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Supplementary Information for

p120-catenin Regulates WNT Signaling and EMT in the Mouse Embryo

Rocío Hernández-Martínez, Nitya Ramkumar and Kathryn V. Anderson

Corresponding author: Kathryn V. Anderson

email: k-anderson@ski.mskcc.org

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Other supplementary materials for this manuscript include the following:

Movies S1 to S4

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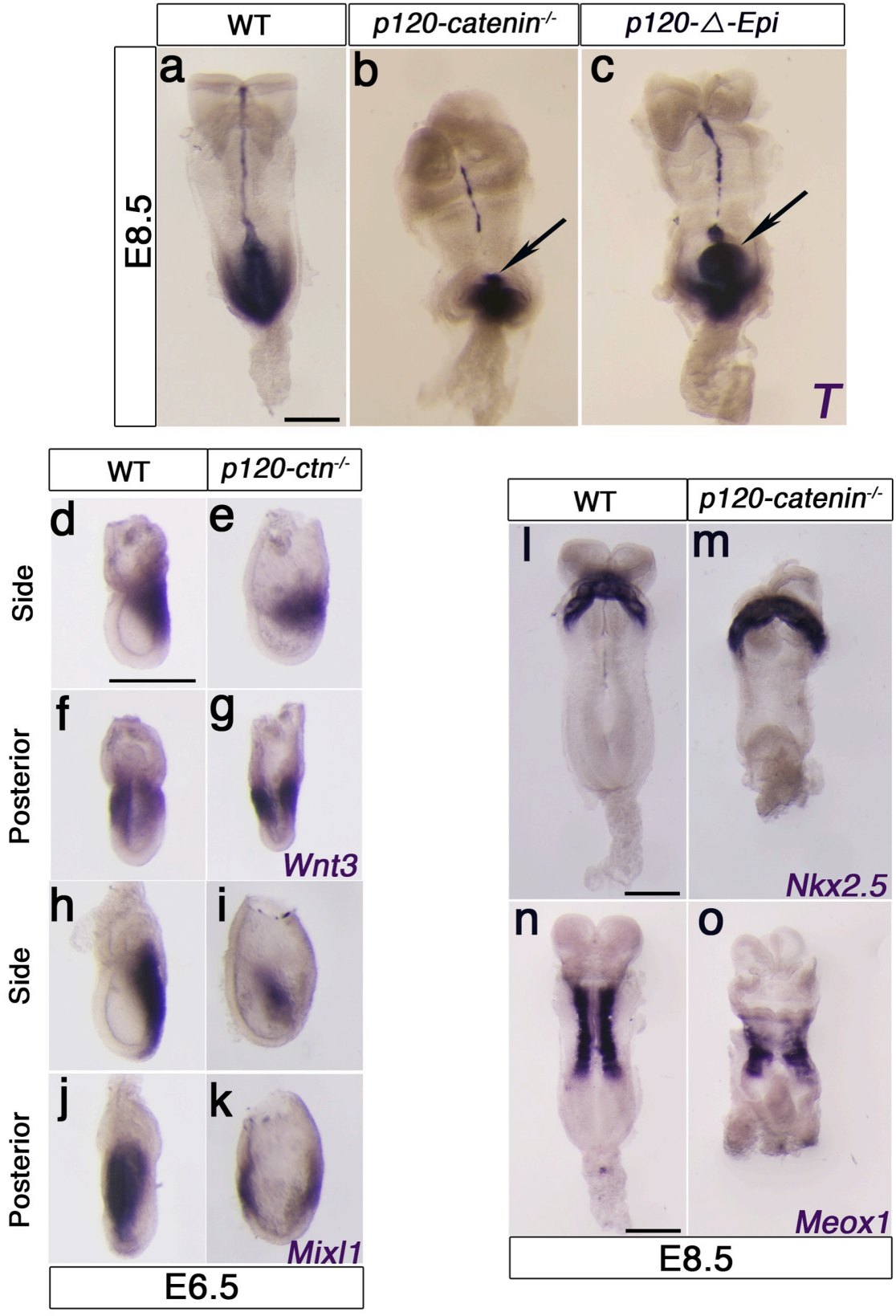
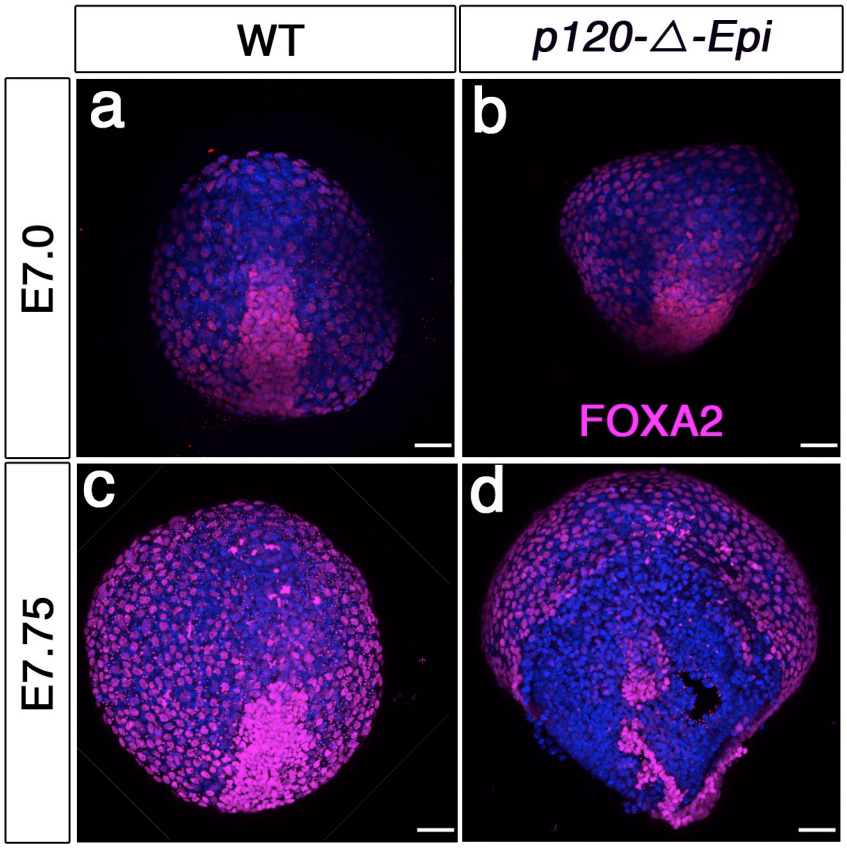
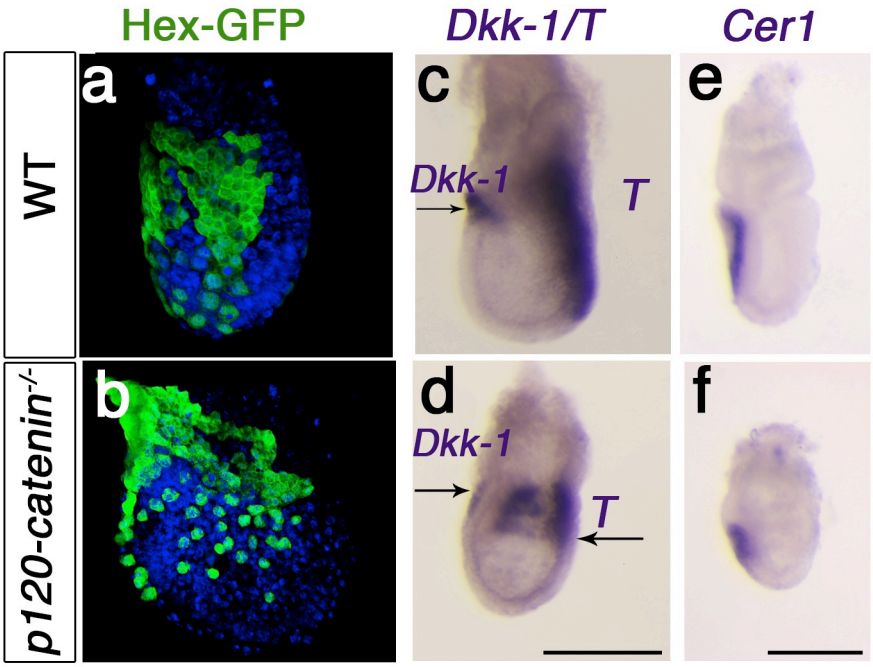

