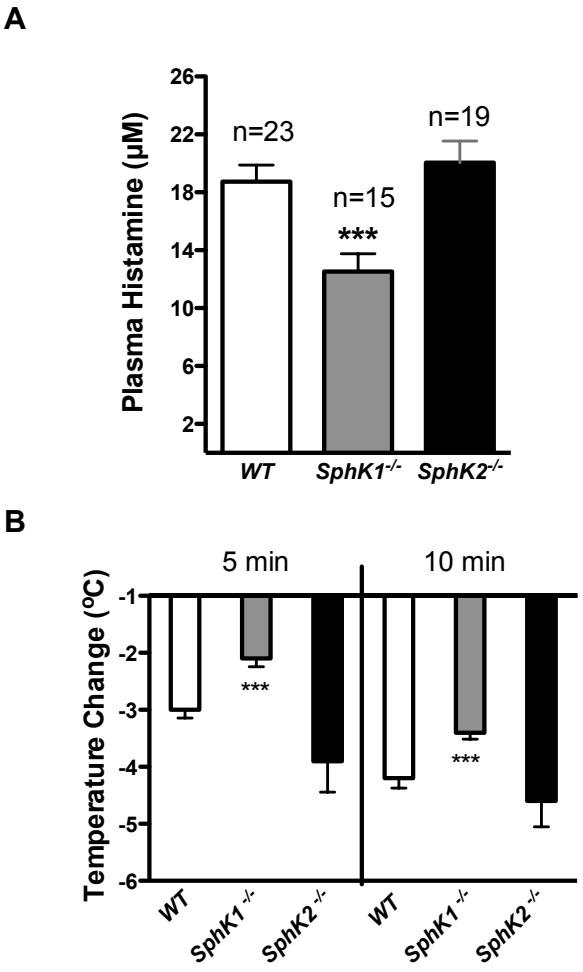
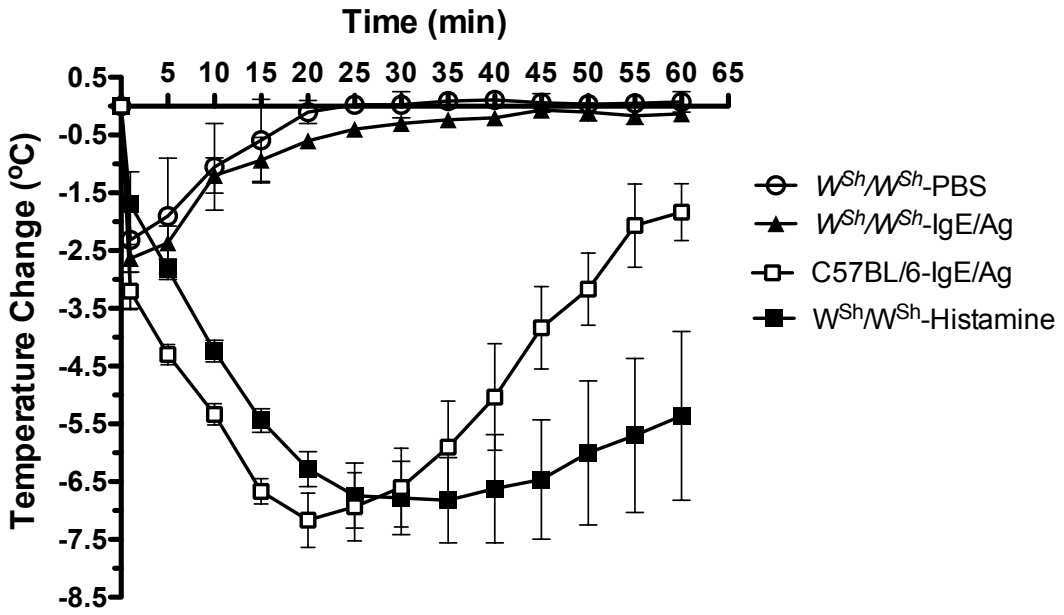
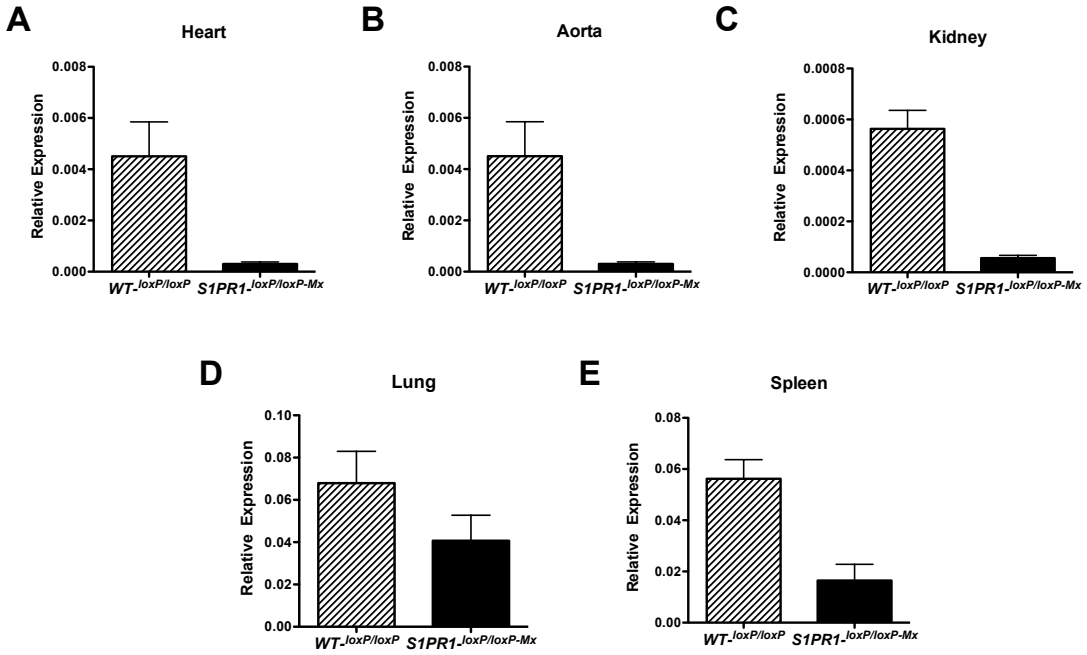


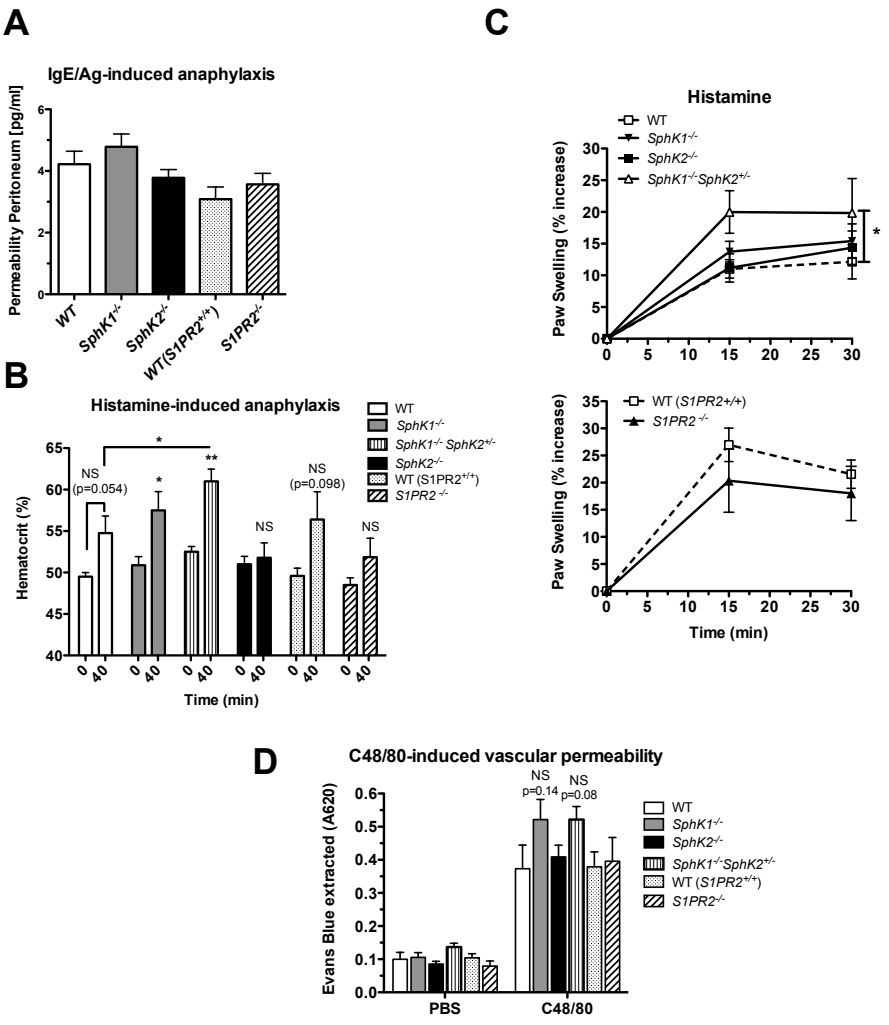
Supplemental Figure 1. *SphK1*$^{-/-}$ and *SphK2*$^{-/-}$ mice differ in the onset of IgE/Ag-induced anaphylaxis. **A**) WT (n=23), *SphK1*$^{-/-}$ (n=15), *Sphk2*$^{-/-}$ (n=19) mice were injected (*i.v.*) with DNP-specific IgE (3 μg). After 24 hrs, mice were challenged with Ag (250 μg DNP_{36}-HSA) to induce systemic anaphylaxis. Plasma histamine concentration was measured from plasma 1.5 min after challenge by ompetitive ELISA. **B**) Body temperature changes during the anaphylactic shock induced by Ag 5 and 10 min after challenge in the WT, *SphK1*$^{-/-}$, *Sphk2*$^{-/-}$ mice (n=12/group), measured as described in Methods. ***$p<0.001$ was determined by Student's t-test.



Supplemental Figure 2. Induction of anaphylaxis by histamine is mast cell-independent. DNP-specific IgE (3 μg) was injected into C57BL/6 or mast cell-deficient (W^{sh}/W^{sh}) mice. After 24 hrs, mice were challenged with Ag (250 μg DNP_{36}-HSA) (C57BL/6 and W^{sh}/W^{sh}) or saline pseudo challenged (W^{sh}/W^{sh}). Anaphylaxis in mast cell-deficient (W^{sh}/W^{sh}) mice was induced by injecting 5 μmol of histamine. Body temperature during anaphylaxis was monitored by an electronic transponder implanted under the skin and read at 1 min and subsequently at 5 min intervals for a total of 60 min using an electronic probe.



