Supporting Information

Sadik et al. 10.1073/pnas.1213797109

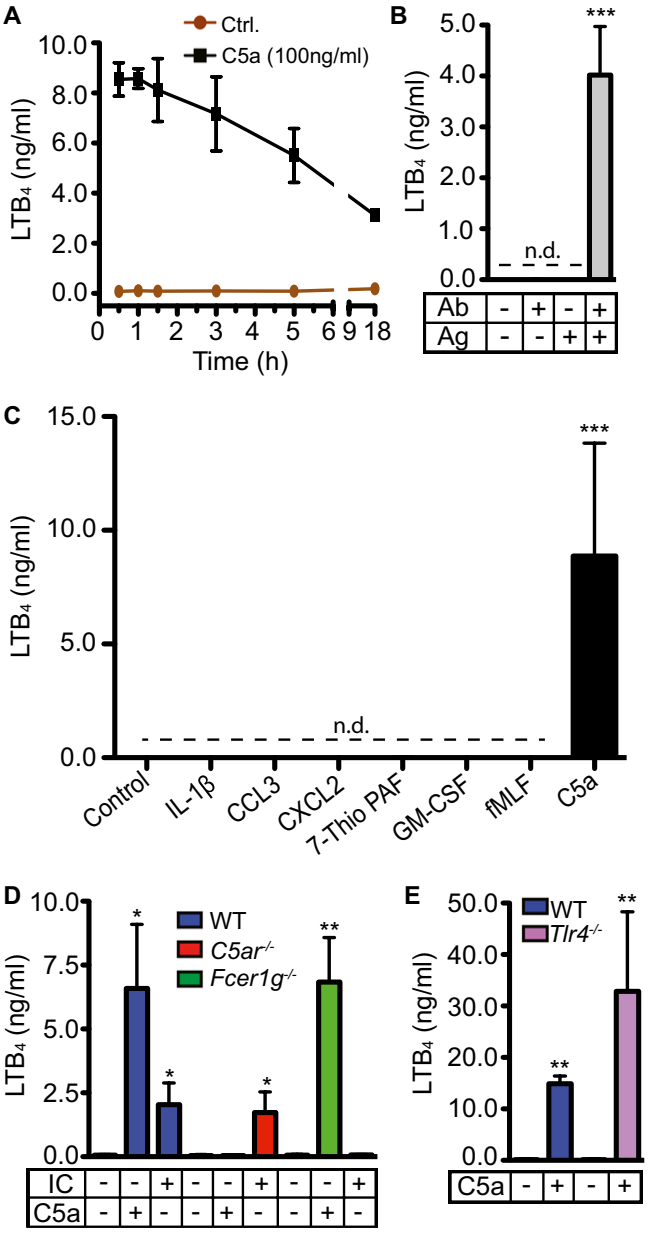

Fig. S1. Induction of leukotriene B_4 (LTB_4) release from resting PMNs in vitro. (*A*) Kinetic analysis of the release of LTB_4 from polymorphonuclear leukocytes (PMNs) after stimulation with recombinant murine C5a (100 ng/mL) or when left unstimulated as a control. (*B*) Release of LTB_4 from PMNs after stimulation with Ab, Ag, or Ab plus Ag [immune complex (IC)] for 1 h. (*C*) Release of LTB_4 from PMNs after stimulation with IL-1β (10 ng/mL), CCL3 (100 ng/mL), CXCL2 (100 ng/mL), 7-thio-PAF (1 nM), GM-CSF (100 ng/mL), fMLF (100 nM), or C5a (100 ng/mL) for 1 h. (*D*) Release of LTB_4 from WT, *C5ar*$^{-/-}$, and *Fcer1g*$^{-/-}$ PMNs after stimulation with C5a (100 ng/mL) or ICs for 18 h. (*E*) Release of LTB_4 from WT and *Tlr4*$^{-/-}$ PMNs after stimulation with C5a (100 ng/mL) for 1 h. All data represent concentrations in the supernatant determined by ELISA, shown as mean ± SD (n = 3 independent experiments). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with unstimulated control. n.d., not detectable.

