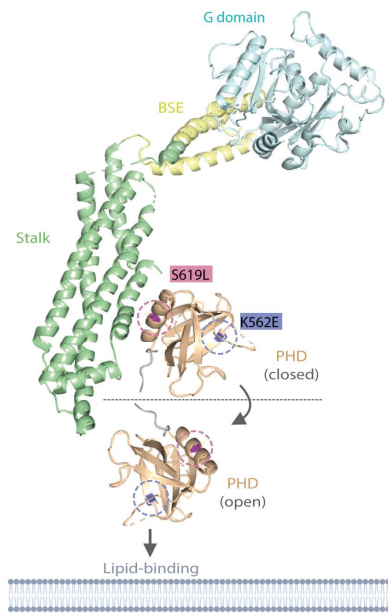


Supplementary information

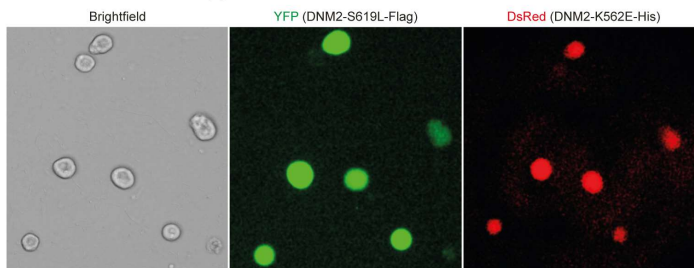
Supplementary Fig. 1 to 12.

Supplementary Table 1. Reagents used.

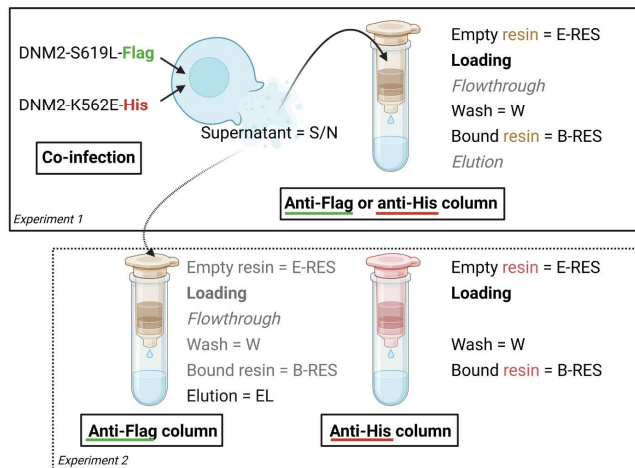
A Mutations localization on dynamin 3D structure



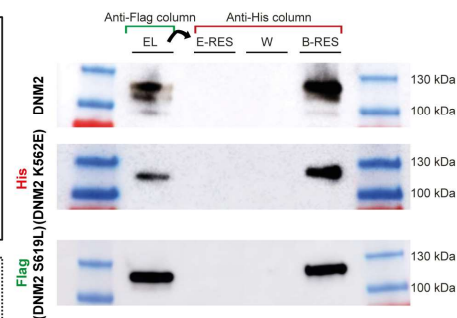
B Fluorescent microscopy of Sf21 cells after co-infection



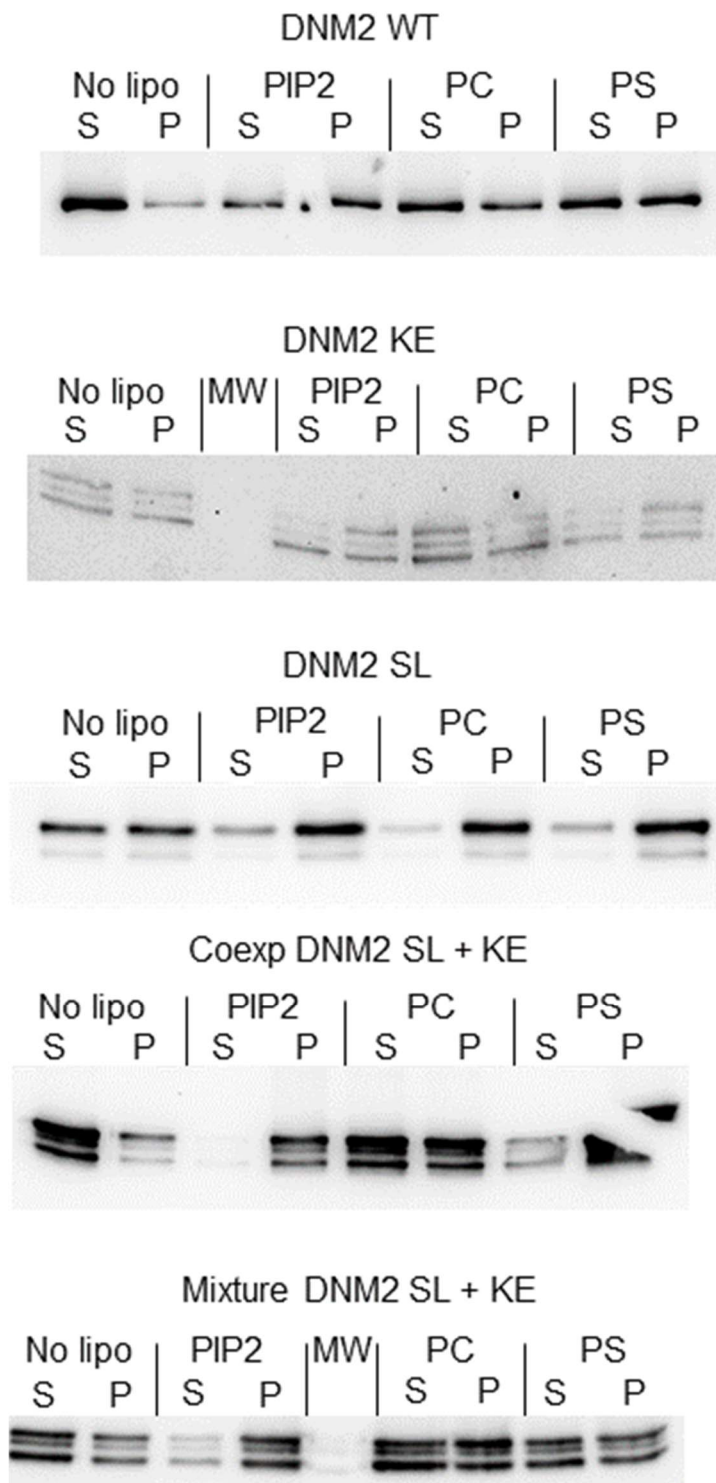
C Principle of the pull-down experiments performed



D Western blot - pull down experiment 2

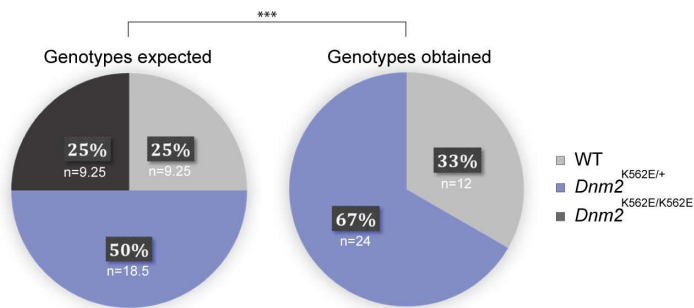


Supplementary Fig 1. Additional *in vitro* data on rDNM2 mutants. (A) Localization of both mutations on DNM1 3D structure (PDB identifier: 3SNH). The S619L mutation is located at the PH-stalk interface, the K562E mutation is located in the lipid-binding region. **(B)** Fluorescent microscopy images of Sf21 cells after co-infection, showing co-expression of both rDNM2-S619L and rDNM2-K562E mutants. **(C)** Principle of the pull-down experiments 1 and 2 performed to assess rDNM2 mutants interaction. Created in BioRender. Laporte, J. (2025) <https://BioRender.com/lupcet8>. **(D)** Representative western blots of elution (EL), empty-resin (E-RES), wash (W) and bound-resin (B-RES) from pull-down experiment 2 incubated with anti-DNM2, anti-His and anti-Flag antibodies.

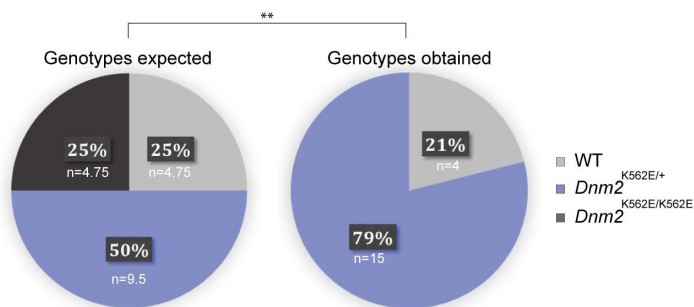


Supplementary Fig 2. DNM2 binding to liposomes of different compositions assessed by liposome co-sedimentation. The binding to liposomes of the indicated DNM2 protein was determined by co-sedimentation assays prior revelation by anti-DNM2 western blot. For the different liposomes composition, PC 100% (PC lipo) or PC based liposomes bearing 3% of PI(4,5)P2 (PIP2 lipo) or 3% of PS (PS lipo), the binding was determined by the ratio of proteins in the pellet fraction (P) compared to total proteins (pellet P + supernatant S), and normalized to the conditions without liposomes (No lipo). MW represents the well containing molecular weight ladder.

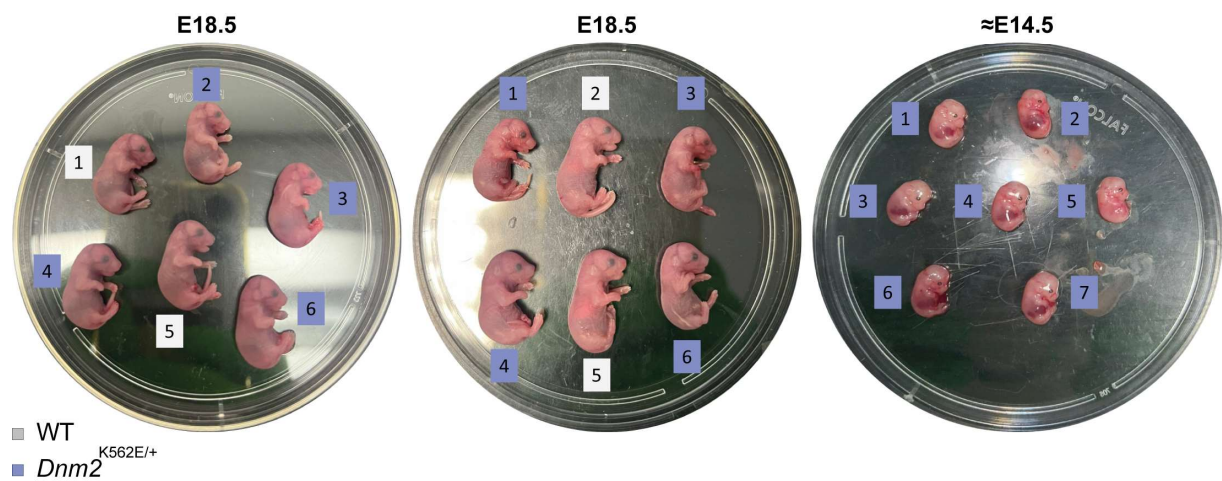
A Birth ratios K562E homozygous at P10



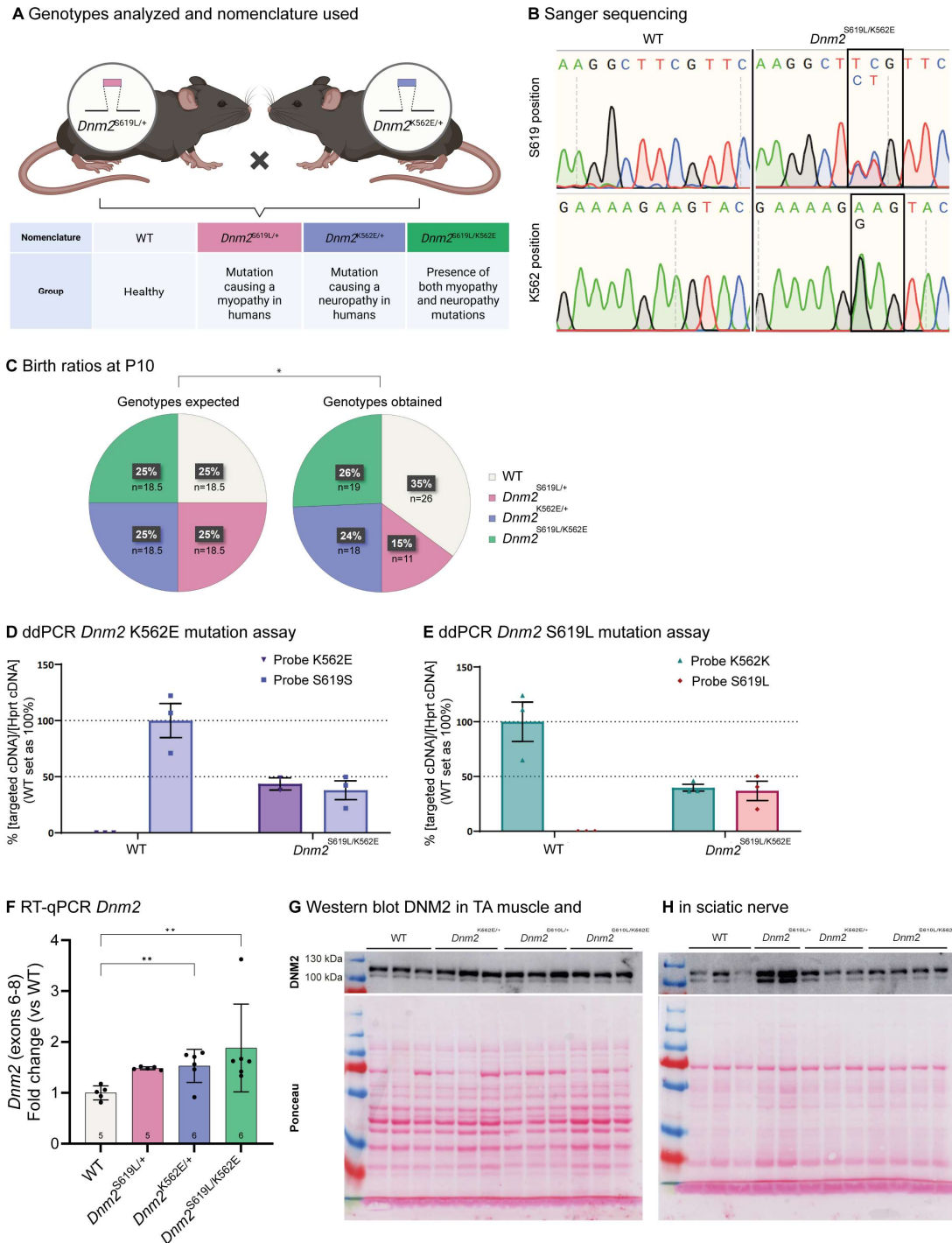
B Birth ratios K562E homozygous $\leq$ E18.5



