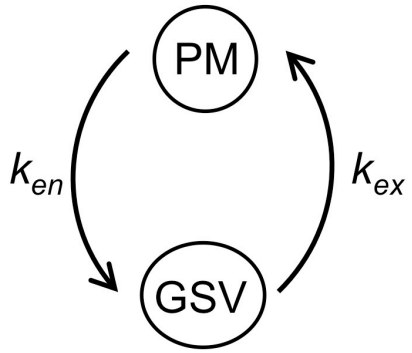
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Mathematical Models of Glut4 Trafficking. A: Dynamic Equilibrium- a single pool of Glut4 cycling between the plasma membrane (PM) and GSVs with a single rate limiting step for endocytosis (k_{en}) and exocytosis (k_{ex}). In this model, *Total* is insulin-independent. B: Static Retention- two intracellular pools of Glut4, one in sequestered, non-cycling (static) GSVs, the other in the sorting endosomal recycling pathway (*SE*), with single rate limiting steps for endocytosis and exocytosis. In this model, *Total* is insulin-dependent. Release of GSVs in response to insulin is quantal (increasing amounts are released with increasing concentrations of insulin) and instantaneous. In addition, Glut4 does not reenter the GSV pathway again until insulin withdrawal. C: Two Exocytic Pathways- there are two intracellular pools of Glut4 with independent pathways of exocytosis, the constitutive endosomal pool which cycles through endosomal recycling intermediate compartments (*ERC*) and the sequestered pool that cycles through GSVs. Each pool has a single rate-limiting step for exocytosis (k_{fuseE} or k_{fuseG}). D: Three-Step- a single intracellular pool of Glut4 that cycles through a pathway with two sequential rate-limiting steps, trafficking from sorting endosomes (*SE*) into the *ERC* (k_{sort}) and fusion of *ERC* to the plasma membrane (k_{fuseE}). This model also can simulate trafficking in AS160 KD cells, although Glut4 may be trafficking predominantly from the sorting endosomes into partially unregulated GSVs rather than into the *ERC* in these cells. E: Dynamic Retention- there are two intracellular pools of Glut4 with independent pathways of exocytosis, the constitutive endosomal pool (through the *ERC*) and the GSV pool. These pools are both derived from sorting endosomes via two independent processes with single rate-limiting steps- Glut4 is either sorted into the ERC (k_{sort}) or sequestered in GSVs (k_{seq}). Each pool has a single rate-limiting step for exocytosis (k_{fuseE} or k_{fuseG}). F: Dynamic Retention with Tf Receptor recycling- an additional direct recycling pathway from sorting endosomes to the plasma membrane with a single rate-limiting step (k_{rec}) was added to the model to allow for very rapid recycling of the Tf receptor and LRP1. Glut4 does not enter into this pathway ($k_{rec\ Glut4}$ = 0).

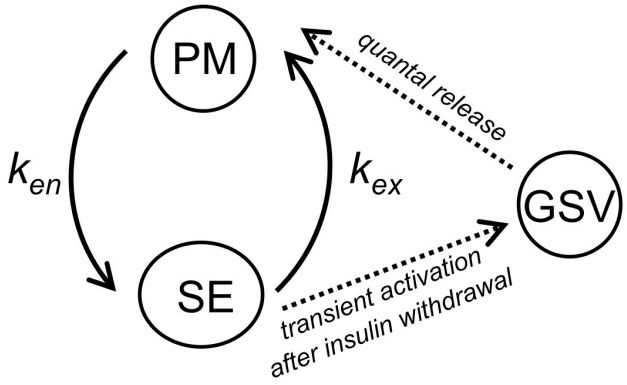
Supplemental Figure 2: Simulations of α-HA uptake data in basal (circles) or insulin-stimulated (squares) 3T3-L1 Control adipocytes (white) or AS160 knockdown (KD) adipocytes (black). Solid lines- simulations using rate constants from simultaneous fits to the data using the models in Supplemental Figs. 1A-E (as described in the text and in Supplemental Materials). Dot-dashed lines- simulations using alternate (primary constrained) fits. Simulations of Supplemental Fig. 1F were used to test hypotheses about LRP1 trafficking (Figs 5F, 6F, and Table 3).

