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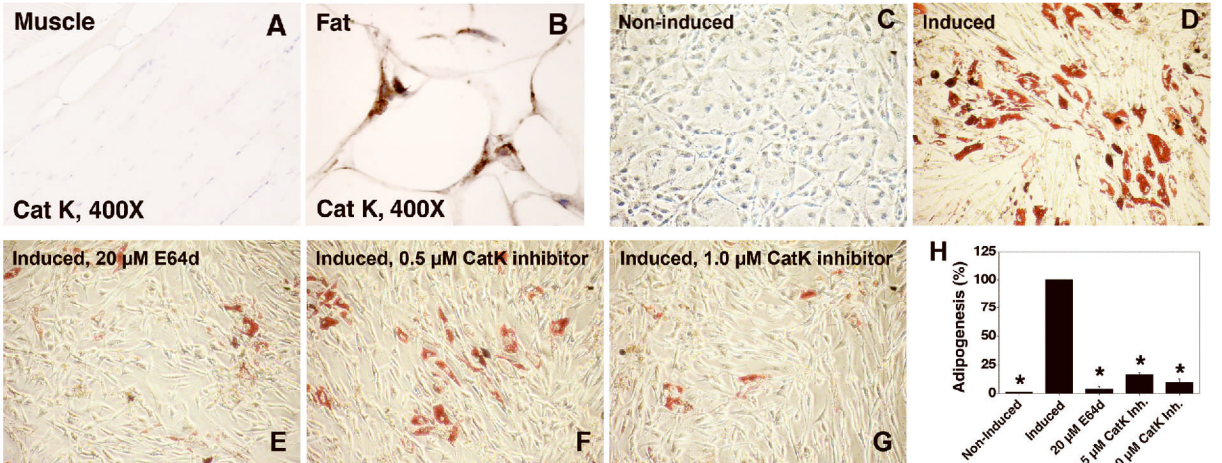

Supplemental Figure I. CatK expression in human adipose tissue and its role in human preadipocyte differentiation. Immunohistology with human CatK antibody revealed negligible CatK expression in normal human muscle (**A**) (n=9), but high CatK expression in human visceral fat (n=9) (**B**). Oil-red O staining of non-differentiated human pre-adipocytes (**C**), differentiated adipocytes (**D**), and differentiated adipocytes in the presence of non-selective cathepsin inhibitor E64d (**E**), 0.5 μM (**F**) and 1 μM (**G**) of a CatK-selective inhibitor. **H**. Quantification of oil-red O staining relative to the positive control (Induced). Data are mean±SE of four experiments. *P<0.005.

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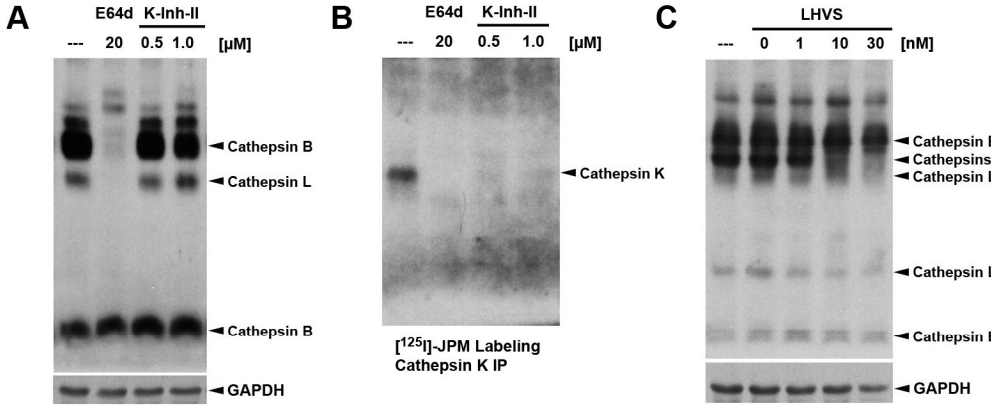

Supplemental Figure II. Cysteine protease cathepsin active site labeling. **A.** 3T3-L1 adipocytes were incubated with CatK-selective inhibitor (K-Inh-II, Calbiochem, La Jolla, CA) or E64d for 6 hrs. Cells were lysed and equal amount of cell lysate (50 μg/sample) were labeled with [^{125}I]-JPM for 1 hr at 37 °C followed by separation on a 12% SDS-PAGE (*J Biol Chem.* 1992;267:7258-7262). At 0.5~1 μM, CatK inhibitor did not inhibit cathepsins L or B. E64d (20 μM) was used as positive control for complete inhibition of all cathepsins. **B.** 3T3-L1 adipocytes were incubated with K-Inh-II and E64d for 6 hrs. Cell lysates were prepared and equal amount of protein (200 μg/sample) was labeled with [^{125}I]-JPM. Cell lysates were then neutralized with 1 M Tris.HCl, pH10.0, boiled, and immunoprecipitated with CatK monoclonal antibody (Santa Cruz) followed by separation on a 12% SDS-PAGE. **C.** Mouse peritoneal macrophages were incubated with CatS-selective inhibitor LHVS (*J Exp Med.* 1997;186:549-560) under indicated concentrations overnight at 37 °C. Equal protein loading for panels **A** and **C** was viewed by immunoblot analysis for GAPDH (bottom panels).

