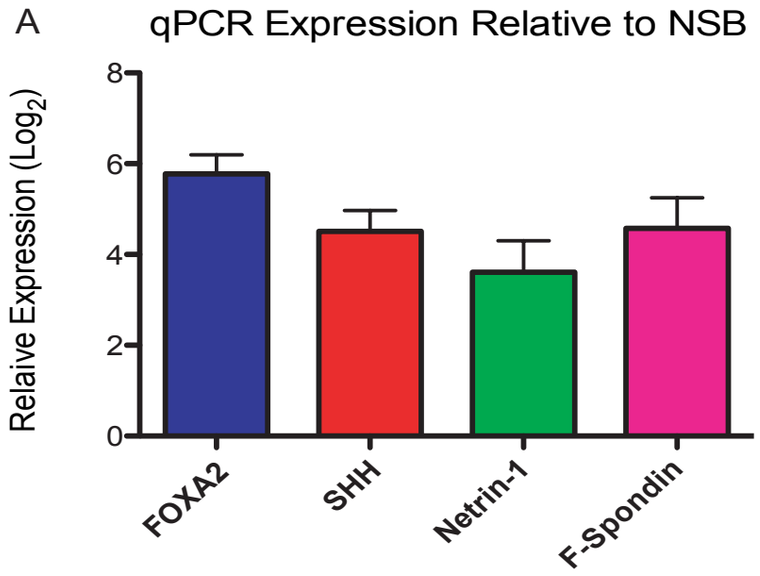
Figure S1 (Fasano et al)

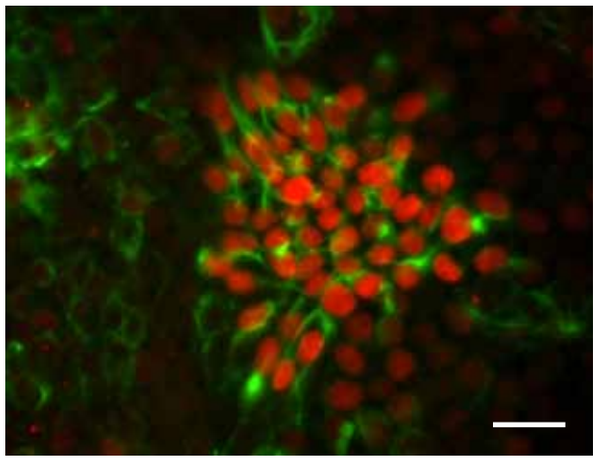
A



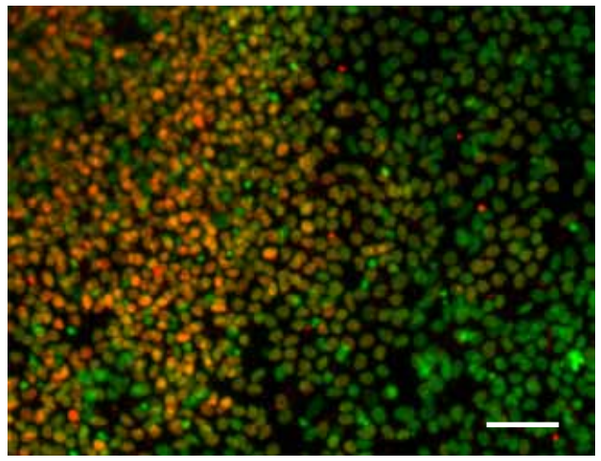
B

Antibody Name	Staining	Percentage
Nestin	+++	86 ± 6
Tuj1	++	37 ± 7
Nkx2.2	+	13 ± 4
TH	+	4 ± 1
SOX2	+	17 ± 5
SOX17	-	
AFP	-	
Albumin	-	
BF1	-	
DCX	-	
S100B	-	
PAX6	-	
SOX1	-	
Brachury	-	
Musashi	-	