Supplemental Figure 3: Mathematical Model of Dynamic Retention, with Tf receptor recycling and AS160-regulated priming of GSVs (release) (Fig. 7). This is the minimal mathematical model required to accurately simulate all of the data in 3T3-L1 cells and primary adipocytes. In this model there are two cycling pools of Glut4, one through GSVs and the other through the ERC (default fibroblast pathway) with a dynamic balance between sequestered and released (primed) GSVs. *PM*- plasma membrane, *SE*- sorting endosomes, *ERC*- endosomal recycling intermediate compartment (constitutive endosomal pathway), GSV_{seq}- sequestered GSVs (vesicles are not fusion competent due to inhibition by the Rab GTPase AS160), GSV_{rel}- released GSVs (vesicles are fusion competent due to GTP-loading of a Rab protein). This model has six rate constants, three of which are highly regulated by insulin (*). Fits to the control and AS160 KD data were done using this model (Supplemental Materials- Supplemental Table 3i and data not shown). However, k_{fuseG} basal and insulin could not be accurately determined in these fits ($k_{fuseG} \gg k_{rel}$; with large standard errors).

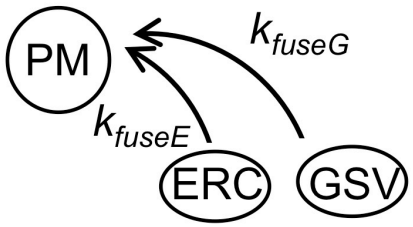
A. **Dynamic Equilibrium-**
single exponent to 1



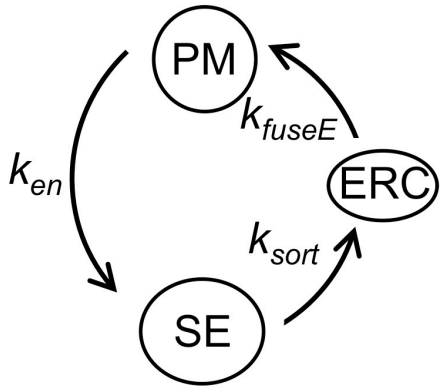
B. **Static Retention-**
single exponent to MFR_{max}



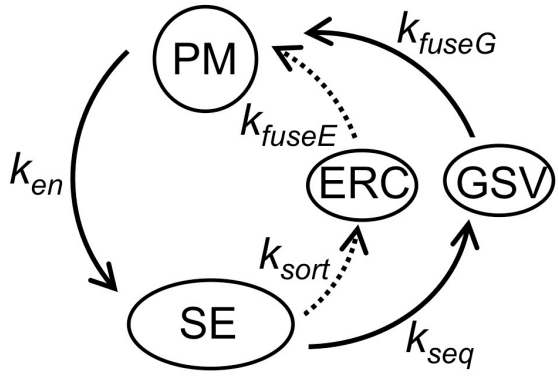
C. **Two Exocytic Pathways-**
two exponents to 1



