Supplementary Figure 1.

Latrunculin B treatment inhibits insulin-induced cell spreading and lamellopodia formation in adipocytes.

3T3-L1 adipocytes expressing IRAP-pH (green) ± 1 h pre-treatment with 10 μM Latrunculin B were imaged before and after a 30 min insulin stimulation. Representative images (combined phase and TIRFM) of live 3T3-L1 adipocytes expressing IRAP-pH (green) ± 1 h pre-treatment with 10 μM Latrunculin B before and after a 30 min insulin stimulation are shown (A). The white line describes the boundary of the 'cell footprint' prior to insulin stimulation. Scale bars, 10 μm. The percentage of cells that show process formation following stimulation with insulin is shown (B). Data is presented as the mean ± SEM of 6 independent experiments (10 cells/expt).***$p<0.001$ vs DMSO control insulin, ### $p<0.001$ vs DMSO control basal.

Supplementary Movie 1.

Insulin induced actin rearrangement and membrane ruffling in an adipocyte.

TIRF images acquired at 10 Hz showing the basal membrane of an adipocyte expressing actin-eGFP. Insulin (100 nM) is added at the 1 minute timepoint.

Supplementary Movie 2.

Insulin stimulates IRAP-pH vesicle fusion and accumulation of fluorescence on the PM.

TIRFM images acquired at 10 Hz showing the basal membrane of an adipocyte expressing IRAP-pH after treatment with 100 nM insulin.

Supplementary Movie 3. ***Representative fusion of an IRAPpH containing vesicle with the PM.***

TIRFM images acquired at 10 Hz showing a characteristic fusion of an IRAP-pH containing vesicle with the PM following treatment with 100 nM insulin. Scale bar, 1 μm.

Supplementary Figure 1