Brief Definitive Report

The Transcription Factor Interferon Regulatory Factor 1 (IRF-1) Is Important during the Maturation of Natural Killer 1.1$^+$ T Cell Receptor–α/β^+ (NK1$^+$ T) Cells, Natural Killer Cells, and Intestinal Intraepithelial T Cells

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Summary

In contrast to conventional T cells, natural killer (NK) 1.1$^+$ T cell receptor (TCR)-α/β^+ (NK1$^+$T) cells, NK cells, and intestinal intraepithelial lymphocytes (IELs) bearing CD8-α/α chains constitutively express the interleukin (IL)-2 receptor (R)β/15Rβ chain. Recent studies have indicated that IL-2Rβ/15Rβ chain is required for the development of these lymphocyte subsets, outlining the importance of IL-15. In this study, we investigated the development of these lymphocyte subsets in interferon regulatory factor 1–deficient (IRF-1$^{-/-}$) mice. Surprisingly, all of these lymphocyte subsets were severely reduced in IRF-1$^{-/-}$ mice. Within CD8-α/α^+ intestinal IEL subset, TCR-γ/δ^+ cells and TCR-α/β^+ cells were equally affected by IRF gene disruption. In contrast to intestinal TCR-γ/δ^+ cells, thymic TCR-γ/δ^+ cells developed normally in IRF-1$^{-/-}$ mice. Northern blot analysis further revealed that the induction of IL-15 messenger RNA was impaired in IRF-1$^{-/-}$ bone marrow cells, and the recovery of these lymphocyte subsets was observed when IRF-1$^{-/-}$ cells were cultured with IL-15 in vitro. These data indicate that IRF-1 regulates IL-15 gene expression, which may control the development of NK1$^+$T cells, NK cells, and CD8-α/α^+ IELs.

In addition to the conventional lymphocyte subsets, other lineages have been identified as NK1.1$^+$TCR-α/β^+ (NK1$^+$T) cells, NK cells, and intestinal intraepithelial lymphocytes (IELs). NK1$^+$T cells have been recently classified as a lymphocyte subset that shares common features with both NK cells and conventional T cells. This lineage expresses NK markers including NKR-P1, Ly-49, and IL-2Rβ/15Rβ as well as an invariant Vα14J281TCR-α chain in combination with Vβ8, Vβ7, or Vβ2 (1, 2). Expression of these TCRs is required for NK1$^+$ T cell development (3, 4). They are positively selected by MHC class I–related CD1 or thymic leukemia (TL) molecules (5–7). The majority of TCR-α/β^+ or TCR-γ/δ^+ intestinal IEL expresses CD8-α/α homodimers. Both NK1$^+$T cells and CD8-α/α^+ intestinal IELs can develop through either extrathymic or alternative thymic pathways (1, 2, 8). Notably, the IL-2Rβ/15Rβ chain is required for the development of NK1$^+$T cells, NK cells, and CD8-α/α^+ intestinal IELs (9, 10), and IL-15 preferentially promotes the proliferation of these lymphocyte subsets (10–12).

IFN regulatory factor 1 (IRF-1), an IFN-inducible transcriptional activator, was initially identified as a protein that binds *cis*-acting DNA elements in the IFN-β promoter (13–15) and the IFN-stimulated response element of IFN-α/β–stimulated genes (16, 17). Recent studies with IRF-deficient (IRF-1$^{-/-}$) mice demonstrated a reduction of CD8$^+$TCR-α/β^+ cells and decreased MHC class I levels as a consequence of reduced expression of transporter associated with antigen processing 1 (TAP-1) and low molecular weight protein 2 (LMP-2; 18, 19).

Since IRF-1 deficiency has been related to T cell maturation, we examined the development of NK1$^+$T cells, NK cells, and IELs in IRF-1$^{-/-}$ mice. Data indicated that these lymphocyte subsets were selectively reduced and IL-15 messenger RNA (mRNA) was barely detectable in IRF-1$^{-/-}$ mice. Therefore, IRF-1 regulates the IL-15 gene that is required for survival and/or expansion of these lymphocyte subsets in vivo.

