Supporting Information: Binding of *Kingella kingae* RtxA Toxin Depends on Cell Surface Oligosaccharides, but Not on β_2 Integrins

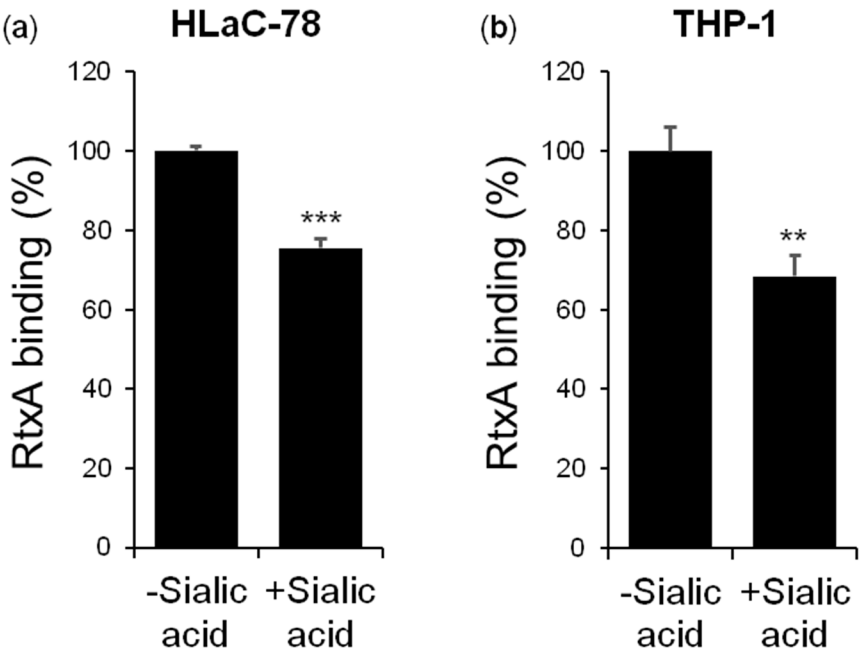

Figure S1. Binding of RtxA to cells is partially inhibited by free sialic acid. The purified and labeled RtxA-Dy495 toxin (2 μg/ml) was preincubated with 10 mM sialic acid or HBSS-Ca/Mg buffer alone for 1 min and then the solutions were added to HLaC-78 (**a**) or THP-1 (**b**) cells (1×10^6/ml). After 10 min at 4°C, the surface-bound RtxA-Dy495 was determined by flow cytometry and binding data were deduced from the MFI values and expressed as percentage of RtxA binding to sialic acid-untreated cells (taken as 100%). Each bar represents the mean value with SD of three independent experiments (**, $p<0.01$; ***, $p<0.001$; Student's t-test).

