Stem Cell Reports, Volume *6*

Supplemental Information

Cyclic AMP Signaling through Epac Axis Modulates Human Hemogenic Endothelium and Enhances Hematopoietic Cell Generation

Shobhit Saxena, Roger E. Rönn, Carolina Guibentif, Roksana Moraghebi, and Niels-Bjarne Woods

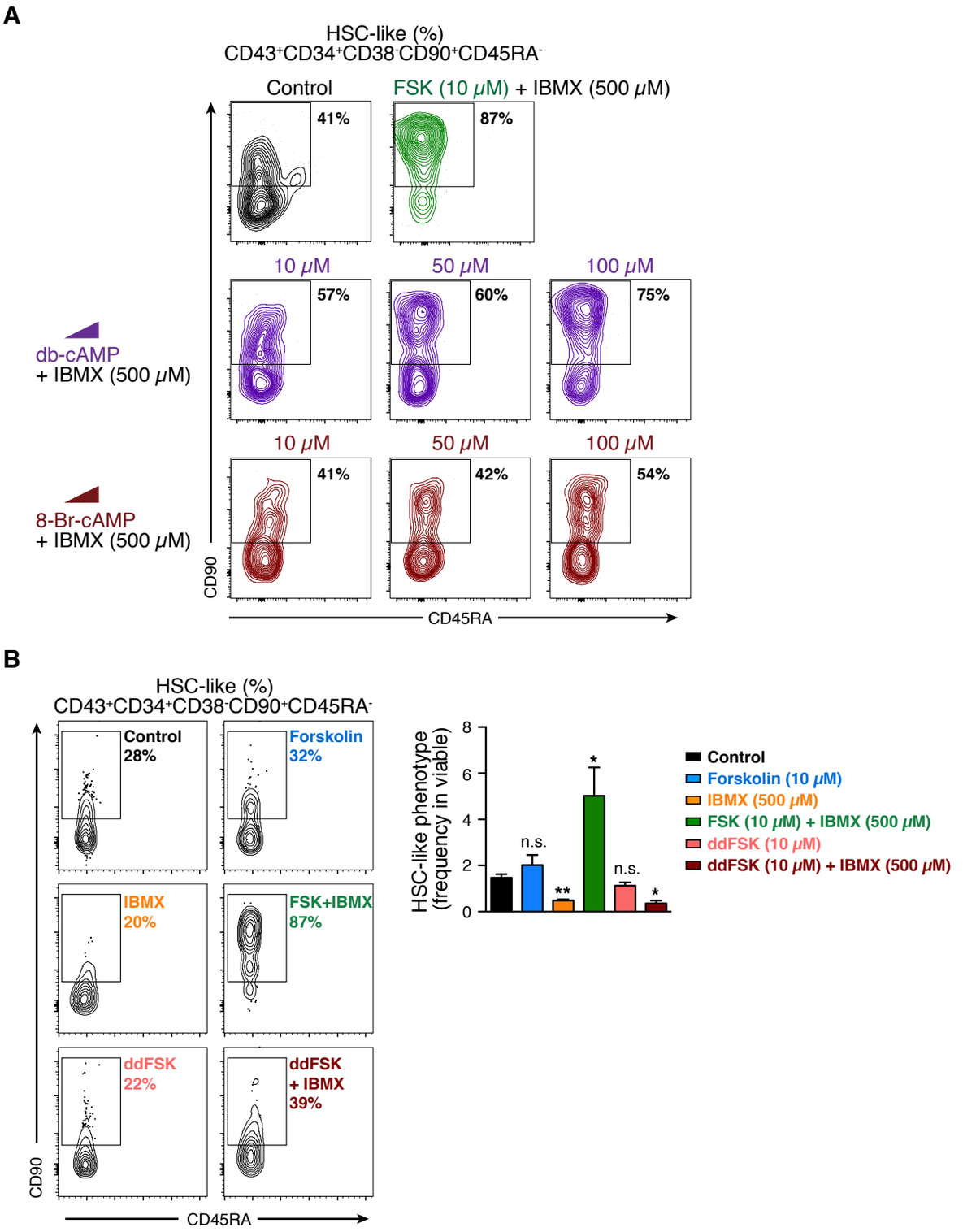

Figure S1, related to Figure 1.

(A) Synthetic cell-permeable cAMP analogs db-cAMP and 8-Br-cAMP increase HSC-like phenotype although a 10-fold higher concentration is required as compared to Forskolin. Flow cytometric plots (biexponential axis) showing the HSC-like surface phenotype in hPSC-derived hematopoietic cells on day 14, a representative experiment is shown.

