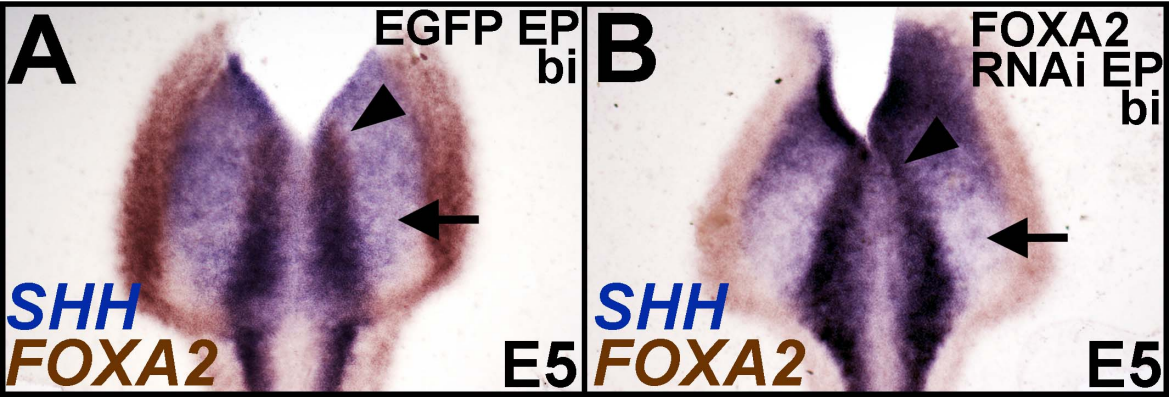
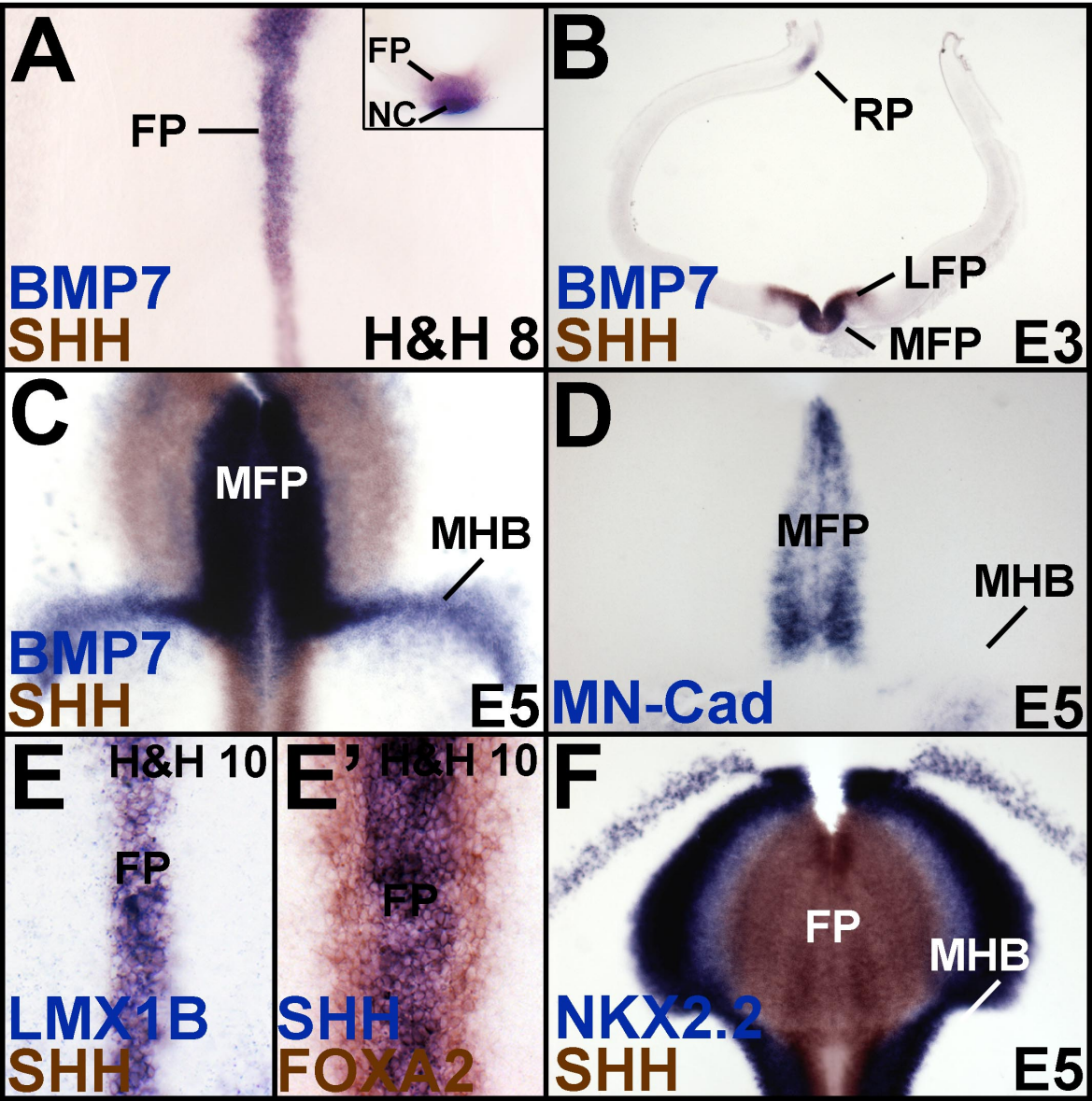
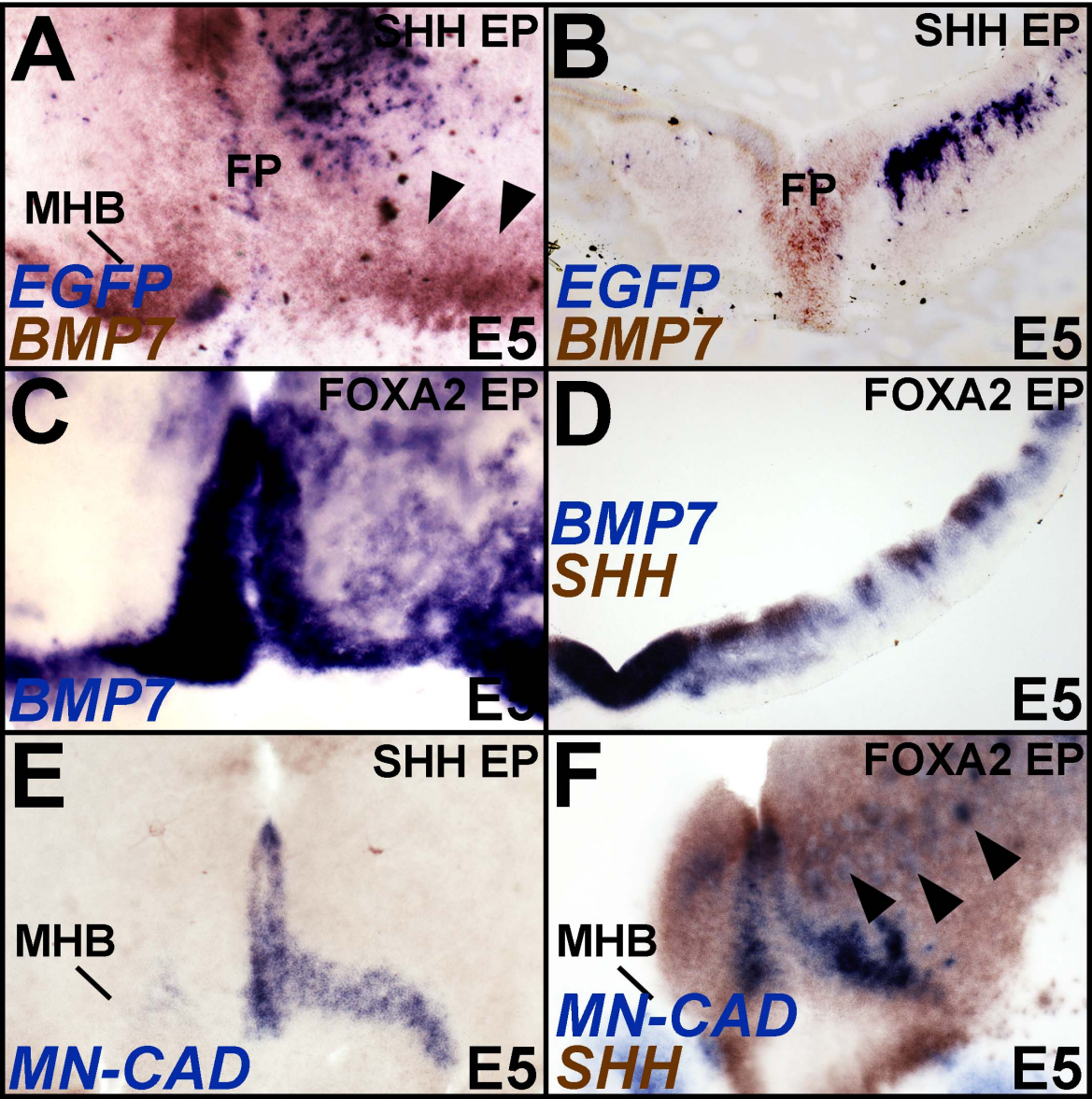
Supplementary Figure S1 - Bayly et al.



