

Reelin promotes adhesion of multiple myeloma cells to bone marrow stromal cells via integrin β 1 signaling

Liang Lin^{a#}, Xinwei Zhang^{a#}, Quanming An^a, Jie Hao^a, Rong Jin^a, Yingjun Chang^b, Xiaojun Huang^b, Jin Lu^{b*}, Qing Ge^{a*}

Supplemental information

Supplemental Figure 1. Reelin promotes the adhesion of U266 cells to HS-5 cells. Calcein-AM-labeled U266 cells were incubated with rReelin, and/or CR-50 or isotype control (Ctrl) mouse IgG antibody for 1 hour. The cells were washed and added in triplicate to HS-5-seeded wells at 37 °C for 1 hour. The unattached cells were removed by three washes and the attached U266 cells were analyzed by laser-scanning confocal microscopy (A) and by calculation of the average numbers of labeled cells per well (B). The values obtained in each group were calculated relative to U266 cells without treatment (Blank) and were shown as relative adhesion (cells) in (B).

Supplemental Figure 2. Overexpression and knockdown of Reelin. (A-B) Overexpression of reelin in H929 cells. H929 cells were transfected with 10 μ g Reelin-expressing plasmid pCrl or control vector pcDNA3. The cells were harvested 24 or 40 hours later, and the mRNA (A) and protein (B) expressions of Reelin were analyzed by quantitative RT-PCR and western blotting. (C-D) Knockdown of Reelin in H929 cells. H929 cells were transfected with 300 pmol Reelin-specific or control siRNA for 24 or 40 hours. The cells were harvested and the mRNA (C) and protein expressions (D) of Reelin were determined. (E-F) Overexpression or knockdown of Reelin changed the adhesion toward HS-5. U266 cells were transfected with Reelin-expressing plasmid pCrl or Reelin-specific siRNAs. Forty hours later, the cells were labeled with Calcein-AM and added in triplicate to HS-5 cells at 37 °C for 1 hour. Cell adhesion was then analyzed by laser-scanning confocal microscopy (E) and

