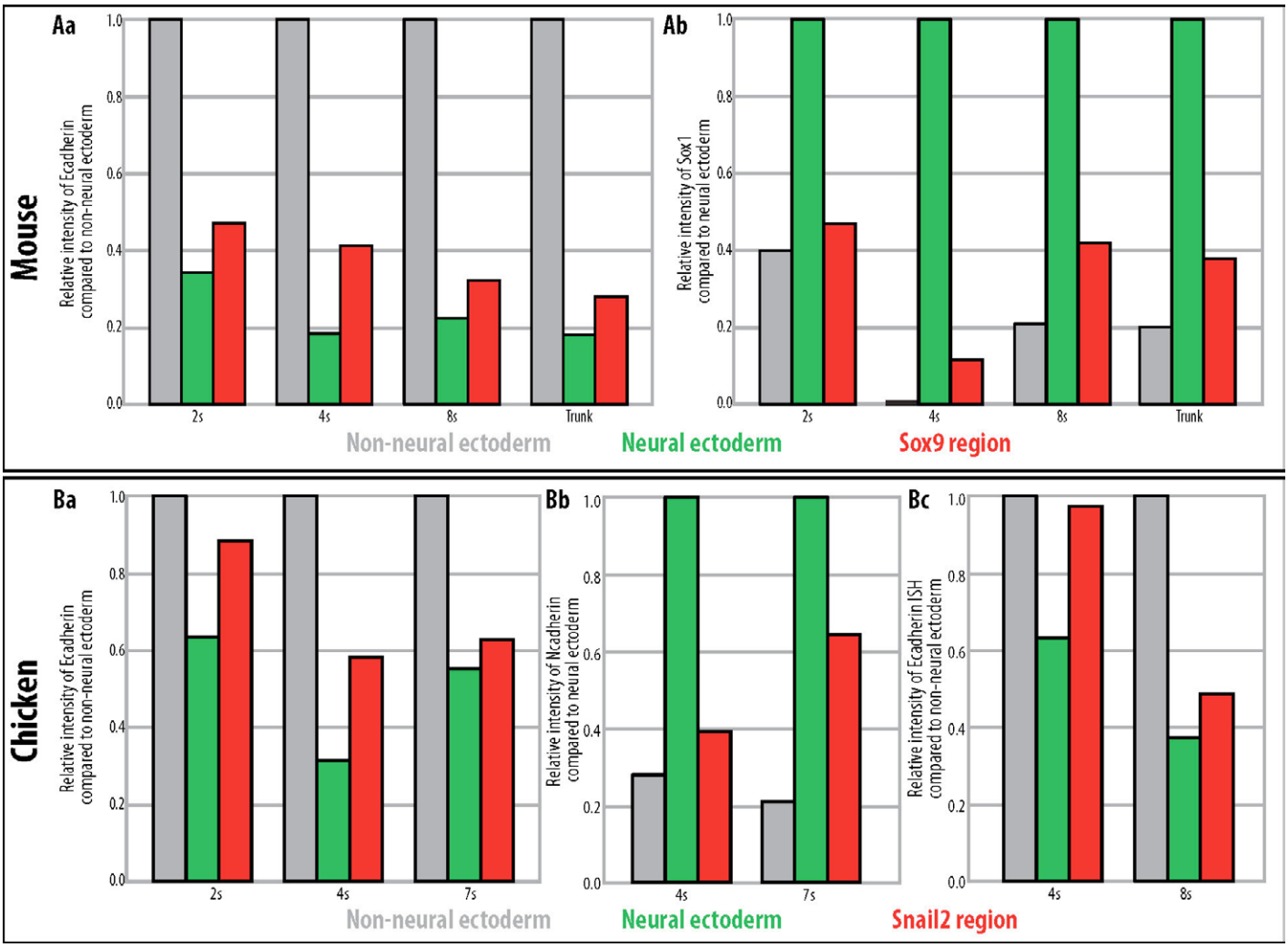

Fig. S1. Quantification of E-cadherin and Sox1/N-cadherin signals in the non-neural ectoderm, neural ectoderm, and in premigratory cells in embryos. The Sox9/Snail2 region is defined as cells remaining in the ectoderm that expresses the respective markers. We compared the expression level of E-cadherin and Sox1/N-cadherin in the region of interest by normalizing it with E-cadherin found in non-neural ectoderm and Sox1/N-cadherin found in neural ectoderm. This allowed us to determine whether the region of interest was more similar to non-neural or neural ectoderm. Quantification of the initial N-cadherin signal in chicken embryos was not performed as it would be misleading because it is expressed mainly apically. (A) Quantification of E-cadherin and Sox1 in different regions of the mouse embryo. At early stages (2 and 4 somites), the region expressing Sox9 shows higher and lower expression levels of E-cadherin (Aa) and Sox1 (Ab) as compared with those expressions