

Differences in the spatiotemporal expression and epistatic gene regulation of the mesodiencephalic dopaminergic precursor marker *Pitx3* during chicken and mouse development

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Supplementary methods

Vector constructs

pRNAT-U6.3-*siPITX3-cGFP* (*pRNAT-siPITX3-GFP*) was generated by ligating a small segment of chick *PITX3* into pRNAT-U6.3 (Finsterwald et al 2011). In short sense and antisense from different segments of cPitx3 DNA sequence were annealed and ligated into the HindIII/BamHI digested pRNAT-U6.3. pRNAT-U6.3 that contains an enhanced U6 promoter and a coral GFP under a SV40 promoter. The different constructs were tested by simultaneous injection of *pMES-PITX3-IRES-eGFP* and *pRNAT-siPITX3-GFP*. The *siPITX3* construct that downregulated *PITX3* in comparison to GFP (Fig. SF,G) was used in our experiments and has the following sequence containing a HindIII and BamHI restriction site (sense:5'-gatccCCAGACAGACGACTCCAACCTTCAAGAGAGTTGGAGTCGTCTGTCTGGTT TTTTga-3; antisense: agcttcAAAAAACCAGACAGACGACTCCAACCTCTCTTGAAGTTGGAG TCGTCTGTCTGG GGCC g -3'; restriction sites are indicated in small letters)

Supplementary Figures

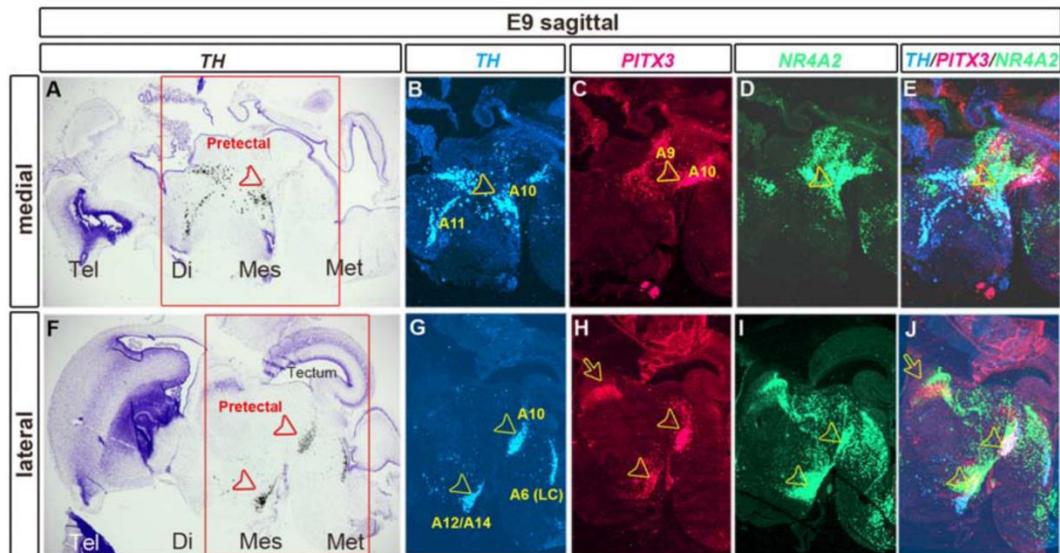


Figure S1: Expression of *TH*, *PITX3* and *NR4A2* in the E9 chick embryo. (A-J) Representative cresyl violet (Nissl)-stained and consecutive midsagittal (A-E) and parasagittal (F-J) paraffin sections from the brain of an E9 chick embryo, hybridized with riboprobes for *TH* (A,B,F,G), *PITX3* (C,H) or *NR4A2* (D,I). (A,F) are the brightfield views of the darkfield images in (B,G). (E,J) are overlays of the pseudo-colored sections in (B-D) and (G-I), respectively; overlapping expression domains appear in white/yellow. Open red/yellow arrowheads in (A-J) point at the mesodiencephalic A9/10 and A12/14 DA cell populations with cells expressing all three genes. Open yellow arrows indicate cells that express only *PITX3*. Abbreviations: Di, diencephalon; Mes, mesencephalon; Met, metencephalon; Tel, telencephalon.

