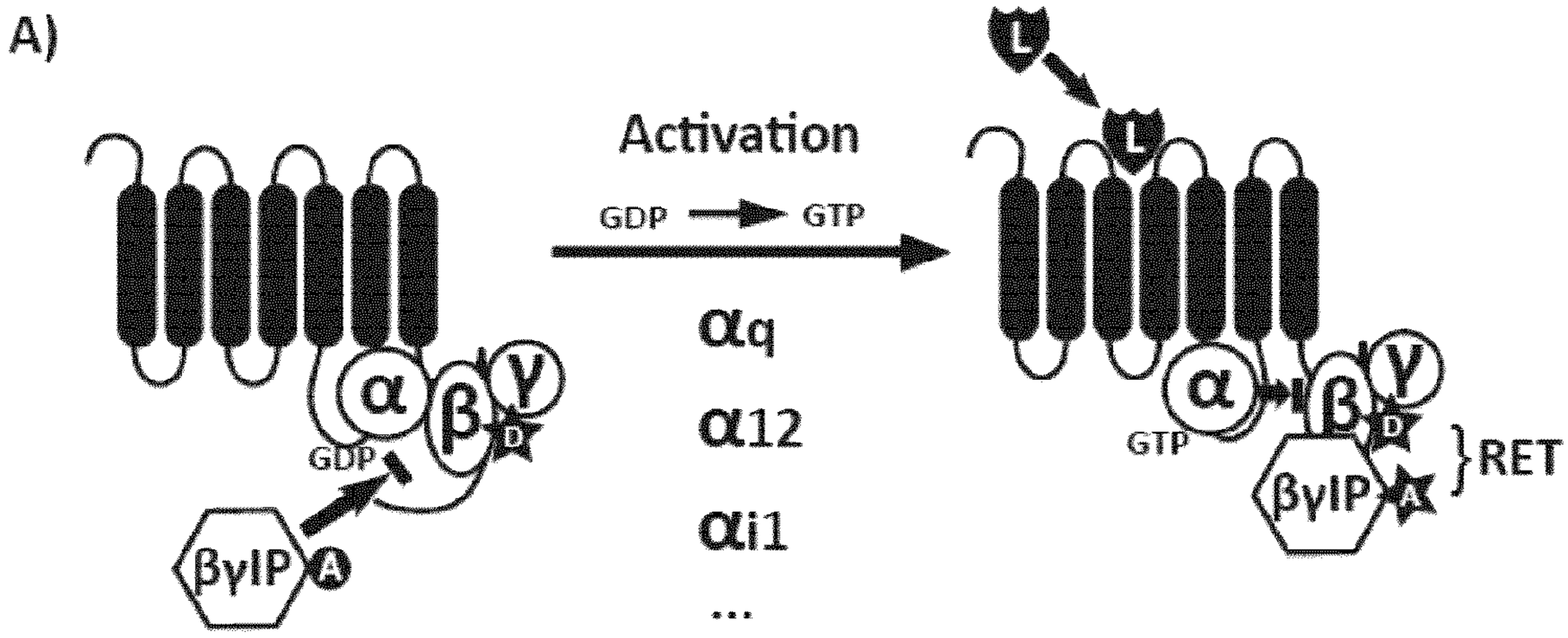

A)
Activation
GDP → GTP
αq
α12
αi1
...
GDP
GTP
βγIP
A
RET
Legend: D =RET donor A =RET acceptor A =Excited RET acceptor L = GPCR ligand

FIG. 1A

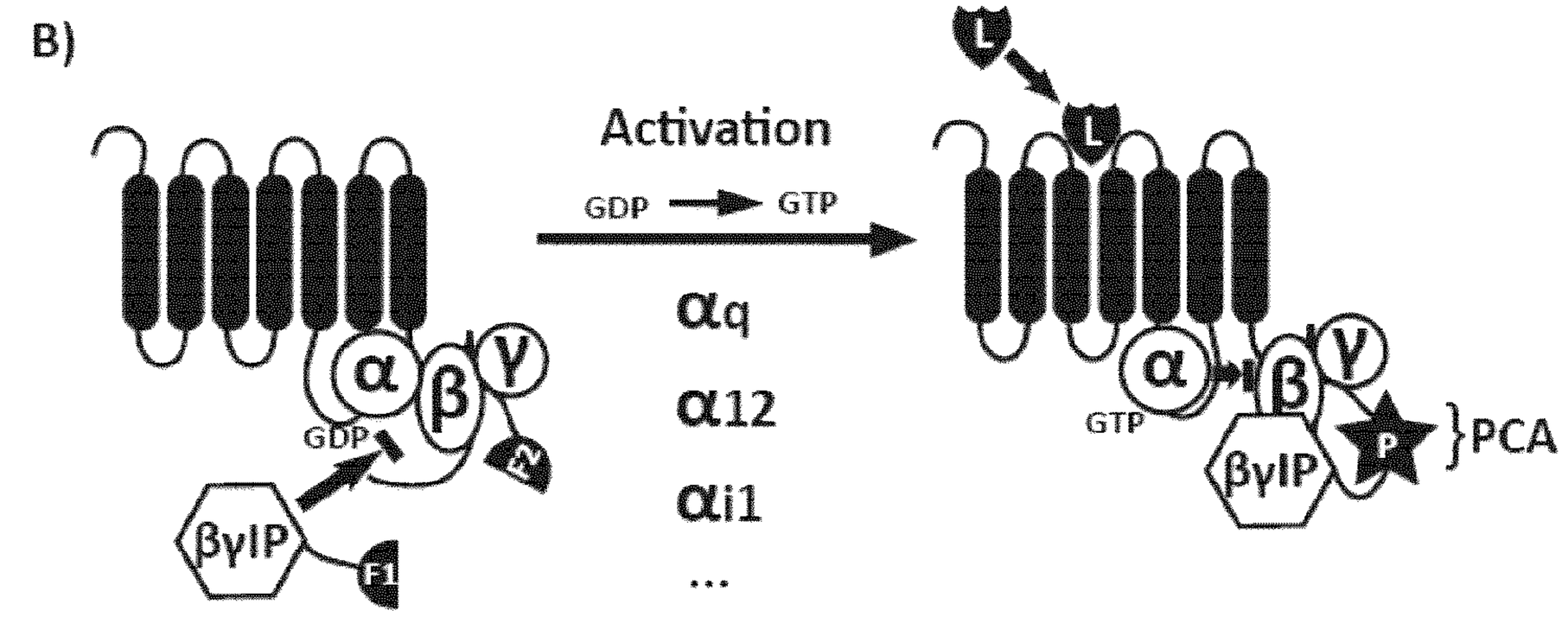

B)
Activation
GDP → GTP
αq
α12
αi1
...
GDP
GTP
βγIP
F1
F2
PCA
Legend: F1 F2 = fragments from a fluorescent or luminescent protein L = GPCR ligand
P = Fluorescent or luminescent protein reconstituted from fragments 1 and 2

FIG. 1B

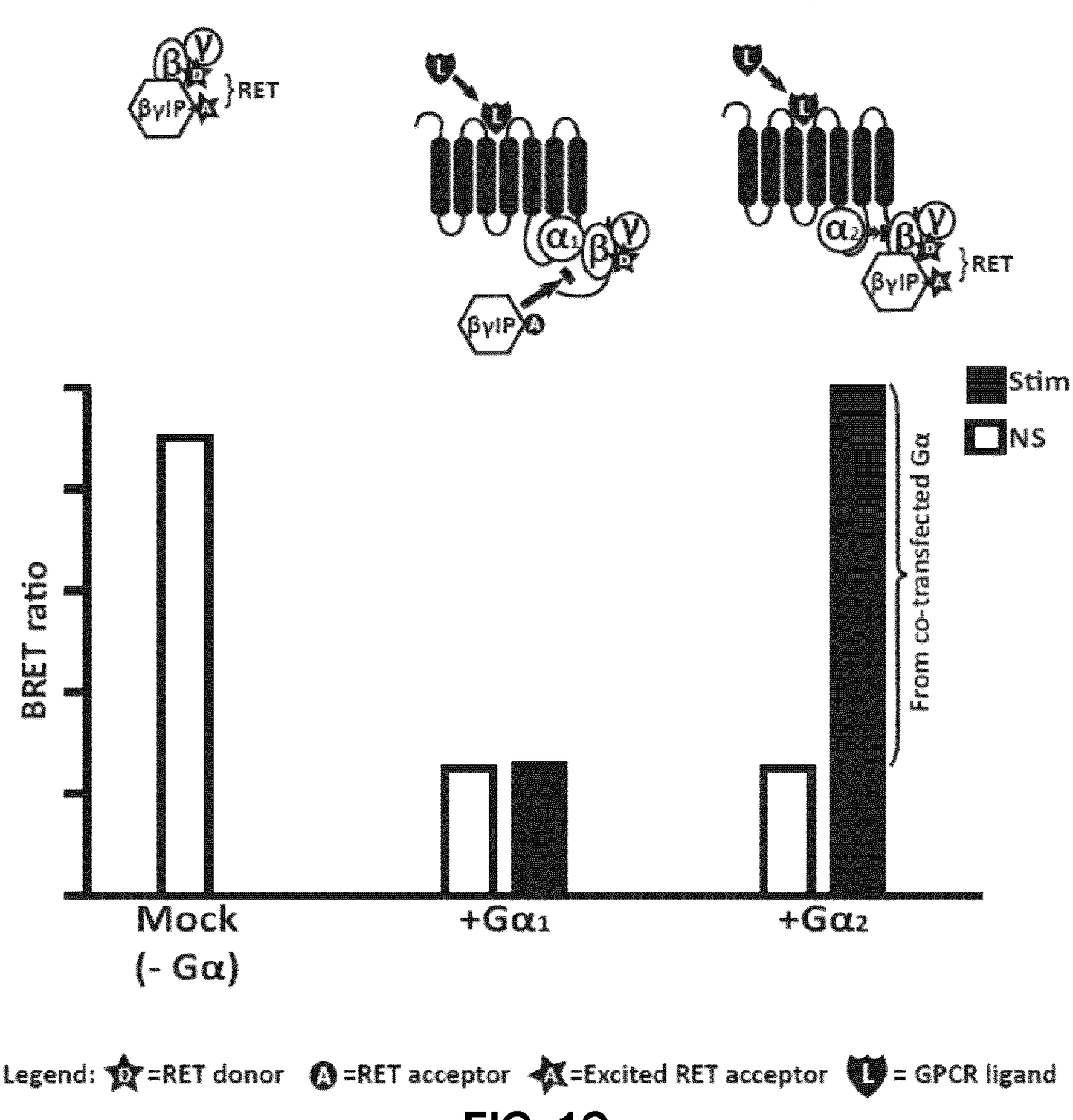
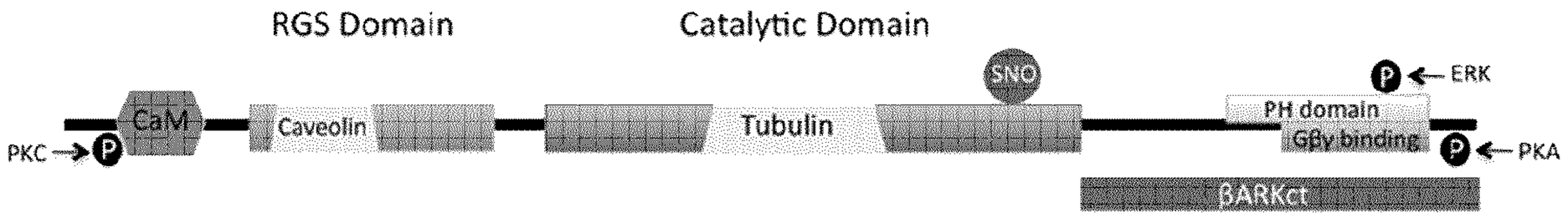

Scenario 1:
Without over-expressed Gα
Scenario 2:
Receptor is not fonctionally coupled to over-expressed Gα1
Scenario 3:
Receptor is functionally coupled to over-expressed Gα2
βγ
D
βγIP
A
}RET
L
α1
α2
Stim
NS
From co-transfected Gα
BRET ratio
Mock
(- Gα)
+Gα1
+Gα2
Legend: D =RET donor A =RET acceptor A =Excited RET acceptor L = GPCR ligand

FIG. 1C

## Structure of GRK2 & 3:




## Constructs:

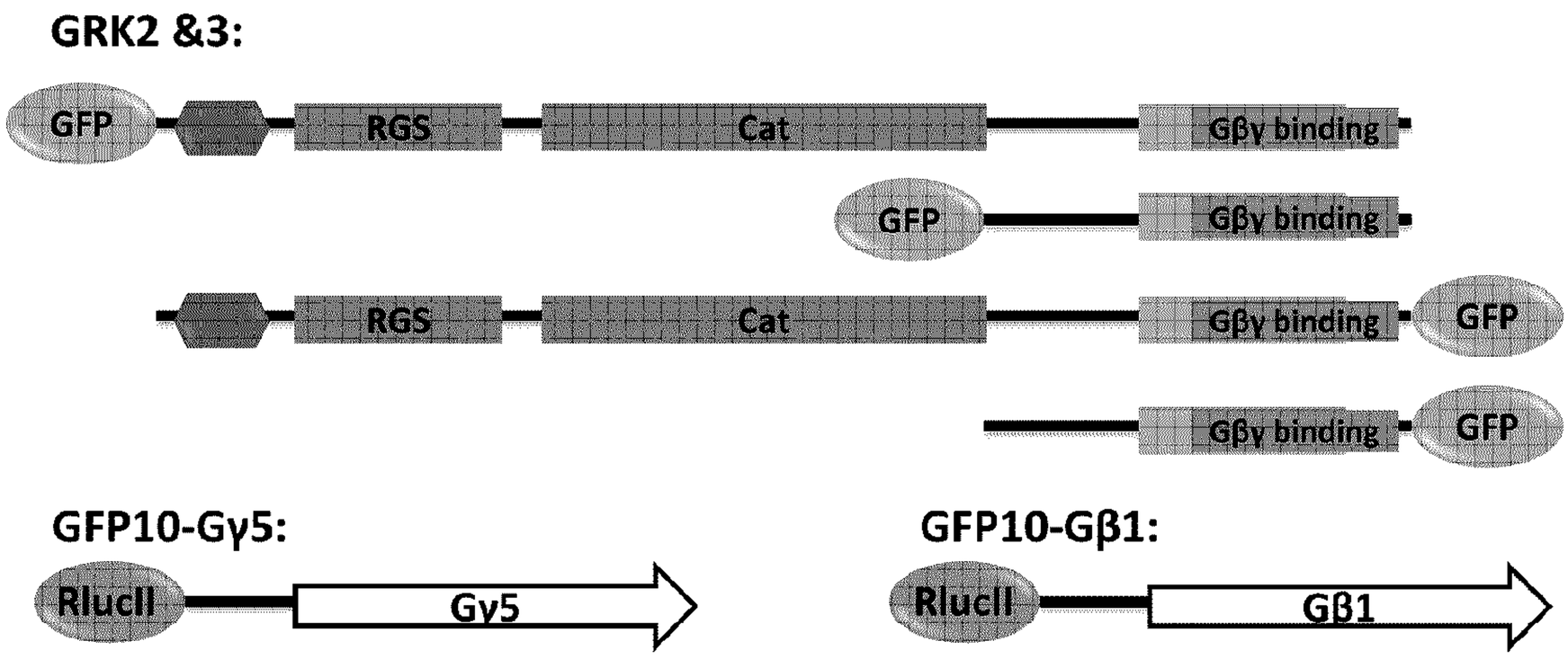



FIG. 2A

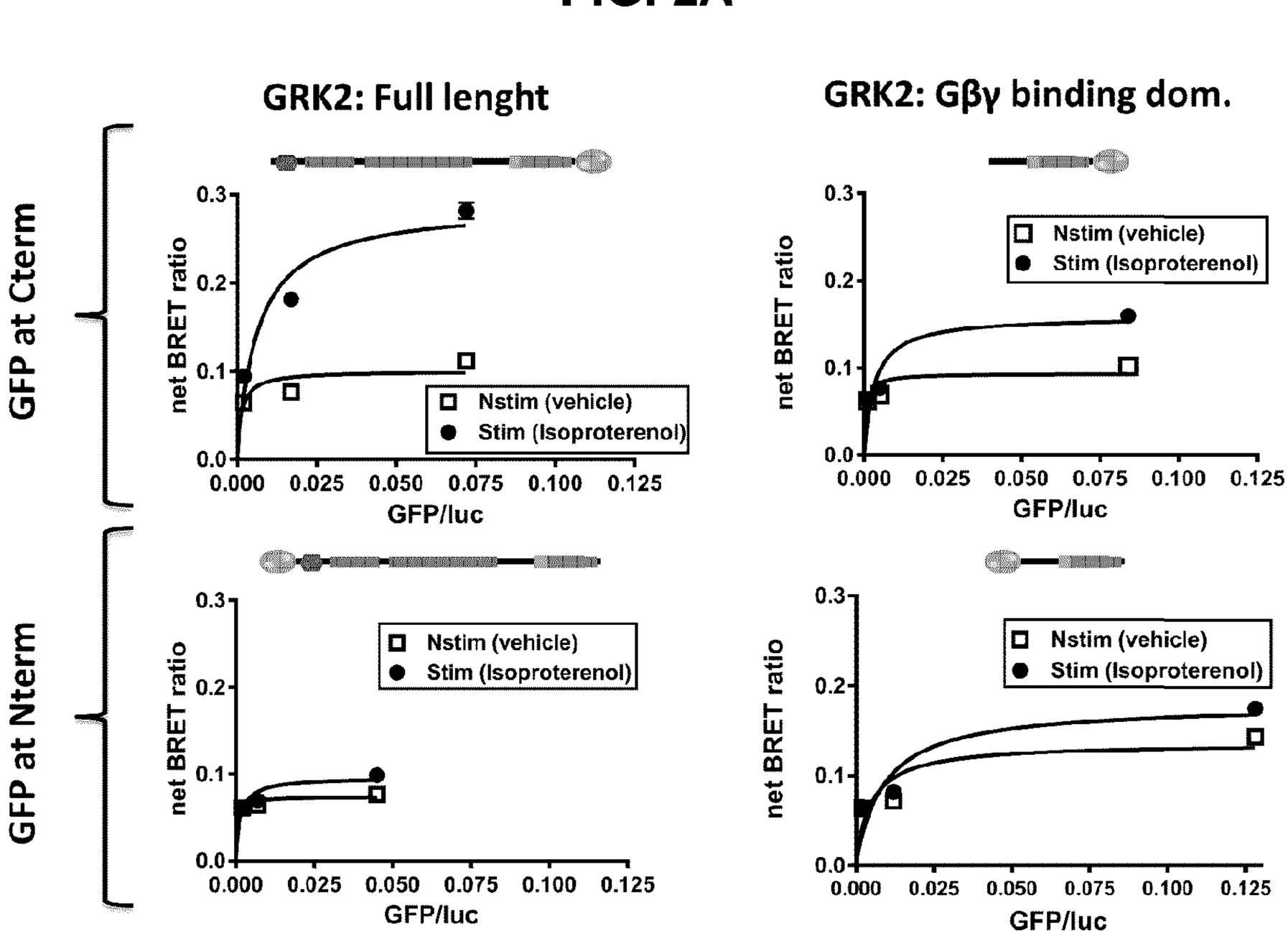



FIG. 2B

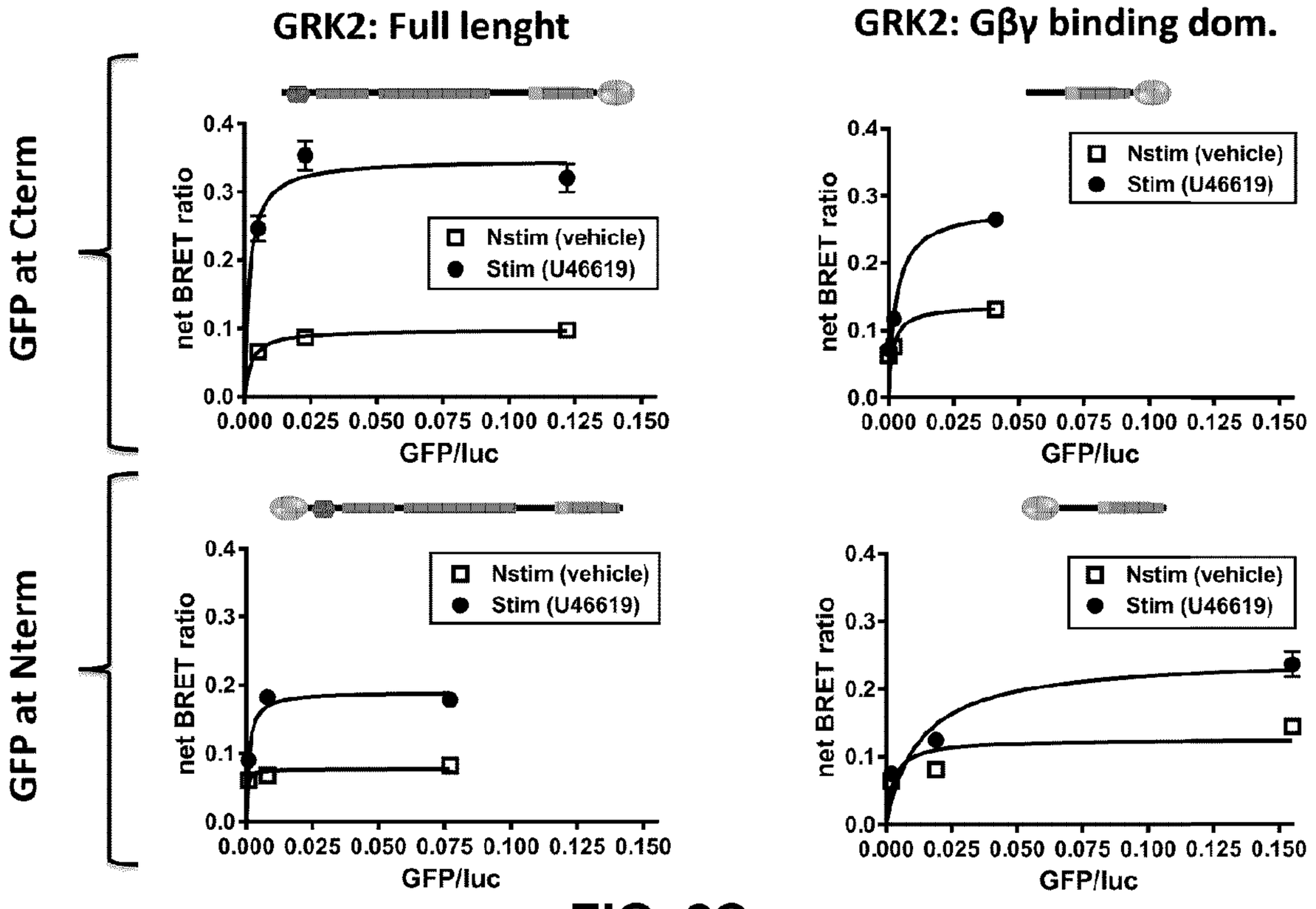

GRK2: Full lenght
GRK2: Gβγ binding dom.
GFP at Cterm
GFP at Nterm
net BRET ratio
GFP/luc
Nstim (vehicle)
Stim (U46619)
0.0
0.1
0.2
0.3
0.4
0.000 0.025 0.050 0.075 0.100 0.125 0.150

