SUPPLEMENTAL

Supplemental Table 1
Clinical and pathological characteristics of AML patient samples used in the current study.

Supplemental Figure 1
(A) AML CD300f gene expression by ELN risk status. Mean with SEM. (B) MFI ratios of CD33 and CD300f on the $CD34^+$ $CD38^-$ subset of patient derived AML (n = 22) are compared with linear regression. (C) TiSNE of CD300f expression on AML samples from 9 patients. (D) TiSNE of CD33 expression on AML samples from 9 patients.

Supplemental Figure 2
MFI ratios of CD300f on the peripheral blood subsets from healthy donors (n = 3). Mean with SEM .

Supplemental Figure 3
Gating strategy to identify HSPC groups via flow cytometry.

Supplemental Figure 4
(A) Viability of MoDC treated with either DCR-2-PBD or isotype-PBD after 96 hours compared to PBS control. Representative figures of activation markers CD83 (B) or CD80 (C) on non-activated or activated myeloid DC with or without DCR-2-PBD. T cell division using a one way MLR with DCR-2-PBD or isotype-PBD, readout after 96 hours, both groups compared to PBS control.

SUPPLEMENTAL METHODS

Antibodies

AML samples were phenotyped with a monoclonal antibody (mAb) panel containing CD300f -efluor660 (clone UP-D1) (ThermoFisher) CD45-V500 (HI30), CD34-PE-CY7 (581), CD38-V450 (HB7), CD33-PE (WM53) and CD11c-APC-AF700 (B-Ly6) from BD Biosciences and HLA-DR-APC (L243) (Biolegend). Healthy BM samples were phenotyped with a lineage (Lin) cocktail containing V450 conjugated CD235a (GA-R2), CD14 (MφP9), CD20 (2H7), CD19 (HIB19), CD56 (NCAM16.2) and CD3 (SK) (BD Biosciences). A mAb panel containing CD3-V450 (SP34-2), CD19-V450 (HIB19), CD56-V450 (B159), CD14-V450 (M5E2), CD11c-AF700 (B-ly6), CD80-PECy7 (L307.4) all from BD biosciences, HLA-DR-APC and CD83-FITC (HB15a) (Beckman Coulter) was used to assess CD83 and CD80 expression on myeloid cell populations. Healthy PB was phenotyped using CD45-AF488 (HI30), CD3-AF700 (SP34-2), CD56-PECy7 (NCAM16.2) and CD19-V450 all from BD Biosciences. Cell populations from mouse xenografts were phenotyped with anti-human CD45-AF488, CD33-PE (WM53), and anti-mouse CD45-PerCP Cy5.5 (30-F11) (BD Biosciences). Samples from the humanised NSG mice (Hu-NSG) were phenotyped with CD3-V450 (SP34-2), CD19-V450, CD56-V450, CD45-AF488, CD34-PE-CF594 (581), CD33-PE, CD38-APC (HIT2), CD90-AF700 (5E10) and anti-mouse CD45-PerCP Cy5.5 (BD Biosciences). Binding is displayed as a geometric mean fluorescence intensity (geoMFI) ratio which was calculated by: geoMFI test antibody/geoMFI isotype control.

Internalisation Assays

DCR-2 (IgG_1), and as an isotype control, the anti-tetanus toxoid mAb, CMRF-81 (IgG_1) which were both produced, purified and directly conjugated to phycoerythrin (PE) in house. Cells were incubated with DCR-2-PE or CMRF-81-PE (10μg/ml) on ice for 20 min, washed to

remove unbound mAb then incubated at 37°C/5% CO_2 to allow internalisation for the indicated times. After incubation, residual mAb remaining on the cell surface was detected with a secondary goat anti-mouse (GAM) IgG-AF488 antibody. Cells were fixed in 1% paraformaldehyde/PBS for flow cytometry analysis. The surface and total relative Mean Fluorescent Index (MFI) was calculated as the (MFI of binding antibody at time point – MFI of isotype control at time point) at 37°C/ (MFI of binding antibody at time point– MFI of isotype control at time point) at 0ºC. The percent internalised was assessed by 100- (relative MFI surface staining / relative MFI total staining). In immunofluorescent microscopy experiments cells were stained with DCR-2 then air-dried and fixed with 4% paraformaldehyde or incubated at 37°C for 30 minutes then fixed. Rehydration was performed using 1% BSA/PBS prior to staining with GAM IgG-AF488 antibody and 18μM DAPI (ThermoFisher).

MoDC Toxicity Assay
MoDC were incubated with DCR-2-PBD or isotype-PBD (200pmol) 72 hours prior to enumeration of live cells by flow cytometry.

