Supplementary information

Basolateral localization of MMP14 drives apicobasal polarity change during EMT independently of its catalytic activity

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Supplementary Figures

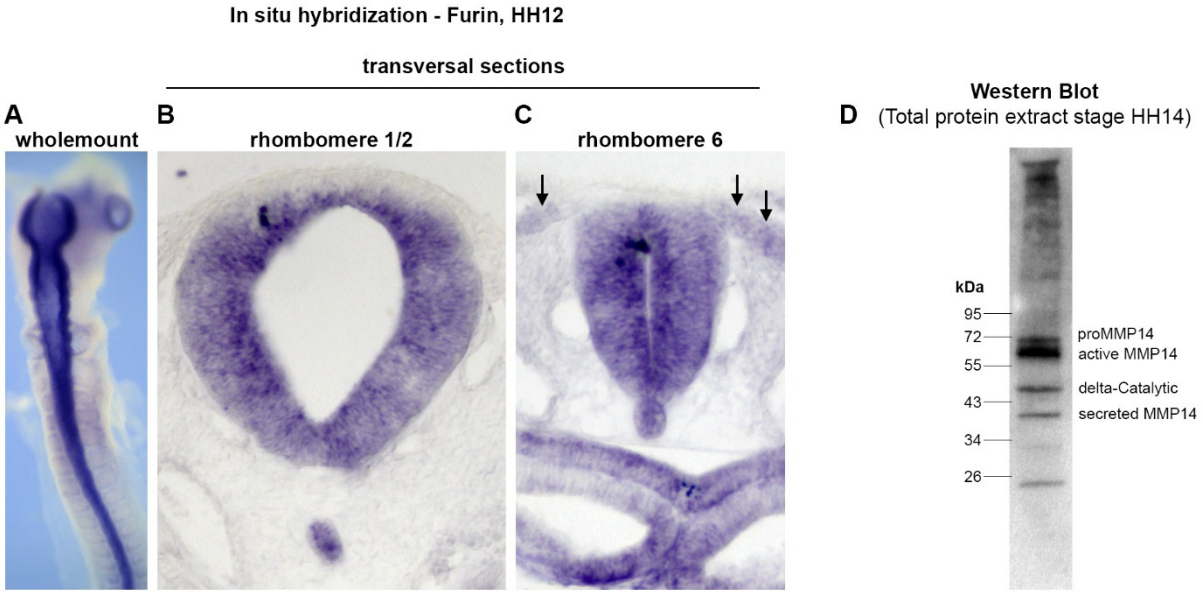

Figure S1 (related to Figure 1). Furin expression and MMP14 processing

A-C. In situ hybridization for chicken Furin on a stage HH12 embryo: whole mount (A), transversal cryosections through rhombomere 1/2 (B) and 6 (C). Expression is seen in the entire neural tube including the NC domain and expression is maintained in migratory NC cells from r6 (C, arrows). D, western blot against chicken MMP14. Bands corresponding to the full length and activated forms are clearly detected just below the 72kDa marker. Two lower bands at around 45 and 38 kDa likely correspond to the forms generated by autocatalysis (delta-catalytic) and shedding of the extracellular domain (secreted MMP14). The last band underneath the 26kDa marker may correspond to a cleavage fragment of the N-terminal portion of the protein.