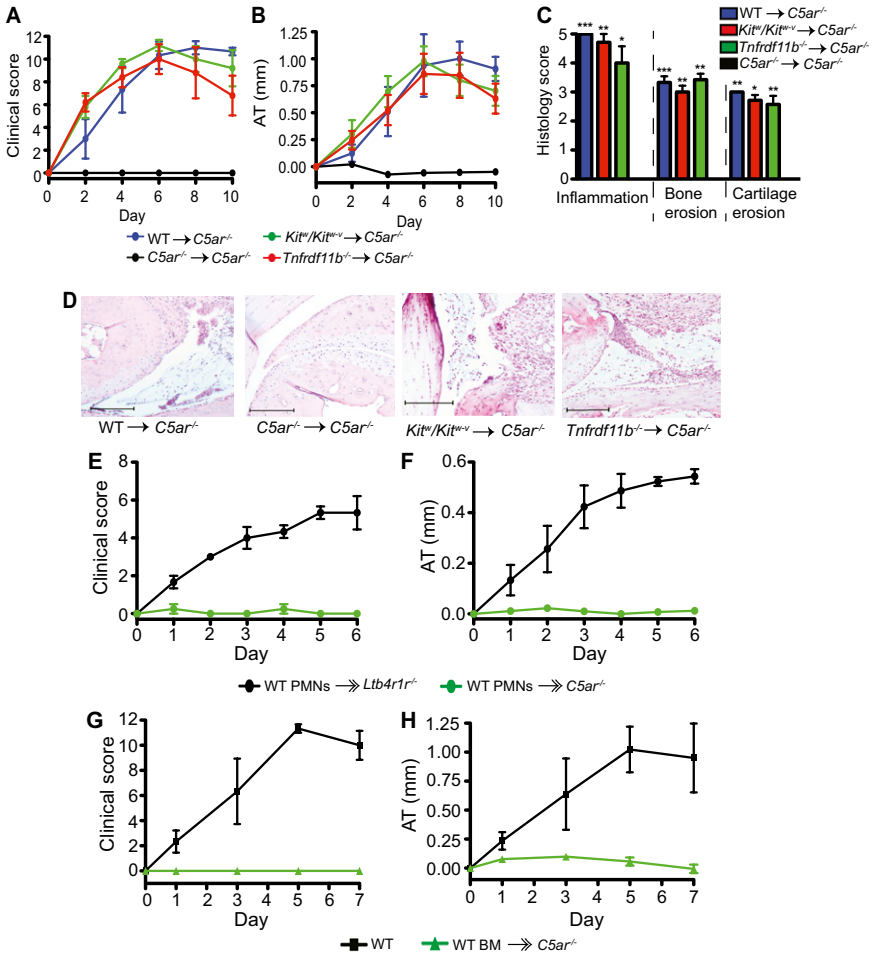

Fig. S2. C5a receptor (C5aR) on mast cells and osteoblasts does not contribute to autoantibody-induced arthritis. (*A* and *B*) Arthritis in WT → $C5ar^{-/-}$, $Kit^{w}/Kit^{w\text{-}v}$ → $C5ar^{-/-}$, $Tnfrdf11b^{-/-}$ → $C5ar^{-/-}$, and $C5ar^{-/-}$ → $C5ar^{-/-}$ bone marrow chimera (BMC) (n = 3–4 mice per group). Each group vs. $C5ar^{-/-}$ → $C5ar^{-/-}$, $P < 0.001$ in clinical score; WT → $C5ar^{-/-}$ or $Kit^{w}/Kit^{w\text{-}v}$ → $C5ar^{-/-}$ vs. $C5ar^{-/-}$ → $C5ar^{-/-}$, $P < 0.001$; and $Tnfrdf11b^{-/-}$ → $C5ar^{-/-}$ vs. $C5ar^{-/-}$ → $C5ar^{-/-}$, $P < 0.01$ in ankle thickness (AT). (*C*) Histological score of ankles from mice in *A* and *B*. WT → $C5ar^{-/-}$, $Kit^{w}/Kit^{w\text{-}v}$ → $C5ar^{-/-}$, $Tnfrdf11b^{-/-}$ → $C5ar^{-/-}$, and $C5ar^{-/-}$ → $C5ar^{-/-}$ BMC were scored (n = 3–4 mice per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for indicated group compared with $C5ar^{-/-}$ → $C5ar^{-/-}$ control. (*D*) Respective representative sections. One representative of two independent experiments is shown. (*E* and *F*) Arthritis in $C5ar^{-/-}$ and $Ltb4r1^{-/-}$ mice after adoptive transfer of 10^7 WT PMNs daily on days 0–3 (n = 3–4 mice per group). Adoptive transfer of PMNs into $Ltb4r1^{-/-}$ served as positive control for the effectivity of the K/BxN serum and the adoptive transfer of WT PMNs. $P < 0.001$ for clinical score of AT. One representative of three independent experiments is shown. (*G* and *H*) Arthritis in $C5ar^{-/-}$ mice after adoptive transfer of WT bone marrow (BM). Each $C5ar^{-/-}$ mouse received the complete BM of a WT mouse daily on days 0–3. WT mice injected only with K/BxN serum served as control (n = 3 mice per group). One representative of three independent experiments is shown. $P < 0.001$ for clinical score and $P < 0.01$ for AT. Data are presented as mean ± SEM. (Scale bars, 100 μm.)