DC Activation Assay
Peripheral Blood Mononuclear Cells (PBMC) were incubated in complete RPMI media with or without DCR-2-PBD (200pmol). After 12 hours, washed cells were incubated for a further 12 hours with or without LPS then DCR-2-PBD or isotype-PBD (200pmol) were added for 72 hours prior to enumeration of live cells by flow cytometry. The expression of CD83 and CD80 was assessed on Lineage$^-$ HLADR$^+$CD11c$^+$ myeloid DC.

Mixed Leucocyte Reaction
T cells were depleted from PBMC by magnetic selection using an AutoMACS Pro with anti-CD3 mAb (HIT3a, Biolegend) with greater than 90% depletion in all samples. The CD3-depleted PBMC were incubated in complete RPMI media with DCR-2-PBD (200pmol), isotype-PBD (200pmol) or PBS for 24 hours. After washing to remove unbound ADC, the CD3-depleted PBMC were used to stimulate allogeneic *Carboxyfluorescein Succinimidyl Ester* (CFSE) labelled naive CD4$^+$ T cells which had been prepared using a RosetteSep Kit (Stem Cell Technologies 17555). On day 5 the proliferation of T cells, identified using anti-CD3 AF700 (SP34-2), was assessed by CFSE reduction using flow cytometry. The results of the DCR-2-PBD and isotype-PBD groups were normalised to the PBS control group. Stimulator populations were prepared from three PBMC donors and experiments were performed in duplicate.

Cytotoxicity Assays
Cytotoxicity was determined by incubating 5000 target cells with DCR-2-PBD, isotype-PBD or PBS in 200μl total volume of complete RPMI for 96 hours at 37°C/5% CO_2 after which DAPI$^-$ viable cells were enumerated using flow cytometry. Events per condition were compared to the mean of the control group to obtain the % viable. Bystander killing of CD300f$^-$ Mino cells was performed as above. After incubation, live bystander cells were identified with CD20-PE (2H7) and DAPI. Kinetic analysis was performed as above. At the indicated time points, washed cells were resuspended in complete RPMI and cultured for 96 hours before analysis. Synergy between ADC and fludarabine was assessed as above by combining DCR-2-PBD and/or fludarabine in samples. All samples were tested in triplicate.

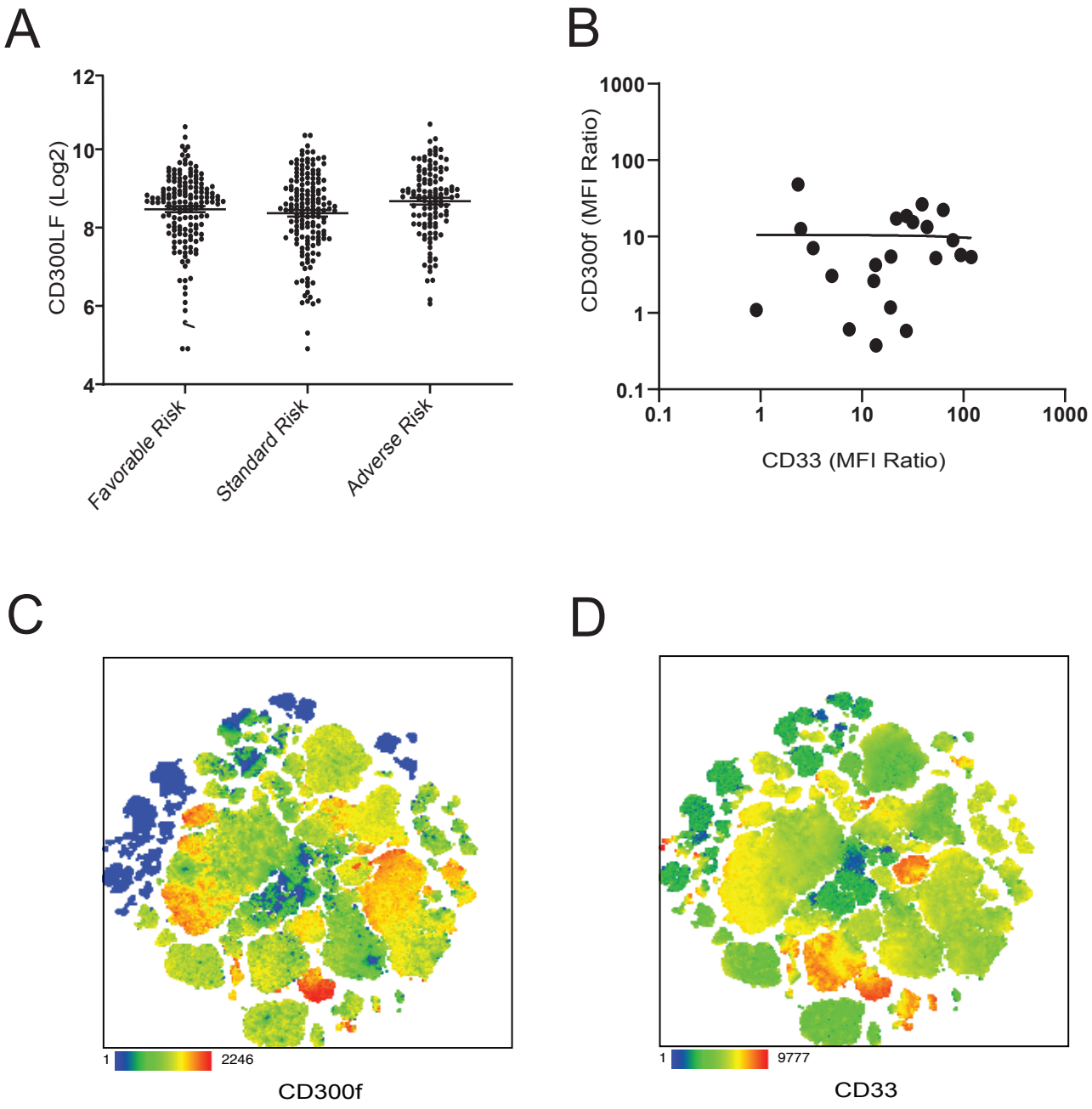
A
CD300LF (Log2)
12
10
8
6
4
Favorable Risk
Standard Risk
Adverse Risk
B
CD300f (MFI Ratio)
1000
100
10
1
0.1
0.1
1
10
100
1000
CD33 (MFI Ratio)
C
1
2246
CD300f
D
1
9777
CD33