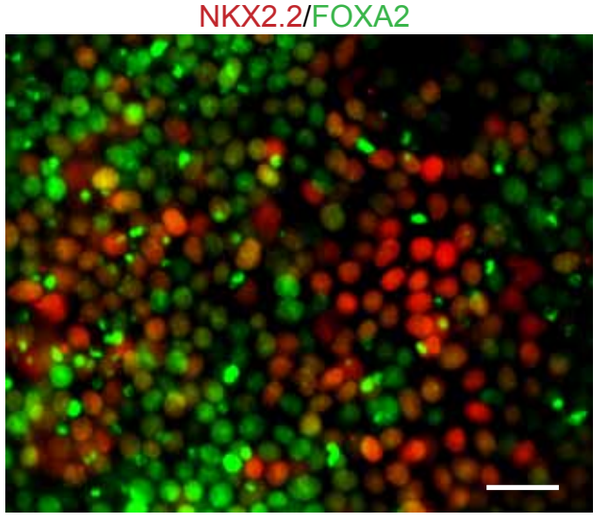
C



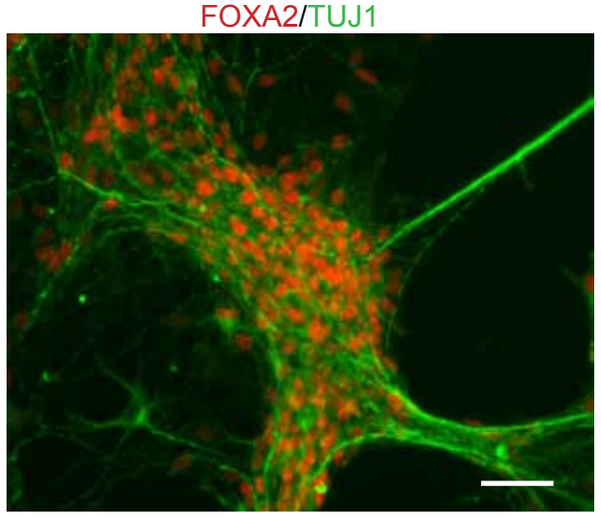
D



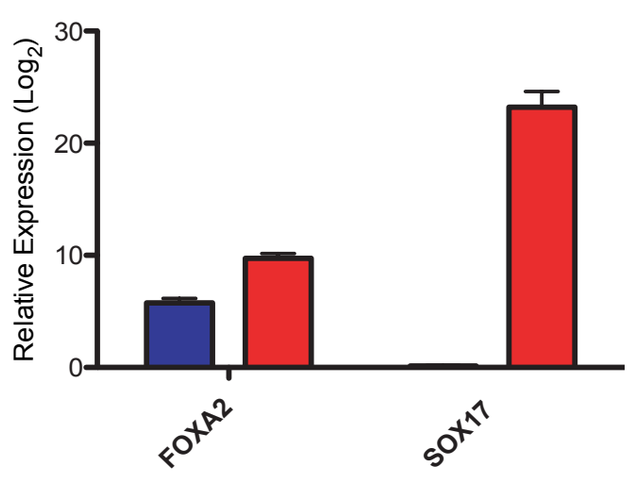
