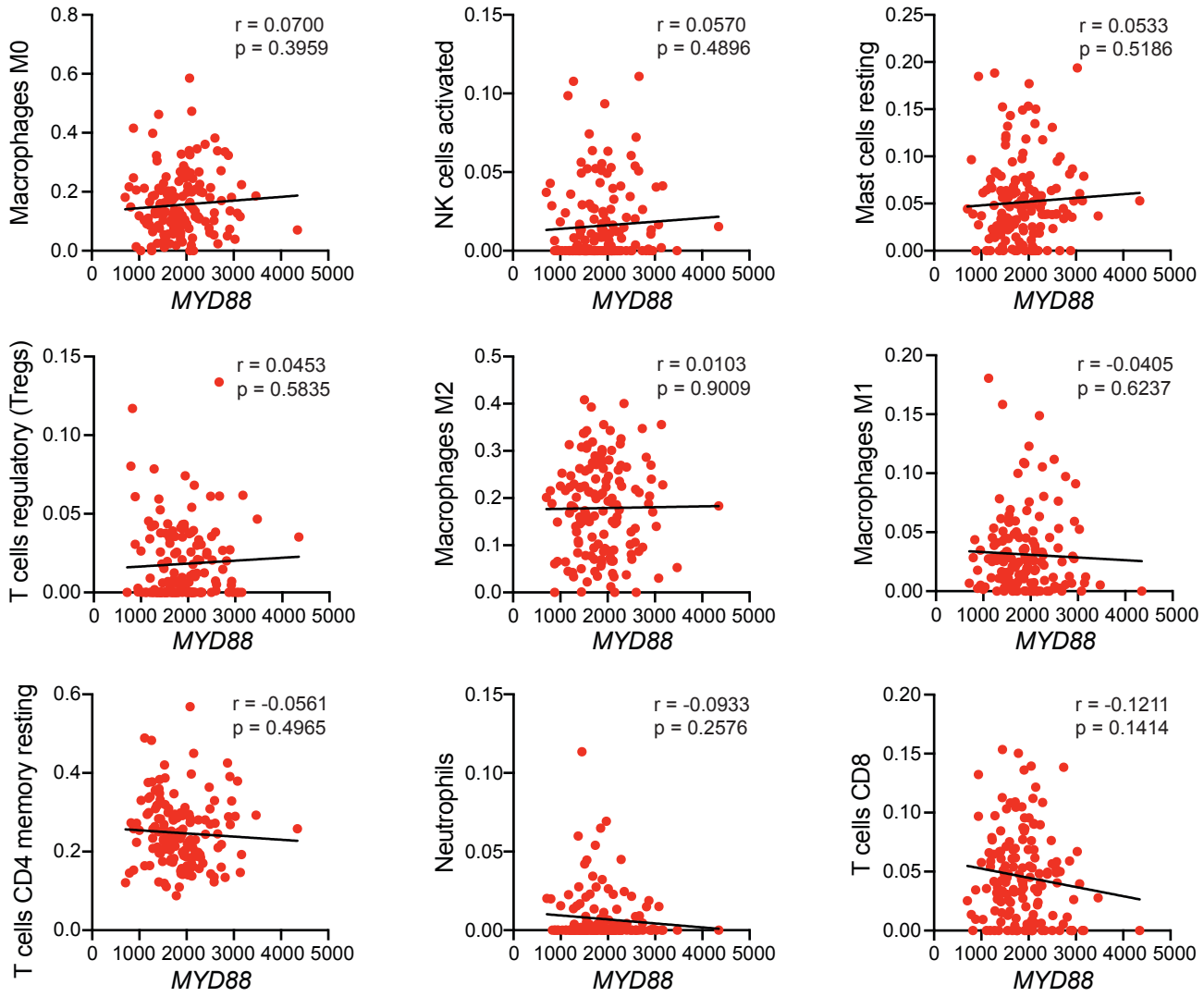
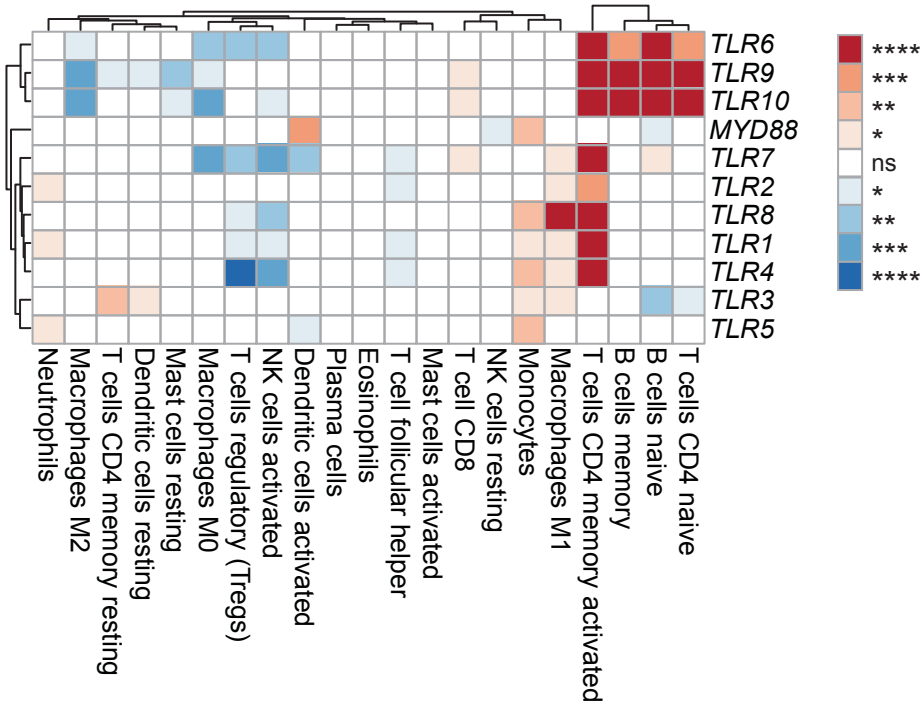


