Supporting Information

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SI Materials and Methods

Constructs. The expression constructs were made by cloning the appropriate gene sequences (GAL4/mof, GAL4/msl2, msl2 and GAL4/mof1) into the KpnI site of the pHT4 expression vector with the Hsp70 promoter to drive expression (1), and the *rosy*$^+$ gene as a transformation marker. The GAL4/mof sequence was PCR-amplified from a plasmid provided by Peter Becker, and the GAL4/msl2 constructs were made by fusing the appropriate sequence amplified from cDNA or plasmids to the GAL4 (+ linker) sequence from the original GAL4/mof construct. Male-specific lethal 2 (MSL2) was PCR-amplified and inserted into pHT4. The correct orientation and absence of mutations were verified by sequencing. The constructs were P-element transformed into *rosy*$^-$ flies and transformants were subsequently placed in a *white* background to allow analysis of the effects of expression in both sexes when crossed to the *miniwhite*-containing reporter lines (Fig. S8).

The target reporter plasmid was constructed by placing five copies of the upstream activation sequence for GAL4, PCR-amplified from the pUAST vector (2) 5′ to a minimal promoter in the CaspeR4 vector (3). Five autosomal reporter lines (M9, M1, M5, M7, and M14) and four X-chromosomal reporter lines (M30, M4, M54, and M76) were obtained. Two GAL4-MOF transgenes were used: one on the second chromosome and the other on the third. One GAL4-MSL2 transgene was used, which is located on chromosome 2. Five transgenes expressing MSL2 in females (and males) were recovered at sites on the autosomes. The one used was located on chromosome 2.

Genetic Crosses. Males of the genotypes (GAL4 fusion protein or MSL2)/SM6a (or MKRS) were crossed with homozygous reporter females, and the progenies containing the transgenic protein were further crossed to each other to obtain the homozygous reporter lines.

We performed crosses to combine GAL4-MOF with the *mof1* mutation (Fig. S1*A*). Because *mof1*, a hemizygous lethal mutation in males, is balanced by *Basc* (*Basc*/*mof1*), with an easily identified phenotype, a smaller bar eye shape in males and kidney-shaped eyes in females (Fig. S1 *B*, 1 and 3), we made the cross using *Basc*/*mof1* as the maternal parent and homozygous GAL4-MOF (four copies) fusion protein stock (*y w*; MOF/MOF; MOF/MOF) as the paternal line. The genotypes of male siblings from this cross are shown in Fig. S1. If the GAL4-MOF fusion protein cannot rescue the *mof1* mutation, only the phenotype of *Basc* will be observed, because the larvae (*mof1*/y; MOF/+; MOF/+) will die because of the lethality of *mof1* mutation. However, we found normal eyed males in the next generation (Fig. S1*B*). The same results were found from different stocks derived from three independent recombinants of *y w* and *mof1*. The recovery of viable *mof1* males illustrates that the GAL4-MOF transgene is capable of supplying MOF function for recovery of adult flies.

We performed crosses to combine GAL4-MSL2 with the *msl2* mutation (Fig. S1*C*). Because the *msl2* mutation is lethal in males, which is usually balanced by CyO, there is only the CyO phenotype in males. We followed the diagrammed strategy, and recovered 39 individual flies from which to amplify a stock that potentially combines the transgene and the *msl2* mutation. We screened all these lines using FISH, and kept 17 stocks with two signals of *msl2*: one is the transgene and the other one is the endogenous gene (mutant or wild-type). Among these GAL4-MSL2 transgenic stocks, 10 stocks had both males and females with non-CyO wings at normal temperature and 7 included nonCyO females but only Curly males, which is indicative of the presence of the *msl2* mutation. Heat shock was performed on vials from the latter seven stocks twice a day for 30 min each. After the process of heat shock, we found that non-CyO adult males appeared in the vials containing the transgene but not in the control with only the *msl2* mutation. This result indicates that the GAL4-MSL2 fusion is capable of supplying MSL2 function for recovery of adult flies.

Mutations, genes and chromosomal balancers are described in Flybase (http://flybase.org). All flies were cultured on cornmeal dextrose medium at 25 °C. For RNAseq, ectopically expressing MSL2 females and males were obtained by using a transformed P-element *msl2* construct with a *miniwhite* marker gene, [(w^+) *H83M2-6I*] (4). The *y w*/Y; (w^+) *H83M2-6I*/*TM6, Hu Tb e* males were crossed with *y w*; *SM6*/*Gla*; *TM3*/*MKRS* females. In the F1 progeny, the males *y w*/Y; (w^+) *H83M2-6I*/*TM3* were crossed to y w;+/+;+/+ females. From this cross, four different classes of progeny were produced, two of which with the presence of (w^+) *H83M2-6I* based on the phenotype of w^+; the other two without (w^+) *H83M2-6I* have white eye color.

Eye Color. Because we use *miniwhite* as the reporter gene, we could easily evaluate the expression level of *white* based on eye colors of the adult flies. Virgins were collected and aged 1 d before photography. The eye pictures were taken using a digital camera with a fluorescent dissecting microscope in white light and blue background. The same intensity of white light and same zoom were used for all pictures.

