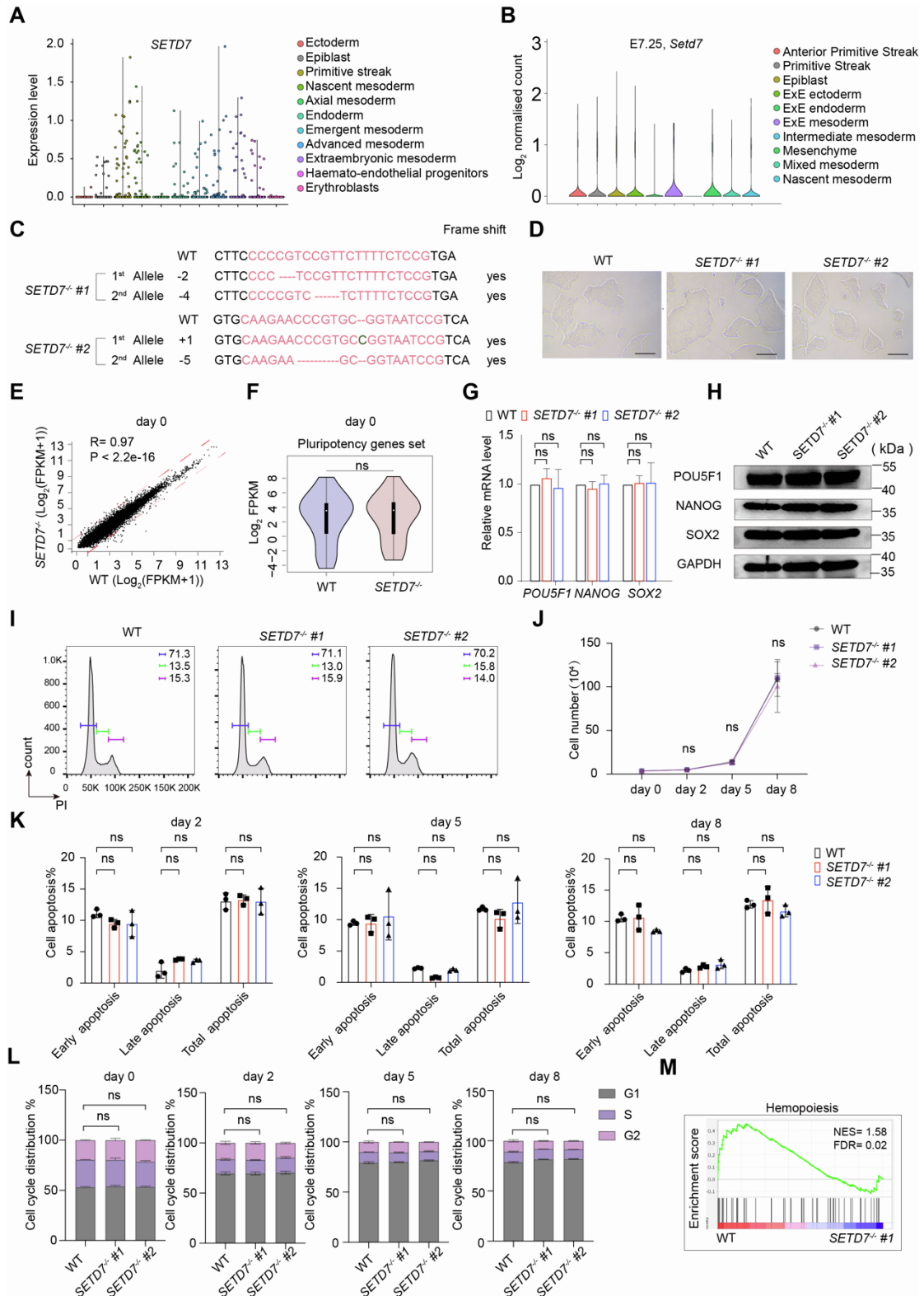


**Supplemental information**

***SETD7* promotes lateral plate mesoderm formation  
by modulating the Wnt/ $\beta$ -catenin signaling pathway**

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**Figure S1. *SETD7* knockout affects differentiation rather than proliferation and apoptosis. Related to Figure 2.**

(A-B) The Expression level of *SETD7* in human and murine embryonic development.

