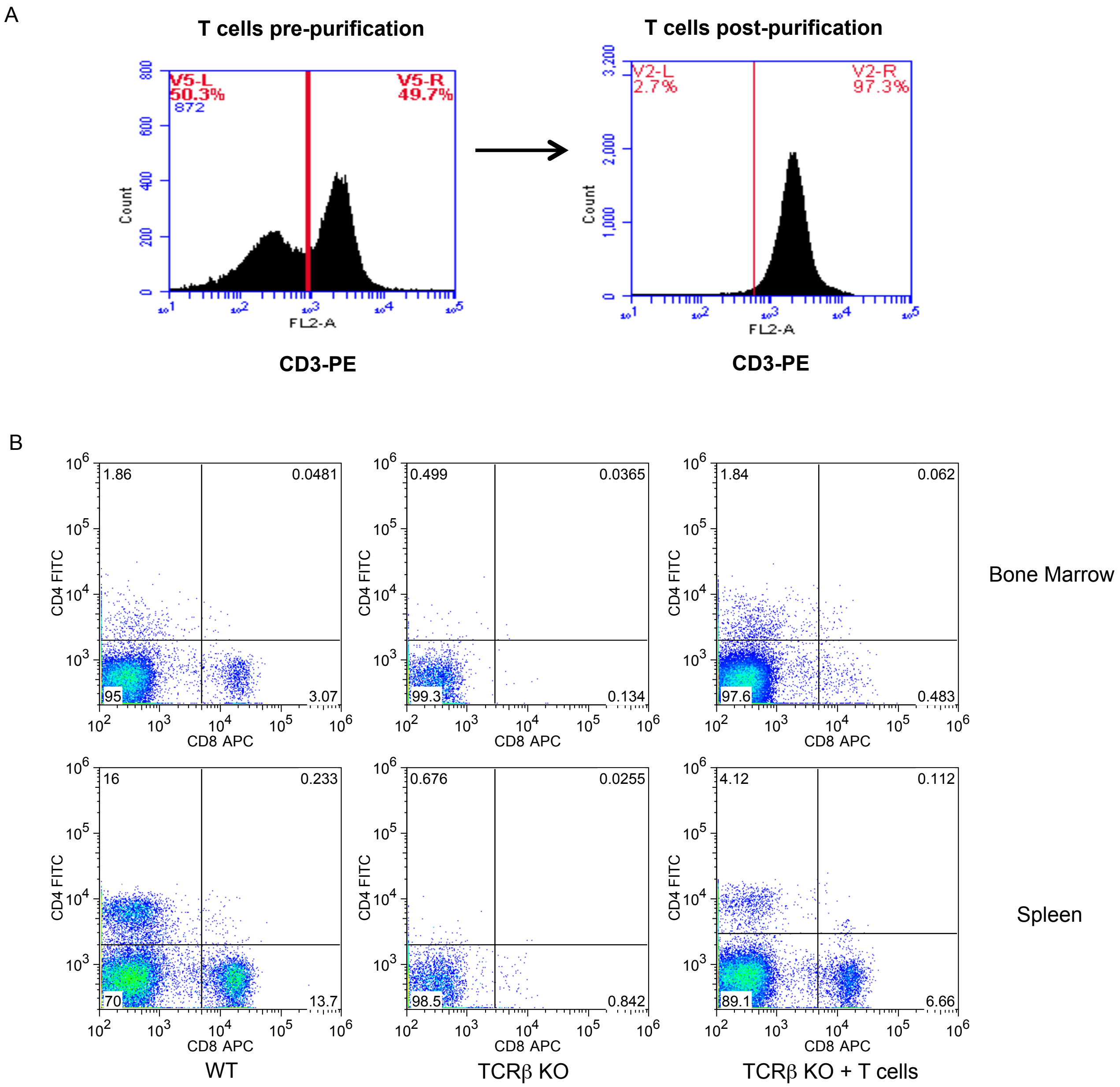
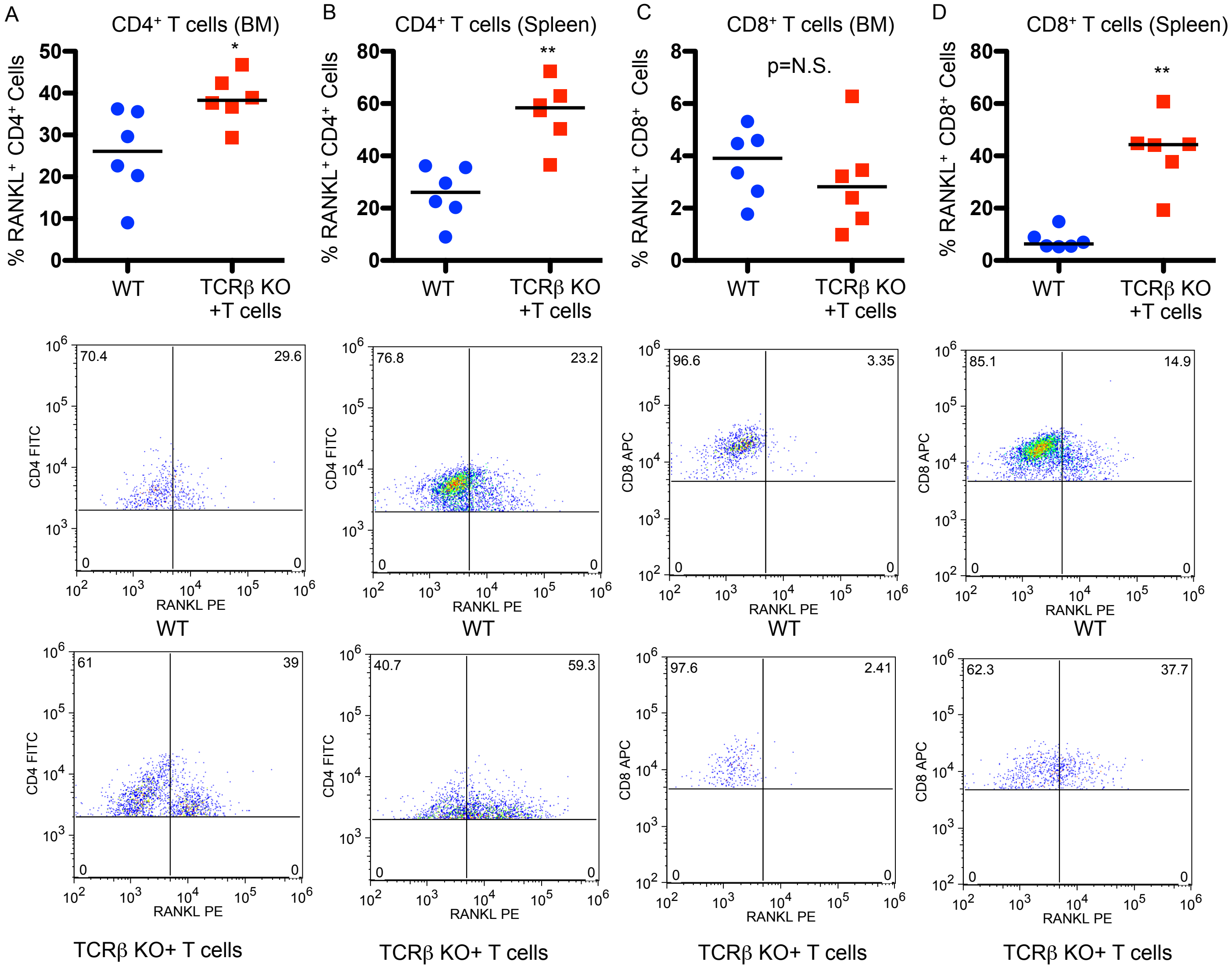
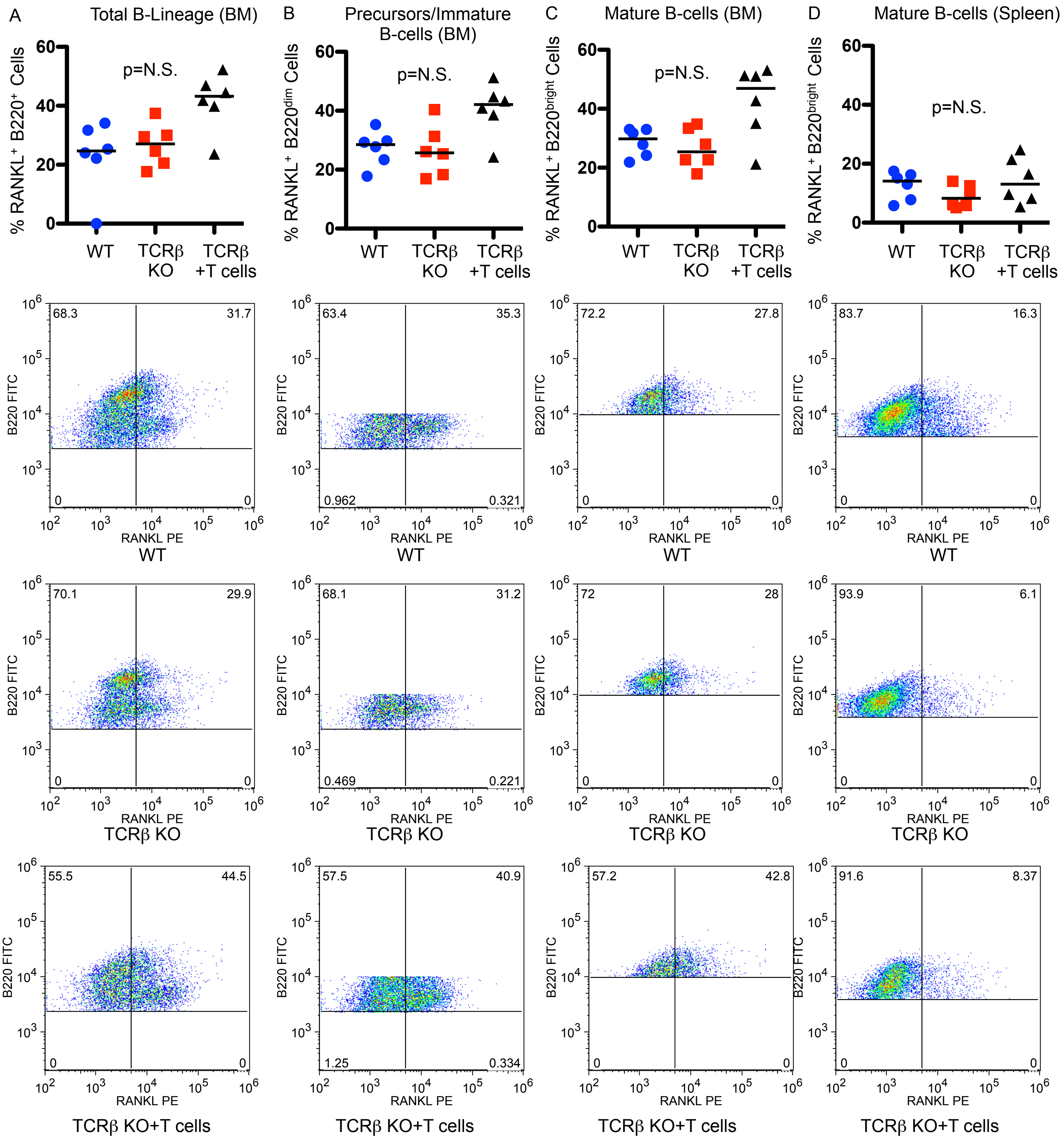


