SUPPLEMENTARY MATERIAL

Allosteric transitions direct protein tagging by PafA, the prokaryotic ubiquitin-like protein (Pup) ligase

Naomi Ofer, Nadav Forer, Maayan Korman, Marina Wishkautzan, Isam Khalaila, Eyal Gur

SUPPLEMENTARY FIGURES

Figure S1. MS-MS analysis of PanB pupylation. Product ion spectrum of pupylated TLTLQ**K**WK (m/z = 630.85) aquired by CID. Abundant b and y ions are indicated. Pup indicates the puppylated lysine, K34.

Figure S2. PafA pupylation of the human titin-I27 domain. *A*, SDS-PAGE analysis of folded and unfolded (i.e., carboxymethylated) titin-I27 (12 μM) by PafA (1 μM) and Pup^E (15 μM). Coomassie staining was carried out following electrophoresis. *B*, Detection of the titin-I27 pupylation site by MS-MS analysis. Product ion spectrum of puuppylated L**K**GQPLAASPDDEIIEDGK (m/z = 747) aquired by CID. Abundant b and y ions are indicated. Pup indicates the puppylated lysine.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

LC/MS analysis – MS analysis was performed using an Eksigent nano-HPLC connected to the LTQ Orbitrap XL (Thermo Fisher Scientific). Reverse-phase chromatography for peptides was performed using an in-house-made C-18 column (15 cm long, 75 µm ID), packed with Jupiter C18, 300Å, 5 µm beads (Phenomenex). Peptides were separated by a 90-min linear gradient, starting with 100% buffer A (5% acetonitrile, 0.1% formic acid) and ending with 80% buffer B (80% acetonitrile, 0.1% formic acid), at a flow rate of 300 nl/min. A full scan, acquired at 60,000 resolution, was followed by CID and HCD MS/MS analysis performed

for the top most abundant 3 peaks, in a data dependent mode. Fragmentation (with minimum signal trigger threshold 1000) and detection of fragments were carried out in the linear ion trap for CID and in the Orbitrap for HCD. Maximum ion fill time settings were 300 ms for the high resolution full scan in the Orbitrap analyzer and 100 ms for MS/MS analysis in the ion trap. The AGC settings were 5×10^5 and 1×10^4 for Orbitrap and linear ion trap analyzers, respectively.

Bioinformatics and pupylation site identification – Proteins were identified on the basis of their precursor mass and the sequence information included in their fragmentation spectra, by using the Proteome Discoverer 1.1 software package (Thermo Fisher Scientific). The acquired spectra were searched against the specific protein database, by using the SEQUEST search engine. The following search parameters were used: enzyme specificity is trypsin; maximum three missed cleavage sites; cysteine carbamidomethylation, methionine oxidation; Lys, Pupylation (GGE= 243.086 Da); and a maximum 10 ppm or 0.8 Da error tolerance for the full scan and MS/MS analysis, respectively. Xscore threshold criteria for peptide identification were defined as >2.2, each with a false discovery rate (FDR) p-value <0.01.

