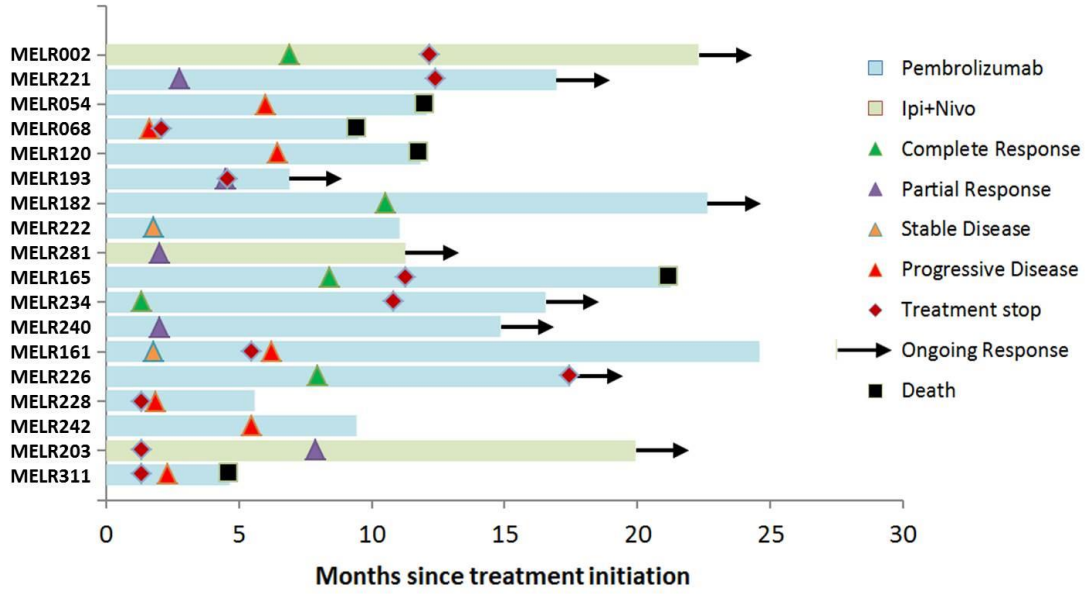
Human CD141^{+} DCs can be targeted to enhance anti-PD-1 efficacy in melanoma Supplementary Data



Supplementary Figure 1: Swimmer's plot of advanced melanoma patients undergoing anti-PD-1 and/or anti-CTLA-4 immunotherapy

Treatment type, duration of response, dates of complete/partial response or progressive disease, and presence of ongoing response or death are indicated accordingly.

