

Supplementary Materials: Human Cytomegalovirus Nuclear Egress Proteins Ectopically Expressed in the Heterologous Environment of Plant Cells are Strictly Targeted to the Nuclear Envelope

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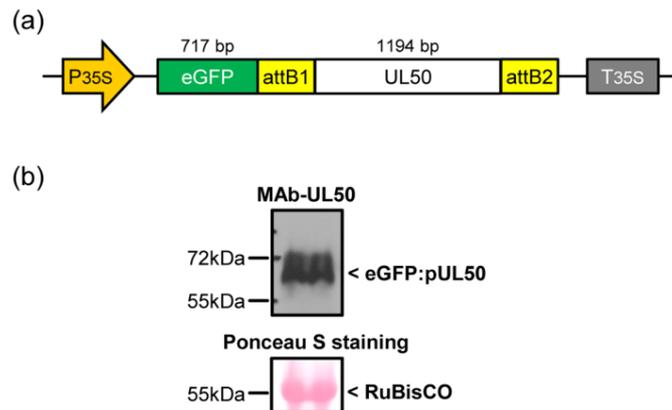


Figure S1. eGFP::pUL50 construct and Western blot detection. (a) Schematic representation of the eGFP::pUL50 construct. P35S, CaMV 35S promoter; attB1/2, Gateway® technology recombination sites; T35S, CaMV 35S terminator; values over scheme indicate size of the respective fragment in basepairs; (b) eGFP::pUL50 protein expression verified via Western blot detection using a monoclonal antibody directed against pUL50; Ponceau S staining as protein loading control showed a proper band for the highly abundant ribulose-1,5-bisphosphat-carboxylase/-oxygenase (RuBisCO).

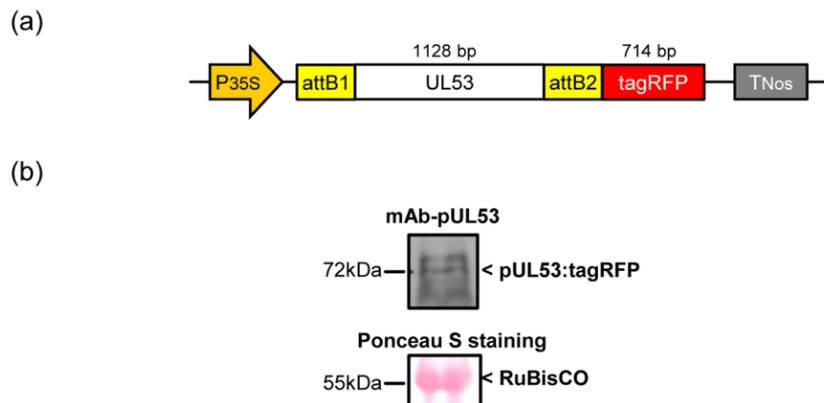


Figure S2. pUL53::tagRFP construct and Western blot detection. (a) Schematic representation of the pUL53::tagRFP-construct. P35S, CaMV 35S promoter; attB1/2, Gateway® technology recombination sites; TNos, nopaline synthase terminator; values over schemes indicate size of the respective fragment in basepairs; (b) pUL53::tagRFP protein expression verified via Western blot detection using a monoclonal antibody directed against pUL53; Ponceau S staining as protein loading control showed a proper band for the highly abundant ribulose-1,5-bisphosphat-carboxylase/-oxygenase (RuBisCO).

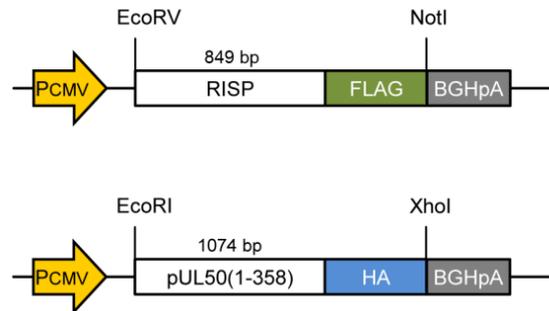


Figure S3. CoIP constructs. Constructs encoding FLAG-tagged RISP and HA-tagged pUL50 (amino acids 1–358) for expression in mammalian cells. PCMV, HCMV immediate early promoter/enhancer; BGHpA, bovine growth hormone polyadenylation signal; restriction sites were used as indicated; values over schemes indicate size of the respective fragment in basepairs.

