PLOS PATHOGENS

CORRECTION

Correction: pUL21 is a viral phosphatase adaptor that promotes herpes simplex virus replication and spread

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The axis labels for Fig 3F are incorrect: the horizontal axis should have the range 0->100 nM. Please see the correct Fig 3 here.

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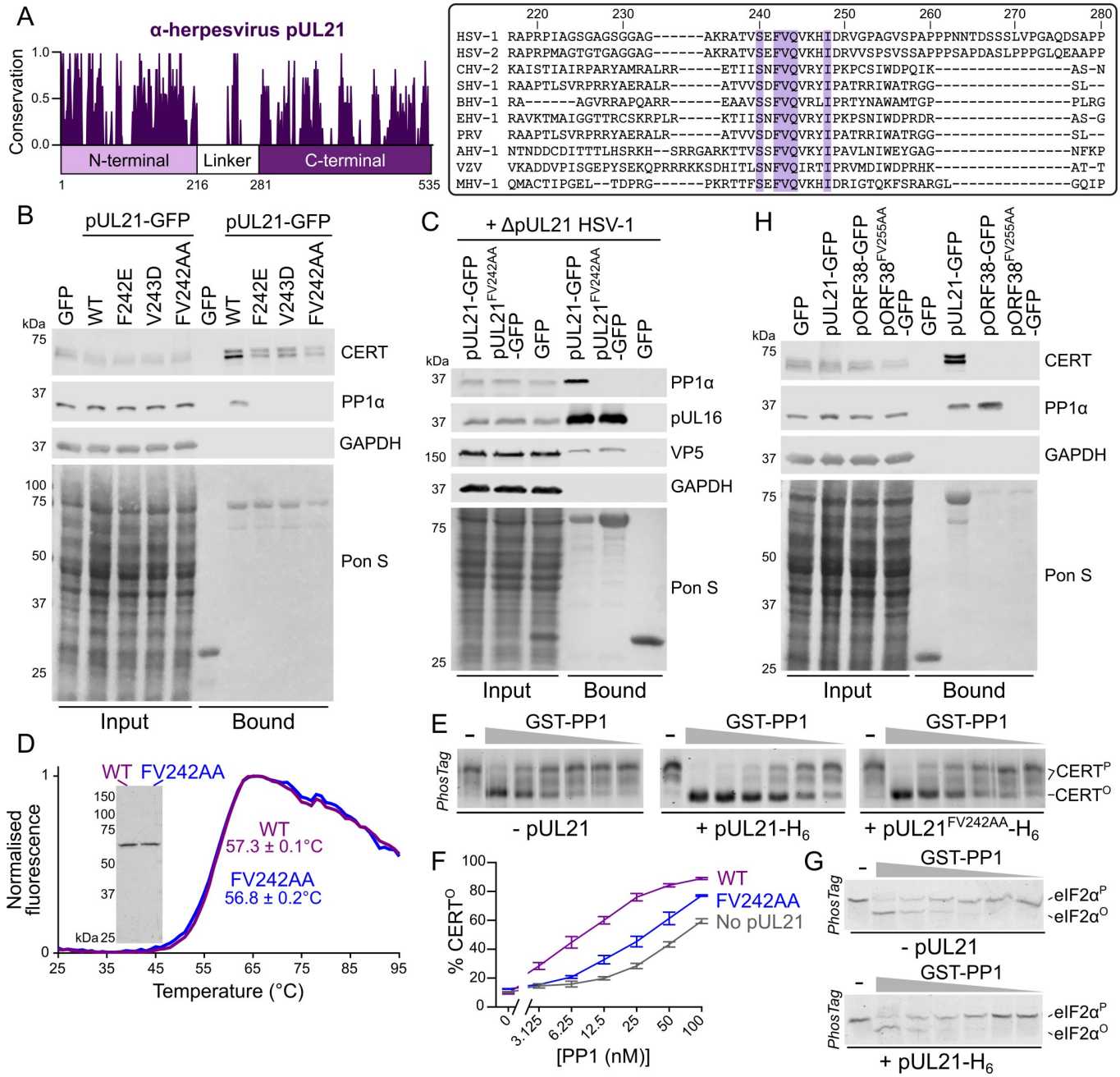

