SUPPORTING INFORMATION

A chemical genetic method for monitoring genome-wide dynamics of O-GlcNAc turnover on chromatin-associated proteins.

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SUPPORTING INFORMATION

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Figure S2. AHA labeling in yeast for use as spiked-in internal control. The left panel shows a blot using streptavidin to detected biotin conjugates proteins from AHA fed (+) and vehicle fed (-) yeast, indicative of AHA labeling of proteins occurring within two different yeast strains, CBY4865 and CBY676. Corresponding coomassie protein loading control is shown at right (ladder in kDa).
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Figure S10: OGA ChIP-seq analysis in Drosophila and overlap with O-GlcNAc. Example loci at which one finds both OGA and O-GlcNAc modified proteins.
Figure S11. OGA is capable of removing O-GlcNac and O-GlcNAz from Ac4GalNAz fed larvae lysates. Ac4GalNAz fed WT larvae lysates were incubated with *Bacteroides thetaiotaomicron* OGA (BtGH84) or mutant BtGH84 (D242A) at 37 °C for 2 h with the OGA specific inhibitor, Thiamet-G in BtGH84 treatments. The left panel shows an O-GlcNac blot with the CTD110.6 antibody, indicative of O-GlcNac quantity for different treatments. Corresponding Streptavidin (Strvn) blot is shown to the right. After enzymatic digestion, proteins were extracted and conjugated by click chemistry to biotin and blotted using Streptavidin. Intensity of bands are indicative of O-GlcNAz bearing proteins.
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Table S1. Sequencing depth of AHA-tagged yeast chromatin for each TC O-GlcNAc-seq experiment. Normalization factors were calculated based on the sequencing depth of sacCer3 aligned reads for each biological replicate. We blinded the study by encoding each experiment with an number (encoding ID) and added a random mix of synthetic DNA to each sample (probe mix ID). See Table S2 for the composition of the probe mixes.

Table S2. Composition of the probe mixes used for blinding. Each TC O-GlcNAc-seq experiment was randomly assigned one of 7 probe mixes with compositions as shown.

Table S3. O-GlcNAc-seq loci in WT and OGA-null Drosophila larvae. Approximately 3% (56/1883) of the TC O-GlcNAc-seq peaks exhibited OGA resistant behavior, calculated from significantly (p < 0.05) high standard scores of the O-GlcNAc modification half-life values obtained using TDCA from the WT TC experiment.

Table S4. Overlap of non-redundant FlyBase Drosophila genes at OGA resistant loci as well as the remaining 1827 loci. Some genes contain loci that were characterizes as both OGA sensitive and OGA resistant.
Supplemental Methods

General procedures

Time course Ac4GalNAz labeling
Wild-type Oregon R (WT) and OGA knockout (OGA-null)\(^1\) Drosophila were expanded in 50 mL bottles of cornmeal molasses media at 25°C. Drosophila parents from approximately 25 of these bottles were required for each time course replicate. Time course Ac4GalNAz feeding was performed on 10 mL petri dishes. Just under 10 mL of cornmeal molasses media supplemented with Ac4GalNAz dissolved in DMSO to a final concentration of 150 uM or DMSO only in the same volume added to the Ac4GalNAz plates (control) were made. Adult Drosophila parents from the bottles were then transferred to the control plates for 2 hours (on cages) for a pre-egg laying period. This facilitated the laying of eggs that were not synchronized. These adult Drosophila were then transferred to either 150 uM Ac4GalNAz or control plates for 2 hours (enabling the laying of synchronized eggs). After a 36 h incubation period, the resulting larvae were transferred to control plates or collected and snap frozen (0 h time point). Larvae that were initially grown on control plates were collected at this time as well (no feed control). The larvae transferred from Ac4GalNAz plates to control plates were then collected after 4, 8, 12, 16, 20, 24, and 36 hours of feeding on these control plates. This process was repeated for two replicates for both WT and OGA-null Drosophila larvae. However, only one no feed control was performed for each genetic strain but we do provide an additional replicate for both WT and OGA-null DMSO control from an earlier pilot study (see NCBI GEO submission data).

