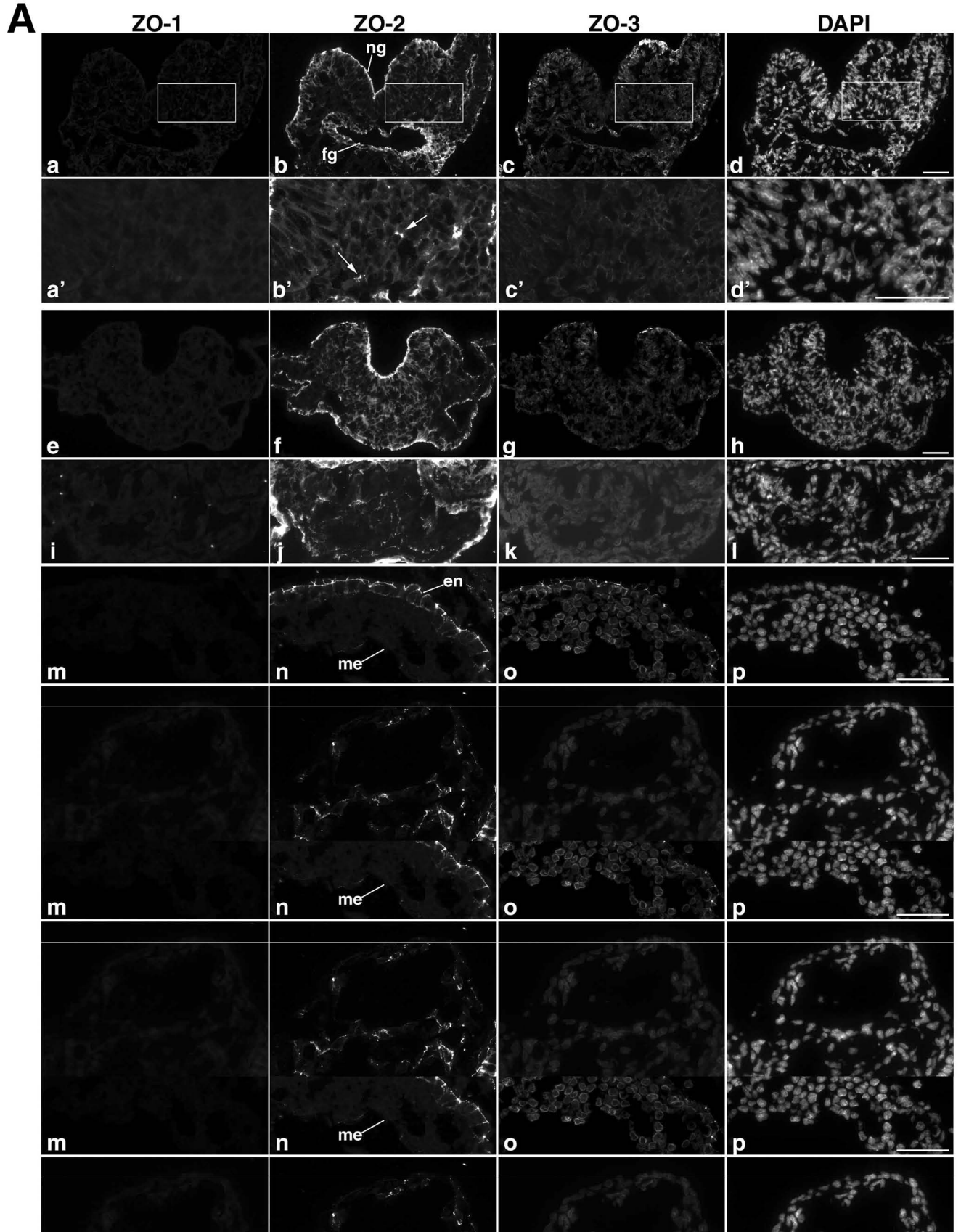
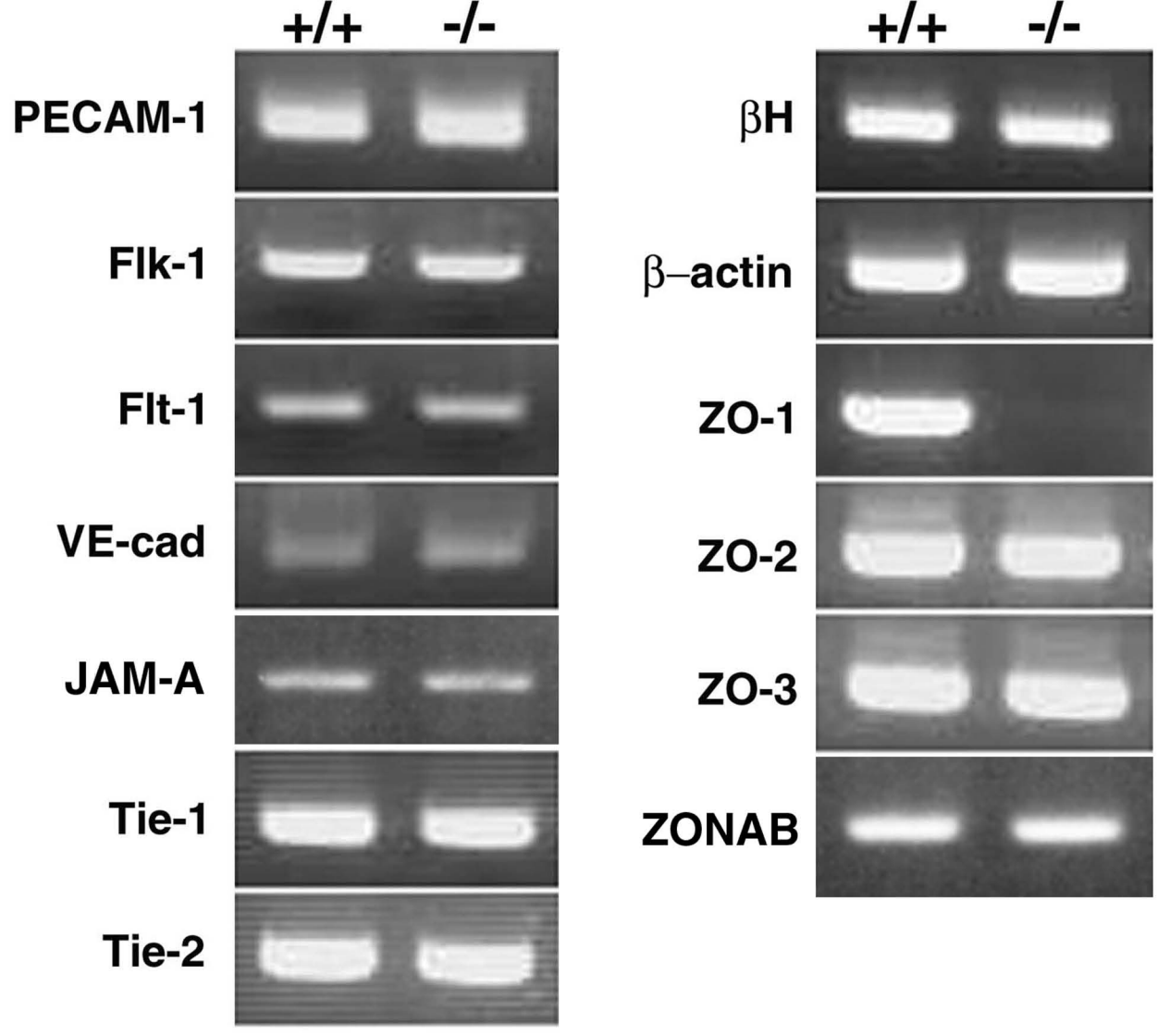
E07-12-1215 Tsukita