SUPPLEMENTAL INFORMATION

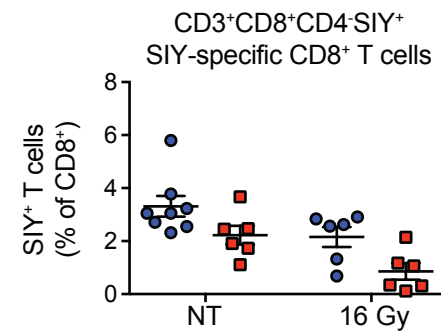
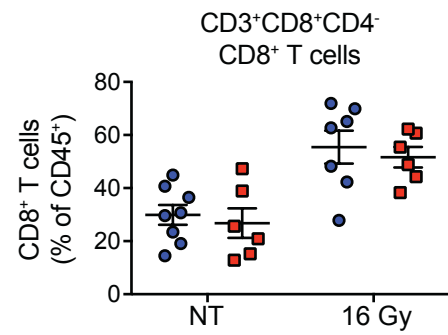
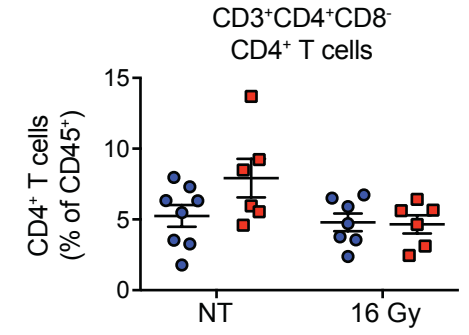
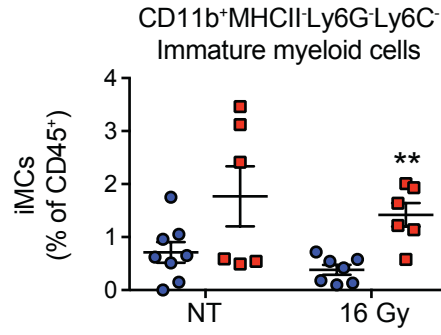
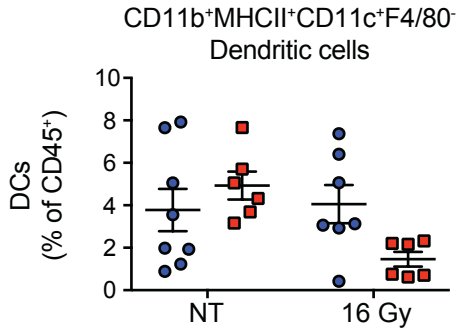
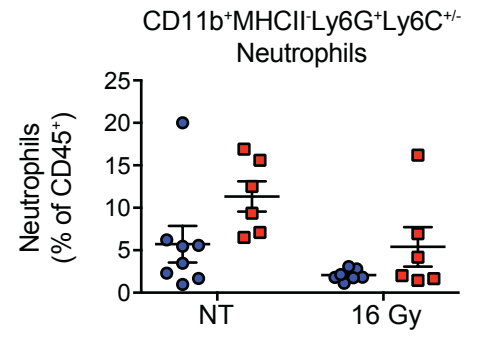
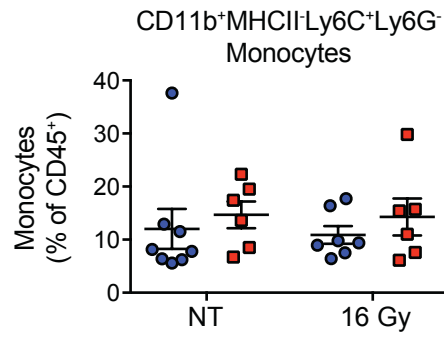
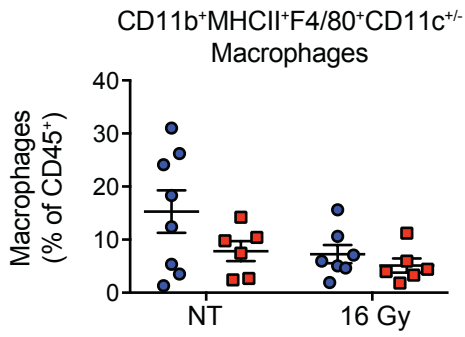
Myeloid MyD88 restricts CD8⁺ T cell response to radiation therapy in pancreatic cancer

Terry R. Medler¹, Tiffany C. Blair^{1,2}, Alejandro F. Alice¹, Alexa K. Dowdell¹, Brian D. Piening¹, Marka R. Crittenden^{1,3}, Michael J. Gough^{1,*}

A**B**

Supplemental Figure S1. *MYD88* and *TLR* expression in human pancreatic cancer.

(A) Linear regression analysis showing Pearson correlation between *MYD88* expression and inferred presence of indicated immune subsets as estimated by CIBERSORT in the PAAD TCGA dataset (n = 149). **(B)** Heatmap of p values for linear regression analysis of *TLR* and *MYD88* expression with presence of immune subsets as inferred by CIBERSORT in the PAAD TCGA dataset (n = 149). Positive p values are indicated in shades of red, while negative p values appear in blue. Data were visualized using ClustVis. See **Figure 1C** for Pearson correlation values.

