SUPPLEMENTAL MATERIAL

Epigenetic Reprogramming Induces the Expansion of Cord Blood Stem Cells

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Supplemental Figure Legends

Supplemental Figure 1: Phenotypic analysis of primary cord blood (CB) $CD34^+$ cells (PC) and $CD34^+$ cells cultured in the serum-free (SF) media without cytokines (Media alone) or in the presence of VPA alone without cytokines for 7 days. CD34, CD90, CXCR4 (CD184), CD49f and CD45RA expression by cultured cells is shown. The co-expression of CD184, CD49f and CD45RA by $CD34^+CD90^+$ cells (red box) is depicted. (n=4)

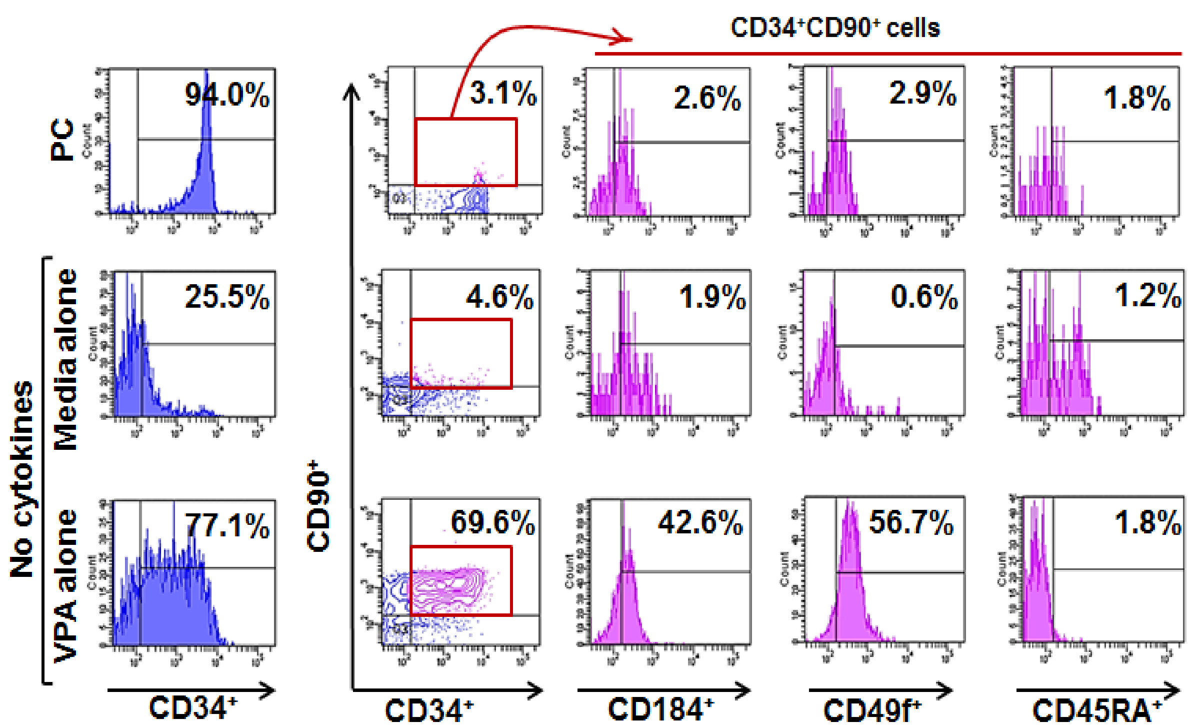
Supplemental Figure 2: Effect of HDACIs on HDAC protein levels in HEK293 cells. HEK293 cells were treated with SCR, C433 and VPA for 2 hr (**T1**) and 24 hr (**T2**). Western blots were probed with primary monoclonal antibodies (mAb) to several Class I (1, 2 and 3), Class IIa (4 and 5) and Class IIb (6) HDACs as described in the Methods section. Each HDACI uniformly affected the expression of HDAC2 and HDAC4. β-actin was used as a loading control. (n=4)

Supplemental Figure 3: Confocal microscopic analysis of pluripotency genes in ES cells. ES(H9) cells were fixed, permeabilized and stained with OCT4/SOX2/NANOG/ZIC3 antibodies (FITC-green) as described in the Methods section. The nuclei were stained with DAPI (blue). Pluripotency gene proteins including SOX2, OCT4, NANOG and ZIC3 (green) were more prominent in the nuclei than cytoplasm of ES cells. A single optical section of the confocal z-series (scale bar= 25 μm (63 X magnification, with optical zoom)) is shown.

