Supplementary Figure Legends

S1. S1PR2-deficient mice on a 129S/B6 background do not exhibit sexually dimorphism in BBB permeability. A. Western blot analysis of $S1PR_2$ in brain extracts from wild-type (WT) and $S1PR_2^{-/-}$ mice**. B.** Male (light and dark blue bars) and female (pink and red bars) $S1PR_2^{-/-}$ mice were examined for BBB NaFluorescein permeability within CTX, CB, and SC, normalized to serum values for individual mice (5-8 mice per group). Data are reported as arbitrary fluorescence values, normalized to mean $\pm$ S.E.M. values for naïve male C57BL/6 in each CNS region.

S2. Sexually dimorphic expression of S1PR2 in SJL cerebella is not controlled by the Y chromosome. Protein lysates derived from CNS regions of naïve male and female SJL and male SJL carrying a B10.S Y chromosome ($SJL.Y^{B10.S}$) were probed for S1PR2 (red), with β-Tubulin (green) probed as a loading control. Data are reported as density values of S1PR2 normalized to density values for β-Tubulin. Group data are normalized to mean values for normal male SJL mice in each CNS region. Data are mean values of 3-4 mice per group. Error bars represent +/- 1 SEM. All data were analyzed via 1-way ANOVA. *$p<0.05$.

S3. Sex and strain differences in BBB permeability are not due to dimorphic expression of Claudin-5. Protein lysates derived from CNS regions of naïve male and female C57/Bl6 and SJL mice were probed for S1PR2 (red), with β-Tubulin (green) probed as a loading control. Data are reported as density values of Claudin-5 normalized to density values for β-Tubulin. Group data are normalized to mean values for male C57/Bl6 mice in each CNS region. Data are mean values of 3 mice per group. Error bars represent +/- 1 SEM. All data were analyzed via 2-way ANOVA.

S4. Sexually dimorphic expression of S1PR2 in female SJL cerebella is not controlled by gonadal hormones. A. Representative images of uterine horns and upper 1/3 of the vagina of placebo- versus 17β-estradiol(17βE2)-treated, ovariectomized female SJL mice, and sham-operated, placebo- and 17βE2-treated, ovariectomized female SJL mice after immunization with PLP peptide (PLP immunization). **B.** Mean distribution of the uterine weights and upper 1/3 of the vagina in same groups of mice as in **(A)**. Uterine weights of 17βE2-treated, ovariectomized SJL females with or without PLP immunization were significantly increased compared with sham and placebo tissues, as assessed via one-way ANOVA; $p < 0.0001$, followed by Bonferroni post-hoc test, for 5 mice per group. **C.** Sham- and placebo-treated, PLP-immunized mice exhibited similar cumulative body weights while 17βE2-treated, PLP immunized SJL females differ significantly. One-way ANOVA; $p<0.0001$, followed by Bonferroni post-hoc test, for 5 mice per group. **D.** Mean of cumulative clinical scores for sham-ovariectomized, and placebo-, and 17β-estradiol-treated, ovariectomized mice after PLP immunization. Note that 17β-estradiol-treated, ovariectomized mice do not develop EAE. One-way ANOVA $p < 0.0001$, followed by Bonferroni post-hoc test, for 5 mice per group. **E.** Protein lysates derived from CNS regions of sham and ovariectomized naïve SJL female mice and from those immunized with PLP, with and without 17β-estradiol (17βE2) replacement, were probed for S1PR2 (red), with β-Tubulin (green) probed as a loading control. Data are reported as density values of S1PR2 normalized to density values for β-Tubulin. Group data are normalized to mean values for normal male SJL mice in each CNS region. Data are mean values of 5 mice per group. Error bars represent +/- 1 SEM. All data were analyzed via 1-way ANOVA.

S5. Inhibition of $S1PR_2$ signaling does not improve EAE progression in male SJL. **A.** Clinical score of male SJL immunized with PLP and treated with either vehicle (black) or 1.5 mg/kg JTE-013 (white) when mice reached a score of 2, for 10 consecutive days (gray bar). **B.** Mean of highest severity score and **(c)** mean of cumulative scores of mice in **(A)**. Tx: treatment; error bars ± 1 S.E.M.

S6. Pharmacological blockade of $S1PR_2$ signaling does not enhance retention of body weight during EAE. A-C. Body weight of SJL immunized with PLP and treated with either vehicle (black) or 1.5 mg/kg JTE-013 (white) when mice reached a score of 2, for either **(A)** females treated 10 or **(B)** 30 consecutive days or **(C)** males treated for 10 consecutive days (gray bar). **D.** Transformed body weight of WT (black) or $S1PR_2$ -/- or wild type mice immunized with MOG. Error bars ± 1 S.E.M.

S7. $S1PR_2$ inactivation ameliorates alterations in BBB permeability. A. Immunohistochemical detection of albumen in the CTX, CB and SC of vehicle- (veh) versus JTE0-13-treated female SJL mice with EAE at peak of disease. Images are representative of 3 images each from 4-5 mice/group. **B.** Immunohistochemical detection of albumen in the CTX, CB and SC of wild-type (WT) versus $S1PR_2$ -/- mice mice. Images are representative of 3 images each from 3-4 mice/group. Scale bars = 50 μm.

S8. S1P dysregulates CNS endothelial barrier structure and function independent of S1PR1, Rac1, clathrin-dependent endocytic, and macropinocytic pathways. A-D. Paracellular permeability of *in vitro* BBB cultures was assessed by electrode recording of transendothelial electrical resistance (TEER), reported in ohm/cm^2 **(A)** TEER after treatment of *in vitro* BBB cultures with 1000 nM exogenous S1P for 4h, +/- treatment

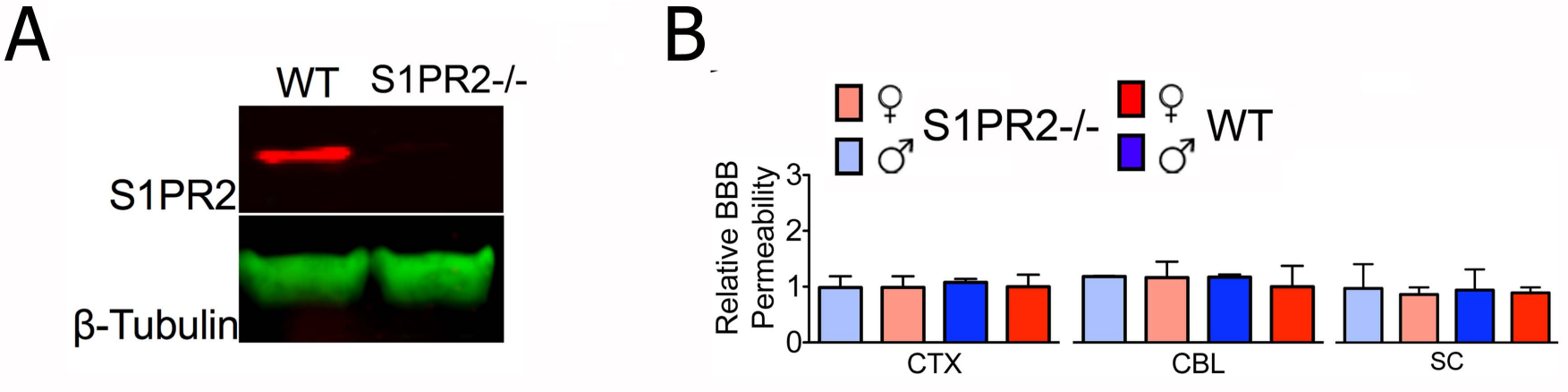
with the S1PR1 specific agonist SEW2871 (1000 nM) at 2h **(B-D)** TEER after 1000 nM S1P treatment of BBB cultures pretreated for 2h with **(B)** the clathrin endocytosis inhibitor chlorpromazine (CPMZ, 10ug/ml), (**C)** the macropinocytosis/PI3K inhibitor wortmannin (100nM) or **(D)** Rac1 inhibitor Z62954982 (1000 nM). **E-H.** Immunocytochemical (ICC) staining of AJs in HCMEC/D3 cells via labeling of VE-cadherin (red, left and middle panels) and confocal z-stack reconstruction of HCMEC/D3 cells (right panels) demonstrating polarized expression of the canonical apical marker gamma-glutamyltranserfase-1 (GGT1, green) and basolateral CXCL12 (red) were performed after treatment with vehicle (left panels) or 1000 nM S1P (middle panels) for 4h, both with SEW2871 **(E)**, CPMZ **(F)**, wortmannin **(G)**, or Z62954982 **(H)** treatment at 2h. Inhibitor concentrations in **E-H** are identical to those in **A-D.** TEER values are means of 6-9 replicates taken from 2-3 independent experiments, analyzed via repeated measures two-way ANOVA. ICC images are representative images taken from 2-3 independent experiments. *p<0.05; **p<0.01; ***p<0.001.