Type of file: figure
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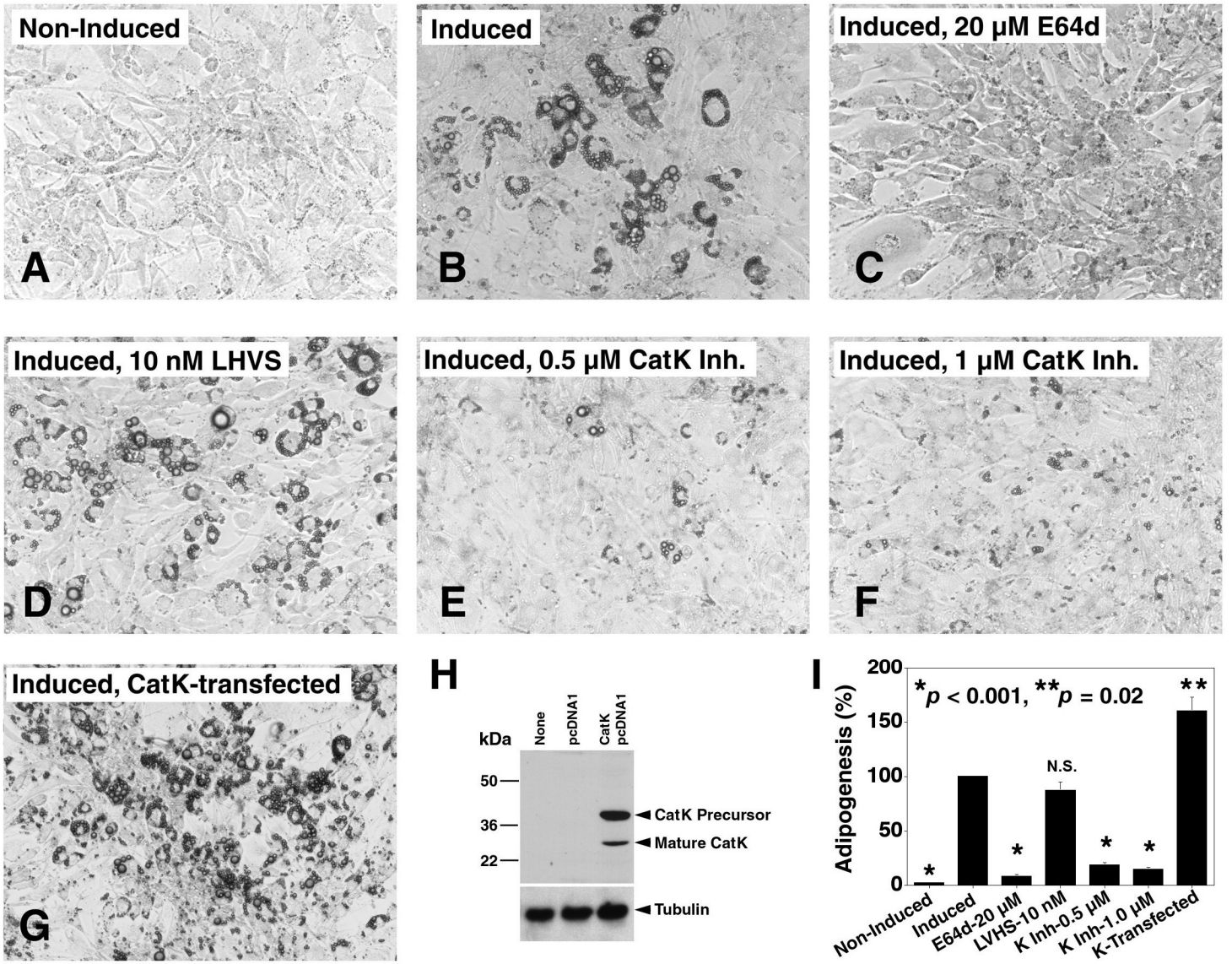

Supplemental Figure III. Inhibition of CatK reduces 3T3-L1 cell adipogenesis. Oil-red O staining revealed negligible lipid deposition in non-induced 3T3-L1 cells (**A**) but increased oil-red O-positive staining in cells differentiated into adipocytes (**B**). 3T3-L1 cell differentiation was blocked with 20 μM E64d (**C**), but much less by 10 nM of CatS-selective inhibitor LHVS (**D**). Strong inhibition of adipogenesis was detected when cells were incubated with 0.5 μM (**E**) or 1 μM (**F**) of CatK-selective inhibitor. In contrast, CatK over-expression in 3T3-L1 cells enhanced adipogenesis (**G**). **H**. Human CatK immunoblot revealed mature and pro-CatK in transfected cells, but not in those non-transected or transfected with empty vector. A mouse tubulin immunoblot was used for protein loading control. **I**. Oil-red O staining levels relative to differentiated positive control cells (N.S.=no significant difference).