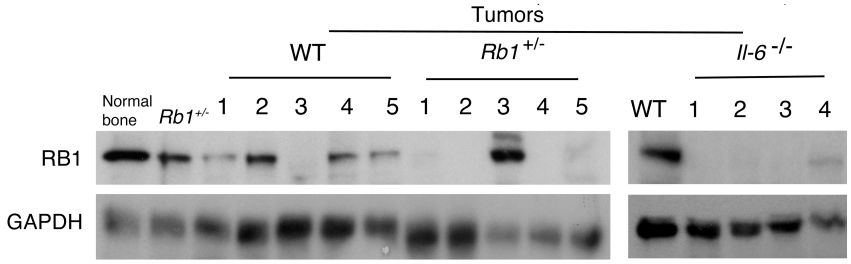
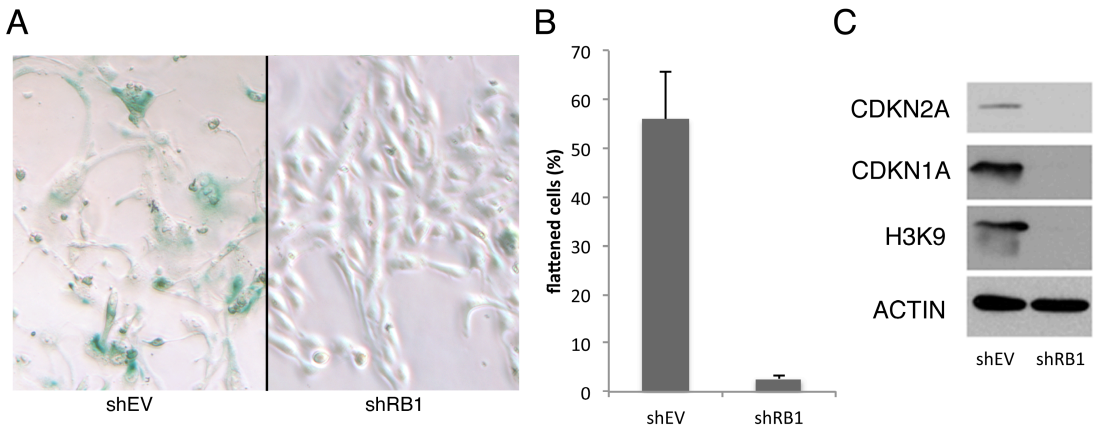
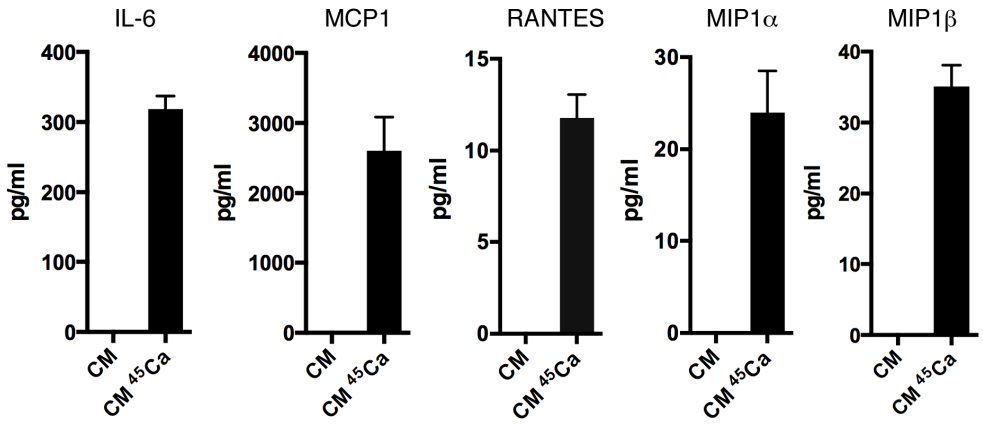
**Supplementary Figure 1.** Reduced RB1 protein expression in osteosarcomas derived in WT, *Rb1*$^{+/-}$ and *Il-6*$^{-/-}$ mice. Protein (25 $\mu$g) from wild-type normal bone, *Rb1*$^{+/-}$ tissue and tumors was resolved on a 7% SDS-PAGE gel and probed for the presence of RB1.



