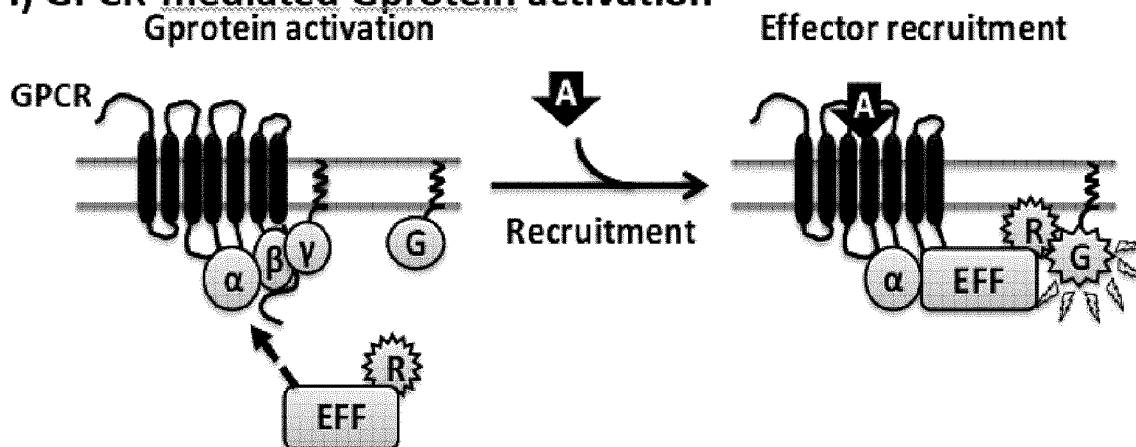
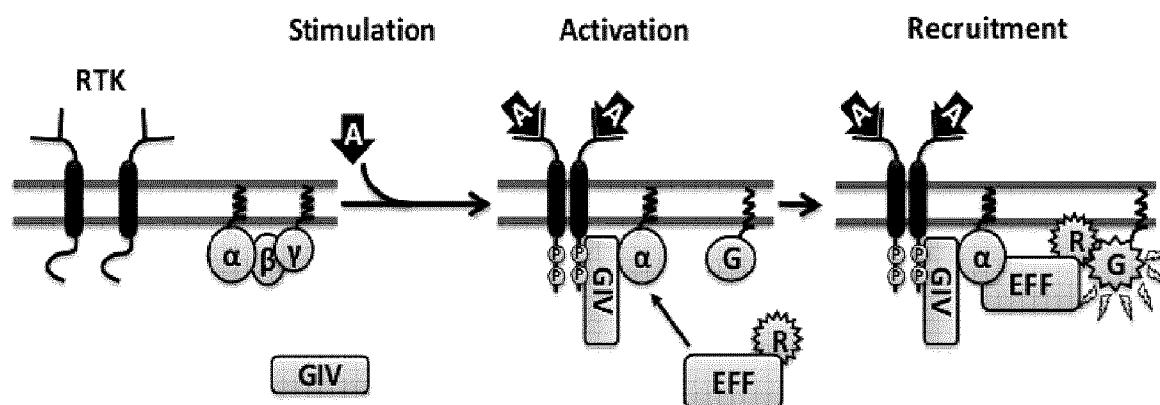
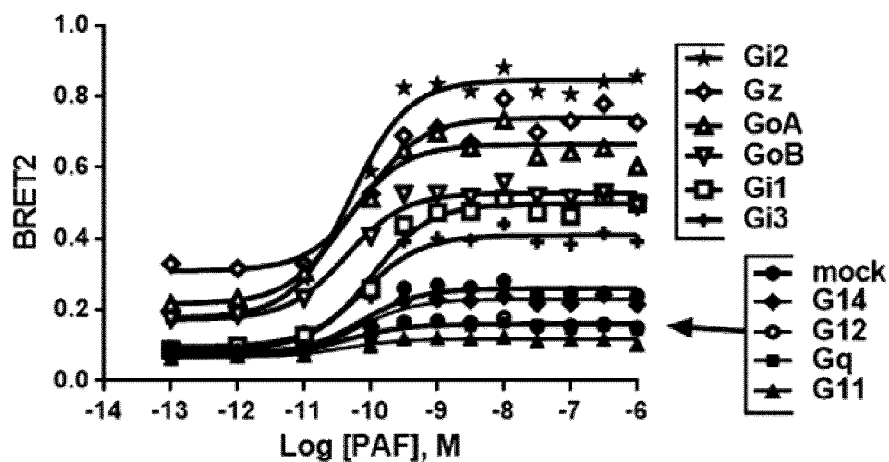


1/42

i) GPCR-mediated Gprotein activation**ii) GEF-mediated Gprotein activation****FIG. 1A**

DRC:PAF/PAFR, RAP1GAP(SSS-AAA) sensor

**FIG. 1B**

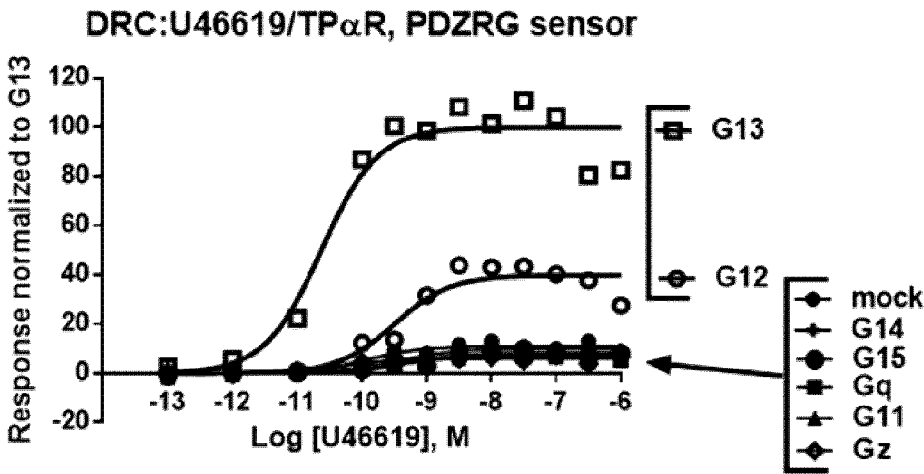


FIG. 1C

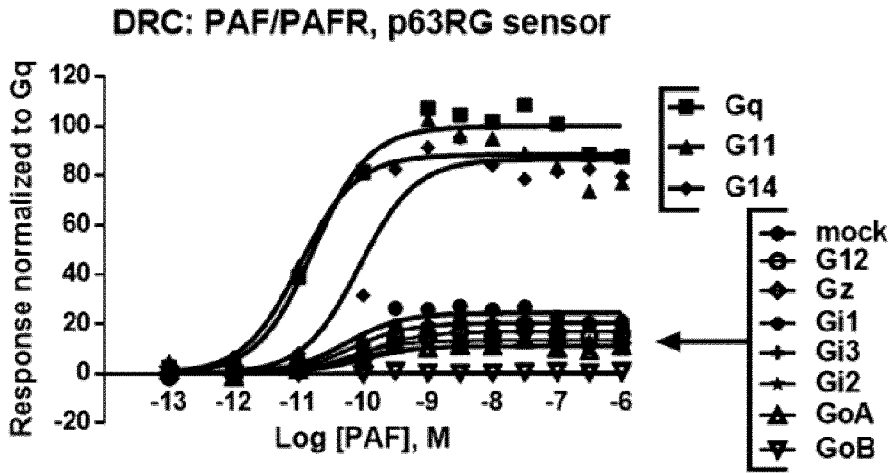


FIG. 1D

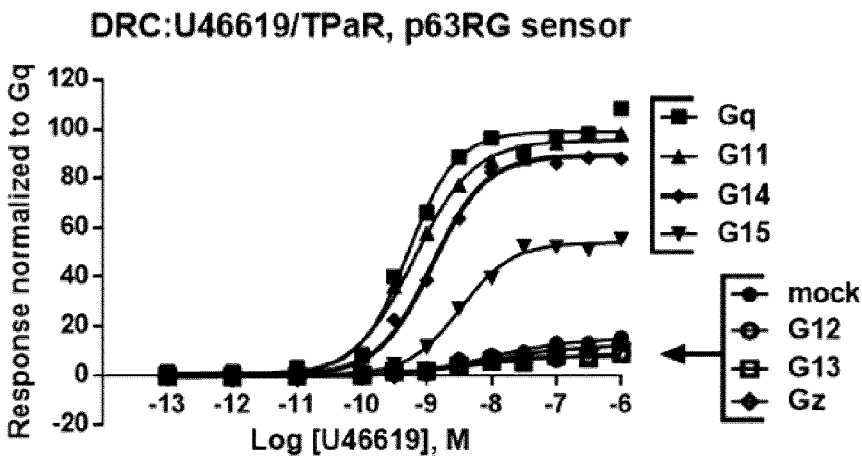
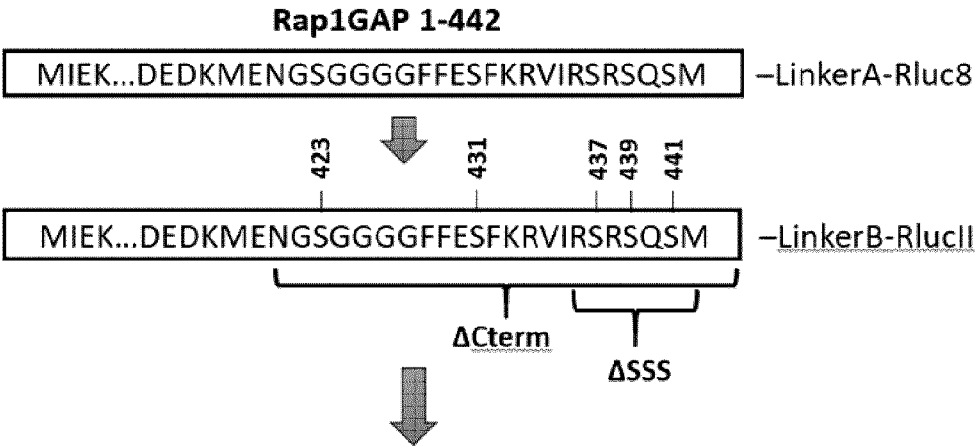


FIG. 1E

3/42



Substitutions and deletions were made to reduce sensitivity to phosphorylation

- Mutants

Rap1GAP ΔCterm (=1-420)

Rap1GAP ΔSSS (=1-436)

Rap1GAP SS-AA (=S437A/S441A)

Rap1GAP SS-DA (=S437D/S441A)

Rap1GAP SS-AD(=S437A/S441D)

Rap1GAP SS-DD (=S437D/S441D)

Rap1GAP SSS-AAA (=S437A/S439A/S441A)

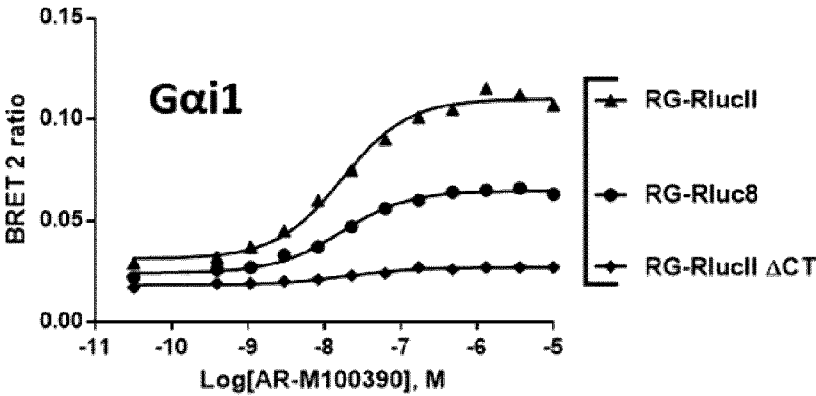
Rap1GAP SSS-TTT (=S437T/S439T/S441T)

LinkerA=GSGGGSGGGA

LinkerB=GSAGTGGRAIDIKLPAT

FIG. 2A

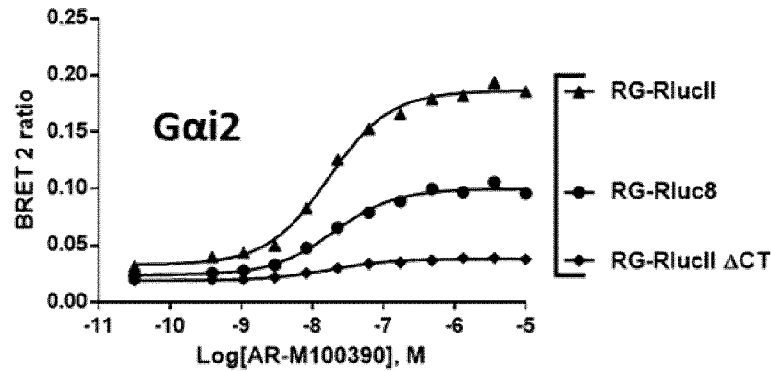
DRC hMOR1/ARM: Rap1GAP-Rluc +Gαi1/rGFP-CAAX



	Rluc8	RlucII	RlucII ΔCT
EC50	1.671e-008	1.696e-008	1.846e-008

FIG. 2B

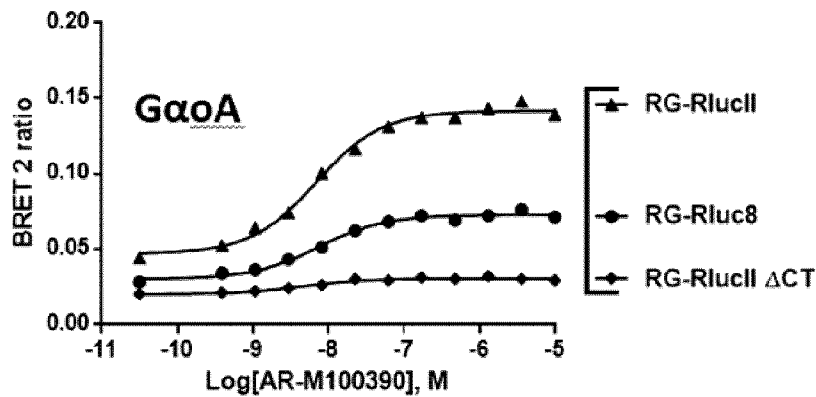
DRC hMOR1/ARM: Rap1GAP-Rluc +Gαi2/rGFP-CAAX



	Rluc8	RlucII	RlucII ΔCT
EC50	1.981e-008	1.693e-008	1.739e-008

FIG. 2C

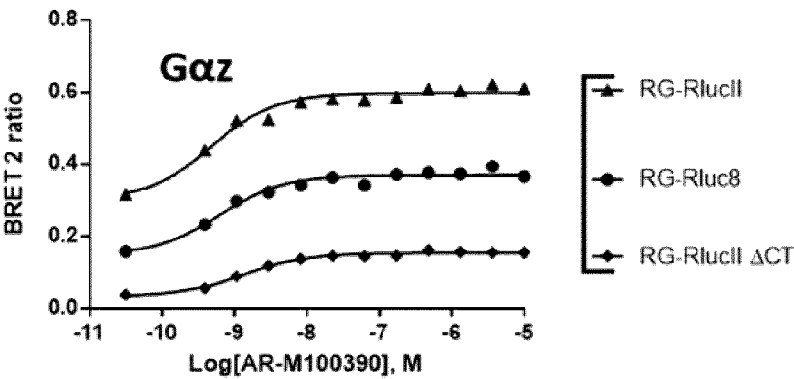
DRC hMOR1/ARM: Rap1GAP-Rluc +GαoA/rGFP-CAAX



	Rluc8	RlucII	RlucII ΔCT
EC50	7.442e-009	6.936e-009	4.671e-009

FIG. 2D

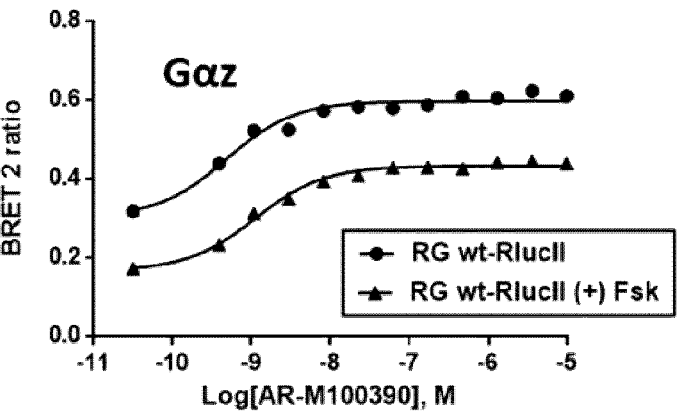
DRC hMOR1/ARM: Rap1GAP-Rluc +Gαz/rGFP-CAAX



	Rluc8	RlucII	RlucII ΔCT
EC50	6.273e-010	4.825e-010	1.328e-009

FIG. 2E

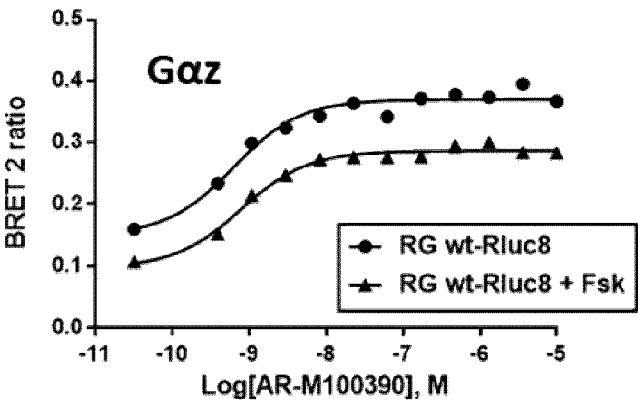
DRC hMOR1/ARM: Rap1GAP-RlucII +Gαz/rGFP-CAAX



	RlucII (-) Fsk	RlucII (+) Fsk
EC50	4.825e-010	1.123e-009

FIG. 2F

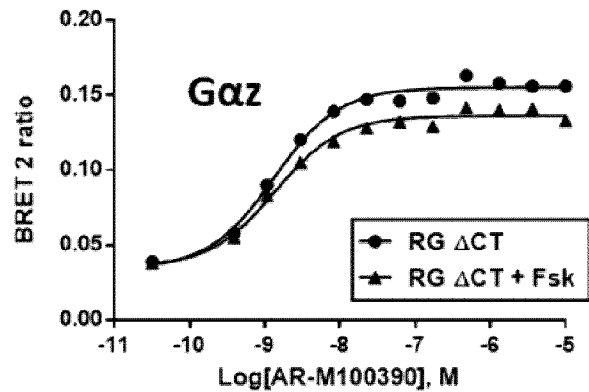
DRC hMOR1/ARM: Rap1GAP-Rluc8 +Gαz/rGFP-CAAX



	Rluc8 (-) Fsk	Rluc8 (+) Fsk
EC50	6.273e-010	7.811e-010

FIG. 2G

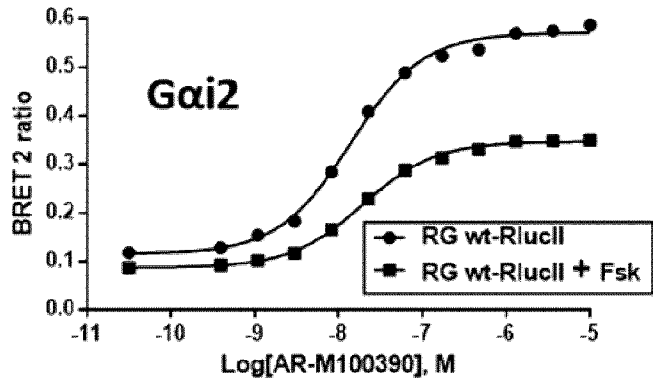
DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gz/rGFP-CAAX



