

Fig. 2

|          | WT1 (WT/WT)                                                                      | Patient (WT/MUT)                                                                  | MUT-int1 (TPM1-F18L, MUT-int/MUT-int)                                            | WT2 (WT/WT)                                                           | MUT-int2 (TnT-R141W, MUT-int/MUT-int)                                 | what data points were used in analyses | Passed normality test (alpha=0.05)?                      | Statistics method                                                                                                                                                                                                                                                                  | n56? | sensitivity analysis                                                                                                                                                                                                                |
|----------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------|----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>C</b> | n=84 cells (348 ROIs); n=3 experiments (n=3 independent cardiac differentiation) | n= 92 cells (421 ROIs); n=3 experiments (n=3 independent cardiac differentiation) | n=95 cells (329 ROIs); n=3 experiments (n=3 independent cardiac differentiation) |                                                                       |                                                                       | ROIs                                   | no                                                       | ***, P < 0.001 for WT1 vs. Pat (Adjusted P value <1 × 10 <sup>-6</sup> ; Yes 15) and WT1 vs. MUT-int1 (Adjusted P value=1.56 × 10 <sup>-9</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                      |      | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.0507) and WT1 vs. MUT-int1 (Adjusted P Value=0.147) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                             |
| <b>D</b> | n=76 cells (n=3 experiments/n=3 independent cardiac differentiation)             | n= 44 cells (n=4 experiments/n=4 independent cardiac differentiation)             | n=80 cells (n=3 experiments/n=3 independent cardiac differentiation).            |                                                                       |                                                                       | cells                                  | no                                                       | ***, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.35 × 10 <sup>-3</sup> ); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=1.36 × 10 <sup>-4</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                             | Yes  | *, P < 0.05 for WT1 vs. Pat (Adjusted P Value=0.0511); *, P < 0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.052) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                        |
| <b>E</b> | n=65 movies (n=3 experiments/n=3 independent cardiac differentiation);           | n=62 movies (n=2 experiments/n=2 independent cardiac differentiation);            | n=72 movies (n=2 experiments/n=2 independent cardiac differentiation)            |                                                                       |                                                                       | movies                                 | no                                                       | *, P < 0.05 for WT1 vs. Pat (Adjusted P Value=2.65 × 10 <sup>-6</sup> ; Yes 2); ***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P Value=1 × 10 <sup>-15</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                          |      | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.104) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                              |
| <b>G</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for WT vs. Pat and WT vs. MUT-int1 iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                                                                        | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.929) and WT vs. MUT-int1 iPSC-CMs (Adjusted P Value=0.657) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                       |
| <b>H</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for WT vs. Pat (Adjusted P Value=0.208); *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0178) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                 | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.208); *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0178) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                  |
| <b>I</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test) | *, P < 0.05 for WT vs. Pat (Adjusted P Value=0.0267) and ***, P < 0.01 for WT vs. MUT-int1 (Adjusted P Value=9.36 × 10 <sup>-3</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                 | Yes  | *, P < 0.05 for WT vs. Pat (Adjusted P Value=0.0267) and ***, P < 0.01 for WT vs. MUT-int1 (Adjusted P Value=9.36 × 10 <sup>-3</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                  |
| <b>K</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | no                                                       | ns, not significant for WT vs. Pat (Adjusted P Value=0.363); WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.929) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                          | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.363); WT vs. MUT-int1 (Adjusted P Value=0.999) and WT vs. igg (Adjusted P Value=0.929) (Kruskal-Wallis test and Dunn's multiple comparisons test).                           |
| <b>L</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | no                                                       | ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.999) iPSC-CMs. *, P < 0.05 for WT vs. Igg (Adjusted P Value=0.0418) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.999) iPSC-CMs. *, P < 0.05 for WT vs. Igg (Adjusted P Value=0.0418) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| <b>M</b> | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)                         | n=5 experiments (n=4 independent cardiac differentiation)                        | n=5 experiments (n=4 independent cardiac differentiation)             |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for WT vs. Pat (Adjusted P Value=0.326); WT vs. MUT-int1 (Adjusted P Value=0.599) iPSC-CMs and WT vs. igg (Adjusted P Value=0.855) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                 | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.326); WT vs. MUT-int1 (Adjusted P Value=0.599) iPSC-CMs and WT vs. igg (Adjusted P Value=0.855) (Kruskal-Wallis test and Dunn's multiple comparisons test).                  |
| <b>O</b> | n=3 experiments (n=1 independent cardiac differentiation).                       | n=3 experiments (n=1 independent cardiac differentiation).                        | n=3 experiments (n=1 independent cardiac differentiation).                       | n=3 experiments (n=1 independent cardiac differentiation).            |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test)                             | ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.423) iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                      | Yes  | ns, not significant for WT vs. Pat (Adjusted P Value=0.999) and WT vs. MUT-int1 (Adjusted P Value=0.423) iPSC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                       |
| <b>P</b> | n=3 experiments (n=1 independent cardiac differentiation).                       | n=3 experiments (n=1 independent cardiac differentiation).                        | n=3 experiments (n=1 independent cardiac differentiation).                       | n=3 experiments (n=1 independent cardiac differentiation).            |                                                                       | independent experiments                | Yes (pass Shapiro-Wilk test)                             | ns, not significant (Adjusted P value=0.202) for WT vs. Pat; *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0341) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                 | Yes  | ns, not significant (Adjusted P value=0.202) for WT vs. Pat; *, P < 0.05 for WT vs. MUT-int1 (Adjusted P Value=0.0341) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                  |
| <b>R</b> | n= 46 cells (n=3 experiments (n=3 independent cardiac differentiation)           | n=63 cells; n=3 experiments (n=3 independent cardiac differentiation)             | n=54 cells; n=3 experiments (n=3 independent cardiac differentiation)            | n=43 cells; n=3 experiments (n=3 independent cardiac differentiation) | n=42 cells; n=3 experiments (n=3 independent cardiac differentiation) | cells                                  | no                                                       | ***, P < 0.01 for WT1 vs. Pat (Adjusted P Value=3.58 × 10 <sup>-3</sup> ); *, P < 0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.0122); ***, P < 0.001 for WT2 vs. MUT-int2 (Adjusted P Value=8.47 × 10 <sup>-8</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.604); WT1 vs. MUT-int1 (Adjusted P Value=0.946) and WT2 vs. MUT-int2 (Adjusted P Value=0.166) (Kruskal-Wallis test and Dunn's multiple comparisons test).                   |