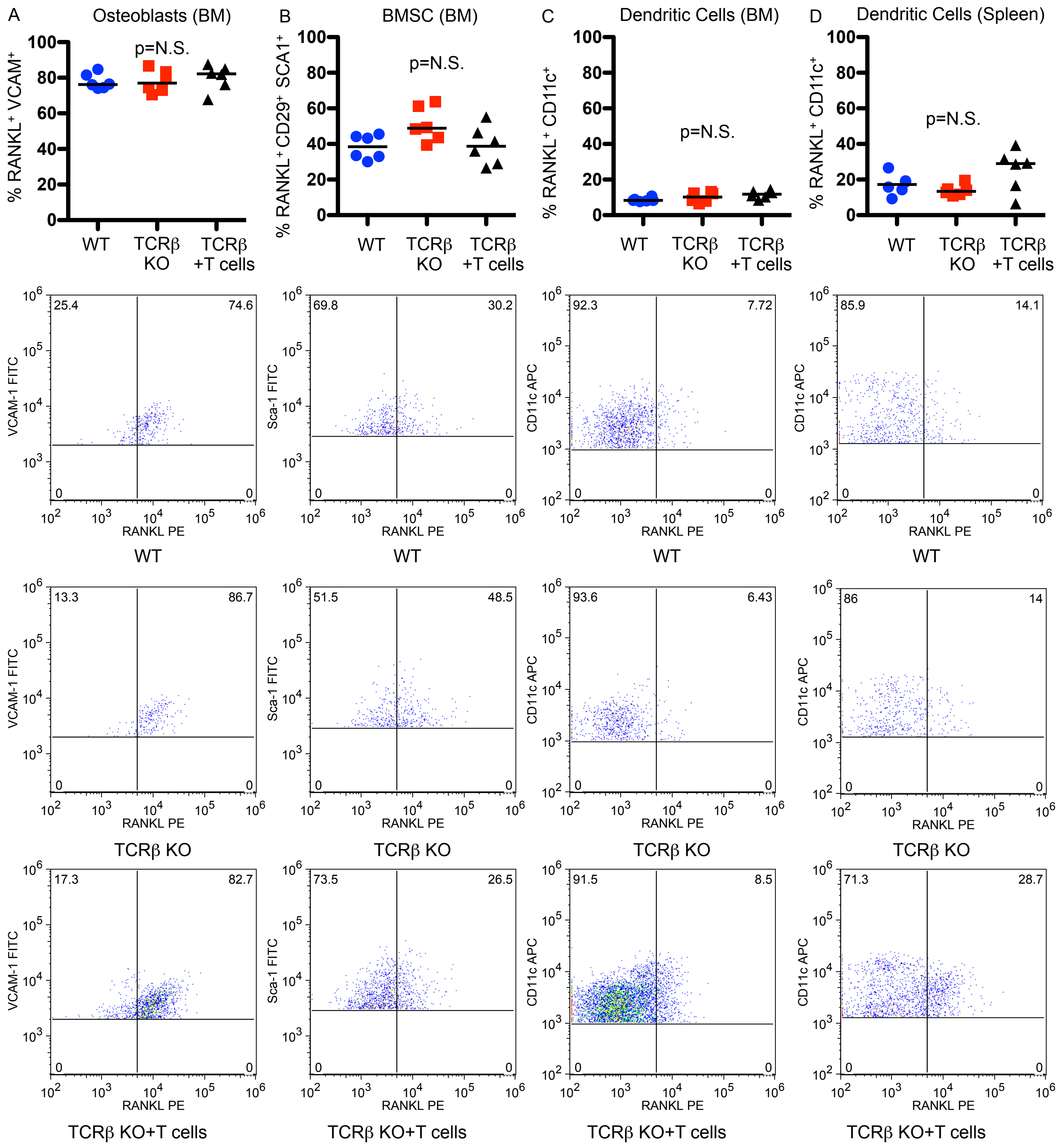
Supplementary Figure 1. Representative T cell purification, proliferation and repopulation in TCRβ KO mice following adoptive transfer. (A) Splenic CD3⁺ T cells prior to purification (left panel) and following immunomagnetic enrichment (Right Panel). (B) CD3⁺ T cell repopulation of spleen and bone marrow following adoptive transfer. T cells quantified using flow cytometry.



