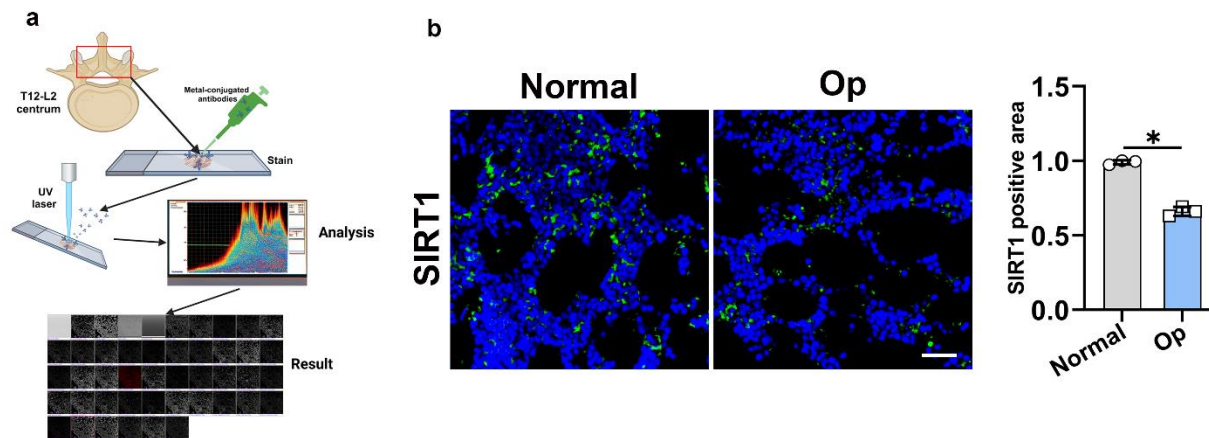
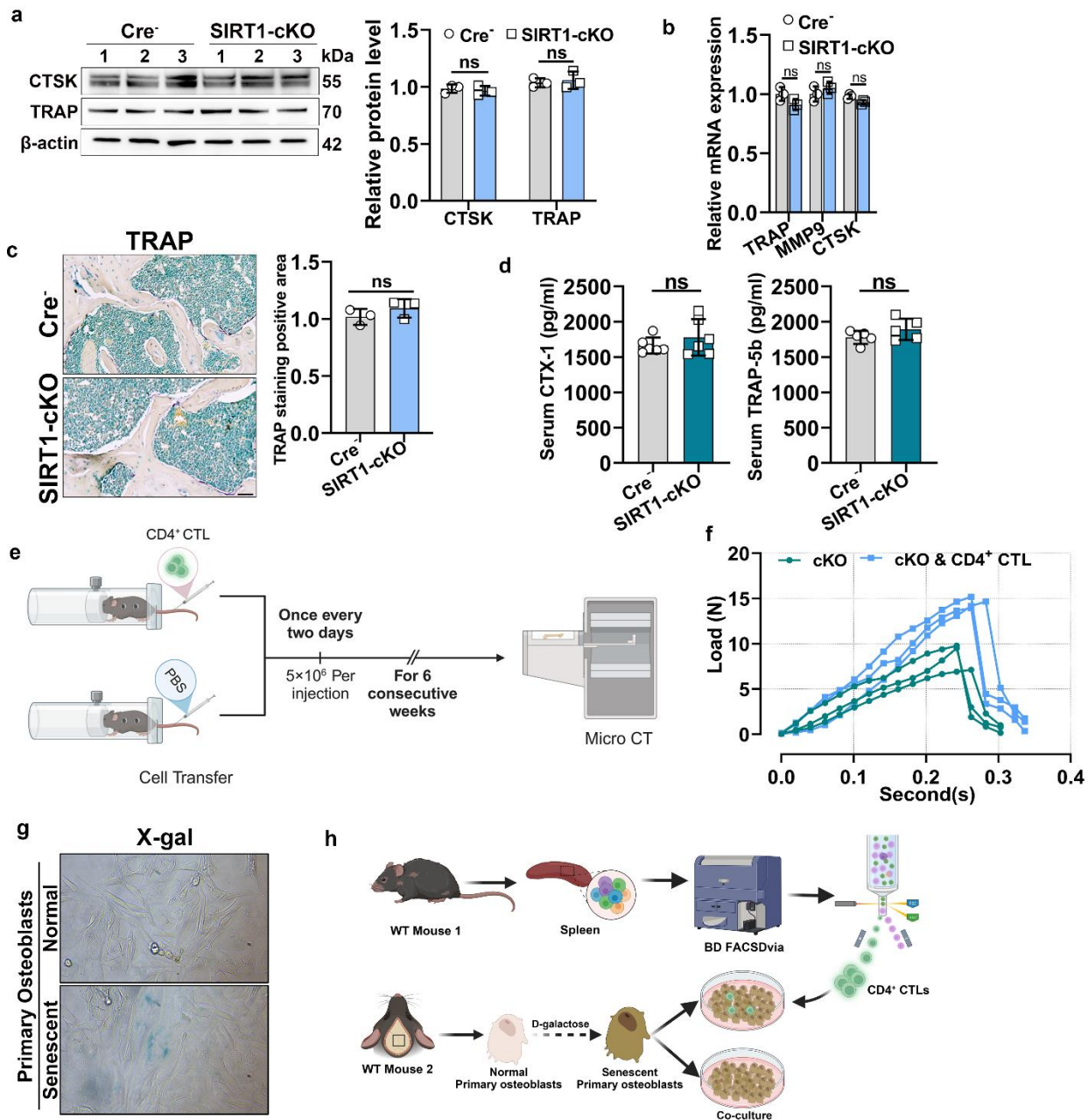