	RlucII ΔCT(-) Fsk	RlucII ΔCT(+) Fsk
EC50	1.328e-009	1.369e-009

FIG. 2H

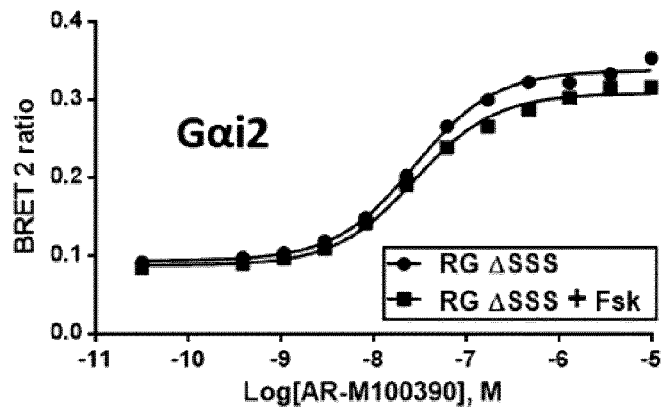
DRC hMOR1/ARM: Rap1GAP-RlucII +Gαi2/rGFP-CAAX



	wt (-) Fsk	wt (+) Fsk
EC50	1.389e-008	1.934e-008

FIG. 2I

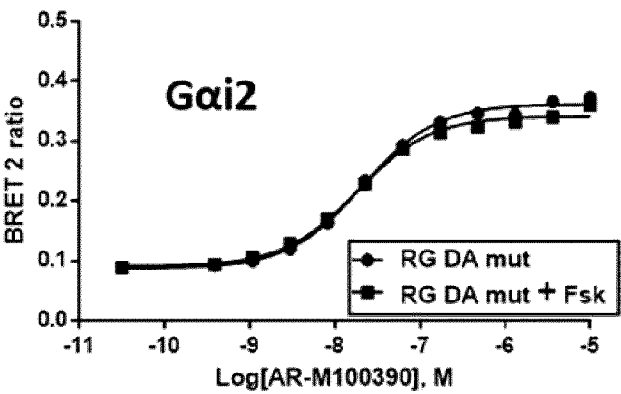
DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gαi2/rGFP-CAAX



	ΔSSS mutant (-) Fsk	ΔSSS mutant (+) Fsk
EC50	2.798e-008	2.819e-008

FIG. 2J

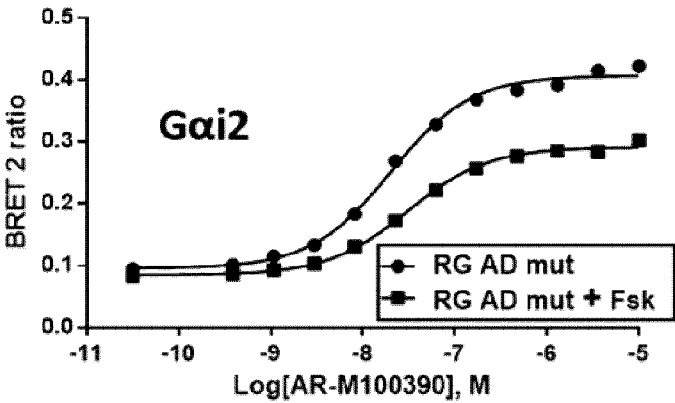
DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gαi2/rGFP-CAAX



	DA mutant (-) Fsk	DA mutant (+) Fsk
EC50	2.088e-008	1.829e-008

FIG. 2K

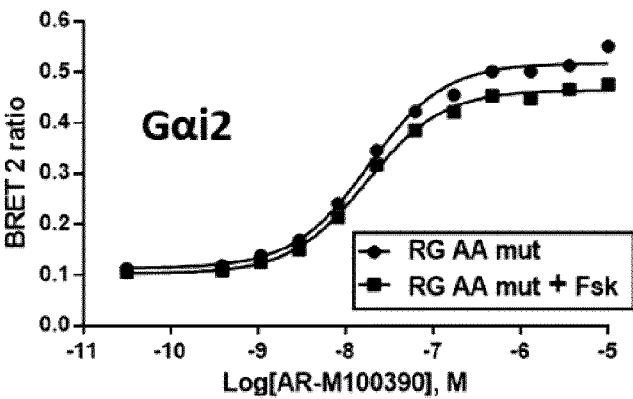
DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gαi2/rGFP-CAAX



	AD mutant (-) Fsk	AD mutant (+) Fsk
EC50	2.023e-008	3.082e-008

FIG. 2L

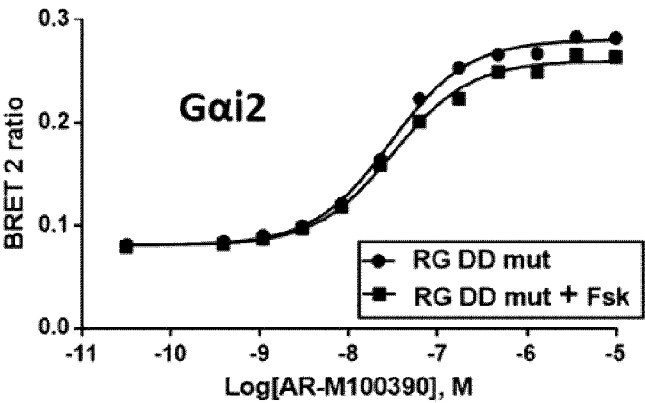
DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gαi2/rGFP-CAAX



	AA mutant (-) Fsk	AA mutant (+) Fsk
EC50	1.841e-008	1.737e-008

FIG. 2M

DRC hMOR1/ARM: Rap1GAP mut-RlucII +Gαi2/rGFP-CAAX



	DD mutant (-) Fsk	DD mutant (+) Fsk
EC50	2.930e-008	3.145e-008

FIG. 2N

DRC hDOR/SNC80: Rap1GAP-RlucII vs GαoB/rGFP-CAAX

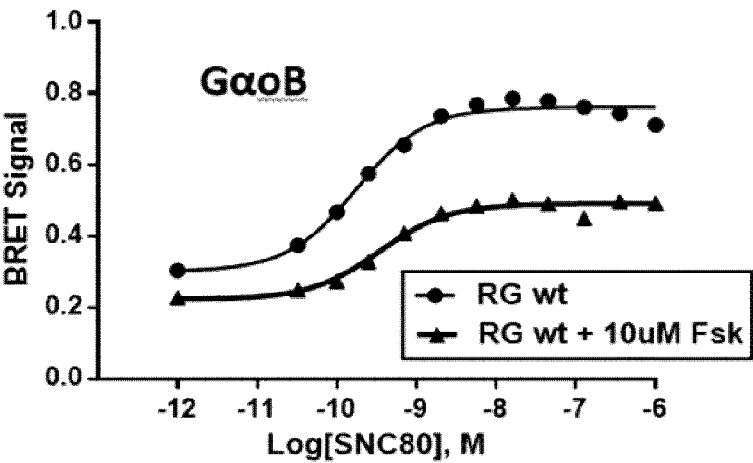


FIG. 2O

DRC hDOR/SNC80: Rap1GAP-RlucII vs GαoB/rGFP-CAAX

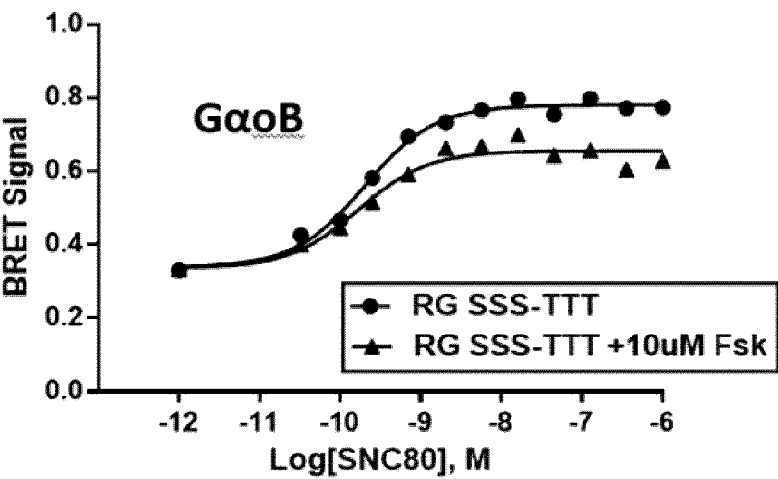


FIG. 2P

DRC hDOR/SNC80: Rap1GAP-RlucII vs $G\alpha_o$ B/rGFP-CAAX

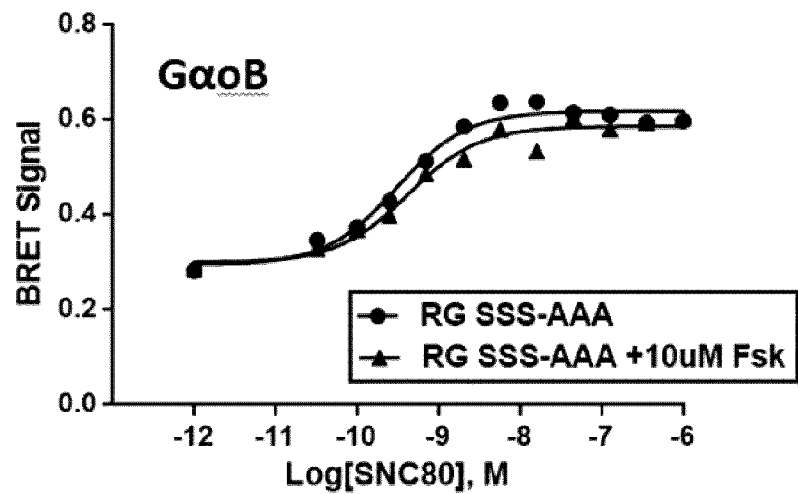
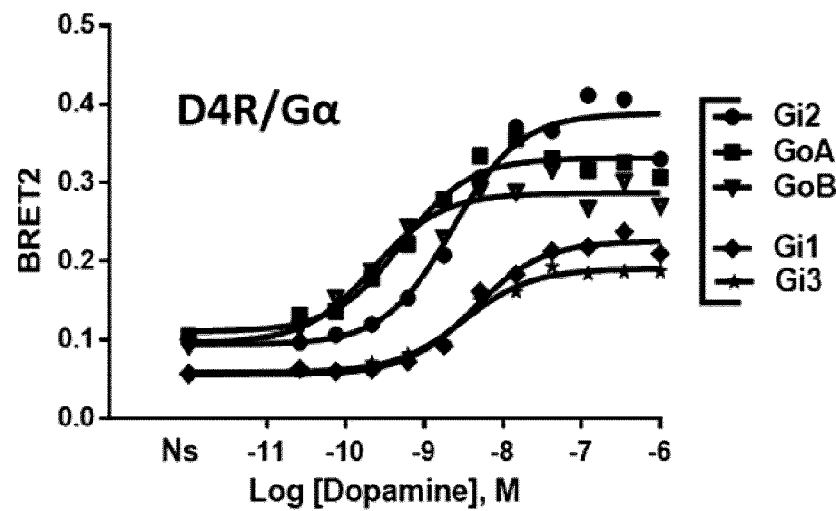


FIG. 2Q

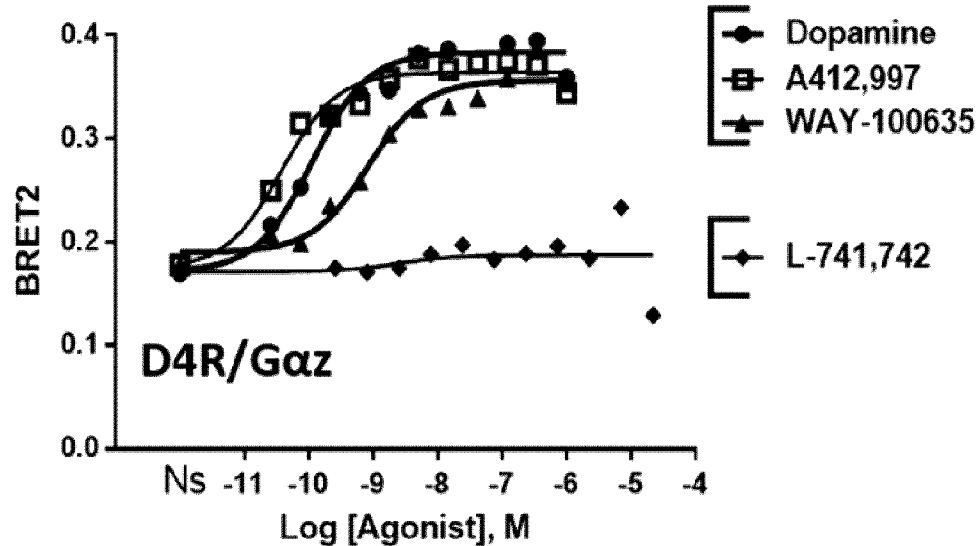
DRC D4R, RAPGAP (SSS-AAA)-RlucII vs $G\alpha$ /rGFP-CAAX



	GoA	GoB	Gi1	Gi2	Gi3
LogEC50	-9.300	-9.651	-8.365	-8.647	-8.505

FIG. 2R

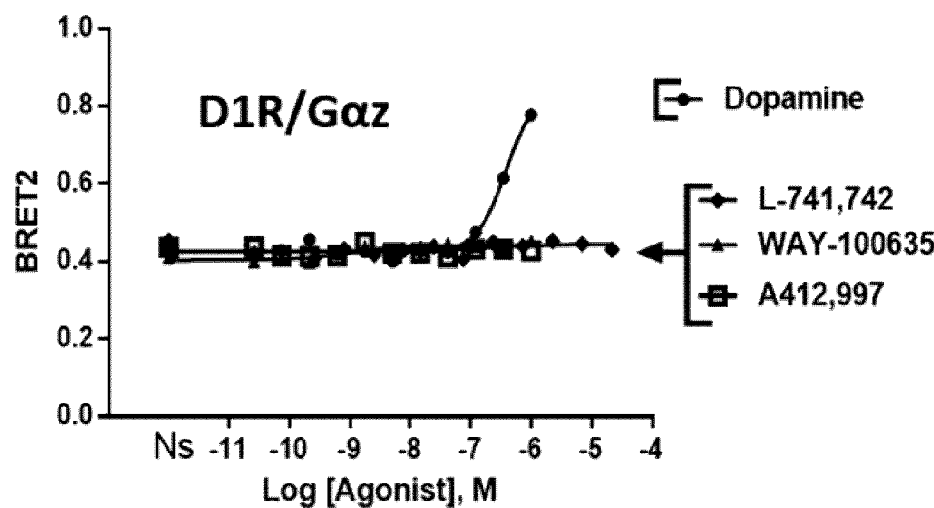
DRC D4R, RAPGAP (SSS-AAA)-RlucII vs Gαz/rGFP-CAAX



	A412,997	Dopamine	L-741,742	WAY-100635
LogEC50	-10.42	-9.945	-8.583	-9.079

FIG. 2S

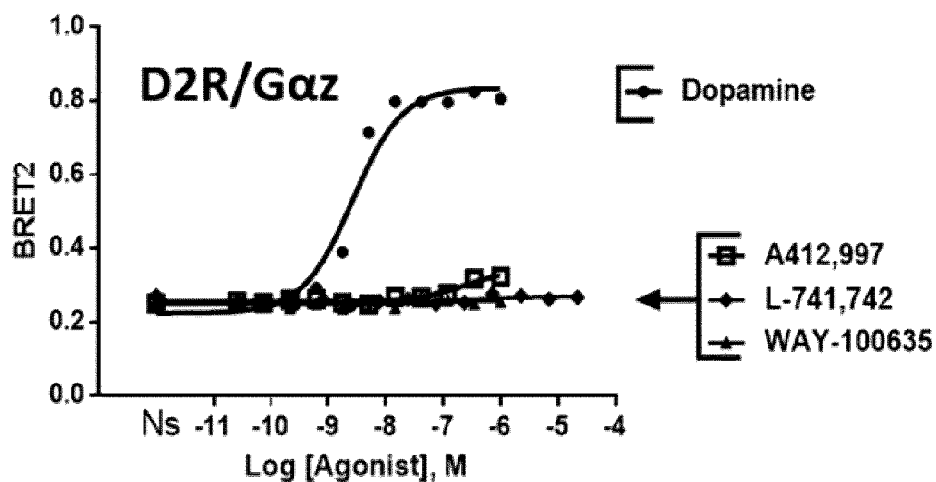
DRC D1R, RAPGAP (SSS-AAA)-RlucII vs Gαz/rGFP-CAAX



