

# Supplementary figure S1

UniProt KB	S173	S422
D5DC07	168 -TQEW	SGSGT-LT- 178 418 -FTY SHFAVP- 426
A0A0B6AQX6	168 -TQEW	SGSAT-LT- 178 418 -FTY SHFAVP- 426
A0A0B6AX67	168 -TQEW	SGSAT-LT- 178 420 ---Y SHFAVP- 426
D5DC38	205 -TQEW	SGSAT-LT- 215 457 ---Y SHFAVP- 463
A0A0M0L610	178 -TQEW	SGSAT-LT- 187 428 -FTY SHFAIP- 436
M5P1E7	168 -TQEW	SGSAT-LT- 178 419 -FTY SHFAVP- 427
A0A1C3SJ80	168 -TQEW	SGSAT-LT- 178 419 -FTY SHFAVP- 427
A0A1J6GYT2	166 -TQEW	SGSAT-LT- 176 417 -FTY SHFAVP- 425
H1AD49	166 -TQEW	SGSAT-LT- 176 417 -FTY SHFAVP- 425
A0A0J6EL77	168 -TQEW	SGSAT-LT- 178 419 -FTY SHFAVP- 427
A0A150L1X8	160 -TQEW	SGSAT-LT- 170 412 ---Y SHFAVP- 418
A0A0H3E6H5	159 -TQEW	SGSAT-LT- 169 411 ---Y SHFAVP- 417
X5A0M2	168 -TQEW	SGSAT-LT- 178 424 -FTY SHFAVP- 432
P05655	159 -TQEW	SGSAT-FT- 169 410 ---Y SHFAVP- 416
F9WBB0	161 -TQEW	SGSAT-FT- 171 412 ---Y SHFAVP- 418
A0A089IVM5	168 -TQEW	SGSAT-LT- 178 424 -FTY SHFAVP- 432
A0A150F2K5	159 -TQEW	SGSAT-LT- 169 410 ---Y SHFAVP- 416
A0A0D1KBL9	159 -TQEW	SGSAT-FT- 169 410 ---Y SHFAVP- 416
A0A0A0TXJ4	159 -TQEW	SGSAT-LT- 169 410 ---Y SHFAVP- 416
A0A0B2FGL3	168 -AEEW	SGSAT-LT- 178 423 ---Y SHFAIP- 429
A0A0Q9MRD7	168 -AEEW	SGSAT-LT- 178 424 ---Y SHFAVP- 430
R4K861	167 -TQEW	SGSAT-LT- 177 425 ---Y SHFAVP- 431
A0A0F7F8Z7	168 -TQEW	SGSAT-LT- 178 424 -FTY SHFAVP- 432
M9TNM8	159 -TQEW	SGSAT-LT- 169 410 ---Y SHFAVP- 416
A0A1J6F5C5	159 -TQEW	SGSAT-FT- 169 410 ---Y SHFAVP- 416
A0A172XPM6	179 -TQEW	SGSAT-FT- 189 430 ---Y SHFAVP- 436
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A0A0Q9K3K8	168 -AEEW	SGSAT-LT- 178 424 ---Y SHFAVP- 430
A0A0D7XQE2	159 -TQEW	SGSAT-FT- 169 410 ---Y SHFAVP- 416
R4K9X0	167 -AEEW	SGSAT-LT- 177 423 TFTY SHFAVP- 432
X4ZKX5	168 -AEEW	SGSAT-LT- 178 423 ---Y SHFAVP- 429
A0A089HVL6	168 -AEEW	SGSAT-LT- 178 423 ---Y SHFAIP- 429
A0A0E4CZK7	176 -AEEW	SGSAT-LT- 186 431 ---Y SHFAIP- 437
A0A0Q9S332	168 -AEEW	SGSAT-LT- 178 422 ---Y SHFAVP- 428
A0A089LUD0	168 -AEEW	SGSAT-LT- 178 423 ---Y SHFAIP- 429
A0A0F7F9T7	168 -AEEW	SGSAT-LT- 178 423 ---Y SHFAIP- 429
A0A0H3J194	168 -TQEW	SGSAT-LT- 177 425 -FTY SHFAIP- 433
A0A0M3RAN0	168 -TQEW	SGSAT-LT- 178 426 FN-Y AHFVIP- 434
A0A089HRP3	168 -GEEW	SGSAT-LT- 178 423 ---Y SHFAIP- 429
R4KCF3	171 -AEEW	SGSAT-LT- 181 425 -FTY SHFAVP- 433