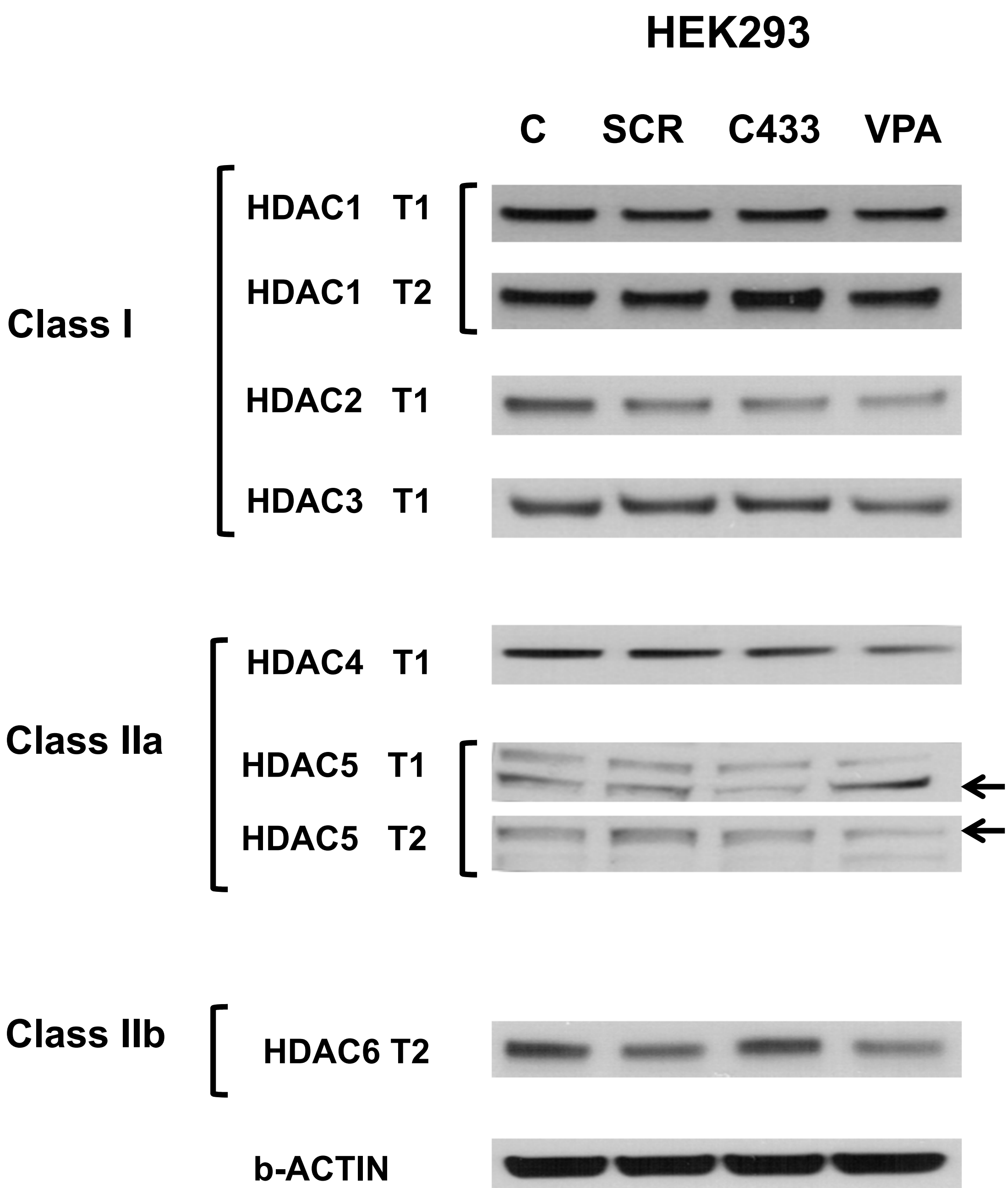
Supplemental Figure 4: Teratoma formation assay. $1x10^6$ of ES (H9) cells or CD34+ cells that were reisolated from control cultures or cultures containing VPA were mixed with Matrigel and injected subcutaneously into the right hind limb of NSG mice (n=9). After 8 weeks, the mice were sacrificed and the masses were dissected, fixed and stained with hematoxylin & eosin. (A and B) Only ES (H9) cells formed teratomas in each of the three mice (left panel), (C) neither control nor VPA treated CD34$^+$ cells formed teratomas (right panel), (D) A photomicrograph of the stained section showing three different germ layers (small arrow head- Ectoderm, solid arrow- Mesoderm and broken arrow- Endoderm) (4x),

