Figure S1

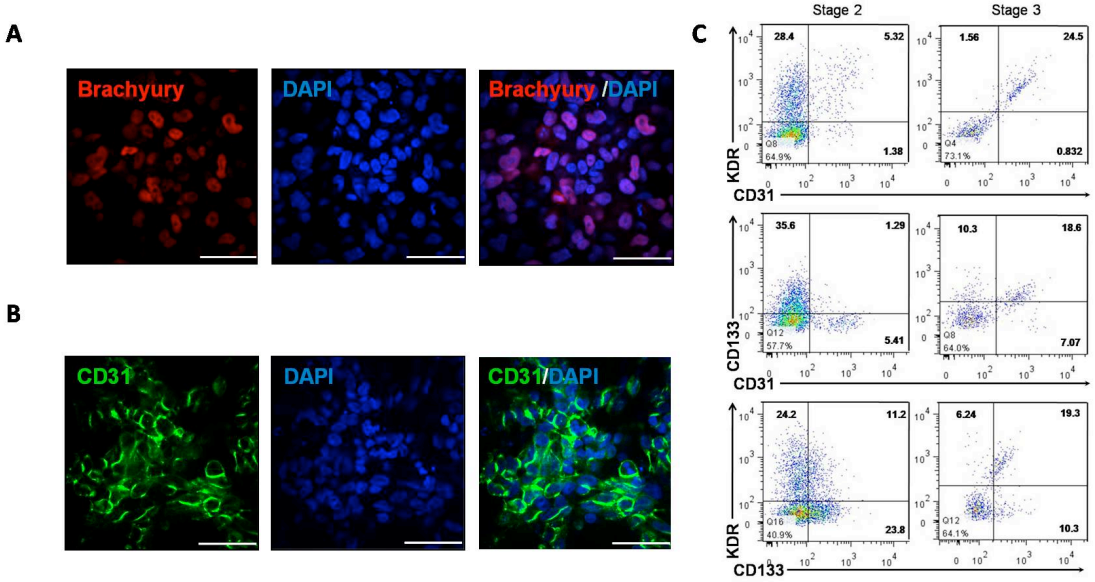

Fig. S1. Stepwise derivation of endothelial lineages from human ESCs.

(A) At 48 hours posted hES cell differentiation by BMP-4 treatment, the induced cells in stage1 expressed mesoderm mark-Brachyury. (Bar=50μm)

(B) Pictures display the morphology of the differentiated hES cells at days 6 with bFGF and VEGF treatment (stage2). Clusters of cell monolayer were positive of endothelial marker-CD31. (Bar=50μm)

(C) Flow cytometry demonstrated the development of endothelial lineages at induction stage 3. At differentiation stage 2, cells expressed endothelial progenitor markers KDR, CD31 and CD133. Higher $CD31^{+}KDR^{+}$ cells merged at the differentiation stage 3, together with lower $CD133^{+}$ population percentage suggesting the maturation of endothelial lineages.

