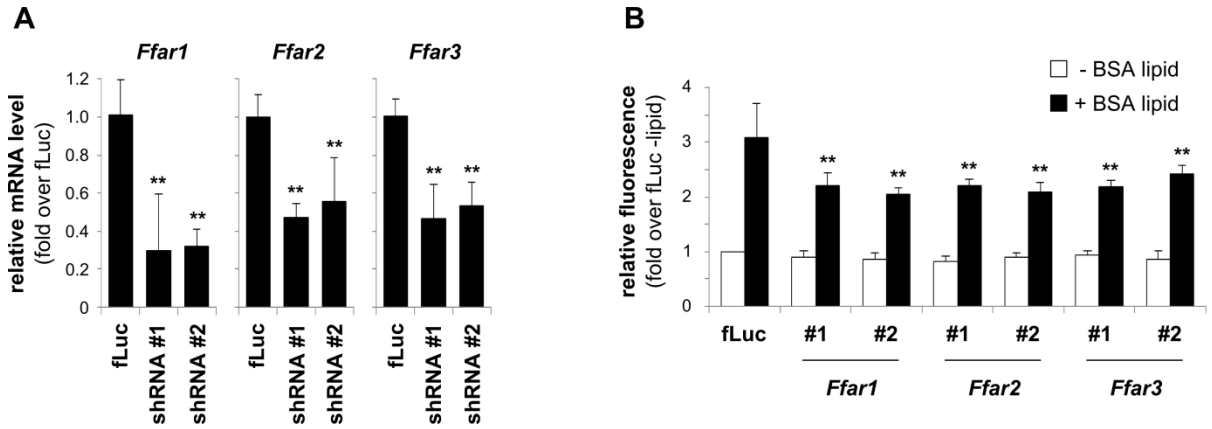
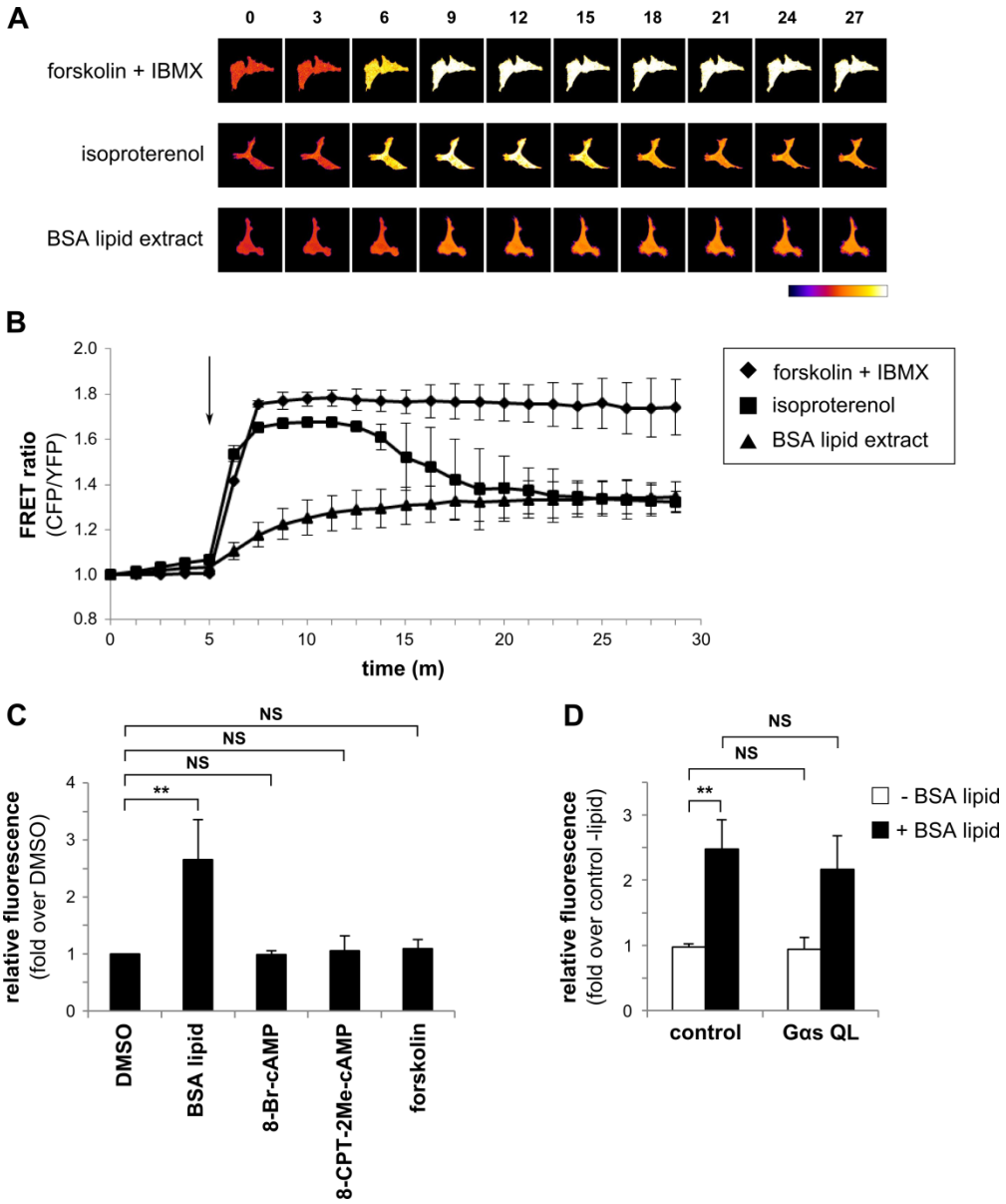