A0A0D5NQ05	168 -AEEW	SGSAT-LT- 178 424 ---Y AHFAIP- 430
V6IV04	167 -TQEW	SGSAT-LT- 176 424 -FTY SHFAVP- 432
A0A0M2P9I7	169 -TEEW	SGSAT-LT- 179 425 ---Y AHFAIP- 431
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A0A0L8F229	165 -TQEW	SGSAT-LT- 175 422 -FTY SHFAVP- 430
A0A117T1Y9	166 -SEEW	SGSAT-LT- 176 421 ---Y AHFATP- 427
V6J0B5	168 -AEEW	SGSAT-LT- 178 421 ---Y SHFAVP- 427
A0A179T2D2	169 -AEEW	SGSAT-FT- 179 424 ---Y AHFAIP- 430
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A0A0H3J026	167 -AEEW	SGSAT-LT- 177 427 ---Y SHFAVP- 433
A0A0J1LQ9	169 -AEEW	SGSAT-FT- 179 425 ---Y AHFAIP- 431
A0A1D9MY97	152 -AEEW	SGSAT-LT- 162 412 ---Y SHFAVP- 418
A0A176J9D4	167 -DQDQ	SGSAI-MT- 177 418 FN-Y AHYAIP- 426
Q97I81	165 -TQEW	SGSAT-LT- 175 425 ---Y SHFAVP- 431
W4D9G7	170 -AEEW	SGSAT-MT- 180 427 ---Y AHFAIP- 433
A0A0U1QN00	158 -AEEW	SGSAT-LT- 168 413 ---Y SHFAVP- 419
A0A0M4GC52	157 -SEEW	SGSAT-LT- 167 412 ---Y AHFVIP- 418
A0A098FE22	169 -AEEW	SGSAT-FT- 179 425 ---Y AHYAIP- 431
A0A0H4KA09	169 -AEEW	SGSAT-FT- 179 426 ---Y AHYAIP- 432
G7VYN5	168 -SEEW	SGSAT-LT- 178 422 ---Y AHFAIP- 428
A0A172ZF36	189 -SEEW	SGSAT-LT- 198 444 ---Y AHFAIP- 450
E0RG62	221 -SEEW	SGSAT-LT- 231 475 ---Y AHFAIP- 481
A0A160IQP4	168 -SEEW	SGSAT-FT- 178 424 ---Y AHYAIP- 430
A0A074LH66	168 -SEEW	SGSAT-LT- 178 422 ---Y AHFAIP- 428
A0A084GQ00	169 -SEEW	SGSAT-FT- 179 425 ---Y AHYAIP- 431
A0A0V8IYB9	168 -SEEW	SGSAT-FT- 178 424 ---Y AHYVIP- 430
O82854	169 -TQEW	SGSAT-LT- 179 426 HTY SHCGIP- 434
A0A1E3L5N1	186 -SEEW	SGSAT-MT- 196 441 ---Y AHFAIP- 447
A0A0D7X3V9	168 -AEEW	SGSAT-LT- 178 422 ---Y AHFAIP- 428
A0A0H3J4D7	339 -TEEW	SGSAT-LT- 348 597 FT-Y SHFAIP- 605
A0A0M4FKS3	147 -TQEW	SGSAT-LT- 156 406 ---Y AHFVIP- 412
E6U567	163 -TEEW	SGSAT-LT- 172 415 TFTY SYAIP- 424
A0A0V8JJC4	174 -IEEW	SGSAL-FT- 184 430 ---Y AHYVIP- 436
A0A024PB57	168 -SEEW	SGSAT-FT- 178 426 ---Y AHYVIP- 432
A0A0M0G105	166 -VEEW	SGSAT-LT- 176 422 ---Y AHYVIP- 428
A0A075LN24	170 -QEEW	SGSAA-FI- 180 425 ---Y AHYVIP- 431
A0A0N1T3P2	300 -KQWQ	SGSAT-V- 309 552 TDTY SYAYVP- 561
A0A0N1U2Q0	313 -EQWQ	SGSAT-V- 322 565 TDTY SYAYVP- 574
A0A192S224	312 -EQWQ	SGSAT-V- 321 564 TDTY SYAYVP- 573
Q03WB9	315 -QQWQ	SGSAT-V- 324 567 TDTY SYAYVP- 576
