SUPPLEMENTAL MATERIALS:

DYNAMICAL MODELS

The Glut4 in the cell was considered to be distributed between different compartments, with various processes connecting and transporting Glut4 between the compartments. Each transport process was considered as first order, and mass action kinetics employed to describe the dynamics. In this we have assumed that it was the amount or proportion of Glut4 in the compartment which was the mass action driver. (Glut4 is present in very different membranous structures in the different locations). The different experimental modalities perturb and/or measure the distribution of Glut4 in the cells as a function of time. The assumptions and underlying principles of the two main experimental protocols are outlined below.

Uptake experiment

Here the cell was assumed to be in a steady state, with a constant insulin level (or none). The HA-Glut4 was exposed to the AF647-α-HA antibody when it was expressed on the cell surface whereupon it became fluorescently labeled. We assumed in the models that this process was instantaneous compared with other timescales operating (the antibody concentration was sufficient high to ensure that this was correct). The experiments measured the total amount of labeled Glut4 in the cell, $L(t)$, as a function of time, t, after the application of the antibody. In these experiments, Glut4 labeling saturates once all HA-Glut4 cycles through the plasma membrane (PM). Thus, the maximal value attained (or approximated by exponential fits of basal data) equals the total amount of Glut4 that was cycling between the cell surface and intracellular compartments under each experimental condition.

Translocation experiments

Here the system was not in steady state, but rather started in one steady state (either basal or insulin-stimulated) and then evolved to a new steady state after perturbation of the system (addition of insulin or inhibitor). The Glut4 was allowed to traffic for varying amounts of time after perturbation, the process was stopped by incubation at low temperature and the surface level of Glut4 measured by the application of the antibody. Thus the experimental measure was the surface level as a function of time since the perturbation, $P(t)$.

In these experiments, the assumption was made that the rate constants in the system instantaneously (compared with other timescales operating) change to the new perturbed values.

Experimental data sets

Data was collected from 2 different types of cells – fibroblasts and adipocytes. (Data from AS160KD adipocytes was also analyzed.) For each cell types four data sets were collected- two uptake experiments and two translocation experiments:

1. Basal uptake
2. Insulin stimulated uptake
3. Insulin + LYi transition
4. Basal to insulin stimulated state transition.

Experiments were replicated 5-19 times. Individual data points from each replicate experiment were included in the fits.

Using the dynamical models and hypotheses for each experiment (Supplementary Tables 1-3), the output for each experiment was simulated and the parameters to best fit, in a least-squares sense, all of the data, with equal weighting for each data set (irrespective of the number of points within that set). The number of points and their spread at different times within each data set provided a bias for the fitting of that data. In fits of the Three-Step model, it was necessary to bias the weighing of the basal to insulin transition 100X relative to the uptake data in order to accurately simulate all four of the data sets. In the case of the Insulin + LYi transition experiments, the cells are initially in the insulin-stimulated steady state. The system then evolves to internalize the Glut4, as the application of LYi inhibits the exocytic pathways. This was modeled by allowing the exocytic rate constants in these data sets to differ from the exocytic rate constants in either the presence or absence of insulin. The initial values of the exocytic rate constants in the presence of LYi were initialized near zero in the optimization process.

MODELS AND HYPOTHESES

Different networks of compartments containing Glut4 in the cell were considered – Supplemental Figure 1. The dynamics based on mass action kinetics, for the Three-Step and Dynamic Retention Models are shown in Supplemental Tables 1 and 2. The dynamics for the Dynamic Equilibrium, Static Retention and Two Exocytic Pathways Models are described in the main text. (The Two Exocytic Pathways Model was only employed to fit the uptake experiments). The dynamics defines the surface level for the basal to insulin-stimulated translocation experiments, with the initial condition given by the steady state PM level, P, in the basal state. In these models, different combinations of the rate constants between the compartments were hypothesized to be insulin dependent. The corresponding dynamics for the uptake experiments given by the different models are shown in Supplementary Table 3.

The variables for each model in Supplemental Fig. 1 are the amounts in the compartments as functions of time, t. The rate constants and $Total$ are assumed to be constants. The variables in the models are P, E, R, G_s and G_r the amount in the PM, endosomes, ERC, GSV_{seq} and GSV_{rel} respectively. For the Four Step Dynamic Retention model, the GSVs were considered to be a single compartment, amount G. All these are dependent on time.

For the uptake experiments, the labeled Glut4 was denoted L and was a function of time. The unlabeled Glut4 in each compartment was denoted with a subscript U. It was assumed that all unlabeled Glut4 was instantaneously labeled upon exocytosis to the PM.

