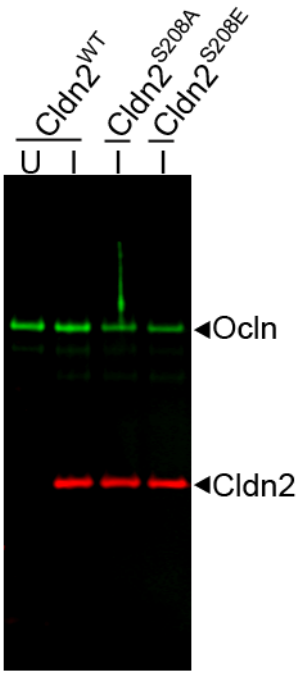

Fig. S1. Treatment of stably transfected MDCK II cells with claudin-2 S208A and S208E with 0.05ng/ml doxycycline results in transgene induction to equivalent levels (U=uninduced, I=induced); ratio of transgene to occludin is 3.4 for S208A and 3.2 for S208E.

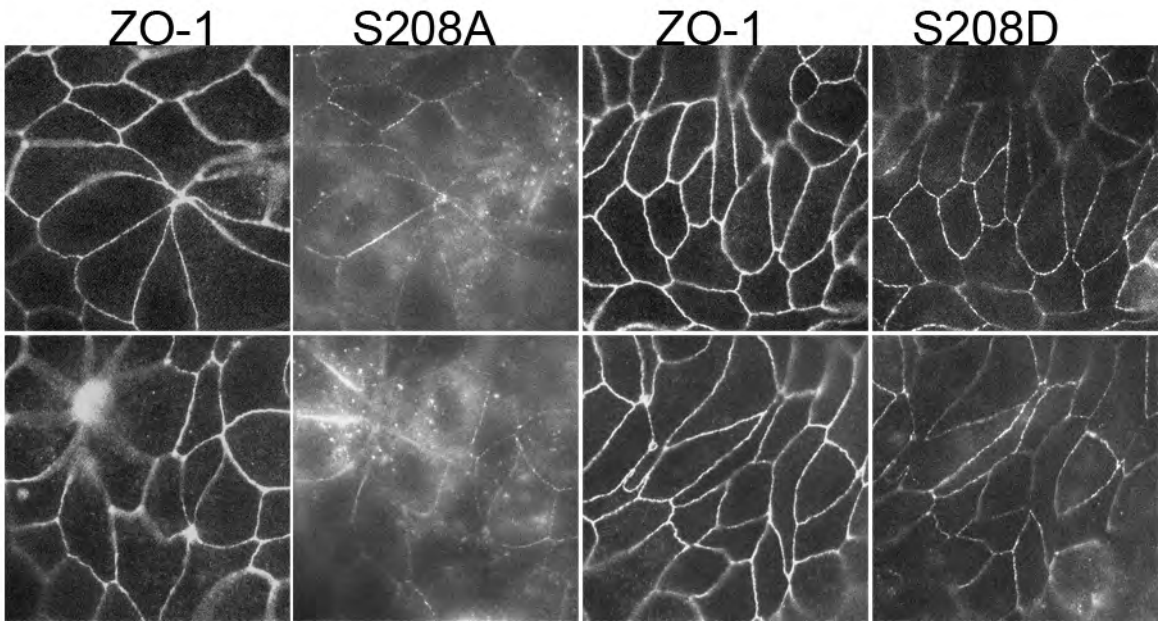

Fig. S2. Like S208E, claudin-2 S208D localizes to cell-cell contacts better than non-phosphorylatable claudin-2 mutant S208A. (Left 4 panels) ZO-1 immunofluorescence marks cell contacts while claudin-2 S208A is localized to cell contacts but also to abundant intracellular puncta. In contrast, (Right 4 panels) both ZO-1 and claudin-2 S208D are largely localized to cell membranes.

