GLUT4 translocation

[Graph D) and E) showing the effects of insulin and basal conditions on GLUT4 translocation over time (in hours).]
**Figure 1 Supplement.** D) Differentiated 3T3L1 adipocytes were electroporated with 400 µg myc-GLUT4-EGFP cDNA and incubated for 3-24 h. The cells were then either left untreated (squares, Basal) or stimulated with 100 nM insulin (circles, Insulin) for 30 min. The cells were then incubated with 2 mM KCN for 5 min followed by myc antibody for 1 h. The cells were then incubated with a HRP-conjugated rabbit anti-mouse antibody, washed and incubated with the substrate as described under “Materials and Methods”. These are the average values obtained from two independent experiments setting the maximal insulin stimulation to 100%. E) Differentiated 3T3L1 adipocytes were electroporated with 50 µg myc-GLUT4-EGFP cDNA and incubated for 3-12 h. The cells were then either left untreated (squares, Basal) or stimulated with 100 nM insulin (circles, Insulin) for 30 min. The cells were fixed and subjected to immunofluorescent confocal fluorescent microscopy for the myc and EGFP fluorescent signals. The pixel intensity of the myc signal was divided by the pixel intensity of the EGFP signal from 10 randomly selected cells. These numbers were averaged from three independent experiments setting the maximal insulin stimulation to 100%.