in the neural ectoderm. By comparison, at 8 somites, the region expressing Sox9 shows lower levels of E-cadherin expression (Aa) and higher levels of Sox1 expression (Ab). This later population of Sox9-expressing cells in the cranial region is similar to Sox9-expressing cells in the trunk. In the cranial region, the expression level of Sox1 in the Sox9-expressing region is consistently lower as compared with the neural ectoderm at all stages, whereas the expression level of E-cadherin is initially higher but falls to levels similar to the neural ectoderm at later stages. Quantification of immunostaining for E-cadherin (Ba) and N-cadherin (Bb) in chicken embryos. Quantification of *in situ* hybridization of E-cadherin in chicken embryos (Bc). At early stages, between 2 and 4 somites, the Snail2-expressing region shows a similar pattern of E-cadherin and N-cadherin expression as that seen in non-neural ectoderm (high expression of E-cadherin and low expression of N-cadherin) as compared with that observed in the neural ectoderm. The pattern of E-cadherin mRNA expression in Snail2-expressing cells at 4 somites is also similar to the non-neural ectoderm (Bc). However, at 7 somites, the Snail2-expressing region shows a more similar pattern of E-cadherin and N-cadherin expression to that of the neural ectoderm (low expression of E-cadherin (Ba) and higher expression of N-cadherin (Bb) as compared with that of the non-neural ectoderm. At 8 somites, the pattern of E-cadherin mRNA expression in Snail2-expressing cells is no longer similar to the non-neural ectoderm (Bc). There is a clear difference in the mRNA expression of E-cadherin between the non-neural and neural ectoderm (Bc).

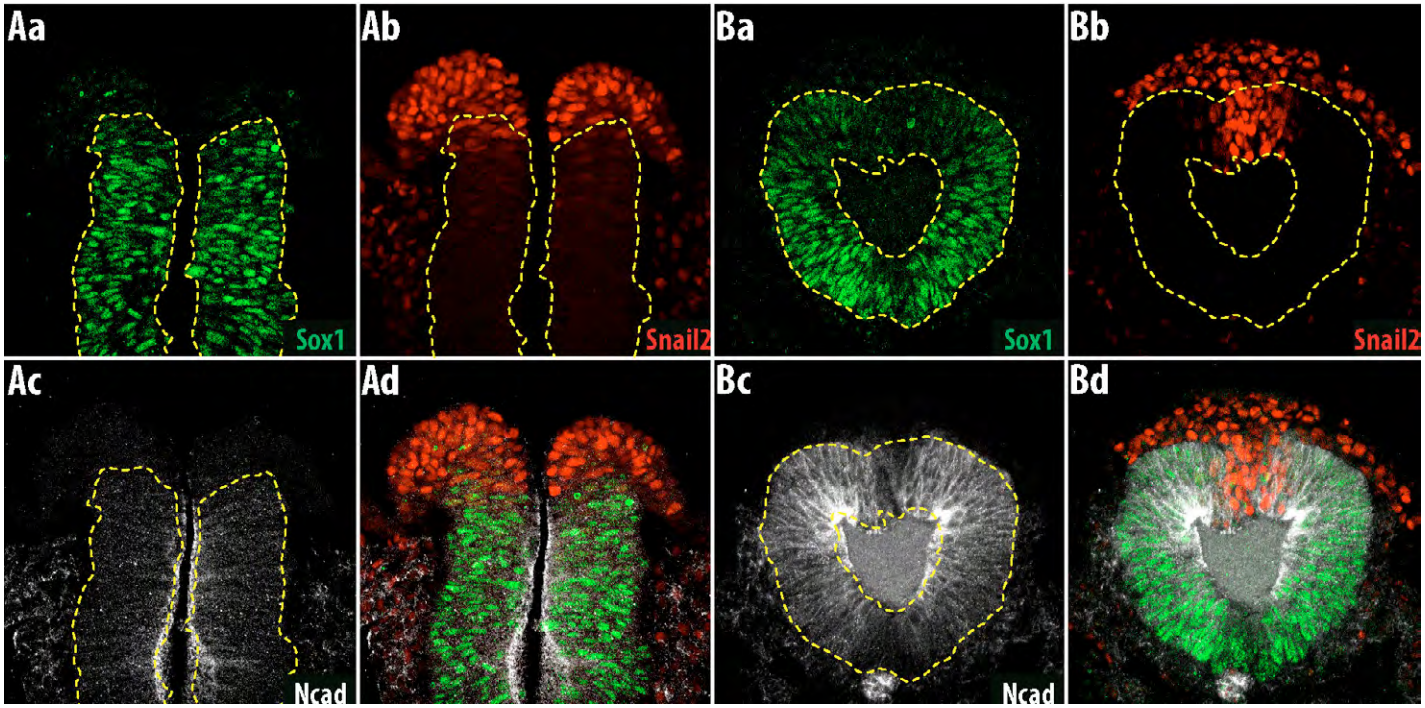

Fig. S2. Sox1 and N-cadherin are similarly expressed in the neural epithelia. Sox1 and N-cadherin expression in the neural epithelia. (A,B) Sox1-expressing cells in the neural epithelia also co-express N-cadherin (yellow dotted line represent Sox1 expressing region). (A) Chicken embryo