

SUPPLEMENTAL MATERIAL

Acceptors and Effectors Alter Substrate Inhibition Kinetics of a Plant Glucosyltransferase NbUGT72AY1 and Its Mutants

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Figure S1. Schemes of the generated mutants.

Figure S2. LC-MS analysis of substrates and products of NbUGT72AY1.

Figure S3. Enzyme activity of NbUGT72AY1 toward vanillin in the presence of fatty acids as determined by LC-MS analysis.

Figure S4. Enzymatic reaction in the presence of micelles and vesicles formed by fatty acid effectors.

Figure S5: Verification of NbUGT72AY1 mutant protein by SDS-PAGE.

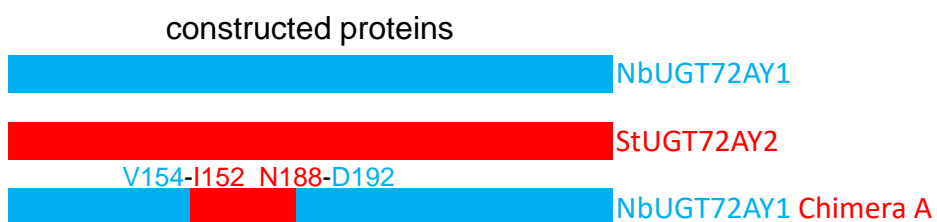


Figure S1. Schemes of the generated mutants. Chimera A contains a part of StUGT72AY2. StUGT72AY2 is a homolog of NbUGT72AY1 but shows no substrate inhibition with scopoletin [19]

