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Supplementary Materials for

Development of a physiological insulin resistance model in human stem cell–derived adipocytes

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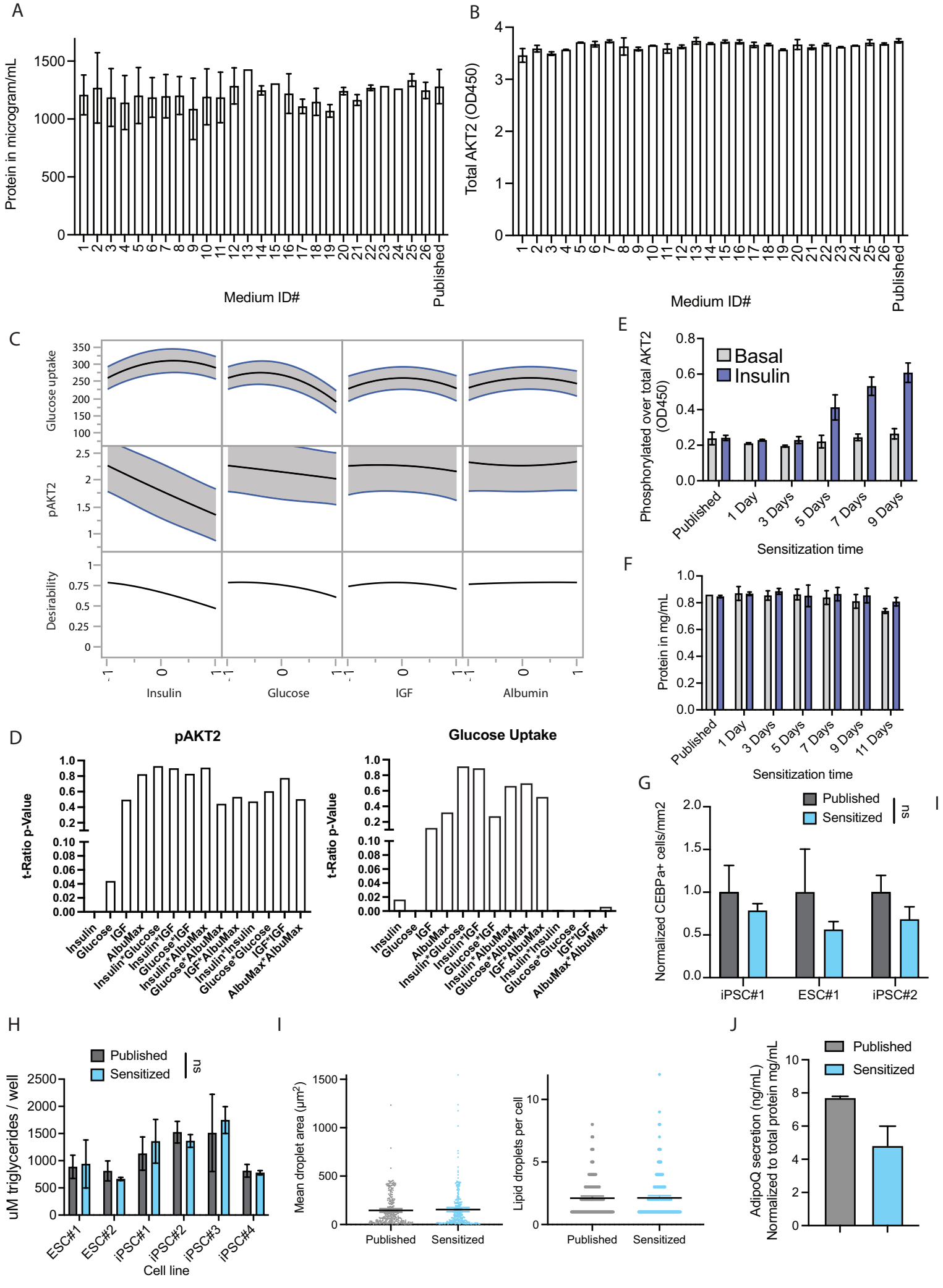
The PDF file includes:

Figs. S1 to S4
Legends for tables S1 and S2

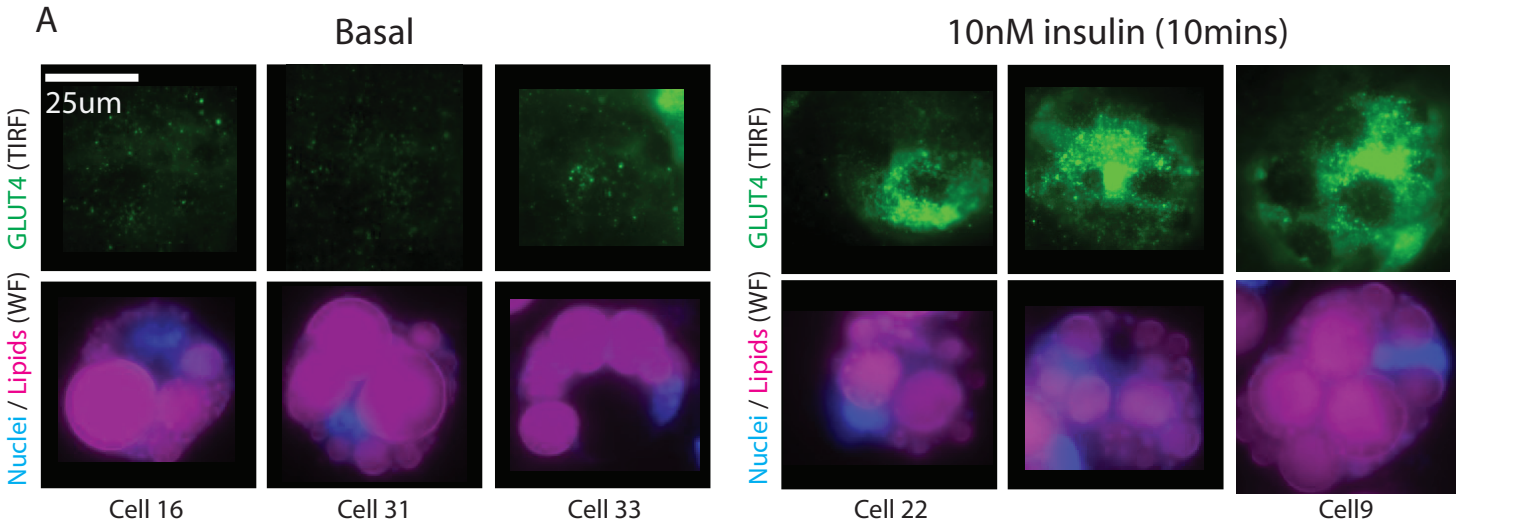
Other Supplementary Material for this manuscript includes the following:

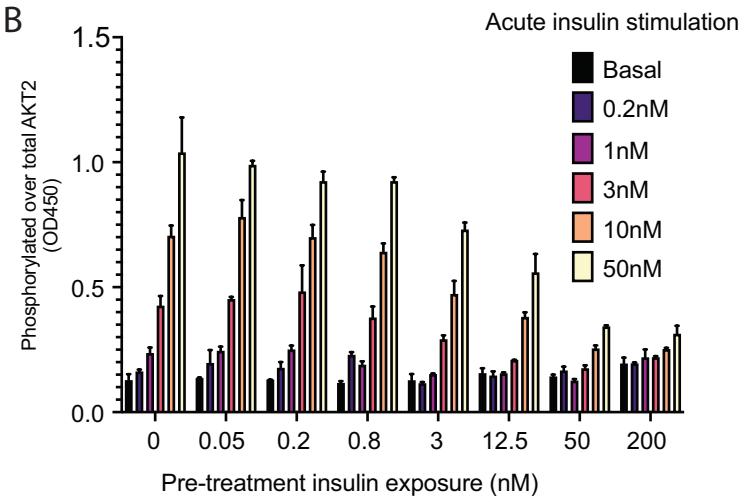
Tables S1 and S2

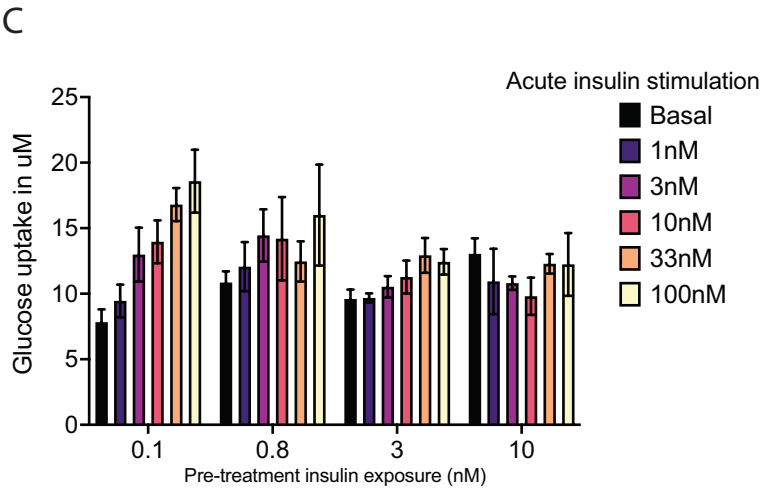
Supp Figure 1 Sensitized adipocytes retain viability and adipocyte features

