

1/40

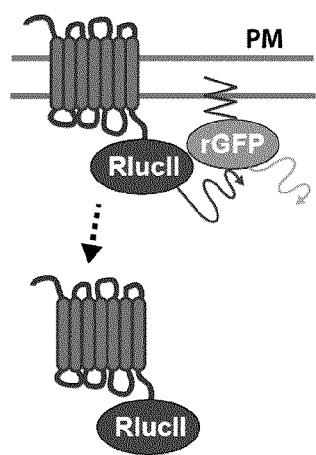


FIG. 1A

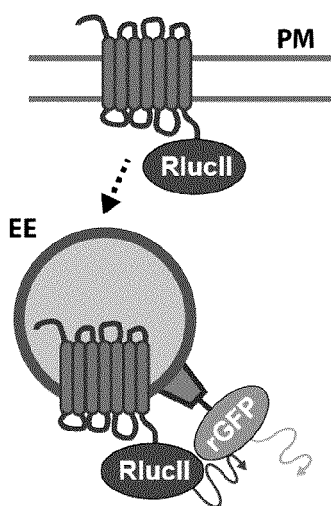


FIG. 1B

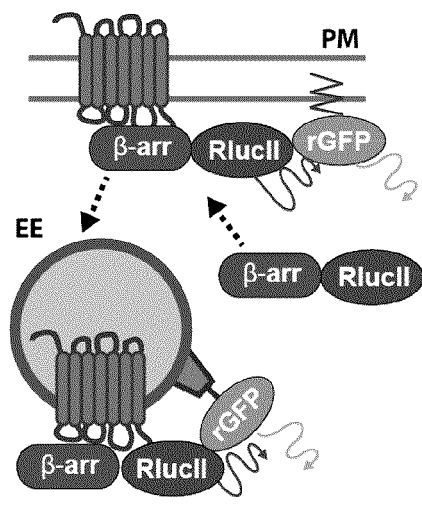


FIG. 1C

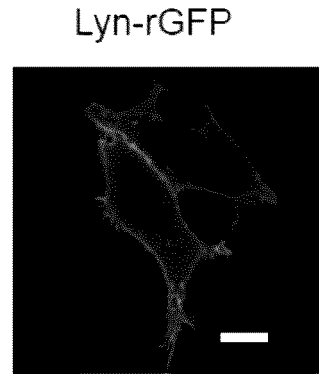


FIG. 1D

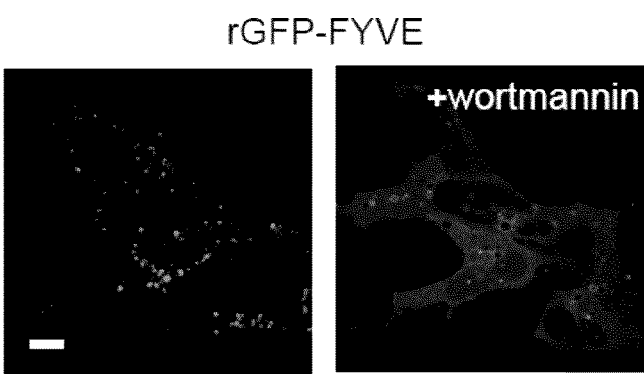


FIG. 1E

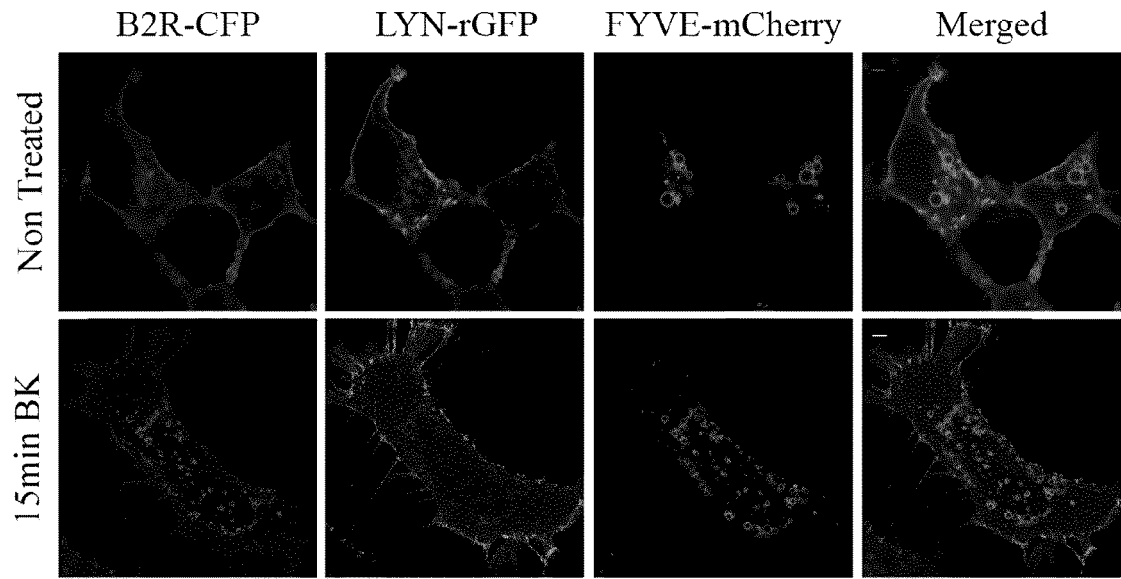


FIG. 1F

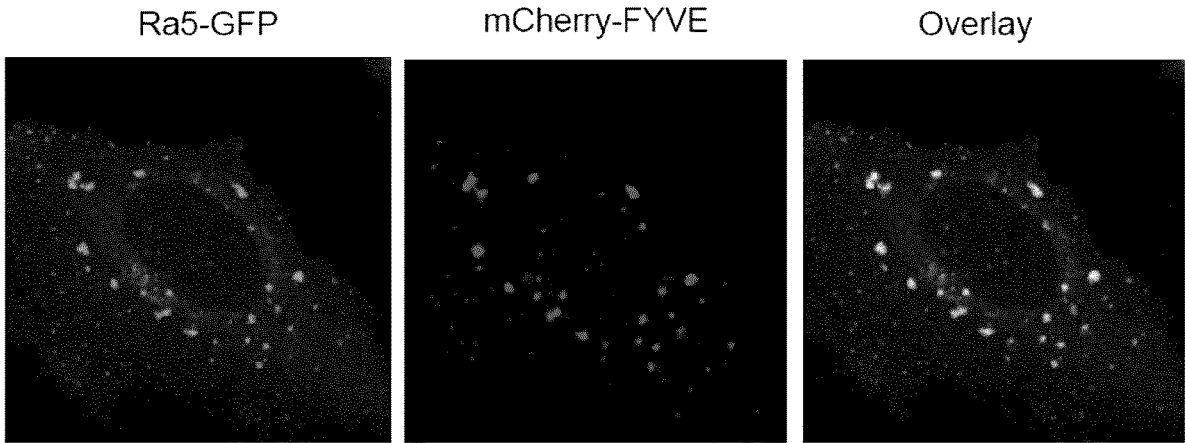


FIG. 1G

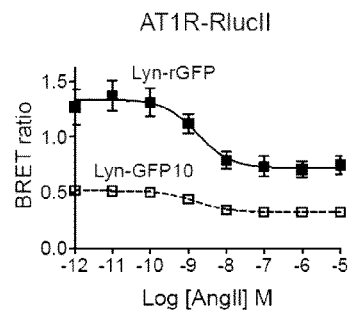


FIG. 2A

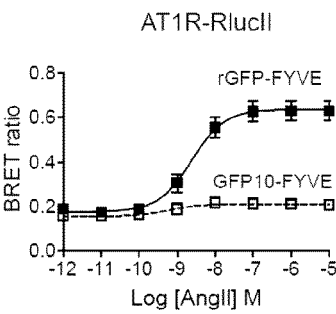


FIG. 2B

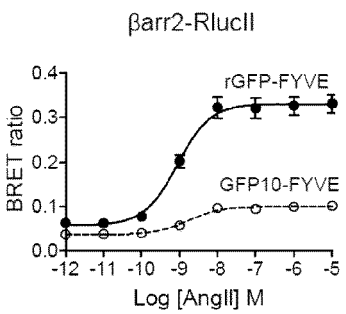


FIG. 2C

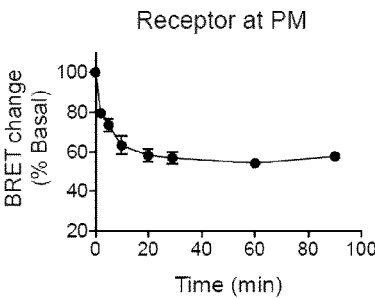


FIG. 2D

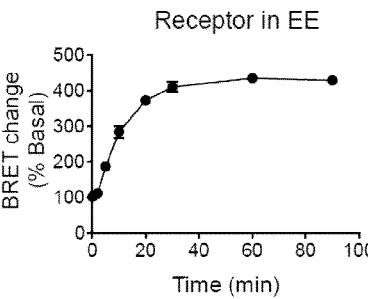


FIG. 2E

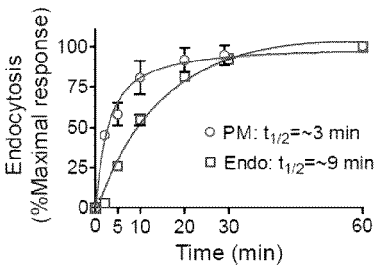
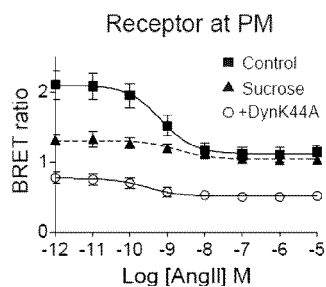
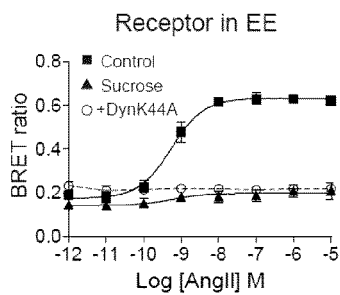
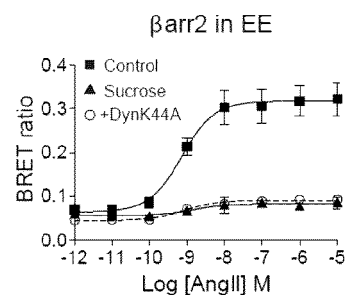
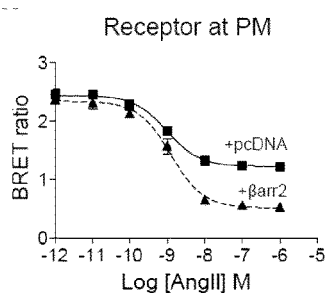
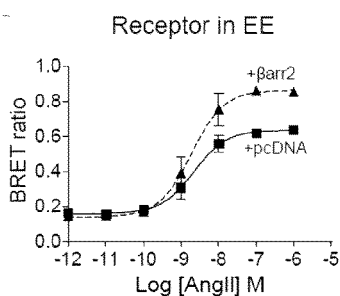
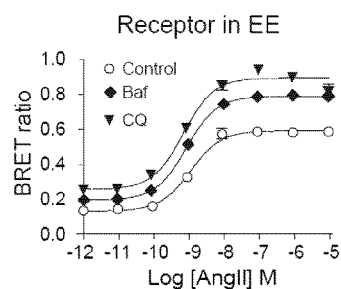
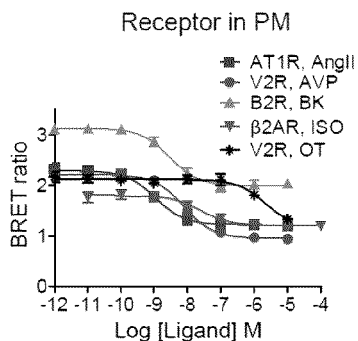
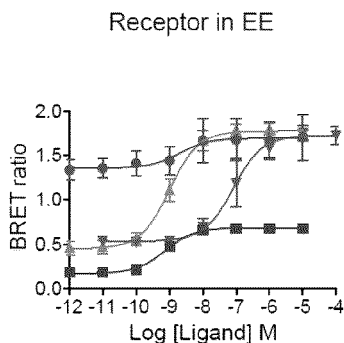
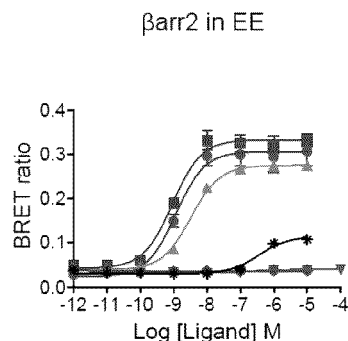
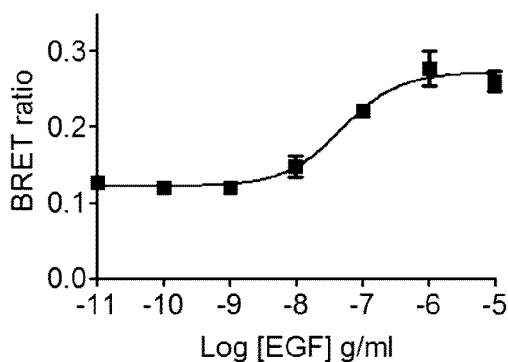


FIG. 2F

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**FIG. 3A****FIG. 3B****FIG. 3C****FIG. 3D****FIG. 3E****FIG. 3F****FIG. 4A****FIG. 4B****FIG. 4C****FIG. 4D**

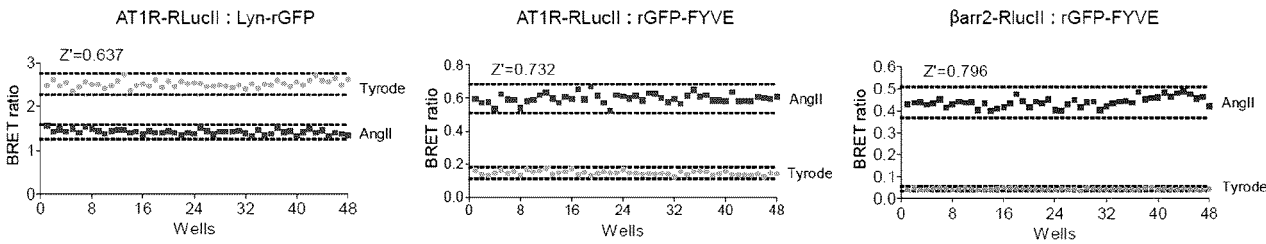


FIG. 4E

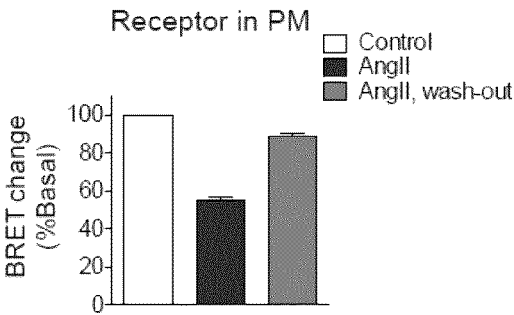


FIG. 5A

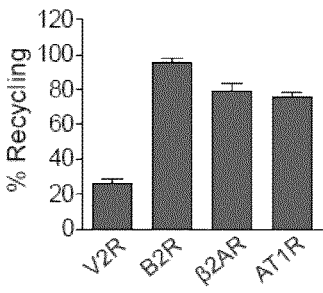


FIG. 5B

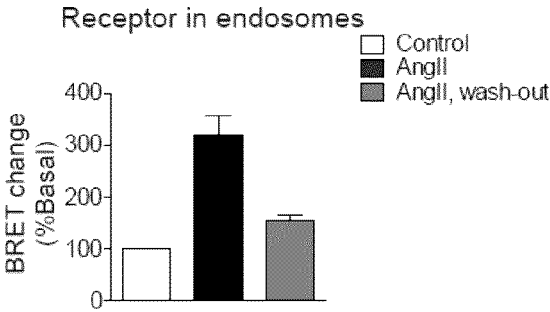


FIG. 5C

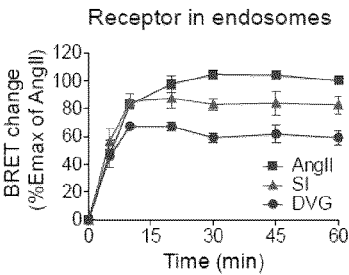


FIG. 6A

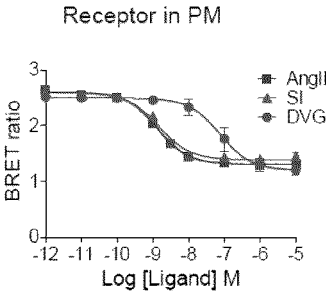


FIG. 6B

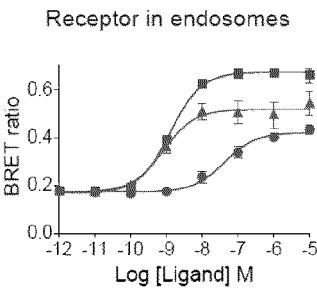


FIG. 6C

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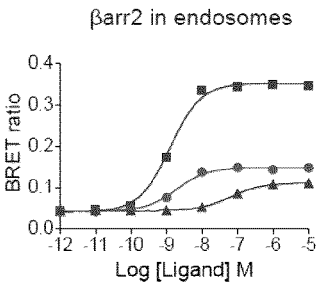