Immunostaining and FISH. The immunostaining with antibodies was combined with the FISH technique (5). Salivary glands from third-instar larvae were dissected and immunostained with antibodies as described (6). Chromosomes were probed with anti-MSL2, anti-MOF and anti-H4K16Ac (Santa Cruz) antibodies at a dilution of 1:100, and probed with an appropriate conjugated secondary antibody at a 1:200 dilution. Slides were fixed in 10% formaldehyde for 10 min after washing the slides twice in PBS following secondary antibody probing. DNA probes based on the exon 3 template sequence of the *white* gene using nick translation (7) with Texas red CTP (red) or Alexa Fluor 488-dUTP (green) were hybridized with chromosome preparations that were denatured for 5 min at 100 °C, with subsequent hybridization and kept at 55 °C overnight (5). The next day, the wash was carried out with prewarmed 2× SSC (55 °C) buffer (8). Chromosomes were mounted in Vectashield mounting medium containing DAPI and viewed with a Zeiss fluorescence microscope.

RNA Preparation and Northern Blot. Total RNA was isolated using TRIZOL Reagent (Invitrogen) and separated on formaldehyde-agarose gels (1.5%) (9) at 20 µg per lane. Gels were run at 50 V for about 16 h. The samples were transferred to a nylon membrane (Ambion) by the Turbo-Blotter downward transfer system (Schleicher & Schuell) using 20× SSC (3 M NaCl, 300 mM sodium citrate) (9). Then the membranes were cross-linked under UV and hybridized with the antisense RNA probes (*white* and rRNA) under the conditions described in previous studies (6, 9, 10) with the ULTRAhyb Ultrasensitive Hybridization Buffer (AM8669). Three washes at 75 °C were applied to the membranes with buffer (0.2× SSC and 0.05% SDS). The membranes were subjected to autoradiography with Kodak XAR film or to a Fujifilm Fluorescent Image Analyzer FLA-2000 to detect the mRNA levels. The signals were measured using the Fujifilm

Image Gauge v3.3 program. The expression levels of all genotype samples were calibrated to the level of the individual reporter in females, which was set to 1.0.

The P^{32}-labeled antisense probes produced by the MAXIscript T7 Kit with Manual (AM1312M) from Ambion (9) were purified by using the column composed of Sephadex G-50 and hybridized with the blots to measure the targeted gene, *miniwhite*, and the endogenous control gene, rRNA, with the proper concentration.

ChIP. For ChIP experiments, 150–200 mg of flies for each sample were homogenized, which is sufficient for four ChIP experiments, with 5 mL of buffer A1 + 1.8% formaldehyde using a Potter homogenizer and then Douncer with type A (Tight) pestle (5, 11). The detailed procedures were described previously (5). The homogenates were centrifuged at 4 °C after adding glycine. Buffer A1 was added to wash the pellet, followed by a wash by lysis buffer without SDS. The pellets were dissolved in 1 mL of lysis buffer + SDS.

The DNAs were sheared by the use of a sonicator. After centrifugation the supernatants were transferred to new tubes, which were repeated several times. The solutions for each sample were loaded into centricon YM-10 columns and washed by ChIP dilution. Eighty microliters of Protein G agarose (Protein A applied for some antibodies) suspension was added to the amounts of chromatin corresponding to 150–200 mg of biological materials for precleaning. The starting materials (150–200 mg) were separated into four different chromatin samples. The primary antibodies of interest (MSL2 and H4K16Ac) were added into each ChIP solution and incubated at 4 °C on a rotating wheel overnight with the mock samples without antibodies. Next, 60 μL of Protein G agarose suspension were added into the previous solutions to be incubated for 4 h at 4 °C on a rotating wheel. The beads were collected and washed with a low salt-wash buffer, a high salt-wash buffer, a lithium chloride buffer, and TE twice. The immune complexes were eluted by adding elution buffer. The samples were reverse cross-linked and incubated overnight. The DNA was extracted by using phenol/chloroform. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the solutions from the previous step. The chloroform extractions were also performed twice via adding 500 μL of chloroform into the tubes. Glycogen (20 mg/mL) and 1/10 volume (40 μL) of 3 M sodium acetate pH 5.0 were added to the tubes followed by mixing well, which was followed by a wash using ethanol. The visible white pellets were washed with 70% ethanol and resuspended in 20 μL of water after drying in air. One microliter of DNA was used in the real-time PCR reactions.

The real-time PCR reactions were processed by using the Power SYBR Green PCR Master Mix Kit (ABI). The primers for different sequences of the *white* reporter were labeled as: mw-left, mw-middle, and mw- right. The left primer of mw-left is CTGCGTCCGCTATCTCTTTC and the right one is GAGAGGAGTTTTGGCACAGC, with a 199 bp DNA product; the left primer of mw-middle is CGCTTCTGATCTGCGATGAG and the right one is CTGGGAGTGCCCAAGAAA, with a 199 bp DNA product; the left primer of mw-right is TGGCGGCTTCTTCTTGAACT and the right one is GTGTTCGACGATGTGCAGCTA, which produces a 150-bp product. The 207-bp β-tubulin amplified from the left primer (AGCTCAGCACCCTCTGTGTAAT) and the right one (AGCTGGAGCGCATCAATGTGTA) was used as an internal control for each ChIP reaction to normalize the amount of immunoprecipitated DNA based on the different Ct value. Each ChIP sample was normalized to the mock input control. The individual female reporter samples were applied as the calibrator in all comparisons.

