Adaptor protein-2 interaction with arrestin regulates GPCR recycling and apoptosis

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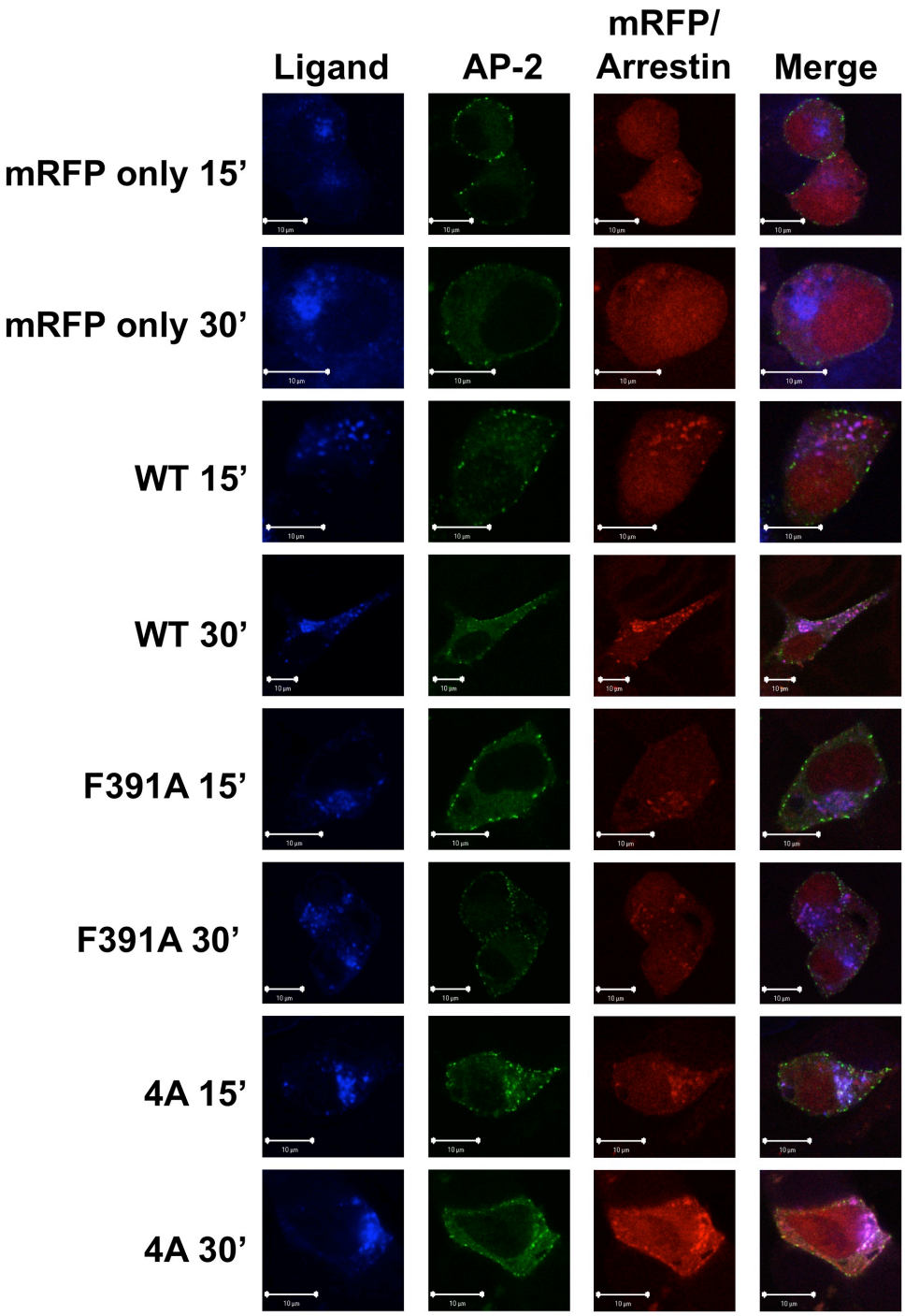
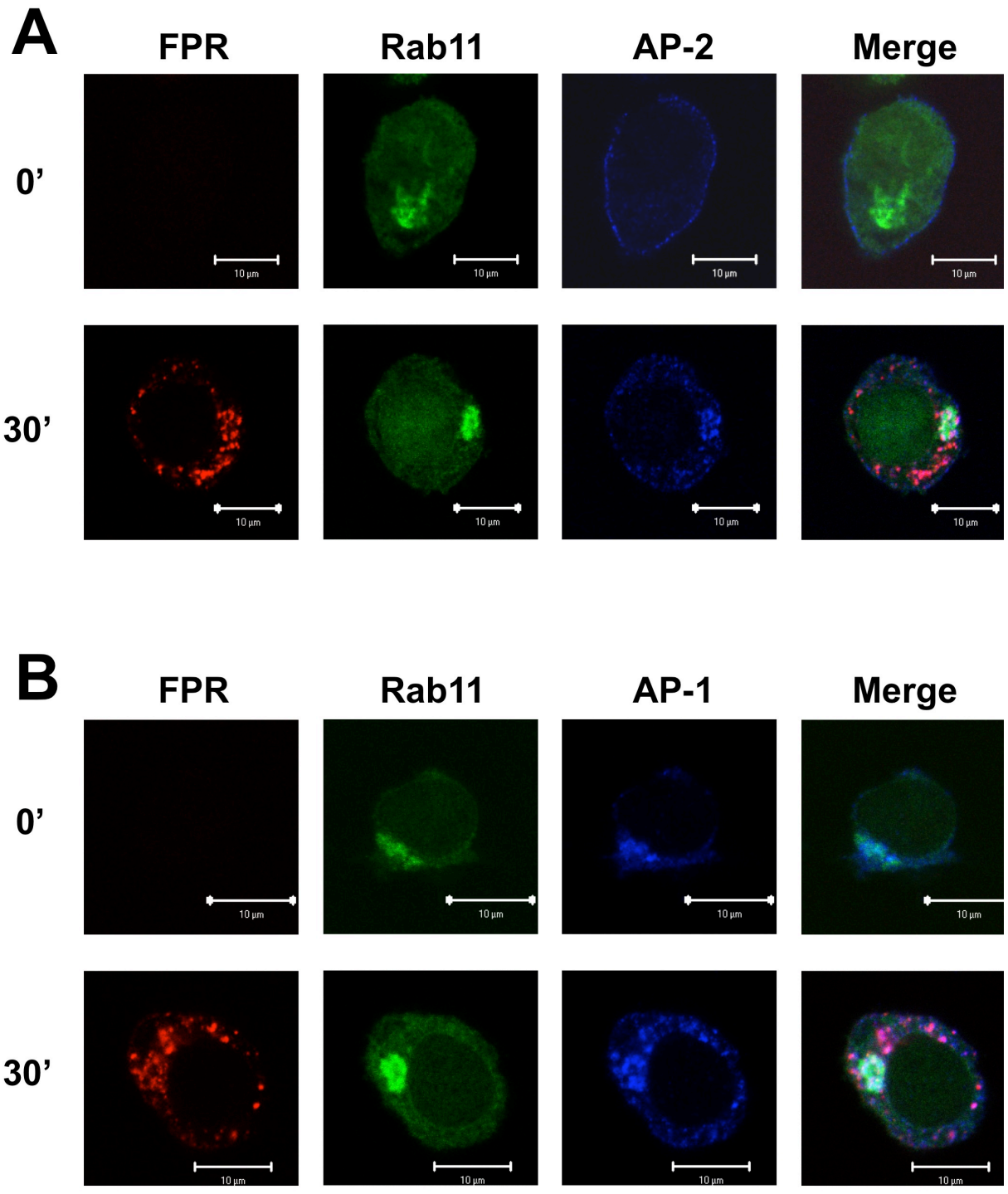
Wagener et al. Suppl. Figure 1



