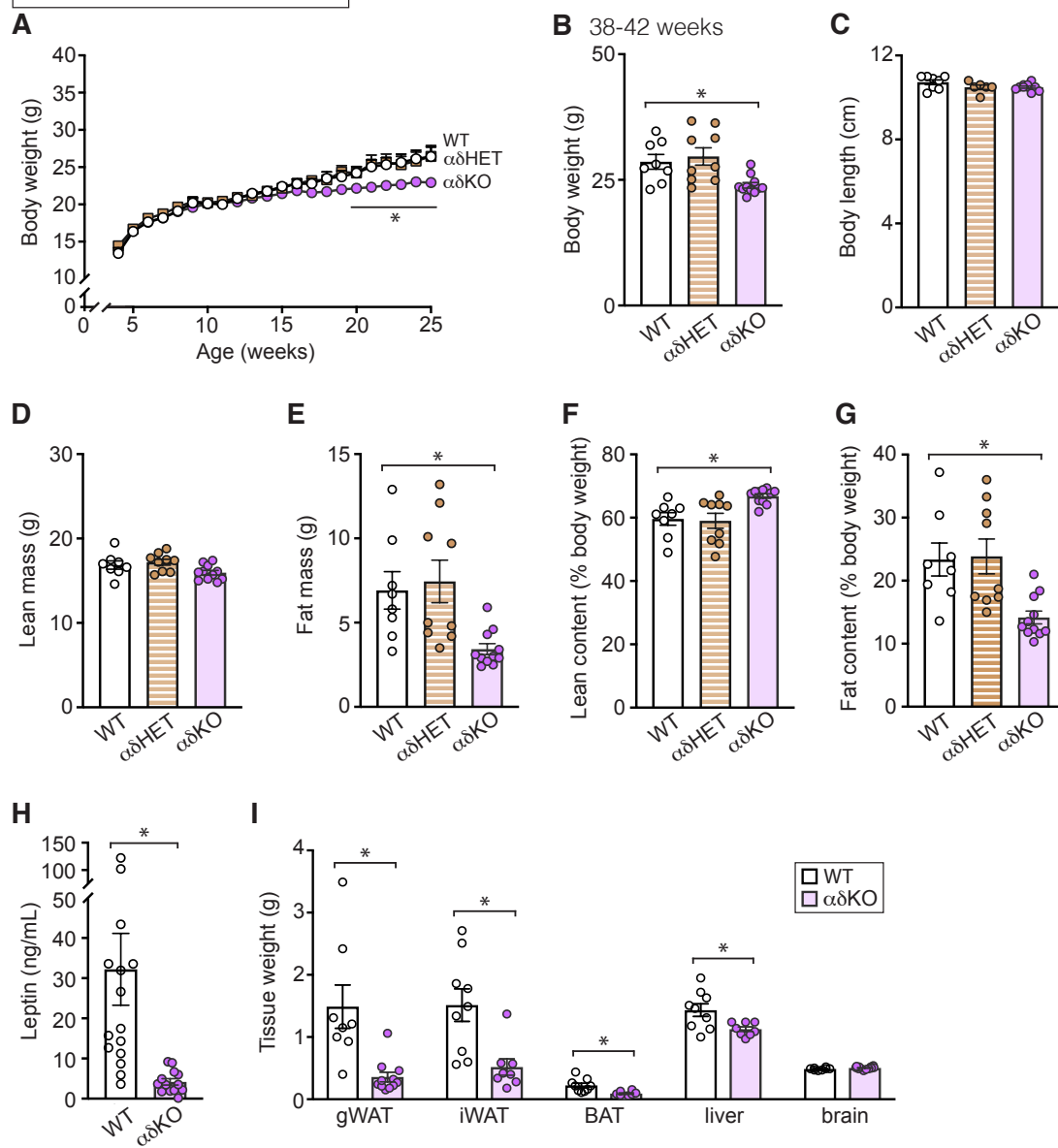


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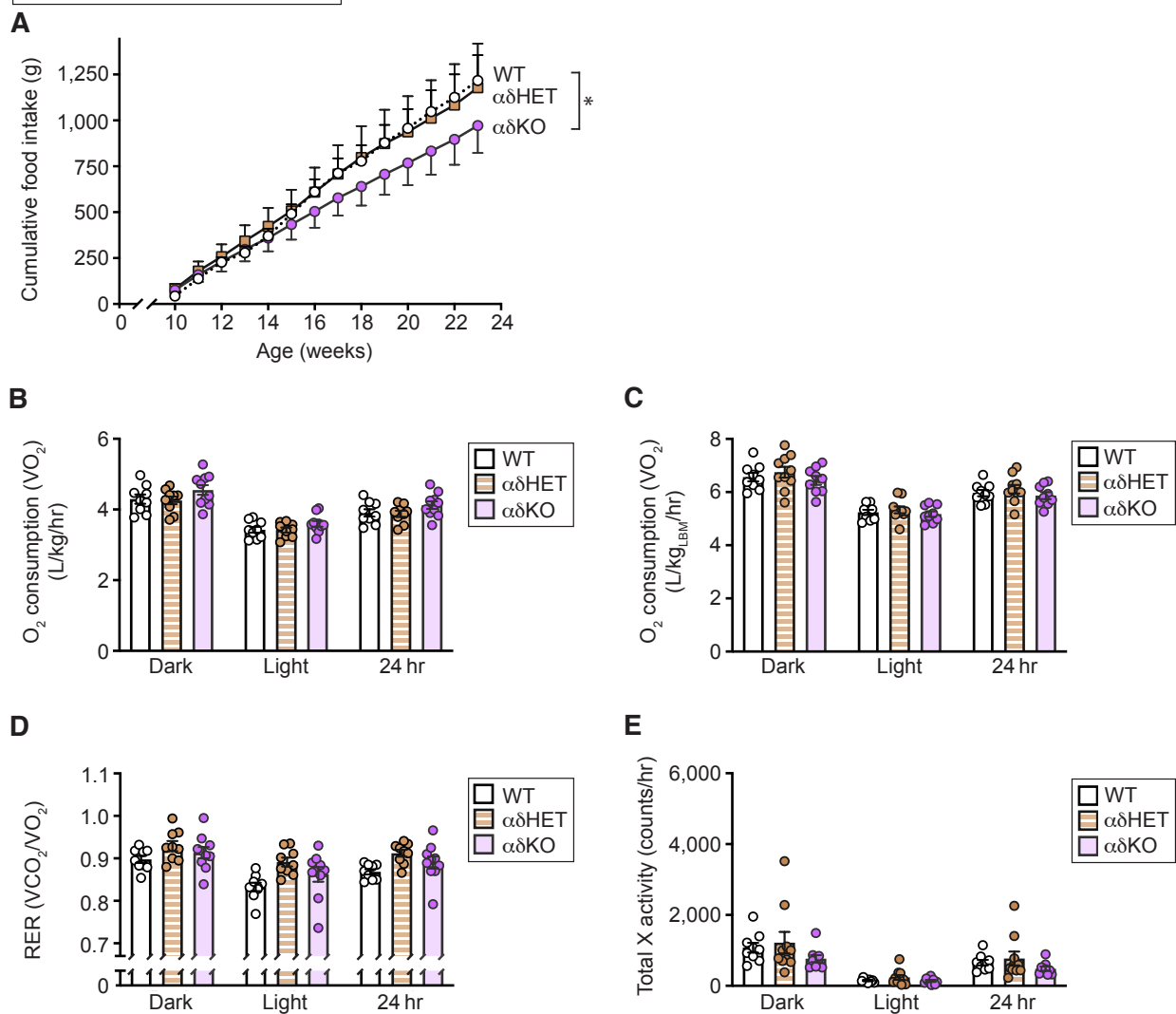
Females on standard chow



