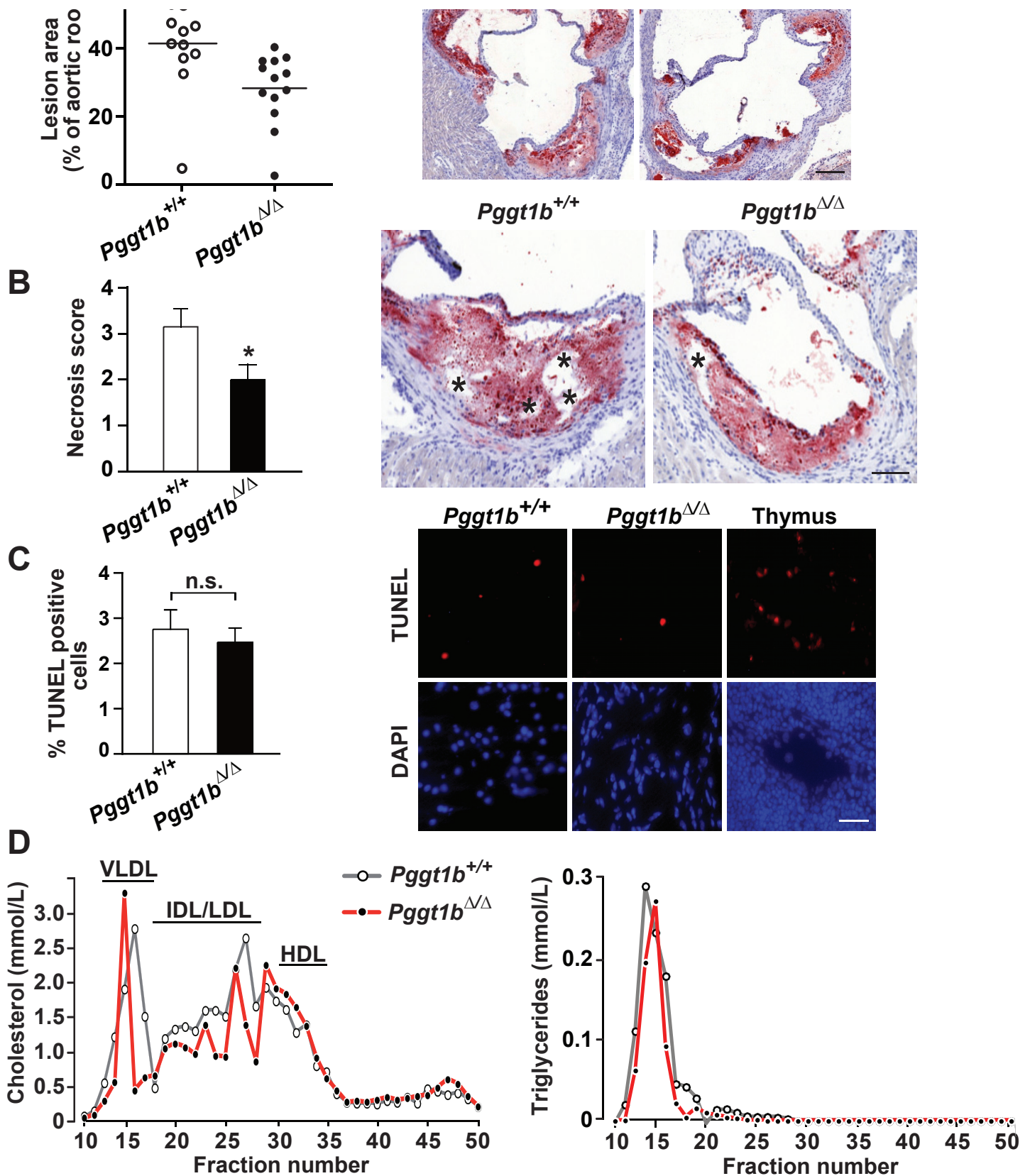


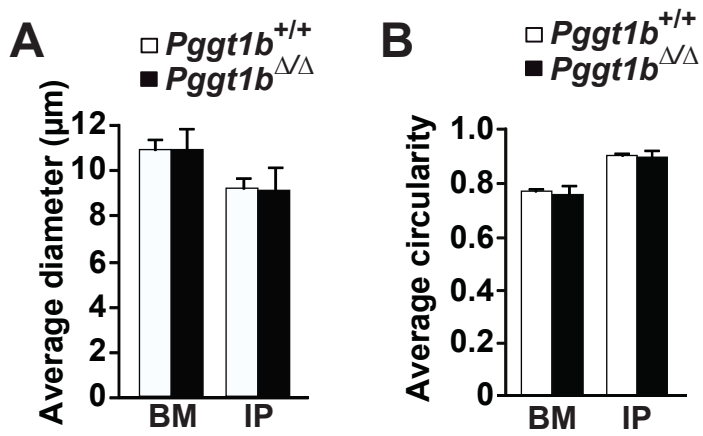
Supplemental Material

**Targeting GGTase-I activates RHOA, increases macrophage reverse
cholesterol transport, and reduces atherosclerosis in mice**

