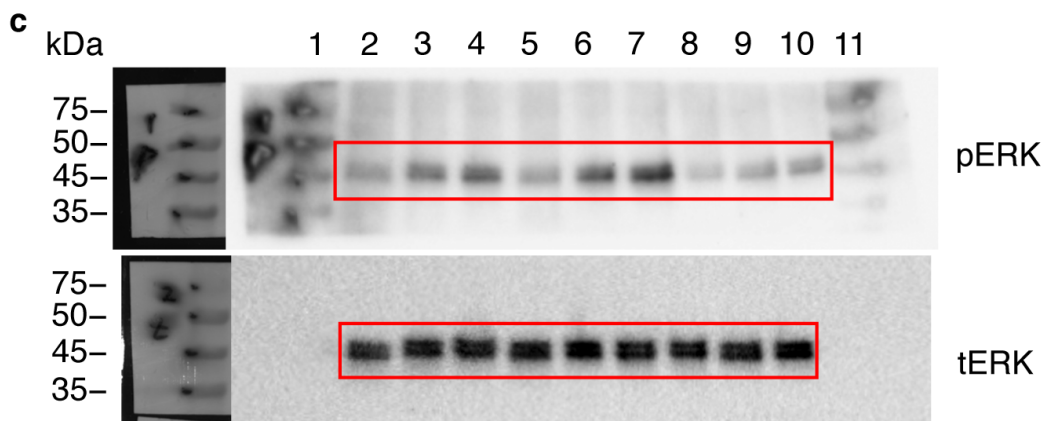
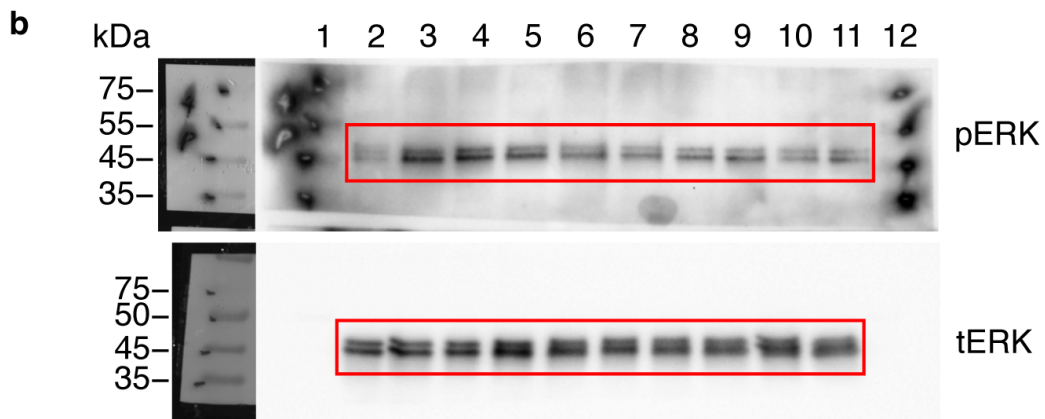
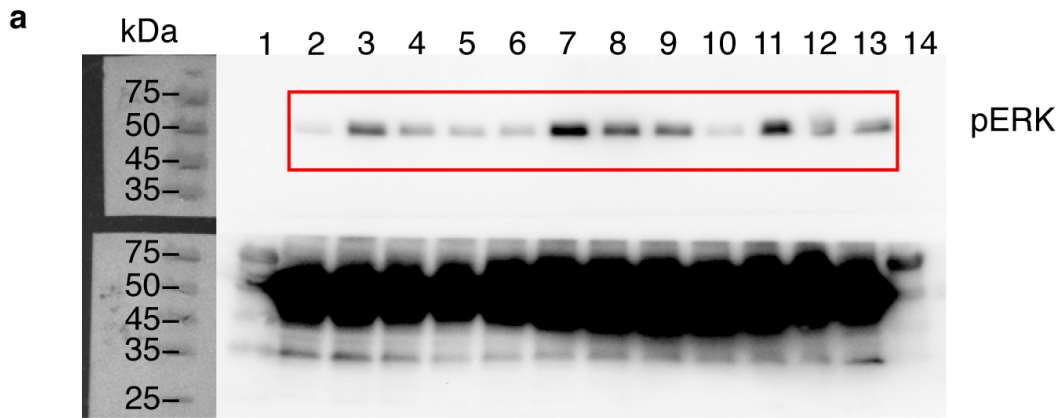


913

914 **Supplementary Figure 1. Epinephrine-induced phosphorylation of ERK at early time**
 915 **points, regarding Extended Data Fig. 2g**

916 Western blot analysis of phospho-ERK (pERK) and total ERK (tERK) levels in HEK293T
 917 cells expressing vector, SNAP-β₂AR or SNAP-β₂AR 341T following stimulation with Epi
 918 for 0, 2, or 5 min. Representative blot (upper) and quantification of normalized pERK/tERK
 919 levels at each time point (lower). *P < 0.05, ***P < 0.001; ordinary one-way ANOVA
 920 followed by Dunnett's multiple-comparisons test. Lines in scatter plots represent mean ±
 921 SEM. Full blots are shown in Supplementary Fig. 2.

