Supplemental Information:

Immunocytochemistry and Histology

Pancreatic paraffin slides from diabetic patients were purchased from BioChain (Newark, CA). Immunostaining was performed as previously described with minor modifications [1]. To block non-specific staining, sections were incubated in a buffer containing 2.5% horse serum (Vector Laboratories) for 20 min at room temperature. Primary antibodies included guinea pig polyclonal anti-insulin Ab (DakoCytomation, Carpinteria, CA), platelet marker FITC-conjugated CD42a (Beckman Coulter), platelet α granule marker von Willebrand factor (vWf) Ab (Sigma), and platelet dense granule marker ADP Ab (GenScript, Piscataway, NJ). The second Ab included Cy3-conjugated AffiniPure donkey anti-guinea pig IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). For isotype-matched controls, mouse $IgG_1\kappa$ was purchased from BD Biosciences, and guinea pig serum was purchased from Santa Cruz Biotechnology. For every experiment, isotype-matched antibodies were used as negative controls. Cells were photographed with a ZEISS Imager M1 equipped with an AxioCam MRc camera and AxioCam MR Rev 3 software or photographed with a Nikon A1R confocal microscope.

To compare the size of pancreatic islets after the treatment with mitochondria in transwells, islets were photographed with a Nikon Diaphot equipped with an EC500 digital camera. The size of pancreatic islets was measured and calculated by point-counting morphometric analysis [2] using Image J software downloaded from the NIH website (http://rsbweb.nih.gov/ij/).

Flow cytometry

Flow cytometric analyses of surface and intra-cellular markers were performed as previously described [3]. Platelets were washed with PBS at 3000 rpm for 15 mins. Human islets were

dissociated with 0.25% trypsin/EDTA for five mins at room temperature with repeatedly pipetting, followed by a wash with PBS. Samples are pre-incubated with human BD Fc Block (BD Pharmingen) for 15 mins at room temperature, and then directly aliquoted for different antibody staining. Cells were incubated with mouse anti-human monoclonal antibodies (mAb; Beckman Coulter, Brea, CA), including FITC-conjugated anti-CD42a, anti-CD61, phycoerythrin (PE)-conjugated anti-CD8β, anti-CXCR4, anti-CCR7, and phycoerythrin-Cy7 (PE-Cy7)-conjugated anti-CD41, anti-CD56, and anti-CCR7, APC-conjugated anti-ICOS, APC-Alexa Fluor 750-conjugated anti-CD4 and anti-CD66b, pacific blue (PB)-conjugated anti-CD38, Krome Orange-conjugated anti-CD8α, anti-CD14, and anti-CD19. From BD Biosciences (San Jose, CA), the investigator purchased AF647-conjugated anti-FoxP3, PerCP-Cy5.5-conjugated mouse anti-human PDX-1, Alexa Fluor 488-conjugated mouse anti-human somatostatin and anti-PDX-1, PE-conjugated mouse anti-NEUROD1 and anti-Glucagon, and anti-FOXA2, and anti-NKX6.1, BV421-conjugated mouse anti-human glucagon, Alexa Fluor 647-conjugated mouse anti-human insulin, anti-human C-peptide, anti-NKX6.1, anti-SOX9, anti-PTFA1, anti-NANOG, and PerCP-Cy5.5-conjugated mouse anti-human SOX17 mAb. The FITC-conjugated mouse anti-human integrin β1 (CD29), PE-conjugated mouse anti-human CD270 (HVEM) mAb, anti-TLR4, anti-TLR6 and anti-CXCL10, APC-conjugated anti-TGF-β1, and APC/Fire 750-conjugated anti-CD36 mAbs were purchased from Biolegend (San Diego, CA). Rabbit anti-AIRE polyclonal antibody was purchased from Abcam (Cambridge, MA). FITC-conjugated anti-CXCR1 and anti-SOX2, PE-conjugated anti-CD274 (PD-L1), anti-CXCR2, anti-CCR3, anti-CCR5, and anti-fibronectin, APC-conjugated anti-CCL2, eFluor660-conjugated anti-Galectin 9, CXCR3, and anti-CXCL1, PerCP-eFluor710-conjugated anti-CCR4, Alexa Fluor 647-conjugated rat anti-human Oct 3/4 mAb and unconjugated mouse anti-human NANOG were purchased from

