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Supplementary Materials for

A modelling-experimental approach reveals IRS dependent regulation of AMPK by Insulin

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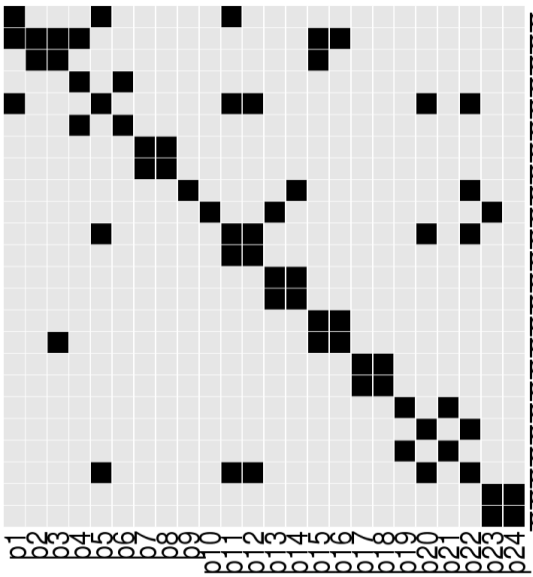

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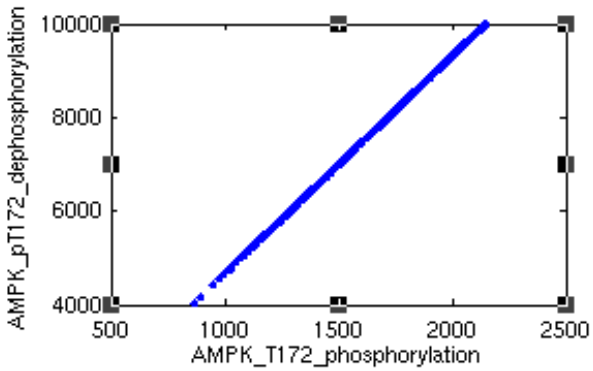
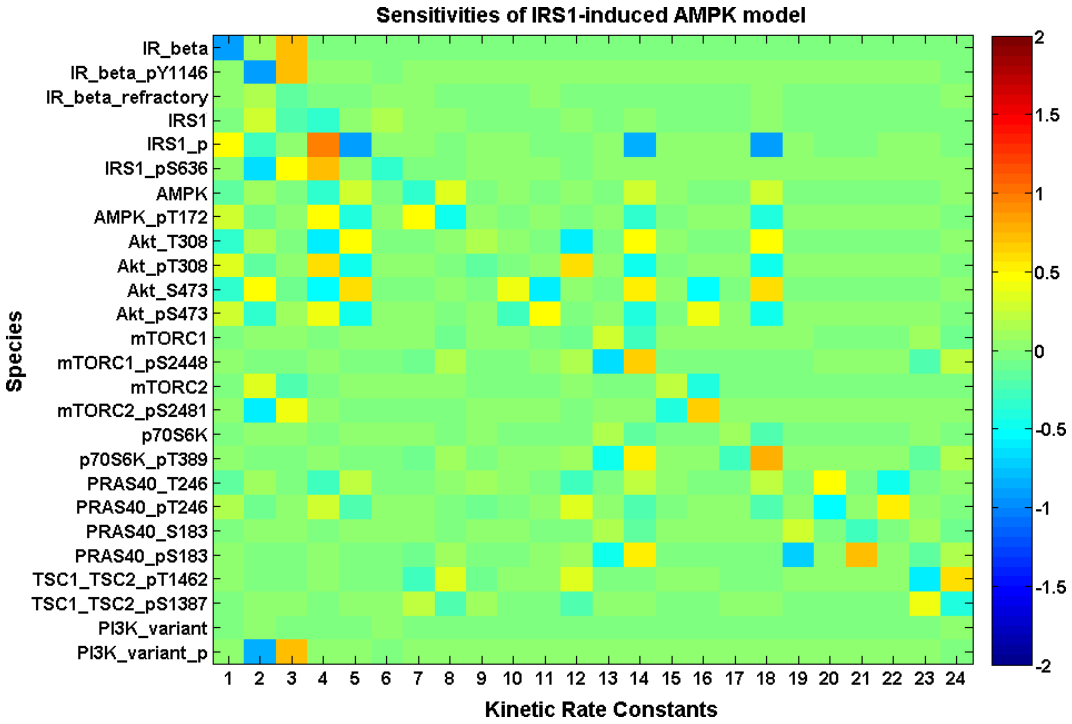


