

Supplementary Information

In mTOR regulatory module, the activation of mTOR involves release of mTOR-raptor complex from the inhibitor complex mTOR-raptor-FKBP38-PRAS40. RhebGTP relieves the inhibition of mTOR from FKBP38 by forming complex FKBP38-RhebGTP. Insulin signaling pathway reduces the GAP activity of RhebGTP, thereby helps to increase the concentration of RhebGTP. Further, the inhibitory action of PRAS40 on mTOR is eliminated by dual phosphorylation of PRAS40 at T246 by Akt and at S183 by mTOR (see Fig. 2).⁵⁰⁻⁵² Further, mTOR activity primarily involves the activation of S6K1, which depends on the phosphorylation at multiple sites controlled by mTOR-raptor and PDK1. mTOR-raptor binds to TOS motif present on the amino terminal end of S6K1 to phosphorylate T389, which helps the PDK1 mediated phosphorylation of T229 on the catalytic domain.⁷⁰⁻⁷² Also, mTOR antagonizes the phosphatase of S6K1, as observed in yeast.^{64, 73} Thus, mTOR can perform dual function with respect to the activation of S6K1.

Insulin signaling pathway is broken down into three modules involving activation of IRS1-PI3K, activation of Akt/PKC- ζ and translocation of glucose transporter GLUT4 to cell membrane. In IRS1-PI3K module, binding of insulin to insulin receptor triggers the autophosphorylation of receptor and tyrosine kinase activity. This leads to the phosphorylation and activation of IRS1. Tyrosine phosphorylated IRS1 serve as a docking site for PI3K.^{75, 76} In the Akt/PKC- ζ activation module, IRS1-PI3K helps in the production of PtdIns (3,4,5)P3 from PtdIns(4,5)P2. This in turn plays a role in recruiting Akt to membrane, where it undergoes phosphorylation at multiple sites controlled by PDK1 and PDK2 (mTOR-rictor complex).^{13, 16, 38} In GLUT4 translocation module, Akt controls the translocation of GLUT4 to membrane.^{77, 78} In addition, insulin signaling is

also subjected to multiple feedback loops involving positive and negative feedback loops. Tyrosine phosphorylation of IRS1 is required to promote insulin signaling through formation of a complex with PI3K. Phosphorylation of serine residues by Akt within the PTB domain of IRS1, a site where tyrosine phosphatase PTP1B binds to dephosphorylate tyrosine residues, serves as a positive feedback for insulin signaling.⁶⁵ Further, Akt mediated phosphorylation of PTP1B at S50 negatively modulates the phosphatase creating a positive feedback for insulin signaling.⁶⁶ Recent evidence also suggests that phosphorylation of IRS1 at Akt consensus phosphorylation motif participates in negative feedback regulation of insulin signaling.⁶⁸ Insulin activated PKC- ζ is involved in negative feedback through phosphorylation of specific serine residues present in IRS1, which prevents the complex formation with PI3K.⁷⁹ Furthermore, insulin also activates other serine/threonine kinases, JNK, mTOR, S6K1, and ERK, which exert negative feedback through serine phosphorylation of IRS1. Thus constituting multiple negative feedback loops in the insulin signaling pathway.^{60, 67} Studies have been carried out to characterize the different serine residues, which are capable of inhibiting the insulin signaling.⁶⁷

