Supplemental Material

Supplemental Material and Methods

Generation of miR-21 knockout mice

A targeting vector was constructed to flank the miR-21 coding sequence with loxP sites. The targeting vector consisted of an 1837 bp 5' homology arm, loxP site, FRT-flanked neomycin resistance cassette (PGK promoter, neomycin resistance coding sequence, bovine growth hormone polyadenylation sequence), 1273 bp segment including *miR-21*, loxP site, 3935 bp 3' homology arm, pUC vector backbone and PolII promoter-driven diphtheria toxin negative selection cassette. The targeting vector was linearized and electroporated into E14 (129P2/ola) embryonic stem cells. Targeted cells were identified by PCR and Southern blot analysis. Two positive clones were microinjected into blastocysts from C57BL/6 mice for chimera production. Chimeras from a single positive clone were mated to Flpe deleter mice and pups were genotyped by PCR for the absence of the neomycin resistance cassette. To generate global miR-21 deficient mice, *miR-21*$^{flox/+,}$ mice were then crossed with Ella-Cre transgenic mice (Jackson laboratories). Offspring carrying Ella-Cre and the floxed *miR-21* allele were used to generate the colony.

Evaluation of Tmem49 mRNA and protein

Total RNA was isolated from the spleen and bone marrow from *miR-21*$^{+/+}$ and *miR-21*$^{-/-}$ mice. After a Trizol-Chloroform extraction (Thermofisher), cDNA was generated (M-MLV transcriptase kit; Promega), and qPCR was performed (Quantitect SYBR green) following manufacturers' protocols. The Tmem49-specific forward primer sequence was 5'-GAGGGAGCCATTTCTTTGTGG and the reverse was 5' – CCAGTTTAGCCCGTGATGC. β-actin was a control. For western blotting, spleen and bone marrow from *miR-21*$^{+/+}$ and *miR-21*$^{-/-}$

mice were lysed in T-PER tissue protein extraction reagent containing protease and phosphatase inhibitor cocktail (both from Thermo Fisher Scientific) according to manufacturer's protocol. Protein concentration was determined using BCA assay (Thermo Fisher Scientific). Equal amount of protein was loaded on a gradient SDS-PAGE pre-cast gel (Life technologies), transferred to PVDF membrane, and blotted with the TMEM49 antibody (Cell Signaling) followed by HRP-conjugated secondary antibody (Agilent DAKO). Proteins were visualized by chemiluminescence using ECL detection kit (Thermo Fisher Scientific) and autoradiography.

Immunohistochemistry

Small intestines were harvested from mice 72 hours post-TBI, fixed in formalin, paraffin-embedded, and sectioned. One section was subjected to H&E staining. Another section was prepared for IHC by performing antigen retrieval by heating the sections in 10 mmol/L citrate buffer pH 6.0 for 25 minutes at 95ºC. Cleaved caspase 3 primary antibody (Cell Signaling) was incubated overnight at 4ºC followed by incubation with goat anti-rabbit-HRP secondary antibody (Dako). Immune complexes were visualized with the peroxidase substrate ImmPACT DAB (Vector) and Hematoxylin counterstain.

Supplemental Figure Legends

Supplemental Figure 1. Generation of *miR-21* knockout mice. A) Targeting strategy of miR-21 and the steps used to obtain global *miR-21* knockout mice. (miR-21 location in yellow in the 3'UTR of Tmem49 gene). B) qRT-PCR (left) and Western blot (right) for Tmem49 in bone marrow and spleen from the genotype of the mice indicated.