(C) DNA sequencing analysis of *SETD7*<sup>-/-</sup> #1 and #2 cell lines. Red dashed lines represent the location of base deletion, and the green character represents the location of base insertion.

(D) Cell morphology of WT, *SETD7*<sup>-/-</sup> #1 and #2 colonies of hESCs. Scale bar, 100 μm.

(E) Correlation analysis of the transcriptome between *SETD7*<sup>-/-</sup> #1 and WT cells at day 0 of hematopoietic differentiation.

(F) Violin plot showing the expression of the pluripotency gene set of *SETD7*<sup>-/-</sup> #1 and WT cells at day 0 of hematopoietic differentiation. (ns, no significance, Wilcoxon rank-sum test)

(G) RT-qPCR assay showing the relative mRNA expression of *POU5F1*, *NANOG* and *SOX2* in WT, *SETD7*<sup>-/-</sup> #1 and #2 cell lines at day 0 of hematopoietic differentiation. (n=3, ns, no significance unpaired two-tailed Student's *t*-test). Data are means ± SD.

(H) Western blotting showing the expression of *POU5F1*, *NANOG*, and *SOX2* in WT, *SETD7*<sup>-/-</sup> #1 and *SETD7*<sup>-/-</sup> #2 cell lines. GAPDH served as the loading control.

(I) Representative flow cytometry histogram plots showing PI staining of WT and *SETD7*<sup>-/-</sup> cells on day 2 of differentiation.

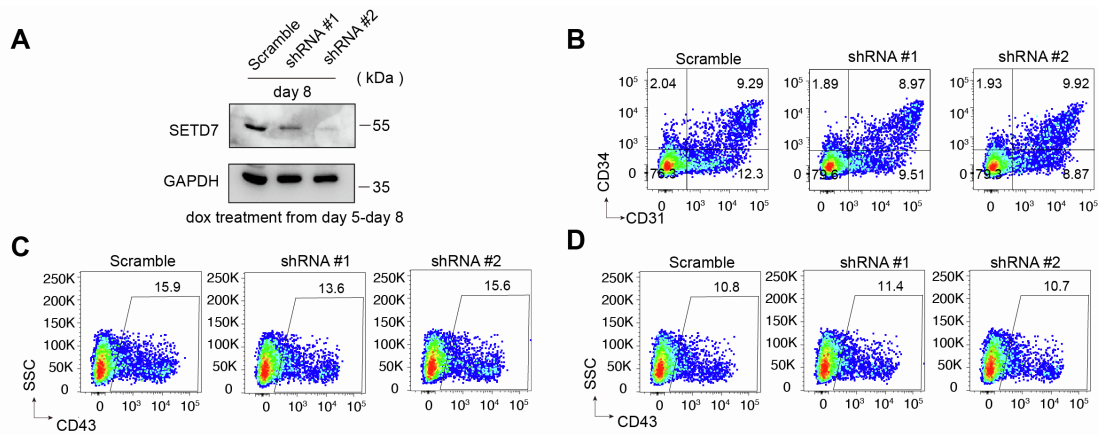
(J) The total cell numbers of WT and *SETD7*<sup>-/-</sup> cells during hematopoietic differentiation.

(K) Bar graphs showing apoptosis on days 0, 2, 5 and 8 of hematopoietic differentiation of WT and *SETD7*<sup>-/-</sup> cells. The number of APLNR<sup>+</sup> LPM cells, CD31<sup>+</sup>CD34<sup>+</sup> EPCs and CD43<sup>+</sup> HPCs during hematopoietic differentiation derived from WT and *SETD7*<sup>-/-</sup> cells. (n=3, ns, no significance, ordinary one-way ANOVA). Data are means ± SD.

(L) Bar graphs showing the cell cycle on days 0, 2, 5 and 8 of hematopoietic differentiation of WT and *SETD7*<sup>-/-</sup> cells. (n=3, ns, no significance, ordinary one-way ANOVA). Data are means ± SD.