Figure S2

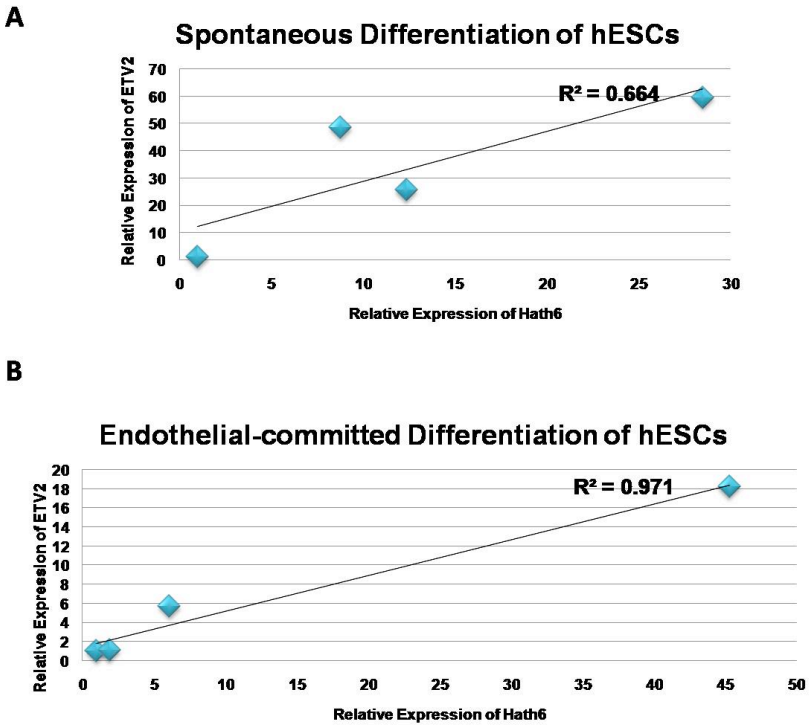

Fig. S2. The expression of *Hath6* and an endothelial marker *ETV2* has a robust correlation both in the spontaneous and stage-depended EC differentiation.

(A) Correlation of the expression of *Hath6* and *ETV2* in the hESC spontaneous differentiation. The coefficient of determination R^2=0.664.

(B) Correlation of the expression of *Hath6* and *ETV2* in the stage-depended hESC-EC differentiation. The coefficient of determination R^2=0.971, which indicated the close relevance of *Hath6* and *ETV2*.

Figure S3

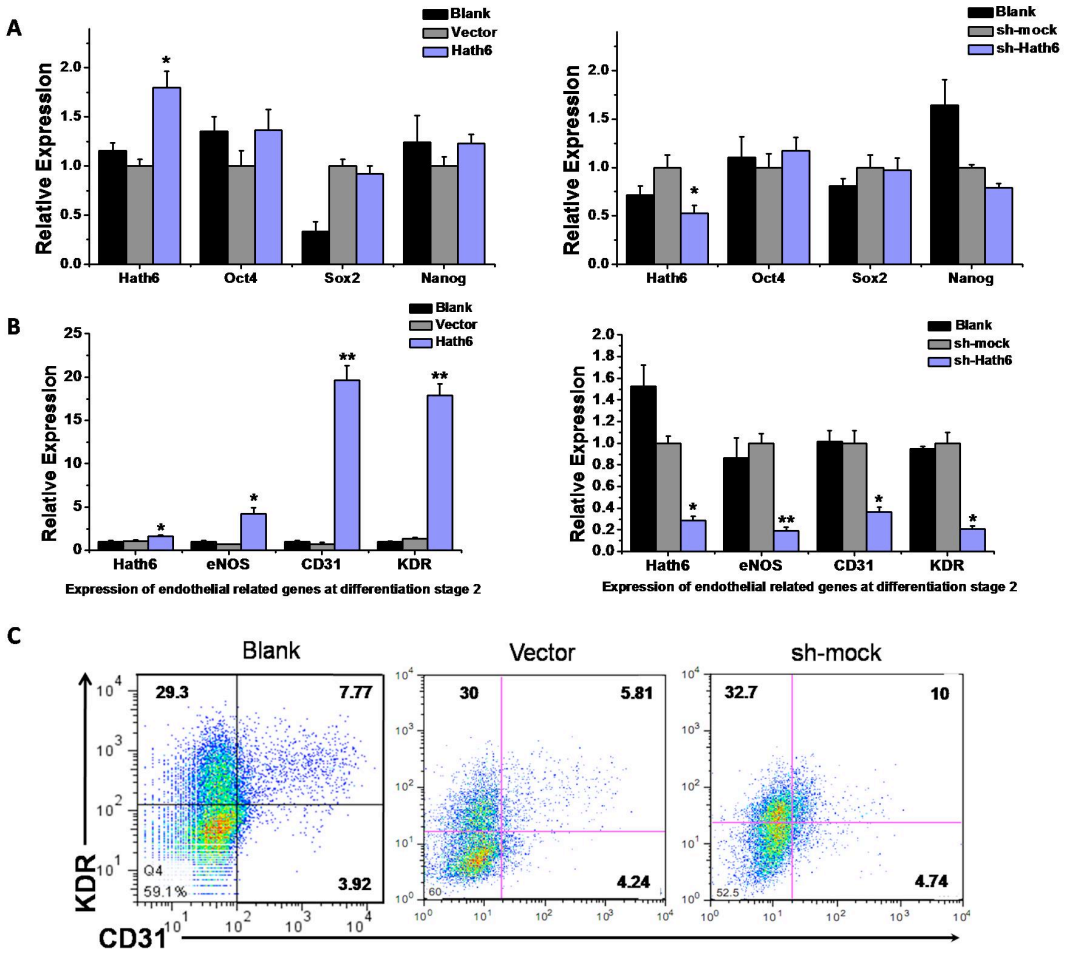

Fig. S3. The transfection of the empty vector and sh-mock control has minute impact on the characteristics of hESCs and the endothelial derivations from these cells.

