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## Supplemental Information

# A Conserved Splicing Silencer Dynamically Regulates O-GlcNAc Transferase Intron Retention and O-GlcNAc Homeostasis

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**SUPPLEMENTAL FIGURES**

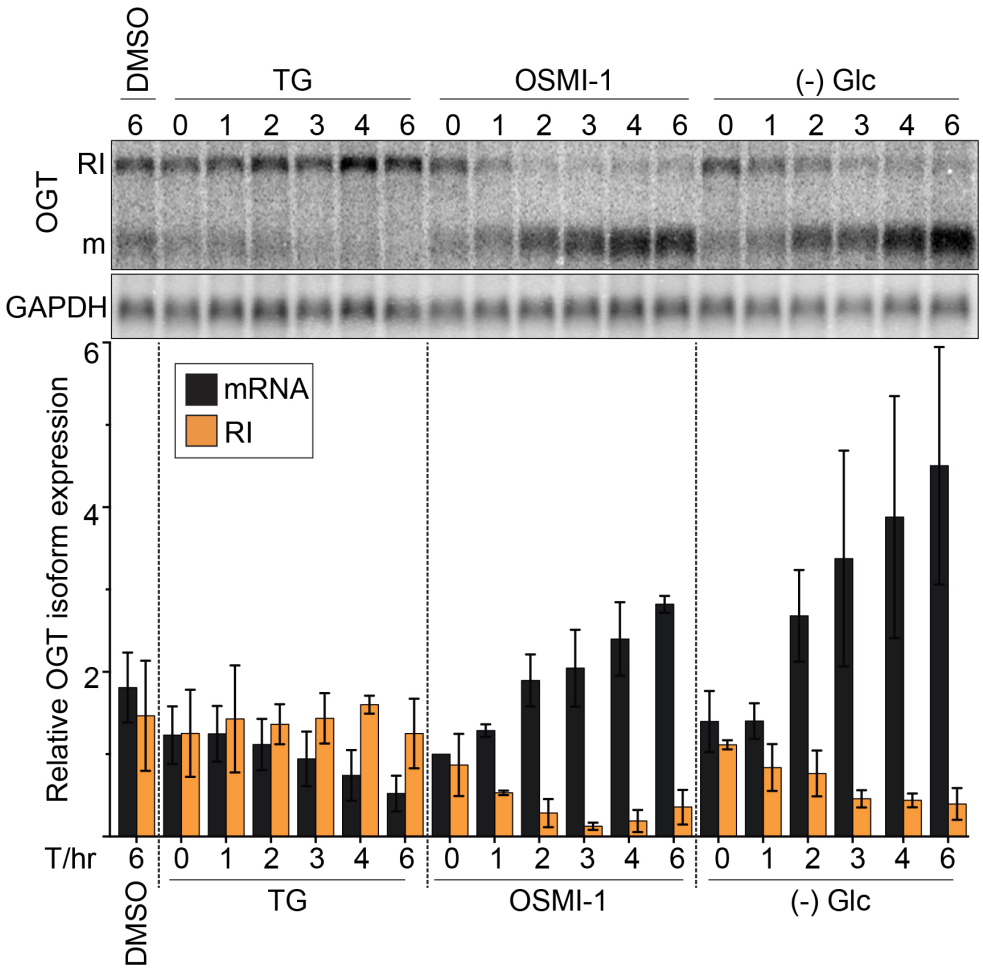


**Figure S1. Time course of OGT intron retention following cell treatments in HCT116 cells, Related to Figure 1.** Representative northern blot and quantification of a time course experiment of TG, OSMI-1 and glucose depletion in HCT116 cells. Data were normalized to the OSMI-1, mRNA signal prior to treatment (t=0). Data are represented as mean ± SD (*n = 3*).

