Supplemental Materials

Molecular Biology of the Cell

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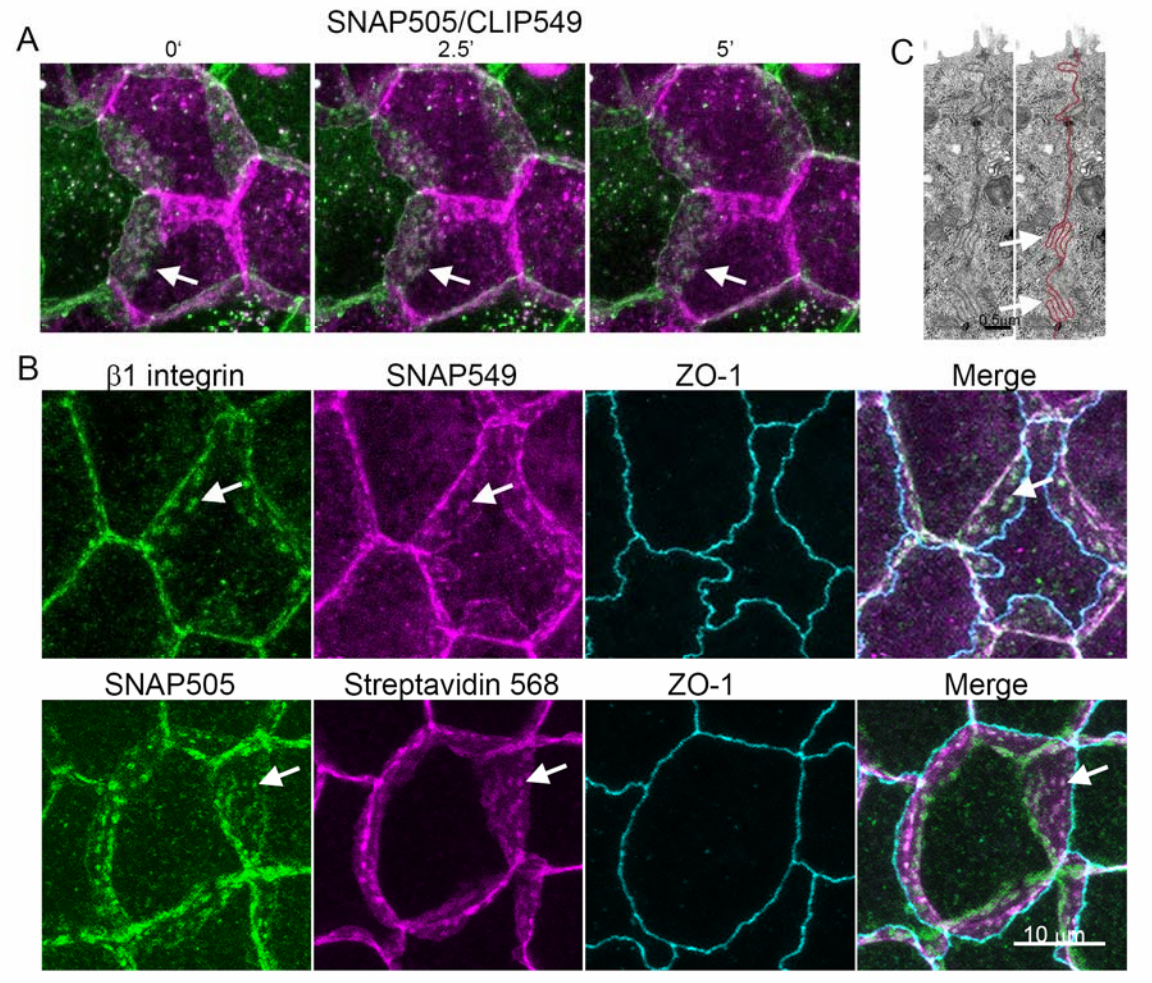
Supplementary Figure Legends

Supplementary Fig. S1. Apparent patchy lateral cldn4 distribution is likely due to infoldings of the lateral plasma membrane. (A) Live cell imaging of MDCK cells separately stably expressing SNAP cldn4 or CLIP cldn4 were co-cultured, labeled with SNAP-cell 505* and JF549 CLIP-ligand and imaged using a Zeiss 880 Airyscan super resolution microscope. Lateral patches of SNAP and CLIP cell cldns moved together over the 5' imaging period (arrows). (B) (Top panels) Patches of β-integrin co-localize with patches of lateral cldn; arrows point to examples of co-localization (Bottom panels) Biotinylation of lateral membrane proteins reveals similar concentration to that seen for lateral SNAP cldn4; arrows point to areas of co-concentration. (C) Transmission electron microscopic image of MDCK cells shows infoldings in the lateral membrane (outlined in red).

