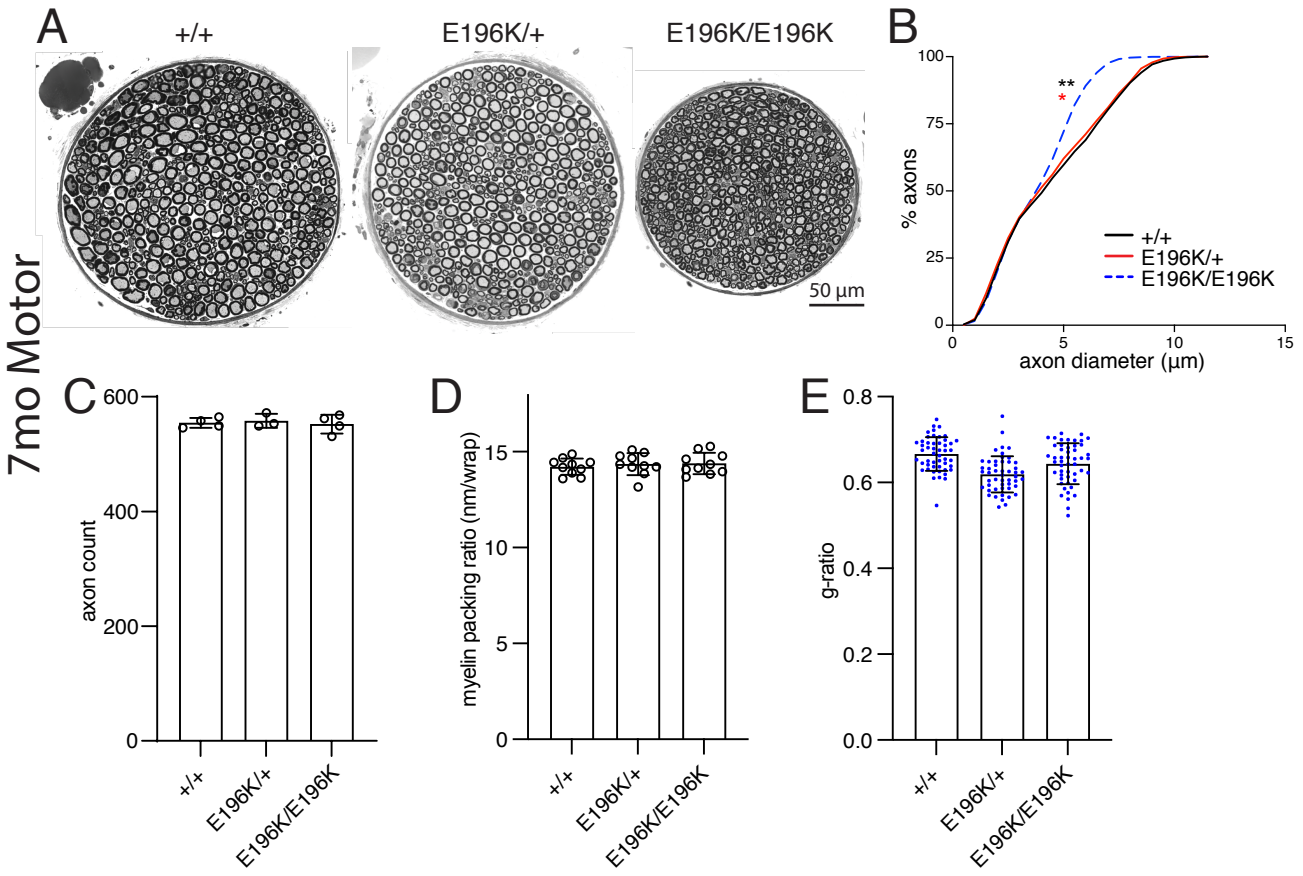
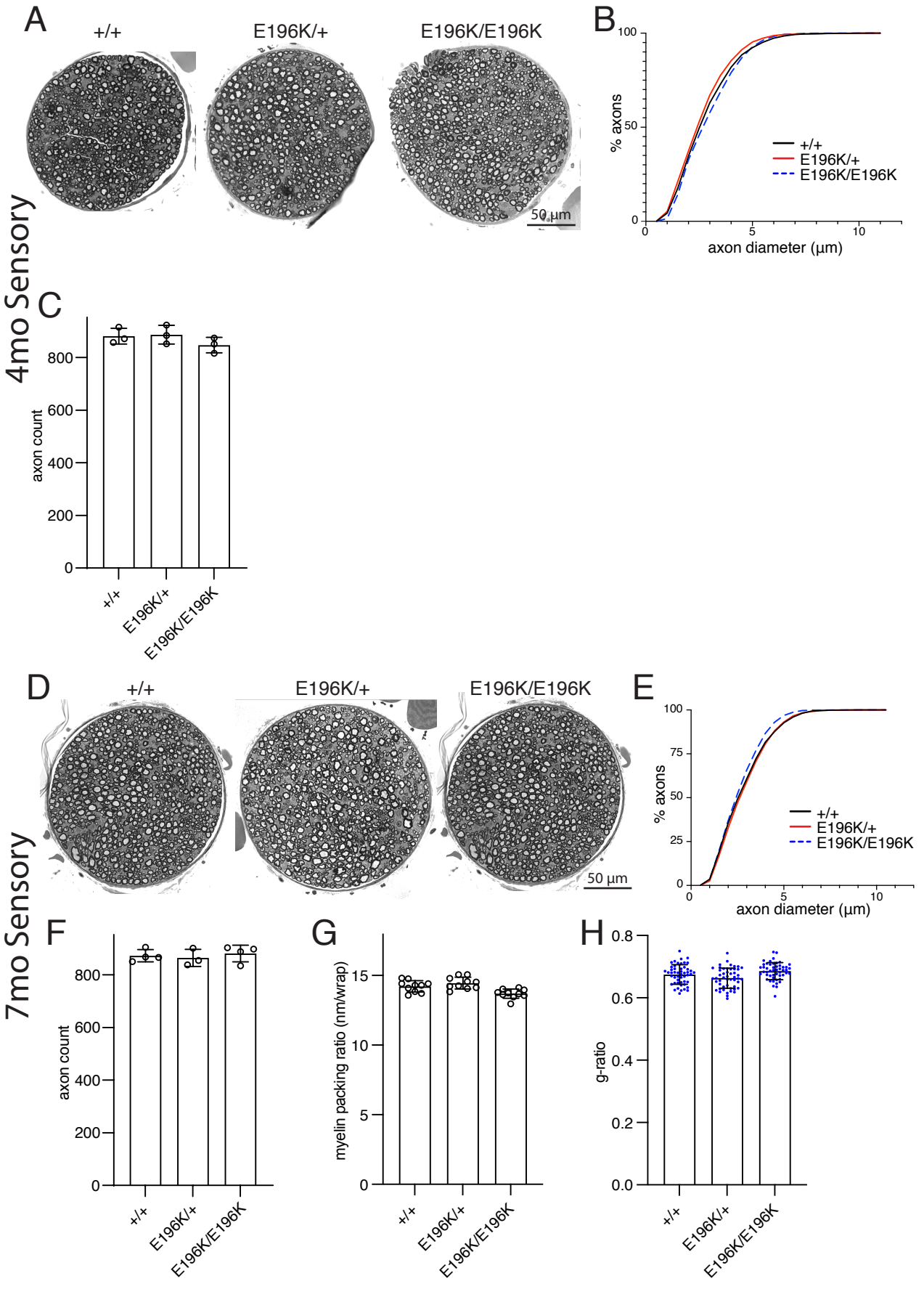
