Supplemental materials

Figure S1

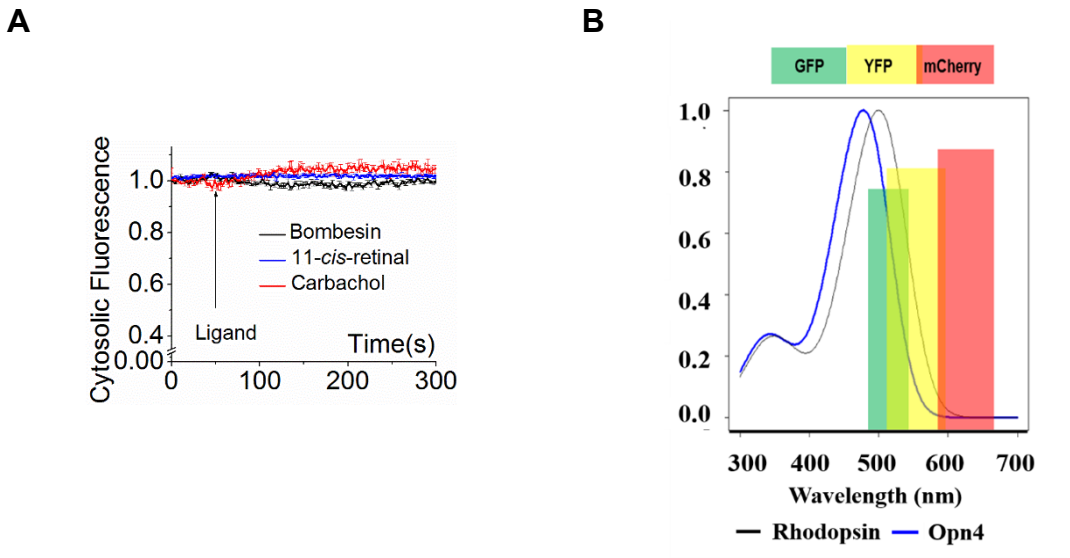


Fig. S1: (A) Without expressing corresponding receptors, the cognate ligands do not induce PIP_2 hydrolysis in HeLa cells. Cytosolic mCherry-PH fluorescence in HeLa cells after addition of Opn4, M3, and GRP receptors agonists without transfecting their respective receptors. Average curves plotted using n=10 cells from ≥3 independent experiments. Error bars: SEM. (**B) Broad absorption profile of Opn4 allow activation by blue (445nm), green (488nm), and yellow (515nm) light.** Simulated absorption spectrum of Opn4, showing that wavelengths below ~590nm can activate it.