eBioscience (San Diego, CA). FITC-conjugated anti-human MAFA ab was obtained from United States Biological (Salem, MA). DyLight 405-conjugated anti-ki67 was purchased from Novus Biologicals (Littleton, CO). Cells were stained for 30 min at room temperature and then washed with PBS prior to flow analysis. Isotype-matched mouse anti-human IgG antibodies (Beckman Coulter) served as a negative control for all fluorescein-conjugated IgG mAb. For intra-cellular staining, cells were fixed and permeablized using a PerFix-nc kit (Beckman Coulter). After staining, cells were collected and analyzed using a Gallios Flow Cytometer (Beckman Coulter) equipped with three lasers (488 nm blue, 638 red, and 405 violet lasers) for the concurrent reading of up to 10 colors. The final data were analyzed using the Kaluza Flow Cytometry Analysis Software (Beckman Coulter).

To detect the basal release of mitochondria, initially, human cord blood-derived 40 ml platelet-rich plasmas (PRP, > 98% purity of $CD41^+$ platelets) were labeled with MitoTracker Deep Red FM (100 nM) (Thermo Fisher Scientific, Waltham, MA) at 37°C for 15 mins according to the manufacturer's recommended protocol, followed by two washes with PBS at 3000 rpm × 15 mins. To examine the basal release of mitochondria from platelets, MitoTracker Deep Red-labeled platelets were re-suspended in 2 ml serum-free X-VIVO 15 culture medium (Lonza) and incubated at 37°C for 15 mins. Consequently, platelets were removed by centrifuging at 2.7kg for 15 mins, 4°C; supernatants were collected and re-centrifuged at 14kg for 15 mins at 4°C to harvest released mitochondria for flow analysis.

For mitochondrial staining of platelets with fluorescent dyes, platelet-rich plasmas (PRP) were labeled with MitoTracker Deep Red FM (100 nM) or MitoTracker Green FM (100 nM) (Thermo Fisher Scientific, Waltham, MA) at 37°C for 15 mins according to the manufacturer's recommended protocol, followed by two washes with PBS at 3000 rpm × 15 mins. To examine

the release of mitochondria by platelets, MitoTracker Deep Red-labeled platelets were treated with different platelet aggregators such as 20 μM ADP, 0.5 mM arachidonic acid (ARA), and 1 Unit/ml thrombin in 0.5 ml serum-free X-VIVO 15 culture medium (Lonza) at 37°C for 15 mins. After these treatments, platelets were removed by centrifuging at 2.7kg for 15 mins, 4°C; supernatants were collected and re-centrifuged at 14kg for 15 mins at 4°C to harvest released mitochondria for flow analysis.

Western blot

Platelets were collected from human cord blood units (Cord:Use Cord Blood Bank, Orlando, FL) and from adult peripheral blood samples (New York Blood Bank, New York). Platelets were solubilized with Cell Extraction buffer (Invitrogen) with a cocktail of protease inhibitors (Sigma). Platelet samples (20 μg protein each) were mixed with a Laemmli sample buffer (Bio-Rad) in a volume ratio of 1:1, boiled, loaded, and separated by electrophoresis on 10% Tris-HCl Criterion Precast Gel (Bio-Rad). The separated proteins were then transferred to a nitrocellulose membrane, blocked with 5% non-fat dry milk in TBST for one hour and incubated with different antibodies. These included rabbit anti-AIRE polyclonal Ab, anti-CRIPTO pAb, and anti-GATA4 pAb (Abcam), rat anti-human OCT4 Ab, rat anti-human SOX2 Ab, mouse anti-human NANOG Ab, mouse anti-human C-myc Ab (eBiosciences), and rabbit anti-MAFA pAb (Novus Biologicals) at 1:1,000 dilution in 5% milk-TBS for two hours at room temperature. After washing, the blot was exposed to a horseradish peroxidase-conjugated secondary antibody (1:2,000; Pierce) in 5% milk-TBS. Immunocomplexes were visualized by the enhanced chemiluminescence (ECL, GE healthcare) method. β-actin served as an internal loading control.

Quantitative real time PCR array

The expression of different mRNAs in platelets was analyzed by quantitative real-time PCR. Total RNAs from each sample were extracted using a Qiagen kit (Valencia, CA). First-strand cDNAs were synthesized from total RNA using an iScript gDNA Clear cDNA Synthesis Kit according to the manufacturer's instructions (Bio-Rad, Hercules, CA). Real-time PCR was performed on each sample in triplicate using the StepOnePlus Real-Time PCR System (Applied Biosystems, CA) under the following conditions: 95 °C for 10 min, then 40 cycles of 95°C for 15 s, and 60 °C for 60 s, using the validated gene-specific RT^2 PCR Primer sets for each gene including ES cell-related markers (e.g., OCT4, NANOG, SOX2, KLF4, and C-myc) and pancreatic islet cell-related markers (e.g., insulin, glucagon, somatostatin, PPY, Ghrelin, GCK, Sur1, Kir6.2, MAFA, NKX6.1, PDX-1, NEUROD1, and NGN3) (Qiagen Valencia, CA). The expression level of each gene was determined relative to β-actin as an internal control. To confirm gene expression, real time PCR products were examined with 1.5% agarose gel electrophoresis. For RT^2 Profiler real time PCR Arrays, the human Stem Cell kit and the human stem cell transcription factors kit (96-well format) were used according to the manufacturer's instructions. The data were analyzed using PrimePCR array analysis software (Bio-Rad).

Figure Legends for Supporting Information:

Figure S1. Flow cytometry showed the expression of chemokine receptors and ligands at various levels on PB-platelets. Isotype-matched IgGs served as negative controls.

Figure S2. Real time PCR Array for the expression of human T cell anergy and immune tolerance-related genes in mitochondria purified from CB- (lanes 1-4) and PB-platelets (lanes 5-8).

Figure S3. Real time PCR Array for human stem cell transcription factors in mitochondria purified from CB- (lanes 1-3) and PB-platelets (lanes 4-6). Colors represent the levels of gene expressions as shown in the key.

Figure S4. Real time PCR Array for human stem cell markers in mitochondria purified from CB- (lanes 1-3) and PB-platelets (lanes 4-6).

Figure S5. Immunohistochemistry of human pancreatic tissues from healthy donors (n = 2) showed the scattered distribution of platelets in pancreatic tissues, with no or a few platelets (green color, a platelet marker CD42a) located in or around islets (red color, a β-cell marker insulin). Scale bar, 22 μm.

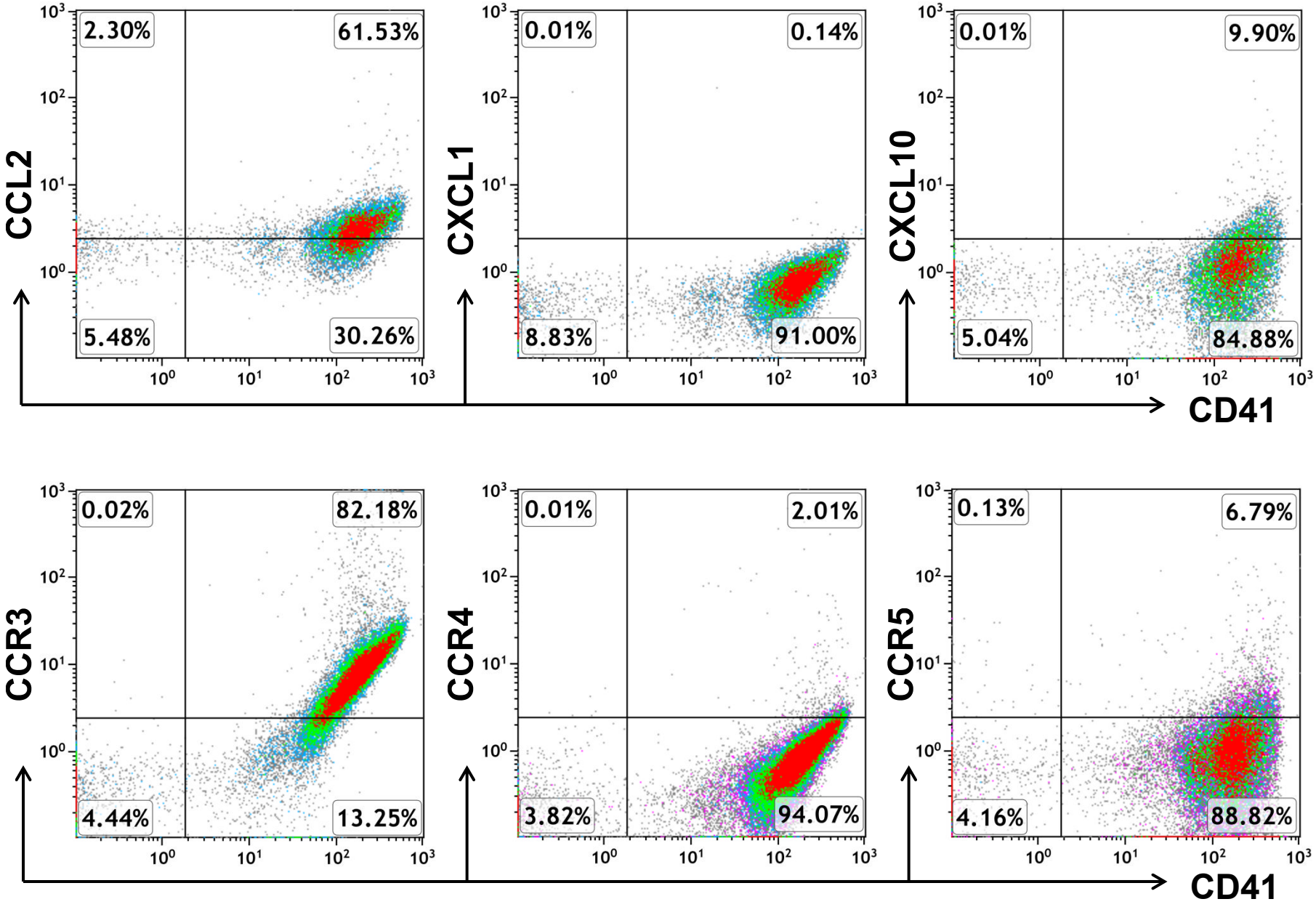
References

1. Zhao Y, Lin B, Darflinger R et al. Human cord blood stem cell-modulated regulatory T lymphocytes reverse the autoimmune-caused type 1 diabetes in nonobese diabetic (NOD) mice. PLOS. ONE. 2009;4:e4226.

2. Meier JJ, Lin JC, Butler AE et al. Direct evidence of attempted beta cell regeneration in an 89-year-old patient with recent-onset type 1 diabetes. DIABETOLOGIA 2006;49:1838-1844.

3. Li Y, Yan B, Wang H et al. Hair regrowth in alopecia areata patients following Stem Cell Educator therapy. BMC. MED. 2015;13:87.

Figure S1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5.04%
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CD41
CCR3
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CD41

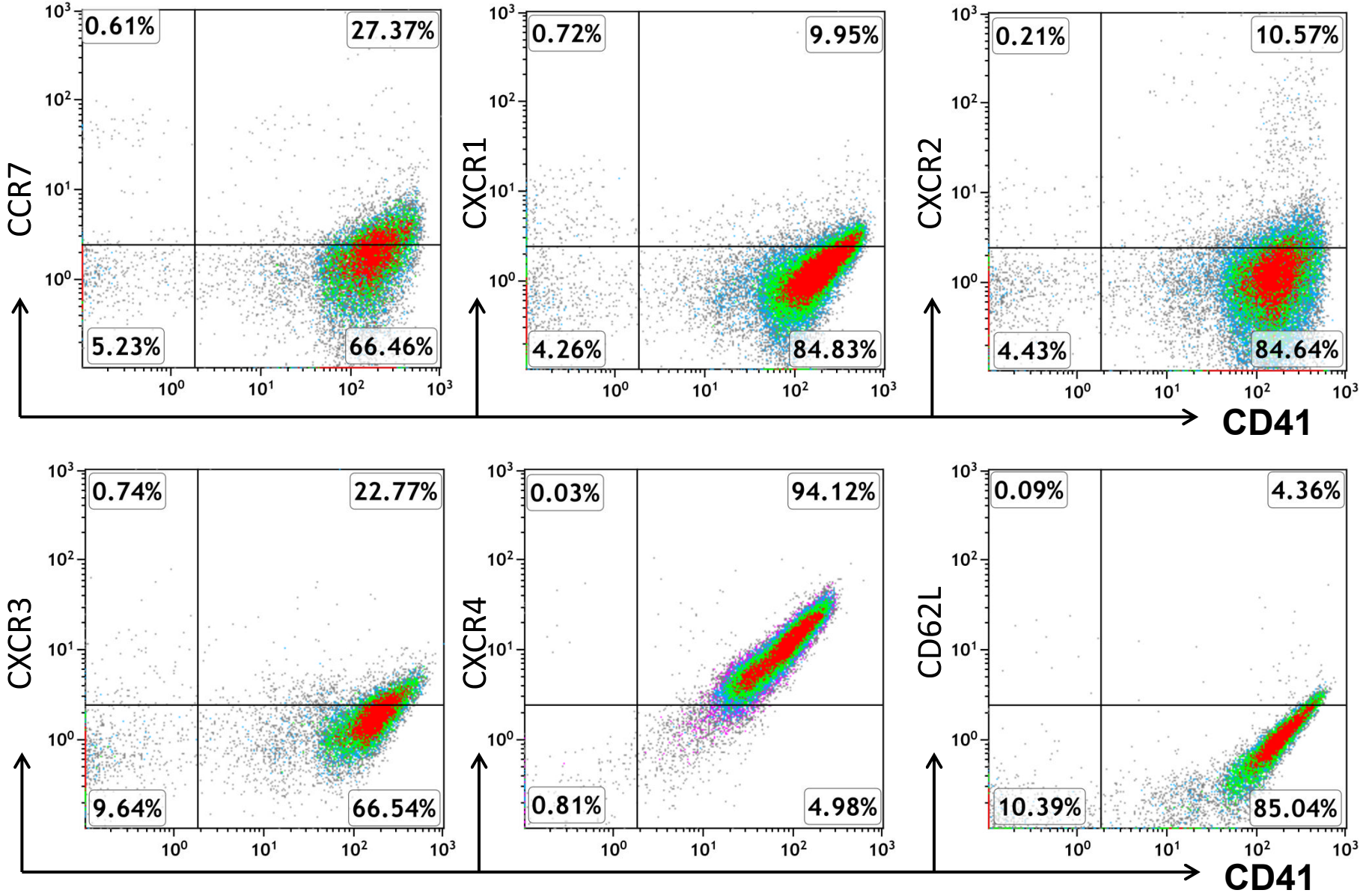
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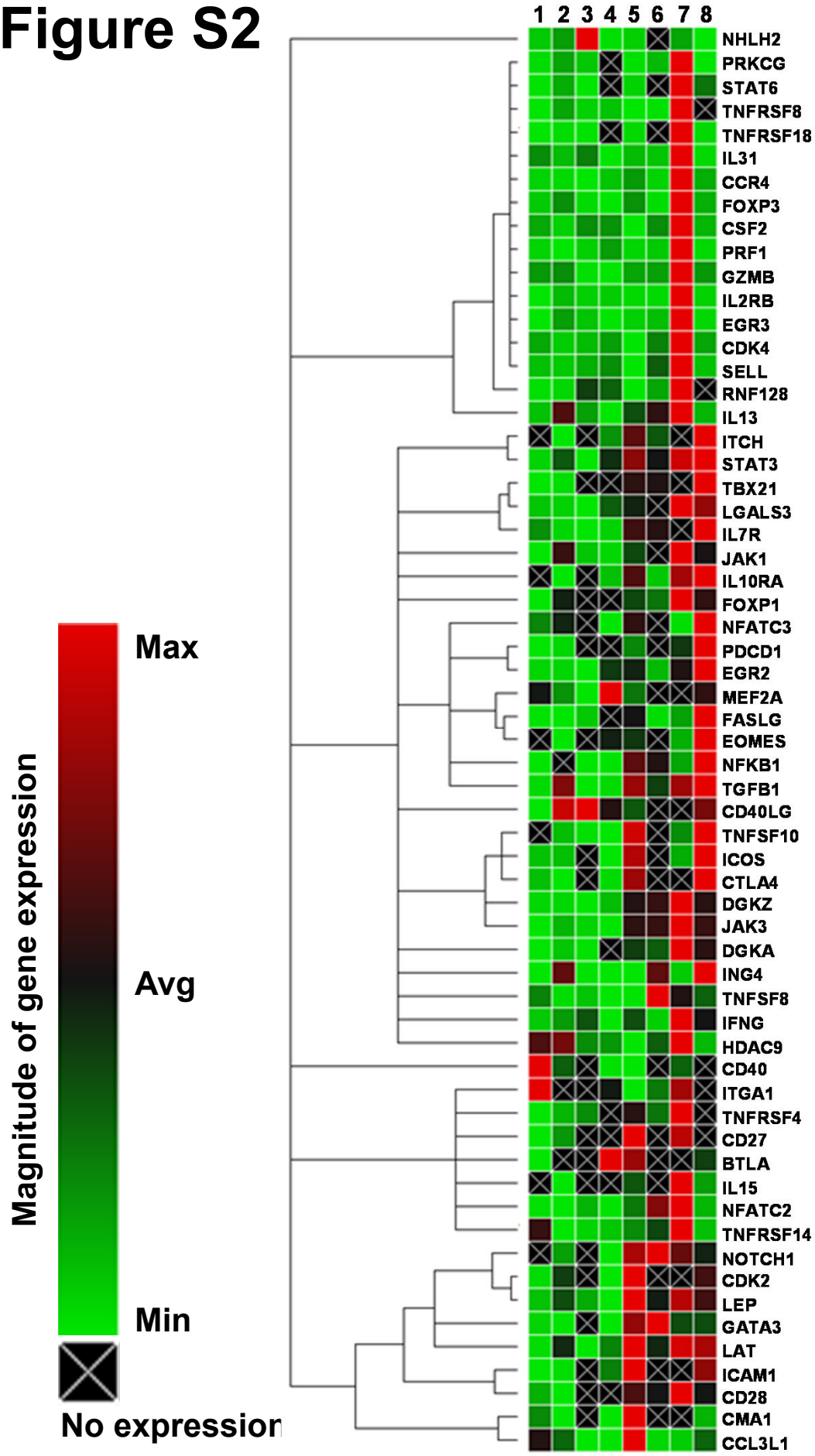
Figure S2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Magnitude of gene expression
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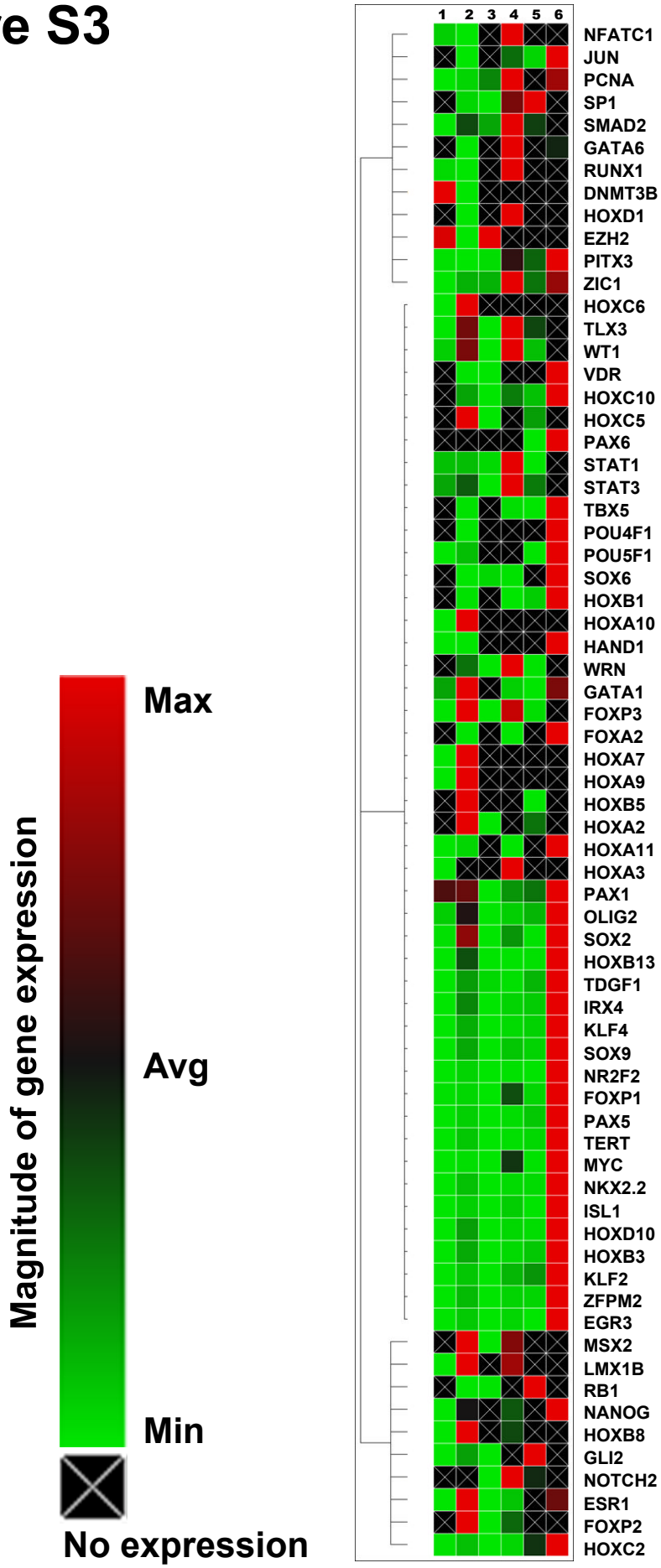
Figure S3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PAX6
STAT1
STAT3
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Magnitude of gene expression
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Avg
Min
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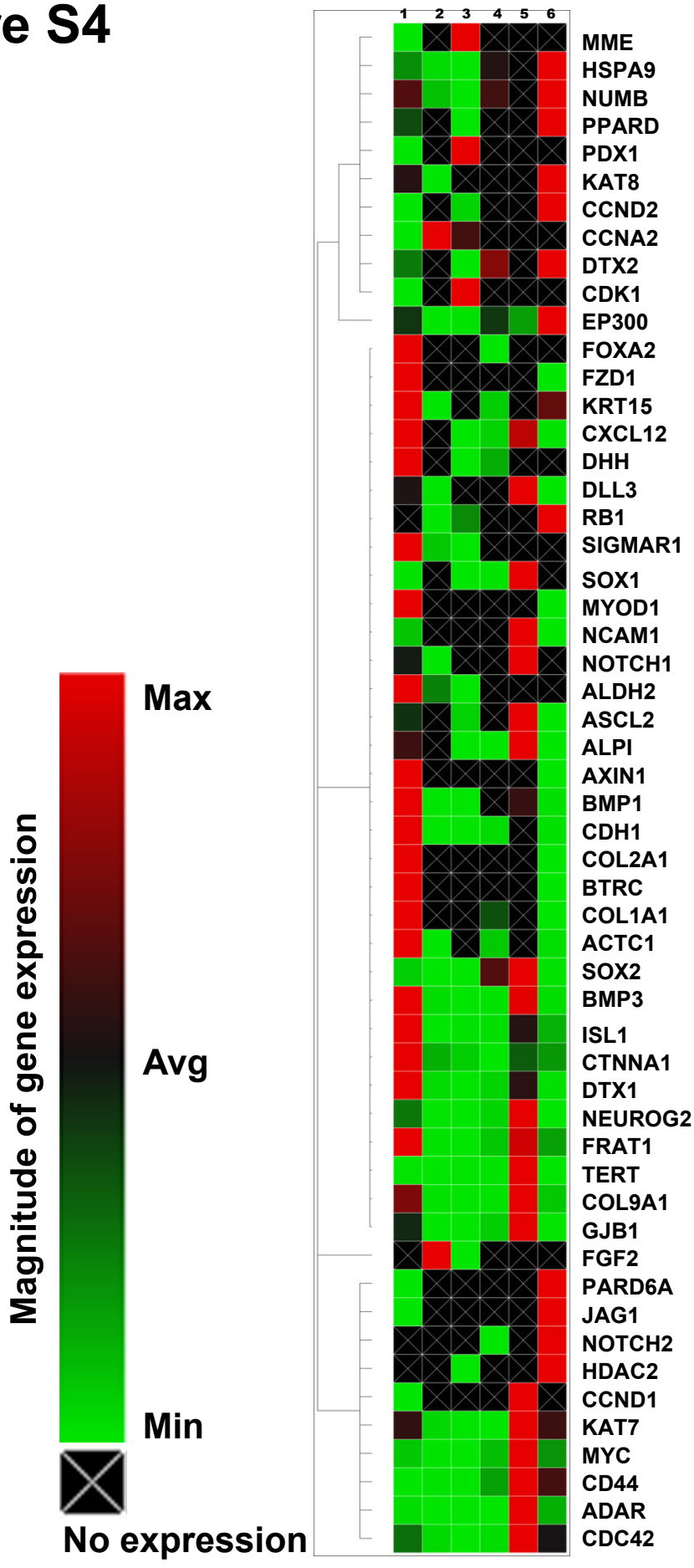
Figure S4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NCAM1
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AXIN1
BMP1
CDH1
COL2A1
BTRC
COL1A1
ACTC1
SOX2
BMP3
ISL1
CTNNA1
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FRAT1
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COL9A1
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CD44
ADAR
CDC42
Magnitude of gene expression
Max
Avg
Min
No expression

Figure S5