Type I interferon activates MHC class I-dressed CD11b+ conventional dendritic cells to promote protective anti-tumor CD8+ T cell immunity

**Graphical abstract**

**Highlights**

- IFN-I induces a stimulatory DC2 state (ISG+ DCs) that activates CD8+ T cells
- $B2M^{-/-}$ ISG+ DCs acquire and present tumor-derived pMHC class I complexes
- Precluding MHC class I transfer to ISG+ DCs ablates T cell responses in $Batf3^{-/-}$ mice
- ISG+ DCs can be induced by exogenous IFN-β addition to drive anti-tumor immunity

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**In brief**

Tumor-infiltrating dendritic cells (DCs) are central to the anti-tumor immune response. Duong et al. reveal an activation state of CD11b+ conventional DCs (DC2) characterized by expression of interferon (IFN)-stimulated genes (ISG+ DCs) and capable of acquiring and presenting intact tumor-derived peptide-MHC class I complexes. ISG+ DCs can activate CD8+ T cells and promote protective anti-tumor immunity in the absence of DC1.
Type I interferon activates MHC class I-dressed CD11b+ conventional dendritic cells to promote protective anti-tumor CD8+ T cell immunity

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SUMMARY

Tumor-infiltrating dendritic cells (DCs) assume varied functional states that impact anti-tumor immunity. To delineate the DC states associated with productive anti-tumor T cell immunity, we compared spontaneously regressing and progressing tumors. Tumor-reactive CD8+ T cell responses in Batf3−/− mice lacking type 1 DCs (DC1s) were lost in progressor tumors but preserved in regressor tumors. Transcriptional profiling of intra-tumoral DCs within regressor tumors revealed an activation state of CD11b+ conventional DCs (DC2s) characterized by expression of interferon (IFN)-stimulated genes (ISGs) (ISG+ DCs). ISG+ DC-activated CD8+ T cells ex vivo comparably to DC1. Unlike cross-presenting DC1, ISG+ DCs acquired and presented intact tumor-derived peptide-major histocompatibility complex class I (MHC class I) complexes. Constitutive type I IFN production by regressor tumors drove the ISG+ DC state, and activation of MHC class I-dressed ISG+ DCs by exogenous IFN-β rescued anti-tumor immunity against progressor tumors in Batf3−/− mice. The ISG+ DC gene signature is detectable in human tumors. Engaging this functional DC state may present an approach for the treatment of human disease.

INTRODUCTION

Cytotoxic CD8+ T cell responses are critical for potent anti-tumor immunity (Fridman et al., 2012). The generation of tumor-specific CD8+ T cells (priming) occurs in the tumor-draining lymph node (tdLN) through interactions of naive T cells with dendritic cells (DCs) (Chen and Mellman, 2013). During these interactions, DCs present tumor antigens on major histocompatibility complex class I (MHC class I) and provide costimulation and cytokine signaling (Inaba et al., 1987).

The DC compartment is heterogeneous but has been defined as comprising conventional DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs can be further subdivided into two populations, CD8α+/CD11c DCs and CD11b+/Sirpα+ DCs, with distinct developmental requirements and functional specialization (Eisenbarth, 2019; Guilliams et al., 2014, 2016; Merad et al., 2013; Mildner and Jung, 2014; Murphy et al., 2016). DC1s require the transcription factors IRF8 and Batf3 for development and are adept at cross-presenting cell-associated antigens to CD8+ T cells (den Haan et al., 2000; Edelson et al., 2010; Hildner et al., 2008; Lyoda et al., 2002; Schulz and Reis e Sousa, 2002; Tamura et al., 2005). In contrast, DC2s are driven by IRF4 and are more potent at stimulating CD4+ T cells (Gao et al., 2013; Krishnaswamy et al., 2017; Tamura et al., 2005; Tussiwand et al., 2015; Williams et al., 2013). The inclusion of monocytes that are recruited to inflammatory sites and differentiate into DC-like cells (moDCs) has further increased the diversity of the DC compartment (Briseno et al., 2016; Leon et al., 2007; Mezenezes et al., 2016; Serbina et al., 2003).

Recent studies indicate that these DC subsets are generally conserved across species (Gerhard et al., 2021; Zilionis et al., 2019) and can be found in solid tumors (Broz et al., 2014; Laoui et al., 2016). Different tumor types harbor distinct compositions of DCs (Laoui et al., 2016) that can impact the resultant anti-tumor T cell response. In murine tumor models, DC1s are regarded as the most critical DC subset driving anti-tumor immunity given their specialized ability to cross-present antigens (Broz et al., 2014; Hildner et al., 2008; Roberts et al., 2016). Accordingly, tumors with a greater DC1 infiltrate tend to be better controlled (Salmon et al., 2016; Spranger et al., 2015), and the presence of the DC1 signature in patient tumors is associated with the response to immunotherapy (Barry et al., 2018; Boettcher et al., 2018; Broz et al., 2014; Michea et al., 2018).

However, there is increasing evidence that tumor-infiltrating DCs can exist in distinct functional states with tremendous implications for the anti-tumor immune response. It was recently reported that activated DC1s in lung tumors (mregDCs) expressed an immunoregulatory program that dampened their ability to...
activate T cells (Maier et al., 2020). While progress has been made in understanding the role and function of DC1s, the contributions of other DCs to anti-tumor immunity remain poorly described. Notably, some reports demonstrate that under specific therapeutic settings, DC subsets distinct from DC1s can be harnessed to enhance anti-tumor T cell immunity and calls for more nuanced investigation into the functional DC states driving anti-tumor immunity. In this study, we aimed to dissect the contributions of distinct DC states and their influence on anti-tumor T cell responses during a productive or dysfunctional anti-tumor immune response. By comparing the DC compartment of a spontaneously regressing tumor and a progressing tumor, we identified an activation signature (ISG+ DCs) that was enriched in regressor tumors and a progressing tumor, we identified an activation

**RESULTS**

**The regression of MC57-SIY tumors is independent of Batf3-driven DC1s**

To identify functionally relevant DC states associated with productive anti-tumor immune responses, we established a comparative model system of a spontaneously regressor tumor (MC57-SIY fibrosarcoma) and a progressively growing tumor (MC38-SIY colon carcinoma) (Figure 1A), both expressing the model antigen SIYRYGGL (SIY).

Our initial analysis focused on the CDC compartment given its reported impact on anti-tumor immunity (Broz et al., 2014; Roberts et al., 2016; Spranger et al., 2015, 2017). At day 7 after tumor inoculation, we detected a greater proportion of CD11b+ DC1s in MC57-SIY tumors, whereas the DC compartment was skewed toward CD11b+ DC2s in MC38-SIY tumors (Figures 1B and 1C). This phenotype was conserved in Rag2−/− mice. Data were pooled from two independent experiments (n = 3–5 mice/group; three independent repeats). (H) Quantification of SIY-specific CD8+ T cells in tumors at day 7 after tumor implantation in Batf3−/− mice. Data were pooled from two independent experiments (n = 3–5 mice/group). Data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant; Mann-Whitney U (MWU) test (C, F, H, and I) or two-way ANOVA (A, D, and G).

To determine whether DC1s were necessary for tumor rejection, we implanted both cell lines into Batf3−/− mice lacking DC1s (Hildner et al., 2008). Consistent with published data (Broz et al., 2014; Hildner et al., 2008), growth of MC38-SIY tumors in Batf3−/− mice was accelerated compared to WT mice (Figure 1G). In contrast, MC57-SIY tumors were rejected in Batf3−/− mice with similar kinetics as in WT mice (Figure 1G), suggesting that regression was independent of DC1s. This notion was supported by the observation that only MC57-SIY tumors harbored SIY-specific and granzyme B-expressing T cells (Figures 1H and S1D–S1G). Furthermore, while systemic anti-tumor
T cell responses against MC38-SIY tumors were completely ablated in Batf3−/− mice (Figure 1), those against MC57-SIY tumors were preserved in Batf3−/− mice but reduced by 58% when compared to responses in WT mice (Figure 1). These data indicate that DC1s are not the sole drivers of anti-tumor CD8+ T cell responses in MC57-SIY tumors.

DC1s selectively express Clec9a, and signaling through this receptor promotes the cross-presentation of dead cell-associated antigens (Sancho et al., 2008, 2009; Zelenay et al., 2012; Zhang et al., 2012). Using Clec9a−/− mice, we affirmed that Clec9a-mediated cross-presentation by DC1s is not required for the rejection of MC57-SIY tumors (Figure S1H). One possibility bypassing the need for cross-presentation is tumor control by CD4+ T cells (Mumberg et al., 1999). To assess whether regression of MC57-SIY is dependent on CD8+ or CD4+ T cells, we depleted each T cell subset alone or in combination and identified that tumor control was driven by CD8+ T cells (Figure S1I). These data indicate that cross-priming of CD8+ T cells is an essential component of anti-tumor immunity but that in certain contexts it can be induced independent of cross-presenting DC1s.

Functional assays and scRNA-seq identify a DC cluster characterized by an IFN-I gene signature in MC57-SIY tumors

Next we aimed to identify the antigen-presenting cell (APC) type(s) mediating the induction of protective immunity against MC57-SIY tumors in Batf3−/− mice. We established a functional ex vivo co-culture assay using naive SIY-reactive 2C T cell receptor (TCR) transgenic T cells (Figure 2A). Myeloid APCs were sorted from MC57-SIY tumors in WT and Batf3−/− mice and co-cultured with dye-labeled 2C T cells. In this assay, T cell activation was solely dependent on spontaneous antigen presentation by APCs in vivo, as no exogenous SIY peptide was added. In both WT and Batf3−/− settings, only CD11c+ DCs, but not Ly6C+ monocytes or F4/80+ macrophages, were able to induce 2C T cell proliferation (Figures 2B and 2C). In the Batf3−/− setting, this implied the presence of stimulatory DCs in the tumor that were distinct from DC1s. To confirm the requirement of CD11c+ DCs for control of MC57-SIY tumors, we generated Itgax−diphtheria toxin receptor bone marrow chimeras (Itgax-DTR BMCs), a model where all CD11c+ (encoded by Itgax) cells expressed DTRs. Specific depletion of CD11c+ cells via DT administration completely abrogated anti-tumor CD8+ T cell responses against MC57-SIY tumors (Figure 2D), which confirmed an absolute requirement for CD11c+ DCs for anti-tumor immunity. To definitively confirm that bona fide DCs are required for anti-tumor immunity against MC57-SIY tumors, we also evaluated the immune response in zDC-DTR BMCs, a mouse model where only Zbtb46-dependent cDCs expressed DTRs (Meredith et al., 2012). Consistent with our observations in Itgax-DTR BMCs, selective depletion of cDCs completely ablated functional tumor-reactive T cell responses against MC57-SIY tumors (Figures 2E, S2A, and S2B). Collectively, these data provided a strong rationale for narrowing our search for stimulatory cells within the intratumoral DC compartment.

