

Structural basis for phosphatidylcholine synthesis by bacterial phospholipid *N*-methyltransferases

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Materials included:

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

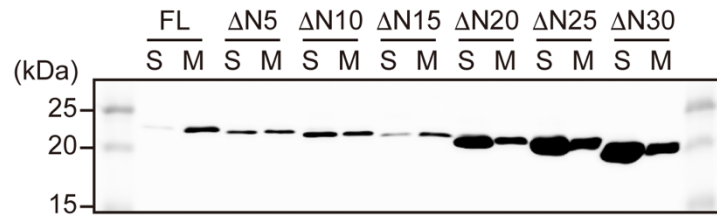
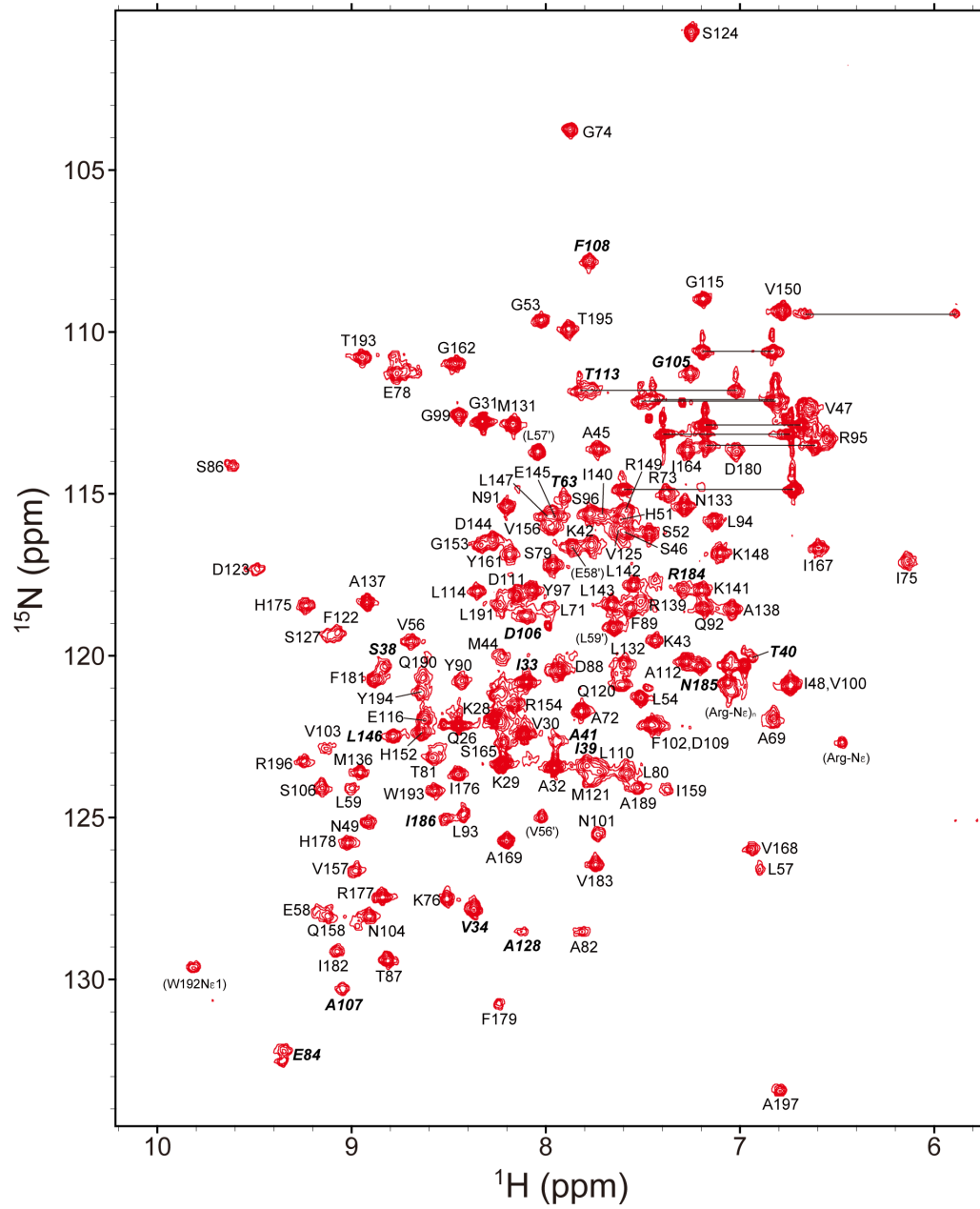