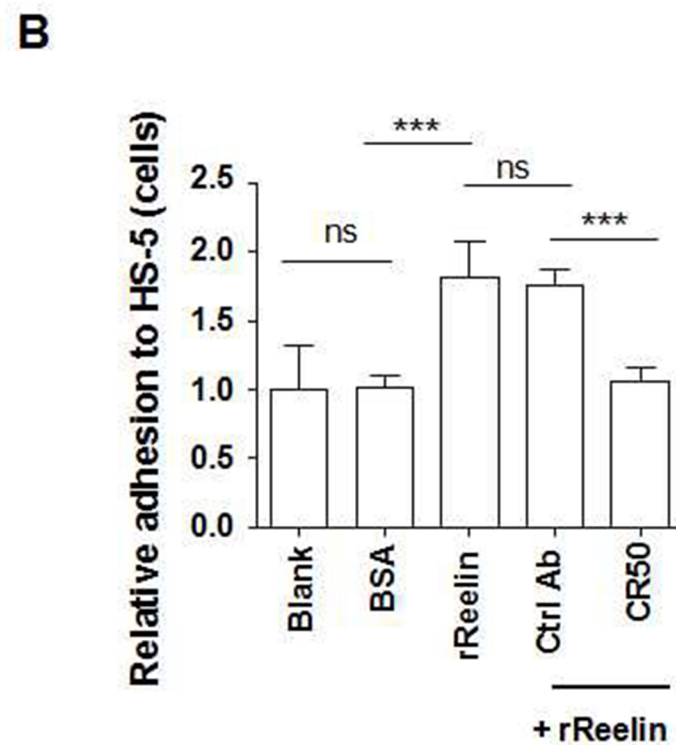
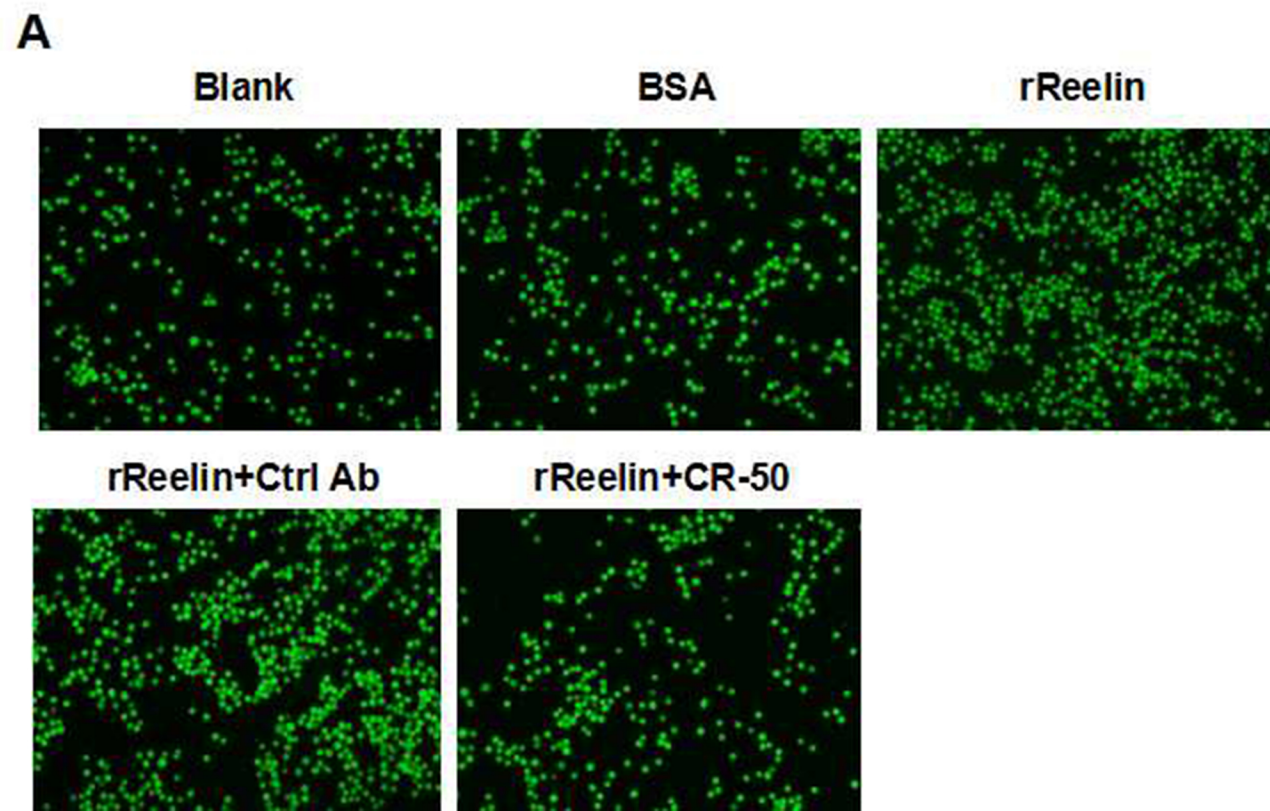
by calculation of the average numbers of labeled cells per well (F). (G-H) The promotion of U266 cell adhesion to HS-5 cells is Reelin specific. U266 cells transfected with Reelin-specific siRNAs or m-siRNA1 were cultured in HS-5-seeded plates in the presence of BSA or rReelin. Cell adhesion was then analyzed by laser-scanning confocal microscopy (G) and by calculation of the average numbers of labeled cells per well (H).

Supplemental Figure 3. Integrin β 1 is involved in Reelin-mediated myeloma cell adhesion. (A) Reelin and integrin β 1 expression in transfected H929 cells. H929 cells were transfected with Reelin-expressing plasmid pCrl or control plasmid for 40 hours. The cells were cultured in FN-coated plates overnight and stained with antibodies against integrin β 1 (red) and Reelin (green). Nuclei were stained with Hoechst 33342. The cells were analyzed by laser-scanning confocal microscopy (40x). (B) Reelin-mediated U266 adhesion to HS-5 cells is blocked by P4C10. Labeled U266 cells were cultured in HS-5-seeded plate in the presence of BSA, rReelin, rReelin with control antibody or rReelin with β 1 blocking antibody P4C10 for 1 hour. Cell adhesion was then analyzed by laser-scanning confocal microscopy (left panel) and by average adhesion cell numbers per well (right panel). (C) Reelin and ApoER2 expression in transfected H929 cells. H929 cells were transfected with Reelin-expressing plasmid pCrl or control plasmid for 40 hours. The cells were cultured in FN-coated plates overnight, and stained with antibodies against ApoER2 (red) and Reelin (green). Nuclei were stained with Hoechst 33342. The cells were analyzed by laser-scanning confocal microscopy (40x).

