

Supplementary figures and tables

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Supplementary tables:

- Table S1:** Parameter values for each of the 4 best parameter sets.

- Table S2:** Simulated *versus* experimental activation/deactivation half-lives measured in independent studies.

SBML model file: separate file

```

MA(k0)           for   G => G_a.
MA(k1)           for   G =[R~{P1}-H]=> G_a.
MA(k2)           for   G =[R-H]=> G_a.

MA(k3)           for   PIP2 =[G_a]=> DAG.
MA(k4)           for   PKC =[DAG]=> PKC_a.
MA(k5)           for   ERK =[PKC_a]=> GpERK.

MA(k6)           for   G_a => G.
MA(k7)           for   DAG => PIP2.
MA(k8)           for   PKC_a => PKC.

MA(k9)           for   GpERK => ERK.

MA(k10*GRK23)    for   R-H => R~{P1}-H.

(MA(k11),MA(k13)) for   R~{P1}-H + barr1 <=> R~{P1}-H-barr1.
(MA(k12),MA(k14)) for   R~{P1}-H + barr2 <=> R~{P1}-H-barr2.
MA(k15)          for   R~{P1}-H-barr1 => R-H + barr1.
MA(k16)          for   R~{P1}-H-barr2 => R-H + barr2.

MA(k18*GRK56)    for   R-H => R~{P2}-H.
MA(k17)          for   R~{P2}-H => R-H.

(MA(k19),MA(k23)) for   barr2 + R-H <=> barr2-R-H.
(MA(k20),MA(k24)) for   barr2 + R~{P2}-H <=> barr2-H-R~{P2}.
MA(k21)          for   ERK =[barr2-R-H]=> bpERK.
MA(k22)          for   ERK =[barr2-H-R~{P2}]=> bpERK.

MA(k25)          for   bpERK => ERK.

```

Figure S1: The minimal model written with BIOCHAM reaction rules (<http://contraintes.inria.fr/BIOCHAM/>).

$$\begin{aligned}
\frac{d[G_a]}{dt} &= k0 \cdot [G] + k1 \cdot [G] \cdot [HRP1] + k2 \cdot [G] \cdot [HR] - k6 \cdot [G_a] \\
\frac{d[DAG]}{dt} &= k3 \cdot [PIP2] \cdot [G_a] - k7 \cdot [DAG] \\
\frac{d[PKC_a]}{dt} &= k4 \cdot [DAG] \cdot [PKC] - k8 \cdot [PKC_a] \\
\frac{d[GpERK]}{dt} &= k5 \cdot [ERK] \cdot [PKC_a] - k9 \cdot [GpERK] \\
\frac{d[HRP1]}{dt} &= k10 \cdot GRK23 \cdot [HR] - k11 \cdot [barr1] \cdot [HRP1] - k12 \cdot [barr2] \cdot [HRP1] \\
&\quad + k13 \cdot [HRP1barr1] + k14 \cdot [HRP1barr2] \\
\frac{d[HRP1barr1]}{dt} &= k11 \cdot [barr1] \cdot [HRP1] - (k13 + k15) \cdot [HRP1barr1] \\
\frac{d[HRP1barr2]}{dt} &= k12 \cdot [barr2] \cdot [HRP1] - (k14 + k16) \cdot [HRP1barr2] \\
\frac{d[HRP2]}{dt} &= -k17 \cdot [HRP2] + k18 \cdot GRK56 \cdot [HR] - k20 \cdot [barr2] \cdot [HRP2] + k24 \cdot [HRP2barr2] \\
\frac{d[HRbarr2]}{dt} &= k19 \cdot [barr2] \cdot [HR] - k23 \cdot [HRbarr2] \\
\frac{d[HRP2barr2]}{dt} &= k20 \cdot [barr2] \cdot [HRP2] - k24 \cdot [HRP2barr2] \\
\frac{d[bpERK]}{dt} &= k21 \cdot [ERK] \cdot [HRbarr2] + k22 \cdot [ERK] \cdot [HRP2barr2] - k25 \cdot [bpERK]
\end{aligned}$$

Figure S2: Ordinary differential equation (ODE) set of the model.

Conservation laws:

$$H_total = HR + HRP1 + HRP2 + HRbarr2 + HRP1barr1 + HRP1barr2 + HRP2barr2$$

$$G_total = G + G_a$$

$$DAG_total = PIP2 + DAG$$

$$PKC_total = PKC + PKC_a$$

$$barr1_total = barr1 + HRP1barr1$$

$$barr2_total = barr2 + HRbarr2 + HRP1barr2 + HRP2barr2$$

$$ERK_total = ERK + GpERK + bpERK$$

Observed variables:

$$pERKtotal_observed = \frac{[GpERK] + [bpERK]}{erknorm}$$

$$DAG_observed = \frac{[DAG] - D0_a}{DAGnorm} + 1$$

$$PKC_observed = \frac{[PKC_a] - P0_a}{PKCnorm} + 1$$

Figure S3: Conservation laws and observed variables of the model.

Simulated

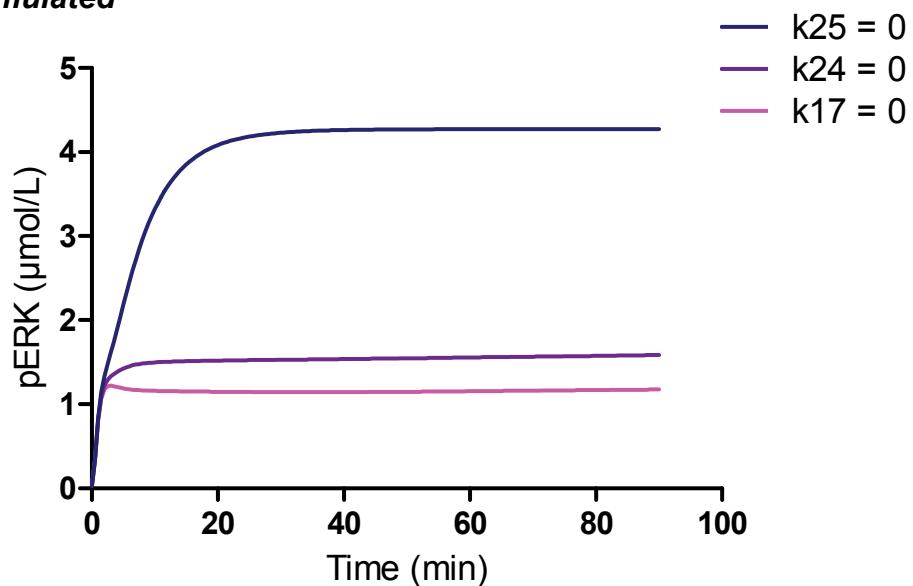


Figure S4: Simulation of the consequences of the absence of HRP2 dephosphorylation ($k_{17} = 0$, pink), HRP2barr2 dissociation ($k_{24} = 0$, purple) or bpERK dephosphorylation ($k_{25} = 0$, blue).

