Figure S1

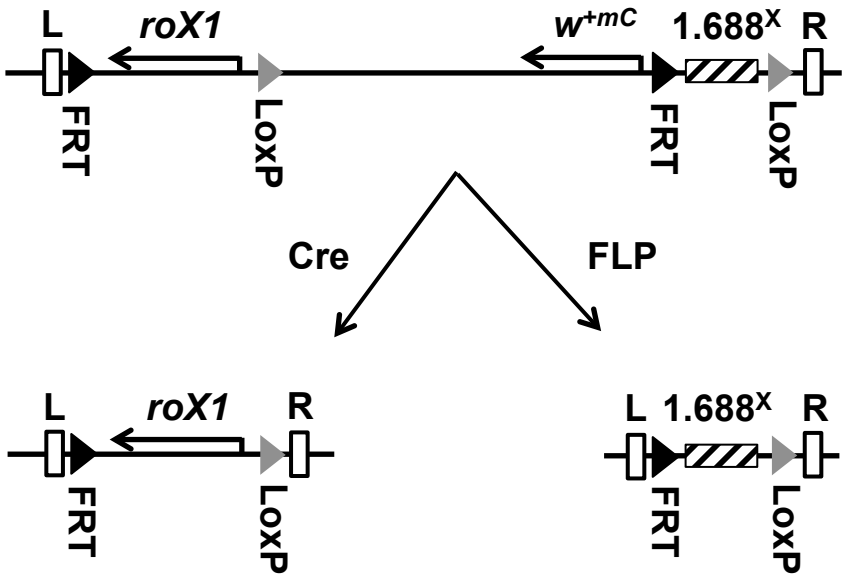

L
roX1
w+mC
1.688X
R
FRT
LoxP
FRT
LoxP
Cre
FLP
L
roX1
R
FRT
LoxP
L
1.688X
R
FRT
LoxP

