



Figure 1. Biochemical characterization of SC ZIKV E variants. (A) Sequences of ZIKV E (PDB 5LBS), and stabilizing combination (SC) ZIKV E variants (SC12, SC12m53, SC30, SC30m53). Positions of installed EDE-binding positive glycan mutations (**yellow**), and stabilizing combination mutations (**purple**) are indicated. (B) Model of SC12m53 and SC30m53 with positions of installed EDE-binding positive glycan positions (**yellow**) and stabilizing combination mutations (**purple**). (C) Mass photometry showing comparison between expected molecular weights of sE proteins to molar mass of species and distribution of oligometric states in solution of SC12, SC12m53, SC30, and SC30m53. (D) SDS PAGE mobility gel shift assay of SC12, SC12m53, SC30, and SC30m53 under reducing and non-reducing conditions with and without PNGase. (E) Binding reactivity of EDE mAbs C8 and C10, and non-EDE mAbs ZV-67 and 4G2 to SC12, SC12m53, SC30, and SC30m53 determined by ELISA. Representative data from two independent experiments completed in duplicate are plotted as the (average absorbance at 450 nm) \pm SD. (F) Reactivity profiles for SC30, and SC30m53 to EDE mAb C10, and non-EDE mAbs ZV-67 and 4G2 as determined by biolayer interferometry (BLI). Shown are representative data from two independent experiments with consistent results. Apparent KD (KDapp) values are indicated for each sensorgram, weak binding (WB), no binding (NB).