

## SUPPLEMENTAL FIGURE LEGENDS

### Supplemental figure 1:

$\beta$ -Arrestin localization in HEK-293 cells transfected with either  $\beta$ -arrestin-1-GFP or  $\beta$ -arrestin-2-YFP.

(A) The top lane shows HEK-293 cells transfected with  $\beta$ -arrestin-1-GFP prior to stimulation with ADP, ATP, UDP, or UTP at final concentrations of 100 $\mu$ M. The bottom lane shows the same cells after 15 min agonist exposure. (B) The top row shows HEK-293 cells transfected with  $\beta$ -arrestin-2-YFP prior to stimulation with ADP, ATP, UDP, or UTP at final concentrations of 100 $\mu$ M. The bottom row shows the same cells 15 min after agonist exposure. Data are representative examples of at least 3 individual experiments. White scale bars represent 10 $\mu$ m.

### Supplemental figure 2:

$\beta$ -Arrestin-1 translocation induced by stimulation of P2Y<sub>1,2,4,6,11,12</sub> receptor. HEK-293 cells were cotransfected with the indicated P2Y-receptor and  $\beta$ -arrestin-1-GFP construct. In case of the P2Y<sub>6</sub>- P2Y<sub>11</sub>-, and P2Y<sub>12</sub>-receptor cells were additionally co-transfected with GRK2. The left column represents cells prior to stimulation with the indicated agonist. The right column shows the same cells 15 min after exposure to the indicated agonist.  $\beta$ -arrestin-1-GFP translocation could be observed for all P2Y-receptors except the P2Y<sub>12</sub>-receptor. All agonist concentrations were 100 $\mu$ M final. Data are representative examples of at least 3 individual experiments. White scale bars represent 10 $\mu$ m.

### Supplemental figure 3:

Antagonistic effect of MRS2179 on ADP induced  $\beta$ -arrestin-2-YFP translocation mediated by the P2Y<sub>1</sub> receptor. Left column, 10 minutes prior to ADP stimulation cells were incubated with MRS2179 at final concentration of 100nM or 1 $\mu$ M respectively. No translocation was observed by MRS2179 alone, middle column. 15 min after addition of ADP (100 $\mu$ M final concentrations) a marked and concentration dependent difference was observed for those cells that were pre-treated with MRS2179. Compare cells in figure 2A for translocation in the absence of inhibitor. Data are representative examples of at least 3 individual experiments. White scale bars represent 10 $\mu$ m.

