



**Figure S5. Correlation of the channel activity of MLKL<sup>2-178</sup> with cell death.**(A) Transfection of wild type MLKL<sup>2-178</sup>, K22E, C24A and C86A induced comparable cell death in the *Mkl* gene knock out HeLa cells, whereas the K22A, L45A and L45C exhibited reduced capability to induce cell death. The cell death was examined 20-24 h after transfection. (B) The mutations in the helices H1 to H6 that block the channel activity rescue the MLKL<sup>E/D</sup>-induced HEK293 cell death. The cell death was examined 30 h after transfection. 'ED' indicates MLKL<sup>E/D</sup>; 'AA' indicates MLKL<sup>A/A</sup>; 'ED-L45C' and 'ED-L45A' indicate triple mutations in the background of MLKL<sup>E/D</sup>; 'ED/ED-L45A' and 'ED/ED-L45C' indicate coexpression of wild type MLKL<sup>E/D</sup> with the corresponding mutant with triple mutations.