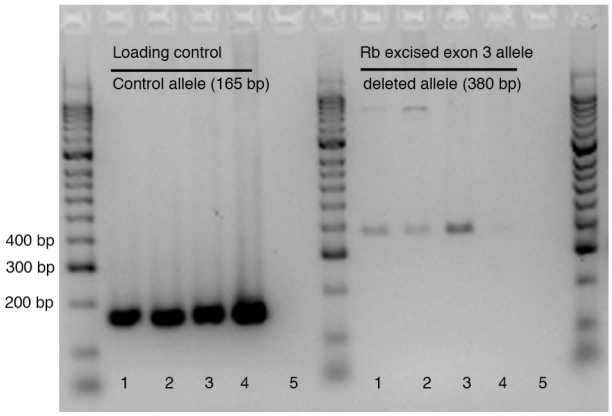
**Supplementary Figure 2.** Markers of senescence 10 days after exposure to 4Gy radiation. A) SA-β-Gal staining of hOB shEV (left panel), and shRB1 (right panel) 10X magnification. B) Percentage of flattened cells shEV and shRB1 10 days after 4Gy IR (4 panels were counted for each cell line with 53-301 cells per field), STDEV is shown. C) Western blot analysis of cyclin dependent inhibitors: p16 and p21 (CDKN2A and CDKN1A), anti-Histone H3 (tri-methyl K9) (H3K9) and ACTIN.



**Supplementary Figure 3.** Changes in cytokine/chemokine protein expression by bone cells following IR using BD bead arrays and FACS analysis. Condition media was collected from control cells (CM) and cells irradiated at 4Gy (CM $^{45}$Ca) after 7 days. Of the 20 proteins analyzed (IL-1$\alpha$, IL-1$\beta$, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-21, G-CSF, GM-CSF, RANTES, MIG, MCP1, MIP1$\alpha$, MIP1$\beta$, TNF$\alpha$ and IFN$\gamma$), only 5 proteins were detectable following irradiation from condition media: IL-6, MCP1, RANTES, MIP1$\alpha$ and MIP1$\beta$.