FIG. 6D

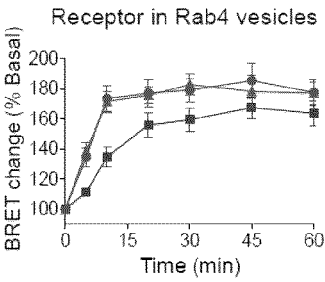


FIG. 6E

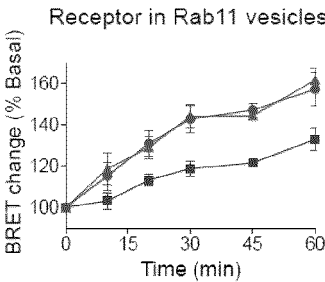


FIG. 6F

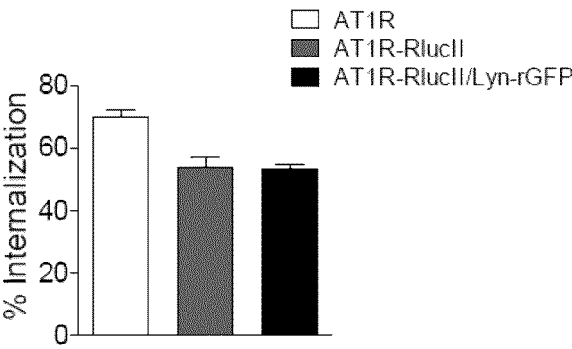


FIG. 7A

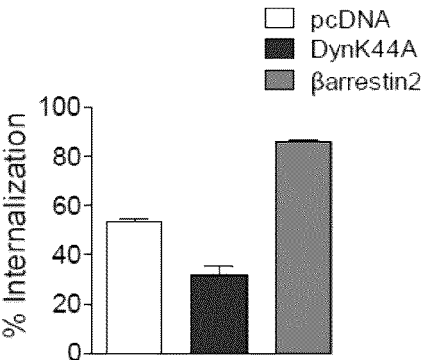


FIG. 7B

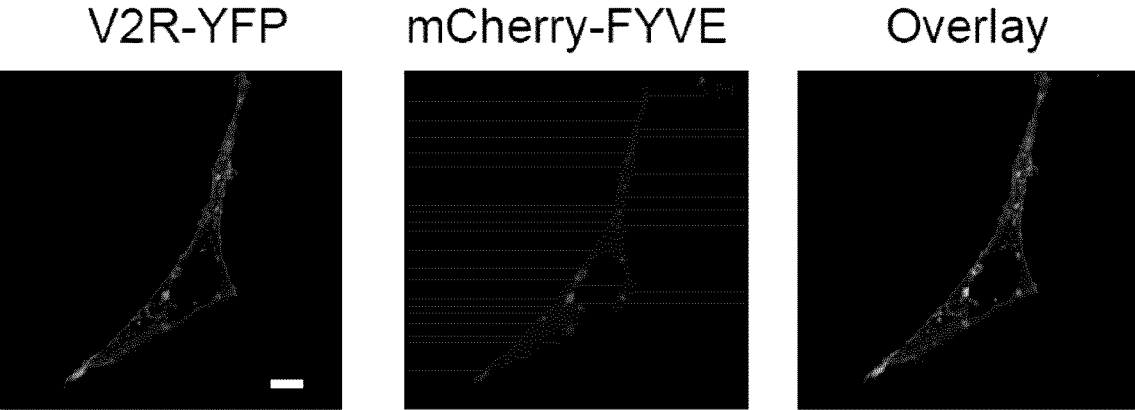
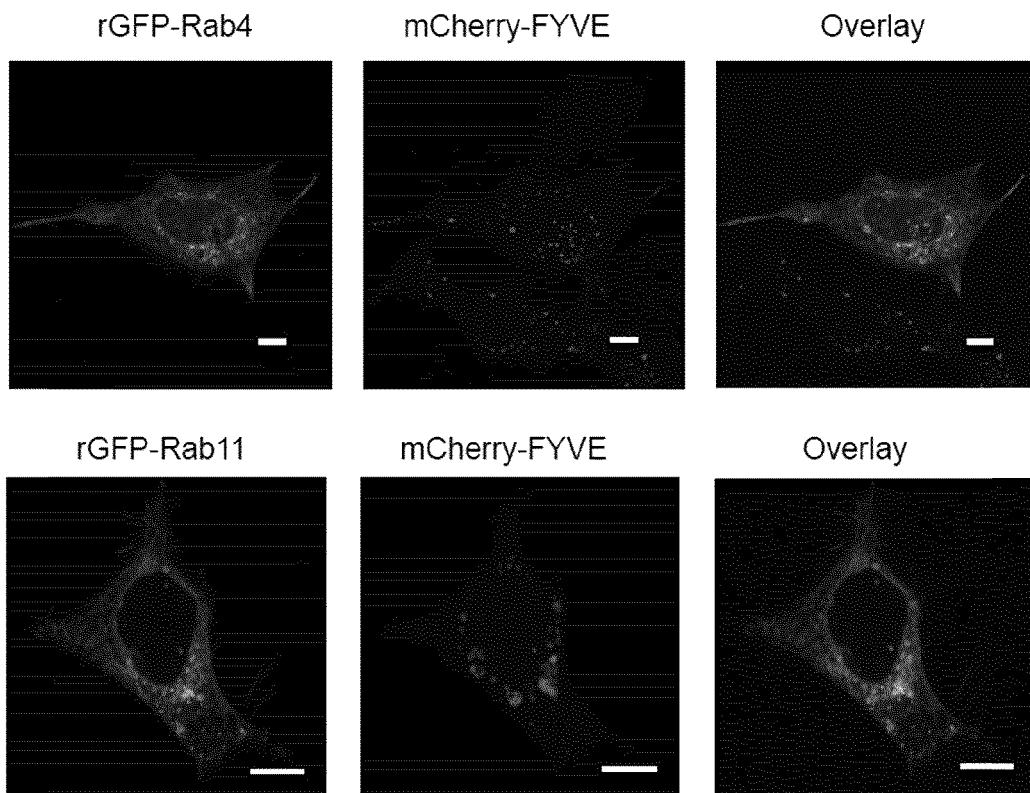
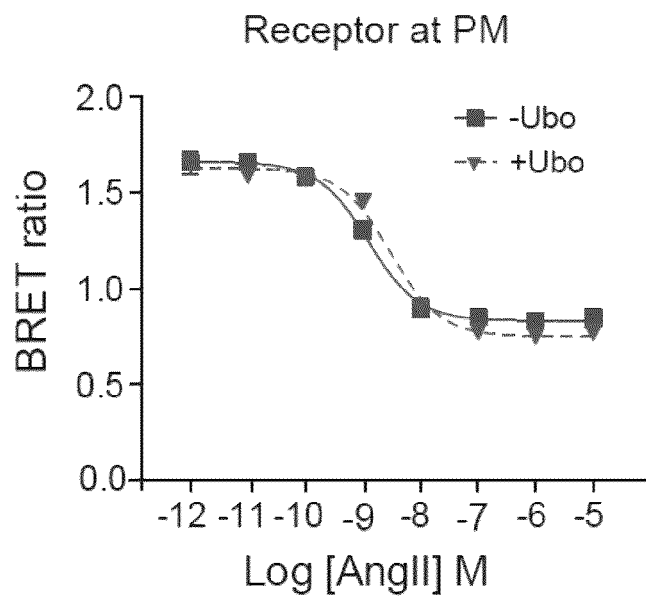


FIG. 8

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**FIG. 9A****FIG. 9B**

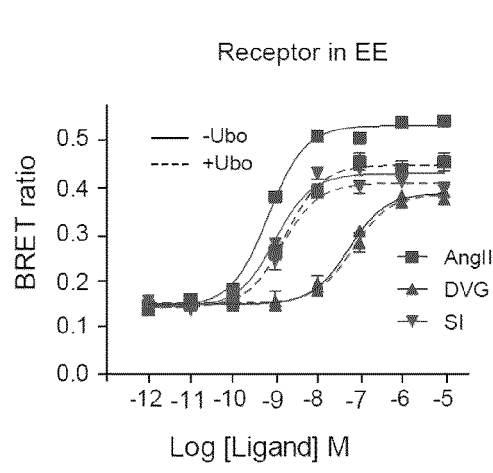


FIG. 9C

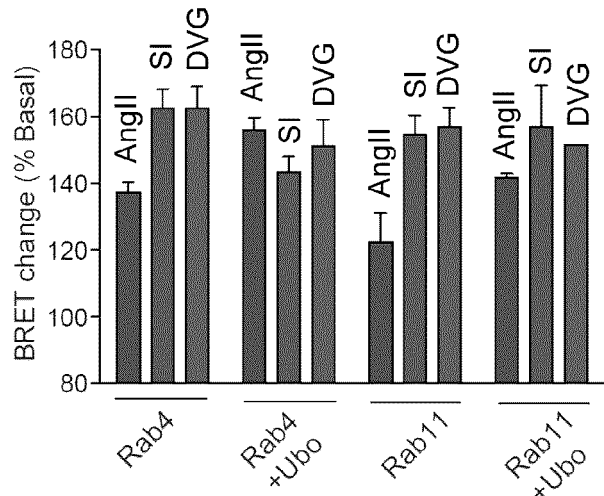


FIG. 9D

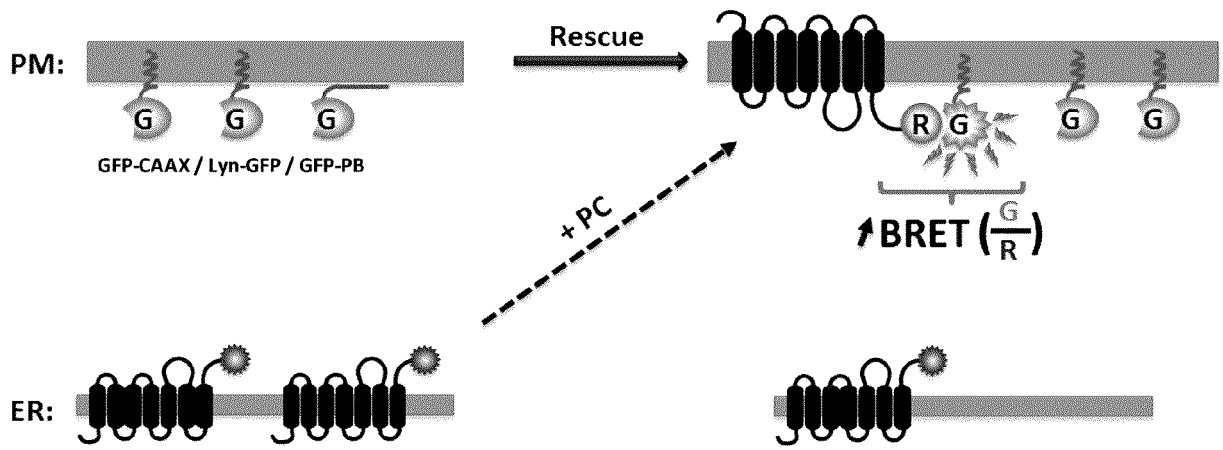


FIG. 10A

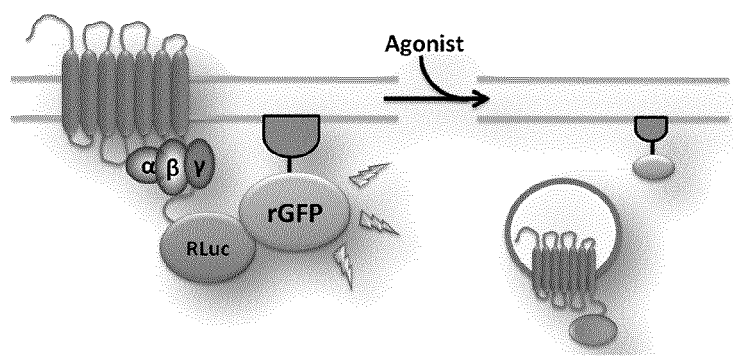


FIG. 10B

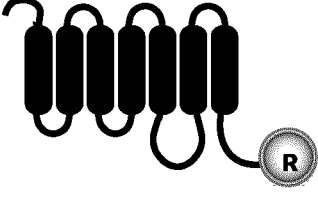
a) MC4R 	1) hMC4R wt-RlucII: human wild type Melanocortin 4 receptor tagged with RlucII 2) hMC4R (R165Q)-RlucII: mutant R165Q-hMC4R tagged with RlucII, intracellularly retained and PC-rescuable 3) hMC4R (P299H)-RlucII: mutant P299H-hMC4R tagged with RlucII, intracellularly retained and not PC-rescuable Linker sequence between the hMC4R and RlucII: hMC4R-<u>VGGGGSKLPAT</u>-RlucII Linker1
---	--

FIG. 11A

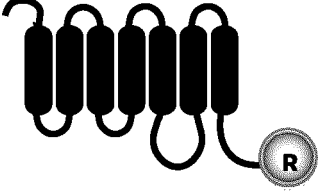
b) V2R 	1) hV2R wt-RlucII: human wild type Vasopressin 2 receptor tagged with RlucII 2) hV2R (Y128S)-RlucII: mutant Y128S-hV2R tagged with RlucII, intracellularly retained and PC-rescuable Linker sequence between the hV2R and RlucII: hV2R-<u>GGSGGLKLPAT</u>-RlucII Linker2
--	--

FIG. 11B

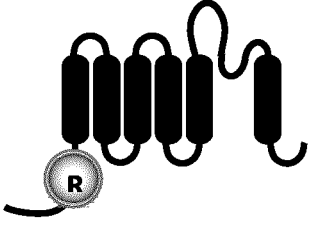
c) hERG int RlucII 	1) hERK wt-RlucII: human wild type voltage-gated Potassium channel H2 (also known as hERG) with an internal RlucII-tag at position 379 with duplication of residues 373 to 379 2) hERG (G601S): mutant G601S-hERG tagged with RlucII (pos 379) intracellularly retained and PC-rescuable Linkers and region duplicated from hERG: ...EKVTQVL³⁷⁹- <u>NAAIRSGG</u>-RlucII- <u>GGNAAIRS</u>- EKVTQVL³⁷⁹ ... hERG Linker 3 Linker 4 hERG
--	---