Q70XJ9	377 -YQEW	SGSAI-V- 386 638 TATY SYAYVP- 647
G2KVB82	378 -YQEW	SGSAI-V- 387 639 TATY SYAYVP- 648
A0A0K8MV50	181 -VGEW	SGSTV-LM- 191 446 ---Y SYLVIP- 452
A0A0K8MPC0	181 -VGEW	SGSTV-LM- 192 446 ---Y SYLVIP- 452
Q8GGV4	315 -FQEW	SGSAI-V- 324 576 TATY SYAYVP- 585
Q03WB8	297 -SQWQ	SGSAT-V- 306 548 TNSY SYAYVP- 557
I0QHA9	344 -DQWQ	SGSAT-V- 353 613 TSTY SYAYVP- 622
B3XLE9	331 -TQEW	SGSAI-V- 340 591 TATY SYAYVP- 600
A0A0R1PF10	258 -YQEW	SGSAI-V- 267 519 TATY SYAYVP- 528
F8DRCO	129 -TQEW	SGSAI-V- 138 388 TATY SYAYVP- 397
Q683P2	336 -TQEW	SGSAI-V- 345 595 TATY SYAYVP- 604
E9DPX9	323 -DQWQ	SGSAT-V- 332 592 TSTY SYAYVP- 601
D3WYWO	315 -LQEW	SGSAI-V- 324 578 TATY SYAYVP- 587
A0A0R1X7N7	245 -FQEW	SGSAI-V- 254 506 TATY SYAYVP- 515
A0A081NSP0	129 -SQEW	SGSAV-L- 138 389 TATY SYAYVP- 398
TOVB16	150 -DQWQ	SGSAT-V- 159 419 TSTY SYAYVP- 428
A0A0U5K6W0	336 -SQEW	SGSAV-L- 345 596 TATY SYAYVP- 605
A0A074IZ03	351 -DQWQ	SGSAT-V- 360 620 TSTY SYAYVP- 629
A0A0E2EKC5	310 -TQEW	SGSAT-V- 319 572 TATY SYAYVP- 581
A0A0R1WJH5	270 -LQWQ	SGSAT-L- 279 535 TATY SYAYVP- 544
D6S431	318 -LQWQ	SGSAV-V- 327 577 TATY SYAYVP- 586
V5P4J0	335 -IQWQ	SGSAT-L- 344 597 TATY SYAYVP- 606
J3JQ55	340 -TQWQ	SGSAT-V- 349 602 TATY SYAYVP- 611
F4AFU1	294 -LQEW	SGSAI-V- 303 557 TATY SYAYVP- 566
A0A1B3PWG6	135 -LQEW	SGSAI-V- 144 398 TATY SYAYVP- 407
A0A0U5JV84	327 -SQEW	SGSAV-L- 336 587 TATY SYAYVP- 596
C2EU18	276 -HQWQ	SGSAT-K- 285 536 TSTY SYAYVP- 545
A0A0R1YAN1	365 -HQRW	SGSAC-V- 374 624 TATY SYAYVP- 633
G5JYF4	360 -TQWQ	SGSAT-V- 369 622 TATY SYAYVP- 631
J9YHQ1	299 -NQEWE	SGSAT-L- 308 555 ADTY SYAYVP- 564
A0A0R1S7U0	293 -LQWQ	SGSAV-V- 302 552 TATY SYAYVP- 561
A0A087QE01	137 -IQWQ	SGSAT-L- 146 399 TATY SYAYVP- 408
D3WYV9	329 -IQWQ	SGSAT-L- 338 591 TATY SYAYVP- 600
Q7X481	420 -SQEW	SGSAV-V- 429 670 TNTY SYAYVP- 679
A0A0U3F832	366 -EQWQ	SGSAT-V- 375 635 TSTY SYAYVP- 644
A0A175D284	342 -NQEWE	SGSAT-L- 351 598 TDTY SYAYVP- 607
A0A175CSP9	277 -NQEWE	SGSAT-L- 286 533 TDTY SYAYVP- 542
A0A175CN55	167 -NQEWE	SGSAT-L- 176 423 TDTY SYAYVP- 432
A0A0R1XZ34	292 -RQEWE	SGSAT-V- 301 554 TATY SYAYVP- 563
A0A0R1XTI2	333 -AQEW	SGSAT-V- 342 593 TSTY SYAYVP- 602
A0A139NH8	342 -TQWQ	SGSAA-V- 351 604 TATY SYAYVP- 613
A0A0R1UH55	226 -DQEWE	SGSAT-V- 235 484 TATY SYAYVP- 493