(A-B) RT-PCR indicates transfection of empty expression vector and sh-mock do not alter the "stemness" gene expression of hESCs (A) and some endothelial specific gene expression by the cells at induction stage 2.

(C) Flow cytometry analysis shows the empty vector and sh-mock transfection have not affected endothelial gene expression at induction stage 2.

Figure S4

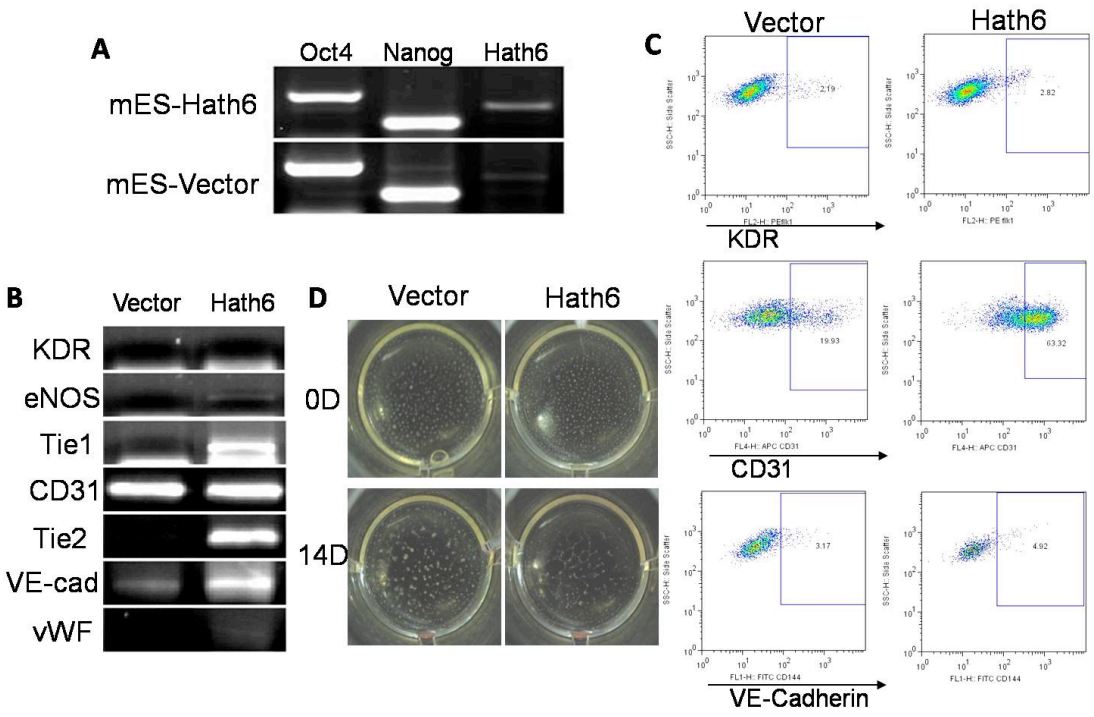

Fig. S4. The overexpression of *Hath6* in mouse ESCs promotes endothelial characters in the differentiation.

(A) The overexpression of *Hath6* in mESCs does not affect embryonic stem cell's totipotency, as shown by the expression of "stemness" marker Oct4 and Nanog.

(B) Agarose gel electrophoresis shows the expression of *Hath6* in mESCs upregulate some endothelial genes in the process of differentiation.

(C) Mouse ESCs were induced to endothelial lineages on collagen IV. The expression of *CD31, KDR* and *VE-cadherin* was observed after 4 days induction by FACS analysis. *CD31* positive endothelial populations are highly upregulated by *Hath6* overexpression.