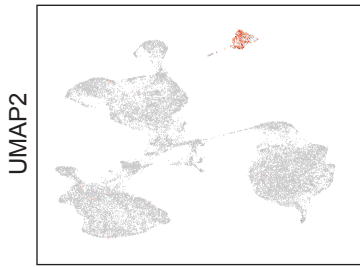


● *Myd88^{fl/fl}*
■ *Lyz2-cre/Myd88^{fl/fl}*

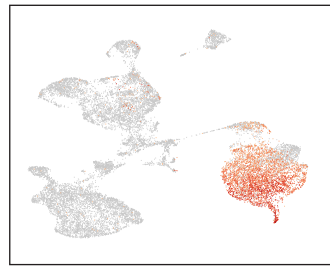
Supplemental Figure S2. Infiltrating immune cells are largely unchanged in *Lyz2-Cre/Myd88^{fl/fl}* mice.

Flow cytometric analysis of Panc02-SIY tumors isolated 7d post-RT using the indicated markers, represented as a percentage of live CD45⁺ cells. Each data point represents a single mouse (n = 6-8 mice per group). Significant differences between genotypes were determined by multiple t tests corrected using the Holm-Sidak method, with ** p < 0.01.

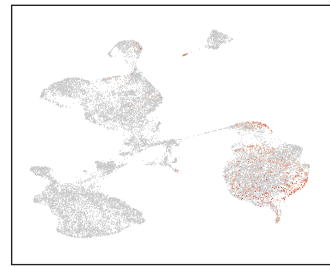
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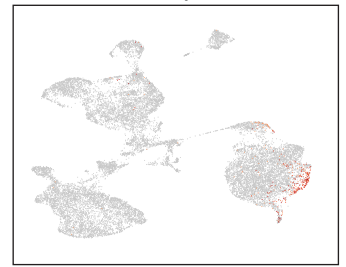
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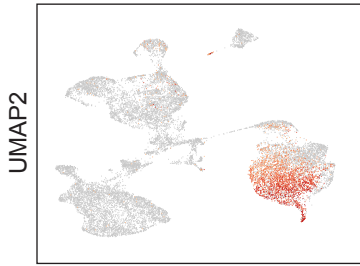
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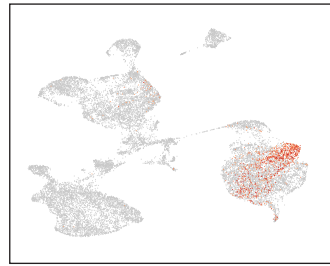
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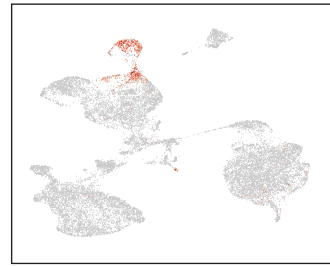
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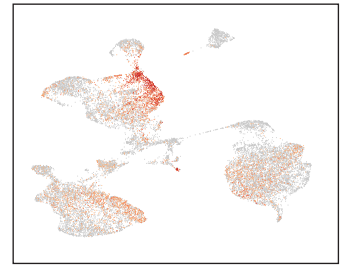