Chromatin preparation
The larvae (WT and OGA-null) were collected and rinsed with PBST (PBS + 0.1% Triton X-100) three times to remove adhering corn food. One volume of hexanes (a mixture of isomers) was equilibrated for 30 min against 0.175 vol of 37% formaldehyde and 0.130 vol of 10X PBS (1.37 M NaCl, 27 mM KCl, 43 mM Na2HPO4 and 14 mM KH2PO4), pH 7.5, to make the mixture 5% formaldehyde and 1X PBS. Only the upper organic phase was used for crosslinking. Larvae were fixed in 10 ml of buffered 5% formaldehyde/hexanes per gram of larvae by vigorous shaking for 5 min at room temperature. The larvae were allowed to settle, and the formaldehyde/hexanes solution was removed by centrifugation (3,000 x g for 5 min at RT). The larvae were washed twice in a solution containing 1X PBS and 0.5% Triton X-100 (0.5% PBST), using the five volumes as used for fixing. They were then stored at -80°C. Chromatin was purified by quickly thawing frozen larvae and resuspending them in buffer A [0.3 M sucrose, 15 mM NaCl, 5 mM MgCl2, 15 mM Tris pH 7.5, 60 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF)]. They were homogenized with 20-25 strokes of a hand-held type B Dounce homogenizer. Triton X-100 was added to 0.3%, and the homogenate was centrifuged (2,000 x g for 15 min at 4°C). The pellet was resuspended in buffer B (100 mM NaCl, 10 mM Tris pH 7.9, 1 mM EDTA, 0.1% v/v NP-40 and 1 mM PMSF) and homogenized with ~5 strokes of a hand-held type B Dounce homogenizer. The homogenate was then sheared by 20 cycles (20 × 20 s on and 50 s off cycles, 40% power settings) through a sonicator (Sonic Dismembrator Model 500, Fisher Scientific) to an average size of ~200 to 700 bp chromatin fragments. After sonication, debris was removed by centrifugation (7,500 r.p.m., 2 min). The chromatin solution was pre-cleared with 100 µl Streptavidin-agarose slurry (Sigma) beads for O-GlcNAz modified or vehicle-only chromatin separately and incubated for 1 hour at 4°C on a rotating wheel. The pre-cleared chromatin was aliquoted after centrifugation at 4°C for 2 min at 7,500 r.p.m., snap-frozen in liquid nitrogen and stored at −80°C for future use. For click chemistry, 20-50 µg chromatin DNA was incubated with iodoacetamide (IAA) to a final concentration of 15 mM, agitate mildly for 30 min at −80°C for future use. For click chemistry, 20-50 µg chromatin DNA was incubated with iodoacetamide (IAA) to a final concentration of 15 mM, agitate mildly for 30 min at
RT then added DMSO solution of dibenzylcyclooctyne-S-S-PEG3-Biotin (DBCO-S-S-PEG3-Biotin, Jena Bioscience GmbH) to a final concentration of 40 μM. The samples were protected from light and agitated mildly for 1 hour at RT. The unreacted probe was filtered off by the Amicon Ultra-0.5 mL centrifugal filter (15K cut-off) (EMD Millipore). Biotinylated-probe-chromatin complexes were captured by incubation with 50 μl Streptavidin magnetic beads (NEB) at 4°C for 2 hours. Beads were washed with 0.5 ml of the following buffers: three washes with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.0, 150 mM NaCl), followed by three washes with high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.0, 500 mM NaCl), then three washes with lithium wash buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.0); then two washes with TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Beads were resuspended in 100 μl TE containing 0.5% SDS, 15 mM DTT and incubated with 0.15 mg/ml proteinase K at 55°C for 3-4 hours. DNA-protein complex was de-crosslinked by incubating overnight at 65°C (not more than 15 hours). ChIP-DNA was purified with QIAquick PCR Purification Kit and stored at -20°C.

