

Supplemental information

**A microscopy-based screen identifies cellular
kinases modulating mitochondrial translation**

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Supplementary Figures

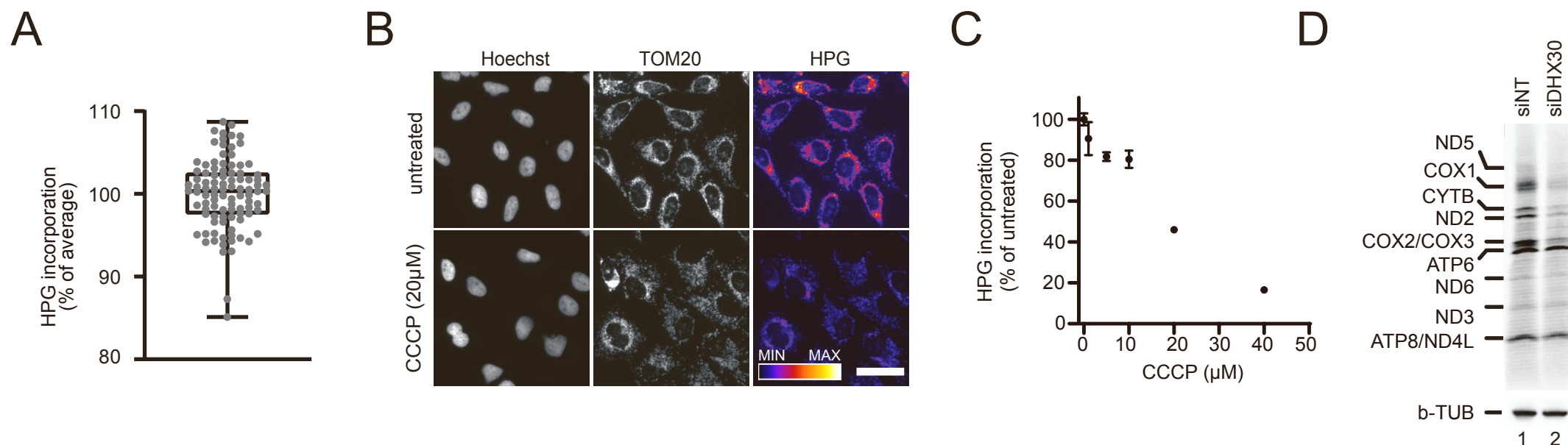


Figure S1. Validation of screen to monitor mitochondrial translation. Related to Figure 1.

(A) HPG incorporation values in each well of a 96-well plate labeled as in (1B). SEM between wells = 4.2%.

(B) Representative fluorescent images of control and CCCP treated cells labeled as in (1B). Bar, 40 μ m.

(C) Quantitative analysis of HPG incorporation in cells treated with different concentrations of CCCP. Each replicate, approx. 2,000 cells analyzed in 20 images. (SEM, n = 3).

(D) Radiolabeling of mitochondrial translation in cells transfected as in (1E). Western blot (b-tubulin) as loading control.

