Supplementary Data

Identification of ILK as a new partner of the ADAM12 disintegrin and metalloprotease in cell adhesion and survival

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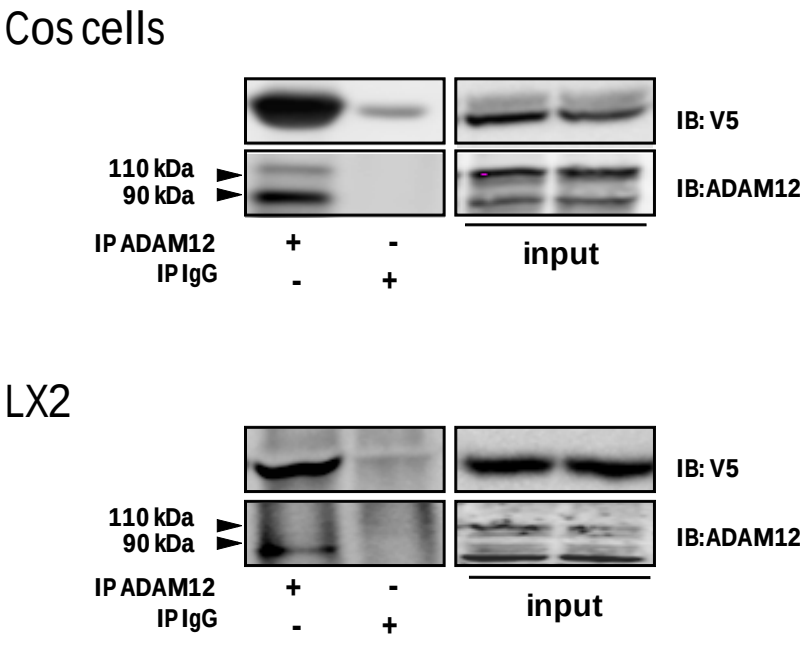
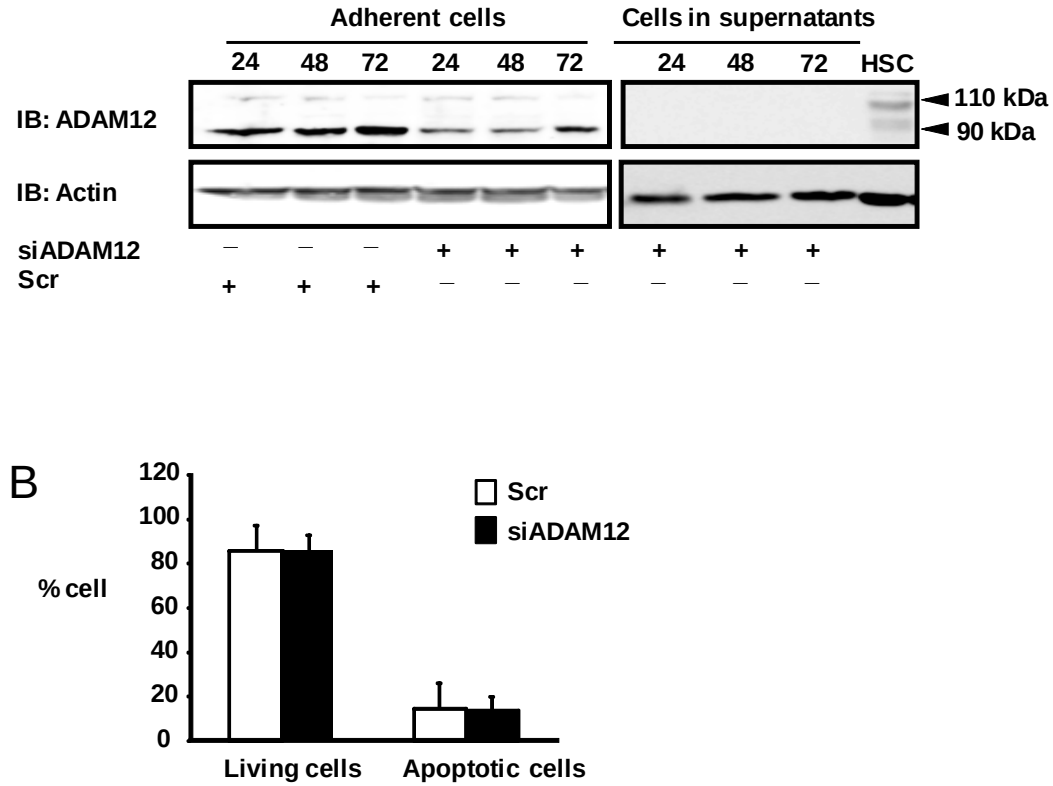
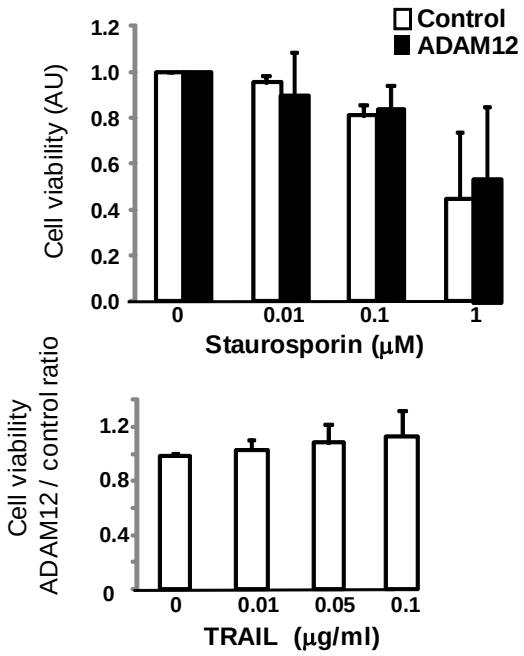
This section contains Supplementary Figure S1, Figure S2, Figure S3 and Figure S4.