FIG. 11C

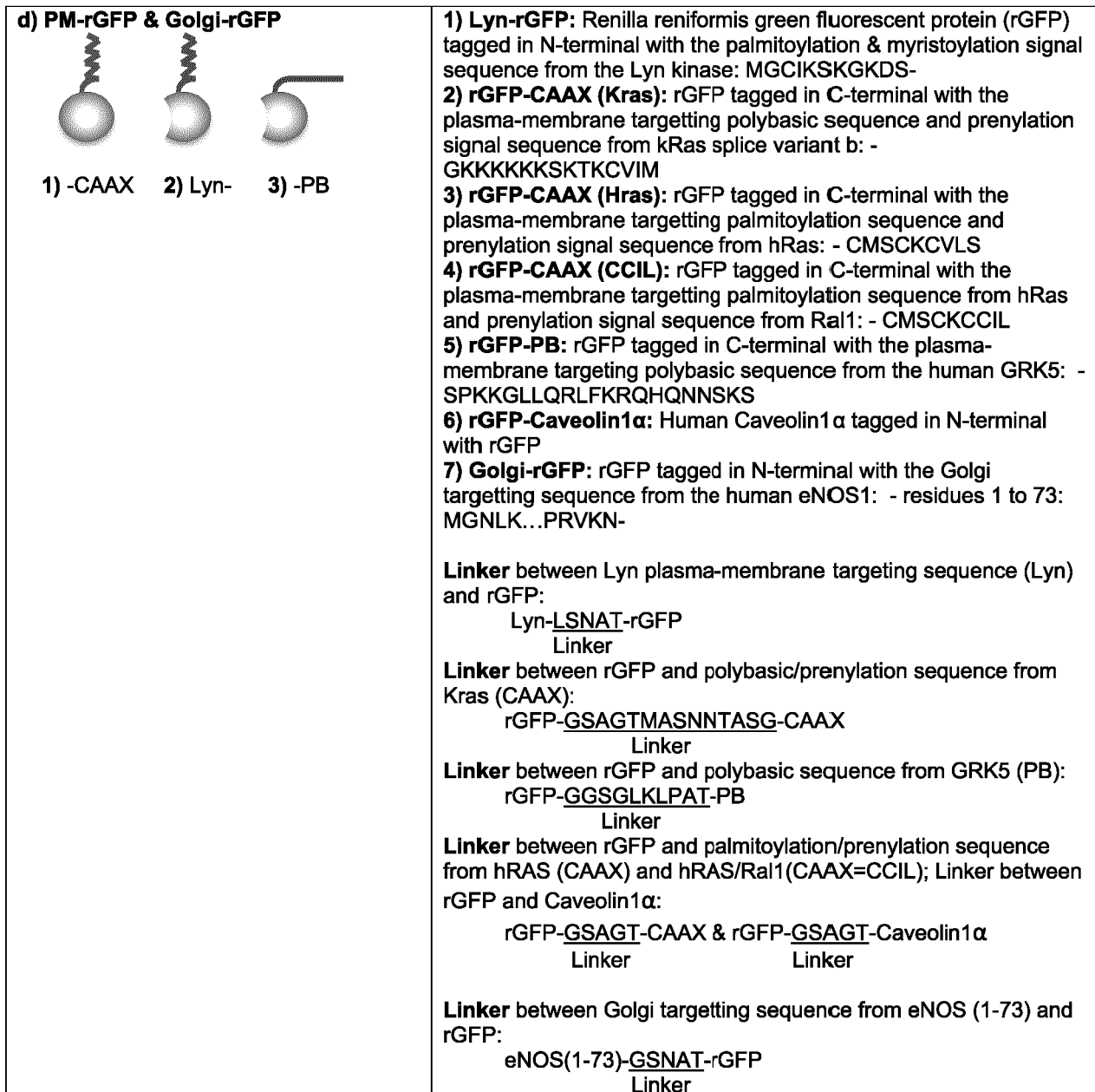


FIG. 11D

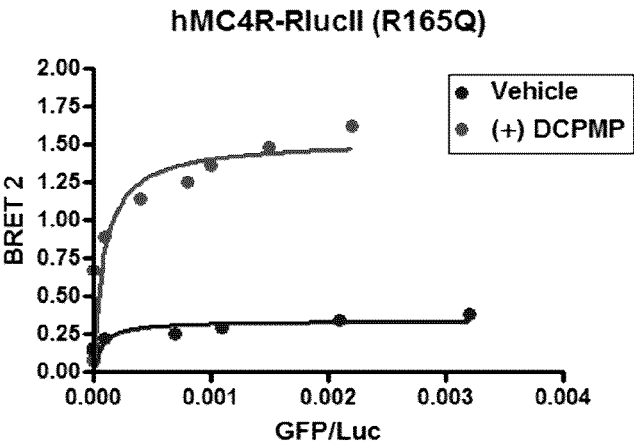


FIG. 12A

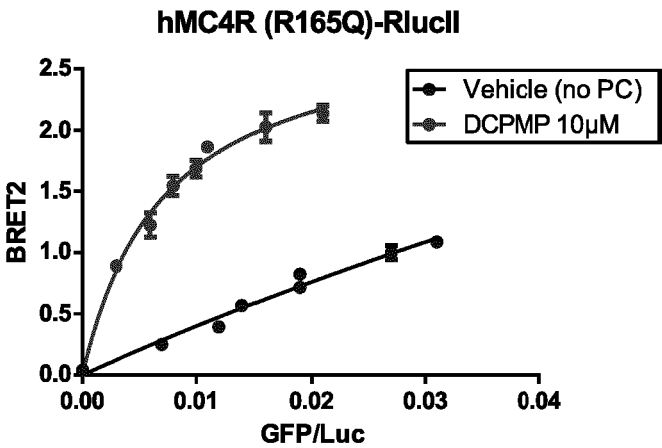


FIG. 12B

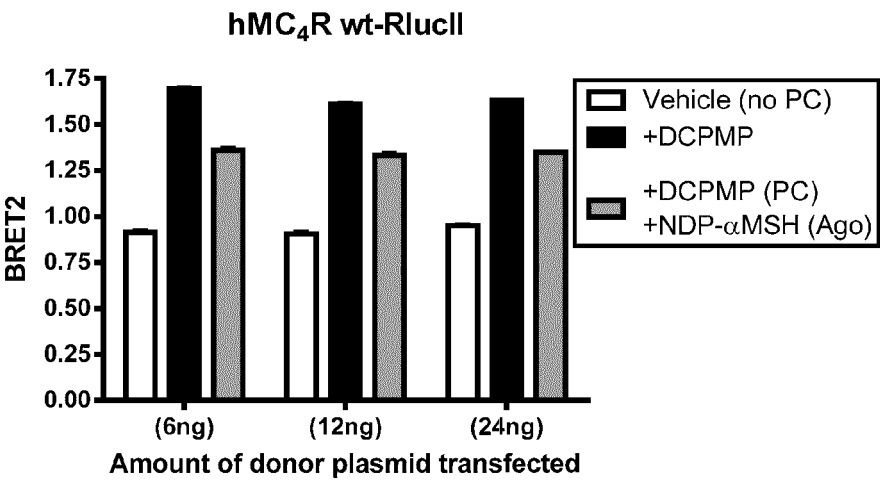


FIG. 13A

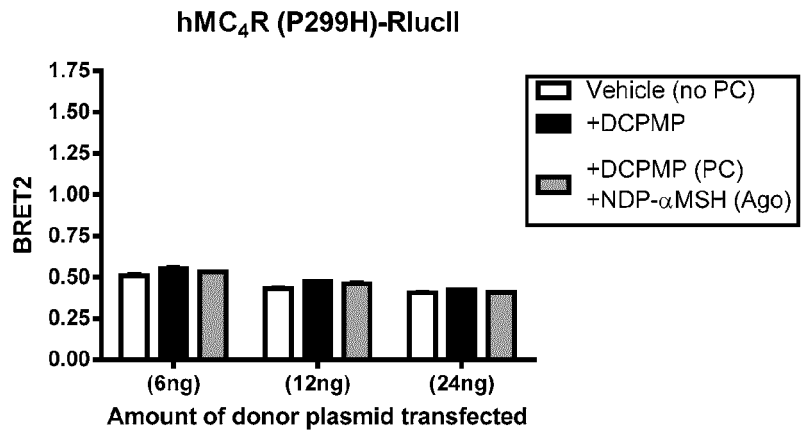


FIG. 13B

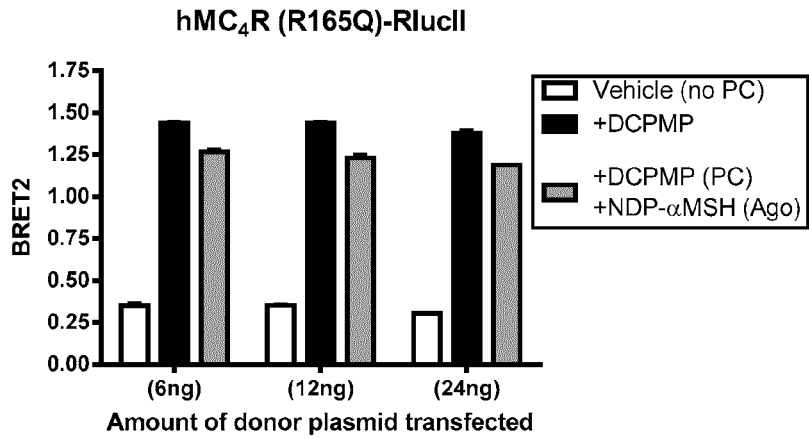


FIG. 13C

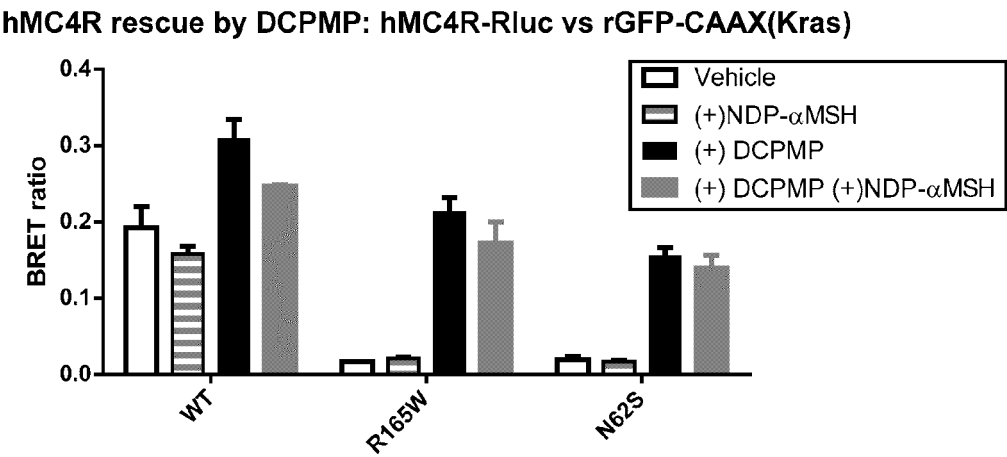


FIG. 13D

hV2R rescue by SR121463: hV2R-Rluc vs rGFP-CAAX(Kras)

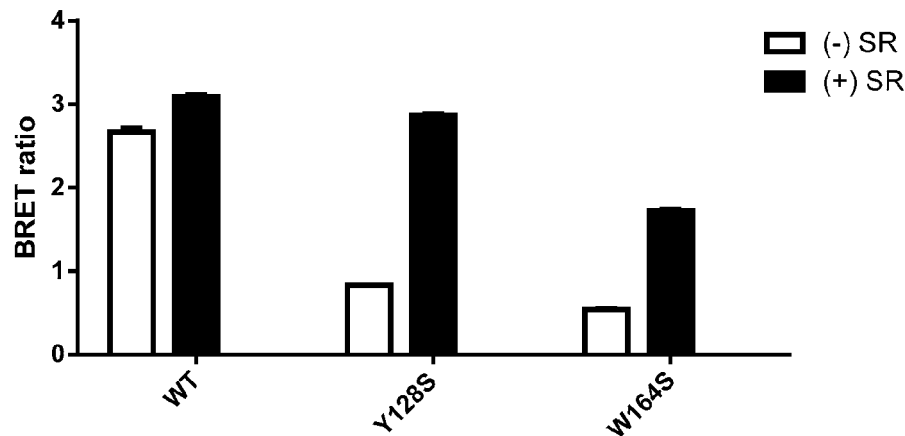
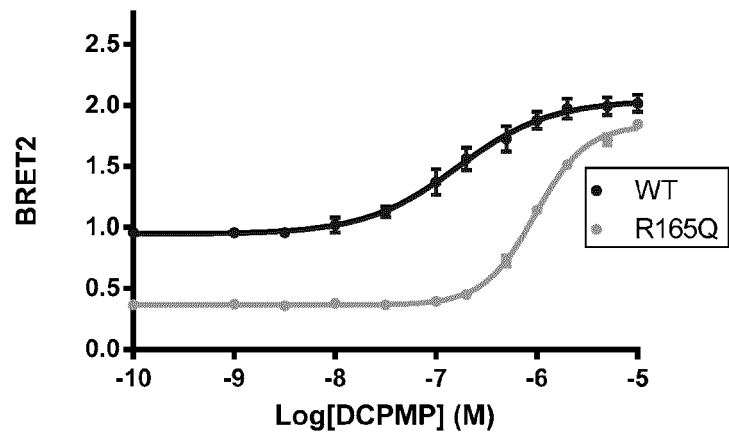


FIG. 13E

Dose-response-curves: DCPMP



	WT	R165Q
Sigmoidal dose-response (variable slope)		
Best-fit values		
Bottom	0.9454	0.3654
Top	2.042	1.840
LogEC50	-6.784	-6.018
HillSlope	0.9448	1.708
EC50	1.644e-007	9.590e-007

FIG. 14A

Dose-response-curves: Cmpd1

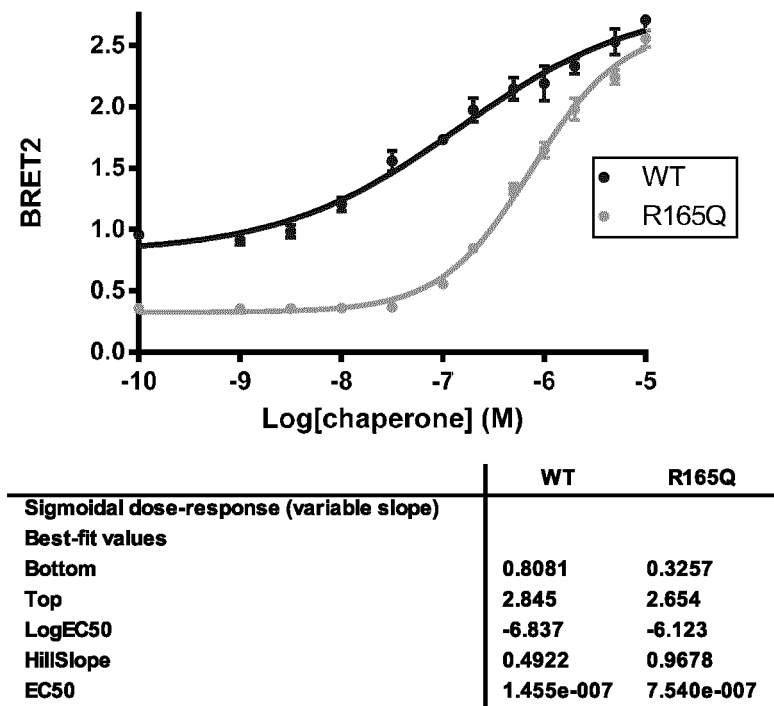


FIG. 14B

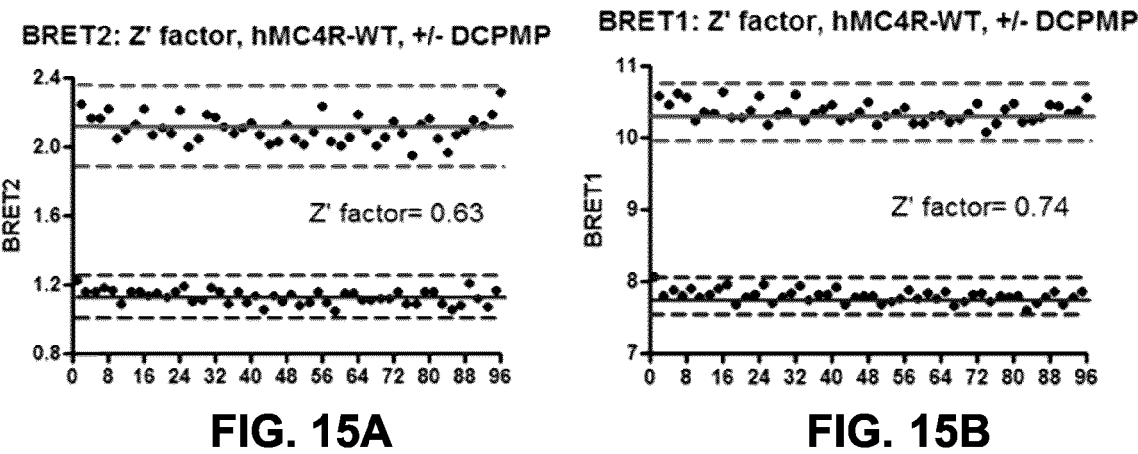
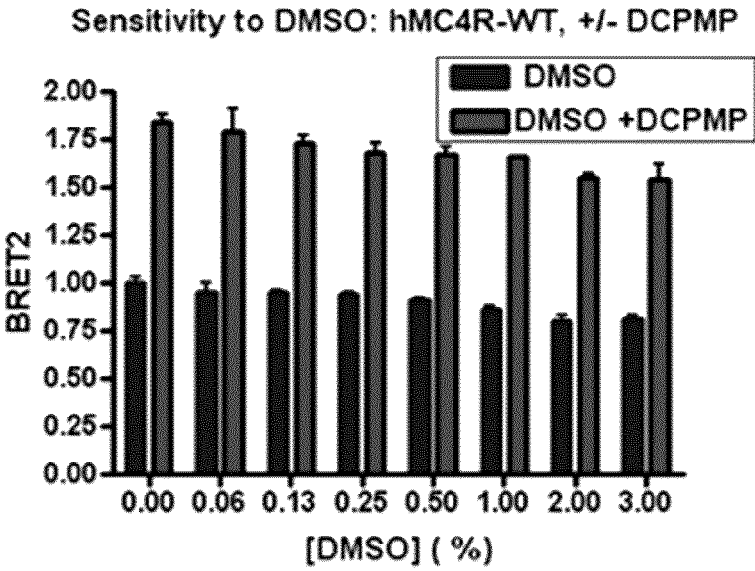
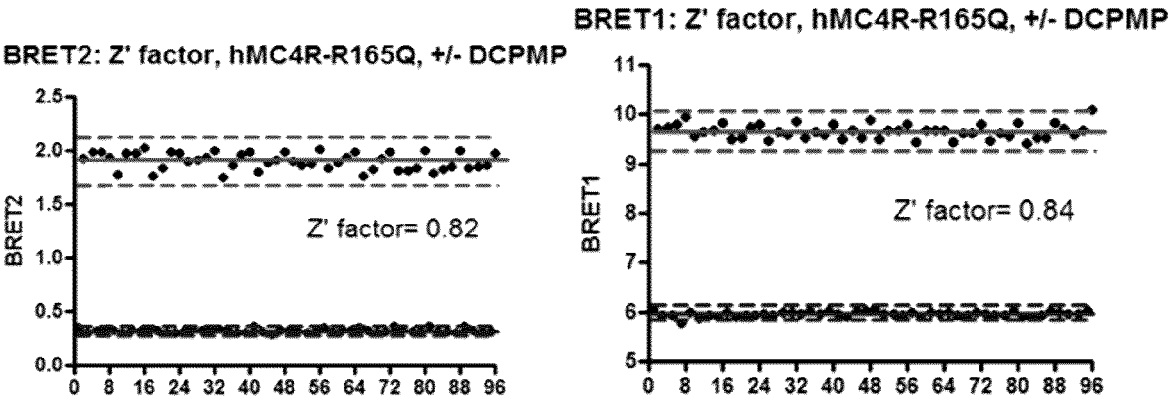


