Cellular and Molecular Life Sciences

In Vivo Regulation of the A Disintegrin And Metalloproteinase 10 (ADAM10) by the Tetraspanin 15

Supplementary Material

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Supplementary figure S1

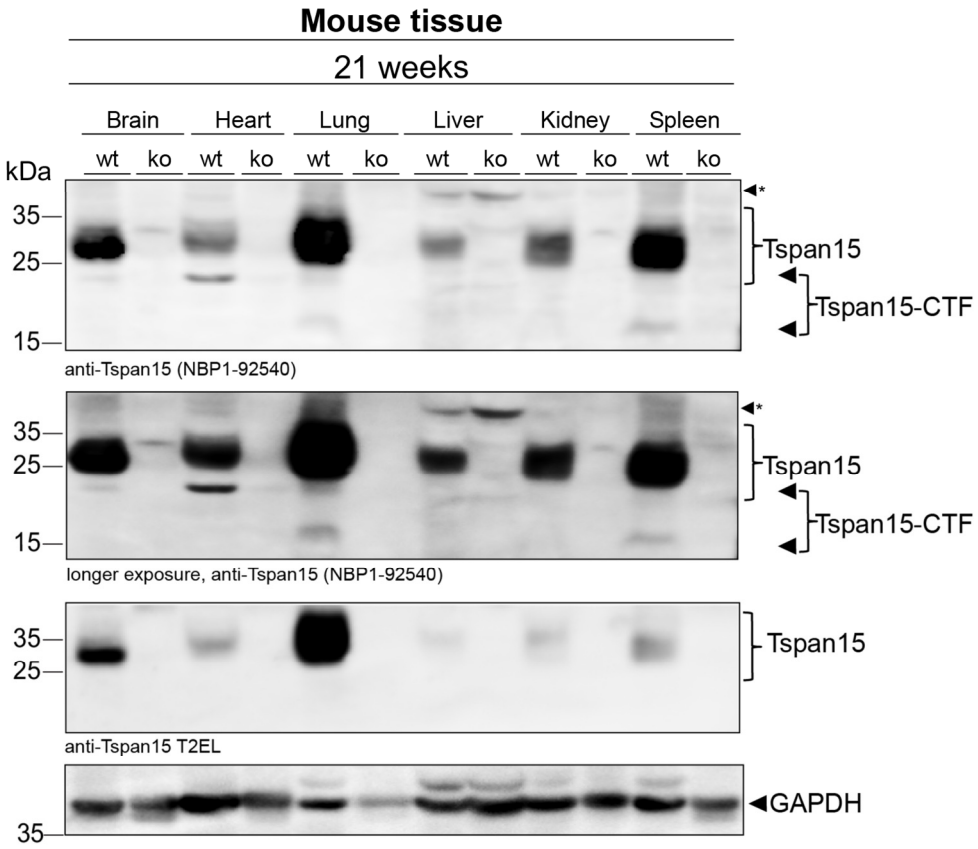
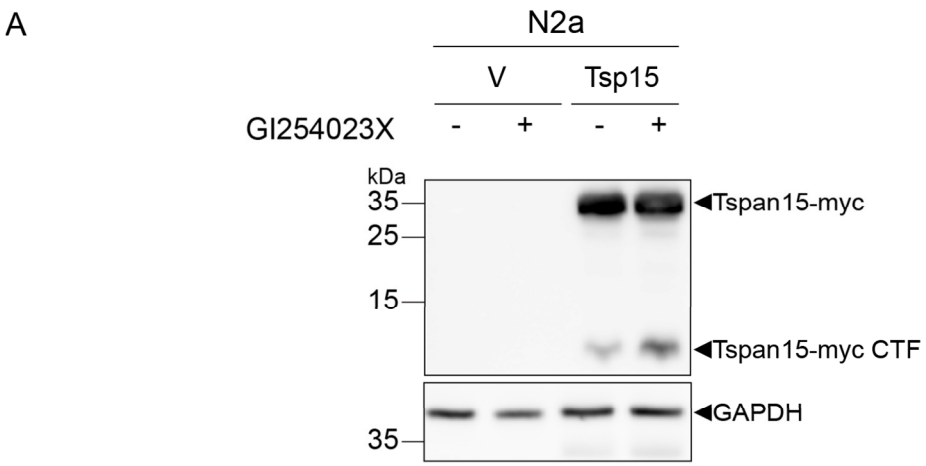


