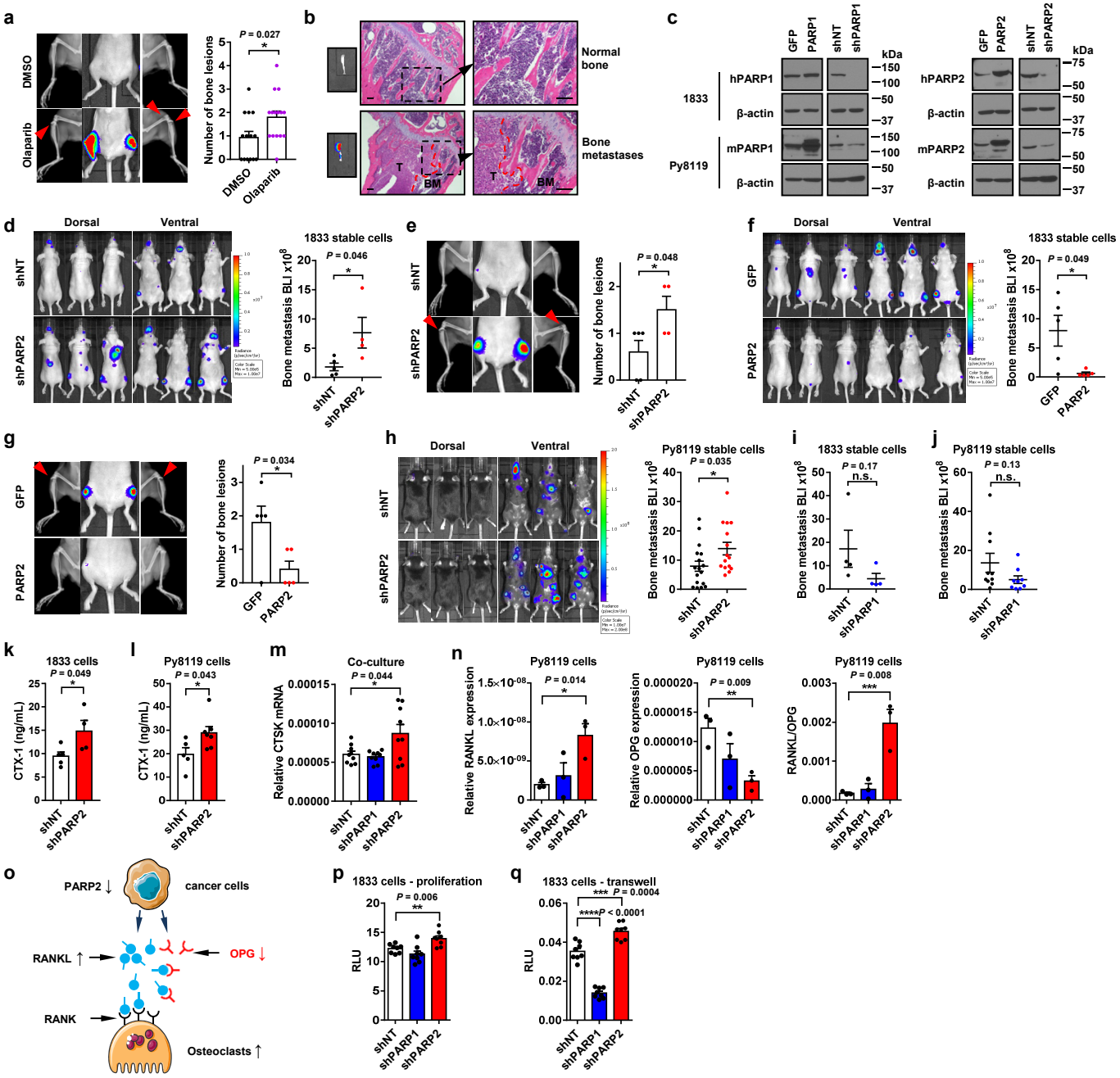
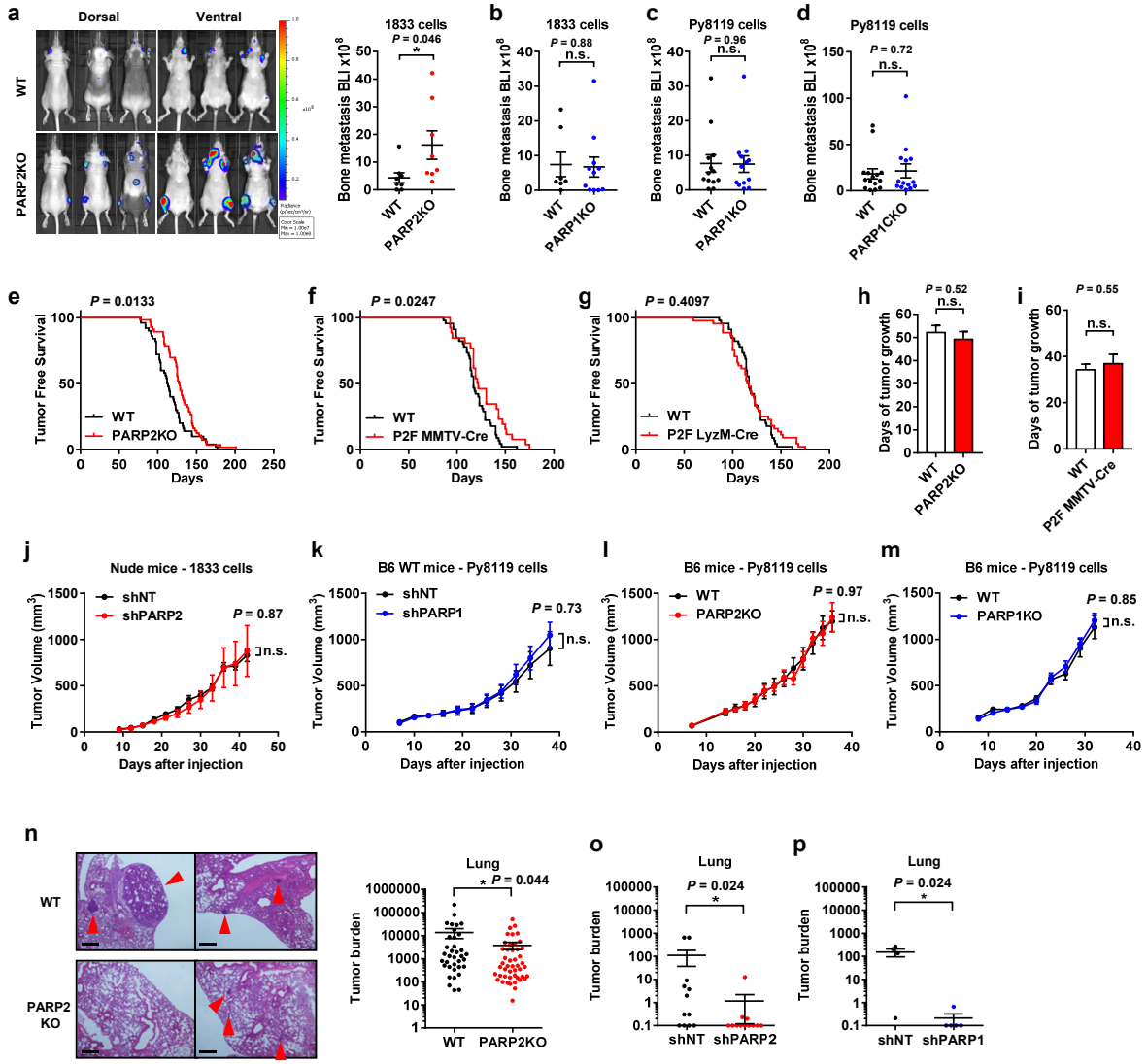
Supplementary Information

Differential Regulation of Breast Cancer Bone Metastasis by PARP1 and PARP2

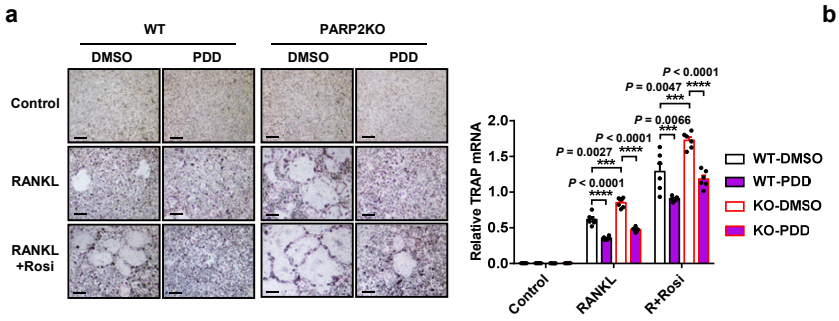
Zuo et al.



