cells

MDPI

Supplementary

Fig.S1.

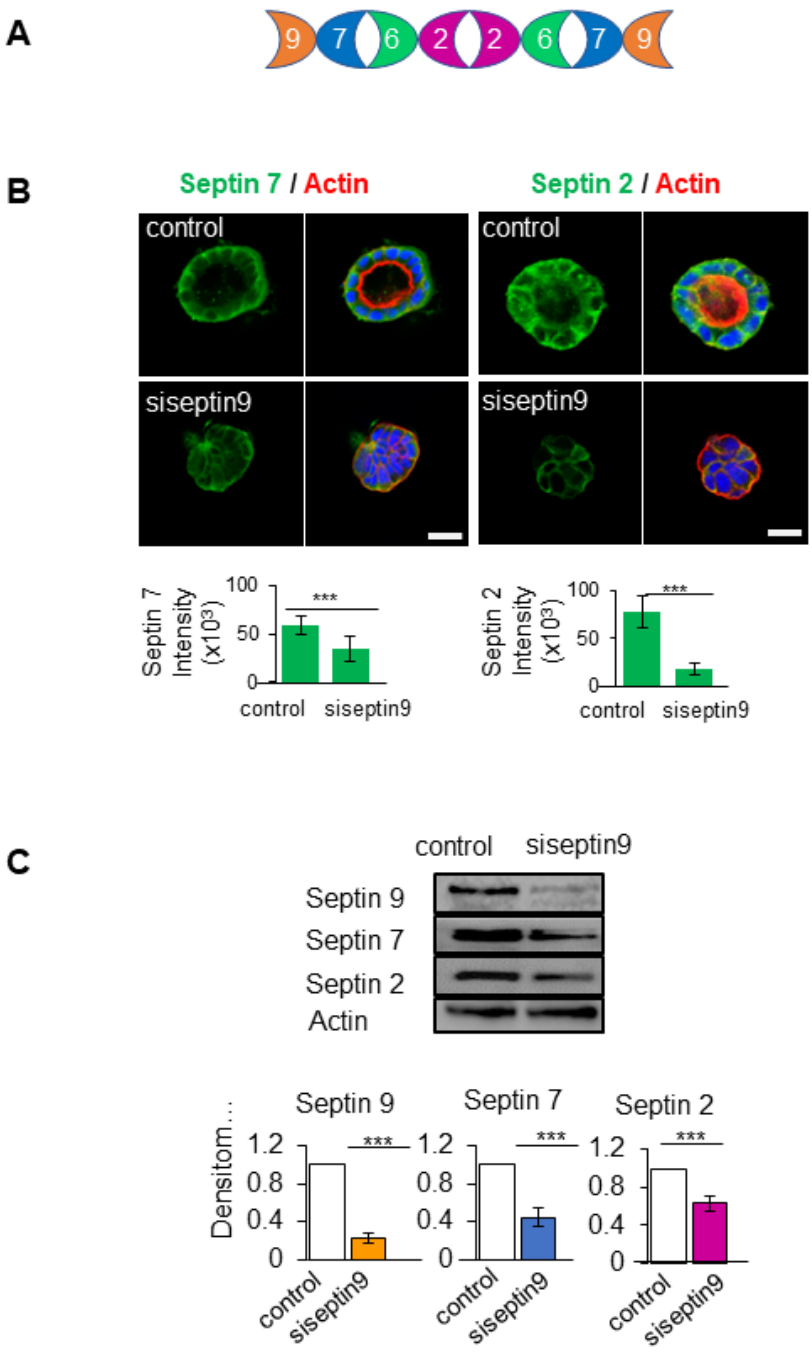

Figure S1. Septin 9 regulates septin2 and septin7 expressions and their localizations at BM. (**A**). The schematic representation of the interaction between septin 9 and other septins. (**B**). MDCK cells were transfected or not with siRNA of septin 9 for 24 h and plated on Matrigel for 4 days to form cysts, and then stained for septin 7 (green), septin 2 (green) and actin (red). A single confocal section through the middle of a cyst is shown. Scale bar 10 μm. Quantification of the fluorescence intensity of each protein expression, septin 2 (green), and septin 7 (green). (**C**). MDCK cells were transfected

or not with siRNA of septin 9 for 24 h then grown on the plate for 3 days. Cells were lysed and analyzed by western blot for septin 9, septin 7 and septin 2 proteins expressions. Data information: Data are at least two times replicates and cysts (n>10) for 3D staining, and three times for immunoblotting. The statistics values are means ±s.e.m. Student's t-test was used. *P<0.05, **P<0.01, ***$P < 0.001$.

