

SUPPLEMENTAL MATERIAL

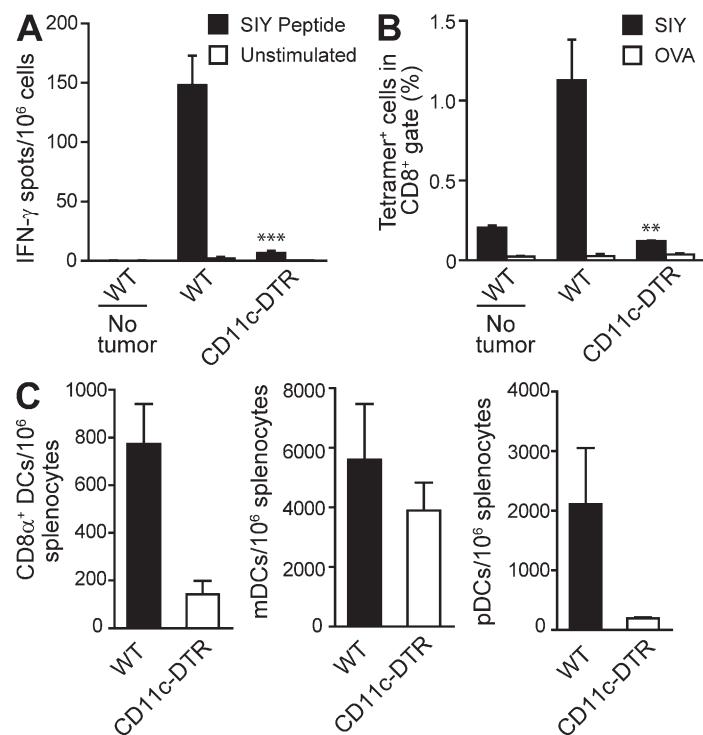
Fuertes et al., <http://www.jem.org/cgi/content/full/jem.20101159/DC1>

Figure S1. Host DCs are critical for spontaneous CD8⁺ T cell priming to tumor-associated antigens. Wild-type C57BL/6 mice (expressing the congenic marker CD45.1⁺) were lethally irradiated and reconstituted with either wild-type (CD45.2⁺) or CD11c-DTR (CD45.2⁺) BM cells. Mice were allowed to reconstitute for 3 mo and were injected i.p. with diphtheria toxin (100 ng in 100 μ l of DPBS) once a day for 8 d starting 2 d before s.c challenge with 10^6 B16.SIY tumor cells in the left flank ($n = 5$). Splenocytes were harvested 6 d after tumor challenge and restimulated for 16 h in the presence or absence of soluble SIY peptide (A). The frequency of tumor-specific IFN- γ -producing cells was assessed by ELISPOT. ***, P < 0.0001 versus WT. (B) Cells were gated on CD8⁺CD4⁻B220⁻ and the frequency of SIY-specific CD8⁺ T cells was assessed by FACS using specific tetramers. **, P = 0.0053 versus WT. Successful reconstitution was confirmed by FACS for CD45.1 and CD45.2 congenic markers in splenocytes from chimeric mice. (C) Efficacy of diphtheria toxin-mediated depletion was assessed by FACS. CD3⁺ cells were gated out and the different DC subpopulations were identified as follows: mDCs, CD11C⁺B220⁻CD8α⁻CD11b⁺; CD8α⁺DCs, CD11C⁺B220⁻CD8α⁺CD11b⁻; and pDCs, CD11C^{int}B220⁺PDCA⁺. Results are shown as mean \pm SEM of 2 independent experiments ($n = 5$ each).

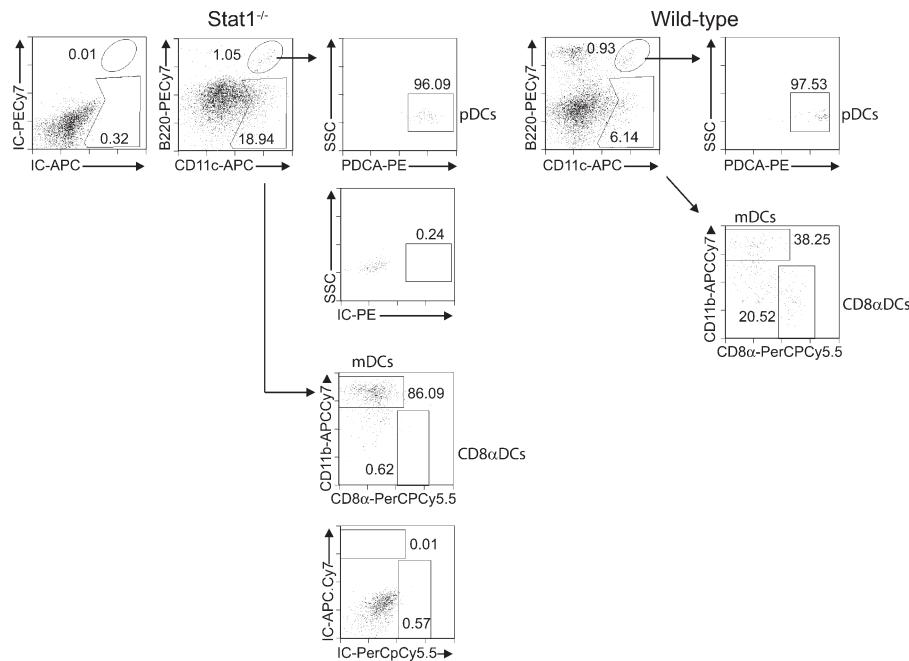


Figure S2. Tumor-infiltrating DCs in wild-type and *Stat1*^{-/-} mice. Wild-type and *Stat1*^{-/-} mice were inoculated s.c. with 10⁶ B16.SIY cells, and 15 d later tumors were harvested, stained, and analyzed by FACS. For the analysis, GFP⁺ DAPI⁺ and CD3⁺ cells were gated out and the different DCs subpopulations were identified as follows: mDCs, CD11c⁺B220⁻CD8α⁺CD11b⁺; CD8α⁺DCs, CD11c⁺B220⁻CD8α⁺CD11b⁻; and pDCs, CD11c^{int}B220⁺PDCA⁺. Representative dot plots of tumor-infiltrating DCs are shown. Numbers indicate the percentage of cells in the gate. Isotype controls (IC) are shown for *Stat1*^{-/-}. The experiment was repeated three times, with similar results.

