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Supplementary Table S1: **Comparison (rmsd) of energy minimised structures, the relative accessible surface area and the change of relative accessible surface area of each affected residue**

	WT vs Mutant rmsd (Å)		**UDP-Glc vs UDP-GlcNAc rmsd (Å)**	**Relative Accessible Surface Area of Residue (%)**					
hGALE Variant	**UDP-Glc**	**UDP-GlcNAc**		**WT $^{\text{UDP-Glc}}$**	**p.A89V $^{\text{UDP-Glc}}$**	**$\Delta^{\text{UDP-Glc}}$**	**WT $^{\text{UDP-GlcNAc}}$**	**p.A89V $^{\text{UDP-GlcNAc}}$**	**$\Delta^{\text{UDP-GlcNAc}}$**
WT	NA	NA	0.662	NA	NA	NA	NA	NA	NA
p.A89V	0.297	0.345	0.671	15.4	15.1	-0.3	15.6	14.0	-1.6

Relative accessible surface area of each residue was determined using the appropriate energy minimised structure and GETAREA and R.M.S.D. was determined using the align function in PyMol. WT, wild-type; UDP-Glc, UDP-glucose; UDP-GlcNAc, UDP-*N*-acetylglucosamine.

<u>Supplementary Table S2:</u> **SNP effect 4.0 and I-Mutant 3.0 predictions for GALE variant monomers**

	Predictions (TANGO, WALTZ, LIMBO and FoldX)	**FoldX kcal/mol UDP-Glc**	**FoldX kcal/mol UDP-GlcNAc**	**I-Mutant 3.0 kcal/mol UDP-Glc**	**I-Mutant 3.0 kcal/mol UDP-GlcNAc**
p.A89V	Increased aggregation tendency Decreased stability	-2.27	-2.04	-0.16 (0)	-0.29 (2)

Reliability index of I-Mutant 3.0 predictions is indicated in brackets.

