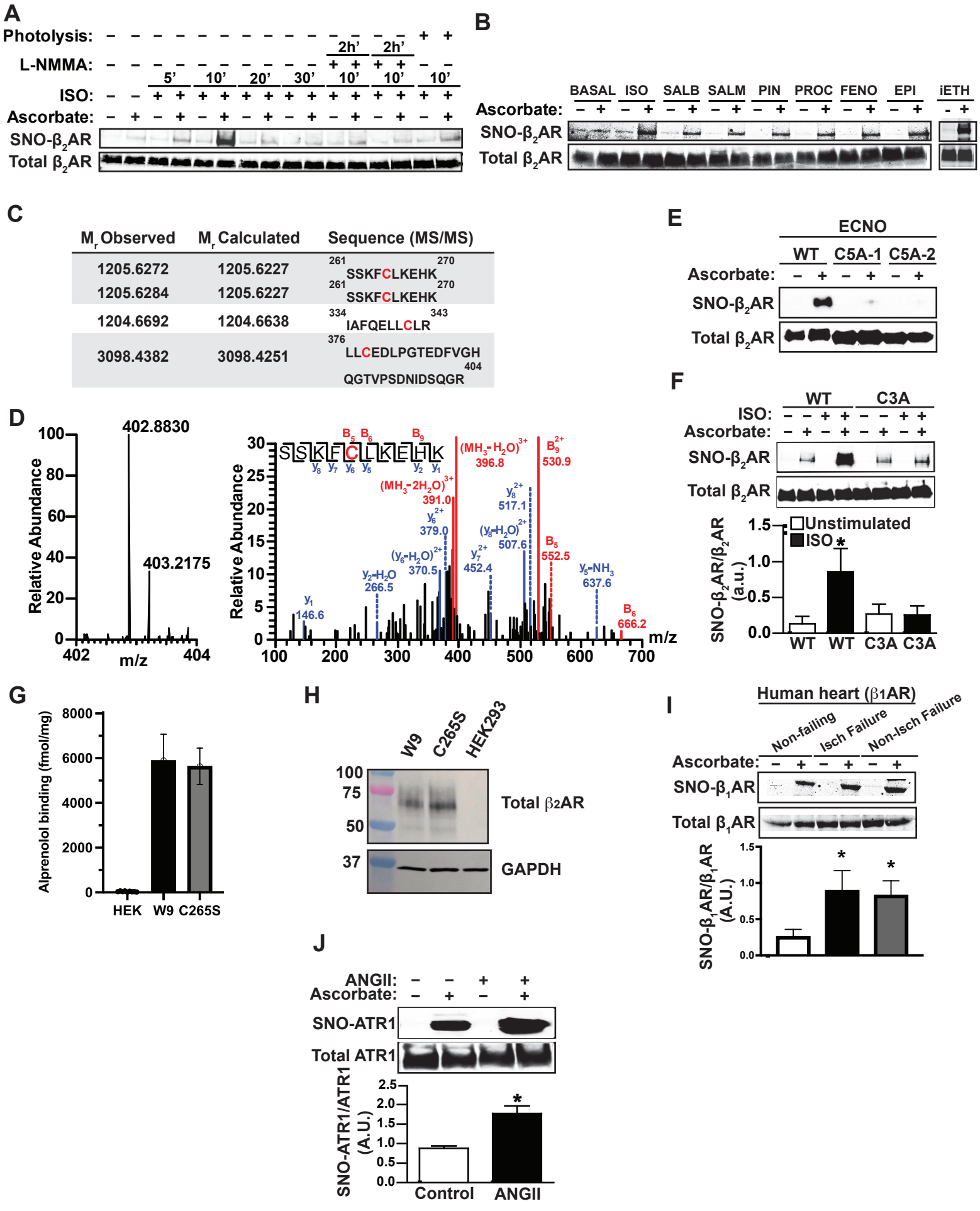
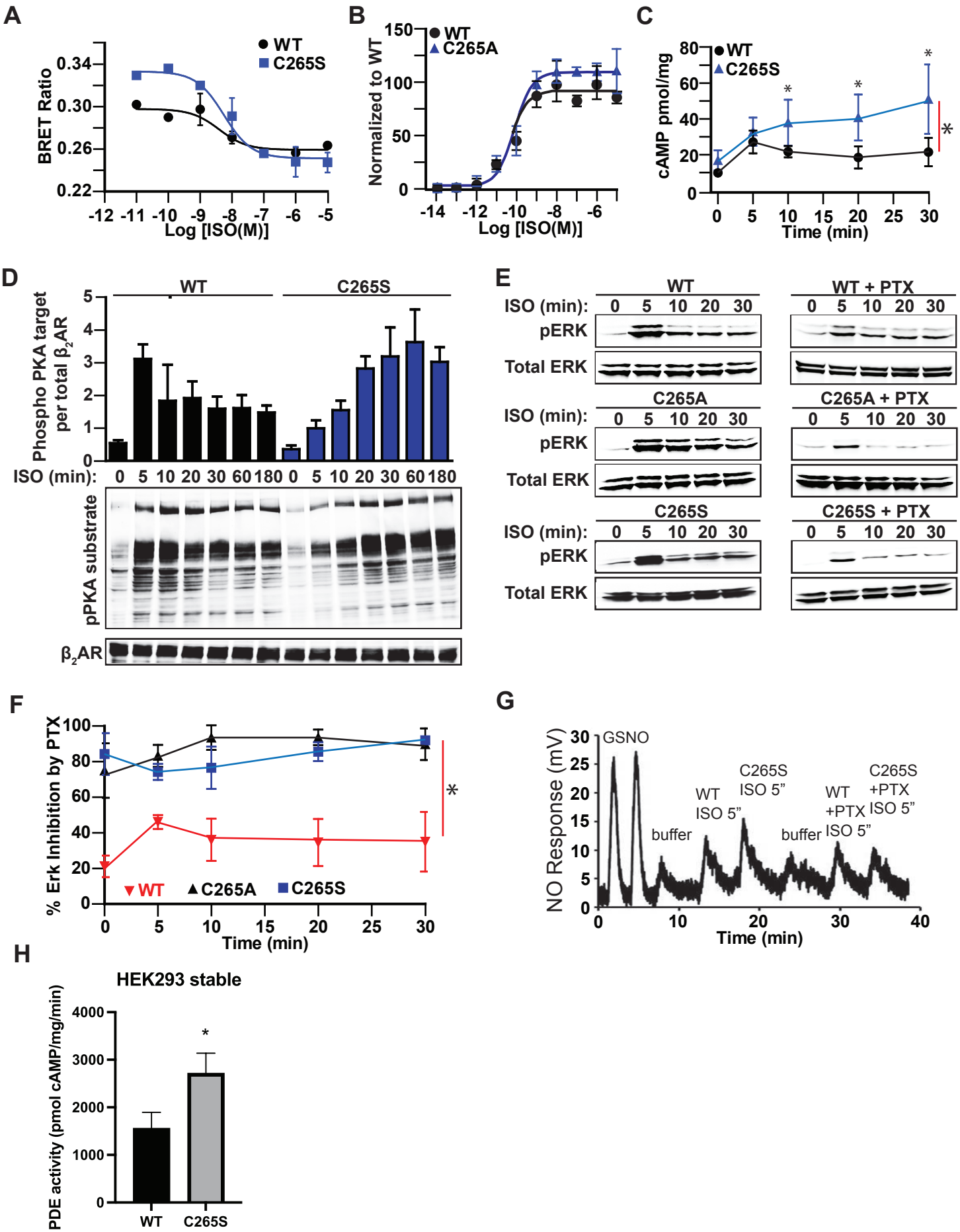

