Supplemental Tables

A high-throughput drug screening strategy for detecting rhodopsin P23H mutant rescue and degradation

Yuanyuan Chen[1], Hong Tang[2], William Seibel[2,3], Ruben Papoian[2,4], Xiaoyu Li[1], Nevin A. Lambert[5], Krzysztof Palczewski[1,#]

1. Department of Pharmacology, Case Western Reserve University, 2109 Euclid Ave., Cleveland, OH 44106-4965, USA
2. Drug Discovery Center, College of Medicine, University of Cincinnati, Cincinnati, OH 45237, USA
3. Current address: Oncology Department, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave., Cincinnati, OH 45229, USA
4. Current address: Department of Neurology and Rehabilitation Medicine, College of Medicine, University of Cincinnati, Cincinnati, OH 45267, USA
5. Department of Pharmacology and Toxicology, Georgia Regents University, 1459 Laney Walker Blvd., Augusta, GA 30912, USA

Supplemental Table 1. Dose-response data for 9-*cis*-retinal in the β-Gal fragment complementation assay plotted in Figure 2B.

[9-*cis*-Retinal]	Log concentration	Mean RLU[a]	Standard deviation[b]
(μM)	(M)		
20	-4.69897	1972	1272
15	-4.82391	6587	5479
10	-5.00000	29038	7889
7.5	-5.12494	39254	3852
5.0	-5.30103	33956	490
2.5	-5.60206	29152	1532
1.0	-6.00000	16039	1157
0.75	-6.12494	17124	2740
0.50	-6.30103	11744	777
0.25	-6.60206	7901	1096
0.10	-7.00000	4227	53

0.05	-7.30103	3116	66
0.01	-8.00000	2142	171
0.005	-8.30103	1592	43
0.001	-9.00000	1317	484

[a] Average readings of relative luminescence units from triplicate determinations

[b]Standard deviations of luminescence readings from triplicate determinations.

Supplemental Table 2. Dose-response data for 9-*cis*-retinal in the BRET assay plotted in Figure 3B.

[9-*cis*-Retinal] (μM)	Log concentration (M)	BRET[a] (ratio of emissions at 530 nm/480 nm)	Standard deviation
20	-4.699	0.853	0.003
10	-5.000	0.843	0.004
2.5	-5.6021	0.814	0.011
1.3	-5.9031	0.804	0.006
0.63	-6.2041	0.784	0.013
0.31	-6.5052	0.776	0.006
0.16	-6.8062	0.77	0.013
0.078	-7.1072	0.766	0.013
0.039	-7.4082	0.756	0.015
0.010	-8.0103	0.748	0.003
0.0050	-8.3113	0.738	0.016
0.0025	-8.6124	0.743	0.004

[a] Average of BRET ratio (emission ratio at 530 to 480 nm) from triplicate determinations.

Supplemental Table 3. Dose response data for 9-*cis*-retinal obtained by high-content imaging plotted in Figure 4B.

[9-*cis*-Retinal] (μM)	Log concentration (M)	Mean INT ratio PM-to-ER[a]	Std. dev	Mean number of nuclei	Standard deviation
20	-4.699	0.134	0.038	66	12
10	-5	0.479	0.000	400	45
5	-5.301	0.756	0.090	467	375
2.5	-5.6021	0.492	0.027	1234	20
1.25	-5.9031	0.390	0.010	2430	323
0.625	-6.2041	0.381	0.028	2426	69
0.3125	-6.5051	0.459	0.032	3474	662
0.15625	-6.8062	0.443	0.011	3020	532

[a] Means of triplicate determinations, each representing the averaged intensity ratio per cell of P23H staining on the PM region to that in the ER region.

Supplemental Table 4. Measurements of band intensities from immunoblots plotted in Figure 5B.

		P23H mutant opsin		WT		P23H mutant opsin	
Treatment		Mean of normalized total INT[a]	Standard deviation	Mean of normalized total INT	Standard deviation	Mean INT ratio of mature glycosylated opsintototal opsin[b]	Standard deviation
DMSO	0.1%	1.000	0.076	1.000	0.002	0.091	0.041
None[c]		0.595	0.151	0.971	0.263	0.102	0.075
9-*cis*-Retinal (μM)	20	0.833	0.265	1.874	0.153	0.762	0.009
	10	4.548	0.088	2.373	0.093	0.870	0.034
	5	4.524	0.105	3.200	0.007	0.910	0.016
	2.5	3.190	0.025	3.347	0.069	0.888	0.038
	1.25	2.857	0.041	2.679	0.141	0.747	0.007
	0.625	1.286	0.228	2.962	0.126	0.399	0.020

[a] The averaged total intensities of immunoblots from 2 individual treatments of P23H mutant opsin normalized by immunoblot intensities of β-tubulin.

