# upporting information

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H. sapiens        DLSQIPCNGKAADRIHQDGIHILVNMNGYTKGARNELFALRPAPIQAMWLGYPGTSGALF    673
M. musculus       DLSQIPCNGKAADRIHQDGIHILVNMNGYTKGARNELFALRPAPIQAMWLGYPGTSGALF    673
R. Norvegicus     DLSQIPCNGKAADRIHQDGIHILVNMNGYTKGARNELFALRPAPIQAMWLGYPGTSGALF    663
D. melanogaster   DLSQIPCNGKAADKIFNDGIHILVNMNGYTKGARNEIFALRPAPIQVMWLGYPGTSGASF    701
C. elegans        DLSQIPCNGKAAEKIAQDGIHILINMNGYTKGARNEIFALRPAPIQVMWLGYPSTSGATF    780
                  *************::*  :*******:************:*********.******.**** *
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Supplementary figure S1: Sequence alignment shows the highly conserved Asparagine 648 from *H. sapiens* to *C. elegans*.

Sequence alignment analysis showing that the Asp648 is highly conserved across species. Clustal Omega was used to generate alignments between sequences (Madeira et al., 2019).
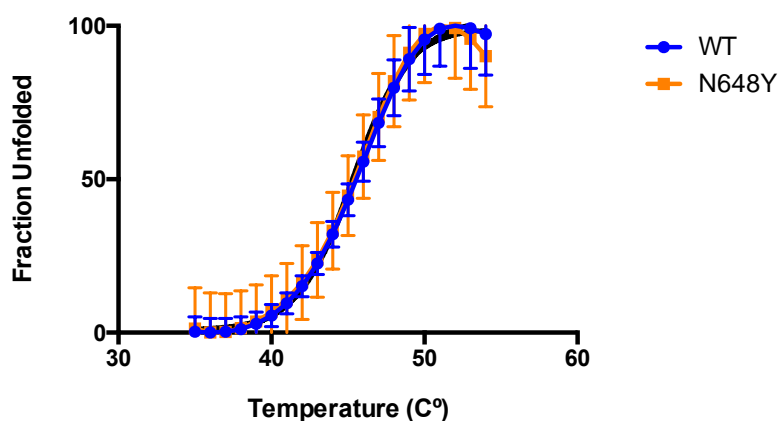
Supplementary figure S2: Effects of the N648Y mutation on unfolding temperature.

Thermal denaturation curve showing fraction of unfolded OGT_{WT} and OGT_{N648Y} as a function of temperature as measured with differential scanning fluorimetry. Data averaged from 12 replicates were fitted to a Boltzmann sigmoidal curve equation, with error bars representing SD.

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                          BfmI    PstI
                           ↓       ↓
WT        GAGCGACTTTATCTGCAGATGTGGGAGCATTATGCAGCTGGCAAC
           E   R   L   Y   L   Q   M   W   E   H   Y   A   A   G   N

          AAACCTGACCACATGATTAAGCCTGTTGAAGTCACCGAGTCAGCC
           K   P   D   H   M   I   K   P   V   E   V   T   E   S   A

          TGAATAAAGACTGCGCACAGGAGAATTGCCCTATACC
           *   I   K   T   A   H   R   R   I   A   L   Y   L
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3HA       GAGCGACTGTACCTCCAAATGTGGGAGCATTATGCAGCTGGCAAC
           E   R   L   Y   L   Q   M   W   E   H   Y   A   A   G   N

          AAACCTGACCACATGATTAAGCCTGTTGAAGTCACCGAGTCAGCC
           K   P   D   H   M   I   K   P   V   E   V   T   E   S   A

          TCTAGATACCCATACGATGTTCCTGACTATGCGGGCTATCCCTAT
           S   R   Y   P   Y   D   V   P   D   Y   A   G   Y   P   Y

          GACGTCCCGGACTATGCAGGATCCTATCCATATGACGTTCCAGAT
           D   V   P   D   Y   A   G   S   Y   P   Y   D   V   P   D

          TACGCTTGAATAAAGACTGCGCACAGGAGAATTGCCCTATACC
           Y   A   *   I   K   T   A   H   R   R   I   A   L   Y   L
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Supplementary Figure S3: Gene-editing of mouse ES cells to introduce 3HA-tag into the endogenous OGT gene.