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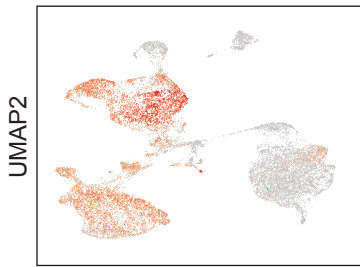
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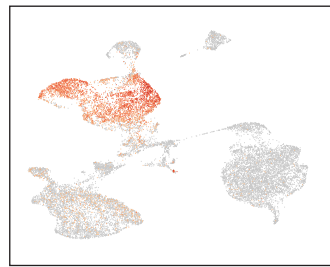
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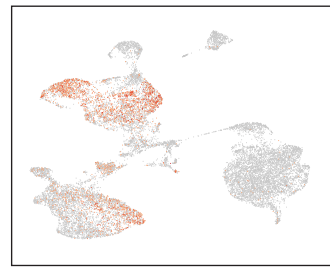
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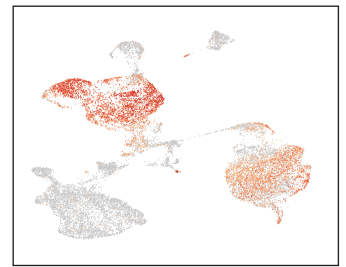
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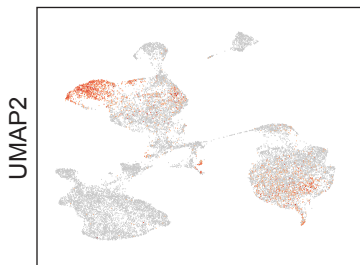
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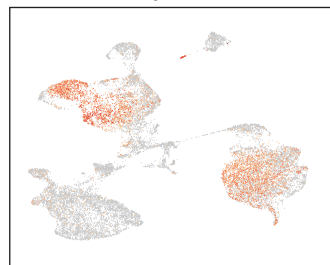
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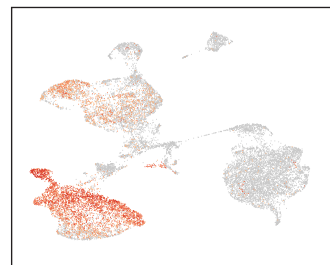
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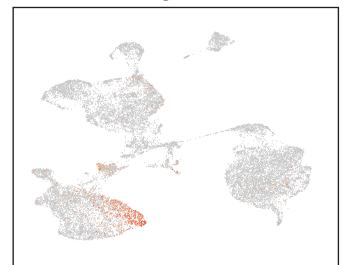
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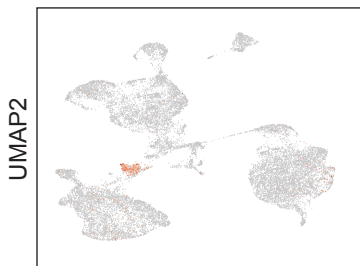
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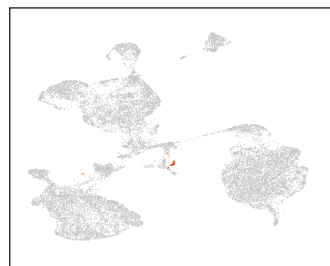
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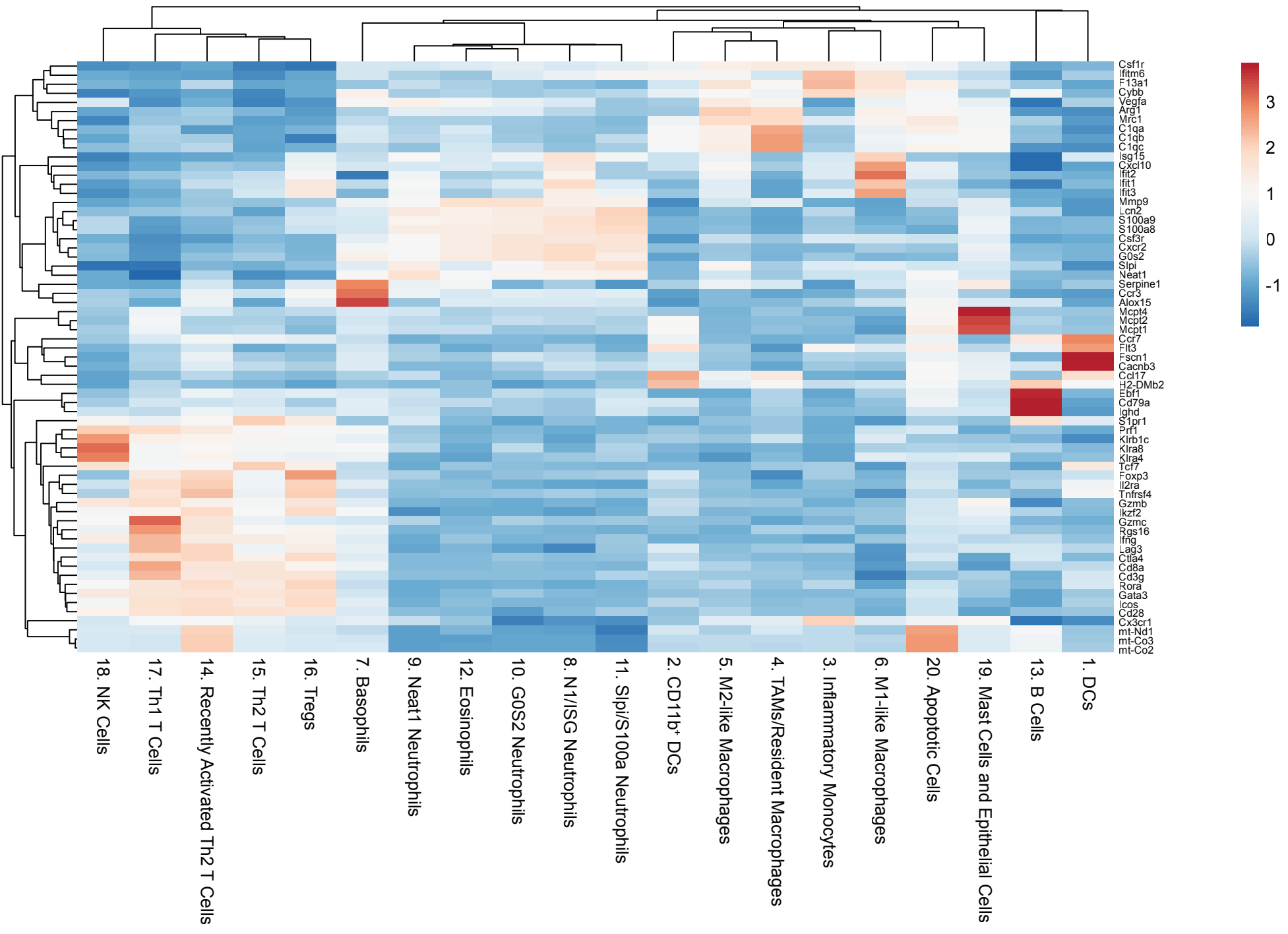


