

Supplementary Information

Genetic recoding to dissect the roles of site-specific protein O-GlcNAcylation

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Supplementary Table 1: List of primers

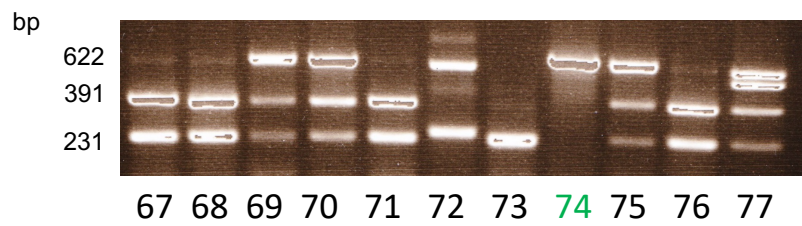
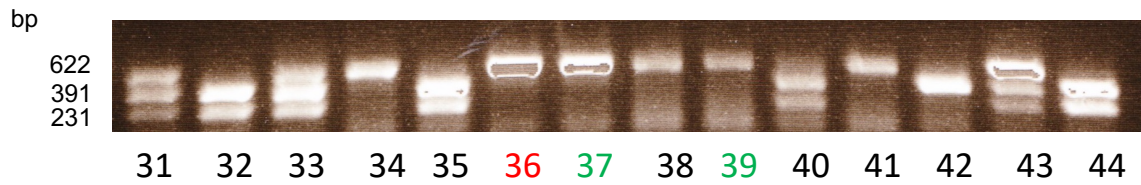
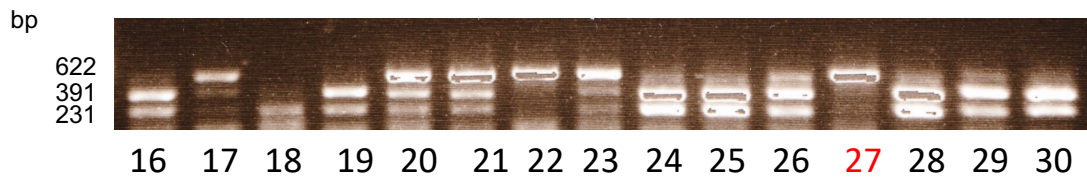
Primer	Description
GTGTCTGTGCCATACTGCAGCGCCAGAGCAC	TAB1_S395C_F
GTGCTCTGGGCGCTGCAGTATGGCACAGACAC	TAB1_S395C_R
AAAGGATCCATGGCGGCGCAGAGGAGGAGCTTG	FLAG_TAB1_BamHI_F
AAAGCGGCCGCTTACTACGGTGTGTACCACGCTC	FLAG_TAB1_NotI_R
CATCACACGCCCGTCTGCAGCGCCAATATG	CK2_peptide_S347C_F
CATATTGGCGCTGCAGACGGGCGTGTGATG	CK2_peptide_S347C_R
CACCGTGTAGTTACAACAGTTTACC	Mm405_gRNA_RIGHT_F
AAACGGTAAACTGTTGTAACACTACAC	Mm405_gRNA_RIGHT_R
CACCGACAGTTGTGGCATTAAAGA	Mm405_gRNA_LEFT_F
AAACTCTTAAATGCCACAACACTGTC	Mm405_gRNA_LEFT_R
AAAGGATCCTGGTCTCTGAGGGTACCAGGCATAC	Mm405_2kb region_F
AAAGCGGCCCGCAGGACTTAATCACAAACTGTAAAGCTGG	Mm405_2kb region_R
GCTCGCACTTATGTCGTTGAGCTTGG	Mm405_PCR_Check_F
CACAGGCTCTGCGGTGTACAGAGG	Mm405_PCR_Check_R
GTAGGTAGGCAAGTTGCACACTGCGGAGCTAAGACAAGTGTAGTTG	RF_Wobble_S405C_F
CTGGTTGTCATTCTTGTGCTCTG	RF_Wobble_405_R
CTTTTCATTTGGACCTGGCAC	MmOGA_405_sequencing
AAAGGATCCTCAGTTTGAGGAGATATGATACCCTCC	Mm405_outside_check_F
AGGTCATCTGGAACACTACCTGCCATTCC	Mm405_outside_check_R
GACTGACAGTGAAGACAGTACTGTATC	Mm405 RTPCR_check_F
CCTAACCACTGCACAAAAGATTTACC	Mm405 RTPCR_check_R
ATGAAGGCAGTGACGAAGAC	Mm405 RTPCR_seq_F
ATAGACATTATACTCTTGATGTC	Mm405 RTPCR_seq_R

