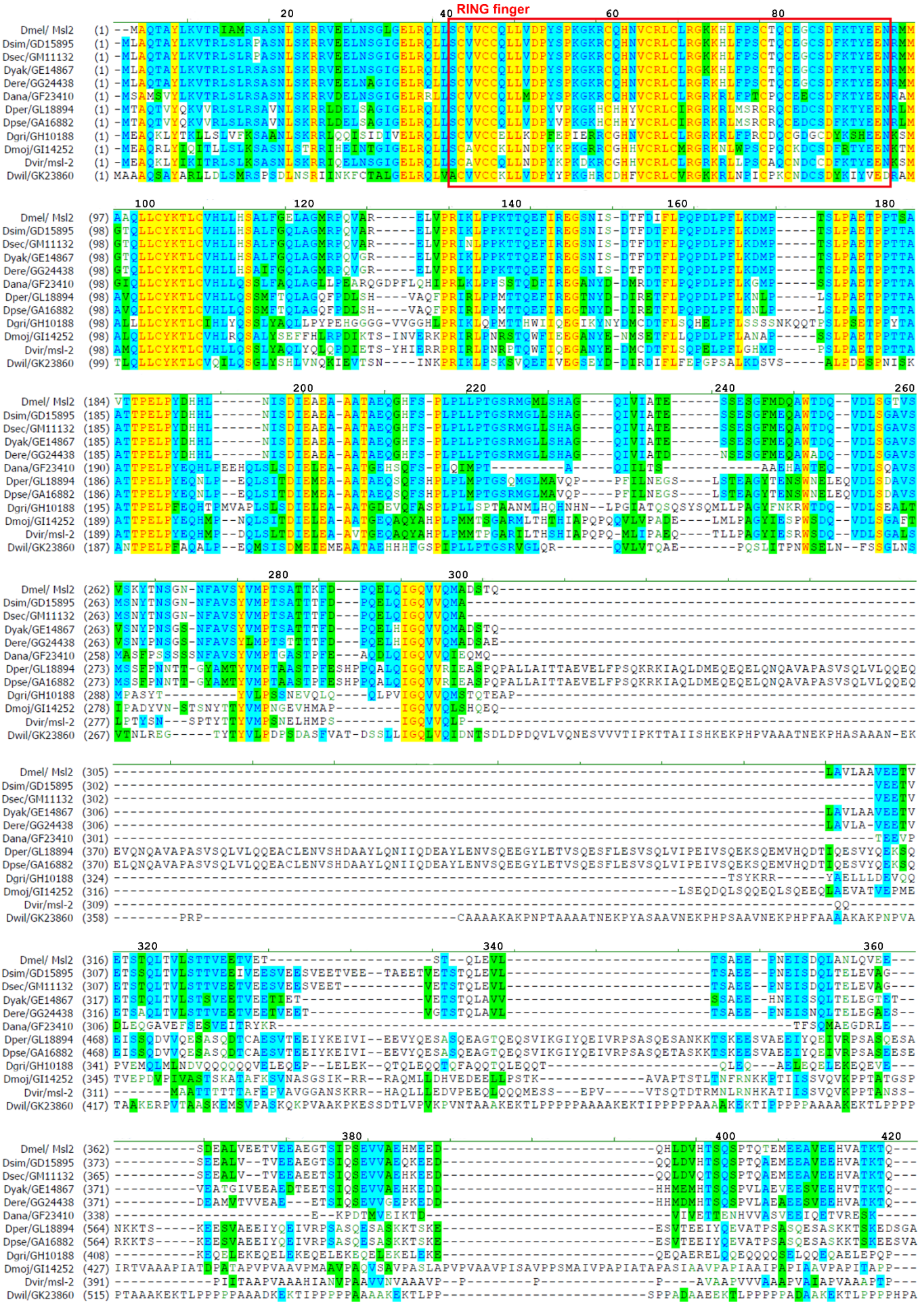
Supplementary Figures



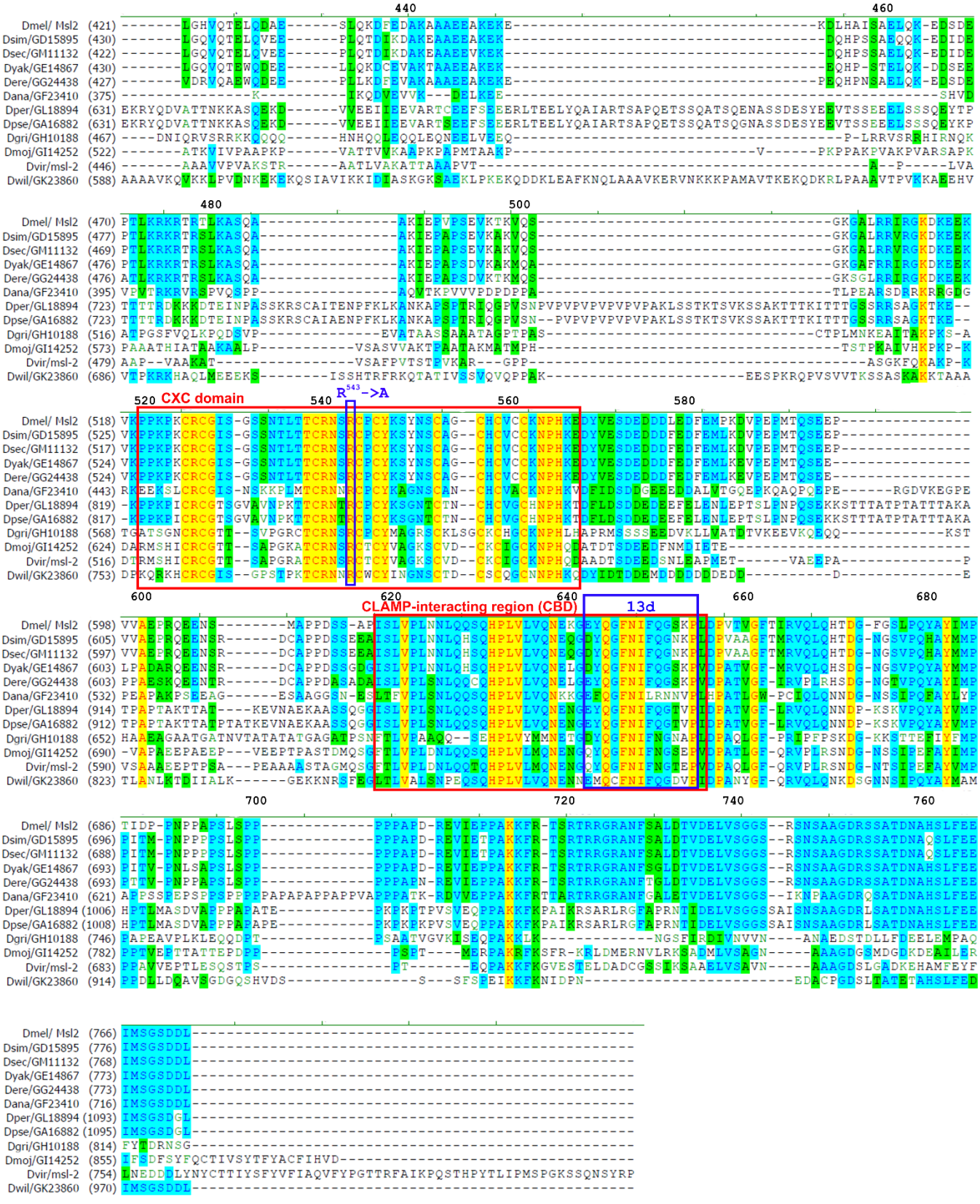


Figure S1. Alignment of MSL2 protein among Drosophilidae. Different regions are shown in the red frames. Also R543 amino acid residue and minimal 13 aa CLAMP-interacting region are marked in blue frames.

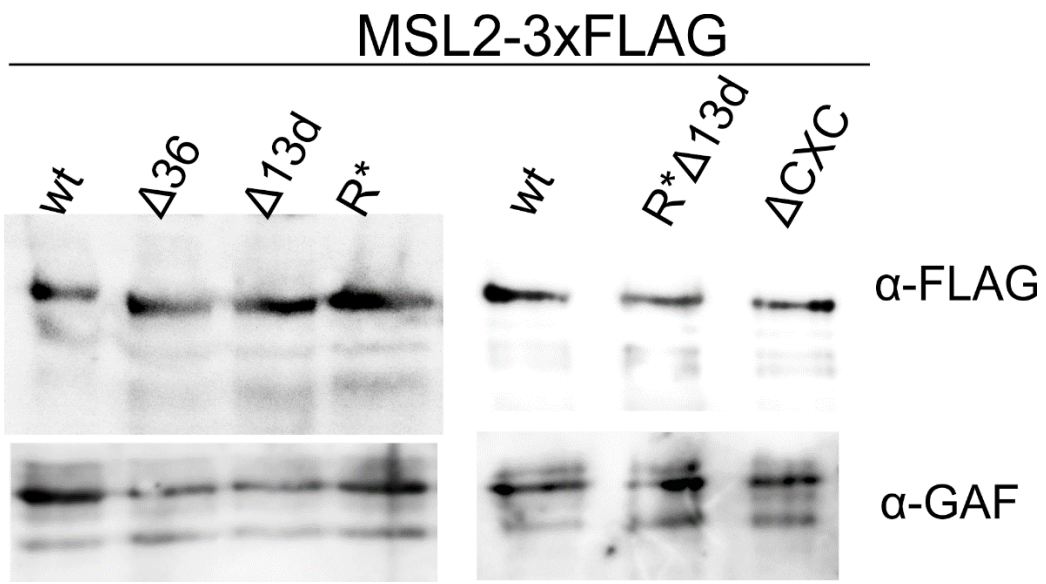


Figure S2.

Western blotting of protein extracts prepared from adult flies expressing different variants of 3xFLAG-tagged MSL2 protein (wild-type (wt), deletion of 36 (Δ36) and 13 (Δ13d) aa from CLAMP-binding region, substitution of arginine at the 543 position by alanine (R*), simultaneous substitution of arginine at the 543 position by alanine and deletion of 13 aa from CLAMP-binding region (R*Δ13d), deletion of CXC domain (ΔCXC)).

Immunoblot analysis was performed with FLAG and GAF antibodies (internal control).