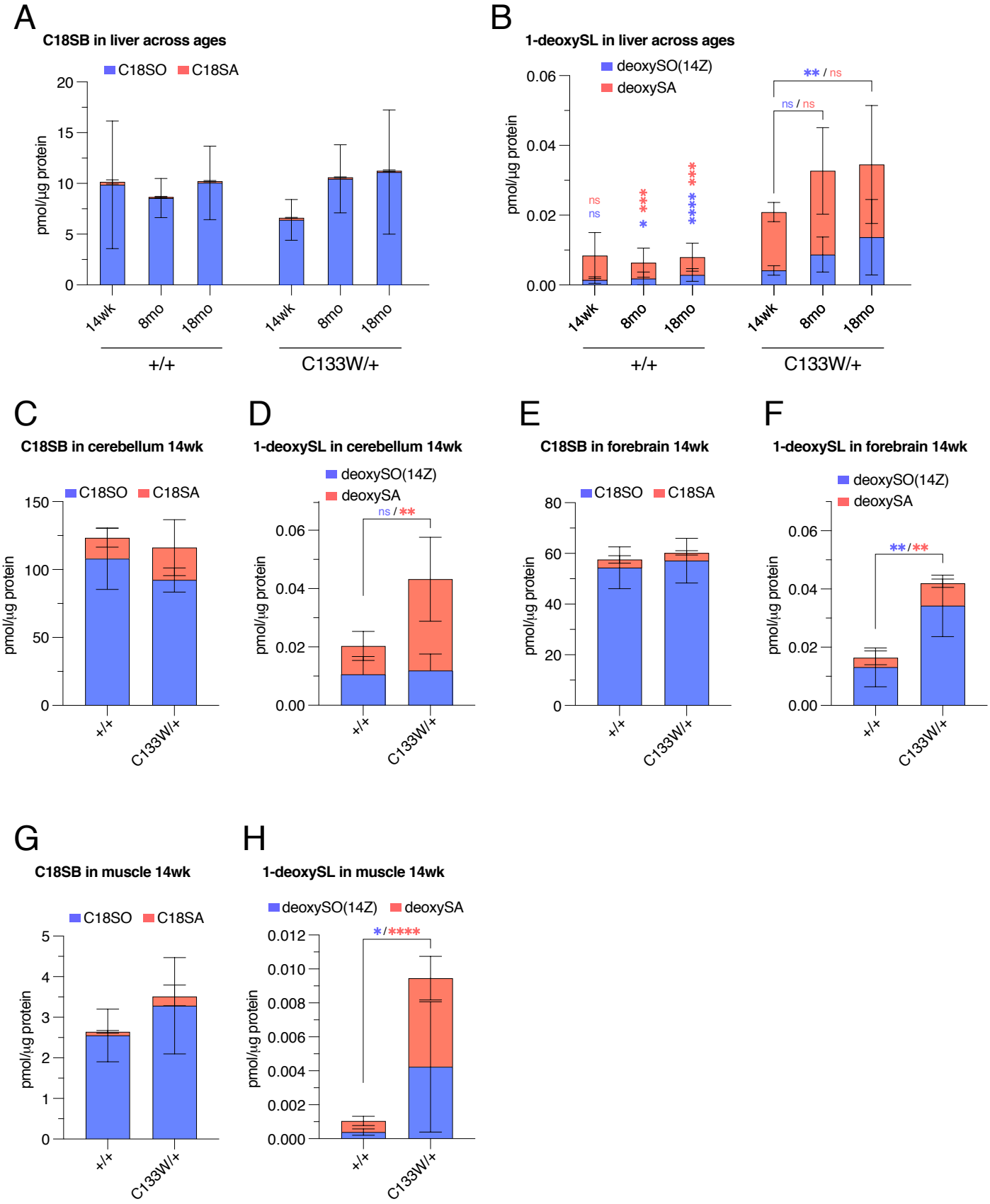

