Supplemental Information

Epstein-Barr Viral BNLF2a Hijacks the Tail-anchored Protein Insertion Machinery to Block Antigen Processing by the TAP Complex

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Running title: Inhibition mechanism of EBV-BNLF2a

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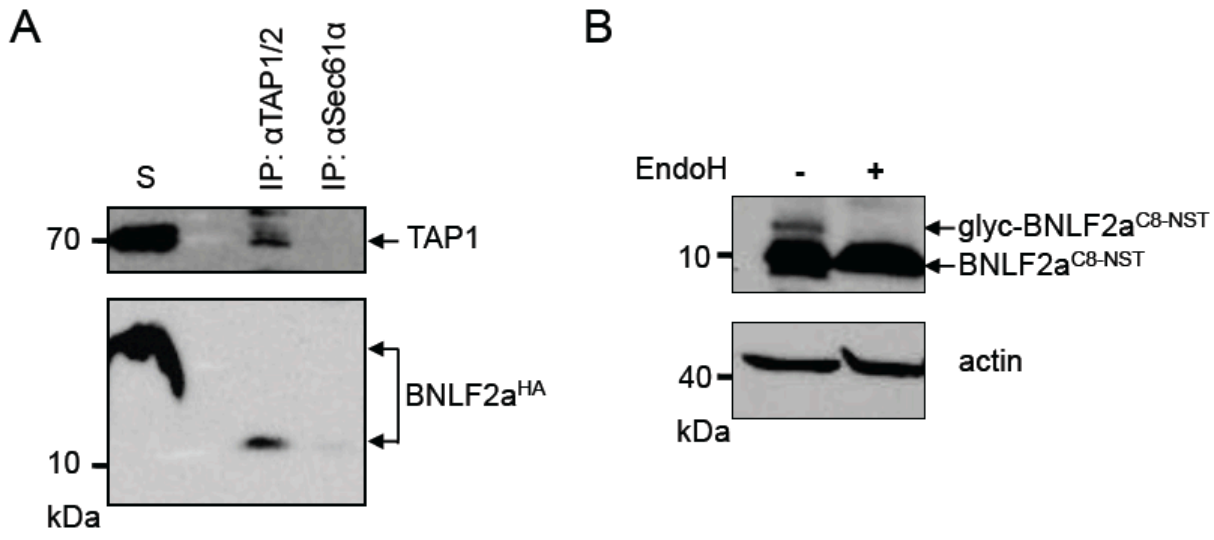
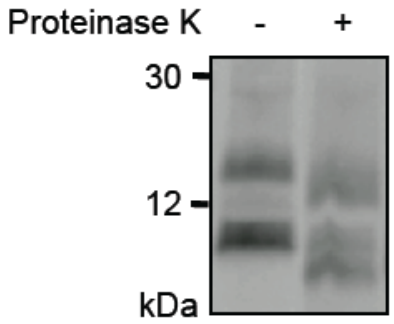
Supplemental Figure 1

BNLF2a is targeted to the ER and associates with the PLC ***A,*** BNLF2a^{HA} associates with the PLC. γ-IFN stimulated HeLa cells were solubilized with 2% digitonin. Solubilized proteins were immunoprecipitated (IP) using TAP1/2 specific antibodies. As negative controls, an antibody specific for the ER translocon protein Sec61α was used. Samples were analyzed by SDS-PAGE (10%) and immunoblotting with the corresponding antibodies. An aliquot (1/20) of the solubilized input (S) is shown. Due to the presence of mixed detergent micelles, the molecular weight of BNLF2a$^{C8\text{-}NST}$ appears to be higher in the solubilized input. ***B***, BNLF2a$^{C8\text{-}NST}$ transiently expressed in HeLa cells is targeted to the ER, as indicated by glycosylation (glyc). ER targeting and membrane insertion was verified by treatment with endoglycosidase H (EndoH). Equal sample loading was verified by immunoblotting against actin.



