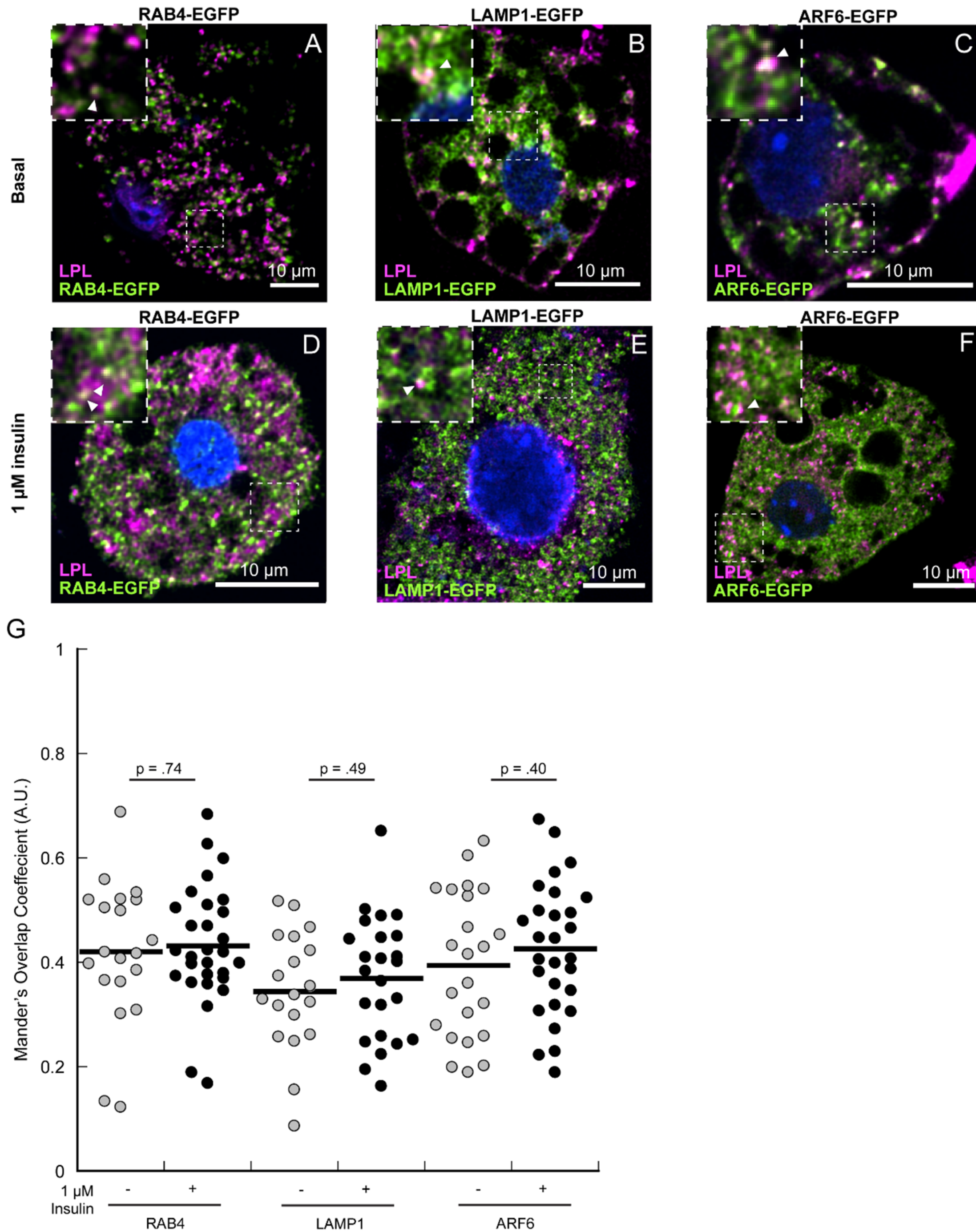

Fig. S1. **LPL colocalization with RAB4, LAMP1, and ARF6**

A-C) Adipocytes were treated with vehicle (basal) or (D-F) with insulin for 120 minutes before being fixed and stained for the indicated target. Insets show selected zoomed-in views. Scale bars = 10 µm. Examples of colocalizing puncta are indicated with arrows. G) The colocalization of LPL with the indicated markers by the Mander's coefficient is shown. All datasets include 3 biological replicate sets: RAB4 (basal n=20; IS n=29), LAMP1 (basal n=19; IS n=24), ARF6 (basal n=23; IS n=28). P-values were calculated with a Student's t-test.