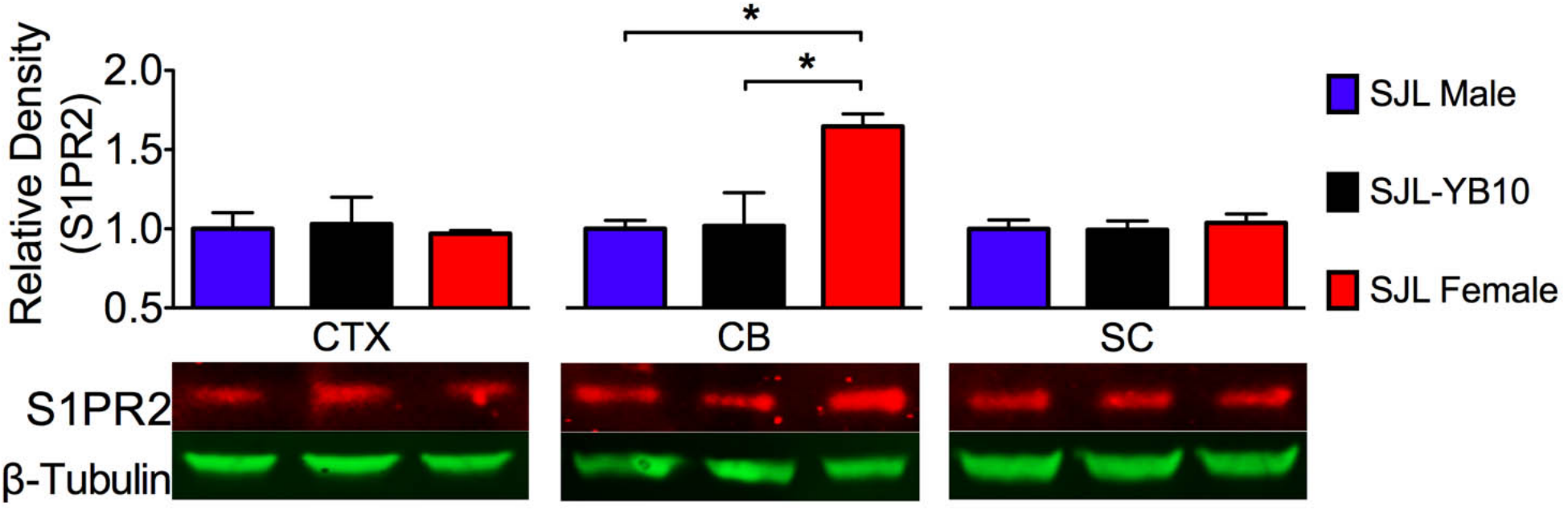
S9. SiRNA knockdown confirms pharmacological data showing S1PR2-mediated dysregulation of CNS endothelial barriers *in vitro*. A. Immunocytochemical staining in HCMEC/D3 showing specificity of knockdown for S1PR1 (top) and S1PR2 (bottom) after 2d incubation in normal culture medium, or with 25nM siRNA targeting S1PR1, S1PR2, or a non-targeting control siRNA. Protein levels are assessed by fluorescence intensity, normalized to mean values for untreated cells. **B.** Paracellular permeability of *in vitro* BBB cultures was assessed by electrode recording of transendothelial electrical resistance (TEER), reported in ohm/cm^2. Cultures were incubated for 2d with 25nM siRNA targeting S1PR1, S1PR2, or a non-targeting control siRNA. After this, cultures were treated with either vehicle or 1000nM S1P for 4h, with TEER measurements every 2h. **C.** Immunocytochemical (ICC) staining of adherens junctions in HCMEC/D3 cells via

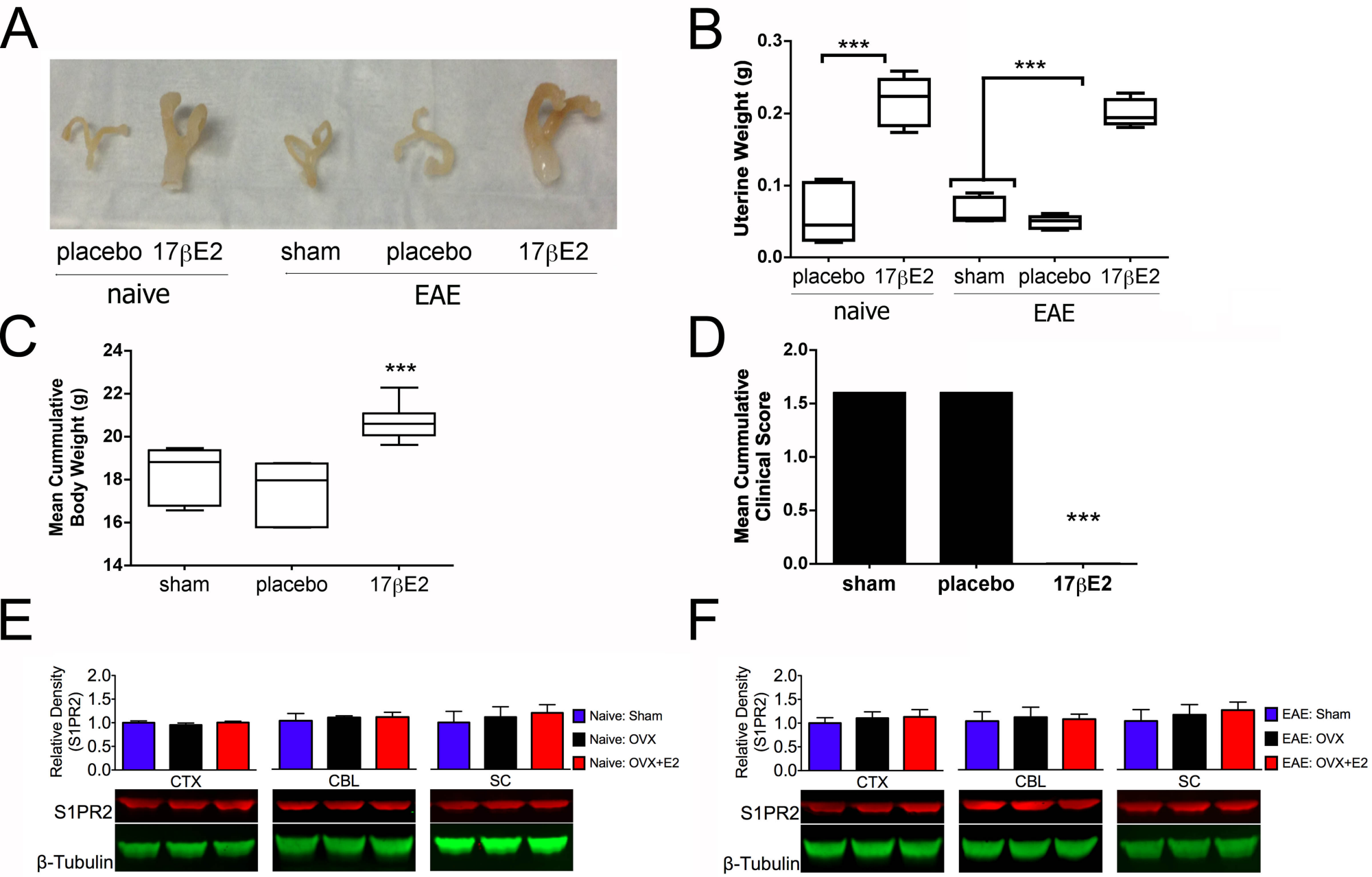
labeling of VE-cadherin (red). Cultures were incubated for 2d in normal culture medium, or with 25nM siRNA targeting S1PR1, S1PR2, or a non-targeting control siRNA. After this, cultures were treated with either vehicle or 1000nM S1P for 4h, followed by fixation and immunostaining. Fluorescence intensities are means of 3 replicates, +/- SEM, compared via one-way ANOVA. TEER values are means of 6 replicates taken from 2 independent experiments, analyzed via repeated measures two-way ANOVA. ICC images are representative images of 6 replicates, taken from 2 independent experiments. *p<0.05; **p<0.01; ***p<0.001.