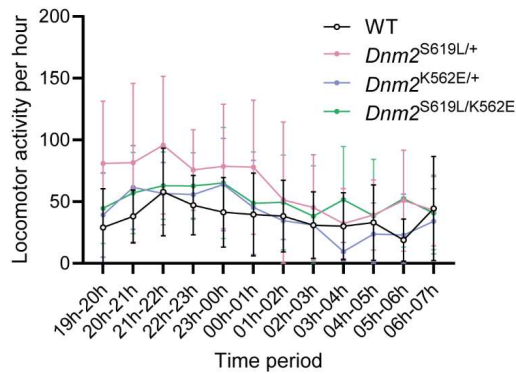
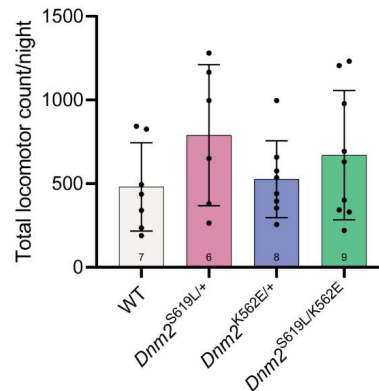
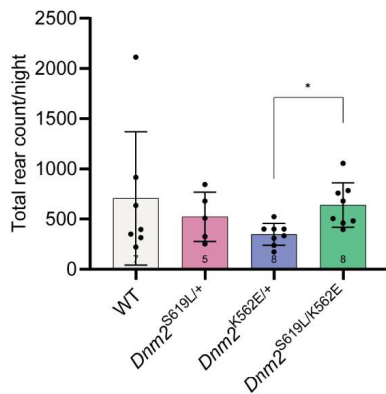
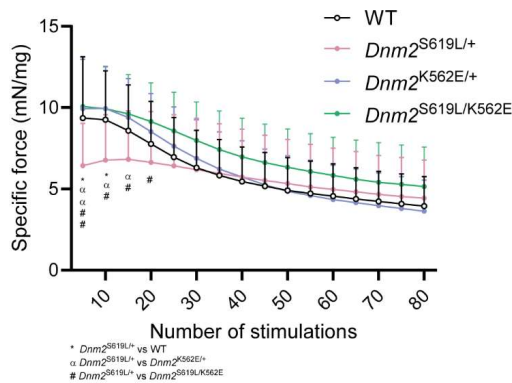
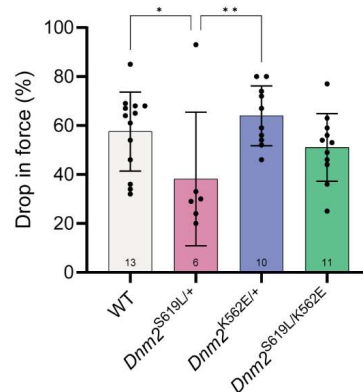
C Three different prenatal litters from $Dnm2^{K562E/+}$ heterozygous parents



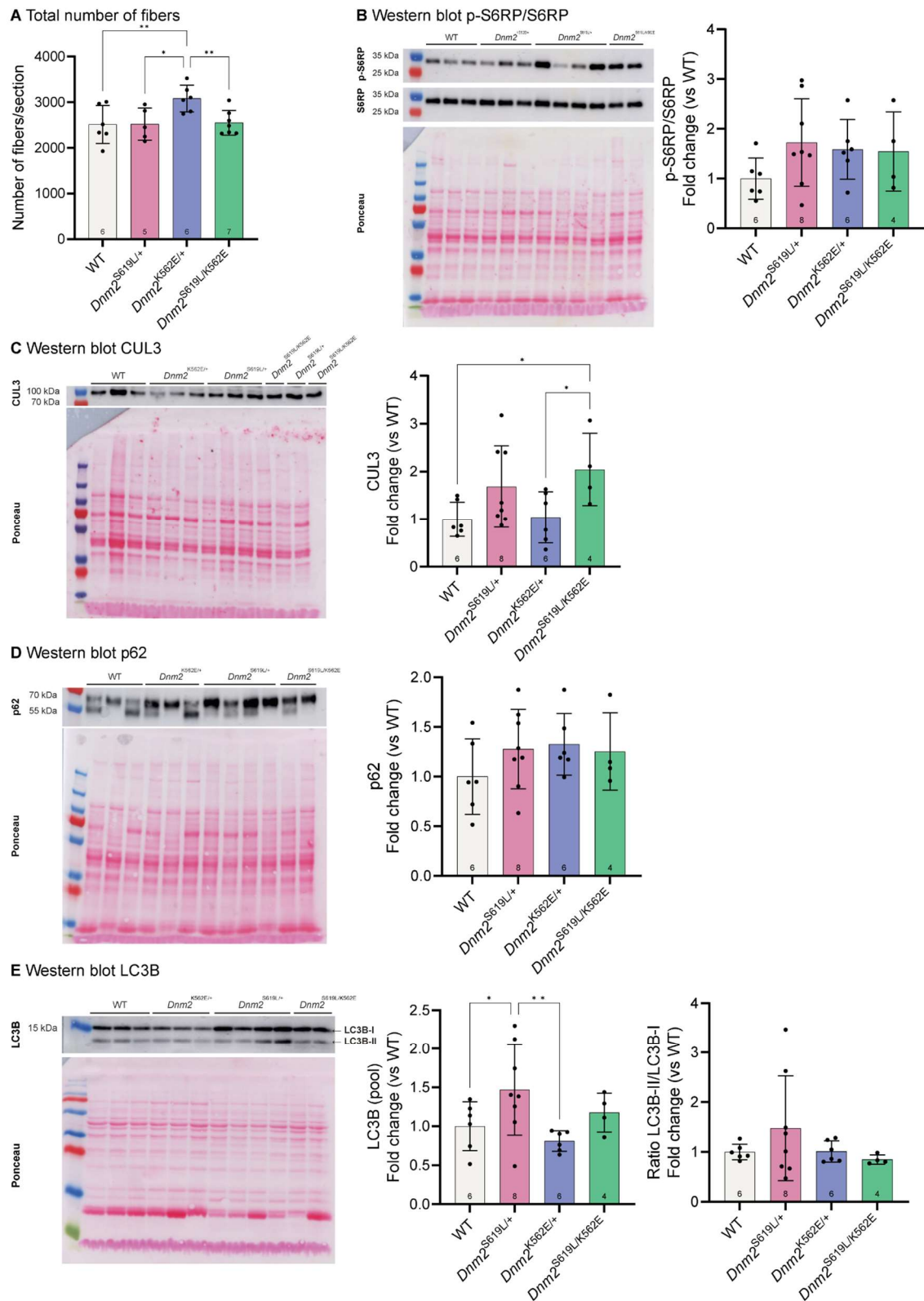
Supplementary Fig 3. Homozygosity for $Dnm2^{K562E/K562E}$ results in early embryonic lethality. (A) Expected and observed birth ratios by crossing $Dnm2^{K562E/+}$ mice together, shown as percentages and absolute numbers; genotyping performed at postnatal day 10 (P10, n=36, *** $P=0.0004281$). **(B)** Expected and observed birth ratios by crossing $Dnm2^{K562E/+}$ mice together, shown as percentages and absolute numbers; genotyping performed on or before embryonic day 18.5 (E18.5, n=19, ** $P=0.0045438$). **(C)** Genotype distribution of pups from three litters collected on or before E18.5. Source data are provided as a Source Data file.



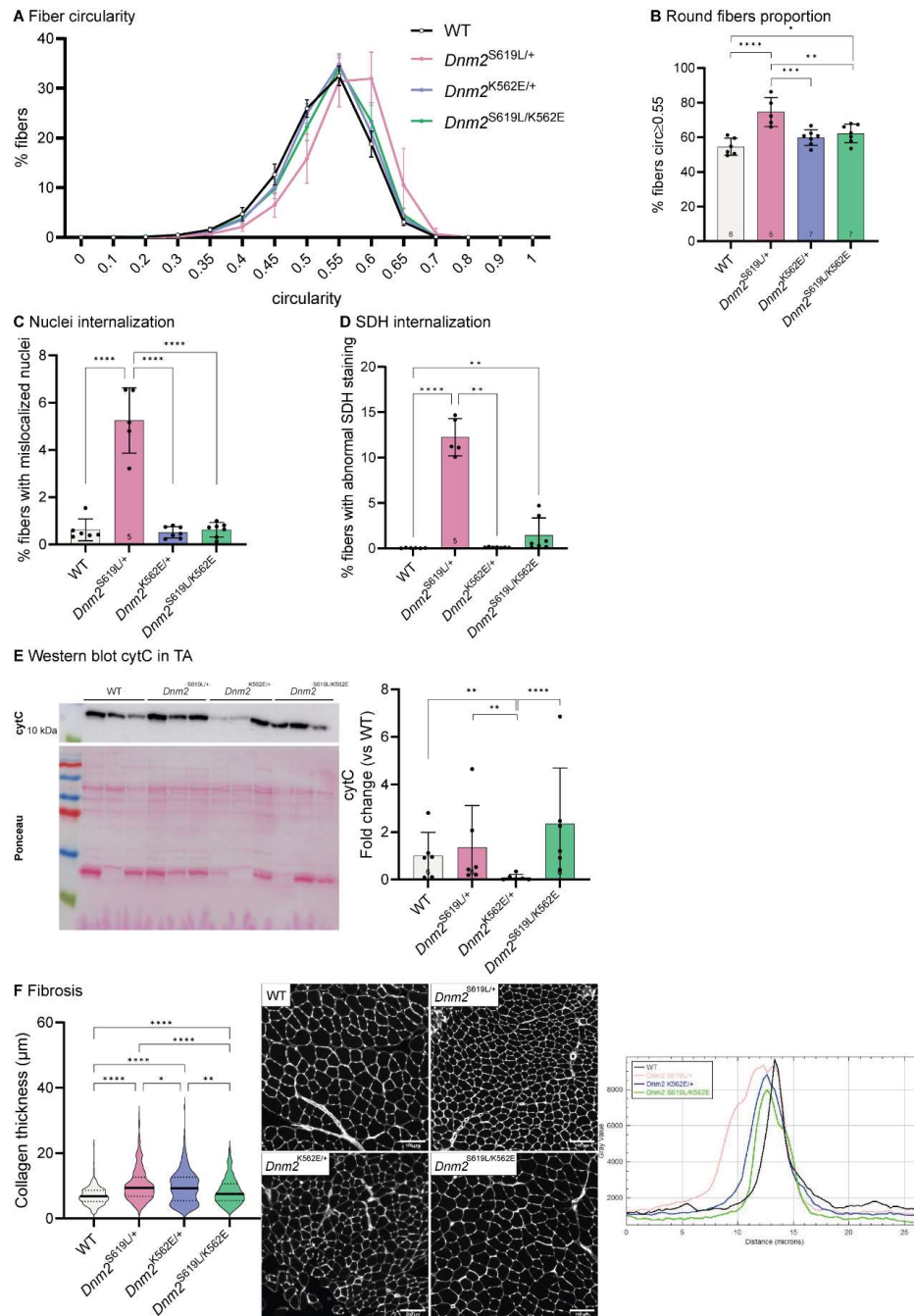
Supplementary Fig 4. Additional characterization of *Dnm2*^{S619L/K562E} mice. (A) Genotypes analyzed and nomenclature used, created in BioRender. Laporte, J. (2025) <https://BioRender.com/mpl1idh>. (B) Sanger sequencing around S619 and K562 position for WT and *Dnm2*^{S619L/K562E} mice. (C) Birth ratios expected and obtained for the four groups in percentage and n number (n=74). (D-E) Droplet digital PCR (ddPCR) analysis of (D) K562E mutation and WT S619 position, and (E) S619L mutation and WT K562 position in TA RNA samples from WT and *Dnm2*^{S619L/K562E} mice (2≤n≤3). (F) RT-qPCR analysis of *Dnm2* expression in TA (5≤n≤6, from left to right **P=0.0065, **P=0.0057). (G-H) Representative western blots of DNM2 protein in (G) TA and (H) sciatic nerve. Each dot represents a mouse. Values are represented as mean ± SD. (C) Chi-squared test. (F) Two-sided Kruskal-Wallis test. Source data are provided as a Source Data file.

A Hour by hour locomotor activity**B** Total locomotor activity**C** Total rear activity**D** Fatigue analysis**E** Drop in force

Supplementary Fig 5. Additional data on motor function. (A-B) (A) Hour by hour (n WT=7, $Dnm2^{S619L/+}$ =6, n $Dnm2^{K562E/+}$ =8, n $Dnm2^{S619L/K562E}$ =9) and (B) total locomotor activity during the night (6≤n≤9). One locomotor count corresponds to one back-and-forth movement in the cage. (C) Total rear activity during the night (5≤n≤8, * P =0.0239). (D) Force developed by TA muscle over 80 consecutive TA stimulations at 40Hz, normalized to TA mass (n WT=13, $Dnm2^{S619L/+}$ =6, n $Dnm2^{K562E/+}$ =10, n $Dnm2^{S619L/K562E}$ =11, from left to right * P =0.0146, αP =0.0053, $\#P$ =0.0032, * P =0.0373, αP =0.0112, $\#P$ =0.0102, αP =0.0393, $\#P$ =0.0230, $\#P$ =0.0414). (E) Drop in force between 5 first and 5 last stimulations at 40Hz (6≤n≤13, * P =0.0216, ** P =0.0053). (A, D) Each n represents a mouse, and the graph shows mean ± SD. (B-C, E) Each dot represents a mouse. Values are represented as mean ± SD. In D, significant differences between $Dnm2^{S619L/+}$ and WT, $Dnm2^{K562E/+}$ or $Dnm2^{S619L/K562E}$ groups were indicated with *, α , and # respectively. (B, C, D) Two-sided ANOVA test. (E) Two-sided Kruskal-Wallis test. Source data are provided as a Source Data file.

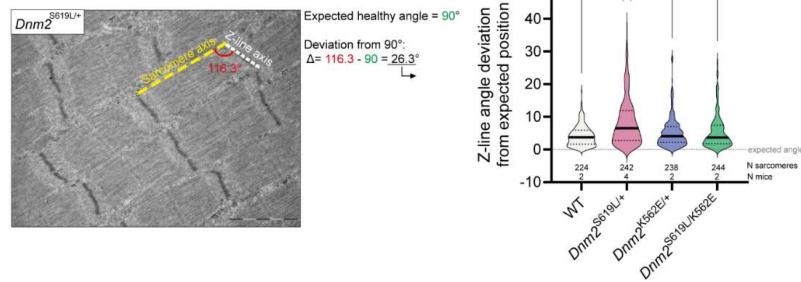


Supplementary Fig 6. Further analysis of muscle atrophy. (A) Total number of fibers in TA muscle sections ($5 \leq n \leq 7$, from left to right $^{**}P=0.0078$, $^{*}P=0.0118$, $^{**}P=0.0098$). (B-E) Representative western blots and quantifications of (B) p-S6RP and S6RP, (C) Cullin3 (CUL3, from left to right $^{*}P=0.0254$, $^{*}P=0.0306$), (D) p62, and (E) LC3B proteins level in TA ($4 \leq n \leq 8$, $^{*}P=0.0408$, $^{**}P=0.0061$). (A-E) Each dot represents a mouse. Values are represented as mean $\pm$ SD. (A-E) Two-sided ANOVA test. Source data are provided as a Source Data file.

