# Supporting Information

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### SI Materials and Methods

**Primary Mouse Brain Endothelial Cell Cultures.** Microvascular endothelial cells were isolated and cultured as previously described (1). After isolation, cells were resuspended in DMEM containing 10% (vol/vol) dialyzed "histamine-free" FBS (2), 100 U/mL penicillin, 100 μg/mL streptomycin, 4 μg/mL puromycin (InvivoGen), 100 μg/mL heparin, and 50 μg/mL endothelial cell growth factor supplement (BD Biosciences); plated onto rat tail collagen (Roche Diagnostics)-coated dishes; and incubated at 37 °C in a humidified 5% $CO_2$/95% air environment for 2 d. The medium was then refreshed with medium without puromycin, and cells were used for experiments 2 d later.

**RT-PCR.** Total RNA was extracted from primary mouse brain endothelial cells and from lymphoid stromal tissues using RNeasy RNA isolation reagent (Qiagen). Stromal tissue from spleen, lymph node, and thymus was isolated by disrupting harvested tissue between plastic mesh to release and eliminate suspension cells. The remaining "stromal" tissue was used for RNA isolation. cDNA was prepared using SuperScript$^{II}$ RT (Invitrogen), and expression of the *HA-Hrh1-hGH* transgene, endogenous *Hrh1*, *Vwf*, and *Gapdh* was determined by RT-PCR using the following primers:

*HA-Hrh1-hGH* transgene:
Forward: 5′-CTC CCG GAC CAC AGA CTC AGA-3′
Reverse: 5′-GAC GGA GGT CTG GGG GTT CTG-3′
*Hrh1*:
Forward: 5′-AGA TGT GTG AGG GGA ACA GG-3′
Reverse: 5′-TAC AGC ACC AGC AGG TTG AG-3′
*Vwf*:
Forward: 5′-GAC CGG GTA GAG GCA CCT AAC CT-3′
Reverse: 5′-TGC ATG AGG TCC ACC AAA CTC T-3′
*Gapdh*:
Forward: 5′-ACC ACA GTC CAT GCC ATC AC-3′
Reverse: 5′-TCC ACC ACC CTG TTG CTG TA-3′

PCR products were resolved on 1% agarose gels and visualized by ethidium bromide staining.

**CSF Collection and BBB Permeability.** Mice were injected i.v. with 200 ng of PTX in buffer containing 25 mM Tris, 0.5 M NaCl, and 0.017% Triton X-100. Ten days later, mice were given 50 μg/g of FITC-labeled BSA (Sigma–Aldrich) by i.v. injection. Four hours later, mice were anesthetized using inhalational isoflurane (1–3% vol/vol) and whole blood was recovered by orbital bleed. Following an overdose of ketamine (100 mg/kg), the dura overlying the posterior fossa was exposed surgically and CSF (typically 1–3 μL) was collected by careful aspiration into glass capillary pipettes. CSF samples with visible blood contamination were discarded. Whole blood was spun for 10 min at 13,000 × *g* at 4 °C in a microcentrifuge, and the resulting supernatant (i.e., plasma) was recovered. One microliter of CSF or plasma was then diluted in 25 μL of PBS and centrifuged at 835 × *g* for 15 min to remove cells. The FI (excitation wavelength of 485 nm, emission wavelength of 528 nm) of FITC in the CSF and serum samples was determined with a microplate fluorescence reader (Flx-800-I; Bio-Tek Instruments, Inc.). The BBB permeability index is expressed as the ratio of the CSF FI divided by the plasma FI.

**Induction and Evaluation of EAE.** For the 1× EAE protocol, mice were injected with 0.2 mL of an emulsion containing 200 μg of $MOG_{35–55}$ in PBS and an equal volume of CFA containing 200 μg of *Mycobacterium tuberculosis* H37RA (Difco Laboratories) distributed equally by s.c. injections into the dorsal right and left flanks and scruff of the neck. Additionally, each animal received 200 ng of PTX i.v. on the same day. For the 2× EAE protocol, mice were injected s.c. with 0.1 mL of an emulsion containing 100 μg of $MOG_{35–55}$ and 100 μg of *M. tuberculosis* H37RA in CFA divided equally over two dorsal flank sites (right and left) on day 0. On day 7, 0.1 mL of $MOG_{35–55}$/*M. tuberculosis* + CFA emulsion (100 μg of $MOG_{35–55}$ to 100 μg of *M. tuberculosis*) was injected s.c. on the dorsal side at two sites anterior or posterior to the sites used on day 0. For both protocols, mice were scored daily, starting on day 10 as previously described (2). Regression analysis (3) revealed that the disease course in mouse strains fit a variable slope sigmoidal curve, and statistical significance in disease course among strains was assessed by the extra sum-of-squares *F* test. Clinical quantitative trait variables, including disease incidence, mean day of onset, cumulative disease score, number of days affected, overall severity index, and peak score, were determined as previously described (2).