To identify the relevant DC states driving anti-tumor immunity, we performed single-cell RNA-sequencing (scRNA-seq) of the CD45+ immune infiltrate of regressor MC57-SIY tumors in Rag2−/− mice. The use of Rag2−/− mice improved cell yield, as MC57-SIY tumors could grow progressively in these mice. Based on expression of a canonical DC signature (H2-Ab1, Ifi33, Ifgax) (Figure 2F) and absence of marker genes corresponding to other lineages (Figure S2C; Table S1), we identified a global DC cluster that was computationally isolated and further subclustered (Figure S2D). We detected a contaminating macrophage cluster that was excluded from a second round of filtering (Figures S2D and S2E). These analyses led to the identification of seven distinct DC clusters (Figures 2F and 2G). By mapping the differentially expressed genes (DEGs) of each DC cluster to the literature (Guilliams et al., 2016; Merad et al., 2013; Mildner and Jung, 2014; Murphy et al., 2016; Zilionis et al., 2019), we identified several classically described subsets: DC1s, migratory DCs, DC2s, moDCs, and two distinct pDC clusters (Figure 2G; Table S2). To validate these assigned cluster identities, we scored the cells in our dataset for their expression of published DC subset-specific signatures and found that our assignments agreed with the published signatures (Table S3; Figure S2F) (Zilionis et al., 2019). Intriguingly, our DEG analysis identified one cluster, c2, that was enriched in ISGs for which we did not observe a comparable counterpart in the published DC signatures, although some cells in this cluster expressed a DC2 signature (Figure S2F). Based on its ISG expression, c2 likely represents an IFN-γ-induced activation state, and we refer to this cluster as ISG+ DCs. Of note, a recent study identified an inflammatory cDC2 state (Inf-cDC2s) that was induced by IFN-I during viral infection (Bosteels et al., 2020), and the Inf-cDC2 signature (generated in-house) was enriched in the ISG+ DC cluster (Figures S2G and S2H; Table S3). The similarity in their transcriptional profiles might suggest that ISG+ DCs and Inf-cDC2s are related activation states; however, direct comparative studies are needed to confirm this notion.

To determine whether induction of the ISG+ DC state was required for anti-tumor immunity against our regressor model, we implanted MC57-SIY cells into Ifnar1−/− mice, wherein host cells are deficient in IFN-I sensing. We observed failed tumor control (Figure 2H), as well as reduced tumor-reactive T cell responses by IFN-γ enzyme-linked immunospot (ELISpot) (Figure S2I), thus confirming the necessity of host IFNAR signaling for anti-tumor immunity. Previous studies have shown that T cell activation can be impacted by defects in T cell-intrinsic IFN-I sensing (Hervas-Stubbs et al., 2011; Huber and Farrar, 2011). To determine whether IFN-I sensing is specifically required in the CD11c+ DC compartment, we generated Itgax-DTR:Ifnar1−/− mixed BMCs using WT or Ifnar1−/− hosts (Figure 2I). DT administration in this model specifically ablated IFN-I sensing in the CD11c+ DC compartment, while other immune compartments were unperturbed. We observed significant reductions in the systemic anti-tumor T cell response when DCs lacked the ability to sense IFN-I (Figure 2J). These data indicate that DC-intrinsic IFN-I sensing is required for induction of a potent anti-tumor T cell response, thus confirming a role for the ISG+ DC state in anti-tumor immunity.

ISG+ DCs are present in Batf3−/− mice and comprise an activation state of CD11b+ DCs

To study the ISG+ DCs, we identified Axl (Schmid et al., 2016) as a surface-expressed marker that differentiated the ISG+ DC
Figure 2. Functional assays and scRNA-seq identify a DC cluster characterized by an IFN-I gene signature in MC57-SIY tumors
(A) Experimental design for (B) and (C).
(B and C) Percentage of 2C T cell proliferation after co-culture with tumor-sorted APCs in WT (B) or Batf3−/− (C) mice at day 5 after tumor inoculation (n = 5 mice/experiment; two independent repeats).
(D) ELISpot of IFN-γ-producing splenocytes from DT-treated or PBS-treated Itgax-DTR BMC mice at day 5 after tumor inoculation. Data were pooled from two independent experiments (n = 3 mice/group).
(E) Number of SIY-reactive tumor-infiltrating lymphocytes (TILs) in tumors from cDC-depleted (zcDC-DTR) or non-depleted (WT) BMC mice at day 7 after tumor inoculation (n = 5 mice/group).
(F) (Top) UMAP plot of cells from MC57-SIY tumors colored by expression module score of a DC signature. (Bottom) UMAP plot of cells contained in the highlighted DC cluster.
(G) Heatmap of top 15 DEGs for each DC cluster identified in (F).
(H) Tumor outgrowth (mm²) in WT or Ifnar1−/− mice (n = 3–4 mice/group; three independent experiments).
(I) Experimental design for (J).
(J) ELISpot of IFN-γ-producing splenocytes from Itgax-DTR:Ifnar1−/− mixed BMC mice (WT hosts, left; Ifnar1−/− hosts, right) at day 7 after tumor inoculation. Data were pooled from two independent experiments (n = 2–3 mice/group).

Data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: MWU test (B–E and J) or two-way ANOVA (H).
Figure 3. ISG+ DCs are present in Batf3−/− mice and comprise an activation state of CD11b+ DCs

(A) Violin plots of expression in DC clusters.

(B and C) Flow gating strategy for APCs and DCs in WT (B) or Batf3−/− (C) mice, pre-gated on live CD45+CD19−CD3e−NK1.1− cells.

(legend continued on next page)
cluster from other DC clusters (Figure 3A; Table S4). As the ISG+ DC cluster also expressed Itgam encoding CD11b (Figure 3A), we used the co-expression of AXL and CD11b to identify ISG+ DCs. Given that AXL can be expressed on other immune cell types, we took measures in our gating strategy to ensure a specific definition of DCs (Figures 3B, 3C, and S3A). This gating strategy enabled us to detect the presence of ISG+ DCs in MC57-SIY tumors in WT and Rag2−/− mice, and, importantly, in Batf3−/− mice (Figures 3B–3I and S3A).

As ISG+ DCs were originally identified in MC57-SIY tumors from Rag2−/− mice, we sought to confirm that ISG+ DCs from both immunocompetent and Rag2−/− mice expressed similar transcriptional signatures using bulk RNA-seq (Figure 3J; Table S3). Cells from the scRNA-seq dataset that scored highly for either the Rag2−/− ISG+ DC or DC1 signatures were significantly enriched (p = 3.22 × 10−5) in their corresponding clusters of our scRNA-seq dataset (Figures 3K, 3L, S3B, and S3C), which validates our flow panel and gating strategy. Cells that scored highly for the WT ISG+ DC signature were also significantly enriched (p = 7.79 × 10−5) in the c2 ISG+ DC scRNA-seq cluster (Figures 3K and 3L). In a pairwise analysis against other clusters, the c2 ISG+ DC cluster consistently scored higher for both the Rag2−/− and WT ISG+ DC signatures (Figure S3D) with p ≤ 7.47 × 10−7, further confirming significant enrichment for the Rag2−/− ISG+ DC signature (adjusted p [p-adj] ≤ 0.05; log2 FC (fold change) cut-off = 1) in the WT ISG+ DC signature by gene set enrichment analysis (GSEA) (Mootha et al., 2003; Subramanian et al., 2005) (Figure 3M). These data enabled us to conclude that ISG+ DCs isolated from immunocompetent and Rag2−/− mice shared similar transcriptional profiles with each other and with the ISG+ DCs originally identified from scRNA-seq.

To phenotypically characterize ISG+ DCs, we assessed expression of myeloid markers. Given the difficulty of distinguishing DC2s from moDCs by flow cytometry (Guilliams et al., 2014; Merad et al., 2013), we refer to them collectively as DC2/moDCs. Consistent with our sequencing analyses, ISG+ DCs were phenotypically distinct from DC1s, migratory DCs, and pDCs, lacking expression of CD24 and CD103, CCR7, and Siglec H, respectively (Figures 3N and S3E). Rather, they more closely resembled DC2/moDCs, expressing high levels of CD11b and Sirpα (Figures 3N and S3E). Given their transcriptional similarity to Inf-cDC2s (Figures S2G and S2H), we assessed the expression of Inf-cDC2 markers and observed that ISG+ DCs also expressed CD64 and MAR-1, as well as the DC-specific marker CD26 (Figure 3N). This observation suggests that ISG+ DCs might comprise a specific activation state of DC2s. To confirm the ontogenicity of ISG+ DCs, we performed a fate-mapping experiment wherein we transferred sorted CD45.1+ granulocyte-macrophage progenitors (GMPs) or precursor (pre-)DCs into MC57-SIY tumors and assessed their fates at day 3 post-transfer (Figures S3F and S3G). Only the transferred pre-DCs but not GMPs gave rise to ISG+ DCs (Figures S3H–S3L). We further affirmed this observation using zDC-DTR BMC mice. Selective depletion of cDCs via DT administration resulted in an 83% reduction of ISG+ DC numbers (Figures S3M and S3N), confirming their ontogenicity as cDCs. To probe whether the ISG+ DC state encompassed DC2s, we used the Irf4f/fxItgaxCre mouse model and validated depletion of splenic DC2s (Figures S3O and S3P). We observed a 62% reduction in ISG+ DCs infiltrating MC57-SIY tumors in Irf4f/fxItgaxCre mice compared to littermate controls (Figures 3O and 3P). The remaining ISG+ DCs in Irf4f/fxItgaxCre mice are likely attributable to incomplete Cre recombination efficiency, although IRF4-independent DC2s may also contribute to the ISG+ DC cluster. Thus, these results indicate that most ISG+ DCs are indeed IRF4-driven DC2s.