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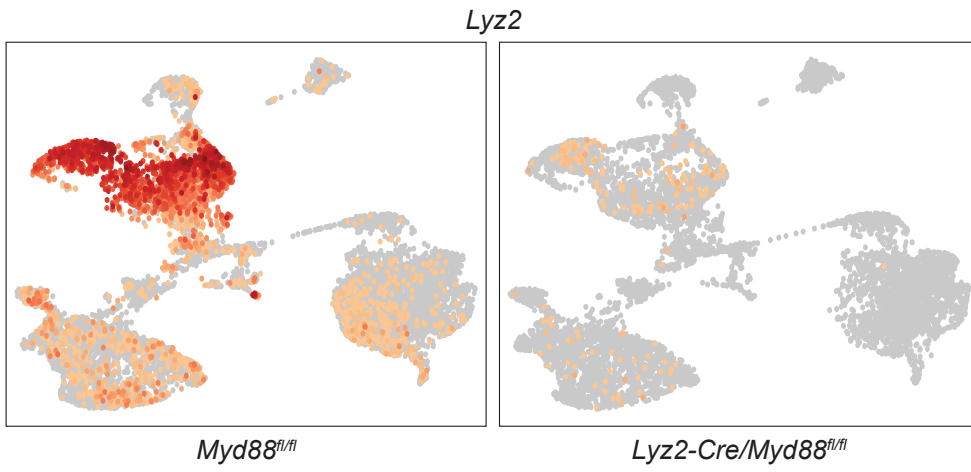
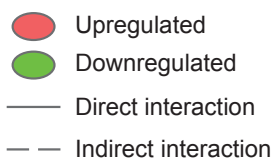
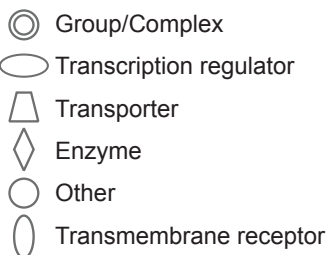
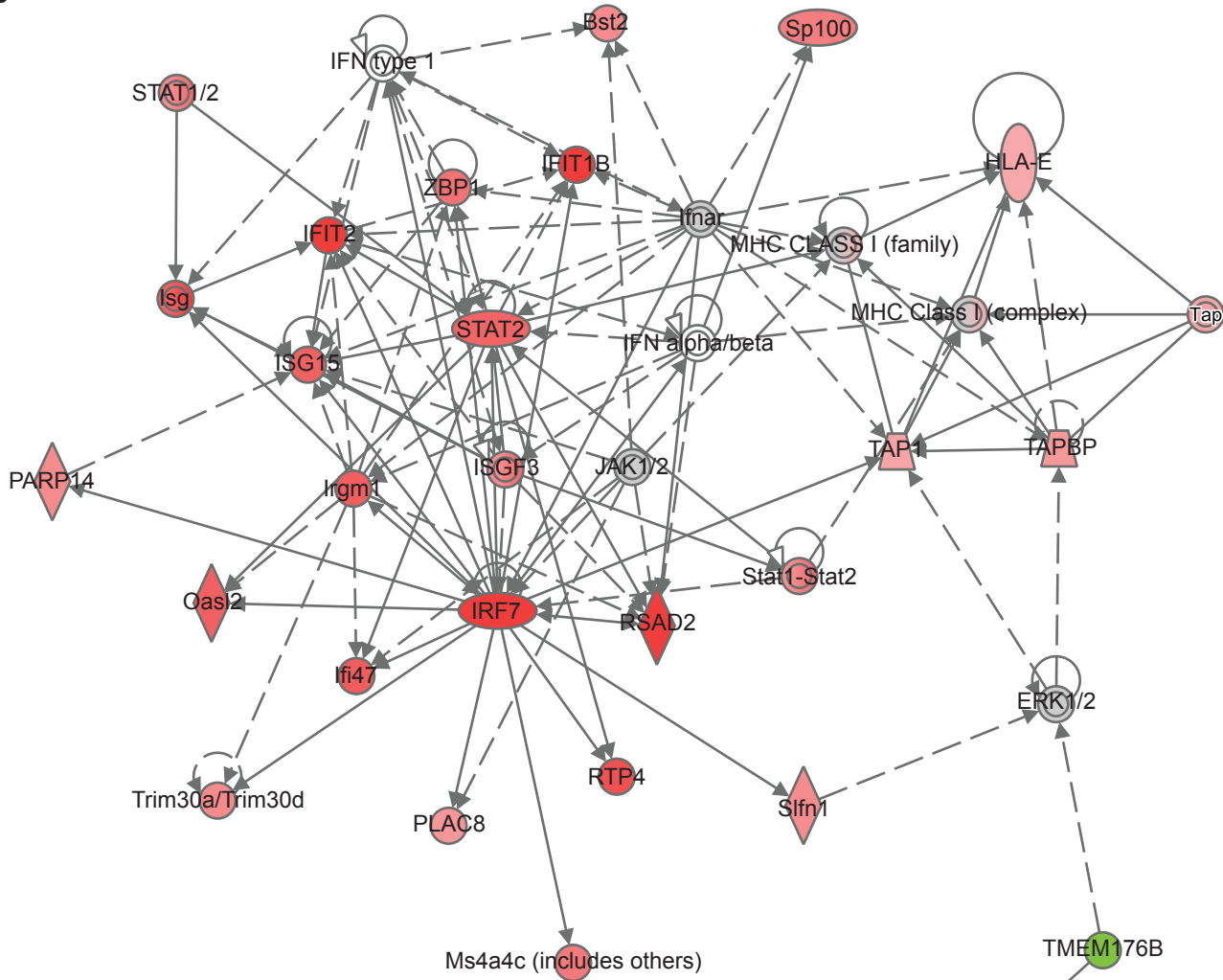
Supplemental Figure S3. Expression of lineage markers used to identify dominant immune populations in scRNAseq.

scRNAseq was performed on CD45⁺ cells isolated from untreated tumors or 3d post-RT in *Lyz2-Cre/Myd88^{fl/fl}* mice and *Myd88^{fl/fl}* mice. Expression of indicated genes in UMAP is shown (see also **Figure 3 and Supplemental Figure S4**), with the intensity of red directly proportional to the degree of expression.



