Supplementary information

Experimentally-driven mathematical model to understand the effects of matrix deprivation in breast cancer metastasis

Sayoni Maiti[1], Annapoorni Rangarajan[2*] and Venkatesh Kareenhalli[3*]

[1] Interdisciplinary Programme in Mathematical Sciences, IISc Mathematics Initiative, Indian Institute of Science, Bengaluru, India

[2] Department of Developmental Biology and Genetics, Indian Institute of Science, Bengaluru, India

[3] Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India

[*]co-corresponding authors

[*]Name and address for correspondence: Prof. Annapoorni Rangarajan, Department of Developmental Biology and Genetics, Indian Institute of Science, Bengaluru, Karnataka, India

Email ID: anu@iisc.ac.in

[*]Name and address for correspondence: Prof. Venkatesh Kareenhalli, Department of Chemical Engineering, Indian Institute of Technology Bombay, Mumbai, India

Email ID: venks@iitb.ac.in

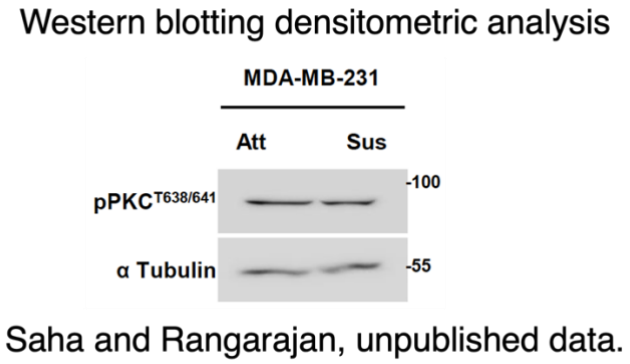
Description of contents

