

## Supporting Information

### Structural basis of substrate recognition and catalysis by fucosyltransferase 8

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HsFUT8      .....RNLGKGDHEILRRRIENGAKELWFFLQSELKCLKLHLEGNELQRHADEFLLD
MmFUT8      EFRRIPEGPIDQGTATGRVRLVEEQLVKAKEQIENYKKAARNLGLGKGDHEILRRRIENGAKELWFFLQSELKCLKLHLEGNELQRHADEFLLD
consensus>70 .....RNLGKGDHEILRRRIENGAKELWFFLQSELKCLKLHLEGNELQRHADE.LLD

                60          70          80          90          100         110         120         130         140
HsFUT8      LGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRRIITYLQNPKDCSKAKLVCNINKCGGYGCQLHHVVYCFMIAYGTORTLTLES
MmFUT8      LGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRRIITYLQNPKDCSKAKLVCNINKCGGYGCQLHHVVYCFMIAYGTORTLTLES
consensus>70 LGHHERSIMTDLYYLSQTDGAGDWREKEAKDLTELVRRIITYLQNPKDCSKA.KLVCNINKCGGYGCQLHHVVYCFMIAYGTORTLTLES

                150         160         170         180         190         200         210         220         230
HsFUT8      QNWRYPATGGWETVFRPVSETCTDRSGESTGHWSGEVNDKNVQVVELPIVDSLHPRPPYLPPLAVPEDLADRLRVHGDPAVWVVSQFVKYL
MmFUT8      QNWRYPATGGWETVFRPVSETCTDRSGESTGHWSGEVNDKNVQVVELPIVDSLHPRPPYLPPLAVPEDLADRLRVHGDPAVWVVSQFVKYL
consensus>70 QNWRYPATGGWETVFRPVSETCTDRSGESTGHWSGEV.DKNVQVVELPIVDSLHPRPPYLPPLAVPEDLADRLRVHGDPAVWVVSQFVKYL

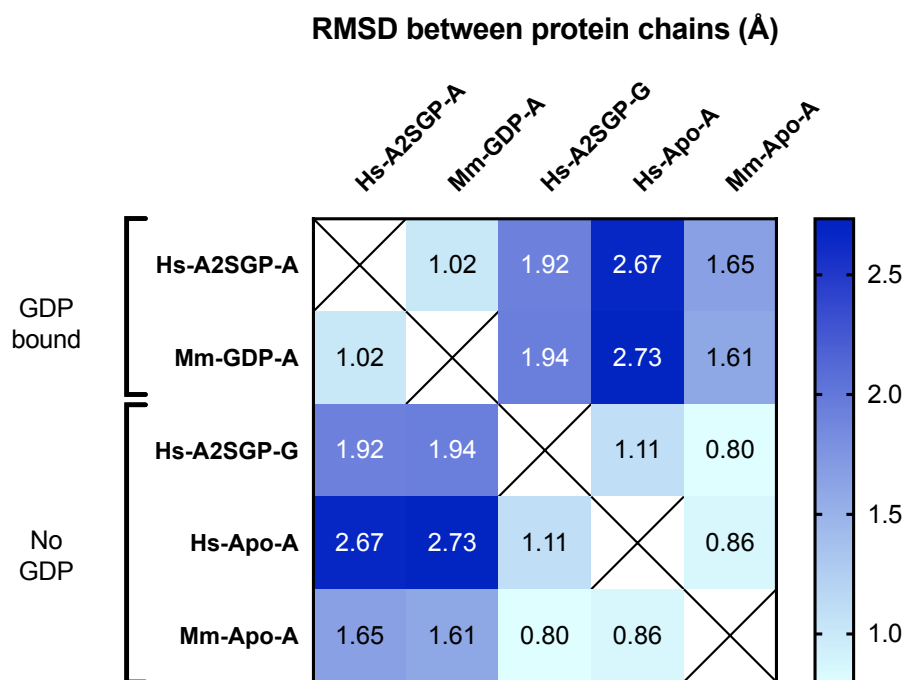
                240         250         260         270         280         290         300         310         320
HsFUT8      IRPQPWLEKEIEEATKKGFKHPVIGVHVRRTDKVGTAAAFHPIEEYMVHVEHFQOLLARRMQVDKRVYLATDDPILLKEANTKYBNYE
MmFUT8      IRPQPWLEKEIEEATKKGFKHPVIGVHVRRTDKVGTAAAFHPIEEYMVHVEHFQOLLARRMQVDKRVYLATDDPILLKEANTKYBNYE
consensus>70 IRPQPWLEKEIEEATKKGFKHPVIGVHVRRTDKVGTAAAFHPIEEYMVHVEHFQOLLARRMQVDKRVYLATDDP.LLKEA.TKY.NYE