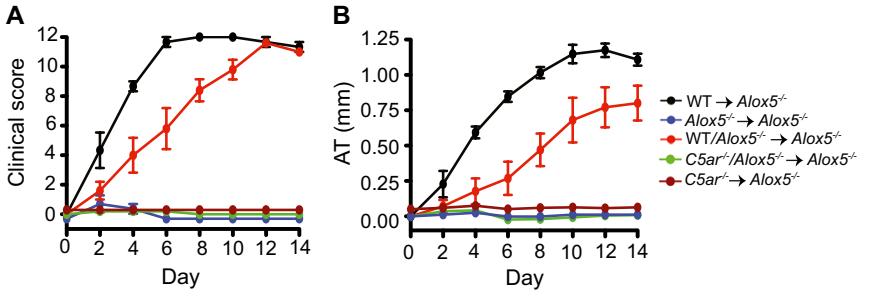

Fig. S3. 5-Lipoxygenase (5-LO) in radioresistant cells does not contribute to autoantibody-induced arthritis. (*A* and *B*) WT → $Alox5^{-/-}$, $C5ar^{-/-}$ → $Alox5^{-/-}$, $Alox5^{-/-}$ → $Alox5^{-/-}$, $Alox5^{-/-}/C5ar^{-/-}$ → WT, and $Alox5^{-/-}$/WT → WT chimeras were generated as described in *Methods*, but in this particular series of experiments $Alox5^{-/-}/C5ar^{-/-}$ and $Alox5^{-/-}$/WT were mixed in a ratio of 3:1 in favor of the $Alox5^{-/-}$ bone marrow for reconstitution. Four weeks after irradiation chimera were injected with K/BxN serum on days 0 and 2 (n = 3–5 mice per group), and the development of arthritis was evaluated every other day. One representative of two independent experiments is shown. $P < 0.001$ for $Alox5^{-/-}$/WT → WT chimera compared with all other groups in clinical score and AT. Data are presented as mean ± SEM.