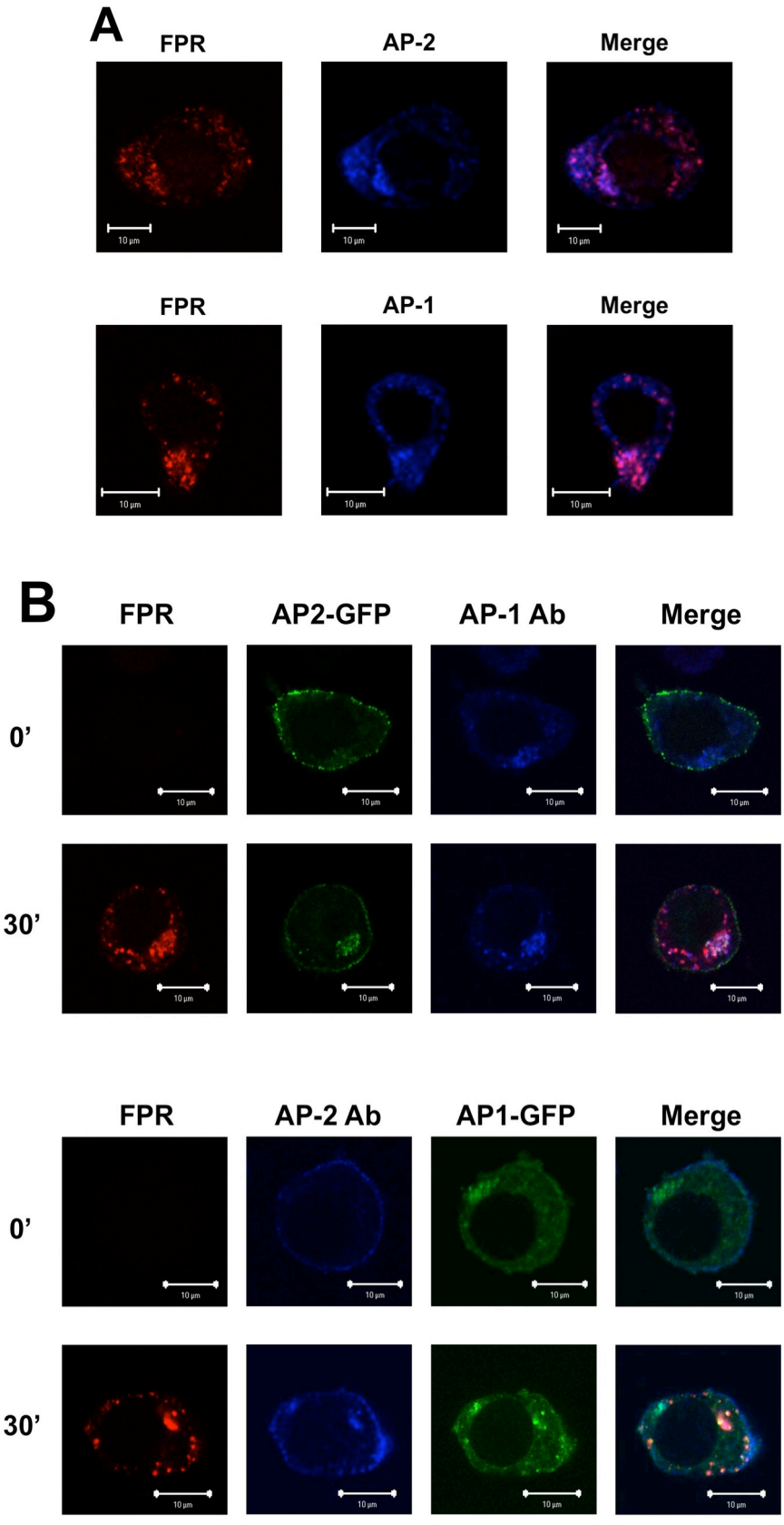
Supplemental Figure 1. Intermediate time point images of wild type, arr2-F391A and arr2-4A. Arr2$^{-/-}$/3$^{-/-}$ FPR MEF cells were transiently transfected with mRFP-fused arrestins (wild type, arr2-F391A and arr2-4A) and GFP-fused α subunit of AP-2. Cells were stimulated with the 633-6pep ligand for intermediate times (15 and 30 min, *cf*. Fig. 3A) and imaged by confocal fluorescence microscopy. Representative images are shown and are representative of three independent experiments. Scale bars, 10μm.

