

## SUPPLEMENTAL MATERIAL

**Fig. 1S. *MsPimA* is a monomer in solution.** Gel filtration chromatography was performed using a BioSuite 250 5  $\mu\text{m}$  HR SEC column (Waters Corporation) equilibrated in 50 mM Tris-HCl pH 6.8 and 150 mM NaCl at 1 ml/min. The column was previously calibrated using gel filtration standards (Sigma) including  $\beta$ -amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and cytochrome c (12.4 kDa).

**Fig. 2S. Limited proteolysis of *MsPimA*.** The N-terminal residues of the two prominent proteolytic fragments are indicated.

**Fig. 3S. Thermal unfolding of *MsPimA*-guanosine complex monitored by DSC.** The excess heat capacity curve of *MsPimA* in the presence of a saturation concentration of guanosine ( $\bullet$ ) is shown with its low and high temperature contribution peaks ( $\bullet$ ). The  $C_{p,\text{ex}}$  curve of free *MsPimA* (-) and its low and high temperature contribution peaks (-) are reported for comparison. *MsPimA* with guanosine shows no significant changes in  $T_{1/2}$  and  $\Delta H_u$  with respect to the free *MsPimA* protein. Guanosine increases  $\Delta H_{u,1}$  by  $6.0 \pm 1.6$  kcal mol $^{-1}$  and decreases  $\Delta H_{u,2}$  by  $5.6 \pm 1.6$  kcal mol $^{-1}$  (Table 1), in agreement with the low guanosine binding affinity for *MsPimA* (see Figure 2D).

**Fig. 4S. Structural alignment of GT-B peripheral-membrane binding GTs.** Molecular surface representation of *MsPimA* and several GT-B fold lipid-binding GTs, showing the position of the positive-charged clusters in the N-terminal domains. The structures are displayed in the same orientation after structural alignment.

**Table 1S.** Complementary oligonucleotide used to introduce mutations in *MsPimA* gene.

<b>Mutation</b>	<b>5'-3' oligonucleotide sequence</b>	<b>Plasmid name</b>
<b>Q18A</b>	F GTGCCGGGTGGCGTGGCGTCCCACGTGCTCCAG R CTGGAGCACGTGGGACGCCACGCCACCCGGCAC	pET29MsPimA- <b>Q18A</b>
<b>Y62A</b>	F GCCGTGCCGATCCCGGCCAACGGCTCGGTGGCG R CGCCACCGAGCCGTTGGCCGGGATCGGCACGGC	pET29MsPimA- <b>Y62A</b>
<b>N63A</b>	F GTGCCGATCCCGTACGCCGGCTCGGTGGCGCGG R CCGCGCCACCGAGCCGGCGTACGGGATCGGCAC	pET29MsPimA- <b>N63A</b>
<b>S65A</b>	F ATCCCGTACAACGGCGCGGTGGCGCGGCTGCGG R CCGCAGCCGCGCCACCGCGCCGTTGTACGGGAT	pET29MsPimA- <b>S65A</b>
<b>R68A</b>	F AACGGCTCGGTGGCGGGCGCTGCGGTTCCGGGCCG R CGGCCCGAACCAGCGCCGCCACCGAGCCGTT	pET29MsPimA- <b>R68A</b>
<b>R70A</b>	F TCGGTGGCGCGGCTGGCGTTCGGGCCGGCCACG R CGTGGCCGGCCCGAACGCCAGCCGCGCCACCGA	pET29MsPimA- <b>R70A</b>
<b>N97A</b>	F ACATCCACGAGCCGGCCGCGCCGAGCCTGTCTG R CGACAGGCTCGGCGCGGCCGGCTCGTGGATGT	pET29MsPimA- <b>N97A</b>
<b>T119A</b>	F TCGCGACGTTCCACGCGTCGACCACGAAGTCG R CGACTTCGTGGTTCGACGCGTGGAAACGTCGCGA	pET29MsPimA- <b>T119A</b>
<b>K123A</b>	F CACACGTCGACCACGGCGTCGTTGACGCTCAGC R GCTGAGCGTCAACGACGCCGTCGGTTCGACGTGTG	pET29MsPimA- <b>K123A</b>
<b>R196A</b>	F TGTTGTTCTGGGGGCGTACGACGAACCGCGC R GCGCGGTTTCGTCTGACGCCCCAGGAACAACA	pET29MsPimA- <b>R196A</b>
<b>E199A</b>	F TGGGGCGGTACGACGCACCGCGCAAGGGCATGG R CCATGCCCTTGCGCGGTGCGTCTGACCGCCCA	pET29MsPimA- <b>E199A</b>
<b>R77E/K78E/ K80E/K81E</b>	F GGGCCGGCCACGCACGCCGCGTTCGCGGCGTGGATCGCCGAGGGC R GCCCTCGGCGATCCACGCCGCGACCGCGGCGTTCGCGTGGCCGGCCC	pET29MsPimA- <b>R77E/K78E/ K80E/K81E</b>