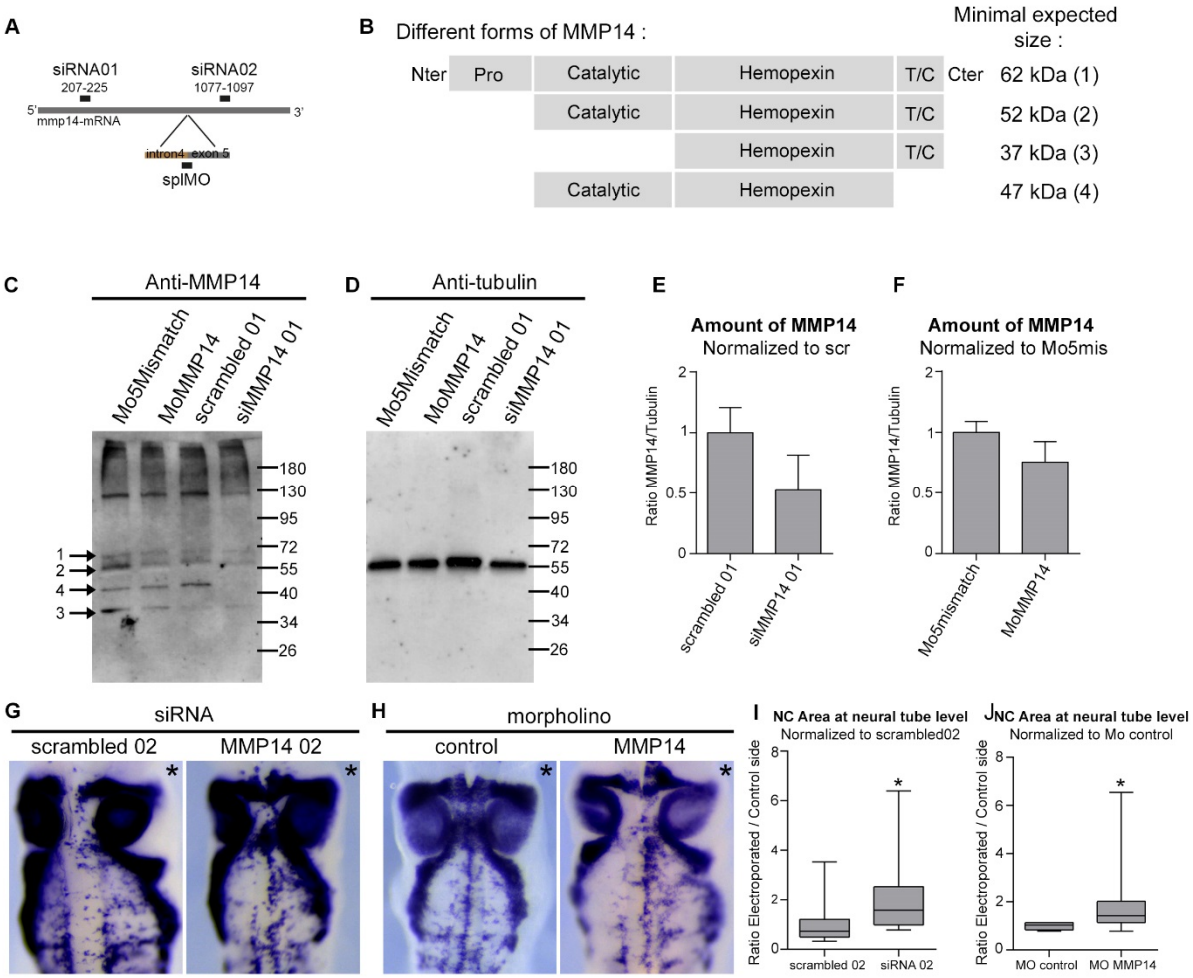

Figure S2 (related to Figure 2). Controls for MO and siRNA efficiency

A, diagram showing the position of the antisplicing Morpholino (splMO) and the two siRNA designed against chick MMP14. **B**, diagram depicting the expected minimal size of activated and processed MMP14. **C**, western blots for MMP14 from total neural tube extracts of embryos electroporated on one side with the antisplicing MO, its cognate control, the siRNA01 and its scrambled control at stage HH12 and harvested 24 hours later. **D**, western blots for Tubulin from the samples shown in B. **E-F**, relative amount of MMP14 to Tubulin in each condition normalized the control sample. Western blots were performed twice from independent protein samples. Note that since neural tubes were electroporated on one side only the maximum knockdown efficiency corresponds to a 50% drop of the amount of MMP14. **G-H**, in situ hybridization for Foxd3/Sox10 on embryos electroporated by siRNA-02 and its scrambled control (F) and MMP14-MO and its control (G). **I-J**, ratio of NC area above the neural tube on the electroporated and the control side and normalized to the control vector (si/scrambled; MO/control). For si/scr, $n_{scrambled}$=13 embryos, $n_{siRNA\text{-}MMP14}$=7 embryos from 2 experiments, Mann-Whitney test, *, p=0.0145. For ctlMO/MO, $n_{controlMO}$=5 embryos from one experiment, $n_{MOMMP14}$=25 from 5 experiments, Mann-Whitney test, *, p=0.0108.