Supplementary Figure 1. Female $\alpha\delta$ KO mice fed standard chow exhibit reduced body weight, fat content, circulating leptin levels, adipose tissue weight, and liver weight. **(A)** Body weight was measured weekly from 4 to 25 weeks (n: WT = 6-8; $\alpha\delta$ HET = 7-9; $\alpha\delta$ KO = 11-13). **(B)** Body weight was measured between 38 and 42 weeks of age (n: WT = 8; $\alpha\delta$ HET = 9; $\alpha\delta$ KO = 11). **(C)** Body length was measured from nose to anus in 38-to-42-week-old mice (n: WT = 8; $\alpha\delta$ HET = 6; $\alpha\delta$ KO = 10). **(D-G)** Body composition of 38-to-42-week-old mice was analyzed by NMR between 9:00_{AM} and 11:00_{AM} (n: WT = 8; $\alpha\delta$ HET = 9; $\alpha\delta$ KO = 11). **(H)** Blood was collected from 38-to-42-week-old mice fed *ad libitum*, and serum leptin levels were measured by ELISA (n: WT = 15; $\alpha\delta$ KO = 13). **(I)** Tissues of 38-to-42-week-old mice were dissected and weighed (n: WT = 8-10; $\alpha\delta$ KO = 8-12). gWAT, gonadal white adipose tissue; iWAT, inguinal white adipose tissue; BAT, brown adipose tissue. **Statistics:** **A**, two-way repeated measures ANOVA (weeks 7 to 25); **B-G**, one-way ANOVA; **H-I**, unpaired, two-tailed Student t test. * $P < 0.05$. Data are means \pm SEM.

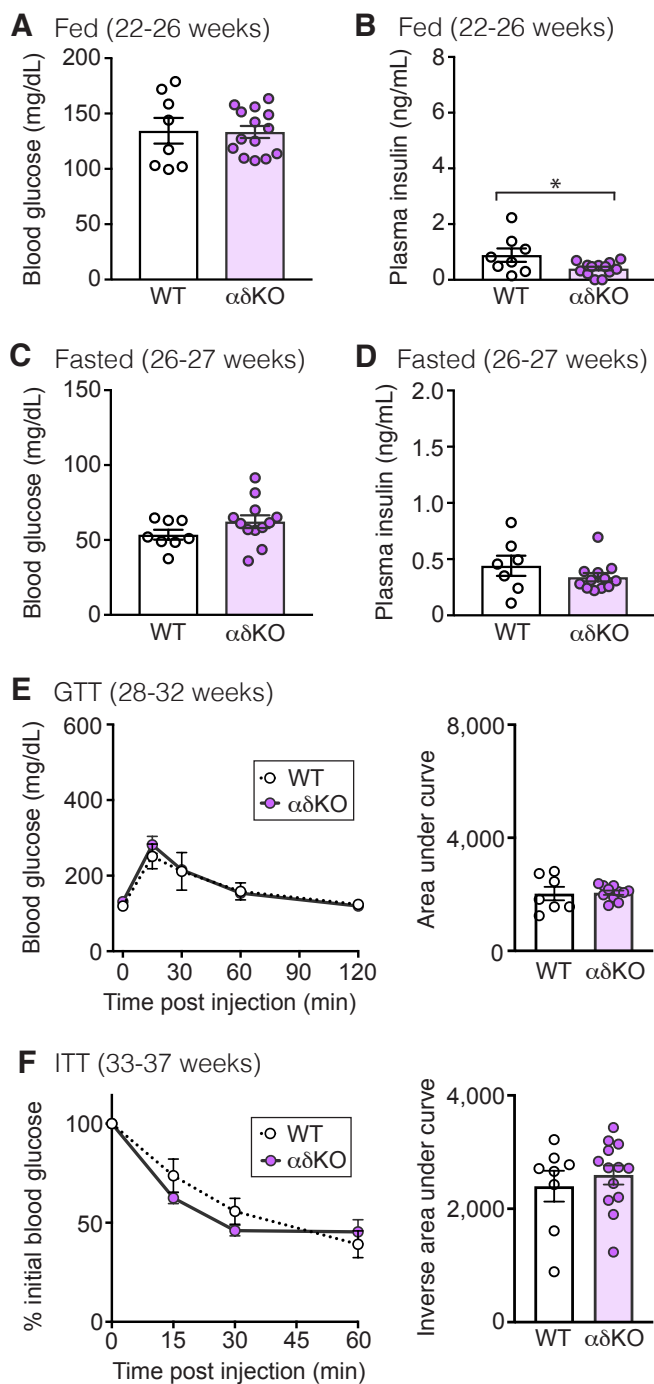
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Females on standard chow



