Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figures

FIGURE S1: Expression of Borg5 in postnatal capillary ECs. Frozen sections from postnatal day 5 mouse brain (A) or heart (B) were co-stained with Borg5 rabbit polyclonal antibodies and PECAM-1 antibody. Nuclei in the merged images were labeled with DAPI. Scale bar, 100 μm.

FIGURE S2: Borg5-null mice exhibit reduced body weight. Measurements of wild-type and Borg5-null mice body weight at E18.5 (A) and P0 (B). Dead pups at birth were excluded from the measurement. (C and D) Growth curve of male (C) and female (D) wild-type and Borg5-null mice from 4 to 12 weeks of age. n>= 6. Error bars, standard error of the mean (SEM). Statistical analysis by two-tailed Student's t-test, *p<0.05, **p<0.01.

FIGURE S3: (A and B) Proliferation of Borg5-null MCECs was similar to wild-type MCECs as quantified by EdU (red) incorporation. Nuclei were labeled with DAPI. Scale bar, 100 μm. (C) A method to plate primary MCECs on glass coverslips. Drops of cells are loaded on the inside surface of the lid from a Petri dish (bacteria grade). Gelatin-coated 12-mm round glass coverslips are then inverted on the cells. Next, the lid is carefully placed back on the Petri dish, which contained 5-10 ml PBS to prevent cells from drying. Cells attached onto the coverslips after overnight incubation are fixed for immunofluorescence staining or continually cultured in 24-well plate for other manipulations. (D) Immunofluorescence staining of VE-cadherin in wild-type and Borg-null MCECs. Both cells formed adherens junctions. Regions in the white dashed squares were enlarged to the right of each image for better visualization of VE-cadherin staining. Scale bars, 100 μm. (E) Immunofluorescence staining of centrosomes (pericentrin, red), Golgi (GM130, green), and nuclei (DAPI, blue) in wild-type and Borg-null MCECs 1.5 h after scratch wound. Scale bar, 50 μm. (F) An illustration of the centrosome angle quantification. (G and H) Histogram of centrosome angles in wild-type (G) and Borg5-null cells (H) at 0-6 h post scratching. No defect in centrosome reorientation was detected in Borg5-null cells.

FIGURE S4: (A) Confocal Z-sections showing co-localization of Borg5 with Sept7 above the nuclei. Scale bar, 25 μm. (B) Quantification of Borg5-Sept7 co-localization by Pearson's coefficient in 3D reconstructed images. Borg5-null cells were included to control for non-specific background staining by Borg5 antibody. (C) Western blotting analysis of MCECs treated with control or Sept7 siRNA oligos. Loading controls, tubulin. (D) Confocal images showing a Sept7-depleted cell in comparison to a cell treated with control siRNA. Scale bar, 25 μm. (E) Quantification of migration speed (path/time) of wild-type and Borg5-null MCECs using a scratch wound-healing migration assay. Error bars, SEM. Statistical analysis was done by two-tailed Student's t-test.

FIGURE S5: Confocal Z-sections showing F-actin and Sept7 in NIH3T3, C3H10T1/2, NMuMG, immortalized MCECs, and HUVECs. Primary MCECs were included for comparison. Note that NIH3T3, C3H10T1/2, and immortalized MCECs contain organized F-actin/Septin filaments underneath but not above the nuclei, while in primary MCECs, prominent parallel F-actin/Septin filaments can be found right above the nuclei. We did not examine Borg5 localization in HUVECs because our antibodies, which were generated against mouse Borg5, poorly recognized the human homolog.