FIG. 2C

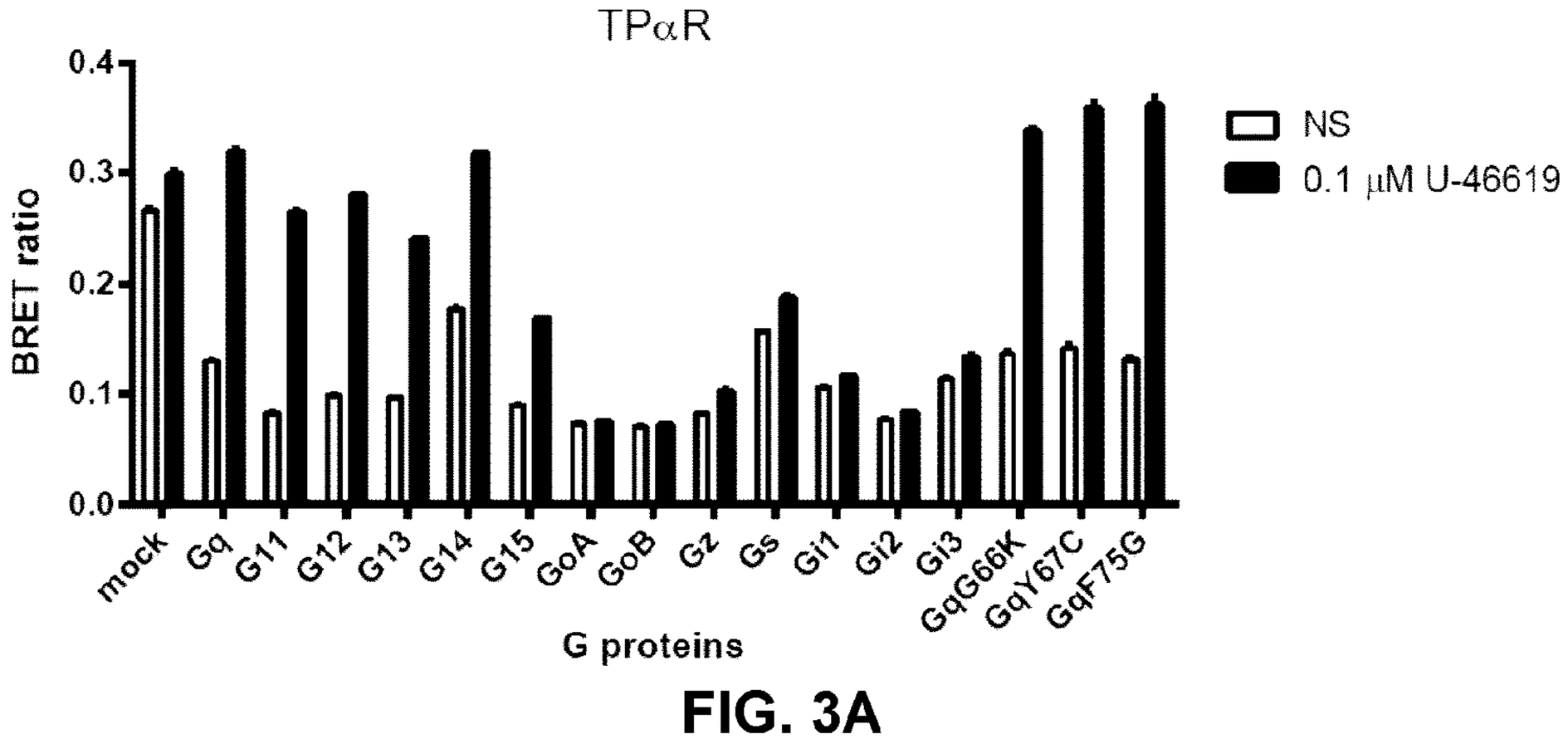

TPαR
BRET ratio
NS
0.1 μM U-46619
mock
Gq
G11
G12
G13
G14
G15
GoA
GoB
Gz
Gs
Gi1
Gi2
Gi3
GqG66K
GqY67C
GqF75G
G proteins

FIG. 3A

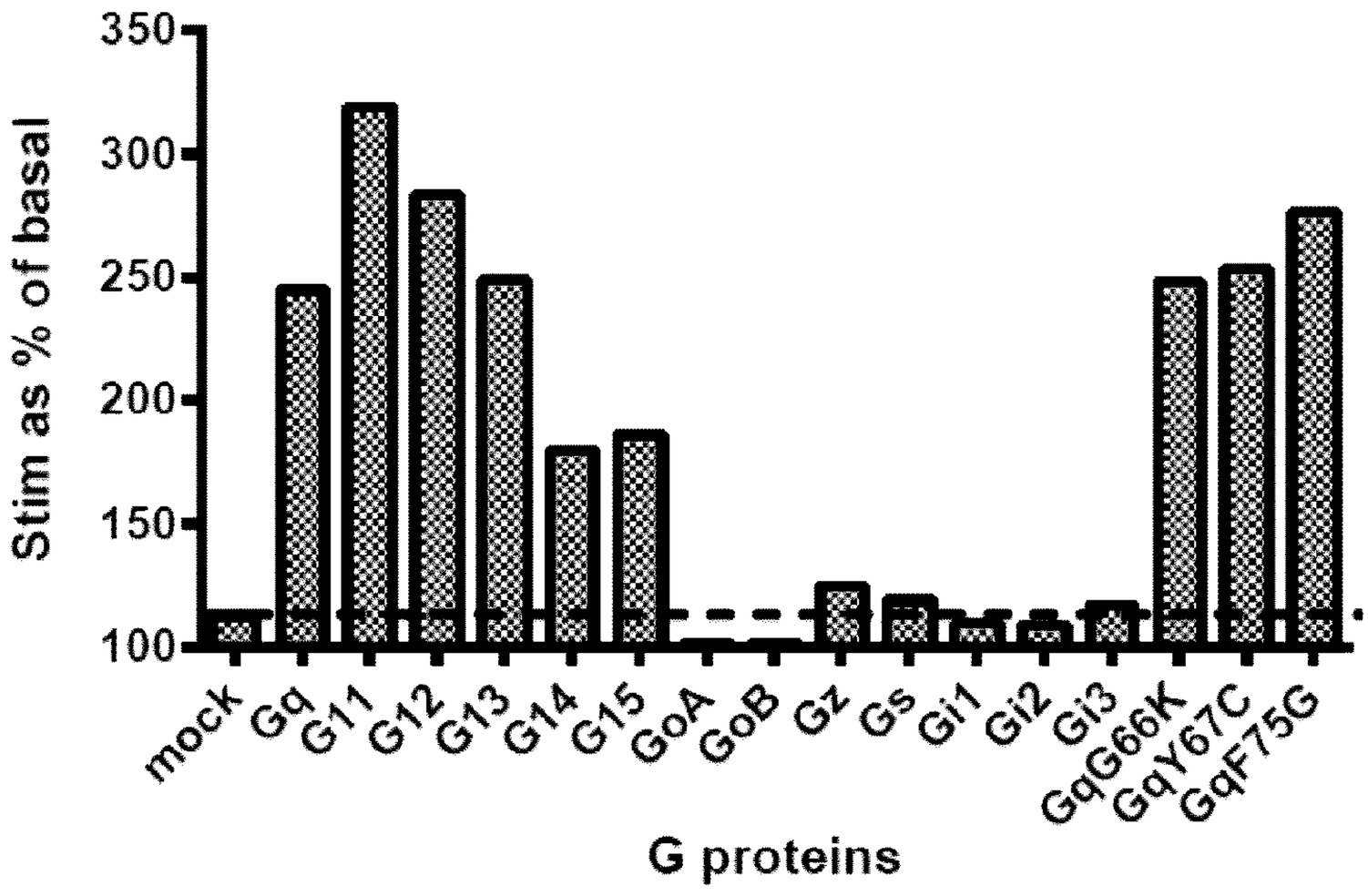

TPαR: U-46619 (0.1 μM)
Stim as % of basal
350
300
250
200
150
100
mock
Gq
G11
G12
G13
G14
G15
GoA
GoB
Gz
Gs
Gi1
Gi2
Gi3
GqG66K
GqY67C
GqF75G
G proteins

FIG. 3B

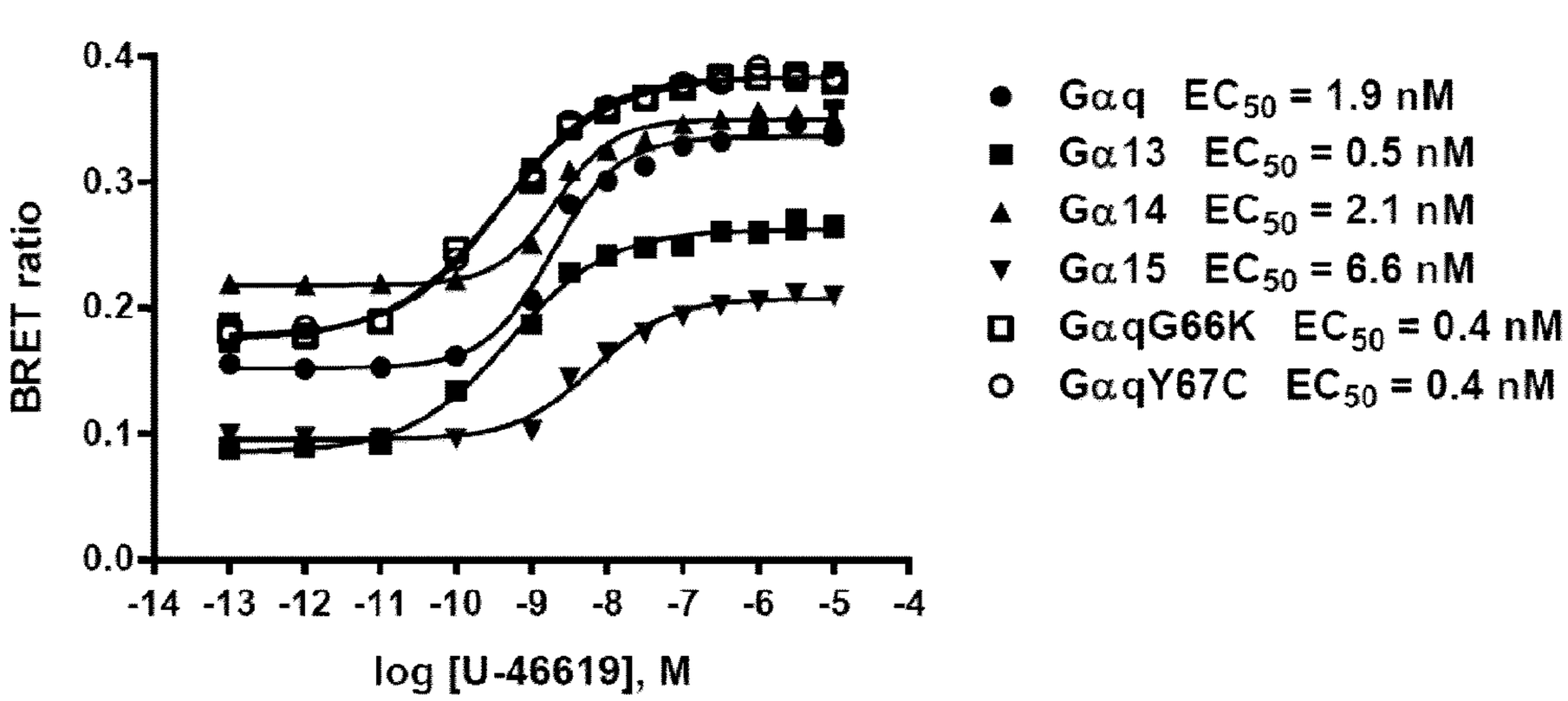

TPαR
BRET ratio
0.4
0.3
0.2
0.1
0.0
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
log [U-46619], M
Gαq EC50 = 1.9 nM
Gα13 EC50 = 0.5 nM
Gα14 EC50 = 2.1 nM
Gα15 EC50 = 6.6 nM
GαqG66K EC50 = 0.4 nM
GαqY67C EC50 = 0.4 nM

FIG. 3C

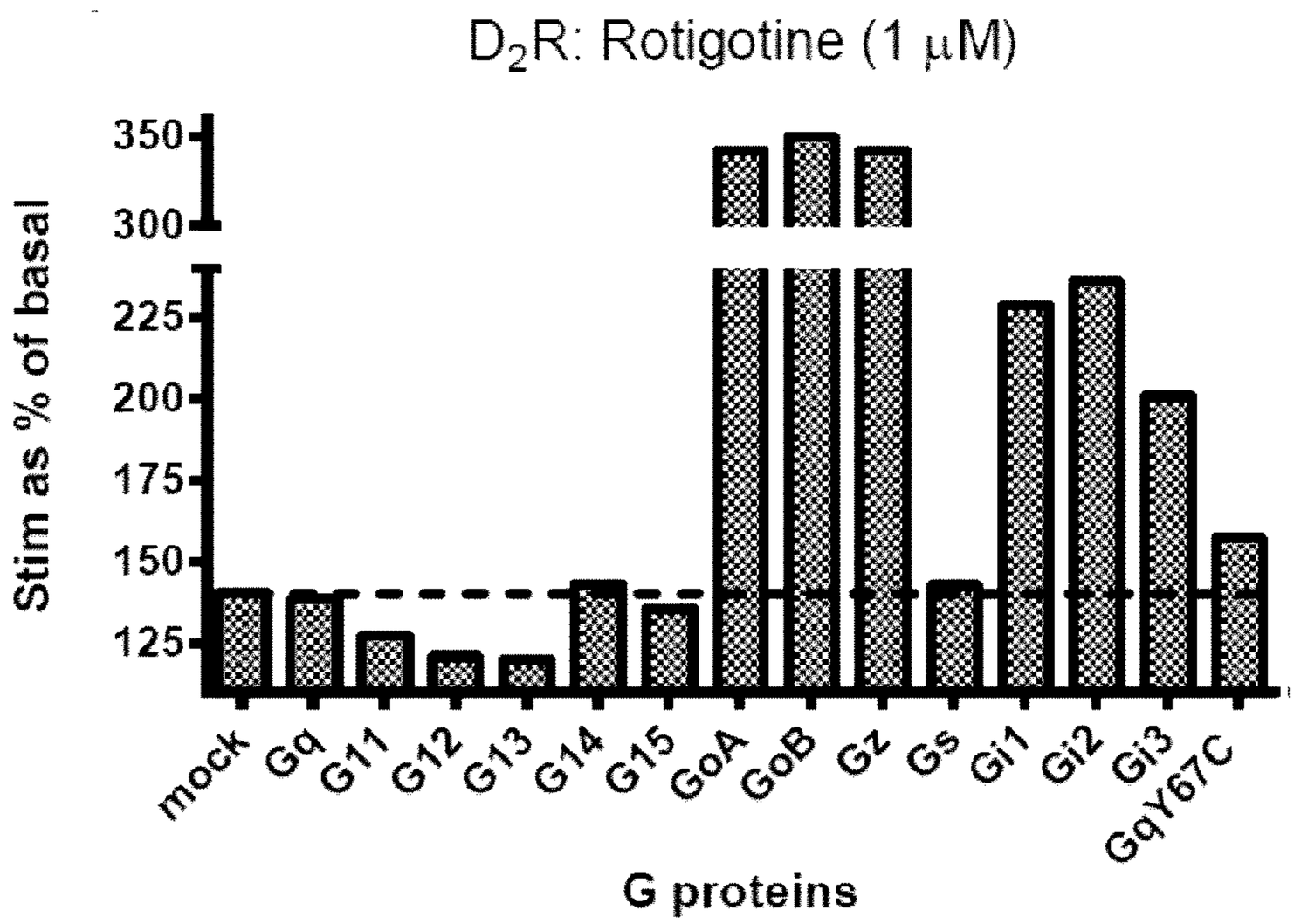
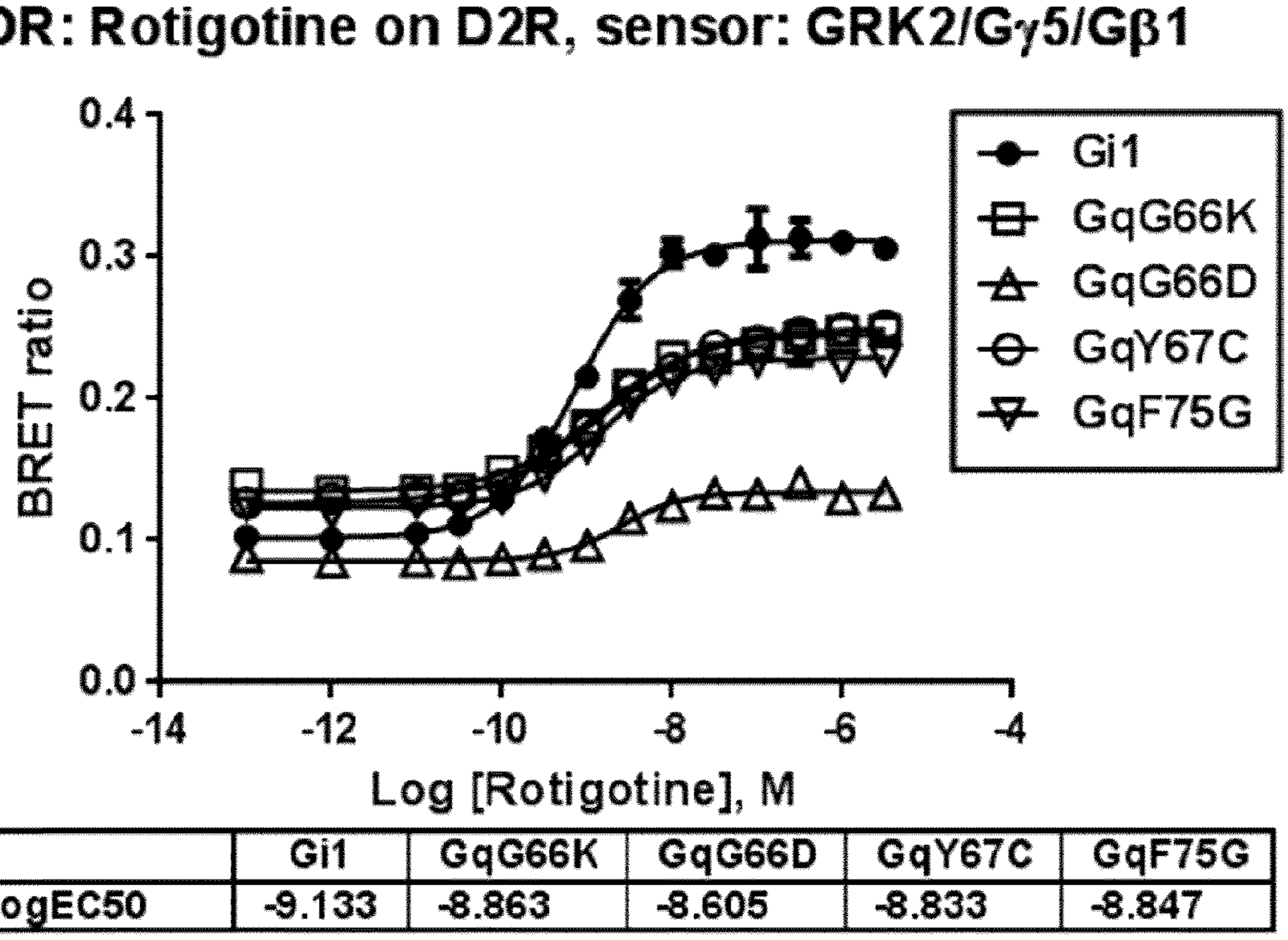



FIG. 4A




| | Gi1 | GqG66K | GqG66D | GqY67C | GqF75G |
|---|---|---|---|---|---|
| LogEC50 | -9.133 | -8.863 | -8.605 | -8.833 | -8.847 |

FIG. 4B

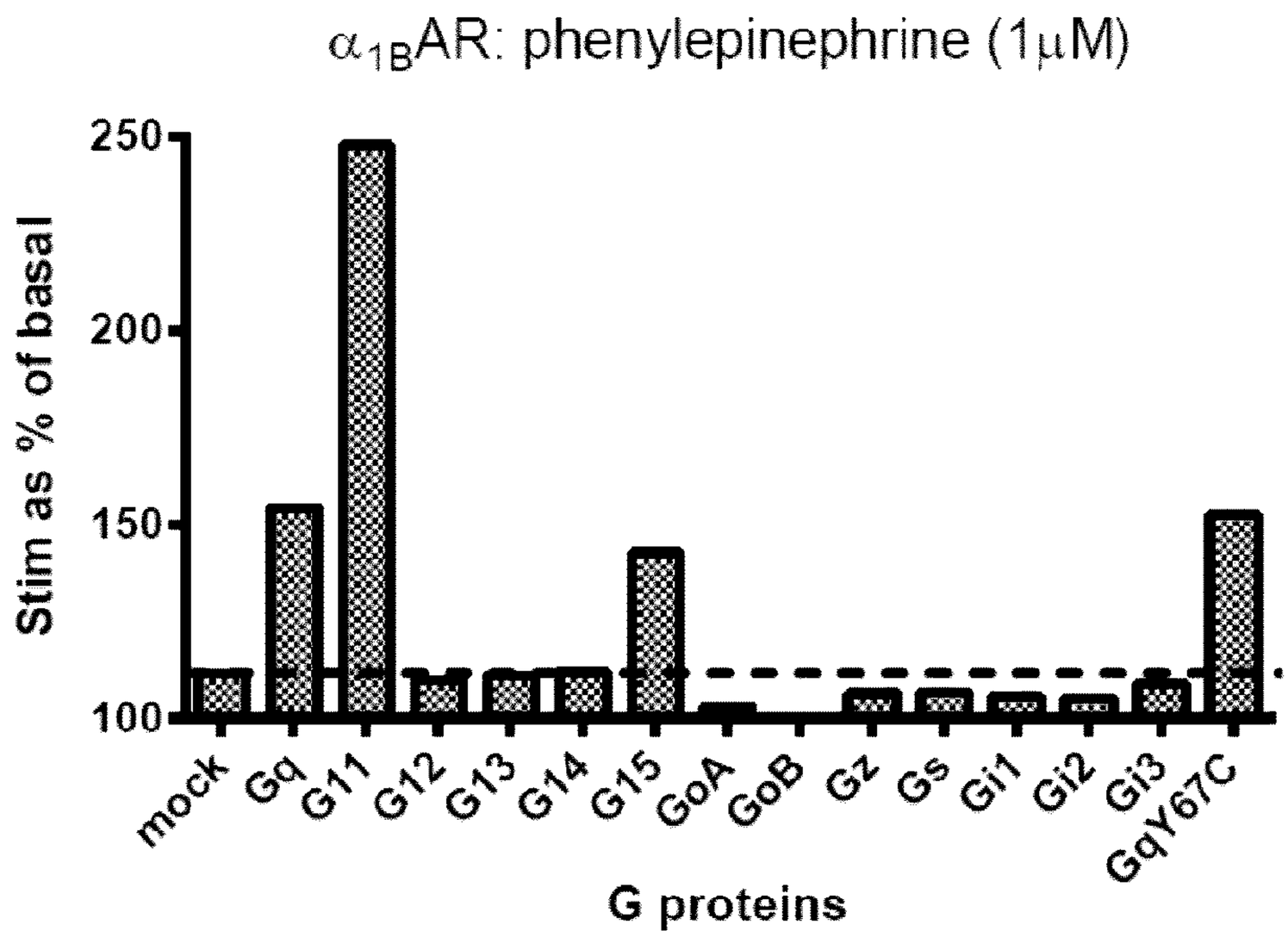

$\alpha_{1B}$AR: phenylepinephrine (1μM)
Stim as % of basal
250
200
150
100
mock
Gq
G11
G12
G13
G14
G15
GoA
GoB
Gz
Gs
Gi1
Gi2
Gi3
GqY67C
G proteins

