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Supplemental Information

The Matricellular Protein R-Spondin 2 Promotes Midbrain Dopaminergic Neurogenesis and Differentiation

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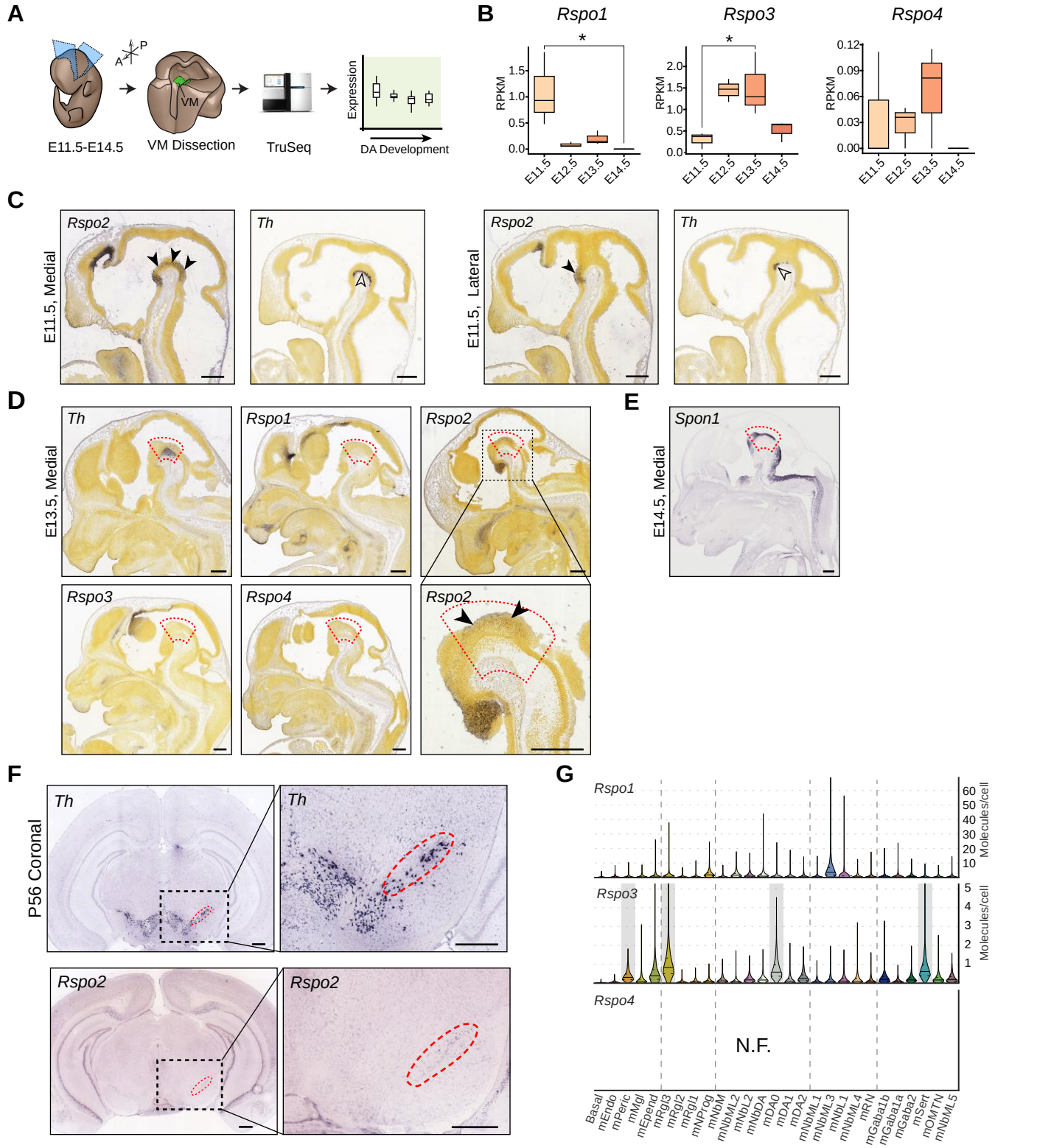

Figure S1. Expression of Spondins in ventral midbrain development. Related to Figure 1.

(A) Scheme of TruSeq experiment, VM region dissected from *TH-GFP* mice followed by Illumina sequencing.