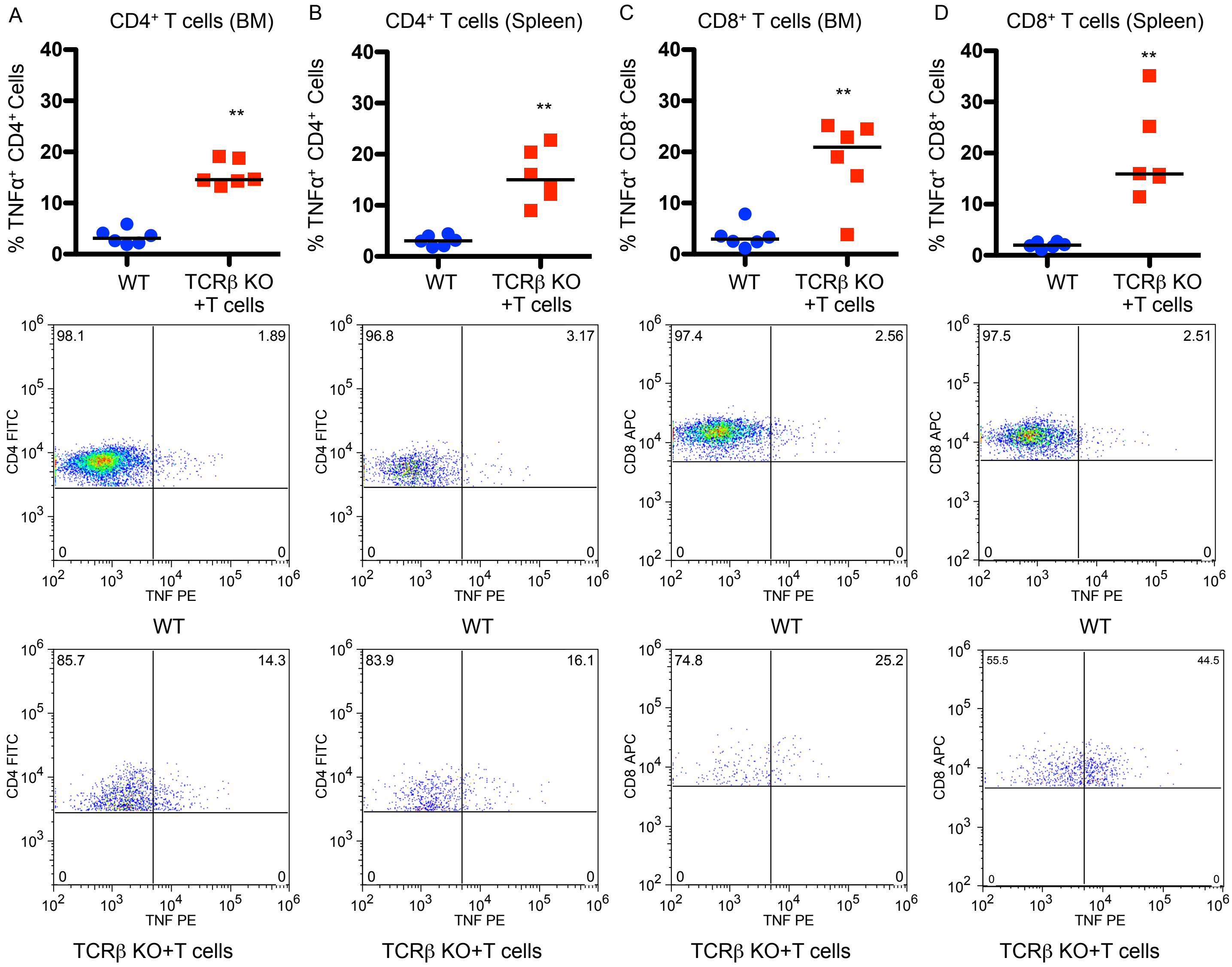
Supplementary Figure 2: The proportion of T cell subsets producing RANKL following CD3$^+$ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/ group. Expression of RANKL was quantified by flow cytometry in WT control mice and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) CD4$^+$ in bone marrow (BM); (B) CD4$^+$ in Spleen; (C) CD8$^+$ in bone marrow (BM); (D) CD8$^+$ in Spleen; One representative dot plot for each WT control and TCRβ+T cells is presented below each bar graph.



