Supplemental Material

Detailed Methods

Cell culture and drug treatment. Lineage - Sca-1+ CD31+ EPCs were cultured on 5μg/mL human fibronectin coated plates in DMEM supplemented with 10% FBS and penicillin/streptomycin at 37°C with 5% CO_2. Human CD34+ cells were cultured in X-VIVO (Lonza) media supplemented with 0.5% human serum albumin, 50ng/mL Flt3 ligand, 20ng/mL stem cell factor, 50ng/mL VEGF, and 10ng/mL thrombopoietin. Either cell type was treated with VPA for 24 hours then 500nM 5'Aza was added to the culture media and cells remained in culture for an additional 24 hours (48 hours total). Mouse EPCs were also cultured with 1μM BIX-01294 for 48 hours in the same culture conditions.

Mice. Eight - ten week old C57BL/6J (stock number 000664), C57BL/6-Tg eGFP (stock number 003291) or nude (stock number 00819) mice were purchased from Jackson Laboratory. All experiments conform to protocols approved by the Institutional Animal Care and Use Committee at Northwestern University (Chicago, IL) in compliance with all state and federal regulations governing the use of experimental animals.

Western blot analysis. Cell lysate from $1.5x10^7$ SVEC cells was prepared using whole cell lysate buffer (50mM Tris-HCl, 0.5% Igepal (NP-40), 150nM NaCl). Proteins (90 μg) were electrophoresed by SDS-PAGE and analyzed using antibodies against acetyl-histone H3K9 (C5B11), di-methyl-histone H3K9 (Cell Signaling), H3 (Cell Signaling), pan acetyl H4 (Active Motif) or H4 (Abcam). Equal protein loading in each lane was verified using antibodies against β-actin and changes in modified histone levels were quantified by first normalization to total H3 or H4 protein.

Immunofluorescence. Immunofluorescence was performed as previously described [1]. Deparaffinized tissue sections were stained for anti-CD31 antibody (BD Biosciences) for capillary density. Donor cells in host tissue were detected by anti-eGFP. Cardiomyocytes were detected by alpha sarcomeric actin antibody (Sigma). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) (1:5000, Sigma Aldrich), and sections were examined with a fluorescent microscope (Nikon ECLIPSE TE200).

Pyrosequencing. PCR reactions were carried out using the Hotstart Taq polymerase kit (Qiagen). For each PCR reaction, 50ng of the bisulfite converted DNA was used as a template with 50pm of forward and reverse primers. After 5 min of initial denaturation at 95°C, the cycling conditions of 44 cycles consisted of denaturation at 95°C for 15 s, annealing at 65°C for 30 s and elongation at 72°C for 45 s. Pyrosequencing was performed using the PyroMark MD Pyrosequencing System (Biotage) as described previously [2]. Pyrosequencing primer at a concentration of 0.3 μM was annealed to the purified single-stranded PCR product at 28°C. Methylation quantification was performed using the manufacturer-provided software.