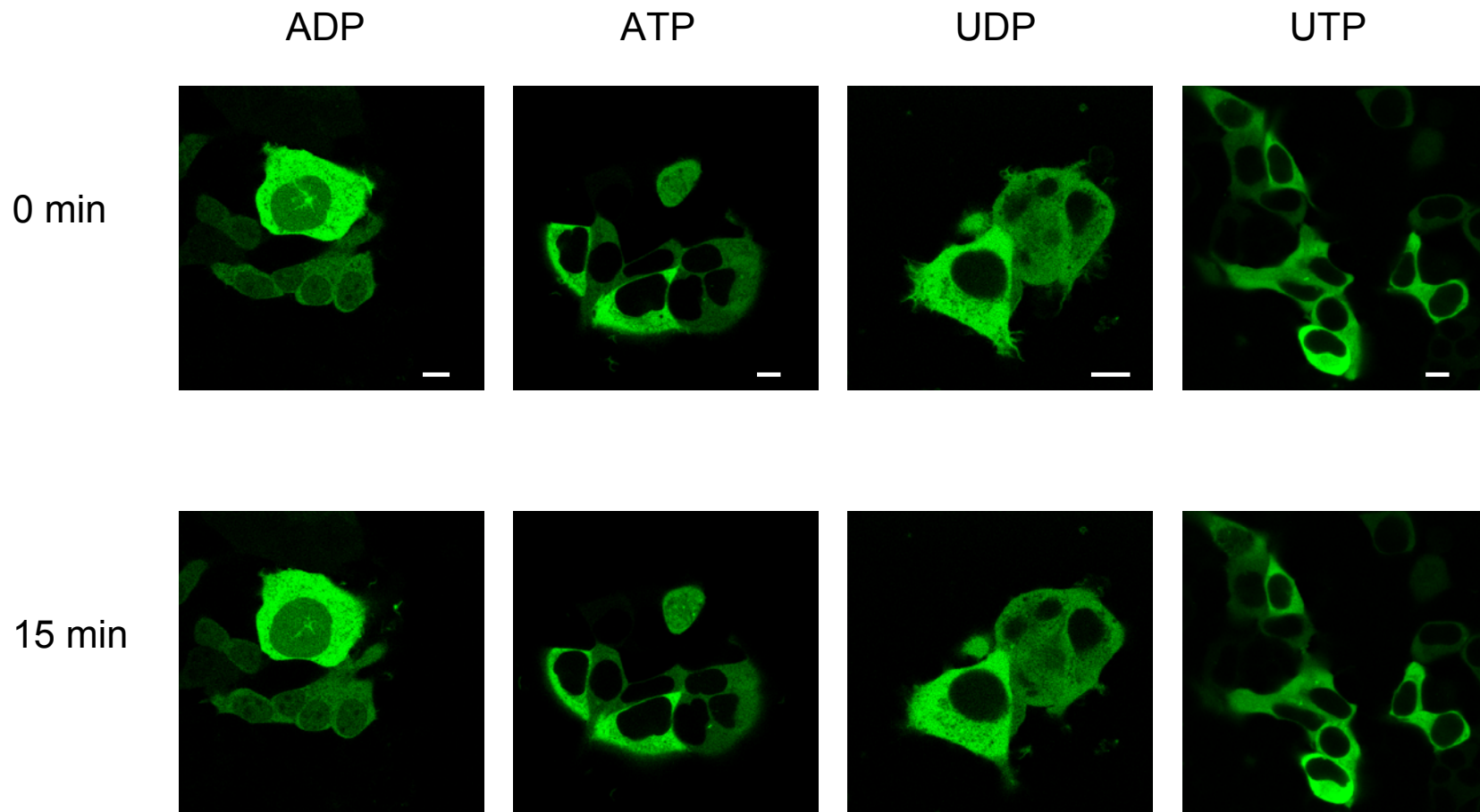
### Supplemental Figure 4:

The cells shown in this figure represent the same cells as presented in Fig. 2B for the P2Y<sub>12</sub>-receptor tagged with CFP and co-transfected with  $\beta$ -arrestin-2-YFP, and GRK2. The left panel shows receptor localization prior to ADP stimulation, while the right panel shows the same cells 15min after ADP exposure. A punctuate pattern indicates receptor internalization of the P2Y<sub>12</sub>-receptor. White scale bars represent 10 $\mu$ m.