                330         340         350         360         370         380         390         400         410
HsFUT8      FISDNSTSWSAGLHNRRTENSLRGVILDIHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSISLDDIYFYGQNAHNOIAIYHPKQ
MmFUT8      FISDNSTSWSAGLHNRRTENSLRGVILDIHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSISLDDIYFYGQNAHNOIAIYHPKQ
consensus>70 FISDNSTSWSAGLHNRRTENSLRGVILDIHFLSQADFLVCTFSSQVCRVAYEIMQTLHPDASANFHSISLDDIYFYGQNAHNOIAIY.H.P

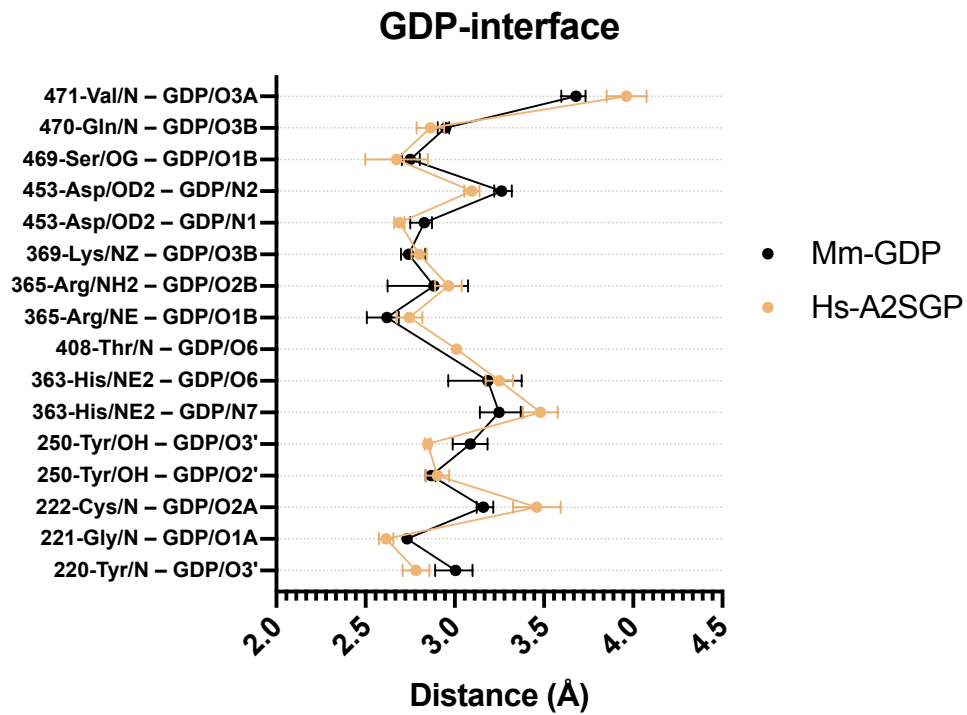
                420         430         440         450         460         470
HsFUT8      RTADEIPMEPGDIIGVAGNHWDGYSKGNRRLGRTGLYPSYKVRREKIEYTKVPTYPEAEK
MmFUT8      RTADEIPMEPGDIIGVAGNHWDGYSKGNRRLGRTGLYPSYKVRREKIEYTKVPTYPEAEK
consensus>70 RT.#EIPMEPGDIIGVAGNHWDGYSKGNRRLGRTGLYPSYKVRREKIEYTKVPTYPEAEK