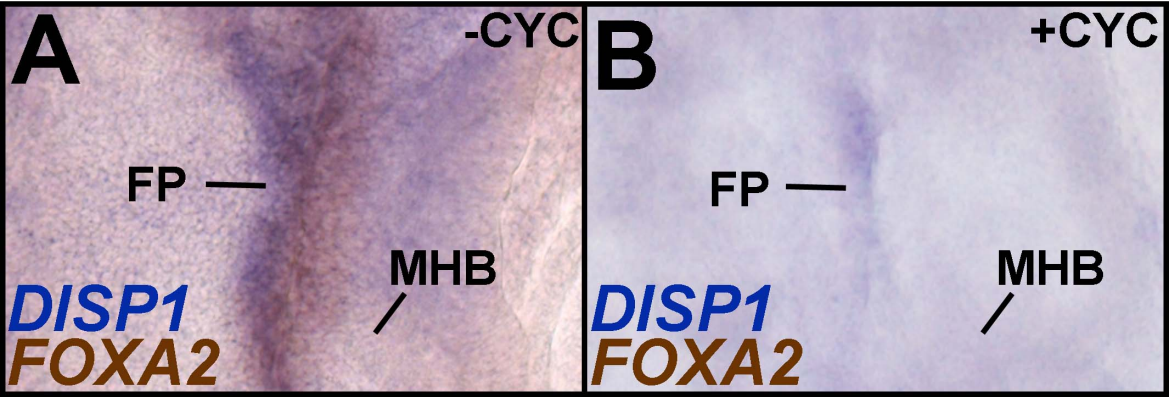
Supplementary Figure S2 - Bayly et al.



