Electronic Supplementary Material (ESI) for Molecular BioSystems

Electronic Supplementary Information

Control of the G-protein Cascade Dynamics by GDP Dissociation Inhibitors

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Supplementary Figures.

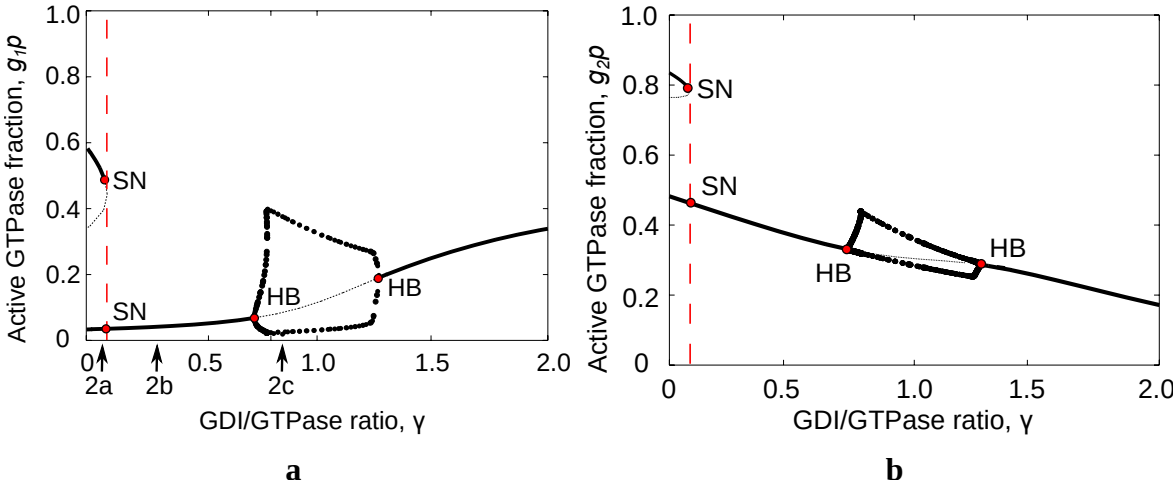


Fig. S1. Regulation of RhoA behavior by RhoGDI/GTPase ratio. Bifurcation diagrams illustrating the behavior of active fractions of RhoA (a) and Rac1 (b) in response to increasing the GDI/GTPase ratio (γ). Solid and dotted lines indicate stable and unstable steady states, respectively and circles indicate the maximums and minimums of stable periodic solutions. The bistable regime shown at the leftmost edge is characterized by the existence of three fixed points; stable "on" and "off" states indicated by the upper and lower solid lines and an intermediate unstable fixed point as indicated by the dotted line. When GDI/GTPase ratio increases, the bistable behavior is succeeded by an excitable regime shown by the solid line. The oscillatory regime is earmarked by the upper and lower solid circles which indicate the amplitude of the limit cycle. Red dots indicate bifurcation points at which the dynamic behavior changes qualitatively. SN indicates a Saddle Node bifurcation and HB a supercritical Hopf bifurcation point. Arrows on the x-axis indicate the γ values used to simulate the corresponding system responses in Fig.2.