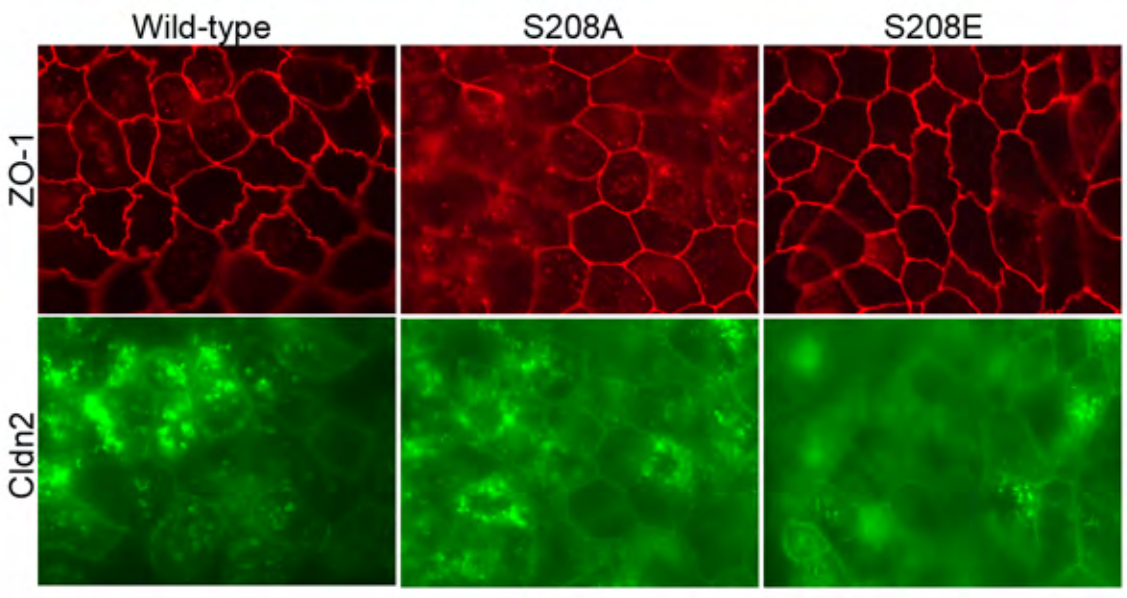

Fig. S3. Maximal induction of wild-type claudin-2 (left panels), S208A (middle) and S208E (right) mutants results in similar localization to both cell contacts and intracellular vesicles for all claudin forms. ZO-1 localization is mostly unaffected, although there is a small increase in intracellular accumulation in all clones.