Figure S2

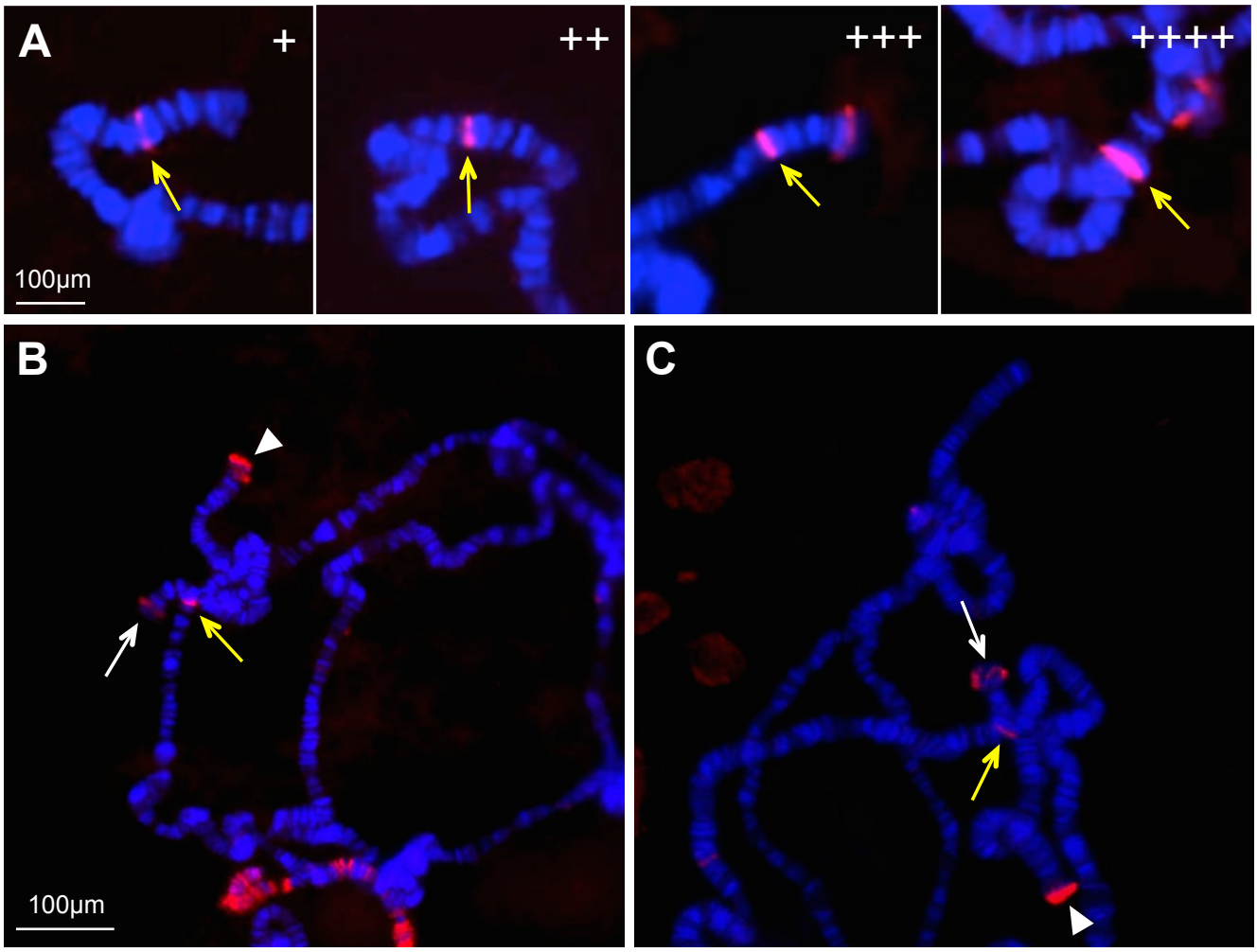


Figure S3