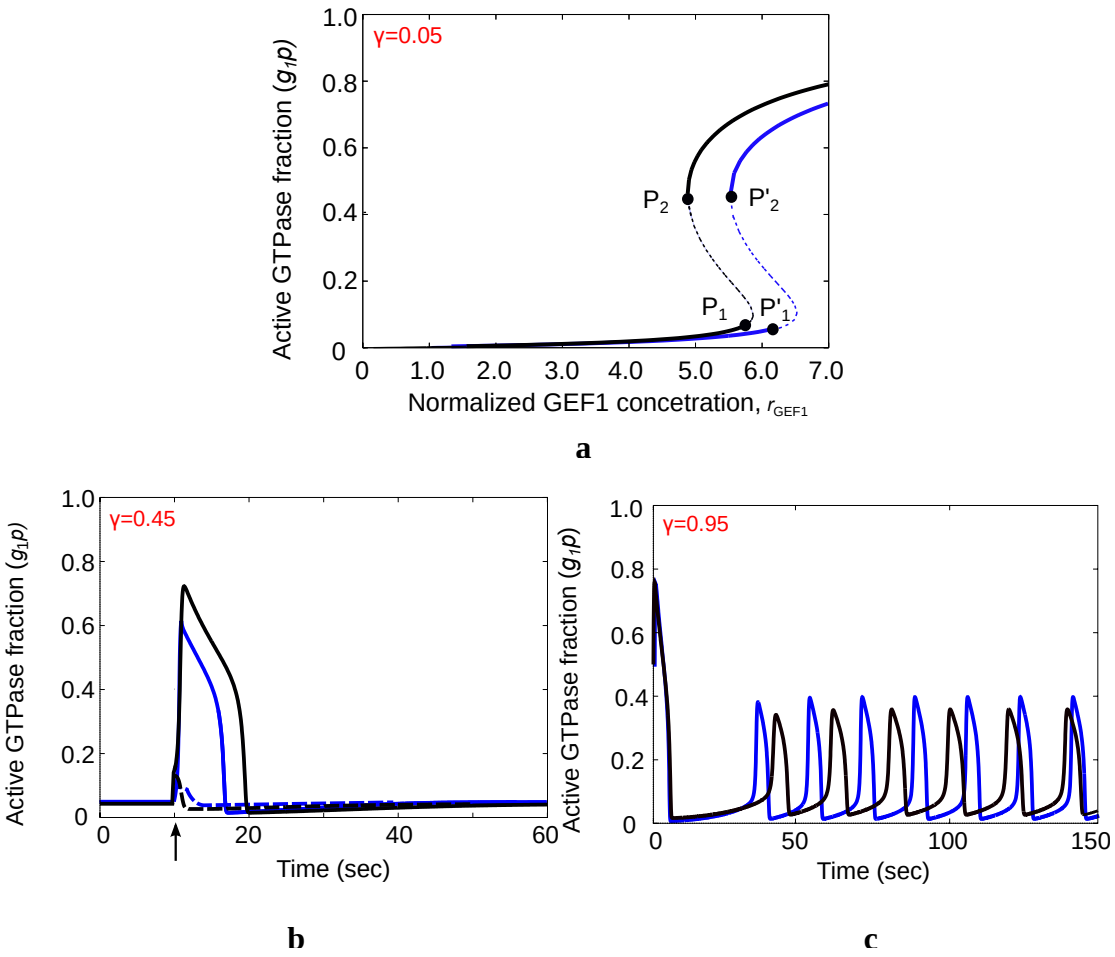


Fig. S2. Bistable, oscillatory and excitable behaviors observed in the RhoA/Rac1 model with perturbed kinetic parameters. Responses of the active GTPase fraction g_1p using the original parameter set in Table S1 (black) and perturbed parameter set in Table S2 (blue) at different GDI/GTPase ratios (shown in the upper left corner). (a) Bistability and hysteresis in response to changing GEF1 activity. The values of the stable (solid lines) and unstable steady states (dotted lines) are shown for different GEF1 activities. Here, RhoA fraction for original and perturbed parameter sets is bistable inbetween the turning points (P_1, P_2) and (P'_1, P'_2) respectively. (b) Excitable overshoot transitions. Initially, a cascade resides in a stable, but excitable steady state. At time t = 10 s (marked by black arrow on the time axis), the GEF concentration of the active GTPase at the first layer is perturbed for 1 second. The threshold for excitable pulse is at r_{GEF1}=5.15. Sub-threshold perturbations result in responses rapidly returning to the basal steady state (shown by dashed lines). Over-threshold perturbations result in large overshoot responses where the activity of the GTPase fraction markedly increases before returning to the (basal) steady state. (c) Sustained oscillations of the active GTPase fraction

