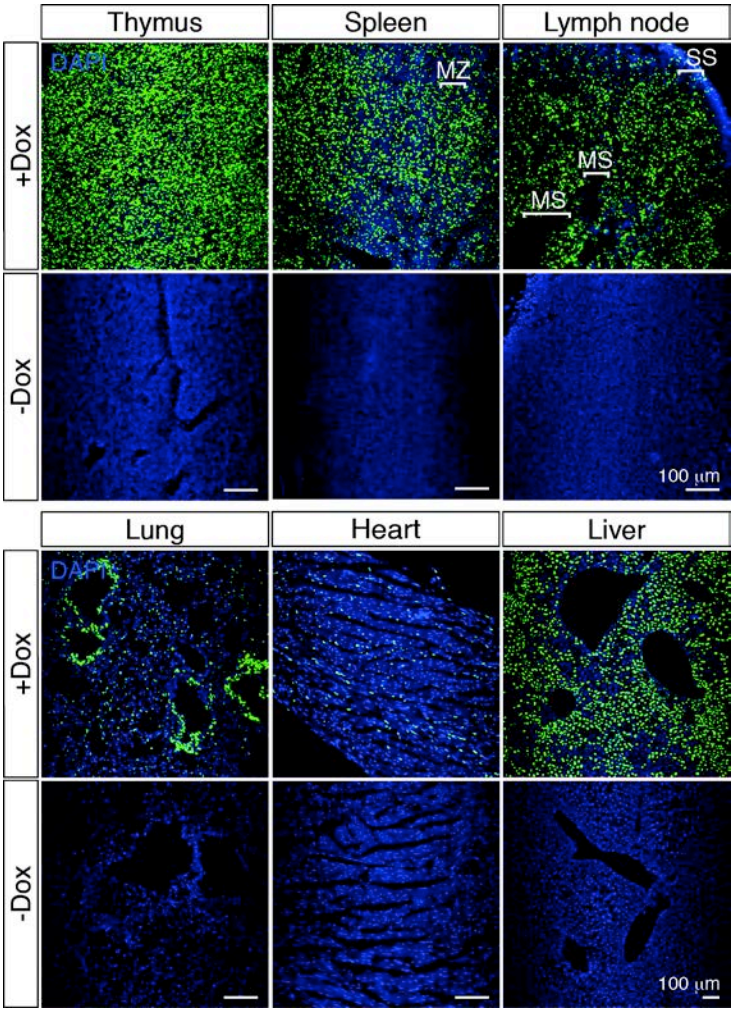
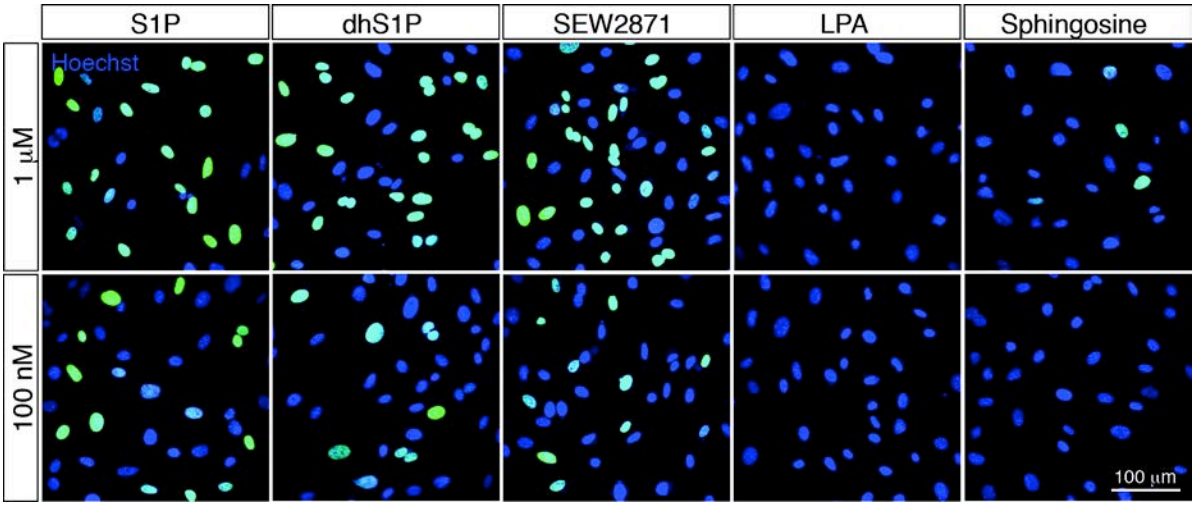
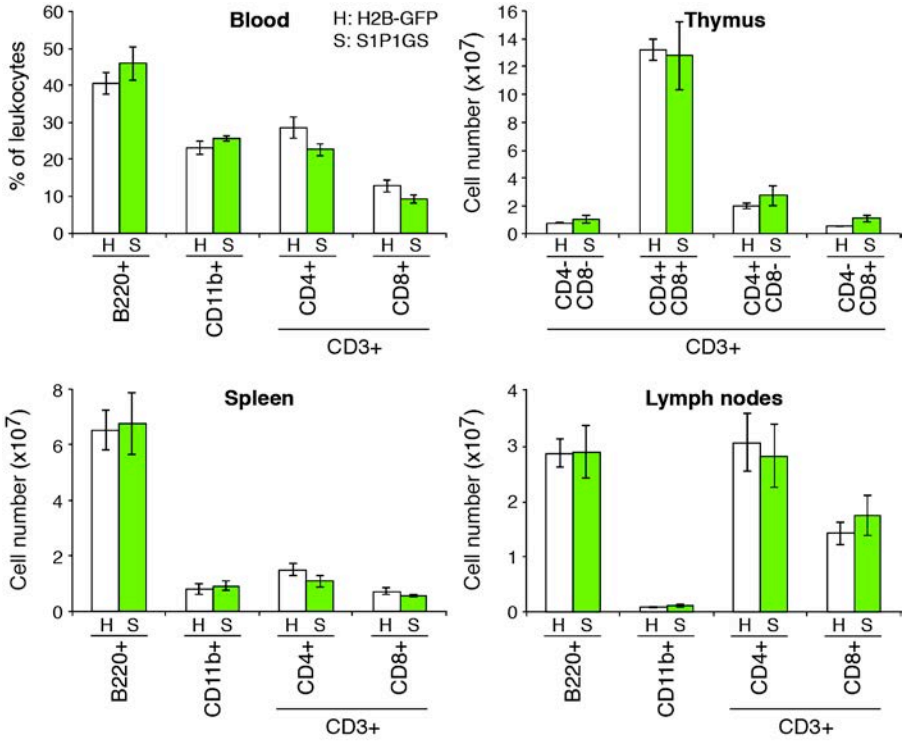
Supplemental Figures



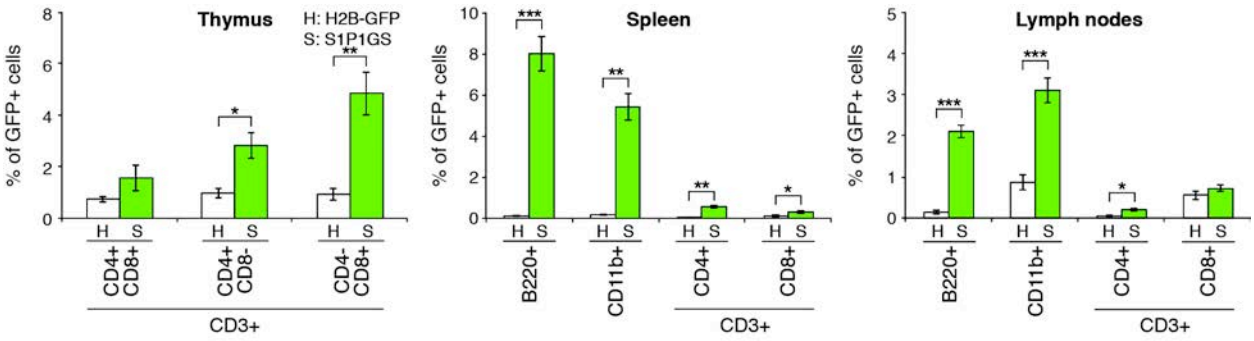
Supplemental Figure 1. Widespread reporter gene expression in tissues of H2B-GFP mice. H2B-GFP mice were crossed with Rosa26-M2rtTA mice. The offspring, which carried histone-GFP and rtTA-M2 genes, received drinking water with (+Dox) or without (-Dox) 0.1% doxycycline (in 5% sucrose in water) for 2 weeks. The tissues were harvested, and the histological sections were prepared. Nuclei were stained with DAPI, and the images were captured using an inverted laser-scanning confocal microscope. Bars indicate the scale of images. MZ, marginal zone; MS, medullary sinuses; SS, subcapsular sinuses. The tissues of three mice for each treatment were examined.



