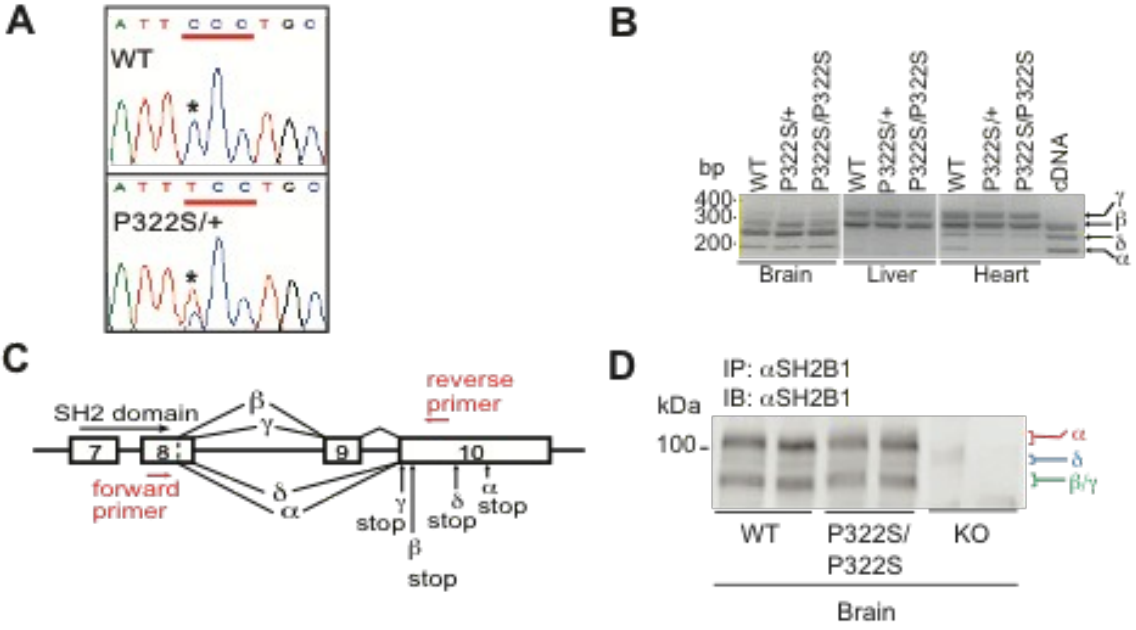
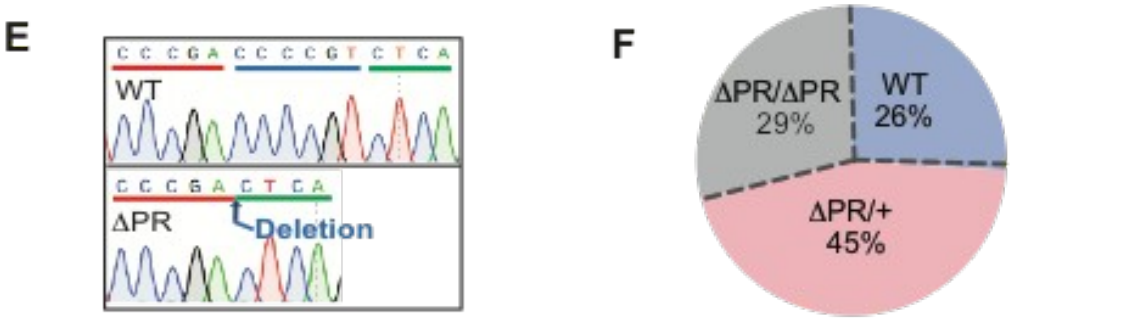
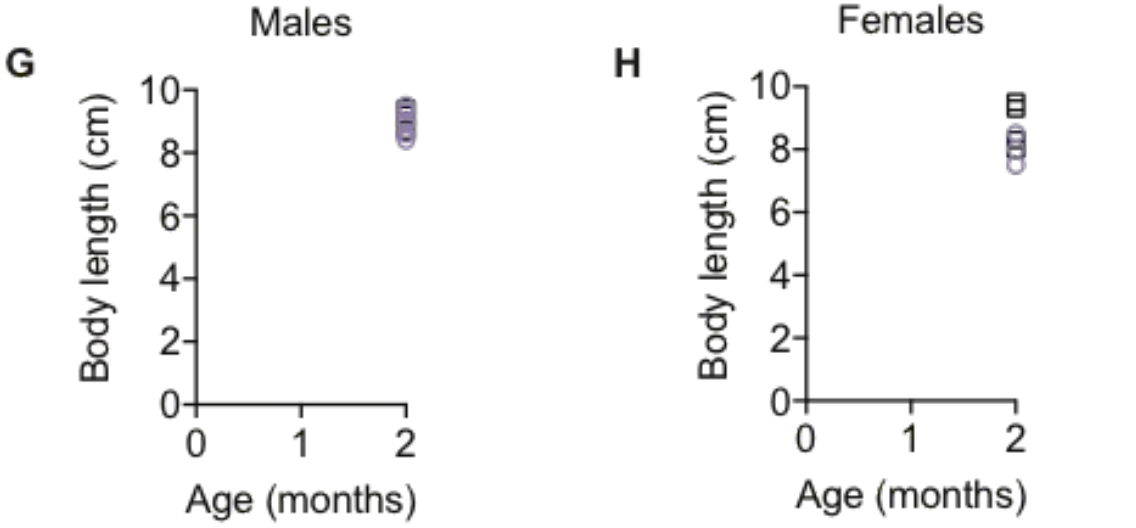
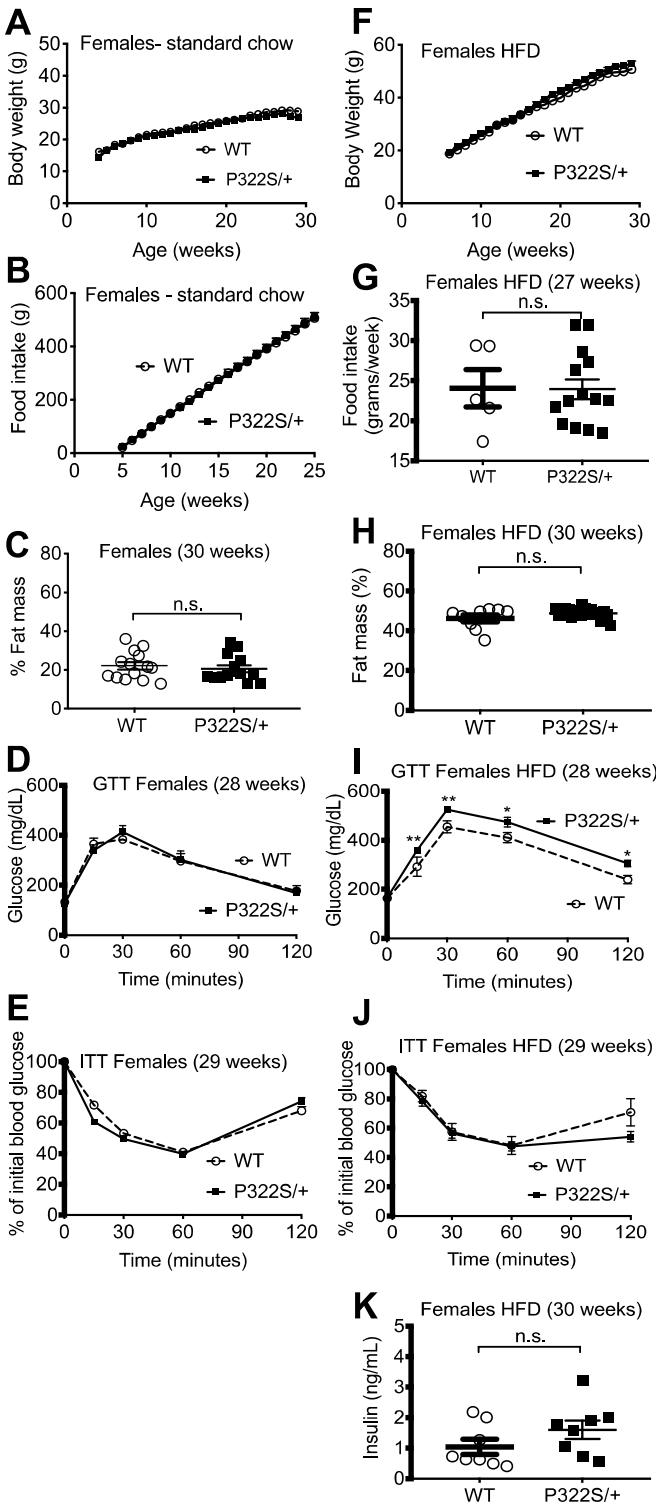
Online Supplementary Data