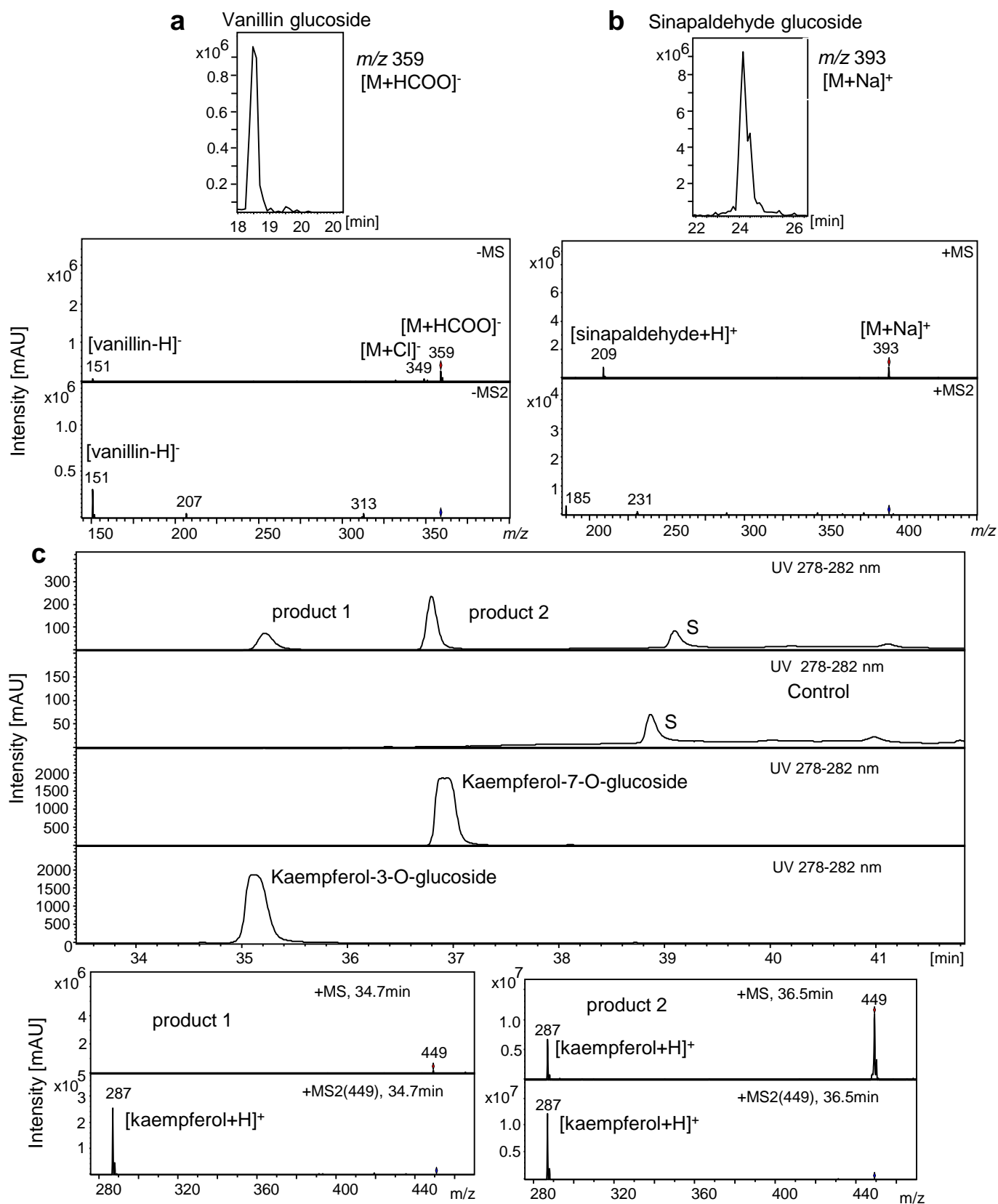


Figure S2. LC-MS analysis of substrates and products of NbUGT72AY1. Chromatogram (top), mass spectrum (MS) and product ion spectra (MS2) acquired in the positive (+) and negative mode (-). **(a)** Vanillin glucoside and **(b)** sinapaldehyde glucoside. **(c)** Ultraviolet detection (UV) at 278–282 nm of kaempferol (S) and two products. The products were identified as product 1 kaempferol-3-O-glucoside and product 2 kaempferol-7-O-glucoside by comparison with chromatographic data obtained for the reference compounds.

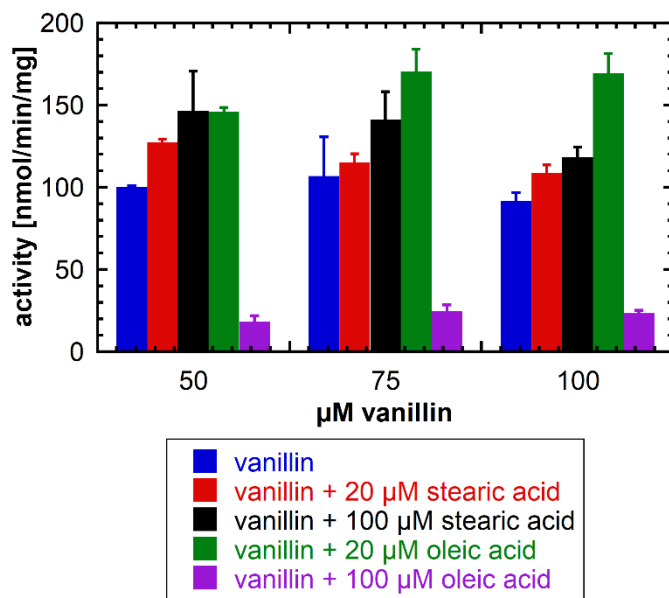


Figure S3. Enzyme activity of NbUGT72AY1 toward vanillin in the presence of fatty acids as determined by LC-MS analysis. NbUGT72AY1 was used to glucosylate vanillin in the presence of stearic acid and oleic acid. Experimental data were determined by LC-MS analysis.

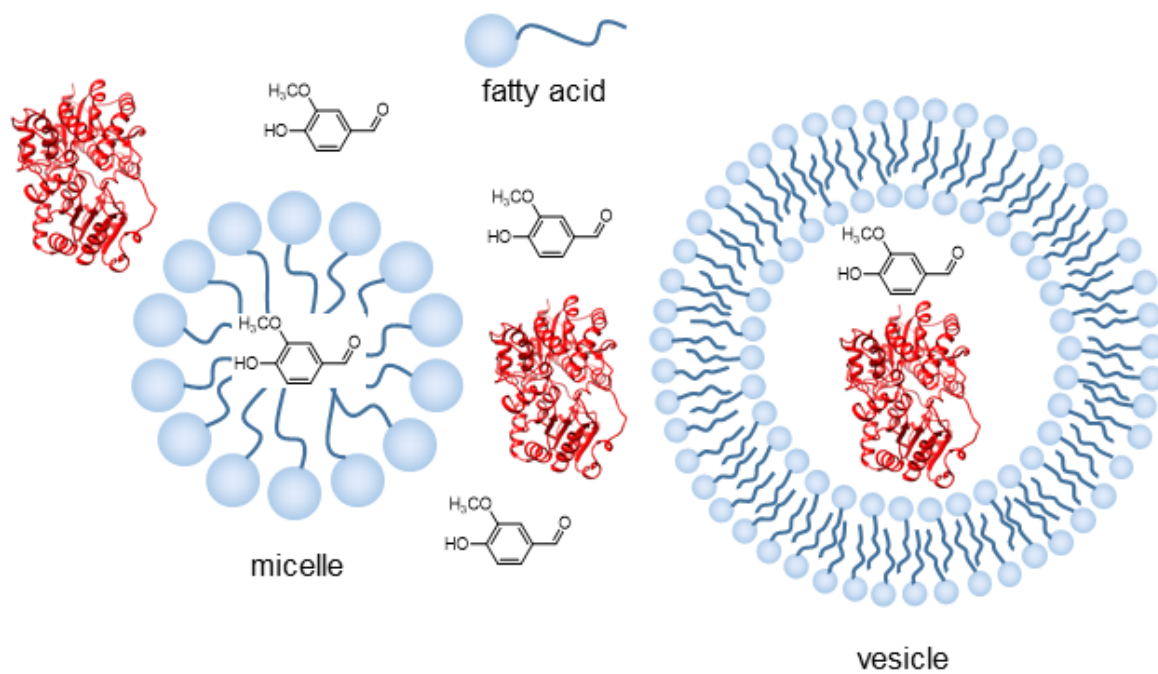


Figure S4. Enzymatic reaction in the presence of micelles and vesicles formed by fatty acid effectors.

