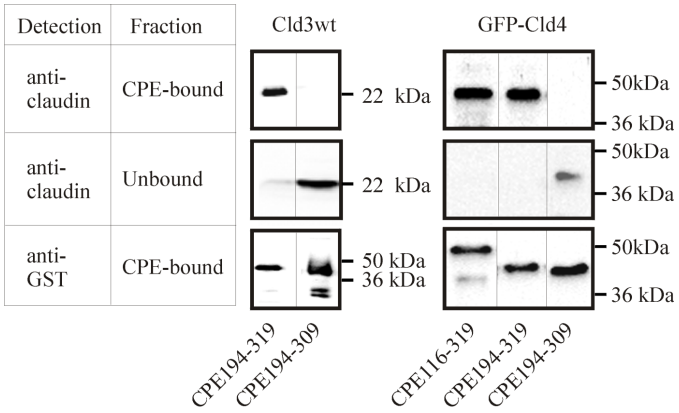
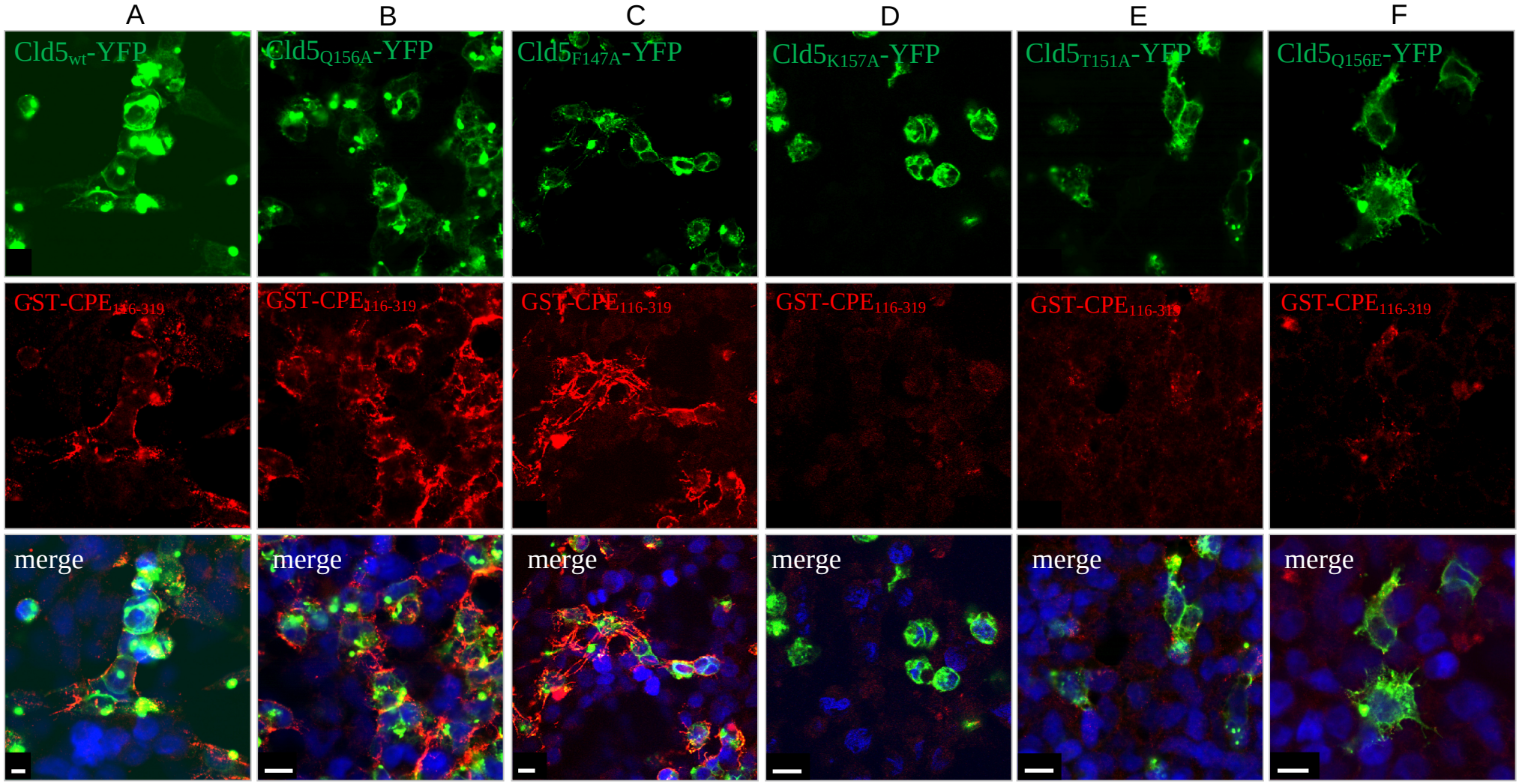


Sup. Fig 1. Deletion of the last 10 C-terminal amino acids diminishes the binding of GST-CPE to full length Cld3 and Cld4. HEK cells were transfected with Cld3wt (left) or GFP-Cld4 (right). Lysates thereof were used for pull down assays with GST-$CPE_{116-319}$, GST-$CPE_{194-319}$ or GST-$CPE_{194-309}$. Bound and unbound fractions were analyzed by SDS-PAGE and Western blot. Representative blots are shown. For GST-$CPE_{194-319}$, Cld3wt was found in the CPE-bound fraction but was barely detectable in the unbound fraction. In contrast, for GST-$CPE_{194-309}$ Cld3wt was barely detectable in the CPE-bound fraction but found in the unbound fraction. Similar, GFP-Cld4 was found in the CPE-bound fraction for GST-$CPE_{116-319}$ and GST-$CPE_{194-319}$ but in the unbound fraction for GST-$CPE_{194-309}$. The amounts of the different GST-CPE constructs found in the CPE-bound fractions were similar.

Sup. Fig 2: Binding of $CPE_{116-319}$ to $Cld5_{wt}$ and strong reduction of this binding by the substitutions T151A and Q156E but not Q156A and F147A detected by immunostaining. HEK293 cells were transfected with $Cld5_{wt}$-YFP or $Cld5_{mutant}$-YFP. Three days after transfection cells were incubated with 10 µg/ml GST-$CPE_{116-319}$ (1h, 37°C), washed with PBS and fixed. GST-$CPE_{116-319}$ (red) was detected by anti-GST antibodies, nuclei (blue) were stained with DAPI, Cld5 was detected by YFP-fluorescence. GST-$CPE_{116-319}$ bound to cells expressing $Cld5_{wt}$-YFP as well as to cells expressing $Cld5_{Q156A}$-YFP and $Cld5_{F147A}$-YFP that localize to the plasma membrane similar as $Cld5_{wt}$ (21). However, non-expressing cells as well as cells that express $Cld5_{K157A}$-YFP, which is strongly affected in plasma membrane targeting, did not bind GST-$CPE_{116-319}$. Moreover, substitutions T151A and Q156E, that did not affect the plasma membrane targeting of Cld5 (21), diminished the binding of GST-$CPE_{116-319}$.. Bar= 10 µm.


A
B
C
D
E
F
Cld5wt-YFP
Cld5Q156A-YFP
Cld5F147A-YFP
Cld5K157A-YFP
Cld5T151A-YFP
Cld5Q156E-YFP
GST-CPE116-319
GST-CPE116-319
GST-CPE116-319
GST-CPE116-319
GST-CPE116-319
GST-CPE116-319
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