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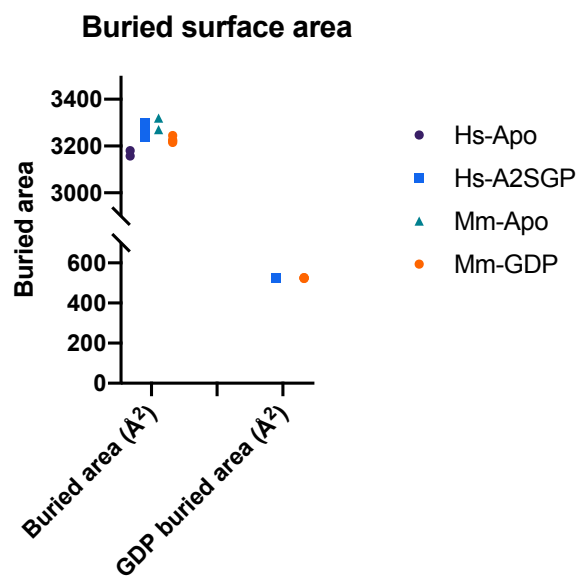
Supplementary Figure 1. Sequence alignment of HsFUT8<sub>105-575</sub> and MmFut8<sub>68-575</sub>. The proteins are  $\approx 97\%$  identical over this region.



**Supplementary Figure 2.** Heatmap illustrating the RMSD between protein chains in the FUT8 structures reported in this study.



**Supplementary Figure 3.** A map of hydrogen bond distances between GDP and FUT8 for all relevant structures reported in this work.



**Supplementary Figure 4.** Buried surface area of FUT8 upon dimerisation, and of GDP upon binding FUT8, for each of the structures reported in this work.

**Table S1.** The sequences of nucleotides and proteins used in this study. Restrictions sites used for cloning are underlined; signal peptides are shaded in grey; His<sub>10</sub> tags are shaded in yellow; V5 epitope tags are shaded in blue; and factor Xa sites are shaded in pink.