Figure S1

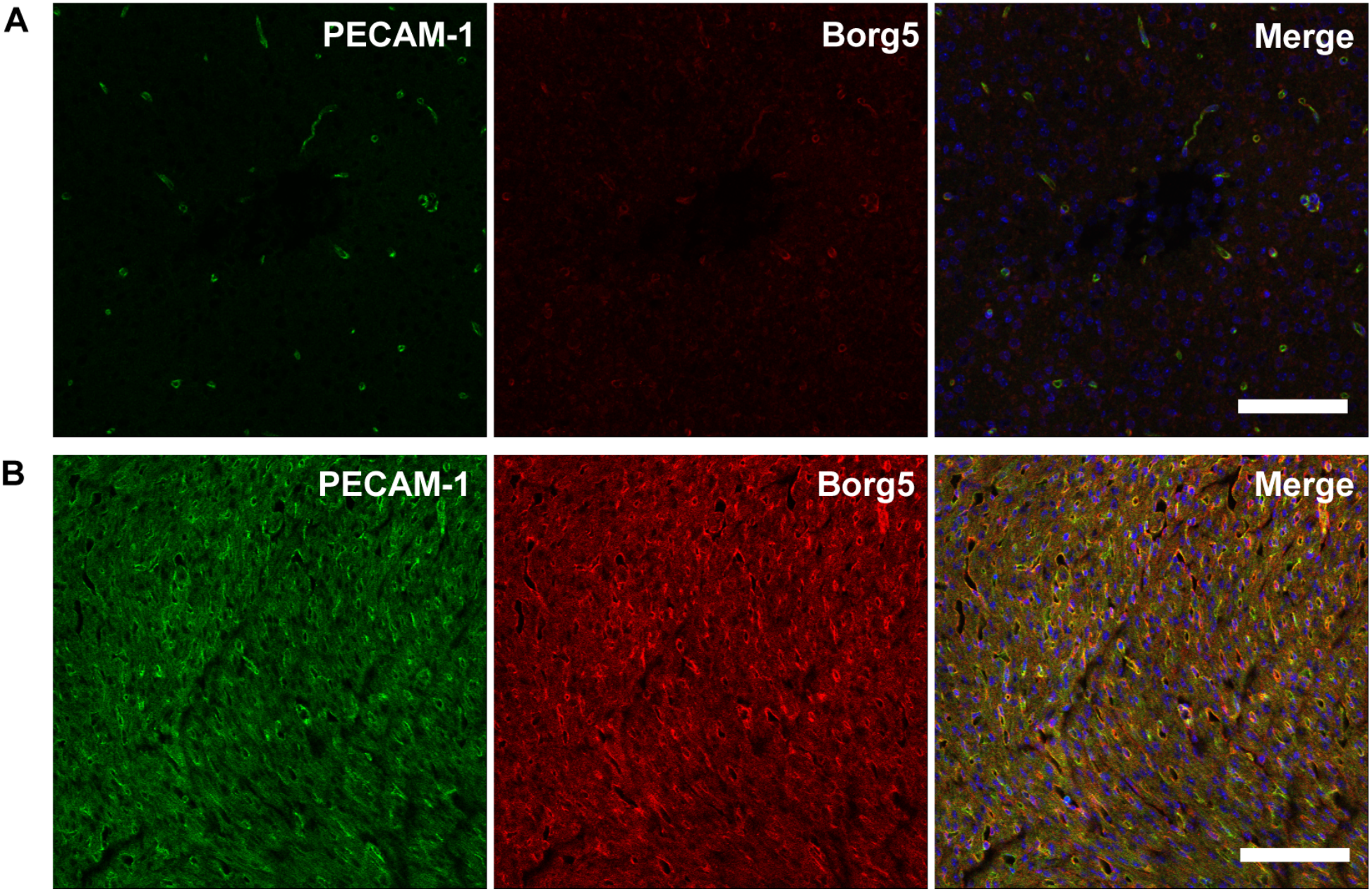


Figure S2

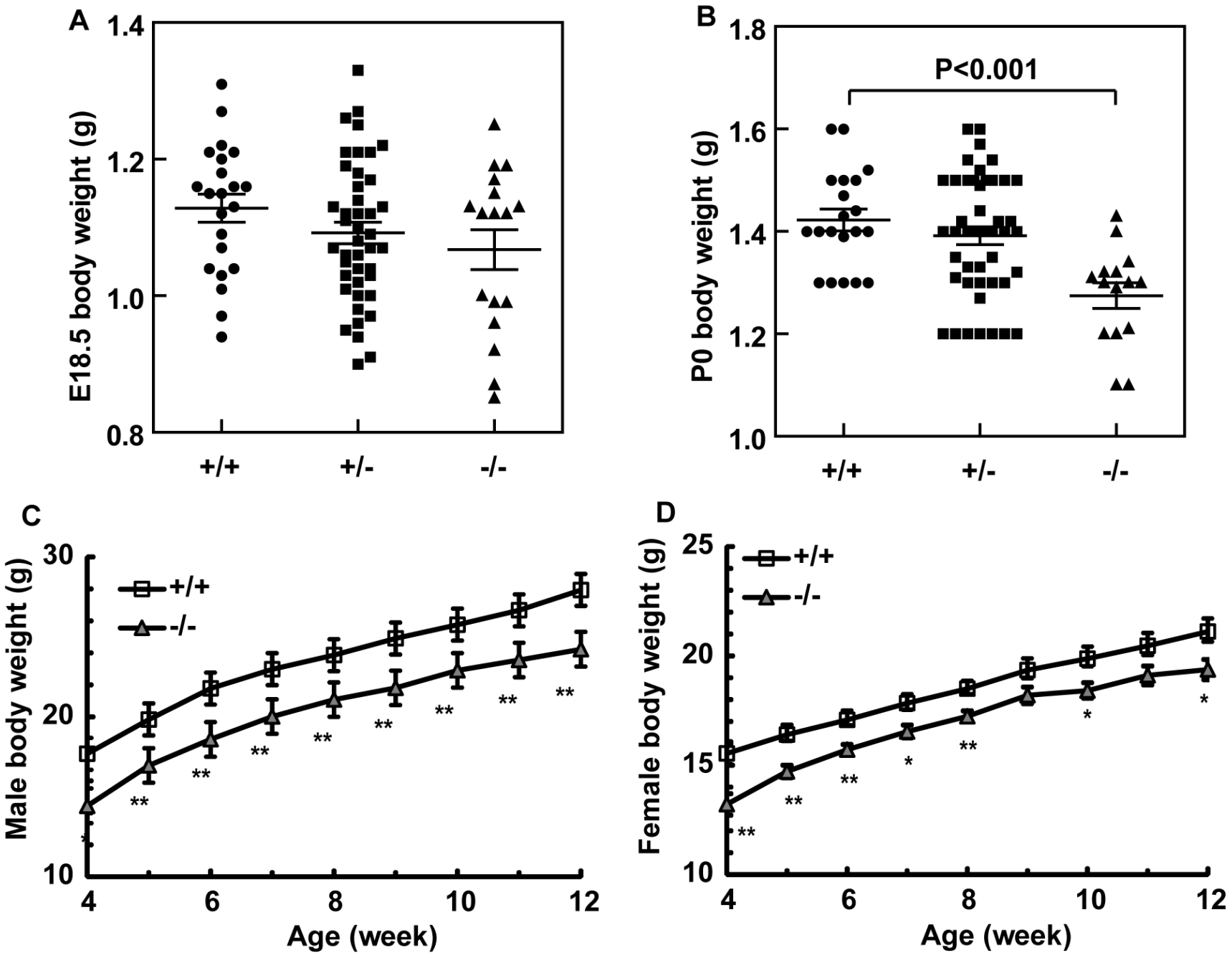

A
E18.5 body weight (g)
1.4
1.2
1.0
0.8
+/+
+/-
-/-
B
P0 body weight (g)
P<0.001
1.8
1.6
1.4
1.2
1.0
+/+
+/-
-/-
C
Male body weight (g)
+/+
-/-
30
20
10
4
6
8
10
12
Age (week)
D
Female body weight (g)
+/+
-/-
25
20
15
10
4
6
8
10
12
Age (week)