Mouse OGA geneblock:

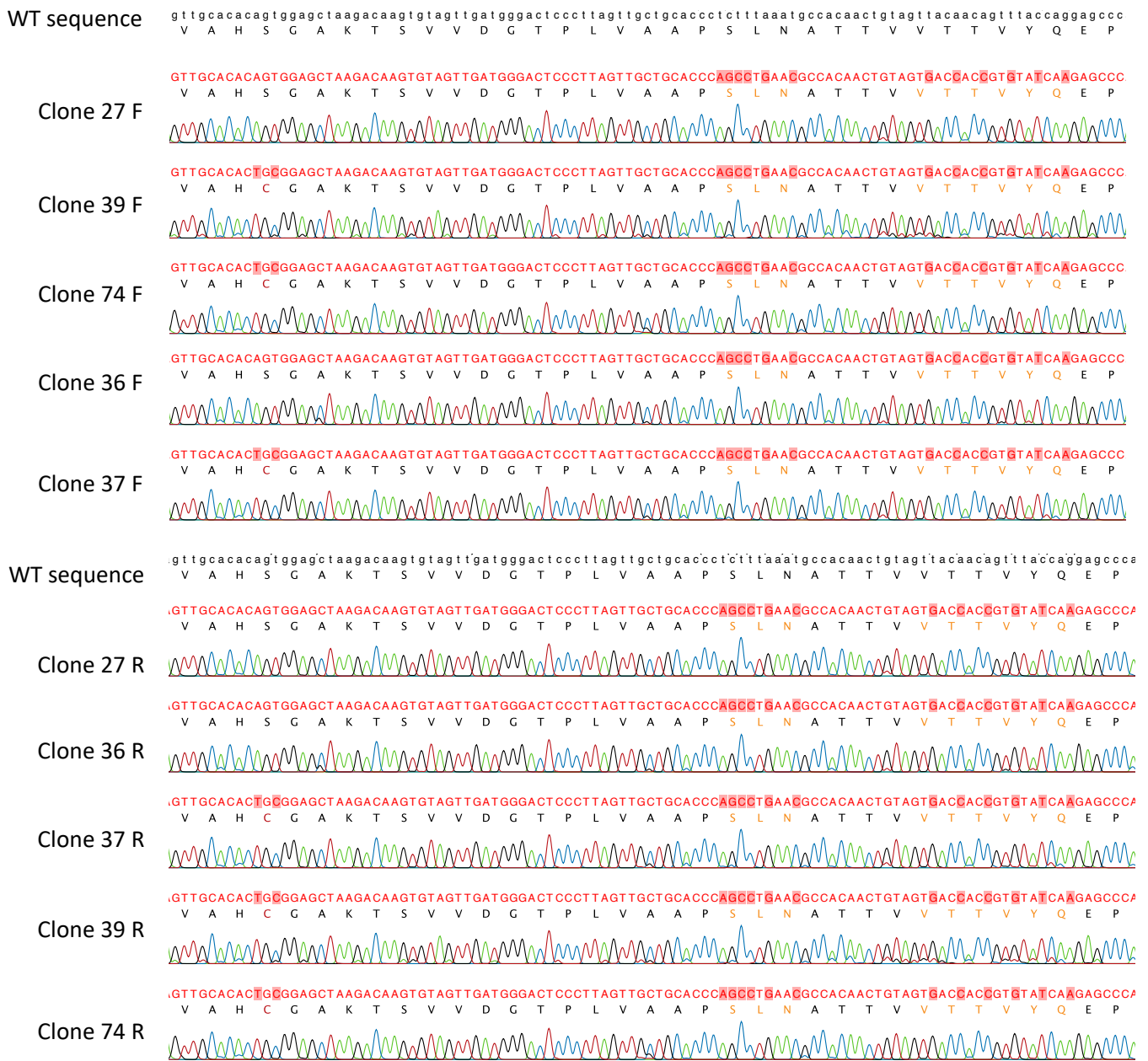
AGGCAAGTTGCACACAGTGGAGCTAAGACAAGTGTAGTTGATGGGACTCCCTTAGTTGCTGCACCCA
GCCTGAACGCCACAACTGTAGT**GACCACCGTGTATCA**AGAGCCCATTATGAGCCAGGGAGCAGCTTT
GAGTGGTGAACCCTCAGTTCTGACCAAGGAAGAGGAAAAGAAACAGCCAGATGAGGAACCCATGGAT
ATGGTGGTCGAAAAGCAAGAAGAAGCAGAGCACAAAGAATGACAACCCAG