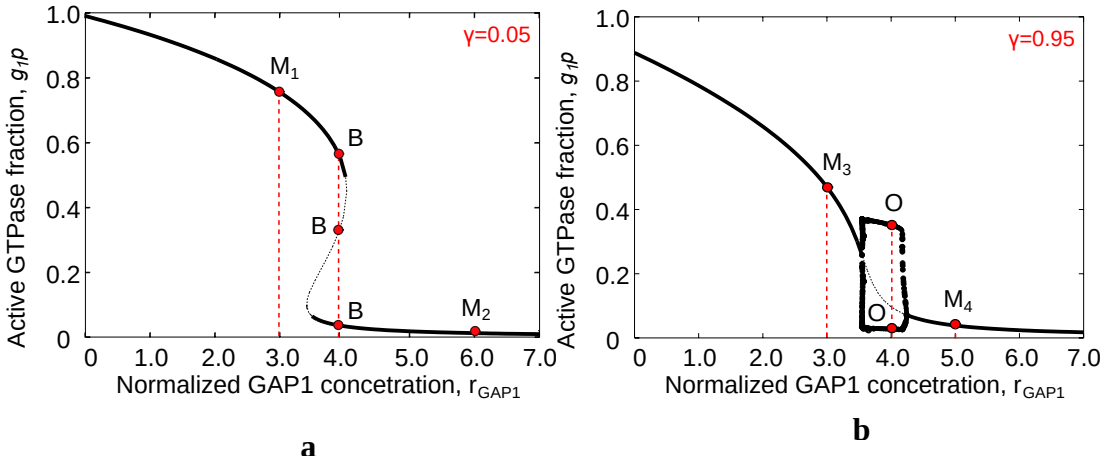


Fig. S3. Qualitative changes in the RhoA asymptotic behavior in response to GAP1 depends on the GDI/GTPase ratio. The RhoA asymptotic behavior changes in response to GAP1 levels showing a bistable regime for a low GDI/GTPase ratio (a) and an oscillatory regime for a high GDI/GTPase ratio (b). Thick lines indicate stable fixed points, dashed lines indicate unstable fixed points, circles indicate stable periodic solutions. Red points indicate the dynamics of the system at the corresponding GAP1 values with M_i, i = 1-4 referring to monostability, O to oscillations and B to bistability.

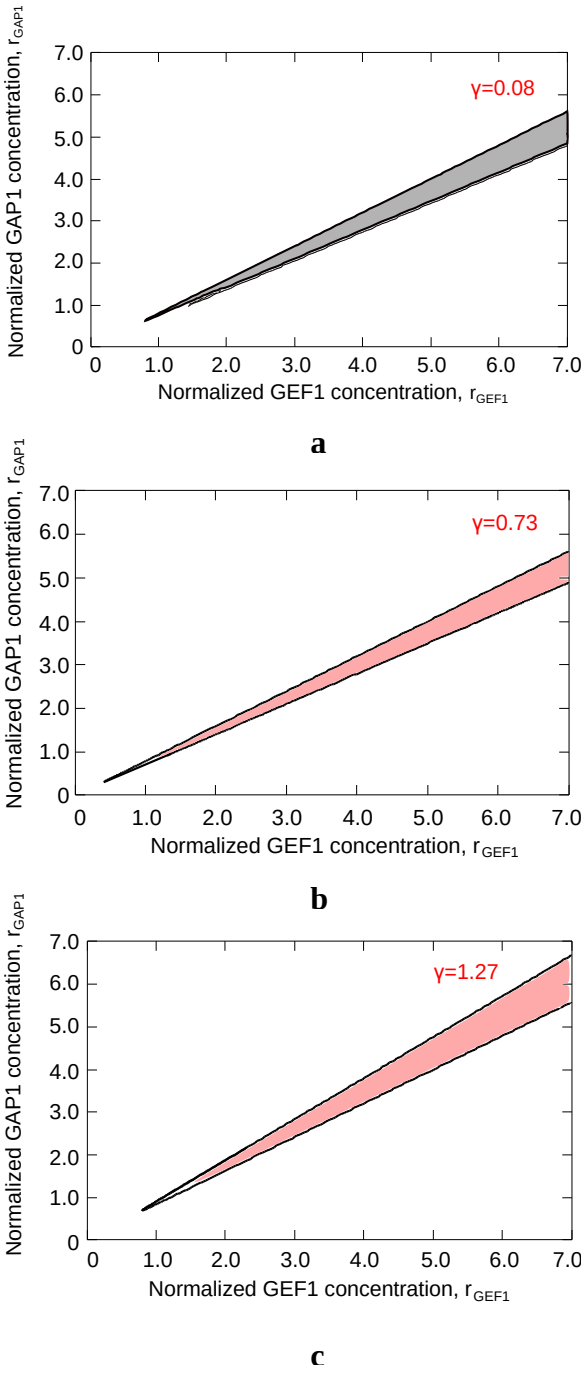


Fig.S4. Separation of the parameter space into regions of qualitatively different behaviors. For different GDI/GTPase ratios (γ) which are set at bifurcation points (a-c), the parameter spaces are divided between GAP1 and GEF1 abundances, into three regions of qualitatively distinct behaviors: monostable and excitable region (white), bistable region (grey) and oscillatory region (pink).