[b] The averaged immunoblot intensity ratios of P23H mutant opsin with mature glycosylation to total P23H mutant opsin $(INT_{band1}+INT_{band3})/(INT_{band1}+INT_{band2}+INT_{band3}+INT_{band4})$, measured from triplicate samples.

[c] None, no treatment.

Supplemental Table 5. Dose-response data for Evans Blue in the Rluc reporter assay plotted in Figure 6B.

[Evans Blue][a] (μM)	Log concentration (M)	Mean RLU[b]	Standard deviation
2.5	-5.60206	11613	155
5	-5.30103	10528	126
10	-5.00000	8668	202
25	-4.60206	5568	312
50	-4.30103	4309	648
75	-4.12494	3750	475
100	-4.00000	3561	517
150	-3.82391	2958	694
200	-3.69897	1989	266
0	N/A	9749	82

[a] Readings from the 0 μM Evans Blue samples treated with 0.1% DMSO are not plotted in the dose-response curve;

[b] Average relative luminescence units read from experiments performed in triplicate.

Supplemental Table 6. Standard curve data from the ALPHA assay plotted in Figure 8A.

Bovine opsin (pg)	Mean ALPHA signal[a]	Standard deviation
0.0075	2450	2584
0.025	3113	2679
0.075	4556	2916
0.25	6558	2900
0.75	9150	3303
2.5	15158	8363
7.5	20868	2906
25	48042	4092
75	72810	2239
250	117416	7986

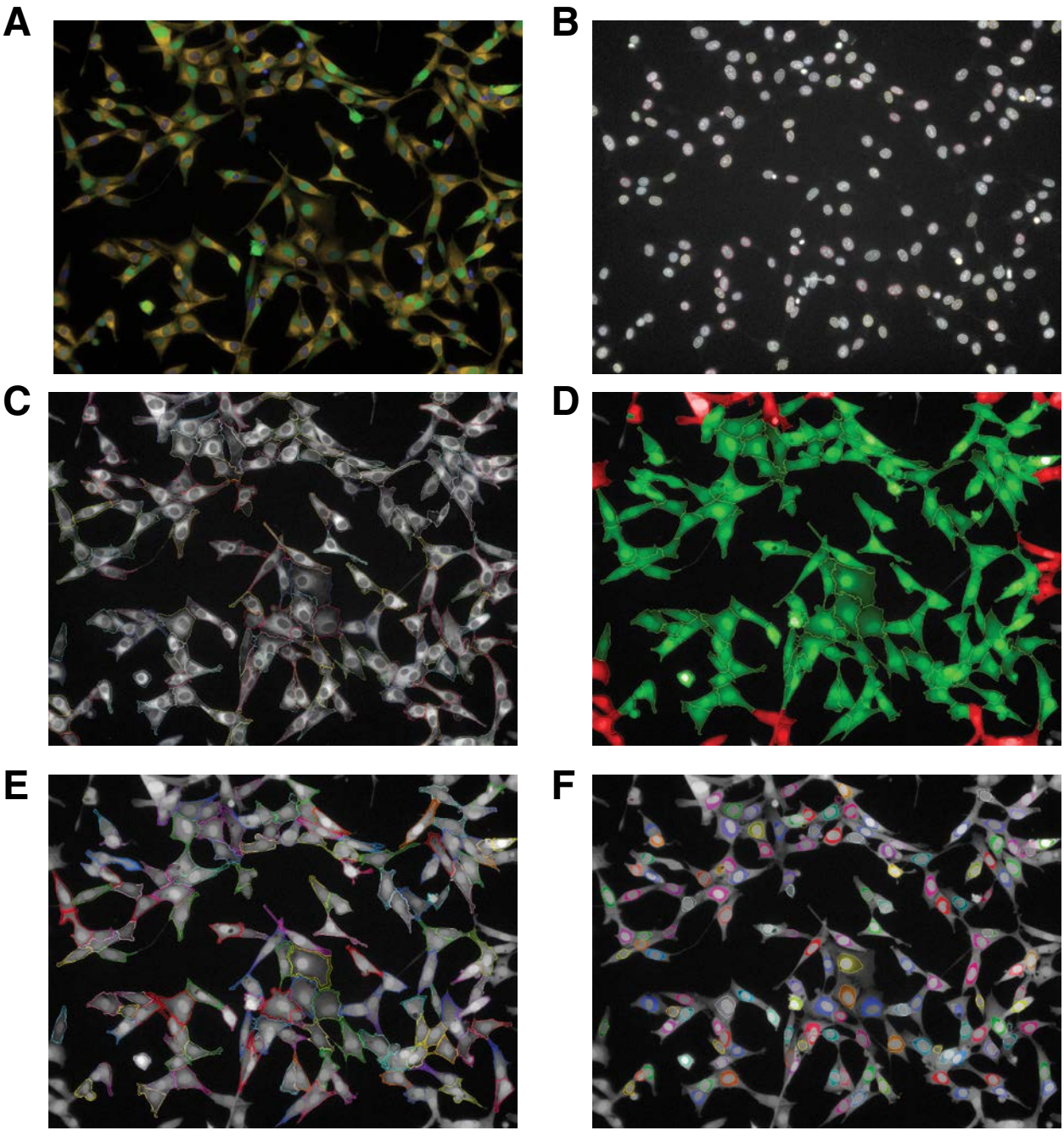
[a] Average of ALPHA signals from 3 experiments.