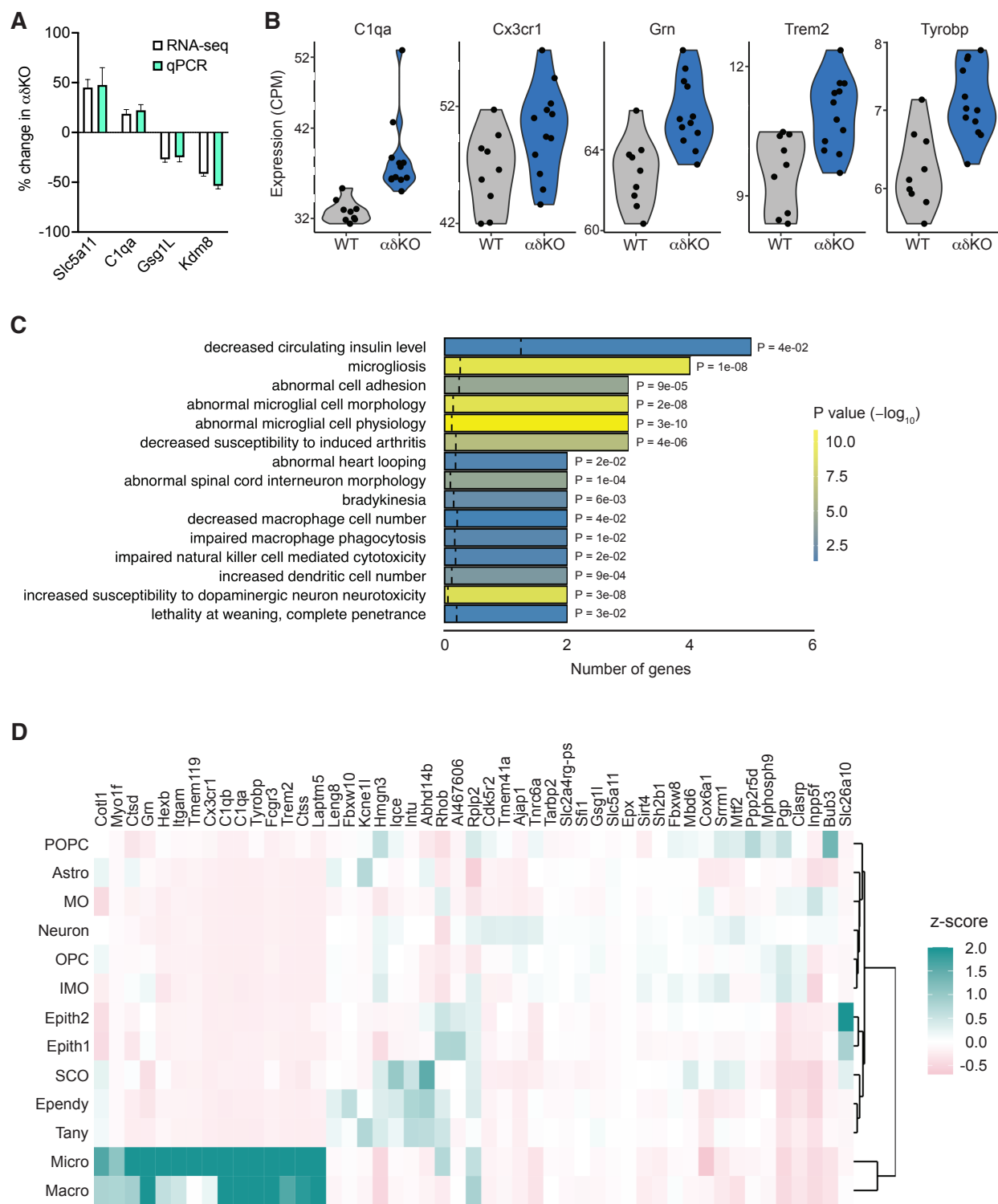
Supplementary Figure 2. Female $\alpha\delta$ KO mice fed standard chow exhibit decreased food consumption but normal energy expenditure. **(A)** Food intake was measured weekly from 10 to 23 weeks (n: WT = 7; $\alpha\delta$ HET = 8; $\alpha\delta$ KO = 11). **(B-E)** Energy expenditure was measured in 10- to 12-week-old mice by CLAMS (n: WT = 9; $\alpha\delta$ HET = 10; $\alpha\delta$ KO = 9). Parameters included: **(B)** oxygen consumption corrected for total body weight or **(C)** lean body mass (LBM); **(D)** respiratory exchange ratio (RER); and **(E)** total motor activity in the X dimension. RER was calculated as VCO_2/VO_2 . The final 24 hours of recordings are presented. **Statistics:** **A**, linear regression analysis (weeks 10 to 23); **B-E**, one-way ANOVA. * $P < 0.05$. Data are means \pm SEM.

Females on standard chow



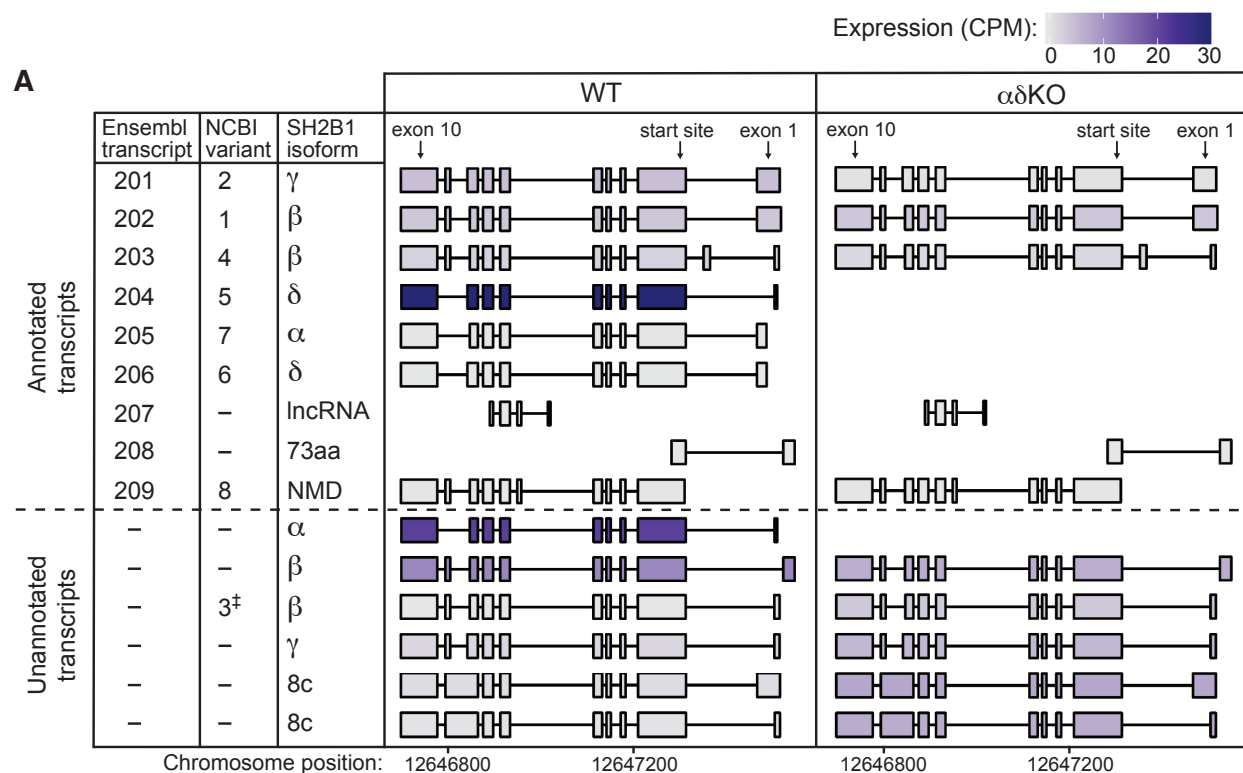
Supplementary Figure 3. Older female $\alpha\delta$ KO mice exhibit modestly improved glucose homeostasis. Blood was collected from 22-to-26-week-old mice fed *ad libitum* and **(A)** blood glucose levels were measured by glucometer (n: WT = 8; $\alpha\delta$ KO = 14) and **(B)** plasma insulin levels were measured by ELISA (n: WT = 8; $\alpha\delta$ KO = 13). Blood was collected from 26-to-27-week-old mice fasted overnight (16 hours) and **(C)** blood glucose levels were measured by glucometer (n: WT = 8; $\alpha\delta$ KO = 12) and **(D)** plasma insulin levels were measured by ELISA (n: WT = 8; $\alpha\delta$ KO = 12). **(E)** Glucose tolerance tests (GTTs) were performed on 28-to-32-week-old-mice following i.p. injection with glucose (2 g/kg) (n: WT = 7; $\alpha\delta$ KO = 11). Area under the curve for each animal was calculated using a baseline of y = 0. **(F)** Insulin tolerance tests (ITTs) were performed on 33-to-37-week-old mice following i.p. injection with insulin (1 unit/kg) (n: WT = 8; $\alpha\delta$ KO = 13). Data are reported as % initial blood glucose values. Inverse area under the curve for each animal was calculated using a baseline of y = 100. **Statistics:** **A-F**, unpaired, two-tailed Student t test. * P < 0.05. Data are means \pm SEM.