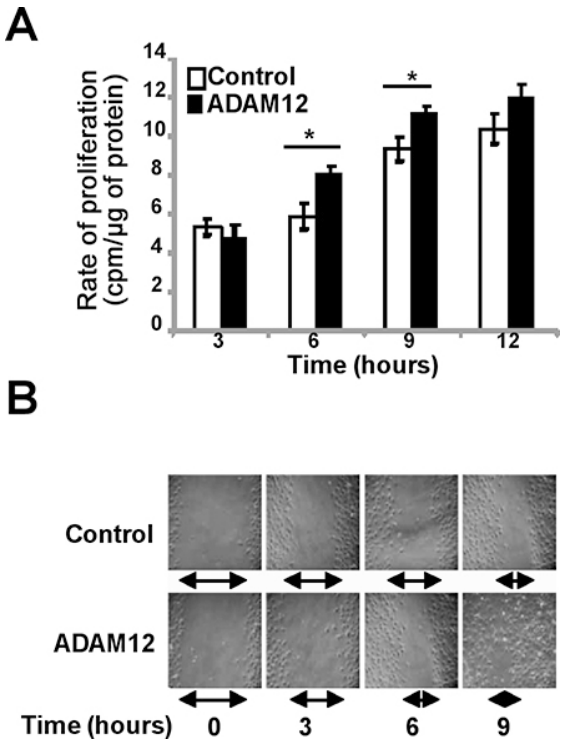
Supplementary Figure S1. ADAM12L interactions with ILK in Cos7 and LX2 cells. Cell extracts were prepared from Cos7 cells co-tranfected with the long form of ADAM12, ADAM12L and V5-tagged ILK, or from hepatic stellate LX2 cells transfected with V5-tagged ILK. Extracts were immunoprecipitated (IP) with anti-ADAM12 and immunoblotted (IB) with anti-V5. The precursor and processed (active) forms of ADAM12 correspond to the 110 and 90-kDa species, respectively, detected by the antibody used.



Supplementary Figure S2: ADAM12 silencing in HSCs is associated with loss of cell adhesion without induction of cell death. HSCs were transfected with 2 nM non-targeted siRNA (Scr) or ADAM12L siRNA (siADAM12) for the indicated times. A) Protein extracts from cells collected in culture supernatants and from adherent cells were immunoblotted with anti-ADAM12 antibodies (IB). Immunoblots for actin are shown as controls. Cell extracts from non-transfected HSCs were used as control (HSC). B) Apoptosis was measured after 48 hrs of transfection by microscopic detection using Hoechst 33342 labeling (0.5 µg/ml). Cells with apoptotic nuclei were counted relative to the total population (n = 200).



Supplementary Figure S3: Overexpression of ADAM12 does not protect cells from Staurosprine- and TRAIL-induced cell death. Chinese hamster ovary (CHO) cells were transfected with ADAM12L or an empty vector (control). At 48 hours post transfection, cells are further treated for 24 hours with A) Staurosporine (0-1 μM) and B) Flag-tagged TRAIL (0-0.1 μg/ml) from Alexis Biochemicals. A total of 2 μg/mL anti-Flag M2 (Sigma-Aldrich, Saint-Quentin Fallavier, France) was added to induce TRAIL oligomerization. Cell viability was assessed by methylene blue staining. Data represent the mean ± SD from three independent experiments performed in sextuplet.



Supplementary Figure S4. Expression of ADAM12 favors cell proliferation and migration. Cos7 cells were transfected with empty vector (control) or ADAM12 constructs and cultured for 48 hrs. The cells were then plated on dishes coated with type I collagen for the indicated times. (A) Cell proliferation was evaluated by ^{3}[H]-thymidine incorporation. Results are expressed as the mean ± s.d. of three independent experiments (*, $p < 0.05$). (B) Cell migration was evaluated by the scratch wound-healing assay, in which a confluent cell monolayer is scratched with a pipette tip and the bare area is subsequently assessed for relocation of cells. Representative data from three independent experiments are shown and arrows indicate the width of the wound.