Section I: Supplementary Figures and Table

Section II: Details on mathematical modeling

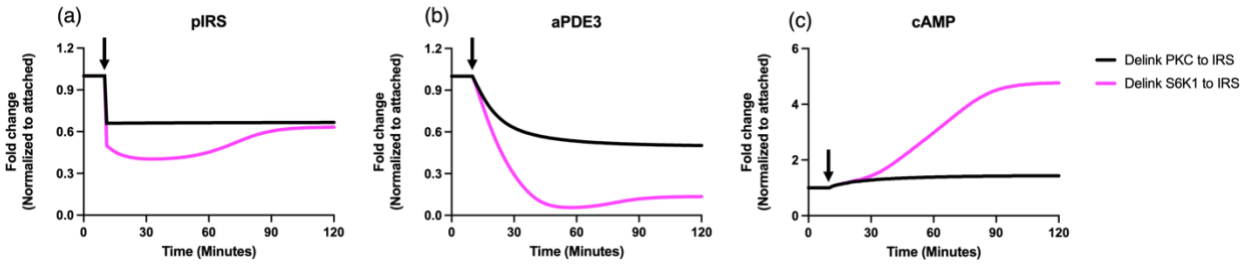
Section I: Supplementary Figures

Supplementary Figure 1



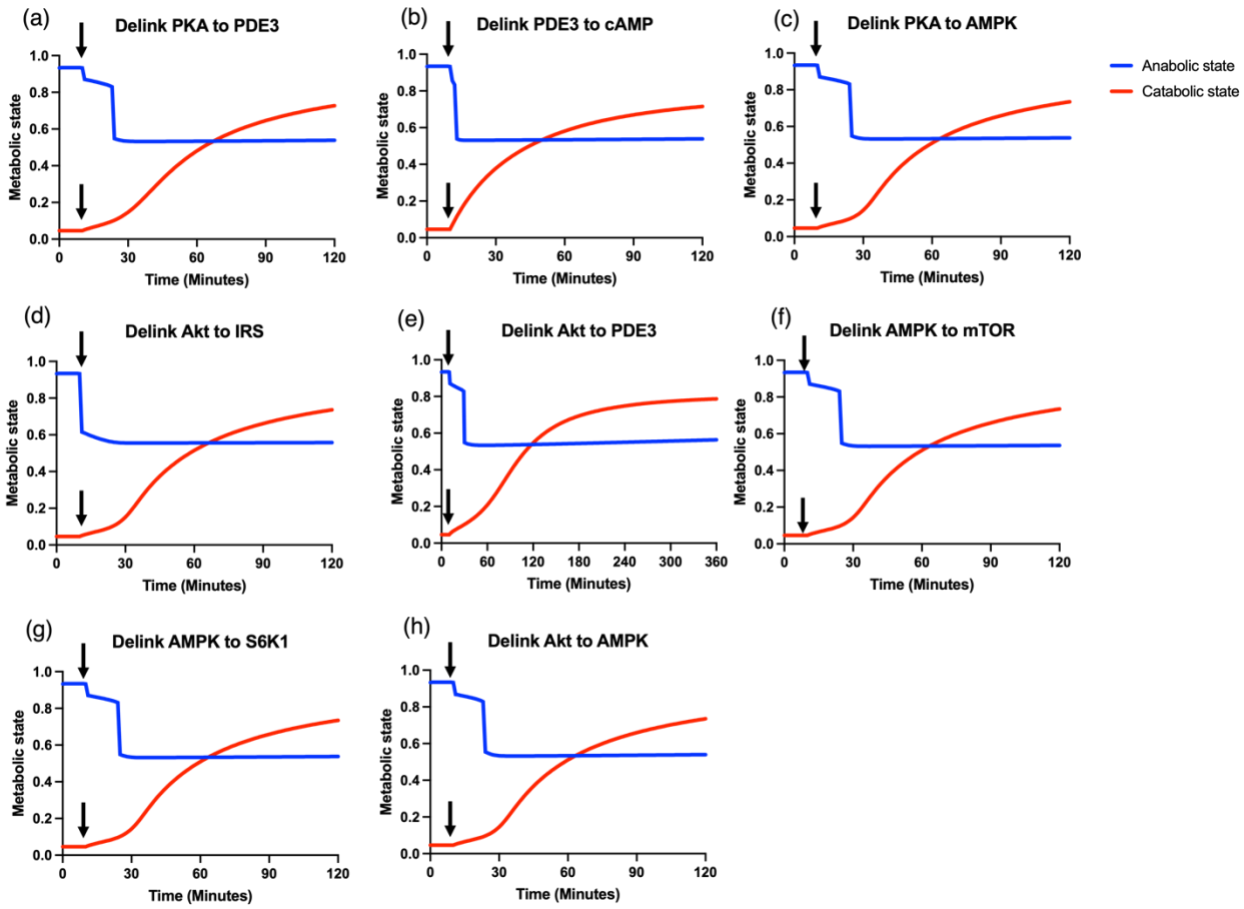
Supplementary Figure 1. Western blot data on pPKC levels when exposed to 8 hours of suspension as compared to attached condition

Supplementary Figure 2



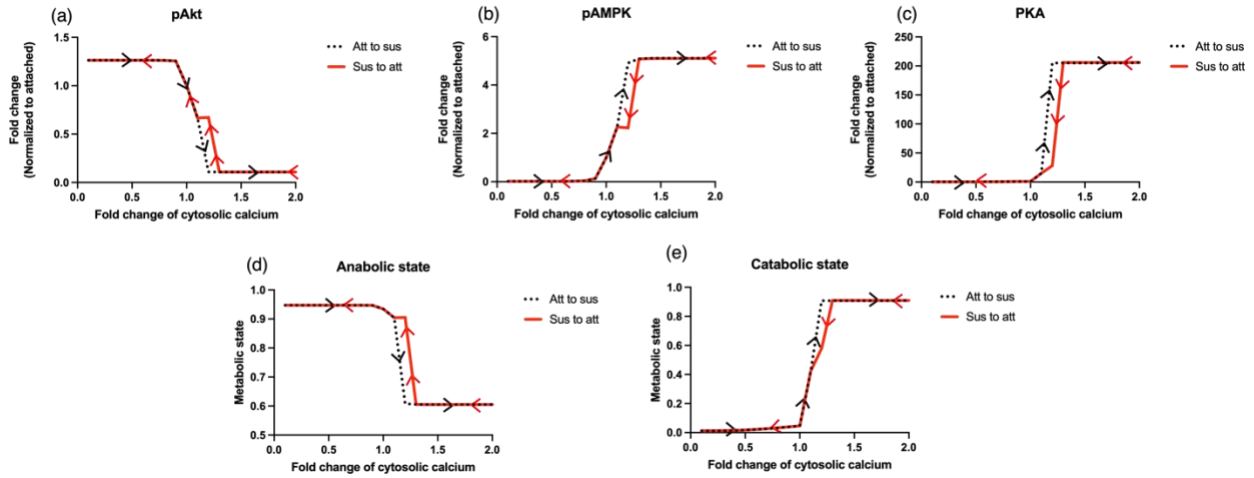
Supplementary Figure 2. Comparing the effects of molecular perturbation on temporal dynamics of molecular players (a) pIRS, (b) aPDE3 and (c) cAMP. The black arrow indicates the time point when matrix deprivation is induced via the spike in cytosolic calcium. The x-axis depicts time in minutes where the first 10 minutes is the matrix-attached state and remaining is the matrix-deprived state. The y-axis depicts the fold change of protein levels, normalized to protein levels in the matrix-attached condition. Black line shows the delink of PKC to IRS and pink line shows the delink of S6K1 to IRS.