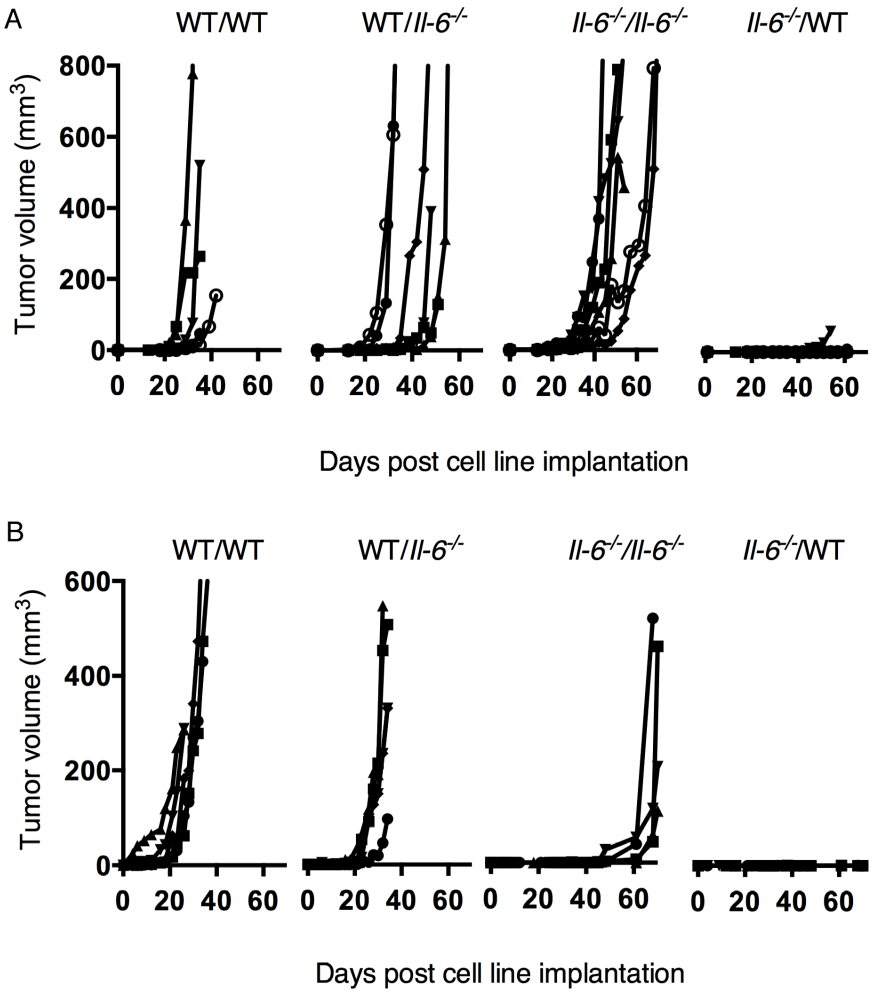


**Supplementary Figure 4.** Recombination in *Osx-Cre+ Rb1*$^{fl/fl}$ mice. Genomic DNA was extracted from calvaria, tibia (flushed of bone marrow cells) an osteosarcoma (derived from *Osx-Cre p53*$^{fl/fl}$ *pRb*$^{fl/fl}$) and muscle. PCR was performed with control (*Recql4* WT allele PCR) and *Rb1* deletion PCR (% loxP and RbCre3-2 primers) to detect the exon 3-deleted allele. For quantification the unexcised allele shows up as a band at approximately 1200-1500 bp. In non-tumor bone approximately 50% excision was observed. In tumor 100% excision was observed.

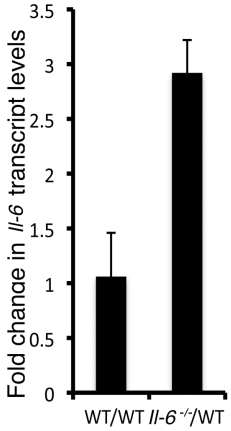



gDNA
1. Calvaria
2. Tibia
3. Osteosarcoma
4. Muscle
5. Blank lane

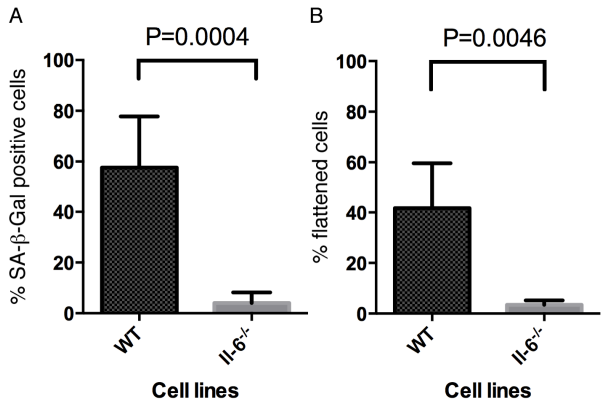