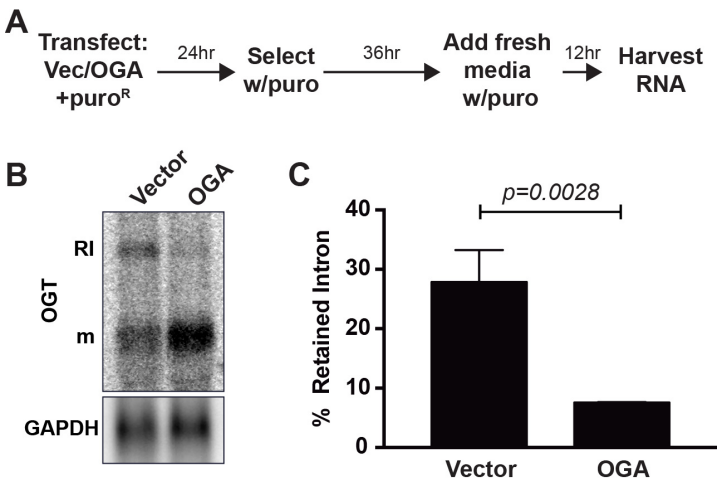


**Figure S2. OGA overexpression decreases intron retention, Related to Figure 2.**
(A) Flow chart of the experiment. We note that if fresh media was not added to cells ~12hrs prior, the OGT-RI isoform was largely lost. Presumably, this was due to glucose depletion, but that has not been tested directly.
(B) Representative northern blot of OGA overexpression experiment.
(C) Quantification of OGA overexpression northern blots; data are represented as mean ± SD (*n = 3*).