at 4 somites. At this stage the majority of Snail2 expressing cells do not express Ncad or Sox1. At these early stages, we observed some Snail2 expressing cells in the neural ectoderm. (B) Chicken embryo at 7 somites. Premigratory Snail2-expressing cells are found in the neural ectoderm.

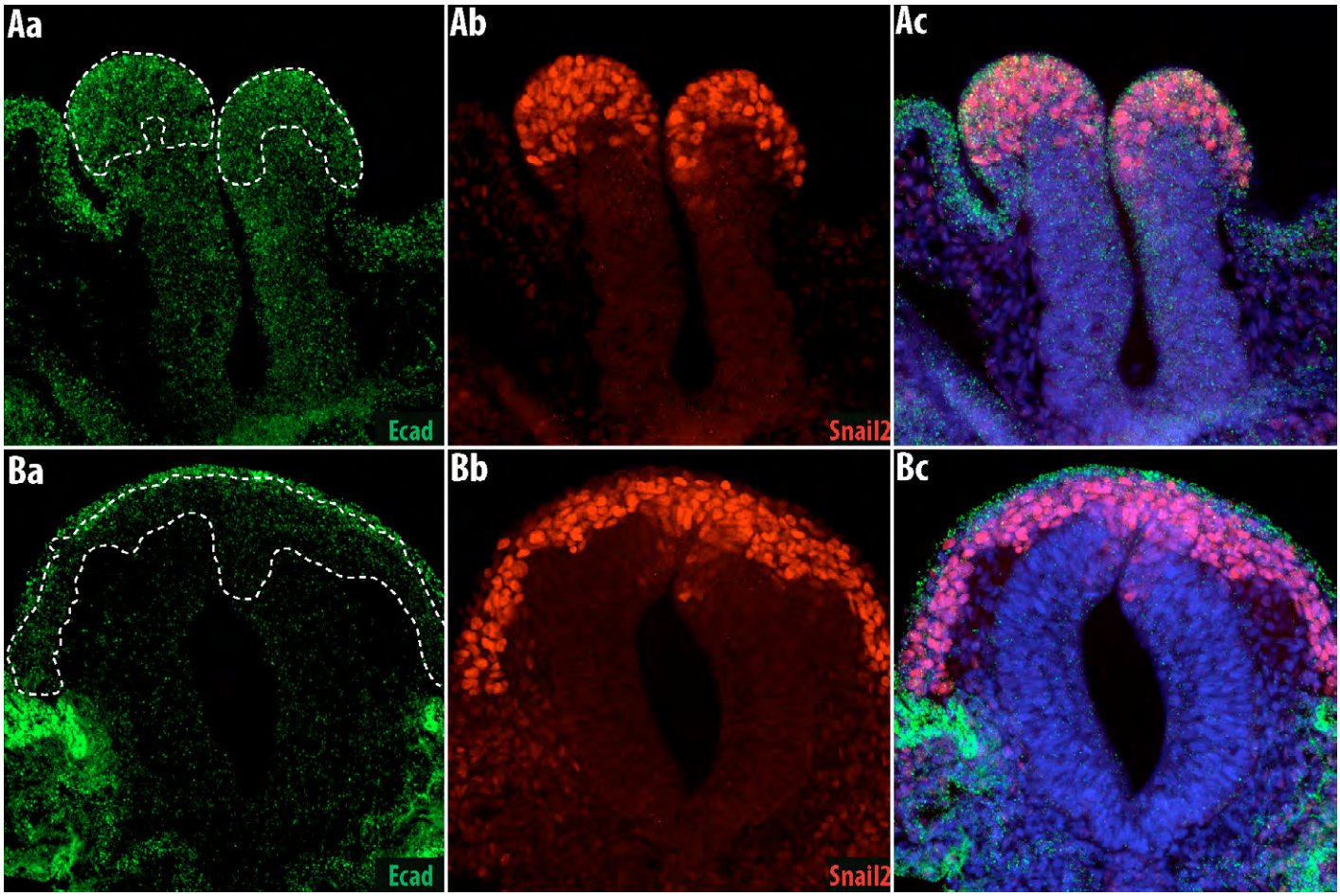

Fig. S3. E-cadherin in-situ shows a similar pattern to E-cadherin immunostaining. Fluorescent in situ hybridization of E-cadherin and Snail2 immunostaining in chicken embryos (A,B). (A) At 4 somites, Snail2 expressing cells still expressed relatively high levels of E-cadherin as compared with those in the neural ectoderm (white dotted area represent Snail2 expressing region). (B) At 8 somites this changes, with only the overlying non-neural ectoderm expressing E-cadherin (white dotted area represents the Snail2 expressing region). E-cadherin expression in the neural ectoderm and Snail2-expressing cells is different from what we observed for E-cadherin immunostaining (Fig. 4B).