E



F



G



H

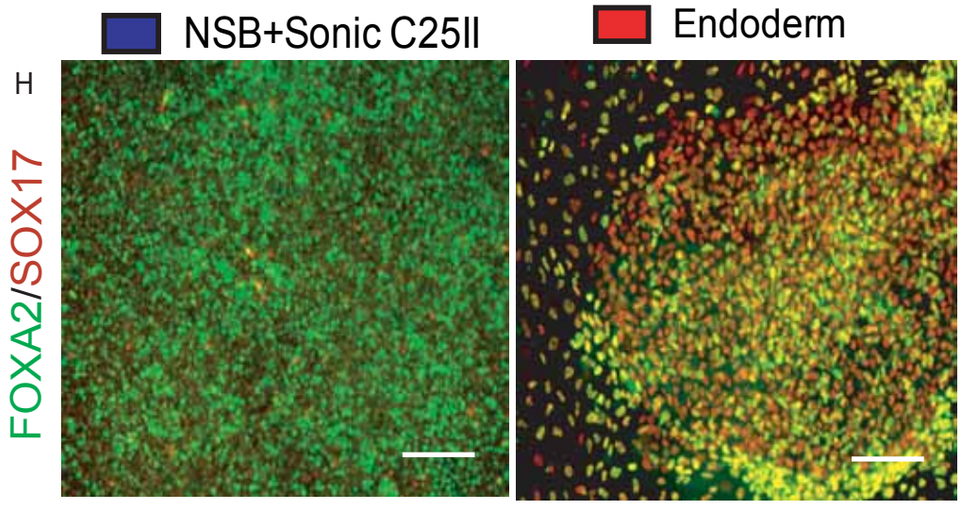
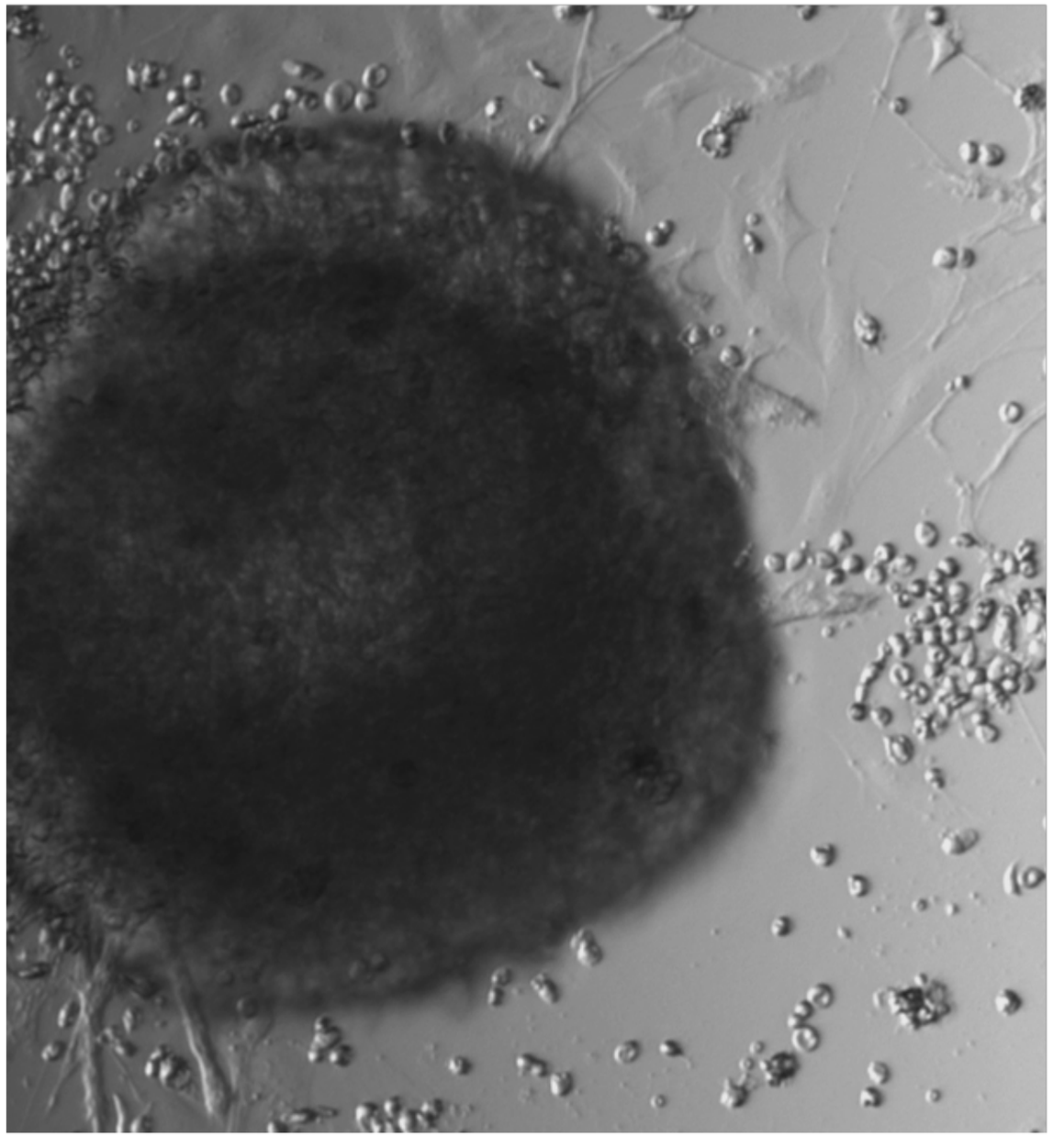


Figure S2 (Fasano et al)