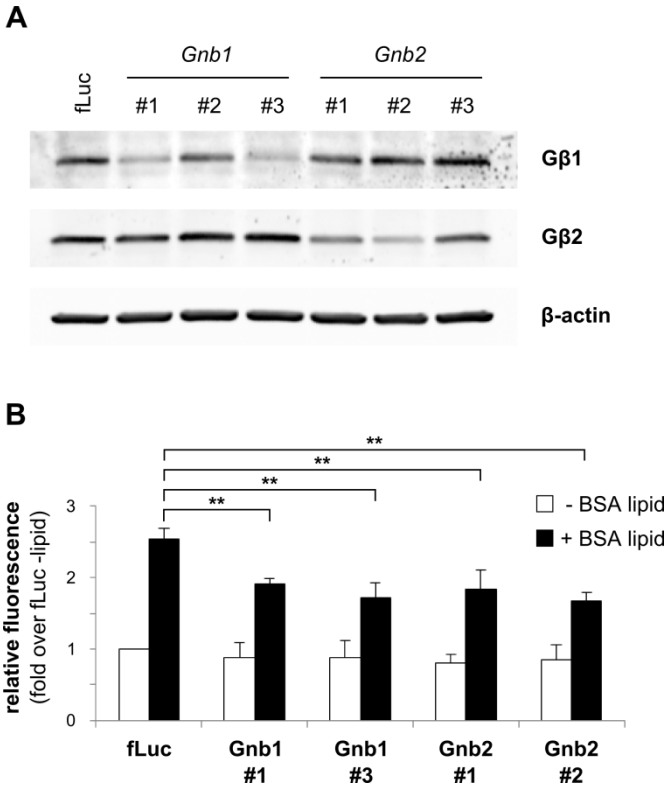
Supplemental Figure 1. Stimulation of macropinocytosis by albumin-associated lipids is mediated by free fatty acid receptors

(A) *Ffar* knockdown podocytes were analyzed by real time quantitative PCR. Results are shown as fold change of mRNA levels compared to control (firefly luciferase) knockdown cells (fLuc). (B) Control and *Ffar* knockdown podocytes were incubated with Rhodamine B-dextran and BSA lipid extracts for 30 min and analyzed by flow cytometry. Results are shown as fold change in mean fluorescence intensity. The data represent mean ± SD of 3 independent experiments analyzed by ANOVA followed by Bonferroni's post hoc analysis. ** $p \leq 0.01$