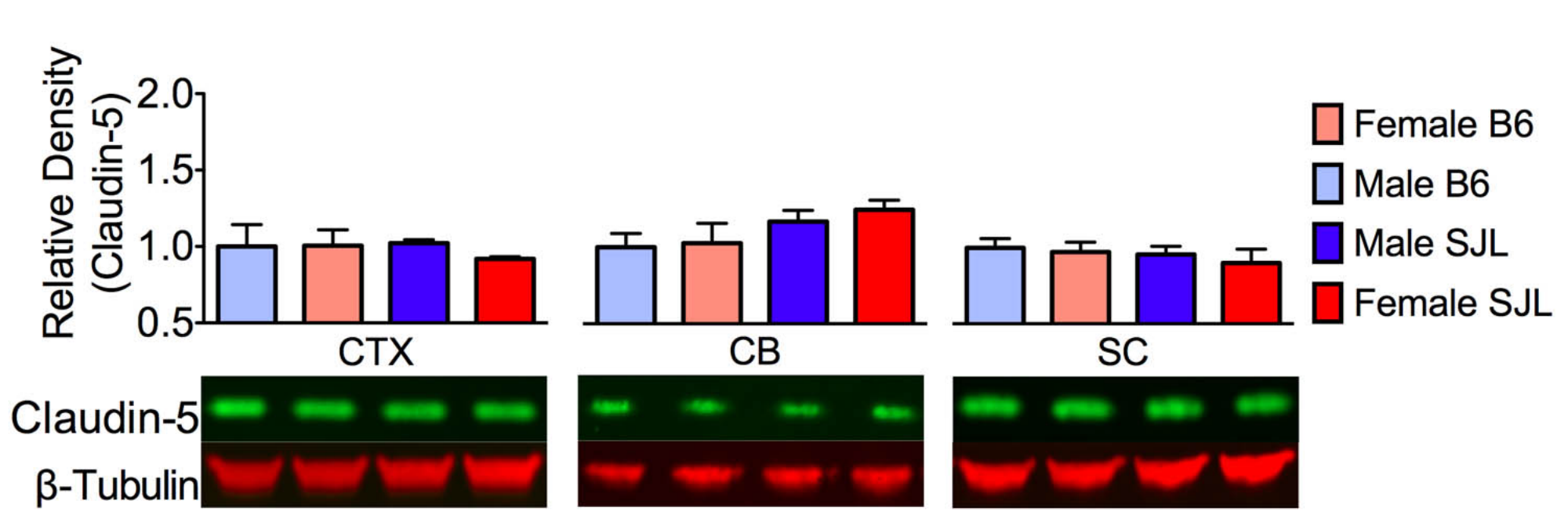
S10. CXCL12 exhibits a sexually dimorphic expression in female SJL in the same CNS regions where $S1PR_2$ is also dimorphic. A-B. Spinal cord expression of CXCL12 (red) and CD31 (green) in naïve male and female mice from three different mouse strains, commonly used for EAE: **A.** B10.PL (top row), C57BL/6 (middle row), and SJL (lower row), nuclei stained with ToPro (blue) nuclear stain (IC, isotype control). **B.** Percentage of apicobasal distribution of CXCL12 in relation to CD31 in spinal cord venules. **C.** Supra-spinal expression of CXCL12 (red) and CD31 (green) in naïve male and female SJL, nuclei stained with ToPro (blue) nuclear stain. **D.** Percentage of apicobasal distribution of CXCL12 on supra-spinal CNS regions: A: prelimbic cortex, B: motor cortex, C: retrosplenial cortex, D: hippocampus, E: corpus callosum, F: colliculi, G: = pons, H: cerebellum; white columns: abluminal, black columns: lumenal. Data derived from venules analyzed within 30 images per brain region for 3-5 mice/treatment group and are expressed as the mean percentages of vessels with abluminal (white box), lumenal (black box) or absent (gray box) CXCL12 signal; *p<0.05; **p<0.01; ***p<0.001for chi-squared comparisons between CXCL12 locations within each brain region.

