Supplemental Figure Legends

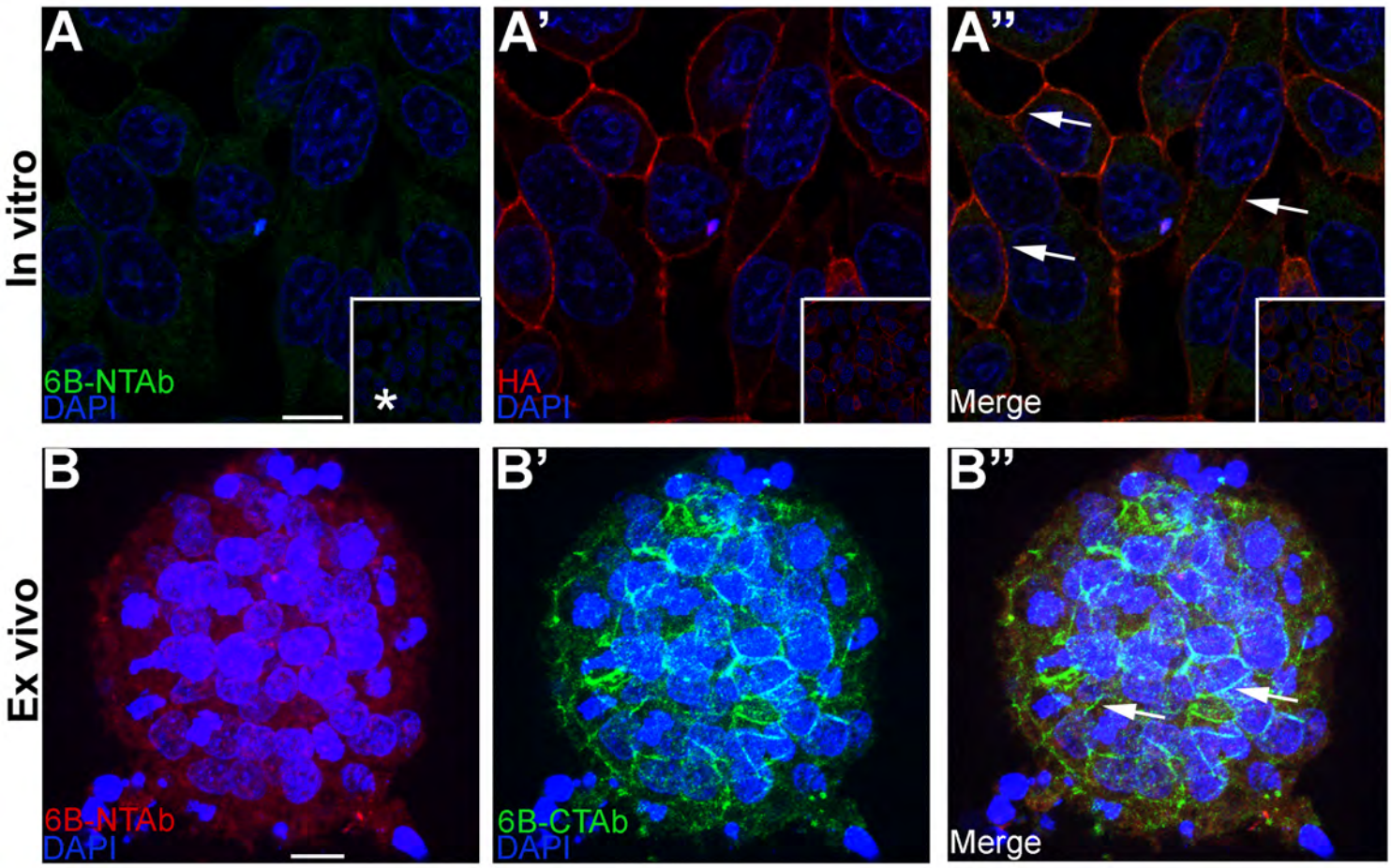
Figure S1: Addition of the Cad6B antibody does not affect Cad6B internalization in feeding assays carried out at 4˚C. The NT-6B antibody was added to the media of FlpIn-wtCad6B cells (A-A") or to cranial dorsal neural fold explants containing premigratory neural crest cells (B-B"), following which time explants were incubated to allow for EMT. In both instances, cells and explants were washed with a low pH buffer, fixed, and immunostained for NT-6B and HA (A') or CT-Ab (B'). Arrows in (A", B") denote membrane-localized Cad6B. Panels represent the 3D composite of several Z-stack images acquired with the confocal. Scale bars: 10μm.