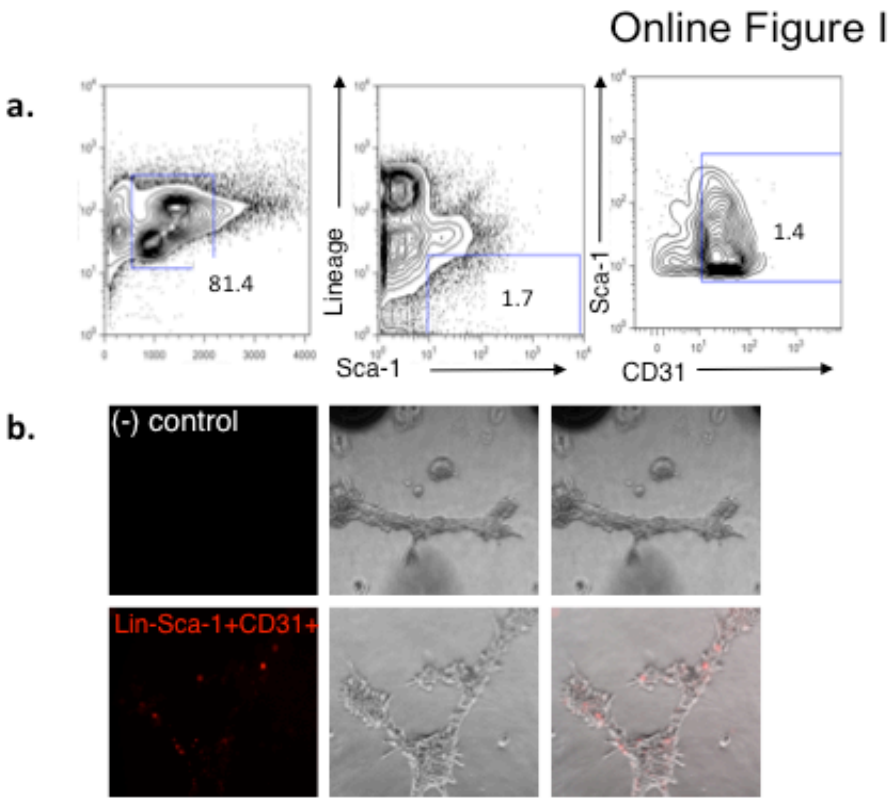
Microarray. Genome-wide expression analysis was preformed on RNA isolated from $1x10^6$ human CD34+ cells cultured 48 hours in X-VIVO (Lonza) media supplemented with 50ng/mL Flt3L, 20ng/mL stem cell factor, 50ng/mL VEGF, 10ng/mL TPO, 0.5% human serum albumin or 24 hours in the same media + 2.5mM VPA followed by an additional 24 hours with 500nM 5'Azacytidine. RNA was isolated and purified using RNA Stat-60 (Tel-test) as per the manufacturer's suggested protocol. RNA quality and purity was assessed using NanoDrop ND-1000. Using the Human HOA5.1 OneArray (Phalanx Biotech Group), data was collected then analyzed on the Rosetta Resolved System (Rosetta Biosoftware). Differentially expressed gene list was produced by a

standard selection criteria as established by $|\text{Fold Change}| \geq 1$ and $P < 0.05$. Clustering analysis was performed to visualize the correlations among the replicates and different sample conditions. A subset of 398 genes was selected based on an intensity filter set such that the difference between the maximum and minimum intensity values exceeds 6000 among all microarrays.

Angiogenesis ELISA array. Two million human CD34+ cells were cultured as described for 48 hours at $1x10^6$ cells/mL. Conditioned media was first collected by removal of cells by centrifugation (400 x g for 15 minutes) then applied to the human angiogenesis array (R&D Systems) as per the manufacturer's suggested protocol. Data were analyzed by ImageJ 1.43u software (NIH). Trends were consistent with a second independent experiment.

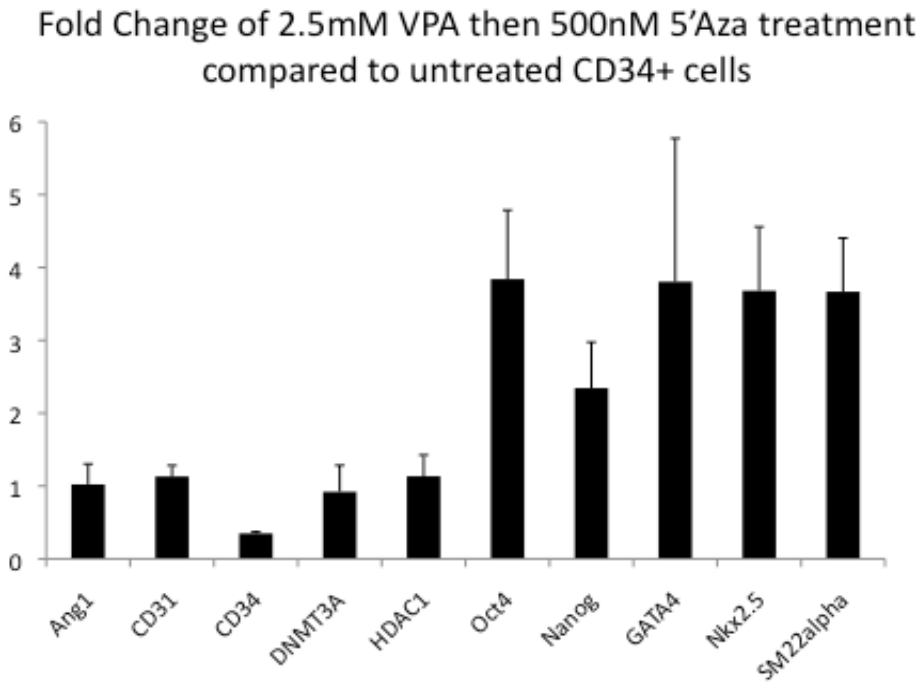
References

1. Krishnamurthy P, Rajasingh J, Lambers E, Qin G, Losordo DW, Kishore R. Il-10 inhibits inflammation and attenuates left ventricular remodeling after myocardial infarction via activation of stat3 and suppression of hur. *Circ Res*. 2009;104:e9-18
2. Xie H, Wang M, Bonaldo Mde F, Smith C, Rajaram V, Goldman S, Tomita T, Soares MB. High-throughput sequence-based epigenomic analysis of alu repeats in human cerebellum. *Nucleic Acids Res*. 2009;37:4331-4340