Figure S1: Expression of Tspan15 in different mouse tissues.
Tspan15 expresseion was analysed in indictaed organs of a 21-weeks-old wild-type and a Tspan15 knockout (ko) mouse. Using a commercial C-terminal Tspan15 antibody (NBP1-92540) Tspan15 expression was detecetd in all tested wild-type tissues, but not in the respective knockout samples, confirming specificity of the Tspan15 antibody. After a longer exposure time, additional signals at a molecular weight of 18-20 kDa were observed in wild-type brain, heart, lung and spleen, but not in the respective knockout controls and likely correspond to a Tspan15 carboxyterminal fragment (CTF). Similar to the commercial Tspan15 antibody (NBP1-92540), staining with our self-made Tspan15 antibody T2EL showed specific signals in all tested wild-type tissues, but not in the respective knockout samples. Light signals, likely corresponding to a Tspan15-CTF were observed in brain and heart. Protein loading was controlled by anti-GAPDH staining.

Supplementary figure S2



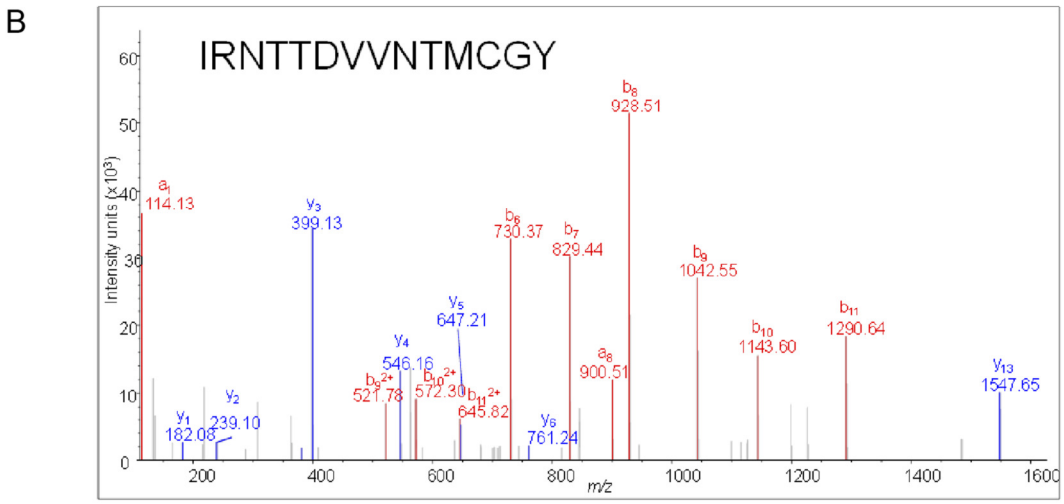
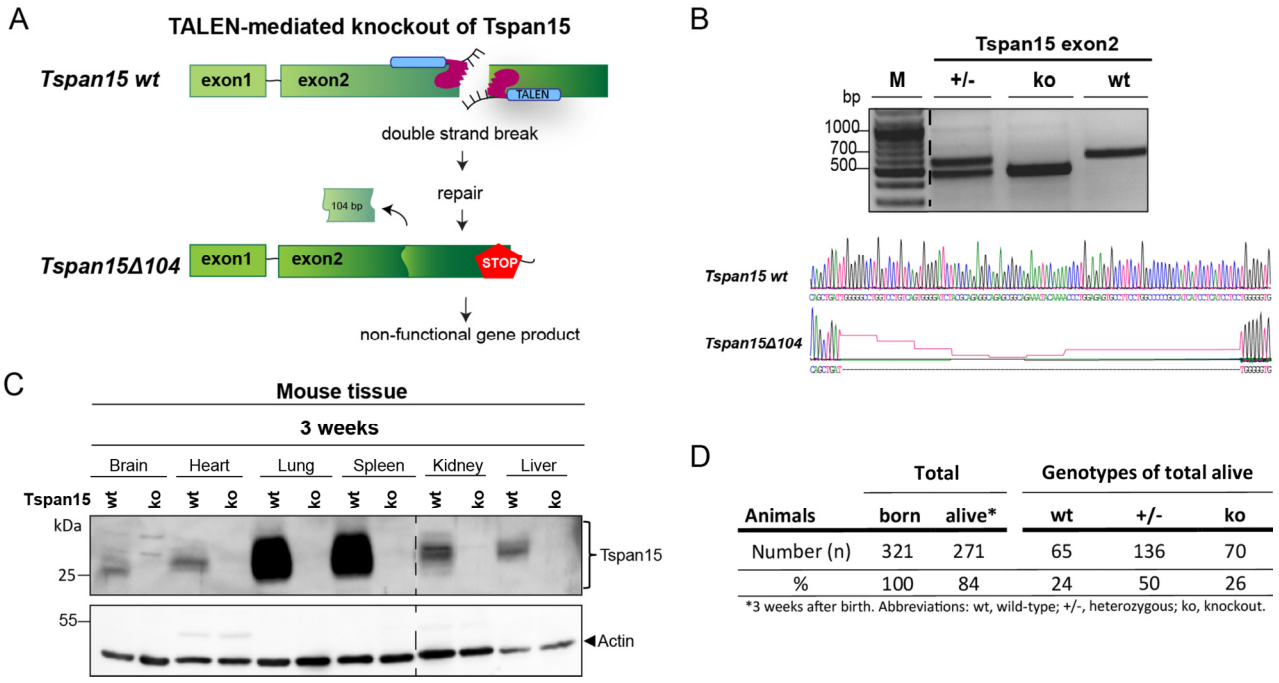


