Plant multifunctional nuclease TBN1 with unexpected phospholipase activity – structural study and reaction mechanism analyses

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SUPPLEMENTARY MATERIAL

Figure S1 Size exclusion chromatography (HiLoad™ Superdex 75 prep grade column) absorbance profiles measured at 280 nm for A) native R-TBN1wt and B) R-TBN1wt treated with EDTA. Peak 1 represents aggregates of R-TBN1wt molecules. Peak 2 belongs to dimers and peak 3 to monomers of R-TBN1wt. In both cases 50 μg of R-TBN1wt was used. Molecular mass estimates are shown.

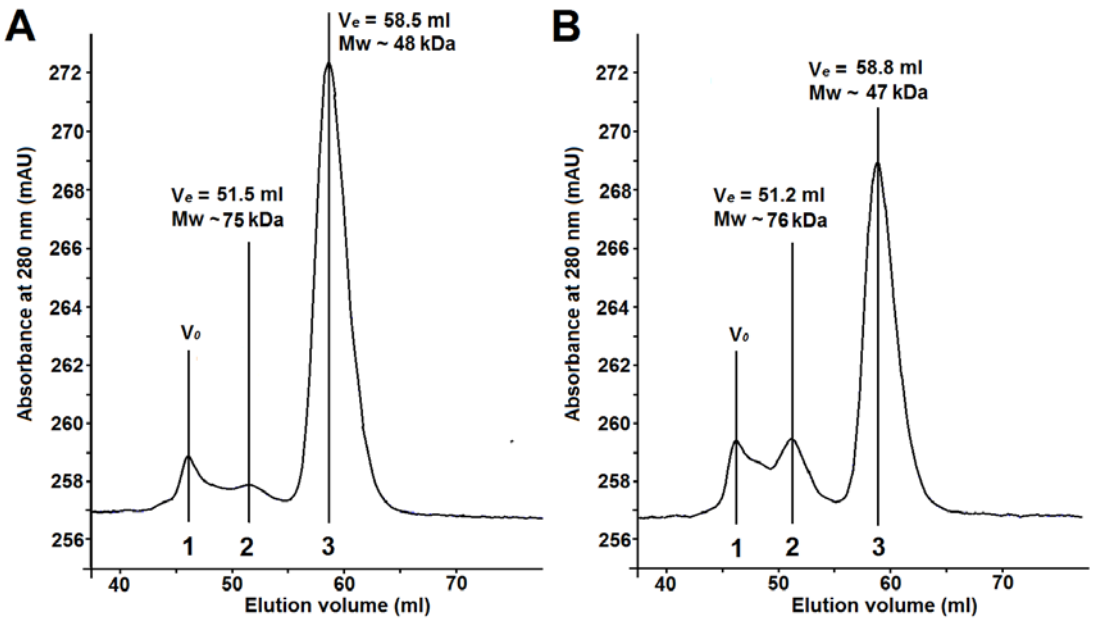


Figure S2 Size exclusion chromatography (HiLoad™ Superdex 200 prep grade column) absorbance profile collected at 280 nm for R-TBN1wt sample treated with PNGase F. Peak 1 represents aggregates of deglycosylated R-TBN1wt. Peak 2 belongs to dimers and peak 3 to monomers of R-TBN1wt, which were not successfully deglycosylated and thus retained their solubility. Molecular mass estimates are shown.

