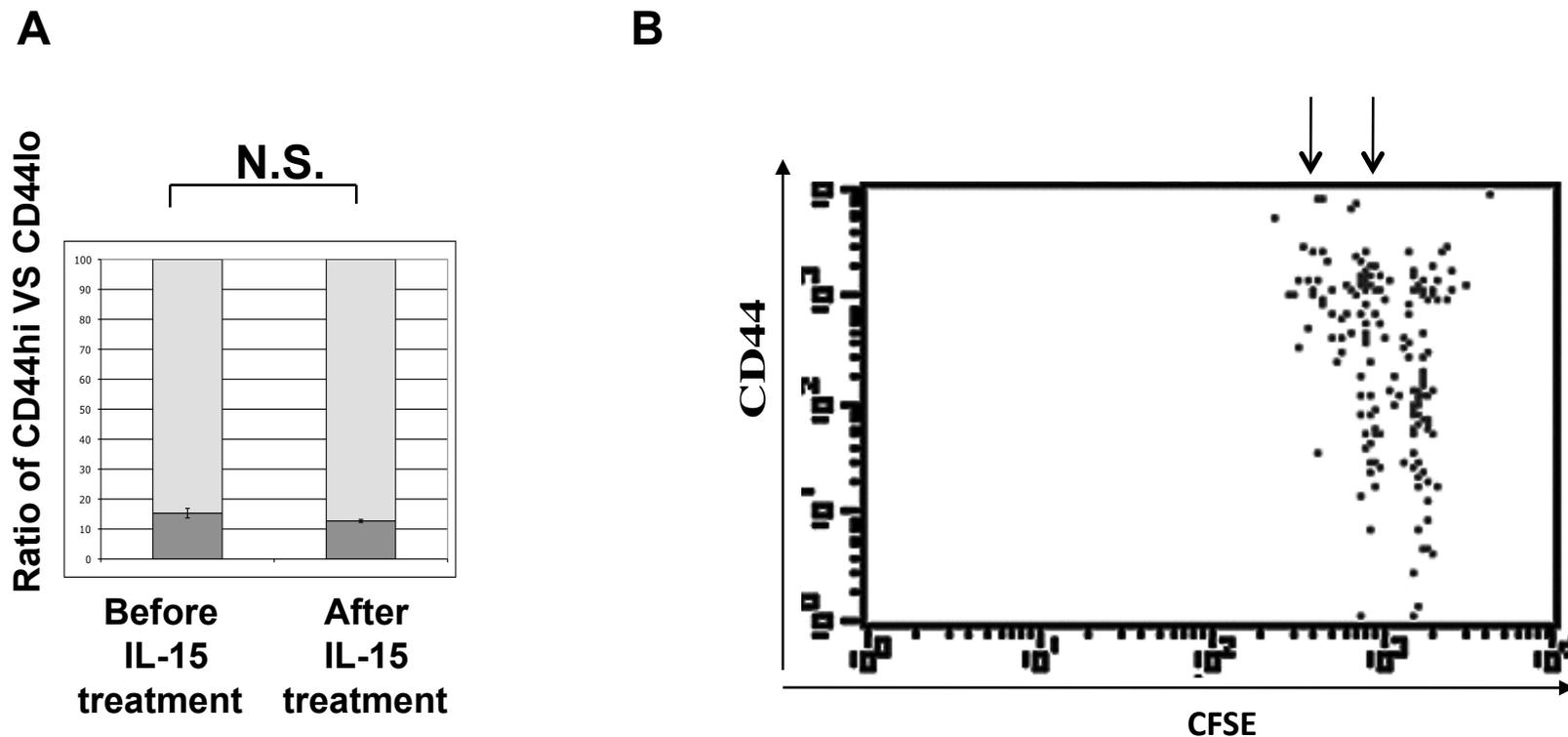
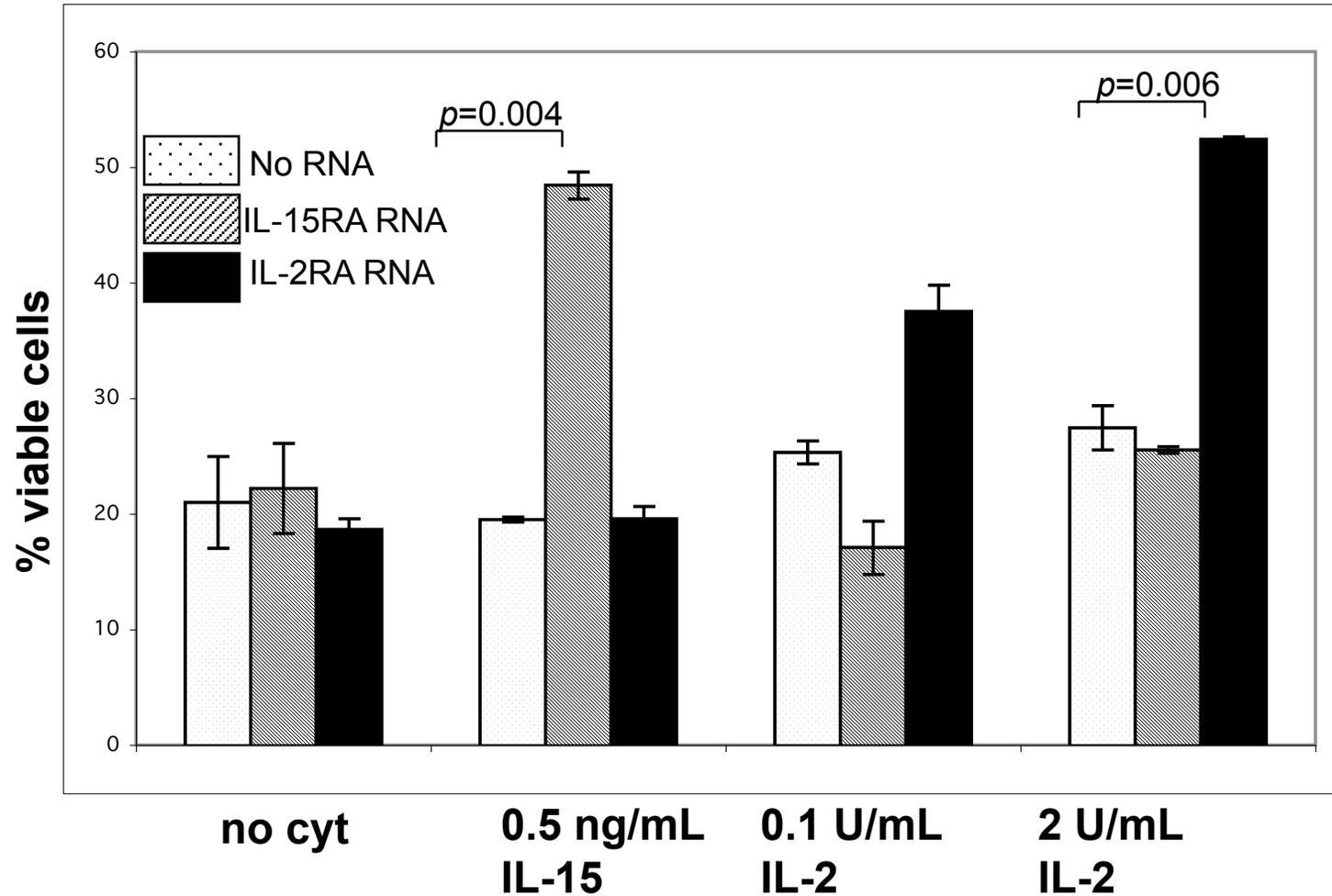


Supporting Information Figure 1. Characterization of the viability of CD8+ T cells from WT mice transfected with varying amounts of IL-15RA RNA. Unstimulated CD8+ T cells were isolated from naive WT C57BL/6 mice. 1×10^6 cells were transfected with increasing amounts IL-15RA RNA. **A)** Histogram representation of IL-15RA expression in CD8+ T cells transfected with increasing amounts of IL-15RA RNA. **B)** Bar graph representing the percentage of viable CD8+ T cells that were transfected with increasing doses of IL-15RA RNA and cultured for 4 days post transfection in the presence of increasing doses of IL-15 (means \pm s.e.). **C)** Histogram representation of IL-15RA expression in various CD8+ T cells. Unstimulated CD8+ T cells transfected with 2.5 µg IL-15RA RNA were compared with CD44 high gated CD8+ splenocytes obtained from 3 days stimulation with 60 ng/mL LPS + 60 ng/mL IFN gamma, or with activated CD8+ T cells from WT mice or IL-15RA $-/-$ mice, which were stimulated for 3 days with CD3, CD28, and IL-2. Activated IL-15RA $-/-$ T cells and activated T cells stained with isotype antibody were used as negative controls. The data shown here are representative of two experiments performed.