Cerebellar Plate explant

with NSB cells



with NSB + Sonic cells

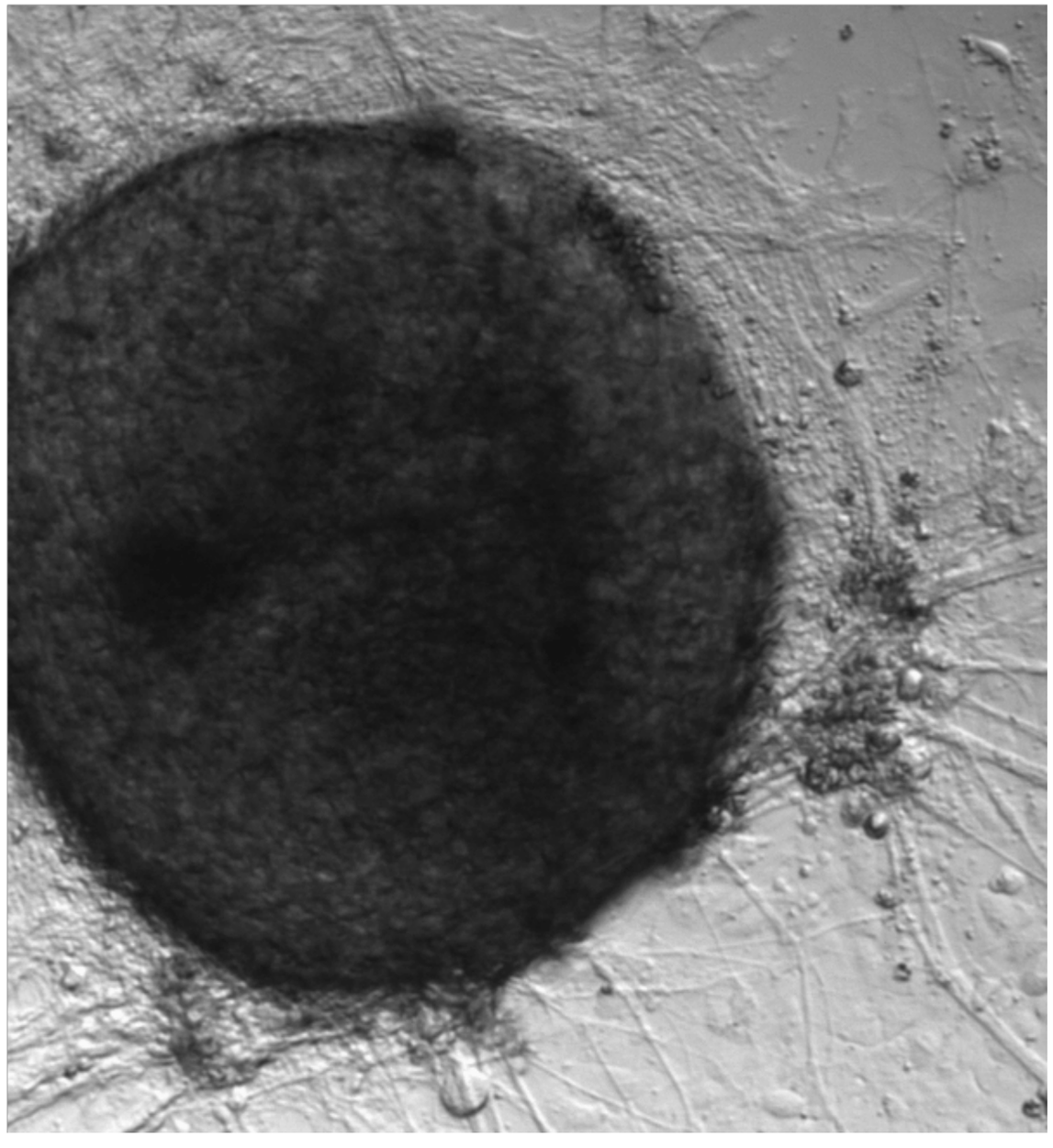


Figure S3 (Fasano et al)