FIG. 15A

FIG. 15B



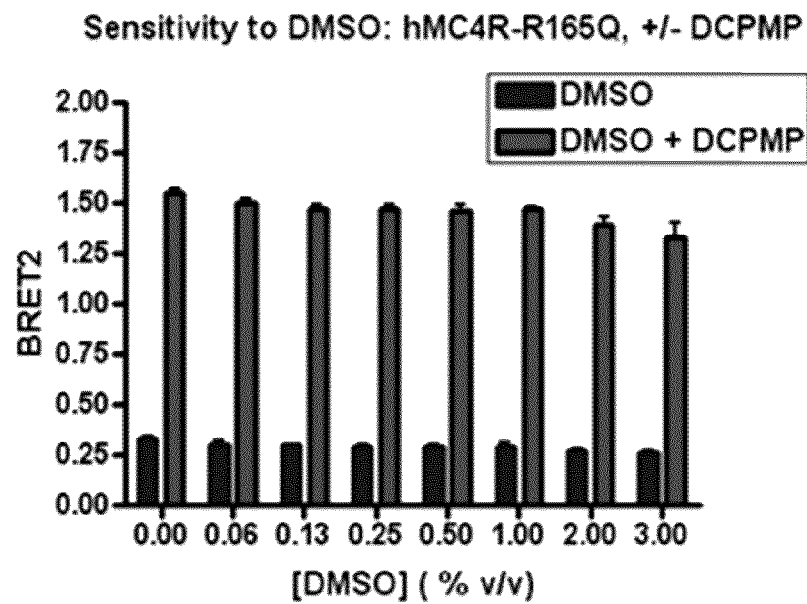


FIG. 16B

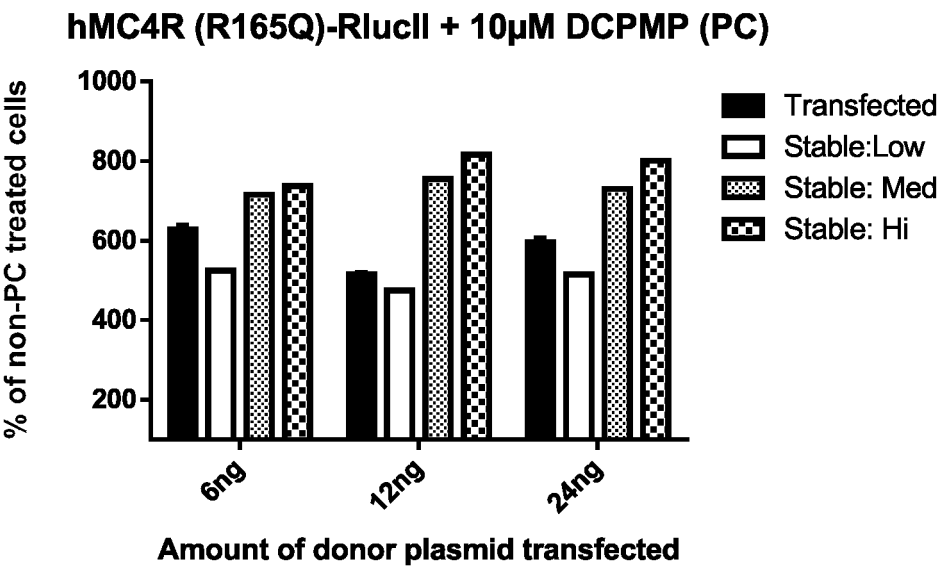


FIG. 17A

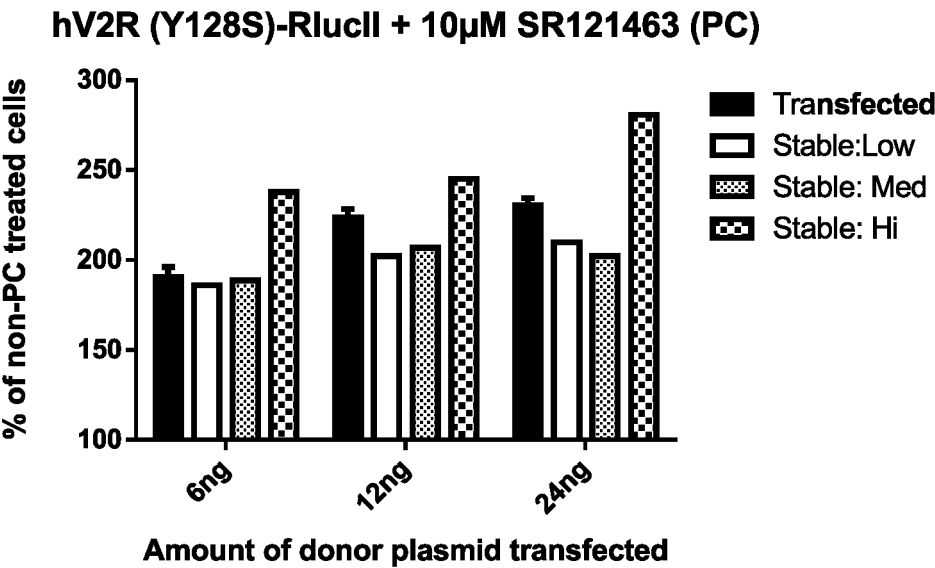


FIG. 17B

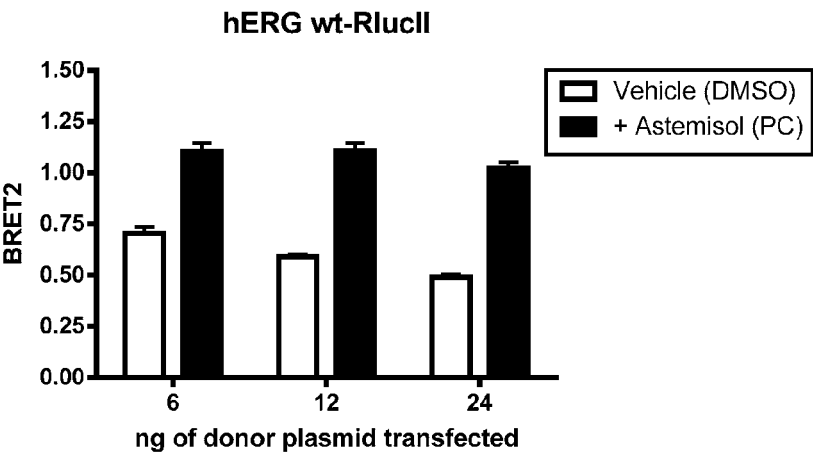


FIG. 18A

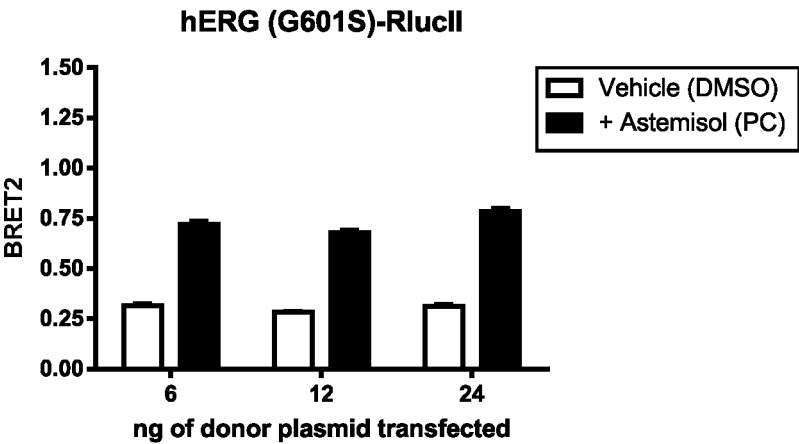
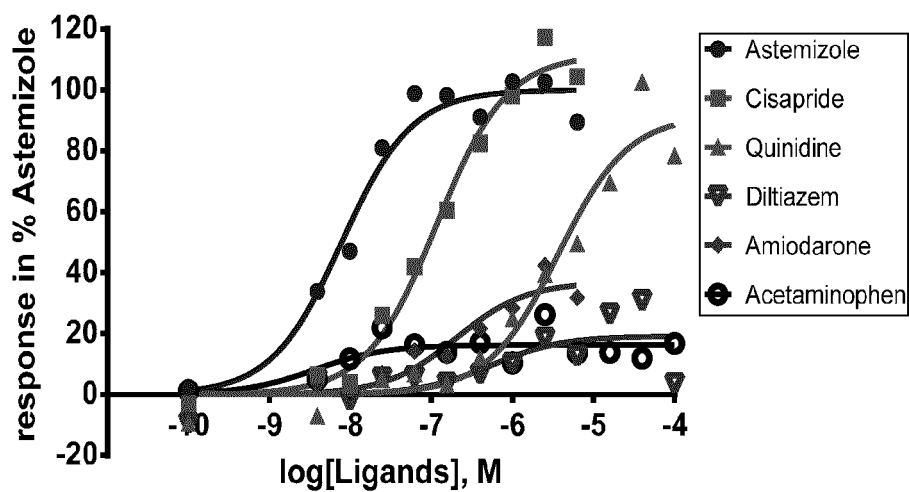
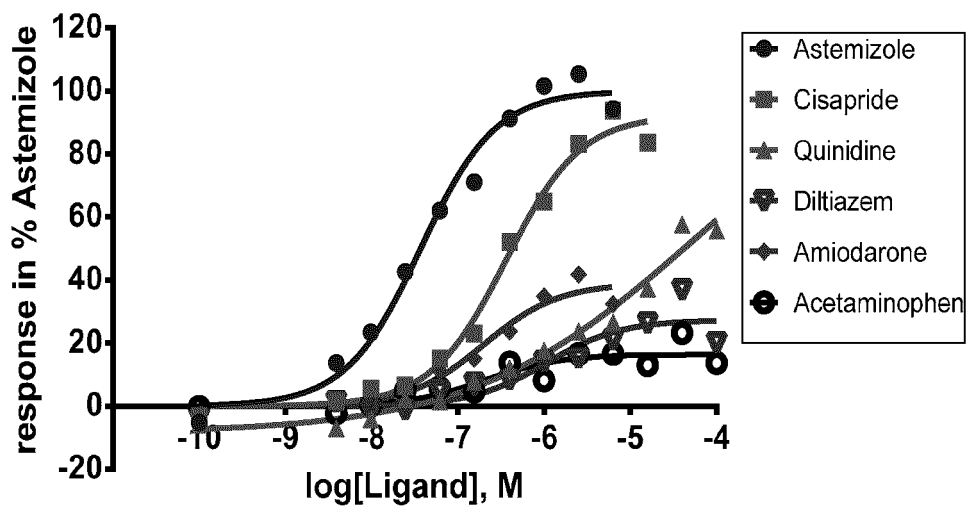


FIG. 18B

Dose-response: hERG-WT-RlucII**FIG. 18C****Dose-response: hERG-(G601S)-RlucII****FIG. 18D**

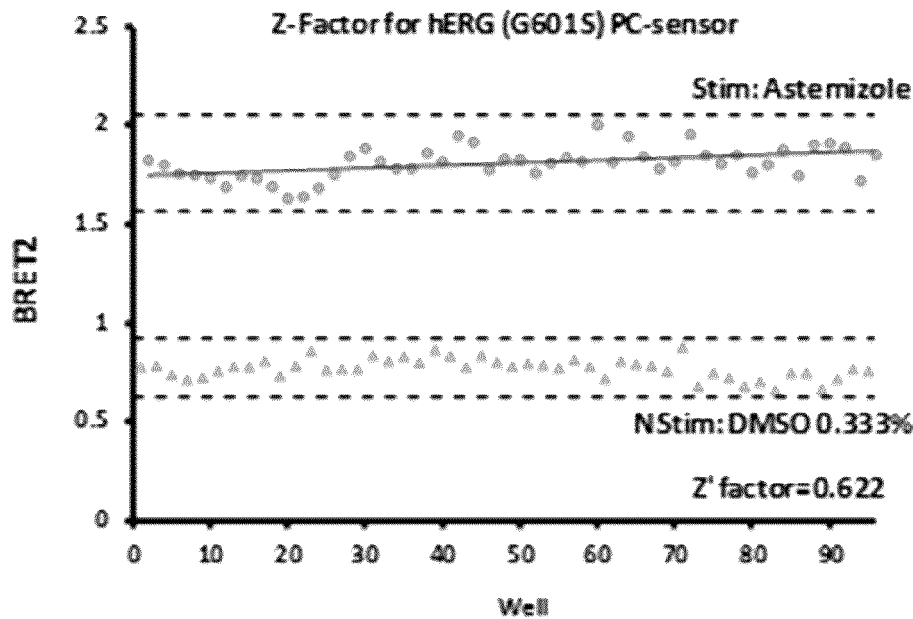


FIG. 18E

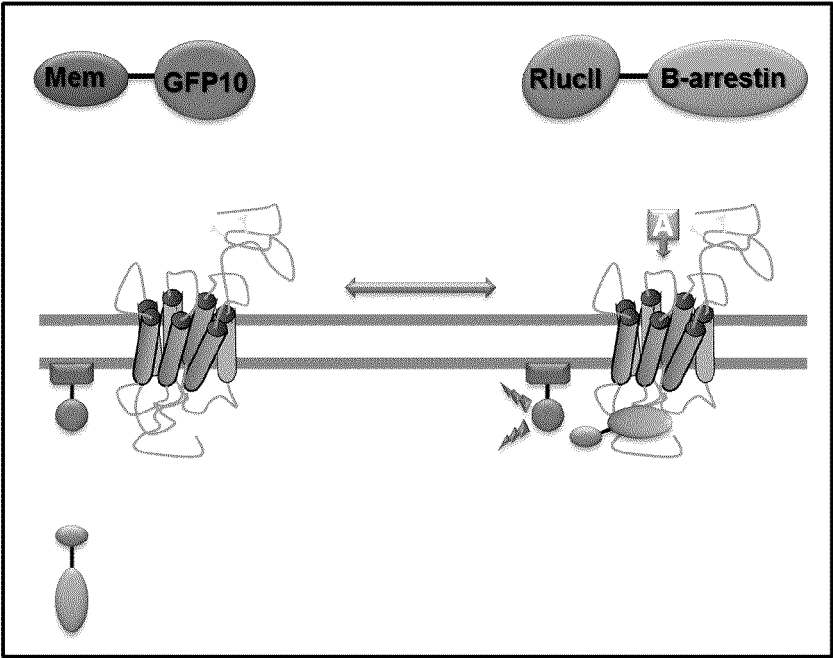
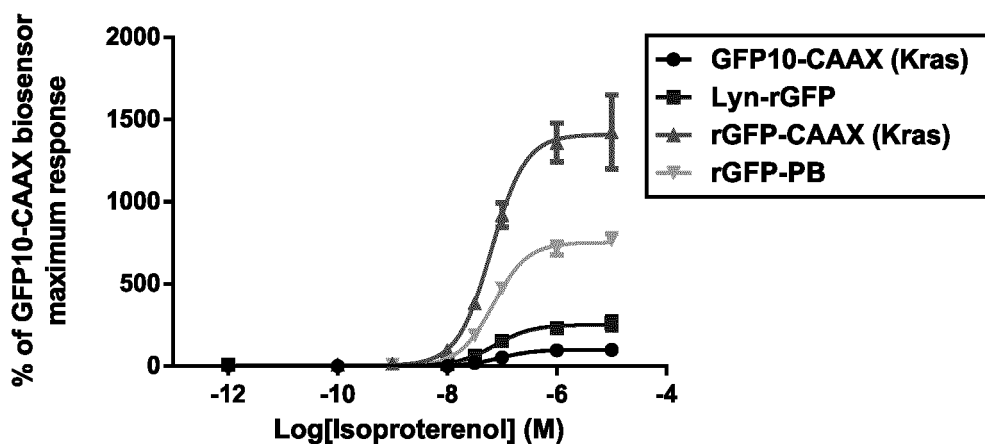
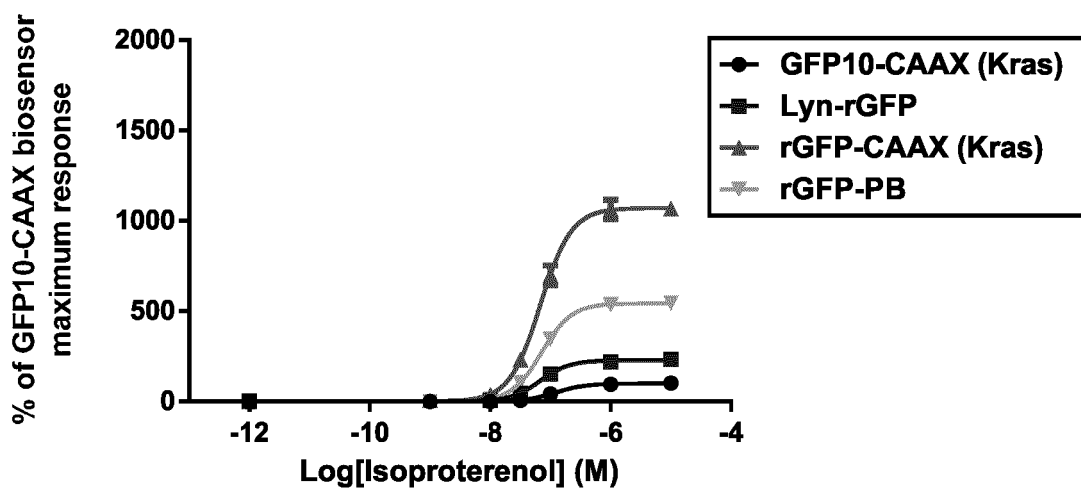


FIG. 19A

DR: β arrestin₁ recruitment to β_2 AR**FIG. 19B****DR: β arrestin₂ recruitment to β_2 AR****FIG. 19C**