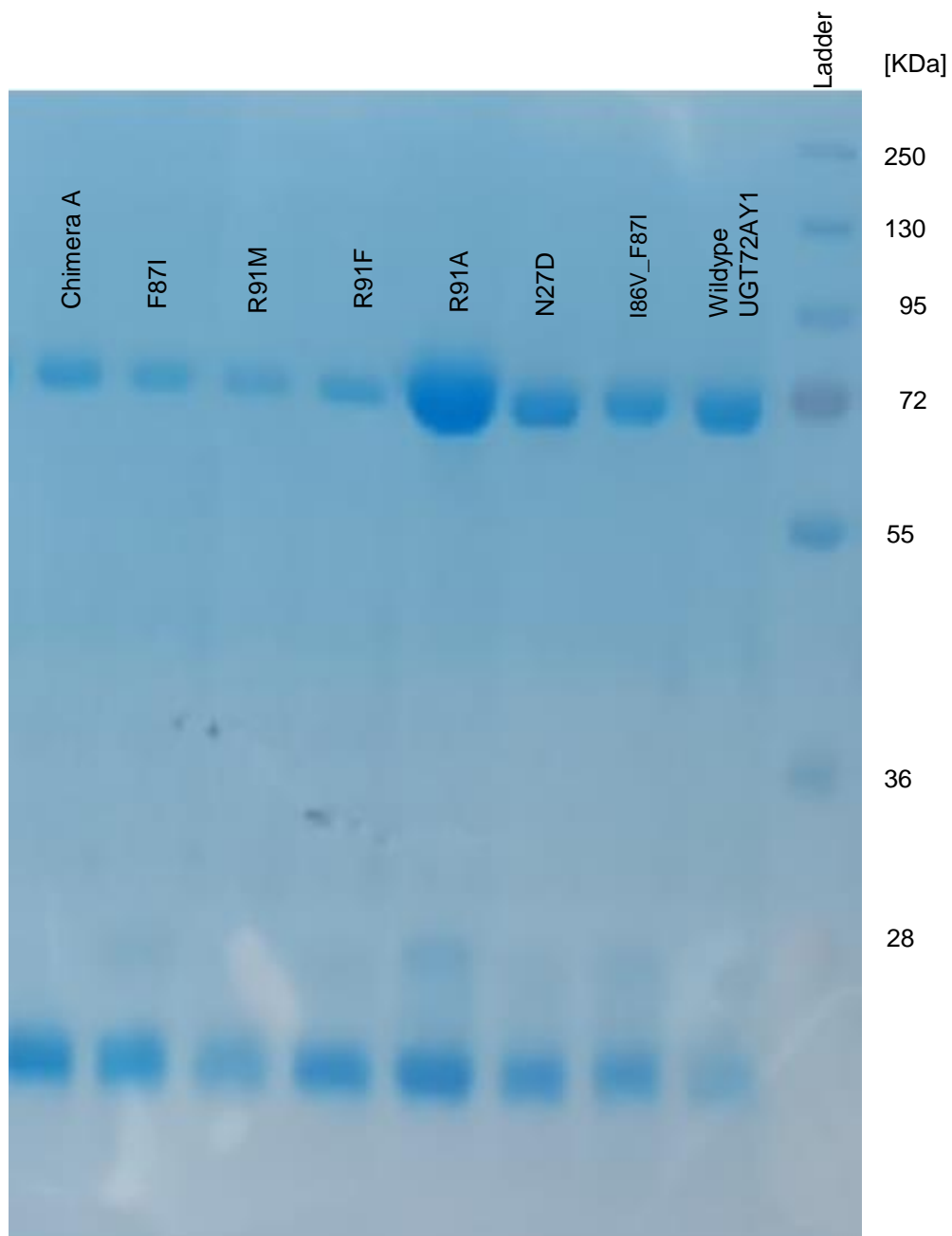


Figure S5: Verification of NbUGT72AY1 mutant protein by SDS-PAGE.
The gel shows the result of the SDS-PAGE that was obtained after GST-affinity purification of the recombinant proteins. Ladder, PageRuler Plus Prestained Protein Ladder