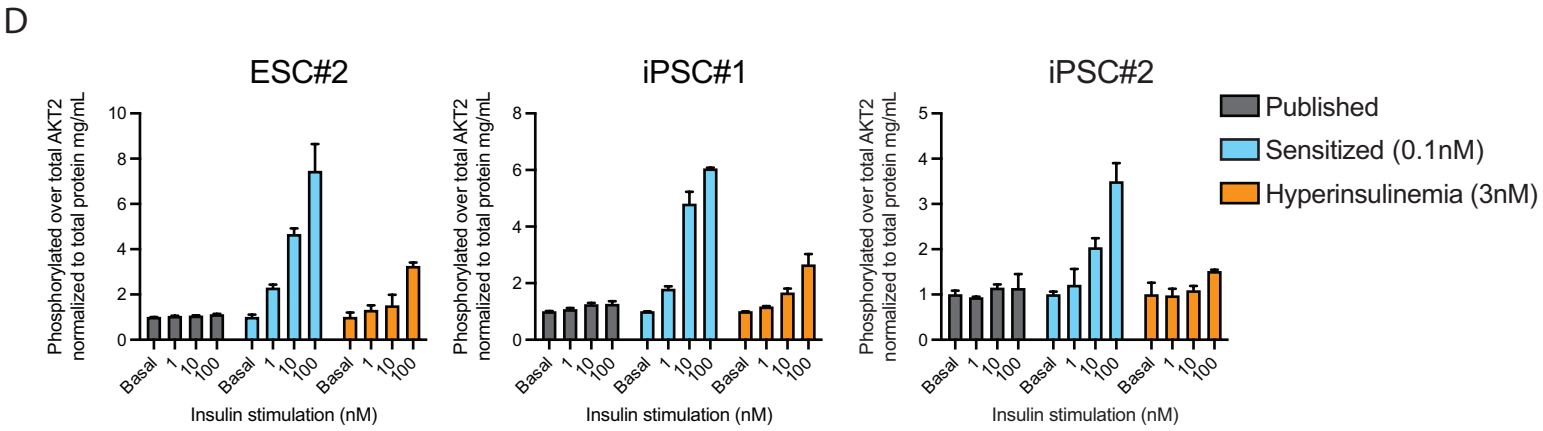


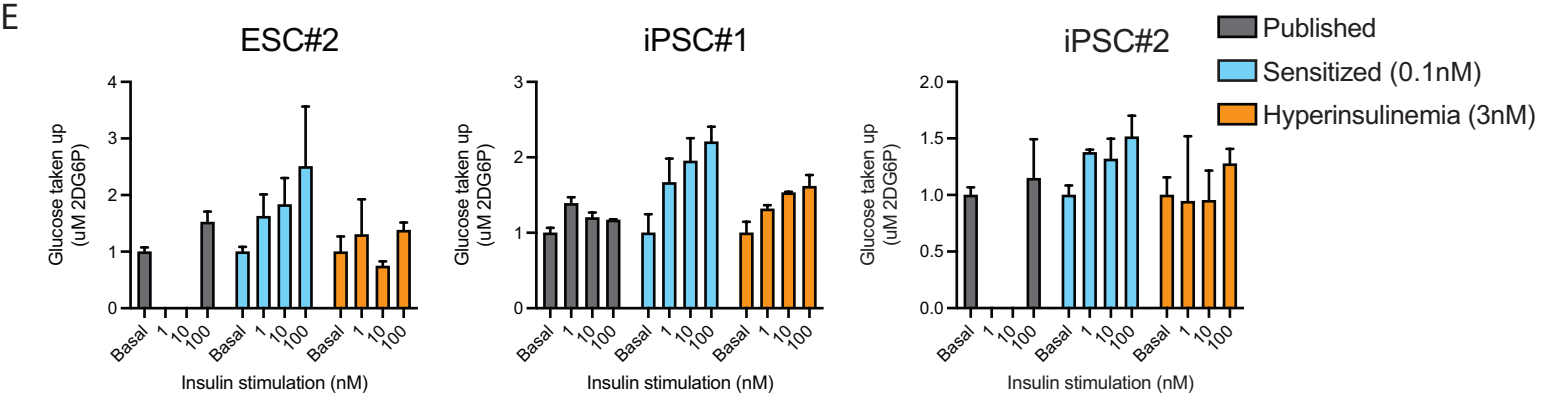
Supp Figure 2 Sensitization and hyperinsulinemia-induced resistance is recapitulated in multiple hPSC lines



