Supporting Information

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SI Materials and Methods

Three-Point Bending Test. Femurs from five wild-type and four *Esl-1*$^{-/-}$ 4-wk-old mice were collected, stripped of soft tissues and frozen for mechanical testing to assess the material properties. The femurs were tested in three-point bending using a span of 6 mm on a computer-controlled testing apparatus (model 5848; Instron). All of the femurs were tested wet at room temperature. Following the preloading to 1 N for 5 s, the femurs were compressed to failure at a rate of 0.1 mm/s. Load and displacement data were captured at rate of 40 Hz by using BLUEHILL software (Instron). Extrinsic material properties were assessed from the load-displacement curve including maximum load, stiffness, and energy. Geometric data obtained from the micro-computed tomography analysis of the femoral midshaft were used to calculate the intrinsic material properties: ultimate strength, elastic modulus, and fracture toughness.

RNA Isolation and Quantitative RT-PCR. Flash-frozen mouse calvaria are collected from P1 *Esl-1*$^{-/-}$ or WT mice ($n = 5$) for total RNA extraction and first-strand cDNA synthesis. Quantitative RT-PCR is performed on a LightCycler.5 (Roche) using SYBR Green I reagent (Roche). β_2-Microglobulin is used as a reference gene for cDNA concentration normalization.

Primary OB Culture. BMSCs and BMMCs are cultured from total bone marrow isolated from the femurs and tibias of 2-mo-old mice. The 7.5×10^4 per well BMSCs are reseeded to 24-well plates and cultured in osteogenic medium (α-MEM supplied with 10% (vol/vol) FBS, 500 μM ascorbic acid, and 10 mM β-glycerophosphate). ALP staining or Alizarin Red S staining was performed on the cultures. The Alizarin Red S stains are dissolved by 10% (g/ml) cetylpyridinium chloride and quantified on a plate reader.

Primary OC Cultures. BMMCs are seeded in 96-well plates at a density of 1.4×10^4 per well and treated with M-CSF (15 μg/mL) and RANKL (30 ng/mL) for 6 d, and visualized and quantified by TRAP staining.

OB–OC Coculture. The 7.5×10^4 BMSCs and 7.5×10^5 splenocytes were cocultured in each well of 24-well plates with α-MEM supplied with 10% (vol/vol) FBS, 100 nM dexamethasone, 100 μM ascorbic acid phosphate, and 10 nM 1α25-dihydroxyvitamin D3. The formation of OCs was detected at day 5 using TRAP staining.

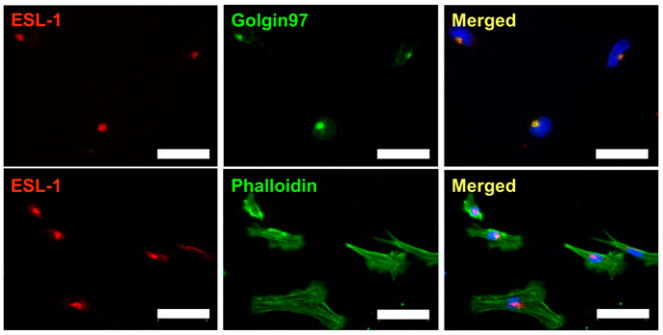

Fig. S1. ESL-1 is expressed in primary mouse osteoblasts and localized in the Golgi apparatus. Golgin 97 antibody or phalloidin indicates Golgi apparatus or actin cytoskeleton for contrast. (Scale bars: 50 μm.)

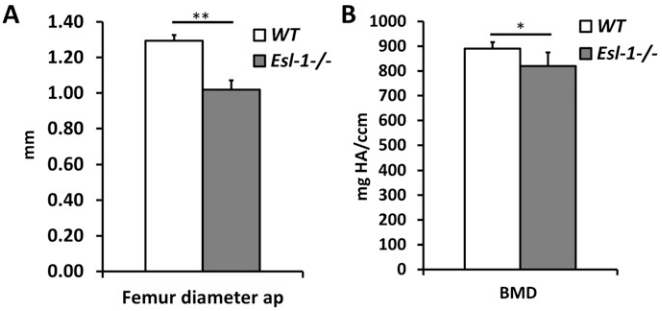

Fig. S2. Midfemurs of 1-mo-old male *Esl-1*$^{-/-}$ mice show significantly decreased anterior–posterior diameter (*A*) and cortical bone mineral density (*B*), compared with WT. $n = 4$; $^*P < 0.05$; $^{**}P < 0.01$.

Table S1. Bone material properties obtained from three-point bending at the femoral midshaft

Property	Wild-type mice	*Esl-1*$^{-/-}$ mice	*P* value
Maximum load, N	11.83 ± 3.22	5.78 ± 2.29	0.013
Stiffness, N/mm	78.21 ± 24.92	48.74 ± 20.59	0.093
Energy to failure, N*mm	8.94 ± 2.24	3.75 ± 1.33	0.004
Ultimate strength, MPa	54.78 ± 13.78	57.66 ± 10.46	0.732
Elastic modulus, GPa	1.70 ± 0.41	2.92 ± 0.59	0.016
Toughness to failure, MPa	8.60 ± 0.77	6.42 ± 1.91	0.103

Results are given as mean ± SDs.