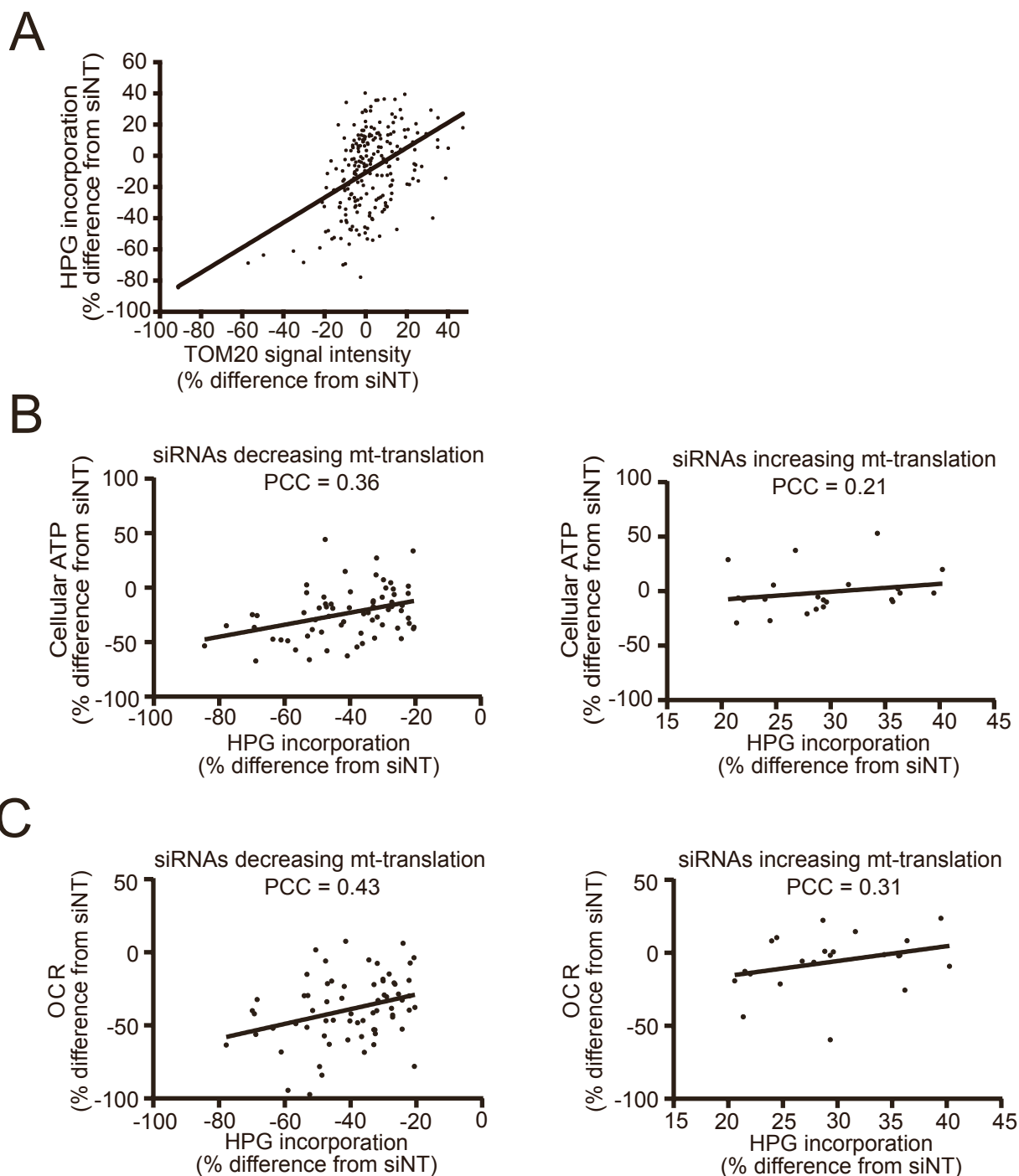


Figure S2. Linking HPG incorporation to bioenergetic parameters. Related to Figure 2 (A) HPG incorporation was plotted against TOM20 staining for each siRNA from (2A). (Pearson correlation coefficient (PCC) = 0.29). (B) cellular ATP and (C) OCR values from (2C) and (2D) plotted against HPG incorporation. (PCCs indicated on the graphs).