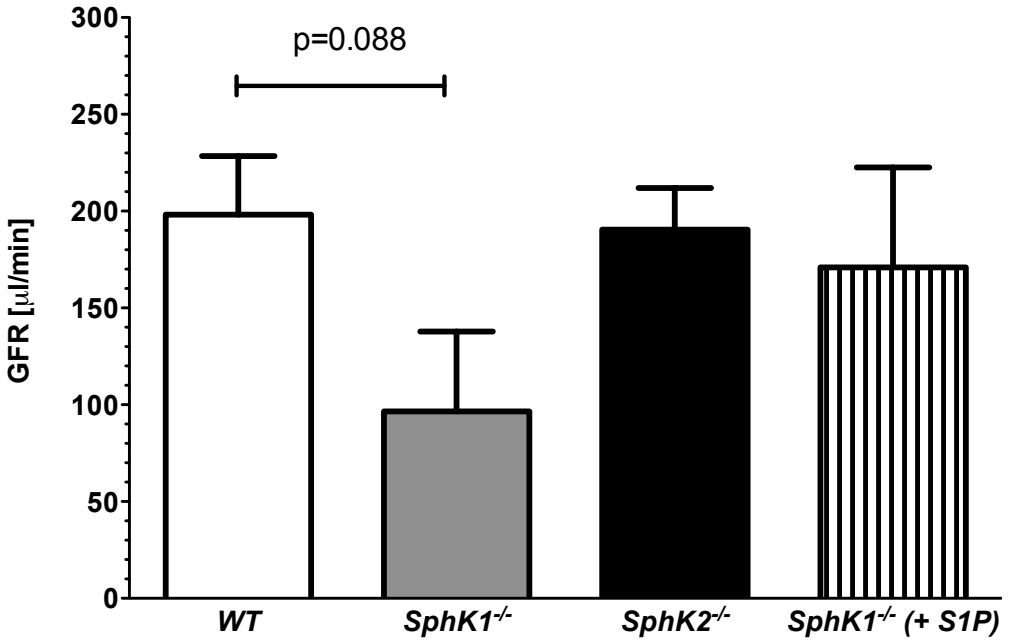
Supplemental Figure 3. Levels of S1PR1 expression in different organs from *S1PR1*$^{loxP/loxP\text{-}Mx}$ and *S1PR1*$^{loxP/loxP}$ mice. Mice were injected with pIpC for 4 weeks and subsequently used for anaphylaxis studies. Organs were collected 7-14 days after anaphylaxis studies to confirm excision of the floxed *S1PR1* gene in the mice carrying the Mx-Cre transgene. RNA was extracted from the indicated organs and expression of *S1PR1* mRNA was determined by quantitative real time PCR (TaqMan® Gene Expression Assays, Applied Biosystems).



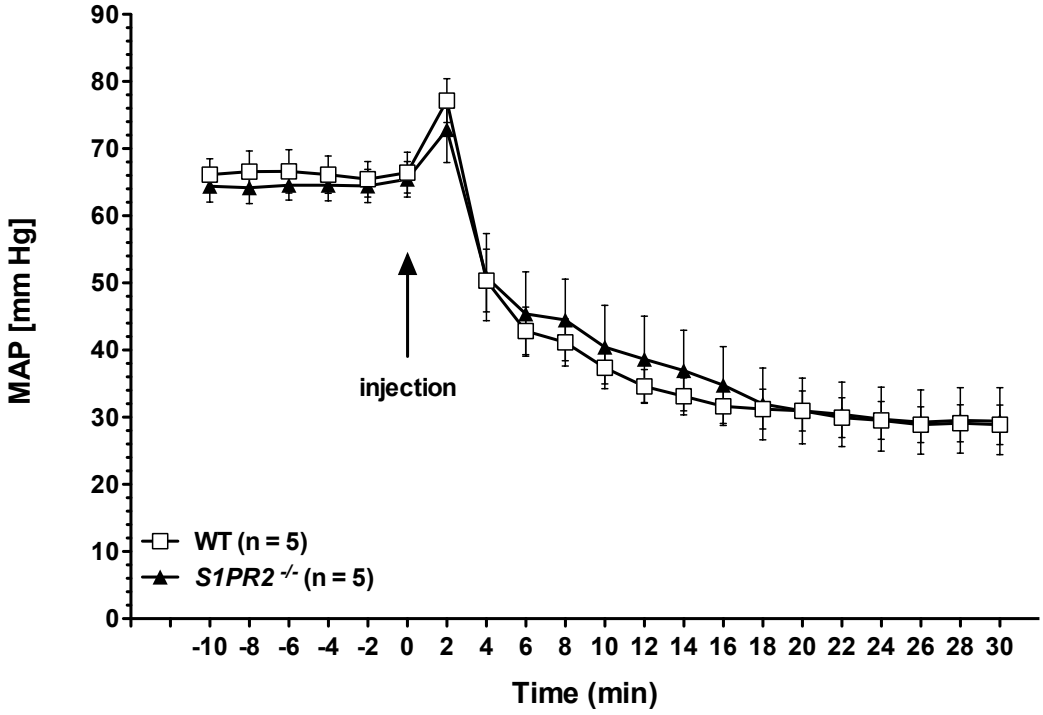
Supplemental Figure 4. Changes in vascular permeability in *SphK*$^{-/-}$ and *S1PR2*$^{-/-}$ mice during systemic anaphylaxis and local inflammation. **A)** Exudation of vascular fluids into the peritoneum after induction of systemic anaphylaxis by IgE/Ag. Mice from the different strains (n=4 to 5) were injected (*i.v.*) with DNP-specific IgE (3 μg) and after 24 hrs, mice were challenged with Ag (500 μg DNP_{36}-HSA) to induce systemic anaphylaxis and 20 mg/Kg Evans blue dye to examine plasma leakage. After 30 min, the peritoneal lavage was collected and mice were perfused with saline though the right ventricle until blood was visibly eliminated. The peritoneal lavage was centrifuged for 10 min at 3000xg and the amount of Evans blue in the supernatants was determined by absorbance at 620 nm minus absorbance at 720 nm. Lungs were collected, weighted and homogenized in 1