**Table 2S.** Structural comparison of GT-B enzymes.

GT-B Family	Catalytic mechanism	Membrane associated	Non-membrane associated
GT1	Inverting	<b>Alg13/Alg14</b> (2JZC, <i>PPI<sup>a</sup></i> ), <b>Ugt2b7</b> (2O6L, <i>A<math>\alpha</math>H</i> )	<b>OGT/NGT</b> (2VCH), <b>GtfA</b> (1PN3), <b>GtfB</b> (1IIR), <b>GtfD</b> (1RRV), <b>CalG3</b> (3DOR), <b>OleD</b> (2IYF), <b>OleI</b> (2IYA), <b>UrdGT2</b> (2P6P), <b>UGT85H2</b> (2PQ6), <b>UGT71G1</b> (2ACV), <b>Ufgt</b> (2C1Z)
GT4	Retaining	<b>PimA</b> (2GEJ, <i>A<math>\alpha</math>H<sup>c</sup></i> ), <b>WaaG</b> (2IV7, <i>A<math>\alpha</math>H</i> )	<b>MshA</b> (3C4V), <b>SpsA</b> (2R68),
GT5	Retaining	NO	<b>AtGlgA</b> (1RZU), <b>PaGlgA</b> (2BIS)
GT9	Inverting	<b>WaaF</b> (1PSW, <i>A<math>\alpha</math>H</i> ), <b>WaaC</b> (2H1F, <i>A<math>\alpha</math>H</i> )	NO
GT10	Inverting	<b>FucT</b> (2NZY, <i>TM<sup>b</sup></i> )	NO
GT20	Retaining	NO	<b>OtsA</b> (1GZ5)
GT23	Inverting	<b>FUT8</b> (1DE0, <i>TM</i> ), <b>NodZ</b> (1HHC, <i>TM</i> )	NO
GT28	Inverting	<b>MurG</b> (1NLM, <i>A<math>\alpha</math>H</i> )	NO
GT35	Retaining	NO	<b>GP</b> (3GPB), <b>MalP</b> (1AHP)
GT63	Inverting	NO	<b>BGT</b> (1BGT)
GT70	Inverting	<b>GumK</b> (3CV3, <i>A<math>\alpha</math>H</i> )	NO
GT72	Retaining	NO	<b>AGT</b> (1XV5)
GT80	Inverting	<b>ST1</b> (2IHZ, <i>TM</i> ), <b>ST</b> (2Z4T, <i>TM</i> )	NO

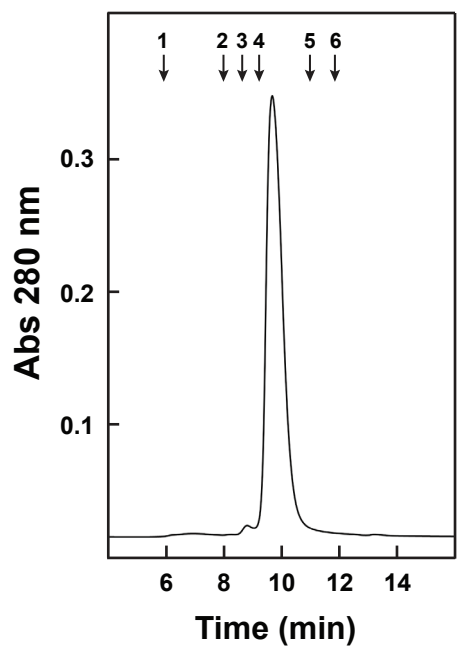
<sup>a</sup>*PPI*: protein-protein interaction.

<sup>b</sup>*TM*: transmembrane peptide.

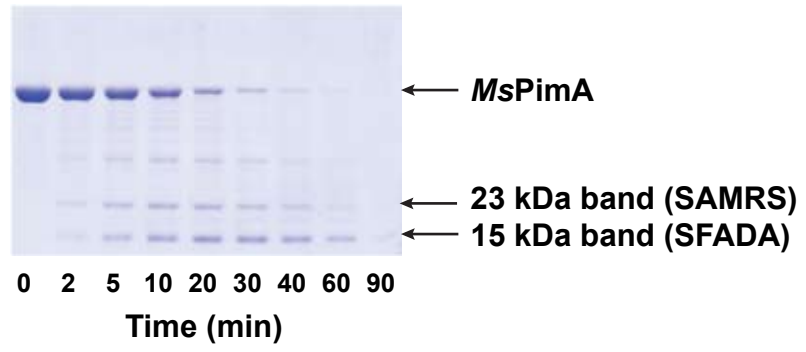
<sup>c</sup>*A $\alpha$ H*: amphiphatic alpha helix.

