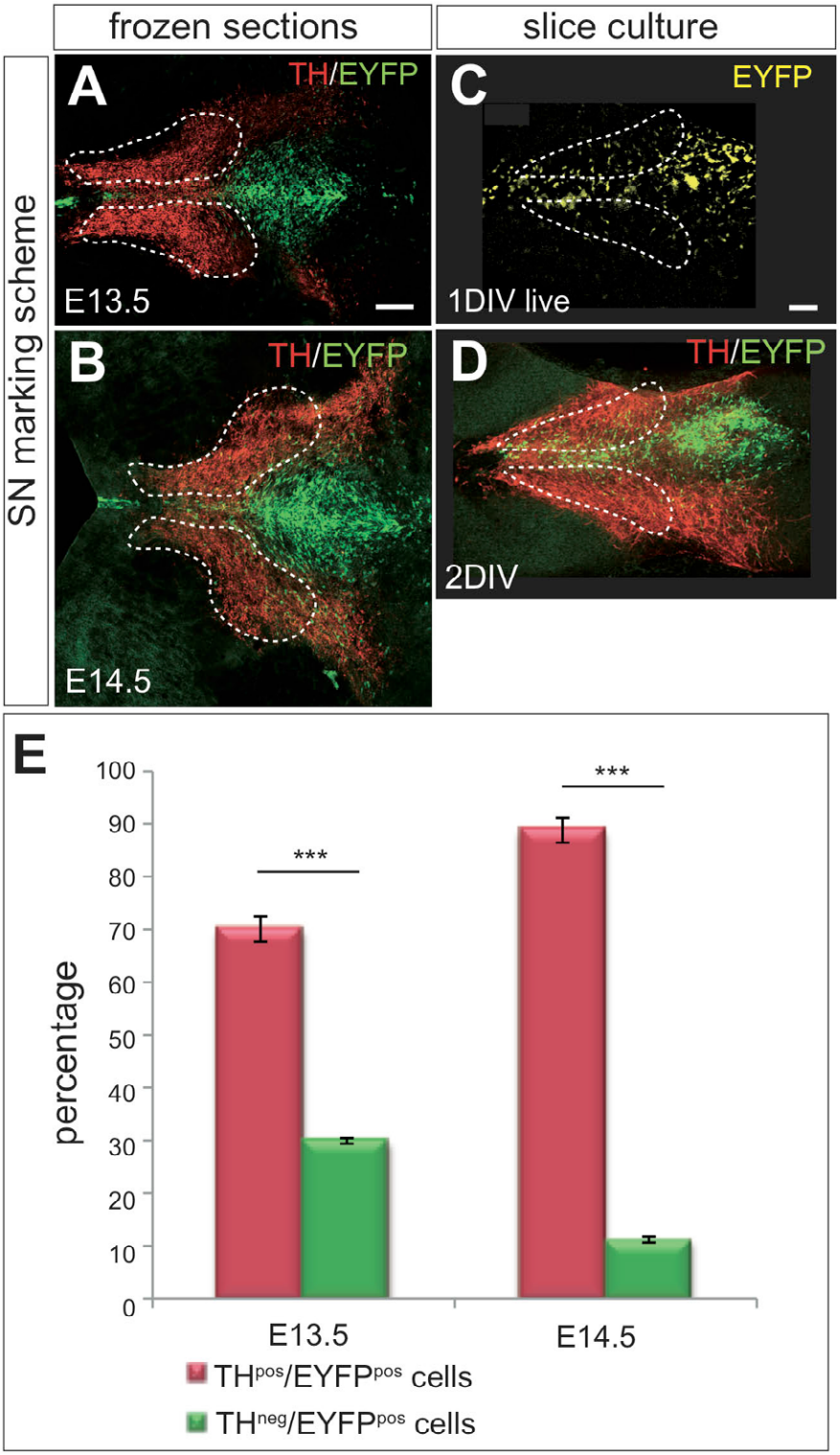


Figure S1. More than two-thirds of differentiated cells labeled with the SN marking scheme express TH. (A, B) Immunostaining for TH and EYFP on horizontal sections. Outlined areas contain differentiated DA neurons (TH^{pos}). (C, D) Organotypic slice cultures, horizontal section plane. The levels and stages are comparable to the frozen sections shown in A and B. (C) EYFP positive cells after 1 day *in vitro* (DIV). (D) Immunostaining for TH and EYFP after 2 DIV. (E) Quantification of $TH^{pos}/EYFP^{pos}$ and $TH^{neg}/EYFP^{pos}$ in the areas outlined in A and B. Significance was determined by Students's t-test, $p < 0.001$

