SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Subcellular localization of claudins and occludin in Caco-2 cells. Cells were treated for 48 h with 100 nM 1α,25$(OH)_2D_3$, and subjected to double immunostaining with the corresponding antibodies, followed by observation under a laser-scanning confocal microscope. Bars, 20 μm.

Figure S2. Suppression of claudin-2 expression in Caco-2 cells prevents the 1α,25$(OH)_2D_3$-dependent decrease in TER levels and increase in $^{45}Ca^{2+}$ transport. Cells were transfected with negative control siRNA or Cldn2 siRNA (#3), and treated as in Figure 5A, subjected to measurement of TER (A) and calcium transport studies (B). The values represent the mean ± SD (error bars; n = 3). *, $P<0.05$. **, $P<0.01$ compared with values of cells grown without 1α,25$(OH)_2D_3$.

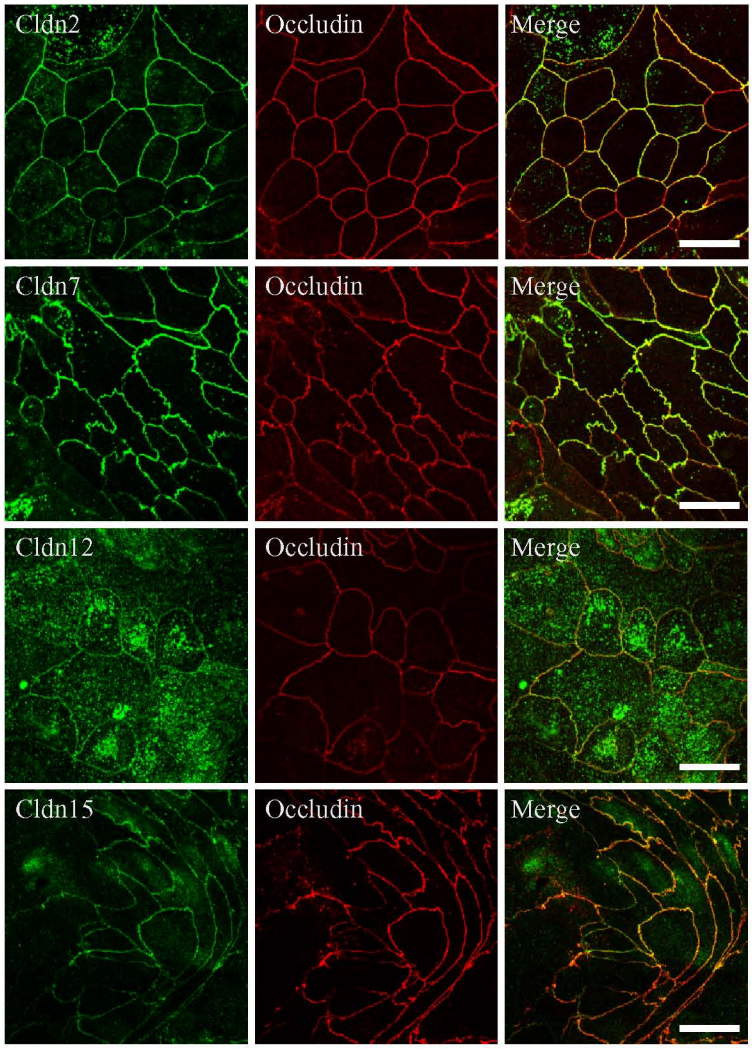
Figure S3. Altered expression of claudin-2 or claudin-12 in Caco-2 cells does not affect TRPV6 expression or apicobasal polarity. (A) Cells were transfected with negative control siRNA (lanes 1 and 3) or siRNAs against Cldn2 (#1) (lane 2) and Cldn12 (#1) (lane 4), incubated for 12 h after transfection, and then treated for 48 h with 100 nM 1α,25$(OH)_2D_3$. 25 μg of whole cell extract from the cells was separated by SDS-PAGE and immunoblotted with an antiTRPV6 antibody, followed by chemiluminescence detection. The blots were stripped and immunoprobed with an antiactin antibody. (B) Expression of TRPV6 proteins in Caco-2, Caco-2:Cldn2 (clone #1) and Caco-2:Cldn12 (clone #1) cells. Western blot analysis was performed as in A. (C) Cells were transfected and treated as in A, subjected to immunostaining

