Supplementary Figure 1. Gene modification design

A Wildtype sequence (**TAO kinase 2** Exon 2):

TACCTACCTTTCCTCAGCAGTACCTCCCCCTCATCAGTAAATGAGGGGAGGAGGAGGTAGAAACCTGAGGGA
GGACCTCTCTCTCCCTCTGGGCCCTATCTTAGCTCTAAGGGTCCTATGTCCTTTTTCCAGGCAAGATCCCAATC
TCAGGGCCCCCTGGGGCCATCATGCCAGCTGGGGGCCGGGCCGGGAGCCTGAAGGACCCTGATGTGGCT
GAGCTCTTCTTCAAGGATGACCCTGAGAAGCTCTTCTCTGACCTCCGGGAGATCGGCCATGGCAGCTTTGG
AGCAGTGTACTTTGTGAGTTGGGTCTTGAAAAGGGTAAAGCAGGGCTCAGTCTCTTTCAACCTGTGGGTCT
CCAGGCCTCTGCACCACTCCACCAAAATAATCCTTCCCACCCTCTTCTAATAGCTCAGCGGGTCCTCTTTCACC
CCATGCCCAAGGTGGTCTTTTCCATCCTCCAATCTGGTCCTCTAGGCCCGGGATGTCCGGAACAGTGAGGTG
GTGGCCATCAAGAAGATGTCCTATAGTGGGAAGCAATCAAATGAGGTGAGTCAGGTTGATTAACATCAGGTT
GTGGAGGG

Mutant Sequence:

TACCTACCTTTCCTCAGCAGTACCTCCCCCTCATCAGTAAATGAGGGGAGGAGGAGGTAGAAACCTGAGGGA
GGACCTCTCTCTCCCTCTGGGCCCTATCTTAGCTCTAAGGGTCCTATGTCCTTTTTCCAGGCAAGATCCCAATC
TCAGGGCCCCCTGGGGCCATCATGCCAGCTGGGGGCCGGGCCGGGAGCTCAGCCACTxxxxxxxxxxCTGA
GCTCTTCTTCAAGGATGACCCTGAGAAGCTCTTCTCTGACCTCCGGGAGATCGGCCATGGCAGCTTTGGAG
CAGTGTACTTTGTGAGTTGGGTCTTGAAAAGGGTAAAGCAGGGCTCAGTCTCTTTCAACCTGTGGGTCTCCA
GGCCTCTGCACCACTCCACCAAAATAATCCTTCCCACCCTCTTCTAATAGCTCAGCGGGTCCTCTTTCACCCCAT
GCCCAAGGTGGTCTTTTCCATCCTCCAATCTGGTCCTCTAGGCCCGGGATGTCCGGAACAGTGAGGTGGTGG
CCATCAAGAAGATGTCCTATAGTGGGAAGCAATCAAATGAGGTGAGTCAGGTTGATTAACATCAGGTTGTGG
AGGG

TCAGCCACT – inversion of part of the deleted sequence
xxxxxxxxxx – 10 bp deletion = out of frame mutation; premature stop in exon 4

B Wildtype sequence (**seizure related 6 homolog like 2 (Sez6l2)** Exon 2 **ATG in Exon 1**):

AGGATGTAGAGGAATGGAGAGGTTCACAGGACTTCACCTCCTAGGTCTGCCCCTGAAGGAGGATGAGATG
ATGCCAGAGCCTGGAAGTGAGACTCCCACAGTGGCCTCTGAGGACCTGGCTGAGCTGCTCCATGGGGCTT
TGCTGCGGAAGGGCCCAGAGATCGGCTTCTTGCCGGGTGAGGCCCACAGTGTGGCATAGGAGTAGAGAG
AGAGGCTATGTCGCTGAGAGCTGGAGTGCCTGGCTAGAGGGAAGGCGGGTTAGAGAGAGTTCTGTGGGA
GAGACCCCCTAGGAAGCTGAGAAAGAGTCAAAGCTGGCC

Mutant Sequence:

AGGATGTAGAGGAATGGAGAGGTTCACAGGACTTCACCTCCTAGGTCTGCCCCTGAAGGAGGATGAGATG
ATGCCAGAGCCTGGAAGTGAGACTCCCACAGTGGCCTCTGAGGACCTGGCTGAGCTGCTCCATGGGG::::::
::::::::::CCAGAGATCGGCTTCTTGCCGGGTGAGGCCCACAGTGTGGCATAGGAGTAGAGAGAGAGGCTATG
TCGCTGAGAGCTGGAGTGCCTGGCTAGAGGGAAGGCGGGTTAGAGAGAGTTCTGTGGGAGAGACCCCCTA
GGAAGCTGAGAAAGAGTCAAAGCTGGCC
:::::::::::::::: **– 17 bp deletion = out of frame mutation; premature stop in exon 3**

C

Wildtype sequence (**major vault protein (Mvp)** Exon 2; ***Exon 3;*** **ATG in Exon 1**):

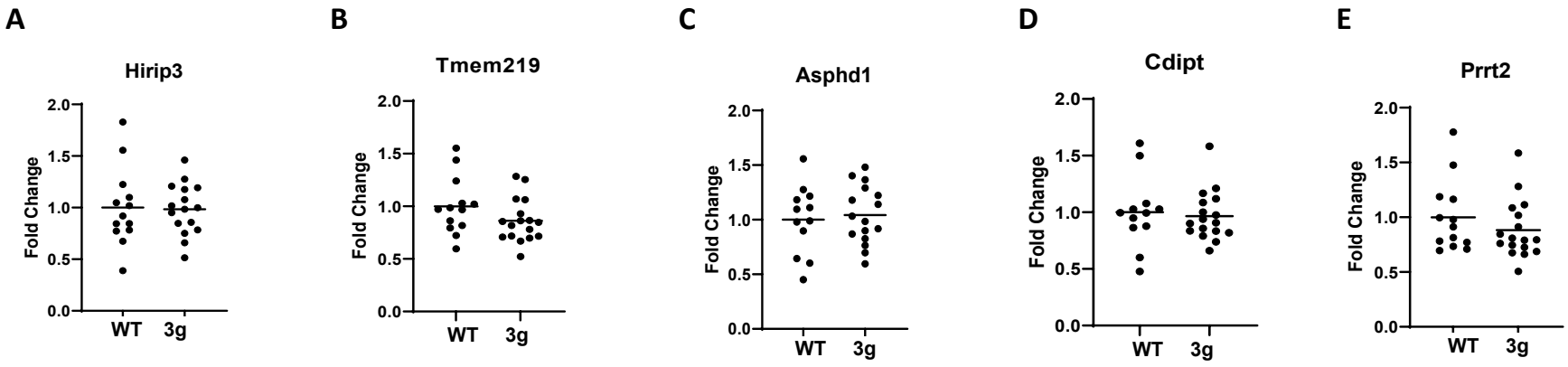
TCACCATGGCAACTGAAGAGGCCATCATCCGCATCCCCCCATACCACTACATCCATGTGCTGGACCAGAACA
GTAATGTGTCCCGTGTAGAGGTTGGACCAAAGACCTAC**ATC**CGGCAGGACAATGAGAGGTTGGTGTAGAG
CTGTCCCAGCCTGGCTGGTGGGAATGACCCTCATCTGGGTGGCCGGGAGGTTTCTCTTGCTTTTACTGTCTCC
TTTGGAACATCATCCTGGCTCCTCACGCCCCTTCTTATCTTACAACAG***GGTACTGTTTGCCCCAGTTCGCATGG***
TGACGGTCCCACCACGCCACTACTGCATAGTGGCCAACCCTGTGTCCCGGGACGCCCAGAGTTCTGTGTTGT
TTGACGTCAC

Mutant Sequence:

TCACCATGGCAACTGAAGAGGCCATCATCCGCATCCCCCCATACCACTACATCCATGTGCTGGACCAGAACA
GTAATGTGTCCCGTGTAGAGGTTGGACCAAAGACCTAC**T****ATC**CGGCAGGACAA**TGA**GAGGTTGGTGTAGA
GCTGTCCCAGCCTGGCTGGTGGGAATGACCCTCATCTGGGTGGCCGGGAGGTTTCTCTTGCTTTTACTGTCT
CCTTTGGAACATCATCCTGGCTCCTCACGCCCCTTCTTATCTTACAACAG***GGTACTGTTTGCCCCAGTTCGCAT***
GGTGACGGTCCCACCACGCCACTACTGCATAGTGGCCAACCCTGTGTCCCGGGACGCCCAGAGTTCTGTGTT
GTTTGACGTCAC

T – 1 bp insertion = out of frame mutation; premature stop (TGA) in exon 2

Supplementary Figure 2. Expression of genes adjacent to those modified in 3g del/+ mice