C

Parameter Name (AMPK induced by IR-Beta Model) (Kinetic rate constants [min^{-1}])	*Calibration Rounds (mean, sd, %) Round 1*	*Round 2*	*Value*
p1=IR_beta_phosphorylation_by_Insulin	0.127895 ± 1.87856e-05 (0%)	fixed	0.127892
p2=IR_beta_pY1146_dephosphorylation	0.421933 ± 6.72417e-05 (0%)	fixed	0.421835
p3=IR_beta_ready	0.0613301 ± 1.39536e-05 (0%)	fixed	0.0613094
p4=IRS1_phosphorylation_by_IR_beta_pY1146	0.00506354 ± 9.74753e-07 (0%)	fixed	0.00506217
p5=IRS1_p_phosphorylation_by_p70S6K_pT389	2166.01 ± 7.00991 (0%)	fixed	2164.5
p6=IRS1_pS636_dephosphorylation	0.0150341 ± 7.28345e-06 (0%)	fixed	0.0150241
p7=AMPK_T172_phosphorylation	1726.2 ± 342.304 (20%)	fixed	2145.27
p8=AMPK_pT172_dephosphorylation	8034.56 ± 1593.25 (20%)	9985.01 ± 1.63853 (0%)	9983.42
p9=Akt_pT308_dephosphorylation	0.00255589 ± 4.5546e-07 (0%)	fixed	0.00255584
p10=Akt_pS473_dephosphorylation	0.00624137 ± 2.30368e-07 (0%)	fixed	0.00624136
p11=Akt_S473_phosphorylation_by_mTORC2_pS2481_n_IRS1_p	14.2914 ± 0.0471528 (0%)	fixed	14.2858
p12=Akt_T308_phosphorylation_by_IRS1_p	7.16228 ± 0.018444 (0%)	fixed	7.16055
p13=mTORC1_pS2448_dephosphorylation_by_TSC1_TSC2_pS1387	0.010212 ± 2.66286e-06 (0%)	fixed	0.0102132
p14=mTORC1_S2448_activation_by_Amino_Acids	0.00414963 ± 8.15228e-07 (0%)	fixed	0.00414999
p15=mTORC2_pS2481_dephosphorylation	0.0204082 ± 2.94073e-06 (0%)	fixed	0.0204037
p16=mTORC2_S2481_phosphorylation_by_PI3K_variant_p	0.385463 ± 3.02274e-05 (0%)	fixed	0.385417
p17=p70S6K_pT389_dephosphorylation	0.0099792 ± 1.57728e-06 (0%)	fixed	0.00997852
p18=p70S6K_T389_phosphorylation_by_mTORC1_pS2448	0.00171206 ± 7.63572e-08 (0%)	fixed	0.00171203
p19=PRAS40_pS183_dephosphorylation	2.79774 ± 0.00410708 (0%)	fixed	2.80277
p20=PRAS40_pT246_dephosphorylation	1.61657 ± 0.00185418 (0%)	fixed	1.61721
p21=PRAS40_S183_phosphorylation_by_mTORC1_pS2448	0.224023 ± 0.00032552 (0%)	fixed	0.224421
p22=PRAS40_T246_phosphorylation_by_Akt_pT308	0.136366 ± 0.000154248 (0%)	fixed	0.136417
p23=TSC1_TSC2_S1387_phosphorylation_by_AMPK_pT172	0.0232386 ± 2.30029e-06 (0%)	fixed	0.0232365
p24=TSC1_TSC2_T1462_phosphorylation_by_Akt_pT308	0.00994593 ± 1.45625e-06 (0%)	fixed	0.00994482
p25=PI3K_variant_p_dephosphorylation	assumed		10
p26=PI3K_variant_phosphorylation_by_IR_beta_pY1146	assumed		0.01

Fig. S1. Identifiability and parameter estimation for the IR-beta-induced AMPK model (hypothesis No.2). (A) Identifiability analysis for the IR-beta-induced AMPK model indicated non identifiability issues for the parameters regulating AMPK dynamics (p7, p8). (B) Correlation plot between the two parameters (p7, p8) confirms non-identifiability of the parameters. (C) Finally, the first round of the parameter estimation reported a standard deviation percentage higher than 5% for the two parameters. P8 was further recalibrated in a second round in which it was correctly identified.