For EAE pathology, brains and spinal cords were dissected from calvaria and vertebral columns, respectively, and fixed in 10% phosphate-buffered formalin (pH 7.2). Representative transverse tissue sections were paraffin-embedded, sectioned at 5 μm, and mounted on glass slides. Sections were stained with H&E for routine evaluation and with Luxol fast blue-periodic acid–Schiff for assessment of demyelination. Sections from representative areas were scored in a semiquantitative fashion for the various lesions, as previously described (2). The total brain and spinal cord pathology indices for each lesion were obtained by summing the scores for all lesions observed in the respective tissue.

**Cytokine/Chemokine Measurement.** Mice were immunized using the 1× EAE protocol, and spleens and draining lymph nodes were harvested 10 d later. Single-cell suspensions of $1 \times 10^6$ cells/mL were cultured with 50 μg/mL $MOG_{35–55}$. After 72 h, the levels of IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12 (p70), IL-13, eotaxin-1/CCL11, G-CSF, GM-CSF, KC/CXCL1 (murine IL-8 homolog), MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, and TNF-α in culture supernatants were quantified by Bio-Plex multiplex assay (Bio-Rad) as described in the manufacturer's protocol. The significance of differences in cytokine/chemokine levels assessed by multiplex analysis was determined by one-way ANOVA using the Kruskal–Wallis test and a Bonferroni-corrected *P* value of 0.002 for multiple comparisons. Significant differences in the main effect of strain were followed by Dunn's post hoc multiple comparison test. IL-4, IL-17, and IFN-γ levels were analyzed by ELISA as described previously (2). Serum vWF levels were determined by ELISA using polyclonal rabbit IgG capture and peroxidase-conjugated detection Abs (Dako).

**ICAM-1 and vWF Immunohistochemistry.** Formalin-fixed paraffin-embedded brain sections from mice immunized 30 d previously with $MOG_{35–55}$ + CFA + PTX were prepared as described above. Sections were deparaffinized and rehydrated in 100% xylene (3× 5-min washes), 100% ethanol (2×), 95% (vol/vol) ethyl alcohol (EtOH) in $H_2O$ (2×), 70% (vol/vol) EtOH in $H_2O$ (2×), 50% (vol/vol) EtOH in $H_2O$ (1×), and distilled $H_2O$ (1×). Antigen retrieval was performed by incubation of slides with Target Retrieval Solution (Dako) in a covered Coplin jar at 95–98 °C in a water bath, and slides were then cooled for 20 min at room temperature. All the following steps were carried out in a covered humidified chamber. After washing with Tris-buffered saline + 0.01% Tween-20 (TBS-T), endogenous peroxidase activity was

blocked with 3% (vol/vol) methanol in hydrogen peroxide (Sigma) for 15 min at room temperature. Sections were blocked for 30 min at room temperature in a covered humidified chamber with 3% (vol/vol) normal rabbit or goat serum (Jackson Immunoresearch) in PBS with avidin reagent (Vectastain Elite ABC kit; Vector Laboratories). Primary Abs (anti-ICAM-1; eBioKAT-1, Rat IgG2a, k; eBioscience and anti-vWF; polyclonal rabbit IgG; Dako) were diluted 1/200 in 3% normal rabbit (for ICAM-1 staining) or goat (for vWF staining) serum in PBS with biotin reagent (Vectastain Elite ABC kit) for 1 h at room temperature. After a TBS-T wash, biotinylated secondary Abs (rabbit anti-rat IgG; Jackson Immunoresearch or goat anti-rabbit IgG; Vectastain Elite ABC kit) was incubated in 1% (mass/vol) BSA in PBS for 1 h at room temperature, humidified. After the TBS-T wash, sections were incubated with Vectastain ABC reagent (Vector Laboratories) for 30 min at room temperature, washed again with TBS-T, and then incubated for 4 min at room temperature with DAB substrate buffer (Dako). Sections were rinsed in $H_2O$, counterstained with hematoxylin for 4 min, rinsed in TBS-T, and mounted with Permount (Fisher). Representative images were captured using an Olympus BX50 light microscope, and brightness and contrast were adjusted uniformly among images using Adobe Photoshop CS.