(B) Effect of cAMP induction using Forskolin+IBMX is specific for upregulation of the HSC-like phenotype, as 1,9-Dideoxy Forskolin (ddFSK), a negative control of Forskolin cannot induce HSC-like phenotype. Left panel, Flow cytometric plots (biexponential axis) showing the HSC-like surface phenotype on day 14, a representative experiment is shown. Right panel, quantification of HSC-like (frequency in viable) is shown. Data represents mean±S.E.M., n=3. Statistical analysis was performed using the t-test. Significance is shown compared to the control setting. *, $p<0.05$, **, $p<0.01$, n.s., not significant.

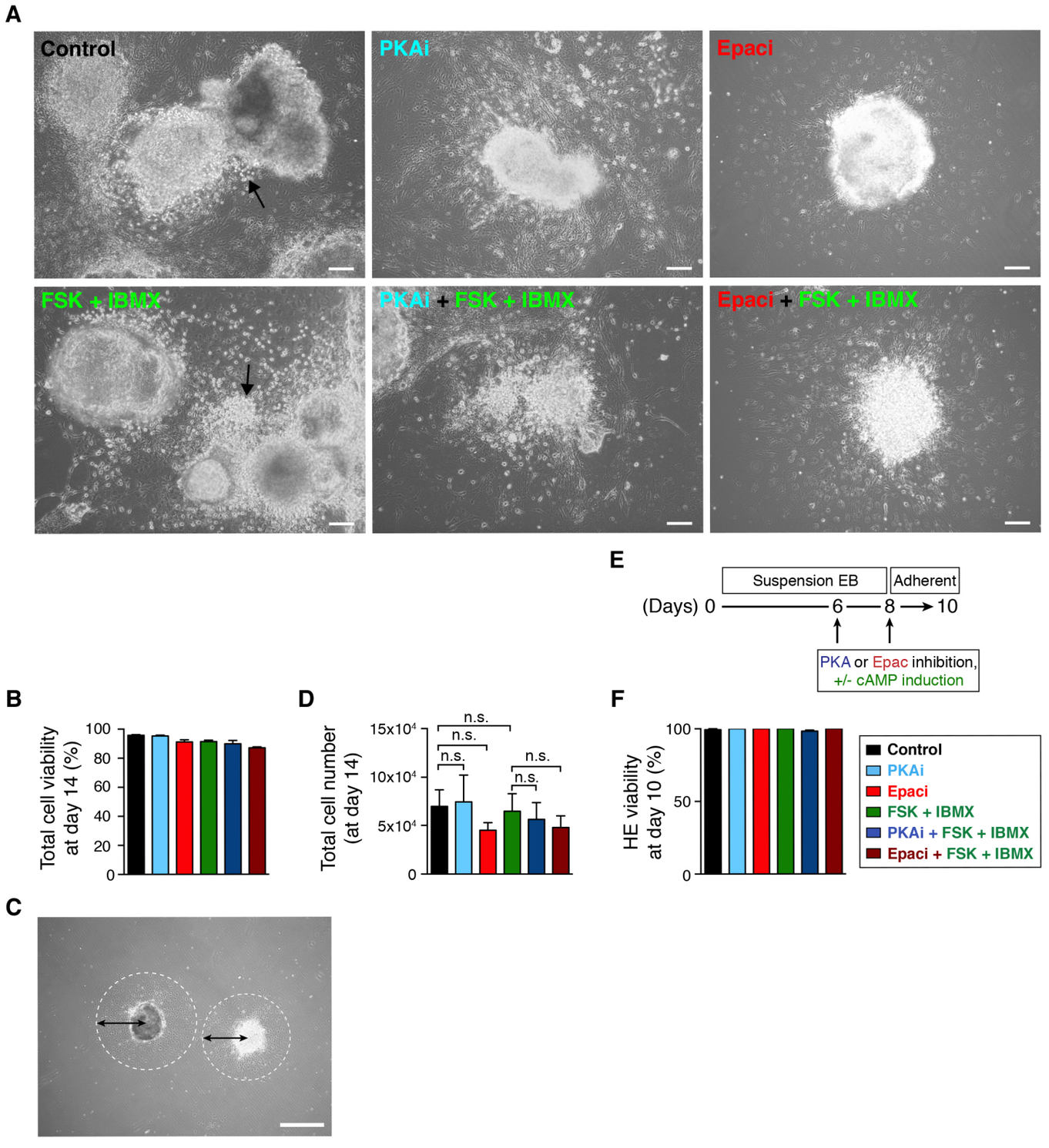

Figure S2, related to Figure 2.