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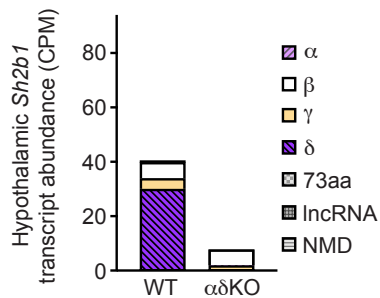


Supplementary Figure 4. Transcriptional changes in $\alpha\delta$ KO hypothalami are significantly associated with terms linked to microglial function. **(A)** mRNA analyzed by RNA-seq was assessed by qPCR for expression levels of indicated genes (n: 4 animals per genotype). Graph depicts the % change in gene expression in $\alpha\delta$ KO compared to WT mice. **(B)** Violin plots depict expression of genes identified by Gene Ontology analysis. CPM, counts per million. **(C)** Bar graph displays mouse phenotypes that significantly associate with genes that have statistically significant differential expression in $\alpha\delta$ KO mice. The dashed vertical line on each bar corresponds to the frequency of finding that phenotype associated with all genes. **(D)** Heat map depicts cell type-specific expression of genes with statistically significant differential regulation in $\alpha\delta$ KO mice. POPC, proliferating oligodendrocyte precursor cell; Astro, astrocyte; MO, myelinating oligodendrocyte; OPC, oligodendrocyte precursor cell; IMO, immature myelinating oligodendrocyte; Epith, epithelial cell; SCO, subcommissural organ cells; Ependy, ependymocyte; Tany, tanycyte; Micro, microglia; Macro, macrophages. **Statistics: B-D**, see Supplementary Materials. Data are means.

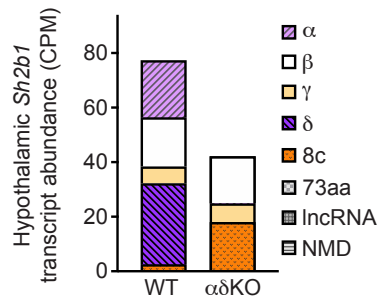
Supplementary Material – Cote et al.