A
Photolysis:
L-NMMA:
ISO:
Ascorbate:
2h'
5'
10'
20'
30'
SNO-β2AR
Total β2AR
B
BASAL ISO SALB SALM PIN PROC FENO EPI iETH
Ascorbate:
SNO-β2AR
Total β2AR
C
Mr Observed
Mr Calculated
Sequence (MS/MS)
1205.6272
1205.6227
261 SSKFCLKEHK 270
1205.6284
1205.6227
261 SSKFCLKEHK 270
1204.6692
1204.6638
334 IAFQELLCLR 343
3098.4382
3098.4251
376 LLCEDLPGTEDFVGH 404
QGTVPSDNIDSQGR
D
Relative Abundance
m/z
402.8830
403.2175
SSKFCLLKEHK
(MH3-H2O)3+ 396.8
B9 530.9
(MH3-2H2O)3+ 391.0
y8 517.1
y6 379.0
(y8-H2O)2+ 507.6
(y6-H2O)2+ 370.5
y7 452.4
B5 552.5
y2-H2O 266.5
y5-NH3 637.6
y1 146.6
B6 666.2
E
ECNO
WT C5A-1 C5A-2
Ascorbate:
SNO-β2AR
Total β2AR
F
WT C3A
ISO:
Ascorbate:
SNO-β2AR
Total β2AR
SNO-β2AR/β2AR (a.u.)
Unstimulated
ISO
WT WT C3A C3A
G
Alprenolol binding (fmol/mg)
HEK W9 C265S
H
W9 C265S HEK293
Total β2AR
GAPDH
I
Human heart (β1AR)
Non-failing Isch Failure Non-Isch Failure
Ascorbate:
SNO-β1AR
Total β1AR
SNO-β1AR/β1AR (A.U.)
J
ANGII:
Ascorbate:
SNO-ATR1
Total ATR1
SNO-ATR1/ATR1 (A.U.)
Control ANGII

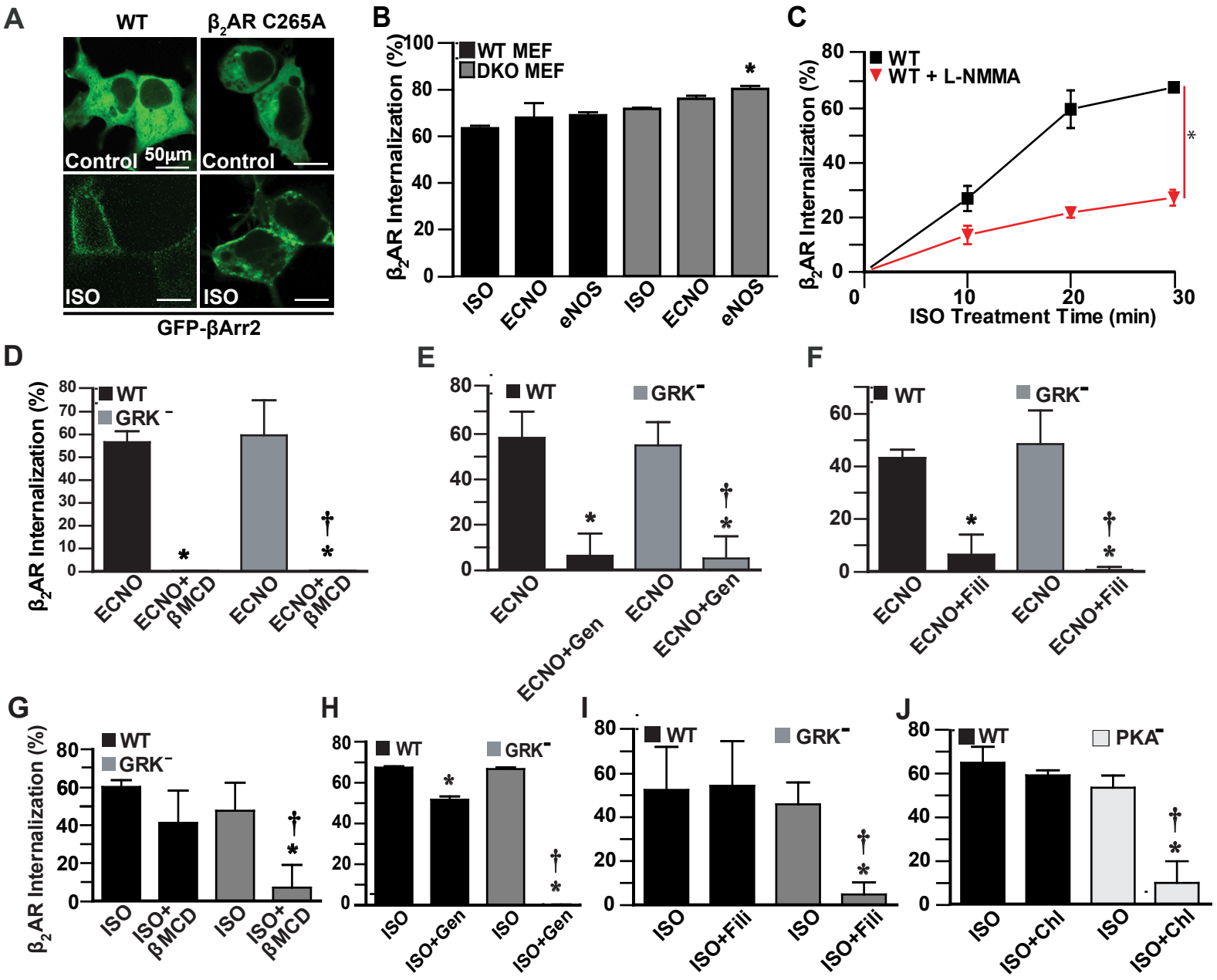
Supplemental Figure 1. ***Identification and validation of SNO-sites in*** **β_2*AR*, Related to Figure 1.** A. SNO-β_2AR detected by SNO-RAC of lysates from HEK293 cells stably expressing Flag-β_2AR, untreated or treated with ISO (10 μM) for the indicated time prior to harvesting cells. The NOS inhibitor L-NMMA was pre-incubated with cells for 2h in indicated lanes. Specificity controls include the absence of ascorbate after free thiol blocking, or photolysis of the nitrosothiol bond during thiol blocking by UV exposure. Total β_2AR control is from Flag immunoblot of lysate. Blot is representative of 3 replicates. B. SNO-β_2AR detected by SNO-RAC of lysates from HEK293 cells stably expressing Flag-β_2AR, untreated or treated with agonists or partial agonists isoproterenol (ISO), salbutamol (SALB), salmeterol (SALM), pindolol (PIN), procaterol (PROC), fenoterol (FEN), epinephrine (EPI) or isoetharine (iETH) for 10 min at 10 μM prior to harvesting cells. Total β_2AR control is from Flag immunoblot of lysate. Blot is representative of 3 replicates. C. Mass spectroscopic identification of β_2AR peptides that contain SNO, following CysNO treatment (100 μM, 10 min) of lysates from HEK293 cells stably expressing Flag-β_2AR. After SNO-RAC, bead-bound proteins were digested with trypsin, peptides were eluted and SNO-site cysteines were labeled with iodoacetamide prior to mass spectrometry identification. Observed and predicted masses are shown for each identified peptide, and SNO-modified Cys residues are highlighted. D. Mass spectrometry identification of β_2AR peptide containing SNO at Cys265. MS/MS spectra identifying the β_2AR peptide containing Cys265 is shown. E. SNO-β_2AR detected by SNO-RAC of lysates from HEK293 cells stably expressing Flag-β_2AR WT (W9) or Flag-β_2AR 5-Cys mutant (C5A: Cys265/328/341/378/406Ala – all of the intracellular cysteine residues, in two cell clones C5A-1 and C5A-2), untreated or treated with ECNO (100 μM, 10 min) prior to harvesting cells. Total β_2AR control is from Flag immunoblot of lysate. Blot shown is representative of 6 replicates. F. SNO-β_2AR detected by SNO-RAC of lysates from HEK293 cells stably expressing Flag-β_2AR WT (W9) or Flag-β_2AR 3-Cys mutant (C3A: Cys265/341/378Ala, the three sites identified by mass spectrometry), untreated or treated with ISO (10 μM, 10 min) prior to harvesting cells. Total β_2AR control is from Flag immunoblot of lysate. Blot shown is representative of 7 replicates. $p<0.0001$ by one-way ANOVA for treatment; * $p<0.0001$ basal vs ISO by Tukey test. G. β_2AR in HEK cell membranes quantified by [^{3}H]-alprenolol binding, from untransfected HEK 293 cells (HEK), wildtype β_2AR -expressing cells (W9), and C265S β_2AR -expressing cells (C265S). Specific binding