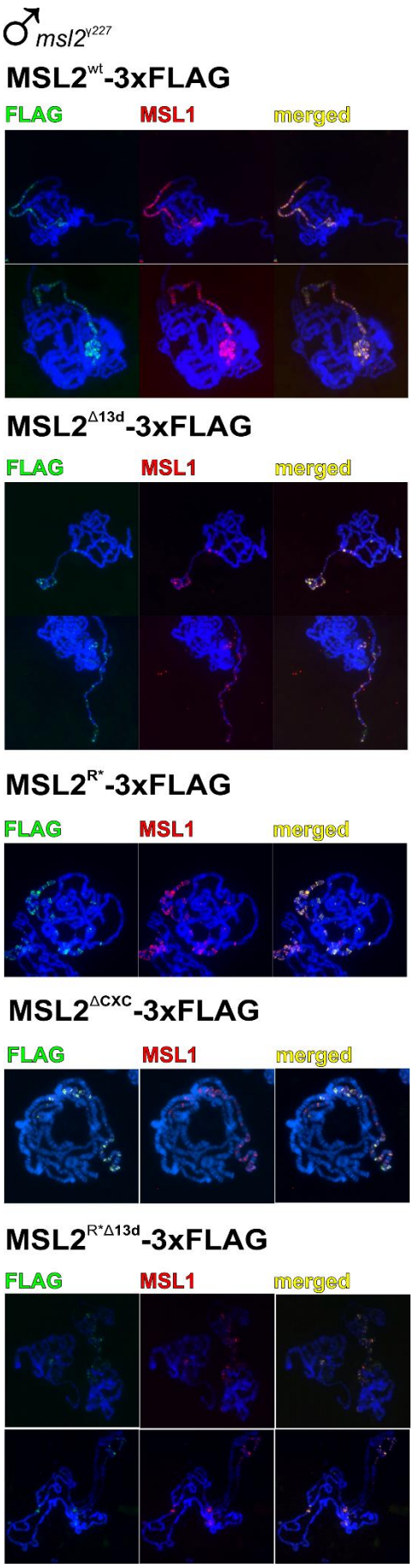


Figure S3. MSL1 and MSL2 localization on the polytene chromosomes from salivary glands of male 3rd instar larvae in the *msl2*-null (*msl2*$^{\gamma 227}$) background that express different FLAG-tagged variants of MSL2 protein: wild-type (wt), deletion of 13 aa from CLAMP-binding region (Δ13d), substitution of arginine at the 543 position by alanine (R*), deletion of CXC domain

(ΔCXC), simultaneous substitution of arginine at the 543 position by alanine and deletion of 13 aa from CLAMP-binding region (R*Δ13d). Panels show immunostaining of 3xFLAG-MSL2 protein with mouse anti-FLAG antibody (green) and MSL1 protein with corresponding rabbit antibody (red) on polytene chromosomes. DNA is stained with DAPI (blue).

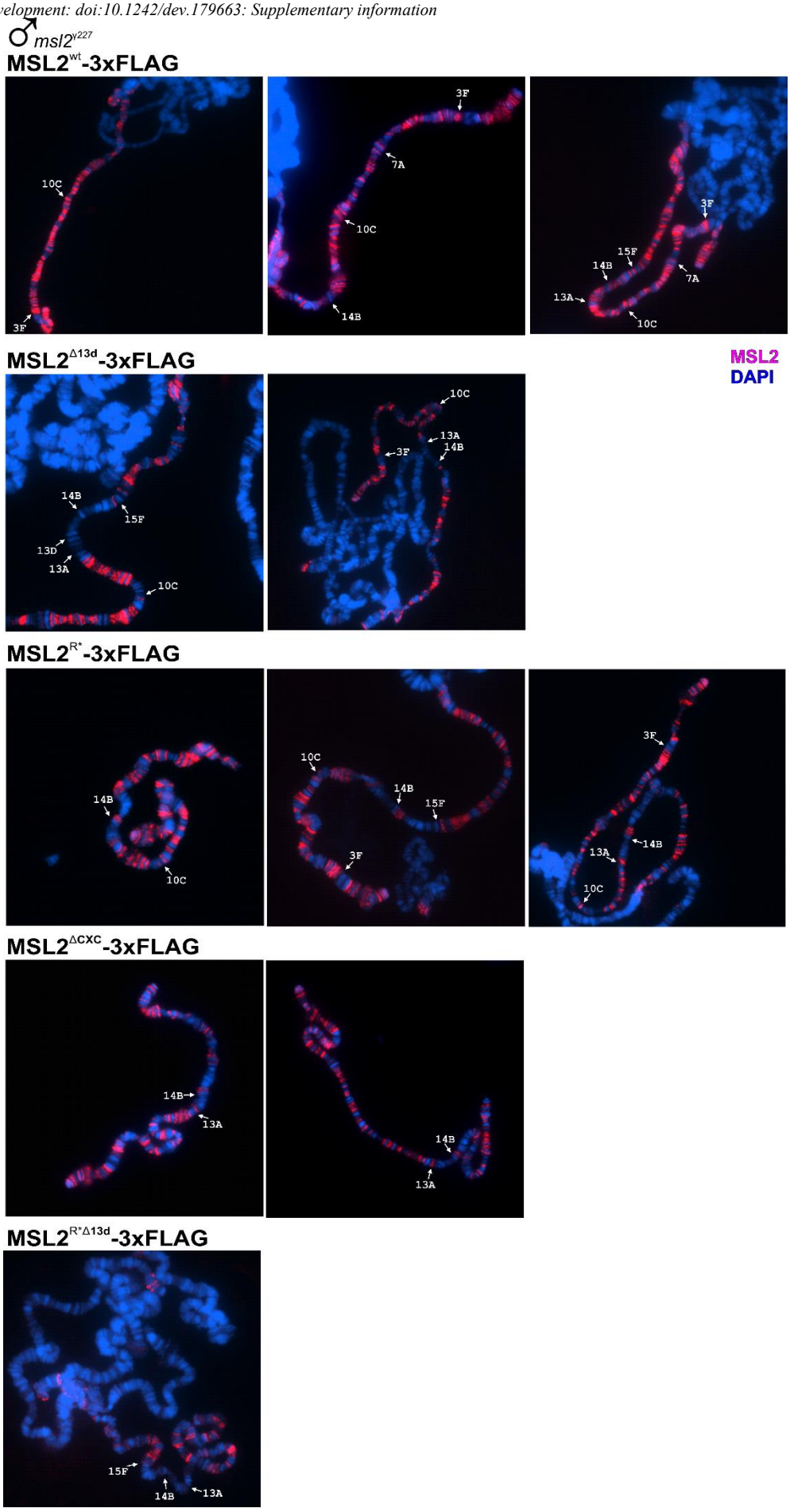


Figure S4. Comparing several sites of MSL complex binding at the high resolution cytological map on the polytene chromosomes from salivary glands of male 3rd instar larvae in the *msl2*-null (*msl2*$^{\gamma 227}$) background that expressed different FLAG-tagged variants of MSL2 protein: wild-

type (wt), deletion of 13 aa from CLAMP-binding region (Δ13d), substitution of arginine at the 543 position by alanine (R*), deletion of CXC domain (ΔCXC), simultaneous substitution of arginine at the 543 position by alanine and deletion of 13 aa from CLAMP-binding region (R*Δ13d). Panels show immunostaining of 3xFLAG-MSL2 protein with rabbit anti-MSL2 antibody (red) on polytene chromosomes. DNA is stained with DAPI (blue).