Section A: Supplementary Figures

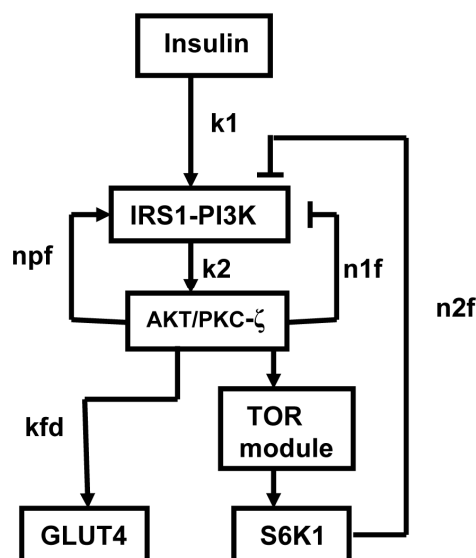


Figure S1: Modules of insulin signaling pathway. IRS1-PI3K module involves binding of insulin to insulin receptor, which undergoes autophosphorylation and increase in tyrosine kinase activity to phosphorylate insulin receptor protein (IRS1). Phosphorylated IRS1 serve as a docking site for PI3K. In the Akt/PKC- ζ activation module, IRS1-PI3K helps in the production of PtdIns (3,4,5)P3 from PtdIns(4,5)P2. This in turn plays a role in recruiting Akt to membrane, where it undergoes phosphorylation at multiple sites controlled by PDK1 and PDK2 (mTOR-ricor complex). In GLUT4 translocation module, Akt controls the translocation of GLUT4 to membrane. Akt mediated phosphorylation at specific serine residues on IRS1 can both activate and inactivate insulin signaling response. Akt exerts a positive feedback on insulin signaling by phosphorylating the PTB domain of IRS1, which prevents the binding of phosphatase PTP1B and by directly phosphorylating PTP1B. Akt can also activate the negative feedback directly and also through activation of downstream kinases such as mTOR/S6K1. Further, insulin can also activate other kinases such as PKC- ζ , which in turn exerts the negative feedback on insulin signaling through phosphorylation of specific serine residues.

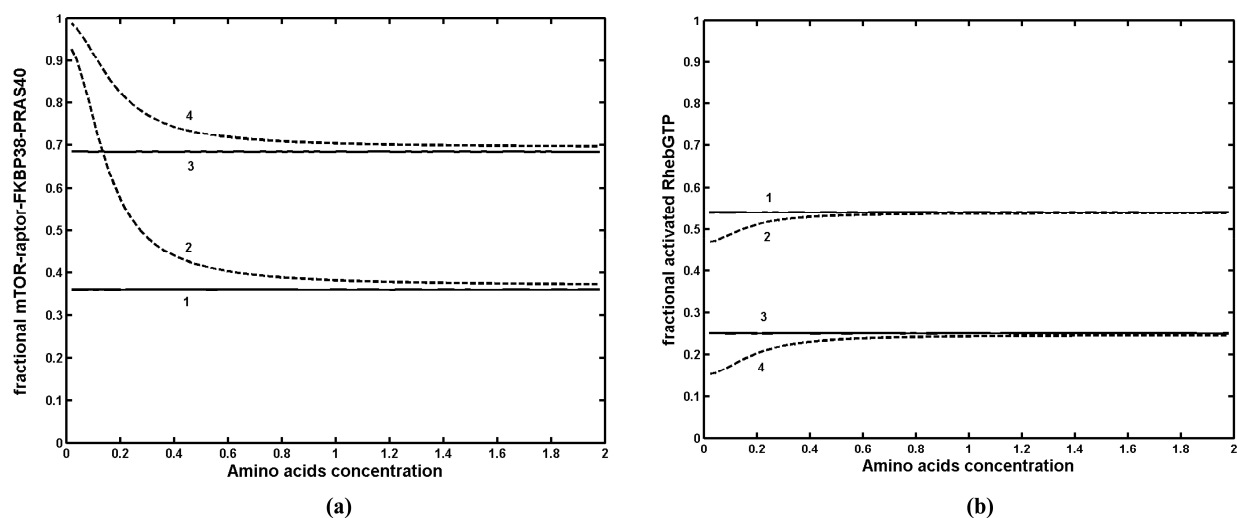


Figure S2: The fractional concentration of **(a)** mTOR-raptor-FKBP38-PRAS40 and **(b)** total RhebGTP with respect to the concentration of amino acids in the presence or absence of insulin input. The input from insulin pathway was fixed at 90%. Curve 1 and curve 2 represent the responses obtained in the presence of insulin input, while curve 3 and curve 4 represent the responses obtained in the absence of insulin input. These responses are shown in the presence (curve 2 and curve 4) and absence (curve 1 and curve 3) of amino acid input '1', which corresponds to the control of localization of RhebGTP by amino acids. The concentration of amino acids mixture is expressed as multiple of reference concentration '1X', which is μM concentration of different amino acids.