Materials and Methods

Mice. Mice deficient in IRF-1 (18) were backcrossed five times with C57BL/6 mice. Homozygous IRF-1$^{-/-}$ mice were bred and identified by staining blood with anti-CD8 and -CD4 mAb. Wild-type or heterozygous mice were used as controls. All mice were maintained in our animal facility according to institutional guidelines, and experiments were done between 8 and 14 wk of age.

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Cell Preparation and Culture. Liver mononuclear cells (MNCs) and IELs were prepared as previously described (20). In some experiments, liver MNCs or IELs obtained from IRF-$1^{-/-}$ mice were cultured with 100 ng/ml mouse IL-15 (provided by Immunex Co., Seattle, WA) for 7 d.

Antibodies and Flow Cytometric Analysis. The following mAb conjugates were purchased from PharMingen (San Diego, CA) and used in this study: M1/69-FITC (anti-HSA), 53-5.8-FITC (anti-CD8β), H57-597-FITC and -PE (anti–TCR-β), TM-β1-PE (anti–IL-2Rβ), GL-3-PE (anti–TCR-δ), 53-6.7-PE (anti-CD8), PK136-PE and -biotin (anti-NK1.1), 1B1-PE (anti-CD1), 27D-biotin (anti–LFA-1), IM7-biotin (anti-CD44), and KJ16-biotin (anti-Vβ8.1,8.2). B22-purified mAb (anti–H-$2D^b$) was prepared in our laboratory. Biotinylated mAbs were detected with streptavidin red 670 (GIBCO BRL, Gaithersburg, MD) and purified mAbs were detected with goat anti–mouse IgG-FITC or goat anti–rat IgM-FITC; 10^6 cells were stained in 2% FCS PBS, washed, and analyzed by FACScan® using the Lysis II program (Becton Dickinson, Mountain View, CA).

Analysis for IL-15 mRNA Expression. Bone marrow (BM) cells were isolated and stimulated by 30 μg/ml LPS and 100 U/ml IFN-γ for 6 h. Total cellular RNA was isolated with TRIZOL (GIBCO BRL) according to the manufacturer's protocol. 10 μg of total RNA were subjected to electrophoresis in a denaturing 1.0% agarose gel containing 2% formaldehyde and transferred to Hybond N+ nylon membrane (Amersham Corp., Arlington Heights, IL). The filter was hybridized with mouse IL-15 cDNA probe radioactively labeled with [^{32}P]dCTP. The mouse IL-15 cDNA used as a probe was obtained by polymerase chain reaction using specific primers: sense primer 5′-GCC AGC TCA TCT TCA ACA-3′ and antisense primer 5′-TAA GTC TGA GAC GAG CTC TTT-3′. Radioactivity was assessed using phosphorimager (Molecular Dynamics, Sunnyvale, CA). The filter was stripped and rehybridized with a β-actin cDNA probe.

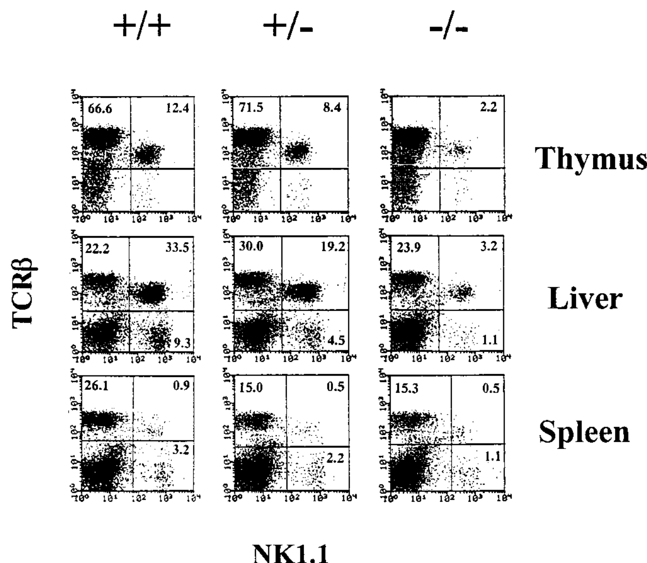

Figure 1. IRF-1 is important for NK1$^+$T cell and NK cell maturation. Thymocytes, liver, and spleen MNCs from indicated strains were stained with M1/69-FITC (anti-HSA), H57-597-PE (anti–TCR-β), and PK136-biotin (anti-NK1.1) plus streptavidin 670. HSA$^-$ cells are shown.