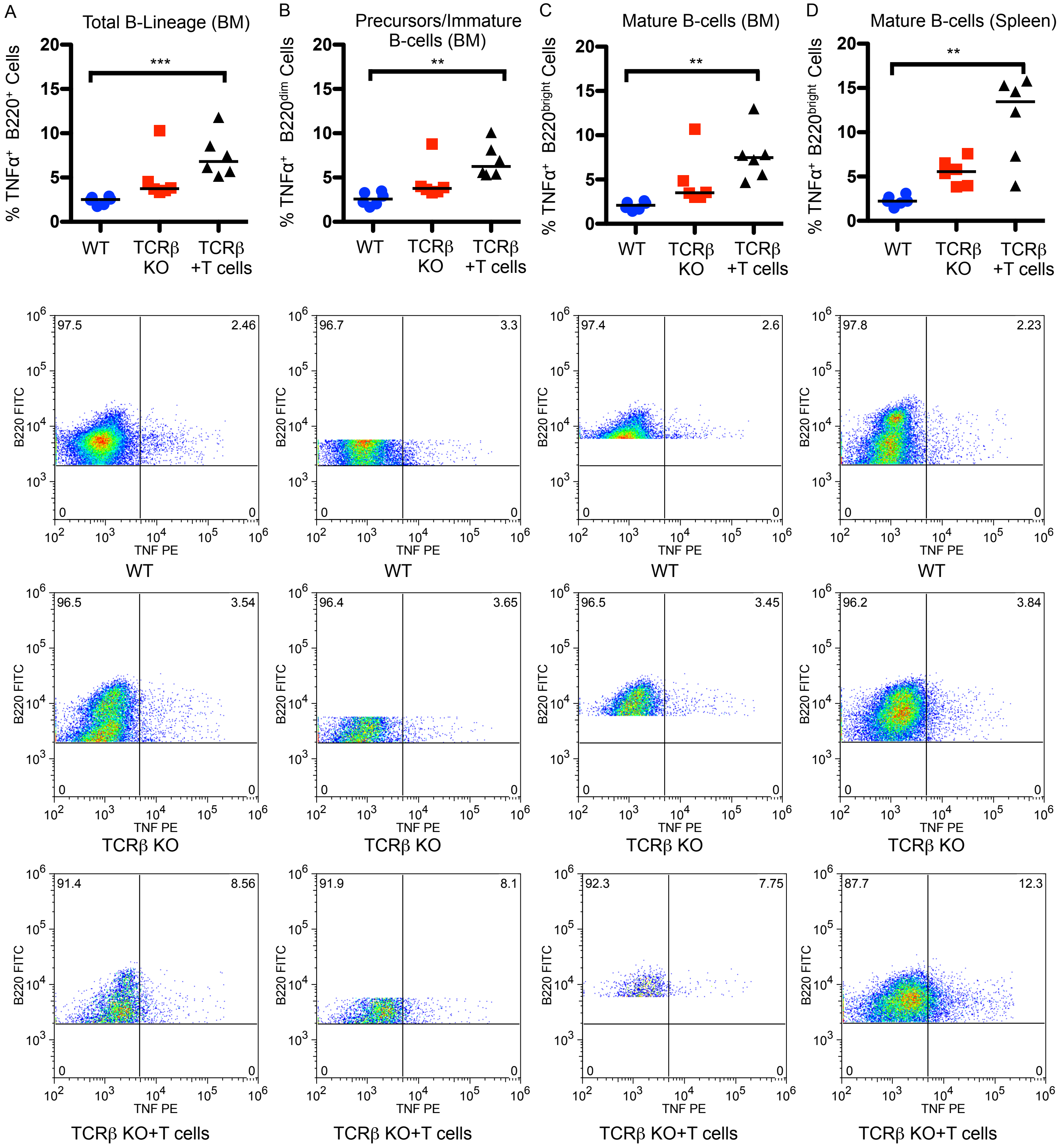
Supplementary Figure 3: The proportion of B-lineage cells producing RANKL following CD3⁺ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/ group. Expression of RANKL was quantified by flow cytometry in WT control mice and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) total bone marrow (BM) B-lineage; (B) bone marrow (BM) immature B-cells and B-cell precursors; (C) Mature bone marrow (BM) B-cells; (D) Mature B-cells in spleen. One representative dot plot for each WT control and TCRβ+T cells is presented below each bar graph.



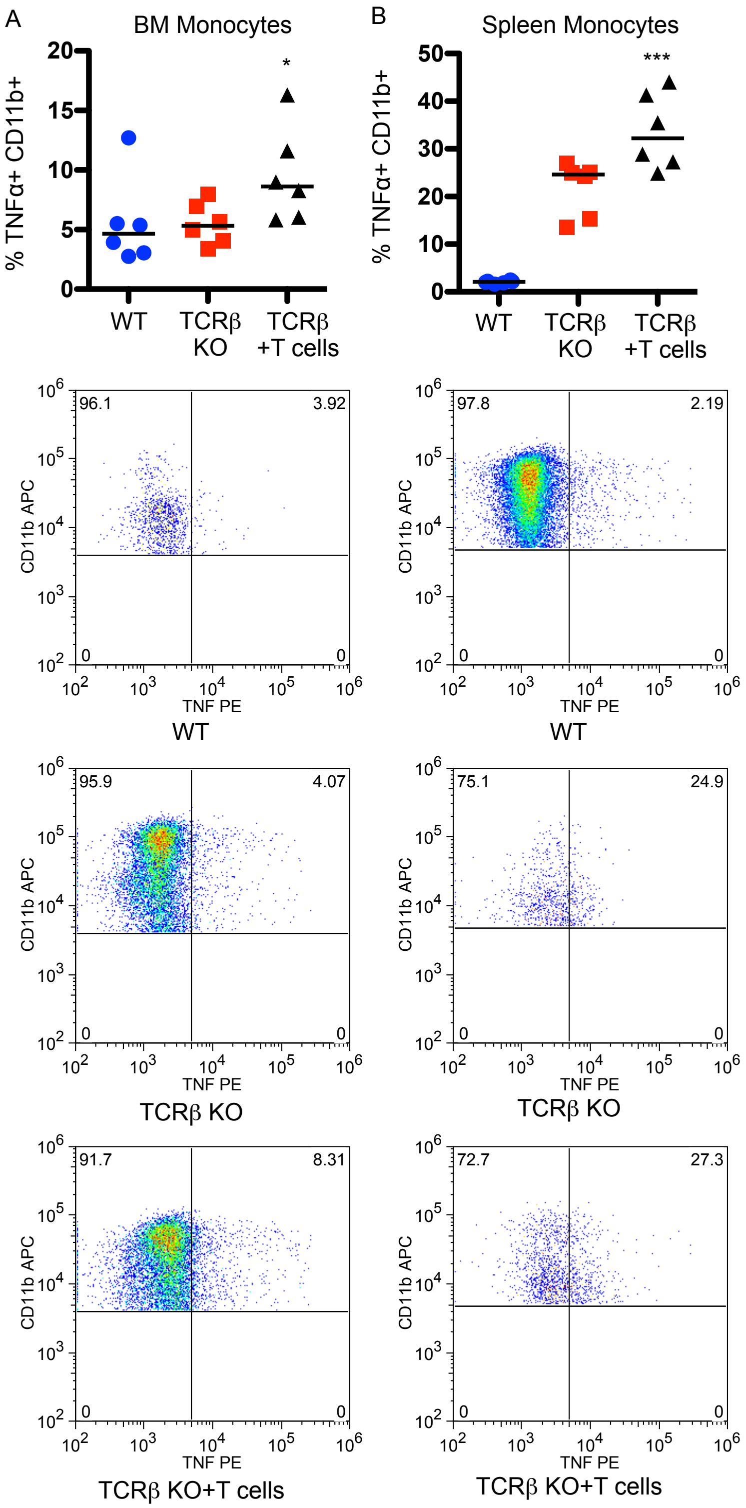
Supplementary Figure 4: The proportion of Osteoblasts, bone marrow stromal cells (BMSC) and dendritic cells producing RANKL following CD3⁺ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/group. Expression of RANKL was quantified by flow cytometry in WT control mice and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) bone marrow osteoblasts; (B) bone marrow stromal cells (BMSC); (C) bone marrow (BM) dendritic cells and (D) dendritic cells in spleen. One representative dot plot for each WT control and TCRβ+T cells is presented below each bar graph.