FIG. 4C

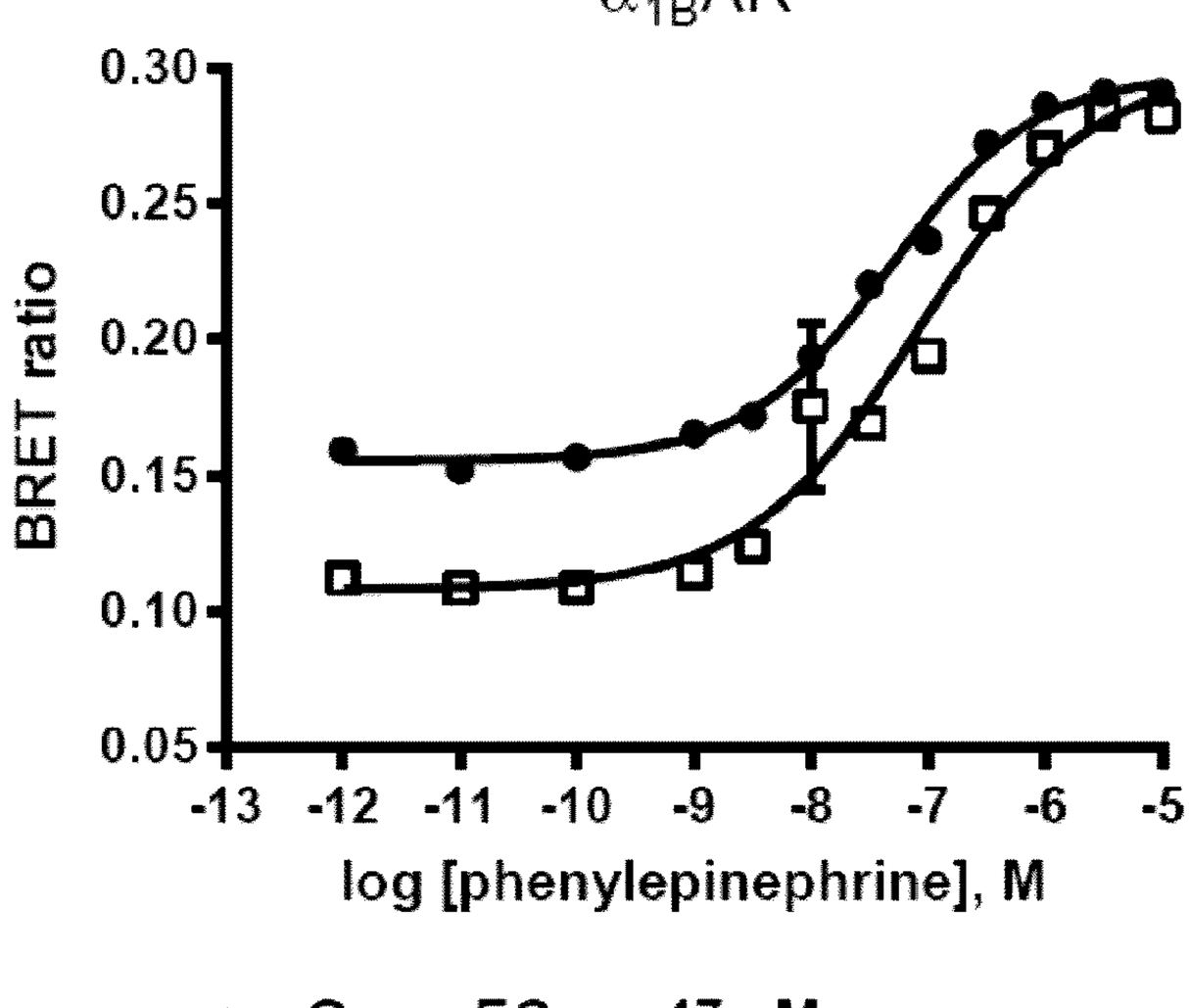
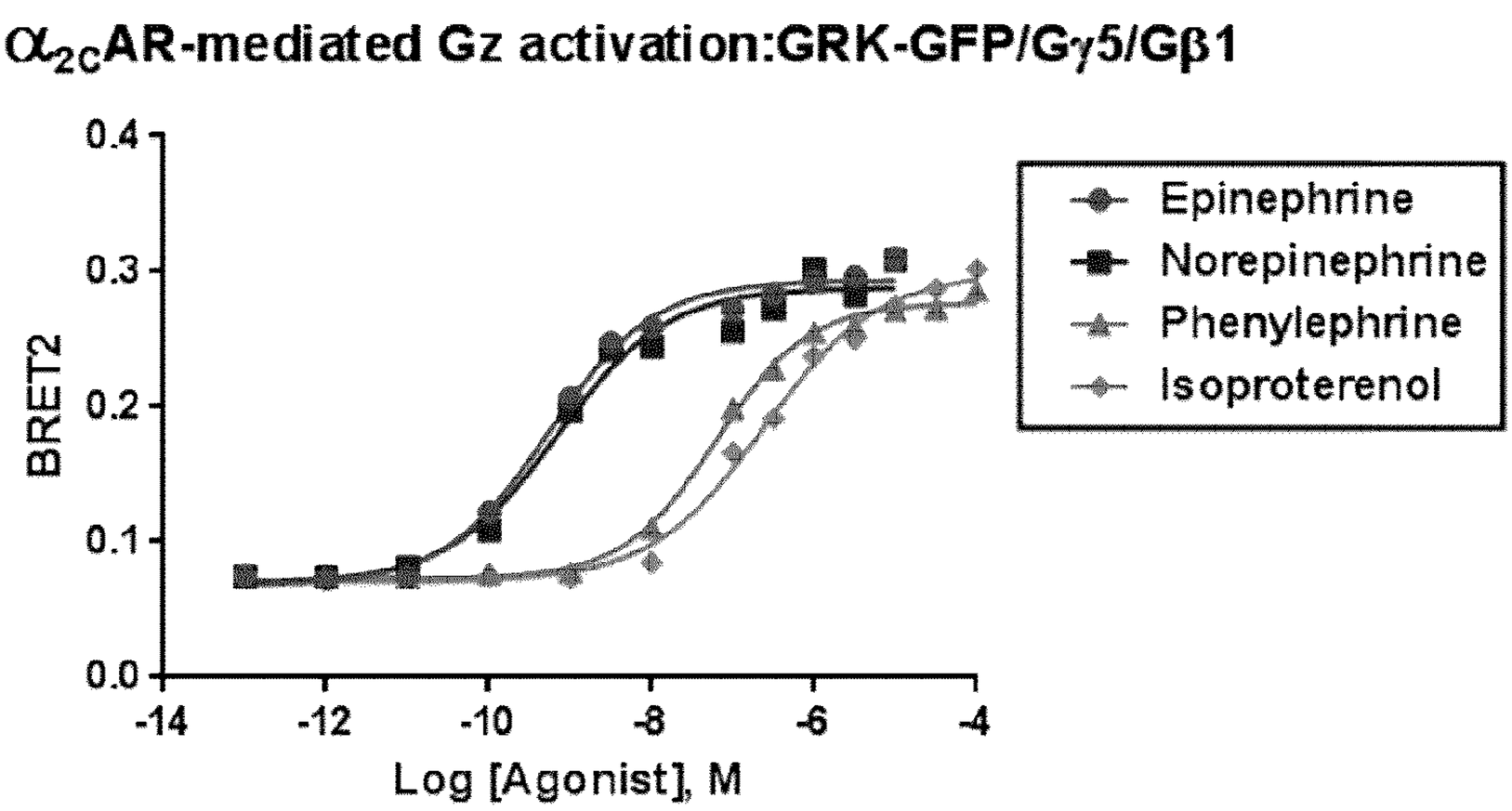

$\alpha_{1B}$AR
BRET ratio
0.30
0.25
0.20
0.15
0.10
0.05
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [phenylepinephrine], M
● Gαq EC50 = 47 nM
□ Gα11 EC50 = 82 nM

FIG. 4D




| | Epinephrine | Norepinephrine | Phenylephrine | Isoproterenol |
|---|---|---|---|---|
| LogEC50 | -9.271 | -9.160 | -7.206 | -6.617 |

FIG. 4E

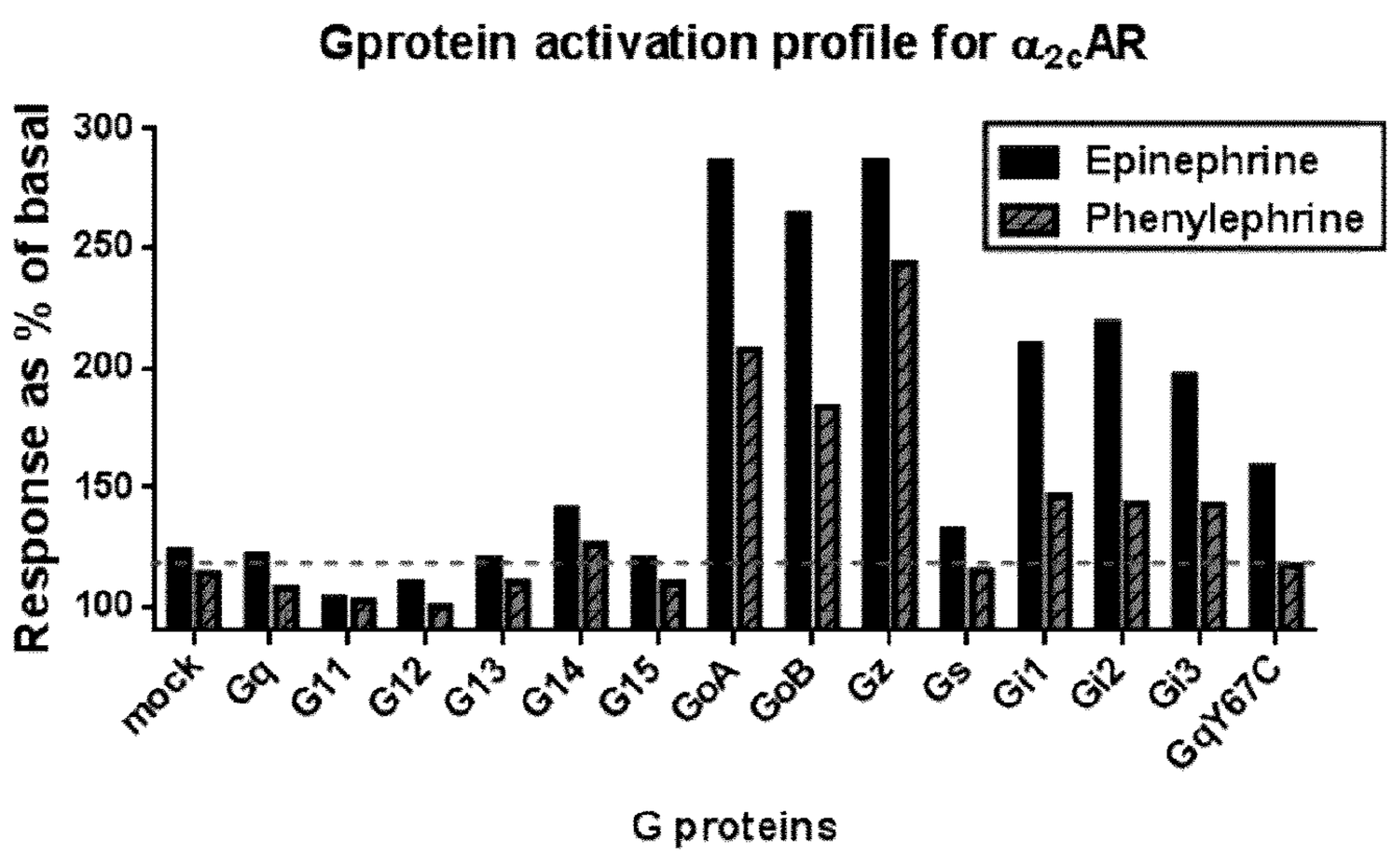



FIG. 4F

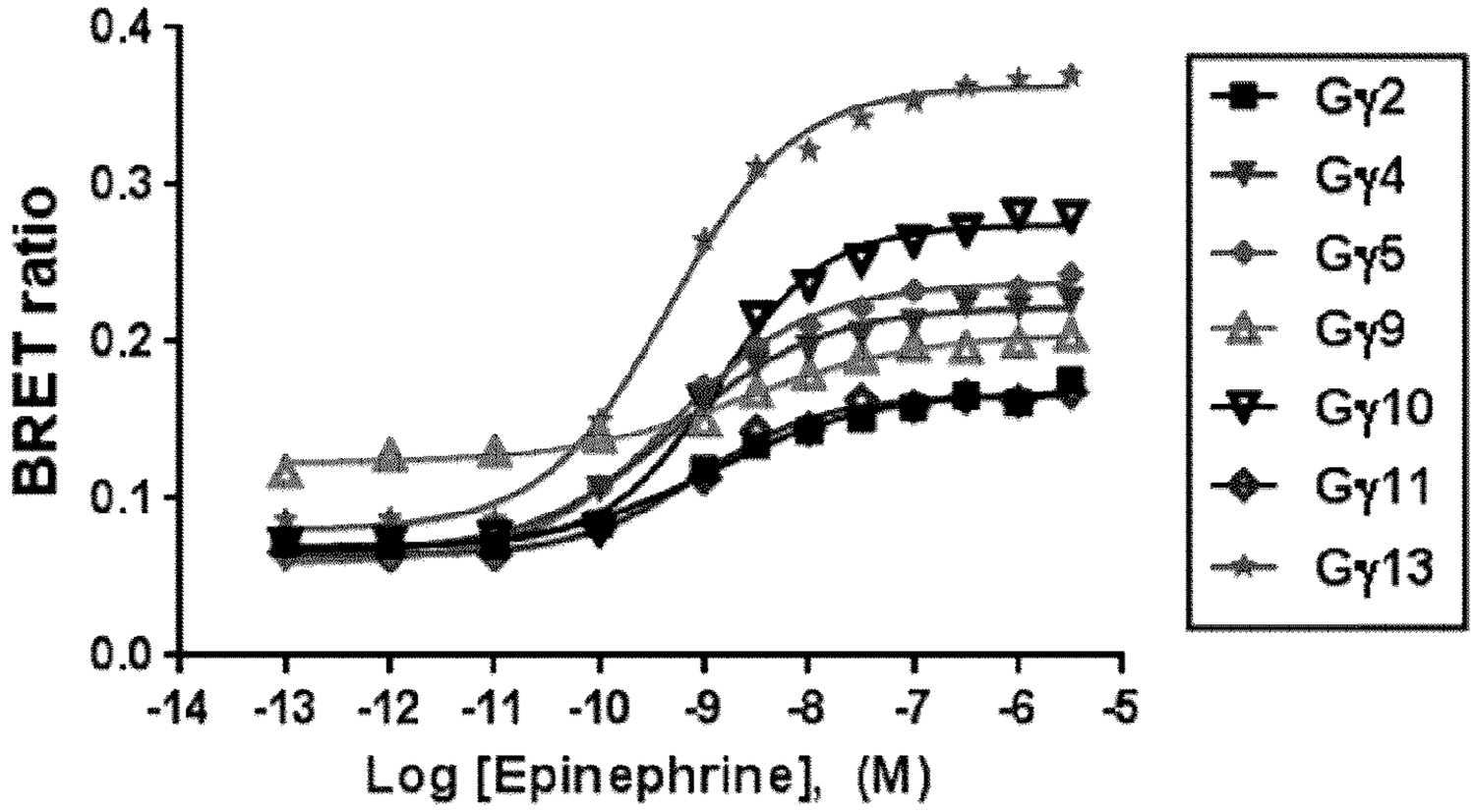

DR: $\alpha_{2C}$AR-mediated Gz activation:GRK-GFP/RlucII-Gγ/Gβ1
BRET ratio
0.4
0.3
0.2
0.1
0.0
-14
-13
-12
-11
-10
-9
-8
-7
-6
-5
Log [Epinephrine], (M)
Gγ2
Gγ4
Gγ5
Gγ9
Gγ10
Gγ11
Gγ13

FIG. 4G

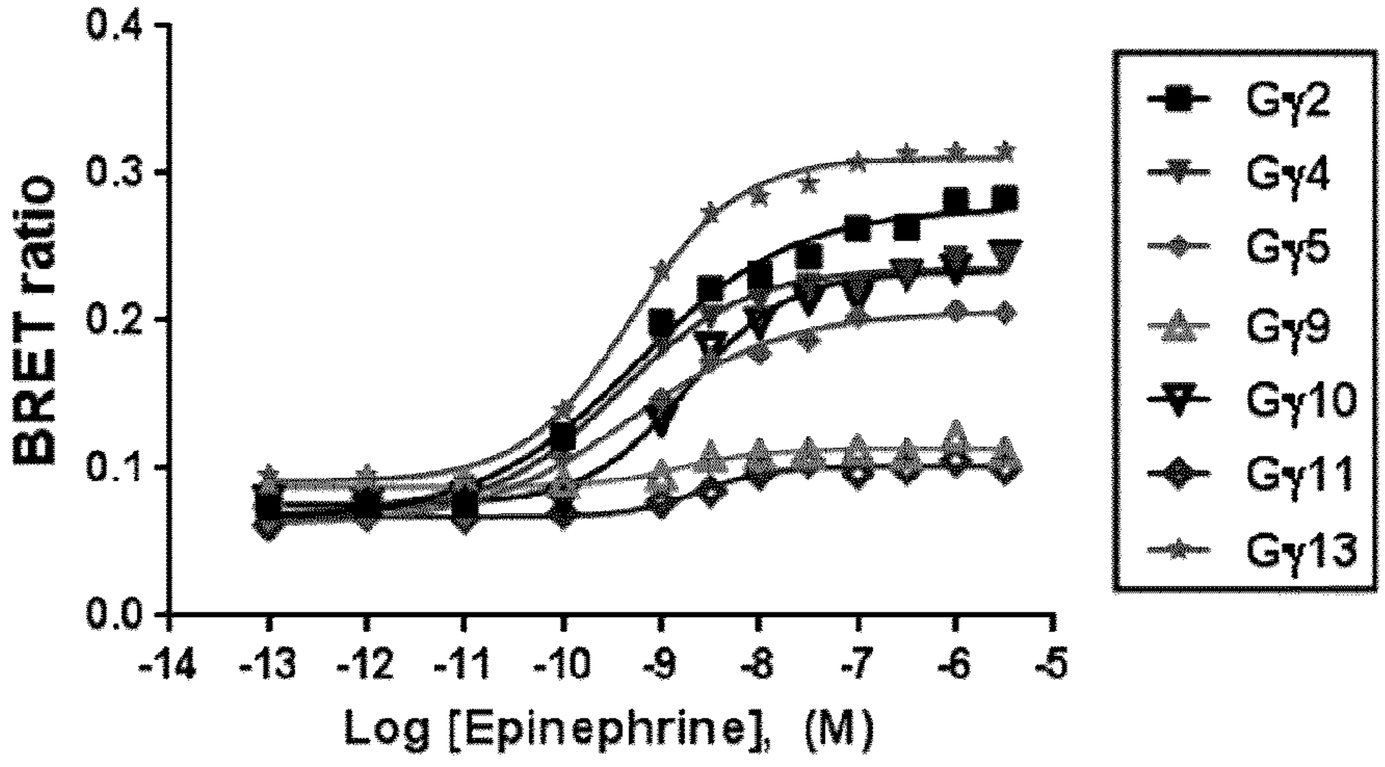

DR: $\alpha_{2C}$AR-mediated Gz activation:GRK-GFP/RlucII-Gγ/Gβ3sh
BRET ratio
0.4
0.3
0.2
0.1
0.0
-14
-13
-12
-11
-10
-9
-8
-7
-6
-5
Log [Epinephrine], (M)
Gγ2
Gγ4
Gγ5
Gγ9
Gγ10
Gγ11
Gγ13

FIG. 4H

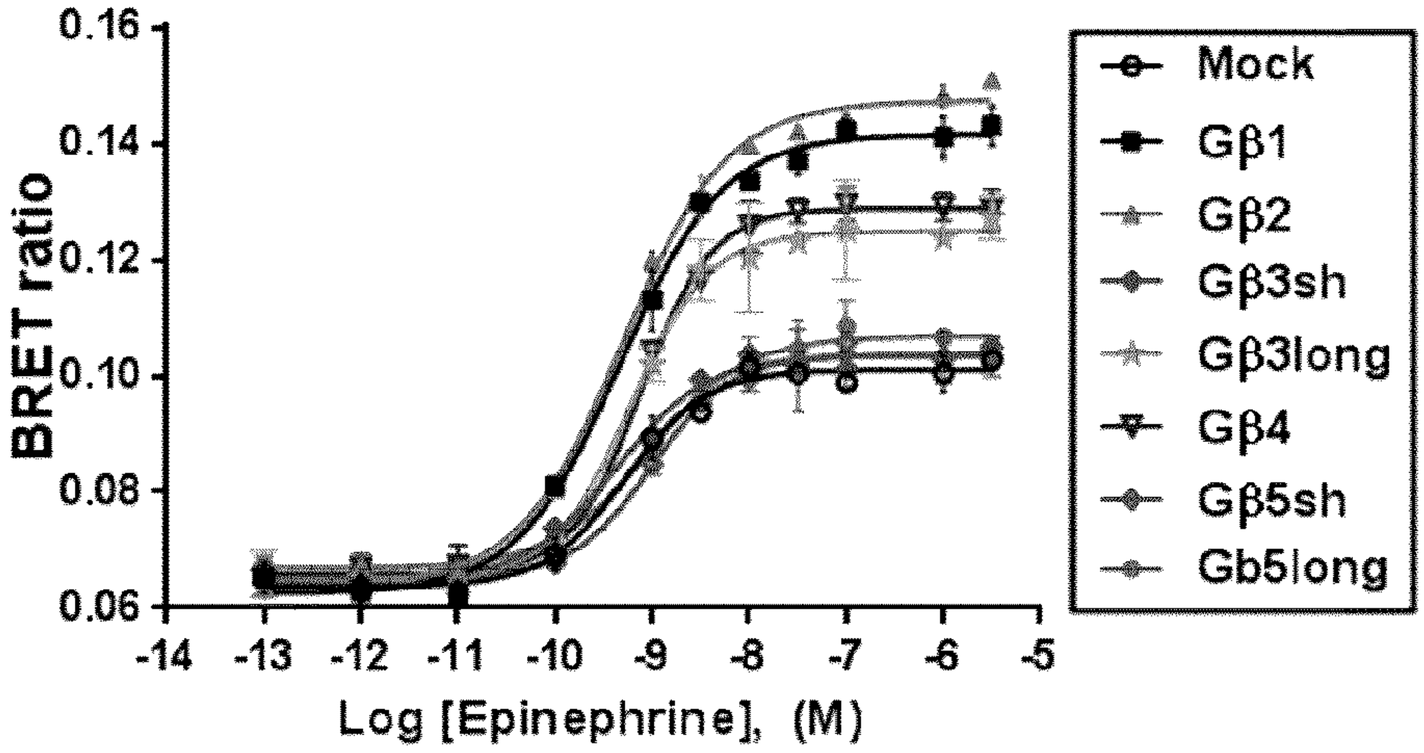

DR: α2cAR-mediated Gz activation:GRK-GFP/RlucII-Gγ1/Gβ
BRET ratio
0.16
0.14
0.12
0.10
0.08
0.06
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5
Log [Epinephrine], (M)
Mock
Gβ1
Gβ2
Gβ3sh
Gβ3long
Gβ4
Gβ5sh
Gb5long

FIG. 4I

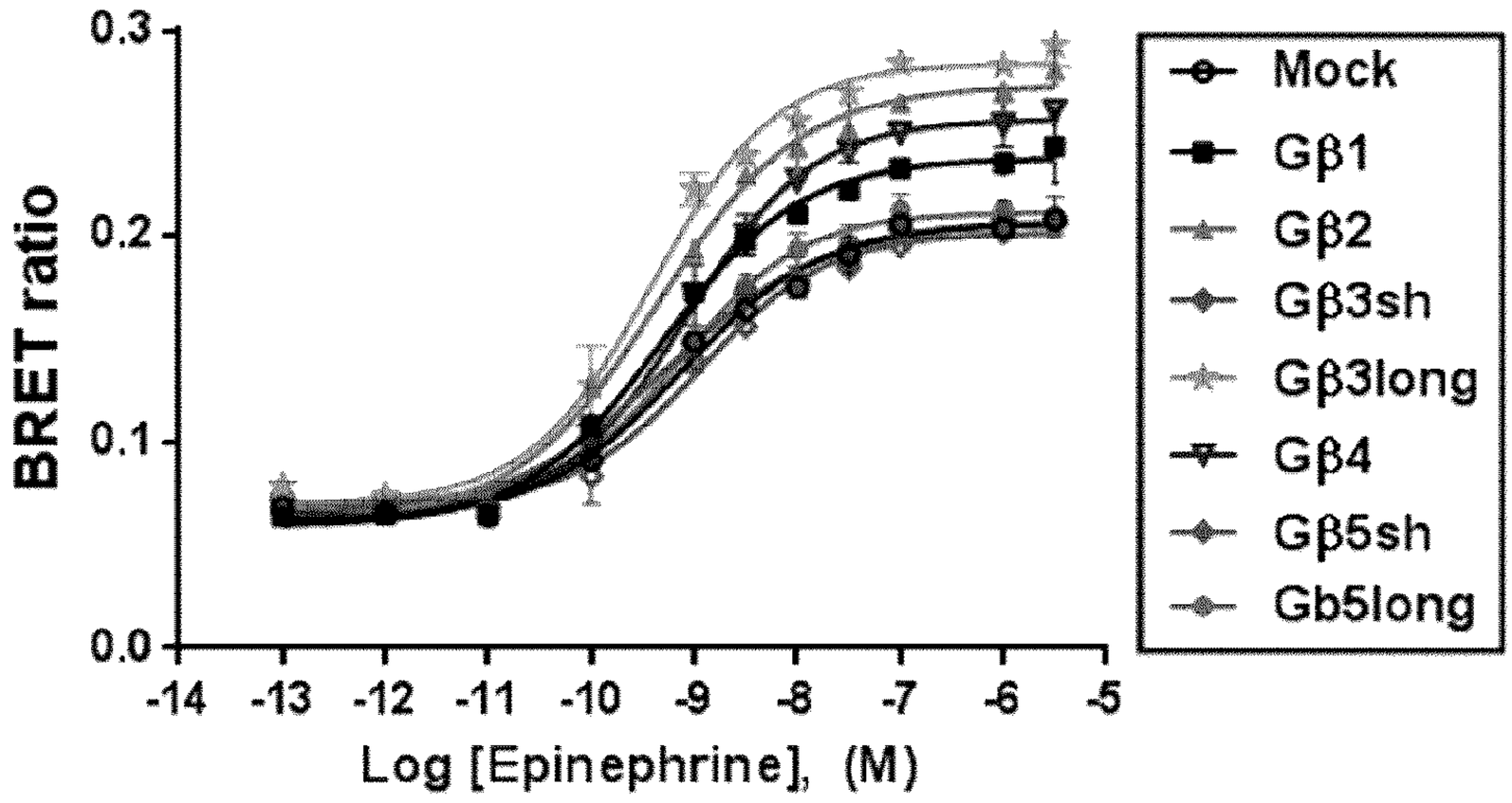

DR: α2cAR-mediated Gz activation:GRK-GFP/RlucII-Gγ5/Gβ
BRET ratio
0.3
0.2
0.1
0.0
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5
Log [Epinephrine], (M)
Mock
Gβ1
Gβ2
Gβ3sh
Gβ3long
Gβ4
Gβ5sh
Gb5long