Results and Discussion

Impaired NK1$^+$T Cell and NK Cell Development in IRF-$1^{-/-}$ Mice. Mouse NK1$^+$T cells are generally either CD4$^+$8$^-$ or CD4$^-$8$^-$ cells that are primarily found in the thymus, liver, and BM (1, 2). We examined the NK1$^+$T cell subset in mice deficient for IRF-$1^{-/-}$. Surprisingly, the percentages of thymic and liver NK1$^+$T cells were decreased by 4–5 fold and 8–10 fold, respectively, in IRF-$1^{-/-}$ mice. The total number of thymic NK1$^+$T cells obtained from IRF-$1^{-/-}$ mice was 10-fold lower than in wild-type control mice. Interestingly, a partial reduction of NK1$^+$T cells was also seen in IRF-$1^{+/-}$ mice (Fig. 1, Table 1). The IL-2Rβ/15Rβ$^+$TCR-α/β$^+$ cells were also decreased, suggesting that the pronounced reduction of NK1$^+$T cells detected in IRF-$1^{-/-}$ mice was not simply due to the loss of NK1.1 molecules from the cell surface (data not shown). The small number of NK1$^+$T cells detected in IRF-$1^{-/-}$ mice expressed the IL-2Rβ/15Rβ chain and preferentially expressed Vβ8$^+$ TCR as seen in control mice (data not shown). In addition, analysis of the thymus, liver, and spleen using IRF-$1^{+/+}$, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice clearly demonstrated a reduction of NK cells (TCR-β$^-$NK1.1$^+$) in IRF-$1^{-/-}$ mice (Fig. 1). This is consistent with the lack of NK cell function previously reported in IRF-$1^{-/-}$ mice (21). Interestingly, IRF-$1^{+/-}$ mice consistently showed an intermediate phenotype, reflecting the dose-dependent requirement for genes regulated by IRF-1. These analysis showed that IRF-1 is important for NK cell and NK1$^+$T cell development.

Previous reports have shown that CD4$^-$8$^+$TCRα/β$^+$ cells were selectively reduced in thymus and periphery of IRF-$1^{-/-}$ mice (18). The data demonstrated a crucial role for IRF-1 in T cell development for the first time. A recent paper suggested that IRF-1 controls MHC class I expression through the regulation of transporter associated with antigen 1 and low molecular weight protein (19). Since mouse NK1$^+$T cells require β2-microgloblin–associated CD1 and TL molecules for development (5–7, 20, 22, 23),

Table 1. *Impaired Maturation of Thymic NK1$^+$T Cells in IRF-$1^{-/-}$ Mice*

Mice	Total thymocytes (× 10^6)	HSA$^-$ cell	NK1$^+$T cell	Total NK1$^+$T cell (× 10^4)
		%	%	
IRF-$1^{+/+}$	81.7 ± 4.7	3.4 ± 0.4	11.9 ± 1.4	39.5 ± 8.8
IRF-$1^{+/-}$	87.3 ± 10.8	3.2 ± 0.6	8.1 ± 0.6	22.6 ± 7.5
IRF-$1^{-/-}$	86.0 ± 11.8	1.6 ± 0.4	2.4 ± 0.4	3.3 ± 0.6

Four to six mice from each group were individually analyzed. Thymocytes were stained with M1/69-FITC (anti-HSA), H57-PE (anti–TCR-β), and PK136-biotin (anti-NK1.1) plus streptavidin 670. The percentage of NK1$^+$T cells was calculated in the HSA$^-$ thymocyte population.