	A412,997	Dopamine	L-741,742	WAY-100635
LogEC50		-5.883	-6.661	-9.022

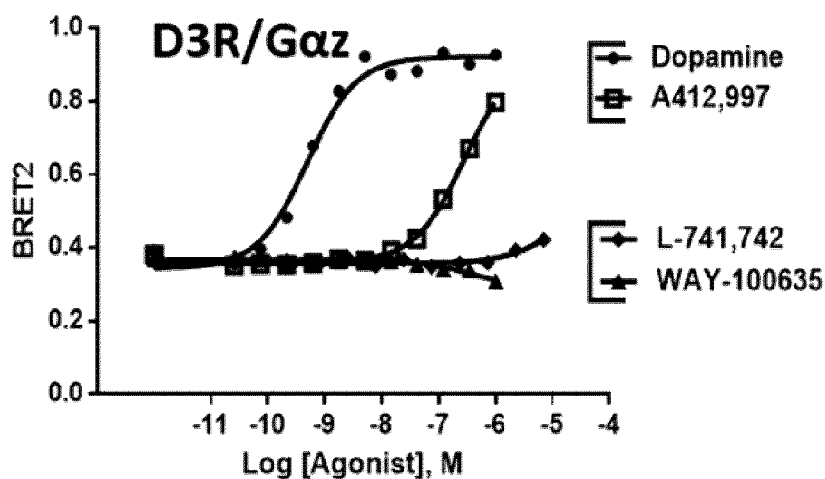
FIG. 2T

11/42

DRC D2R, RAPGAP (SSS-AAA)-RlucII vs $G\alpha z$ /rGFP-CAAX

	A412,997	Dopamine	L-741,742	WAY-100635
LogEC50	-6.626	-8.577	-6.387	-7.487

FIG. 2U

DRC D3R, RAPGAP (SSS-AAA)-RlucII vs $G\alpha z$ /rGFP-CAAX

	A412,997	Dopamine	L-741,742	WAY-100635
LogEC50	-6.551	-9.309	-5.006	-6.476

FIG. 2V

DRC D5R, RAPGAP (SSS-AAA)-RlucII vs Gαz/rGFP-CAAX

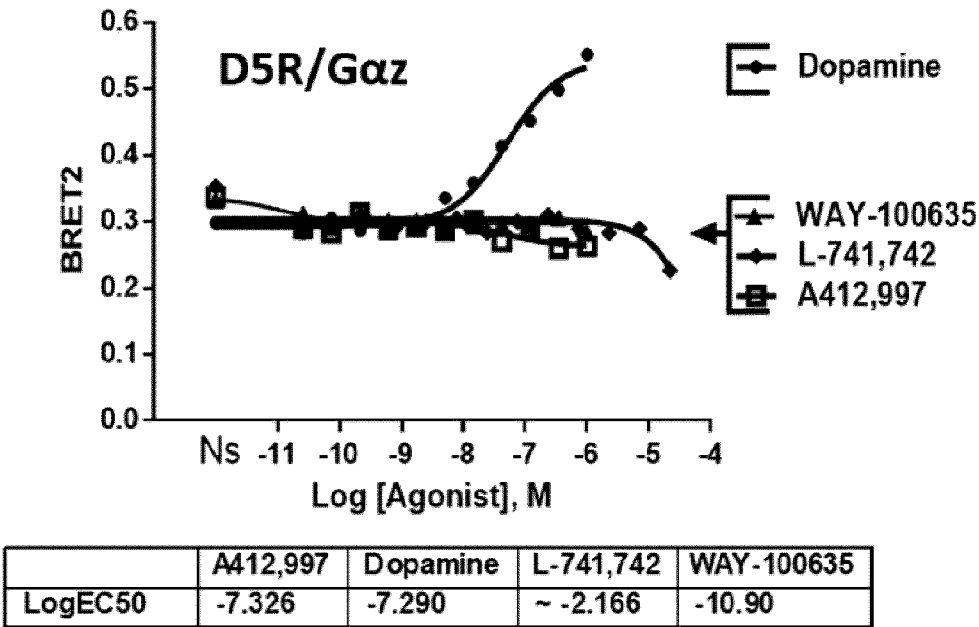


FIG. 2W

Z' factor: D4R, Rap1GAP (SSS-AAA)/Gi2 sensor

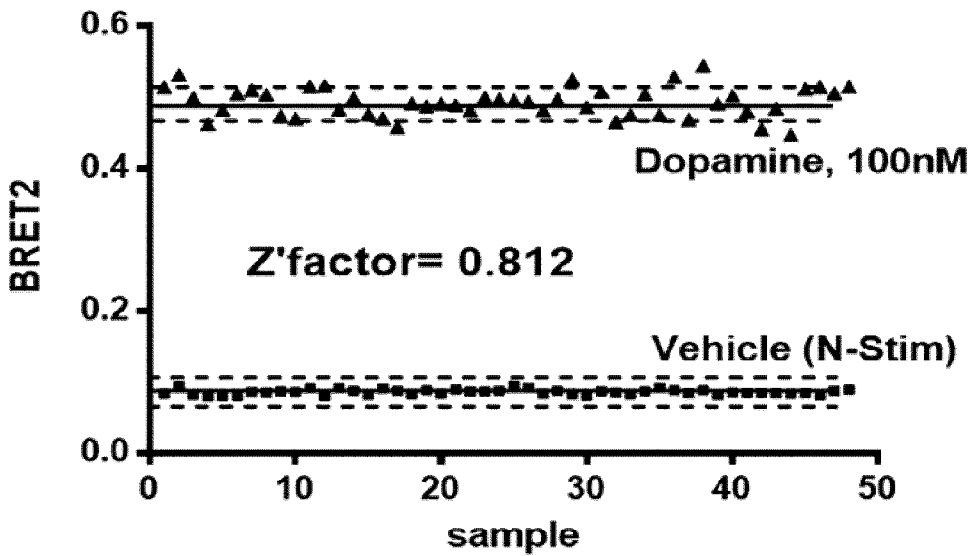
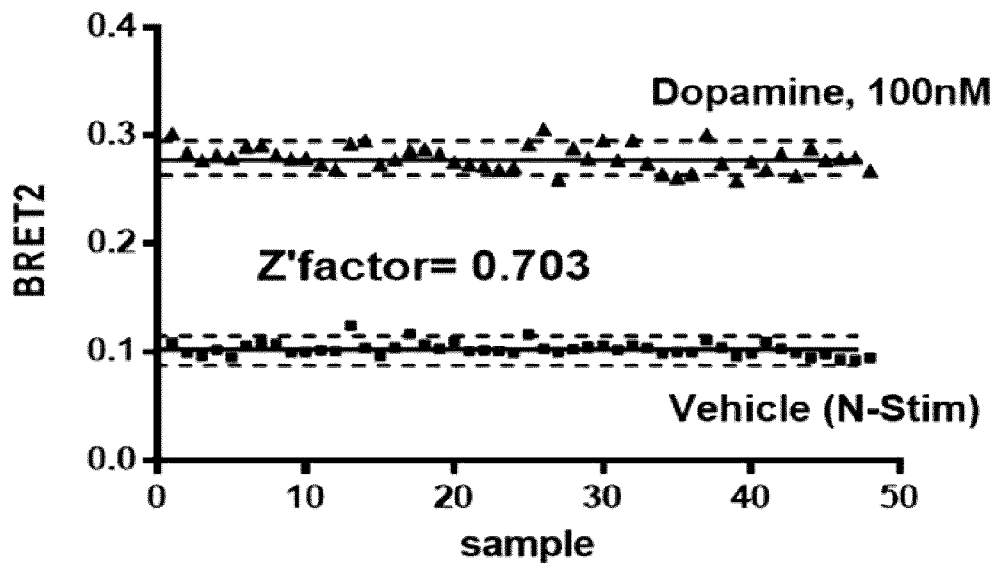
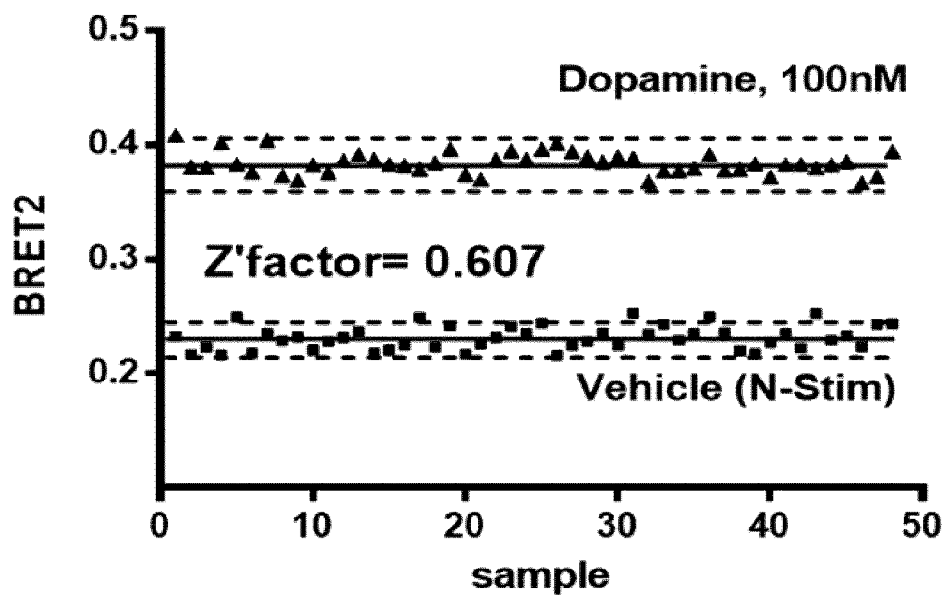
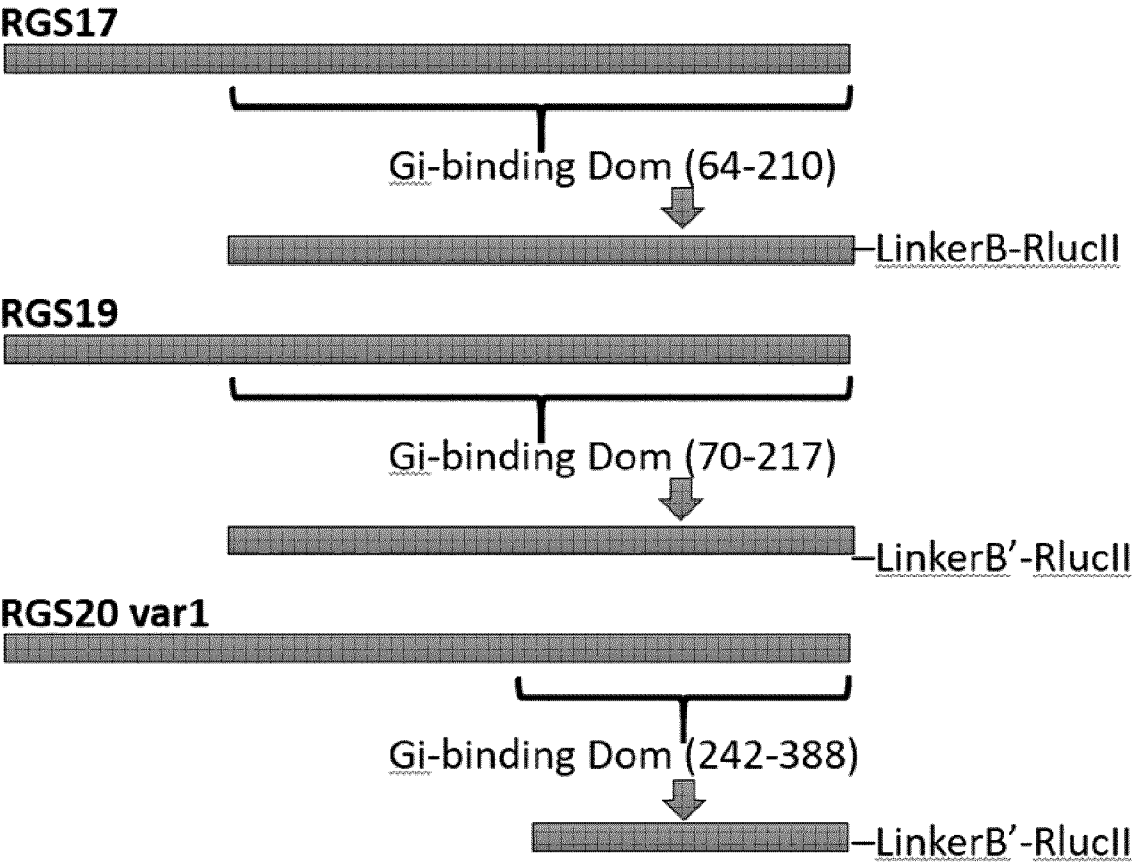


FIG. 2X

13/42

Z' factor: D4R, Rap1GAP (SSS-AAA)/GoA sensor**FIG. 2Y****Z' factor: D4R, Rap1GAP (SSS-AAA)/Gz sensor****FIG. 2Z**



LinkerB'=GSAGTGGRAIDIKLASAT

FIG. 3A

DRC: Dopamine/D4R, RGS17-based sensor

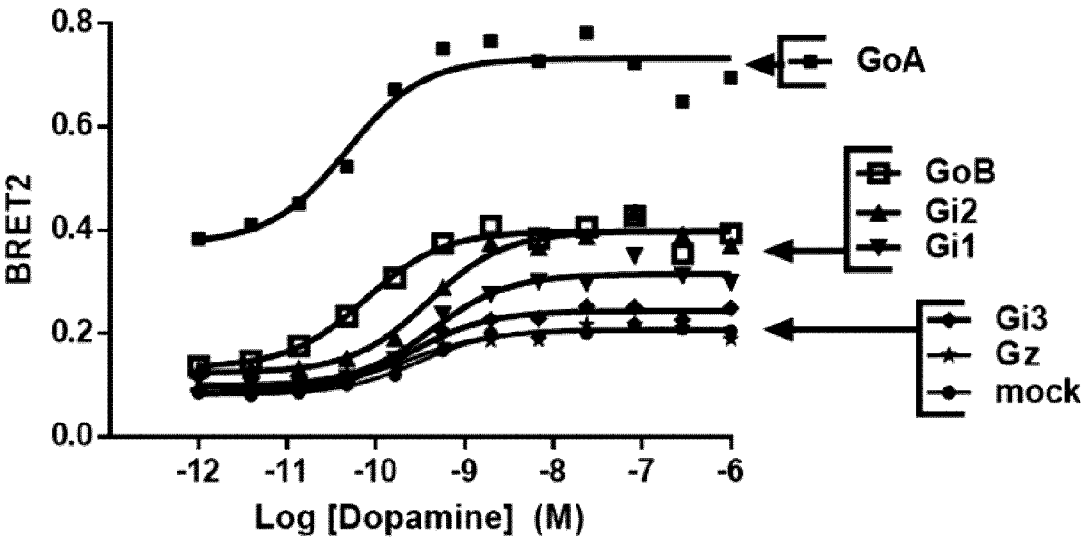


FIG. 3B

DRC: Dopamine/D4R, RGS19-based sensor

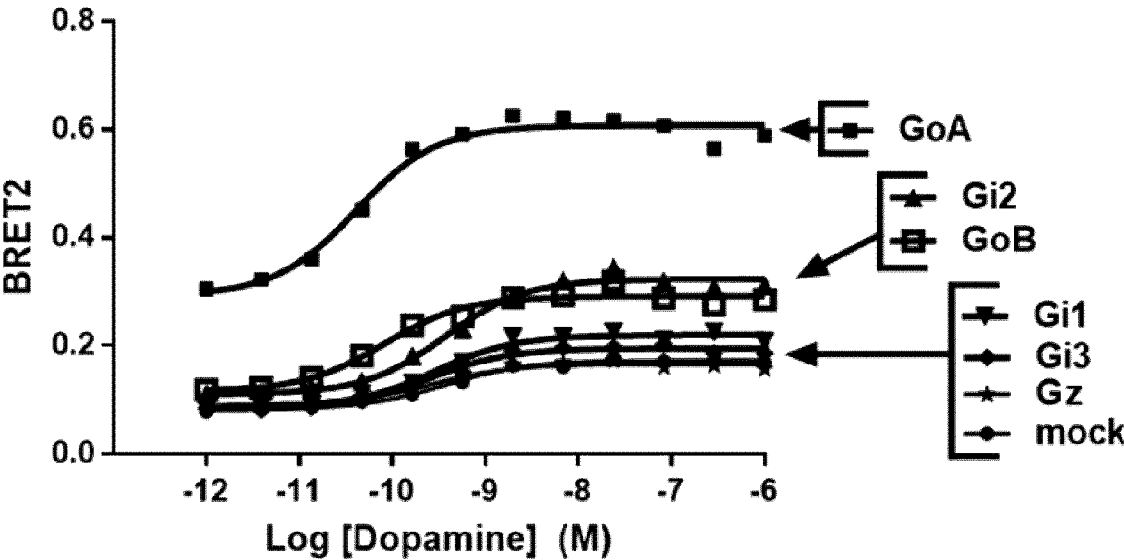


FIG. 3C

DRC: Dopamine/D4R, RGS20-based sensor

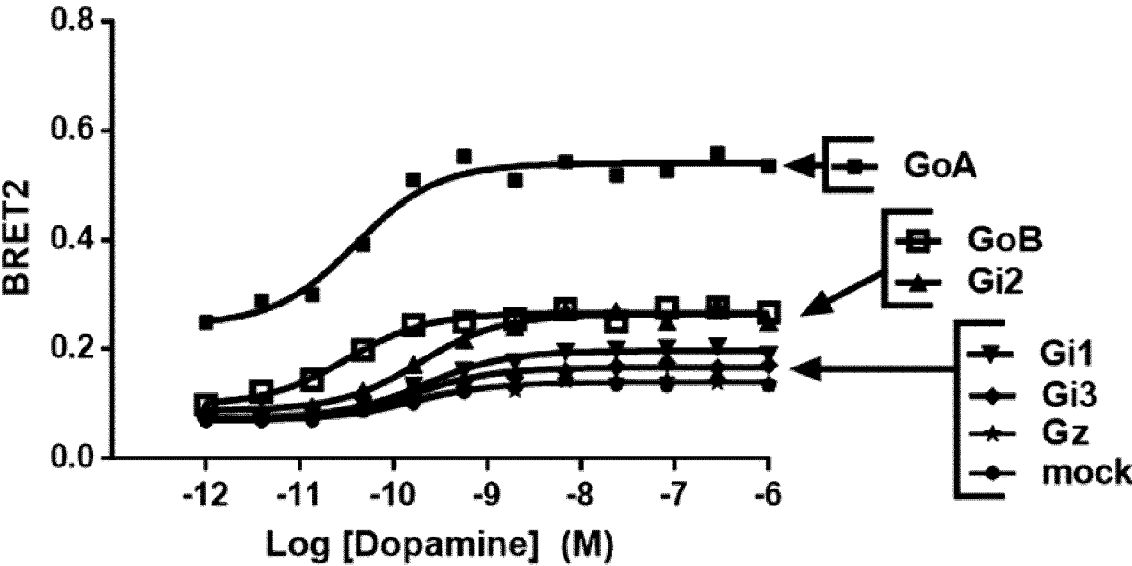
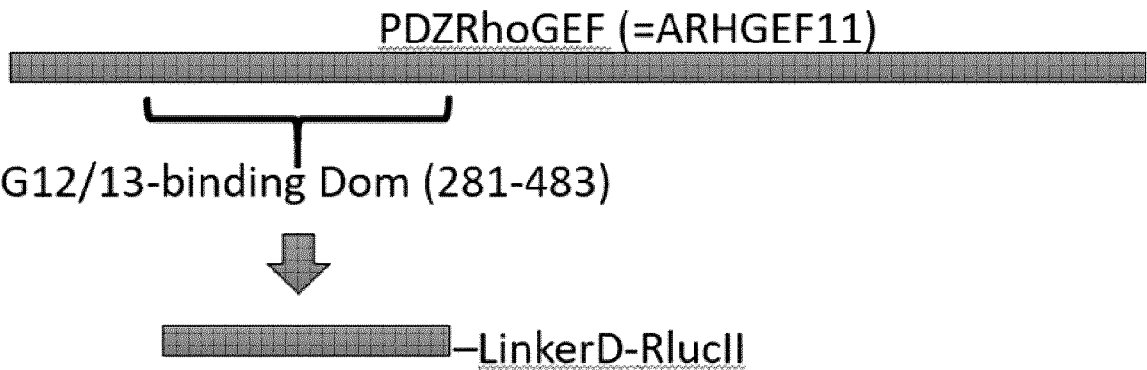


FIG. 3D

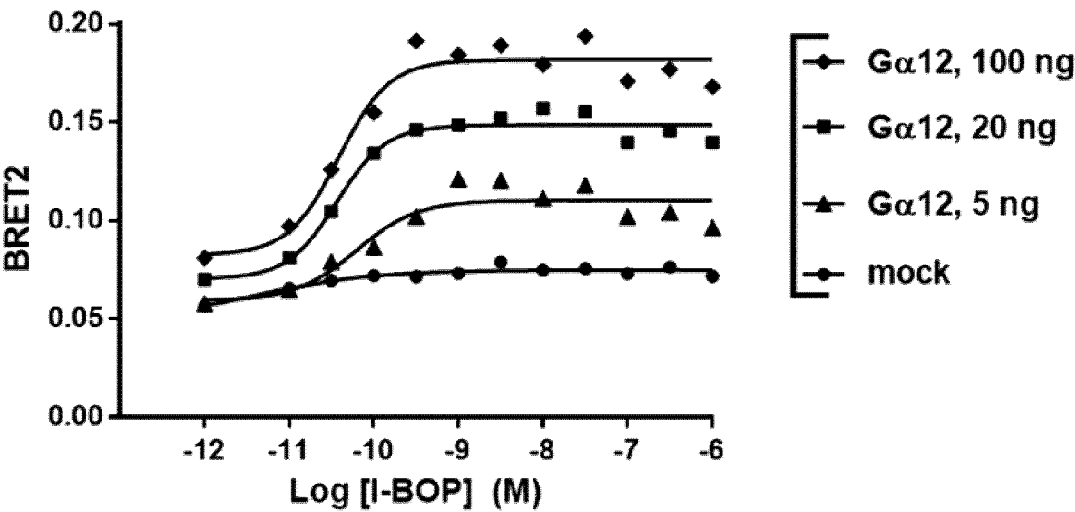


RlucII-tagged cytosolic G12/13 interacting domain of PDZ Rho GEF

LinkerD= GIRLREALKLPAT

FIG. 4A

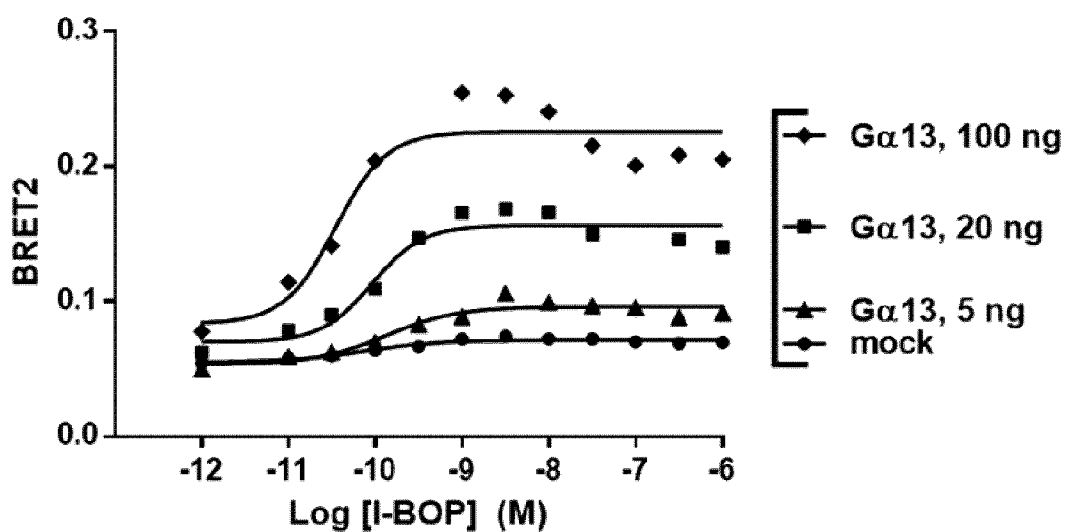
DRC TP α R/I-BOP, PDZRG-RlucII vs G12/rGFP-CAAX



	mock	G α 12, 5 ng	G α 12, 20 ng	G α 12, 100 ng
LogEC50	-11.37	-10.21	-10.44	-10.41