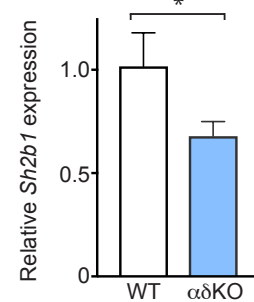
B Annotated transcripts only



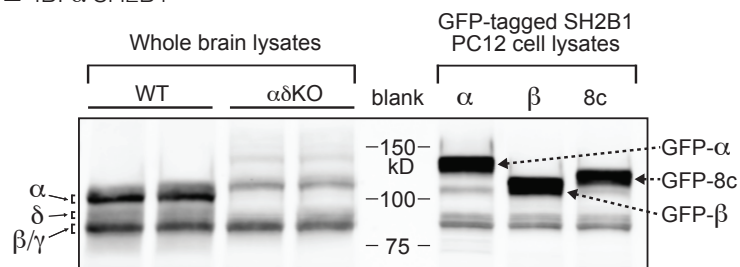
C Annotated & unannotated transcripts



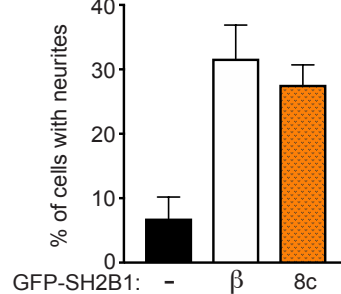
D qPCR



E IB: α SH2B1



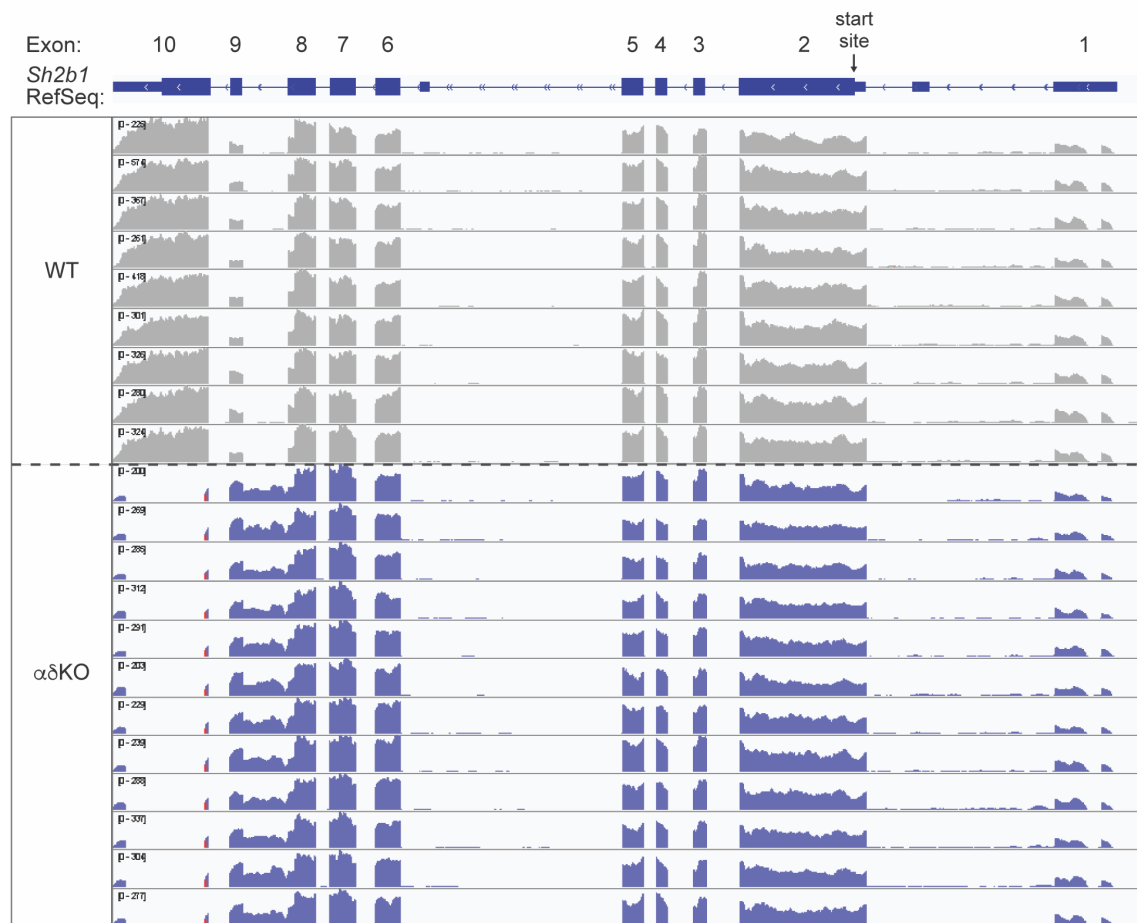
F



Supplementary Figure 5. RNA-seq data analysis reveals potential novel transcripts of *Sh2b1*.

(A) Schematic of *Sh2b1* transcripts and their expression levels in hypothalami of 10-to-12-week-old male mice as identified by RNA-seq (n: WT = 9; $\alpha\delta$ KO = 12). The dotted horizontal line separates annotated (top) versus unannotated (bottom) Ensembl transcripts. *Sh2b1*8c transcripts have an extended 8th exon. Validated NCBI variant numbers are listed. † SH2B1 isoform assignment based on transcript schematic. CPM, counts per million; lncRNA, long non-coding RNA; aa, amino acids; NMD, nonsense-mediated decay. **(B)** *Sh2b1* transcript abundance was calculated by RNA-seq data analysis using annotated Ensembl transcripts only or **(C)** both annotated and unannotated Ensembl transcripts. Data for α , β , γ , and δ transcripts in **C** are the same as in Figure 1F. **(D)** Levels of total *Sh2b1* mRNA were measured by qPCR using an aliquot of the same hypothalamic RNA as in Fig. 8A (n: WT = 9; $\alpha\delta$ KO = 12). **(E)** Proteins in brain tissue homogenates from WT and $\alpha\delta$ KO adult male mice and lysates of PC12 cells expressing GFP-tagged SH2B1 α , β , or 8c were immunoblotted with antibody to SH2B1 (α SH2B1). The migration of molecular weight standards is shown in the center of the blot. The expected migration of the different isoforms is indicated on the left (endogenous) and right (GFP-tagged). IB, immunoblot. **(F)** PC12 cells transiently expressing GFP (-), GFP-SH2B1 β (β), or GFP-SH2B18c (8c) were treated with 25 ng/mL NGF for 2 days, at which point neurite outgrowth was assessed. GFP-positive cells were scored for the presence of neurites at least 2 times the length of the cell body (n: 3 experiments, 200-300 cells/condition/experiment). **Statistics:** **D**, unpaired, two-tailed Student t test. * $P < 0.05$. Data are means \pm SEM.

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Supplementary Figure 6. RNA-seq reads from WT and $\alpha\delta$ KO hypothalami mapped to mouse *Sh2b1* Reference Sequence (RefSeq). In the *Sh2b1* RefSeq schematic at the top of the figure, blue rectangles represent exons; non-coding regions have less height than coding regions. The height of the reads tracks from individual WT (light gray) and $\alpha\delta$ KO mice (dark blue) depicts the level of mRNA expression at each nucleotide of the RefSeq for each mouse. Note that $\alpha\delta$ KO reads tracks are lacking expression for most of exon 10 as expected. However, additional expression is present between exons 8 and 9, which was unexpected and corresponds to the predicted *Sh2b18c* transcripts (Supplementary Fig. 5). Numbers in brackets on the left side of each track (e.g., [0-225]) are the range of expression values for that track.

Supplementary Table 1. Primers for genotyping

Primer	Direction	Sequence (5' → 3')
WT	Forward	CAAAGGGGAGGTCACCATAAGAACTCAC
WT	Reverse	GTGGGCAGGTATCTCACACAAATGAGTA
Edit	Forward	GGGAATGTGCAGAACTGGACCCA
Edit	Reverse	GCGAGTGACTGTGTAACGGAGCA

Supplementary Table 2. Primers for *Sh2b1δ* and β -actin cDNA

Primer	Direction	Sequence (5' → 3')
<i>Sh2b1δ</i>	Forward	GTGCACCCAAGAAGTGAGAAC
<i>Sh2b1δ</i>	Reverse	CTTGTACCAACATACACACCCTTG
β -actin	Forward	CGTCTGGACCTGGCTGGCCGGGACC
β -actin	Reverse	CTAGAAGCATTGCGGTGGACGATG

Supplementary Table 3. TaqMan Gene Expression Assays from Applied Biosystems

Gene	TaqMan Gene Expression Assay
<i>Agrp</i>	Mm00475829_g1
<i>Asb4</i>	Mm00480830_m1
<i>Clqa</i>	Mm00432142_m1
<i>Cartpt</i>	Mm04210469_m1
<i>Ghrh</i>	Mm00439100_m1
<i>Gsg1l</i>	Mm01278519_m1
<i>Irf9</i>	Mm00492679_m1
<i>Kdm8</i>	Mm00513079_m1
<i>Pomc</i>	Mm00435874_m1
<i>Npy</i>	Mm00445771_m1
<i>Serpina3n</i>	Mm00776439_m1
<i>Sh2b1α</i>	Mm01275190_m1
<i>Sh2b1β</i>	Mm01163373_g1
<i>Sh2b1γ</i>	Mm00488153_m1
<i>Sh2b1δ</i>	Mm01163374_m1
<i>Slc5a1l</i>	Mm00461434_m1
<i>36b4 (reference gene)</i>	Mm00725448_s1
<i>Gapdh (reference gene)</i>	Mm99999915_g1
<i>Tbp (reference gene)</i>	Mm01277042_m1