Supplementary Figure S1: Validation of the P322S and ΔPR mice. A) Sequencing of DNA from mice from the N1 generation confirms germline transmission of the SH2B1 P322S mutation. The relevant region of DNA sequence from P322S/+ and WT littermates are shown. The codons for P322 (upper panel) and S322 (lower panel) are underlined. The peaks corresponding to the C >T mutation are denoted by asterisks. **B**) mRNA was extracted from brain, liver, and heart tissue of SH2B1 WT, P322S/+, and P322S/P322S 31-week old male mice. The migration of DNA standards (left) and isoform-specific PCR products (right) are shown. **C**) Location of forward and reverse primers used for PCR in Panel S1B. **D**) Proteins in lysates from brain tissues from WT, P322S/P322S and KO mice were immunoprecipitated using αSH2B1 and immunoblotted with αSH2B1. The migration of the 100 kDa protein standard (left) and four known isoforms of SH2B1 (right) are shown. **E**) The relevant DNA sequences from ΔPR/ΔPR and WT littermates are shown. The sequence corresponding to the codons for P317, R318 is underlined in blue. The location of the missing codons for P317, R318 in the *Sh2b1* ΔPR/ΔPR mice is indicated by an arrow. **F**) The ΔPR mice are born at the expected (1 out of 4) Mendelian ratio. n = 183 mice. **G** and **H**) Body length was assessed at weeks 8-10. Males n=7 (WT), 9 (ΔPR/ ΔPR) and females n=5 (WT), 5 (ΔPR/ ΔPR).