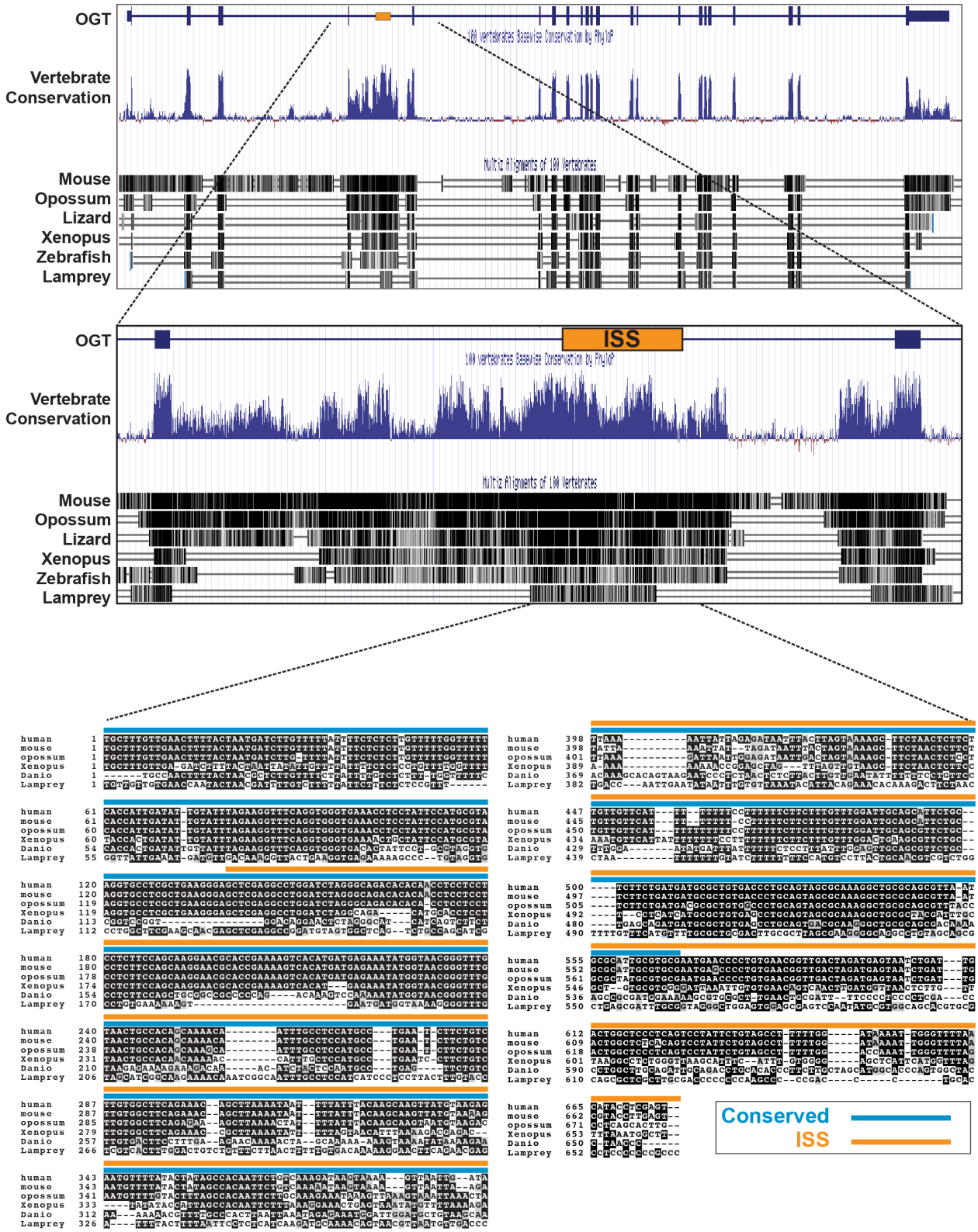


**Figure S3. Evolutionary conservation of OGT ISS, Related to Figure 3.**
*Top,* vertebrate conservation track from UCSC Genome Browser (*http://genome.ucsc.edu*). Position of the ISS is approximated in orange. *Middle*, zoomed in view of the OGT intron four and flanking exons. *Bottom*, sequence alignments (Clustal Omega)(McWilliam et al., 2013). The blue bar highlights the region of conservation that extends to lamprey and the orange bar highlights the ISS.

