Supplemental data for:

IL-1β-driven osteoclastogenic T regulatory cells accelerate bone erosion in arthritis

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All authors declare no related conflicts of interest.

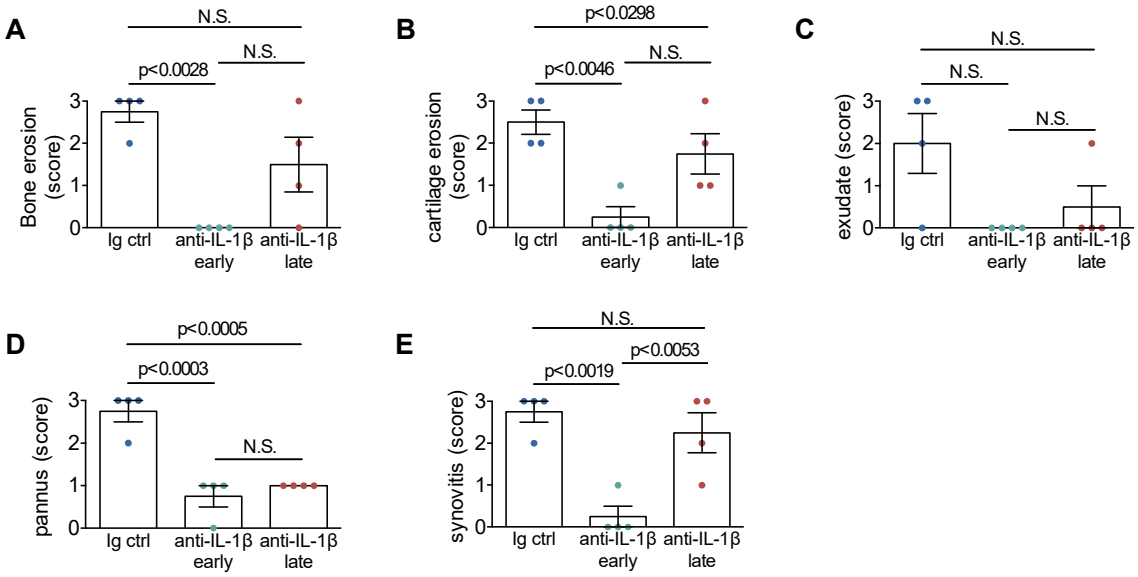
Extended Data Table 1 | Mass cytometry panels for analysis of *in vitro* differentiated Tregs

Synovial Panel		
Target	**Clone**	**Metal**
pSTAT3(Y705)	D3A7	169Tm
pSTAT1 (Y701)	58D6	160Gd
pSTAT5 (Y694)	D47E7	174Yb
CD62L	MEL-14	146Nd
Tbet	4B10	158Gd
CCR6	G034E3	169Tm
CTLA-4	UC10-4B9	176Yb
Eomes	Dan11mag	156Gd
RORgt	AFKJS-9	160Gd
CXCR4	L276F12	144Nd
Helios	22F6	168Er
CD44	IM7	115In
CD73	TY/11.8	143Nd
CD25	3C7	167Er
Foxp3	FJK-16s	162Dy
PD-1	29F.1A12	171Yb
CD39	Duha59	159Tb
CD69	H1.2F3	173Yb
RANKL	IK22/5	166Er
CD103	2E7	154Sm

Extended Data Table 2 | Mass cytometry panels for analysis of human synovial cells