Hypotheses

Hypotheses were tested for the dependence of the different rates on insulin. All hypotheses were tested with two variants – with the $Total$ either regulated by insulin (two parameters in the fit – $Total_{basal}$ and $Total_{ins}$), or unregulated, (one parameter, $Total$). The values for the totals under different conditions were found to only differ significantly for the Static Retention and the Three-Step Models, which allowed for insulin-regulated sequestration within the model structure. Thus fits assuming that $Total = 1$ were employed in the Dynamic Retention models to refine the parameter estimates.

The parameter values were held in common and optimized simultaneously across each sub-set of experimental data set except for the LYi transition data set which had separate exocytosis rates. Those rates which were perturbed by insulin were represented in the fit by two independent parameters for the basal and insulin-stimulated values. Some rates were constrained to test whether they remained independent of the cell type, or to constrain the rate when LYi was applied. For those experiments where an obvious overshoot occurred in the transition experiments, these data were biased in the fits to retain this feature.

The fit initial conditions, constraints, optimized output and goodness of fit are shown in Supplemental Table 3 for the hypotheses mentioned in the main text.

Fits were also performed using the 6-step Dynamic Retention model (Figure 7) with excellent correspondence with the data (Supplementary Table 3 (h)). Due to the increased number of parameters however, it was not possible to precisely determine all of the rates independently, with large confidence intervals resulting for the processes between the sequestration and release steps of the GSVs. This model represents all of the data well, as well as embodying the known docking and fusion steps of GSVs (eg, Lopez, J. A., Burchfield, J. G., Blair, D. H., Mele, K., Ng, Y., Vallotton, P. A., James, D. E. and Hughes, W. E. (2009) Identification of a Distal GLUT4 Trafficking Event Controlled by Actin Polymerization. *Mol Biol Cell*. **20**, 3918-3929). Thus this model is the candidate for the underlying structure of the processes.

Supplemental Table 1 The dynamics of the Three-Step and Dynamic Retention Models.