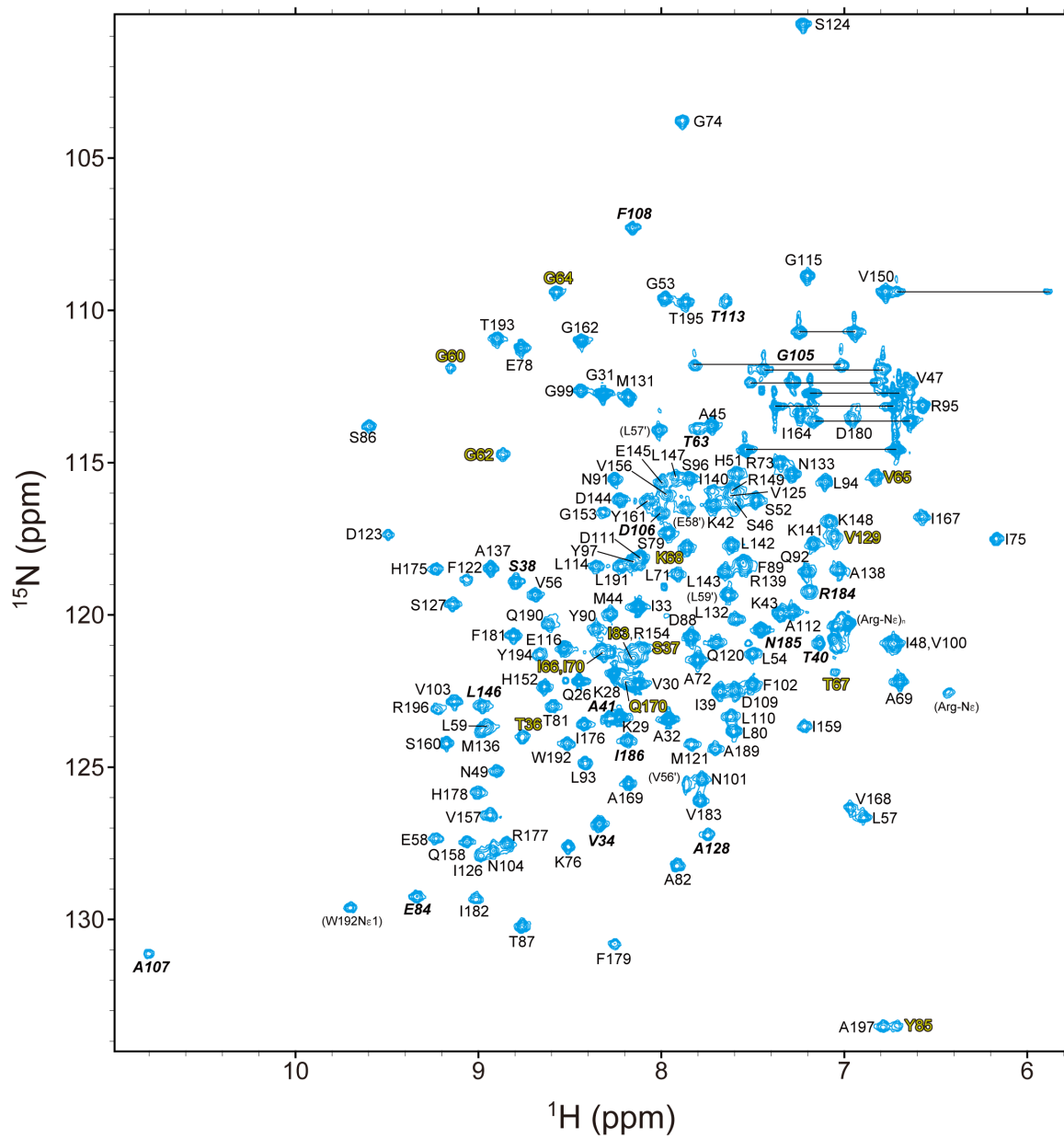
Figure S1. Membrane-binding activity of the N-terminal region lacking AtPmtA mutants Soluble (S) and membrane (M) fractions were separated from *E. coli* cells expressing full-length AtPmtA (FL) or the N-terminal truncated mutants of AtPmtA (Δ N5, Δ N10, Δ N15, Δ N20, Δ N25, and Δ N30) and subjected to SDS-PAGE, followed by immunoblotting with anti-6x histidine antibody.