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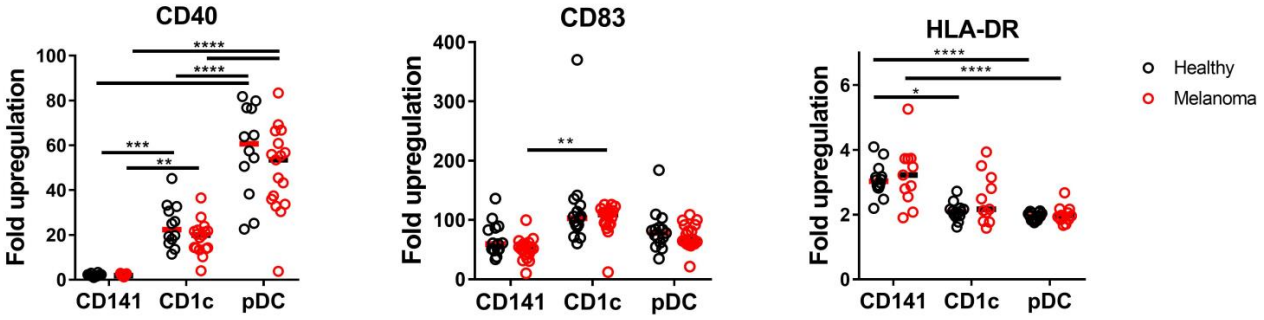
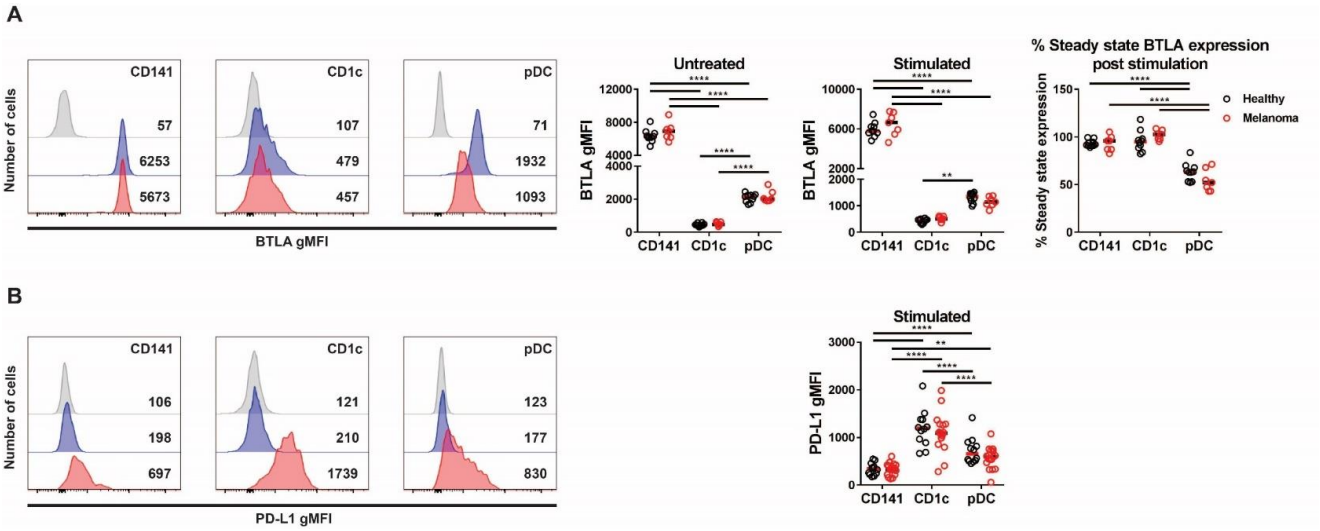


Supplementary Figure 2: Fold increase of costimulatory molecule expression of activated DC relative to non-activated

Data shown is the Geometric Mean Fluorescence Intensity (gMFI) values (after FMO subtraction) of costimulatory molecules on DC subsets following ex vivo whole blood culture activation with PolyIC + R848 expressed as the fold increase relative to the matched untreated sample for each donor (healthy controls, n = 10-12, melanoma patients, n = 7-18). Median values are shown. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by 2-way ANOVA.



Supplementary Figure 3: BTLA and PD-L1 expression on DC subsets in advanced melanoma patients

Expression of BTLA (A) and PD-L1 (B) by DC subsets from advanced (Stage 3/4) melanoma patients and healthy donor controls after ex vivo whole blood culture alone (untreated) or after activation with PolyIC + R848. Left panels: Representative histograms showing fluorescence minus one (FMO) control (grey), and marker expression on untreated (blue) or activated (red) DC subsets. Number represents geometric mean fluorescence intensity (gMFI) values. Right panels: Compiled background-subtracted gMFI of expression of each marker (healthy controls (black), n = 10-12, melanoma patients (red), n = 7-18). Median values are shown. ** $p < 0.01$, **** $p < 0.0001$ by 2-way ANOVA.

