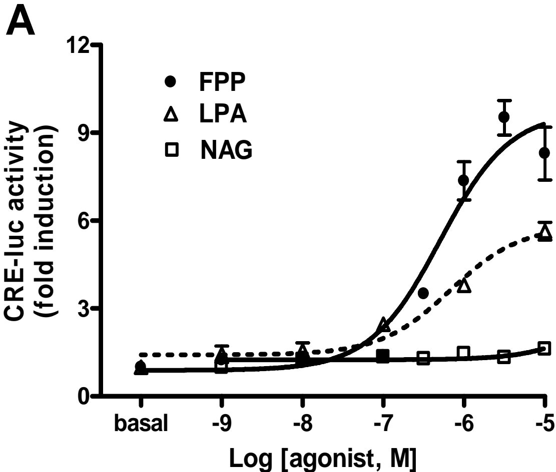
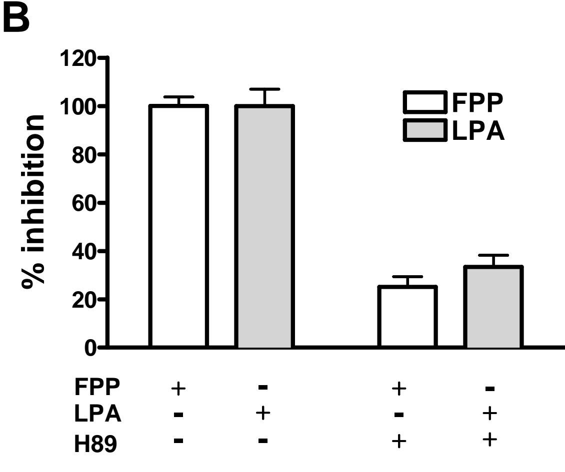
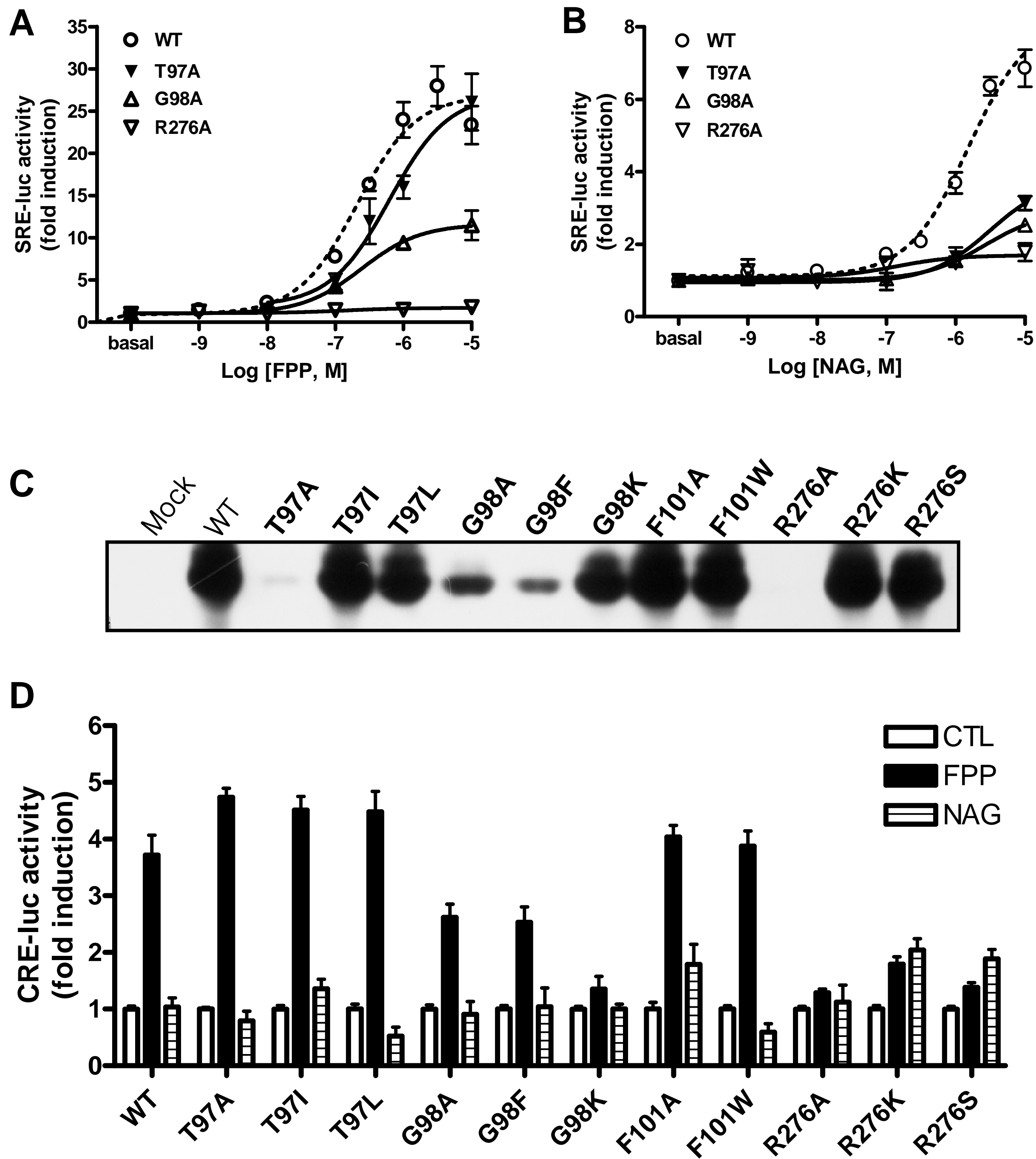
Suppl-Figure 1. Oh *et al.*