ml formamide. The dye accumulated in the extravascular space was extracted by incubation for 18 hrs at 55^{o}C and the amount extracted was determined by absorbance. The amounts of dye extracted by g of wet weight were as follows: *WT*, 8.5±1.0 pg/g; *SphK1*$^{-/-}$, 9.8±2.1 pg/g; *SphK2*$^{-/-}$, 12.8±4.2 pg/g; WT*(S1PR2*$^{+/+}$*),* 9.8±1.9 pg/g, and *S1PR2*$^{-/-}$*,* 12.78±3.8 pg/g. Direct observation of the ears, limbs and nose, did not reveal visible differences in the intensity of blue between the different genotypes. **B**) Changes in hematocrit during histamine-induced anaphylaxis. A baseline hematocrit was taken by withdrawing 50 µl of blood via the tail vein into a heparinized microhematocrit tube 2 h to 24 h before the induction of anaphylaxis. Anaphylaxis was induced by injecting histamine (5 µmol) into the various mice strains (n=4-5/group). After 40 min, mice were euthanized and blood was collected by cardiac puncture and 50 µl transferred into heparinized microhematocrit tubes. Blood was centrifuged and the percentage of packed blood cell volume (hematocrit) determined using a microhematocrit capillary tube reader. **C)** Paw edema induced by injection of histamine into the footpad. Mice (n=5 to 6) were anesthetized (isoflurane (2%):oxygen (98%) mix for 2-3 min) and injected in the footpad with 20 µl of histamine (90 µg) or 