PCR amplification and fluorescence detection were performed in a 25-μL final volume containing 100 ng of each primer and 1 μL DNA product from immunoprecipitation by using the Applied Biosystems 7300 real-time PCR System with a thermocycler profile of 50 °C for 2 min, 95 °C for 10 min, and followed 40 cycles of 95 °C for 15 s, 60 °C for 1 min. Each DNA sample was processed three times in 96-well plates. The relative quantification (RQ) for each pair of primers was measured based on the ΔCt analysis according to the instructions from the manufacturer (Applied Biosystems 7300 Real-time PCR system, sequence detection software v1.3.1).

Illumina TruSeq RNA Library Preparation and Sequencing. Libraries were constructed following the manufacturer's protocol with reagents supplied in Illumina's TruSeq RNA sample preparation kit (#RS-930-2001). Briefly, the poly-A containing mRNA is purified from total RNA, RNA is fragmented, double-stranded cDNA is generated from fragmented RNA, and the index containing adapters are ligated to the ends. Total RNA (2 μg) was first incubated in a thermal cycler for 5 min at 65 °C in a total volume of 50 μL in a 96-well PCR plate. The plate was removed and incubated an additional 5 min at room temperature allowing RNA to bind to the poly-T oligo-attached magnetic beads. Beads were washed by placing the PCR plate on the magnetic stand at room temperature for 5 min and discarding supernatant. Bead Washing Buffer (200 μL) was added and returned to the magnetic stand for 5 min. Supernatant was removed and discarded. The plate was removed from the magnetic stand and Elution Buffer (50 μL) was added to each well. The plate was incubated at 80 °C for 2 min and then placed at room temperature. RNA was rebound to beads with the addition of Bead Binding Buffer (50 μL) and incubated for 5 min at room temperature. Beads were washed as previously described. First-strand cDNA synthesis was performed by adding the Elute, Prime, Fragment Mix (19.5 μL) to each well. The mixture was incubated for 8 min at 94 °C. The plate was placed on the magnetic stand at room temperature for 5 min. From the plate, 17 μL of the fragmented and primed RNA was transferred to a new PCR plate. First Strand Master Mix and SuperScript II mix (8 μL) was added to each well and gently mixed. Incubation was performed in a thermal cycler with the program: 25 $°C^{(10:00)}$+42 $°C^{(50:00)}$+70 $°C^{(15:00)}$. Second-strand cDNA synthesis was performed by the addition of Second Strand Master Mix (25 μL) to each well. Mixture was incubated at 16 °C for 1 h. Aline PCRClean beads (90 μL) were added to each well containing 50 μL of double-stranded cDNA. The plate was incubated at room temperature for 15 min and placed on the magnetic stand for 5 min. The supernatant (135 μL) was removed and discarded. Each well was washed by addition of 200 μL of 80% EtOH, incubation at room temperature for 30 s, and removal of supernatant. Wash steps were repeated once and plate was allowed to dry on a magnetic stand for 15 min. Resuspension Buffer (52.5 μL) was added to each well. The plate was returned to the magnetic stand at room temperature for 5 min and 50 μL of supernatant was transferred to a new PCR plate. Fragment overhang ends were converted to blunt ends by the addition of the End Repair Mix (40 μL) to each well and incubation at 30 °C for 30 min. Aline PCRClean beads (160 μL) were added to each well, which contained 100 μL of End Repair Mix. The plate was incubated at room temperature for 15 min. Supernatant (127.5 μL) was removed and discarded. Each well was washed with 80% EtOH as previously described. The dried pellet was resuspended in Resuspension Buffer (20 μL) and 15 μL was transferred to a new PCR plate. The 3′ ends of the fragments were adenylated with the addition of A-Tailing Mix (12.5 μL) to each well and then incubated for 30 min at 37 °C. DNA Ligase Mix (2.5 μL) and a single RNA Adapter Mix (2.5 μL) were added to each well and then incubated for 10 min at 37 °C. The ligation reaction was stopped with the addition of Stop Ligase Mix (5 μL). Aline PCRClean beads (42 μL) were added to each well. The plate was incubated at room temperature for 15 min. Supernatant (79.5 μL) was removed and discarded. Each well was washed with 80% EtOH as previously described. The