**OGA ChIP**

50 larvae were cross-linked in 5% formaldehyde in PBS with 0.5% Triton X-100 (0.5% PBST) for 5 min by vortexing occasionally at room temperature. The flies were then washed three times in 0.1% PBST, using the five volumes as used for fixing, and glycine was added to a final concentration of 0.125 M. The samples were washed once with PBS, 1 mM PMSF, protease inhibitor cocktail (Roche) followed by two washes in PBS, 1 mM PMSF and protease inhibitor cocktail. These flies were homogenized in 500 μl Lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1). To ensure shearing of the cross-linked DNA into 200-500 bp long fragments, 120 μl glass beads were added prior to sonication for 4 min using a sonicator (Sonic Dismembrator Model 500, Fisher Scientific) at high setting (30 s ON, 30 s OFF). The samples were cleared by centrifuging for 10 min at 14,000 x g at 4 °C. For each ChIP (WT and OGA-null) and Input, 150 μl of cell lysate was diluted by a factor of ten in ChIP Dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.0, 167 mM NaCl), and protein inhibitors were added. To reduce nonspecific background the diluted lysate was pre-cleared by incubation with 60 μl of equilibrated Dynabeads Protein A (Thermo Fisher Scientific) for 30 min at 4 °C with agitation. For immunoprecipitation, the cleared lysates were incubated with 5 μl of normal rabbit IgG (Control; Santa Cruz, sc-2027) and rabbit antibodies (MEGA5, Proteintech #14711-1-AP) raised against full-length Drosophila OGA protein overnight at 4 °C on a rotating platform. The antibody complexes were precipitated by incubation with equilibrated Protein A Dynabeads for 1 h at 4 °C. The beads were washed for 4 min with agitation at 4 °C with the following buffers: once with low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM Tris-HCl pH 8.0, 150 mM NaCl), once with high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM Tris-HCl pH 8.0, 500 mM NaCl), once with LiCl-containing buffer (250 mM LiCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1% NP-40, 1% sodium deoxycholate), and twice with TE Buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). The protein/DNA complexes were eluted from the antibody by incubating for 15 min at room temperature in 250 μl Elution buffer (1% SDS, 0.1 M NaHCO₃) with rotation. The elution was repeated once, and the eluates were combined to a total volume of 500 μl. NaCl was added to a final concentration of 200 mM, and protein/DNA crosslinks were reversed by heating at 65 °C for 4 h. A total of 10 μl of 0.5 M EDTA, 20 μl of 1 M Tris-HCl pH 6.5, and 1 μl of 20 mg/ml proteinase K were added before an additional incubation at 45 °C for 1 h. The DNA was recovered by phenol/chloroform extraction followed by ethanol precipitation. The immunoprecipitated DNA was then dissolved in 24 μl water.

**DNA library preparation and sequencing**

The libraries were prepared according to the manufacturer’s instructions using the NEBNext kit (E6200). Briefly, DNA was fragmented by sonication to a maximum of 300 bp. Next, the ends of the fragments were repaired with a combination of fill-in reactions and exonuclease activity to produce blunt ends that were then tailed with an A-base. Illumina-specific adaptors were ligated followed by
removal of unligated adaptors using AMpure XP beads (Beckman Coulter). Finally, a PCR with 12-15 cycles for both kits was performed to enrich final adaptor-ligated fragments. Quality and quantity were assessed on High Sensitivity dsDNA Agilent Bioanalyzer Chips and Quibit. WT and OGA-null O-GlcNAc-seq libraries were sequenced on the Illumina NextSeq 500 High Output (75 cycles, 400M reads) platform. The additional replicates for both WT and OGA-null DMSO control from an earlier pilot study were sequenced on the MiSeq platform, using v3, 150-cycle reagent kits (Illumina).

**Immunoblots and streptavidin blots**

10 of each WT and OGA-null larvae were washed with PBS, dislodged and pelleted by centrifugation and homogenized around 20 times in the 500 μL RIPA buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 0.5% sodium deoxycholate, 10% glycerol and 0.1% SDS) containing protease inhibitor cocktail (Roche). Cells were mixed and incubated on ice for 30 min, after which cell debris were collected by centrifugation (30 min) at 14,000 rpm (4°C). The supernatant was treated with methanol/chloroform/H₂O (4:1:3), and the precipitate was then resuspended in SDS lysis buffer (1% SDS, 50 mM Tris pH 7.0 and 1 mM EDTA). Run SDS-PAGE according to standard procedures. The primary antibodies used include CTD110.6 (Millipore #MABS1254), OGA (MEGA5, Proteintech #14711-1-AP) and β-Actin (Santa Cruz #sc-47778). Immunoreactivity was imaged using the Odyssey Infrared Imaging System (LI-COR Biosciences).