A0A0R1NSL5	320 -SQEW	SGSAT-V- 329 580 TSTY SYAYVP- 589
A0A0R2HHK9	192 -KSEWE	SGSTTMMH-203 455 ---Y AYLAVR- 461
A0A1C2D650	308 -DQEWE	SGSAT-M- 317 568 TSTY SYAYVP- 577
A0A109DGA4	302 -DQEWE	SGSAT-V- 311 562 TSTY SYAYVP- 571
A0N159	353 -AEQW	SGSAV-L- 362 609 TFTY SYAIP- 618
A0A0R2DDE0	84 -IQWQ	SGSAT-L- 93 360 TATY SYAYVP- 369
A0A0R1WQB6	365 -TGHW	SGSAT-V- 374 624 TATY SYFAVK- 633
A0A0R2HFP4	339 -QQEW	SGSTVMLH- 350 605 TETV SFYPI- 614
GRWIM3	375 -SQWQ	SGSAI-L- 384 636 TATY SYFAIK- 644
R9X6M9	140 -DQEWE	SGSAY-PL- 150 435 TATY SYAYVP- 445
D2WS87	354 -DQEWE	SGSAY-PL- 364 650 TATY SYAYVP- 659
A0A165YU9	355 -DQEWE	SGSAY-PL- 365 651 TATY SYAYVP- 660
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A0A1JOCKW0	406 -DQEWE	SGSAY- 414 702 TATY SYAYVP- 711
D8FQV7	384 -TGWQ	SGSAM-L- 393 653 TATY SYAYVP- 662
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F0HVA2	387 -TGWQ	SGSAM-L- 396 656 TATY SYAYVP- 665
A0A0R1MDC7	366 -TGWQ	SGSAM-L- 375 635 TATY SYAYVP- 644
A0A0N0CQ12	296 -TQWQ	SGSAVKLD- 307 627 ---Y SYIMPT- 634
A0A0R1FW02	83 -TGWQ	SGSAM-L- 92 352 TATY SYAYVP- 361
L5NBP0	547 -SRQW	AGSAM-V- 556 770 YQTY SWTVMP- 779
A0A0Q9LAQ7	142 -SRQW	AGSSV-V- 151 374 YQTY SWTVMP- 383
A0A0J5X6D2	190 -DNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
B1T3G3	174 -NNAEW	SGSAR-L- 184 434 FQSY SHYVMP- 443
A0A1B4B4C5	175 -NNAEW	SGSAR-L- 185 435 FQSY SHYVMP- 444
A0A118HU84	191 -NNAEW	SGSAR-L- 201 451 FQSY SHYVMP- 460
A0A0B0D7L4	142 -SRQW	AGSAM-M- 151 376 FQSY SHYVMP- 385
A0A087N2I9	191 -NNAEW	SGSAR-L- 201 451 FQSY SHYVMP- 460
A0A0B0D3D8	123 -SRQW	AGSAM-I- 132 357 YQTY SWTVMP- 366
A0A0D0GZ86	191 -NNAEW	SGSAR-L- 201 451 FQSY SHYVMP- 460
A0A071MG51	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
L5N6Q3	139 -SRQW	AGSAM-L- 148 366 FQTY SHVLP- 375
A0A081VAY8	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
L5N874	137 -SRQW	AGSAM-M- 146 372 YQTY SWTVMP- 381
A2ZV58	206 -NNAEW	SGSAR-L- 216 466 FQSY SHYVMP- 475
Q2T433	178 -NNAEW	SGSAR-L- 188 438 FQSY SHYVMP- 447
A0A103U6L5	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
A0A096YXB8	192 -NNAEW	SGSAR-L- 202 452 FQSY SHYVMP- 461
I2DV73	174 -NNAEW	SGSAR-L- 184 434 FQSY SHYVMP- 443
A0A088TRL1	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