A
+/+
E196K/+
E196K/E196K
50 µm
7mo Motor
B
100
75
50
25
0
% axons
**
*
+/+
E196K/+
E196K/E196K
0
5
10
15
axon diameter (µm)
C
600
400
200
0
axon count
+/+
E196K/+
E196K/E196K
D
15
10
5
0
myelin packing ratio (nm/wrap)
+/+
E196K/+
E196K/E196K
E
0.8
0.6
0.4
0.2
0.0
g-ratio
+/+
E196K/+
E196K/E196K

Supplemental figure 1: *Yars*^{E196K} femoral motor nerves at 7-months-of-age. (A) 40X images of femoral motor nerves taken at 7 months of age from WT, *Yars*$^{E196K/+}$ and *Yars*$^{E196K/E196K}$ mice. (B) Cumulative histogram of axon areas demonstrates that homozygous mutant mice have smaller axons. (C) Myelinated axon number remains unchanged across genotypes at 7months of age. (D) Myelin packing is unchanged across genotypes. (E) The g-ratio remains unchanged across genotypes. N=4 WT, 3 heterozygous and 4 homozygous mice, data points in C are individual mice, values are mean±SD. Data were analyzed by nested one-way ANOVA (B, D, E) or by one way ANOVA (C) with Tukey's multiple comparisons. Asterisks in B denote comparison between WT and *Yars*$^{E196K/E196K}$ (black) or *Yars*$^{E196K/+}$ and *Yars*$^{E196K/E196K}$ (red). *$p<0.05$, **$p<0.01$.