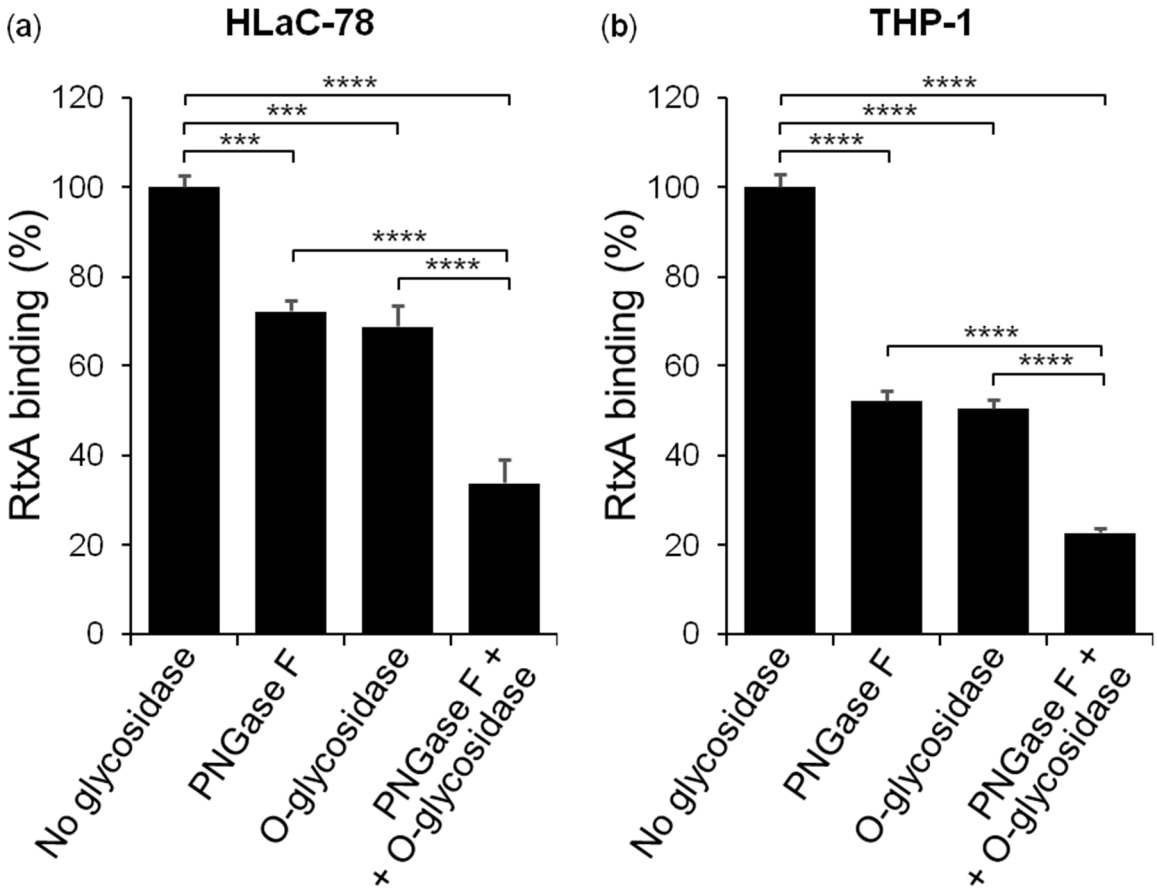

Figure S2. RtxA binding to cells deglycosylated with a combination of PNGase F and O-glycosidase was substantially decreased compared to cells deglycosylated individually with PNGase F or O-glycosidase. Human HLaC-78 (**a**) and THP-1 (**b**) cells were treated with PNGase F, O-glycosidase, a combination of PNGase F and O-glycosidase or buffer alone (no glycosidase) for 1 h at 37 °C. Then the cells (1×10^6/ml) were incubated with 2 μg/ml of the purified and labeled RtxA-Dy495 toxin for 10 min at 4 °C and the surface-bound RtxA-Dy495 was determined by flow cytometry. Binding data were deduced from the MFI values and expressed as percentage of RtxA binding to glycosidase-untreated cells (taken as 100%). Each bar represents the mean value with SD of three independent experiments (***, $p<0.001$; ****, $p<0.0001$; ANOVA).

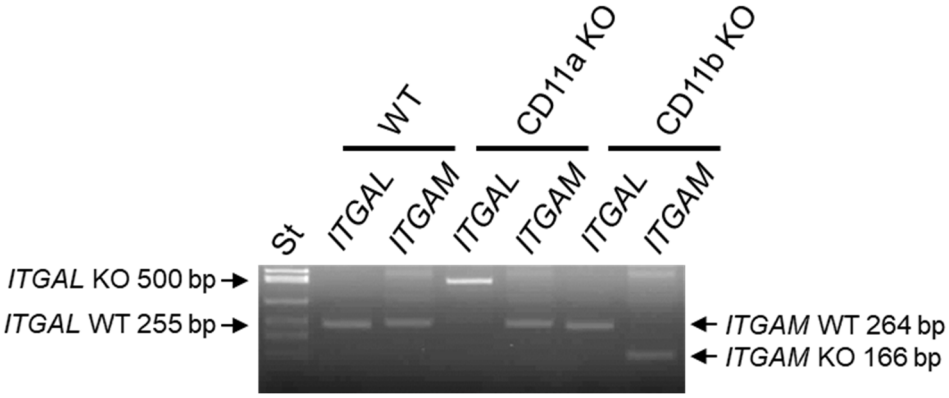

Figure S3. Genotyping of bone marrow-derived macrophages. DNA isolated from 1×10^6 bone marrow-derived macrophages of WT, CD11a KO and CD11b KO mice was amplified by PCR with primer pairs specific for *ITGAL* (encoding CD11a) and *ITGAM* (encoding CD11b) alleles. The product size of *ITGAL* and *ITGAM* WT alleles is 255 bp and 264 bp, respectively, of *ITGAL* KO allele is 500 bp, and of *ITGAM* KO allele is 166 bp.

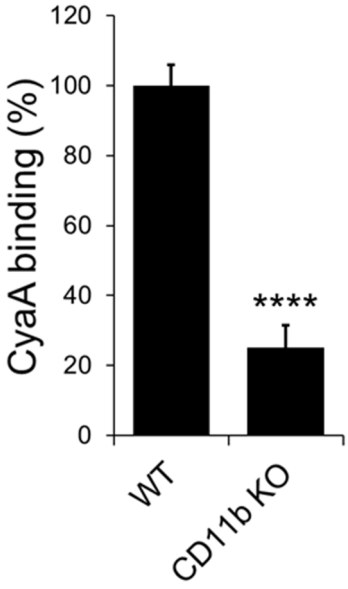

Figure S4. Bone marrow-derived macrophages of CD11b KO mice bind substantially lower amounts of CyaA than macrophages of WT mice. CD11b KO and WT macrophages (1×10^6/ml) were incubated with 5 μg/ml of the purified and Dy647-labeled CyaA toxoid (CyaA-AC$^-$, unable to covert ATP to cAMP) for 30 min at 4 °C and analyzed by flow cytometry. Binding data were deduced from the MFI values and expressed as percentage of CyaA binding to WT macrophages (taken as 100%). Each bar represents the mean value with SD of at least five independent experiments (****, p-value < 0.0001; Student's t-test).