1. Lu C, et al. (2008) Pertussis toxin induces angiogenesis in brain microvascular endothelial cells. *J Neurosci Res* 86:2624–2640.
2. Noubade R, et al. (2007) Histamine receptor H1 is required for TCR-mediated p38 MAPK activation and optimal IFN-gamma production in mice. *J Clin Invest* 117:3507–3518.
3. Teuscher C, et al. (2006) Evidence that the Y chromosome influences autoimmune disease in male and female mice. *Proc Natl Acad Sci USA* 103:8024–8029.


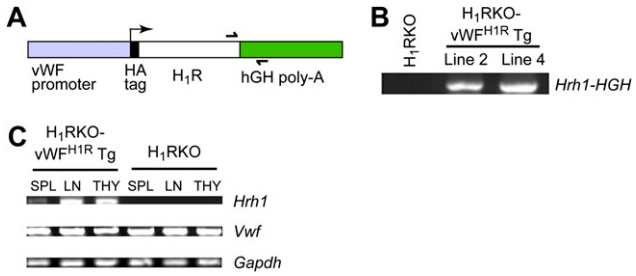


**Fig. S1.** Generation of $H_1$RKO-vWF$^{H1R}$ Tg mice. (*A*) *Vwf* promoter-*HA-Hrh1-hGH* transgenic construct with screening primers indicated. (*B*) Expression of the *HA-Hrh1-hGH* transgene in brain endothelial cells from $H_1$RKO mice and two founder lines of $H_1$RKO-vWF$^{H1R}$ Tg mice was analyzed by RT-PCR. (*C*) *Hrh1* expression in spleen (SPL), lymph node (LN), and thymus (THY) stromal tissue from $H_1$RKO and $H_1$RKO-vWF$^{H1R}$ Tg mice was analyzed by RT-PCR.

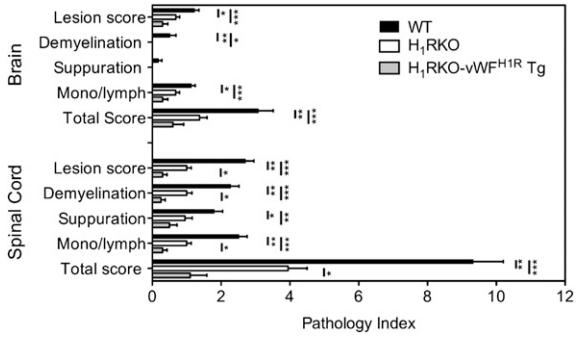


**Fig. S2.** Histopathological quantification of CNS disease traits. EAE was elicited in WT ($n$ = 21), $H_1$RKO ($n$ = 19), and $H_1$RKO-vWF$^{H1R}$ Tg ($n$ = 10) mice using the 1× $MOG_{35–55}$ + CFA + PTX immunization protocol. The indicated parameters were quantified by histological analysis in formalin-fixed brain and spinal cord tissue. Statistical significance was determined using one-way ANOVA with Kruskal–Wallis and Dunn's multiple comparison tests (*$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001).