(E) mesoderm (cartilage) (4X), (F) pigmented ectoderm (20X) and (G) endoderm (20X). 3 mice were utilized per group including ES (H9), Control and VPA (n= 9 mice).

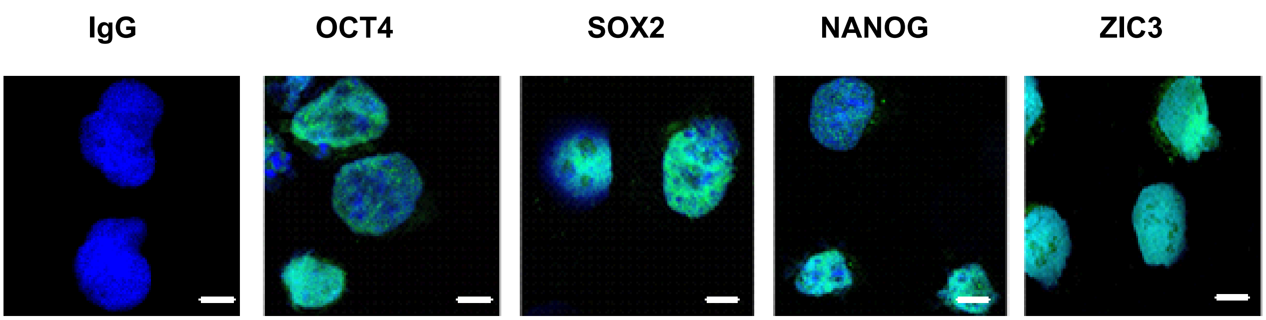
Supplemental Figure 1



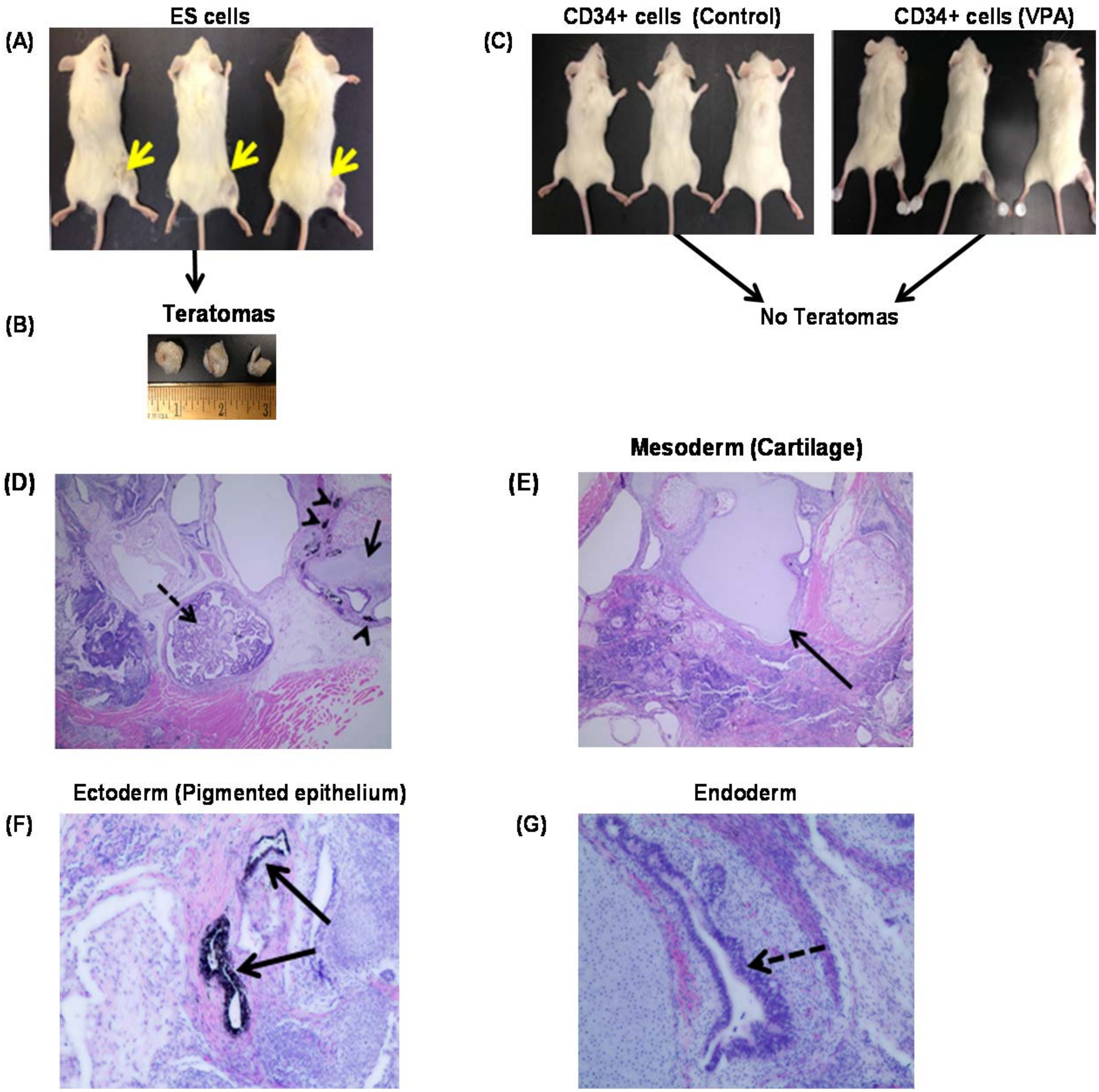
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Table 1