dried pellet was resuspended in Resuspension Buffer (52.5 μL) and 50 μL was transferred to a new PCR plate. Aline PCRClean beads (50 μL) were added to each well. The plate was incubated at room temperature for 15 min. Supernatant (95 μL) was removed and discarded. Each well was washed with 80% EtOH as previously described. The dried pellet was resuspended in Resuspension Buffer (22.5 μL) and 20 μL was transferred to a new PCR plate. DNA fragments were enriched by adding PCR Primer Mixture (5 μL) and PCR Master Mix (25 μL) to each well. PCR amplification was performed as follows: 98 °C$^{(0:30)}$+[98 °C$^{(0:10)}$+60 °C$^{(0:30)}$+72 °C$^{(0:30)}$] × 15 cycles +72 °C$^{(5:00)}$. The amplified cDNA construct was purified by addition of Aline PCRClean beads (50 μL) to each well. The plate was incubated at room temperature for 15 min. Supernatant (95 μL) was removed and discarded. Each well was washed with 80% EtOH as previously described. The dried pellet was resuspended in Resuspension Buffer (32.5 μL), incubated at room temperature for 2 min, and then placed on the magnetic stand for 5 min. Supernatant (30 μL) was transferred to a low binding microcentrifuge tube for storage. The final construct of each purified library was evaluated using the BioAnalyzer 2100 automated electrophoresis system, quantified with the Qubit flourometer using the quant-iT HS dsDNA reagent kit (Invitrogen), and diluted according to Illumina's standard sequencing protocol for sequencing on the HiSEq. 2000.

RNA Sequencing Analyses. We used TopHat (12) to map the RNA-Seq reads of each replicate of each sample to the *Drosophila melanogaster* genome (dm3) in the University of California at Santa Cruz genome browser (13) in conjunction with the RefSeq genome reference annotation (14). The threshold of mismatch was set to 2. Overall, ~80% of reads of each replicate were mapped to genes. The reads that were mapped to a unique location on the genome were used to calculate the RNA counts per gene. We used both our in-house tool (MULTICOM) and a public tool (HTseq) (14) to calculate read counts of the genes in a replicate according to the genome mapping outputs and the RefSeq genome reference annotation (15). The counts of the genes in a replicate calculated by MULTICOM or HTseq were normalized by dividing them by the total number of uniquely mapped reads in the replicate. The normalized count of a gene was an indicator of the relative expression level of the gene in the replicate. The normalized counts of a gene in multiple replicates of a sample were further averaged and used as the measure of the relative expression level of the gene in the sample.

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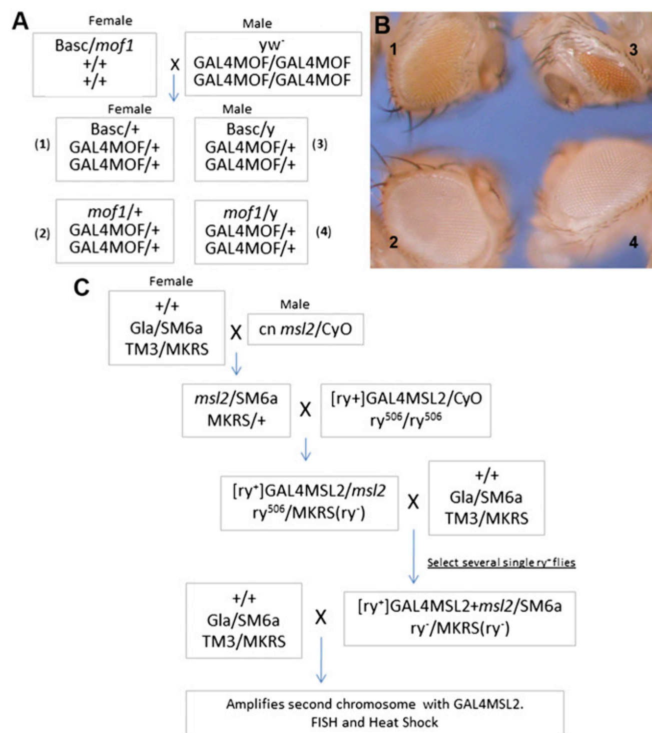

Fig. S1. Complementation experiments for GAL4-MOF and GAL4-MSL2 constructs. (*A* and *B*) Complementation experiment for the GAL4-MOF construct. (*A*) Females with heterozygous *mof1* mutation (*mof1*/Basc), which has reduced acetylase activity and is lethal in males, were crossed to males with autosomally inserted GAL4-MOF constructs (y w^-; GAL4MOF/GAL4MOF; GAL4MOF/GAL4MOF). The genotypes of the F1 progenies were listed as 1, 2, 3, and 4. (*B*) The eye colors of the adult flies of the F1 progenies. Because of the lethality of the *mof1* mutation in males, the survival of genotype 4 to adult males demonstrates that the GAL4-MOF fusion protein is capable of supplying the MOF function. This conclusion is supported by the facts that targeting reporters increases H4 acetylation and that in males brings the whole MSL complex to reporters (see main text). The table below shows results of crosses shown in panel *A* of the figure. Bar eye is the phenotype of Basc/Y; normal eye is the phenotype of *mof1*/Y; GAL4-MOF; GAL4-MOF. Ratio is the comparison of normal eye (genotype 4) vs. Bar eye (genotype 3).