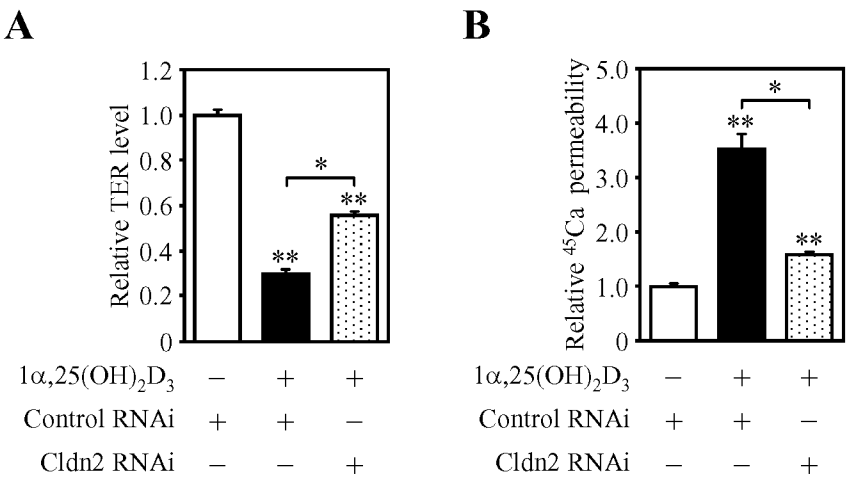
with the corresponding antibodies, and observed under a laser-scanning confocal microscope. Bar, 10 μm. (D) Caco-2, Caco-2:Cldn2 (clone #1) and Caco-2:Cldn12 (clone #1) cells were subjected to double immunostaining with the corresponding antibodies. Bar, 10 μm.

