Supplemental Materials for manuscript M803894

Multiple Membrane-Cytoplasmic Domain Contacts in CFTR Mediate Regulation of Channel Gating

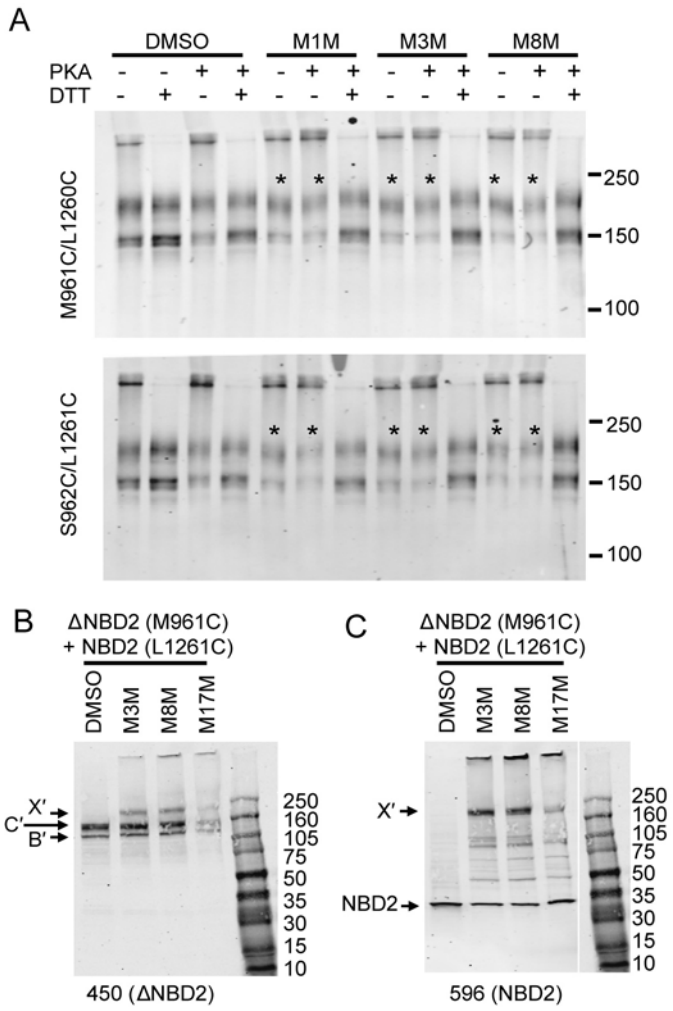
He/Alesksandrov/Serohijos/Hegedus/Aleksandrov/Cui/Dokholyan/Riordan

Supplemental Figure Legends

Supplement Fig. 1 CL3-NBD2 interface. A). Membrane vesicles were prepared from HEK cells transiently transfected with Cys-less CFTR plus Cys pair M961C/L1260C or S962C/L1261C. Control or PKA treated membrane vesicles were incubated with 20 μM M8M and proteins were resolved with 7.5% SDS-PAGE and detected with CFTR mAb 596. * denotes the faster moving band of cross-linked CFTR. PKA phosphorylation of CFTR did not affect cross-linking. **B&C).** Confirmation of CL3 and NBD2 interface with co-expressed ΔNBD2 and NBD2. HEK cells were transiently co-transfected with a Cys-less ΔNBD2 construct with M961C and a Cys-less NBD2 construct with L1261C. Cross-linking was carried out using the same protocol described in Fig. 2 legend, and the N-half and C-half fragments of CFTR were detected using mAb 450 (B) and mAb 596 (C), respectively. B′: immature core glycosylated ΔNBD2-CFTR; C′: mature complex glycosylated ΔNBD2-CFTR; X′: cross-linked ΔNBD2-CFTR and NBD2 fragment.

Supplement Fig. 2 Interface between CL1 and NBD1 at RI. A). HEK 293 cells transiently transfected with Cys-less CFTR with Cys pairs introduced at V171 of CL1 and E407 or L408 of the RI of NBD1 were incubated with MTS reagents and cross-linking was carried out as described in Fig. 2 legend. No cross-linked band was detected. **B).** Membrane vesicles were prepared from stable BHK cells with Cys-less CFTR plus Cys pair V171C/E407C or V171C/L408C. Control or PKA treated membrane vesicles were incubated with 20 μM M8M before subjecting to limited trypsin digestion as described in Fig. 3 legend. Partially digested fragments were resolved with 4-20% SDS-PAGE and Western blotting with CFTR mAb 13-4. The band highlighted in the rectangle is shown at the bottom. * denotes the cross-linked fragment that moved faster than its uncross-linked counterpart. **C).** Membrane vesicles were prepared from stable BHK cells with a Cys pair introduced at Q958 and L1261. After incubation with 20 μM M8M, membranes were subjected to limited trypsin digestion with concentrations indicated in the figure. Digested CFTR fragments were detected as described in B). No cross-linking was detected as predicted by the model.