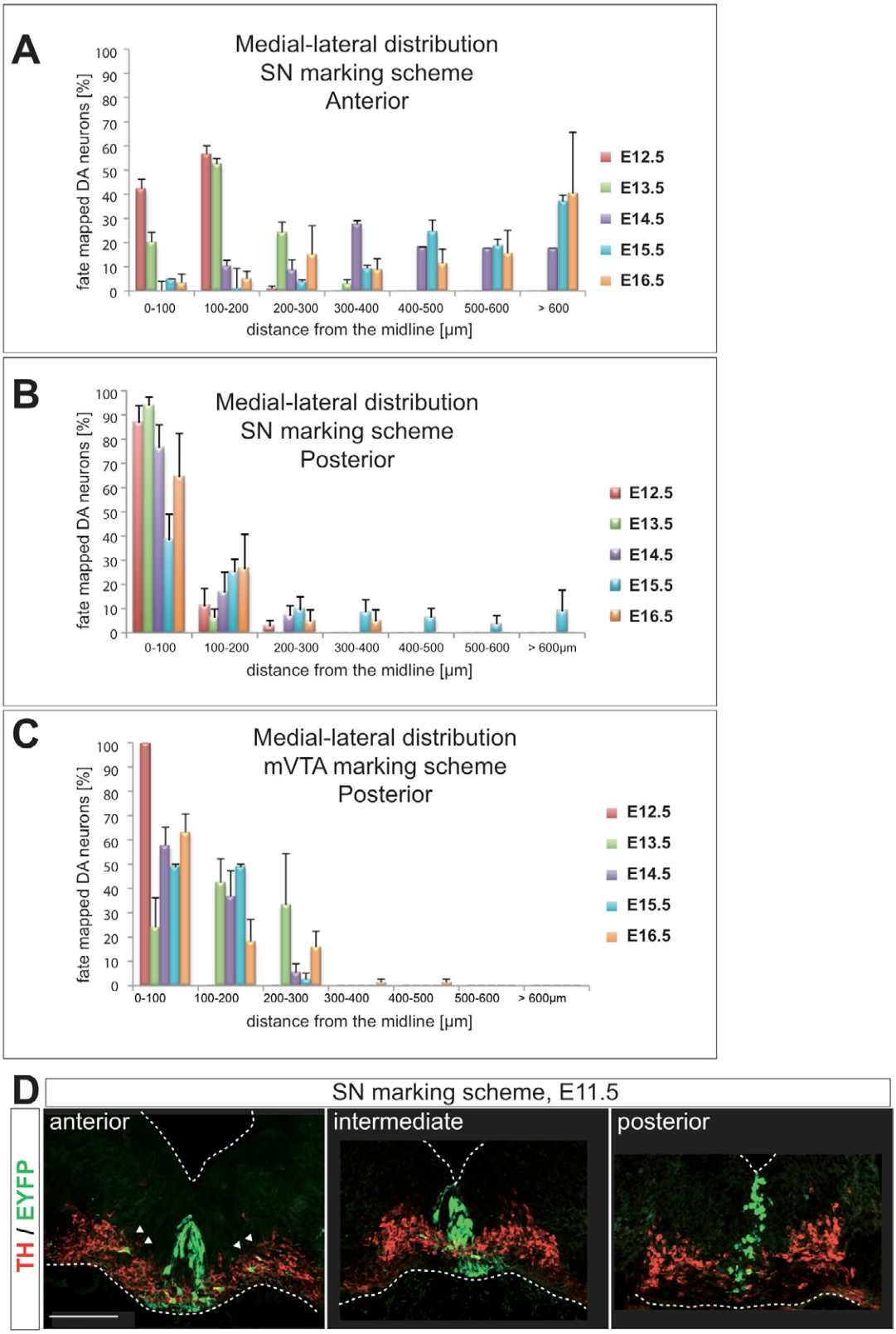


Figure S2. Distribution of fate-mapped DA neurons at anterior and posterior section levels. (A, B) Mediolateral distribution of DA neurons labeled with the SN marking scheme between E12.5 and E16.5 at anterior and posterior section levels. The ventral midbrain was divided in 100 µm wide bins from medial to lateral; fate-mapped DA neurons were counted in each bin. There is a shift from medial to lateral (≥ 600 µm from the midline) in the distribution of DA neurons between E12.5 and E16.5 in anterior sections. In posterior sections, only few DA neurons were found in lateral positions. (C) Mediolateral distribution of DA neurons labeled with the mVTA marking scheme between E12.5 and E16.5 at posterior section levels. There is no significant lateral shift over time. Data are presented as ± SEM, n=3. Statistical significance determined by ANOVA and post-hoc Bonferroni analysis. (D) SN marking scheme. E11.5 coronal sections at three anteroposterior levels were immunostained for TH and EYFP. Note that DA neurons at anterior levels are located at some distance from the midline (arrowheads). Scale bars: 100 µm

