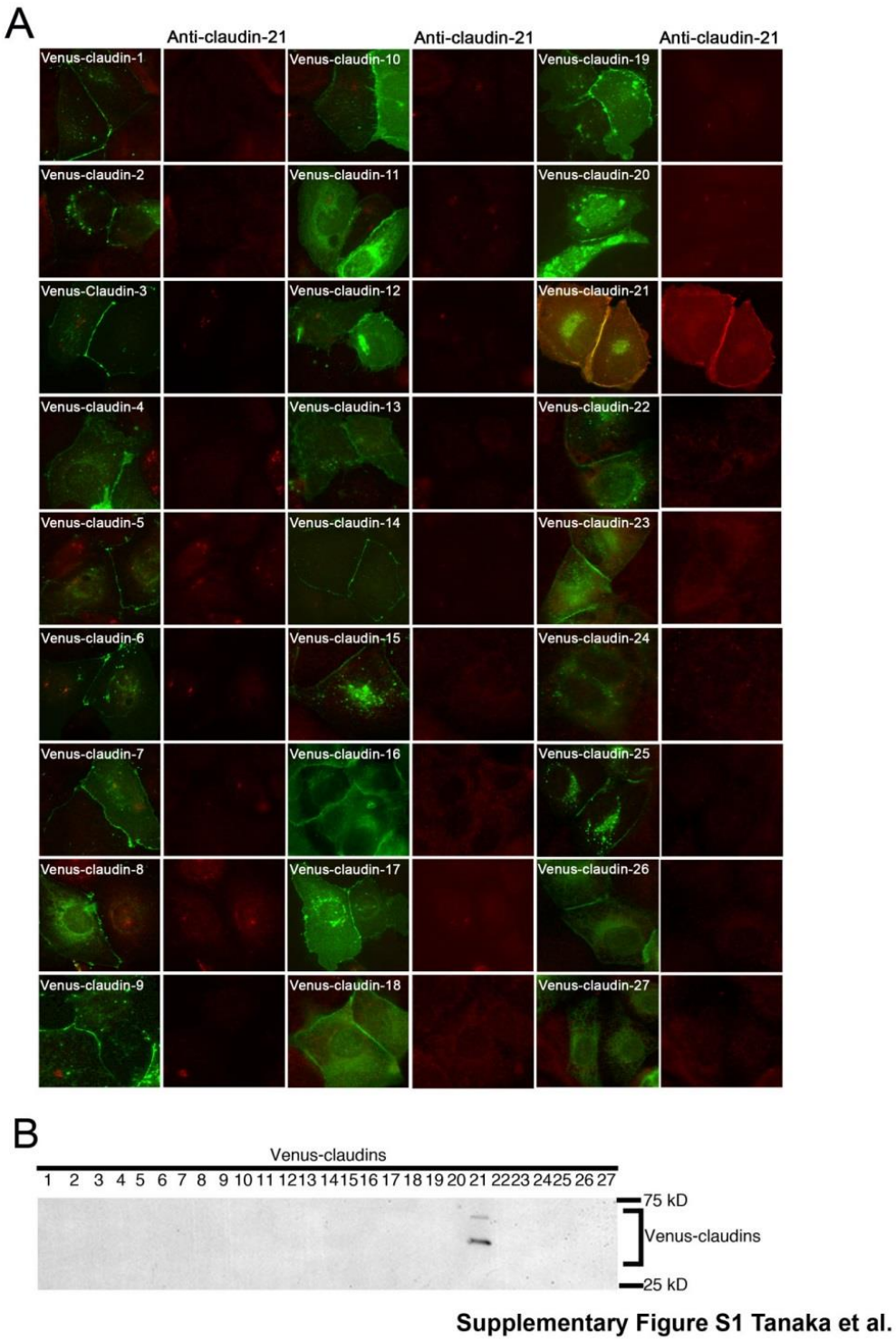
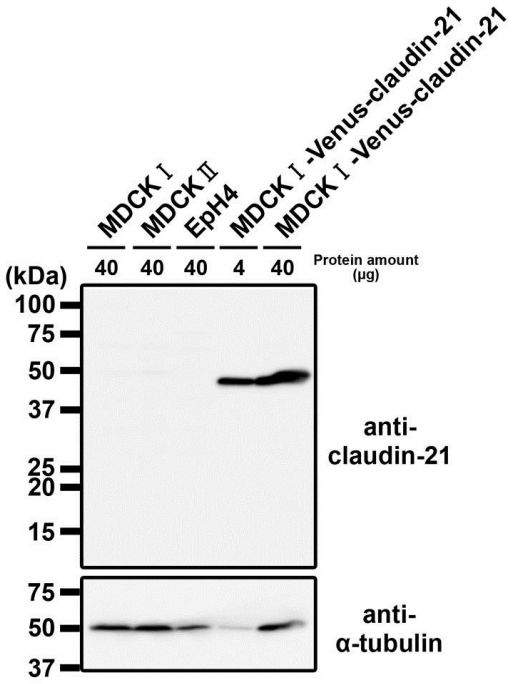