Cross	Bar eye male	Normal eye male	Ratio
Basc/*mof1* X y w; MOF/MOF;MOF/MOF	88	18	20.5%
Basc/*mof1* X Basc/Y	97	0	0

(*C*) Complementation experiment for GAL4-MSL2 construct. The genetic strategy to combine the *msl2* mutation (homozygous lethal in adult males) with the GAL4-MSL2 construct, which also carries the selection marker ry^+. When the combined lines were stabilized with the balancer chromosome (SM6a), the individual ry^+ flies were selected to amplify a stock. Heat-shock experiments were performed to induce the expression of GAL4-MSL2 using lines with two FISH signals: one of the endogenous *msl2* gene and one from the transgenic signal. Homozygous *msl2* and GAL4-MSL2 male adult flies obtained after the heat shock demonstrate that the GAL4-MSL2 fusion protein can perform the functions of MSL2. This conclusion is supported by the facts that GAL4-MSL2 coats the X chromosomes in females, increases the level of H4 acetylation and brings the whole complex to autosomal reporters concordant with increased acetylation (see text). The table below shows results of crosses shown in panel *C* of the figure. Curly wing is the phenotype of the SM6 balancer chromosome. Ratio is the comparison of homozygous (non-Curly) vs. one-half of heterozygous (Curly) males.

Cross	Curly wing males	Non-Curly wing males	Ratio
GAL4-MSL2+*msl2*/SM6	301	28	18.6%
msl2/SM6	224	0	0

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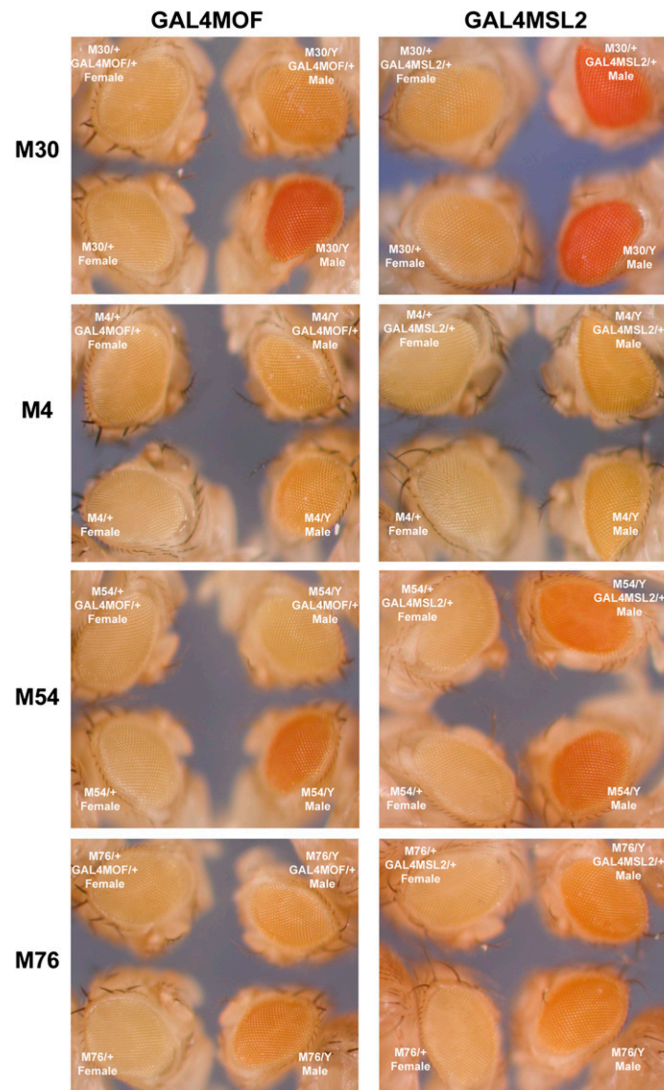

Fig. S2. X-linked reporters with GAL4-MOF and GAL4-MSL2 constructs. The X-linked reporters (M30, M4, M54, and M76) were combined with GAL4-MOF (*Left*) and GAL4-MSL2 (*Right*). In each comparison, the respective reporter genotypes are listed at the bottom; the female (*Lower Left*) and the male (*Lower Right*) and the GAL4 combined genotypes are shown at the top; female (*Upper Left*) and male (*Upper Right*). GAL4-MOF targeted reporters showed an increased expression in females and a reduced expression in males with all of the reporters (M30, M4, M54, and M76). GAL4-MSL2 targeted reporters do not induce dosage compensation in females (M30, M4, M54, and M76). All of the adult flies were collected at similar ages and the comparison pictures taken under the same conditions.

Fig. S3. Different copy number of the GAL4-MOF construct with heterozygous X-linked reporters. Different copy numbers of GAL4-MOF constructs were combined with the heterozygous X-linked reporters (M30/+; M4/+; M54/+, and M76/+) to test the phenotypic changes with increasing copy number of GAL4-MOF. In each comparison, females are on the *Left* and males on the *Right*. Individual reporter flies were listed on the *Upper Left*. One copy of GAL4-MOF with the reporter is at the *Upper Right*. The image of two copies of GAL4-MOF with the reporter is at the *Lower* area. In females, the expression level of the reporters was elevated with increasing copy number of GAL4-MOF, but in males, the expression level of the reporters is reduced when the copy number of GAL4-MOF is increased. Both of these results were consistent with the previous results that the GAL4-MOF fusion protein induces the up-regulation in females and down-regulation of the X-linked reporter gene in males. All of the adult flies were collected at similar ages and comparison pictures taken under the same conditions.