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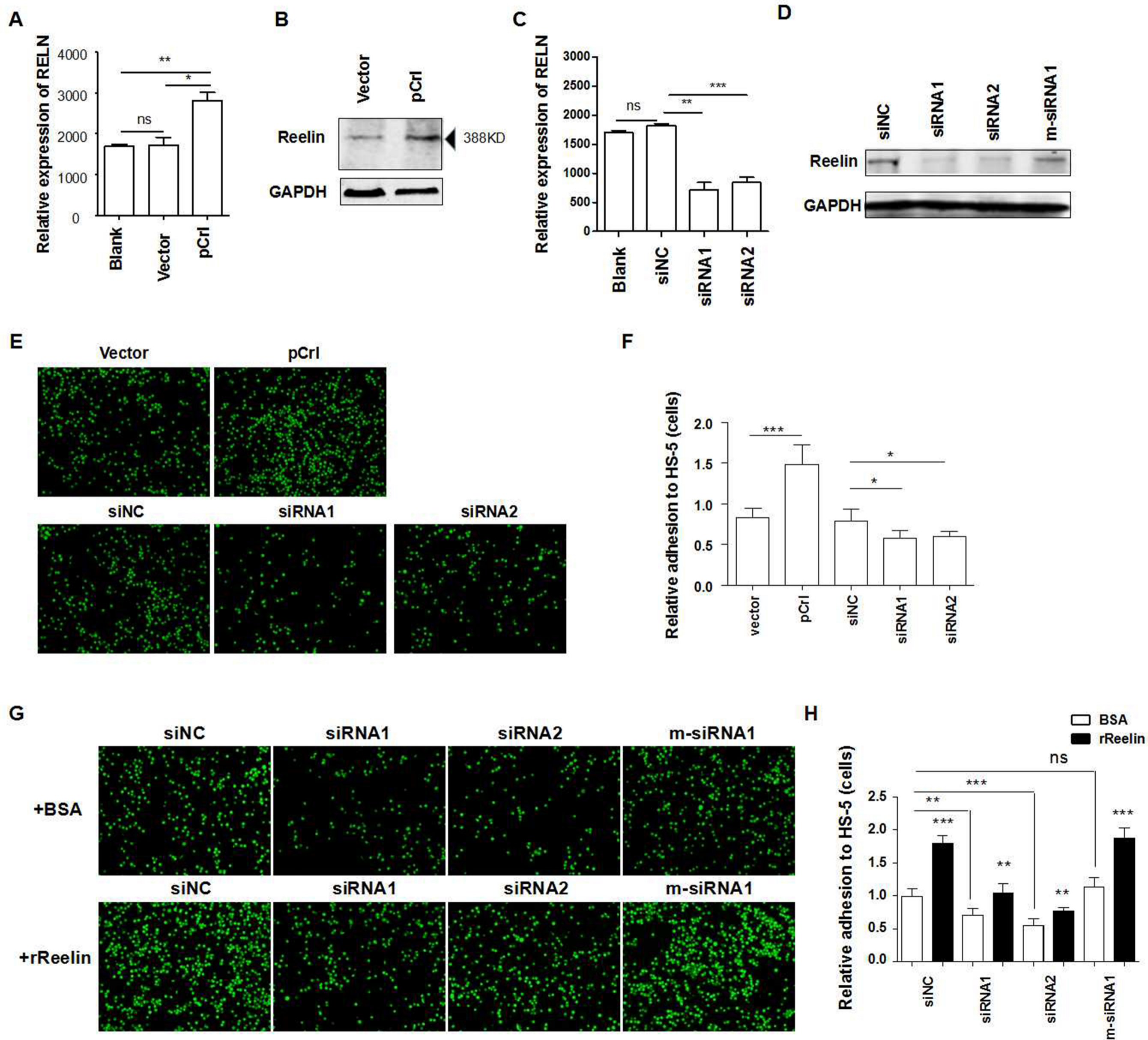
sFigure 1



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sFigure 2



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sFigure 3