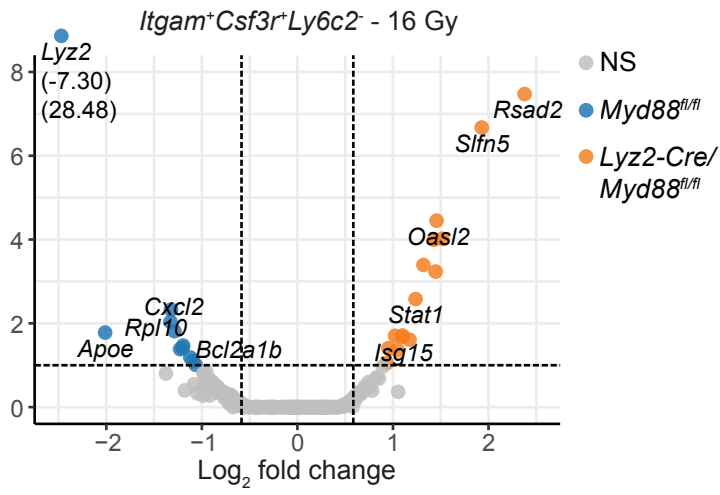
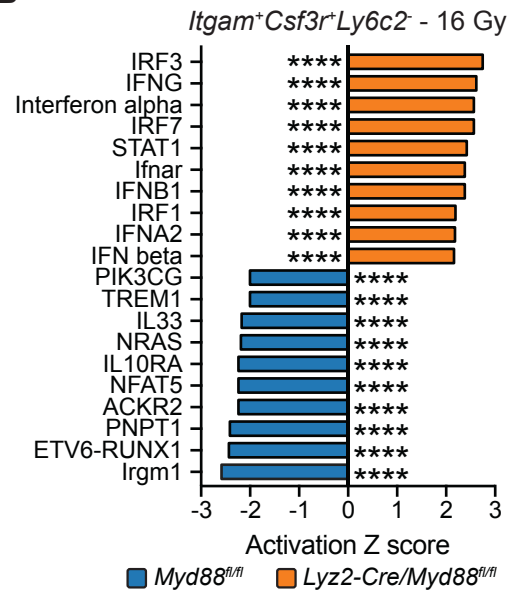
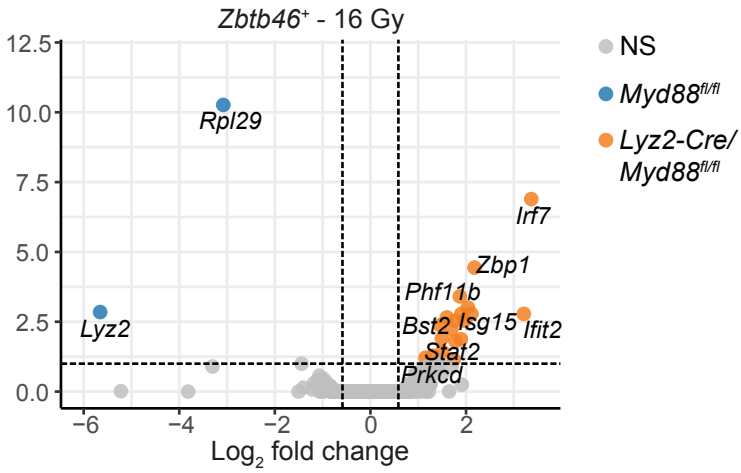
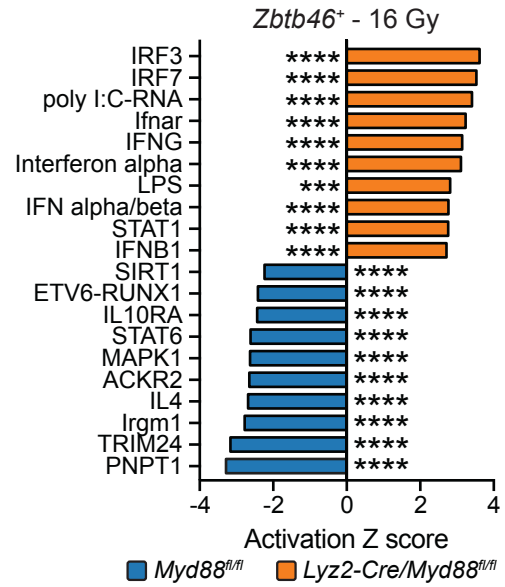
Supplemental Figure S4. Expression of lineage markers used to identify dominant immune populations in scRNAseq.

scRNAseq was performed on CD45⁺ cells isolated from untreated tumors or 3d post-RT in *Lyz2-Cre/Myd88^{fl/fl}* mice and *Myd88^{fl/fl}* mice. Unsupervised hierarchical clustering of differential gene expression of indicated genes using ClustVis is shown (see also **Figure 3** and **Supplemental Figure S3**).