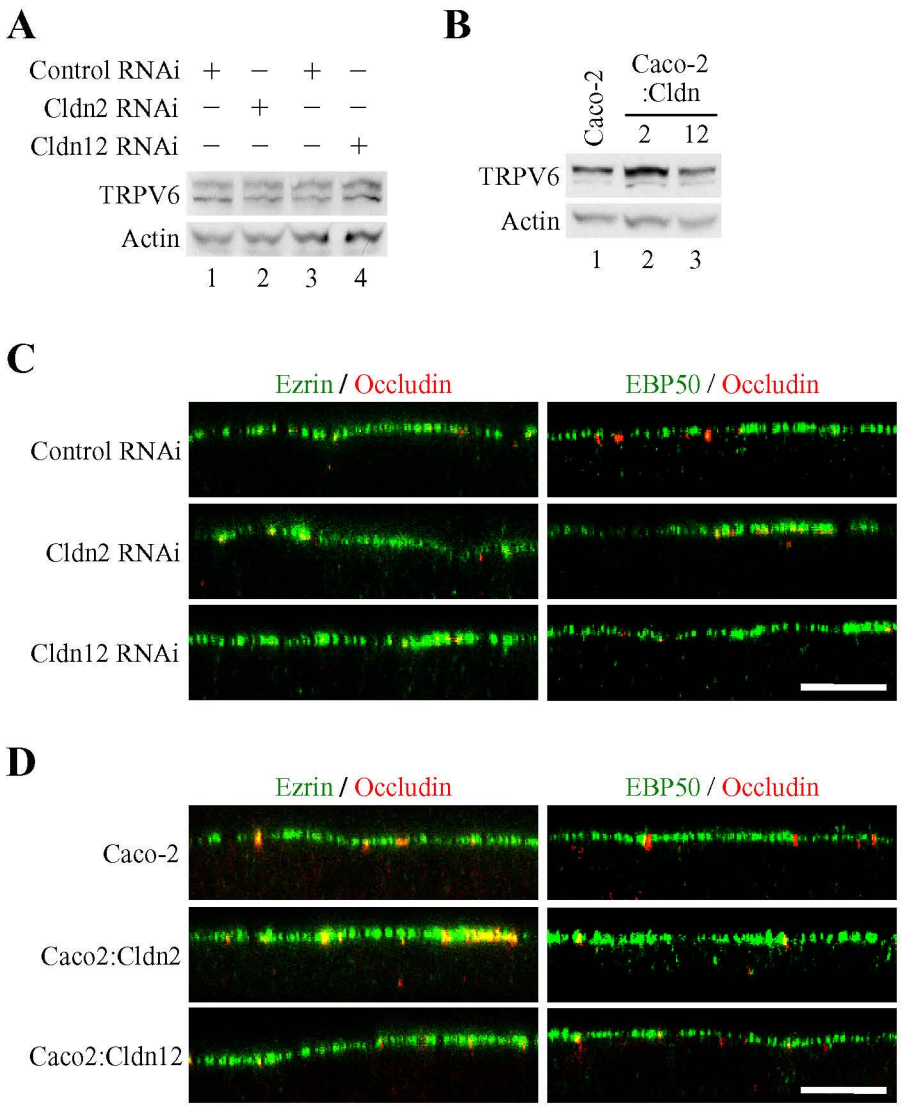
Figure S4. Knockdown of Cldn7 expression in Caco-2 cells does not alter levels of TER or $^{45}Ca^{2+}$ permeability. (A) Cells were transfected with negative control siRNA (lane 1) or Cldn7 siRNAs (#1-3; lanes 2-4, respectively), incubated for 12 h after transfection, and grown for 48 h without $1\alpha,25(OH)_2D_3$. 25 μg of whole cell extract from the cells was separated by SDS-PAGE and immunoblotted with an antiCldn7 antibody, followed by chemiluminescence detection. The blots were stripped and immunoprobed with an antiactin antibody. (B, C) Cells were transfected with negative control siRNA or the Cldn7 siRNA #1, and incubated and grown as in A. They were subjected to measurement of TER (B) and calcium transport studies (C). The values represent the mean ± SD (error bars; n = 6). $P>0.05$ compared with values of cells transfected with control siRNA.

Figure S5. Staining pattern of claudin-15 (Cldn15), and the levels of TER and $^{45}Ca^{2+}$ permeability in Caco-2 and Caco-2:Cldn15 cells. The values represent the mean ± SD (error bars; n = 4). *, $P<0.05$ compared with values of the mock-transfected cells.

Figure. S1


Cldn2
Occludin
Merge
Cldn7
Occludin
Merge
Cldn12
Occludin
Merge
Cldn15
Occludin
Merge


A
Relative TER level
1.2
1.0
0.8
0.6
0.4
0.2
0
**
*
**
1α,25(OH)2D3 – + +
Control RNAi + + –
Cldn2 RNAi – – +
B
Relative 45Ca permeability
5.0
4.0
3.0
2.0
1.0
0
**
*
**
1α,25(OH)2D3 – + +
Control RNAi + + –
Cldn2 RNAi – – +


A
Control RNAi + − + −
Cldn2 RNAi − + − −
Cldn12 RNAi − − − +
TRPV6
Actin
1 2 3 4
B
Caco-2
Caco-2 :Cldn
2 12
TRPV6
Actin
1 2 3
C
Ezrin / Occludin
EBP50 / Occludin
Control RNAi
Cldn2 RNAi
Cldn12 RNAi
D
Ezrin / Occludin
EBP50 / Occludin
Caco-2
Caco2:Cldn2
Caco2:Cldn12