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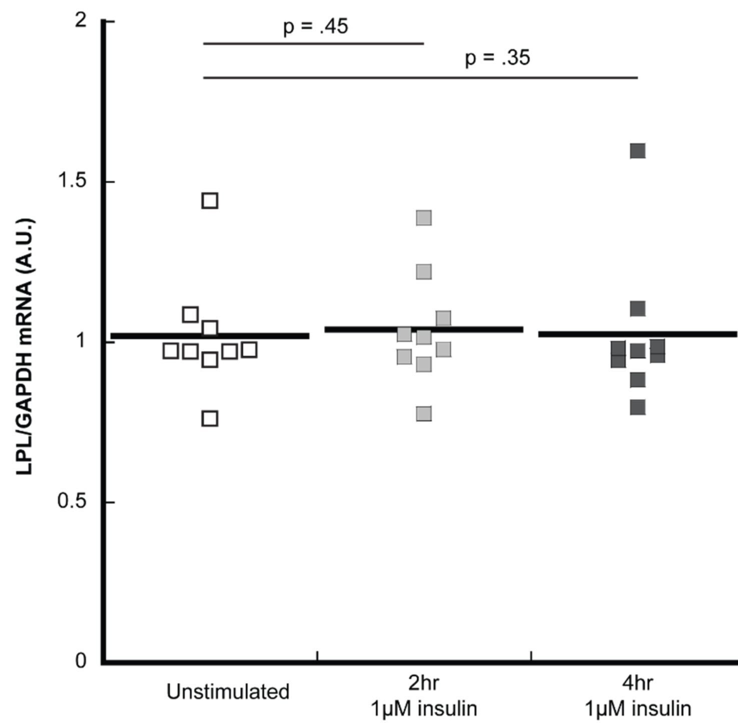

Fig. S2. Acute insulin treatment does not affect LPL mRNA levels

mRNA was collected from cells grown under cell culture conditions and RT-qPCR was used to measure LPL mRNA relative to GAPDH mRNA. Each point represents a technical replicate. All experiments completed in triplicate. P-values were calculated with a one-way ANOVA with Dunnet's post-hoc test.

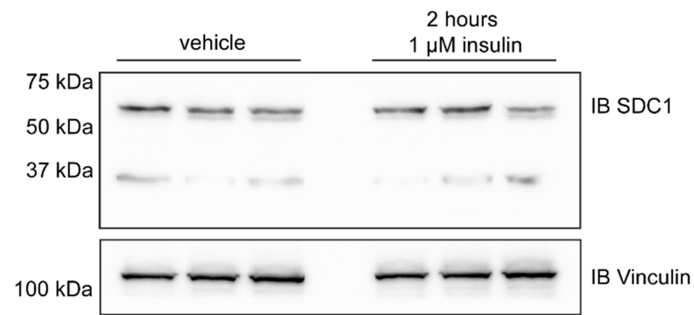

Fig. S3. Acute insulin treatment does not affect SDC1 levels

Differentiated 3T3-L1 adipocytes were treated with either vehicle or insulin for 2 hours. Cell lysates were analyzed by Western blot and blotted for SDC1 and vinculin. Samples were collected and analyzed in triplicate.