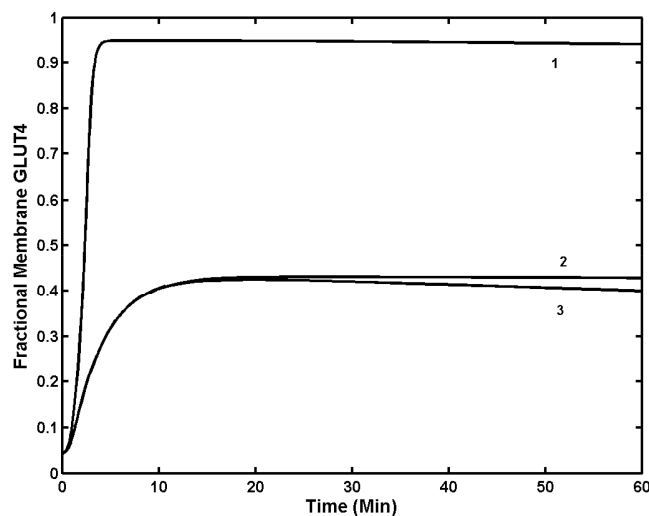
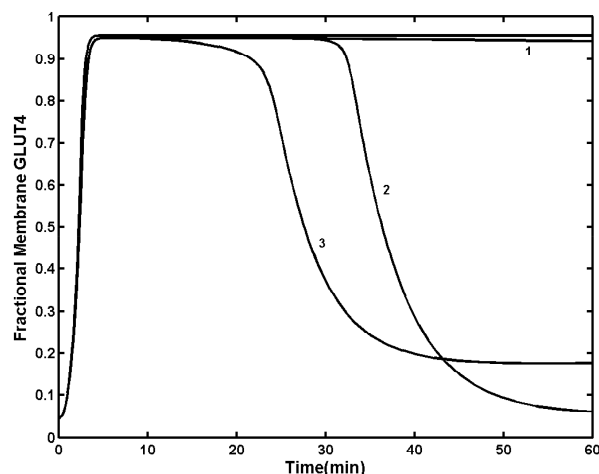
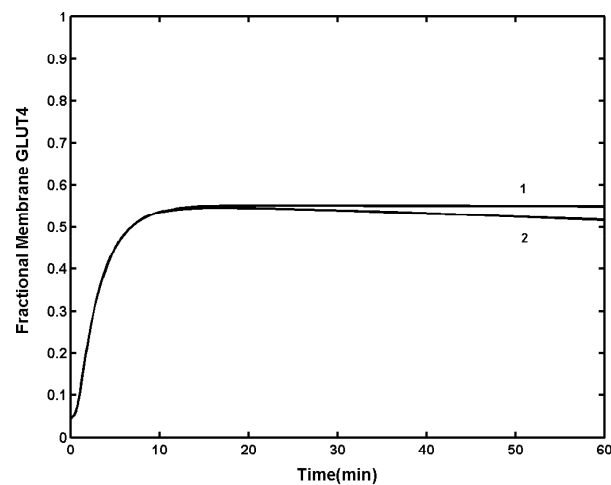


Figure S3: The dynamics of GLUT4 translocation in the presence (curve 1) and absence (curve 2) of positive feedback. Curve 3 represents the response in the absence of positive feedback under higher concentration of amino acids.



(a)



(b)

Figure S4: (a) The dynamics of GLUT4 translocation in the absence of negative feedback. The response was unaffected in the presence or absence of negative feedback at lower concentration of amino acids (curve 1). Under higher concentration of amino acids, the duration of insulin response in the absence of negative feedback increased (curve 2) in comparison to the response in the presence of negative feedback (curve 3). (b) The dynamics of GLUT4 translocation on increasing the strength of negative feedback at lower (curve 2) and higher (curve 3) concentration of amino acids.

Section B: Model equations

mTOR Regulatory Module:

Rate Expressions

$$\begin{aligned}
 v1 &= k1(mTOR)(raptor) \\
 v2 &= k2(mTOR_raptor) \\
 v3 &= k3(mTOR_raptor)(FKBP38) \\
 v4 &= k4(mTOR_raptor_FKBP38)(RhebGTP) \\
 v5 &= k5(mTOR_raptor_FKBP38_RhebGTP) \\
 v6 &= k6(FKBP38_RhebGTP) \\
 v7 &= k7(GTP)(Rheb) \\
 v8 &= k8(Rheb_GTP) \\
 v9 &= k9(TSC) \left[\frac{RhebGTP}{km2 + RhebGTP} \right] \\
 v10 &= k10(GEF) \left[\frac{RhebGDP}{km1 + RhebGDP} \right] \\
 v11 &= k11(RhebGDP) \\
 v12 &= k12(GDP)(Rheb) \\
 v13 &= k13(mTOR_raptor)(PRAS40) \\
 v14 &= k14(mTOR_raptor_PRAS40) \\
 v15 &= k15(mTOR_raptor_PRAS40)(FKBP38) \\
 v16 &= k16(mTOR_raptor_FKBP38)(PRAS40) \\
 v17 &= k17(mTOR_raptor_PRAS40_FKBP38)(RhebGTP) \\
 v18 &= k18(PRAS40p) \\
 v19 &= k19(mTOR_raptor_PRAS40_FKBP38) \\
 v20 &= k20(mTOR_raptor)(df1)(S6K1) \\
 v21 &= k21(S6K1p)(PP2A)
 \end{aligned}$$