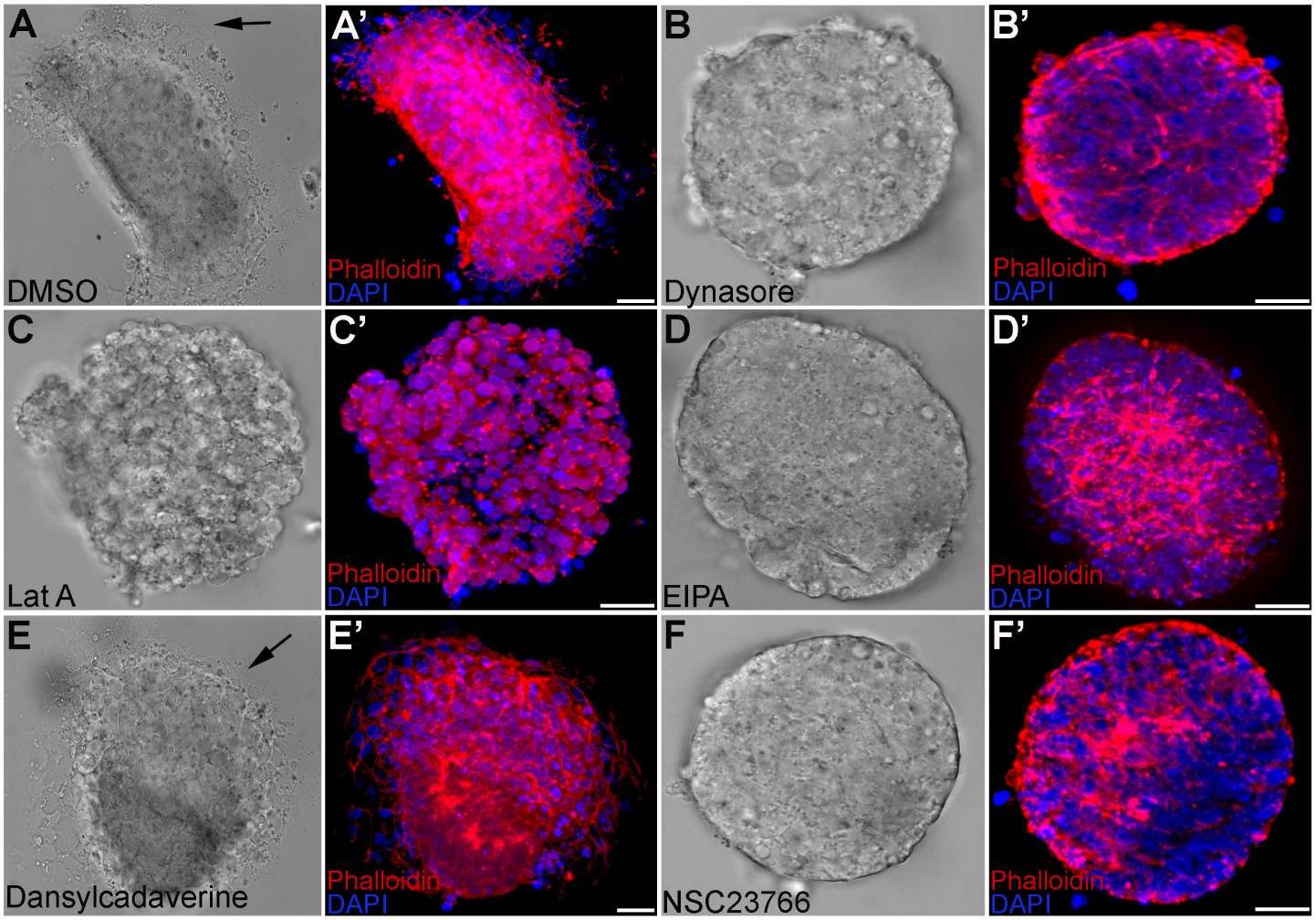
Figure S2: Internalization inhibitors except Dansylcadaverine negatively impact neural crest cell EMT. Dorsal neural folds were explanted into media containing 0.5% (vol/vol) DMSO or appropriate quantities of inhibitors. Explants were then incubated to allow for EMT, fixed, and stained for phalloidin (red). (A - F) and (A'-F') represent brightfield and phalloidin-stained images of the explants, respectively. Immunofluorescence images represent the 3D composite of several Z-stack images acquired with an inverted fluorescence microscope. (G) Quantification of number of Cad6B puncta in explants larger than or equal to 2μm (manually measured in 3D at its greatest extent). The number of explants used for quantification were as follows: DMSO: 8, EIPA: 8, Dynasore: 7, Dansylcadaverine: 6, and NSC23766: 8. Asterisk denotes a statistically significant difference compared to the DMSO-treated control as determined by the Student's t-test. Scale bars: 10μm.