A



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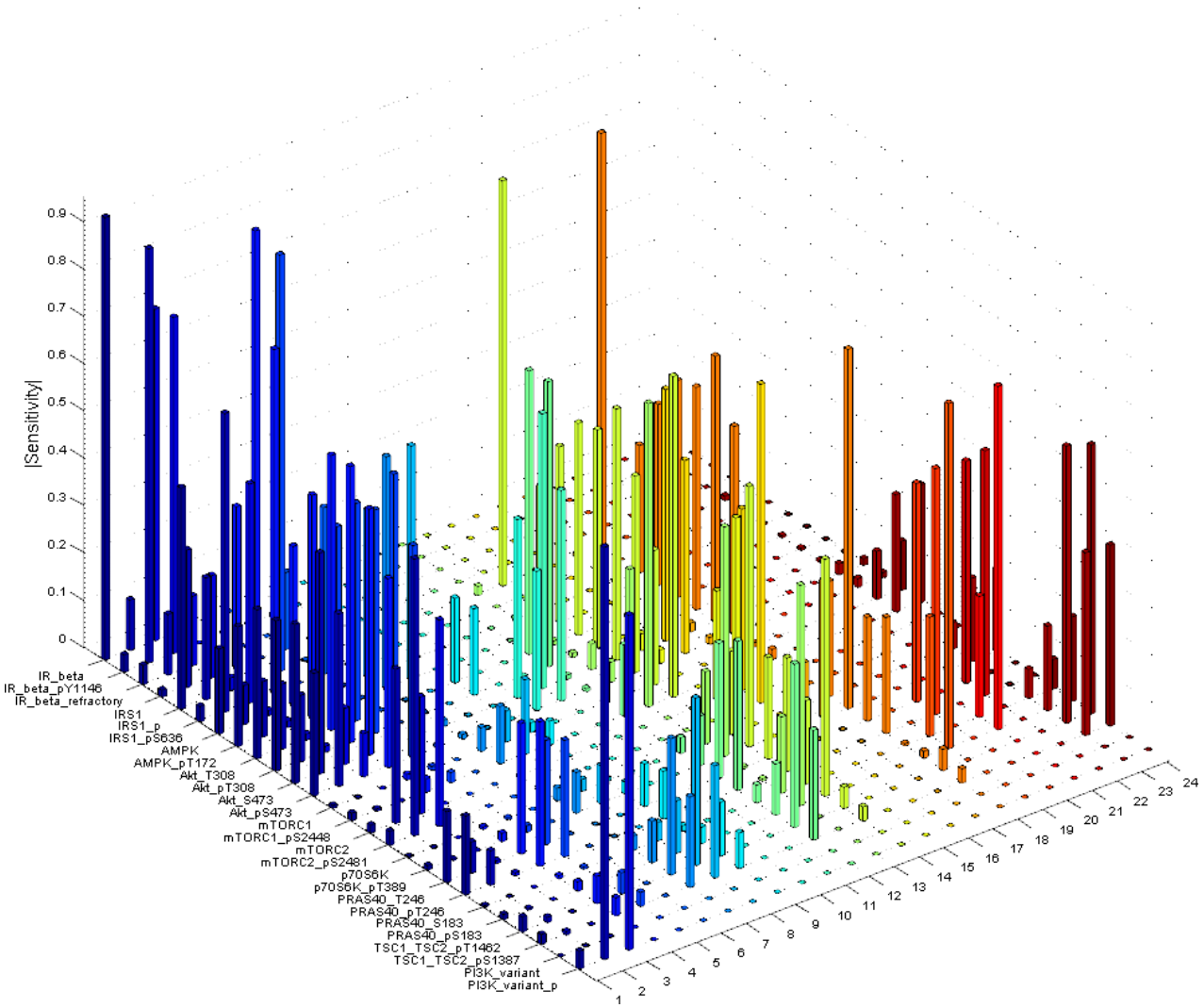
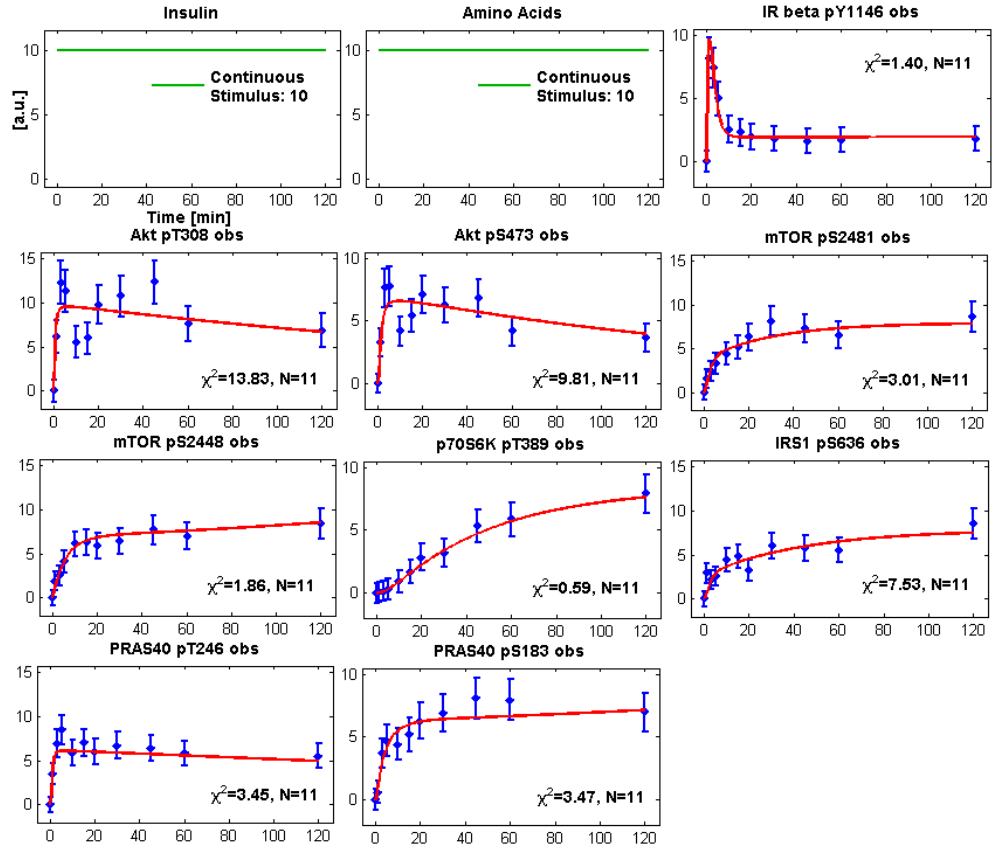