Fig. S1

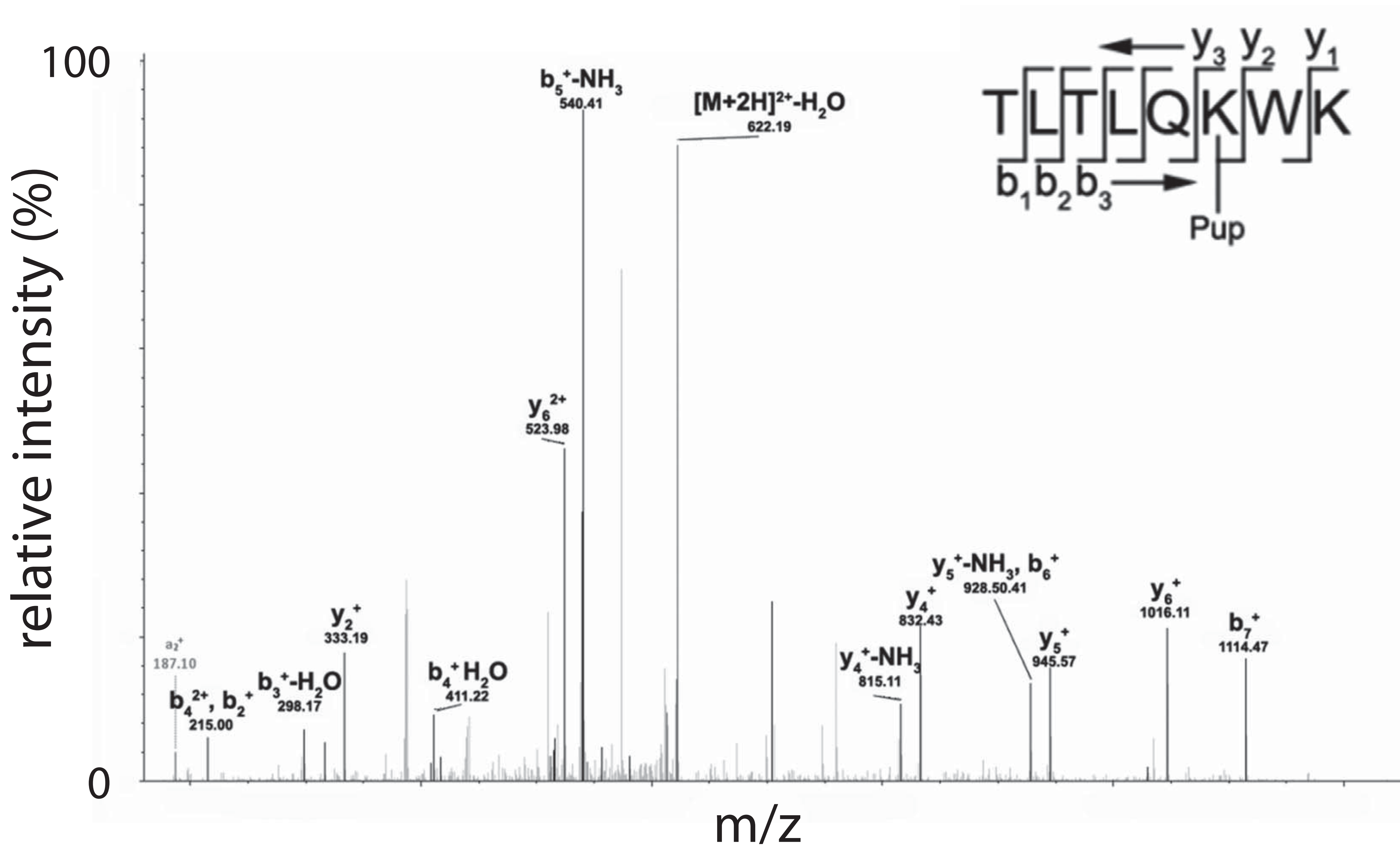
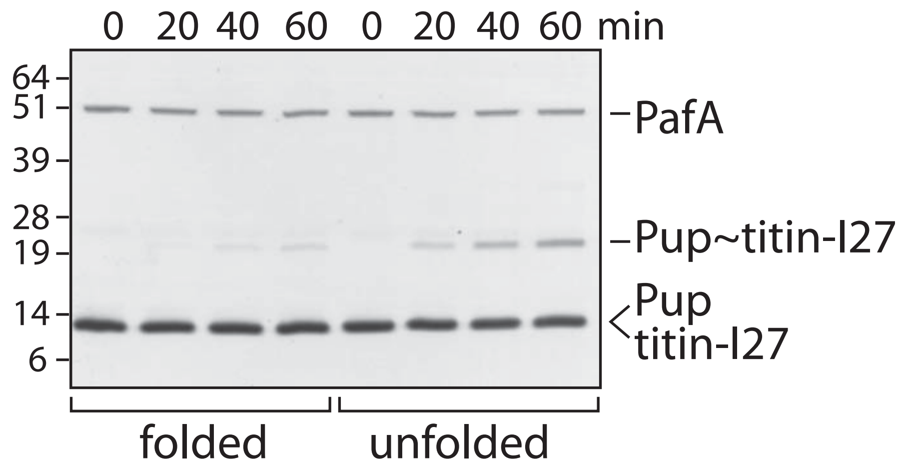


Fig. S2

A.



B.