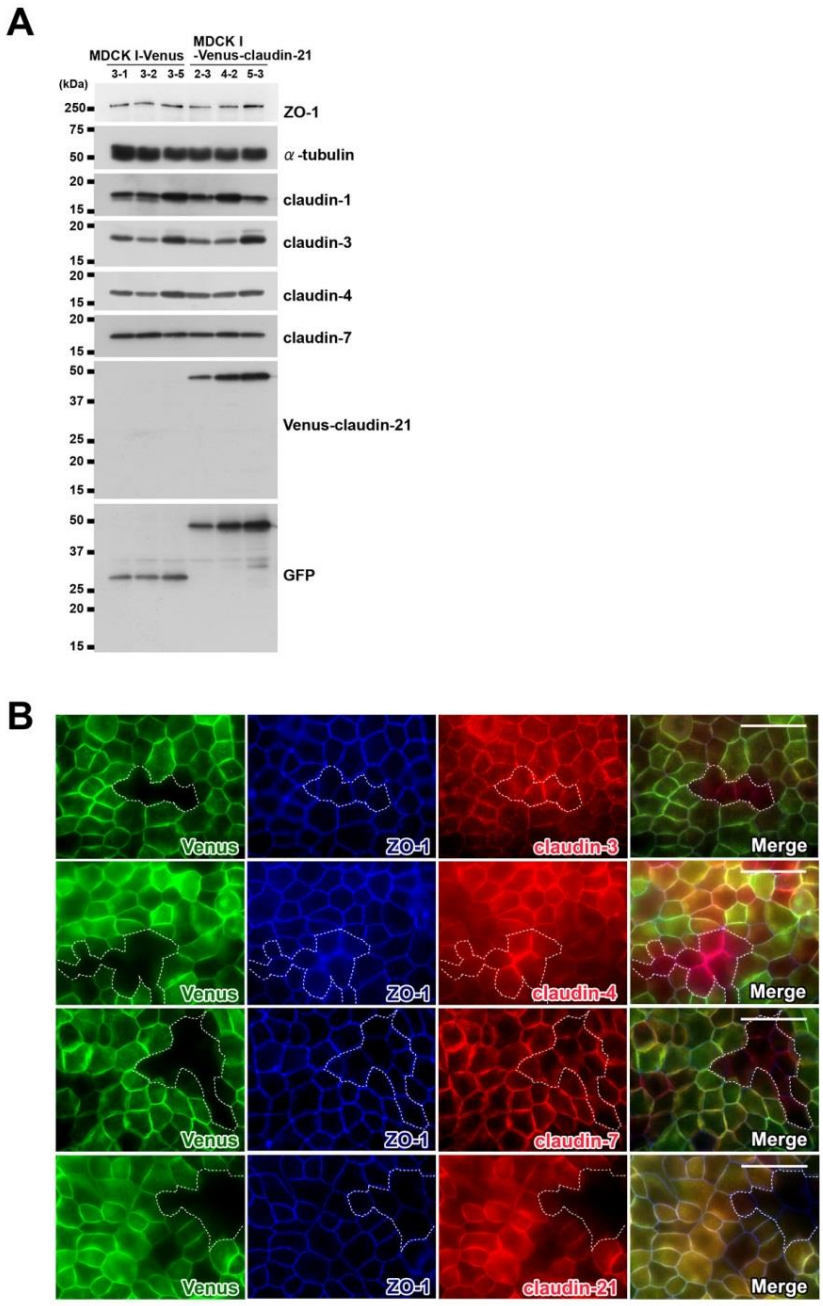