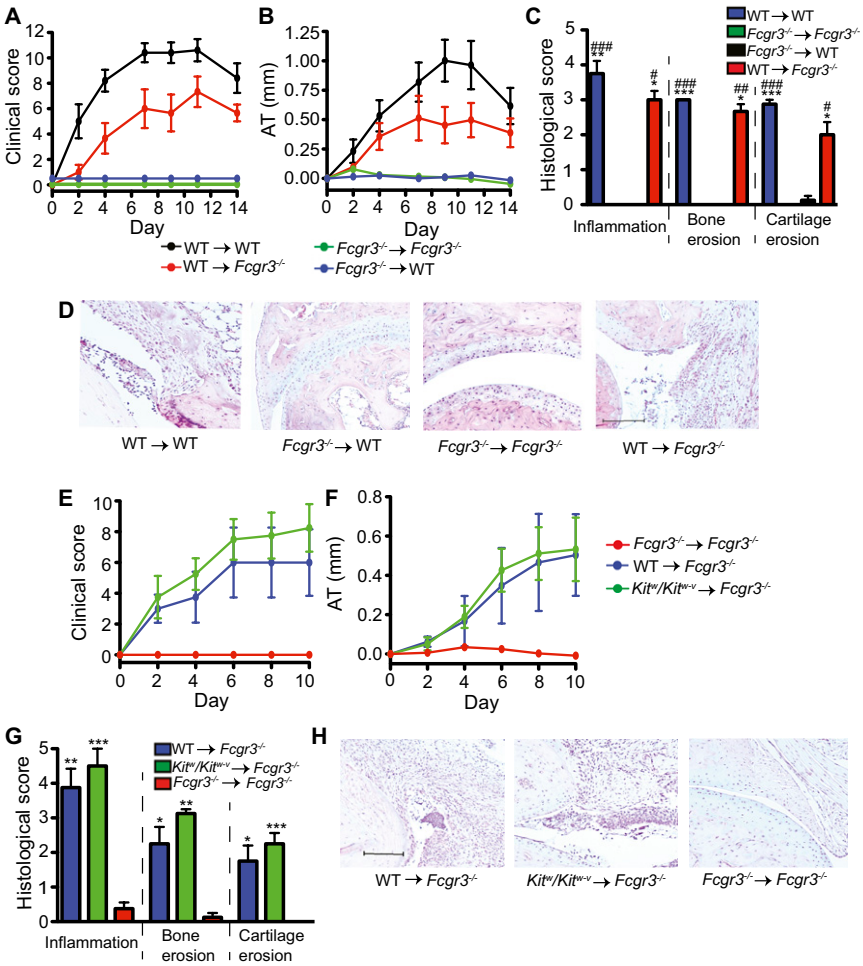

Fig. S4. FcγRIII on radiosensitive cells is necessary and sufficient for arthritis. (*A* and *B*) Arthritis in all "criss-cross" BMCs of WT and *Fcgr3*$^{-/-}$ mice (n = 4–5 mice per group). One representative of two independent experiments is presented. $P < 0.001$ for WT → WT or WT → *Fcgr3*$^{-/-}$ vs. *Fcgr3*$^{-/-}$ → WT or *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$ in clinical score and ankle thickening (AT), and $P < 0.001$ and $P < 0.05$ for WT → WT vs. WT → *Fcgr3*$^{-/-}$ in clinical score and AT, respectively. (*C*) Histological score of ankles from mice corresponding to *A* and *B* (n = 8–10 mice per group; data compiled from two independent experiments). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicated for group vs. *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$. (*D*) Respective representative sections. (*E* and *F*) Arthritis in WT → *Fcgr3*$^{-/-}$, *Kit*^{w}/*Kit*$^{w\text{-}v\text{-}/-}$ → *Fcgr3*$^{-/-}$, and *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$ chimera (n = 4 mice per group). WT → *Fcgr3*$^{-/-}$ vs. *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$, $P < 0.001$ in clinical score (CS) and $P < 0.01$ in AT; *Kit*^{w}/*Kit*$^{w\text{-}v}$ → *Fcgr3*$^{-/-}$ vs. *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$, $P < 0.01$ in CS and $P < 0.05$ in AT. One representative of two independent experiments is shown. (*G*) Histological score of ankles from mice in *E* and *F* (n = 8–10 mice per group; data compiled from two independent experiments). *$P < 0.05$, **$P < 0.01$ for indicated group vs. *Fcgr3*$^{-/-}$ → *Fcgr3*$^{-/-}$; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ for indicated group vs. *Fcgr3*$^{-/-}$ → WT. (*H*) Respective representative sections. All data are presented as mean ± SEM. (Scale bars, 100 μm.)