Supplementary Figure 3



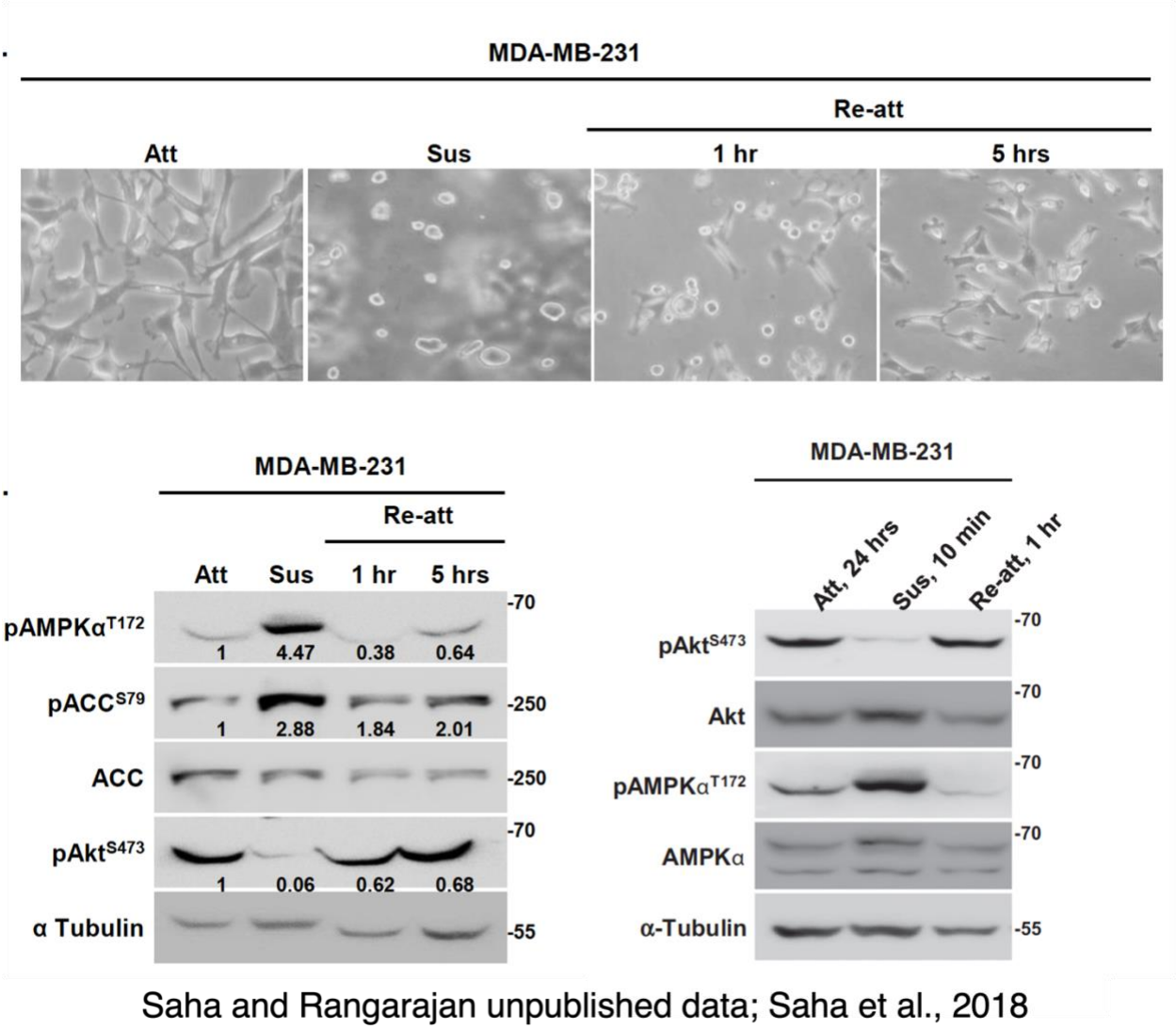
Supplementary Figure 3. Effects of molecular perturbations on the metabolic state. (a) PKA to PDE3, (b) PDE3 to cAMP, (c) PKA to AMPK, (d) Akt to IRS, (e) Akt to PDE3, (f) AMPK to mTOR, (g) AMPK to S6K1 and (h) Akt to AMPK. The black arrow indicates the time point when matrix deprivation is induced via the spike in cytosolic calcium. The x-axis depicts time in minutes, where, first 10 minutes is the matrix-attached state and remaining is the matrix-deprived state. The y-axis depicts the metabolic state. Blue line shows the anabolic state and red line shows the catabolic state.