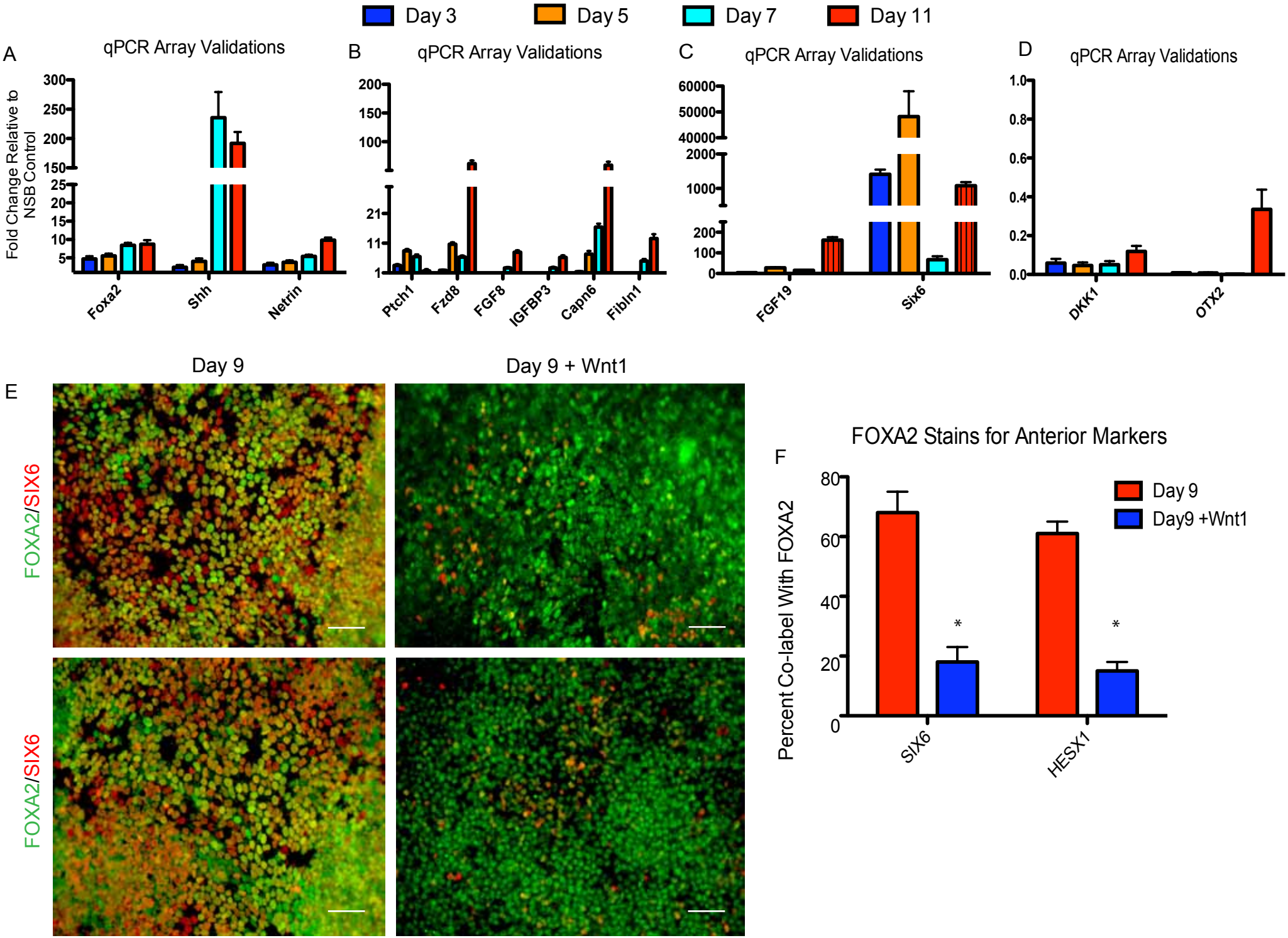


Figure S4 (Fasano et al)