20 µl PBS into the contralateral hindpaw. Paw thickness in both hindpaws was determined using a caliper (Mitutoyo) before injection and at 15 and 30 min post-injection. The fold increase in thickness was calculated for both paws (histamine- and PBS-injected) and the difference in swelling between the histamine-injected paw as compared to the PBS-injected paw calculated for each time point and expressed as percentage. *$p<0.05$ between *WT* and *SphK1*$^{-/-}$*SphK2*$^{+/-}$ mice using a two way ANOVA test. **D**) Changes in vascular permeability in the skin. Evans blue dye (200 µl of 0.2%) was injected *i.v.* into the various mice strains. Compound 48/80 (C48/80) (100 µg in 20 µl), a known secretagogue for mast cells, was then injected intradermally in one of the ears of the various mice strains, while the contralateral ear was injected with 20 µl of PBS. C48/80 induces the release of mediators from mast cells that increase the permeability of the local vascular beds and cause edema. After 30 min,

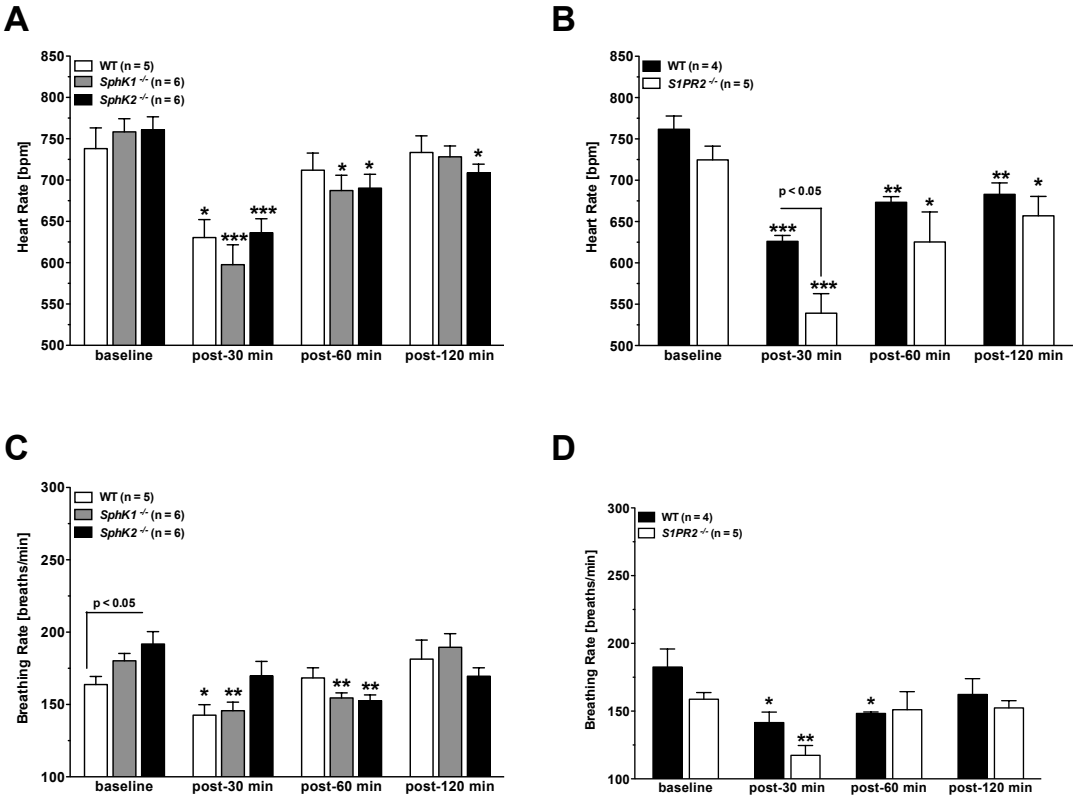
mice were euthanized and their ears collected, and plasma exudation measured by the content of blue dye in the ears. The dye was extracted from the minced ears by incubation for 1h at 55^{o}C with 700 µl of formamide, and the amount of dye determined by absorbance at 620 nm. *$p<0.05$ and **$p<0.01$ compared to the respective baseline measurements as determined by Student's t-test.