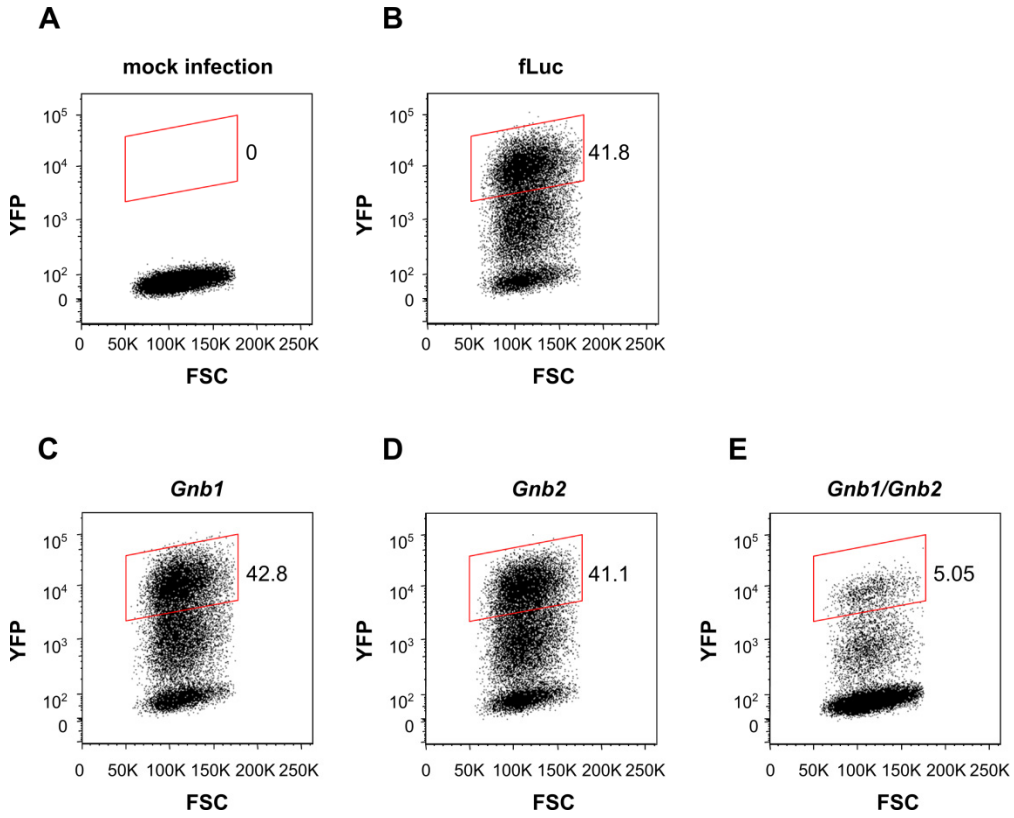
Supplemental Figure 2. Stimulation of macropinocytosis by albumin-associated lipids is not mediated by Gα activation

(A) Podocytes expressing the cAMP FRET sensor $^{T}Epac^{VV}$ were treated with forskolin (25 µM) + IBMX (100 µM), isoproterenol (10 µM), or 3 µl/ml BSA lipid extract and analyzed by real time confocal microscopy. Cells are pseudocolored to display CFP/YFP ratios. Changes in CFP/YFP ratios over time are shown in (B). Arrow indicates time of treatment. (n= 4, Forskolin + IBMX; 4, Isoproterenol; 10, BSA lipid extract) (C) Podocytes were incubated with 3 µl/ml BSA lipid extract, forskolin (10 µM), 8-Br-cAMP (10 µM), or 8-CPT-2Me-cAMP (10 µM) in the presence of FITC-dextran for 30 min and analyzed by flow cytometry. (D) Control cells or podocytes expressing constitutively active GαS (Q227L) were incubated with BSA lipid extract in the presence of FITC-dextran for 30 min and analyzed by flow cytometry. Results are shown as fold change in mean fluorescence intensity. The data represent mean ± SD of 3 independent experiments analyzed by ANOVA followed by Bonferroni's post hoc analysis. ** $p \leq 0.01$, NS (not statistically significant)