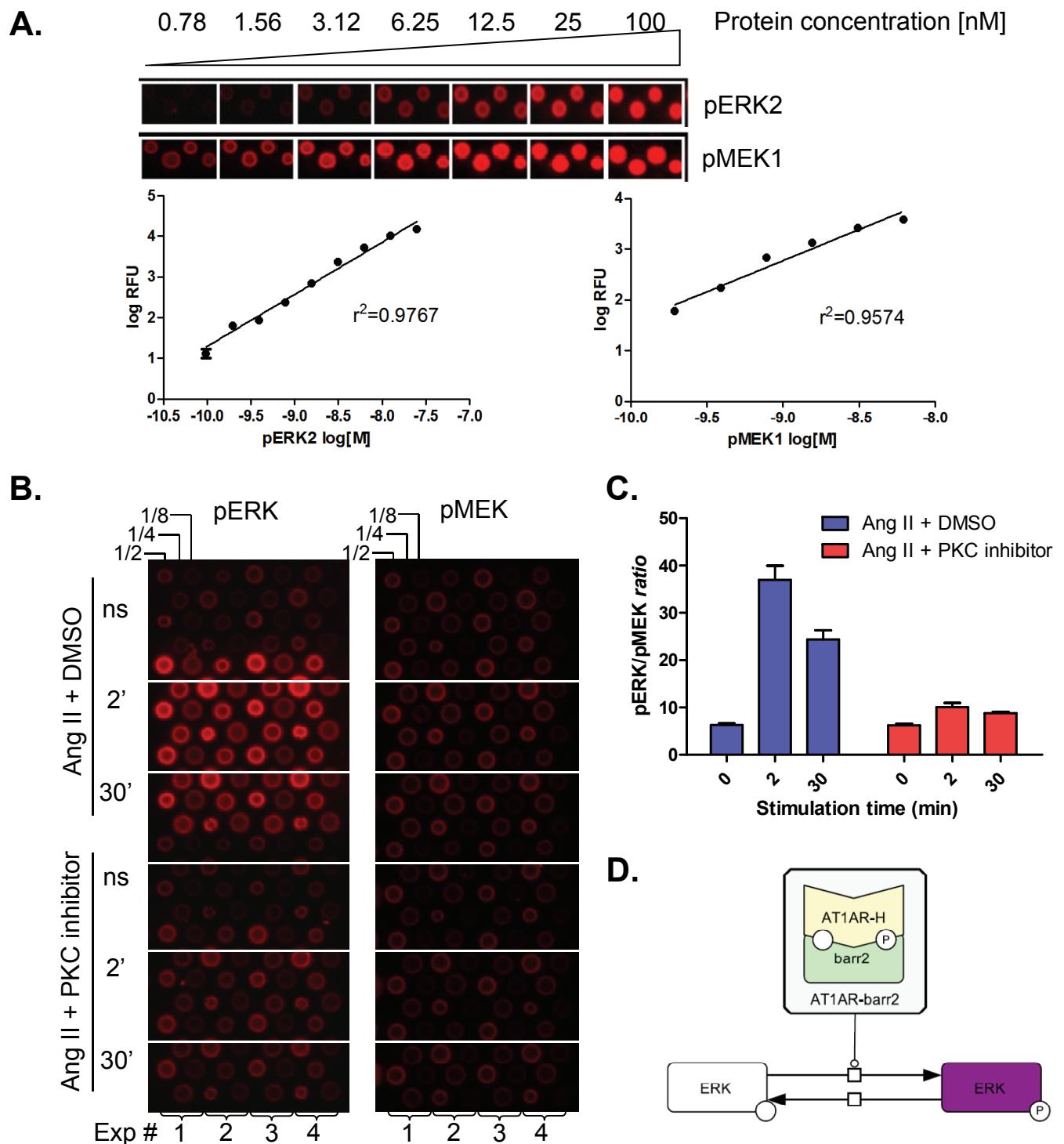


Figure S5: Quantitative assessment of ERK1/2 and MEK1/2 phosphorylation in HEK293 cells expressing the AT_{1A}R using a reverse phase protein array (RPPA) approach. **(A)** Wide ranges of recombinant pERK2 and pMEK1 dilutions were arrayed in four replicates and revealed in near infrared using anti-pERK and anti-pMEK primary antibodies. Linear range was determined for both standard curves. **(B)** Cells, pre-treated or not with PKC inhibitor (Ro-31-8425, 1 μ M), were stimulated for 2 or 30 minutes with 100 nM angiotensin. Whole cell lysates corresponding to four independent experiments were arrayed in 3 dilutions (i.e.: 1/2, 1/4, 1/8). **(C)** The molar quantities of pERK1/2 and of pMEK1/2 were calculated according to each standard curves and the pERK/pMEK ratios were determined for each condition. **(D)** ERK phosphorylation catalyzed by the AT_{1A}R/β-arrestin 2 complex in SBGN.

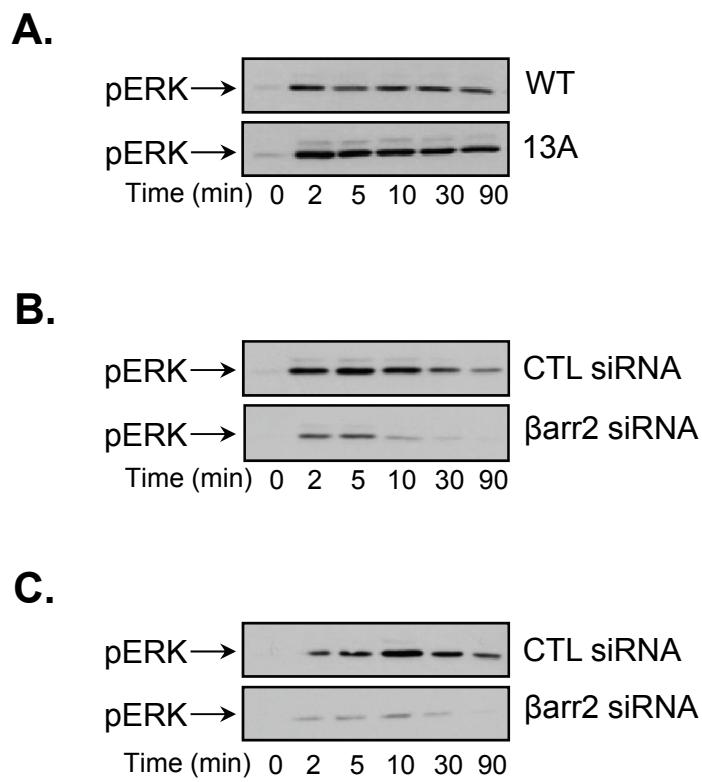


Figure S6: Representative Western blots of HEK293 cells transiently co-transfected with wild-type (WT) or 13A AT_{1A}R and either control (CTL) or β-arrestin 2 (barr2) siRNAs. Cells were stimulated with 100 nM AngII. **(A)** Kinetics of ERK phosphorylation in WT versus 13A-transfected cells. **(B)** Kinetics of ERK phosphorylation in WT-transfected cells depleted or not in β-arrestin 2. **(C)** Kinetics of ERK phosphorylation in 13A-transfected cells depleted or not in β-arrestin 2.

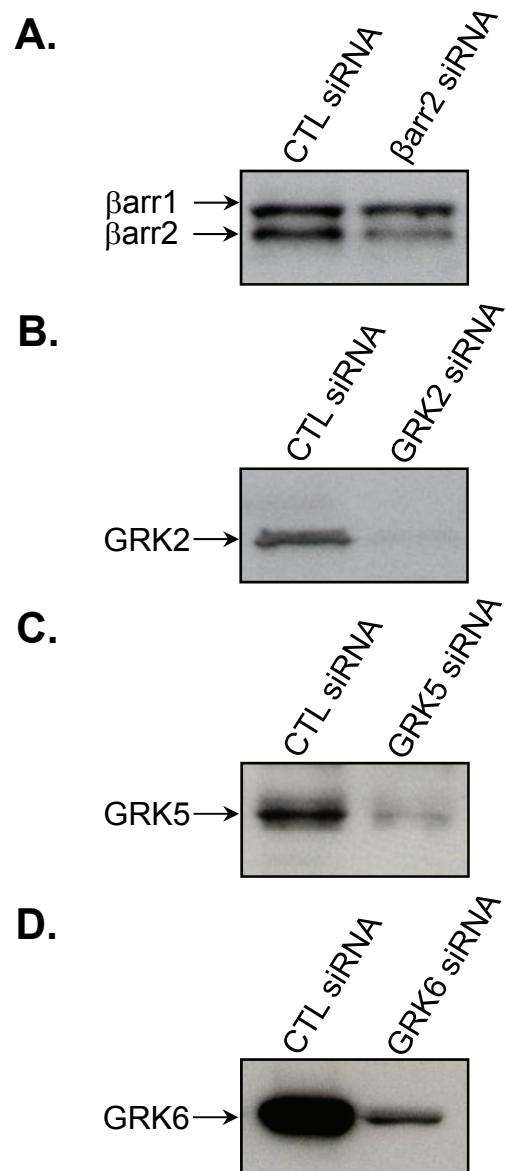


Figure S7: Representative Western blots of HEK293 cells transiently transfected with the different siRNA used in this study. Endogenous proteins are shown. **(A)** Control versus β-arrestin 2-depleted cells. **(B)** Control versus GRK2-depleted cells. **(C)** Control versus GRK5-depleted cells. **(D)** Control versus GRK6-depleted cells.

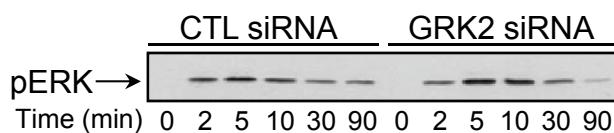
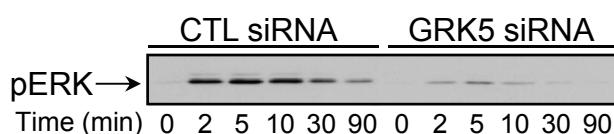
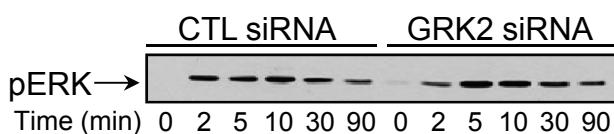
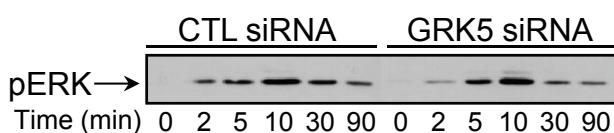
A.**B.****C.****D.**

Figure S8: Representative Western blots of HEK293 cells transiently co-transfected with wild-type (WT) or 13AAT_{1A}R and either control (CTL), GRK2 or GRK5 siRNAs. **(A)** Effect of GRK2 depletion on the kinetics of ERK phosphorylation upon AngII (100 nM) stimulation in WT-transfected cells. **(B)** Effect of GRK5 depletion on the kinetics of ERK phosphorylation upon AngII stimulation in WT-transfected cells. **(C)** Effect of GRK2 depletion on the kinetics of ERK phosphorylation upon AngII stimulation in 13A-transfected cells. **(D)** Effect of GRK5 depletion on the kinetics of ERK phosphorylation upon AngII stimulation in 13A-transfected cells.

