Supplemental Materials

Molecular Biology of the Cell

To et al.

SUPPLEMENTAL MATERIALS

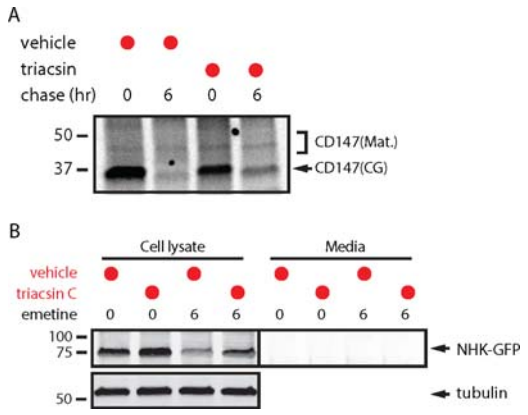

FIGURE S1. Analysis of CD147 maturation and NHK secretion. (**A**) HEK293 cells were pretreated with vehicle or 1 µg/mL triacsin C for 16 hr, pulse labeled, and samples collected at 0 hr and 6 hr. CD147 was immunoprecipitated, separated by SDS-PAGE, and radioactivity detected using a Typhoon 9400. (**B**) HEK293 cells expressing NHK-GFP were treated with vehicle or 1 µg/mL triacsin C for 16 hr. Cells were washed with PBS, and the media was replaced with serum-free OPTI-MEM containing vehicle or 1 µg/mL triacsin C for the remaining 6 hr. Lysates and NHK-GFP immunoprecipitated from the media were analyzed by immunoblotting.

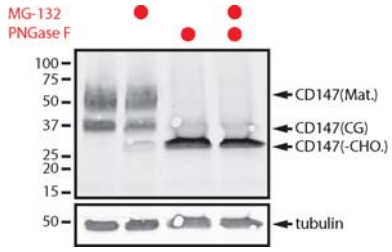

FIGURE S2. Proteasome inhibition causes accumulation of CD147 in a deglycosylated form. HEK293 cells incubated with vehicle or 10 µM MG-132 for 6 hr were lysed in 1% SDS. Lysates were then incubated in the presence and absence of PNGase F for 30 min at 37^{o}C. Proteins were separated by SDS-PAGE and analyzed by immunoblotting.

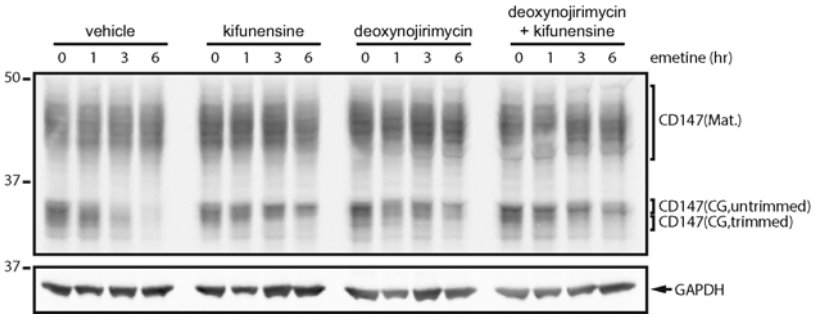

FIGURE S3. Analysis of glucosidases and mannosidases in CD147 glycan trimming and degradation. HEK293 cells were incubated with 75 µM emetine in the presence and absence of 5 µg/mL kifunensine and 50 µM deoxynojirimycin as indicated. SDS lysates were separated on large format SDS-PAGE gels and analyzed by immunoblotting to visualize the different CD147 glycoforms.

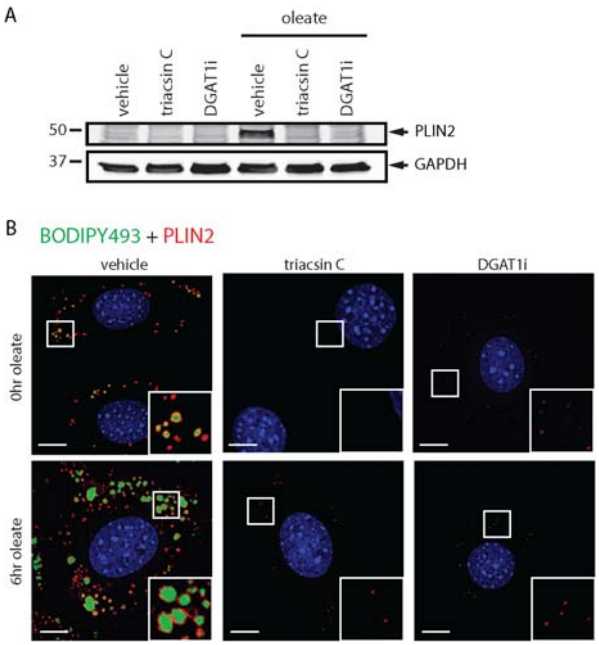

FIGURE S4. Triacsin C and DGAT1 reduce the amount of PLIN2-positive lipid droplets. (**A**) DGAT2-/- MEFs were pretreated with 1 µg/mL triacsin C or 20 µM DGAT1i for 3 hr and then incubated with 200 µM oleate for 0 hr or 6 hr as indicated. Cells were lysed in 1% SDS and PLIN2 levels were analyzed by immunoblotting. (**B**) Cells were treated as in panel A and immunofluorescence microscopy employed to visualize PLIN2 (red), LDs (green), and nuclei (blue). Scale bar = 10 µm.

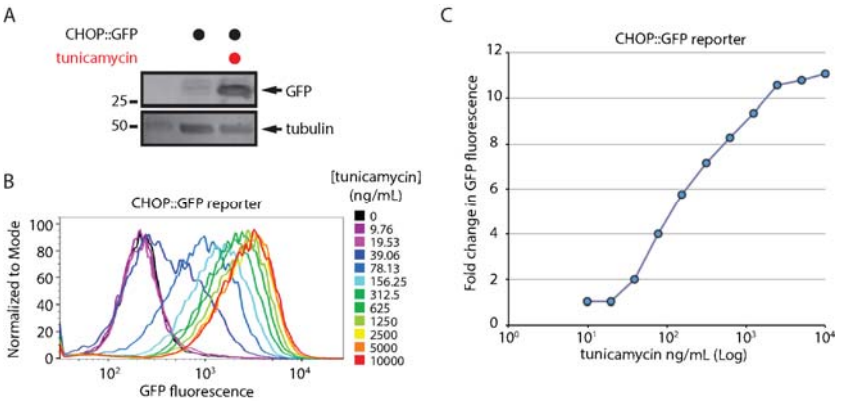

FIGURE S5. Characterization of a CHOP::GFP reporter cell line. (**A**) Untransfected HEK293 cells or HEK293 cells stably expressing the CHOP::GFP reporter plasmid were incubated in the presence or absence of 5 µg/mL tunicamycin as indicated. GFP levels were analyzed by immunoblotting. (**B**) HEK293 cells stably expressing a CHOP::GFP construct were treated with increasing concentrations of tunicamycin. GFP levels were measured using flow cytometry and are represented as a histogram normalized to the mode. (**C**) The fold change in GFP fluorescence levels relative to time 0 hr from cells treated as in panel B is shown.

SUPPLEMENTAL TABLES

TABLE S1. High confidence Hrd1 interacting proteins.

TABLE S2. SILAC proteomic analysis of Hrd1 interacting proteins.

TABLE S3. Metabolomic Profiling of triacsin C treated cells.