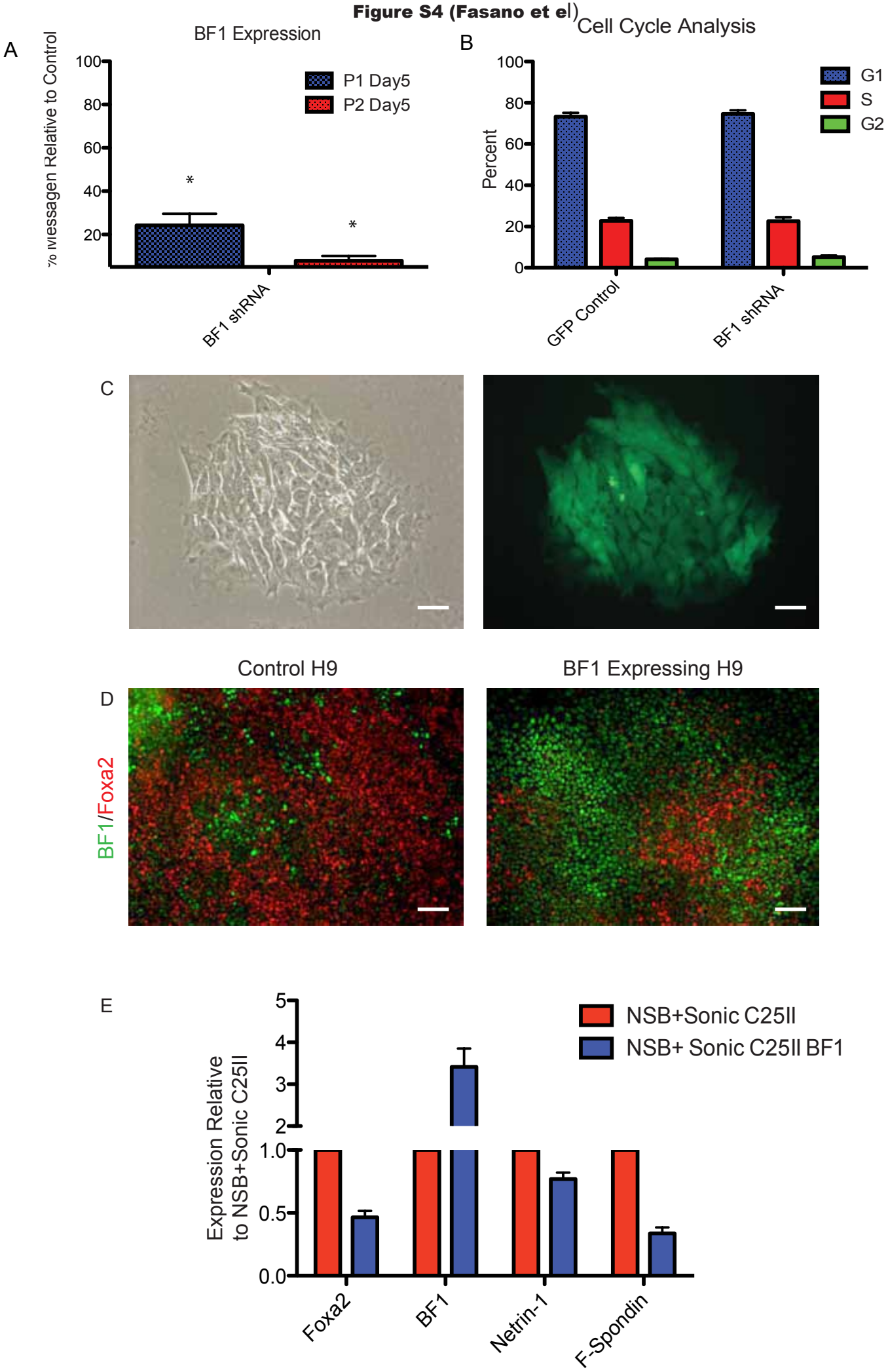
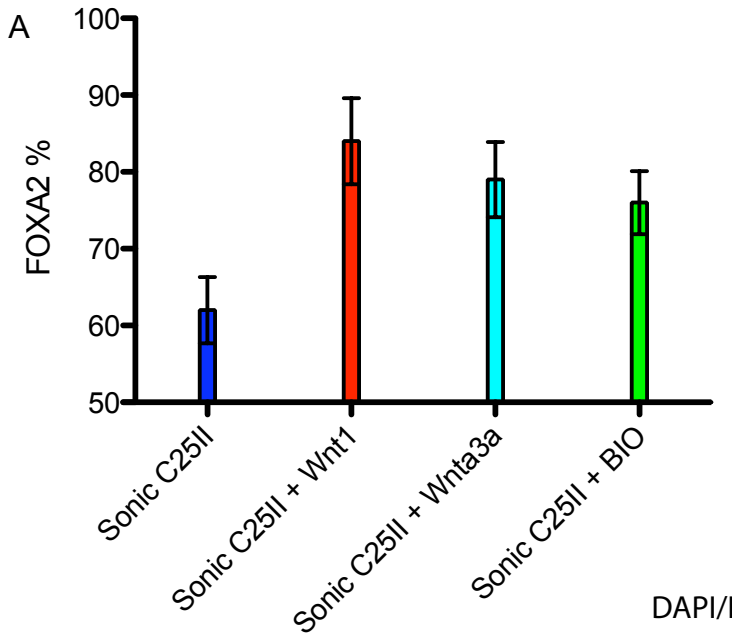