Fig.S2.

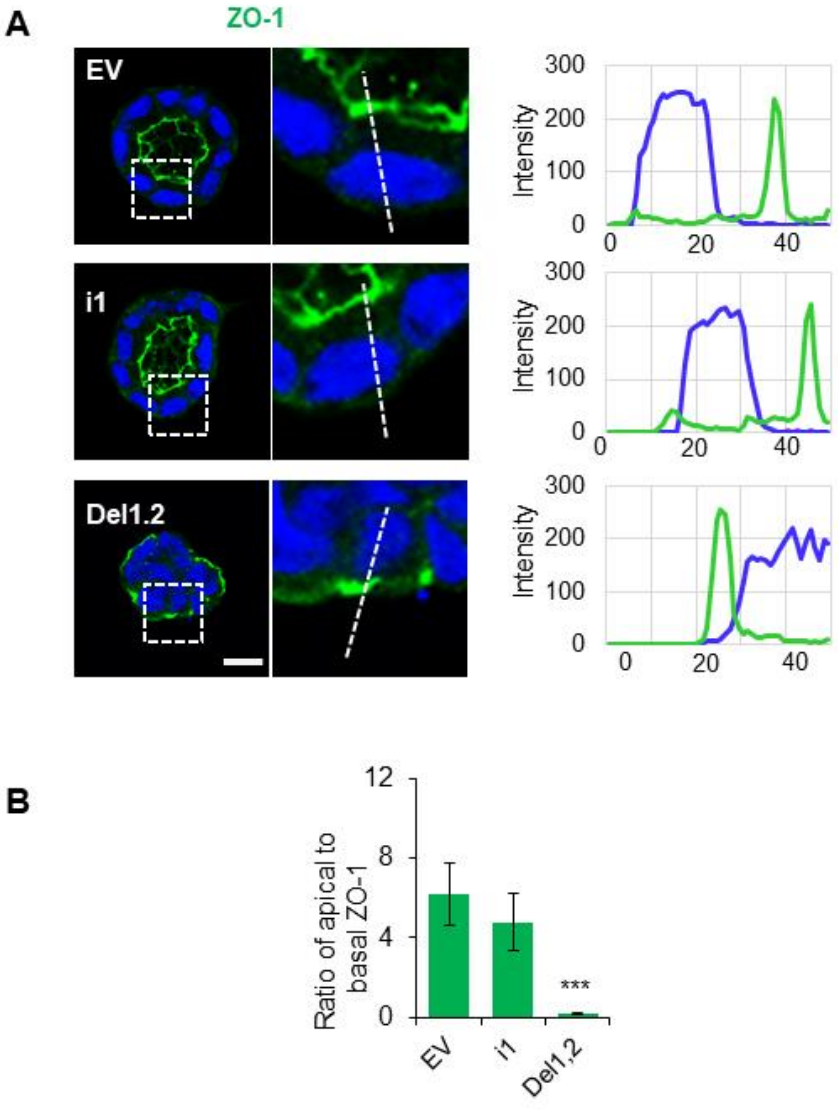

Figure S2. Deletion of septin 9_i1 PB domains impacts tight junctions. (**A**). MDCK cells expressing EV, i1 and del1.2 plated on Matrigel for 4 days to form cysts, and then stained for ZO-1 (green). A single confocal section through the middle of a cyst is shown. Scale bar 10 μm. (**B**). Line profiles showing ZO-1 distribution from basal to apical area, using Image J. (**C**). Quantification of ZO-1 distribution: the ratio of maximal fluorescence intensity at the apical side versus the maximal fluorescence intensity at the basal side, ***P <0.001. Data information: Data are at least two times replicates and cysts (n>10) for 3D staining. The statistics values are means ±s.e.m. Student's t-test was used. ***$P < 0.001$.