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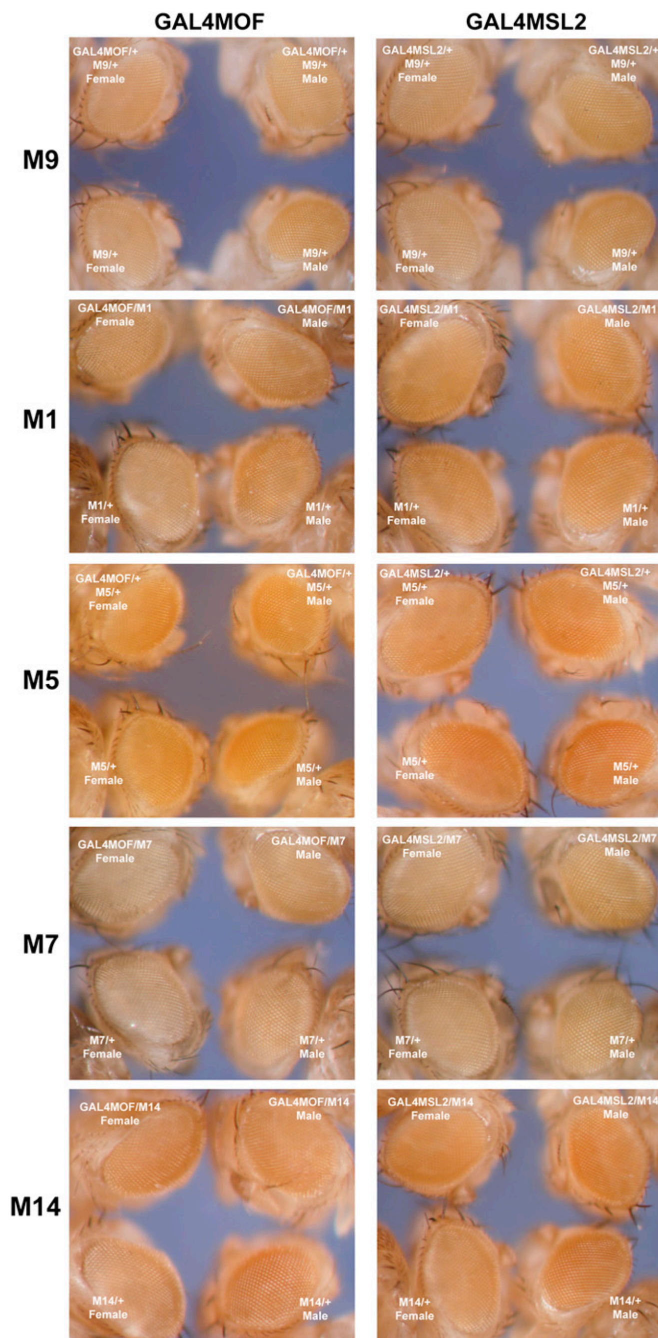

Fig. S4. Autosomal reporters with GAL4-MOF and GAL4-MSL2 constructs. The autosomal reporters (M9, M1, M5, M7, and M14) were combined with the GAL4-MOF (*Left*) and GAL4-MSL2 (*Right*) constructs. In each comparison, the respective reporter genotypes are listed at the *Lower area*; the female (*Lower Left*) and the male (*Lower Right*) and the GAL4 combined flies are shown in the *Upper* area; female (*Upper Left*) and male (*Upper Right*). GAL4-MOF targeted reporters show an increased expression in females (M9, M1, M5, and M14) and a reduced expression in males (M9, M1, and M14). GAL4-MSL2 targeted reporters do not induce dosage compensation in females (M9, M1, M5, and M14). The eye color of the M7 reporter is too light to be distinguished in the comparisons. All of the adult flies were collected at similar ages and comparison pictures taken under the same conditions.

Fig. S5. GAL4-*mof1* fusion protein with reporter genes. (*A*) Constructs used in the GAL4-UAS (upstream activating sequence) system. GAL4-DBD (DNA binding domain) fused with *mof1* protein is inserted in the expression plasmid pHT4 with the Hsp70 promoter; the 5× UAS sequences are followed by the *miniwhite* reporter gene. (*B*) Expression levels of the X-linked reporter gene, M30, with GAL4-*mof1* fusion protein. Samples: 1, M30 female ($n = 6$); 2, M30 male ($n = 6$); 3, M30; GAL4-*mof1*(1) female ($n = 4$); 4, M30; GAL4-*mof1*(1) male ($n = 4$). n, the number of replicates for each sample. The error shown is the SD of the independent replicates. A t test is applied to each comparison and P value is shown. Normal female levels are set at 1.0. Target gene (*miniwhite*): the upper bands are from P^{32}-labeled *white* probe and endogenous control gene (rRNA) from Northern blot. Comparisons of eye colors (*miniwhite*) from the individual reporter