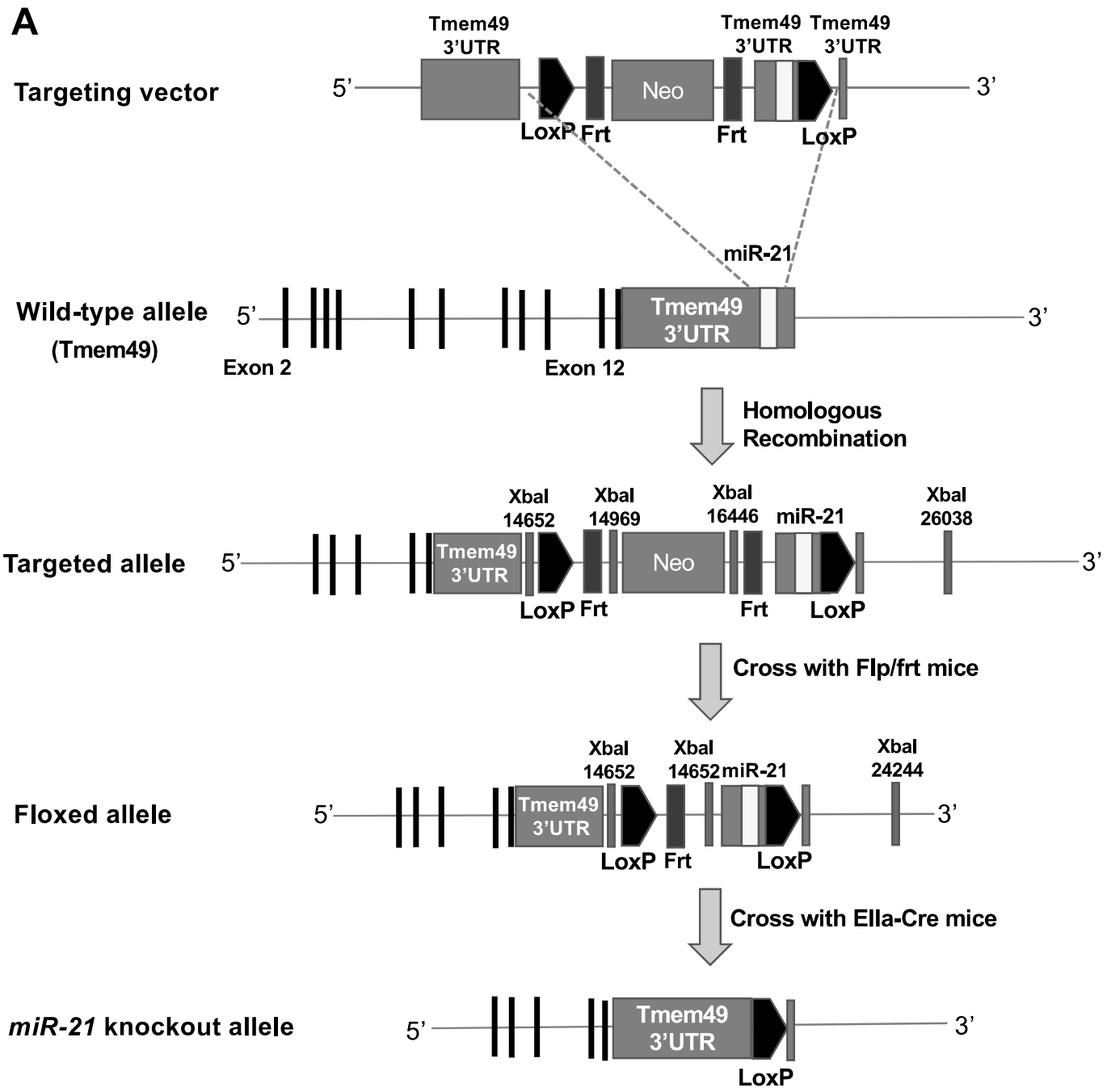
Supplemental Figure 2. miR-21 loss in Ola129/B6 mice results in decreased survival following radiation. Kaplan-Meier survival curves following a single 6.5 Gy dose of radiation of a cohort of mice of the indicated genotypes backcrossed onto the Ola129/B6 background; log-rank test.

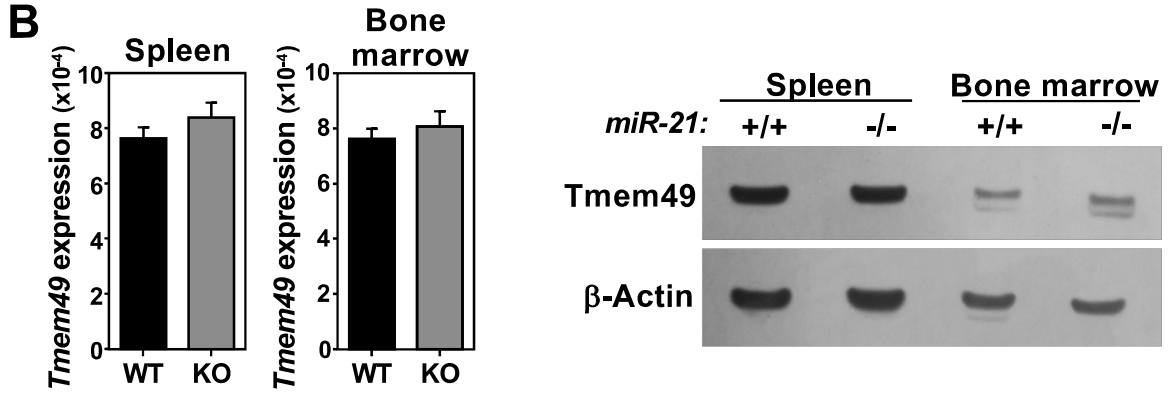
Supplemental Figure 3. Loss of miR-21 leads to minor villous blunting and modest apoptosis of intestinal crypt cells. A) Representative H&E images of small intestine from *miR-21*$^{+/+}$ and *miR-21*$^{-/-}$ mice prior to or 72 hours after a single 6.5 Gy dose of radiation. B) Immunohistochemistry for cleaved Caspase 3 in samples from (A, 10X objective).