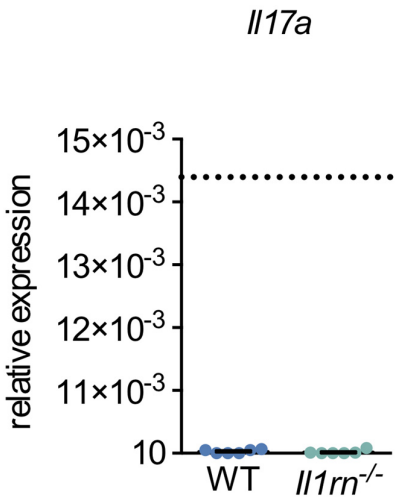
Synovial Panel

Target	Clone	Metal
CD45	HI30	141Pr
CD19	HIB19	142Nd
RANKL	MIH24	143Nd
CD64	10,1	144Nd
CD16	3G8	145Nd
CD8a	RPA T8	146Nd
FAP	Poly	147Sm
CD20	2H7	148Nd
CD45RO	UCHL1	149Sm
CD38	HIT2	150Nd
CD279/PD-1	EH12.2H7	151Eu
CD14	M5E2	152Sm
CD69	FN50	153Eu
CD185/CXCR5	J252D4	154Sm
CD4	RPA T4	155Gd
Podoplanin	NC-08	156Gd
CD3	UCHT1	158Gd
CD11c	Bu15	159Tb
CD307d/FcRL4	413D12	160Gd
CD138	MI15	161Dy
CD90	5E10	162Dy
CCR2	K036C2	163Dy
Cadherin 11	3C10	164Dy
FoxP3	PCH101	165Ho
CD34	581	166Er
CD146/MCAM	SHM-57	167Er
IgA	9H9H11	168Er
TCRgd	B1	169Tm
ICOS	C398.4A	170Er
CD66b	G10F5	171Yb
IgM	MHM-88	172Yb
CD144/VE-Cadherin	BV9	173Yb
HLA-DR	L243	174Yb
IgD	IA6-2	175Lu
CD106/VCAM-1	STA	176Yb