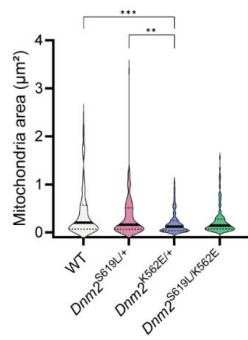


Supplementary Fig 7. Additional data on muscle histology. (A) TA myofibers repartition based on their circularity, 0 represents a line and 1 a circle (n WT=6, *Dnm2*^{S619L/+}=5, n *Dnm2*^{K562E/+}=7, n *Dnm2*^{S619L/K562E}=7). (B) Proportion of round fibers (circularity ≥ 0.55) in TA sections (5 ≤ n ≤ 7, **P*=0.0260, ***P*=0.0014, ****P*=0.0003, *****P*<0.0001). (C-D) Proportion of fibers with abnormal localization of (C) nuclei (*****P*<0.0001), and (D) oxidative staining in TA sections (5 ≤ n ≤ 7, from top to bottom ***P*=0.0022, ***P*=0.0033, *****P*<0.0001). (E) Representative western blot and quantification of cytochrome C (cytC) protein level in TA (n=6, from left to right ***P*=0.0020, ***P*=0.0011, *****P*<0.0001). (A) Each n represents a mouse, and the graph shows mean ± SD. (B-E) Each dot represents a mouse. Values are represented as mean ± SD. (F) Collagen interfiber thickness for each group, representative immunofluorescence images of collagen staining between muscle fibers, and example of the plots obtained when measuring interfiber distance based on collagen fluorescence staining for each group (300 ≤ n = interfibers ≤ 336, n = mice = 6, **P*=0.0251, ***P*=0.0018, *****P*<0.0001). Each interfiber is plotted. (B-C, E) Two-sided ANOVA test. (D, F) Two-sided Kruskal-Wallis test. Source data are provided as a Source Data file.

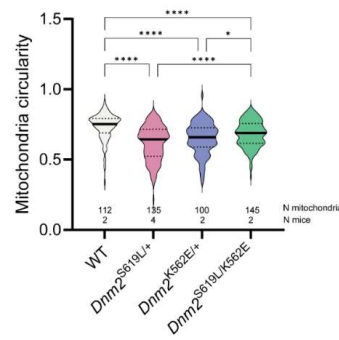
A Z-line misalignment analysis



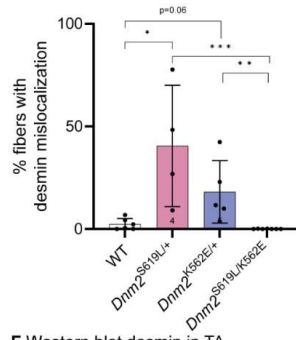
B Mitochondria area



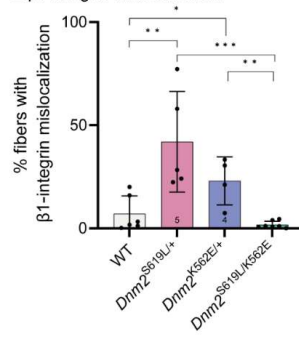
C Mitochondria circularity



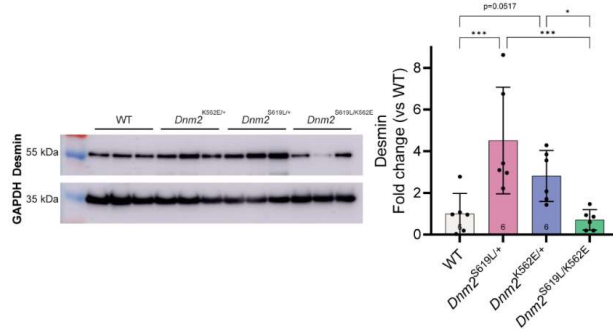
D Desmin mislocalization



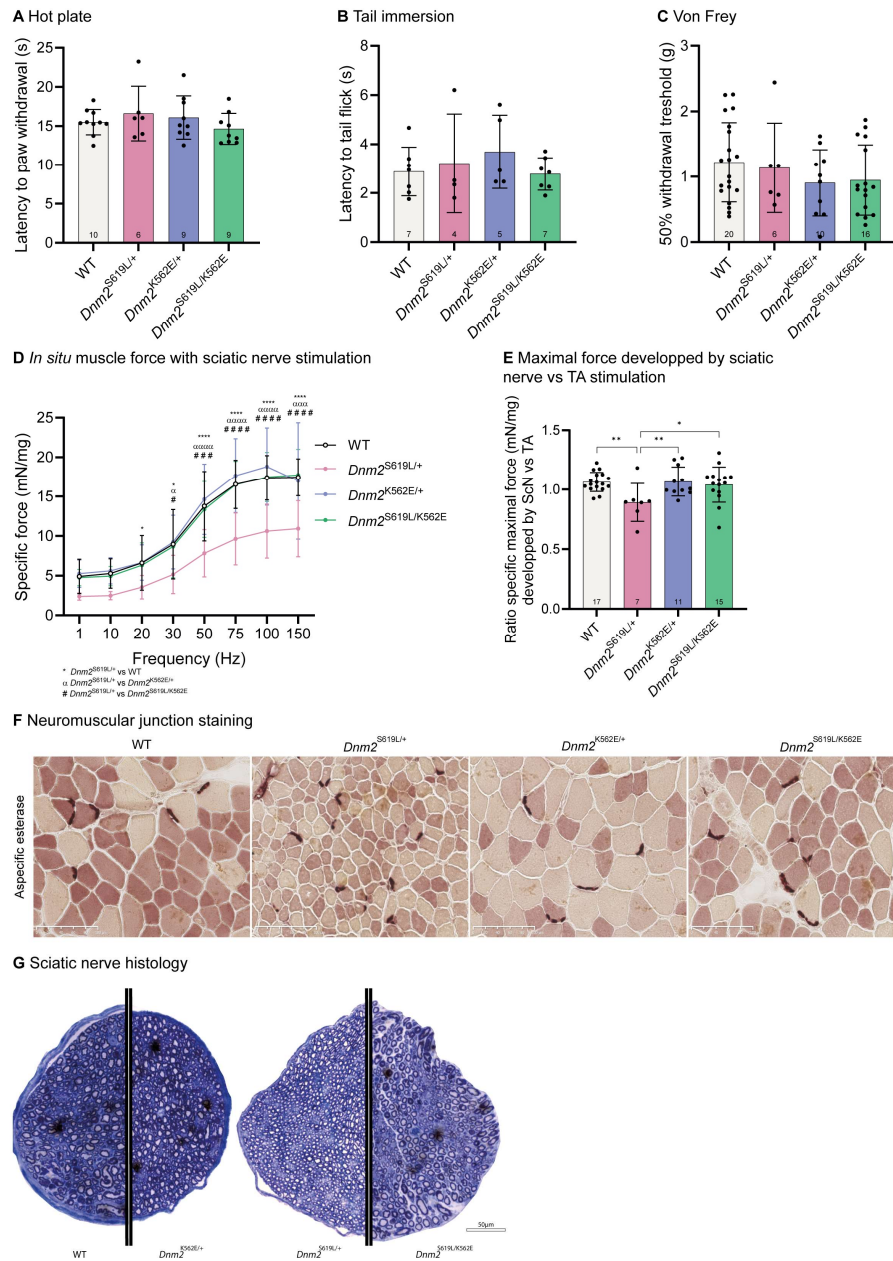
E β1-integrin mislocalization



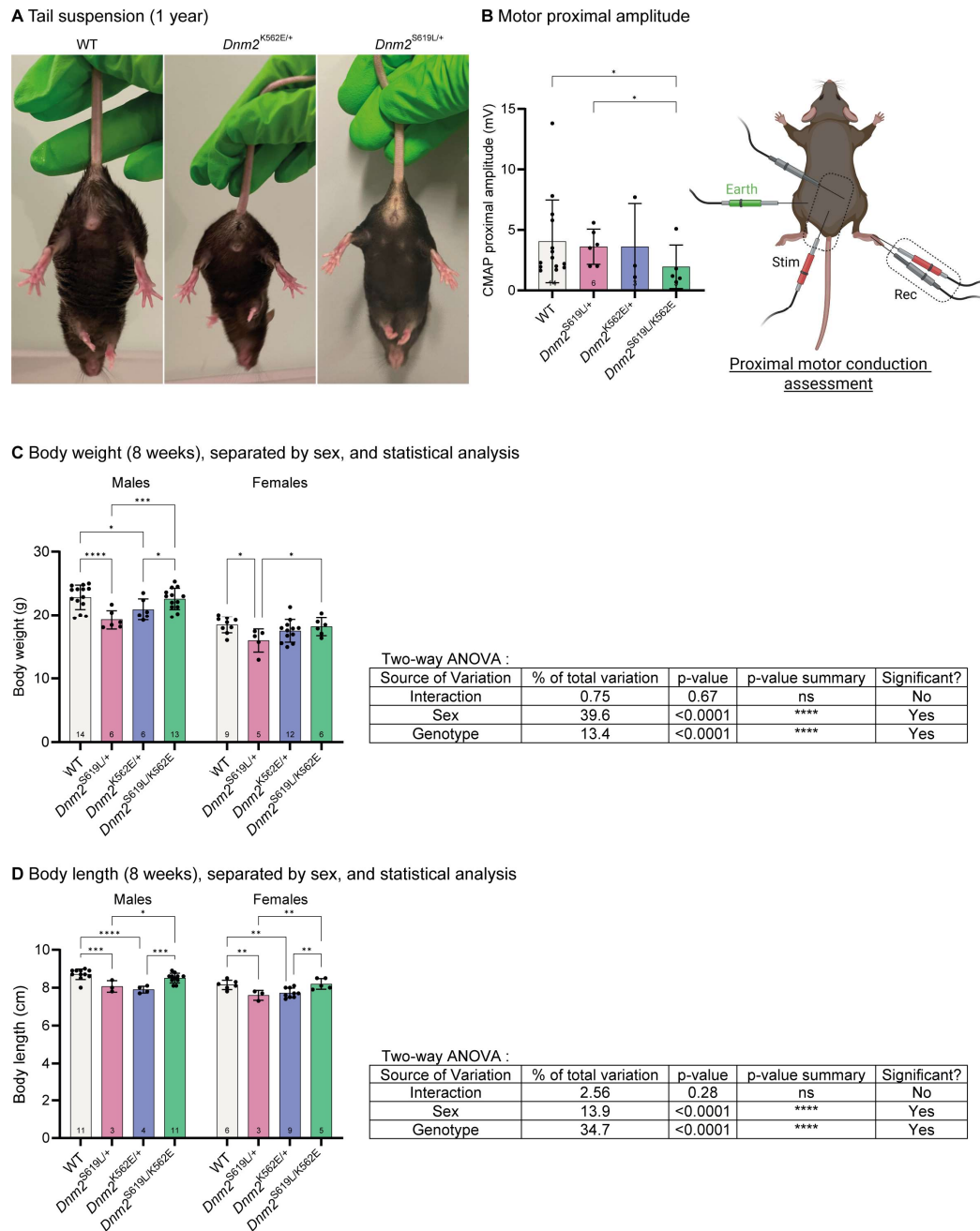
F Western blot desmin in TA



Supplementary Fig 8. Additional data on muscle ultrastructure and histology. (A) Z-line angle deviation from the expected 90° alignment with sarcomere axis, measured on electron microscopy images as illustrated (224≤n=Z lines≤244, 2≤n=mice≤4, *****P*<0.0001). Each Z line is plotted. **(B-C)** Quantification of mitochondria **(B)** area (**P*=0.0069, ***P*=0.0003), and **(C)** circularity (**P*=0.0363, *****P*<0.0001) from electron microscopy images (101≤n=mitochondria≤145, 2≤n=mice≤4). Each mitochondria is plotted. **(D-E)** Proportion of fibers presenting an abnormal localization of **(D)** desmin (**P*=0.0162, ***P*=0.0025, ****P*=0.0005) and **(E)** β1-integrin (from left to right ***P*=0.0048, **P*=0.0341, ****P*=0.0001, ***P*=0.0012) in TA sections at 8 weeks (4≤n≤7). **(H)** Representative western blot and quantification of desmin protein level in TA (n=6, from left to right ****P*=0.0007, ****P*=0.0003, **P*=0.0261). (D-F) Each dot represents a mouse. Values are represented as mean ± SD. (E-F) Two-sided ANOVA test. (A-D) Two-sided Kruskal-Wallis test. Source data are provided as a Source Data file.



Supplementary Fig 9. Evaluation of neurological phenotypes in the four groups of mice. (A) Hot plate test (52°C) at 8 weeks. Reaction latency is recorded (6≤n≤10). **(B)** Tail immersion test (48°C) at 8 weeks. Reaction latency is recorded (4≤n≤7). **(C)** Von Frey test at 8 weeks. Reaction to mechanic stimuli is recorded. The data presents the gram value at which the mouse is predicted to react 50% of the time (6≤n≤20). **(D)** Force developed by TA muscle when stimulating the sciatic nerve at different incremental frequencies, from 1 to 150Hz, normalized to TA mass (n WT=17, *Dnm2*^{S619L/+}=7, n *Dnm2*^{K562E/+}=11, n *Dnm2*^{S619L/K562E}=15, from left to right *P=0.0394, *P=0.0105, ^aP=0.0108, #P=0.0196, ###P=0.0003, ^{ααα}P=0.0002, ****/αααα/####P<0.0001). **(E)** Ratio of the maximal force developed from sciatic nerve vs. TA muscle stimulation (7≤n≤17, from left to right **P=0.0035, **P=0.0054, *P=0.0114). **(F)** TA transversal sections stained with aspecific esterase to observe neuromuscular junctions. Scale= 100μm. **(G)** Half-sciatic nerve transversal section stained with toluidine blue, further used for analysis. Scale= 50μm. (D) Each n represents a mouse, and the graphs show mean ± SD. (A-C, E) Each dot represents a mouse. Values are represented as mean ± SD. In E, significant differences between *Dnm2*^{S619L/+} and WT, *Dnm2*^{K562E/+} or *Dnm2*^{S619L/K562E} groups were indicated with *, α, and # respectively. (A-E) Two-sided ANOVA test. Source data are provided as a Source Data file.



Supplementary Fig 10. Additional *in vivo* phenotyping of 1-year old mice; and sex-specific graphs for body size at 8 weeks. (A) Tail suspension of 1-year-old mice. (B) Electrophysiological analysis of compound muscle action potential (CMAP) amplitude, measured proximally as illustrated (3≤n≤14, from left to right E*P=0.0407, *P=0.0401). Stim= stimulation electrodes. Rec= recording electrodes. Created in BioRender. Laporte, J. (2025) <https://BioRender.com/sk689pl>. (C) Body weight of 8 weeks-old mice, separated by sex, and statistical analysis performed (5≤n≤14). (D) Body length of 8 weeks-old mice, measured with gait analysis. Measurement from the nose of the animal to the start of the tail (6≤n≤17, from left to right ***P=0.0005, ****P<0.0001, *P=0.0123, ***P=0.0002, **P=0.0045, **P=0.0032, **P=0.0029, **P=0.0020). Results separated by sex, and statistical analysis performed (3≤n≤11). (B-D) Each dot represents a mouse. Values are represented as mean ± SD. (C, D) Two-sided Two-way ANOVA test. Source data are provided as a Source Data file.

