Isatuximab Acts Through Fc-Dependent, Independent, and Direct Pathways to Kill Multiple Myeloma Cells

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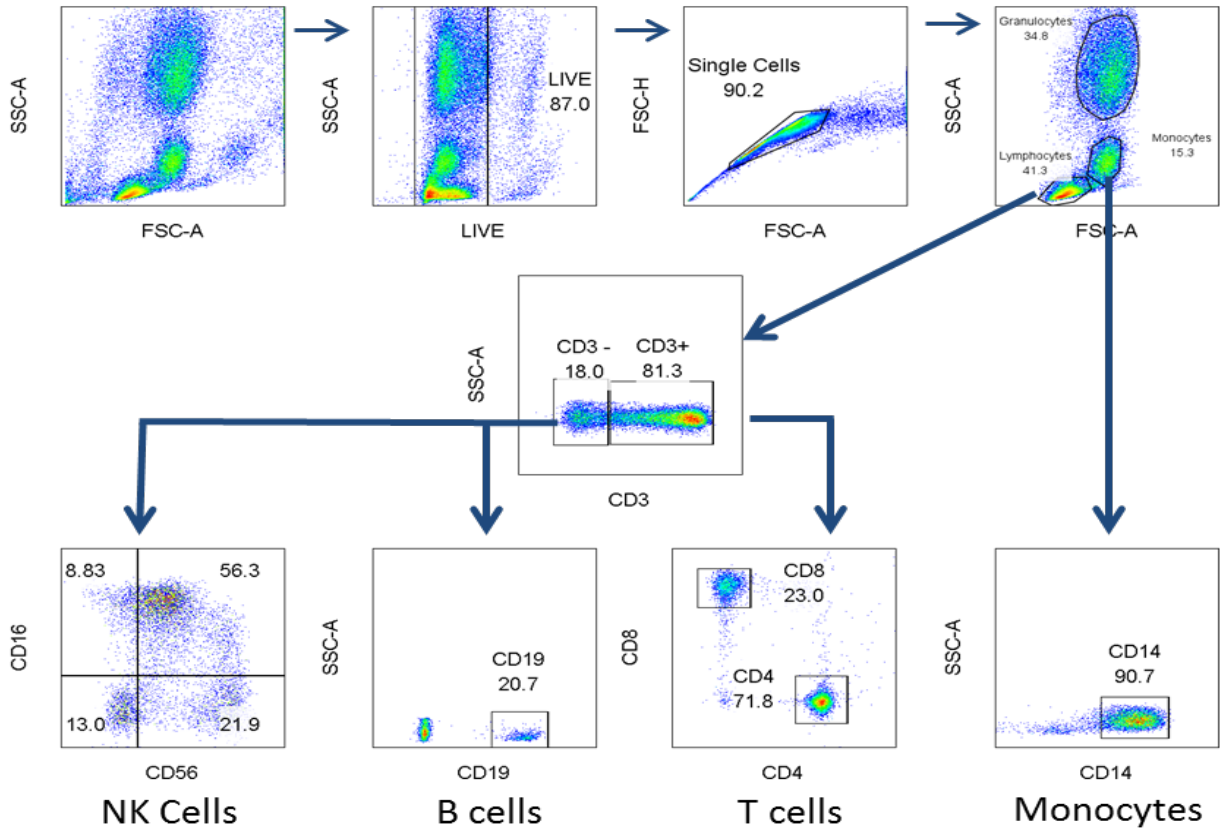
SUPPLEMENTARY MATERIAL

Supplementary Table 1. CCLE (1) cell lines used in this study.

CCLE cell line	Indication
BJAB	Diffuse large B cell lymphoma
JURKAT	Acute lymphoblastic T cell leukemia
Daudi	Burkitt lymphoma
EJM	Plasma cell myeloma
HBL-1	Diffuse large B cell lymphoma
HT	B cell lymphoma (unspecified)
HuNS1	Plasma cell myeloma
JJN-3	Plasma cell myeloma
KARPAS-620	Plasma cell myeloma
KMS-11	Plasma cell myeloma
KMS-12-BM	Plasma cell myeloma
KMS-20	Plasma cell myeloma
KMS-26	Plasma cell myeloma
L-363	Plasma cell myeloma
LP-1	Plasma cell myeloma
MM.1S	Plasma cell myeloma
MOLP-2	Plasma cell myeloma
MOLP-8	Plasma cell myeloma
NCI-H929	Plasma cell myeloma
OCI-LY10	Diffuse large B cell lymphoma
OCI-LY19	Diffuse large B cell lymphoma
OCI-LY3	Diffuse large B cell lymphoma
OPM-2	Plasma cell myeloma
RPMI-8226	Plasma cell myeloma
SK-MM-2	Plasma cell myeloma
SUDHL-10	Diffuse large B cell lymphoma
SUDHL-4	Diffuse large B cell lymphoma
SUDHL-6	Diffuse large B cell lymphoma
SUDHL-8	Diffuse large B cell lymphoma
TMD-8	Diffuse large B cell lymphoma
U266	Plasma cell myeloma