Equations:

$$\frac{d(mTOR_raptor)}{dt} = v1 - v2 - v3 + v5 - v13 + v14 + v19 + v22 \quad S1$$

$$\frac{d(mTOR_raptor_FKBP38)}{dt} = v3 - v4 - v16 \quad S2$$

$$\frac{d(FKBP38_RhebGTP)}{dt} = v5 - v6 + v17 \quad S3$$

$$\frac{d(mTOR_raptor_PRAS40)}{dt} = v13 + v17 - v14 - v15 - v22 \quad S4$$

$$\frac{d(mTOR_raptor_FKBP38_PRAS40)}{dt} = v16 + v15 - v17 - v19 \quad S5$$

$$\frac{d(S6K1p)}{dt} = v_{20} - v_{21} \quad S6$$

$$\frac{d(mTOR_raptor_FKBP38_RhebGTP)}{dt} = v_4 - v_5 \quad S7$$

$$\frac{d(PRAS40p)}{dt} = v_{14} - v_{18} \quad S8$$

$$\frac{d(RhebGTP)}{dt} = v_7 - v_8 - v_9 + v_{10} + v_6 \quad S9$$

$$\frac{d(RhebGDP)}{dt} = v_9 - v_{10} - v_{11} + v_{12} \quad S10$$

<i>Species</i>	<i>Total molar balances</i>	<i>S11</i>
<i>Rhebt</i>	$= Rheb + RhebGTP + Rheba + RhebGDP + FKBP38_RhebGTP + mTOR_raptor_FKBP38_RhebGTP$	
<i>PRAS40t</i>	$= PRAS40 + PRAS40p + mTOR_raptor_PRAS40 + mTOR_raptor_FKBP38_PRAS40$	
<i>mTORt</i>	$= mTOR + mTOR_raptor + mTOR_rictor + mTOR_raptor_FKBP38 + mTOR_raptor_FKBP38_RhebGTP + mTOR_raptor_PRAS40 + mTOR_raptor_FKBP38_PRAS40$	
<i>Raptort</i>	$= Raptor + mTOR_raptor + mTOR_raptor_FKBP38 + mTOR_raptor_FKBP38_RhebGTP + mTOR_raptor_PRAS40 + mTOR_raptor_FKBP38_PRAS40$	
<i>Rictort</i>	$= Rictor + mTOR_rictor$	
<i>FKBP38t</i>	$= FKBP38 + FKBP38_RhebGTP + mTOR_raptor_FKBP38 + mTOR_raptor_FKBP38_RhebGTP + mTOR_raptor_FKBP38_PRAS40$	
<i>S6K1t</i>	$= S6K1 + S6K1p$	

(1) Disassociation reaction for *mTOR_rictor*

$$mTOR_rictor = \frac{(mTOR \times rictor)}{kd} \quad S12$$

(2) Amino acids Influence the translocation of RhebGTP

$$ktf = kt \left[\frac{AA^{na}}{AA^{na} + kad^{na}} \right] \quad S13$$

Translocated RhebGTP, Rhebat = *ktf* (RhebGTP)

Rhebat = *Rheba* + *FKBP38_RhebGTP* + *mTOR_raptor_FKBP38_RhebGTP*

Rhebt = *Rheb* + *RhebGTP* + *RhebGDP* + *Rhebat*

Rheba binds instead of *RhebGTP* to *mTOR* inhibitor complex

(3) Amino acids influence the binding of RhebGTP to *mTOR_raptor_FKBP38*

$$v4 = k4 \left[\frac{AA^{na}}{AA^{na} + kad^{na}} \right] (mTOR_raptor_FKBP38)(RhebGTP) \quad S14$$