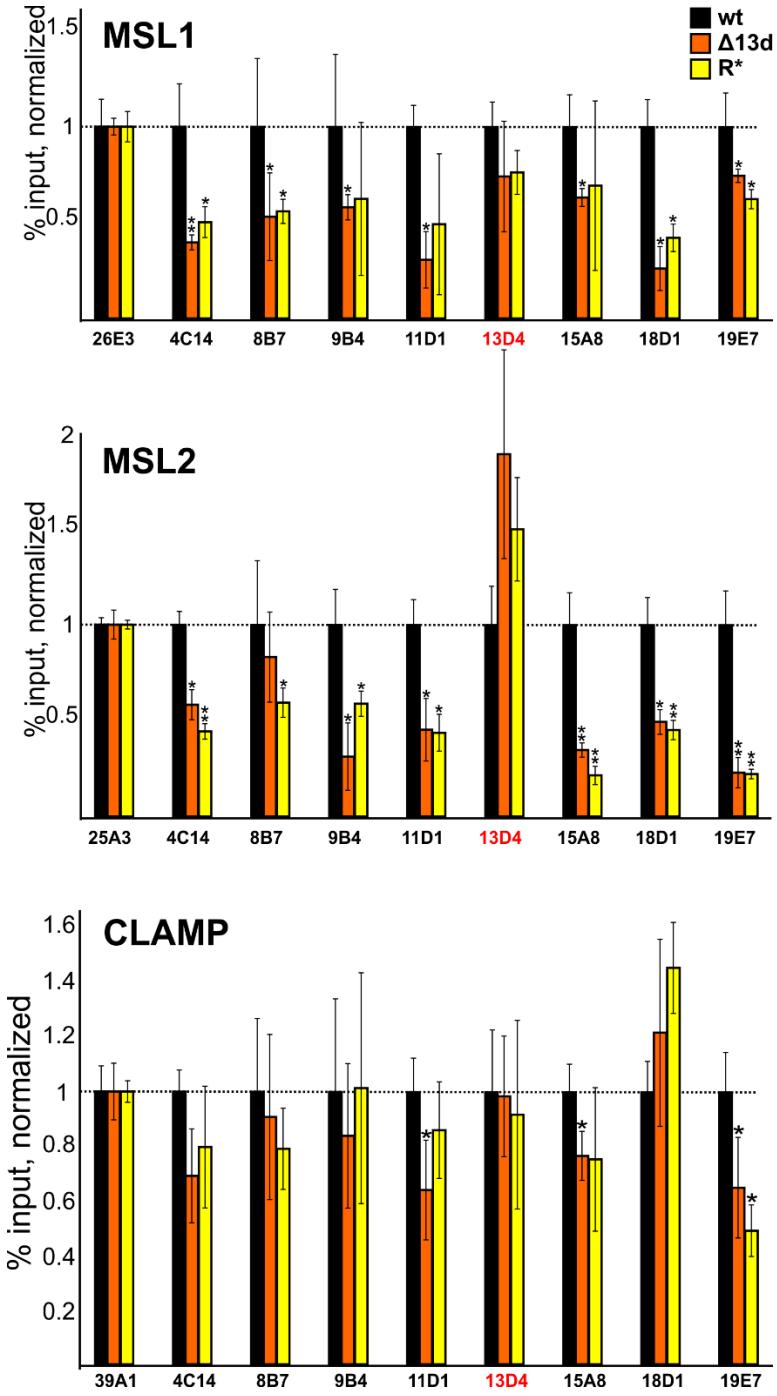


Figure S5. Comparing binding of MSL1, MSL2, and CLAMP at different CES and PionX (marked with red) regions in the MSL2-expressing males in the *msl2*$^{\gamma 227}$ background. Histograms show ChIP enrichments at the CES regions on chromatin isolated from male flies expressing different variants (wt, Δ13d, R*) of MSL2 protein. The results are presented as a percentage of input genomic DNA normalized to corresponding positive autosomal genome regions for MSL1(26E3) and MSL2 (25A3), and compared with binding levels in the flies with expressed wild type MSL2 protein (corresponding to the "1" on the scale). Error bars show standard deviations of quadruplicate PCR measurements for three independent experiments. Asterisks indicate significance levels: *$p < 0.05$, **$p < 0.01$.