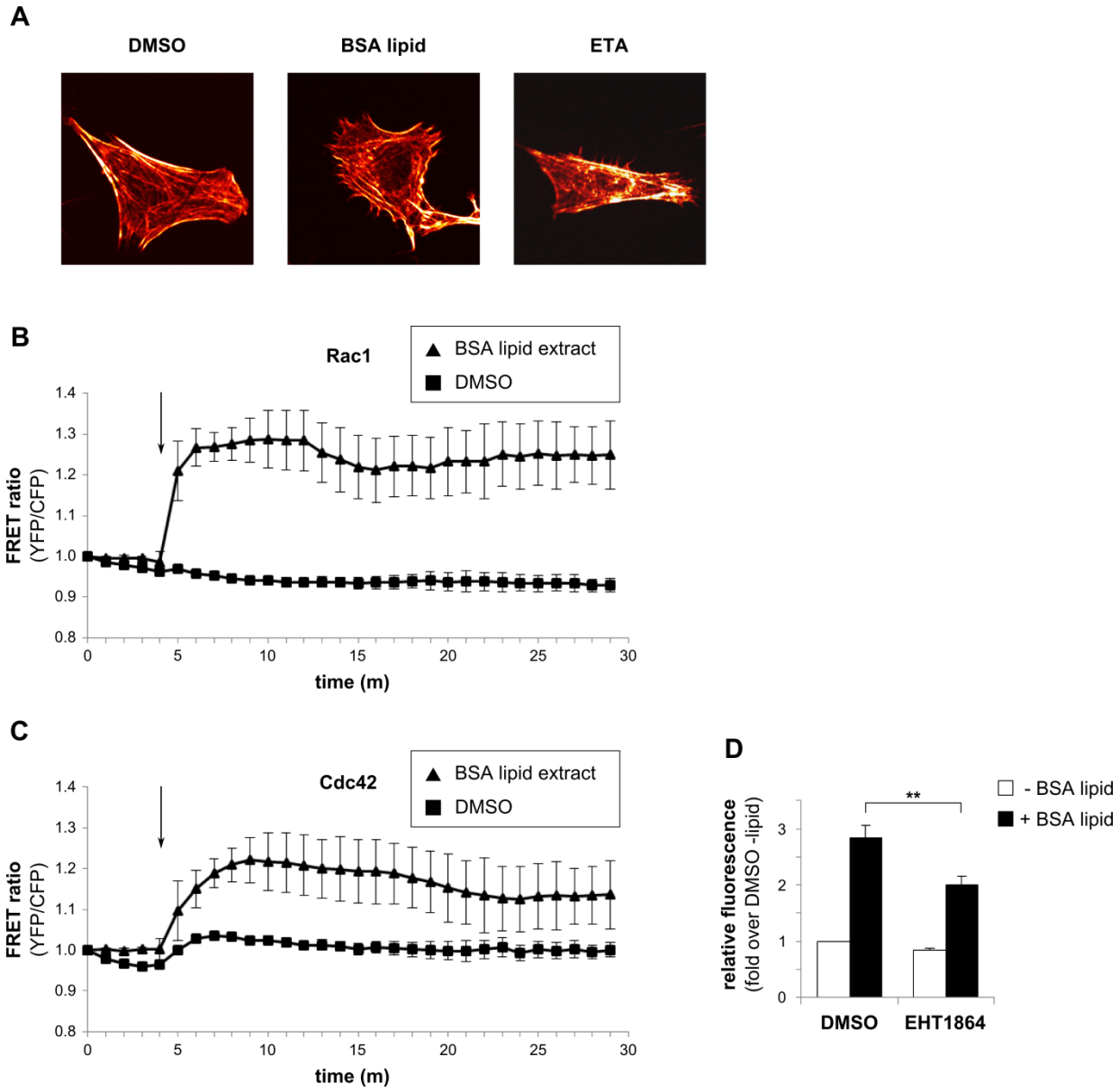
Supplemental Figure 3. Stimulation of macropinocytosis by albumin-associated lipids is mediated by Gβ activation

