

Supplemental table 1

Blood Lineage Counts for the Reconstituted Mice

Parameter	Whole blood cells counts			
	<i>ire1α^{-/-}</i> reconstituted mice	<i>ire1α^{+/+}</i> reconstituted mice	Control 1: <i>rag2^{-/-}</i> (non-irradiated)	Control 2: wild-type C57B/129S mice
WBC (K/uL)	5.97 ± 2.1	7.12 ± 2.5	5.12 ± 1.7	14.0 ± 2.3
NE (K/uL; %)	4.2 ± 0.9; 70.4 ± 2.4	3.92 ± 0.8 ; 55 ± 1.5	3.93 ± 0.6; 76.8 ± 3.5	3.59 ± 1.0 ; 25.6 ± 0.9
LY (K/uL; %)	1.44 ± 0.6; 24.1 ± 3.7	2.75 ± 1.3; 38.7 ± 5.9	0.73 ± 0.2; 14.2 ± 1.3	10.10 ± 4.5; 72.3 ± 10.1
MO (K/uL; %)	0.13 ± 0.05; 2.2 ± 0.3	0.29 ± 0.06; 4.1 ± 0.9	0.22 ± 0.05; 4.3 ± 0.8	0.13 ± 0.07; 0.9 ± 0.4
EO (K/uL; %)	0.16 ± 0.1; 2.7 ± 1.7	0.14 ± 0.09; 1.9 ± 0.9	0.18 ± 0.06; 3.5 ± 1.7	0.12 ± 0.08; 0.9 ± 0.5
BA (K/uL; %)	0.04 ± 0.02; 0.67 ± 0.3	0.02 ± 0.01; 0.3 ± 0.19	0.06 ± 0.01; 1.2 ± 0.4	0.04 ± 0.02; 0.3 ± 0.2
RBC (M/uL)	5.24 ± 1.2	8.17 ± 1.5	9.54 ± 1.9	8.80 ± 2.0
HB (g/dL)	5.3 ± 1.3	12.9 ± 2.4	14.9 ± 2.0	14.0 ± 2.6
PLT (K/uL)	403 ± 51	461 ± 55	608 ± 49	586 ± 67

Note: Mice whole blood counts were determined by using the Drew Scientific HEMAVET Multispecies Hematology Analyzer according to the manufacturer's instructions (Delta Scientific, Inc).

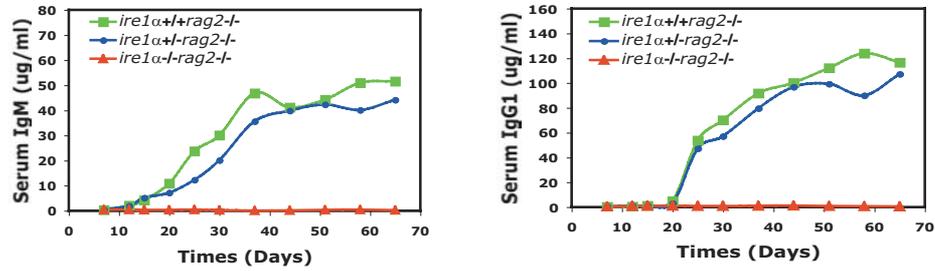
WBC, White blood cell or leukocyte count; NE, Neutrophil count and percent;
LY, lymphocyte count and percent; MO, monocyte count and percent;
EO, eosinophil count and percent; BA, basophil count and percent;
RBC, red blood cell and/or erythrocyte count;
HB, hemoglobin concentration; PLT, platelet and/or thrombocyte count