FIG. 4J

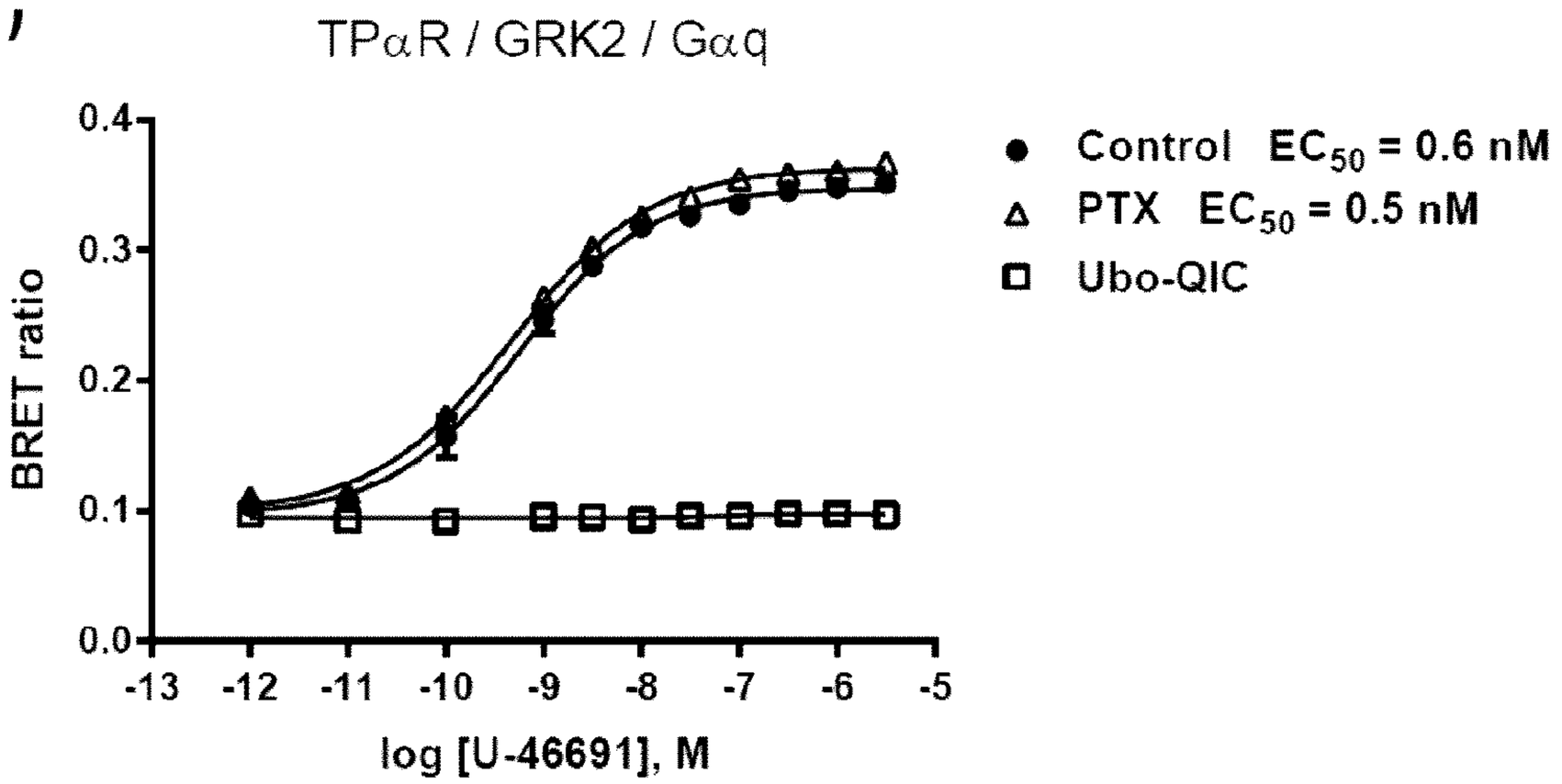

I
TPαR / GRK2 / Gαq
BRET ratio
0.4
0.3
0.2
0.1
0.0
-13 -12 -11 -10 -9 -8 -7 -6 -5
log [U-46691], M
Control EC50 = 0.6 nM
PTX EC50 = 0.5 nM
Ubo-QIC

FIG. 5A

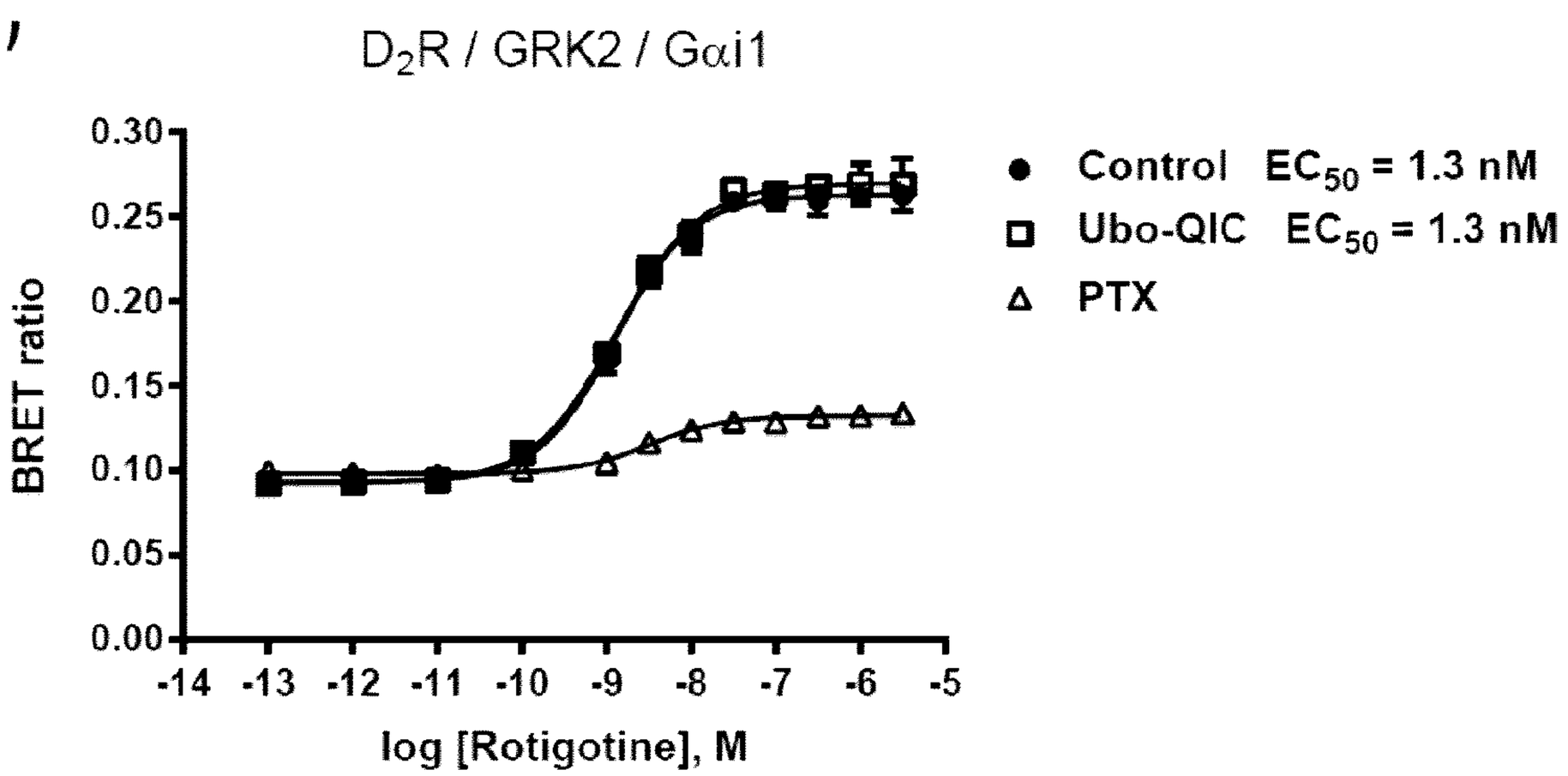

I
D2R / GRK2 / Gαi1
BRET ratio
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5
log [Rotigotine], M
Control EC50 = 1.3 nM
Ubo-QIC EC50 = 1.3 nM
PTX

FIG. 5B

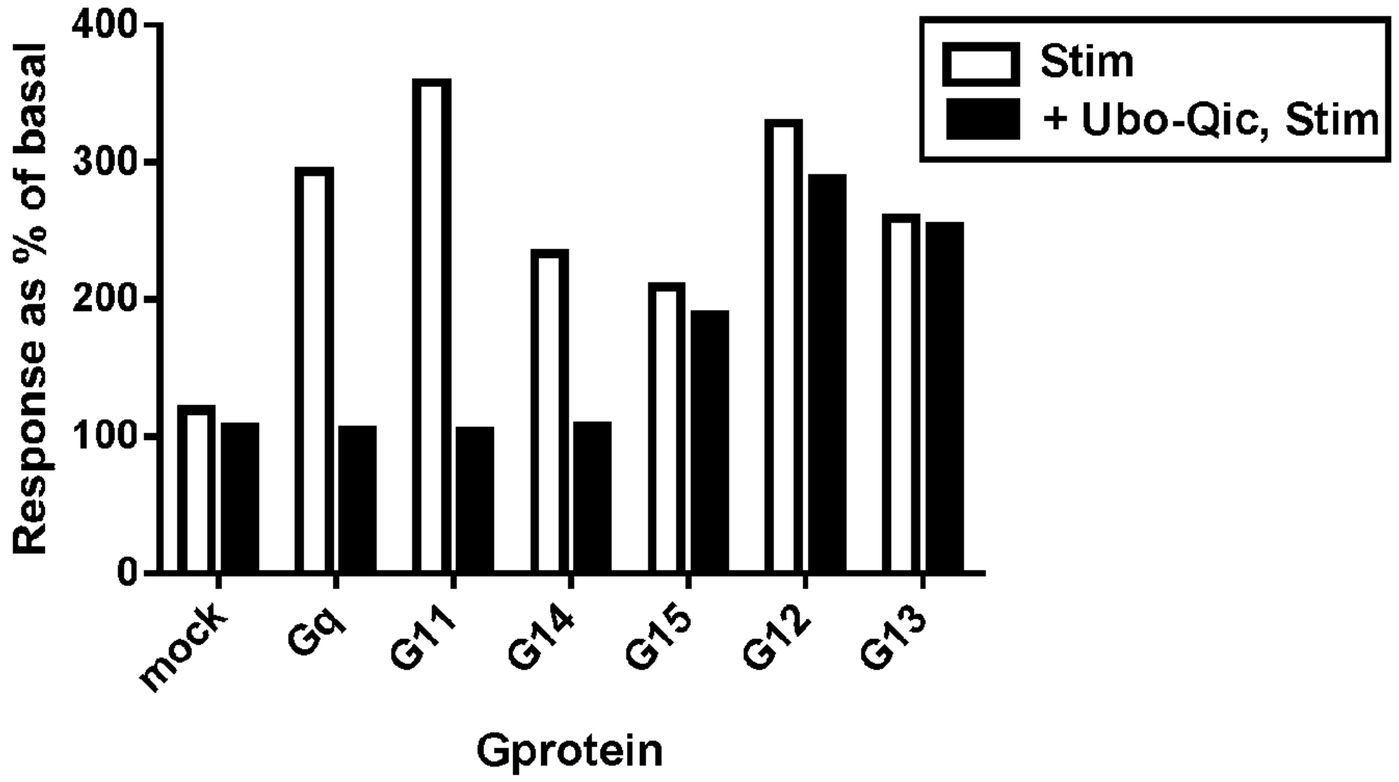

Ubo-Qic inhibition profile of Gproteins: TPαR/U46619
Response as % of basal
400
300
200
100
0
Stim
+ Ubo-Qic, Stim
mock
Gq
G11
G14
G15
G12
G13
Gprotein

FIG. 5C

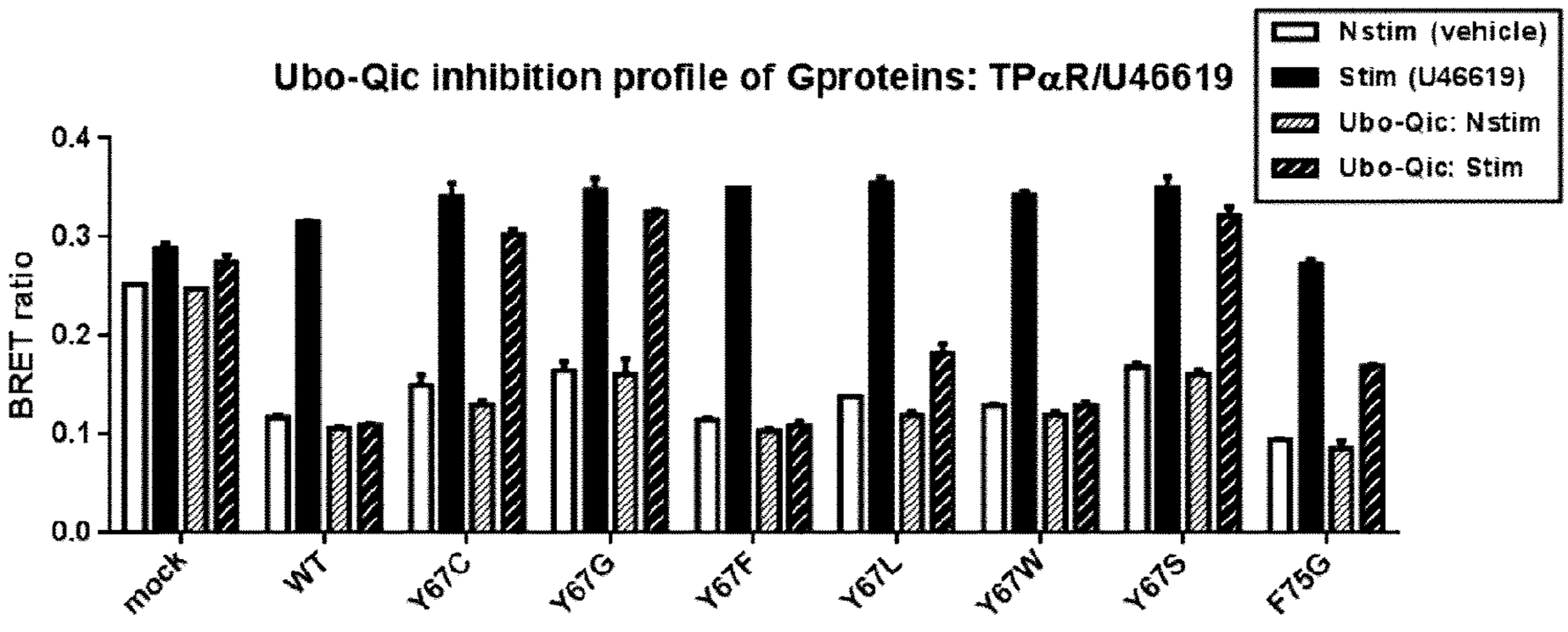

Ubo-Qic inhibition profile of Gproteins: TPαR/U46619
Nstim (vehicle)
Stim (U46619)
Ubo-Qic: Nstim
Ubo-Qic: Stim
BRET ratio
0.4
0.3
0.2
0.1
0.0
mock
WT
Y67C
Y67G
Y67F
Y67L
Y67W
Y67S
F75G
Gαq proteins

FIG. 5D

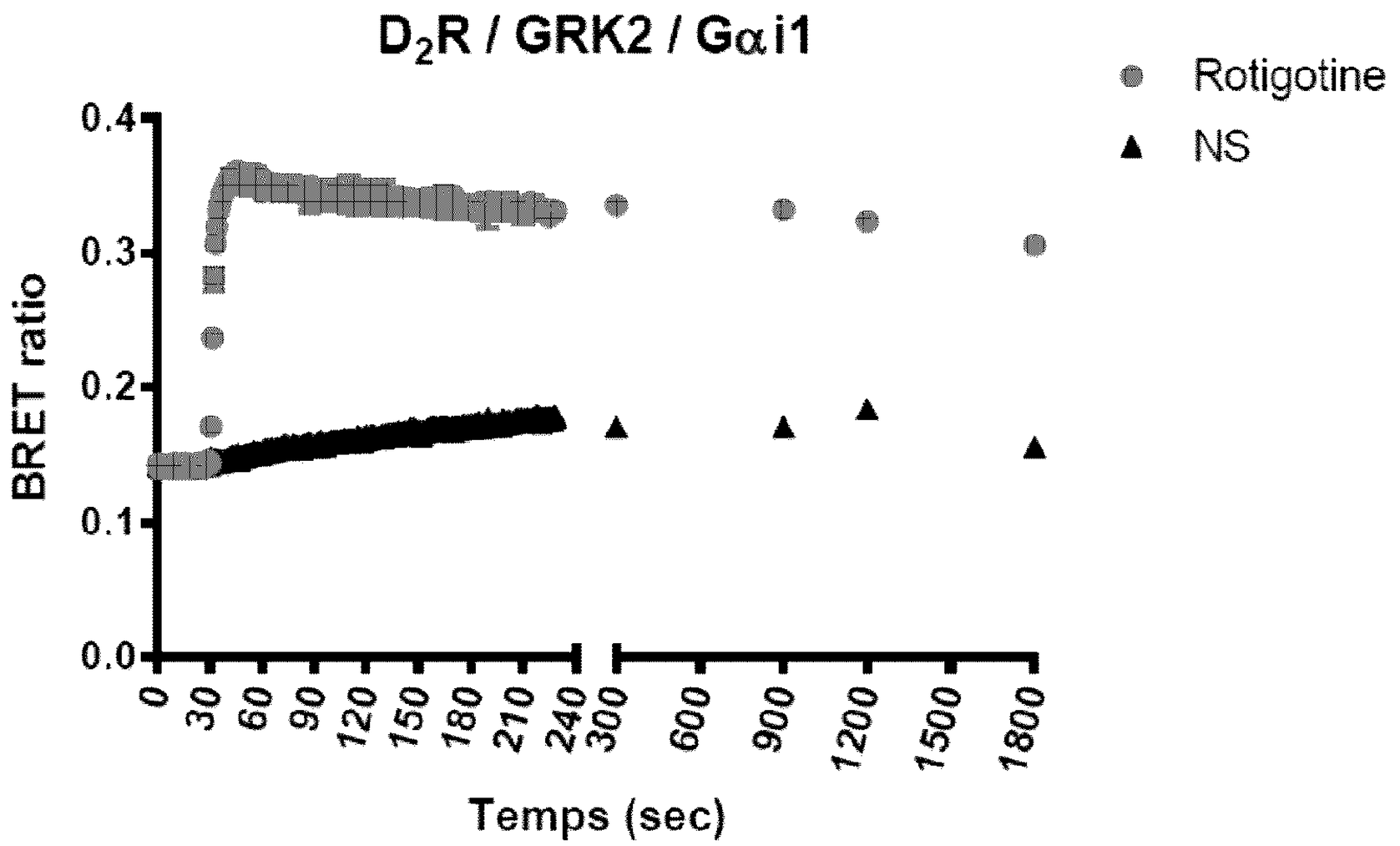

D2R / GRK2 / Gαi1
Rotigotine
NS
BRET ratio
0.0
0.1
0.2
0.3
0.4
0
30
60
90
120
150
180
210
240
300
600
900
1200
1500
1800
Temps (sec)

FIG. 6A

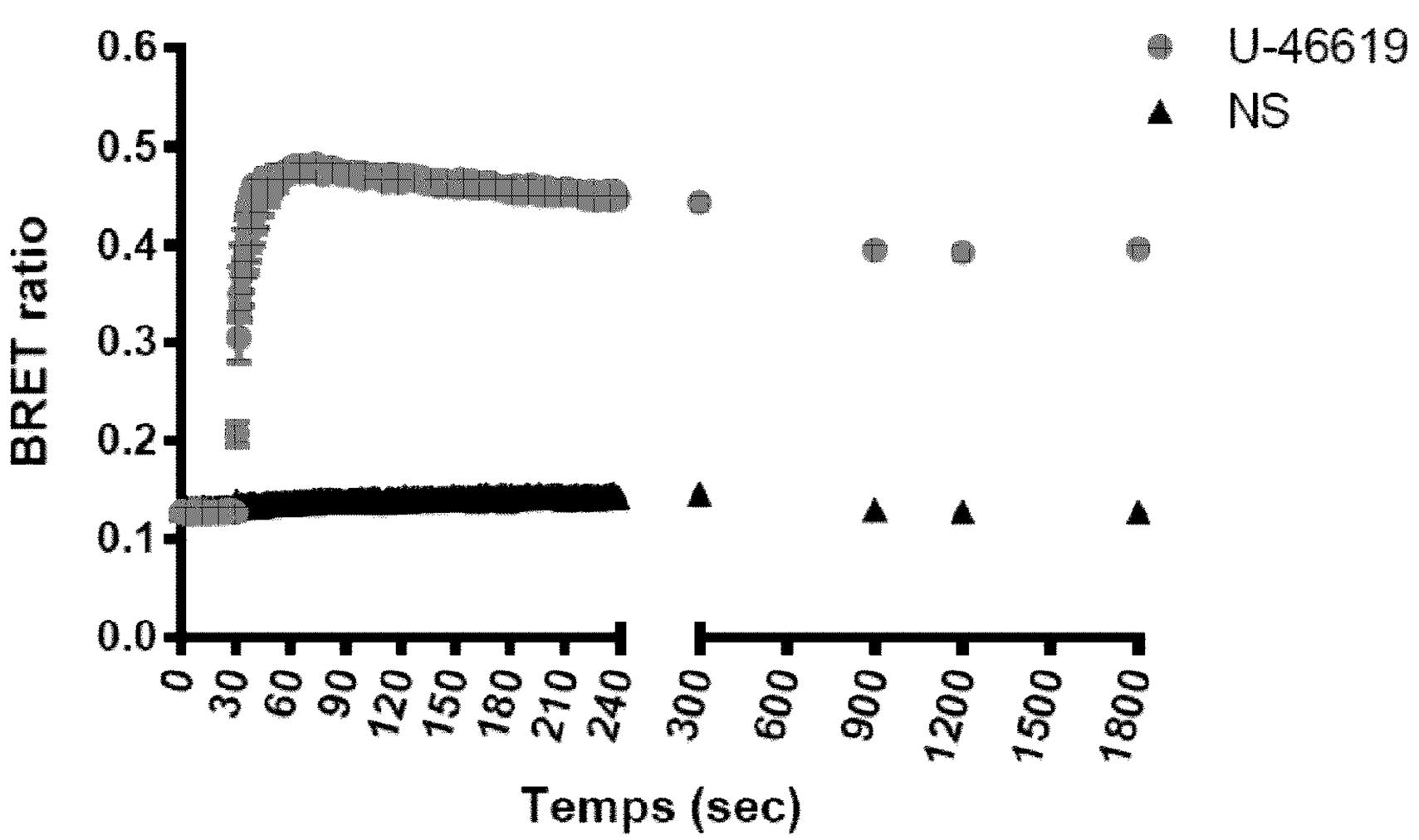

TPαR / GRK2 / Gα11
U-46619
NS
BRET ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
30
60
90
120
150
180
210
240
300
600
900
1200
1500
1800
Temps (sec)

FIG. 6B

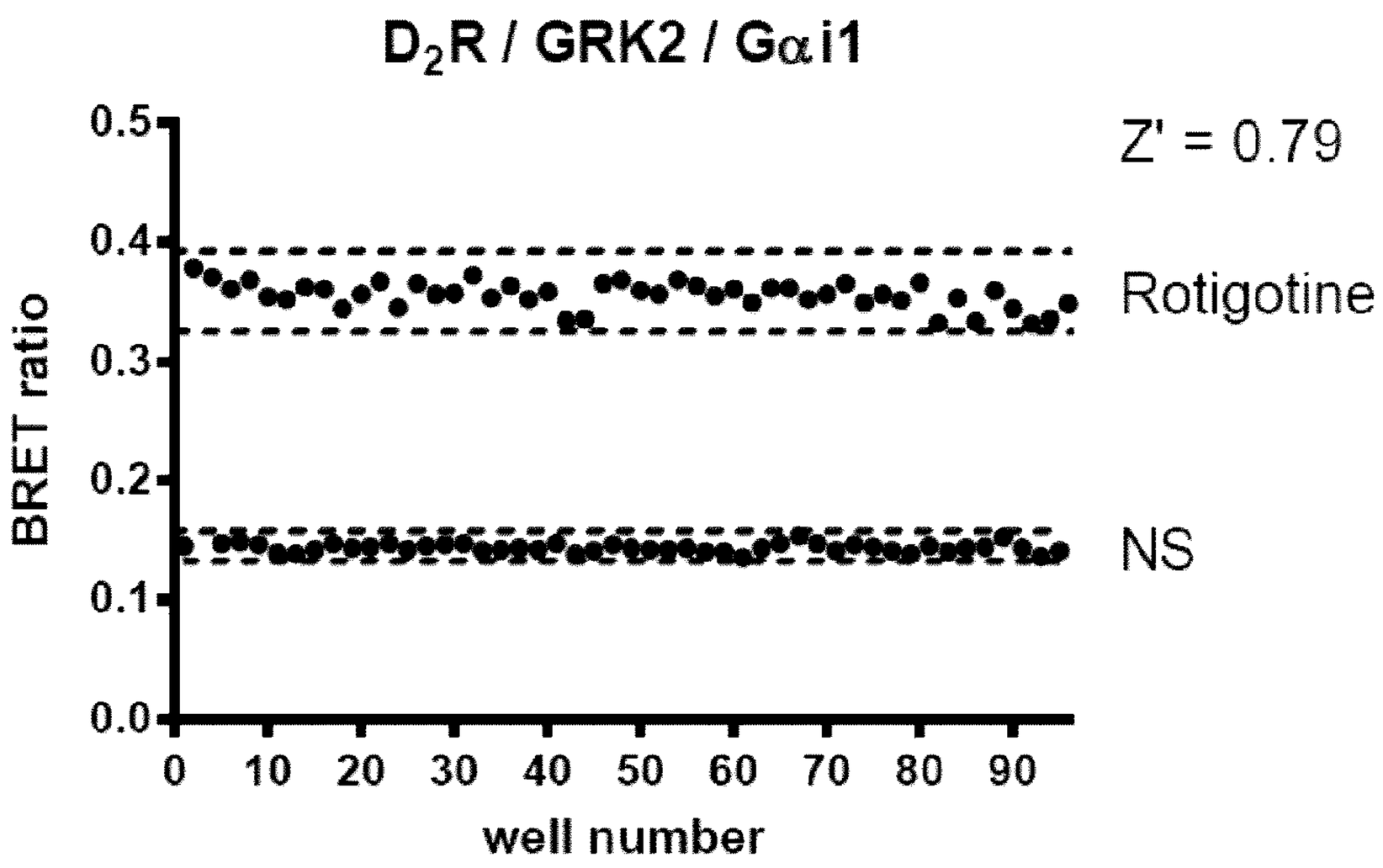

D2R / GRK2 / Gαi1
Z' = 0.79
Rotigotine
NS
BRET ratio
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
well number