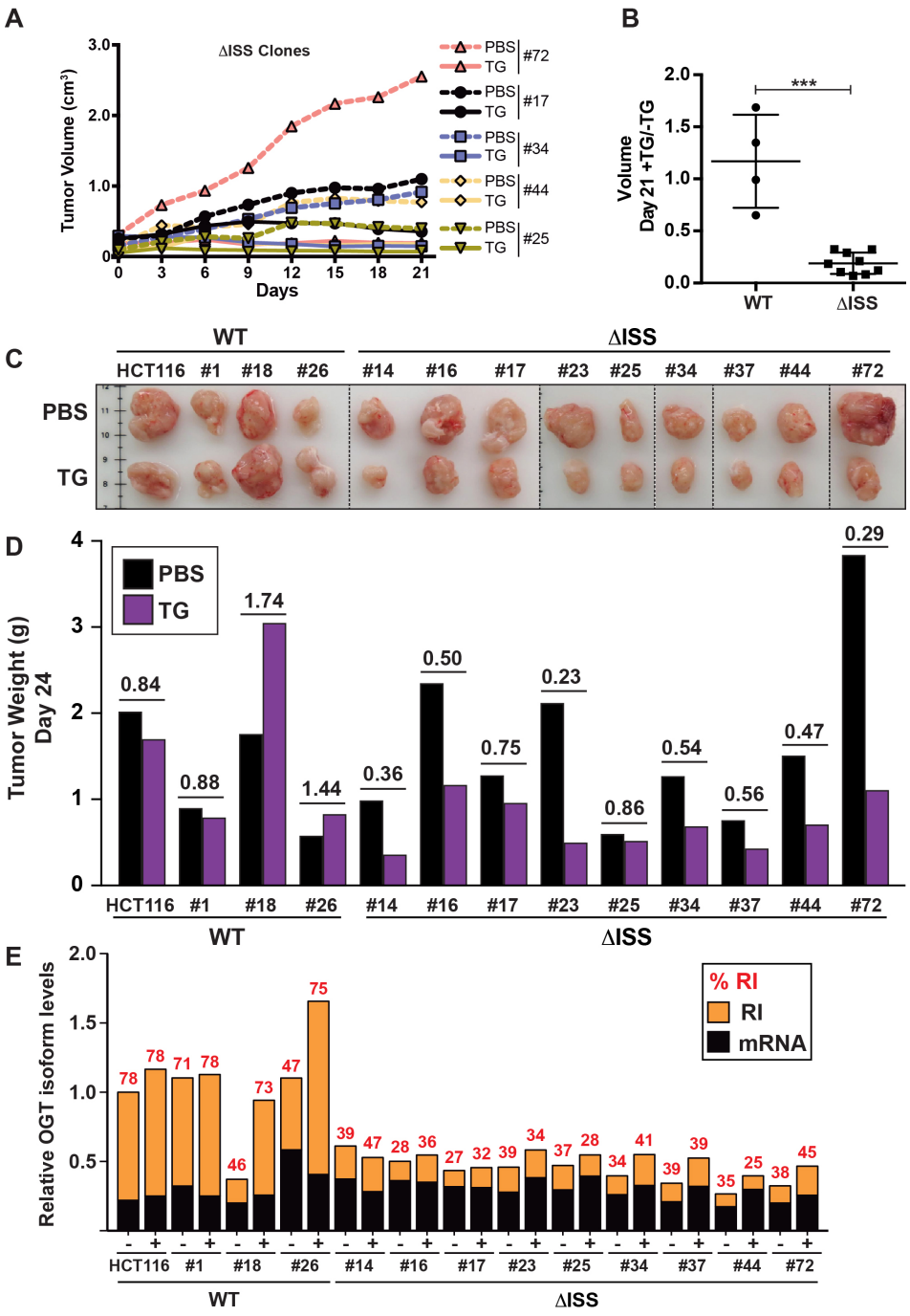


**Figure S4. The ISS is necessary for O-GlcNAc homeostasis in vivo tumors derived from colorectal cancer cells, Related to Figure 6.**

(A) Growth curves of mutant tumors volume in vivo. Growth of control (PBS) and TG-treated mice are in dashed and solid lines, respectively.

(B) Plot of the TG/PBS ratios of estimated tumor volumes from day 21 after treatment.

(C) Pictures of tumors. All are shown at the same scale; dotted lines represent regions cropped for presentation.

(D) Tumor weights of each of the individual samples. The number above each matched pair is the ratio of the weights from the TG/PBS-treated samples.

(E) Quantitative RT-PCR results from all tumors. Each sample was first normalized to b-actin mRNA to control for differences in RNA in the sample. The sum of the normalized RI and mRNA levels was set to one for the HCT116 DMSO sample and the values for each isoform were referenced to that value. As a result, the relative level of both OGT isoforms can be assessed in each tumor as well as that of each individual isoform. The red number above each bar indicates the percent OGT-RI.

**Supplemental Table S1. Primers used in this study, Related to Experimental Procedures.**

| Name | Description | Use | Sequence (5'-3') |
|---|---|---|---|
| NC2053 | FOR-β-globin exon 2 (inserted BglII) | cloning | gtcctttggAGATCTgtccactc |
| NC2054 | REV-β-globin exon 3 (inserted BglII) | cloning | cttggtacctgtAGATCTggccctc |
| NC1876 | FOR-OGT-exon 4 | cloning | ctgcactgaagtttgtcaggc |
| NC1877 | REV-OGT-exon 5 | cloning | catactgcctataccgccagag |
| NC1864 | FOR-OGT-exon 4 (inserted EcoRI) | cloning | gtctctgggtGAATTCgatttgtac |
| NC1860 | REV-OGT-exon 5 (inserted BglII) | cloning | ttaatttaaAGATCTggcttttcaaagtg |
| NC2144 | FOR-OGT-ISS_upstream (inserted BsrGI) | cloning | GATATTGTATgTAcAAGGTTTCAGG |
| NC2145 | REV-OGT-ISS_downstream (inserted BsrGI) | cloning | GAGAATATTTGTacAATGAGATTAGTTGG |
| NC2249 | FOR-OGT-ISS_upstream | sgRNA annealing | caccGCGTAAGGTGCCTCGCTGAA |
| NC2250 | REV-OGT-ISS_upstream | sgRNA annealing | aaacTTCAGCGAGGCACCTTACGC |
| NC2251 | FOR-OGT-ISS_downstream | sgRNA annealing | caccgAATGAGATTAGTTGGACTCG |
| NC2252 | REV-OGT-ISS_downstream | sgRNA annealing | aaacCGAGTCCAACTAATCTCATTc |
| NC2065 | LKO1_5' (human U6 promoter) | sequencing | GACTATCATATGCTTACCGT |
| NC2260 | FOR-Genotyping for ΔOGT-ISS clone | PCR | CTTGAGACTGTCCTCATTCCC |
| NC2261 | REV-Genotyping for ΔOGT-ISS clone | PCR | CATTCTCACAATGCCCCGTC |
| NC2018 | FOR-OGT 3'-UTR | Northern blotting | cagtgtaaatcacggaatatc |
| NC1248 | REV-OGT 3'-UTR (T7) | Northern blotting | TAATACGACTCACTATAGGGaaggatcgcaagacaacatct |
| NC2071 | FOR-OGT-RI | Northern blotting | gtaggtgtttgatagaacacatttaaac |
| NC2072 | REV-OGT-RI (T7) | Northern blotting | TAATACGACTCACTATAGggaacttgggaagggagtgg |
| NC781 | FOR-β-globin | Northern blotting | aaacctAAGCTTgacaccatgGACTACAAGGACGACGATGACAAG |
| NC780 | REV-β-globin (T7) | Northern blotting | TAATACGACTCACTATAGGGttagtgatacttgtgggccaggg |
| NC236 | FOR-β-globin intron 2 | Northern blotting | AATCCGAAGCTTCCTGAGAACTTCAGGGTGAG |
| NC303 | REV-β-globin intron 2 (T7) | Northern blotting | GACCAATTAATACGACTCACTATAGGGctgtgggaggaagataagag |
| NC1549 | FOR-GAPDH probe | Northern blotting | CCTGCCGTCTAGAAAAACCTG |
| NC1550 | REV-GAPDH probe | Northern blotting | CGCGTAATACGACTCACTATAGGGGGTTGAGCACAGGGTACTTTA |
| NC897 | FOR-MALAT1 probe | Northern blotting | CCCAAATCTCAAGCGGTGCTT |
| NC898 | REV-MALAT1 probe | Northern blotting | CGCGTAATACGACTCACTATAGGGAAGCTAGAAAAAGGCCAAAAAGC |