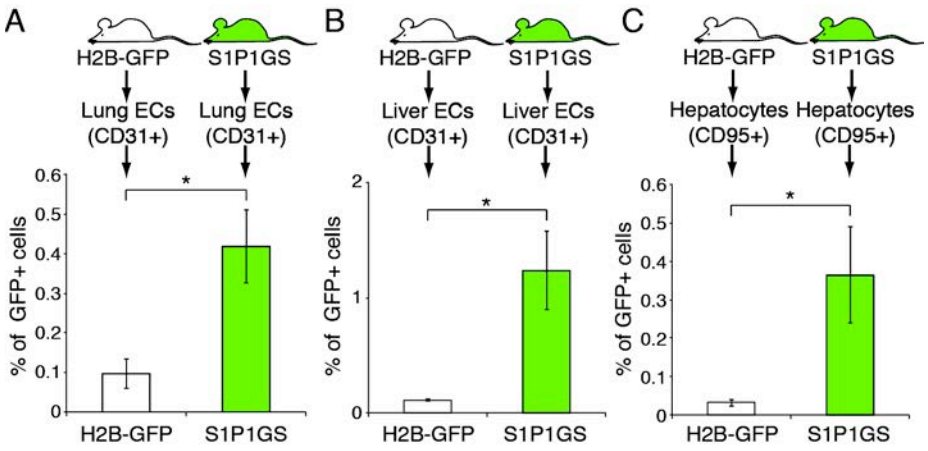
Supplemental Figure 2. Specificity of S1P1 activation in S1P1GS MEFs. S1P1GS MEFs were cultured for 16 h in medium containing 10 % charcoal-stripped FBS, and received various lipids, S1P, dhS1P, SEW2871, LPA or sphingosine. After 24 h, the nuclei were stained with Hoechst, and MEF cultures were imaged under an inverted laser-scanning confocal microscope. Bar indicates the scale of images. The experiment was performed twice, in duplicate, and representative images are shown.



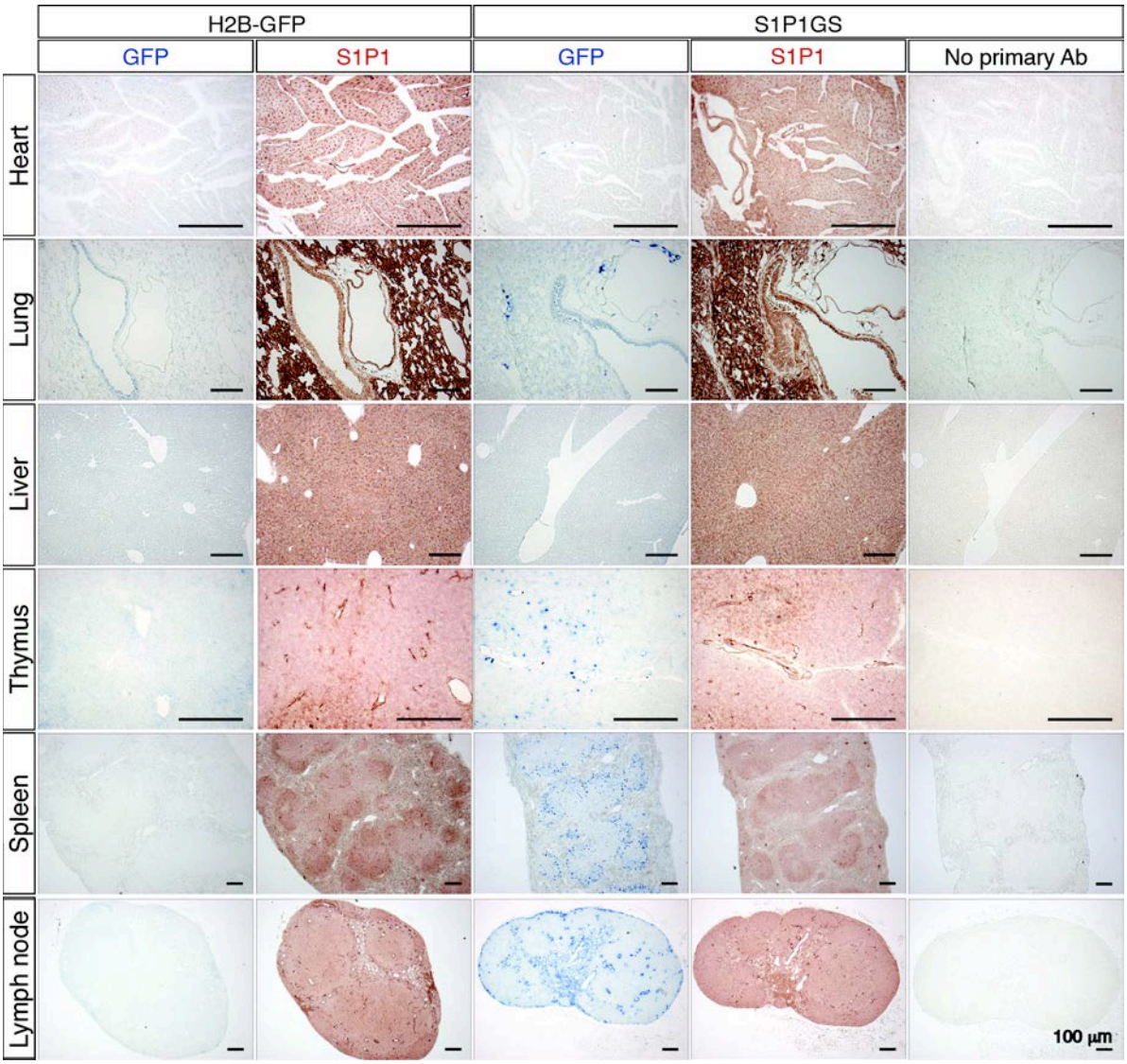
Supplemental Figure 3. Quantification of immune cells in S1P1 GFP signaling mice. Cells were isolated from the blood, thymus, spleen, and lymph nodes (pool of inguinal, brachial, and superficial cervical lymph nodes) of S1P1GS and H2B-GFP mice, immunostained