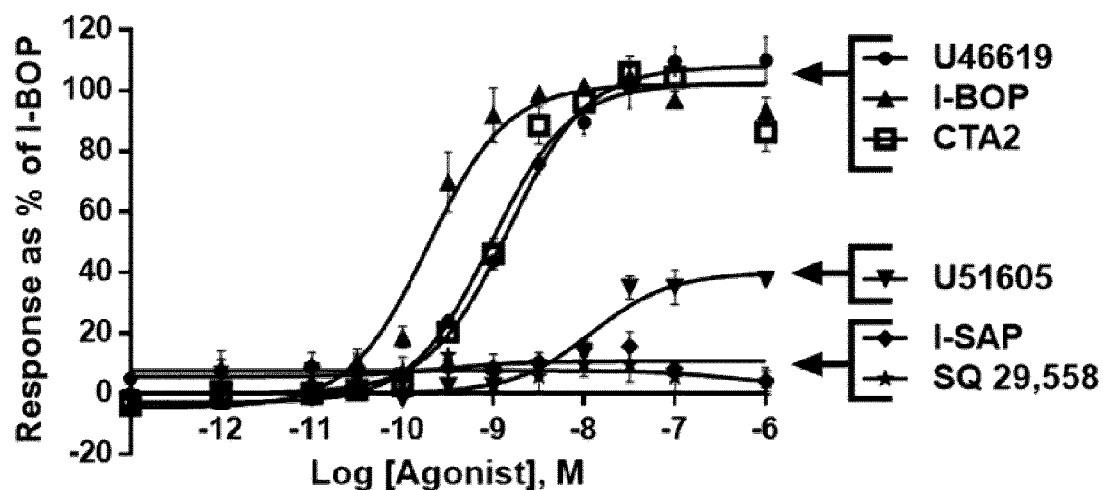
FIG. 4B

17/42

DRC TP α R/I-BOP, PDZRG-RlucII vs G13/rGFP-CAAX

	mock	G α 13, 5 ng	G α 13, 20 ng	G α 13, 100 ng
LogEC50	-10.13	-9.875	-10.04	-10.46

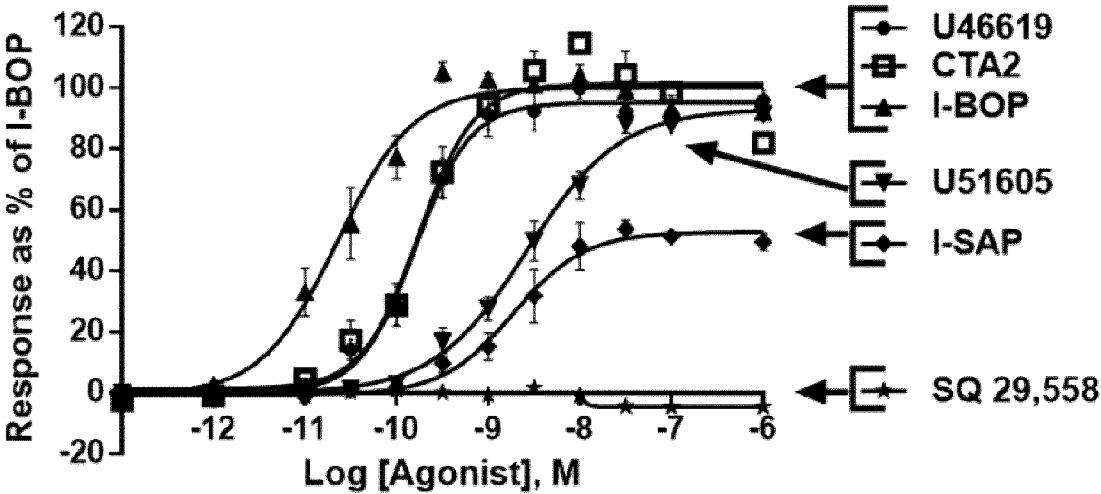
FIG. 4C

DRC TP α R, PDZRG-RlucII vs G12/rGFP-CAAX

	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29,558
LogEC50	-8.835	-9.720	-9.027	-7.920	-9.582	-6.396

FIG. 4D

DRC TP α R, PDZRG-RlucII vs G13/rGFP-CAAX



	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29,558
LogEC50	-9.796	-10.65	-9.765	-8.583	-8.727	~ -7.959

FIG. 4E

Z'Factor: TP α R, pDZRG-RlucII/G12 sensor

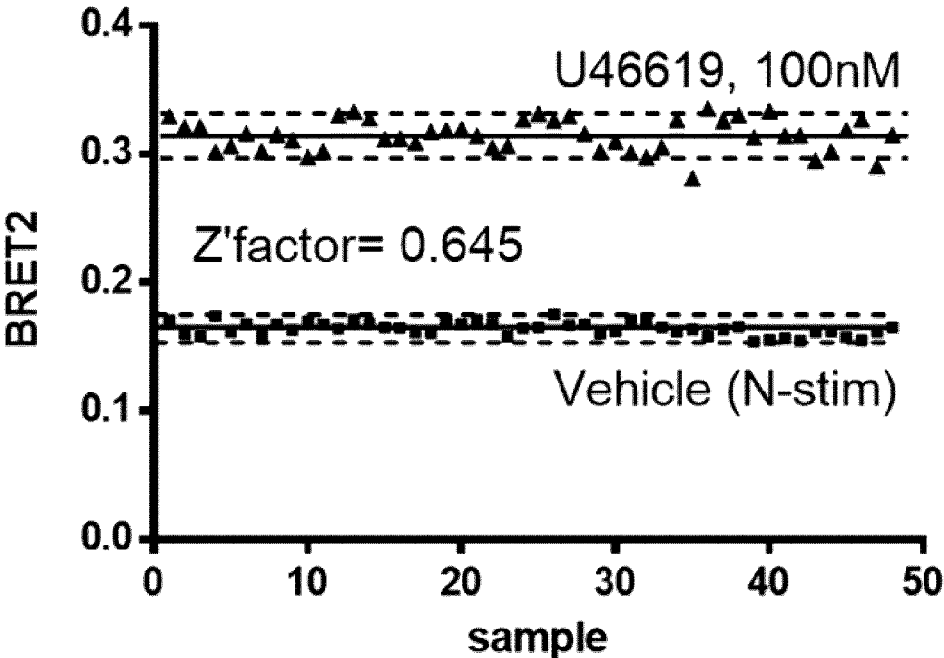
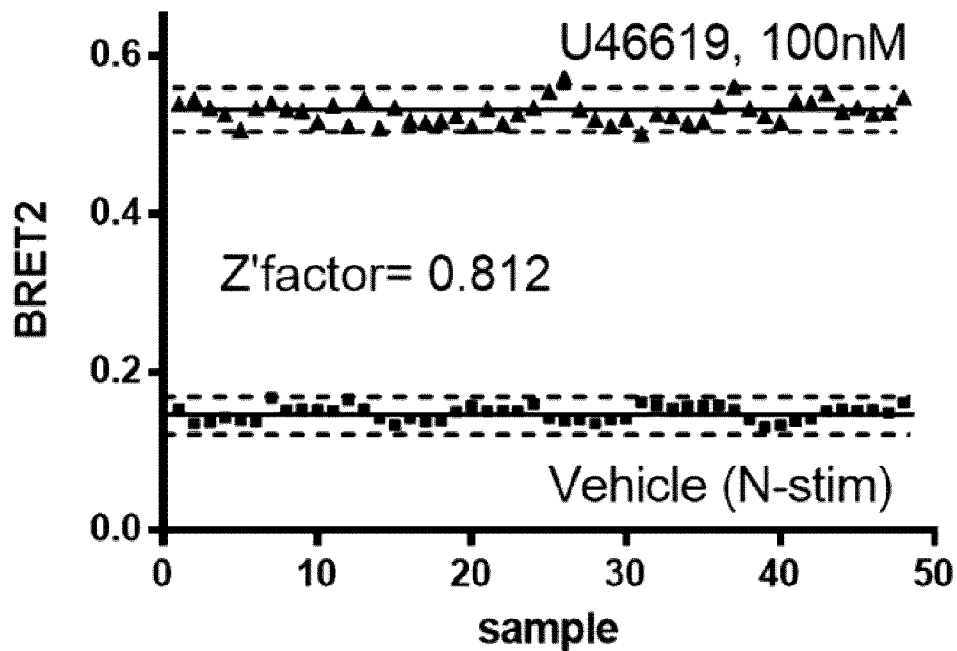
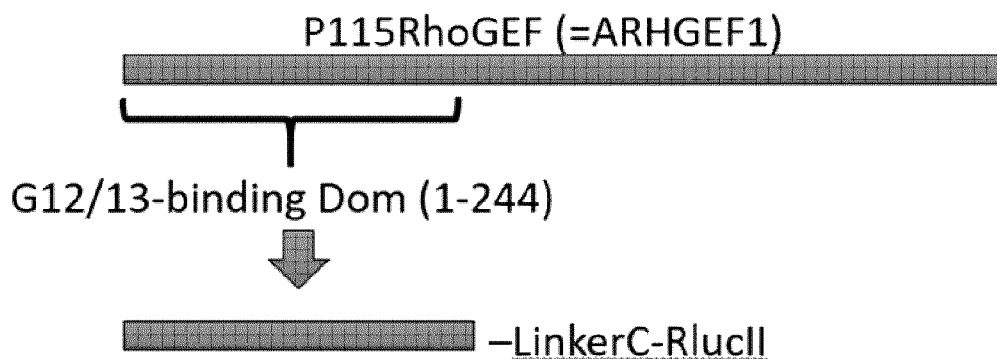


FIG. 4F

19/42

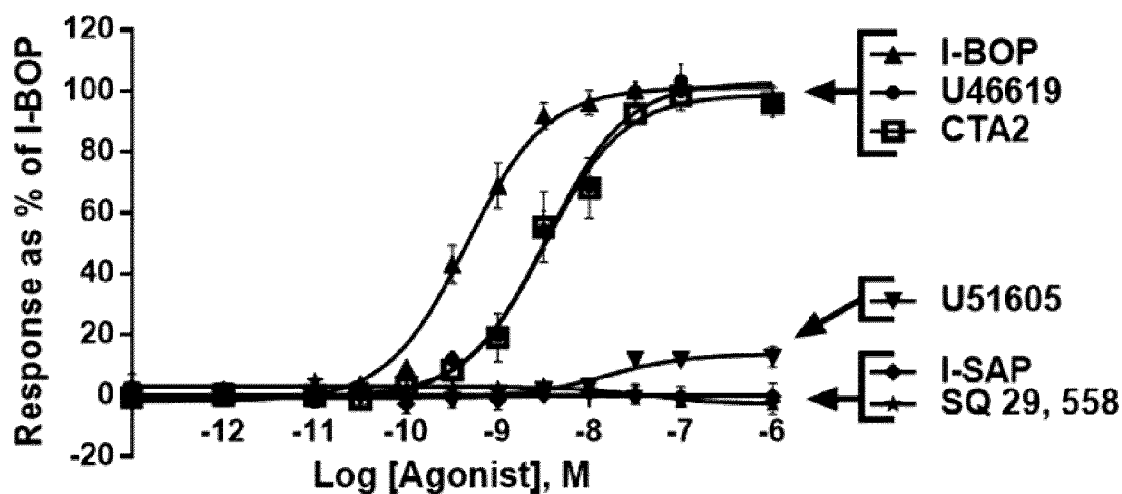
Z'Factor: TP α R, PDZRG-RlucII/G13 sensor**FIG. 4G**

RlucII-tagged cytosolic G12/13 interacting domain of P115 Rho GEF

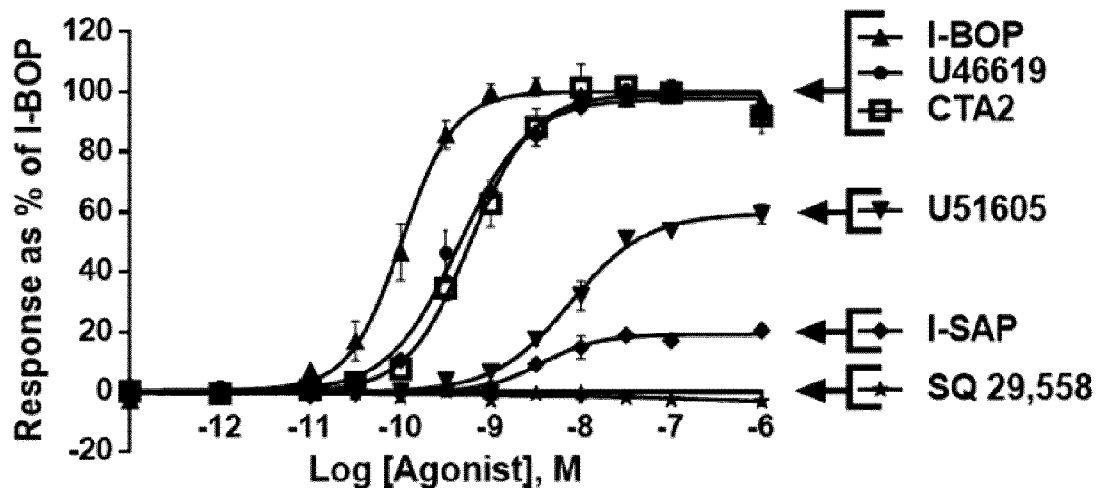
LinkerC= RLKLPAT

FIG. 5A

20/42

DRC TP α R, P115RG-RlucII vs G12/rGFP-CAAX

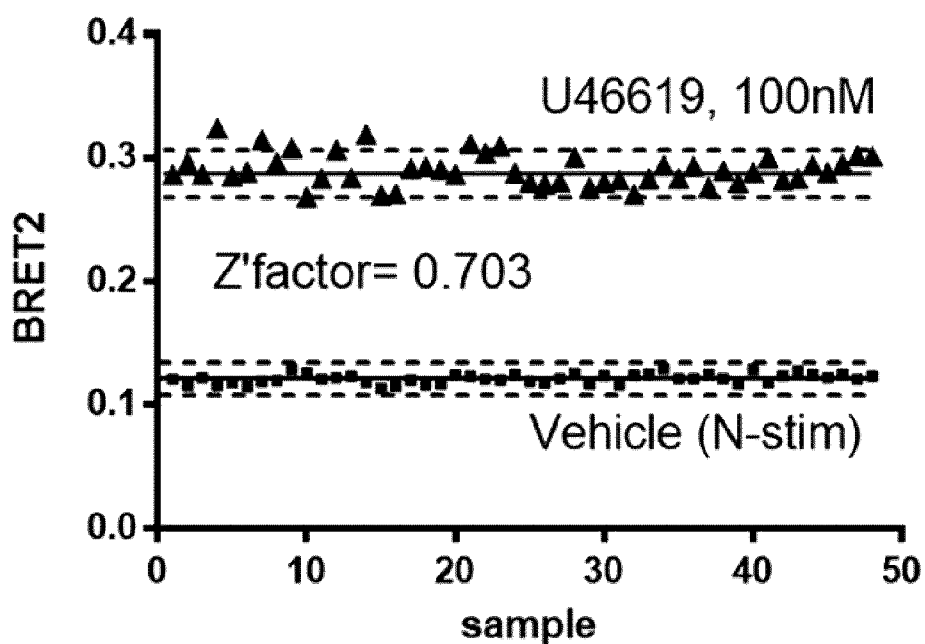
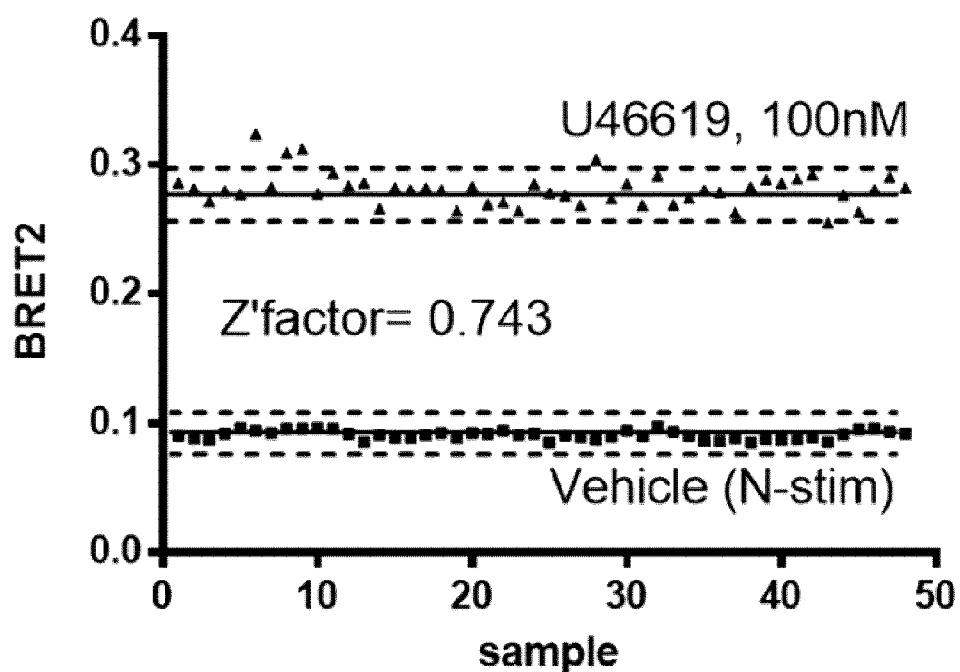
	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29, 558
LogEC50	-8.470	-9.341	-8.499	-7.804	-8.620	-7.373

FIG. 5B**DRC TP α R, P115RG-RlucII vs G13/rGFP-CAAX**

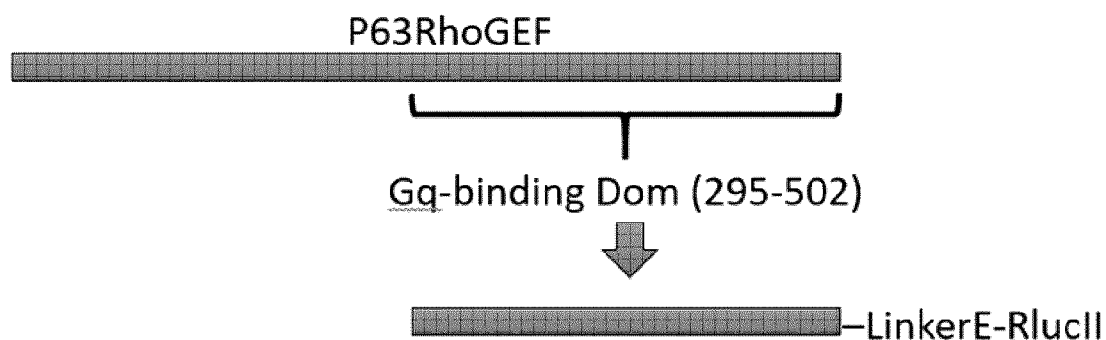
	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29,558
LogEC50	-9.356	-9.986	-9.232	-8.119	-8.444	17.94

FIG. 5C

21/42

Z'Factor:TP α R, p115RG-RlucII/G12 sensor**FIG. 5D****Z'Factor:TP α R, p115RG-RlucII/G13 sensor****FIG. 5E**

22/42



RlucII-tagged cytosolic fragment of p63 (lacking the membrane tethering portion of the protein) containing only the Gq binding domain