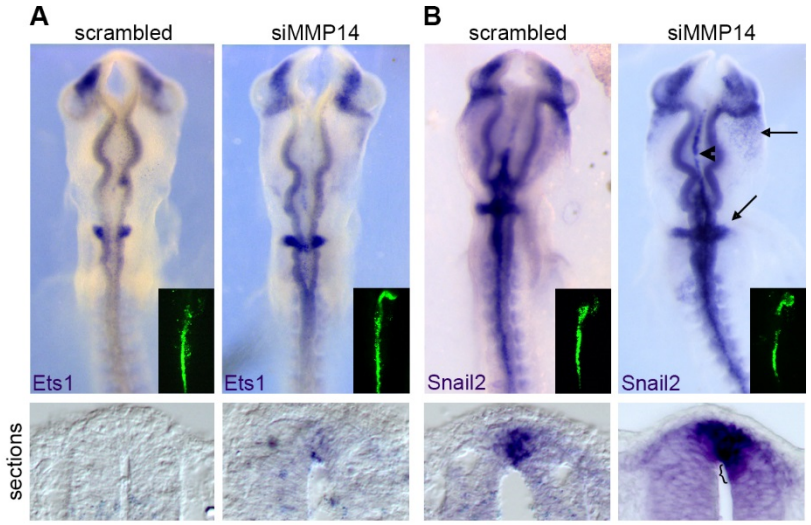

Figure S3 (related to Figure 3). MMP14 is not required for maintenance of Ets1 and Snail2 expressions.

A, in situ hybridization for Ets1 in embryos electroporated with scrambled and siRNA for MMP14. **B**, in situ hybridization for Snail2 in embryos electroporated with scrambled and siRNA for MMP14. Arrowhead indicate regions where NC cells are retained in the dorsal neural tube. Arrows indicate delayed migration compared to the control side. Bracket indicates the expansion of Snail2 domain in siMMP14.

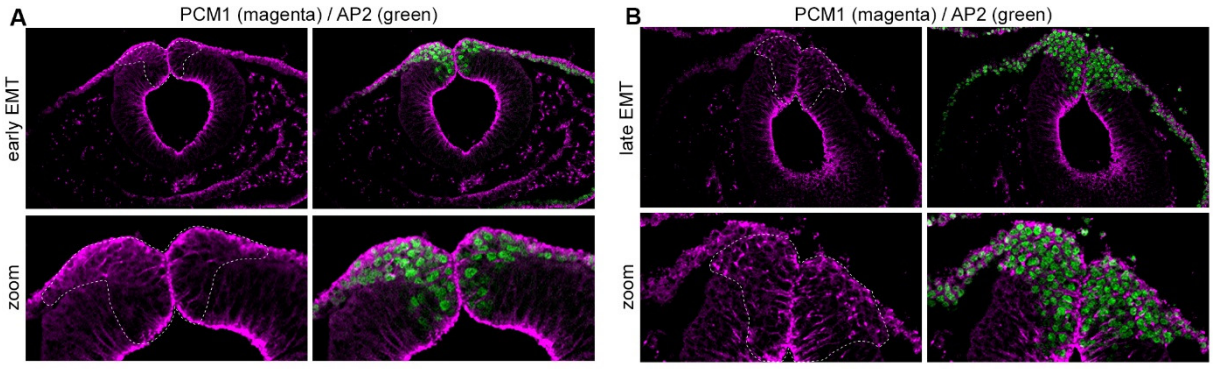

Figure S4 (related to Figure 5). Subcellular localizations of PCM1 changes during EMT of cephalic NC cells.