Importantly, using a recent resource publication on human tumor-infiltrating myeloid cells (Cheng et al., 2021), we observed that cells scoring highly for the ISG+ DC signature were significantly enriched (p = 3.86 × 10−3) in the c5_cDC2_IRF15 cluster (Figure 3Q), indicating that an ISG+ DC-like population can be found in human tumors. Furthermore, the c5_cDC2_IRF15 cluster was significantly enriched for the ISG+ DC signature (p ≤ 1.57 × 10−26) in a pairwise comparison against other clusters. In our re-analysis of the Cheng et al. (2021) dataset restricted to tumor-derived cells, we also observed a similar enrichment (p = 3.04 × 10−18) in the c5_cDC2_IRF15 cluster (Figures 3R and S3Q). It is noteworthy that most cells in Cheng et al. (2021) c5_cDC2_IRF15 cluster were derived from a single patient with renal cell carcinoma, which is a relatively immunogenic cancer type (Heidegger et al., 2019). As we also identified ISG+ DCs in the immunogenic MC57-SIY tumor, it is conceivable that the presence of ISG+ DCs may be restricted to highly immunogenic tumors. This notion might explain why they are not detected in...
most of the previously published human tumor scRNA-seq data-sets. Nonetheless, the observation of the ISG+ DC signature in a cluster of human tumor-infiltrating DC2s indicates that they may contribute to anti-tumor immunity against human tumors.

**ISG+ DCs acquire and present tumor antigens by MHC class I dressing**

To elucidate whether ISG+ DCs were capable of activating CD8+ T cells, we evaluated their stimulatory ability using the *ex vivo* co-culture assay (Figure 4A). ISG+ DCs induced similar levels of 2C T cell expansion as DC1s and to a significantly higher degree than DC2/moDCs (Figure 4B). This observation suggests that in MC57-SIY tumors, the stimulatory DC fraction primarily comprises DC1s and ISG+ DCs. As it is well-established that DC1s excel at cross-presenting cell-associated antigens to prime CD8+ T cells compared to DC2s and other subsets (Broz et al., 2014; Edelson et al., 2010; Hildner et al., 2008; Iyoda et al., 2002), our observations from the co-culture assays prompted us to interrogate the mechanism of antigen presentation used by ISG+ DCs. Two routes of cross-priming of CD8+ T cells have been reported: (1) cross-presentation of exogenously derived antigens (i.e., dead cell debris) and (2) MHC class I dressing, wherein DCs acquire and display intact pMHC class I complexes derived from adjacent cells (Embgenbroich and Burgdorf, 2018). Studies initially described the phenomenon of MHC class I-dressing between virally infected and non-infected DCs *in vivo* (Wakim and Bevan, 2011), but there is increasing evidence for MHC class I dressing as a means of antigen presentation in the tumor context (Das Mohapatra et al., 2020; Nakayama et al., 2021; Squadrito et al., 2018).

We have thus far demonstrated that systemic anti-tumor T cell responses against MC57-SIY tumors are preserved in DC1-deficient *Batf3*−/− mice (Figures 1I and 4C and 4D, #1 and 2). As the only other major stimulatory DC in the tumor (Figure 4B), ISG+ DCs likely drive these responses in *Batf3*−/− mice. Therefore, by using the T cell response in *Batf3*−/− mice as a readout of activation by ISG+ DCs, we could infer their specific mode of antigen presentation. To probe whether the preserved T cell responses in *Batf3*−/− mice were attributable to MHC class I dressing, we generated MC57-SIY tumor cells lacking MHC class I expression by CRISPR-Cas9-mediated deletion of *B2M* encoding the β2-microglobulin (β2M) subunit. We validated outgrowth of this line in WT mice (Figure S4A). In contrast to MC57-SIY, implantation of MC57-SIY-*B2M*−/− into *Batf3*−/− mice led to complete loss of systemic anti-tumor T cell responses by IFN-γ ELISpot (Figures 4C and 4D, #3). This observation suggested that ISG+ DCs failed to induce a T cell response when they were precluded from acquiring pMHC class I complexes from the tumor. One possible...
alternative explanation is the contribution from direct priming by the tumor cells themselves; however, our previous data demonstrating complete ablation of anti-tumor T cell responses in Itgax- DTR BMC and zDC-DTR BMC mice effectively excluded this possibility (Figures 2D, 2E, S2A, and S2B). As an additional control to ensure that antigen from MC57-SIY-B2M/-/- cells could be cross-presented in a WT host, we implanted MC57-SIY-B2M/-/- cells into WT mice and indeed observed induction of a systemic T cell response (Figures 4C and 4D, #4). Taken together, these data led us to hypothesize that ISG+ DCs were activating CD8+ T cells by MHC class I dressing with tumor-derived pMHC class I complexes.

We used several complementary approaches to validate that ISG+ DCs were indeed capable of MHC class I dressing. We generated B2M/-/- BMC mice wherein the host immune cells lacked MHC class I molecules (Figures 4E and S4B). Here, cross-presentation is not possible due to the lack of host MHC class I, and therefore CD8+ T cell activation is dependent on MHC class I dressing by DCs. Implanting MC57-SIY cells into B2M/-/- BMC mice and profiling the tumor-infiltrating DCs, we detected the highest levels of tumor-derived H-2Kb complexes on the surface of B2M/-/- ISG+ DCs compared to other DC subsets (Figures 4F and 4G). Furthermore, the systemic anti-tumor T cell response in B2M/-/- BMC mice was comparable to the response in WT BMC mice (Figure 4H), thus providing additional evidence for the contribution of MHC class I-dressed ISG+ DCs to anti-tumor immunity.

To affirm that ISG+ DCs are MHC class I dressing, we established a complementary in vivo transfer assay in which we implanted the MC57-SIY tumor line (H-2b) into MHC class I haplotype mismatched BALB/c mice (H-2d) (Figure 5A). For these experiments, MC57-SIY cells were engineered to express the ovalbumin-derived model antigen SIINFEKL (SIIN) to allow detection of transferred tumor-derived H-2Kb:SIIN complexes to BALB/c DCs using the antibody 25-D1.16 (Porgador et al., 1997). Specificity of the 25-D1.16 antibody was validated using
Figure 6. IFNAR signaling in the MC57-SIY TME drives ISG+ DC activation
(A) Number of ISG+ DCs in tumors at day 11 following implantation in Rag2<sup>−/−</sup> mice. Data were pooled from two independent experiments (n = 2–3 mice/group).
(B) Experimental design for (C).
(C) (Left) Replication index of 2C T cells after co-culture with tumor-sorted DCs from Rag2<sup>−/−</sup> mice at day 11 after tumor inoculation. Data were pooled from two independent experiments (n = 5 mice/experiment). (Right) Representative example.
(D) Representative tumor outgrowth (mm<sup>2</sup>) in WT or Sting1<sup>−/−</sup> mice (n = 3–5 mice/group; three independent repeats).
(E) Relative expression of Ifnb1, Irf7, and Isg15 in tumor cells. Data were pooled from Ifnb1 (n = 6), Irf7 (n = 7), and Isg15 (n = 3) independent experiments.
(F) Expression level of Ifnb1, Irf7, and Isg15 in BM-DCs that were unstimulated or cultured with tumor-conditioned media. Data were pooled from two independent experiments.
(G) Representative tumor outgrowth in Batf3<sup>−/−</sup> mice (n = 3–4 mice/group; three independent repeats).
(H) Representative tumor outgrowth in CD86<sup>−/−</sup> mice (n = 3–4 mice/group; three independent repeats).
(I) Representative tumor outgrowth in NSG mice (n = 3–4 mice/group; three independent repeats).
(J) Ratio of the gMFI values of CD86 expressed by CD45.1<sup>+</sup>:CD45.2<sup>+</sup> DC subsets. Data were pooled from two independent experiments (n = 5 mice/experiment).
an isotype control and a SIIN-negative tumor cell line (Figures 5B and 5C). Following implantation of MC57-SIIN-SIY cells into BALB/c mice, we detected the highest levels of tumor-derived H-2Kb:SIIN complexes on the surface of BALB/c ISG+ DCs, indicating that they are indeed most efficient at MHC class I dressing (Figures 5B and 5C). Of note, DC1s were able to acquire some appreciable amount of H-2Kb:SIIN complexes, but this was significantly lower compared to the levels on ISG+ DCs. Implanta-
tion of MC57 parental cells into BALB/c mice, the highest levels of H-2Kb complexes were again detected on the surface of BALB/c ISG+ DCs compared to other DC subsets (Figures S5A–S5C), showing that MHC class I dressing was independent of a model antigen. To visualize the transfer of tumor-derived pMHC class I complexes ex vivo, we sorted ISG+ DCs from BALB/c mice bearing parental MC57 tumors and co-cultured them ex vivo with MC57-SIIN-SIY tumor cells (Figure S5D). Using immunofluorescence microscopy, we confirmed the presence of tumor-derived H-2Kb:SIIN complexes on BALB/c ISG+ DCs, indicating they can MHC class I dress ex vivo (Figure S5E).