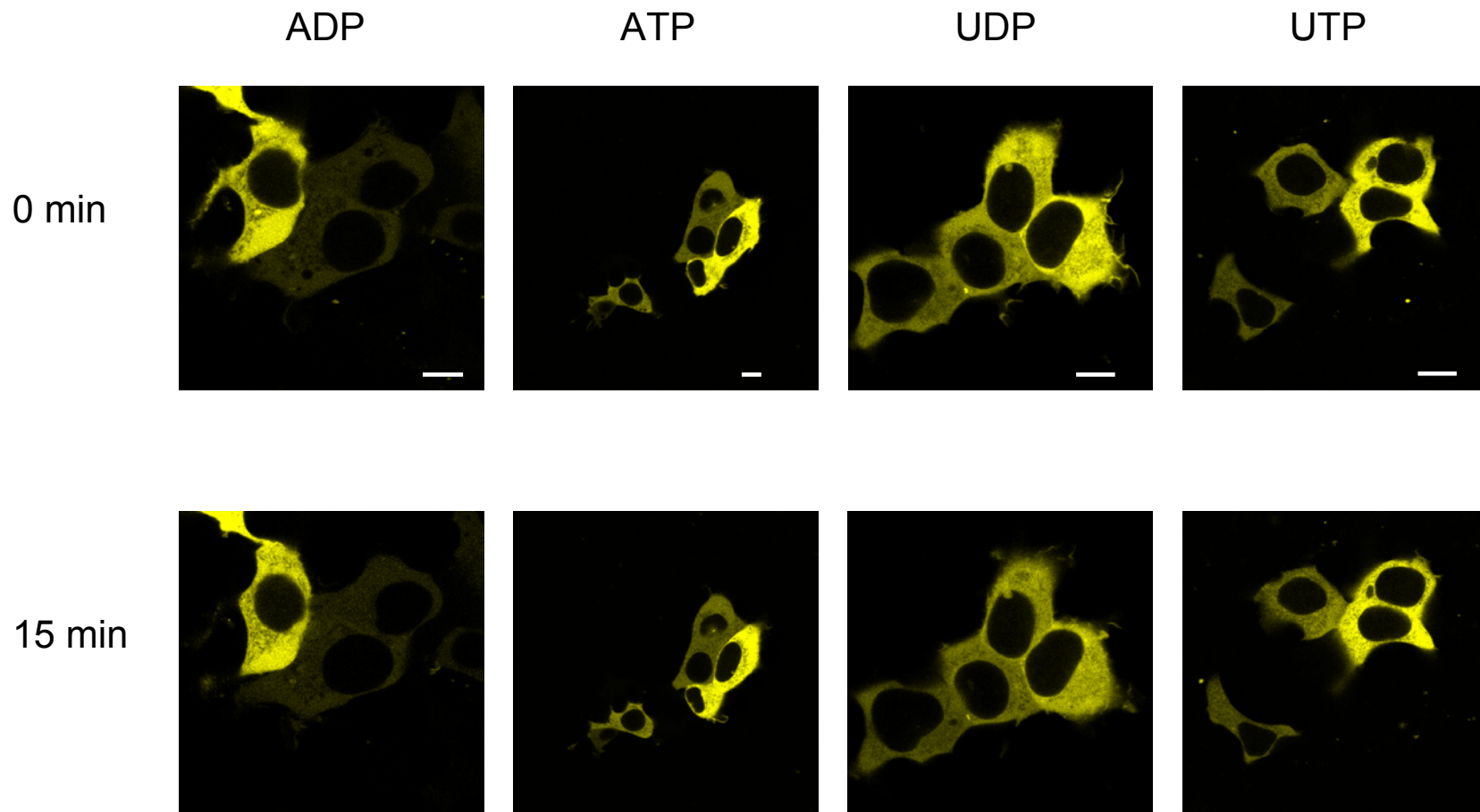
### Supplemental Figure 5:

Left: Normalized FRET-ratio of a single cells co-transfected with P2Y<sub>12</sub>-YFP and  $\beta$ -arrestin-2-CFP. Right: Normalized FRET-ratio of a single cells co-transfected with P2Y<sub>12</sub>-YFP,  $\beta$ -arrestin-2-CFP and GRK2. Cells were constantly superfused with buffer or buffer supplemented with ADP