LinkerE=ASGSAGTGGRAIDIKLPAT

FIG. 6A

DRC TP α R/U46619, p63RG-RlucII/Gq vs PM

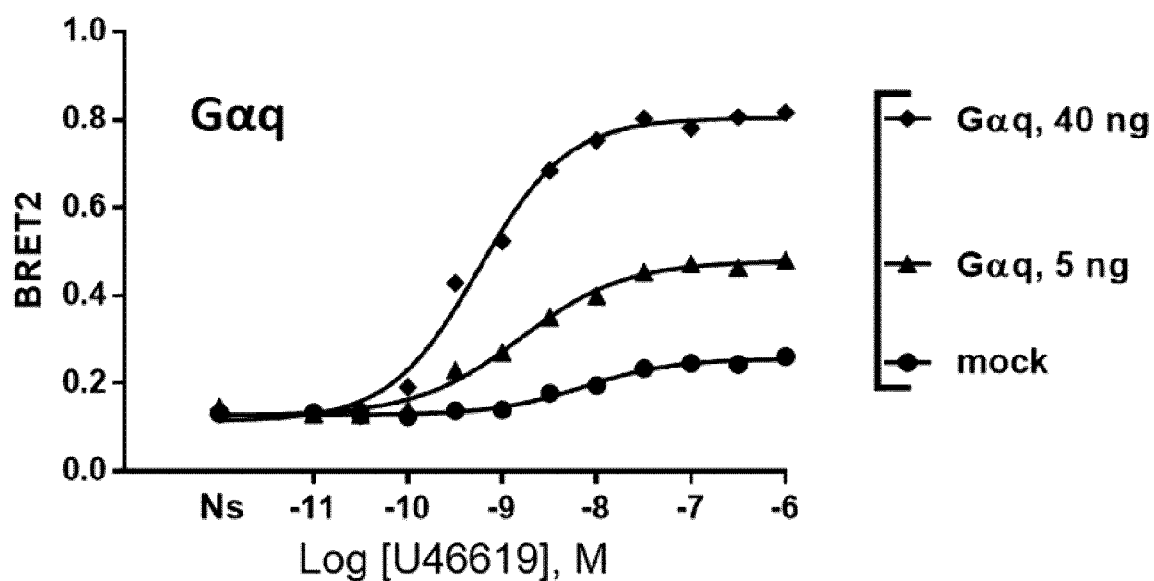


FIG. 6B

DRC:TP α R/U46619, p63RG-RlucII/G11 vs PM

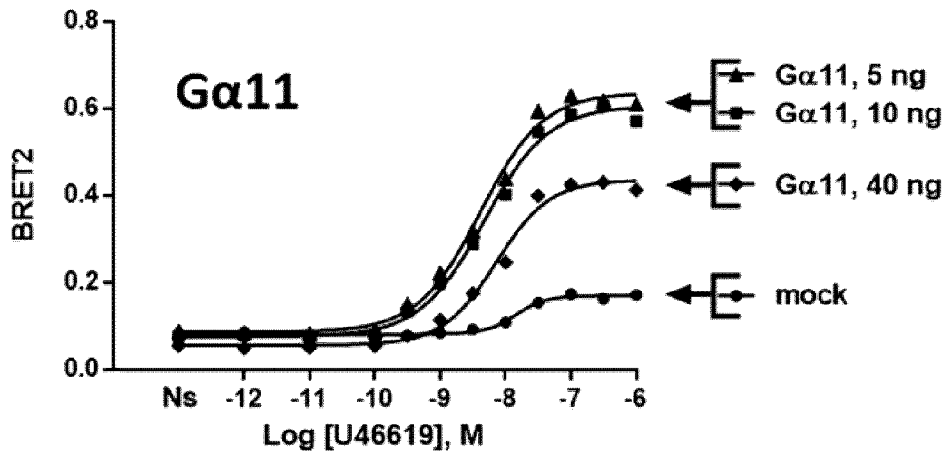


FIG. 6C

DRC:TP α R/U46619, p63RG-RlucII/G14 vs PM

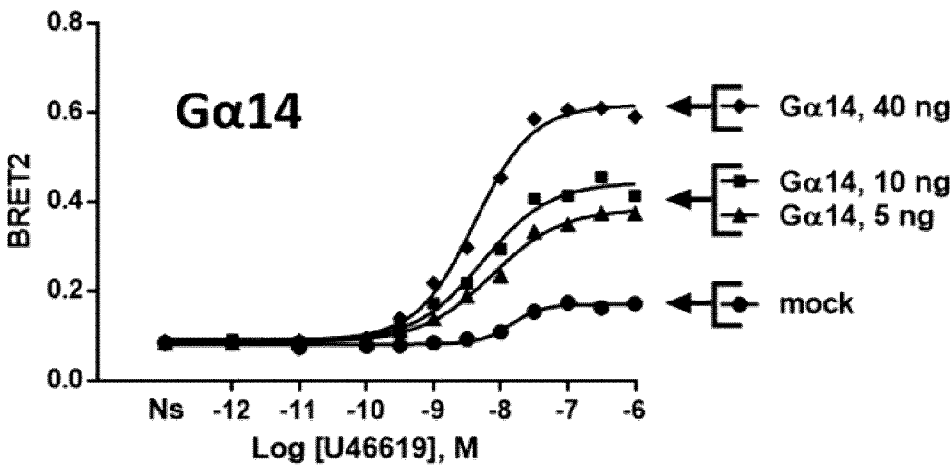


FIG. 6D

DRC:TP α R/U46619, p63RG-RlucII/G15 vs PM

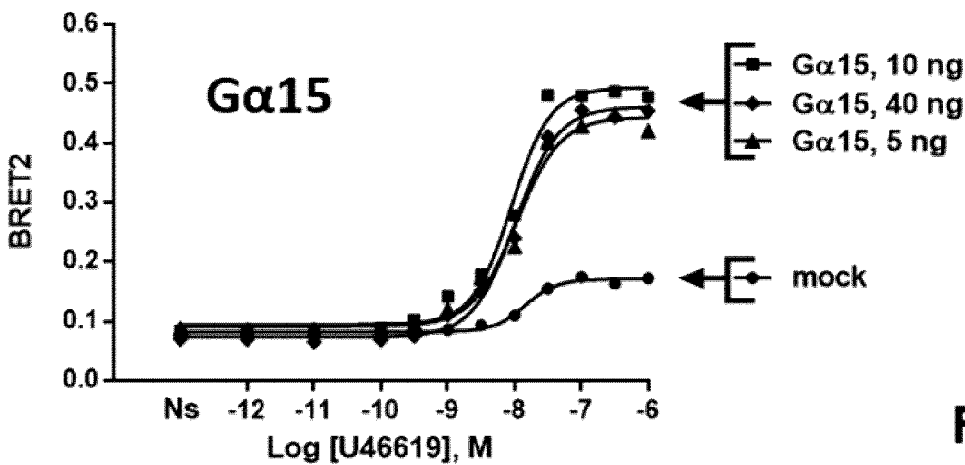


FIG. 6E

DRC: TP α R/U46619, p63RG-RlucII/Gq vs EE

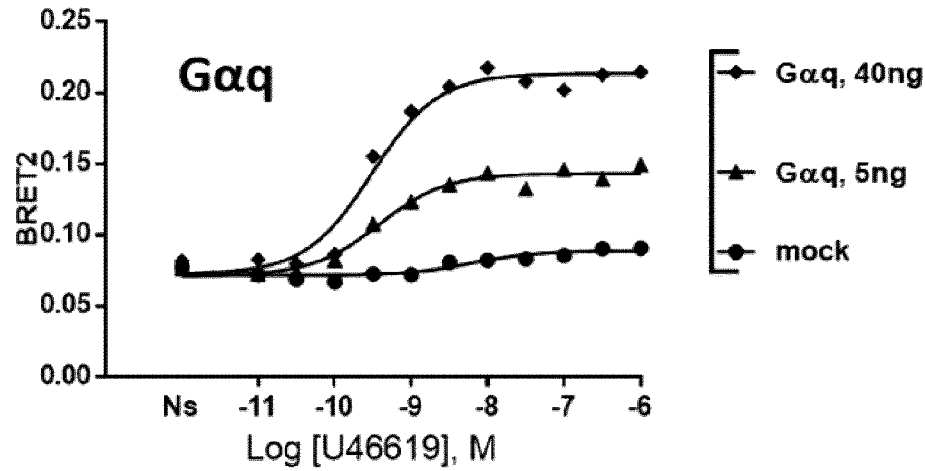


FIG. 6F

DRC: TP α R/U46619, p63RG-RlucII/G11 vs EE

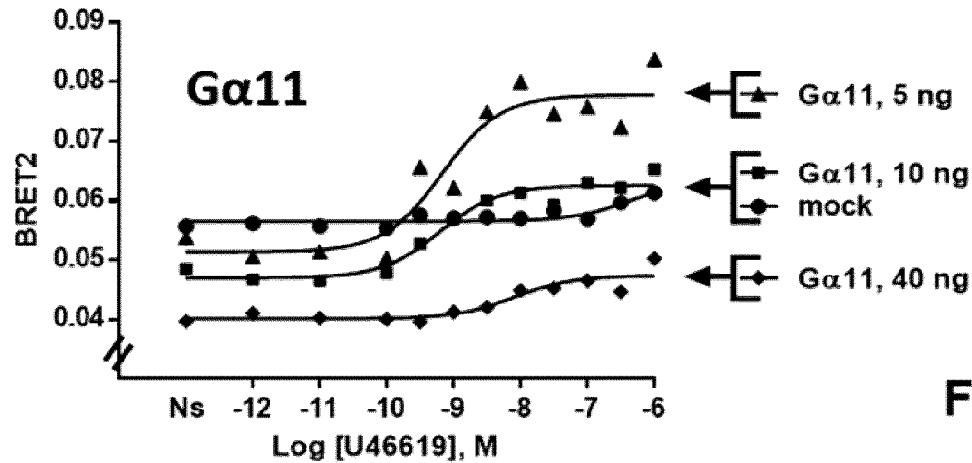


FIG. 6G

DRC: TP α R/U46619, p63RG-RlucII/G14 vs EE

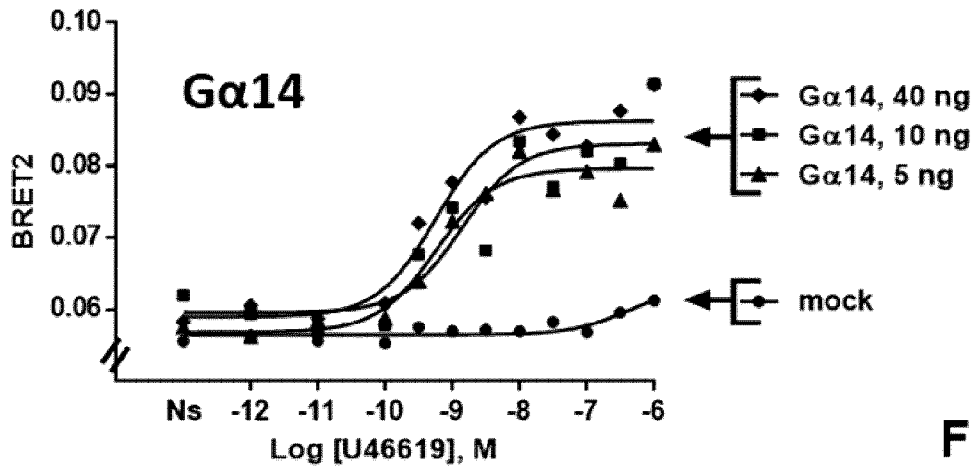


FIG. 6H

25/42

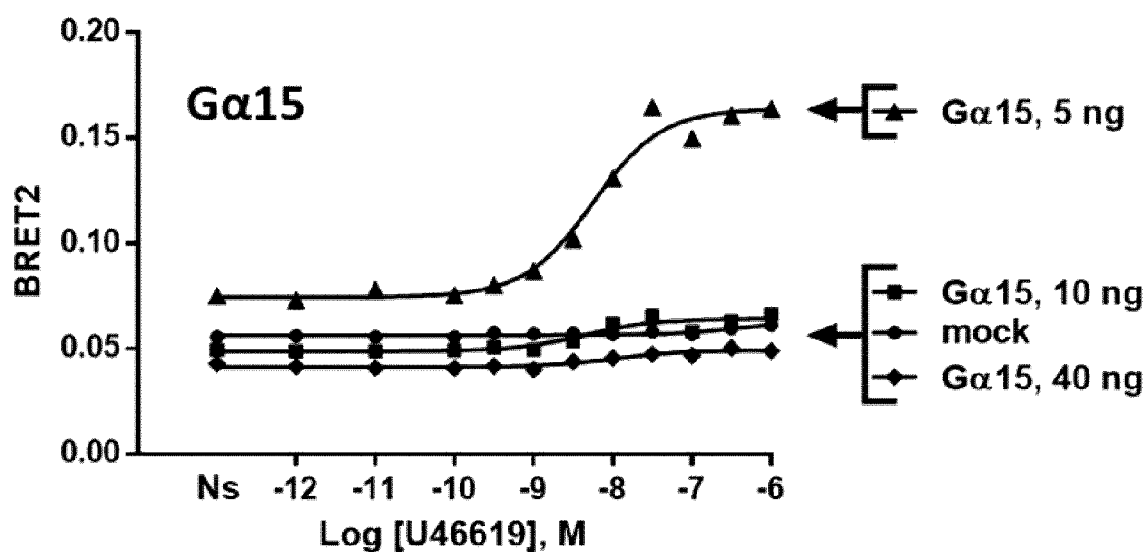
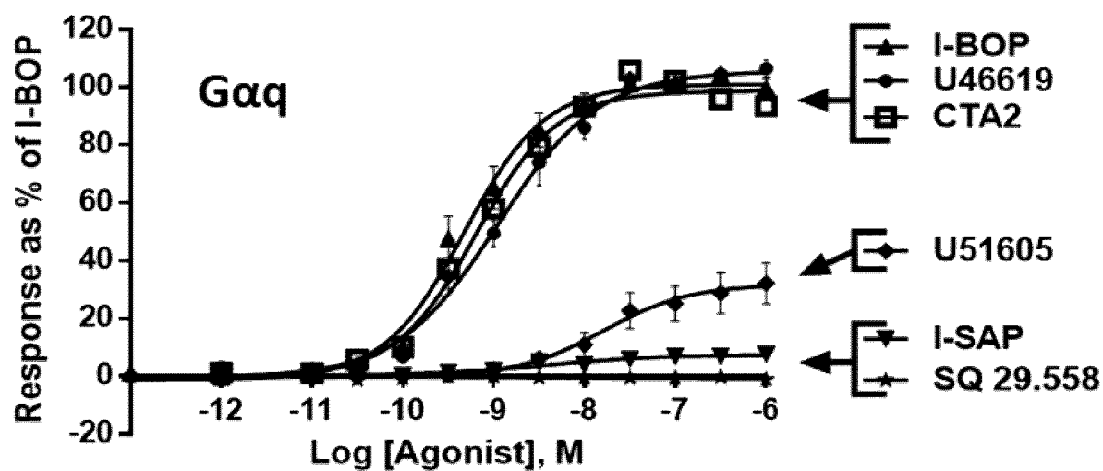
DRC:TP α R/U46619, p63RG-RlucII/G15 vs EE

FIG. 6I

DRC TP α R, p63-RlucII vs Gαq/rGFP-CAAX

	U46619	I-BOP	I-SAP	CTA2	U51605	SQ 29.558
LogEC ₅₀	-8.955	-9.324	-8.404	-9.192	-7.787	-94.50

FIG. 6J

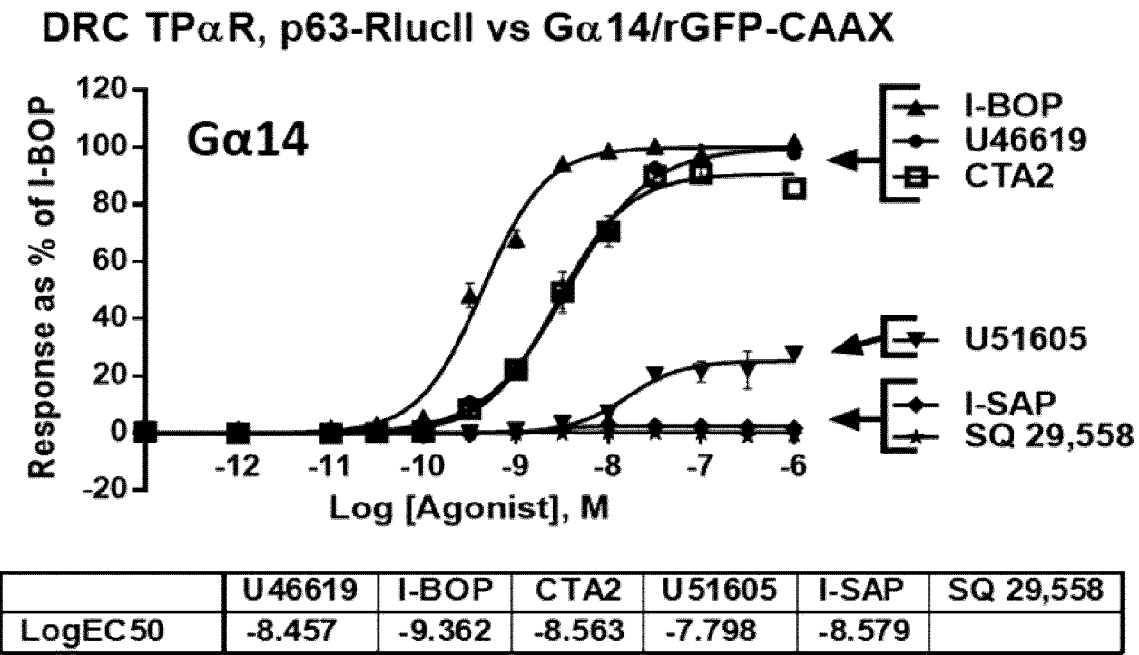


FIG. 6K

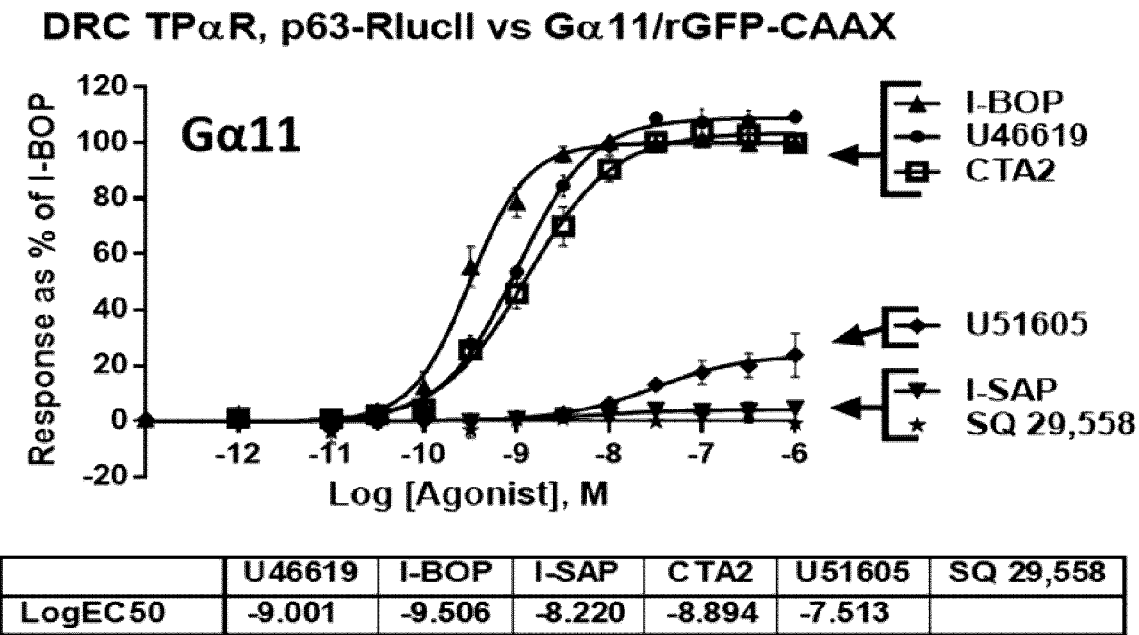
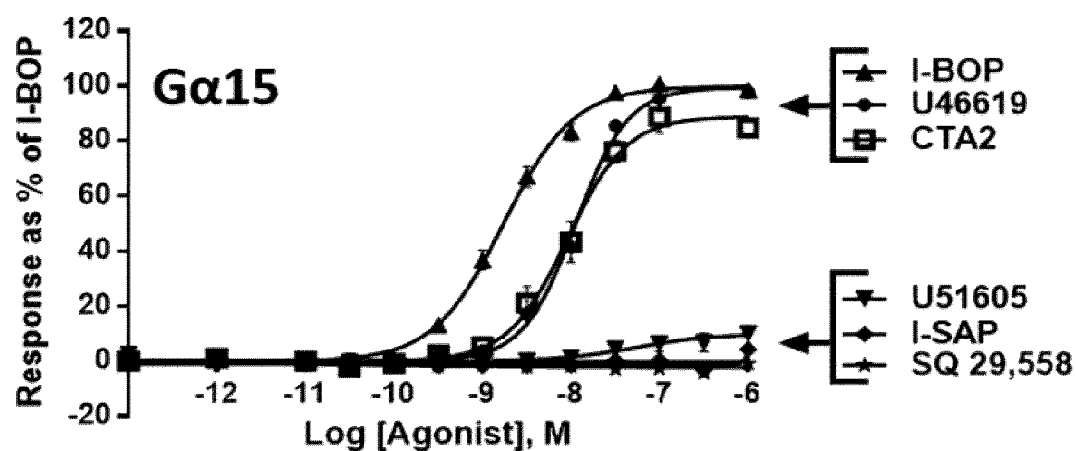
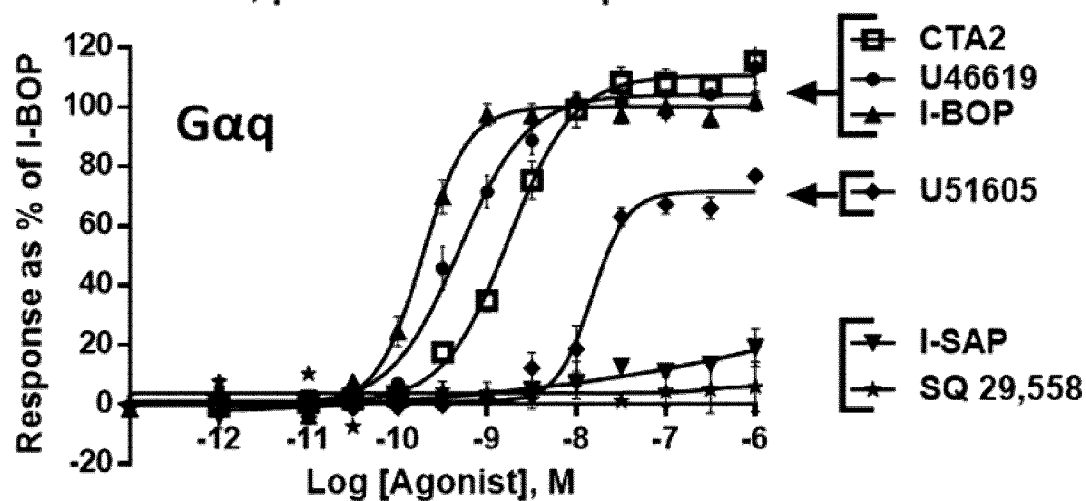