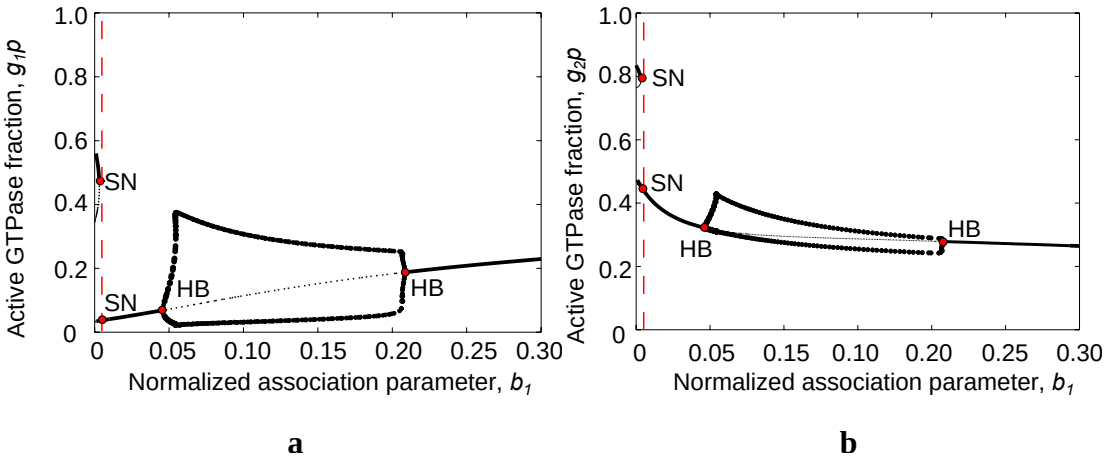


Fig. S5. Regulation of GDI-GTPase affinity affects GTPase dynamics. Bifurcation diagrams illustrate the behavior of active fractions of RhoA (a) and Rac1 (b) in response to increasing the second order binding rate constant of RhoA to RhoGDI (denoted b_1), while holding all other parameters constant (See Table 1 in SI). Solid and dotted lines indicate stable and unstable steady states, respectively and circles indicate stable periodic solutions. The bistable regime shown at the leftmost edge is characterized by the existence of three fixed points; stable "on" and "off" states indicated by the upper and lower solid lines and an intermediate unstable fixed point as indicated by the dotted line. When the affinity increases, the bistable behavior is succeeded by an excitable regime shown by the solid line. The oscillatory regime is indicated by the upper and lower solid circles which delineate the amplitude of the limit cycle. Red dots indicate bifurcation points at which the dynamic behavior changes qualitatively. SN indicates a Saddle Node bifurcation and HB a supercritial Hopf bifurcation point.

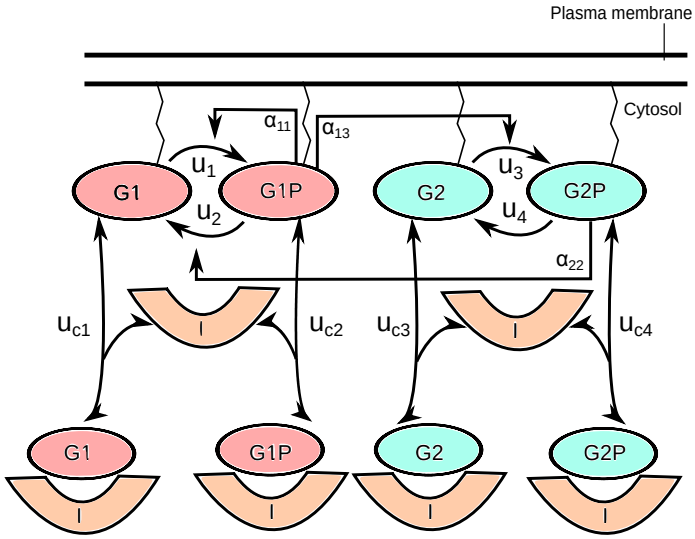


Fig. S6. Alternative model for the inhibition of RhoA by Rac1. Here inhibition of RhoA ($\boldsymbol{G_1}$) occurs via activation of GAP for RhoA (vs inhibition of GEF for RhoA in the original model, Fig.1b)**.** $\boldsymbol{G_1}$ and $\boldsymbol{G_2}$ correspond to the inactive states and $\boldsymbol{G_1P}$ and $\boldsymbol{G_2P}$ correspond to the active states of RhoA and Rac1, respectively. GEF and GAP catalyzed reactions are denoted by $\boldsymbol{u_i}$ ($\boldsymbol{i}$ denotes a reaction number), which are modified by activating and inhibiting interactions ($\boldsymbol{\alpha_{ij}}$). GDI (denoted $\boldsymbol{I}$) binds to inactive and active forms of both GTPases with the corresponding rates $\boldsymbol{u_{c1}, u_{c2}, u_{c3}, u_{c4}}$, producing inactive complexes in the cytosol.