WT
p120-catenin-/-
p120-△-Epi
E8.5
a
b
c
T
WT
p120-ctn-/-
Side
d
e
Posterior
f
g
Wnt3
Side
h
i
Posterior
j
k
Mixl1
E6.5
WT
p120-catenin-/-
l
m
Nkx2.5
n
o
Meox1
E8.5

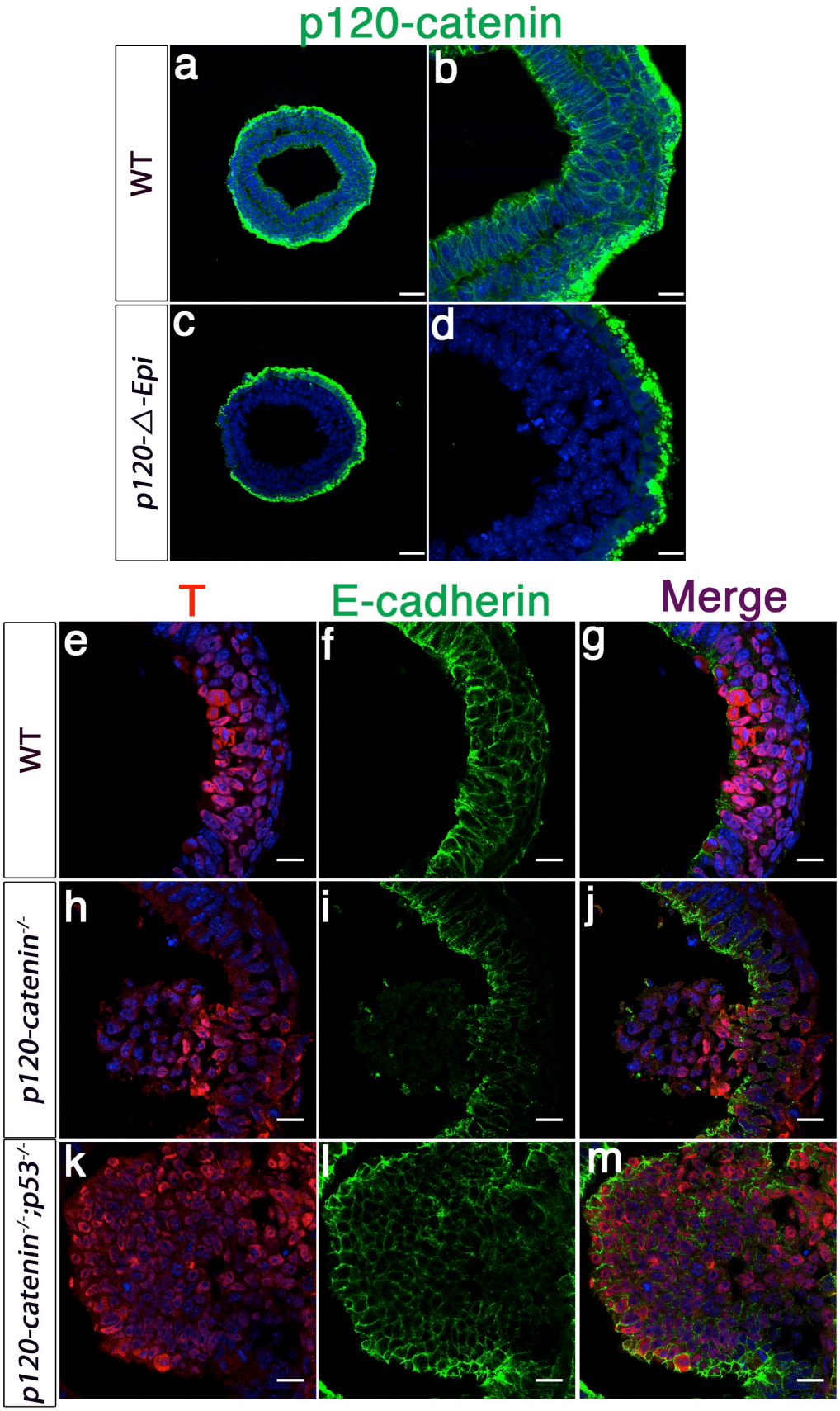
Supplementary Figure 1. The posterior body axis is split in *p120-catenin* mutant embryos. (a-**d***) In situ* hybridization for *T* in E8.5 embryos. (**a**) Wild-type embryos express *T* in the posterior streak and axial midline. (**b**) While ~80% of *p120-catenin* null mutants have a posterior bifurcation of the streak, the remaining null accumulate *T*+ cells in a bulge at the primitive streak (arrow). (**c**) *p120-ΔEpi* mutants accumulate *T*+ cells in a bulge at the primitive streak (arrow). (**d**-**k**) *In situ* hybridization for the primitive streak markers *Wnt3* and *Mixl1* in E6.5 embryos. Side views and the posterior of the embryo are shown. (**d**,**f,h,j**) Expression of *Wnt3* and *Mixl1* is restricted to the posterior of wild-type embryos. (**e**,**g**,**i**,**k**) *Wnt3* and *Mixl1* expression in *p120-catenin* mutant embryos is split into two domains. Scale bars, 50μm. (**l**-**m**) *In situ* hybridization for *Nkx2.5* in E8.5 embryos shows that the cardiac anlage is specified in both (**l**) wild-type and (**m**) the mutant. (**n**-**o**) *In situ* hybridization for the paraxial mesoderm marker *Meox1* in E8.5 embryos. (**n**) Wild-type embryos show segmented paraxial mesoderm. (**o**) *p120-catenin* mutant embryos show a smaller, unsegmented paraxial mesoderm domain.