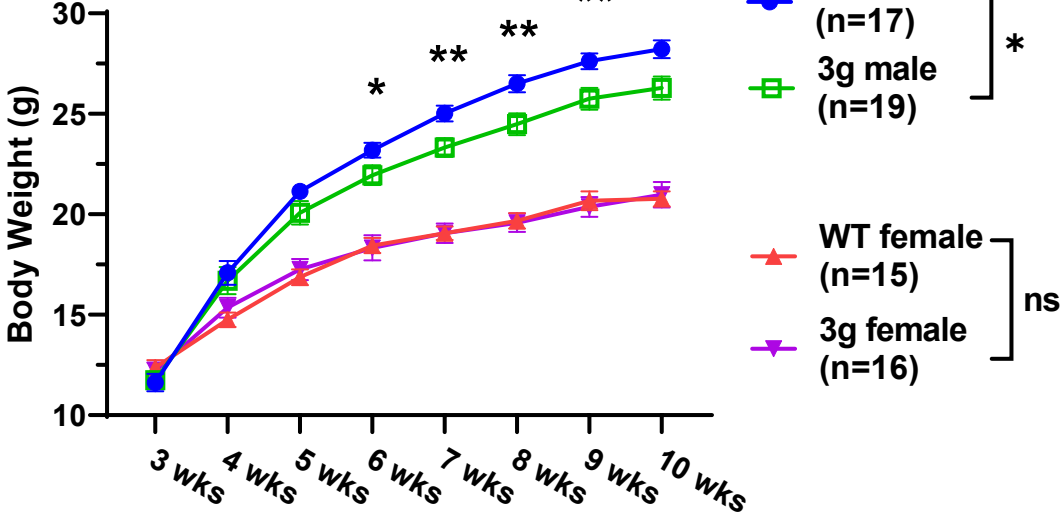
Supplementary Figure 3. 3g del/+ mice are obtained at a Mendelian frequency and male 3g del/+ mice show a decrease in body weight

A

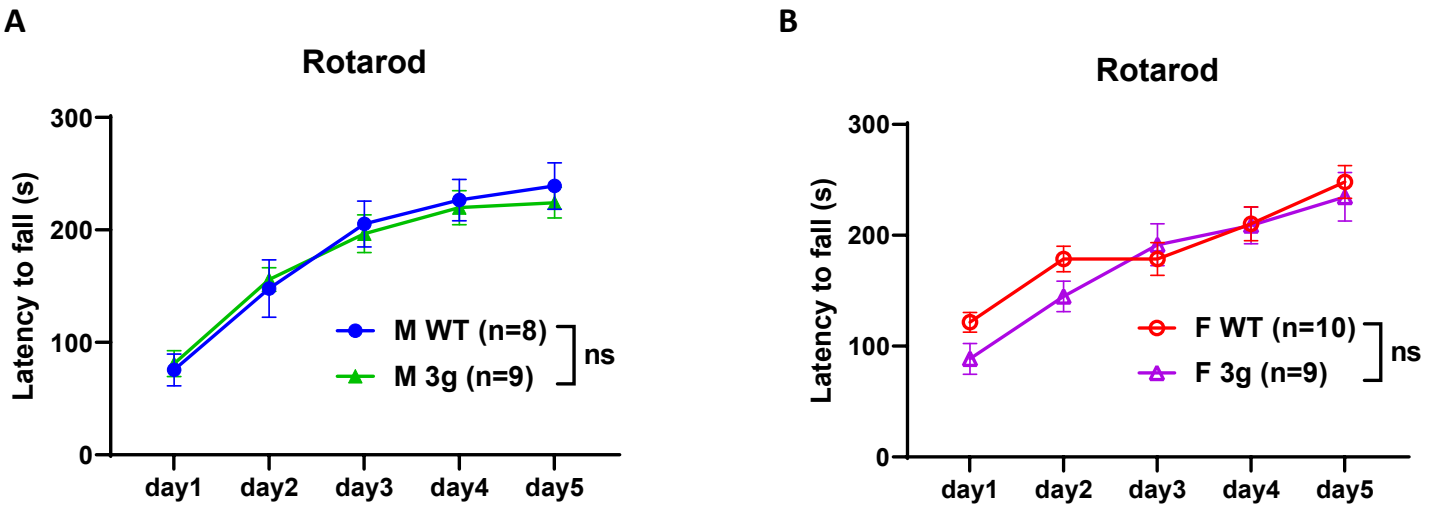
3g del/+ male mouse x wt female mouse

	WT (number of animals)	3g del/+ (number of animals)	3g del/+ inheritance (%)
Male	62	58	48.33
Female	47	44	48.35

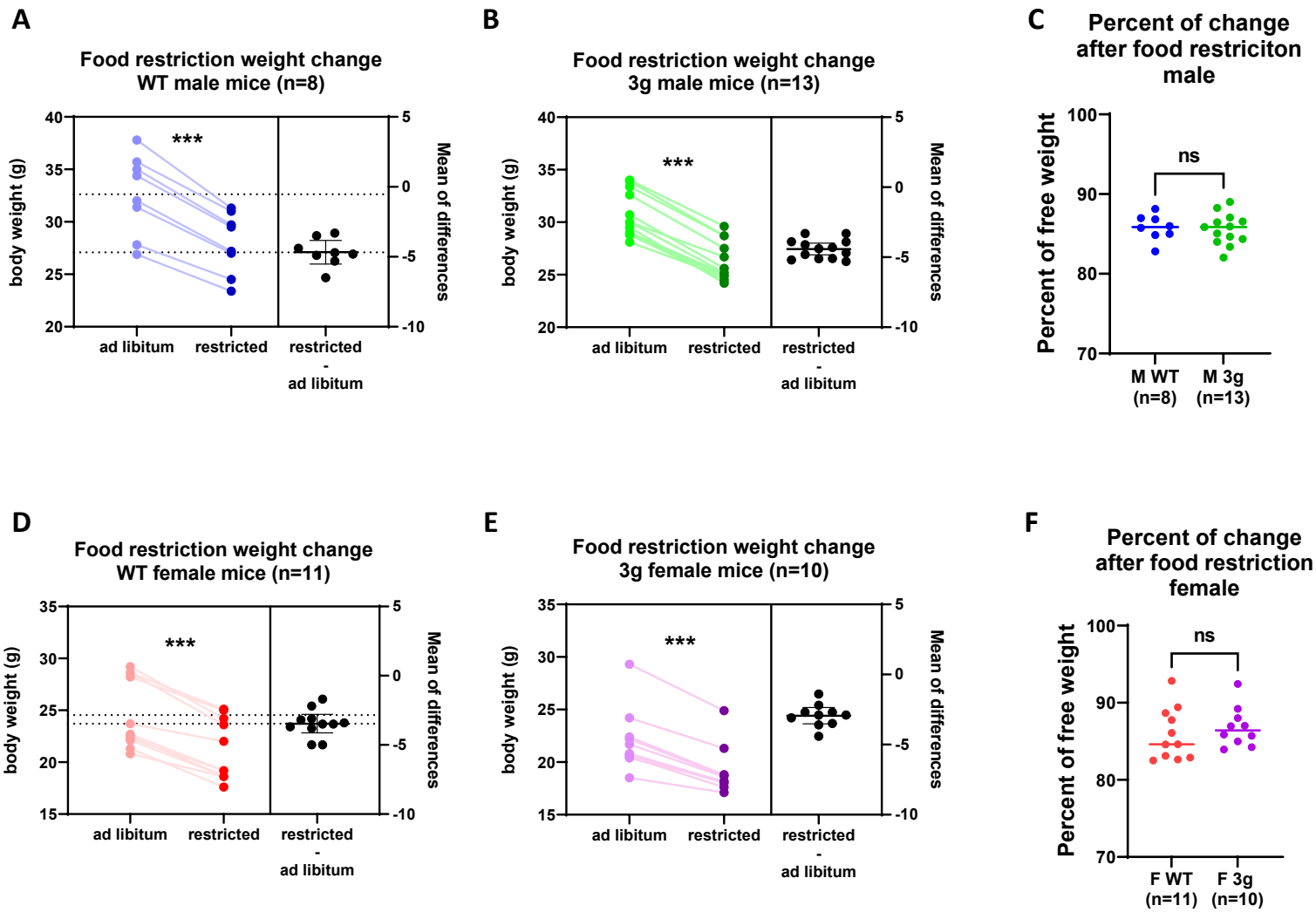
B



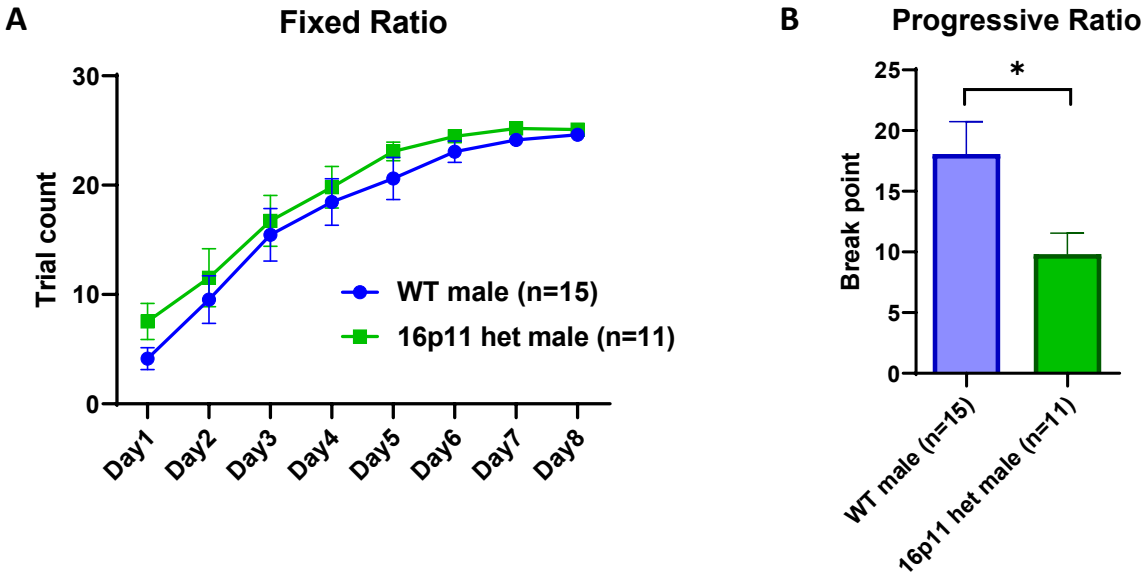
Supplementary Figure 4. Rotarod test did not show any difference between 3g del/+ mice and wt mice



