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Supplemental tables

Supplementary Table 1. Patient characteristics

Patient	Disease	Sample time point	Sample type	Extra information	MiHA	% MiHA-specific T cells day 0
A	Multiple myeloma	22 mo post alloSCT	PB	DLI: 6 mo post alloSCT	HA-1	0.06
B	Myeloid dysplastic syndrome	9 mo post alloSCT	PB	-	HA-1	0.25
C	Acute myeloid leukemia	12 mo post alloSCT	PB	-	HA-1	0.02
D	Myeloid dysplastic syndrome	9 mo post alloSCT	PB	DLI: 4 mo post alloSCT	LRH-1	0.05
E	Acute myeloid leukemia	36 mo post alloSCT	PB	-	LRH-1	0.02
F	Acute myeloid leukemia	76 mo post alloSCT	PB	DLI: 13 and 36 mo post alloSCT	LRH-1	0.03
G	Myeloid dysplastic syndrome	3 mon post alloSCT	PB	-	HA-1	< 0.01
H	Acute myeloid leukemia M0	Diagnosis	BM	-	-	-
I	Acute myeloid leukemia M1	Diagnosis	BM	-	-	-
J	Acute myeloid leukemia M2	Diagnosis	BM	-	-	-
K	Acute myeloid leukemia M0	Diagnosis	BM	-	-	-

Mo, months; DLI, donor lymphocyte infusion; alloSCT, allogeneic stem cell transplantation; MiHA; Minor histocompatibility antigen

Supplementary Table 2. Overview of used antibodies in flow cytometry analyses.

Specificity	Fluorochrome	Clone	Isotype	Company	Cat#
HPC Purity					
CD34	PE-Cy7	581.0	IgG1κ	Biolegend	A21691
CD45	ECD	J.33	IgG1κ	Beckman Coulter	A007784
Phenotype and sort *ex vivo* generated DCs					
CD1c	APC-Cy7	L161	IgG1κ	Biolegend	331520
CD14	PE-Cy7	HCD14	IgG1κ	Biolegend	325618
CD123	BV510	6H6	IgG1κ	Biolegend	306022
CD141	APC	M80	IgG1κ	Biolegend	344106
CD303	FITC	201A	IgG2aκ	Biolegend	353208
CD370/CLEC9A	PE	8F9	IgG2a	Biolegend	353804
HLA-DR	ECD	Immu359	IgG1κ	Beckman Coulter	IM3636
Phenotype and sort *in vivo* DCs					
CD1c	PE-Cy7	L161	IgG1κ	Biolegend	331516
CD11c	AF700	Bu15	IgG1κ	Biolegend	337220
CD14	BV785	M5F2	IgG2aκ	Biolegend	301841
CD19	PE	HIB19	IgG1κ	eBioscience	12-0193-82
CD141	APC	M80	IgG1κ	Biolegend	344106
CD303	FITC	201A	IgG2aκ	Biolegend	353208
HLA-DR	APC Cy7	G45-6	IgG2aκ	BD Bioscience	335976
DC maturation assays					
CD80	PE-Cy7	L307.4	IgG1κ	BD Bioscience	561135
CD83	PE	HB15e	IgG1κ	Biolegend	305308
CD86	AF488	IT2.2	IgG2bκ	Biolegend	305414
CCR7	FITC	G043H7	IgG2aκ	Biolegend	353216
Allogeneic T cell proliferation assays					
CD3	PE-Cy7	UCHT1	IgG1κ	Biolegend	300420
CD8	AF700	RPA-T8	IgG2aκ	Invitrogen	MCHD0829
Antigen-specific T cell expansion assays					
CD3	PE-Cy7	UCHT1	IgG1κ	Biolegend	300420
CD8	AF700	RPA-T8	IgG2aκ	Invitrogen	MCHD0829
Tetramer	PE	-	-	Custom	
Tetramer	APC	-	-	Custom	
Cross-presentation assays					
CD8	AF700	RPA-T8	IgG2aκ	Invitrogen	MCHD0829

CD69	BV421	FN50	IgG1κ	Biolegend	310930
CD107a	PE Cy7	H4A3	IgG1κ	Biolegend	328618
CD137	APC	4B4-1	IgG1κ	BD Bioscience	550890
IFN-γ (intracellular)	FITC	B27	IgG1κ	BD Bioscience	554700
TNF-α (intracellular)	PE	MAb11	IgG1κ	BD Bioscience	554513
NK cell assays					
CD69	FITC	FN50	IgG1κ	Biolegend	310904
CD107a	PE-Cy7	H4A3	IgG1κ	Biolegend	328618
TRAIL/CD253	APC	RIK-2	IgG1κ	Biolegend	308210
Viability Dyes					
-	Sytox Blue	-	-	Invitrogen	S34857
-	7-AAD	-	-	Sigma Aldrich	A9400
-	eFluor780	-	-	Thermo Fisher	65-0865-14
-	DAPI	-	-	Sigma Aldrich	D9532-50MG

Data acquisition and analyses were performed using Gallios or FC500 flow cytometers and Kaluza version 2.1 software (all Beckman Coulter).

Abbreviations

CD: Cluster of differentiation

HPC: Hematopoietic progenitor cell

PE: Phycoerythrin

Cy: Cyanine

ECD: Electron coupled dye (PE-Texas red)