922



924 **Supplementary Figure 2. Uncropped western blots**

925 Western blots from HEK293T cells (a) expressing (lane 2-5) vector, (lane 6-9) SNAP- β_2 AR
926 or (lane 10-13) SNAP- β_2 AR 341T stimulated with Epi for 0, 5, 15, 30 min. Protein ladder
927 loaded in lanes 1 and 14 (regarding Extended Data Fig. 2g). (b) Western blots from
928 HEK293T cells stimulated with Epi for 0, 2, 5, 15, 30 min either without (lane 2-6) or with
929 (lane 7-11) Dyngo4a pretreatment . Protein ladder loaded in lanes 1 and 12 (regarding
930 Extended Data Fig. 7g), (c) Western blots from HEK293T cells expressing (lane 2-4)
931 vector, (lane 5-7) SNAP- β_2 AR or (lane 8-10) SNAP- β_2 AR 341T stimulated with Epi for 0, 2,
932 or 5 min. Protein ladder loaded in lanes 1 and 11 (regarding Supplementary Fig. 1).
933

Figure	DNA amount (ng)	DNA Ratio	List of transfected DNA	siRNA	Reagent (μ l)
Fig.1a, b, c, 2a, 4b, Extended Data Fig. 2b, c, 3a, b, 6a	600	1:1:1	Vector, SNAP- β_2 AR and biosensors	-	Polyjet, 1.8
Fig.2b, Extended Data Fig.3c, and 6b	600	1:1:1	Vector or β -arr2 (β -arr2 cDNA is resistant to siRNA targets β -arr2), plus SNAP- β_2 AR and EKAR	500 pmol (siARRB1, siARRB2 mixture, or siScramble)	Polyjet, 1.8
Fig.2c, e, f, g, 3a, 4c, d, e, g Extended Data Fig.4g, h, 6c, d, 9b, c and S.Fig.1	600	1:1	SNAP- β_2 AR-T2A-GsL/S, SNAP- β_2 AR-T2A-Nb37-FYVEx2, SNAP- β_2 AR-T2A-Nb37-NLS, SNAP- β_2 AR-T2A-Nb80-FYVEx2, SNAP- β_2 AR-T2A-Nb80-NLS, SNAP- β_2 AR-T2A-GsCT-FYVEx2 or SNAP- β_2 AR-T2A-GsCT-NLS plus EKAR	-	Polyjet, 1.8
Fig 2d, Extended Data Fig.2d, e, and 3e, f	600	1:1:1	Vector, PTX, PKI or β -arr2, plus SNAP- β_2 AR and EKAR	-	Polyjet, 1.8
Extended Data Fig. 2g	50	1	Vector, SNAP- β_2 AR or SNAP- β_2 AR341T	-	Polyjet 1.5
Fig.3c, d and Extended Data Fig.1, 5	300	1	SNAP- β_2 AR, SNAP- β_2 AR-T2A-Nb37-FYVEx2 or EKAR	-	Polyjet, 0.9
Extended Data Fig.2f	350	1:6	Vector or GPCR plus ICUE4	-	Polyjet, 1.8
Fig 4f, Extended Data Fig.2a, e, 9a	50	1	Vector or GPCR	-	Polyjet, 1.8
Extended Data Fig.2h and 4a-d	600	1:1	Vector or endo-Nb37 plus SNAP- β_2 AR	-	Polyjet, 1.8
Extended Data Fig.3f	300	1	Vector or PTX	-	Polyjet, 0.9
Extended Data Fig. 3g-i	500	1:3:1	vector or β -arr2 plus SNAP- β_2 AR and $G\alpha_s$	500 pmol (siARRB1, siARRB2 mixture, or siScramble)	Polyjet, 1.5
Extended Data Fig.7a-f	300	1	EKAR	-	(a-c) Polyjet, 0.9 (d-f) Lipofectamine 2000, 0.9
Extended Data Fig. 8	600	1:1	GPCR plus biosensors	-	Polyjet, 1.8

934

935 **Supplementary Table 1. Transfection information**

936 Detailed information on transfections performed for all experiments shown in the indicated
937 figures, including the total amount, ratio and type of DNA, siRNA and transfection
938 reagents.