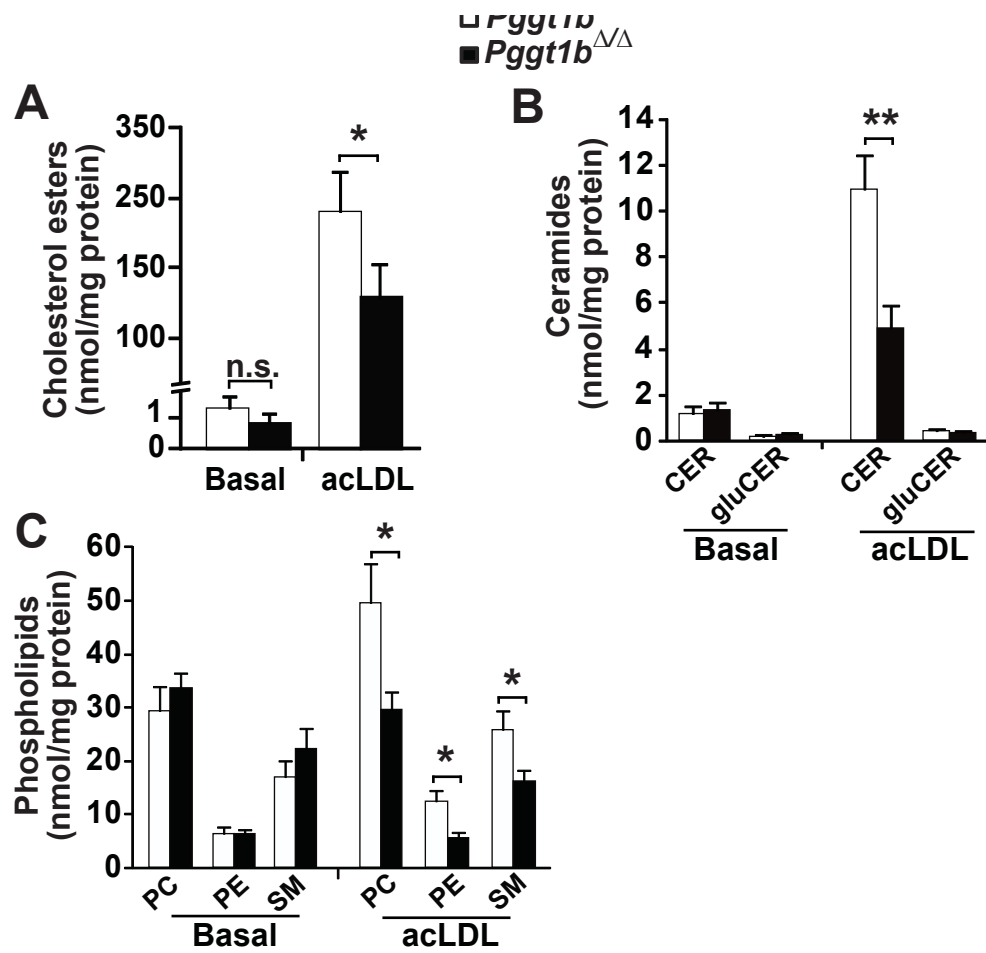
**Omar M. Khan,¹ Murali K. Akula,¹ Kristina Skålen,² Christin Karlsson,¹ Marcus
Ståhlman,² Stephen G. Young,³ Jan Borén,² and Martin O. Bergo¹**