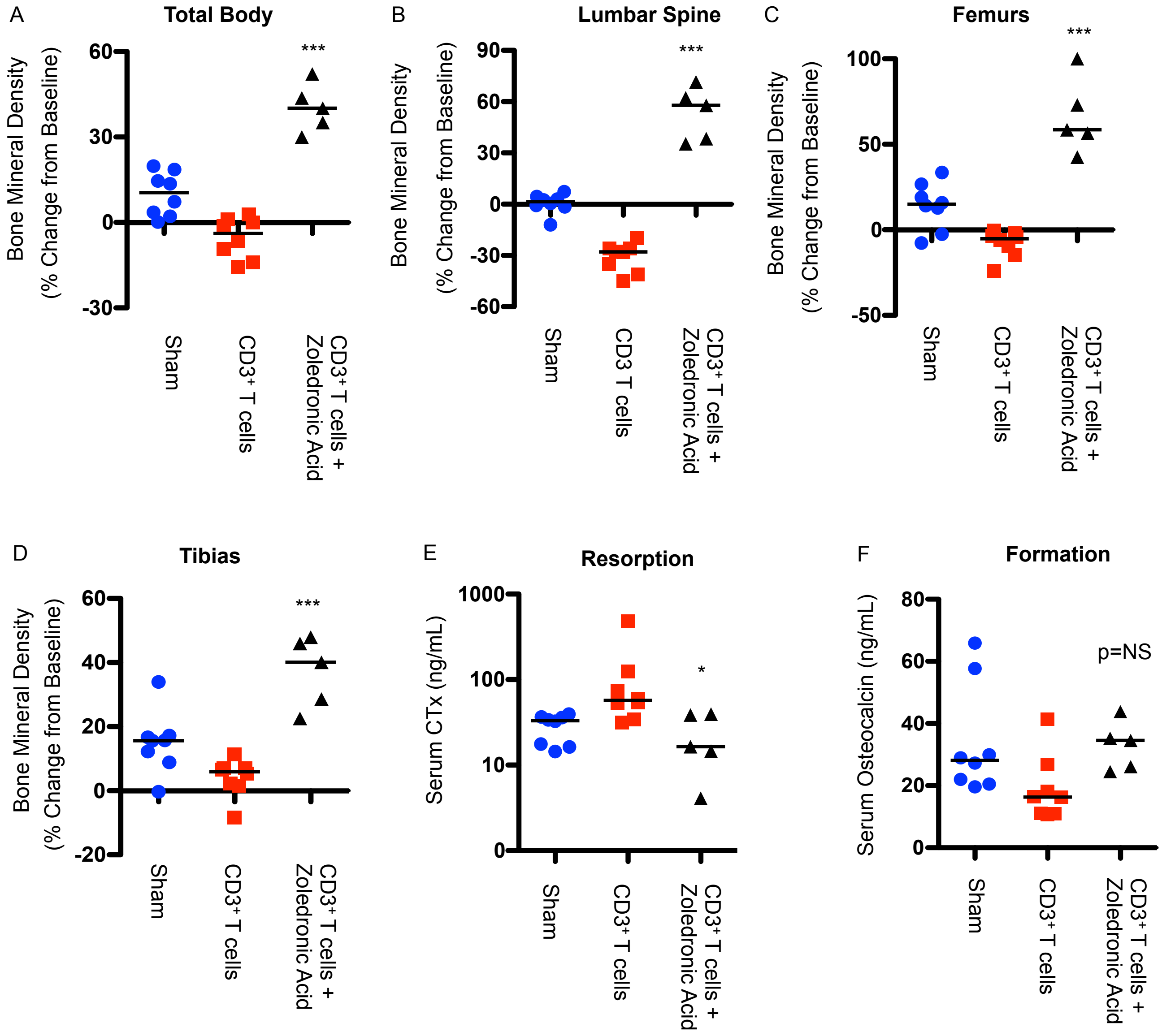
Supplementary Figure 5: The proportion of T cell subsets producing TNFα following CD3⁺ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/ group. Expression of TNFα was quantified by flow cytometry in WT control mice and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) CD4⁺ in bone marrow (BM); (B) CD4⁺ in Spleen; (C) CD8⁺ in bone marrow (BM); (D) CD8⁺ in Spleen; One representative dot plot for each WT control and TCRβ+T cells is presented below each bar graph.