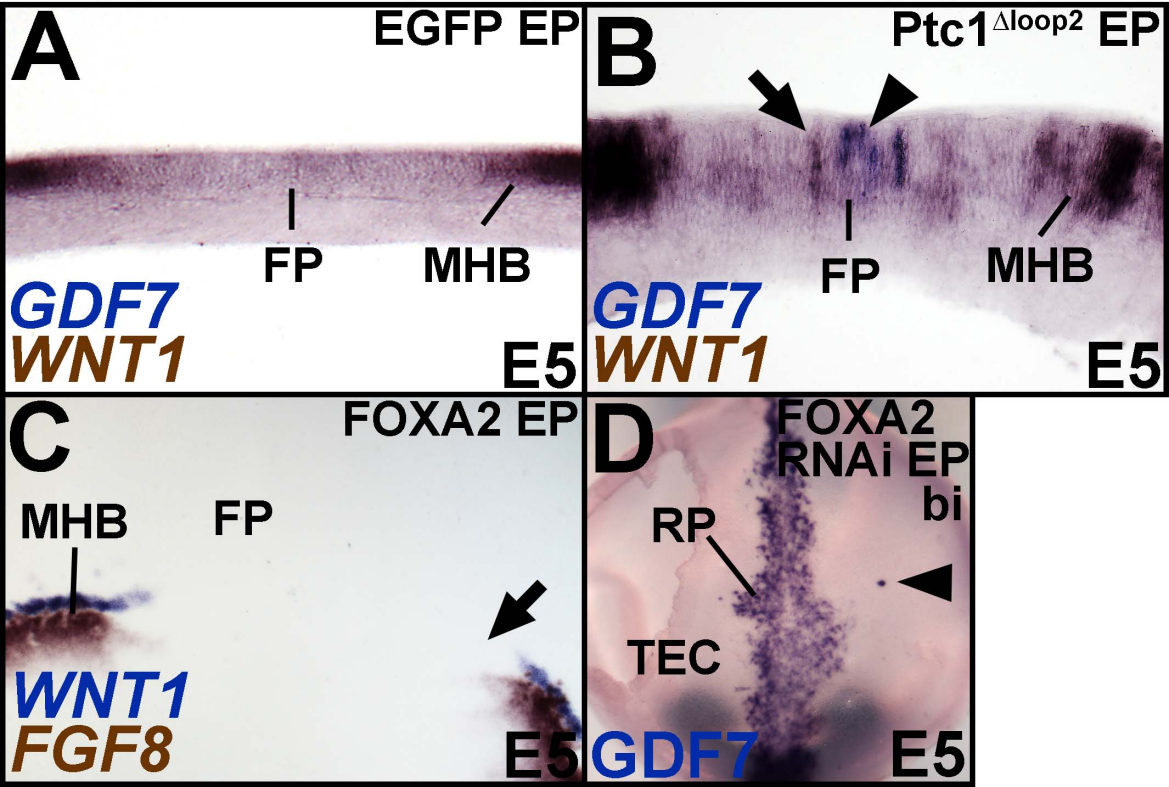
Supplementary Figure S3 - Bayly et al.