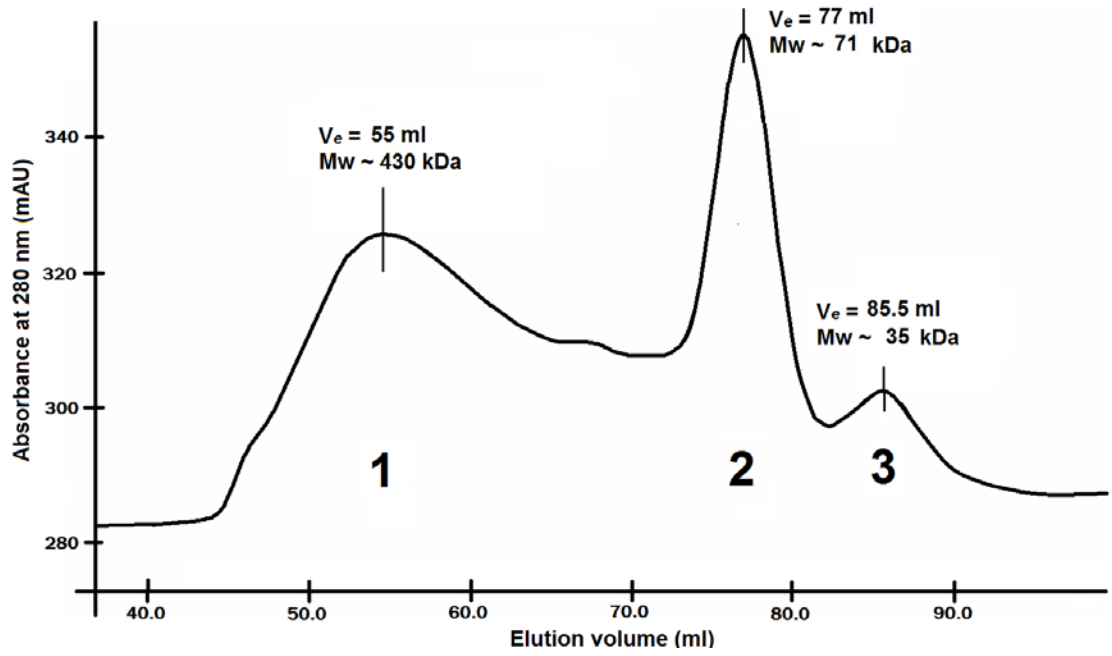


Figure S3 Structure-based sequence alignment of TBN1 (PDB ID: 3SNG) and P1 (PDB ID: 1AK0, Romier *et al*., 1998). The superposition of the structures was calculated using PDBeFold (Krissinel & Henrick, 2004). The numbering corresponds to the full-length sequence of TBN1. Identical residues are marked by red background and similarities by red lettering. The superimposed parts of the protein chains are marked by the solid line below. The graphic representation of the alignment was prepared using ESPript 2.2 (Gouet *et al*., 1999).