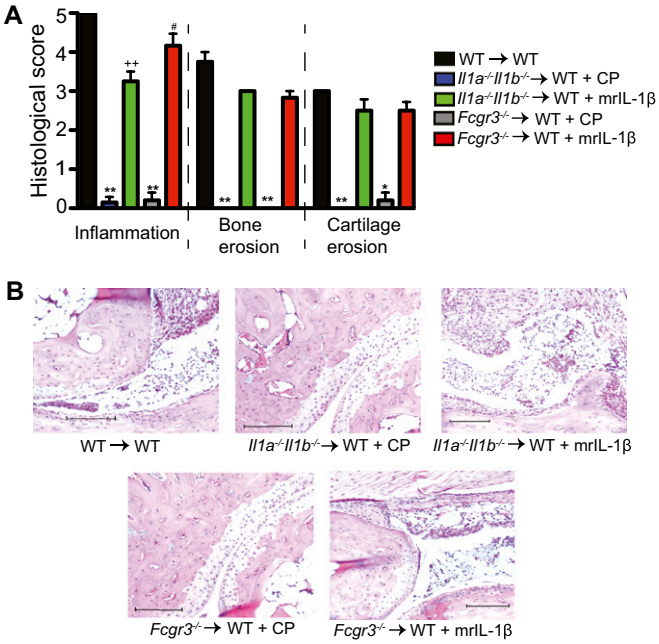

Fig. S5. IL-1β is downstream of FcγRIII activation. (*A*) Histological score of ankles from WT → WT, *Fcgr3*$^{-/-}$ → WT, and *Il1a*$^{-/-}$*Il1b*$^{-/-}$ → WT chimera on day 9 d after i.p. injection of K/BxN serum (This histology corresponds to the experiment shown in Fig. 6 *C* and *D*) (n = 6–10 mice per group; data compiled from two independent experiments). *$P < 0.05$, **$P < 0.01$ for indicated group vs. WT → WT; $^{\#}P < 0.05$ for indicated group vs. *Fcgr3*$^{-/-}$ → WT; $^{++}P < 0.01$ for indicated group vs. *Il1a*$^{-/-}$*Il1b*$^{-/-}$ → WT. (*B*) Respective representative sections. All data are presented as mean ± SEM. (Scale bars, 100 μm.)

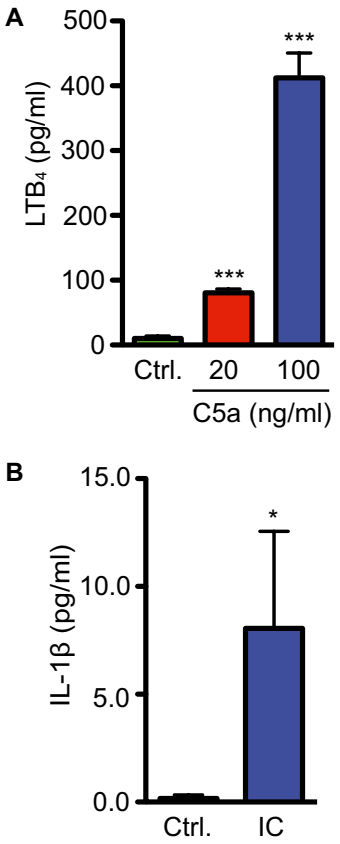

Fig. S6. C5a and ICs induce the release of LTB_4 and IL-1β, respectively, from human peripheral PMNs. Human PMNs were freshly isolated using dextrane and Ficoll from heparinized peripheral blood of healthy donors and resuspended to a cell concentration of 5×10^6/mL in RPMI, 1% human serum, 10 mM Hepes. (*A*) PMNs were seeded into round-bottom polypropylene tubes. Cells were left as unstimulated controls or stimulated with 20 or 100 ng/mL human recombinant C5a, as indicated, for 1 h. Afterward, cell-free supernatants were harvested and LTB_4 contents assayed by ELISA (n = 6 blood donors compiled from two independent experiments). (*B*) PMNs were prestimulated with 50 ng/mL GM-CSF for 30 min. Afterward, PMNs were either left unstimulated or stimulated with immobilized ICs, as described in *Methods*. After 20 h cell-free supernatants were harvested and IL-1β was determined by ELISA (n = 7 blood donors compiled from two independent experiments). All data are presented as mean ± SEM. $*P < 0.05$, $***P < 0.001$ for indicated group vs. control.