with antibodies to B220, CD11b, CD3, CD4, and CD8, and quantified by flow cytometry. Open bars, H2B-GFP mice; green bars, S1P1GS mice. Bars represent mean values ± SE. n = 5 for each genotype.



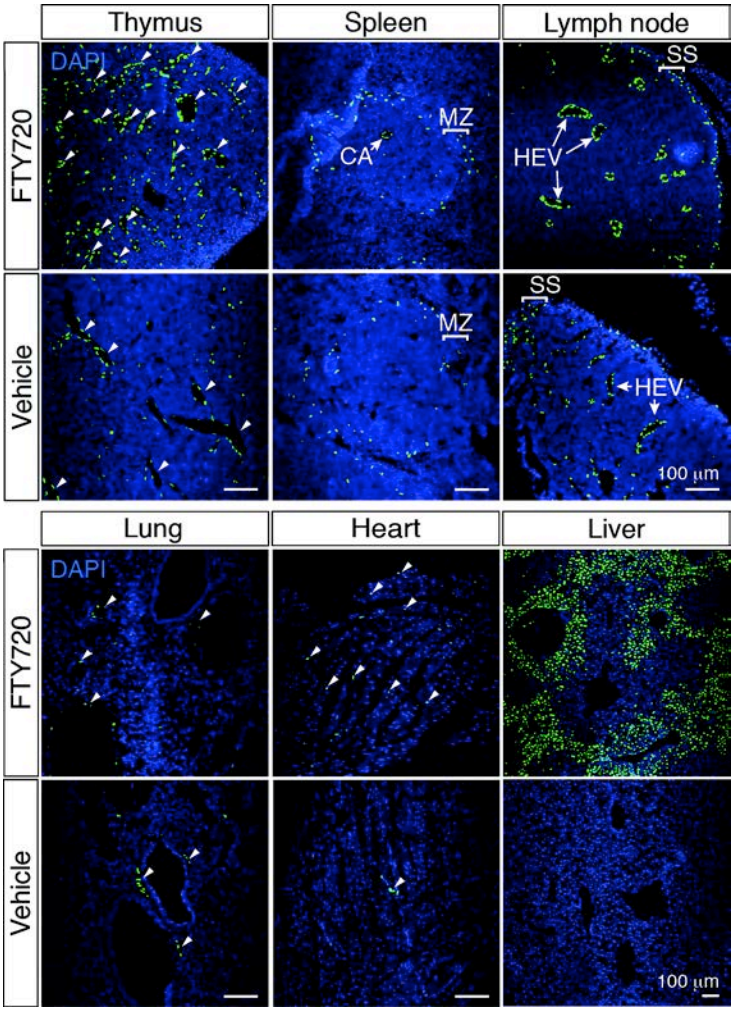
Supplemental Figure 4. Immune cell expression of activated S1P1. Cells were isolated from the thymus, spleen, and lymph nodes (pool of inguinal, brachial, and superficial cervical lymph nodes) of S1P1GS and H2B-GFP mice, immunostained with antibodies to B220, CD11b, CD3, CD4, and CD8, and analyzed by flow cytometry to determine the percentage of each cell population that was positive for GFP expression. Open bars, H2B-GFP mice; green bars, S1P1GS mice. Bars represent means values ± SE. n = 5 for each genotype. Student's *t* test; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.



