Supplemental Figure Legends:

Fig. S1. (A) Western blot analysis of the surface biotinylation of GPR56 and GPR56ΔNT. As shown in the panel on the right, both the wild-type and truncated mutant were expressed at roughly comparable values. As shown on the left, surface biotinylation of GPR56 and GPR56ΔNT was similar, revealing both receptors to be found equally at the plasma membrane. Interestingly, the main surface-expressed form of each receptor is the GPS-cleaved species at just under 50 kDa. None of the lower or higher molecular weight bands, representing further cleaved species or uncleaved receptors, were detectably expressed at the plasma membrane. (B) Surface luminometry assays revealed that Flag-GPR56 wild-type, Flag-GPR56ΔNT, and Flag-GPR56-NT are all expressed at roughly equal levels (per microgram of plasmid transfected) on the surface of transfected HEK-293 cells. (C) The Flag-GPR56-NT fragment remains cell-associated. HEK-293 cells were transfected with the GPR56 Flag-NT construct and incubated for 24 hours. The next day, the conditioned medium was collected and immunoprecipitation with anti-Flag antibody was performed in order to concentrate any Flag-NT that might be in the medium. Also, a whole cell lysate sample from the transfected cells was prepared. Western blot analysis revealed that very little of the Flag-GPR56-NT fragment was secreted into the medium, but rather the vast majority of the Flag-NT remained associated with the cells.

Supplemental Figure 1