(B) Expression levels of *Rspo1*, *Rspo3* and *Rspo4* by TruSeq RNA-seq analysis of VM tissue obtained from *TH-GFP* mice over time points E11.5 to E14.5 (Toledo et al., 2017a).

(C) Lower magnification of Figure 1D of E11.5 mouse VM ISH (image data from Allen Institute for Brain Science: Allen Developing Mouse Brain Atlas) for *Rspo2* (solid arrowheads) and *Th* (open arrowheads). Sagittal sections. Scale bar, 500 µm.

(D) E13.5 mouse head ISH (image data from Allen Institute for Brain Science: Allen Developing Mouse Brain Atlas) for *Th* (VM outlined in red) and *Rspo(1-4)* at E13.5. Sagittal sections through the midline and lateral. Scale bars, 500 µm.

(E) E14.5 mouse head ISH (image data from GenePaint, Set ID:EB2268) for *Spon1*. VM outlined in red. Sagittal section through midline. Scale bar, 500 µm.

(F) P56 mouse brain ISH (image data from Allen Institute for Brain Science: Allen Mouse Brain Atlas) for *Th* and *Rspo2*. SNc outlined in red. Coronal sections. Scale bars, 500 µm.

(G) Violin plots generated from single-cell RNA-seq data of the developing mouse VM. *Rspo1*, *Rspo3* and *Rspo4* expression levels are shown across all known cell types. Right axis shows absolute molecule counts (N.F. = not found). Grey, enriched over baseline with posterior probability >99.8%. For cell type nomenclature, see La Manno et al., 2016.