was determined using 1 nM [^{3}H]-alprenolol in the absence or presence of 1 μM propranolol. No significant difference between W9 and C265S was detected in assays in triplicate using 3 distinct membrane preparations. H. Overexpressed β_2AR in HEK293 cell membranes detected by western blot using anti-β_2AR antisera (Santa Cruz H-20, cat # SC-569), from wildtype β_2AR-expressing cells (W9) and C265S β_2AR-expressing cells (C265S). Untransfected HEK 293 cells (HEK) were used as a negative control, and GAPDH was detected as a loading control. I. SNO-β_1-adrenergic receptor (β_1AR) detected by SNO-RAC of lysates from human heart samples, from normal non-failing heart, ischemic failing heart, and non-ischemic failing heart. Total β_1AR control is from anti-β_1AR immunoblot of lysate. Representative blot is shown, of 3 non-failing, 3 ischemic and 3 non-ischemic failing heart samples tested, which are all quantified in the bar graph. * p<0.05 vs non-failing heart by one-way ANOVA with Tukey post-hoc test. J. SNO-Angiotensin II receptor 1 (ATR1) detected by SNO-RAC of lysates from HEK293 cells stably expressing HA-tagged ATR1, without or with angiotensin II peptide (AngII, 10 μm, 10 min). Total ATR1 control is from anti-HA immunoblot of lysate. Blot shown is representative of 3 replicates, which are all quantified in the bar graph. * p<0.05 by t-test.


A
BRET Ratio
WT
C265S
Log [ISO(M)]
B
Normalized to WT
WT
C265A
Log [ISO(M)]
C
cAMP pmol/mg
WT
C265S
Time (min)
D
Phospho PKA target per total β2AR
WT
C265S
ISO (min): 0 5 10 20 30 60 180 0 5 10 20 30 60 180
pPKA substrate
β2AR
E
WT
WT + PTX
ISO (min): 0 5 10 20 30
pERK
Total ERK
C265A
C265A + PTX
C265S
C265S + PTX
F
% Erk Inhibition by PTX
WT
C265A
C265S
Time (min)
G
NO Response (mV)
GSNO
buffer
WT ISO 5”
C265S ISO 5”
buffer
WT +PTX ISO 5”
C265S +PTX ISO 5”
Time (min)
H
HEK293 stable
PDE activity (pmol cAMP/mg/min)
WT
C265S

Supplemental Figure 2. β_2*AR S-nitrosylation at Cys265 does not affect short-term cAMP signaling*, Related to Figure 2. A. Dose response of ISO-stimulated (10 min) cAMP accumulation in HEK293 cells stably expressing Flag-β_2AR WT or Flag-β_2AR-C265S mutation, measured using the cAMP BRET biosensor CAMYEL. Data are shown as mean±SD for 2 assays (*p*=ns). B. Dose response of ISO-stimulated (5 min) cAMP accumulation in HEK293 cells stably expressing Flag-β_2AR WT or Flag-β_2AR -C265A mutation, measured using the GloSensor cAMP assay. Data are shown as mean±SD for 3 assays in triplicate (*p*=ns). C. Time course of ISO (10 μM)-stimulated cAMP accumulation in HEK293 cells stably expressing Flag-β_2AR WT or Flag-β_2AR-C265S mutation for the indicated times, measured using ELISA assay from cell lysates. Data are shown as mean±SD for 6 assays. $p<0.005$ by repeated measures ANOVA for genotype, time and time x genotype; * $p<0.05$ for individual time-points by Sidak test. D. Time course of ISO (10 μM)-stimulated PKA activity in HEK293 cells stably expressing Flag-β_2AR WT or Flag-β_2AR-C265S mutation for the indicated times, measured using immunoblotting for pan-PKA phosphorylated target site from cell lysates. Total β_2AR control is from Flag immunoblot of lysate. Image is representative of 4 assays. E. Time course of ISO (10 μM)-stimulated Erk activation in HEK293 cells stably expressing Flag-β_2AR WT (W9) or Flag-β_2AR-C265S or -C265A mutations for the indicated times, measured using immunoblotting for phospho-Thr202/Tyr204-Erk1/2 from cell lysates. Cells were preincubated with 100 ng/ml PTX overnight, or untreated. Total β_2AR control is from Flag immunoblot of lysate. Images are representative of 4 assays. F. Quantification of pErk data (from panel E, and alternately plotted in Fig 1H), shown as % activity that is inhibited by PTX pretreatment. Data are shown as mean±SD for 4 assays. $p<0.0001$ in a one-way ANOVA among genotypes ($F(2,9) = 115.3$); * $p<0.0001$ for cumulative response (area under curve) for WT vs mutants using Tukey test. G. Raw traces for SNO levels measured by Hg-coupled photolysis/chemiluminescence, in lysates of HEK293 cells expressing WT or C265S β_2AR, pretreated with PTX (100 ng/ml for 16h) or not, and stimulated with ISO (10 μM for 5 min), vs GSNO control. Area under curves was quantified by comparison to buffer control and to a standard curve of responses to defined concentrations of GSNO, and are plotted in Fig 2C. H. Basal cAMP phosphodiesterase activity is elevated in HEK293 cells stably expressing β_2AR C265S

mutant. HEK293 cells stably expressing Flag-β_2AR WT (W9) or Flag-β_2AR-C265S (C265S) were lysed and assayed for cAMP phosphodiesterase activity. N=3; *, *p*=0.0004 by t-test.


