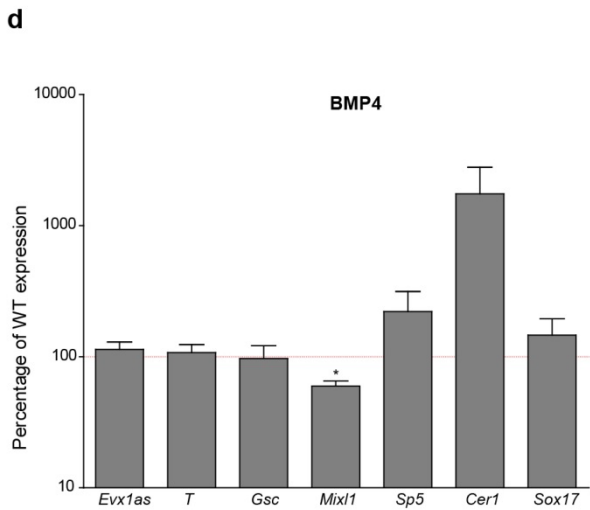
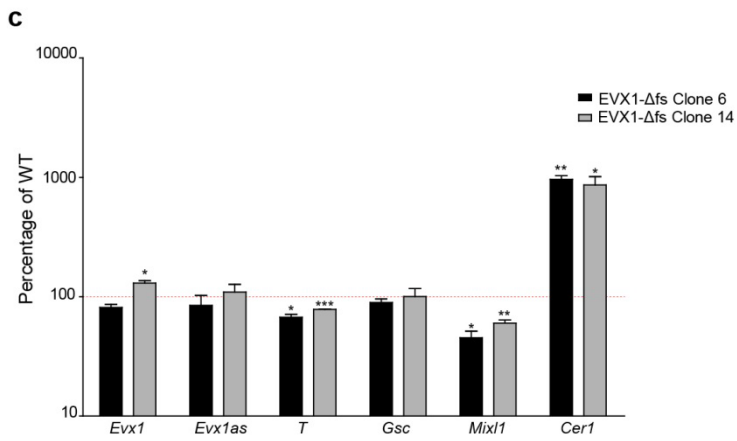
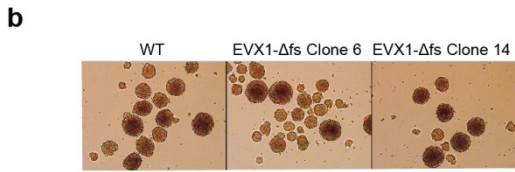
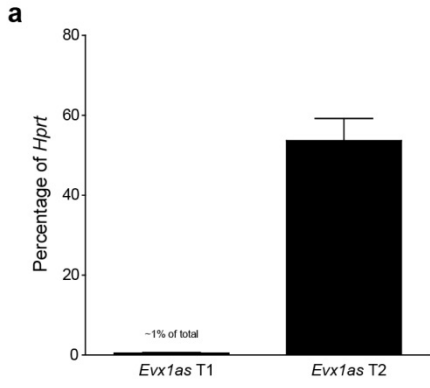


Supplementary Information:

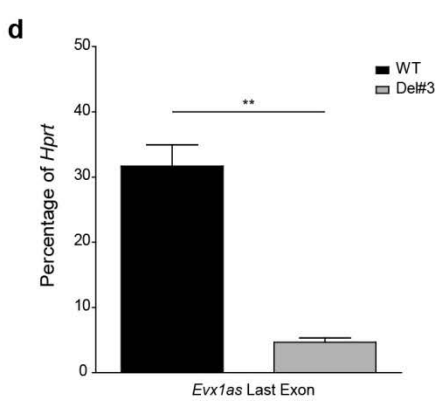
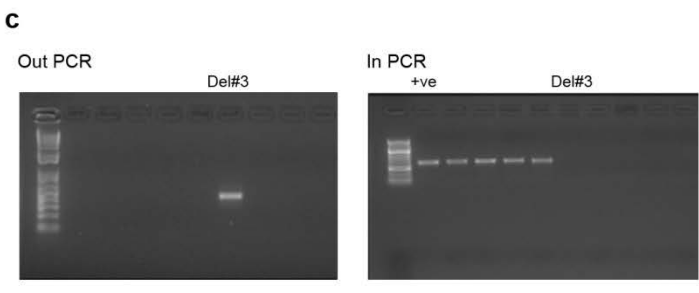
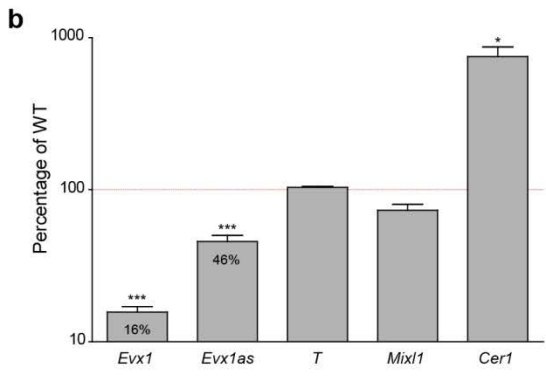
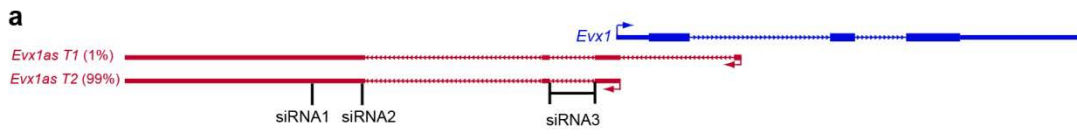
The *Evx1/Evx1as* gene locus regulates anterior-posterior patterning during gastrulation

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Supplementary Figure 1:

A) qRT-PCR of each isoform of *Evx1as*. Standard curves were performed for each transcript to assess primer efficiency. The relative abundance of each transcript is expressed as a percentage of *Hprt*. T1=transcript 1, T2=transcript 2. **B)** Images of D4 EBs at 10x magnification. **C)** Expression profiling of EVX1-Δfs clone 6 and EVX1-Δfs clone 14. Expression of each gene in each sample was first normalized to *Hprt* then normalized to WT expression. 4 biological replicates were performed. No significant differences were found when comparing EVX1-Δfs clone 6 to EVX1-Δfs clone 14. **D)** Expression profiling of EVX1-Δfs in SFM supplemented with BMP4. Expression of each gene in each sample was first normalized to *Hprt* then normalized to WT expression. 4 biological replicates were performed. **C)** and **D)** * indicates a p-value <0.05, ** indicates a p-value <0.01, *** indicates a p-value <0.001 when compared to WT. All error bars show SEM.



Supplementary Figure 2:

A) UCSC browser schematic showing the locations of the three *Evx1as* siRNAs used. **B)** Expression profiling of Del#2. Expression of each gene in each sample was first normalized to *Hprt* then normalized to WT expression. 3 biological replicates were performed. * indicates a p-value <0.05, ** indicates a p-value <0.01, *** indicates a p-value <0.001 when compared to WT. **C)** Ethidium Bromide gel demonstrating presence of band using Out PCR primers and lack of a band using In PCR primers on Del#3 cells. **D)** qRT-PCR of WT and Del#3 cells using primers designed against the last exon of *Evx1as*. ** indicates a p-value <0.01. All error bars show SEM.