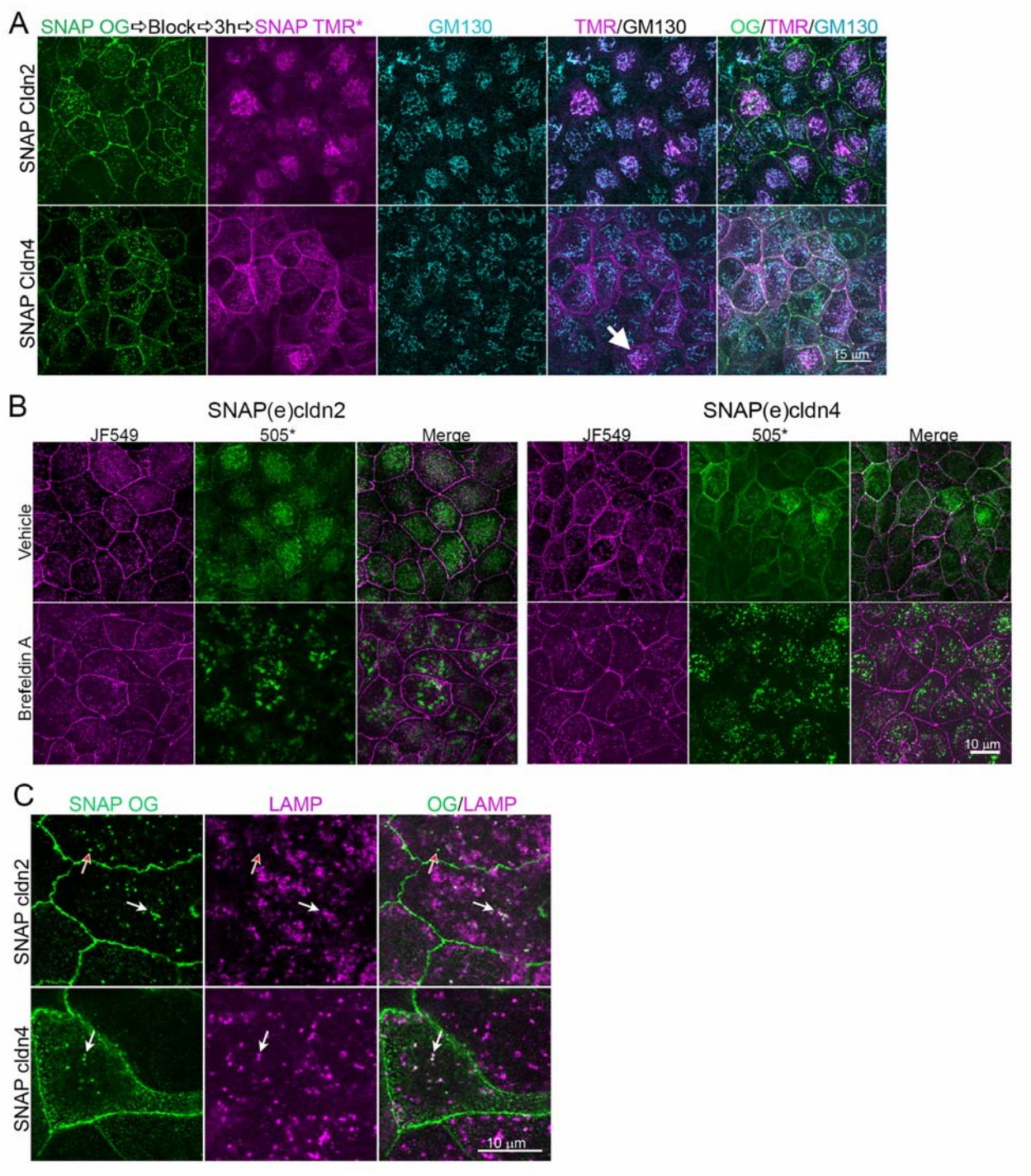
Supplementary Fig. S2. Intracellular cldns co-localize with a Golgi marker early in biosynthetic pathway and later partially co-localize with a lysosomal marker. A. MDCK cells expressing SNAP cldn2 (top panels) or SNAP cldn4 were labeled with SNAP-cell Oregon Green (OG), blocked and then labeled 3h post-block with SNAP-cell TMR*, then fixed and stained for the Golgi marker GM130. At this time, almost all newly synthesized SNAP cldn2 co-localizes with GM130; a fraction of the SNAP cldn2 also co-localizes with GM130 (arrow), but much of newly synthesize SNAP cldn4 is already on the lateral membrane. B. MDCK cells expressing SNAP(e)cldn2 (left panels) and SNAP(e)cldn4 (right panels) were incubated with JF549 SNAP ligand, blocked with SNAP cell block and vehicle or Brefeldin A (5 μg/ml) added. After 4h, cells were incubated with SNAP-cell 505*, fixed and imaged. Maximum intensity projections reveal that Brefeldin A treatment results in loss of membrane localization of new cldns. C. When MDCK cells expressing SNAP cldn2 (top panels) and SNAP cldn4 (bottom panels) are incubated with OG, some (white arrows) but not all (red arrows) vesicular cldns co-localize with the lysomal membrane marker, LAMP.