**Supplementary Figure S1**
(A, B) Immunofluorescence (A) and immunoblots (B) of mouse claudins expressed in MDCK I cells. The polyclonal antibody (pAb) for claudin-21 specifically recognized claudin-21 among the 27 mouse claudins. Green, Venus-tagged claudins (Venus-claudins); red, claudin-21 antibody labeling.




**Supplementary Figure S2 Tanaka et al.**

**Supplementary Figure S2**
Immunoblots for claudin-21 in MDCK I, MDCK Ⅱ, EpH4 or claudin-21-expressing transfectant of MDCK I (MDCK I-Venus-claudin-21) cells . Claudin-21 was detected by the pAb for claudin-21. Tubulin was detected as loading controls. The amounts of loaded proteins were shown.

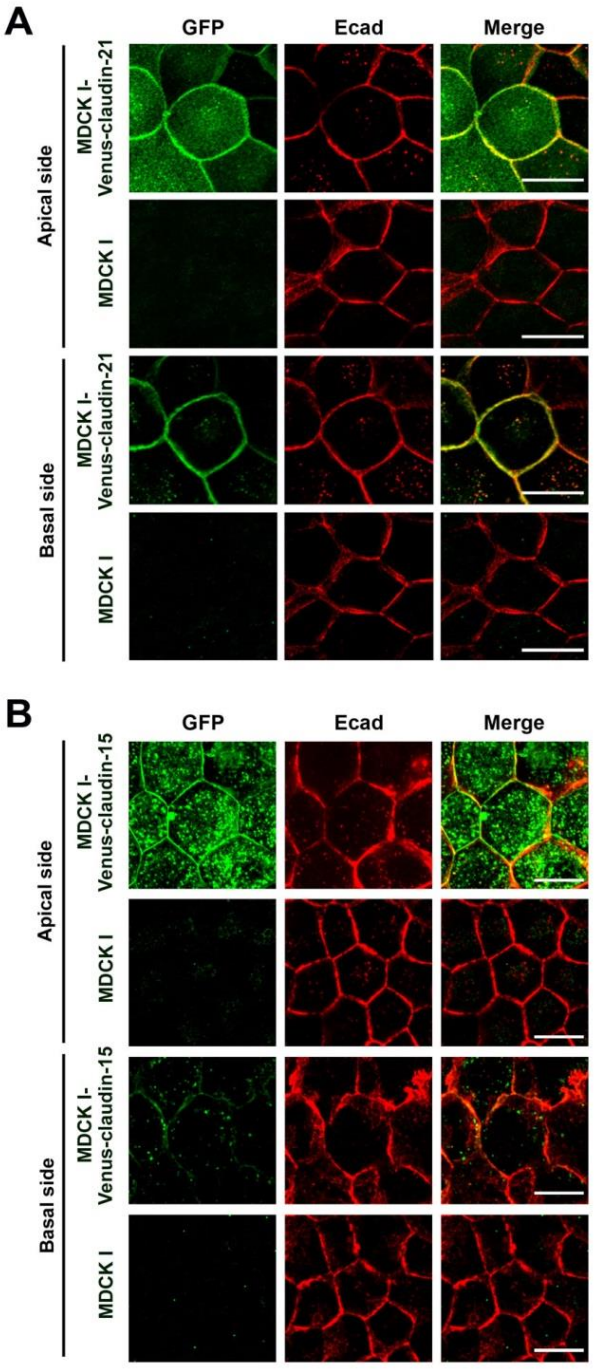



**Supplementary Figure S3 Tanaka et al.**

**Supplementary Figure S3**

(A) Immunoblots for TJ-related proteins in mock-transfected MDCK I (MDCK I-Venus) or claudin-21-expressing transfectant of MDCK I (MDCK I-Venus-claudin-21) cells.