A
WT
β2AR C265A
Control
50μm
Control
ISO
ISO
GFP-βArr2
B
WT MEF
DKO MEF
β2AR Internalization (%)
ISO
ECNO
eNOS
ISO
ECNO
eNOS
C
WT
WT + L-NMMA
β2AR Internalization (%)
ISO Treatment Time (min)
D
WT
GRK⁻
β2AR Internalization (%)
ECNO
ECNO+ βMCD
ECNO
ECNO+ βMCD
E
WT
GRK⁻
ECNO
ECNO+Gen
ECNO
ECNO+Gen
F
WT
GRK⁻
ECNO
ECNO+Fili
ECNO
ECNO+Fili
G
WT
GRK⁻
β2AR Internalization (%)
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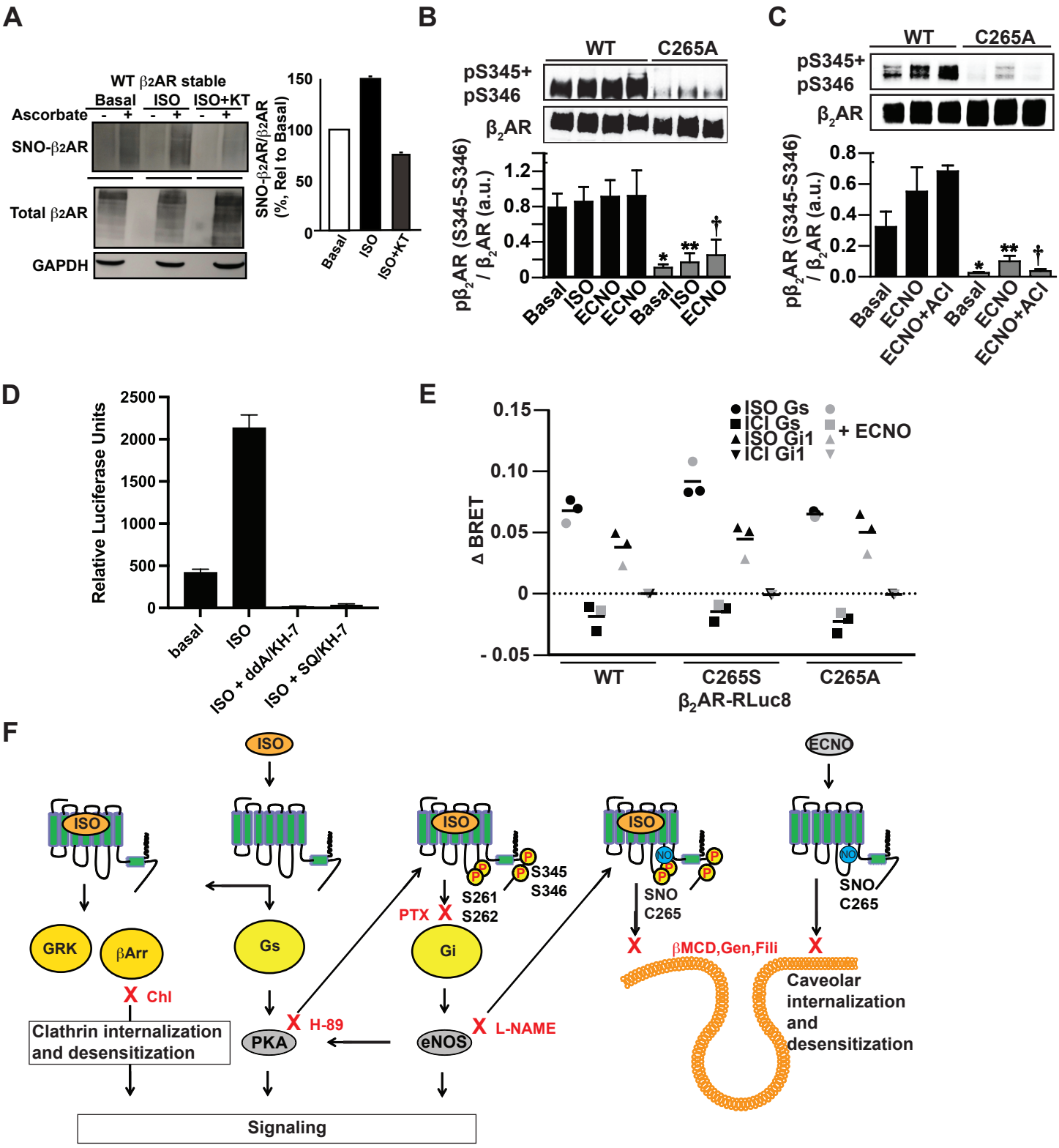
ISO+ βMCD
ISO
ISO+ βMCD
H
WT
GRK⁻
ISO
ISO+Gen
ISO
ISO+Gen
I
WT
GRK⁻
ISO
ISO+Fili
ISO
ISO+Fili
J
WT
PKA⁻
ISO
ISO+Chl
ISO
ISO+Chl

Supplemental Figure 3. β_2*AR S-nitrosylation drives agonist-independent receptor internalization through the caveolae pathway, but not the GRK/β-arrestin2/clathrin pathway,* Related to Figure 2. A. Translocation of β-arrestin2-GFP in Flag-β_2AR-expressing HEK293 cells stimulated with ISO (10 μM) or ECNO (100 μM) for 10 min, visualized by confocal microscopy. Data are representative of 15 cells surveyed in 3 experiments. B. Flag-β_2AR internalization 10 min after ISO (10 μM) or ECNO (100 μM), or with eNOS overexpression, in WT MEFs vs βarr1/βarr2 double knockout MEFs (DKO), measured by flow cytometry. Data are shown as mean±SD for 3 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(5, 12) = 14.60$); * $p<0.0097$ vs DKO ISO group by Sidak test. C. ISO (10 μM) induced time-dependent internalization of cell surface WT Flag-β_2AR in stable W9 HEK293 cells is reduced by pretreatment with the eNOS inhibitor L-NMMA (100 μM, 16 hours), quantified by flow cytometry. Data are shown as mean±SD for 3 assays. * $p<0.0001$ for cumulative response (area under curve) for WT vs drug-treated using two-tail t-test test. D. ECNO-driven (50 μM, 20 min) internalization of WT β_2AR or β_2AR deficient in GRK phosphorylation sites (GRK$^-$) is inhibited by the caveolae inhibitor β-methyl cyclodextrin (βMCD, 1.5 mM 1h), as measured by flow cytometry. Data are shown as mean±SD for 6 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,20) = 104.1$); * $p<0.0001$ vs WT ECNO and † $p<0.0001$ vs GRK$^-$ ECNO by Tukey test. E. ECNO-driven (50 μM, 20 min) internalization of WT β_2AR and GRK$^-$ β_2AR is inhibited by pre-incubation with the tyrosine kinase inhibitor genistein (Gen, 200 μM for 1h) to inhibit the caveolae pathway, as measured by flow cytometry. Data are shown as mean±SD for 6 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,20) = 48.23$); * $p<0.0001$ vs WT ECNO and † $p<0.0001$ vs GRK$^-$ ECNO by Tukey test. F. ECNO-driven (50 μM, 20 min) internalization of WT β_2AR and GRK$^-$ β_2AR is inhibited preincubation by the cholesterol-sequestering caveolae inhibitor filipin III (Fili, 800 ng/mL for 1h) used to inhibit the caveolae pathway, as measured by flow cytometry. Data are shown as mean±SD for 6 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,20) = 62.82$); * $p<0.0001$ vs WT ECNO and † $p<0.001$ vs GRK$^-$ ECNO by Tukey test. G. ISO-driven (10 μM, 20 min) internalization of WT β_2AR is not inhibited by the caveolae inhibitor β-methyl cyclodextrin (βMCD, 1.5 mM 1h), while internalization of GRK$^-$ mutant β_2AR is markedly inhibited, as measured by flow cytometry. Data are shown as mean±SD for 6 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,20) = 52.05$); * $p<0.0001$ vs WT

