

Unbiased complexome profiling and global proteomics analysis reveals mitochondrial impairment and potential changes at the intercalated disk in presymptomatic R14^{Δ/+} mice hearts

Supplemental Methods and Materials

Brian Foo^{1,2*¶}, Hugo Amedei^{3¶}, Surmeet Kaur^{3,#a}, Samir Jaawan^{1,2}, Angela Boshnakovska⁴, Tanja Gall⁴, Rudolf A. de Boer^{5,6}, Herman H.W. Silljé⁵, Henning Urlaub^{3,7}, Peter Rehling⁴, Christof Lenz^{3,7*}, Stephan E. Lehnart^{1,2*}

¹ University Medical Center Göttingen, Heart Research Center Göttingen, Cellular Biophysics and Translational Cardiology Section, Göttingen, Germany.

² Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Germany.

³ University Medical Center Göttingen, Department of Clinical Chemistry, Göttingen, Germany.

⁴ University Medical Center Göttingen, Department of Cellular Biochemistry, Göttingen, Germany.

⁵ Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.

⁶ Erasmus MC, Cardiovascular Institute, Thorax Center, Department of Cardiology, Rotterdam, the Netherlands.

⁷ Max Planck Institute for Multidisciplinary Sciences, Bioanalytical Mass Spectrometry Group, Göttingen, Germany.

^{#a}Current Address: Institute of Medical Virology, Justus Liebig University, Giessen, Germany.

*Corresponding authors:

Email: brian.foo@med.uni-goettingen.de (BF), christof.lenz@mpinat.mpg.de (CL) and slehnart@med.uni-goettingen.de (SEL)

¶These authors contributed equally to this work.