Supplementary Figure 1S. (A) Immunolabeling of ZO-1, ZO-2, and ZO-3 in E 8.5 *Tjp1*$^{-/-}$ embryos. (A) (a-d) Caudal parts of embryos. (a'-d') High magnification of the boxed region of caudal parts of embryos, each corresponding to a-d, respectively. ZO-2 localized at cell-cell junctions in the mesenchymal cells (arrows). (i-l) Primitive heart. (m-p) Yolk sac. (q-t) Allantois. Specific structures were indicated as follows; neural groove (ng), fore gut (fg), somite (so), extraembryonic endoderm (en), extraembryonic mesoderm (me). In *Tjp1* $^{-/-}$ embryos, signals for ZO-1 was undetectable. ZO-2 and ZO-3 localized in the cell-cell junctions in the same as *Tjp1*$^{+/+}$ embryos. Scale bars, 50μm in a-d and e-t 20μm in a'-d'. (B) Immunoblotting for ZO-1/2/3 and β-actin in of *Tjp1*$^{+/+}$ and *Tjp1*$^{-/-}$ yolk sacs. ZO-1 did not detect in *Tjp1*$^{-/-}$ yolk sacs. ZO-2/3 did not increase in *Tjp1*$^{-/-}$ yolk sacs

Supplementary Figure 2S. RT-PCR analyses of total RNA of yolk sacs at E9.5. Total RNA was prepared and subjected to RT-PCR analyses with a variety of angiogenesis related molecules and junction molecules primers, for PECAM-1, Flk-1, Flt-1, JAM-A, VE-cadherin, Tie-1, Tie-2, βH, β-actin, ZO-1, ZO-2, ZO-3, and ZONAB, respectively. Note that there are no differences in the intensities of signals for these proteins except ZO-1 between *Tjp1*$^{+/+}$ and *Tjp1*$^{-/-}$ yolk sacs in RT-PCR.


A
ZO-1
ZO-2
ZO-3
DAPI
ng
fg
en
me
a
b
c
d
a'
b'
c'
d'
e
f
g
h
i
j
k
l
m
n
o
p


+/+
-/-
PECAM-1
Flk-1
Flt-1
VE-cad
JAM-A
Tie-1
Tie-2
+/+
-/-
βH
β–actin
ZO-1
ZO-2
ZO-3
ZONAB