Supplementary Figures

20 4 <u>0</u> RING finger 60 80
Dmel/Msl2 (1)MAQTAYLKVTRIAMRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dsim/GD15895 (1) -WLAQTAYLKVTRISLRPASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dsec/GM11132 (1)MLAQTAYLKVTRISLRPASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dyak/GE14867 (1)MLAQTAYLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dana/GF23410 (1)MSAMSVVLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dana/GF23410 (1)MSAMSVVLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKHLEPSCTQCGCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKLEPSCTQCGCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKLEPSCTQCGCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYLKVTRISLRSASNISKRVEELNSGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGKKLEPSCTQCGCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYCKVVRISIRSAVNISKRRIDELSGGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLMSRCRQCEDCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYCKVVRISIRSAVNISKRRIDELSGGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLMSRCRQCEDCSDFKTYEENRAM Dper/GL8894 (1)MAQTAYCKVVRISIRSAVNISKRRIDELSGGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLMSRCRQCEDCSDFKTYEENRAM Dper/GL8892 (1)MAQTAYCKVVRISIRSAVNISKRRIDELSGGIGELRQLISCVVCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLMSRCRQCEDCSDFKTYEENRAM Dper/GL8892 (1)MAQTAYCKVCRSISSANNISKRRIDQISIGELRQLISCVCCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLEPRCDQCGDCSDFKTYEENRAM Dgri/GH10188 (1)MAQKLYTKIISINSANNISKRRIDQISIGELRQLISCVCCQLIVDPYSPKGKRCQHNVCRLCLRGRKRLEPRCDQCGDCSDFKTYEENRAM Dwi/GK23860 (1) MAAAQKAYAKIIDISCRLEPRCDUSTKFKDKCGHNVCRLCLRGRKRLIPSCAQCNDCCDFKTYEENRAM Dvir/msl-2 (1)MAAQKLYTKIISINSNISKRRIQELNSGIGELRQLISCAVCCQLISCOCCLINDPYKFKDKCGHNVCRLCLRGRKRLIPSCAQCNDCCDFKTYEENRAM Dwi/GK23860 (1) MAAAQXAYAKIIDISKRIQENSISTRIAGELSCAUCCCALLVDPYSFKGHRCDHFVCRLCWRGKKRINFICPKCNDCSDFKTYEENRAM
100 120 140 160 180 Demol/Msi2 (97) AAC LLCYKTLC VHLH SALFG LAGM RPOVAR BLVPR IK LPPKTYQEFIR BC SNIS - DTFDIFL OPPDLPE KOMP TS LPAET PTTA Dsim/GD15895 (98) GTC LLCYKTLC VHLH SALFG LAGM RPOVAR ELVPR IK LPPKTYQEFIR BC SNIS - DTFDTFL OPPDLPE KOMP TS LPAET PTTA Dsec/GM1112 (98) GTC LLCYKTLC VHLH SALFG QLAGM RPOVAR ELVPR IN LPPKTYQEFIR BG SNIS - DTFDTFL OPPDLPE KOMP
Dmel/Msl2 (184) VT TPELFYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGMLSHAG QIVIATESSESGFMDQAWTDQVDLSGAVS 260 Dsim/GD15895 (185) AT TPELPYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGLLSHAG QIVIATE SSESGFMDQAWTDQVDLSGAVS 260 Dsec/GM11132 (185) AT TPELPYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGLLSHAG QIVIATESSESGFMEQAWTDQVDLSGAVS 260 Dsec/GM11132 (185) AT TPELPYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGLLSHAG QIVIATESSESGFMEQAWTDQVDLSGAVS 260 Dysk/GE14867 (185) AT TPELPYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGLLSHAG QIVIATESSESGFMEQAWTDQVDLSGAVS 260 Dere/GC24438 (185) AT TPELPYD HHINISDIABA - AATAEQGHFS - PLPLLPTGSRMGLLSHAG QIVIATESSESGFMEQAWTDQVDLSGAVS 260 Dara/GF23410 (190) AT TPELPYD HI PEHOLS ISDIZEA - AATAEQGFFS - PLULPTGSRMGLISHAG QIVIATESSESGFMEQAWTDQVDLSGAVS 20 Dper/GL38894 (186) AT TPELPYEQHLPEQLSITD IEMEA - AATAEQGFFS -PLULPTGSRMGLISHAG QIVIATE
280 300 Dsm/G/15895 (262) MS KY TN S GN - NFAVS YVM TS ATT TF D POELO IG QV QVA DS TQ
Dmel/ Msl2 (305) IAVLAAVEETV Dsim/GD15895 (302) VEETV Dsec/GM11132 (302) VEETV Dyak/GE14867 (306) VEETV Dere/G24438 (306) IAVLAAVEETV Dere/G24438 (306) IAVLAAVEETV Dere/G24438 (306) IAVLAAVEETV Dere/G24438 (306) IAVLA-VEETV Dere/G24438 (306) IAVLA-VEETV Dere/G24438 (307) EVQNQAVAPASVSQLVLQQEACLENVSHDAAVLQNIIQDEAYLENVSQEEGYLETVSQESFLESVSQLVIPEIVSQEKSQEMVHQDTTQESVYQEKSQ Dpse/GA16882 (370) EVQNQAVAPASVSQLVLQQEACLENVSHDAAYLQNIIQDEAYLENVSQEEGYLETVSQESFLESVSQLVIPEIVSQEKSQEMVHQDTTQESVYQEKSQ Dpse/G118894 (370) EVQNQAVAPASVSQLVLQQEACLENVSHDAAYLQNIIQDEAYLENVSQEEGYLETVSQESFLESVSQLVIPEIVSQEKSQEMVHQDTTQESVYQEKSQ Dpse/G11888 (324) TSYKRRYAELLDEVQQ Domj/G114252 (316) S24 Dvii/msl-2 (309) LAEVATWE PME Dvii/msl-2 (309) QQ Dwii/GK23860 (358) PRP
320 340 340 340 340 340 340 340 34
380 400 420 Dmel/ Msl2 (362)