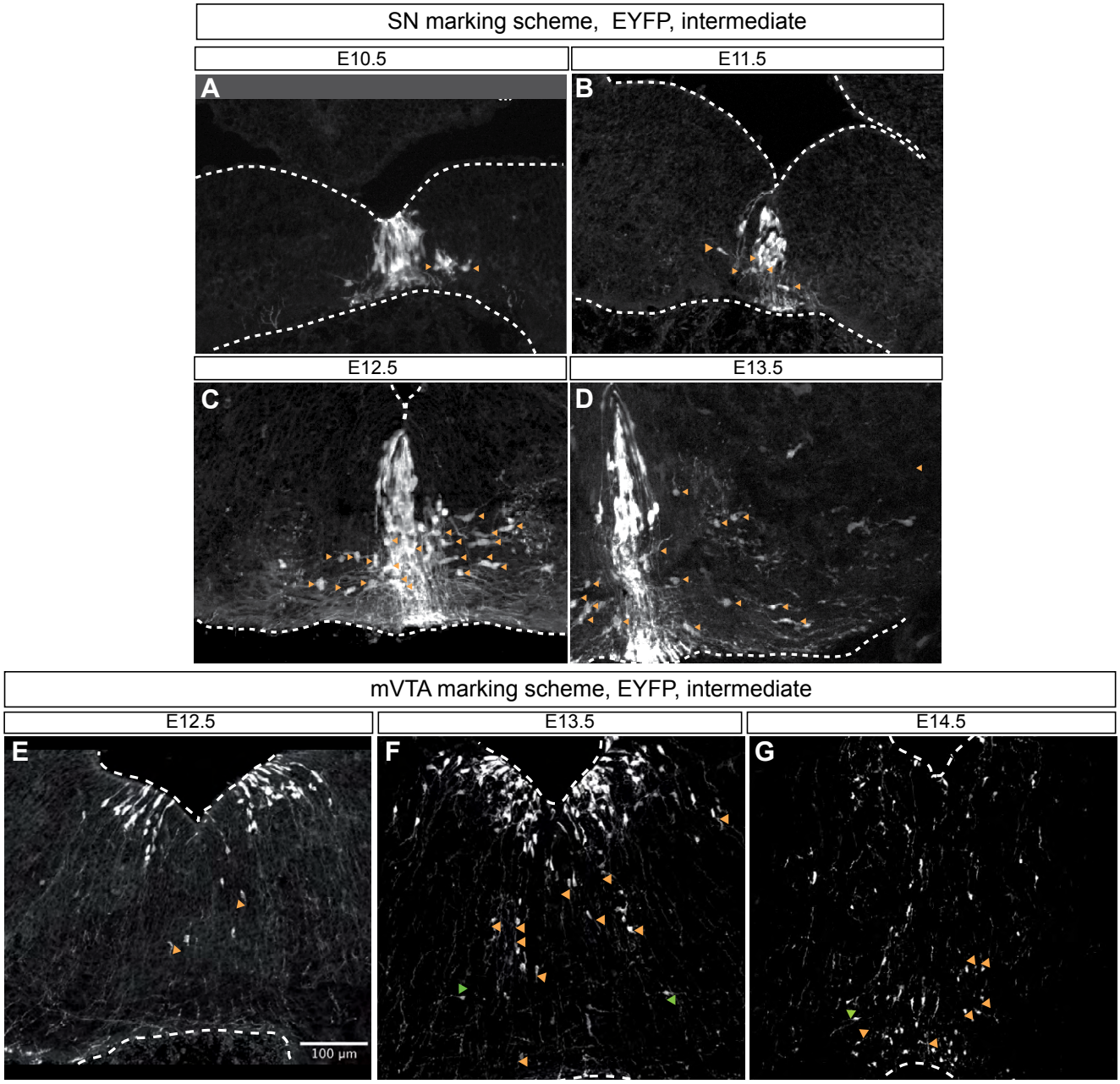


Figure S3. DA neurons labeled with the SN marking scheme, but not the mVTA marking scheme show many laterally oriented processes. (A-G) Coronal sections at an intermediate level were stained with antibodies against TH and EYFP, but only EYFP immunostaining is shown to visualize processes. The ventricle and the pial surface are outlined. (A-D) Orange arrowheads indicate TH^{pos} neurons. (E-G) Orange arrowheads indicate radially oriented TH^{pos} neurons, green arrowheads indicate tangentially oriented TH^{pos} neurons. Scale bars: 100 µm