A



26 QPKKVGAI^{VP}TSSITAKKMASVINPHSGLPVL^{EL}GP^{GT}GVITKAILARGIKPESL 80
 81 TAIEYSTDFYNQL^{LR}SYPGVNFVNGDAFDL^{DAT}LG^{EH}KGQMFDSVISAVPMLN^{FP} 135
 136 MAARIKLLDELLKRV^{PH}GRPVVQISYGPISPIVAQ^{PH}LYHIRH^{FD}FIVRNIPPAQ 190
 191 LWTYTRA 197

B



26 QPKKVGAIVPTSSITAKKMASVINPHSGLPVLELGPVITKAILARGIKPESL 80
 81 TAIEYSTDFYNQLLRSYPGVNFVNGDAFDLDTLGEHKGMFDSVISAVPMLNFP 135
 136 MAARIKLLDELLKRVPHGRPVVQISYGPISPIVAQPHLYHIRHFDFIVRNIPPAQ 190
 191 LWYYTRA 197

C

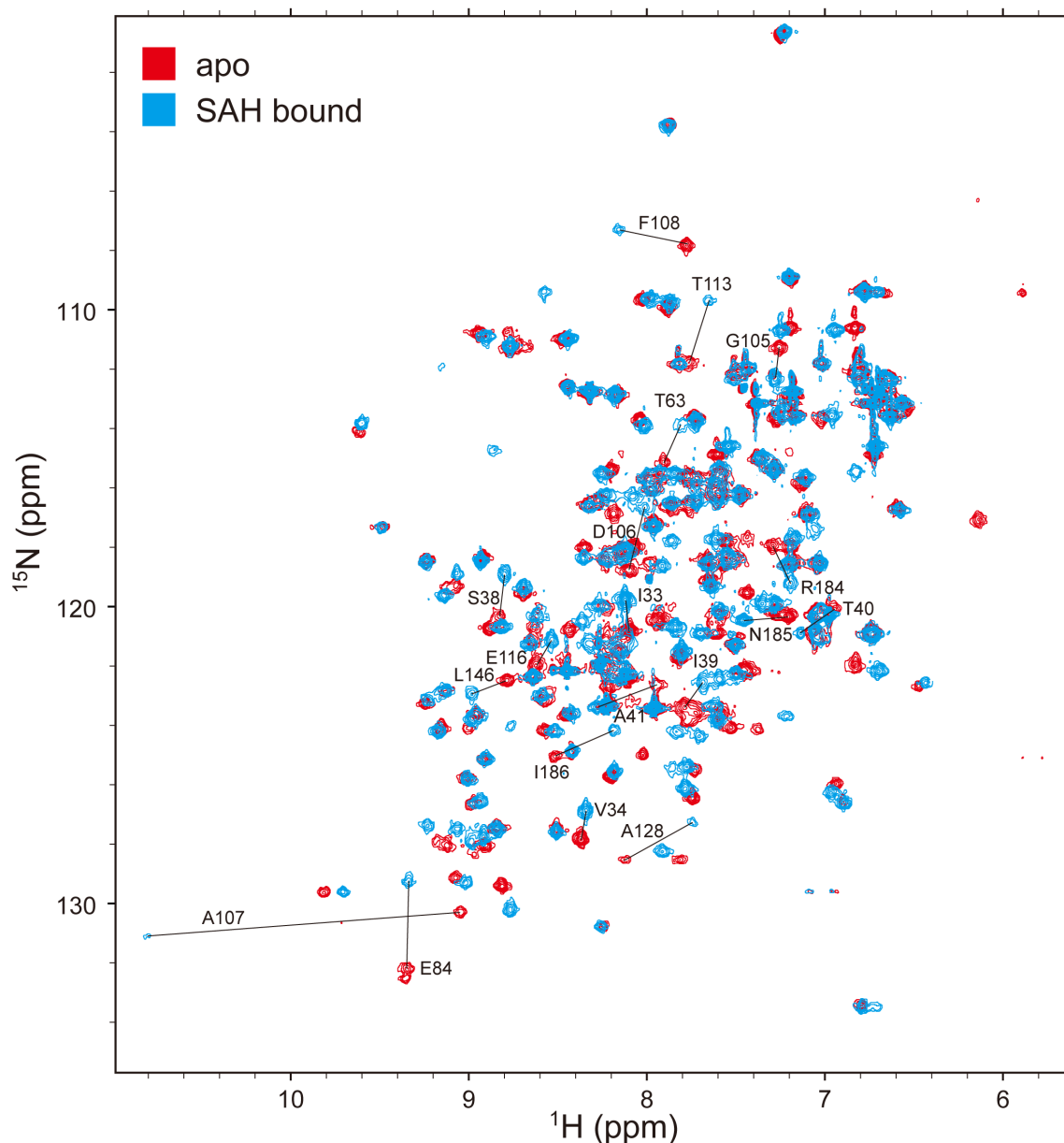
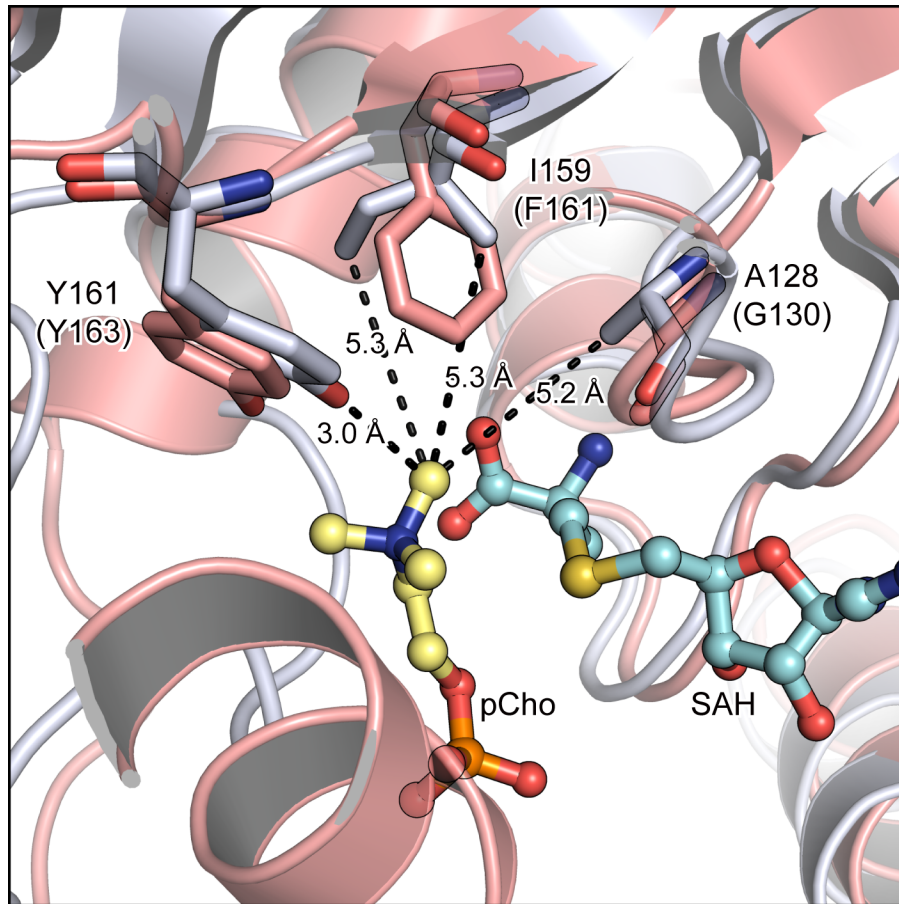


