



Figure S4. The mutants in MLKL²⁻¹⁷⁸ lack sensitivity to MTSET.(A) Schematic representation of the residues examined and the corresponding six α -helices revealed by solution MLKL structure. The dotted lines indicate the hypothetical lipid bilayer. MTSET was applied from either *cis* or *trans* side. Mutations for MTSET test from *cis* (small lilac balls) or *trans* (small yellow balls) side are indicated. (B and C) Responses of indicated mutants to MTSET. The mutants C24A, L165C/C86A, K22A/R30A, L45C and L45A cannot induce currents; the mutants E2C, N3C, A131C, S132C and L151C/C86A lack sensitivity to MTSET. The currents were recorded in asymmetric 15:150 mM (*cis:trans*) Na⁺ solutions (n > 4 for each

group). (D) The proteint samples were analyzed by SDS PAGE and coomassie blue staining. The positions of molecular weight markers (kDa) are indicated on the left.