A0A0D6LVE5	163 -NNAEW	SGSAR-L- 173 423 FQSY SHYVMP- 432
A0A1J0T6D0	168 -HQTQW	SGSAR-I- 178 428 FQSY SHYVMP- 437
A0A1J0SIU6	201 -HQTQW	SGSAR-I- 211 461 FQSY SHYVMP- 470
A0A1J0S6W7	187 -HQTQW	SGSAR-I- 197 447 FQSY SHYVMP- 456
A0A0J5GNX9	121 -HMWQ	AGSAM-L- 130 366 FQAY SWVAAP- 375
A0A095E6N9	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
U2G5V0	151 -NNAEW	SGSAR-L- 161 411 FQSY SHYVMP- 420
B1G3X6	195 -QNAEW	SGSAR-L- 205 455 FQSY SHYVMP- 464
A0A0J6MDB3	190 -NNAEW	SGSAR-L- 200 450 FQSY SHYVMP- 459
A0A0D5L6T3	188 -NNAEW	SGSAR-L- 198 448 FQSY SHYVMP- 457
A0A0S3EPZ1	185 -HQTQW	SGSAR-I- 195 444 FQSY SHYVMP- 453
R4KDH7	146 -SRQW	AGSAM-L- 155 378 YQTY SWTVMP- 387
A0A151A9R2	144 -HHQW	AGSAM-Y- 153 358 FQAY SWLAMP- 367
A0A157ZW82	189 -NNAEW	SGSAR-L- 199 449 FQSY SHYVMP- 458
A0A0P9EBQ6	145 -HMWQ	AGTAM-L- 154 389 YQAY SWMLVP- 398
A0A0X0SMM9	93 -SRQW	AGSAM-M- 102 328 YQAY SWLVP- 337
W0JW88	143 -HHQW	AGSAM-Y- 152 357 FQYS SWLAMP- 366
A0A0A6Q5Q9	184 -NNAEW	SGSAR-F- 194 444 FQSY SHYVMP- 453
L9XHV2	143 -HHQW	AGSAM-Y- 152 357 FQYS SWLAMP- 366
BZJYV2	195 -DNAEW	SGSAR-L- 205 455 FQSY SHYVMP- 464
MOGZM4	115 -HHQW	AGSAM-Y- 124 329 FQAY SWLAMP- 338
A0A154UZ40	187 -QQAWE	SGSTR-I- 197 446 FQAY SSYLP- 455
A0A0W1R697	118 -SRQW	AGSAL-Y- 127 335 FQAY SWLAYD- 344
MOBV67	135 -HHQW	AGSAM-Y- 144 349 FQAY SWLAMP- 358
G7LSK3	149 -AREW	AGTPV-LL- 159 371 YQTY SHYVMP- 380
A0A0H0SLX3	123 -HMWQ	AGTAM-L- 132 359 YQAY SWMLVP- 368
A0A1H8VTP1	144 -HHQW	AGSAM-Y- 153 358 FQAY SWLAMP- 367
D2S0N4	144 -HHQW	AGSAM-Y- 153 358 FQAY SWLAMP- 367
A0A0U3PY74	181 -HQTQW	SGSAR-V- 191 440 FQAY SHYVMP- 449
A0A0M2HA26	200 -HQTQW	SGSAR-I- 210 459 FQAY SHYVMP- 468
LZ2Z73	144 -HHQW	AGSAM-Y- 153 358 FQYS SWLAMP- 367
E7QM02	109 -HHQW	AGSAM-Y- 118 323 FQAY SWLAMP- 332
A0A166RTL7	111 -SRQW	AGSAL-L- 120 327 FQAY SWLAMP- 336
A0A0N9JHF5	191 -HQTQW	SGSAR-V- 201 450 FQAY SHYVMP- 459
A0A1B1BID6	194 -HQTQW	SGSAR-V- 204 452 FQAY SHYVMP- 461
A0A0G4JRN2	146 -TREW	AGTPI-LL- 156 368 FQTY SHYVMP- 377



**Supplementary figure S1.** Extract of the ranked multiple sequence alignment (367 sequences) created for family 68 showing positions S173 and S422; highlighted sequences: D5DC07 *B. megaterium* levansucrase (orange); P05655 *B. subtilis* levansucrase (green).