A**B**

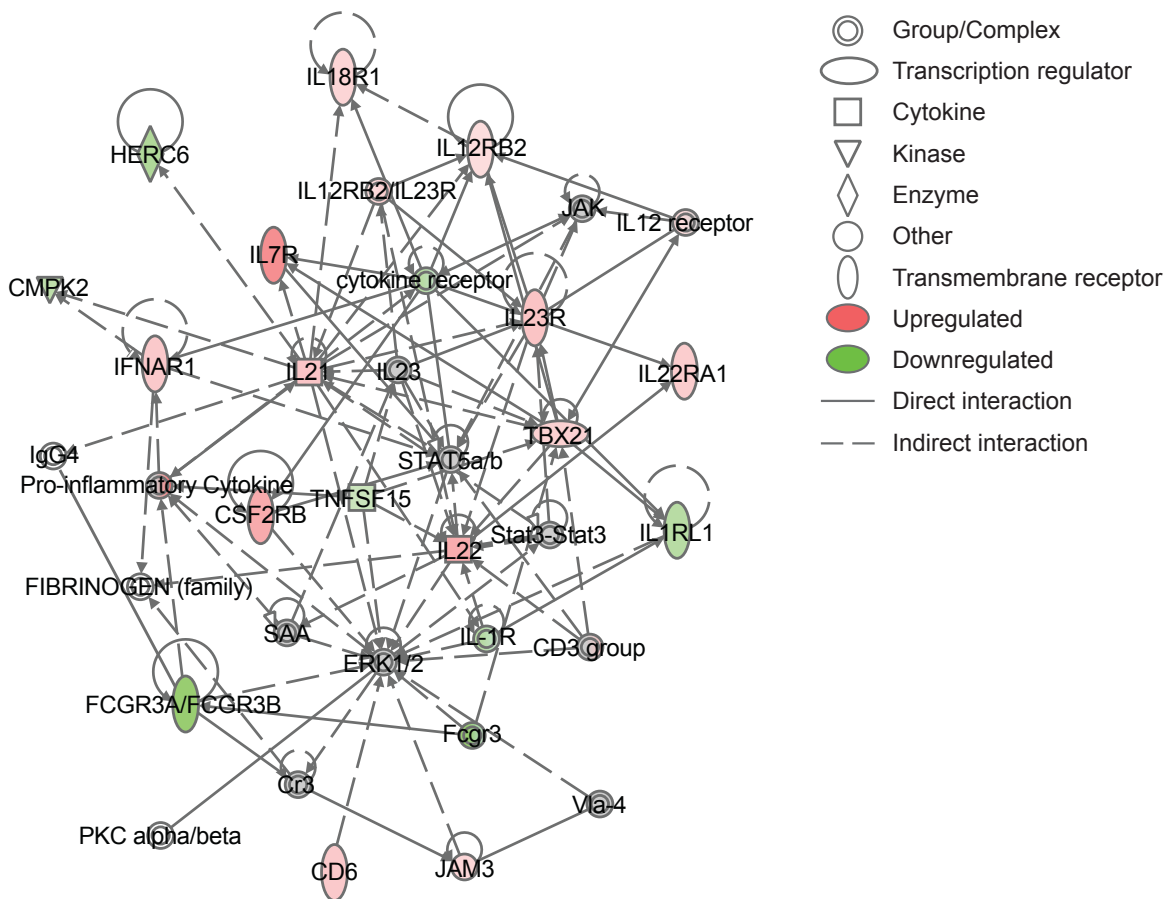
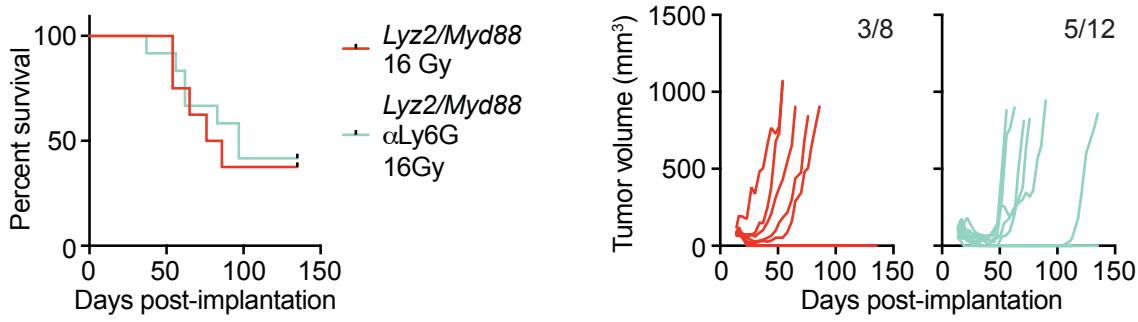
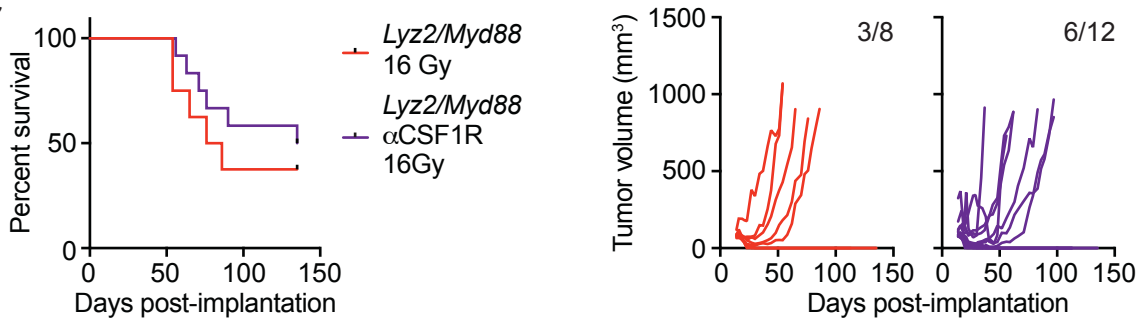
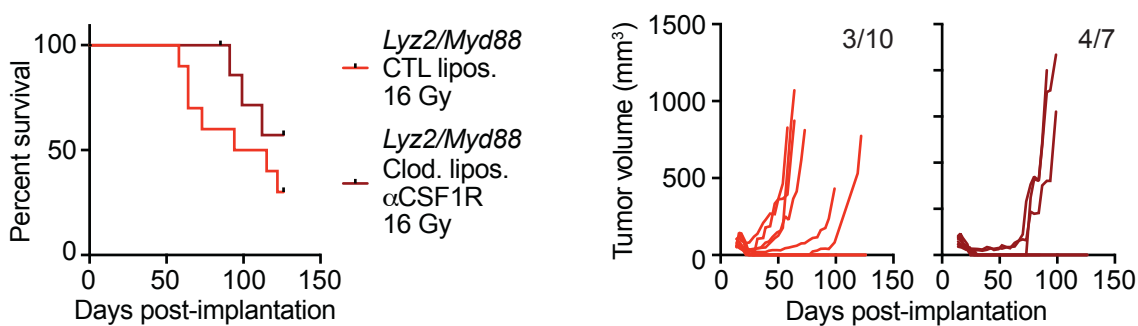
Supplemental Figure S5. Enhanced interferon responses characterize infiltrating macrophages and monocytes in tumors from *Lyz2-Cre/Myd88^{fl/fl}* mice.