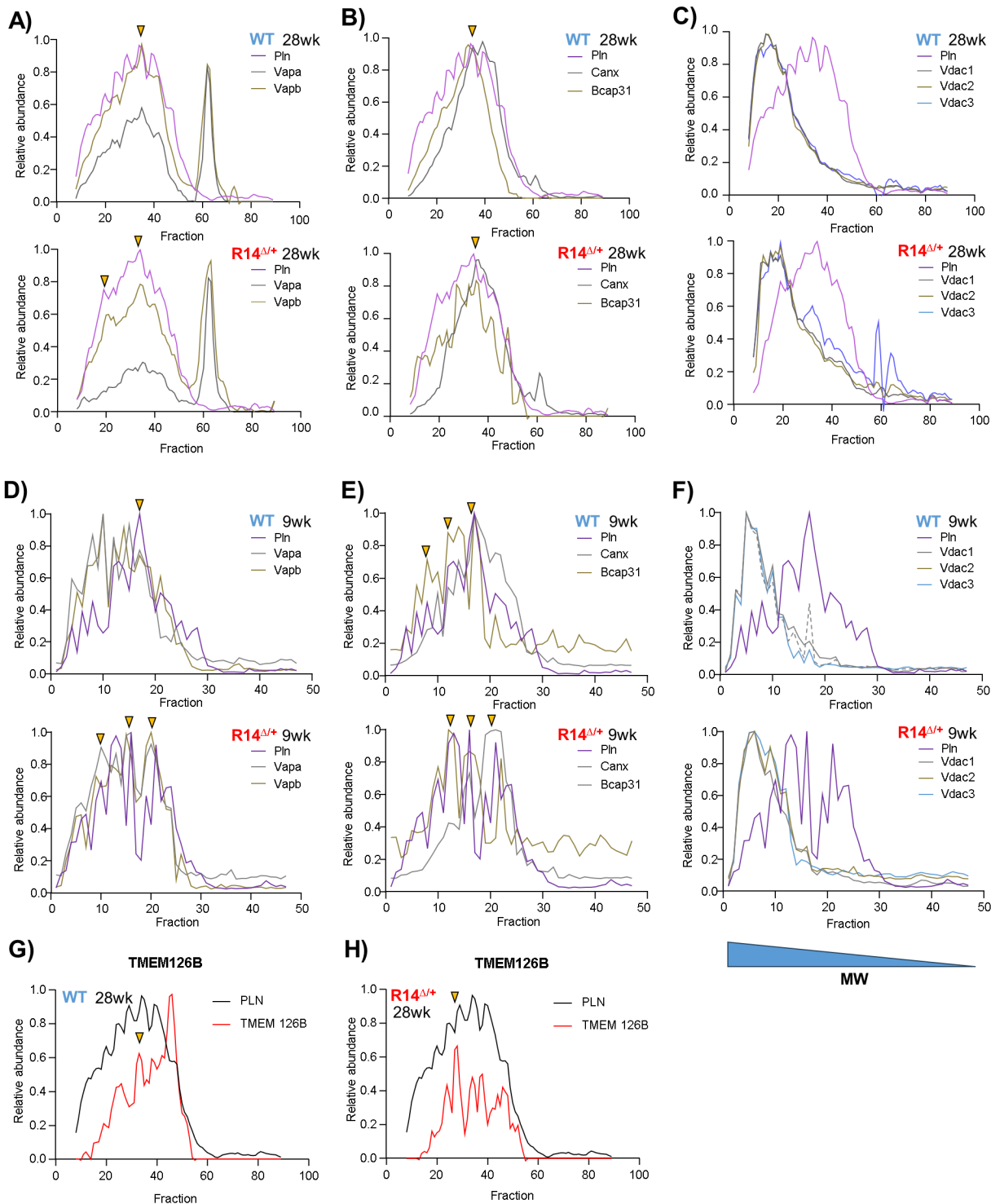
Supplemental Materials and Methods

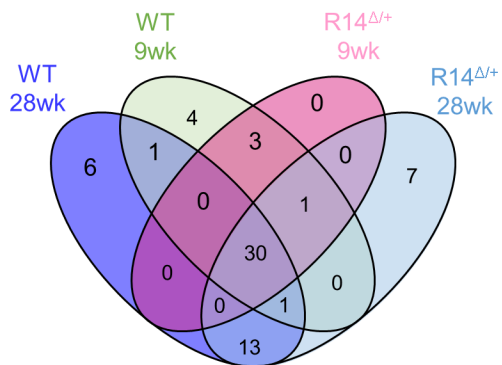
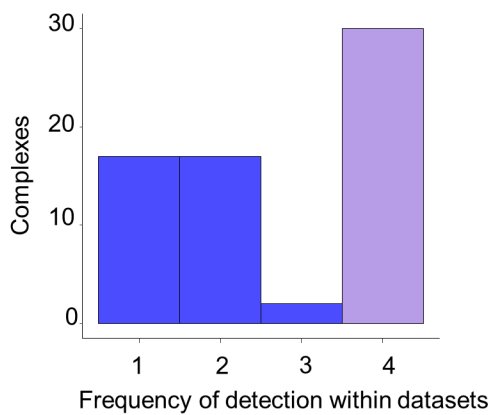
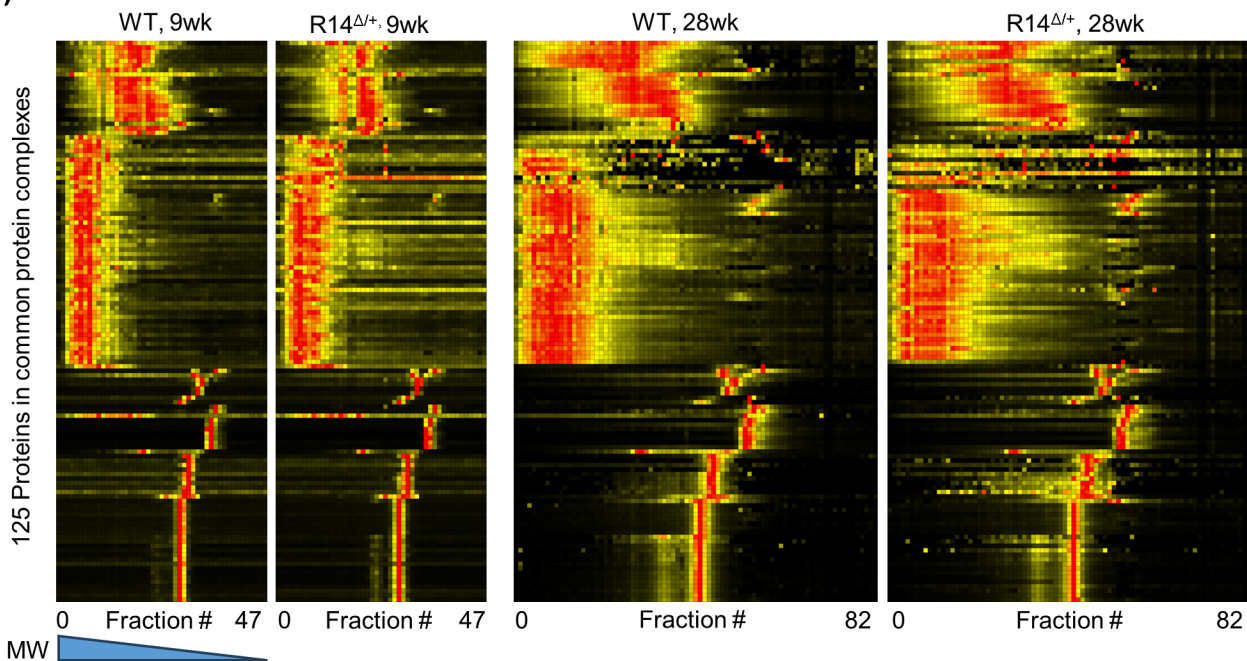
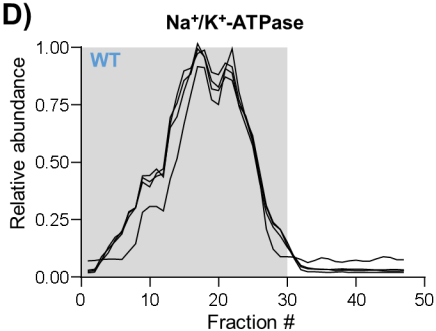
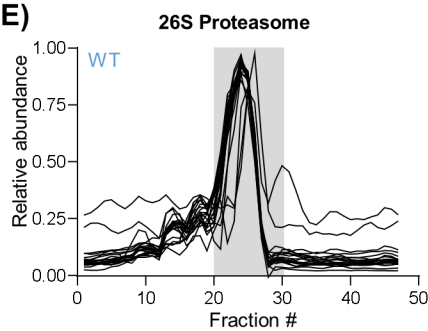
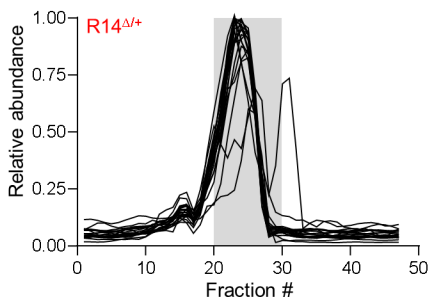
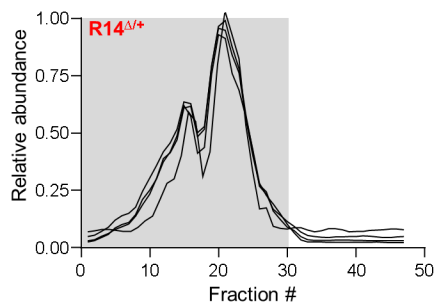
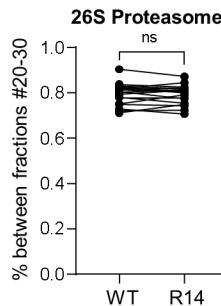
Super-resolution confocal microscopy. Cardiomyocytes were isolated from mouse left-ventricle as described in the main text and seeded onto fibronectin-coated glass cover slips. PLN, SERCA2a and RyR2 were labelled using the following antibodies: monoclonal mouse α -PLN (Thermo), polyclonal rabbit α -SERCA2a (Badrilla) and rabbit α -RyR2 (Sigma). Secondary antibodies conjugated to STED-compatible fluorophores (STAR 580 or STAR 635P, Abberoir) were used for labelling. Images were acquired using a Leica TCS SP8 STED system using an HC PL APO C2S 100x/1.40 oil immersion lens. Image analysis was performed on Fiji using a custom macro similar to those used in our earlier works[1,2].

