

Supplement Figure 6

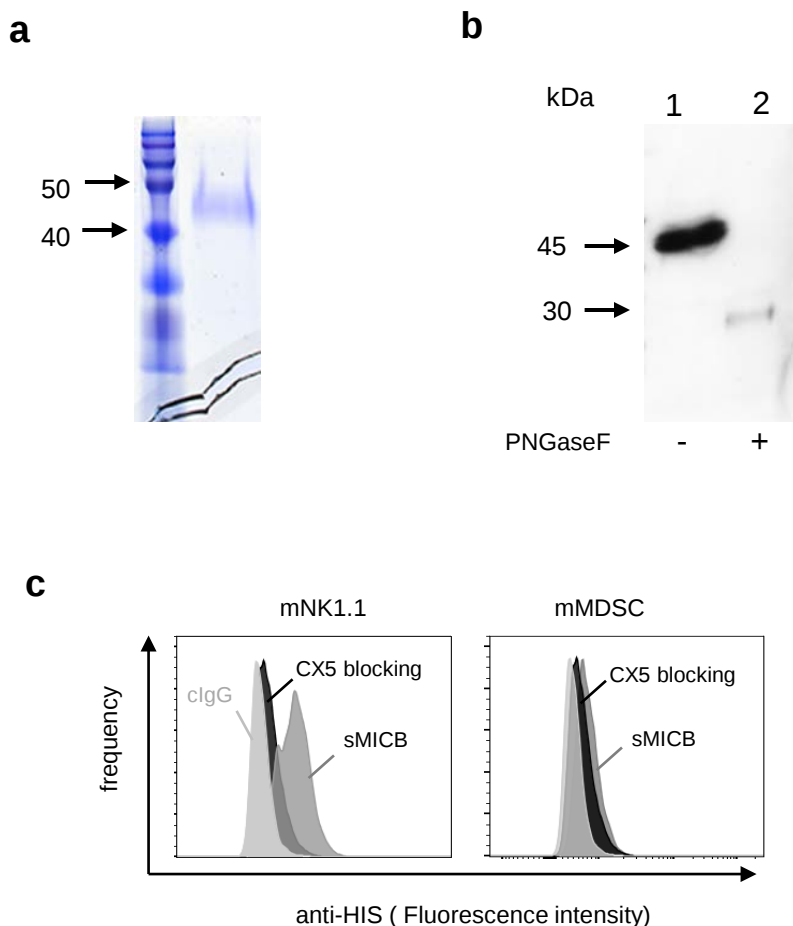


Figure S6. Validation of purified sMICB. **a**, Coomassie blue staining of SDS gel of sMICB elution after PBS buffer exchange. **b**, Western-blot probed with anti-MIC antibody H-300 (Santa Cruz) and HRP-conjugated donkey anti-rabbit secondary antibody. Bioactive sMIC produced in mammalian system is highly glycosylated. Lane 1, without PNGaseF de-glycosylation. Lane 2, after PNGaseF de-glycosylation. **c**, Histogram of flow cytometry showing specific binding of purified sMICB to mouse NKG2D on mouse NK1.1 (mNK1.1) and MDSC (mMDSC). Mouse (B6) splenic NK cells or bone marrow-derived MDSCs were stained with purified sMICB (HIS-Tagged) (20 μ g/ml) followed by rabbit anti-HIS antibody and PE-conjugated goat anti-rabbit antibody. Dark grey profile represents sMICB binding. Black profile represents reduced sMICB binding when cells were pre-incubated with NKG2D blocking antibody CX5 (10 μ g/ml). Light grey profile represents control rabbit IgG staining.