Supplemental Materials

Molecular Biology of the Cell

Schiffmacher et al.

SUPPLEMENTAL FIGURE LEGENDS

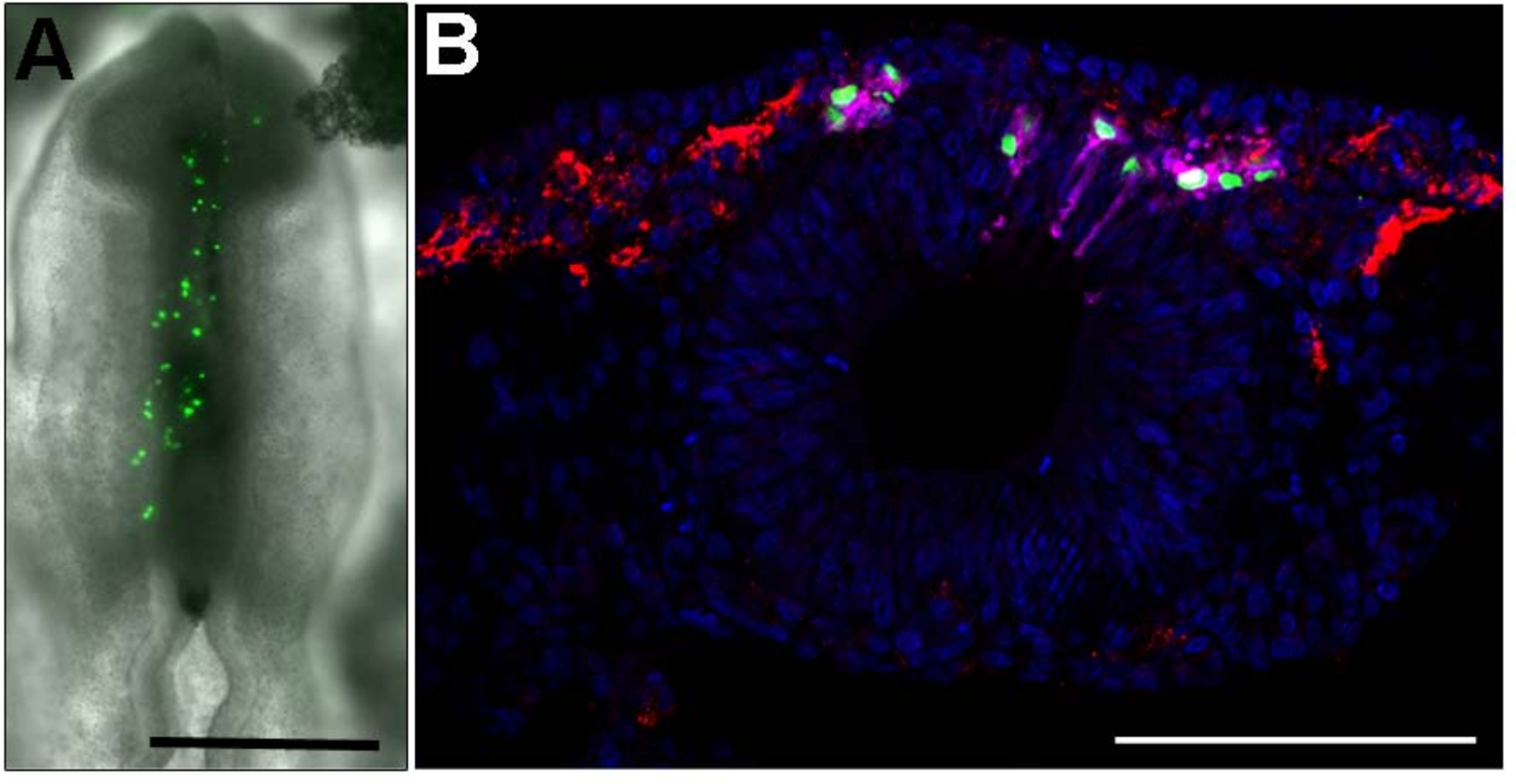
Supplemental Figure 1. Electroporation of trace amounts of C-terminal HA-tagged Cad6B (pCIG.Cad6B.HA) into the dorsal neural tube does not affect neural tube morphology or elicit a Cad6B overexpression phenotype. (A) Whole-mount brightfield merge image demonstrating successful dorsal/ventral electroporation (GFP fluorescence, green) of trace amounts of pCIG.Cad6B.HA into the dorsal neural tube of the chick head at the 4ss, followed by re-incubation and imaging at the 8ss. (B) Representative transverse section taken through the same embryo after whole-mount immunohistochemistry for Cad6B:HA (purple) and section immunohistochemistry for HNK-1 (red) and GFP (green). Neither head morphology nor bilateral neural crest cell migration is affected, as observed when Cad6B is overexpressed at higher levels (Coles et al., 2007). Scale bar in (A) and (B) is 500 μm and 100 μm, respectively. DAPI (blue) labels cell nuclei.