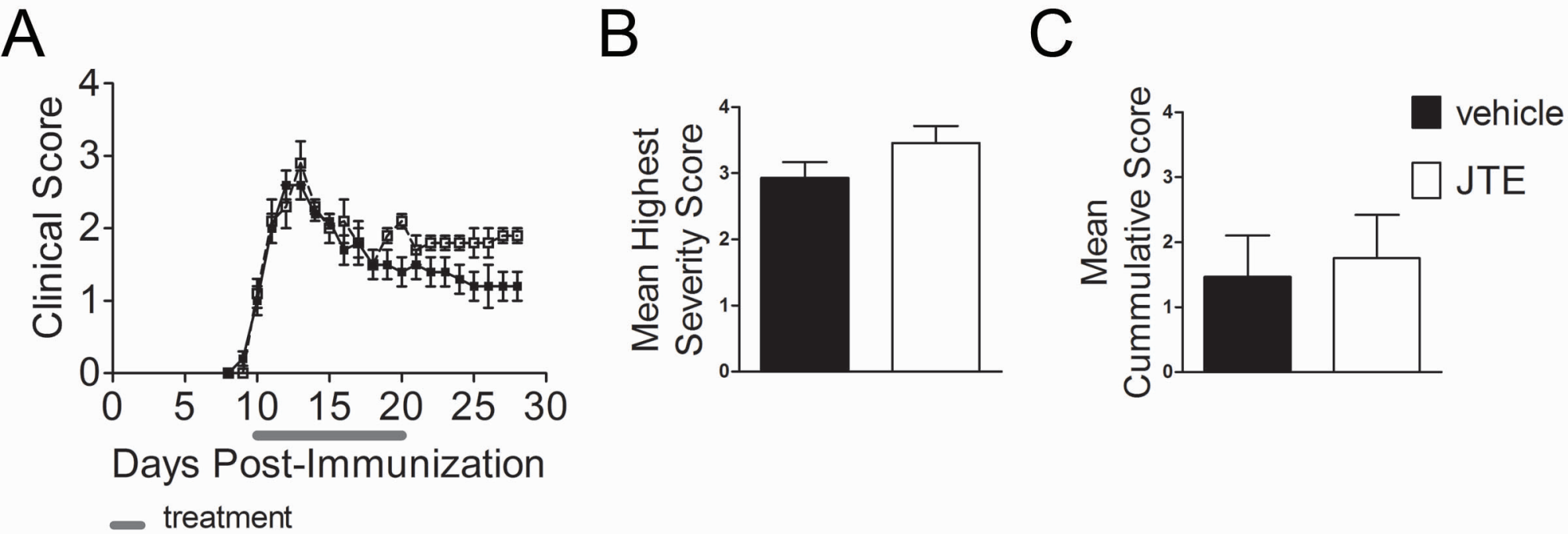
S11. Distribution of CXCL12 at peak of EAE in SJL is also sexually dimorphic. (**a)** Expression of CXCL12 (red) and CD31 (green) in brainstem (BS), cerebellum (CB) and spinal cord (SC) with ToPro (blue) nuclear staining. IC: isotype control. (**b)** Percentage of apicobasal distribution of CXCL12 in relation to CD31 in spinal cord venules. Data derived from venules analyzed within 30 images per brain region for 3-5 mice/treatment group and are expressed as the mean percentages of vessels with abluminal (white box), lumenal (black box) or absent (gray box) CXCL12 signal; *p<0.05; **p<0.01; ***p<0.001for chi-squared comparisons between CXCL12 locations within each brain region.

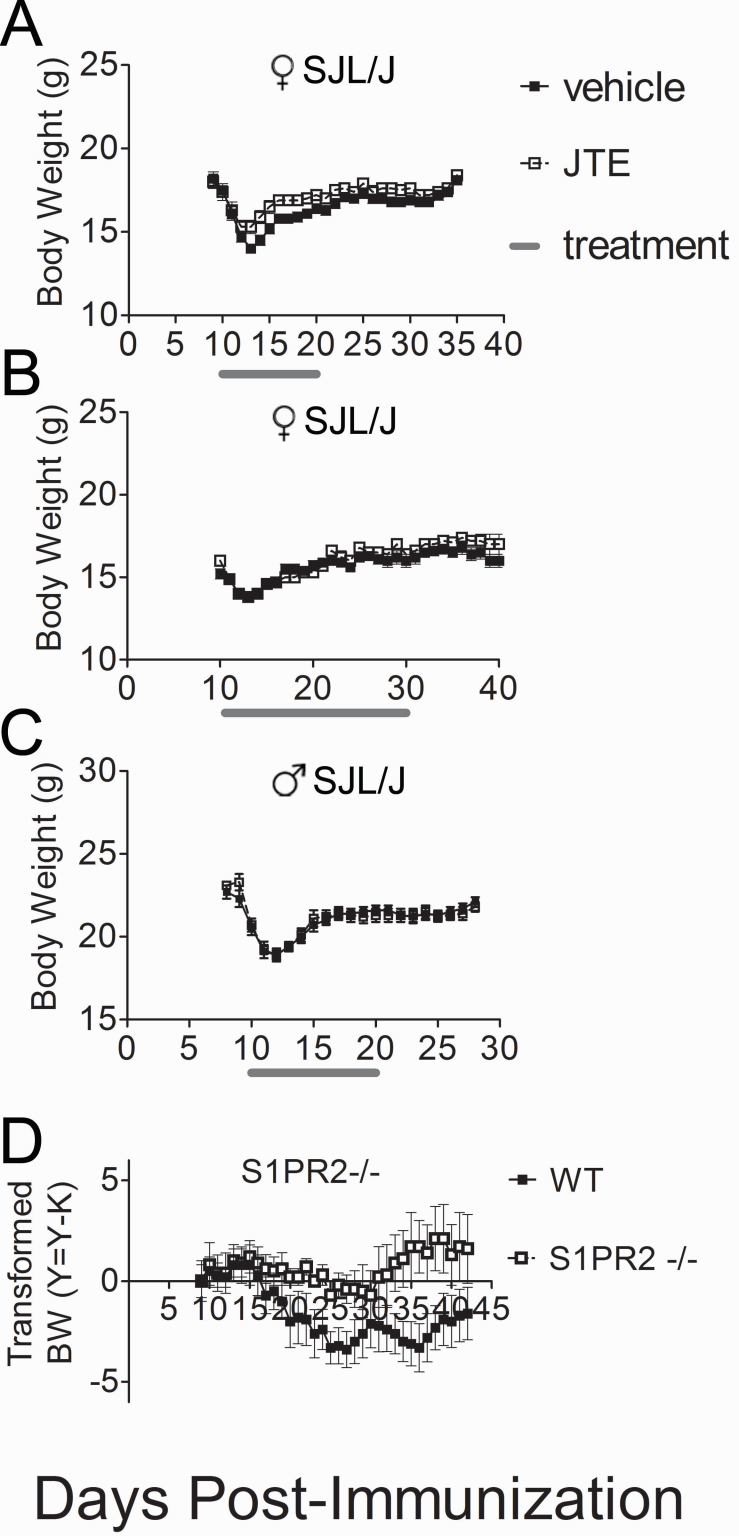

A
WT
S1PR2-/-
S1PR2
β-Tubulin
B
S1PR2-/-
WT
Relative BBB Permeability
0
1
2
3
CTX
CBL
SC