939

Figure	Replication for each panel
Fig. 1	(a-c) 3
Fig. 2	(a) 2 (b-e) 3, (f) 2
Fig. 3	(c) -Epi -endo-Nb37: 3, +Epi -endo-Nb37: 7, -Epi +endo-Nb37: 3, and +Epi +endo-Nb37: 7, respectively, (d) -Epi -endo-Nb37: 7, +Epi -endo-Nb37: 5, -Epi +endo-Nb37: 6, +Epi +endo-Nb37: 5
Fig. 4	(b) Mock: 2, Epi: 4, (c): 3, (d): 2, (e): 2, (h): 3
Extended Data Fig. 1	(a - d, f): 2, (e, g): 3
Extended Data Fig. 2	(a - c), (f - i): 3, (d): 2, (e): 4
Extended Data Fig. 3	(a) NT: 3, Dyngo4a: 2, (b): 2, (c-e): 3, (f) 2 (g-i): 2
Extended Data Fig. 4	(a - f): 3
Extended Data Fig. 5	3
Extended Data Fig. 6	(a) NT: 3, Nb37: 3, Barbadin : 3, Dyngo4a: 2, (b): 3, (c) NT: 3, Barbadin:3, endo-GsCT: 2, (d) NT: 3, Barbadin: 2, endoGsCT: 2
Extended Data Fig. 7	(a) NT: 3, Dyngo4a: 3, (b) NT: 3, Dyngo4a, ICI118,551: 2, (c) NT: 2, Dyngo4a: 2, (d) NT: 2, Dyngo4a: 2, (e) NT: 4, Dyngo4a, ICI118,551: 2, (f) NT: 3, Dyngo4a: 3, (g) 4
Extended Data Fig. 8	(a) PM: 3, endo: 2, nuc: 2, (b) NT: 2, Barbadin: 3, Dyngo4a: 2, (c) NT: 2, Barbadin: 2, Dyngo4a: 2, (d) PM: 2, endo: 3, nuc: 3, (e) NT: 3, Barbadin: 3, Dyngo4a: 3, (f) NT: 3, Barbadin: 2, Dyngo4a: 2, (g) PM: 2, endo: 3, nuc: 2, (h) NT: 3, Barbadin: 3, Dyngo4a: 2, (i) NT: 2, Barbadin: 2, Dyngo4a: 2
Extended Data Fig. 9	(a-c) 3
Supplementary Fig. 1	4

940

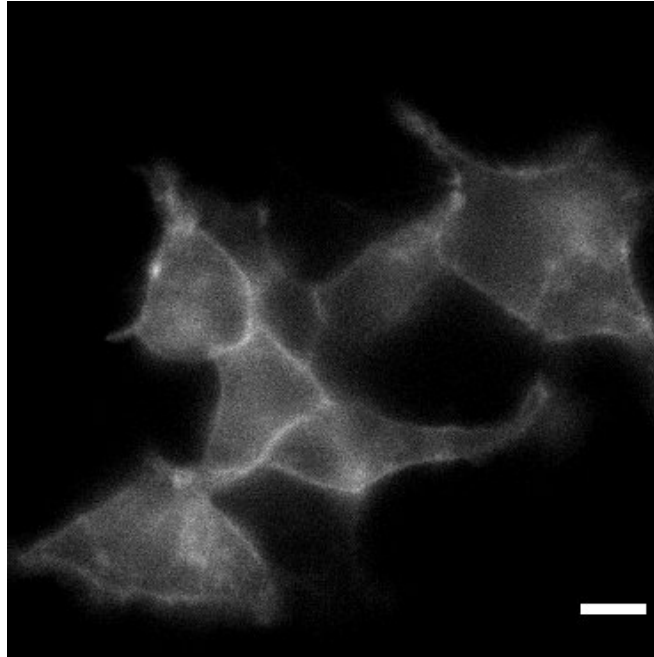
941 **Supplementary Table 2. Replication information for Extended Data Figures**

942 Details on the number of experimental replicates performed for all data shown in the

943 Extended Data Figures.

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947 **Supplementary Movie 1. Epinephrine-induced β_2 AR internalization**

948 Time lapse imaging of HEK293T cells expressing SNAP- β_2 AR and labeled with JF646

949 stimulated with 10 μ M epinephrine. Scale bar, 10 μ m.

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951