Spatial analysis of the bone microenvironment reveals a novel osteoblast-CD4⁺ CTL crosstalk mediated by the SIRT1/DAAM2 axis

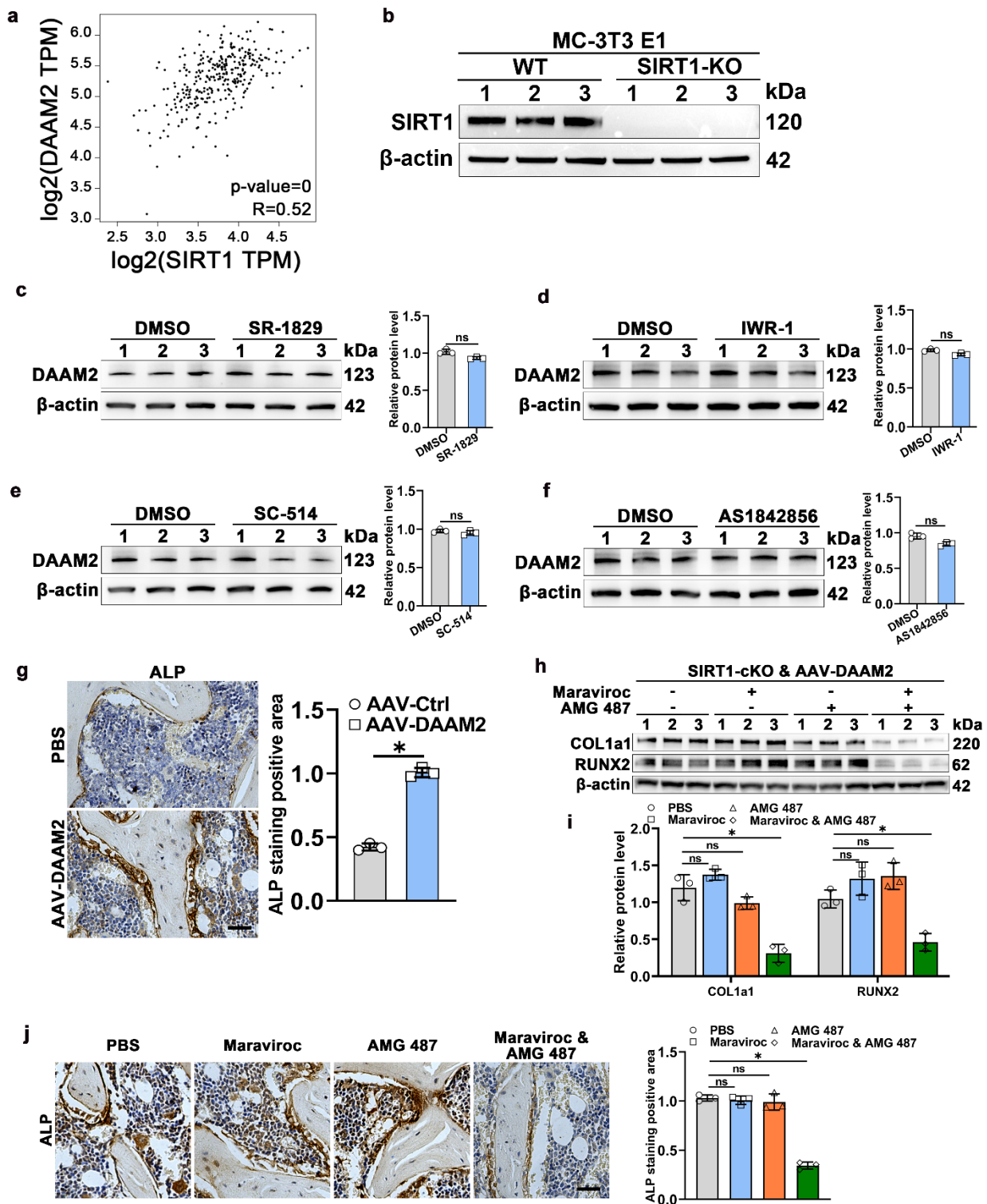


Extended Data Fig.1|a, The workflow of IMC. Patients' OP and healthy donors' samples were acquired by IMC. IMC images went through preprocessing before cell segmentation, followed by batch effect removing and downstream bioinformatics analysis. **b**, Representative IF staining and quantitative analyses of SIRT1 in human normal and OP bone samples. (scale bar, 50 μ m) (Normal, n = 3; Op, n = 3). Data are compared with the control group as the mean $\pm$ SD. Statistical analysis: *p < 0.05.