protein	Synthetic dsDNA used for cloning	Protein sequence
HsFUT8	<p>GGATCT<u>CGGGTC</u>CGAAACCATGCTACTAGTAAATCAGTCACACCAAGGCTTCA  ATAAGGAACACACAAGCAAGATGGTAAAGCGCTATTGTTTTATATGTGCTTTT  GGCGGCGGCGCGCATCTGCCTTTGCGGAGAATGGTCTGGGGAAGGATCAT  GAAATCCTGAGGAGGAGGATTGAAAATGGAGCTAAAGAGCTCTGGTTTTCC  TACAGAGTGAATTGAAGAAATTAAGAAGCTTAGAAGGAAATGAACCCAAAG  ACATGCAGATGAATTTCTTTGGATTAGGACATCATGAAAGGTCTATAATG  ACGGATCTATACTACCTCAGTCAGACAGATGGAGCAGGTGATTTGGCGGGAAA  AAGAGCCAAAGATCTGACAGAAGCTGGTTCAGCGGAGAATAACATATCTTCA  GAATCCCAAGGACTGCAGCAAAGCCAAAAGCTGGTGTGTAATATCAACAAA  GGCTGTGGCTATGGCTGTGACGTCATCATGTTGGTCTACTGCTTCATGATTG  CATATGGCACCAGCGAACACTCATCTTGGAAATCTCAGAATTGGCGCTATGC  TACTGGTGGATGGGAGACTGTATTTAGGCCCTGTAAGTGAGACATGCACAGAC  AGATCTGGCATCTCCACTGGACACTGGTCAAGTGAAGTGAAGGACAAAAATG  TTCAAGTGGTGGAGCTTCCCATTTGTAGACAGTCTTCATCCCGCTCCCTCATA  TTTACCCTTGGCTGTACCAGAAGACCTCGCAGATCGACTTGTACGAGTGCAT  GGTAGCCCTGAGTGTGGTGGTGTCTCAGATTGTCAAAATGACTTGTATCCGCC  CACAGCCTTGGCTAGAAAAGAAATAGAAGAAGCCACCAAGAAGCTTGGCTT  CAAAATCCAGTTATTTGGAGTCCATGTCAGACGCACAGACAAAGTGGGAACA  GAAGCTGCCTCCATCCCATGAAGAGTACATGGTGCATGTTGAAGAATTT  TTACAGCTTCTTGCACGCAGAATCAAGTGGACAAAAAAGAGTGTATTTGGC  CACAGATGACCTTCTTTATTAAGGAGGCCAAAACAAGTACCCCAATTAT  GAATTTATTAGTGATAACTCTATTTCTGGTCAAGTGGACTGCACAAATCGAT  ACACGAAAAATCACTTCGTGGAGTGATCCTGGATATACATTTCTCTCTCA  GGCAGACTTCTAGTGTGTACTTTTTTCATCCAGGTCTGTGAGTTGCTTAT  GAAATATGCAAACTACATCCTGTGCTTGCAGAACTTCCATTCATTTAG  ATGACATCTACTATTTTGGGGGCCAGAATGCCCAATCAAAATGCCATTTA  TGCTCACAAACCCGAAGTGCAGATGAAATCCCATGGAACCTGGAGATATC  ATTGGTGTGGCTGGAAATCATTTGGGATGGCTATTCTAAAGGTGTCAACAGGA  AATTGGGAAGGACGGGCTATATCCCTCCTACAAAGTTCGAGAGAAGATAGA  AACGGTCAAGTACCCACATATCCTGAGGCTGAGAAACACCACCATCACCAT  CACCATCACCATCACTGACTCGAGGCATG</p>	<p>MLLVNQSHQGFNKEHTSKMVSAILV  YVLLAAAAHSFAFARNGLGKDHEILR  RRIENGAKELWFFLQSELKCLKNLE  GNELQRHADEFLLDLGHHERSIMTD  LYYLSQTDGAGDWREKEAKDLTEL  QRRITYLQNPKDCSKAKKLVCNINK  GCGYGCQLHHVVYCFMAYGTQRTL  ILESQNWRYATGGWETVFRPVSETC  TDRSGISTGHWSEVVDKKNVQVVEL  PIVDSLHPRPPYLPVAVPEDLADRL  VRVHGDPAVWVWSQFVKYLIRPQPV  LEKEIEEATKKLGFKHPVIGVHVR  TDKVGTEAAFHPVIEEYMVHVEEHFQ  LLARRMQVDDKRVYLATDDPSSLKE  AKTKYPNYEFISDNSISWSAGLHNR  YTENSLRGVILDIHFLSQDFLVCT  FSSQVCRVAYEIMQTLHPDASANFH  SLDDIYFYGQNAHNQIAIYAHQPR  TADEIPMEPGDIIIGVAGNHWDGYSK  GVNRKLGRTGLYPSYKVRKIEYTVK  YPTYPEAEKHHHHHHHHHH</p>