Dynamics	**Steady State Levels**
Three-Step (Supplemental Figure 1D)	
$\frac{dP}{dt} = k_{fuseE}R - k_{en}P$ $\frac{dE}{dt} = k_{en}P - k_{sort}E$ $\frac{dR}{dt} = k_{sort}E - k_{fuseE}R$ $Total = P + E + R$	$P_\infty = \frac{k_{fuseE}k_{sort}Total}{k_{fuseE}k_{en} + k_{fuseE}k_{sort} + k_{sort}k_{en}}$ $E_\infty = \frac{k_{fuseE}k_{en}Total}{k_{fuseE}k_{en} + k_{fuseE}k_{sort} + k_{sort}k_{en}}$ $R_\infty = \frac{k_{sort}k_{en}Total}{k_{fuseE}k_{en} + k_{fuseE}k_{sort} + k_{sort}k_{en}}$
Dynamic Retention, 4 step (Supplemental Figure 1E) Note that the GSVs are considered to be a single compartment, amount G, in this model.	
$\frac{dP}{dt} = k_{fuseE}R + k_{fuseG}G - k_{en}P$ $\frac{dE}{dt} = k_{en}P - (k_{sort} + k_{seq})E$ $\frac{dR}{dt} = k_{sort}E - k_{fuseE}R$ $\frac{dG}{dt} = k_{seq}E - k_{fuseG}G$ $Total = P + E + R + G$	$P_\infty = \frac{k_{fuseE}(k_{sort} + k_{seq})Total}{k_{en}k_{seq}k_{fuseE} + k_{en}k_{sort}k_{fuseG} + k_{en}k_{fuseE}k_{fuseG} + k_{seq}k_{fuseE}k_{fuseG} + k_{fuseG}k_{sort}k_{fuseE}}$ $E_\infty = \frac{k_{fuseG}k_{fuseE}k_{en}Total}{k_{en}k_{seq}k_{fuseE} + k_{en}k_{sort}k_{fuseG} + k_{en}k_{fuseE}k_{fuseG} + k_{seq}k_{fuseE}k_{fuseG} + k_{fuseG}k_{sort}k_{fuseE}}$ $R_\infty = \frac{k_{en}k_{sort}k_{fuseG}Total}{k_{en}k_{seq}k_{fuseE} + k_{en}k_{sort}k_{fuseG} + k_{en}k_{fuseE}k_{fuseG} + k_{seq}k_{fuseE}k_{fuseG} + k_{fuseG}k_{sort}k_{fuseE}}$ $G_\infty = \frac{k_{seq}k_{fuseE}k_{en}Total}{k_{en}k_{seq}k_{fuseE} + k_{en}k_{sort}k_{fuseG} + k_{en}k_{fuseE}k_{fuseG} + k_{seq}k_{fuseE}k_{fuseG} + k_{fuseG}k_{sort}k_{fuseE}}$
Dynamic Retention, 6 step (Supplemental Figure 3). Note that for fibroblasts $k_{seq} = 0$, so the state variables are P, E and R only ($G_s = G_r = 0$).	
$\frac{dP}{dt} = k_{fuseE}R + k_{fuseG}G_r - k_{en}P$ $\frac{dE}{dt} = k_{en}P - (k_{sort} + k_{seq})E$ $\frac{dR}{dt} = k_{sort}E - k_{fuseE}R$ $\frac{dG_s}{dt} = k_{seq}E - k_{rel}G_s + k_{rev}G_r$ $\frac{dG_r}{dt} = k_{rel}G_s - (k_{rev} + k_{fuseG})G_r$ $Total = P + E + R + G_s + G_r$	$P_\infty = \frac{(k_{sort} + k_{seq})k_{rel}k_{fuseG}k_{fuseE}Total}{\left(\begin{array}{c}k_{rel}k_{fuseG}k_{en}k_{fuseE} + k_{rel}k_{fuseG}k_{en}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{seq}\\ +k_{rel}k_{en}k_{fuseE}k_{seq} + k_{en}k_{fuseE}k_{seq}k_{rev} + k_{en}k_{fuseE}k_{seq}k_{fuseG}\end{array}\right)}$ $E_\infty = \frac{k_{rel}k_{fuseG}k_{en}k_{fuseE}Total}{\left(\begin{array}{c}k_{rel}k_{fuseG}k_{en}k_{fuseE} + k_{rel}k_{fuseG}k_{en}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{seq}\\ +k_{rel}k_{en}k_{fuseE}k_{seq} + k_{en}k_{fuseE}k_{seq}k_{rev} + k_{en}k_{fuseE}k_{seq}k_{fuseG}\end{array}\right)}$ $R_\infty = \frac{k_{sort}k_{rel}k_{fuseG}k_{en}Total}{\left(\begin{array}{c}k_{rel}k_{fuseG}k_{en}k_{fuseE} + k_{rel}k_{fuseG}k_{en}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{seq}\\ +k_{rel}k_{en}k_{fuseE}k_{seq} + k_{en}k_{fuseE}k_{seq}k_{rev} + k_{en}k_{fuseE}k_{seq}k_{fuseG}\end{array}\right)}$ $G_{s\infty} = \frac{k_{seq}(k_{rev} + k_{fuseG})k_{en}k_{fuseE}Total}{\left(\begin{array}{c}k_{rel}k_{fuseG}k_{en}k_{fuseE} + k_{rel}k_{fuseG}k_{en}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{seq}\\ +k_{rel}k_{en}k_{fuseE}k_{seq} + k_{en}k_{fuseE}k_{seq}k_{rev} + k_{en}k_{fuseE}k_{seq}k_{fuseG}\end{array}\right)}$ $G_{r\infty} = \frac{k_{seq}k_{rel}k_{en}k_{fuseE}Total}{\left(\begin{array}{c}k_{rel}k_{fuseG}k_{en}k_{fuseE} + k_{rel}k_{fuseG}k_{en}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{sort} + k_{rel}k_{fuseG}k_{fuseE}k_{seq}\\ +k_{rel}k_{en}k_{fuseE}k_{seq} + k_{en}k_{fuseE}k_{seq}k_{rev} + k_{en}k_{fuseE}k_{seq}k_{fuseG}\end{array}\right)}$

The variables in the models are P, E, R and G (G_s and G_r), the amount in the PM, sorting endosomes, ERC, and GSVs (GSV_{seq} and GSV_{rel}) respectively. All these are dependent on time, t. P_∞, E_∞, R_∞ and G_∞ ($G_{s\infty}$ and $G_{r\infty}$) are the steady state (long term) levels in those compartments. The rates, k_{fuseE}, k_{en}, k_{sort}, k_{seq} and k_{fuseG}, and the total amount of Glut4, $Total$, are assumed constant. For the transition experiments the dynamics take the values for the rate in the perturbed (insulin-stimulated) state. The initial conditions are calculated using the rate constants of the initial (basal) state.

Supplemental Table 2

The dynamics of the uptake experiments, Three-Step and Dynamic Retention Models.