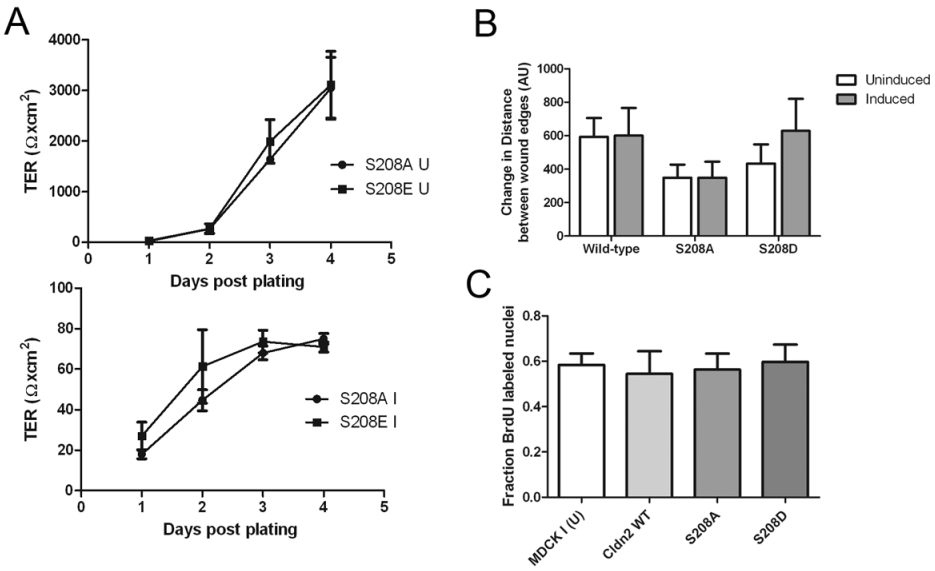

Fig. S4. Expression of claudin-2 S208 mutants has little effect on physiologic parameters. (A) MDCK I Tet-off cells not induced to express claudin-2 mutants develop normal TER levels over 4 days in culture to approximately 3000 Ωcm^2 (top panel), while cells induced to express either claudin-2 S208A or S208E develop maximal TER below 100 Ωcm^2 over a similar time course. (B) MDCK I Tet-off cells inducibly expressing wild-type claudin-2 or S208A or S208E mutants show similar wound healing behavior independent of claudin-2 induction. (C) Uninduced MDCK I cells or cells induced to express wild-type claudin-2 or S208A or S208E mutants show identical levels of nuclear labeling for BrdU.

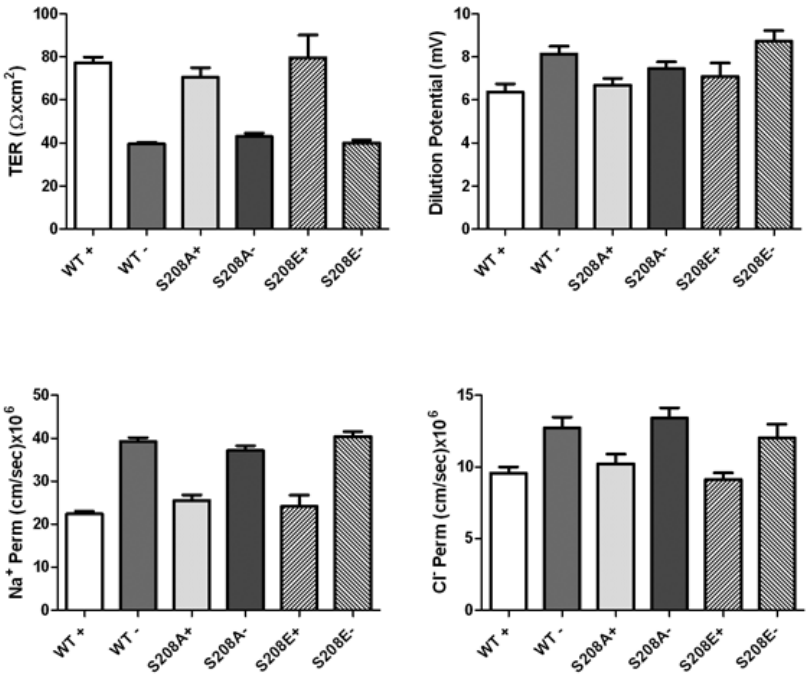

Fig. S5. Induction of wild-type claudin-2, S208A and S208E in claudin-2 KD background results in similar changes in TER, dilution potential and Na^+ and Cl^- permeability.