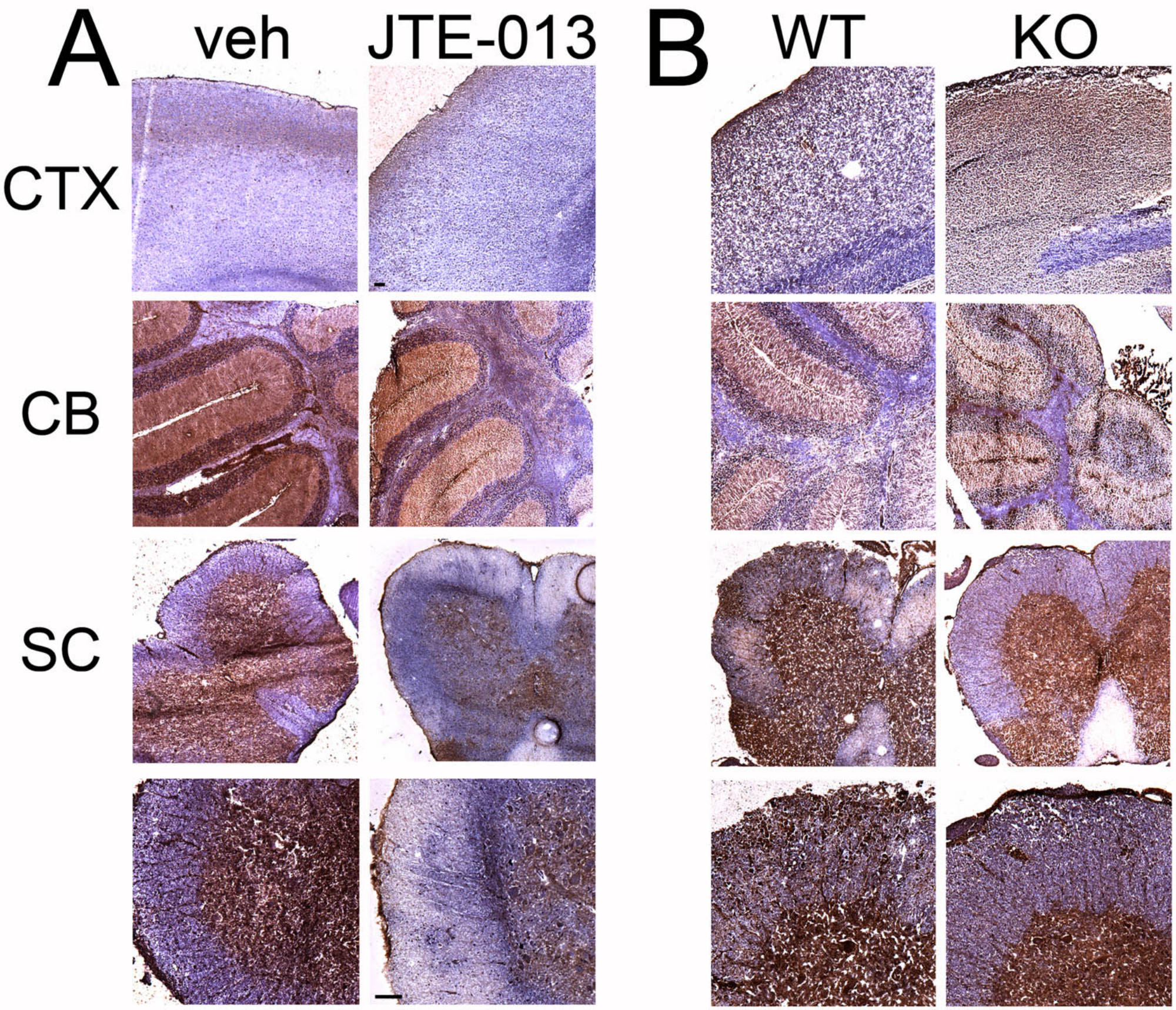

Relative Density (S1PR2)
2.0
1.5
1.0
0.5
*
*
SJL Male
SJL-YB10
SJL Female
CTX
CB
SC
S1PR2
β-Tubulin

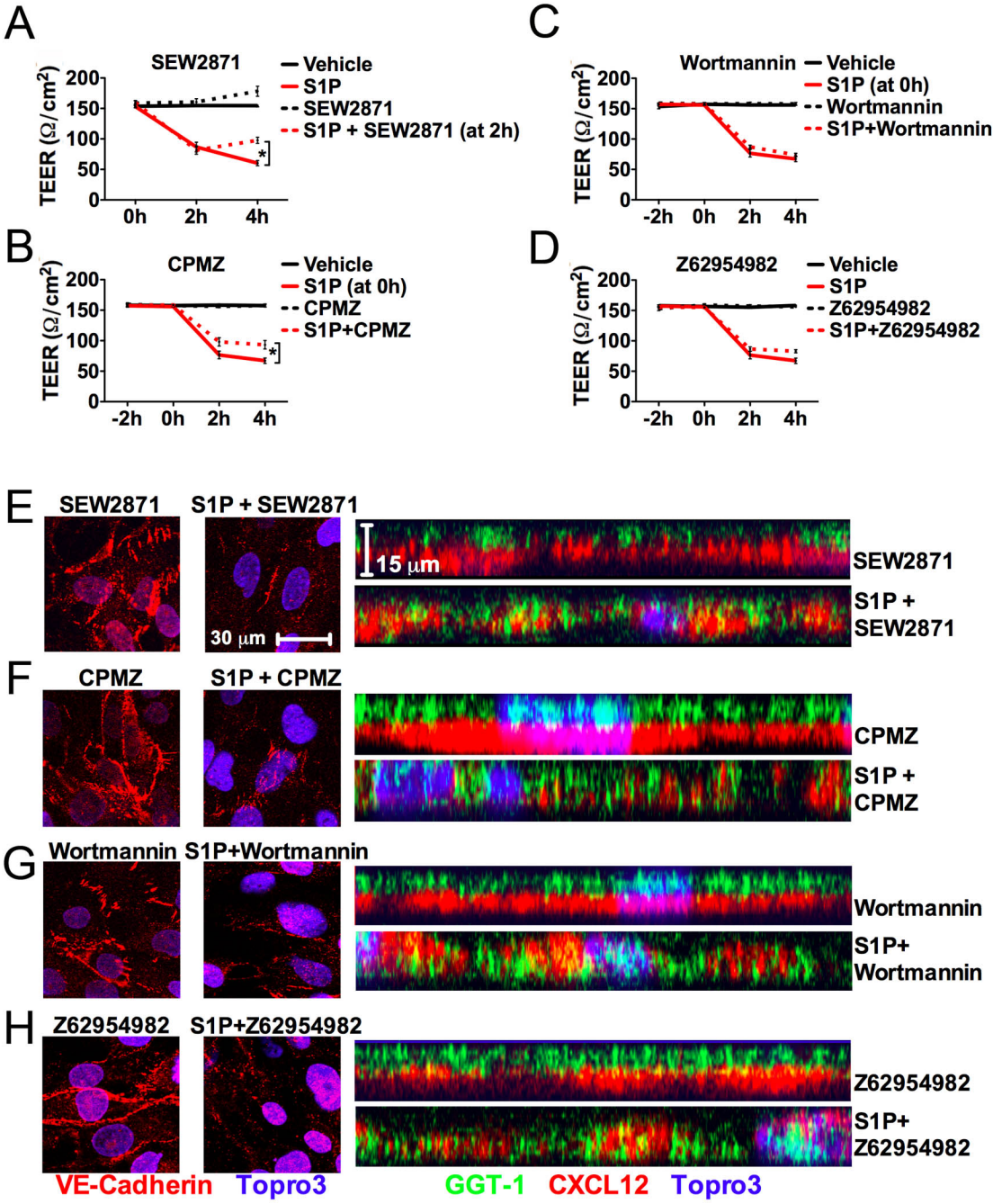

A
placebo
17βE2
sham
placebo
17βE2
naive
EAE
B
Uterine Weight (g)
0.3
0.2
0.1
0.0
***
***
placebo
17βE2
sham
placebo
17βE2
naive
EAE
C
Mean Cummulative Body Weight (g)
24
22
20
18
16
14
***
sham
placebo
17βE2
D
Mean Cummulative Clinical Score
2.0
1.5
1.0
0.5
0.0
***
sham
placebo
17βE2
E
Relative Density (S1PR2)
2.0
1.5
1.0
0.5
0.0
CTX
CBL
SC
Naive: Sham
Naive: OVX
Naive: OVX+E2
S1PR2
β-Tubulin
F
Relative Density (S1PR2)
2.0
1.5
1.0
0.5
0.0
CTX
CBL
SC
EAE: Sham
EAE: OVX
EAE: OVX+E2
S1PR2
β-Tubulin