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                                                                           A89
Q45291          ---REAVPA----------------DA-RLIEGDVNDVV---EEVLSEGGFEGVVHFAAR
P33119          ---RDAVPL----------------GA-TFVEGDIKDVA---DNVLSSDSFDAVLHFAAR
P21977          ---RAAVHP----------------DA-IFYQGDLSDQDFMRKVFKENPDVDAVIHFAAY
P96995          ---RAAVHP----------------AA-KFYQGDLADREFMSMVFRENPDVDAVIHFAAY
Q59745          ---REFVRW----------------G--PAEEGDIRDRARLDEVLAK-HKPAAILHFAAL
P26503          ---EEFVKW----------------G--VLEKGDIRDRQRLDEVLAR-HKPRAILHFAAM
Q14376          --LPESLRRVQELTGR---------SV-EFEEMDILDQGALQRLFKK-YSFMAVIHFAGL
Q5R8D0          --LPESLRRVQELTGR---------SV-EFEEMDILDQGALQRLFKK-HSFMAVIHFAGL
Q8R059          --MPESLRRVQELTGR---------SV-EFEEMDILDQAALQHLFKK-HSFKAVIHFAGL
P18645          --MPESLRRVQELTGR---------SV-EFEEMDILDQAALQHLFKK-HNFKAVIHFAGL
Q42605          ---IEAVDRVRELVGPD-----LSKKL-DFNLGDLRNKGDIEKLFSK-QRFDAVIHFAGL
Q43070          ---MEAVERVREVVGSN-----LSQNL-EFTLGDLRNKDDLEKLFSK-SKFDAVIHFAGL
O65780          ---IDAVHRVRLLVGPL-----LSSNL-HFHHGDLRNIHDLDILFSK-TKFDAVIHFAGL
Q9T0A7          ---AASLQRVKKLAGE------NGNRL-SFHQVDLRDRPALEKIFSE-TKFDAVIHFAGL
Q9SN58          ---AVSLQRVKKLAAE------HGERL-SFHQVDLRDRSALEKIFSE-TKFDAVIHFAGL
O65781          ---ETAIHRVKELAGK------FAGNL-SFHKLDLRDRDALEKIFSS-TKFDSVIHFAGL
P56985          ---INILPRLKTITGQ---------EI-PFYQGDIRDREILRRIFAE-NRIDSVIHFAGL
P56986          ---INILPRLKTITGQ---------EI-PFYQGDIRDREILRRIFAE-NRIDSVIHFAGL
P56997          ---INILPRLKTITGQ---------EI-PFYQGDIRDREILRRIFAE-NRIDSVIHFAGL
Q05026          ---AAVLPRLRQITGR---------NI-PFYQGDIRDCQILRQIFSE-HEIESVIHFAGL
P24325          ---PKSLERVKQITGK---------EA-KFYEGDILDRALLQKIFAE-NEINSVIHFAGL
Q9CNY5          ---PKSLERVAQITGK---------QV-KFYQGDILDTALLQKIFAE-NQIQSVIHFAGL
Q59678          ---EVSLERVKQITGK---------SV-KFYQGDILDRDILRKIFAE-NQIESVIHFAGL
P22715          ---RSVLPVIERLGGK---------HP-TFVEGDIRNEALITEILHD-HAIDTVIHFAGL
Q56093          ---RSVLPVIERLGGK---------HP-TFVEGDIRNEALITEILHD-HAIDTVIHFAGL
P09147          ---RSVLPVIERLGGK---------HP-TFVEGDIRNEALMTEILHD-HAIDTVIHFAGL
Q9F7D4          ---SSVLARIHSLTGY---------TP-ELYAGDIRDRTLLDSIFAA-HPIHAVIHFAGL
P04397          ---YDSVARLEVLTKH---------HI-PFYEVDLCDRKGLEKVFKE-YKIDSVIHFAGL
P09609          ---YESVARMELLTGQ---------EI-KFAKIDLCELEPLNKLFDD-YKIDSVLHFAGL
P40801          ---YDVIVRIEVLTRK---------QI-PFFKIDLNDHDALDQVFKL-YPIQAVLHFAAL
Q9HDU3          ---YDAVARVEFIVRK---------SI-KFFKLDLRDKEGLAQIFDT-FKIKGVIHFAAL
Q8L2J6          ---KQEDMRIALNH----------SKL-KFYIGDVRNYQSIDDAM---HGVDYVFHAAAL
Q9ZDJ5          ---KQEDMRIALNN----------SKL-KFYIGDVRNYQSIDDAM---HGVDYVFHAAAL
Q92IG3          ---KQEDMRIALNN----------PKL-KFYIGDVRNYKSIDEAM---HGVDYVFHAAAL
A8F1A5          ---KQEDMRIAFNN----------PKL-KCYIGDVRNYKSIDEAM---HGVDYVFHAAAL
A8GRN9          ---KQEDMRIALNN----------PKL-KFYIGDVRNYKSIDEAM---HGVNYVFHAAAL
Q4UM33          ---KQEDMRIALNN----------PKL-KFYIGDVRNYKSIDEAM---HGVDYVFHAAAL