Supplement Fig. 3 Negative cross-linking controls. Cross-linking was performed on transiently transfected HEK cells as described in Fig. 2 figure legend. Cys pairs that do not associate according to the structural model were introduced in Cys-less CFTR at different CLs and NBDs. A). L172C/E543C at CL1/NBD1; B). T966C/D1341C (CL3/NBD2); C). V171C/L1261C (CL1/NBD2); and D). M961C/L408C (CL3/NBD1). No cross-linking was detected.



Supplementary Figure 1
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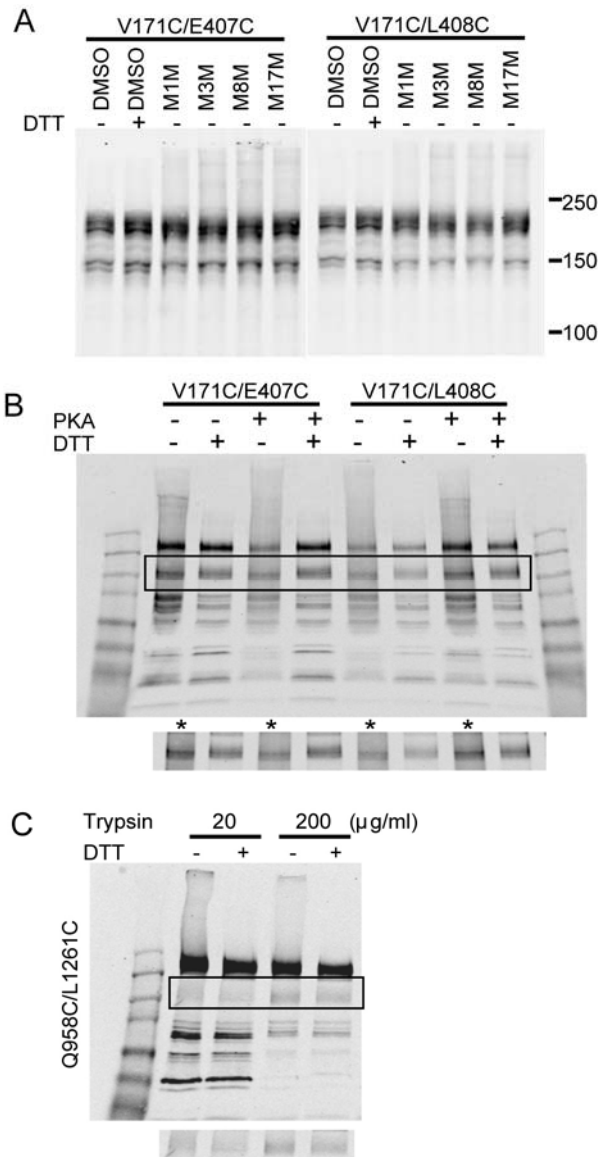

Supplementary Figure 2
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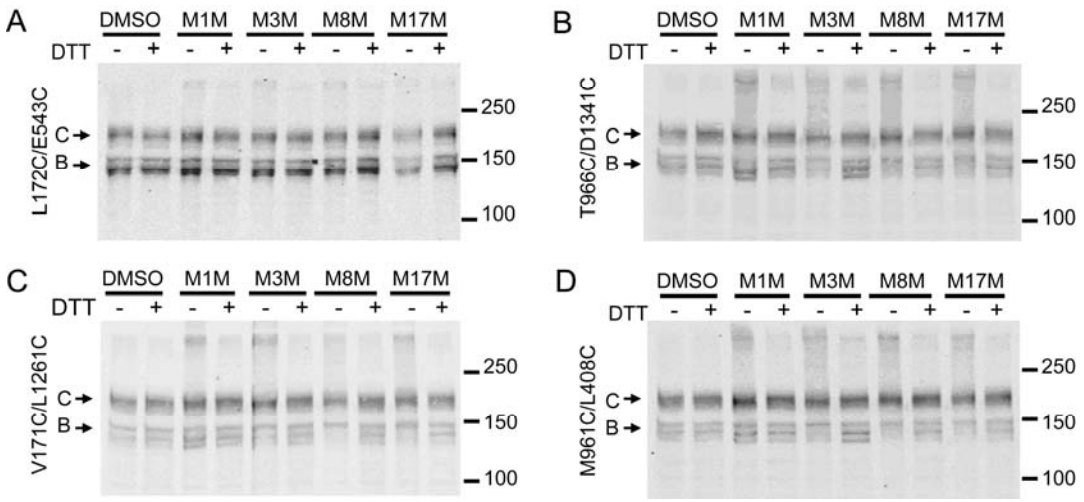

Supplementary Figure 3
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