SUPPLEMENTAL MATERIAL

Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells

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Supplemental Figure 1 shows TLR expression and cytokine induction of Imiquimod treated skin as well as gating strategies to identify pDCs in the skin.

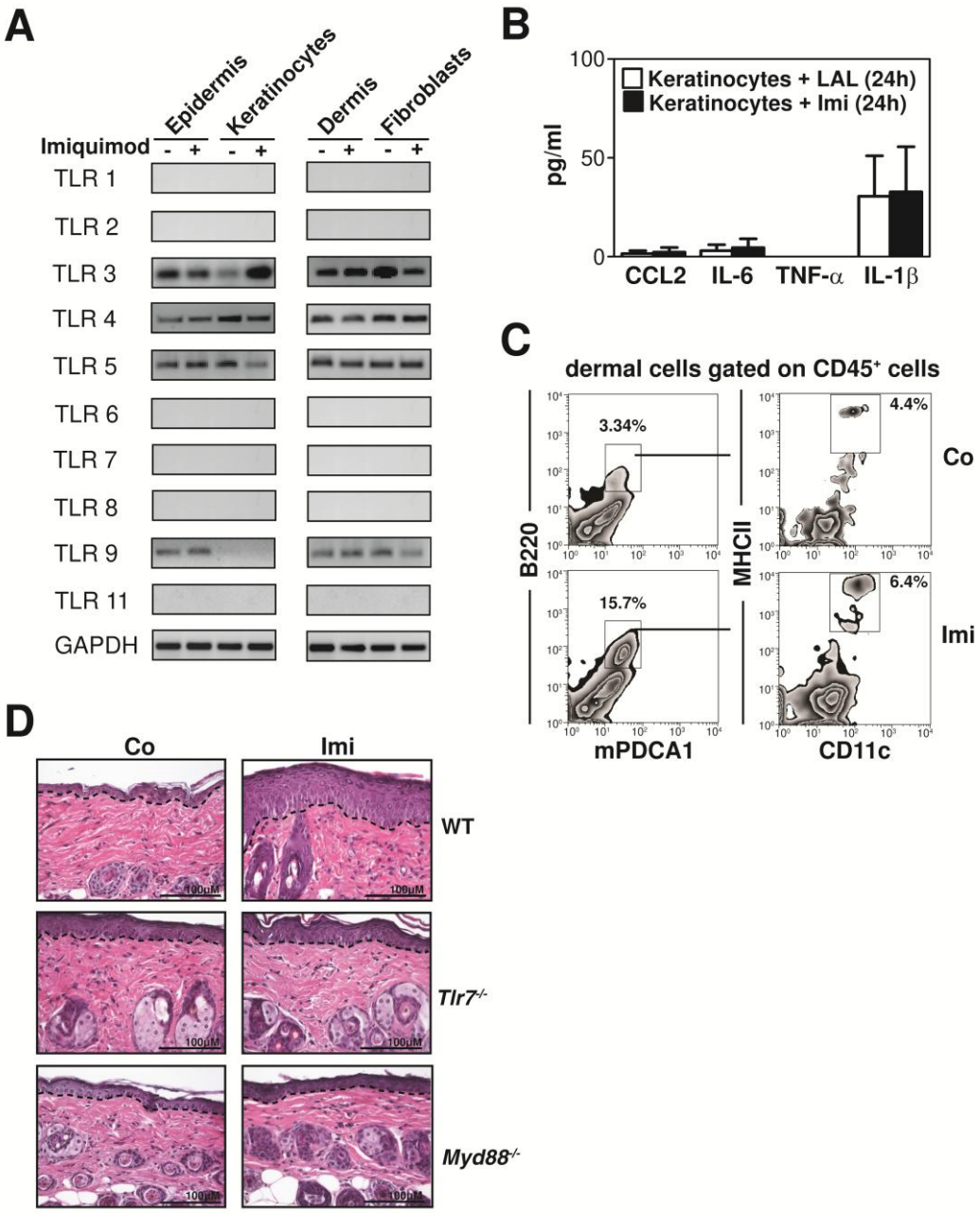
Supplemental Figure 2 demonstrates the bone marrow chimerism by molecular analysis (PCR) and shows tumor growth in *Myd88*$^{-/-}$ chimeric mice

Supplemental Figure 3 confirms the efficient and specific depletion of the respective immune cell population in tumor-bearing mice. It also shows that pDCs upregulate CD8α after Imiquimod treatment.