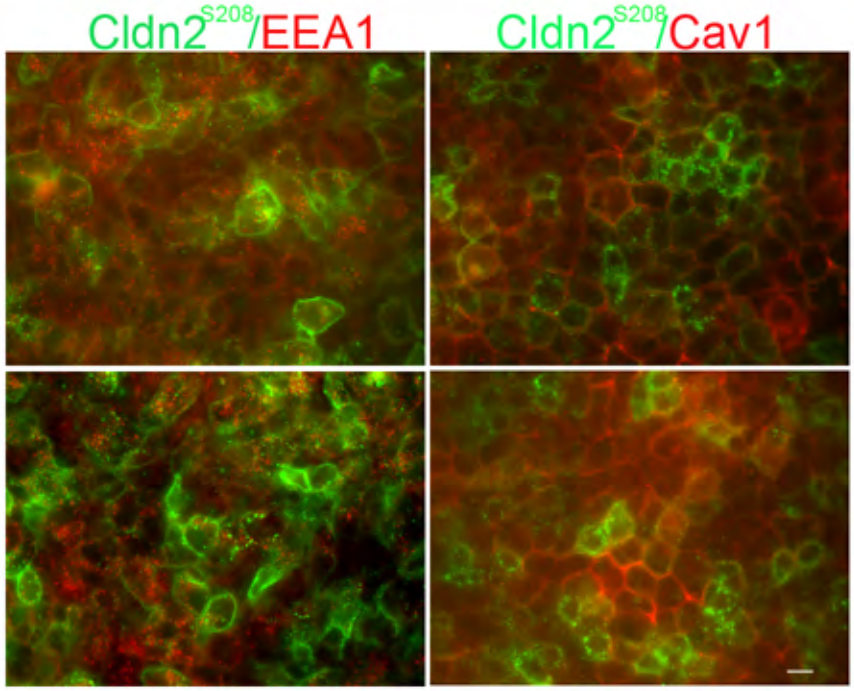

Fig. S6. Immunofluorescent analysis of the localization of claudin-2 S208 (green and the endosomal marker EEA-1 (red, left) or with caveolin-1 (red, right) does not reveal significant colocalization; two examples of each (top and bottom) is shown.

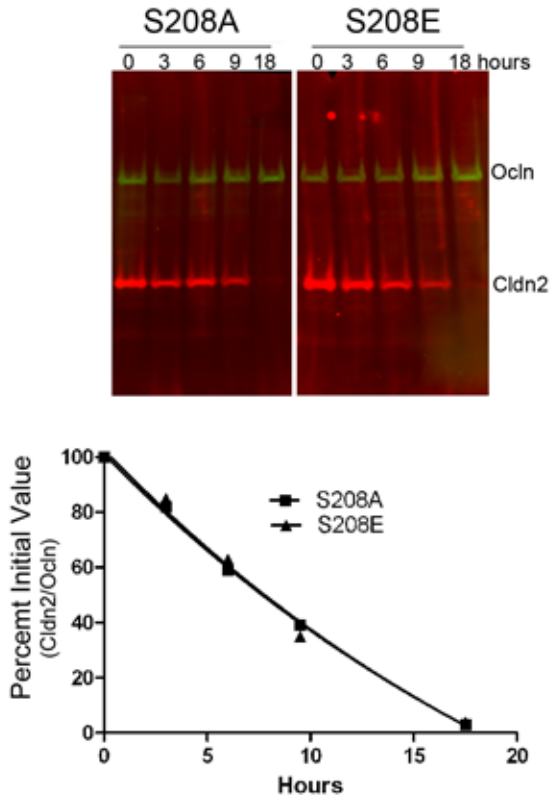

Fig. S7. Half-lives of claudin-2 S208A and S208E mutants are identical. Stable cell lines expressing each mutant were induced for 4 days in the absence of doxycyline; at time=0 50ng/ml doxycycline was added and cells were collected at the indicated intervals. Samples were immunoblotted for claudin-2 and occludin and the ratio of claudin-2/occludin plotted. This experiment was repeated three times with different mutant clones with similar results.