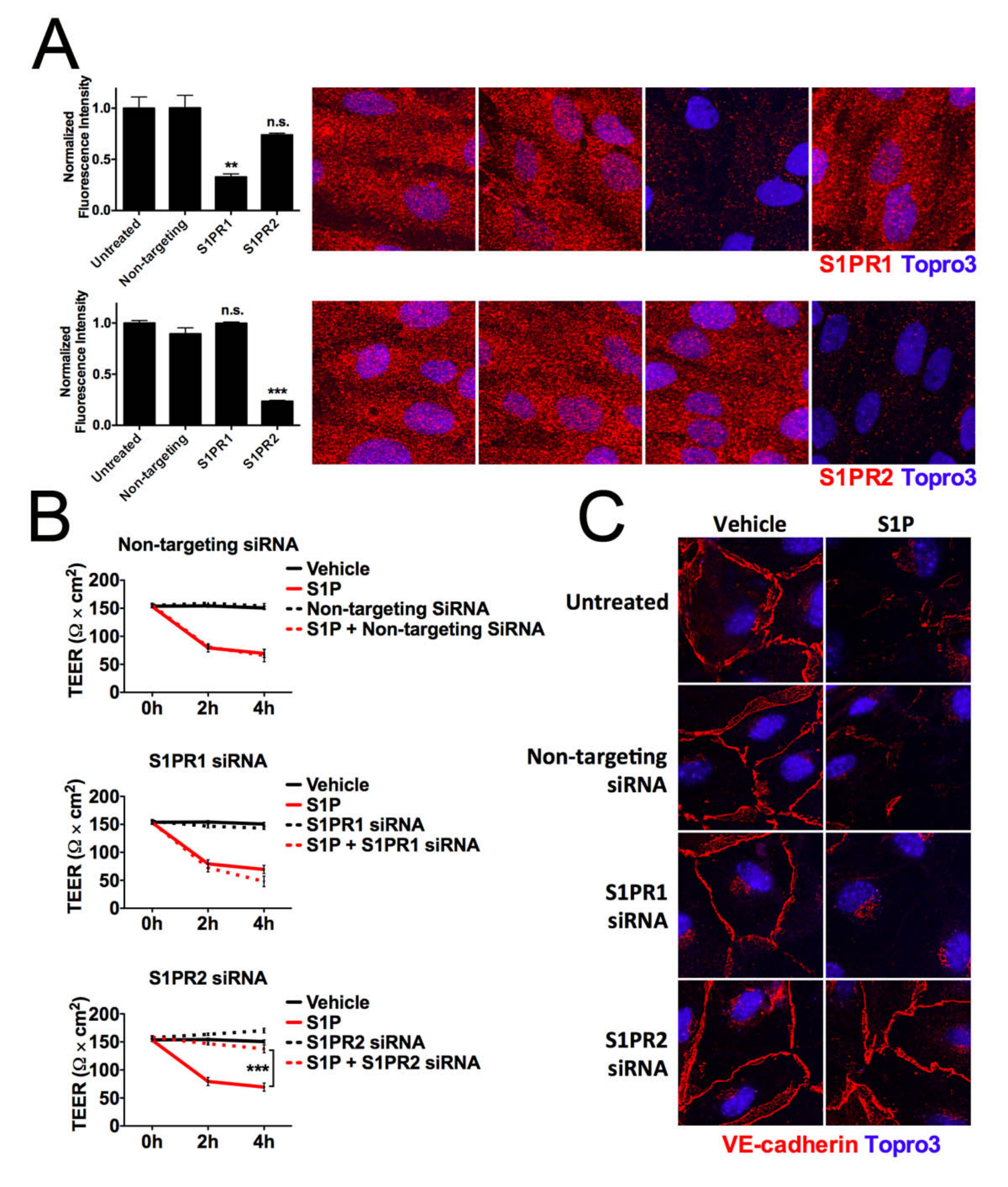

Relative Density
(Claudin-5)
2.0
1.5
1.0
0.5
CTX
CB
SC
Female B6
Male B6
Male SJL
Female SJL
Claudin-5
β-Tubulin

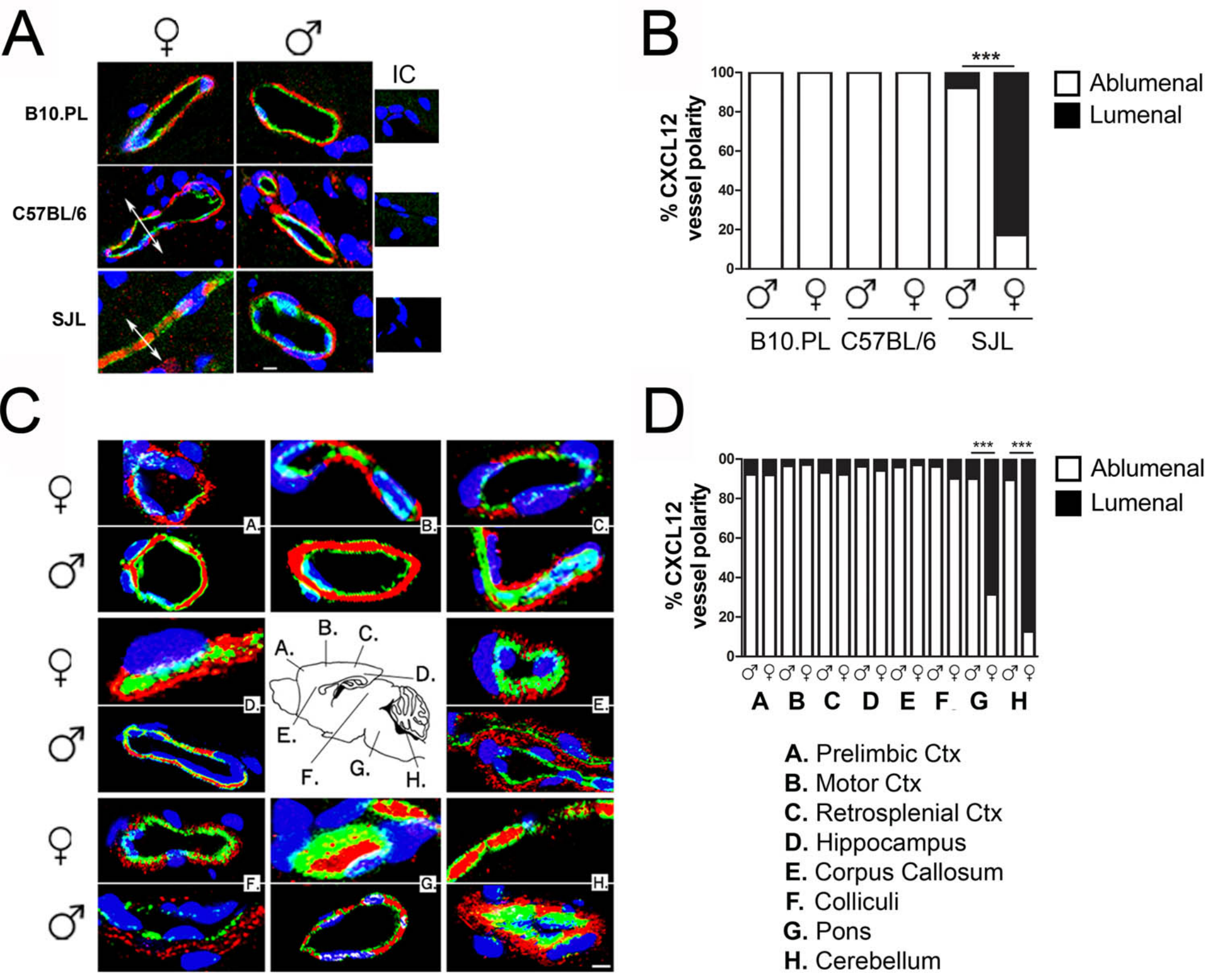

A
Clinical Score
0
1
2
3
4
0
5
10
15
20
25
30
Days Post-Immunization
treatment
B
Mean Highest Severity Score
0
1
2
3
4
C
Mean Cummulative Score
0
1
2
3
4
vehicle
JTE