Dynamics	**Initial Condition**
Uptake: Three-Step Model (Supplemental Figure 1D)	
$\frac{dL}{dt} = k_{fuseE} R_U$ $\frac{dE_U}{dt} = k_{fuseE} R_U$ $\frac{dR_U}{dt} = k_{sort} E_U - k_{fuseE} R_U$ $Total = L + E_U + R_U$	$L(0) = \frac{k_{fuseE} k_{sort} Total}{k_{fuseE} k_{en} + k_{fuseE} k_{sort} + k_{sort} k_{en}}$
Uptake: Dynamic Retention, 4 step (Supplemental Figure 1E) Note that the GSVs are considered to be a single compartment, unlabeled amount G_U, in this model.	
$\frac{dL}{dt} = k_{fuseE} R_U + k_{fuseG} G_U$ $\frac{dE_U}{dt} = -(k_{sort} + k_{seq}) E_U$ $\frac{dR_U}{dt} = k_{sort} E_U - k_{fuseE} R_U$ $\frac{dG_U}{dt} = k_{seq} E_U - k_{fuseG} G_U$ $Total = L + E_U + R_U + G_U$	$L(0) = \frac{k_{fuseE}(k_{sort} + k_{seq}) Total}{\left(\begin{array}{c} k_{en} k_{seq} k_{fuseE} + k_{en} k_{sort} k_{fuseG} + \\ k_{en} k_{fuseE} k_{fuseG} + k_{seq} k_{fuseE} k_{fuseG} + k_{fuseG} k_{sort} k_{fuseE} \end{array}\right)}$
Uptake: Dynamic Retention, 6 step (Supplemental Figure 3). Note that for fibroblasts $k_{seq} = 0$, so the state variables are L, E_U and R_U only ($G_{sU} = G_{rU} = 0$).	
$\frac{dL}{dt} = k_{fuseE} R_U + k_{fuseG} G_{rU}$ $\frac{dE_U}{dt} = -(k_{sort} + k_{seq}) E_U$ $\frac{dR_U}{dt} = k_{sort} E_U - k_{fuseE} R_U$ $\frac{dG_{sU}}{dt} = k_{seq} E_U - k_{rel} G_{sU} + k_{rev} G_{rU}$ $\frac{dG_{rU}}{dt} = k_{rel} G_{sU} - (k_{rev} + k_{fuseG}) G_{rU}$ $Total = L + E_U + R_U + G_{sU} + G_{rU}$	$L(0) = \frac{(k_{sort} + k_{seq}) k_{rel} k_{fuseG} k_{fuseE} Total}{\left(\begin{array}{c} k_{rel} k_{fuseG} k_{en} k_{fuseE} + k_{rel} k_{fuseG} k_{en} k_{sort} \\ + k_{rel} k_{fuseG} k_{fuseE} k_{sort} + k_{rel} k_{fuseG} k_{fuseE} k_{seq} \\ + k_{rel} k_{en} k_{fuseE} k_{seq} + k_{en} k_{fuseE} k_{seq} k_{rev} \\ + k_{en} k_{fuseE} k_{seq} k_{fuseG} \end{array}\right)}$

The variables in the models are L, the total amount of labeled Glut4, and E_U, R_U and G (G_{sU} and G_{rU}), the unlabeled amount in the sorting endosomes, ERC, and GSVs (GSV_{seq} and GSV_{rel}) respectively. All these are dependent on time, t. As the system was in steady-state, the rates, k_{fuseE}, k_{en}, k_{sort}, k_{seq}, k_{rel}, k_{rev} and k_{fuseG}, and the total amount of Glut4, $Total$, are assumed constant.

Supplemental Table 3
RESULTS OF FITS

3a) FIBROBLAST Three-Step Model – k_{fuseE} depends on insulin

Free Fit, Transition data weighted x100

Start point and constraints

k_{en}	0.1200	0.0000	≤	k_{en}	≤	∞
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	∞
$k_{fuseE\ basal}$	0.0050	0.0000	≤	$k_{fuseE\ basal}$	≤	∞
$k_{fuseE\ ins}$	0.0300	0.0000	≤	$k_{fuseE\ ins}$	≤	∞
k_{sort}	0.0900	0.0000	≤	k_{sort}	≤	∞

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1826	0.1642	0.2009	0.0093
$k_{fuseE\ YLi}$	0.0029	-0.0084	0.0142	0.0057
$k_{fuseE\ basal}$	0.0329	0.0293	0.0365	0.0018
$k_{fuseE\ ins}$	0.0804	0.0719	0.0889	0.0043
k_{sort}	0.0528	0.0433	0.0622	0.0048

Goodness of Fit:

sse	1.7165
R^2	0.9483
dfe	213.0000
Adj R^2	0.9473
rmse	0.0898

Inferred Steady State Values

	Basal	*Insulin*
P	0.0999	0.1486
S	0.3457	0.5141
R	0.5544	0.3373

Supplemental Table 3

3b) FIBROBLAST Three-Step Model – k_{fuseE} and k_{sort} depend on insulin

Free Fit, Transition data weighted x100.