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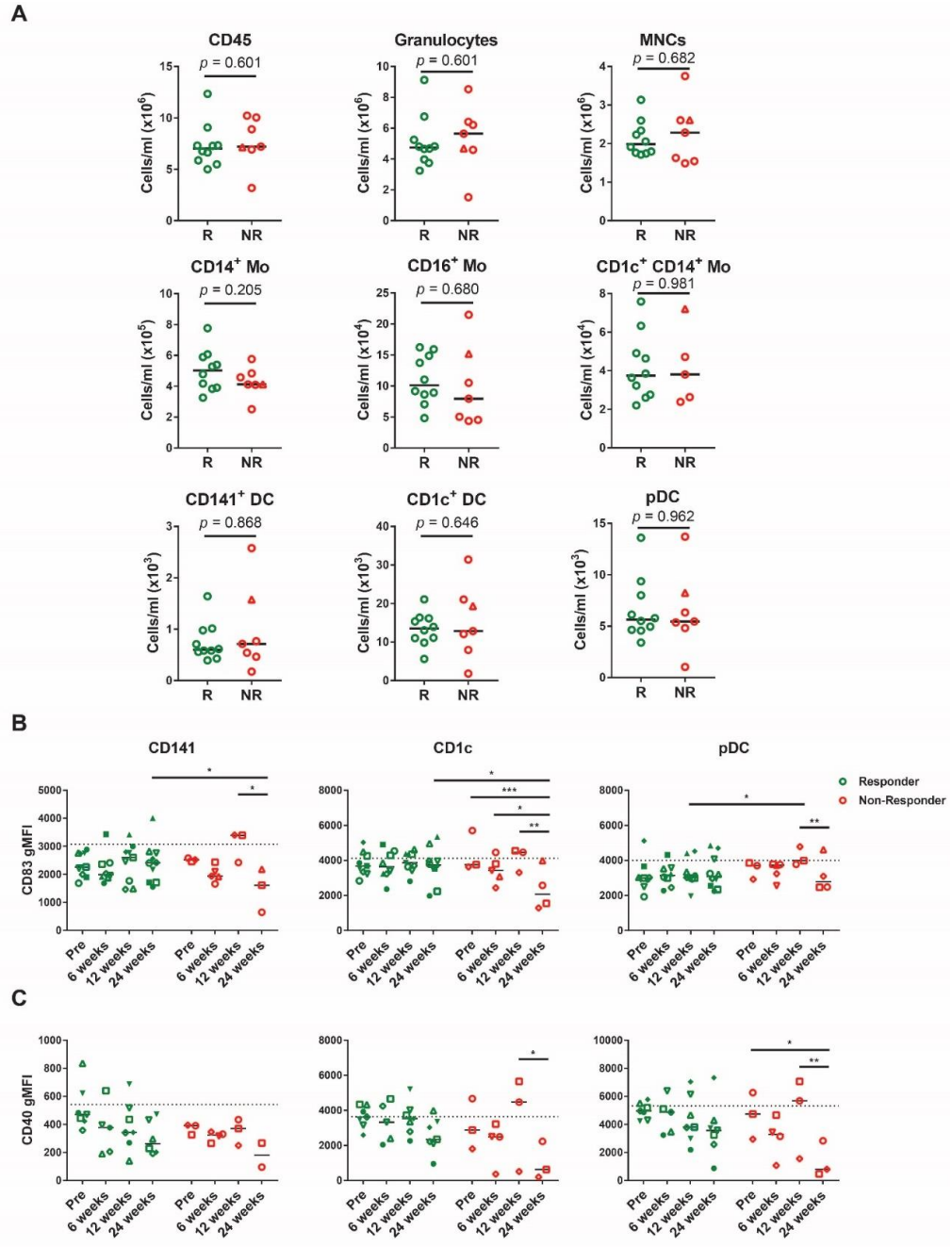

Supplementary Figure 4: DC numbers and activation in melanoma patients treated with immunotherapy. A. Numbers of leukocyte subsets in advanced melanoma patients prior to treatment with immunotherapy segregated into responders (R, green, n = 9-10) and non-responders (NR, red, n = 5-7). A patient in the NR group who had disease progression on anti-PD-1 alone was switched to anti-CTLA-4 alone and had stable disease, denoted in triangle. B. CD83 and C. CD40 expression on CD141+ DCs, CD1c+ DCs, and pDCs (left to right) post PolyIC and R848 stimulation in advanced melanoma patients prior (Pre) and during immunotherapy at the indicated time points. CD83: n = 6-10 responders, n = 3-5 non-responders; CD40: n = 4-7 responders, n = 2-4 non-responders). gMFI, geometric mean fluorescence intensity. Dotted lines represent median values of marker expression based on healthy controls. Each symbol represents an individual patient.

