Supporting Information

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SI Experimental Procedures

Chromatin Preparation. A modification of the protocol described in ref. 1 as used to prepare cross-linked chromatin from embryonic progeny from parents of wild-type (*Ore R*) flies at 25 °C. One- to 3.5-h embryos were collected, dechorionated in 50% bleach, washed in PBS (150 mM sodium chloride (NaCl), 10 mM sodium phosphate, pH 7.6) and 0.1% Triton X-100 (PBST), and then ground in homogenization buffer (50 mM Hepes at pH 7.6, 60 mM potassium chloride, 0.25 M sucrose, protease arrest) (786-108; G Biosciences). The homogenate was first clarified by centrifugation at 500 × *g* for 6 min before the addition of formaldehyde to 1% and a 15-min rotation. Glycine was added to a 250-mM final concentration followed by a 6-min spin at 3,000 × *g*. Cross-linked chromatin was then washed three times in PBS, resuspended in RIPA buffer (10 mM Hepes, pH 7.6, 1 mM ethylene diamine tetraacetic acid (EDTA), 0.5 mM ethylene glycol tetraacetic acid (EGTA), 140 mM NaCl, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS), and sonicated to an average length of ~500 bp.

Chromatin Immunoprecipitations. Clarified chromatin from ~120 μL of starting embryos was split into two and incubated with either anti-Dm or preimmune serum (1) (0.225 μL each). Washes were performed as in Alekseyenko et al. (2). Immunoprecipitated material was eluted from the beads by incubation at 37 °C for 1 h in 500 μL TE (1 mM EDTA, 10 mM Tris, pH 8.0) containing 0.5% SDS and proteinase K (0.1 mg/mL), followed by 12 h at 65 °C after the addition of NaCl to 0.3 M and SDS to 1%. Samples were then extracted twice with phenol/chloroform before ethanol precipitation in the presence of glycogen.

Protocol for Quantitative Real-Time PCR. The iCycler iQ real-time PCR detection system (Bio-Rad) was used to quantitate sequences in the precipitated DNA from each ChIP fraction. Primer pairs used for PPAN and Nop60b have been described (3). *Sxl* primers for −96 were 5′-GCC ACG TTC CAC CTT TCG GC-3′ and 5′-TGC GCG TGC CAG TCG CGT GG-3′ and +1,171 5′-GTG GTT ATC CCC CAT ATG GC-3′ and 5′-GCC AAA GAG GTA TGG GTA GC-3′. To calculate relative enrichment of immune over nonimmune serum, the amount in the ChIP as a percentage of total was used. PCR amplification was performed in triplicate in 20 μL SYBR Green qPCR SuperMix (Bio-Rad) on two biological replicates of each ChIP fraction. Dissociation curve analysis was performed at the end of 40 cycles, and quantification was carried out by Bio-Rad comparative C_T methodology with standard curves constructed for each primer pair with a serial dilution of template DNA, which had PCR efficiencies of 87–97%. An unpaired *t* test was used to determine significance of differences between two samples using GraphPad.

Embryo Staining. Collected embryos 0- to 4-h old, paraformaldehyde fixed, were stained with anti-Sxl monoclonal m114 and m5 as described in ref 4. Embryos 0- to 2-h old, collected and then aged 5 h at 25 °C were fixed and stained for β-galactosidase as described in ref 5.

Fly Stocks. Most fly stocks are referenced in FlyBase. Stocks used were as follows: w(1)sc(3-1)/FM7c; y(1)w(1)Df(1)Sxl(7B0)/FM7c; y(1)w(1)sis-a(1)/FM7c; dpn(7)/CyO; Sxl(M4)/FM7c; w(1118); P{Sxl Pe3.0 lacZ}; w(1118);P{Sxl Pe0.4lacZ}; w(1118);P{Sxl Pe0.4GOFlacZ}; w(1)snf(1621); Df(1)N71/FM6, y[31d] sc[8] dm [1] B[1]; Df(1)Pgd-kz/FM6, dm[+]; dm(1)/FM7c; w(1)dm(P0)/FM7c; dm(P1)/FM7c; hairy-GAL-4/TM3ser.

1. Maines JZ, Stevens LM, Tong X, Stein D (2004) Drosophila dMyc is required for ovary cell growth and endoreplication. *Development* 131:775–786.
2. Alekseyenko AA, Larschan E, Lai WR, Park PJ, Kuroda MI (2006) High-resolution ChIP-chip analysis reveals that the Drosophila MSL complex selectively identifies active genes on the male X chromosome. *Genes Dev* 20:848–857.
3. Teleman AA, Hietakangas V, Sayadian AC, Cohen SM (2008) Nutritional control of protein biosynthetic capacity by insulin via Myc in Drosophila. *Cell Metab* 7:21–32.
4. Bopp D, Bell LR, Cline TW, Schedl P (1991) Developmental distribution of female-specific Sex-lethal proteins in Drosophila melanogaster. *Genes Dev* 5:403–415.
5. Keyes LN, Cline TW, Schedl P (1992) The primary sex determination signal of Drosophila acts at the level of transcription. *Cell* 68:933–943.