Figure S3: Specific inhibitors of clathrin and Rac1 (targets of Dynasore and EIPA, respectively) recapitulate accumulation of large Cad6B puncta as observed after EIPA and Dynasore treatment. (A, B) Dorsal neural folds were explanted into media containing 100μM Dansylcadaverine and 160μM NSC23766. Explants were then incubated to allow for EMT, fixed, and immunostained for Cad6B (purple). (A', B') are higher magnification views of the boxed region in (A, B), respectively. Panels represent the 3D composite of several Z-stack images acquired with the confocal. Arrows in (A', B') represent large Cad6B puncta. Scale bars: 10μm.

Figure S4: Cad6B is localized as cytoplasmic puncta at various axial levels of the chick embryo. 15ss embryos were fixed and immunostained for Cad6B (green), with representative images of transverse sections from the rostral hindbrain (A), hindbrain-gut (B), and trunk (C) regions shown. Similar to the crania, Cad6B localizes as cytoplasmic puncta in these other axial levels (arrows). Scale bars: 10μm.

Figure S1


A
A'
A''
In vitro
6B-NTAb
DAPI
*
HA
DAPI
Merge
B
B'
B''
Ex vivo
6B-NTAb
DAPI
6B-CTAb
DAPI
Merge


A
DMSO
A'
Phalloidin
DAPI
B
Dynasore
B'
Phalloidin
DAPI
C
Lat A
C'
Phalloidin
DAPI
D
EIPA
D'
Phalloidin
DAPI
E
Dansylcadaverine
E'
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F
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F'
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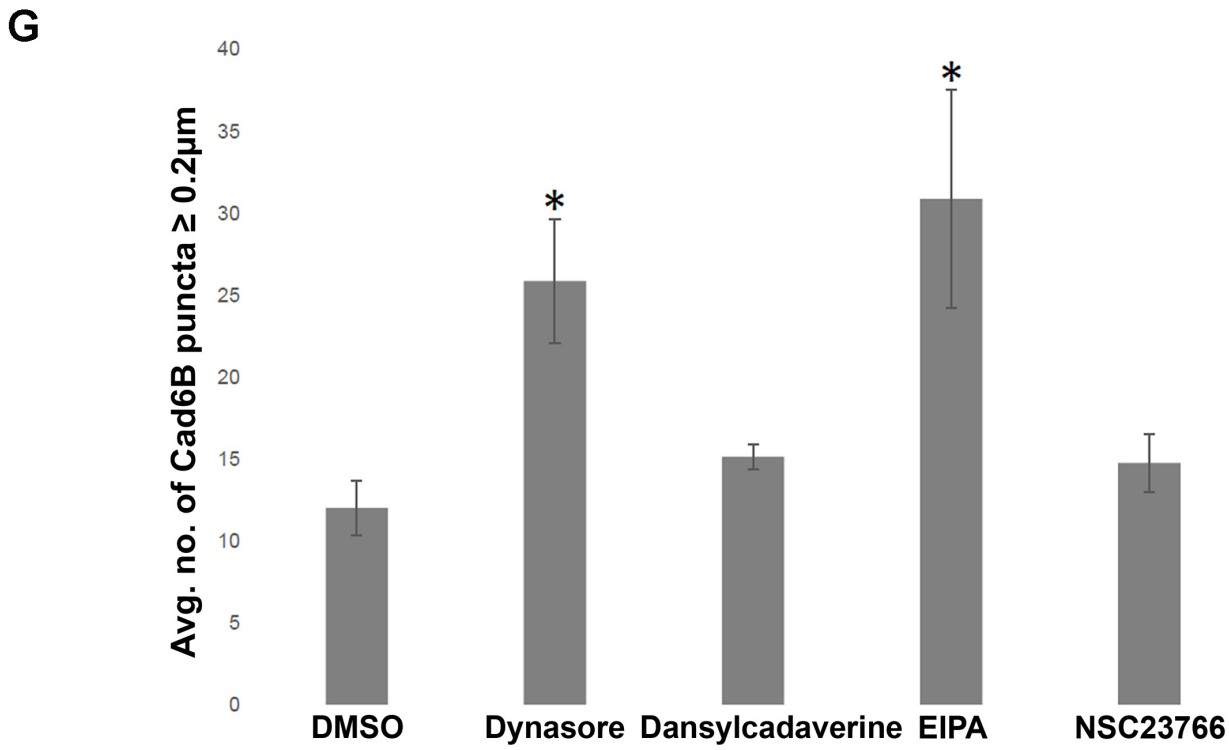