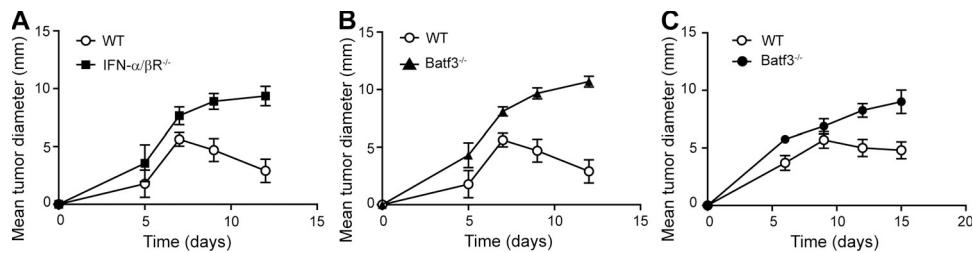


Figure S3. Tumor-growth in wild-type, IFN-α/βR^{-/-}, and Batf3^{-/-} mice. Wild-type and IFN-α/βR^{-/-} mice (A) and wild-type and Batf3^{-/-} mice (B) were inoculated s.c. with 10⁶ B16.SIY cells in the left flank ($n = 5$) and tumor size was measured every 2–3 d. (C) Wild-type and Batf3^{-/-} mice were inoculated s.c. with 10⁶ parental B16 cells in the left flank ($n = 5$) and tumor size was measured every 2–3 d.

Table S1. Selected immune-related genes up-regulated with B16 tumors

Cell type	Gene	Fold increase ^a
Vasculature and red blood cells	<i>Hemoglobin α</i>	1605
	<i>β-globin</i>	282
	<i>endothelial-specific 1</i>	1194
	<i>VCAM-1</i>	53
	<i>Ephrin B2</i>	18
	<i>angiopoietin 2</i>	3
	<i>endothelin R β</i>	3
Macrophages and other myeloid cells	<i>Ly68</i>	277
	<i>Lysozyme</i>	248
	<i>CD36</i>	111
	<i>Jak3</i>	95
	<i>CD53</i>	33
	<i>Hematopoietic cell specific phosphatase</i>	29
	<i>CD34</i>	17
	<i>c-Kit</i>	12
	<i>chitinase-like 3</i>	9
	<i>CD48</i>	8
	<i>class II MHC</i>	6
	<i>CD1d</i>	5
	<i>Macrophage activation 2</i>	3
	<i>ICAM-2</i>	3
	<i>TLR8</i>	3
	<i>IL-18</i>	2
NK cells	<i>CTLA2</i>	94
	<i>IL-2Rγ chain</i>	26
	<i>CRIP</i>	10
	<i>granzyme D</i>	9
	<i>Ly49</i>	6
	<i>Killer lectin R B1A</i>	5
	<i>CD52</i>	4

Table S1. Selected immune-related genes up-regulated with B16 tumors (Continued)

Cell type	Gene	Fold increase ^a
Fibroblasts	<i>Collagen pro-α1 collagen IV</i>	129 14
	<i>fibroblast-inducible secreted protein</i>	12
	<i>procollagen XI</i>	6
	<i>procollagen Iα</i>	5
	<i>procollagen VIα</i>	5
Interferon pathway	<i>IFN-activated 204</i>	169
	<i>IFN-activated 205</i>	81
	<i>IFN-inducible 47</i>	6
	<i>IFN-induced 12</i>	5
	<i>IFN-inducible 30</i>	3
	<i>Interferon α</i>	3
	<i>IFN-αR2b</i>	2
	<i>IFN-inducible GTPase</i>	2
IL-1 pathway	<i>IL-1R II</i>	12
	<i>IL-1R I</i>	4
	<i>IL-1R antagonist</i>	2
	<i>IRAK-1</i>	2
Complement factors	<i>Complement H</i>	30
	<i>Complement C1q</i>	27

^aFold increase over value obtained from B16 grown in vitro.

Table S2. Expression of co-stimulatory molecules on DCs subsets from tumor draining LNs

DC subset	Molecule	Mean fluorescence intensity \pm SD	
		WT	IFN α / β R $^{-/-}$
mDCs	CD40	1,456.00 \pm 280.98	1,154.67 \pm 325.18
	CD80	244.00 \pm 14.42	354.33 \pm 107.43
	CD86	781.33 \pm 43.29	657.33 \pm 190.16
CD8 α^+ DCs	CD40	350.67 \pm 70.31	366.67 \pm 227.30
	CD80	57.73 \pm 6.33	84.50 \pm 28.21
	CD86	370.00 \pm 83.21	626.00 \pm 504.36
pDCs	CD40	750.67 \pm 178.62	876.00 \pm 176.69
	CD80	433.00 \pm 47.03	404.67 \pm 125.37
	CD86	1,237.67 \pm 161.78	1,262.00 \pm 481.76