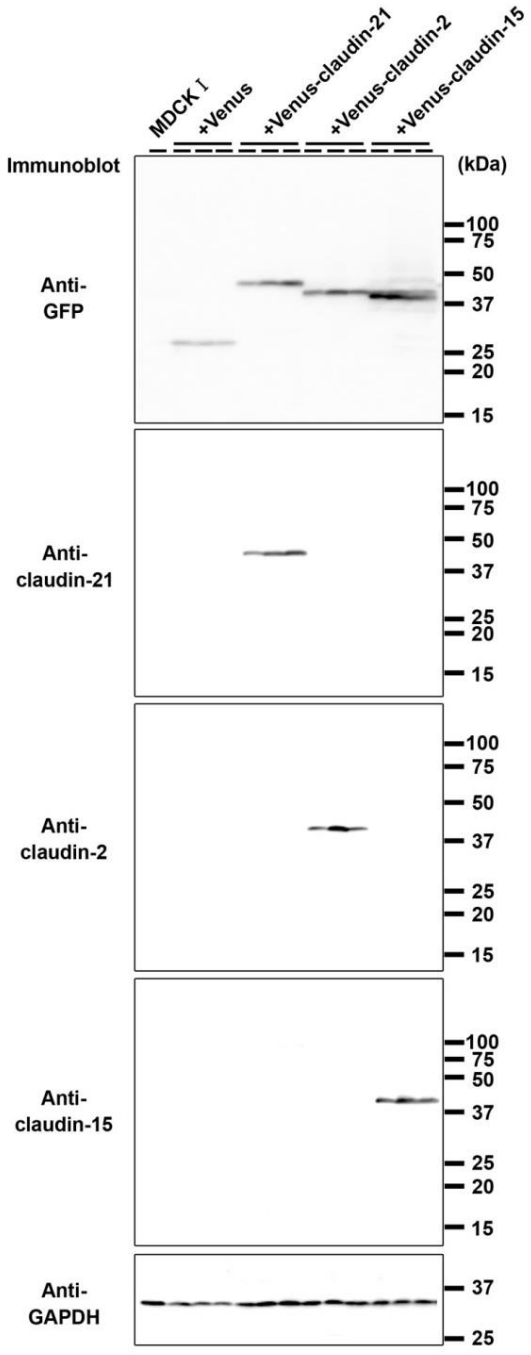
(B) Immunofluorescence micrographs of TJ-related proteins in a mixed culture of MDCK I-Venus and MDCK I-Venus-claudin-21 cells co-stained with an anti-GFP pAb, an anti-ZO-1 Ab and each anti-TJ-related proteins pAb. The anti-GFP- (green), the anti-ZO-1- (blue) and the anti-TJ-related proteins-positive signals (red) are shown. Bar: 50 μm




SUpplementary Figure S4 Tanaka et al.