(M) GSEA plots depicting the enrichment of hematopoiesis in WT and *SETD7*<sup>-/-</sup>

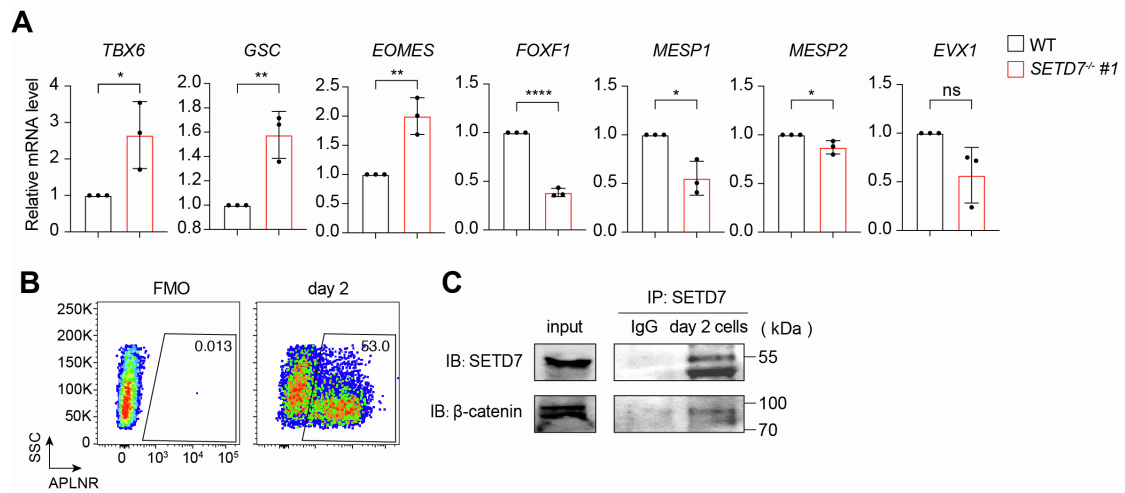
<sup>-/-</sup> #1 cells at day 8 of differentiation.



**Figure S2. Generation of EPCs and HPCs upon stage-specific *SETD7* abrogation. Related to Figure 3.**

(A) Western blot showing SETD7 protein levels after Dox induction from days 5 to 8 of differentiation. GAPDH served as the loading control.

(B-D) Representative flow cytometry plots showing the generation of CD31<sup>+</sup>CD34<sup>+</sup> EPCs (B), CD43<sup>+</sup> HPCs (C) after Dox treatment from days 2 to 8 and CD43<sup>+</sup> HPCs after Dox treatment from days 5 to 8 (D) in WT and *SETD7*-knockdown cell lines.



**Figure S3. SETD7 is involved in mid-primitive and anterior primitive streak progenitor formation. Related to Figure 6 and Table S3.**

(A) Relative mRNA expression of the indicated genes in WT and *SETD7*<sup>-/-</sup> cells on day 1 of hematopoietic differentiation. (n=3, \*P < 0.05, \*\*P < 0.01, \*\*\*\*P < 0.0001, ns, no significance, unpaired two-tailed Student's *t*-test). Data are means ± SD.

(B) The frequency of APLNR<sup>+</sup> cells on day 2 of hematopoietic differentiation.

(C) Coimmunoprecipitation of endogenous SETD7 and β-catenin on day 2 of hematopoietic differentiation. Ten percent of the input was used as the total protein control.

**Table S1. Primers for constructing inducible overexpression hESC lines, related to Figure 4 and STAR METHODS.**

NAME	Sequence (5' to 3')
SETD7-F	ATCGAAGCTTATGGACTACAAGGACGACGATGACAAG
SETD7-R	ATCGACTAGTCTTTTGCTGGGTGGCCTGGAAGGCC
CTNNB1-F	ATCGAAGCTTATGGACTACAAGGACGACGATGACAAGGCT ACTCAAGCTGATTTGATGG
CTNNB1-R	ATCGACTAGTTTACAGGTCAGTATCAAACCAGGCC
CTNNB1-K180R <sup>mu</sup> -F	TTTCTAAAAGGGAAGCTTCCAGACACG
CTNNB1-K180R <sup>mu</sup> -R	TAGCGTGTCTGGAAGCTTCCCTTTTAGAAA