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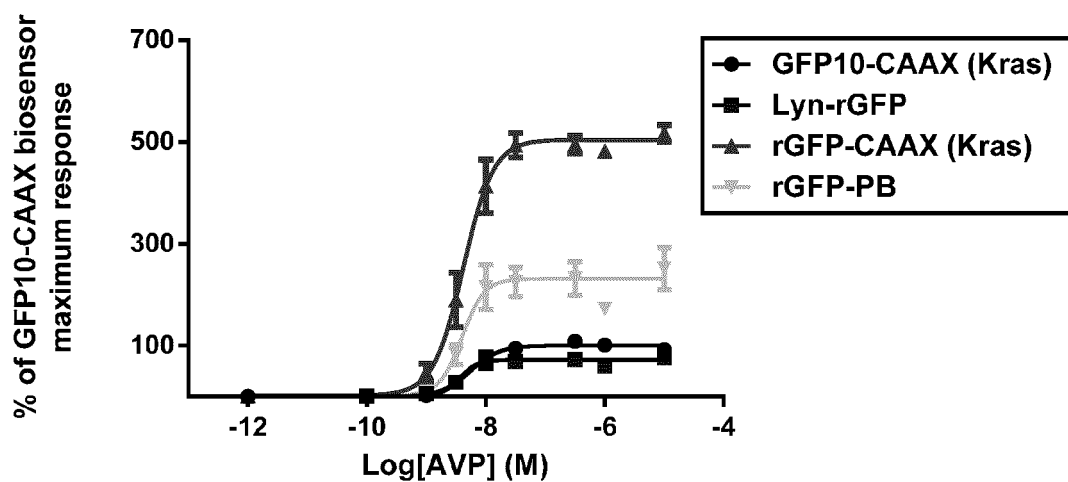
DR: β arrestin₁ recruitment to V₂R

FIG. 19D

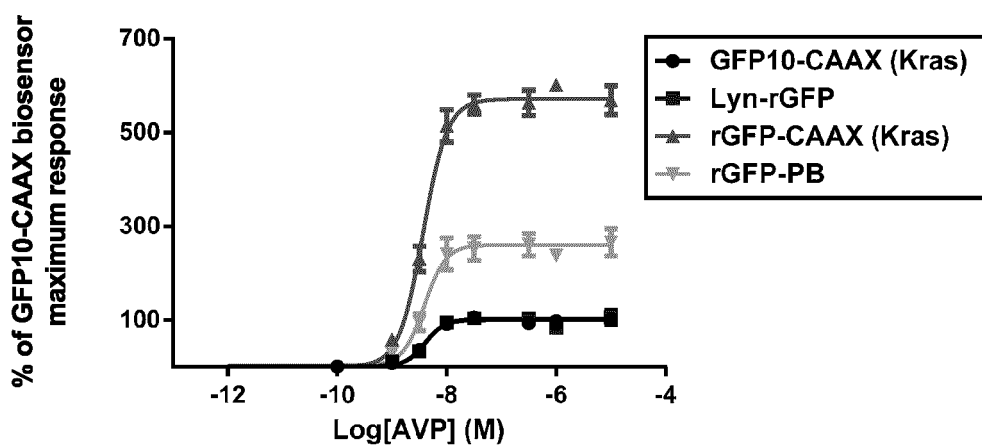
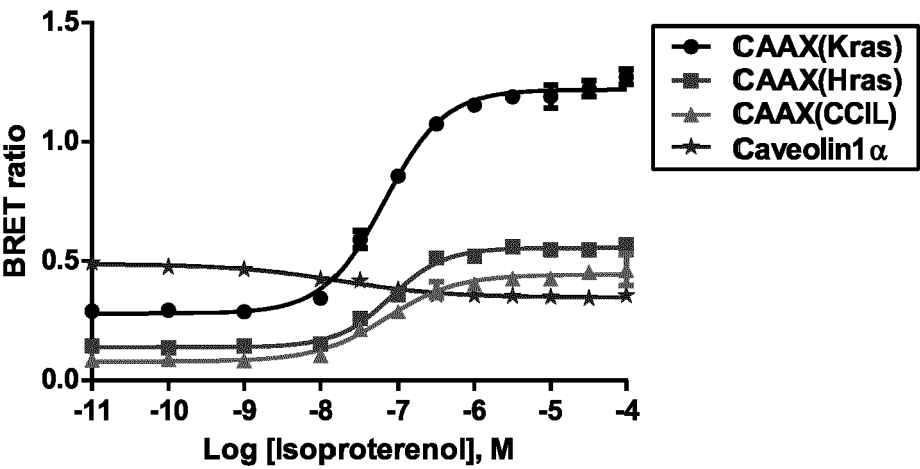
 β arrestin₂ recruitment to V₂R

FIG. 19E

DR: β 2AR, β -arrestin2 recruitment to rGFP-CAAX/Cav1 α



	CAAX(Kras)	CAAX(CCIL)	CAAX(Hras)	Caveolin1 α
LogEC50	-7.179	-7.155	-7.103	-7.767

FIG. 19F

AT1R
 β arr2-RlucII

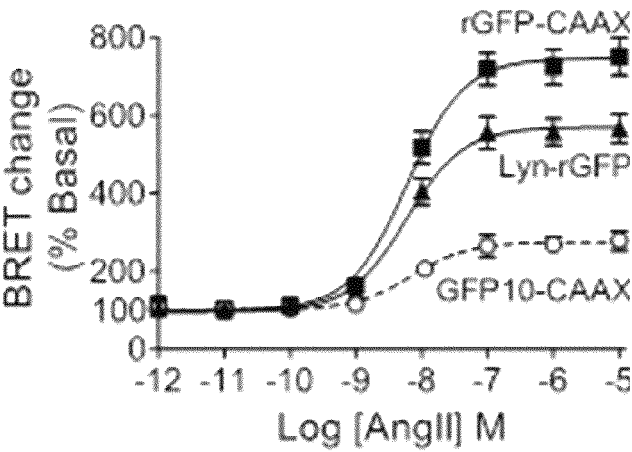
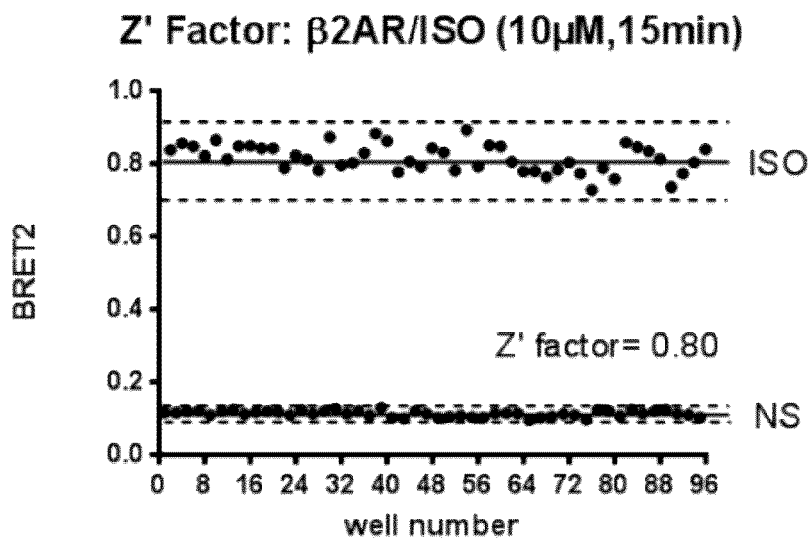
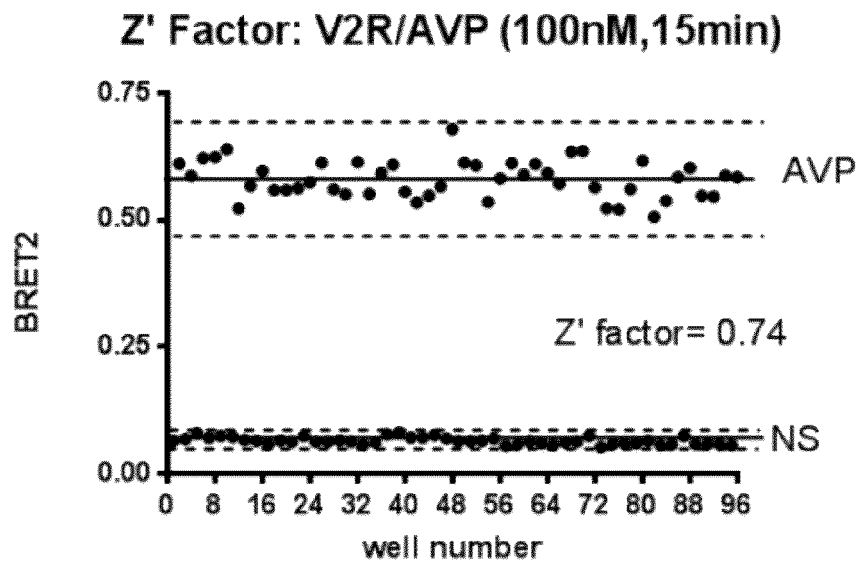
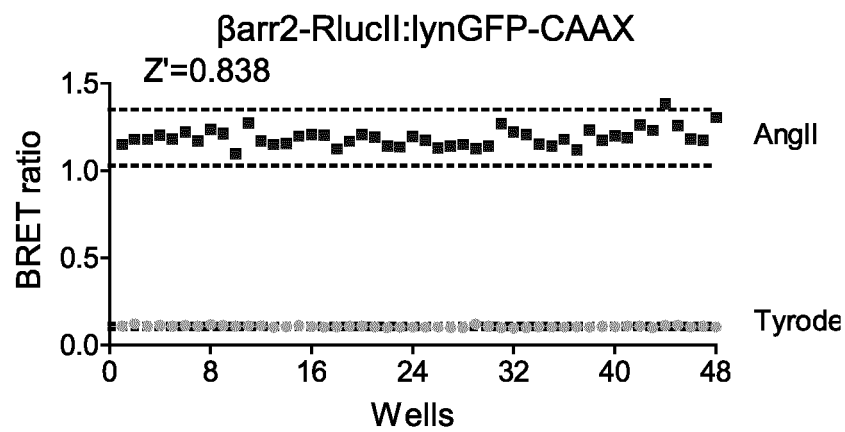
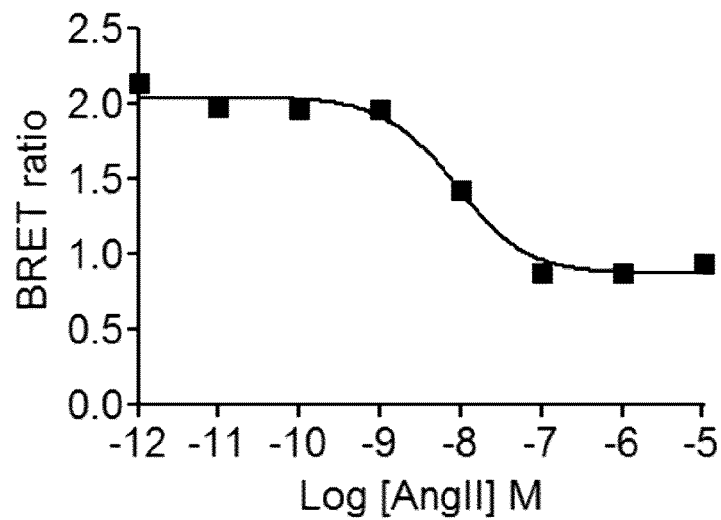
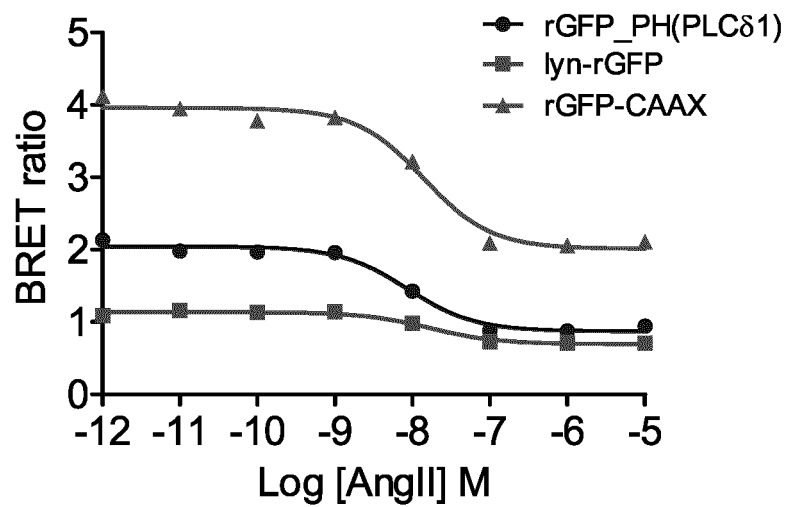


FIG. 19G

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Class A receptor: β 2AR**FIG. 19H****Class B receptor: V2R****FIG. 19I**

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**FIG. 19J****FIG. 20A****FIG. 20B**

