

SPR Analysis. SPR analysis was performed similarly as described previously for other cell-surface receptors (42). Briefly, Mincle and MCL were each dissolved in 10 mM sodium acetate (pH 4) containing 5 mM CaCl₂. SPR experiments were performed with a BIACore T3000 (GE Healthcare). All of the proteins were covalently immobilized on a CM5 sensor chip by amine coupling (GE Healthcare). β 2-Microglobuline was used as a negative control protein. All glycolipids (C12, C10, and C8), and trehalose as a negative control, were injected in 10 mM Hepes (pH 7.4) containing 150 mM NaCl and 5 mM CaCl₂ with or without 5% (vol/vol) dimethyl sulfoxide. The data were analyzed using BIAevaluation software, version 4.1 (GE Healthcare).

Note Added in Proof. While this paper was under revision, Feinberg et al. (43) reported the crystal structure of bovine Mincle complexed with trehalose, whose binding mode is similar to that of human Mincle for glycolipids we proposed here.

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