Supplemental Figures

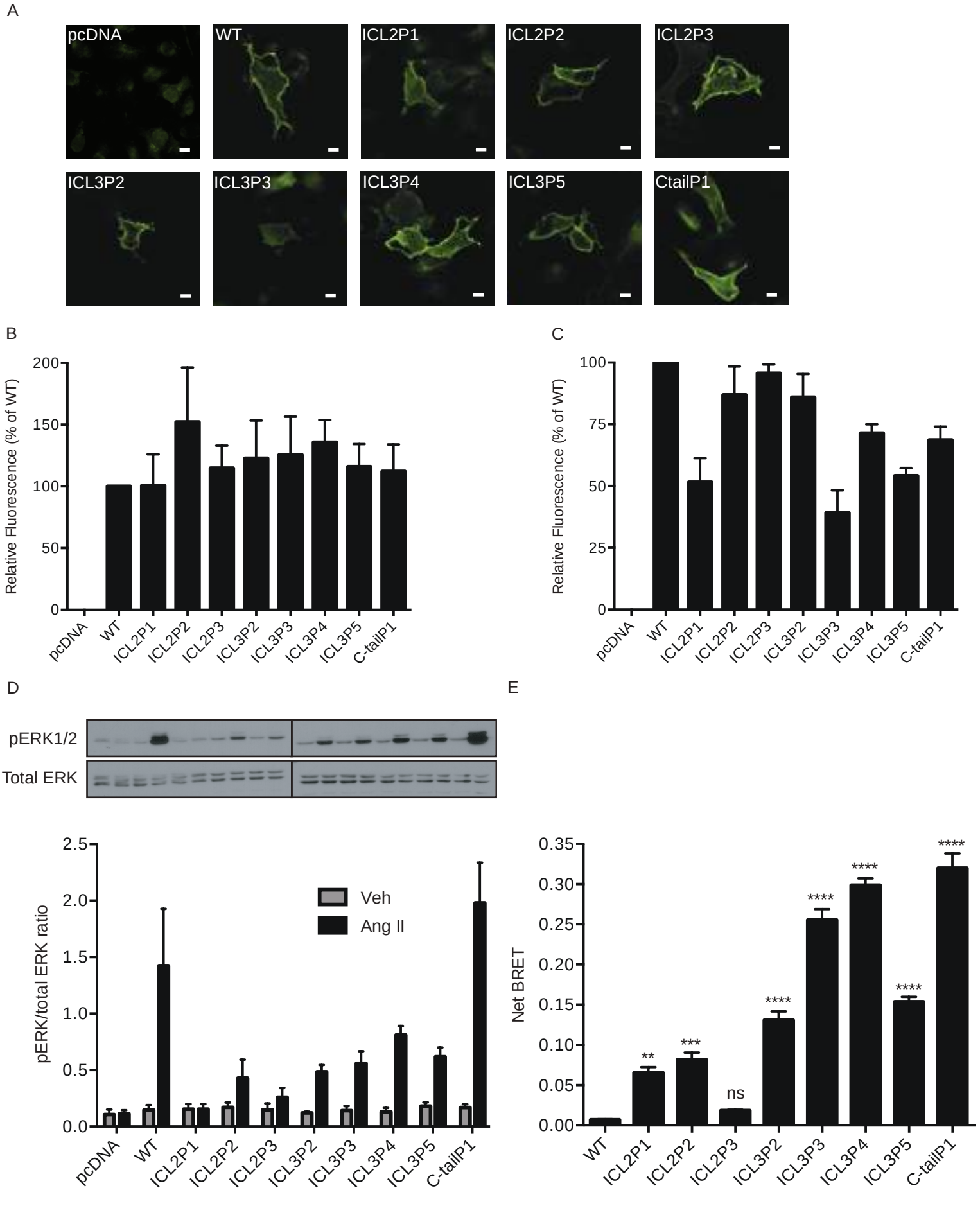
Supplemental Figure 1. *Validation of conformational sensors expressed in HEK 293 cells*. A) Cell surface expression by immunofluorescence imaging; scale bar represents 10 μm. B) Total and C) cell surface sensor level expression monitored by quantitative immunofluorescence as described in Materials and Methods. D) ERK1/2 MAPK activation following Ang II stimulation for 5 minutes. Detection and quantification were as described in *Experimental Procedures*: top panel is a representative blot and bottom graph represents averages of fold stimulation; data represent mean +/- SEM from 3 independent experiments. E) Basal BRET of our sensor panel was recorded when expressed transiently in HEK 293 cells. To monitor for non-specific labeling a construct that did not have the FlAsH binding sequence (WT) was expressed in parallel. Net BRET was calculated as described in *Experimental Procedures*. Bars represent mean +/- SEM of net BRET of 48 to 49 replicates from 3 independent experiments. Statistical analysis was performed as described in *Experimental Procedures*, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ and ns is not significant.