Supplementary Fig. 1 | PARP2 deficiency in cancer cells increases breast cancer bone metastasis. a, Representative X-ray images and bone lesion quantification of nude mice intracardiacally injected with 1833 cells and treated with DMSO or olaparib (n = 14). **b**, H&E staining of the distal femur with or without bone metastases. Staining was repeated twice with different mice and showed similar results. Scale bar, 0.1 mm. **c**, Western blot analyses showing the efficiency of stable overexpression and knocking down of PARP1 or PARP2 in 1833 cells and Py8119 cells. Western blot analyses were repeated twice and showed similar results. **d**, **e**, Nude mice injected with PARP2KD 1833 cells (n = 4) developed more bone metastases (**d**) and bone lesions (**e**) than control 1833 cells (n = 5). **f**, **g**, Bone metastasis (**f**) and bone lesions (**g**) of PARP2-overexpressing 1833 cells in nude mice (n = 5) were reduced compared with control cells (n = 4). **h**, B6 mice injected with PARP2KD Py8119 cells developed more bone metastases than control Py8119 cells (n = 15). **i**, **j**, Nude mice injected with PARP1KD 1833 cells (**i**, n = 5) or B6 mice injected with PARP1KD Py8119 cells (**j**, n = 9) had similar bone metastases as respective control 1833 cells (n = 4) or Py8119 cells (n = 10). **k**, **l**, Serum CTX-1 levels in nude mice (**k**) or B6 mice (**l**) injected with PARP2KD 1833 cells (n = 4) or Py8119 cells (n = 7) were higher than mice injected with control cells. **m**, Osteoclast precursors had increased expression of osteoclast marker gene CTSK after co-culturing with Py8119-shPARP2 cells for 3 days as compared to Py8119-shNT or shPARP1 cells (n = 9). **n**, Py8119-shPARP2 cells had increased RANKL mRNA (left), decreased OPG mRNA (middle) and elevated RANKL/OPG ratio (right) as compared to Py8119-shNT or shPARP1 cells (n = 3). **o**, A diagram showing that PARP2KD in breast cancer cells increases osteoclast differentiation. **p**, PARP2 levels negatively correlated with the proliferation rate of 1833 cells (n = 8). **q**, Migration of PARP2KD 1833 cells through transwell was increased (n = 8). Data represent mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n.s., non-significant. Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.



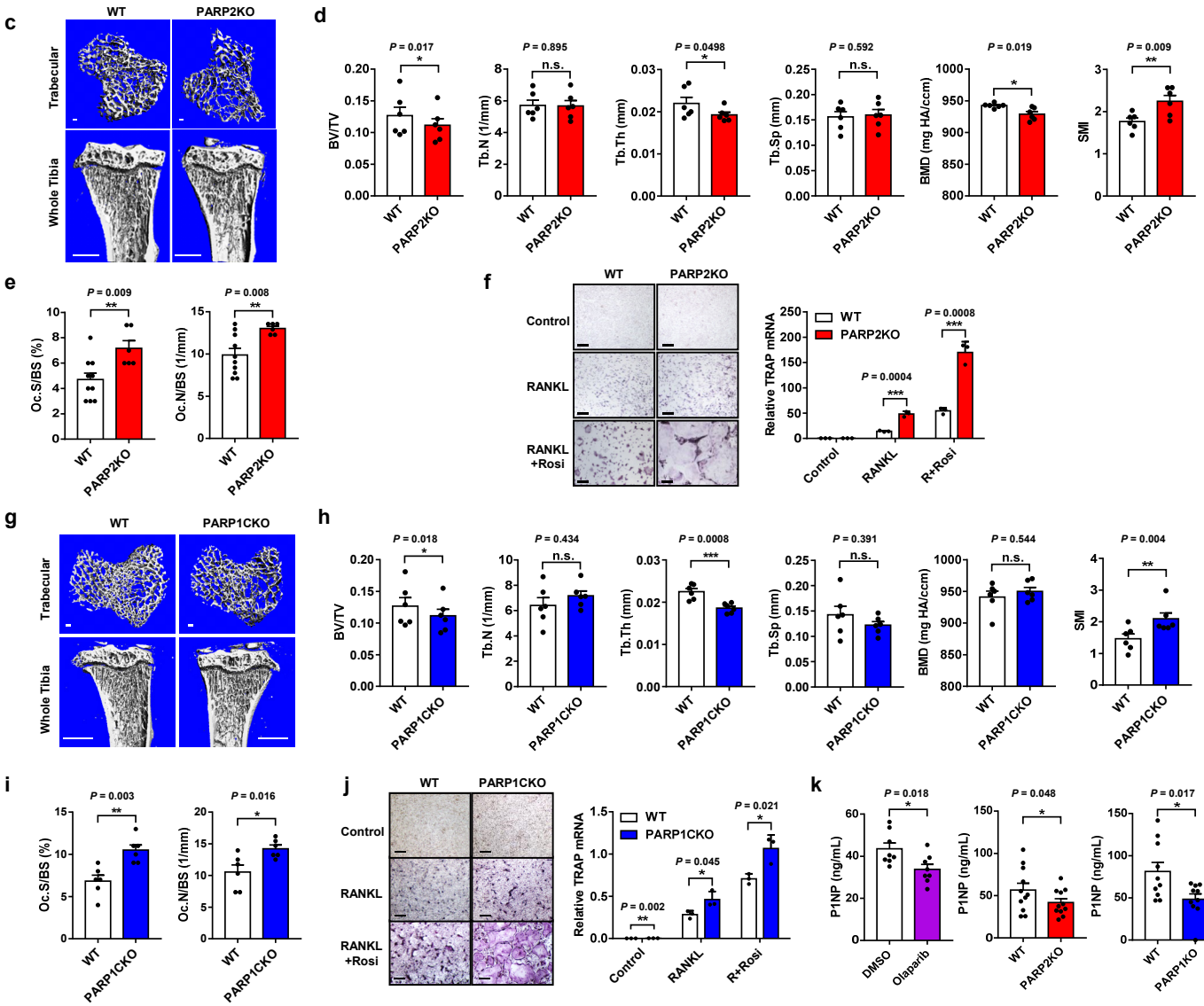
Supplementary Fig. 2 | PARP2 deficiency does not affect primary tumor growth but suppresses lung metastasis. **a**, Bone metastasis of 1833 cells was increased in PARP2KO nude mice compared with WT nude mice (n = 8). **b**, Bone metastasis of 1833 cells was similar in nude WT (n = 7) or PARP1KO mice (n = 11). **c**, Bone metastasis of Py8119 cells was similar in B6 WT or PARP1KO mice (n = 13). **d**, Bone metastasis of Py8119 cells was similar in B6 WT or PARP1CKO (n = 14) mice. **e-g**, PARP2KO (**e**, n = 56) and PARP2 flox/flox (P2F) + MMTV-Cre (**f**, n = 26), but not P2F + lyzM-Cre (**g**, n = 44), increased tumor free survival of MMTV-PyMT mice compared with respective control PARP2WT (**e**, n = 50) or P2F (**f**, **g**, n = 45) mice. Two-sided log-rank test was used to calculate statistical difference. **h**, **i**, The time of primary tumor growth between tumor onset and end point (maximal volume limit) of mice in **e** and **f** was not altered by PARP2 deletion. **j-m**, Primary mammary tumor growth were similar for PARP2KD 1833 cells vs. control 1833 cells in nude mice (**j**, n = 5), PARP1KD Py8119 cells vs control Py8119 cells in B6 WT mice (**k**, n = 13), Py8119 cells in WT vs. PARP2KO B6 mice (**l**, n = 7), and Py8119 cells in WT vs. PARP1KO mice (**m**, n = 10). Statistical analysis was performed using two-way ANOVA with Sidak's multiple comparison. **n**, Left, H&E staining of spontaneous lung metastases in PARP2KO MMTV-PyMT mice (scale bar, 25 μm). Arrows, lung metastases. Right, PARP2KO MMTV-PyMT mice had reduced tumor burden of spontaneous lung metastases compared with WT MMTV-PyMT mice (n = 36). **o**, **p**, Tumor burden of spontaneous lung metastases was less in B6 mice injected with PARP2KD (**o**, n = 12) or PARP1KD (**p**, n = 5) Py8119 cells at mammary fat pad than mice with control cells. Data represent mean ± SEM, *P < 0.05; n.s., non-significant. **a-d**, **h**, **i**, Two-sided Student's t-test was used to calculate statistical difference. **n-p**, Two-sided Mann-Whitney U test was performed (non-normally distributed data). Source data are provided as a Source Data file.