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.

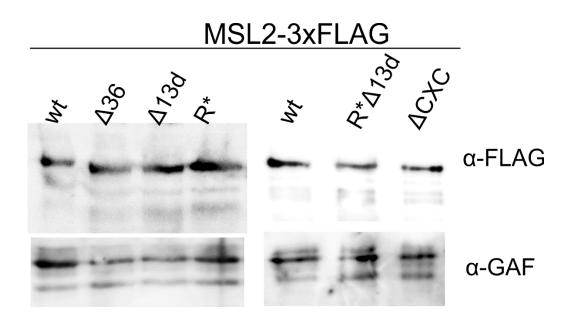
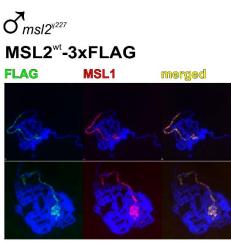
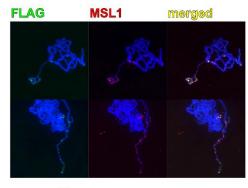


Figure S2.

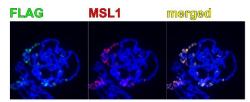
Western blotting of protein extracts prepared from adult flies expressing different variants of 3xFLAG-tagged MSL2 protein (wild-type (wt), deletion of 36 (Δ 36) and 13 (Δ 13d) aa from CLAMP-binding region, substitution of arginine at the 543 position by alanine (R*), simultaneous substitution of arginine at the 543 position by alanine and deletion of 13 aa from CLAMP-binding region (R* Δ 13d), deletion of CXC domain (Δ CXC)). Immunoblot analysis was performed with FLAG and GAF antibodies (internal control).



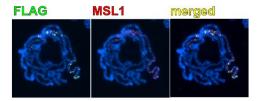
MSL2^{∆13d}-3xFLAG



MSL2^{R*}-3xFLAG



MSL2^{∆cxc}-3xFLAG



MSL2^{R*Δ13d}-3xFLAG

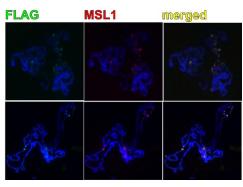
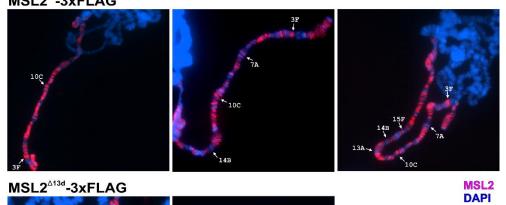