| Fig. 3                                                                                   |                                                                              |                                                                              |                                                                              |                                                                           |                                                                                                           |                                                                                                                     |                                                                                                                                                                                                                                                                                                                  |      |                                                                                                                                                                                                                                                                         |
|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WT1 (WT/WT)                                                                              | Patient (WT/MUT)                                                             | MUT-int1<br>(TPM1-FHSL MUT-<br>int/MUT-int)                                  | WT2<br>(WT/WT)                                                               | MUT-int2<br>(Tst-RE141W MUT-<br>int/MUT-int)                              | what data points<br>were used in analyses                                                                 | Passed normality test<br>(alpha=0.05)?                                                                              | Statistics method                                                                                                                                                                                                                                                                                                | n50? | sensitivity analysis                                                                                                                                                                                                                                                    |
| <b>A</b><br>n=4 experiments<br>/4 independent cardiac<br>differentiation                 | n=4 experiments<br>/4 independent cardiac<br>differentiation                 | n=4 experiments<br>/4 independent cardiac<br>differentiation                 | n=3 experiments<br>/3 independent cardiac<br>differentiation                 | n=4 experiments<br>/4 independent cardiac<br>differentiation              | Technical replicates from<br>independent experiments (Each experiment<br>contains 4 technical replicates) | Yes (pass Anderson-Darling test,<br>D'Agostino & Pearson test,<br>Shapiro-Wilk test and<br>Kolmogorov-Smirnov test) | **, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.25 × 10 <sup>-3</sup> ); ***, P < 0.001 for WT1<br>vs. MUT-int1 (Adjusted P Value=4.77 × 10 <sup>-8</sup> ); ns, not significant for WT2 vs. MUT-int2<br>(Adjusted P Value=0.061) (Kruskal-Wallis test and Dunn's multiple comparisons test).                   | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.235); *, P < 0.05 for WT1 vs. MUT-<br>int1 (Adjusted P Value=0.0115); ns, not<br>significant for WT2 vs. MUT-int2 (Adjusted P<br>Value=0.999) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |
| <b>C</b><br>n=78 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)  | n=50 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation); | n=101 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation) | n=39 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation); | n=35 cells (n=2<br>experiments/2 independent<br>cardiac differentiation). | cells                                                                                                     | no                                                                                                                  | ***, P < 0.001 for WT1 vs. Pat (Adjusted P Value=4.23 × 10 <sup>-12</sup> ), WT1 vs. MUT-int1<br>(Adjusted P Value=1.23 × 10 <sup>-4</sup> ) and WT2 vs. MUT-int2 (Adjusted P Value=6.92 × 10 <sup>-7</sup> )<br>(Kruskal-Wallis test and Dunn's multiple comparisons test).                                     | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.291), WT1 vs. MUT-int1 (Adjusted P<br>Value=0.999) and WT2 vs. MUT-int2 (Adjusted<br>P Value=0.380) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test).                                           |
| <b>E</b><br>n=23 cells<br>(n=2 experiments/2<br>independent cardiac<br>differentiation); | n=23 cells<br>(n=2 experiments/2<br>independent cardiac<br>differentiation); | n=28 cells<br>(n=2 experiments/2<br>independent cardiac<br>differentiation)  | n=42 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)  | n=28 cells (n=3<br>experiments/3 independent<br>cardiac differentiation)  | cells                                                                                                     | no                                                                                                                  | ***, P < 0.001 for WT1 vs. Pat (Adjusted P Value=2 × 10 <sup>-15</sup> ), WT1 vs. MUT-int1<br>(Adjusted P Value=5.88 × 10 <sup>-6</sup> ), and WT2 vs. MUT-int2 (Adjusted P Value=2.24 × 10 <sup>-4</sup> )<br>(Kruskal-Wallis test and Dunn's multiple comparisons test).                                       | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.497), WT1 vs. MUT-int1 (Adjusted P<br>Value=0.075) and WT2 vs. MUT-int2<br>(Adjusted P Value=0.999) (Kruskal-Wallis test<br>and Dunn's multiple comparisons test).                                           |
| <b>G</b><br>n=52 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)  | n=49 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)  | n=59 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)  | n=26 cells<br>(n=2 experiments/2<br>independent cardiac<br>differentiation)  | n=31 cells (n=2<br>experiments/2 independent<br>cardiac differentiation)  | cells                                                                                                     | Yes (pass Anderson-Darling test,<br>D'Agostino & Pearson test,<br>Shapiro-Wilk test and<br>Kolmogorov-Smirnov test) | **, P < 0.01 for WT1 vs. Pat (Adjusted P Value=1.347 × 10 <sup>-3</sup> ); ***, P < 0.001 for WT1<br>vs. MUT-int1 (Adjusted P Value=8.16 × 10 <sup>-11</sup> ); **, P < 0.01 for WT2 vs. MUT-int2<br>(Adjusted P Value=1.105 × 10 <sup>-3</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons<br>test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.224), WT1 vs. MUT-int1 (Adjusted P<br>Value=0.139) and WT2 vs. MUT-int2 (Adjusted<br>P Value=0.598) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test).                                           |

| Fig. 4 |                                                                             |                                                                                                        |                                                                                                          |                                                                             |                                                                                                        |                                                      |                                        |                                                                                                                                                                                                                                                                                                  |      |                                                                                                                                                                                                                              |
|--------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|        | WT1 (WT/WT)                                                                 | Patient (WT/MUT)                                                                                       | MUT-int1 (TPM1-F185L<br>MUT-int/MUT-int)                                                                 | WT2 (WT/WT)                                                                 | MUT-int2 (TaT-R141W<br>MUT-int/MUT-int)                                                                | what data points were used<br>in analyses            | Passed normality<br>test (alpha=0.05)? | Statistics method                                                                                                                                                                                                                                                                                | n567 | sensitivity analysis                                                                                                                                                                                                         |
| B      | n=87 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation) | n=60 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)                            | n=120 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation)                             | n=55 cells<br>(n=3 experiments/3<br>independent cardiac<br>differentiation) | n=50 cells<br>(n=2 experiments/2<br>independent cardiac<br>differentiation).                           | cells                                                | no                                     | *, P<0.05 for WT1 vs. Pat (Adjusted P Value=1.86 × 10 <sup>-2</sup> );<br>***, P < 0.001 for WT1 vs. MUT-int1 (Adjusted P<br>Value=9.88 × 10 <sup>-6</sup> ) and WT2 vs. MUT-int2 (Adjusted P<br>Value=2.15 × 10 <sup>-10</sup> ) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value<0.999), WT1 vs.MUT-int1 (Adjusted P<br>Value<0.999) and WT2 vs. MUT-int2 (Adjusted P<br>Value=0.528) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |
| C      | n=4 experiments/4<br>independent<br>cardiac differentiation                 | n=4 experiments/4<br>independent<br>cardiac differentiation                                            | n=4 experiments/4<br>independent<br>cardiac differentiation                                              |                                                                             |                                                                                                        | Technical replicates from<br>independent experiments | no                                     | ***, P<0.001 for WT1 vs. patient (Adjusted P Value=8.17 ×<br>10 <sup>-6</sup> ) and WT1 vs. mut-int1 (Adjusted P Value=1 × 10 <sup>-15</sup> )<br>iPSC-CMs, (Kruskal-Wallis test and Dunn's multiple<br>comparisons test).                                                                       | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value<0.999) and WT1 vs.MUT-int1 (Adjusted P<br>Value=0.232) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test).                                               |
| F      |                                                                             | CV, n=47 cells; Fe(II), n=50<br>cells<br>n=2 experiments/2<br>independent cardiac<br>differentiation   |                                                                                                          |                                                                             |                                                                                                        | cells                                                | no                                     | ***, P < 0.001 for CV vs. Fe(II) Pat iPSC-CMs (P<br>value=1.65 × 10 <sup>-8</sup> ) (Mann-Whitney test).                                                                                                                                                                                         | Yes  | ns, not significant for CV vs. Fe(II) Pat iPSC-CMs<br>(P Value=0.667) (Mann-Whitney test).                                                                                                                                   |
| H      |                                                                             |                                                                                                        | CV, n=48 cells; Fe(II), n=45<br>cells<br>n=2 experiments/2<br>independent cardiac<br>differentiation     |                                                                             |                                                                                                        | cells                                                | no                                     | ***, P < 0.001 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P<br>value=1 × 10 <sup>-15</sup> ) (Mann-Whitney test).                                                                                                                                                                                      | Yes  | ns, not significant for CV vs. Fe(II) MUT-int1 iPSC-<br>CMs (P Value=0.333) (Mann-Whitney test).                                                                                                                             |
| I      |                                                                             | CV, n=21 movies; Fe(II),<br>n=21 movies<br>n=2 experiments/2<br>independent cardiac<br>differentiation |                                                                                                          |                                                                             |                                                                                                        | Technical replicates from<br>independent experiments | no                                     | ***, P < 0.001 for CV vs. Fe (II) in Pat iPSC-CMs (P<br>value=8.80 × 10 <sup>-4</sup> ) (Mann-Whitney test).                                                                                                                                                                                     | Yes  | ns, not significant for CV vs. Fe(II) Pat iPSC-CMs<br>(P Value=0.333) (Mann-Whitney test).                                                                                                                                   |
| J      |                                                                             |                                                                                                        | CV, n=41 movies; Fe (II),<br>n=115 movies<br>n=6 experiments/6<br>independent cardiac<br>differentiation |                                                                             |                                                                                                        | Technical replicates from<br>independent experiments | no                                     | ***, P < 0.001 for CV vs. Fe(II) in MUT-int1 iPSC-CMs (P<br>value=1 × 10 <sup>-15</sup> ) (Mann-Whitney test).                                                                                                                                                                                   | Yes  | *, P < 0.05 for CV vs. Fe(II) MUT-int1 iPSC-CMs<br>(P value=0.0411) (Mann-Whitney test).                                                                                                                                     |
| K      |                                                                             |                                                                                                        |                                                                                                          |                                                                             | CV, n=29 movies; Fe(II),<br>n=63 movies<br>n=5 experiments/5<br>independent cardiac<br>differentiation | Technical replicates from<br>independent experiments | no                                     | ***, P<0.001 for CV vs. Fe(II) MUT-int2 (P value=1.60 ×<br>10 <sup>-4</sup> ) (Mann-Whitney test).                                                                                                                                                                                               | Yes  | ns, not significant for CV vs. Fe(II) MUT-int2 iPSC-<br>CMs (P Value=0.1508) (Mann-Whitney test).                                                                                                                            |

