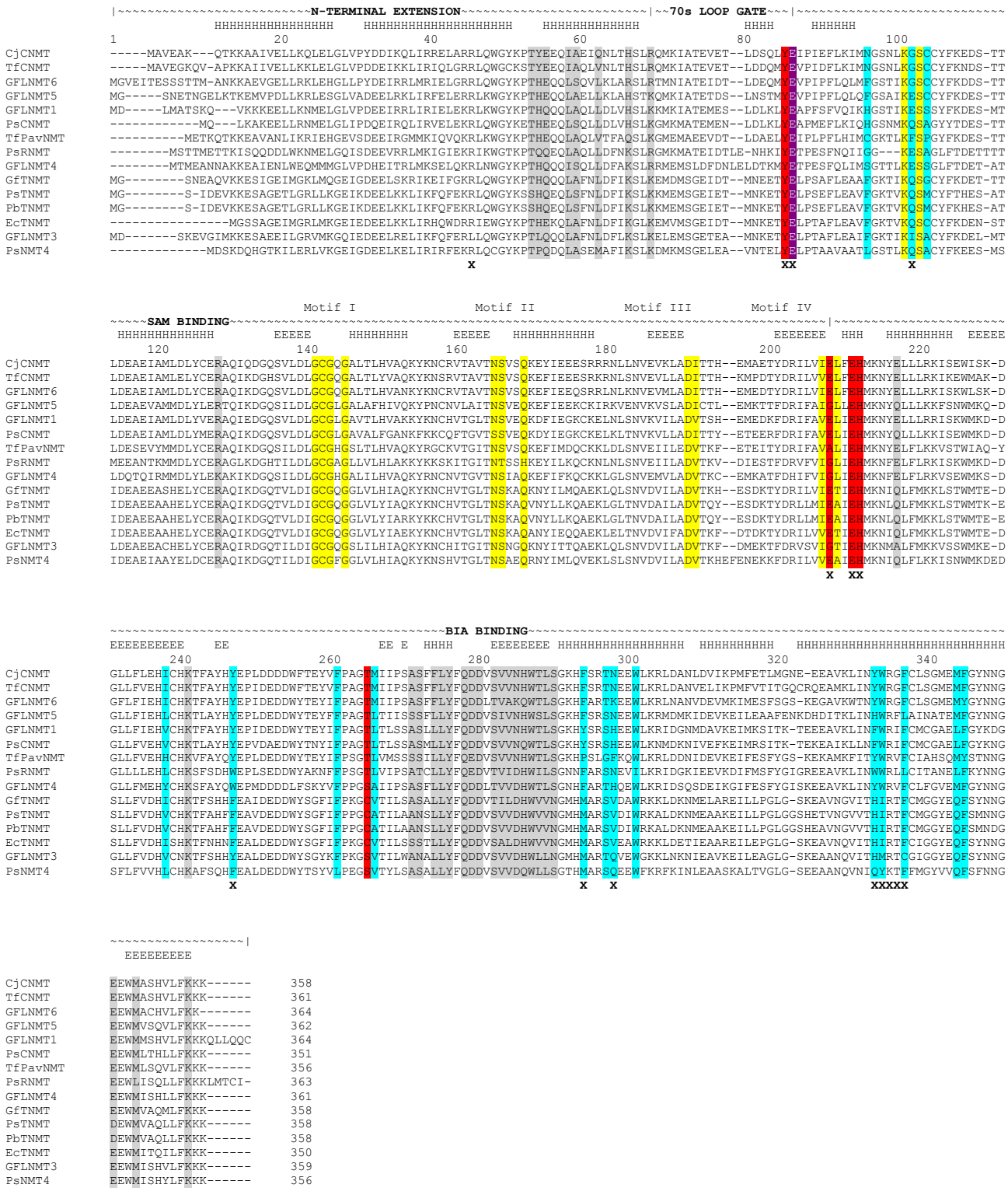

Supplementary Figure 6. Amino acid sequence alignment of select BIA *N*-methyltransferases characterized *in vitro*. Protein sequences were obtained by translation of nucleotide sequences deposited in Genbank (accession numbers listed in Supplementary Table 1) and aligned using Clustal Omega under default parameters (Chojnacki et al., 2017). Major domains and key residues are adapted from structural studies of TfPavNMT and CjCNMT (Torres et al., 2016; Bennett et al., 2018). Residues shaded in *yellow* form hydrogen bonds (directly or water-mediated) with SAM/SAH. Residues shaded in *grey* form part of the dimerization interface. Residues shaded in *cyan* interact with the alkaloid substrate. Residues

(*continued on next page*)

shaded in *red* contribute to catalysis and/or hydrogen bond with the target nitrogen atom. Residues shaded in *purple* contribute to positioning the active site gate upon substrate binding. Secondary structure is given according to DSSP analysis of CjCNMT (Touw et al., 2015). "X" indicates residues subjected to mutational analysis. Percentage identities are provided in Supplementary Figure 2.

Bennett, M. R., Thompson, M. L., Shepherd, S. A., Dunstan, M. S., Herbert, A. J., Smith, D. R. M., et al. (2018). Structure and biocatalytic scope of coclaurine *N*-methyltransferase. *Angew. Chemie - Int. Ed.* 57, 10600–10604. doi:10.1002/anie.201805060.

Chojnacki, S., Cowley, A., Lee, J., Foix, A., and Lopez, R. (2017). Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Res.* 45, W550–W553. doi:10.1093/nar/gkx273.

Torres, M. A., Hoffarth, E., Eugenio, L., Savtchouk, J., Chen, X., Morris, J. S., et al. (2016). Structural and functional studies of pavine *N*-methyltransferase from *Thalictrum flavum* reveal novel insights into substrate recognition and catalytic mechanism. *J. Biol. Chem.* 291, 23403–23415. doi:10.1074/jbc.M116.747261.

Touw, W. G., Baakman, C., Black, J., te Beek, T. A. H., Krieger, E., Joosten, R. P., et al. (2015). A series of PDB-related databanks for everyday needs. *Nucleic Acids Res.* 43, D364–D368. doi:10.1093/nar/gku1028.