Genomic DNA sequence of mouse wild type and 3HA OGT gene and protein product is shown (highlighted in yellow). *Bfm*I and *Pst*I restriction sites are lost in 3HA cells (silent mutations highlighted in green)

Eco130I
                                        ↓
WT   ATGAATGGGTATACCAAGGGTGCTCGGAATGAGCTCTTT
              M   N   G   Y   T   K   G   A   R   N   E   L   F

              GCTCTTAGGCCAGCTCCTATTCAGGTAAAAGAACAA
              A   L   R   P   A   P   I   Q   V   K   E   Q

N648Y   ATGAATGGGTATAC**AAA**AGG**C**GC**C**CG**CT**ATGAGCTCTTT
              M   N   G   Y   T   K   G   A   R   Y   E   L   F

              GCTCTTAGGCC**T**GC**C**CC**C**AT**C**CAGGTAAAAGAACAA
              A   L   R   P   A   P   I   Q   V   K   E   Q

Supplementary Figure S4: Gene-editing of mouse ES cells to introduce N648Y mutation into endogenous OGT gene.

Genomic DNA sequence of mouse wild type and N648Y OGT gene and translated protein product is shown. Modification highlighted in orange is responsible for the N648Y mutation, nucleotides highlighted in green are silent wobble sites. *Eco*130I restriction site is lost in N567K cells.

Guide RNA	MmEsLEF_F	CACC gagctcattccgagcaccct
Guide RNA	L3HA_R	AAACaacaatacacaatggaattaC
Guide RNA	MmEsLEF_R	AAAC agggtgctcggaatgagctc
Guide RNA	3HAR_F	CACCGagcgactttatctgcagatg
Guide RNA	MmEsRIG_F	CACCG tcttaggccagctcctattc
Genomic DNA	MmOGT_HA_F	aaaGGATCCagcaagagaattccttaagttttagg
Guide RNA	MmEsRIG_R	AAAC gaataggagctggcctaagaC
Genomic DNA	MmOGT_HA_R	aaaGCGGCCGCgaaagaaatgggaagaacaggactcac
Sequencing	V3_MmEsto_Bam HI_F	aaaGGATCC gagtctgttatagtcctctgtttatacc
Genomic DNA	OGT_HA_seq2	gaatctcttagagttttgcagc
Sequencing		
Mutagenic GB*	MmOGT_HA_GB	ctgatttgtaaatttggttctctttgtttttaccacctagcctgaagaaatt
		cgtggcaaagtctggaaacagagaatatctagccctctgttcaacacAaaGcaGtaTacCatggaattagagcgactGtaCctCcaAatgtgggagcattatgcagctggcaacaaacctgaccacatgattaagcctgttgaagtcaccgagtcagccTCTAGATACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGATCCTATCCATATGACGTTCCAGATTACGCTtgaataaagactgcgcacaggagaattgccctatacctgagcctcaaccttctgggggaagggaactagataacatgctttgtgtgtatctgtgtagttctgtgttgcag
Mutagenesis	3xHA_fwd	ctgatttgtaaatttggttctctttg
Mutagenesis	3xHA_rev	ctgcaacacagaactacacagatac
Genotyping	3HAdiag_F	CTGGCCTGGGTCATTCCTCATTAATC
Genotyping	3HAdiag_R	CAATTCTCCTGTGCGCAGTCTTTATTC

Supplementary Table S1: Primers and geneblock used for introducing 3HA-tag to OGT gene and genotyping candidate 3HA-OGT^{WT} mES cell line.

* - upper case letters denote introduced changes in Geneblocks (GB).

Genomic DNA	V3_MmEsto_NotI_R	aaaGCGGCCGC ctgcaatcgtattcatgggaataacgg
Sequencing	MmEsto_seq1	Ttgctgggaattgaactcaag
Sequencing	MmEsto_seq2	Atcagtggaaacaggcatttc
Mutagenic GB*	MmOGT_HA_GB	attccttgtaatggaaaagcagccgaccgcatccaccaagatggaattcacatccttgtgaatatgaatgggtatacAaaAggCgcCcgCTatgagctctttgctcttaggccTgcCccCatCcaggtaaaagaacaatcacttacaatgtctattggtctgaaaagatagtgggttttgggttttctccatctggctaactgctct
Mutagenesis	MmEstoPatch_F	gatggaattCacatccttgtgaatatg
Mutagenesis	MmEstoPatch_R	gaccaatagacattgtaagtgattg
Genotyping	MmESTO_DIG_F	tacagatggttgtgagccaccacgtg
Genotyping	MmESTO_DIG_R	cacctaaaaatctatcctcgtccattcc

Supplementary Table S2: Primers and geneblock used for introducing N648Ymutation to OGT gene and genotyping candidate 3HA-OGT^{N648Y} mES cell line.

* - upper case letters denote introduced changes in Geneblocks (GB).