Heirarchical Clustering. Heirarchical clustering performed using NOVA v. 0.8.0.0[3]. Clustering was performed using Pearson correlation for distance calculation, default parameters, average linkage, without normalization, without leaf order optimization, and without elimination of empty rows.

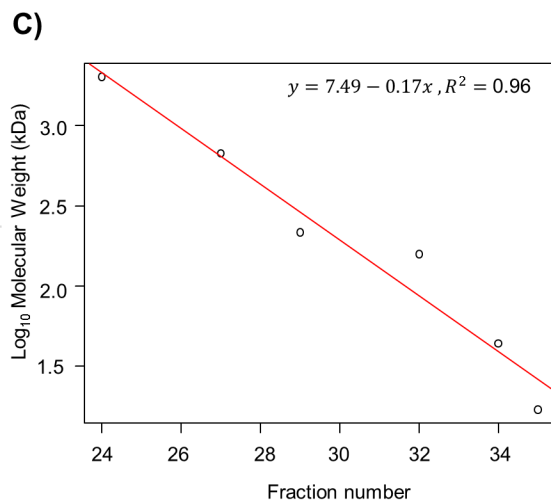
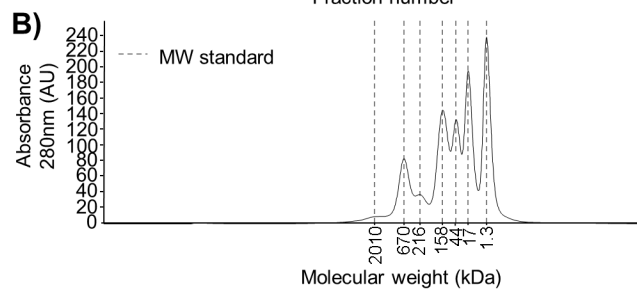
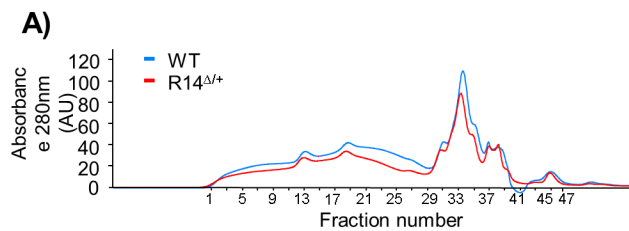