Supplementary Figure 4



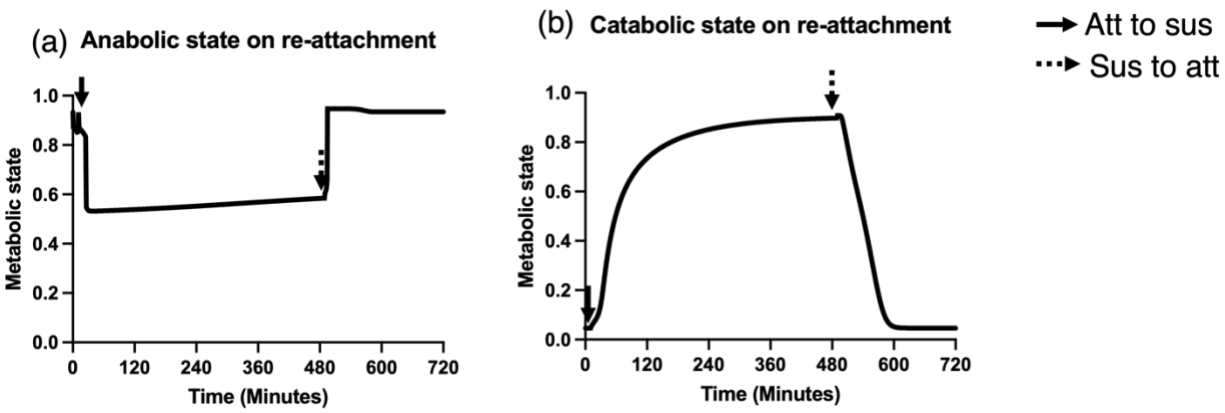
Supplementary Figure 4. Bistability analysis: The steady state responses for (a) pAkt, (b) pAMPK, (c) PKA, (d) Anabolic state and (e) Catabolic state with varying levels of cytosolic calcium. The x-axis depicts the fold change of cytosolic calcium. The y-axis depicts the fold change of protein levels, normalized to protein levels in the matrix-attached condition and the metabolic state. The black dotted line shows transition from attached to suspension state and red solid lines shows transition from suspension to attached state.

Supplementary Figure 5



Supplementary Figure 5. Experimental data on pAMPK and pAkt for re-attachment

Supplementary Figure 6



Supplementary Figure 6. Effects of re-attachment on the metabolic state. (a) Anabolic state on re-attachment **(b)** Catabolic state on re-attachment. The dynamics of metabolic states are plotted for 720 minutes. The solid black arrow indicates the time point when matrix deprivation is induced via the spike in cytosolic calcium. The dashed black arrow indicates the time point when re-attachment is induced by resetting the fold change of cytosolic calcium. The x-axis depicts time in minutes and y-axis depicts the metabolic state.

Supplementary Table 1. Summary of data used for model calibration and validation, and testable prediction

Model calibration (These events capture the key events of matrix detachment and these data were used for model building)	1. The spike in cytosolic calcium due to matrix detachment 2. Levels of pAMPK, pAkt, p-mTOR in matrix-deprived condition compared to matrix-attached.
Model Validation (Model outcomes that conform to known observation)	1. Levels of pS6K1, pPKC, and aPKA in matrix-detached condition as compared to matrix-attached. 2. Metabolic state in matrix-detached condition as compared to matrix-attached. 3. Levels of pAMPK, pAkt, p-mTOR and pS6K1 upon matrix deprivation in AMPK knockdown condition as compared to unperturbed AMPK. 4. Metabolic state upon matrix deprivation in AMPK knockdown condition as compared to unperturbed AMPK.
Testable predictions	1. Levels of pIRS, aPI3K, sGLUT1, IP3, DAG, cAMP and aPDE3 in matrix-attached vs. matrix-deprived conditions. 2. Levels of pAkt, aPKA, pAMPK and metabolic state in matrix-attached vs. matrix-deprived conditions upon delinking several feedbacks/crosstalks (DAG to PKC, PKC to IRS, S6K1 to IRS, cAMP to PKA, AMPK to Akt, PKA to PDE3, PKA to AMPK, PDE3 to cAMP, Akt to PDE3, AMPK to mTOR, AMPK to S6K1, Akt to AMPK, Akt to IRS) 3. Effect of AMPK knockdown on Levels of pIRS, aPI3K, pPKC, sGLUT1, IP3, DAG, aPKA, cAMP and aPDE3 upon matrix deprivation