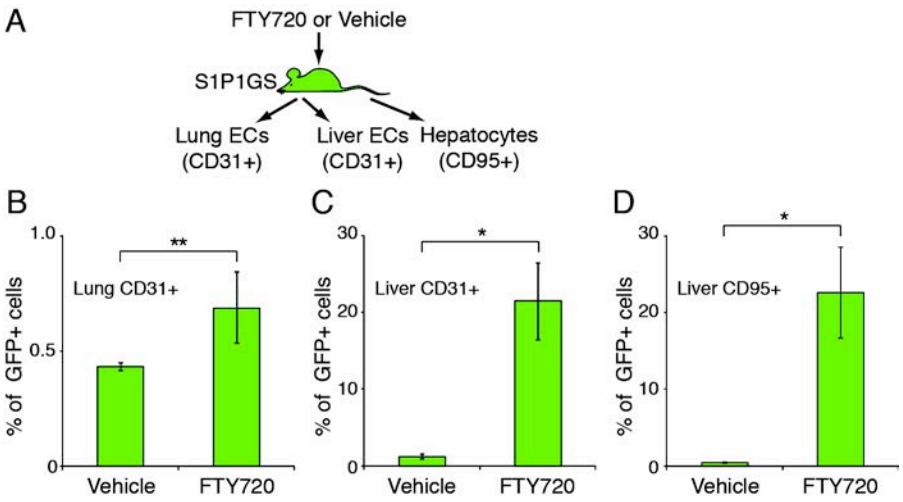
Supplemental Figure 5. S1P1 activation in endothelial cells and hepatocytes. (A–C) Schematic and the result of flow cytometry experiment. (A) Cells were isolated from the lung of S1P1GS and H2B-GFP mice, immunostained with antibody to CD31 and CD45, and analyzed by flow cytometry to determine the percentage of GFP+ cells in the CD45- CD31+ population for each genotype (B–C) Cells were isolated from the liver of S1P1GS and H2B-GFP mice, immunostained with antibodies against CD31 and CD95, and analyzed by flow cytometry to determine the percentage of GFP+ cells in CD31 or CD95+ populations for each genotype. Bars represent mean values ± SE. n = 3 for each genotype in (A). n = 5 for each genotype in (B-C). Student's *t* test; *, $p < 0.05$. EC, endothelial cells.



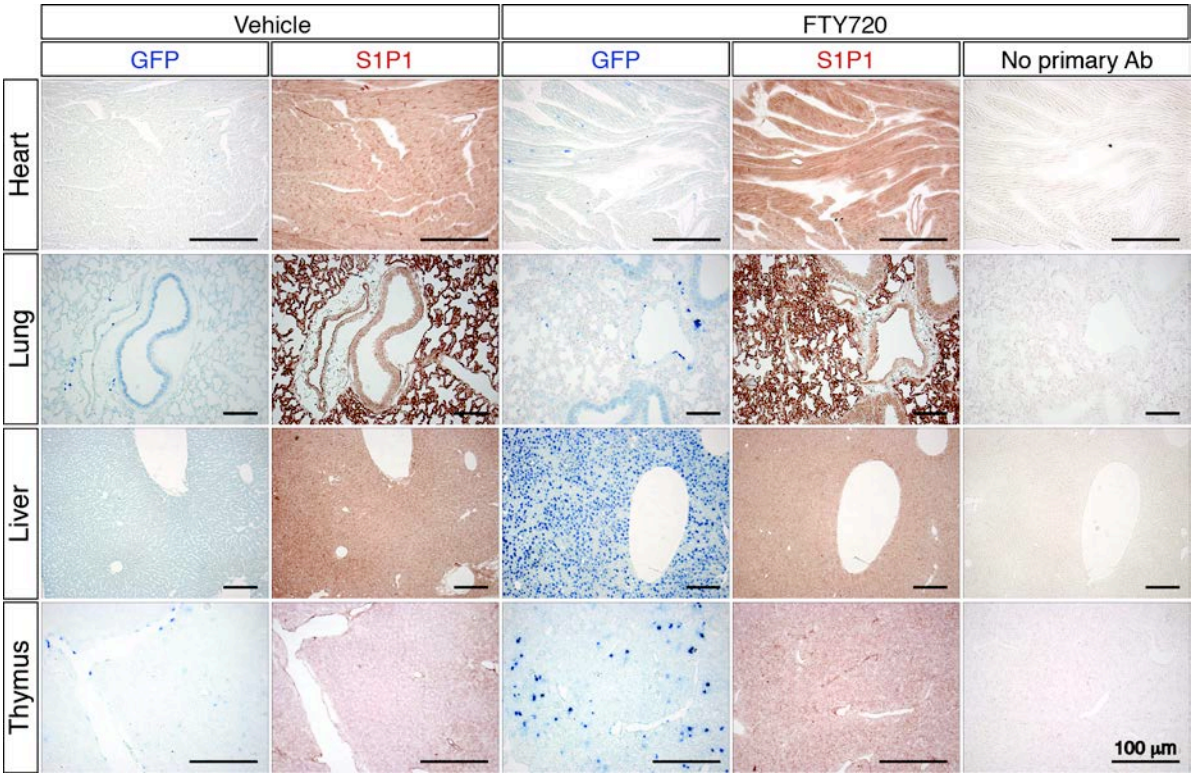
Supplemental Figure 6.
