

Fig S1. Quantitative RT-PCR analysis confirms that gene expression of MCORF 821 is not decreased in CL-deficient strains of O35E. Shown are the results of the qRT-PCR experiments examining gene expression of MCORF 821, which is located immediately downstream of *mclS*. The results are expressed as the mean fold expression (\pm standard error) of MCORF 819. Values are normalized to the expression of the reference gene *mcaP*.

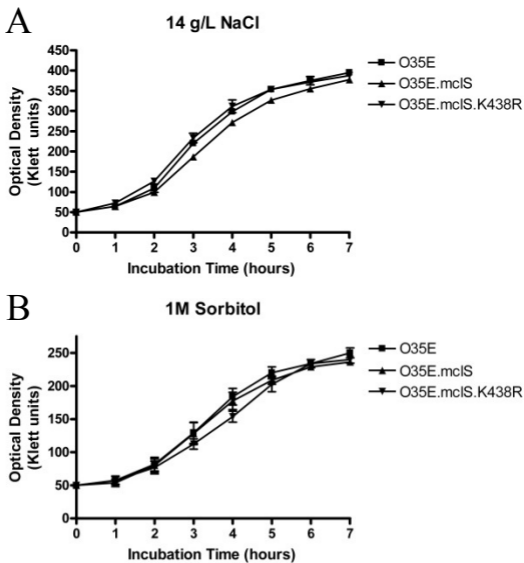


Fig S2. The CL-deficient strains do not exhibit reduced viability in conditions of osmotic stress. Growth curves of WT, *mclS* insertion mutant, and *mclS* point mutant strains of O35E when grown in TH broth containing 14 g/L sodium chloride (A) or 1M sorbitol (B).

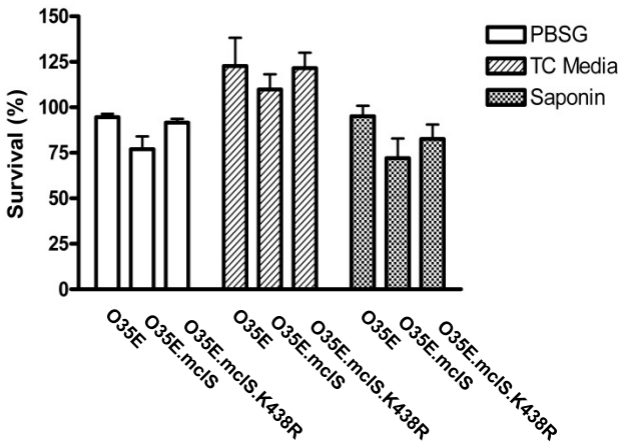


Fig S3. The absence of CL in *M. catarrhalis* does not affect its ability to survive in PBSG, TC media, and saponin solution. WT, *mclS* insertion mutant, and *mclS* point mutant strains of O35E were incubated in PBSG, TC media, and saponin solution for 15-30 minutes. The results are expressed as the mean percentage survival (\pm standard error) following incubation.

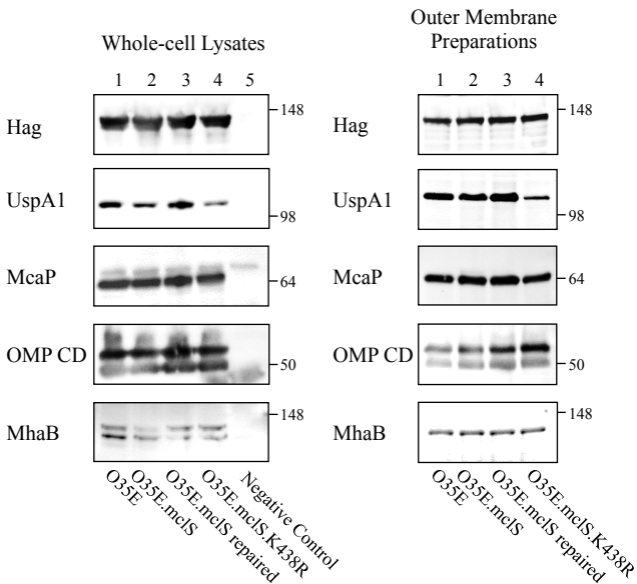


Fig S4. Adhesins are expressed and localized to the outer membrane in CL-deficient strains of *M. catarrhalis*. Western blot analysis of the whole-cell lysate (A) and outer membrane preparation (B) of strains O35E, O35E.mclS, O35E.mclS repaired, and O35E.mclS.K438R demonstrates expression and localization of the following adhesins: Hag, UspA1, McaP, OMP CD, and MhaB1/MhaB2. The negative control in lane 5 of panel A is O35E.ZCSM for Hag, UspA1, and McaP; O35E.CD1 for OMP CD; and O35E.B1B2 for MhaB1/MhaB2. The antibodies used for detection of the adhesins are described in the Materials and Methods.