Start point and constraints

k_{en}	0.1200	0.0000	≤	k_{en}	≤	∞
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	∞
$k_{fuseE\ basal}$	0.0050	0.0000	≤	$k_{fuseE\ basal}$	≤	∞
$k_{fuseE\ ins}$	0.0300	0.0000	≤	$k_{fuseE\ ins}$	≤	∞
$k_{sort\ basal}$	0.0900	0.0000	≤	$k_{sort\ basal}$	≤	∞
$k_{sort\ ins}$	0.0900	0.0000	≤	$k_{sort\ ins}$	≤	∞

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1863	0.1673	0.2052	0.0096
$k_{fuseE\ YLi}$	0.0028	-0.0077	0.0132	0.0053
$k_{fuseE\ basal}$	0.0268	0.0176	0.0359	0.0046
$k_{fuseE\ ins}$	0.0646	0.0415	0.0877	0.0117
$k_{sort\ basal}$	0.0923	-0.0316	0.2162	0.0628
$k_{sort\ ins}$	0.0651	0.0358	0.0944	0.0149

Goodness of Fit:

sse	1.7074
R^2	0.9485
dfe	212.0000
Adj R^2	0.9473
rmse	0.0897

Inferred Steady State Values

	Basal	*Insulin*
P	0.1002	0.1482
E	0.2024	0.4242
R	0.4276	0.4276

Supplemental Table 3

3c) ADIPOCYTE Three-Step Model – k_{fuseE} depends on insulin

Free Fit, Transition data weighted x100

Start point and constraints

k_{en}	0.1200	0.0000	≤	k_{en}	≤	∞
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	∞
$k_{fuseE\ basal}$	0.0050	0.0000	≤	$k_{fuseE\ basal}$	≤	∞
$k_{fuseE\ ins}$	0.0300	0.0000	≤	$k_{fuseE\ ins}$	≤	∞
k_{sort}	0.0900	0.0000	≤	k_{sort}	≤	∞

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1133	0.1002	0.1265	0.0067
$k_{fuseE\ YLi}$	0.0002	-0.0027	0.0031	0.0015
$k_{fuseE\ basal}$	0.0011	0.0009	0.0013	0.0001
$k_{fuseE\ ins}$	0.0273	0.0257	0.0290	0.0008
k_{sort}	0.3502	-0.0015	0.7019	0.1790

Goodness of Fit:

sse	3.7872
R^2	0.9638
dfe	461.0000
Adj R^2	0.9635
rmse	0.0906

Inferred Steady State Values

	Basal	*Insulin*
P	0.0095	0.1827
E	0.0031	0.0591
R	0.9874	0.7582

Supplemental Table 3

3d) AS160KD ADIPOCYTES Three-Step Model – k_{fuseE} depends on insulin

Free Fit, Transition data weighted x100

Start point and constraints

k_{en}	0.1200	0.0000	≤	k_{en}	≤	∞
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	∞
$k_{fuseE\ basal}$	0.0050	0.0000	≤	$k_{fuseE\ basal}$	≤	∞
$k_{fuseE\ ins}$	0.0300	0.0000	≤	$k_{fuseE\ ins}$	≤	∞
k_{sort}	0.0900	0.0000	≤	k_{sort}	≤	∞

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1220	0.1069	0.1371	0.0076
$k_{fuseE\ YLi}$	0.0063	-0.0024	0.0150	0.0044
$k_{fuseE\ basal}$	0.0057	0.0051	0.0063	0.0003
$k_{fuseE\ ins}$	0.0319	0.0285	0.0354	0.0018
k_{sort}	0.0550	0.0380	0.0720	0.0086

Goodness of Fit:

sse	1.5698
R^2	0.9565
dfe	155.0000
Adj R^2	0.9554
rmse	0.1006

Inferred Steady State Values

	Basal	*Insulin*
P	0.0407	0.1421
E	0.0904	0.3151
R	0.8689	0.5428

Supplemental Table 3

3e) ADIPOCYTES Dynamic Retention, 4 Step Model – Fibroblast constraints, k_{fuseE}, k_{seq} and k_{fuseG} depend on insulin

Fit with rates constrained to fibroblast values for common rates from the Three Step Model (LYi rates constrained).