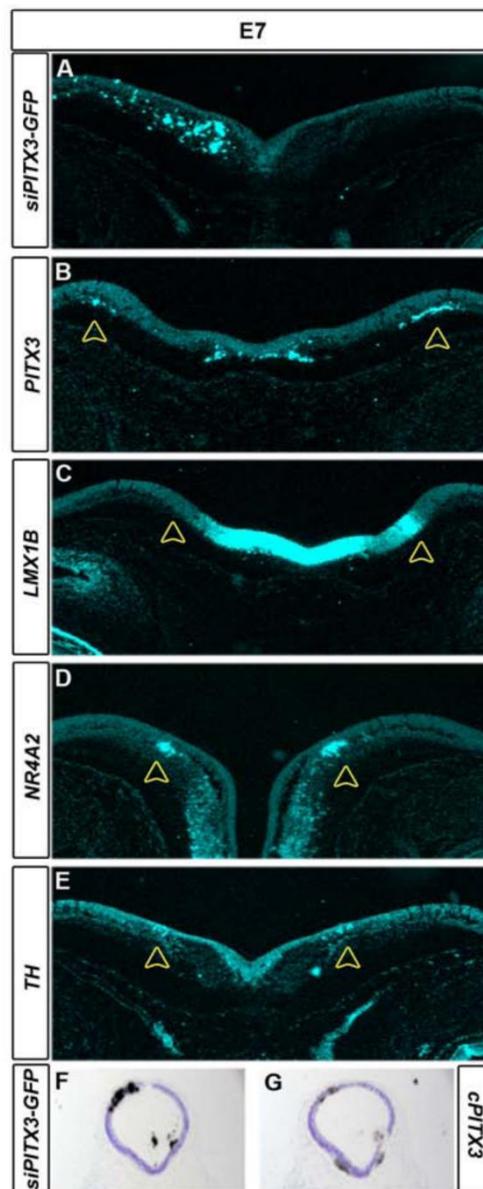


Figure S2: Reduced expression of mdDA-related genes after siRNA-mediated knockdown of *PITX3*. (A-G) Consecutive coronal sections of an E7 chick mesencephalon electroporated with pRNAT-siPitx3-GFP in the left half of the mesencephalon (A). Right half serves as control. Brains were electroporated at HH 10-12 and fixed after 6 days post-electroporation. *RISH* for *GFP* (A,F), *PITX3* (B,G), *LMX1B* (C), *NR4A2* (D) and *TH* (E). Open yellow arrowheads in (B-E) point at the reduced or missing expression in the electroporated left half or endogenous expression in the control right half of the mesencephalon for the corresponding marker gene. Note, that *siPITX3* overexpression resulted in a reduced expression (knockdown) of the co-electroporated *PITX3* (F,G), thus demonstrating efficacy of the *siPITX3* construct.