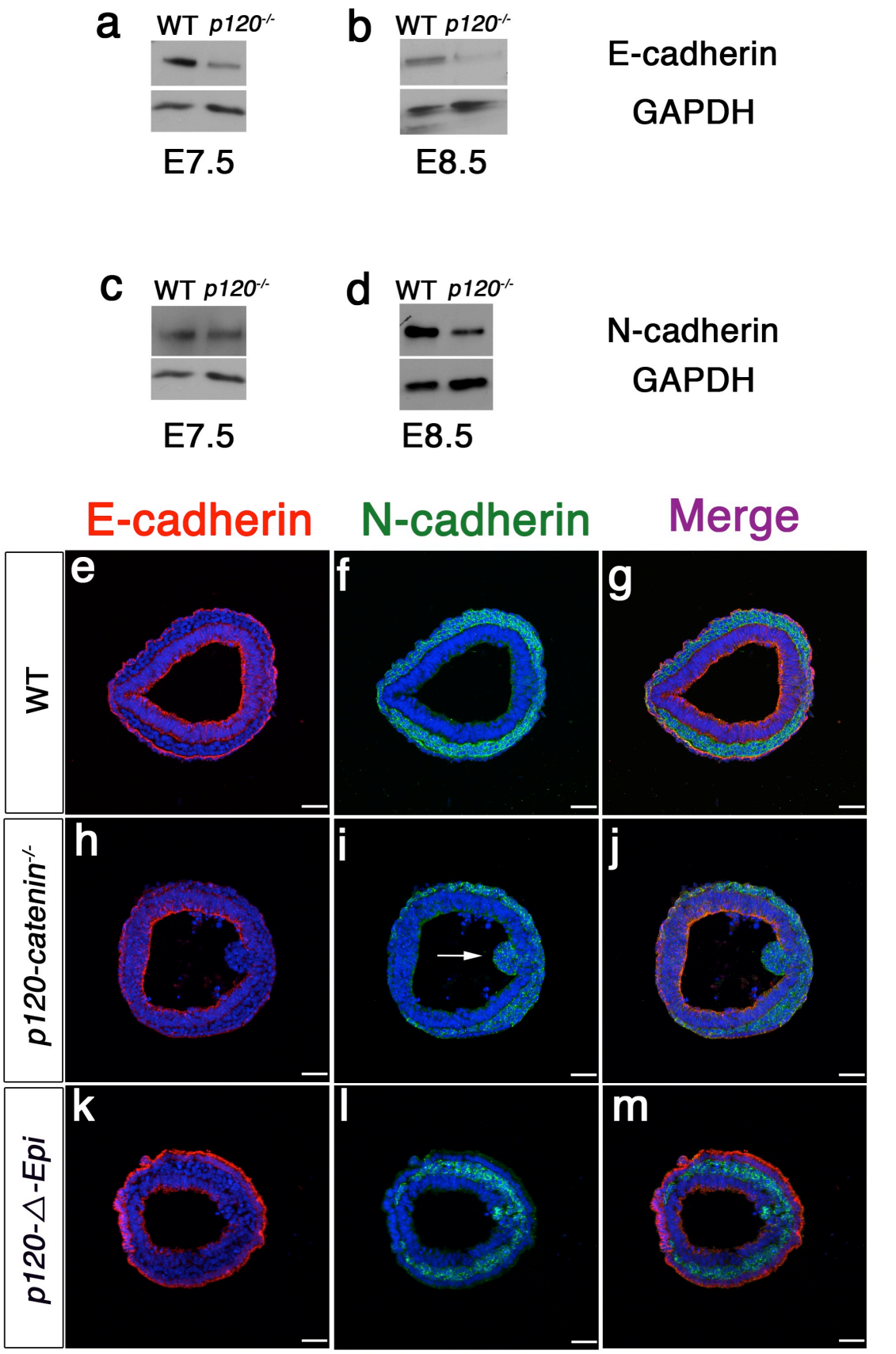
Supplementary Figure 2. *p120-catenin* mutant embryos have a distorted midline and node. (**a**,**b**) Whole-mount immunofluorescence for FOXA2 (magenta) in wild-type and *p120-catenin* mutant embryos; anterior is to the top (**a,c**) Immunofluorescent staining in wild-type embryos for FOXA2, which is express in the definitive endoderm, the midline and the node. (**b,d**) FOXA2 expression reveals a distorted node and discontinuous midline in *p120-ΔEpi* mutant embryos. Scale bar 50μm.