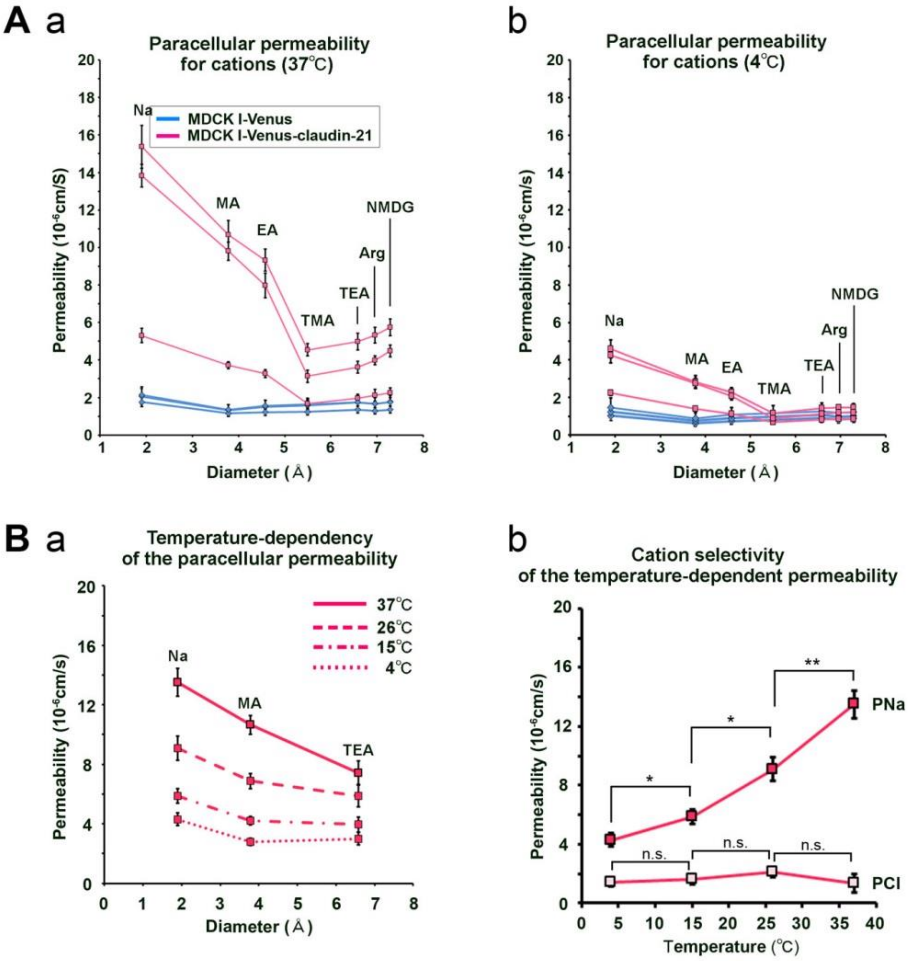
**plementary Figure S4**

MDCK I, claudin-21-expressing transfectant of MDCK I (MDCK I-Venus-claudin-21) or claudin-15-expressing transfectant of MDCK I (MDCK I-Venus-claudin-15) were cultured to confluence in glass coverslips and examined by the confocal laser scanning microscopy. (A) MDCK I and MDCK I-Venus-claudin-21 (B) MDCK I and MDCK I-Venus-claudin-15 cells were double stained with an anti-GFP pAb and an anti-E-cadherin mAb. The anti-GFP-positive signals (green) are overlapped with the anti-E-cadherin-positive signals (red). Stacked images of apical or basal side of the epithelial cells are shown respectively. Green, GFP; Red, E-cadherin (Ecad). Bar: 10 μm.




Supplementary Figure S5 Tanaka et al.

**Suplementary Figure S5**
Immunoblots for claudin-21, -2 or -15 in MDCK I, mock-transfected MDCK I (MDCK I-Venus), claudin-21-expressing transfectant of MDCK I (MDCK I-Venus-claudin-21) and claudin-15-expressing transfectant of MDCK I (MDCK I-Venus-claudin-15) cells. Venus or venus-tagged claudins were detected by the anti-GFP monoclonal antibody (mAb). Claudin-21 was detected by the anti-claudin-21 pAb. GAPDH was detected as loading controls.