A-B, immunostaining against PCM1 and TFAP2α at early EMT stage (A) and late EMT stage (B) on transversal cryosections through the mesencephalon of a stage HH8 (A) and HH9 (B) embryos.

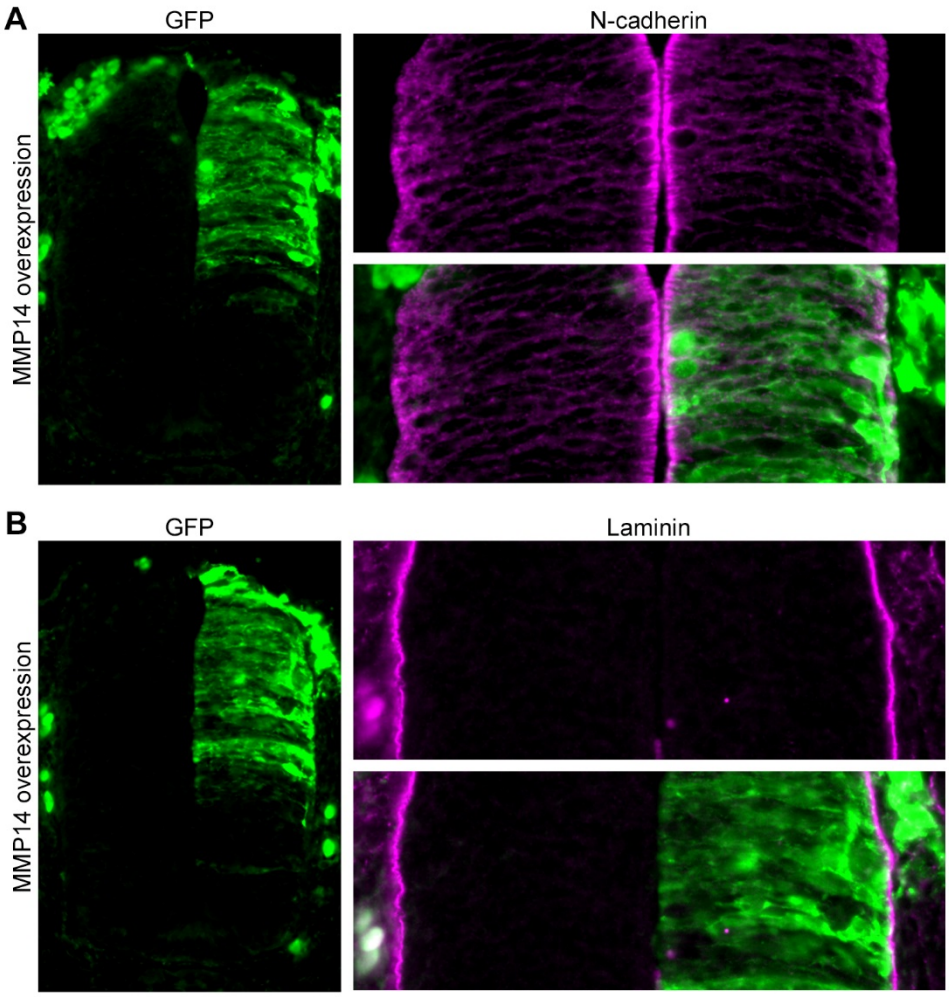

Figure S5 (related to Figure 6). MMP14 overexpression is not sufficient to induce EMT-like phenotypes in the neuroepithelium

A, immunostaining on cryosections against N-cadherin (magenta) after MMP14 overexpression (GFP). B, immunostaining on cryosections against Laminin (magenta) after MMP14 overexpression (green). Note that N-cadherin distribution is normal with a strong accumulation in the apical domain and that laminin is continuous along the basal domain.