Two *S. cerevisiae* strains CBY4865 and CBY676 were the gifts from Prof. Christopher Beh (Simon Fraser University, Canada). Both were 30°C grown in minimal medium (0.7% Yeast Nitrogen Base, 2% glucose and appropriate amino acids) to mid-log phase (OD₆₀₀ nm of 0.6 to 0.8) with shaking at 250 rpm. Both fresh culture pellets were transferred into 30 mL minimal medium w/o methionine then fed with and without 150 μM L-azidohomoalanine (AHA, AnaSpec Inc.) for 4 hours as shown in the **Figure S2**. We extracted the yeast proteins followed a previous study² and blocked the free cysteine thiols by addition of iodoacetamide (IAA) to a final concentration of 15 mM (from the fresh made 0.5 M stock) for 30 min at RT. After degassing of the solution, CuAAC coupling was performed using Biotin-PEG4-Alkyne (Sigma). Detection of AHA modified proteins was done using streptavidin-conjugated IRDye 800CW (1:50,000) and imaged using the Odyssey Infrared Imaging System (LI-COR Biosciences).

**Bacterial O-GlcNAcase (BrGH84) assay**

Total cell lysates (~200 μg, in PBS at pH 7.4) were incubated with 10 μM *Bacteroides thetaiotaomicron* OGA (BrGH84) or mutant BrGH84 (D242A) in 200 μL reaction mixtures and allowed to incubate at 37 °C for 2 h with the OGA specific inhibitor, Thiamet-G in BrGH84 treatment samples. Following incubation, BrGH84 was denatured by being heated at 100 °C for 5 min. Immediately thereafter, reaction mixtures were cooled and analyzed by immunoblotting.

**RT-PCR**

Semi-quantitative PCR analyses using a primer set specific for the last exon in OGA mRNA was performed in 10 μL, using 3 ng DNA per reaction and 12 pmol of each primer. The PCR conditions were: 1 min 94°C initial denaturation, followed by 25 cycles of 94°C for 30 sec, 52°C for 35 sec, 72°C for 30 sec, with a final extension of 2 min at 72°C and storage at 16°C. The following PCR primer pairs were used: 5’-TACGACGAGGTAACCGTATCA-3’ and 5’-ATCAACACGGCAGGGAAG-3’.

**Yeast spike-in normalization (Input DNA)**

To isolate input DNA, we reserved an aliquot of AHA-feeding CBY4865 yeast culture from previous AHA-labeling preparation; proteins were crosslinked to DNA by adding formaldehyde to the culture (final concentration of 1%). Crosslinking was done for 15 min and quenched with glycin (final concentration of 0.125 M) for 5 min. Yeast cells were re-suspended with lysis buffer (50 mM HEPES, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X, 0.1% SDS, 0.1% Na Deoxycholate + PMSF) and
disrupted by agitation with glass beads using a Bead beater (BioSpec Products). The cell lysates were sheared using a Sonic Dismembrator (Model 500, Fisher Scientific), blocked the free cysteine thiols by addition of iodoacetamide (IAA) to a final concentration of 15 mM (from the fresh made 0.5 M stock) for 30 min at RT. After Copper-free clicking was performed using DBCO-S-S-PEG3-Biotin (Jena Bioscience GmbH) to a final concentration of 40 μM. The samples were protected from light and agitated mildly for 1 hour at RT. The unreacted probe was filtered off by the Amicon Ultra-0.5 mL centrifugal filter (15K cut-off) (EMD Millipore) and the rest of procedures were carried out as described above in the Chromatin preparation and DNA library preparation and sequencing sections.