Supplementary Fig. S3. RTqPCR demonstrates that exogenously expressed SNAP cldn2 and SNAP cldn4 mRNAs (red symbols) are expressed at approximately the same levels as endogenous MDCK cell cldn2 and cldn4 mRNAs (blue symbols). Values are normalized to endogenous ZO-1 levels; it is notable that cldn2 mRNA levels (both endogenous and exogenous) are more than 6-fold higher than ZO-1 or cldn4. The mechanism is unknown but might reflect differences in mRNA stability.

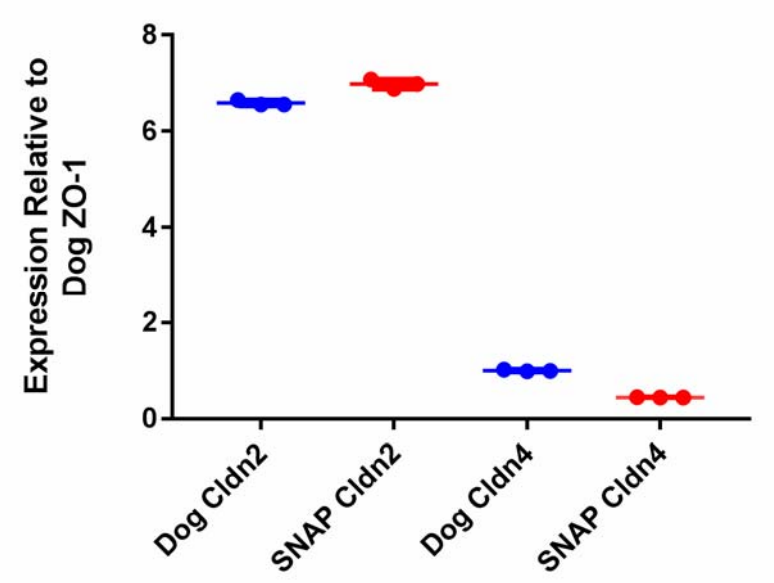
Supplementary Fig. S4. SNAPcldn4 localization and half-life are unaffected in Z1/Z2 dKd cells. (A) Time course of localization of old (JF549, left panels) and new SNAPcldn4 (505*, middle panels) are similar between wild-type MDCK cells and Z1/Z2 dKd cells at 7h. (B) Fluorescent imaging (upper panel) of duplicate samples of JF549-labeled SNAPcldn4 in wildtype cells and in Z1/Z2 dKd cells at various times after labeling reveals similar decay curves (quantified in (C) in both cell lines. Ocln (lower panel) is included as a loading control.