**Supplementary Figure 5.** Growth of *Il-6*$^{-/-}$ osteosarcoma cell lines is suppressed when implanted into a wild-type background. A) Shows a repeat cross transplantation experiment: cell lines wild-type #18 and *Il-6*$^{-/-}$ #12. B) Shows an independently derived set of cell lines WT#5 and *Il-6*$^{-/-}$#13. Cells (1 x $10^6$) were implanted subcutaneously into flanks of mice. Tumor volumes plotted for each cohort (WT/WT, WT/*Il-6*$^{-/-}$, *Il-6*$^{-/-}$/*Il-6*$^{-/-}$ and *Il-6*$^{-/-}$/WT) over 60 days.



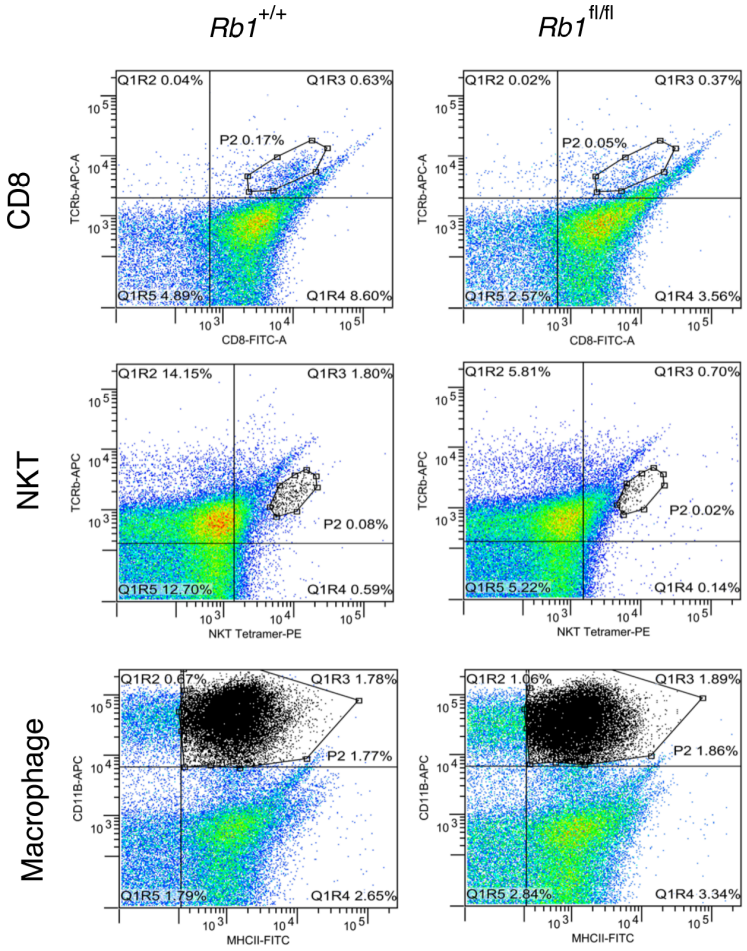
**Supplementary Figure 6.** Increased *Il-6* transcript levels in *Il-6*$^{-/-}$ osteosarcomas grown in WT mice. qRT-PCR was carried out on RNA derived from WT tumors implanted in WT mice and in *Il-6*$^{-/-}$ tumors implanted in WT mice. Values are shown as expression relative to WT cell line ± SEM and are representative of three independent experiments.