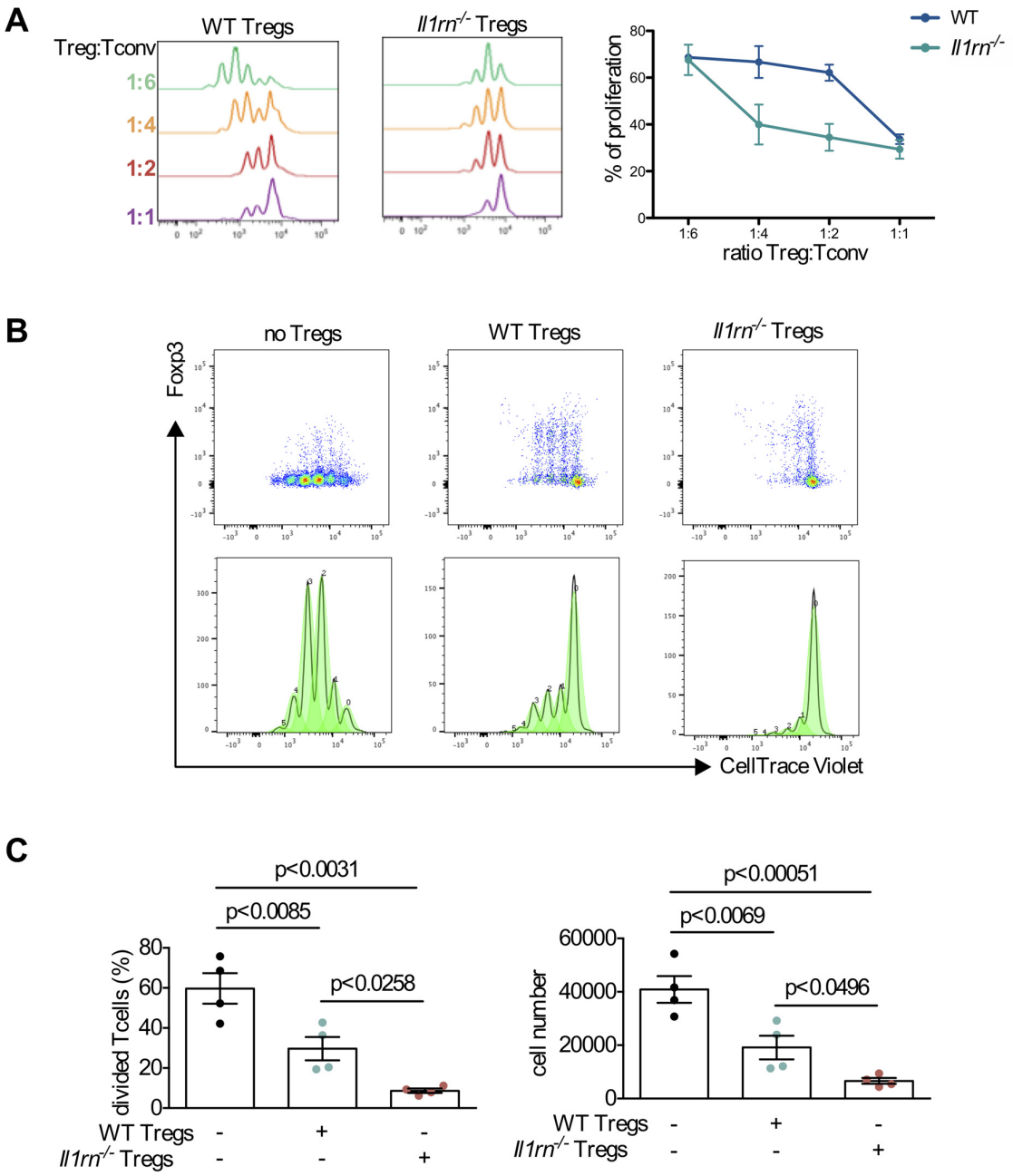


Supplemental Figure 1

(**A,E**) *Il1rn*$^{-/-}$ mice were treated with anti-IL-1β or isotype-matched IgG (n=4) (5mg/kg i.p. 1 time per week) for 2 weeks either at weaning (early treatment, n=4) or 14 days after weaning (late treatment n=4). d, Histological evaluation of bone erosion in knee joints (**A**), cartilage erosion (**B**), exudate (**C**), pannus (**D**) and synovitis (**E**).

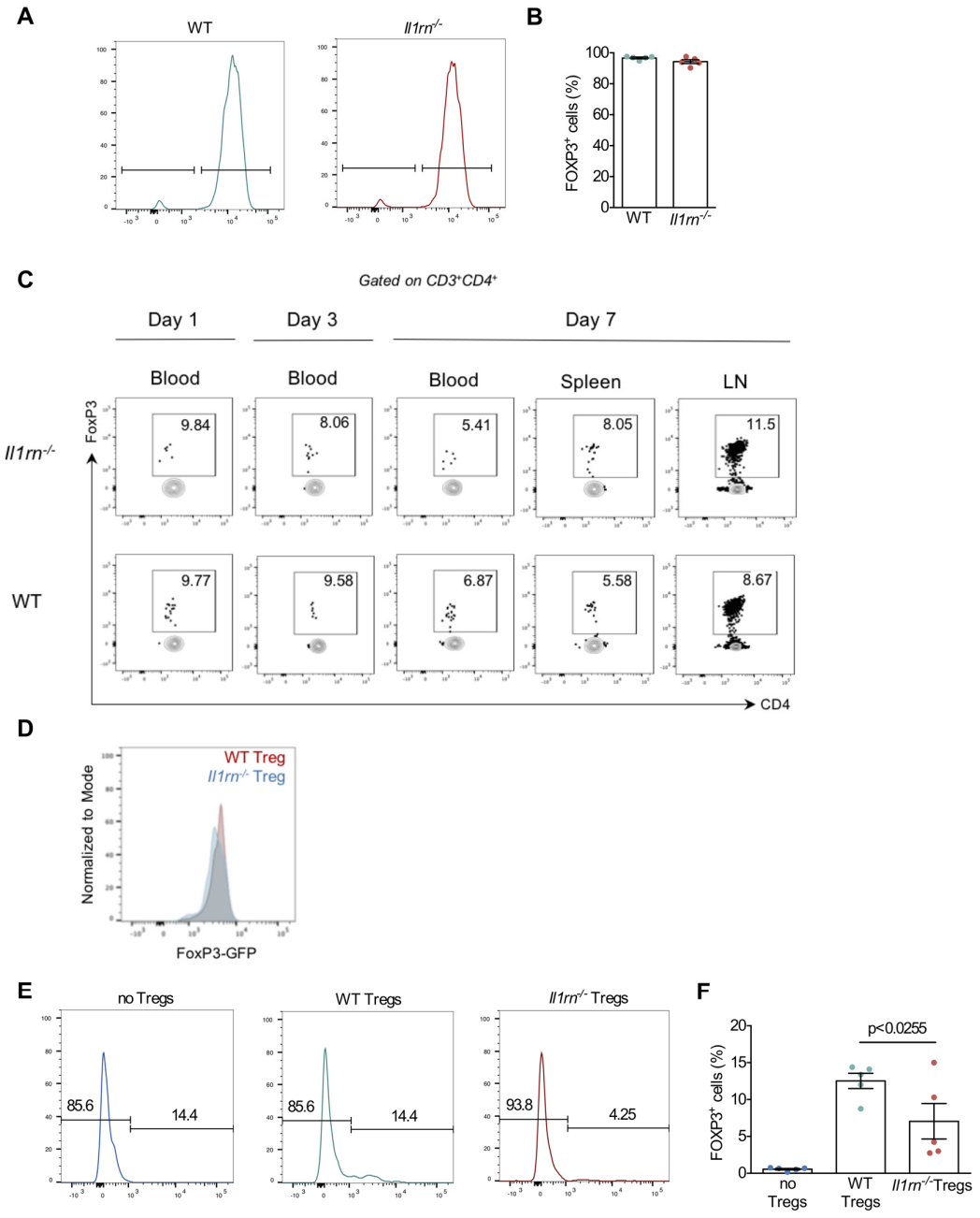


Supplemental Figure 2
RT-PCR of Il17a in sorted CD4+Foxp3eGFP+ Treg cells from WT and *Il1rn*$^{-/-}$ mice, n=6 per group (dotted line: limit of detection).