A8GN21          ---KQEDMRIALSN----------PKL-KFYIGDVRNYKSIDEAM---RGVDYVFHAAAL
A8EZA9          ---KQEDMRIALSN----------PKL-KFYIGDVRNYKSVDEAM---HGVDYVFHAAAL
Q1RIM4          ---KQEDMRIALNN----------PKI-KFYIGDVRNYNSIDDAM---KGVDYVFHAAAL
A8GWP0          ---KQEDMRIALNN----------PKI-KFYIGDVRNYNSIDDAM---KDVDYVFHAAAL
O84903          ---RKAVDP----------------RA-RFYQGDIRDYHFLSQVFSQ-EKIDGIVHFAAF
P55180          ---AEALNRVKEITGK---------DL-TFYEADLLDREAVDSVFAE-NEIEAVIHFAGL
Q9W0P5          --LPEALSRVQEITGK---------KV-NFYRVDITDREQVRSVFQE-HKIDMVAHFAAL
Q57301          ---AESLARVSKICGR---------KP-NFYHGDILDRSCLKLIFSS-HKIDSVIHFAGL
Q7WTB1          ---RKAVDP----------------KA-KFYQGDIEDTFLVSKILRD-EKIDAVMHFAAY
P35673          ---RESINRVEKLTGK---------TA-TFFEGDLLDRSCLRSVFSA-HRISAVIHFAGL
Q553X7          ---LEAIKRVESITGK---------EI-EFHHVDIMNEKALDEIFET-GNIRSVIHFAGL
P13226          ---RAGVPA----------------GA-SFYRGDIRDQDFMRKVFRGRLSFDGVLHFAAF
Q9KDV3          ---AGAL-S----------------DV-TFYHGDIRDDQLLDTIFTT-HSIDTVIHFAAN
Q59083          ---REAIPA----------------AV-PLVEGDIGSAELLDRVMRD-HRVDAVMHFAGS
P75517          --ALQLLPR----------------QV-NVHFVNLLDRAQLTDTIAQ-INPDVVFHFAAK
P47364          -----VIKLLKKI------------GI-EFYFADLLDRHKLTEVIAA-IQPDVVFHFAAK
Q57664          ---KNNINP----------------KA-EFVNADIRDKDLDEKINF--KDVEVVIHQAAQ
Q56623          ---RHAVNK-------------DDGL-LFEVGDINASTDFELPL---KNTTVVVHCAAR
P45602          ---RRILPVIERLGGK---------EA-TFIEGDIRNEARMTEILHD-HAIEAVIHFAGL
P56600          ---YDAVARIEFIVKQ---------HVSHFIKVDIRNEKELNQVFSN-HKISGVIHFAAL
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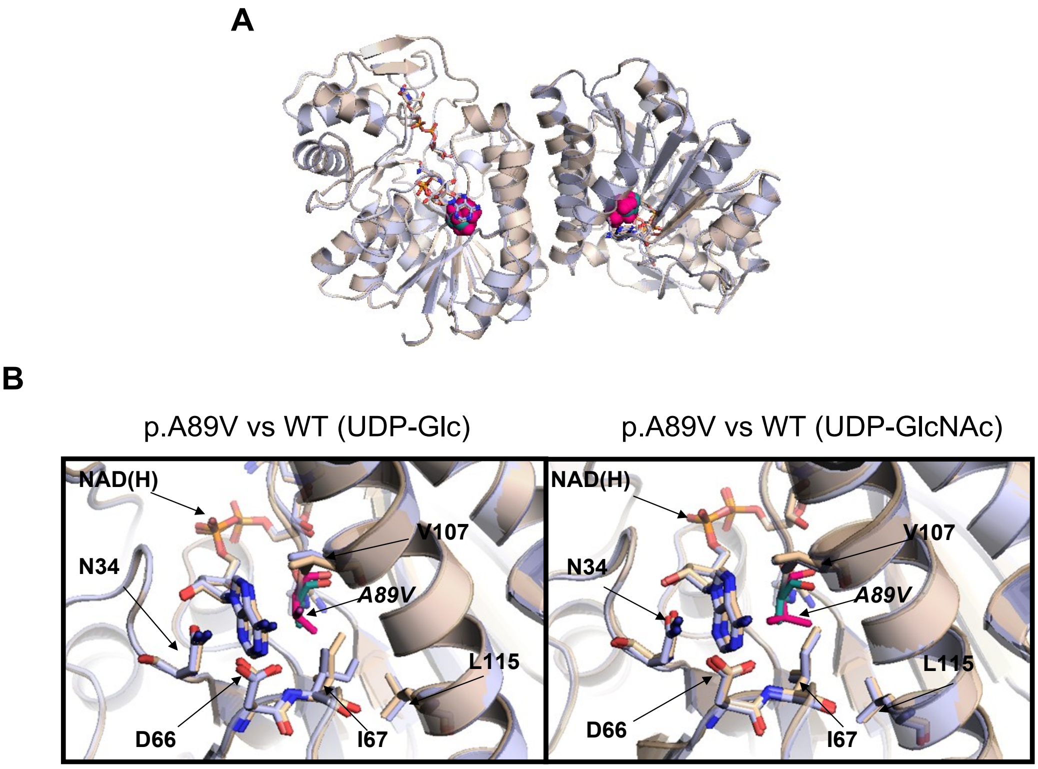
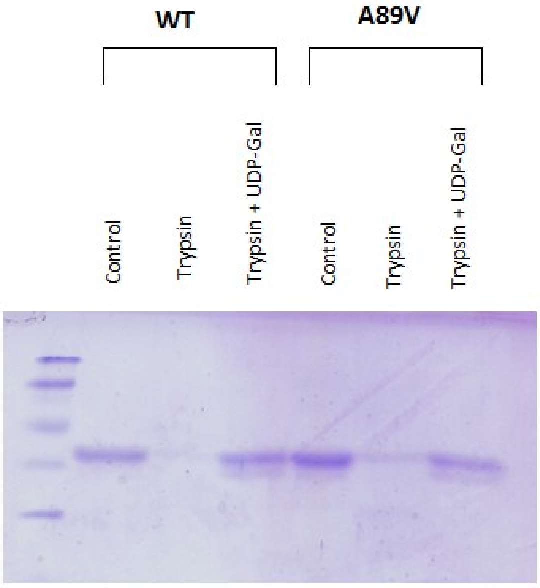
<u>Supplementary Figure S1:</u> **Multiple sequence alignment of GALE enzymes from different species showing the region around Ala-89.** Sequence alignment was carried out using ClustalW2 in combination with reviewed GALE sequences from the UniProt database (www.uniprot.com).



Supplementary Figure S2: **Aligned UDP-Glc and UDP-GlcNAc bound energy minimised structures. (A)** Aligned p.A89V and WT UDP-Glc bound structures show few differences in overall fold. **(B)** Close-up of both aligned structures of p.A89V and wild-type GALE bound to UDP-Glc and UDP-GlcNAc. Original residues are coloured deep teal while altered residue is coloured pink. Highlighted residues, cofactors and substrates are depicted as stick figures or spheres. Wild-type and mutant structures are depicted as cartoon figures and are coloured wheat and light blue respectively. Oxygen, nitrogen and phosphate atoms are coloured red, blue and orange respectively. Figures were created using PyMol (www.pymol.com) and the appropriate energy minimised structures of 1EK5 and 1HZJ.



Supplementary Figure S3: **UDP-galactose (1 mM) protects wild-type and p.A89V GALE (16 µM) from proteolysis by trypsin (630 nM).** Results were analysed by 10% SDS-PAGE and the sizes of molecular mass markers (left most lane) are 116, 66, 45, 35 and 25 kDa.