Supplementary Table 4. Genes with significantly increased expression in $\alpha\delta$ KO mice

(sorted by % increase, largest to smallest)

Gene name	Gene product	Average CPM		% increase in $\alpha\delta$ KO	P value
		WT	$\alpha\delta$ KO		
<i>Slc5a11</i>	Solute carrier family 5 (sodium/glucose cotransporter), member 11 (a.k.a. KST1, SMIT2)	1.663	2.413	45.087	0.0318
<i>AI467606</i>	Expressed sequence AI467606	3.974	5.599	40.882	0.0012
<i>Myo1f</i>	Myosin IF (unconventional)	2.428	3.185	31.195	0.0320
<i>C1qa</i>	Complement component 1, q subcomponent, α polypeptide	32.498	38.561	18.658	0.0127
<i>Itgam</i>	Integrin α M (a.k.a. CD11b/CD18, CR3, CR3 α , Mac-1, Mac-1 α)	4.279	5.033	17.613	0.0407
<i>Ctss</i>	Cathepsin S	49.920	58.084	16.355	0.0117
<i>Fcgr3</i>	Fc receptor, IgG, low affinity III (a.k.a. CD16)	7.314	8.459	15.648	0.0168
<i>Tyrobp</i>	TYRO protein tyrosine kinase binding protein (a.k.a. DAP12, KARAP)	6.190	7.141	15.370	0.0288
<i>C1qb</i>	Complement component 1, q subcomponent, β polypeptide	39.367	45.255	14.957	0.0048
<i>Trem2</i>	Triggering receptor expressed on myeloid cells 2 (a.k.a. TREM2a, TREM2b, TREM2c)	9.441	10.832	14.732	0.0166
<i>Tmem41a</i>	Transmembrane protein 41a	19.207	21.883	13.936	0.0155
<i>Kcne1l</i>	Potassium voltage-gated channel, Isk-related family, member 1-like, pseudogene (a.k.a. MINK, KCNE5)	10.386	11.626	11.943	0.0337
<i>Laptm5</i>	Lysosomal-associated protein transmembrane 5 (a.k.a. E3)	27.053	30.221	11.708	0.0273
<i>Tmem119</i>	Transmembrane protein 119 (a.k.a. OBIF)	19.186	21.245	10.732	0.0413
<i>Pgp</i>	Phosphoglycolate phosphatase (a.k.a. AUM, G3PP)	64.349	70.578	9.680	2.53E-04
<i>Cx3cr1</i>	Chemokine (C-X3-C motif) receptor 1	46.008	49.929	8.521	0.0127
<i>Hexb</i>	Hexosaminidase B	98.714	106.838	8.230	0.0062
<i>Ajap1</i>	Adherens junction associated protein 1	44.643	47.881	7.252	0.0443
<i>Cox6a1</i>	Cytochrome c oxidase subunit 6A1	166.605	178.519	7.151	0.0145
<i>Ppp2r5d</i>	Protein phosphatase 2, regulatory subunit B', δ (a.k.a. B' δ , TEG-271)	131.952	140.590	6.546	0.0407
<i>Ctsd</i>	Cathepsin D (a.k.a. CD, CatD)	143.229	152.248	6.297	0.0381
<i>Cdk5r2</i>	Cyclin-dependent kinase 5, regulatory subunit 2 (p39)	199.699	212.086	6.203	0.0159
<i>Rplp2</i>	Ribosomal protein, large P2	97.732	103.728	6.135	0.0159
<i>Abhd17a</i>	$\alpha\beta$ hydrolase domain containing 17A (a.k.a. FAM108A)	96.430	102.187	5.971	0.0121
<i>Grn</i>	Progranulin (a.k.a. GP88, PEPI, PCDGF)	62.386	65.965	5.736	0.0336
<i>Gpat4</i>	Glycerol-3-phosphate acyltransferase 4 (a.k.a. AGPAT6, TSARG7)	107.142	113.061	5.525	0.0166
<i>Cotl1</i>	Coactosin-like 1 (Dictyostelium) (a.k.a. CLP)	73.903	77.760	5.218	0.0119
<i>Rhob</i>	Ras homolog family member B (a.k.a. ARH6, ARHB)	390.237	409.486	4.933	0.0449
<i>Inpp5f</i>	Inositol polyphosphate-5-phosphatase F (a.k.a. SAC2)	181.284	189.614	4.595	0.0145
<i>Fbxw8</i>	F-box and WD-40 domain protein 8 (a.k.a. FBW6, FBW8, FBX29, FBXO29)	66.920	69.887	4.435	0.0354
<i>Map7d1</i>	MAP7 domain containing 1 (a.k.a. RPRC1, PARCC1, MTAP7D1)	341.982	354.815	3.752	0.0127

CPM, counts per million

Supplementary Table 5. Genes with significantly decreased expression in $\alpha\delta$ KO mice

(sorted by % decrease, largest to smallest)

