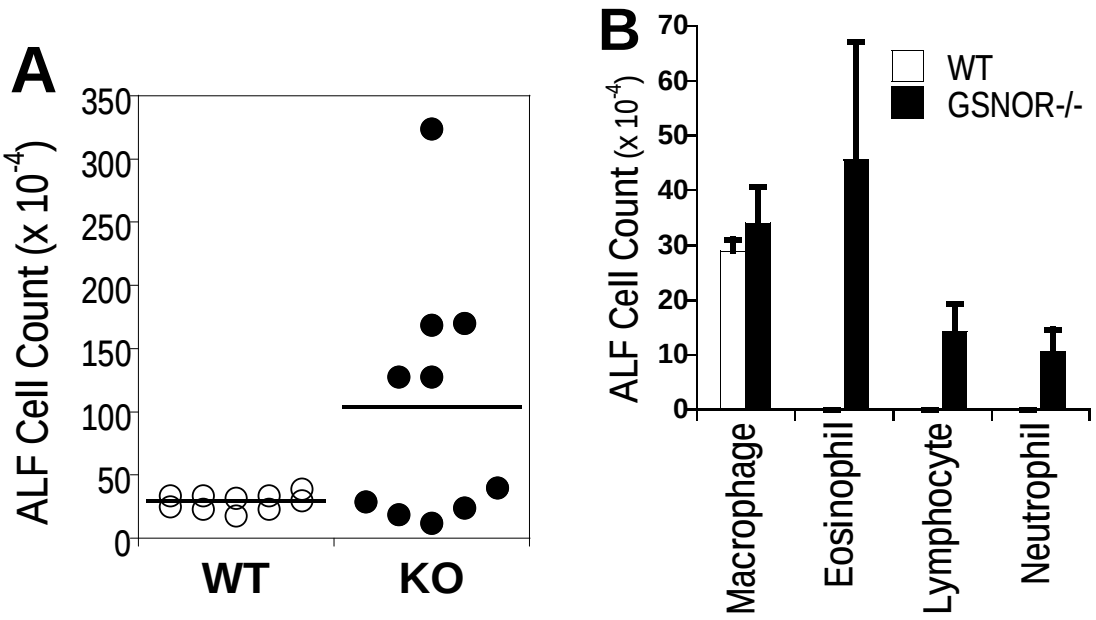
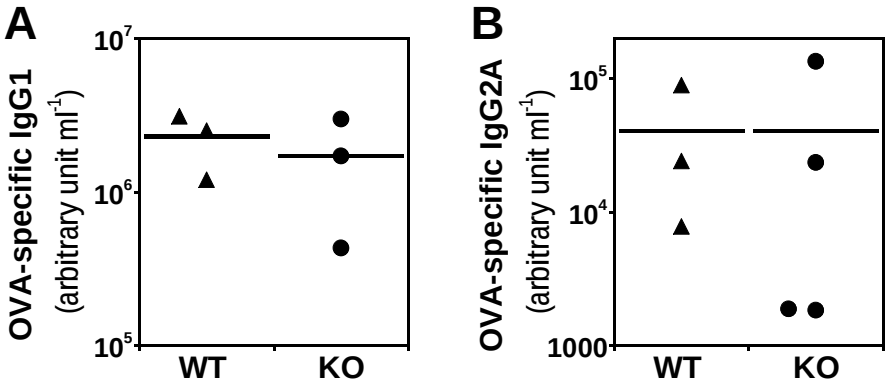
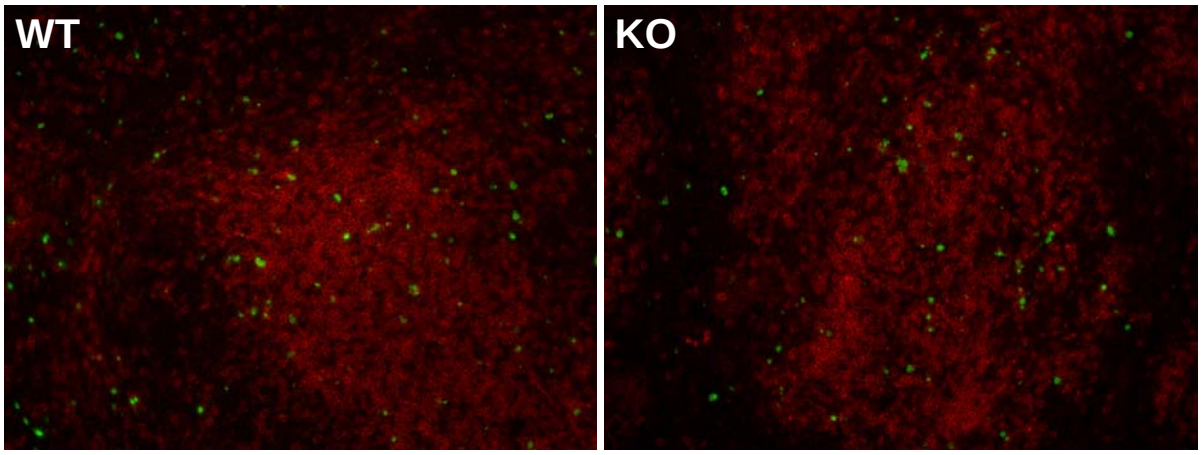