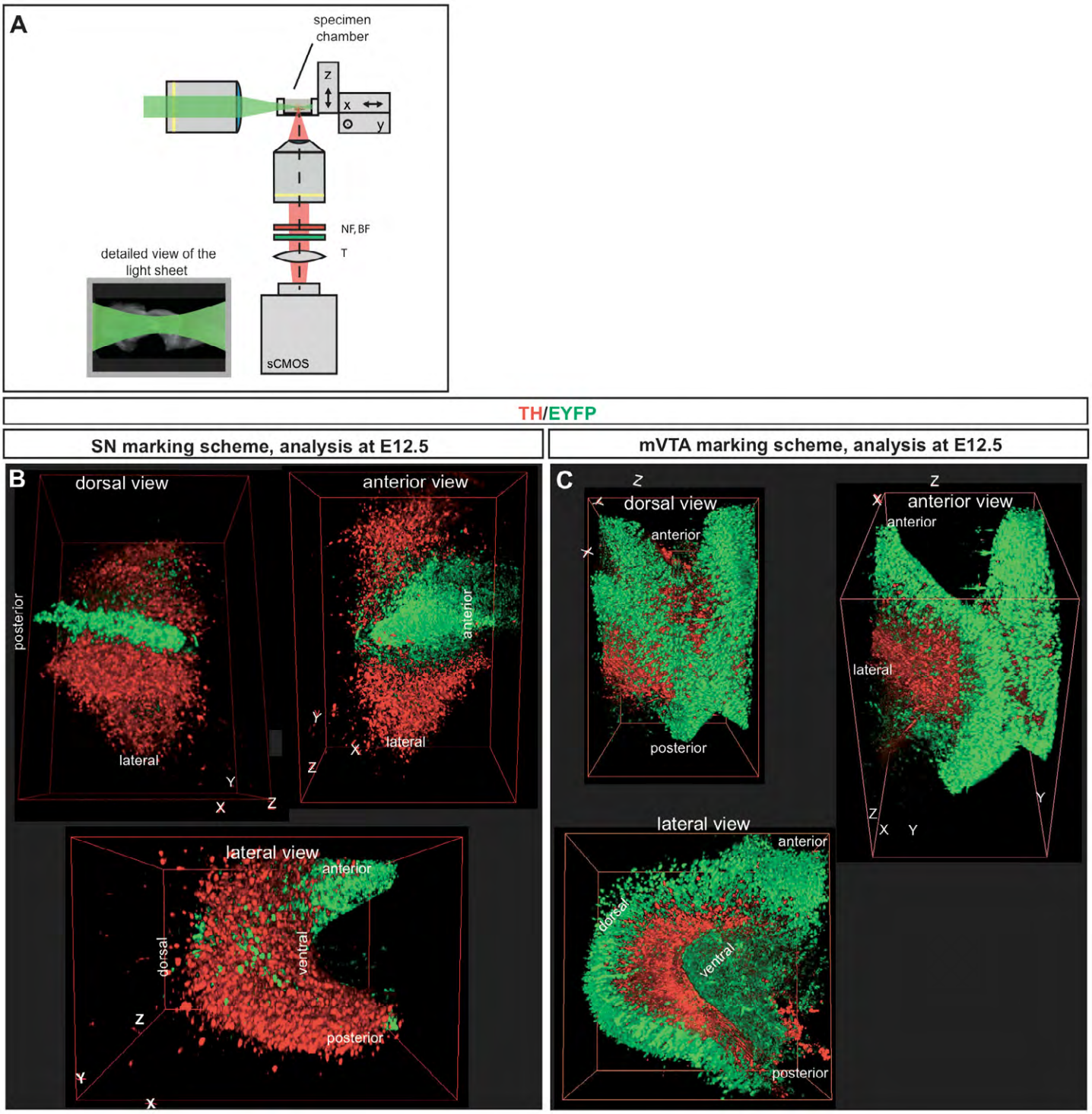


Figure S4. Ultramicroscopy and 3D renderings of the ventral midbrain (vMb) labeled with the SN or mVTA marking scheme. (A) Ultramicroscopy set-up. The sample is illuminated from one side by a laser forming a thin sheet of light. (B) 3D-rendering of the vMb labeled with the SN marking scheme. Brains were immunostained for TH and EYFP. Top, anterior and lateral views of the vMb show fate-mapped cells in the medial progenitor domain (only $EYFP^{pos}$) and laterally-positioned fate-mapped cells within the TH^{pos} area. (C) 3D-rendering of the vMb labeled with the mVTA marking scheme. Top, anterior and lateral views of the vMb show the two lateral progenitor domains (only $EYFP^{pos}$) and fate-mapped cells spread medially within the TH^{pos} domain. Fiji 3D-viewer software was used for 3D-reconstruction.