Gene name	Gene product	Average CPM		% decrease in $\alpha\delta$ KO	P value
		WT	$\alpha\delta$ KO		
<i>Kdm8</i>	Lysine (K)-specific demethylase 8 (a.k.a. JMJD5)	5.606	3.277	-41.546	2.23E-20
<i>Epx</i>	Eosinophil peroxidase (a.k.a. EPO)	1.271	0.842	-33.792	0.0414
<i>Slc26a10</i>	Solute carrier family 26, member 10	2.566	1.789	-30.264	0.0145
<i>Fbxw10</i>	F-box and WD-40 domain protein 10 (a.k.a. FBW10, HREP, SM25H2, SM2SH2)	1.268	0.900	-29.027	0.0407
<i>Gsg1l</i>	Germ cell-specific gene 1-like	44.831	32.760	-26.926	5.52E-07
<i>Slc2a4rg-ps</i>	Slc2a4 regulator, pseudogene	6.401	5.206	-18.662	0.0119
<i>Gm15446</i>		18.392	15.394	-16.305	0.0336
<i>C78859</i>	Expressed sequence C78859, long non-coding RNA	8.953	7.497	-16.259	0.0145
<i>Bub3</i>	BUB3 mitotic checkpoint protein	85.535	71.881	-15.963	9.00E-07
<i>Sfi1</i>	SFI1 centrin binding protein	15.313	13.211	-13.727	0.0145
<i>Leng8</i>	Leukocyte receptor cluster (LRC) member 8	135.504	117.415	-13.349	0.0434
<i>Mbd6</i>	Methyl-CpG binding domain protein 6	34.378	29.797	-13.327	0.0127
<i>Intu</i>	Inturned planar cell polarity protein (a.k.a. PDZD6, PDZK6)	9.416	8.162	-13.316	0.0240
<i>Sirt4</i>	Sirtuin 4	13.946	12.167	-12.762	0.0407
<i>Mir124a-1hg</i>	Mir124-1 host gene (non-protein coding)	46.598	40.709	-12.639	0.0152
<i>Uvssa</i>	UV stimulated scaffold protein A	10.853	9.535	-12.141	0.0263
<i>Rsrp1</i>	Arginine/serine rich protein 1	221.905	198.823	-10.402	0.0166
<i>Abhd14b</i>	$\alpha\beta$ hydrolase domain containing 14b	16.624	14.957	-10.026	0.0151
<i>Tarbp2</i>	TARBP2, RISC loading complex RNA binding subunit (a.k.a. PrBP)	15.433	13.893	-9.975	0.0337
<i>Clasrp</i>	CLK4-associating serine/arginine rich protein (a.k.a. CLASP, SFRS16, SRSF16, SWAP2)	54.974	49.735	-9.531	0.0337
<i>Zfp512b</i>	Zinc finger protein 512B (a.k.a. ZNF512b)	52.156	47.248	-9.410	0.0337
<i>Mtf2</i>	Metal response element binding transcription factor 2 (a.k.a. M96, PCL2)	28.200	25.661	-9.002	0.0288
<i>Mphosph9</i>	M-phase phosphoprotein 9 (a.k.a. MPP9)	17.364	15.892	-8.476	0.0155
<i>Hmgn3</i>	High mobility group nucleosomal binding domain 3 (a.k.a. TRIP7)	28.740	26.527	-7.700	0.0293
<i>Iqce</i>	IQ motif containing E	32.067	29.635	-7.582	0.0218
<i>Kmt2b</i>	Lysine (K)-specific methyltransferase 2B (a.k.a. MLL2, WBP7)	64.157	59.958	-6.544	0.0489
<i>Tnrc6a</i>	Trinucleotide repeat containing 6a (a.k.a. GW182)	121.310	113.487	-6.449	0.0166
<i>Srrm1</i>	Serine/arginine repetitive matrix 1 (a.k.a. SRM160)	108.441	101.786	-6.137	0.0192

CPM, counts per million

Supplementary Table 6. Oligos for guide RNAs

Guide	Nucleotide #s	Strand	Sequence (5' → 3') *
C	7203-7222	1	AAACccgcccattgattcatcttcc
C	7203-7222	2	CACCggaagatgaatcatgggcgg
D	8024-8043	1	AAACaaccacaaccaagggtgagggC
D	8024-8043	2	CACCGccctcacccttggtgggtt

* The capitalized nucleotides correspond to the recognition sites for the BbsI restriction enzymes. The lowercase nucleotides correspond to the guide sequences.

Supplementary Table 7. Primers used to generate a vector encoding GFP-SH2B18c

Primer	Direction	Sequence (5' → 3')
AarI	Forward	GCTGACGGATCCACCTGCGCTTG TCACTAAATGAG
HindIII (reverse complement)	Reverse	GGATCCGTCAGCAAGCTTTCAGGC CATGAATCCCCCAAAGG

Animal care

Mice were weaned at 3 weeks of age. Mice were fed standard chow or a HFD starting at 3 or 4 weeks of age, respectively. Mice included in standard chow experiments were individually housed at 5 weeks of age (leptin sensitivity experiments, Fig. 7B-D) or 9 weeks of age (all other experiments). Mice included in HFD experiments were individually housed at 4 weeks of age. Breeders were fed a standard chow containing 6.5% fat (#5008; LabDiet) or 9% fat (#5058; LabDiet). When fed *ad libitum*, mice were housed in cages with corncob bedding (Bed-o'Cobs ¼", #4B; The Andersons). When fasted, mice were temporarily housed in cages with corn-free natural soft cellulose bedding (Comfort Bedding; BioFresh) to prevent them from eating the bedding and thus disrupting their fasting.

Generation of $\alpha\delta$ KO mice

The reverse complement of the genomic *Sh2b1* sequence in C57BL/6J mice (GenBank accession #NC_000073, GRCm38) was used when designing CRISPR reagents. The selected guides were designed to direct Cas9 to cut close to the ends of the desired deleted regions (Fig. 1B, top) and have a low likelihood of off-target binding. Two sets of RNA guides, C and D, were selected. Guide D is located 3' of the α stop codon at the 3' end of the deleted region. Guide C was used alone or Guides C and D were used together. The guides were expressed using the pX330 vector [1, 2], which contains a chimeric guide RNA expression cassette and an hSpCas9 expression cassette. Sense and anti-sense oligos (Supplementary Table 6) corresponding to either Guide C or Guides C and D were annealed and subcloned between the two Bbs1 sites in the pX330 vector. The resulting construct was expressed in DH5 α cells. Colonies were selected, DNA isolated, and the sequence verified in the region of the inserted guide sequence. The pX330

plasmid containing the guide sequence and the Zero blunt TOPO vector containing the donor template were purified as previously described [3].

The guide(s) and donor template were tested in blastocysts of C57BL/6J x SJL F2 mice. Sequencing of DNA from blastocysts showed that 6 of 20 blastocysts (30%) injected with Guide C and 16 of 30 blastocysts (53%) injected with both Guides C and D contained the correctly edited *Sh2b1* sequence. Of the 17 pups born from mice implanted with oocytes injected with Guide C, 3 (18%) contained the correctly edited *Sh2b1* sequence and 14 were WT. Of the 58 pups born from mice implanted with oocytes injected with both Guides C and D, 7 (12%) contained the correctly edited sequence, 2 had insertions/deletions, and 52 were WT. DNA sequencing revealed germline transmission from all founders. All experiments were performed using progeny of one founder generated from Guide C.

Body weight and food intake

Body weight was assessed starting at 4 weeks of age. Food intake was determined weekly starting at 10 weeks of age for standard chow experiments or twice weekly starting at 5 weeks of age for the HFD experiment. Food intake was determined by weighing the food remaining in the cage and subtracting the value from the weight of the food initially added to the hopper. Following determination of food intake, old food was removed and fresh food was added. Mice were excluded from food intake data analysis if one or more weekly measurement was missing and/or they were identified as outliers by the following rule: their final cumulative food intake value was greater than two standard deviations from the mean. This quantitative identification of outliers matched with qualitative notes from individuals blinded to mouse genotypes that

identified “extreme nibblers”—mice that ground food pellets into powder and spread it around their cage, making it difficult to accurately measure their food intake.