Each table shows the effect of the combination of the two mutants, S619L and K562E, on each individual mutant:

A Summary of rDNM2 mutants functionality & provided rescue

rDNM2	S619L	K562E
Interaction	✓	
Basal GTPase activity		
Induced GTPase activity		
Liposomes binding		

B Summary of molecular DNM2 levels & provided rescue

Level	<i>Dnm2</i> ^{S619L/+}	<i>Dnm2</i> ^{K562E/+}
<i>Dnm2</i> RNA TA		
DNM2 TA		
DNM2 ScN		

C Summary of muscle phenotypes & provided rescue at 8w

Test	<i>Dnm2</i> ^{S619L/+}	<i>Dnm2</i> ^{K562E/+}
Body weight		
Hanging time		
Body length		
Angle feet		
Stride		
Locomotor activity		
Rear activity		
<i>In situ</i> muscle force		
TA mass		
Fiber size		
Nuclei localization		
Mitochondria localization		
Desmin localization		
β1-integrin localization		
Ultrastructural organization		
Fiber circularity		
CytC level		
Desmin level		

D Summary of neurological phenotypes & provided rescue at 8w

Test	<i>Dnm2</i> ^{S619L/+}	<i>Dnm2</i> ^{K562E/+}
Von Frey		
Hot plate		
Tail immersion		
Tail suspension		
NMJ morphology		
<i>Chma1</i> RNA		
<i>Chmg</i> RNA		
Myelin anomalies		
G-ratio		
Axon diameter		
Myelin thickness		

Model presented:	
No phenotype	
<i>Dnm2</i> ^{S619L/K562E} provided:	
Opposite or exacerbated phenotype	
No rescue	
Tendency to rescue (ns)	
Partial rescue	
Rescue	

* Qualitative assessment

E Summary of muscle phenotypes & provided rescue at 1y

Test	<i>Dnm2</i> ^{S619L/+}	<i>Dnm2</i> ^{K562E/+}
Body weight		
Hanging time		
Body length		
Angle feet		
Stride		
Locomotor activity		
Rear activity		
<i>In situ</i> muscle force		
TA mass		
Gastrocnemius mass		
Soleus mass		
Fiber size		
X ray kyphosis *		

F Summary of neurological phenotypes & provided rescue at 1y

Test	<i>Dnm2</i> ^{S619L/+}	<i>Dnm2</i> ^{K562E/+}
Von Frey		
Hot plate		
Tail immersion		
Tail suspension *		
Sensory NCV		
Sensory amplitude		
Motor NCV		
Motor proximal amplitude (data not shown)		
Motor distal amplitude		
G-ratio		
Axon diameter		
Myelin thickness		

Supplementary Fig 11. Overview of the improvements observed with combined CNM-S619L and CMT-K562E DNM2 mutations over each individual mouse models. (A-D) Summary of (A) *in vitro* assays with recombinant DNM2 (rDNM2), and (B) molecular, (C) muscular, and (D) neurological assessments in mouse models, indicating the effect provided by the concomitant presence of both mutations compared to each individual mutant, at 8 weeks. (E-F) Summary of (E) muscular, and (F) neurological assessments in mouse models, indicating the effect provided by the concomitant presence of both mutations compared to each individual mutant, at 1 year. Tendency to rescue= lack of difference between double mutant and single mutant or WT mice. Partial rescue= double mutant different from single mutant and WT mice. Rescue= double mutant different from single mutant but similar to WT. TA = tibialis anterior. ScN= sciatic nerve.

Supplementary Fig. 12 : Western blot
Unedited membranes

Figure 1B
DNM2

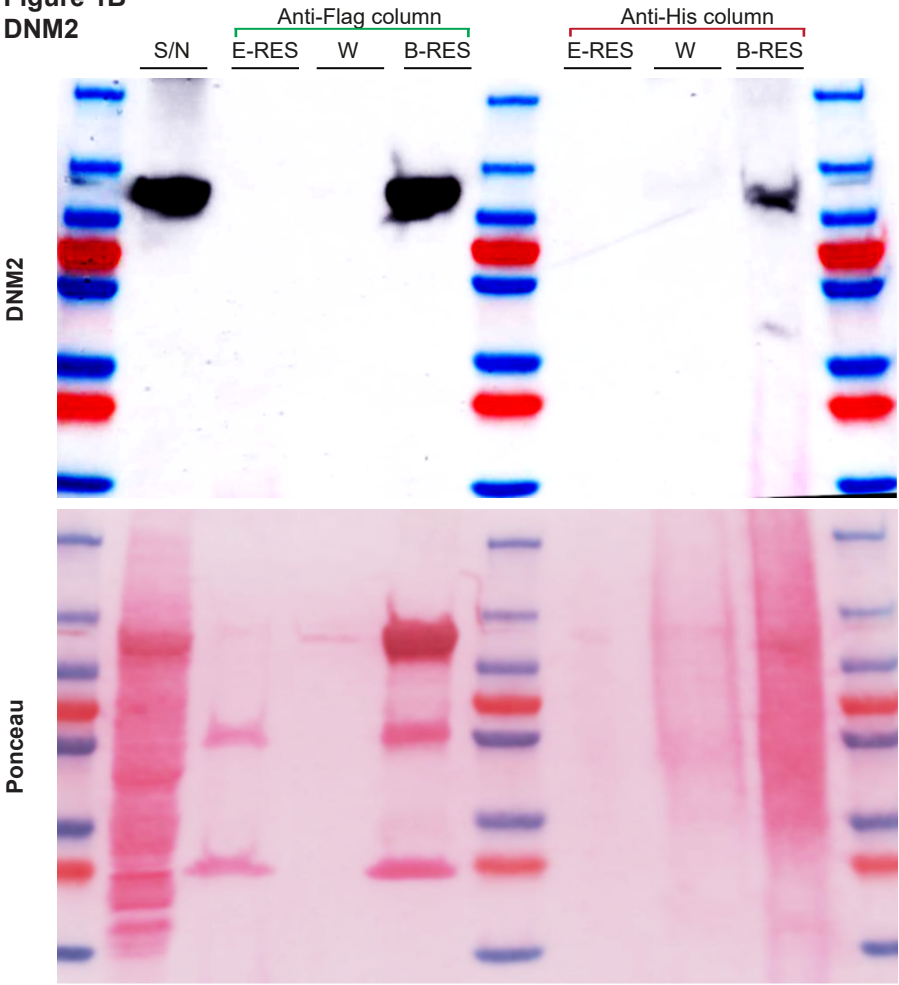
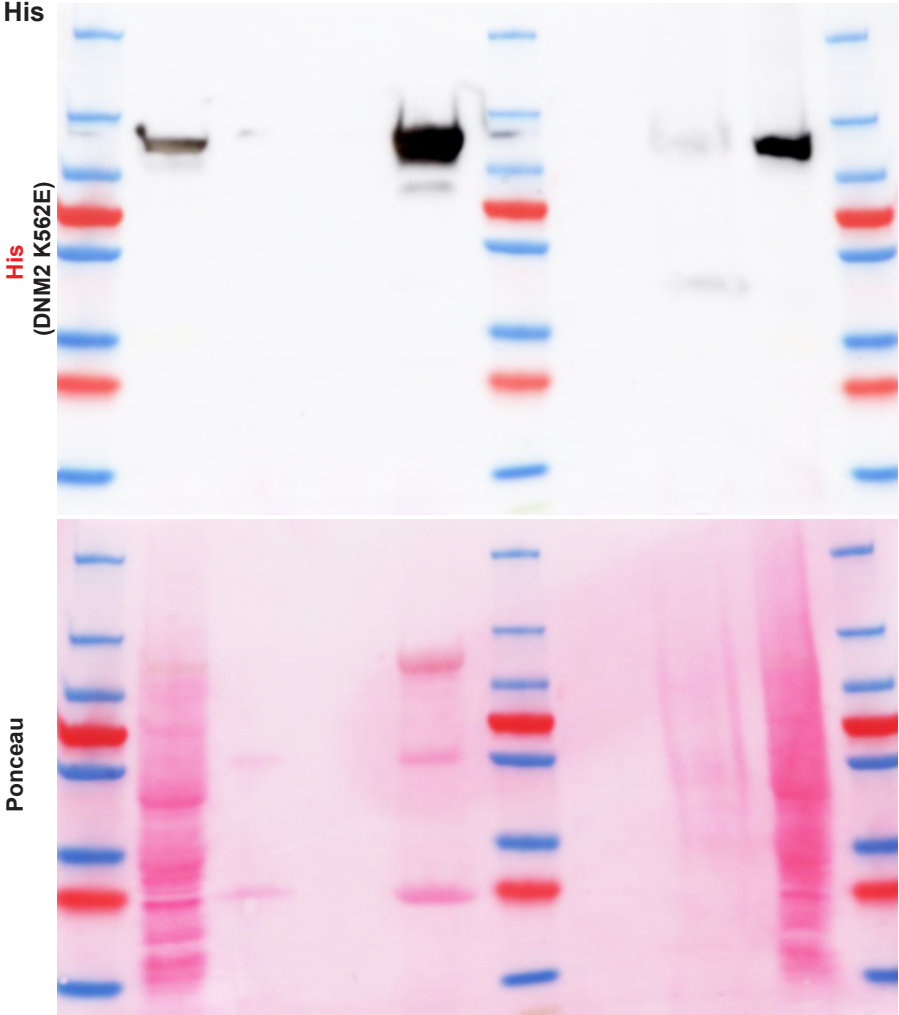
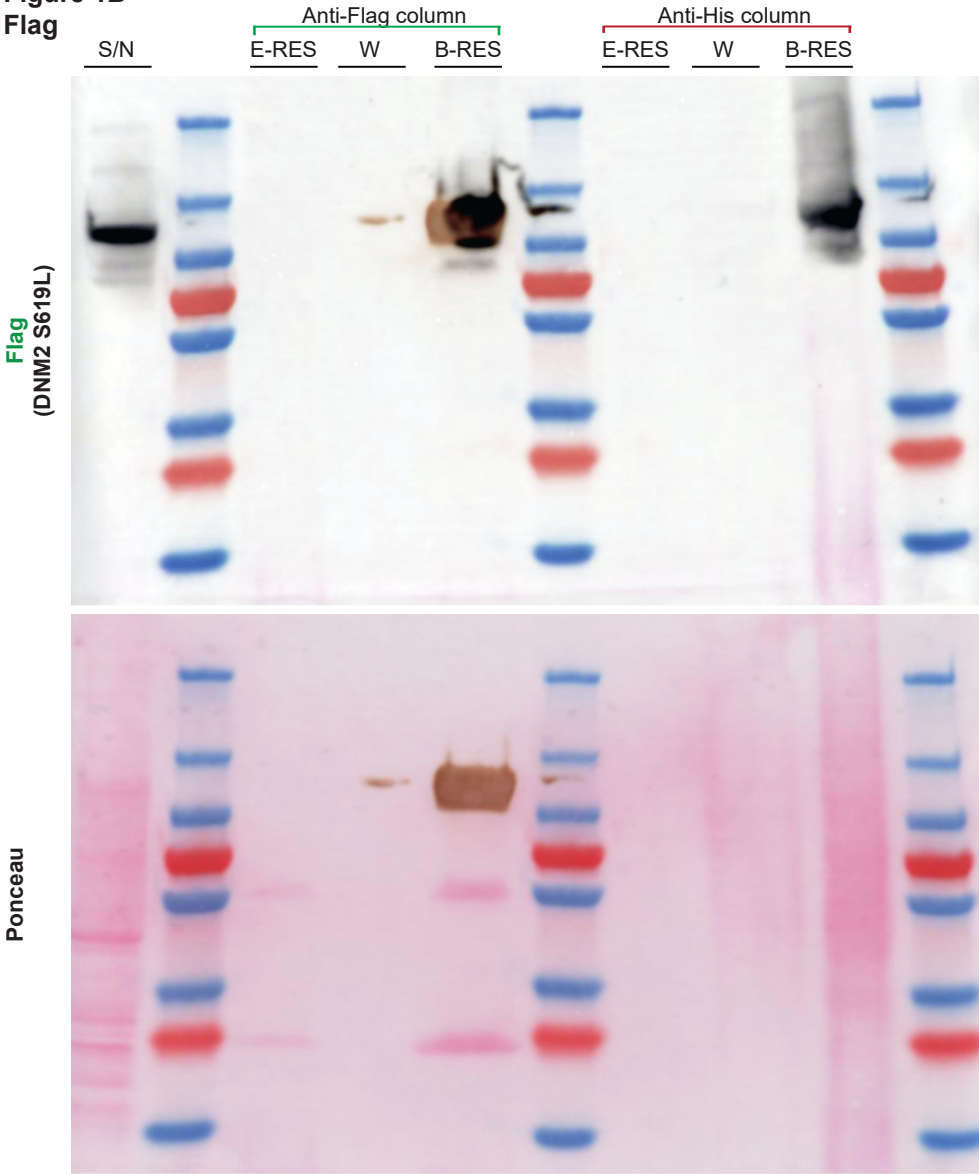


Figure 1B
His



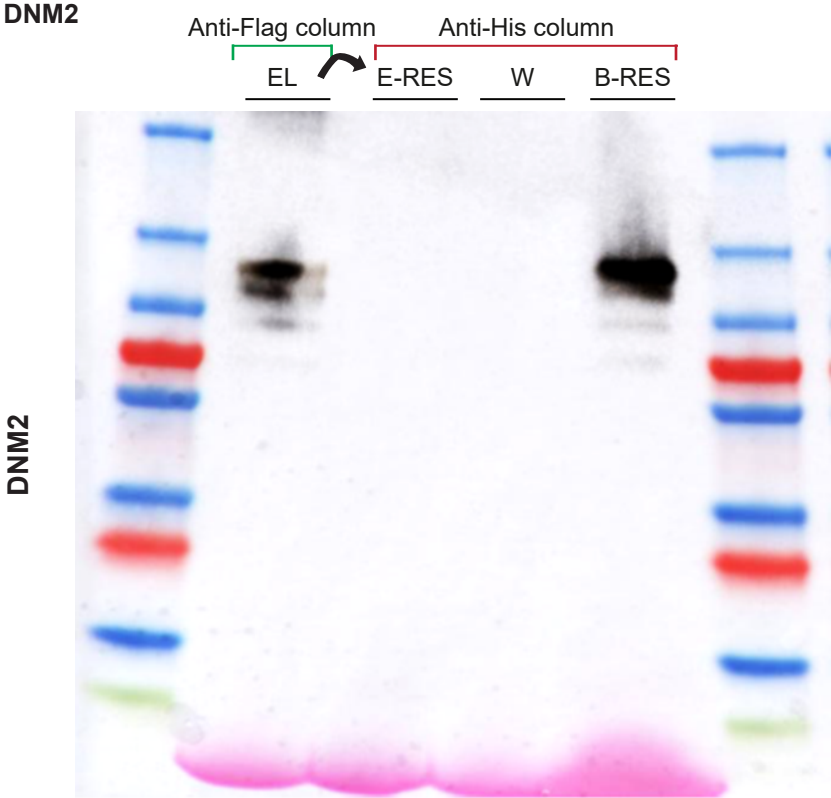
Supplementary Fig.12.

Figure 1B
Flag

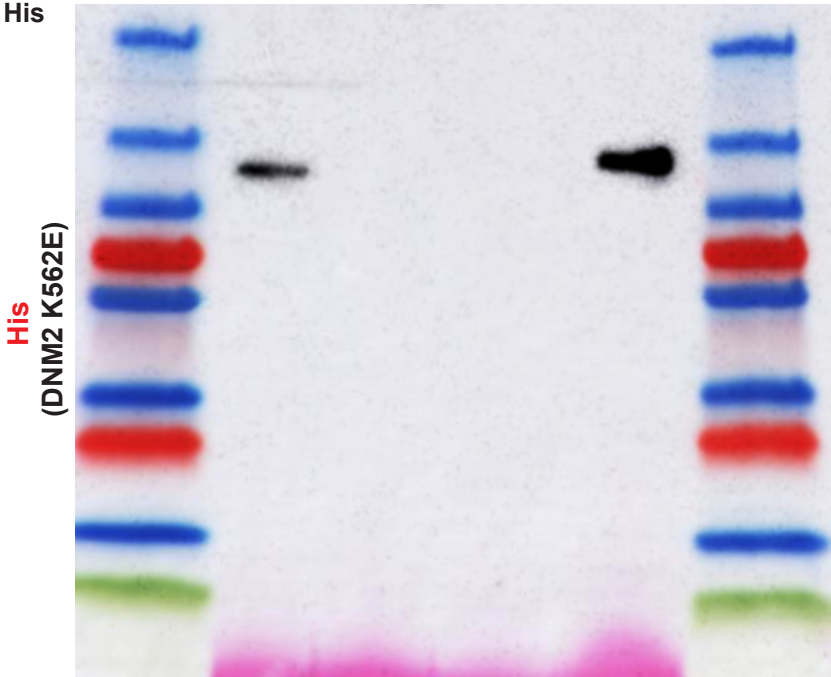


Supplementary Fig. 12.