**Supplementary table S1.** Amino acid distribution of the mutated positions in the created datasets of GH families 68 and 32; values are given in %.

Family 68 (367 sequences)						Family 32 (3951 sequences)				
position	Ser	Ala	Gly	Thr	other	Ser	Ala	Gly	Thr	other
S173	67	33	-	-	-	80	<1	<1	17	3
S422	92	8	-	-	-	<1	94	4	<1	1

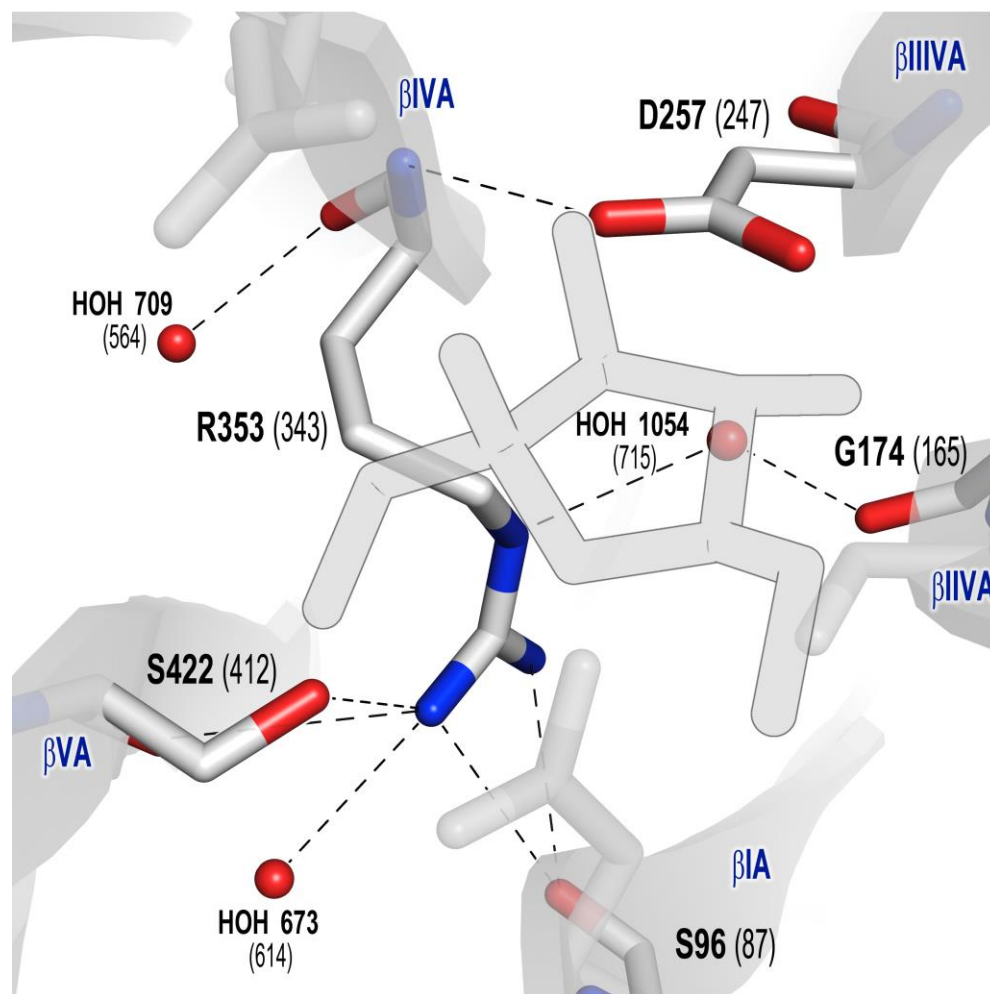
  

position	Tyr	Phe	Trp	other	Tyr	Phe	Trp	other
Y421	100	-	-	-	97	1	-	2
Y439	38	61	-	1	2	<1	96	2