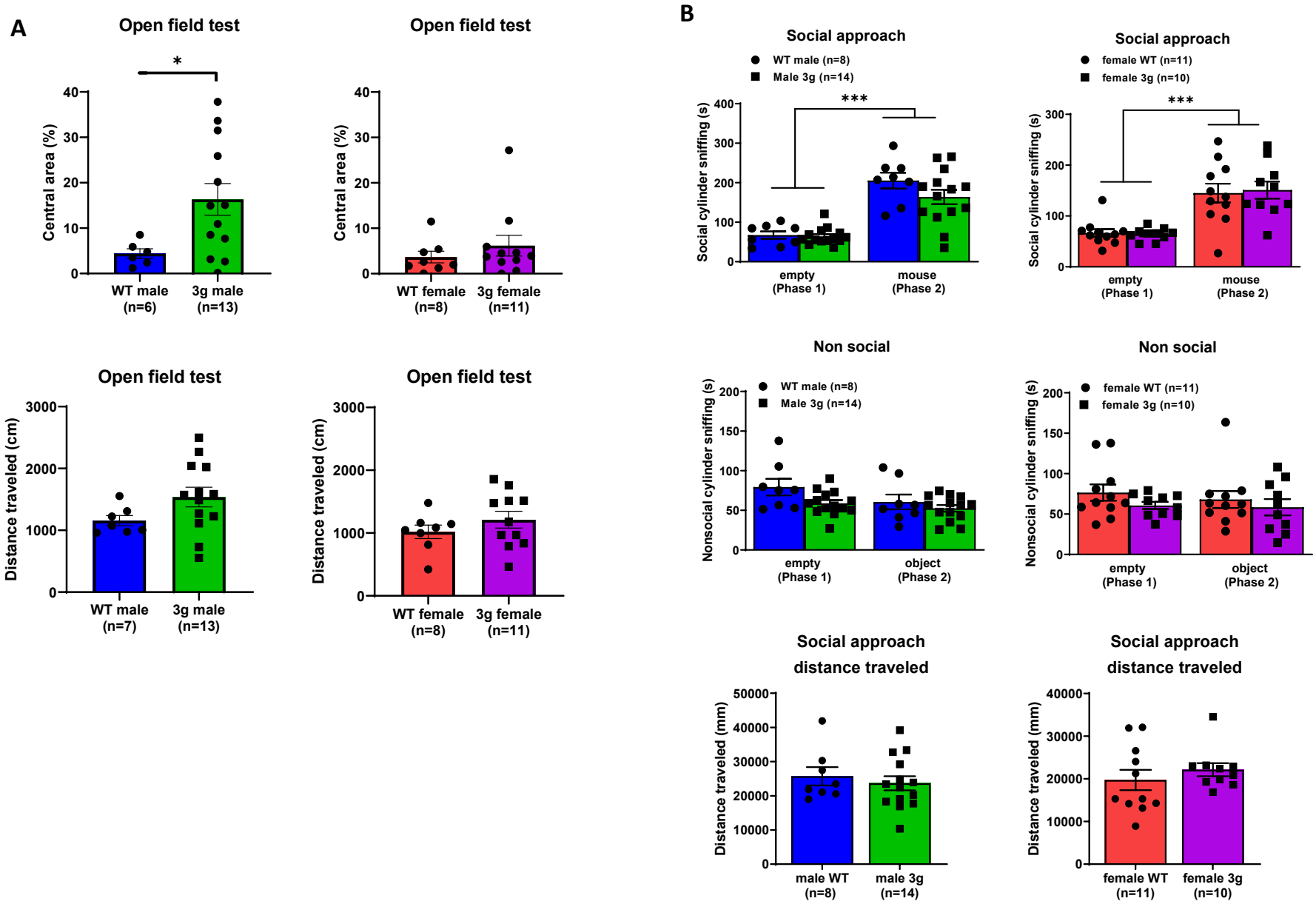
Supplementary Figure 5. Body weight during food restriction



Supplementary Figure 6. 16p11.2 del/+ male mice show decreased break points in progressive ratio compared to sex- and age- matched wt mice

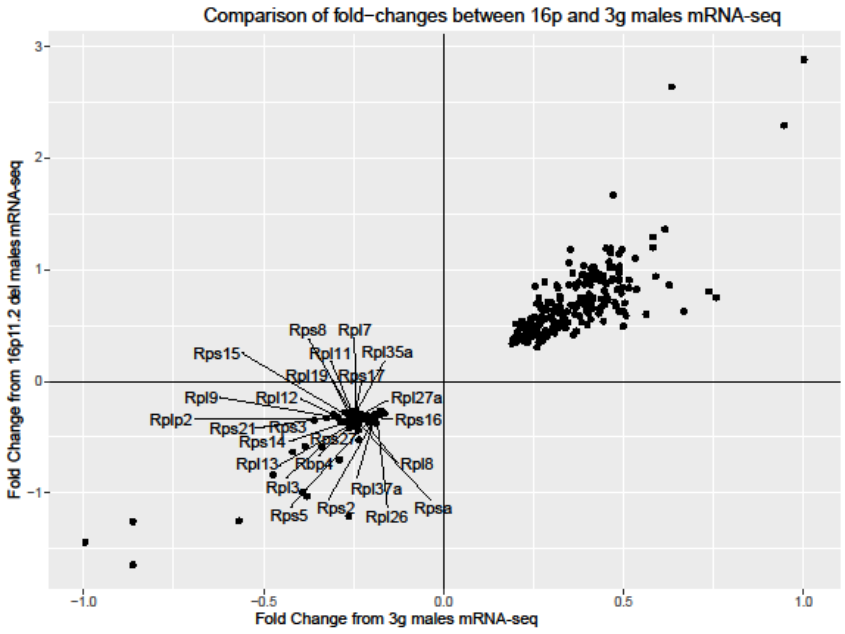


Supplementary Figure 7. Additional behavioral phenotypes of 3g del/+ mice



Supplementary Figure 8. Labeling ribosomal genes in 248 DEGs and overlapped genes with SFARI genes