ECNO and † $p<0.0001$ vs GRK^- ECNO by Tukey test. H. ISO-driven (10 μM, 20 min) internalization of WT β_2AR is partially inhibited by the caveolae inhibitor genistein (Gen, 200 μM for 1h), while internalization of GRK^- β_2AR is markedly inhibited, as measured by flow cytometry. Data are shown as mean±SD for 3 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,8) = 3214$); * $p<0.0001$ vs WT ISO and † $p<0.0001$ vs GRK^- ISO by Tukey test. I. ISO-driven (10 μM, 20 min) internalization of WT β_2AR is not inhibited by the caveolae inhibitor filipin III (Fili, 800 ng/mL for 1h), while internalization of GRK^- β_2AR is markedly inhibited, as measured by flow cytometry. Data are shown as mean±SD for 6 assays. $p<0.0001$ by one-way ANOVA for treatment ($F(3,20) = 14.09$); * $p<0.0001$ vs WT ISO and † $p<0.001$ vs GRK^- ISO by Tukey test. J. ISO-driven (10 μM, 20 min) internalization of WT β_2AR is not inhibited by the clathrin inhibitor chlorpromazine (Chl, 10 μM 1h), while internalization of β_2AR deficient in PKA phosphorylation sites (PKA^-) is markedly inhibited, as measured by flow cytometry. Data are shown as mean±SD for 3 assays in triplicate. $p<0.0001$ by one-way ANOVA for treatment ($F(3,32) = 75.29$); * $p<0.0001$ vs WT ISO and † $p<0.0001$ vs PKA^- ISO by Tukey test.


A
WT β2AR stable
Basal
ISO
ISO+KT
Ascorbate
SNO-β2AR
Total β2AR
GAPDH
SNO-β2AR/β2AR (%, Rel to Basal)
Basal
ISO
ISO+KT
B
WT
C265A
pS345+ pS346
β2AR
pβ2AR (S345-S346) / β2AR (a.u.)
Basal
ISO
ECNO
ECNO
Basal
ISO
ECNO
C
WT
C265A
pS345+ pS346
β2AR
pβ2AR (S345-S346) / β2AR (a.u.)
Basal
ECNO
ECNO+ACI
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D
Relative Luciferase Units
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ISO Gi1
ICI Gi1
+ ECNO
Δ BRET
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C265A
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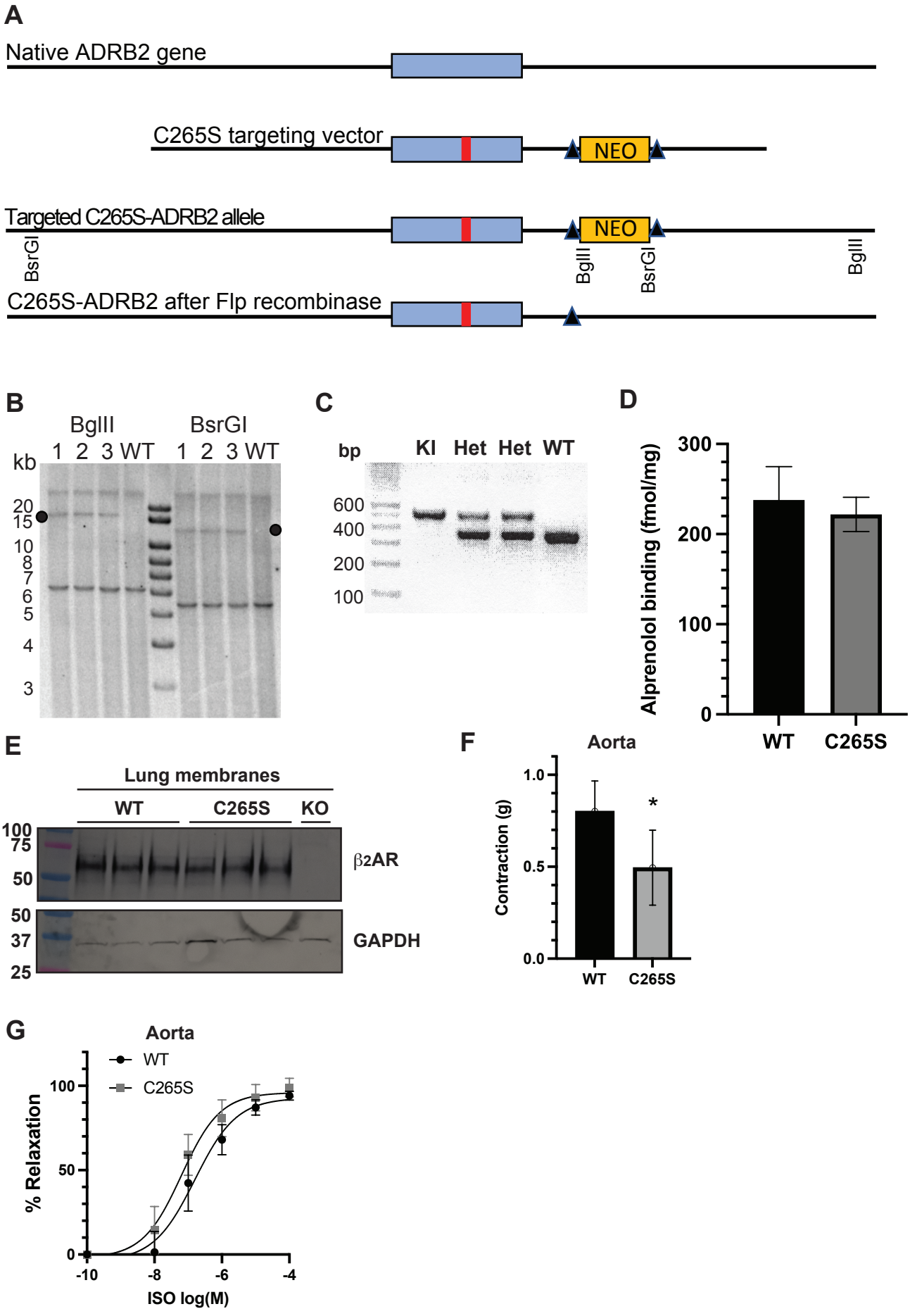
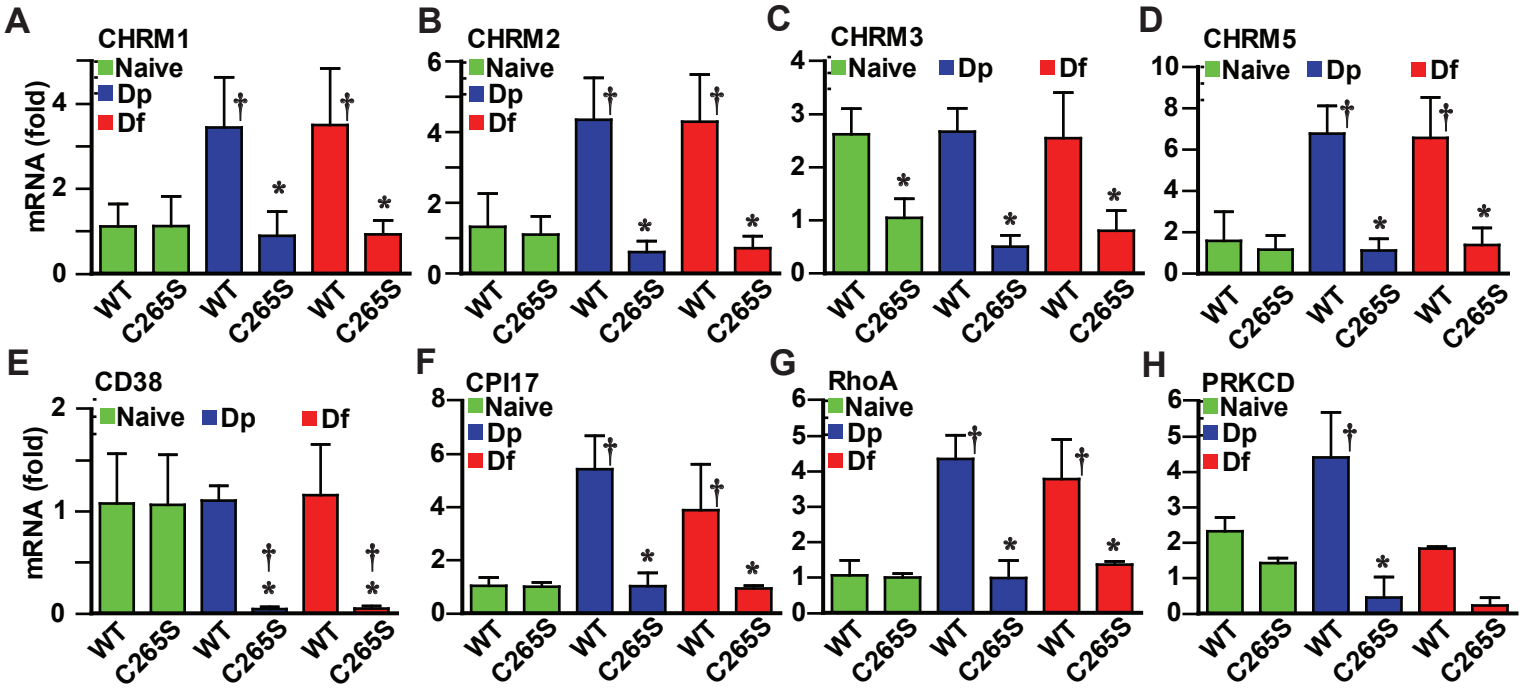
βArr
Gs
Gi
PKA
eNOS
PTX
S261
S262
S345
S346
SNO C265
βMCD,Gen,Fili
Caveolar internalization and desensitization
Chl
Clathrin internalization and desensitization
H-89
L-NAME
Signaling