(4) *mTOR* regulation of phosphatase of S6K1

$$PP2A = PP2A \max \left[\frac{kpp^{npp}}{kpp^{npp} + mTOR_raptor^{npp}} \right] \quad S15$$

(5) *PP2A* regulation of *mTOR_raptor*

$$df1 = \left[\frac{kto^{nto}}{kto^{nto} + PP2A^{nto}} \right] \quad S16$$

(6) Amino acids influence of phosphorylation of PRAS40, which undergo multiple phosphorylation controlled by amino acids and insulin.

$$k14 = k14r \left[\frac{AA^{na}}{AA^{na} + kad^{na}} \right] \left(\frac{Akt}{Akt_T} \right) \quad S17$$

(7) Overexpression of Rheb can overcome the amino acid starvation only if it can bring about multiple effects in mTOR activation similar to the effects brought about by amino acids.

Effect 1: Overexpression of Rheb should bring about localization of RhebGTP

$$ktf = kt \left[\left(\frac{RhebGTP^{nr}}{RhebGTP^{nr} + knr^{nr}} \right) + \left(\frac{AA^{na}}{AA^{na} + kad^{na}} \right) \right] \quad S18$$

Effect 2: Higher concentration of RhebGTP should be able to help in the removal of inhibitor PRAS40 from the complex mTOR_raptor_PRAS40

$$v22 = k22 (Rheba)(mTOR_raptor_PRAS40) \quad S19$$

(8) mTOR-raptor activation by amino acids in the absence of insulin input as observed in CHO cells

$$k19 = k19r \times \left[\frac{AA^{ns}}{AA^{ns} + kaa^{ns}} \right] \quad S20$$

Insulin Regulatory Module:

IRS1-PI3K module

$$\frac{d(IRS1-PI3K)}{dt} = k1 \left[\frac{I^{nH1}}{I^{nH1} + K_1^{nH1}} \right] \left(1 + npf \left(\frac{AKTp}{AKT_t} \right)^{n1} \right) \left[\frac{Kn1f^{nH2}}{Kn1f^{nH2} + PKC - \xi^{nH2}} \right] \left[\frac{Kn2f^{nH4}}{Kn2f^{nH4} + S6K1^{nH4}} \right] - kd1[IRS1-PI3K] \quad S21$$

AKT module

$$\frac{d(AKTp)}{dt} = k2 \left[\frac{IRS1-PI3K^{nH3}}{IRS1-PI3K^{nH3} + K_2^{nH3}} \right] - kd2[AKTp] \quad S22$$

GLUT4 module (Gm: Membrane GLUT4)

$$\frac{d(Gm)}{dt} = kf(Gt - Gm) - kb(Gm) + kfd(Gt - Gm) \left(\frac{AKTp}{AKT_t} \right) \quad S23$$

In Eqn S21-S23, 'I' represents the insulin concentration, AKTp represents the phosphorylated Akt, 'Gm' represents the membrane concentration of GLUT4, 'npf' represents the degree of positive feedback, 'K_{nf}' represents the degree of negative

feedback, 'nH' represents Hills coefficient, 'k' represents the reaction rate constant, 'K' represents the half saturation constant and kd represents the degradation rate. 'kfd' represents the reaction rate for GLUT4 translocation controlled by Akt. 'kb' and 'kf' represents the basal reaction rates for GLUT4 translocation from and towards the membrane, respectively.

Section C: Parameters used for the simulations

mTOR module

The parameters for mTOR complex formation are largely unknown and hence most of the parameters were suitably assumed to be in the range of $1 \text{ min}^{-1} / \text{nM}^{-1} \text{min}^{-1}$ depending on unimolecular or bimolecular reactions [81]. The parameter values were calculated to reproduce the experimentally observed fold change in complex concentrations in the presence of insulin input and to match the steady state and time course profile of mTOR substrate, S6K1 activation.