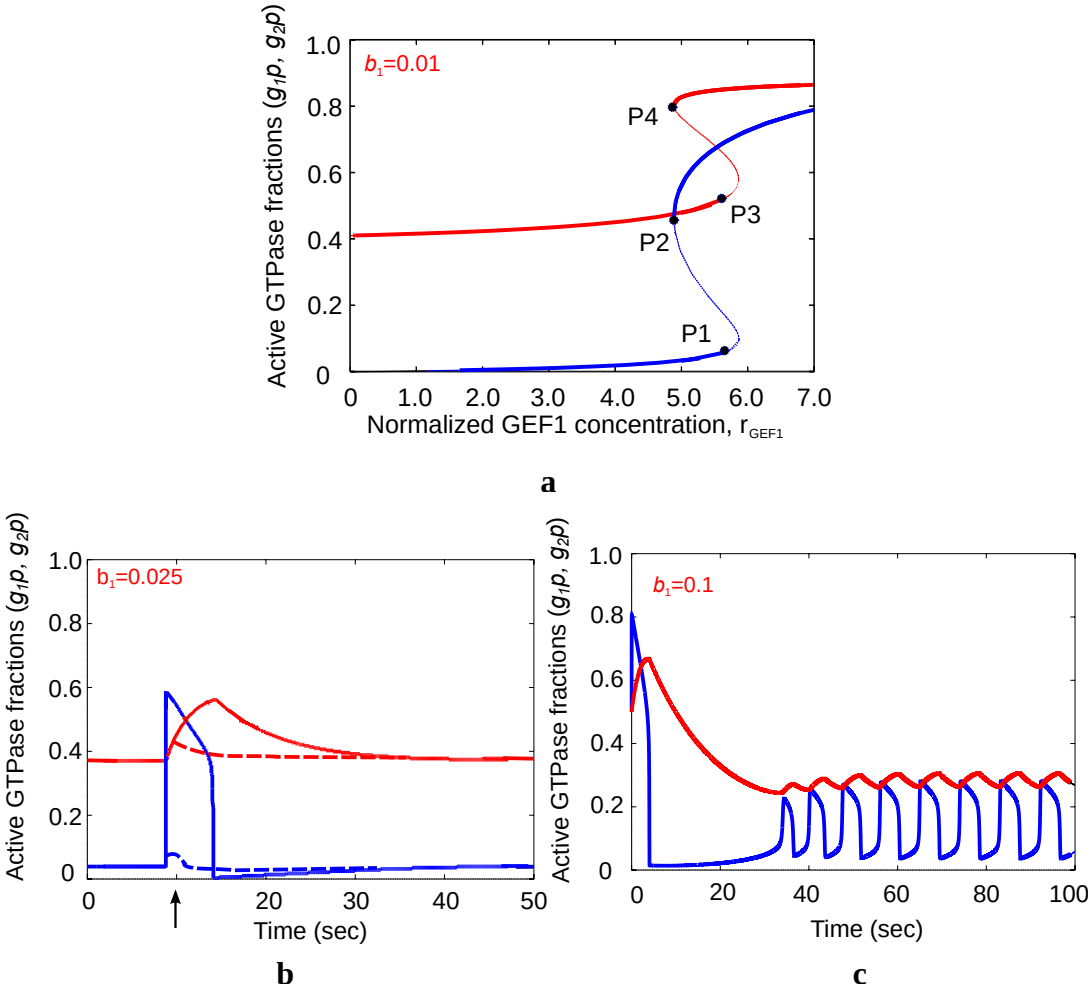


Fig. S7. Bistable, oscillatory and excitable behaviors observed in the alternative RhoA/Rac1 model at different GDI-RhoA association parameters. Responses of active GTPase fractions, g_1p (blue) and g_2p (red) at different GDI-RhoA association parameters (b_1, shown in the upper left corner) and at fixed kinetic parameters given in Table 3 in the SI. (a) Bistability and hysteresis in response to changing GEF1 activity. The values of the stable (solid lines) and unstable steady states (dotted lines) are shown for different GEF1 activities. Both active RhoA and active Rac1 fractions are bistable inbetween the turning points (P_1, P_2) and (P_3, P_4) respectively. (b) Excitable overshoot transitions. Initially, a cascade resides in a stable, but excitable steady state. At time t = 10 s (marked by black arrow on the time axis), the concentration of GEF1 for the GTPase at the first layer is perturbed for 1 second. The threshold for excitable pulse is at r_{GEF1}=5.15. Sub-threshold perturbations result in responses rapidly returning to the basal steady state (shown by dashed lines). Over-threshold perturbations result in large overshoot responses where the activity of both GTPase fractions markedly increases before returning to the (basal) steady state. (c) Sustained oscillations of active GTPase fractions