(A) UMAP of *Lyz2* expression in *Lyz2-Cre/Myd88^{fl/fl}* mice and *Myd88^{fl/fl}* mice. **(B)** IPA Network analysis of significant differentially regulated genes in *Lyz2-Cre/Myd88^{fl/fl}* mice compared to *Myd88^{fl/fl}* mice. Genes upregulated in *Lyz2-Cre/Myd88^{fl/fl}* mice are depicted in red, while genes upregulated in *Myd88^{fl/fl}* mice are depicted in green, with color intensity associated with degree of upregulation. Chosen network consists of the top scoring network (score = 52). Significance of differential gene expression was defined as fold change >1.5 and $p < 0.1$.

A**B****C****D**

Supplemental Fig. S6. Enhanced interferon responses characterize granulocytes and dendritic cells in tumors from *Lyz2-Cre/Myd88^{fl/fl}* mice.

(A) Volcano plot of differential gene expression in granulocytes (*Itgam⁺Csf3r⁺Ly6c2⁺* cells) that are upregulated in *Lyz2-Cre/Myd88^{fl/fl}* mice (right, orange) or upregulated in *Myd88^{fl/fl}* mice (left, blue). **(B)** Upstream analysis *Itgam⁺Csf3r⁺Ly6c2⁺* cells granulocytes using Ingenuity Pathway Analysis identifying likely regulators of increased differential gene expression observed in *Lyz2-Cre/Myd88^{fl/fl}* mice (top, orange) or *Myd88^{fl/fl}* mice (bottom, blue). **(C)** Volcano plot of differential gene expression in dendritic cells (*Zbtb46⁺* cells) that are upregulated in *Lyz2-Cre/Myd88^{fl/fl}* mice (right, orange) or upregulated in *Myd88^{fl/fl}* mice (left, blue). **(D)** Upstream analysis *Zbtb46⁺* using Ingenuity Pathway Analysis identifying likely regulators of increased differential gene expression observed in *Lyz2-Cre/Myd88^{fl/fl}* mice (top, orange) or *Myd88^{fl/fl}* mice (bottom, blue). Significance of differential gene expression in **(A-D)** was defined as fold change >1.5 and $p < 0.1$. **** $p < 0.0001$.

A**B****C****D**

Supplemental Figure S7. Depletion of Macrophages or Granulocytes does not reverse efficacy of RT in *Lyz2-Cre/Myd88^{fl/fl}* mice.

(A) IPA network analysis of significantly differentially regulated genes identified using the PanCancer Immune Profiling gene set by NanoString. Genes upregulated in *Lyz2-Cre/Myd88^{fl/fl}* mice are depicted in red, while genes upregulated in *Myd88^{fl/fl}* mice are depicted in green, with color intensity associated with degree of upregulation. Chosen network consists of the top scoring network (score = 37). **(B)** Survival curves (left) and individual Panc02-SIY tumor growth curves (right) in control *Lyz2-Cre/Myd88^{fl/fl}* mice treated with 16 Gy RT or depleted of granulocytes with α Ly6G (n = 8-12 mice per group). **(C)** Survival curves (left) and individual Panc02-SIY tumor growth curves (right) in control *Lyz2-Cre/Myd88^{fl/fl}* mice treated with 16 Gy RT or depleted of macrophages with α CSF1R (n = 8-12 mice per group). **(D)** Survival curves (left) and individual Panc02-SIY tumor growth curves (right) in control *Lyz2-Cre/Myd88^{fl/fl}* mice treated with 16 Gy RT and control liposomes or depleted of macrophages with α CSF1R and clodronate liposomes (n = 7-10 mice per group). Significance of differentially regulated genes in **(A)** was defined as fold change >1.5 and p < 0.1. Numbers in the upper right of individual tumor growth curves in **(B-D)** represent the proportion of mice cured by RT. Significance in **(B-D)** was assessed log-rank test.