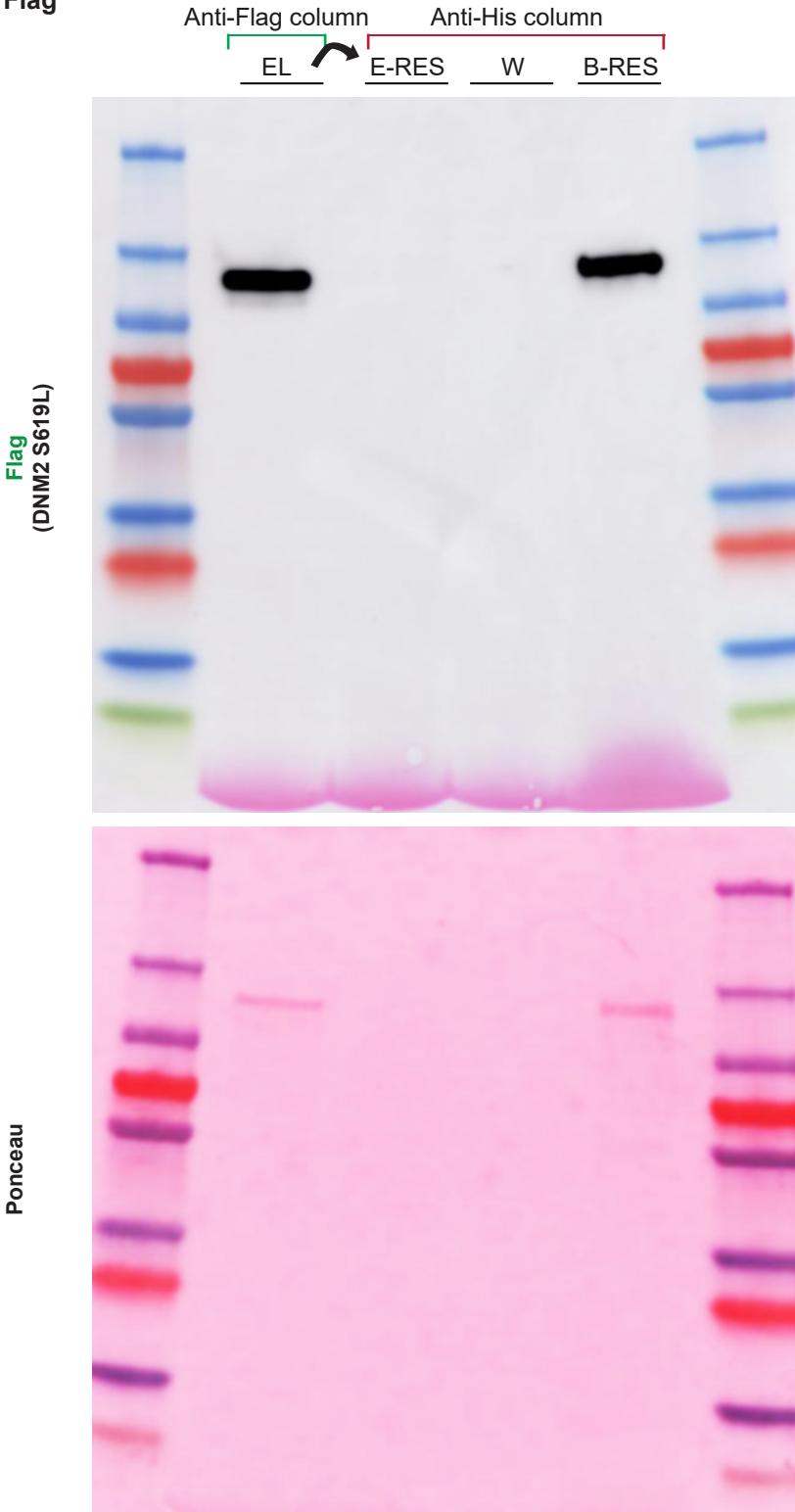
Supplementary Figure 1D



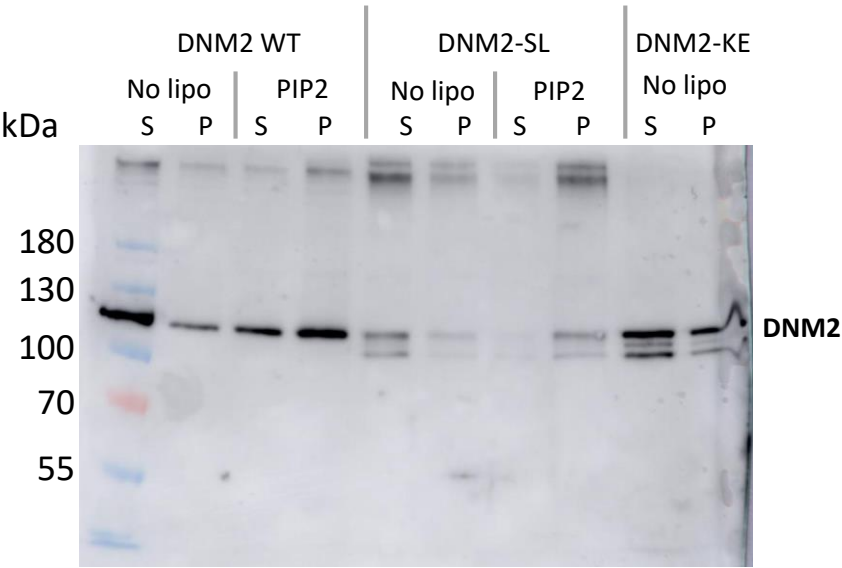
Supplementary Figure 1D



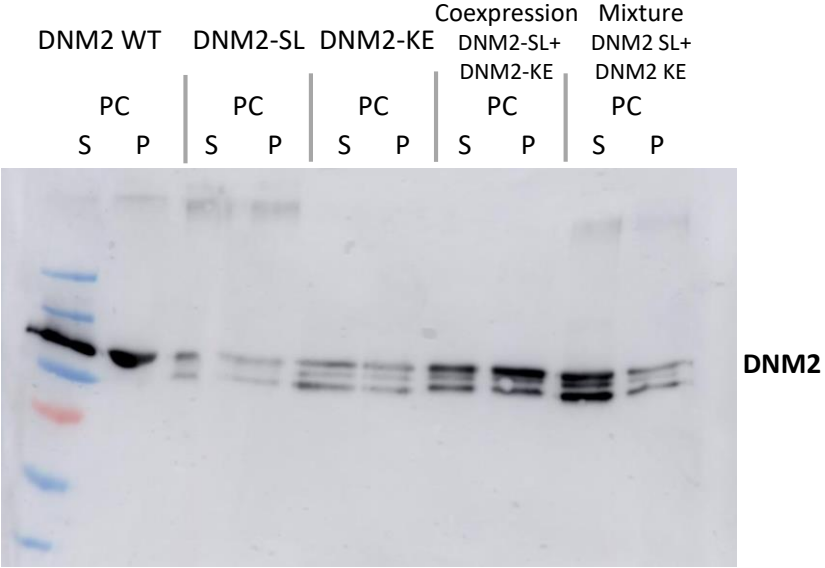
Supplementary Figure 1D
Flag



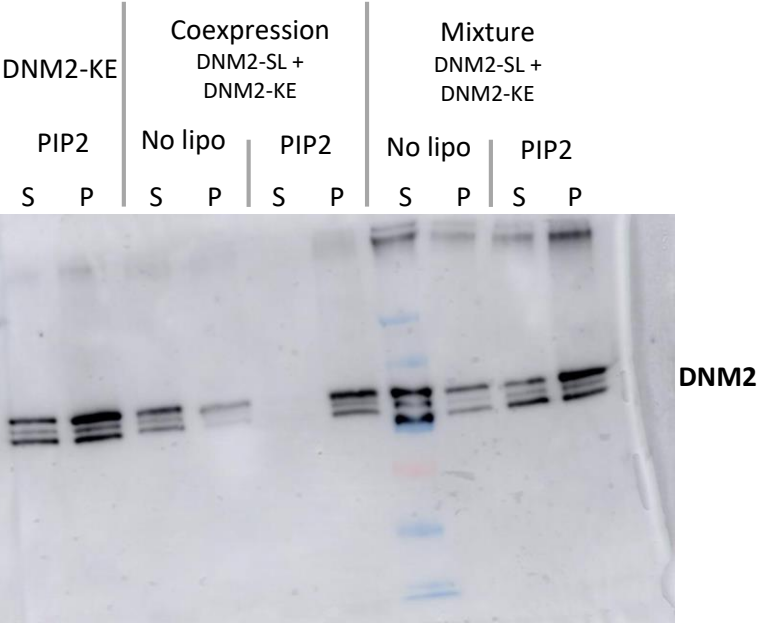
Liposome binding assay N=1 PIP2, PC and PS liposomes



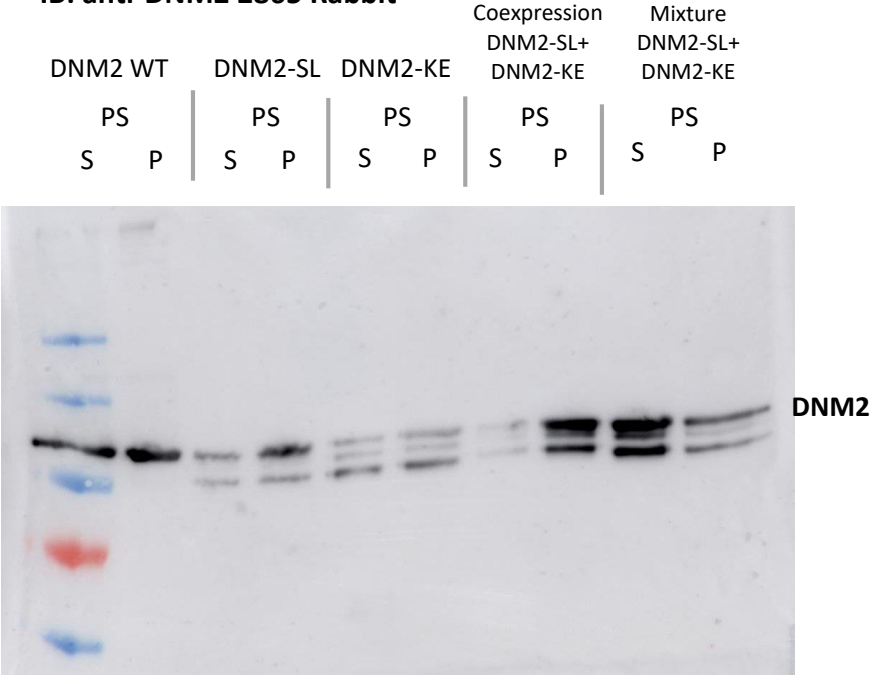
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IB: anti-DNM2 2865 Rabbit

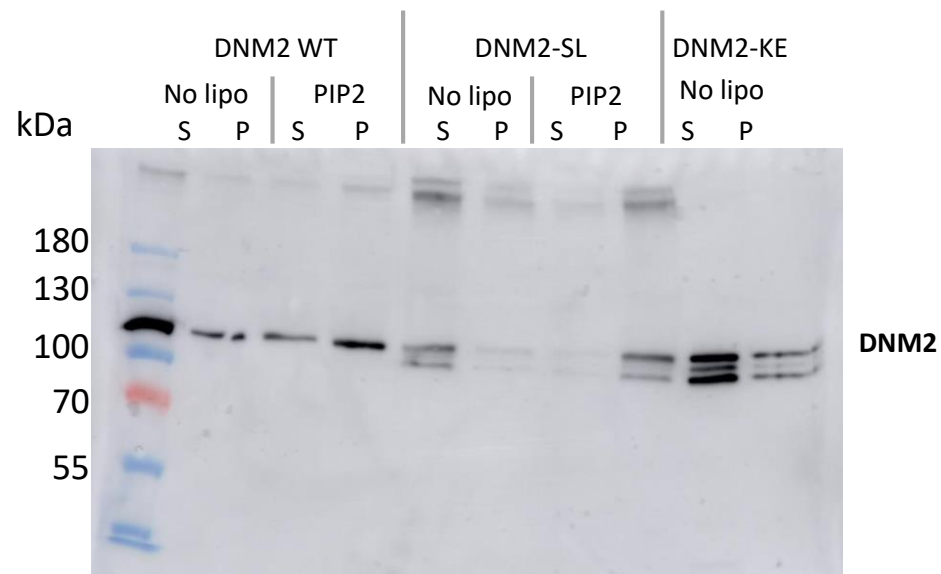


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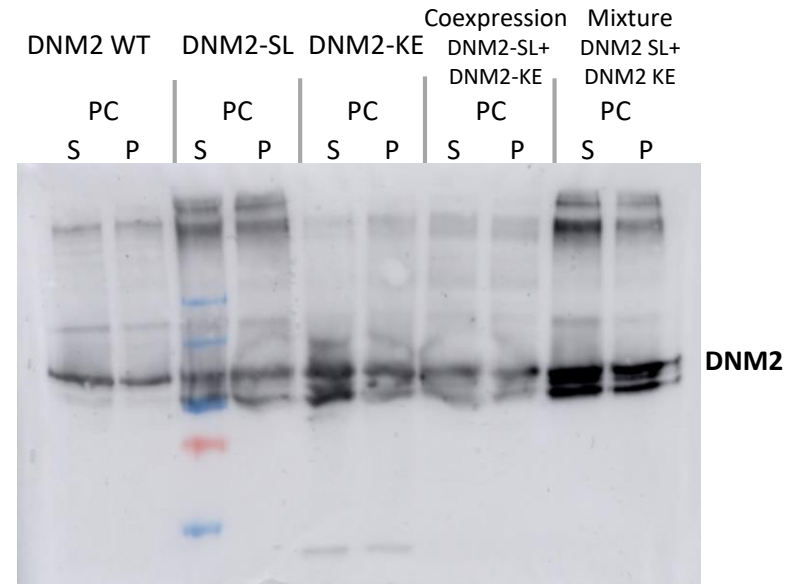