**Supplementary Figure 7.** *Il-6*$^{-/-}$ tumor cells had reduced capacity to undergo senescence compared to WT tumor cells following IR. Cell lines derived from WT and *Il-6*$^{-/-}$ $^{45}$Ca induced tumors were cultured ex vivo and irradiated at 4 Gy. Seven days after irradiation, cells were stained for (A) SA-β-Gal and (B) the number of flattened cells were quantitated. Values show % of cells that are SA-β-Gal positive and/or flattened ± SEM and are representative of two independent experiments.



**Supplementary Figure 8.** Dot plot showing differential infiltration of immune cells post-IR in spines of *Rb*$^{+/+}$ compared to *Rb*$^{fl/fl}$ mice. Dot plots showing representative images for CD8, NKT cells and macrophages.



**Supplementary Table 1. Enrichment of expression of SASP genes among genes regulated by RB1 following ionizing radiation**. The SASP signature derived from Coppe *et al* (Coppe et al., 2008) was significantly enriched in hOB compared to RB1 knockdown cells following IR (FDR q-value 0.0054). RANK = rank in gene list; ES = enrichment score, which reflects the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes.

| PROBE | SYMBOL | GENE TITLE | RANK | ES | ENRICHED |
|---|---|---|---|---|---|
| 8131803 | *IL6* | interleukin 6 (interferon, beta 2) | 13 | 0.0903 | Yes |
| 8006433 | *CCL2* | chemokine (C-C motif) ligand 2 | 19 | 0.1771 | Yes |
| 8112139 | *IL6ST* | interleukin 6 signal transducer | 52 | 0.2474 | Yes |
| 8152512 | *TNFRSF11B* | TNF receptor superfamily, member 11b | 65 | 0.3165 | Yes |
| 8054722 | *IL1B* | interleukin 1, beta | 108 | 0.3746 | Yes |
| 7965322 | *KITLG* | KIT ligand | 568 | 0.3955 | Yes |
| 8095680 | *IL8* | interleukin 8 | 634 | 0.4287 | Yes |
| 7929032 | *FAS* | Fas (TNF receptor superfamily, member 6) | 671 | 0.4623 | Yes |
| 7960518 | *TNFRSF1A* | TNF receptor superfamily, member 1A | 713 | 0.4949 | Yes |
| 8018972 | *TIMP2* | TIMP metallopeptidase inhibitor 2 | 829 | 0.5232 | Yes |
| 8018966 | *TIMP2* | TIMP metallopeptidase inhibitor 2 | 882 | 0.5531 | Yes |
| 8138542 | *IL6* | interleukin 6 (interferon, beta 2) | 949 | 0.5817 | Yes |
| 8048864 | *CCL20* | chemokine (C-C motif) ligand 20 | 1885 | 0.5725 | Yes |
| 8107887 | *CSF2* | colony stimulating factor 2 | 2234 | 0.5818 | Yes |
| 8135909 | *LEP* | leptin | 2517 | 0.5920 | Yes |
| 8033987 | *ICAM3* | intercellular adhesion molecule 3 | 3569 | 0.5717 | No |
| 8097256 | *FGF2* | fibroblast growth factor 2 (basic) | 3941 | 0.5740 | No |
| 8095697 | *CXCL1* | chemokine (C-X-C motif) ligand 1 | 4031 | 0.5859 | No |
| 8014342 | *CCL16* | chemokine (C-C motif) ligand 16 | 4938 | 0.5674 | No |
| 8032899 | *TICAM1* | toll-like receptor adaptor molecule 1 | 5147 | 0.5727 | No |
| 7955694 | *IGFBP6* | insulin-like growth factor binding protein 6 | 6317 | 0.5425 | No |
| 8039484 | *IL11* | interleukin 11 | 7104 | 0.5244 | No |
| 8075316 | *OSM* | oncostatin M | 10770 | 0.4015 | No |
| 8007100 | *IGFBP4* | insulin-like growth factor binding protein 4 | 10773 | 0.4058 | No |
| 8145244 | *TNFRSF10C* | TNF receptor superfamily, member 10c | 10980 | 0.4028 | No |
| 8097553 | *IL15* | interleukin 15 | 11456 | 0.3899 | No |
| 8014369 | *CCL3* | chemokine (C-C motif) ligand 3 | 11643 | 0.3869 | No |
| 7983630 | *FGF7* | fibroblast growth factor 7 | 12252 | 0.3685 | No |
| 8107970 | *IL13* | interleukin 13 | 15173 | 0.2672 | No |
| 8006453 | *CCL8* | chemokine (C-C motif) ligand 8 | 15918 | 0.2423 | No |
| 8140556 | *HGF* | hepatocyte growth factor | 18146 | 0.1682 | No |
| 8132694 | *IGFBP1* | insulin-like growth factor binding protein 1 | 19023 | 0.1421 | No |
| 7973084 | *ANG* | angiogenin | 20531 | 0.0959 | No |
| 8006459 | *CCL13* | chemokine (C-C motif) ligand 13 | 20739 | 0.0952 | No |
| 7897877 | *TNFRSF1B* | TNF receptor superfamily, member 1B | 22164 | 0.0544 | No |
| 7980233 | *PGF* | placental growth factor | 23313 | 0.0254 | No |
| 8151447 | *IL7* | interleukin 7 | 23643 | 0.0255 | No |
| 8029006 | *AXL* | AXL receptor tyrosine kinase | 26729 | -0.0594 | No |
| 8037374 | *PLAUR* | plasminogen activator, urokinase receptor | 26976 | -0.0441 | No |
| 8048205 | *IGFBP2* | insulin-like growth factor binding protein 2 | 27094 | -0.0235 | No |
| 8140358 | *CCL26* | chemokine (C-C motif) ligand 26 | 27619 | -0.0123 | No |
| 8071737 | *MIF* | macrophage migration inhibitory factor | 28619 | 0.0073 | No |

**Supplementary Table 2. Differential infiltration of immune cells into mouse tumors.** Tumors from cross-transplantation experiments (3-4/genotype) (tumor genotype/mouse genotype: WT/WT, *Il-6*$^{-/-}$/*Il-6*$^{-/-}$, WT/*Il-6*$^{-/-}$ and *Il-6*$^{-/-}$/WT) were excised and tumors digested and immune cell subsets residing in spine analyzed using FACS analysis at days 14 and day 27. Mean and SEM are shown. Significance was determined by 2-tailed student's *t*-test.

| | | | | *P-value* |
|---|---|---|---|---|
| | *WT/WT* | *Il-6*$^{-/-}$/*Il-6*$^{-/-}$ | *Il-6*$^{-/-}$/*WT* | *WT/WT compared to Il-6*$^{-/-}$/*WT* |
| CD4 | 0.09 ± 0.01 | 0.19 ± 0.03 | 0.75 ± 0.07 | 0.01 |
| CD8 | 0.06 ± 0.02 | 0.14 ± 0.04 | 0.41 ± 0.07 | 0.04 |
| NK | 24.9 ± 1.02 | 8.74 ± 1.31 | 18.5 ± 1.0 | 0.02 |
| NKT | 0.04 ± 0.05 | 0.09 ± 0.01 | 0.18 ± 0.04 | 0.04 |
| B-cell | 2.65 ± 1.79 | 2.06 ± 0.22 | 2.04 ± 0.39 | NS |
| Macrophage | 4.55 ± 2.51 | 5.70 ± 0.33 | 7.27 ± 2.13 | NS |
| Dendritic cell | 5.10 ± 2.90 | 5.80 ± 0.29 | 7.70 ± 2.41 | NS |
| Neutrophil | 1.28 ± 0.50 | 2.39 ± 0.30 | 4.60 ± 0.20 | 0.002 |