Supplementary Figure S2: The P322S SH2B1 mutation leads to impaired glucose homeostasis in female mice challenged with a HFD. Panels A-E: female mice fed standard chow. **A**) Body weight was assessed at weeks 4-29. n=10 (WT), 14 (P322S/+). **B**) Food intake was assessed at weeks 5-25 and cumulative food intake graphed. Females n=7 (WT), 9

(P322S/+). **C**) Body fat mass was determined at week 30. Percent fat mass was determined by dividing fat mass by body weight. n=14 (WT, P322S/+). **D**) GTT was assessed at 28 weeks. After a 4-hour fast (9:00 – 13:00), mice were injected intraperitoneally with D-glucose (2 mg/kg of body weight). Blood glucose was monitored at indicated times. n=9 (WT), 14 (P322S/+). **E**) ITT was assessed at 29 weeks. After a 6-hour fast (8:00 – 14:00), mice were injected intraperitoneally with insulin (1 IU/kg of body weight). Blood glucose was monitored at indicated times. n=9 (WT), 13 (P322S/+). Panels **F-K**: female mice fed a HFD: **F**) Starting at week 6, body weight was assessed weekly. n=9 (WT), 20 (P322S/+). **G**) Food intake was measured during week 27. n=5 (WT), 14 (P322S/+). **H**) Body fat mass was determined at week 30. n=9 (WT), 20 (P322S/+). **I**) GTT was assessed at 28 weeks. Mice were fasted for 4 hours (9:00-13:00) and then injected intraperitoneally with D-glucose (2 mg/kg of body weight). Blood glucose was monitored at times indicated. Females: n=9 (WT), 20 (P322S/+). **J**) ITT was assessed at 29 weeks. Mice were fasted for 6 hours (8:00 – 14:00) and then injected intraperitoneally with human insulin (1 IU/kg of body weight). Blood glucose was monitored at the times indicated. n=9 (WT), 20 (P322S/+). **K**) At week 30, mice were fasted overnight and insulin levels were determined. n=8 (WT, P322S/+). For all comparisons: Means ± SEM, *P<0.05, **P<0.01, n.s. = not significant.