Figure S2: A) ADAM10 inhibition increases the appearance of the Tspan15 CTF in N2a cells. N2a Cells were transfected with a murine, myc-tagged Tspan15 (Tsp15) expression construct or an empty vector (V). Before harvesting, the cells were treated with 3 µM of the selective ADAM10 inhibitor GI254023X (+) or equal amounts of DMSO as control (-) for 24 h. After immunoblotting, Tspan15-myc and the Tspan15 carboxyterminal fragment (CTF) were observed in transfected cells. Application of the ADAM10 inhibitor did not reduce, but rather increased the appearance of the Tspan15 CTF. **B) MS/MS spectra of the peptide C.IRNTTDVVNTMCGY with a *m/z* of 844.8848 ($[M+2H]^{2+}$).** The mass of the peptide corresponds to a deamidated (0.98402 Da) asparagine residue (N189), a carbamidomethylated (57.02146 Da) cysteine residue, an oxidated (15.99492 Da) methionine residue and a dimethylated N-terminus.

Supplementary figure S3



	Total		Genotypes of total alive		
Animals	born	alive*	wt	+/-	ko
Number (n)	321	271	65	136	70
%	100	84	24	50	26

*3 weeks after birth. Abbreviations: wt, wild-type; +/-, heterozygous; ko, knockout.

Figure S3: Generation of Tspan15-deficient mice. A) Schematic drawing showing the Transcription Activator-Like Effector Nuclease (TALEN)-mediated knockout strategy for the generation of Tspan15-deficient mice. TALENs were designed to induce a site-specific double strand break within exon2 of the *tspan15* gene locus. Subsequent DNA repair led to a 104-base pair (bp) deletion mutation (*Tspan15Δ104*), which generated a premature translational stop codon at the end of *Tspan15* exon2. **B)** Agarose gel electrophoresis of PCR products amplified from genomic DNA obtained from Tspan15 heterozygous (+/-), knockout (ko) and wild-type (wt) littermate mice using a primer pair (forward and reverse) surrounding the sequence of *Tspan15* exon2. Sequencing chromatogram of the TALEN-targeted region within the wild-type (*Tspan15 wt*) and mutated Tspan15 exon2 (*Tspan15Δ104*). **C)** Immunoblot analysis of Tspan15 knockout efficiency in indicated organs. Tissue lysates of a wild-type (wt) and a Tspan15 knockout (ko) mouse were prepared and subjected to immunoblot analysis. Using an anti-Tspan15 (NBP1-92540) antibody, endogenous Tspan15 was detected in all wild-type tissues. No Tspan15-specific signal was observed in the respective knockout samples. **D)** Table showing the calculated survival rate and genotype distribution resulting from the Tspan15Δ104 breeding.