**Supplementary table SII.** Oligonucleotides for creation of libraries S173/S422, Y421 and Y439.

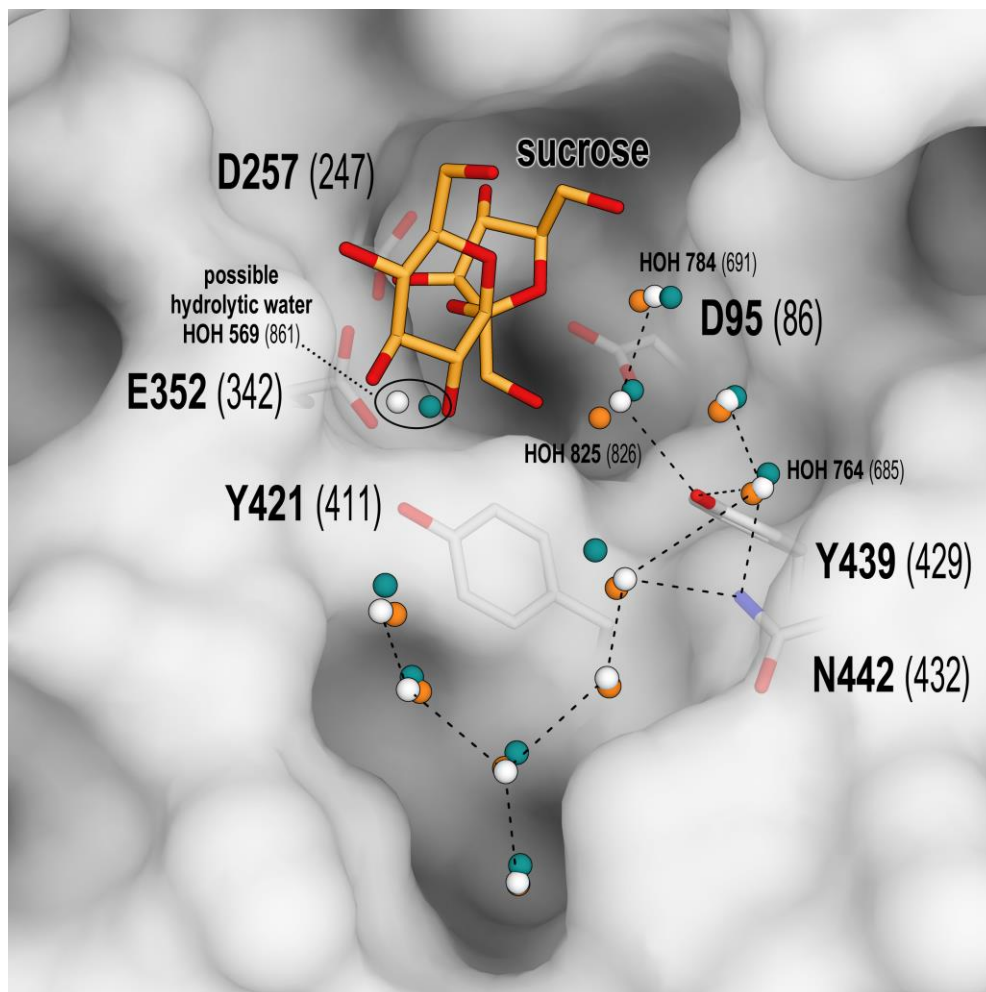
Oligonucleotide	Library	Sequence
Frag1_S173S_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCAGACCATTCTTGTTTTG
Frag1_S173A_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCCGCCATTCTTGTTTTG
Frag1_S173T_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCCGTCCATTCTTGTTTTG
Frag1_S173D_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCCTCCATTCTTGTTTTG
Frag1_S173V_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCAACCATTCTTGTTTTG
Frag1_S173G_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCACCCATTCTTGTTTTG
Frag1_S173L_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCAGCCATTCTTGTTTTG
Frag1_S173W_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCCACCATTCTTGTTTTG
Frag1_S173F_rev	S173/S422	GATCGGTCTCCGTGCCAGAACCGAACCCATTCTTGTTTTG
Frag2_fwd	S173/S422	GATCGGTCTCGGCACGTTAACAAAAGATGGTAAAGTTCG
Frag2_S422S_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGAGAATACGTAATG
Frag2_S422A_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGCGCATACGTAATG
Frag2_S422H_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGGTGATACGTAATG
Frag2_S422F_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGAAAATACGTAATG
Frag2_S422G_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGACCATACGTAATG
Frag2_S422Q_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGCTGATACGTAATG
Frag2_S422T_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGGGTATACGTAATG
Frag2_S422V_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGAACATACGTAATG
Frag2_S422L_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGCAGATACGTAATG
Frag2_S422W_rev	S173/S422	GATCGGTCTCGAGGTACAGCAAAGTGCCAATACGTAATG
Frag3_fwd	S173/S422	GATCGGTCTCTACCTCAAACAAAAGGCG
Y421A-f	Y421	GATAAAACATTTACGcgTCTCACTTTGCTGTACCTCA