Supplemental Figure 2. ADAM10 proteolytically processes N-cadherin *in vitro*. Transient transfection experiments in CHO cells followed by immunoblotting for full length N-cadherin (N-cad). (A) A reduction in N-cad is observed upon co-transfection of N-cad with ADAM10, with no observable difference detected for other ADAMs including ADAM19, or the control pCIG construct. Immunoblot analysis of HA-tagged proteases was performed to ensure adequate expression of proteases. β-Actin was performed as a loading control.

Supplemental Figure 3. Cad6B proteolytic processing is sensitive to ADAM and γ-secretase inhibition. Co-transfection of Cad6B and ADAM10 into CHO cells (which contain endogenous metalloproteinases/sheddases and γ-secretase that act on Cad6B, see lane 3 for background levels of proteolysis), leads to a reduction in FL-Cad6B and the appearance of Cad6B CTFs at levels above background and those observed in the presence of E/A ADAM10. Co-transfection of Cad6B with ADAM10 in the presence of L-685,458, a γ-secretase inhibitor (Shearman *et al.*, 2000), results in higher levels of CTF1 compared to CTF2, which is even further reduced upon inhibitor addition to cells co-

transfected with Cad6B and E/A ADAM10 (compare lanes 5 and 6). No bands are apparent in the absence of any transfected constructs.