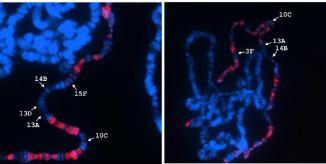
Figure S3. MSL1 and MSL2 localization on the polytene chromosomes from salivary glands of male 3rd instar larvae in the *msl2*-null (*msl2*^{γ 227}) background that express different FLAG-tagged variants of MSL2 protein: wild-type (wt), deletion of 13 aa from CLAMP-binding region (Δ 13d), substitution 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* Δ 13d). 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). Development: doi:10.1242/dev.179663: Supplementary information

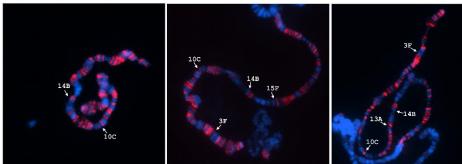
O^msl2^{v27} MSL2^{wt}-3xFLAG



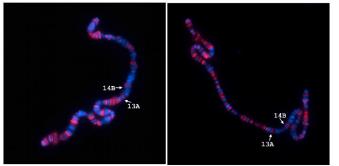
MSL2^{∆13d}-3xFLAG



MSL2^{R*}-3xFLAG



MSL2^{∆CXC}-3xFLAG



MSL2^{R*A13d}-3xFLAG

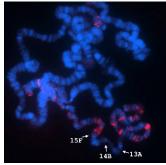


Figure S4. Comparing several sites of MSL complex binding at the high resolution cytological map on the polytene chromosomes from salivary glands of male 3rd instar larvae in the *msl2*-null $(msl2^{\gamma 227})$ background that expressed different FLAG-tagged variants of MSL2 protein: wildDevelopment: doi:10.1242/dev.179663: Supplementary information

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

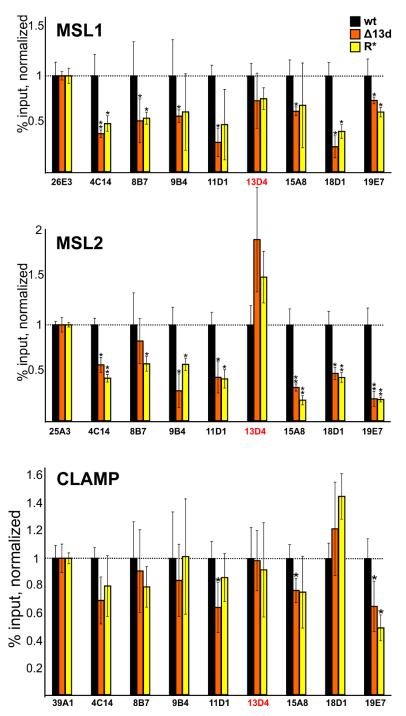


Figure S5. Comparing binding of MSL1, MSL2, and CLAMP at different CES and PionX (marked with red) regions in the MSL2-expressing males in the *msl2*^{γ 227} background. Histograms show ChIP enrichments at the CES regions on chromatin isolated from male flies expressing different variants (wt, Δ 13d, 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.

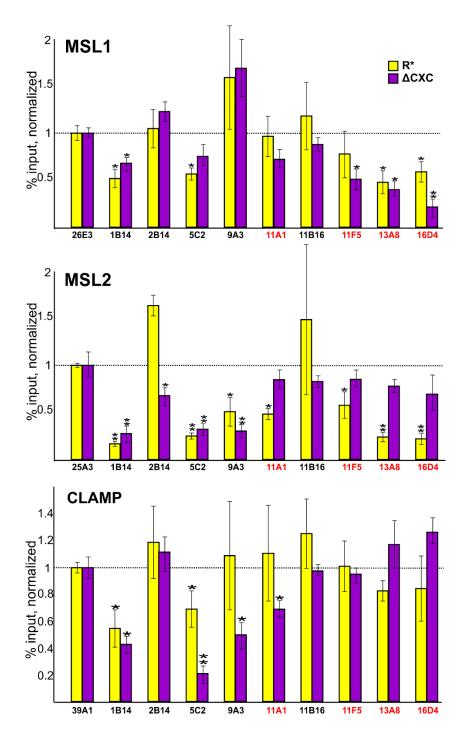


Figure S6. Comparing binding of MSL1, MSL2, and CLAMP at different CES and PionX (marked with red) regions in the MSL2-expressing flies in the *msl2*^{γ 227} background. Histograms show comparison of ChIP enrichments at the CES regions on chromatin isolated from male flies expressed MSL2 protein with substitution of arginine at the 543 position by alanine (R*) and deletion of CXC domain (Δ CXC). 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.