FIG. 6L

27/42

DRC TP α R, p63-RlucII vs G α 15/rGFP-CAAX

	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29,558
LogEC50	-7.952	-8.766	-8.041	-7.257	~ -5.983	-10.89

FIG. 6M**DRC TP α R, p63-RlucII vs G α q/rGFP-FYVE**

	U46619	I-BOP	I-SAP	CTA2	U51605	SQ 29,558
LogEC50	-9.313	-9.723	~ 3.747	-8.759	-7.827	-6.554

FIG. 6N

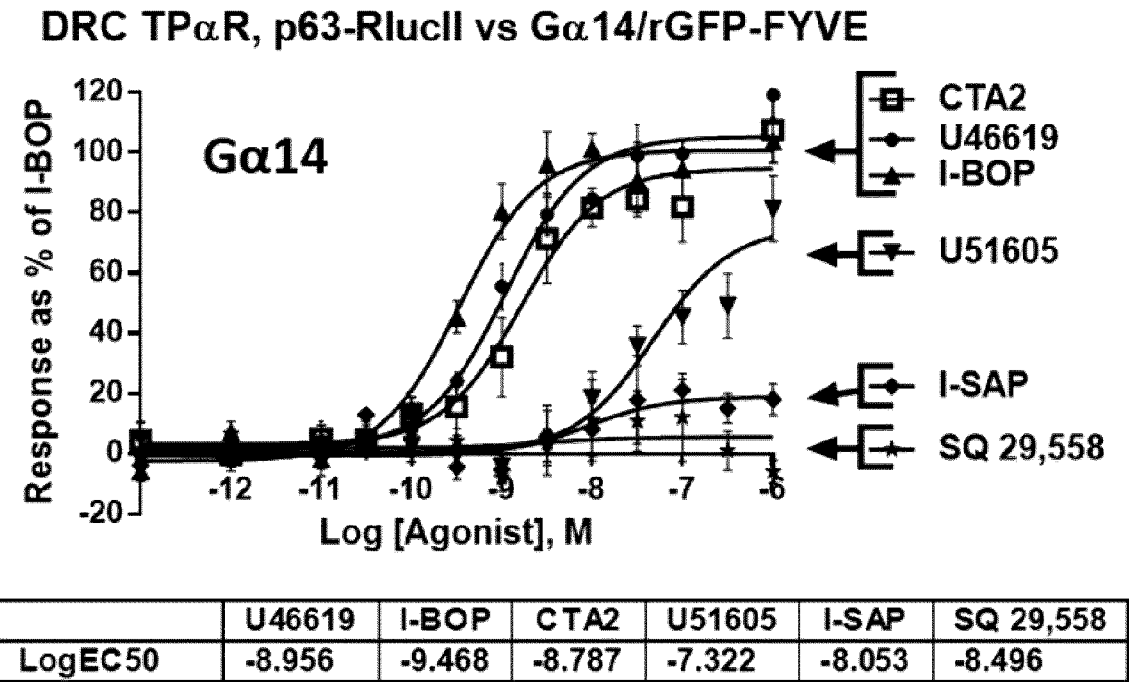


FIG. 6O

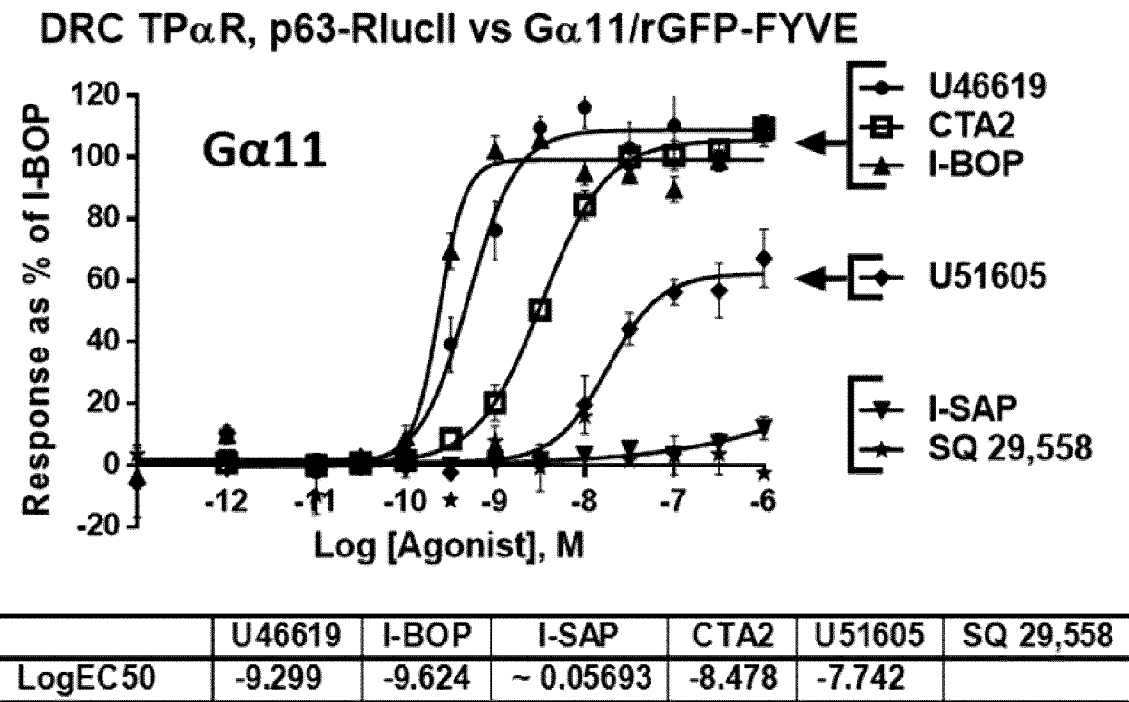
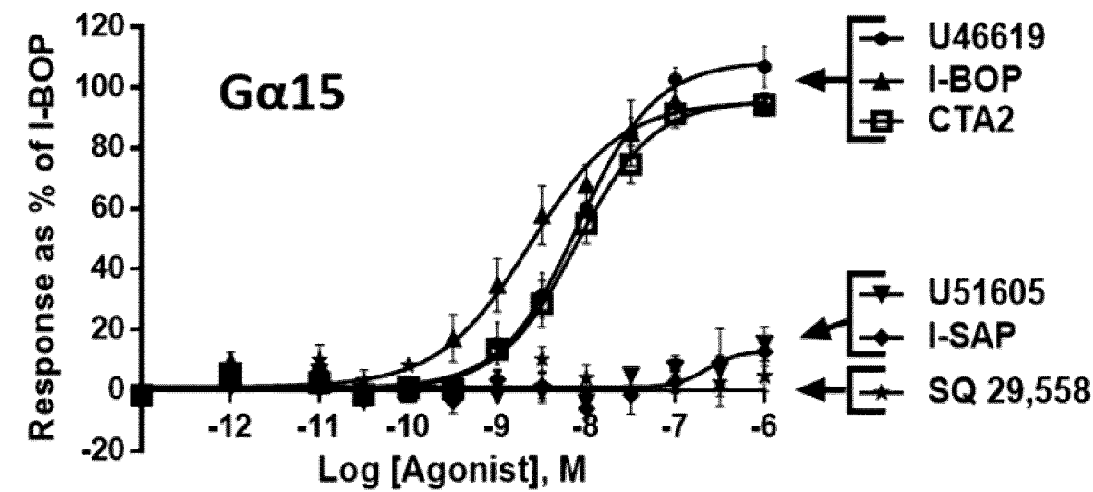


FIG. 6P

DRC TPαR, p63-RlucII vs Gα15/rGFP-FYVE



	U46619	I-BOP	CTA2	U51605	I-SAP	SQ 29,558
LogEC50	-8.089	-8.633	-8.115		-6.644	

FIG. 6Q

Z'Factor: TPαR, p63RG-RlucII/Gq sensor

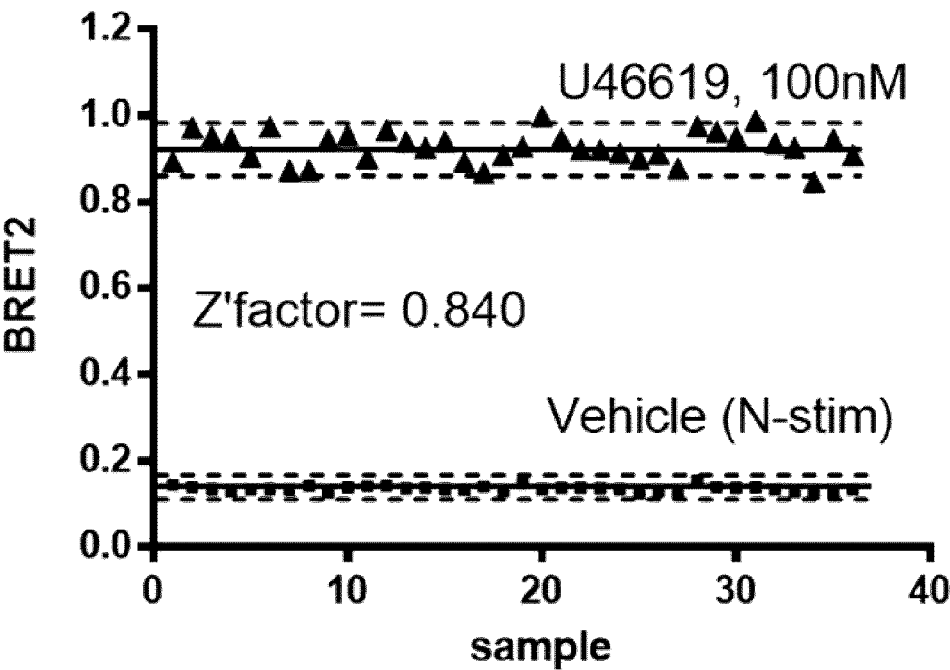
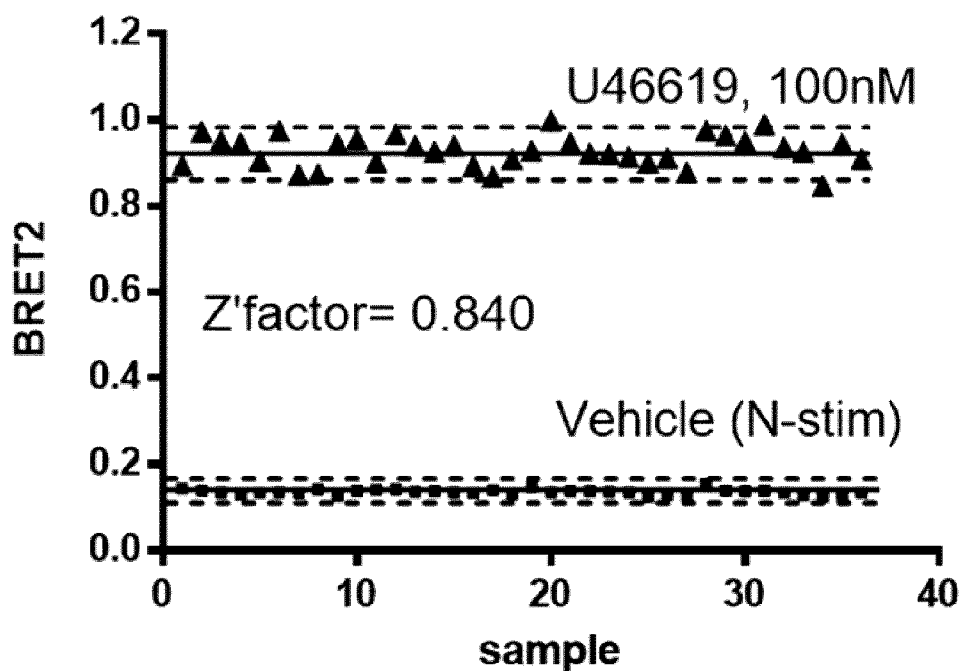
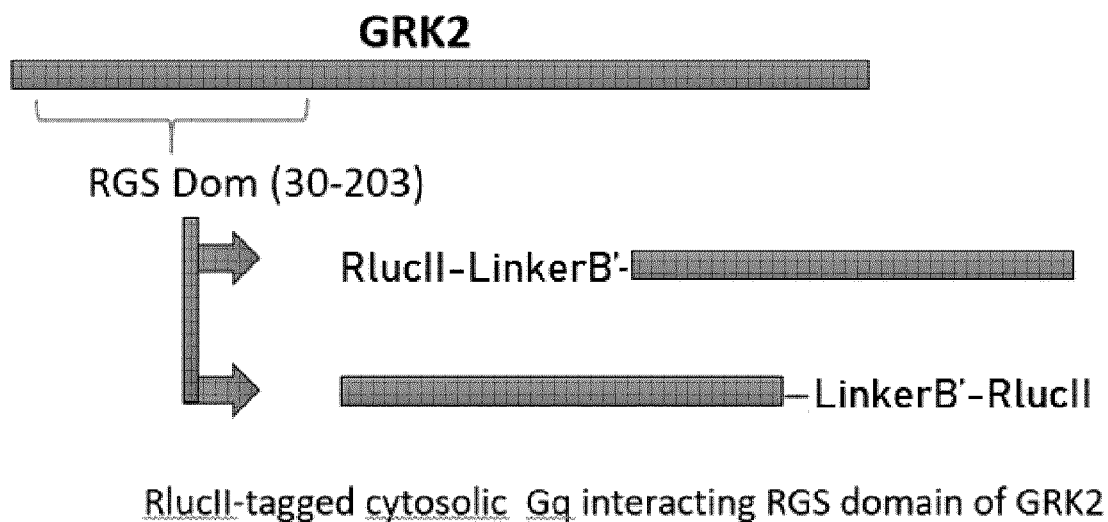
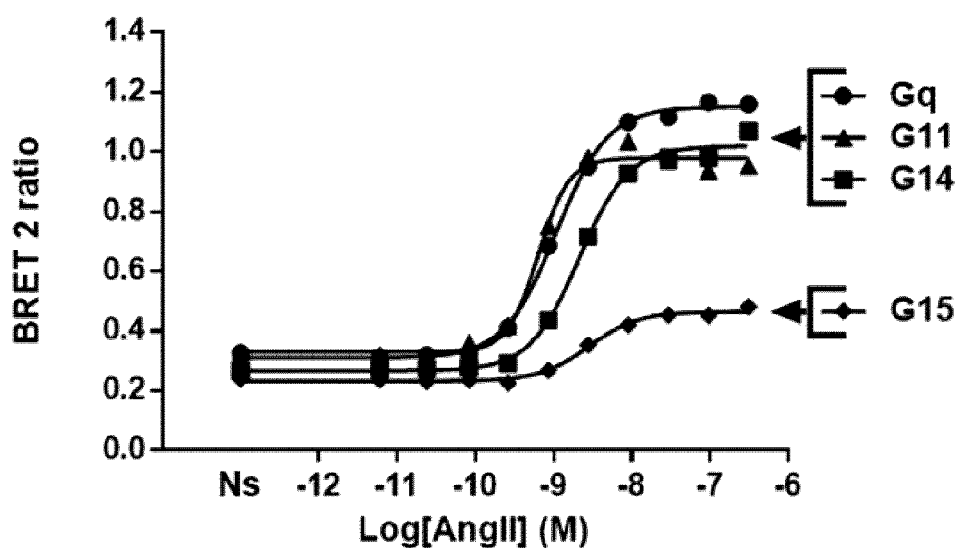
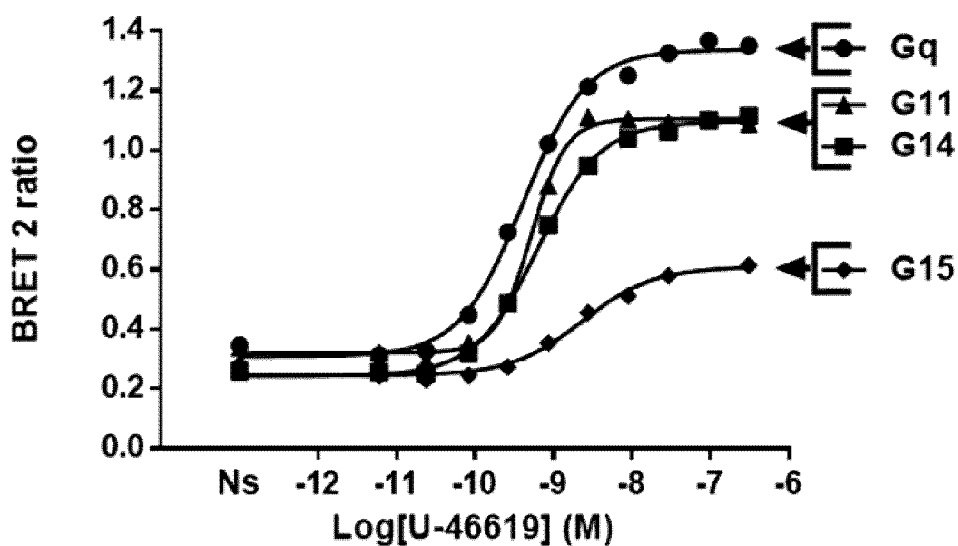