Figure S2

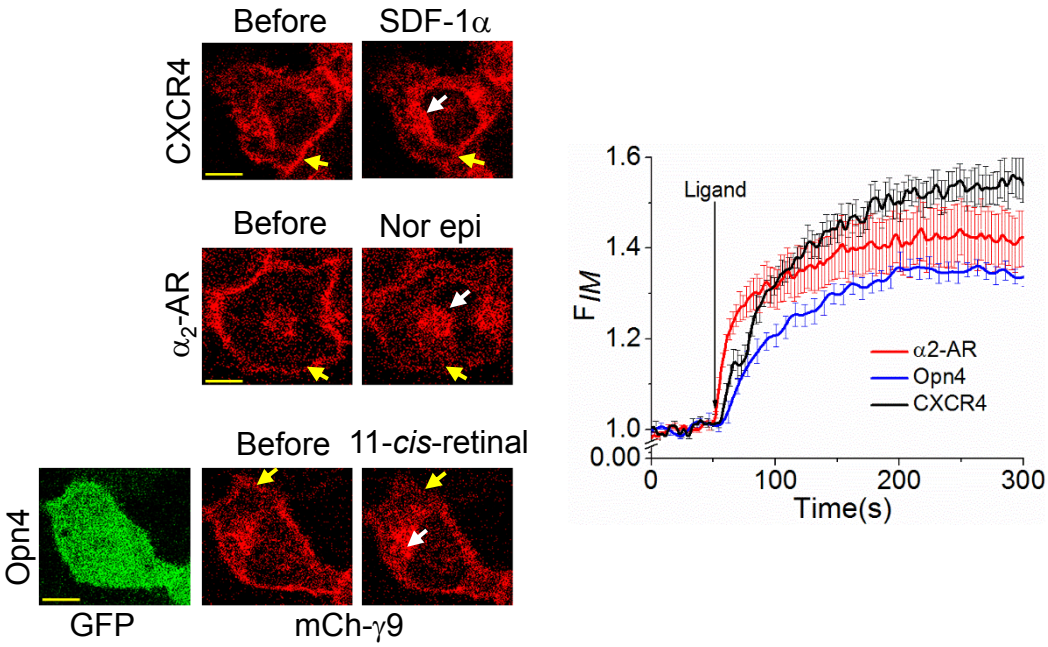


Fig. S2: Opn4 induces comparable mCherry-γ_9 translocation to G_i-coupled CXCR4 and α_2-AR. mCherry-γ_9 translocation in HeLa cells after addition of 50ng/ mL SDF-1α (top), 100μM norepinephrine (middle) to activate their respective receptors (CXCR4, α_2-AR) endogenously expressed, 50μM 11-*cis*-retinal added, imaged for mCherry and YFP to phoactivate bi-cistronic Opn4 GFP (bottom) transiently transfected in HeLa. Yellow arrows (PM), white arrows (IMs). Scale bar 10μm. Average curves plotted using n=10 cells from ≥3 independent experiments. Error bars: SEM.

Figure S3

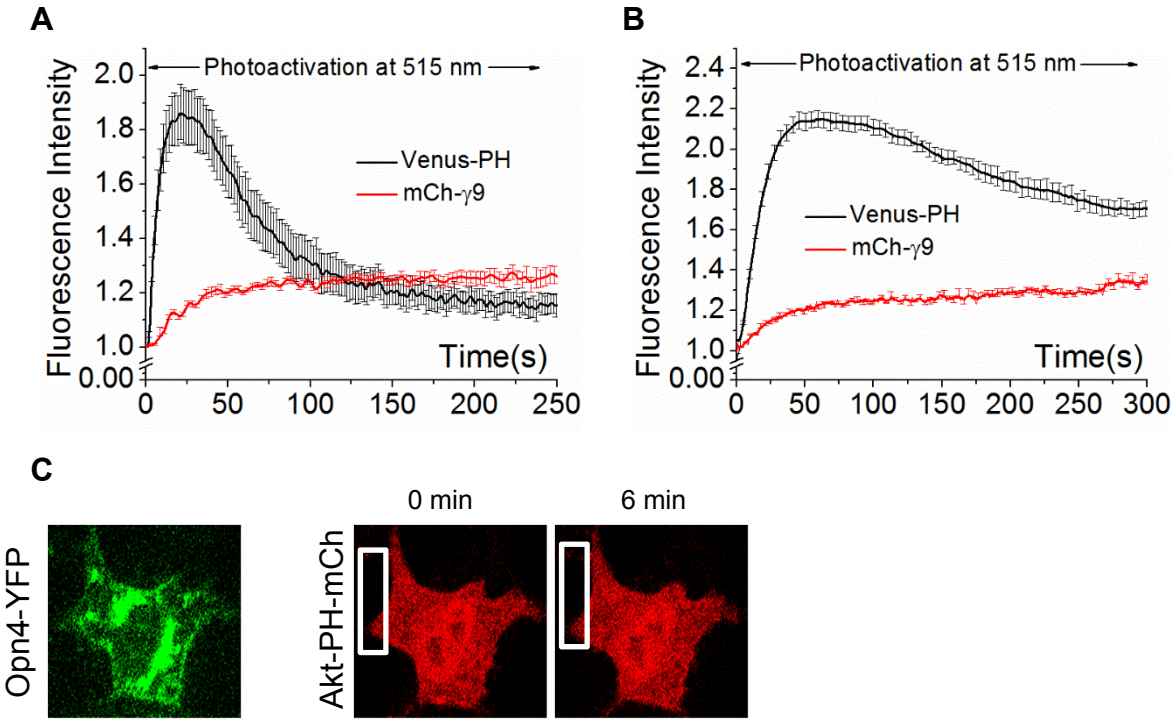


Fig. S3: (A,B) Control cells show PIP_2 hydrolysis and γ_9 translocation upon Opn4 activation. PIP_2 hydrolysis and $G\gamma_9$ translocation in HeLa cells treated with vehicle solvent used to dissolve **A**. PTx and **B.** YM-254890 after activation of Opn4. Average curves plotted using n=8 cells from ≥3 independent experiments. Error bars: SEM. **(C)** PIP3 generation in RAW264.7 in the presence of 50 μM gallein after photoactivating Opn4-YFP supplemented with 11-*cis*-retinal.