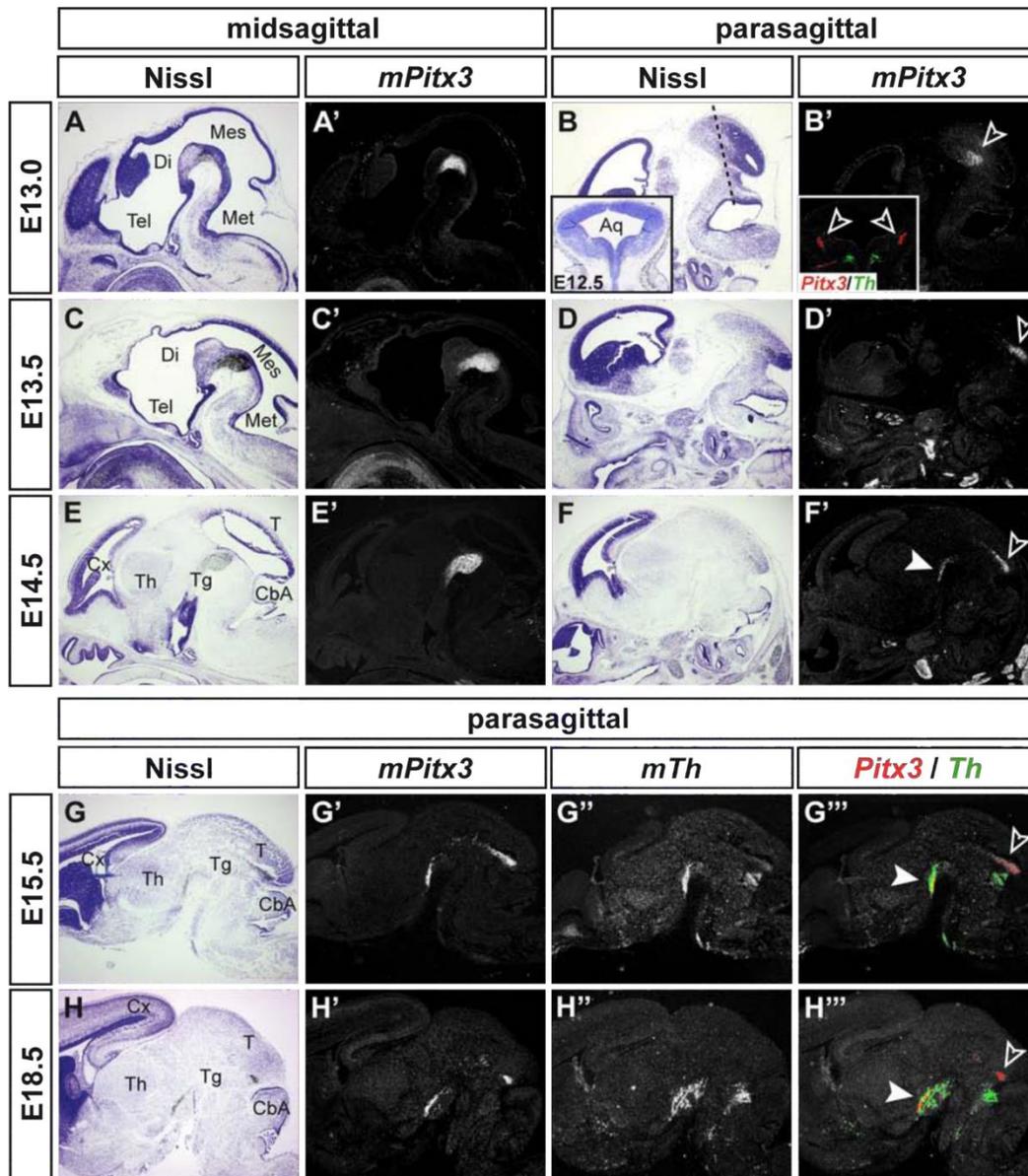


Figure S3: Expression of *Pitx3* in the mid- to late gestational mouse embryo. (A-H''') Representative cresyl violet (Nissl)-stained midsagittal (A,A',C,C',E,E') and parasagittal (B,B',D,D',F,F',G-H''') paraffin sections from the head or brain of E13.0 (A-B'), E13.5 (C-D'), E14.5 (E-F'), E15.5 (G-G''') and E18.5 (H-H''') CD-1 mouse embryos, hybridized with

riboprobes for *mPitx3* (A-F',G',H') or *Th* (G'',H''). (A-H) are the brightfield views of the darkfield images in (A'-H'). Insets in (B,B') show a coronal section from the caudal midbrain of an E12.5 mouse embryo. Inset in (B') and sections in (G''',H''') are pseudo-colored overlays of consecutive sections hybridized with riboprobes for *Pitx3* (red) and *mTh* (green); overlapping expression domains appear in yellow. White open arrowheads in (B',D',F',G''',H''') point at the dorsolateral *Pitx3*-positive but *Th*-negative domain in the caudal mesencephalon. White filled arrowheads in (F',G''',H''') point at the rostralateral *Pitx3*-positive domain in the ventrocaudal diencephalon. Abbreviations: Aq, aqueduct; CbA, cerebellar anlage; Cx, cortex; Di, diencephalon; Mes, mesencephalon; Met, metencephalon; T, tectum (dorsal mesencephalon); Tel, telencephalon; Tg, tegmentum (ventral mesencephalon); Th, thalamus (diencephalon).

