Supplementary Information

CYTL1 regulates bone homeostasis in mice by modulating osteogenesis of mesenchymal stem cells and osteoclastogenesis of bone marrow-derived macrophages

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**Fig. S1 Knockdown of CYTL1 promotes the osteogenesis of hMSCs.** hMSCs were transfected with 100 nM of control or CYTL1-targeting siRNAs and cultured under osteogenic-differentiating conditions for the indicated number of days (a, d) or for 18 days (b, c). a CYTL1 mRNA levels were quantified by qRT-PCR analysis (n = 8). b, c Representative images of alizarin red S staining (b, n = 6). The absorbance of the released alizarin red S was normalized with respect to the protein content (left panel), and the relative value was obtained by dividing the value of Ad-CYTL1 by that of Ad-C (right panel) (c, n = 6). d Relative ALPL activity (n = 5). Data represent the means ± SEM of the indicated number of independent experiments; *p < 0.05, **p < 0.005, ***p < 0.0005 determined by two-tailed t-test. ns, not significant.
| RNA    | Species | Sequence (5’-3’)                          |
|--------|---------|-------------------------------------------|
| CYTL1  | Human   | CCGGGTACCTGGACATACACAATTACTCGAGTAATTGTGTAT|
| shRNA-1|         | GTCCAGGTACTTTTTTG                          |
| CYTL1  | Human   | CCGGTAGATTCCATGAAGGACAAACTCGAGTTTGTTCTTC  |
| shRNA-2|         | AAGGAATCTACTTTTTG                          |
| CYTL1  | Human   | Sense: GGUUUAUGGAGUAUGGU                   |
| siRNA-1|         | Antisense: ACCAUUACUCAUUAAACC               |
| CYTL1  | Human   | Sense: GUUAGAUACACAGCAUGUU                 |
| siRNA-2|         | Antisense: AACAUGCUGUGUAUCUAAC              |
| CYTL1  | Human   | Sense: ACACCAUAUGAACUCGU                  |
| siRNA-3|         | Antisense: AACGAGUUAUGAGGUGU               |
| BAX    | Human   | Sense: GAGUGGCAGCUCUGACGUUUUCUGA          |
| siRNA-1|         | Antisense: GGUCACCCGUUCGUACUAACAGACU       |
| BAX    | Human   | Sense: GUGGGCAUUUUUCUCUACUUUGUAA          |
| siRNA-2|         | Antisense: UGCACCCGUAAGAUGAAACAUAUU       |
Table S2. PCR primers and conditions

| Gene Symbol | Species | Strand  | Sequence (5'-3') | At (°C) | Size (bp) |
|-------------|---------|---------|------------------|---------|-----------|
| ACAN        | Human   | Sense   | GCCTTGAGCAGGTTCACCTTCCTCTTCTACGGGAGCAG | 60      | 395       |
|             |         | Antisense | CTCTTCTACGGGAGCAG |         |           |
| ALPL        | Human   | Sense   | AGACTCGCGCTGGTATGTTGTCACCTACATTTTGTCG | 58      | 196       |
|             |         | Antisense | CCACGTCTTCACATTTTCTCCAG |         |           |
| BAX         | Human   | Sense   | AACTGTTGTCACAGGCTCTTCACATTTTCTCCAG | 63      | 276       |
|             |         | Antisense | GCCTCAACCCATCTTCTCCAG |         |           |
| CEBPA       | Human   | Sense   | GCAAGGCAAGAAGTCGCTCAACATTTTCTCCAG | 58      | 252       |
|             |         | Antisense | TCCTCAAGCAGTGTTGTCG |         |           |
| COL2A1      | Human   | Sense   | CAGTGGGAGTAATGCAAG | 58      | 300       |
|             |         | Antisense | GCCTGGGTAACCTTCTCCAG |         |           |
| CYTL1       | Human   | Sense   | AGATCACCACGCTCTTACATTTTCTCCAG | 60      | 302       |
|             |         | Antisense | TTAGCGCTGACGCTGCTCCAG |         |           |
| FABP4       | Human   | Sense   | TATGAAAGAAGTAGGAGTGG | 58      | 288       |
|             |         | Antisense | ACCACCATTTTATCATTTCTCCAG |         |           |
| GAPDH       | Human   | Sense   | CGTCTTCACCCACCACATTTTCTCCAG | 62      | 300       |
|             |         | Antisense | GCCGATTACAGCCACCTTCTCCAG |         |           |
| IBSP        | Human   | Sense   | AGTGAGGGGAGGAGAGAATAC | 57      | 331       |
|             |         | Antisense | CCTTCCTCCTCTTCTTCACTTCG |         |           |
| OCN         | Human   | Sense   | GAGGGTATAAACAGTGCTGAGAGAATAC | 53      | 244       |
|             |         | Antisense | CCCAGCATGTTGACATTACAGGATAGA |         |           |
| OPN         | Human   | Sense   | TTGACAGTATTGCTTCCTTCG | 60      | 439       |
|             |         | Antisense | ACATATCACCCTCGCCACCTC |         |           |
| PPARG       | Human   | Sense   | AGACAAACAGACAAACATACCAT | 58      | 401       |
|             |         | Antisense | CTTACAGCAAAACTCACAACCTT |         |           |
| RUNX2       | Human   | Sense   | CTCACCTCCACACCTACCTCCTGCAAATG | 58      | 270       |
|             |         | Antisense | TCAATATGCTCAGCAGACCTAG |         |           |
| SOX9        | Human   | Sense   | GGCAGCTGTGAACTGGGAGA | 62      | 408       |
|             |         | Antisense | GCCACGGGAGAAGGACGTCGA |         |           |
| TAZ         | Human   | Sense   | AGTACATGAACACCACCTGACG | 60      | 321       |
|             |         | Antisense | TGTCCTGTACCTGTCTTCGAAGA |         |           |
| Acp5        | Mouse   | Sense   | CACGATGGGAGGAGGGCAGAGGAGGTGTC | 58      | 366       |
|             |         | Antisense | AAACGTAGTGTCCTCCTGGGCTGTCG |         |           |
| Gapdh       | Mouse   | Sense   | TCAGCCACACAGAAGAC | 60      | 450       |
|             |         | Antisense | TGTAGGCACTGAGGCTCC |         |           |
| Nfat1       | Mouse   | Sense   | ACCACCTCCACACCTTCTGACTTC | 58      | 349       |
|             |         | Antisense | AGCTGTAGGCTGAGGCTTC |         |           |
| Gene  | Species | Strand  | RNA Sequence | ATC | Tm |
|-------|---------|---------|--------------|-----|----|
| *Ocstamp* | Mouse | Sense | CTCTCTCTGTGGTCTTCTC | 60 | 343 |
|  |  | Antisense | TGTGAAGGGCGGAAAGGCTGAG |  |  |

S, sense; As, antisense; At, annealing temperature