

Pooled overexpression screening identifies PIPPI as a novel microprotein involved in the ER stress response

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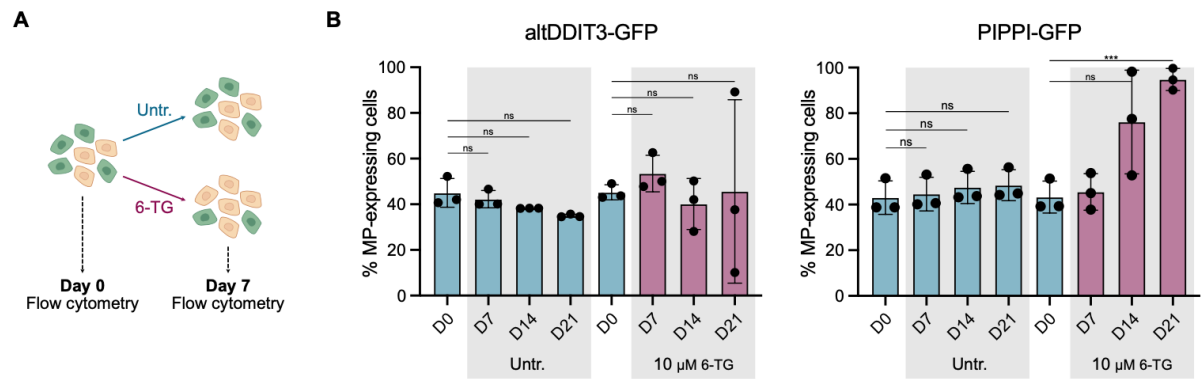
SUPPLEMENTARY INFORMATION