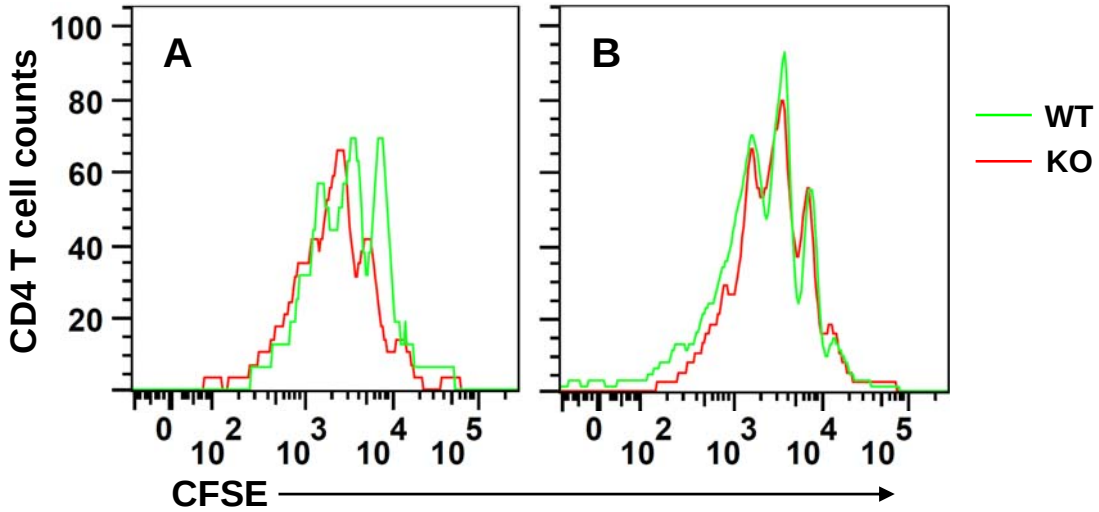
Supplemental Figure 1. Increase in leukocyte counts in lung of unchallenged GSNOR$^{-/-}$ mice. Total leukocyte counts (*A*) and counts of major leukocytes (*B*; mean + SE) in airway lining fluid (ALF) were from 10 wildtype (WT) and 10 GSNOR$^{-/-}$ (KO) mice.



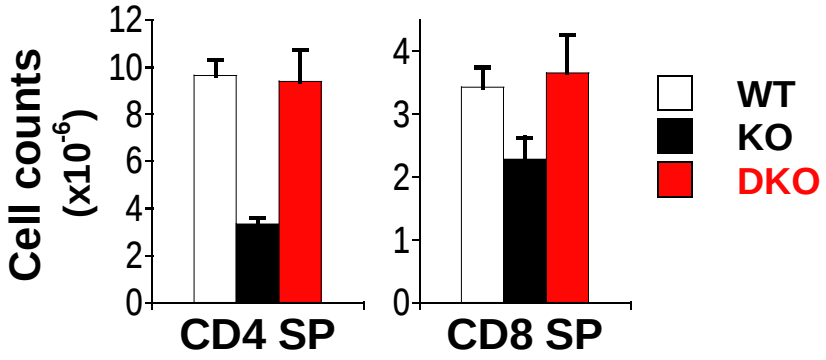
Supplemental Figure 2. Antibody production is unimpaired in GSNOR$^{-/-}$ mice. Six-week-old mice were immunized with ovalbumin (OVA) and boosted 3 weeks after immunization. Five weeks after the immunization, serum titers of IgG1 (*A*) and IgG2A (*B*) specific to OVA were measured using Mouse MonoAB ID KIT (HRP; Invitrogen).