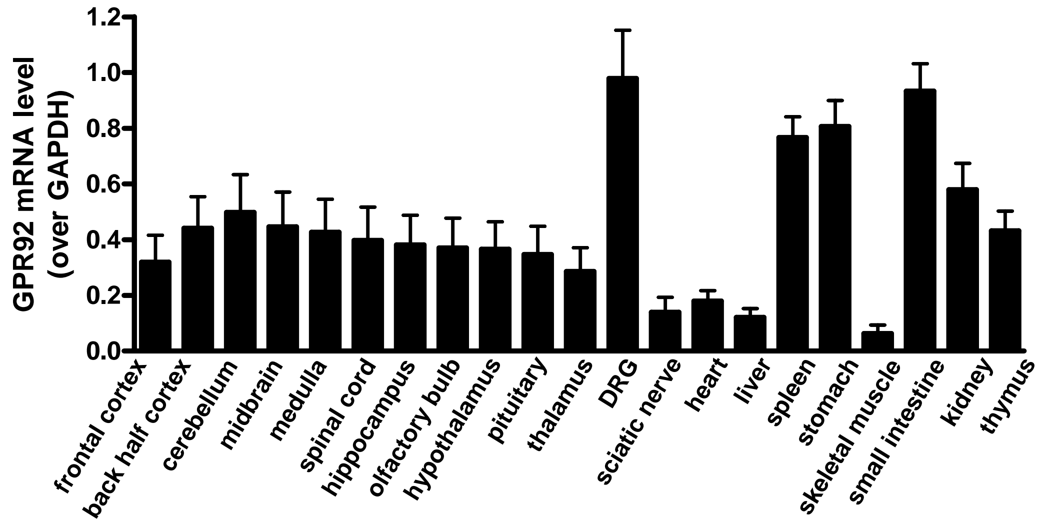


Suppl-Figure 2. Oh *et al.*



Suppl-Figure 3. Oh *et al.*

A



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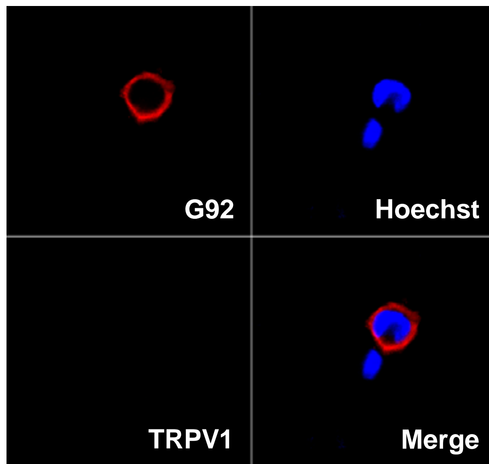