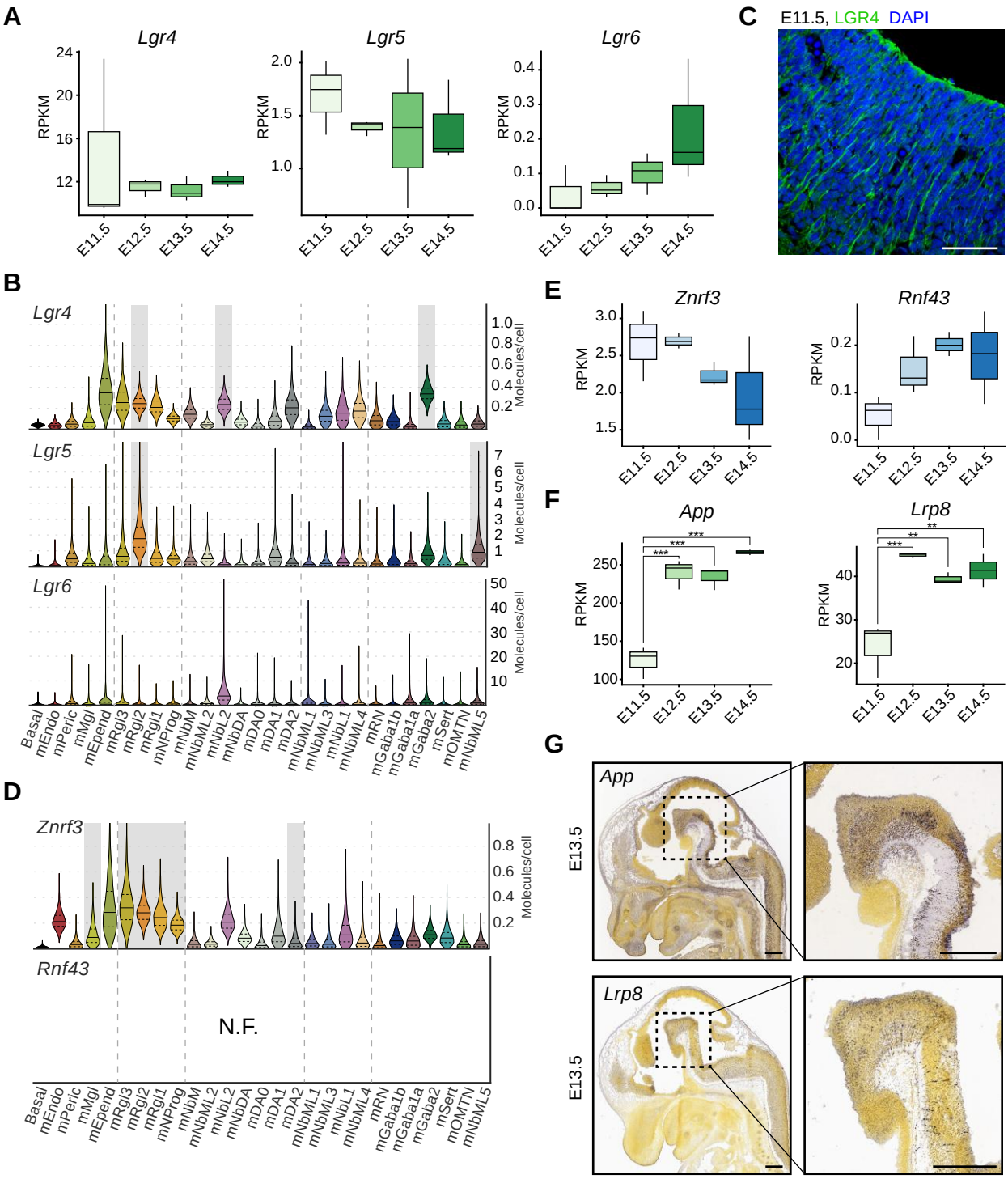

Figure S2. Expression patterns of Spondin-associated receptors. Related to Figure 2.

(A) Expression levels of *Lgr4*, *Lgr5*, and *Lgr6* by TruSeq RNA-seq analysis of VM tissue obtained from *TH-GFP* mice over timepoints E11.5 to E14.5 (Toledo et al., 2017a).

(B) Violin plots generated from single-cell RNA-seq data of the developing mouse VM. *Lgr4*, *Lgr5*, and *Lgr6* expression levels are shown across all known cell types. Right axis shows absolute molecule counts. Grey, enriched over baseline with posterior probability >99.8%. For cell type nomenclature, see La Manno et al., 2016.

(C) Detection of LGR4 in the midbrain by IHC, at E11.5. Scale bar, 50 µm.

(D) Violin plots generated from single-cell RNA-seq data of the developing mouse VM. *Znrf3* and *Rnf43* expression levels are shown across all known cell types. Right axis shows absolute molecule counts (N.F. = not found). Grey, enriched over baseline with posterior probability >99.8%. For cell type nomenclature, see La Manno et al., 2016.

(E) Expression levels of *Znrf3* and *Rnf43* by TruSeq RNA-seq analysis of VM tissue obtained from *TH-GFP* mice over timepoints E11.5 to E14.5 (Toledo et al., 2017a).

(F) Expression levels of *App* and *Lrp8* by TruSeq RNA-seq analysis of VM tissue obtained from *TH-GFP* mice over timepoints E11.5 to E14.5 (Toledo et al., 2017a).

(G) E13.5 mouse head ISH (image data from Allen Institute for Brain Science: Allen Developing Mouse Brain Atlas) for *App* and *Lrp8* at E13.5. Sagittal sections through the midline. Scale bars, 500 µm.