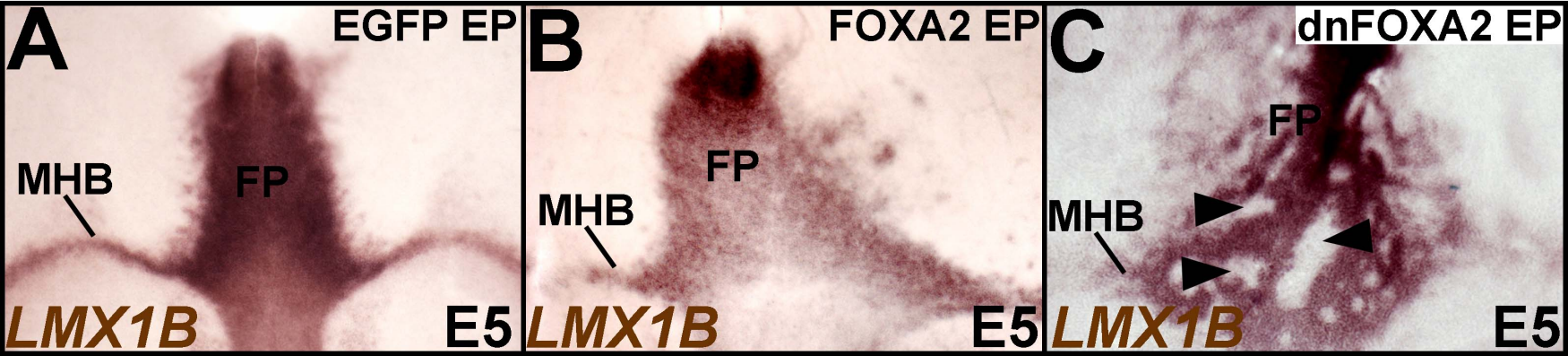
Supplementary Figure S4 - Bayly et al.