Densitometric analysis of Western blots (Figure 5)*:* **Upper panel**: HDAC protein levels were evaluated by densitometry and were normalized to b-ACTIN expression. **Lower panel**: Percent downregulation of class I and class II HDACs with respect to control. A reduction in HDAC1, -3 and -5 occurred in the presence of each HDACI. (Mean ± SE, n=4)

HDAC expression relative to b-ACTIN (%)

	Control	SCR	VPA	C433
HDAC1	46.1±4.6	21.0±20.0	25.48±7.78	24.3±0.4
HDAC2	67.0±25.2	33.0±7.0	45.8±25.2	40.0±8.0
HDAC3	40.5±7.4	4.7±3.1	11.4±12.4	28.0±20.0
HDAC4	43.4±11.8	16.6±7.8	30.5±9.3	23.5±16.5
HDAC5	54.8±9.4	22.8±30.6	21.4±10.7	12.6±1.9
HDAC6	59.5±5.6	41.3±7.9	54.2±7.9	35.8±7.2

HDAC down-regulation relative to control (%)

	SCR	VPA	C433
HDAC1	54.3±49.2	45.3±11.5	40.6±15.0
HDAC2	49.2±8.6	16.5±12.0	41.7±5.3
HDAC3	89.0±5.7	40.6±19.9	51.4±13.6
HDAC4	62.78±7.9	30.0±2.4	47.2±22.5
HDAC5	62.6±49.4	58.6±26.7	70.3±1.0
HDAC6	30.9±6.8	9.1±4.8	49.1±19.1

Supplemental Table 2

Effects of siRNA transfection on the control culture and VPA treated culture.

Upper panel: Control cultures were transfected with siRNA as previously described for VPA cultures in the Methods section. No significant difference in the total cell numbers and percent of $CD34^{+}CD90^{+}$ cells was observed following 72 hr after transfection with Scrambled and SON siRNA. (n=4) (*p<0.5, ns)

Lower panel: VPA treated cultures were transfected with Scrambled and *GAPDH* siRNA as previously described in the Methods section. No significant difference in the cell number or percent of $CD34^{+}CD90^{+}$ cells was observed following 72 hr after *GAPDH* siRNA transfection. (n=3) (*p<0.25, ns)

	siRNA	**(Control culture) Total number of cells/well**	**(Control culture) CD34+CD90+ cells (%)**
1.	**No -transfection**	$6.6 \times 10^{6} \pm 1.5 \times 10^{6}$*	17.1±7.8*
2.	**Scrambled**	$6.6 \times 10^{6} \pm 1.6 \times 10^{6}$*	16.7±4.7*
3.	***GAPDH***	$6.3 \times 10^{6} \pm 2.0 \times 10^{6}$*	18.3±3.5*
4.	***SON* (*SOX2, OCT4* and *NANOG*)**	$5.9 \times 10^{6} \pm 2.0 \times 10^{6}$*	21.2±2.5*