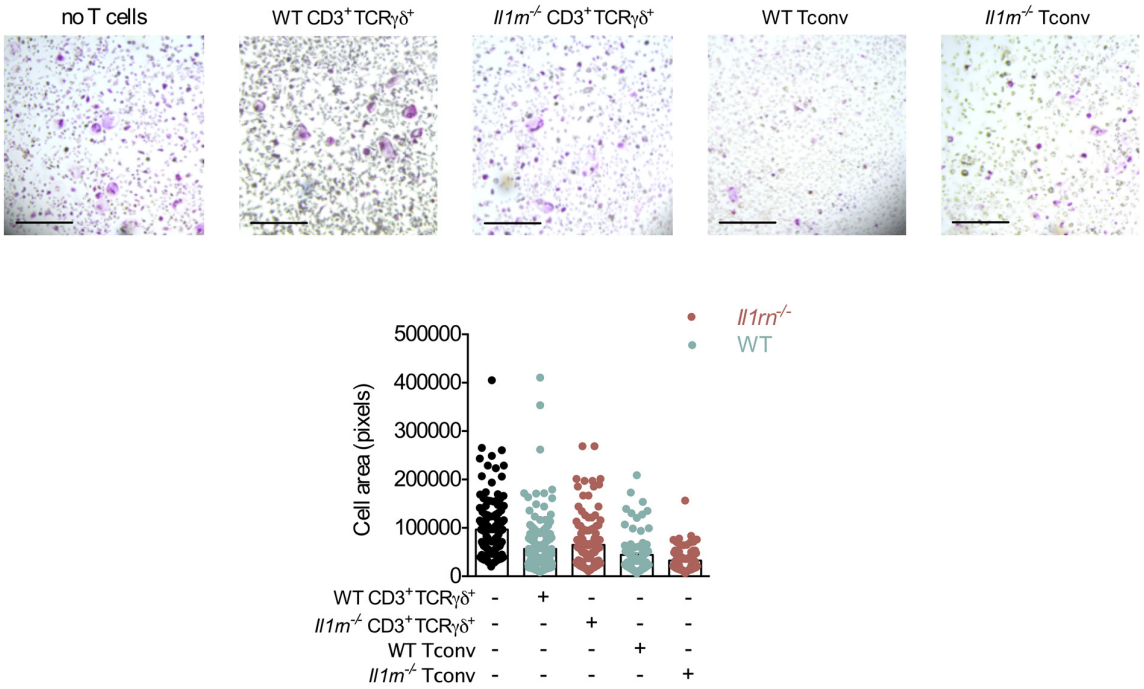
Supplemental Figure 3

(**A**) Foxp3^{+} cells from WT mice or *Il1rn*$^{-/-}$ mice were cocultured with WT Foxp3^{-} cells stained with CellTrace Violet (n=4 per group). Tregs (CD3^{+}Foxp3^{+}) and Tconv (CD3^{+}Foxp3^{-}) were co-cultured in different ratios. (1:6, 1:4, 1:2, 1:1). Proliferation of WT and *Il1rn*$^{-/-}$ CD3^{+}Foxp3^{-} cells was measured following 72 h of coculture. (**B,C**) Tconv were stained with CellTrace Violet and cultured without Tregs, with WT Tregs or *Il1rn*$^{-/-}$ Tregs and CellTrace Violet staining was assayed by flow cytometry. (**C**) Percentage of divided Tconv and cell number after. 72h of co-culture (n=4 per group). (**C**) One-way ANOVA.