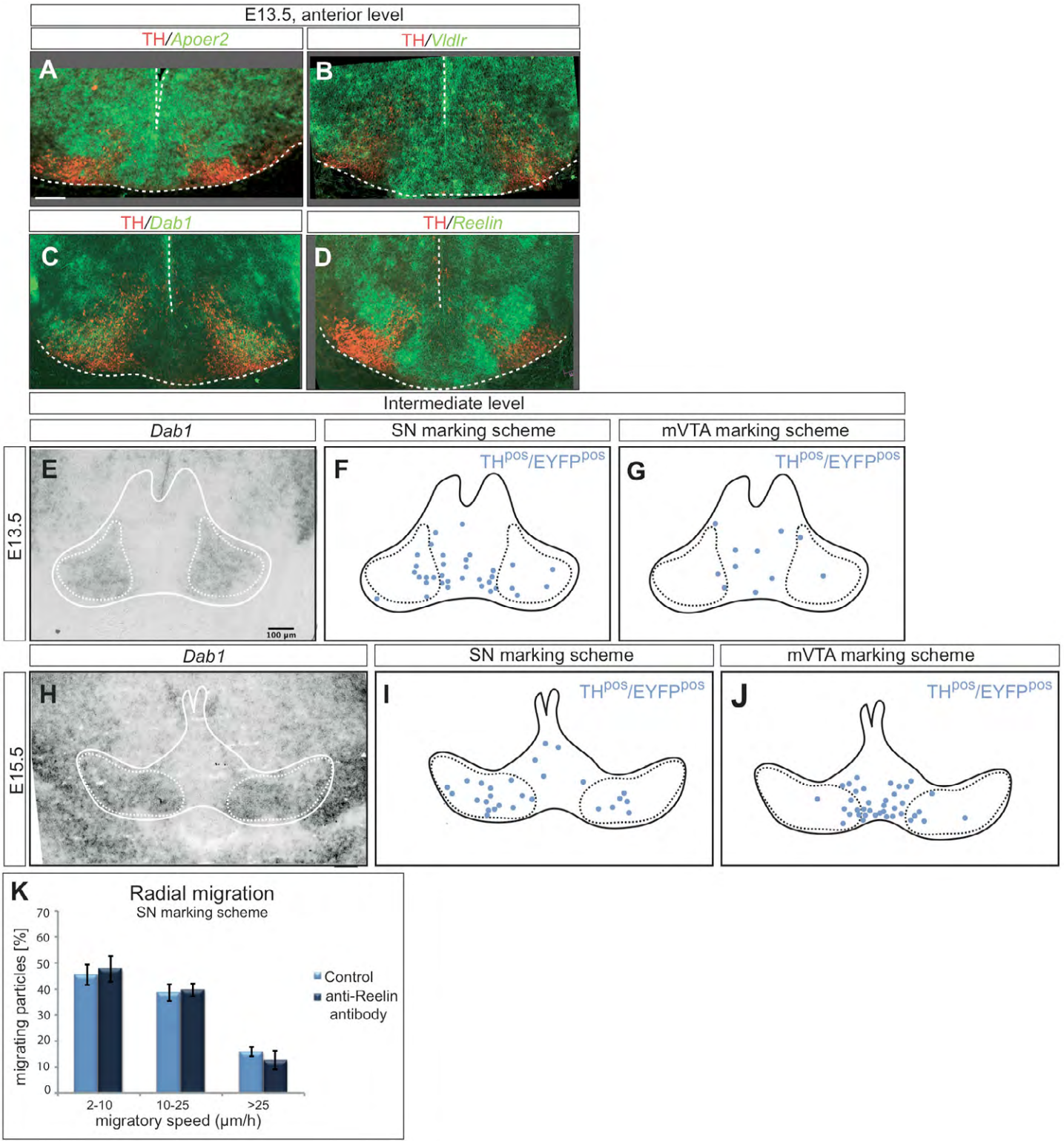


Figure S5. Expression of Reelin signaling pathway components and effect of Reelin inhibition on radial migration of cells marked with the SN marking scheme. (A-D) RNA *in situ* hybridization for components of the Reelin signaling pathway and immunostaining for TH on anterior sections. *Apoer2, Vldlr and Dab1* are expressed in the anterior SN. *Reelin* is expressed dorsally to the *Dab1*^{pos} cells. (E,H) RNA in situ hybridization for Dab1 on intermediate secions at E13.5 and E15.5. (F,G,I,J) Overlay of the *Dab1*-expression domain onto representative schematics showing the distribution of DA neurons labeled with the SN marking scheme (F, I) or the mVTA marking scheme (G, J) at intermediate section levels. Scale bars: 100 µm. (K) Inhibition of Reelin has no effect on the migratory speed of radially migrating cells labeled with the SN marking scheme and imaged in a sagittal slice. Note that the migratory speed of radially migrating cells labeled with the SN marking scheme is slower than the migratory speed of cells labeled with the mVTA marking scheme (Figure 6G) showing another aspect in which their migratory behavior is distinct.

