Supporting Information

Guenther *et al.* 10.1073/pnas.0802781105

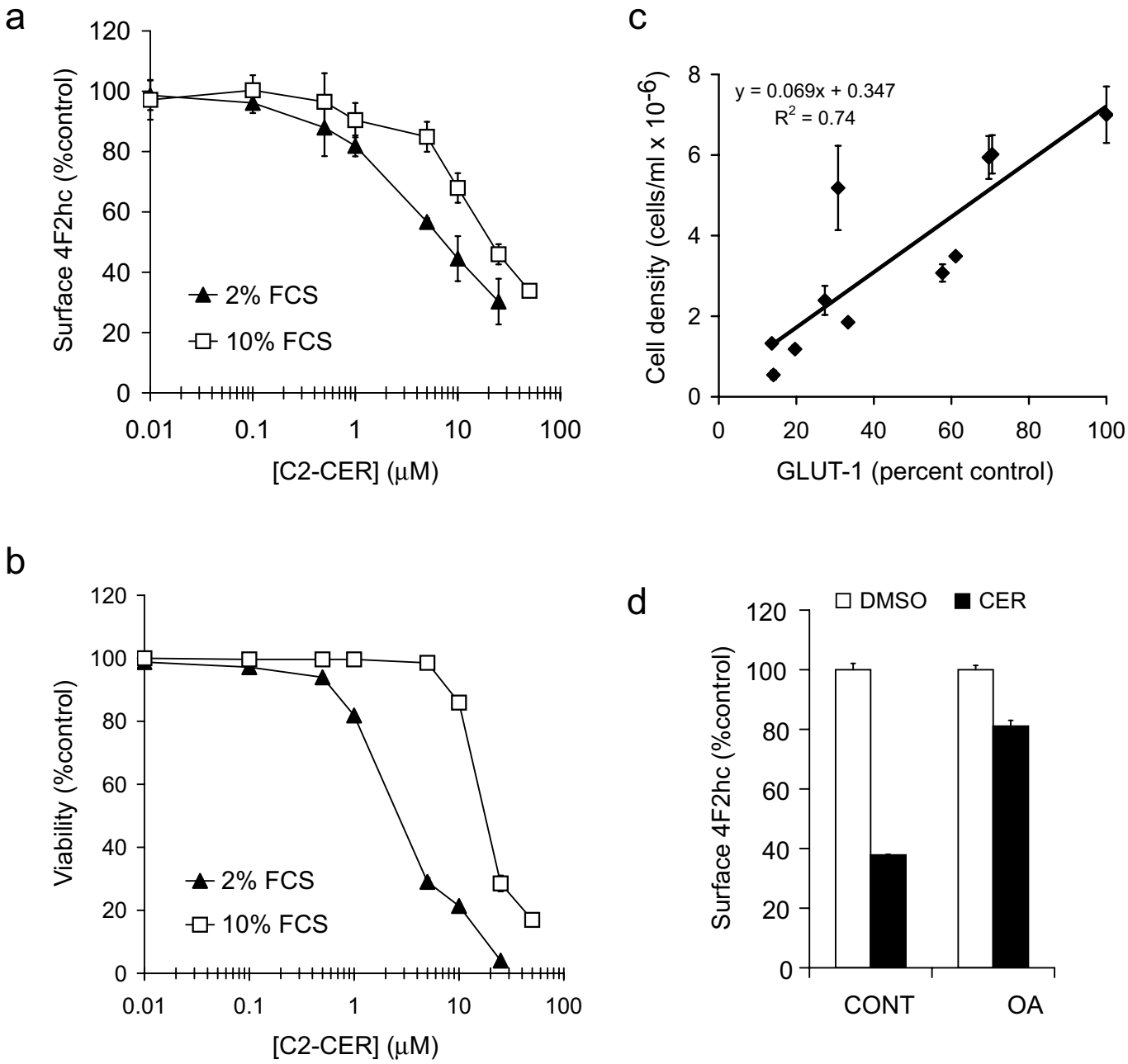

Fig. S1. Ceramide induces intracellular nutrient stress by causing nutrient transporter protein downregulation. (*a*) Surface expression of 4F2hc was evaluated by flow cytometry 8 h after FL5.12 cells were treated with C2-cer in media containing either 2% or 10% FCS. Error bars, SD. (*b*) Viability of the cells in *a* was determined at 24 h. Error bars, SD. (*c*) Independent FL5.12 clonal lines stably expressing one of four different shRNA targeting GLUT-1 were plated at 25,000 cells/ml and cell density was measured 4 days later (error bars, SEM). GLUT-1 expression level was quantified by Western blot using an Odyssey Infrared Imaging System. (*d*) FL5.12 cells were treated with DMSO or 50 μM C2-cer in the presence or absence of 1 μM okadaic acid (OA) and 4F2hc expression measured at 4 h. Error bars, SD.

