Supplemental Figure

Supplementary Fig. 1. Injection of S1P is unable to protect against histamine-induced venular leakage. Representative images of before, 5 min after 10^{-5}M, and 5 min after 10^{-4}M of histamine application in either control vehicle (left panels) or S1P (0.38 mg/ kg body weight, right panels) injected SD rats.

Supplementary Fig. 2. *A*: $S1P_1$ and $S1P_2$ are expressed in the endothelium of cremaster muscle circulatory bed. Specimens of cremaster muscle were immunostained with mouse anti-rat PECAM followed by Alexa488 conjugated donkey anti-mouse antibody to identify endothelium (left panels). The sections were doubly-stained with rabbit polyclonal anti-$S1P_1$ (upper right panel) or anti-$S1P_2$ (middle right panel) antibodies followed by Alexa647 conjugated donkey anti-rabbit antibody. Note that $S1P_1$ and $S1P_2$ receptors are expressed in the endothelium of cremaster muscle microvessels (arrows). PECAM and $S1P_1$/$S1P_2$ Ab control, sections were incubated in the absence of primary antibody followed by Alexa488-donkey anti-mouse and alexa647-donkey anti-rabbit, respectively. *B*: $S1P_1$ and $S1P_2$ receptors are expressed in the endothelium (arrows) and smooth muscle cells (arrowheads) of arterial (Av) and venular (Ve) microvessels. Specimens of cremaster muscle were immunostained with goat anti-$S1P_1$ and rabbit anti-$S1P_2$ followed by incubating with Alexa488 conjugated donkey anti-goat and Alexa647 conjugated donkey anti-rabbit antibodies. Bottom panels, sections were incubated with mouse anti-□-actin followed by Alexa647-donkey anti-mouse to identify smooth muscle cells. Note that anti-□-actin only stains smooth muscle cells (arrowheads) but not endothelium (arrows), indicating the immunostaining is specific. DIC, images acquired by differential interference analyzer.

Supplementary Fig. 3. Sew2871 treatment diminishes histamine-induced venular leakage. Representative images of venular leakage in cremaster muscle vasculature of SD rats injected with vehicle (left panels), 0.05 mg/ kg body weight of sew2871 (middle panels), or 0.5 mg/ kg body weight of sew2871 (right panels).

Supplementary Fig. 4. Co-injection of VPC 23019 and S1P enhances venular leakage after the addition of 10^{-5}M histamine. Representative images of venular leakage in the cremaster muscle of SD rats injected with VPC 23019 alone (0.05 mg/ kg body weight, left panels), or VPC (0.05 mg/ kg body weight) and S1P (0.5 mg/ kg body weight, right panels). Note that co-injection of VPC plus S1P markedly enhances venular leakage after addition of 10^{-5}M histamine.

Supplementary Fig. 5. FTY720 prevents histamine-induced venular leakage. Representative images of microvessel leakage in cremaster muscle of SD rats injected with vehicle (left panels), or FTY720 (1 mg/ kg body weight, right panels). Note that FTY720 treatment greatly diminishes venular leakage at both 10^{-5}M and 10^{-4}M histamine addition.

Supplementary Fig. 6. S1P prevents histamine-induced venular leakage in the presence of JTE-013. *A*: Representative images of venular leakage in the cremaster muscle microvessels of SD rats injected with vehicle (left panels), JTE-013 (0.25 mg/ kg body weight, middle panels), or JTE-013 (0.25 mg/ kg body weight) plus S1P (0.5 mg/ kg body weight) (right panels). *B*: Representative images of microvascular leakage in SD rats injected with vehicle (first row), JTE-013 (0.025 mg/ kg body weight) plus S1P (0.025 mg/ kg body weight) (second row), JTE-013 (0.025 mg/ kg body weight) plus S1P (0.5 mg/ kg body weight) (third row), or JTE-013 (0.25 mg/ kg body weight) plus S1P (0. 5 mg/ kg body weight) (bottom row). Note that co-injection of JTE-013 and S1P markedly inhibits histamine-induced venular leakage, whereas injection of vehicle control or JTE-013 alone has no effect.