A



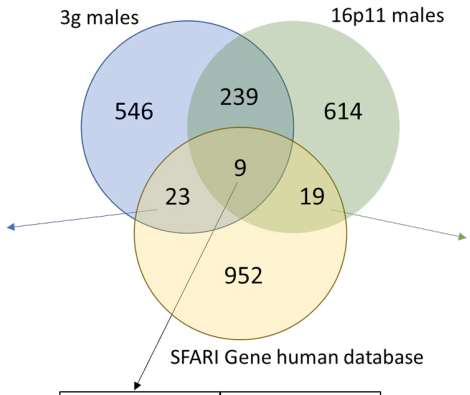
B

Syndromic:
KAT6A
NTNG1

High Confidence:
KMT2E
TEK
SON
AP2S1

Strong Candidate:
CEP135
GRIK2
UBR5
WWOX
LRP1
CPEB4
CHMP1A
BTAF1

Suggestive Evidence:
REEP3
PTPRB
MEGF10
RIMS3
LRFN2
RAPGEF4
COL28A1
SF3B1
THRA



High Confidence:
DMPK

Strong Candidate:
UBN2
CTTNBP2
TET2

Suggestive Evidence:
UIMC1
PRPF39
DUSP15
GPX1
MSANTD2

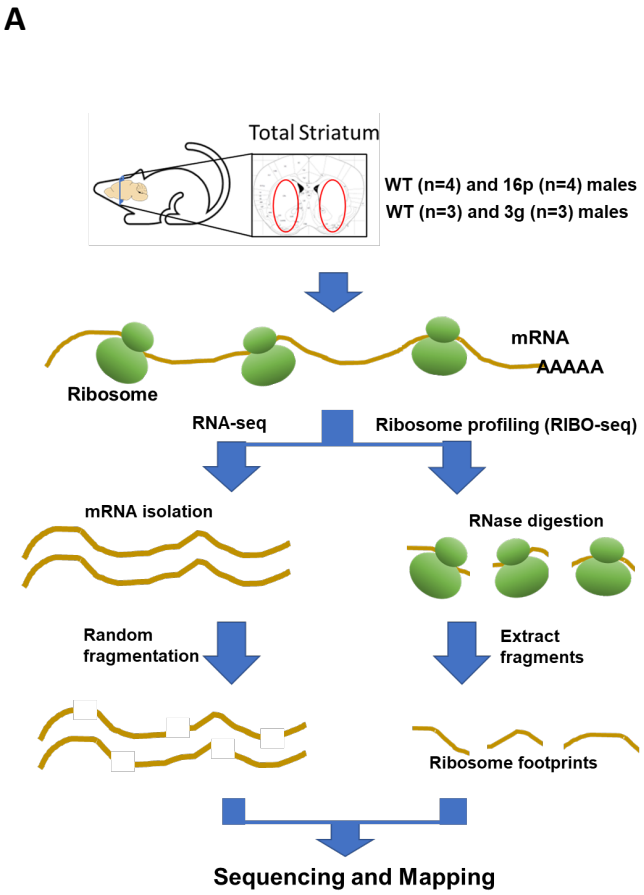
Syndromic:
TTN

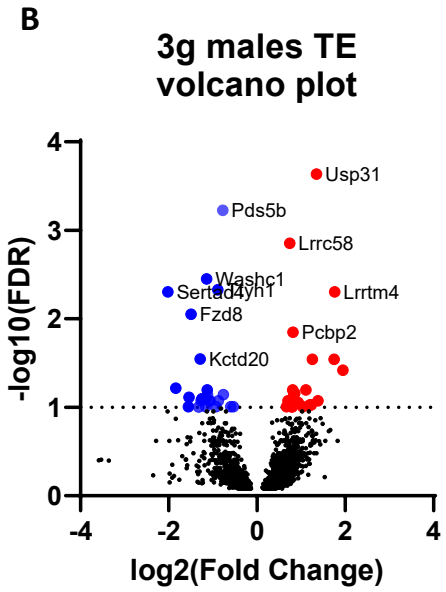
High Confidence:
DNMT3A
HRAS
CORO1A
GFAP

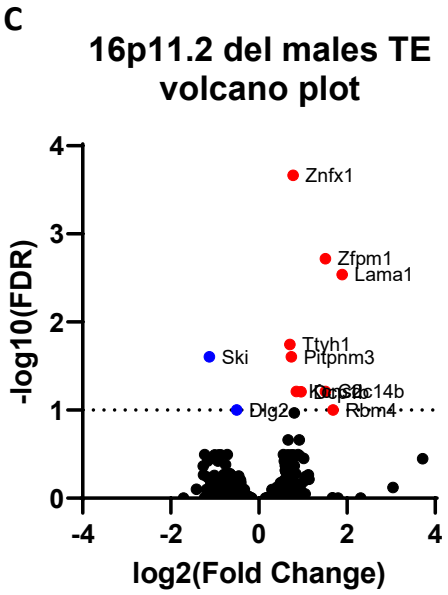
Strong Candidate:
MYO9B
SRSF11
ETFB
SCN9A

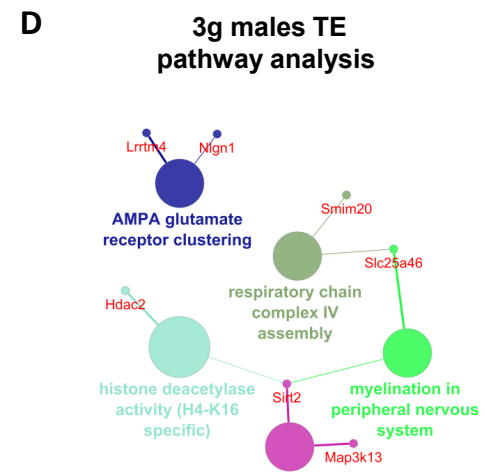
Suggestive Evidence:
KLF16
YEATS2
DRD3
MYO5C
PCDHA11
KCTD13
TSPOAP1
GSTM1
MAPK3
ZC3H11A

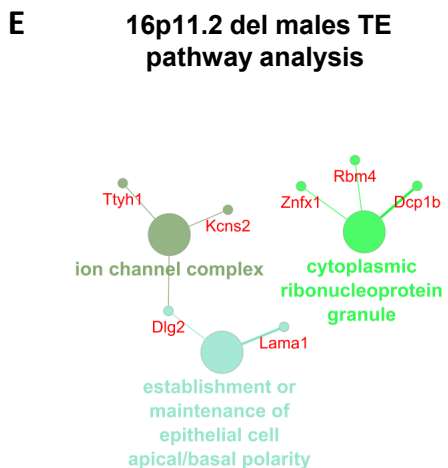
Supplementary Figure 9. Ribosome profiling in the striatum of 3g del/+ and 16p11.2 del/+ male mice and mTOR in 3g del/+ mice