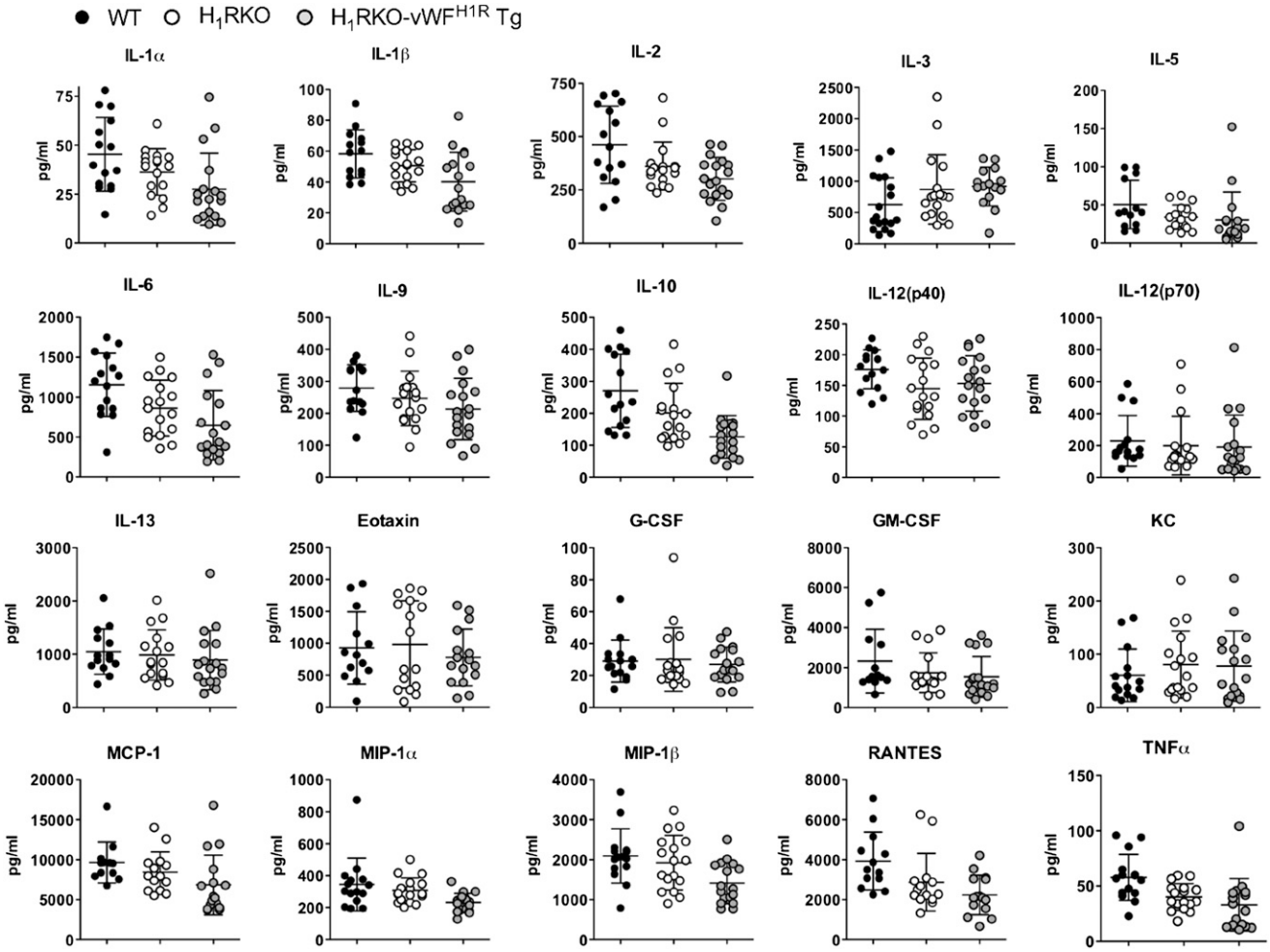


**Fig. S3.** Endothelial $H_1R$ expression did not alter the antigen-specific cytokine response in 1× $MOG_{35–55}$ + CFA-immunized mice. Spleen and draining lymph node cells were isolated from WT, $H_1RKO$, and $H_1RKO$-$vWF^{H1R}$ Tg ($n$ = 15–17 per strain) mice that were immunized 10 d previously with the 1× $MOG_{35–55}$ + CFA + PTX protocol. Cells were restimulated ex vivo with 50 μg/mL $MOG_{35–55}$ for 72 h. Supernatants were harvested, and the indicated cytokines [IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, eotaxin-1/CCL11, G-CSF, GM-CSF, KC/CXCL1, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, and TNF-α] were analyzed by Bio-Plex multiplex analysis. No significance was determined using one-way ANOVA with the Kruskal–Wallis test and a Bonferroni-corrected $P$ value of 0.002.