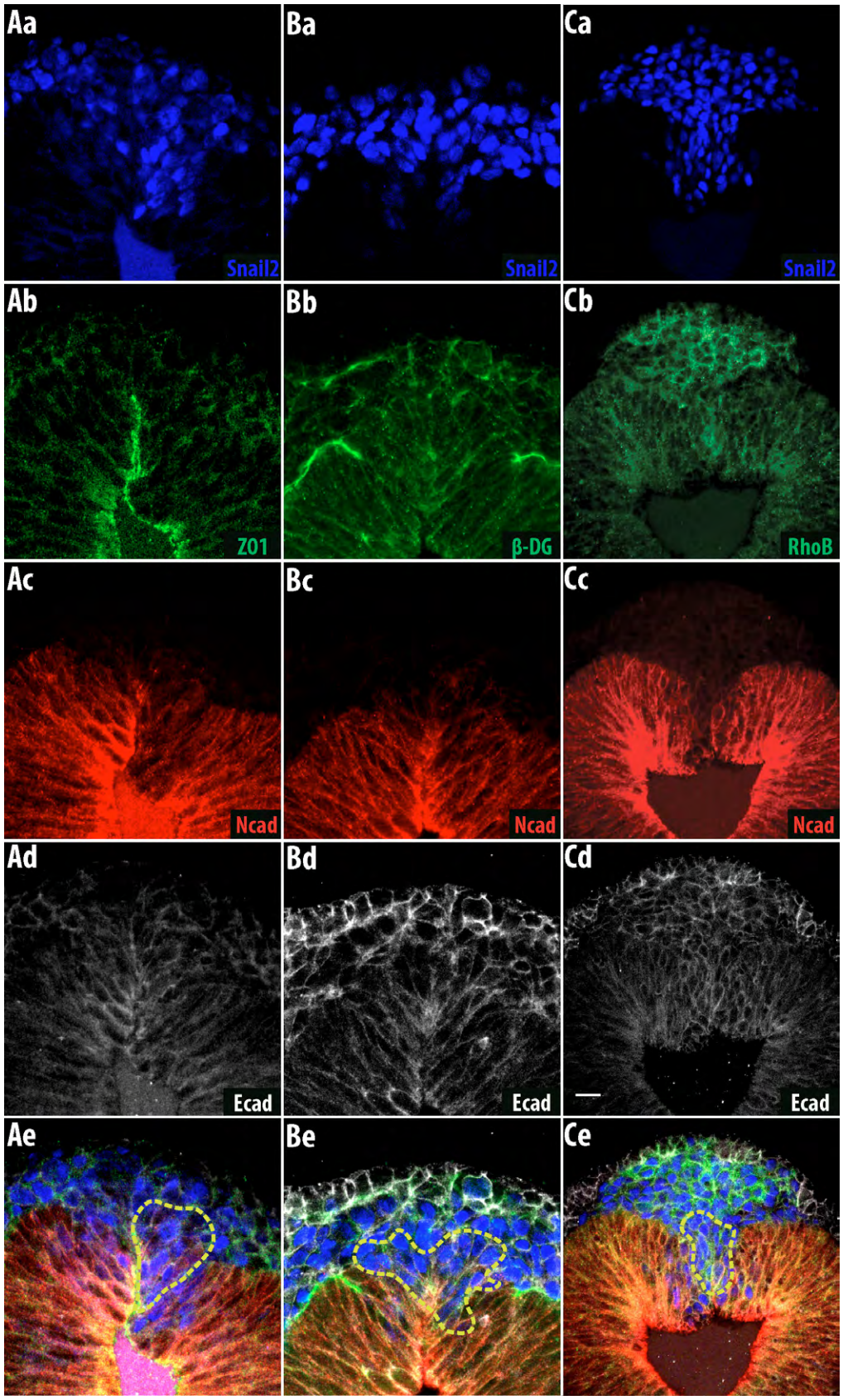

Fig. S4. In chicken embryos cells delaminate from the neural ectoderm at 7 somites. Snail2-expressing cells in the neural epithelia delaminate at about 7 somites in chicken embryos. (A-C) Chicken embryos (7 somites). Snail2-expressing cells in the neural epithelia are undergoing EMT, as they have lost apical and basal polarity as shown by the cortical localization of ZO1 (A) and β-Dystroglycan (B) (yellow dotted line). RhoB is expressed in Snail2-positive cells found in the neural ectoderm (yellow dotted line), further indicating that these cells are undergoing EMT (C).