Glucose tolerance tests

When HFD-fed mice were subjected to glucose tolerance tests, six WT mice reached the maximum value of the glucometer range (600 mg/dL) at one or more time points. As a result, the average WT curve depicted (Fig. 5G) is lower than it would be if the meter had a higher maximum value.

qPCR

Before reverse transcription, RNA quality was confirmed using a Nanodrop spectrophotometer. qPCR was performed with an Eppendorf Realplex2 using Mastercycler software and PCR parameters recommended by Applied Biosystems. Three reference genes (*36b4*, *Gapdh*, *Tbp*) were used for all assays. All cDNA samples were analyzed in triplicate and a non-template control was included for each gene of interest. Cycle threshold (Ct) values were normalized to the geometric mean of the Ct values of the reference genes, as in Vandesompele et al. [4]. The expression of the reference genes did not differ between the control and experimental samples (data not shown).

RNA-seq and data analysis

RNA samples were treated with DNase I. RNA was assessed for quality using Agilent 4200 TapeStation. RNA samples were enriched for mRNA transcripts using NEBNext Poly(A) mRNA Magnetic Isolation Module (#E7490; New England BioLabs). cDNA libraries were prepared

from mRNA using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (#E7760; New England BioLabs). Libraries were assessed for quality and quantity using Agilent TapeStation and qPCR by KAPA Library Quantification Kit for Illumina Platforms (#KK4835; Kapa Biosystems). Libraries were sequenced on the Illumina NovaSeq 6000 S4 Flow Cell for 200 cycles with 100 base pair paired-end reads.

FASTQ files were inspected using FastQC 0.11.7 [5] to ensure quality reads. Fastq_quality_filter (from the fastx_toolkit 0.0.14) [6] was used to remove data with Phred score < 20. Reads that made it through filtering were then aligned to the genome using STAR 2.5.3a_modified [7] and mouse genome GRCm38 (version 92). Count tables were generated using STAR with flag --quantMode GeneCounts. Count tables were analyzed in R 3.5.2 [8] and another round of quality control was performed. Samples were inspected for library size (all samples had > 20 million mapped reads), downregulation of *Sh2b1* in $\alpha\delta$ KO samples, and dimension reduction for detection of batch effects. RNA samples were prepared in multiple batches, which were accounted for in the analysis. Differential expression of genes was determined using DESeq2 1.22.2 [9]. GO analysis was performed using PANTHER DB [10]. *Sh2b1* transcripts were assembled using Ensembl annotation (version 92) [11]. Novel *Sh2b1* transcripts were identified using StringTie 1.3.6 [12]. WT and $\alpha\delta$ KO samples were merged during StringTie analysis. Read sequences misaligned by StringTie were excluded from further analysis. Misalignments were identified by their robust outlier quality (e.g., all samples of the same genotype measured 0 counts per million except one sample, which measured 11 counts per million). Statistics for differential gene expression and GO analysis were calculated using the default parameters of DESeq2 and PANTHER DB, respectively. $P < 0.05$ was considered

significant. Differentially expressed genes were visualized using ggplot2 3.3.0 [13] in RStudio [14].

To identify phenotype enrichment of differentially expressed genes, the differentially expressed genes were used to query the MouseMine database through the python Application Programming Interface (<http://www.mousemine.org/mousemine/>). The associated phenotype corresponds to the MouseMine category “ontologyTerm.name”. To determine significant enrichment, all protein-coding genes for which a P value was measured by DESeq2 were fed into the same MouseMine query. Enrichment significance of phenotypes in differentially expressed genes versus all genes was determined using prop.test() in R 3.6.3. P values were corrected for multiple comparisons using the Benjamini-Hochberg method.

To determine cell type enrichment of differentially expressed genes, data from GSE87544 was downloaded from GEO as a count matrix (GSE87544_Merged_17samples_14437cells_count.txt.gz) and metadata (GSE87544_1443737Cells.SVM.cluster.identity.renamed.csv.gz). Data were imported into R and analyzed with Seurat 3.1.5 [15, 16]. Count data was scaled with NormalizeData() followed by ScaleData(), then averaged by cell type as defined by the GSE87544 metadata. Heatmap and dendrogram were plotted with ggplot2 3.3.0 and ggdendro 0.1-20.

Plasmid expressing GFP-SH2B18c

The genomic sequence of *Sh2b1* between the end of exon 8 and the start of exon 9 (nt 6658-7181) was isolated from a pCR-Blunt II-TOPO vector (Invitrogen) containing a segment of genomic *Sh2b1* sequence corresponding to nt 4579-8807 using primers containing the AarI or the HindIII restriction sites (primers listed in Supplementary Table 7) and PfuUltra High-Fidelity

DNA Polymerase (#600380; Agilent). cDNA encoding mouse SH2B1 γ [17] was subcloned into pEGFPC1 (Clontech). The resulting vector encoding GFP-SH2B1 γ was then cleaved with the AarI and HindII restriction sites and the novel PCR product (*Sh2b1* nt 6658-7181) was inserted into the GFP-SH2B1 γ vector. The sequence was confirmed by the University of Michigan DNA Sequencing Core.

PC12 cell neurite outgrowth assay

PC12 cells were plated in tissue culture dishes coated with rat tail type I collagen (#354236; Corning) in RPMI 1640 (#A10491-01; Gibco) supplemented with 10% horse serum (HS) (#16050114; Gibco) and 5% fetal bovine serum (FBS) (#S11150; Atlanta Biologicals). For the neurite outgrowth assay, PC12 cells were plated in 6-well collagen-coated plates and transiently transfected with the indicated construct for 24 hours using Lipofectamine LTX (#15338030; Invitrogen). Cells were treated with 25 ng/mL mouse NGF 2.5S (BT.5025; Envigo) in RPMI 1640 medium containing 2% HS and 1% FBS. After 2 days, GFP-positive cells were visualized by fluorescence microscopy (20X or 40X objective, Nikon Eclipse TE200) and scored for the presence of neurites ≥ 2 times the length of the cell body. N: 3 experiments (300 cells per condition counted in 1st replicate; 200 cells per condition counted in 2nd and 3rd replicates).

Immunoblotting

Blots were incubated overnight at 4°C with mouse monoclonal antibody to SH2B1 (1:1,000) (#sc-136065; Santa Cruz) or β -tubulin (1:1,000) (#sc-55529; Santa Cruz) or ERK1/2 (1:1,000) (#4695S; Cell Signaling) in 10 mM Tris, 150 mM NaCl, pH 7.4, 0.1% Tween 20, and 3%

ovalbumin from chicken egg white, followed by IRDye-800CW goat anti-mouse IgG secondary antibody (1:20,000) (#926-32210; Li-Cor) for 1 hour at room temperature.

Supplemental Material References

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