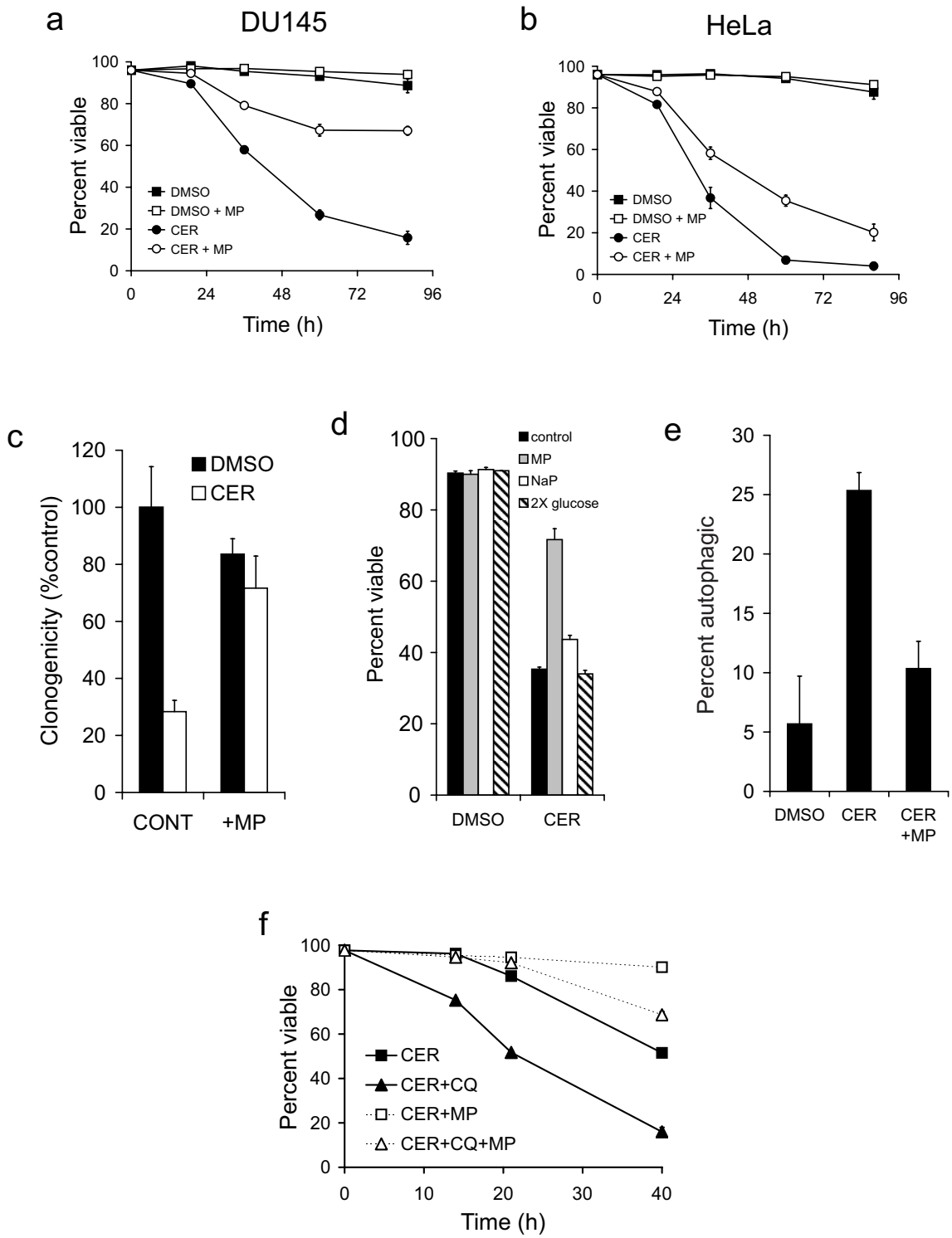

Fig. S2. Methyl pyruvate supplementation rescues cells from ceramide. (*a*) Viability of DU145 cells treated with DMSO or 50 μM C2-cer in the presence or absence of methyl pyruvate. Error bars, SD. (*b*) Viability of HeLa cells evaluated as in *a*. (*c*) Clonogenicity of cells treated for 24 h with DMSO or with 25 μM C2-cer in the presence or absence of methyl pyruvate. Error bars, SEM. (*d*) Cells were maintained for 16 h in medium containing DMSO or 25 μM C2-cer and supplemented with 11 mM methyl pyruvate (MP), sodium pyruvate, or glucose as indicated. Error bars, SD. (*e*) The fraction of autophagic cells was quantified on the basis of GFP-LC3 localization in FL5.12 cells treated with DMSO or 50 μM C2-cer for 4 h in the presence or absence of methyl pyruvate. Error bars, SD. (*f*) Viability of FL5.12 cells treated with 10 μM C2-cer in the presence or absence of 10 μM CQ and methyl pyruvate (MP). Error bars, SD.

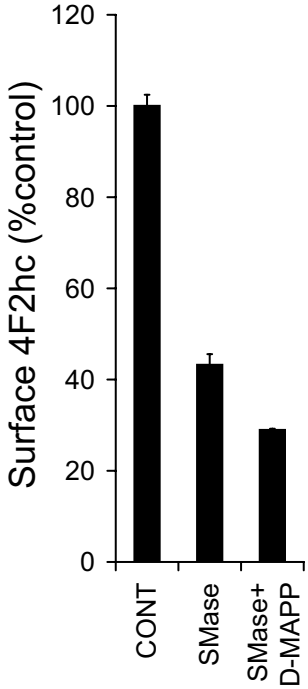

Fig. S3. Ceramide produced from sphingomyelin degradation causes nutrient transporter downregulation. Surface expression of 4F2hc by flow cytometry in cells left untreated (CONT) or incubated for 2 h with 1 U/ml of bacterial SMase in the presence or absence of 10 μM D-MAPP. Error bars, SD.