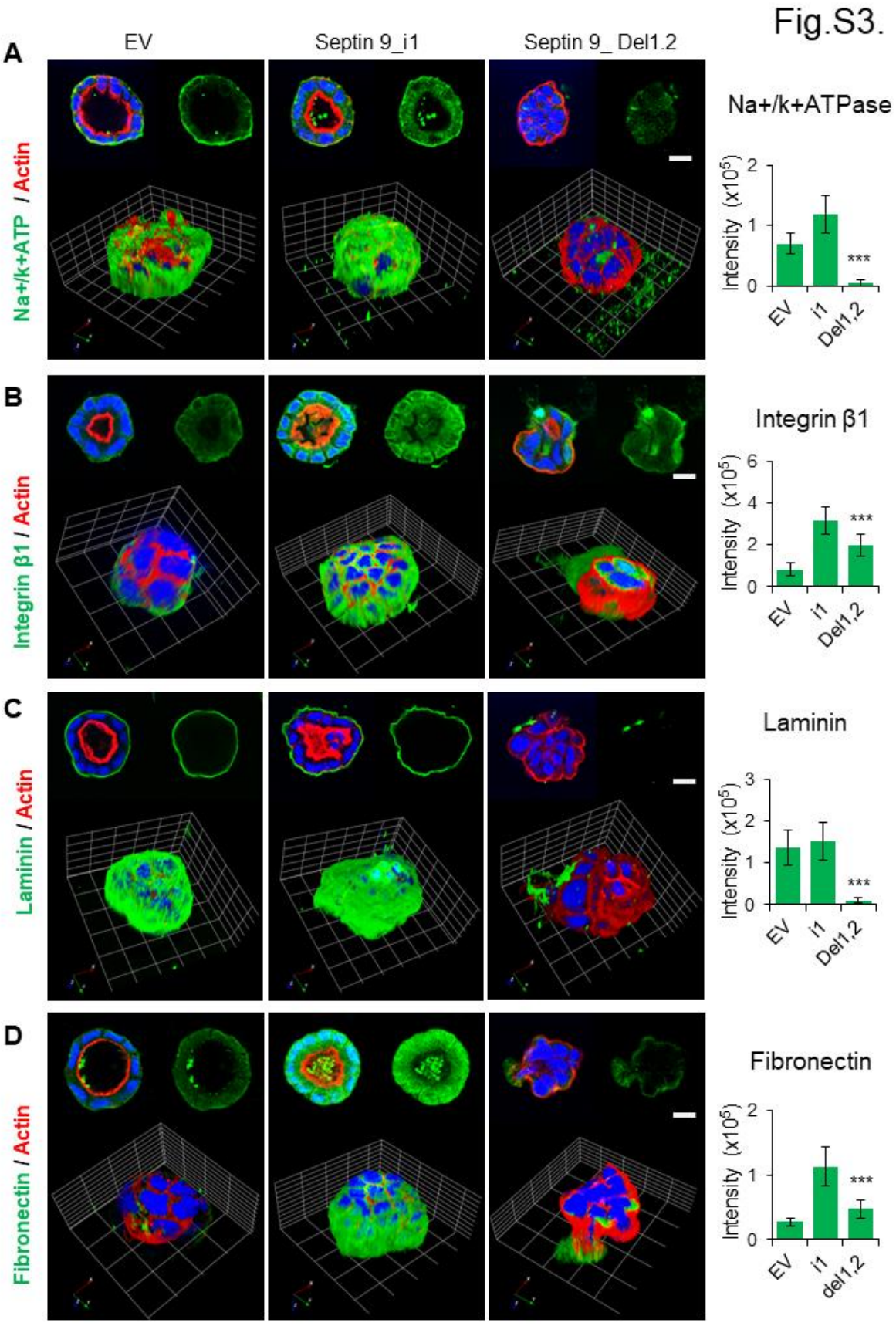

Figure S3. Deletion of septin 9_i1 PB domains impacts cell-ECM adhesion. (**A**)–(**D**). MDCK cells expressing EV, i1 and del1.2 plated on Matrigel for 6 days to form cysts were stained for Na+/K+

ATPase (green) and actin (red), integrin β1 (green) and actin (red), laminin (green) and actin(red), and fibronectin (green) and actin (red). A single confocal section through the middle of a cyst is shown. Scale bar 10 μm. The 3D reconstructions of all the cysts were presented. The quantification of the fluorescence intensity of Na+/K+ ATPase, integrin β1, laminin, and fibronectin were showed. The data are means ±s.e.m. Student's t-test was used. ***$p < 0.001$. Data information: Data are at least two times replicates and cysts (n>10) for 3D staining. The statistics values are means ±s.e.m. Student's t-test was used. ***$P < 0.001$.