Online Figure I: Isolation of Lineage-Sca-1+CD31+ EPCs from mouse bone marrow. (a) Sorting strategy for obtaining EPCs directly from mouse bone marrow. Percentages are of total bone marrow. (b) Tube formation assay shows incorporation of DiI labeled Lin-Sca-1+CD31+ cells into tubes formed by 5.0×10^4 SVEC cells. 10x magnification.

Online Figure II



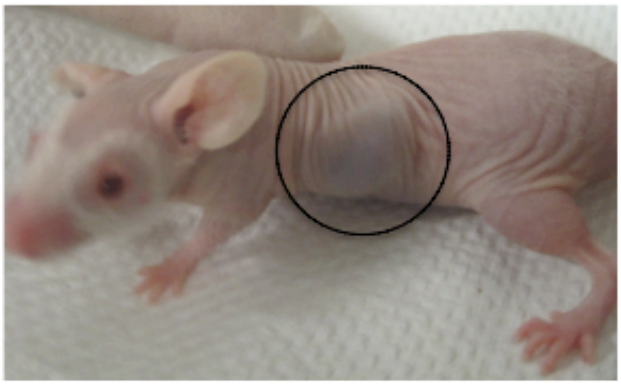
Online Figure II: Real-time PCR data represented as fold difference in mRNA expression in $5.0x10^5$ VPA then 5'Aza treated CD34+ cells compared to untreated control cells. (n=3)