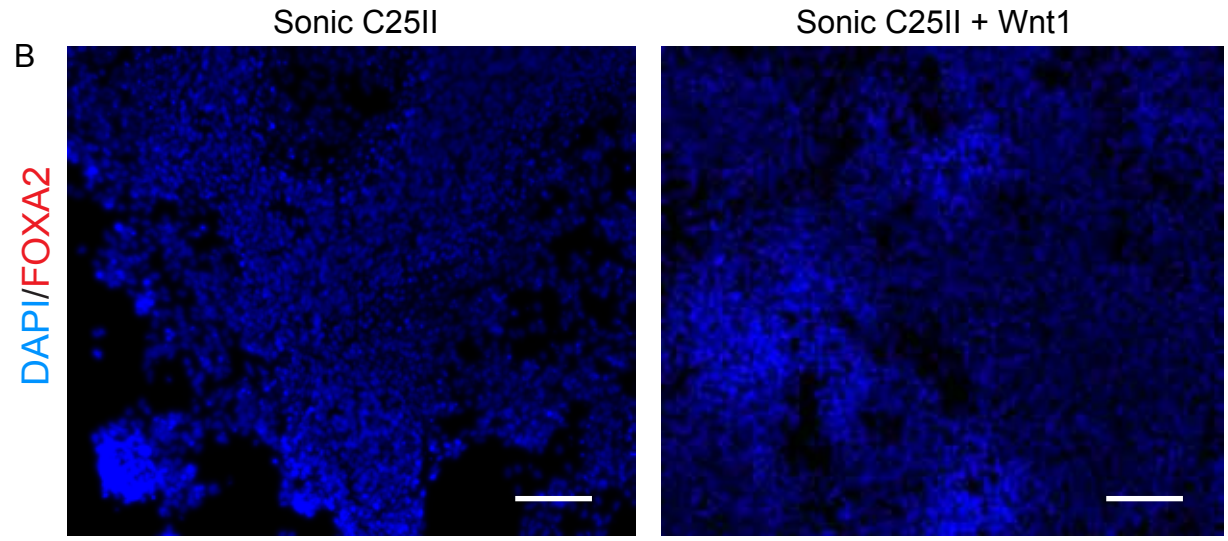