Fig.S4.

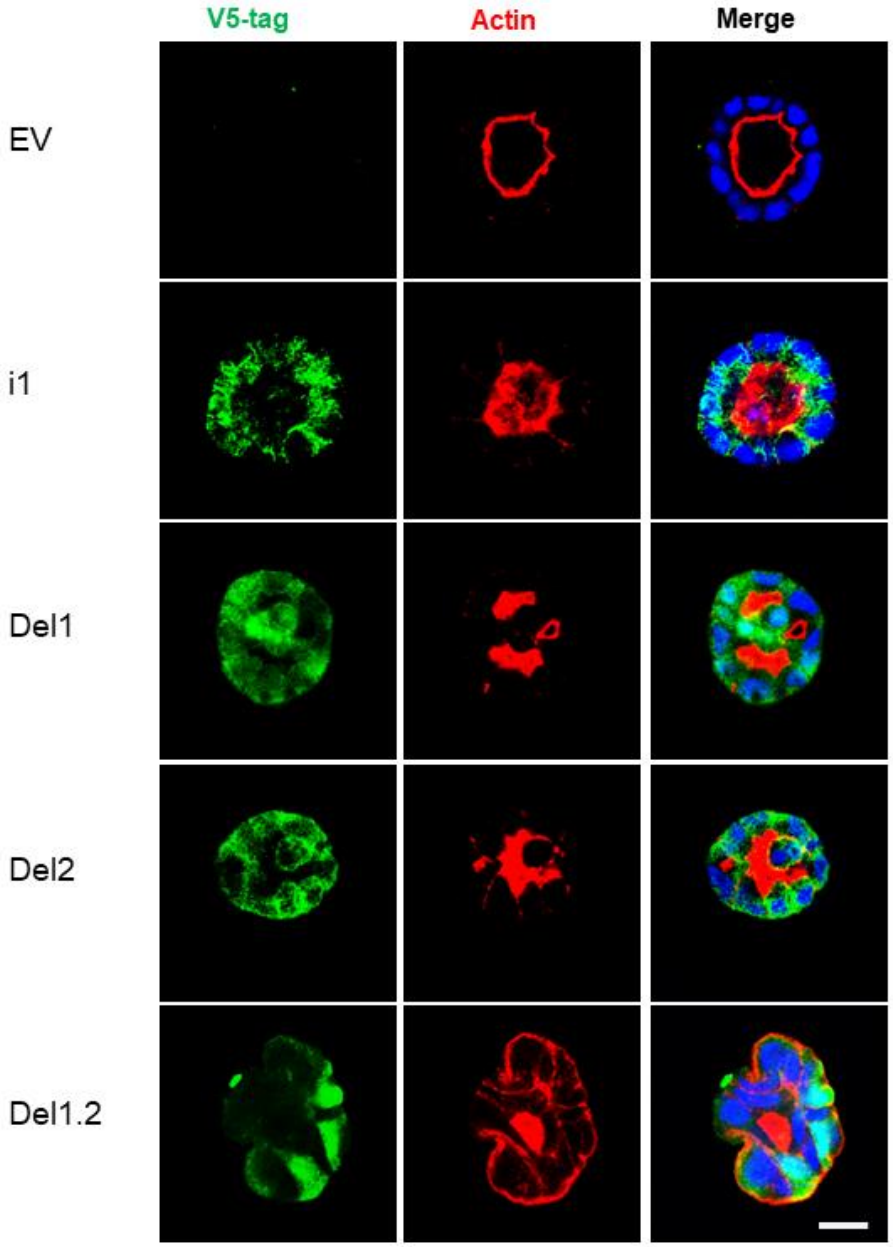

Figure S4. Septin 9-V5 tag expression in MDCK stable cell lines in day 6. MDCK cells expressing EV, i1, del1, del2, and del1.2 plated on Matrigel for 6 days to form cysts were stained for septin 9-V5 tag antibody (green), and actin (red). A single confocal section through the middle of a cyst is

shown. Scale bar 10 μm. Data information: Data are at least two times replicates and cysts (n>10) for 3D staining.

Fig.S5.