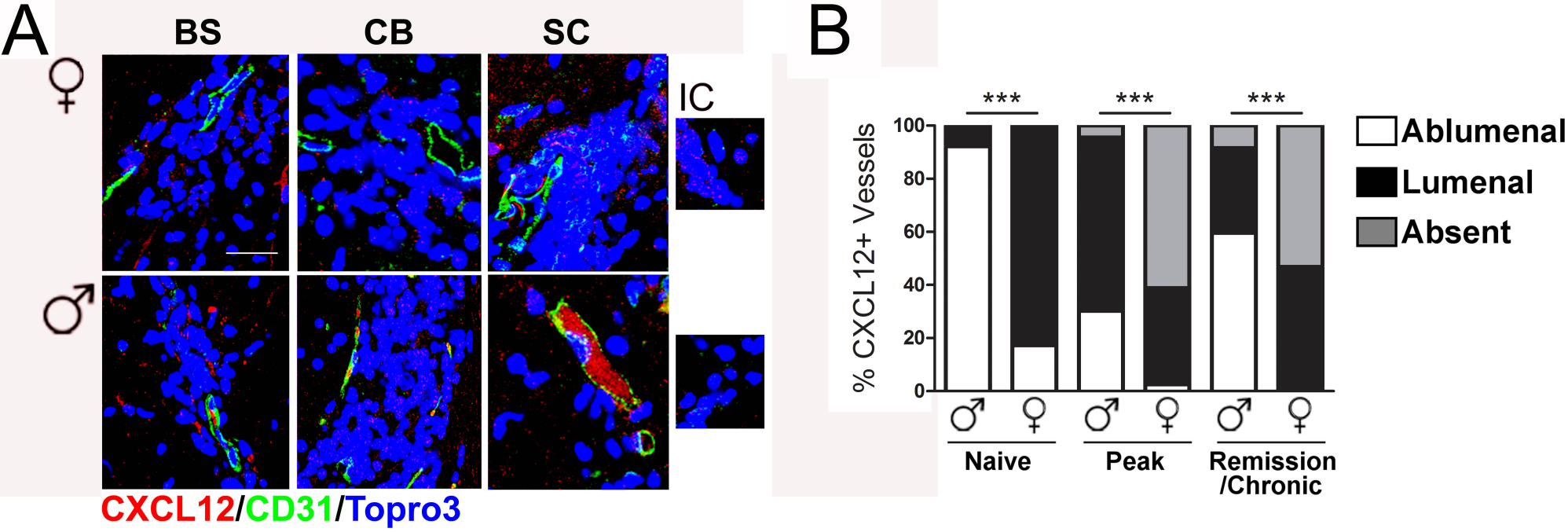

A
♀ SJL/J
Body Weight (g)
25
20
15
10
0 5 10 15 20 25 30 35 40
vehicle
JTE
treatment
B
♀ SJL/J
Body Weight (g)
25
20
15
10
0 10 20 30 40
C
♂ SJL/J
Body Weight (g)
30
25
20
15
0 5 10 15 20 25 30
D
S1PR2-/-
Transformed BW (Y=Y-K)
5
0
-5
5 10 15 20 25 30 35 40 45
WT
S1PR2 -/-
Days Post-Immunization


A
veh
JTE-013
B
WT
KO
CTX
CB
SC


A
SEW2871
Vehicle
S1P
SEW2871
S1P + SEW2871 (at 2h)
TEER (Ω/cm²)
200
150
100
50
0
0h
2h
4h
*
B
CPMZ
Vehicle
S1P (at 0h)
CPMZ
S1P+CPMZ
-2h
C
Wortmannin
S1P+Wortmannin
D
Z62954982
S1P+Z62954982
E
SEW2871
S1P + SEW2871
15 μm
30 μm
F
CPMZ
S1P + CPMZ
G
Wortmannin
S1P+Wortmannin
H
Z62954982
S1P+Z62954982
VE-Cadherin
Topro3
GGT-1
CXCL12


A
Normalized Fluorescence Intensity
1.0
0.5
0.0
n.s.
**
Untreated
Non-targeting
S1PR1
S1PR2
S1PR1 Topro3
n.s.
***
S1PR2 Topro3
B
Non-targeting siRNA
TEER (Ω × cm²)
200
150
100
50
0
0h
2h
4h
Vehicle
S1P
Non-targeting SiRNA
S1P + Non-targeting SiRNA
S1PR1 siRNA
S1P + S1PR1 siRNA
S1PR2 siRNA
S1P + S1PR2 siRNA
C
Vehicle
S1P
Untreated
Non-targeting siRNA
S1PR1 siRNA
S1PR2 siRNA
VE-cadherin Topro3


A
♀
♂
IC
B10.PL
C57BL/6
SJL
B
***
100
80
60
40
20
0
% CXCL12 vessel polarity
Ablumenal
Lumenal
♂ ♀ ♂ ♀ ♂ ♀
B10.PL C57BL/6 SJL
C
A.
B.
C.
D.
E.
F.
G.
H.
D
***
***
00
80
60
40
20
0
% CXCL12 vessel polarity
Ablumenal
Lumenal
A B C D E F G H
A. Prelimbic Ctx
B. Motor Ctx
C. Retrosplenial Ctx
D. Hippocampus
E. Corpus Callosum
F. Colliculi
G. Pons
H. Cerebellum


A
BS
CB
SC
IC
♀
♂
CXCL12/CD31/Topro3
B
% CXCL12+ Vessels
100
80
60
40
20
0
***
***
***
♂
♀
♂
♀
♂
♀
Naive
Peak
Remission
/Chronic
Ablumenal
Lumenal
Absent

Supplemental Table 1. Summary of microarray results looking for sexually dimorphic genes among adult SJL cortex and cerebellum.

	microarray hits	unique to	X or Y -linked	autosomal
CB	23	20	15	5
CTX	44	41	5	36
both	3		3	
TOTAL	64	61	23	41