Supplemental Figure 4 shows that pDC were specifically depleted in tumor-bearing *Bdca2*-DTR mice after DT treatment. Moreover, it shows the apoptosis observed in Imiquimod treated tumor tissue.

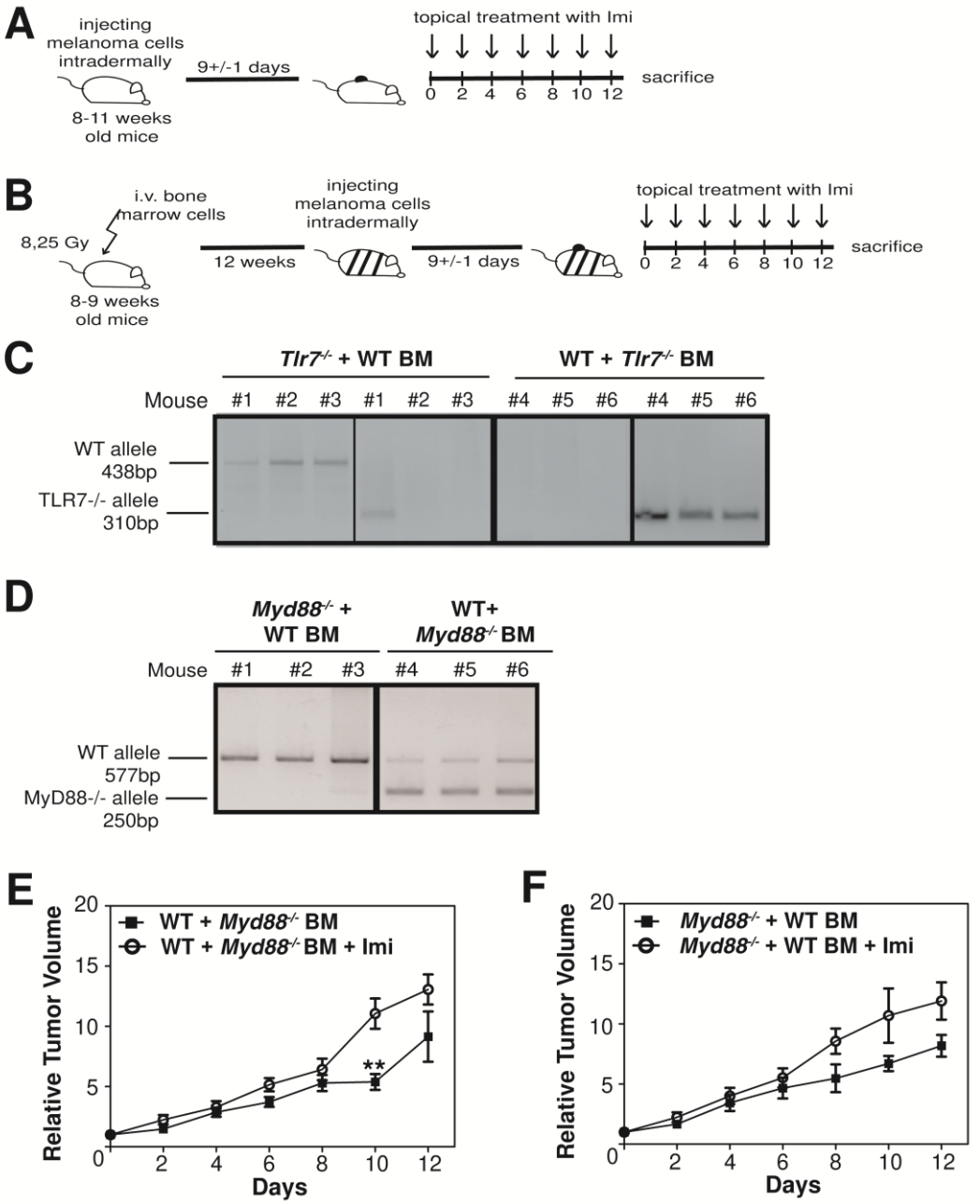
Supplemental Figure 5 confirms cell purity after FACS sorting and shows that Imiquimod-activated pDCs upregulate cytotoxic molecules in a TLR7- and IFNAR1-dependent manner.