Supplemental Figure 4. ***Co-regulation of β_2AR by S-nitrosylation and PKA phosphorylation, and receptor–G protein coupling*, Related to Figure 3.** A. SNO-β_2AR detected by SNO-RAC of membrane lysates from HEK293 cells stably expressing Flag-β_2AR WT (W9), preincubated in the absence or presence of the PKA inhibitor KT-5720 (10 μM, 30 min), and treated with ECNO (100 μM, 10 min). Total β_2AR control is from Flag immunoblot of membrane lysate. Blot shown on left is representative of 2 replicates, which are quantified in the graph on the right. B. PKA phosphorylation of Flag-β_2AR WT and Flag-β_2AR C265A mutant in stable HEK293 cells, stimulated with ISO (10 μM, 10 min) or ECNO (100 μM, 10 min), detected by immunoblotting of cell lysates using anti-phospho-Ser345/Ser346-β_2AR antiserum. Blots are representative of 3 replicates that were quantified by densitometry and plotted as mean±SD. p<0.0001 by one-way ANOVA for treatment F (6, 14) = 13.86; * p<0.0043 comparing basal, ** p<0.0040 comparing ISO, † p<0.0054 comparing ECNO by Tukey test. C. Protein kinase A-mediated phosphorylation of the Flag-β_2AR WT and Flag-β_2AR C265A mutant in stable HEK293 cells, pretreated with adenylyl cyclase inhibitor cocktail for 1h, stimulated with ECNO (100 μM, 10 min), detected by immunoblotting of cell lysates using anti phospho-Ser345/Ser346-β_2AR antiserum. Blot shown is representative of 3 replicates that were quantified by densitometry and plotted as mean±SD. p<0.0001 by one-way ANOVA for treatment (F (5,12) = 37.79); * p=0.0063 vs basal, ** p=0.0002 vs WT ECNO and † p<0.001 vs ECNO+ACI by Tukey test. D. Isoproterenol-stimulated cAMP accumulation in Flag-β_2AR WT HEK293 cells is ablated in the presence of adenylyl cyclase inhibitors. W9 cells were transfected with pGlo-20F GloSensor plasmid, and preincubated with DMSO vehicle or with adenylyl cyclase inhibitors 2',5' dideoxyadenosine 3'-triphosphate 10 μM + KH-7 50 μM (ddA/KH7) or SQ-22536 10 μM + KH-7 50 μM (SQ/KH7) for 1h. cAMP accumulation was assessed after stimulation with 10 μM isoproterenol (ISO) for 10 min on a Promega GloMax instrument. Data from 3 assays are plotted mean±SD. †, p<0.0001 vs. ISO alone by one-way ANOVA and Tukey test. E. Coupling of RLuc8-fused β_2AR WT or C265S/C265A mutants to overexpressed G_s or G_{i1} in HEK293 cells, measured by BRET to split Venus-tagged (Venus 156-239)-Gβ1 and (Venus 1-155)-Gγ2. Data for 3 experiments is shown, with mean value indicated as a line. F. Model for β_2AR activation of eNOS leading to SNO- and PKA-mediated internalization of the β_2AR. In the traditional model of ISO-stimulated β_2AR action, the active receptor couples to G_s to activate PKA,