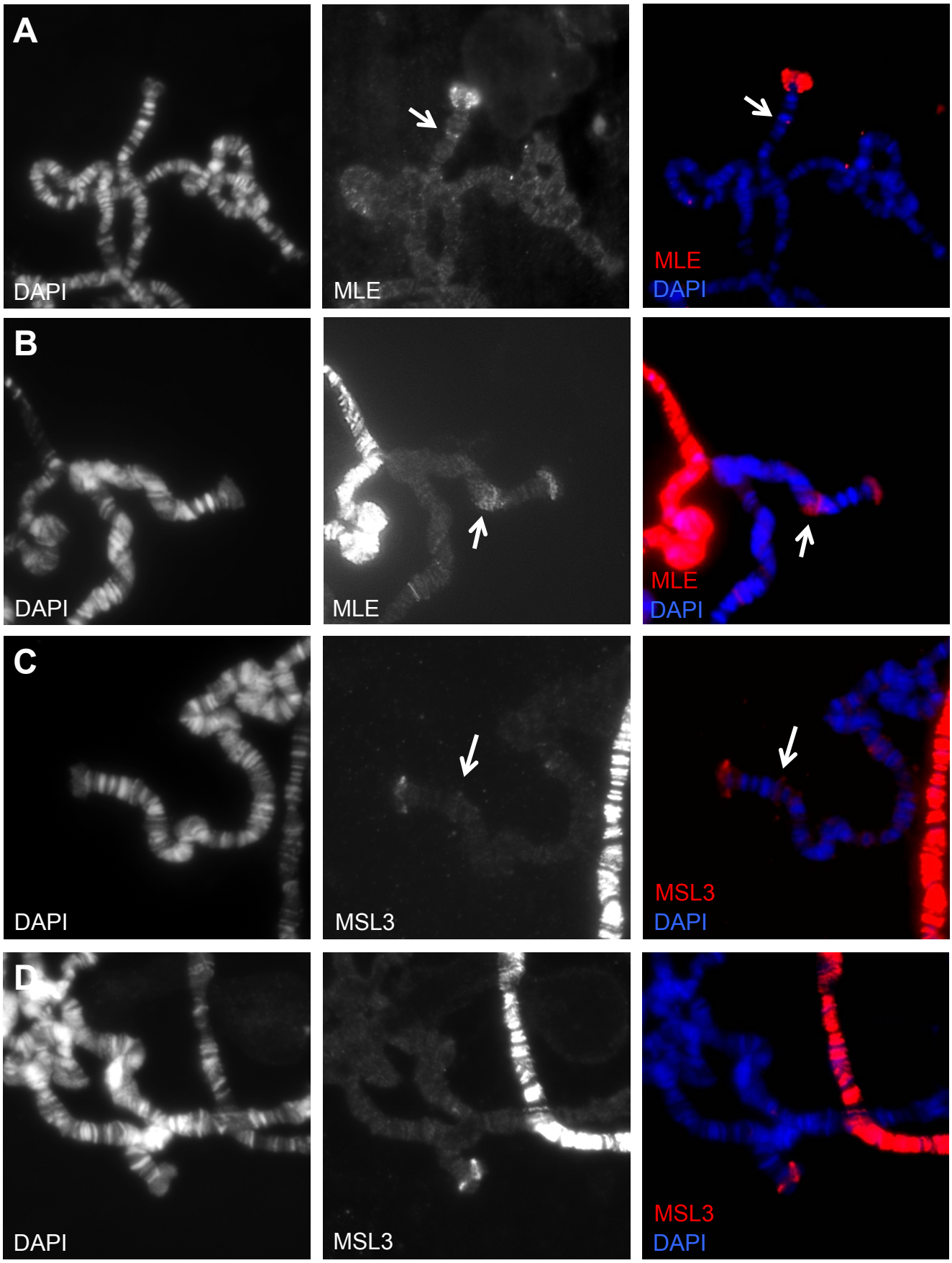
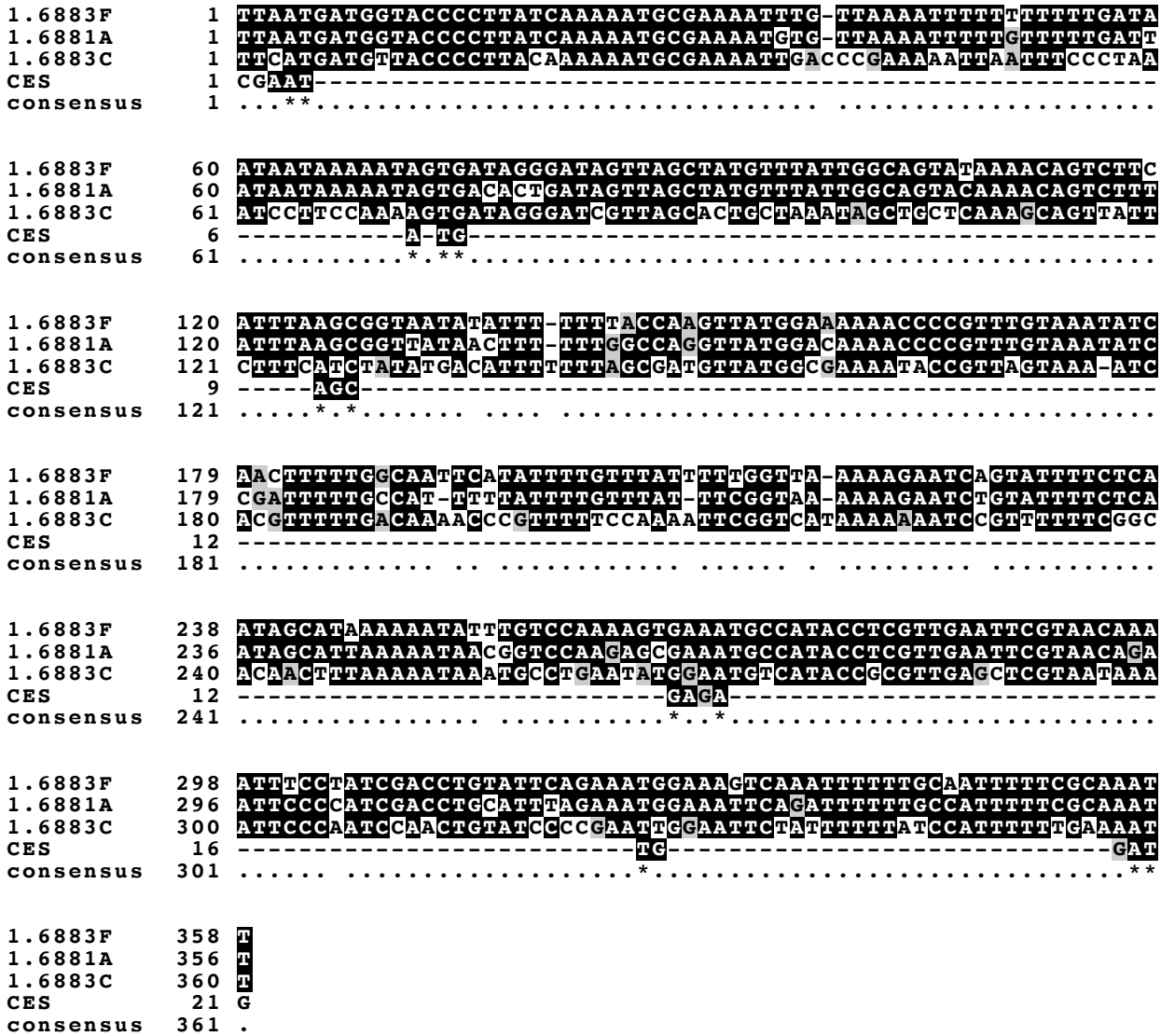