Fig 3. pUL21 recruits PP1 via a conserved motif in the linker region to accelerate CERT dephosphorylation. (**A**). Conservation of pUL21 across *Alphaverpesvirinae*. The following sequences were aligned using ClustalW and conservation calculated using Jalview (Abbreviation and Uniprot ID are shown in parentheses): HSV-1 (HSV1, P10205), HSV-2 (HSV2, G9I242), cercopithecine herpesvirus 2 (CHV2, Q5Y0T2), saimiriine herpesvirus 1 (SHV1, E2IUE9), bovine alphaherpesvirus 1 (BHV1, Q65563), equine herpesvirus 1 (EHV1, P28972), pseudorabies virus (PRV, Q04532), anatid herpesvirus 1 (AHV1, A4GRJ2), varicella-zoster virus (VZV, Q6QCT9), turkey herpesvirus (MHV1, Q9DPR5). Alignment across the linker region (residues 217–280 of HSV-1 pUL21) is shown with conserved residues highlighted. (**B**) HEK293T cells were transfected with plasmids expressing GFP, wild-type (WT) pUL21-GFP or pUL21-GFP with amino acid substitutions in the conserved motif. At 24 hours post-transfection the cells were lysed, subjected to immunoprecipitation using a GFP affinity resin, and captured proteins were subjected to SDS-PAGE and immunoblotting using the listed antibodies. Ponceau S (Pon S) staining of the nitrocellulose membrane before blocking is shown, confirming efficient capture of GFP-tagged proteins. (**C**) Plasmids expressing wild-type or mutant pUL21-GFP, or GFP alone, were transfected into HEK293T cells. At 24 hours post-transfection cells were infected with ΔpUL21 HSV-1 (MOI = 5). Cells were lysed 16 hours post-infection and subjected to immunoprecipitation, SDS-PAGE and immunoblotting as in (**B**). (**D**) Differential scanning fluorimetry of WT (purple) and FV242AA substituted (blue) pUL21-H_6. Representative curves are shown. Melting temperatures (T_m) is mean ± standard deviation (n = 3). Inset shows Coomassie-stained SDS-PAGE of the purified protein samples. (**E**) *In vitro* dephosphorylation assays using all-purified reagents. 0.5 μM CERT was incubated with varying concentrations of GST-PP1 (two-fold serial dilution from 100–3.1 nM) in the absence or presence of 2 μM pUL21-H_6 (WT or FV242AA) for 30 min at 30˚C. Proteins were

resolved using SDS-PAGE where PhosTag reagent was added to enhance separation of CERT that is hyper- ($CERT^P$) or hypo-phosphorylated ($CERT^O$) and gels were stained with Coomassie. Images are representative of three independent experiments. (**F**) Quantitation of pUL21-mediated stimulation of CERT dephosphorylation, as determined by densitometry. Ratio of $CERT^O$ to total CERT ($CERT^O$ + $CERT^P$) for three independent experiments is shown (mean ± SEM). (**G**) 0.5 μM phosphorylated eIF2α ($eIF2\alpha^P$) was subjected to *in vitro* dephosphorylation using varying concentrations of GST-PP1 (two-fold serial dilution from 200–6.3 nM) in the absence or presence of 2 μM pUL21-H_6 as in (**E**). pUL21 does not enhance PP1-mediated dephosphorylation of eIF2α. (**H**) HEK293T cells were transfected with GFP, pUL21-GFP, the VZV homologue of pUL21 with a C-terminal GFP tag (pORF38-GFP), or with pORF38-GFP where amino acid in the conserved motif had been substituted with alanine. Cells were lysed at 24 hours post-transfection and subjected to IP, SDS-PAGE and immunoblotting as in (**B**).

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Reference

1. Benedyk TH, Muenzner J, Connor V, Han Y, Brown K, Wijesinghe KJ, et al. (2021) pUL21 is a viral phosphatase adaptor that promotes herpes simplex virus replication and spread. PLoS Pathog 17(8): e1009824. https://doi.org/10.1371/journal.ppat.1009824 PMID: 34398933