Supplemental Table 1 shows primer sequences for RT- and qRT-PCRs.



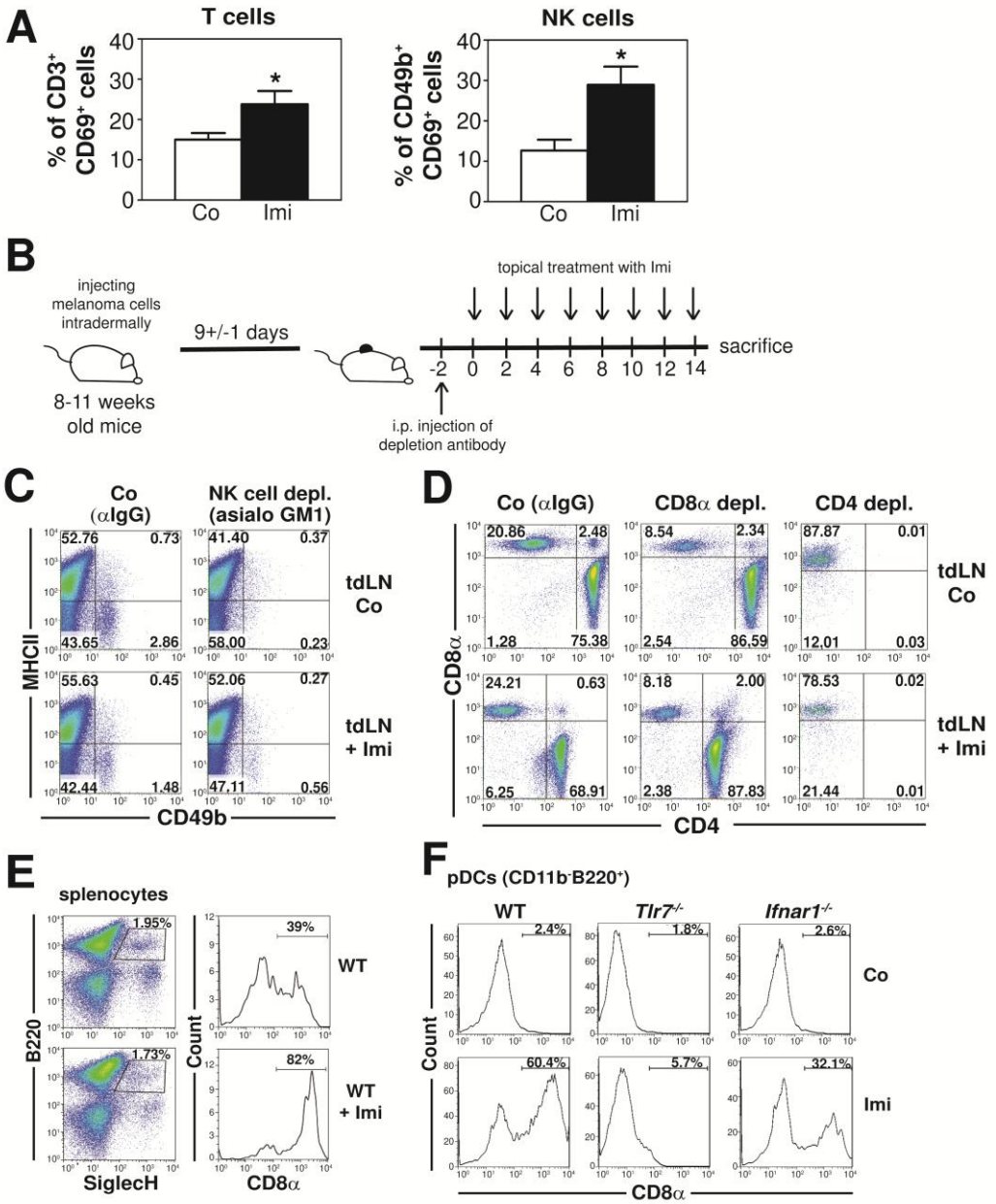
Supplemental Figure 1 Effects of Imiquimod in the skin