Figure S1

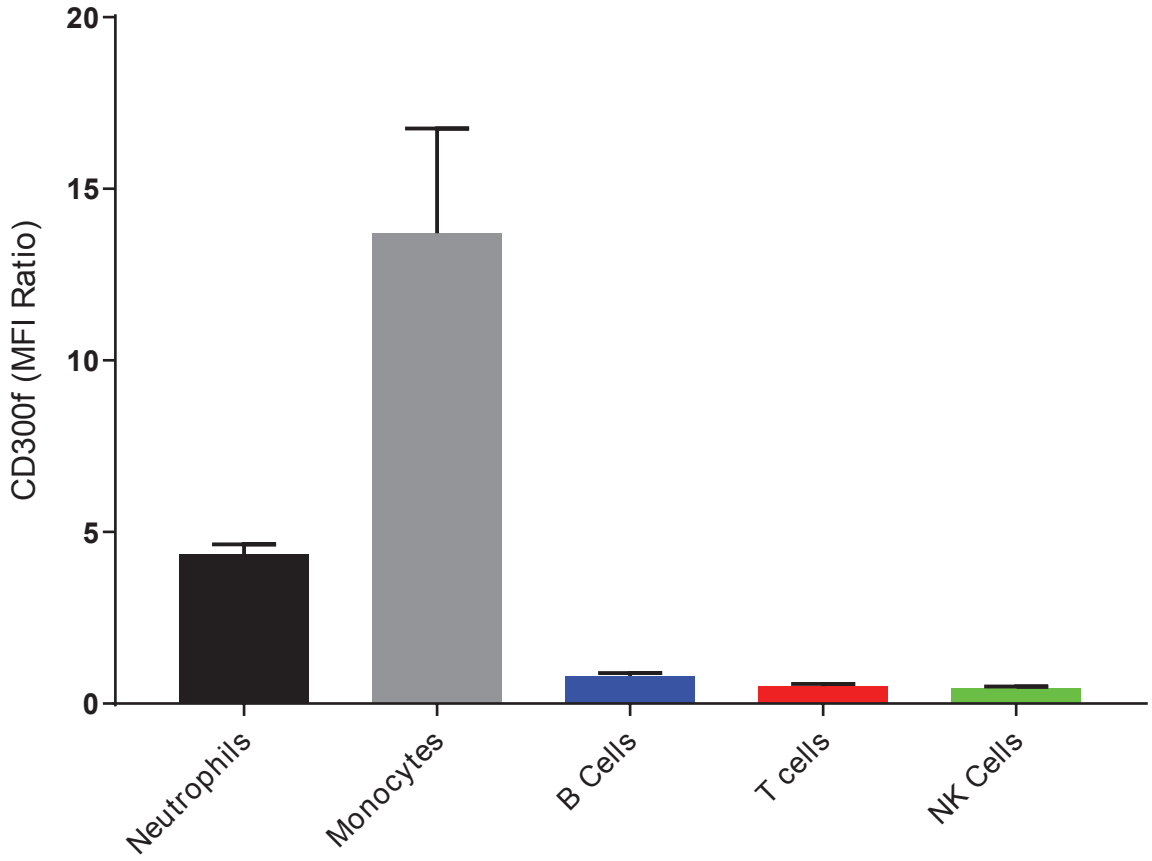
20
15
10
5
0
CD300f (MFI Ratio)
Neutrophils
Monocytes
B Cells
T cells
NK Cells