Fig. S2. Sensitivity analysis for the IRS1-induced AMPK model (hypothesis No.3). (A) 2-dimensional sensitivity analysis between the estimated kinetic rate constants versus the protein concentrations. The table shows that all the parameters are essential for describing the model and the IRS1-p regulation is the most important as it mediates the insulin signalling as well as the p70-S6K-negative feedback loop. Colours indicate sensitivity levels. (B) 3-dimensional sensitivity analysis as normalised in [0,1]. Colours distinguish different estimated kinetic rate constant parameters.

A



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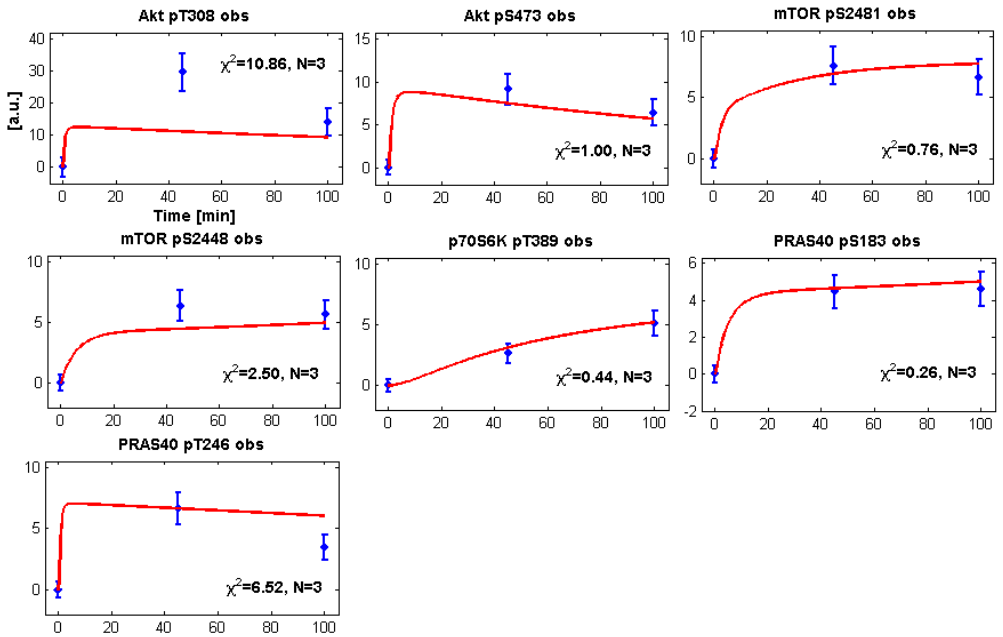

Fig. S3. Additional simulated versus experimental time courses for the IRS1-induced AMPK model (hypothesis No.3). (A) Main data set used for parameter estimation. Simulated (red lines) versus experimental data (blue points) are plotted for nine wild type (WT) readouts along the insulin-TOR network upon insulin/aa induction. (B) Additional data set used for parameter estimation. Experimental data for seven readouts for a Raptor knock down (KD) upon insulin/aa induction. Experimental mean +/- SEM calculated from four repetitions. Goodness-of-fit χ^2 is reported for each plot along with the number of measured time points.