| | | | |
|---|---|---|---|
| NC1772 | FOR-OGT mRNA | qPCR | ACTGTGTTCGCAGTGACCTG |
| NC1773 | REV-OGT mRNA | qPCR | TCAAATAACATGCCTTGGCT |
| NC1770 | FOR-OGT-RI | qPCR | AGTGCGATGCTGTTAGGTTG |
| NC1771 | REV-OGT-RI | qPCR | GGAGGACTGAAGCTGAAACC |
| NC1224 | FOR-ACTB spliced | qPCR | ACCCAGCACAATGAAGATCA |
| NC1225 | REV-ACTB spliced | qPCR | CTCGTCATACTCCTGCTTGC |
| NC1862 | FOR-OGA cDNA (inserted KpnI) | cloning | ccgagctGGTACCatggtgcag |
| NC1875 | REV-OGA cDNA | cloning | CTAGATGCATGCTCGAGCGGCCG |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### RNA isolation, northern blotting, and qRT-PCR

RNAs were prepared from cells or tissues using TRI Reagent (Molecular Research Center) following the manufacturer's protocols. For nucleocytoplasmic fractionation, cell pellets from a 6-well plate were resuspended in 100 µL Buffer I [10 mM Tris-HCl (pH 8.0), 0.32 M Sucrose, 3 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.4 U/mL RNasin, and 0.5% Triton X-100)], incubated for 5 min on ice and then centrifuged at 500 g for 5 min at 4°C. RNAs in the supernatant (cytoplasmic fraction) were added to 1 mL TRI Reagent. The pellet was washed once in 100 µL Buffer I and centrifuged again at 500 g for 5 min at 4°C. The resulting supernatant was discarded and the remaining pellet (nuclear fraction) was extracted in 1 mL TRI Reagent. Northern blot analyses for OGT (3' UTR or RI), GAPDH and β-globin (CDS or RI) were performed using standard techniques (Conrad and Steitz, 2005). RNA probes were generated by incorporation of $\alpha^{32}$P-UTP in vitro using templates generated by PCR. All primers and oligonucleotides used in this study are listed in Table S1. Stripping and re-probing of the membranes were performed as previously described (Bresson and Conrad, 2013). RNA was quantified using ImageQuant software (GE Healthcare). Quantitative RT-PCR for OGT isoforms was performed as previously described (Bresson et al., 2015).

### Western blotting

Proteins were harvested from cells using RSB100-T [10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2.5 mM $MgCl_2$ and 0.5% Triton X-100] with PMSF and 1x protease inhibitor cocktail followed by brief sonication. Harvested proteins were mixed with 2x SDS loading buffer [100 mM Tris-HCl (pH 6.8), 20% glycerol, 4% SDS, 2% β-mercaptoethanol and 0.1% bromophenol blue] and separated by SDS-PAGE using standard procedures. Proteins from mouse tissues were prepared using TRI Reagent after RNA isolation following the manufacturer's protocols.