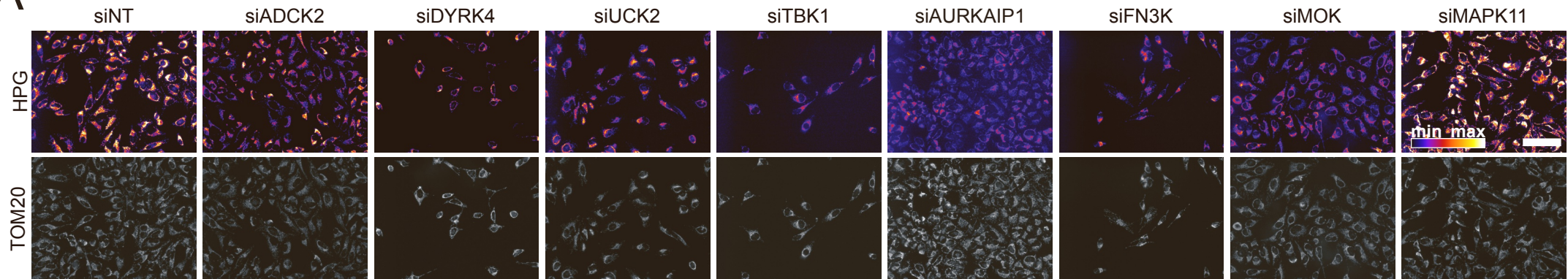
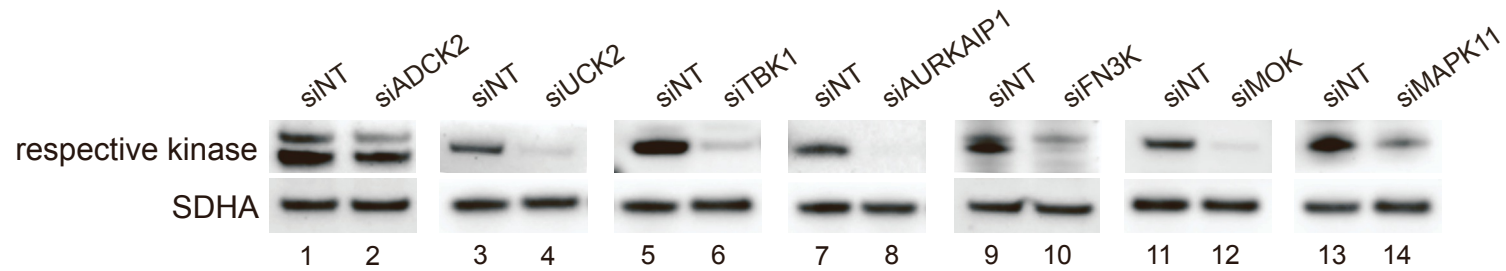
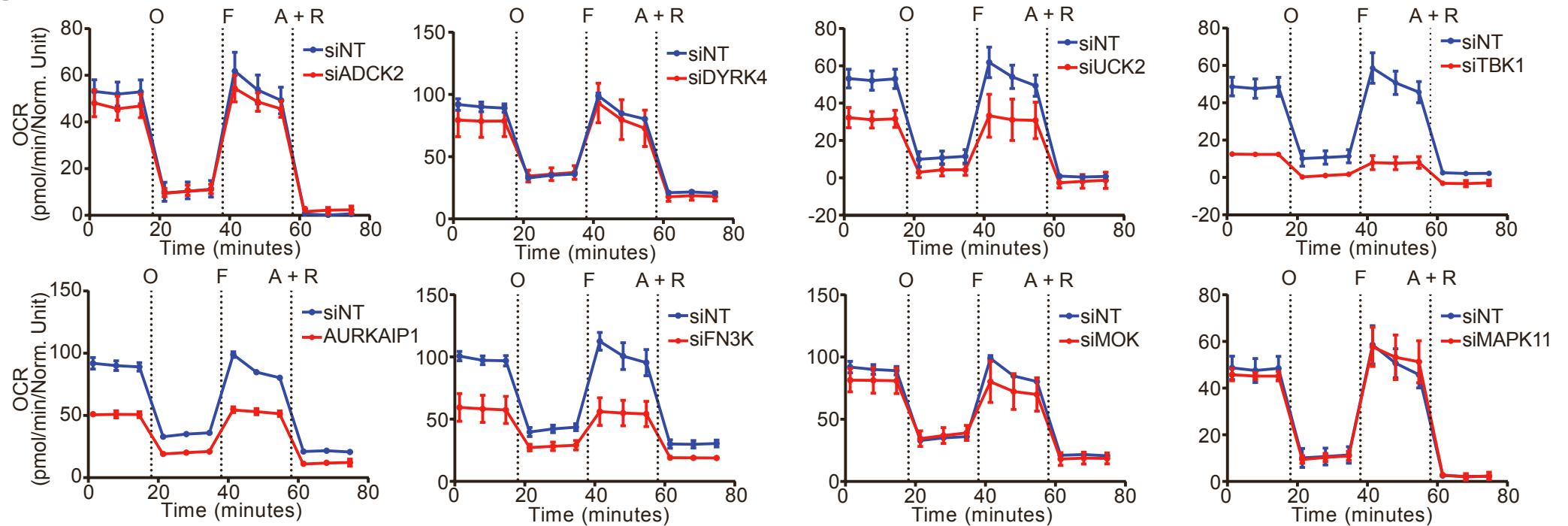
A**B****C**

Figure S3. Effect of selected kinases downregulation on mitochondrial translation and oxygen consumption. Related to Figure 3

(A) Representative fluorescent images of HPG incorporation and TOM20 signal after knockdown of the selected kinases (images partially used for quantification of (2A)).

(B) Western blot based steady-state protein analysis of the kinases after transfection with siNT (non targeting) and candidate siRNAs.