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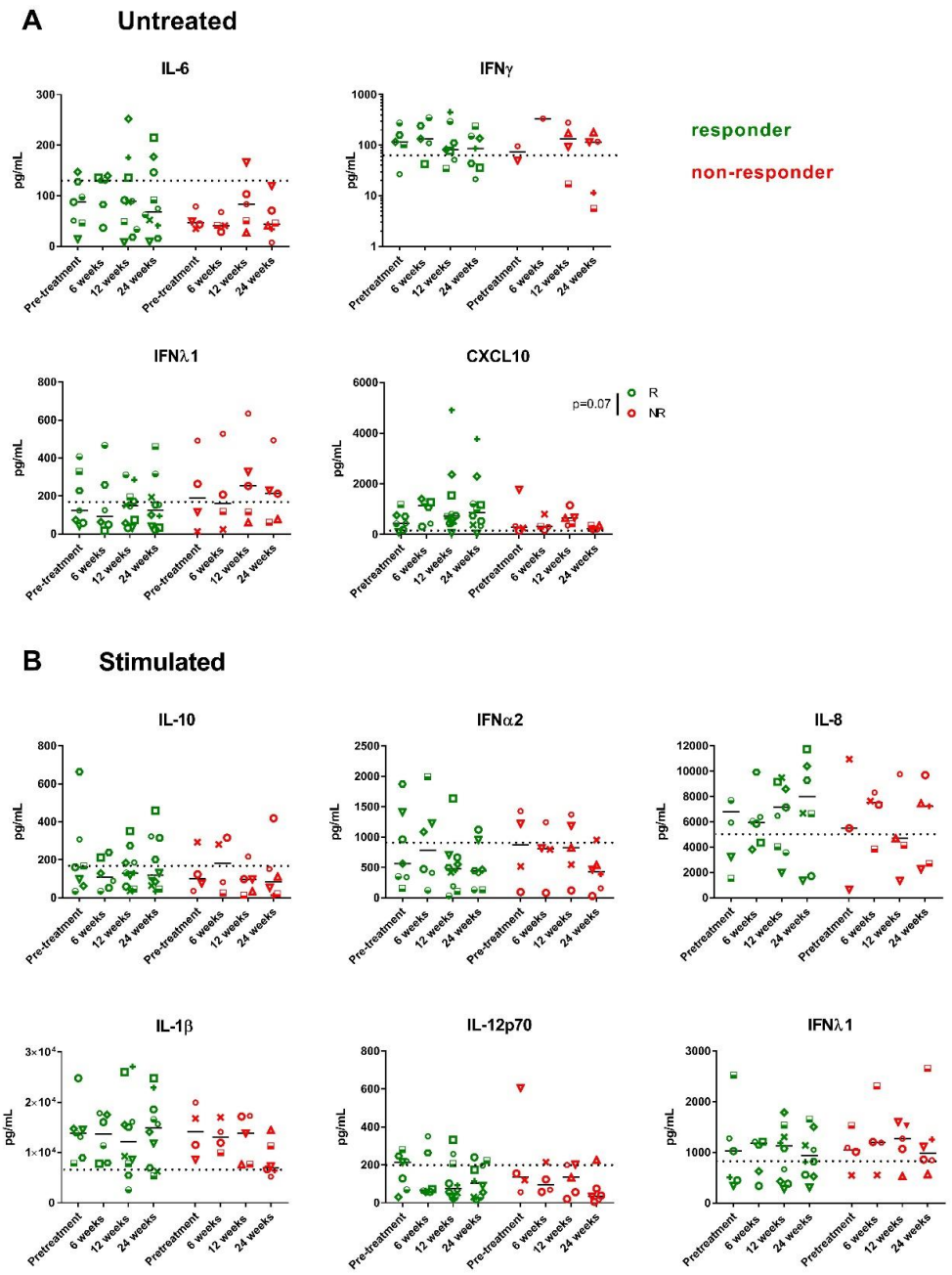

Supplementary Figure 5: Cytokine production in melanoma patients treated with immunotherapy. Plasma cytokines in advanced melanoma patients prior (Pretreatment) and during immunotherapy at the indicated time points, segregated into responders (R, green, *n* = 4-10) and non responders (NR, red, n=4-6). A. Plasma IL-6, IFNγ, IFNλ1 and CXCL10 in the absence of stimulation. B. Plasma IL-10, IFNα2, IL-8, IL-1β, IL-12p70 and IFNλ1 post stimulation with PolyIC + R848. Median values are shown with Bars representing median, dotted lines indicate median healthy donor levels. Statistical significance assessed using 2-way ANOVA. No significant differences were found between responders and non-responders at any time points.