Online Figure III

No Drug

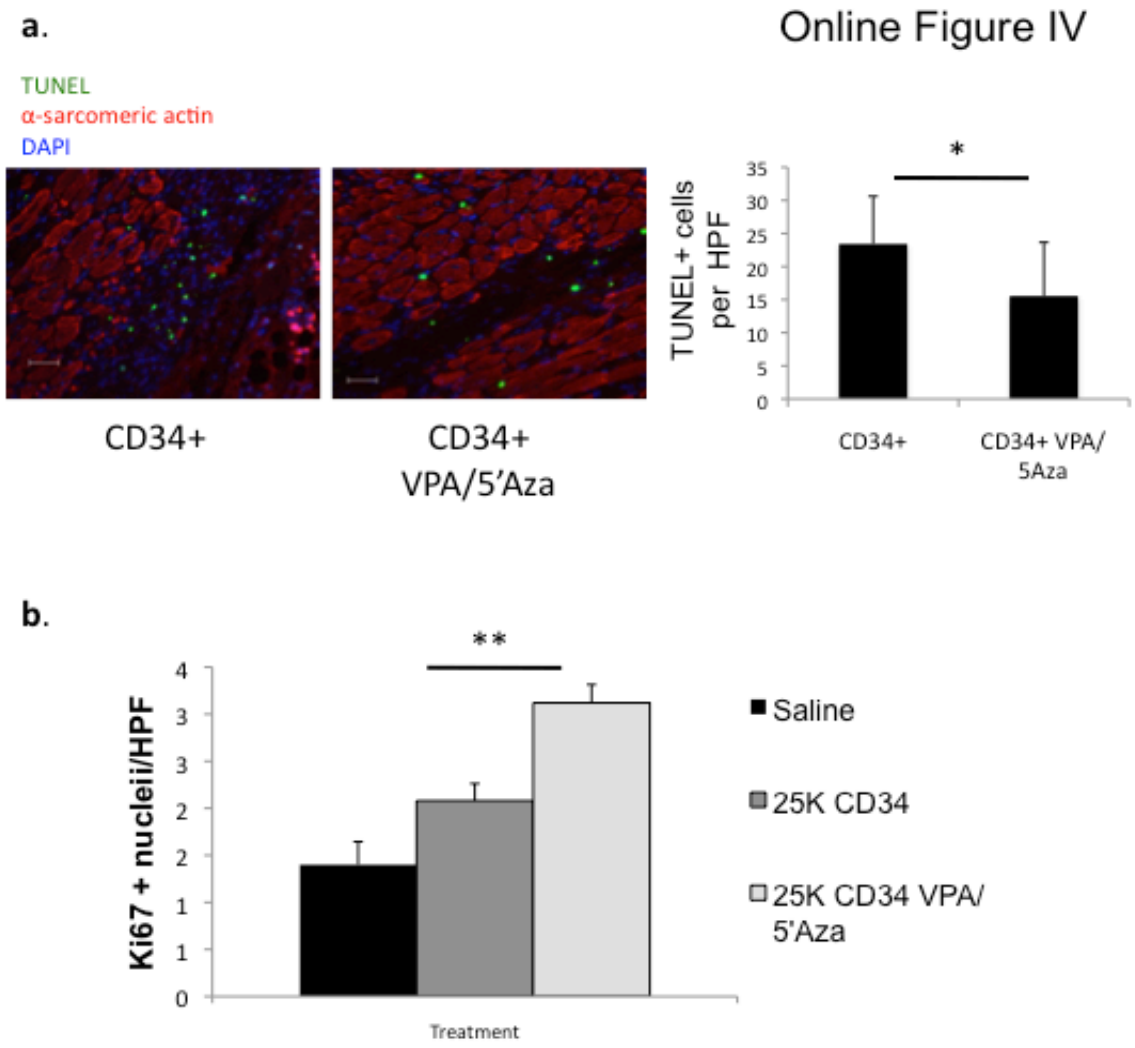
VPA/ 5'Aza

BIX-01294



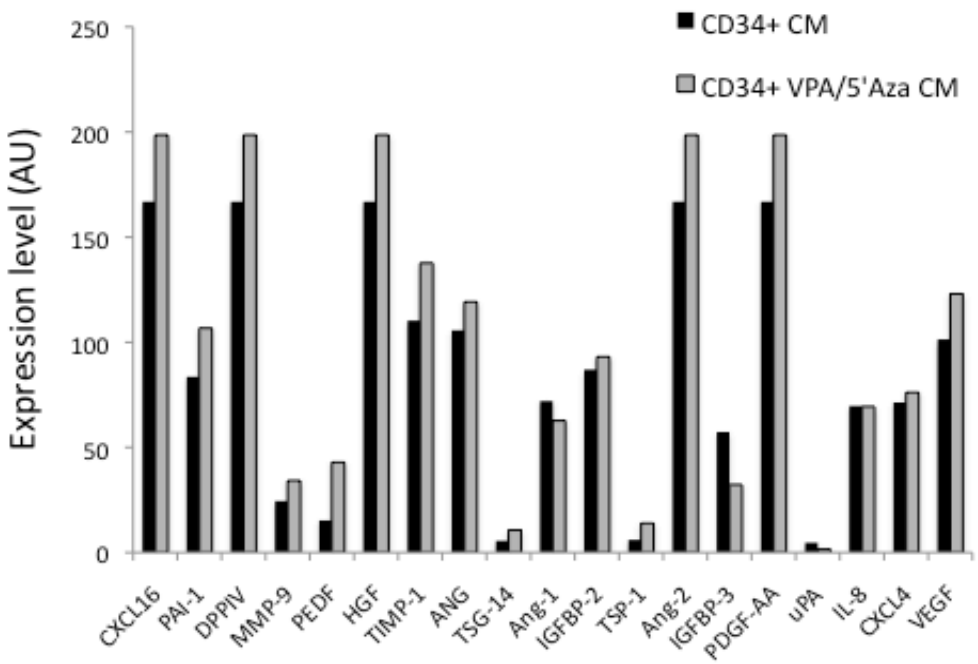
+ control: mouse embryonic stem cells

Online Figure III: No teratoma formation occurred when 1 million untreated, VPA/5'Aza or BIX-01294 treated bone marrow cells were injected into the flanks of nude mice. Positive teratoma formation with 1 million mouse embryonic stem cells (mES). (n=3)



Online Figure IV: VPA/5'Aza treated CD34+ cell therapy in mouse AMI results in less apoptosis and increased proliferation in the border zone. (a) Representative TUNEL stained sections with alpha-sarcomeric actin (red) and DAPI (blue). Scale bar is 20 μm. Quantification of TUNEL+ cells from 3 high power fields per heart of the border zone of myocardial infarcted mice, minimally 4 mice per condition, 14 days post-AMI. (b) Quantification of Ki67+ nuclei per high power field (HPF) at day 28 post-AMI. *$p \leq 0.05$, **$p \leq 0.01$

Online Figure V



Online Figure V: Reprogrammed human CD34+ cells have increased angiogenic protein secretion. Human angiogenesis ELISA array shows a trend of increased angiogenesis protein levels in conditioned media [3] from VPA/5'Aza treated CD34+ cells compared to the untreated cells. Representative results from one array, confirmed by an independent experiment.