A
DAPI
MLE
MLE
DAPI
B
DAPI
MLE
MLE
DAPI
C
DAPI
MSL3
MSL3
DAPI
D
DAPI
MSL3
MSL3
DAPI

Figure S4


1.6883F 1 TTAATGATGGTACCCCTTATCAAAAATGCGAAAATTTG-TTAAAATTTTTTTTTTTTGATA
1.6881A 1 TTAATGATGGTACCCCTTATCAAAAATGCGAAAATGTG-TTAAAATTTTTGTTTTTGATT
1.6883C 1 TTCATGATGTTACCCCTTACAAAAAATGCGAAAATTGACCCGAAAAATTAATTTCCCTAA
CES 1 CGAAT----------
consensus 1 ...**.....
1.6883F 60 ATAATAAAAATAGTGATAGGGATAGTTAGCTATGTTTATTGGCAGTATAAAACAGTCTTC
1.6881A 60 ATAATAAAAATAGTGACACTGATAGTTAGCTATGTTTATTGGCAGTACAAAACAGTCTTT
1.6883C 61 ATCCTTCCAAAAGTGATAGGGATCGTTAGCACTGCTAAATAGCTGCTCAAAGCAGTTATT
CES 6 -----------A-TG----------
consensus 61 ...........*.**.....
1.6883F 120 ATTTAAGCGGTAATATATTT-TTTTACCAAGTTATGGAAAAACCCCGTTTGTAAATATC
1.6881A 120 ATTTAAGCGGTTATAACTTT-TTTGGCCAGGTTATGGACAAAACCCCGTTTGTAAATATC
1.6883C 121 CTTTCATCTATATGACATTTTTTTAGCGATGTTATGGCGAAAATACCGTTAGTAAA-ATC
CES 9 -----AGC----------
consensus 121 .....*.*.....
1.6883F 179 AACTTTTTTGGCAATTCATATTTTGTTTATTTTTGGTTA-AAAAGAATCAGTATTTTCTCA
1.6881A 179 CGATTTTTTGCCAT-TTTTATTTTGTTTAT-TTCGGTAA-AAAAGAATCTGTATTTTCTCA
1.6883C 180 ACGTTTTTTGACAAAACCCGTTTTTCCAAAATTCGGTCATAAAAAAATCCGTTTTTTCGGC
CES 12 ----------
consensus 181 .....
1.6883F 238 ATAGCATAAAAAATATTTGTCCAAAAGTGAAATGCCATACCTCGTTGAATTCGTAACAAA
1.6881A 236 ATAGCATTAAAAAATAACGGTCCAAGAGCGAAATGCCATACCTCGTTGAATTCGTAACAGA
1.6883C 240 ACAACTTTAAAAATAAATGCCTGAATATGGAATGTCATACCGCGTTGAGCTCGTAATAAA
CES 12 ----------GAGA----------
consensus 241 .....*..*.....
1.6883F 298 ATTTCCTATCGACCTGTATTCAGAAATGGAAAGTCAAATTTTTTGCAATTTTTCGCAAAT
1.6881A 296 ATTCCCCATCGACCTGCATTTAGAAATGGAAATTCAGATTTTTTGCCATTTTTCGCAAAT
1.6883C 300 ATTCCCAATCCAACTGTATCCCCGAATTGGAATTCTATTTTTTATCCATTTTTTGAAAAT
CES 16 ----------TG----------GAT
consensus 301 ....*.....**
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1.6881A 356 T
1.6883C 360 T
CES 21 G
consensus 361 .