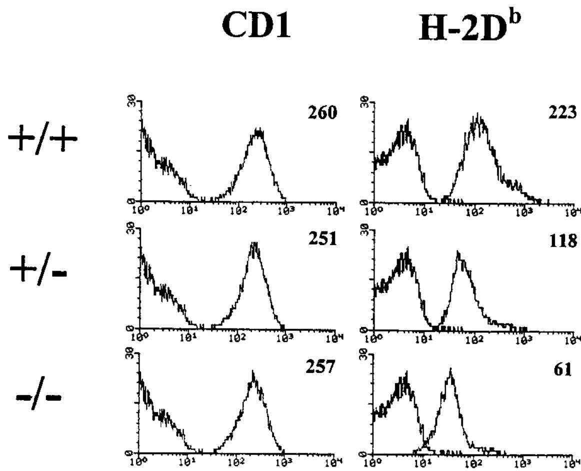

Figure 2. Normal CD1 expression on IRF-$1^{-/-}$ thymocytes. Thymocytes from the indicated strains were stained with 1B1-FITC (anti-CD1), 57.6.7-PE (anti-CD8), and L3T4-biotin (anti-CD4) plus streptavidin 670, and double-positive $CD4^+8^+$ thymocytes were analyzed for CD1 expression. For H-$2D^b$ expression, total thymocytes were stained with B22 (anti–H-$2D^b$) plus goat anti–mouse Ig-FITC.

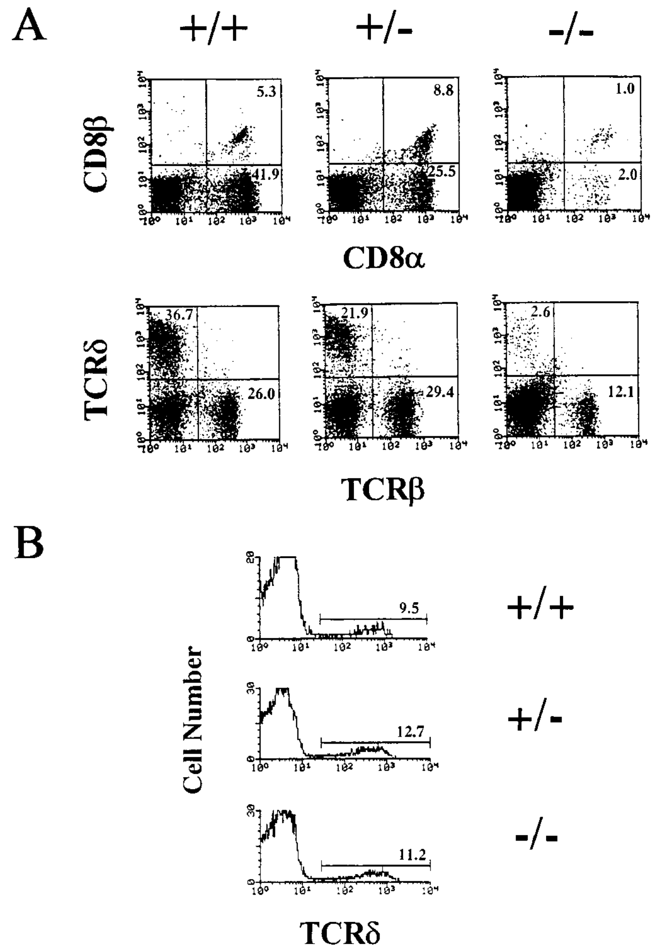

Figure 3. IRF-1 controls intestinal IEL development. (*A*) Intestinal IELs were obtained from either IRF-$1^{+/+}$ mice, IRF-$1^{+/-}$ mice, or IRF-$1^{-/-}$ mice and stained with H57-597 (anti–TCR-β) and GL-3-PE (anti–TCR-δ), or 53.6.7-FITC (anti–CD8α) and Lyt3-PE (anti-CD8β). (*B*) Thymocytes were stained with L3T4-FITC (anti-CD4), GL-3-PE (anti–TCR-δ), and 53.6.7-FITC (anti-CD8α). Histograms are gated on double-negative $CD4^-8^-$ thymocytes and TCR-δ expression is shown.

we examined CD1 expression on thymocytes from IRF-$1^{-/-}$ mice. Consistent with a recent paper (19), Fig. 2 showed that the lack of the IRF-1 gene clearly resulted in reduced H-$2D^b$ expression. However, the mean intensities of CD1 on IRF-$1^{-/-}$ thymocytes was comparable to littermate controls, suggesting that the IRF-1 gene does not control $NK1^+T$ cell development through CD1 expression. In addition, we can further exclude the role of the TL antigen in $NK1^+T$ cell development, since both IRF-$1^{-/-}$ and control mice are of the C57B1/6 background and do not express TL.