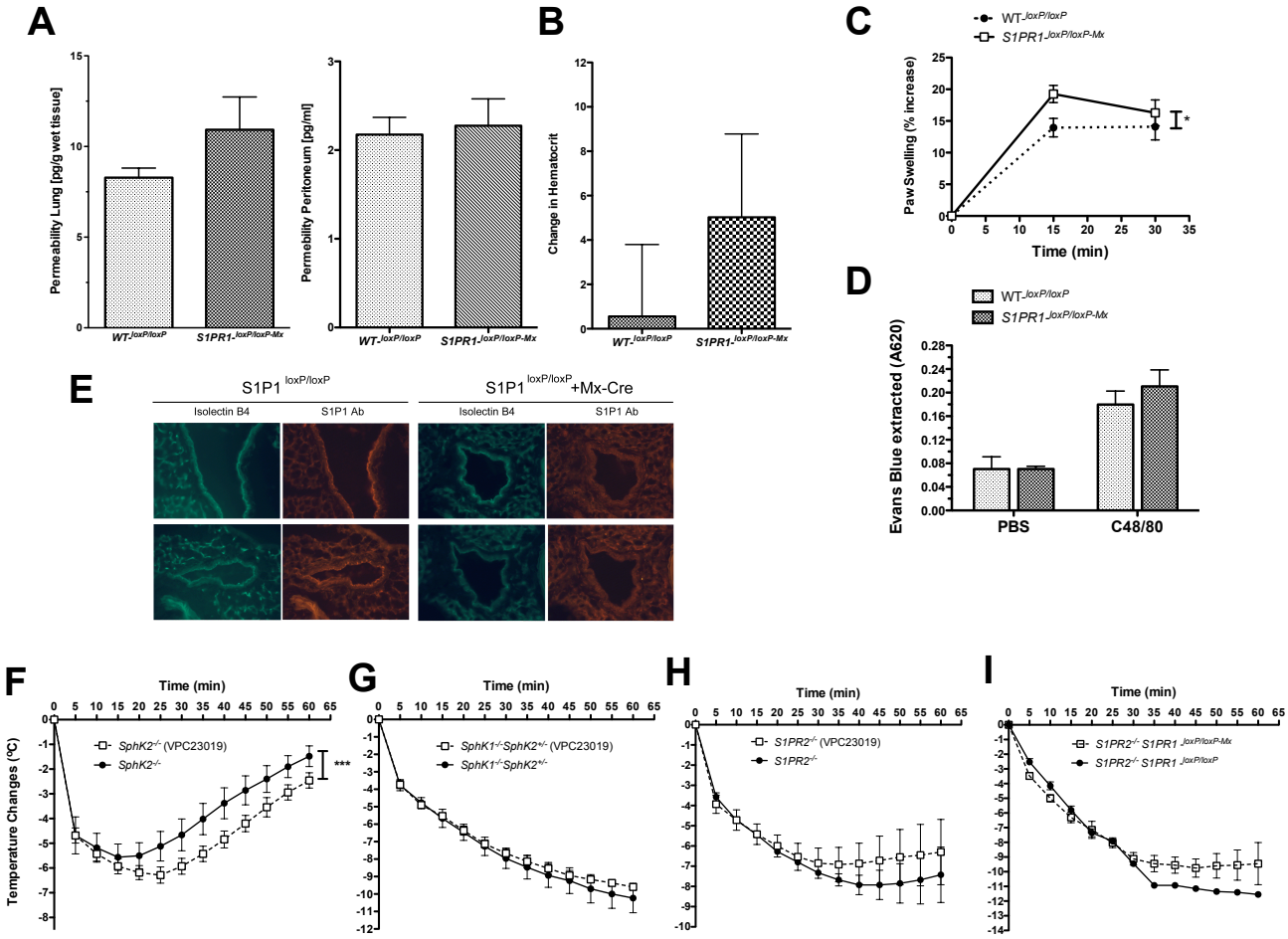
Supplemental Figure 5. Glomerular filtration rates in anesthetized mice during histamine-induced anaphylaxis. Bar graph of GFR values measured between 5 to 60 min after histamine injection (5 μmol) in anesthetized WT (n=6), *SphK1*$^{-/-}$ (n=6) and *SphK2*$^{-/-}$ mice (n=6). Also shown is the GFR of *SphK1*$^{-/-}$ mice that were treated with S1P post-histamine (10 min) administration (n=3). It is important to note that 3 out of the 6 *SphK1*$^{-/-}$ mice showed no measurable GFR while all of the WT had values between 131 and 273 μl/min.



Supplemental Figure 6. Blood pressure of anesthetized WT and *S1PR2*$^{-/-}$ mice. Mean arterial pressure (MAP) before (10 min) and after (30 min) histamine injection (5 μmol) did not reveal any differences in anesthetized *S1PR2*$^{-/-}$ (n=5) versus WT mice (n=5). Graph lines connect mean values of MAP measured at 2 min intervals. *$p<0.05$ compared to baseline measurements.