Section II: Details on Mathematical modeling

Supplementary Table 2. List of variables (molecular players) in the breast cancer mathematical model

Protein ID	Protein Species	Description
1.	IRS pIRS	Insulin receptor substrate Phosphorylated insulin receptor substrate
2.	PI3K PI3Ka	Phosphoinositide 3 kinase Activated Phosphoinositide 3 kinase
3.	Akt pAkt	Protein kinase B Phosphorylated protein kinase B
4.	PKC pPKC	Protein kinase C Phosphorylated protein kinase C
5.	GLUT1c GLUT1s	Glucose transporter type 1 (cytosol) Glucose transporter type 1 (surface)
6.	mTOR p-mTOR	Mammalian target of rapamycin Phosphorylated mammalian target of rapamycin
7.	S6K1 pS6K1	Ribosomal protein S6 kinase 1 Phosphorylated ribosomal protein S6 kinase 1
8.	Ccal	Cytosolic calcium
9.	IP3	Inositol tri-phosphate
10.	DAG	Diacyl-glycerol
11.	aPKA	Active Protein kinase A
12.	cAMP	Cyclic adenosine monophosphate
13.	aPDE3a	Active Phosphodiesterase 3

14.	pAMPK	Phosphorylated AMP-activated protein kinase
15.	CaMKKβ	Calcium/calmodulin dependent protein kinase kinase beta

Supplementary Table 3. List of parameter values

Sr. No.	Parameter values	Values	Units	References
1	**Dynamics of IRS**			
	k2f	141.254*10	/min	Calibrated
	k2b	3533330	/min	Somvanshi et al., 2019
	k21f	9178.23*12	/min	Calibrated
2.	**Dynamics of PI3K**			
	K3f	226300*6	/min	Calibrated
	K3b	1493960	/min	Somvanshi et al., 2019
3.	**Dynamics of Akt**			
	k4f	513844*7.5	/min	Calibrated
	k4b	320822	/min	Somvanshi et al., 2019
4.	**Dynamics of PKC**			
	K5f	1337290/2	/min	Somvanshi et al., 2019
	K5b	1629.22*20	/min	Somvanshi et al., 2019
5.	**Dynamics of GLUT1**			
	K6f	0.06308	/min	Somvanshi et al., 2019
	K6b	0.212	/min	Somvanshi et al., 2019
6.	**Dynamics of mTOR**			

	K7f1	856353/2	/min	Somvanshi et al., 2019
	K7b	1430390*1.5	/min	Somvanshi et al., 2019
	K7f	856353*5	/min	Calibrated
7.	**Dynamics of S6K1**			
	K8f	1.5E-3	/min	Somvanshi et al., 2019
	K8b	5E-4	/min	Somvanshi et al., 2019
8.	**Dynamics of Ccal**			Somvanshi et al., 2019
	Ccal (attached)	0.002	AU	Somvanshi et al., 2019
	Ccal (Suspension)	0.004	AU	Calibrated
9.	**IP3**			
	ks	$1x10^{-4}x0.01$	μM	Calibrated
10.	**DAG**			
	Vi	120	/min	Somvanshi et al., 2019
	Vf	60	/min	Somvanshi et al., 2019
	Bd	0.5*60	/min	Somvanshi et al., 2019
11.	**cAMP**			
	kc1	$2*10^{-6}$	μM/min	Somvanshi et al., 2019
	kcm1	$25*10^{-12}$	M	Somvanshi et al., 2019
	kc2	1.5	/min	Somvanshi et al., 2019
	kcm2	1	AU	Somvanshi et al., 2019
	cqi	2	AU	Somvanshi et al., 2019