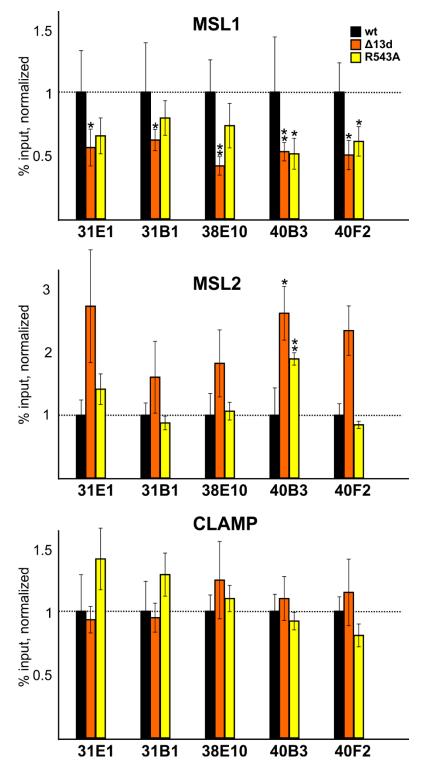


Figure S7. Comparing binding of MSL1, MSL2 and CLAMP at different autosomal genome regions in the MSL2-expressing flies in the *msl2*^{γ 227} background. Histograms show ChIP enrichments at the CES regions on chromatin isolated from male flies expressing different variants (wt, Δ 13d, R543A) of MSL2 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.

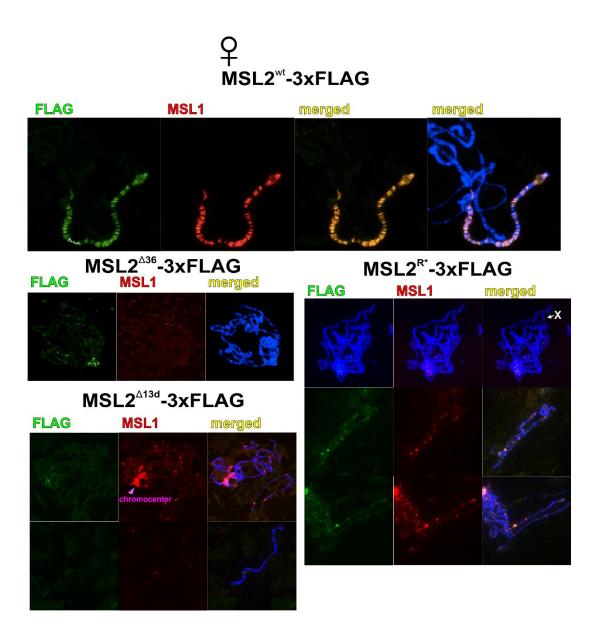


Figure S8. Distribution of MSL complex on the polytene chromosomes extracted from salivary glands of female 3^{rd} instar larvae that express different FLAG-tagged variants of MSL2 protein: wild-type (wt), deletion of 36 (Δ 36) and 13 (Δ 13d) aa from CLAMP-binding region, substitution of arginine at 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).

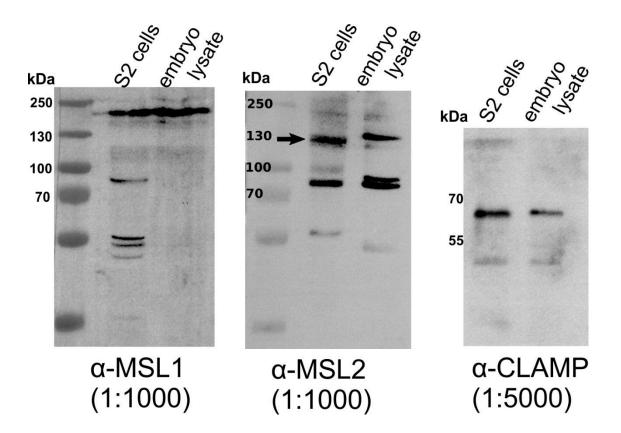


Figure S9. Immunoblot analysis of affinity-purified antibodies for MSL1, MSL2, CLAMP proteins.