Figure S5 (Fasano et al)





DAPI/FOXA2

TH/FOXA2

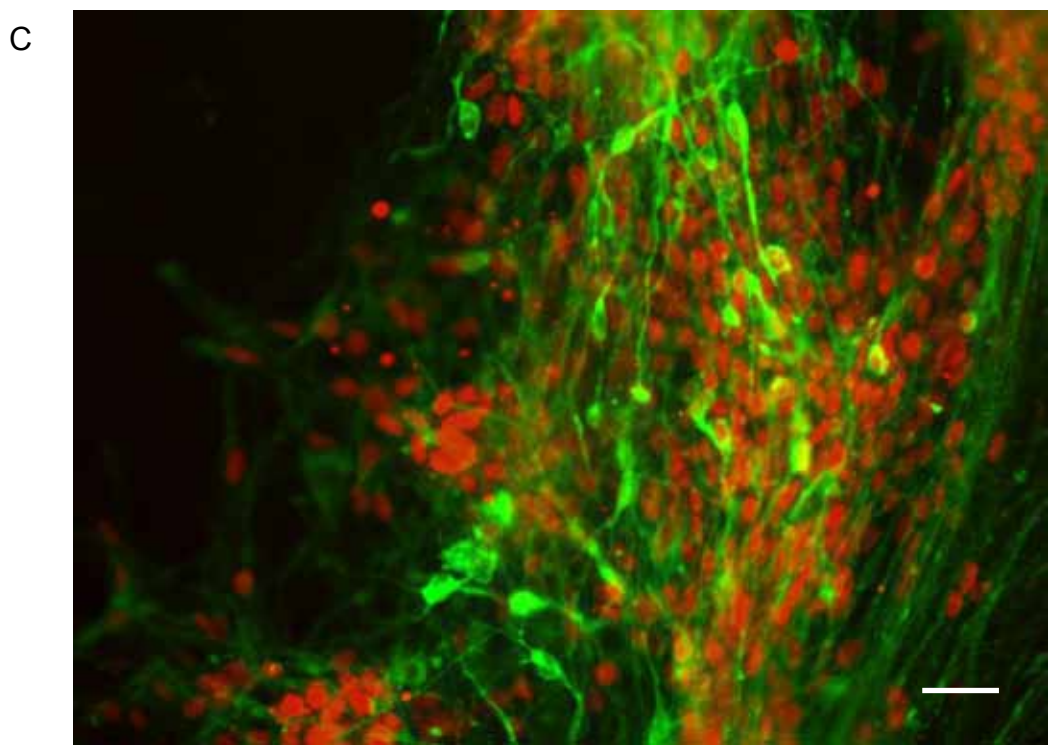


Figure S1. hESC derived FP expresses the appropriate markers, related to Figure 1.
(A) qRT-PCR data at Day 11 showing an increase in floor plate markers *FOXA2*, *SHH*, *Netrin-1*, and *F-Spondin* relative to control NSB conditions.
(B) Table quantifying results of immunostaining experiments.
(C-F) Immunostaining of FOXA2+ cells reveals co-labelling with few markers such as (C) Nestin, (D) SOX2, (E) Nkx2.2, and (F) Tuj1. Scale bar, (D and F, 50um) (E and G, 100um).
(G-H) qRT-PCR data at Day 11 showing levels of *FOXA2* and *SOX17* cells differentiated with NSB+Sonic C25II treatment and cells differentiated towards an endodermal lineage. *SOX*17 is not expressed in Sonic C25II conditions but is highly expressed in the endoderm. This is shown at the level of the protein by immunostaining (H). Scale bar, 200um.

Figure S2. Co-culture of cerebellar plate explants on FP cells induces neurite outgrowth, related to Figure 3.
Cerebellar explants from E8.5 mouse were plated on NSB neural cells or NSB + Sonic (FP) cells. After 3 days considerable neurite outgrowth was observed in the NSB+Sonic (FP) condition compared to control.

Figure S3. qPCR validates genes changing in microarray, related to Figure 4.
(A) qPCR for FP genes showing an enrichment in Sonic C25II condition compared to NSB control.
(B-D) qPCR validating novel genes that changes in the Sonic C25II condition compared to NSB control condition.
(E and F) After 9 Days FOXA2+ FP cells co-stain for the anterior markers SIX6 and HESX1. This co-localization is lost after treatment of Wnt-1. Scale bar, 50um. *$p<0.01$ N=3.

Figure S4. BF1 expression inhibits FP induction, related to Figure 5.
(A) qRT-PCR at two points during neural differentiation showing a decrease in BF1 levels in the BF shRNA hESC line compared to control, *$p<0.01$ N=3.

(B) Cell cycle analysis revealed no differences in the cell cycle kinetics of the two lines.
(C) hESC expressing BF1 visualized by GFP. Scale bar, 20um.
(D) Cells overexpressing BF lack FOXA2+ expression. Scale bar, 200um.
(E) qRT-PCR data at Day 11 showing a lack of FP induction in BF1 expressing hESC after Sonic C25II treatment.

Figure S5. Early WNT1 addition along FP differentiation can cause DA neuron differentiation, related to Figure 6.
(A) Adding WNTs or GSK3β-Inhibitor (BIO 100nM) early can increase *FOXA2* expression.
(B) Addition of WNT1 to later stage neural rosette cells has no effect on FOXA2 induction, scale bar 200um.
(C) WNT1 treated FP cultures can give rise to DA Neurons expressing FOXA2, scale bar 50um.

All Supplemental Tables related to Figure 4.
Table S1. Complete gene list of from microarray analysis performed at Day 3, Day5, Day7, and Day 11 in control NSB and NSB+Sonic C25II conditions.

Table S2. GO terms upregulated in NSB+Sonic C25II condition at Day 7.
Table S3. GO terms downregulated in NSB+Sonic C25II condition at Day 7.

Table S4. GO terms upregulated in NSB+Sonic C25II condition at Day 11.

Table S5. GO terms downregulated in NSB+Sonic C25II condition at Day 7.

Table S6. Pairwise comparison showing genes up and down regulated in NSB+Sonic C25II condition compared to NSB control at Day 3.

Table S7. Pairwise comparison showing genes up and down regulated in NSB+Sonic C25II condition compared to NSB control at Day 5.

Table S8. Pairwise comparison showing genes up and down regulated in NSB+Sonic C25II condition compared to NSB control at Day 7.

Table S9. Pairwise comparison showing genes up and down regulated in NSB+Sonic C25II condition compared to NSB control at Day 11.

Table S10. Analysis showing times of maximum (TOM) and times of minimum gene expression along the differentiation protocol.

Table S11. GO terms for genes expressed maximally at Day 3.

Table S12. GO terms for genes expressed minimally at Day 3.

Table S13. GO terms for genes expressed maximally at Day 5.

Table S14. GO terms for genes expressed minimally at Day 5.

Table S15. GO terms for genes expressed maximally at Day 7.

Table S16. GO terms for genes expressed minimally at Day 7.

Table S17. GO terms for genes expressed maximally at Day 11.

Table S18. GO terms for genes expressed minimally at Day 11.