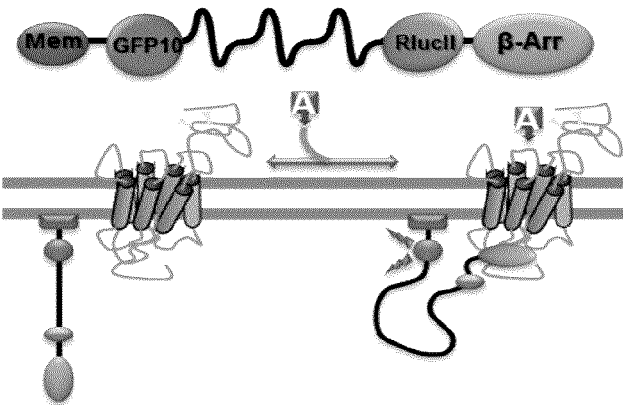


FIG. 21A

Opt:V2R, unimolecular-βarr2 recruitment sensor

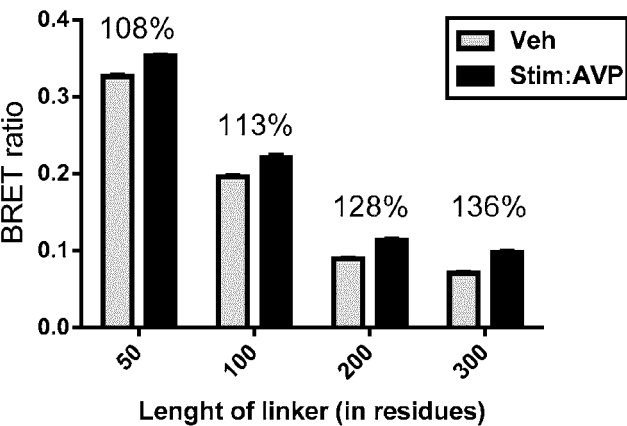


FIG. 21B

DR: At1AR, unimolecular-βarr2 recruitment sensor

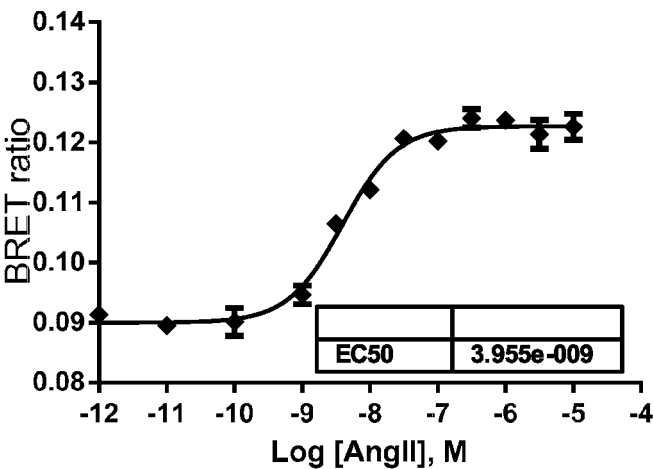


FIG. 21C

DR: V2R, unimolecular-βarr2 recruitment sensor

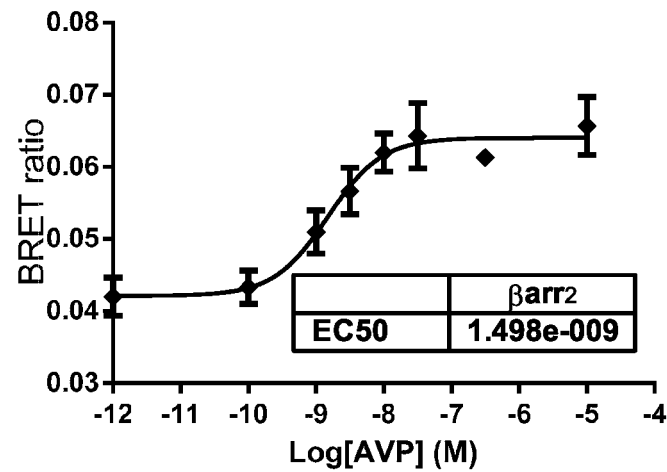


FIG. 21D

DR: β2AR, unimolecular-βarr2 recruitment sensor

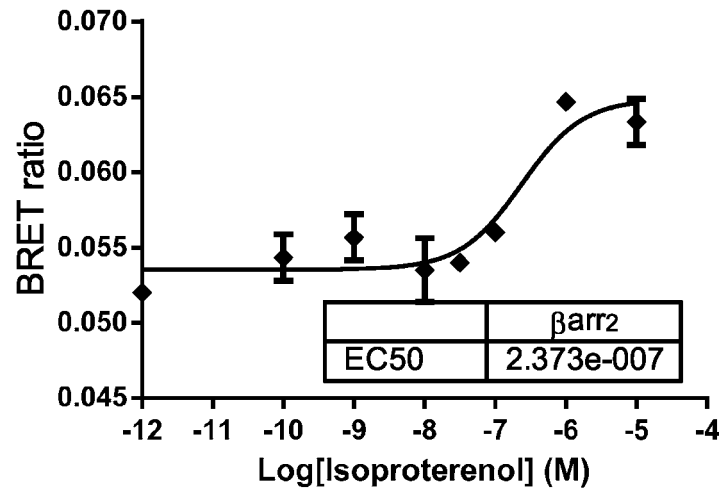


FIG. 21E

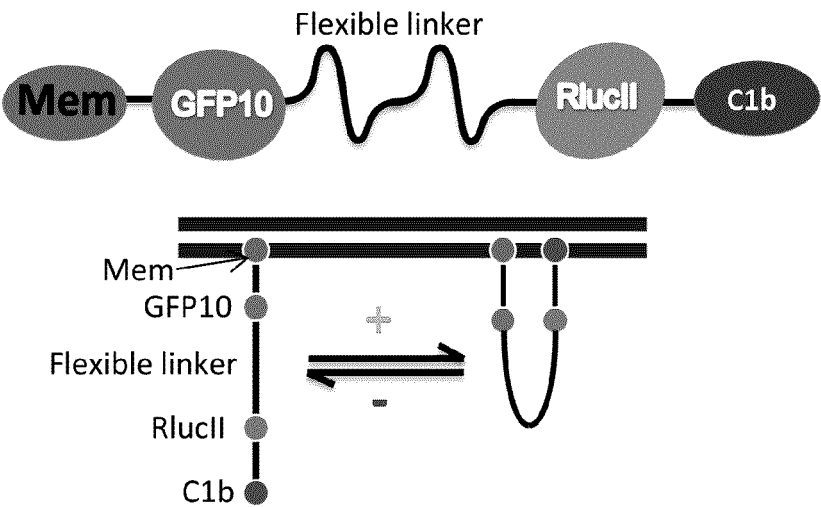


FIG. 22A

Kin: At1AR, unimolecular DAG sensor

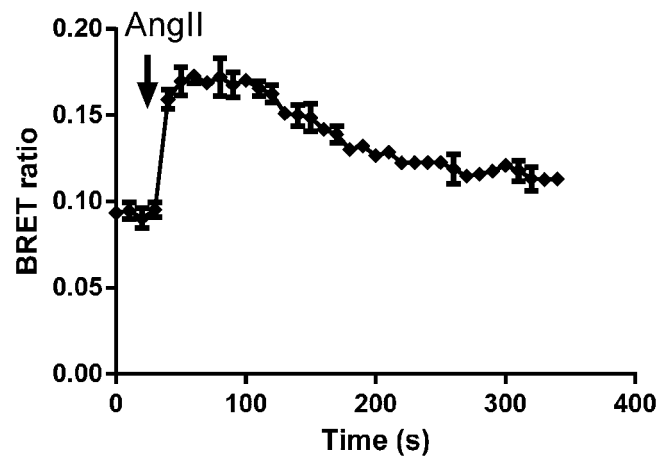


FIG. 22B

DR: At1AR, unimolecular-DAG sensor

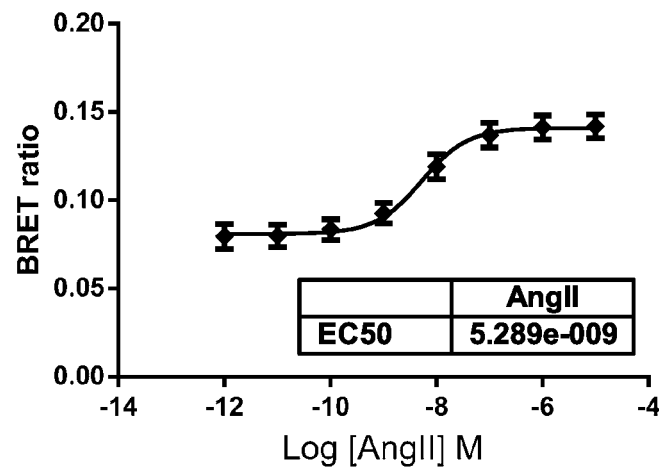


FIG. 22C

DR: FP, unimolecular DAG sensor

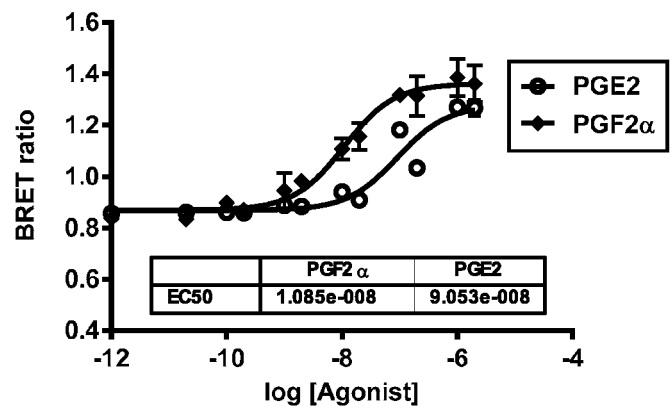


FIG. 22D

DR: GPR14, unimolecular DAG sensor

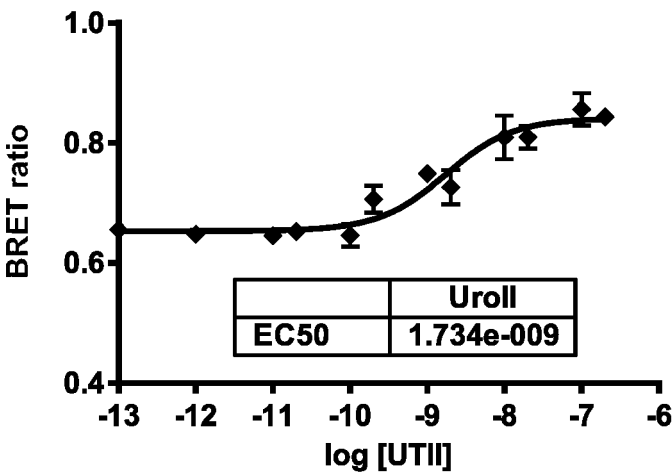


FIG. 22E

Kin: PLC activator, unimolecular DAG sensor

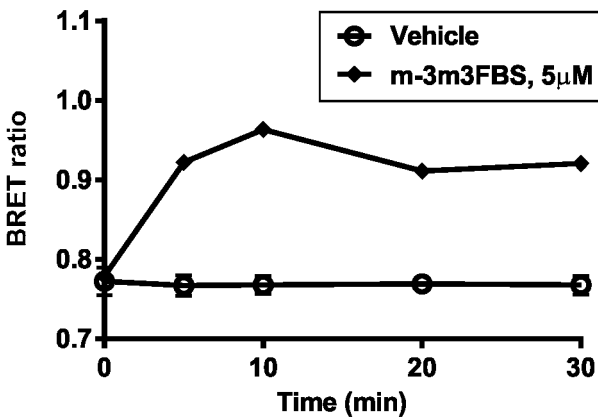


FIG. 22F

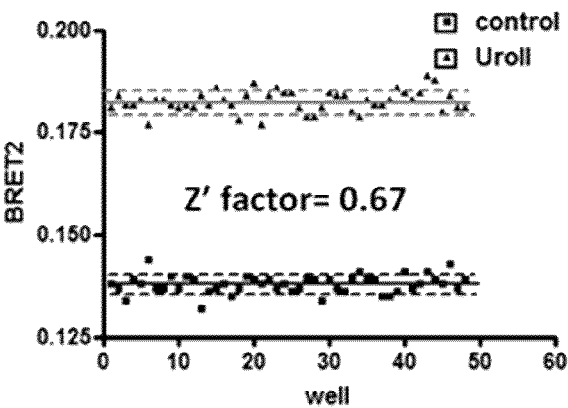


FIG. 22G

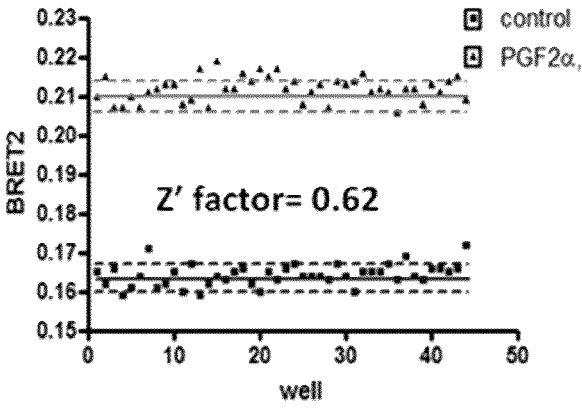


FIG. 22H

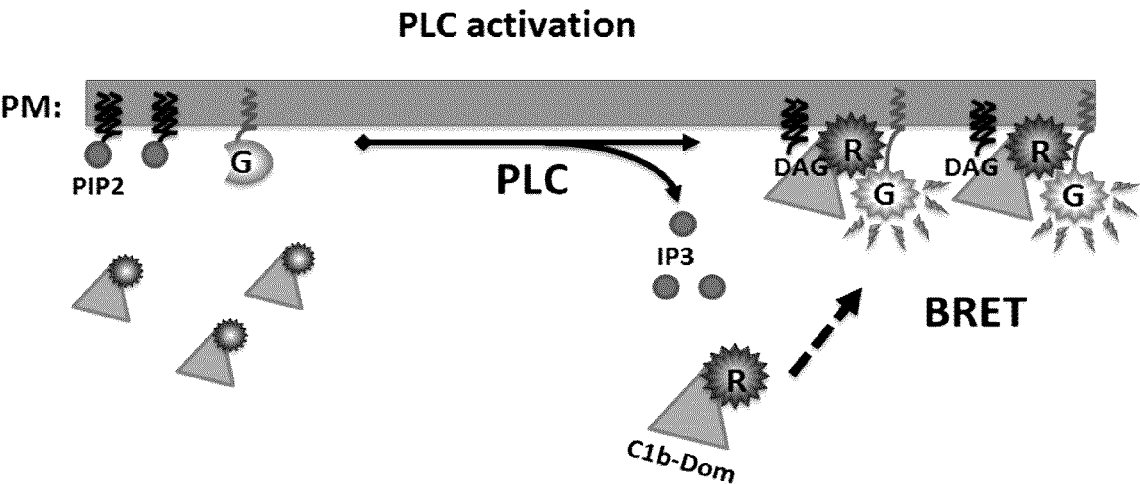


FIG. 23A

DR: H1R, DAG sensor (RlucII-C1b vs rGFP-CAAX(Kras))

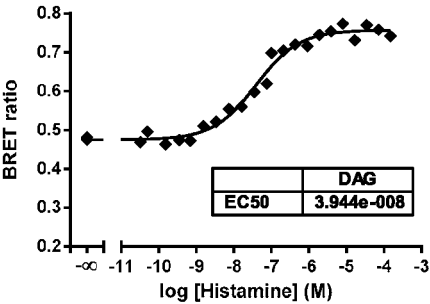


FIG. 23B

DR: BKRB2, DAG sensor (RlucII-C1b vs rGFP-CAAX(Kras))

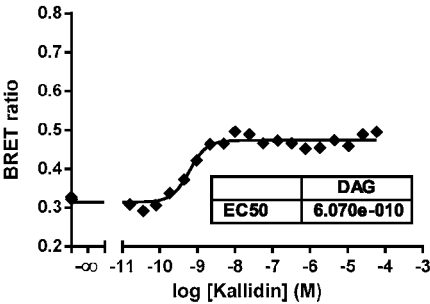


FIG. 23C

DR: D2R, DAG sensor (RlucII-C1b vs rGFP-CAAX(Kras))

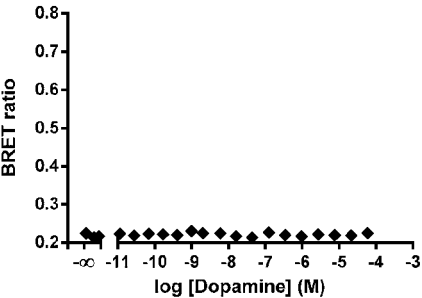


FIG. 23D

DR: β 2AR, DAG sensor (RlucII-C1b vs rGFP-CAAX(Kras))

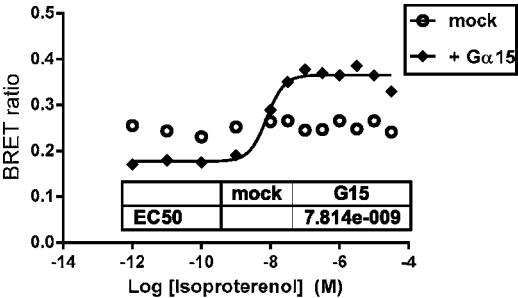
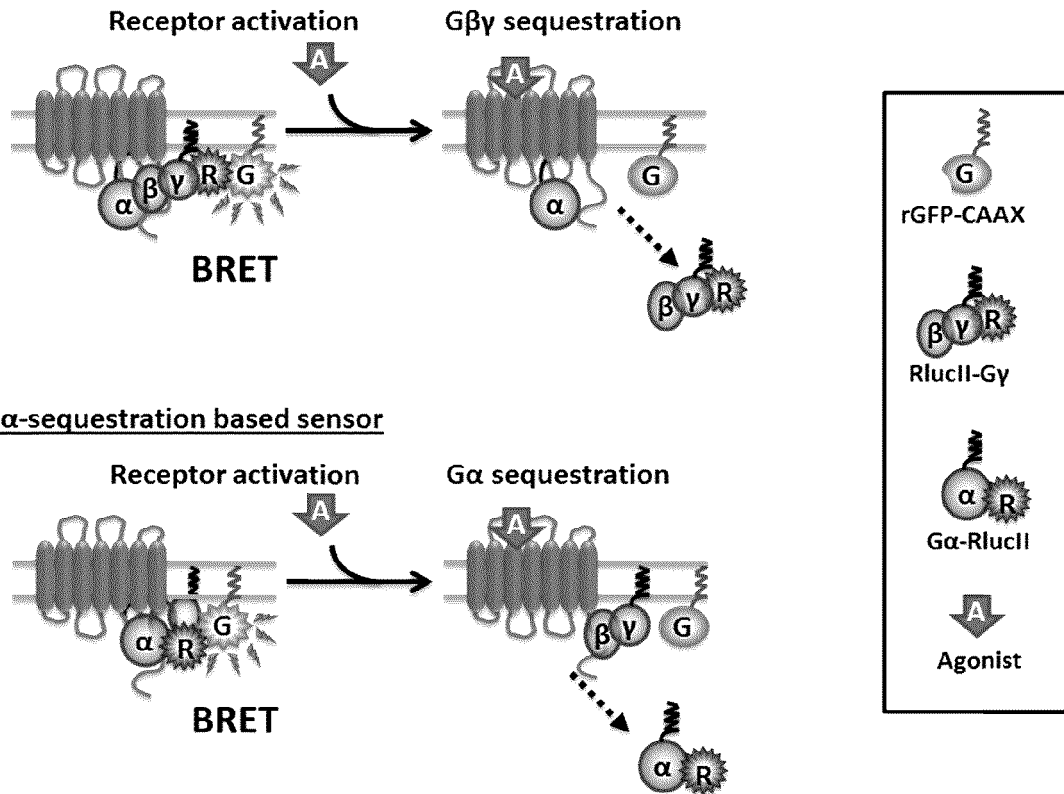
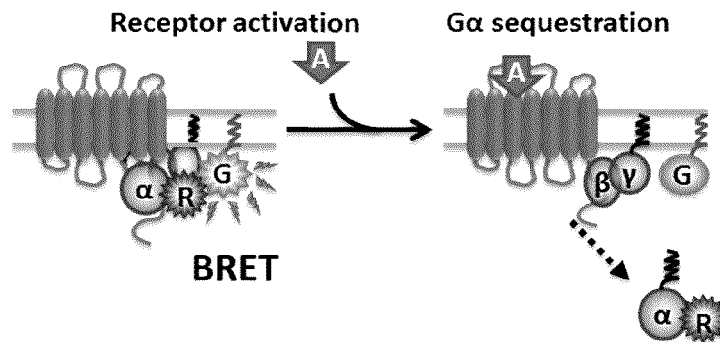
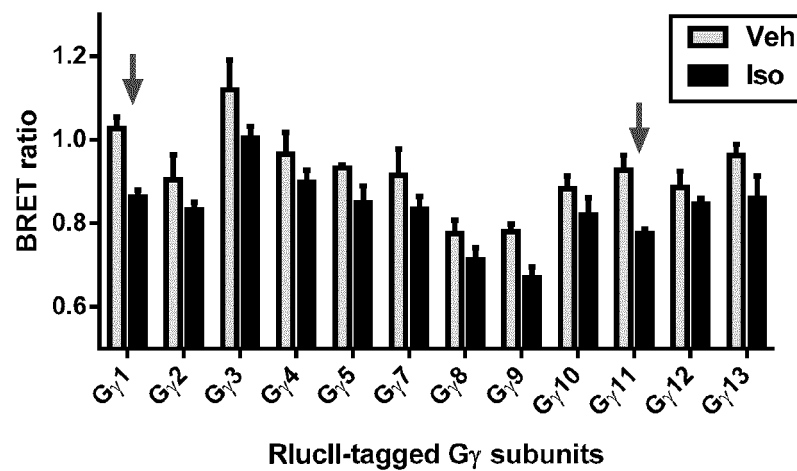


FIG. 23E

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G $\beta\gamma$ -sequestration based sensorG α -sequestration based sensor**FIG. 24A****Sequestration of G γ -RlucII from rGFP-CAAX (Kras) in response to β 1AR stimulation****FIG. 24B**

Sequestration of $G\gamma$ -RlucII from rGFP-CAAX (Kras)
in response to β 2AR stimulation