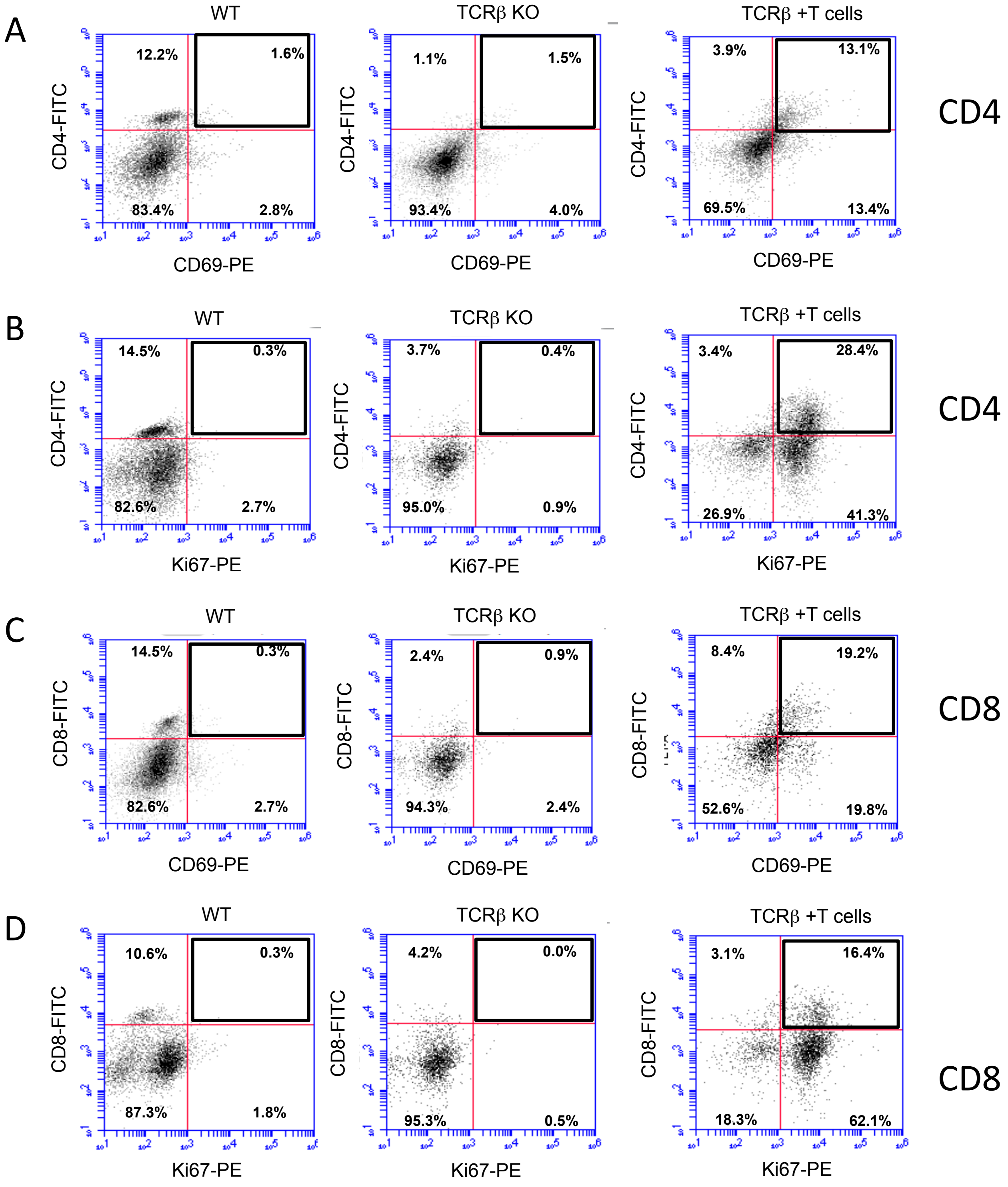
Supplementary Figure 6: The proportion of B-lineage cells producing TNFα following CD3$^+$ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/group. Expression of TNFα was quantified by flow cytometry in WT control mice and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) total bone marrow (BM) B-lineage; (B) bone marrow (BM) immature B-cells and B-cell precursors; (C) Mature bone marrow (BM) B-cells; (D) Mature B-cells in spleen. One representative dot plot for each WT control and TCRβ +T cells is presented below each bar graph.



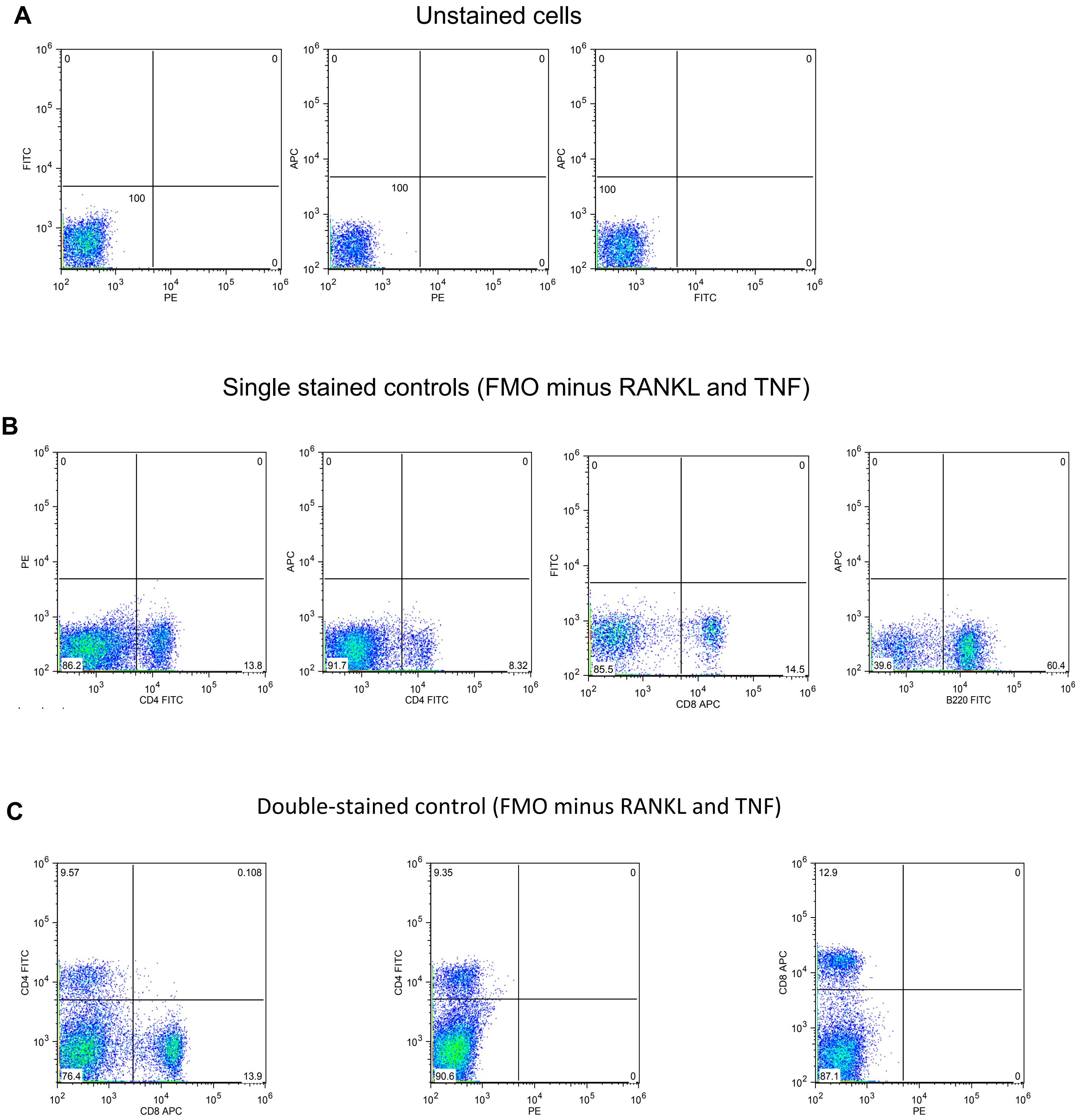
Supplementary Figure 7: The proportion of monocytes/macrophages producing TNFα following CD3$^+$ T cell reconstitution. All data are expressed as Mean ± SEM of 6 independent mice/group. Expression of TNFα by monocytes/macrophages was quantified by flow cytometry in WT control mice, TCRβ KO and TCRβ KO mice reconstituted with T cells (TCRβ+T cells) in: (A) total bone marrow (BM) and (B) spleen. One representative dot plot for each WT control and TCRβ+T cells is presented below each bar graph.