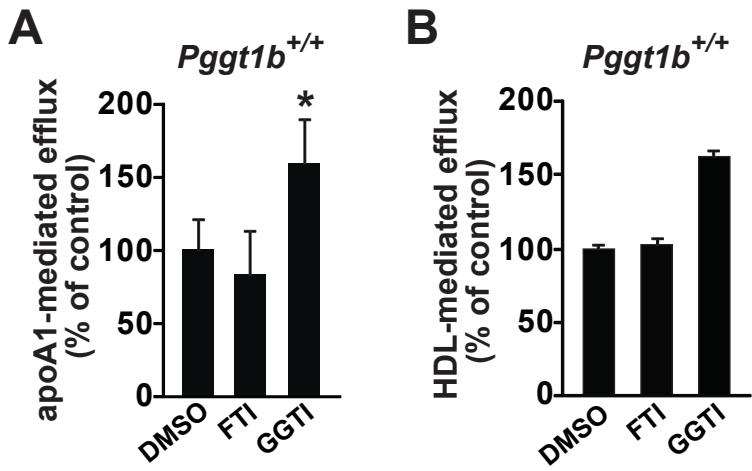
Supplementary Figure 1 (A) Quantification of Oil Red O staining of lipid lesions in aortic root sections expressed as percent of total aortic root area. Right panel, representative photographs of Oil Red O-stained aortic root sections. Scale bar, 50 μ m. The mice were fed a high-fat diet for 12 weeks. (B) Quantification of necrosis in aortic root sections. The mice were fed a high-fat diet for 24 weeks. Right panel, photographs of Oil Red O-stained aortic root lesions; areas of necrosis are indicated. Scale bar, 20 μ m (C) Quantification of TUNEL positive cells in aortic root lesions from mice shown in panel B. Right panel, representative immunofluorescence images. Thymus was used as a positive control. Scale bar,



Supplementary Figure 2 (A) Average diameter of BM and IP macrophages in suspension. (B) Average circularity of BM and IP macrophages in suspension. Values were obtained in a Vi-Cell XR cell counter (Beckman Coulter).



