Molecular Medicine

Supplemental Data

TRPV1 Gates Tissue Access and Sustains Pathogenicity in Autoimmune Encephalitis

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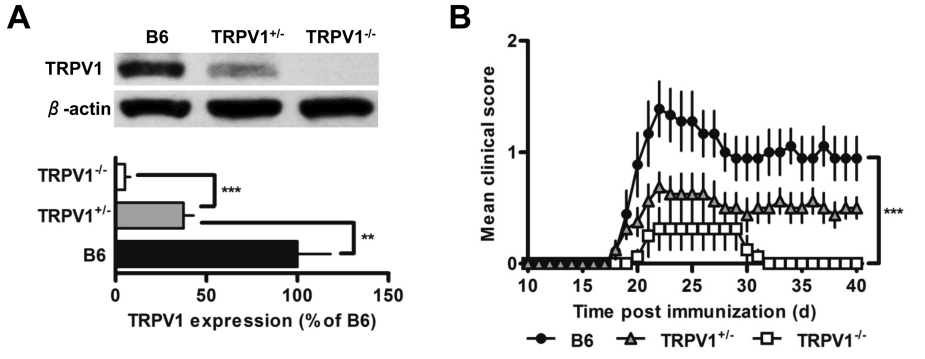
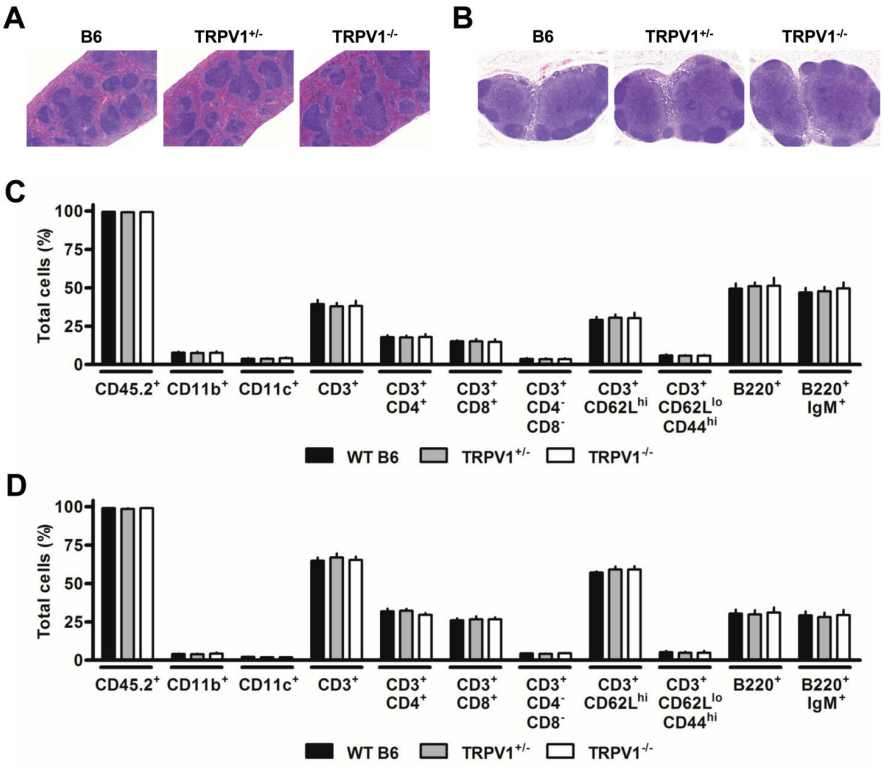
Supplementary Table S1. Summary of EAE severity in B6, TRPV1$^{+/-}$, and TRPV1$^{-/-}$ mice.

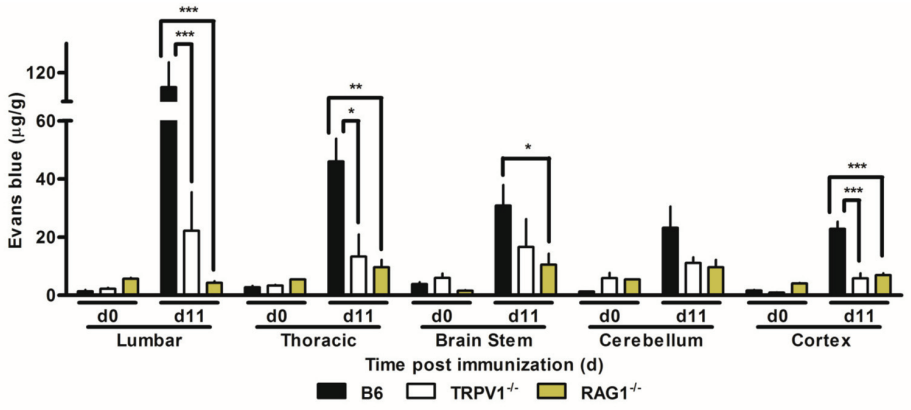
	Incidence	*Mean Day of Onset*	*Mean Maximum Score*
With Pertussis Toxin			
B6	20/20 (100%)	9.9 ± 0.390	4.425 ± 0.171
TRPV1$^{+/-}$	20/20 (100%)	10.8 ± 0.427	4.325 ± 0.196
TRPV1$^{-/-}$	19/20 (95%)	14.7 ± 0.831	2.675 ± 0.352
Without Pertussis Toxin			
B6	9/9 (100%)	19.4 ± 0.377	1.444 ± 0.256
TRPV1$^{+/-}$	7/8 (87.5%)	19.4 ± 0.571	0.750 ± 0.164
TRPV1$^{-/-}$	3/8 (37.5%)	21.0 ± 0.577	0.313 ± 0.187