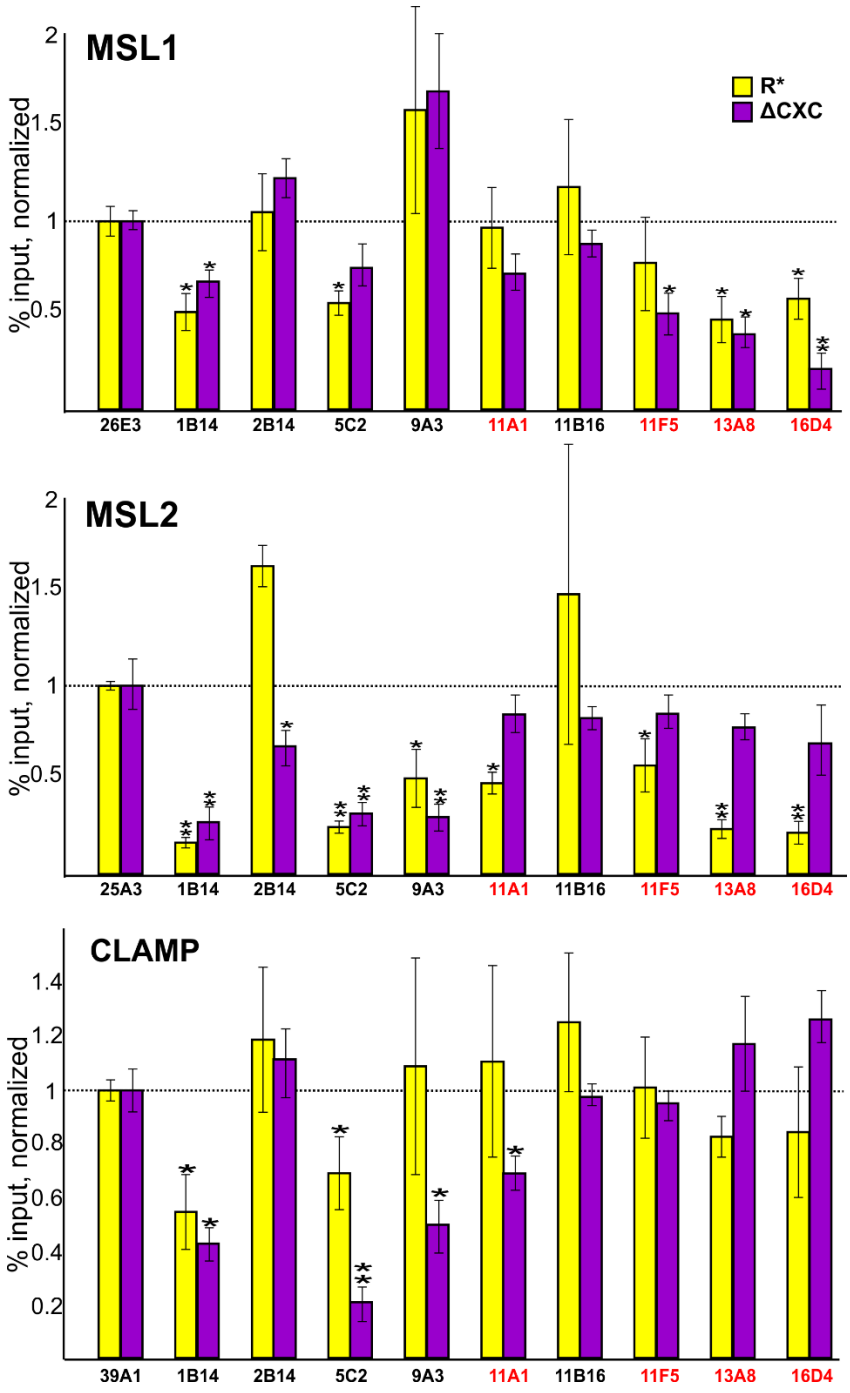


Figure S6. Comparing binding of MSL1, MSL2, and CLAMP at different CES and PionX (marked with red) regions in the MSL2-expressing flies in the *msl2*$^{\gamma 227}$ background. Histograms show comparison of ChIP enrichments at the CES regions on chromatin isolated from male flies expressed MSL2 protein with substitution of arginine at the 543 position by alanine (R*) and deletion of CXC domain (ΔCXC). The results are presented as a percentage of input genomic DNA normalized to corresponding positive autosomal genome regions for MSL1(26E3), MSL2 (25A3) and CLAMP (39A1), and compared with binding levels in the flies with expressed wild type MSL2 protein (corresponding to the "1" on the scale). Error bars show standard deviations of quadruplicate PCR measurements for three independent experiments. Asterisks indicate significance levels: *$p < 0.05$, **$p < 0.01$.