**MHC class I-dressed ISG+ DCs can induce protective systemic anti-tumor T cell immunity**

We next aimed to determine whether MHC class I dressing could activate CD8+ T cells. DC subsets were sorted from MC57-SIIN-SIY (H-2d) tumors in BALB/c mice (H-2b) and co-cultured with OTI TCR transgenic CD8+ T cells (Figure 5D). Due to the mismatched haplotypes in this co-culture system, OTI T cells can only be acti-
vated by MHC class I-dressed BALB/c DCs. Consistent with their higher degree of MHC class I dressing with H-2Kb:SIIN complexes (Figures 5B and 5C), BALB/c ISG+ DCs induced the greatest OTI T cell activation (Figures 5E and 5F). In contrast, OTI T cell activation by BALB/c DC1s and DC2/moDCs was weaker (Fig-
ures 5E and 5F) in accordance with the lower levels of H-2Kb:SIIN complexes on these DC subsets (Figures 5B and 5C).

To assess whether MHC class I-dressed ISG+ DCs could induce systemic immunity in the absence of DC1s, we performed contralateral flank experiments. MC57-SIY cells were implanted into the flank of Batf3<−/−> mice to initiate the anti-tumor immune response by ISG+ DCs. Six days later, we implanted secondary MC38-SIY cells on the contralateral flank and evaluated their outgrowth (Figure 5G). SIY-specific CD8+ T cells induced by ISG+ DCs were able to control the growth of MC38-SIY tumors, with a 90% decrease in average tumor burden compared to control at endpoint (Figure 5H). While none of the mice in the control group was tumor-free, 4 out of 15 mice from the MC57-SIY group had completely eradicated their MC38-SIY tumors. The eventual outgrowth of MC38-SIY tumors in all analyzed mice from the MC57-SIY group was due to loss of the shared SIY antigen (Figure S5F). Importantly, implanting MC57-SIY-B2M<−/−> tumor cells (lacking MHC class I for MHC class I dressing) in Batf3<−/−> mice (Figure 5I) completely failed to induce protective immunity, which enabled MC38-SIY tumors on the contralateral flank to grow similar to the control (Figure 5J). Collectively, these observations confirmed that MHC class I-dressed ISG+ DCs contribute to anti-tumor CD8+ T cell immunity.

**IFNAR signaling in the MC57-SIY tumor microenvironment (TME) drives ISG+ DC activation**

We next aimed to understand how the functional ISG+ DC state was induced. Our data demonstrated that while anti-tumor T cell responses in MC57-SIY tumors were driven by both DC1s and ISG+ DCs, those against MC38-SIY tumors were solely dependent on DC1s (Figures 1H and 1I). This observation prompted us to interrogate whether ISG+ DCs could be found in MC38-
SIY tumors. While ISG+ DCs could be detected, MC38-SIY tumors showed 5.6-fold higher numbers of ISG+ DCs compared to MC38-SIY tumors (Figure 6A). When DCs sorted from MC38-SIY tumors were evaluated in our ex vivo co-culture assay (Figure 6B), only DC1s and not ISG+ DCs or DC2/moDCs were stimulatory, which is consistent with our data indicating that T cell responses are fully dependent on DC1 (Figure 6C).

The strong IFN response signature characterizing ISG+ DCs (Figure 2G) indicates that they are likely sensing IFN-I. IFN-I pro-
teins are secreted by many cell types upon engagement of pattern recognition receptors (PRRs) (Fuertes et al., 2013; Musella et al., 2017; Zitvogel et al., 2015). In the tumor context, activation of STING-dependent cytosolic DNA sensing in innate immune cells is reported to be the predominant PRR that drives IFN-I production (Fuertes et al., 2013; Liu et al., 2018; Woo et al., 2014). To assess the contribution of the host STING pathway to anti-tumor immunity, we implanted MC57-SIY cells into Sting1<−/−> mice but found that the tumors still regressed with similar kinetics as in WT mice (Figure 6D). This observation suggests that the IFN-I response in MC57-SIY tumors likely does not derive from STING-activated immune cells.

Several studies have demonstrated that tumor cell-derived IFN-I can induce an inflamed tumor microenvironment (Musella et al., 2017; Trujillo et al., 2018; Zitvogel et al., 2019). Accord-
ingly, tumor cells can evolve to suppress cell-intrinsic IFNAR signaling to favor immune evasion (Albacker et al., 2017; Bidwell et al., 2012; Katlinskaya et al., 2016; Katlinski et al., 2017; Linsley et al., 2014). The differences in ISG+ DCs from MC57-SIY and MC38-SIY tumors together with our finding that anti-tumor immunity against MC57-SIY was STING-independent prompted us to interrogate whether differential tumor cell-intrinsic IFNAR signaling was a contributing factor. We analyzed the expression of IFN-I and ISG transcripts and observed that MC57-SIY cells expressed higher transcripts of Ifnb1, Irf7, and Isg15 compared to MC38-SIY cells at steady state, indicating constitutive IFNAR signaling (Figure 6E). As IFN-I is a secreted cytokine, we next determined whether the amount of IFN-I present in tumor cell-
conditioned media was enough to elicite a DC-intrinsic IFN-I response. To this end, we used WT bone marrow-derived DCs (BM-DCs) (Mayer et al., 2014) and stimulated them overnight with tumorconditioned media. Consistent with their higher expression of IFN-related transcripts, the MC57-SIY conditioned media strongly induced the expression of Ifnb1, Irf7, and Isg15 transcripts in BM-DCs, similar to levels induced by the STING agonist DMXAA (Figure 6F). In contrast, the expression in BM-
DCs cultured in MC38-SIY conditioned media was more comparable to unstimulated BM-DCs (Figure 6F). To confirm the role of

(K and L) Number of SIY-specific TILs (K) and ELISpot quantification of IFN-γ-producing splenocytes (L) from WT and Batf3<−/−> mice at day 7 after tumor im-
plantation. Data were pooled from three independent experiments (n = 3 mice/group).

Data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001: MWU test (A, C, E, F, H, and J–L) or two-way ANOVA (D and G).
tumor-derived IFN-I, we generated IRF3-deficient MC57-SIY cells using CRISPR-Cas9, which rendered them incapable of producing IFN-I (Figures S6A and S6B) (Sato et al., 2000; Tamura et al., 2008). Importantly, MC57-SIY-IRF3−/− tumors progressively in Batf3−/− mice, which was in contrast to MC57-SIY tumors (Figure 6G). As ISG+ DCs are the predominant stimulatory DCs in Batf3−/− mice, this observation suggests that tumor-derived IFN-I is critical for driving ISG+ DC function. It is possible that IFN-I may be required for the differentiation or recruitment of ISG+ DCs. When we analyzed DCs in MC57-SIY and MC57-SIY-IRF3−/− tumors, however, no significant differences in numbers or proportion for any DC subset were observed, including ISG+ DCs (Figures S6D and S6E). We validated these observations using Ifnar1−/− mice. Despite a trend toward reduced frequency of tumor-infiltrating DCs in Ifnar1−/− mice compared to WT mice (Figures S6F and S6G), it was not significant, indicating that DC differentiation or recruitment is likely not impacted by IFN-I. Alternatively, IFN-I might induce ISG+ DC maturation (Hervas-Stubbs et al., 2011). In MC57-SIY tumors, ISG+ DCs are the most mature DCs, expressing the highest levels of CD86, CD80, and CD40 (Figures 6H, 6I, and S6E). To determine whether their maturation state was driven by IFN-I, we generated WT:Ifnar1−/−/mixed BMC mice (Figure 6I). Indeed, the enhanced maturation of ISG+ DCs, and to a lesser degree DC1s and DC2/moDCs, was intrinsically dependent on IFNAR signaling, as indicated by higher expression of CD86 and MHC class II on cells derived from WT BM compared to Ifnar1−/− BM (Figures 6J, 6K, and S6K).

As costimulatory signaling is critical for successful T cell activation (Chen and Flies, 2013; Lenschow et al., 1996), we next assessed how impaired maturation of ISG+ DCs from MC57-SIY-IRF3−/− tumors impacted anti-tumor T cell responses. While anti-tumor T cell responses were still induced in WT mice, likely by DC1s, ISG+ DCs from MC57-SIY-IRF3−/− tumors failed to mount anti-tumor T cell responses in Batf3−/− mice (Figures 6K and 6L). Taken together, these data indicate that in MC57-SIY tumors ISG+ DCs are activated by tumor-derived IFN-I to drive anti-tumor CD8+ T cell responses.

Exogenous addition of IFN-β to progressor tumors restores anti-tumor T cell responses in Batf3−/− mice via activation of MHC class I-dressed ISG+ DCs

Our data suggest that tumor cell-derived IFN-I is a driving factor for the induction of the functional ISG+ DC state. To determine whether this observation was generalizable, we screened a panel of murine and human tumor lines for constitutive IFNAR signaling at baseline. While the vast majority of tumor lines did not exhibit spontaneous IFNAR signaling, a handful did express IFN-I and ISG transcripts at steady state, similar to MC57-SIY cells (Figures 7A, 7B, S7A, and S7B). We sorted DC subsets from such IFN-I/ISG-expressing tumor, the fibrosarcoma 1969-SIY, and evaluated them in our ex vivo co-culture assay (Figure S7C). Similar to our observations from MC57-SIY tumors, both DC1s and ISG+ DCs from 1969-SIY tumors induced robust 2C T cell proliferation (Figure S7D). Systemic anti-tumor T cell responses against 1969-SIY were also preserved in the absence of DC1 (Figure S7E). Thus, these data indicate that ISG+ DCs are contributors to anti-tumor T cell responses in tumors that constitutively produce IFN-I.