Supplemental Figure 7. Changes in the heart and breathing rates of *SphK*$^{-/-}$ and *S1PR2*$^{-/-}$ mice during histamine-induced-anaphylaxis. Heart (**A** and **B**) and breathing (**C** and **D**) rates were recorded using a pulse oximeter with a collar clip sensor both at baseline and after injection of histamine (5 μmol) at 30, 60 and 120 min in conscious *SphK1*$^{-/-}$, $^{/-}$ (**A** and **C**, n=6) and *S1PR2*$^{-/-}$ mice (**B** and **D**, n=5) and compared to their appropriate WT counterparts (n=4 or 5). *p<0.05 compared to baseline measurements.



Supplemental Figure 8- Changes in vascular permeability in *S1PR1*$^{loxP/loxP\text{-}Mx}$ mice during systemic anaphylaxis and local inflammation and effects of S1PR1 inhibition in *SphK*$^{-/-}$ and *S1PR2*$^{-/-}$ mice during histamine-induced anaphylaxis. **A)** Exudation of vascular fluids into the lungs and peritoneum after induction of systemic anaphylaxis by IgE/Ag. Mice (n=4) were injected (*i.v.*) with DNP-specific IgE (3 μg) and after 24 hrs, mice were challenged with Ag (500 μg DNP_{36}-HSA) to induce systemic anaphylaxis and 20 mg/Kg Evans blue dye to examine plasma leakage. After 30 min, the peritoneal lavage was collected and mice were perfused with saline though the right ventricle until blood was visibly eliminated. The amount of Evans blue in the peritoneal lavage and lungs was determined as described in Supplemental figure 4. **B**) Changes in hematocrit during

histamine-induced anaphylaxis. Hematocrits before and after 30 min of histamine-induced anaphylaxis were measured as described in Supplemental figure 6. The results are expressed as Δ increase in hematocrit (n= 4 to 5). **C)** Paw edema induced by injection of histamine into the footpad. Mice (n=7 to 9) were anesthetized (isoflurane (2%):oxygen (98%) mix for 2-3 min) and injected in the footpad with 20 µl of histamine (90 µg) or 20 µl PBS into the contralateral hindpaw. Paw thickness were measured as described in Supplemental figure 6. *p<0.05 between $WT^{loxP/loxP\text{-}Mx}$ and $S1PR1^{loxP/loxP\text{-}Mx}$ mice using a two way ANOVA test. **D**) Changes in vascular permeability in the skin. Vascular leakage was determined by quantifying the Evans blue dye extravasated into the ear 30 min after injection of the secretagogue C48/80 as described in Supplemental figure 4. **E)** Immunostaining showing reduced staining of S1PR1 in the endothelium of the aorta. Cross sections of aorta from $WT^{loxP/loxP\text{-}Mx}$ and $S1PR1^{loxP/loxP\text{-}Mx}$ were immunostained for isolectin B4 (green), a marker for endothelial cells, and S1PR1 as described previously (Allende, M.L, Yamashita, T., and Proia, R.L. Blood 15:3665-3667, 2003). **F-H)** Effects of the inhibition of S1PR1 by VPC23019 on histamine-induced anaphylaxis in $SphK2^{-/-}$ (F)(n=5-7), $SphK1^{-/-}$ $SphK2^{+/-}$ (G)(n=3) and $S1PR2^{-/-}$ mice (H) (n=4-6). VPC23019, an S1PR1/S1PR3 inhibitor, was injected into the mice *i.v.* (0.4 mM in 200µl) 10 min prior to the histamine injection. Body temperature was monitored as described in Methods. VPC23019 at 0.8 mM did not induce any further effects on body temperature on $SphK2^{-/-}$ mice as compared to 0.4 mM VPC23019. **I)** Effect of histamine on body temperature in $S1PR2^{-/-}$ /$S1PR1^{loxP/loxP\text{-}Mx}$ double knockout mice. Anaphylaxis by histamine in these mice was determined as described in Methods. (*p<0.05 and **p<0.01 was determined using a two way ANOVA).