

Supplementary Figure 1. Uncropped Western blots with molecular weight markers and indication of cropped region.

Supplementary Video 1 sptPALM video of PA-mCherry- β 1AR. HEK 293 cells were co-transfected with PA-mCherry- β 1AR and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of PA-mCherry- β 1AR. This is a representative video from 2 independent experiments.

Supplementary Video 2 Representative sptPALM video of PA-mCherry- β 2AR. HEK 293 cells were co-transfected with FLAG- β 1AR, PA-mCherry- β 2AR, and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of PA-mCherry- β 2AR. This is a representative video from at least 3 independent experiments.

Supplementary Video 3 Representative sptPALM video of β -arrestin-2-PA-mCherry after activation of β 1AR. HEK 293 cells were co-transfected with FLAG- β 1AR, β -arrestin-2-PA-mCherry, and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of β -arrestin-2-PA-mCherry. This is a representative video from at least 3 independent experiments.

Supplementary Video 4 Representative sptPALM video of β -arrestin-2-PA-mCherry after activation of β 2AR. HEK 293 cells were co-transfected with FLAG- β 2AR, β -arrestin-2-PA-mCherry, and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of β -arrestin-2-PA-mCherry. This is a representative video from at least 3 independent experiments.

Supplementary Video 5 Representative sptPALM video of β -arrestin-2-PA-mCherry (CCS mutant) after activation of β 1AR. HEK 293 cells were co-transfected with FLAG- β 1AR, β -arrestin-2-PA-mCherry (CCS mutant), and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of β -arrestin-2-PA-mCherry (CCS mutant). This is a representative video from at least 3 independent experiments.

Supplementary Video 6 Representative sptPALM video of β -arrestin-2-PA-mCherry (CCS mutant) after activation of β 2AR. HEK 293 cells were co-transfected with FLAG- β 2AR, β -arrestin-2-PA-mCherry (CCS mutant), and CLC-GFP and treated with 10 μ M isoproterenol. Shown are single-molecule images of β -arrestin-2-PA-mCherry (CCS mutant). This is a representative video from at least 3 independent experiments.