Supplementary Figure 2. Potency of LMP1 to interact with PRA1, traffic, and activate NF-κB signaling is mainly impaired by alterations through LMP1 transmembrane segments 3-6

(A) Schematic representations of FLAG-tagged LMP1 derivatives with specific transmembrane alterations. The transmembrane-spanning domain of LMP1 was deleted segment to segment (ΔTM3/4, ΔTM5/6, or ΔTM1/2), or substituted with the transmembrane domain derived from CD40 (aa 1-214, termed CD40-CT) or from β-1,4-galactosyltransferase (GT; aa 1-81, termed GT-CT). (B) Impaired interaction of LMP1ΔTM derivatives with PRA1 but not with TRAF3. HEK293 cells were transfected with the constructs as indicated. Cell lysate was harvested at 24 h post-transfection and subjected to co-precipitation with anti-FLAG affinity matrix. Protein expression and immunocomplexes were analyzed by Western blotting with Ab specific to FLAG, PRA1, or TRAF3. (C) LMP1ΔTM3/4 and LMP1ΔTM5/6 were defective in ER-Golgi transport. NPC117 cells transfected with the plasmids for FLAG-LMP1ΔTM3/4 or -LMP1ΔTM5/6 were fixed at 24 h post-transfection and stained with Abs specific to LMP1 and PRA1. The numbers of cells in which LMP1ΔTM derivatives were significantly retained in the ER (marked as ▼) were individually calculated (%). Bar, 20 µm. (D) Impaired NF-κB activation by LMP1ΔTM derivatives. NPC117 cells were transfected with 250 ng of the plasmid encoding LMP1 or its derivatives in conjunction with 100 ng of NF-κB-luciferase reporter plasmid and 100 ng of expression plasmid for Renilla luciferase. Cell extracts were harvested at 24 h after transfection and applied to the NF-κB activity assay as described in Materials and Methods.