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Title: Structural insights of the ssDNA binding site in the bifunctional endonuclease AtBFN2 from Arabidopsis thaliana

Supplementary materials

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Alignment of S1/P1-type nucleases.

Amino acid sequences of AtBFN1 (UnitProt: Q9SXA6), AtBFN2 (Q9C9G4) from Arabidopsis thaliana, BEN1 (O81958) from Hordeum vulgare, ABN1 (E3PQH5) from Fourraea alpine, ZEN1 (O80326) from Zinnia elegans, CEL1 (Q9LL59) from Apium graveolens, HBN1 (O80326) from Humulus lupulus, TBN1 (Q0KFV0) from Solanum lycopersicum, HBN1 (B4ERM5) from Humulus lupulus, TBN1 (Q0KFV0) from Solanum lycopersicum,
P1 nuclease (P24289) from *Penicillium citrinum* and BcPLC (P09598) from *Bacillus cereus* were aligned using the ClustalW program. Binding pocket 1 (yellow), pocket2 (green), pocket3 (cyan) and pocket4 (pink) are labeled. The tri-metallic zinc active site is labeled in red and (*). Finally, the highly variable Tyr-site amino-acids are labeled with black outlines.
Figure S2. Comparison of secondary binding site between AtBFN2/A5T (red) and TBN1 (3SNG, orange) with conserved protein residues. Tyr90 in AtBFN2 is replaced by a glutamine in TBN1. However, an extension of α helix 11 in TBN1, which AtBFN2 is missing, supplies Phe231.
Table S1. Primers used for PCR-amplification of constructs applied in this study.

For the overexpression construct of

**ENDO2**

<table>
<thead>
<tr>
<th>Sequences of primer pairs (5' → 3')</th>
<th>Enzyme site</th>
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<tbody>
<tr>
<td>B2-11F:</td>
<td>BamHI</td>
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<tr>
<td>CGGGATCCATGGCAAAACCAA</td>
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<tr>
<td>B2-12R:</td>
<td>SaeI</td>
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<tr>
<td>TGGTGACCGAAAATCCT</td>
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