G
Avg. no. of Cad6B puncta ≥ 0.2µm
0
5
10
15
20
25
30
35
40
*
*
DMSO
Dynasore
Dansylcadaverine
EIPA
NSC23766

Figure S3

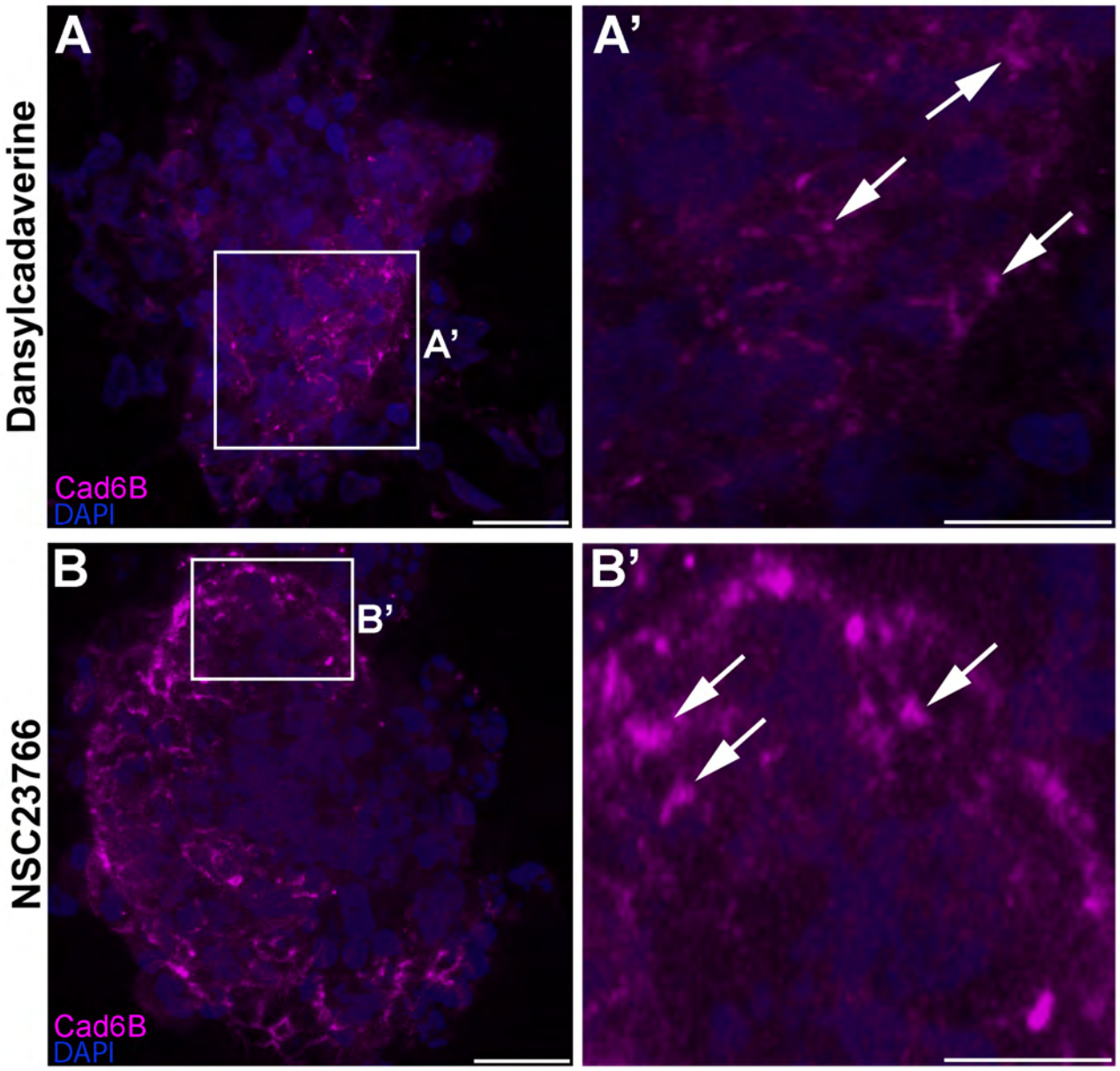