Figure S2

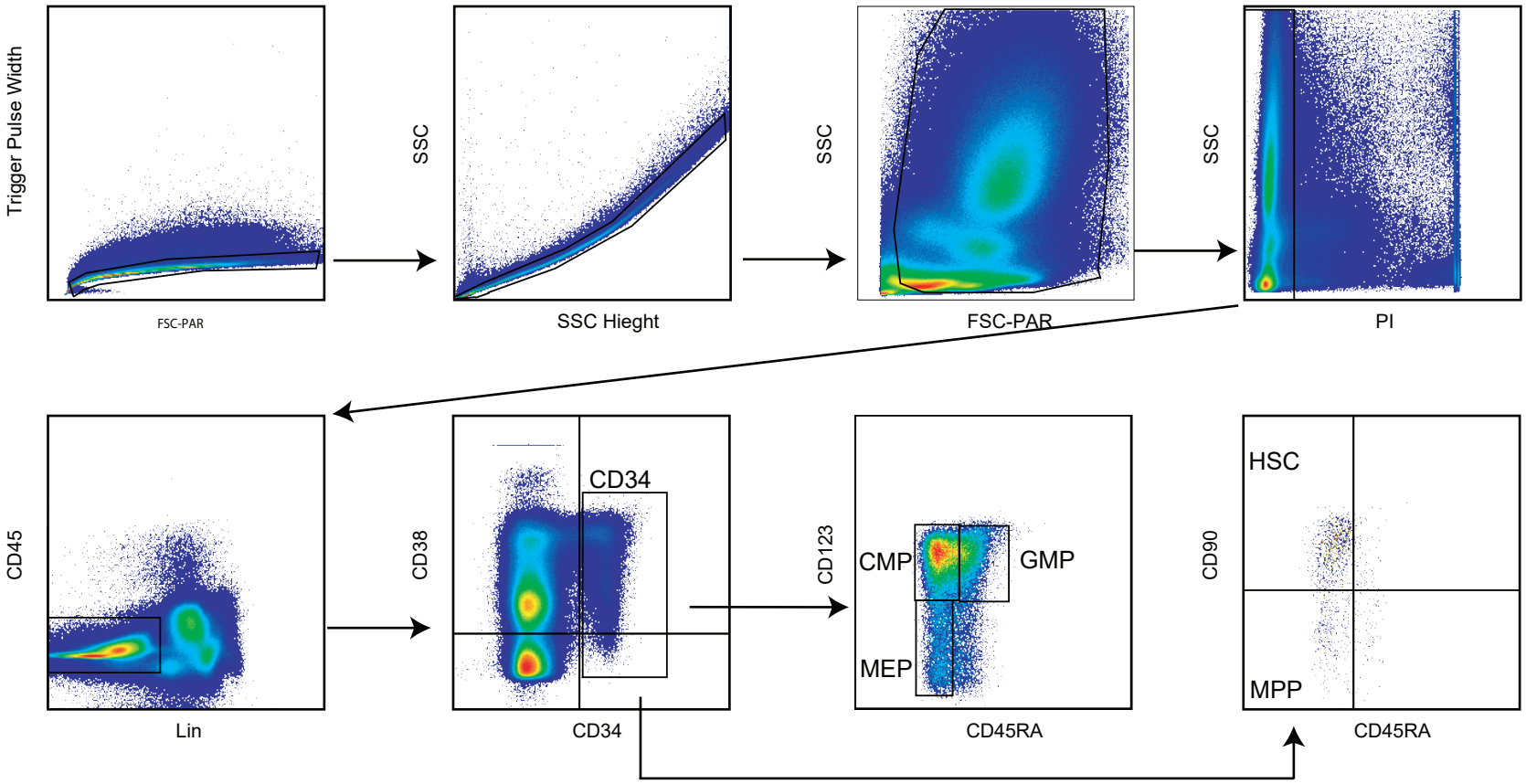
Trigger Pulse Width
FSC-PAR
SSC
SSC Hieght
SSC
FSC-PAR
SSC
PI
CD45
Lin
CD38
CD34
CD34
CD123
CMP
GMP
MEP
CD45RA
CD90
HSC
MPP
CD45RA