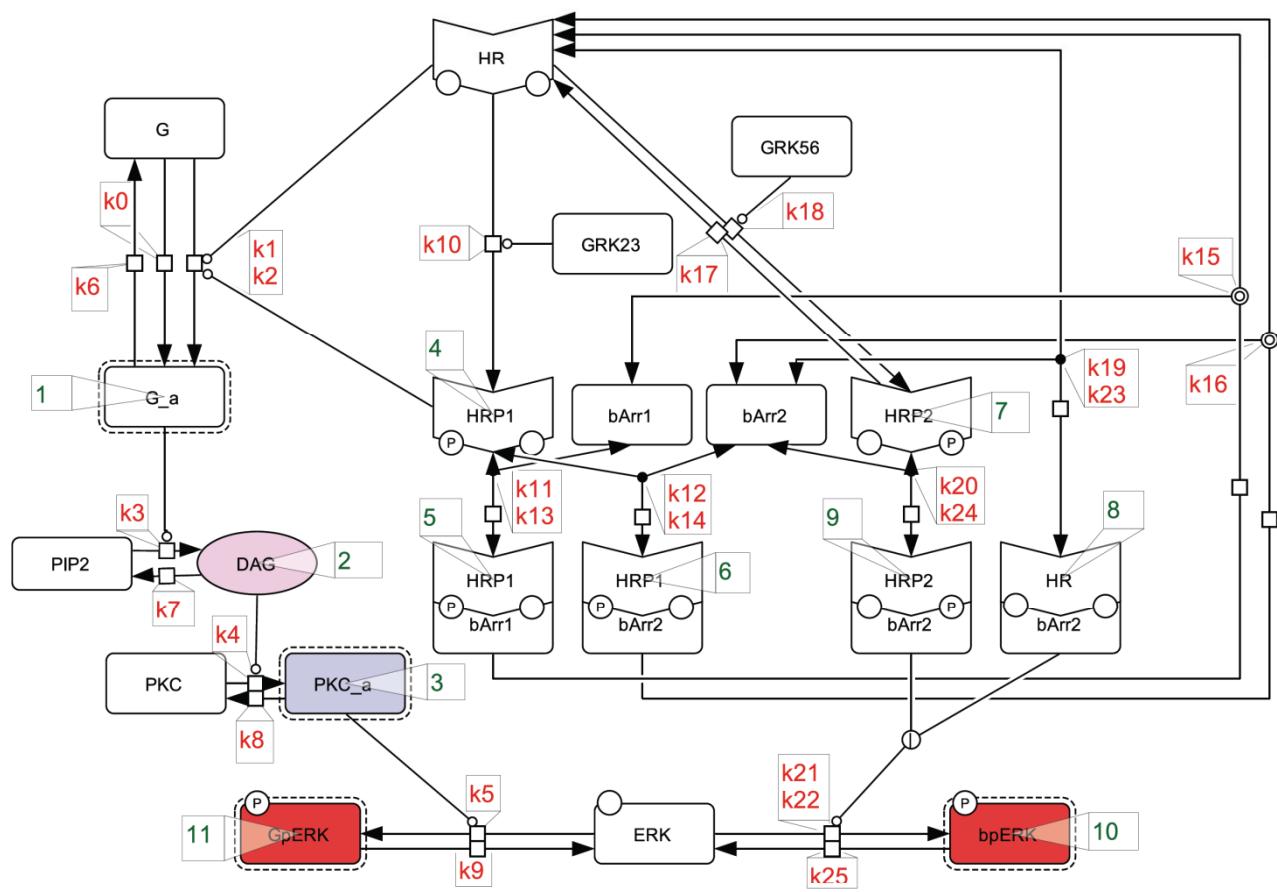


Figure S9: CellDesigner representation of the network structure (as presented in figure 1) with rate constants (in red) and parameter numbers (in green) indicated on the corresponding species and reactions.

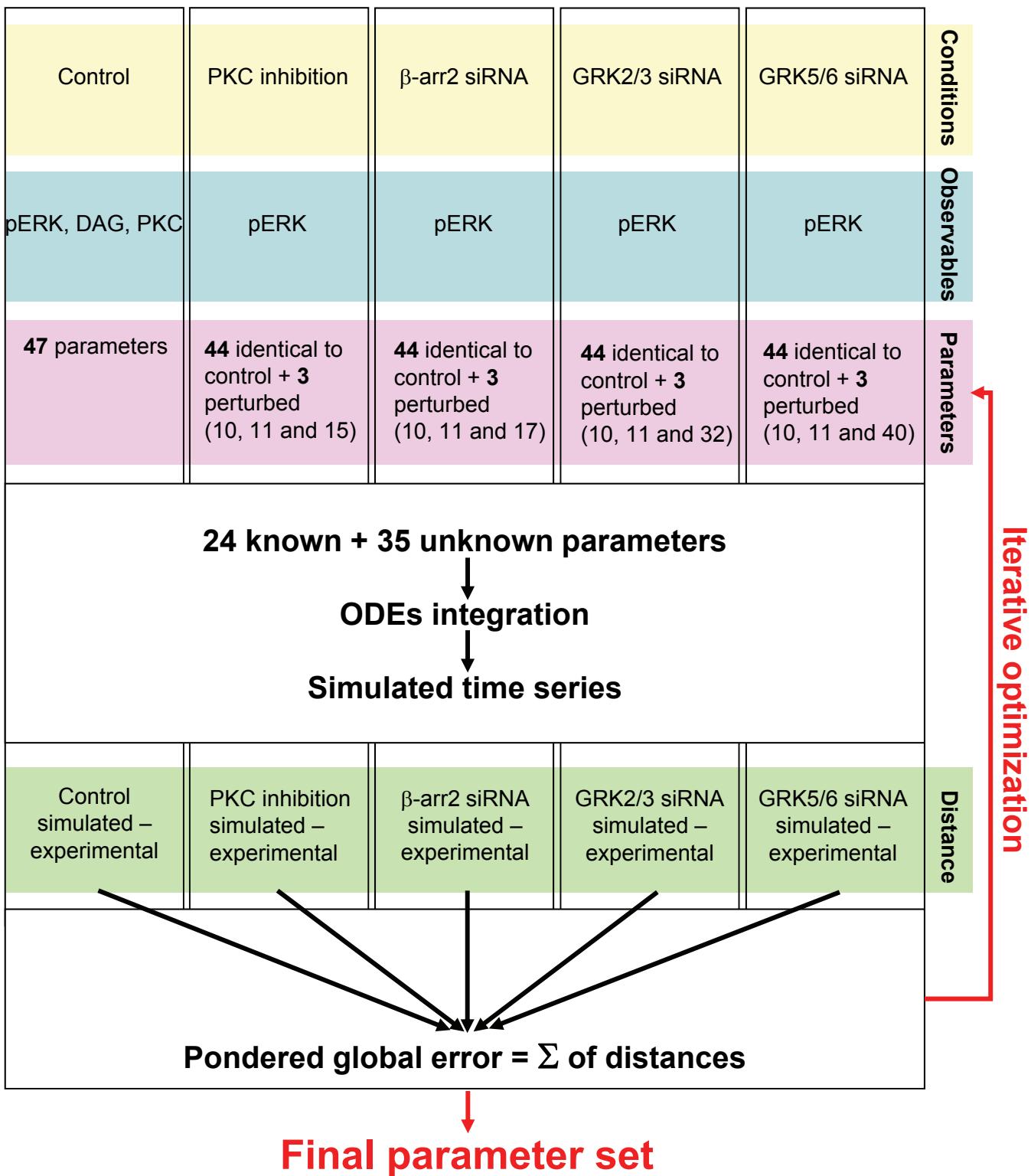


Figure S10: Principle of the global parameter optimization strategy. One control and four perturbed data sets (in yellow) were used. Each data set corresponds to different observables (in blue) and to a number of unknown parameters (in pink). Only the parameter targeted by the biological perturbation was modified within the control parameter set, all the others being identical to control. A total of 38 unknown parameters was optimized. The optimization algorithm minimizes the global error (*i.e.*: the distance of the simulated model with respect to the different experimental curves, either control or perturbed [in green]) through an iterative and auto-adaptative optimization algorithm.