Figure S3

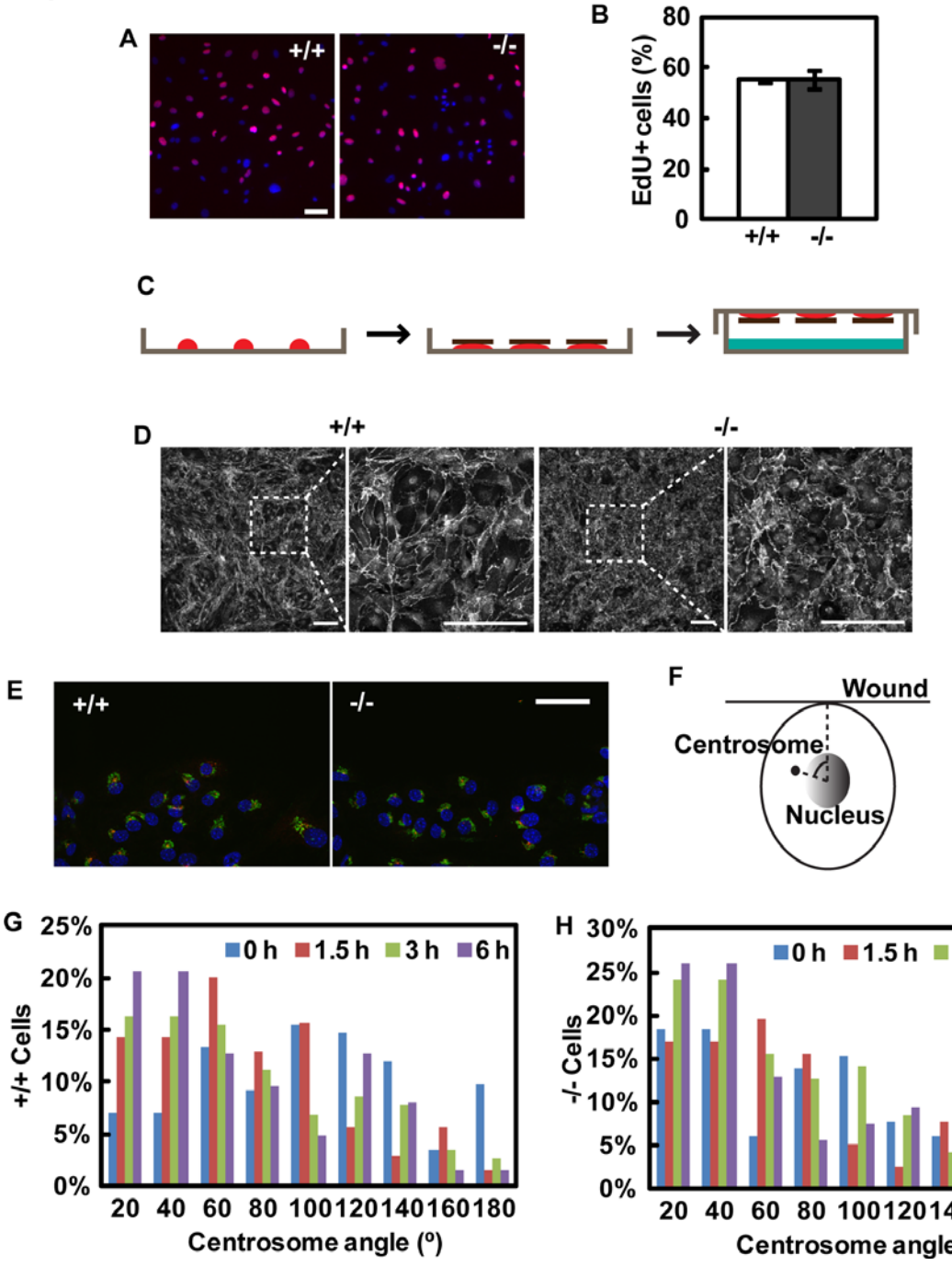

A
+/+
-/-
B
EdU+ cells (%)
80
60
40
20
0
+/+
-/-
C
D
+/+
-/-
E
+/+
-/-
F
Wound
Centrosome
Nucleus
G
+/+ Cells
25%
20%
15%
10%
5%
0%
0 h
1.5 h
3 h
6 h
20 40 60 80 100 120 140 160 180
Centrosome angle (º)
H
-/- Cells
30%
25%
20%
15%
10%
5%
0%
0 h
1.5 h
3 h
6 h
20 40 60 80 100 120 140 160 180
Centrosome angle (º)