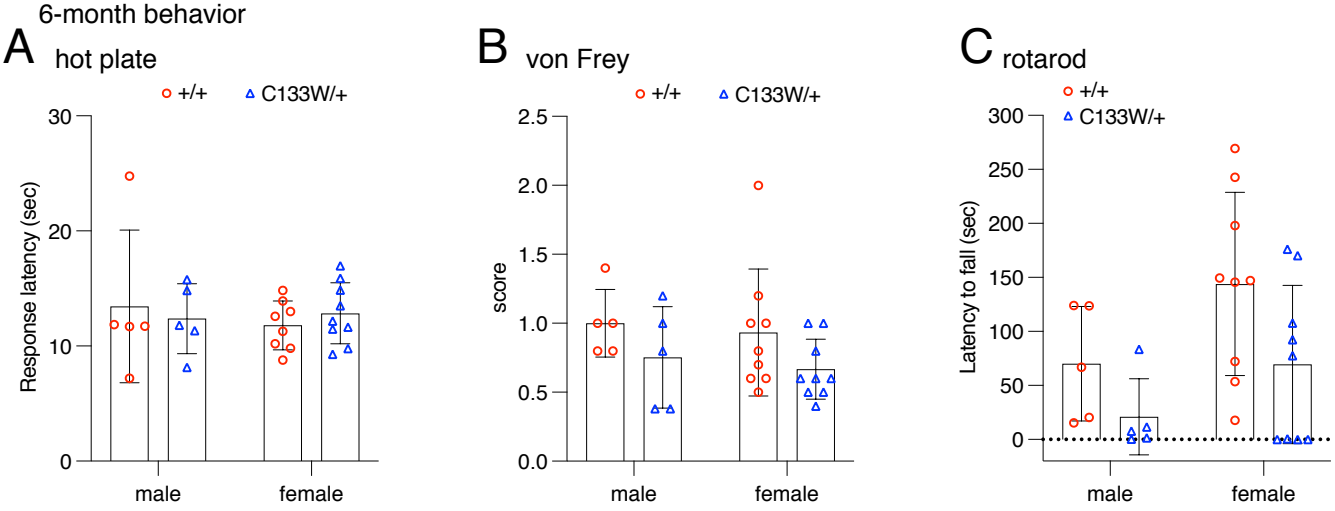

A
+/+
E196K/+
E196K/E196K
50 µm
B
% axons
axon diameter (µm)
+/+
E196K/+
E196K/E196K
4mo Sensory
C
axon count
+/+
E196K/+
E196K/E196K
D
+/+
E196K/+
E196K/E196K
50 µm
E
% axons
axon diameter (µm)
+/+
E196K/+
E196K/E196K
7mo Sensory
F
axon count
+/+
E196K/+
E196K/E196K
G
myelin packing ratio (nm/wrap)
+/+
E196K/+
E196K/E196K
H
g-ratio
+/+
E196K/+
E196K/E196K

Supplemental figure 2: *Yars*^{E196K} femoral sensory nerves at 4- and 7-months-of-age. (A) 40X images of femoral sensory nerve branches from 4-month-old WT, *Yars*$^{E196K/+}$ and *Yars*$^{E196K/E196K}$ mice. At 4-months-of-age there are no differences across genotypes in axon diameter (B) or axon number (C). N=3 mice per genotype. (D) 40X images of femoral sensory nerve branches from 7-month-old WT, *Yars*$^{E196K/+}$ and *Yars*$^{E196K/E196K}$ mice. At 7-months-of-age there are no differences across genotypes in axon diameter (E), axon number (F), myelin packing (G), or g-ratio (H). N=4 WT, 3 heterozygous, and 4 homozygous mice. Values are mean ± SD except B and E, which are cumulative histograms showing the distribution of axon sizes in each genotype. Data were analyzed by one-way ANOVA (C,F) or nested one-way ANOVA (B, E, G, and H) with Tukey's multiple comparisons test.