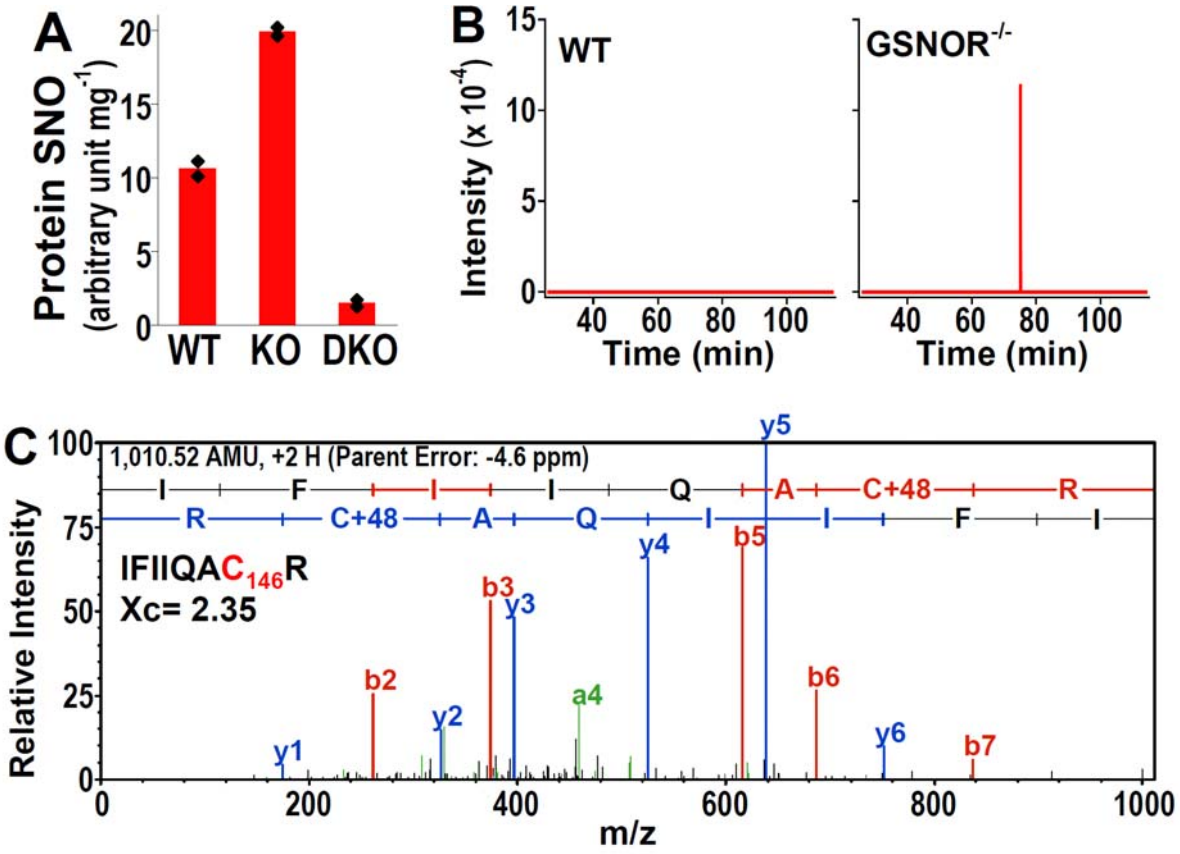
Supplemental Figure 3. Apoptosis in spleen of wildtype (WT) and GSNOR$^{-/-}$ (KO) mice. Apoptotic cells (green) were detected by TUNEL assay. The tissue sections were also stained with anti-CD4 antibody. The data represent 3 independent experiments.



Supplemental Figure 4. T cell proliferation in vitro. CD4 T cells, isolated from spleen and mesenteric lymph nodes of wildtype and GSNOR$^{-/-}$ mice, were labeled with CFSE and incubated for 3 days with anti-CD3 Ab, anti-CD28 Ab, and IL-2 supplemented with either IL-12 (*A*) or IL-4 (*B*). The data represent 2 independent experiments.



Supplemental Figure 5. CD4 SP and CD8 SP cell counts in thymuses of wildtype (WT), GSNOR$^{-/-}$ (KO), and GSNOR$^{-/-}$iNOS$^{-/-}$ (DKO) mice. The data (mean + SE) are from 6 mice of each strain. The CD4 SP ($P < 0.001$) and CD8 SP ($P < 0.05$) counts in GSNOR$^{-/-}$ thymus are significantly lower than wildtype controls.



Supplemental Figure 6. Protein S-nitrosylation is increased in thymus of GSNOR$^{-/-}$ mice. *A*, Content of protein S-nitrosothiols in mouse thymus. *B*, Extracted ion chromatographs at m/z 506.2683 of wildtype and GSNOR$^{-/-}$ thymuses. The m/z value corresponds to the expected m/z value for doubly charged SNO peptide IFIIQAC146R from caspase-6. C146 is the enzyme active site Cys. *C*, Establishment of the sequence and sulfonation site of the peptide in (*B*) from GSNOR$^{-/-}$ thymus by tandem mass spectrometry. The modified caspase-6 peptide is undetectable when the sample is first exposed to UV or treated with copper-ascorbate to destroy SNO.