FIG. 7A

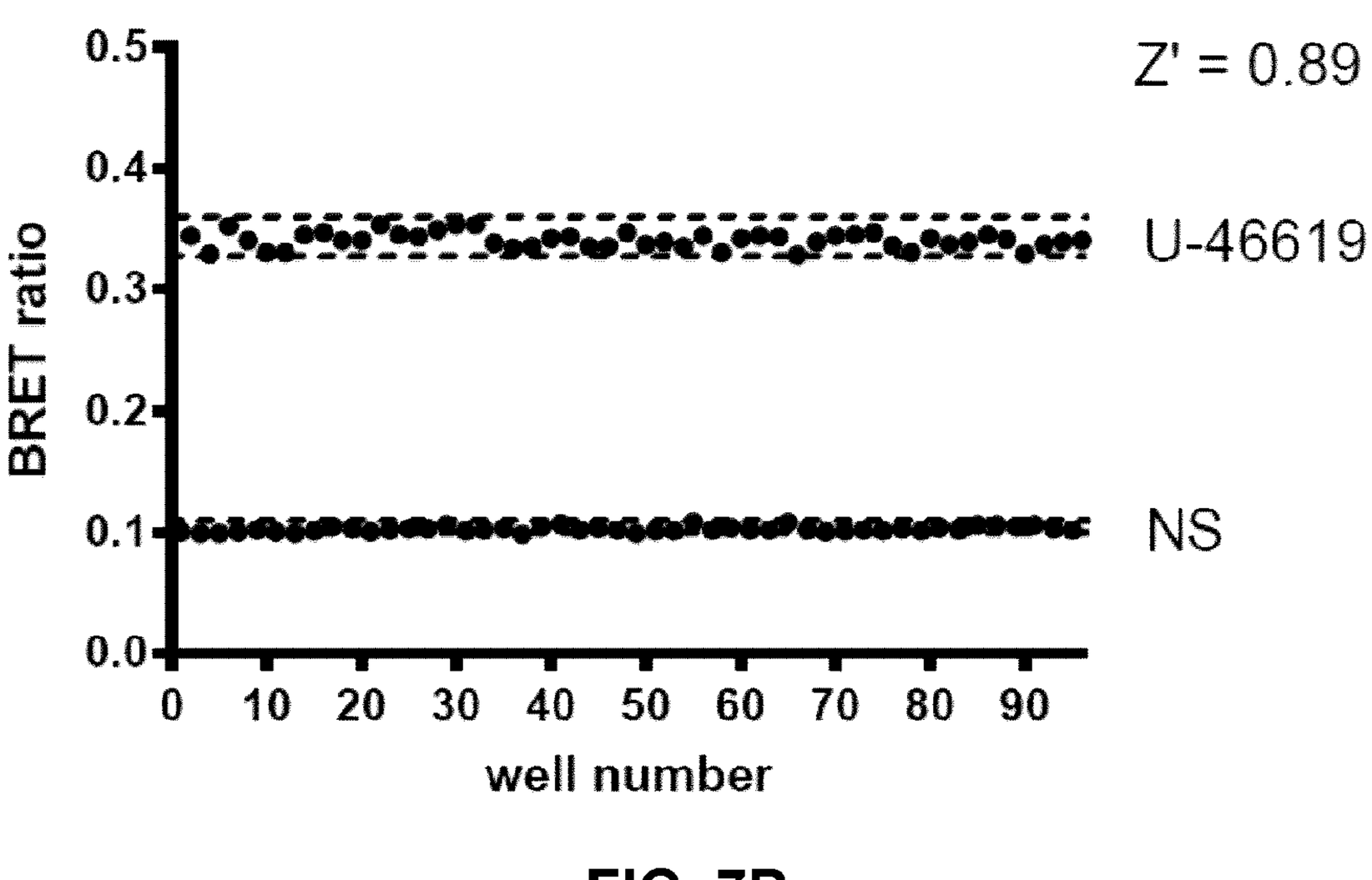

TPαR / GRK2 / Gα11
Z' = 0.89
U-46619
NS
BRET ratio
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
well number

FIG. 7B

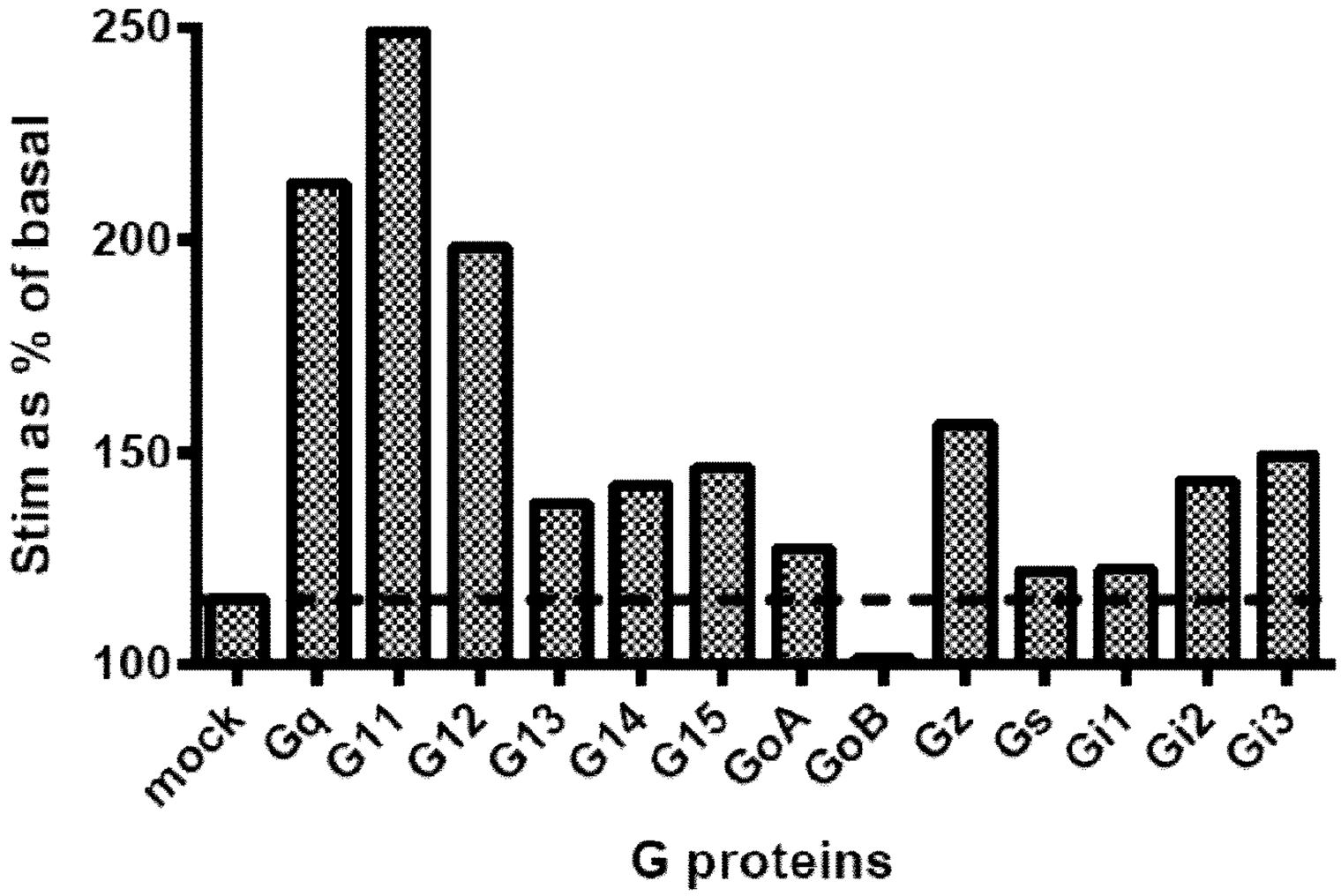

AT1aR: AngII (1μM)
Stim as % of basal
250
200
150
100
mock
Gq
G11
G12
G13
G14
G15
GoA
GoB
Gz
Gs
Gi1
Gi2
Gi3
G proteins

FIG. 8A

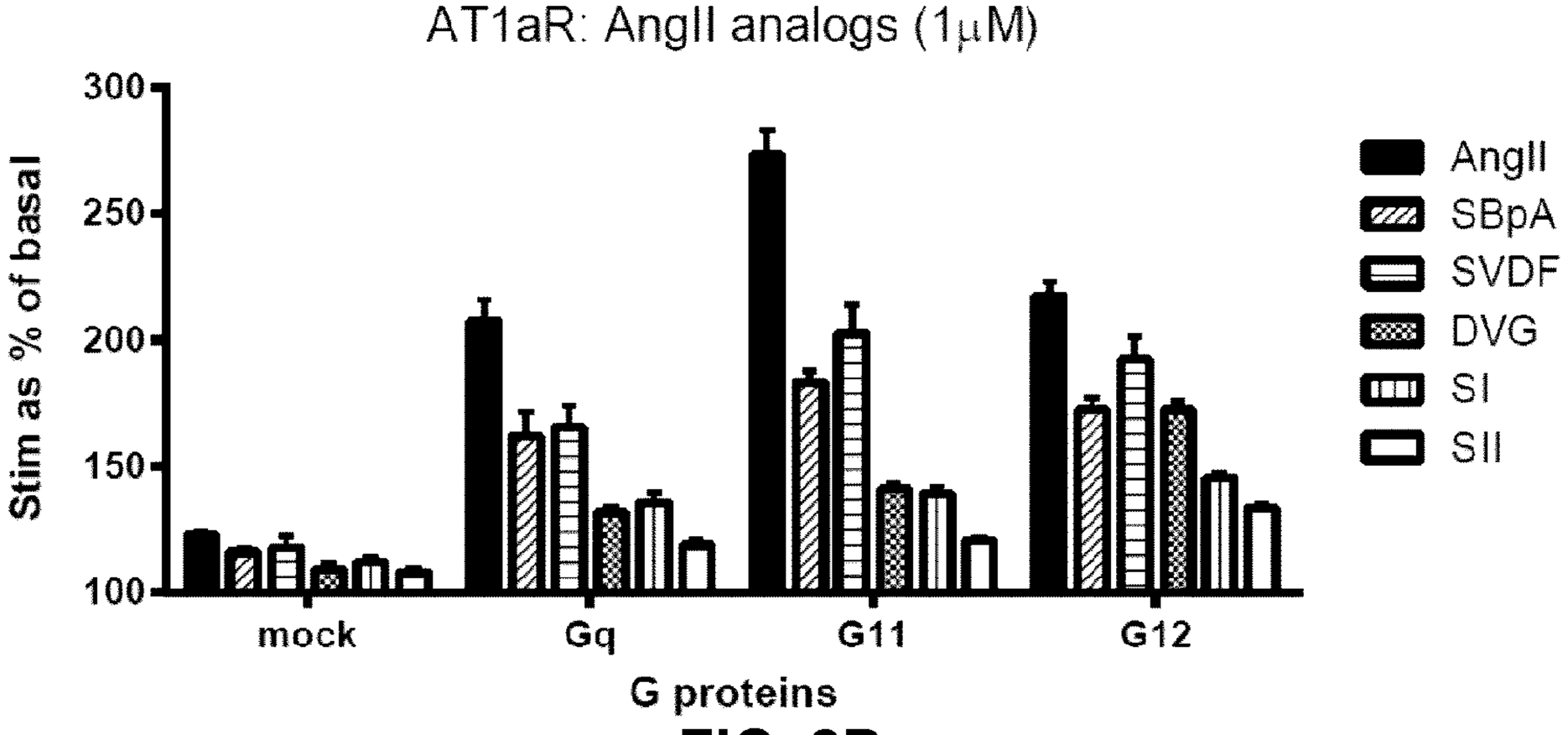

AT1aR: AngII analogs (1μM)
Stim as % of basal
300
250
200
150
100
mock
Gq
G11
G12
G proteins
AngII
SBpA
SVDF
DVG
SI
SII

FIG. 8B

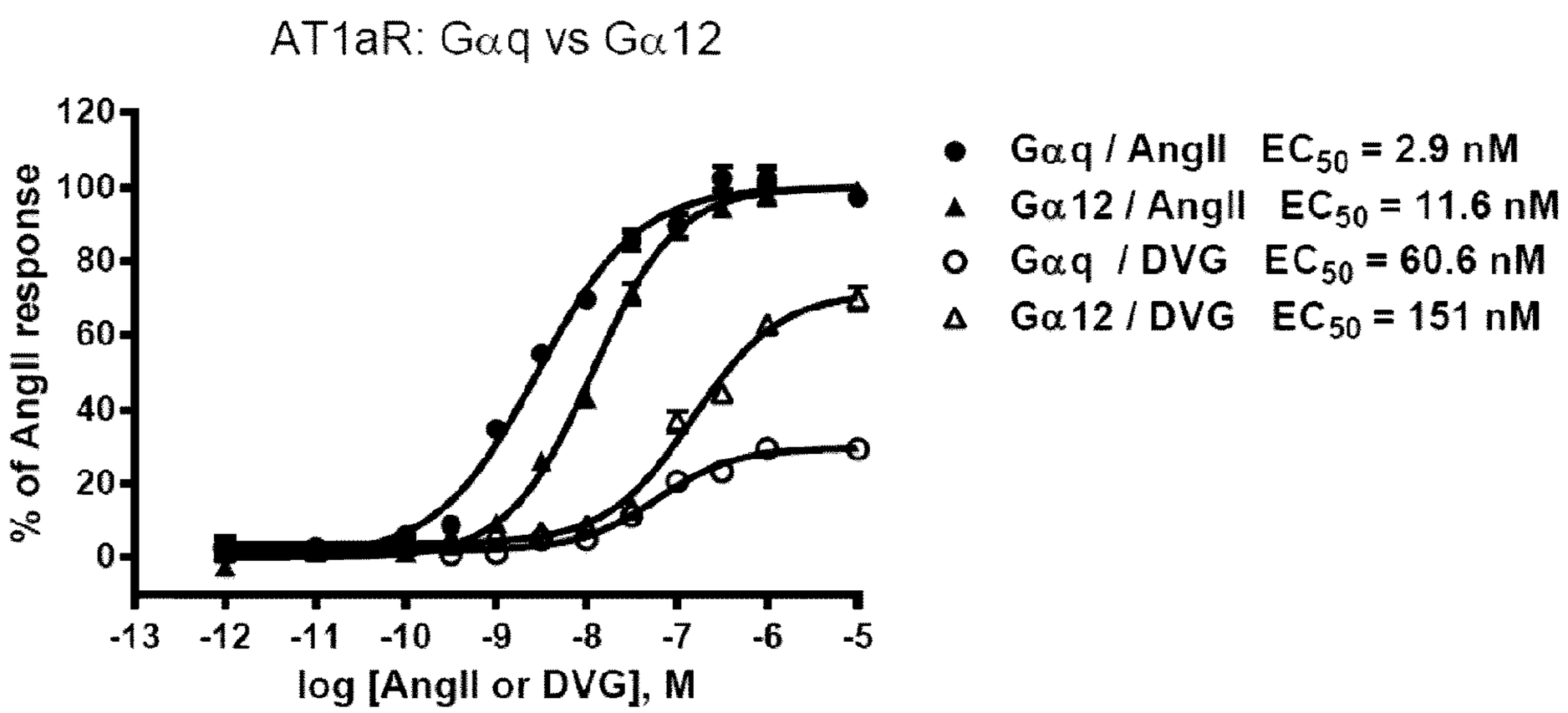

AT1aR: Gαq vs Gα12
% of AngII response
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
-5
log [AngII or DVG], M
Gαq / AngII EC50 = 2.9 nM
Gα12 / AngII EC50 = 11.6 nM
Gαq / DVG EC50 = 60.6 nM
Gα12 / DVG EC50 = 151 nM

FIG. 8C

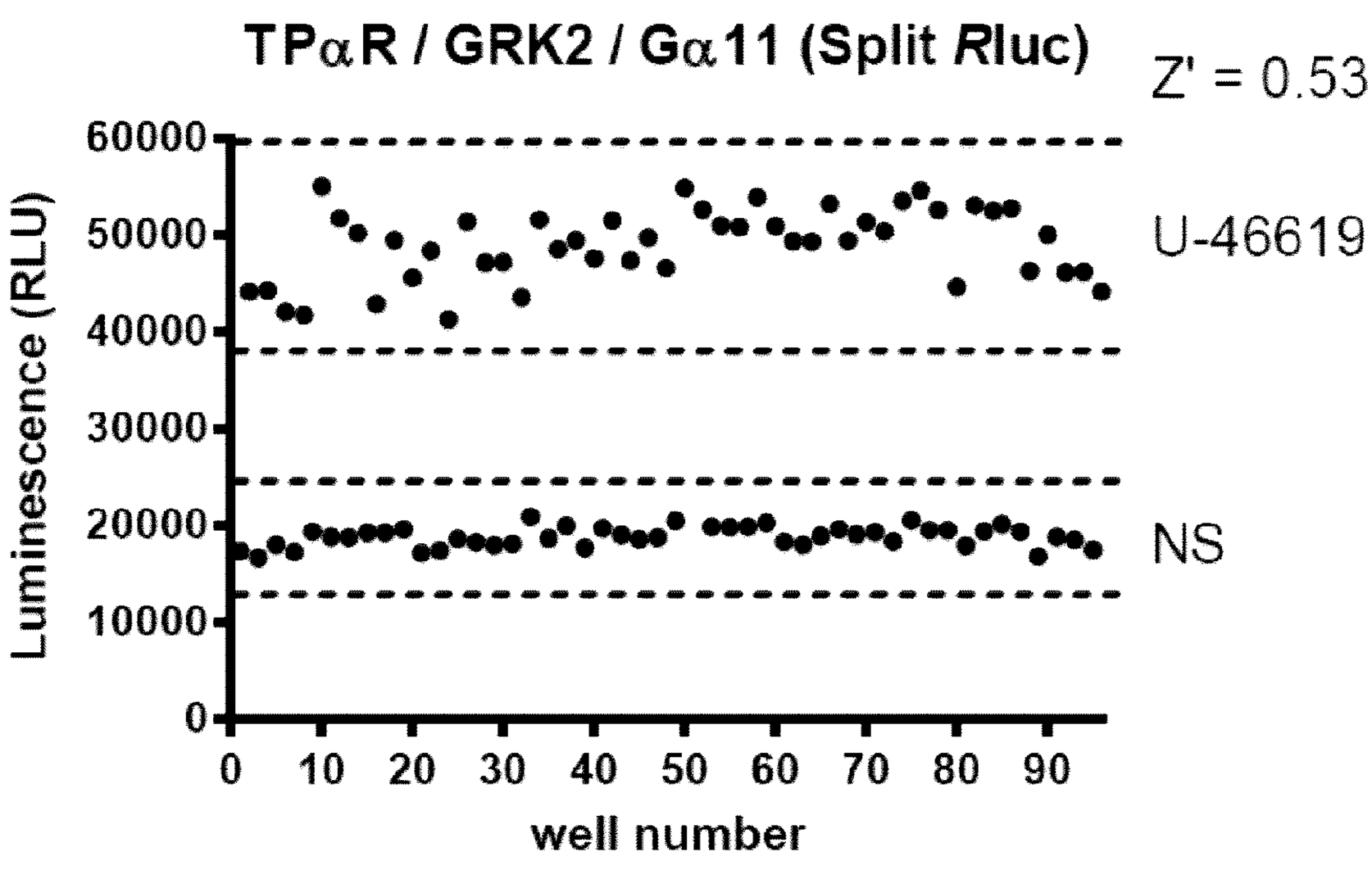

TPαR / GRK2 / Gα11 (Split Rluc)
Z' = 0.53
U-46619
NS
Luminescence (RLU)
60000
50000
40000
30000
20000
10000
0
0
10
20
30
40
50
60
70
80
90
well number