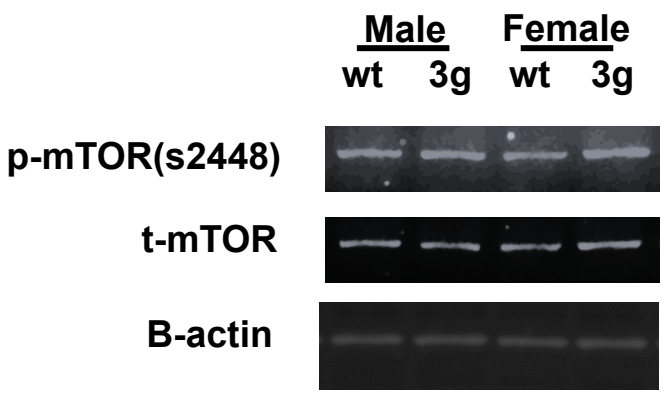


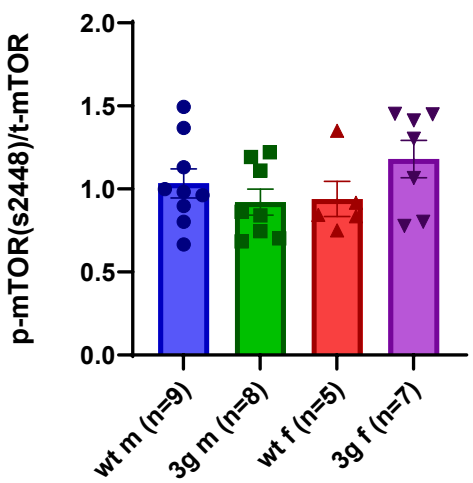


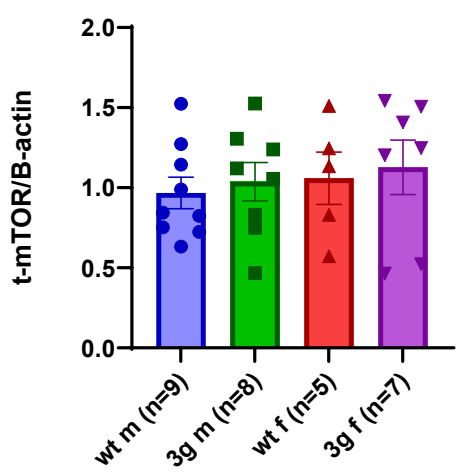






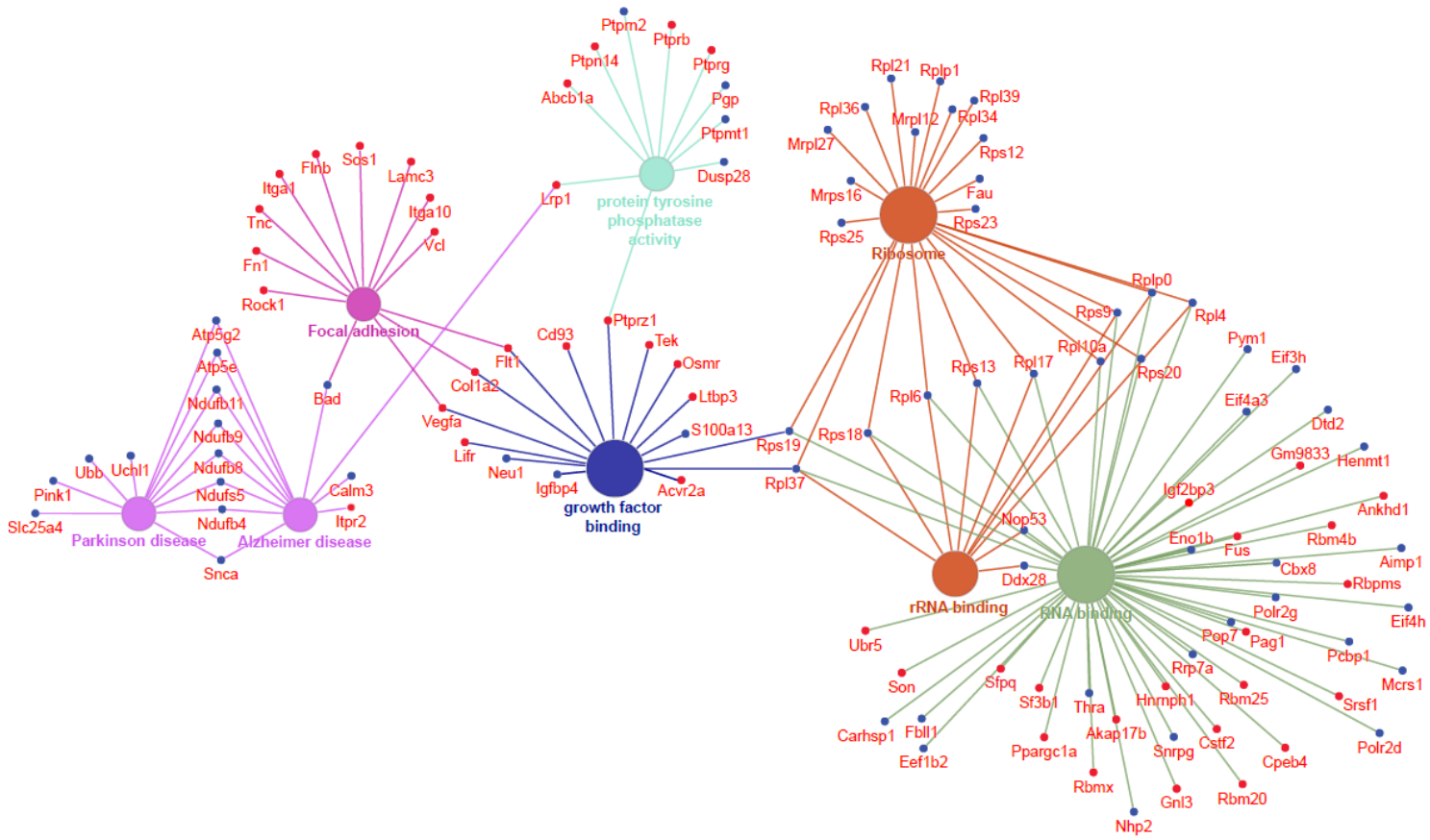




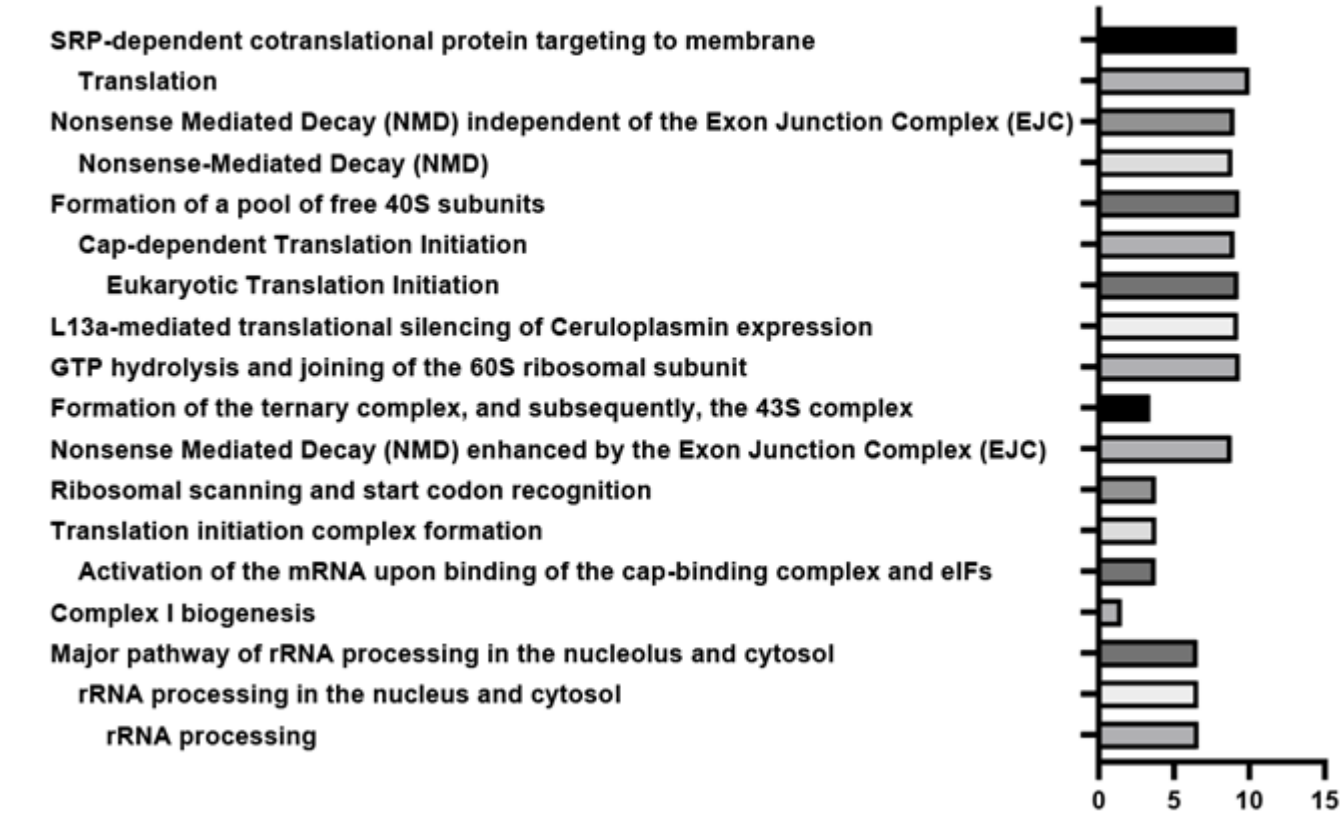


Supplementary Figure 10. Pathway analysis of RNAseq results from 3g males (569 DEGs)

A

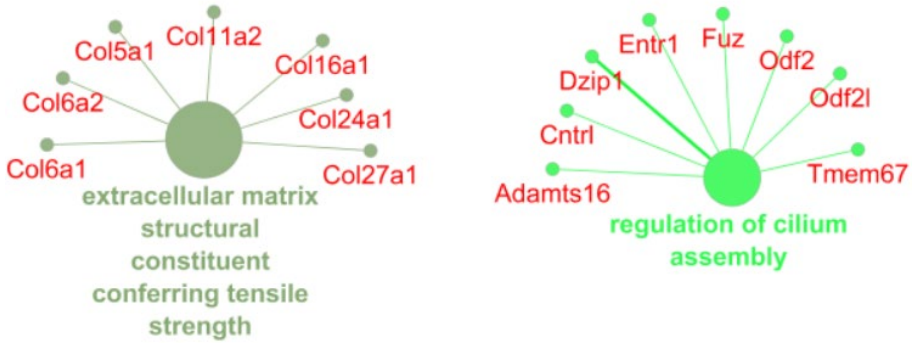


B

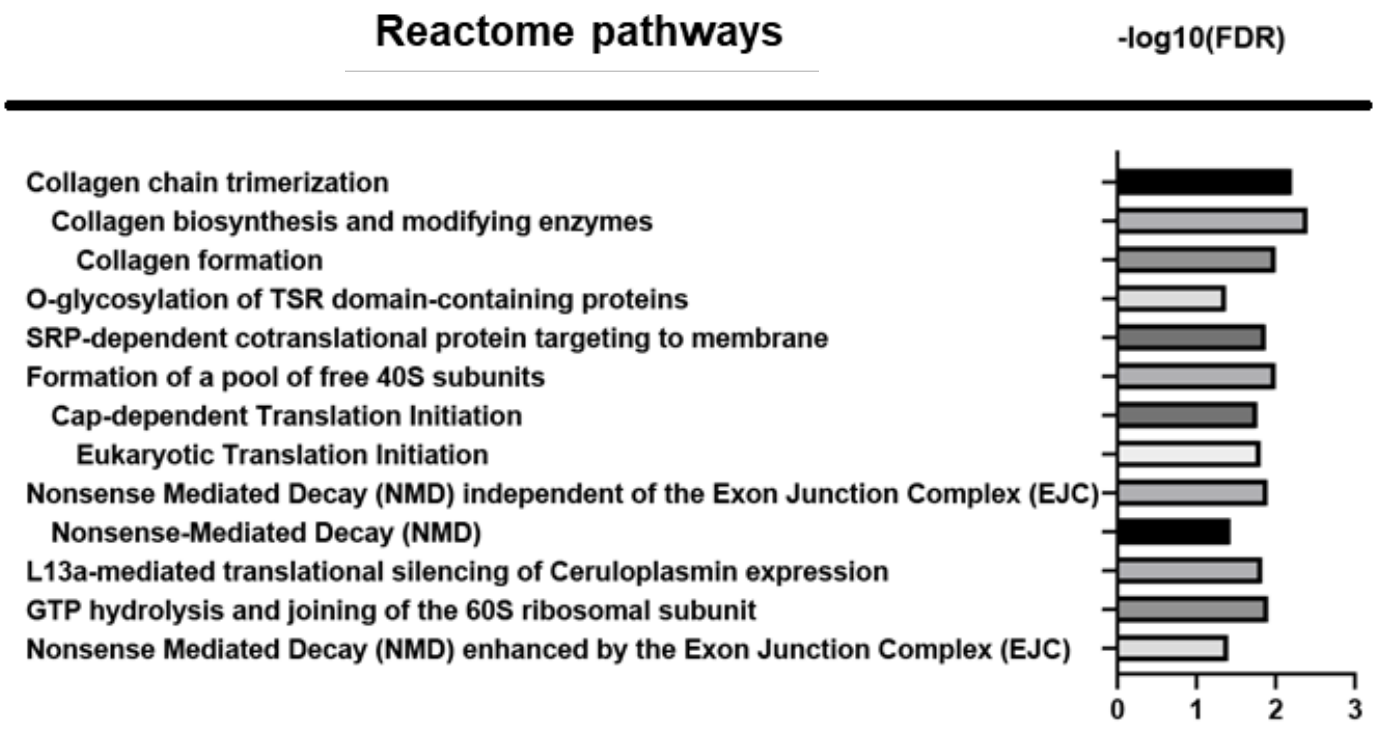


Supplementary Figure 11. Pathway analysis of RNAseq results from 16p11.2 males (633 DEGs)