To determine whether MC57-SIY-derived IFN-I could drive the functional ISG+ DC state, we assessed how ablation of IFN-I production via IRF3 deletion would impact anti-tumor immunity. While we still observed rejection in WT mice that was likely mediated by DC1 (Figure S6C), MC57-SIY-IRF3−/− tumors grew progressively in Batf3−/− mice, which was in contrast to MC57-SIY tumors (Figure 6G). As ISG+ DCs are the predominant stimulatory DCs in Batf3−/− mice, this observation suggests that tumor-derived IFN-I is critical for driving ISG+ DC function. It is possible that IFN-I may be required for the differentiation or recruitment of ISG+ DCs. When we analyzed DCs in MC57-SIY and MC57-SIY-IRF3−/− tumors, however, no significant differences in numbers or proportion for any DC subset were observed, including ISG+ DCs (Figures S6D and S6E). We validated these observations using Ifnar1−/− mice. Despite a trend toward reduced frequency of tumor-infiltrating DCs in Ifnar1−/− mice compared to WT mice (Figures S6F and S6G), it was not significant, indicating that DC differentiation or recruitment is likely not impacted by IFN-I. Alternatively, IFN-I might induce ISG+ DC maturation (Hervas-Stubbs et al., 2011). In MC57-SIY tumors, ISG+ DCs are the most mature DCs, expressing the highest levels of CD86, CD80, and CD40 (Figures 6H, 6I, and S6E). To determine whether their maturation state was driven by IFN-I, we generated WT:Ifnar1−/−/mixed BMC mice (Figure 6I). Indeed, the enhanced maturation of ISG+ DCs, and to a lesser degree DC1s and DC2/moDCs, was intrinsically dependent on IFNAR signaling, as indicated by higher expression of CD86 and MHC class II on cells derived from WT BM compared to Ifnar1−/− BM (Figures 6J, 6K, and S6K).

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DISCUSSION

We identified a novel IFN-I-induced activation state of DC11b+ cDCs, which we called ISG+ DCs, that was capable of driving anti-tumor CD8+ T cell immunity by MHC class I dressing with tumor-derived pMHC class I complexes. The contribution of ISG+ DCs to anti-tumor immunity was best discerned using Batf3−/− mice. Whereas the absence of DC1 completely ablated anti-tumor CD8+ T cell responses against MC38-SIY tumors, those against MC57-SIY tumors were still induced and capable of driving tumor rejection in Batf3−/− mice. We provide evidence that ISG+ DCs were activated by IFNAR signaling in the TME of MC57-SIY tumors. Thus, we speculate that they are most relevant to the...
immune response in disease settings that trigger a strong IFN-I response, such as in viral infections. Importantly, ISG+ DCs could be induced by addition of exogenous IFN-β to drive anti-tumor CD8+ T cell responses in poorly immunogenic tumors lacking DC1.

Numerous studies have delved into elucidating tumor-intrinsic signaling pathways that suppress anti-tumor immune responses (Nguyen and Spranger, 2020; Spranger and Gajewski, 2018; Yang et al., 2019). We demonstrate in the present study that it is also critical to dissect tumor-intrinsic pathways that are immunostimulatory, as they may yield insights toward modulating the TME to promote productive anti-tumor immune responses. The study of spontaneously regressing tumors proves particularly useful in this context. The stark contrast in the anti-tumor immune response between MC38-SIY and MC57-SIY tumors in Batf3−/− mice was mediated by differential tumor cell-intrinsic IFNAR signaling at baseline. MC57-SIY tumor cells exhibited constitutive IFN-I production, whereas MC38-SIY tumor cells did not. The increased presence of IFN-I in the MC57-SIY TME was sufficient to drive the maturation and activation of stimulatory ISG+ DCs. Our screen of murine and human tumor lines revealed that constitutive IFNAR signaling in tumor cells at steady-state is a rather rare phenotype. Accordingly, this may be why the ISG+ DC state has not been widely described in the tumor context.

While we have yet to determine the upstream pathways triggering IFNAR signaling in regressor MC57-SIY tumors, several reports indicate that tumor-intrinsic IFN-I can be induced by the aberrant accumulation of intracellular double-stranded RNA (dsRNA) or cytosolic DNA in tumor cells (Ishizuka et al., 2019; Liu et al., 2019; Schadt et al., 2019; Takahashi et al., 2021). In line with the immunostimulatory effects of IFN-I, these tumors generated more inflamed microenvironments and were more sensitive to immunotherapy (Ishizuka et al., 2019; Liu et al., 2019; Schadt et al., 2019; Takahashi et al., 2021). Although IFN-I has been described to modulate multiple facets of the immune response, it is conceivable that ISG+ DCs activated by IFNAR signaling may contribute to anti-tumor CD8+ T cell responses in these settings. This observation is consistent with the recent report describing the induction of Inf-cDC2s in the context of an IFN-I response triggered by viral infection (Bosteels et al., 2020).

While direct comparative studies are needed to determine their degree of relatedness, ISG+ DCs expressed the Inf-cDC2 markers CD26, CD64, and MAR-1. The expression of CD64 and MAR-1 receptors on Inf-cDC2s was reported to be critical for their uptake of viral antigens in the form of immune complexes. While it is possible that CD64 and MAR-1 may also contribute to antigen uptake by ISG+ DCs via Fc receptor-mediated endocytosis, we demonstrate through several complementary experiments that the major mode of antigen presentation by ISG+ DCs occurs via MHC class I dressing. We offer a couple of lines of reasoning to suggest why ISG+ DCs are particularly adept at MHC class I dressing. First, the phenomenon of CD8+ T cell activation by MHC class I dressing depends on the acquisition of pMHC class I complexes from tumor cells. Thus, the
number of pMHC class I complexes expressed on tumor cells is inherently an important factor for whether MHC class I dressing occurs. As IFN-I is a positive regulator of MHC class I expression (Raval et al., 1998), it is conceivable that MCS7-SIY cells express higher levels of MHC class I, thereby increasing the probability of MHC class I dressing. Second, MHC class I dressing by DCs has been reported by Wakim and Bevan (2011) during viral infection, which is associated with strong IFN-I induction. The downstream effects of IFNAR signaling in ISG+ DCs might explain their enhanced ability to MHC class I dress. We identified and used AXL solely as a phenotypic marker for ISG+ DCs. However, given that AXL is IFN-inducible and has been reported to be an endocytic receptor (Schmid et al., 2016; Subramanian et al., 2014), it is plausible that AXL might be involved in MHC class I dressing.

It is increasingly important to delineate the individual contributions of distinct DC states to the anti-tumor immune response, as they may be non-redundant. Our work is another example that the functional dichotomy between DC1s and DC2s is not black and white and changes under inflammatory conditions, wherein DC2s and other CD11b+ cDCs can acquire the ability to activate CD8+ T cells. The differing modalities of antigen presentation used by DC1s and ISG+ DCs, cross-presentation and MHC class I dressing, respectively, can have major implications for the resultant anti-tumor T cell response. The density of pMHC class I complexes on DCs, for instance, has been described to impact memory and effector T cell responses (Bullock et al., 2003; Sykulev et al., 2012). It is conceivable that MHC class I dressing might yield a lower density of pMHC class I complexes on the surface of DCs compared to direct or cross-presentation, which would therefore influence T cell priming. ISG+ DCs also express higher levels of costimulatory molecules compared to DC1s, which can diversify T cell activation phenotypes. Additionally, the high expression of Cxcl10 (an IFN-induced gene) by ISG+ DCs identified through scRNA-seq suggests that they may contribute to T cell recruitment, which is a function that has recently been ascribed to tumor-resident DC1 (Spranger et al., 2017). Accordingly, it is of great interest to investigate the range of T cell responses induced by DC1s or ISG+ DCs and their impact on anti-tumor immunity.

The contribution of ISG+ DCs in the context of cancer therapy warrants further investigation. There is substantial evidence that the success of radiation therapy, chemotherapy, and immunotherapy is dependent on intact IFNAR signaling (Bunnett et al., 2011; Sistigu et al., 2014; Zaretsky et al., 2016). As ISG+ DCs are activated by IFN-I, they are likely relevant to the anti-tumor immune response induced by these therapies. Importantly, our data suggest that IFN-I does not have to derive from tumor cells per se. Rather, the total intratumoral IFN-I concentration appears to be a critical factor. This presents an opportunity for therapeutic intervention using tumor-localized IFN-β. It will be interesting to determine whether PRR agonists that drive strong IFN-I responses, such as RIG-I and STING agonists that are currently in clinical development (Iurescia et al., 2020; Le Naour et al., 2020), can also activate ISG+ DCs and enhance anti-tumor T cell responses. Our work suggests that these IFN-I-related therapies might be most effective in DC1-excluded tumors (Barry et al., 2018; Böttcher et al., 2018) or poorly immunogenic tumors with defective tumor cell-intrinsic IFNAR signaling (Kalbasi and Ribas, 2020; Zaretsky et al., 2016; Zitvogel et al., 2015). Taken together, our work broadens the current knowledge of functional DC states distinct from DC1s that are capable of driving anti-tumor CD8+ T cell responses.

**Limitations of study**

While our comparative model of a regressor and a progressor tumor facilitated the study of stimulatory DC states, it also has limitations. To broaden our findings, we screened additional murine tumor lines and found that our observations could be extended beyond our model system. Moving forward, it will be critical to assess for ISG+ DCs in additional murine tumor models and, more importantly, to establish the relevance of ISG+ DCs in human tumors. Nonetheless, our study demonstrates that IFN-β can be used to induce ISG+ DCs in poorly immunogenic murine tumors, and it will be interesting to determine whether this therapeutic implication holds true in the human setting.

**STAR METHODS**

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.immuni.2021.10.020.

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AUTHOR CONTRIBUTIONS

E.D. and S.S. conceptualized the study, designed experiments, and interpreted data. E.D. performed experiments, analyzed data, and wrote the manuscript. T.B.F. generated CRISPR-modified cell lines. T.B.F. and E.D. performed image analyses. E.L. generated recombinant IFN-β proteins. T.D. and L.Y. performed human and mouse tumor line screens and assisted with experiments. A.B., S.B., and E.D. performed computational analyses. E.D., T.B.F., T.D., E.L., and S.S. reviewed and edited the manuscript. S.S. acquired funding and supervised the study.

DECLARATION OF INTERESTS

The authors declare no competing interests. Part of this work contributed to the US patent 17/348,704 with E.D. and S.S. as inventors. S.S. is a co-founder of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and a consultant or SAB member of Arcus Biosciences, Draghi of Danger Bio and 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ment in renal cell cancer biology and therapy. Front. Oncol. 9, 490.


ment harbours ontogenically distinct dendritic cell populations with opposing effects on tumour immunity. Nat. Commun. 7, 13720.