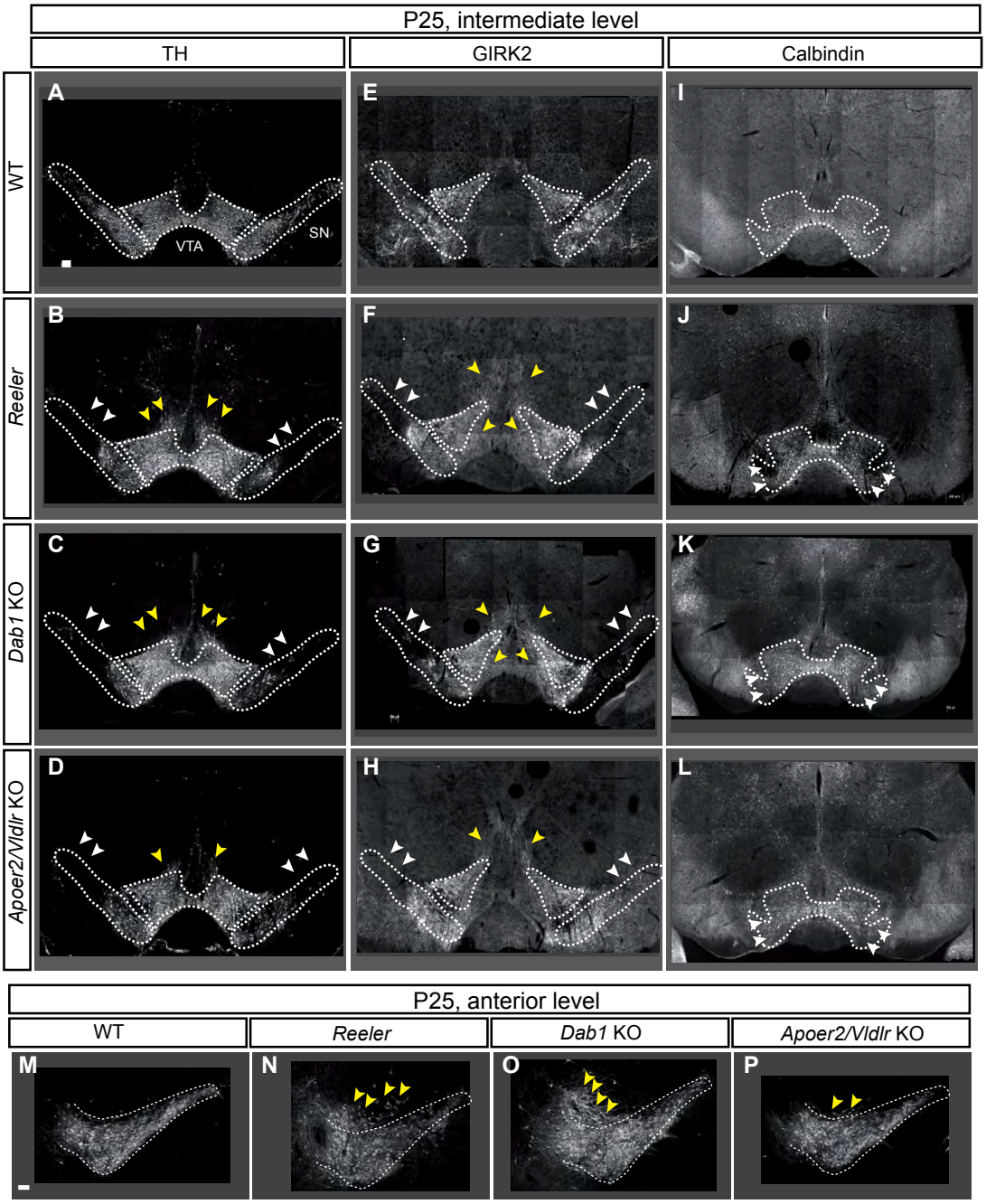


Figure S6. Canonical Reelin signaling pathway regulates the formation of the SN. Immunohistochemistry for TH on P25 brains of *Reeler* mutant, *Dab1* KO and *Vldlr/Apoer2* double KO mice. (A-L) At intermediate section levels, the lateral SN does not form in the mutants. The GIRK2^{pos} (E-H) and Calbindin^{pos} (I-L) DA neurons are not intermingled in the mutant animals, indicating that Reelin signaling is not required for the separation of these two populations. (M-P) At anterior section levels, the SN forms, but the neurons are disorganized. Yellow arrowheads indicate ectopic cells in the SN or VTA, white arrowheads indicate missing cells in the SN. Scale bar: 100 µm.