S1P1 activation and S1P1 expression in tissues from S1P1GS and H2B-GFP mice. Histological sections were prepared from S1P1GS and H2B-GFP mice and immunostained for the S1P1 receptor (red) and GFP (blue). The images were captured using a light microscope. Bars indicate the scale of images. The left two columns and the right three columns are serial sections.



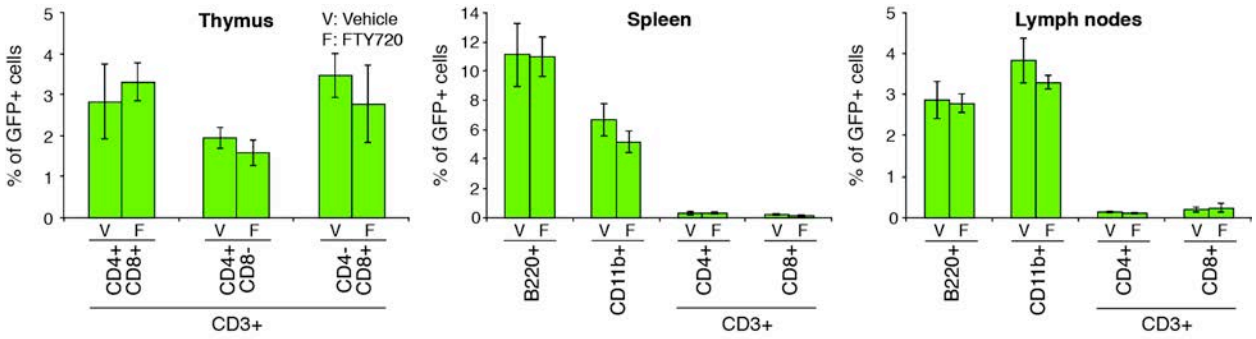
Supplemental Figure 7. FTY720-induced S1P1 activation in tissues. FTY720 (1 mg/kg) or vehicle (ethanol:PBS, 1:1) was intraperitoneally injected into S1P1GS mice. The tissues were harvested 1 day after injection, and histological sections were prepared. The nuclei were stained with DAPI, and the images were captured using an inverted laser-scanning confocal microscope. Small arrowheads point to GFP+ vasculature. Bars indicate the scale of images. CA, central arteries; MZ, marginal zone; HEV, high endothelial venules; SS, subcapsular sinuses. The tissues of six mice injected with FTY720 and five mice injected with vehicle were examined.