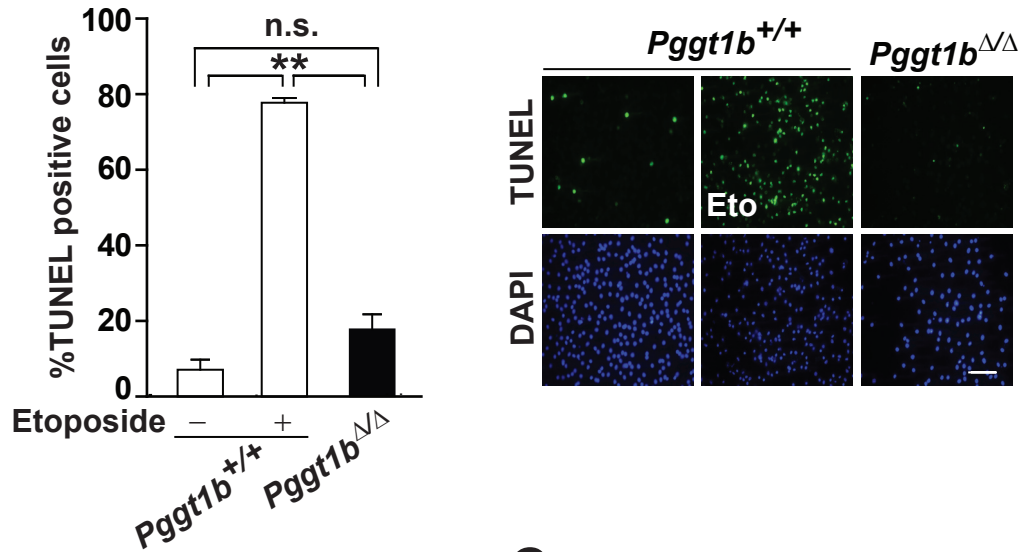
Supplementary Figure 3 HPLC- and mass spectrometry-based lipidomic analyses of cholesterol esters (**A**); ceramide (CER) and glucosylceramide (gluCER) (**B**); phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) (**C**) in BM macrophages before and after a 36-h incubation with acLDL (50 μg/ml). * $P < 0.05$ and ** $P < 0.01$.



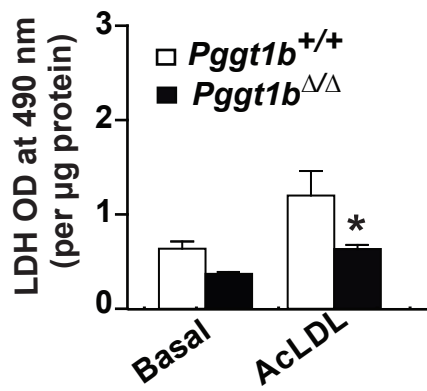
Supplementary Figure 4 (A) ApoA1-mediated cholesterol efflux in BM macrophages incubated with DMSO, FTI (10 μ M), and GGTI (10 μ M) ($n = 3-4$ /treatment). **(B)** HDL-mediated cholesterol efflux in BM macrophages incubated with DMSO, FTI, and GGTI ($n = 2$ /treatment).