Supplementary Table 2: List of antibodies used for Western blotting

Antibody	Species	Dilution	Company	Catalogue number
RL2 (O-GlcNAc)	Mouse	1:1000	Thermo Fisher Scientific	MA1-072
α -tubulin	Rabbit	1:1000	CST/Proteintech	2125/11224-1-AP
TAB1	Sheep	1:1000	DSTT (Dundee)	-
O-GlcNAc TAB1	Rabbit	1:1000	DSTT (Dundee)	-
OGT	Rabbit	1:1000	Abcam	ab96718
CTD 110.6 (O-GlcNAc)	Mouse (IgM)	1:1000	CST	9875
FLAG (M2)	Mouse	1:1000	Sigma-Aldrich	F1804
OGA	Rabbit	1:1000	Sigma-Aldrich	HPA036141



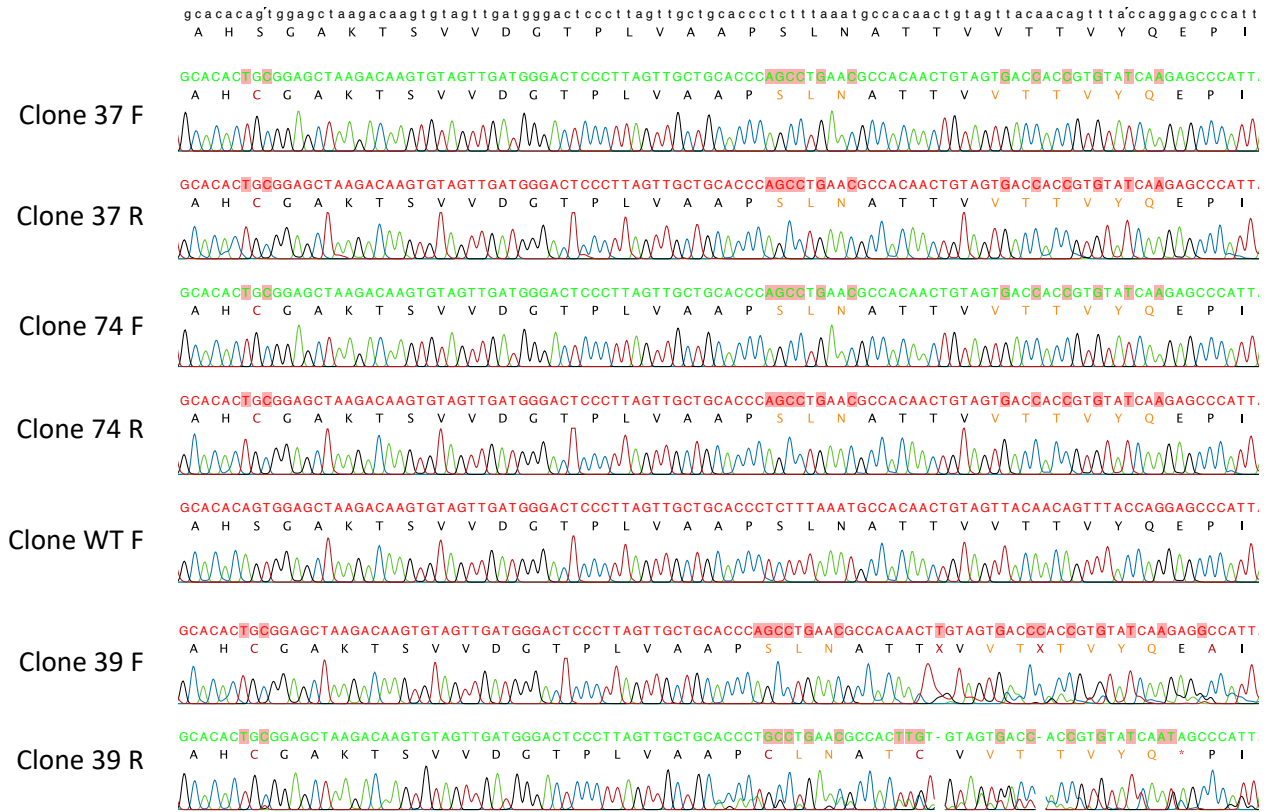
Supplementary Figure 1: Restriction digestion of PCR products of a 622 bp OGA region covering the S405 site in mouse embryonic stem cells that underwent CRISPR-Cas9. Wild type clones further confirmed by sequencing are highlighted in red. OGA^{S405C} knock-ins are highlighted in green.



