

Fig. S1. Nukacin ISK-1 production by NukT mutants. Production of nukacin ISK-1 was monitored by LC/MS analysis of culture supernatant of *L. lactis* recombinant strains carrying pInukdT/pNZ8048, pInukdT/pNZT, and pInukdT/pNZT mutants. The extracted ion chromatogram at  $m/z = 1,480$  corresponding to the double charge of nukacin ISK-1 (2,960 Da), is displayed for each analysis. The experiments were performed at least twice and the representative data are shown.

Fig. S2. MALDI-TOF/MS spectrum of the cell-free extract of *L. lactis* recombinant strains expressing the NukT mutant. Modified NukA accumulated inside the cell was monitored by MALDI-TOF/MS. The experiments were performed at least twice and the representative data are shown.