Supplementary Figure 5

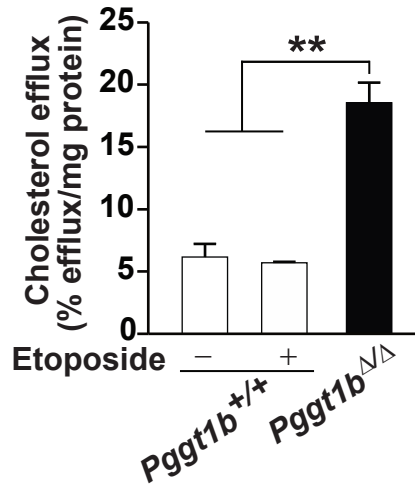
A



B

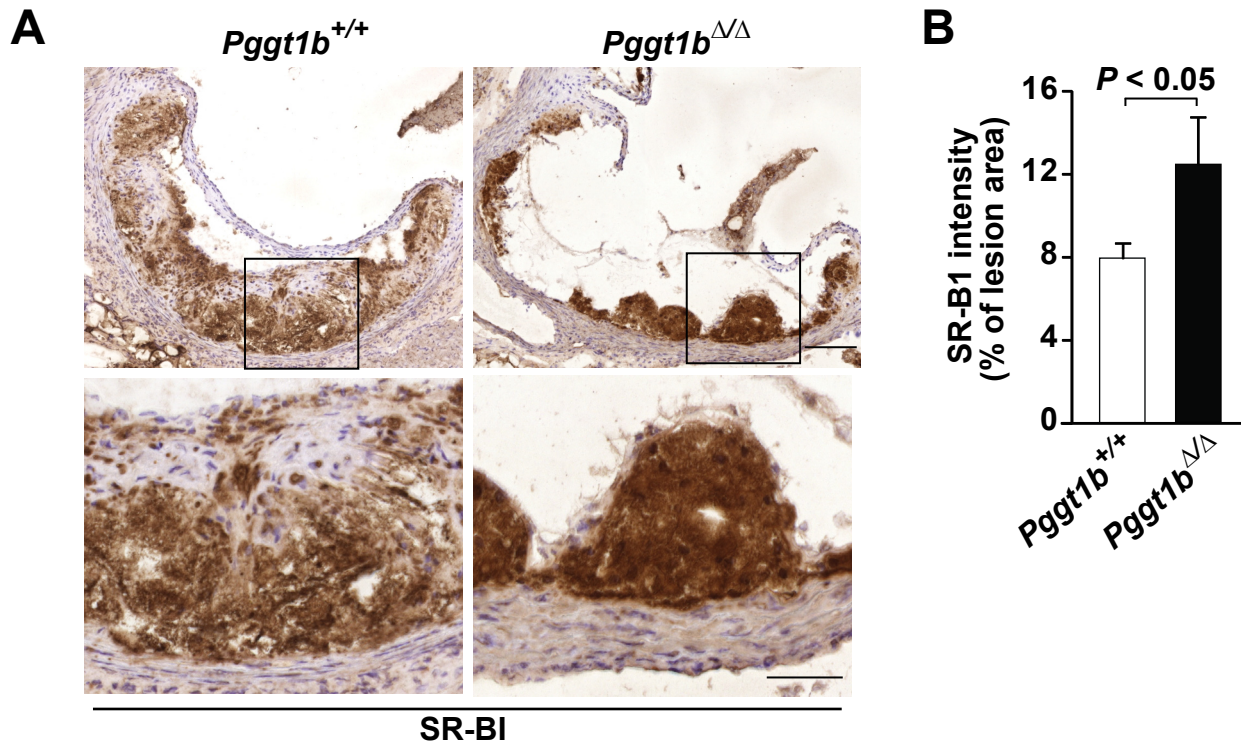


C

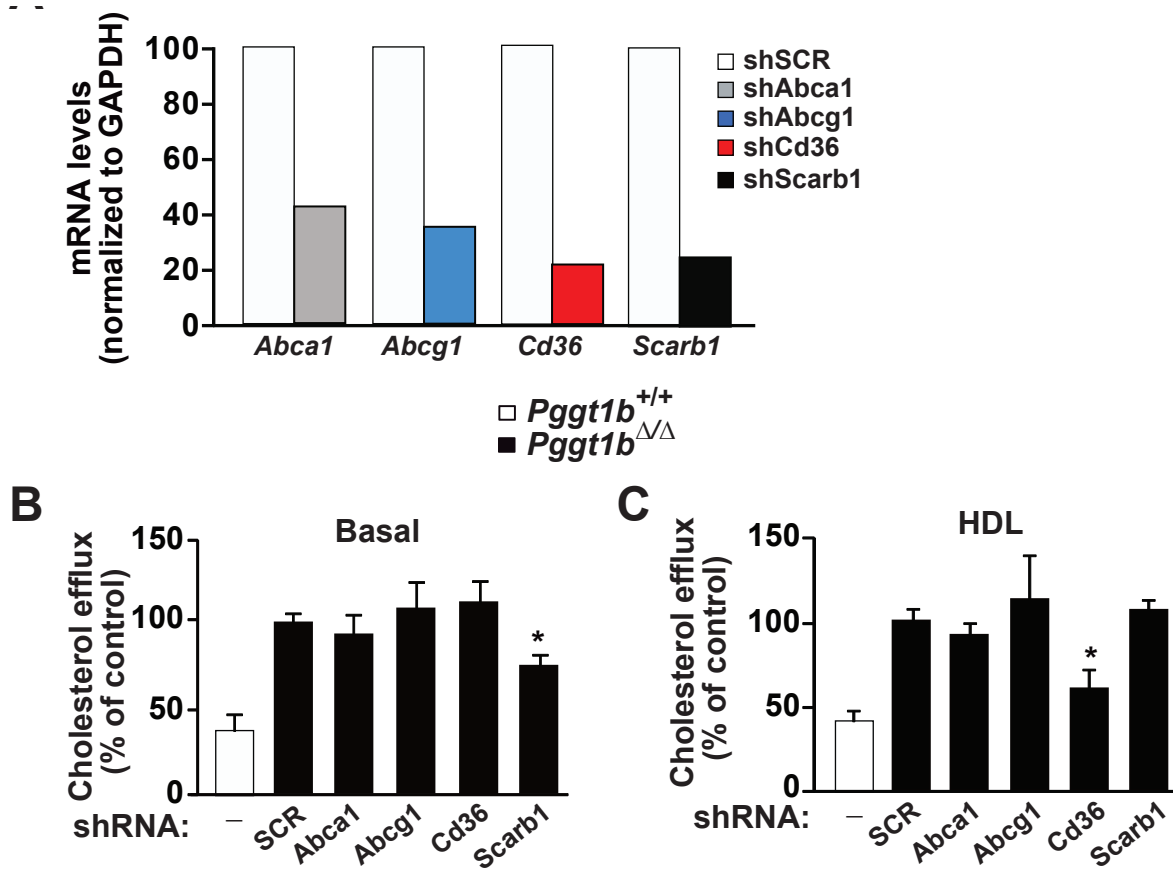


Supplementary Figure 5 (A) TUNEL staining of BM macrophages incubated for 24 h with 50 μg acLDL. Etoposide (25 μM) was used as a positive control. Right panel, representative immunofluorescence images. Scale bar, 10 μm. **(B)** Levels of lactate dehydrogenase (LDH; cytotoxicity assay) in cell culture media of BM macrophages from the efflux phase of the cholesterol efflux assay ($n = 3/\text{genotype}$). **(C)** Basal cholesterol efflux of BM macrophages incubated with etoposide (25 μM) or DMSO during the equilibration and efflux phases of the cholesterol efflux assay ($n = 3$ *Pggt1b*^{ΔΔ} and 4 *Pggt1b*^{+/+} cell lines).