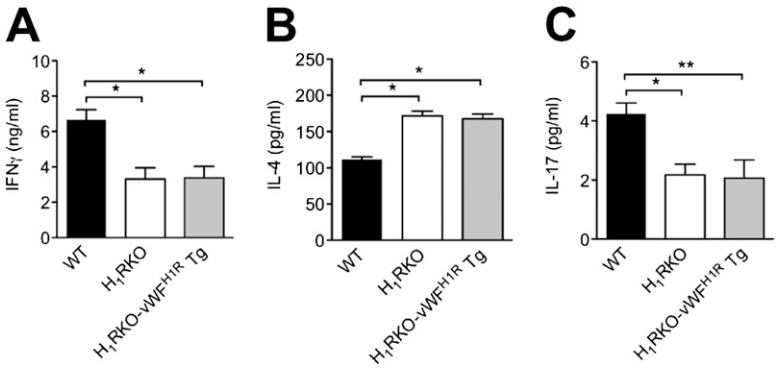


**Fig. S4.** Endothelial $H_1R$ expression did not alter the antigen-specific cytokine response in 2× $MOG_{35–55}$ + CFA-immunized mice. C57BL/6J (WT), $H_1RKO$, and $H_1RKO$-$vWF^{H1R}$ Tg ($n$ = 4–6 per strain) mice were immunized with 2× $MOG_{35–55}$ + CFA. At 10 d postimmunization, spleen and draining lymph node cells were isolated and restimulated with 50 μg/mL $MOG_{35–55}$ for 72 h. Supernatants were collected and cytokines were determined by ELISA. IFN-γ (*A*), IL-4 (*B*), and IL-17 (*C*) levels are shown. The significance of the differences in cytokine expression by cells from WT, $H_1RKO$, and $H_1RKO$-$vWF^{H1R}$ mice was determined using one-way ANOVA and the Kruskal–Wallis test. Significant differences in the main effect of strain were detected by Dunn's post hoc multiple comparison test (*$P < 0.05$; **$P < 0.01$).

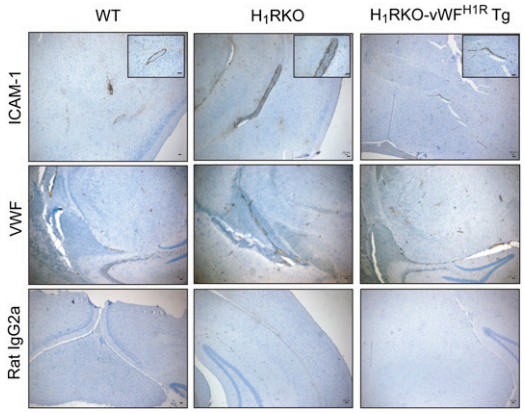


**Fig. S5.** $H_1R$ does not regulate brain endothelial ICAM-1 expression during EAE. EAE was elicited in WT, $H_1RKO$, and $H_1RKO$-$vWF^{H1R}$ Tg mice using the 1× $MOG_{35–55}$ + CFA + PTX immunization protocol. At day 30 after immunization, expression of the endothelial activation marker ICAM-1 and the endothelial marker vWF was analyzed in formalin-fixed paraffin-embedded brain tissue by DAB immunohistochemistry with hematoxylin counterstaining. (Scale bar = 50 μm.) All panels are shown at 40× magnification except for the insets in the upper right of ICAM-1 panels, which are shown at 200× magnification. Results are representative of three animals of each genotype analyzed.

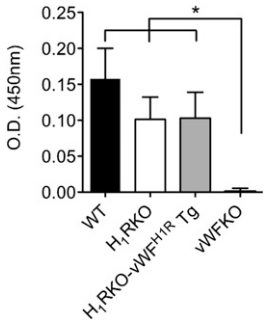


**Fig. S6.** $H_1R$ does not affect endothelial degranulation during EAE. EAE was elicited in WT, $H_1RKO$, $H_1RKO$-$vWF^{H1R}$ Tg, and vWF-deficient (vWFKO) (1) ($n = 7$ for each genotype) mice using the 1× $MOG_{35–55}$ + CFA + PTX immunization protocol. Thirty days after immunization, serum vWF levels were determined by ELISA. *$P < 0.05$, as determined using one-way ANOVA and the Kruskal–Wallis test. Significant differences in the main effect of strain were determined by Dunn's post hoc multiple comparison test.

1. Denis C, et al. (1998) A mouse model of severe von Willebrand disease: defects in hemostasis and thrombosis. *Proc Natl Acad Sci USA* 95:9524–9529.