U2932	Diffuse large B cell lymphoma
U2973	Diffuse large B cell lymphoma
WSU-DLCL2	Diffuse large B cell lymphoma

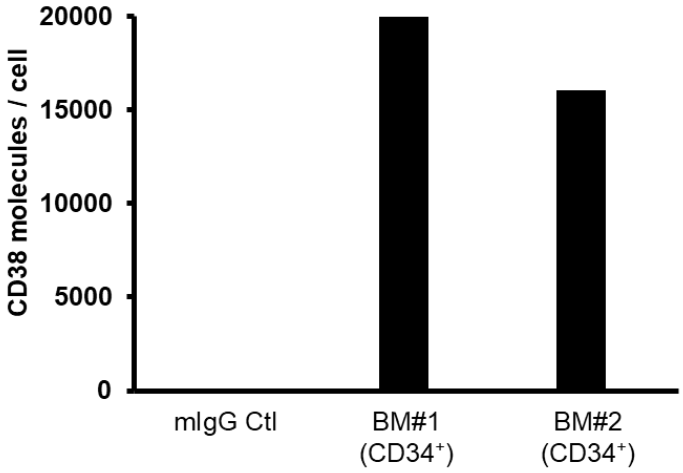
CCLE, Cancer Cell Line Encyclopedia.



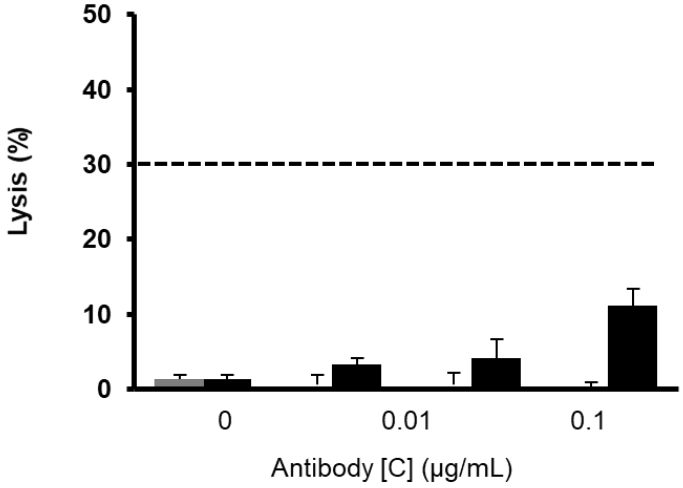
Supplementary Figure 1. Gating strategy for granulocytes, NK cells, B cells, monocytes, and CD4 and CD8 T cells in human PBMCs.

FSC-A, forward scatter area; FSC-H, forward scatter height; NK, natural killer; PBMC, peripheral blood mononuclear cell; SSC-A, side scatter area

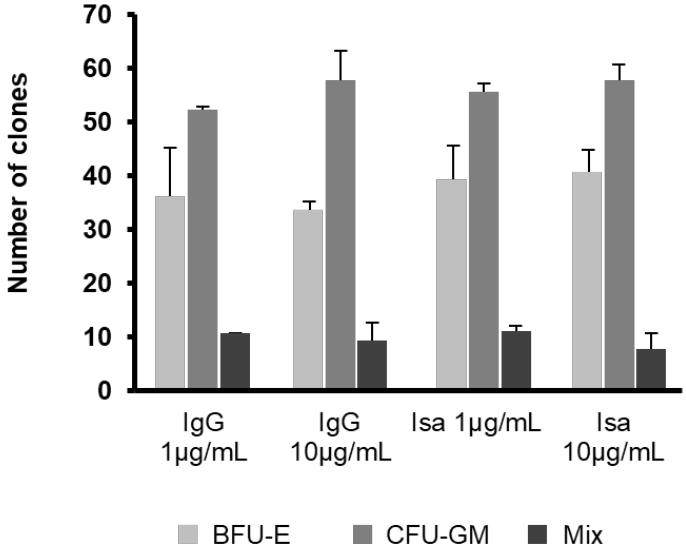
A


20000
15000
10000
5000
0
CD38 molecules / cell
mIgG Ctl
BM#1
(CD34+)
BM#2
(CD34+)