(A) RT-PCR analysis showing TLR1-9, 11 expression in the indicated cells and tissues of mice treated for 7 days with Imiquimod (Imi) or left untreated. Results are representative of at least three independent experiments. **(B)** Primary keratinocyte cultures isolated from C57BL/6 (WT) mice were stimulated with Imi (12 µg/ml) or LAL reagent water (control) for 24 hours and cytokines such as IL-6, TNF-α, IL-1β or CCL2 were measured in the supernatants. **(C)** FACS analysis showing representative dot plots of pDCs infiltrating the dermis of WT mice 3 days after Imi treatment. $CD45^+$ cells within the dermal cell suspension were further gated for $mPDCA1^+B220^+$ and $CD11c^+MHCII^+$. Data are representative for at least 2 independent samples. **(D)** H&E staining of skin sections from mice of the indicated genotype. Skin was treated with Imi for 7 days or left untreated (Co). Magnification x400. The dotted lines delineate the dermal-epidermal junction. Data are representative for at least 3 independent samples.



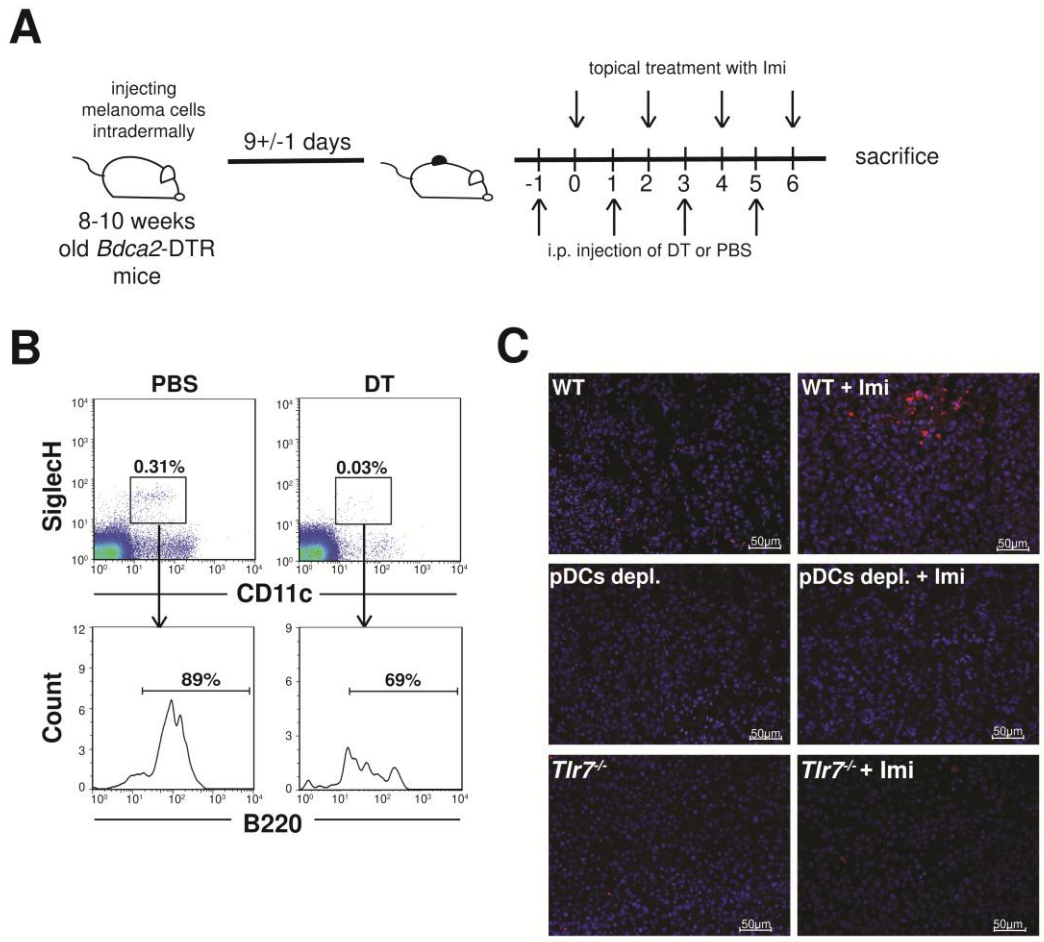
Supplemental Figure 2 Tumor induction and generation of bone marrow chimeras