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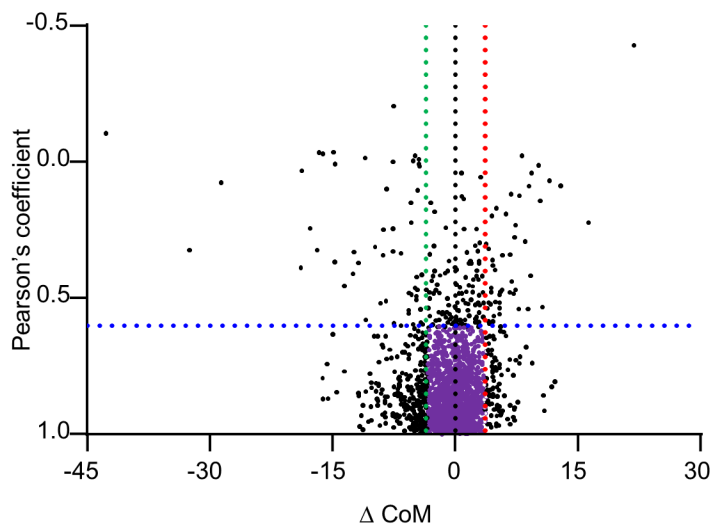
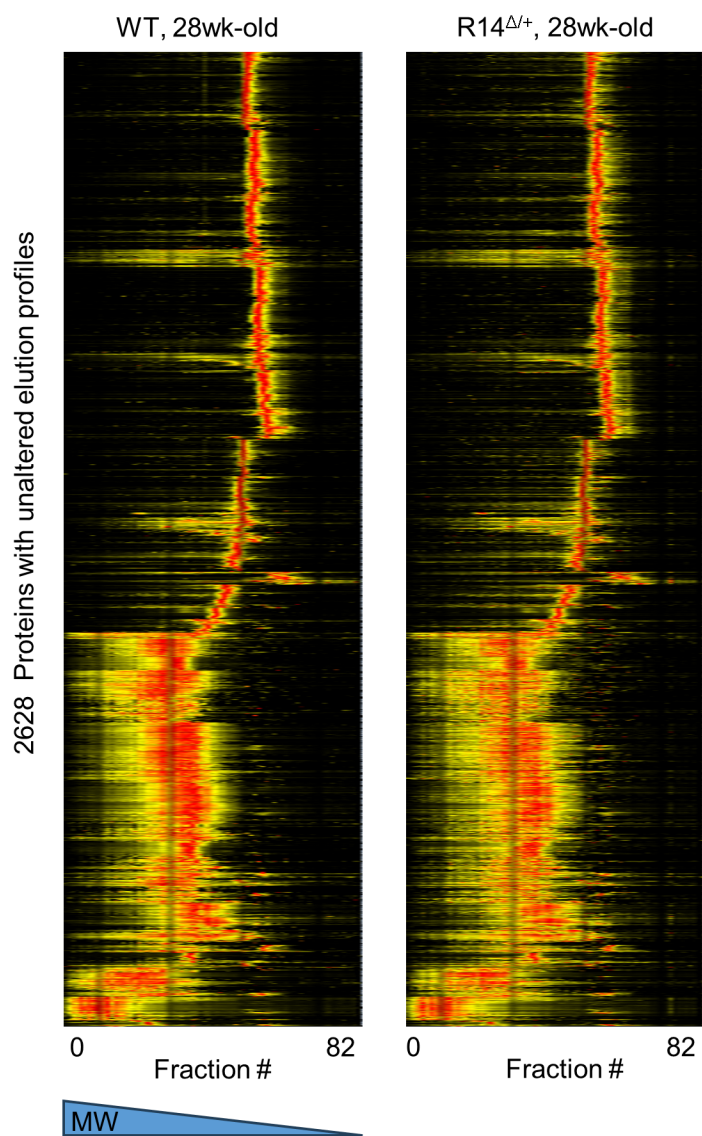
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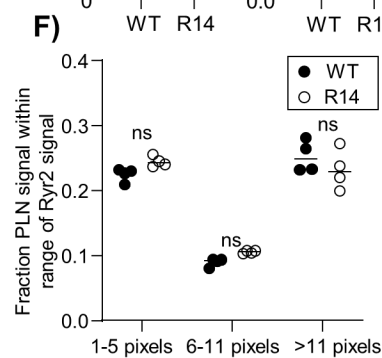
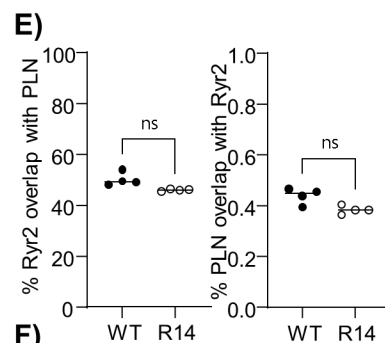
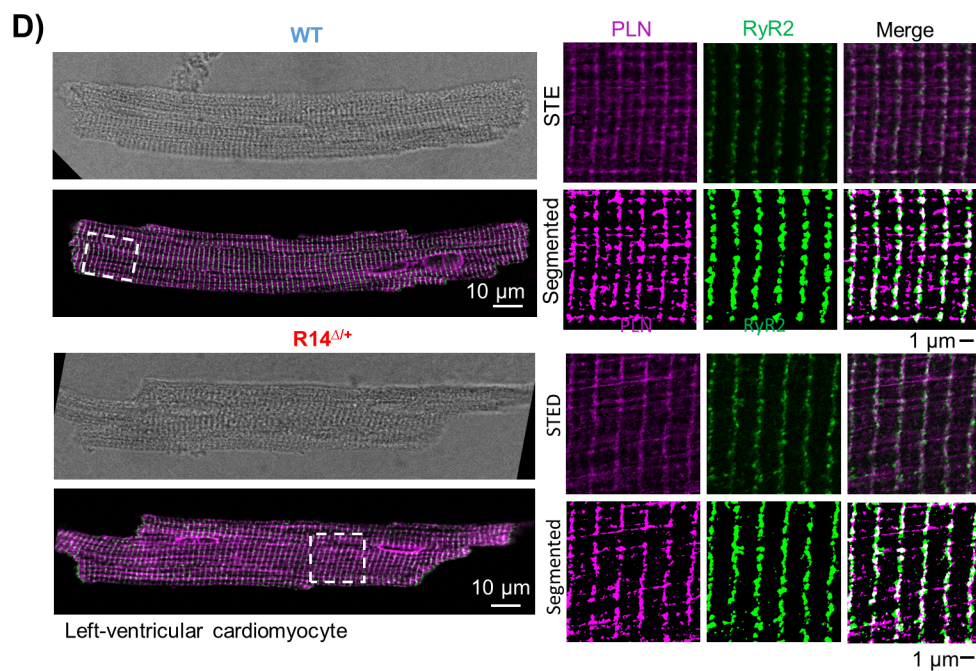
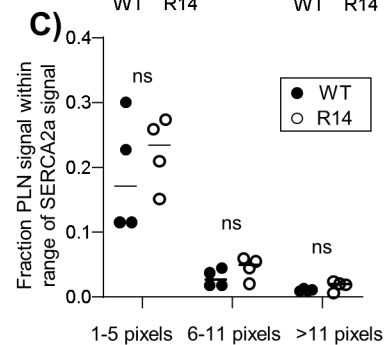
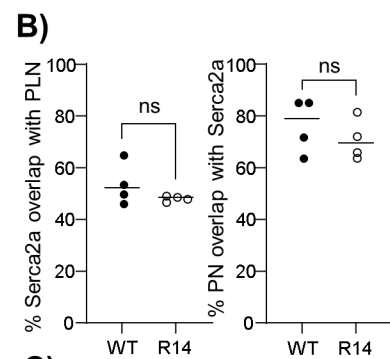
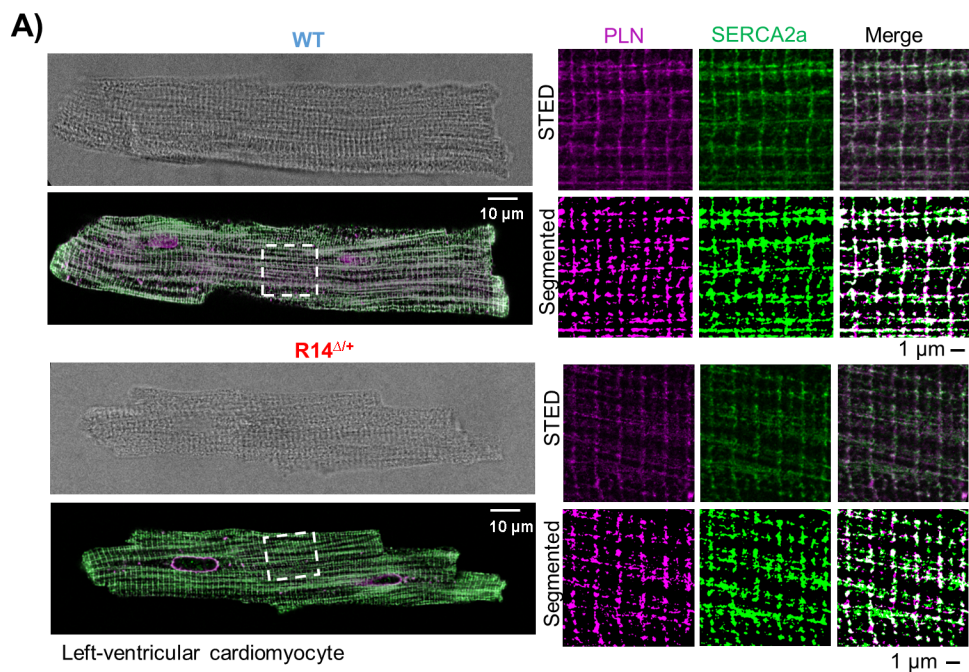
A)**B)****C)****D)****E)****F)**

