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SUPPLEMENTARY ONLINE DATA

Doppel and PrP^{C} co-immunoprecipitate in detergent-resistant membrane domains of epithelial FRT cells

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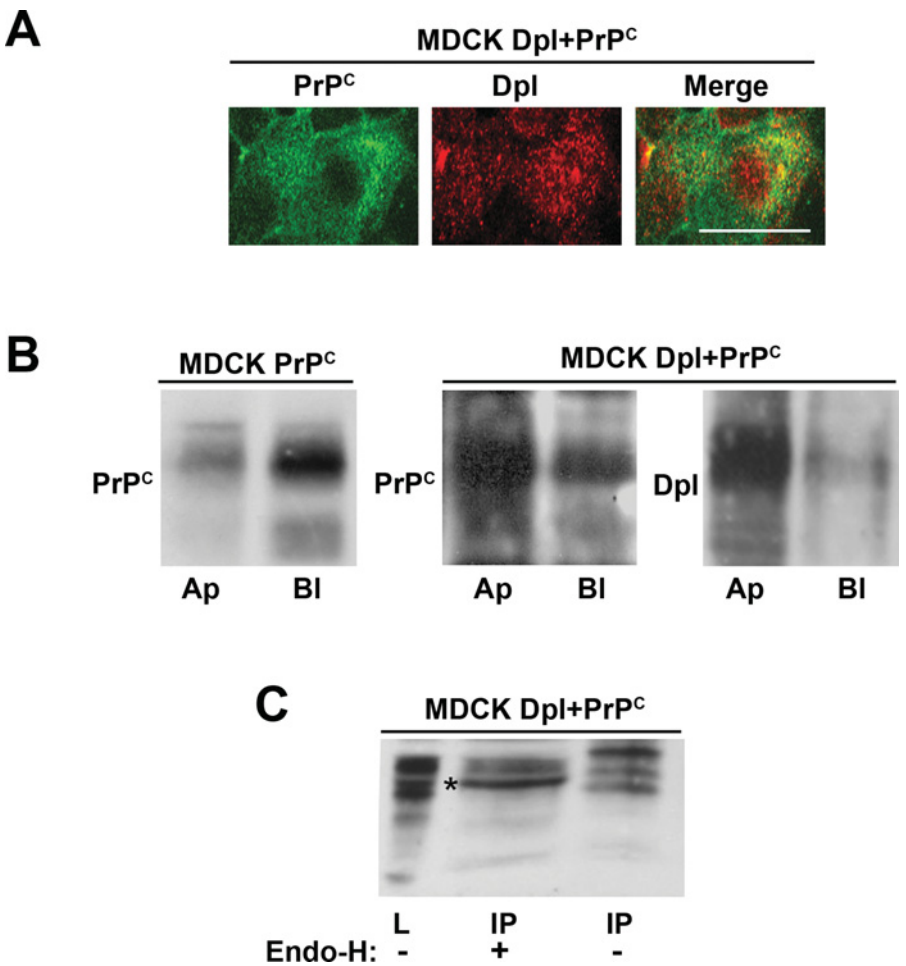

Figure S1 Co-expression of Dpl directs PrP^{C} to the apical cell surface of polarized MDCK cells

(**A**) MDCK cells expressing Dpl and PrP^{C} (MDCK Dpl + PrP^{C}) cells were fixed with 2% (w/v) paraformaldehyde and incubated for 20 min with the monoclonal antibody SAF-32, against PrP, and the polyclonal antibody Q55, against Dpl, and secondary FITC- and TRITC-conjugated antibodies were used to reveal PrP^{C} and Dpl respectively. Images were acquired with a Zeiss laser confocal microscope (LSCM 510). Scale bar, 10 μm. (**B**) After growth on transwell filters for 4 days, singly (MDCK PrP^{C}), or doubly (MDCK Dpl + PrP^{C}), transfected MDCK cells were selectively biotinylated from the apical (Ap) or basolateral (Bl) surface of the plasma membrane. Biotinylated Dpl and PrP^{C} were then recovered from cell lysates by immunoprecipitation with streptavidin beads and detected by immunoblotting with specific antibodies (Dpl 151 and SAF-32 antibodies respectively). Note that when PrP^{C} is co-transfected with Dpl its polarity is reversed (from basolateral to apical) and it is found localized on the apical surface like Dpl. (**C**) Native Dpl immunoprecipitated (IP) from MDCK Dpl + PrP^{C} cell lysates was digested with (+) and without (−) endo H (Endo-H) at 37 °C for 16 h and subjected to SDS/PAGE followed by Western blot analysis with the anti-Dpl Q55 antibody. (*) indicates the band resulting from the enzymatic digestion, indicating a partial sensitivity of Dpl to endo H. L, lysate.

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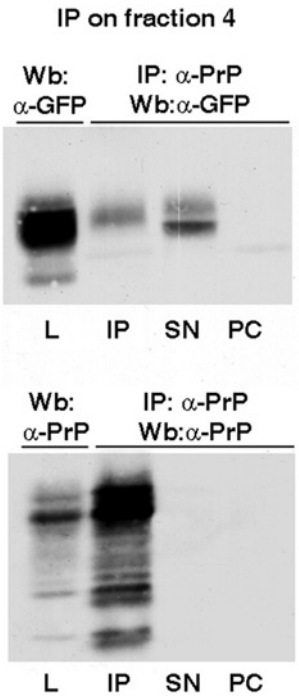

Figure S2 Co-immunoprecipitation between GFP–Dpl and PrP^C using an anti-PrP antibody in the precipitation step

The OptiPrep™ density gradient DRM fractions (4–5) of FRT GFP–Dpl + PrP^C clone lysate were first immunoprecipitated (IP) with the anti-PrP antibody and then revealed by Western blotting (Wb) with anti-GFP antibody (to reveal the co-immunoprecipitation) or anti-PrP antibody (to reveal the immmunoprecipitation). The loading control (L, 60 μg of cell lysate), the pre-clearing (PC) and 1/10 of the supernatants (SN) were also analysed.

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