A

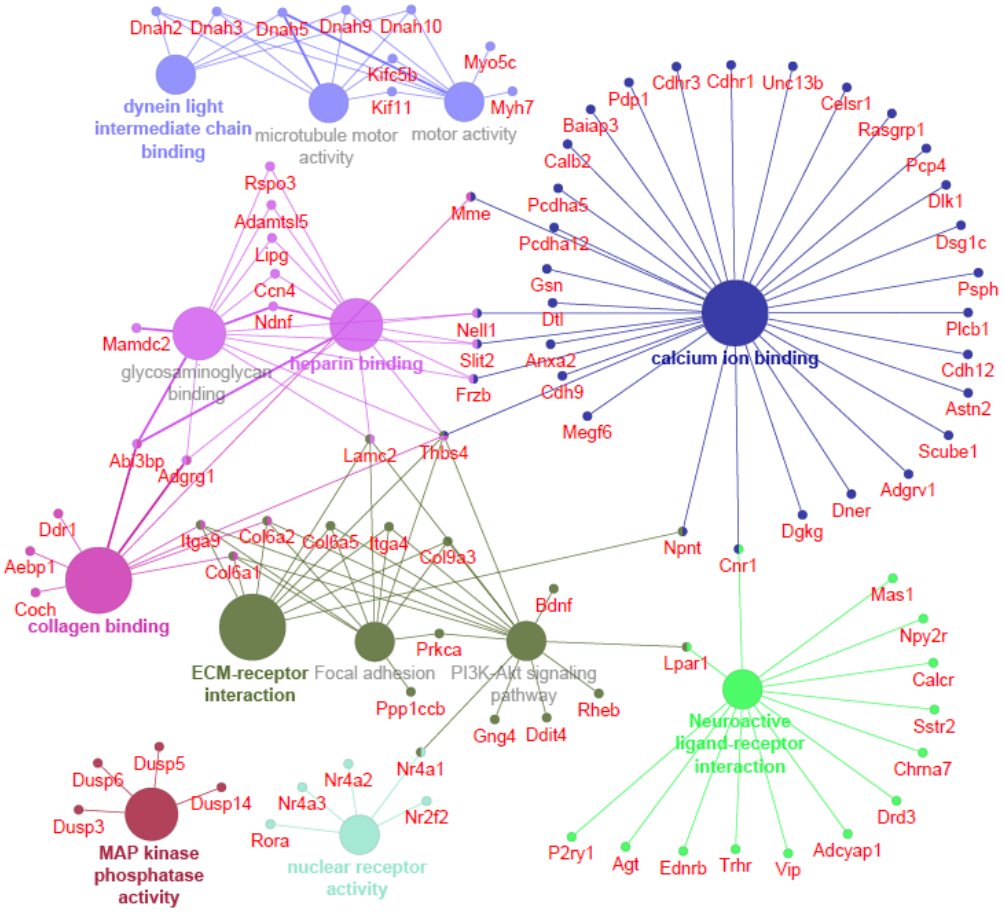


B

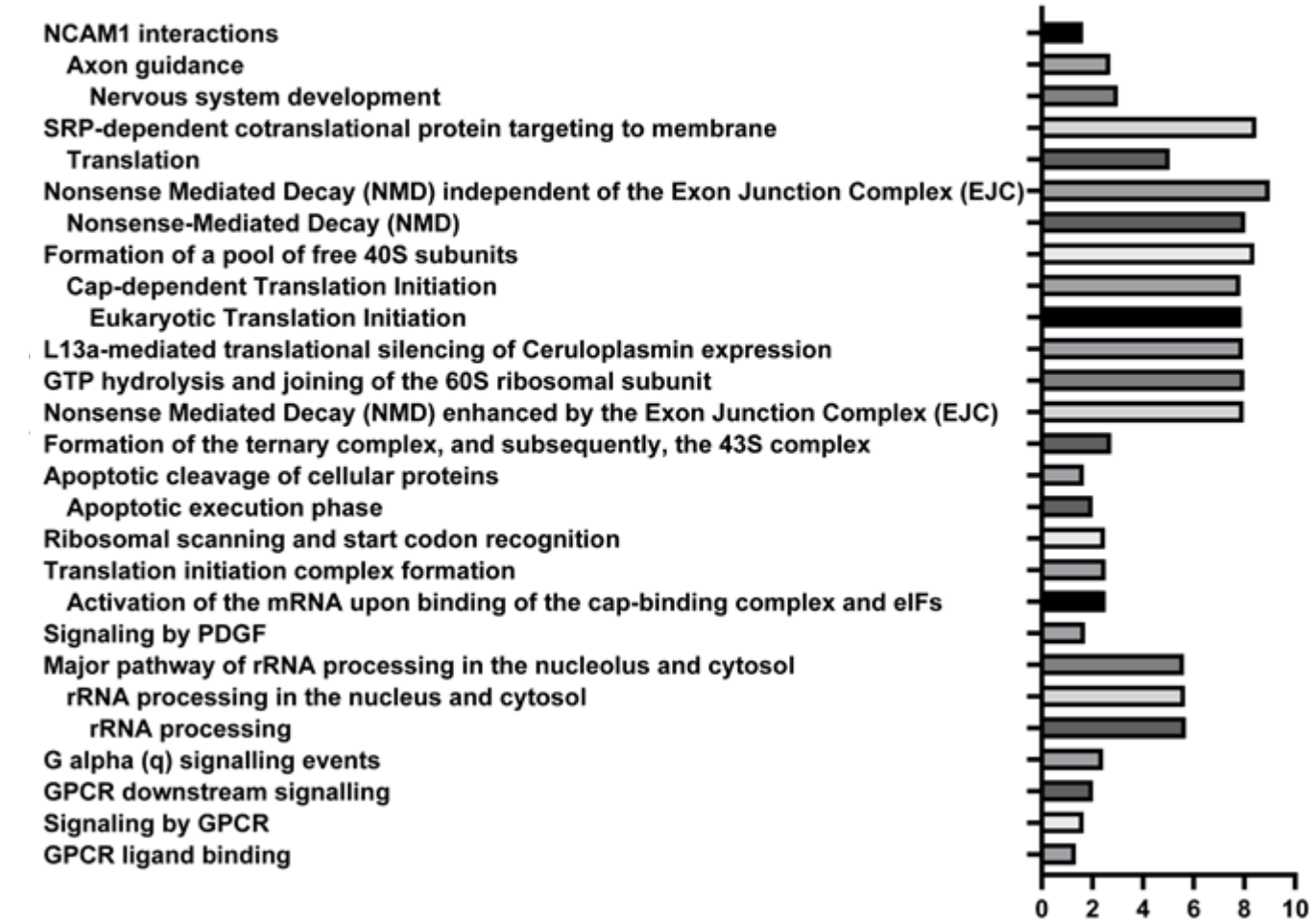


Supplementary Figure 12. Pathway analysis of RNAseq results from 3g females (340 DEGs)

A

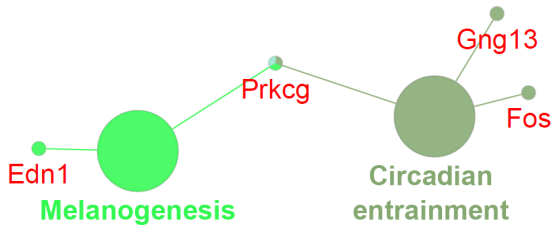


B

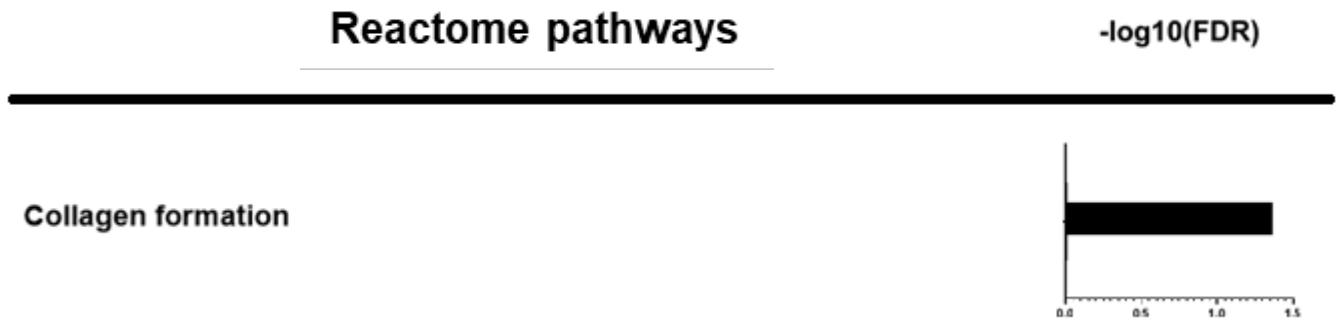


Supplementary Figure 13. Pathway analysis of RNAseq results from 16p11.2 females (121 DEGs)

