A. 8 hrs after Electroporation WT. WT FA OF CELLS % OF CELLS 1.3 1.6 1.9 2.2 2.5 2.8 GFP FLUORESCENCE (AFU) GFP FLUORESCENCE (AFU) WT % OF CELLS EEAA OF CELLS 1.3 1.6 1.9 2.2 2.5 2.8 0.1 0.4 0.7 1 1.3 1.6 1.9 2.2 2.5 2.8 GFP FLUORESCENCE (AFU) GFP FLUORESCENCE (AFU) B. 24 hrs after Electroporation WT % OF CELLS OF CELLS 1.3 1.6 1.9 2.2 2.5 2.8 1.3 1.6 1.9 2.2 2.5 2.8 GFP FLUORESCENCE (AFU) GFP FLUORESCENCE (AFU) WT **EEAA** % OF CELLS % OF CELLS

Supplemental Figure 1. Cell-by-cell analysis of the expression levels of WT and mutated HA-GLUT4-GFP in adipocytes 8 hrs (A) or 24 hrs (B) after electroporation. Results are from 150-300 cells from at least 6 independent experiments. Within each experiments, GFP fluorescence intensities are normalized to the average fluorescence intensity of the WT constructs.

0.1 0.4 0.7

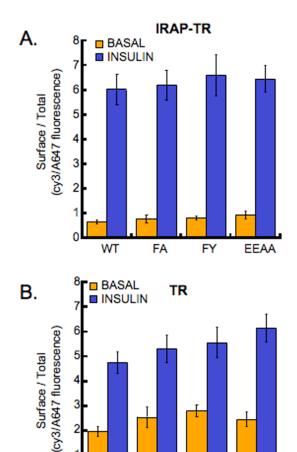
GFP FLUORESCENCE (AFU)

1.3 1.6 1.9 2.2 2.5 2.8

Supplemental FIGURE 1

0.1 0.4 0.7 1 1.3 1.6 1.9 2.2 2.5 2.8

GFP FLUORESCENCE (AFU)



WT

FΑ

Supplemental Figure 2. HA-GLUT4-GFP mutants do not alter IRAP-TR or transferrin receptor trafficking. (A) IRAP-TR and (B) Transferrin receptor (TR) basal and insulin-stimulated surface-to-total distributions in adipocytes co-expressing either WT or mutated HA-GLUT4-GFP. Results are from an individual experiments and are the average Surface-to-total value ± SEM from at least 20 cells. IRAP-TR and TR surface-to-total distributions were measured as previously described (Zeigerer et al., 2002. GLUT4 retention in adipocytes requires two intracellular insulin-regulated transport steps. Mol Biol Cell 13, 2421-2435.

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