Supplementary Figures 1-5

Supplementary Data

Location and genomic sequence of PIPPI sORFs

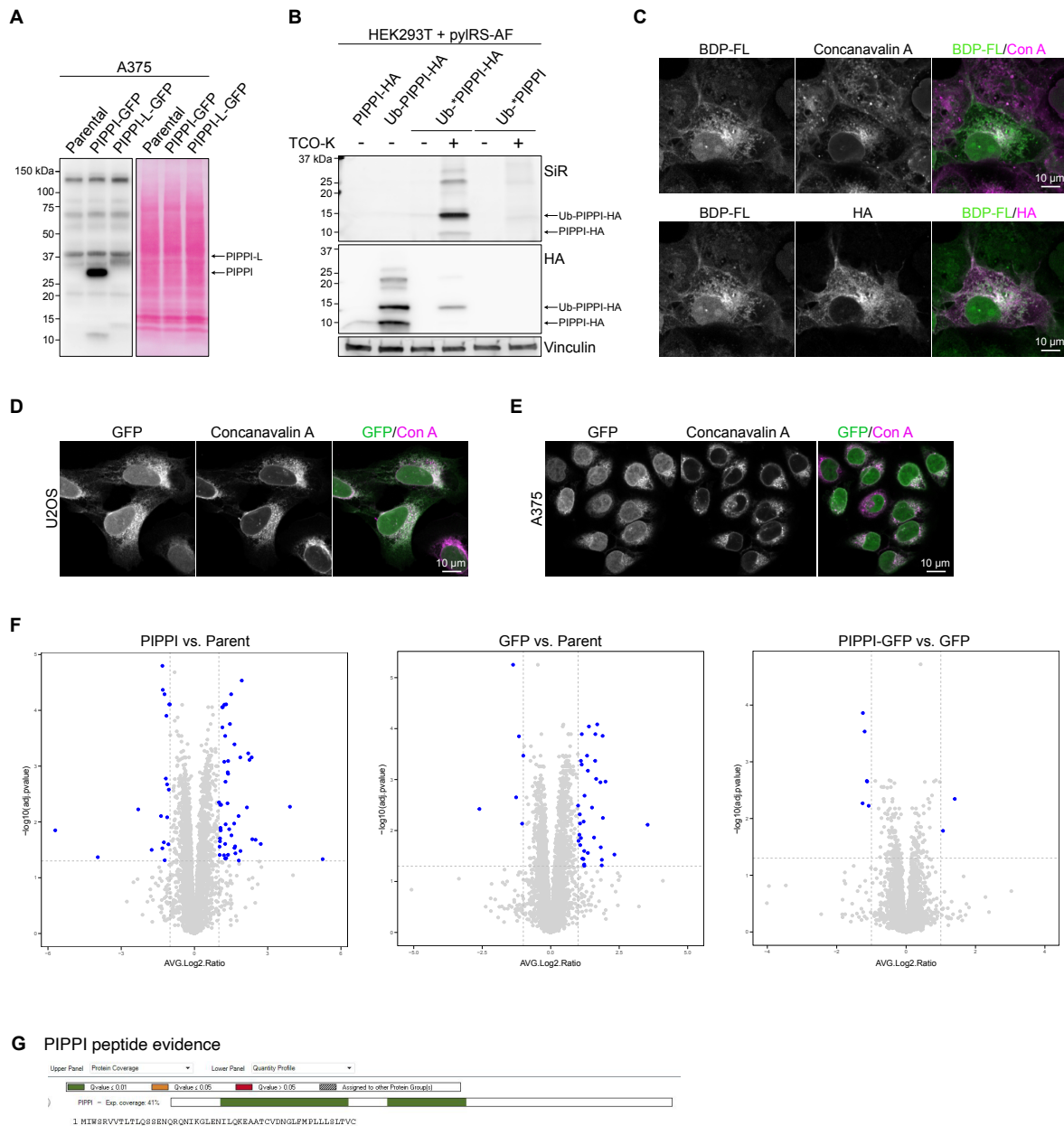
Supplementary Tables 1-4



Supplementary Figure 1

A) Schema illustrating the design of the growth competition assays performed to validate the results of the pooled overexpression screen. In short, microprotein-expressing cells (beige cells) are mixed 1:1 with their parental counterparts expressing a fluorescent marker (green cells). The mixed populations are either cultured untreated for 7 days or left recover for the same period following a 24 hours 6-TG treatment. At day 0 and day 7 of the assay, cells are collected, and the amount of GFP-positive and GFP-negative cells present in each sample is assessed by flow cytometry. B) Results of the growth competition assays comparing the growth of altDDIT3-GFP or PIPPI-GFP to A375 parental cells. Where indicated, cells were continuously treated with 10 mM 6-TG, which was renewed every 2-3 days until the end of the assay. Height of the bars represents the fraction of microprotein-expressing cells present in the total cell population. Values were averaged based on 3 independent biological replicates (black circles). p-values were calculated using unpaired Student's t-test (ns = $p > 0.05$, *** = $p < 0.001$).