12.	**PKA**			
	Va1	0.9	/min	Somvanshi et al., 2019
	Va2	8	/min	Somvanshi et al., 2019
	kcamp1	$2*3.2*10^{-6}$	μM	Somvanshi et al., 2019
	kcamp2	$4*3.2*10^{-6}$	μM	Somvanshi et al., 2019
	tPKA	$0.6*10^{-3}$	μM	Somvanshi et al., 2019
13.	**Dynamics of PDE3**			
	kPDE3	1.5*1.1	/min	Calibrated
	tPDE3	5	AU	Somvanshi et al., 2019
14.	**36. Dynamics of AMPK**			
	tAMPK	1	AU	Somvanshi et al., 2019
	AMP_ATP	1	AU	Somvanshi et al., 2019
	kam1	1.5	mM	Calibrated
	Kam2	2.25	mM	Somvanshi et al., 2019

Supplementary Table 4. Initial conditions of all variables (molecular players)

Protein ID	Protein Species	Initial conditions
1.	IRS pIRS	10 0
2.	PI3K PI3Ka	10 0
3.	Akt pAkt	10 0
4.	PKC	10

	pPKC	0
5.	GLUT1c GLUT1s	10 0
6.	mTOR pmTOR	10 0
7.	S6K1 pS6K1	10 0
8.	IP3	0.1
9.	Ccal (Attached)	0.0022
10.	Ccal (suspension)	0.0044
11.	DAG	1
12.	PKA	$8x10^{-6}$
13.	cAMP	$3.16x10^{-6}$
14.	PDE3a	1
15.	pAMPK	0.16
16.	CaMKKβ	-

The interplay of the above proteins captures the dynamics of breast cancer cells in matrix attached and matrix deprived conditions. However, the complete mathematical model used in this study is adapted from Somvanshi et al., which consists of additional variables. The prior model can be referred for further details (Somvanshi et al., 2019).

Model equations

A. Forward-backward reactions and essential Hill functions

1. Dynamics of IRS

The mass balance for activation of IRS is written as:

$$\mathrm{IR_p^a} + \mathrm{IRS} \underset{v_{2b}}{\overset{v_{2f}}{\rightleftharpoons}} \mathrm{IRS_p^a} + \mathrm{IR} \quad (1)$$

The rate of activation of IRS by active IR can be written as:

$$v_{2f} = k_{2f} + k_{21f} * \mathrm{IR_p} * \mathrm{IRS} * \mathrm{pAkt_{ptv}IRS} * \mathrm{pPKC_{ntv}IRS} \quad (2)$$

pAkt has a positive effect on IRS and pPKC has a negative effect on IRS. The respective hill functions are written in the following manner:

Effect of pAkt on IRS

$$\mathrm{pAkt}_{\mathrm{ptv}}\mathrm{IRS} = \left(\frac{\mathrm{pAkt}^2}{\mathrm{pAkt}^2+7.1^2}\right) \quad (3)$$

Effect of pPKC on IRS

$$\mathrm{pPKC}_{\mathrm{ntv}}\mathrm{IRS} = \left(\frac{7}{7+\left(\frac{\mathrm{pPKC}}{1.5}\right)}\right) \quad (4)$$

IRS is phosphorylated and it is activated.

The rate of deactivation of pIRS is written as:

$$\mathrm{v}_{2\mathrm{b}} = \mathrm{k}_{2\mathrm{b}} * \mathrm{IRS}_{\mathrm{p}}^{\mathrm{a}} * (1 + \mathrm{pS6K1}_{\mathrm{ntv}}\mathrm{pIRS}) \quad (5)$$

pS6K inhibits pIRS. Since this negative feedback is included in deactivated term, it is written as a positive hill function as follows:

Effect of S6K1 on pIRS

$$\mathrm{pS6K1}_{\mathrm{ntv}}\mathrm{pIRS} = 0.2 * \left(\frac{\mathrm{pS6K1}^4}{\mathrm{pS6K1}^4+6^4}\right) \quad (6)$$

The mass balance, forward and backward reactions of the remaining proteins can be understood similarly.

The forward and backward reactions for dynamics of IR are v_{1f} an v_{1b} (Somvanshi et al., 2019).