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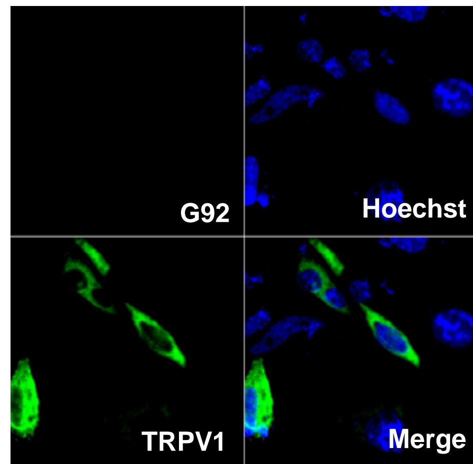
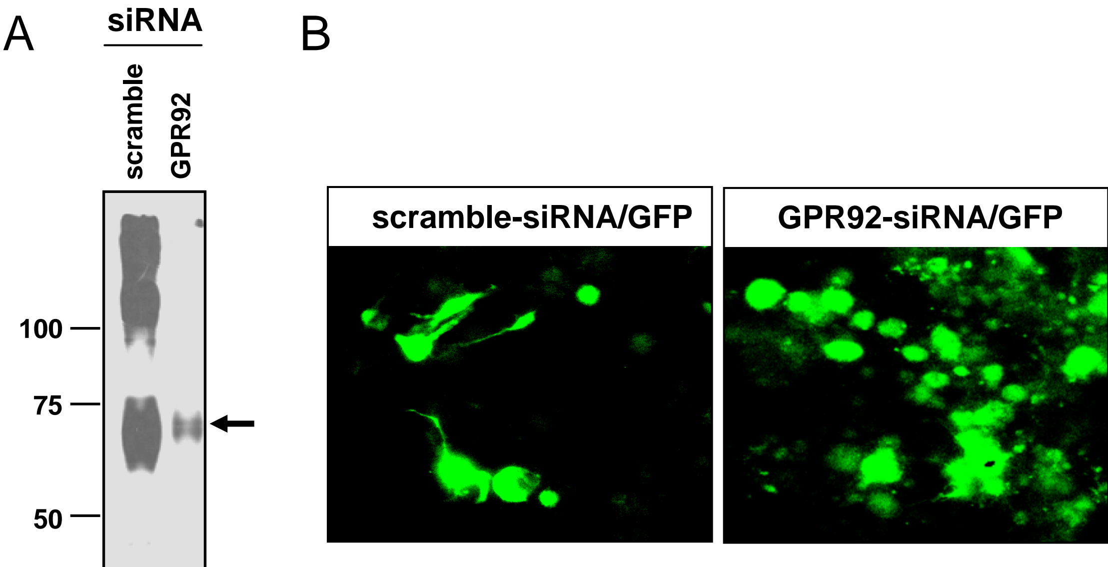


Suppl-Figure 4. Oh *et al*.



Legends for Supplemental Figures

S-Fig. 1. CRE-luc activity of cells expressing GPR92. *A,* CV-1 cells were coinfected with adenoviruses containing human GPR92 and CRE-luc in 96-well plates. Forty-eight hours after infection, cells were treated with the indicated concentration of FPP, NAG or LPA for 6 h. Cells were harvested and luciferase activity of the cell extracts was measured. *B,* The specificity of the signaling pathway was verified by pretreating with H89 (10 μM), a protein kinase A inhibitor 15 min before the treatment of 10 μM of FPP or LPA. Results are plotted as fold activity over basal activity. Each bar represents means ± S.E. of three independent experiments performed in triplicate.

S-Fig. 2. Dose-response activities of wild type and mutant GPR92s stimulated by FPP or NAG. Wild type and mutant GPR92 were co-transfected with SRE-luc in CV-1 cells. Cells were then treated with the indicated concentrations of FPP or NAG for 6 h. Luciferase activity was measured and normalized against β-galactosidase activity. The results are plotted as fold activity over basal luciferase activity. SRE-luc activity of wild type and mutant receptors T97A, G98A, R276A in response to FPP (*A*) and NAG (*B*) are shown. Protein expression levels of wild type and mutant GPR92-GFP were determined by Western blot using an anti-GFP antibody (*C*). CRE-luc activity of cells expressing wild type and mutant GPR92s were determined in the presence of FPP (1 μM) and NAG (10 μM)

S-Fig. 3. Tissue distribution of GPR92 mRNA and immunohistochemistry of GPR92 in mouse and human DRG. *A,* Total RNA levels from 6-week old C57B/6 male mice in the indicated tissues were assessed by RT-PCR. GAPDH was used as a loading control. GPR92 mRNA levels over GAPDH mRNA are presented. Data represent means ± S.E. of three independent experiments. *B and C,* For validation of the anti-GPR92 antibody and anti-TRPV1 antibody, an immunofluorescence assay was performed in HeLa cells expressing mouse GPR92 (B) and rat TRP V1 (C). Mouse GPR92 and rat TRP V1 were detected using anti-rabbit alexa594 and anti-guniea pig alexa488 secondary antibodies, respectively. No cross-reactivity between GPR92 and TRP V1 antibodies has been observed.

S-Fig. 4. Knock-down of GPR92. *A,* HeLa cells were transfected with GPR92-GFP in combination with scramble or GPR92 siRNA. Protein levels of GPR92-GFP were determined by Western blot using an anti-GFP antibody. The arrow indicates a GPR92-GFP signal. Signals with a molecular weight over 100 kDa likely represent dimeric or oligomeric forms of GPR92-GFP. *B & C*, Rat DRG cells were co-transfected with the GFP expression vector pEGFP-N1 and the scramble- or GPR92-siRNA, to monitor transfection efficiency (>80%).