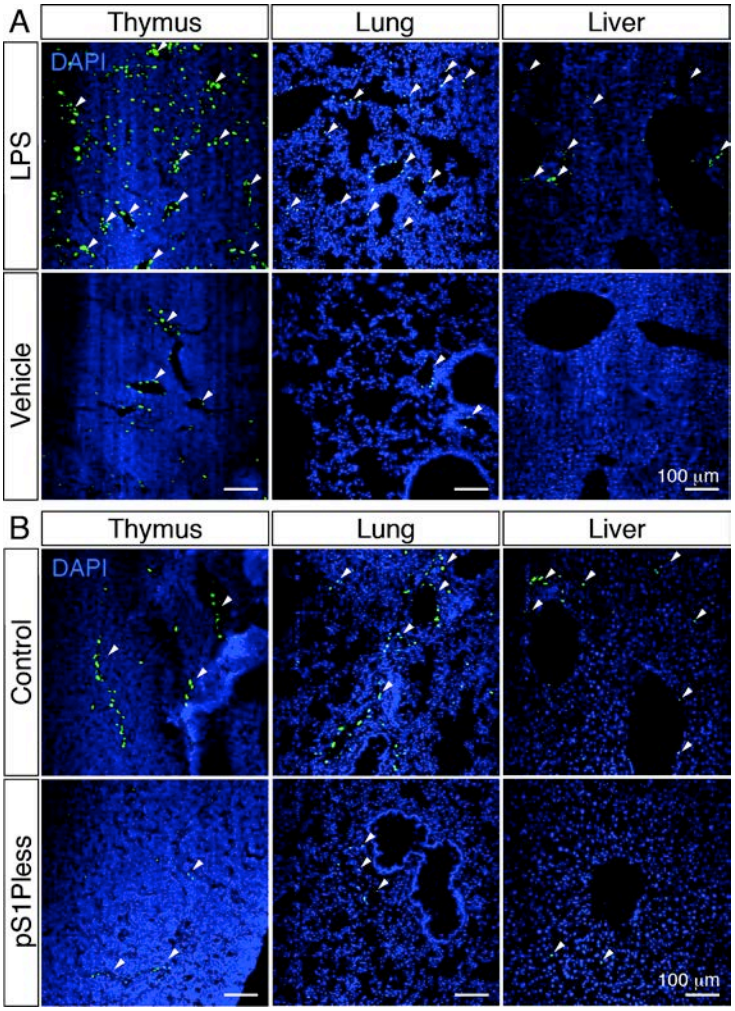
Supplemental Figure 8. FTY720-induced S1P1 activation in endothelial cells and hepatocytes. (A) Schematic of the experiment. FTY720 (1 mg/kg) or vehicle (ethanol:PBS, 1:1) was intraperitoneally injected into S1P1GS mice. After 24 h, cells were isolated from the lung and liver and immunostained with antibodies to CD31 and CD45 for lung, and CD31 and CD95 for liver. (B–D) Flow cytometry analysis was used to determine the percentage of GFP+ cells in the lung CD45- CD31+ population (B) and GFP+ cells in the liver CD31+ population (C) or CD95+ population (D). Bars represent mean values ± SE. n = 3 for each group in (B). n = 6 for FTY720 injected group and n = 5 for vehicle injected group in (C-D). Student's *t* test; *, $p < 0.05$, **, $p < 0.01$. EC, endothelial cells.



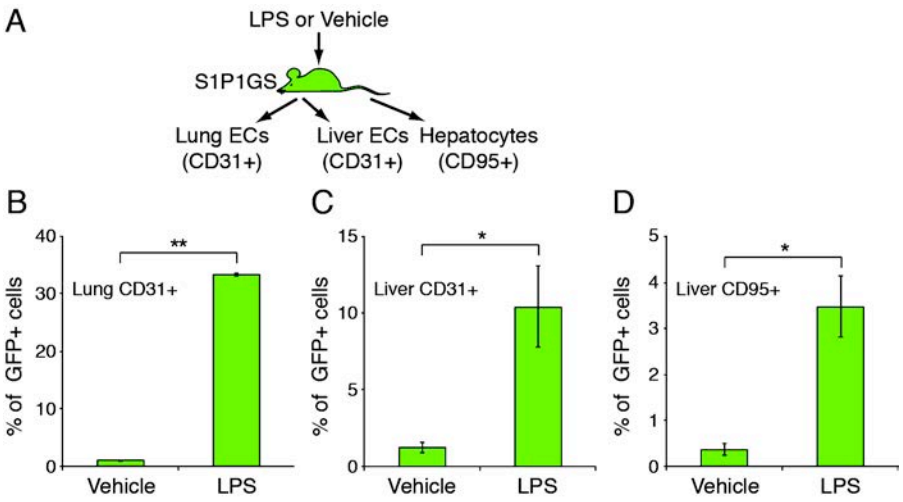
Supplemental Figure 9.
S1P1 activation and S1P1 expression in tissues from S1P1GS and H2B-GFP mice after FTY720 treatment. FTY720 (1 mg/kg) or vehicle (ethanol:PBS, 1:1) was intraperitoneally injected into S1P1GS mice. The tissues were harvested 1 day after injection, and histological sections were prepared and immunostained for GFP (blue) and S1P1 receptor (red). The images were captured using a light microscope. Bars indicate the scale of images. The left two columns and the right three columns are serial sections.