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stocks (M30) and the combined stocks including GAL4-MOF1 fusion protein and M30. (*C*) Expression levels of the autosomal reporter gene, M9, with GAL4-*mof1* fusion protein. Samples: 1, M9 female (n = 5); 2, M9 male (n = 5); 3, M9; GAL4-*mof1* (1) female (n = 4); 4, M9; GAL4-*mof1* (1) male (n = 4). n, number of replicates for each sample. Error: SD of independent replicates. A t test is applied to each comparison and P value is shown. Target gene (*miniwhite*), the upper bands are from P^{32}-labeled *white* probe and endogenous control gene (rRNA) from Northern blot. Comparisons of eye colors (*miniwhite* reporter) from the individual reporter stocks (M9) and the combined stocks with GAL4-MOF1 fusion protein and M9. (*D*) Immunolocalization and FISH of polytene chromosome from third instar larvae of females (*Left*) and males (*Right*) in the M9 reporter. The red channel (*Right Top* box) is the signal from FISH; the green channel (*Right Middle* box) is the signal from the respective antibody against the noted protein. (Scale bars, 10 μm.)

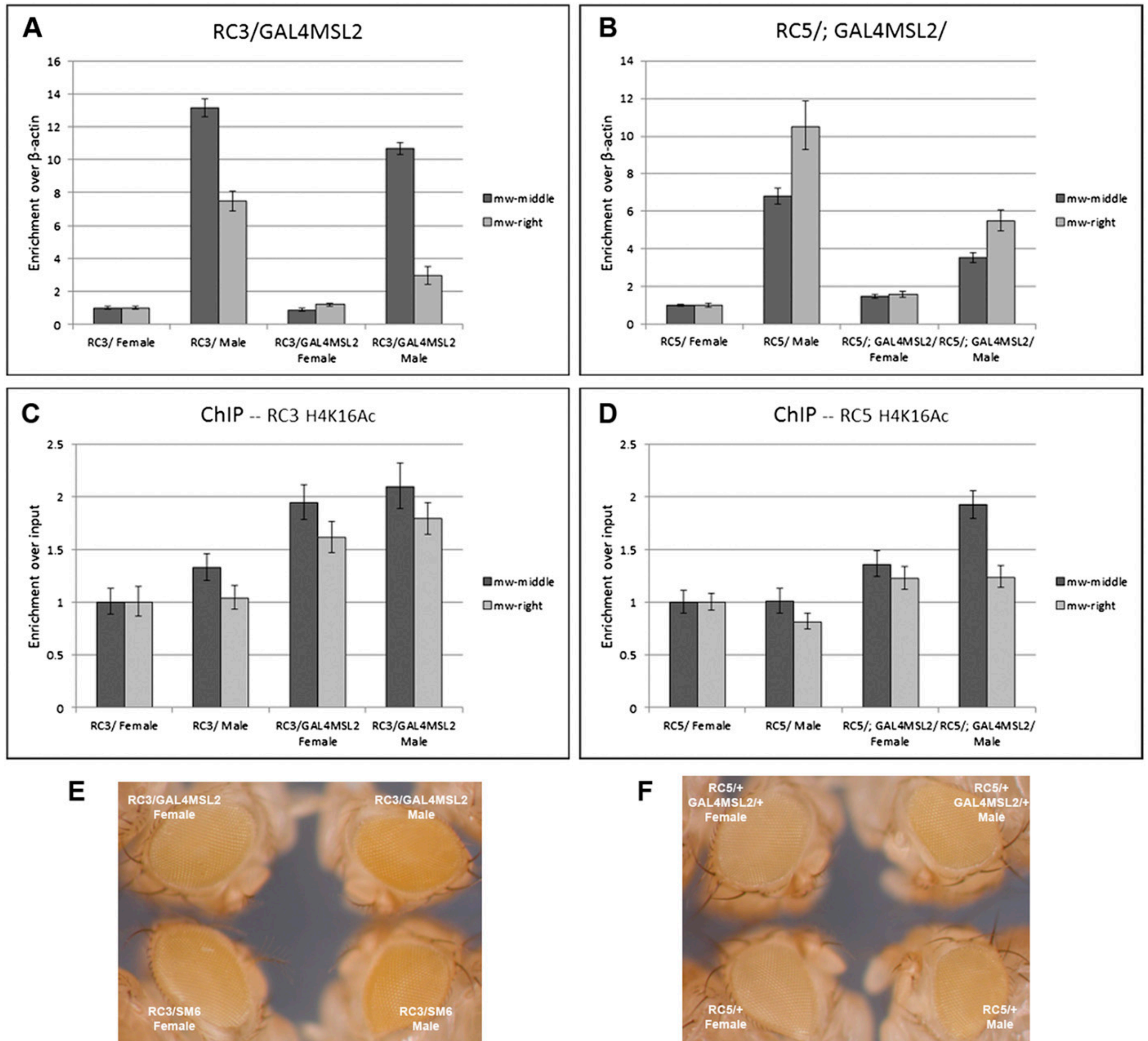

Fig. S6. RC3 and RC5 reporter genes targeted with GAL4-MSL2. (*A* and *B*) Expression levels of the *miniwhite* reporter genes from Prestel et al. (1). (*A*) RC3 reporter gene on chromosome 2; (*B*) RC5 reporter gene on chromosome 3. The quantitative real-time PCR was applied to analyze their expression levels with the exogenous input human β-actin as the control. For all determinations, n = 3. (*C* and *D*) ChIP binding pattern of H4K16Ac protein on the reporter genes. The real-time data were analyzed with Applied Biosystems 7300 system SDS software with calculation of the 95% confidence interval. Bar: positive represents the maximum RQ; negative represents the minimal RQ. For all determinations, n = 3. (*E* and *F*) Comparisons of eye colors in females and males from the individual reporter stocks (RC3 and RC5) and the combined stocks with the targeting GAL4-MSL2 fusion construct.