### Plasmid constructs

The pcDNA-OGA construct was generated by PCR amplification of OGA cDNA sequence from CMV-OGA using primers NC1862 and NC1875 followed by insertion of the amplified product into pcDNA3 using Kpn I and Xho I sites and standard molecular biology techniques. The β–OGT wild-type fusion construct was created by inserting PCR products of OGT intron 4 with adjacent exons into the EcoRI and BglII sites of βΔ1 (Conrad and Steitz, 2005) and then re-inserting the amplified product of βΔ1 exon 2 - intron 2 - exon 3 using primers NC2053 and NC2054 into the BglII site. The PCR products of OGT exon 4 - intron 4 - exon 5 were obtained by two-step PCR, first amplification by 15 cycles using NC1876 and NC1877 and then second amplification using NC1864 with EcoRI site and NC1860 with BglII site from the first products. The β–OGT deletions were constructed using available restriction enzyme sites. To remove nt 798-2576 or 798-3162, the β–OGT was digested with Pac I, partially digested with EcoRV, the ends were filled with Klenow polymerase, and re-ligated to create β–OGTΔ798-2576 and β–OGTΔ798-3162. To remove nt 798-1285 or 798-2297, β–OGT was double-digested with PacI and EcoNI or PspXI, respectively, and re-ligated. Similarly, PspXI was used to delete nt 1771-2297 from the β–OGT. βΔ1-ISS-F and βΔ1-ISS-R were constructed by insertion of the OGT-ISS amplified using primers NC2144 and NC2145, into the BsrGI site of βΔ1 intron 2. Primers used for PCR amplification are listed in Table S1. To generate the sgRNA expression constructs (pX458-OGT-ISS-upstream and pX459-OGT-ISS-downstream), the oligonucleotides NC2249/NC2250 or NC2251/NC2252 (Table S1), were phosphorylated and annealed. The resulting annealed oligo pairs were ligated into BbsI site of pX458 or pX459, gifts from Feng Zhang (Addgene plasmid # 48138 or 48139, respectively). These plasmids express hSpCas9 and the chimeric guide RNA containing +85nt of tracrRNA with GFP (pX458) or puromycin (pX459) selectable markers (Ran et al., 2013). The resulting plasmids were sequence verified using primer NC2065.

### FISH

Cells were grown to ~75% confluency on cover slips pre-coated by Poly-L-Lysine solution (Sigma, 1:10 dilution in water) and then fixed in fixation buffer [3.7% (v/v) formaldehyde in 1x PBS] for 10 min at RT. Fixed cells were permeabilized and stored in 70% EtOH at 4°C. To prepare the hybridization buffer containing probes for one cover slip, 1 µL of probe stock solution (12.5 µM) was added to 90 µL of Stellaris® RNA FISH Hybridization Buffer (SMF-HB1-10) plus 10 µL of formamide. The hybridization mixture was applied to fixed cells in the dark for 4~6 hours at 37°C. After hybridization, cells were washed two times in wash buffer [20% of Stellaris® RNA FISH Wash Buffer A (SMF-WA1-60) and 10% formamide in water] for 20 min at 37°C and then mounted on the slide-glass with SlowFade® Gold Antifade reagent with DAPI (Invitrogen). For simultaneous immunostaining, primary antibody (anti-OGA) was added in the hybridization mixture and secondary antibody (goat anti-rabbit IgG-Alexa Fluor 488) was added to the first wash buffer and incubated for 30 min at 37°C, followed two additional washes for 20 min at 37°C each. Deltavision Deconvolution Microscope equipped with cooled CCD camera (Live Cell Imaging Core Facility, UT Southwestern Medical Center) was used for imaging, and all raw-images with Z-slice sections separated by 0.2 µm were 3D-deconvoluted through AutoQuant X and Imaris software

to remove the out-of-focus signals. After deconvolution, representative images generated using Z-project function with maximum intensity in ImageJ program were presented here.

**Statistical analyses**

Unpaired Student's t-test were used to determine statistical significance. Comparison groups are indicated in each figure and/or legend. Unless otherwise noted, all statistical analyses are unpaired Student's t tests, and significance is annotated as $p \leq 0.05$, $**p \leq 0.01$, or $***p \leq 0.001$. GraphPad or Excel were used to perform the analyses.

**SUPPLEMENTAL REFERENCES**

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