**Supplementary Figure S6 Tanaka et al.**

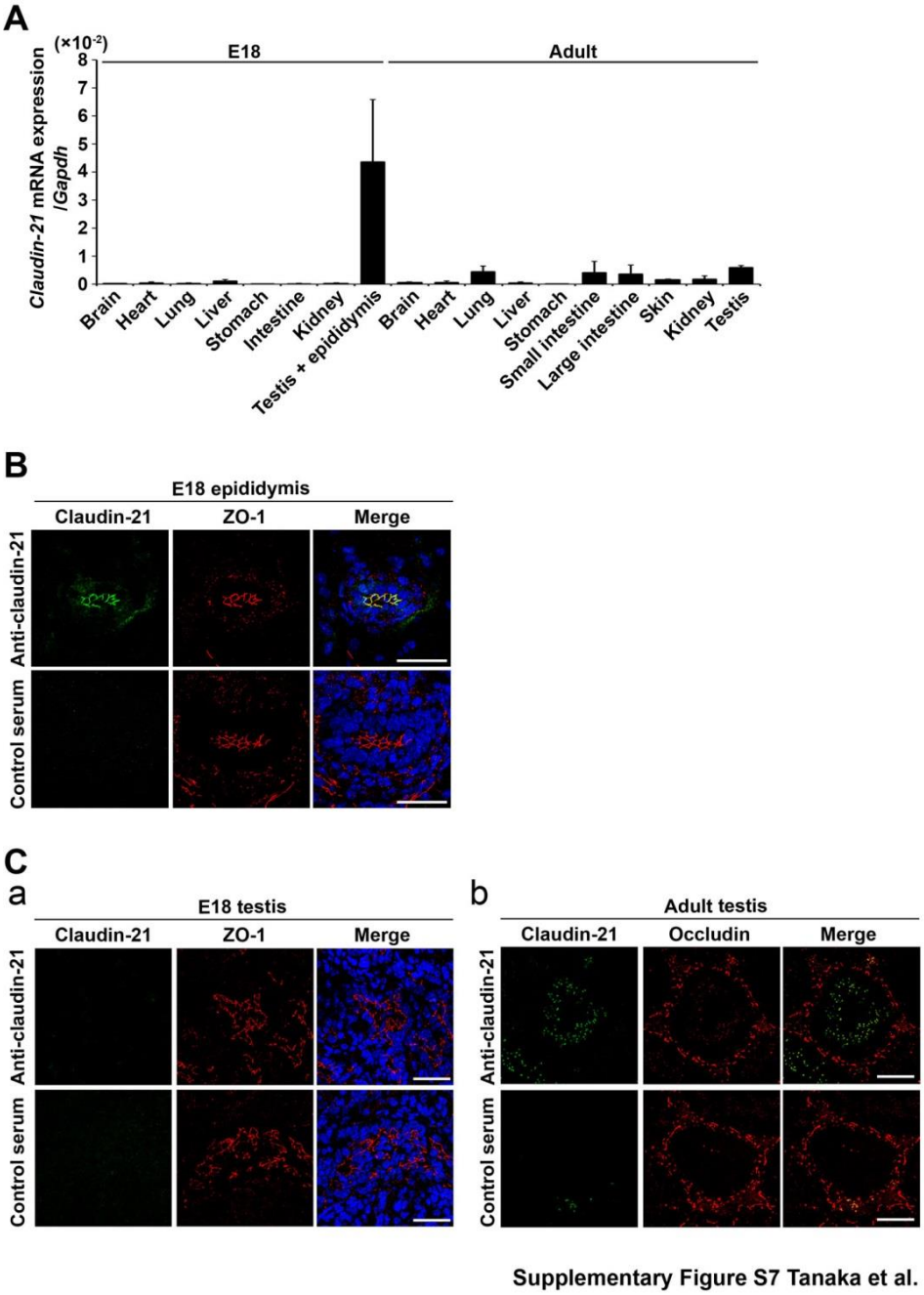
**Supplementary Figure S6**

(A) Transepithelial ion permeabilities for $Na^+$, $MA^+$, $EA^+$, $TMA^+$, $TEA^+$, $Arg^+$ and $NMDG^+$ in all of 3 clones of mock-transfected MDCK I (MDCK I-Venus) and claudin-21-expressing transfectant of MDCK I (MDCK I-Venus-claudin-21) cells at 37℃ (a) or 4℃ (b) (n=4/group).

(B) (a) Transepithelial permeabilities for $Na^+$, $MA^+$, and $TEA^+$ at 37 ℃, 26 ℃, 15 ℃ or 4 ℃ of MDCK I-Venus-claudin-21 cells. The averaged results from 2 clones of claudin-21 highly expressing transfectant MDCK I cells (n=6/group) are shown.

(b) Transepithelial ion permeabilities for $Na^+$ and $Cl^-$ at 37 ℃, 26 ℃, 15 ℃ or 4 ℃of MDCK I-Venus-claudin-21 cells. The averaged results from 2 clones of claudin-21 highly-expressing transfectant of MDCK I cells (n=6/group) are shown.

n.s., not significant; *, $p<0.05$.; **, $p<0.01$.