Maturation of Intestinal IELs Is Reduced in IRF-$1^{-/-}$ Mice. The majority of IELs express CD8 and can be divided into two subsets. One population bears CD8-α/$β^+$ heterodimers and expresses TCR-α/$β^+$, whereas the other expresses CD8-α/$α^+$ homodimers consisting of TCR-α/$β^+$ and TCR-γ/$δ^+$ cells. Using thymectomized recombinase activating gene (RAG)-deficient mice reconstituted with BM cells from athymic (nude) mice, thymus-independent development of CD8-α/$α^+$ IELs has been clearly demonstrated to occur (8). Surprisingly, in IRF-$1^{-/-}$ mice, the percentage of intestinal CD8-α/$α^+$ IELs was approximately eight- to ninefold less than in wild-type control mice. As seen with $NK1^+T$ cells, mice heterozygous for IRF-$1^{+/-}$ showed altered CD8-α/$α^+$ IEL development. TCR-γ/$δ^+$ IELs were profoundly reduced by IRF gene disruption (Fig. 3 *A*, Table 2). In addition, CD8-α/$β^+T$ cells were also reduced as seen in periphery. The total cell numbers of IELs from IRF-$1^{-/-}$ mice ($0.4 \pm 0.1 \times 10^6$) were three- to fourfold lower than those from littermate controls ($1.5 \pm 0.3 \times 10^6$). Therefore, IRF-1 controls the expression of genes important for IEL T cell development. It is likely that the reduced development of intestinal γ/$δ^+T$ cells is controlled by other mechanisms unrelated to MHC class I expression in IRF-$1^{-/-}$ mice. Previous studies using β2-microglobulin–deficient (MHC class $I^{-/-}$) mice showed a reduction in TCR-α/$β^+$ IELs, but not TCR-γ/$δ^+$ IELs (24), demonstrating that TCR-α/$β^+$ and TCR-γ/$δ^+$ IELs have differential requirements for β2-microglobulin dependent selection.

Since the majority of thymus-independent intestinal TCR-γ/$δ^+$ cells were absent in IRF-$1^{-/-}$ mice, we also examined whether thymic TCR-γ/$δ^+$ cells were present in these mice. Although the number of intestinal TCR-γ/$δ^+$ cells were decreased by 10-fold in IRF-$1^{-/-}$ mice, thymic TCR-γ/$δ^+$ cells were normal (Fig. 3 *B*, Table 2). Thus, IRF-1 selectively affected the development of intestinal TCR-γ/$δ^+$ cells.

IL-15 mRNA Expression Is Impaired in IRF-$1^{-/-}$ BM Cells. As certain cytokines are crucial for lymphocyte development, it is possible that a reduction in the expression of cytokine receptors or cytokines may result in poor selection, survival, or expansion of $NK1^+T$ cells, NK cells, and

Table 2. *Intestinal and Thymic T Cell Subsets in IRF-1$^{-/-}$ Mice*

Mice	Intestine				Thymus*
	CD8-α/α$^+$	CD8-α/β$^+$	TCR-α/β$^+$	TCR-γ/δ$^+$	TCR-γ/δ$^+$
IRF-1$^{+/+}$	42.5 ± 4.3	7.5 ± 3.0	26.7 ± 2.6	34.7 ± 4.0	9.3 ± 0.5
IRF-1$^{+/-}$	27.0 ± 2.6	10.5 ± 2.3	29.5 ± 3.6	21.0 ± 4.4	10.7 ± 1.8
IRF-1$^{-/-}$	5.0 ± 3.4	3.2 ± 1.7	15.8 ± 8.9	4.6 ± 2.8	9.6 ± 0.8

Four mice in each group were individually analyzed.
*Total thymocytes were stained with GK1.5-FITC (anti-CD4), GL-3-PE (anti–TCR-γ/δ), and 53.6.7-biotin (anti-CD8) plus streptavidin 670. TCR-γ/δ$^+$ cells were analyzed on gated double-negative (CD4$^-$8$^-$) thymocytes.

intestinal IEL subsets in IRF-1$^{-/-}$ mice. IL-15 is one of the most likely targets because NK1$^+$T cells, NK cells and intestinal IEL subsets are severely reduced in IL-2Rβ/15Rβ$^{-/-}$ mice (9, 10), while present in normal numbers in IL-2, IL-7Rα, or IL-7–deficient mice (10, 25–27). Interestingly, IL-15 preferentially promotes the proliferation of these T cell subsets (10–12). Thus, we examined IL-15 mRNA expression by Northern blot analysis (Fig. 4 *A*). Wild-type BM cells cultured in the presence of LPS and IFN-γ for 6 h, clearly increased IL-15 mRNA levels. In contrast, IL-15 mRNA remained undetectable in IRF-1$^{-/-}$ BM cells, even after induction with LPS and IFN-γ. These data demonstrate that IRF-1 regulates the expression of IL-15.