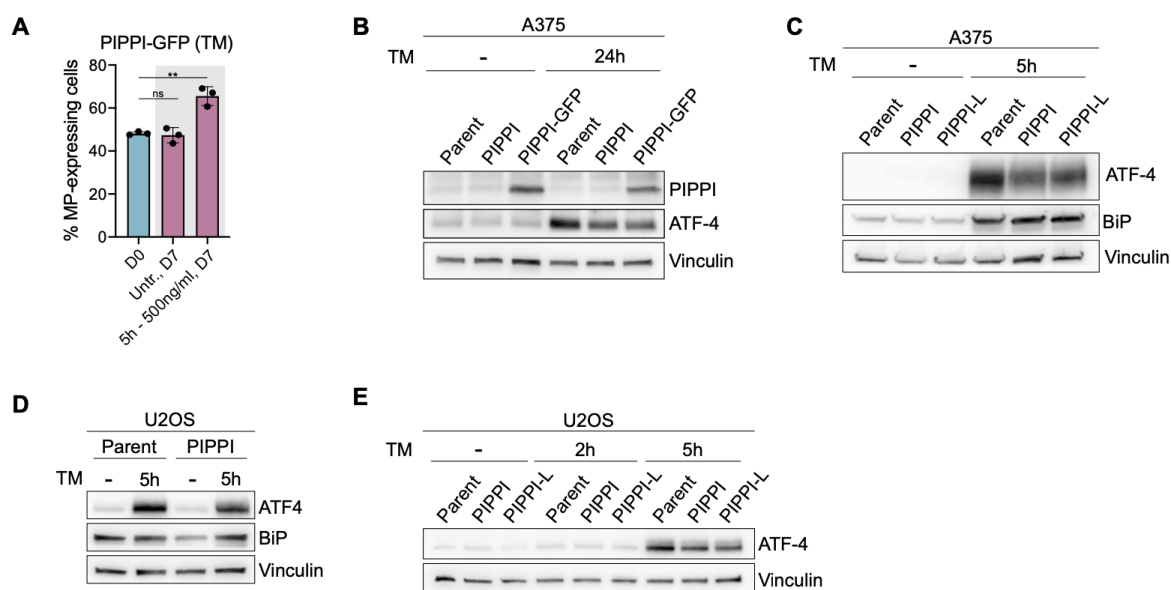
Figure S2



Supplementary Figure 2

A) Immunoblots showing the levels of PIPPI-GFP and PIPPI-L-GFP overexpression in A375 cells. B) Various PIPPI-HA constructs were transiently expressed in HEK293T cells. Where indicated, the pyrrolysine tRNA synthetase (pyIRS) and the non-canonical amino acid TCO*K were provided to allow suppression of the amber stop codon present in some of the constructs. Lysates were labelled using SiR tetrazine and analysed by SDS-PAGE followed by immunoblotting. C) Images presented in Figure 2C are overlapped to highlight the extent of colocalization existing between the BDP-FL and HA signals, as well as the BFP-FL and Concanavalin A (Con A) signals. D-E) Confocal images of U2OS (D) and A375 (E) cells stably expressing a PIPPI-GFP transgene. Concanavalin A (Con A) staining was included to visualize the endoplasmic reticulum. F) Volcano plots presenting the pairwise comparisons of overexpression and the control cell lines. Thresholds are set at $\text{Log}_2(\text{FC}) = 1$ and adjusted p-value = 0.05 (dashed lines). G) Peptide evidence for PIPPI.