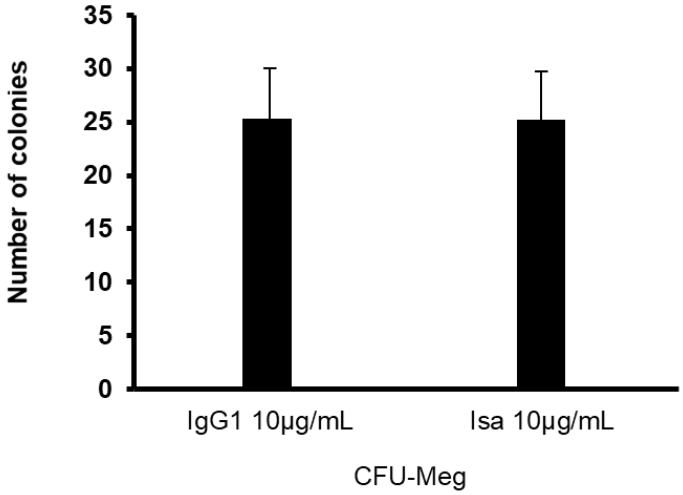
B


50
40
30
20
10
0
Lysis (%)
0
0.01
0.1
Antibody [C] (µg/mL)

C



D

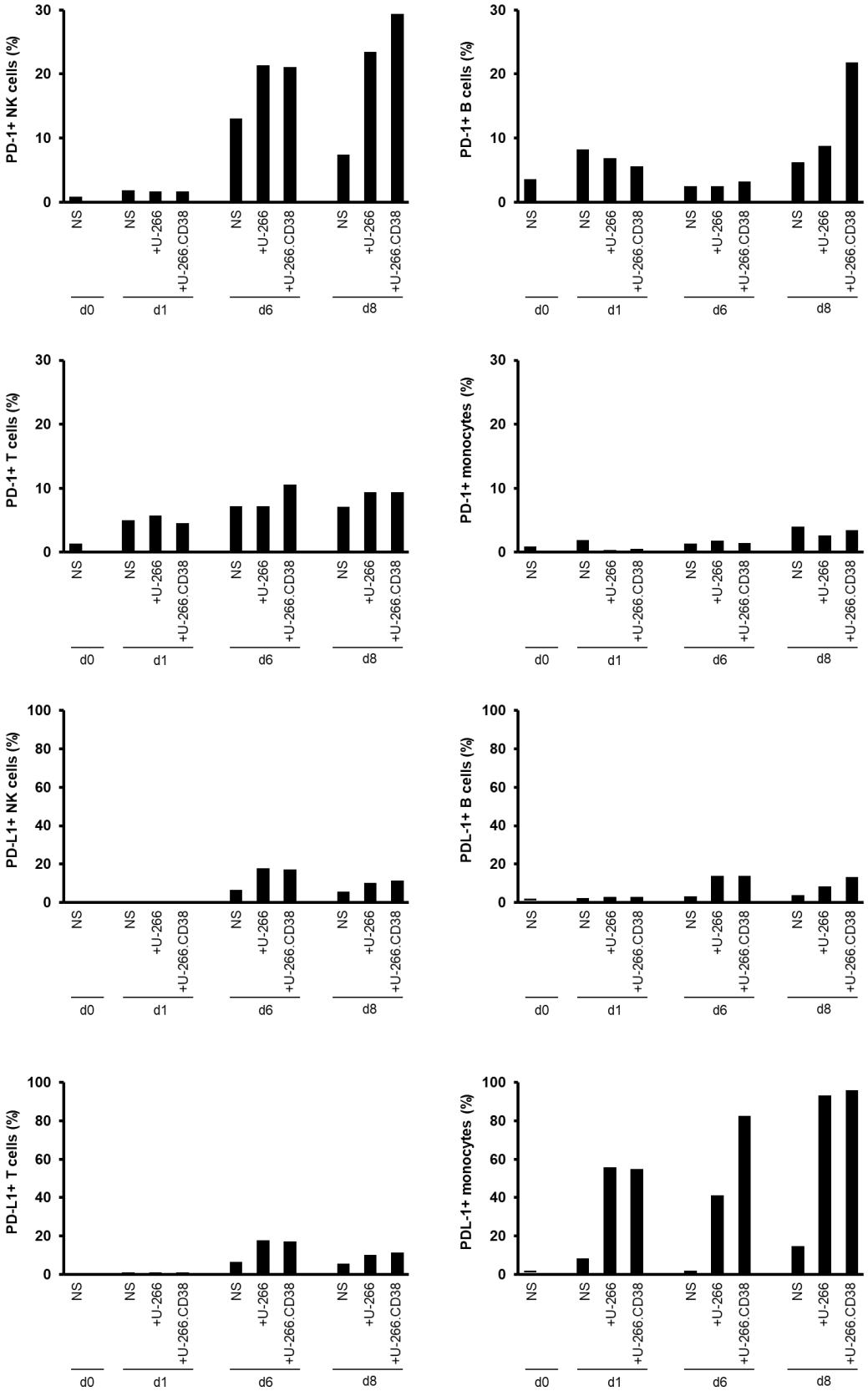


Supplementary Figure 2. Effect of isatuximab on human bone marrow $CD34^+$ progenitor cells. (**A**) Human bone marrow $CD34^+$ progenitor cells from two donors were stained with equal amount of mouse IgG1, k isotype control antibody (clone MOPC-21) or mouse anti-human CD38 antibody (clone AT13/5). The surface density of CD38 was quantified by QIFIKIT and FACS analysis. (**B**) Purified BM stem cells were tested for their sensitivity to isatuximab-induced ADCC. The ratio of effector cells (NK-92.$CD16^{V/V}$ cells) and target cells (BM stem cells) was 5:1. The percentage of ADCC

lysis was measured by calcein AM release from the stem cells. Black dashed line indicates a threshold for isatuximab induced ADCC in the assay.

