

Supplemental Figure 1

HEK cells co-transfected with GFPvarr and either the R135 mutant (**A** and **B**) or WT rhodopsin (**C** and **D**) were PFA fixed ~48 h after transfection. GFPv-arr was visualized directly by way of the GFP signal (**A** and **C**) and nuclei were visualized by DAPI labeling (**B** and **D**). Bar = 20 μ m.



Supplemental Figure 2

Differential binding ability of mAb3A6 to the WT and R135L rhodopsins in the presence of GFPv-arr. HEK cells co-transfected with GFPv-arr and either the WT rhodopsin (A and B) or R135L mutant (C and D) were PFA fixed ~24h after transfection and immunolabeled with mAb3A6 followed by Alexa594-conjugated anti-mouse Ab (B and **D**). GFPv-arr was visualized directly by the GFP signal (A and C). mAb3A6 gave rise to prominent PM labeling of WT rhodopsin in cells co-expressed with GFPv-arr, similar to the staining results obtained by mAbB6-30 immunolableing in Figure 3A. However, mAb3A6 failed to detect most of the R135L signal except for a few intracellular areas that were highly enriched with the transfected proteins (arrow). Ba $r = 20 \mu m$.

Supplemental Table 1

	24 h	48 h
R135L/GFPv-arr	$7.3 \pm 1.2^{*}$	$12.3 \pm 1.3^*$
WT/GFPv-arr	0.19 ± 0.01	0.34 ± 0.13
GFPv-arr	0.17 ± 0.01	0.9 ± 0.2
GFP	0.33 ± 0.01	0.23 ± 0.13

Cell death of HEK cells transfected with R135L/GFPv-arr, WT/GFPv-arr, GFPv-arr and GFP was measured by the Annexin V-binding (53). 24h or 48h-post transfection using the calcium phosphate method, living HEK cells grown on gelatin-coated slips were incubated with Annexin V-biotin (DB Biosciences Pharmingen) for 20 min and followed by Alexa 594-streptavidin (Molecular Probes) for an additional 20 min. All incubations and rinsing were carried out in ice-cold binding buffer (10 mM HEPES, 150 mM NaCl, 2.5 mM CaCl₂). Counting was carried out under the epifluroescent microscope (Zeiss Axioscope II) in a blind-test fashion. 500 GFP⁺ cells were counted in each of three independent experiments. Cell death was expressed as the percentage of Annexin V-positive cells among the GFP⁺ transfected cells. **P* < 0.001 in Student's *t* test.