(A) Representative micrographs of differentiation assay at day 13, in the presence or absence of PKA and Epac inhibitors, +/- cAMP induction. Arrows indicate areas where hematopoietic clusters are present. Scale bars = 100 µm.

(B) Total cell viability was not compromised after PKA or Epac inhibition. Viability of cells (day 14) as determined by flow cytometry is shown, live cells were quantified ($7AAD^-$ %). Data represents mean±S.E.M., n=3.

(C) Measurement of radial spread was carried out on day 13 of differentiation (after inhibitor treatment at day 10 and day 12). ImageJ (NIH, USA) was used to quantify the spread (dashed circles), wherein for each individual EB, the distance from the center of an embryoid body to the outer edge of its cellular spread (arrows) was measured in µm. Mean±S.E.M. of more than 100 embryoid bodies analyzed such (n=3) is depicted in Fig. 2C. Scale bar = 200 µm.

(D) Total cell number was reduced after Epac inhibition, in accordance with reduced endothelial cell types (Fig. 2D-F) and EB radial spread after Epac inhibition (Fig. 2C), the data represents total cell number at day 14, values are mean±S.E.M., n=3.

(E) Schematic regimen for inhibitor treatment, to analyze hemogenic endothelium, non-hemogenic endothelium, and arterial endothelium. After applying the factors on day 6 and day 8, the analysis of endothelial cells was carried out at day 10. EB, embryoid body.

(F) Viability of hemogenic endothelium ($CD43^-CD34^+CXCR4^-CD73^-VEcad^+$) at day 10 is shown. Data represents mean±S.E.M., n=3.

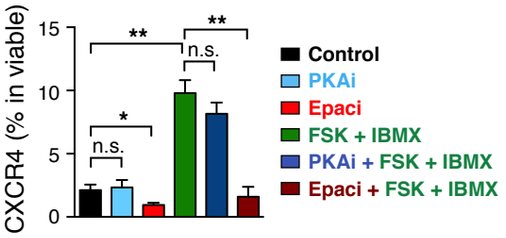

Figure S3, related to Figure 3.

Cyclic AMP-Epac axis regulates CXCR4 expression. Assessment of CXCR4 expression levels, after Forskolin+IBMX mediated cAMP induction and PKA or Epac inhibition (PKAi, Epaci) with or without Forskolin+IBMX is shown (day 14). Data represents mean±S.E.M., n=3. Statistical analysis was performed using the t-test. *, $p<0.05$, n.s., not significant.

Table S1. Primer sequences, related to experimental procedures.

Gene name	Primer sequence
NFE2L2.F	TGACAATGAGGTTTCTTCGGCT
NFE2L2.R	GACTGGGCTCTCGATGTGAC
***SOD2*.F**	GCTCCGGTTTTGGGGTATCTG
***SOD2*.R**	GCGTTGATGTGAGGTTCCAG
***SOD1*.F**	GGTGGGCCAAAGGATGAAGAG
***SOD1*.R**	CCACAAGCCAAACGACTTCC
***GPX2*.F**	GGTAGATTTCAATACGTTCCGGG
***GPX2*.R**	TGACAGTTCTCCTGATGTCCAAA
***CAT*.F**	TGTTGCTGGAGAATCGGGTTC
***CAT*.R**	TCCCAGTTACCATCTTCTGTGTA
***GSR*.F**	TTCCAGAATACCAACGTCAAAGG
***GSR*.R**	GTTTTCGGCCAGCAGCTATTG
***P38MAPKα*.F**	GCTTCAGCAGATTATGCGTCTG
***P38MAPKα*.R**	GTTTCTTGCCTCATGGCTTGG
***P38MAPKδ*.F**	AAGCTGAGCCGACCCTTTC
***P38MAPKδ*.R**	CCAATGACGTTCTCATGCTGC
***P38MAPKγ*.F**	ACATGAGAAGCTAGGCGAGGA
***P38MAPKγ*.R**	GGCAGCGTGGATATACCTCAG
***β-ACTIN*.F**	CCCCGCGAGCACAGAG
***β-ACTIN*.R**	ATCATCCATGGTGAGCTGGC