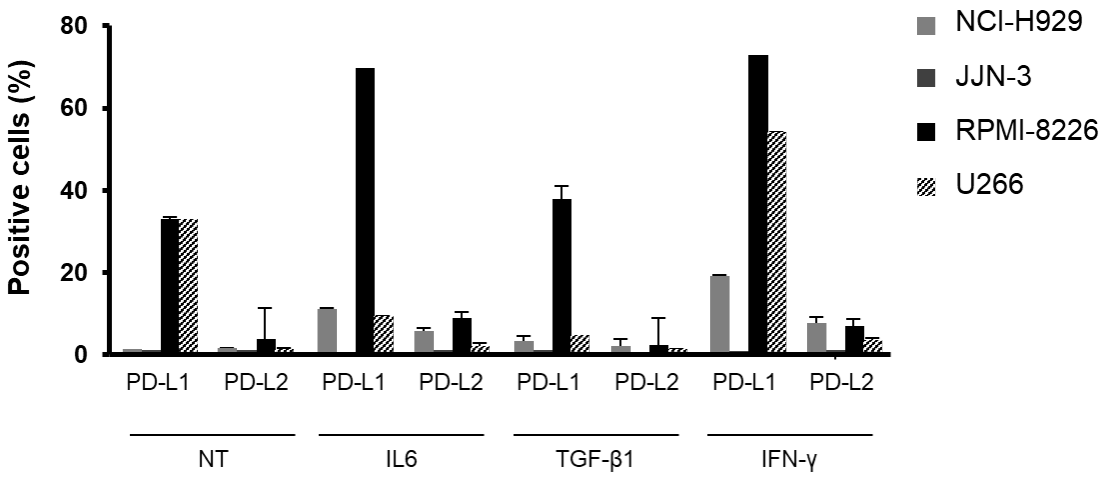
(**C**) 5×10^2 purified BM stem cells were cultured in 35 mm culture dishes for 14 to 16 days with the presence of indicated concentrations of isatuximab or IgG1 isotype control in MethoCult™ H4034 Optimum medium. BFU-E, CFU-GM and mix colonies were quantified under a microscopy. Each treatment was analyzed in duplicate. Results are Mean (SD). (**D**) 5×10^3 purified BM stem cells were cultured in Double Chamber Slides for 10 to 12 days at 37°C with the presence of indicated concentrations of isatuximab or IgG1 isotype control in MegaCult™-C Medium with Cytokines, followed by applying anti-CD41 immunostaining process. CFU-Meg colonies then were quantified under a microscopy. Each treatment was analyzed in duplicate. Results are Mean (SD).