	siRNA	**(VPA culture) Total number of cells/well**	**(VPA culture) CD34+CD90+ cells (%)**
1.	**No -transfection**	$4.0 \times 10^{6} \pm 0.4 \times 10^{6}$*	78.6±2.0*
2.	**Scrambled**	$3.9 \times 10^{6} \pm 0.5 \times 10^{6}$*	76.0±2.4*
3.	***GAPDH***	$3.3 \times 10^{6} \pm 0.8 \times 10^{6}$*	75.0±1.7*
4.	***SON* (*SOX2, OCT4* and *NANOG*)**	$3.4 \times 10^{6} \pm 0.5 \times 10^{6}$*	73.2±3.5*

Supplemental Table 3

In vivo functional behavior of VPA-treated CD34+ cells cultured under serum-free (SF) conditions without cytokines in NOD/SCID/γc^{null} (NSG) mice.

Bone marrow analysis of NSG mice receiving $2.0x10^5$ **primary CB CD34+ cells (PC)** or $CD34^+$ cells reisolated after 7 days from cultures containing media alone (no cytokines) and cultures containing VPA alone (no cytokines) under serum-free (SF) conditions. The percentage of human cell chimerism ($CD45^+$, $CD33^+$ and $CD34^+$) and multilineage hematopoietic cell engraftment including B cells ($CD19^+$), granulocytes ($CD14^+$), erythroid cells (Glycophorin A (GPA^+) and megakaryocytes ($CD41^+$) after 12-13 weeks of transplantation is shown. (Mean±SE, *$p<0.05$,(ANOVA $P<0.0001$)**.** NSG mice recipients (n=15).

% Human cell engraftment in primary NSG mice							
	CD45	**CD33**	**CD34**	**CD19**	**CD14**	**GPA**	**CD41a**
PC (n=5)	19.4±4.8	4.5±6.8	7.4±1.9	5.7±3.3	6.6±1.6	3.7±0.7	1.2±0.2
Media Alone (n=5)	8.2±2.2	0.7±0.2*	0.76±0.2*	5.2±1.6	5.4±1.3*	9.9±2.2	0.62±0.6
VPA Alone (n=5)	12.5±2.2	3.5±0.6*	1.6±0.4*	1.9±1.1	2.1±0.2*	9.8±0.8	0.90±0.6

Supplemental Table 4

Primer sequences for RT-PCR and Q-PCR

Gene	Forward primer sequence (5’-3’)	Reverse primer sequence (5’-3’)
Pseudo OCT4*	GAAGGTATTCAGCCAAAC	CTTAATCCAAAAACCCTGG
Pseudo OCT4*	CGACCATCTGCCGCTTTGAG	CCCCCTGTCCCCCATTCCTA
OCT4	AACCTGGAGTTTGTGCCAGGGTTT	TGAACTTCACCTTCCCTCCAACCA
SOX2	AGAAGAGGAGAGAGAAAGAAAGGGAGAGA	GAGAGAGGCAAACTGGAATCAGGATCAAA
NANOG	CCTGAAGACGTGTGAAGATGAG	GCTGATTAGGCTCCAACCATAC
TERT	TGAAAGCCAAGAACGCAGGGATG	TGTCGAGTCAGCTTGAGCAGGAATG
CD34	ACAAACATCACAGAAACGACAGT	TGACAGGCTAGGCTTCAAGGT
SET	GCAAGAAGCGATTGAACACA	GCAGTGCCTCTTCATCTTCC
MYST3	ACTCCACCACCTACGAATGC	CTCCTTCCTCAGCCTCCTCT
SMARCAD1	TGGAAGACCTTTCGGAATTG	CACCTGCATCACCAAACATC
ZIC3	GCAAGTCTTTCAAGGCGAAG	CATGCATGTGCTTCTTACGG
GAPDH	from Qiagen	

*Redshaw, Z., and Strain, A.J. 2010. Human haematopoietic stem cells express Oct4 pseudogenes and lack the ability to initiate Oct4 promoter-driven gene expression. *J Negat Results Biomed* 9:2-8.