SUPPLEMENTARY FIGURES

GFPPTHR (+ NHERF)



^{mCherry}NHERF (+ PTHR)



Supplementary Figure 1. **PTHR clusters after PTH stimulation dissociate from NHERF1**. TIRF images corresponding to ^{GFP}PTHR (top panel) or ^{mCherry}NHERF (bottom panel) at the cell membrane after stimulation with 100 nM hPTH(1-34). Representative images from 10 cells corresponding to 3 independent experiments are shown.



Supplementary Figure 2. **NHERF1-B-arrestin interactions**. Donor dequenching after acceptor photobleaching was used as described in Materials and Methods to analyze interactions between ^{CFP}B-arrestin and ^{YFP}NHERF or PTHR^{CFP}ETVM. *A*, ^{CFP}Membrane protein and ^{YFP}NHERF were used to exclude nonspecific ^{YFP}NHERF FRET interactions at the cell membrane. *B and C*, ^{CFP}EPAC^{YFP} or ^{YFP}NHERF and PTHR^{CFP}ETVM pair were used as positive interaction controls for donor dequenching after acceptor photobleaching. *D and E*, ^{CFP}β-arrestin-2 and ^{YFP}NHERF or ^{YFP}β-arrestin-2 and hPTH(1-34)-stimulated PTHR^{CFP}ETVM FRET pairs were used to test interactions between β-arrestin and NHERF or PTH-stimulated PTHR respectively. Data shown correspond to $\geq 8-10$ cells from 3 independent experiments.



Supplementary Figure 3. Recruitment of β -arrestin2 to PTHR at the cell membrane upon PTH stimulation. ROS cells were transfected with ^{YFP} β -arrestin2 and PTHR^{CFP} and analyzed by confocal microscopy 24 h after transfection. Representative images from 6 independent experiments are shown in "false" color (CFP=green, YFP=red).



Supplementary Fig. 4. Cell surface colocalization of NHERF1 and B-Arrestin1 or PTHR and B-Arrestin1. ROS cells were transfected with ^{YFP}NHERF or ^{GFP}PTHR and ^{Tomato}B-Arrestin1 and analyzed by TIRF microscopy 24 h after transfection. Colocalization of NHERF1 or PTHR with B-Arrestin1 was quantified by calculating Pearson's correlation coefficient as described in Materials and Methods.