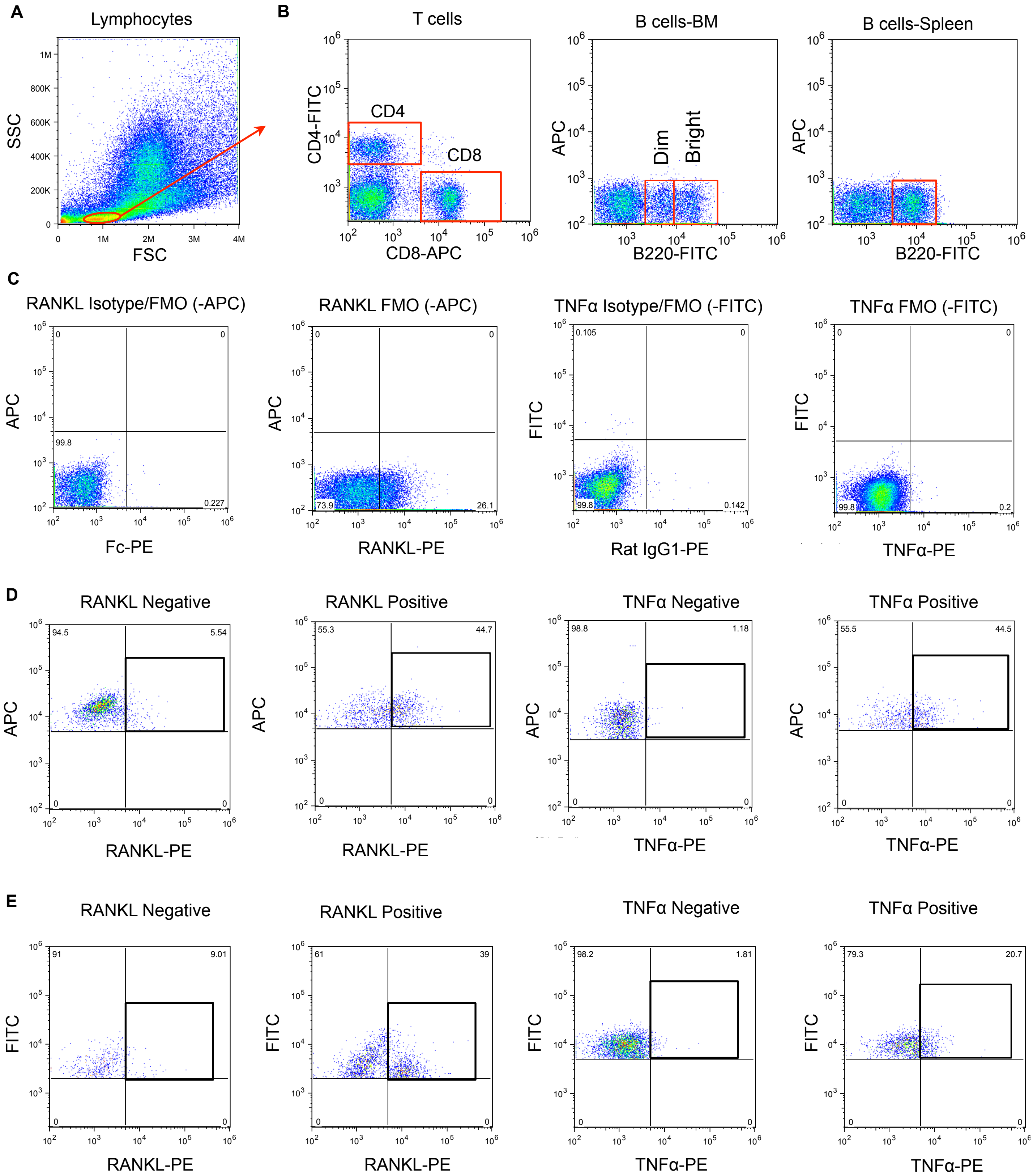
Supplementary Figure 8. Zoledronic acid prevents bone loss associated with T cell reconstitution. BMD was quantitated by DXA (% change from baseline) for (A) total body, (B) lumbar spine, (C) femurs and (D) tibias in Sham, 1 x 10^5 CD3$^+$ T cell reconstituted and CD3$^+$ T cell reconstituted mice receiving a single IP injection of zoledronic acid. Data presented as parentage change in BMD from baseline at 12 weeks. ***P<0.001 vs. CD3$^+$ T cells. (E) CTx ***P<0.001 vs. Sham and (F) Serum osteocalcin *p<0.05 vs. Sham by Kruskal-Wallis 1-way ANOVA with Dunn's Multiple Comparison post-test. Data points represent individual animals with median (black line), N=8 Sham and CD3$^+$ T cell mice/group and 5 CD3 T cell + zoledronic acid mice.



Supplementary Figure 9: T cell activation/proliferation state 3 months after adoptive transfer. Splenic T cells were harvested from WT mice, TCRβKO mice and TCRβ KO mice reconstituted with T cells,3 months after adoptive transfer. A) CD4 and C) CD8 T cells were analyzed for expression of CD69, an activation marker and B) CD4 and D) CD8 T cells analyzed for Ki67, a marker of proliferation. Lymphocytes were segregated on SSC and FSC and gates set using isotype controls and FMO staining for PE and FITC. Black boxes represent CD4 or CD8 double positive for CD69 or Ki67.



Supplementary Figure 10: Examples of Single and Double FMO gating strategies. (A) Non-specific fluorescence for FITC, PE and APC was controlled with reference to unstained cells. Effective compensation was verified based on lack of spillover of CD4-FITC, B220-FITC and CD8-APC into the RANKL and TNFα channel (PE) using (B) single stained FMO controls and double stained (C) controls.