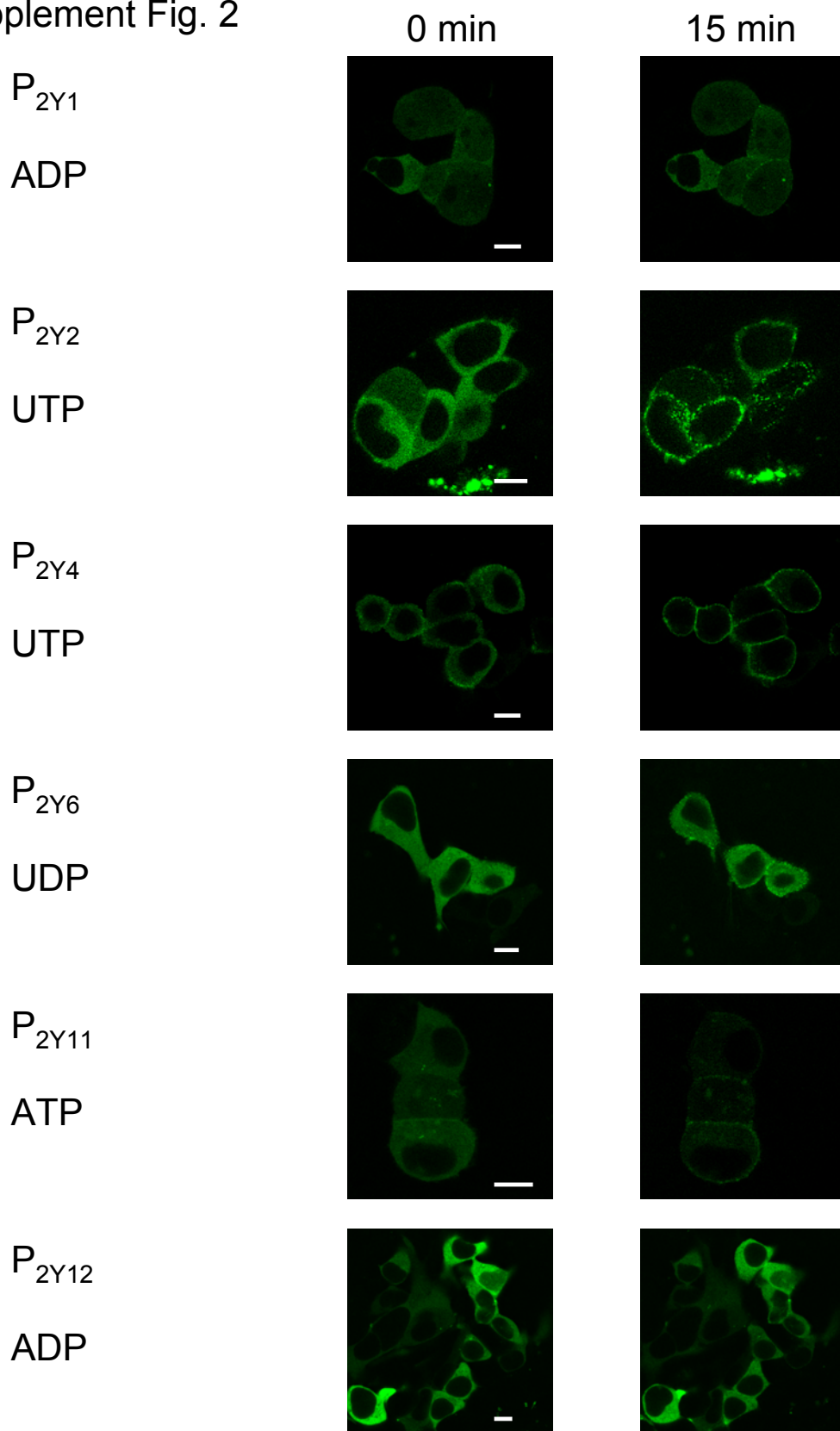
Supplement Fig. 1A:  $\beta$ -arrestin-1-GFP in HEK-293 control cells



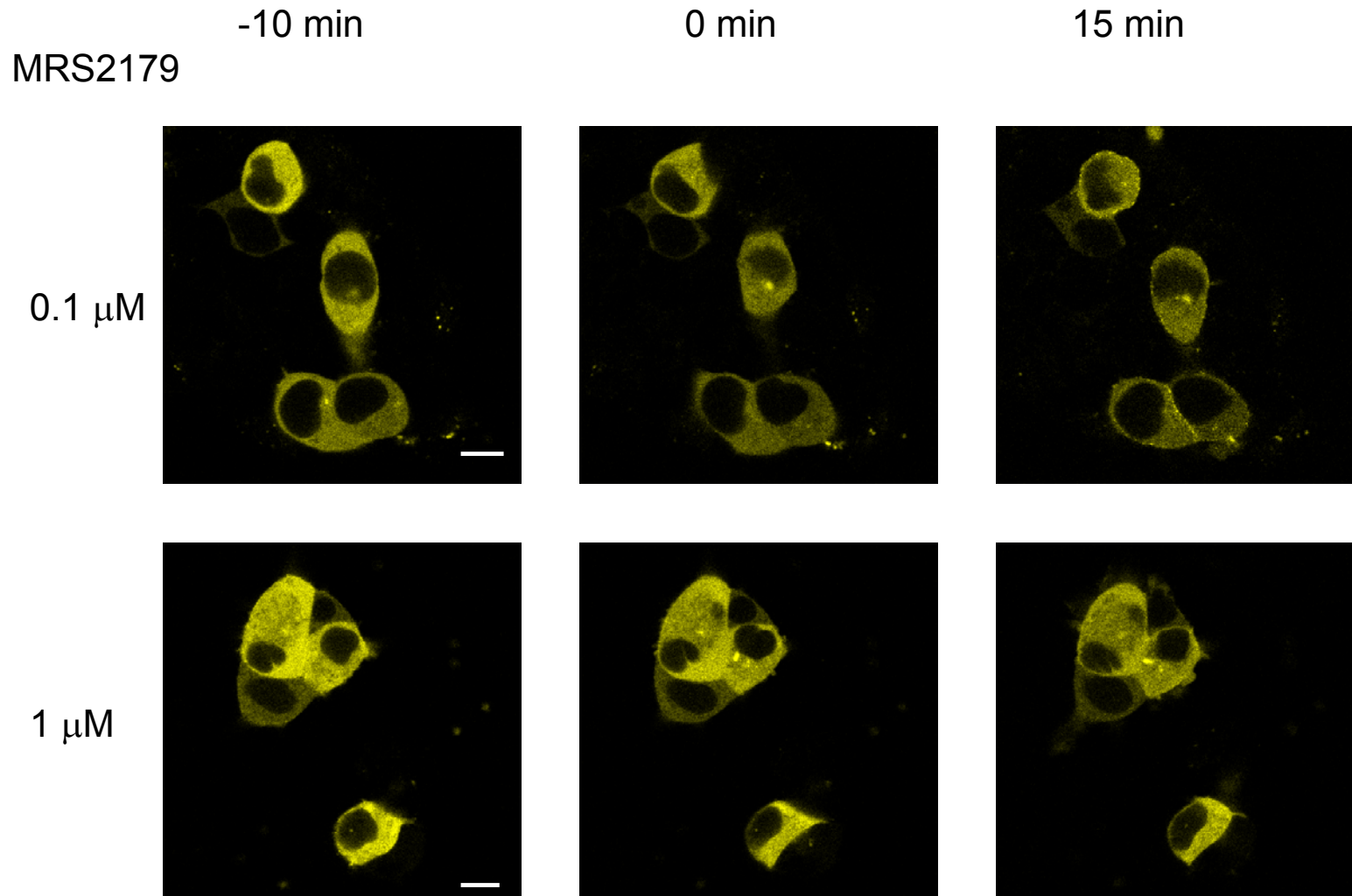
Supplement Fig. 1B:  $\beta$ -arrestin-2-YFP in HEK-293 control cells