Supplemental Figure 2. *Agonist-induced BRET changes in conformational sensors expressed in HEK 293 cells where minimal or common responses were detected*. (A) ICL2P3, (B) ICL3P2, (C) ICL3P5 and (D) ICL3P4. Bars are averages +/- SEM of ΔBRET of 3 to 6 replicates from 3 independent experiments. For each sensor, statistical analysis was performed as described in *Experimental Procedures*. * $P < 0.05$ and ** $P < 0.01$. *Inset*-controls for FlAsH labelling. Ang II-induced BRET changes in unlabelled (WT) negative control sensor expressed in HEK 293 cells and labelled with the FlAsH reagent as described in *Experimental Procedures*. Bars represent mean +/- SEM of ΔBRET from 3 independent experiments. A two-tailed unpaired t-test showed no statistically differences between buffer- and Ang II-treated cells.

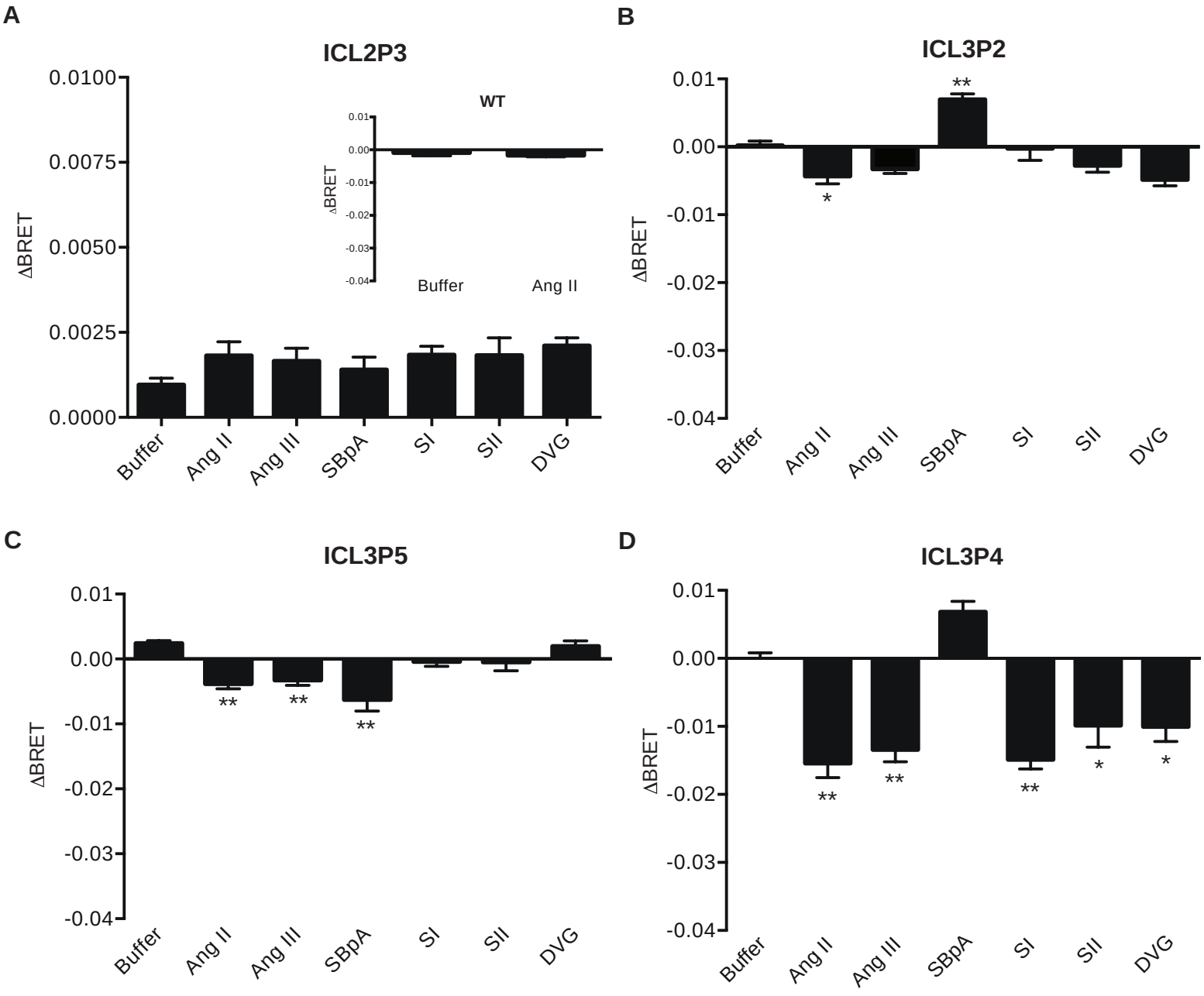
Supplemental Figure 3. *Monitoring β-arrestin levels by western blot.* (A) Top: a representative blot (n=3) of β-arrestin expression levels from β-arrestin KO cell line transiently expressing β-arrestin-1, β-arrestin-2, both or vector control (pcDNA). Parental cell line lysates were loaded next to the samples to show endogenous levels. Bottom: GAPDH detection from the same membrane after stripping to control for loading. (B) Top: a representative blot (n=6) of β-arrestin expression levels from HEK 293 cells transfected with either control or a mixture of β-arrestin-1 and β-arrestin-2 siRNA (knockdown efficiency of 75 +/- 4% of n=6). Bottom: as in (A).