Supplementary Figure 11: RANKL and TNFα gating Schemes. (A) Lymphocytes were presorted based on SSC and FSC, and (B) T cell and B cell populations segregated based on CD4 and CD8 (T cell) or B220 (B cell) expression. (C) Lymphocytes were segregated for RANKL and TNFα staining with gates positioned with respect to Isotype and FMO controls and verified with reference to empirical visual cues based on positive and negative staining for (D) APC and (E) FITC. Additional single and double stained FMO controls are shown in Supplemental Fig. 3

Supplementary Table 1

Baseline BMD (g/cm^2) for all mice in each group

Figure #	Group	Site				
1A,B,C,D			Total Body	Femur	Spine	Tibia
	Sham		0.0414	0.0595	0.0679	0.0514
			0.0425	0.0648	0.0662	0.0527
			0.0447	0.0658	0.0693	0.0572
			0.0460	0.0535	0.0652	0.0526
			0.0403	0.0595	0.0679	0.0514
			0.0438	0.0648	0.0662	0.0527
			0.0442	0.0658	0.0693	0.0572
			0.0465	0.0535	0.0652	0.0526
		Mean	0.0437	0.0609	0.0672	0.0535
		Median	0.0438	0.0648	0.0679	0.0527
	T cells		0.0430	0.0621	0.0655	0.0498
			0.0431	0.0507	0.0695	0.0483
			0.0441	0.0669	0.0680	0.0529
			0.0456	0.0733	0.0671	0.0576
			0.0418	0.0621	0.0655	0.0498
			0.0451	0.0507	0.0695	0.0483
			0.0433	0.0669	0.0680	0.0529
			0.0416	0.0733	0.0671	0.0576
		Mean	0.0435	0.0632	0.0675	0.0522
		Median	0.0433	0.0621	0.0680	0.0498

Supplementary Table 2

Baseline BMD (g/cm^2) for all mice in each group

Figure #	**Group**	**Site**				
3A,B,C			**Total Body**	**Femur**	**Spine**	**Tibia**
	Sham		0.0428	0.0657	0.0600	0.0530
			0.0432	0.0643	0.0618	0.0520
			0.0435	0.0650	0.0623	0.0517
			0.0434	0.0655	0.0587	0.0507
			0.0410	0.0615	0.0540	0.0480
			0.0426	0.0646	0.0589	0.0496
			0.0435	0.0648	0.0644	0.0543
			0.0410	0.0626	0.0520	0.0518
			0.0413	0.0649	0.0567	0.0511
			0.0426	0.0649	0.0579	0.0525
			0.0451	0.0655	0.0719	0.0515
			0.0433	0.0585	0.0574	0.0511
		Mean	0.0428	0.0640	0.0597	0.0514
		Median	0.0426	0.0648	0.0579	0.0515
	WT T cells		0.0446	0.0671	0.0676	0.0526
			0.0437	0.0633	0.0624	0.0498
			0.0453	0.0648	0.0567	0.0485
			0.0437	0.0641	0.0639	0.0509
			0.0446	0.0656	0.0578	0.0499
			0.0450	0.0647	0.0629	0.0496
			0.0445	0.0666	0.0558	0.0530
			0.0390	0.0580	0.0453	0.0501
			0.0390	0.0596	0.0503	0.0514
			0.0390	0.0579	0.0522	0.0483
			0.0384	0.0576	0.0527	0.0497
		Mean	0.0414	0.0614	0.0539	0.0503
		Median	0.0390	0.0588	0.0525	0.0499
	TNFα KO T cells		0.0369	0.0561	0.0434	0.0482
			0.0385	0.0560	0.0474	0.0473
			0.0367	0.0505	0.0485	0.0479
			0.0375	0.0545	0.0545	0.0482
			0.0402	0.0620	0.0515	0.0524
			0.0389	0.0505	0.0518	0.0495
			0.0397	0.0581	0.0520	0.0506
			0.0385	0.0612	0.0474	0.0469
		Mean	0.0384	0.0561	0.0496	0.0488
		Median	0.0389	0.0581	0.0518	0.0495

Figure #			**Total Body**	**Femur**	**Spine**	**Tibia**
3A,B,C Continued	**RANKL KO T cells**		0.0407	0.0604	0.0501	0.0511
			0.0674	0.0637	0.0529	0.0539
			0.0420	0.0620	0.0577	0.0482
			0.0422	0.0629	0.0634	0.0494
			0.0427	0.0621	0.0555	0.0519
			0.0418	0.0624	0.0531	0.0531
			0.0430	0.0666	0.0636	0.0554
		Mean	0.0457	0.0622	0.0555	0.0512
		Median	0.0424	0.0645	0.0584	0.0542

Supplementary Table 3

Baseline BMD (g/cm^2) for all mice in each group

Figure #	Group	Site				
4A,B,C,D			Total Body	Femur	Spine	Tibia
	Sham		0.0447	0.0616	0.0609	0.0500
			0.0454	0.0665	0.0590	0.0506
			0.0454	0.0705	0.0603	0.0527
			0.0431	0.0659	0.0628	0.0512
			0.0430	0.0652	0.0558	0.0498
			0.0435	0.0649	0.0587	0.0500
			0.0433	0.0639	0.0674	0.0487
		Mean	0.0441	0.0655	0.0607	0.0504
		Median	0.0435	0.0652	0.0603	0.0500
	T cells		0.0446	0.0671	0.0676	0.0526
			0.0437	0.0633	0.0624	0.0498
			0.0453	0.0648	0.0567	0.0485
			0.0437	0.0641	0.0639	0.0509
			0.0446	0.0656	0.0578	0.0499
			0.0450	0.0647	0.0629	0.0496
			0.0445	0.0666	0.0558	0.0530
		Mean	0.0445	0.0652	0.0610	0.0506
		Median	0.0446	0.0648	0.0624	0.0499
	T cells + Etanercept		0.0427	0.0635	0.0588	0.0503
			0.0437	0.0653	0.0586	0.0535
			0.0443	0.0630	0.0705	0.0520
			0.0444	0.0686	0.0644	0.0543
			0.0435	0.0616	0.0675	0.0489
			0.0438	0.0654	0.0624	0.0507
			0.0430	0.0652	0.0583	0.0480
		Mean	0.0436	0.0646	0.0629	0.0511
		Median	0.0437	0.0652	0.0624	0.0507

Supplementary Table 4

Baseline BMD (g/cm^2) for all mice in each group

Figure #	Group	Site		
5A,B.			Femur	Spine
	0 T cells		0.0595	0.0679
			0.0648	0.0662
			0.0658	0.0693
			0.0535	0.0652
			0.0595	0.0679
			0.0648	0.0662
			0.0658	0.0693
			0.0535	0.0652
		Mean	0.0609	0.0672
		Median	0.0648	0.0679
	1X10^3 T cells		0.0616	0.0673
			0.0648	0.0692
			0.0487	0.0658
			0.0658	0.0651
			0.0616	0.0673
			0.0648	0.0692
			0.0487	0.0658
			0.0658	0.0651
		Mean	0.0602	0.0669
		Median	0.0616	0.0673
	1X10^5 T cells		0.0621	0.0655
			0.0507	0.0695
			0.0669	0.0680
			0.0733	0.0671
			0.0621	0.0655
			0.0507	0.0695
			0.0669	0.0680
			0.0733	0.0671
		Mean	0.0632	0.0675
		Median	0.0621	0.0680
	1X10^7 T cells		0.0621	0.0688
			0.0633	0.0662
			0.0686	0.0718
			0.0708	0.0732
			0.0621	0.0688
			0.0633	0.0662
			0.0686	0.0718
			0.0708	0.0732
		Mean	0.0662	0.0700
		Median	0.0633	0.0688