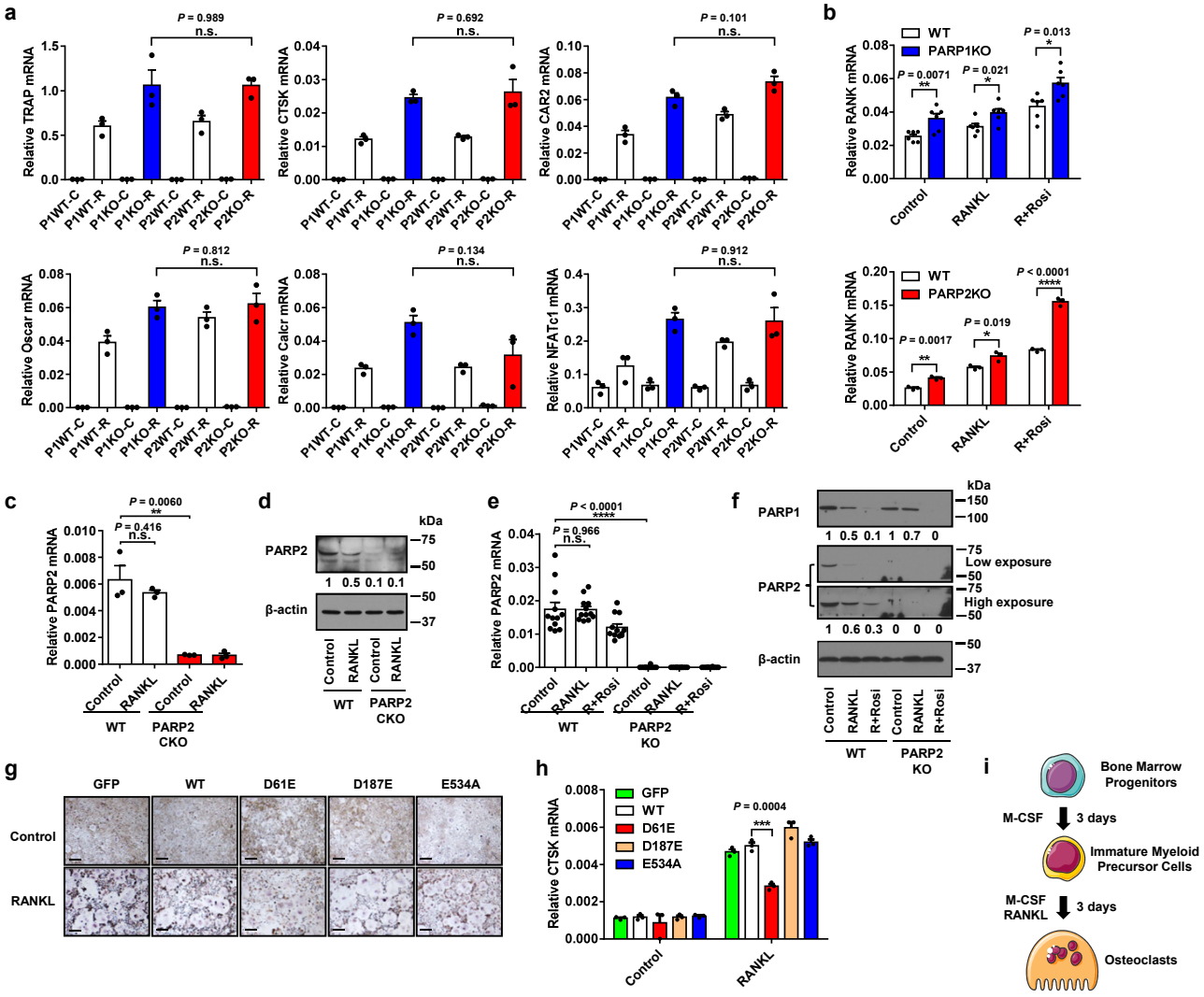
b

Parameters	DMSO	Olaparib	WT	PARP2CKO
BS (mm^2)	25.09±2.03	19.30±0.86 *	24.38±1.40	18.66±1.93 *
BS/BV (1/mm)	77.46±2.28	80.98±1.91	90.22±3.74	105.4±4.25 *
Tb. Porosity (%)	82.96±1.04	86.13±0.42 *	84.59±1.09	89.09±1.02 *
Cortical BV/TV	0.89±0.00	0.88±0.00	0.89±0.01	0.88±0.00 ***
Cortical Porosity (%)	10.85±0.14	11.57±0.25 *	10.52±0.50	12.18±0.39 ***
Cortical Thickness (mm)	0.13±0.00	0.11±0.00 *	0.12±0.00	0.11±0.00 **

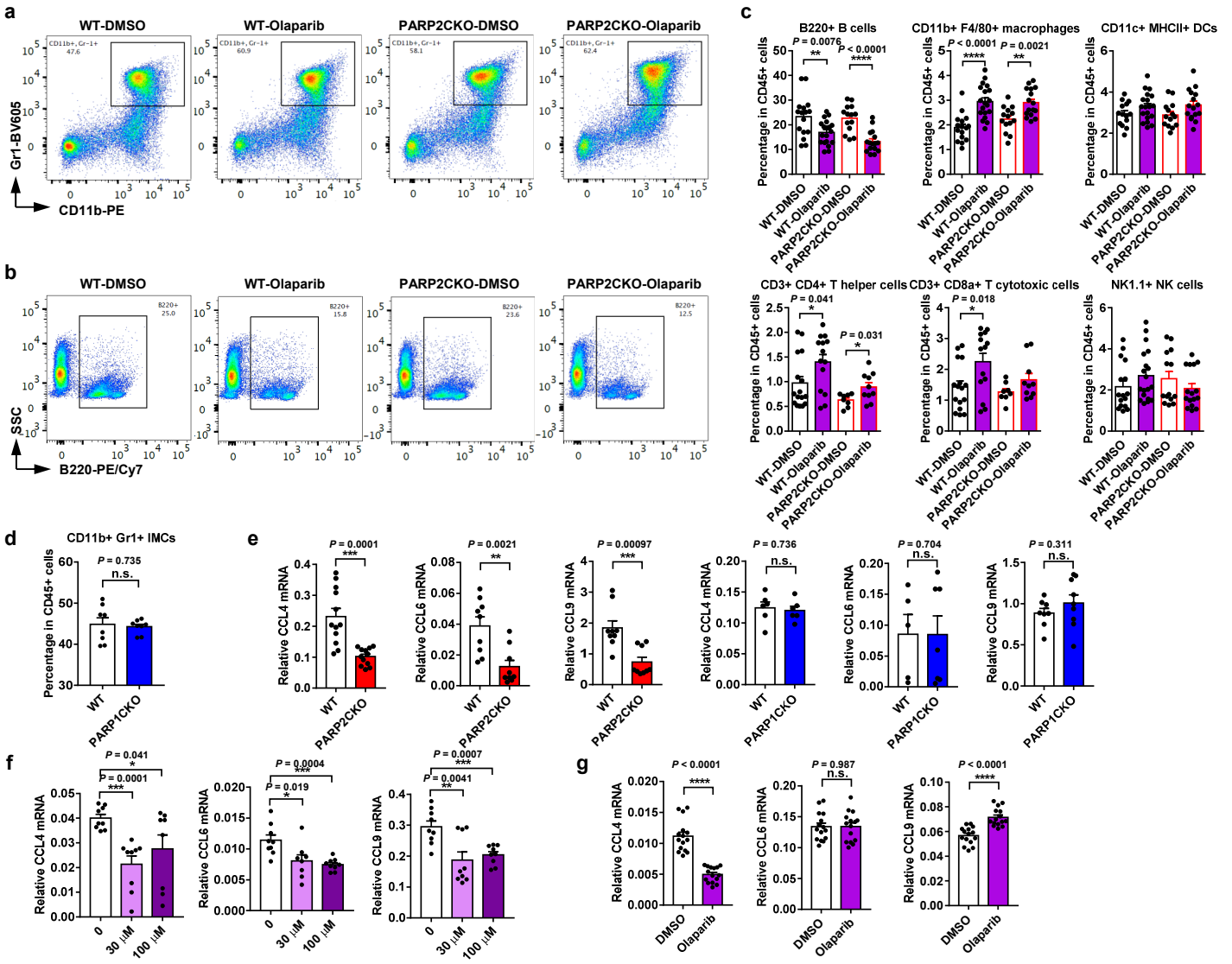
Parameters	WT	PARP2KO	WT	PARP1CKO
BS (mm^2)	22.89±1.76	19.81±1.73 *	22.32±2.58	26.04±1.46
BS/BV (1/mm)	92.88±5.21	103.45±3.05	93.55±3.50	103.7±4.36
Tb. Porosity (%)	87.34±1.23	88.86±0.95 *	86.02±1.66	86.06±0.88
Cortical BV/TV	0.87±0.01	0.87±0.00	0.88±0.00	0.87±0.01
Cortical Porosity (%)	12.81±0.73	12.57±0.37	12.47±0.37	13.07±0.35
Cortical Thickness (mm)	0.11±0.00	0.11±0.00	0.11±0.00	0.11±0.00