Figure S3

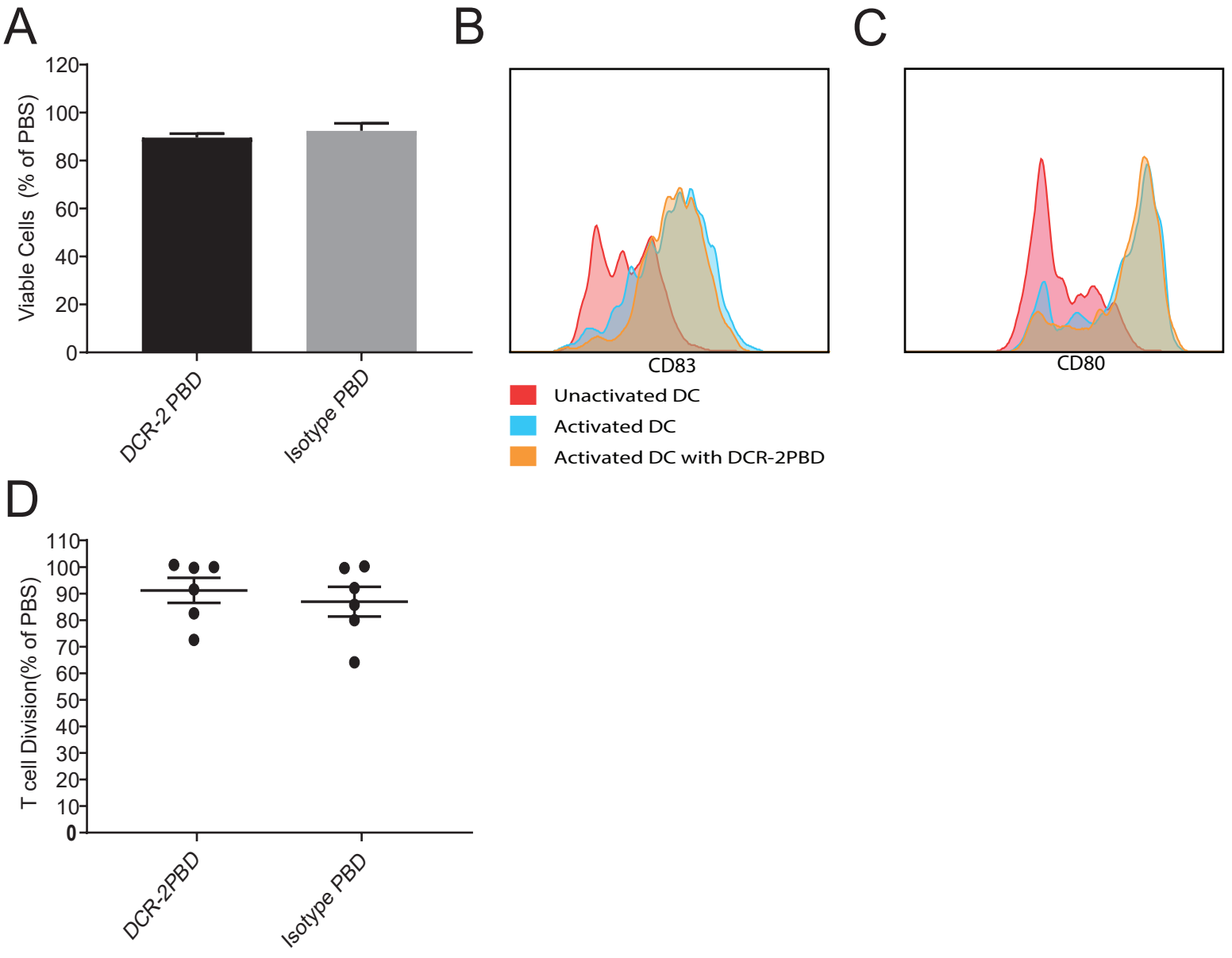
A
B
C
Viable Cells (% of PBS)
120
100
80
60
40
20
0
DCR-2 PBD
Isotype PBD
CD83
CD80
Unactivated DC
Activated DC
Activated DC with DCR-2PBD
D
T cell Division(% of PBS)
110
100
90
80
70
60
50
40
30
20
10
0
DCR-2PBD
Isotype PBD

Figure S4