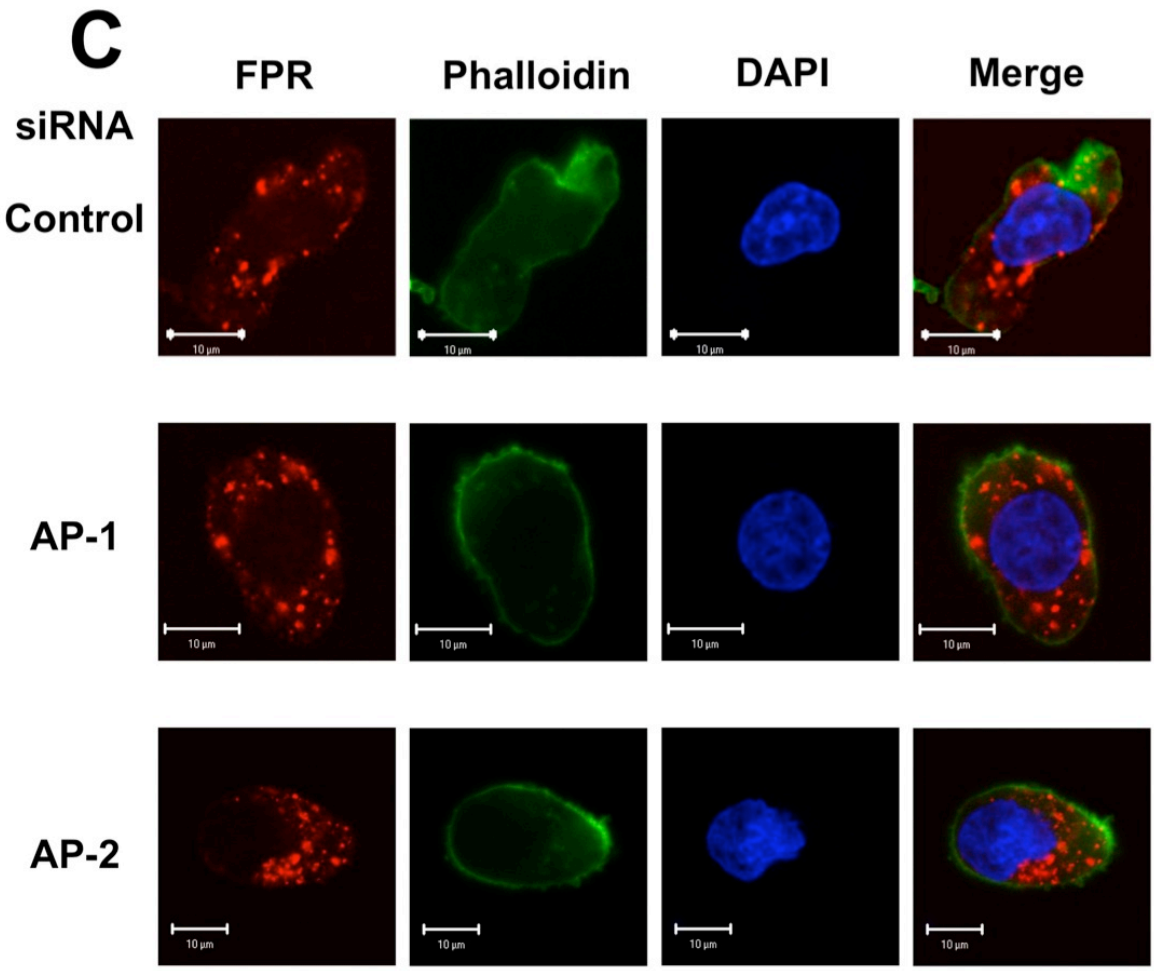
Wagener et al. Suppl. Fig. 2



Supplemental Figure 2. Antibody staining of AP-2 and AP-1 in U937 FPR cells. U937 FPR cells were transiently transfected with GFP-fused Rab11. Cells were then stimulated with 546-6pep for the indicated times, stained with antibodies to the α or γ subunits (AP-2 **(A)** or AP-1 **(B)**, respectively) and imaged by confocal fluorescence microscopy. Representative images are shown from three independent experiments. Scale bars, 10µm.

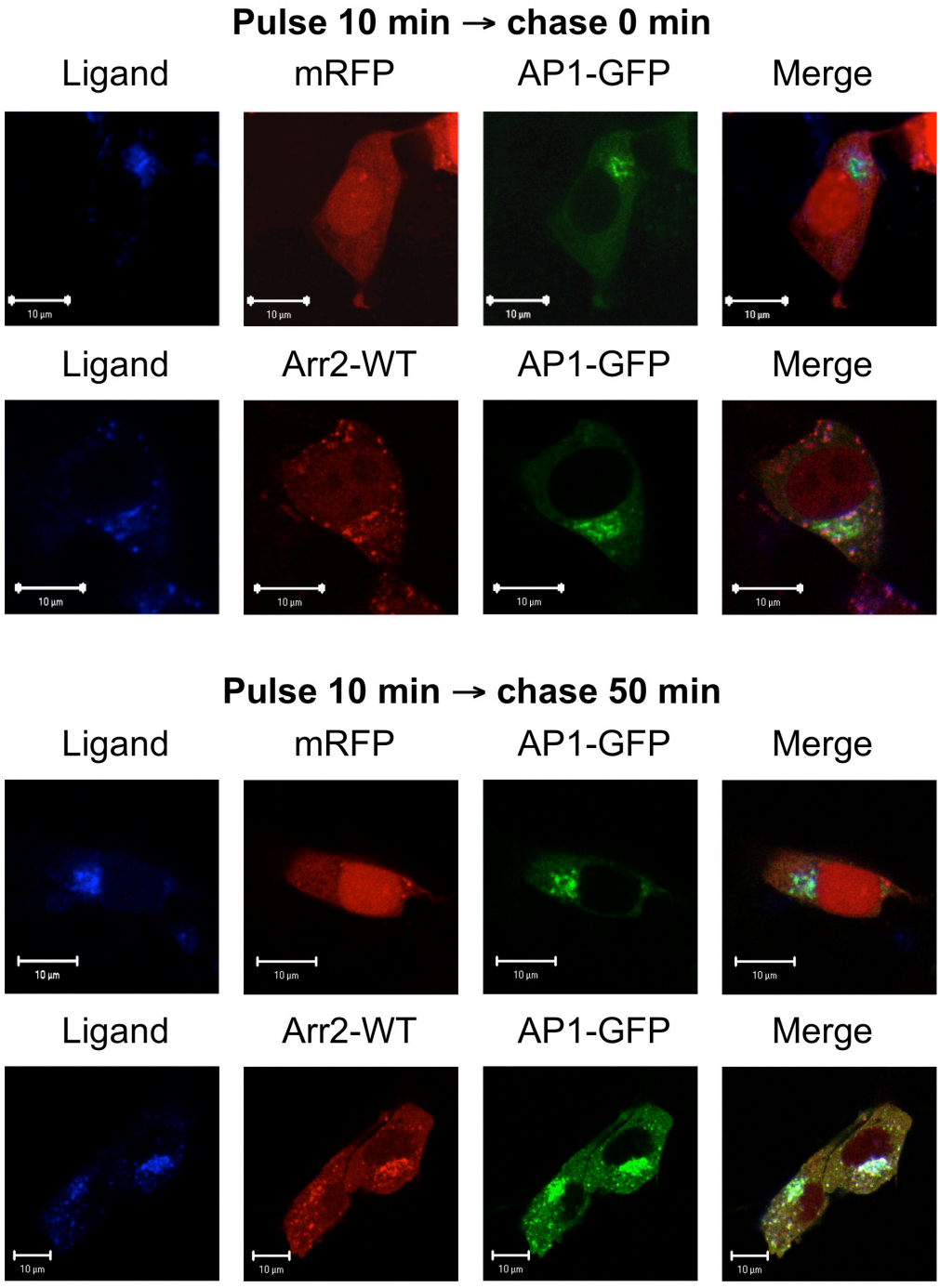
Wagener et al. Suppl. Figure 3





Supplemental Figure 3. Antibody staining of adaptor proteins in U937 FPR cells depleted of AP-1 or AP-2. (A) Individual colocalization of AP-1 and AP-2 with the FPR in untransfected cells. U937 FPR cells were stimulated with 546-6pep for 30 min, stained with antibodies to the α or γ subunits (AP-2 or AP-1, respectively) and imaged by confocal fluorescence microscopy. The colocalization of the FPR with both AP-1 and AP-2 demonstrate that expression of Rab11-GFP (*cf.* Suppl. Fig. 2) does not alter receptor trafficking. **(B)** Simultaneous colocalization of AP-1 and AP-2 with the FPR in perinuclear endosomes. U937 FPR cells were transiently transfected with either the GFP-fused γ subunit of AP-1 or the GFP-fused α subunit of AP-2. Cells were then stimulated with 10nM 546-6pep