Data fitting

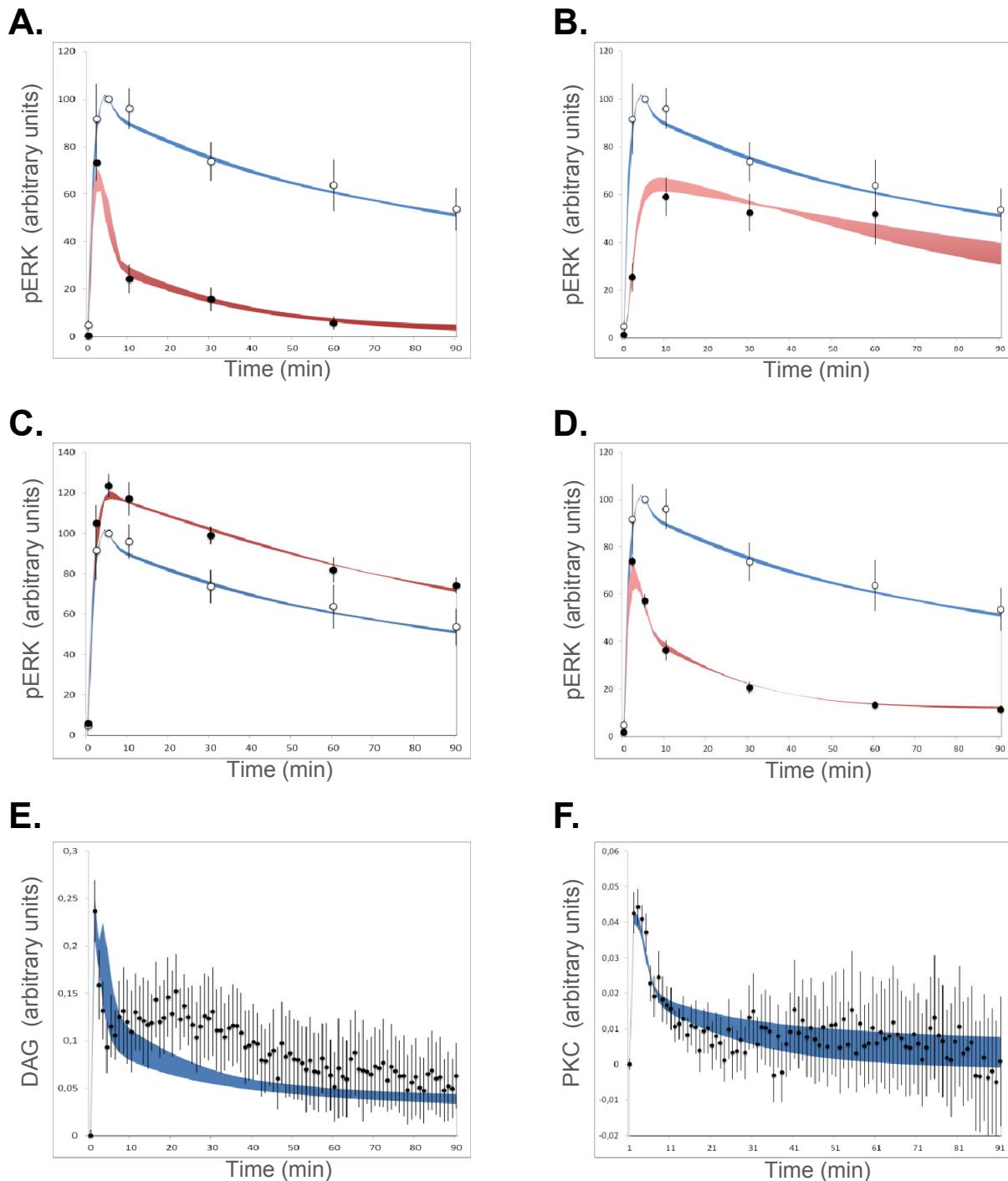


Figure S11: Standard errors on the fits generated with parameter sets 1, 2, 3 and 4. Experimental data are represented as mean \pm S.E.M (in black). **A**, pERK control siRNA (open circles) versus βarr2 siRNA (black circles); **B**, pERK control (open circles) versus PKC inhibition (black circles); **C** pERK control (open circles) versus GRK2/3 siRNA (black circles); **D**, pERK control (open circles) versus GRK5/6 siRNA (black circles); **E** control DAG and **F** control PKC activity. All the colored curves were generated through model simulations: control conditions are in blue and perturbed conditions in red. The curve widths represent the mean \pm standard error on the four parameter sets.

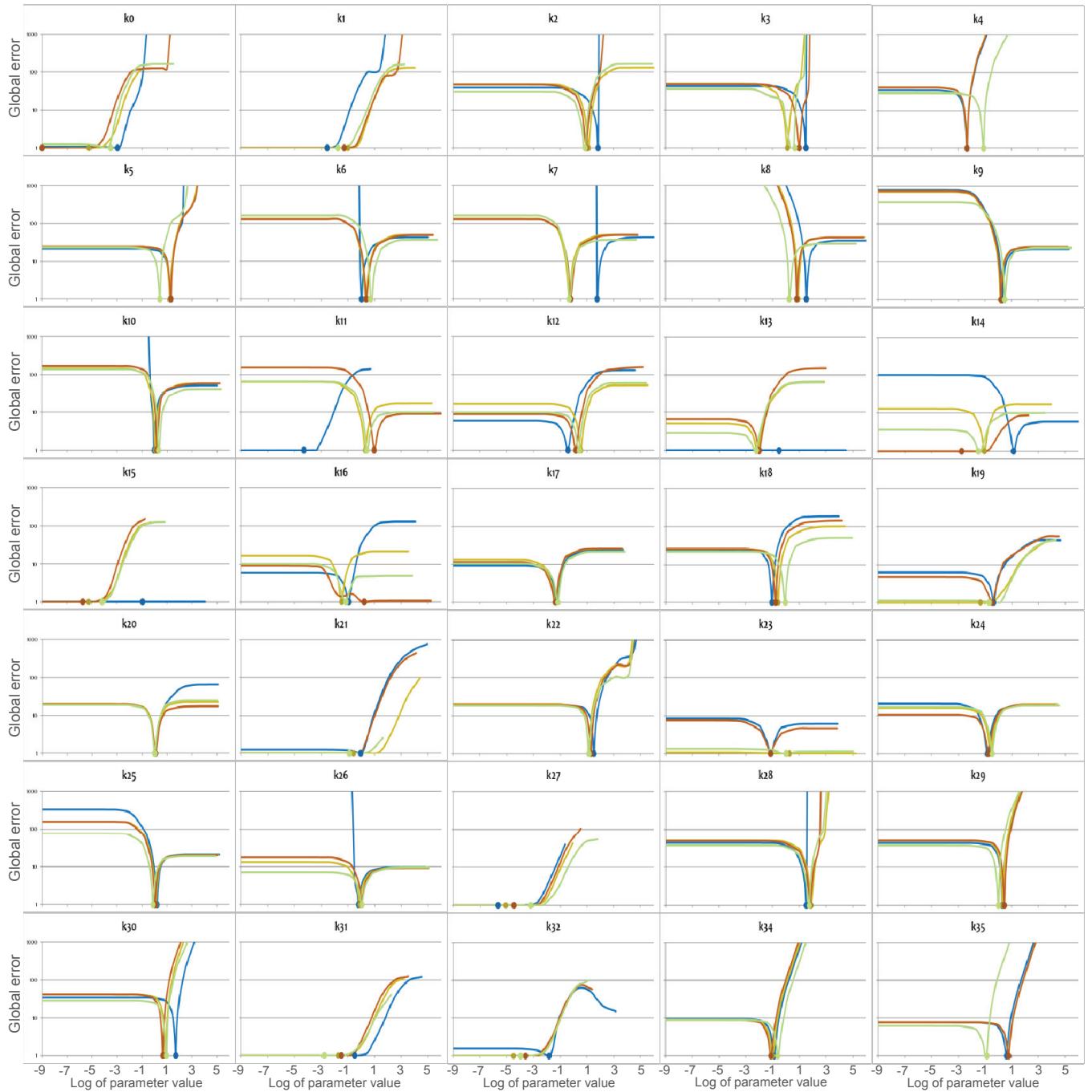


Figure S12: Sensitivity analysis for each parameter of the 4 optimized parameter sets. Each parameter value was scanned across 14 logs (from 10^{-9} to 10^5) and plotted against the normalized global error for each of the 4 parameter sets (Set1 in blue, Set2 in yellow, Set3 in red and Set4 in green). The optimal parameter value is figured by a color dot. Optimized parameter numbers correspond to Table 1.