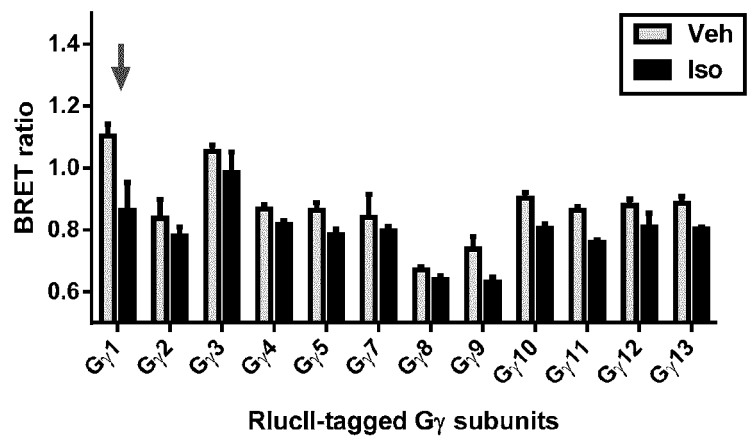
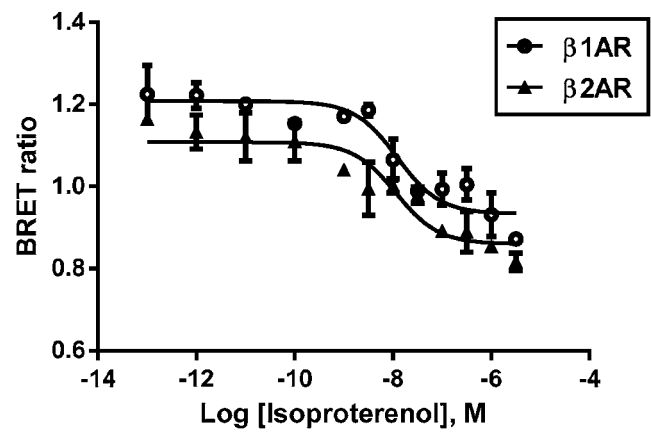


FIG. 24C

DR: agonist-promoted RlucII- $G\gamma$ 1 sequestration
from rGFP-CAAX(Kras)



	β 1AR	β 2AR
LogEC50	-7.911	-7.936

FIG. 24D

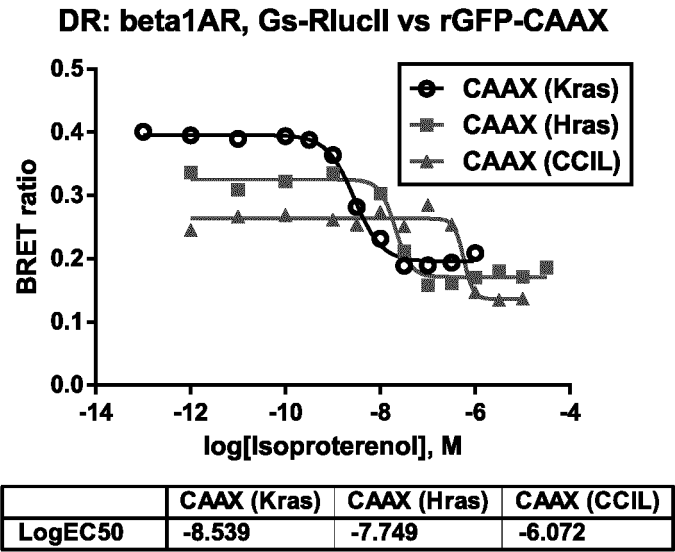


FIG. 24E

Kinetics:beta1AR/iso 1μM, Gs-RlucII vs rGFP-CAAX

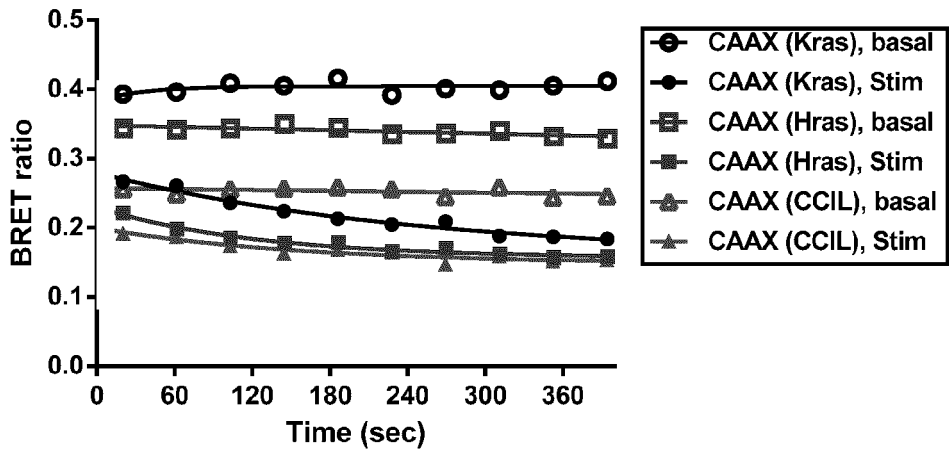
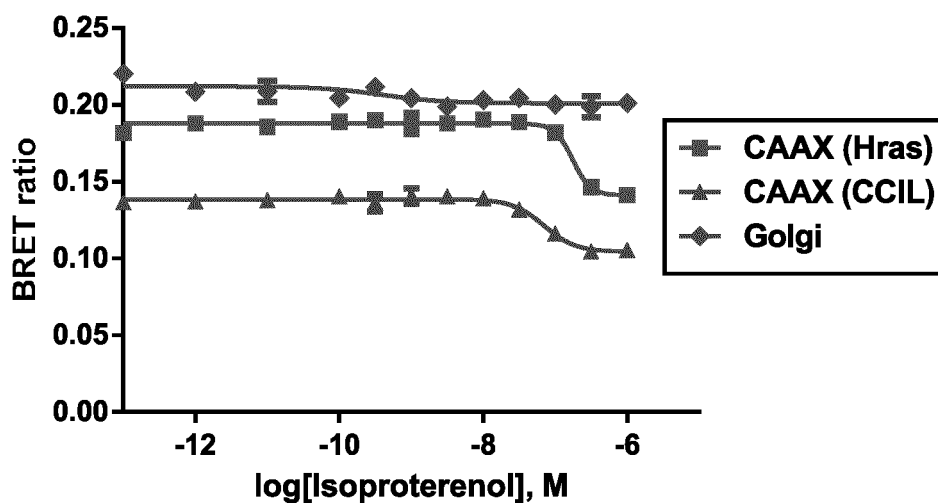
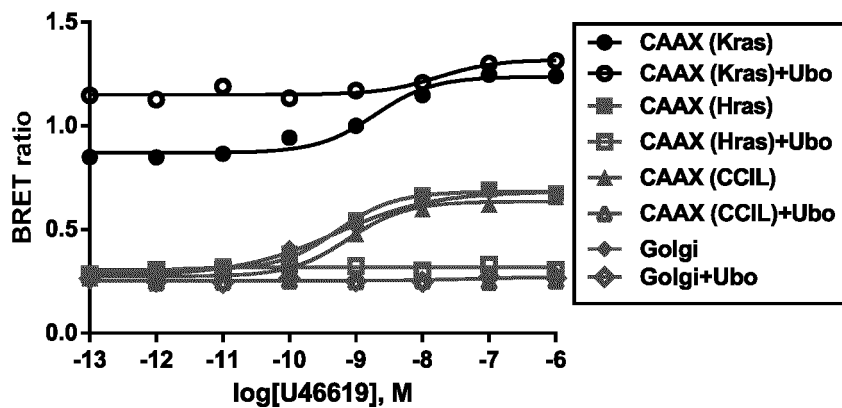


FIG. 24F

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DR: beta1AR, G12-RlucII vs rGFP-CAAX/Golgi

	CAAX (Hras)	CAAX (CCIL)	Golgi
LogEC50	-6.441	-7.033	-9.396

FIG. 24G**DR: TPalphaR, Gq-RlucII vs rGFP-CAAX/Golgi**

	CAAX (Kras)	CAAX (Hras)	CAAX (CCIL)	Golgi
LogEC50	-8.712	-9.281	-9.115	-9.295

FIG. 24H

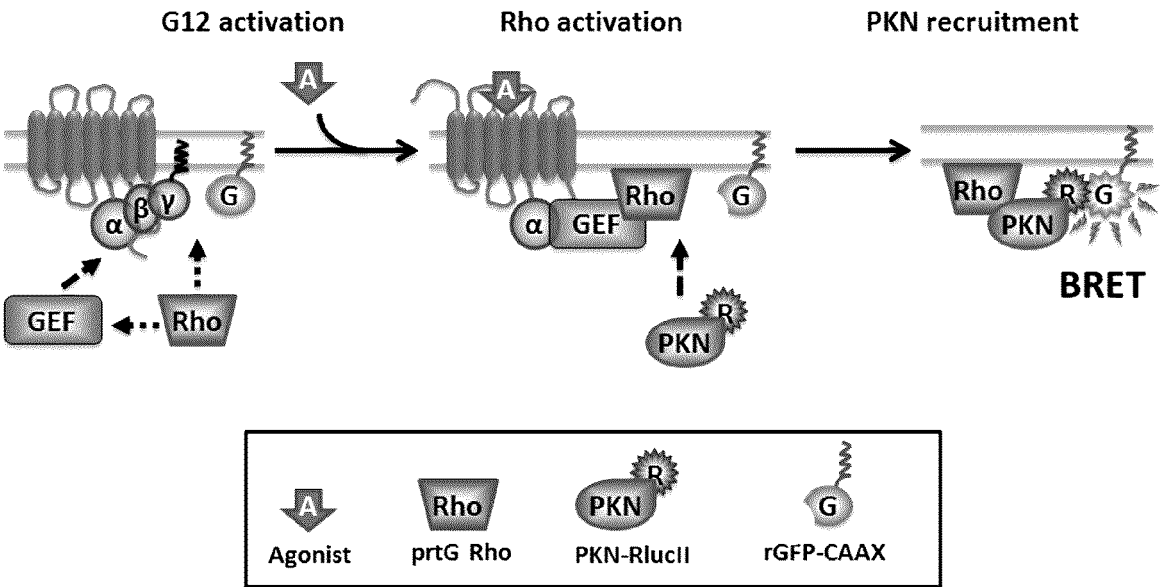
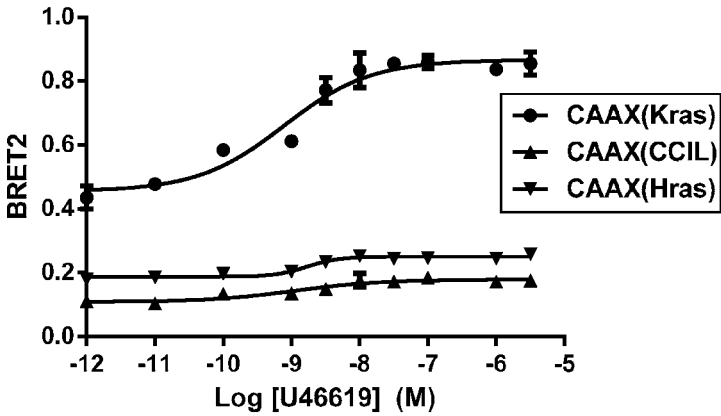


FIG. 25A

DR: for TP α R with PKN-RlucII vs rGFP-CAAX



	CAAX(Kras)	CAAX(CCIL)	CAAX(Hras)
LogEC50	-9.107	-8.943	-8.773

FIG. 25B

Kinetics of PKN sensor's response to At1AR Stim

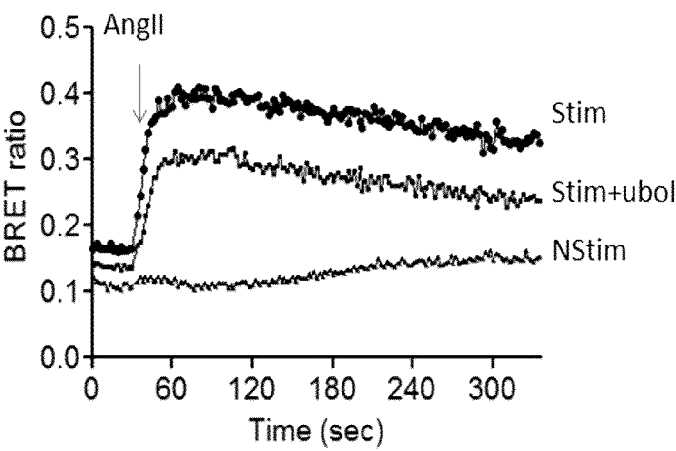


FIG. 25C

DR: At1AR, Rho activation sensor: PKN-RlucII vs rGFP-CAAX(Kras)

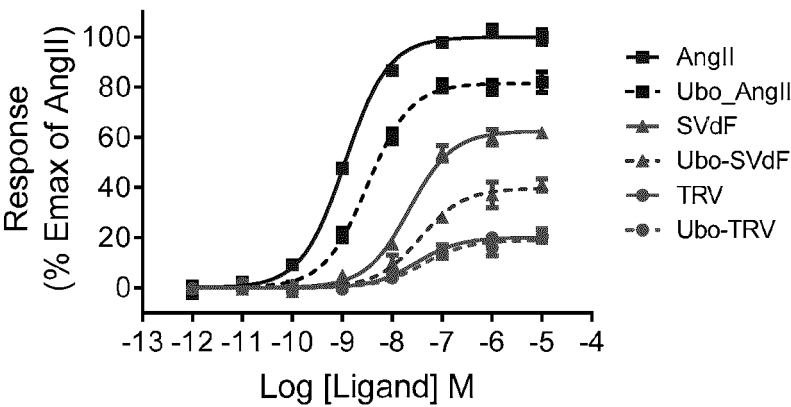


FIG. 25D

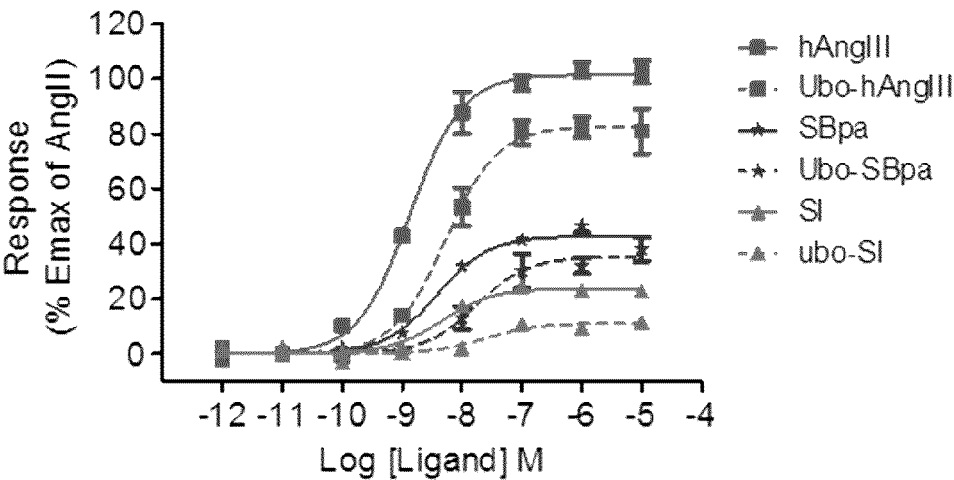


FIG. 25E

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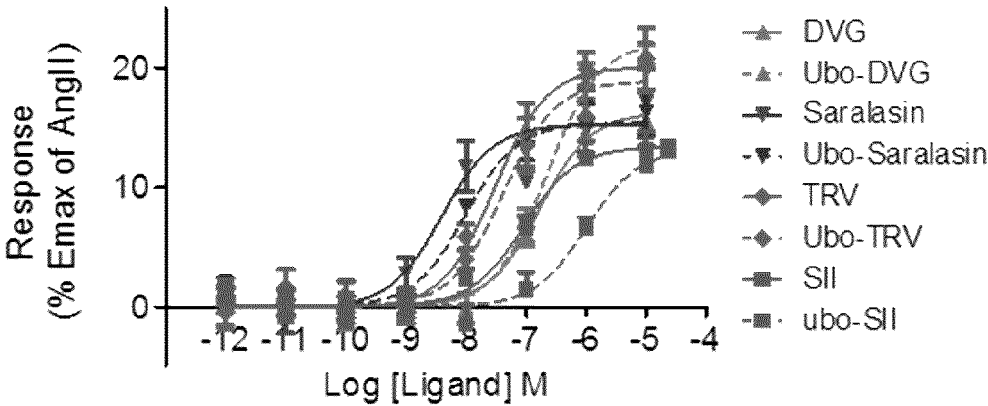


FIG. 25F

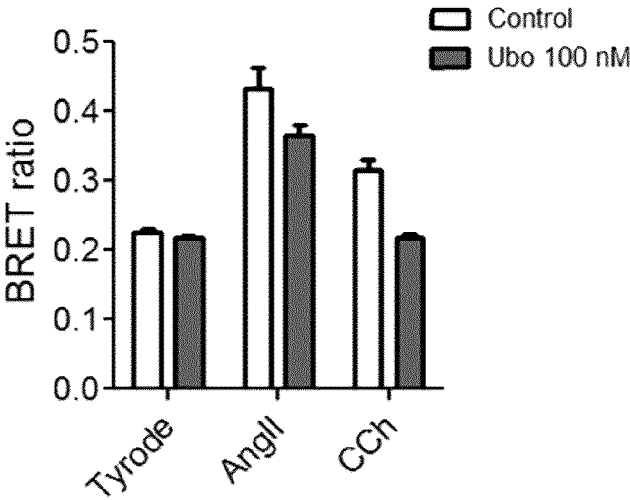


FIG. 25G

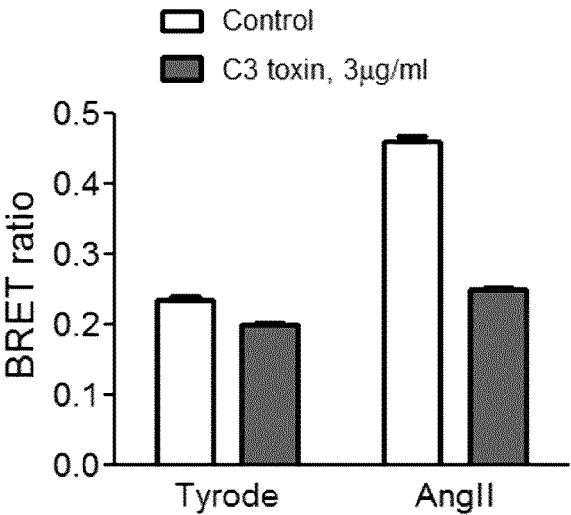


FIG. 25H

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Effect of Rho modulators on PKN sensor's response