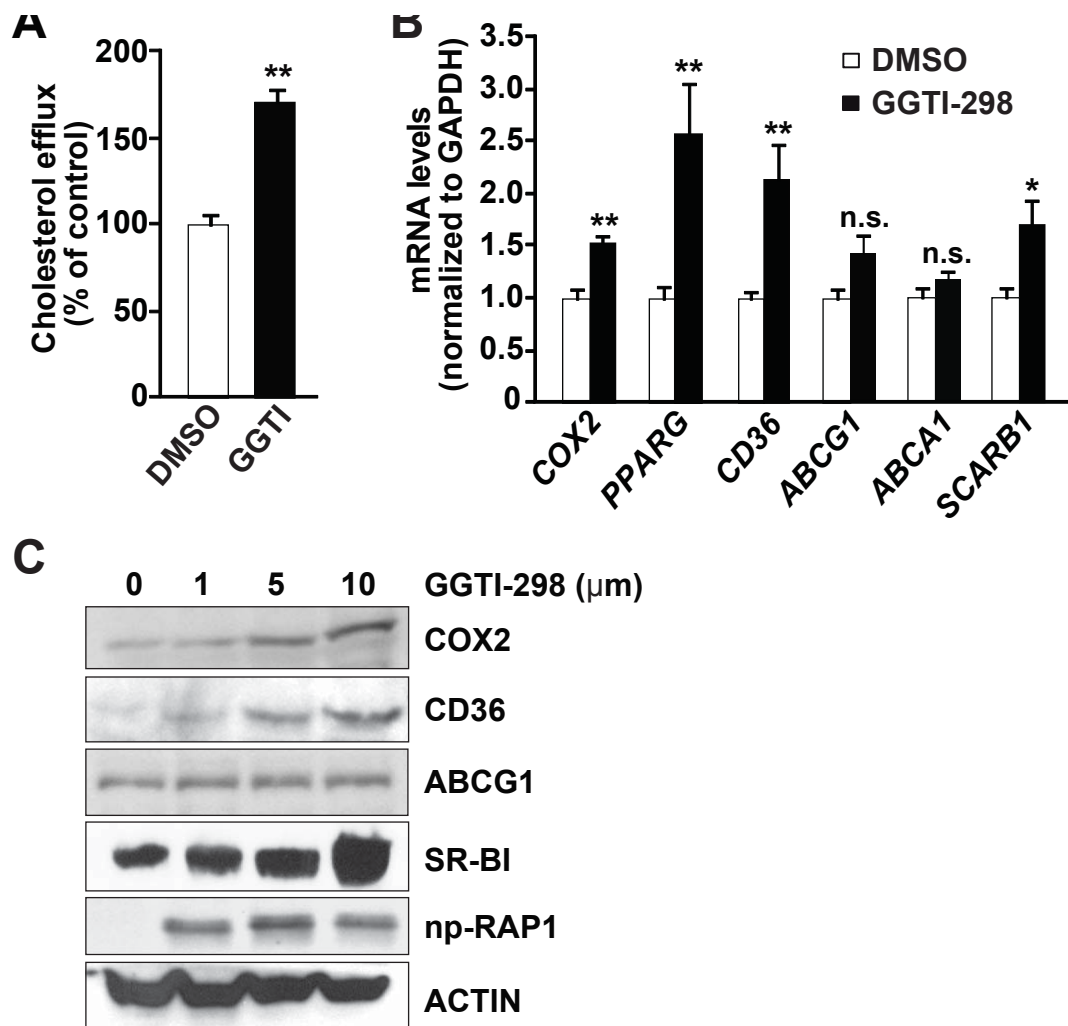
Supplementary Figure 6



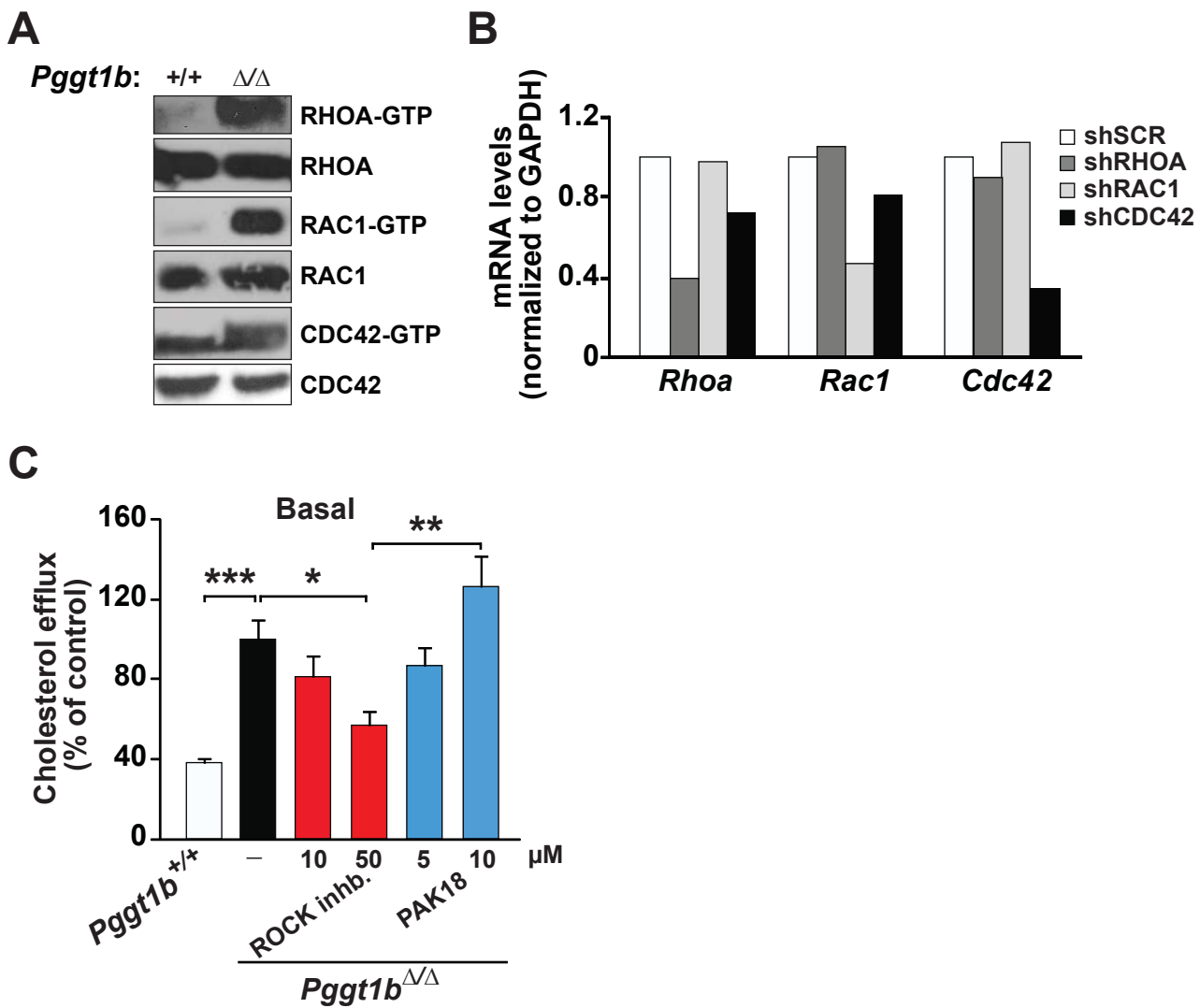
Supplementary Figure 6 (A) Immunohistochemical staining of SR-B1 in aortic root sections of mice fed a high-fat diet for 12 weeks. Scale bar, 10 μ m **(B)** Quantification of SR-B1 staining in aortic root lesions ($n = 10$ /genotype).



Supplementary Figure 7 (A) Taqman analyses showing gene expression in BM macrophages incubated with lentiviruses expressing shRNAs for *Abca1*, *Abcg1*, *Cd36*, and *Scarb1*, or containing a scrambled (SCR) sequence ($n = 2/\text{treatment}$). (B, C) Basal (B) and HDL-mediated (C) cholesterol efflux in BM macrophages incubated with lentiviruses described in A ($n = 6-9/\text{treatment}$). * $P < 0.05$.



Supplementary Figure 8 (A) Basal cholesterol efflux in THP-1 human macrophages incubated with DMSO or GGTI (10 μ M) for 48 h. Values are the mean of two independent experiments performed in triplicate. (B) TaqMan analysis showing gene expression in THP-1 macrophages incubated with DMSO or GGTI (10 μ M) for 48 h ($n = 4$ /treatment). (C) Western blots of lysates from THP-1 macrophages incubated with DMSO or GGTI for 48 h. The experiment was repeated three times with similar results. * $P < 0.05$ and ** $P < 0.01$.



Supplementary Figure 9 (A) Western blots showing levels of GTP-bound and total RHOA, RAC1, and CDC42 in lysates of BM macrophages. **(B)** Taqman analyses showing gene expression in *Pggt1b* ^{Δ/Δ} BM macrophages incubated with lentiviruses expressing shRNAs targeting RHOA, RAC1, and CDC42, or containing a scrambled (SCR) sequence ($n = 3/\text{treatment}$). **(C)** Basal cholesterol efflux in BM macrophages incubated with DMSO, ROCK inhibitor, and PAK kinase inhibitor ($n = 6\text{--}8/\text{genotype}$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.