Fig. 5

|   | Patient (WT/MUT)                                                                                                    | MUT-int1 (TPM1-F18SL MUT-int/MUT-int)                                                                              | what data points were used in analyses            | Passed normality test (alpha=0.05)?                                                                        | Statistics method                                                                                                       | n56? | sensitivity analysis                                                        |
|---|---------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------|-----------------------------------------------------------------------------|
| A |                                                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                              | Values from independent experiments               | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns. for CV vs. RhoA II (P value=0.100) (Mann-Whitney test).                                                             | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test). |
| C | CV, n=39 cells; RhoA II, n=38 cells; n=2 experiments/2 independent cardiac differentiation                          |                                                                                                                    | cells                                             | no                                                                                                         | ***, P < 0.001 (P value=6.36 × 10 <sup>-3</sup> ) for CV vs. RhoA II Patient (Mann-Whitney test).                       | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.200) (Mann-Whitney test). |
| D |                                                                                                                     | CV, n=19 cells; RhoA II, n=26 cells; n=2 experiments/2 independent cardiac differentiation                         | cells                                             | no                                                                                                         | ***, P < 0.001 (P value=6.68 × 10 <sup>-5</sup> ) for CV vs. RhoA II MUT-int1 (Mann-Whitney test).                      | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.333) (Mann-Whitney test). |
| F |                                                                                                                     | CV, n=37 cells; RhoA II, n=87 cells; n=3 experiments/3 independent cardiac differentiation                         | cells                                             | no                                                                                                         | ** P < 0.01 (P value=2.30 × 10 <sup>-3</sup> ) for CV vs. RhoA II in MUT-int1 (Mann-Whitney test).                      | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test). |
| H |                                                                                                                     | CV, n=40 cells; RhoA II, n=37 cells; n=3 experiments/3 independent cardiac differentiation                         | cells                                             | Yes (pass Shapiro-Wilk test)                                                                               | ***, P < 0.001 for CV vs. Rho activator II in PM-localized EEA1 (P value=2.51 × 10 <sup>-5</sup> ) (Mann-Whitney test). | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test). |
| J | CV, n=45 cells; RhoA II, n=36 cells; n=2 experiments/2 independent cardiac differentiation                          |                                                                                                                    | cells                                             | no                                                                                                         | ***, P < 0.001 for CV Pat vs. RhoA II Pat (P value=1.19 × 10 <sup>-5</sup> ) (Mann-Whitney test)                        | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.667) (Mann-Whitney test). |
| L |                                                                                                                     | CV, n=50 cells; RhoA II, n=59 cells; n=3 experiments/3 independent cardiac differentiation                         | cells                                             | no                                                                                                         | ***, P < 0.001 for CV vs. RhoA II MUT-int1 (P value=6.34 × 10 <sup>-4</sup> ) (Mann-Whitney test)                       | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test). |
| M | n=2 experiments/2 independent cardiac differentiation                                                               |                                                                                                                    | Technical replicates from independent experiments | no                                                                                                         | ** P<0.01 for patient CV vs. patient RhoA II (P value=2.44 × 10 <sup>-4</sup> ) (Mann Whitney test).                    | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.667) (Mann-Whitney test). |
| O |                                                                                                                     | n=2 experiments/2 independent cardiac differentiation                                                              | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for mut-int1 CV vs. mut-int1 RhoA II (P value<1 × 10 <sup>-15</sup> ) (Mann Whitney test).                 | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.333) (Mann-Whitney test). |
| Q |                                                                                                                     | CV, n=88 movies; RhoA II, n=46 movies; n=3 experiments/3 independent cardiac differentiation                       | Technical replicates from independent experiments | no                                                                                                         | ***, P < 0.001 CV vs. Rho activator II MUT-int1 (P value=5.07 × 10 <sup>-7</sup> ) (Mann-Whitney test).                 | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.400) (Mann-Whitney test). |
| S | CV, n=56 cells, n=287 ROIs; RhoA II, n= 67 cells, n=292 ROIs; n=3 experiments/3 independent cardiac differentiation |                                                                                                                    | ROIs                                              | no                                                                                                         | *, P < 0.05 (P value=0.0363) CV vs. RhoA II Pat (Mann-Whitney test).                                                    | Yes  | ns, not significant for CV vs. RhoA II (P Value>0.999) (Mann-Whitney test). |
| T |                                                                                                                     | CV, n=52 cells, n=142 ROIs; RhoA II, n=51 cells, n=165 ROIs; n=3 experiments/3 independent cardiac differentiation | ROIs                                              | no                                                                                                         | *, P < 0.05 (P value=0.0231) CV vs. RhoA II MUT-int1 (Mann-Whitney test).                                               | Yes  | ns, not significant for CV vs. RhoA II (P Value=0.100) (Mann-Whitney test). |

