



Supplemental Figure S1: Definitive endoderm is successfully induced in human ESC line HUES8.

- (A) RT-qPCR analysis of signature gene expression verifies successful definitive endoderm differentiation of human ESCs. Expression of definitive endoderm genes (*SOX17*, *FOXA2*, *GATA6* and *GSC*), pluripotency genes (e.g. *NANOG* and *OCT4*), and mesendoderm genes (*T* and *MIXL1*) were analyzed during the differentiation process.
- (B) Immunofluorescence staining of human ESCs after 1 and 4-day of activin A treatment with the endoderm marker SOX17 and the ESC marker OCT4.
- (C) CD184-positive cells enriched definitive endoderm cells compared to CD184-negative cells for the expression of endoderm marker genes *SOX17*, *FOXA2*, and *GATA6*.
- (D) The great majority of CD184-positive are also CD117-positive in our differentiation system.