2. Dynamics of PI3K

$$\mathrm{v}_{3\mathrm{f}} = \mathrm{k}_{3\mathrm{f}} * \mathrm{PI3K} * \mathrm{pIRS} \quad (7)$$

$$\mathrm{v}_{3\mathrm{b}} = \mathrm{k}_{3\mathrm{b}} * \mathrm{aPI3K} \quad (8)$$

3. Dynamics of Akt

$$\mathrm{v}_{4\mathrm{f}} = \mathrm{k}_{4\mathrm{f}} * \mathrm{Akt} * \mathrm{aPI3K} \quad (9)$$

$$\mathrm{pAMPK}_{\mathrm{ntv}}\mathrm{pAkt} = \left(\frac{\mathrm{pAMPK}^2}{\mathrm{pAMPK}^2+0.28^2}\right) \quad (10)$$

$$\mathrm{v}_{4\mathrm{b}} = \mathrm{k}_{4\mathrm{b}} * \mathrm{pAkt} * (1 + 49 * \mathrm{pAMPK}_{\mathrm{ntv}}\mathrm{pAkt}) \quad (11)$$

4. Dynamics of PKC

$$\mathrm{DAG}_{\mathrm{ptv}}\mathrm{PKC} = \left(\frac{\mathrm{DAG}^3}{\mathrm{DAG}^3+3.74^3}\right) \quad (12)$$

$$\mathrm{FFA}_{\mathrm{ptv}}\mathrm{PKC} = 1 + 0.5 * \left(\frac{\mathrm{FFA}^3}{\mathrm{FFA}^3+1.5^3}\right) \quad (13)$$

$$\mathrm{v}_{5\mathrm{f}} = \mathrm{k}_{5\mathrm{f}} * \mathrm{DAG}_{\mathrm{ptv}}\mathrm{PKC} * \mathrm{FFA}_{\mathrm{ptv}}\mathrm{PKC} * \mathrm{aPI3K} * \mathrm{PKC} \quad (14)$$

$$v_{5b} = k_{5b} * pPKC \quad (15)$$

5. Dynamics of GLUT1

$$v_{6f} = k_{6f} * (0.9 * pAkt + 0.1 * pPKC) * GLUT1c \quad (16)$$

$$v_{6b} = k_{6b} * GLUT1s \quad (17)$$

6. Dynamics of mTOR

$$AA_{ptv}mTOR = 2.5 * \left(\frac{AA^3}{AA^3+0.75^3}\right) \quad (18)$$

$$v_{7f} = k_{7f} * mTOR * pAkt + k_{7f1} * (1 + AA_{ptv}mTOR) * mTOR$$

$$AMPK_{ntv}mTOR = \left(\frac{AMPK^{0.5}}{AMPK^{0.5}+0.3^{0.5}}\right) \quad (19)$$

$$v_{7b} = k_{7b} * pmTOR * (1 + 1.5 * AMPK_{ntv}mTOR) \quad (20)$$

7. Dynamics of S6K1

$$AMPK_{ntv}S6K1 = 7.5 * \left(\frac{0.26^2}{0.26^2+AMPK^2}\right) \quad (21)$$

$$v_{8f} = k_{8f} * aPDK1 * pmTOR * PP2A_{ntv}S6K1 * AMPK_{ntv}S6K1 * S6K1 \quad (22)$$

$$v_{8b} = k_{8b} * PP2A * pS6K1 \quad (23)$$

8. IP3

$$kc1p = kc1 + csi * \left(\frac{PKA^4}{PKA^4+k_S^4}\right) \quad (24)$$

9. Ccal

The ordinary differential equation of Ccal is set to zero because the dynamics of Ccal are not captured in this study. Instead the initial conditions of Ccal are directly used as the input to the system.

10. DAG

$$kc2p = 1 - \frac{PKA^4}{k_S^4+PKA^4} \quad (25)$$

12. cAMP

$$kck = 1 + 0.5 * \left(cqi * \left(\frac{C_{Cal}^3}{0.00216_{cal}^3+C_{Cal}^3}\right)\right) \quad (26)$$

13. PDE3

$$Akt_{ptv}PDE3 = 0.3 * \left(\frac{Akt^6}{Akt^6+6^6}\right) \quad (27)$$

$$PKA_L = PKA/8e^{-6} \quad (28)$$

$$\mathrm{PKA_{ptv}PDE3} = \left(\frac{\mathrm{PKA_L}}{\mathrm{PKA_L}+12}\right) \quad (29)$$

14. AMPK

$$\mathrm{Akt_{ntv}AMPK} = 4 * \left(\frac{\mathrm{Akt}^4}{\mathrm{Akt}^4+4^4}\right) \quad (30)$$

$$\mathrm{PKA_{ntv}AMPK} = \left(\frac{3^2}{3^2+\mathrm{PKA_L^2}}\right) \quad (31)$$

