

Description of Additional Supplementary Files

Supplementary Data Legends

Supplementary Data: 1 (SupplementaryDataFile_1_HMT_Primers.xlsx) Primers used for mutagenesis of *AtHMT*.

Supplementary Data: 2 (SupplementaryDataFile_2_Phytase_Primers.xlsx) Primers used for mutagenesis of *YmPhytase*.

Supplementary Data: 3 (SupplementaryDataFile_3_HMT_Screening_Data.xlsx) Data underlying figure S5 and the templates used for SDM for each round. The results from four rounds of crude cell lysate screening for *AtHMT*.

Supplementary Data: 4 (SupplementaryDataFile_4_Phytase_Screening_Data.xlsx) Data underlying figure S8 and the templates used for SDM for each round. The results from four rounds of crude cell lysate screening for *YmPhytase*.

Supplementary Data: 5 (SupplementaryDataFile_5_DNA_sequencing_files.zip) The DNA sequences for wild-type *AtHMT* and *YmPhytase*.

Supplementary Movie Legends

Supplementary Movie 1: The mutation sites from the fourth round of autonomous protein engineering of *AtHMT* marked in red. (PDB ID: 3LCC)

Supplementary Movie 2: The mutation sites from the fourth round of autonomous protein engineering of *YmPhytase* marked in red in the predicted structure of *YmPhytase* from AlphaFold2.