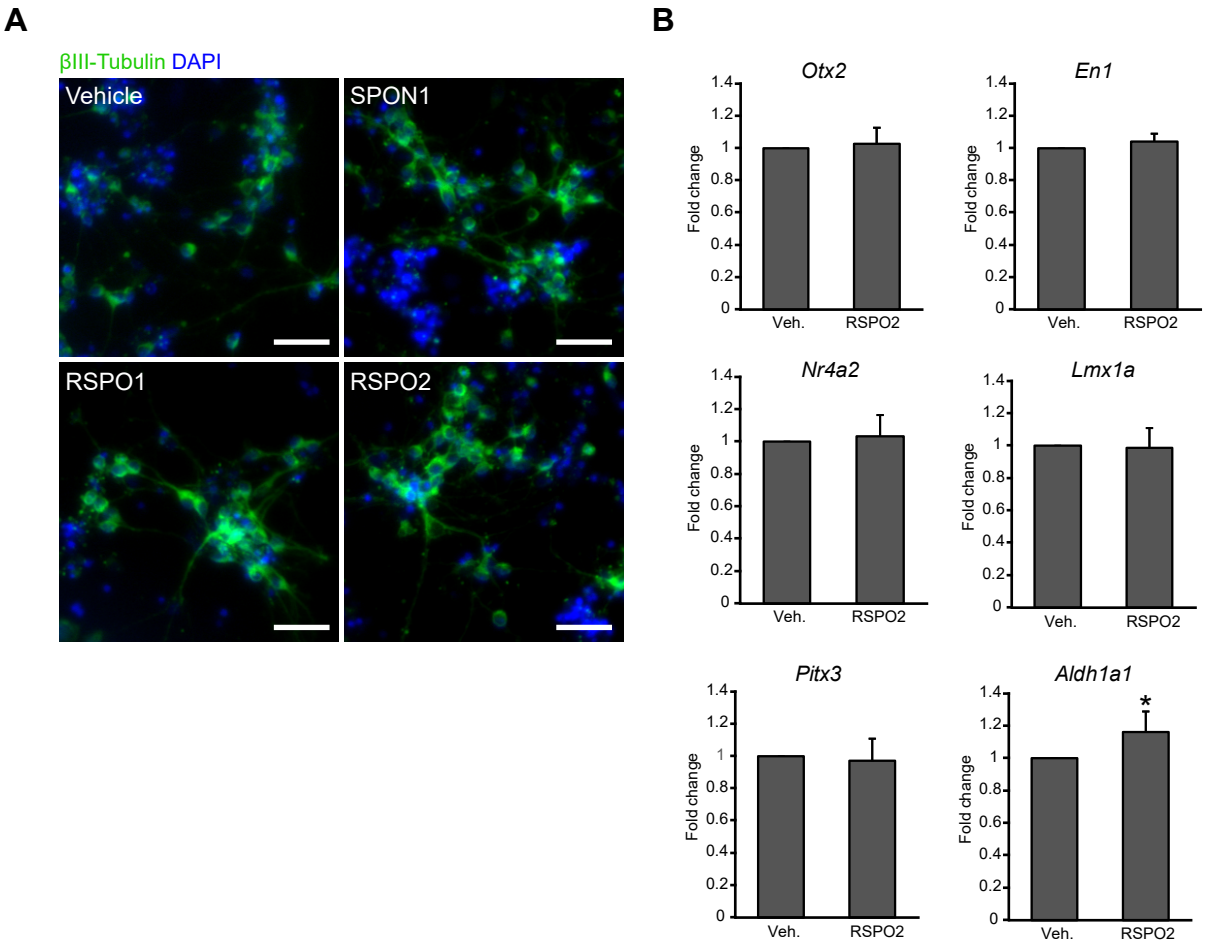

Figure S3. Primary mouse cell culture. Related to Figure 3 and Table S1.
(A) ICC staining with βIII-tubulin antibodies and DAPI showed no differences between conditions. Scale bar, 50 μm.
(B) Expression of *Otx2*, *En1*, *Nr4a2*, *Lmx1a*, *Pitx3*, and *Aldh1a1* measured by qPCR at the end primary cell culture. Statistical analysis compared to Vehicle treated control. *$p < 0.05$ by t-test. Data presented as means ± standard deviation measured in arbitrary units.