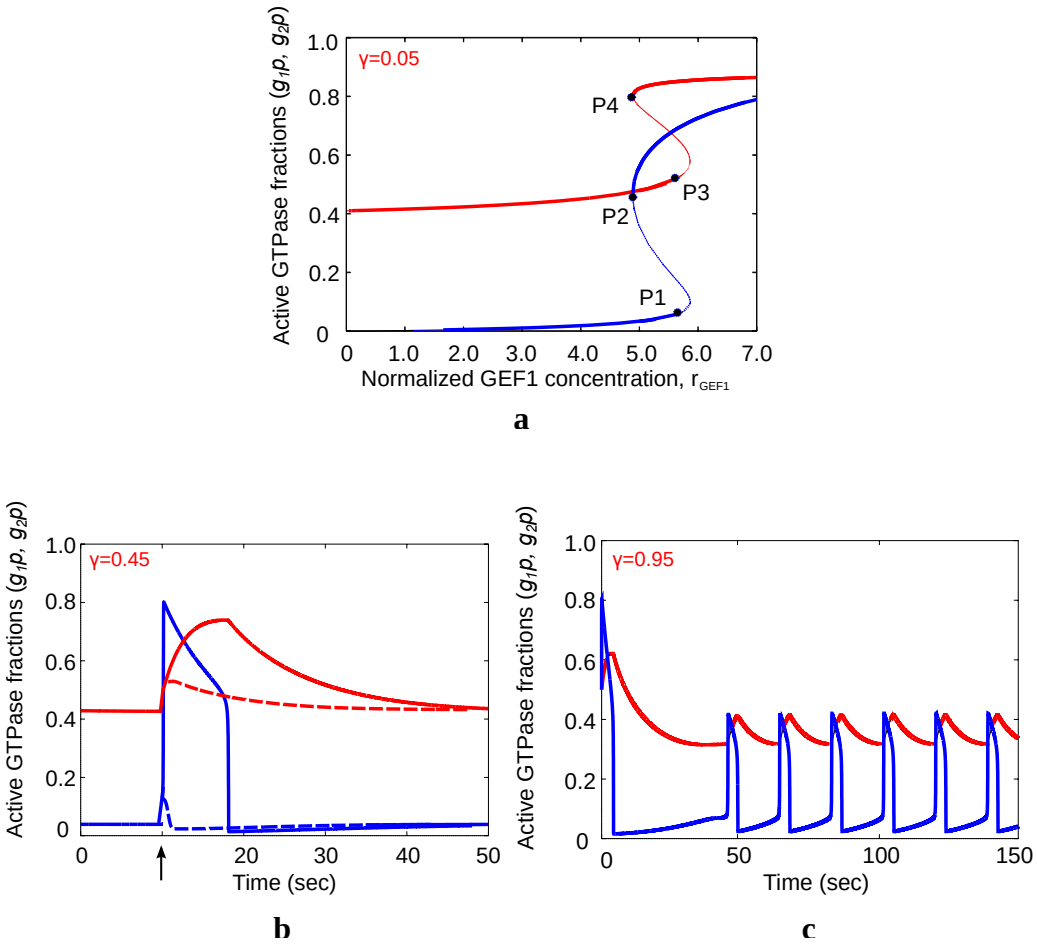
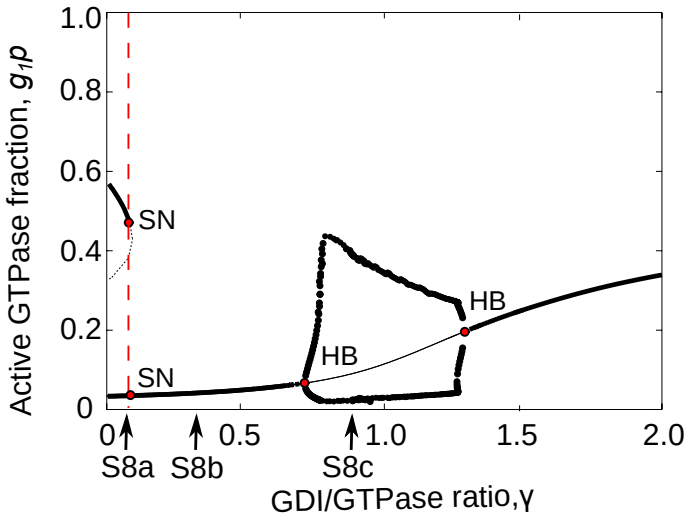
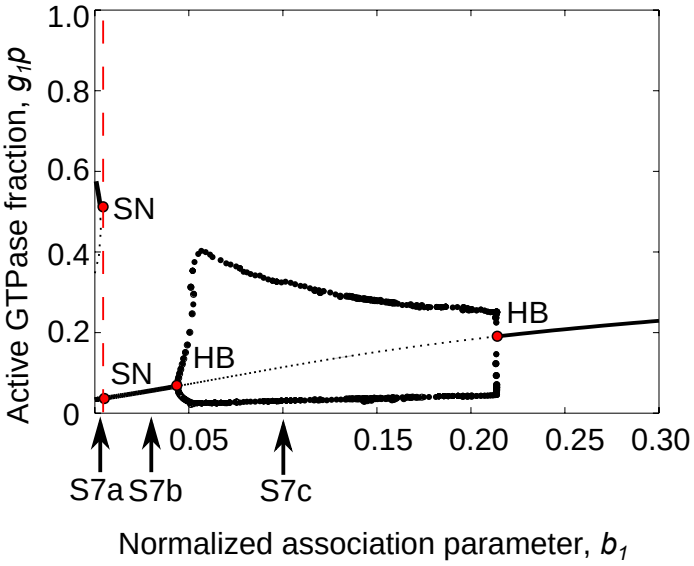