		Fold-Change (female vs. male)			
#	Gene symbol	CB	CTX	Chromosome	Gene ID
	Known X or Y -linked				
1	Xist	3.837	3.544	X	Inactive X specific transcripts
	Xist	3.829	2.733	X	Inactive X specific transcripts
	Xist	2.970	2.762	X	Inactive X specific transcripts
	Xist	2.070	1.495	X	Inactive X specific transcripts
2	Utx	1.341	1.317	X	Lysine (K)-specific demethylase 6A
3	Eif2s3x	1.277	1.348	X	Eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked
4	Igsf1	1.101	-1.694	X	Immunoglobulin superfamily, member 1
	Igsf1	1.043	-1.897	X	Immunoglobulin superfamily, member 1
5	Timp1	-1.215	-1.519	X	Tissue inhibitor of metalloproteinase 1
6	Pisd-ps3	1.287	1.622	Y	Phosphatidylserine decarboxylase, pseudogene 3
7	LOC382297	-1.311	-1.074	Y	similar to RIKEN cDNA 1700029H17
8	LOC100042351	-1.377	-1.087	Y	Y-linked testis-specific protein 1-like
9	LOC665746	-1.431	1.013	Y	Y-linked testis-specific protein 1-like
10	LOC382133	-1.494	1.002	Y	Spermiogenesis specific transcript on the Y 1, family member
11	LOC665918	-1.619	-1.115	Y	Y-linked testis-specific protein 1-like

12	LOC100039829	-1.727	-1.065	Y	Y-linked testis-specific protein 1-like
13	LOC435023	-1.762	-1.026	Y	similar to Spermiogenesis specific transcript on the Y 2
14	LOC100039516	-1.918	-1.100	Y	Y-linked testis-specific protein 1-like
15	LOC100042550	-2.208	-1.104	Y	Y-linked testis-specific protein 1-like
16	LOC100041256	-2.220	-1.139	Y	uncharacterized
17	LOC385575	-2.225	-1.059	Y	similar to RIKEN cDNA 1700029H17
18	LOC100041934	-2.420	-1.122	Y	Y-linked testis-specific protein 1-like
19	Ddx3y	-11.042	-9.345	Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
20	4921530F17Rik	-2.410	-1.204	Y-linked	Reinius et al, 2010
	Novel X or Y -linked				
21	A830055I09Rik	-1.203	1.524	X-linked	uncharacterized
22	LOC673556	-1.598	-1.072	Y-linked	hypothetical LOC673556
23	4930579N16Rik	-1.978	-1.100	Y-linked	uncharacterized
	Autosomal				
24	Myl1	1.042	1.338	1	Myosin, light polypeptide 1
25	Rgs4	1.032	1.309	1	Regulator of G-protein signaling 4
26	R3hdm1	-1.035	1.441	1	R3H domain containing 1
27	Ppp1r1c	1.039	1.373	2	Protein phosphatase 1, regulatory (inhibitor) subunit 1C
28	Hdc	1.029	-1.429	2	Histidine decarboxylase
29	Frmd4a	1.021	1.332	2	FERM domain containing 4A
30	Cugbp2	-1.039	1.453	2	CUGBP, Elav-like family member 2
31	Negr1	1.023	1.314	3	Neuronal growth regulator 1
32	Lhx8	-1.017	-1.333	3	LIM homeobox protein
33	Hsd3b2	-1.037	1.400	3	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2
34	Cort	1.021	1.327	4	Cortistatin
35	Emp1	-1.186	-1.426	6	Epithelial membrane protein 1

36	Csda [Ybx3]	-1.564	-1.166	6	Cold shock domain protein A
37	Apoc1	-1.004	-1.411	7	Apolipoprotein C-I
38	LOC384552	-1.306	-1.020	7	similar to OBOX4
39	Sorbs2	1.075	1.347	8	Sorbin and SH3 domain containing 2
40	Rasd2	1.054	-1.375	8	RASD family, member 2
41	Tppp3	1.005	-1.304	8	Tubulin polymerization-promoting protein family member 3
42	Zfhx3	-1.012	-1.301	8	Zinc finger homeobox 3
43	LOC100046883	-1.055	-1.320	8	similar to CKLF-like MARVEL transmembrane domain containing 3
44	Necab2	-1.076	-1.325	8	N-terminal EF-hand calcium binding protein 2
45	Calb2	-1.090	-1.636	8	Calbindin 2
46	S1pr2	1.311	-1.092	9	Sphingosine-1-phosphate receptor 2
47	BC038167	-1.071	-1.310	9	Coiled-coil domain containing 153
48	Anln	-1.126	-1.400	9	Anillin, actin binding protein
49	Il20rb	-1.367	1.006	9	Interleukin 20 receptor beta
50	Myl4	1.157	1.482	11	Myosin, light polypeptide 4
51	Foxk2	1.016	1.415	11	Forkhead box K2
52	Slc47a1	-1.047	1.386	11	Solute carrier family 47, member
53	Doc2b	-1.057	-1.312	11	Double C2, beta
54	Samd14	-1.064	-1.393	11	Sterile alpha motif domain containing 14
55	A230065H16Rik	1.025	-1.517	12	RIKEN cDNA A230065H16 gene
56	Tmem90a	-1.083	-1.405	12	Transmembrane protein 90a
57	Idb2	-1.168	1.377	12	Inhibitor of DNA binding 2
58	Nefm	1.337	-1.038	14	Neurofilament, medium polypeptide
59	Tnnc1	1.079	1.582	14	Troponin C, cardiac/slow skeletal
60	Lgals3	-1.142	-1.312	14	Lectin, galactose binding, soluble 3
61	Sdf2l1	-1.017	-1.339	16	Stromal cell-derived factor 2-like 1
62	Adcyap1	1.076	1.365	17	Adenylate cyclase activating polypeptide 1
63	2900055J20Rik	1.033	1.390	18	RIKEN cDNA 2900055J20 gene
64	Cd6	1.015	1.347	19	CD6 antigen

Patient number	Age (yrs)	Sex	PMI (hrs)	Diagnosis	CNS level	Inflammation	Demyelination	ORO
non-MS								
1	62	female	unknown	post-surgical dehiscence sepsis	cerebellum	—	—	—
94	73	male	3	CA, CAD, HTN, MI, atypical lower MND*	cerebrum	—	—	—
210	41	male	24	non-ischemic cardiomyopathy, HTN, small bowel ischemia	cerebellum	—	**	—
509	71	female	7	pancreatic cancer	cerebrum	—	—	—
525	81	male	3	MI	periventricular white matter	—	—	—
561	77	male	6	CAD***	cerebellum	—	—	—
632	56	female	18	emphysema & artherosclerosis	cerebellum	—	—	—
908	84	female	6	pulmonary embolism	cerebellum	—	—	—
938	39	female	4	CNS lymphoma	cerebellum	—	—	—
MS cases								
58	64	female	10	RRMS	cerebellum	—	†	—
71	69	male	6	PPMS	cerebellum	—	—	—
101	46	female	6	SPMS	cerebellum	—	—	—
107	88	male	6	PRMS	cerebellum	—	—	—
234	95	female	8	SPMS	cerebellum	—	—	—
271	50	female	20	SPMS	cerebellum	—	††††	—
303	45	female	3	PPMS	cerebellum	††††	†	††††
305	70	male	8	SPMS	cerebellum	—	—	—
388	69	female	9	RRMS	cerebellum	—	—	—
650	35	male	10	PPMS	cerebellum	†	†	††
1101	54	male	7	SPMS	cerebellum	—	—	—

PMI: post-mortem interval, PPMS: primary progressive MS, PRMS: progressive-relapsing MS, RRMS: relapsing-remitting MS, SPMS: secondary progressive MS, CA: cardiac arrest, CAD: coronary artery disease, HTN: hypertension, MI: myocardial infraction, MND: motor neuron disease, *Patient received intravenous immunoglobulin treatment. **Biopsy showed a slightly pale site, presumably an artifact as assessed by its location and shape. ***Biopsy showed some features similar to Alzheimer's but patient has no clinical history of AD.