(C) Individual OCR measurements (quantified in Figure 2D) after transfection with siNT and selected siRNAs. O, oligomycin. F, Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP). A + R, Antimycin plus Rotenone)

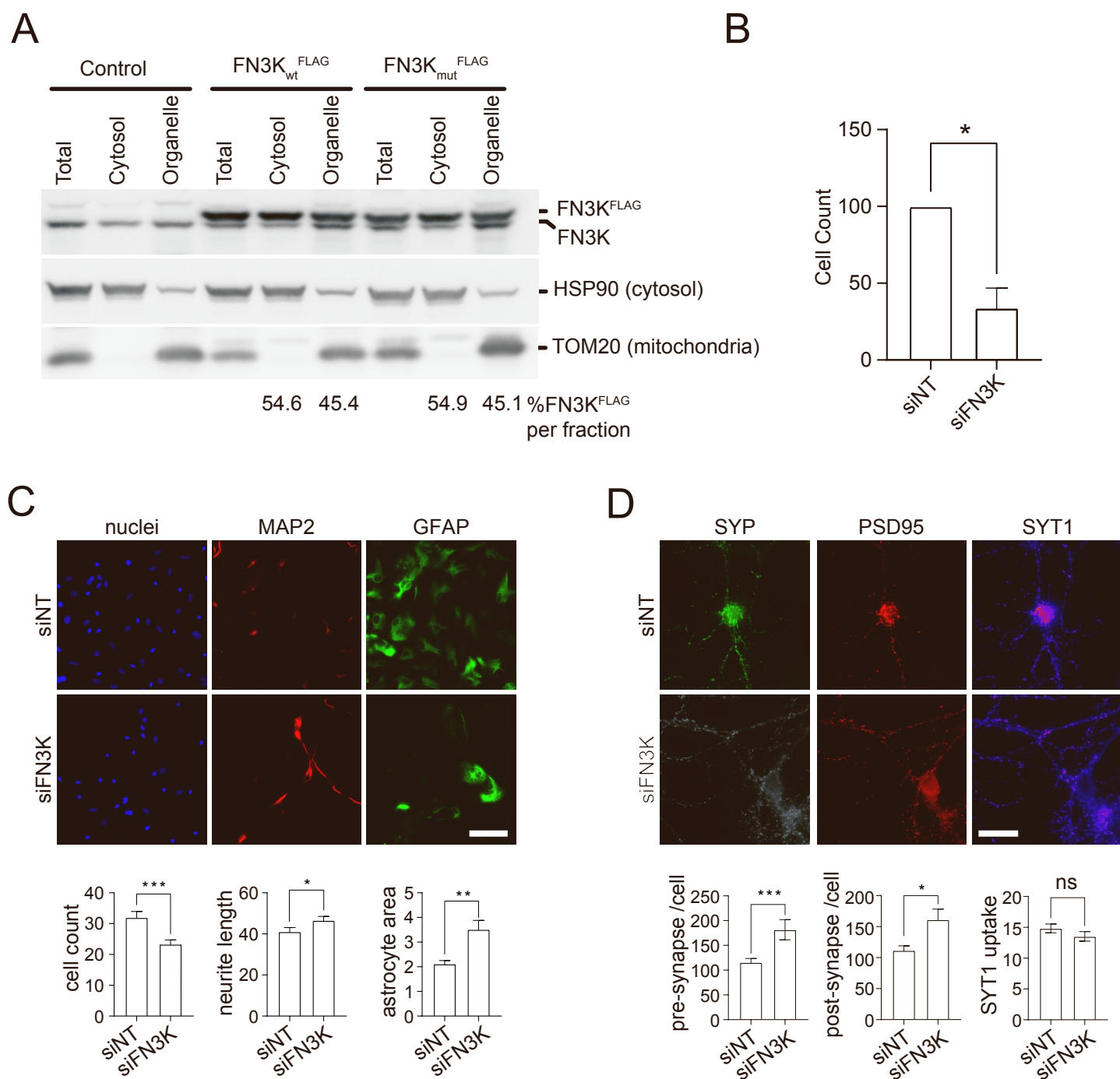


Figure S4. Subcellular localization of FN3K and functional relevance in primary neuronal cultures. Related to Figures 5 and 6.

(A) Cell fractionation of Hela Cells expressing WT or mutant FN3K^{FLAG}.

(B) Cell count after transfection with siNT and siFN3K using Hoechst from images in (2A). (SEM, n=3. Student t test, *P<0.05).

(C) Representative epifluorescent images of MAP2 and GFAP (upper panels) and cell number, neurite length and astrocyte area quantification (lower panels) in primary hippocampal culture transfected with siNT and siFN3K. Hoechst, nuclei. Bar, 75 μ m. (SEM, n>30 from 3 experiments).

(D) Representative epifluorescent images (upper panels) for Cy3-SYT1, SYP and PSD95 and quantification (lower panels) of signal intensity in presynapses (SYP) and postsynapses (PSD95), as well as vesicle recycling capacity (SYT1 uptake) in hippocampal cultures treated as in (B). Hoechst, nuclei. Bar, 25 μ m. (SEM, n>30 cells from 3 experiments).

Significance (B and C). (Mann-Whitney test, *P<0.05, **P<0.01, ***P<0.001).