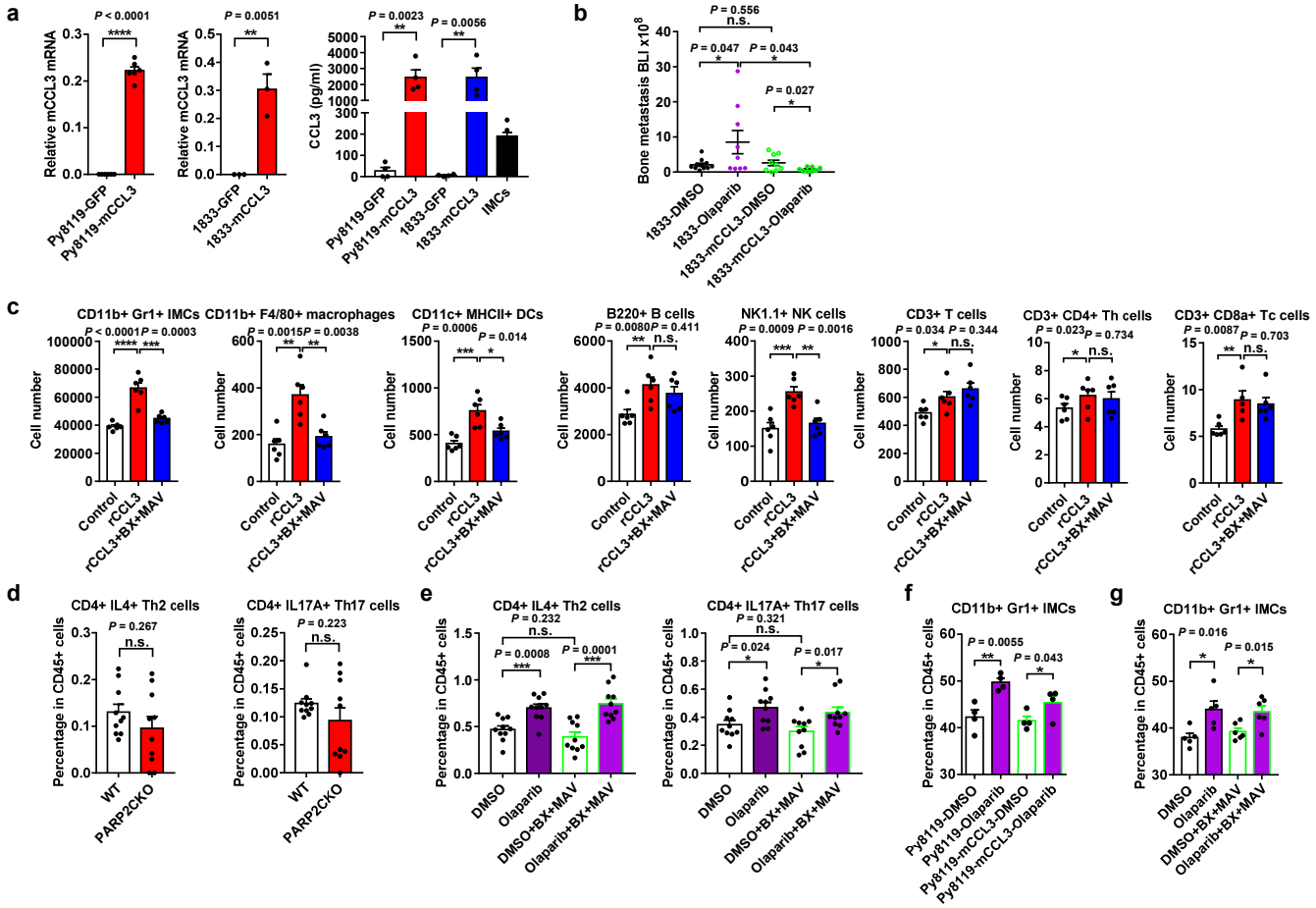
Supplementary Fig. 3 | PARP2KO and PARP1CKO increase osteoclast differentiation. a, Treatment with 1 μM PARG inhibitor, PDD 00017273 (PDD), suppressed *in vitro* osteoclast differentiation of WT or PARP2KO cultures (n = 3). Left, TRAP staining (scale bar, 25 μm); right, TRAP expression. **b**, Other trabecular and cortical bone parameters in μCT. BS, bone surface. **c**, **d**, μCT of the tibiae from PARP2KO mice showed reduced BV, Tb.Th, BMD and increased SMI compared with littermate WT control mice (2-month-old, male, n = 6). **c**, Images of the trabecular bone of the tibial metaphysis (top) (scale bar, 100 μm) and the entire proximal tibia (bottom) (scale bar, 1 mm) from WT or PARP2KO mice. **d**, Trabecular bone parameters. **e**, Histomorphometry of TRAP-stained bone sections from the distal femur showed that PARP2KO mice had more osteoclasts than WT mice (2-month-old, male, n = 6). **f**, PARP2KO cultures showed increased osteoclast differentiation (n = 3). Left, TRAP staining (scale bar, 25 μm); right, TRAP expression. **g**, Images of the trabecular bone of the tibial metaphysis (top) (scale bar, 100 μm) and the entire proximal tibia (bottom) (scale bar, 1 mm) from WT or PARP1CKO mice. **h**, μCT of the tibiae showed that PARP1CKO mice had decreased BV, Tb.Th and increased SMI compared with WT littermates (2-month-old, male, n = 6). **i**, Histomorphometry of the distal femur showed more osteoclasts in PARP1CKO mice than WT littermate controls (2-month-old, male, n = 6). **j**, PARP1CKO cultures showed increased osteoclast differentiation (n = 3). Left, TRAP staining (scale bar, 25 μm); right, TRAP expression. **k**, Serum bone formation marker P1NP levels of olaparib treated (n = 8), PARP2KO (n = 11) or PARP1KO (n = 10) mice were lower. Data represent mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n.s., non-significant. Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.



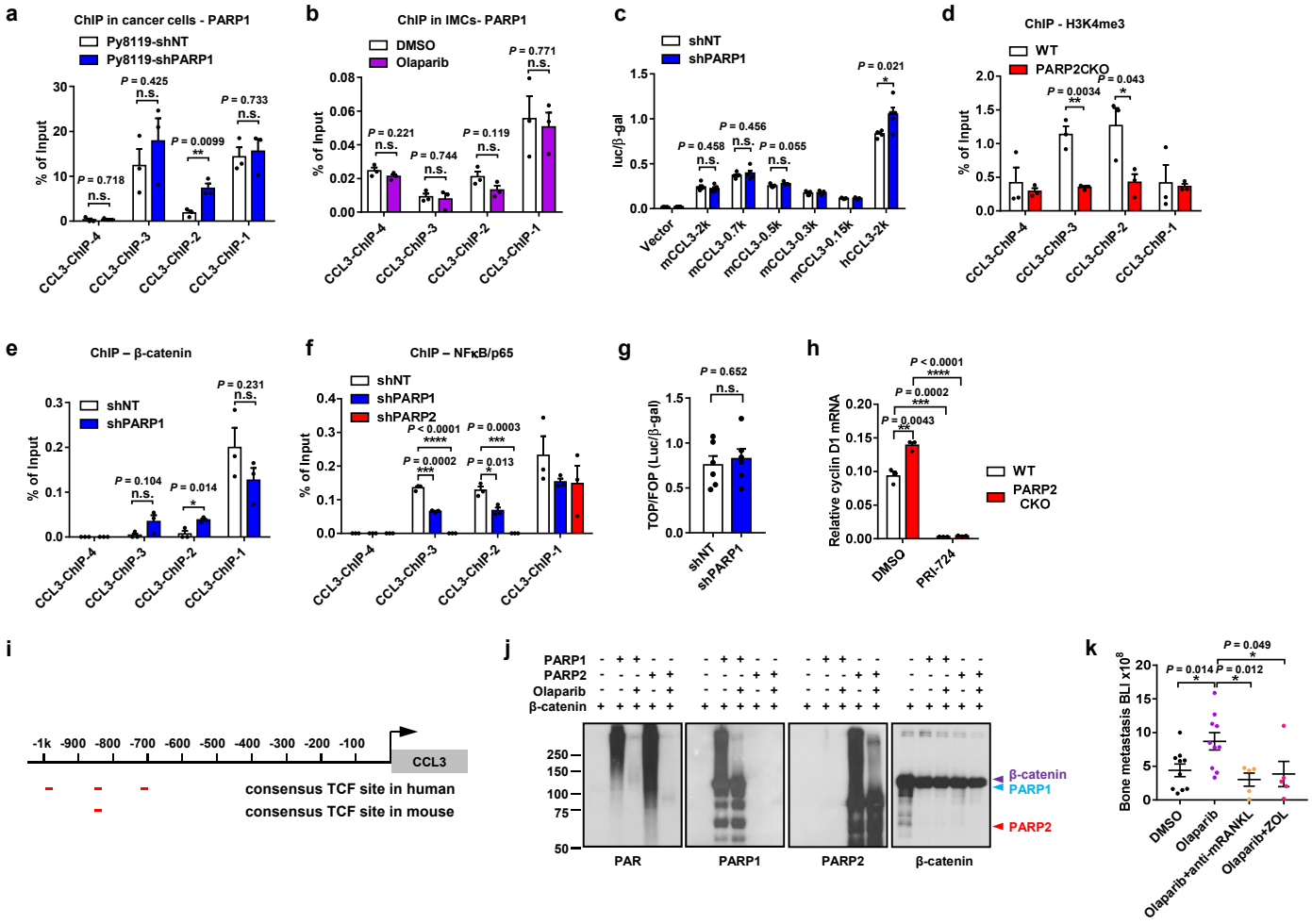
Supplementary Fig. 4 | PARP1 and PARP2 regulate osteoclast similarly and are both cleaved during osteoclast differentiation. a, **b**, The expression of osteoclast marker genes (**a**, *n* = 3) and RANK gene (**b**, *n* = 6 for PARP1KO, *n* = 3 for PARP2KO) in osteoclast differentiation assay was similarly up-regulated by PARP1KO or PARP2KO. P1, PARP1; P2, PARP2; C, control; R, RANKL; TRAP, tartrate-resistant acid phosphatase; CTSK, cathepsin K; CAR2, carbonic anhydrase 2; Oscar, osteoclast-associated receptor; Calcr, calcitonin receptor; NFATc1, Nuclear factor of activated T-cells, cytoplasmic 1; Rosi, rosiglitazone. **c**, Cultured PARP2CKO bone marrow cells had ~85% PARP2 deletion and RANKL did not alter PARP2 mRNA levels (*n* = 3). **d**, PARP2 protein level was reduced by RANKL in cultured WT bone marrow cells. Western blot analyses were repeated twice and showed similar results. **e**, **f**, PARP2KO bone marrow cells had complete PARP2 deletion. RANKL did not alter PARP2 mRNA (**e**, *n* = 12) but reduced PARP2 protein levels (**f**). Western blot analyses were repeated twice and showed similar results. **g**, **h**, Osteoclast differentiation assay with overexpression of control GFP, PARP2 WT, uncleavable mutants D61E or D187E, or enzymatic activity dead mutant E534A in RAW 264.7 murine macrophage cells. Only D61E mutant suppressed osteoclast differentiation. **g**, TRAP staining was repeated three times and showed similar results. **h**, CTSK expression (*n* = 3). **i**, A diagram showing osteoclast differentiation. Data represent mean ± SEM, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001; n.s., non-significant. Two-sided Student's *t*-test was used to calculate statistical difference. Source data are provided as a Source Data file.