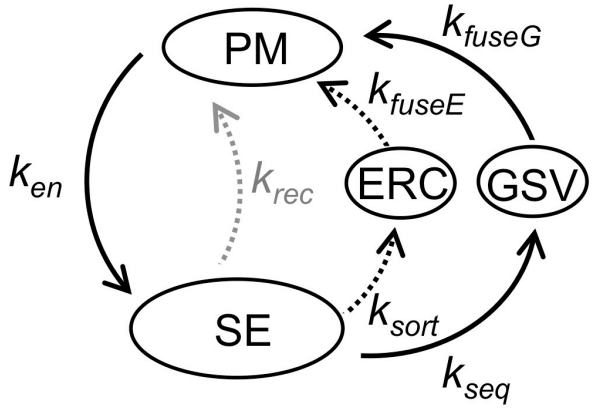
D. **Three-Step**



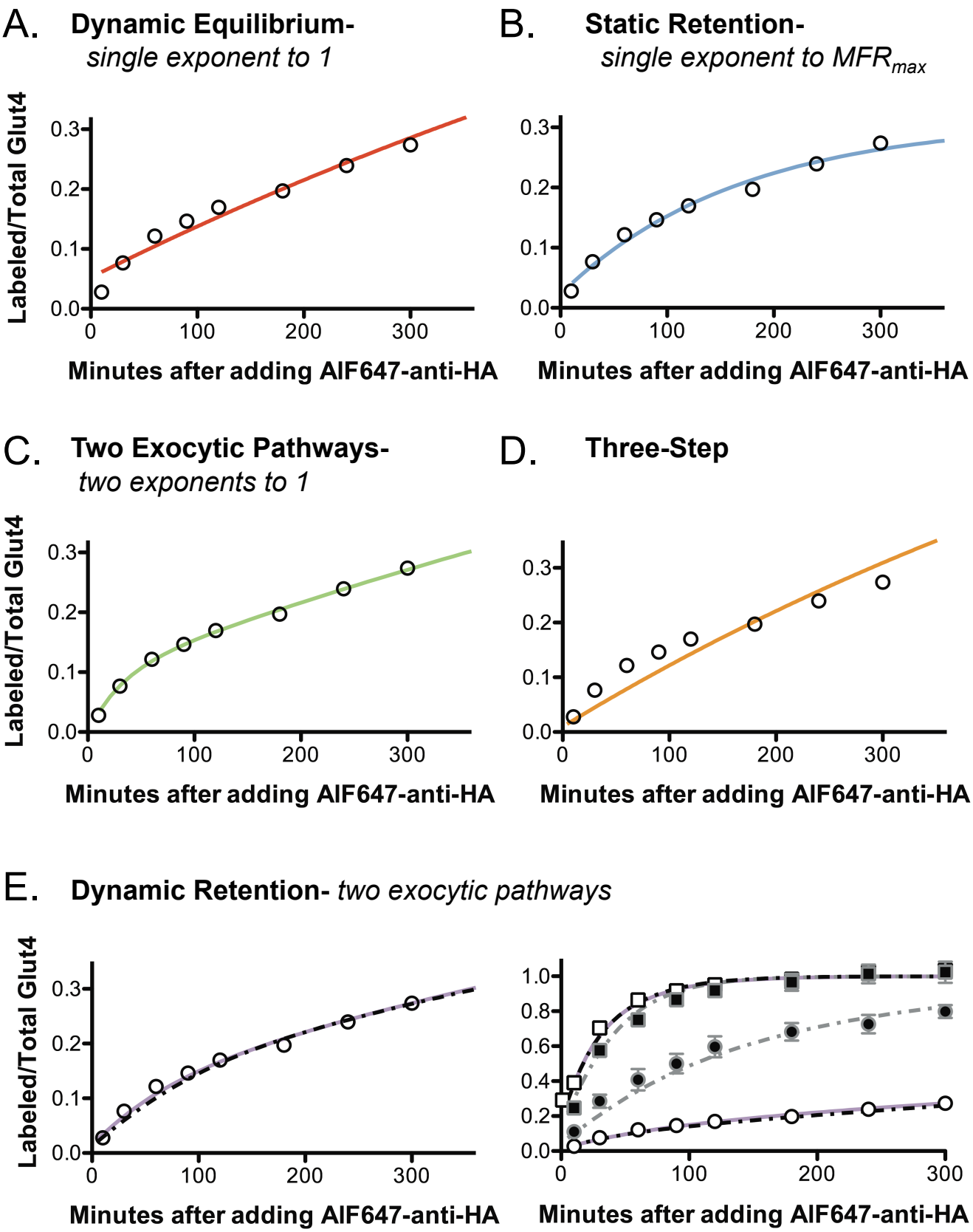
E. **Dynamic Retention-**
two exocytic pathways



F. **Dynamic Retention-**
with Tf receptor recycling

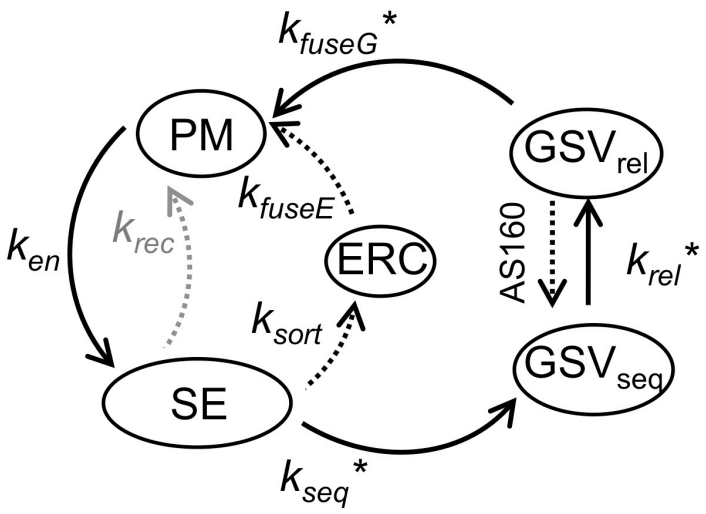


Supplemental Fig. 1- Brewer et al



Supplemental Fig. 2- Brewer et al

Dynamic Retention- *two exocytic pathways*



with Tf receptor recycling and AS160-regulated priming of GSVs (release)

Supplemental Fig. 3- Brewer et al