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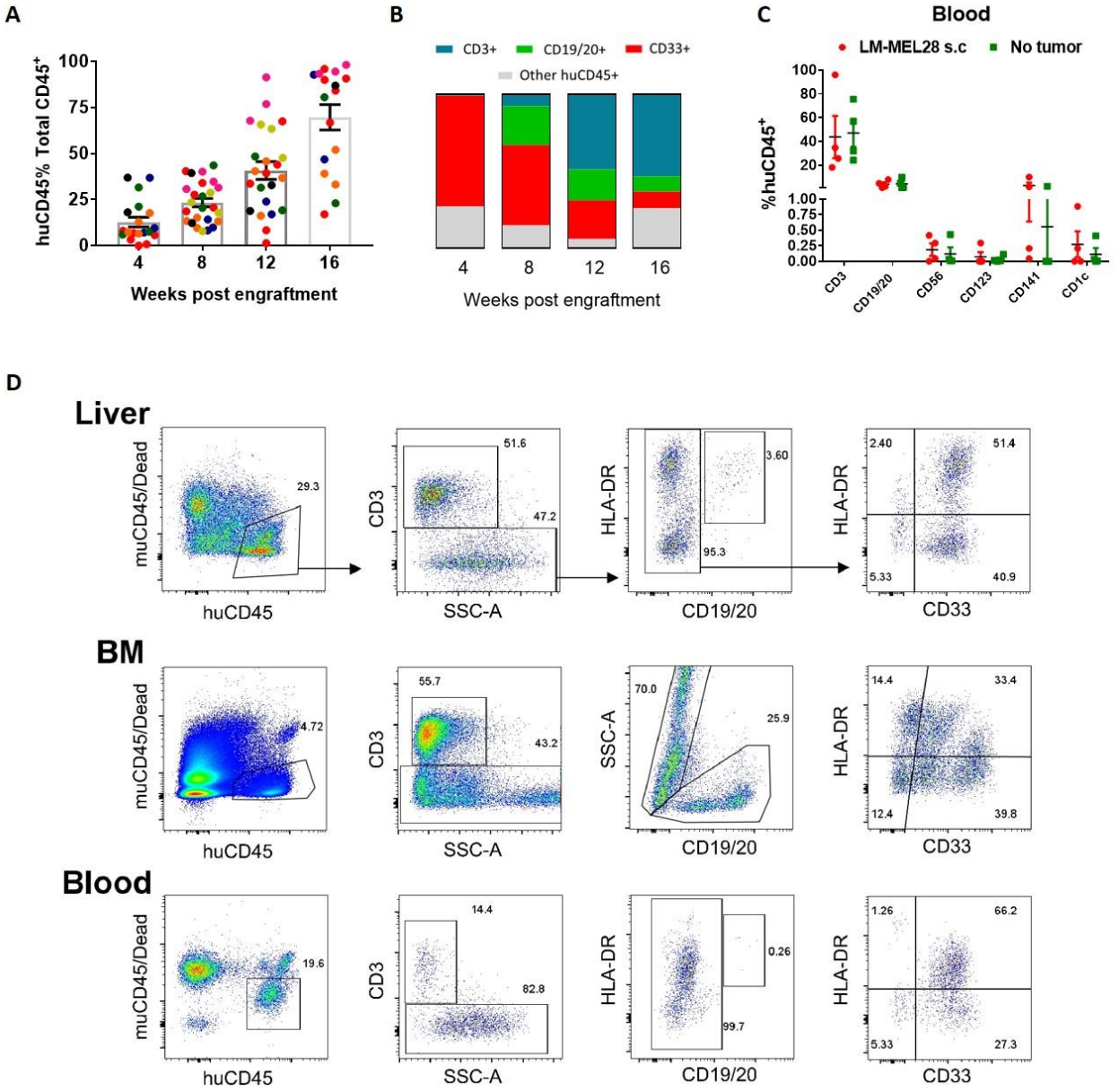