15. CAMKKB

$$\mathrm{CAMKKB} = \ \mathrm{Ccal_{ptv}CAMKKB} = (1 + 37 * \left(\frac{\mathrm{Ccal}^{10}}{\mathrm{Ccal}^{10}+0.003^{10}}\right) \quad (32)$$

$$\mathrm{CAMKKB_{hf}} = 40 * \left(\frac{\mathrm{CAMKKB}^4}{\mathrm{CAMKKB}^4+7.1^4}\right) \quad (33)$$

B. Mass balance equations (Ordinary differential equations)

$$\frac{\mathrm{dIRS}}{\mathrm{dt}} = \mathrm{v_{2b}} - \mathrm{v_{2f}} \quad (34)$$

$$\frac{\mathrm{dpIRS}}{\mathrm{dt}} = \mathrm{v_{2f}} - \mathrm{v_{2b}} \quad (35)$$

$$\frac{\mathrm{dPI3K}}{\mathrm{dt}} = \mathrm{v_{3b}} - \mathrm{v_{3f}} \quad (36)$$

$$\frac{\mathrm{dPI3Ka}}{\mathrm{dt}} = \mathrm{v_{3f}} - \mathrm{v_{3b}} \quad (37)$$

$$\frac{\mathrm{dAkt}}{\mathrm{dt}} = \mathrm{v_{4b}} - \mathrm{v_{4f}} \quad (38)$$

$$\frac{\mathrm{dpAkt}}{\mathrm{dt}} = \mathrm{v_{4f}} - \mathrm{v_{4b}} \quad (39)$$

$$\frac{\mathrm{dPKC}}{\mathrm{dt}} = \mathrm{v_{5b}} - \mathrm{v_{5f}} \quad (40)$$

$$\frac{\mathrm{dpPKC}}{\mathrm{dt}} = \mathrm{v_{5f}} - \mathrm{v_{5b}} \quad (41)$$

$$\frac{\mathrm{dGLUT1c}}{\mathrm{dt}} = \mathrm{v_{6b}} - \mathrm{v_{6f}} \quad (42)$$

$$\frac{\mathrm{dGLUT1s}}{\mathrm{dt}} = \mathrm{v_{6f}} - \mathrm{v_{6b}} \quad (43)$$

$$\frac{\mathrm{dmTOR}}{\mathrm{dt}} = \mathrm{v_{7b}} - \mathrm{v_{7f}} \quad (44)$$

$$\frac{\mathrm{dpmTOR}}{\mathrm{dt}} = \mathrm{v_{7f}} - \mathrm{v_{7b}} \quad (45)$$

$$\frac{\mathrm{dS6K1}}{\mathrm{dt}} = \mathrm{v_{8b}} - \mathrm{v_{8f}} \quad (46)$$

$$\frac{\mathrm{dpS6K1}}{\mathrm{dt}} = \mathrm{v_{8f}} - \mathrm{v_{8b}} \quad (47)$$

$$\frac{dIP3}{dt} = \frac{Ccal*PLC}{kc1p+Ccal} - bi * IP3 \quad (48)$$

$$\frac{dDAG}{dt} = v_i * \left(\frac{0.5*Ccal*PLC}{Kc2p+0.1*Ccal}\right) + v_f * FFA - bd * DAG \quad (49)$$

$$\frac{dcAMP}{dt} = kck * kc1 * \left(1 + \left(\frac{GlnP^2}{kcm1^2+GlnP^2}\right)\right) - kc2 * \left(\frac{PDEa^2}{kcm2^2+PDE3a^2}\right) * cAMP - 2 * \frac{dPKA}{dt} \quad (50)$$

$$\frac{dPKA}{dt} = Va_1 * \left(\frac{cAMP^3}{k^3+cAMP^3}\right) * (tPKA - PKA) - Va_2 * PKA * \left(\frac{k^1}{k^1+cAMP^1}\right) \quad (51)$$

$$\frac{dPDE3a}{dt} = Akt_{ptv}PDE3 * (tPDE3 - PDE3a) - (kPDE3 * PKA_{ptv}PDE3 * PDE3a) \quad (52)$$

$$\frac{dpAMPK}{dt} = k_{am1} * AMP_{ATP} * CAMKKB_{hf} * PKA_{ntv}AMPK * (tAMPK - pAMPK) - k_{am2} * pAMPK * Akt_{ntv}AMPK \quad (53)$$

The additional proteins, secondary messengers and their ordinary differential equations can be referred to from the previous model (Somvanshi et al., 2019).