León, B., López-Bravo, M., and Ardaun, C. (2007). Monocyte-derived dendritic cells formed at the infection site control the induction of protective T help-
er responses against Leishmania. Immunity 26, 519–531.


Sancho, D., Mourão-Sá, D., Joffre, O.P., Schulz, O., Rogers, N.C., Pennington, D.J., Carlyle, J.R., and Reis e Sousa, C. (2008). Tumor therapy in mice via antigens by CD8+ dendritic cells is attributable to their ability to internalize dead cells. Immunology 107, 183–189.


# STAR METHODS

## KEY RESOURCES TABLE

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**Critical commercial assays**

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**Deposited data**

| Murine scRNA-seq data (CD45+ cells in MC57-SIY tumors) | This paper | GSE181939 |
| Murine bulk RNA-seq data (sorted WT and Rag2⁻/⁻ DC subsets) | This paper | GSE181939 |

**Experimental models: Cell lines**

| MC57 parental | Thomas Gajewski Lab (UChicago) | N/A |
| MC57-SIY-GFP | Thomas Gajewski Lab (UChicago) | N/A |
| MC57-SIY-GFP-β2M⁻/⁻ | This paper | N/A |
| MC57-SIY-GFP-IRF3⁻/⁻ | This paper | N/A |
| MC57-cerulean-SILN-SIY | This paper | N/A |
| MC38 parental | Thomas Gajewski Lab (UChicago) | N/A |
| MC38-SIY-GFP | Thomas Gajewski Lab (UChicago) | N/A |
| MC38-SIY-GFP-β2M⁻/⁻ | This paper | N/A |
| 1969-SIY-GFP | Thomas Gajewski Lab (UChicago) | N/A |
| B16-SIY-dsRed | Thomas Gajewski Lab (UChicago) | N/A |

Please refer to Table S6 for cell lines used in mouse/human *Ifnb1* qPCR screens.

**Experimental models: Organisms/strains**

| Mouse: C57BL/6 | Taconic | Stock# B6-F |
| Mouse: BALB/c | Taconic | Stock# BALB-F |
| Mouse: Rag2⁻/⁻ | Taconic | Stock# RAGN12-F |
| Mouse: Batf3⁻/⁻ | Jackson Laboratory | Stock# 013755 |
| Mouse: β2M⁻/⁻ | Jackson Laboratory | Stock# 002087 |
| Mouse: CD11c<sup>Cre</sup> | Jackson Laboratory | Stock# 008068 |
| Mouse: CD11c-DTR | Jackson Laboratory | Stock# 004509 |
| Mouse: Clec9a⁻/⁻ | Jackson Laboratory | Stock# 017696 |
| Mouse: Ifnar1⁻/⁻ | Jackson Laboratory | Stock# 028288 |
| Mouse: Irf4<sup>Cre</sup> | Jackson Laboratory | Stock# 009380 |
| Mouse: 2C Rag2⁻/⁻ TCR-transgenic | Thomas Gajewski Lab (UChicago) | N/A |
| Mouse: OTI TCR-transgenic | Jackson Laboratory | Stock# 003831 |
| Mouse: zDC-DTR | Jason Cyster (UCSF) and Thorsten Mempel (Harvard/MGH) | Meredith et al., 2012 |

**Oligonucleotides**

Please refer to Table S5.

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Stefani Spranger (spranger@mit.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
All data is available in the main text or the supplementary materials. The RNA-seq data has been deposited to the GEO database (GSE181939). Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
C57BL/6, BALB/c, and Rag2−/− mice were purchased from Taconic Biosciences. Batf3−/−, B2M−/−, ItgaxCre, Itgax-DTR, Clec9a−/−, Ifnar1−/−, and Irf4f/f mice were purchased from Jackson Laboratories and bred in-house. Irf4f/f×CD11cCre mice were obtained by breeding Irf4f/f mice and ItgaxCre mice to specifically ablate IRF4 in the CD11c+ compartment. T cell receptor transgenic (TCR-tg) 2C Rag2−/− and OTI Rag2−/− mice were bred and maintained in-house. Zbtb46-DTR (zDC-DTR) mice were a gift from the Cyster Lab at UCSF and the Mempel Lab at Harvard/MGH. All mice were housed and bred under specific pathogen free (SPF) conditions at the Koch Institute animal facility. Ifnar1−/− mice were initially housed and bred at the Koch Biology Building animal facility. Following rederivation, Ifnar1−/− mice were bred and maintained at the Koch Institute animal facility. For experiments with Ifnar1−/− mice, only female mice 6-8 weeks old were used. For all other strains, mice were gender-matched and age-matched to be 6-12 weeks old at the time of experimentation. All experimental animal procedures were approved by the Committee on Animal Care (CAC/IA-CUC) at MIT.

Tumor cell lines
Parental and SIY-GFP expressing MC38 colon carcinoma, MC57 fibrosarcoma, 1969 fibrosarcoma, and B16 melanoma tumor cell lines were a gift from the Gajewski laboratory at The University of Chicago. Tumor cell lines were cultured at 37°C and 5% CO2 in DMEM (GIBCO) supplemented with 10% FBS (Atlanta Biologicals), 1% penicillin/streptomycin (GIBCO), and 1X HEPES (GIBCO). All cell lines were regularly subjected to mycoplasma testing.

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METHOD DETAILS

Generation of cerulean-SIIN-SIY expression vector
The pLV-EF1α-IRES-puro vector (Addgene #85132) was digested with BamHI and EcoRI restriction enzymes (NEB) to linearize the vector. The cerulean-SIIN-SIY insert was generated using the Cerulean-N1 vector (Addgene #54742) linked to a codon-optimized sequence of the SIINFEKL (SIIN) and SIYRYYG (SIY) peptides. The insert was then cloned into the linearized pLV-EF1α-IRES-puro vector (final construct referred to as pLV-EF1α-cerulean-SIIN-SIY-IRES-puro) using the In-Fusion cloning kit (Takara Bio), amplified, and sequenced for accuracy.

Generation of CRISPR knockout constructs
The px459-Cas9-puro vector (Addgene #62988) was digested with the BbsI restriction enzyme (NEB) to linearize the vector. CRISPR guides targeting exon 2 of murine β2-microglobulin (β2M) and exons 1-3 of Irf3 were designed using Benchling (Table S5). Forward and reverse oligos (Integrated DNA Technologies) for each guide were annealed together with a standard annealing protocol, cloned into the px459-Cas9-puro vector by T4 ligation (NEB), amplified, and sequenced for accuracy.

Generation of modified tumor cell lines
Parental and SIY-GFP expressing MC38 colon carcinoma, MC57 fibrosarcoma, and B16 melanoma tumor cell lines were a gift from the Gajewski laboratory at The University of Chicago. The MC57 tumor line stably expressing cerulean-SIIN-SIY was generated by lentiviral transduction of the parental tumor line with the pLV-EF1α-cerulean-SIIN-SIY-IRES-puro construct and puromycin (GIBCO) selected. Expression was confirmed using flow cytometry for cerulean-expressing cells. CRISPR-Cas9-mediated knockout tumor cell lines for B2M and Irf3 were generated by transient transfection with the pooled guide constructs and selected with puromycin for 48 hr. Cells surviving puromycin treatment were expanded, and the ablation of the target gene was confirmed by sequencing, qPCR, and/or western blot.

Tumor outgrowth studies
Tumor cells were harvested by trypsinization (GIBCO) and washed 3 times with 1X PBS (GIBCO). Cells were resuspended in PBS, and 2x10⁶ tumor cells were injected subcutaneously into the flanks of mice. Subcutaneous tumor area measurements (calculated as length x width) were collected 2-3 times a week using digital calipers until the endpoint of the study.

Generation of recombinant IFNβ
Murine Ifnb1 was cloned with C-terminal His-tags into the gWiz expression vector (Gelantis) using the In-Fusion HD cloning kit (Takara Bio). HEK293 cells were transfected with endotoxin free plasmid DNA (Macherey-Nagel) using OptiPRO serum-free media (GIBCO) and polyethylenimine 25K (Polysciences). Six days later, proteins were purified from filtered supernatant using TALON metal affinity resin (Takara Bio), eluted with PBS 200 mM imidazole, buffer exchanged into PBS, and sterile-filtered. IFNβ activity was confirmed using RAW-Lucia ISG Cells (InvivoGen).

In vivo IFNβ co-injection
For in vivo experiments involving IFNβ co-injection, 2x10⁶ tumor cells were resuspended with 50 μg IFNβ (generated as described above by the Wittrup Lab) in PBS and injected subcutaneously into the flanks of mice.

In vivo depletion of cytolytic cells
To deplete Natural Killer (NK) cells, 50 μg of anti-NK1.1 (Bio X Cell) or an isotype control antibody (Bio X Cell) was injected intraperitoneally 2 days prior to tumor implantation and subsequently every 3-4 days thereafter for the duration of the study. To deplete T cells, 200 μg of anti-CD8 (Bio X Cell), anti-CD4 (Bio X Cell), combined anti-CD8/anti-CD4, or an equal volume of PBS was injected intraperitoneally 2 days prior to tumor implantation, and 100 μg was subsequently injected every 3-4 days thereafter for the duration of the study.

IFNγ ELISpot
ELISpot plates (EMD Millipore) were coated overnight at 4°C with anti-IFNγ (BD Biosciences). Plates were washed and blocked with DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1X HEPES for 2 hr at room temperature (RT). Spleens were harvested from mice at day 5 or day 7 post-tumor inoculation and mashed through a 70 μm filter with a 1 mL syringe plunger to generate a single cell suspension. Red blood cells were lysed with 500 μL of ACK Lysing Buffer (GIBCO) on ice for 2 min and splenocytes were washed 3 times with chilled PBS. For IFNγ-ELISpot assays using SIY peptide restimulation, 1x10⁶ splenocytes were assayed per well in the presence or absence of 160 nM SIY peptide. For IFNγ-ELISpot assays using irradiated parental tumor cell debris for restimulation (Figures S7I–S7K), 3x10⁶ splenocytes were assayed per well in the presence or absence of 1x10⁶ parental tumor cells that were irradiated a day prior with 4000 rad. As a positive control, splenocytes were incubated with a mixture of 100 ng/mL PMA (Sigma-Aldrich) and 1 μg/mL ionomycin (Sigma-Aldrich). Following an overnight incubation at 37°C and 5% CO2, plates were developed using the BD mouse IFNγ-ELISpot kit, following manufacturer’s protocol.