MmFUT8	<p>GGATCT<u>CGGGTC</u>CGAAACCATGCTACTAGTAAATCAGTCACACCAAGGCTTCA  ATAAGGAACACACAAGCAAGATGGTAAAGCGCTATTGTTTTATATGTGCTTTT  GGCGGCGGCGCGCATCTGCCTTTGCGGCGGATCTTGGATCCCAACCATCAT  CACCACCATCACCACCATCAGCGCAAACCAATTTCCCAACCTTTGCTGGGAC  TGGATTCACATATAGACGGCGTGAATTCGGAATACAGAAGGCCCAATTTGA  CCAGGGGACAGCTACAGGAAGAGTCCCGTGTTTAGAAGAAGAGCTTGTAAAG  GCCAAAGAACAGATTGAAAATTAACAAGAAACAGCTAGAAAATGGTCTGGGGA  AGGATCATGAAATCTTAAGAAGGAGGATTGAAAATGGAGCTAAAGAGCTCTG  GTTTTTCTACAAAGCGAAGTGAAGAAATTAAGCATTTAGAAGGAAATGAA  CTCAAAGACATGCAGATGAAATCTTTGGATTAGGACACCATGAAAGGT  CTATCATGACAGATCTATACTACCTCAGTCAAACAGATGGAGCAGGGGATTG  GCGTGAAGAAAGAGGCAAAGATCTGACAGAGCTGGTCCAGCGGAGAATAACA  TATCTCCAGAATCTTAAGGACTGCAGCAAAGCCAGGAAGCTGGTGTGTAACA  TCAATAAAGGCTGTGGCTATGGTGTCAACTCCATCAGTGGTCTACTGTTT  CATGATTGCTTATGGCACCCAGCGAACACTCATCTTGAATCTCAGAATTGG  CGCTATGCTACTGGTGGATGGGAGACTGTGTTTAGACCTGTAAGTGAGACAT  GTACAGACAGATCTGGCCTCTCCACTGGACACTGGTCAAGTGAAGTAAATGA  CAAAAACATTCAGTGGTGGTCCGAGCTCCCATTTGTAGACAGCTCCATCCTCGG  CCTCCTTACTTACCCTGGCTGTCCAGAAGACCTTGCAGACCGACTCCTAA  GAGTCCATGGTGACCTGCAGTGTGGTGGGTGTCCAGTTTGTCAAATACTT  GATTCGTCCACAACCTTGGCTGGAAAAGGAAATAGAAGAAGCCCAAGAAG  CTTGGCTTCAAACATCCAGTTATTGGAGTCCATGTCAGACGCACAGACAAAG  TGGGAACAGAAGCAGCTTCCACCCATCGAGGAGTACATGGTACACGTTGA  ACAACATTTTCACTTCTCGCAGCAGAATGCAAGTGGATAAAAAAGAGTA  TATCTGGCTACTGATGATCCTACTTTGTTAAAGGAGGCCAAACACAAAGTACT  CCAATGAAATTTATAGTGATAACTCTATTTCTTGGTCACTGGACTGACTACA  CAATCGGTACACAGAAAATCACTTCCGGGTGTGATCCTGGATATACACTTT  CTCTCACAGGCTGACTTTCTAGTGTGACTTTTTTCATCCAGGTCTGTGGG  TTGCTTATGAAATCATGCAAACCTGCATCCTGATGCTCTGCGAATCTCCA  TTCTTTGGATGACATCTACTATTTTGGAGGCCAAAATGCCACAATCAGATT  GCTGTTTATCCTCACAAACCTCGAAGTGAAGAGGAAATCCAAATGGAACCTG  GAGATATCATTTGGTGTGGCTGGAAACCATTTGGGATGGTTATTTCAAAGGTAT  CAACGAAAACCTTGGAAAACAGGCTTATATCCCTCCTACAAAGTCCGAGAG  AAGATAGAAACAGTCAAGTATCCACATATCTGAAGCTGAAAAATAGCTCG  AGGCATG</p>	<p>MLLVNQSHQGFNKEHTSKMVSAILV  YVLLAAAAHSFAADLGSHHHHHHH  HHHGKPIPNPLGLDSTIDGRFRI  PEGPIDQGTATGRVVRVLEEQLVKAK  EQIENYKQARNGLGKDHEILRRRI  ENGAKELWFFLQSELKCLKHLEGN  LQRHADEFLLDLGHHERSIMTDLY  LSQTDGAGDWREKEAKDLTELQRR  ITYLQNPKDCSKARKLVCNINKGCG  YGCQLHHVVYCFMAYGTQRTLILE  SQNWRYATGGWETVFRPVSETCTDR  SGLSTGHWSEVVDKNIQVVELPIV  DSLHPRPPYLPVAVPEDLADRLR  HGDPVWVWSQFVKYLIRPQVLEK  EIEEATKKLGFKHPVIGVHVRRTDK  VGTEAAFHPVIEEYMVHVEQHFQLLA  RRMQVDDKRVYLATDDPPLLKEANT  KYSNYEFISDNSISWSAGLHNRYTE  NSLRGVILDIHFLSQADFLVCTFSS  QVCRVAYEIMQTLHPDASANFHSLD  DIYFYGQNAHNQIAVYHPKPRTEE  EIPMEPGDIIIGVAGNHWDGYSKIN  RKLKGTGLYPSYKVRKIEYTVKYPT  YPEAEK</p>