**Table S2. Primers for SETD7 sgRNAs, related to Figure 2 and STAR METHODS.**

NAME	Sequence (5' to 3')
sgRNA1-F-5'	CACCACGATGACGGATTACCGCAC
sgRNA1-R-3'	AAACGTGCGGTAATCCGTCATCGT
sgRNA2-F-5'	CACCACGGAGAAAAGAACGGACGG
sgRNA2-R-3'	AAACCCGTCCGTTCTTTTCTCCGT

**Table S3. Primers for constructing inducible knockdown hESC lines, related to Figure 3, S2 and STAR METHODS.**

NAME	Sequence (5' to 3')
Scramble-J-F	CCGGCTTCTCCGAACGTGTCACGTTCTCGAGAACGTGACACGTTCCGAGAACTTTTTG
Scramble-J-R	AATTCAAAAAGTTCTCCGAACGTGTCACGTTCTCGAGAACGTGACACGTTCCGAGAAC
SETD7 sh1-F	CCGGGCCAGGGTATTATTATAGAATCTCGAGATTCTATAATAATACCCTGGCTTTTTG
SETD7 sh1-R	AATTCAAAAAGCCAGGGTATTATTATAGAATCTCGAGATTCTATAATAATACCCTGGC
SETD7 sh2-F	CCGGCTTATGAATCAGAAAGGGTTTCTCGAGAAACCCTTTCTGATTCATAAGTTTTTG
SETD7 sh2-R	AATTCAAAACTTATGAATCAGAAAGGGTTTCTCGAGAAACCCTTTCTGATTCATAAG

**Table S4. Primers for RT-qPCR, related to Figure 1, 6, S3 and STAR METHODS.**

NAME	Sequence (5' to 3')
CTNNB1-F	GCCCTGGTGAAAATGCTTGG
CTNNB1-R	CGCACTGCCATTTTAGCTCC
SETD7-F	TCATTGATGTGCCTGAGCCC
SETD7-R	GGGTGCGGATGCATTTGATG
OCT4-F	GCCCGAAAGAGAAAGCGAAC
OCT4-R	ATCTGCTGCAGTGTGGGTTT
SOX2-F	AGGATAAGTACACGCTGCCC
SOX2-R	TAAGTGTCCATGCGCTGGTT
NANOG-F	CAGAAGGCCTCAGCACCTAC
NANOG-R	ATTGGAAGGTTCCCAGTCGG
HAND1-F	CGTGAGAGCAAGCGGAAAAG
HAND1-R	CCGGTGCCTCCTTTAATCCT
FOXF1-F	CATGATGAACGGCCACTTGC
FOXF1-R	TGTCGTGGTGC GG TACT
BMP4-F	AGCGTAGCCCTAAGCATCAC
BMP4-R	AGTCATTCCAGCCACATCG
MESP1-F	CGAGTCCTGGATGCTCTCTG
MESP1-R	ATGAGTCTGGGGACGAGACG
DUSP6-F	TTCTACCTGGAAGATGAAGCCC
DUSP6-R	AGGCATCGTTCATCGACAGA
MSGN1-F	CTGCACACCCTCCGGAATTA
MSGN1-R	AGGAGGTCTGTGAGTTCCCC
TBX6-F	GAGGCCCGCTACTTGTTTCT
TBX6-R	CAGGAGAGTCGGGGTGAATG
EOMES-F	TCCTTTCACCCAACAGAGC
EOMES-R	TTTGTTGGTCCCAGGTTGCT
GSC-F	AACGCGGAGAAGTGGAACAA
GSC-R	AGCTGTCCGAGTCCAAATCG
MESP2-F	ACTGCCCCAAGATACAGTCG
MESP2-R	GGGTGGCGTCCAAAGAGAC
EVX1-F	TACCAGCACAGCAAAGGGTC
EVX1-R	GGTAACGACGCATCTGGTCA