Fig. S1. Sxl expression depends on *dm*. Progeny of wild-type parents or of dm^1/+ mothers crossed to dm^1/Y fathers were probed with Sxl antibody. Two equal classes of embryos are seen in wild type: no Sxl (male) and (*A*) a high uniform level of Sxl (female). As in wild type, approximately one-half (48%) of the embryos from the *dm* cross were negative (male). The Sxl^+ embryos fell into three classes: class I, 13% had a uniform but intermediate level of Sxl compared to wild type; class 2, 15% had an intermediate level of Sxl but the distribution was patchy; and class 3 (*B*), 24% had a low level of Sxl and the distribution was patchy. On the basis of the frequency of the different classes (and the classes seen when WT females are mated to dm^1 males), we believed that embryos in classes 1 and 2 are dm^1/+, whereas embryos in class 3 are dm^1/dm^1.

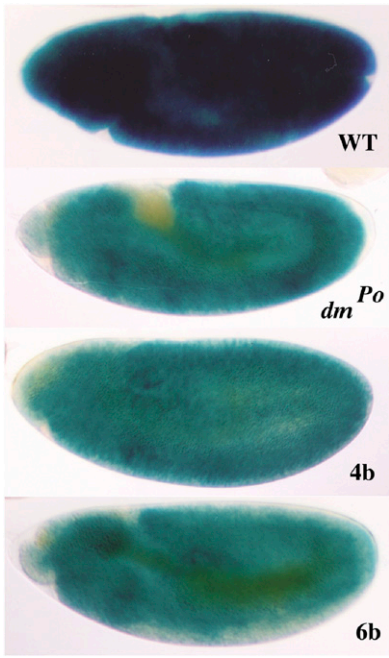

Fig. S2. Sxl^{M4} suppresses the female lethal effects of dm^{P0} by a mechanism that bypasses the *dm*-dependent defects in *Sxl-Pe* activity. The *hopper* transposon insertion into the male exon in Sxl^{M4} mutation induces "constitutive" female-specific splicing of the late *Sxl-Pm* premRNAs independent of Sxl protein. However, the mutation has no effect on the expression or splicing of the *Sxl-Pe* mRNAs. Thus, if the female lethal effects of *dm* arise, as we suppose, because the dMyc protein is required to activate *Sxl-Pe*, Sxl^{M4} must suppress these female lethal effects by a mechanism that bypasses the defects in *Sxl-Pe* activity. To confirm that this is the case, we examined *Sxl-Pe* activity in dm^{Po}/dm^{P0} Sxl^{M4} female embryos. As shown for two of the recombinants, 4b and 6b, the defects in *Sxl-Pe* activity evident in dm^{P0}/dm^{P0} are *still* present in the recombinants, even though they are fully female viable.

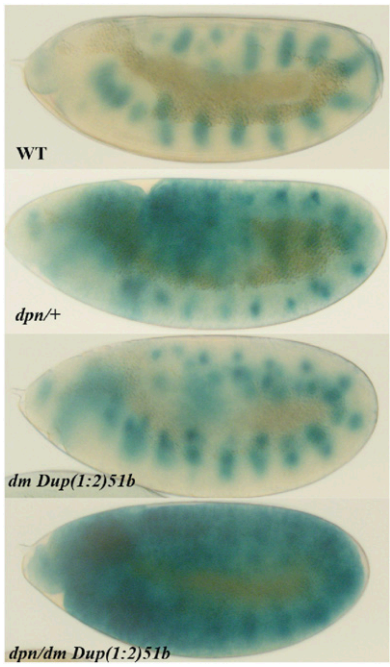

Fig. S3. Effects of changing relative dose of *dpn* and *dm* on *Sxl-Pe*$_{5xDbox}$ *Sxl-Pe*$_{5xDbox}$ drives only a very low level of LacZ expression in wild-type females. In embryos heterozygous for *dpn*^{7}, where *dm*/*dpn* ratio is 2/1, *Sxl-Pe*$_{5xDbox}$ activity is up-regulated. In *dm Dup(11:2)51b* embryos, where the *dm*/*dpn* ratio is 3/2, there is a slight but noticeable increase in *Sxl-Pe*$_{5xDbox}$ activity. *Sxl-Pe*$_{5xDbox}$ drives a much higher level of LacZ expression in *dpn*/*dm Dup(11:2)51b* embryos where the ratio of *dm*/*dpn* is 3/1.