Supplemental Figure 4. *Cell context for basal BRET in AT1R biosensors*. A) Effect of $G\alpha_q$ overexpression on total expression of ICL2P2, ICL3P3 and C-tailP1 monitored by total luminescence emission from the data acquired in (C); luminescence levels are expressed as relative values in which the luminescence from pcDNA transfected cells is set to 1 (dotted line in red). Black lines represent average values. B) Data represent net *basal* BRET for a set of independent experiments with ICL2P2, ICL3P3 and C-tailP1 biosensors. BRET was monitored in native HEK 293 cells (n=3) and VSMCs (n=3). C) Data represent net *basal* BRET for a set of independent experiments with ICL2P2, ICL3P3 and C-tailP1 biosensors. BRET was monitored in absence (pcDNA) or presence of $G\alpha_q$ in the $\Delta G\alpha_{q/11/12/13}$ line. Data represent mean Net BRET +/- SEM. D) Sensor expression levels in HEK 293 cells and VSMCs. Total luminescence of ICL2P2 or ICLDP3 biosensors calculated as the combined Relative Luminescence Unit (RLU) from both donor and acceptor moieties. Bars represent average of 7 independent experiments for HEK 293 cells and 3 independent experiments for VSMCs and error bars represent SEM.

Devost *et al.* Supplemental Figure 1

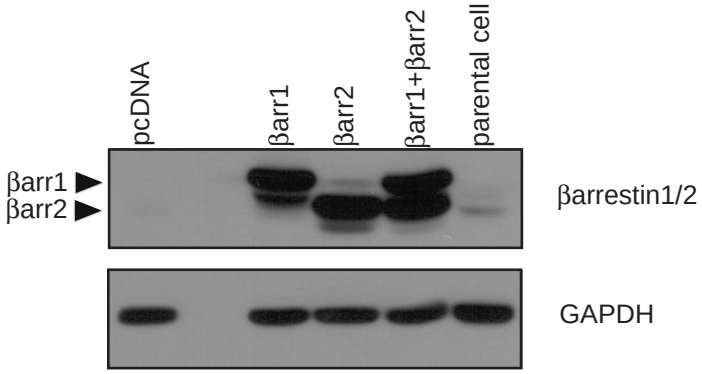


Devost *et al.* Supplemental Figure 2



Devost *et al.* Supplemental Figure 3

A



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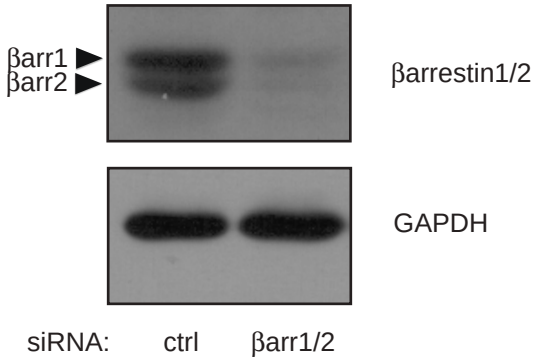

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