Supplementary Figure 6: Human immune reconstitution in NSG-SGM3 mice.
A. Percentage of human CD45$^+$ cells among total peripheral blood CD45$^+$ cells of non-tumour-bearing NSG-SGM3 mice injected with CD34$^+$ HSCs 2-5 days post-partum. Blood was analyzed every 4 weeks by flow cytometry. Symbols depict individual mice and colours denote mice engrafted with the same human CD34$^+$ HSC sample (n = 5-7 HSC donors and 19-25 mice per time point). B. Proportions of CD3, CD19/20, CD33 lineages within human CD45 compartment of blood for mice shown in (A). C. Percentage of each immune subset of total huCD45+ cells in blood of tumor and non-tumor bearing huNSG-SGM3 mice at 12-16 weeks post-engraftment. Tumour-bearing mice culled at day 35. Human CD34$^+$ cell donors were split evenly between tumor and non-tumor bearing littermates. D. Human immune reconstitution in liver, bone marrow and blood of NSG-SGM3 mice 8-16 weeks post-engraftment.

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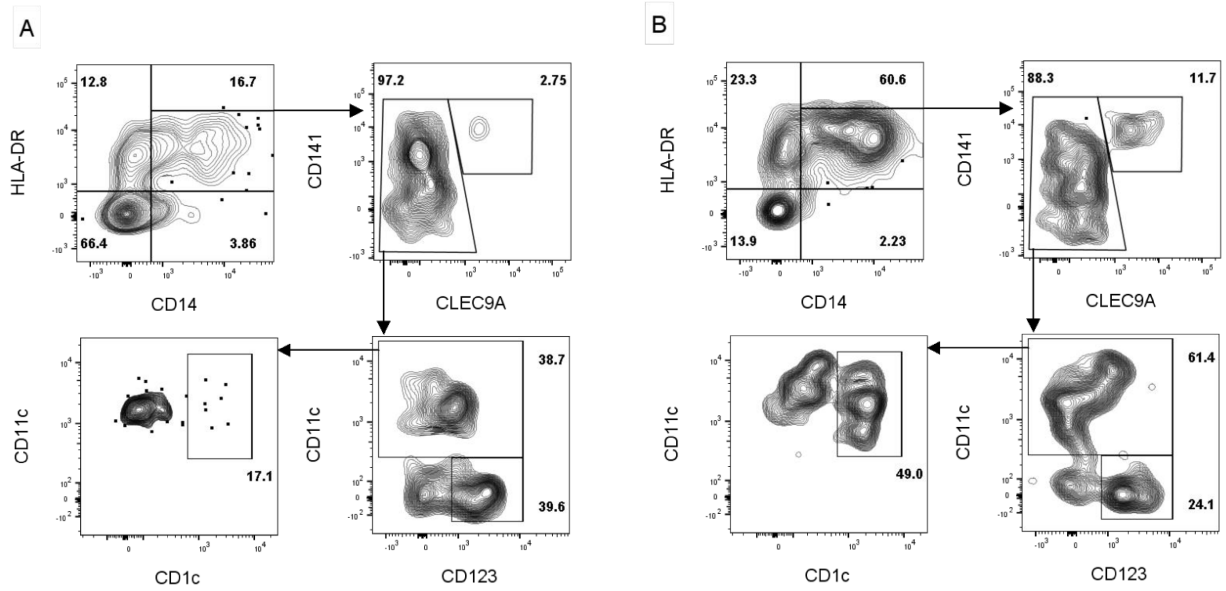

Supplementary Figure 7: Detection of DC subsets in melanoma tumors of humanized mice.
A. CD141⁺ (CD141⁺Clec9A⁺), CD1c⁺ (CD11c⁺CD1c⁺), and pDCs (CD11c⁻CD123⁺) within the human CD45+ leukocyte compartment of a vehicle treated LM-MEL28 tumor in humanized mice. DC were not detected in 3 of 4 tumors in the control group. B. DC subsets infiltrating tumors in Flt3L+PIC treated mice. Representative of 2 mice, DC were undetectable in another 2 treated mice. Tumors were harvested 10 days after the first Flt3L treatment (day 19 after tumor injection). Numbers near gates refer to percentage of parent population. Cells were pre-gated on muCD45⁻/Live/huCD45⁺/CD3⁻/CD19/20⁻ events.