**gBlocks spike-in blinding probes**

Three 150 bp gBlocks® Gene Fragments (Integrated DNA Technologies) were designed such that no 20 consecutive base pairs (bp) within each gBlock or its complement would align to any other gBlock or the *D. melanogaster* dm3 reference genome or the *S. cerevisiae* sacCer3 reference genome using the help of BLAST³. gBlock sequences used were as follows:

>`gBlock1
5' - CAGATATCTTTAATGTGGTAAATTGGAAGGACTCTTTGCCCCTCCACCCCTTAGACAGTGTA TACTCTTCCATAAAAAAGCAGCTTAGGCGACAADAAATGCTCAGCATCATTATTTAAACCAGACTGAT ACGATTACCGGCAATCTATCTGATCGCGACGAC-3'

>`gBlock2
5' - ATTCGCGCCCACCTCTCCCCATTAACGCTAGTAGAATCTAAAGTGGCGATCGAAGTTGCAAGATGCTCAC GAGTACTGGAGCAGGAGAGGTAACTATGATGCC

>`gBlock3
5' - ACACTCCGTAATTGACTACGCATTCTCTCTAGACCTTTACTCTGACCAGATACAGTGACTTT GACACGTGTTTATGGAAGATTAGCAGAACATACGACGATGCCTATGGAGGAGGACACACTCT TGAGTGTGAATATGTTGACTCTCGTATGAGGC-3'

Each gBlock was prepared according to the manufacturer's protocol to 10 ng/uL and serially diluted to 1 pg/uL (two 2/200 dilutions). From these three 1 pg/uL gBlock solutions, seven different gBlock mixes were created as specified in Figure 2b and Figure S4 (two biological replicates in WT and OGA-null larvae). Random 1 pg/uL gBlock mixes were then added to each time course chromatin preparation.

**Bioinformatics**

**Sequence alignment and pre-processing**

FASTQ sequencing files were aligned to a concatenated reference genome of *D. melanogaster* (dm3), *S. cerevisiae* (sacCer3), and the three gBlock fragments using the snap-aligner single command from SNAP version 1.0beta.18⁴. The yeast chromosomes were re-name with the prefix “yeast” to avoid conflicts with the same chromosome names as *Drosophila*. The resulting BAM-format alignment files were sorted, cleared of duplicates and indexed using the SAMtools commands sort, rmdup -s, and index, respectively, using SAMTools version 0.1.19-96b5f2294a⁵. The sum of sequencing coverage at each chromosome was calculated using the SAMtools depth -r command piped to the command awk ‘{ sum += $3 } END { print sum } ‘. The percent alignment of each gBlocks fragment was determined in the same way and reported as a percent of total base pairs that aligned to all gBlock
fragment. BDG files were generated from the BAM files using the command bedtools genomcov -ibam <$bam> -bg from bedtools v2.17.0, where the <$bam> indicates the input BAM file. Yeast spike-in normalized BDG files were generated by multiplying by the fourth column, which specifies the sequencing coverage, of the BDG file by the normalization constants. These normalization constants were calculated by dividing the number of base pairs that aligned to the sacCer3 genome from the time point that had the least sacCer3 aligned base pairs by the number of base pairs that aligned to the sacCer3 reference genome from each time point. This was done within each replicate separately. The command awk -F'\t' '{$4=$4*a;print}' a=NF OFS='\t' <$bdg> was used to do this, where NF is the normalization factor, which are specified in Table S1, and <$bdg> is the appropriate time course input BDG file. Appropriate headers were concatenated to the beginning of each normalized BDG file and visualized using UCSC genome browser. For large time course studies such as this one, visualization of select coordinates of tracks is much more time efficient and can be accomplished by uploading the intersected coordinates of BDG files with a BED file of choice using the bedtools intersect command.

**O-GlcNAc-seq peak calling**

Peaks were called by calculating the coordinates that contained 5 times the average base pair sequencing coverage within each time course experiment. This was done for the no feed controls as well. The coordinates of base pairs that satisfied this requirement were then merged if they were within 30 base pairs of each other. This was done with the command awk '{if( $4 > 5X) print}' <$bdg> | cut -f 1,2,3 | bedtools merge -d 30, where 5X indicates 5 times the average base pair sequencing coverage within each time course experiment and <$bdg> indicates the appropriate time course BDG file. For each time point, the peaks that occurred from the no feed controls were eliminated from the time course experiment peaks using the bedtools intersect -v command. This effectively removed false positives from the analysis. Peaks resulting from the intersection of replicate time points were kept using the regular bedtools intersect command. For the Jaccard heatmap analysis, these peaks were compared with each other in all combinations using the bedtools Jaccard command, and the results were plotted using R. For all other analyses using peaks, we took only the peaks at time point 0 for WT and OGA-null and intersected them using the bedtools intersect command. Peaks that were less than 20 base pairs were then expanded from their midpoint by 10 base pairs on each side to a peak length of 20 base pairs. This strategy resulted in 1883 peaks and enabled focus on coordinates that consistently contained O-GlcNAz peaks within both WT and OGA-null and each replicate.