Supplementary Table S1. Oligo / Primer Table

Description	Location of guide / primer sequence *	Oligo / primer
guide 1, reverse complement	nt 3325-3344	5'-AAACcgtaggcgtcccgaccccgtC-3' †
guide 1, guide sequence strand	nt 3325-3344	5'-CACCGacggggtcgggacgcctacg-3' ‡ §
Guide 2, reverse complement	nt 3337-3356	5'-AAACgaccccgtctcagcattccc-3'
Guide 2, guide sequence strand	nt 3337-3356	5'-CACCgggaatgctgagacggggtc-3'
Donor (sense strand)	nt 3264-3443	5′-gaatggagggagatacagttagatttgacacaccaaaaactgattcttcttccctttcccgcgtaggcgtctagacctagactcagcatttcctgctctactattactgatgtccgcacagccacagccctagagatgcctgacagggagaacacgtttgtggttaaggtaggaacccca-3'
Donor (antisense strand)	nt 3264-3443	5'-tggggttcctaccttaaccacaaacgtgttctccctgtcaggcatctctagggctgtggctgtgcggacatcagtaatagtagagcaggaaatgctgagtctaggtctagacgcctacgcgggaaagggaagaagaatcagtttttggtgtgtcaaatctaactgtatctccctccattc-3'
Genotyping SH2B1 P322S and ΔPR mice	nt 3156-3185	5'-tattgctgtcctgggttcagtgctaactgt-3'
	nt 3518-3547	5'-aagactcaaagcccccgacatatactcatc-3'
Genotyping SH2B1 KO mice	nt 6280-6303	5'- ggaggcactggctcccatggtgtc-3'
	nt 6460-6483	5'-gcaggatgacaagtgaggtgggag-3'
	From neo cassette	5'-attcctcccactcatgatctatagatc-3'
SH2B1 gene expression	nt 2387-2410	5'-ttcgatatgcttgagcacttccgg-3'
	nt 2651-2671	5'-gcctcttctgccccaggatgt-3'

* Nucleotide location in reverse complement of genomic *Sh2b1* (accession # NC_000073, GRC m38). † AAAC overhang for subcloning into a Bbs1 site with a C for binding to the complementary G. ‡ CACCG overhang for subcloning into the second Bbs1 site with a G for initiating transcription by U6 polymerase. § guide sequence is in lower case.

Supplementary Data

Generation of SH2B1 P322S and ΔP317, R318 mice

Two sets of RNA guides (Guides 1 and 2) that cut within 40 nt of the codon for P322 and had a high inverse likelihood of off-target binding were tested. The oligos required for expressing the guides (Supplementary Table S1) (Integrated DNA Technologies IDT) were subcloned into the chimeric guide RNA expression cassette in the pX330 vector, which also contained a hSpCas9 expression cassette (1,2). The sequence in the region of the guide sequence was verified. The donor to direct homology-directed repair (HDR) spanned the 180 nt region from nucleotide 3264-3443 of *Sh2b1*(from IDT). The donor template introduced the C>T mutation into the codon for P322 and silent mutations to create a diagnostic Xbal site and disrupt guide RNA binding following HDR (Fig. 1C). The donor and the pX330 plasmid containing the guide sequence were purified initially using the University of Michigan Transgenic Animal Model Core procedure and subsequently Qiaprep miniprep columns (Qiagen, # 27106) using filtered buffers (Anotop 0.02 μm filters, Whatman, # 6809-1002). The University of Michigan Transgenic Animal Model Core tested the purified donor and guides in blastocysts. They then injected each guide/donor combination into ~75 oocytes from C57BL/6J mice, and implanted the oocytes into C57BL/6J mice. The resulting pups were genotyped and the sequence confirmed by DNA sequencing. Guide 1 produced 4 wild-type (WT) pups and 1 pup containing an indel. Guide 2 produced 2 pups containing the P322S edit and 4 pups containing indels. The same Guide 2 founder was used for all experiments. N2 mice were used for experiments in Fig. 2A-G and Supplementary Fig. S2A-E, N5 mice for Fig. 2H-M and Supplementary Fig. S2F-K. One of the indels encoded SH2B1 ΔP317, R318 (ΔPR). N3 mice were used for experiments in Figs. 4, 5A, 5C-E, N4 mice for experiments in Figs. 5B and 6.