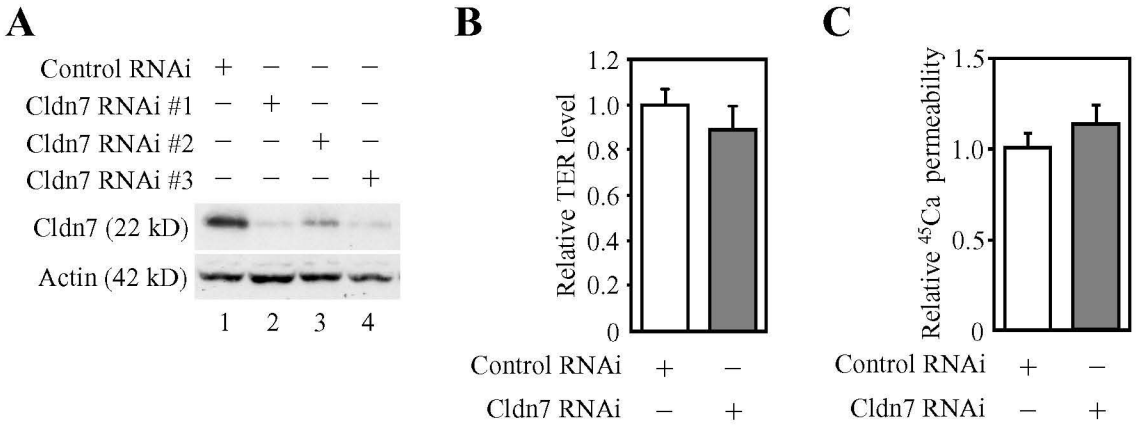
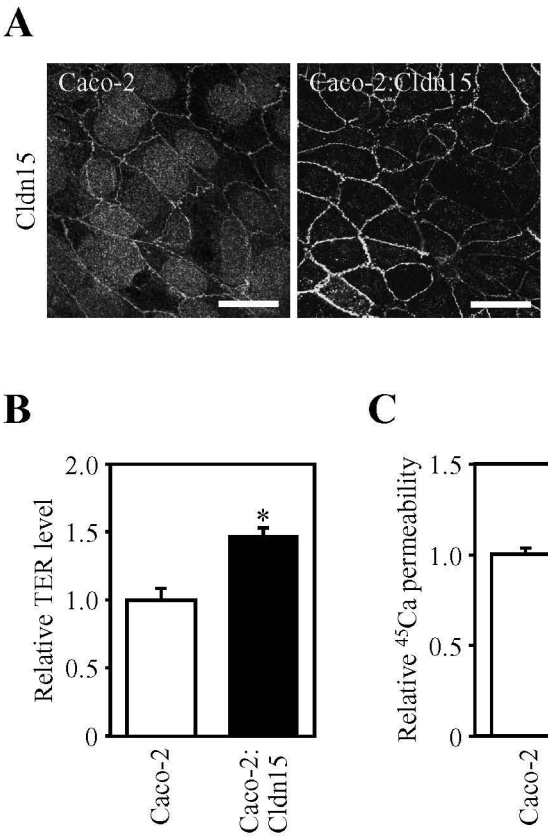

A
Control RNAi + − − −
Cldn7 RNAi #1 − + − −
Cldn7 RNAi #2 − − + −
Cldn7 RNAi #3 − − − +
Cldn7 (22 kD)
Actin (42 kD)
1 2 3 4
B
Relative TER level
1.2
1.0
0.8
0.6
0.4
0.2
0
Control RNAi + −
Cldn7 RNAi − +
C
Relative 45Ca permeability
1.5
1.0
0.5
0
Control RNAi + −
Cldn7 RNAi − +

Figure. S5


A
Caco-2
Caco-2:Cldn15
Cldn15
B
Relative TER level
2.0
1.5
1.0
0.5
0
*
Caco-2
Caco-2: Cldn15
C
Relative ^{45}Ca permeability
1.5
1.0
0.5
0
Caco-2
Caco-2: Cldn15