Fig. 6

|          | Patient (WT/MUT)                                                                 | Mut-corrected (WT-cor/WT-cor )                                                                                                    | what data points were used in analyses            | Passed normality test (alpha=0.05)?                                                                        | Statistics method                                                                                                                                                                                                                                                                                     | n567 | sensitivity analysis                                                                                                                                                                                                               |
|----------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>B</b> | n=4 experiments/<br>4 independent cardiac differentiation                        | n=5 experiments/<br>5 independent cardiac differentiation                                                                         | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ***, $P < 0.001$ (P value= $9.011 \times 10^{-4}$ ) for Pat vs. mut-corrected (Mann-Whitney test).                                                                                                                                                                                                    | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.111) (Mann-Whitney test).                                                                                                                                                 |
| <b>D</b> | n=23 cells; n=2 experiments/<br>2 independent cardiac differentiation            | n=52 cells; n=3 experiments/<br>3 independent cardiac differentiation                                                             | cells                                             | no                                                                                                         | ***, $P < 0.001$ (P value= $1.63 \times 10^{-4}$ ) for Pat vs. mut-corrected (Mann-Whitney test).                                                                                                                                                                                                     | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.800) (Mann-Whitney test).                                                                                                                                                 |
| <b>F</b> | n=49 cells; n=3 experiments/<br>3 independent cardiac differentiation            | n=41 cells; n=3 experiments/<br>3 independent cardiac differentiation                                                             | cells                                             | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ***, $P < 0.001$ for Pat vs. mut-corrected in PM-localized EEA1 (P value= $1.308 \times 10^{-5}$ ) (Mann-Whitney test).                                                                                                                                                                               | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.200) (Mann-Whitney test).                                                                                                                                                 |
| <b>H</b> | n=44 cells; n=2 experiments/<br>2 independent cardiac differentiation            | n=43 cells; n=2 experiments/<br>2 independent cardiac differentiation                                                             | cells                                             | no                                                                                                         | ***, $P < 0.001$ for Pat vs. mut-corrected (P value= $1 \times 10^{-15}$ ) (Mann-Whitney test).                                                                                                                                                                                                       | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.333) (Mann-Whitney test).                                                                                                                                                 |
| <b>I</b> | n=4 experiments/<br>4 independent cardiac differentiation                        | n=2 experiments/<br>2 independent cardiac differentiation                                                                         | values from independent experiments               | no                                                                                                         | ***, $P < 0.001$ for patient vs. mut-cor iPSC-CMs (P value= $3.54 \times 10^{-6}$ ) (Mann-Whitney test).                                                                                                                                                                                              | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.800) (Mann-Whitney test).                                                                                                                                                 |
| <b>L</b> | n=92 cells; n=421 ROI; n=3 experiments/<br>3 independent cardiac differentiation | n=36 cells; n=299 ROI; n=2 experiments/<br>2 independent cardiac differentiation                                                  | ROIs                                              | no                                                                                                         | P value= $6.62 \times 10^{-3}$ , **, $P < 0.01$ for Pat vs. mut-corrected (Mann-Whitney test).                                                                                                                                                                                                        | Yes  | ns, not significant for Pat vs. mut-corrected (P Value=0.400) (Mann-Whitney test).                                                                                                                                                 |
| <b>M</b> | n=44 cells; n=4 experiments/<br>4 independent cardiac differentiation            | n=58 cells; n=3 experiments/<br>3 independent cardiac differentiation                                                             | cells                                             | no                                                                                                         | ***, $P < 0.001$ for Pat vs. mut-corrected (P value= $5.36 \times 10^{-5}$ ) (Mann-Whitney test).                                                                                                                                                                                                     | Yes  | *, $P < 0.05$ for Pat vs. mut-corrected (P value=0.0286) (Mann-Whitney test).                                                                                                                                                      |
| <b>O</b> |                                                                                  | CV, n=33 cells; DFO, n=34 cells;<br>DFO+RhoA II, n=38 cells; n=2<br>experiments/2 independent cardiac<br>differentiation          | cells                                             | no                                                                                                         | ***, $P < 0.001$ for CV vs. DFO (P value= $1.47 \times 10^{-8}$ ) and DFO vs. DFO+RhoA II (P value= $8.41 \times 10^{-11}$ ). ns, not significant for CV vs. DFO +RhoA II (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                 | Yes  | ns, not significant for CV vs. DFO (Adjusted P Value=0.326), DFO vs. DFO+RhoA II (Adjusted P Value=0.326) and CV vs. DFO +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).             |
| <b>P</b> |                                                                                  | CV, n=24 movies; DFO, n=23 movies;<br>DFO+RhoA II, n=24 movies; n=2<br>experiments/2 independent cardiac<br>differentiation       | Technical replicates from independent experiments | no                                                                                                         | ***, $P < 0.001$ for CV vs. DFO (Adjusted P Value= $3.47 \times 10^{-12}$ ) and DFO vs. DFO+RhoA II (Adjusted P Value= $1 \times 10^{-15}$ ). ns, not significant for CV vs. DFO+RhoA II (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                  | Yes  | ns, not significant for CV vs. DFO (Adjusted P Value=0.999), DFO vs. DFO+RhoA II (Adjusted P Value=0.326) and CV vs. DFO +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).             |
| <b>R</b> |                                                                                  | CV, n=34 cells; TFR Ab, n=33 cells;<br>TFR Ab+RhoA II, n=39 cells; n=2<br>experiments/2 independent cardiac<br>differentiation    | cells                                             | no                                                                                                         | ***, $P < 0.001$ for CV vs. TFR Ab (Adjusted P Value= $1.19 \times 10^{-4}$ ) and TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value= $9 \times 10^{-15}$ ); **, $P < 0.01$ for CV vs. TFR Ab+RhoA II (Adjusted P Value= $7.05 \times 10^{-4}$ ) (Kruskal-Wallis test and Dunn's multiple comparisons test); | Yes  | ns, not significant for CV vs. TFR Ab (Adjusted P Value=0.999), TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value=0.326) and CV vs. TFR Ab +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| <b>S</b> |                                                                                  | CV, n=29 movies; TFR Ab, n=30<br>movies; TFR Ab+RhoA II, n=29<br>movies; n=2 experiments/2 independent<br>cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ***, $P < 0.001$ for CV vs. TFR Ab (Adjusted P Value= $1 \times 10^{-15}$ ) and TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value= $1 \times 10^{-15}$ ); *, $P < 0.05$ for CV vs. TFR Ab +RhoA II (Adjusted P Value=0.047) (Kruskal-Wallis test and Dunn's multiple comparisons test)                      | Yes  | ns, not significant for CV vs. TFR Ab (Adjusted P Value=0.184), TFR Ab vs. TFR Ab+RhoA II (Adjusted P Value=0.544) and CV vs. TFR Ab +RhoA II (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |

| Fig. 7 |                           |                           |                                        |                                     |                                                                        |                                                                               |
|--------|---------------------------|---------------------------|----------------------------------------|-------------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------|
|        | Control                   | HF                        | what data points were used in analyses | Passed normality test (alpha=0.05)? | Statistics method                                                      | n≤6? sensitivity analysis                                                     |
| C      | n=45 images, n=4 patients | n=52 images, n=5 patients | images                                 | no                                  | **<br>P<0.01 for control vs. HF (P value=0.00187) (Mann-Whitney test). | Yes<br>**<br>P<0.01 for control vs. HF (P value=0.00187) (Mann-Whitney test). |
| F      | n=61 images, n=4 patients | n=76 images, n=5 patients | images                                 | no                                  | **<br>P<0.01 for control vs. HF (P value=0.00121) (Mann-Whitney test). | Yes<br>**<br>P<0.01 for control vs. HF (P value=0.00121) (Mann-Whitney test). |

| Fig. 8   |                          |                          |                                        |                                     |                                                                                        |                                                                                            |
|----------|--------------------------|--------------------------|----------------------------------------|-------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
|          | CV                       | RhoAII                   | what data points were used in analyses | Passed normality test (alpha=0.05)? | Statistics method                                                                      | n≤6? sensitivity analysis                                                                  |
| <b>B</b> | n=42 cells; n=3 patients | n=47 cells; n=3 patients | cells                                  | no                                  | ***, P<0.001 for CV vs. RhoA II (P value= $1.21 \times 10^{-5}$ ) (Mann Whitney test). | Yes ***, P<0.001 for CV vs. RhoA II (P value= $1.21 \times 10^{-5}$ ) (Mann Whitney test). |

| Fig. S2                                                                 |                                                             |                                                             |                                                      |                                                                                                                      |                                                                                                                                                                                        |      |                                                                                                                                                                                  |
|-------------------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WT1 (WT/WT)                                                             | Patient (WT/MUT)                                            | MUT-int1 (TPM1-F18SL<br>MUT-int/MUT-int)                    | what data points were<br>used in analyses            | Passed normality test<br>(alpha=0.05)?                                                                               | Statistics method                                                                                                                                                                      | n≤6? | sensitivity analysis                                                                                                                                                             |
| <b>C</b><br>n=2 experiments/2<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | Technical replicates from<br>independent experiments | no                                                                                                                   | ***, P < 0.001 for WT1 vs. Pat (Adjusted P Value=9.41 × 10 <sup>-4</sup> ); ns, not<br>significant for WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple<br>comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.0883) and WT1 vs. MUT-int1<br>(Adjusted P Value>0.999) (Kruskal-Wallis test<br>and Dunn's multiple comparisons test). |
| <b>D</b><br>n=3 experiments/3<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | Technical replicates from<br>independent experiments | Yes (pass Anderson-Darling test,<br>D'Agostino & Pearson test, Shapiro-<br>Wilk test and Kolmogorov-Smirnov<br>test) | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis<br>test and Dunn's multiple comparisons test).                                                                | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value=0.0507) and WT1 vs. MUT-int1<br>(Adjusted P Value=0.742) (Kruskal-Wallis test<br>and Dunn's multiple comparisons test). |
| <b>E</b><br>n=3 experiments/3<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | n=3 experiments/3<br>independent cardiac<br>differentiation | Technical replicates from<br>independent experiments | no                                                                                                                   | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis<br>test and Dunn's multiple comparisons test).                                                                | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value>0.999) and WT1 vs. MUT-int1 (Adjusted<br>P Value>0.999) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test).  |