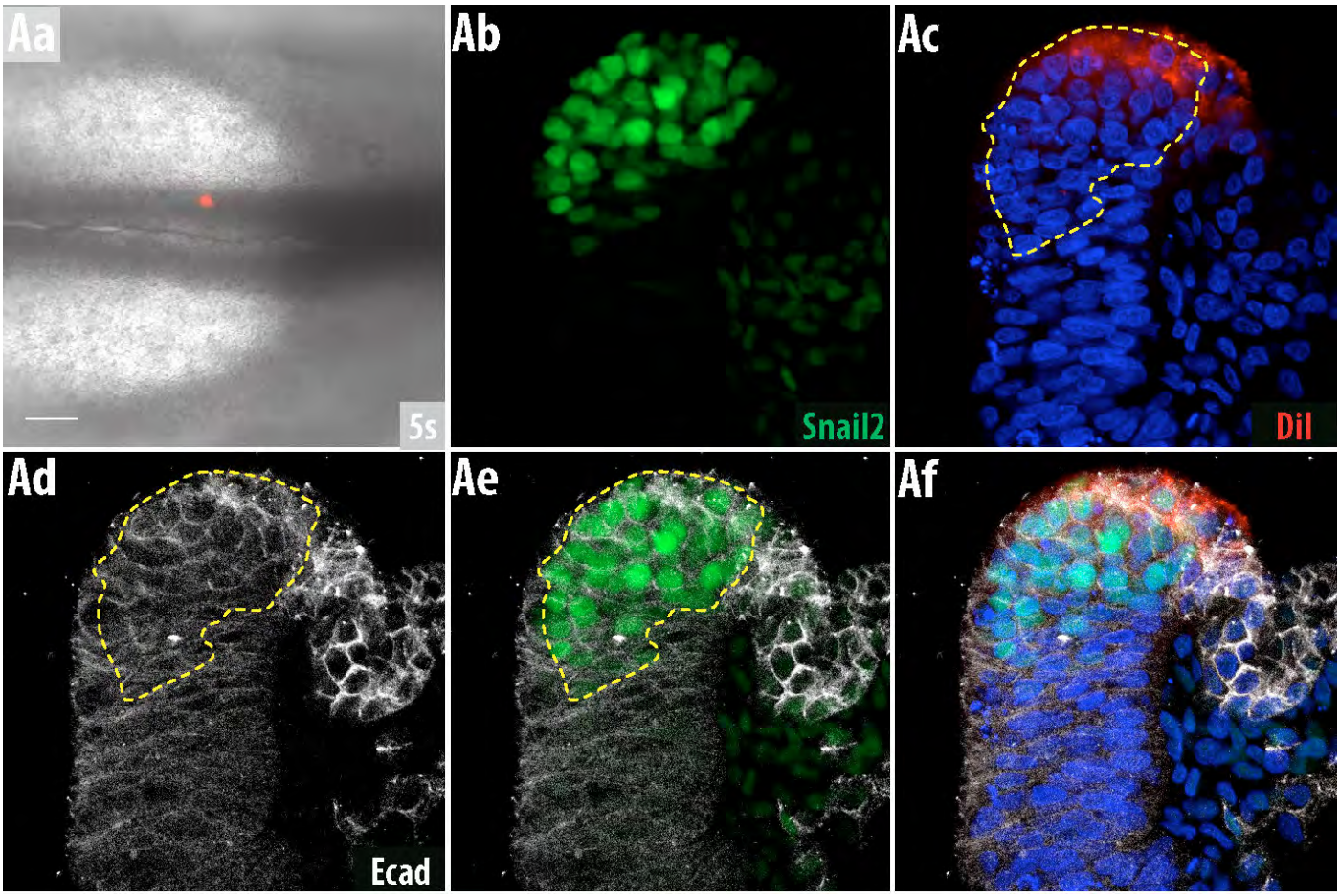

Fig. S5. DiI labeling of the non-neural ectoderm. A chicken embryo used for lineage analysis (between 4 and 5 somites and 6 embryos were analyzed) with the non-neural ectoderm labeled with DiI. All DiI-labeled cells were found in the non-neural ectoderm expressing Snail2 and E-cadherin (Ad,Ae), yellow dotted lines represent the region where Snail2 is expressed.