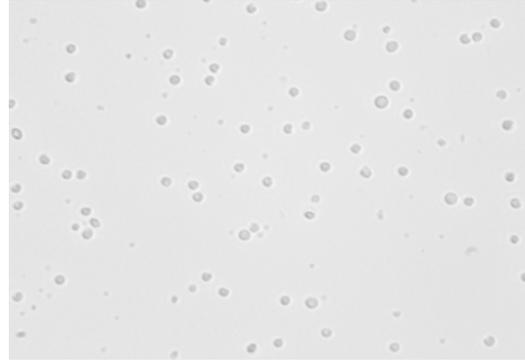


Supporting Information Figure 2. Characterization of CD44 expression on transfected CD8⁺ T cells in culture or after adoptive transfer. **A.** Bar graph representing the ratio of CD44^{hi} versus CD44^{lo} cells after IL-15RA transfection and before and after 4 days culture in the presence IL-15. CD8⁺ T cells were isolated from C57BL/6 mice. Cells were transfected with IL-15RA. Following transfection cells were stained for CD44 expression before or after culture for 4 days in the presence of 5 ng/mL IL-15 **B.** Flow cytometry data representing CD44 expression on THY1.1 positive and 7-AAD negative gated cells. Arrows indicate first and second divisions. Splenocytes were isolated from naive C57BL/6 THY1.1 congenic mice and were differentially labeled with or without CFSE. CD8⁺ cells were transfected with IL-15/IL-15RA RNA. 1×10^6 /mouse of CFSE labeled CD8⁺ T cells transfected with IL-15/IL-15RA RNA were transferred i.v. into C57BL/6 THY1.2 mice. 7 days later, splenocytes were isolated and stained with 7-AAD, CD44, and THY1.1 and analyzed for CD44 expression and CFSE dilution by flow cytometry. Experiments were performed in triplicate. N.S., not significant according to student T test.



Supporting Information Figure 3. Flow cytometry analysis to characterize the % viability of CD8+ T cells transfected with IL-2RA RNA or IL-15RA RNA and cultured in IL-2 or IL-15. CD8+ T cells were purified from the spleens of C57BL/6 mice and transfected with no RNA (dotted bars), IL-15RA RNA (hatched bars), or IL-2RA RNA (solid bars). Within 3 hours of transfection, cells were plated in 96 well U bottom plate with 0 or 0.5 ng/mL IL-15 or 0, 0.1, or 2 units/mL IL-2. 4 days later cells were analyzed for viability by 7 AAD staining. Undadjusted P value determined by student T test.