| Fig. S3 |                                                                                                                                           |                                                                                                                                                     |                                                       |                                                   |                                                                                                            |                                                                                                                                     |                                                                                                                                                                              |
|---------|-------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | WT1 (WT/WT)                                                                                                                               | Patient (WT/MUT)                                                                                                                                    | MUT-int1 (TPM1-F18SL MUT-int/MUT-int)                 | what data points were used in analyses            | Passed normality test (alpha=0.05)?                                                                        | Statistics method                                                                                                                   | n56? sensitivity analysis                                                                                                                                                    |
| A       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| B       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.912) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| C       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.202) and WT1 vs.MUT-int1 (Adjusted P Value=0.593) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| D       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for WT1 vs. pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.912) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| H       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| I       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| J       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.742) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                              |
| K       | n=3 experiments/3 independent cardiac differentiation                                                                                     | n=3 experiments/3 independent cardiac differentiation                                                                                               | n=3 experiments/3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 $\beta$ SC-CMs (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes<br>ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| M       | WT (WT1+WT2)<br>n=2 cell lines, n=3 independent experiments/3 independent cardiac differentiation (each experiment contains 6 replicates) | DCM (Patient+Mut-int1)<br>n=2 cell lines, n=3 independent experiments/3 independent cardiac differentiation (each experiment contains 6 replicates) |                                                       | Technical replicates from independent experiments | no                                                                                                         | *, P<0.05 for WT vs. DCM (P value=0.0206) (Mann Whitney test).                                                                      | Yes<br>ns, not significant for WT vs. DCM (P Value=0.400)(Mann Whitney test).                                                                                                |
| N       | WT1 (WT/WT)<br>n=2 independent experiments were performed, each experiment contained n=3 technical replicates.                            | Mut-int1 (MUT/MUT)<br>n=2 independent experiments were performed, each experiment contained n=3 technical replicates.                               |                                                       | Technical replicates from independent experiments | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)                                                   | **, P<0.01 for WT1 vs. Mut-int1 (P value=0.00216) (Mann Whitney test).                                                              | Yes<br>ns, not significant for WT1 vs. Mut-int1 (P Value=0.333) (Mann Whitney test).                                                                                         |
| O       | n=2 independent experiments (2 independent cardiac differentiation) were performed, each experiment contained n=3 technical replicates.   | n=2 independent experiments (2 independent cardiac differentiation) were performed, each experiment contained n=3 technical replicates.             |                                                       | Technical replicates from independent experiments | Yes (pass Shapiro-Wilk test and Kolmogorov-Smirnov test)                                                   | **, P<0.01 for WT1 vs. Mut-int1 (P value=0.00216) (Mann Whitney test).                                                              | Yes<br>ns, not significant for WT1 vs. Mut-int1 (P Value=0.333) (Mann Whitney test).                                                                                         |

| Fig. S4                                                                        |                                                                       |                                                                       |                                                   |                                     |                                                                                                                                                                                                      |      |                                                                                                                                                                       |
|--------------------------------------------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------|---------------------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WT1 (WT/WT)                                                                    | Patient (WT/MUT)                                                      | MUT-int1 (TPM1-F18SL MUT-int/MUT-int)                                 | what data points were used in analyses            | Passed normality test (alpha=0.05)? | Statistics method                                                                                                                                                                                    | n56? | sensitivity analysis                                                                                                                                                  |
| <b>D</b> n=65 movies (n=3 experiments/n=3 independent cardiac differentiation) | n=62 movies (n=2 experiments/n=2 independent cardiac differentiation) | n=72 movies (n=2 experiments/n=2 independent cardiac differentiation) | movies                                            | no                                  | ns, not significant for WT1 vs. Pat; **, $P < 0.01$ WT1 vs. MUT-int1 (Adjusted P Value= $1.36 \times 10^{-3}$ ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                          | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.894) and WT1 vs.MUT-int1 (Adjusted P Value=0.621) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| <b>E</b> n=65 movies (n=3 experiments/n=3 independent cardiac differentiation) | n=62 movies (n=2 experiments/n=2 independent cardiac differentiation) | n=72 movies (n=2 experiments/n=2 independent cardiac differentiation) | movies                                            | no                                  | ns, not significant for WT1 vs. Pat; ***, $P < 0.001$ WT1 vs. MUT-int1 (Adjusted P Value= $5.81 \times 10^{-6}$ ) (Kruskal-Wallis test and Dunn's multiple comparisons test).                        | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.410) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| <b>F</b> n=4 experiments/n=4 independent cardiac differentiation               | n=3 experiments/n=3 independent cardiac differentiation               | n=4 experiments/n=4 independent cardiac differentiation               | Technical replicates from independent experiments | no                                  | ***, $P < 0.001$ for WT1 vs. Pat (Adjusted P Value= $1.20 \times 10^{-6}$ ) and WT1 vs. Mut-int1 (Adjusted P Value= $1 \times 10^{-15}$ ) (Kruskal-Wallis test and Dunn's multiple comparisons test) | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.859) and WT1 vs.MUT-int1 (Adjusted P Value=0.176) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| <b>G</b> n=4 experiments/n=4 independent cardiac differentiation               | n=3 experiments/n=3 independent cardiac differentiation               | n=4 experiments/n=4 independent cardiac differentiation               | Technical replicates from independent experiments | no                                  | ***, $P < 0.001$ for WT1 vs. Pat (Adjusted P Value= $1 \times 10^{-14}$ ) and WT1 vs. Mut-int1 (Adjusted P Value= $1.28 \times 10^{-8}$ ) (Kruskal-Wallis test and Dunn's multiple comparisons test) | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.999) and WT1 vs.MUT-int1 (Adjusted P Value=0.482) (Kruskal-Wallis test and Dunn's multiple comparisons test). |

| Fig. S5    |                                                                                |                                                               |                                                               |                                                               |                                           |                                                                |                                                                                                                                      |      |                                                                                                                                                                                                                       |
|------------|--------------------------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|-------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|            | WT (WT/WT)                                                                     | Patient (WT/MUT)                                              | MUT-int1 (TPM1-F185L)<br>MUT-int/MUT-int                      | WT2 (WT/WT)                                                   | what data points were used<br>in analyses | Passed normality test<br>(alpha=0.05)?                         | Statistics method                                                                                                                    | n56? | sensitivity analysis                                                                                                                                                                                                  |
| <b>A</b>   | WT1, n=3 experiments/n=3<br>independent cardiac<br>differentiation             | n=3 experiments/n=3<br>independent cardiac<br>differentiation | n=3 experiments/n=3<br>independent cardiac<br>differentiation |                                                               | independent experiments                   | Yes (pass Shapiro-Wilk test)                                   | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1<br>(Kruskal-Wallis test and Dunn's multiple comparisons test).              | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value<0.999) and WT1 vs. MUT-int1 (Adjusted<br>P Value<0.999) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test).                                       |
| <b>B-C</b> | WT (WT1+WT2), n=5<br>experiments/n=4<br>independent cardiac<br>differentiation |                                                               |                                                               |                                                               | independent experiments                   | Yes (pass Shapiro-Wilk test<br>and Kolmogorov-Smirnov<br>test) | ** P < 0.01 for WT vs. Igg (P value=7.94 × 10 <sup>-3</sup> ) (Mann<br>Whitney test).                                                | Yes  | ** P < 0.01 for WT vs. Igg (P value=7.94 × 10 <sup>-3</sup> )<br>(Mann Whitney test).                                                                                                                                 |
| <b>E</b>   | WT (WT1+WT2), n=3<br>experiments/n=1<br>independent cardiac<br>differentiation |                                                               |                                                               |                                                               | independent experiments                   | Yes (pass Shapiro-Wilk test)                                   | P value=0.100. ns, not significant for WT vs. igg (Mann<br>Whitney test).                                                            | Yes  | P value=0.100. ns, not significant for WT vs. igg<br>(Mann Whitney test).                                                                                                                                             |
| <b>F</b>   | WT (WT1+WT2), n=3<br>experiments/n=1<br>independent cardiac<br>differentiation |                                                               |                                                               |                                                               | independent experiments                   | Yes (pass Shapiro-Wilk test)                                   | P value=0.100. ns, not significant for WT vs. igg (Mann<br>Whitney test).                                                            | Yes  | P value=0.100. ns, not significant for WT vs. igg<br>(Mann Whitney test).                                                                                                                                             |
| <b>H</b>   | WT (WT1+WT2), n=3<br>experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | independent experiments                   | Yes (pass Shapiro-Wilk test)                                   | ns, not significant for WT vs. Pat, WT vs. MUT-int1 and WT<br>vs. igg (Kruskal-Wallis test and Dunn's multiple comparisons<br>test). | Yes  | ns, not significant for WT vs. Pat (Adjusted P<br>Value<0.999), WT vs. MUT-int1 (Adjusted P<br>Value<0.999) and WT vs. igg (Adjusted P<br>Value<0.999) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |
| <b>I</b>   | WT (WT1+WT2), n=3<br>experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | n=3 experiments/n=1<br>independent cardiac<br>differentiation | independent experiments                   | no                                                             | ns, not significant for WT vs. Pat, WT vs. MUT-int1 and WT<br>vs. igg (Kruskal-Wallis test and Dunn's multiple comparisons<br>test). | Yes  | ns, not significant for WT vs. Pat (Adjusted P<br>Value<0.999), WT vs. MUT-int1 (Adjusted P<br>Value<0.999) and WT vs. igg (Adjusted P<br>Value<0.773) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |

Fig. S6

|   | WT1 (WT/WT)                                             | Patient (WT/MUT)                                        | MUT-int1 (TPM1-F18SL MUT-int/MUT-int)                   | WT2 (WT/WT)                                                                                  | MUT-int2 (TnT-R141W MUT-int/MUT-int)                    | what data points were used in analyses            | Passed normality test (alpha=0.05)? | Statistics method                                                                                                    | n56? | sensitivity analysis                                                                                                                                                   |
|---|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|----------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| A | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation |                                                                                              |                                                         | independent experiments                           | Yes (pass Shapiro-Wilk test)        | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value>0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| C |                                                         |                                                         |                                                         | CV, n=45 cells; UNC3230, n=51 cells; n=3 experiments/n=3 independent cardiac differentiation |                                                         | cells                                             | no                                  | ***, P < 0.001 (P value<1 × 10 <sup>-15</sup> ) for CV WT2 vs. UNC3230 WT2 (Mann-Whitney test).                      | Yes  | ns, not significant for CV WT2 vs. UNC3230 WT2 (P Value=0.100) (Mann-Whitney test).                                                                                    |
| D | n=3 experiments/n=3 independent cardiac differentiation |                                                         |                                                         |                                                                                              |                                                         | Technical replicates from independent experiments | no                                  | **, P<0.01 (P value=2.94 × 10 <sup>-3</sup> ) for CV WT1 vs. UNC3230 WT1 (Mann-Whitney test).                        | Yes  | ns, not significant for CV WT1 vs. UNC3230 WT1 (P Value=0.400) (Mann-Whitney test).                                                                                    |
| E |                                                         |                                                         |                                                         | n=3 experiments/n=3 independent cardiac differentiation                                      |                                                         | Technical replicates from independent experiments | no                                  | ***, P<0.001 (Adjusted P Value=2.22 × 10 <sup>-5</sup> ) for CV vs. UNC3230 WT2 iPSC-CMs (Mann-Whitney test).        | Yes  | ns, not significant for CV WT2 vs. UNC3230 WT2 (P Value=0.100) (Mann-Whitney test).                                                                                    |
| F | n=2 experiments/n=2 independent cardiac differentiation | n=2 experiments/n=2 independent cardiac differentiation | n=2 experiments/n=2 independent cardiac differentiation | n=2 experiments/n=2 independent cardiac differentiation                                      | n=2 experiments/n=2 independent cardiac differentiation |                                                   |                                     |                                                                                                                      |      |                                                                                                                                                                        |
| G | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation |                                                                                              |                                                         | independent experiments                           | Yes (pass Shapiro-Wilk test)        | ns, not significant for WT1 vs. Pat and WT1 vs. MUT-int1 (Kruskal-Wallis test and Dunn's multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value>0.999) and WT1 vs. MUT-int1 (Adjusted P Value=0.912) (Kruskal-Wallis test and Dunn's multiple comparisons test). |

| Fig. S7                                                                              |                                                               |                                                                          |                                           |                                                                                                                      |                                                                                                                            |      |                                                                                                                                                                                 |
|--------------------------------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WT1 (WT/WT)                                                                          | Patient (WT/MUT)                                              | MUT-int1 (TPM1-F18SL<br>MUT-int/MUT-int)                                 | what data points were<br>used in analyses | Passed normality test<br>(alpha=0.05)?                                                                               | Statistics method                                                                                                          | n≤6? | sensitivity analysis                                                                                                                                                            |
| <b>B</b><br>n=36 cells;n=3<br>experiments/n=3 independent<br>cardiac differentiation |                                                               | n=40 cells;n=3<br>experiments/n=3 independent<br>cardiac differentiation | cells                                     | Yes (pass Anderson-Darling test,<br>D'Agostino & Pearson test, Shapiro<br>Wilk test and Kolmogorov-<br>Smirnov test) | <b>**</b> , P < 0.01 for WT1 vs. MUT-int1 iPSC-<br>CMs (P value= $1.31 \times 10^{-3}$ ) (Mann<br>Whitney test).           | Yes  | ns, not significant for WT1 vs. MUT-int1 iPSC-<br>CMs (P Value=0.100) (Mann Whitney test).                                                                                      |
| <b>C</b><br>n=3 experiments/n=3<br>independent cardiac<br>differentiation            | n=3 experiments/n=3<br>independent cardiac<br>differentiation | n=3 experiments/n=3<br>independent cardiac<br>differentiation            | independent experiments                   | Yes (pass Shapiro-Wilk test)                                                                                         | ns, not significant for WT1 vs. Pat and<br>WT1 vs. MUT-int1 (Kruskal-Wallis test and<br>Dunn's multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value>0.999) and WT1 vs. MUT-int1 (Adjusted P<br>Value=0.742) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |
| <b>D</b><br>n=2 experiments/n=2<br>independent cardiac<br>differentiation            |                                                               | n=2 experiments/n=2<br>independent cardiac<br>differentiation            | cells                                     | Yes (pass Anderson-Darling test,<br>D'Agostino & Pearson test, Shapiro<br>Wilk test and Kolmogorov-<br>Smirnov test) | <b>**</b> , P<0.01 for WT1 vs. Mut-int1 iPSC-<br>CMs (P value=0.00649) (Mann Whitney<br>test).                             | Yes  | ns, not significant for WT1 vs. MUT-int1 iPSC-<br>CMs (P Value=0.333) (Mann Whitney test).                                                                                      |
| <b>G</b><br>n=3 experiments/n=3<br>independent cardiac<br>differentiation            | n=3 experiments/n=3<br>independent cardiac<br>differentiation | n=3 experiments/n=3<br>independent cardiac<br>differentiation            | independent experiments                   | Yes (pass Shapiro-Wilk test)                                                                                         | ns, not significant for WT1 vs. Pat and<br>WT1 vs. MUT-int1 (Kruskal-Wallis test and<br>Dunn's multiple comparisons test). | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P<br>Value>0.999) and WT1 vs. MUT-int1 (Adjusted P<br>Value=0.999) (Kruskal-Wallis test and Dunn's<br>multiple comparisons test). |

**Fig. S8**

|          | WT1 (WT/WT)                                             | WT2 (WT/WT)                                                                                | what data points were used in analyses            | Passed normality test (alpha=0.05)? | Statistics method                                                                                                         | n≤6? | sensitivity analysis                                                                                        |
|----------|---------------------------------------------------------|--------------------------------------------------------------------------------------------|---------------------------------------------------|-------------------------------------|---------------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------------------------------|
| <b>A</b> | n=2 experiments/n=2 independent cardiac differentiation |                                                                                            | Technical replicates from independent experiments | no                                  | *, $P < 0.05$ ( $P$ value = $1.89 \times 10^{-2}$ ) for CV WT1 vs. Lat-A WT1 (Mann-Whitney test).                         | Yes  | ns, not significant for CV WT1 vs. Lat-A WT1 ( $P$ Value = 0.667) (Mann-Whitney test).                      |
| <b>C</b> |                                                         | CV, n=61 cells; Lat-A, n=65 cells; n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                  | ***, $P < 0.001$ for CV WT2 vs. Lat-A WT2 in PM-localized EEA1 ( $P$ value = $6.02 \times 10^{-8}$ ) (Mann-Whitney test). | Yes  | ns, not significant for CV WT2 vs. Lat-A WT2 in PM-localized EEA1 ( $P$ Value = 0.200) (Mann-Whitney test). |