Supplementary Table

Table S1. The list of used oligonucleotides.

Primers for clo	ning of msl2 deletions]	
Name dir		rev	1
msl2_full-sized	TTCGATATCATGGCCCAGACGGCATACTT	CCCGGGCAAGTCATCCGAGCCCGA	1
msl2_d13	AAAGGCGAGGATCCGGTCACCGTTGG	GACCGGATCCTCGCCTTTCTCATTCT	1
msl2_dB	CCACCGGCCGCCAACTTCTCGGCCCT	GAAGTTGGCGGCCGGTGGCTCGATGA	1
msl2_dP	TATATAATGGCCAAGAAATTCAGGACC	TTTCTTGGCCATTATATAAGCATACT	1
Primers for RT	-qPCR	1	1
Name	dir	rev	Taqman probe
RoX1	CTTGTGCTTTCTCCTGAATGTG	TGTATTAGGCGGAGCTTCTTG	FAM-AGCCTATGAAATCCGGTCCAACCC-BHQ1
RoX2	TTCGAAACGTTCTCCGAAGC	AGTCGTACTCATCTCACTGTCC	FAM-AGCAAGAGTAACGATTTCCGCATAGTCG-BHQ
RpL34	ACAACACGCTCCAACA	GGGTGATACCCTTCAACTTCTC	FAM-TGGTAAACCAGACGACCACCGG-BHQ
Primers for Ch	IP analysis	ł	
Genome region	dir	rev	1
RoX1	AGGTCTGCAAGGTTCAGTTTAT	ATTCTTAAGGGTGGCGTCTTC	1
RoX2	GGCTTAGAGAGAGATGGCAATAC	AGTTCTGGTCACCCTGGA	1
CES1B14	CAGGACAAGACTAGGACAAAGG	GGTTTGCGGATTGAGGATTATTG	1
CES2B14	CCCTCATATGCTTCTTCTGGTC	ACGTACGATCACGCACATATC	1
CES4C14	ATTGGTATTCGGGCAAGGG	GAGCATATTTCTCTCGAGGATGG	1
CES5C2	CAGAAATTCGAAGCGATCTCAAC	AATCGACTGCTAGGTTGGTAAA	1
CES8B7	CAGCAGAACGCTCTTTGATTT	CCAACATTCGCTTCACACAC	1
CES9A3	TAATGCTGGATCTCGCTCAC	CTGATCGCCGGTCATAGAAA	1
CES9B4	CGTGCCGCCTAACTATCTAAC	GGGATGGAAAGAGAGAGAGAAA	1
CES11A1	GCCCGGAACTCCTTTAGTATG	TGATGCCACTGGATGAGTATG	1
CES11B16	GGTGGTGGACATCTCGTTAAT	ACCAGCGAATATCGAGCATAAA	1
CES11D1	AAGCCACTGATGCGTACAA	CAGAACGGCTGGCAAGATA	1
CES11F5	TACAGTAGCTGAGAGCTGTACT	GTTTGGACTTGCGCCTTTAC	1
CES13A8	AATCTGAGCGAGATGGAAGAAC	GTGTCTAGTGGTTGGCTATGAC	1
CES13D4	GCCGTGATTGTGGATCTCTT	CTCCAGACGTGCTGAATTGT	1
CES15A8	CCAGGCTAAATAGTTCGCTACA	CGTAGTTGCATCTCGCTCTAAT	1
CES16D4	CATCAACGACCTTGTCACATTATC	GCACGAATCAGACAGAGAAGTA	1
CES18D11	GCGCTTGCAATAGCTTCAATTA	GGAAATCCAAACAGTACAACTTCG	1
CES19E7	GAGCGAGAGAGAGATTGCCAAATA	CGAATCGTGGAGTCTGAGAAAT	1
26E3	CGTAACGGCACCCCTCAA	ACCGCACCGCACTACAAG	1
25A3	GCTCCAGGAACCGATCTATTG	CTTGGCTTCCACTGAAGTTAGA	1
39A1	CTCTTGTTCACACAGCCATTTC	TACTCTCTTCGGGCGGTATAA	1
31E1	TGGATATGGCTTCTGGTTCATC	GGGCTGCATTCGGAGTTTA	1
31B1	TTGTAAGGCGACTCGACTATTT	TGCTTTCGGCTGACTAATGA	1
38E10	CGACACAAGTACCAGCTCTAAT	CGGCGAGGTACTTACCATATTC	1
40B3	GCAGAAGGGAGAACTGTGAAATA	CTTCGCGGAGGGTTAATTGT	1
40F2	CAGAACAACGCTCAGAGATAGA	GGGCTCTAAGAAATCCTACCAG	1