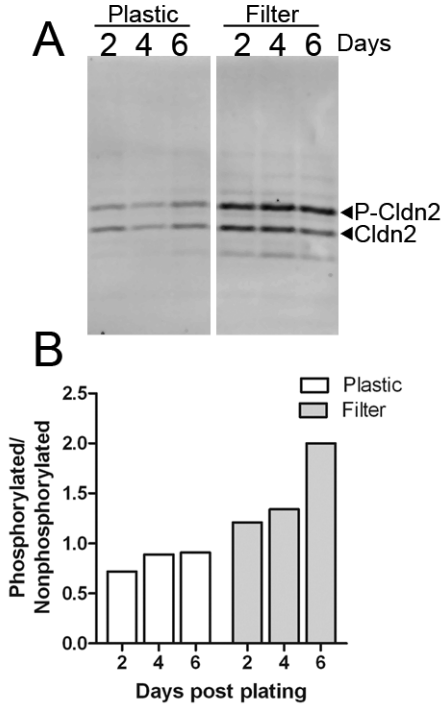

Fig. S8. Wild-type MDCK II cells were plated at $5x10^4$ cells/cm^2 on 24-well tissue culture plates or transwell filters and collected at the indicated times. Samples were immunoblotted for claudin-2 after phos-tag gel electrophoresis (A) and the ratio of phosphorylated to nonphosphorylated claudin-2 was determined.

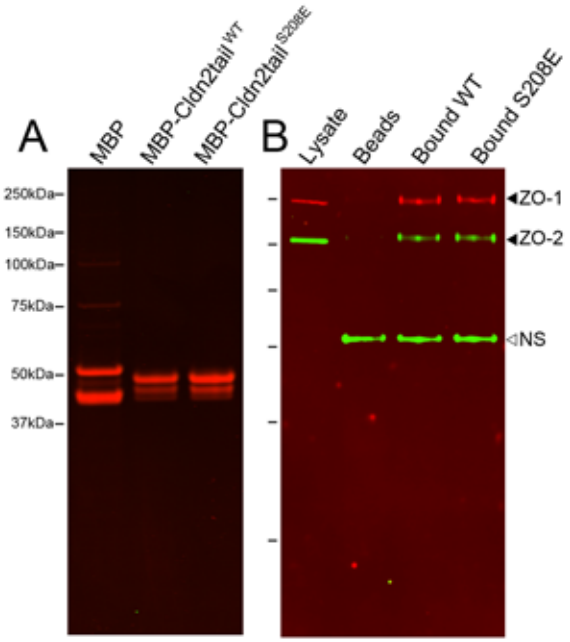

Fig. S9. Phosphorylation state does not alter interaction of claudin-2 with ZO-1 or ZO-2. (A) SDS-PAGE of bacterially-expressed, metal affinity resin-purified fusion proteins; left, MBP, middle, MBP-tail claudin-2, right MBP tail claudin-2 S208E. (B) Immunoblot for ZO-1 (red) and ZO-2 (green) after fusion protein pull-down; left lane, MDCK II lysate; Beads, non specific binding to MBP alone; Bound WT, ZO-1 and ZO-2 bound to MBP-cld2, Bound S208E, ZO-1 and ZO-2 bound to MBP-mutant cldn2. NS, non-specific band binding to all fusion proteins.