Figure S5

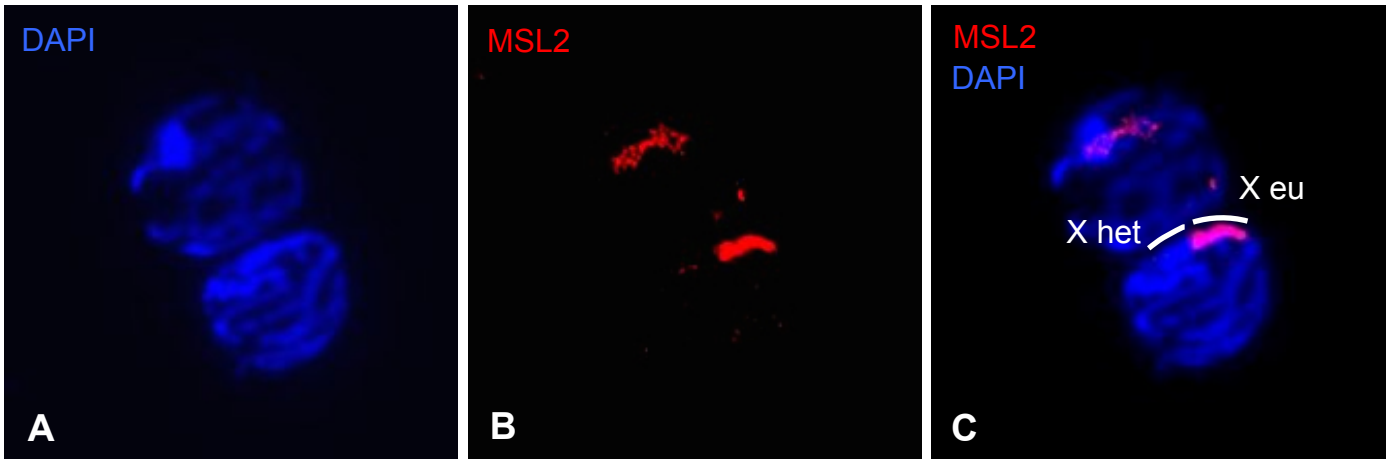
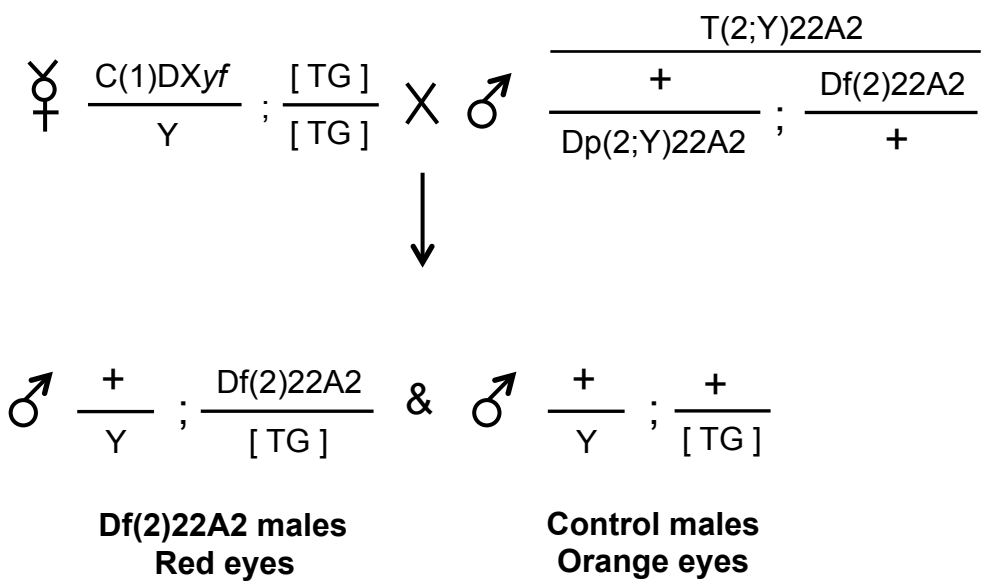


Figure S6

A


♀ C(1)DXyf / Y ; [TG] / [TG] X ♂ T(2;Y)22A2 / + / Dp(2;Y)22A2 ; Df(2)22A2 / +
♂ + / Y ; Df(2)22A2 / [TG] & ♂ + / Y ; + / [TG]
Df(2)22A2 males
Red eyes
Control males
Orange eyes

