Figure 4. PST is required for efficient hPSC differentiation to endoderm. **(A)** Immunostaining of PSA and SOX17 expression in DE cells transduced with control shRNA (Left) and PST shRNA (Right). Scale bar, 100μm. **(B)** Schematic of CRISPR gene editing approach of the PST first exon. The 300 bp region containing the PST TSS was cleaved and replaced by a BFP-IRES-Zeo cassette via homology directed repair. **(C)** qPCR of genes in WT and $PST^{-/-}$ cells differentiated to DE. **(D)** Flow cytometry of CXCR4 and PSA expression in WT and $PST^{-/-}$ cells differentiated to DE. **(E)** Immunoblot comparisons of WT and $PST^{-/-}$ cells differentiated to DE. **(F)** Immunostaining of PSA with SOX17 (Top) and FOXA2 (Bottom) in WT and $PST^{-/-}$ cells differentiated to DE. Abbreviations: CTL, control; hPSC, human pluripotent stem cell; DE, definitive endoderm; TSS, transcription start site; sgRNA, single guide RNA.

Supplemental Figure Legends

Figure S1. Polysialic acid is expressed upon differentiation in multiple hPSC lines. **(A-B)** Flow cytometry showing surface expression of PSA and CXCR4 in hPSC **(A)** and DE **(B)** in WA01, WA07, WA09, and TE03 cell lines. **(C)** Immunostaining of PSA and SOX17 in DE cells. Scale bar, 100μm. **(D)** qPCR expression profile of hPSC endoderm differentiation. **(E)** Immnunostaining of mESC differentiation with retinoic acid. Abbreviations: mESC, mouse embryonic stem cells; RA, retinoic acid.

Figure S2. Transcript levels of PST and STX during PST shRNA knockdown in DE day 4 cells. Data are normalized to WA09 hPSC. Abbreviations: CTL, control; DE, definitive endoderm.

Figure S3. Cell surface polysialylation requires expression of both PST/STX and NCAM. **(A)** Immunostaining of PSA expression hPSCs transfected with PST and NCAM overexpression

constructs. Scale bar, 100μm. **(B)** Immunostaining of PSA and Golgi marker Giantin in hPSCs overexpressing PST. Scale bar, 100μm. **(C)** Immnunoblot of NCAM expression in hPSCs transfected with PST, STX, and NCAM overexpression constructs. CDK2 is shown as control. Abbreviations: hPSC, human pluripotent stem cell.

Figure S4. Polysialylation is regulated by bivalent histone modifications. **(A-C)** ChIP PCR showing changes in H3K4me3 and H3K27me3 on the PST **(A)**, STX **(B)**, and NCAM **(C)** loci during differentiation to DE, mesoderm, and NCC. Abbreviations: hPSC, human pluripotent stem cell.

Figure S5. PST is under the control of the T/GSC regulatory network in mesendoderm. **(A)** Transcript levels of PST, ISL1, and GATA6 in T-GR cells after 6h Dex addition. **(B)** Time course expression levels of PST, T, and GSC trancsripts during 4 day differentiation to DE. **(C)** Illustration of luciferase constructs. LUC-control (Top) has no promoter while PST-LUC (Middle) and STX-LUC (Bottom) contain the 5kb upstream promoters of the respective genes. **(D)** Luciferase assay comparing PST-LUC activity to LUC-control in T-GR cells induced with Dex for 6-12h. **(E)** Luciferase assay comparing PST-LUC activity to LUC-control in hPSC differentiation to DE and mesoderm. **(F)** Luciferase assay showing activity of LUC-control (Left), PST-LUC (Middle), and STX-LUC (Right) in GSC-GR and T-GR cells induced with Dex for 24h. **(G)** Transcript levels of T in shRNA knockdown of GSC in DE day 4 cells. Abbreviations: Dex, dexamethasone; hPSC, human pluripotent stem cell; LUC, luciferase; DE, definitive endoderm; mes, mesoderm; CTL, control.

Figure S6. shRNA knockdown of PST inhibits DE differentiation while NCAM knockdown does not. **(A)** Immunostaining of PSA and FOXA2 expression in DE cells transduced with control shRNA (Left) and PST shRNA (Right). Scale bar, 100μm. **(B)** qPCR of DE cells transduced with control shRNA and two shRNAs targeting PST. **(C)** Immunostaining of PSA expression in

DE cells transduced with control shRNA (Top) and NCAM shRNA (Bottom). Scale bar, 100μm. **(D)** qPCR of NCAM and endoderm markers in DE cells transduced with control shRNA and NCAM shRNA. **(E)** Immunostaining of E-cadherin and FOXA2 expression in DE cells transduced with GFP control shRNA and NCAM shRNA. Scale bar, 100μm. Abbreviations: hPSC, human pluripotent stem cell; CTL, control; DE, definitive endoderm.

Figure S7. Disruption of the PST gene using CRISPR-Cas9 genome editing. **(A)** Diagram of PST WT genomic locus. sgRNAs were designed to target the PST peptide start codon in the first exon. **(B)** Diagram of the PST CRISPR knockdout genomic locus. The region between sgRNAs has been replaced with a resistance cassette via homology directed repair. **(C-D)** genomic PCR of PST locus in hPSC and $PST^{-/-}$. Primers were designed to amplify the WT region excised by CRISPR-Cas9 **(C)** and the knockout locus containing the resistance cassette **(D)**. **(E-F)** Expected PCR product sizes for WT PCR test (E) and CRISPR-Cas9 knockout PCR test **(F)**. Abbreviations: sgRNA, single guide RNA; L, ladder.

Figure S8. Overexpression of polysialyltransferases restores endoderm marks in $PST^{-/-}$ DE. **(A-D)** qPCR of endoderm marks in $PST^{-/-}$ DE cells overexpressing PST_{v5} or STX_{v5} expression constructs. Cells were sorted for PSA^{+}/PSA^{-} 3 days after transfection.

Figure S9. $PST^{-/-}$ cells are capable of forming mesoderm and ectoderm. **(A-B)** Flow cytometry showing surface expression of PSA **(A)** and SSEA3 **(B)** in hPSCs and $PST^{-/-}$ cells differentiated to mesoderm. **(C)** qPCR comparison of hPSCs and $PST^{-/-}$ cells differentiated to mesoderm. **(D)** Flow cytometry showing PSA expression in hPSCs and $PST^{-/-}$ cells differentiated to NCC. **(E-F)** qPCR comparison of hPSCs and $PST^{-/-}$ cells differentiated to ectoderm lineages NCC **(E)** and neural progenitor cells **(F)**. **(G)** ChIP PCR of $PST^{-/-}$ mesoderm

cells showing levels of H3K4me3 and H3K27me3 epigenetic marks on the STX promoter. Abbreviations: hPSC, human pluripotent stem cell; meso, mesoderm; NCC, neural crest cell; NPC, neural progenitor cell.

Figure S10. Ectopic expression of PSA in hPSCs is not sufficient to drive differentiation. **(A)** qPCR of hPSCs electroporated with PST and NCAM compared to a GFP control plasmid. **(B-D)** Immunostaining of PSA **(B-D)**, FOXA2 **(B)**, T **(C)**, and OCT4 **(D)** in hPSCs electroporated with PST and NCAM overexpression construct. Scale bar, 100μm. Abbreviations: CTL, control; hPSC, human pluripotent stem cell.