**GtfA**, dTDP- $\beta$ -L-4-epi-epivancosamine: epivancosaminyltransferase from *Amycolatopsis orientalis* A82846 ; **GtfB**, TDP/UDP-glucose:aglycosyl-vancomycin glucosyltransferase from *Amycolatopsis orientalis* A82846 ; **GtfD**, UDP- $\beta$ -L-4-epi-vancosamine:vancomycin-pseudoaglycone vancosaminyltransferase from *Amycolatopsis orientalis* ATCC19795 ; **CalG3**, enediyne glycosyltransferase from *Micromonospora echinospora* ; **OleD**, oleandomycin glycosyltransferase from *Streptomyces antibioticus* ATCC11891 ; **OleI**, oleandomycin glycosyltransferase from *Streptomyces antibioticus* ATCC11891 ; **UrdGT2**, urdamycin A glycosyltransferase II from *Streptomyces fradiae* T2717 ; **OGT/NGT**, 2,5-DHBA-, 3,4-DHBA-glucosyltransferase, from *Agrobacterium tumefaciens* ; **Ugt2b7**, UDP-glucuronosyltransferase 2B7 from *Homo sapiens* ; **UGT85H2**, UDP-glucose : (iso)flavonoid  $\beta$ -glucosyltransferase from *Medicago truncatula* ; **UGT71G1**, UDP-glucose : flavonoid  $\beta$ -glucosyltransferase from *Medicago truncatula* ; **Alg13**, UDP-GlcNAc : Dol-PP-GlcNAc N-acetylglucosaminyltransferase from *Saccharomyces cerevisiae* S288C ; **Ufgt**, UDP-Glc: anthocyanidin 3-O-glucosyltransferase from *Vitis vinifera* ; **PimA**, GDP-Man: phosphatidylinositol mannosyltransferase from *Mycobacterium smegmatis* ; **MshA**, UDP-GlcNAc : inositol-P N-acetylglucosaminyltransferase from *Corynebacterium glutamicum* ; **WaaG**, UDP-Glc: L-glycero-D-manno-heptose II -1,3-glucosyltransferase I. from *Escherichia coli* K12 ; **SpsA**, sucrose phosphate synthase from *Halothermothrix orenii* ; **AviGT4**, eurekaate-attachment enzyme from *Streptomyces viridochromogenes* Tue57 ; **GS**, glycogen synthase from *Pyrococcus abyssi* GE5 Orsay ; **GS**, glycogen synthase from *Agrobacterium tumefaciens* ; **WaaF**, heptosyltransferase II from *Escherichia coli* K12 ; **WaaC**, heptosyltransferase I from *Escherichia coli* K12 ; **FucT**  $\alpha$ -1,3-fucosyltransferase (FucT) from *Helicobacter pylori* NCTC 11639 ; **OtsA**,  $\alpha$ , $\alpha$ -trehalose-phosphate synthase from *Escherichia coli* ; **Fut8**, N-acetyl-D-glucosaminide -1,6-L-