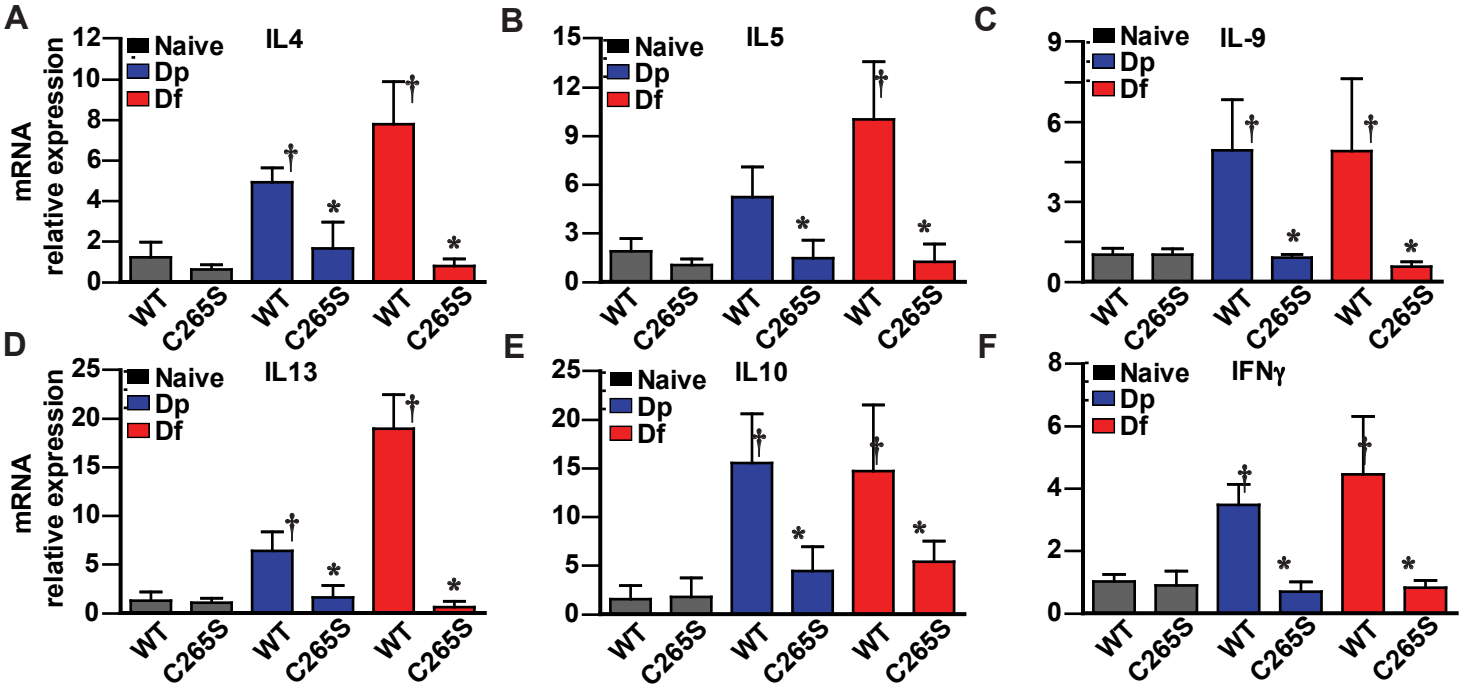
and is phosphorylated by GRKs leading to β-arrestin-mediated signaling and receptor internalization through clathrin coated pits (left side). PKA can phosphorylate the β_2AR at Ser261/262 and Ser345/246 (center), promoting activation of G_i and reducing coupling to G_s. Among the G_i-activated effectors is eNOS, which produces NO. In our study, we find that β_2AR activation (or the NO donor ECNO alone, far right) promotes S-nitrosylation of β_2AR at Cys265 and trafficking of the receptor in caveolae, even in the absence of agonist. This trafficking is inhibited by the caveolae inhibitors β-methyl-D-cyclodextrin (βMCD), genistein (Gen) and Filipin III (Fili), the eNOS inhibitor L-NAME (or L-NMMA), the G_i inhibitor pertussis toxin (PTX) and the PKA inhibitor H-89, as well as by mutation of β_2AR C265S, Ser261,262,345,346Ala (PKA^-), but not by the clathrin inhibitor chlorpromazine (Chl) or the GRK^- β_2AR mutant.


A
Native ADRB2 gene
C265S targeting vector
NEO
Targeted C265S-ADRB2 allele
NEO
BsrGI
BglII
BsrGI
BglII
C265S-ADRB2 after Flp recombinase
B
BglII
BsrGI
kb
1 2 3 WT 1 2 3 WT
20
15
10
8
7
6
5
4
3
C
bp
KI Het Het WT
600
400
200
100
D
Alprenolol binding (fmol/mg)
300
200
100
0
WT
C265S
E
Lung membranes
WT
C265S
KO
100
75
50
β2AR
50
37
25
GAPDH
F
Aorta
Contraction (g)
1.0
0.5
0.0
*
WT
C265S
G
Aorta
WT
C265S
% Relaxation
100
50
0
-10
-8
-6
-4
ISO log(M)

Supplemental Figure 5. ***Generation of β_2AR C265S knock-in mouse model*, Related to STAR Methods ("Animals") and Figure 4.** A. Strategy for generating β_2AR C265S knock-in mice. Mice were generated by homologous recombination in C57BL/6 ES cells using a targeting vector bearing the ADRB2 C265S gene (mutated TGC to TCC, red line) with a loxP/Frt-flanked NEO cassette followed by injection of targeted ES cells into mouse embryos, and the NEO cassette was removed in vivo by breeding heterozygote mice with Flp deleter mice to recombine at Frt sites (black triangles) and selecting for loss of NEO selection marker. B. Validation of integration and point mutation. Proper targeted integration was confirmed by Southern blot. Genomic DNA from targeted ES cell clones (1-3) and a WT clone was digested with BglII or BsrGI and probed with NEO to identify 16kb and 12kb bands, respectively (marked with black circles), indicating proper targeting in ES cell clones for embryo injection. In addition, DNA product bands from PCR amplification across the mutation site in genomic DNA from selected ES clones was sequenced to verify the C265S mutation. C. Genotyping of β_2AR C265S knock-in mice. PCR amplification across the residual loxP/FRT site yields a larger product band in the C265S knock-in (552 bp) compared to WT (372 bp). A representative genotyping reaction is shown identifying homozygous knock-in (KI), heterozygote (Het) and wildtype (WT) pups. D. β_2AR in mouse lung membranes quantified by [^{3}H]-alprenolol binding, from wildtype mice (WT), and β_2AR C265S knock-in mice (C265S). Specific binding was determined using 1 nM [^{3}H]-alprenolol in the absence or presence of 1 μM propranolol. No significant difference between WT and C265S was detected in assays in triplicate assays using lung membrane preparations from 3 distinct mice. E. Native β_2AR in lung membranes detected by western blot using anti-β_2AR antisera (Santa Cruz H-20, cat # SC-569), from 3 wildtype mice (WT) and 3 β_2AR C265S knock-in mice (C265S). A single β_2AR knockout mouse (KO) was used as a negative control, and GAPDH was detected as a loading control. F. Phenylephrine-stimulated contraction of wildtype versus C265S aorta ex vivo. Tension generated by aortic rings differed between genotypes. N=8 each; *, $p<0.005$ by t-test. G. ISO dose-response for relaxation of aorta ex vivo. Wildtype and C265S mouse aortas pretensioned with phenylephrine in (F) were tested for relaxation to increasing doses of ISO, as indicated. No significant difference, $p=0.134$ by 2-way ANOVA. n=7 WT and n=8 C265S aortas tested.