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Generation of bone marrow (BM) chimeric mice

Host mice were injected with 500 rad, allowed to recover for 3 hr, and subsequently injected again with 550 rad. The next day, BM was harvested from the femur and tibia of donor mice, washed and resuspended in PBS, and 1x10^7 cells were injected retro-orbitally into the irradiated host mice. For mixed BM chimeras, 1x10^7 total cells of a 50:50 mixture of BM from donor mice was transferred. A period of 8 weeks was allowed for engraftment prior to the start of experiments.

DT-mediated depletion

For depletion of DC in Itgax-DTR, Itgax-DTR:Ifnar1^−/−, and zDC-DTR BM chimeras, 500 ng diphtheria toxin (DT) (Sigma-Aldrich) (or an equivalent volume of PBS for control mice) was injected intraperitoneally 2 days prior to tumor implantation and subsequently injected every other day thereafter for 7 days.

Tumor dissociation

Tumors were dissected from mice, weighed, and collected in 500 µL RPMI (GIBCO) containing 250 µg/ml Liberase (Sigma-Aldrich) and 50 µg/mL DNase (Sigma-Aldrich). Tumors were minced with dissection scissors and incubated for 20 min at 37° C for enzymatic digestion. Following the digestion, tumor pieces were washed through a 70 µm filter with a 1 mL syringe plunger to generate a single cell suspension. The dissociated cells were washed 3 times with chilled PBS containing 1% BFS and 2 mM EDTA (GIBCO).

Flow cytometry and cell sorting

Prior to staining, cells were washed with FACS staining buffer (chilled PBS containing 1% BFS and 2 mM EDTA). Cells were stained for 15 min on ice with eBioscience Fixable Viability Dye eFluor 780 to prevent non-specific antibody binding. Cells were then washed once and cell surface proteins were stained for 30 min on ice with fluorophore-conjugated antibodies at the specified dilutions (Table S5). For stains that used biotinylated primary antibodies, cells were washed twice and subsequently stained with a streptavidin–conjugated fluorophore for 30 min on ice. Following the surface staining, cells were washed twice and analyzed directly or fixed with IC Fixation Buffer (eBioscience) for 20 min at RT for analysis the next day. To obtain absolute counts of cells, Precision Count Beads BioLegend were added to samples following manufacturer’s instructions. Flow cytometry sample acquisition was performed on a BD LSRII Fortessa cytometer, and the collected data was analyzed using FlowJo v10.5.3 software (TreeStar). For cell sorting, the surface staining was performed as described above under sterile conditions, and cells were acquired and sorted into RPMI containing 10% FBS, 1% penicillin/streptomycin, and 1X HEPES using a BD FACSAria III sorter.

SIY-pentamer staining

To identify SIY-reactive CD8^+ T cells, samples were stained with a 1:100 dilution of a PE-conjugated SIY pentamer (ProImmune) for 30 min on ice. The pentamer was added during the surface staining step in the flow cytometry methods described above.

Progenitor transfer fate-mapping experiment

To expand pre-DC, CD45.1^+ C57BL/6 mice were injected every other day with 10 µg recombinant human Flt3L-Ig (Bio X Cell). BM was then harvested from the femur and tibia of mice by flushing the bones with RPMI using a 1 mL syringe. Cells were passed through a 70 µm filter, washed twice with PBS, and subjected to flow cytometry staining and sorting as described above. GMP were sorted as live CD45.1^+, lineage^− (CD19, CD3e, NK1.1, MHC-II), Sca-1^−, c-Kit^+, Flt3^−, CD16/32^+, CD11b^+, Ly6C^+. Pre-DC were sorted as live CD45.1^+, lineage^− (CD19, CD3e, NK1.1, MHC-II), Sca-1^−, c-Kit^−, CD16/32^+, Flt3^+, CD11c^+. Equal numbers of GMP or pre-DC were injected i.t. into MC57-SIY tumor-bearing CD45.2^+ Rag2^−/− host mice at day 11 post-tumor implantation. At 3 days post-transfer, MC57-SIY tumors were harvested and the fates of the transferred cells were analyzed by flow cytometry.

Staining for in vivo MHC class I dressing assay

Related to Figures 4E–4G, 5A–5C, and SSA–SSC. Surface flow staining for H-2K^b or H-2K^b:SIIN on the DC infiltrate of dissociated tumors was performed as follows: Cells were stained for 15 min on ice with eBioscience Fixable Viability Dye eFluor 780 and blocked with anti-CD16/CD32 (clone 93, BioLegend) to prevent non-specific antibody binding. Cells were then washed once and cell surface proteins were stained as described above. For detection of H-2K^b, PE-Cy7 anti-H-2K^b (clone AF6-88.5, BioLegend) was used at a 1:200 dilution. For detection of H-2K^b:SIIN, biotinylated anti-H-2K^b:SIIN (clone 25-D1.16, eBioscience) (Porzador et al., 1997) or a biotinylated isotype control (clone 2016875, eBioscience) was used at a 1:200 dilution, followed by a streptavidin-BV711 secondary at a 1:400 dilution. Specificity for H-2K^b or H-2K^b:SIIN staining was validated using a Fluorescence Minus One (FMO) control, an antigen-irrelevant tumor line, and/or an isotype control.

MHC class I dressing visualization by immunofluorescence microscopy

MC57-SIY (H-2^b) tumor cells were implanted into the flanks of WT BALB/c mice (H-2^d). On day 5 post-tumor inoculation, BALB/c ISG^+ tumors were dissected using a Fluorescence Minus One (FMO) control, an antigen-irrelevant tumor line, and/or an isotype control.
and 1X β-mercaptoethanol. After 24 hr, cells were fixed with 4% paraformaldehyde for 20 min at RT and gently permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) for 10 min at RT. Coverslips were then blocked with 2.5% bovine serum albumin (Research Products International) and anti-CD16/CD32 (clone 93, BioLegend) for 20 min at RT. Primary antibodies at a 1:200 or a 1:400 dilution (Table S4) were added to the coverslips and incubated for 1 hr at RT. Coverslips were then washed 3 times with Dulbecco’s PBS with calcium and magnesium (GIBCO) for 5 min each. Secondary antibodies (Table S5) were added at 1:400 dilution and incubated for 20 min at RT. Following washes, coverslips were mounted on glass slides using ProLong Gold Antifade with DAPI (Invitrogen). Slides were dried overnight, sealed with clear nail polish, and imaged using a Leica TCS SP8 confocal laser scanning microscope.

**Ex vivo APC/DC-T cell co-culture assay**

To obtain antigen-presenting cell compartments or specific DC subsets, cells were FACS-sorted from tumors as described above. To obtain CD8+ T cells, TCR transgenic CD8+ T cells were isolated from spleen and lymph nodes of naïve 2C or OTI TCR transgenic Rag2^{-/-} mice using a CD8+ T cell isolation kit (Miltenyi Biotec), following manufacturer’s instructions. Isolated CD8+ T cells were washed twice with PBS and stained with 2.5 µM CFSE (eBioscience) in PBS for 8 min at 37°C or 5 µM CellTrace Violet (Thermo Fisher Scientific) in PBS for 20 min at 37°C. The dye was then quenched with FBS, and the cells were washed 3 times with RPMI containing 10% FBS. For the co-culture, 5x10^5 dye-labeled TCR transgenic CD8+ T cells and 1x10^5 sorted antigen-presenting cells or DCs (5:1 DC:T cell ratio) were mixed and then added to wells of a V-bottom tissue culture-treated 96-well plate in RPMI supplemented with 10% FBS, 1% penicillin/streptomycin, 1X HEPES, 1X MEM Non-Essential Amino Acids (GIBCO), and 1X β-mercaptoethanol (GIBCO). The cells were cultured at 37°C and 5% CO2 for 72 hr at which point T cell proliferation was measured by dye dilution via flow cytometry as a proxy for T cell activation. To determine the replication indices per condition, gates for each individual proliferation peak were manually drawn in FlowJo to obtain cell counts per round of division. The replication indices per condition were then calculated as total number of divided cells / total number of cells that underwent division. Expression of T cell activation markers and cytokines was also assessed by flow staining as described.

**scRNA-seq and analysis**

Live intratumoral CD45+ cells from Rag2^{-/-} mice bearing MC57-SIY tumors at day 7 post-tumor implantation were FACS-sorted as described above. Sorted cells were washed twice and resuspended at a final concentration of 1x10^5 cells/µL in chilled PBS containing 0.04% BSA (Thermo Fisher Scientific). The cellular suspension was submitted to the Whitehead Institute Genome Technology Core for cDNA library preparation. Briefly, single cells were encapsulated into droplets using the 10X Genomics Chromium Controller, and the cDNA library was prepared using the Chromium Single Cell 3’ Reagent Kits v2 (10X Genomics) following manufacturer’s instructions. The resultant cDNA library was then sequenced by the MIT BioMicro Center using an Illumina HiSeq2000. Demultiplexing, mapping to the mm10 genome, and barcode and UMI counting were performed with 10X Genomics Cell Ranger v3.0.1, and the resultant count matrix was loaded into Seurat v3.2.2 (Butler et al., 2018) for further processing. Cells expressing less than 200 genes or more than 4500 genes, as well as cells expressing more than 25% mitochondrial transcripts were excluded, which left 6262 cells for downstream analysis. The data was normalized using the Seurat LogNormalize function with the default scale factor of 10^4. The data was then scaled using the Seurat ScaleData function and latent variables (number of UMIs and percentage of mitochondrial transcripts) were regressed out. The Seurat FindVariableGenes function was used to identify 2000 variable genes for principal component analysis (PCA). The Seurat FindClusters function, which implements the shared nearest neighbor (SNN) clustering algorithm, identified 15 clusters using the top 17 PCA components and a resolution of 0.8. The Seurat FindAllMarkers function was used to identify the differentially expressed genes (DEG) for each cluster compared to all other clusters with default parameters that required genes to be expressed in more than 25% of cells with a minimum 0.5-fold difference (Table S1). To identify clusters, we manually compared the DEG lists of our clusters to reports in the literature.