Figure S4

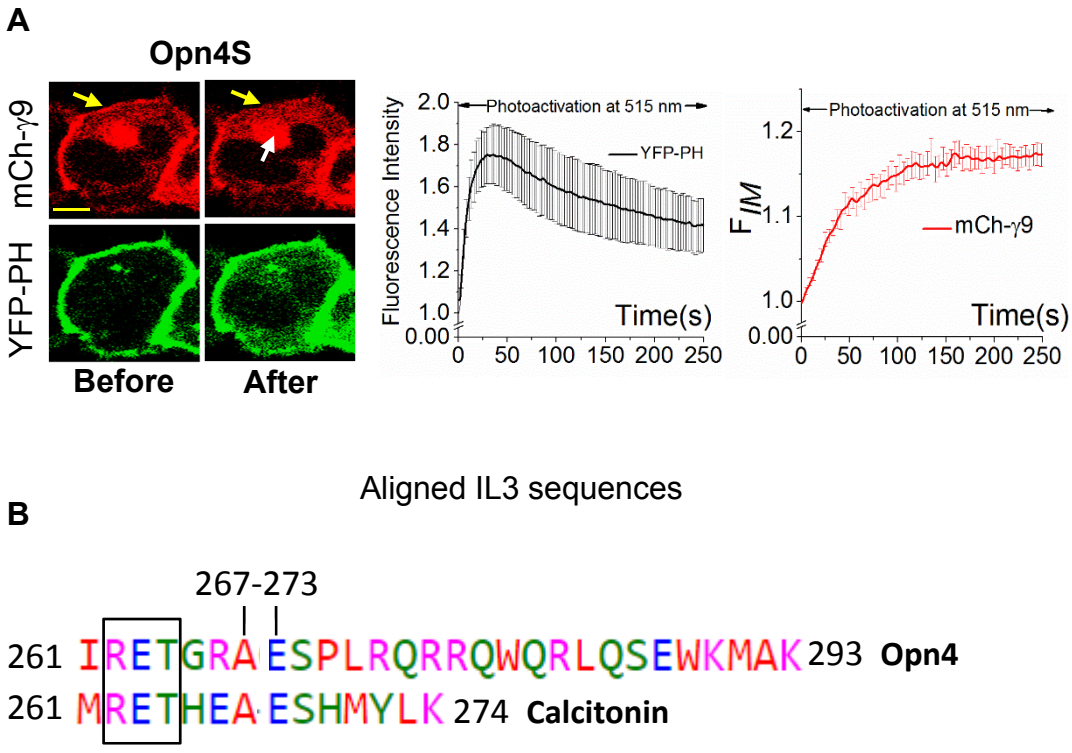
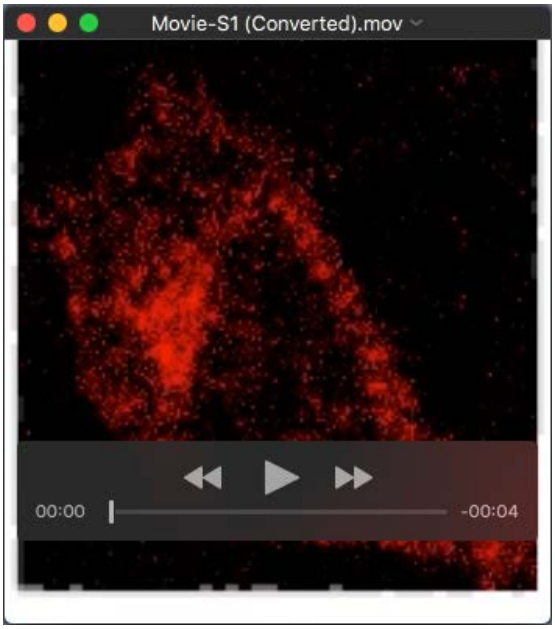