**Time course O-GlcNAc-seq analysis using TDCA**

TDCA version 1.1.0_21-10-2017 was used to analyze data. WT and OGA-null replicates were analyzed together using the spike-in aligned yeast sequences as normalization constants. The TDCA command was run as follows, in a directory containing the appropriate files: tdca -bed <BED file of 5X enriched peaks> -bam <folder containing BAM files from first replicate TC O-GlcNAc-seq> -bam <folder containing BAM files from second replicate TC O-GlcNAc-seq> -g dm3 -dm <Text file with yeast aligned sequences in chronological order with replicate 1 first>. See Table S1 for the -dm file.

**DiffBind heatmap**

DiffBind version 2.4.8 under R version 3.4.0 (2017-04-21). The raw BAM files from both replicates of WT were analyzed together including the no feed control as well as the inclusion of a no feed control from a pilot study performed on the Illumina MiSeq platform. OGA-null was analyzed separately. For each BAM file, the same 1883 loci that were originally computed were used for the DiffBind analysis for every time point.

**Venn diagram of FlyBase genes in different Drosophila stages**
FlyBase genes were curated from UCSC table browser and intersected with peaks from \(O\)-GlcNAz bound loci in S2 cells and pupae from our previous study and in larvae from this study. Redundant gene names were removed. Unix text manipulation tools were used to count the overlap of genes in these different \emph{Drosophila} stages.

**Stage-specific FlyBase gene expression analysis**

Stage-specific \emph{Drosophila} genes categorized into different expression levels were curated from FlyBase. Our null hypothesis was that genes surrounding \(O\)-GlcNAz peaks should exhibit the same distribution of expression levels compared to all genes in both larvae and pupae stages. The two classes used in calculating the chi-squared values were genes in a particular expression category and genes not in that expression category. Chi-squared values were calculated from the number of \(O\)-GlcNAz bound genes (observed) and the total number of genes (expected) for each of the two classes. Therefore, p-values were obtained using 1 degree of freedom.

**Determination of loci that are most greatly influenced by loss of OGA**

Z-scores were calculated from the TDCA turnover values from the WT TC data (both replicates). Loci that exhibited significant high standard scores (\(p < 0.05; z\)-score > 1.96) were considered resistant to removal of \(O\)-GlcNAz by OGA (56/1883 loci and Table S3). We report the overlap of non-redundant FlyBase genes \emph{Drosophila} genes curated from UCSC table browser at each of these 1883 loci (Table S4) using bedtools.

**OGA ChIP-seq data**

Fastq files were aligned to the dm3 genome the Burrows–Wheeler Aligner version 0.7.5a-r405 algorithm. The resulting BAM-format alignment files were sorted, cleared of duplicates and indexed using the SAMtools commands sort, rmdup -s, and index, respectively, using SAMtools version 0.1.19-96b5f2294a. BAM files were converted to BDG files using bedtools. Appropriate headers were concatenated to the beginning of each normalized BDG file and visualized using UCSC genome browser. Peaks were called by calculating the coordinates that contained 5 times the average base pair sequencing coverage within both OGA ChIP-seq experiments (WT and OGA-null). Peaks within the OGA-null experiment were considered false positives and were subtracted from the peaks called in the WT experiment using the bedtools intersect -v command to yield the final OGA ChIP-seq peaks. The OGA ChIP-seq peaks were then intersected with FlyBase genes curated from UCSC table browser yielding 1846 non-redundant genes bound to OGA or OGA containing complexes. Venn diagrams of the OGA containing genes and \(O\)-GlcNAz bound genes in S2 cells and pupae from our previous study and in larvae from this study were created using Unix text manipulation tools.

**External data and accession numbers**

Next generation sequencing data was submitted to NCBI GEO under accession numbers: GSE124785.
Supplemental References:

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