**scRNA-seq DC population analysis and ISG+ DC surface marker identification**

To examine DC at higher resolution, we computationally isolated cell clusters that expressed a canonical DC signature (H2-Ab1 and Itgax and Flt3) using the Seurat SubsetData function (clusters 4, 12, 13, 15). The initial analysis using Seurat identified a contaminating macrophage cluster expressing Adgre1, Mafb, and C5ar1 (cluster 3), which was subsequently excluded during another round of filtering. The remaining cells were then passed through the Seurat analysis pipeline as described above which led to the identification of seven DC clusters (711 cells) using 2000 variable genes, the top 12 PCA components, and a resolution of 0.8 (Table S2). To identify clusters, we manually cross-referenced the DEG lists of the DC clusters to the DC signatures recently reported in the literature (Giulliams et al., 2016; Merad et al., 2013; Mildner and Jung, 2014; Murphy et al., 2016; Zilionis et al., 2019). To validate our manual cluster assignments, we scored each cell in our dataset using the AddModuleScore function (Tirosh et al., 2016) for expression of DC subset gene signatures that were either published (Zilionis et al., 2019) or generated from an analysis of a publicly available dataset (GSM4505993) (Bosteels et al., 2020) (Table S3). To identify surface markers for cluster 2 (ISG+ DC) for downstream functional studies, we filtered the DEG list for cluster 2 and required that marker genes must (1) have a minimum avg_logFC threshold of 0.5; (2) have an adjusted p-value < 0.05; (3) be unique to cluster 2; (4) have an enrichment score < 0.5, defined as the ratio of percent expression in all other clusters (pct.2) versus percent expression in cluster 2 (pct.1), (5) be surface-expressed; and (6) have a commercially available antibody (Table S4).
Generation of Bosteels et al. DC signatures
To generate the Bosteels DC signatures for non-mig. cDC2 and Inf-cDC2, we downloaded the “CD45.1 WT derived cells from WT:WT chimeric mice” dataset from the Gene Expression Omnibus database under accession number GSM4505993 (Bosteels et al., 2020). The dataset was analyzed with the Seurat package as described above, using the top 15 PCA components and a resolution of 0.4 to cluster the cells. Cluster identities were assigned by cross-referencing DEG from each cluster with the published marker genes (Bosteels et al., 2020). The top 20 DEG for non-mig. cDC2 and Inf-cDC2 (Table S3) were used to generate the signatures used in the AddModuleScore analysis.

Bulk RNA-seq and analysis
ISG+ DC and CD103+ DC1 were FACS-sorted from MC57-SIY tumors in WT and Rag2-/- mice at day 7 post-implantation as described above using the gating strategies shown in Figures 3B and S3B. Cells were sorted directly into TRIzol (Invitrogen), and RNA was isolated using a TRIzol-chloroform extraction. The RNA-containing aqueous layer was collected, purified using the RNeasy MinElute Cleanup Kit (QIAGEN) following manufacturer’s instructions, and submitted to the MIT BioMicro Center for library preparation (Clontech ZapR) and sequencing (Illumina NextSeq500). Paired-ended 38-mer RNA-seq reads were pre-processed to trim five low-quality read positions from the second read (R2) of each pair, using the FASTX-Toolkit (Hannon Lab, CSHL). Reads were then mapped to the USCC mm9 mouse genome build (genome.ucsc.edu) using Bowtie v1.2.3 (Langmead et al., 2009) and gene counts were quantified using RSEM v1.3.1 (Li and Dewey, 2011). Estimated expression counts generated by RSEM were used to detect differentially expressed (DE) genes (p-adj \leq 0.05) between pairwise conditions (Rag2-/- ISG+ DC versus Rag2-/- DC1; WT ISG+ DC versus Rag2-/- DC1) using DESeq2 v1.26.0 (Love et al., 2014) with a 2X fold-change cutoff per comparison (Table S3). Pairwise signature enrichment analysis was conducted using the pre-ranked mode in GSEA v4.1.0 (Subramanian et al., 2005). The AddModuleScore function (Tirosh et al., 2016) in Seurat (Butler et al., 2018) was used to score cells from the scRNA-seq dataset for enrichment of the bulk RNA-seq-derived DC signatures. Each cell in the scRNA-seq UMAP plot was then colored by its enrichment score for the bulk RNA-seq signatures.

Re-analysis of dataset from Cheng et al. (Cell 2021)
A normalized expression matrix of scRNA-seq counts for the cDC2 dataset was retrieved from GEO (accession GSE154763) along with associated metadata per cell and processed with Seurat v3.2 (Stuart et al., 2019). A Seurat object was created such that the counts slot and data slot were populated with the library-size corrected counts and log-transformed normalized counts, respectively. The dataset was filtered (based on metadata annotation) to retain only cells from tumor samples. Only twenty patient samples with over 30 cells were selected and used for downstream analyses. Seurat’s reference-based integration approach (Stuart et al., 2019) was used to hierarchically integrate samples, first per patient and then across patients. Dimensionality reduction with PCA and UMAP embeddings (with the top 35 principal components) were generated using the integrated dataset. The Seurat default “RNA” assay with log-transformed normalized counts was used for expression-based analyses.

Statistical tests for enrichment of external signatures in scRNA-seq clusters
Related to Figures 3L, 3R, S3C, and S3D. Statistical significance for the enrichment of high-scoring cells (standardized module score: > 2 for bulk RNA-seq signatures, > 2.5 for ISG+ DC scRNA-seq signature) was assessed using an upper-tailed hypergeometric test of proportions (phyper, Stats R package; alpha = 0.05). To compare signature module scores for all cells within a given cluster with cells in every other cluster in a pairwise fashion, a two-sided Wilcoxon rank sum test (wilcox.test, Stats R package; alpha = 0.05) was used.

Collection of tumor-conditioned media
Tumor-conditioned media was collected when flasks containing MC38-SIY, MC57-SIY, or MC57-SIY-Inf3-/- tumor cells reached 100% confluency. Tumor supernatant was centrifuged at 500 g for 3 min to pellet cell debris and subsequently filtered through a 0.45 µm PVDF syringe filter (EMD Millipore). The resultant cell-free tumor-conditioned media was aliquoted and stored at –20°C.

Collection of BM-DCs
Adapted from (Mayer et al., 2014). BM was harvested from the femur and tibia of mice by flushing the bones with RPMI using a 1 mL syringe. Cells were passed through a 70 µm filter, washed twice with PBS, and cultured at a density of 1.5x10^6 cells/mL in RPMI supplemented with 10% FBS, 1% penicillin/streptomycin, 1X HEPES, 1X MEM Non-Essential Amino Acids, 1X β-mercaptoethanol, 100 ng/mL recombinant human FLT3-L (Bio X Cell), and 5 ng/mL recombinant mouse GM-CSF (BioLegend) for 7 days at 37°C.
and 5% CO₂. BM-DCs at day 7 of culture were either used directly in assays or frozen in 10% DMSO in FBS and stored in liquid nitrogen.

**BM-DC IFN-I and ISG induction assay**

BM-DCs at day 7 of culture were plated at 3x10⁶ cells per well of a 6-well tissue culture-treated plate and cultured with 2 mL of tumor-conditioned media. BM-DC were cultured with 2 mL of fresh complete DMEM media as a negative control or with complete DMEM media containing 20 μg/mL DMXAA (InvivoGen) as a positive control. Following 24 hr incubation at 37°C and 5% CO₂, BM-DC were washed, lysed with RLT Buffer (QIAGEN), and frozen at −80°C for subsequent RNA extraction.

**Baseline IFN-I and ISG screen with mouse and human tumor cell lines**

Murine and human tumor cell lines used for the IFN-I and ISG qPCR screen were either available in-house, gifts, or purchased from ATCC or the High Throughput Sciences Core at the Koch Institute Swanson Biotechnology Center and cultured at the indicated conditions (Table S6). For the experiment, 3x10⁵ cells were washed with PBS, lysed with RLT Buffer, and frozen at −80°C for subsequent RNA extraction.

**RNA isolation, cDNA reaction, and qRT-PCR**

RNA was isolated using the QIAGEN RNeasy Kit (QIAGEN) following manufacturer’s instructions. Extracted RNA was quantified by NanoDrop and 500 ng of RNA was reverse transcribed into cDNA using the Applied Biosystems Reverse Transcriptase Kit, following manufacturer’s instructions. For each qRT-PCR reaction, 1 μL of the cDNA was assayed using the Applied Biosystems SYBR Green PCR Master Mix with defined primer sets for each target gene (Table S5). Reactions were run on the StepOne Real-Time PCR System (Applied Biosystems) and the expression level was calculated as 2⁻ΔCT, where ΔCT is the difference between the CT values of the target gene and 18S.

**Protective systemic immunity assay**

Batf3⁻/⁻ mice were implanted subcutaneously in the flank with 2x10⁶ MC57-SIY tumor cells or 2x10⁶ MC57-SIY-B2M⁻/- tumor cells to induce the immune response by ISG⁺ DC. As a control, a cohort of Batf3⁻/⁻ mice was injected with equal volume of PBS. Six days after the initial implantation with MC57 tumor cells or PBS control, 2x10⁶ MC38-SIY tumor cells were implanted on the contralateral flanks of the mice, and tumor outgrowth was measured as previously described.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All statistical analyses were performed using GraphPad Prism (GraphPad). All data are shown as mean ± s.e.m. Unless stated otherwise, statistical analyses were performed with MWU test (for comparison of two groups) or two-way ANOVA (for multiple comparisons) with *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns = not significant.