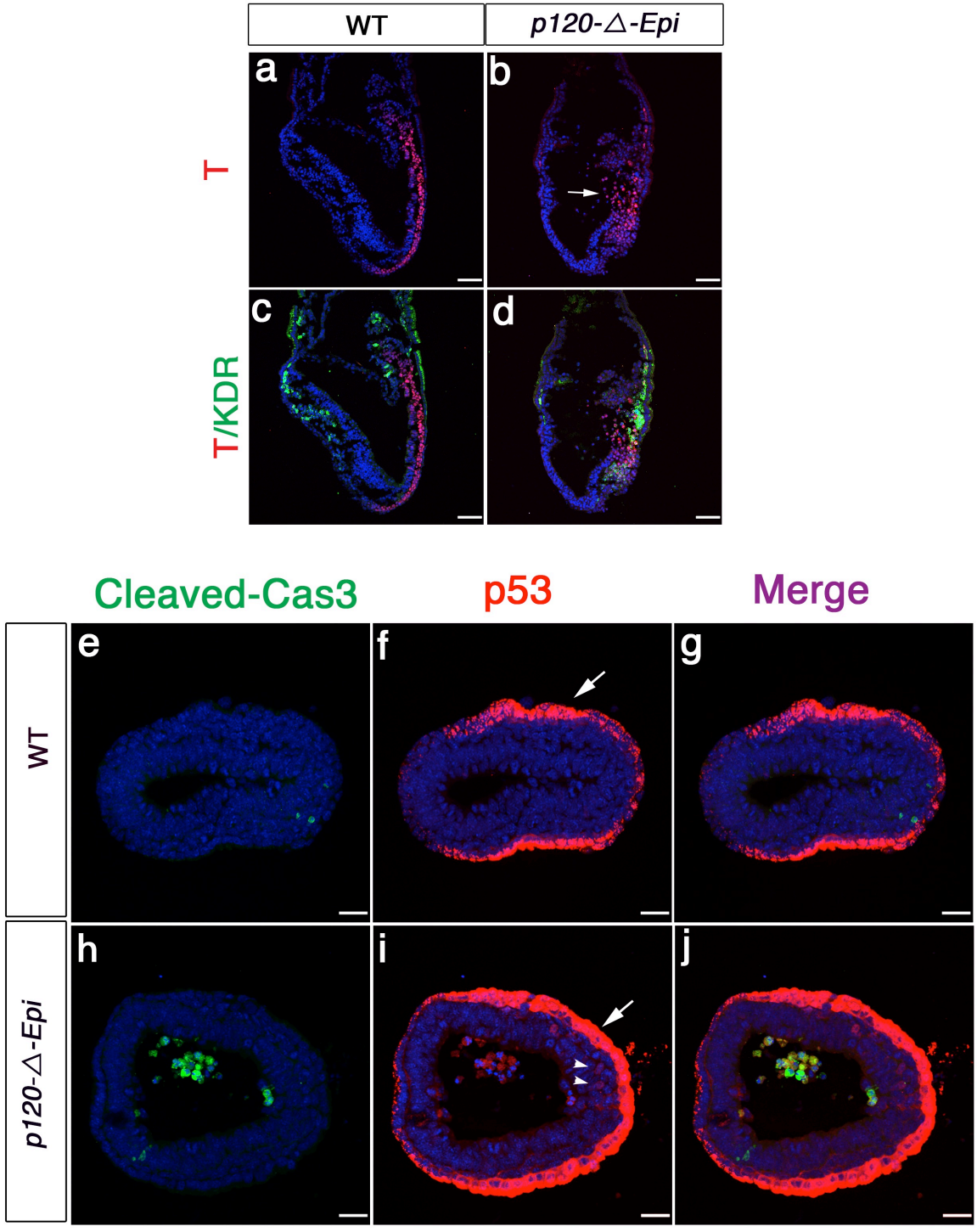
Supplementary Figure 3. *p120-catenin* is not required for anterior visceral endoderm (AVE) migration. (**a**,**b**) 3D reconstruction of confocal Z-stacks of immunofluorescence in E6.5 whole-mount embryos. Expression of Hex-GFP (green) cells in E6.5 embryos at the end of AVE cell migration. Most Hex-GFP cells have reached the embryonic/extraembryonic border in both (**a**) wild-type and (**b**) mutant embryos. (**c**,**d**) Double *in situ* hybridization for *Dkk1* and *T* expression in E6.5 wild-type and *p120-catenin* null embryos. (**c**) *T* is expressed in the posterior and *Dkk1* in the anterior of the wild-type embryo. (**d**) *Dkk1* is restricted to the anterior, whereas *T* is split into two-domains, in the *p120-catenin* mutant embryo. (**e**,**f**) *Cerberus1* (*Cer1*) is expressed at E6.5 in the anterior visceral endoderm in both E6.5 (**e**) wild-type and (**f**) *p120-catenin* null embryos. Scale bar 50μm.


p120-catenin
WT
p120-Δ-Epi
a
b
c
d
T
E-cadherin
Merge
e
f
g
h
i
j
k
l
m
p120-catenin-/-
p120-catenin-/-;p53-/-