Gene expression

Total RNA was extracted using TRIzol Reagent (Ambion, Life Technologies). cDNA was synthesized from the RNA using Taqman Reverse Transcription Reagent Kit (Applied Bio-Systems, # N808-0234). PCR was performed using GoTaq DNA polymerase (Promega, Catalog #M3005). Primers for genotyping SH2B1 P322S and ΔPR mice (Supplementary Table S1) and the PCR parameters (95°C 2 minutes, 95°C 15 seconds, 63°C 30 seconds, 72°C 1:20 minutes, repeat 42 cycles, 72°C 4 minutes, 4°C) were chosen to optimize detection of all four SH2B1 isoforms.

Mouse body weight and food intake

Body weight was assessed weekly at the same time and day of the week. To assess food intake, food was added and weighed at the start of each week. At the end of the week, the food that remained was removed and weighed, including any food in the bedding. Bedding was changed each week. Mice that nibble on the food but do not necessarily eat it, were excluded from the study. Food intake shown is cumulative intake. Food intake, glucose tolerance tests, insulin tolerance tests, and blood draws for determination of hormone levels were also performed on these cohorts of mice.

Mouse body length

Body length was measured in mice anesthetized with isoflurane in order to avoid inaccuracy from movement. Body length was measured with a ruler from tip of nose to middle of anus as previously described in (3).

Plasmids

GFP-tagged rat SH2B1β (4) and SH2B1β P322S (5) (GenBank accession number NM_001048180) have been described previously. The ΔPR deletion was introduced into GFP-SH2B1β using a QuikChange II site-directed mutagenesis kit (Stratagene) and the deletion confirmed by sequencing. For Fig. 1B, human SH2B1β in pcDNA3.1(+) (GenBank accession number NM_015503) was purchased from Origene (# RC222333) and GFP from Addgene (plasmid no. 13031). Human SH2B1β was mutated using QuikChange II site-directed mutagenesis and the sequence confirmed by Sanger sequencing (3730 DNA Analyser, Thermo Scientific).

Neurite outgrowth

For Fig. 1B, PC12 cells (from Harvey McMahon, Laboratory of Molecular Biology, Cambridge, UK) were grown in suspension and maintained in PC12 growth medium B (RPMI growth medium, 7.5% FBS, 7.5% HS). The PC12 cells were transferred into serum-free Opti-MEM medium (Thermo Scientific) and passed through a 21G needle four times to break up lumps of cells. The cells were seeded into collagen IV (Corning, #354429) coated 96-well plates at a density of 2500 cells per well. The next day, cells were co-transfected with 50 ng/well of each construct encoding GFP and either WT or mutant SH2B1β using Lipofectamine 2000 (Thermo Scientific). Four hours later, rat NGF (20 ng/ml) (R&D Systems) was added. After 72 hours, cells were photographed (EVOS FL Cell imaging system, ThermoFisher Scientific) under blue laser and the number of GFP-expressing cells were assessed for the presence of neurites greater than two times the length of the cell body using the ImageJ (6) 'Cell Counter' plugin (https://imagej.nih.gov).

Supplementary Data References

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