Supplemental Table 7. Data from the ALPHA immunoassay used to calculate the P23H mutant opsin amounts plotted in Figure 8B.

Amount of cell lysate	Mean ALPHA signal	Standard deviation	Mean calculated P23H mutant opsin amount[a]	Standard deviation
(%)			(pg)	(pg)
100	83976	7919	101.6	20.8
75	79321	2737	88.2	7.4
50	60254	5255	47.2	9.0
25	29082	6522	10.8	4.7

[a] Average of calculated P23H mutant opsin amounts calibrated by the standard curve plotted in Figure 8A and derived from experiments performed in triplicate.

Supplementary Figure 1. Illustration of image-based analysis for subcellular localization of P23H opsin. Here the immunostained image of DMSO treated NIH3T3 cells expressing P23H opsin was used to demonstrate the individual steps involved in image-based analysis. (**A**) Overlay of 3 channels in an image field. Yellow, opsin; green, GFP; blue, DAPI. (**B**) Nuclei defined by DAPI staining. The boundary of each nucleus was defined as the 50% line of the cell. (**C**) Cytoplasm defined by GFP fluorescence. The boundary of GFP fluorescence in each cell was defined as the 0% line of the cell. Only cells with intact cytoplasmic images were selected in the population for this image analysis. Numbers of intact cells in the 5 imaged fields of each well were recorded as the 'Cell number'. Intensity of Cy3 immunostaining of opsin with the 0% line of each cell was measured and averaged through the 5 image fields taken in each well of the 384-well plate, and the result was recorded as 'Cy3-INT-Total'. (**D**) The plasma membrane region was defined as the area between -5 (outside) to +5 % (inside) lines of each cell. Average intensity of Cy3 immunostaining of opsin within this region per cell was recorded as 'Cy3-INT-PM'. (**E**) The endoplamic reticulum was defined as the area between the 25% and 50% lines of the cell. The average intensity of Cy3 staining of opsin within this region per cell was recorded as Cy3-INT-ER. The PM-ER INT ratio was calculated as: Cy3-INT-PM/Cy3-INT-ER.


A
B
C
D
E
F

Supplementary Figure 2. WT-opsin-Rluc reporter assay for selection of compounds which specifically degrade P23H opsin but not affect the WT opsin amount. (**A**) Immunoblot of opsin from lysate of HEK293 stable cells expressing WT-opsin-Rluc (left, molecular mass of opsin monomer is 75 kDa) or P23H-opsin –Rluc (right). Bands from bottom to up are dimer and higher molecular weight oligomers of opsin-Rluc fusion proteins. Due to the difference of glycosylation between WT and P23H opsin, band sizes of the opsins are different. (**B**) Chart of luciferase activity in relationship to number of cells expressing WT-opsin-Rluc. Different number of cells (12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100%) was seeded to each well of a 384-well plate to represent proportional amount of WT-opsin. The 100% cell amount equals to 8000 cells/well. The luminescence readouts were calibrated to WT-opsin amount by sigmoidal curve fitting. Parameters of fitted curve were listed in the inset of (**B**). This chart will be used to calculate WT-opsin amount after treatment of compounds of interest.