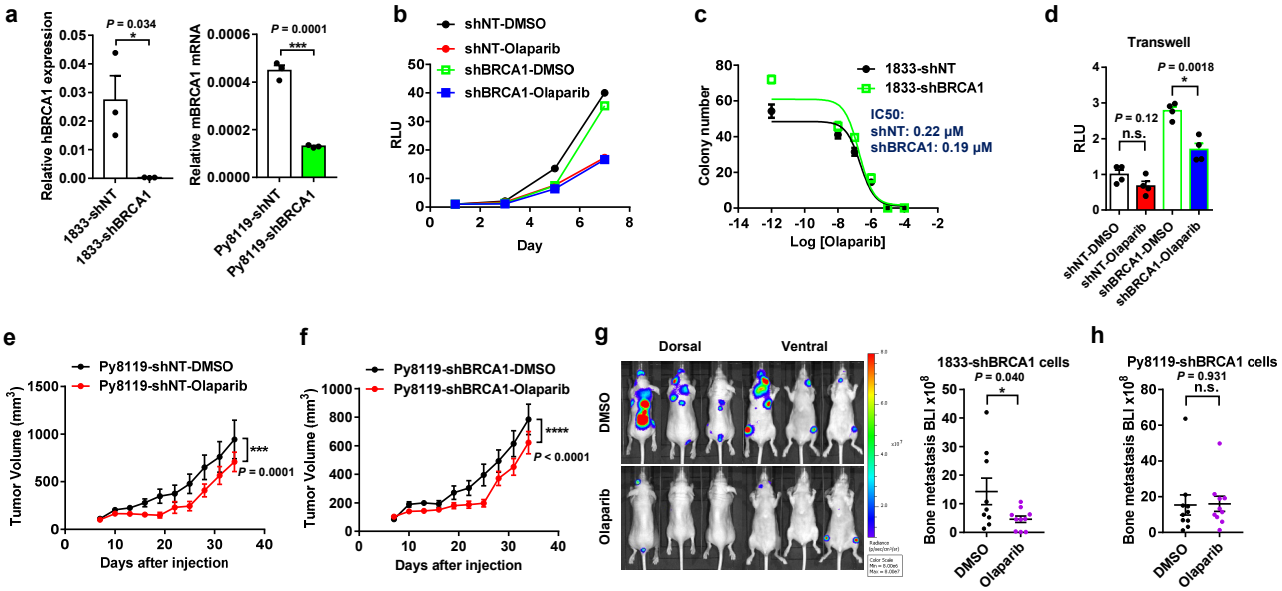
Supplementary Fig. 5 | Additional analyses of bone marrow population and chemokine expression regulated by PARP. a, b, Representative images of FACS analyses of CD11b+ Gr1+ IMC cells (**a**) and B220+ B cells (**b**) in bone marrow. **c**, Olaparib and PARP2CKO did not show similar effects on B220+ B cells, CD11b+ F4/80+ macrophages, CD11c+ MHCII+ DCs (dendritic cells), CD3+ CD4+ Th (T helper) cells, CD3+ CD8+ Tc (T cytotoxic) cells and NK1.1+ NK cells (natural killer cells) in FACS analyses of bone marrow cells from WT or PARP2CKO mice treated with DMSO or olaparib after injection with Py8119 cells (n = 16). **d**, PARP1CKO did not alter IMCs in FACS analyses of bone marrow cells from WT or PARP1CKO mice intracardiacally injected with Py8119 cells (n = 8). **e**, RT-qPCR showed that mRNA levels of CCL4, CCL6 and CCL9 were reduced in PARP2CKO IMCs (n = 9) but not in PARP1CKO IMCs (n = 6). **f**, The expression of CCL4, CCL6 and CCL9 mRNA in IMCs was suppressed by the treatment of 30 μM or 100 μM olaparib (n = 9). **g**, CCL4 mRNA, but not CCL6 and CCL9 mRNA, was reduced in bone marrow cells of olaparib-treated B6 mice compared with DMSO-treated mice (2-month-old, female, 5-week daily treatment, n = 5). Data represent mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n.s., non-significant. Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.



Supplementary Fig. 6 | Effects of CCL3 on immune cells in bone marrow. a, RT-qPCR and ELISA analyses showing the efficiency of stable overexpression of mCCL3 in Py8119 cells (n = 6) and 1833 cells (n = 3). CCL3 ELISA analyses were performed on conditioned medium cultured for 24 hours (n = 4). **b**, 1833-mCCL3 cells caused similar bone metastasis as 1833 cells after intracardiacally injection into nude mice, and olaparib increased bone metastasis of 1833 cells but decreased bone metastasis of 1833-mCCL3 cells (n = 10). **c**, 200 ng/mL recombinant murine CCL3 (rCCL3) triggered transwell migration of bone marrow IMCs, macrophages, DCs, NK, B and T cells. BX-471 and Maraviroc (BX and MAV, CCR1/5 antagonists, 1 μM) suppressed the CCL3-induced migration of IMCs, macrophages, DCs and NK cells (n = 6). **d**, PARP2CKO did not affect CD4+ IL4+ type 2 T helper cells (Th2) or CD4+ IL17A+ T-helper 17 cells (Th17) in FACS analyses of bone marrow cells from WT or PARP2CKO mice intracardiacally injected with Py8119 cells (n = 8). **e**, Th2 and Th17 cells were increased by olaparib but unaffected by CCR1/5 antagonists BX plus MAV in FACS analyses of bone marrow cells from B6 mice intracardiacally injected with Py8119 cells (n = 10). **f**, **g**, Neither CCL3 overexpression in Py8119 (**f**, n = 4) nor CCR1/5 antagonists BX plus MAV (**g**, n = 6) affected olaparib-induced IMC accumulation in bone marrow. Data represent mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; n.s., non-significant. Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.



Supplementary Fig. 7 | PARP1 does not bind to CCL3 promoter or β-catenin transcription factor. a, ChIP analyses of PARP1KD Py8119 cells vs. control cells showed that PARP1 did not associate with CCL3 promoter (n = 3). **b**, Olaparib (3 μM) did not affect PARP1 signal at CCL3 promoter in IMCs (n = 3). **c**, PARP1KD did not affect CCL3 promoter driven luciferase expression (n = 5). **d**, ChIP analyses showed that H3K4me3 (Histone H3 trimethyl Lys4) level at CCL3 promoter was lower in PARP2CKO IMCs compared with WT control IMCs (n = 3). **e**, In ChIP, PARP1KD did not alter β-catenin binding to CCL3 promoter in Py8119 cells (n = 3). **f**, ChIP of p65/NFκB showed that both PARP1KD and PARP2KD reduced p65/NFκB binding to CCL3 promoter (n = 3). **g**, TOP/FOP flash assay revealed that PARP1 did not regulate β-catenin transcriptional activity (n = 6). **h**, Treatment with β-catenin inhibitor PRI-724 eliminated the expression of β-catenin target gene cyclin D1 (n = 3). **i**, Consensus TCF binding sites CTTTG(A/T)(A/T) identified at human or mouse CCL3 promoter. **j**, *In vitro* PARylation assay with recombinant human PARP1, PARP2 and GST-β-catenin with or without olaparib showed no PARylation of β-catenin. Western blot analyses were repeated twice and showed similar results. **k**, Both anti-RANKL and zoledronate (ZOL) (n = 5) diminished olaparib-induced bone metastasis (n = 10). Data represent mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; n.s., non-significant. Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.