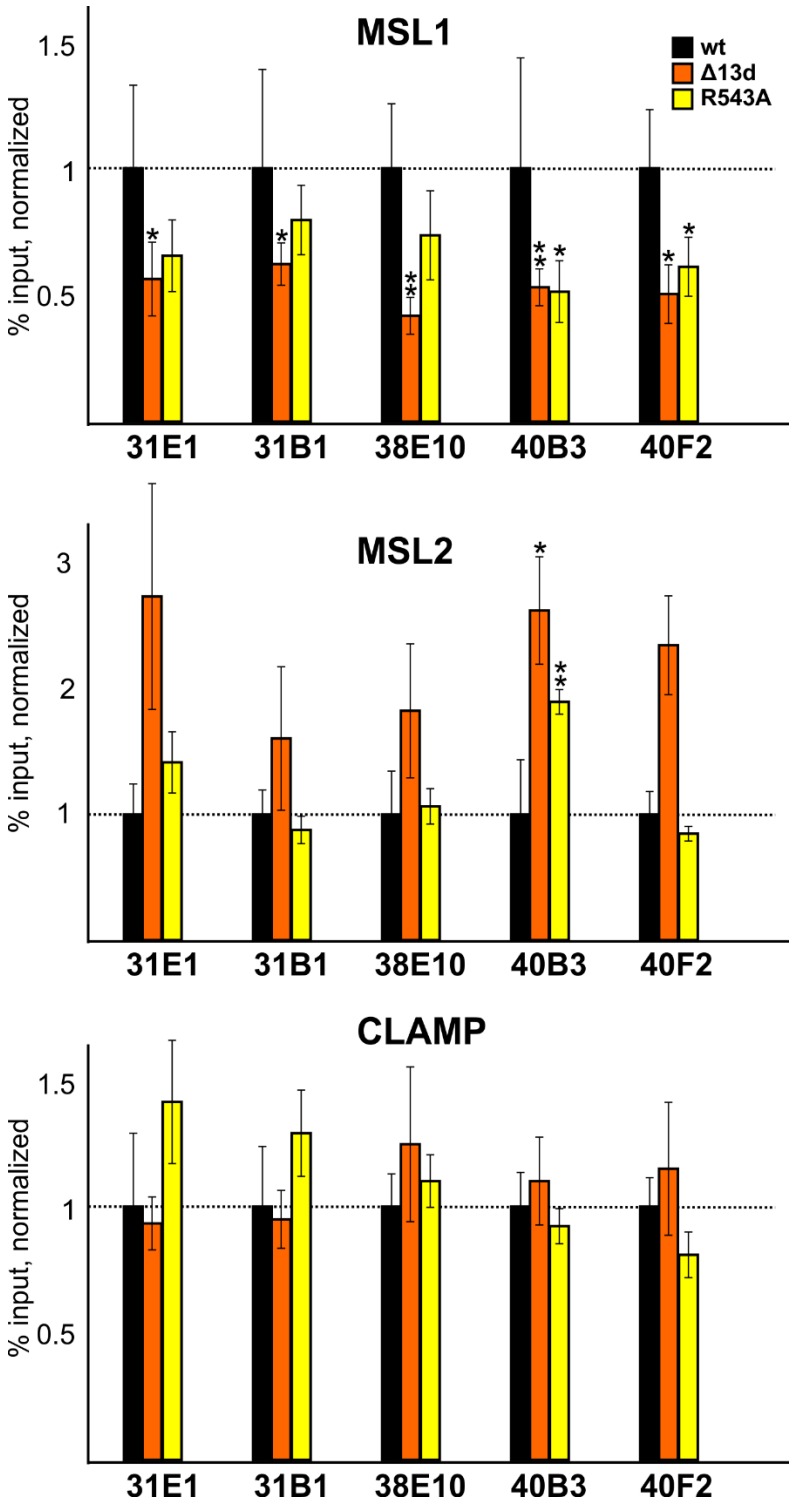


Figure S7. Comparing binding of MSL1, MSL2 and CLAMP at different autosomal genome regions in the MSL2-expressing flies in the *msl2*$^{\gamma 227}$ background. Histograms show ChIP enrichments at the CES regions on chromatin isolated from male flies expressing different variants (wt, Δ13d, R543A) of MSL2 protein. The results are presented as a percentage of input genomic DNA normalized to corresponding positive autosomal genome regions for MSL1(26E3), MSL2 (25A3), and compared with binding levels in the flies with expressed wild type MSL2 protein (corresponding to the "1" on the scale). Error bars show standard deviations of quadruplicate PCR measurements for three independent experiments. Asterisks indicate significance levels: *$p < 0.05$, **$p < 0.01$.

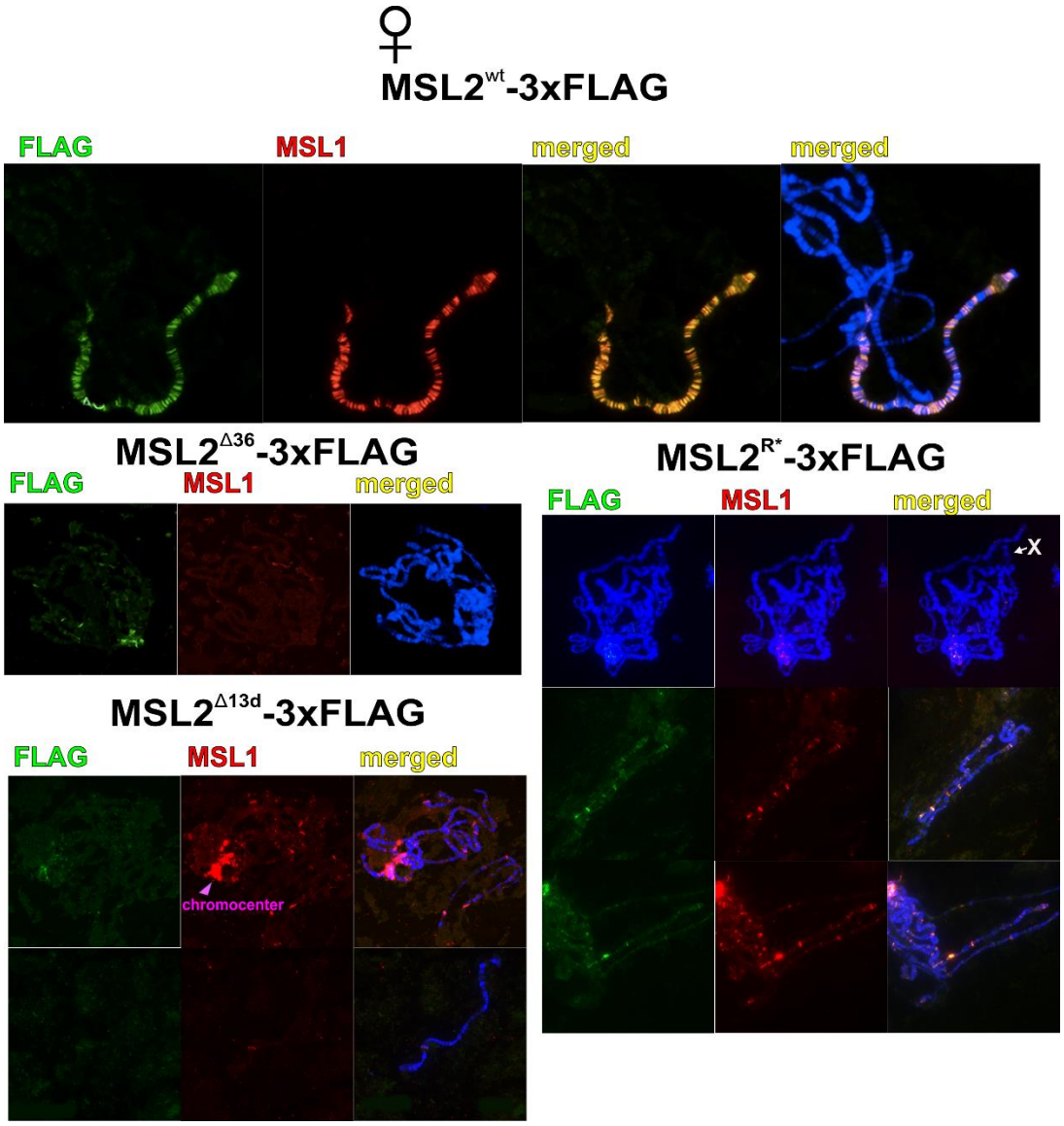


Figure S8. Distribution of MSL complex on the polytene chromosomes extracted from salivary glands of female 3rd instar larvae that express different FLAG-tagged variants of MSL2 protein: wild-type (wt), deletion of 36 (Δ36) and 13 (Δ13d) aa from CLAMP-binding region, substitution of arginine at the 543 position by alanine (R*). Panels show immunostaining of 3xFLAG-MSL2 protein with mouse anti-FLAG antibody (green) and MSL1 protein with corresponding rabbit antibody (red) on polytene chromosomes. DNA is stained with DAPI (blue).