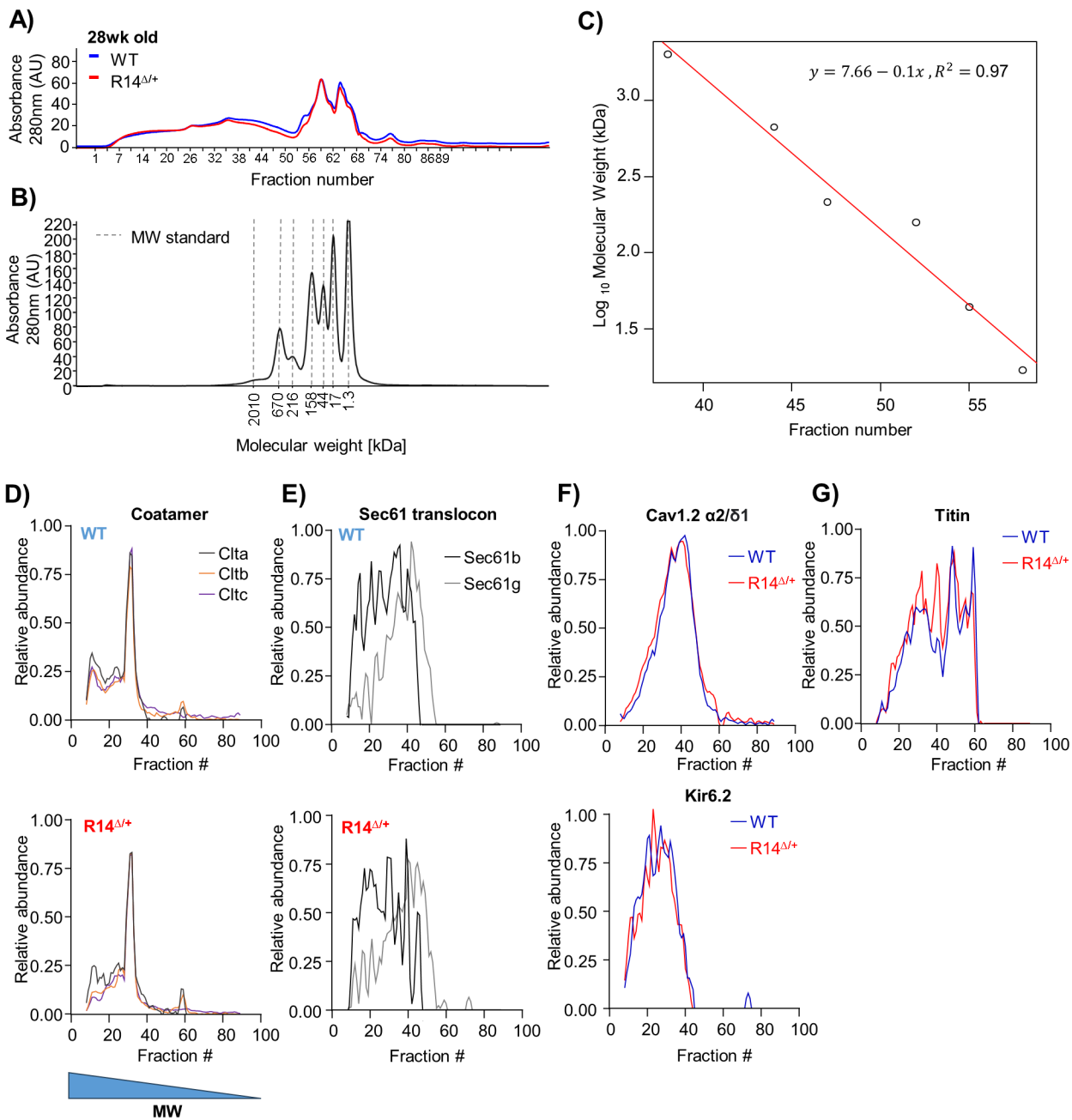
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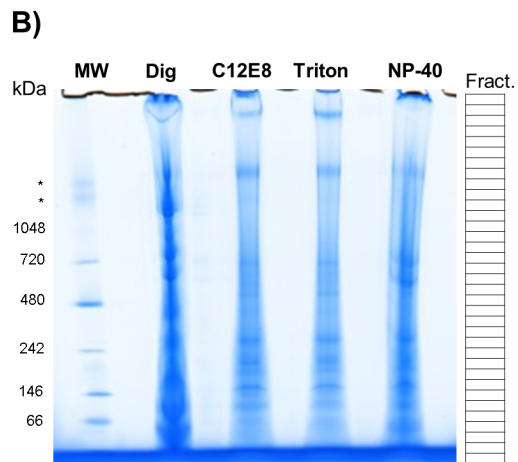
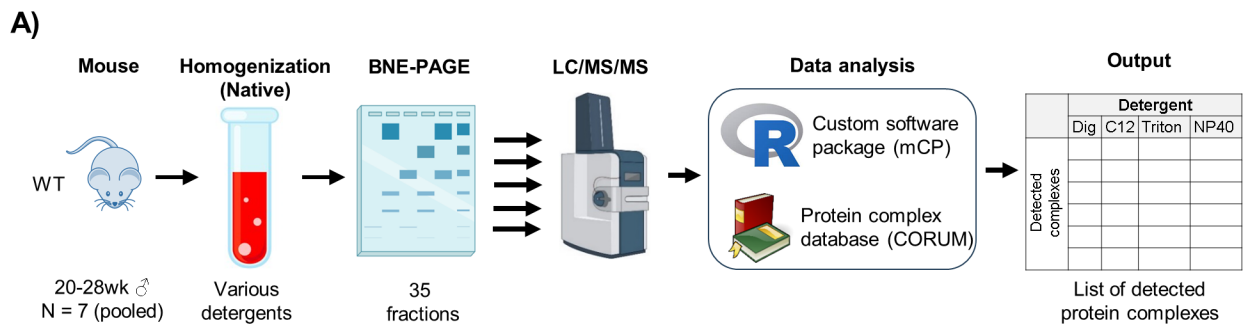
A)**B)**