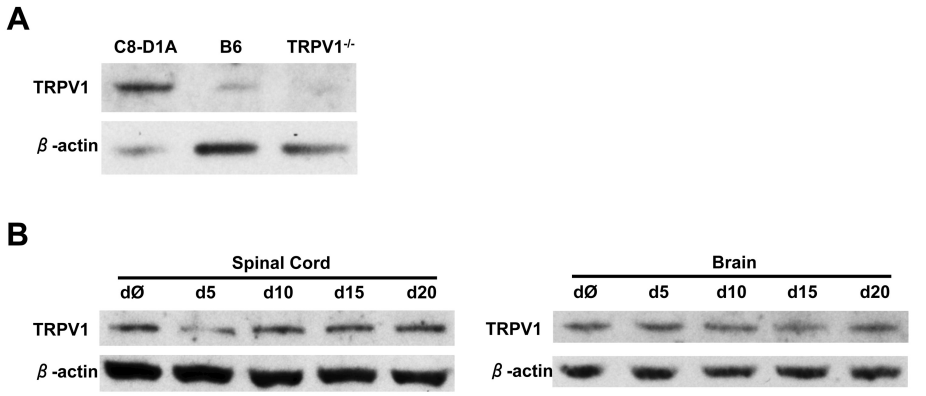
Supplementary Figure S1. TRPV1$^{+/-}$ mice progress to intermediate disease. (A) Representative Western blot of TRPV1 protein in the spinal cord of B6, TRPV1$^{+/-}$, and TRPV1$^{-/-}$ mice 6-8wk of age, with β-actin as a loading control. Quantification was performed by analysis of pixel density (n≥5 per group, **p<0.01, ***p<0.001). (B) Daily clinical scores of B6, TRPV1$^{+/-}$, and TRPV1$^{-/-}$ mice following immunization with MOG35-55 peptide without pertussis toxin (n≥8 per group, ***p<0.0001).



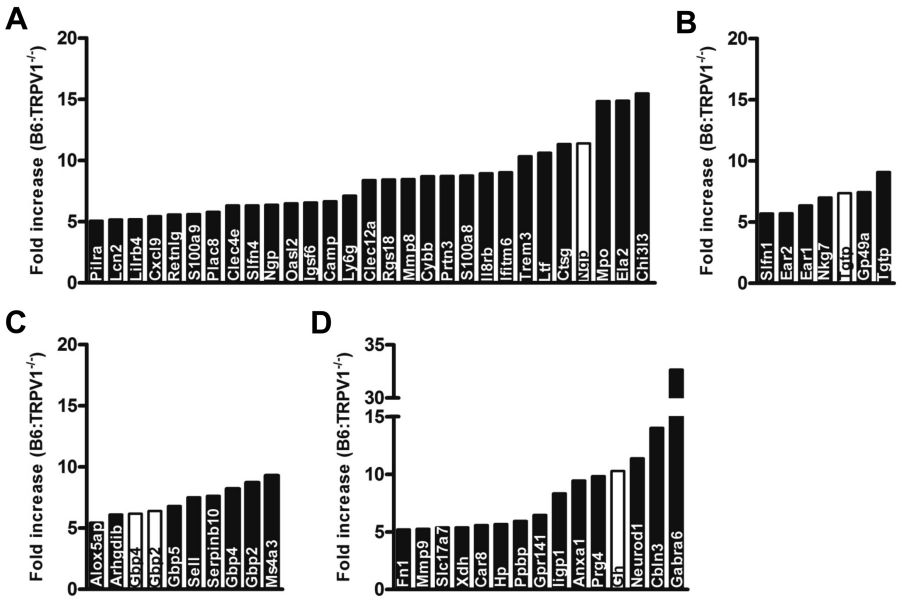
Supplementary Figure S2. TRPV1 congenic mice are immunocompetent. (A-B) Representative H&E stained spleen (A) and inguinal lymph node (B) sections from B6, TRPV1$^{+/-}$, and TRPV1$^{-/-}$ mice. (C-D) Haematopoietic composition of B6, TRPV1$^{+/-}$, and TRPV1$^{-/-}$ mice spleen (C, n=4 per group) and lymph node (D, n=4 per group).