B

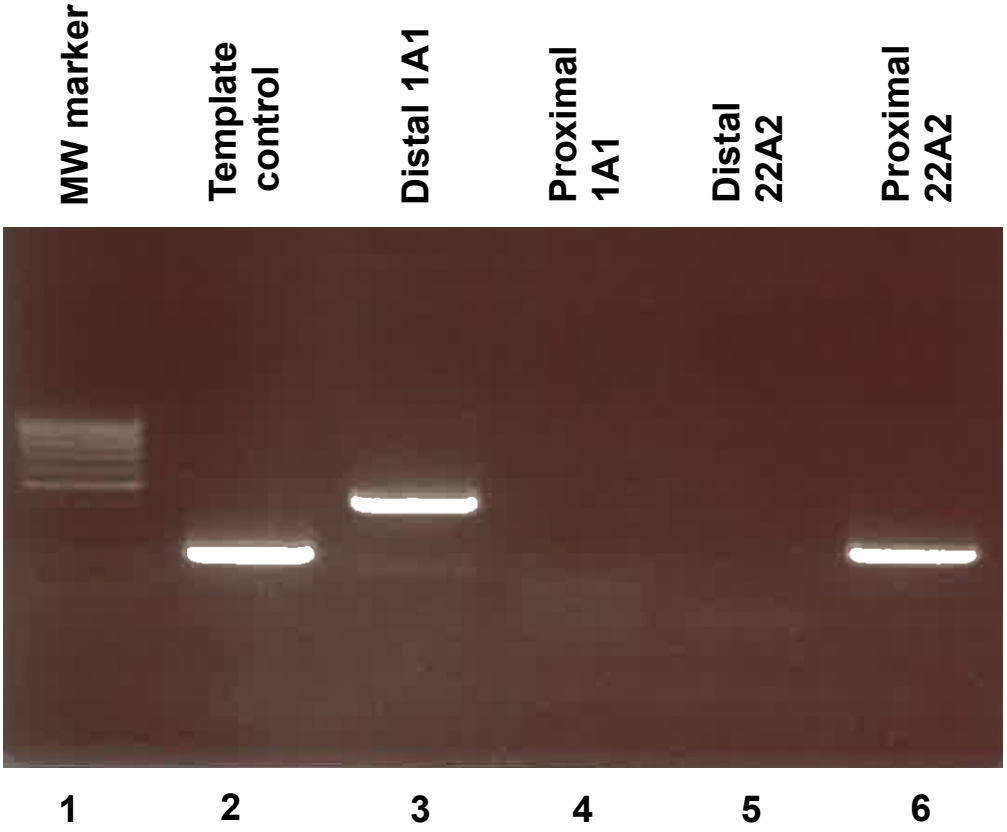

MW marker
Template control
Distal 1A1
Proximal 1A1
Distal 22A2
Proximal 22A2
1
2
3
4
5
6