(A) Podocytes expressing shRNAs targeting fLuc, *Gnb1*, or *Gnb2* were immunoblotted for Gβ1 and Gβ2 expression. β-actin was used as loading control. A representative blot of 3 independent experiments is shown. Gnb1 #3 and Gnb2 #2 shRNAs were used for double knockdown experiments in Figure 6. (B) *Gnb* knockdown podocytes were incubated with BSA lipid extracts in the presence of Rhodamine B-dextran for 30 min and analyzed by flow cytometry. Results in (B) are shown as fold change in mean fluorescence intensity. The data represent mean ± SD of 3 independent experiments analyzed by ANOVA followed by Bonferroni's post hoc analysis. ** $p \leq 0.01$



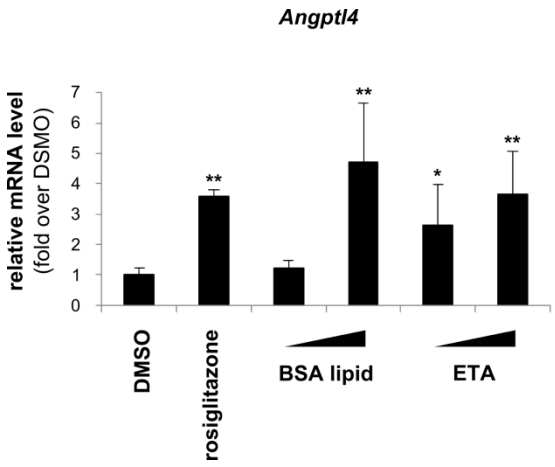
Supplemental Figure 4. Flow cytometry analysis of podocytes infected with *Gnb* knockdown lentivirus

Podocytes were infected with lentiviral vectors expressing YFP and shRNAs targeting firefly luciferase (fLuc) or different *Gnb* isoforms. Cells expressing high levels of YFP (gated region) were sorted and used for further analysis. Note the lower number of YFP-positive cells, especially the significant reduction of the YFP^{hi} populations, in podocytes infected with *Gnb1*/*Gnb2* shRNA lentivirus (panel E). Representative results from 3 independent experiments are shown.



Supplemental Figure 5. Stimulation of podocytes with albumin-associated lipids leads to changes in actin structures and activation of Rac1 and Cdc42

(A) Podocytes were incubated with DMSO, 3 µl/ml BSA lipid extract, or 20 µM ETA for 20 min, fixed, stained with phalloidin, and analyzed by confocal microscopy. Images are representative of 3 independent experiments (B, C) Podocytes expressing FRET biosensors of Rac1 (B) and Cdc42 (C) were treated with 3 µl/ml BSA lipid extract and analyzed by live cell imaging on a confocal microscope. Changes in YFP/CFP ratios are plotted against time. Arrows indicates time of treatment. Results are representative of 3 independent experiments. (n= 3 each) (D) Podocytes were pretreated with DMSO or EHT1864 (5 µM) for 30 min. Subsequently, the cells were incubated with BSA lipid extract in the presence of FITC-dextran for 30 min and analyzed by flow cytometry. Results are shown as fold change in mean fluorescence intensity. The data represent mean ± SD of 3 independent experiments analyzed by ANOVA followed by Bonferroni's post hoc analysis. ** $p \leq 0.01$



Supplemental Figure 6. Free fatty acids stimulate expression of Angptl4 in podocytes

Podocytes were treated with 5 μM rosiglitazone, 2 - 5 μL/ml BSA lipid extract, or 2 - 10 μM ETA for 24 h and analyzed by real time quantitative PCR. Results are shown as fold change of mRNA levels compared to vehicle (DMSO)-treated cells. The data represent mean ± SD of 3 independent experiments analyzed by ANOVA followed by Bonferroni's post hoc analysis. * $p \leq 0.05$, ** $p < 0.01$