Table S1. *dm* alleles are female lethal when *trans* to a deficiency or with each other

	% viability
dm^{P0}/*Df(1)dm75319*	61 (*n* = 78)
dm^{P1}/*Df(1)dm75319*	21 (*n* = 138)
dm^{1}/*Df(1)dm75319*	0 (*n* = 143)
dm^{1}/dm^{P0}	78 (*n* = 196)

Percentage surviving *dm*/*Df* or dm^{1}/*dm* female progeny relative to their *dm*/*Bal* sibs. *N* indicates the number of *dm*/*Bal* females scored.

Table S2. The constitutively active Sxl^{M4} mutation rescues the female lethal effects of the dm^{PO} mutation

	% viability	*n*	Female sterile
dm^{P0}/dm^{P0}	35	646	Yes
dm^{P0}/dm^{P0}M4 4b	98	824	Yes
dm^{P0}/dm^{P0}M4 5b	90	506	Yes
dm^{P0}/dm^{P0}M4 6b	108	238	Yes
dm^{P0}/dm^{P0}M4 8b	102	287	Yes

Eleven independent dm^{PO} Sxl^{M4} recombinants were isolated and balanced over *FM7*. Females from the parental dm^{P0}/*FM7* stock and recombinant $dm^{P0}Sxl^{M4}$/*FM7* stocks were crossed to dm^{PO}/Y males and the viability of the *dm*/*dm* females relative to their *dm*/*FM7* sibs determined. All of the lines gave results similar to those for the four lines shown in this table. For these four lines, we outcrossed the Sxl^{M4} allele and recovered the dm^{P0} chromosome as a male viable derivative. For each of these four Sxl^{M4} lines, several independent outcrossed recombinants (2–5) were tested for female lethal effects. As was observed for the parental dm^{P0} chromosome, these Sxl^{+} recombinants are once again female lethal as homozygotes. To further confirm that all 11 $Sxl^{M4}dm^{P0}$ recombinants actually carried dm^{PO}, we tested for the surviving dm^{PO} Sxl^{M4}/dm^{PO} females from the test cross for fertility sterile. As is true for dm^{P0}/dm^{PO} females, the surviving dm^{PO} $Sxl^{M4}/$ dm^{PO} females from each of the 11 recombinants were sterile. We also scored for the characteristic *dm* bristle phenotype. dm^{PO} Sxl^{M4}, dm^{PO} females from all 11 lines had this *dm* phenotype.

Table S3. Female lethal interactions between *dm* and other sex determination genes

Paternal	Sxl^{fP7BO}/Y	sc^{3-1}/Y
Maternal genotype		
Df939/FM7	58% (398)	67% (763)
$dm^{1}/FM7$	79% (682)	84% (1,559)
$dm^{P0}/FM7$	84% (2,145)	86% (349)
$dm^{P1}/FM7$	85% (567)	88% (2,030)

For each cross, the paternal genotype is indicated in the top row and maternal genotype in the first column. The percentage of surviving *trans*-heterozygous females from each cross was calculated relative to the number of balancer females. The total number of flies is indicated in parentheses.

Table S4. Effects of *dm* on *Sxl-Pe$_{0.4kb}$* activity

Maternal	Paternal	+++	++	+	–	*N*
w^{1}/w^{1}	$w^{1}/Y;0.4$	45			55	239
$dm^{1}/+$	$w^{1}/Y;0.4$	22	24		53	439
$dm^{1}/+$	$dm^{1}/Y;0.4$		32		68	424
$sc^{3-1}/+$	$dm^{1}/Y;0.4$		13	14	73	191
$upd^{1}/+$	$dm^{1}/Y;0.4$		20	25	55	187
sisa/ +	$dm^{1}/Y;0.4$		15	23	64	159
$dm^{P1}/+$	$w^{1}/Y;0.4$	24	24		52	327
$dm^{P1}/+$	$sc^{3-1}/Y;\ 0.4$		21	25	54	441

Sxl-Pe$_{0.4kb}$ activity in the progeny from each of the indicated crosses. +++, high level of LacZ; ++, intermediate level; +, low level; –, none. *n* = number scored. Note that a "high" level of LacZ for *Sxl-Pe$_{0.4kb}$* is a good deal less than a "high" level of LacZ for the larger *Sxl-Pe$_{3.0kb}$* reporter. Some indication of the magnitude of the difference between the two reporters can be seen by comparing the female *Sxl-Pe$_{0.4kb}$* embryo in Fig. 3 with the female *Sxl-Pe$_{3.0kb}$* embryo in Fig. 1.