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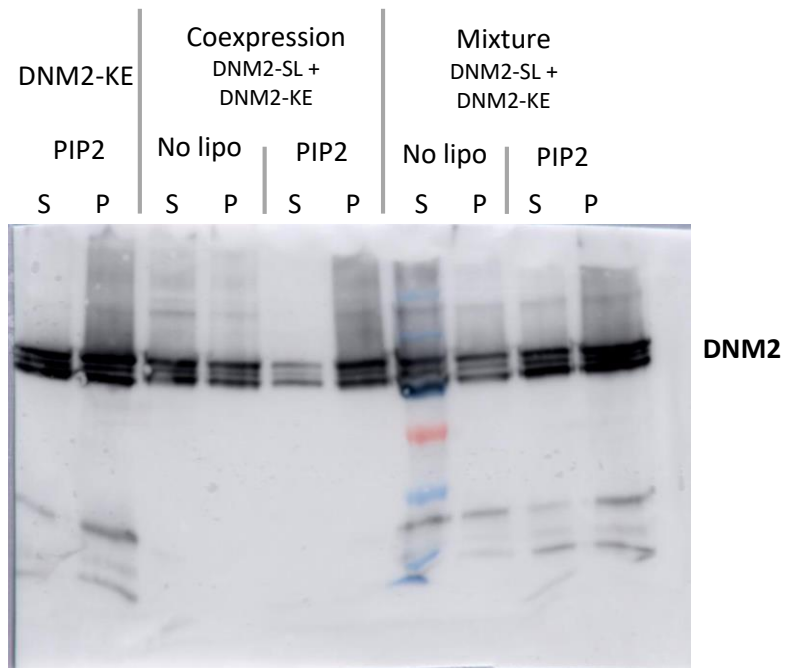
Liposome binding assay N=2 PIP2, PC and PS liposomes



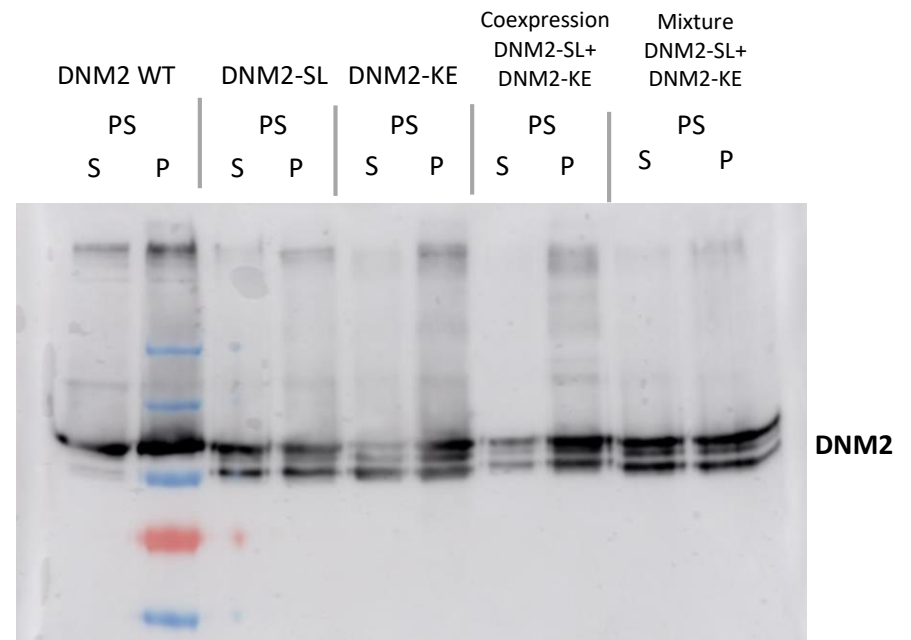
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IB: anti-DNM2 2865 Rabbit

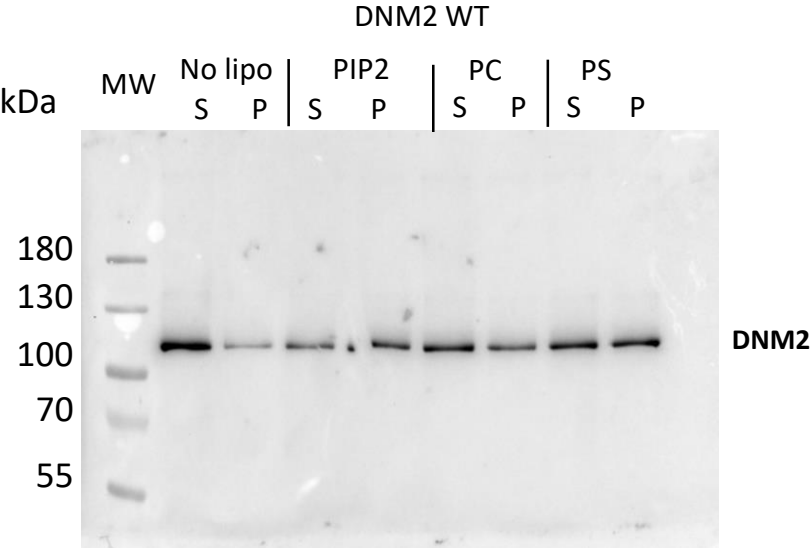


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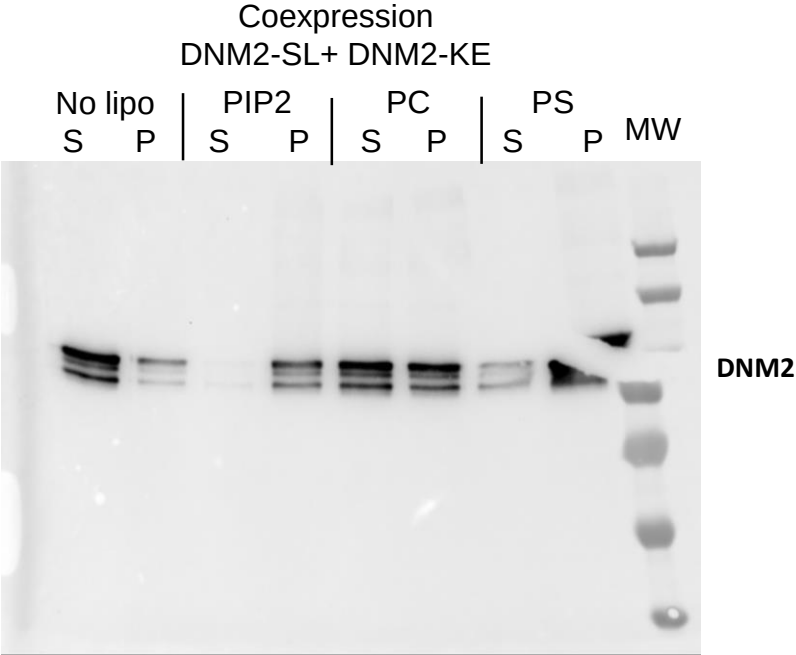


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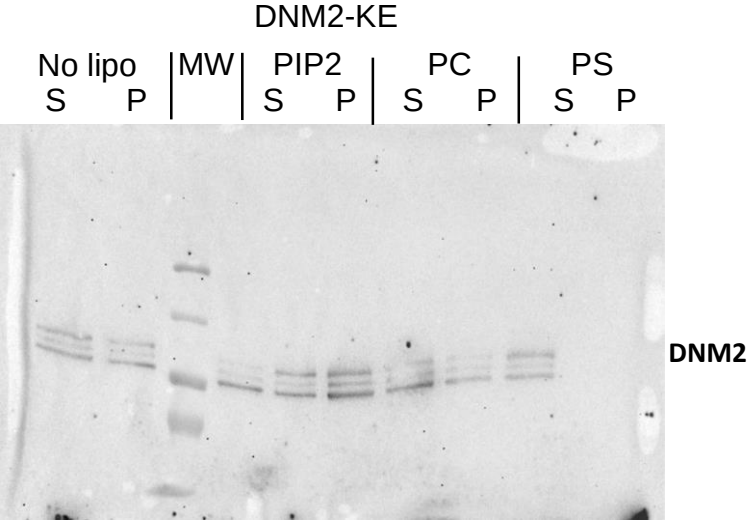
Liposome binding assay N=3 PIP2, PC and PS liposomes



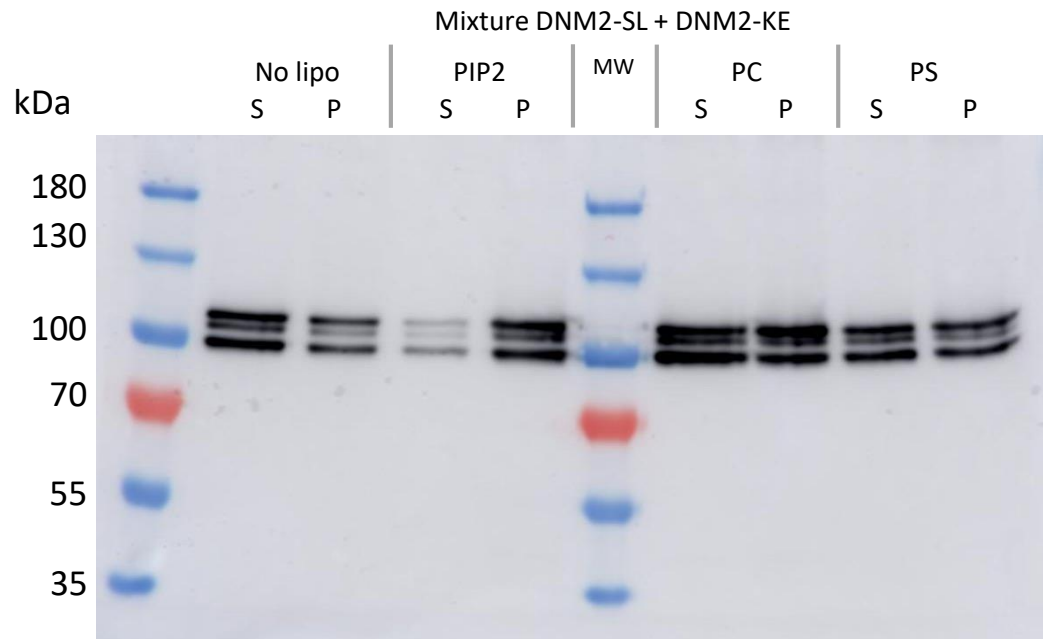
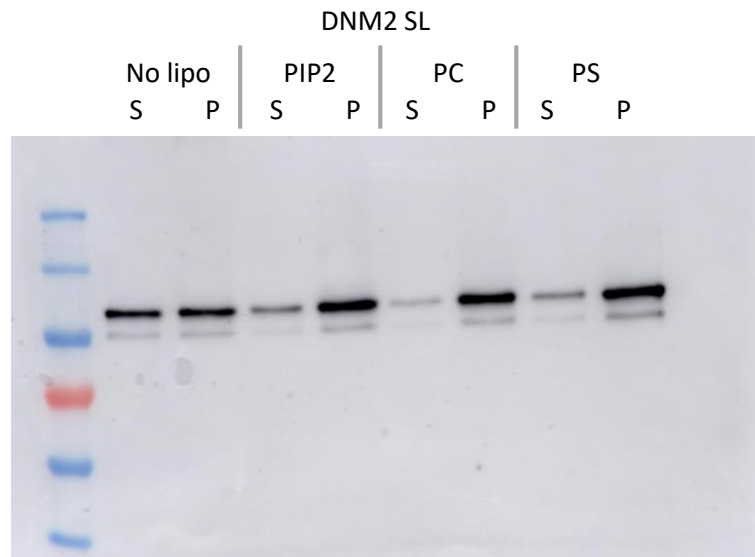
IB: anti-DNM2 2865 Rabbit



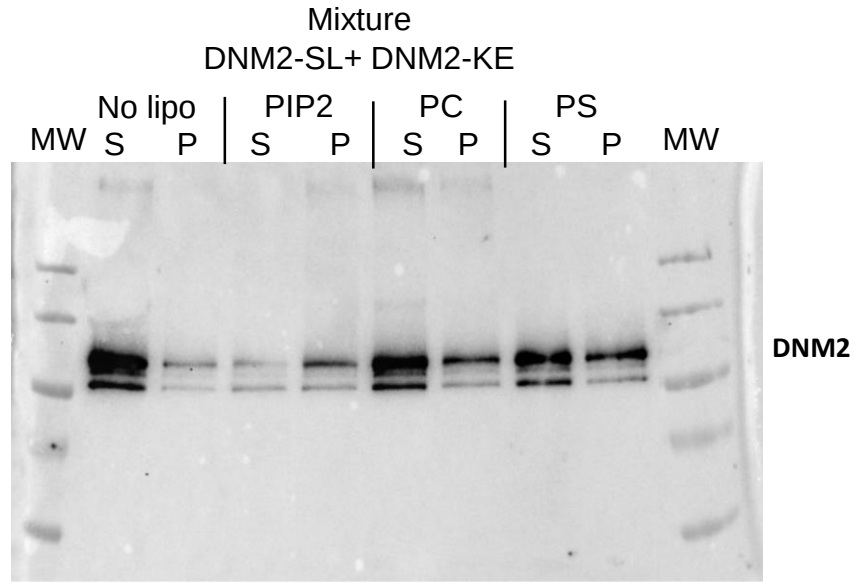
IB: anti-DNM2 2865 Rabbit



IB: anti-DNM2 2865 Rabbit

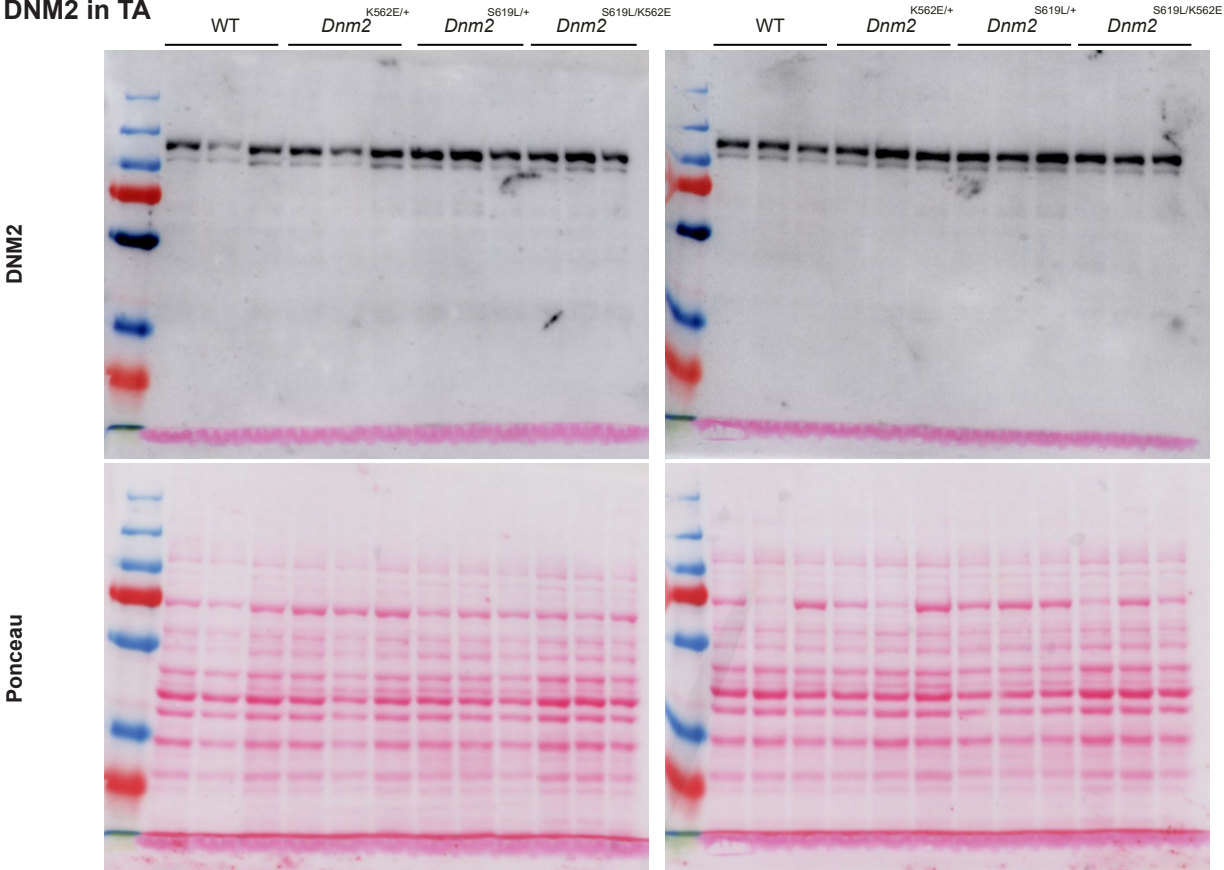
Liposome binding assay N=3 PIP2, PC and PS liposomes**IB: anti-DNM2 2865 Rabbit****IB: anti-DNM2 2865 Rabbit**