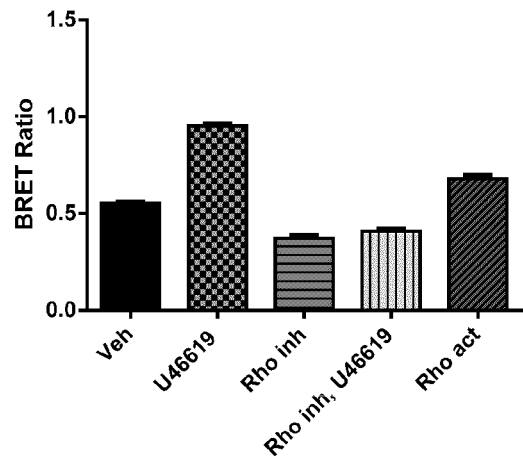
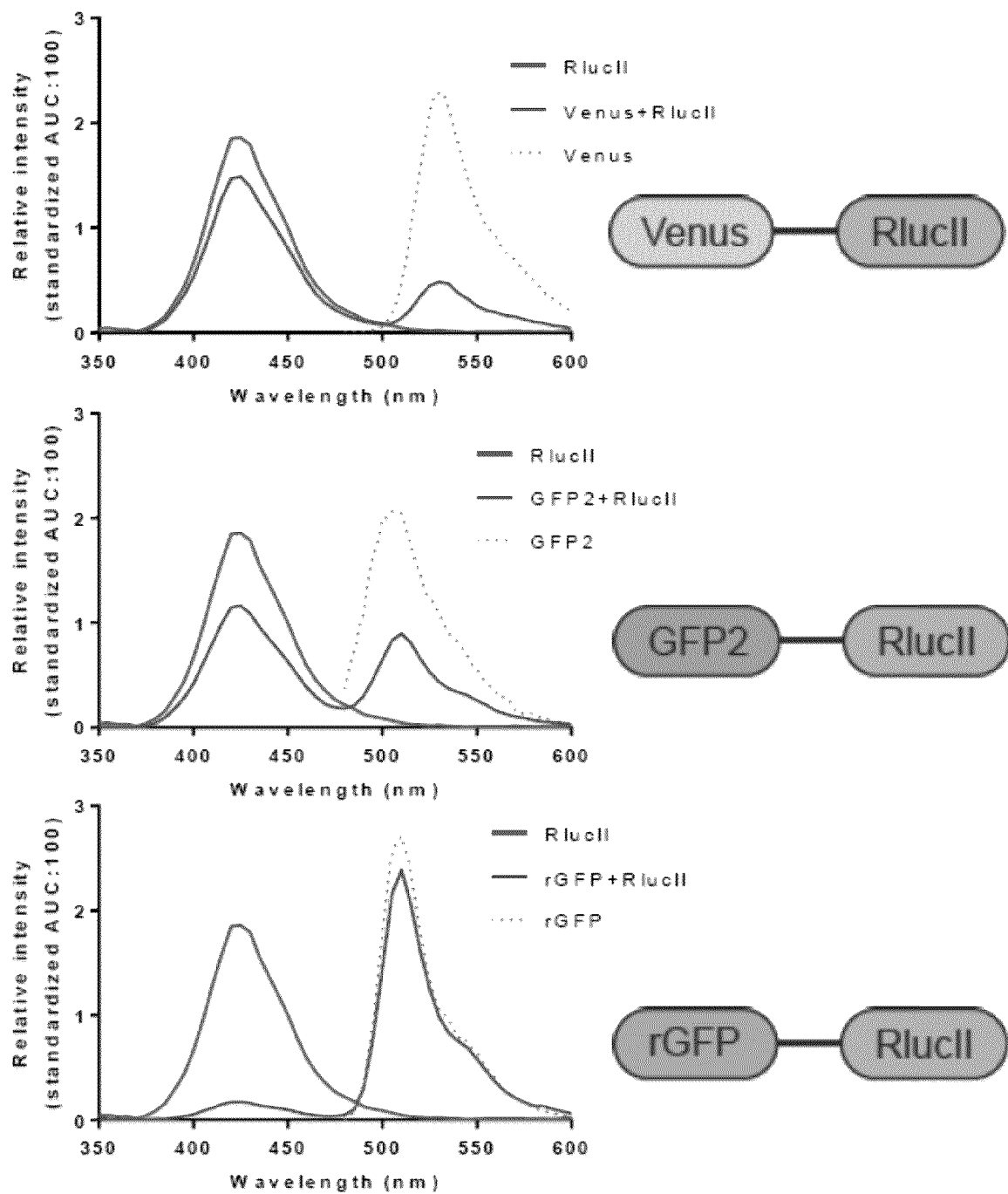


FIG. 25I

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**FIG. 26A**

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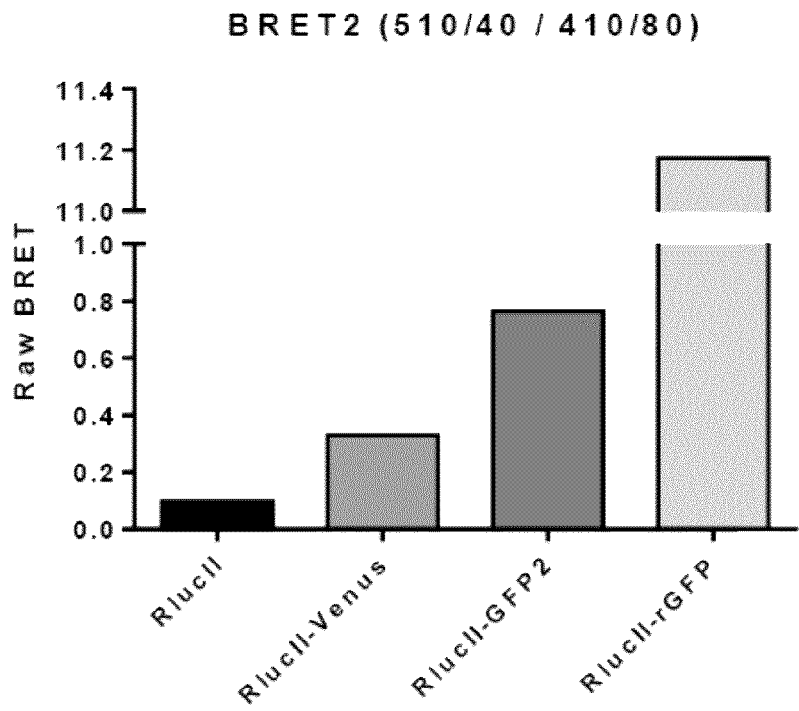


FIG. 26B

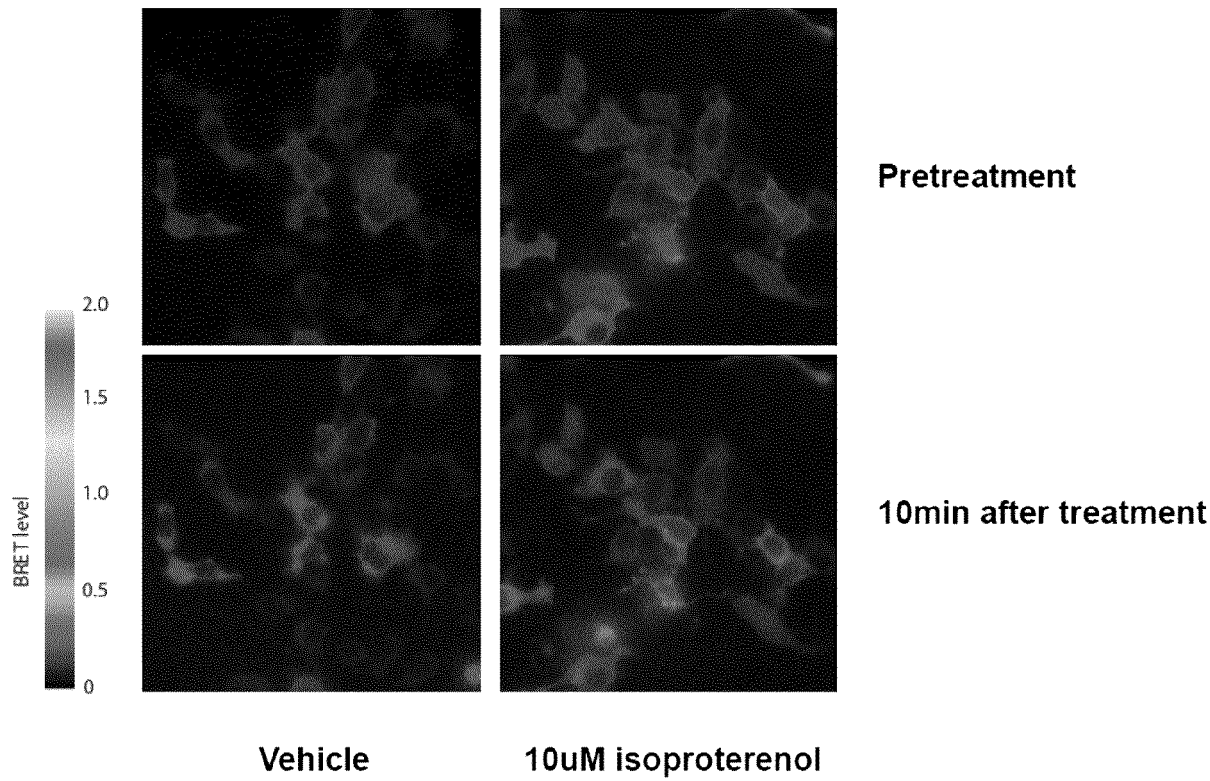


FIG. 26C

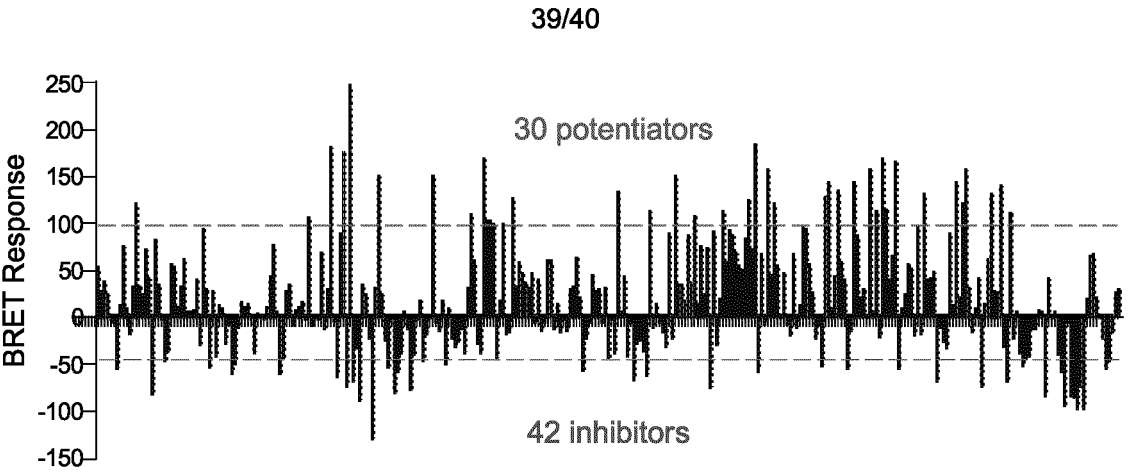


FIG. 27A

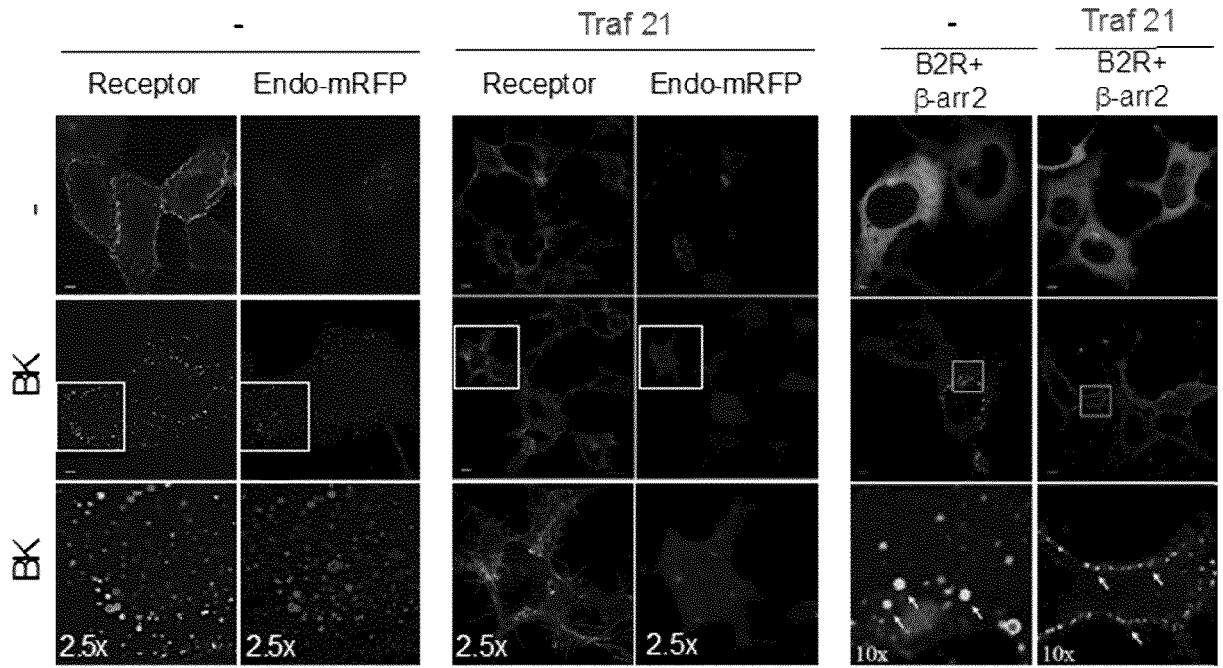


FIG. 27B

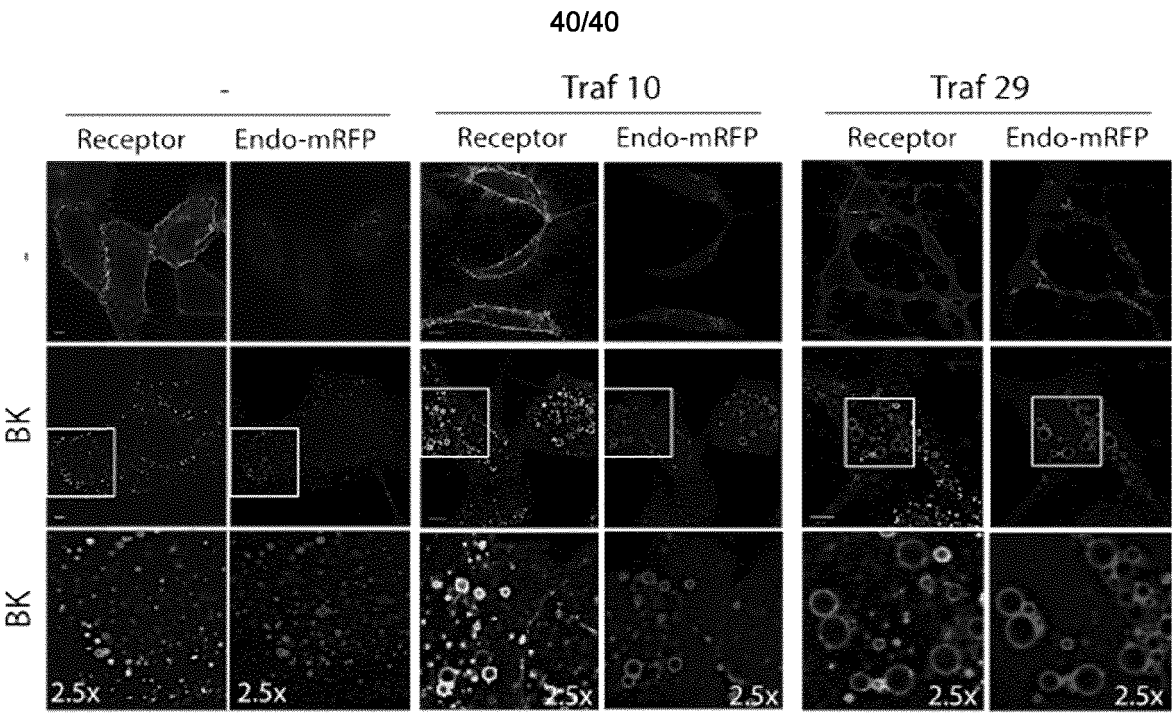


FIG. 27C