Supplementary Data

The DNA binding CXC domain of MSL2 is required for faithful targeting the Dosage Compensation Complex to the X chromosome

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Figure S1

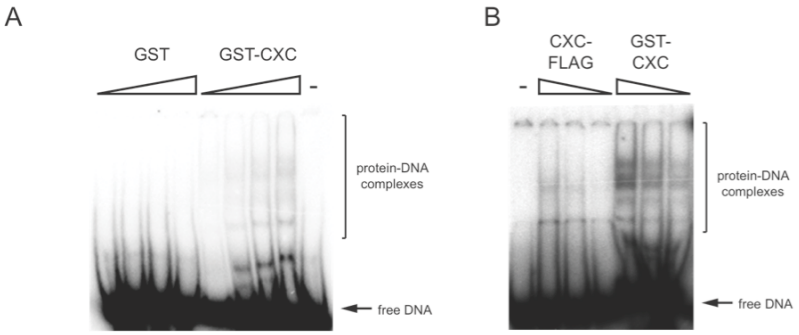

Figure S1: Binding of the isolated recombinant CXC domain to a DNA high affinity site *in vitro*. Increasing concentrations (1 - 8 µM) of (A) GST and GST-CXC or of (B) CXC-FLAG (5 - 20 µM) and GST-CXC (2 - 8 µM) were incubated with radiolabeled 40 bp DBF12-L15 dsDNA and protein-DNA complexes were separated from unbound DNA in non-denaturing polyacrylamide gels.

Figure S2

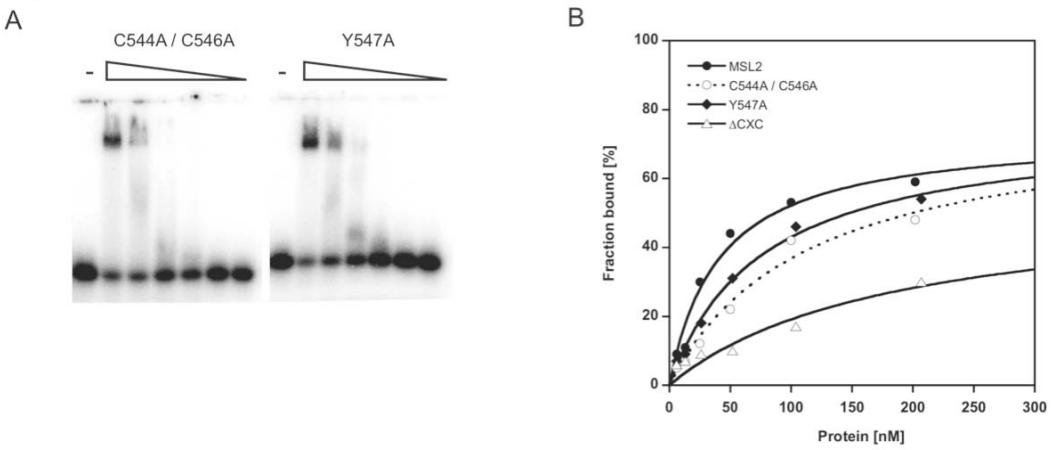

Figure S2: Binding of different recombinant MSL2 derivatives to a DNA high affinity site *in vitro*. Electrophoretic mobility shift assay. Increasing concentrations of MSL2 carrying point mutations in the CXC domain were incubated with radiolabeled 40 bp DBF12-L15 dsDNA and protein-DNA complexes were separated from unbound DNA in non-denaturing agarose gels. (B) Binding curves obtained from quantification of (A) and fitting to a standard bimolecular model. For comparison MSL2ΔCXC is also displayed.

Figure S3

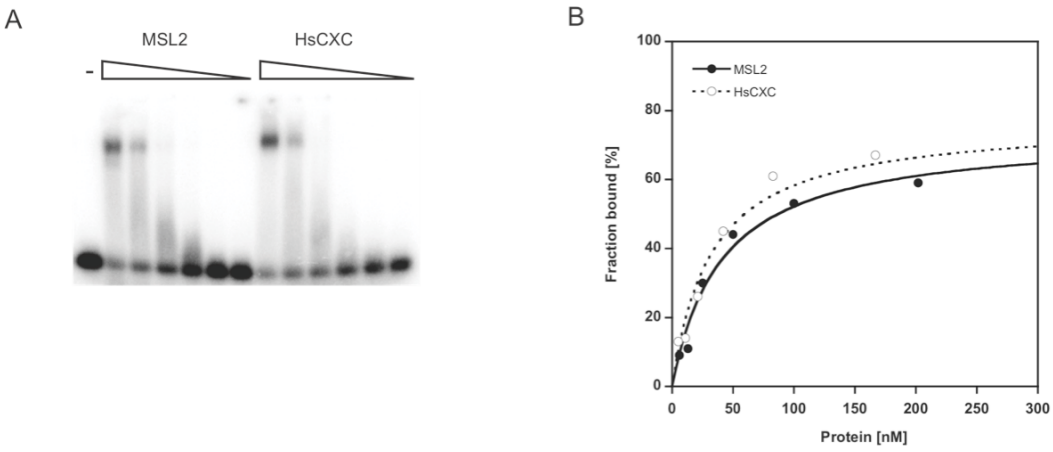

Figure S3: Binding of different recombinant MSL2 derivatives to a DNA high affinity site *in vitro*. Increasing concentrations of MSL2 and of the chimeric HsCXC were assayed as in Fig. S2.