| Fig. S9     |                                                                                                               |                                                                                     |                                                                                                                                            |                                                         |                                                   |                                                                                                            |                                                                                                                                                                                                                                                                                   |      |                                                                                                                                                                                                                                  |
|-------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| WT1 (WT/WT) | Patient (WT/MUT)                                                                                              | MUT-int1 (CPM-F185L MUT-intMUT-int)                                                 | WT2 (WT/WT)                                                                                                                                | MUT-int2 (TnT-R141W MUT-intMUT-int)                     | what data points were used in analysis            | Passed normality test (alpha=0.05)?                                                                        | Statistics method                                                                                                                                                                                                                                                                 | n567 | sensitivity analysis                                                                                                                                                                                                             |
| A           | n=3 experiments/w=3 independent cardiac differentiation                                                       | n=3 experiments/w=3 independent cardiac differentiation                             | n=3 experiments/w=3 independent cardiac differentiation                                                                                    |                                                         | independent experiments                           | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.272) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                            | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.272) and WT1 vs. MUT-int1 (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                           |
| B           | n=3 experiments/w=3 independent cardiac differentiation                                                       | n=3 experiments/w=3 independent cardiac differentiation                             | n=3 experiments/w=3 independent cardiac differentiation                                                                                    |                                                         | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for WT1 vs. Pat (Adjusted P Value=8.40 × 10 <sup>-4</sup> ). ***, P<0.01 for WT1 vs MUT-int1 (Adjusted P Value=0.00469) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                  | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.147) and WT1 vs. MUT-int1 (Adjusted P Value=0.359) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                           |
| C           |                                                                                                               |                                                                                     | n=3 experiments/w=3 independent cardiac differentiation                                                                                    | n=2 experiments/w=2 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for WT2 vs. Mut-int2 iPSC-CMs (P value<1 × 10 <sup>-5</sup> ). Yes 15) (Mann Whitney test).                                                                                                                                                                          |      | ns, not significant for WT2 vs. Mut-int2 iPSC-CMs (P Value=0.133) (Mann Whitney test).                                                                                                                                           |
| F           |                                                                                                               |                                                                                     | n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=42 cells; Fe(II), n=32 cells; Fe(II)-chicken, n=39 cells.    |                                                         | cells                                             | no                                                                                                         | ns, not significant for CV vs. Fe(II), CV vs. Fe(II)-chicken, and Fe(II) vs. Fe(II)-chicken (Kruskal-Wallis test and Dunn's multiple comparison test)                                                                                                                             | Yes  | ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.999), CV vs. Fe(II)-chicken (Adjusted P Value=0.890), and Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparison test) |
| H           |                                                                                                               |                                                                                     | n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=37 cells; Fe(II), n=36 cells; Fe(II)-Ebselen, n=26 cells.    |                                                         | cells                                             | no                                                                                                         | ***, P<0.001 for CV vs. Fe(II) (Adjusted P Value=1.87 × 10 <sup>-13</sup> ) Yes and Fe(II) vs. Fe(II)-chicken (Adjusted P Value=1.18 × 10 <sup>-8</sup> ); ns, not significant for CV vs. Fe(II)-chicken (Kruskal-Wallis test and Dunn's multiple comparisons test)               | Yes  | ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.326), Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.326) and CV vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test) |
| I           |                                                                                                               |                                                                                     | n=2 experiments/w=2 independent cardiac differentiation were performed; CV, n=28 movies; Fe(II), n=55 movies; Fe(II)-chicken, n=37 movies. |                                                         | movies                                            | no                                                                                                         | ***, P<0.001 for CV vs. Fe(II) (Adjusted P Value=1 × 10 <sup>-15</sup> ); Fe(II) vs. Fe(II)-chicken (Adjusted P Value=4.48 × 10 <sup>-10</sup> ) and CV vs. Fe(II)-chicken (Adjusted P Value=4.05 × 10 <sup>-4</sup> ) (Kruskal-Wallis test and Dunn's multiple comparisons test) | Yes  | ns, not significant for CV vs. Fe(II) (Adjusted P Value=0.699), Fe(II) vs. Fe(II)-chicken (Adjusted P Value=0.999) and CV vs. Fe(II)-chicken (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test) |
| K           |                                                                                                               |                                                                                     | n=3 experiments/w=3 independent cardiac differentiation were performed; CV, n=35 cells; Fe(II), n=37 cells.                                |                                                         | cells                                             | no                                                                                                         | ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test).                                                                                                                                                                                                      | Yes  | ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (P Value=0.400) (Mann Whitney test).                                                                                                                                     |
| L           |                                                                                                               |                                                                                     | n=2 experiments/w=2 independent cardiac differentiation                                                                                    |                                                         | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (Mann Whitney test);                                                                                                                                                                                                      | Yes  | ns, not significant for CV vs. Fe(II) Mut-int1 iPSC-CMs (P Value=0.667) (Mann Whitney test);                                                                                                                                     |
| M           | CV=78 cells; Lat-A=72 cells; Lat-A + Fe(II)=78 cells; n=3 experiments/w=3 independent cardiac differentiation |                                                                                     |                                                                                                                                            |                                                         | cells                                             | no                                                                                                         | ***, P < 0.001 for CV vs. Lat-A (Adjusted P Value=1 × 10 <sup>-15</sup> ) Yes and Lat-A vs. Lat-A + Fe(II) (Adjusted P Value=1 × 10 <sup>-15</sup> ); ns, not significant for CV vs. Lat-A + Fe(II) (Kruskal-Wallis test and Dunn's multiple comparisons test).                   | Yes  | ns, not significant for CV vs. Lat-A (Adjusted P Value=0.2309), Lat-A vs. Lat-A + Fe(II) (Adjusted P Value=0.760) and CV vs. Lat-A + Fe(II) (Adjusted P Value=0.999) (Kruskal-Wallis test and Dunn's multiple comparisons test). |
| N           | n=9 experiments/w=9 independent cardiac differentiation; n=36 technical replicates                            | n=9 experiments/w=9 independent cardiac differentiation; n=36 technical replicates  | n=6 experiments/w=6 independent cardiac differentiation; n=28 technical replicates                                                         |                                                         | Technical replicates from independent experiments | no                                                                                                         | ** P < 0.01 for WT1 vs. Pat (Adjusted P Value=0.00596) and WT1 vs. MUT-int1 (Adjusted P Value=0.00538) (Kruskal-Wallis test and Dunn's multiple comparisons test).                                                                                                                | Yes  | ns, not significant for WT1 vs. Pat (Adjusted P Value=0.572) and *, P < 0.05 for WT1 vs. MUT-int1 (Adjusted P Value=0.021) (Kruskal-Wallis test and Dunn's multiple comparisons test)                                            |
| O           |                                                                                                               | n=6 experiments/w=6 independent cardiac differentiation; n=23 technical replicates. |                                                                                                                                            |                                                         | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | **, P<0.01 for CV vs. Fe(II) Par iPSC-CMs (P value=0.0015) (Mann Whitney test);                                                                                                                                                                                                   | Yes  | **, P<0.01 for CV vs. Fe(II) Par iPSC-CMs (P value=0.00216) (Mann Whitney test).                                                                                                                                                 |
| P           |                                                                                                               |                                                                                     | n=6 experiments/w=6 independent cardiac differentiation; n=24 technical replicates                                                         |                                                         | Technical replicates from independent experiments | no                                                                                                         | ***, P < 0.001 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value=1.06 × 10 <sup>-4</sup> ) (Mann-Wilcoxon test).                                                                                                                                                                       | Yes  | **, P<0.01 for CV vs. Fe(II) MUT-int1 iPSC-CMs (P value=0.00216) (Mann Whitney test).                                                                                                                                            |
| Q           | n=3 experiments/w=3 independent cardiac differentiation                                                       |                                                                                     |                                                                                                                                            |                                                         | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for WT1 CV vs. WT1 UNC3230 (P value=7.01 × 10 <sup>-4</sup> ) (Mann Whitney test).                                                                                                                                                                                   | Yes  | ns, not significant for WT1 CV vs. WT1 UNC3230 (P Value=0.700) (Mann Whitney test).                                                                                                                                              |
| S           | n=3 experiments/w=3 independent cardiac differentiation                                                       |                                                                                     |                                                                                                                                            |                                                         | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for WT1 CV vs. WT1 Lat-A (P value<1 × 10 <sup>-15</sup> ) (Mann Whitney test).                                                                                                                                                                                       | Yes  | ns, not significant for WT1 CV vs. WT1 Lat-A (P Value=0.100) (Mann Whitney test).                                                                                                                                                |