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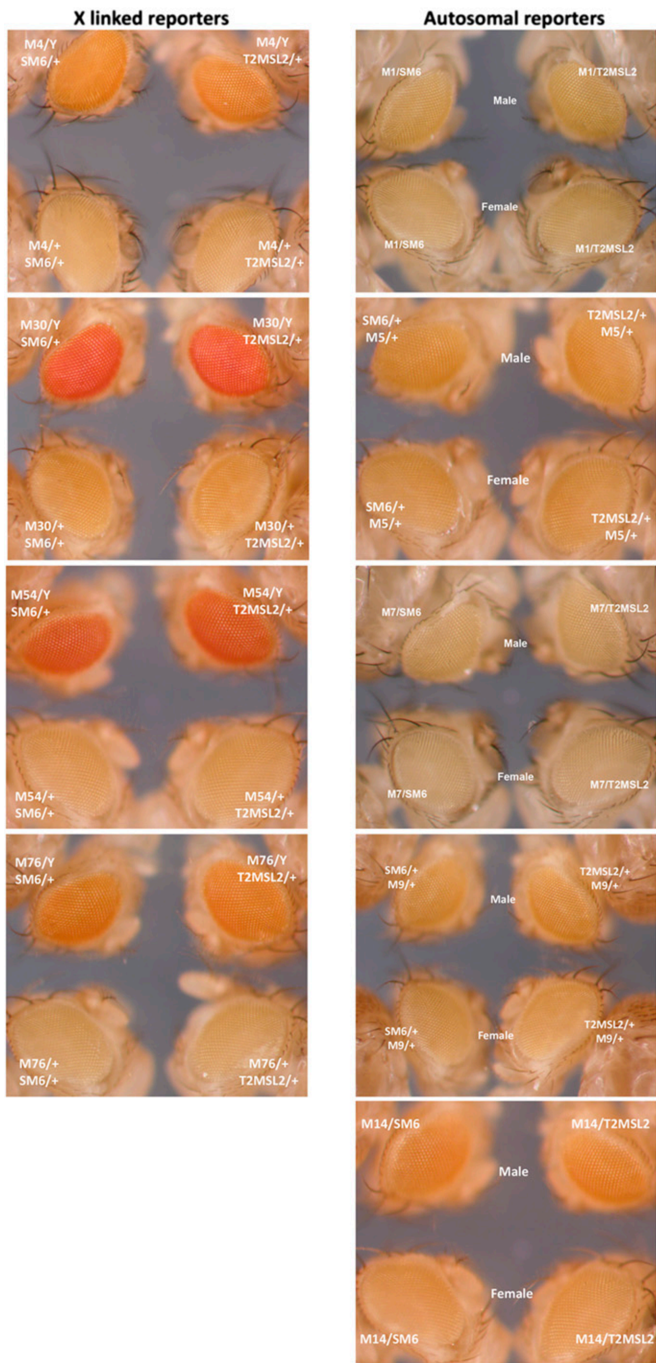

Fig. S7. X and autosomal reporters combined with ectopically expressed MSL2. The X-linked (M30, M4, M54, and M76) and autosomal (M9, M1, M5, M7, and M14) reporters were combined with a transgene ectopically expressing MSL2 (T2MSL2) (*Right*) constructs. Genotypes are labeled. For the induction of dosage compensation, the one-copy X-linked female reporter should be converted by MSL2 to the intensity of the one-copy normal male phenotype, but none of the reporters exhibited this effect. All of the adult flies were collected at similar ages and comparison pictures taken under the same conditions.

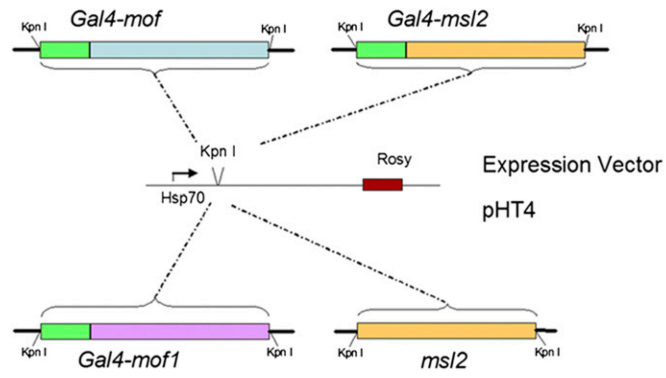

Fig. S8. Expression constructs. Diagrammatic representations of constructs made in this study and their placement in the transformation vector. See description in *SI Materials and Methods* for further details of construction.