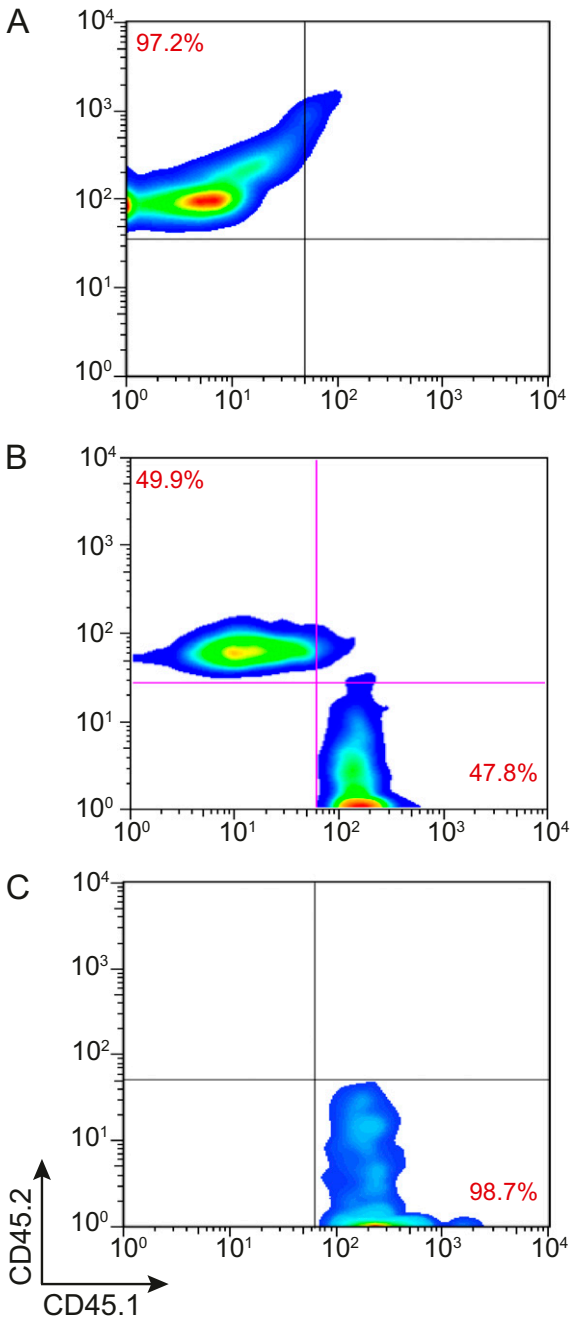

Fig. S7. Origin of PMNs and tissue macrophages in chimeric mice. BMC were generated, as described in *Methods*, and cells for FACS analysis were harvested 4 wk after reconstitution of BM. (*A*) BM cells were isolated from *Fcer1g*$^{-/-}$ → WT (CD45.2$^{+/+}$ → CD45.1$^{+/+}$) BMC and stained with anti-CD45.2 FITC, anti-CD45.1PE, and anti-Ly6G PerCP-Cy5.5. CD45.1 vs. CD45.2 expression in neutrophils (cells first gated for neutrophils in the FSC – SSC plot and then for Ly6G$^{+/+}$ cells) is shown. (*B*) BM cells were isolated from *Fcer1g*$^{-/-}$/WT → WT (CD45.2$^{+/+}$/CD45.1$^{+/+}$ → CD45.1$^{+/+}$) mixed BMC and stained with anti-CD45.2 FITC, anti-CD45.1PE, and anti-Ly6G PerCP-Cy5.5. CD45.1 vs. CD45.2 expression in PMNs (cells first gated for neutrophils in the FSC – SSC plot and then for Ly6G$^{+/+}$ cells) is shown. (*C*) Cells recovered by peritoneal lavage from *Fcer1g*$^{-/-}$ → WT (CD45.2$^{+/+}$ → CD45.1$^{+/+}$) BMC were stained with anti-CD45.2 FITC, anti-CD45.1 PE, and anti-F4/80 PerCP-Cy5.5. CD45.1 vs. CD45.2 expression in macrophages (cells first gated for macrophages in the Forward Scatter Channel vs. Side Scatter Channel plot and then for F4/80$^{+/+}$ cells) is shown.

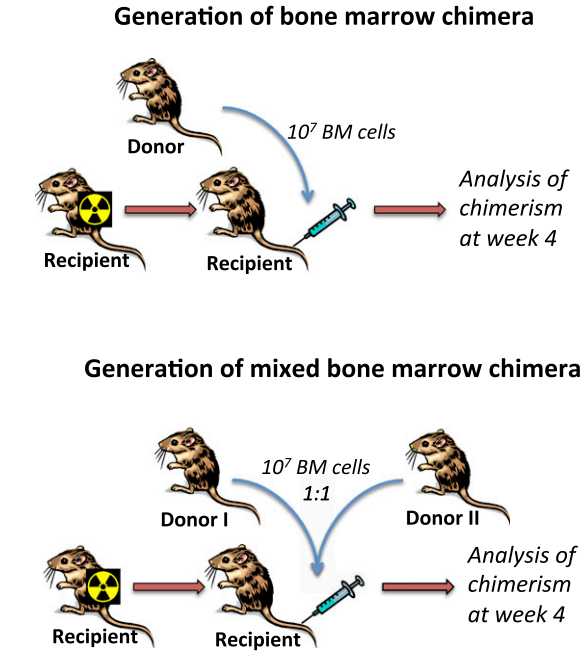

Fig. S8. Schematic of the generation of bone marrow chimeric mice.