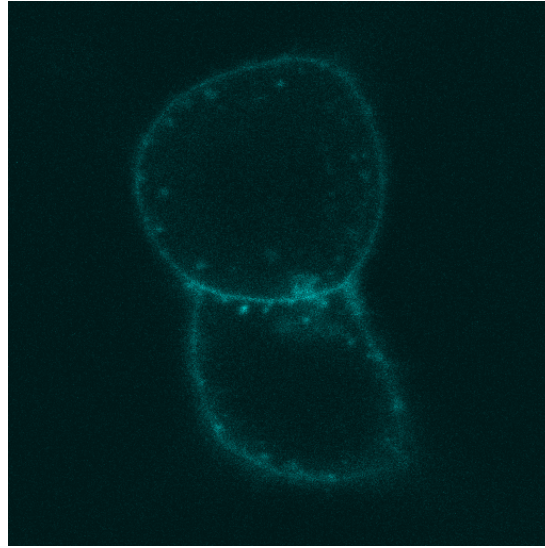
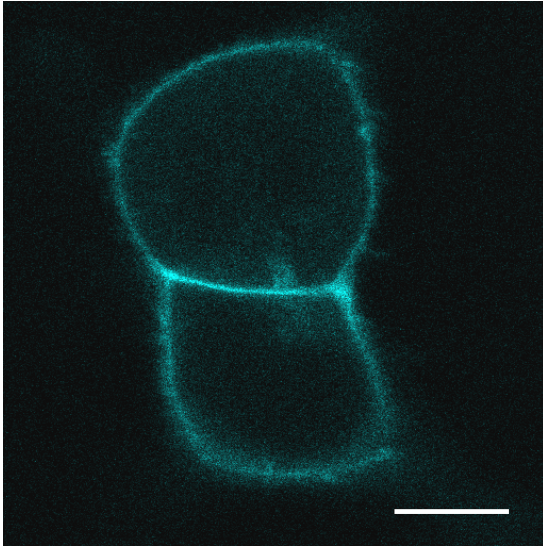
Supplement Fig. 2



Supplement Fig 3: P<sub>2Y1</sub> co-transfected with  $\beta$ -arrestin-2  
preincubated with MRS2179 and stimulated with ADP



Supplement Fig. 4:



Supplement Fig. 5:

