Cell culture

iPSCs were cultured on irradiated MEF feeder layers in standard ESC maintenance media: Knockout DMEM supplemented with 20% KOSR, NEAA, 2-ME, GlutaMAX, 8 ng/ml basic fibroblast growth factor (all from Invitrogen). Alternatively cells were cultured on matrigel using MEF conditioned media or mTeSR (StemCell Technologies). This study was done in accordance with Johns Hopkins ISCRO regulations and following a protocol approved by the Johns Hopkins IRB.

Differentiation of human iPSCs to multistage hepatic cells

Fifty percent confluent cultures were placed in RPMI medium (supplemented with GlutaMAX and 0.5% defined FBS, and 100ng/ml Activin A (R&D Systems) for 5–6 days to induce definitive endoderm stage cells.[21] The medium was replaced every other day. DE cells were passaged with 0.05% trypsin-0.53 mM EDTA and plated on collagen I-coated dishes in minimal MDBK-MM medium (Sigma) supplemented with GlutaMAX and 0.5 mg/ml BSA, 10 ng/ml FGF4, and 10 ng/ml HGF. Day 10 hepatic progenitors were utilized for experiments. Hepatic progenitor cells were switched to complete hepatocyte culture medium (HCM) containing 5% heat-inactivated FBS, 10 ng/ml FGF-4, 10 ng/ml HGF, 10 ng/ml oncostatin M (R&D Systems), and 10^{-7} M dexamethasone (Sigma). Differentiation was continued for another 10 days to generate mature hepatocyte like cells.[21]

Teratoma formation

Ten-week-old male NSG mice (Jackson Laboratories) were anesthetized and ~1 to 2 million iPSCs, resuspended in 20–40 ul of 50% matrigel, were injected subcutaneously. Mice were euthanized 8 to 12 weeks after cell injection and tumors were analyzed following H&E staining. All animal experiments were conducted following experimental protocols previously approved by Johns Hopkins IACUC.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde. The following antibodies were used: TRA-1–60 (Millipore, 1:100); SSEA-4 (Cell Signaling, 1:200); OCT-4 (Millipore, 1:100), NANOG (BD, 1:200). Secondary antibodies used were all of the Alexa Fluor Series from Invitrogen.
Images were taken using the motorized Nikon Ti-E microscope and NIS-Elements software.

Genomic DNA PCR

Total cellular and viral DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen). PCR to detect episomal reprogramming vector and viral DNA was performed using primers previous

described.[4, 25] PCR was run using 100 ng of genomic DNA per reaction and GoTaq polymerase (Promega) for 30 cycles at 95°C for 1 min, 55°C for 30 s, and 72°C for 30 s.

EBV related gene expression analysis

Total RNA was isolated from human fibroblast, iAB5 P40, and the parental EBV–B-cell line, and genomic DNA was removed using RNAqueous®-PCR kit (Applied Biosystems). 2 ug total RNA from each sample was subjected to cDNA using a High Capacity RNA-to-cDNA kit (Applied Biosystems). PCR was run using 100ng of cDNA per reaction and GoTaq polymerase (Promega) for 30 cycles at 95°C for 1 min, 55°C for 30 s, and 72°C for 30 s.

PCR analysis of the presence of genomic rearrangements at IGH loci

Validated PCR primers to analyze V(D)J genomic rearrangements in B cells were purchased from InVivoScribe Technologies (San Diego, CA). Genomic DNA (500 ng-1ug) isolated from human ESCs (a negative control), parental EBV transformed B cells and iPSCs derived from the B-cell lines were used as templates for PCR amplification. These were designed to detect gene rearrangements at immunoglobulin heavy chain (IGH) loci in clonal cell populations. Three sets of primers amplify the rearranged IGH loci as shown when a clonal B-cell genomic DNA is used as positive control.

Table S1. Different transfection reagents and conditions for the two distinct patient cell types

Cell type	Transfection method	Plasmid combinations and dose
Fibroblasts	Basic 96 well Nucleofector kit 2 for primary fibroblasts Solution 2 Program 96-CA-137	EN2L 0.7, ET2K 0.5, EM2K 0.8 (total 2ug)
EBV transformed B-cell lines	Cell Line Nucleofector® Kit V Solution V Program X005	EN2L 3.5, ET2K 2.5, EM2K 4.0 (total 10ug)

Table S2. Transduction and transfection efficiencies for different human cell types

	Fibroblasts	**EBV–B-cell lines**	**Primary B cells**
Transduction efficiency at 72 hours after virus addition (MOI-15)	**88** +⁄– 1.5 (%)	**0.8** +⁄– 0.1 (%)	**0** +⁄– 0.3 (%)
Transfection efficiency at 24 hour after nucleofection	**97** +⁄– 3 (%)	**58** +⁄– 5 (%)	**2** +⁄– 0.8 (%)

Human fibroblasts, EBV-B cells, and primary $CD19^+$ B cells (derived from either peripheral blood or cord blood, Lonza) were either transduced with retrovirus or transfected with episomal plasmid. For transfection, specific nucleofection kits and protocols (described in Table S1 for fibroblasts and EBV–B-cell lines, and VPA-1001, protocol number U-015 for primary B cells) were used. GFP signals were assessed by flow cytometry at indicated times and expressed as mean+⁄–SD.

Figure S1. Characterization of patient iPSC lines derived from multiple other AAT patients

All other iPSC lines derived from AAT patients also expressed pluripotency markers including TRA-1–60, SSEA4, OCT4, and NANOG (A–C) and were able to differentiate into three germ layer tissues (endoderm; left panel, mesoderm; middle panel; ectoderm; right panel) as shown in teratoma analyses (D–F).

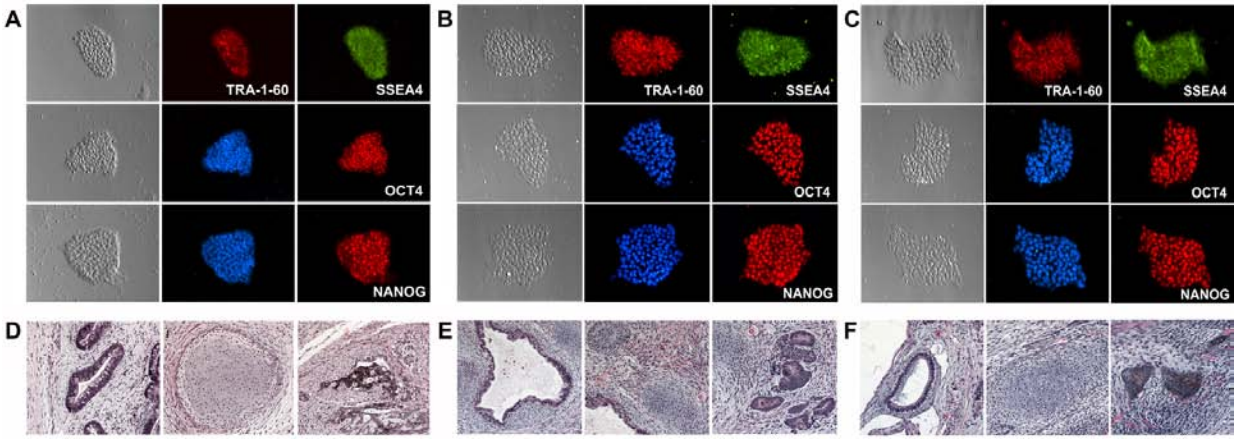

Figure S2. Characterization of patient iPSC lines derived from sickle cell anemia patients
All iPSC lines derived from sickle cell anemia patient derived EBV–B-cell lines also expressed pluripotency markers including TRA-1–60, SSEA4, OCT4, and NANOG (A–D, respectively). Representative images are shown here for iSB3.

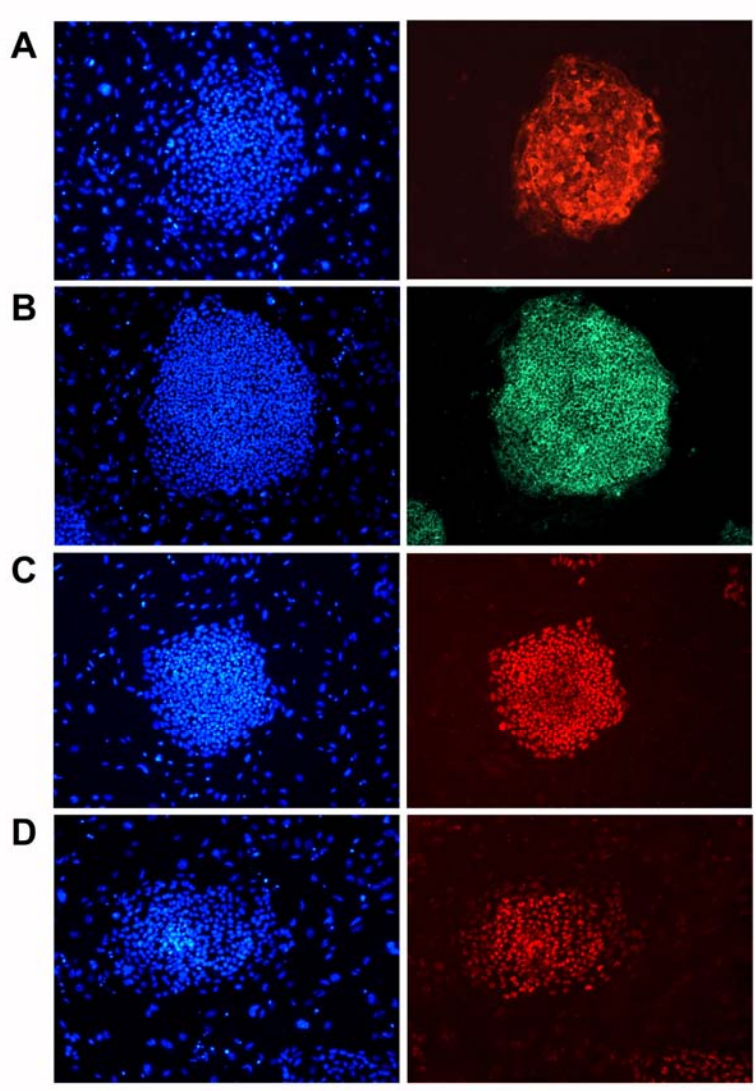

Figure S3. Surface marker analyses for EBV–B-cell lines

(A) Control, (B) Normal peripheral blood derived EBV B-cell line, (C, D) AAT-deficiency patient peripheral blood derived EBV–B-cell lines.

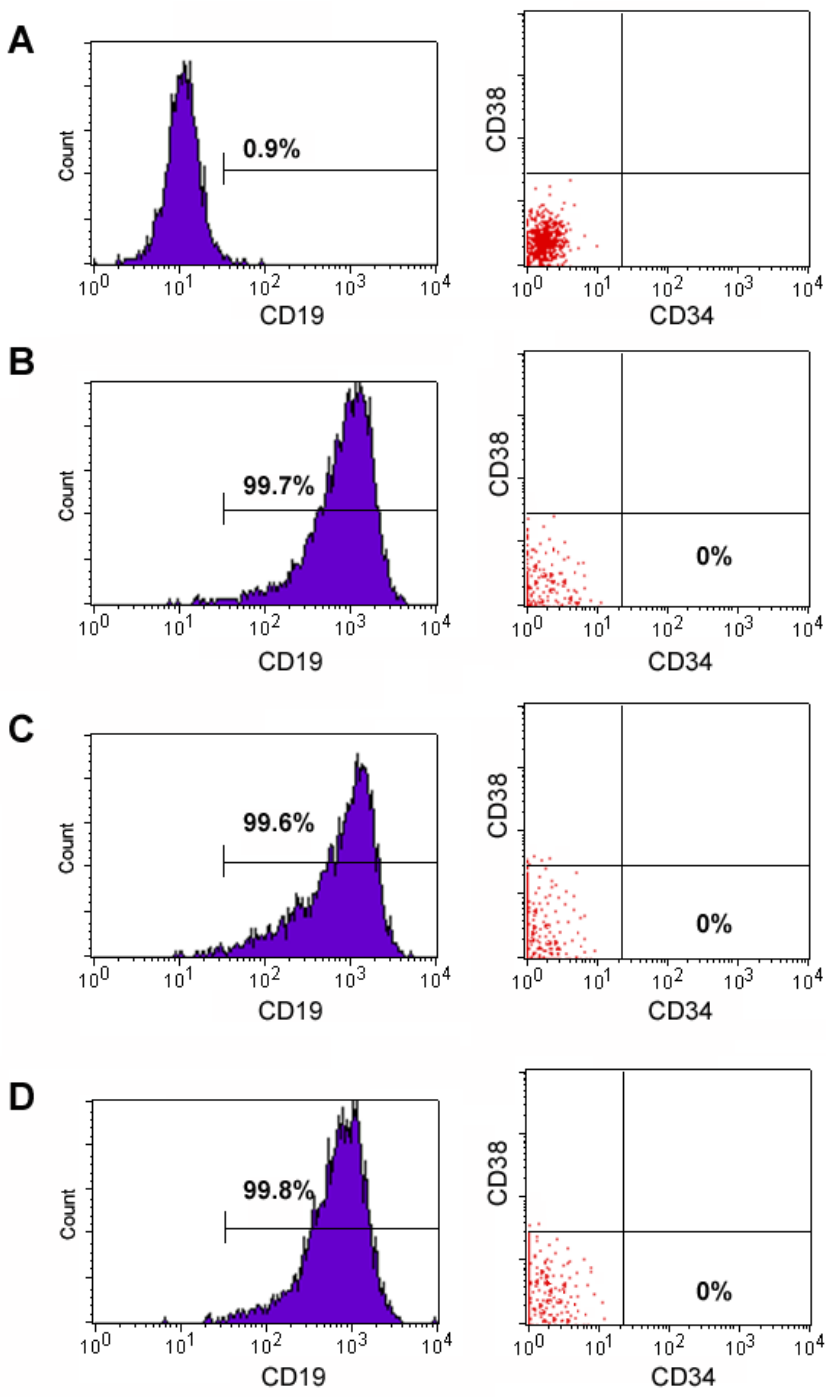

Figure S4. Expression of EBV related genes in EBV–B-cell line derived iPSCs

EBNA-1, EBNA-2, LMP-1 and BZLF-1 were analyzed with RT-PCR analysis of mRNA obtained from human fibroblasts, parental B cells, and the B-cell line derived iPSC line. RT-PCR results reveal that EBV related genes are no longer expressed in reprogrammed and established iPSCs.