Start point and constraints

k_{en}	0.1200	0.1000	≤	k_{en}	≤	0.1300
$k_{fuseE\ YLi}$	0.0002	0.0000	≤	$k_{fuseE\ YLi}$	≤	0.0050
$k_{fuseE\ basal}$	0.0370	0.0250	≤	$k_{fuseE\ basal}$	≤	0.0450
$k_{fuseE\ ins}$	0.0880	0.0500	≤	$k_{fuseE\ ins}$	≤	0.2500
$k_{fuseG\ YLi}$	0.0035	0.0000	≤	$k_{fuseG\ YLi}$	≤	0.0050
$k_{fuseG\ basal}$	0.0368	0.0000	≤	$k_{fuseG\ basal}$	≤	∞
$k_{fuseG\ ins}$	0.0900	0.0000	≤	$k_{fuseG\ ins}$	≤	∞
$k_{seq\ basal}$	0.0200	0.0000	≤	$k_{seq\ basal}$	≤	∞
$k_{seq\ ins}$	0.0200	0.0000	≤	$k_{seq\ ins}$	≤	∞
k_{sort}	0.0630	0.0000	≤	k_{sort}	≤	0.0750

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1165	0.1031	0.1299	0.0068
$k_{fuseE\ YLi}$	0.0012	-0.0041	0.0066	0.0027
$k_{fuseE\ basal}$	0.0250	*Fixed at Bound*		
$k_{fuseE\ ins}$	0.0882	-0.0172	0.1937	0.0537
$k_{fuseG\ YLi}$	0.0000	*Fixed at Bound*		
$k_{fuseG\ basal}$	0.0007	0.0005	0.0009	0.0001
$k_{fuseG\ ins}$	0.0253	0.0228	0.0279	0.0013
$k_{seq\ basal}$	0.0205	0.0031	0.0379	0.0089
$k_{seq\ ins}$	0.0588	-0.0681	0.1857	0.0646
k_{sort}	0.0424	0.0112	0.0737	0.0159

Goodness of Fit:

sse	0.4250
R^2	0.9882
dfe	458.0000
Adj R^2	0.9881
rmse	0.0305

Inferred Steady State Values

	Basal	*Insulin*
P	0.0173	0.1860
E	0.0321	0.2140
R	0.0545	0.1030
G	0.8961	0.4970

Supplemental Table 3

3f) ADIPOCYTES Dynamic Retention, 4 Step Model – Primary Constraints, k_{fuseE}, k_{seq} and k_{fuseG} depend on insulin

Fit with rates constrained to values for primary adipocytes (Table 2) (LYi rates constrained).

Start point and constraints

k_{en}	0.0500	0.0000	≤	k_{en}	≤	0.1300
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	0.0050
$k_{fuseE\ basal}$	0.0180	0.0180	≤	$k_{fuseE\ basal}$	≤	0.0180
$k_{fuseE\ ins}$	0.0780	0.0780	≤	$k_{fuseE\ ins}$	≤	0.0780
$k_{fuseG\ YLi}$	0.0000	0.0000	≤	$k_{fuseG\ YLi}$	≤	0.0050
$k_{fuseG\ basal}$	0.0005	0.0000	≤	$k_{fuseG\ basal}$	≤	∞
$k_{fuseG\ ins}$	0.0500	0.0000	≤	$k_{fuseG\ ins}$	≤	∞
$k_{seq\ basal}$	0.0060	0.0060	≤	$k_{seq\ basal}$	≤	0.0060
$k_{seq\ ins}$	0.1430	0.1430	≤	$k_{seq\ ins}$	≤	0.1430
k_{sort}	0.0130	0.0130	≤	k_{sort}	≤	0.0130

Optimized Coefficients

		95% confidence bounds		*Standard*
	Value	*lower*	*upper*	*Error*
k_{en}	0.1127	0.1057	0.1197	0.0036
$k_{fuseE\ YLi}$	0.0050	-0.0291	0.0391	0.0174
$k_{fuseE\ basal}$	0.0180	-0.0419	0.0779	0.0305
$k_{fuseE\ ins}$	0.0780	-0.1668	0.3227	0.1245
$k_{fuseG\ YLi}$	0.0000	*Fixed at Bound*		
$k_{fuseG\ basal}$	0.0006	-0.0001	0.0013	0.0004
$k_{fuseG\ ins}$	0.0282	0.0261	0.0303	0.0011
$k_{seq\ basal}$	0.0060	*Fixed at Bound*		
$k_{seq\ ins}$	0.1430	*Fixed at Bound*		
k_{sort}	0.0130	-0.0110	0.0370	0.0122