NK1$^+$T Cells, NK Cells, and Intestinal IELs were Recovered by IL-15 In Vitro. To further examine the importance of IL-15 for maturation of NK1$^+$T cells, NK cells, and intestinal IEL subsets in IRF-1$^{-/-}$ mice, liver MNCs and intestinal IELs were isolated from these mice and cultured with 100 ng/ml mouse IL-15 for 7 d (Fig. 4 *B*). Recovery of these lymphocyte subsets was observed. This suggested that IL-15 is essential for the survival or expansion of NK1$^+$T cells, NK cells, and intestinal IELs, and not early development or commitment.

NK1$^+$T cells, NK cells, and intestinal IELs share cell surface markers and other common features during development. In addition to the expression and developmental requirement of IL-2Rβ/15Rβ chain, they also express the NK complex that encompasses NKR-P1 and Ly-49 (1, 2, 28, 29). In contrast, conventional T cells do not express these products. Although the majority of T cells develops in the thymus, NK cells develop normally in athymic nude mice. The developmental origin of NK1$^+$T cells can be either thymus dependent or independent (2, 30, 31). Thymus-independent development of intestinal CD8-α/α$^+$ T cells has been clearly demonstrated to occur (8). Thus, NK1$^+$T cells and intestinal CD8-α/α$^+$ T cells are related to the NK lineage and can be distinguished from mainstream T cells. Our results demonstrate that IRF-1 controls the expression of IL-15, which is likely to be important for the maturation of the related NK1$^+$T cell, NK cell, and CD8-α/α$^+$ IEL lineages.

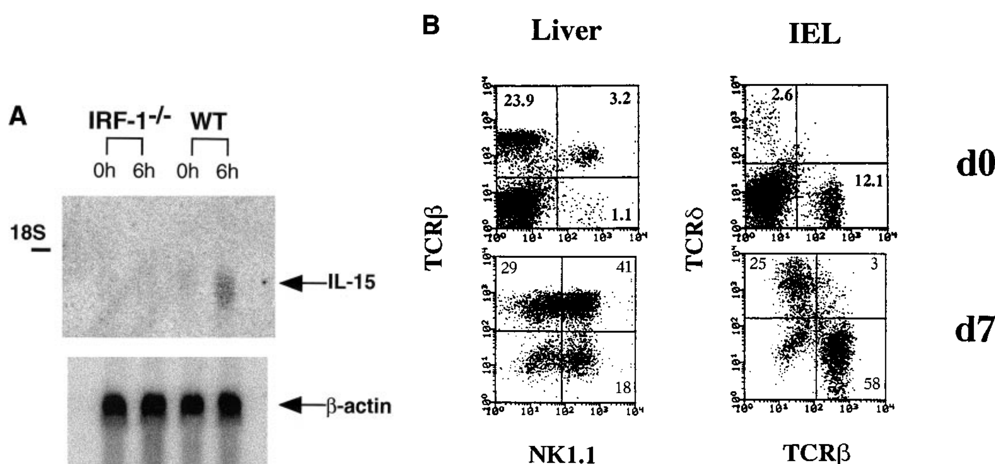

Figure 4. Impaired lineage development correlates with the absence of IL-15. (*A*) Limited IL-15 expression in the absence of IRF-1. BM cells were isolated from IRF-1$^{-/-}$ mice or control wild-type (*WT*) mice. Total RNA was extracted from untreated BM cells or BM cells cultured for 6 h in the presence of LPS (30 μg/ml) and IFN-γ (100 U/ml). Northern blot analysis was performed using IL-15 cDNA and β-actin probes. (*B*) IL-15 induces the expansion of NK1$^+$T cells, NK cells, and IEL subsets. Liver MNCs and intestinal IELs were isolated from IRF-1$^{-/-}$ mice and cultured with 100 ng/ml mouse IL-15 for 7 d.

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