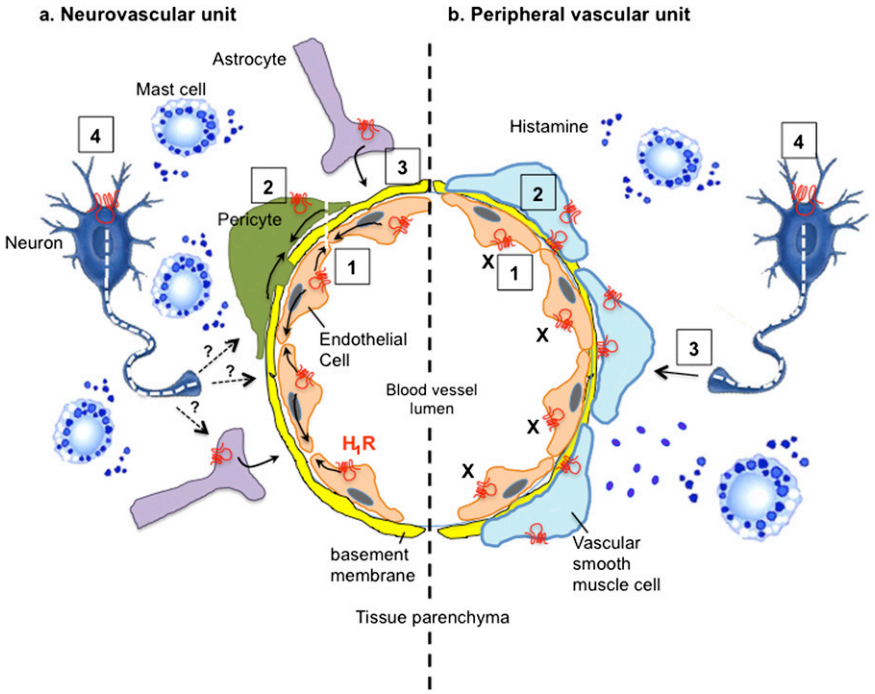


**Fig. S7.** (*A*) Neurovascular unit. (*Box 1*) $H_1R$ activation reduces BBB permeability (this study). (*Box 2*) $H_1R$ activation induces pericyte contraction (1). (*Box 3*) $H_1R$ activation induces secretion of neurotrophic factors from astrocytes (2). (*Box 4*) $H_1R$ is expressed on central neurons (3), but the role of $H_1R$ in direct or indirect control of BBB is unknown. (*B*) Peripheral vascular unit. (*Box 1*) No effect (indicated as "X") of endothelial $H_1R$ in Bphs (this study). (*Box 2*) Vascular smooth muscle cells express $H_1R$ (3). (*Box 3*) $H_1R$ in neurons regulates peripheral vasculature (4). (*Box 4*) $H_1R$ is expressed on peripheral neurons (3).

1. Murphy DD, Wagner RC (1994) Differential contractile response of cultured microvascular pericytes to vasoactive agents. *Microcirculation* 1:121–128.
2. Lipnik-Stangelj M, Carman-Krzan M (2004) Activation of histamine H1-receptor enhances neurotrophic factor secretion from cultured astrocytes. *Inflamm Res* 53:245–252.
3. Akdis CA, Simons FE (2006) Histamine receptors are hot in immunopharmacology. *Eur J Pharmacol* 533:69–76.
4. Jin H, et al. (2006) Histamine-induced vasodilation and vasoconstriction in the mesenteric resistance artery of the rat. *Eur J Pharmacol* 529:136–144.

**Table S1. Nonsedating $H_1$ antihistamines block Bphs**

| Compound, mg/kg | No. dead/no. tested |
|---|---|
| Saline | 8/8 |
| Sedating, mg/kg | |
| Mepyramine | |
| 6.25 | 0/4 |
| 1.56 | 0/4 |
| Clemastine fumarate | |
| 6.25 | 0/4 |
| 1.56 | 0/4 |
| Nonsedating, mg/kg | |
| Cetirizine di-HCl | |
| 6.25 | 0/4 |
| 1.56 | 0/4 |
| Loratadine | |
| 6.25 | 0/4 |
| 1.56 | 0/4 |
| Fexofenadine HCl | |
| 6.25 | 0/4 |
| 1.56 | 0/4 |

C57BL/6J mice (*Bphs*$^S$) were sensitized with 200 ng of purified PTX by i.v. injection on day 0. On day 3, 20 min before histamine challenge, mice were injected i.v. with $H_1$ antihistamines at the doses indicated (mg/kg). Histamine challenges (25 mg/kg) were carried out by i.v. injection, and deaths were recorded at 30 min postchallenge. The results are expressed as the number of animals dead over the number of animals studied.