Figure S2. Assigned ^1H - ^{15}N HSQC spectra of apo and SAH bound AtPmtA Δ N25

(A-B) [^1H - ^{15}N] HSQC spectra with resonance assignments of 300 μM AtPmtA Δ N25 in the absence (A) and presence of 3 mM SAH (B). The residues with significant chemical shift changes upon binding to SAH are labeled in *italic*. The residues whose signals appeared upon binding to SAH are labeled in yellow. Minor form assignments are indicated by the prime symbol ('). Amino group resonances of Asn and Gln side chains are connected by horizontal lines. Amino acid sequence of AtPmtA Δ N25, with assigned residues in black and unassigned residues in gray, is shown below each spectrum. (C) Overlay of the [^1H - ^{15}N] HSQC spectra with resonance assignments of 300 μM AtPmtA Δ N25 in the absence (red) and presence of 3 mM SAH (blue). The residues with significant chemical shift changes upon binding to SAH are indicated.



light blue: AtPmtA Δ N25-SAH (This study)
 pink: BdPmtA (AlphaFold 2)

Figure S3. Substrate binding sites of AtPmtA and BdPmtA

Superposition of the docking model of the AtPmtA Δ N25-SAH complex with pCho and the AlphaFold 2-predicted structure of BdPmtA. AtPmtA Δ N25 and BdPmtA are colored in light blue and pink, respectively. Ala128, Ile159, and Tyr161 in AtPmtA and Gly130, Phe161, and Tyr163 in BdPmtA are shown in stick form. BdPmtA residues are indicated in parentheses. The distances between the methyl group of pCho and side chains of Ala128, Ile159, and Tyr161 in AtPmtA.

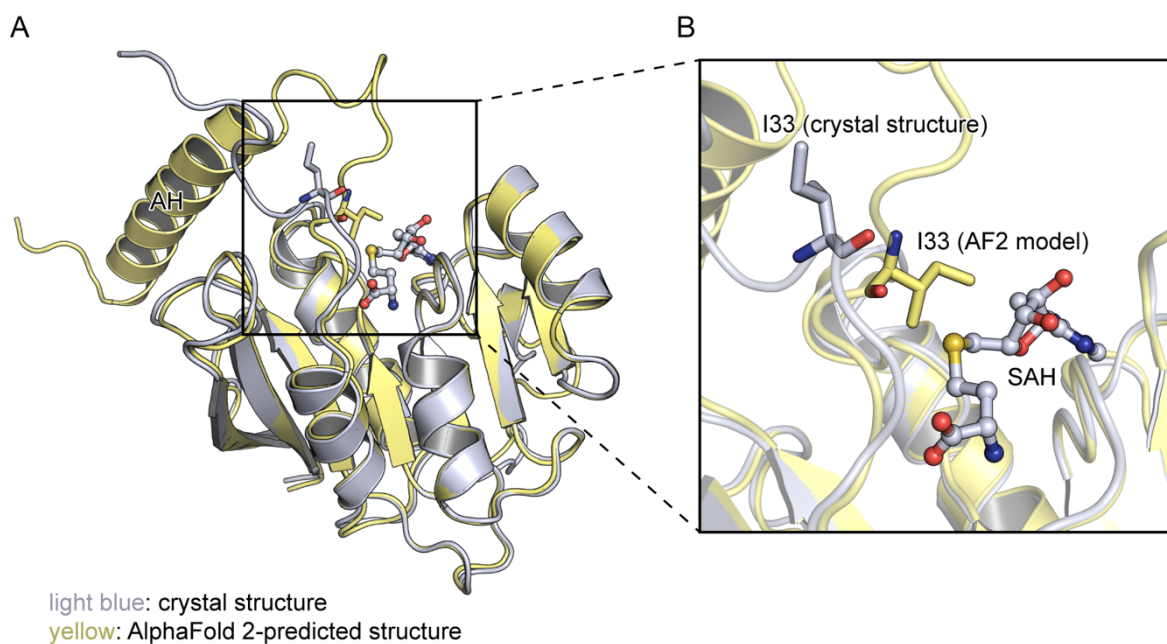


Figure S5. Comparison of AlphaFold 2-predicted and crystal structures of AtPmtA
(A) Superposition of the AlphaFold 2-predicted structure of AtPmtA (yellow) and the crystal structure of AtPmtA Δ N25 (light blue). The N-terminal amphipathic helix of the AlphaFold 2-predicted structure of AtPmtA is labeled as AH. (B) The magnified view around Ile33 of AtPmtA. Ile33 is shown in stick form, whereas SAH is shown in stick and ball form.