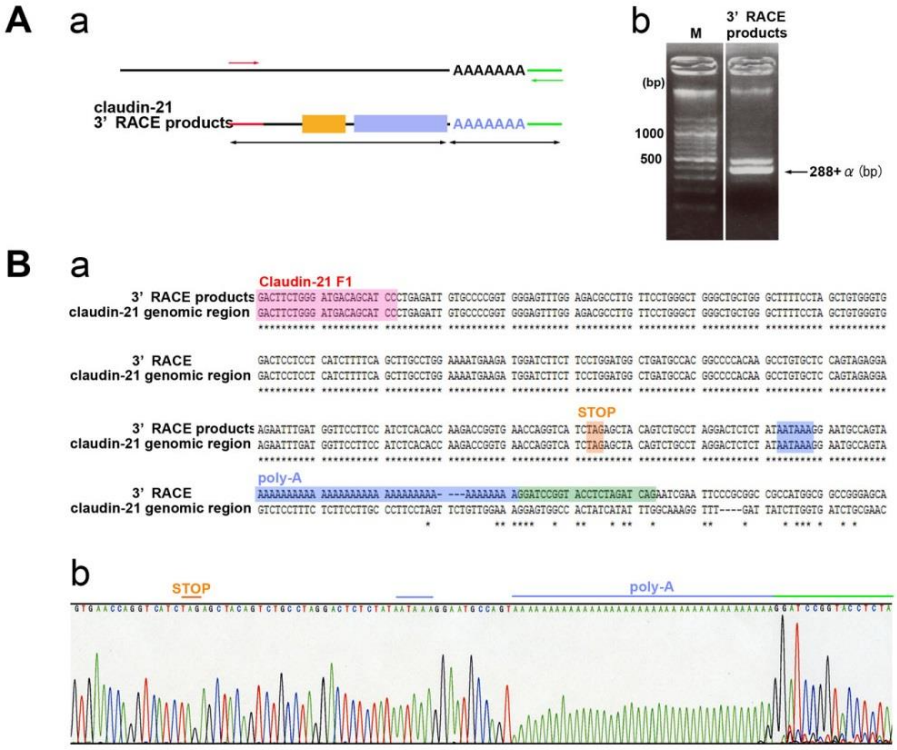

Supplementary Figure S7 Tanaka et al.

**Supplementary Figure S7**

(A) The probe-based quantitative RT-PCR for claudin-21 of E18.5- and adult -mouse tissues (n=3/group). Gapdh mRNA was detected as reference gene mRNA expression and the relative expression levels of claudin-21 mRNA versus gapdh mRNA were shown (n=3/group).

(B) Immunofluorescence micrographs of the epididymis from E18-mouse co-stained with an anti-claudin-21 pAb and an anti-ZO-1 mAb, or anti-serum and anti-ZO-1 mAb. Dapi was used for the detection of nucleus. The anti-claudin-21-positive signals or the anti-serum positive signals (green), the anti-ZO-1-positive signals (red) and nuclei (blue) are shown. Bar: 50 μm.

(C) (a) Immunofluorescence micrographs of the testis from E18-mouse co-stained with an anti-claudin-21 pAb and an anti-ZO-1 mAb, or anti-serum and anti-ZO-1 mAb. Dapi was used for the detection of nucleus. The anti-claudin-21-positive signals or the anti-serum-positive signals (green), the anti-ZO-1-positive signals (red) and nuclei (blue) are shown. Bars: 50 μm. (b) Immunofluorescence micrographs of the testis from adult-mouse co-stained with an anti-claudin-21 pAb and an anti-occludin mAb, or anti-serum and anti-occludin mAb. The anti-claudin-21-positive signals or the anti-serum positive signals (green), the anti-occludin-positive signals (red) are shown. Bar: 50 μm.




**Supplementary Figure S8 Tanaka et al.**

**Supplementary Figure S8**

(A) (a) Schematic diagram of the claudin-21 3'RACE analysis. (b) PCR products resulting from the 3'RACE-PCR. M: 100-bp molecular weight markers.

(B) (a) Alignment of the sequence from 3'RACE products with the sequence of claudin-21's genomic region. (b) Partial electropherogram of claudin-21's 3'RACE products showing the poly-A tail**.**

| Primers | Sequences (5'→3' ) | Purpose |
|---|---|---|
| RT-oligo-dT | CTGATCTAGAGGTACCGGATCC TTTTTTTTTTTTTTT | Reverse transcription |
| Claudin-21 F1 | GACTTCTGGGATGACAGCATCC | 3' RACE |
| 3' RACE Reverse | CTGATCTAGAGGTACCGGATCC | 3' RACE |

**Table1 Tanaka et al.**

**Supplementary Table 1**
Primers list used for claudin-21 3'RACE analysis was shown. The RT-oligo-dT primer was used for reverse transcription. The claudin-21 F1 and 3'RACE Reverse primers were used for amplification for the partial fragment of claudin-21.