FIG. 6R

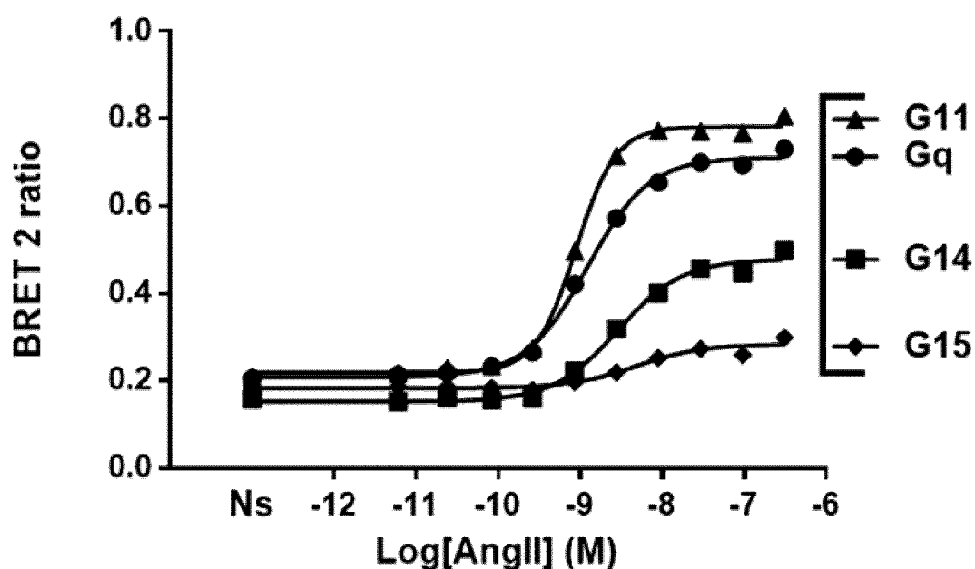
30/42

Z'Factor: TP α R, p63RG-RlucII/Gq sensor**FIG. 6S****LinkerB'** =GSAGTGGRAIDIKLASAT**FIG. 7A**

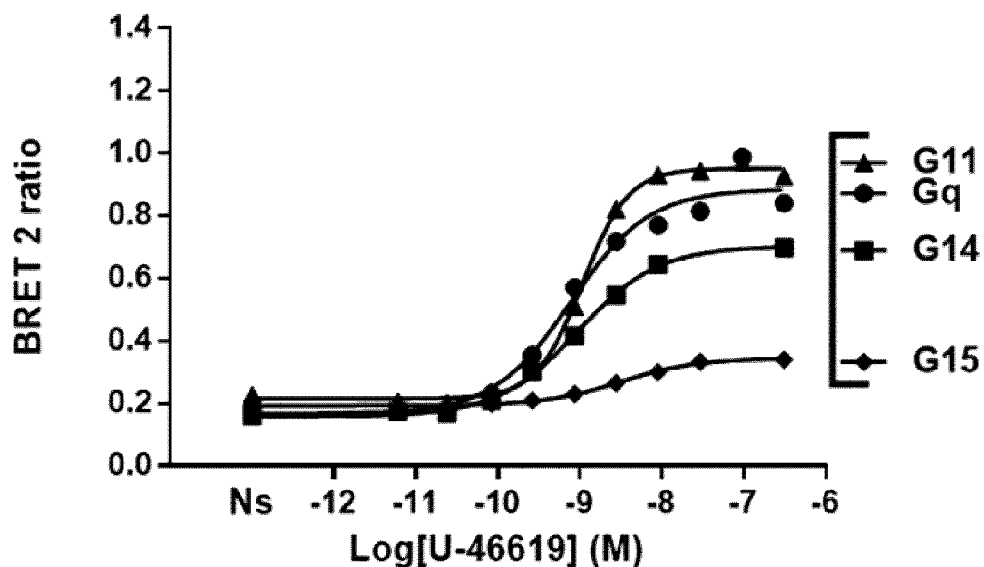
31/42

DRC AT1aR/AngII: RlucII-RGS(GRK2) vs $G\alpha$ /rGFP-CAAX**FIG. 7B****DRC TP α R/U46619: RlucII-RGS(GRK2) vs $G\alpha$ /rGFP-CAAX****FIG. 7C**

32/42

DRC AT1aR/AngII: RGS(GRK2)-RlucII vs G α /rGFP-CAAX

	Gq	G11	G14	G15
LogEC50	-8.914	-9.055	-8.543	-8.324

FIG. 7D**DRC TP α R/U46619: RGS(GRK2)-RlucII vs G α /rGFP-CAAX**

	Gq	G11	G14	G15
LogEC50	-9.107	-8.969	-9.024	-8.514

FIG. 7E

Z'Factor:TP α R, RlucII-RGS(GRK2)/Gq sensor

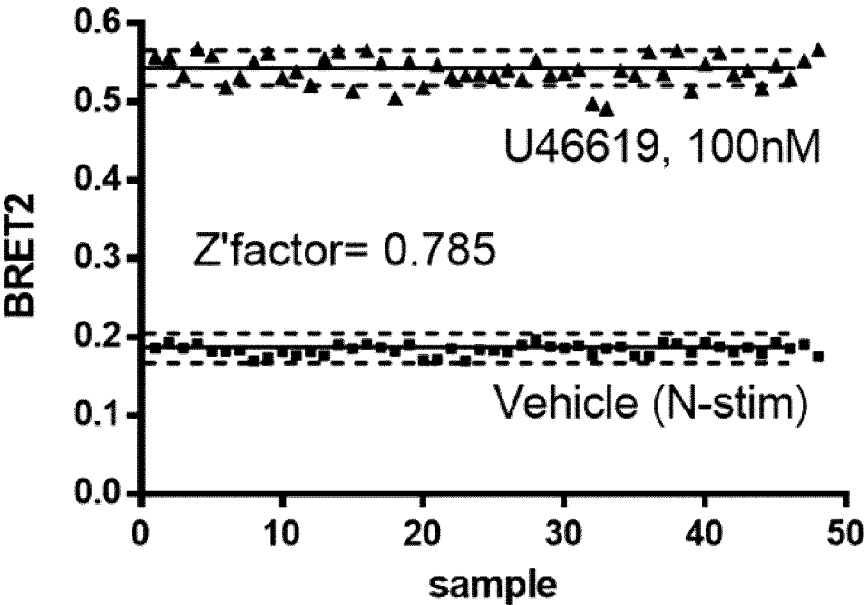
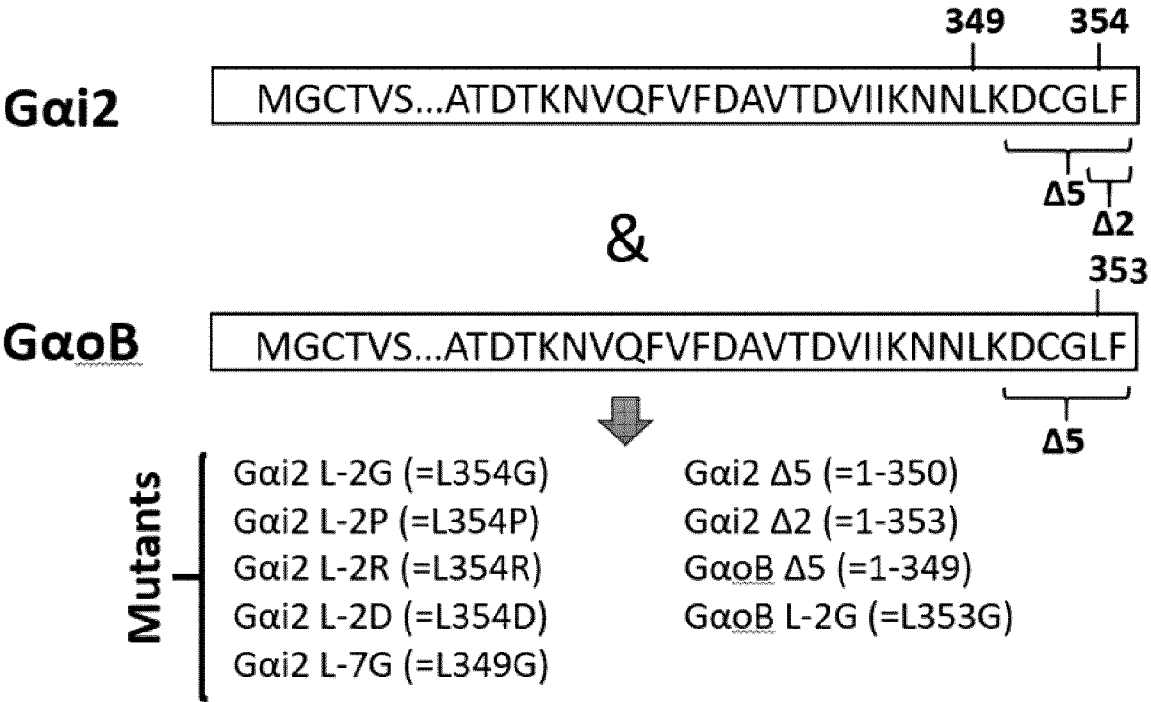
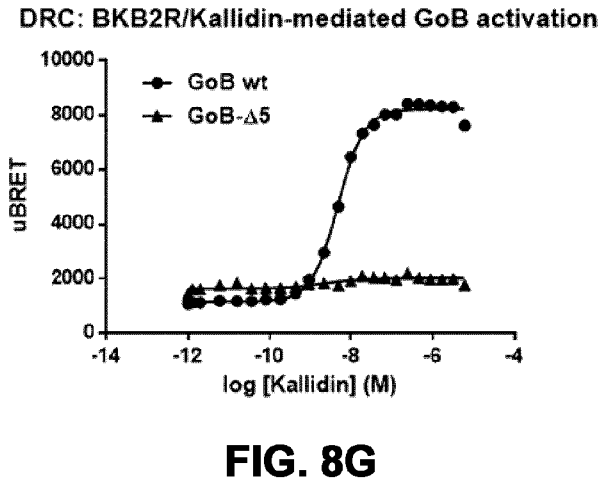
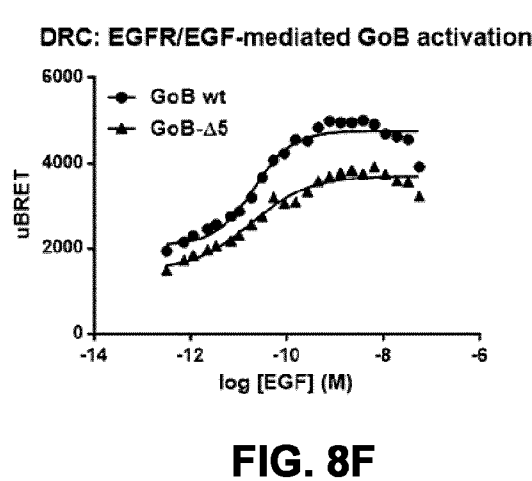
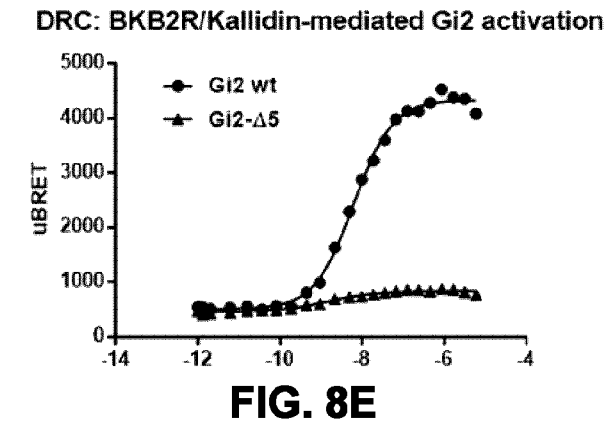
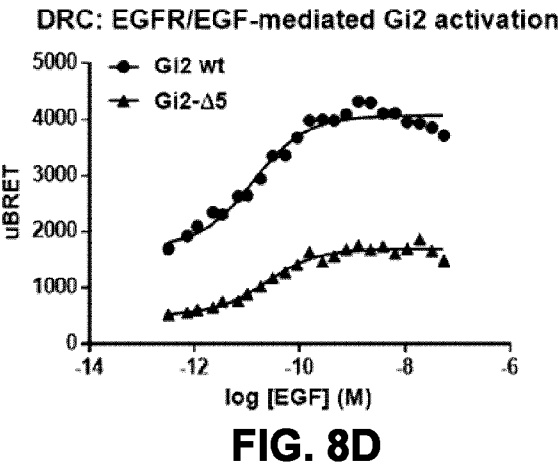
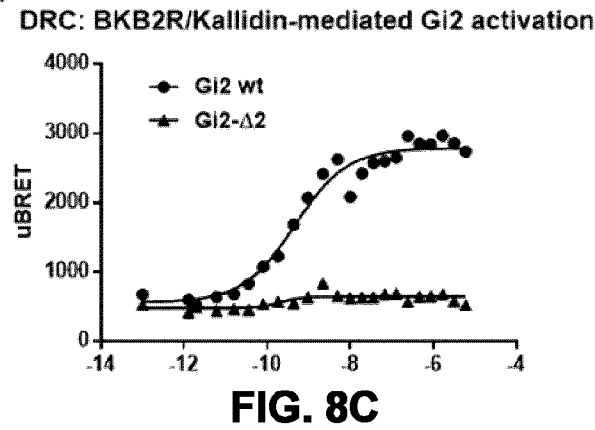
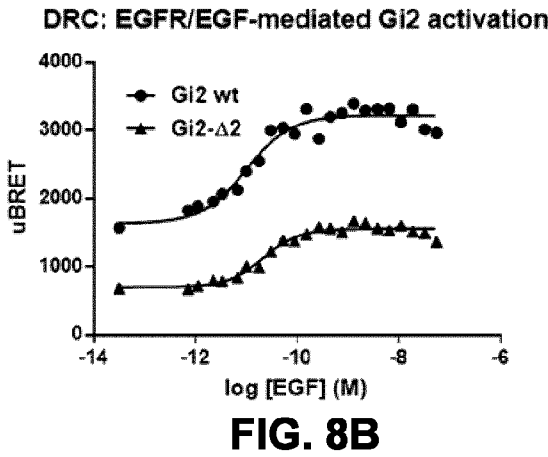


FIG. 7F



Mutations that prevent GPCR-mediated Gprotein activation, while keeping GIV/Girdin-mediated Gprotein activation

