Cardiotrophin-like cytokine (CLCF1) modulates mesenchymal stem cell osteoblastic differentiation.

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Materials: Allophycocyanin (APC)-labelled anti-mouse CD45.2 (17-0454-82, Thermo Fisher Scientific); Phycoerythrin (PE)-labelled CD11b (557397, BD Biosciences); V450-labelled anti-Sca1 (62-5981-82, Thermo Fisher Scientific); FITC-labelled anti-CD44 (11-0441-81, Thermo Fisher Scientific).

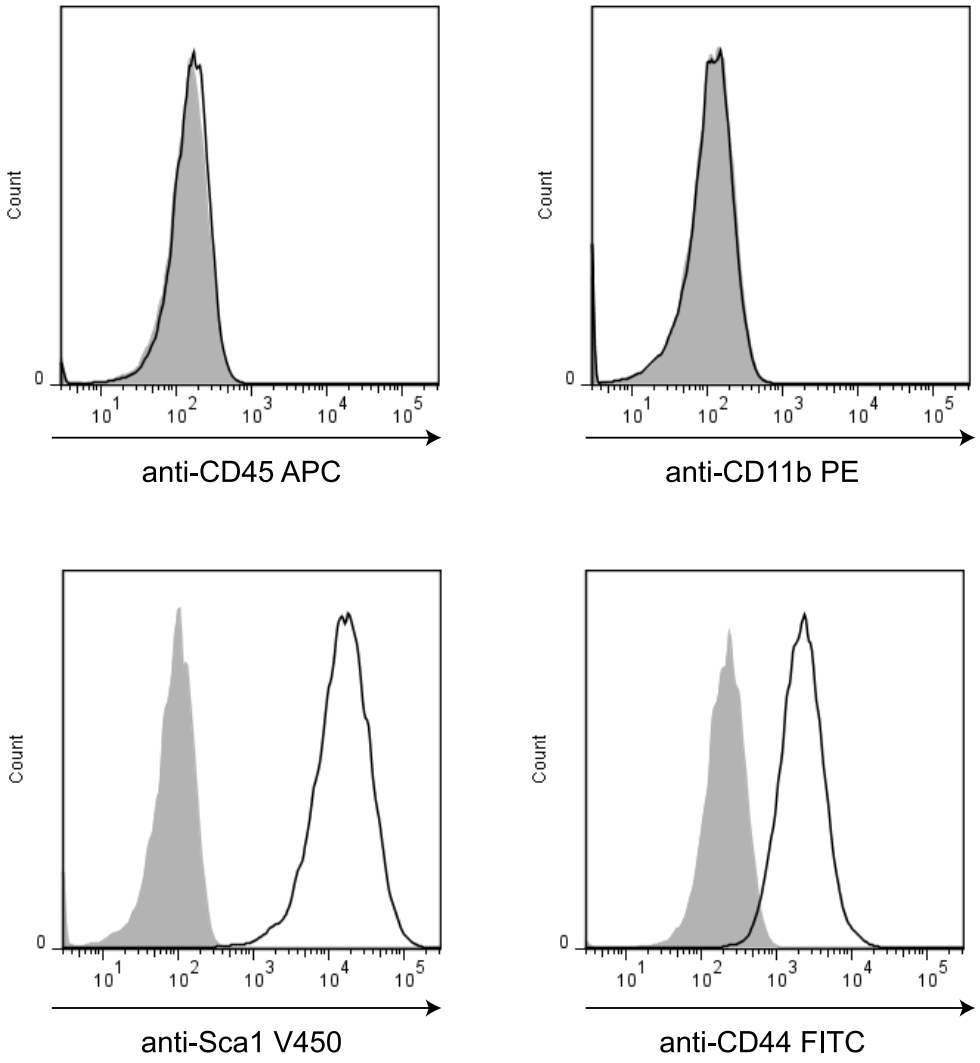

Figure S1. Mesenchymal stem cell surface marker expression. Bone marrow MSC surface marker expression was assessed by flow cytometry. Passage 8 MSC were detached and stained using APC-labelled anti-CD45.2, PE-labelled anti-CD11b, V450-labelled anti-Sca1 and FITC-labelled anti-CD44 antibodies. The gray shading and black line histograms show the unstained and stained MSC, respectively.