Goodness of Fit:

sse	0.4404
R^2	0.9878
dfe	459.0000
Adj R^2	0.9877
rmse	0.0310

Inferred Steady State Values

	Basal	*Insulin*
P	0.0142	0.1815
E	0.0839	0.1311
R	0.0606	0.0219
G	0.8413	0.6655

Supplemental Table 3

3g) AS160KD ADIPOCYTES Dynamic Retention, 4 Step Model – Primary Constraints, k_{fuseE}, k_{seq} and k_{fuseG} depend on insulin

Fit with rates constrained to values for primary adipocytes (Table 2) (LYi rates constrained).

Start point and constraints

k_{en}	0.0500	0.0000	≤	k_{en}	≤	0.1300
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	0.0050
$k_{fuseE\ basal}$	0.0180	0.0180	≤	$k_{fuseE\ basal}$	≤	0.0180
$k_{fuseE\ ins}$	0.0780	0.0780	≤	$k_{fuseE\ ins}$	≤	0.0780
$k_{fuseG\ YLi}$	0.0000	0.0000	≤	$k_{fuseG\ YLi}$	≤	0.0050
$k_{fuseG\ basal}$	0.0005	0.0000	≤	$k_{fuseG\ basal}$	≤	∞
$k_{fuseG\ ins}$	0.0500	0.0000	≤	$k_{fuseG\ ins}$	≤	∞
$k_{seq\ basal}$	0.0060	0.0060	≤	$k_{seq\ basal}$	≤	0.0060
$k_{seq\ ins}$	0.1430	0.1430	≤	$k_{seq\ ins}$	≤	0.1430
k_{sort}	0.0130	0.0130	≤	k_{sort}	≤	0.0130

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1133	0.0724	0.1541	0.0207
$k_{fuseE\ YLi}$	0.0000	*Fixed at Bound*		
$k_{fuseE\ basal}$	0.0180	*Fixed at Bound*		
$k_{fuseE\ ins}$	0.0780	*Fixed at Bound*		
$k_{fuseG\ YLi}$	0.0049	-0.0000	0.0098	0.0025
$k_{fuseG\ basal}$	0.0041	0.0036	0.0046	0.0002
$k_{fuseG\ ins}$	0.0203	0.0180	0.0226	0.0012
$k_{seq\ basal}$	0.0060	*Fixed at Bound*		
$k_{seq\ ins}$	0.1430	-0.4170	0.7030	0.2834
k_{sort}	0.0130	*Fixed at Bound*		

Goodness of Fit:

sse	0.6690
R^2	0.9612
dfe	155.0000
Adj R^2	0.9602
rmse	0.0657

Inferred Steady State Values

	Basal	*Insulin*
P	0.0501	0.1438
E	0.2988	0.1044
R	0.2159	0.0174
G	0.4352	0.7344

Supplemental Table 3

3h) AS160KD ADIPOCYTES Dynamic Retention, 4 Step Model – Primary and LRP1 Constraints, k_{fuseE}, k_{seq} and k_{fuseG} depend on insulin

Fit with rates constrained to values for primary adipocytes (Table 2) (LYi rates constrained). Additionally the values for k_{seq} were set to those determined from fits of the LRP1 surface data (main text).

Start point and constraints

k_{en}	0.0500	0.0000	≤	k_{en}	≤	0.1300
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	0.0050
$k_{fuseE\ basal}$	0.0180	0.0180	≤	$k_{fuseE\ basal}$	≤	0.0180
$k_{fuseE\ ins}$	0.0780	0.0780	≤	$k_{fuseE\ ins}$	≤	0.0780
$k_{fuseG\ YLi}$	0.0000	0.0000	≤	$k_{fuseG\ YLi}$	≤	0.0050
$k_{fuseG\ basal}$	0.0041	0.0000	≤	$k_{fuseG\ basal}$	≤	0.0081
$k_{fuseG\ ins}$	0.0280	0.0280	≤	$k_{fuseG\ ins}$	≤	0.0280
$k_{seq\ basal}$	0.0180	0.0180	≤	$k_{seq\ basal}$	≤	0.0180
$k_{seq\ ins}$	0.0300	0.0300	≤	$k_{seq\ ins}$	≤	0.0300
k_{sort}	0.0130	0.0130	≤	k_{sort}	≤	0.0130