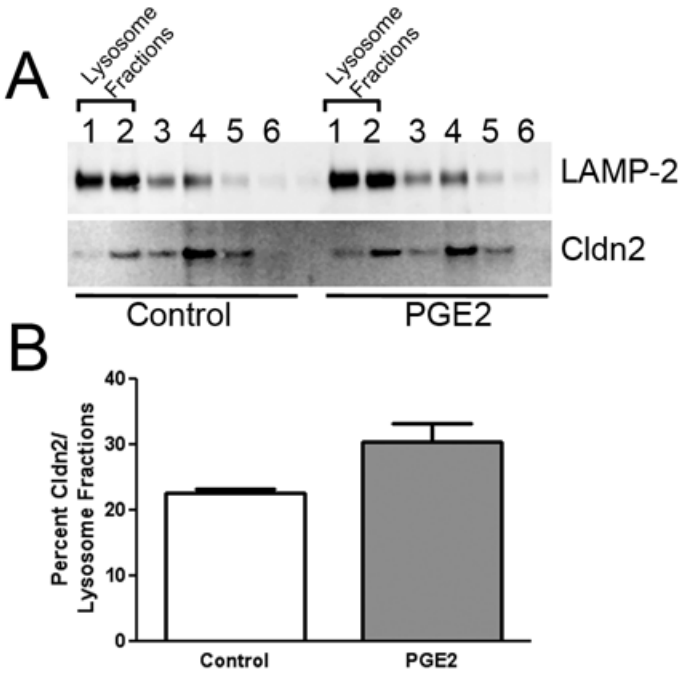

Fig. S10. PGE2 treatment of MDCK II cells results in a small but reproducible shift of claudin-2 into the lysosome fractions. MDCK II cells were cultured on 6-well Transwell filters for 4 days and treated with 1 μM PGE2 for 60 min. Monolayers were washed with PBS and 3 filters of control and treated cells were combined for gradient fractionation. (A) Gradient fractions were subjected to SDS PAGE and immunoblotted for LAMP-2 (top panel) and claudin-2 (lower panel). (B) The amount of claudin-2 in the each fraction was quantified and the percent in the two fractions with the most LAMP-2, were compared between the control and PGE2 treated samples.

Table S1. The percent of serine plus threonine (S/T), proline (P), tyrosine (Y), arginine plus lysine (R/K), glutamic acid plus aspartic acid (E/D) and the isoelectric point (PI) for the whole of several mouse claudins with the C-terminal tail is compared.

	%S/T overall	%S/T tail	%P overall	%P tail	%Y overall	%Y tail	%R/K overall	%R/K tail	%E/D overall	%E/D tail	PI overall	PI tail
cldn1	14.6	25.92	8.06	25.92	4.7	11.11	4.27	18.51	6.16	3.703	7.91	
cldn2	16.52	22.72	3.91	9.09	4.35	11.36	5.65	11.36	4.78	4.54	7.93	
cldn3	13.25	21.21	4.57	9.09	3.2	12.12	6.4	18.18	5.48	9.09	7.77	
cldn4	14.33	21.73	3.81	8.69	2.86	13.04	6.19	17.39	4.28	4.34	8.3	
cldn5	10.55	12.5	4.59	12.5	3.67	9.37	5.96	18.75	4.58	9.37	7.92	
cldn6	14.16	21.875	3.65	12.5	3.65	12.5	5.48	9.375	4.11	3.12	7.96	
cldn7	13.74	24	3.79	8	3.79	12	7.11	20	4.27	8	8.72	
cldn8	13.78	28.94	2.67	5.26	3.56	10.52	8.89	18.42	6.23	5.26	8.56	
cldn9	11.06	11.42	4.15	14.28	2.3	5.71	5.99	17.14	6.99	8.57	6.97	
cldn10a	15.72	22.44	2.18	6.12	3.93	6.12	8.3	14.28	4.8	8.16	8.71	
cldn11	13.53	29.62	3.86	3.7	3.86	11.11	5.8	7.4	4.35	7.4	7.9	
cldn12	15.99	26.08	4.1	10.86	4.1	6.52	6.97	4.34	4.51	4.34	8.47	
cldn14	15.9	17.85	5.44	14.28	4.6	10.71	7.11	10.71	3.35	7.14	8.88	
cldn15	17.18	23.4	3.08	6.38	4.41	6.38	5.72	10.63	5.28	10.63	7.51	
cldn16	12.39	13.33	3.42	8.88	5.13	11.11	8.55	20	8.54	8.88	6.94	
cldn17	9.82	13.51	4.46	10.81	3.57	8.1	8.48	21.62	4.92	5.4	9.15	
cldn18	15.15	16.66	2.27	4.54	3.79	6.06	6.44	15.15	5.69	10.6	7.87	
Average	**13.98**	**20.78**	**4.00**	**10.05**	**3.85**	**9.64**	**6.66**	**14.9**	**5.19**	**6.97**	**8.08**	

Table S1. Comparison of overall claudin amino acid composition with the composition of the C-terminal tail