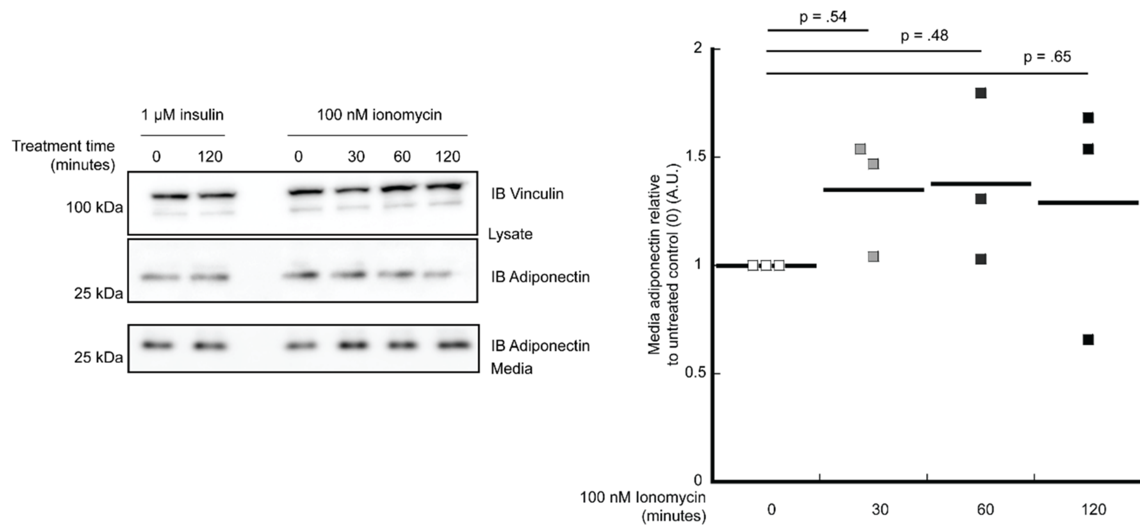

Fig. S4. Ionomycin treatment does not significantly increase adiponectin secretion

Cells were treated with either insulin (left) or ionomycin (right) for the indicated times. Untreated cells (0) were treated with vehicle alone. Media adiponectin was measured and plotted relative to untreated cells (0). P-values were calculated by one-way ANOVA with Dunnett's post-hoc test.

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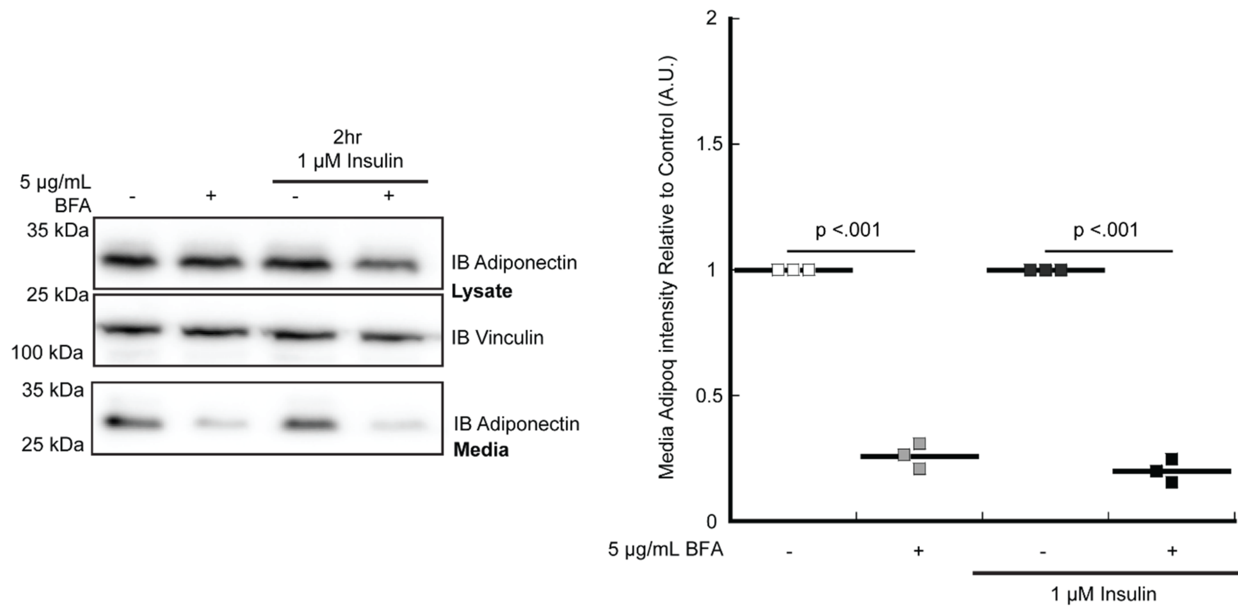