BM, bone marrow; ADCC, antibody-dependent cellular cytotoxicity; BFU-E, furst-forming unit-erythroid; CFU-GM, colony-forming unit-granulocyte macrophage; CFU-Meg, colony-forming unit megakaryocyte; IgG1, immunoglobulin G1; SD, standard deviation


PD-1+ NK cells (%)
PD-1+ B cells (%)
PD-1+ T cells (%)
PD-1+ monocytes (%)
PD-L1+ NK cells (%)
PDL-1+ B cells (%)
PD-L1+ T cells (%)
PDL-1+ monocytes (%)
0
10
20
30
40
60
80
100
NS
+U-266
+U-266.CD38
d0
d1
d6
d8

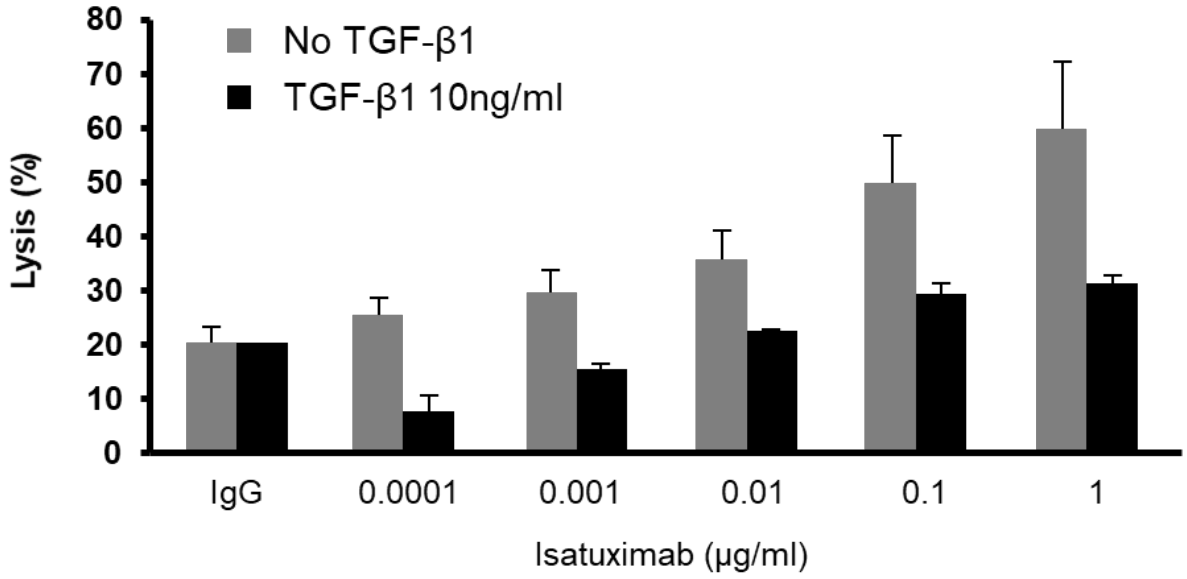
Supplementary Figure 3. Induction of PD-1 and PD-L1 expression on immune cells after co-culture with MM cells. 5×10^6 PBMCs cultured with or without 5×10^5 U266 cells overexpressing CD38 (U266.CD38^{++}) in ultra-low attachment 6-well plates were analyzed for expression of PD-1 and PD-L1 on CD3$^+$ (T cells), CD14$^+$ (monocytes), and CD56$^+$ (NK cells) cells by flow cytometry on days 0, 1, 6, and 8 during the co-culture. Each assay was performed four to six times, using samples from six donors.

MM, multiple myeloma; NK, natural killer; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1.



Supplementary Figure 4. Effect of PD-L1/PD-1 interaction on isatuximab ADCC activity. 2×10^6 MM cells were treated with 10 ng/ml of IL-6 or TGF-β1 or 100 ng/ml of IFN-γ at 37ºC for 48 hours and the expression of PD-L1 and PD-L2 was quantified by flow cytometry. Experiments were performed at least three times in triplicate for each criterion.

ADCC, antibody-dependent cellular cytotoxicity; IFN-γ, interferon-gamma; IL-6, interleukin-6; NT, not treated; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; PD-L2, programmed cell death-ligand 2; TGF-β, transforming growth factor-beta.



Supplementary Figure 5. Pre-conditioning of NK-92.CD16$^{V/V}$ cells with recombinant TGF-β1 reduces isatuximab-induced ADCC. NK-92.CD16$^{V/V}$ cells were treated with 10 ng/ml of TGF-β1 at 37°C for 90 hours and used as effector cells in isatuximab-mediated killing of MOLP-8 target cells and ADCC quantified by calcein AM release. The experiment was performed in technical triplicate.

ADCC, antibody-dependent cellular cytotoxicity; IgG, immunoglobulin G; TGF-β1, transforming growth factor-beta 1.

References

1. Broad Institute. Cancer Cell Line Encyclopedia. Available from: https://portals.broadinstitute.org/ccle.