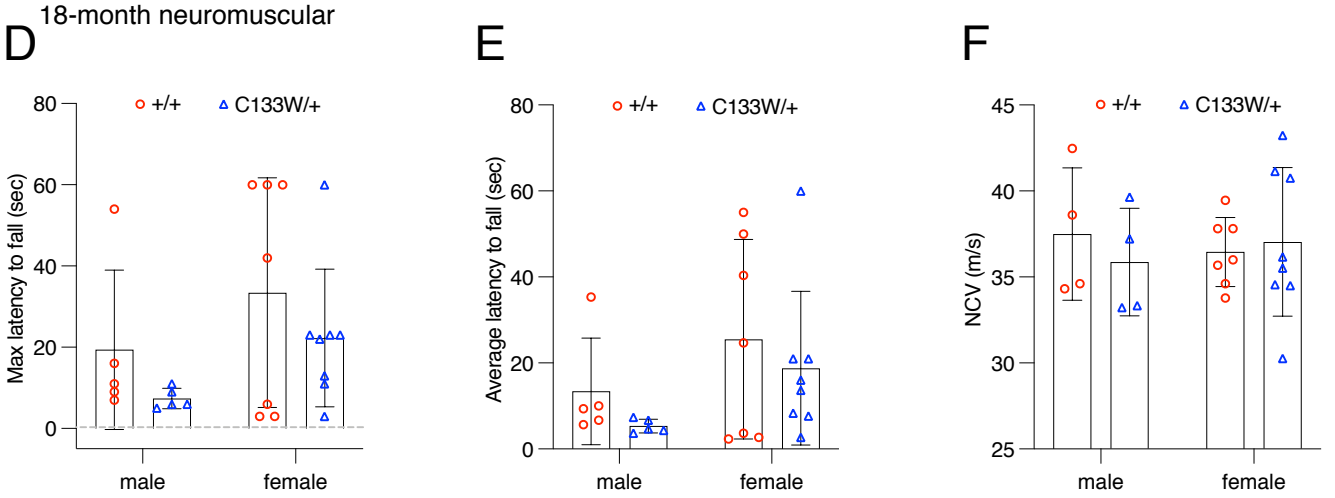

A
C18SB in liver across ages
C18SO
C18SA
pmol/µg protein
20
15
10
5
0
14wk
8mo
18mo
14wk
8mo
18mo
+/+
C133W/+
B
1-deoxySL in liver across ages
deoxySO(14Z)
deoxySA
pmol/µg protein
0.06
0.04
0.02
0.00
ns
ns
**** / ns
ns / ns
14wk
8mo
18mo
14wk
8mo
18mo
+/+
C133W/+
C
C18SB in cerebellum 14wk
C18SO
C18SA
pmol/µg protein
150
100
50
0
+/+
C133W/+
D
1-deoxySL in cerebellum 14wk
deoxySO(14Z)
deoxySA
ns / **
pmol/µg protein
0.06
0.04
0.02
0.00
+/+
C133W/+
E
C18SB in forebrain 14wk
C18SO
C18SA
pmol/µg protein
80
60
40
20
0
+/+
C133W/+
F
1-deoxySL in forebrain 14wk
deoxySO(14Z)
deoxySA
** / **
pmol/µg protein
0.06
0.04
0.02
0.00
+/+
C133W/+
G
C18SB in muscle 14wk
C18SO
C18SA
pmol/µg protein
5
4
3
2
1
0
+/+
C133W/+
H
1-deoxySL in muscle 14wk
deoxySO(14Z)
deoxySA
* / ****
pmol/µg protein
0.012
0.010
0.008
0.006
0.004
0.002
0.000
+/+
C133W/+