Fig. S8. Bistable, oscillatory and excitable behaviors observed in the alternative RhoA/Rac1 model at different GDI/GTPase ratios. Responses of active GTPase fractions, g_1p (blue) and g_2p (red) at different GDI/GTPase ratios (shown in the upper left corner) and at fixed kinetic parameters given in Table 3 in the SI. (a) Bistability and hysteresis in response to changing GEF1 activity. The values of the stable (solid lines) and unstable steady states (dotted lines) are shown for different GEF1 activities. Both active RhoA and active Rac1 fractions are bistable inbetween the turning points (P_1, P_2) and (P_3, P_4) respectively. (b) Excitable overshoot transitions. Initially, a cascade resides in a stable, but excitable steady state. At time t = 10 s (marked by black arrow on the time axis), the concentration of GEF1 for the GTPase at the first layer is perturbed for 1 second. The threshold for excitable pulse is at r_{GEF1}=5.15. Sub-threshold perturbations result in responses rapidly returning to the basal steady state (shown by dashed lines). Over-threshold perturbations result in large overshoot responses where the activity of both GTPase fractions markedly increases before returning to the (basal) steady state. (c) Sustained oscillations of active GTPase fractions



a



b

Fig. S9 Regulation of RhoA behavior by RhoGDI/GTPase ratio and RhoGDI-RhoA affinity in the alternative RhoA/Rac1 model. Bifurcation diagrams illustrating the behavior of active fractions of RhoA in response to increasing the GDI/GTPase ratio (γ) or the second order binding rate constant of RhoA to RhoGDI (b_1). Solid and dotted lines indicate stable and unstable steady states, respectively and circles indicate periodic solutions. The bistable regime shown at the leftmost edge is characterized by the existence of three fixed points; stable "on" and "off" states indicated by the upper and lower solid lines and an intermediate unstable fixed point as indicated by the dotted line. When GDI/GTPase ratio or GDI-GTPase affinity increases, the bistable behavior is succeeded by an excitable regime shown by the solid line. The oscillatory regime is earmarked by the upper and lower solid circles which indicate the amplitude of the limit cycle. Red dots indicate bifurcation points at which the dynamic behavior changes qualitatively. SN indicates a Saddle Node bifurcation and HB a Hopf bifurcation point. Arrows on the x-axis indicate the γ and b_1 values used to simulate the corresponding system responses in Fig.S7

Supplementary Documents.

Supplementary Document I. Kinetic equations describing the alternative RhoA/Rac1/RhoGDI1 interaction model

The kinetic interactions of the alternative RhoA/Rac1 model can be described by the same system of ODEs as in Eqs.2, with the exception of GAP catalyzed reactions for the RhoA cascade. Thus the expressions for the rates u_1 and u_2 in Eqs 2 are replaced by the following:

$$u_1 = \alpha_{11} V_1 \frac{G_1/K_1}{1 + G_1/K_1}, \text{(S1a)}$$

$$u_2 = \alpha_{22} V_2 \frac{G_1 P/K_2}{1 + G_1 P/K_2}. \text{(S1b)}$$

Normalizations are done the same as previously (Eqs. 4, 5), thus (Eq. S1a) and (Eq. S1b) yield the following normalized rates of RhoA GEF-, GAP- catalyzed exchange:

$$\nu_1 = \alpha_{11} r_{GEF1} \frac{g_1/m_1}{1 + g_1/m_1}, \text{(S2a)}$$

$$\nu_2 = \alpha_{22} r_{GAP1} \frac{g_1 p/m_2}{1 + g_1 p/m_2}. \text{(S2b)}$$

In the alternative model, the above expressions (S2a) and (S2b) replace the expressions for ν_1 and ν_2 given in Eqs. 4 of the main text, while all other expressions remain unchanged.