(D) The assay of vascular-like network on Matrigel reveals that *Hath6*-mESC-EPC form better tube-like structure compared to control.

Figure S5

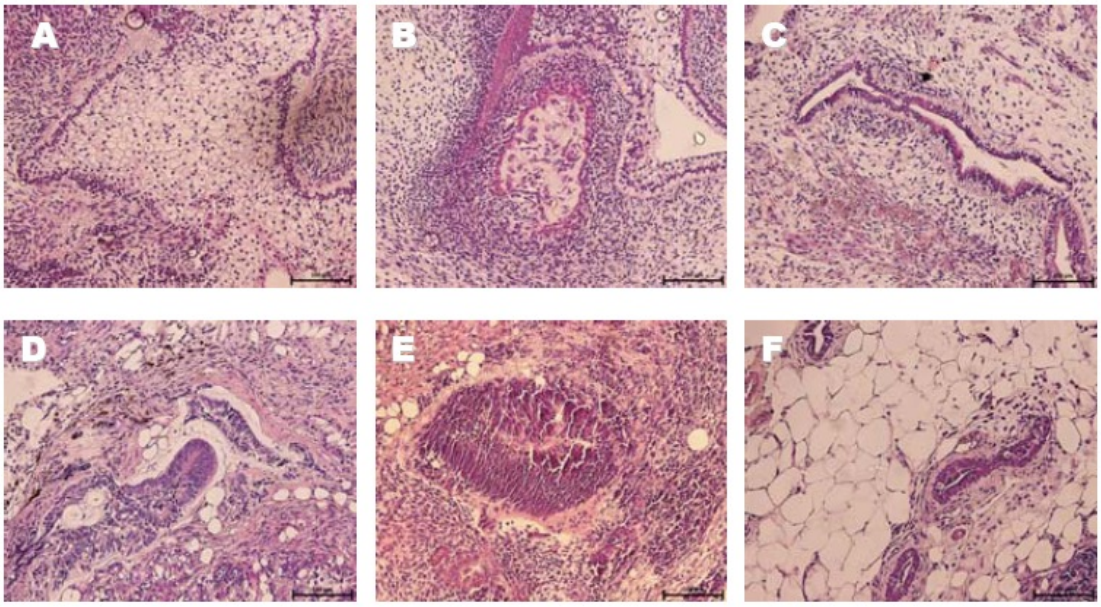

Fig. S5. *Hath6* gene knockdown does not affect *in vivo* teratoma formation from hES cells.

Postmortem histological analysis of subcutaneously transplanted H1 cells shows (A) Squamous epithelium (ectoderm), (B) bone (mesoderm), (C) gut epithelium (endoderm); (D) neural epithelium (ectoderm), (E) muscle (mesoderm), (F) gut epithelium (endoderm). Both *Hath6* gene knockdown H1 hESCs (A-C) and the sh-mock hESCs (D-F) developed all three germ layer morphologies. (Scale bars: 100μm)