**Supplementary Table 3. Radiation induced changes in immune cells numbers in spines of C57/Bl6 mice.** Mice were injected with saline or 4 $\mu$Ci/g of $^{45}$Ca and culled 2 weeks post-injection, spines were digested into single cell suspension and FACs analysis carried out to determine immune cell numbers (7 mice/cohort). Mean and SEM are shown. *P*-values were determined by unpaired 2-tailed student's *t*-test.

| | Saline | $^{45}$Ca | Fold change | P-value |
|---|---|---|---|---|
| CD4 | 25.6 ± 4.80 | 16.2 ± 3.00 | 0.6 | NS |
| CD8 | 18.5 ± 6.60 | 10.9 ± 3.60 | 0.6 | NS |
| NK | 3.00 ± 0.30 | 6.50 ± 1.80 | 2.2 | 0.04 |
| NKT | 0.03 ± 0.01 | 0.23 ± 0.15 | 8.6 | 0.04 |
| B cell | 0.26 ± 0.01 | 0.29 ± 0.09 | 1.1 | NS |
| Macrophage | 11.5 ± 3.00 | 15.4 ± 5.70 | 1.3 | NS |
| Dendritic cell | 0.70 ± 0.10 | 0.69 ± 0.06 | 1.0 | NS |
| Neutrophil | 5.25 ± 0.62 | 7.35 ± 0.55 | 1.4 | 0.04 |

**Supplementary Table 4. Osteoblast specific knockout of RB1 results in differential immune cell infiltration following exposure to $^{45}$Ca.** *Rb1*$^{+/+}$ *and Rb1*$^{fl/fl}$ (6 mice/cohort) were injected with 4 $\mu$Ci/g $^{45}$Ca and mice at 2 weeks post-injection. FACs analysis of immune cell subsets in spines was carried out. This experiment was repeated independently three times. The mean ± SEM are shown. *P*-values were determined by unpaired 2-tailed student's *t*-test.

| | *Rb1*$^{+/+}$ | *Rb1*$^{fl/fl}$ | Fold change | *P-value* |
|---|---|---|---|---|
| CD4 | 0.01 ± 0.02 | 0.01 ± 0.00 | 1 | NS |
| CD8 | 0.15 ± 0.27 | 0.09 ± 0.01 | 0.6 | NS |
| NK | 0.82 ± 0.21 | 0.48 ± 0.05 | 0.58 | NS |
| NKT | 0.09 ± 0.02 | 0.05 ± 0.01 | 0.55 | 0.04* |
| B-cell | 0.71 ± 0.06 | 0.73 ± 0.07 | 1.03 | NS |
| Macrophage | 0.36 ± 0.04 | 0.35 ± 0.03 | 0.97 | NS |
| Dendritic cell | 1.08 ± 0.07 | 1.07 ± 0.13 | 0.98 | NS |
| Neutrophil | 1.71 ± 0.23 | 1.15 ± 0.15 | 0.67 | NS |

**Supplementary Table 5. Differential immune cell infiltration in spines of *Il-6*$^{-/-}$ mice compared to *wildtype* mice following exposure to radiation.** Cohorts of mice C57/Bl6 *Il-6*$^{-/-}$ or WT (n=6 cohort) were injected with $^{45}$Ca at 28 days of age. At 14 days post IR spines were excised, digested to a single cell suspension and analyzed by FACS to determine numbers of resident immune cells. *NKT cells assayed in this experiment by NK1.1 and TCRβ. Mean and SEM are shown. Significance was determined by 2-tailed student's *t*-test. Cell numbers were normalized to differences in baseline differences between immune cell subsets in control compared to *Il-6*$^{-/-}$ spleen.

| | WT | *Il-6*$^{-/-}$ | Fold change | *P-value* |
|---|---|---|---|---|
| CD4 | 0.01 ± 0.00 | 0.01 ± 0.01 | 1 | NS |
| CD8 | 0.65 ± 0.10 | 0.89 ± 0.20 | 1.38 | NS |
| NK | 0.80 ± 0.09 | 0.60 ± 0.20 | 0.75 | NS |
| NKT* | 0.24 ± 0.01 | 0.13 ± 0.02 | 0.53 | <0.01 |
| B cell | 2.27 ± 0.58 | 1.68 ± 0.60 | 0.75 | 0.03 |
| Macrophage | 3.60 ± 0.54 | 2.40 ± 0.30 | 0.66 | NS |
| Dendritic cell | 0.63 ± 0.20 | 0.59 ± 0.30 | 0.93 | NS |
| Neutrophil | 0.17 ± 0.06 | 0.09 ± 0.01 | 0.52 | NS |