FIG. 8A



35/42

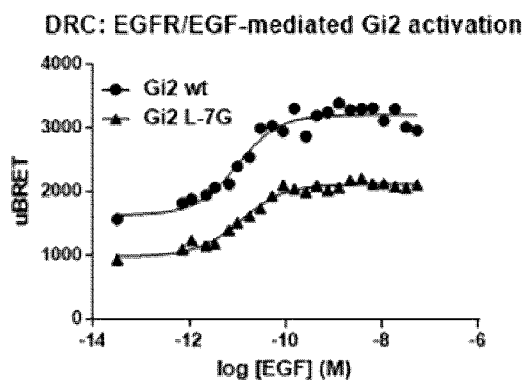


FIG. 8H

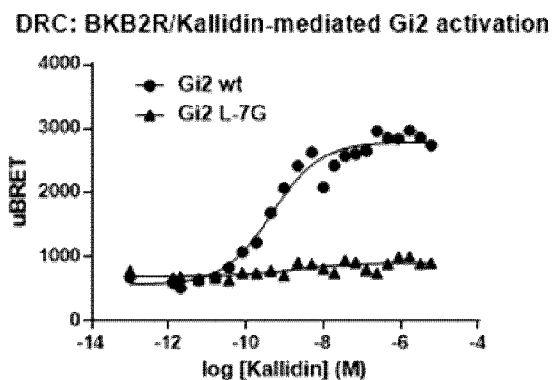


FIG. 8I

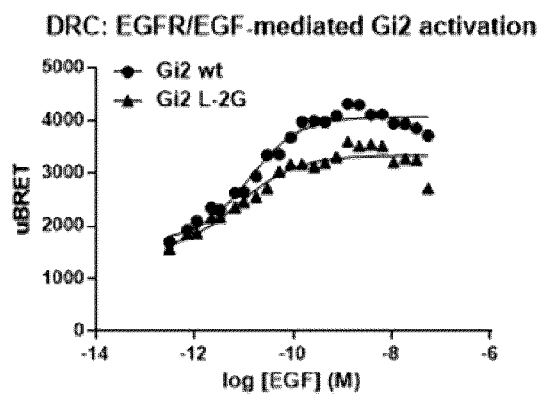


FIG. 8J

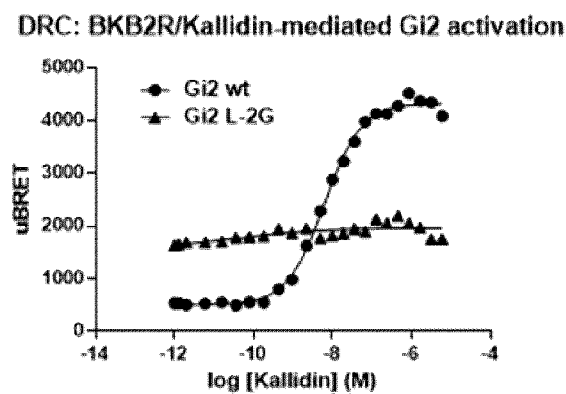


FIG. 8K

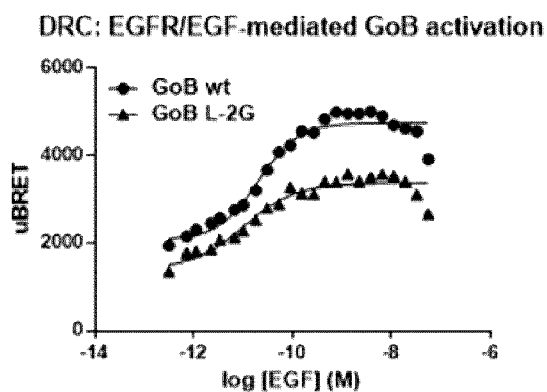


FIG. 8L

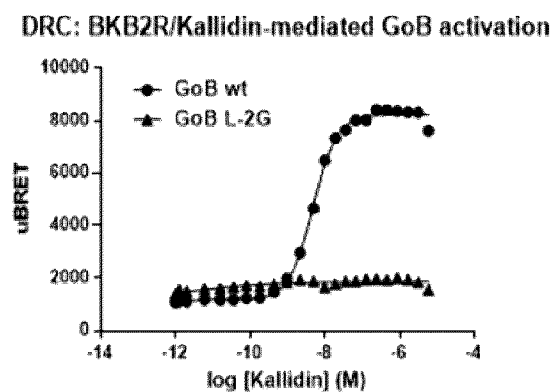


FIG. 8M

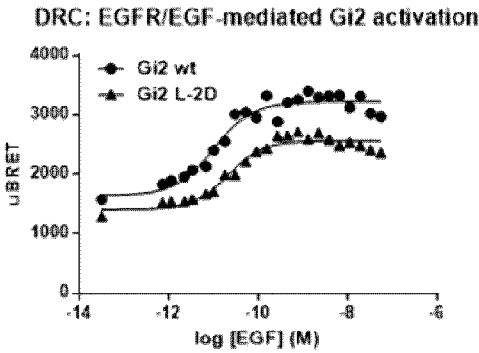


FIG. 8N

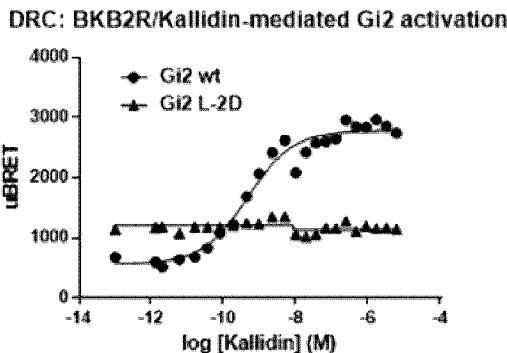


FIG. 8O

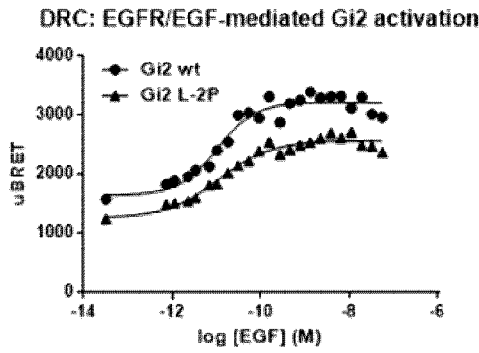


FIG. 8P

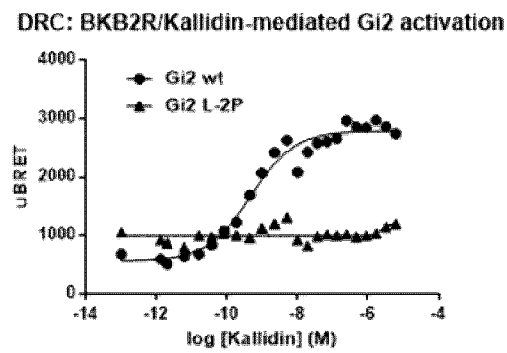


FIG. 8Q

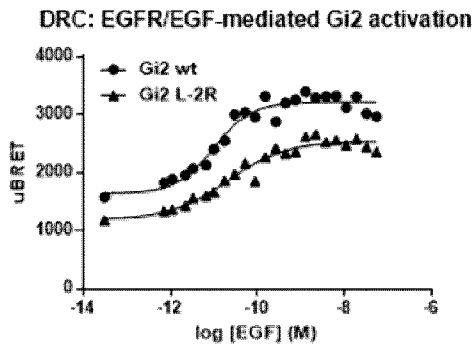


FIG. 8R

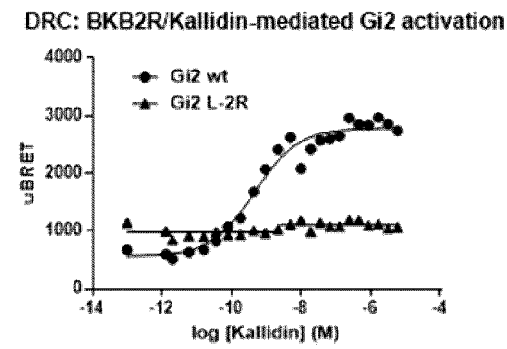
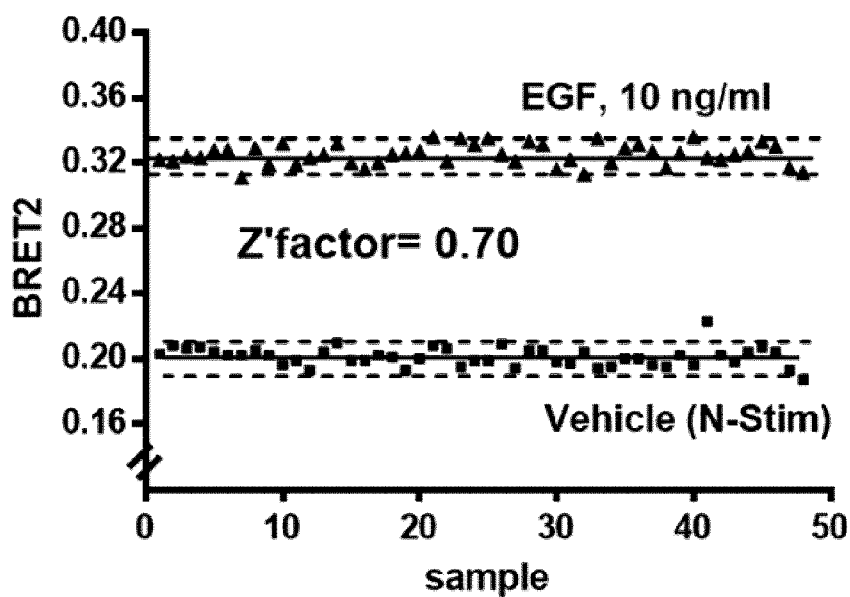
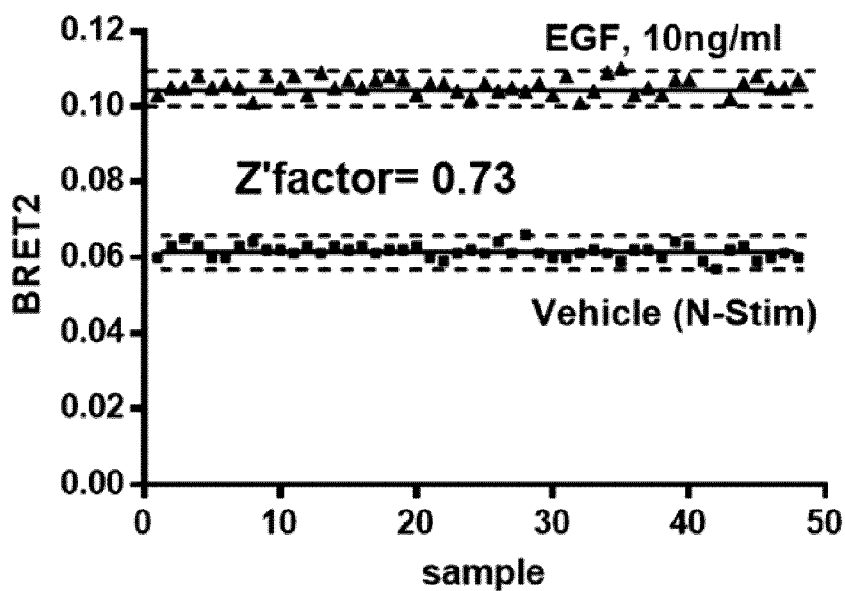


FIG. 8S

37/42

Z' factor: EGFR, Rap1GAP (SSS-AAA)/Gi2-WT**FIG. 8T****Z' factor: EGFR, Rap1GAP (SSS-AAA)/Gi2_L-2P****FIG. 8U**

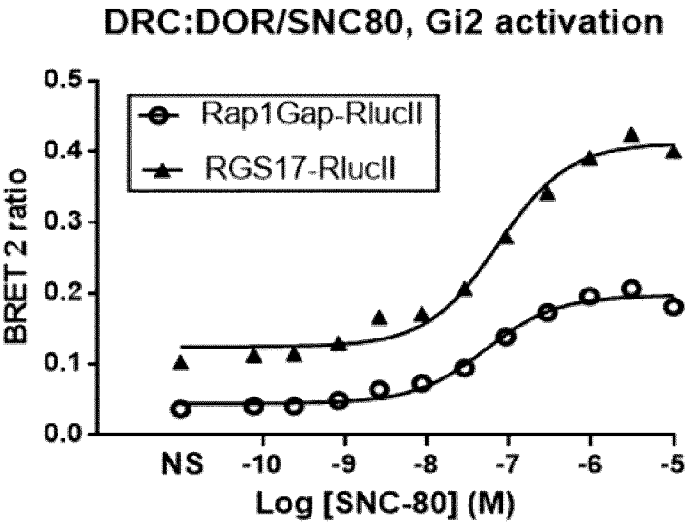


FIG. 8V

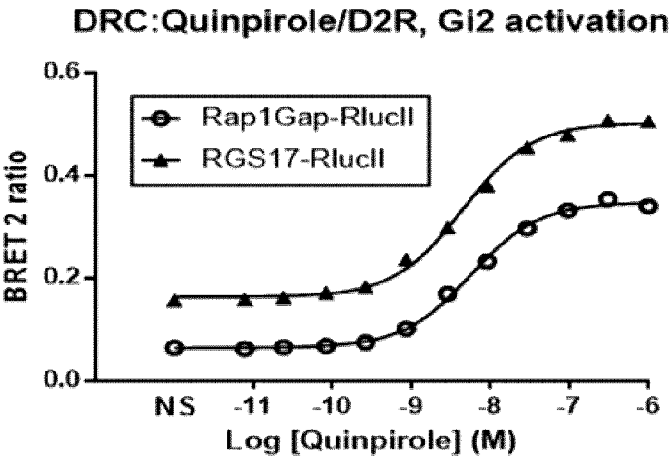


FIG. 8W

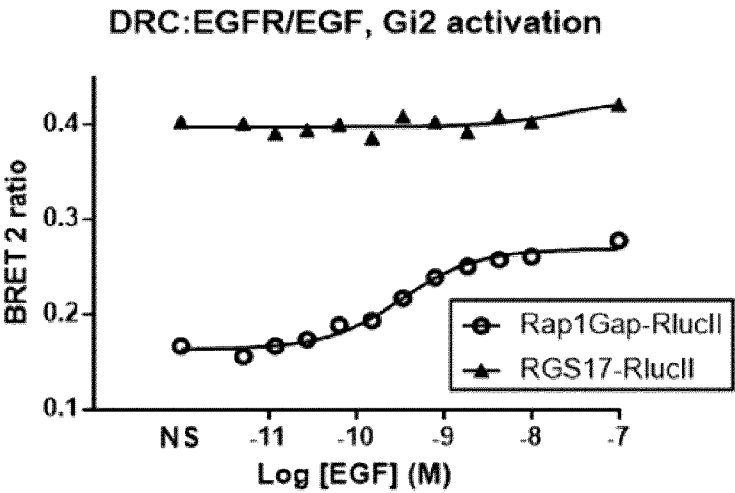


FIG. 8X

39/42

>Rap1GAP (1-442) (SEQ ID NO: 8)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVRSRQSM

>Rap1GAP ΔSSS (1-436) (SEQ ID NO: 9)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVR

>Rap1GAP (1-420) (SEQ ID NO: 10)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMEN

>Rap1GAP SSS-AAA (1-442; S437A/S439A/S441A) (SEQ ID NO: 11)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVIRARAQAM

>Rap1GAP SSS-TTT (1-442; S437T/S439T/S441T) (SEQ ID NO: 12)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVIRTRTQTM

>Rap1GAP SS-AA (1-442; S437A/S441A) (SEQ ID NO: 13)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVIRARSQAM

>Rap1GAP SS-DA (1-442; S437A/S441A) (SEQ ID NO: 14)

MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPTTKVK
 LECNPTARIYRKHFLGKEHFNYSLDAALGHLVFSLYKDVIGDQEHRLRLLRRTKCRTHYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRLFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLQGTSEEEELFSTNEESPAFVEFLEFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVPMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINAEYACYKA EKFAKLEERTRAALLETL YEE
 LHIHSQSMMGLGGDEDKMENGSGGGGFESFKRIVIRDRSQAM

FIG. 9A

40/42

>Rap1GAP SS-AD (1-442; S437A/S441D) (SEQ ID NO: 15)
 MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPPTTKVK
 LECNPTARIYRKHFLGKEHFNYYSLDAALGHLVFSCLKYDVIGDQEHRLRLRLTKCRTYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLGQTSEELFSTNEESPAFVEFLBFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVDPDMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINA EYACYKAEKFAKLEERTRAALLETL YEE
 LHIHSQSMMLGGDEDKMENGSGGGGFFESFKRVIRARSQDM

>Rap1GAP SS-DD (1-442; S437A/S441A) (SEQ ID NO: 16)
 MIEKMQGSRMDEQRCSFPPPLKTEEDYIPYPSVHEVLGREGPFPLILLPQFGGYWIEGTNHEITSIPETEPLQSPPTTKVK
 LECNPTARIYRKHFLGKEHFNYYSLDAALGHLVFSCLKYDVIGDQEHRLRLRLTKCRTYHDVIPISCLTEFPNVVQMAKL
 CEDVNVDRFYPVLYPKASRLIVTFDEHVISNNFKFGVIYQKLGQTSEELFSTNEESPAFVEFLBFLGQKVKLQDFKGR
 GGLDVTHGQTGTESVYCNFRNKEIMFHVSTKLPYTEGDAQQLQKRKHIGAAIVAVVFQDENTPFVDPDMIASNFLHAYVVV
 QAEGGGPDGPLYKVSVTARDDVPFFGPPLPDPAVFRKGPEFQEFLLTKLINA EYACYKAEKFAKLEERTRAALLETL YEE
 LHIHSQSMMLGGDEDKMENGSGGGGFFESFKRVIRDRSQDM

>P63 RGS (residues 295-502 of SEQ ID NO: 25)
 MIMKYQLLLKDFLKYYNRAGMDTADLEQAVEVMCFVPKRCNDMMTLGRLRGFEGKLT AQGKLLGQDTFWVTEPEAGGLLS
 SRGRERRVFLFEQIIIFSEALGGGVRGGTQPGYVYKNSIKVSCLGLEGNLQGDPCR FALTSRGPEGGIQRYVLQAADPAI
 SQAWIKHVAQILESQRDFLNALQSPIEQRRSQTNLSLRPRGPGVGS

>PDZRG (=ARHGEF11) (residues 281-483 of SEQ ID NO: 21)
 QGVDQSPKPLIIGPEEDYDGYFNESDIIFQDLEKLKSRPAHLGVFLRYIFSQADPSPLLFYLC AEVYQASPKDSRSL
 GKDIWNIFLEKNAPLRVKIPEMLQAEIDSRLRNSEDARGVLCEAQEAAMPEIQEQIHDYRTKRTLGLGS LYGENDLLDL
 GDPLRERQVAEKQLAALGDILSKYEEDRSAPMDFALNTYMSHA

>P115RG (=ARHGEF1, isoform var2) (residues 1-244 of SEQ ID NO: 23)
 MEDFARGAASPGSRPGLVPVSIIGAEDEDFENELETNSEEQNSQFQSLEQVKRRPAHLMALLQHVALQFEPGPLLCC LH
 ADMLGSLGPKEAKKAFLDYHSFLEKTAVLRVPVPENAVAFELDRTRADLISEDVQRRFVQEVVQSQQVAVGRQLEDFRSK
 RLMGMTPEWQELAQLAEAWGRDRASYEARERHVAERLLMHLEEMQHTISTDEEKSAAVVNAIGLYMRHLGVRTKSGDKKS
 GRNF

>GRK2 RGS dom (residues 30-203 of SEQ ID NO: 27)
 KILLPEPSIRSVMQKYLEDRGEVTFEKIFSQKLYLLFRDFCLNHLEEARPLVEFYEEIKKYEKLETEEEERVARSR EIFD
 SYIMKELLACSHFSSKSA TEHVQGHGKKQVPPDLFPQPIIEEICQNLRGDVFQKFIESDKFTRFCQWKNVELNIHLTMND
 FSVHRIIGRGGFG

>G α i2 Δ 5(=1-350) (SEQ ID NO: 28)
 MGCTVSAEDKAAAEERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDS AAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTGIVETHFTFKDLHFKMFDVGGQRSEKWKIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLK

>G α i2 Δ 2(=1-353) (SEQ ID NO: 29)
 MGCTVSAEDKAAAEERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDS AAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTGIVETHFTFKDLHFKMFDVGGQRSEKWKIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCG

>G α i2 L-7G(=L349G) (SEQ ID NO: 30)
 MGCTVSAEDKAAAEERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDS AAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTGIVETHFTFKDLHFKMFDVGGQRSEKWKIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCGLF

FIG. 9B

41/42

>Gαi2 L-2G(=L354G) (SEQ ID NO: 31)
 MGCTVSAEDKAAAERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKI IHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDSAAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTIVETHFTFKDLHFHFMFDVGGQRSEKRWIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCGGF

>Gαi2 L-2D(=L354D) (SEQ ID NO: 32)
 MGCTVSAEDKAAAERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKI IHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDSAAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTIVETHFTFKDLHFHFMFDVGGQRSEKRWIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCGGF

>Gαi2 L-2P(=L354P) (SEQ ID NO: 33)
 MGCTVSAEDKAAAERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKI IHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDSAAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTIVETHFTFKDLHFHFMFDVGGQRSEKRWIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCGPF

>Gαi2 L-2R(=L354R) (SEQ ID NO: 34)
 MGCTVSAEDKAAAERSKMIDKNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKI IHEDGYSEEECRQYRAVVYSNTIQS
 IMAIVKAMGNLQIDFADPSRADDARQLFALSCTAEEQGVLPDDLSGVIRRLWADHGVQACFGRSREYQLNDSAAYYLNDL
 ERIAQSDYIPTQQDVLRLTRVKTGTIVETHFTFKDLHFHFMFDVGGQRSEKRWIHC FEGVTAIIFCVALSAYDLVLAEDDEE
 MNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKITHSPLTICFPEYTGANKYDEAASYIQSKFEDLNKRKDTKEI
 YTHFTCATDTKNVQFVFDAVTDV I I KNNLKDCGRF

>GαoB L-2G(=L353G) (SEQ ID NO: 35)
 MGCTLSAEERAALERSKAIEKNLKEDGISAAKDVKLLLLGAGESGKSTIVKQMKI IHEDGFSGEDVKQYKPVVYSNTIQS
 LAATVRAMDTLGIEYGDKERKADAKMVCVVSRMEDTEPFSAELLSSAMMRLWGD SGIQECFNRSREYQLNDSAKYYLDSL
 DRIGAADYQPTQDILRLTRVKTGTIVETHFTFKDLHFRLFDVGGQRSEKRWIHC FEGVTAIIFCVALSGYDQVLHEDET
 TNRMHESLKLFDSCNNKWFTDTSIILFLNKKDIFEEKIKKSPLTICFPEYTGPSAFTEAVAYIQAQYESKNKSAHKEIY
 THVTCATDTNNIQFVFDAVTDV I I AKNLRGCGGY

>GαoB Δ5(=1-349) (SEQ ID NO: 36)
 MGCTLSAEERAALERSKAIEKNLKEDGISAAKDVKLLLLGAGESGKSTIVKQMKI IHEDGFSGEDVKQYKPVVYSNTIQS
 LAATVRAMDTLGIEYGDKERKADAKMVCVVSRMEDTEPFSAELLSSAMMRLWGD SGIQECFNRSREYQLNDSAKYYLDSL
 DRIGAADYQPTQDILRLTRVKTGTIVETHFTFKDLHFRLFDVGGQRSEKRWIHC FEGVTAIIFCVALSGYDQVLHEDET
 TNRMHESLKLFDSCNNKWFTDTSIILFLNKKDIFEEKIKKSPLTICFPEYTGPSAFTEAVAYIQAQYESKNKSAHKEIY
 THVTCATDTNNIQFVFDAVTDV I I AKNLR

>RLucII (SEQ ID NO: 37)
 MTSKVYDPEQRKRMITGPQWWARCKQMNVLDSFINYYDSEKHAENAVIFLHGNATSSYLWRHVVP HIEPVARCIIPDLIG
 MGKSGKSGNGSYRLLDHYKYLTAWFELNLNPKK IIFVGHWDGAALAFHYSYEHQDKI KAIVHAESVVDVIESWDEWPDIE
 EDIALIKSEEKGMVLENNFFVETVLP SKIMRKLEPEEFAAYLEPFKEKGEVRRPTLSWP REIPLVKGKPDVVQIVRNY
 NAYLRASDDLPKMFIESDPGFFSNAIVEGAKKFPNTEFVKVKGHLHFSQEDAPDEMGKYIKSFVERVLKNEQ

>rGFP: (SEQ ID NO: 38)
 MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEKNILEGTQEVKISVTKGAPLPFAFDIVSVAFSYGNRAYTGYPEEIS
 DYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLEDGKFIVNVDFKAKDLRRMGPMQDDIVGMQPSYESMYTNVTSVIGE
 CIIAFKLQTKGHFTYHMRTVYKSKKPVETMPLYHF IQHRLVKTNVDTASGYVQHETAIAAHSTIKKIEGSLP

>endofin's FYVE domain (SEQ ID NO: 39)
 QKQPTWVPDSEAPNCMNCQVKFTFTKRRHHCRACGKVFCGVCCNRKCKLQYLEKEARVCVVCYETISK

>CAAX sequence; plasma-membrane targeting domain from KRAS (SEQ ID NO: 1)
 GKKKKKSKTKCVIM

42/42

FIG. 9C

>RGS(RGS17;residues 64-210 of SEQ ID NO:17)
IQVLEECQNPTAEEVLSWSQNFDKMMKAPAGRNLFRFLRTEYSEENLLFWLACEDLKKEQNKKVIEEKARMIYEDYISI
LSPKEVSLDSRVREVINRNLLDPNPHMYEDAQLQIYTLMHRDSFPRFLNSQIYKSFVESTAGSSSES

>RGS(RGS19;residues 70-217 of SEQ ID NO:18)
LPSCEVCATPSPEEVQSWAQSFDKLMHSPAGRSVFRAFLRTEYSEENMLFWLACEELKAEANQHVVDEKARLIYEDYVSI
LSPKEVSLDSRVREGINKKMQEPSAHTFDDAQLQIYTLMHRDSYPRFLSSPTYRALLLQGPSQSSSEA

>RGS(RGS20;residues 242-388 of SEQ ID NO:19)
LPTWEESPAPTLEEVDNAWAQSFDKLMVTPAGRNAFRFLRTEFSEENMLFWMAEELKKEANKNIIIEEKARIYEDYISI
LSPKEVSLDSRVREVINRNMVEPSQHIFDDAQLQIYTLMHRDSYPRFMNSAVYKDLLQSLSEKSIEA

FIG. 9D