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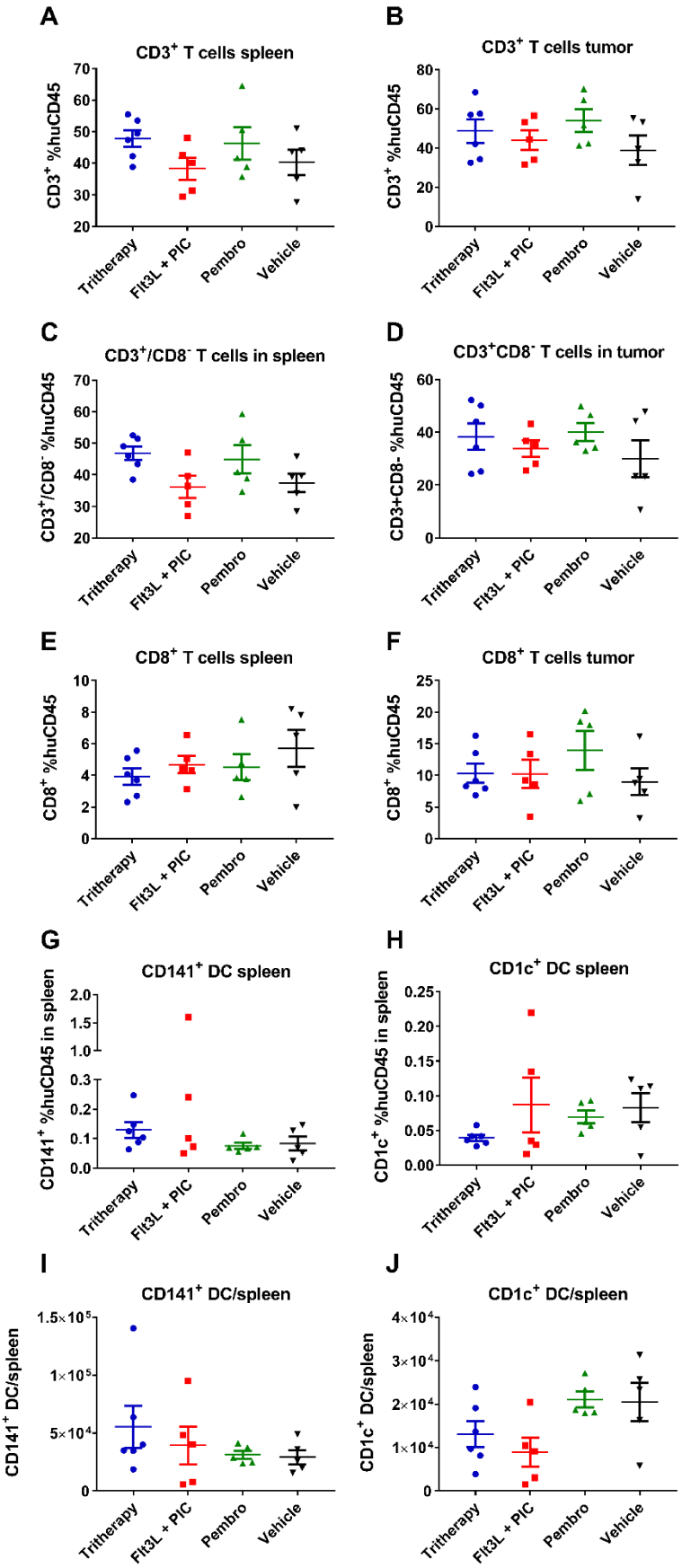