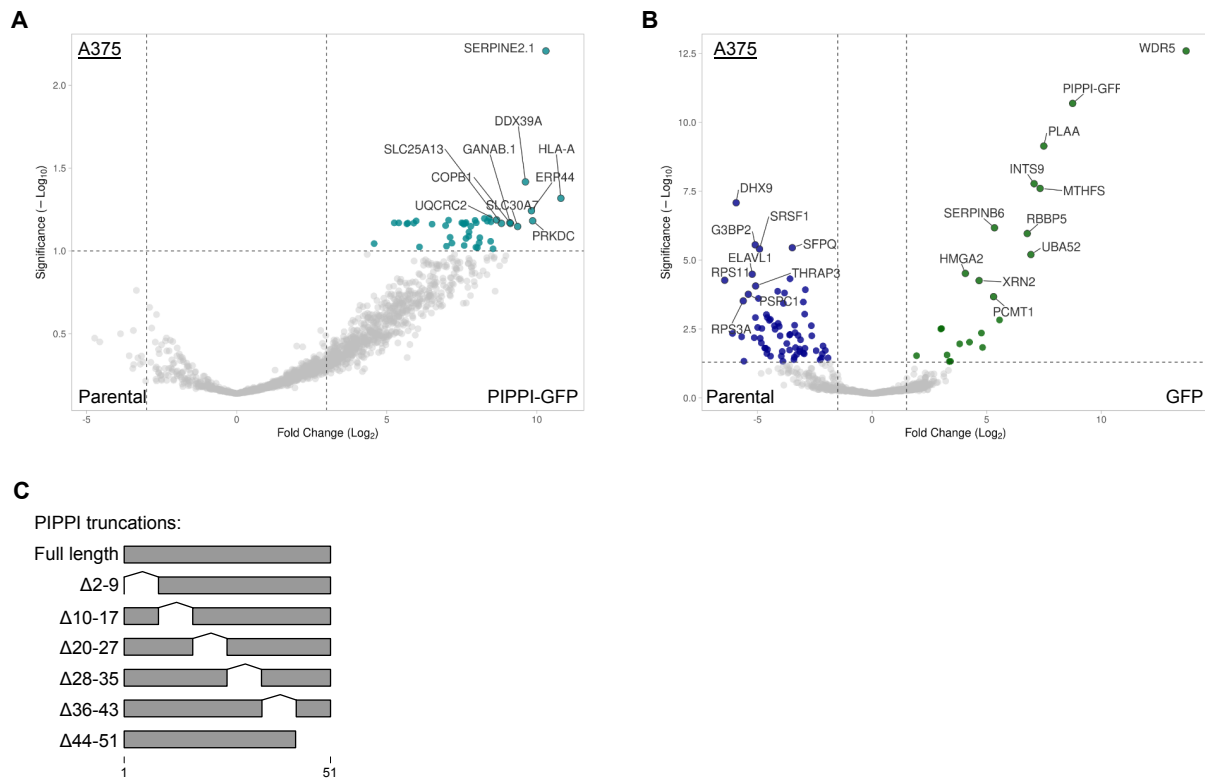
Figure S3



Supplementary Figure 3

A) Bar chart presenting the results of the growth competition assays performed to assess the ability of PIPPI-GFP-expressing cells to overcome tunicamycin treatment. Height of the bars represents the fraction of PIPPI-GFP-expressing cells present in the total cell population. Values were averaged based on 3 independent biological replicates (black circles). p-values were calculated using unpaired Student's t-test (ns = $p > 0.05$; ** = $p < 0.01$). B) Parental, PIPPI- and PIPPI-GFP-expressing A375 cells were treated with 100 ng/ml Tunicamycin for 24 hours. Lysates were then analysed by immunoblotting. C) Parental, PIPPI- and PIPPI-L-expressing A375 cells were treated for 5h with 500 ng/ml Tunicamycin. Induction of the UPR was assessed by immunoblotting. D) Parental and PIPPI-expressing U2OS cells were treated with 500 ng/ml Tunicamycin for 5 hours. After lysis, the levels of ATF4, BiP and vinculin were assessed by immunoblotting. E) Parental, PIPPI- and PIPPI-L-expressing U2OS cells were treated with 200 ng/ml Tunicamycin for either 2 or 5 hours. The extent of ATF4 induction was then assessed by immunoblotting.

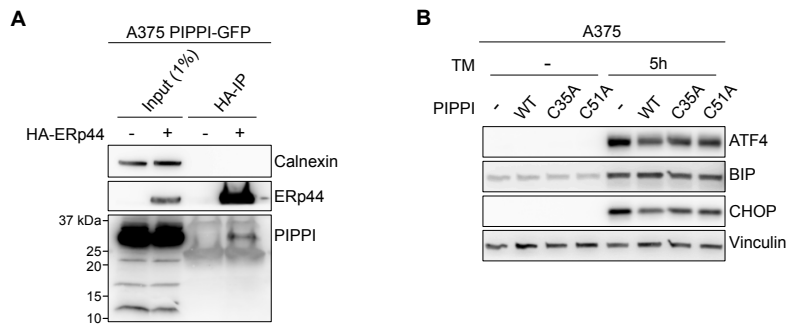
Figure S4



Supplementary Figure 4

A) Volcano plot of co-immunoprecipitation LC-MS/MS (co-IP/MS) experiments performed with parental and PIPPI-GFP-expressing A375 cells. Experiment was conducted in two biological replicates, which were each analyzed three times by mass-spectrometry. Thresholds are set at $\text{Log}_2\text{FC} = 3$ and DEP adjusted $p\text{-value} = 0.1$ (dashed lines). Proteins enriched in PIPPI-GFP lysates are clustering in the right half of the plot and the top ten proteins (assessed by Manhattan distance) are labelled with their names. B) Volcano plot of co-immunoprecipitation LC-MS/MS (co-IP/MS) experiments performed with parental and GFP-expressing A375 cells. Experiment was conducted in two biological replicates, which were each analyzed three times by mass-spectrometry. Thresholds are set at $\text{Log}_2\text{FC} = 1$ and DEP adjusted $p\text{-value} = 0.1$ (dashed lines). Proteins significantly enriched in parental lysates are labeled in blue, whereas proteins enriched in GFP lysates are labeled in green. In both cases, the top ten proteins (assessed by Manhattan distance) are labelled with their names. C) Schema depicting the different PIPPI-GFP truncations that were tested in Figure 4 E-F.

Figure S5



Supplementary Figure 5

A) Lysates from A375 cells either expressing PIPPI-GFP or co-expressing PIPPI-GFP and HA-ERp44 were subjected to immunoprecipitation using HA-beads and analysed by immunoblotting with the antibodies indicated on the right. B) Parental, wild type PIPPI-GFP-, C35A PIPPI-GFP-, and C51A PIPPI-GFP-expressing A375 cells were treated with 500 ng/ml Tunicamycin for 5 hours. After lysis, the level of different proteins involved in the ER stress response were assessed by immunoblotting.

Supplementary Data – Location and genomic sequences of PIPPI sORFs

Ensembl release 110 - July 2023

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