CORUM protein complex detected in 28wk-old ♂ WT and R14 ^{Δ/+} mice	Detected subunits	Total subunits
IDH3G STRING COMPLEX	6	8
9S-cytosolic aryl hydrocarbon (Ah) receptor non-ligand activated complex	10	10
20S proteasome	3	4
Immunoproteasome	14	14
COP9 signalosome complex	13	14
CCT complex (chaperonin containing TCP1 complex)	6	8
CCT complex (chaperonin containing TCP1 complex)	8	24
Gamma-secretase complex (Aph1a, Psen1, Psenen, Ncstn)	8	24
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	2	8
CCT complex (chaperonin containing TCP1 complex)	4	12
Skeletal muscle sarcoglycan complex SGC, beta-gamma-delta-zeta	8	24
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	3	4
Skeletal muscle sarcoglycan complex SGC, alpha-beta-epsilon-gamma	4	12
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	3	4
Skeletal muscle sarcoglycan complex SGC, epsilon-beta-gamma-delta	4	12
Dystrophin-sarcoglycan-syntrophin complex, skeletal muscle	3	4
Sarcoglycan-sarcospan-dystroglycan complex	5	6
Sarcoglycan-sarcospan-complex SG-SPN	5	6
Sarcoglycan-sarcospan-syntrophin-dystrobrevin complex	4	5
Respiratory chain complex I, mitochondrial	5	8
Respiratory chain complex I, mitochondrial	30	77
Succinyl-CoA synthetase, ADP-forming	30	77
Succinyl-CoA synthetase, GDP-forming	2	2
(ER)-localized multiprotein complex, Ig heavy chains associated	2	2
Succinate dehydrogenase complex II, mitochondrial	8	10
Cytochrome bc1-complex, mitochondrial	4	4
Cytochrome c oxidase, mitochondrial	9	10
Gamma-secretase complex (Aph1a, Ncstn, Psen1, Psenen, Tmp21)	10	13
TRPV5-S100A10-annexin 2 complex	3	5
S100A10-annexin 2 complex	2	3
SNARE complex (Vti1b, Stx6, Stx7)	2	2
SNARE complex (Vti1b, Stx7, Stx8, Vamp8)	2	3
SNARE complex (Stx4, Stx6, Stx7, Vamp3, Vamp7, Vamp8, Vti1b)	3	4
Kif13a-AP1 complex	5	7
ERdj3-BiP complex	3	4
Itgav-Itgb3-Gsn complex	2	2
Itga-Itgb1-Ppap2b complex	2	3
Aph1a-Psen1-Ncstn complex	3	3
Gata1-Fog1-MeCP1 complex	2	3
Nkx3.2-SMAD1-SMAD4-HDAC-Sin3A complex	3	13
Drosha complex	2	7
Parvulin-associated pre-rRNP complex	5	8
CCT complex (chaperonin containing TCP1 complex), testis specific	27	62
G protein complex (Hdac4, Gnb1, Gng2)	7	8
G protein complex (Hdac5, Gnb1, Gng2)	2	3
G protein complex (Btk, Gng2, Gnb1)	2	3
Vps29-Vps35-Vps26a complex	2	3
Gamma-secretase complex (Aph1a, Psen1, Psenen, Ncstn)	2	3
Ksr1-PP2A holoenzyme complex (Ppp2r1a, Ppp2r2b, Ppp2ca), PDGF stimulated	2	8
Ksr1-PP2A core enzyme complex (Ppp2r1a, Ppp2ca), untreated	2	4
Ksr1-PP2A core enzyme complex (Ppp2r1a, Ppp2ca), untreated	2	6
Raf1-PP2A holoenzyme complex (Ppp2r1a, Ppp2r2b, Ppp2ca), PDGF stimulated	2	6
Raf1-PP2A core enzyme complex (Ppp2r1a, Ppp2ca), untreated	3	4
p18-p14-Mp1 complex	3	3
Mp1-p14 scaffolding complex	3	3
Ncstn-Psen1 complex	2	2
Rabep1-Ap1g1-Ap1s1 complex	2	2