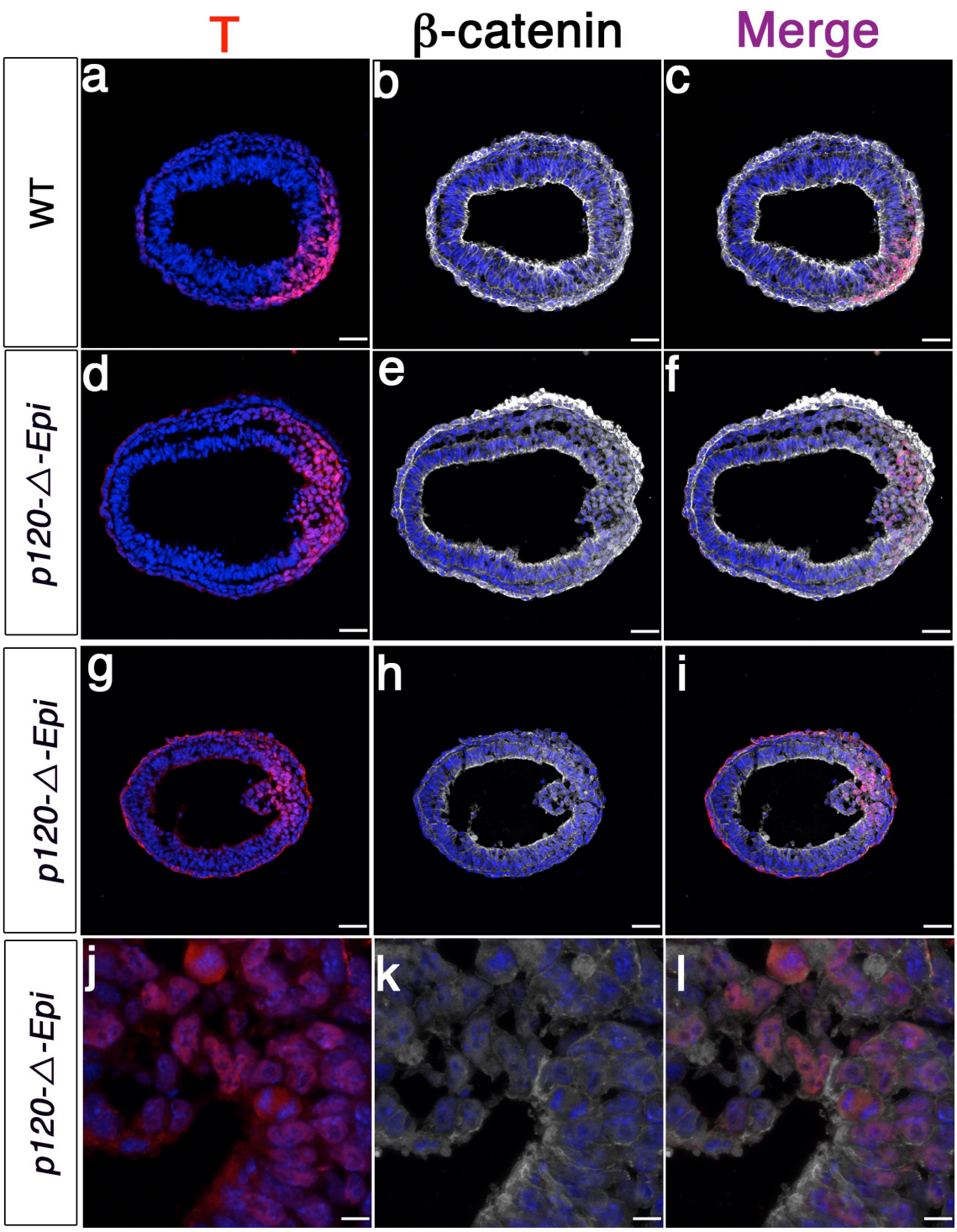
Supplementary Figure 4. p120-catenin is uniformly express in wild-type embryos and optical sections illustrating the quantitation of T+ at the streak. (**a**,**c**) Immunofluorescence on transverse sections of E7.5 for p120-catenin (green) in (**a,b**) wild-type and (**c,d**) *p120-ΔEpi* mutant embryos; posterior to the right. Observe that p120-catenin is expressed in the visceral endoderm. (**a**,**c**) Scale bar 50μm. (**b,d**) Higher magnification views of p120-catenin expression in the primitive streak region of E7.5 (**b**) wild-type and (**d**)*p120-catenin* mutant embryos. Scale bar 16μm. (**e**-**m**) Cryosections of E7.5 embryos stained for T (red) and E-cadherin (green). Each section represents a single optical section taken from a Z-stack. (**e**-**g**) wild-type embryo, (**h**-**j**) *p120-catenin* mutant embryo and (**k**-**m**) *p120-catenin;p53* double mutant embryo. Only cells positive for both T and E-cadherin were consider as part of the streak. Scale bar, 16μm.