$k_1 = k_3 = k_4 = k_{15} = k_{16} = k_{17} = 1 \text{ nM}^{-1} \text{min}^{-1}$ (assumed); $k_6 = k_{18} = 1 \text{ min}^{-1}$ (assumed); $k_2 = k_5 = 5 \text{ min}^{-1}$ (calculated); $k_{13} = 10 \text{ nM}^{-1} \text{min}^{-1}$ (calculated); $k_{14} = k_{19r} = 10 \text{ min}^{-1}$ (calculated); $k_{20} = 1.92 \times 10^{-3} \text{ nM}^{-1} \text{min}^{-1}$ (calculated); $k_{21} = 6 \times 10^{-4} \text{ nM}^{-1} \text{min}^{-1}$ (calculated)

Half saturation constant and Hill coefficient (calculated): $k_{ad} = 0.2$; $k_{aa} = 4$; $k_{pp} = 6 \text{ nM}$; $k_{nr} = 200 \text{ nM}$; $k_{to} = 2 \text{ nM}$; $n_a = 0.8$; $n_{pp} = 1.2$; $n_{to} = 4$; $n_r = 2$; $n_s = 4$;

Parameters of Rheb module [82]

$k_7 = 1.3 \times 10^{-1} \text{ nM}^{-1} \text{min}^{-1}$; $k_8 = 1.5 \times 10^{-2} \text{ min}^{-1}$; $k_{11} = 6.6 \times 10^{-3} \text{ min}^{-1}$; $k_{12} = 1.38 \times 10^{-1} \text{ nM}^{-1} \text{min}^{-1}$; $k_9 = 324 \text{ min}^{-1}$; $k_{10} = 300 \text{ min}^{-1}$; $k_{m1} = 700 \text{ nM}$ (calculated); $k_{m2} = 20 \text{ nM}$ (calculated)

Total Concentration (nM)

mTOR_t = 100; Rheb_t = 100; raptort = rictort = FKBP38_t = PRAS40_t = 50 (assumed to be in equal proportion); S6K1 = 50; GTP = 1.8×100000 [83]; GDP = 1.8×10000 [83]; TSC (GAP) = 0.06 [82]; GEF = 0.2 [82]; AKT_t = 0.02 [84]; G_t = 9 [84];

Different modules of insulin signaling pathway:

The parameters were calculated from Giri et al (2004) [80]

$K_1 = 0.3 \text{ nM}$; $K_2 = 5 \times 10^{-6} \text{ nM}$; $k_1 = 6 \times 10^{-5} \text{ nM min}^{-1}$; $k_2 = 6 \times 10^{-2} \text{ nM min}^{-1}$; $k_f = 6.95 \times 10^{-3} \text{ min}^{-1}$; $k_b = 0.1680 \text{ min}^{-1}$; $k_{d1} = 3 \text{ min}^{-1}$; $k_{d2} = 3 \text{ min}^{-1}$; $k_{fd} = 3.6 \text{ min}^{-1}$; $k_{n1f} = 0.01 \text{ nM}$; $k_{n2f} = 7 \text{ nM}$; $n_{pf} = 20$; $n_{H1} = 1.5$; $n_{H2} = 2-4$; $n_{H3} = 3$; $n_{H4} = 1.3$; $n_1 = 1.5$

Section D: Supplementary Results

The analysis was carried out to study the effect of perturbations on the insulin system comprising of only a positive feedback and counteracting feedback loops. In a system comprising of only a positive feedback, the degree of hysteresis increased with an increase in the rate constant for Akt activation by IRS1-PI3K (region between solid and dashed line in Fig. S5a). At higher values of the rate constants, the threshold concentration of insulin required for switching off decreased indicating that the response was tending towards irreversibility (dashed line in Fig. S5a). However, with an increase in the reaction rate for Akt activation, a system with counteracting feedback loops maintained a constant degree of hysteresis (region between solid and dashed line in Fig. S5c). Similarly, on decreasing the degradation rate constant for Akt, the degree of hysteresis was unaffected for a system with counteracting feedback loops (Fig. S5d). However, it increased for a system comprising of only a positive feedback with switching off threshold decreased to a very low value (Fig. S5b). Thus, in the presence of counteracting feedback loops, the system exhibits a robust degree of hysteresis to perturbations compared to a system with only a positive feedback.

Furthermore, the response of the system containing counteracting feedback loops was less affected under perturbation in comparison to a response without any perturbation. To study the effect of perturbation, the ratio of insulin concentration required to switch on the response under perturbation to that without perturbation was defined as a metric (S_{on}). Similarly, the ratio of insulin concentration required to switch off the response under perturbation to that without perturbation was also defined as another metric (S_{off}). A value of the metric being equal to 'one' implies no deviation from the original system. A system with only a positive feedback demonstrated a stronger deviation from the ratio of one with respect to ' S_{off} ', whereas was less affected with respect to ' S_{on} ' (Fig. S6a and Fig. S6b). However, a system with counteracting feedback loops demonstrated a value closer to one for both S_{on} and S_{off} , indicating that the response was closer to the original system without any perturbation (Fig. S6c and Fig. S6d). It can be observed that a system with counteracting feedback loops offers better resistance to perturbation compared to a system with only a positive feedback. Therefore, we concluded that in the presence of counteracting feedback loops, insulin system can

prevent the response from moving into either of regions involving disease states and also maintain a robust insulin response under perturbation.

