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Supplemental information

Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance

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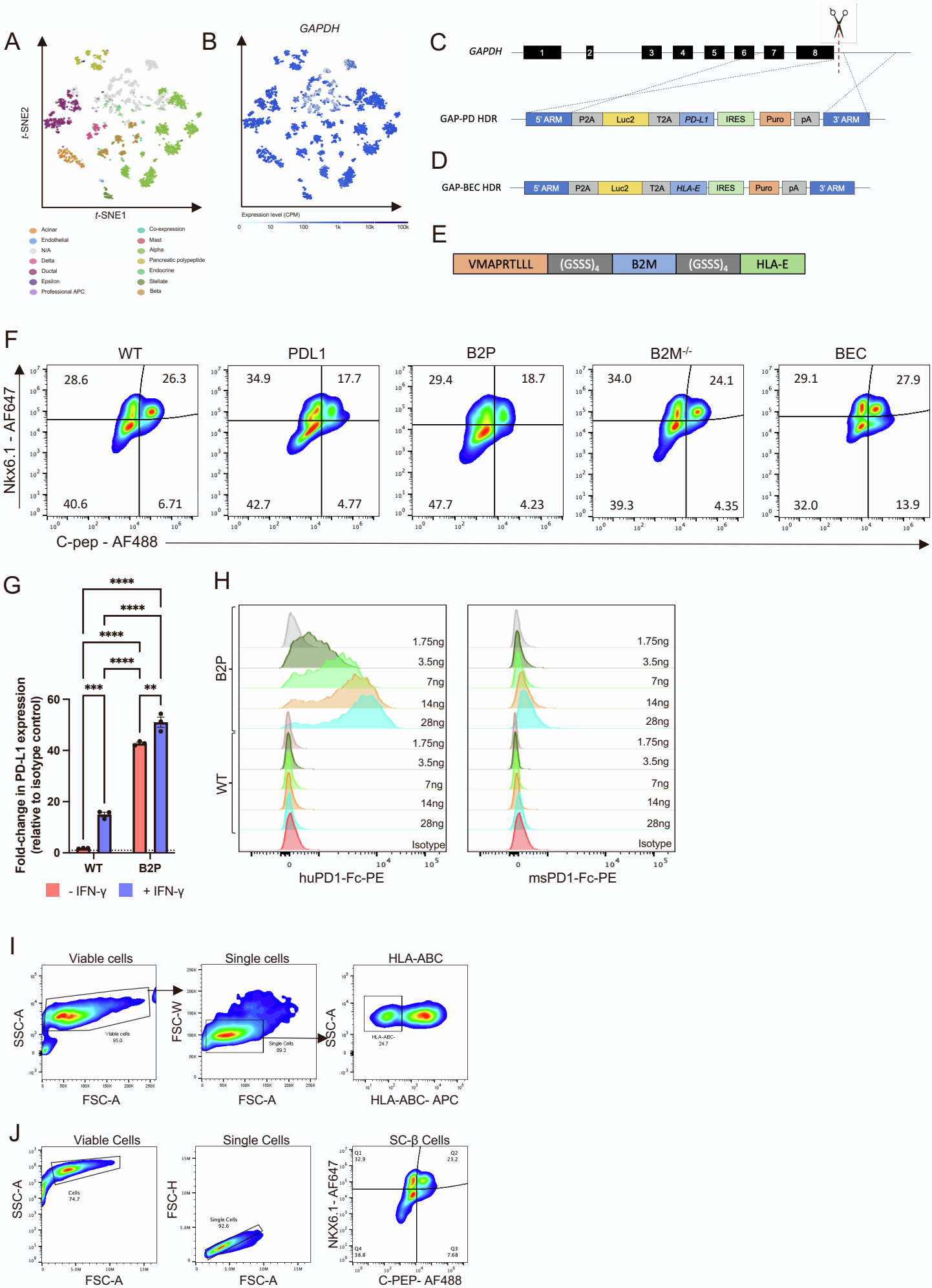
A
t-SNE2
t-SNE1
Acinar
Endothelial
N/A
Delta
Ductal
Epsilon
Professional APC
Co-expression
Mast
Alpha
Pancreatic polypeptide
Endocrine
Stellate
Beta
B
GAPDH
Expression level (CPM)
0
10
100
1k
10k
100k
C
GAPDH
1
2
3
4
5
6
7
8
GAP-PD HDR
5' ARM
P2A
Luc2
T2A
PD-L1
IRES
Puro
pA
3' ARM
D
GAP-BEC HDR
5' ARM
P2A
Luc2
T2A
HLA-E
IRES
Puro
pA
3' ARM
E
VMAPRTLLL
(GSSS)4
B2M
(GSSS)4
HLA-E
F
WT
PDL1
B2P
B2M-/-
BEC
28.6
26.3
40.6
6.71
34.9
17.7
42.7
4.77
29.4
18.7
47.7
4.23
34.0
24.1
39.3
4.35
29.1
27.9
32.0
13.9
Nkx6.1 - AF647
C-pep - AF488
G
Fold-change in PD-L1 expression (relative to isotype control)
WT
B2P
- IFN-γ
+ IFN-γ
****
****
****
****
***
**
H
B2P
WT
1.75ng
3.5ng
7ng
14ng
28ng
Isotype
huPD1-Fc-PE
msPD1-Fc-PE
I
Viable cells
Single cells
HLA-ABC
SSC-A
FSC-A
FSC-W
HLA-ABC- APC
Viable cells 95.0
Single Cells 89.3
HLA-ABC- 24.7
J
Viable Cells
Single Cells
SC-β Cells
SSC-A
FSC-A
FSC-H
NKX6.1 - AF647
C-PEP- AF488
Cells 74.7
Single Cells 92.6
Q1 32.9
Q2 23.2
Q4 38.8
Q3 7.68