Supplemental Figure 4. ADAM control MOs do not affect the size of the Cad6B-positive-premigratory neural crest cell domain. ADAM10 (A-D), ADAM19 (E-H) or an equimolar mixture of ADAM10 + ADAM19 (I-L) MOs were electroporated unilaterally into the developing neural crest cell population. Embryos were re-incubated to the 8-9ss to allow for the onset of neural crest cell EMT and migration and then processed for FoxD3 (B,F,L) and Cad6B whole-mount immunohistochemistry (A,A',E,E',I,I'), followed by transverse sectioning, with merge images shown in (D,H,L). No change in the size of the Cad6B-positive premigratory neural crest cell population is apparent (A',E',I'). Dotted lines demarcate the dorsal neural tube midline and solid lines mark the width of left and right Cad6B-positive-premigratory neural crest cell domains. Extent of MO electroporation is noted in (C,G,K). Scale bar in (A) is 50 μm and applicable to section images (A-L). Scale bar in (A') is 100 μm and is applicable to section images (A',E',I'). DAPI (blue) labels cell nuclei (D,H,L).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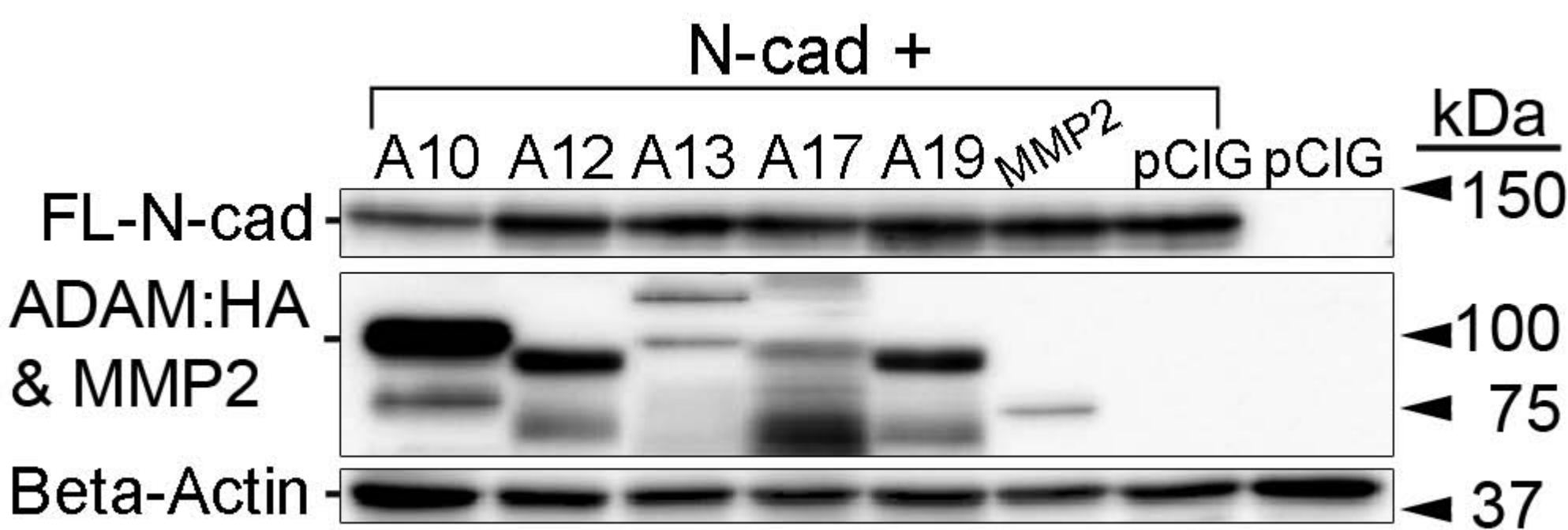
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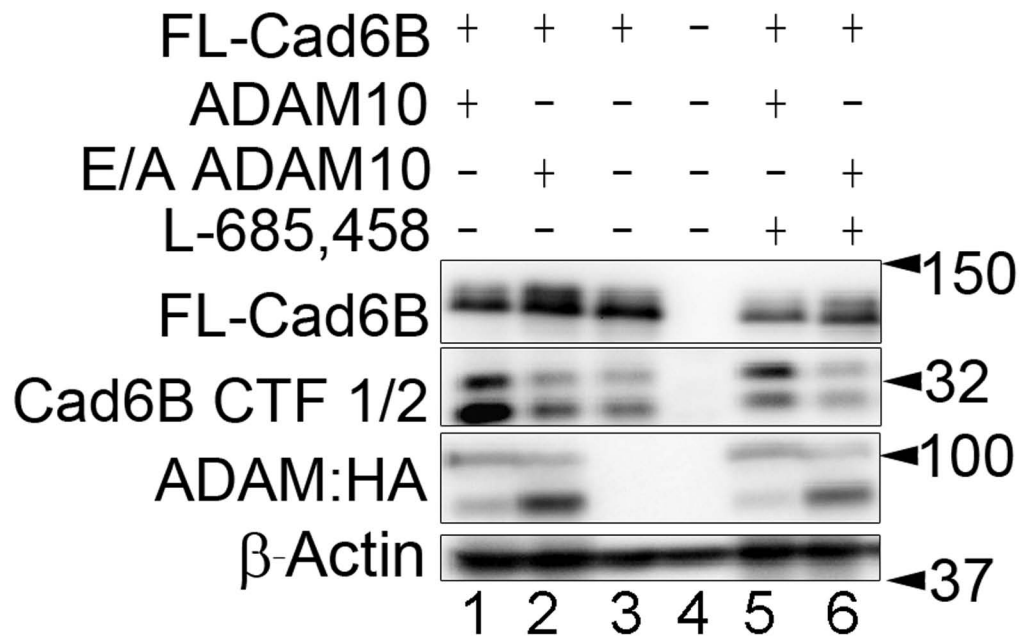
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ADAM10 + − − − + −
E/A ADAM10 − + − − − +
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FL-Cad6B
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ADAM:HA
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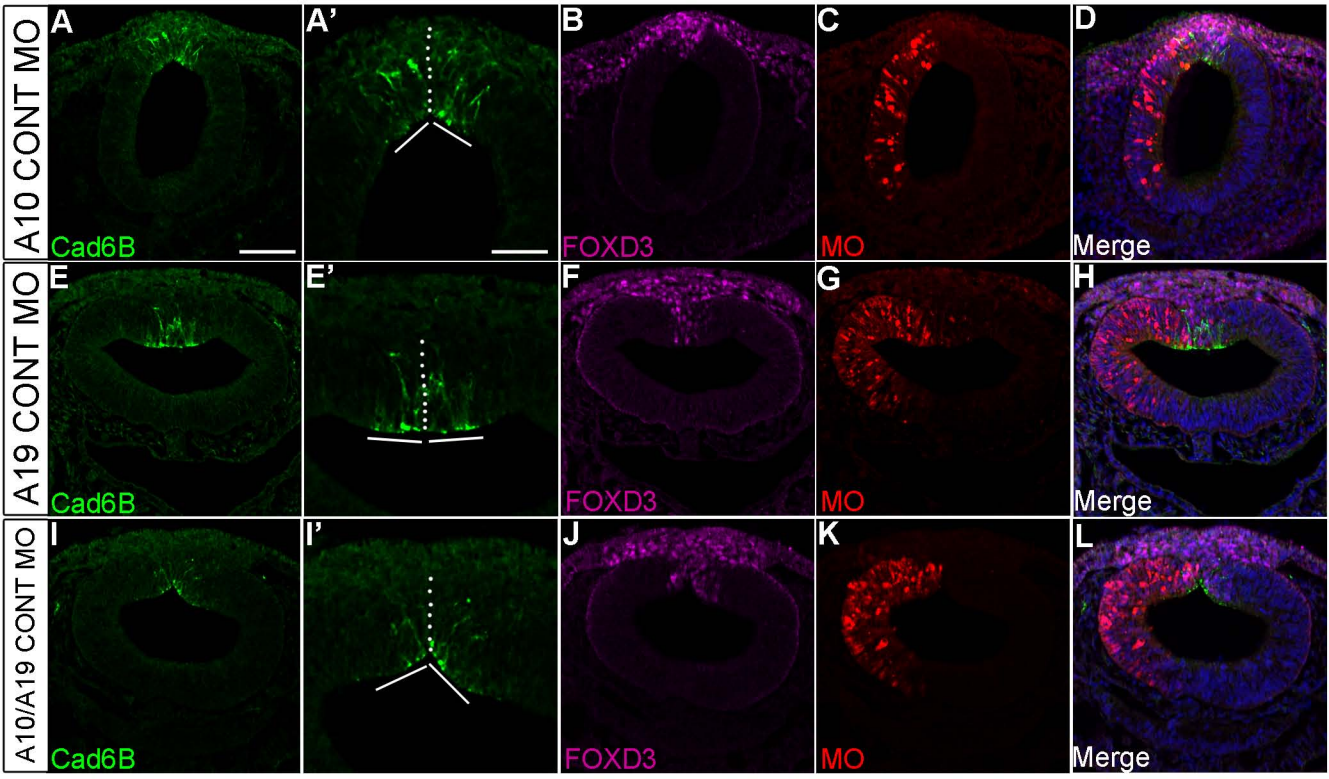
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