a
WT p120-/-
E7.5
b
WT p120-/-
E8.5
E-cadherin
GAPDH
c
WT p120-/-
E7.5
d
WT p120-/-
E8.5
N-cadherin
GAPDH
E-cadherin
N-cadherin
Merge
WT
p120-catenin-/-
p120-△-Epi
e
f
g
h
i
j
k
l
m

Supplementary Figure 5. p120-catenin regulates cadherin levels in gastrulating mouse embryos. (**a**,**b**,**c,d**). Western blots of E7.5 and E8.5 wild-type and *p120* mutant embryo extracts. (**a**) Wild-type embryos have an average of 2.4-fold more E-cadherin than mutant embryos at E7.5, normalized to the loading control (n=4; range 1.3-4.0). (**b**) At E8.5, wild-type embryos have ~1.7 fold more E-cadherin than mutants (n= 3; range 1.2-1.8). N-cadherin protein is reduced in mutant embryos at both (**c**) E7.5 and (**d**) E8.5. At E8.5 wild-type embryos have ~3.5-fold more N-cadherin than in mutants (n=3; range 3.3-4.5). At E7.5, N-cadherin levels were variable from embryo to embryo, reflecting the dynamics of development, as nascent mesodermal cells are generated at this stage in wild type. (**e**-**m**) Transverse sections of E7.5 wild-type and *p120-catenin* mutant embryos stained for E-cadherin (red) and N-cadherin (green), primitive streak to the right. (**i**) Example of a mutant embryo with a cluster of N-cadherin+ cells protruding into the amniotic cavity (arrow). Scale bars, 50μm.