Figure S1. Characterization of genetically-engineered hESCs and their islet derivatives. Related to Figure 1

A. t-SNE projections of primary human islet cells.
B. t-SNE projections of GAPDH expression across the assigned populations. Cells are colored according to their assigned cluster. (Adapted from Segerstolpe et al., 2016)
C. Schematic of homology directed repair plasmids for integration of Luc2 and PD-L1 at the GAPDH locus.
D. Schematic GAPDH-targeting Luc2 and peptide::B2M::HLA-E HDR plasmid.
E. Schematic of the peptide::B2M::HLA-E long-chain fusion.
F. FACS analysis of Nkx6.1^{+}/C-peptide^{+} SC-β cells derived from hypoimmunogenic hESCs (S6d14).
G. Quantitative analysis of surface expression of PD-L1. Data are presented as mean fold-change in MFI ± SD (n = 3 independent differentiations) normalized to isotype control (dashed line). P values were determined by two-way ANOVA, *$p < 0.05$.
H. PD1-Fc binding assay. PD-L1 and WT SC-islet cells were dissociated and stained with a 2-fold dilution series of PE-conjugated human and mouse PD1-Fc. Data are presented as MFI normalized to mode.
I. FACS sorting strategy of HLA-ABC$^{-/-}$ hESCs.
J. FACS gating strategy for quantification of Nkx6.1^{+}/C-pep^{+} SC-β cells following *in vitro* differentiation.

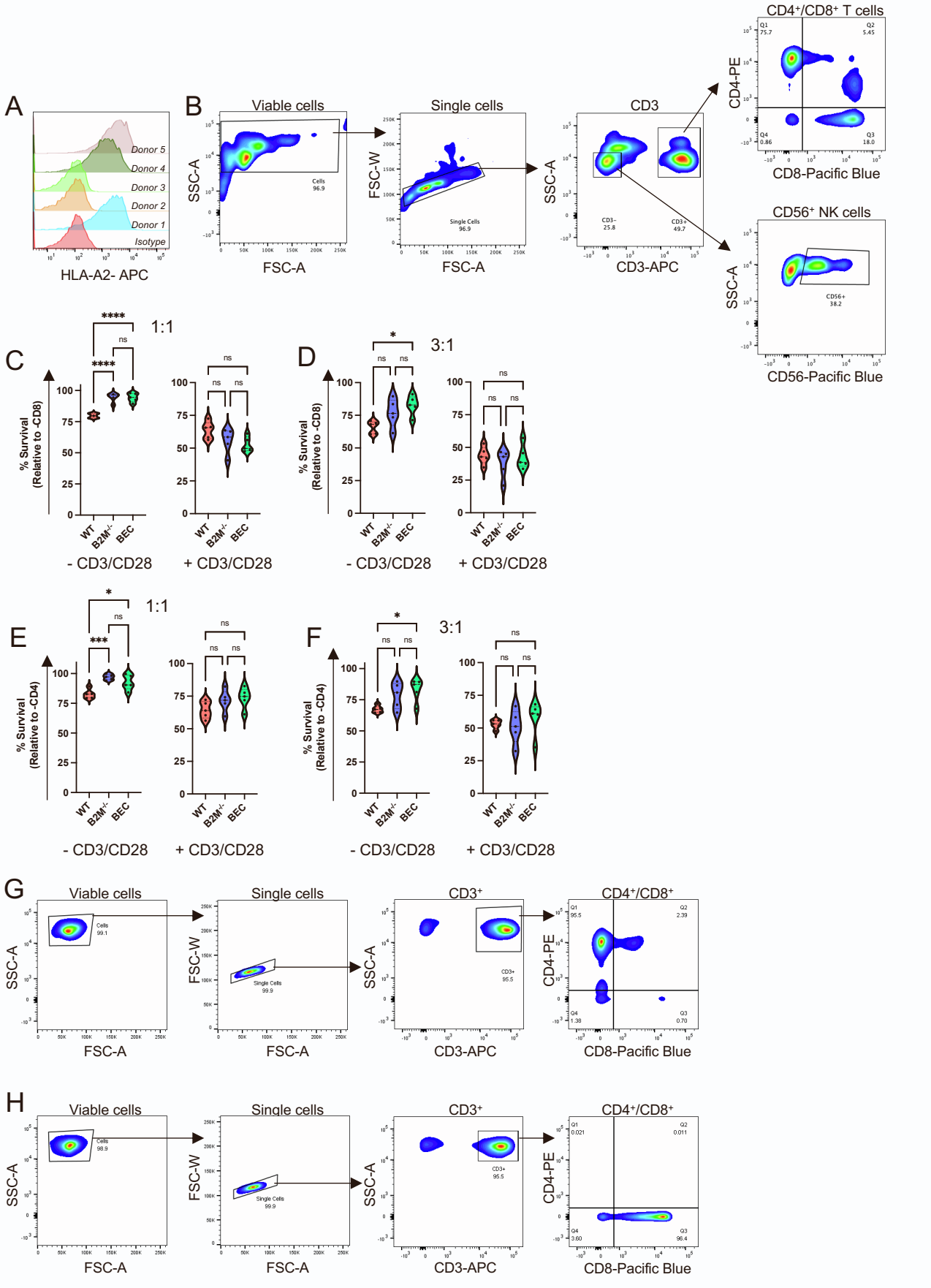
A
Donor 5
Donor 4
Donor 3
Donor 2
Donor 1
Isotype
HLA-A2- APC
B
Viable cells
SSC-A
FSC-A
Cells
96.9
Single cells
FSC-W
FSC-A
Single Cells
96.9
CD3
SSC-A
CD3-APC
CD3-
25.8
CD3+
49.7
CD4+/CD8+ T cells
CD4-PE
CD8-Pacific Blue
Q1
75.7
Q2
5.45
Q4
0.86
Q3
18.0
CD56+ NK cells
SSC-A
CD56-Pacific Blue
CD56+
38.2
C
1:1
% Survival
(Relative to -CD8)
WT
B2M-/-
BEC
- CD3/CD28
+ CD3/CD28
D
3:1
E
1:1
% Survival
(Relative to -CD4)
F
3:1
G
Viable cells
Cells
99.1
Single cells
Single Cells
99.9
CD3+
CD3+
95.5
CD4+/CD8+
Q1
95.5
Q2
2.39
Q4
1.38
Q3
0.70
H
Viable cells
Cells
98.9
Single cells
Single Cells
99.9
CD3+
CD3+
95.5
CD4+/CD8+
Q1
0.021
Q2
0.011
Q4
3.60
Q3
96.4

Figure S2: Characterization of immune subsets within human PBMCs. Related to Figure 2.

A. FACS profiling of HLA-A2 status of 5 PBMC donors.
B. FACS gating strategy of $CD4^+$ and $CD8^+$ T cells and $CD56^+$ NK cells in PBMCs enriched from human apheresis leukoreductions. Plots are representative of 5 donors.
C. Quantification of SC-islet cell survival when co-cultured with purified human $CD8^+$ T cells at a 1:1 ratio. Cell survival is presented as mean ± SD (n = 5 donors in technical triplicate). P values were determined by one-way ANOVA with Tukey's post-hoc test, $*p < 0.05$.
D. Quantification of SC-islet cell survival when co-cultured with purified human $CD8^+$ T cells at a 3:1 ratio. Cell survival is presented as mean ± SD (n = 5 donors in technical triplicate). P values were determined by one-way ANOVA with Tukey's post-hoc test, $*p < 0.05$.
E. Quantification of SC-islet cell survival when co-cultured with purified human $CD4^+$ T cells at a 1:1 ratio. Cell survival is presented as mean ± SD (n = 5 donors in technical triplicate). P values were determined by one-way ANOVA with Tukey's post-hoc test, $*p < 0.05$.
F. Quantification of SC-islet cell survival when co-cultured with purified human $CD4^+$ T cells at a 3:1 ratio. Cell survival is presented as mean ± SD (n = 5 donors in technical triplicate). P values were determined by one-way ANOVA with Tukey's post-hoc test, $*p < 0.05$.
G. FACS gating strategy of $CD4^+$ T cells enriched from human apheresis leukoreductions. Plots are representative of 5 donors.
H. FACS gating strategy of $CD8^+$ T cells enriched from human apheresis leukoreductions. Plots are representative of 5 donors.
I. FACS gating strategy of $CD56^+$ NK cells enriched from human apheresis leukoreductions. Gates for specific population include all $CD56^+$ NK cells (red), $CD56^{high}$ (green) and $CD56^{dim}$ (pink). Plots are representative of 5 donors.
J. *In vivo* NK cell assay. Scid/beige mice (n = 5) were transplanted subcutaneously with 2.5×10^5 WT or $B2M^{-/-}$ SC-islet cells in the presence or absence of a 3:1 effector:target ratio of rhIL-2 pre-treated primary human NK cells. Bioluminescence imaging was performed on day 1 and 5 post-transplantation.

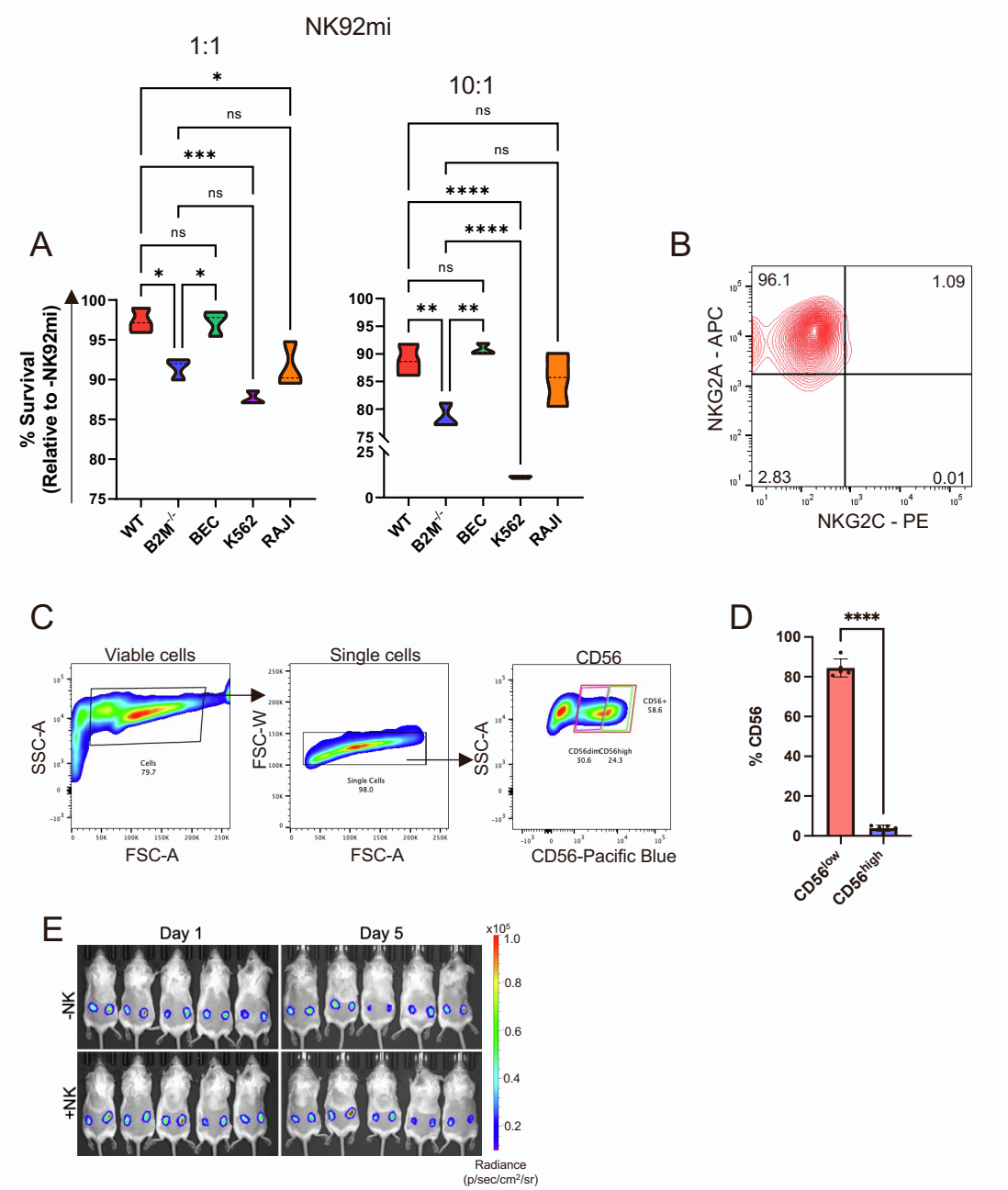
NK92mi
1:1
10:1
A
% Survival
(Relative to -NK92mi)
WT
B2M-/-
BEC
K562
RAJI
ns
*
***
****
**
B
96.1
1.09
2.83
0.01
NKG2A - APC
NKG2C - PE
C
Viable cells
Single cells
CD56
SSC-A
FSC-A
FSC-W
Cells
79.7
Single Cells
98.0
CD56+
58.6
CD56dimCD56high
30.6
24.3
CD56-Pacific Blue
D
% CD56
CD56low
CD56high
E
Day 1
Day 5
-NK
+NK
x10^5
Radiance
(p/sec/cm²/sr)

Figure S3: *In vitro* and *in vivo* co-culture of hypoimmunogenic SC-islet cells with primary and immortalized human NK cells. Related to Figure 3.

A. Quantification of SC-β cell survival when co-cultured with NK92mi cells at 1:1 and 10:1 NK:SC-β cell ratios. K562 and Raji cells were used as positive and negative controls, respectively. Cell survival is presented as mean ± SD (n = 5 donors in technical triplicate).
B. NKG2A/NKG2C expression on NK92mi cells.
C. FACS gating strategy of $CD56^{+}$ NK cells enriched from human apheresis leukoreductions. Gates for specific population include all $CD56^{+}$ NK cells (red), $CD56^{high}$ (green) and $CD56^{dim}$ (pink). Plots are representative of 5 donors.
D. Quantitative analysis of CD56 expression on enriched primary human NK cells. Data is presented as % CD56 expression (n = 5 donors in technical triplicate).
E. *In vivo* NK cell assay. Bioluminescence imaging was performed on day 1 and 5 post-transplantation.

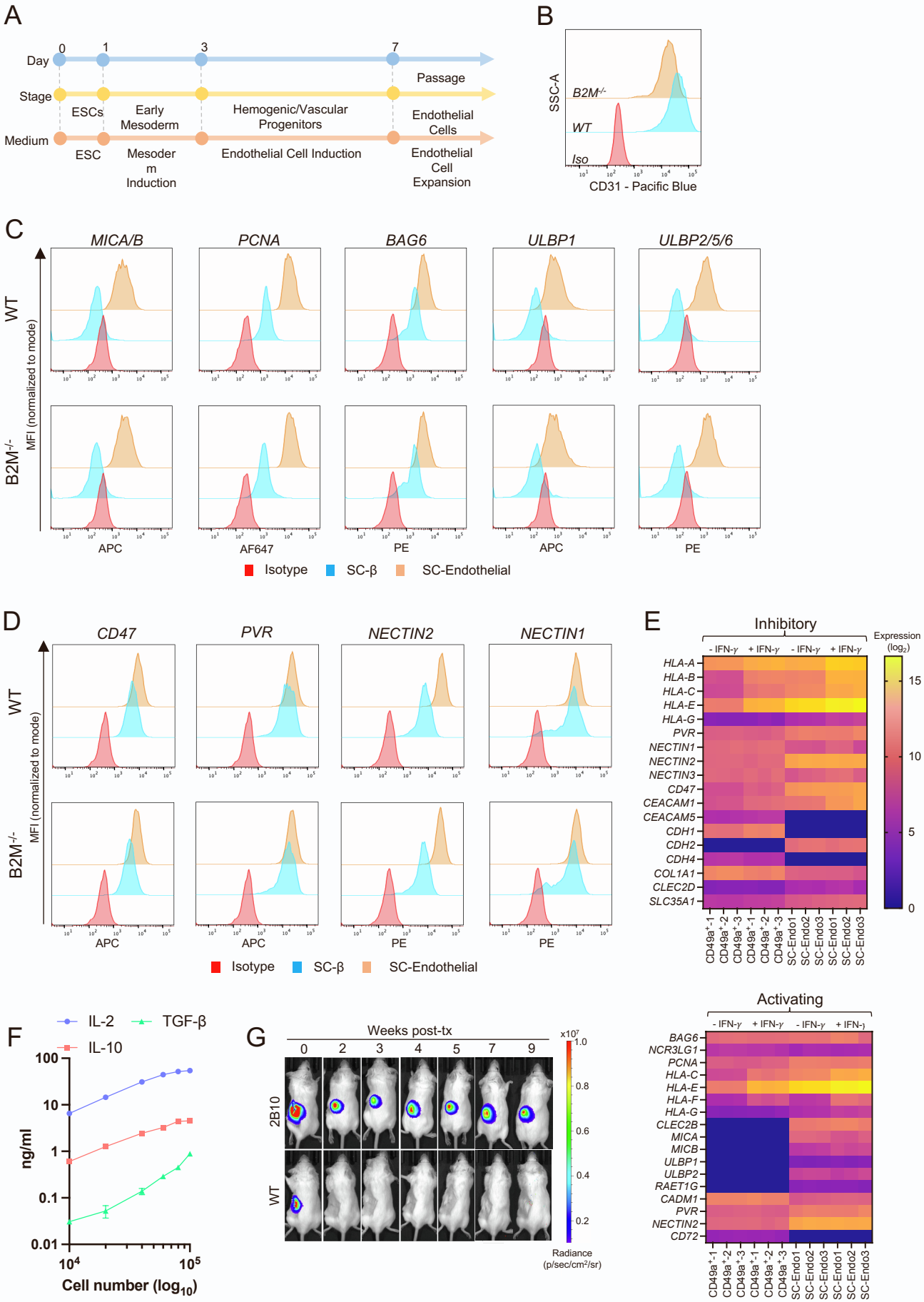
A
Day
Stage
Medium
0
1
3
7
ESCs
ESC
Early Mesoderm
Mesoderm Induction
Hemogenic/Vascular Progenitors
Endothelial Cell Induction
Passage
Endothelial Cells
Endothelial Cell Expansion
B
SSC-A
B2M-/-
WT
Iso
CD31 - Pacific Blue
C
MICA/B
PCNA
BAG6
ULBP1
ULBP2/5/6
WT
B2M-/-
MFI (normalized to mode)
APC
AF647
PE
APC
PE
Isotype
SC-β
SC-Endothelial
D
CD47
PVR
NECTIN2
NECTIN1
WT
B2M-/-
MFI (normalized to mode)
APC
APC
PE
PE
Isotype
SC-β
SC-Endothelial
E
Inhibitory
- IFN-γ
+ IFN-γ
- IFN-γ
+ IFN-γ
Expression (log2)
HLA-A
HLA-B
HLA-C
HLA-E
HLA-G
PVR
NECTIN1
NECTIN2
NECTIN3
CD47
CEACAM1
CEACAM5
CDH1
CDH2
CDH4
COL1A1
CLEC2D
SLC35A1
CD49a+-1
CD49a+-2
CD49a+-3
CD49a--1
CD49a+-2
CD49a+-3
SC-Endo1
SC-Endo2
SC-Endo3
SC-Endo1
SC-Endo2
SC-Endo3
Activating
BAG6
NCR3LG1
PCNA
HLA-C
HLA-E
HLA-F
HLA-G
CLEC2B
MICA
MICB
ULBP1
ULBP2
RAET1G
CADM1
PVR
NECTIN2
CD72
F
IL-2
TGF-β
IL-10
ng/ml
Cell number (log10)
G
Weeks post-tx
0
2
3
4
5
7
9
2B10
WT
x10^7
Radiance (p/sec/cm²/sr)

Figure S4: Characterization of the NK cell ligand profile on SC-islet and SC-endothelial cells. Related to Figures 3 and 4.

A. Schematic of SC-endothelial cell differentiation protocol.
B. FACS analysis of the endothelial cell marker CD31 in SC-endothelial cells derived from WT and B2M$^{-/-}$ hESCs.
C. NK cell activating ligand expression on SC-β and SC-Endothelial cells. Data are presented as MFI normalized to mode and is representative of three independent experiments.
D. NK cell inhibitory ligand expression on SC-β and SC-Endothelial cells. Data are presented as MFI normalized to mode and is representative of three independent experiments.
E. Heatmap of NK cell ligand expression in SC-β and SC-Endothelial cells (-IFN-γ vs +IFN-γ). Ligand expression is presented as fold-change (log_2).
F. Quantification of cytokine secretion from 2B10 SC-islet cells. Data are presented as mean ± SD (n = 2).
G. *In vivo* bioluminescence imaging of B6/albino mice transplanted with WT and 2B10 SC-islet cells (n = 3/group).