Simulated

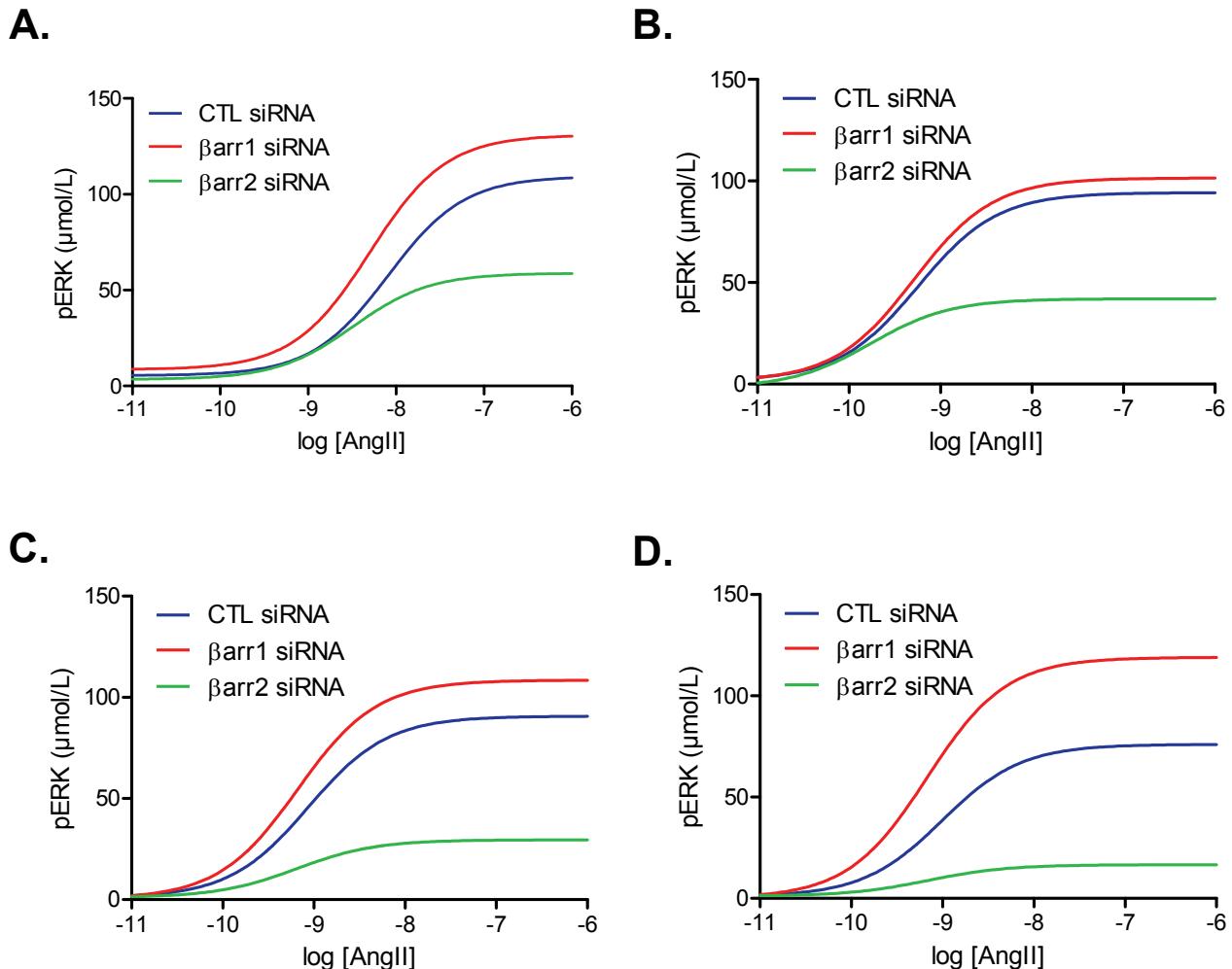


Figure S13: Simulation of the consequences of β -arrestin 1 or 2 depletion on total pERK response to increasing angiotensin concentrations at different time points. Control conditions are presented as the blue curves. Simulations of either β -arrestin 1 (red curve) or β -arrestin 2 (green curve) depletion on phosphorylated ERK response as a function of angiotensin concentration were compared at different time points: **(A)** 5 minutes, parameter set number 3; **(B)** 5 minutes, parameter set number 4; **(C)** 10 minutes, parameter set number 4; **(D)** 30 minutes, parameter set number 4. At the three time points, the model displays antagonistic actions of both β -arrestin isoforms, albeit with different intensities.

Simulated

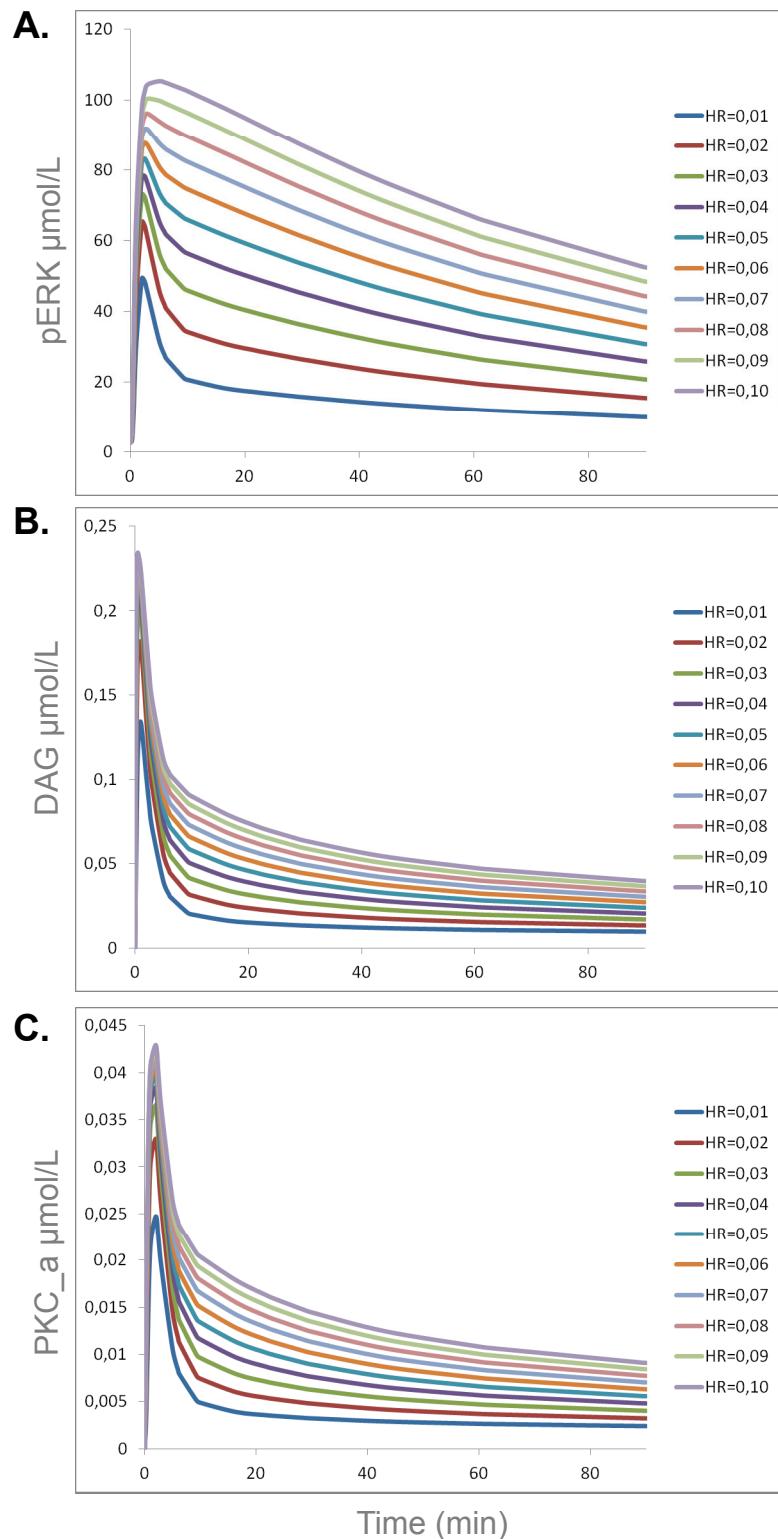


Figure S14: Simulated kinetics (parameter set number 4) of pERK (A), DAG (B) and PKC_a (C) at different HR concentrations (between 0.1 and 0.01 μmol/L).

Simulated

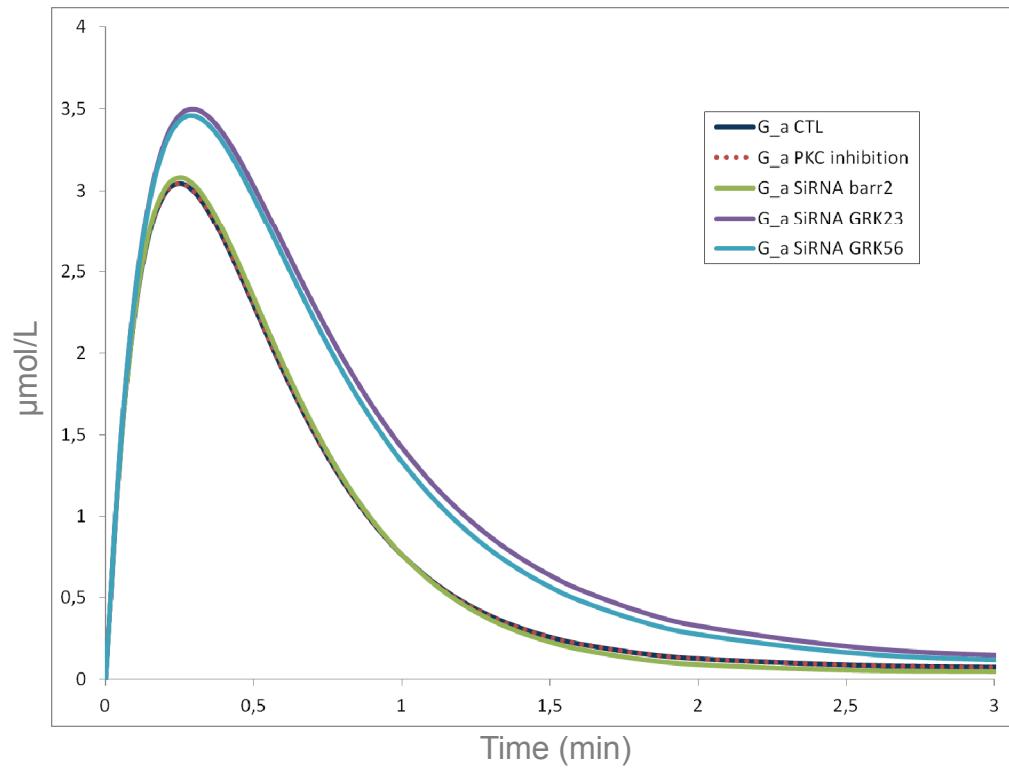


Figure S15: Simulated kinetics (parameter set number 4) of G_a in control *versus* perturbed conditions.