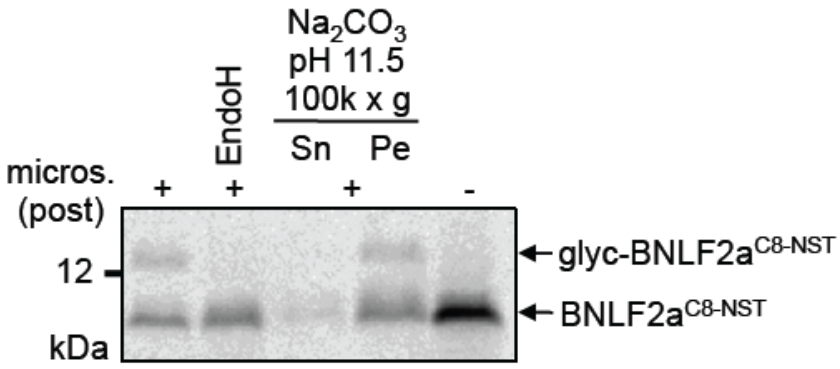
Supplemental Figure 2

Protease K degrades the hydrophilic N-terminal region of BNLF2a. BNLF2a$^{C8\text{-}NST}$ (including three extra methionines at the C-terminus for [^{35}S]-Met labeling) was *in vitro* translated in rabbit reticulocyte lysate in the presence of microsomal membranes. After translation, membranes were collected by sedimentation through a 0.5 M sucrose cushion in HEPES buffer (10 mM HEPES pH 7.5, 100 mM KAc, 1 mM MgAc, 1 mM DTT) at 100,000 x g for 20 min at 4°C. Translation products were incubated either with or without 0.2 mg/ml Protease K (Sigma-Aldrich, Munich, Germany) in HEPES buffer for 30 min at 4°C. Proteolysis was stopped by 2 mM phenylmethylsulfonyl fluoride and proteins were separated by Tricine/SDS-PAGE (10%) and visualized by phosphoimaging.



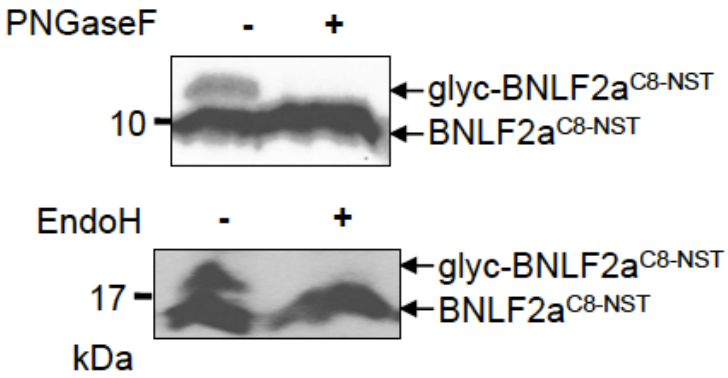
Supplemental Figure 3

BNLF2a inserts post-translationally into ER membranes in the presence of mammalian cytosolic factors. *In vitro* translation reactions were performed in wheat germ extract in the presence of [^{35}S]-Met using truncated BNLF2a$^{C8\text{-}NST}$ mRNA templates lacking a stop codon. BNLF2a$^{C8\text{-}NST}$ containing ribosome-nascent chain complexes were collected by centrifugation through a sucrose cushion, and re-suspended in rabbit reticulocyte lysate with or without of microsomal membranes (micros). After ribosomal release by puromycin and RNAse treatment, samples were further incubated for 30 min at 32°C. Aliquots of the translation product were then analyzed either directly, treated with EndoH, or extracted with alkaline sodium carbonate pH 11.5. The latter aliquot was separated in a membrane pellet (Pe) and supernatant (Sn) fraction by centrifugation at 100.000 x g. Samples were analyzed by Tricine/SDS-PAGE (10%) and visualized by phosphoimaging. glyc: glycosylated protein.



Supplemental Figure 4

ER targeting and membrane insertion of BNLF2a is independent of TAP1/2. BNLF2a$^{C8\text{-}NST}$ was expressed in *Sf*9 insect cell membranes that do not contain endogenous TAP1/2. N-linked glycosylation is demonstrated by peptide: N-endoglycosidase F (PNGaseF) and EndoH treatment.



Supplemental Figure 5

The soluble domain of BNLF2a does not bind to TAP. ***A***, Competition assays. Equal amounts of membranes isolated from full-length TAP1/2 expressing *Sf*9 cells were incubated with 0.5 µM of the fluorescein-labeled TAP substrate peptide $R9L^{Flu}$ (RRYØKSTEL; Ø, fluorescein-labeled cysteine) for 15 min on ice. A 100-fold excess (50 µM) of either unlabeled R9L (RRYQKSTEL) or BNLF2a soluble domain (amino acids 2-42; $BNLF2a^{2-42}$) was used to compete for binding. After washing on filter plates, the amount of membrane-associated peptide was quantified by fluorescence ($\lambda_{ex/em}$= 485/520 nm). Binding of $R9L^{Flu}$ to TAP in the absence of any additional peptide was set to 100%. The mean of at least three independent experiments is shown; error bars indicate the S.D. ***B***, Binding assays. Equal amounts of membranes isolated from either uninfected *Sf*9 insect cells (mock) or from full-length TAP1/2 expressing *Sf*9 cells were incubated with 0.5 µM of either $R9L^{Flu}$ or 0.5 µM of fluorescein-labeled BNLF2a soluble domain ($BNLF2a^{2-42-Flu}$). 100-fold excess of unlabeled R9L was used to probe for unspecific binding (open bars). After washing on filter plates, the amount of membrane-associated peptide was quantified as described above. Binding of $R9L^{Flu}$ to TAP was set to 100%.