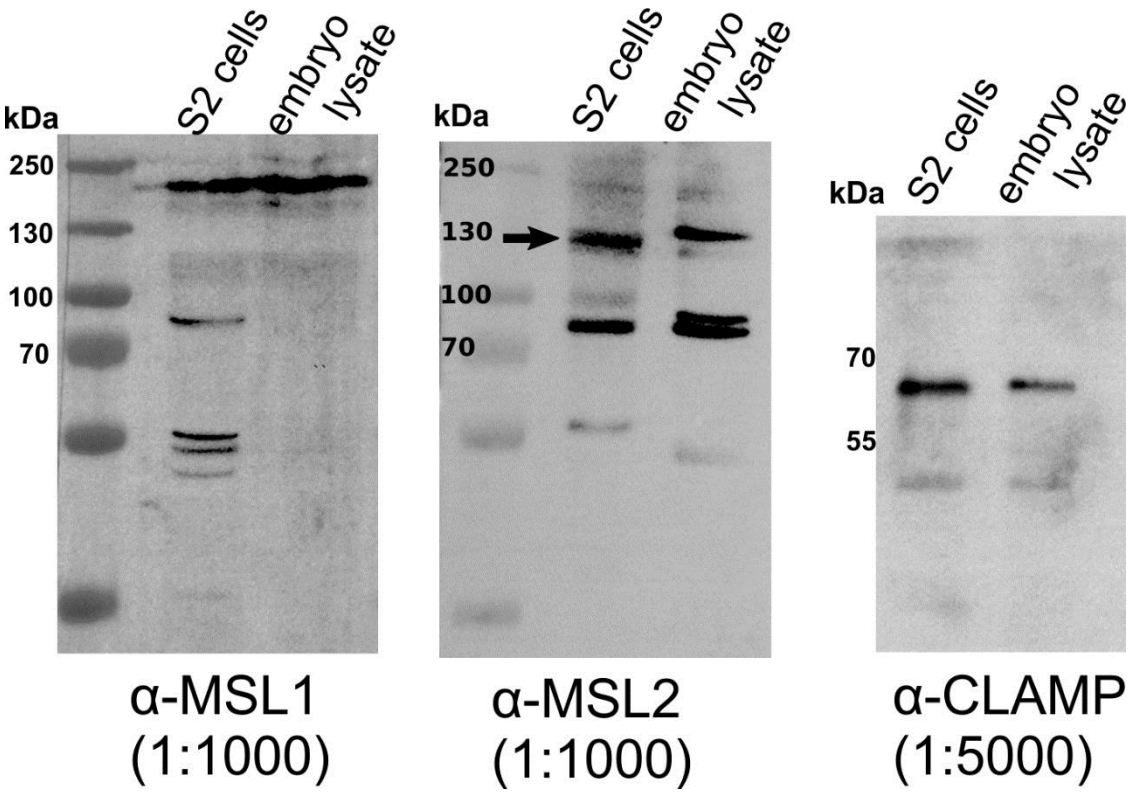


Figure S9. Immunoblot analysis of affinity-purified antibodies for MSL1, MSL2, CLAMP proteins.

Supplementary Table

Table S1. The list of used oligonucleotides.