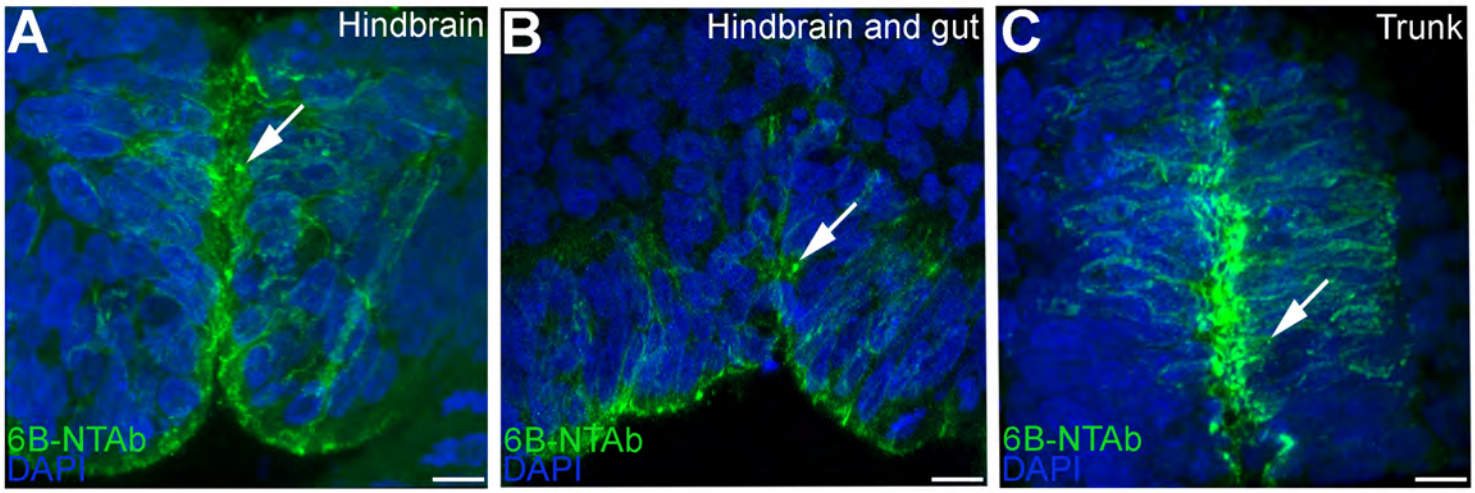


Figure S4


A
Hindbrain
6B-NTAb
DAPI
B
Hindbrain and gut
6B-NTAb
DAPI
C
Trunk
6B-NTAb
DAPI