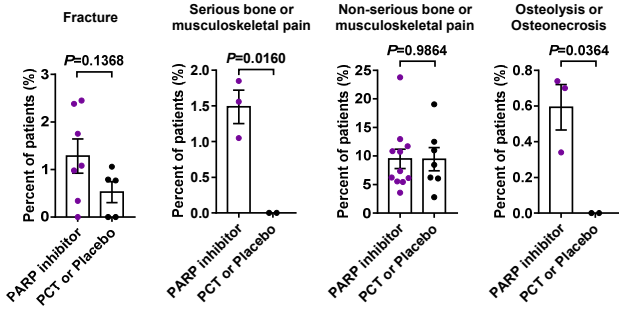


Supplementary Fig. 8 | Olaparib suppresses primary tumor growth and bone metastasis of BRCA1-deficient breast cancer cells. **a**, RT-qPCR showing the efficiency of stable knockdown of BRCA1 in 1833 (~100%) and Py8119 (~70%) cells (n = 3). **b**, 10 μM Olaparib had similar suppressive effect on the proliferation of 1833-shNT and 1833-shBRCA1 cells (n = 6). **c**, Clonogenic assay showed similar IC50s of olaparib to the survival of 1833-shNT and 1833-shBRCA1 cells (n = 4). **d**, 10 μM Olaparib significantly suppressed the migration of 1833-shBRCA1 cells but not control cells (n = 4). **e**, **f**, Olaparib similarly suppressed primary tumor growth of Py8119-shNT cells (**e**, n = 10) or Py8119-shBRCA1 cells (**f**, n = 10) at mammary fat pad. Statistical analysis was performed using two-way ANOVA with Sidak's multiple comparison. **g**, Bone metastasis of 1833-shBRCA1 cells was reduced by olaparib treatment (n = 10). Left, BLI images; right, BLI signals. **h**, Bone metastasis of Py8119-shBRCA1 cells was not altered by olaparib treatment (n = 10). Data represent mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n.s., non-significant. **a**, **d**, **g**, **h**, Two-sided Student's t-test was used to calculate statistical difference. Source data are provided as a Source Data file.

a

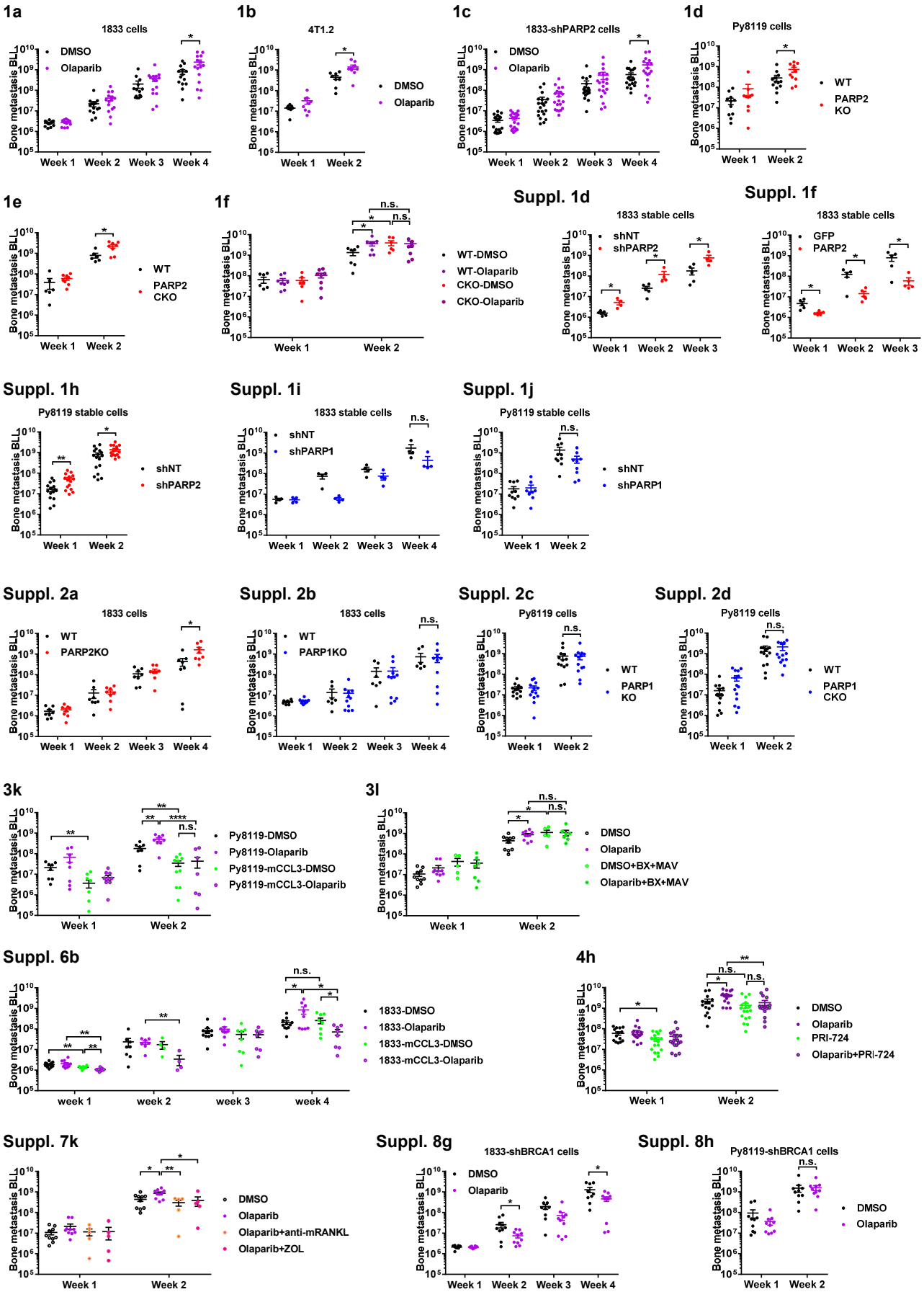
Identifier	Other Name	Cancer types	Inhibitor name	PARP inhibitor				Physician's Choice Treatment or Placebo				Refer-ence
				Fracture	Serious bone or musculoskeletal pain	Non-serious bone or musculoskeletal pain	Osteolysis or Osteonecrosis	Fracture	Serious bone or musculoskeletal pain	Non-serious bone or musculoskeletal pain	Osteolysis or Osteonecrosis	
NCT00494234	ICEBERG 1	Breast	Olaparib	n/a	1/54 (1.85%)	7/54 (12.96%)	n/a	n/a	n/a	n/a	n/a	1
NCT00494442	ICEBERG 2 / study 9	Ovarian	Olaparib	1/57 (1.75%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	2
NCT00628251	ICEBERG 3 / study 12	Ovarian	Olaparib	n/a	1/64 (1.56%)	4/64 (6.25%)	n/a	n/a	0/32 (0.00%)	4/32 (12.5%)	n/a	3
NCT00753545	study 19	Ovarian	Olaparib	3/126 (2.38%)	n/a	10/136 (7.35%)	1/136 (0.74%)	0/128 (0.00%)	n/a	8/128 (6.25%)	0/128 (0.00%)	4
NCT00679783	study 20	Ovarian/Breast	Olaparib	n/a	n/a	5/90 (5.56%)	n/a	n/a	n/a	n/a	n/a	5
NCT01078662	study 42	Ovarian/ Breast/ Prostate/ Pancreatic/ Advanced Tumours	Olaparib	1/298 (0.34%)	n/a	32/298 (10.74%)	1/298 (0.34%)	n/a	n/a	n/a	n/a	6
NCT02000622	OlympiAD	Breast	Olaparib	2/205 (0.98%)	n/a	24/205 (11.71%)	n/a	0/91 (0.00%)	n/a	10/91 (10.99%)	n/a	7
NCT01844986	SOLO-1	Ovarian	Olaparib	0/260 (0.00%)	n/a	16/260 (6.15%)	n/a	1/130 (0.77%)	n/a	11/130 (8.46%)	n/a	8
NCT01874353	SOLO-2	Ovarian	Olaparib	n/a	n/a	7/195 (3.59%)	n/a	n/a	n/a	6/99 (6.06%)	n/a	9
NCT01945775	EMBRACA	Breast	Talazoparib	7/286 (2.45%)	3/286 (1.05%)	68/286 (23.78%)	3/286 (0.70%)	1/126 (0.79%)	0/126 (0.00%)	24/126 (19.05%)	0/126 (0.00%)	10
NCT02034916	ABRAZO	Breast	Talazoparib	n/a	n/a	9/83 (10.84%)	n/a	n/a	n/a	n/a	n/a	11
NCT01891344	ARIEL2	Ovarian	Rucaparib	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	12
NCT01968213	ARIEL3	Ovarian	Rucaparib	4/372 (1.08%)	n/a	n/a	n/a	2/189 (1.06%)	n/a	n/a	n/a	13
NCT01847274	ENGOT-OV16/NOVA	Ovarian	Niraparib	n/a	n/a	20/367 (5.45%)	n/a	n/a	n/a	5/179 (2.79%)	n/a	14