Liposome binding assay N=4 PIP2, PC and PS liposomes

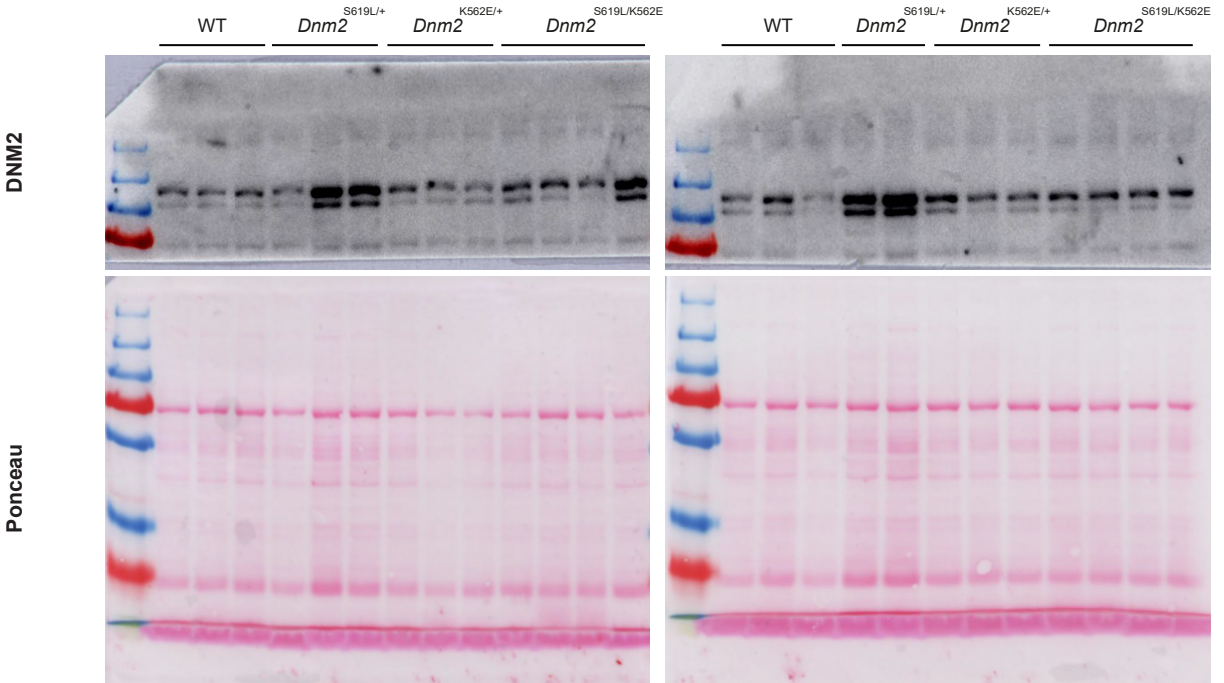


IB: anti-DNM2 2865 Rabbit

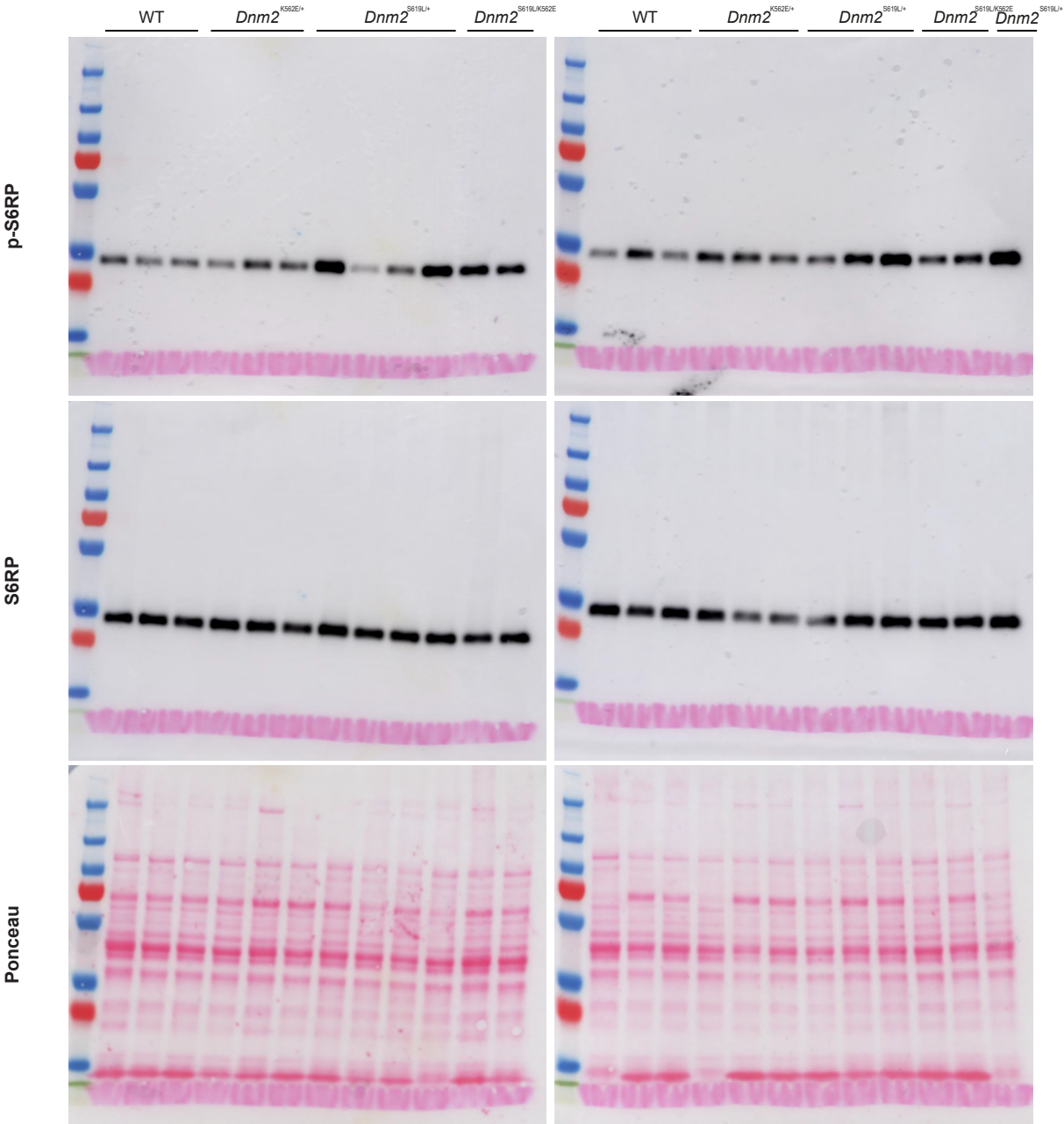
Supplementary Figure 4G
DNM2 in TA



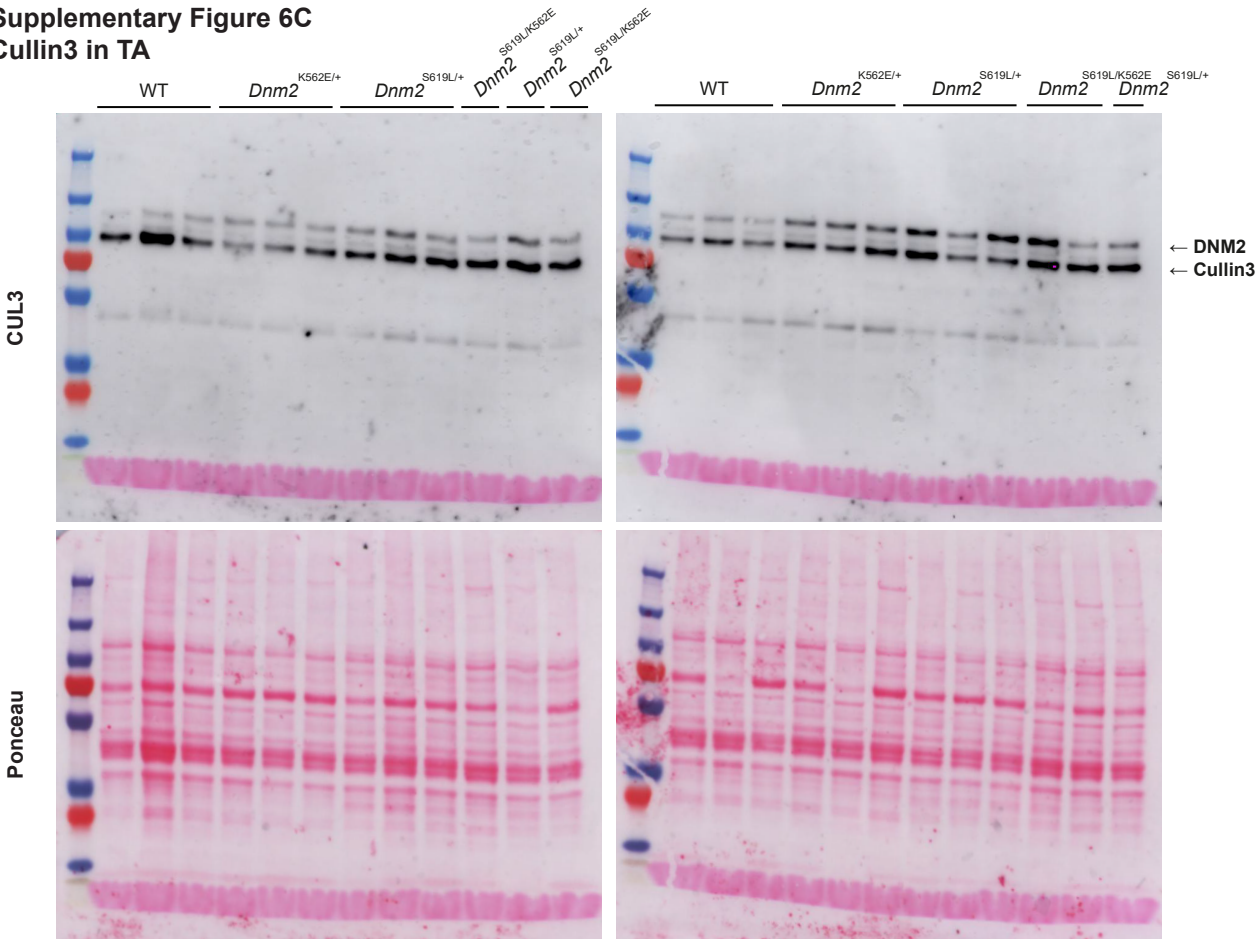
Supplementary Figure 4H
DNM2 in sciatic nerve



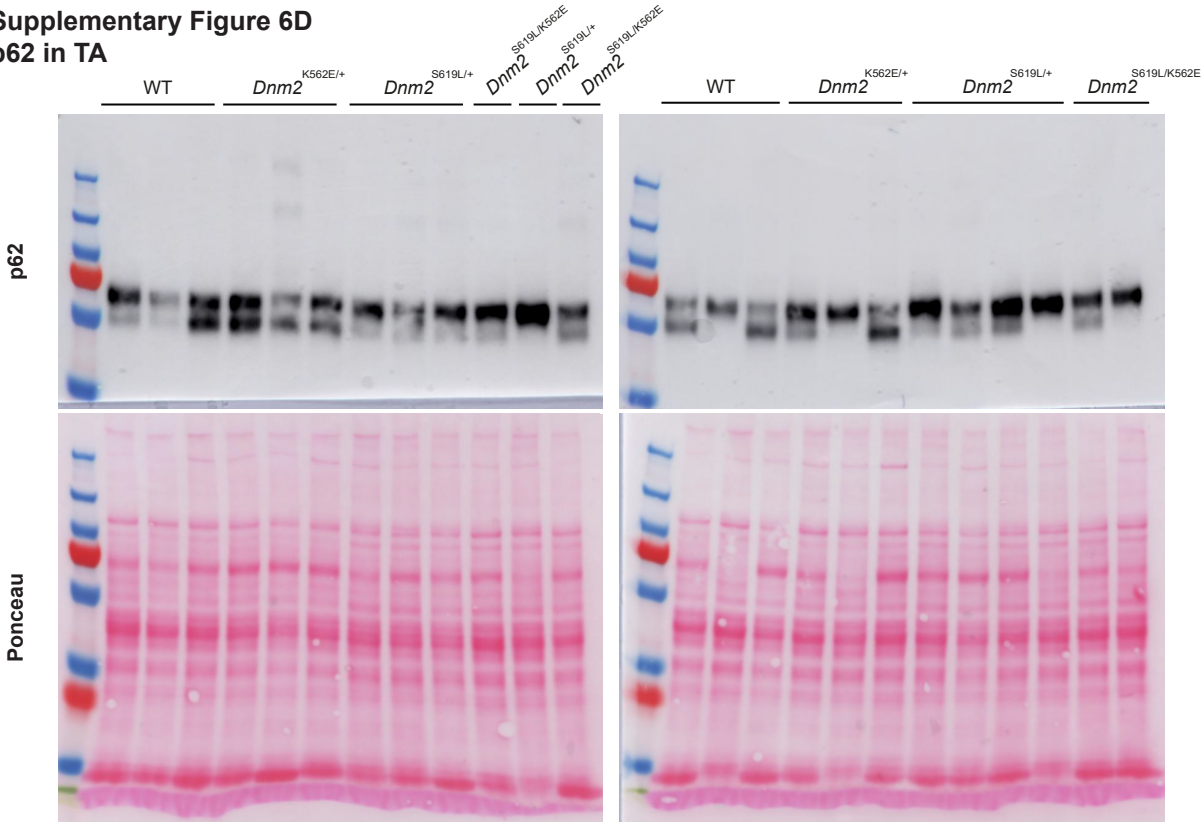
Supplementary Figure 6B
p-S6RP/S6RP in TA