r_{GEFi}, r_{GAPi}, i=1-2 – normalized maximal rates
m_i, i=1-4 – normalized Michaelis constants
m_{ij}, i=1-4, j=1-4 – normalized Michaelis-Menten-type half-activation/half-inhibition constants
a_{ij} , i=1-4, j=1-4 –activation/inhibition of the rate v_j by $g_i p$
b_1 – rate constant for binding GDI to inactive RhoA/Rac1 forms
d_1 – rate constant for dissociation GDI from inactive RhoA/Rac1 forms
b_2 – rate constant for binding GDI to active RhoA/Rac1 forms
d_2 – rate constant for dissociation GDI from active RhoA/Rac1 forms
γ – GDI/GTPase ratio

Supplementary Document II. Parameter values.

All kinetic parameter values in the model were informed by the literature and chosen within reasonable biological bounds. The parameters of the core model relating to the two GTPases and their mutual interactions were taken from *Tsyganov et al*.[1] The GDI-GTPase binding parameters were informed by the literature as follows. The binding strength of GDI to Rho GTPases was reported in the low nanomolar range with dissociation constants (Kd) ranging from 1 nM to 30 nM.[2, 3] Using a GTPase concentration of 0.05 microM (from the model in Falkenberg et al.)[4] and equation (5) we can convert the reported dissociation constants into our normalised coordinates and obtain values between 0.62 and 0.05. Nomanbhoy et al. 1999[5], reported dissociation constants between 6.9 and 10.2 nM with a GTPase concentration of 31 nM, yielding a normalised Kd range between 0.222 and 0.329. In our model we use $d_1/b_1 = 0.25$ for the GDI binding to both inactive GTPases, which lies well within these ranges. Further, RhoGDI binding to the active Rac1-GTP was reported to be one order of magnitude weaker than RhoGDI binding to the inactive Rac1-GDP. In line with this observation, the normalised dissociation constant for the GDI binding to the active GTPases in our model is $d_2/b_2 = 4$, i.e. 16-times higher than d_1/b_1.

Table S1. Kinetic parameters used for the RhoA/Rac1 model with RhoGDI binding

r_{GEF1}	5
r_{GAP1}	4
r_{GEF2}	0.13
r_{GAP2}	0.072
m_1	0.7
m_2	0.15
m_3	0.6
m_4	0.05
a_{11}	200
m_{11}	4
a_{13}	100
m_{13}	40
a_{21}	0.02
m_{21}	0.04
b_1	0.2
d_1	0.05
b_2	0.05
d_2	0.2
γ	1.3

Table S2. Perturbed kinetic parameters used for the RhoA/Rac1 model with RhoGDI binding

r_{GEF1}	5.5
r_{GAP1}	4.5
r_{GEF2}	0.14
r_{GAP2}	0.0792
m_1	0.77
m_2	0.135
m_3	0.66
m_4	0.055
a_{11}	220
m_{11}	4.3
a_{13}	110
m_{13}	36
a_{21}	0.018
m_{21}	0.044
b_1	0.18
d_1	0.055
b_2	0.055
d_2	0.18
γ	1.5

Table S3. Kinetic parameters used for the alternative RhoA/Rac1 model with RhoGDI binding

r_{GEF1}	5
r_{GAP1}	4
r_{GEF2}	0.13
r_{GAP2}	0.072
m_1	0.7
m_2	0.15
m_3	0.6
m_4	0.05
a_{11}	200
m_{11}	4
a_{13}	100
m_{13}	40
a_{22}	50
m_{22}	2
b_1	0.2
d_1	0.05
b_2	0.05
d_2	0.2
γ	1.5

References:

1. M. A. Tsyganov, W. Kolch and B. N. Kholodenko, *Mol Biosyst*, 2012, **8**, 730-743.
2. Y. Q. Gosser, T. K. Nomanbhoy, B. Aghazadeh, D. Manor, C. Combs, R. A. Cerione and M. K. Rosen, *Nature*, 1997, **387**, 814-819.
3. T. K. Nomanbhoy and R. Cerione, *The Journal of biological chemistry*, 1996, **271**, 10004-10009.
4. C. V. Falkenberg and L. M. Loew, *PLoS Comput Biol*, 2013, **9**, e1002831.
5. T. K. Nomanbhoy, J. W. Erickson and R. A. Cerione, *Biochemistry*, 1999, **38**, 1744-1750.