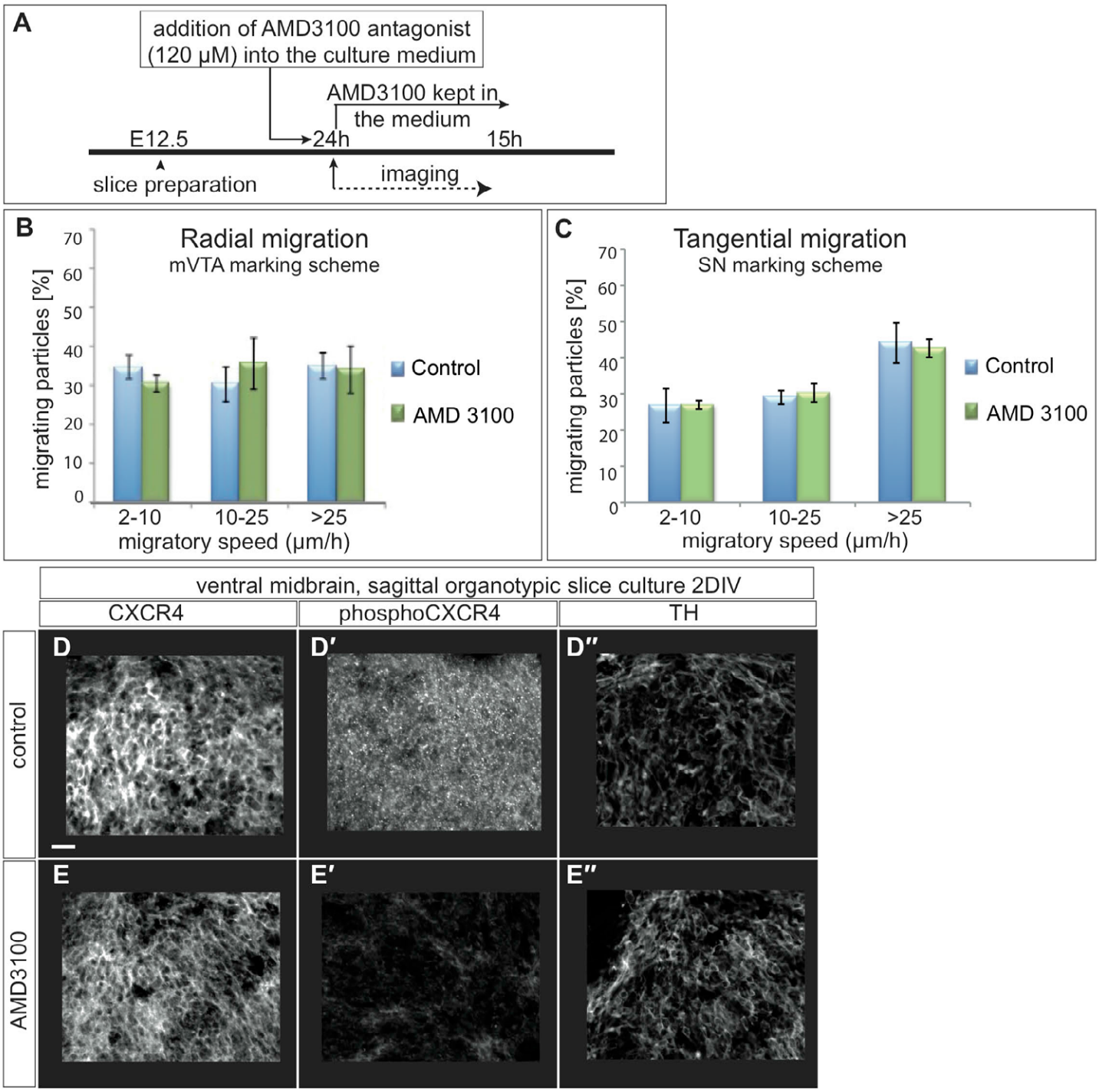
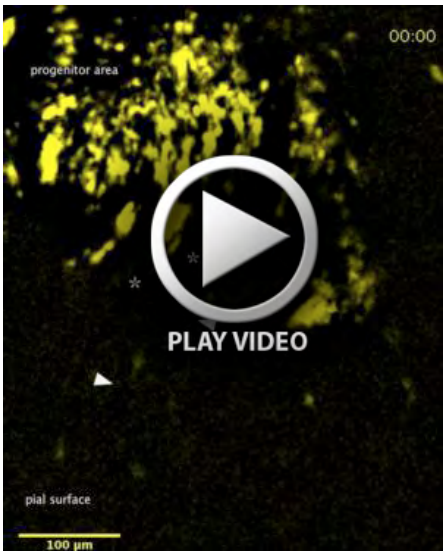
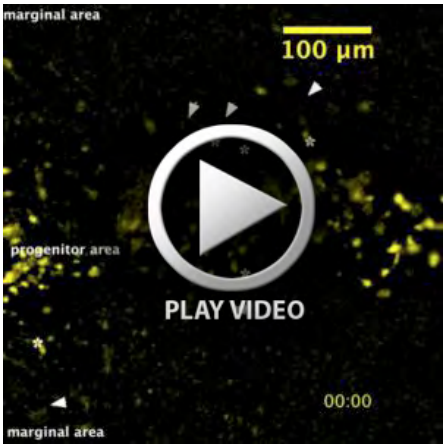


Figure S7. Inhibition of CXCR4 in organotypic slice cultures. (A) Experimental setup: treatment with CXCR4 antagonist AMD3100. (B, C) Quantification of the migratory speed of labeled cells with or without the addition of AMD3100. (B) Radial migration in a sagittal slice (mVTA marking scheme). (C) Tangential migration in a horizontal slice (SN marking scheme). Data are presented as ±SEM, n≥3. (D-E″) Analysis of phosphorylated CXCR4 in sagittal slice cultures. Upon CXCL12-mediated activation, CXCR4 is phosphorylated at its Serine residues-324 and 325. An antibody specific for CXCR4-Ser(P)-324/5 was used for immunostaining of untreated slices or slices treated with AMD3100 for 12 hours. Note that phosphorylated CXCR4 is no longer detected upon AMD3100 treatment.