(A) Mice were injected intradermally with B16-F10 melanoma cells. Mice developed tumors after 9 days which were treated topically with Imi every other day. **(B)** Experimental scheme for tumor induction in bone marrow (BM) chimeras: 8-9 weeks old mice were lethally irradiated and reconstituted intravenously with BM cells. Tumor cells were intradermally injected 12 weeks later and the same experimental protocol as depicted in A was followed. **(C** and **D)** BM chimerism was verified by PCR with primers detecting the respective wild-type (WT) and knock out alleles in BM cells isolated from mice reconstituted with either WT, *Tlr7*$^{-/-}$ or *Myd88*$^{-/-}$ BM. Results are representative for at least 2 independent batches. **(E** and **F)** Relative tumor volume in BM chimeras of the indicated genotypes following Imi treatment. WT and *Myd88*$^{-/-}$ mice were lethally irradiated and reconstituted intravenously with BM cells of the indicated genotypes. After 12 weeks reconstituted mice were injected intradermally with B16-F10 melanoma cells and tumors treated with Imi or left untreated (n = 7-11 per group). Data represent mean ± SEM of at least 2 independent experiments. * $P < 0.05$, ** $P < 0.005$



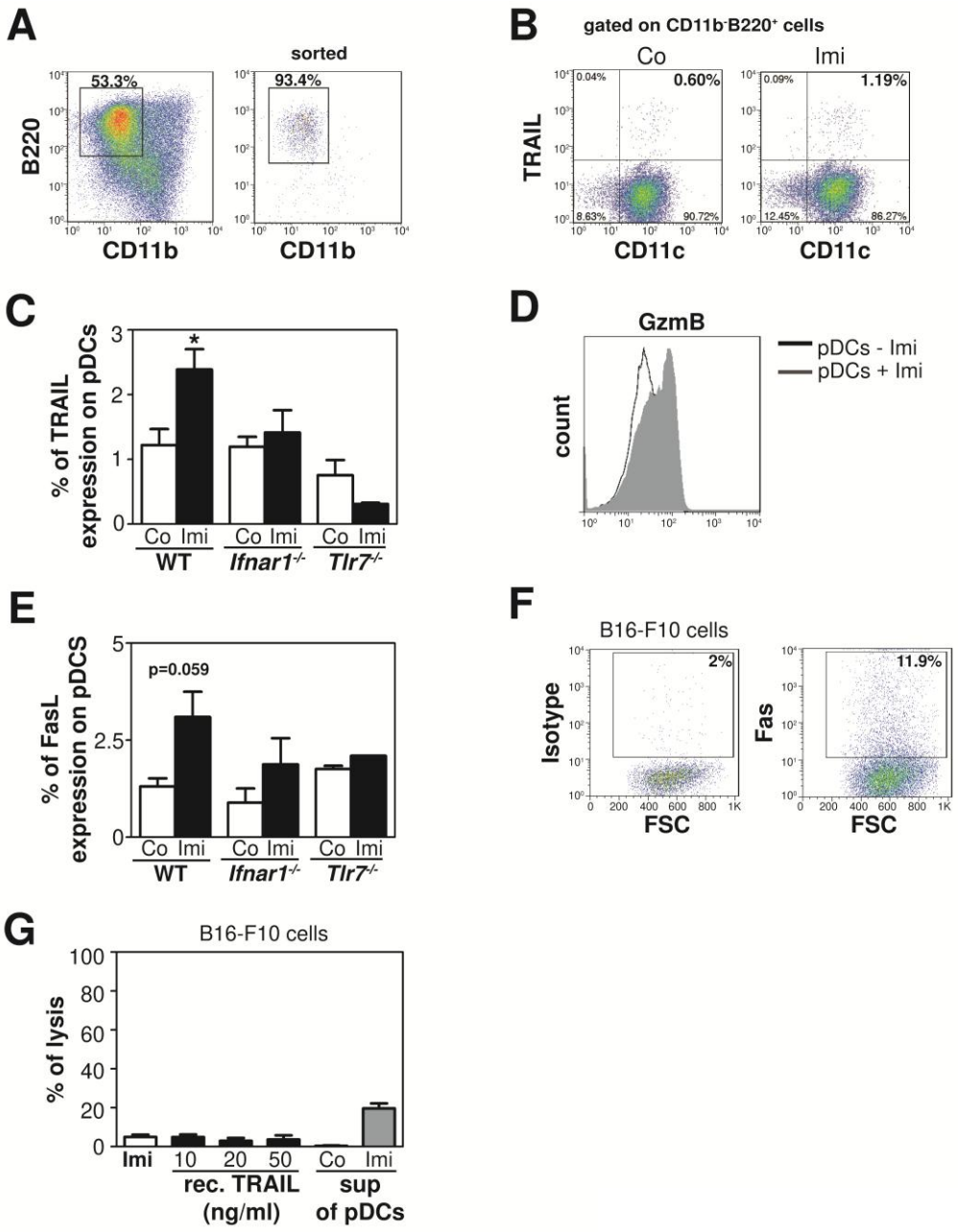
Supplemental Figure 3 Analysis of immune cells in mice depleted of specific immune cell populations