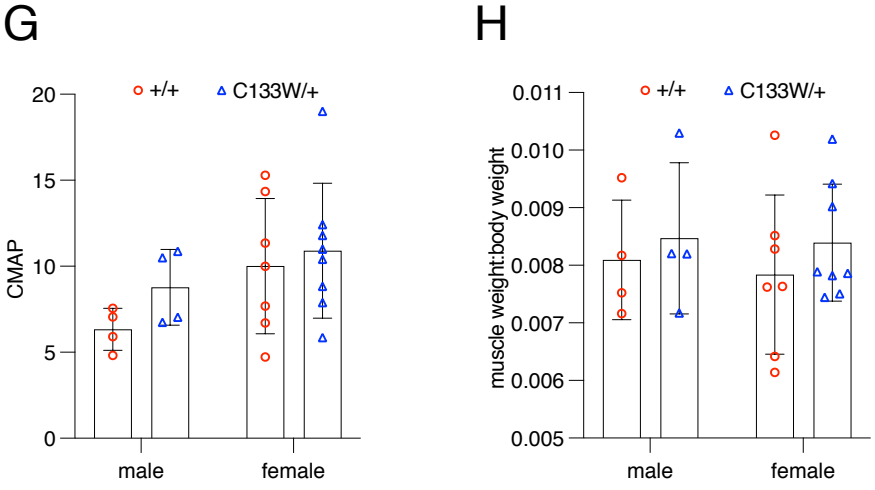
Supplemental figure 3: Canonical and 1-deoxySL levels in additional tissues. (A) Canonical C18SO and C18SA levels were consistent across age and genotype in liver. (B) Levels of deoxySLs were elevated in *Sptlc1*$^{C133W/+}$ liver at 8 and 18 months-of-age, and deoxySO(14Z) rose with age in the mutant samples. At 14-weeks-of-age, canonical C18SO and C18SA levels were unchanged in *Sptlc1*$^{C133W/+}$ cerebellum (C), forebrain (E), or skeletal muscle (G). Levels of deoxySA were elevated in mutant cerebellum (D), whereas both deoxySA and deoxySO(14Z) were increased in forebrain (F) and muscle (H). N=6 mice per genotype at 14-weeks of age for all tissues, 10 mice per genotype were analyzed at 8-months, and 12 mice per genotype were analyzed at 18-months. Values were compared within each metabolite by ordinary two-way ANOVA with Sidak's multiple comparisons test (A,B) or unpaired, two-tailed t-test (C-H). * $p<0.05$, ** $p<0.01$, ***$p<0.001$, ****$p<0.0001$. Vertical asterisks over WT data (B) denote difference compared to C133W mutants at the same age. Asterisks are color coded to match each metabolite. Stacked bar graphs show mean ± S.D.