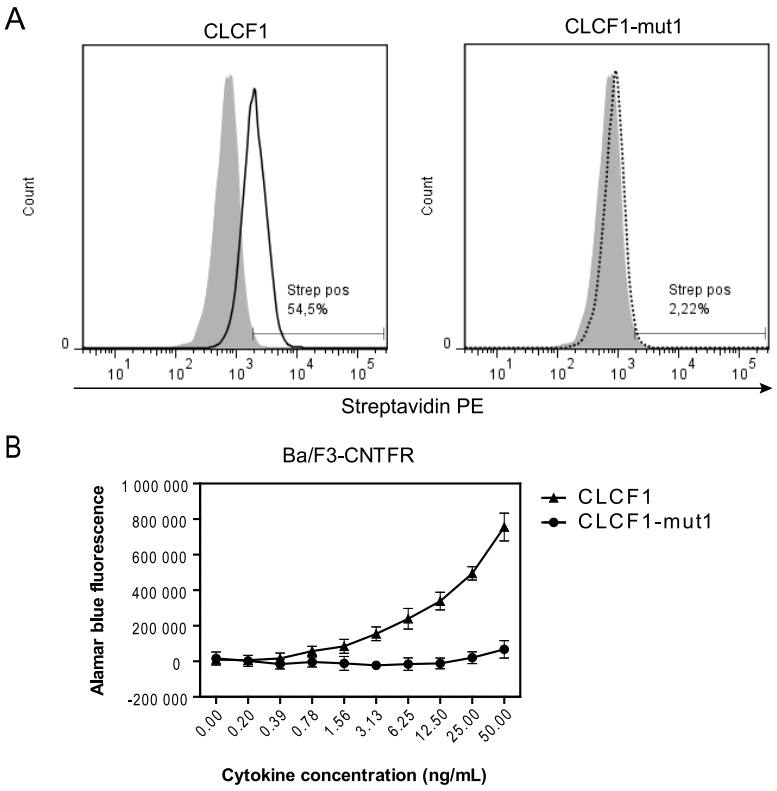

Figure S2. CLCF1-mut1 does not bind or activate CNTFR. (A) Ba/F3 transfectants expressing the tripartite CNTFR (CNTFRα, LIRβ and gp130; 1 x 10^6 cells) were incubated with biotinylated-CLCF1 or CLCF1-mut1 (both at 1 µg/mL) for 1 h then stained with PE-conjugated streptavidin. Fluorescence was assessed by flow cytometry. The gray filled histogram represents the fluorescence of MSCs incubated with streptavidin alone. The solid and dotted lines represent MSC incubated with CLCF1 and CLCF1-mut1, respectively. **(B)** Ba/F3 transfectants expressing CNTFR (50,000 cells/mL) were incubated with CLCF1 or CLCF1-mut1 (0 to 50 ng/mL dilutions) for 72 h. The proliferation was assessed using the Alamar blue test.

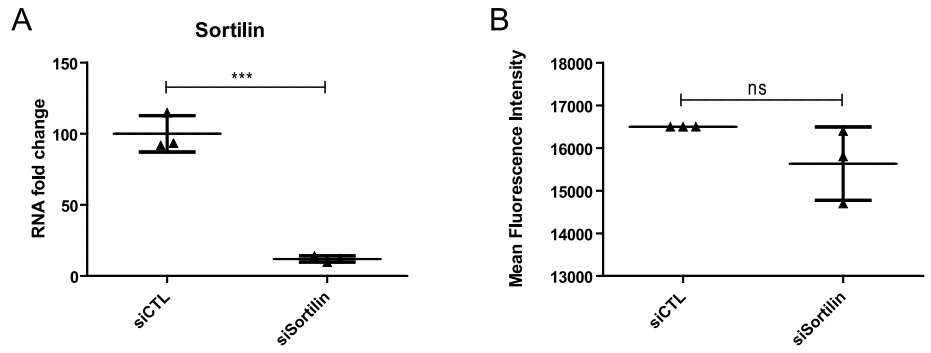

Figure S3. Down regulation of sortilin mRNA using siRNA does not decrease CLCF1-mut1 binding to MSC. (A) MSC were transfected using siRNA NON-targeting pool (siCTL) or siRNA specific for mouse sortilin (siSortilin). Sortilin mRNA expression was quantified using RT-qPCR. Results were normalized using the housekeeping gene GAPDPH mRNA levels. The vertical dot plot indicates mean mRNA fold changes ± SD. Statistical significance was assessed using Student's t-test. ***$p<0.001$, n = 3 technical replicates. **(B)** MSC transfected with control or sortilin specific siRNA (1 x 10^6 cells) were incubated with biotinylated-CLCF1-mut1 (1 µg/mL) for 1 h then stained with a PE-conjugated streptavidin. Fluorescence was measured by flow cytometry. The vertical dot plot shows the mean fluorescence intensity ± SD of the CLCF1-mut1-biot binding. Student's t-test was used to assess statistical significance. ns: not significant, n = 3 technical replicates.

Materials:

siRNA Transfection

MSC were plated at 25,000 cells/cm^2 in a 10 cm petri dish overnight. Cells were transfected in AMEM media containing 100 µM ON-TARGETplus Mouse Sort1 siRNA-SMARTpool (Dharmacon, Lafayette, CO) or ON-TARGETplus NON-targeting Pool (Dharmacon) using lipofectamine RNAiMAX reagent (Thermo Fisher Scientific) following the manufacturer's protocol. After 48 h, cells were detached and used for the binding assay. Aliquots of cells were used for RNA isolation and RT-qPCR.