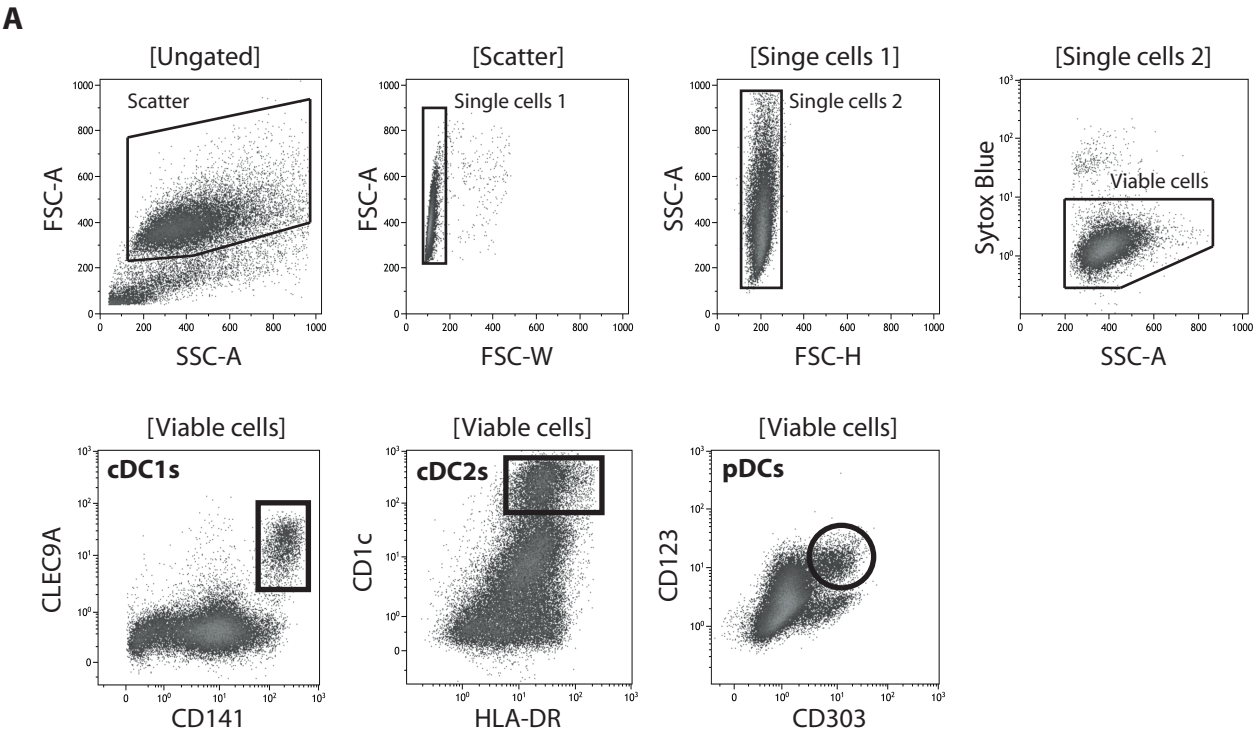
APC: Allophycocyanin

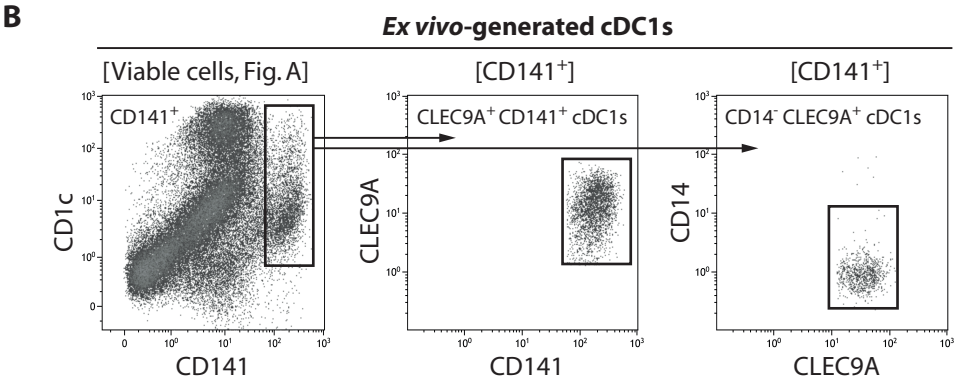
BV: Brilliant violet

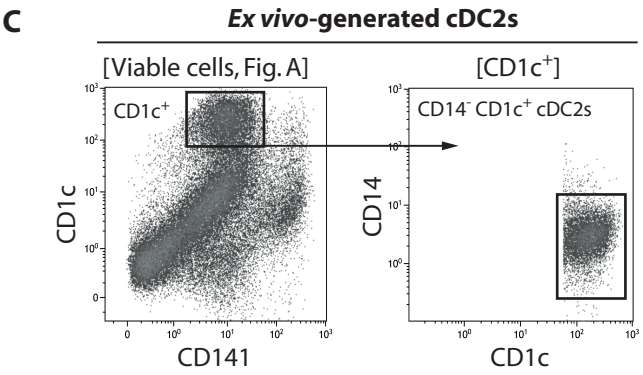
FITC: Fluorescein

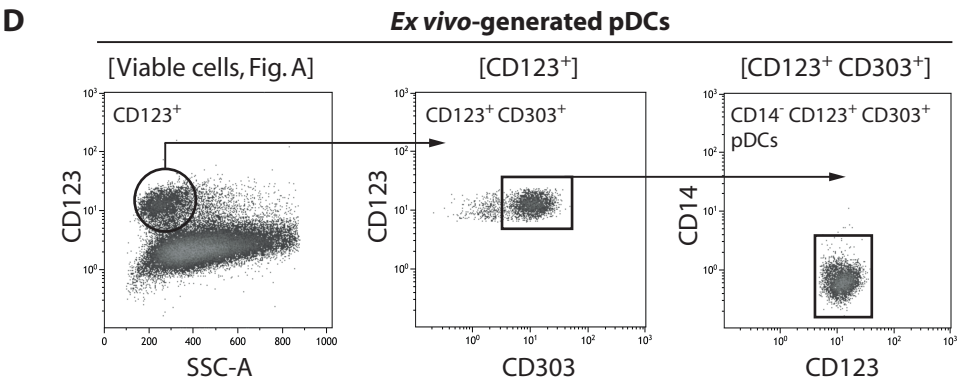
AF: Alexa fluor

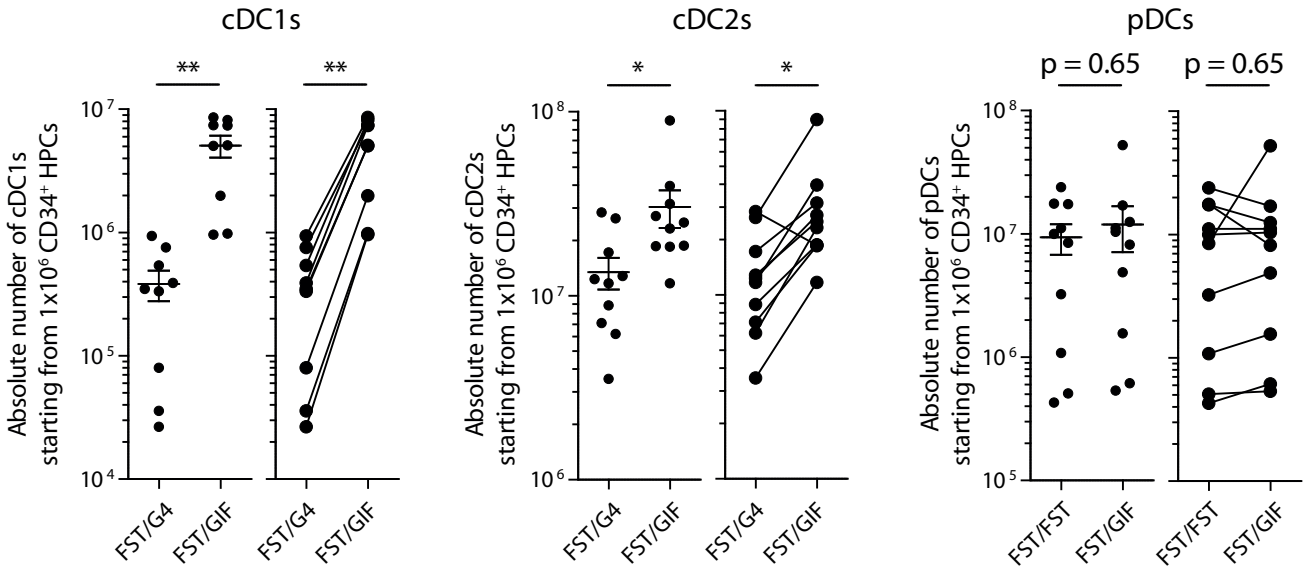
Supplemental Figure 1





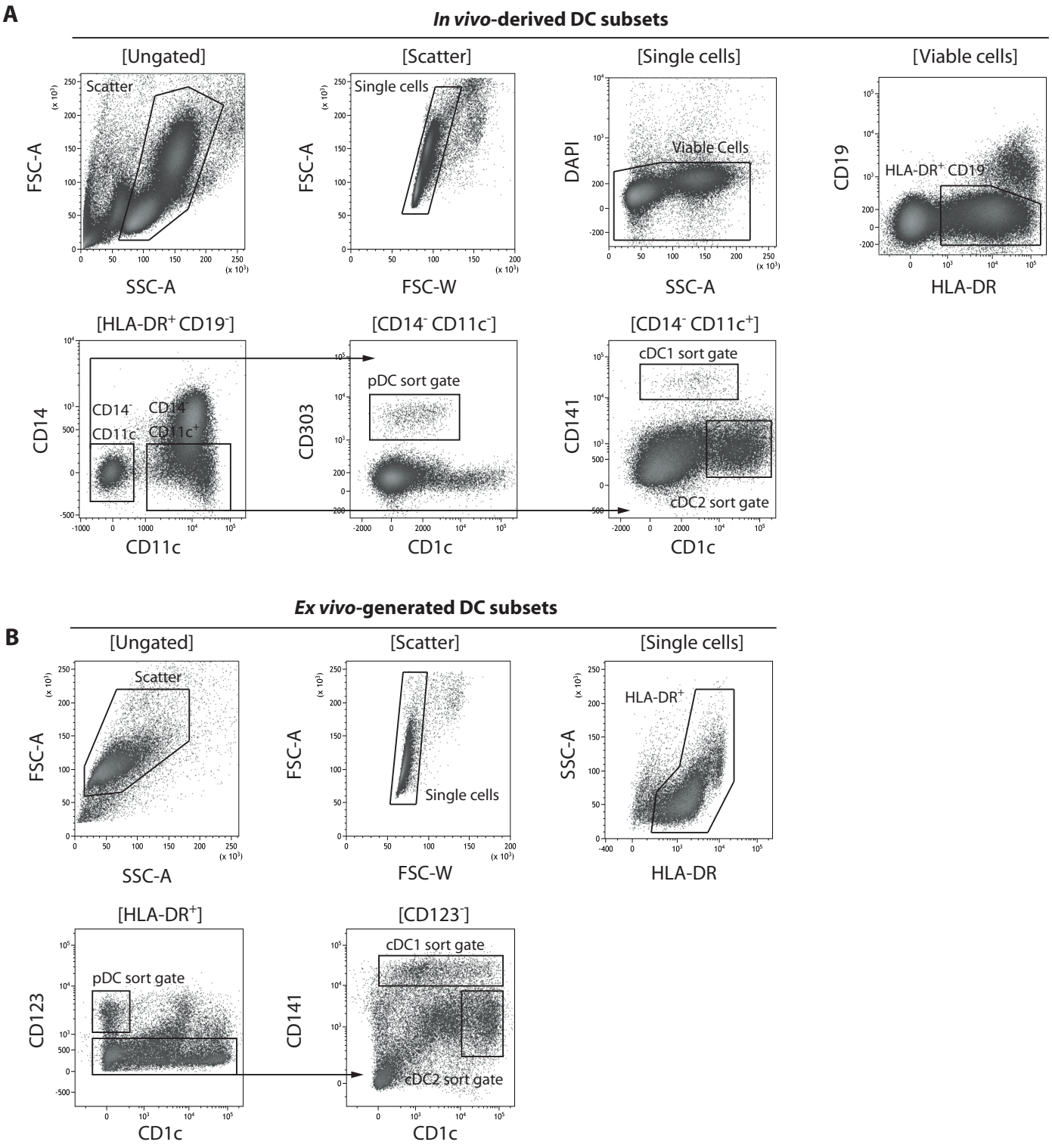






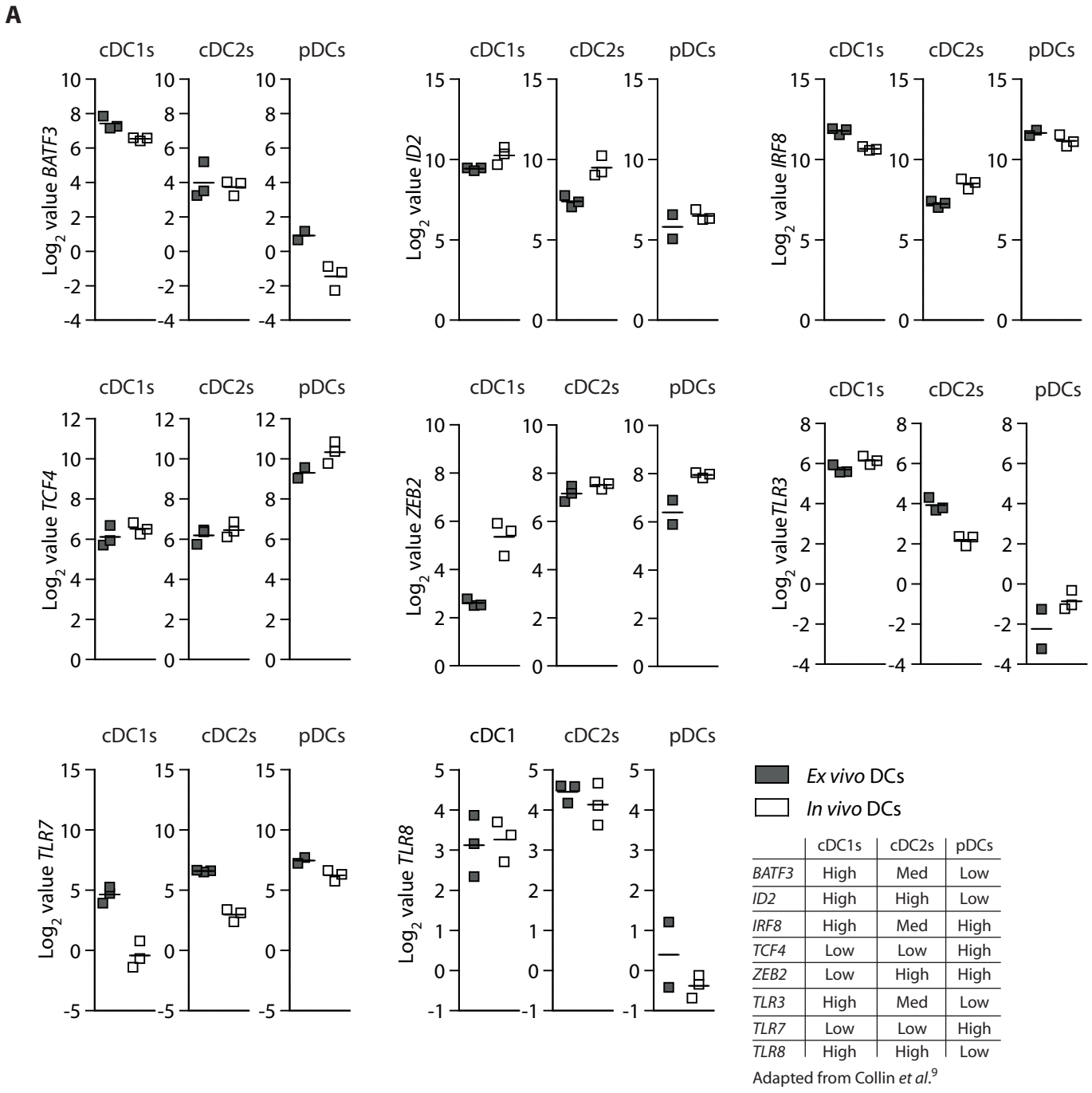
Supplementary Figure 1. Gating strategy and phenotype of *ex vivo*-generated cDC1s, cDC2s and pDCs. (A) Dot plots of a representative FST/GIF *ex vivo* HPC-DC culture showing gating strategy of HPC-derived DC subsets within total cultured cells. Cells are gated based on forward- and side scatter, doublets (FCS-A/SSC-A versus FCS-W/FCS-H) and dead cells (sytox blue positive) were excluded, followed by gating on the particular HPC-derived DC subsets using CLEC9A and CD141 for cDC1s, CD1c and HLA-DR for cDC2s and CD123 and CD303 for pDCs. (B-D) All *ex vivo*-generated DC subsets are $CD14^{-}$. (E) Absolute number of *ex vivo*-generated cDC1s, cDC2s and pDCs in the FST/GIF culture compared with previously reported FST/G4 and FST/FST culture protocols (18).