Online Table I

geneset name	# genes in geneset (K)	p value
LEARNING_AND_OR_MEMORY	14	1.58E-01
PROTEIN_TETRAMERIZATION	14	1.58E-01
SENSORY_ORGAN_DEVELOPMENT	14	1.58E-01
TUBE_MORPHOGENESIS	15	1.91E-01
PEPTIDE_METABOLIC_PROCESS	10	1.92E-01
REGULATION_OF_AXONOGENESIS	10	1.92E-01
RESPONSE_TO_DRUG	21	2.15E-01
ACTIVATION_OF_IMMUNE_RESPONSE	17	2.62E-01
CELLULAR_RESPONSE_TO_EXTRACELLULAR_STIMULUS	12	2.80E-01
TUBE_DEVELOPMENT	18	2.99E-01
NEGATIVE_REGULATION_OF_ANGIOGENESIS	13	3.25E-01
HUMORAL_IMMUNE_RESPONSE	31	3.43E-01
INACTIVATION_OF_MAPK_ACTIVITY	14	3.71E-01
REGULATION_OF_NEUROGENESIS	14	3.71E-01
AMINO_ACID_TRANSPORT	26	3.74E-01
CYTOPLASM_ORGANIZATION_AND_BIOGENESIS	15	4.15E-01
LIPID_HOMEOSTASIS	16	4.59E-01
AMINO_ACID_DERIVATIVE_BIOSYNTHETIC_PROCESS	10	4.72E-01
CELLULAR_RESPONSE_TO_NUTRIENT_LEVELS	10	4.72E-01
CELLULAR_RESPONSE_TO_STRESS	10	4.72E-01
HETEROPHILIC_CELL_ADHESION	10	4.72E-01
NEUTRAL_AMINO_ACID_TRANSPORT	10	4.72E-01
POSITIVE_REGULATION_OF_CELL_MIGRATION	10	4.72E-01
POSITIVE_REGULATION_OF_EPITHELIAL_CELL_PROLIFERATION	10	4.72E-01
SPERMATID_DEVELOPMENT	10	4.72E-01
SPHINGOLIPID_BIOSYNTHETIC_PROCESS	10	4.72E-01
ACTIN_POLYMERIZATION_AND_OR_DEPOLYMERIZATION	23	4.85E-01
ICOSANOID_METABOLIC_PROCESS	17	5.02E-01
NEGATIVE_REGULATION_OF_MAP_KINASE_ACTIVITY	17	5.02E-01
PHAGOCYTOSIS	17	5.02E-01
REGULATION_OF_ACTION_POTENTIAL	17	5.02E-01
REGULATION_OF_ANATOMICAL_STRUCTURE_MORPHOGENESIS	24	5.20E-01
ENDOTHELIAL_CELL_MIGRATION	11	5.25E-01
INTERCELLULAR_JUNCTION_ASSEMBLY	11	5.25E-01
MITOCHONDRIAL_MEMBRANE_ORGANIZATION_AND_BIOGENESIS	11	5.25E-01
PROTEIN_IMPORT_INTO_NUCLEUS_TRANSLOCATION	11	5.25E-01
SPERMATID_DIFFERENTIATION	11	5.25E-01
JAK_STAT_CASCADE	31	5.34E-01
CELL_RECOGNITION	18	5.42E-01
DETECTION_OF_CHEMICAL_STIMULUS	18	5.42E-01
INORGANIC_ANION_TRANSPORT	18	5.42E-01
NEGATIVE_REGULATION_OF_GROWTH	38	5.46E-01
AGING	12	5.74E-01
CDC42_PROTEIN_SIGNAL_TRANSDUCTION	12	5.74E-01
REGULATION_OF_ACTIN_POLYMERIZATION_AND_OR_DEPOLYMERIZATION	12	5.74E-01
REGULATION_OF_JNK_CASCADE	12	5.74E-01
CELLULAR_RESPONSE_TO_STIMULUS	19	5.81E-01
PROTEIN_POLYMERIZATION	19	5.81E-01
REGULATION_OF_DNA_REPLICATION	19	5.81E-01
REGULATION_OF_MYELOID_CELL_DIFFERENTIATION	19	5.81E-01

Online Table I: Gene families of statistically significantly affected genes with VPA then 5'Aza treatment of CD34+ cells.