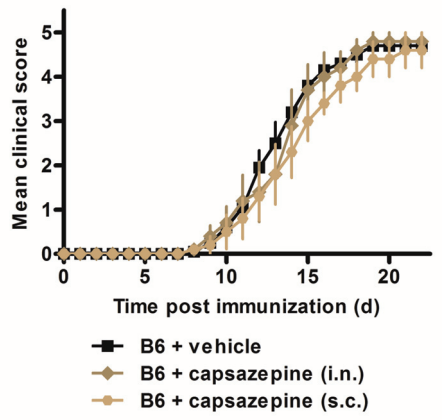
Supplementary Figure S3. Protection from BBB and BSCB breakdown in TRPV1$^{-/-}$ mice is comparable to RAG1$^{-/-}$ mice. Quantification of Evans Blue in regions of the spinal cord and the brain (n=5 per group, *p<0.05, **p<0.01, ***p<0.001) of B6, TRPV1$^{-/-}$, and RAG1$^{-/-}$ mice at indicated time points post-immunization.



Supplementary Figure S4. TRPV1 expression within the CNS during EAE. (A) Representative Western blot of TRPV1 protein from the C8-D1A cell line and isolated astrocytes from B6 and TRPV1$^{-/-}$ mice, with β-actin as a loading control. (B) Representative Western blot of TRPV1 protein during the course of EAE in the spinal cord and brain of B6 mice, with β-actin as a loading control.



Supplementary Figure S5. Gene expression differences between B6 and TRPV1$^{-/-}$ mice during early EAE. (A-D) Microarray results from the brain (white) and spinal cord (black) of B6 and TRPV1$^{-/-}$ mice with a cut-off set at 5-fold or greater. Genes are segregated into those largely expressed by macrophages, dendritic cells, and neutrophils (A), T cells, B cells, NK cells, mast cells, and eosinophils (B), pan-immune (C), or predominantly non-immune (D). Pilra: paired immunoglobin-like type 2 receptor alpha, Lcn2: lipocalin-2, Lilrb4: leukocyte immunoglobulin-like receptor subfamily B (with TM and ITIM domains) member 4, Cxcl9: chemokine (C-X-C motif) ligand 9, Retnlg: resistin like gamma, S100a9: S100 calcium binding protein A9, Plac8: placenta-specific 8, Clec4e: C-type lectin domain family 4 member E, Slfn4: schlafen 4, Ngp: neutrophilic granule protein, Oasl2: 2′-5′ oligoadenylate synthetase-like 2, Igsf6: immunoglobulin superfamily member 6, Camp: cathelicidin antimicrobial peptide, Ly6g: lymphocyte antigen 6 complex locus G, Clec12a: c-type lectin domain family 12 member A, Rgs18: regulator of G protein signalling 18, Mmp8: matrix metallopeptidase 8, Cybb: cytochrome b-245 beta polypeptide, Prtn3: proteinase 3, S100a8: S100 calcium binding protein A8, Il8rb: interleukin 8 receptor beta, Ifitm6: interferon induced transmembrane protein 6, Trem3: triggering receptor expressed on myeloid cells 3, Ltf: lactotransferrin, Ctsg: cathepsin G, Mpo: myeloperoxidase, Ela2: elastase 2, Chi3l3: chitinase 3-like-3, Slfn1: schlafen 1, Ear2: eosinophil-associated ribonuclease A family member 2, Ear1: eosinophil-associated ribonuclease A family member 1, Nkg7: natural killer cell group 7 sequence, Tgtp: T cell specific GTPase, Gp49a: glycoprotein 49 A, Alox5ap: arachidonate 5-lipoxygenase-activating protein, Arhgdlb: rho GDP dissociation inhibitor beta, Gbp4: guanylate binding protein 4, Gbp2: guanylate binding protein 2, Gbp5: guanylate binding protein 5, Sell: L-selectin, Serpinb10: serpin peptidase inhibitor clade B (ovalbumin) member 10, Ms4a3: membrane-spanning 4-domains subfamily A member 3, Fn1: fibronectin 1, Mmp9: matrix metallopeptidase 9, Slc17a7: solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter) member 7, Xdh: xanthine dehydrogenase, Car8: carbonic anhydrase 8, Hp: haptoglobin, Ppbp: proplatelet basic protein, Gpr141: G protein-coupled receptor 141, Iigp1: interferon inducible GTPase1, Anxa1: annexin A1, Prg4: proteoglycan 4, Gh: growth hormone, Neurod1: neurogenic differentiation 1, Cbln3: cerebellin 3 precursor, Gabra6: gamma-aminobutyric acid receptor subunit alpha-6.



Supplementary Figure S6. Early administration of the TRPV1 antagonist capsazepine does not reduce severity of EAE. Daily clinical scores of B6 mice following administration of capsazepine intranasally (i.n.), subcutaneously (s.c.), or vehicle alone, from d 0 to d 6 (n≥5 per group).