Supplemental Figure 2



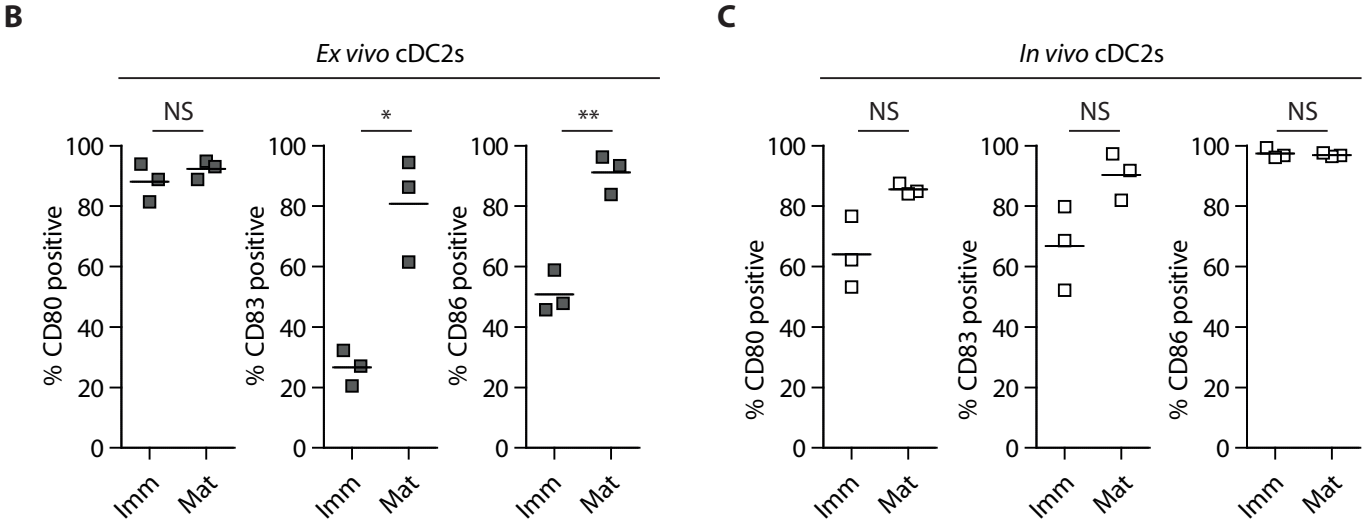
Supplementary Figure 2. Sort strategy *ex vivo*-generated- and *in vivo* DC subsets. (A-B) Dot plots of representative sorting strategy of *in vivo*-derived- (A) and *ex vivo*-generated (B) DC subsets.

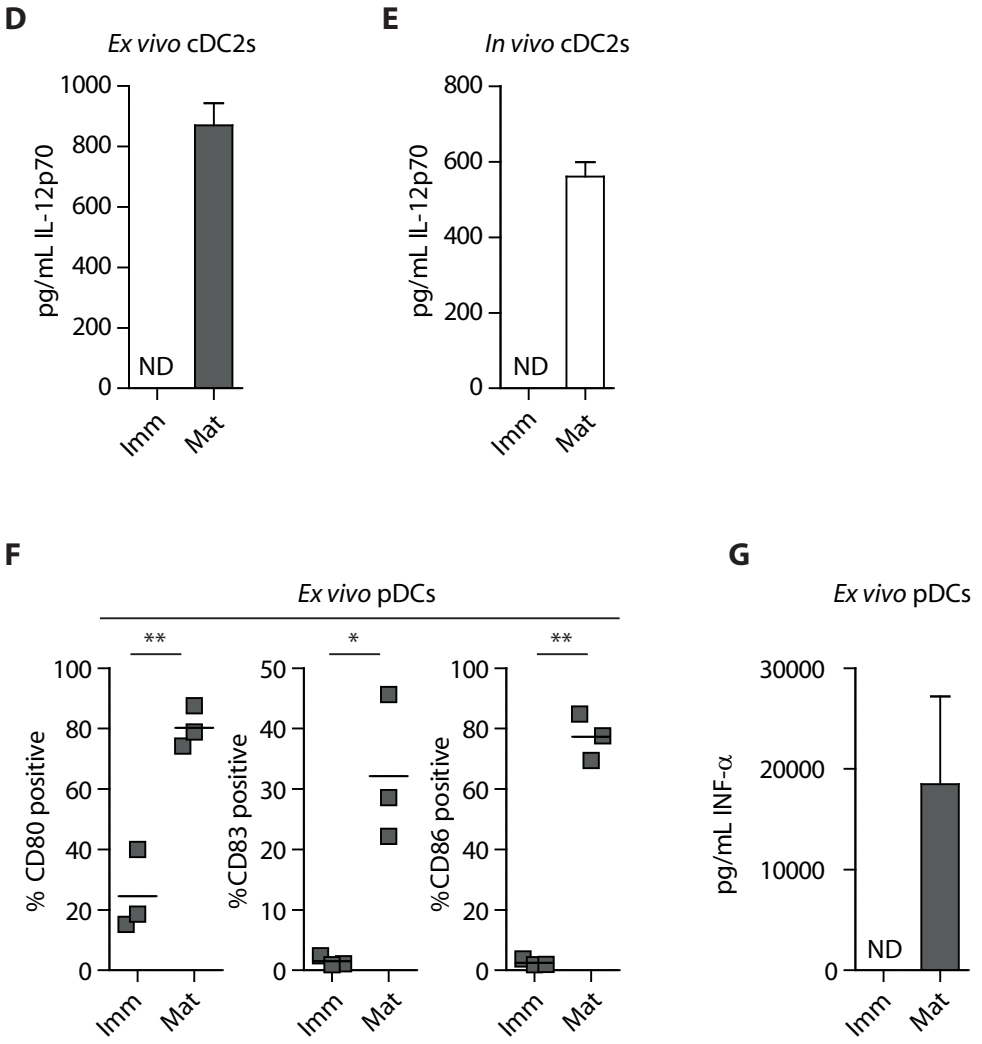
Supplemental Figure 3



	cDC1s	cDC2s	pDCs
BATF3	High	Med	Low
ID2	High	High	Low
IRF8	High	Med	High
TCF4	Low	Low	High
ZEB2	Low	High	High
TLR3	High	Med	Low
TLR7	Low	Low	High
TLR8	High	High	Low