Supplementary figure S4:

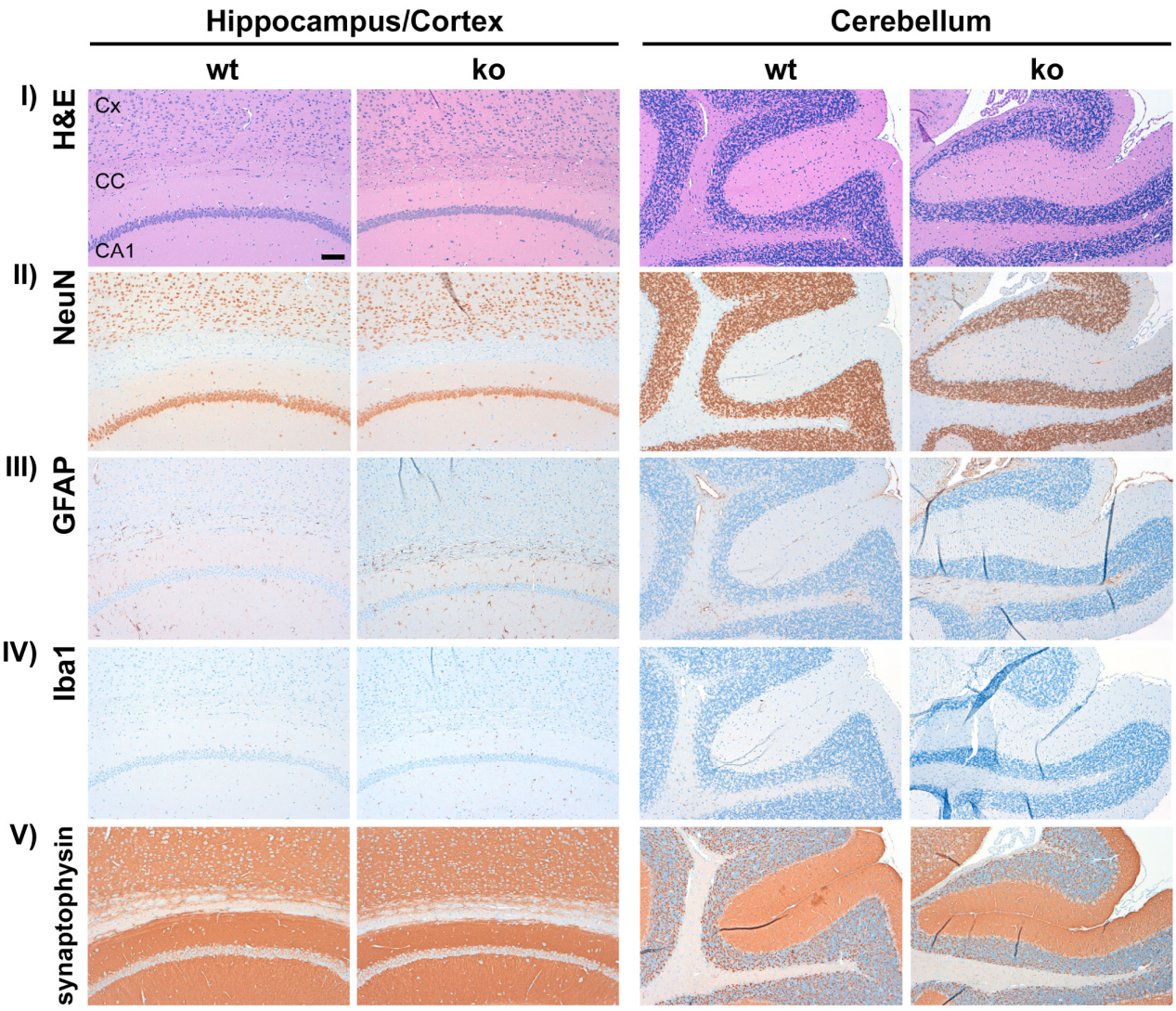


Figure S4: Histological analysis of the hippocampus in Tspan15-deficient mice. Representative IHC stainings of the hippocampal/cortex region and the cerebellum of three wild-type (wt) and Tspan15 knockout (ko) mice **I)** Hematoxylin and eosin (H&E) staining. **II-V)** Diaminobenzidine (DAB) staining of the neuronal neuclei antigen (NeuN, II), the glial fibrillary acidic protein (GFAP, III), the astroglia-specific protein ionized calcium binding adaptor molecule 1 (Iba1, IV) and Synaptophysin (V). Cx: cortex; CC: corpus callosum; CA1: CA1 layer of the hippocampus; Scale bar represents 100 µm.

Supplementary figure S5:

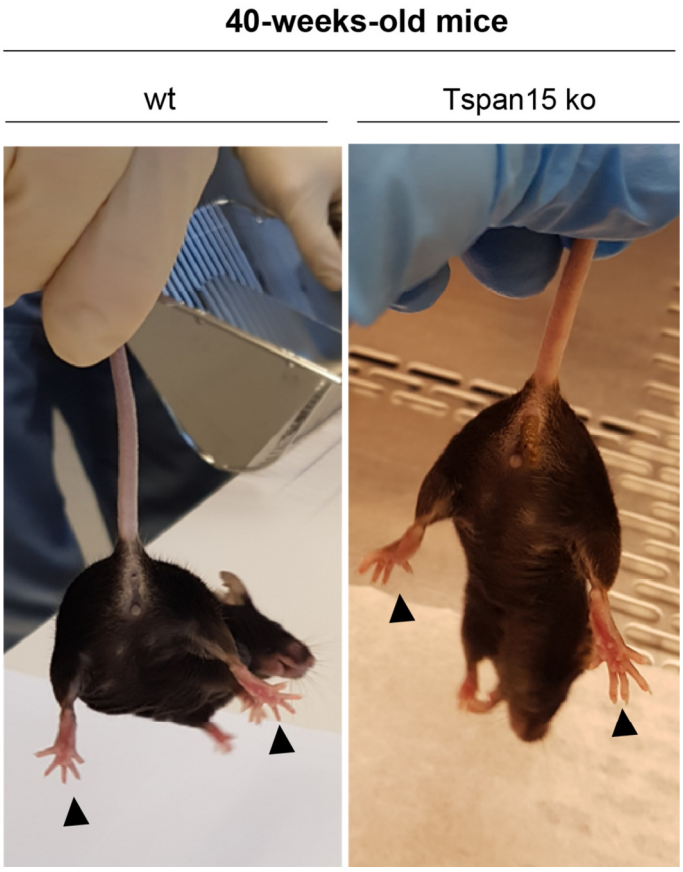


Figure S5: Tspan15 knockout mice do not show signs of a neurological phenotype. Hind limp clasping was analyzed in 10-month-old wild-type and Tspan15 knockout (ko) mice.

Supplementary table S1:

Table S1: Demographics of control and Alzheimer's disease patient samples.

Type	sample number	Age at death (years)	Gender	CERAD	Braak
Ctrl	1	68	m	/	/
	2	68	m	/	/
	3	68	f	/	/
	4	63	m	/	/
	5	86	m	/	/
AD	6	69	m	C	4-5
	7	88	m	C	4-5
	8	74	m	C	3-4
	9	79	m	B	5
	10	63	m	C	4-5
	11	88	f	B	4

Ctrl: Patients without diagnosed neurodegeneration, AD: Alzheimer's disease patients, f: female, m: male.

Supplementary movie legend

Video S1: Transport of Tspan15 in dendrites. Primary hippocampal cultures were transfected with hTspan15-YPET at day in vitro 15, and imaged after 24 h. Images were bleach corrected, slightly adjusted in contrast, and jpg compressed. 20 frames per seconds.