Supplemetary Figure S5 - Bayly et al.



Supplementary Figure S6 - Bayly et al.



Supplementary Figure Legends

Supplementary Figure S1. FOXA2 RNAi suppresses *FOXA2* expression. Compared to electroporated controls (A), bilateral misexpression of FOXA2 RNAi results in lowered *FOXA2* (brown) expression (arrowheads, A, B). The expression of *SHH*, a transcriptional target of *FOXA2* is also reduced (arrows, A, B).

Supplementary Figure S2. Gene expression patterns in the midbrain FP. (A) Flattened wholemount and cross-section (inset, A) displaying *BMP7* and *SHH* co-expression in midbrain FP. **(B, C)** E3 cross-section and E5 wholemount demonstrating that *BMP7* expression distinguishes the MFP from the LFP. Note that *BMP7* is also expressed in the RP (B) and the MHB (C). **(D)** *E5* wholemount demonstrating that *MN-CAD* is exclusively expressed in the MFP. **(E, E')** Wholemounts demonstrating that *LMX1B* (E, blue), *SHH* (E, brown; E', blue) and *FOXA2* (brown, E') are expressed in a mosaic pattern within FP. **(F)** *NKX2.2* expression occurs lateral to the *SHH*+ FP.

Supplementary Figure S3. FOXA2, but not SHH, is sufficient to induce the MFP. (A, B) Wholemount (A) and cross-section (B) demonstrating that unilateral (right side) misexpression of *SHH* is insufficient to induce *BMP7* anywhere in the midbrain except the MHB (arrowheads). **(C, D)** Wholemount (C) and cross-section (D) demonstrating that *FOXA2* misexpression is sufficient to induce *BMP7* expression anywhere in ventral midbrain. **(E, F)** E5 wholemounts demonstrating that unilateral *SHH* misexpression (E) can induce *MN-CAD* only along the MHB (right side), while *FOXA2* misexpression (F) can induce *MN-CAD* anywhere in the ventral midbrain.

Supplementary Figure S4. HH signaling is required for inducing HH-target/pathway genes in the MFP. H&H 3-5 explants (flattened wholemount view) treated with either the vehicle (A) or cyclopamine (B) for 24 hours demonstrate that the HH-target genes, *DISP1* and *FOXA2* are severely reduced following cyclopamine treatment.

Supplementary Figure S5. SHH and FOXA2 regulate midbrain signaling center identity. **(A, B)** Unlike EGFP-electroporated controls (A), *Ptc1*$^{\Delta loop2}$-electroporated brains (B) display ectopically induced *GDF7+/WNT1+* RP cells (blue + brown, arrowhead) and *WNT1+/GDF7-* negative MHB cells (brown, arrow) along the ventral midline. **(C)** Unilateral *FOXA2* misexpression (right side) suppresses MHB expression of *WNT1* and *FGF8* (arrow). **(D)** Top down view of RP demonstrating that compared to EGFP-electroporated controls (see Fig. 7A), the RP is expanded as a consequence of FOXA2 (arrowhead) downregulation in ventral midbrain

Supplementary Figure S6. FOXA2 is necessary and sufficient for MFP induction. (A) H&H 4-6 electroporations of EGFP showing normal *LMX1B* expression in the MFP. FOXA2 misexpression (B) at H&H 4-6 results in increased *LMX1B* expression, while dnFOXA2 misexpression (C) at the same age results in blockade of *LMX1B* in the MFP (arrowheads).