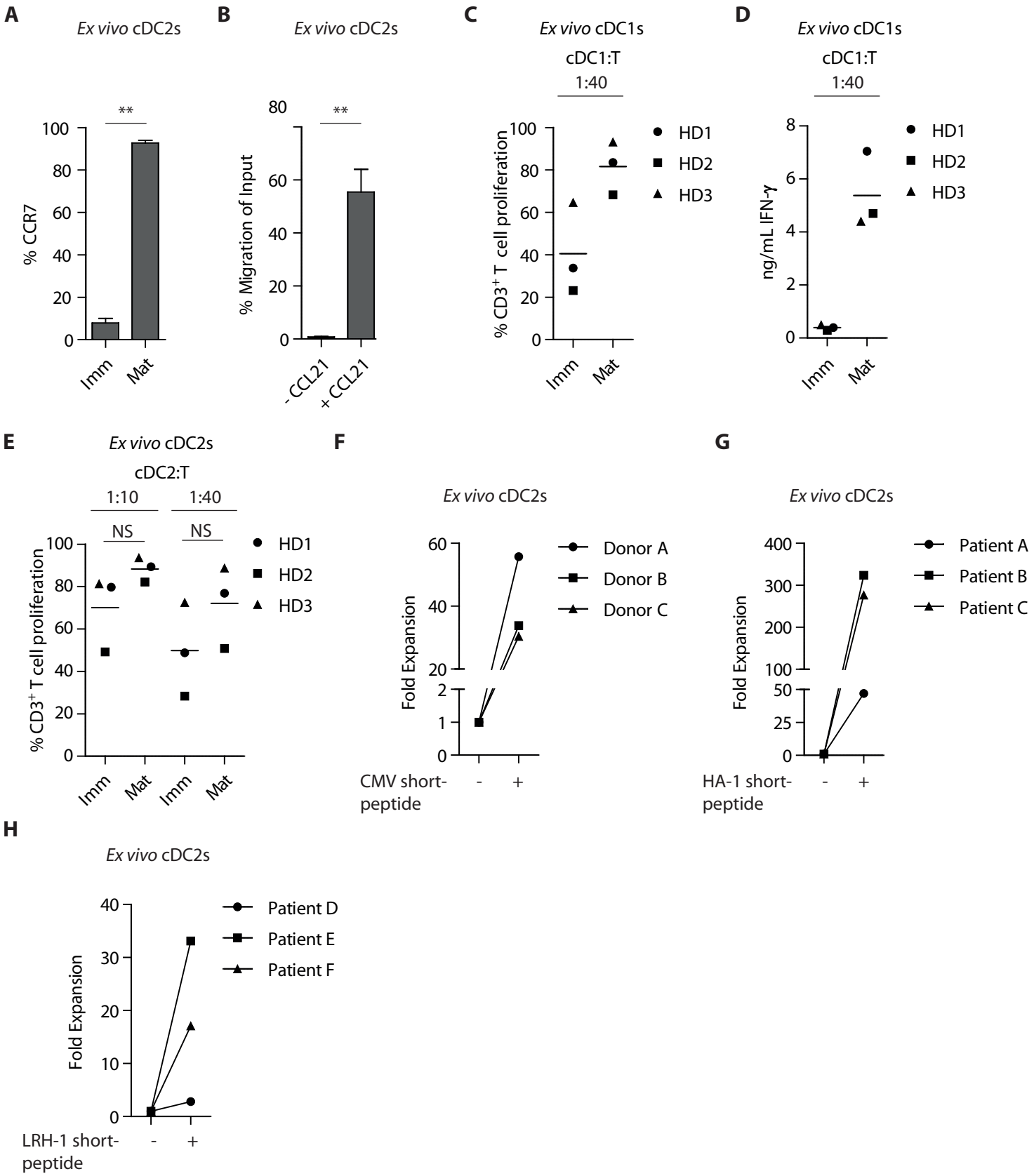
Adapted from Collin *et al.*[9]





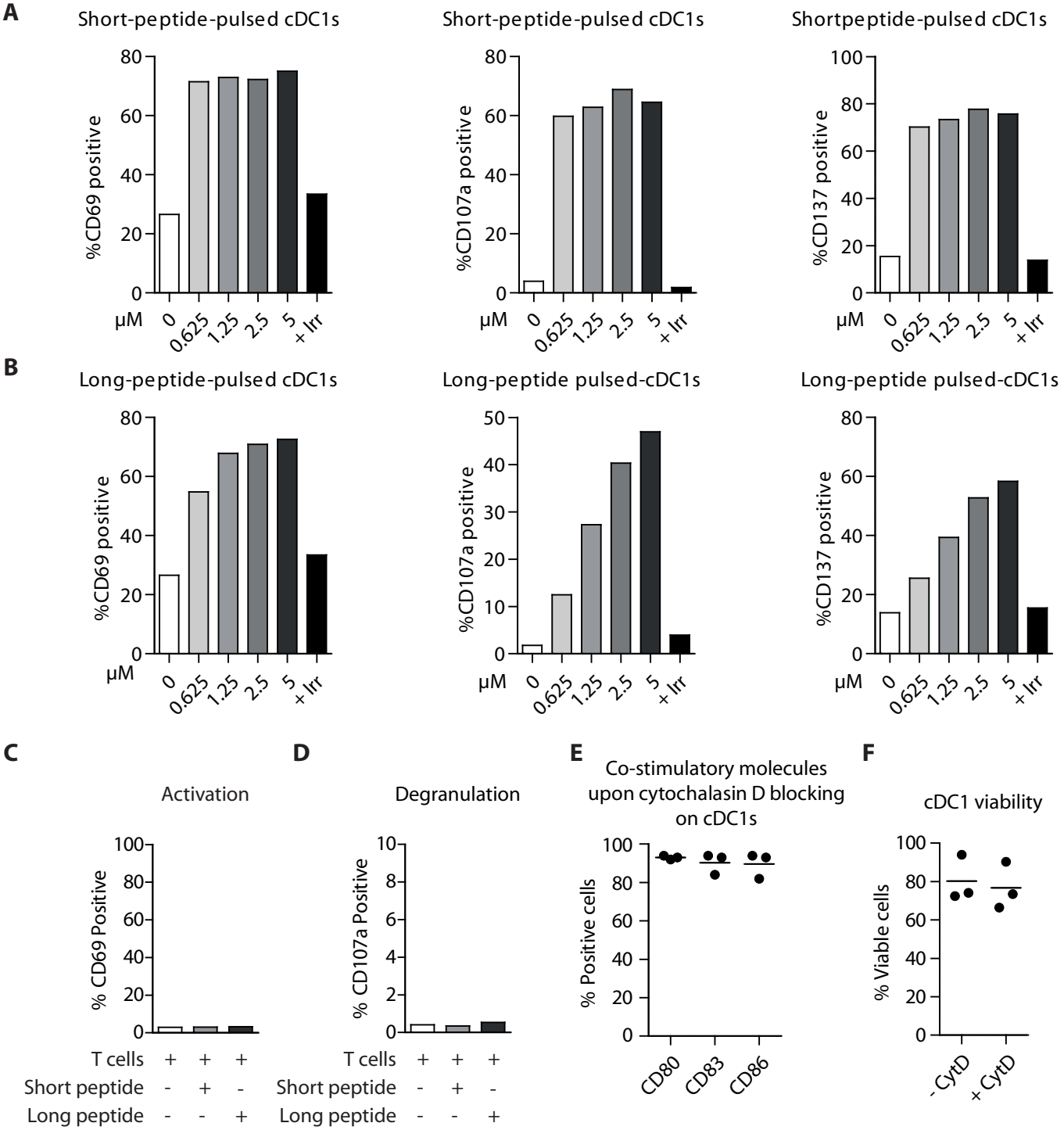
Supplementary Figure 3. *Ex vivo*-generated cDC2 and pDCs possess phenotypic and functional resemblance with their *in vivo* counterpart. (A) Expression of cDC1, cDC2 and pDC-related transcription factors and TLRs. (B-C) Expression of co-stimulatory molecules CD80, CD83 and CD86 on immature and mature *ex vivo*-generated (B) and *in vivo* cDC2s (C). (D-E) Release of pro-inflammatory cytokine IL12p70 by immature and mature *ex vivo*-generated (D) and *in vivo* cDC2s (E). (F-G) Expression of co-stimulatory molecules CD80, CD83 and CD86 on immature and mature *ex vivo*-generated pDCs. (G) Release of pro-inflammatory cytokine IFN-α by immature and mature *ex vivo*-generated pDCs. Data is shown as mean ± SEM (n=3). Statistical analysis was performed using paired T-test (B). TLR, toll like receptor. *** $P < 0.001$, ** $P < 0.01$. ND, non-detectable; SEM, standard error of the mean.