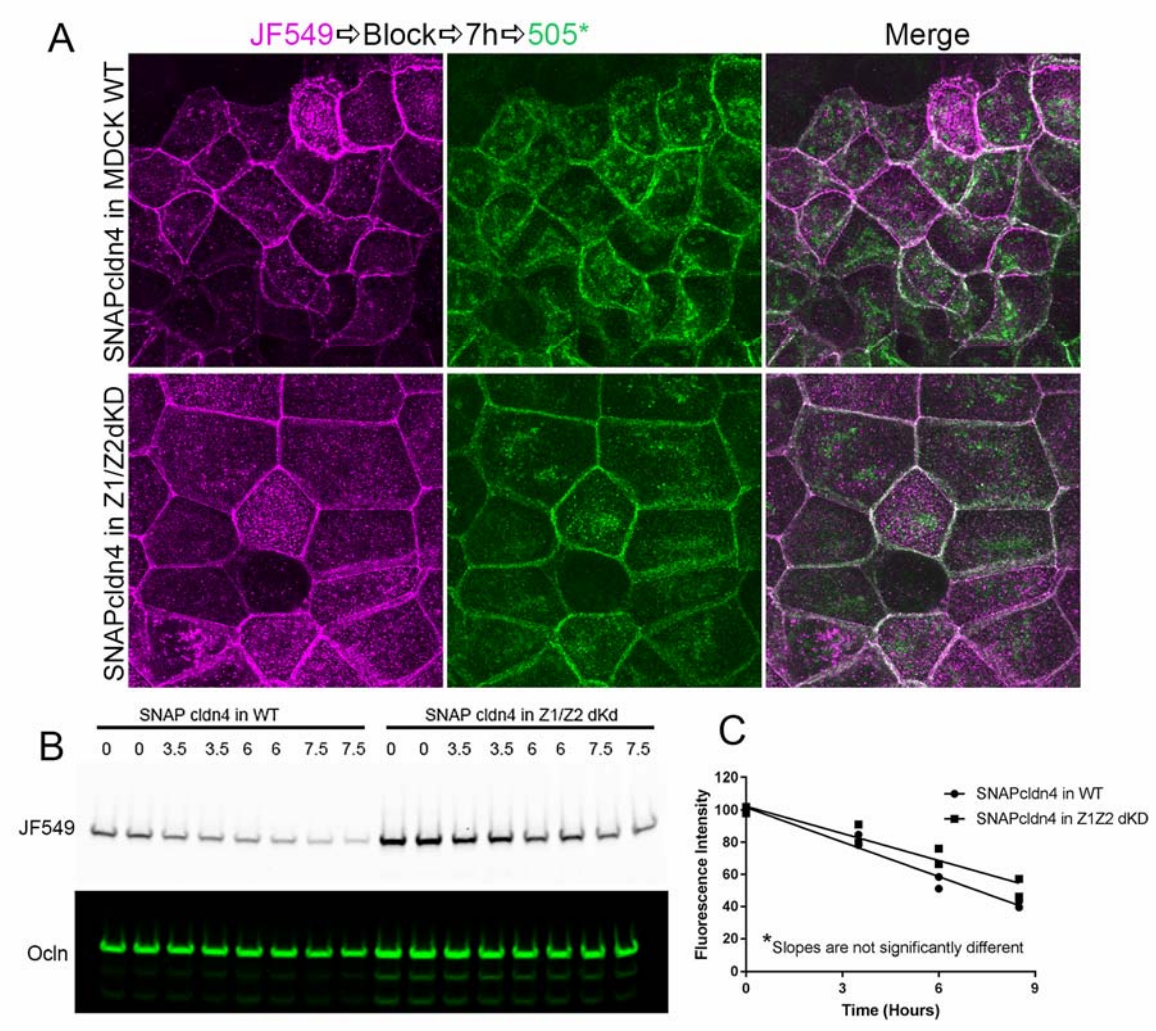
Supplementary Fig. S1.



Supplementary Fig. S2.



Supplementary Fig. S3.