Fig. S5. BFA partly prevents adiponectin secretion

Differentiated 3T3-L1 adipocytes were treated with either vehicle, 1 µM insulin, 5 µg/mL BFA, or a combination of insulin and BFA. Cells were pre-treated with BFA or vehicle for 30 minutes before insulin or vehicle was added for 2 hours. Media and lysates were collected after 2 hours and samples were prepared for analysis by Western blot. Samples were blotted for adiponectin and vinculin. Graphs show media adiponectin levels relative to untreated controls for 3 replicate experiments. P-values were calculated with two-way ANOVA with Šidák-corrected pairwise tests. Media adiponectin levels: +BFA / -BFA = 26%±5; IS +BFA / -BFA = 20%±5.

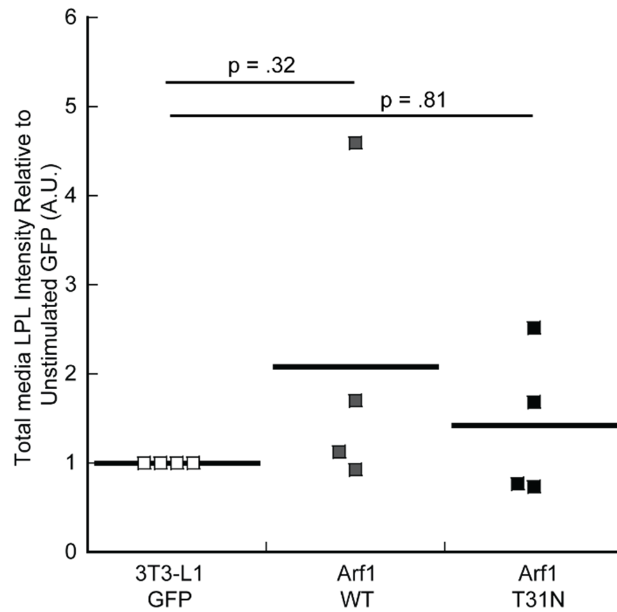

Fig. S6. LPL secretion relative to unstimulated controls

Data from Figure 7B was analyzed to show indicate LPL secretion relative to unstimulated GFP controls. There is no difference in LPL secretion from unstimulated cells expressing GFP, ARF1 WT-GFP, and ARF1 T31N-GFP. Full-length LPL band intensity relative to unstimulated cells for 4 replicate experiments are shown. P-values were calculated with a one-way ANOVA with Dunnett's post-hoc test.