Figure S7

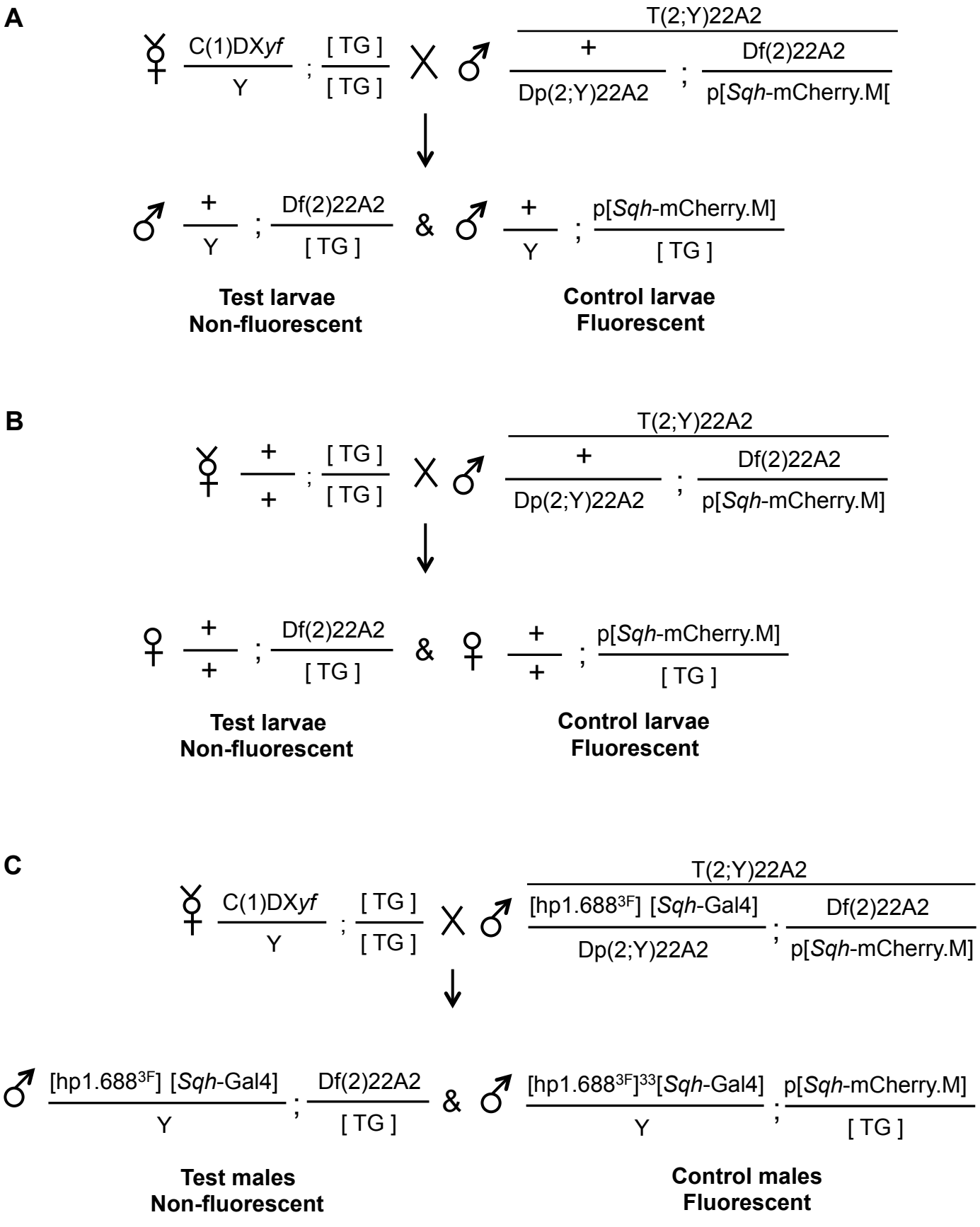

A
♀ C(1)DX*yf* / Y ; [TG] / [TG] × ♂ T(2;Y)22A2 / + / Dp(2;Y)22A2 ; Df(2)22A2 / p[*Sqh*-mCherry.M[
♂ + / Y ; Df(2)22A2 / [TG] & ♂ + / Y ; p[*Sqh*-mCherry.M] / [TG]
Test larvae Non-fluorescent
Control larvae Fluorescent
B
♀ + / + ; [TG] / [TG] × ♂ T(2;Y)22A2 / + / Dp(2;Y)22A2 ; Df(2)22A2 / p[*Sqh*-mCherry.M]
♀ + / + ; Df(2)22A2 / [TG] & ♀ + / + ; p[*Sqh*-mCherry.M] / [TG]
Test larvae Non-fluorescent
Control larvae Fluorescent
C
♀ C(1)DX*yf* / Y ; [TG] / [TG] × ♂ T(2;Y)22A2 / [hp1.688^{3F}] [*Sqh*-Gal4] / Dp(2;Y)22A2 ; Df(2)22A2 / p[*Sqh*-mCherry.M]
♂ [hp1.688^{3F}] [*Sqh*-Gal4] / Y ; Df(2)22A2 / [TG] & ♂ [hp1.688^{3F}]^{33}[*Sqh*-Gal4] / Y ; p[*Sqh*-mCherry.M] / [TG]
Test males Non-fluorescent
Control males Fluorescent

Figure S1. Transgene insertions containing *roX1* and 1.688^{X} repeats. Related to Figures 1-5. Integrated transgenes are flanked by attR and attL sites (L, R). LoxP and FRT sites allow excision of w^{+mC} and 1.688^{X} upon Cre expression (left), or w^{+mC} and *roX1* upon FLP expression (right).

Figure S2. MSL2 recruitment by autosomal transgenes and spreading to the 3L telomere. Related to Figure 1 and Table 1. (**A**) Representative images depicting scoring of MSL2 recruitment from weak (+) to very strong (++++). Scoring data is presented in Table 1. MSL2 recruitment by [1.688^{3F}]^{22A3} (**B**) and [*roX1*+1.688^{3F}]^{22A3} (**C**) detected by immunostaining of polytene preparations and visualized with Texas Red. Recruitment near the integration site (yellow arrows), recruitment at telomere 2L (white arrows) and 3L (white arrowheads) is observed. DNA was counterstained with DAPI (blue).

Figure S3. MSL3 and MLE are recruited by 1.688^{3F} repeats. Related to Figure 1. Polytene preparations from [1.688^{3F}]^{22A3} males were stained with antibodies to the non-core components of the MSL complex MSL3 and MLE. Both display minor binding near the 22A3 integration site (arrows), as well as spreading into subtelomeric regions of 2L.

Figure S4. Sequence alignment detects no similarity between 1.688^{X} repeats and CES. Related to Figures 4 and 5. Multiple sequence alignment was performed using 1.688^{3F}, 1.688^{1A} and 1.688^{3C} (superscript denotes cytological position). Alignment of a Chromatin Entry Site (CES) is also shown. Multiple sequence alignment was generated using T-Coffee [S1].

Figure S5. MSL complex is limited to euchromatic X chromatin. Related to Figure 1. Mitotic embryo nuclei were probed with antibodies to MSL2, detected by Texas Red. A little over half of the X is euchromatic (X eu) and binds MSL2. The remainder is heterochromatic (X het), largely composed of 359 bp repeats that are related to the euchromatic 1.688^{X} repeat family. DNA is counterstained with DAPI.