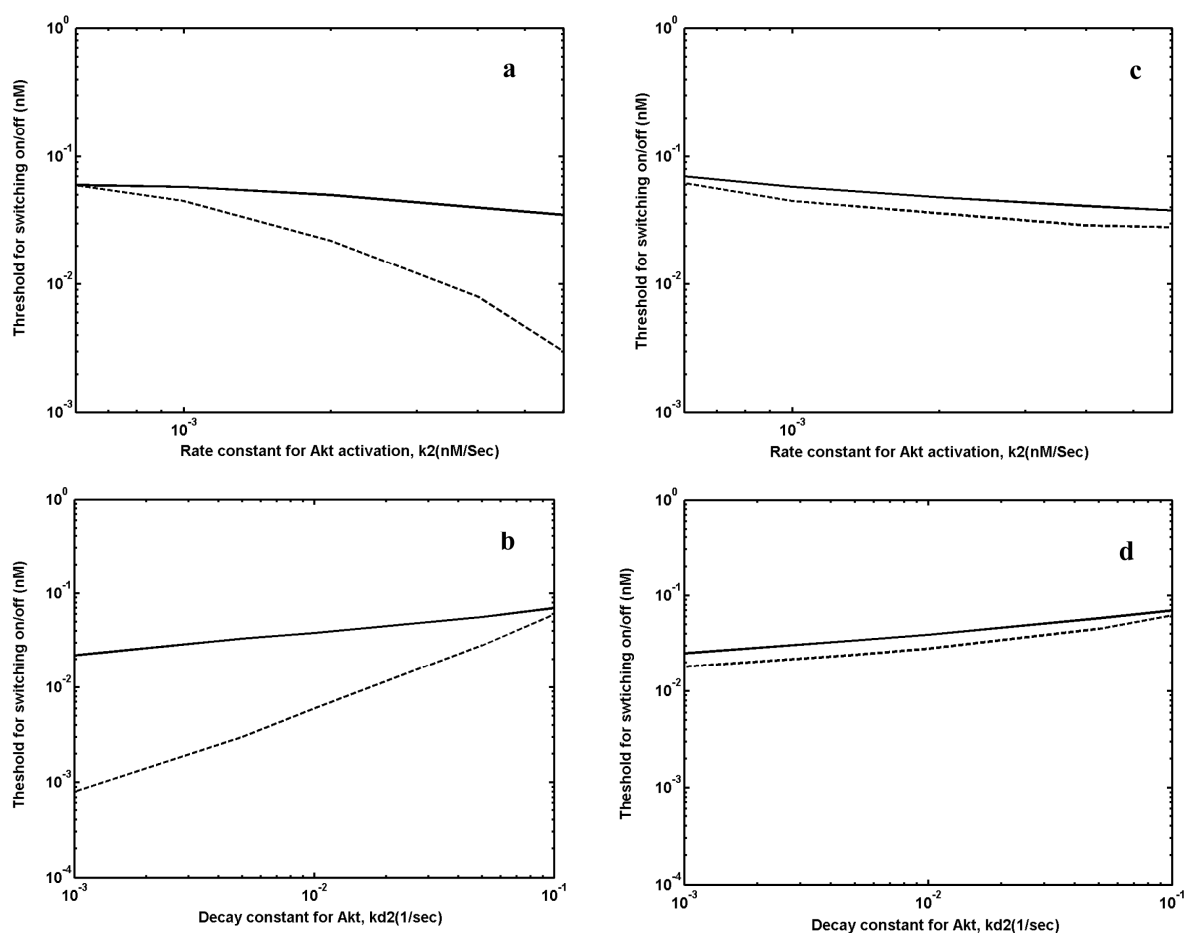


Figure S5: Robust degree of hysteresis in a system with counteracting feedback loops. The variation in the threshold concentration required for switching on (solid line) and switching off (dashed line) the GLUT4 translocation with respect to the variation in the rate constant for Akt activation and decay rate constant of Akt. Cases (a) and (b) represent the system with only a positive feedback, whereas cases (c) and (d) represent the system with counteracting feedback loops.