Supplementary Fig. S4.

Supplementary Table S1.

This table contains a list of all primers used in plasmid construction, for sequencing and for quantitative real-time PCR.

Supplementary Table 1

For generation of SNAP(e)cldn2 and SNAP(e)cldn4 construction

InFusion Primers	**Sequence**
Cldn2_HA1_F	ATCGTCGCTAGCGATATCAGAACAGCTTCCCTTTCCTGTCT
Cldn2_HA1_R	TGTCCATGGTGAATTGGCGGACCTCCCAGCAGA
Cldn2_HA2_F	CGCGTTTAAACTCGAGGCCTCTCTCGGCCTCCAAC
Cldn2_HA2_R	GTTATCTATGCGGCCGCCTTGGCAAGCAATCTTGACAATCG
Cldn4_HA1_F	ATCGTCGCTAGCGATATCGCTTGCTCTGGGTGACCTCC
Cldn4_HA1_R	TGTCCATGGTGAATTGGCTCGCCGTCCGCTGGG
Cldn4_HA2_F	CGCGTTTAAACTCGAGGCCTCCATGGGGCTGCAG
Cldn4_HA2_R	GTTATCTATGCGGCCGCTCCTCCTGGCCAACACAG
gRNA	**Sequence**
Cldn2_F	CACCGAGGCCGAGAGAGGCCATGG
Cldn2_R	AAACCCATGGCCTCTCTCGGCCTC
Cldn4_F	CACCGGCGAGCCATGGCCTCCATG
Cldn4_R	AAACCATGGAGGCCATGGCTCGCC
Sequencing Primers	**Sequence**
Cldn2_F	GGTCAAAAGGCATCCAGCATT
Cldn2_R	GCTGGTACCAACGTAGGAGC
Cldn4_F	GGACCGAAACCCCTCCCTCT
Cldn4_R	CGCTCTCATCCTCCACGCAG

For construction of SNAP/CLIP cldn4 and mutants

Infusion/PCR Primers for cloning and mutants	**Sequence**
Cldn4_F	TCCTGCAGGCGGATCCATGGCCTCCATGGGGCTACAGGTA
Cldn4_R	ATTAATTAACCTCGAGTTACACGTAGTTGCTGGCAGCAGC
Cldn4(-3)_F	CTCTGCTGCTGCCAGCTAACTACGTGTAACTCG
Cldn4(-3)_R	CGAGTTACACGTAGTTAGCTGGCAGCAGCAGAG
Cldn4_S194A_F	GCACAGACAAGCATTACGCCGCCAAGTATTCTG

Cldn4_S194A_R	CAGAATACTTGGCGGCGTAAGGCTTGTCTGTGC
Cldn4_S194E_F	GGGCAGCAGAATACTTGGCTTCGTAAGGCTTGTCTGTGCGG
Cldn4_S194E_R	CCGCACAGACAAGCCTTACGAAGCCAAGTATTCTGCTGCCC
Ocln_F	TCCTGCAGGCGGATCCATGTCATCCAGGCCTCTTGAAAGT
Ocln_R	ATTAATTAACCTCGAGTCATGTTTTCTGTCTATCATAGTC

gRNA (CRISPR/Cas9 KO)	**Sequence**
Cldn2_F	CACCCACGATGCTGGTACCAACGT
Cldn2_R	AAACACGTTGGTACCAGCATCGTG

RTqPCR Primers	Sequence
Dog ZO-1 F	AGTTTGTCTCCACGGTCTGA
Dog ZO-1 R	GGATTTCACCAATGTGACTT
Dog cldn2 F	GTTGGTACCAGCATCGTGAC
Dog cldn2 R	TGTCACACTGGGTTATGCCT
Ms cldn2 F	CTGCCAGGATTCTCGAGCTA
Ms cldn2 R	AGGATGCCACCAAGGATGAA
Dog cldn4 F	CTGGCTTGTCCTTCTGACTG
Dog cldn4 R	GACAGACACTCTGCGTCCAC
Hu Cldn4 F	ATTGTCACCTCGCAGACCATCTGG
Hu Cldn 4 R	AGCAGCCACGATGATGCTGATGAT