Supplemental Figure 8

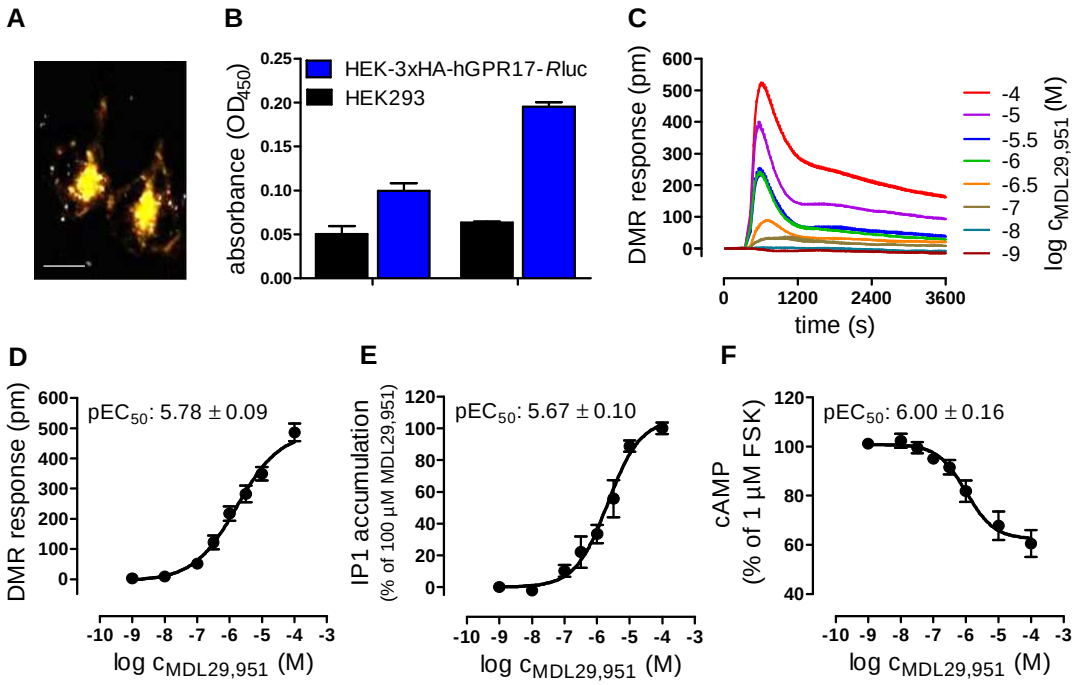

Fig. S8. GPR17-*R*luc fusion protein is targeted to the cell surface and signals via G protein–mediated pathways. (**A**) Immunocytochemical staining. HEK293 cells stably expressing 3xHA-hGPR17-*R*luc were fed with anti-HA monoclonal antibody and were then fixed and stained for the receptor with Alexa Fluor546 goat anti-mouse IgG. Scale bar 10 µM. (**B**) ELISA. Cell surface expression of 3xHA-hGPR17-*R*luc was quantified by ELISA against the HA-epitope tag. Absorbance values obtained for native HEK293 cells reflect nonspecific labeling of the cells. Data shown are mean values + S.E.M. of five independent experiments, each performed in triplicate. (**C**,**D**) Label-free DMR assay. HEK-hGPR17-*R*luc cells were stimulated with increasing concentrations of MDL29,951 and DMR was recorded over time. (C) Representative DMR traces (mean + S.E.); (D) concentration-effect-curve derived from traces in (C), mean ± S.E.M., n= 3, each performed

in triplicate. (**E** and **F**) HEK-hGPR17-*R*luc cells were stimulated with the indicated concentrations of MDL29,951 and levels of intracellular inositolphosphate IP1 (E) or inhibition of forskolin-mediated cAMP production (F) were quantified. Data are mean ± S.E.M. of at least three independent experiments, each performed in triplicate.