(A) Increased activation of tumor infiltrating T cells (CD3) and NK cells (CD49b) shown by upregulation of CD69 on the respective cells in Imi treated tumors **(B)** Experimental protocol for cell depletion experiments. Tumor bearing mice were injected with antibodies depleting the indicated cell population before starting the topical treatment with Imi. **(C** and **D)** FACS analysis showing depletion efficacy in tumor draining lymph nodes (tdLN) of mice treated with specific anti-NK cell antibodies **(C)**, anti-CD4 antibodies or anti-CD8α antibodies **(D)**. **(E** and **F)** FACS analysis for CD8α expression on pDCs ($SiglecH^+B220^+$) in cultured splenocytes **(E)** and BM-derived pDCs ($CD11b^-B220^+$) of WT, *Tlr7*$^{-/-}$ or *Ifnar1*$^{-/-}$ mice **(F)** stimulated with Imi (2.5 µg/ml) for 24 hours. Results are representative for at least 2 independent batches. * $P < 0.05$



Supplemental Figure 4 pDCs are the effector cells in mediating the tumoricidal effect of Imiquimod

(A) Experimental protocol for pDC depletion: Diphteria Doxin (DT) (4.5 ng per g body weight) or PBS (control) was administered intraperitoneally (i.p) in tumor bearing *Bdca2*-DTR^{+} mice every other day. First Imi treatment was performed one day after depletion start. **(B)** FACS analysis showing pDC (CD11c^{+}B220^{+}SiglecH^{+}) depletion efficacy in tumor-bearing *Bdca2*-DTR mice treated with DT. **(C)** Representative immune-fluorescence staining of active caspase-3 in tumor tissue of WT (n = 7-8 per group), *Bdca2*-DTR + DT (pDCs depl.) (n = 8-9 per group) and *Tlr7*$^{-/-}$ mice (n = 3 per group) treated with Imi or left untreated. Magnification x200