A

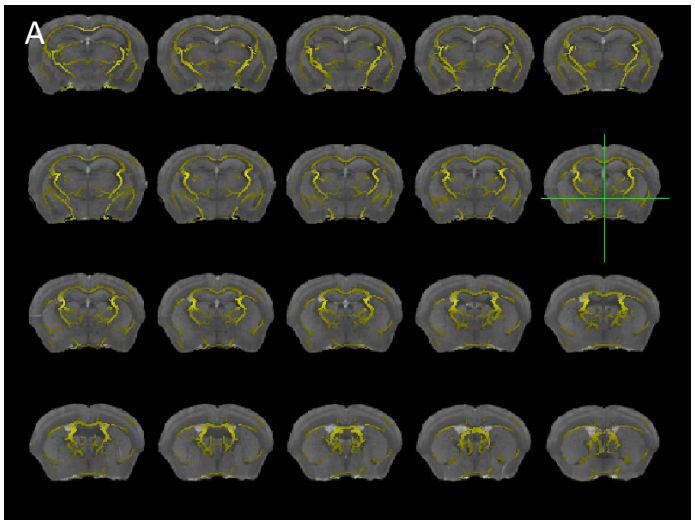


B

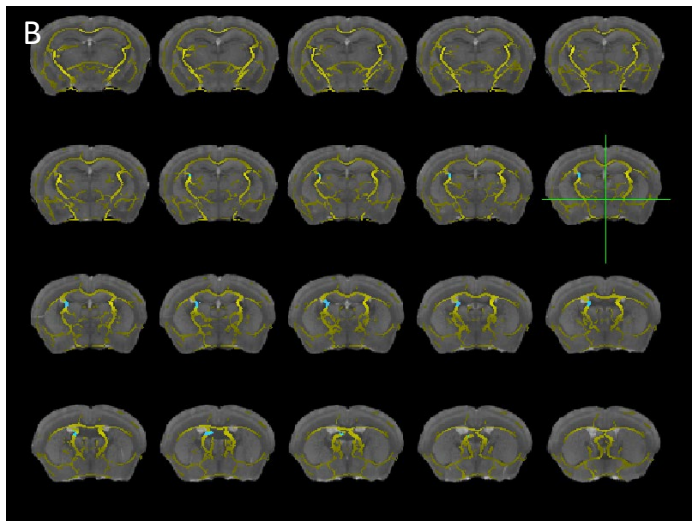


Supplementary Figure 14. Fiber tract changes in 3g mice compared to wild types

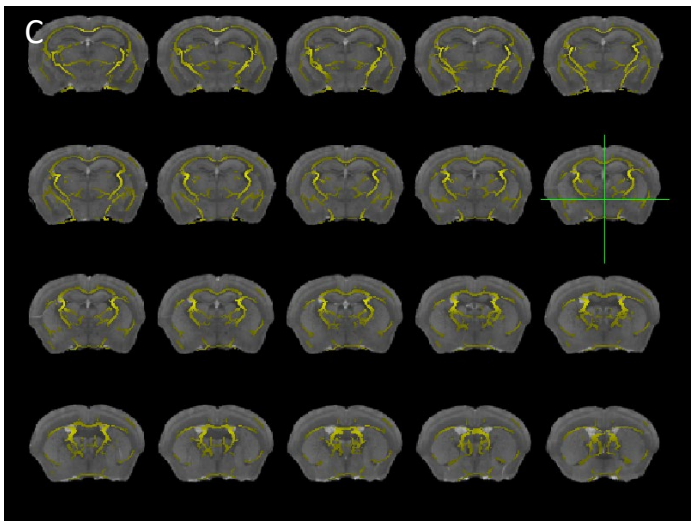
3g males (6-week-old)



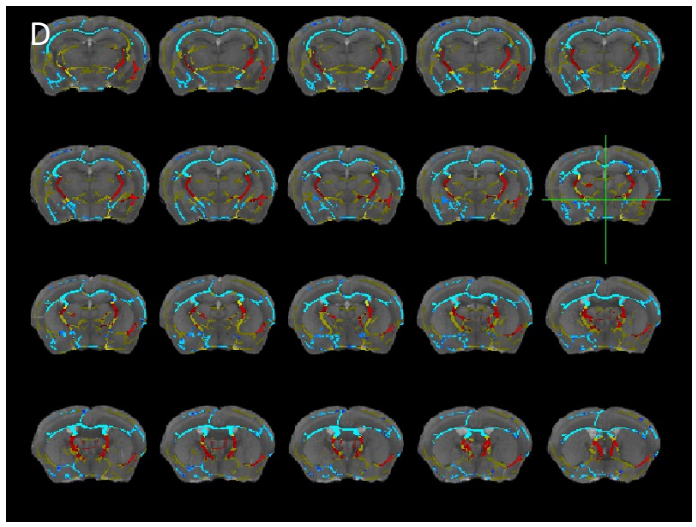
3g females (6-week-old)



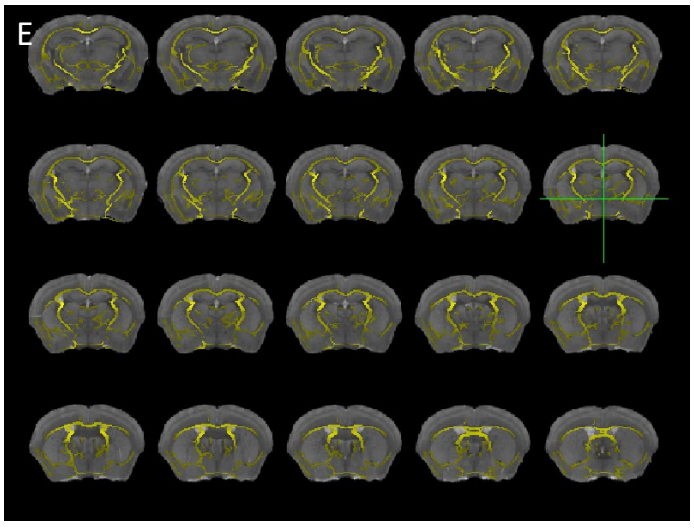
16p11.2 males (6-week-old)



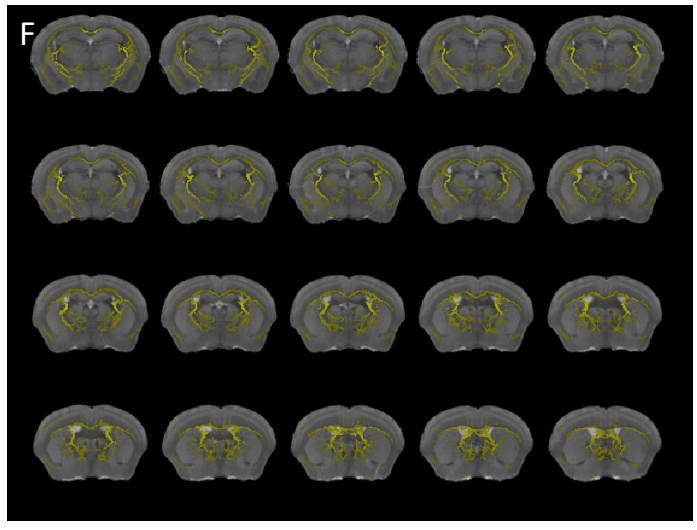
16p11.2 females (6-week-old)



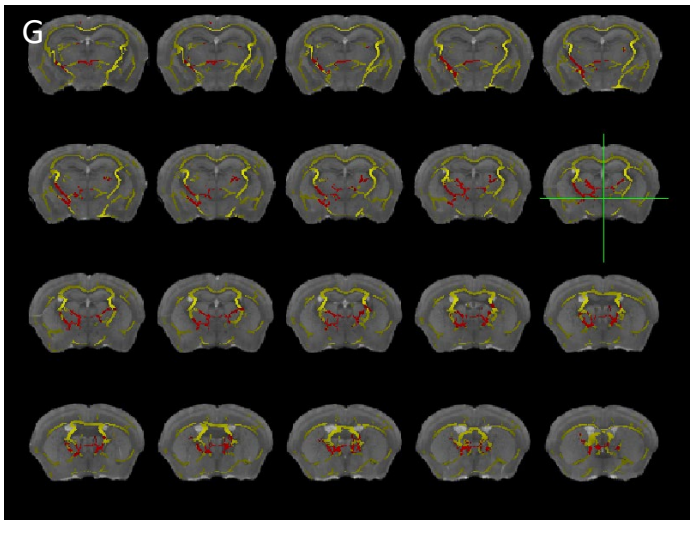
3g males (16-week-old)