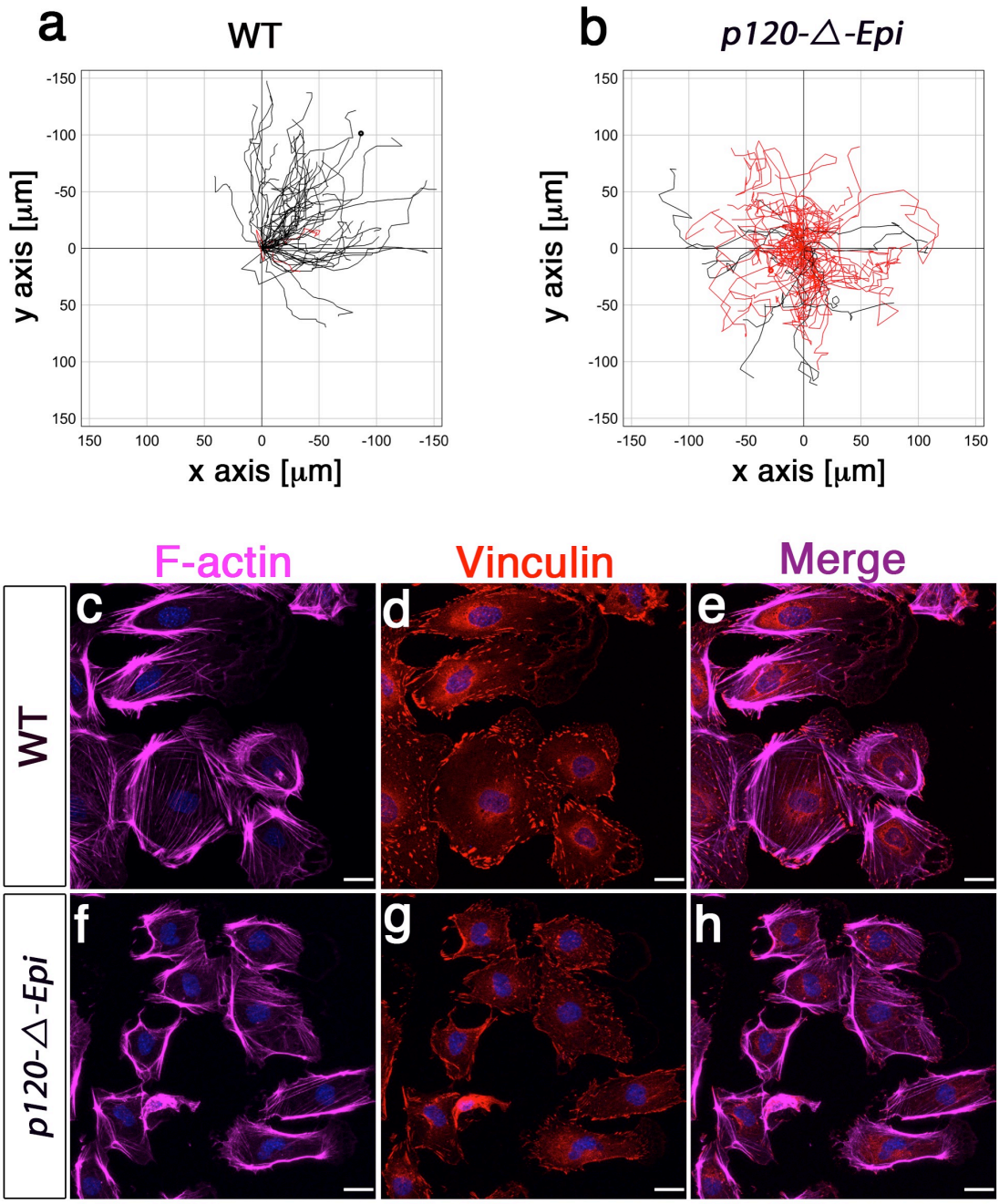

WT
p120-△-Epi
a
b
T
c
d
T/KDR
Cleaved-Cas3
p53
Merge
WT
e
f
g
p120-△-Epi
h
i
j

Supplementary Figure 6. p120-catenin restricts primitive streak size and limits apoptosis during the EMT. (**a**,**b**) Immunostaining for T in E7.5 longitudinal sections. (**a**) Wild-type embryos show T in the posterior whereas (**b**) in the mutant epiblast, some T+ cells have apparently detached and been extruded into the amniotic cavity (arrow). (**c**,**d**) Immunofluorescent staining for T (red) and KDR (green) in (**c**) wild-type and (**d**) *p120-catenin* mutant embryos. KDR is expressed in the extra-embryonic mesoderm and cells of the anterior mesoderm of the wild-type embryo. T marks the posterior of the wild-type embryo. In contrast. KDR+ cells in *p120-catenin* mutants do not migrate properly and remain at the primitive streak. Scale bars, 50μm. (**e**-**j**) Sections of E7.0 embryos stained for Cleaved Caspase-3 (green), and p53 (red). (**e**-**g**) Dead cells are rare in wild-type embryos and no p53 signal is detected. (**h**) *p120-catenin* mutant embryos have apoptotic bodies in the amniotic cavity. (**i**) Some cells in the posterior epiblast of mutants express p53 (arrowheads). (**f**,**i**) Arrows point to non-specific staining for p53 in the visceral endoderm. Scale bar, 24μm.



Supplementary Figure 7. p120-catenin restricts the domain of WNT response in the posterior epiblast. (**a**-**f**) Lower magnification views of the embryos shown in Figure 4**i**-**t** and an additional section of a *p120-ΔEpi* mutant embryo. Transverse sections of wild-type and mutant embryos stained for β-catenin (white) and T (red). (**a**) T is expressed in the streak and

nascent mesoderm of wild-type embryos. (**b**) In wild-type embryos β-catenin is expressed at the junctions. (**d**,**g,j**) The T domain is expanded in the epiblast layer of *p120-catenin* mutant embryos. (**e**,**h,k**) β-catenin relocalizes from the membrane to the nuclei of streak cells in *p120-catenin* mutant embryos. (**j**-**l**) Images are a higher magnification of the embryo streak shown in (**g**-**i**). (**a-i**) Scale bar is 50μm; the scale bar in (**j**-**l**) is 8μm.



Supplementary Figure 8. (**a**-**b**) Tracks of the individual wild-type (left) and mutant (right) mesoderm migrating cells from live imaging of explants (See Videos 1-4). The x and y axes represent the geographic coordinates of cell migration on the slide relative to the starting point of the cell. Black lines indicate cells migrating with persistence above of 0.5, whereas cells migrating with directionality below of 0.5 (i.e. change direction between frames more than half the time) are in red. (**c**-**h**) Mesoderm explants stained for F-Actin (magenta) and Vinculin (red). (**c**-**e**) Wild-type mesoderm explants, (**f**-**h)** mutant mesoderm explants.

Video legends

Video 1. Wild-type mesoderm explants cells migrate collectively. Mesoderm cells expressing membrane GFP (55) from a wild-type embryo plated on coverglass chambers and incubated for 12 hours at 37°C before imaging. Movies comprise images taken every 15 minutes during 7-hour period. Videos are displayed at 5 frames per second. Each color track represents the migration trajectory of each cell during the time-lapse. Tracks from five cells were traced using Manual track macro from Fiji.

Video 2. Wild-type mesoderm explants cells migrate collectively. Video of a wild-type mesoderm explant from a second embryo expressing membrane GFP (55). Imaging as for Video 1. Tracks from four cells were traced using Manual track macro from Fiji.

Video 3. *p120-catenin* mutant mesoderm cells migrate as single cells and lack direction. See Video1 legend for imaging details. The video includes four tracked cells from this mutant explant

Video 4. *p120-catenin* mutant mesoderm cells migrate as single cells and lack direction. See Video1 legend for imaging details. The two video includes four tracked cells from an explant obtained from a second mutant embryo.