Y421V-f	Y421	GATAAAACATTTACGgtTCTCACTTTGCTGTACCTCA
Y421I-f	Y421	GATAAAACATTTACGatcTCTCACTTTGCTGTACCTCA
Y421L-f	Y421	GATAAAACATTTACGctgTCTCACTTTGCTGTACCTCA
Y421M-f	Y421	GATAAAACATTTACGatgTCTCACTTTGCTGTACCTCA
Y421F-f	Y421	GATAAAACATTTACGttcTCTCACTTTGCTGTACCTCA
Y421W-f	Y421	GATAAAACATTTACGtgTCTCACTTTGCTGTACCTCA
Y421G-f	Y421	GATAAAACATTTACGgtTCTCACTTTGCTGTACCTCA
Y421N-f	Y421	GATAAAACATTTACGaacTCTCACTTTGCTGTACCTCA
Y421D-f	Y421	GATAAAACATTTACGgacTCTCACTTTGCTGTACCTCA
Y421K-f	Y421	GATAAAACATTTACGaaaTCTCACTTTGCTGTACCTCA
Y439A-f	Y439	GTCGTTATTACAAGCcgATGACAAATAGAGGC
Y439V-f	Y439	GTCGTTATTACAAGCgttATGACAAATAGAGGC
Y439I-f	Y439	GTCGTTATTACAAGCcatcATGACAAATAGAGGC
Y439L-f	Y439	GTCGTTATTACAAGCctgATGACAAATAGAGGC
Y439M-f	Y439	GTCGTTATTACAAGCcatgATGACAAATAGAGGC
Y439F-f	Y439	GTCGTTATTACAAGCcttcATGACAAATAGAGGC
Y439W-f	Y439	GTCGTTATTACAAGCctggATGACAAATAGAGGC
Y439G-f	Y439	GTCGTTATTACAAGCggtATGACAAATAGAGGC
Y439N-f	Y439	GTCGTTATTACAAGCcaacATGACAAATAGAGGC
Y439D-f	Y439	GTCGTTATTACAAGCgacATGACAAATAGAGGC
Y439K-f	Y439	GTCGTTATTACAAGCaaaATGACAAATAGAGGC

Supplementary figure S2



**Supplementary figure S2.** Contacts of R353 (343 in *B. subtilis* levansucrase) are shown based on PDB 1pt2. Only the fructose moiety is displayed for clarity. Waters (in red) in the active site are conserved in *B. subtilis* (1pt2) and *B. megaterium* (3om2) enzymes.

Supplementary figure S3



**Supplementary figure S3.** Water channel to the active site. Waters from *B. subtilis* sucrose-unbound (1oyg) and bound- (1pt2) levansucrase crystallographic structures are depicted in white and orange, respectively. Waters from *B. megaterium* (3om2) 3D structure are displayed in cyan. Sticks and Surface are from 1oyg, sucrose from 1pt2. Distances are  $< 4 \text{ \AA}$  (only 1oyg distances shown). Only conserved waters are shown.