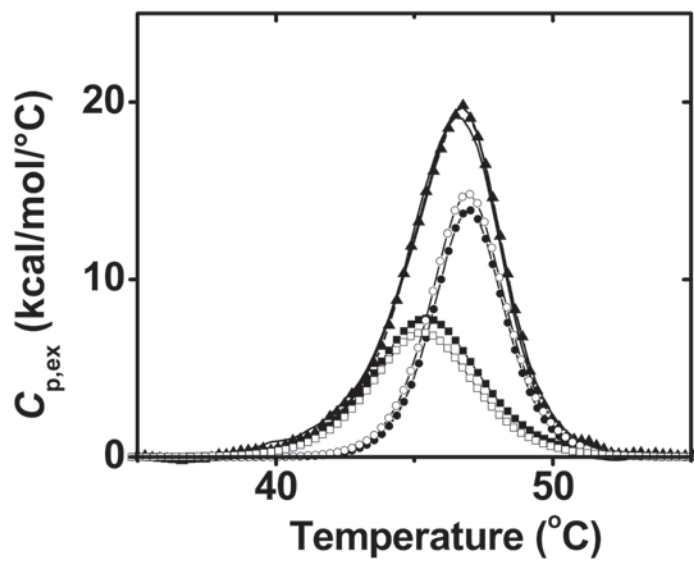
fucosyltransferase from *Homo sapiens* ; **NodZ**,  $\alpha$ -1,6-L-fucosyltransferase from *Bradyrhizobium sp* WM9 ; **MurG**, UDP-GlcNAc: N-acetylmuramyl-(pentapeptide)-PP-C55 N-acetylglucosaminyltransferase from *Escherichia coli* K12 ; **MalP**, maltodextrin phosphorylase from *Escherichia coli* ; **GP**, glycogen phosphorylase from *Oryctolagus cuniculus* ; **BGT**, UDP-Glc: DNA  $\beta$ -glucosyltransferase from *Bacteriophage* T4 ; **GumK**, UDP-GlcA: (xanthan)  $\alpha$ -Man-(1,3)- $\beta$ -Glc-(1,4)-  $\alpha$ -Glc-PP-polyisoprenyl  $\beta$ -1,2-glucuronosyltransferase from *Xanthomonas campestris pv campestris* ; **AGT**, UDP-Glc: DNA  $\alpha$ -glucosyltransferase from *Bacteriophage* T4 ; **ST1**, CMP-NeuA :  $\alpha$ -2,3/2,6-sialyltransferase / sialidase from *Pasteurella multocida* ATCC 15742 ; **ST**, CMP-NeuAc:  $\beta$ -galactoside  $\alpha$ -2,6-sialyltransferase from *Vibrionaceae Photobacterium sp.* JT-ISH-224 (for further details please see [www.cazy.org](http://www.cazy.org)).



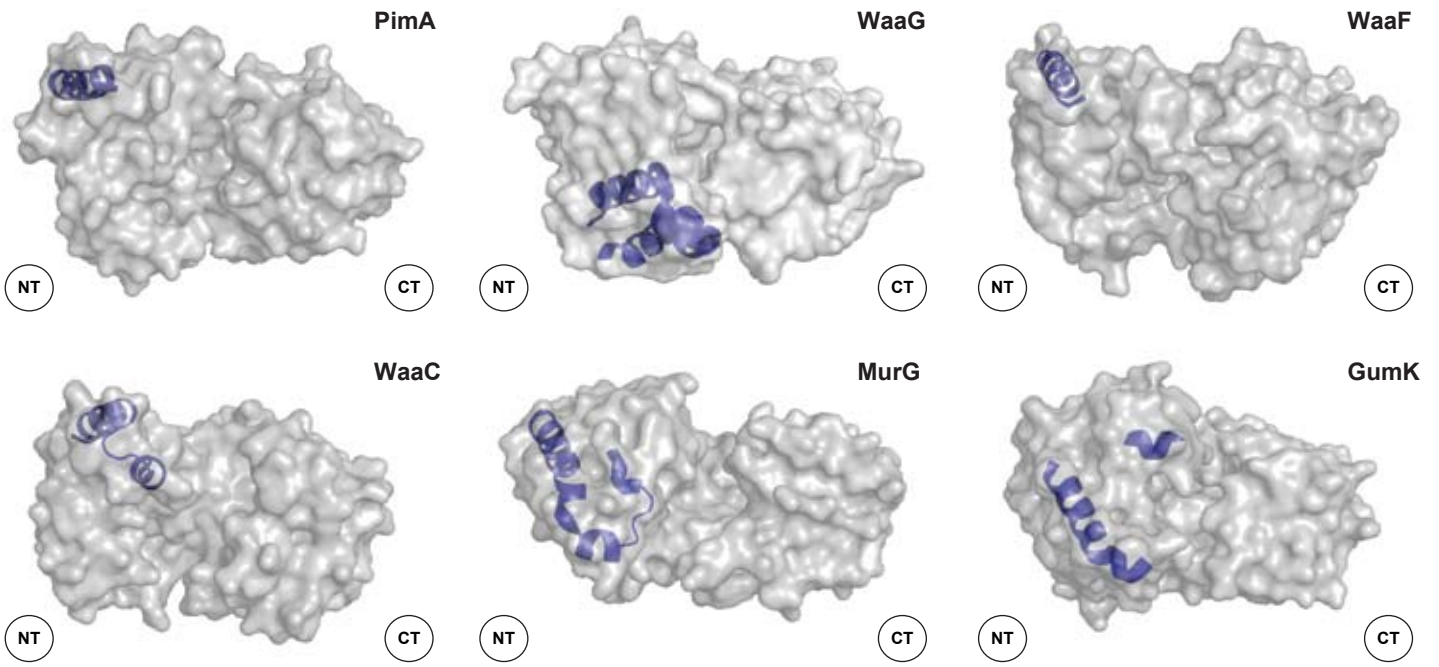
**Figure 1S**



**Figure 2S**



**Figure 3S**



**Figure 4S**