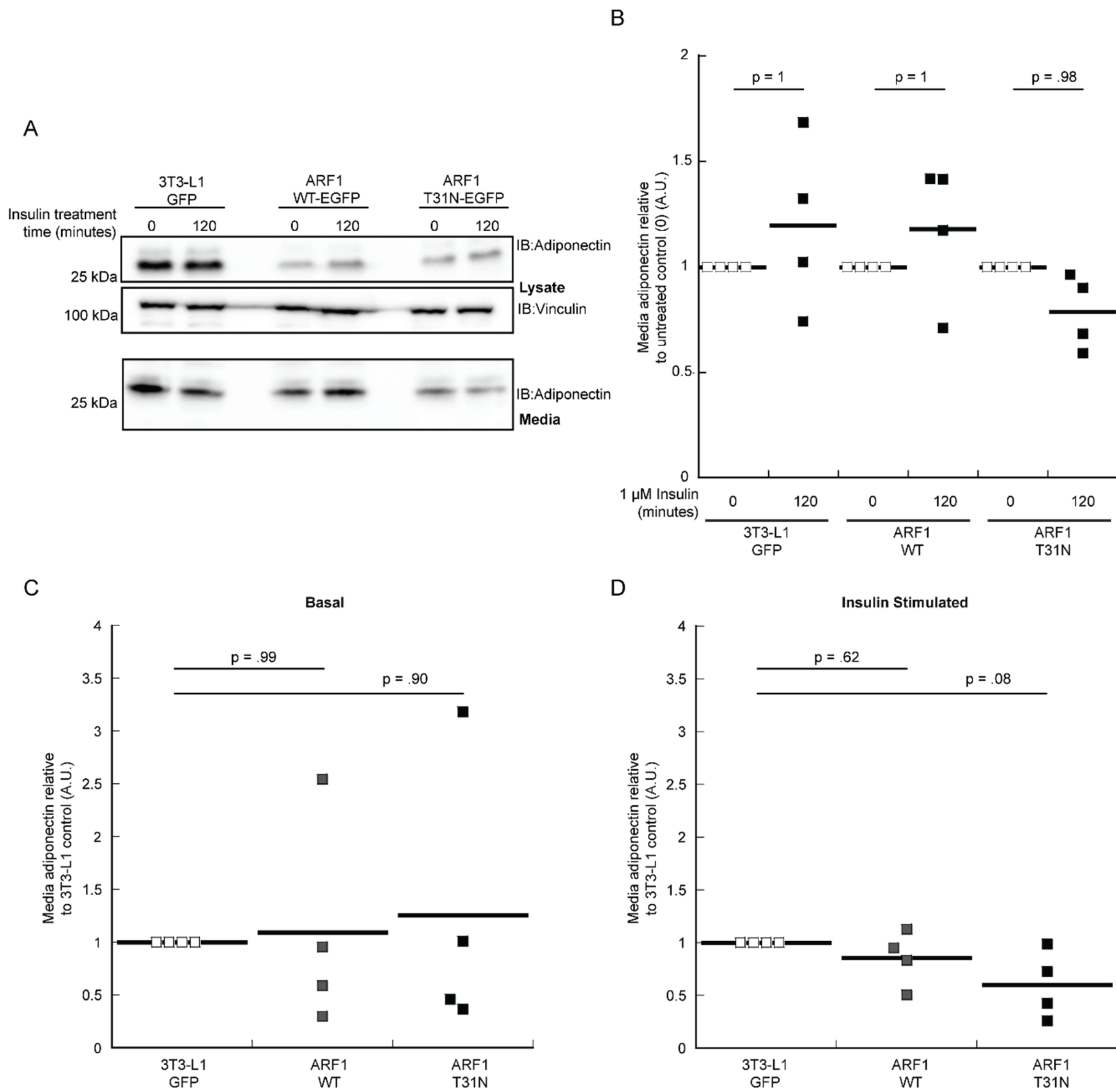

Fig. S7. ARF1 overexpression does not affect adiponectin secretion

A) 3T3-L1 adipocytes expressing either GFP, ARF1-WT, or ARF1-T31N were treated with vehicle or 1μM insulin for the indicated times. Media and lysates were collected and samples were analyzed for Adiponectin and vinculin by Western blot. B) Media adiponectin levels are plotted relative to untreated controls. Significant interactions were calculated with a two-way ANOVA and p-values were calculated with Šidák-corrected pairwise tests. C) Media adiponectin levels from unstimulated (basal, 0 minutes) or D) insulin-stimulated cells (120 minutes) are plotted relative to 3T3-GFP control cells. P-values were calculated with a one-way ANOVA with Dunnett's post-hoc test. Results from 4 replicate experiments are shown.