Fig. S10

| Patient (WT/MUT) | MUT-int1 (TPM1-F185L<br>MUT-int/MUT-int)                                                                            | what data points were used<br>in analyses            | Passed normality test<br>(alpha=0.05)?                                                                              | Statistics method                                                                                                    | n≤6? | sensitivity analysis                                                                                        |
|------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|------|-------------------------------------------------------------------------------------------------------------|
| <b>A</b>         | n=5 experiments/n=5<br>independent cardiac<br>differentiation;each<br>experiment contains 4<br>technical replicates | Technical replicates from<br>independent experiments | Yes (pass Anderson-Darling<br>test, D'Agostino & Pearson<br>test, Shapiro-Wilk test and<br>Kolmogorov-Smirnov test) | <b>**</b> , P<0.01 (P value=0.0051)<br>for CV vs. RhoA II Mut-int1<br>iPSC-CMs (Mann-Whitney<br>test).               | Yes  | ns, not significant for<br>CV vs. RhoA II Mut-int1<br>iPSC-CMs (P<br>Value=0.1508) (Mann-<br>Whitney test). |
| <b>C</b>         | CV, n=42 cells; RhoA II, n=<br>53 cells; n=3 experiments/n=3<br>independent cardiac<br>differentiation              | cells                                                | no                                                                                                                  | <b>**</b> , P < 0.01 (P<br>value=6.36×10 <sup>-3</sup> ) CV vs.<br>RhoAII Patient (Mann-<br>Whitney test).           | Yes  | ns, not significant for<br>CV vs. RhoAII Patient<br>(P Value=0.1000)<br>(Mann-Whitney test).                |
| <b>D</b>         | CV, n=64 cells; RhoA II, n=<br>61 cells; n=3 experiments/n=3<br>independent cardiac<br>differentiation              | cells                                                | no                                                                                                                  | <b>***</b> , P < 0.001 (P<br>value=6.68×10 <sup>-5</sup> ) CV vs.<br>RhoA II MUT-int1 (Mann-<br>Whitney test).       | Yes  | ns, not significant for<br>CV vs. RhoA II MUT-<br>int1 (P Value=0.200)<br>(Mann-Whitney test).              |
| <b>G</b>         | CV , n=88 movies; RhoA II,<br>n=46 movies; n=3<br>experiments/n=3 independent<br>cardiac differentiation            | movies                                               | no                                                                                                                  | <b>***</b> , P < 0.001 for CV vs.<br>RhoA II MUT-int1 (P<br>value=1.32 × 10 <sup>-4</sup> ) (Mann-<br>Whitney test). | Yes  | ns, not significant for<br>CV vs. RhoA II MUT-<br>int1 (P Value=0.400)<br>(Mann-Whitney test).              |

Fig. S11

|   | Patient (WT/MUT)                                        | Mut-corrected (WT-cor/WT-cor )                          | what data points were used in analyses            | Passed normality test (alpha=0.05)?                                                                        | Statistics method                                                                                       | n≤6? | sensitivity analysis                                                                        |
|---|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|------|---------------------------------------------------------------------------------------------|
| C | n=2 experiments/n=2 independent cardiac differentiation | n=2 experiments/n=2 independent cardiac differentiation | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for Patient vs. Mut-cor (Mann Whitney test).                                        | Yes  | ns, not significant for Patient vs. Mut-cor (P Value=0.800) (Mann Whitney test).            |
| D | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for Patient vs. Mut-cor (Mann Whitney test).                                        | Yes  | ns, not significant for Patient vs. Mut-cor (P Value>0.999) (Mann Whitney test).            |
| E | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | Yes (pass Anderson-Darling test, D'Agostino & Pearson test, Shapiro-Wilk test and Kolmogorov-Smirnov test) | ns, not significant for Patient vs. Mut-cor (Mann Whitney test).                                        | Yes  | ns, not significant for Patient vs. Mut-cor (P Value>0.999) (Mann Whitney test).            |
| F | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ns, not significant for Patient vs. Mut-cor iPSC-CMs (Mann Whitney test).                               | Yes  | ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.200) (Mann Whitney test).   |
| G | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | *, P < 0.05 for Patient vs. Mut-cor iPSC-CMs (P value=0.0400) (Mann Whitney test).                      | Yes  | ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.400) (Mann Whitney test).   |
| H | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ***, P < 0.001 for Patient vs. Mut-cor iPSC-CMs (P value=4.11 × 10 <sup>-5</sup> ) (Mann Whitney test). | Yes  | ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.100) (Mann Whitney test).   |
| I | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ns, not significant for Patient vs. Mut-cor iPSC-CMs (Mann Whitney test).                               | Yes  | ns, not significant for Patient vs. Mut-cor iPSC-CMs (P Value=0.700) (Mann Whitney test).   |
| M | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).                                   | Yes  | ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value>0.999) (Mann Whitney test).       |
| N | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).                                   | Yes  | ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value=0.400) (Mann Whitney test).       |
| O | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Data from independent experiments                 | no                                                                                                         | ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).                                   | Yes  | ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value>0.999) (Mann Whitney test).       |
| P | n=3 experiments/n=3 independent cardiac differentiation | n=3 experiments/n=3 independent cardiac differentiation | Data from independent experiments                 | Yes (pass Shapiro-Wilk test)                                                                               | ns, not significant for WT/MUT vs. WT-cor/WT-cor (Mann Whitney test).                                   | Yes  | ns, not significant for WT/MUT vs. WT-cor/WT-cor (P Value=0.700) (Mann Whitney test).       |
| Q |                                                         | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | *, P<0.05 for Mut-cor CV vs. Mut-cor UNC3230 (P value=7.01 × 10 <sup>-4</sup> ) (Mann Whitney test).    | Yes  | ns, not significant for Mut-cor CV vs. Mut-cor UNC3230 (P Value=0.800) (Mann Whitney test). |
| S |                                                         | n=3 experiments/n=3 independent cardiac differentiation | Technical replicates from independent experiments | no                                                                                                         | ***, P<0.001 for Mut-cor CV vs. Mut-cor Lat-A (P value=1.62 × 10 <sup>-2</sup> ) (Mann Whitney test).   | Yes  | ns, not significant for Mut-cor CV vs. Mut-cor Lat-A (P Value=0.400) (Mann Whitney test).   |

Fig. S12

|   | WT1 (WT/WT)                                             | Mut-corrected (WT-cor/WT-cor )                                                                                                                                                                  | what data points were used in analyses            | Passed normality test (alpha=0.05)? | Statistics method                                                                                    | n56? | sensitivity analysis                                                                       |
|---|---------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------|------|--------------------------------------------------------------------------------------------|
| B |                                                         | n=1 experiment/n=1 independent cardiac differentiation; CV, n=27 cells; TFR Ab 20 µg/mL, n=17 cells; TFR Ab 30 µg/mL, n=20 cells.                                                               | cells                                             |                                     |                                                                                                      |      |                                                                                            |
| C | n=3 experiments/n=3 independent cardiac differentiation |                                                                                                                                                                                                 | Technical replicates from independent experiments | no                                  | ***, P<0.001 for WT1 CV vs. WT1 TFR Ab (P value=1.28×10 <sup>-4</sup> ) (Mann Whitney test).         | Yes  | ns, not significant for WT1 CV vs. WT1 TFR Ab (P Value=0.400) (Mann Whitney test).         |
| E |                                                         | n=3 experiments/n=3 independent cardiac differentiation                                                                                                                                         | Technical replicates from independent experiments | no                                  | ***, P<0.001 for Mut-cor CV vs. Mut-cor TFR Ab (P value=1.72×10 <sup>-8</sup> ) (Mann Whitney test). | Yes  | ns, not significant for Mut-cor CV vs. Mut-cor TFR Ab (P Value=0.400) (Mann Whitney test). |
| G |                                                         | CV, n=2 experiments, n=29 movies; TFR Ab 20 µg/mL, n=1 experiment, n=15 movies; TFR Ab 20 µg/mL + RhoA II, n=1 experiment, n=11 movies; TFR Ab 30 µg/mL + RhoA II, n=1 experiment, n=14 movies. | movies                                            | no                                  |                                                                                                      |      |                                                                                            |

| Fig. S14       |                                             |                                             |                                                   |                                     |                                                                                     |                                                                                             |
|----------------|---------------------------------------------|---------------------------------------------|---------------------------------------------------|-------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
|                | Healthy control                             | DCM                                         | what data points were used in analyses            | Passed normality test (alpha=0.05)? | Statistics method                                                                   | n≤6? sensitivity analysis                                                                   |
| <b>G and H</b> | n=3 independent experiments;n=5 individuals | n=3 independent experiments;n=8 individuals | Technical replicates from independent experiments | no                                  | *, P<0.05 for donor (healthy control) vs. DCM (P Value=0.0473) (Mann Whitney test). | Yes<br>**, P<0.01 for donor (healthy control) vs. DCM (P Value=0.0031) (Mann Whitney test). |