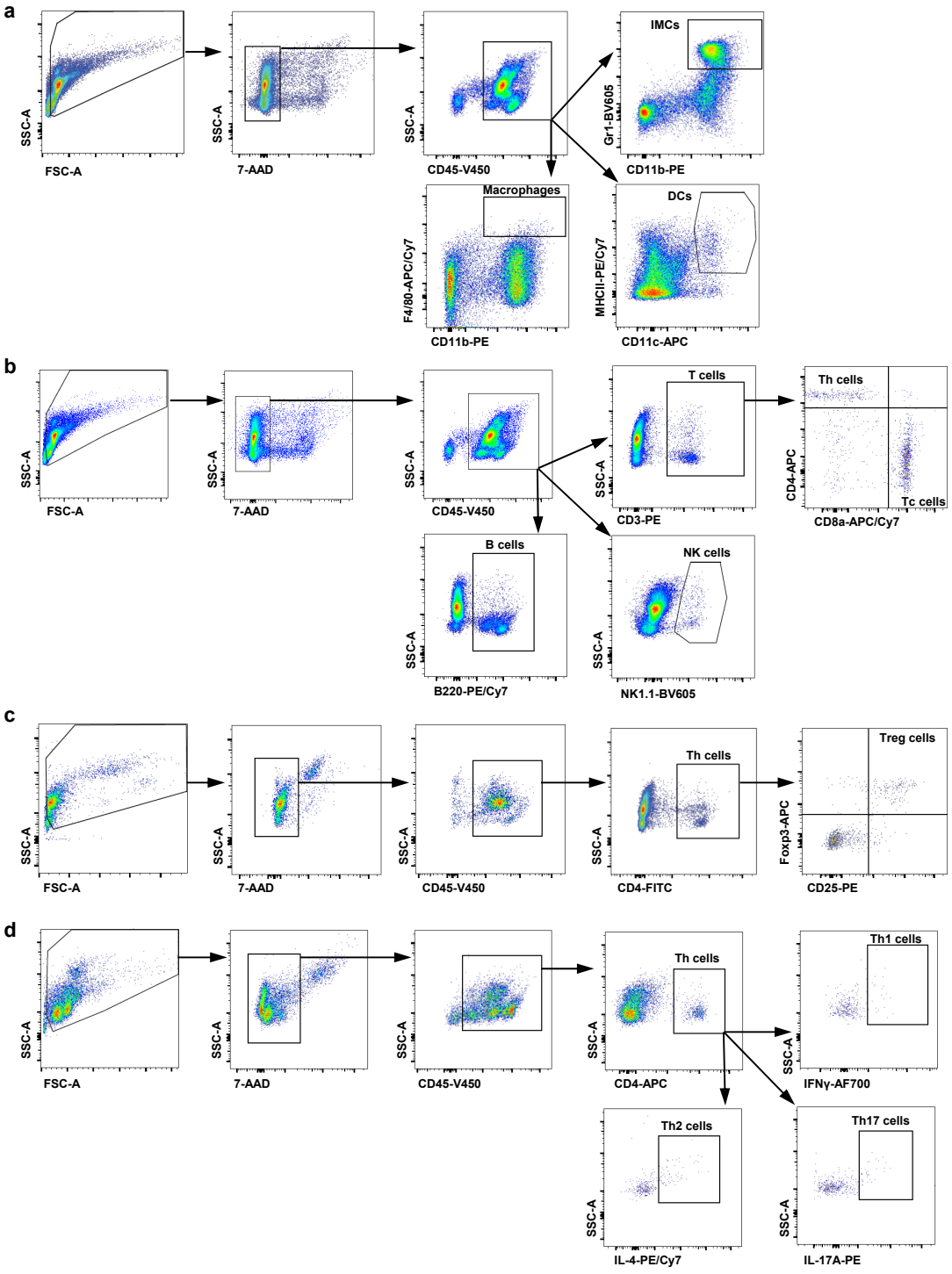
b



Supplementary Fig. 9 | Increased incidence of bone health related adverse events in PARP inhibitor treated patients. a, A summary of available data on adverse events related to bone health in PARP inhibitor monotherapy clinical trials. The clinical results were from https://clinicaltrials.gov/ and published articles. Reported data is presented as adverse events / total patient number (event incidence) in the group for PARP inhibitor treatment and the group for Physician's Choice Treatment or Placebo when available. **b**, Statistic analyses illustrate that patients with PARP inhibitor treatment showed increased incidence of serious bone or musculoskeletal pain and osteolysis/osteonecrosis, as well as an increased risk of bone fracture. The incidence of non-serious bone or musculoskeletal pain are comparable between the two groups of patients. Data represent mean ± SEM. Two-sided Student's *t*-test was used to calculate statistical difference. n/a, not available.

Supplementary Fig. 10 The development of the BLI signal over the entire course in main and Supplementary figures (Suppl.).





Supplementary Fig. 11 | Gating strategies used for FACS analyses. a, Gating strategies for CD11b+ Gr1+ IMCs, CD11b+ F4/80+ macrophages, and CD11c+ MHCII+ DCs in CD45+ mouse bone marrow cells presented on Fig. 3a-c and Supplementary Fig. 5a, 5c-d, 6c, 6f-g. **b**, Gating strategies for B220+ B cells, NK1.1 NK cells, and CD3+ T cells in CD45+ mouse bone marrow cells, and for CD4+ Th cells and CD8a+ Tc cells in CD3+ T cells presented on Supplementary Fig. 5b-c, 6c. **c**, Gating strategies for CD25+ Foxp3+ Treg cells in CD4+ Th cells from mouse bone marrow presented on Fig. 3m-n. **d**, Gating strategies for IFNγ+ Th1 cells, IL-4+ Th2 cells, and IL-17A+ Th17 cells in CD4+ Th cells from mouse bone marrow presented on Fig. 3m-n and Supplementary Fig. 6d-e.

Supplementary Table 1. RAW data of PARP2 regulated genes from RNA-seq of PARP1WT vs. PARP1CKO and PARP2WT vs. PARP2CKO.