CORUM protein complex detected in C12E8 solubilization	Detected subunits	Total subunits
IDH3G STRING COMPLEX	10	10
Caveolins and others	6	14
20S proteasome	14	14
Immunoproteasome	13	14
COP9 signalosome complex	4	8
CCT complex (chaperonin containing TCP1 complex)_1	8	24
CCT complex (chaperonin containing TCP1 complex)_2	8	24
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	4	12
CCT complex (chaperonin containing TCP1 complex)_3	8	24
Skeletal muscle sarcoglycan complex SGC, beta-gamma-delta-zeta	3	4
Smooth muscle sarcoglycan complex SGC, beta-delta-zeta	2	3
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	4	12
Skeletal muscle sarcoglycan complex SGC, alpha-beta-epsilon-gamma	3	4
Skeletal muscle sarcoglycan complex SGC, alpha-beta-gamma-delta	4	12
Skeletal muscle sarcoglycan complex SGC, epsilon-beta-gamma-delta	3	4
Dystrophin-sarcoglycan-syntrophin complex, skeletal muscle	5	6
Sarcoglycan-sarcospan-dystroglycan complex	5	6
Sarcoglycan-sarcospan-complex SG-SPN	4	5
Sarcoglycan-sarcospan-syntrophin-dystrobrevin complex	4	8
Respiratory chain complex I, mitochondrial	29	77
Respiratory chain complex I, mitochondrial	29	77
(ER)-localized multiprotein complex, Ig heavy chains associated	7	10
Succinate dehydrogenase complex II, mitochondrial	4	4
Cytochrome bc1-complex, mitochondrial	9	10
Cytochrome c oxidase, mitochondrial	11	13
Kif13a-AP1 complex	2	4
Itgav-Itgb3-Gsn complex	3	3
Parvulin-associated pre-rRNP complex	23	62
CCT complex (chaperonin containing TCP1 complex), testis specific	7	8
Vps29-Vps35-Vps26a complex	2	3
Dnajc5-Sgta complex	2	3
Ap1g1-Ap2a2-Dcx complex	2	3



C)

Protein complexes	Digitonin	C12E8	Triton	NP-40
Protein complexes detected	26	32	30	30
Unique complexes	1	1	3	1
Shared with all detergents	21	21	21	21
Shared with ≥ 1 other detergent	4	10	6	8