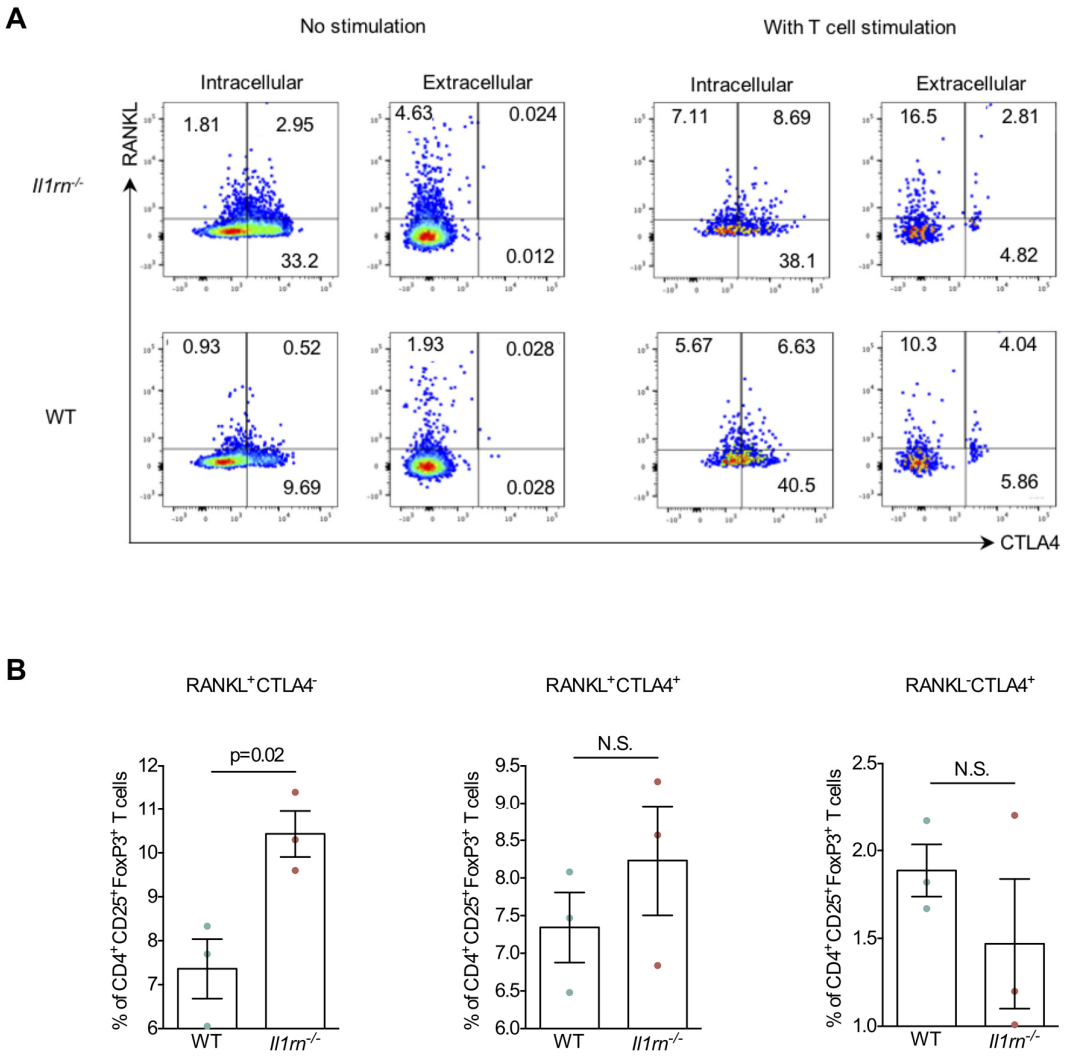


Supplemental Figure 4

(**A,B**) Foxp3^{eGFP+} cells from WT and *Il1rn*$^{-/-}$ mice were sorted and stimulated in vitro from 72h with CD3/CD28 beads. (**C**) T cells from FoxP3-GFP+ WT or *Il1rn*$^{-/-}$ mice were transfused into WT mice at Day 0. Representative flow cytometry plots show percentage of FoxP3+ T cells in blood and tissues at various timepoints after adoptive transfer. (**D**) Histogram depicting FoxP3 protein expression in donor FoxP3+ CD4 T cells at Day 7 after transfer. (**E,F**) Foxp3^{eGFP+} and Foxp3^{-} cells stained with CellTrace Violet from WT mice or *Il1rn*$^{-/-}$ mice were cocultured for 72h with CD3/CD28 beads. Foxp3 expression by CellTrace^{+} cells (n=5 per group).



Supplemental Figure 5
Sorted CD3^{+}TCR$\gamma\delta^{+}$ and CD3^{+}FOXP3^{eGFP-} (Tconv) from WT and *Il1rn*$^{-/-}$ were sorted and co-cultured with macrophage precursor cells (n=4 per group). After 5 days of co-culture, cells were stained with tartrate-resistant acid phosphatase (TRAP) and TRAP^{+} multinucleated cells were measured. Scale bar, 1mm



Supplemental Figure 6

T cells purified from WT or *Il1rn*$^{-/-}$ mice were assessed for RANKL and CTLA4 expression. T cells stimulated with anti-CD3/anti-CD28 beads for 48 hours. (**A**) Representative dot plots showing intracellular and extracellular protein expression. (**B**) RANKL and CTLA4 cell surface expression in stimulated T regs. Mann and Whitney *t* test.