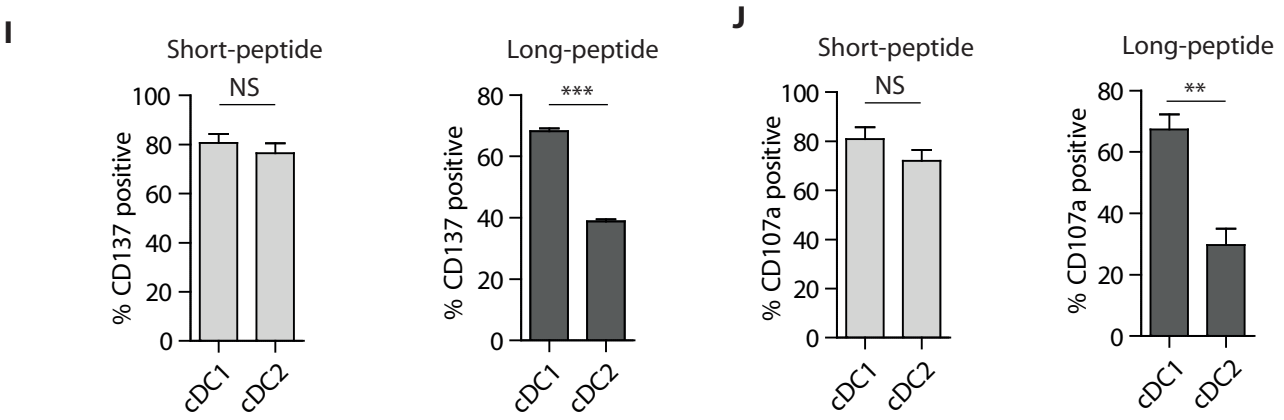
Supplemental Figure 4



Supplementary Figure 4. *Ex vivo*-generated cDC2s and/or cDC1s possess LN homing capacity, efficiently induce alloreactive T cell proliferation and boost expansion of tumor-reactive T cells. (A) Expression of LN homing chemokine receptor CCR7 on immature and mature *ex vivo*-generated cDC2s. Data is shown as mean ± SEM (n=3). (B) Percentage of *ex vivo*-generated cDC2 migration to LN homing chemokine CCL21 (250 ng/mL). Data is shown as mean ± SEM (n=3). (C, E) Percentage of allogeneic T cell proliferation at day 5 of culture induced by *ex vivo*-generated cDC1s (C) or *ex vivo*-generated cDC2s (E) using cDC:T cell ratio of 1:10 or 1:40 respectively. Lines indicate mean value (n=3). (D) Release of IFN-γ upon 5 days T cell stimulation using *ex vivo*-generated cDC1s in alloMLRs using a cDC1:T cell ratio of 1:40. Line indicate mean value (n=3). (F-H) Fold expansion of CMV, HA-1 -and LRH-1 specific $CD8^+$ T cell induced by short-peptide-pulsed *ex vivo*-generated cDC2s. Statistical analyses was performed using an unpaired T test (A, C-D) or paired T test (B) LN, lymph node; CCR7, C-C chemokine receptor 7; CCL21, C-C motif chemokine ligand 21; AlloMLRs, allogeneic mixed leukocyte reactions; SEM, standard error of the mean. ** $P<0.05$.