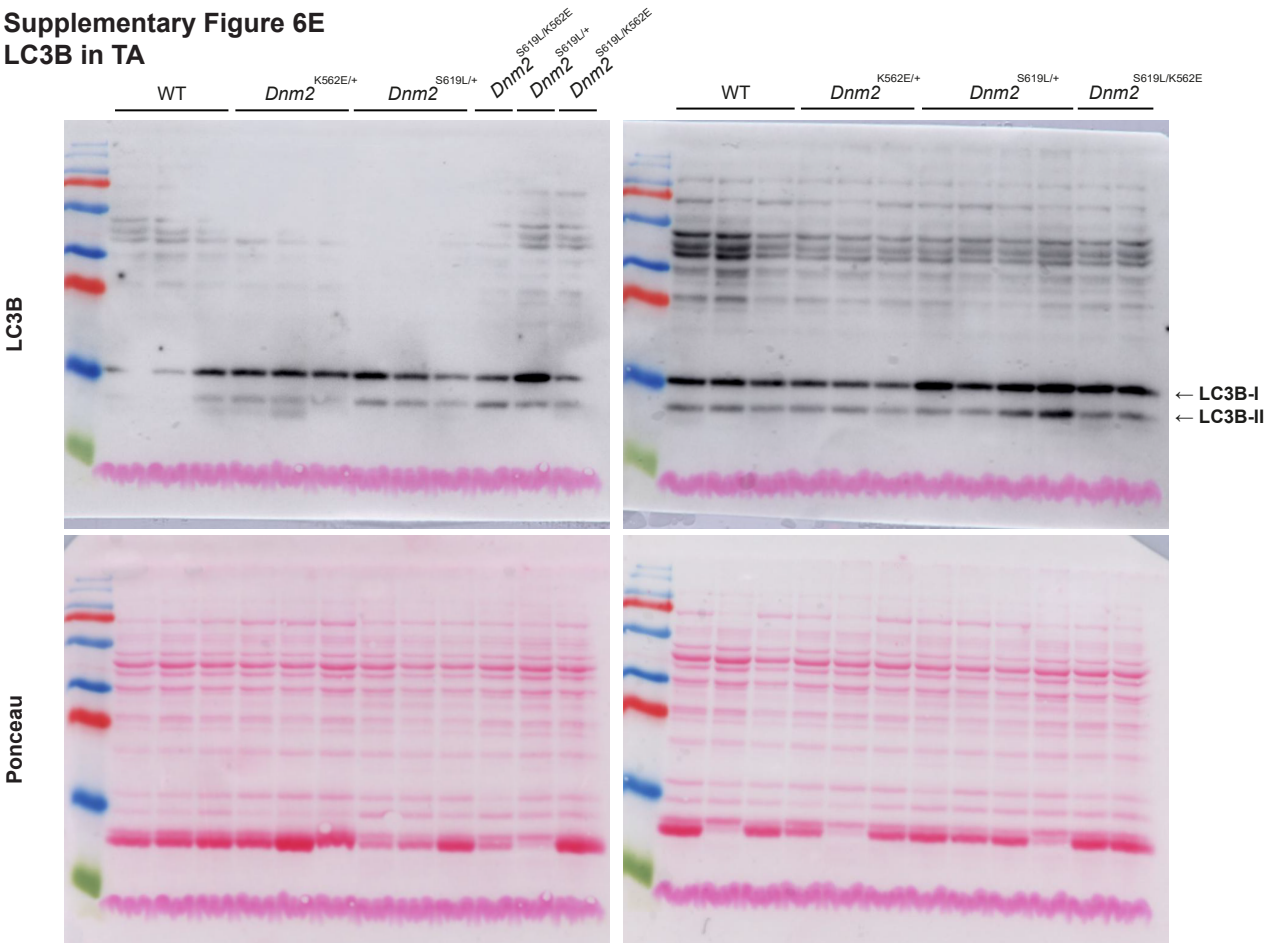
Supplementary Figure 6C
Cullin3 in TA



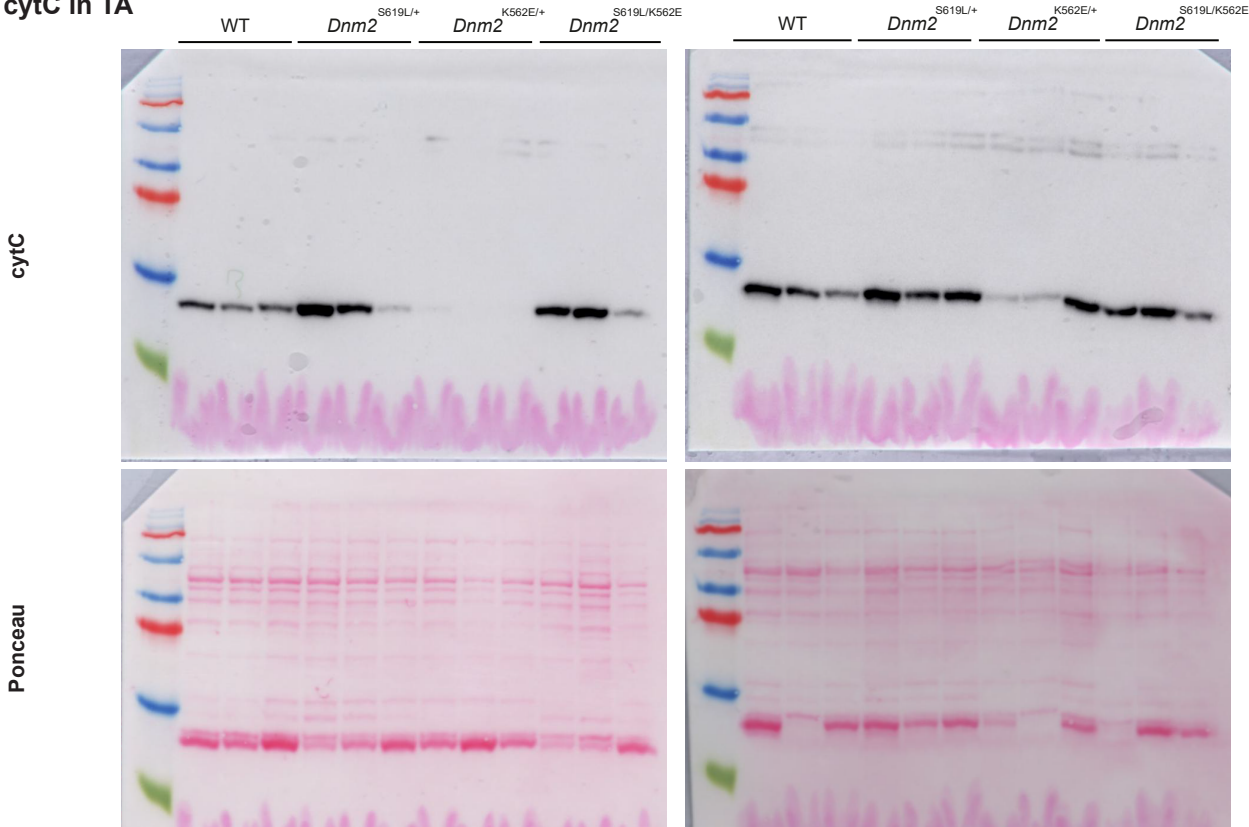
Supplementary Figure 6D
p62 in TA



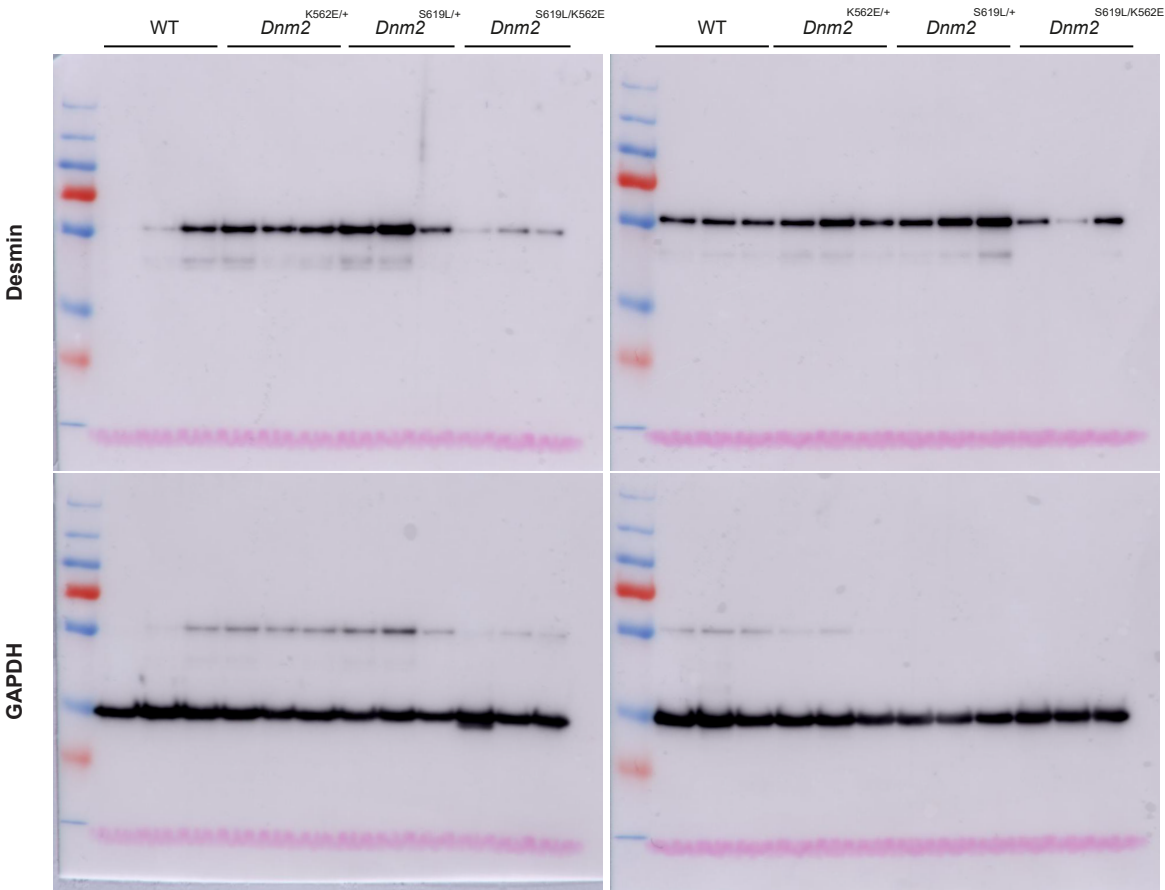
Supplementary Figure 6E
LC3B in TA



Supplementary Figure 7E
cytC in TA



Supplementary Figure 8F
Desmin in TA



Supplementary Figure 12. Uncropped and unprocessed western blot membranes showed in main and supplementary figures. Full membranes with protein ladder.

Reagent/Resource	Reference or Source	Identifier or Catalog Number
Immunofluorescence antibodies (dilution)		
Integrin β 1, rat monoclonal (1:250)	Sigma-Aldrich	MAB1997, lot:3956934
↓ GARat Alexa 488, goat polyclonal (1:250)	Thermo Scientific	A-11006, lot:2480078
Desmin, rabbit polyclonal (1:250)	Abcam	AB15200, lot:1077918-1
↓ GAR Alexa 555, goat polyclonal (1:250)	Invitrogen	A32732, lot:WL332194
Collagen VI, rabbit polyclonal (1 :250)	Novus Biologicals	NB120-6588
↓ GAR Alexa 488, goat polyclonal (1:250)	Thermo Scientific	A-11008, lot:2018309
DAPI (1:1000)	/	/
Western blot antibodies pull-down experiments (dilution)		
DNM2, rabbit polyclonal (1:1000)	Homemade (2865)	N/A
His, mouse monoclonal (1:1000)	Thermo Scientific	MA1-21315-HRP, lot:YK382778
Flag, mouse monoclonal (1:1000)	Thermo Scientific	MA1-91878-HRP, lot:YG377536
GAR perox, goat polyclonal (1:10000)	Jackson ImmunoResearch	111-036-045, lot: 111806
Western blot antibodies on mice tissues (dilution)		
DNM2, rabbit polyclonal (1:1000)	Homemade (2865)	N/A
cytC, rabbit polyclonal (1:1000)	Cell Signaling	#4272
S6RP, rabbit monoclonal (1:1000)	Cell Signaling	#2217, lot:10
p-S6RP, rabbit polyclonal (1:1000)	Cell Signaling	#2211, lot:25
Cullin3, rabbit polyclonal (1:500)	Thermo Scientific	PA5-17397, lot:WG3333458
SQSTM1/ P62, mouse monoclonal (1:1000)	Thermo Scientific	H00008878-M01, lot:I9131-2c11
LC3B, rabbit polyclonal (1:1000)	Cell Signaling	CS2775, lot:14
Cytochrome C, rabbit polyclonal (1:1000)	Cell Signaling	#4272
Desmin, rabbit polyclonal (1:1000)	Abcam	AB15200
GAPDH, mouse monoclonal (1:1000)	Sigma-Aldrich	MAB374, lot:4134614
GAR perox, goat polyclonal (1:10000)	Jackson ImmunoResearch	111-036-045, lot:111806
GAM perox, goat polyclonal (1:10000)	Jackson ImmunoResearch	115-036-068, lot:172700
PCR oligos	5'-sequence-3'	
6115 Er KE	TACACTGTCTGCACTGTCGAGCCCTG	
6116 Ef KE	GCCATCTTCAACACAGAGCAGAGGTG	

9580 Ef SL		CAGAAAGCAGGATCCTCGGTGCC		
9581 Er SL		AGTCCAGCTCTGGCTTTGGATCGC		
Mf KE		AAGGGGACGTTTGAGTAAATGTGGGG		
Mr KE		TGTGCTAGGCAAGACCCTGGCA		
Amplif SL for		TCGTACCCAGATCAGGCAGA		
Amplif SL rev		ACACAGGAACCCCCT		
Mf SL		CCAGAGCCCATGGTCTTAGTGGCC		
qPCR oligos		5'-sequence-3'		
Rsp11 for		CGCGTGGTGAATAAGGAAGC		
Rsp11 rev		GTAAGCACGCTCCGTCTGAA		
Dnm2 for		ACCCACACTTGCAGAAAAC		
Dnm2 rev		CGCTTCTCAAAGTCCACTCC		
Chrna for		CCACAGACTCAGGGGAGAAG		
Chrna rev		AACGGTGGTGTGTGTTGATG		
Chrng for		GAGAGCCACCTCGAAGACAC		
Chrng rev		GACCAACCTCATCTCCCTGA		
Chrne for		CAATGCCAATCCAGACACTG		
Chrne rev		CCCTGCTTCTCCTGACACTC		
ddPCR				
Assay code	Target	Forward primer	Reverse primer	Probe
Probe K562K	Dnm2 Exon 15 to 16 wild-type K562	GTGCTGA CAGCTGA GTCATT	TGTTGGA CATGAAG CCCTTT	ZEN™ Double-Quenched probe** HEX fluorochrome AAAGAAGTACATGCTGCCACTGG A
Probe K562E	Dnm2 Exon 15 to 16 mutation K562E	GTGCTGA CAGCTGA GTCATT	TGTTGGA CATGAAG CCCTTT	ZEN™ Double-Quenched probe** FAM fluorochrome AAAGGAGTACATGCTGCCACTGG A
Probe S619S	Dnm2 Exon 17 to 18 wild-type S619	CTTGTGA CTCCCAG GAAGAT	GTGCTCC ATCCTCA TTCTCT	ZEN™ Double-Quenched probe** HEX fluorochrome TGGAAGGCTCTGTTCTCTGCG
Probe S619L	Dnm2 Exon 17 to 18 mutation S619L	CTTGTGA CTCCCAG GAAGAT	GTGCTCC ATCCTCA TTCTCT	ZEN™ Double-Quenched probe** FAM fluorochrome TGGAAGGCTTCGTTCTCTGCG
RNA reference assay	Hprt	CCCCAAA ATGGTTA AGGTTGC	AACAAAG TCTGGCC TGTATCC	ZEN™ Double-Quenched probe** FAM and/or HEX fluorochrome CTTGCTGGTGAAAAGGACCTCTC GAA

Supplementary Table 1. Reagents used. Antibodies used for immunofluorescences and western blots. Primers used for PCR, qRT-PCR and ddPCR.