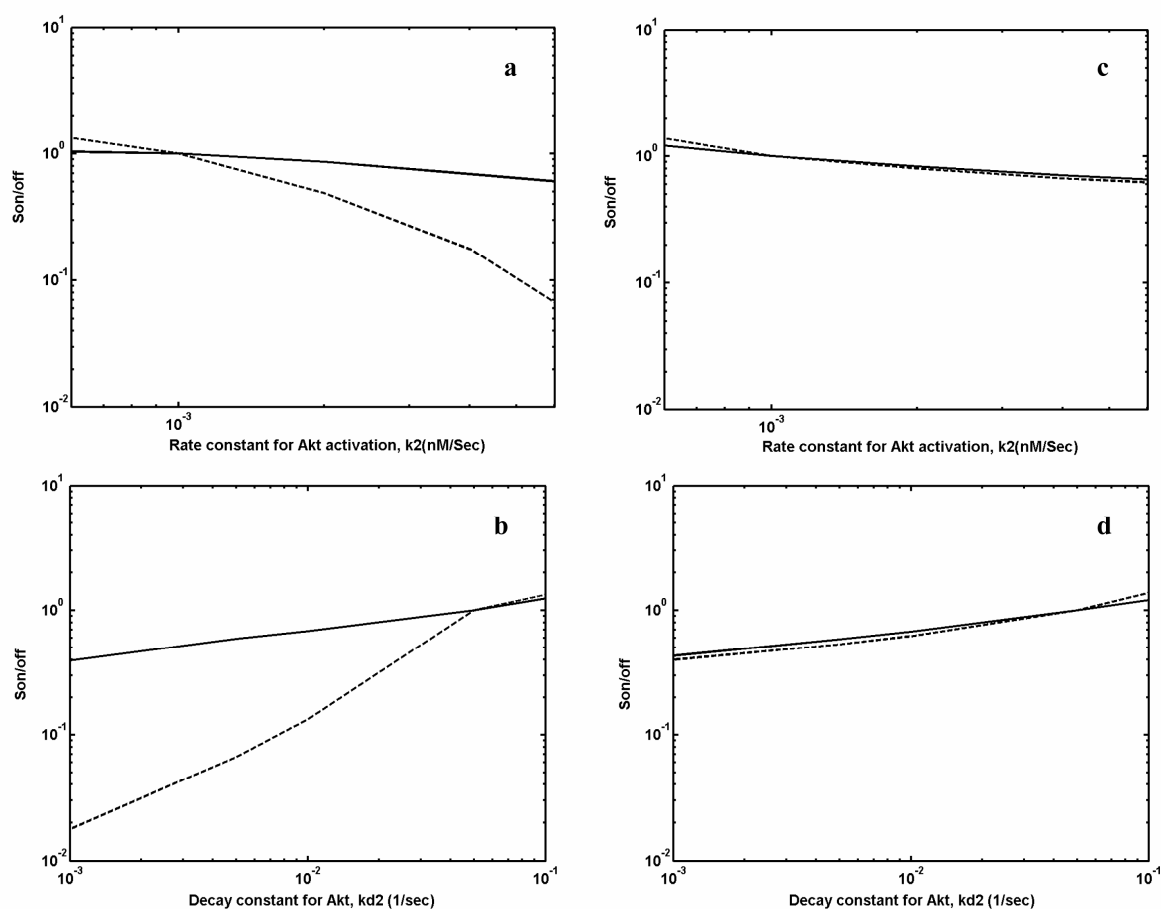


Figure S6: Robust behavior of a system with counteracting feedback loops. The ratio of threshold concentration of insulin required to switch on the response under perturbation to that without perturbation is given by a metric S_{on} (solid line). Similarly, the switching off metric is given by S_{off} (dashed line). The deviation of S_{on} and S_{off} in a system comprising of only a positive feedback with respect to the variation in the **(a)** rate constant for Akt activation and **(b)** decay rate constant of Akt. The deviation of S_{on} and S_{off} in a system comprising of counteracting feedback loops with respect to the variation in the **(c)** rate constant for Akt activation and **(d)** decay rate constant of Akt.

Supplementary References

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