Figure S4

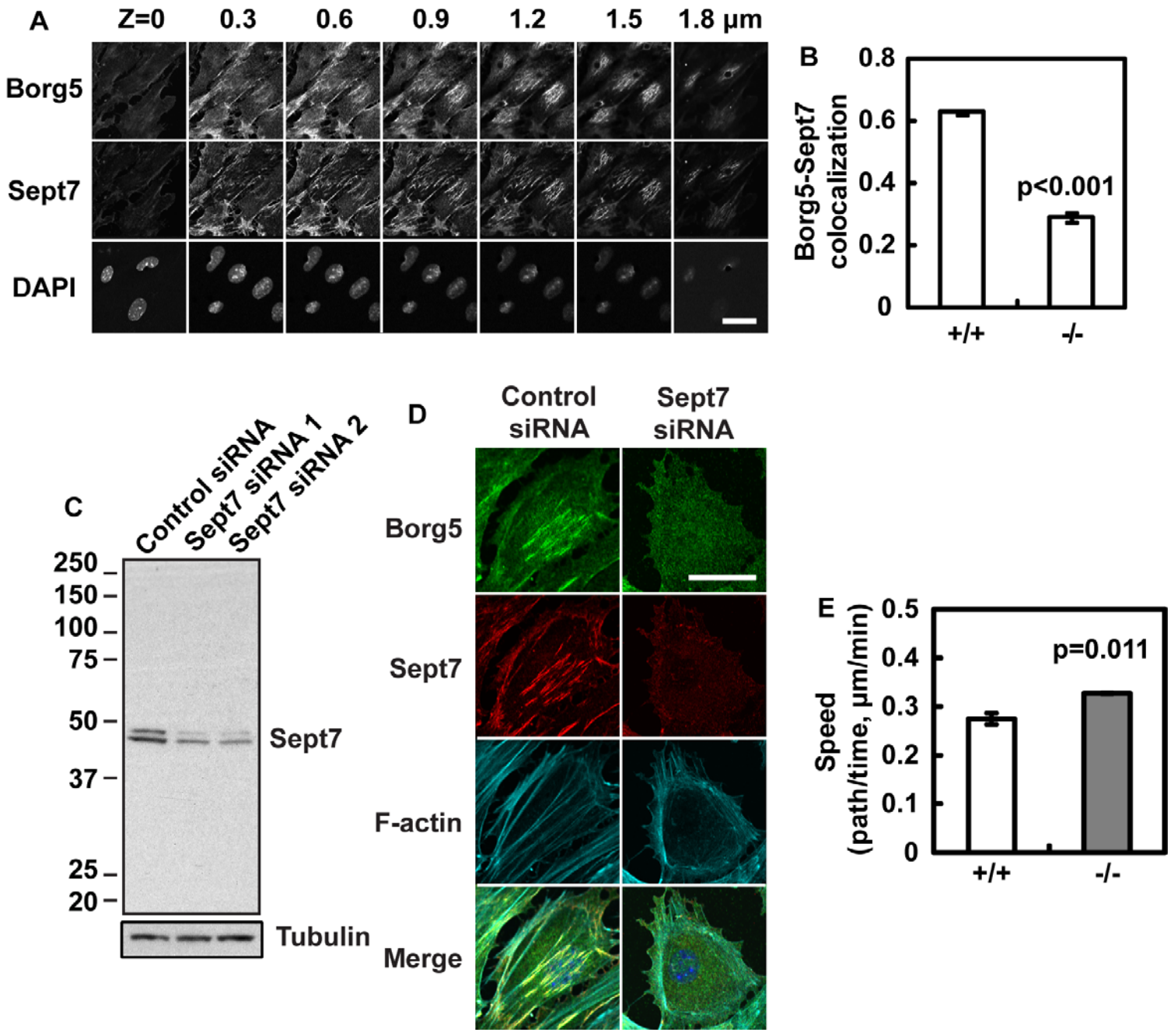
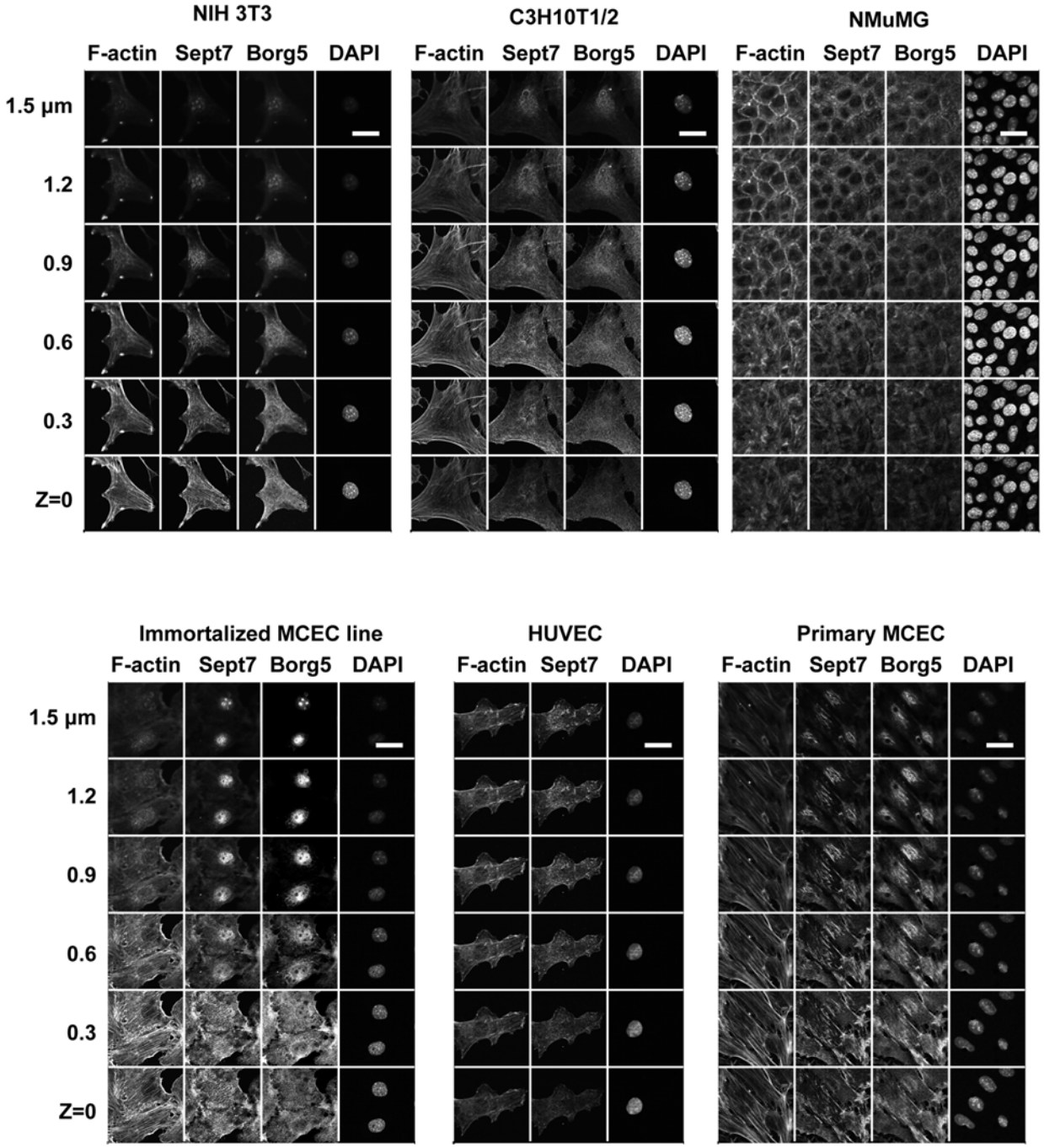

A
Z=0
0.3
0.6
0.9
1.2
1.5
1.8 µm
Borg5
Sept7
DAPI
B
Borg5-Sept7 colocalization
0.8
0.6
0.4
0.2
0
p<0.001
+/+
-/-
C
Control siRNA
Sept7 siRNA 1
Sept7 siRNA 2
250
150
100
75
50
37
25
20
Sept7
Tubulin
D
Control siRNA
Sept7 siRNA
Borg5
Sept7
F-actin
Merge
E
Speed (path/time, µm/min)
0.5
0.4
0.3
0.2
0.1
0
p=0.011
+/+
-/-

Figure S5


NIH 3T3
C3H10T1/2
NMuMG
Immortalized MCEC line
HUVEC
Primary MCEC
F-actin
Sept7
Borg5
DAPI
1.5 µm
1.2
0.9
0.6
0.3
Z=0