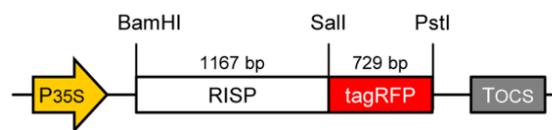


Figure S4. RISP::tagRFP construct. (a) Schematic representation of the RISP::tagRFP construct. P35S, CaMV 35S promoter; TOCS, octopine synthase terminator; restriction sites were used as indicated; values over schemes indicate size of the respective fragment in basepairs.

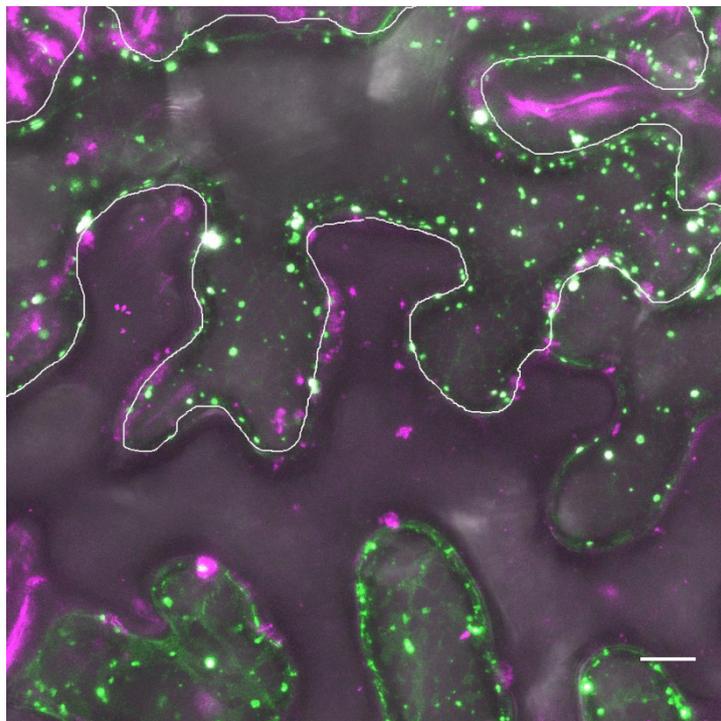


Figure S5. Region of interest used for statistical analysis. A cell coexpressing both RISP::tagRFP and eGFP::pUL50 was selected using the ROI-tool to calculate colocalization rate and Pearson's correlation coefficient using LAS AF software (Leica Microsystems). Scale bar, 10 μ m.

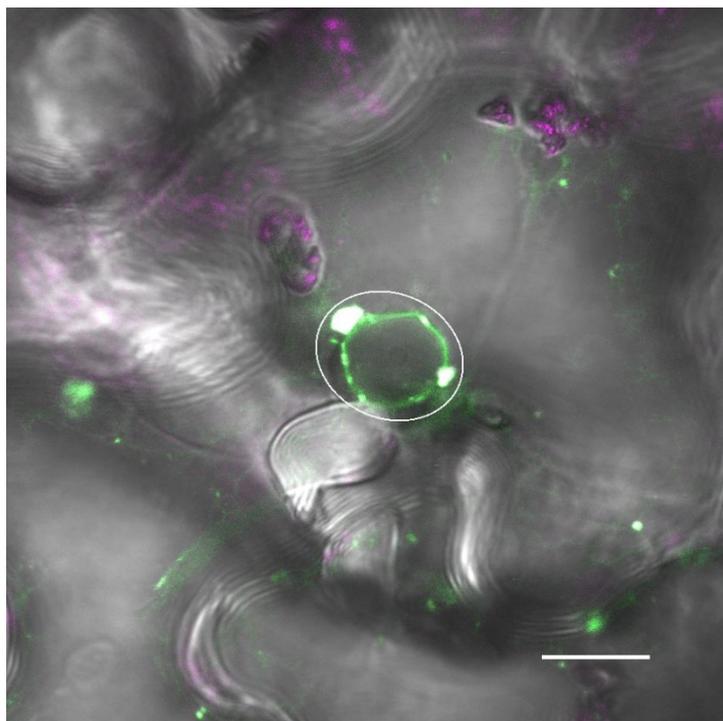


Figure S6. Region of interest used for statistical analysis. A ROI enclosing the nucleus was set, which was used for calculation of colocalization rate and Pearson's correlation coefficient using LAS AF software (Leica Microsystems). Scale bar, 10 μ m.



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