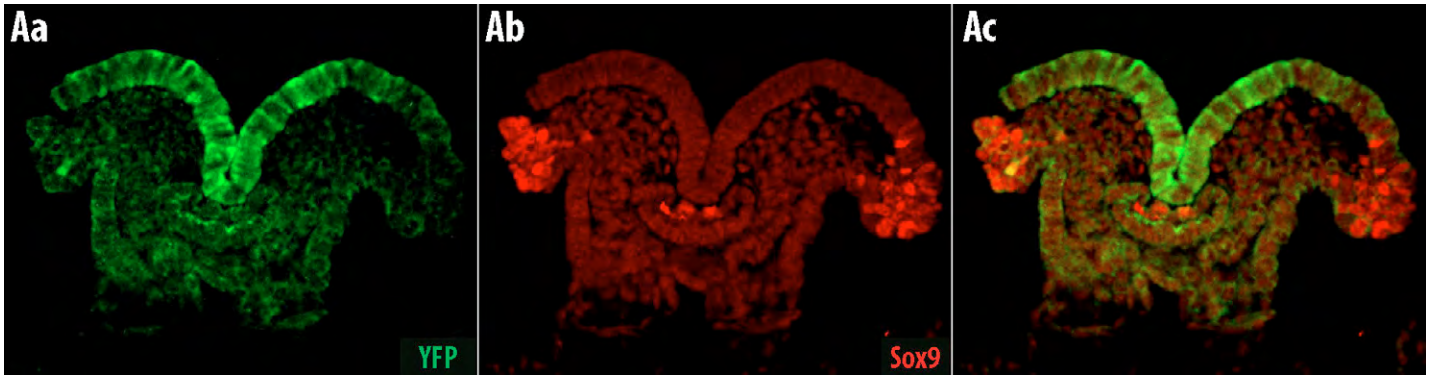

Fig. S6. YFP reporter is expressed in the neural ectoderm prior to the delamination of cells in the neural fold. Mouse embryo (3 somites). Activation of YFP reporter by Sox1 Cre occurs prior to the delamination of cells. YFP (Aa) is expressed in the neural ectoderm prior to the delamination of cells (Ab).

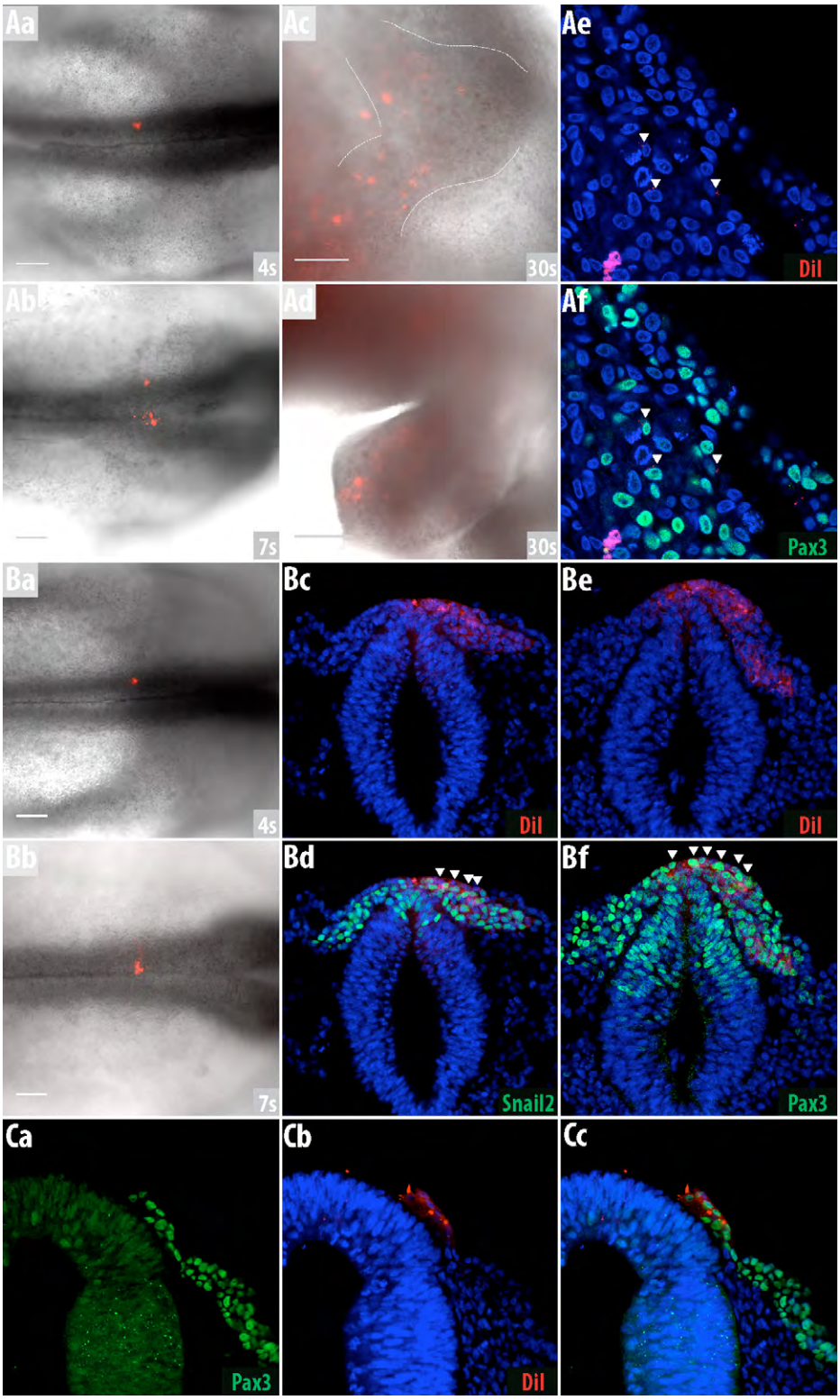

Fig. S7. The trigeminal placode and its derivatives are also labeled in some non-neural DiI-labeled embryos. DiI labeling of the lateral non-neural ectoderm also labels the trigeminal placode. We analyzed 2 embryos at ~30 somites where there was labeled cells in the trigeminal ganglia (A), and analyzed 3 embryos which show DiI labeling on the dorsal midline at 7 somites (B,C). DiI was used to label the non-neural ectoderm at 4 somites (A) and some DiI-labeled cells have migrated close to the midline of the neural tube at 7 somites (Ab). After growing to ~30 somites, DiI-labeled cells can be clearly seen in the trigeminal ganglia (Ac) as well as in the branchial arch (Ad). DiI-labeled cells in the trigeminal ganglia are Pax3-positive, indicating that they are derived from the trigeminal placode (Ae,Af). DiI-labeled cells can be seen in the trigeminal placode when the non-neural ectoderm is labeled at 4 somites (B,C). Chicken embryo at 4 somites (Ba) and 7 somites (Bb-f). DiI can be seen in cells that are Snail2-negative (arrowheads in Bd). These cells on the surface ectoderm are Pax3-positive (arrowheads in Bf). (C) Dil-labeled chicken embryo (13 somites), showing that the Pax3-expressing trigeminal placode (Ca) is labeled with DiI (Cb).

Movie 1. Time-lapse movie of delaminating cells in the mesencephalon labeled with DiI. Chicken embryos were labeled with DiI at 4somites and imaged every 3 minutes. Cells move medially before undergoing EMT and migrating away. Anterior is to the right. The dotted line represents the midline and the line represents regions of the neural tube that have not fused.