Movie 1. DA neurons labeled with the SN marking scheme undergo radial migration. Migrating cells move from the progenitor area (top) towards the pial surface (bottom). The cell bodies elongate and translocate. 300 µm sagittal slice (E12.5) after 1 DIV. Imaging time: 8 h. Asterisks: initial positions of migrating cells. Arrowheads: final positions of migrating cells. Scale bar: 100 µm

Movie 2. DA neurons labeled with the mVTA marking scheme undergo radial migration. Migrating cells move from the progenitor area (top) towards the pial surface (bottom). The cell bodies elongate and translocate. 300 µm sagittal slice (E12.5) after 1 DIV. Imaging time: 14.35 h. Asterisks: initial positions of migrating cells. Arrowheads: final positions of migrating cells. Scale bar: 100 µm



Movie 3. DA neurons labeled with the SN marking scheme undergo tangential migration. Migrating cells move from the progenitor area (middle) towards the lateral, marginal area (bottom and top). The cell bodies elongate and translocate. 300 µm horizontal slice (E12.5) after 1 DIV. Imaging time: 8 h. Asterisks: initial positions of migrating cells. Arrowheads: final positions of migrating cells. Scale bar: 100 µm

Movie 4. DA neurons labeled with the mVTA marking scheme do not undergo tangential migration. Migrating cells appear to move in place and extend processes, but do not leave their position. 300 µm horizontal slice (E12.5) after 1 day in vitro (DIV). Imaging time: 9.12 h. Arrowheads: cells remain in a fixed place. Scale bar: 100 µm

Movie 5. DA neurons labeled with the mVTA marking scheme do not undergo tangential migration. Migrating cells appear to move in place and extend processes, but do not leave their position. 300 µm horizontal slice (E13.5) after 1 day in vitro (DIV). Imaging time: 12 h. Arrowheads: cells remain in a fixed place. Scale bar: 100 µm

Movie 6. DA neurons labeled with the mVTA marking scheme do not undergo tangential migration. Migrating cells appear to move in place and extend processes, but do not leave their position. 300 µm horizontal slice (E14.5) after 1 day in vitro (DIV). Imaging time: 15 h. Arrowheads: cells remain in a fixed place or move very little. Scale bar: 100 µm

Experiment and corresponding Figures	**Age**	**Marking scheme**	**No. of embryos**	**Average number of TH^{pos}/$EYFP^{pos}$ cells analyzed per embryo (±SEM)**
Fate mapping (Figure 3 and Figure S2)	E12.5	SN	3	34.17±3.09
	E13.5	SN	3	26.17±2.62
	E14.5	SN	3	38±4.73
	E15.5	SN	4	30.88±7.72
	E16.5	SN	3	27.5±1.61
	E12.5	mVTA	3	1.33±0.16
	E13.5	mVTA	3	13.33±1.16
	E14.5	mVTA	3	26.67±6.98
	E15.5	mVTA	3	11.5±2.51
	E16.5	mVTA	4	32±16.60
	Marking scheme	**Treatment**	**No. of embryos**	**Total number of cells tracked**
Trajectory in horizontal slices (Figure 6)	SN	Control	3	33
	SN	Reelin inhibitor	4	46
	SN	AMD3100 antagonist	2	18
Trajectory in sagittal slices (Figure 6)	mVTA	Control	3	22
	mVTA	AMD3100 antagonist	3	26
	Marking scheme	**Treatment**	**No. of embryos**	**Average number of particles tracked per embryo (±SEM)**
Speed in horizontal slices (Figure 6)	SN	Control	5	320±178
	SN	Reelin inhibitor	4	619.25±186.19
	SN	AMD3100 antagonist	3	519±157
Speed in sagittal slices (Figure 6)	mVTA	Control	5	262.2±62.11
	mVTA	Reelin inhibitor	3	244±182
	mVTA	AMD3100 antagonist	3	412.5±225.91
	SN	Control	3	229.33±50.24
	SN	Reelin inhibitor	3	371.33±114.83
	Age	**Phenotype**	**No. of embryos**	**Average number of TH^{pos} cells analyzed per embryo (±SEM)**
DA neuron numbers in *Cxcr4* and *Cxcl12* KO mice (Figure 8)	E14.5	Control	4	311.63±28.64
	E14.5	*Cxcl12* KO	4	260.75±9.37
	E14.5	*Cxcr4* KO	4	306.63±14.63
	E16.5	Control	4	228.88±44.84
	E16.5	*Cxcl12* KO	4	250.88±8.51
	E16.5	*Cxcr4* KO	4	294.75±24.69

Table S1: Numbers of quantified cells.