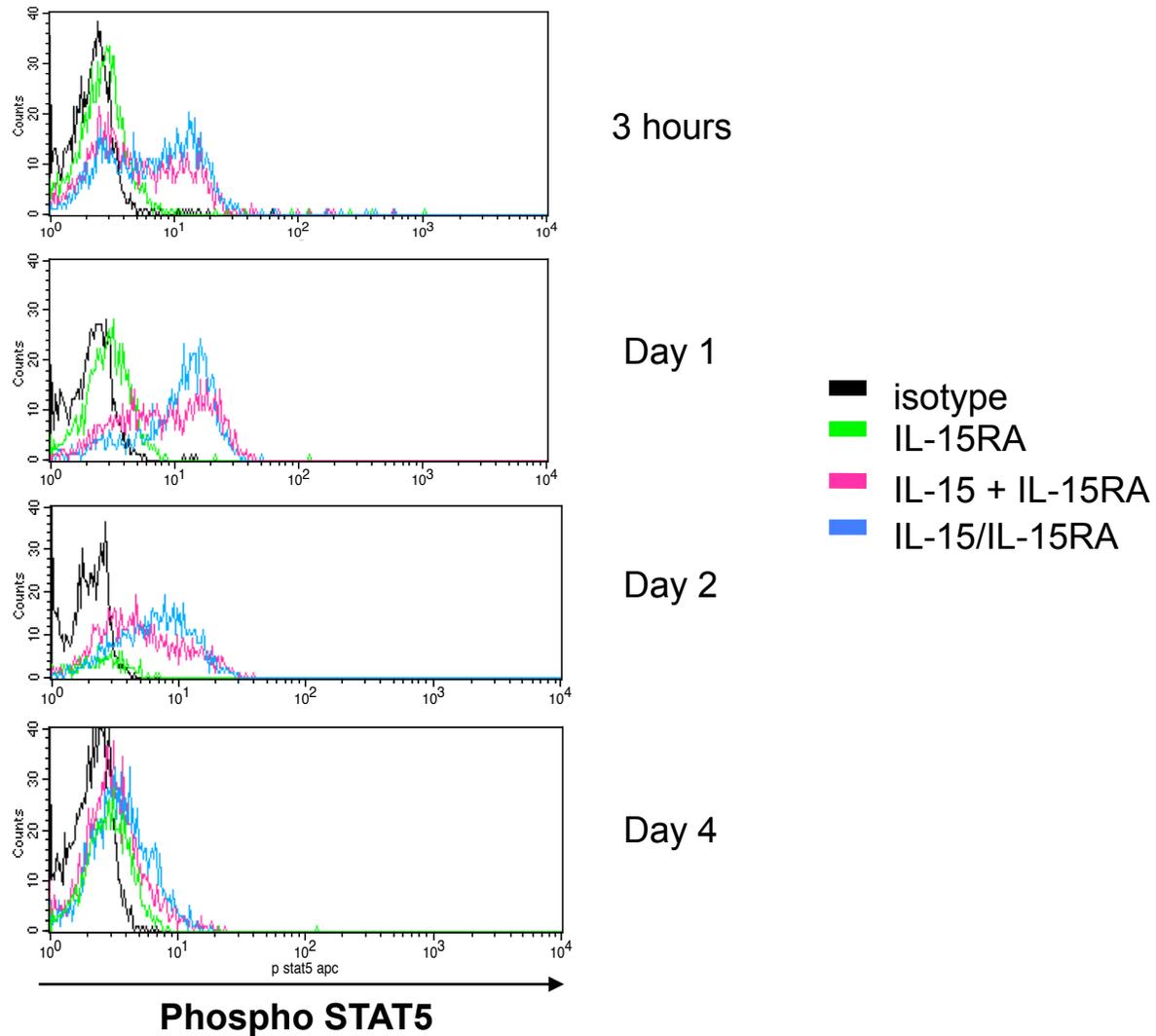
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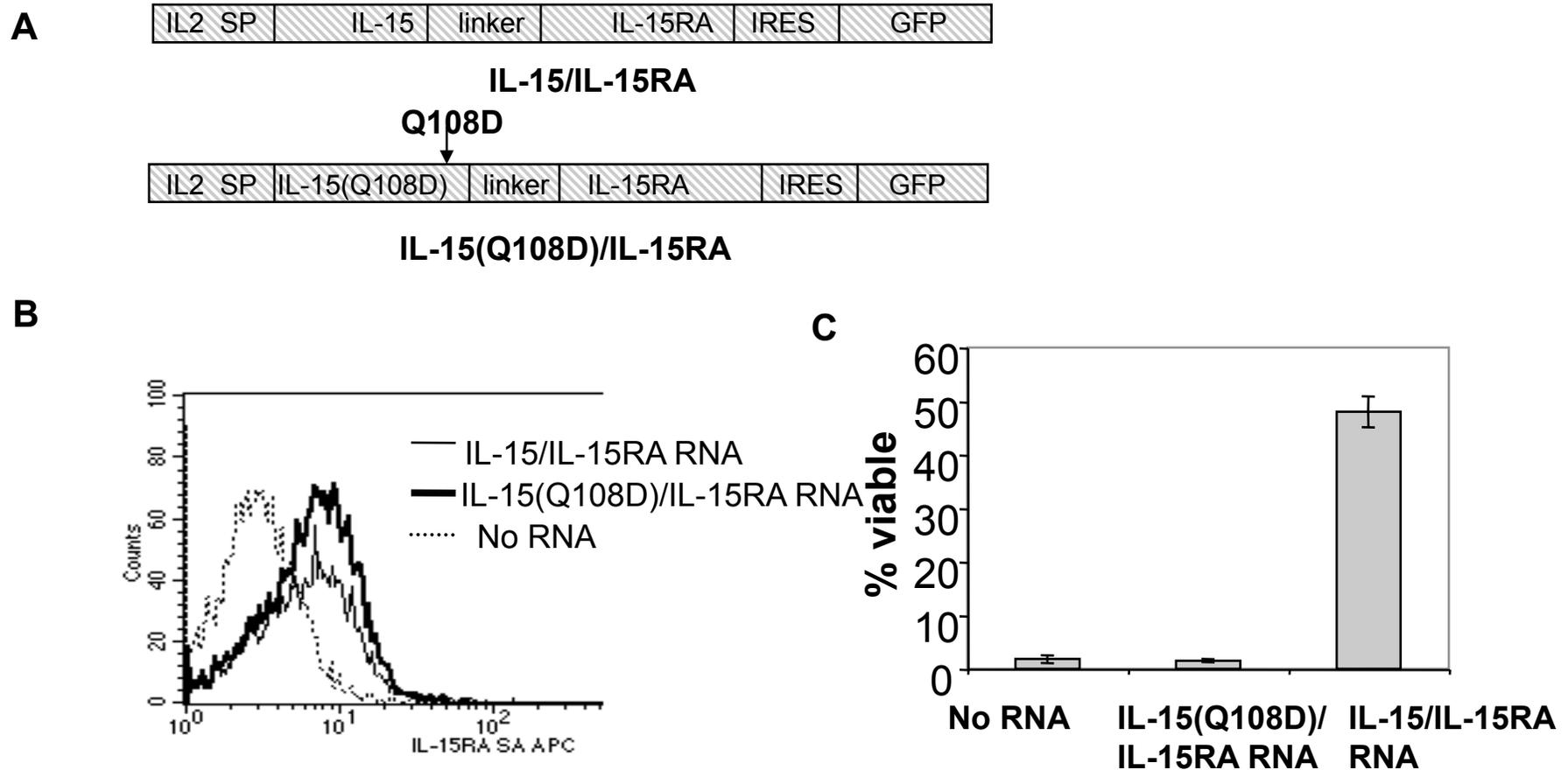
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Supporting Information Figure 4. Photographic images of CD8+ T cells in culture. 1×10^5 transfected cells were cultured in **A)** 6-well plates to minimize cell-cell contact or **B)** 96-well plates and centrifuged briefly to enhance cell-cell contact.



Supporting Information Figure 5. Analysis of STAT5 phosphorylation in cells transfected with IL-15 + IL-15RA RNA or IL-15/IL-15RA RNA. Histograms comparing intensity of phospho-STAT5 staining in transfected cells at different time points after transfection. Unstimulated CD8⁺ T cells were isolated from naïve C57BL/6 mice. 1×10^6 cells were transfected with IL-15RA RNA alone as baseline control, IL-15 + IL-15RA, or IL-15/IL-15RA RNA. 3 hours, 1 day, 2 days or 4 days after transfection, cells were stained intracellularly for phospho-STAT5 and analyzed by flow cytometry.



Supporting Information Figure 6. Characterization of the expression and effect on survival of transfection of IL-15/IL-15RA RNA with a point mutation in the γ c binding region of IL-15 (IL-15 (Q108D)/IL-15RA). Unstimulated CD8⁺ T cells were isolated from naïve C57BL/6 mice. 1×10^6 cells were transfected with IL-15/IL-15RA RNA, IL-15(Q108D)/IL-15RA RNA, or no RNA. Cells were stained for IL-15RA expression 1 day after transfection or stained with 7-AAD and analyzed by flow cytometry for viability 4 days after transfection. A. Depiction of the IL-15(Q108D)/IL-15RA mutant. B. Histograms comparing intensity of IL-15RA surface staining in transfected cells 1 day after transfection. C. Bar graph representing viability of transfected cells 4 days after transfection (means \pm s.e.). Assays were performed in duplicate. Shown is a representative of 2 independent experiments with similar results.