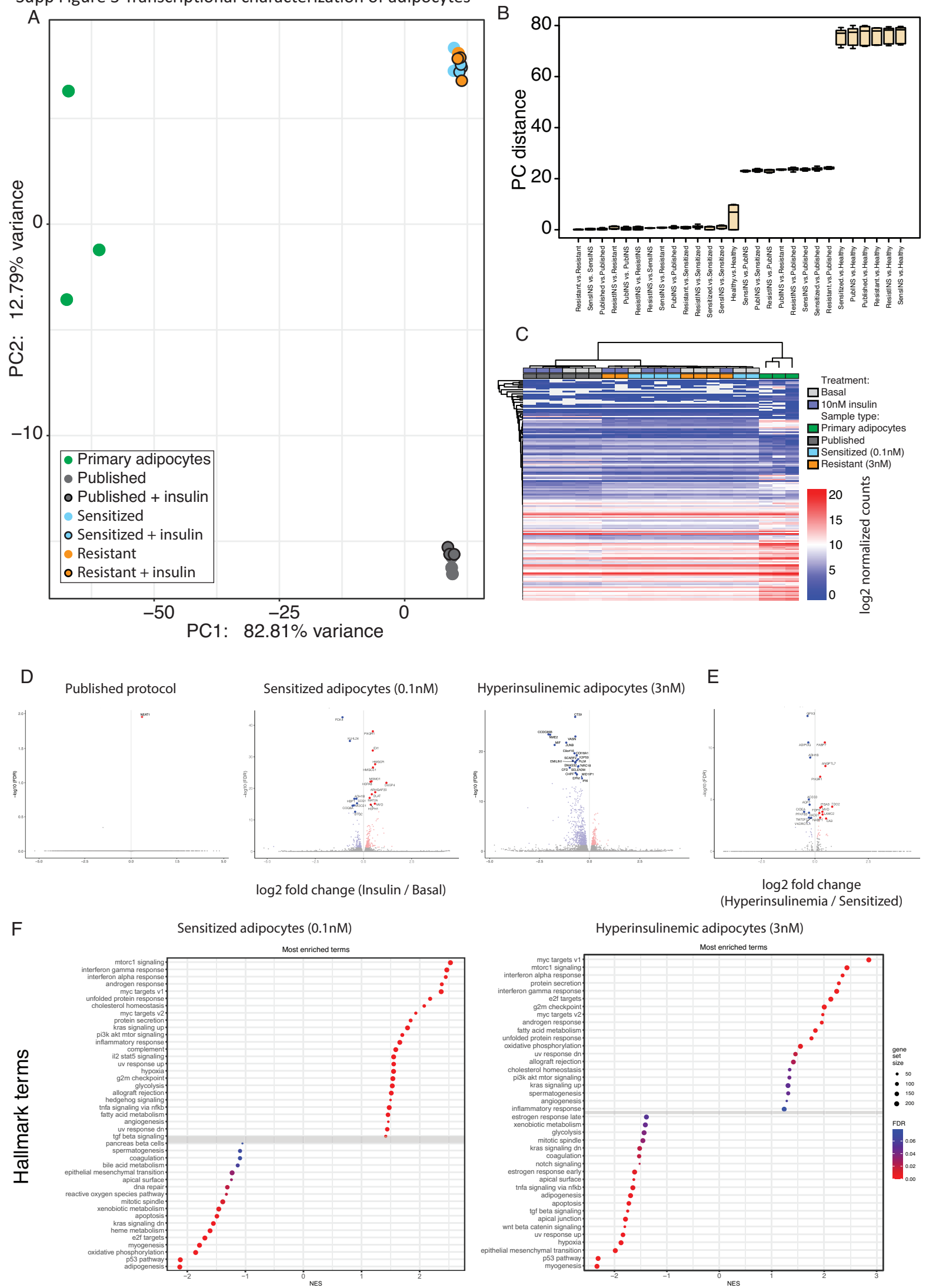






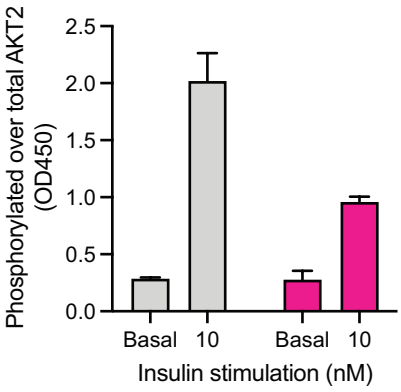


Supp Figure 3 Transcriptional characterization of adipocytes

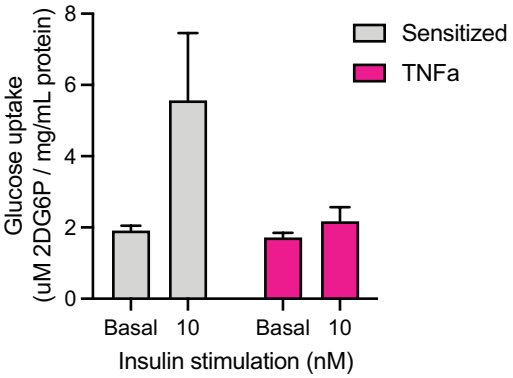


Supp Figure 4 TNFa induces insulin resistance

A



B



Supplemental fig. 1:
A, Total protein content of samples used in the DoE screen and the published protocol medium. B, Total AKT2 ELISA measurement of samples in the DoE screen. C, Profile curves for each factor illustrating the influence on glucose uptake and phosphorylation of AKT2 throughout the experimental space of the DoE. D, t-Ratio p-values indicating the significance of each factor in the DoE screen for phosphorylated AKT2 (left) and glucose uptake (right). E, Timecourse of sensitization with the DoE-optimized media measuring phosphorylation of AKT2 at baseline and after insulin stimulation. Results are normalized to total AKT2 for each sample. F, Total protein content of each sample during the glucose uptake timecourse (Fig. 1f). G, Quantification of adipocyte marker CEBPA-positive nuclei per area in published and sensitized-protocol adipocytes. H, Triglyceride content per sample of published and sensitized adipocytes in several cell lines. I, Mean lipid droplet area and lipid droplets per cell in hPSC-adipocytes in published or senstitization medium. J, Adiponectin secretion of adipocytes in the published or sensitized protocol, normalized to total protein. All bar graphs depict the mean with error bars representing s.d., n=2 biological replicates for A-G, n=3 for G,H,J.

Supplemental fig. 2:
A, Representative images of the TIRF quantification in fig. 1d. For each condition, the three cells closest to the mean are shown. B, Full panel of pre-treatment insulin exposure concentrations and acute stimulation dose-response measuring phosphorylated AKT2 normalized to total AKT2, related to figure 2f. C, As in B but measuring glucose uptake normalized to total protein, related to figure 2g. D, Phosphorylated AKT2 measurements normalized to total AKT2 for 3 additional hPSC lines. E, As in D but measuring glucose uptake normalized to total protein. All bar graphs depict the mean with error bars representing s.d., n=2 biological replicates for B,C, n=3 for D,E.

Supplemental fig. 3:
A, PCA plot of all samples assessed with RNA-sequencing. B, Measurement of PCA distances between all sample groups. C, Total expression heatmap of adipocyte genes identified through the Human Protein Atlas. D, Volcano plots of published, sensitized, and hyperinsulinemic adipocytes showing log2 fold change of genes upon insulin stimulation. Genes with FDR<=0.05 are colored, and the top 20 significant genes are labeled. E, Volcano plot of gene changes between sensitized and hyperinsulinemic adipocytes. Genes with FDR<=0.05 are colored, and the top 20 significant genes are labeled. F, GSEA results of all Hallmark terms that are enriched upon insulin stimulation in the sensitive (left) and resistant (right adipocytes, ranked by NES score, related to figure 3e.

Supplemental fig. 4:
A, Phosphorylation of AKT2 normalized to total AKT2 after TNFa exposure. B, Glucose uptake normalized to total protein after TNFa exposure. All bar graphs depict the mean with error bars representing s.d., n=3 biological replicates.

Supplemental table 1:
Layout of the Design-of-experiments approach. The log pattern and concentration for each of

the four factors are given.

Supplemental table 2:
Significantly different genes between the sensitized and resistant adipocytes, and the DAVID results of this list of genes.