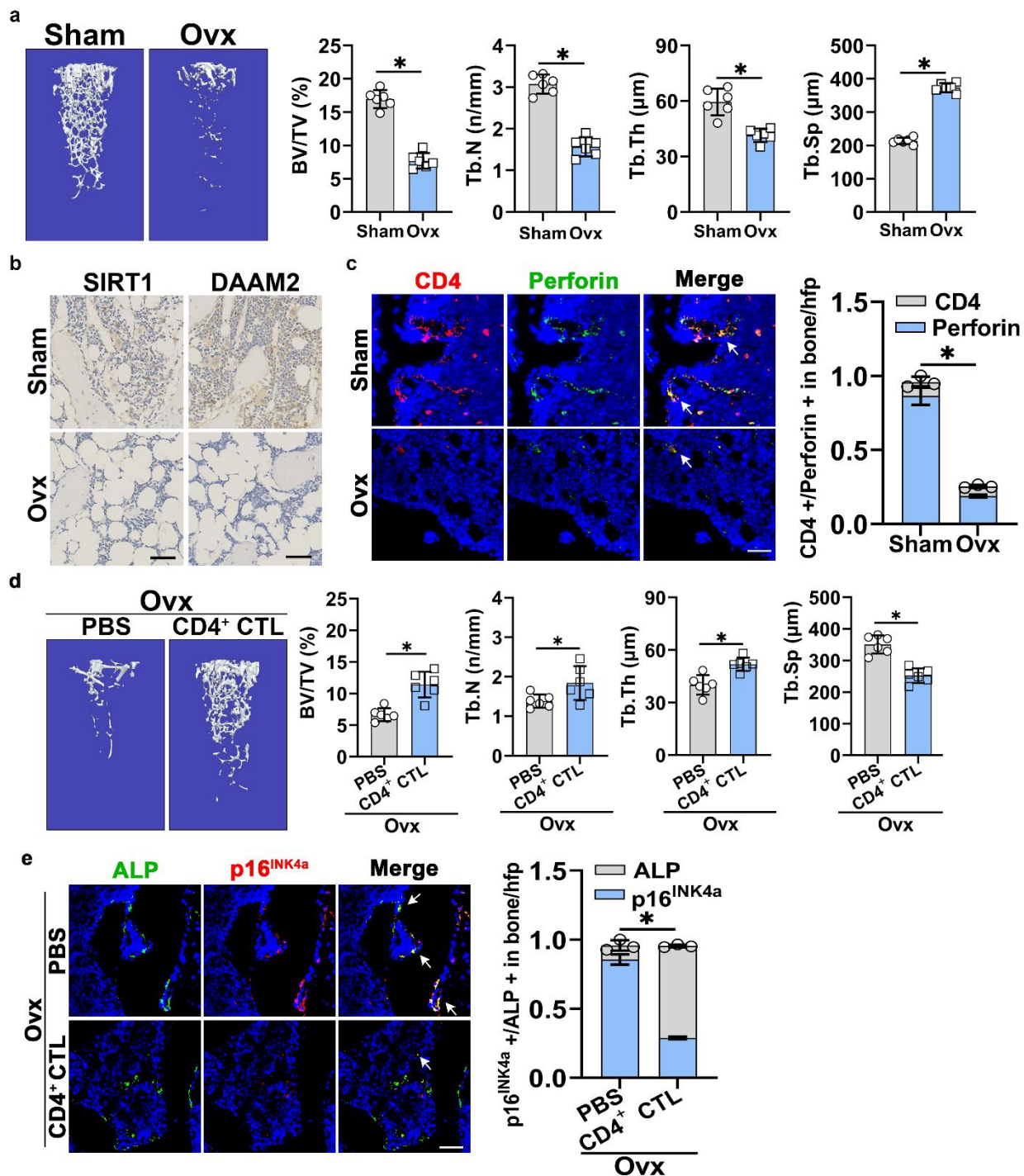
Extended Data Fig. 2 | **a**, Representative western blots and quantification of CTSK and TRAP expression in mouse bone samples (n = 3). **b**, qRT-PCR of relative osteoclast marker gene (TRAP, MMP9, and CTSK) mRNA levels in the groups (n = 3). **c**, Representative images of IHC staining for detecting the TRAP expression and quantitative analyses of TRAP staining positive area (scale bar, 50 μm) (n = 3). **d**, Serum ELISA for bone resorption markers (CTX-1 and TRAP-5b) (n = 6). **e**, Schematic representation of SIRT1-cKO mice split into a sham-operated control group and