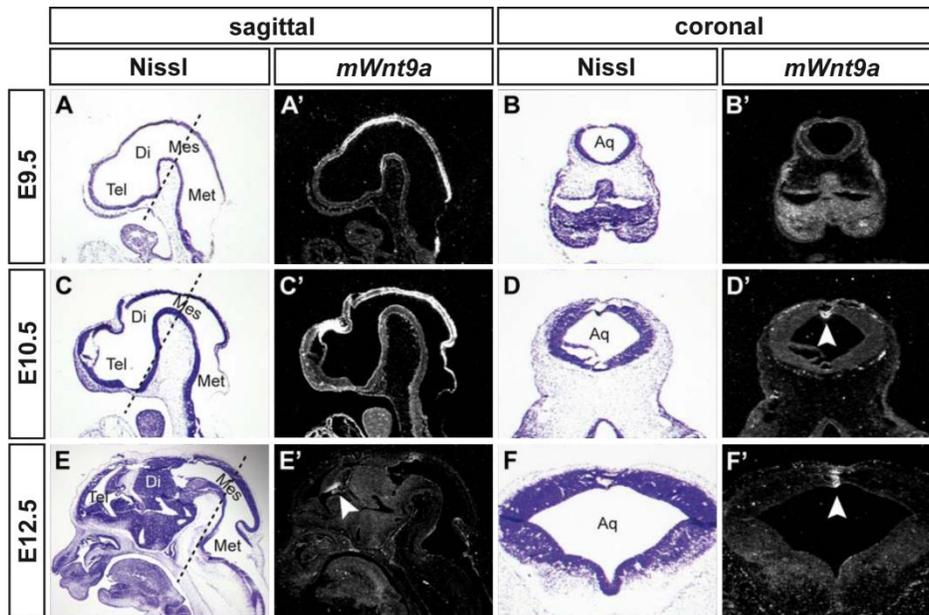


Figure S4: Expression of *Wnt9a* in the mid-gestational mouse embryo. (A-F') Representative cresyl violet (Nissl)-stained midsagittal (A,A',C,C',E,E') and coronal (B,B',D,D',F,F'; levels are depicted by black broken lines in A;C,E) paraffin sections from the head of E9.5 (A-B'), E10.5 (C-D') and E12.5 (E-F') CD-1 mouse embryos, hybridized with a riboprobe for *mWnt9a*. (A-F) are the brightfield views of the darkfield images in (A'-F'). White arrowheads in (D',E',F') point at *Wnt9a* expression in the dorsal midline of the mesencephalon (D',F') or diencephalon (E'). Abbreviations: Aq, aqueduct; Di, diencephalon; Mes, mesencephalon; Met, metencephalon; Tel, telencephalon.

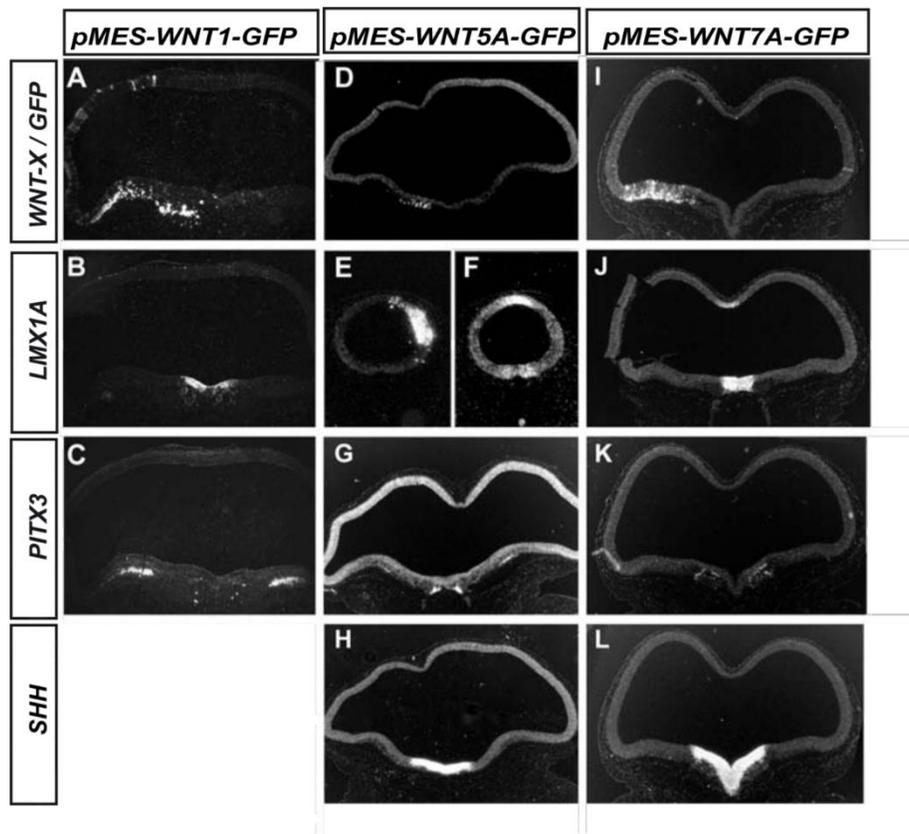


Figure S5: Other members of the *WNT* family have no influence on mdDA-related gene expression. (A-L) Coronal sections of chick mesencephalon electroporated with *mouse Wnt1* (A-C), chick *WNT5A* (D-H) and *WNT7A* (I-L) in the left half of the mesencephalon (A,D,I) and incubated for 2 days post-electroporation. The right side of the brain is the non-electroporated control. *RISHs* against *WNT* or *GFP* (A,D,E,I), *LMX1B* (B,F,J), *PITX3* (C,G,K) or *SHH* (H,L). (E,F) show *GFP/WNT5A* (E) and *LMX1B mRNA* expression (F) after 1dpe. Note that no ectopic expression of marker genes was induced after overexpression of any of these *Wnt* genes in midbrain.