GENE_NAME	FPKM (Fragments Per Kilobase of exon model per Million mapped fragments)								PARP1WT v.s. PARP1CKO			PARP2WT v.s. PARP2CKO		
	PARP1WT		PARP1CKO		PARP2WT		PARP2CKO		log_2FC	P value	FDR	log_2FC	P value	FDR
Ifi202b	0	0	0	0.28667534	19.9229723	19.7773186	0	0	5.80006401	4.45E-05	0.01165844	-12.961331	1.88E-188	3.30E-184
Nlrp1c-ps	8.15231213	8.52532977	9.99637519	8.68846801	3.51683772	6.15636378	0	0	0.16396938	0.43747902	0.89988942	-10.921348	1.59E-69	1.39E-65
Fmr1nb	0.12899228	0.24185333	0.07933631	0	0.2945135	0.26934092	0.01680561	0	-2.1310336	0.00422302	0.24831141	-4.6006639	8.95E-08	9.81E-05
Xlr3b	0.56756603	0.90694998	0.63469049	0.63950653	0.19056756	0.25010228	0.01680561	0	-0.2307358	0.51403366	0.94076369	-4.238461	1.88E-06	0.00150011
Rmrp	0.05159691	0.04030889	0.03966816	0.0882078	8.64483755	0.7310682	0.47055717	0.12404127	0.45346524	0.68763444	0.98535912	-3.9520249	2.96E-06	0.00216618
Spint1	5.03069894	5.38123652	4.00648371	5.88787046	2.97978369	0.17314773	0.18486175	0.16538836	-0.0739577	0.90931038	1	-3.153011	2.88E-05	0.01681118
Wfdc18	0.07739537	0.16123555	0.09917039	0.11025975	0.25986486	0.42325001	0.01680561	0.06202064	-0.2361237	0.728908	0.99709522	-3.1031098	2.58E-06	0.00196924
Nlrp1a	22.4446568	19.3079573	22.4323419	19.979066	9.56302671	17.8726936	2.08389606	1.73657783	0.02354476	0.90754751	1	-2.8364624	1.05E-34	4.61E-31
Hdc	7.97172294	8.76718309	8.60798974	11.0700785	5.5437836	0.98117048	0.80666944	0.86828891	0.23287449	0.60552596	0.96495836	-1.962622	5.55E-05	0.02952358
Ccl6	104.070972	95.9351529	93.3193358	127.239747	54.1731604	104.081025	22.5195219	20.4461366	0.14108917	0.49285568	0.93389947	-1.880297	1.45E-18	4.25E-15
Wfdc17	11.764096	20.476915	11.5632673	13.6722086	38.3907014	67.7777174	16.4022787	14.8436057	-0.3563204	0.1319374	0.76123149	-1.7638378	1.70E-13	3.31E-10
Egr1	72.3388709	51.6558397	14.4590427	7.14483157	41.0586473	79.1669904	17.5954772	22.5341647	-2.5191445	1.41E-16	2.74E-13	-1.5831279	7.35E-08	8.59E-05
Kcnj10	0.4127753	0.46355221	0.39668156	0.50719483	1.62848643	1.38518185	0.67222454	0.37212382	0.03144819	0.93573702	1	-1.5016775	1.42E-06	0.00118682
Fosb	6.81079241	6.44942205	0.93220165	0.61745458	1.73243238	4.69422738	1.39486591	0.9509831	-3.0874727	1.72E-15	3.02E-12	-1.4420894	8.06E-05	0.0392966
Slfn8	27.888131	22.1295794	25.1892787	35.3272227	13.3743779	19.9312277	7.74738777	6.86361712	0.27550015	0.12241772	0.74928527	-1.1860281	1.70E-10	2.98E-07
Ccl4	71.2037388	70.4800903	64.1037393	72.3303936	47.1741336	80.1289223	26.5696747	34.8555978	-0.0545451	0.75469319	1	-1.0517389	2.88E-09	4.22E-06
Ccl9	528.687761	531.371913	506.820189	480.401715	393.054257	554.553706	234.774419	235.74044	-0.1026881	0.37308049	0.86146873	-1.0099683	4.49E-18	9.86E-15
Ccl3	135.493491	131.991453	138.124517	143.469982	104.985402	146.329071	62.7017435	63.8605822	0.07419271	0.49083519	0.93224673	-0.9893628	1.29E-19	4.54E-16
Parp2	38.9814672	40.77244	38.2797701	36.8708592	48.265566	48.3659329	25.8134221	25.5938494	-0.0864414	0.19969231	0.77375933	-0.9102434	1.17E-41	6.83E-38
Scd1	149.656844	85.2331432	107.004849	144.748995	136.238482	140.865299	66.7014795	89.847229	0.10042777	0.61302389	0.96495836	-0.8240635	3.74E-05	0.02113605
Hmga2-ps1	9.54542876	8.50517532	9.32201654	7.6079225	16.7699454	15.8333981	9.99933996	9.34444259	-0.086695	0.49729858	0.93552779	-0.7520351	3.28E-10	5.23E-07
Lyz2	5701.58779	6387.00373	3496.21239	4320.77073	9742.61065	10813.2297	6758.59589	5736.78485	-0.6289721	1.40E-06	0.00066275	-0.7181479	3.72E-08	4.66E-05
Retnlg	19.9422066	19.4087295	17.4738225	18.1046504	9.85754022	12.9860798	7.61294286	7.25641449	-0.1451894	0.21457271	0.77375933	-0.6157784	7.79E-07	0.00075912
Slfn10-ps	23.4507966	23.7419349	24.0587363	23.9925208	18.0519454	21.7973755	13.5117132	13.1690485	0.02579324	0.78958736	1	-0.5766734	6.28E-09	8.47E-06
Cxcr2	19.7616174	19.8521272	19.2787236	24.5217676	13.4609996	16.3913186	10.7387869	10.4401405	0.14361848	0.23848778	0.77375933	-0.4933961	8.56E-05	0.04059047
Lyz1	258.629522	252.857653	149.886126	183.251698	411.002257	462.631499	336.935743	303.96314	-0.6187595	2.99E-11	3.40E-08	-0.4467733	1.29E-06	0.00116878
Mmp9	46.6436086	48.5520553	46.9670961	56.0340031	29.2607828	32.5710121	24.4689731	21.4384667	0.11261606	0.22658698	0.77375933	-0.4272614	8.62E-06	0.00560069
Phgdh	115.2933	88.0346109	98.5753664	108.892526	82.3078622	91.5374339	60.9539597	68.6361712	0.02992738	0.77805241	1	-0.4241433	7.02E-05	0.03516416
Fads2	145.322703	123.788594	124.756349	149.115281	137.918941	134.747412	98.5145056	107.626478	0.02565224	0.77489782	1	-0.4038356	6.85E-06	0.00461992
Chd3	46.6178102	43.6948344	62.8541924	69.1769649	52.3021334	58.6778422	46.2826592	37.6672	0.54825632	3.66E-09	2.47E-06	-0.4006909	1.49E-05	0.00902234
Pck2	39.2652502	34.9881146	39.0731332	38.1498722	34.4927286	36.4572167	27.7628733	29.8112527	0.0588702	0.45132647	0.90751476	-0.3021303	0.00011208	0.04915098
Tfrc	378.334359	385.534357	461.776998	481.614572	531.423631	498.203739	673.955513	593.640861	0.30447088	8.87E-05	0.01945385	0.30003887	0.00010627	0.04779889
Atp6v0d2	67.0759859	64.7562282	57.0626417	55.7252758	106.475294	127.186628	146.360087	142.151299	-0.2246033	0.00438717	0.25370098	0.304679	6.05E-05	0.03120391
Plscr3	37.2013737	39.0391578	41.1358773	39.5391451	46.2559444	41.20916	54.7190772	57.5758244	0.0808945	0.27221404	0.78526208	0.35922645	4.34E-07	0.00044831
Pdpn	6.47541248	7.45714424	6.5055775	6.83610428	16.215567	15.4486253	25.4941155	19.2677445	-0.0671047	0.64354841	0.9709298	0.50132168	9.48E-05	0.04375196
Dusp23	10.4999716	11.7298863	12.0591193	11.5111175	7.70932407	8.54195474	11.8815687	11.1223675	0.08185166	0.47636276	0.92319852	0.50381655	1.22E-05	0.00765151
Folr2	30.4937751	26.7852559	45.5985448	43.7069635	24.4619451	29.8583643	42.770286	35.1450274	0.64241943	3.49E-09	2.45E-06	0.52253691	1.33E-06	0.00116878
Slamf7	128.089335	118.367049	137.926177	143.734605	94.2269969	88.8440247	139.385757	132.165977	0.19313631	0.00316025	0.21759945	0.56886802	4.16E-18	9.86E-15
Sel1l3	5.44347424	5.09907431	6.6840842	7.03457182	5.26659442	4.94432966	9.78086699	6.71890231	0.38097136	0.02985455	0.54401419	0.69616032	4.58E-05	0.02512967
2410003L11Rik	0.33537993	0.08061778	0.25784301	0.19846754	0.08662162	0.03847727	0.55458524	0.35145027	0.20685646	0.72553005	0.99670761	2.79827498	6.13E-06	0.00430134

Red color labels immune chemokines.

Supplementary Table 2. Primer sequences

Primers	Sequences	Primers	Sequences
mL19 qPCR	GGTCTGGTTGGATCCCAATG CCCATCCTTGATCAGCTTCCT	mPARP2 qPCR	ATCTTCGAGTCCAGGAGCTG ACTGTCAGCTTTCCAAGCG
mTRAP qPCR	AAGTATGCCCACACCAACTGATC GAAAGCCCGTTCCCAAGAAA	hPARP1 qPCR	GGCTTAATCCTGTTGGGAGA CCAAACCTTTGACACTGTGC
mCTSK qPCR	AGCAGGCTGGAGGACTAAGGT GATTTGTGCATCTCAGTGGAAGAC	hPARP2 qPCR	GCATGACTTTGGACTCCGTA GCAATTTCAATGTCTCCCAA
mCCL3 qPCR	GTGGAATCTTCCGGCTGTAG ACCATGACACTCTGCAACCA	mRANKL qPCR	TCCATGTTCCTGGCCCTCCT AGGATCCATCTGCGCTCGAA
mCCL4 qPCR	GAAACAGCAGGAAGTGGGAG CATGAAGCTCTGCGTGTCTG	mOPG qPCR	AGGGCGTTACCTGGAGATCG TGCAAACTGTGTTTCGCTCTGG
mCCL6 qPCR	TTGGAGGGTTATAGCGACGA CCAGGAGGATGAGAAACTCC	PyMT qPCR	CCAACAGATACACCCGCACAT GGTCTTGGTCGCTTTCTGGATA
mCCL9 qPCR	GCCTTCAGACTGCTCTGGAC ACTGCCCTCTCCTTCCTCAT	mVimentin qPCR	AGCTGCTAACTACCAGGACACTATTG CGAAGGTGACGAGCCATCTC
mCCL3-ChIP-1	GCCCCTTCTCTTATAGGTAGCCC CACTGTGGACCCTGGGTTG	Luciferase qPCR	CAACTGCATAAGGCTATGAAGAGA ATTTGTATTCAGCCCATATCGTTT
mCCL3-ChIP-2	GTCTCAGCTCTCAACTCGTGACC TTCTCATGAGATGGGACCAGC	mCAR2 qPCR	TGGTCAACTTAGGGCATCTTTTC TCCTATGGCTGTGAAGAGAAGCA
mCCL3-ChIP-3	CTCAGCTTCTCTAACCACATGGA GACCGCGTCTTCCCCATT	mOscar qPCR	AATGCTTTGCCTGTATGCATGTA TTCCAAGAGATCAGATGCCTTCT
mCCL3-ChIP-4	GTGACTAGGCCACTGGGTTGA CTTTCCTAGAAAGGCTATTTCTAGCTT	mCalcr qPCR	TGCCTTTCAGAGGTGATTTAGTTG CTCAGTATCCAAGGTCCACAGTTG
mCCL3-promoter-2k	AGAAAGGGACACATTATAGGAA	mNFATc1 qPCR	AGTCTCTTTCCCCGACATCA GATCCGAAGCTCGTATGGAC
mCCL3-promoter-0.7k	GAGATGGCTTTACATTTGGG		
mCCL3-promoter-0.5k	GGCTGATGATTGGACAAATG	mRANK qPCR	TCGGAGCTCAGCATCCCTTG TCCAAGGAGGGTGCAGTTGG
mCCL3-promoter-0.3k	TTATTCACAGAACGGTTCTCA		
mCCL3-promoter-0.15k	GATGCTATTCTTAGATATCCTGG	mCyclin D1 qPCR	TCCCTAGCAAGCTGCCAAAC TGGACCCACCACCAGTCTATG
mCCL3-promoter+75	GCTTGTCAGCAGAACAGAA		
hCCL3-promoter-2k	CTACAGACACTTAGAAAGGACAGA	hBRCA qPCR	TGTGAAGGCCCTTTCTTCTG TCCCATCTGTCTGGAGTTGA
hCCL3-promoter+75	CTCGAGTGTCAGCAGAGC		
hGAPDH qPCR	AAGGTGAAGGTCGGAGTCAA AATGAAGGGGTCATTGATGG	mBRCA qPCR	TGTGGTCACACTTTGTGGAAA ATCTGCCGTCCAAATTCAAG
mPARP1 qPCR	CTGTCCAAGAAGATGGTGGA CTTGAGCTGACTGGCACTGT		

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