Primers for cloning of msl2 deletions			
Name	**dir**	**rev**	
msl2_full-sized	TTCGATATCATGGCCCAGACGGCATACTT	CCCGGGCAAGTCATCCGAGCCCGA	
msl2_d13	AAAGGCGAGGATCCGGTCACCGTTGG	GACCGGATCCTCGCCTTTCTCATTCT	
msl2_dB	CCACCGGCCGCCAACTTCTCGGCCCT	GAAGTTGGCGGCCGGTGGCTCGATGA	
msl2_dP	TATATAATGGCCAAGAAATTCAGGACC	TTTCTTGGCCATTATATAAGCATACT	
Primers for RT-qPCR			
Name	**dir**	**rev**	**Taqman probe**
RoX1	CTTGTGCTTTCTCCTGAATGTG	TGTATTAGGCGGAGCTTCTTG	FAM-AGCCTATGAAATCCGGTCCAACCC-BHQ1
RoX2	TTCGAAACGTTCTCCGAAGC	AGTCGTACTCATCTCACTGTCC	FAM-AGCAAGAGTAACGATTTCCGCATAGTCG-BHQ1
RpL34	ACAACACACGCTCCAACA	GGGTGATACCCTTCAACTTCTC	FAM-TGGTAAACCAGACGACCACCGG-BHQ
Primers for ChIP analysis			
Genome region	**dir**	**rev**	
RoX1	AGGTCTGCAAGGTTCAGTTTAT	ATTCTTAAGGGTGGCGTCTTC	
RoX2	GGCTTAGAGAGAGATGGCAATAC	AGTTCTGGTCACCCTGGA	
CES1B14	CAGGACAAGACTAGGACAAAGG	GGTTTGCGGATTGAGGATTATTG	
CES2B14	CCCTCATATGCTTCTTCTGGTC	ACGTACGATCACGCACATATC	
CES4C14	ATTGGTATTCGGGCAAGGG	GAGCATATTTCTCTCGAGGATGG	
CES5C2	CAGAAATTCGAAGCGATCTCAAC	AATCGACTGCTAGGTTGGTAAA	
CES8B7	CAGCAGAACGCTCTTTGATTT	CCAACATTCGCTTCACACAC	
CES9A3	TAATGCTGGATCTCGCTCAC	CTGATCGCCGGTCATAGAAA	
CES9B4	CGTGCCGCCTAACTATCTAAC	GGGATGGAAAGAGAGAGCAAA	
CES11A1	GCCCGGAACTCCTTTAGTATG	TGATGCCACTGGATGAGTATG	
CES11B16	GGTGGTGGACATCTCGTTAAT	ACCAGCGAATATCGAGCATAAA	
CES11D1	AAGCCACTGATGCGTACAA	CAGAACGGCTGGCAAGATA	
CES11F5	TACAGTAGCTGAGAGCTGTACT	GTTTGGACTTGCGCCTTTAC	
CES13A8	AATCTGAGCGAGATGGAAGAAC	GTGTCTAGTGGTTGGCTATGAC	
CES13D4	GCCGTGATTGTGGATCTCTT	CTCCAGACGTGCTGAATTGT	
CES15A8	CCAGGCTAAATAGTTCGCTACA	CGTAGTTGCATCTCGCTCTAAT	
CES16D4	CATCAACGACCTTGTCACATTATC	GCACGAATCAGACAGAGAAGTA	
CES18D11	GCGCTTGCAATAGCTTCAATTA	GGAAATCCAAACAGTACAACTTCG	
CES19E7	GAGCGAGAGAGATTGCCAAATA	CGAATCGTGGAGTCTGAGAAAT	
26E3	CGTAACGGCACCCCTCAA	ACCGCACCGCACTACAAG	
25A3	GCTCCAGGAACCGATCTATTG	CTTGGCTTCCACTGAAGTTAGA	
39A1	CTCTTGTTCACACAGCCATTTC	TACTCTCTTCGGGCGGTATAA	
31E1	TGGATATGGCTTCTGGTTCATC	GGGCTGCATTCGGAGTTTA	
31B1	TTGTAAGGCGACTCGACTATTT	TGCTTTCGGCTGACTAATGA	
38E10	CGACACAAGTACCAGCTCTAAT	CGGCGAGGTACTTACCATATTC	
40B3	GCAGAAGGGAGAACTGTGAAATA	CTTCGCGGAGGGTTAATTGT	
40F2	CAGAACAACGCTCAGAGATAGA	GGGCTCTAAGAAATCCTACCAG	

Table S2. Cloning procedures

Pull down assay			
	dir	**rev**	**Restriction sites**
TRX-His-MSL2 [573-708]	ttggaattcatggaggactacgttg	agtgtcgacctattccctgtcaggagca	EcoRI SalI
TRX-His-CLAMP [1-153]	ctggaattcatggaagaccttaccaa	aacgtcgacttccccgtctgtatgcat	EcoRI SalI
TRX-His-CLAMP [40-153]	ctggaattcatgaaaacggagcagcagc	aacgtcgacttccccgtctgtatgcat	EcoRI SalI
TRX-His-CLAMP [86-153]	-	-	HincII SalI
TRX-His-CLAMP [116-153]	-	-	MunI SalI
GST-CLAMP [1-196]	tacccgggatggaagaccttaccaaaaac	aacgtcgacagacacaatctgtatctgg	SmaI SalI
GST-MSL2 [573-708]	ttggaattcatggaggactacgttg	agtgtcgacctattccctgtcaggagca	EcoRI SalI
GST-MSL2 [618-642]	-	-	BamHI RsaI
GST-MSL2 [618-655]	-	-	BamHI DpnI
GST-MSL2 [618-667]	-	-	BamHI PvuII
GST-MSL2 [618-687]	-	-	BamHI MunI
GST-MSL2 [651-708]	cagggatccaagcccttgatccggtc	agtgtcgacctattccctgtcaggagca	BamHI SalI
GST-MSL2 [630-655]	ctcggatccatgcagcatcctttggt	gtggaattcctaatcaaggggcttg	BamHI EcoRI
GST-MSL2 [641-655]	-	gtggaattcctaatcaaggggcttg	BamHI EcoRI
Fly constructs			
pSK msl2-Fx3 full	ttcgatatcatggcccagacggcatactt	tctcccgggcaagtcatccgagcccga	EcoRV SmaI
pSK msl2-Fx3 Δ36	accgatatcatggagggcatggatctg	tggactagttaggcttatgggggcagaa	EcoRV SpeI
	ctcactagtcttgatccggtcaccgt	tctcccgggcaagtcatccgagcccga	SpeI SmaI
pSK msl2-Fx3 Δ13d	ttcgatatcatggcccagacggcatactt	gaccggatcctcgcctttctcattct	EcoRV SmaI
	aaaggcgaggatccggtcaccgttgg	tctcccgggcaagtcatccgagcccga	
pSK msl2-Fx3 R543A	atcgaattccgcctgtcctt	tctcccgggcaagtcatccgagcccga	EcoRI SmaI
pSK msl2-Fx3 ΔCXC	ttcgatatcatggcccagacggcatactt	cggattcttcttcggcttcggaggct	EcoRV SmaI
	aagccgaagaagaatccgcacaagga	cccgggcaagtcatccgagcccga	
pSK msl2-Fx3 R543A ΔCXC	atcgaattccgcctgtcctt	tctcccgggcaagtcatccgagcccga	EcoRI SmaI
yil Ubi msl2-Fx3 full attB	-	-	EcoRV NotI
yil Ubi msl2-Fx3 Δ36 attB	-	-	EcoRV NotI
yil Ubi msl2-Fx3 Δ13d attB	-	-	EcoRV NotI
yil Ubi msl2-Fx3 R543A attB	-	-	EcoRV NotI
yil Ubi msl2-Fx3 ΔCXC attB	-	-	EcoRV NotI
yil Ubi msl2-Fx3 R543A ΔCXC attB	-	-	EcoRV NotI