FIG. 9A

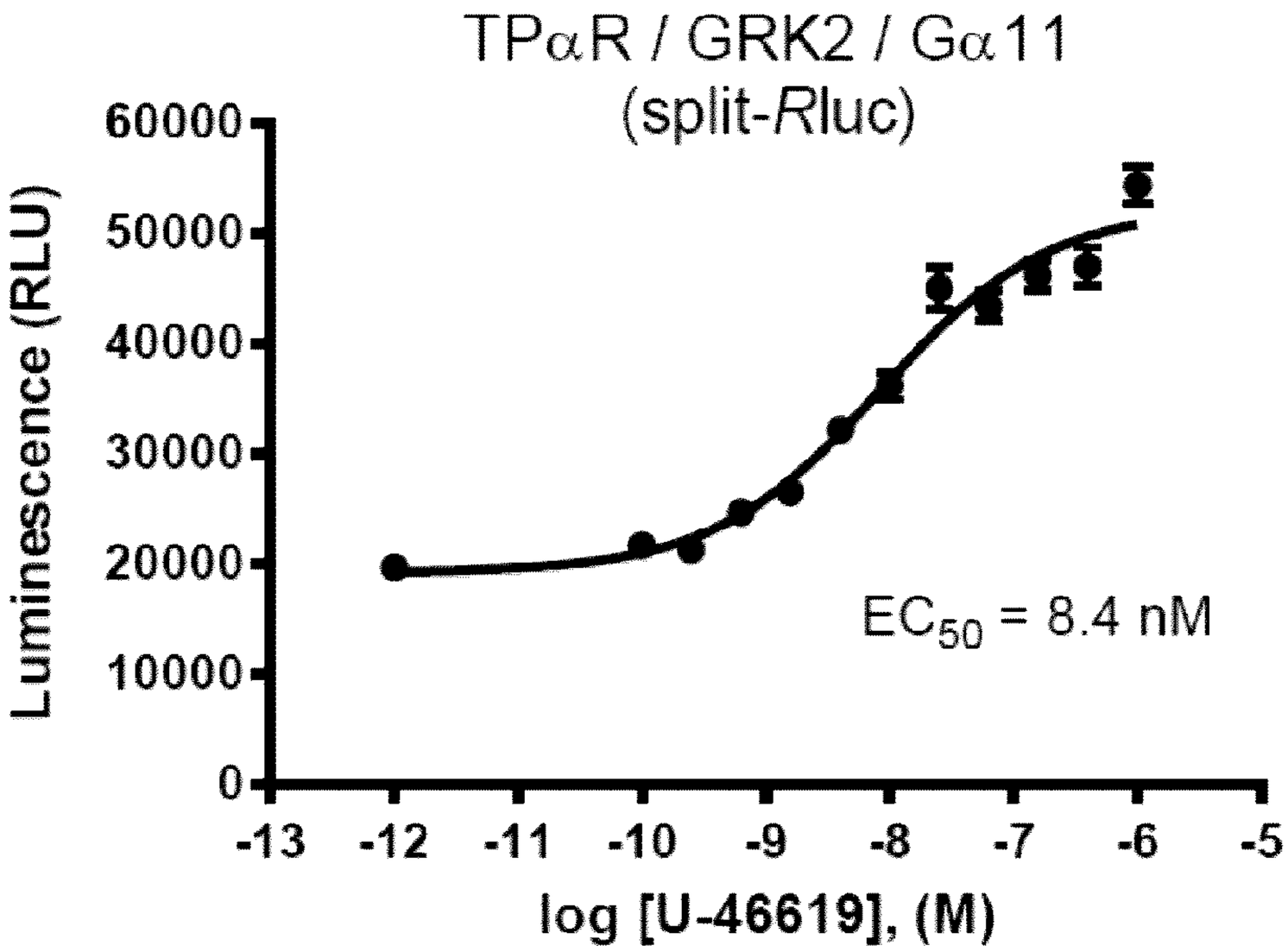



FIG. 9B

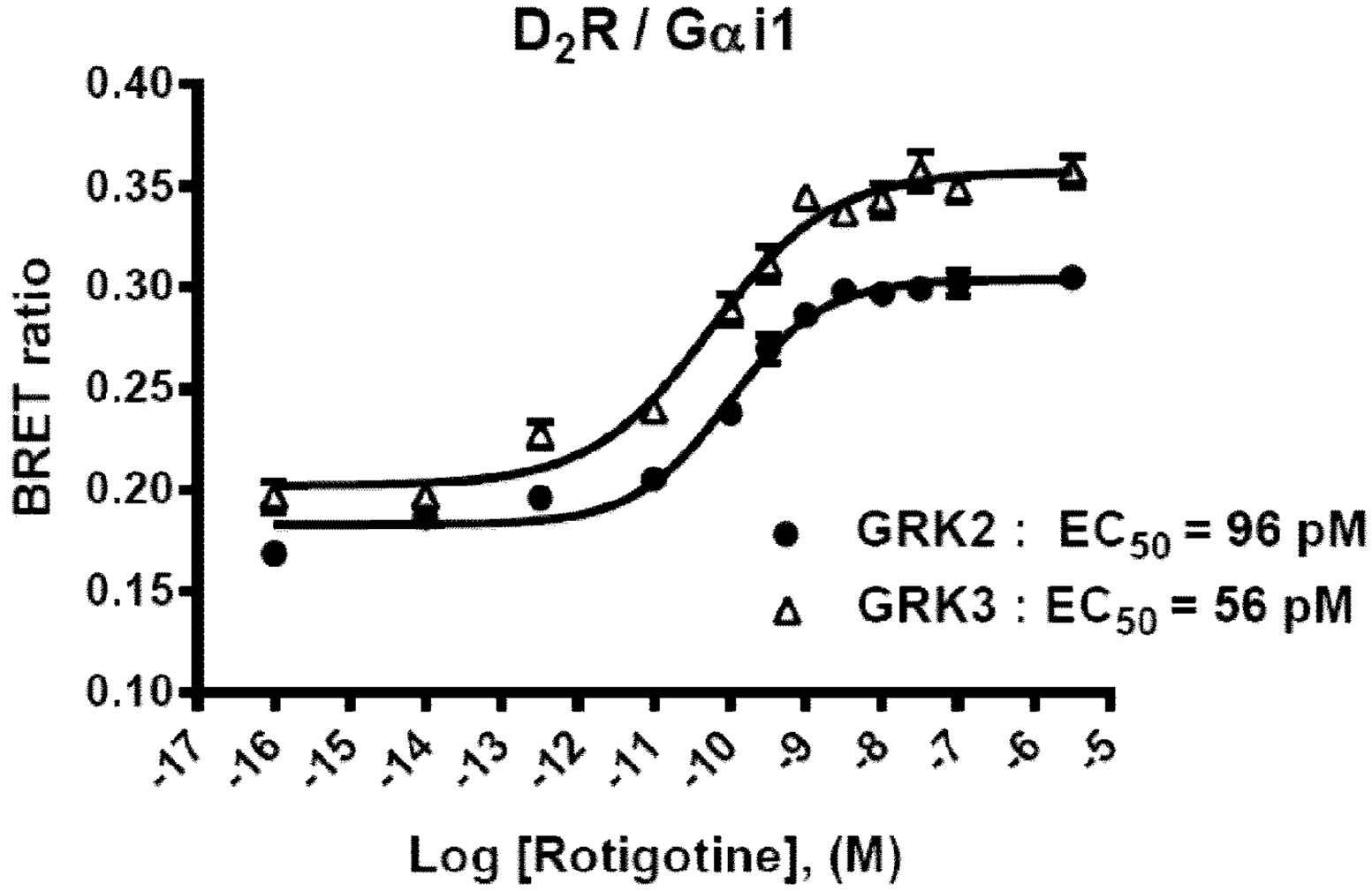



FIG. 10A

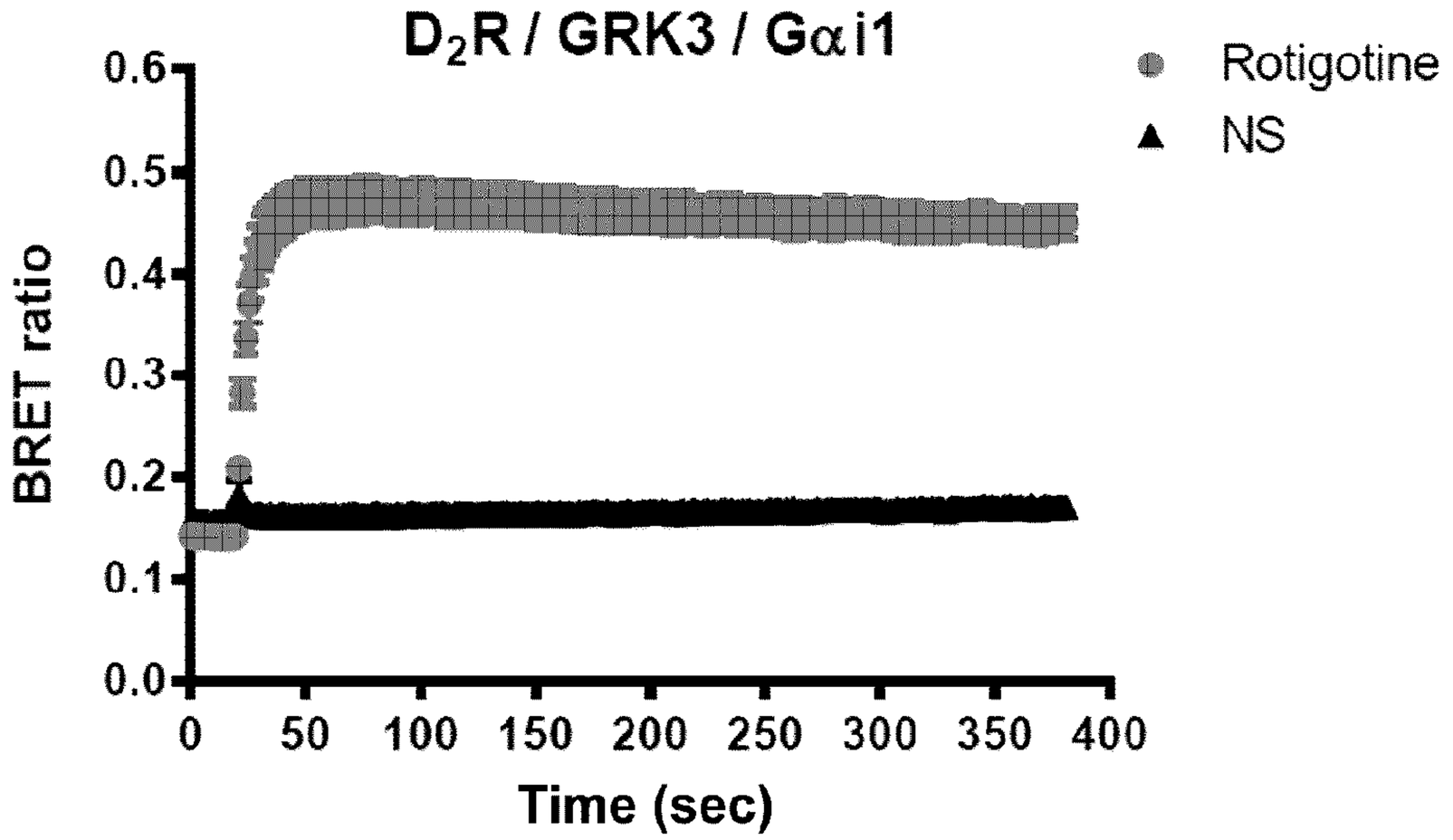



FIG. 10B

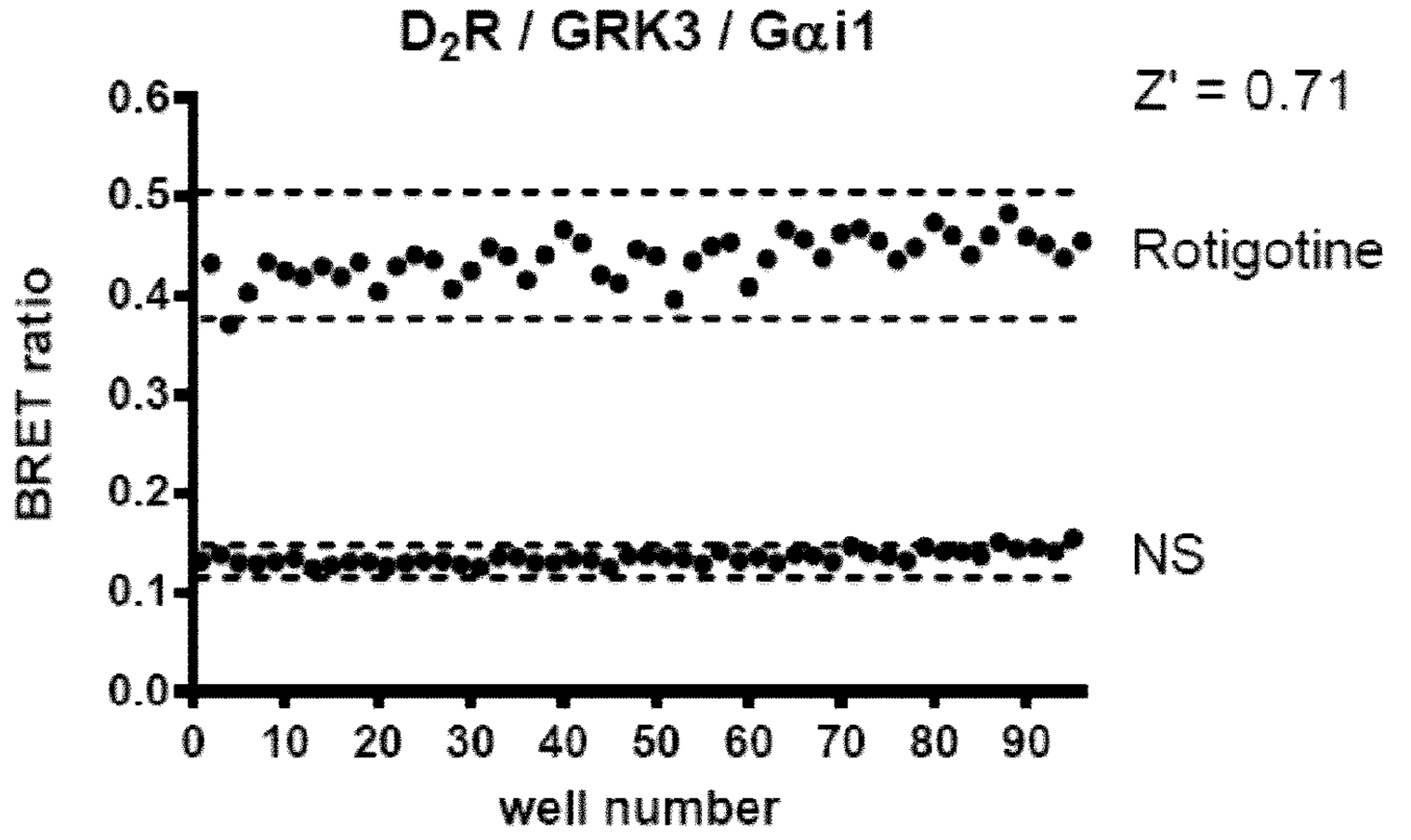
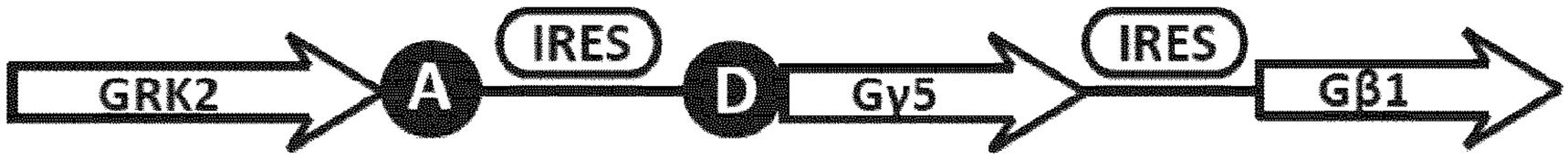