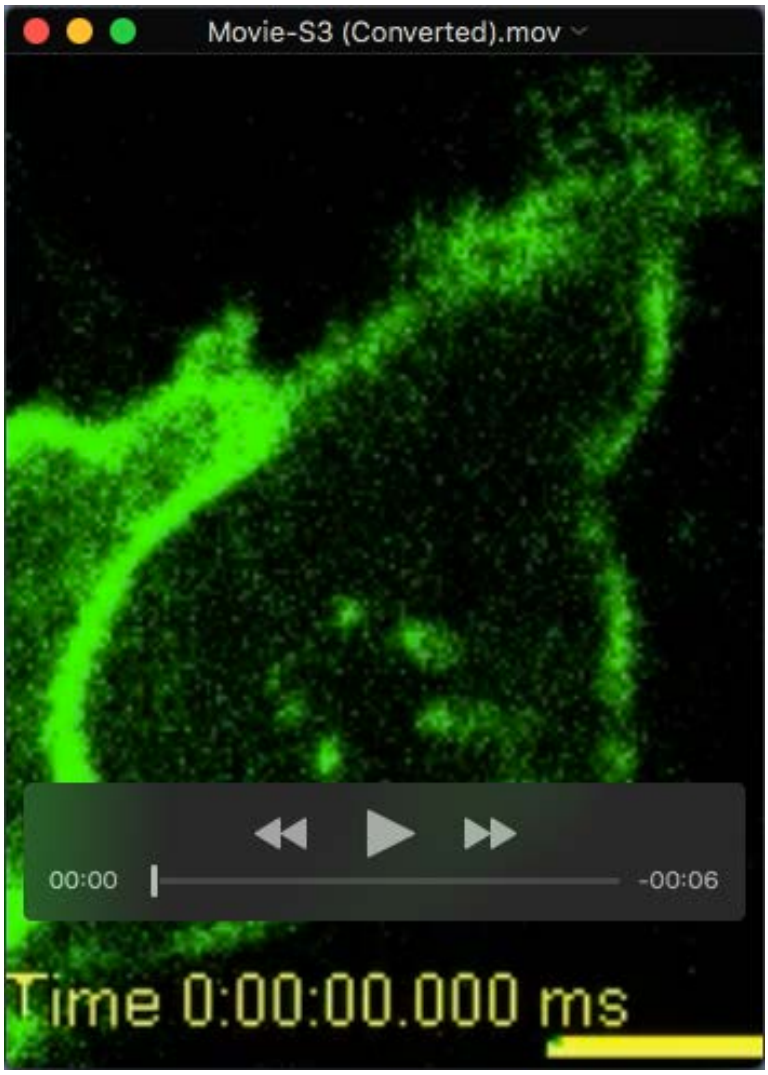
Fig. S4: (A) Similar to Opn4, Opn4S also induced PIP_2 hydrolysis and $G\gamma_9$ translocation. PIP_2 hydrolysis and $G\gamma_9$ translocation in HeLa cells after activation of Opn4S. Yellow arrows (PM), white arrows (IMs). **(B) IL3 Sequence alignments of Opn4 with G_q receptor IL3 regions that are clustered together in phylogenetic analysis.** Sequence alignment for Opn4 and G_q-coupled calcitonin receptors. Mutations in B-box resulted in a non-functional receptor. Average curves plotted using n≥10 cells from ≥3 independent experiments. Error bars: SEM.