Supplemental Figure 5 Imiquimod stimulated pDCs kill tumor cells

(A) FACS sorted B220^{+}CD11b^{-} cells of in vitro generated BM-derived pDCs. **(B)** Representative dot plots of TRAIL expression on WT pDCs (CD11b^{-}B200^{+}) after 6 hours of Imi (2.5 µg/ml) stimulation. **(C)** Quantification of TRAIL expression by FACS on WT, *Ifnar1*$^{-/-}$ and *Tlr7*$^{-/-}$ pDCs after 6 hours of Imi stimulation. **(D)** Flow cytometric analysis showing intracellular granzyme B (GzmB) staining of pDCs after stimulation with Imi for 4 hours. **(E)** Quantification of FasL expression on WT, *Ifnar1*$^{-/-}$ and *Tlr7*$^{-/-}$ pDCs after Imi stimulation for 6 hours analyzed by FACS. **(F)** Representative staining of Isotype (IgG) or Fas antibodies on B16-F10 melanoma cells. **(G)** Killing assay performed with B16-F10 melanoma treated with Imi, recombinant TRAIL (10, 20, 50 ng/ml) or supernatants of Imi stimulated or unstimulated WT pDCs. Graphs are representative for one experiment of at least 2 independent batches.* $P < 0.05$

Supplemental Table 1

Primer Sequences for RT-PCR
Tlr1**:** 5'-CAATGTGGAAACAACGTGGA-3'; 5'-TGTAACTTTGGGGGAAGCTG-3';
Tlr2**:** 5'-AAGAGGAAGCCCAAGAAAGC-3'; 5'-CGATGGAATCGATGATGTTG -3';
Tlr3**:** 5'-CACAGGCTGAGCAGTTTGAA-3'; 5'-TTTCGGCTTCTTTTGATGCT-3';
Tlr4**:** 5'-ACCTGGCTGGTTTACACGTC- 3'; 5'-CTGCCAGAGACATTGCAGAA- 3';
Tlr5**:** 5'-AAGTTCCGGGGAATCTGTTT-3'; 5'-GCATAGCCTGAGCCTGTTTC-3';
Tlr6**:** 5'-TTCCCAATACCACCGTTCTC- 3'; 5'-CTATGTGCTGGAGGGTCACA-3';
Tlr7**:** 5'-AATCCACAGGCTCACCCATA-3'; 5'-CAGGTACCAAGGGATGTCCT-3';
Tlr8**:** 5'-GTTATGTTGGCTGCTCTGGTTCAC-3'; 5'- TCACTCTCTTCAAGGTGGTAGC- 3';
Tlr9**:** 5'-ACTGAGCACCCCTGCTTCTA-3'; 5'-AGATTAGTCAGCGGCAGGAA- 3';
Tlr11**:** 5'-TTGATGTATTCGTGTCCCACTGC-3'; 5'-CCACTCTTTCTCTCCTCTTCCTCG-3';
Gapdh**:** 5'-CTCATGACCACAGTCCATCG-3'; 5'-CACATTGGGGGTAGGAACAC-3'
Primer Sequences for qRT-PCR
Trail**:** 5´-GTGTCTGTGGCTGTGACTTACA-3´; 5'-AATGCCCTTTCCGAGAGGA-3',
Gzmb: 5´-ATTCCCCACCCAGACTATAATCC-3´; 5´-TTACTCTTCAGCTTTAGCAGCATGA-3';
Actin**:** 5´-ACCAACTGGGACGATATGGAGAAGA-3´; 5´TACGACCAGAGGCATACAGGGACAA-3';