FIG. 10C




Legend: D =RET donor A =RET acceptor IRES=Internal Ribosome Entry Site

FIG. 11A

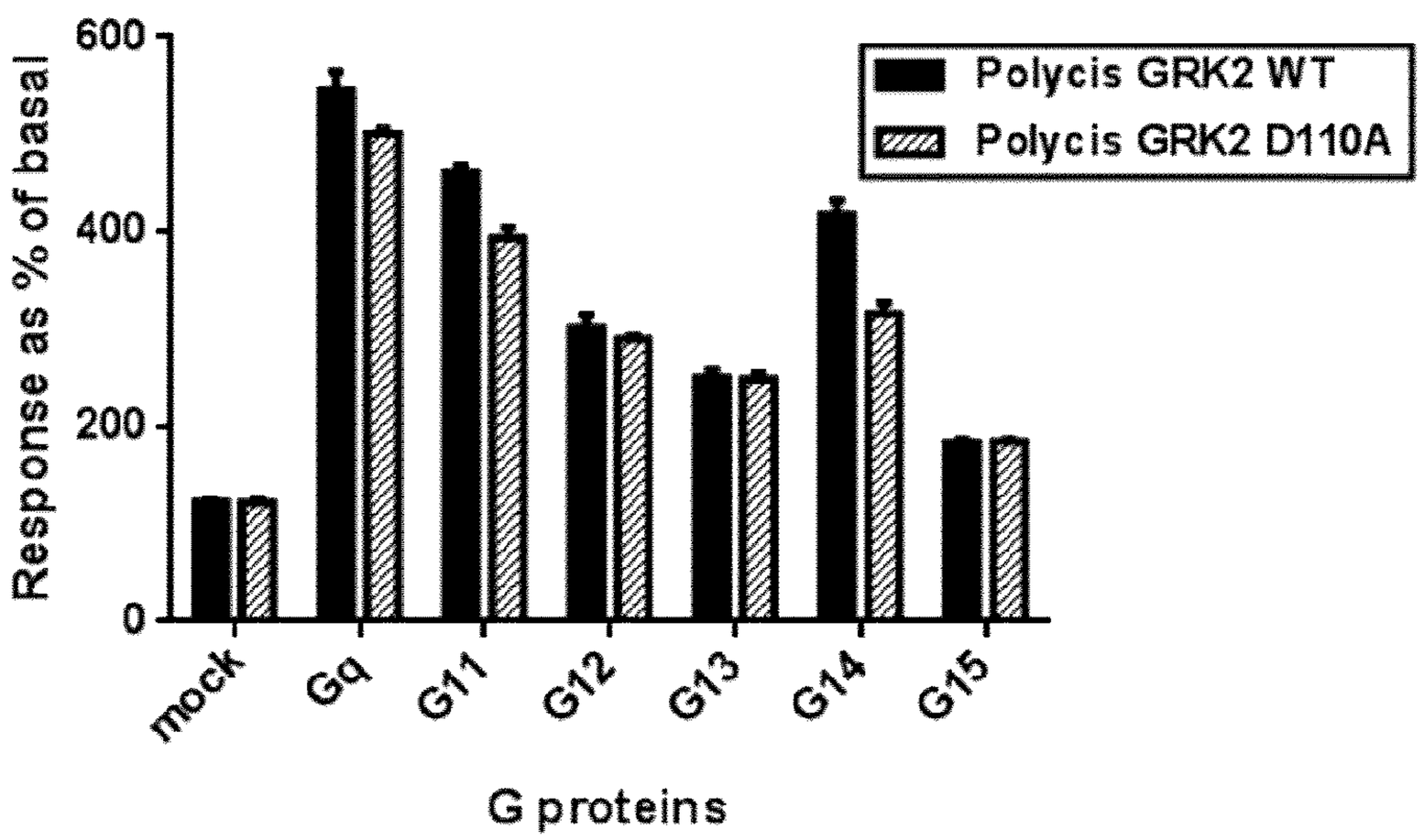



FIG. 11B

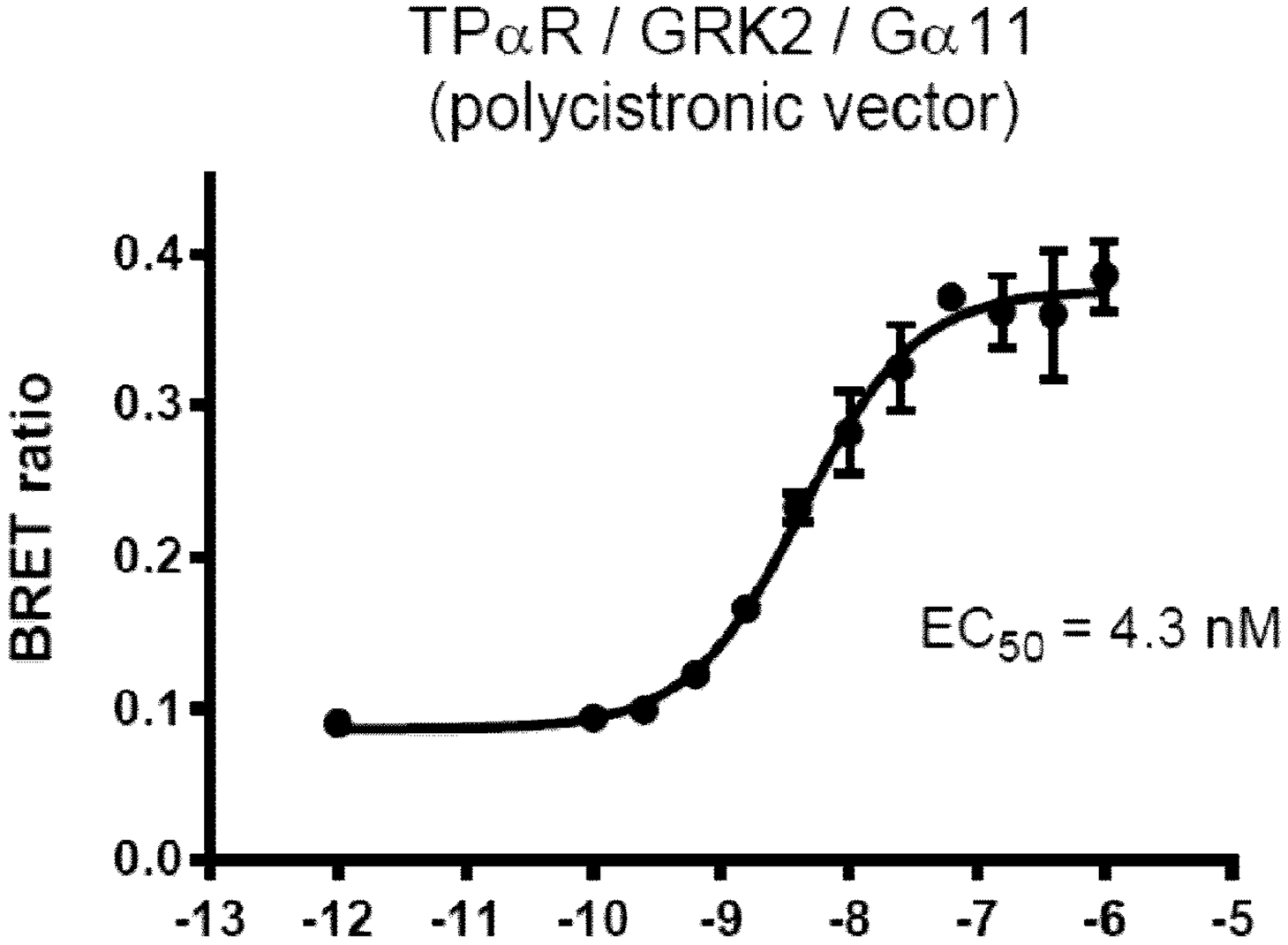



FIG. 11C

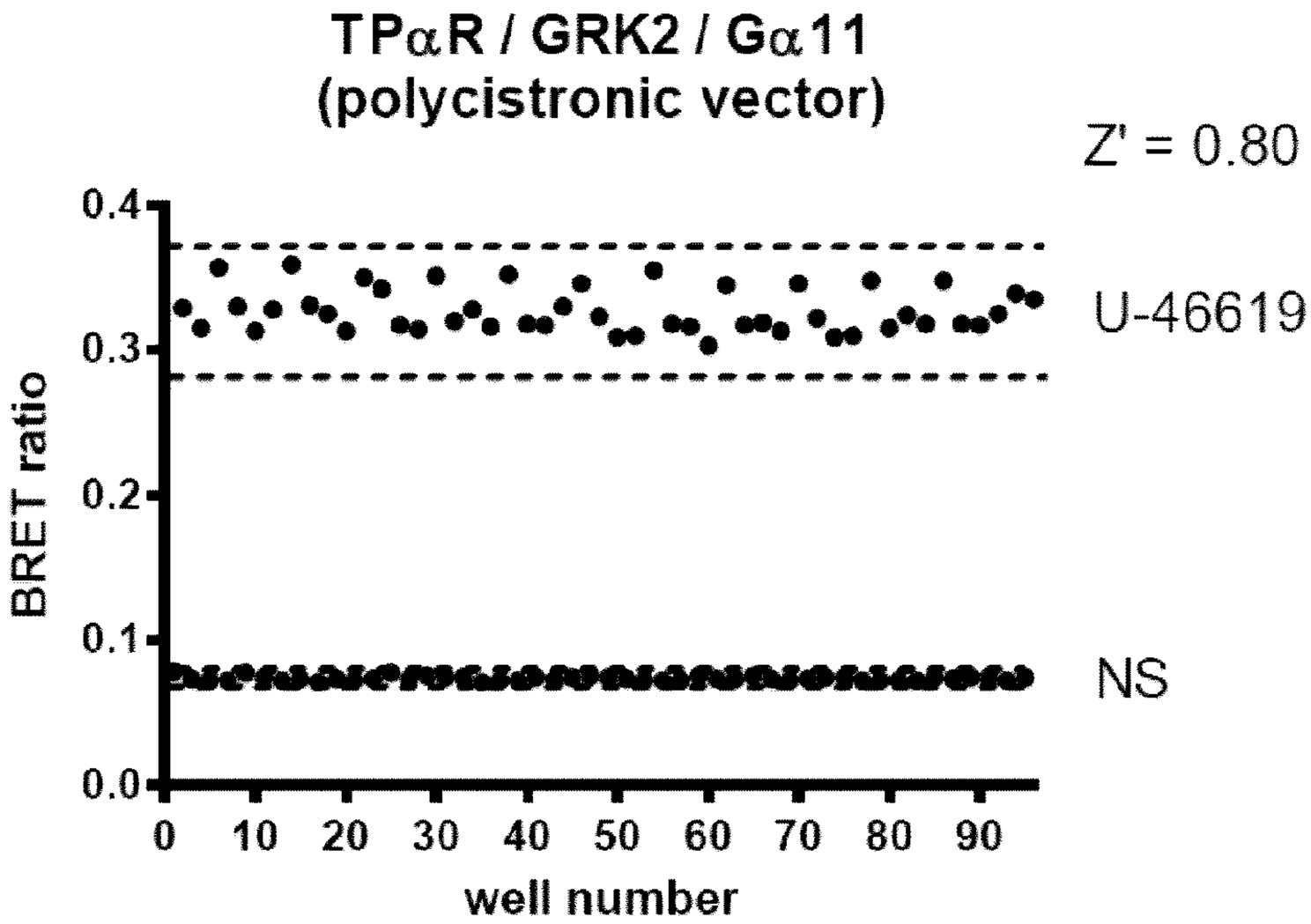



FIG. 11D

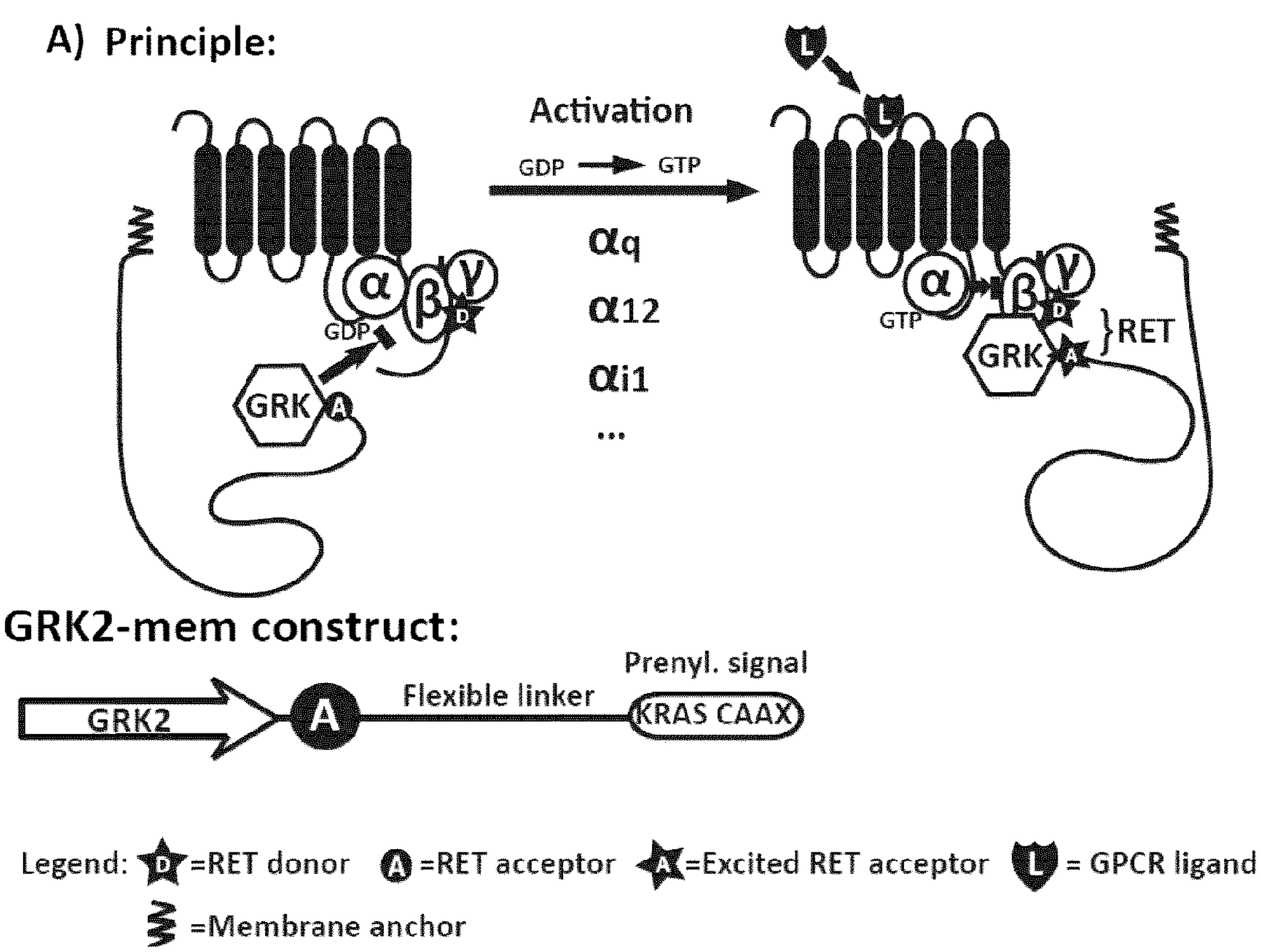



FIG. 12A

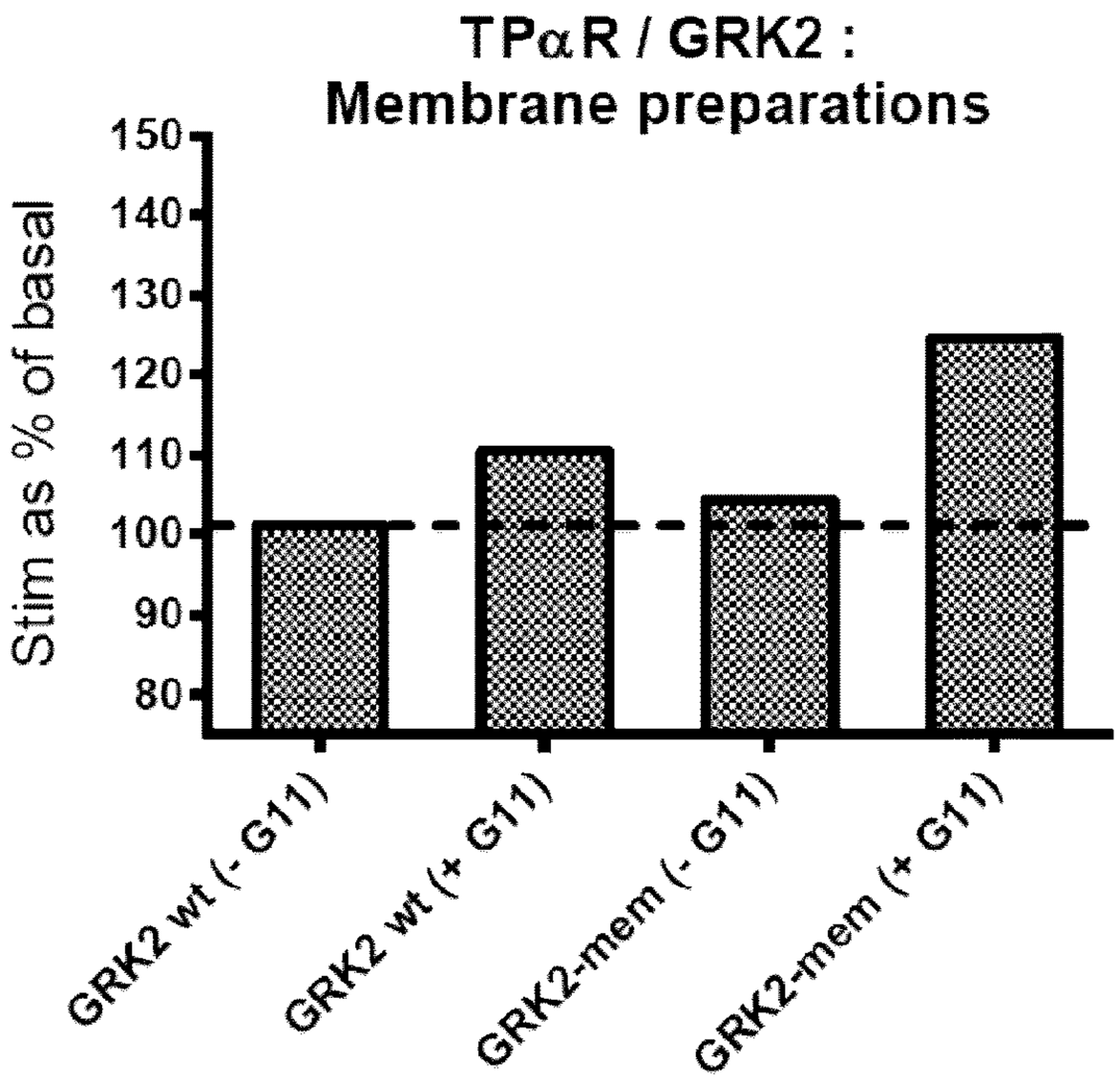



FIG. 12B

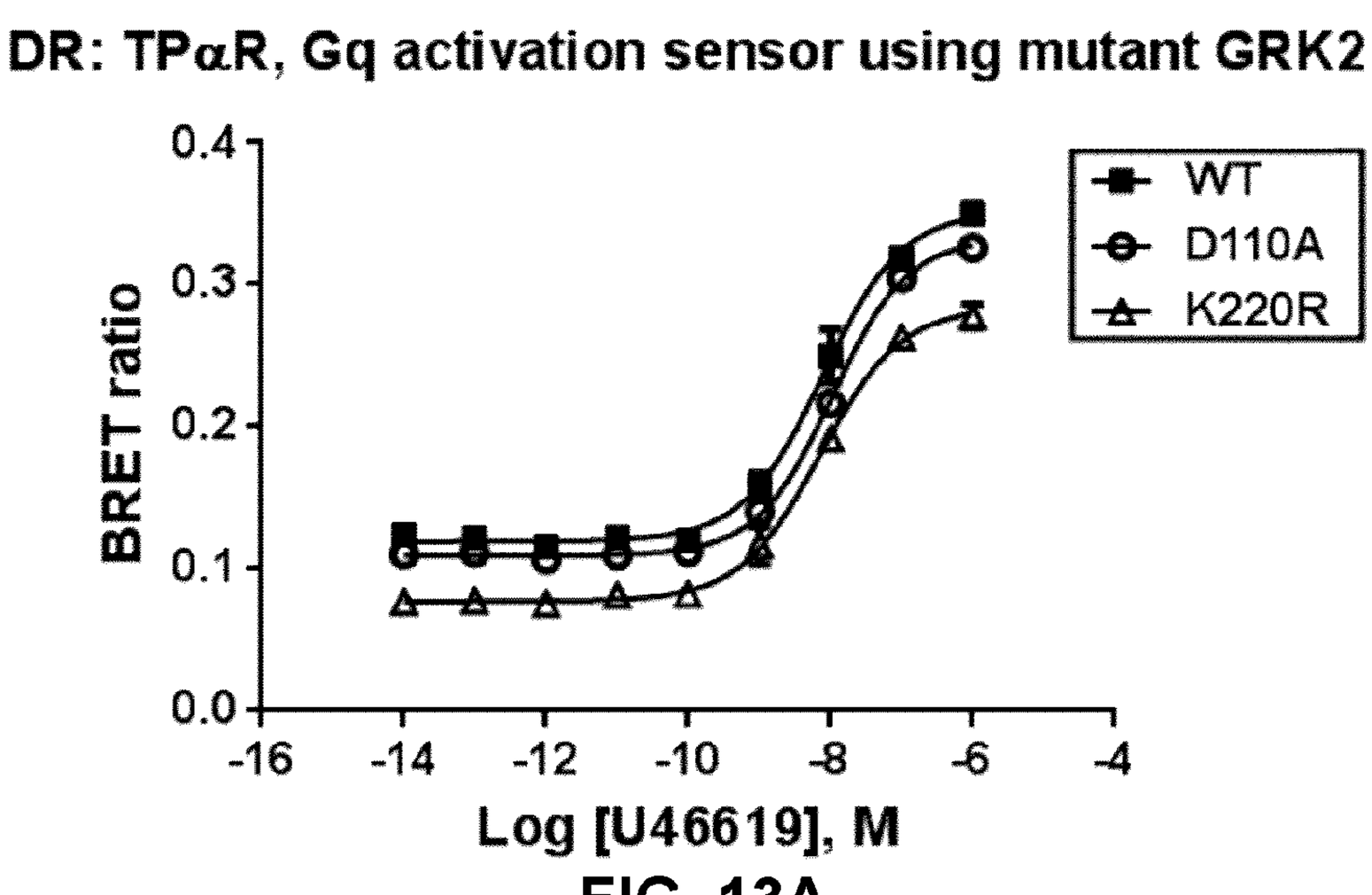



FIG. 13A

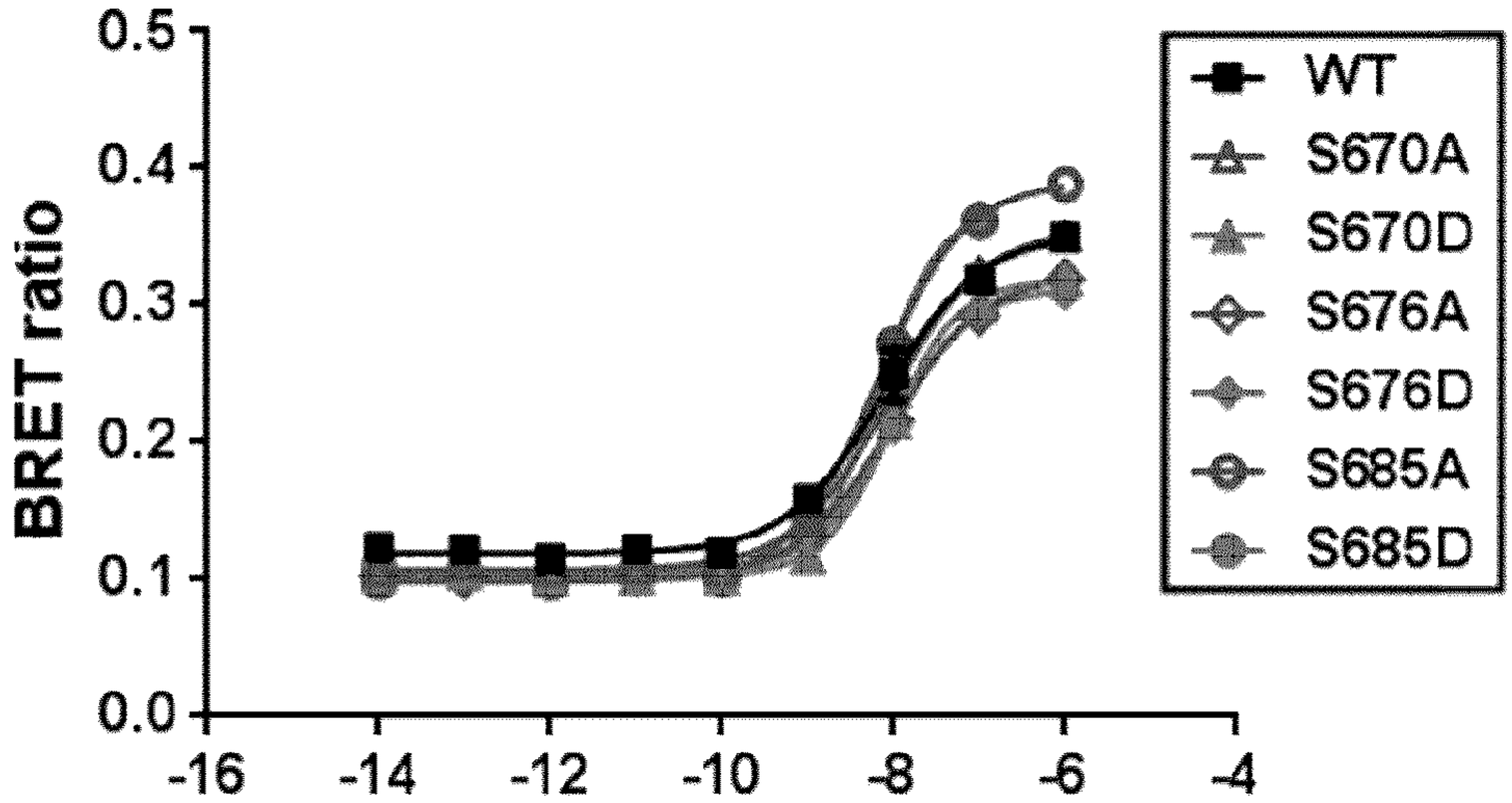



FIG. 13B

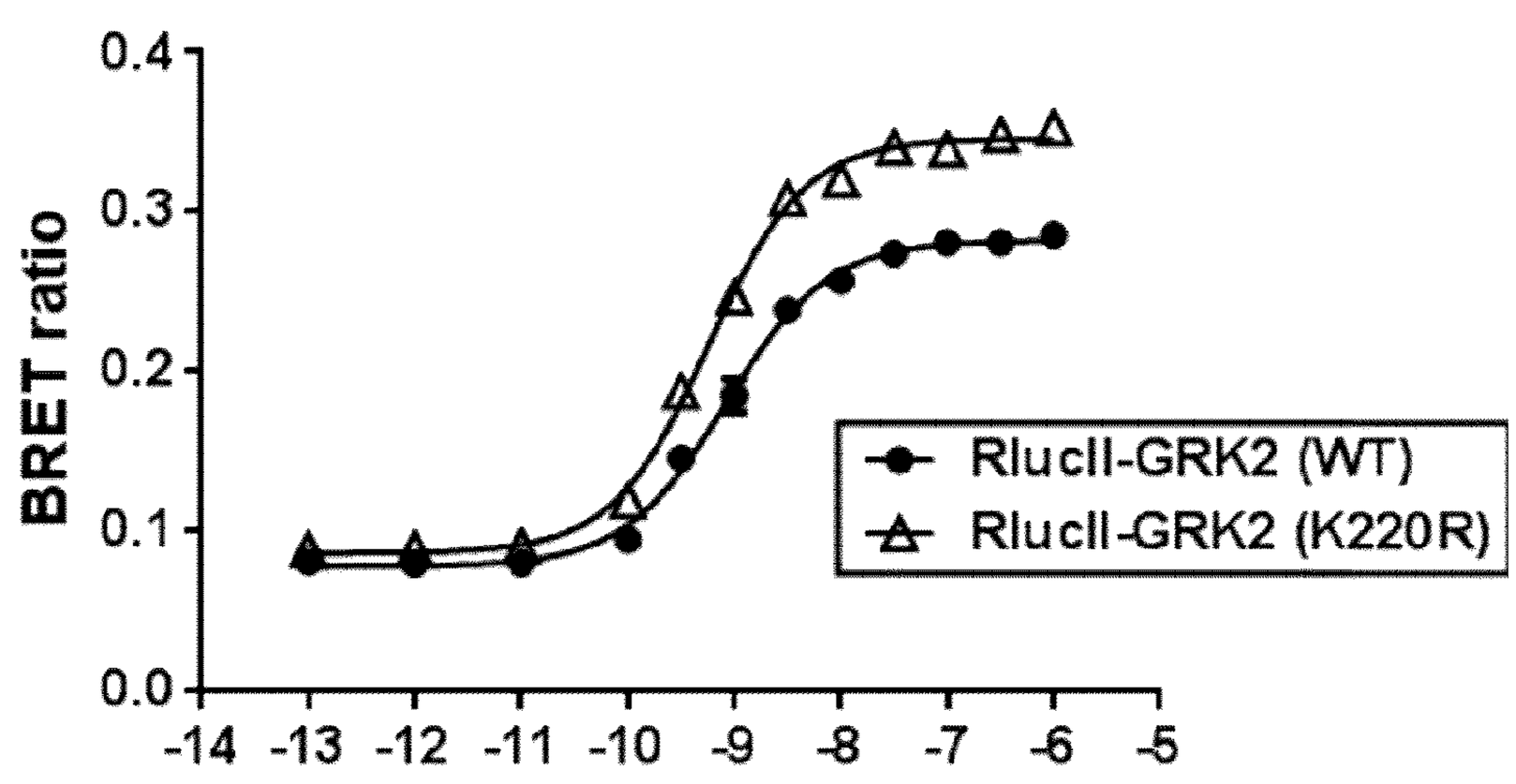



FIG. 13C

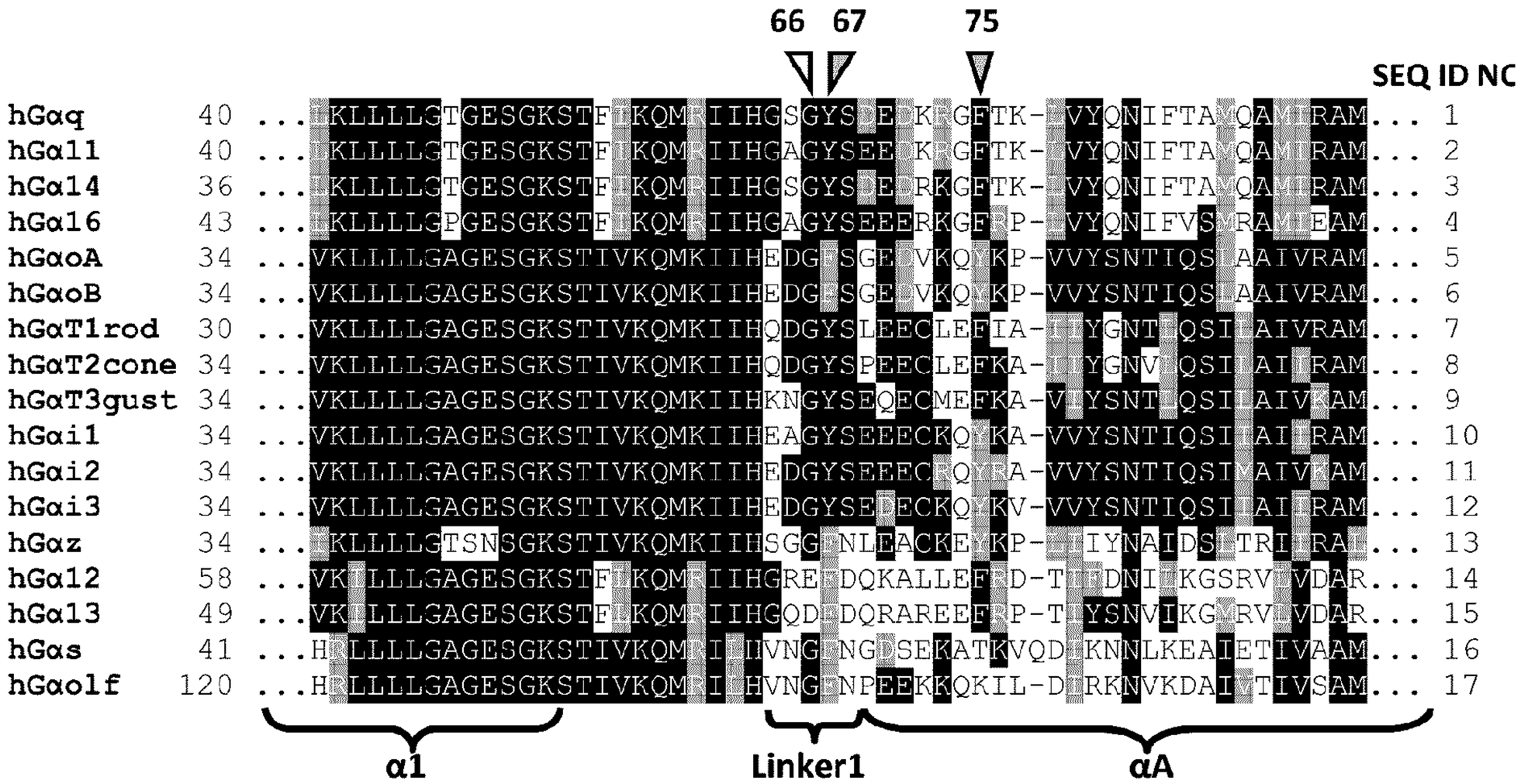
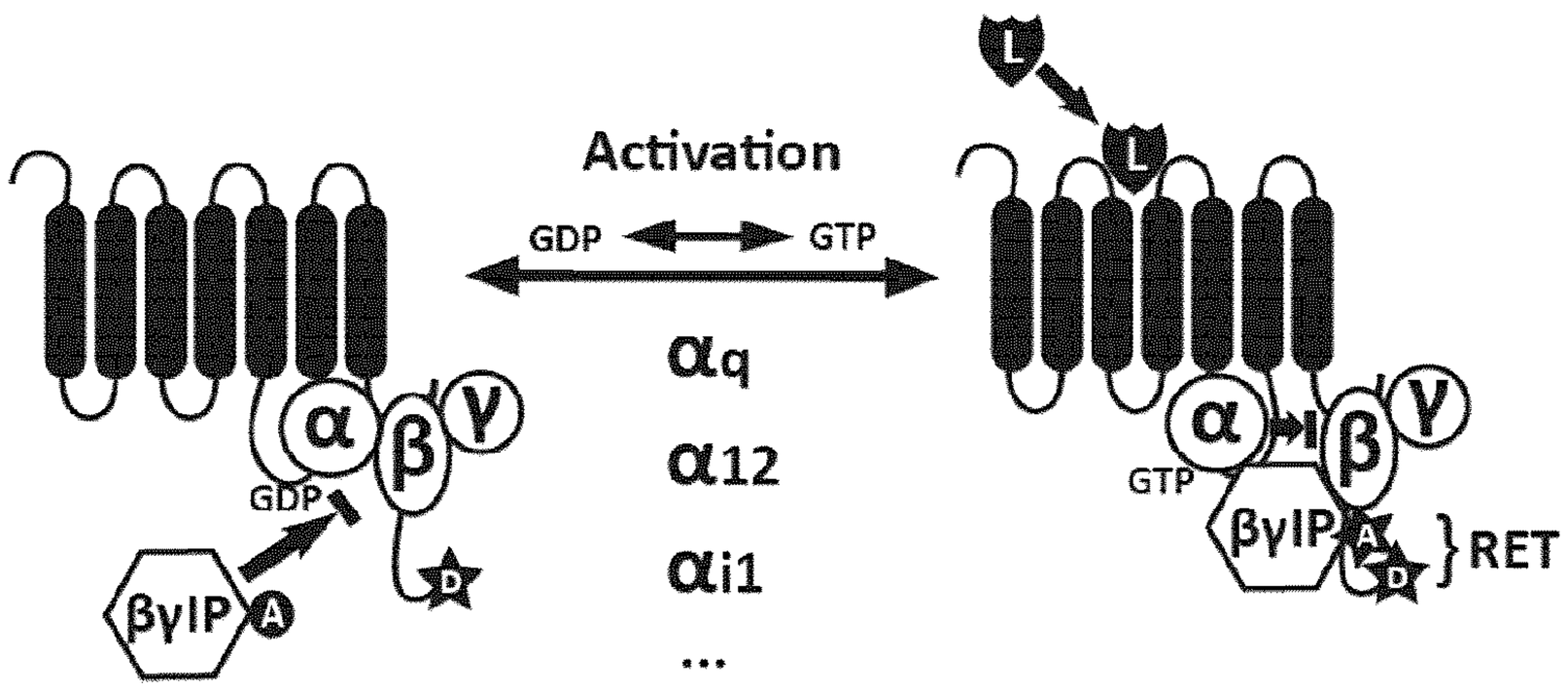
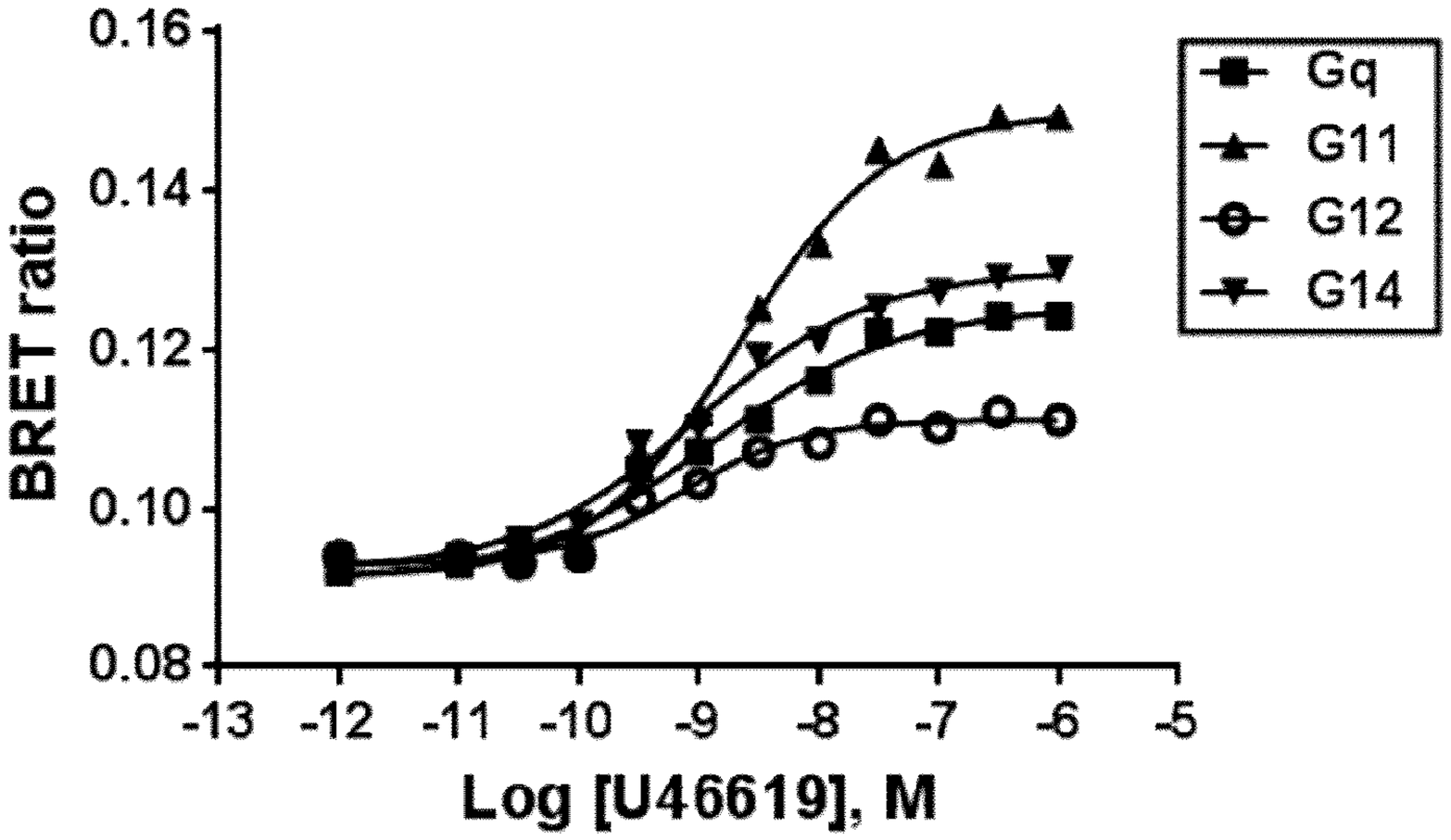