Figure S6

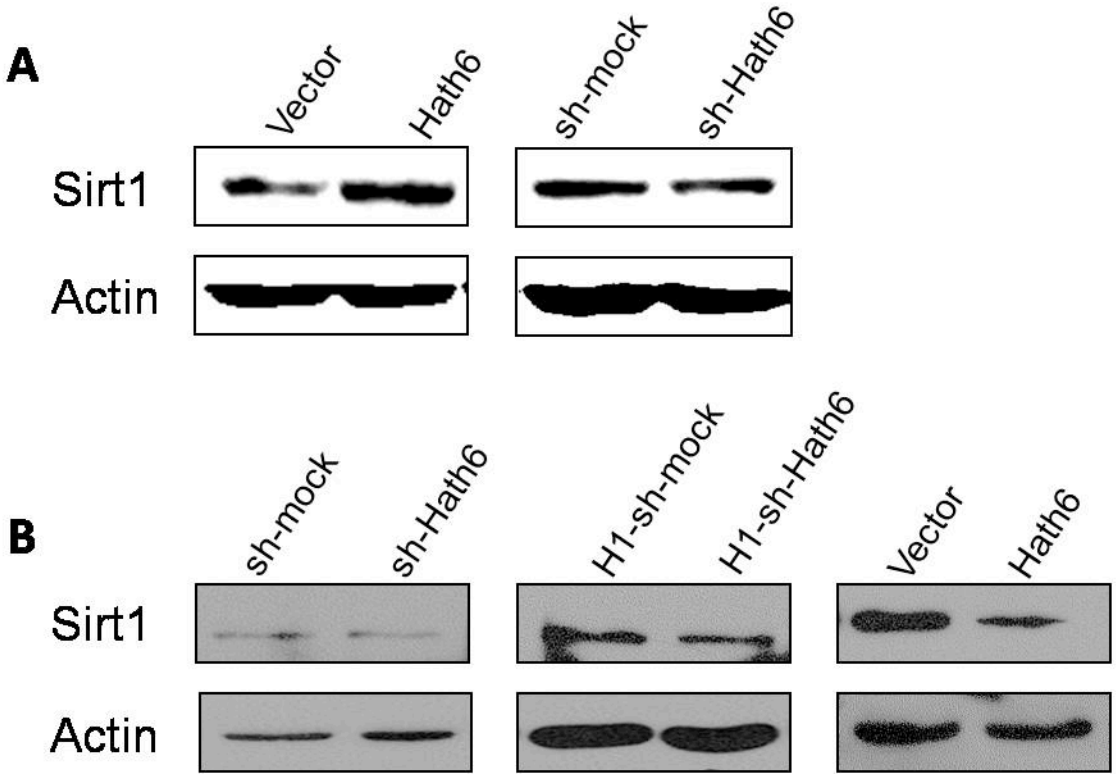

Fig. S6. *Sirt1* is a potential target of *Hath6*.

(A) In ECV-304 cells, the expression of *Sirt1* is upregulated with the overexpression of *Hath6*, and *Sirt1* downregulation is corresponding with the knockdown of *Hath6*.

(B) Western blot depicts the corresponding downexpression of *Sirt1* and *Hath6* in sh-*Hath6* (H9 hESC line) and H1-sh-*Hath6* (H1 hESC line) at stage 2 of phase differentiation. But the overexpression of *Hath6* does not upregulate the expression of *Sirt1*.

Table S1. PCR primers and conditions used for real-time PCR.

Gene	Primers (forward, reverse)	Tm℃	product size（bp）
CD31	ATCATTTCTAGCGCATGGCCTGGT	59	159
	ATTTGTGGAGGGCGAGGTCATAGA		
CD34	AAATCCTCTTCCTCTGAGGCTGGA	59	216
	AAGAGGCAGCTGGTGATAAGGGTT		
KDR	CCTCTACTCCAGTAAACCTGATTGGG	59	219
	TGTTCCCAGCATTTCACACTATGG		
eNOS	AAGATCTCCGCCTCGCTCA	59	336
	GCTGTTGAAGCGGATCTTA		
Hath6	CCTCCTCCGAGATCAAAGC	59	219
	CGGCACTGTAGTCAAGGTCA		
VE-cadherin	TGGAGAAGTGGCATCAGTCAACAG	59	118
	TCTACAATCCCTTGCAGTGTGAG		
ETV2	GAAGGAGCCAAATTAGGCTTCT	60	95
	GAGCTTGTACCTTTCCAGCAT		
Ang2	GCAAGTGCTGGAGAACATCA	61.4	168
	GGCTGTTTGGTTCAACAGGT		
Tie2	TACACCTGCCTCATGCTCAG	55.8	161
	TTCACAAGCCTTCTCACACG		
vWF	CCCACCCTTTGATGAACACA	63.3	366
	CCTCACTTGCTGCACTTCCT		
Oct-4	GGAGGAAGCTGACAACAATGAAA	60	64
	GGCCTGCACGAGGGTTT		
SOX2	TGCGAGCGCTGCACAT	60	72
	TCATGAGCGTCTTGGTTTTCC		
NANOG	ACAACTGGCCGAAGAATAGCA	60	111
	GGTTCCCAGTCGGGTTCAC		
GAPDH	GAGTCAACGGATTTGGTCGT	59	238
	TTGATTTTGGAGGGATCTCG		