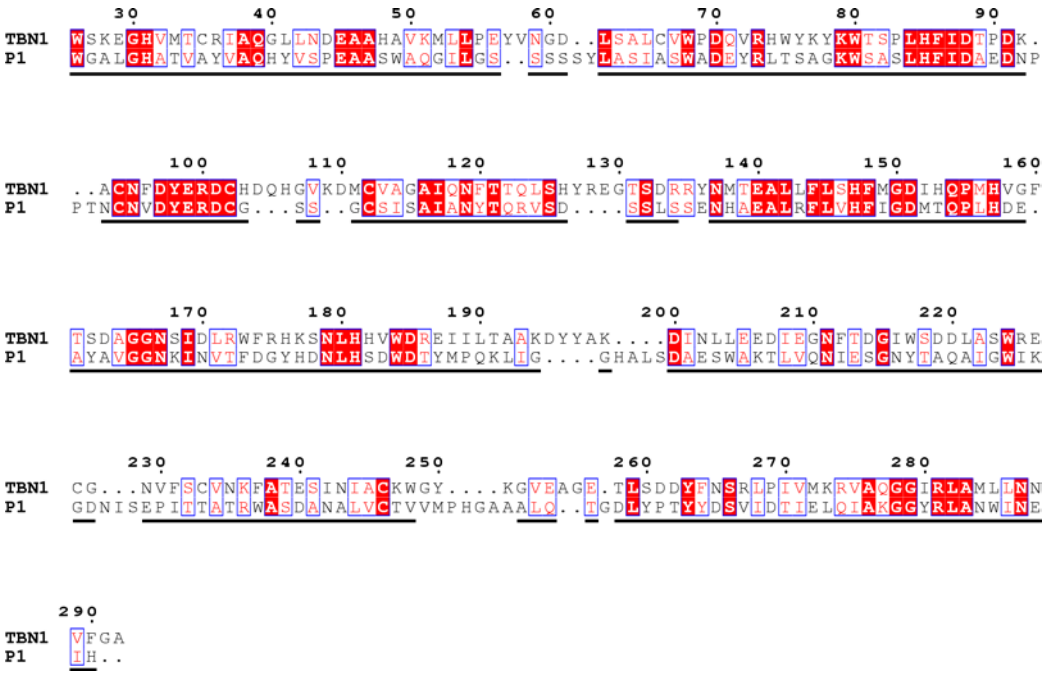
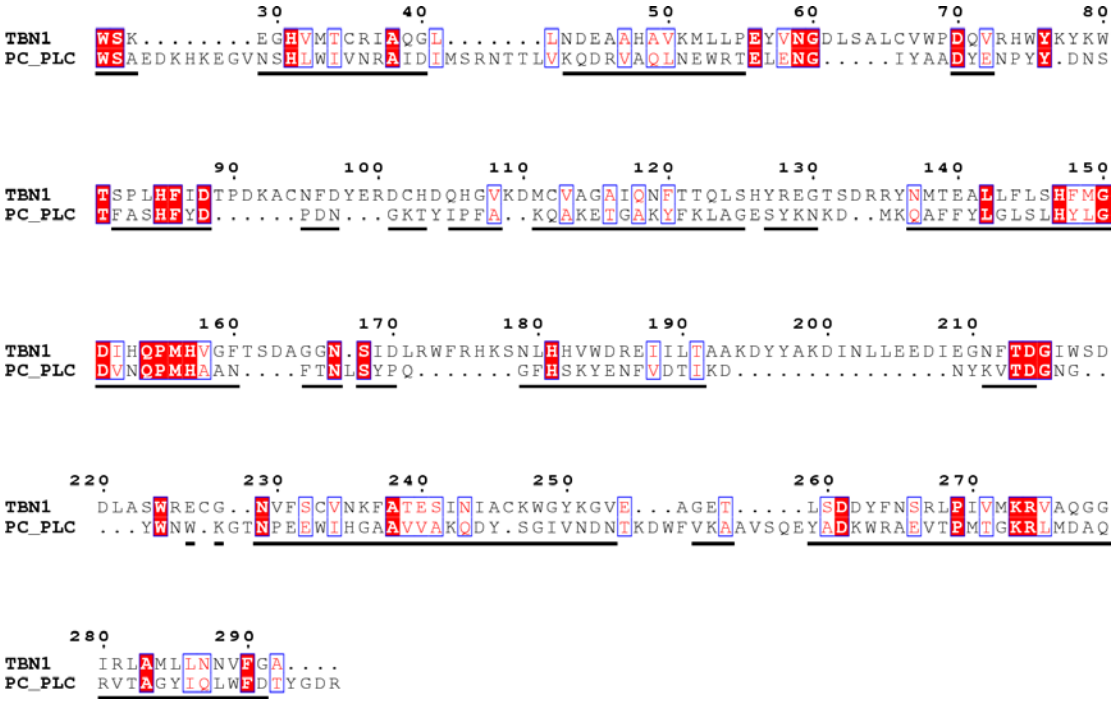


Figure S4 Structure-based sequence alignment of TBN1 (PDB ID: 3SNG) and PC-PLC$_{Bc}$ (PDB ID: 1AH7, Hough *et al.*, 1989). The superposition of the structures was calculated using PDBeFold (Krissinel & Henrick, 2004). The numbering corresponds to the full-length sequence of TBN1. Identical residues are marked by red background and similarities by red lettering. The superimposed parts of the protein chains are marked by the solid line below. The graphic representation of the alignment was prepared using ESPript 2.2 (Gouet *et al.*, 1999).



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Hough, E., Hansen, L. K., Birknes, B., Jynge, K., Hansen, S., Hordvik, A., Little, C., Dodson, E. & Derewenda, Z. (1989). *Nature* **338**, 357-360.

Krissinel, E. & Henrick, K. (2004). *Acta Cryst*. **D60**, 2256-2268.

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