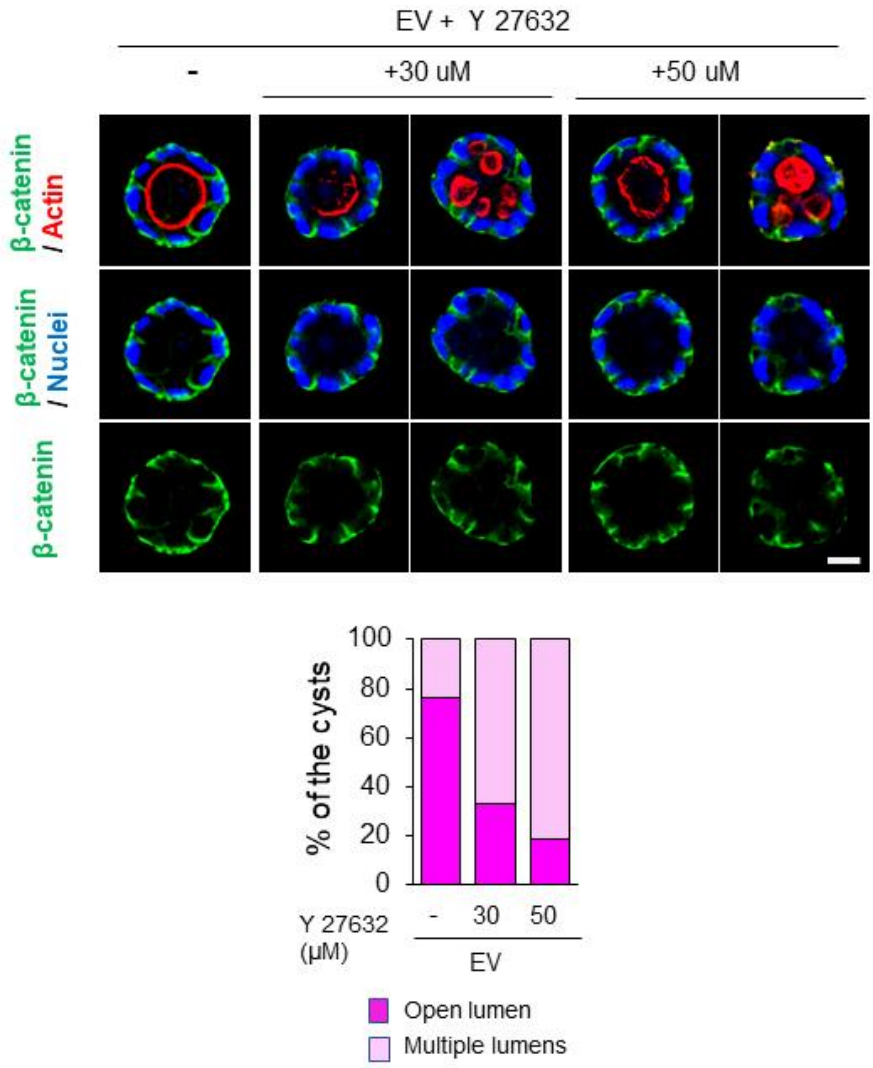

Figure S5. Treatment of septin 9_EV cysts with Y27632 rescues polarity. MDCK septin 9_EV cells were plated on Matrigel for 6 days and treated with 30 μM and 50 μM of Y27632. All the cysts were stained with β-catenin (green) for basolateral membrane, actin (red) for apical surface and Hoechst (blue) for nuclei. The representative confocal images are show in single merge and nuclei. Quantification of polarized and inverted polarity phenotypes in septin 9_EV cysts. Data information: Data are at least two times replicates and cysts (n>100) for 3D staining. The statistics values are means ±s.e.m. Student's t-test was used. ***$P < 0.001$.

Table S1. The sequence for the primers.

Primer name	Forward sequence	Reverse sequence
Canis_SEPTIN9	GTC CAC TGC TGC CTC TAC TTC A	GGA CGA TGT TGA CCA CCT TGC T
Canis_Vimentin	GCC ATC AAC ACC GAG TTC AA	GGA AGC GCA CCT TGT CGA T
Canis_N-cadherin	CAA CTT GCC AGA AAA CTC CAG	ATG AAA CCG GGC TAT CAG CTC
Canis_ZEB1	CAA GGT GGC CAT TCT GTT AT	CTA GGC TGC TCA AGA CTG TAG
Canis_TGF-β1	GGC CAC CAT TCA TGG CAT GA	CGT GTC CAG GCT CCA AAT GT
Canis_GAPDH	CAT CAC TGC CAC CCA GAA G	CAG TGA GCT TCC CGT TCA G