FIG. 14



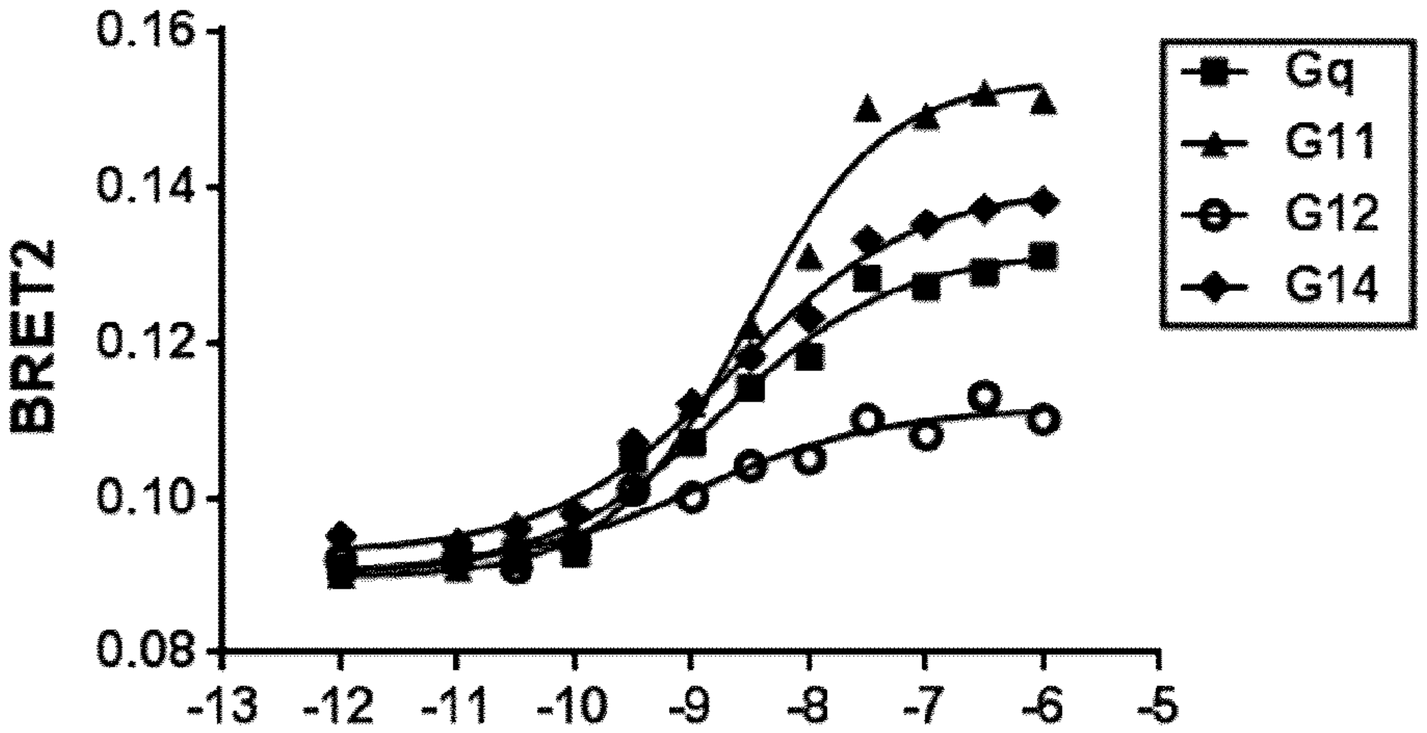

Legend: =RET donor =RET acceptor =Excited RET acceptor = GPCR ligand

FIG. 15A



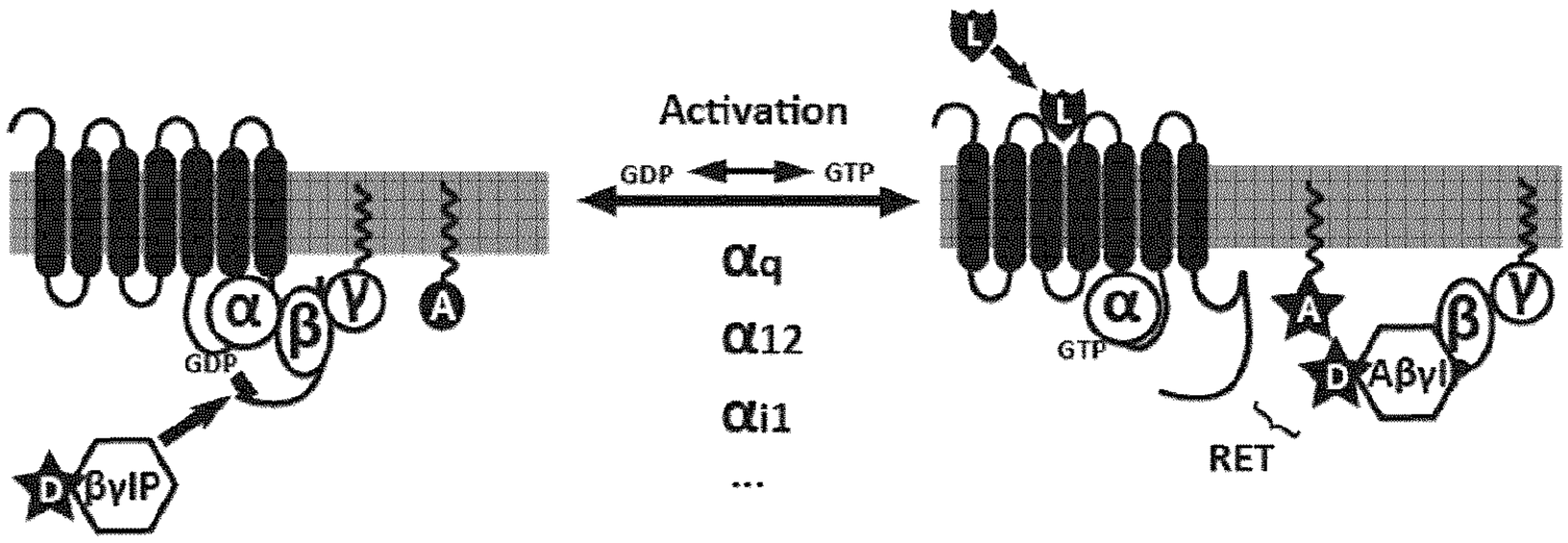

| | Gq | G11 | G12 | G14 |
|---|---|---|---|---|
| LogEC50 | -8.898 | -8.673 | -9.141 | -9.025 |

FIG. 15B



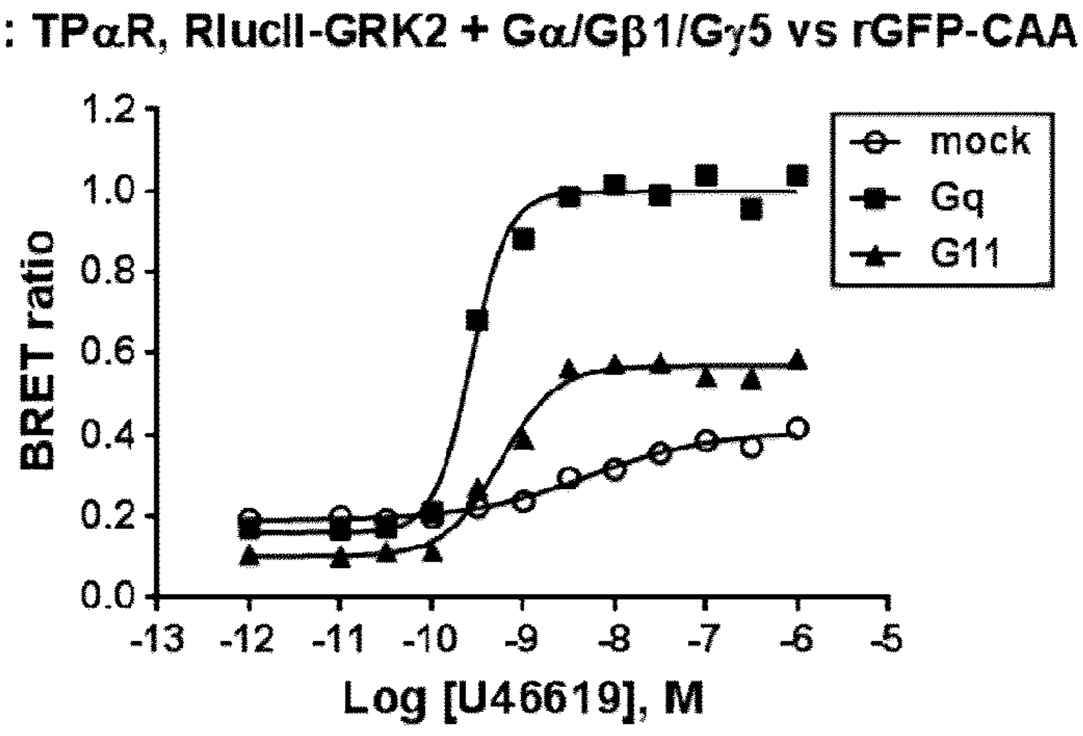

| | Gq | G11 | G12 | G14 |
|---|---|---|---|---|
| LogEC50 | -8.743 | -8.541 | -8.975 | -8.643 |

FIG. 15C



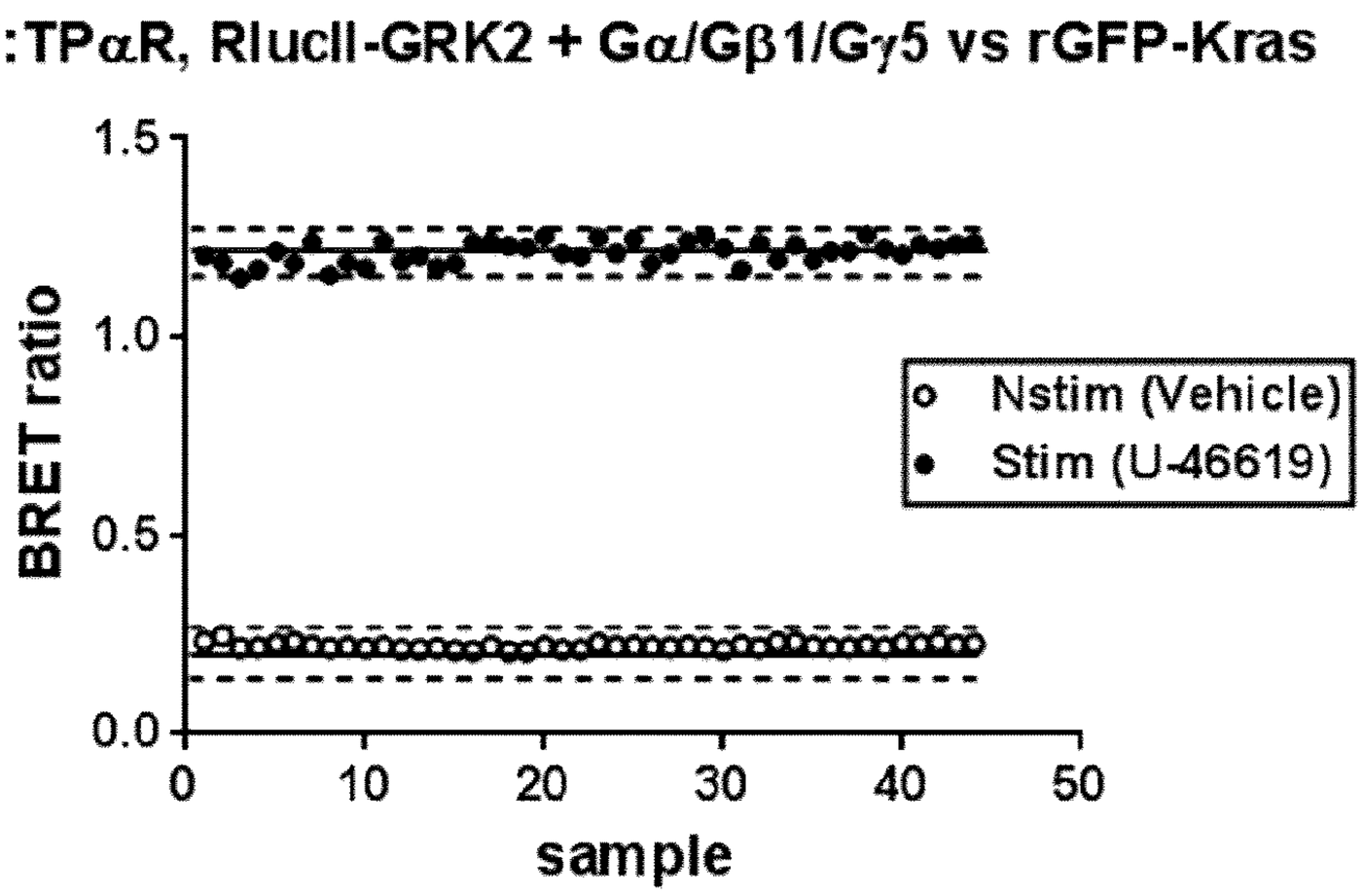

Legend: D =RET donor A =RET acceptor A =Excited RET acceptor L = GPCR ligand

FIG. 16A




| | mock | Gq | G11 |
|---|---|---|---|
| LogEC50 | -8.290 | -9.573 | -9.246 |

FIG. 16B




| | |
|---|---|
| 0.0845 | 3SD |
| 0.1073 | 3SDstim+3SDnstim |
| 0.9919 | stim-NS |
| | |
| 0.8919 | Z' Factor |

FIG. 16C

**GRK2**

MADLEAVLAD VSYLMAMEKS KATPAARASK KILLPEPSIR SVMQKYLEDR GEVTFEKIFS QKLGYLLFRD
FCLNHLEEAR PLVEFYEEIK KYEKLETEEE RVARSREIF**D** SYIMKELLAC SHPFSKSATE HVQGHLGKKQ
VPPDLFQPYI EEICQNLRGD VFQKFIESDK FTRFCQWKNV ELNIHLTMND FSVHRIIGRG GFGEVYGCRK
ADTGKMYAM**K** CLDKKRIKMK QGETLALNER IMLSLVSTGD CPFIVCMSYA FHTPDKLSFI LDLMNGGDLH
YHLSQHGVFS EADMRFYAAE IILGLEHMHN RFVVYRDLKP ANILLDEHGH VRISDLGLAC DFSKKKPHAS
VGTHGYMAPE VLQKGVAYDS SADWFSLGCM LFKLLRGHSP FRQHKTKDKH EIDRMTLTMA VELPDSFSPE
LRSLLEGLLQ RDVNRRLGCL GRGAQEVKES PFFRSLDWQM VFLQKYPPPL IPPRGEVNAA DAFDIGSFDE
EDTKG*IKLLD SDQELYRNFP LTISERWQQE VAETVFDTIN AETDRLEARK KAKNKQLGHE EDYALGKDCI*
*MHGYMSKMGN PFLTQWQRRY FYLFPNRLEW RGEGEAPQSL LTMEEIQSVE ETQIKERKCL LLKIRGGKQF*
*ILQCDSDPEL VQWKKELRDA YREAQQLVQR VPKMKNKPR**S** PVVEL**S**KVPL VQRG**S**ANGL*

FIG. 17A

**GRK3**

MADLEAVLAD VSYLMAMEKS KATPAARASK KIVLPEPSIR SVMQKYLEER HEITFDKIFN QRIGFLLFKD
FCLNEINEAV PQVKFYEEIK EYEKLENEED RLCRSRQIYD TYIMKELLSC SHPFSKQAVE HVQSHLSKKQ
VTSTLFQPYI EEICESLRGS IFQKFMESDK FTRFCQWKNV ELNIHLTMND FSVHRIIGRG GFGEVYGCRK
ADTGKMYAMK CLDKKRIKMK QGETLALNER IMLSLVSTGD CPFIVCMTYA FHTPDKLCFI LDLMNGGDLH
YHLSQHGVFS EKEMRFYATE IILGLEHMHN RFVVYRDLKP ANILLDEHGH VRISDLGLAC DFSKKKPHAS
VGTHGYMAPE VLQKGTAYDS SADWFSLGCM LFKLLRGHSP FRQHKTKDKH EIDRMTLTMN VELPDVFSPE
LKSLLEGLLQ RDVSKRLGCH GGSAQELKTH DFFRGIDWQH VYLQKYPPPL IPPRGEVNAA DAFDIGSFDE
EDTKG*IKLLD CDQELYKNFP LVISERWQQE VAETVYEAVN ADTDKIEARK RAKNKQLGHE EDYALGRDCI*
*VHGYMLKLGN PFLTQWQRRY FYLFPNRLEW RGEGESRQSL LTMEQIVSVE ETQIKDKKCI LLRIKGGKQF*
*VLQCESDPEF VQWKKELTET FMEAQRLLRR APKFLNKSRS AVVELSKPPL CHRNSNGL*

FIG. 17B

**PLEKHG2**

MPEGAQGLSL SKPSPSLGCG RRGEVCDCGT VCETRTAPAA PTMASPRGSG SSTSLSTVGS EGDPAPGPTP
ACSASRPEPL PGPPIRLHLS PVGIPGSARP SRLERVAREI VETERAYVRD LRSIVEDYLG PLLDGGVLGL
SVEQVGTLFA NIEDIYEFSS ELLEDLENSS SAGGIAECFV QRSEDFDIYT LYCMNYPSSL ALLRELSLSP
PAALWLQERQ AQLRHSLPLQ SFLLKPVQRI LKYHLLLQEL GKHWAEGPGT GGREMVEEAI VSMTAVAWYI
NDMKRKQEHA ARLQEVQRRL GGWTGPELSA FGELVLEGAF RGGGGGGPRL RGGERLLFLF SRMLLVAKRR
GLEYTYKGHI FCCNLSVSES PRDPLGFKVS DLTIPKHRHL LQAKNQEEKR LWIHCLQRLF FENHPASIPA
KAKQVLLENS LHCAPKSKPV LEPLTPPLGS PRPRDARSFT PGRRNTAPSP GPSVIRRGRR QSEPVKDPYV
MFPQNAKPGF KHAGSEGELY PPESQPPVSG SAPPEDLEDA GPPTLDPSGT SITEEILELL NQRGLRDPGP
STHDIPKFPG DSQVPGDSET LTFQALPSRD SSEEEEEEEE GLEMDERGPS PLHVLEGLES SIAAEMPSIP
CLTKIPDVPN LPEIPSRCEI PEGSRLPSLS DISDVFEMPC LPAIPSVPNT PSLSSTPTLS CDSWLQGPLQ
EPAEAPATRR ELFSGSNPGK LGEPPSGGKA GPEEDEEGVS FTDFQPQDVT QHQGFPDELA FRSCSEIRSA
WQALEQGQLA RPGFPEPLLI LEDSDLGGDS GSGKAGAPSS ERTASRVREL ARLYSERIQQ MQRAETRASA
NAPRRRPRVL AQPQPSPCLP QEQAEPGLLP AFGHVLVCEL AFPLTCAQES VPLGPAVWVQ AAIPLSKQGG
SPDGQGLHVS NLPKQDLPGI HVSAATLLPE QGGSRHVQAP AATPLPKQEG PLHLQVPALT TFSDQGHPEI
QVPATTPLPE HRSHMVIPAP STAFCPEQGH CADIHVPTTP ALPKEICSDF TVSVTTPVPK QEGHLDSESP
TNIPLTKQGG SRDVQGPDPV CSQPIQPLSW HGSSLDPQGP GDTLPPLPCH LPDLQIPGTS PLPAHGSHLD
HRIPANAPLS LSQELPDTQV PATTPLPLPQ VLTDIWVQAL PTSPKQGSLP DIQGPAAAPP LPEPSLTDTQ
VQKLTPSLEQ KSLIDAHVPA ATPLPERGGS LDIQGLSPTP VQTTMVLSKP GGSLASHVAR LESSDLTPPH
SPPPSSRQLL GPNAAALSRY LAASYISQSL ARRQGPGGGA PAASRGSWSS APTSRASSPP PQPQPPPPPA
RRLSYATTVN IHVGGGGRLR PAKAQVRLNH PALLASTQES MGLHRAQGAP DAPFHM

**FIG. 17C**

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLSYGVQCFSRYPDHMK
QHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNPHNVYIMADKQKN
GIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLFTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

**FIG. 17D**

MDLAKLGLKEVMPTKINLEGLVGDHAFSMEGVGEGNILEGTQEVKISVTKGAPLPFAFDIVSVAFSYGNRAYTGYPEEIS
DYFLQSFPEGFTYERNIRYQDGGTAIVKSDISLEDGKFIVNVDFKAKDLRRMGPVMQQDIVGMQPSYESMYTNVTSVIGE
CIIAFKLQTGKHFTYHMRTVYKSKKPVETMPLYHFIQHRLVKTNVDTASGYVVQHETAIAAHSTIKKIEGSLP

**FIG. 17E**

MTSKVYDPEQRKRMITGPQWWARCKQMNVLDSFINYYDSEKHAENAVIFLHGNATSSYLWRHVVPHIEPVARCIIPDLIG
MGKSGKSGNGSYRLLDHYKYLTAWFELLNLPKKIIFVGHDWGAALAFHYSYEHQDKIKAIVHAESVVDVIESWDEWPDIE
EDIALIKSEEGEKMVLENNFFVETVLPSKIMRKLEPEEFAAYLEPFKEKGEVRRPTLSWPREIPLVKGGKPDVVQIVRNY
NAYLRASDDLPKMFIESDPGFFSNAIVEGAKKFPNTEFVKVKGLHFSQEDAPDEMGKYIKSFVERVLKNEQ

**FIG. 17F**