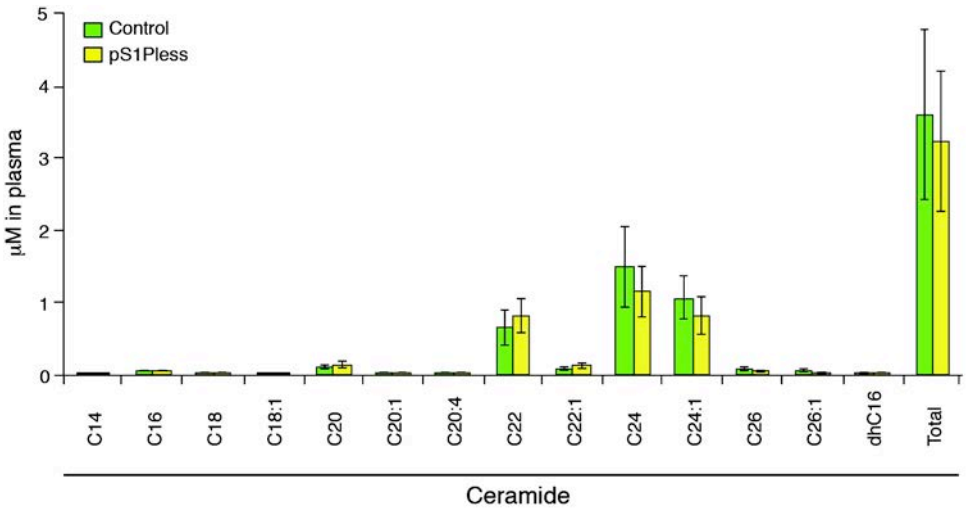
Supplemental Figure 10. FTY720-induced S1P1 activation in immune cells. FTY720 (1 mg/kg) or vehicle (ethanol:PBS, 1:1) was intraperitoneally injected into S1P1GS mice. Cells from thymus, spleen, and lymph nodes (pool of inguinal, brachial, and superficial cervical lymph nodes) were isolated, immunostained with antibodies to B220, CD11b, CD3, CD4, and CD8, and analyzed by flow cytometry to determine the percentage of each cell population that was positive for GFP expression. Bars represent mean values ± SE. n = 7 for FTY720 injected group and n = 5 for vehicle injected group.



Supplemental Figure 11.
S1P1 activation by hematopoietically derived S1P during systemic inflammation. (A) LPS (20 mg/kg) or vehicle (PBS) was intraperitoneally injected into S1P1GS mice. The tissues were harvested 3 days after injection, and histological sections were prepared. The nuclei were stained with DAPI, and the images were captured using an inverted laser-scanning confocal microscope. Small arrowheads point to GFP+ nuclei. Bars indicate the scale of images. The tissues of six mice injected LPS and five mice injected vehicle were examined. (B) Bone-marrow cells from plasma(p)S1Pless or control mice were transplanted into S1P1GS mice. Ten weeks later, LPS (20 mg/kg) was injected intraperitoneally into the mice. After 24 h, the tissues were harvested, and histological sections were prepared. The nuclei were stained with DAPI, and the images were captured using an inverted laser-scanning confocal microscope. Small arrowheads point to GFP+ nuclei. Bars indicate the scale of images. n = 2 for each group of thymus. n = 5 for each group of lung. n = 5 for each group of liver.



Supplemental Figure 12. S1P1 activation in endothelial cells and hepatocytes during systemic inflammation. (A) Schematic of the experiment. LPS (20 mg/kg) or vehicle (PBS) was intraperitoneally injected into S1P1GS mice. After 2 days, cells were isolated from the lung and liver and immunostained with antibodies to CD31 and CD45 for lung, and CD31 and CD95 for liver. (B–D) Flow cytometry analysis was used to determine the percentage of GFP+ cells in lung CD45- CD31+ population (B) and GFP+ cells in the liver CD31+ population (C) and CD95+ population (D). Bars represent mean values ± SE. n = 3 for each group in (B). n = 4 for the LPS injected group and n = 5 for the vehicle injected group in (C-D). Student's *t* test; *, $p < 0.05$, **, $p < 0.01$. EC, endothelial cells.



Supplemental Figure 13.
Quantification of ceramide species in plasma of bone marrow-transplanted mice. Bone marrow cells from plasma(p)S1Pless or control mice were transplanted into S1P1GS mice. Ten weeks later, plasma ceramide levels of species with different acyl-chain lengths were determined by HPLC-tandem mass spectrometry in bone marrow-transplanted mice. Bars represent mean values ± SE. n = 3 for each genotype.

Full unedited gel for Figure 2D