Supplementary Movies

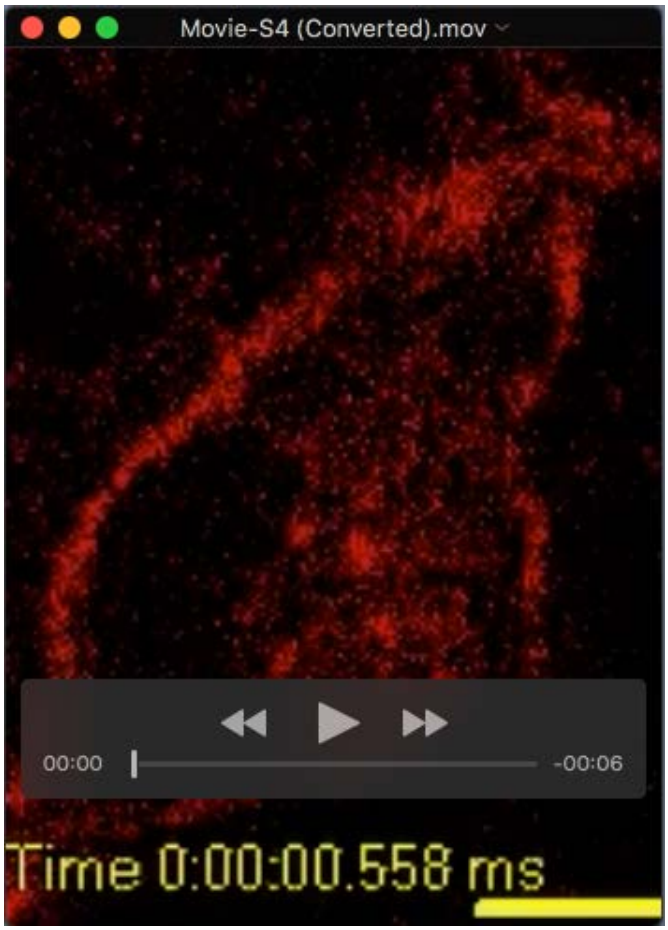


Movie-S1. A HeLa cell expressing Opn4 and PIP_2 sensor (Venus-PH) shows profound PIP_2 hydrolysis upon addition of 50 µM 11-*cis*-retinal. Scale bar 10 mm.

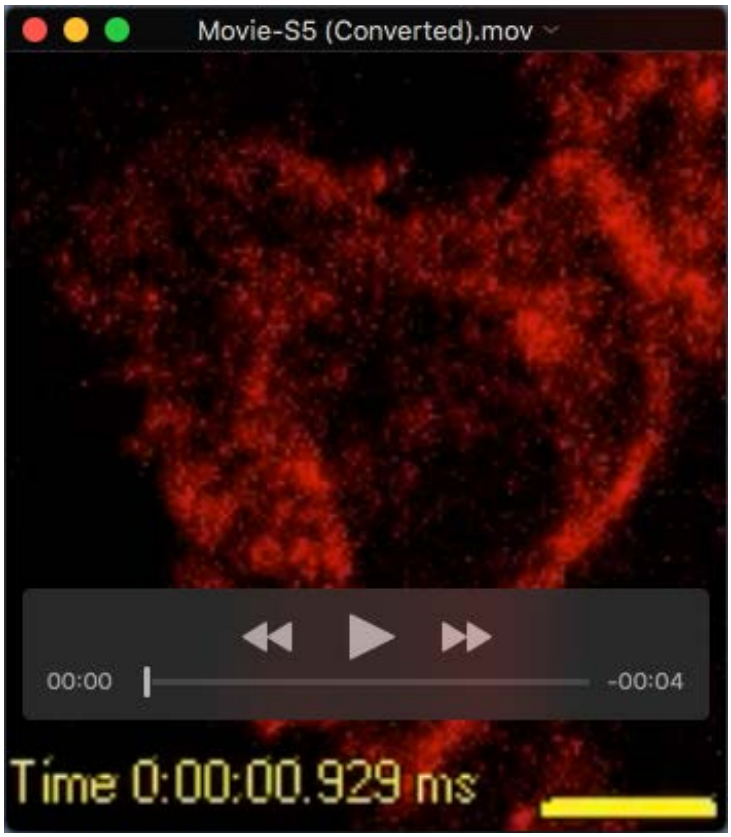
Movie-S2. A HeLa cell expressing Opn4 and mCh- γ_9 shows γ_9 translocation upon addition of 50 µM 11-*cis*-retinal while photoactivation at 515 nm. Scale bar 10 mm.



Movie-S3. A HeLa cell expressing GRPR and and PIP_2 sensor (Venus-PH) shows profound PIP_2 hydrolysis upon addition of 1 μM bombesin. Scale bar 10 mm.



Movie-S4. A HeLa cell expressing GRPR and mCherry- γ_9 does not exhibit γ_9 translocation upon addition of 1 μM bombesin. Scale bar 10 mm.



Movie-S5. A HeLa cell endogenously expressing a_2-AR and mCherry- γ_9 shows γ_9 translocation upon addition of 100 μM nor-epinephrine. Scale bar 10 mm.