ligand, fixed, stained with antibodies to the α or γ subunits (AP-2 or AP-1, respectively) and viewed by confocal fluorescence microscopy. **(C)** U937 FPR cells were electroporated with control, AP-1 or AP-2 siRNA and stimulated with 10nM 546-6pep ligand for 30 min. Depletion of AP-2 using siRNA against the μ2-subunit resulted in moderate perinuclear accumulation of the FPR. Depletion of AP-1 or use of control siRNA had no effect on FPR distribution. For all experiments, representative images are shown from three independent experiments. Scale bars, 10µm.

Wagener et al. Suppl. Figure 4



Supplemental Figure 4. Dispersal of perinuclear AP-1 upon recycling of the FPR. Arr2$^{-/-}$/3$^{-/-}$ FPR MEF cells were transiently transfected with mRFP alone (mRFP) or wild type, arr2 (Arr2-WT) and the GFP-fused γ subunit of AP-1. Cells were then stimulated with 10nM 633-6pep ligand for 10 min at 37°C in PBS, washed extensively (three times with ice-cold PBS) and fixed immediately or incubated for an additional 50 min with pre-warmed PBS at 37°C in the absence of ligand (chase) and fixed. The pulse interval was selected to permit internalization with minimal recycling, whereas the 50 min chase was selected to permit internalized receptor (during the 10 min pulse) to traffic and recycle. Representative images from three independent experiments are shown. Scale bars, 10µm.