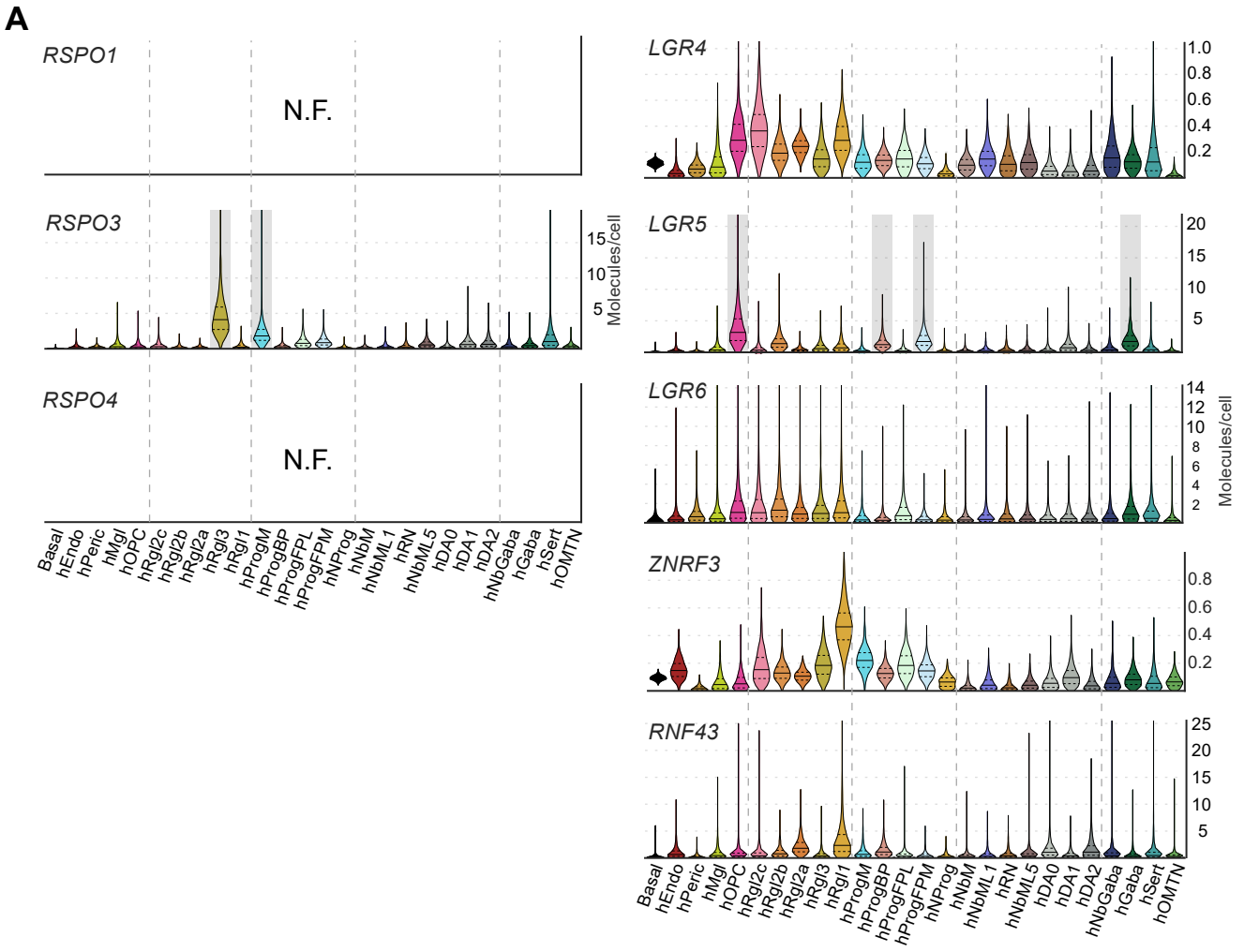

Figure S4. Single-cell expression of Spondin related genes in the human ventral midbrain. Related to Figure 5. (A) Violin plots generated from single-cell RNA-seq data of the developing human VM. *RSPO1*, *RSPO3*, *RSPO4*, *LGR4*, *LGR5*, *LGR6*, *ZNRF3*, and *RNF43* expression levels are shown across all known cell types. Right axis shows absolute molecule counts (N.F. = not found). Grey, enriched over baseline with posterior probability >99.8%. For human cell type nomenclature, see La Manno et al., 2016.

Gene	Forward	Reverse
Rspo1	ggagggagaatgccaaca	actgatgtgagtggccctgt
Rspo2	tgaatgccagatggttttgc	atctgccgtgttctggtttc
Rspo3	agctcctcctctgacagcaa	cttgtctcgggctcttctctt
Rspo4	ggagtgccaggaagagtgtg	ggacccgggtttctaggc
Spon1	actgtgcaacgcaagaagtg	attctgaccaggctgtccac
hOtx2	acaagtggccaattcactcc	gaggtggacaagggatctga
hEn1	gagcgcagggcaccaaata	cgagtcagttttgaccacgg
hPitx3	ccgtgtcctgcccttatgc	gggtcccgatagacgtagg
hNr4a2	gttcaggcgcagtatgggtc	ctcccgaagagtggtaactgt
hAldh1a1	gcacgccagacttacctgtc	cctcctcagttgcaggattaaag
hHPRT	cctggcgtcgtgattagtgat	agacgttcagtcctgtccataa
mNr4a2	gcatacaggtccaacccagt	aatgcaggagaaggcagaaa
mPitx3	gcaactggccgcccaagg	aggccccacgttgaccga
mAldh1a1	gacaggctttccagattggctc	aagactttcccaccattgagtgc
mOtx2	tcgaagagctaagtgccgcc	ggcaatggttgggactgagg
mEn1	tctctgggtacctcctgcac	tccagaaaaggaaggggatt
mLmx1a	ggaccataagcgacccaaac	cctgaaccacacggacactc
mGapdh	tggcctccaaggagtaagaa	tgtgagggagatgctcagtg

Table S1. Primer Sequences for qPCR. Related to Figure 1, 5, and S3.