Supplementary Figure 6. PRA1 affects LMP1 oligomerization and signaling

(A) PRA1\textsubscript{170-164}, but not PRA1\textsubscript{1-78}, impaired LMP1 oligomerization. HEK293 cells were transfected with the plasmids encoding FLAG-tagged LMP1\textsubscript{1-296} and Myc-tagged LMP1, in conjunction with the plasmid for HA-PRA1\textsubscript{170-164} or -PRA1\textsubscript{1-78}, the latter of which fails to interact with LMP1. At 24 h after transfection, cell lysate was harvested and applied to co-precipitation with anti-FLAG affinity matrix. Protein expression and precipitated protein complexes were analyzed by Western blotting with Ab specific to HA or LMP1 (1B, which recognizes the epitope within both FLAG-LMP1\textsubscript{1-296} and Myc-LMP1). *, oligomerized LMP1, the amounts of which are quantified with densitometry and normalized by the amounts of precipitated FLAG-LMP1\textsubscript{1-296}. The resulting ratio in the absence of Myc-LMP1 was set as 1.

(B) Altered localization of LMP1 in the presence of PRA1\textsubscript{70-164}. COS7 cells co-expressing FLAG-LMP1 and GFP-PRA1\textsubscript{70-164} were fixed and stained with Ab specific to LMP1 (S12). The numbers of cells in which FLAG-LMP1 localization was classified into four patterns (ER, ER/Golgi, Golgi, Vesicle) were individually counted. In all, about 100 cells were analyzed. Bar, 24 µm.

(C) PRA1\textsubscript{70-164} impaired LMP1-induced NF-κB activation. Cell extracts from NPC117 cells co-expressing FLAG-LMP1 with HA-PRA1 or -PRA1\textsubscript{70-164} were harvested and subjected to the assay for NF-κB activation as described in Materials and Methods.

(D) PRA1 knockdown impaired LMP1 oligomerization. HEK293 cells pre-expressing control or PRA1 siRNA for 48 h were further transfected with the plasmids encoding FLAG-LMP1\textsubscript{1-296} and Myc-LMP1. Cell lysate was harvested 24 h later and applied to co-immunoprecipitation as described in (A). *, oligomerized LMP1, the amounts of which was calculated as previously described and the ratio in the absence of Myc-LMP1 under control siRNA treatment was set as 1.