Simulated

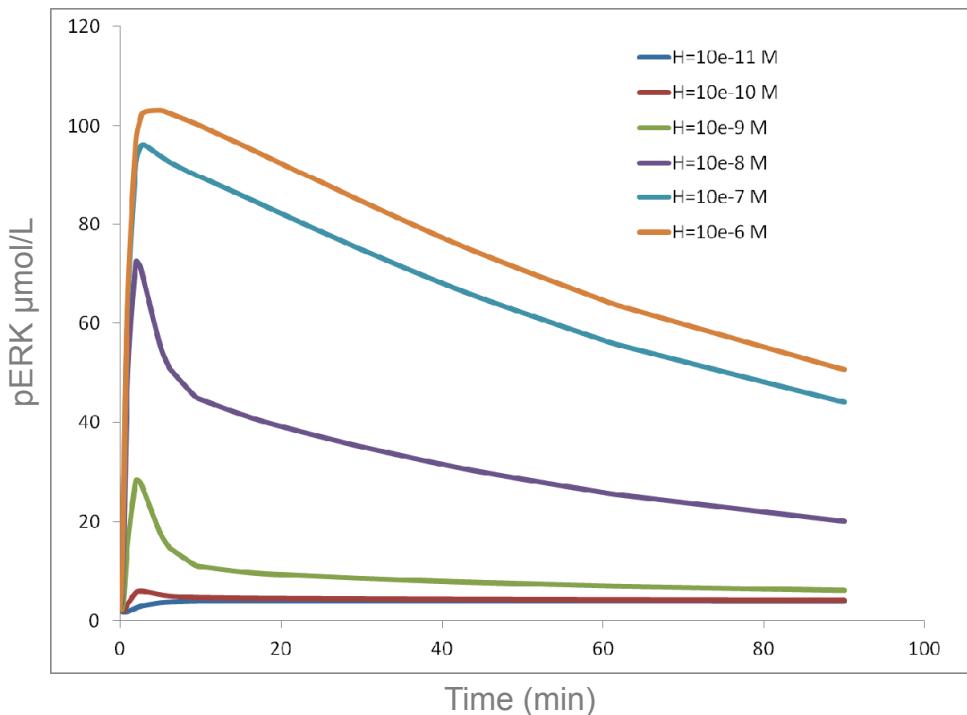


Figure S16: Simulated kinetics (parameter set number 4) of pERK at different H concentrations (between 10^{-11} M and 10^{-6} M).

Simulated

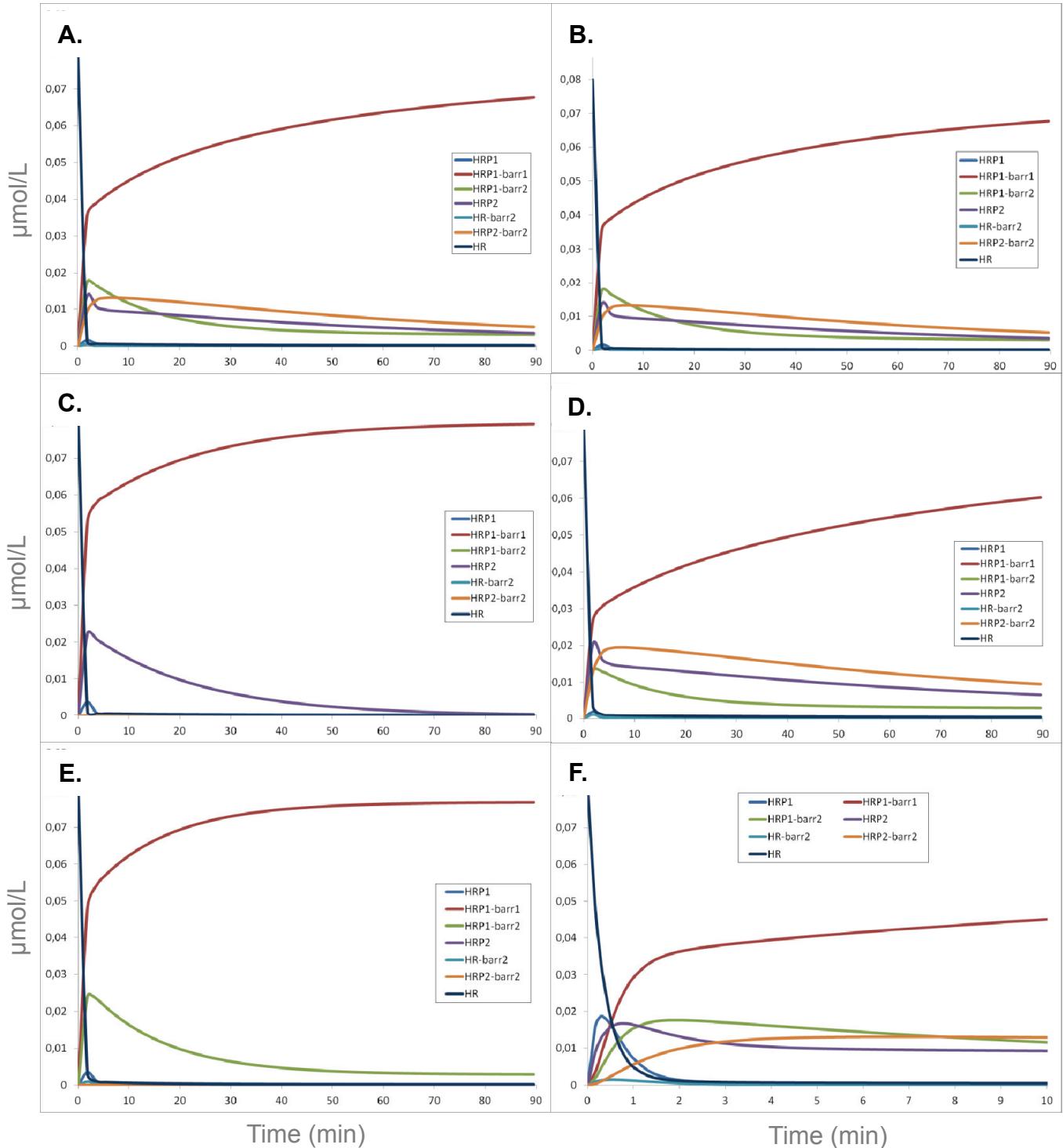
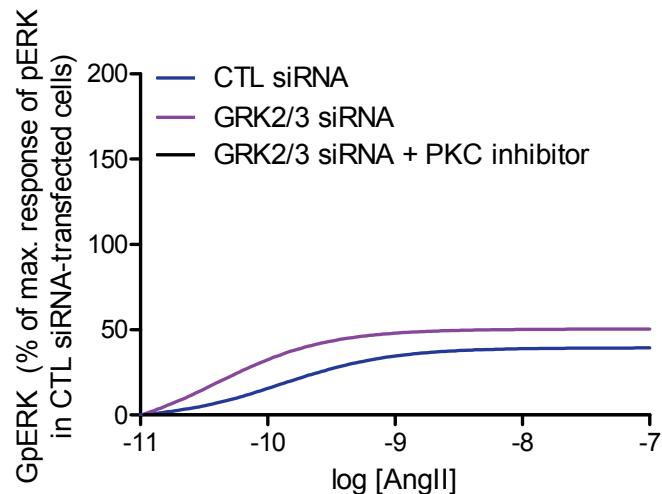


Figure S17: Simulated kinetics (parameter set number 4) of the different receptor forms in control *versus* perturbed conditions. (A) Control from 0 to 90 min; (B) PKC inhibition; (C) barr2 siRNA; (D) GRK23 siRNA; (E) GRK56 siRNA; (F) Control from 0 to 10 min.

Simulated

A.



B.

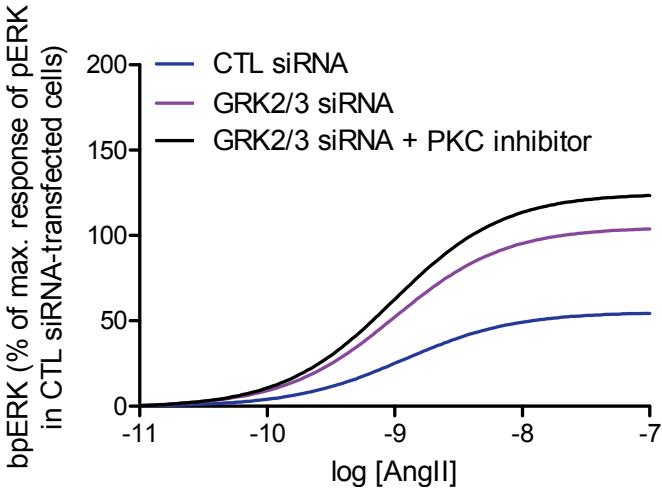


Figure S18: Simulated GpERK (**A**) and bpERK (**B**) responses at 5 min as a function of angiotensin concentration in control (blue), GRK2/3-depleted (purple), or GRK2/3-depleted and PKC-inhibited (black) conditions. Simulated total pERK response is shown at Figure 6C.