Figure S6. Matings to detect autosomal compensation in pharate pupae and adults. Related to Figure 4. (A) Compound X/Y females homozygous for transgene integrations ([TG]) on 2L were mated to T(2;Y)22A2 males. T(2;Y)22A2 can be separated into Df(2)22A2 and Dp(2;Y)22A2 [S2]. All male progeny inherit a wild type maternal Y chromosome, the 2L [TG] and Df(2)22A2 (test males) or the wild type 2nd chromosome (control males). Df(2)22A2 carries a w^{+} marker that confers dark red eye color. **(B)** PCR to detect the proximal and distal ends of the insertions used to generate T(2;Y)22A2. The translocation occurred between FRT sites at 22A2 (2L) and 1A1 of a duplication of X on the Y [S2]. Amplicons from a representative pharate male carrying Df(2)22A2 are shown. Only distal 1A1 (lane 3) and proximal 22A2 (lane 6) PCR products are present in Df(2)22A2. The proximal 1A1 (lane 4) and the distal 22A2 (lane 5) PCR products, indicative of Dp(2;Y)22A2, are not detected. A template control (lane 2) to an unrelated gene is also shown. Primer sequences are available upon request.

Figure S7. Matings to detect autosomal compensation and the effect of 1.688^{3F} siRNA on the recruitment of compensation. Related to Figures 4 and 5. (A) Compound X/Y females with the transgene to be tested ([TG]) were mated to T(2;Y)22A2 / p[Sqh-mCherry.M] males to produce unmarked Df(2)22A2 male larvae. Control males are marked with p[Sqh-mCherry.M] on the intact 2nd chromosome. All male offspring inherit the maternal Y chromosome. Males are identified by gonad morphology. **(B)** Df(2)22A2 female larvae were produced by mating females homozygous for the transgene to T(2;Y)22A2 males carrying p[Sqh-mCherry.M] on the intact 2nd chromosome. All female progeny inherit one copy of the transgene and either Df(2)22A2 (test) or p[Sqh-mCherry.M] (control). Females are identified by gonad morphology. **(C)** Compound X/Y females homozygous for the transgene to be tested ([TG]) were mated to T(2;Y)22A2 males carrying [hp 1.688^{3F}] [S3] and p[Sqh-GAL4] on the X chromosome. p[Sqh-mCherry.M] marks the intact 2nd chromosome. All male progeny produce ectopic 1.688^{3F}siRNA and carry a wild type Y chromosome.

Table S1.

	Gene	Transgene						
		None	*roX1*		1.688^{3F}		*roX1*+1.688^{3F}	
Test Genes on 2L	CG33128	1	1.263	1.11	1.208	1.326	1.425	1.337
	RFeSP	1	1.192	1.242	1.368	1.29	1.63	1.49
	Eno	1	1.096	1.184	1.14	1.26	1.31	1.51
	CG16704	1	1.096	1.06	1.12	1.098	1.204	1.133
	eIF-3p40	1	0.998	1.1	1.09	1.25	1.37	1.26
	His3.3A	1	1.49	1.612	1.45	1.348	1.59	1.474
	Rpn11	1	0.862	0.824	0.96	0.935	0.99	0.92
	RpL37A	1	1.412	1.478	1.762	1.696	1.88	2.069
	Trip1	1	1.408	1.48	1.51	1.592	1.672	1.761
	CG31778	1	1.396	1.484	1.689	1.745	1.533	1.594
Control Genes on 2R and 3R	Xbp1	1	0.954	0.943	0.92	0.894	0.902	0.88
	DipB	1	0.844	0.876	1	0.912	0.912	0.993
	ATPa	1	1.178	1.192	1.107	1.11	1.116	1.143
	Gprk2	1	1.115	1.084	1.098	1.12	1.108	1.033
	GAPDH	1	1.102	1.196	1.086	1.162	1.14	1.092

Table S1. Relative expression of control and test genes in males with *roX1* and 1.688^{3F} transgenes. Related to Figures 2 and 3. Gene expression was measured by quantitative RT-PCR. Expression in male larvae with integrations of [*roX1*], [1.688^{3F}], or [*roX1*+1.688^{3F}] on 2L was measured. A laboratory reference strain with no transgene (*yw*) was used as the calibrator, and *Dmn* was the normalizing gene. Two biological replicates are presented.

SUPPLEMENTAL REFERENCES

S1. Di Tommaso, P., Moretti, S., Xenarios, I., Orobitg, M., Montanyola, A., Chang, J.M., Taly, J.F., and Notredame, C. (2011). T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Nucleic Acids Res *39*, W13-17.

S2. Joshi, S.S., Cheong, H., and Meller, V.H. (2014). A strategy for generation and balancing of autosome: Y chromosome translocations. Fly (Austin) *8*, 58-62.

S3. Menon, D.U., Coarfa, C., Xiao, W., Gunaratne, P.H., and Meller, V.H. (2014). siRNAs from an X-linked satellite repeat promote X-chromosome recognition in Drosophila melanogaster. Proc Natl Acad Sci U S A *111*, 16460-16465.