Supplemental Figure 4. *miR-21*-deficiency does not alter mature hematopoietic cell populations in the spleen, bone marrow, or blood. A, B) Quantification of total nucleated cells in the spleen (A) or bone marrow (B) of *miR-21*$^{+/+}$, *miR-21*$^{+/-}$ and *miR-21*$^{-/-}$ mice determined counting on a hemocytometer. C-F) Total percentage of B cells (C; CD19+/B220+), CD4+ T cells (D; CD4+/CD3+), CD8+ T cells (E; CD8+/CD3+) and myeloid lineage cells (F; CD11b+/Gr1+) in the spleen of the mice in (A) determined by flow cytometry. G-J) Total percentage of B cells

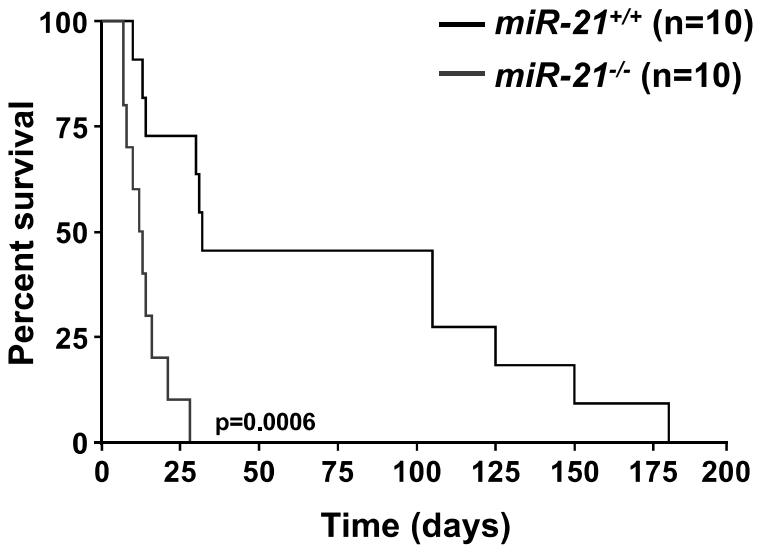
(G; CD19+/B220+), T cells (H; CD90.2); myeloid lineage cells (I; CD11b+/Gr1+) and erythrocytes (J; Ter119+) in the bone marrow of the mice in (B) determined by flow cytometry. I) Numbers of white blood cell populations (LY, lymphocytes; MO, monocytes; NE, neutrophils; EO, eosinophils; BA, basophils) in the blood of the mice determined by analysis on a Genesis veterinary hematology system; S.E.M. graphed.

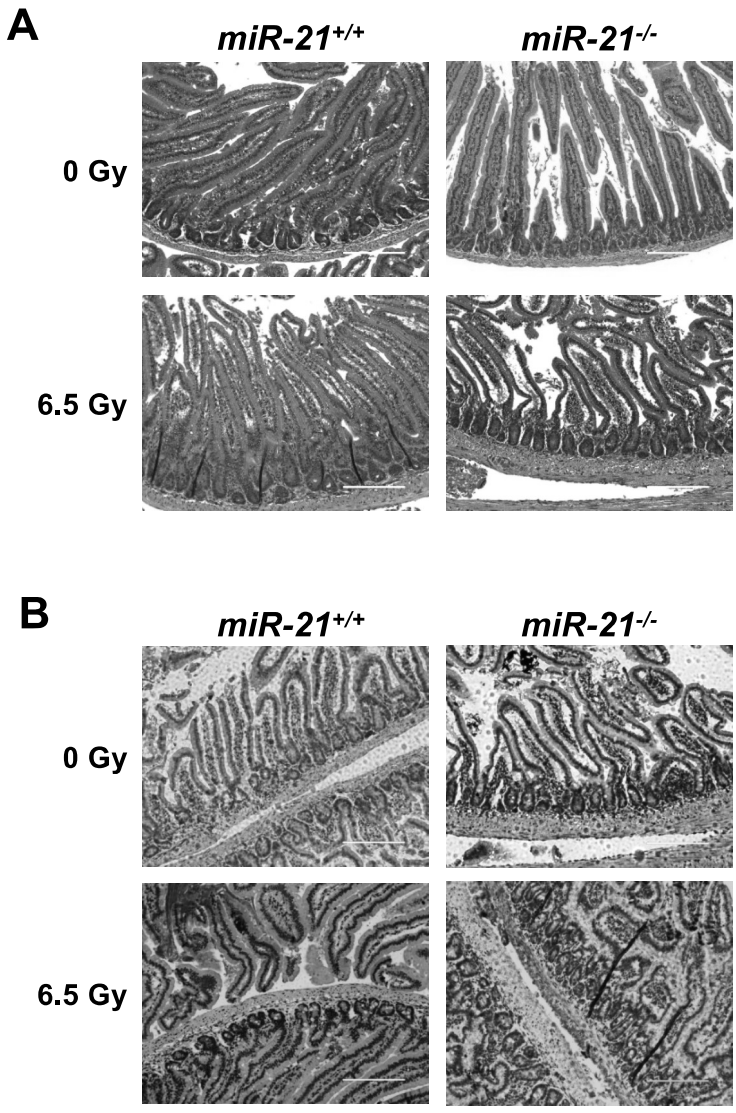
Supplemental Figure 1





Supplemental Figure 2




A
miR-21+/+
miR-21-/-
0 Gy
6.5 Gy
B
miR-21+/+
miR-21-/-
0 Gy
6.5 Gy

Supplemental Figure 4