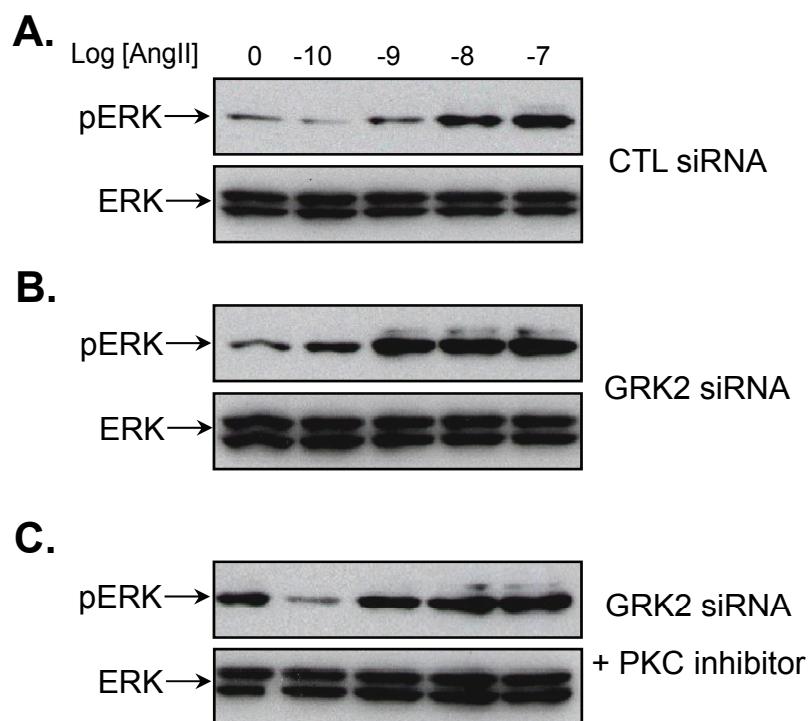


Figure S19: Representative Western blots of HEK293 cells transiently co-transfected with wild-type (WT) AT_{1A}R and either control (CTL) or GRK2 siRNAs. **(A)** ERK phosphorylation as a function of AngII concentration (10^{-10} to 10^{-7} M) in control cells stimulated for 2 min. **(B)** ERK phosphorylation as a function of AngII concentration (10^{-10} to 10^{-7} M) in GRK2-depleted cells stimulated for 2 min. **(C)** ERK phosphorylation as a function of AngII concentration (10^{-10} to 10^{-7} M) in GRK2-depleted cells pre-incubated with PKC inhibitor (Ro-31-8425, 1 μ M) and stimulated for 2 min.

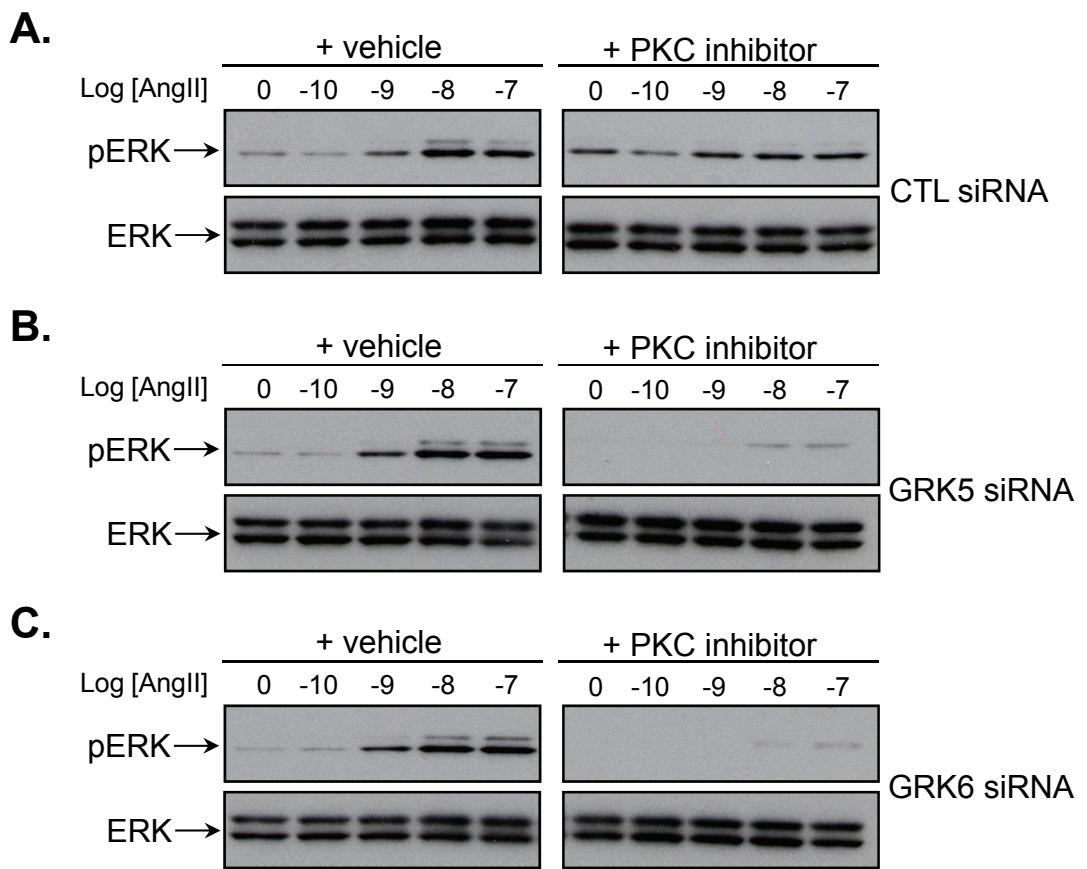


Figure S20: Representative Western blots of HEK293 cells transiently co-transfected with wild-type (WT) AT_{1A}R and either control (CTL), GRK5 or GRK6 siRNAs. **(A)** ERK phosphorylation as a function of AngII concentration (10⁻¹⁰ to 10⁻⁷ M) in control cells pre-treated with DMSO or the PKC inhibitor Ro-31-8425 (as indicated) and stimulated for 2 min. **(B)** ERK phosphorylation as a function of AngII concentration (10⁻¹⁰ to 10⁻⁷ M) in GRK5-depleted cells pre-treated with DMSO or PKC inhibitor (as indicated) and stimulated for 2 min. **(C)** ERK phosphorylation as a function of AngII concentration (10⁻¹⁰ to 10⁻⁷ M) in GRK6-depleted cells pre-treated with DMSO or PKC inhibitor (as indicated) and stimulated for 2 min.