6-month behavior



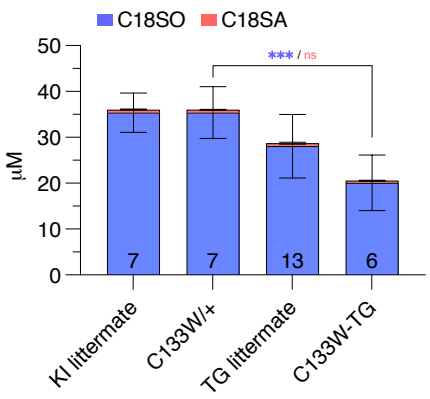
18-month neuromuscular



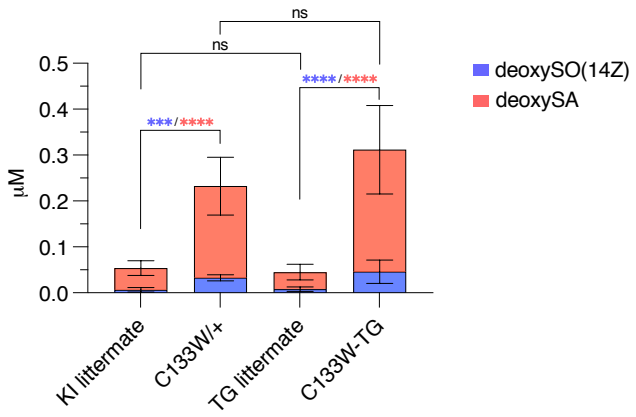


Supplemental figure 4: Phenotyping of *Sptlc1*$^{C133W/+}$ mice at 6- and 18-months-of-age. (A_C) At six-months-of-age, *Sptlc1*$^{C133W/+}$ mice did not show changes in thermal nociception (A), mechanical sensitivity (B), or performance on the accelerating rotarod (C). Behavior and physiology in 18mo *Sptlc1*$^{C133W/+}$ mice. *Sptlc1*$^{C133W/+}$ mice show no late-onset differences in motor or sensory function at 18 months of age compared to WT littermates. (D-E) *Sptlc1* $^{C133W/+}$ mice performed similarly to littermate controls on the wire hang test of grip strength and endurance, maximum (D) or average (E) latency to fall in three trials is shown. (F-H) No differences were observed in sciatic motor nerve conduction velocity (F), compound muscle action potential amplitude (G), or muscle weight to body weight ratio (H). Data points indicate individual mice and were analyzed within each sex by unpaired, two-tailed t-test, bars represent mean ± S.D.

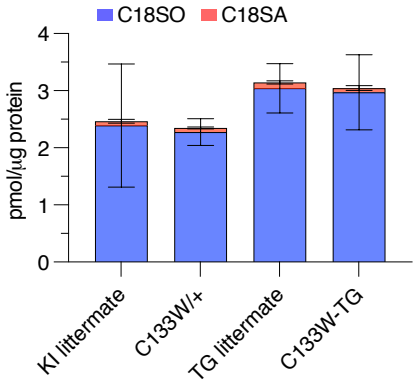
A


C18SB in plasma KI vs TG
C18SO
C18SA
*** / ns
µM
50
40
30
20
10
0
7
7
13
6
KI littermate
C133W/+
TG littermate
C133W-TG

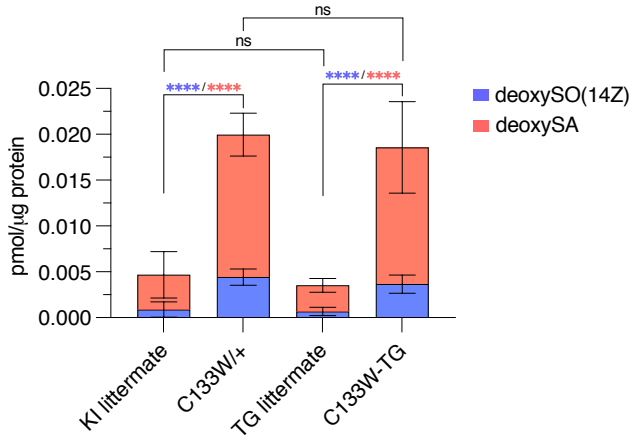
B


1-deoxySL in plasma KI vs TG
ns
ns
/*
****/****
µM
0.5
0.4
0.3
0.2
0.1
0.0
deoxySO(14Z)
deoxySA
KI littermate
C133W/+
TG littermate
C133W-TG

C


C18SB in liver KI vs TG
C18SO
C18SA
pmol/µg protein
4
3
2
1
0
KI littermate
C133W/+
TG littermate
C133W-TG

D


1-deoxySL in liver KI vs TG
ns
ns
****/****
****/****
pmol/µg protein
0.025
0.020
0.015
0.010
0.005
0.000
deoxySO(14Z)
deoxySA
KI littermate
C133W/+
TG littermate
C133W-TG

Supplemental figure 5: Canonical and 1-deoxySL levels in *Sptlc1* $^{C133W/+}$ knockin compared to *Sptlc1-C133W* transgenic mice. (A) Canonical C18SO and C18SA levels in plasma from knockin (KI) and transgenic (TG) mice, as well as control littermates for each. C18SO levels were decreased in the transgenic compared to the knockin, but did not differ significantly from control littermates. (B) Levels of deoxySLs were elevated in plasma from both knockin and transgenic mice compared to their control littermates, but there was no significant difference between the mutant groups. (C) Canonical C18SO and C18SA levels in liver were similar among all genotypes. (D) Levels of deoxySLs were elevated in liver from both knockin and transgenic mice compared to their control littermates, but there was no significant difference between the mutant groups. N is between 6-13 per group and is indicated on the bars in panel A. Samples were collected from adult mice (both sexes, 20-45 weeks of age). Values were compared within each metabolite by ordinary one-way ANOVA with Tukey's multiple comparisons test. ***$p<0.001$, ****$p<0.0001$. Asterisks are color coded to match each metabolite. Stacked bar graphs show mean ± S.D.