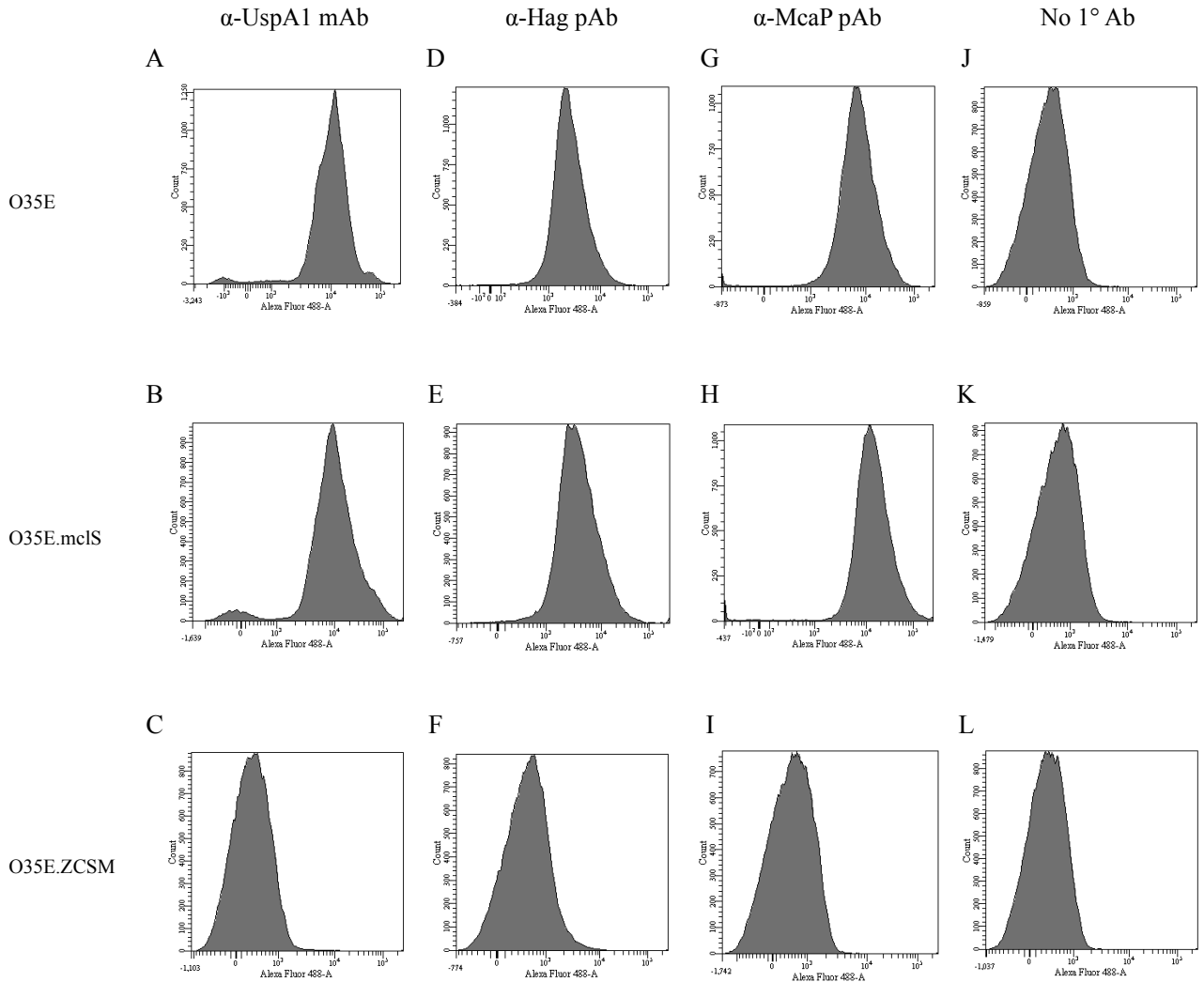


Fig S5. *Moraxella catarrhalis* adhesins are localized to and displayed on the outer membrane of WT and CL-deficient bacteria. Strains O35E (A, D, G, J), O35E.mclS (B, E, H, K), and O35E.ZCSM (C, F, I, L) were incubated with antibodies against UspA1 (A-C), Hag (D-F), and McaP (G-I) followed by incubation with Alexa Fluor 488-conjugated secondary antibodies. As a negative control, *M. catarrhalis* strains were incubated in the absence of primary antibody to determine background fluorescence (J-L).

The O35E.ZCSM strain lacks expression of UspA1, Hag, and McaP and therefore was included as a negative control. Display of adhesins was then analyzed by flow cytometry. A detailed procedure and description of the antibodies used for flow cytometry are found in the Materials and Methods. The x-axes represent the level of fluorescence, and the y-axes indicate the particle count. Shown are representative results of flow cytometry experiments.