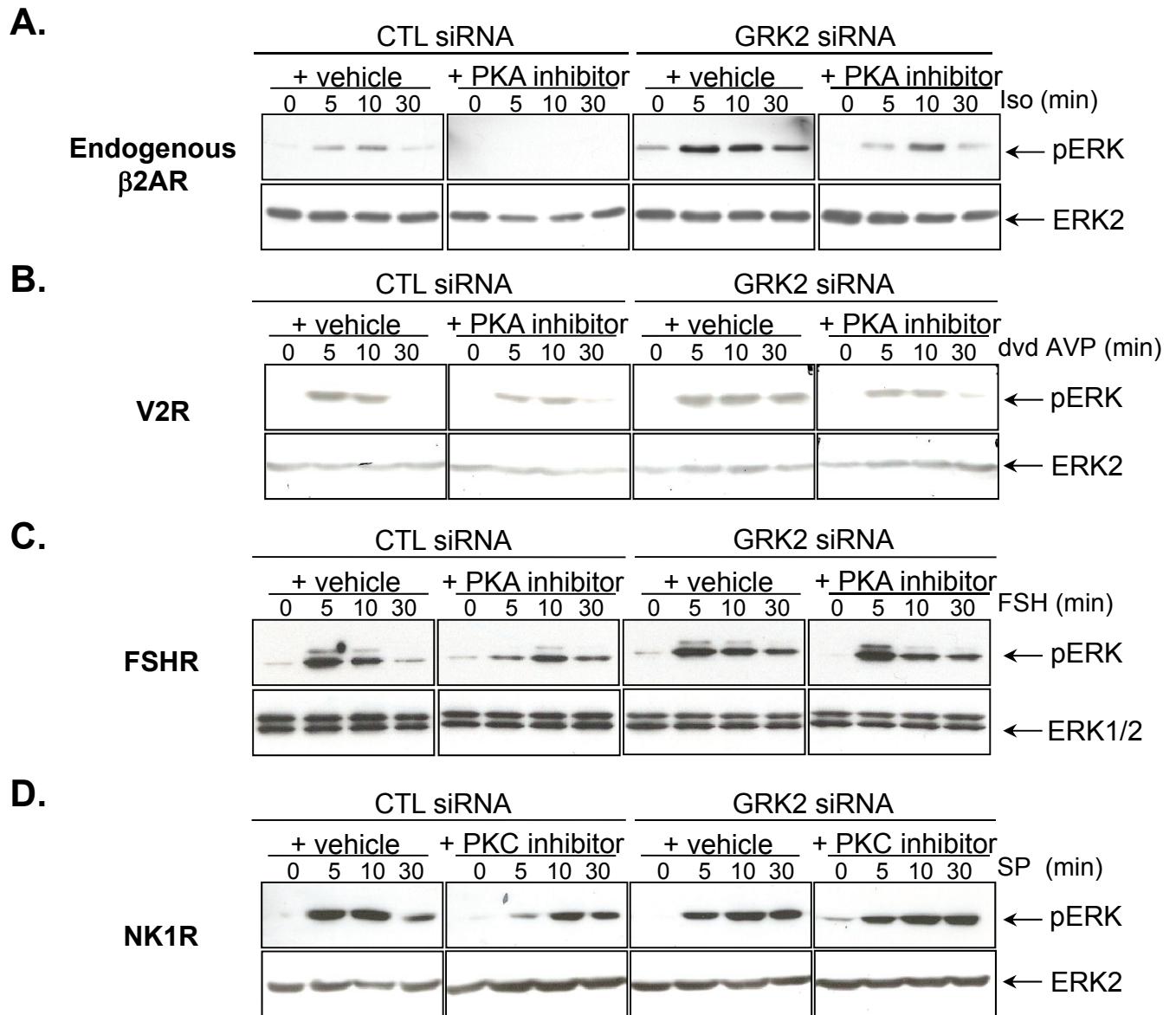


Figure S21: Representative Western blots of HEK293 cells transiently co-transfected with different GPCRs and either control (CTL) or GRK2 siRNAs. **(A)** Time-course of ERK phosphorylation upon stimulation of endogenously expressed β₂AR with isoproterenol (10⁻⁵ M) in cells transfected with CTL or GRK2 siRNA and pre-treated with DMSO or with a PKA inhibitor (H-89, 10 μM). **(B)** Time-course of ERK phosphorylation upon stimulation of transiently transfected V2R with dvd-AVP (10⁻⁷ M) in cells co-transfected with CTL or GRK2 siRNA and pre-treated with DMSO or with a PKA inhibitor (H-89, 10 μM). **(C)** Time-course of ERK phosphorylation upon stimulation of transiently transfected FSHR with FSH (3 10⁻⁹ M) in cells co-transfected with CTL or GRK2 siRNA and pre-treated with DMSO or with a PKA inhibitor (H-89, 10 μM). **(D)** Time-course of ERK phosphorylation upon stimulation of transiently transfected NK1R with Substance P (10⁻⁷ M) in cells co-transfected with CTL or GRK2 siRNA and pre-treated with DMSO or with a PKC inhibitor (Ro-31-8525, 1 μM).

Parameter #	Description	Name in optimization	Unit	set4	set3	set2	set1
Initial quantities							
1	Activated G protein (G_a)		μmol.L-1	0			
2	DAG		μmol.L-1	0.009			
3	Active PKC (PKC_a)		μmol.L-1	0.002			
4	HRP1		μmol.L-1	0			
5	HRP1-β arrestin 1 (HRP1-barr1)		μmol.L-1	0			
6	HRP1-β arrestin 2 (HRP1-barr2)		μmol.L-1	0			
7	HRP2		μmol.L-1	0			
8	HR-β-arrestin 2 (HR-barr2)		μmol.L-1	0			
9	HRP2-β-arrestin 2 (HRP2-barr2)		μmol.L-1	0			
10	bpERK		μmol.L-1	0.015			
11	GpERK		μmol.L-1	0.015			
Total quantities							
12	G protein (G+G_a)	k28	μmol.L-1	56.99	66.36	79.61	34.23
13	Receptor (HR+HRP1+HRP1-barr1+HRP1-barr2+HR-barr2+HRP2+HRP2-barr2)		μmol.L-1	0.08			
14	DAG (PIP2+DAG)	k29	μmol.L-1	1.006	2.81	2.1	2.03
15	PKC (PKC+PKC_a)	k30	μmol.L-1	8.842	4.69	7.81	49.81
16	β-arrestin 1 (barr1+HRP1-barr1)		μmol.L-1	0.858			
17	β-arrestin 2 (barr2+HRP1-barr2+HR-barr2+HRP2-barr2)		μmol.L-1	0.483			
18	ERK (ERK + bpERK + GpERK)		μmol.L-1	4.273			
Norms							
19	ERK norm		L.μmol-1	0.013			
20	DAG norm	k34	L.μmol-1	4.12	0.075	0.1	0.13
21	PKC_a norm	k35	L.μmol-1	7.21	5.98	6.16	4.13
Reaction rates							
22	G auto activation	k0	min-1	3.11e-4	0	5.43e-6	0.001
23	Activation of G by HRP1	k1	L.μmol-1.min-1	0.018	0.053	0.1	0.0028
24	Activation of G by HR	k2	L.μmol-1.min-1	7.6	8.92	13.17	69.86
25	Activation of DAG by G_a	k3	L.μmol-1.min-1	4.63	9.76	1.28	30.53
26	Activation of PKC by DAG	k4	L.μmol-1.min-1	0.079	0.0046	0.0045	0.0044
27	Phosphorylation of ERK by PKC	k5	L.μmol-1.min-1	2.65	19.58	16.98	18.19
28	Deactivation of G_a	k6	min-1	5.1	2.47	2.17	1.08
29	Deactivation of DAG	k7	min-1	0.461	0.61	0.47	58.58
30	Deactivation of PKC	k8	min-1	1.77	6.66	8.06	34.08
31	Dephosphorylation of GpERK	k9	min-1	3.04	1.6	1.41	1.96
32	Phosphorylation of HR by GRK23 x GRK23 quantity	k10	min-1	2.05	1.59	1.14	0.96
33	Association of HRP1 with β-arrestin 1	k11	L.μmol-1.min-1	2.61	9.54	1.99	5.06e-5
34	Association of HRP1 with β-arrestin 2	k12	L.μmol-1.min-1	2.59	1.44	3.41	0.38
35	Dissociation of HRP1-barr1 complex	k13	min-1	0.0062	0.01	0.0072	0.32
36	Dissociation of HRP1-barr2 complex	k14	min-1	0.031	0.0018	0.091	13.77
37	Recycling of HRP1-barr1 complex	k15	min-1	6.54e-5	1.81e-6	5.24e-6	0.11
38	Recycling of HRP1-barr2 complex	k16	min-1	0.072	1.66	0.034	0.12
39	Dephosphorylation of HRP2	k17	min-1	0.067	0.041	0.045	0.049
40	Phosphorylation of HR by GRK56 x GRK56 quantity	k18	min-1	0.896	0.17	0.26	0.092
41	Association of HR with barr2	k19	L.μmol-1.min-1	0.21	0.34	0.045	0.43
42	Association of HRP2 with barr2	k20	L.μmol-1.min-1	1.04	1.23	1.24	1.2
43	Phosphorylation of ERK by HR-barr2	k21	L.μmol-1.min-1	4.2e-4	0.13	0.27	0.89
44	Phosphorylation of ERK by HRP2-barr2	k22	L.μmol-1.min-1	14.44	19.69	13.35	33.2
45	Dissociation of HR-barr2 complex	k23	min-1	1.05	0.07	1.87	0.073
46	Dissociation of HRP2-barr2 complex	k24	min-1	0.35	0.18	0.26	0.15
47	Dephosphorylation of bpERK	k25	min-1	0.76	1.09	1.29	1.52
Inhibited PKC condition							
48	PKC total quantity (PKC + PKC_a)	k31	μmol.L-1	0.0018	0.033	0.019	0.33
49	Initial bpERK quantity		μmol.L-1	0.0037			
50	Initial GpERK quantity		μmol.L-1	0.0037			
Inhibited β-arrestin 2 condition							
51	β-arrestin 2 total quantity	k32	μmol.L-1	1.12e-4	0.00025	3.17e-5	0.015
52	Initial bpERK quantity		μmol.L-1	8.2e-4			
53	Initial GpERK quantity		μmol.L-1	8.2e-4			
Inhibited GRK23 condition							
54	Phosphorylation rate of HR by GRK23 x GRK23 quantity	k26	min-1	1.087	1.08	0.74	0.64
55	Initial bpERK quantity		μmol.L-1	0.0182			
56	Initial GpERK quantity		μmol.L-1	0.0182			
Inhibited GRK56 condition							
57	Phosphorylation rate of HR by GRK56 x GRK56 quantity	k27	min-1	6.13e-4	3.55e-5	8.74e-6	2.31e-6
58	Initial bpERK quantity		μmol.L-1	0.0055			
59	Initial GpERK quantity		μmol.L-1	0.0055			

Table S1: Parameter values for each of the 4 best parameter sets. Parameter set4 has been used throughout the paper. The 4 parameter sets have been compared in sensitivity analyses.

Description	Calculated from simulated kinetics	Independent experimental	References
Activation of G by HRP1	12 sec		
Activation of G by HR	3.3 sec	0.3-2 sec	Lohse et al., 2008; Vilardaga et al. 2010
Activation of DAG by G_a	7.2 sec	5-10 sec	Violin et al., 2006
Activation of PKC by DAG	25 sec	30 sec	Violin et al., 2006
Phosphorylation of ERK by PKC	37 sec	60 sec	Ahn et al., 2004
Deactivation of G_a	27 sec	15-121 sec	Vilardaga et al., 2010
Deactivation of DAG	183 sec	1-2 min	Violin et al., 2006
Deactivation of PKC	204 sec	30 sec	Violin et al., 2006
Dephosphorylation of GpERK	204 sec	3 min	Ahn et al., 2004
Phosphorylation of HR by GRK23 x GRK23 quantity	10 sec		
Association of HRP1 with β-arrestin 1	80 sec	20-50 sec	Rajagopal et al., 2006; Lohse et al., 2008
Association of HRP1 with β-arrestin 2	80 sec	20-50 sec	Rajagopal et al., 2006; Lohse et al., 2008
Dephosphorylation of HRP2	17 min	10-15 min	Pöll et al., 2011
Phosphorylation of HR by GRK56 x GRK56 quantity	13 sec		
Association of HRP2 with barr2	80 sec	20-50 sec	Rajagopal et al., 2006; Lohse et al., 2008
Phosphorylation of ERK by HRP2-barr2	150 sec	3 min	Ahn et al., 2004
Dephosphorylation of bpERK	60 min	> 60 min	Ahn et al., 2004

Table S2: Simulated *versus* experimental activation/deactivation half-lives measured in independent studies.