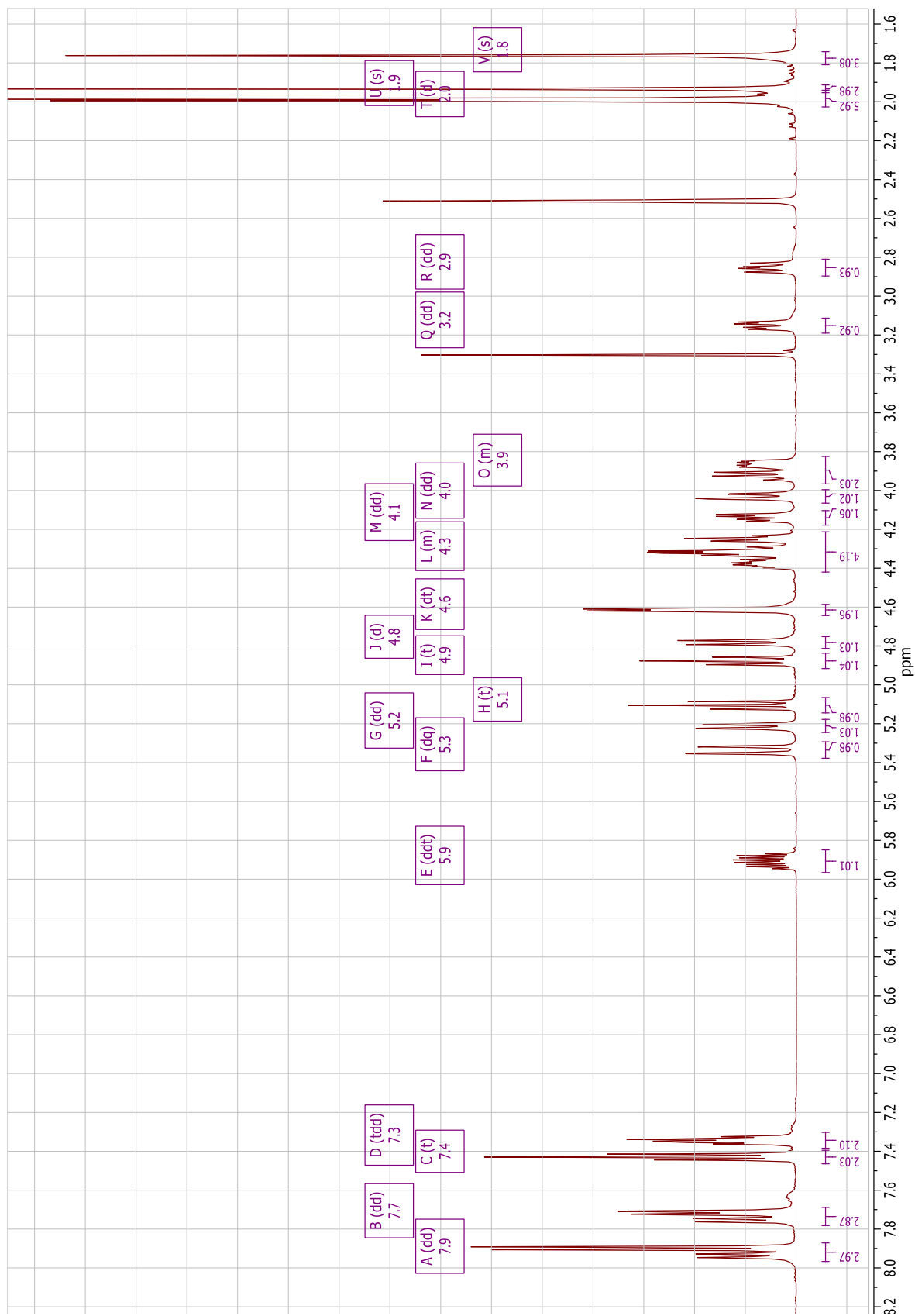
Supplementary Figure 2: DNA sequencing results confirming the introduction of S405C mutation into OGA in mouse embryonic stem cells



Supplementary Figure 3: DNA sequencing results (larger PCR product) confirming the introduction of S405C mutation into OGA in mouse embryonic stem cells



Supplementary Figure 4: cDNA sequencing results confirming the introduction of S405C mutation into OGA in mouse embryonic stem cells



Supplementary Figure 5: ¹H NMR spectrum of compound 2