Table S2. Cloning procedures

Pull down assay						
	dir	rev	Restriction sites			
TRX-His-MSL2 [573-708]	ttggaattcatggaggactacgttg	agtgtcgacctattccctgtcaggagca	EcoRI Sall			
TRX-His-CLAMP [1-153]	ctggaattcatggaagaccttaccaa	aacgtcgacttccccgtctgtatgcat	EcoRI Sall			
TRX-His-CLAMP [40-153]	ctggaattcatgaaaacggagcagcagc	aacgtcgacttccccgtctgtatgcat	EcoRI Sall			
TRX-His-CLAMP [86-153]	-	-	Hincll Sall			
TRX-His-CLAMP [116-153]	-	-	Munl Sall			
GST-CLAMP [1-196]	taccccgggatggaagaccttaccaaaaac	aacgtcgacagacacaatctgtatctgg	Smal Sall			
GST-MSL2 [573-708]	ttggaattcatggaggactacgttg	agtgtcgacctattccctgtcaggagca	EcoRI Sall			
GST-MSL2 [618-642]	-	-	BamHl Rsal			
GST-MSL2 [618-655]	-	-	BamHl Dpnl			
GST-MSL2 [618-667]	-	-	BamHl Pvull			
GST-MSL2 [618-687]	-	-	BamHl Munl			
GST-MSL2 [651-708]	cagggatccaagcccttgatccggtc	agtgtcgacctattccctgtcaggagca	BamHI Sall			
GST-MSL2 [630-655]	ctcggatccatgcagcatcctttggt	gtggaattcctaatcaaggggcttg	BamHI EcoRI			
GST-MSL2 [641-655]	-	gtggaattcctaatcaaggggcttg	BamHI EcoRI			
Fly constructs						
pSK msl2-Fx3 full	ttcgatatcatggcccagacggcatactt	tctcccgggcaagtcatccgagcccga	EcoRV Smal			
nSK mcl2 Ev2 A26	accgatatcatggagggcatggatctg	tggactagttaggcttatgggggcagaa	EcoRV Spel			
pSK msl2-Fx3 ∆36	ctcactagtcttgatccggtcaccgt	tctcccgggcaagtcatccgagcccga	Spel Smal			
pSK msl2-Fx3 ∆13d	ttcgatatcatggcccagacggcatactt	gaccggatcctcgcctttctcattct	EcoRV Smal			
μοκ msiz-Fx5 Δ150	aaaggcgaggatccggtcaccgttgg	tctcccgggcaagtcatccgagcccga				
pSK msl2-Fx3 R543A	atcgaattccgcctgtcctt	tctcccgggcaagtcatccgagcccga	EcoRl Smal			
pSK msl2-Fx3 ∆CXC	ttcgatatcatggcccagacggcatactt	cggattcttcttcggcttcggaggct	EcoRV Smal			
	aagccgaagaagaatccgcacaagga	cccgggcaagtcatccgagcccga				
pSK msl2-Fx3 R543A ΔCXC	atcgaattccgcctgtcctt	tctcccgggcaagtcatccgagcccga	EcoRl Smal			
yil Ubi msl2-Fx3 full attB	-	-	EcoRV Notl			
yil Ubi msl2-Fx3 ∆36 attB	-	-	EcoRV Notl			
yil Ubi msl2-Fx3 ∆13d attB	-	-	EcoRV Notl			
yil Ubi msl2-Fx3 R543A attB	-	-	EcoRV Notl			
yil Ubi msl2-Fx3 ∆CXC attB	-	-	EcoRV Notl			
yil Ubi msl2-Fx3 R543A						
ΔCXC attB	-	-	EcoRV Notl			