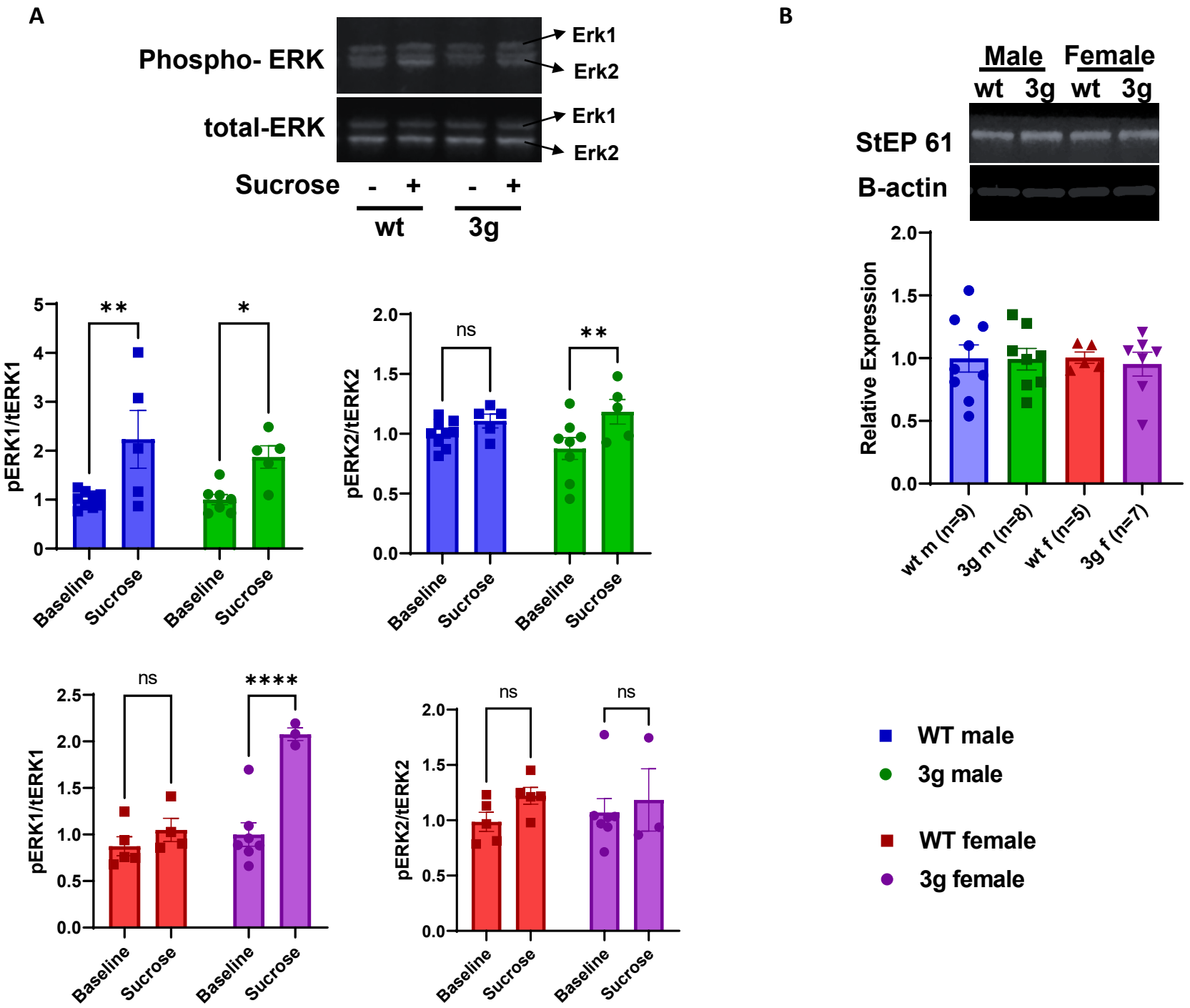
3g females (16-week-old)



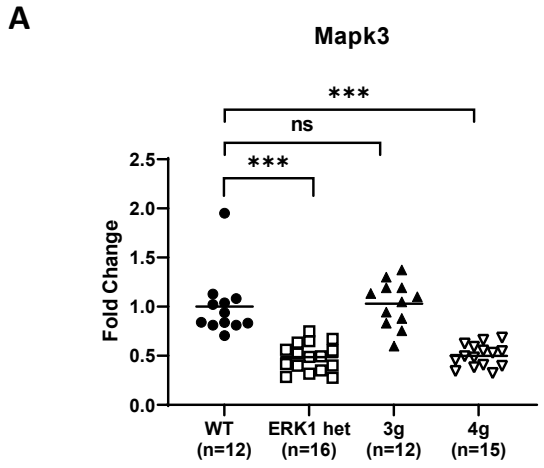
16p11.2 males (10-week-old)

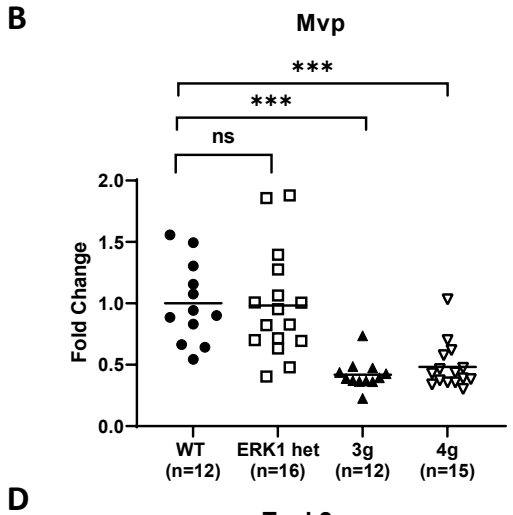


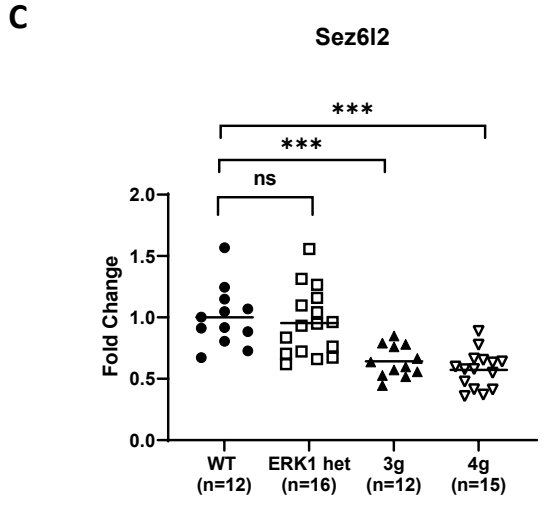
Supplementary Figure 15. ERK activation after sucrose consumption in 3g del/+ and wt mice and StEP expression in 3g del/+ mice

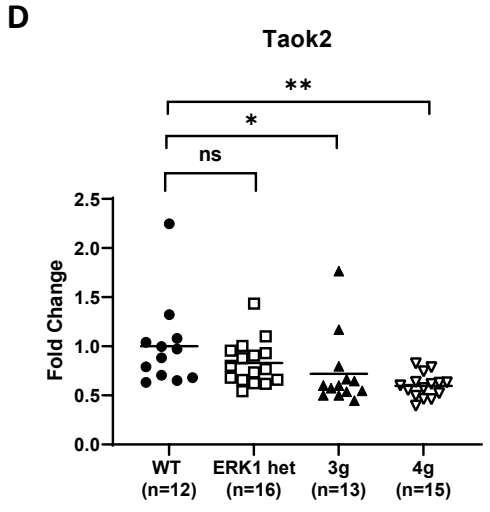


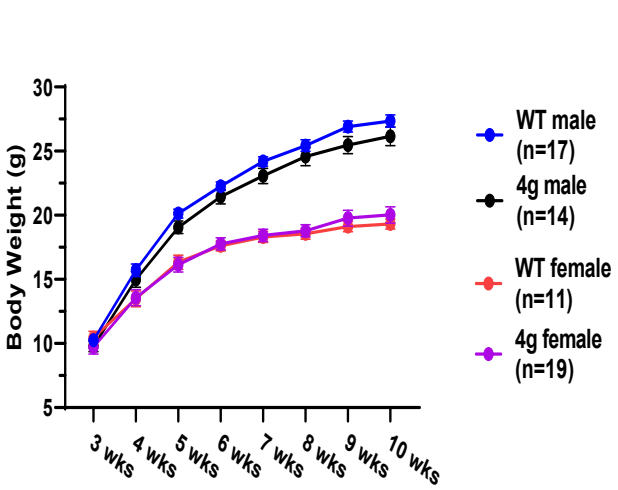
Supplementary Figure 16. 4 gene hemi-deletion validation (qPCR) and additional phenotypes of 4g del/+ mice

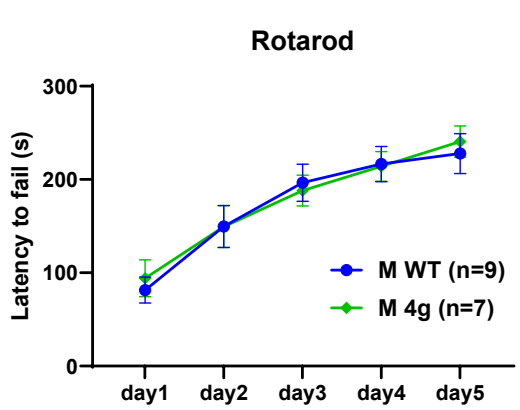


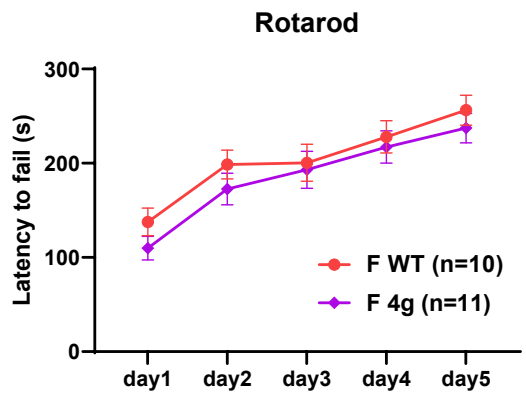












Supplementary Figure 17. Graphic abstract