**Table S2. One-week mortality rates after Bphs histamine challenge in $H_1$RKO vs. $H_1$RKO-vWF$^{H1R}$ Tg mice**

| Strain | No. dead/no. total | % affected | *P* value |
|---|---|---|---|
| $H_1$RKO | 5/15 | 33% | |
| $H_1$RKO-vWF$^{H1R}$ Tg | 13/23 | 57% | 0.1983 |

Mice that survived acute (30 min) Bphs challenge with 100 mg/kg histamine were returned to the vivarium and allowed to rest for up to 7 d. The results are expressed as the number of animals dead divided by the number of animals studied. Fisher's exact test was used to test for significance of differences. The *P* value is indicated (nonsignificant).

**Table S3. Clinical disease traits following immunization of C57BL/6J, $H_1$RKO, and $H_1$RKO-vWF$^{H1R}$ Tg mice with $MOG_{35–55}$ + CFA + PTX**

| | | | Affected animals | | | |
|---|---|---|---|---|---|---|
| Strain | Incidence (%)* | CDS | DO | PS | DA | SI |
| C57BL/6J | 18/19 (95) | 53.2 ± 5.3 | 13.0 ± 0.3 | 3.9 ± 0.3 | 18.0 ± 0.3 | 3.1 ± 0.2 |
| $H_1$RKO | 45/49 (92) | 28.9 ± 2.3 | 13.7 ± 0.2 | 2.8 ± 0.1 | 15.4 ± 0.5 | 1.9 ± 0.1 |
| $H_1$RKO-vWF$^{H1R}$ Tg | 15/25 (60) | 8.7 ± 2.2 | 14.3 ± 0.7 | 1.5 ± 0.2 | 10.7 ± 1.4 | 1.2 ± 0.1 |
| | $\chi^2$ = 14.5 | H = 41.8 | H = 2.9 | H = 34.0 | H = 14.8 | H = 37.8 |
| | *P* = 0.0007 | *P* < 0.0001 | *P* = 0.23 | *P* < 0.0001 | *P* = 0.0006 | *P* < 0.0001 |

*Percent affected. Animals were considered affected if clinical scores ≥1 were apparent for 2 or more consecutive days. CDS, cumulative disease score over 30 d of experiment; DA, days affected; DO, day of onset; PS, peak score; SI, severity index (cumulative disease score/days affected). Means ± SD are shown. The significance of differences for the trait values among the strains was assessed by $\chi^2$ analysis (overall incidence) or the Kruskal–Wallis test (H), followed by Dunn's post hoc multiple comparisons. *P* values are indicated.

**Table S4. Clinical disease traits following immunization of C57BL/6J, $H_1$RKO, and $H_1$RKO-vWF$^{H1R}$ Tg mice with 2× $MOG_{35–55}$ + CFA**

| | | | Affected animals | | | |
|---|---|---|---|---|---|---|
| Strain | Incidence (%)* | CDS | DO | PS | DA | SI |
| C57BL/6J | 18/18 (100) | 37.6 ± 2.9 | 16.6 ± 0.7 | 3.2 ± 0.2 | 14.3 ± 0.7 | 2.5 ± 0.1 |
| $H_1$RKO | 35/36 (97) | 19.7 ± 1.6 | 17.7 ± 0.4 | 2.2 ± 0.1 | 11.8 ± 0.6 | 1.6 ± 0.1 |
| $H_1$RKO-vWF$^{H1R}$ Tg | 9/23 (39) | 6.8 ± 2.4 | 18.2 ± 0.6 | 2.0 ± 0.3 | 9.6 ± 1.7 | 1.6 ± 0.2 |
| | $\chi^2$ = 35.5 | H = 38.8 | H = 6.2 | H = 16.9 | H = 10.3 | H = 28.5 |
| | *P* < 0.0001 | *P* < 0.0001 | *P* = 0.05 | *P* < 0.0002 | *P* < 0.006 | *P* < 0.0001 |

*Percent affected. Animals were considered affected if clinical scores ≥1 were apparent for 2 or more consecutive days. CDS, cumulative disease score over 30 d of experiment; DA, days affected; DO, day of onset; PS, peak score; SI, severity index (cumulative disease score/days affected). Means ± SD are shown. The significance of differences for the trait values among the strains was assessed by $\chi^2$ analysis (overall incidence) or the Kruskal–Wallis test (H), followed by Dunn's post hoc multiple comparisons. *P* values are indicated.