Legend for supplemental figure 1

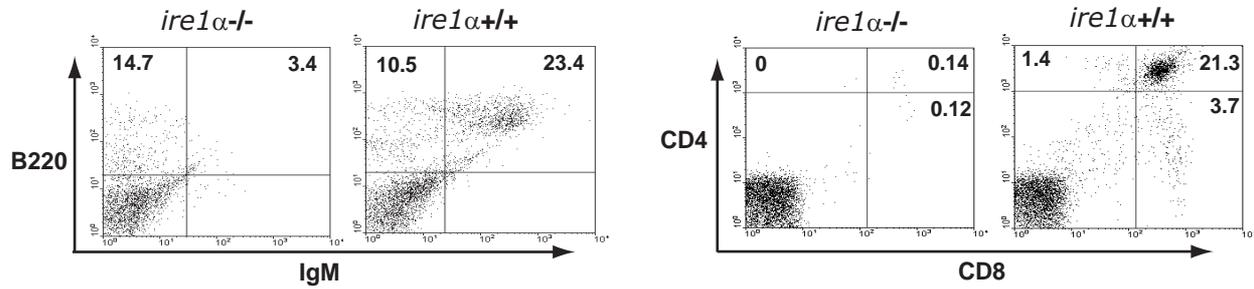
The *ire1 α ^{-/-}* fetal hematopoietic cells (4×10^6) and *rag2^{-/-}* bone marrow cells (2×10^5) were co-transferred intravenously into lethally-irradiated *rag2^{-/-}* mice for long-term reconstitution. All the reconstituted mice survived more than 2 months after transplantation. Blood serum, splenocytes, thymocytes and genomic DNA of various organs from the reconstituted mice were collected for the following analysis. **(A)** Reconstitution of serum IgM and IgG1 in *ire1 α ^{+/+}rag2^{-/-}*, *ire1 α ^{+/-}rag2^{-/-}* and *ire1 α ^{-/-}rag2^{-/-}* chimaeric mice. Blood serum samples were collected from snipped tails of reconstituted *rag2^{-/-}* mice. Levels of serum IgM and IgG1 were determined by ELISA. The *ire1 α ^{+/+}* hematopoietic cells reconstituted significant levels of serum IgM and IgG1 in the *rag2^{-/-}* chimaeric mice. In contrast, no serum IgM and IgG1 was detected in the *ire1 α ^{-/-}* reconstituted *rag2^{-/-}* mice up to 2 months after transplantation. **(B)** FACS analysis with B220, IgM, CD4, and CD8 of splenocytes or thymocytes from the *ire1 α ^{+/+}rag2^{-/-}* and *ire1 α ^{-/-}rag2^{-/-}* chimaeric mice. Neither B220⁺IgM⁺ splenic B cells nor CD4⁺ or CD8⁺ T cells were detected in the *ire1 α ^{-/-}* reconstituted mice. **(C)** Quantification of rates of engraftment of *ire1 α ^{-/-}* or *ire1 α ^{+/-}* cells in the peripheral lymphoid organs after two and half months of reconstitution by Southern blot analysis. The phenotypes of *ire1 α ^{+/-}* reconstituted mice, including serum Ig levels and mature B and T cells in the peripheral lymphoid organs, were similar to those of *ire1 α ^{+/+}* mice. Since both *ire1 α ^{-/-}* and *ire1 α ^{+/-}* cells have the *ire1 α* recombinant allele integrated in their genomes that can be detected by a specific probe (see methods), we utilized *ire1 α ^{+/-}rag2^{-/-}* chimaeric mice in place for

ire1α^{+/+}rag2^{-/-} chimaeric mice in comparing the engraftment rates of external hematopoietic cells. The amounts of the reconstituted *ire1α^{-/-}* or *ire1α^{+/-}* cells in the reconstituted bone marrow, spleen and thymus were determined by quantification of *ire1α* recombinant allele in the genomic DNA isolated from those organs. The engraftment rate of *ire1α^{-/-}* cells in bone marrow was comparable to that of *ire1α^{+/-}*, while very few *ire1α^{-/-}* cells could be detected in the spleen and thymus of the *ire1α^{-/-}* reconstituted mice. Rec. allele, *ire1α* recombinant allele; WT allele, wild-type *ire1α* allele. For panels A-C, representative data from at least 3 separate experiments are shown.

A



B



C