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Supplementary Figure 8: Human T cells and DCs in spleens and tumors of humanized NSG-SGM3 treated with immunotherapy.
A-F. Percentage of CD3$^+$ cells (A, B), CD3$^+$/CD8$^-$ T cells (C, D), and CD3$^+$ /CD8$^+$ T cells (E, F) within the human CD45$^+$ compartment in spleens (A, C and E) and subcutaneous LM-MEL28 tumors (B, D and F) from humanized NSG-SGM3 mice treated with indicated regimen. G-H. Percentage of CD141$^+$ DCs (G) or CD1c$^+$ DCs (H), among human CD45$^+$ cells in spleens of mice. I-J. Total number of CD141$^+$ and CD1c$^+$ DCs per spleen of NSG-SGM3 mice. n = 5-6 mice per group.

Supplementary Table 1. Demographic and clinical characteristics of advanced melanoma patients (7th edition of the American Joint Committee on Cancer (AJCC) staging manual [1]) **and healthy donor controls.**
N/A: Not applicable.

Characteristic	Patients	Controls
Sex – no. (%)		
Male	39 (73.6)	13 (52.0)
Female	14 (26.4)	12 (48.0)
Median age (range) – year	61 (26 – 87)	52 (30 – 63)
Disease stage – no. (%)		
3	32 (60.4)	N/A
4	21 (39.6)	N/A
BRAF mutation status – no. (%)		
Wild type	23 (43.4)	N/A
V600E or V600K mutation	14 (26.4)	N/A
Unknown	16 (30.2)	N/A
Previous treatment – no. (%)		
Surgery	19 (35.8)	N/A
Wide local excision	16 (30.2)	N/A
Nodal	2 (3.8)	N/A
Axillary	1 (1.9)	N/A
Radiation	9 (17.0)	N/A
Systemic therapy	1 (1.9)	N/A

1. Balch, C.M., et al., *Final version of 2009 AJCC melanoma staging and classification.* J Clin Oncol, 2009. **27**(36): p. 6199-206.

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Supplementary Table 2. Patient characteristics (immunotherapy).

Patient ID	Sex	Age	Stage	Treatment	Best response	Prior treatment
MELR161	M	68	4	Pembrolizumab	SD	
MELR165	F	66	4	Pembrolizumab	CR	Surgery
MELR120	M	53	4	Pembrolizumab	PD	Surgery, Radiation
MELR002	M	64	4	Ipilimumab + Nivolumab	CR	
MELR182	M	78	4	Pembrolizumab	CR	
MELR054	M	54	4	Pembrolizumab	PD	Radiation
MELR203	F	57	4	Ipilimumab + Nivolumab	PR	
MELR068	M	38	4	Pembrolizumab	PD	Surgery, Radiation
MELR226	M	63	4	Pembrolizumab	PR	
MELR234	F	53	4	Pembrolizumab	CR	
MELR221	M	49	4	Pembrolizumab	PR	
MELR240	M	77	4	Pembrolizumab	PR	
MELR222	F	57	3*	Pembrolizumab	SD	Radiation
MELR281	M	66	4	Ipilimumab + Nivolumab	PR	
MELR242	M	45	4	Pembrolizumab	PD	Radiation
MELR193	F	86	4	Pembrolizumab	PR	
MELR311	F	74	4	Pembrolizumab	PD	
MELR228	F	61	4	Pembrolizumab	PD	Radiation, BRAFi + MEKi

Patients were treated intravenously with anti-PD-1 (Pembrolizumab, Keytruda®) monotherapy (2mg/kg every 3 weeks), anti-CTLA-4 (Ipilimumab, Yervoy®) monotherapy (3mg/kg every 3 weeks), or anti-PD-1 (Nivolumab, Opdivo®) (1mg/kg every 3 weeks) and anti-CTLA-4 Ipilimumab in combination (3mg/kg every 3 weeks), followed by maintenance Nivolumab at 3mg/kg every 2 weeks. Treatment was allowed to continue unless disease progression or unacceptable toxicity occurred, or at patient decision to stop treatment on complete response. ID: De-identified patient identity. Sex: M, male; F, female. Best response: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. BRAFi, BRAF inhibitor. MEKi, MEK inhibitor. * MELR222 is a patient with unresectable stage 3C melanoma.