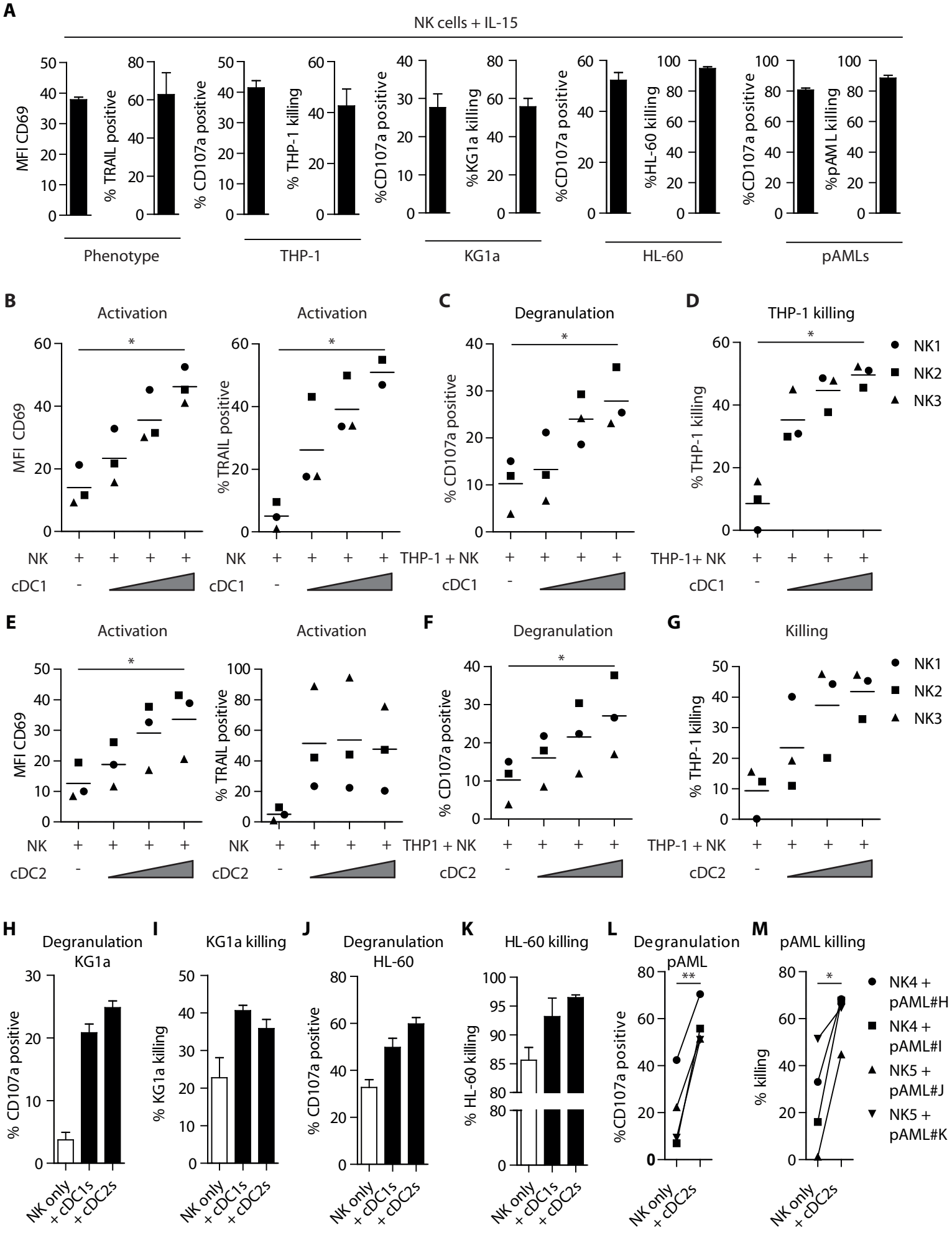
Supplemental Figure 5





Supplementary Figure 5. Short- and long-peptide titration, control conditions in antigen cross-presentation assays and antigen cross-presentation by *ex vivo*-generated cDC2s. (A-B) Activation shown by expression of CD69, CD107a and CD137 of HA-1 T cell receptor (TCR) transduced T cells by cDC1s pulsed with different concentration of short- and long HA-1 peptide. (C-D) Expression of activation marker CD69 (A) and T cell degranulation (B) by HA-1 TCR-transduced T cells in the absence of *ex vivo*-generated cDC1s or presence of HA-1 short- or HA-1 long-peptide only. (E-F) Expression of co-stimulatory molecules CD80, CD83 and CD86 on *ex vivo*-generated cDC1s treated with CytD. Lines indicate mean value (n=3). (D) Viability of *ex vivo*-generated cDC1s treated with/without CytD. Lines incubate mean value (n=3). (G-H) Percentage and fold expansion of patient-derived HA-1-specific T cells induced by long-peptide pulsed cDC2s. (I-J) Graphs showing CD137 expression and degranulation (CD107a expression) by HA-1-transduced TCR T cells induced by short -or long-peptide-pulsed cDC1s and cDC2s. Irr, irrelevant peptide; CytD, cytochalasin D.

Supplemental Figure 6



Supplementary Figure 6. *Ex vivo*-generated cDC1s and cDC2s enhance NK cell leukemia-reactivity. (A) Expression of CD69 and TRAIL, degranulation and tumor cell killing by IL-15-activated NK cell. Data is shown as mean ± SEM (n=3). (B, E) MFI of activation marker CD69 and percentage TRAIL expression on cDC1 and cDC2-activated NK cells after 48 hours co-cultured (cDC:NK cell ratios of 0.02:1, 0.2:5 and 1:1 respectively). Lines indicate mean value (n=3). (C, D, F, G) Degranulation (C, F) and THP-1 killing (D, G) by cDC1 and cDC2-activated NK cells 4 hours stimulated with THP-1 cells (NK cell:THP-1 cells ratio of 2:1). Lines indicate mean value. (H-K) Degranulation (H, J) and killing (I,K) by cDC1 and cDC2-activated NK cells 4 hours stimulated with KG1a (H-I) or HL-60 (J-K). (L, M) Degranulation by (L) and killing of pAML (M) by cDC2-activated NK cells 48 hours stimulated with primary AML cells. Statistical analyses was performed using Friedman test followed by Dunns post-hoc test (B, C, D, E and F) or paired T-test (L, M). * $P < 0.05$. NK, natural killer; TRAIL, TNF-related apoptosis-inducing ligand; pAML, primary acute myeloid leukemia; MFI, median fluorescence intensity.