A
CHRM1
B
CHRM2
C
CHRM3
D
CHRM5
E
CD38
F
CPI17
G
RhoA
H
PRKCD
Naive
Dp
Df
mRNA (fold)
WT
C265S
*
†

Supplemental Figure 6. *β_2AR C265S mouse lung lacks muscarinic compensation in allergic asthma model*, Related to Figure 5. A.-H. Quantitative realtime PCR measurement of expression of muscarinic signaling and contractile machinery genes in RNA isolated from whole lung from WT and β_2AR C265S mice treated with the indicated house dust mite allergens (naïve, D.p., D.f.). (A) Chrm1, (B) Chrm2, (C) Chrm3, (D) Chrm5, (E) Cd38, (F) Cpi17, (G) RhoA, (H) Prkcd. Data is shown as mean±SD from samples from 3-5 mice. $p<0.05$ for all panels B-I by one-way ANOVA; * $p<0.05$ vs corresponding WT treatment control, or † $p<0.05$ from corresponding naïve control, by Tukey test.


A
IL4
mRNA relative expression
Naive
Dp
Df
B
IL5
C
IL-9
D
IL13
E
IL10
F
IFNγ
WT
C265S

Supplemental Figure 7. β_2*AR C265S mice fail to induce cytokine expression in allergic asthma model*, Related to Figure 6. A.-F. Quantitative real time PCR measurement of expression of asthma-associated and Th2 cytokine genes in RNA isolated from whole lung from WT and β_2AR C265S mice treated with the indicated house dust mite allergens (naïve, D.p., D.f.). (A) Il4, (B) Il5, (C) Il9, (D) Il13, (E) Il10, (F) Ifng. Data is shown as mean±SD from samples from 3-7 mice. $p<0.05$ for all panels A-F by one-way ANOVA; * $p<0.05$ vs corresponding WT treatment control, or † $p<0.05$ from corresponding naïve control, by Tukey test.

Supplemental Table I. ***Oligonucleotide Primers used for mutagenesis and qPCR*****, Related to STAR Methods ("Plasmids and Mutagenesis" and "RNA isolation and Realtime reverse transcription-PCR") and Figures 5 and 6***.*

Oligonucleotides		
ADRB2 C265S	5'-TCT TCC AAG TTC TCC TTG AAG GAG CAC	5'-GTG CTC CTT CAA GGA GAA CTT GGA AGA
ADRB2 C265A	5'-TCT TCC AAG TTC GCC TTG AAG GAG CAC	5'-GTG CTC CTT CAA GGC GAA CTT GGA AGA
ADRB2 C327A	5'-CCC CTT ATC TAC GCC CGC AGC CCA GAT	5'-ATC TGG GCT GCG GGC GTA GAT AAG GGG
ADRB2 C341S	5'-CAG GAG CTT CTG TCC CTG CGC AGG TCT	5'-AGA CCT GCG CAG GGA CAG AAG CTC CTG
ABRB2 C341A	5'-CAG GAG CTT CTG GCC CTG CGC AAG TCT	5'-AGA CTT GCG CAG GGC CAG AAG CTC CTG
ADRB2 C378A	5'-AAT AAA CG CTG GCT GAA GAC CTC CCA	5'-TGG GAG GTC TTC AGC CAG CAG TTT ATT
ABRB2 C406A	5'-CAA GGG AGG AAT GCT AGT ACA AAT GAC	5'-GTC ATT TGT ACT AGC ATT CCT CCC TTG
Adrb2 qPCR	5'-TGCTATCACATCGCCCTTC	5'-ACCACTCGGGCCTTATTCTT
Chrm1 qPCR	5'-GGCTGGGCAGTGCTACAT	5'-ATGGCTGTGCCAAAAGTGAT
Chrm2 qPCR	5'-ACTAGTGGGATCGTCAGGTCA	5'-ATTTTGCGGGCTACAATGTT
Chrm3 qPCR	5'-TGATGAAGAGGATATTGGCTCA	5'-GGCAGCTTGAGTACAATGGAA
Chrm5 qPCR	5'-GGCCCAGAGAGTACGGAAC	5'-GTTGTTGAGGTGCTTCTACGG
Cd38 qPCR	5'-AAGATGTTCACCCTGGAGGA	5'-ACTCCAATGTGGGCAAGAGA
Cpi17 qPCR	5'-GAGAAGTGGATCGACGGATG	5'-TCCGGCATGTCTGACTCC
RhoA qPCR	5'-GAATGACGAGCACACGAGAC	5'-TCCTGTTTGCCATATCTCTGC
Prkcd qPCR	5'-CAAGAAGAACAACGGCAAGG	5'-TGCACACACATCAGCACCT
IL1b qPCR	5'-AGTTGACGGACCCCAAAAG	5'-AGCTGGATGCTCTCATCAGG
IL4 qPCR	5'-CATCGGCATTTTGAACGAG	5'-CGAGCTCACTCTCTGTGGTG
IL5 qPCR	5'-ACATTGACCGCCAAAAAGAG	5'-ATCCAGGAACTGCCTCGTC
IL9 qPCR	5'-GCCTCTGTTTTGCTCTTCAGTT	5'-GCATTTTGACGGTGGATCAT
IL10 qPCR	5'-ACTGCACCCACTTCCCAGT	5'-TGTCCAGCTGGTCCTTTGTT
IL12a qPCR	5'-TCAGAATCACAACCATCAGCA	5'-CGCCATTATGATTCAGAGACTG
IL12b qPCR	5'-TTGCTGGTGTCTCCACTCAT	5'-GGGAGTCCAGTCCACCTCTAC
IL13 qPCR	5'-CCTCTGACCCTTAAGGAGCTTAT	5'-CGTTGCACAGGGGAGTCT
Ifng qPCR	5'-ATCTGGAGGAACTGGCAAAA	5'-TTCAAGACTTCAAAGAGTCTGA GGTA
TNFa qPCR	5'-TCTTCTCATTCCTGCTTGTGG	5'-GGTCTGGGCCATAGAACTGA
Actb qPCR	5'-GGCTGTATTCCCCTCCATCG	5'-CCAGTTGGTAACAATGCCATGT

Plcb3 qPCR	5’-AAAAAGCCCACCACTGATGA	5’-ACATCTCCTCCGTGGCATT
Pkca qPCR	5’-ACCGCCGACTGTCTGTAGAA	5’-CCCATGAAGTCATTCCGAGT
RhoK qPCR	5’-AACGGAGGTGACGTGAGGTA	5’-CGGTAGACAATGCGTCTCTG