Optimized Coefficients

	Value	*95% confidence bounds* *lower*	*upper*	*Standard Error*
k_{en}	0.1160	0.0984	0.1335	0.0089
$k_{fuseE\ YLi}$	0.0050	*Fixed at Bound*		
$k_{fuseE\ basal}$	0.0180	*Fixed at Bound*		
$k_{fuseE\ ins}$	0.0780	*Fixed at Bound*		
$k_{fuseG\ YLi}$	0.0050	*Fixed at Bound*		
$k_{fuseG\ basal}$	0.0053	0.0049	0.0058	0.0002
$k_{fuseG\ ins}$	0.0280	*Fixed at Bound*		
$k_{seq\ basal}$	0.0018	*Fixed at Bound*		
$k_{seq\ ins}$	0.0300	*Fixed at Bound*		
k_{sort}	0.0130	*Fixed at Bound*		

Goodness of Fit:

sse	0.7032
R^2	0.9592
dfe	158.0000
Adj R^2	0.9590
rmse	0.0667

Inferred Steady State Values

	Basal	*Insulin*
P	0.0498	0.1421
E	0.1865	0.3833
R	0.1345	0.0638
G	0.6292	0.4107

Supplemental Table 3

3i) ADIPOCYTES Dynamic Retention, 6 Step Model – Primary Constraints, k_{fuseE}, k_{seq} and k_{fuseG} depend on insulin

Fit with rates constrained to values for primary adipocytes (Table 2) (LYi rates constrained). k_{rev} was fixed to be 0.001 to reduce the degrees of freedom.

Start point and constraints

k_{en}	0.0500	0.0000	≤	k_{en}	≤	0.1300
$k_{fuseE\ YLi}$	0.0000	0.0000	≤	$k_{fuseE\ YLi}$	≤	0.0050
$k_{fuseE\ basal}$	0.0180	0.0180	≤	$k_{fuseE\ basal}$	≤	0.0180
$k_{fuseE\ ins}$	0.0780	0.0780	≤	$k_{fuseE\ ins}$	≤	0.0780
$k_{fuseG\ YLi}$	0.0000	0.0000	≤	$k_{fuseG\ YLi}$	≤	0.0050
$k_{fuseG\ basal}$	0.0041	0.0000	≤	$k_{fuseG\ basal}$	≤	∞
$k_{fuseG\ ins}$	0.0286	0.0000	≤	$k_{fuseG\ ins}$	≤	∞
$k_{rel\ basal}$	0.0000	0.0000	≤	$k_{rel\ basal}$	≤	∞
$k_{rel\ ins}$	0.0000	0.0000	≤	$k_{rel\ ins}$	≤	∞
$k_{seq\ basal}$	0.0060	0.0060	≤	$k_{seq\ basal}$	≤	0.0060
$k_{seq\ ins}$	0.1430	0.1430	≤	$k_{seq\ ins}$	≤	0.1430
k_{sort}	0.0130	0.0130	≤	k_{sort}	≤	0.0130

Optimized Coefficients

	Value	*95% confidence bounds lower*	*upper*	*Standard Error*
k_{en}	0.1137	0.1062	0.1212	0.0038
$k_{fuseE\ YLi}$	0.0000	*Fixed at Bound*		
$k_{fuseE\ basal}$	0.0180	*Fixed at Bound*		
$k_{fuseE\ ins}$	0.0780	*Fixed at Bound*		
$k_{fuseG\ YLi}$	0.0009	-0.0014	0.0032	0.0012
$k_{fuseG\ basal}$	0.0768	-0.5272	0.6809	0.3074
$k_{fuseG\ ins}$	0.8839	-0.8813	2.6491	0.8982
$k_{rel\ basal}$	0.0006	0.0005	0.0007	0.0001
$k_{rel\ ins}$	0.0292	0.0278	0.0306	0.0007
$k_{seq\ basal}$	0.0060	*Fixed at Bound*		
$k_{seq\ ins}$	0.1430	*Fixed at Bound*		
k_{sort}	0.0130	*Fixed at Bound*		

Goodness of Fit:

sse	0.4320
R^2	0.9881
dfe	460.0000
Adj R^2	0.9879
rmse	0.0306

Inferred Steady State Values

	Basal	*Insulin*
P	0.0141	0.1803
E	0.0844	0.1315
R	0.0609	0.0219
G_s	0.8340	0.6450
G_r	0.0066	0.0213