another receiving bidaily tail vein injections of 5×10^6 CD4⁺ CTLs. **f**, Stress test used to detect the femoral cortex ability to withstand stress (n = 3). **g**, Representative senescence-associated β -galactosidase (SA- β -Gal or X-gal) staining of normal and senescent primary osteoblasts. **h**, Schematic representation of CD4⁺ CTL isolated from splenic lymphocytes by flow cytometry and co-cultured with senescent primary osteoblasts. Data are compared with the control group as the mean $\pm$ SD. Statistical analysis: *p < 0.05.



Extended Data Fig. 3| a, GEPIA2 revealing a correlation between SIRT1 and DAAM2. **b** Representative western blots of SIRT1 expression in WT and SIRT1-KO MC3T3-E1 cells (n = 3). **c-f** Representative western blots of DAAM2 expression in MC3T3-E1 cells treated with different

inhibitors (SR-1829, IWR-1, SC-514, and AS1842856) and their quantification (n = 3). **g**, Representative images of IHC staining for detecting the ALP expression and quantitative analyses of ALP staining positive area (scale bar, 50 μ m) (n = 3). **h,i** Representative western blots of COL1a1 and RUNX2 expression and quantification in bone samples of SIRT1-cKO mice injected with AAV-DAAM2 into four groups: Control, CCR5 antagonist (Maraviroc), CXCR3 antagonist (AMG 487), and mixed antagonists (Maraviroc and AMG 487) (n = 3). **j**, Representative images of IHC staining for detecting the ALP expression and quantitative analyses of ALP staining positive area (scale bar, 50 μ m) (n = 3). Data are compared with the control group as the mean $\pm$ SD. Statistical analysis: *p < 0.05.



Extended Data Fig. 4 **a**, Representative micro-CT images and quantitative analyses of the distal femora in Sham and OvX mice ($n = 6$). **b**, Representative images of IHC staining to detect SIRT1 and DAAM2 expression (scale bar, 50 μm) ($n = 3$). **c**, Representative IF staining and quantitative analyses of CD4 and Perforin in Sham and OvX mouse bone samples. (scale bar = 50 μm). **d**, Representative micro-CT images and quantitative analyses of the distal femora of OvX mice

injected with CD4⁺ CTLs (n = 6). **e**, Representative images and quantitative analyses of the double staining of ALP and p16^{INK4a} in the distal femora of Ovx mice injected with CD4⁺ CTLs (scale bar, 50 μm) (n = 3). Data are compared with the control group as the mean ± SD. Statistical analysis: *p < 0.05.

Extended Data Table. 1| Primer sequences used in siRNA-mediated knockdown of SIRT1 and DAAM2

siRNA	Sequence
si-SIRT1#1	CCGATGGACTCCTCACTAA
si-SIRT1#2	GCTGTTCGTGGAGACATTT
si-DAAM2#1	GGACACAACCCATGAGGTT
si-DAAM2#2	GACCGAATCCTTCAACAGA

Extended Data Table. 2| Primer sequences used in qRT-PCR.

Gene	Sequence	
	Forward	Reverse
RUNX2	GCCACCTTTACCTACACCCC	ACTCTGGCTTTGGGAAGAGC
COL1a1	GGAGAGAGCATGACCGATGG	CGATCTCGTTGGATCCCTGG
OCN	CTGCGCTCTGTCTCTCTGAC	CTCTGGCCACTTACCCAAGG
OSX	TTCGCATCTGAAAGCCCACT	TGCGCTGATGTTTGCTCAAG
ALP	TAACACCAACGCTCAGGTCC	TGGATGTGACCTCATTGCC
TRAP	CACTCCCACCCTGAGATTTGT	CATCGTCTGCACGGTTCTG
MMP9	CTGGACAGCCAGACACTAAAG	CTCGCGGCAAGTCTTCAGAG
CTSK	TACCCATATGTGGGCCAGGA	TTCAGGGCTTTCTCGTTCCC
GAPDH	GGTGAAGGTCGGTGTGTGAACG	CTCGCTCCTGGAAGATGGTG