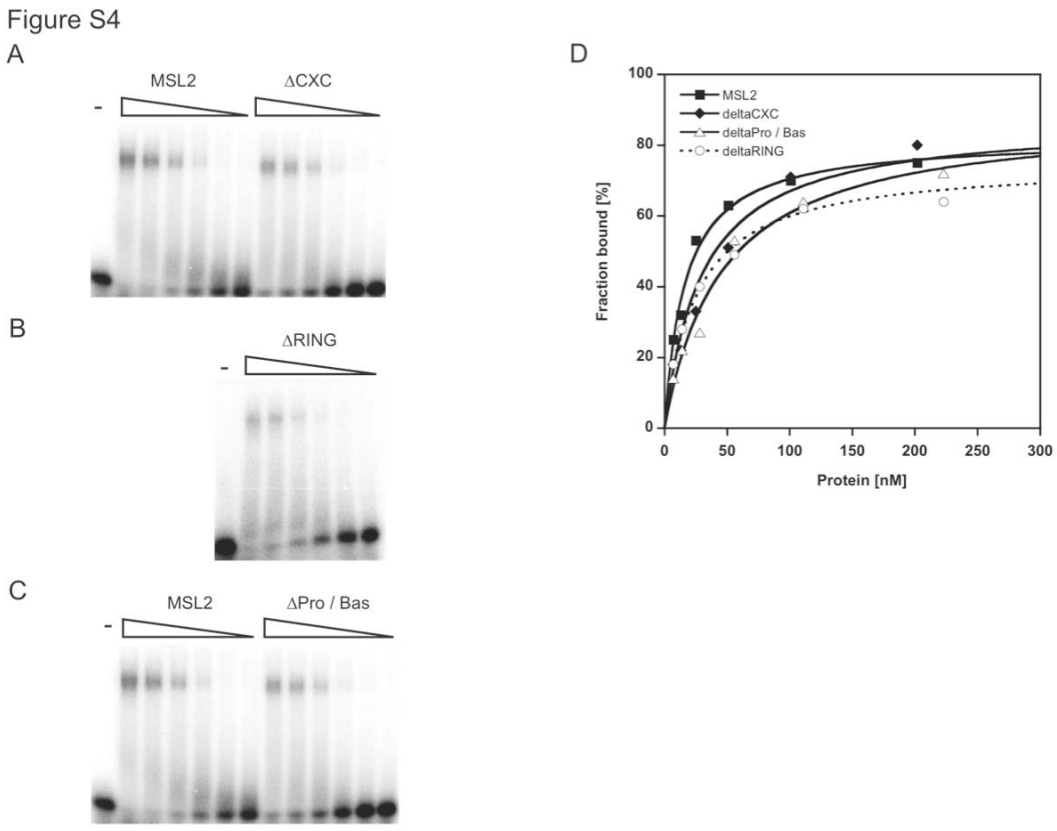

Figure S4: Binding of different recombinant MSL2 derivatives to RNA. Electrophoretic mobility shift assays. Increasing concentrations of MSL2 and MSL2-ΔCXC (A), MSL2-ΔRING (B) or MSL2 and MSL2-ΔPro/Bas (C) were incubated with radiolabeled dsRNA representing the DBF12-L15 sequence and protein-RNA complexes were separated from unbound RNA in non-denaturing agarose gels. (D) Binding curves obtained from quantification of EMSAs and fitting to a standard bimolecular model.

Figure S5

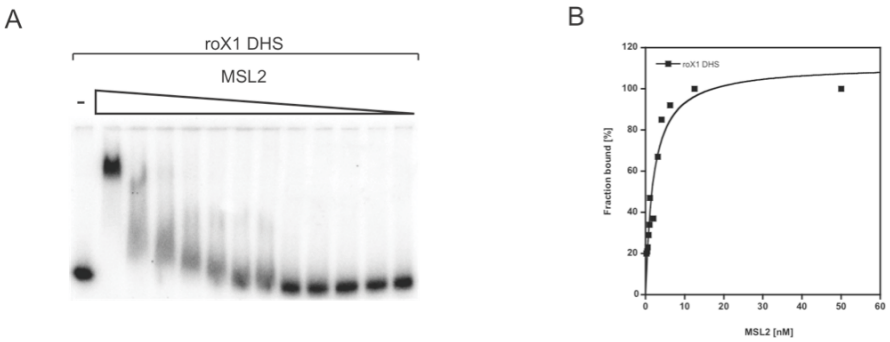

Figure S5: Binding of MSL2 to the HAS within the *roX*1 gene *in vitro*. Electrophoretic mobility shift assays. (A) Increasing concentrations of MSL2 were incubated with radiolabeled 226 bp DNA fragment representing the HAS within the *roX*1 gene (22) and protein-DNA complexes were separated from unbound DNA in non-denaturing agarose gels. (B) Binding curves obtained from quantification of (A) and fitting to a standard bimolecular model.

Figure S6

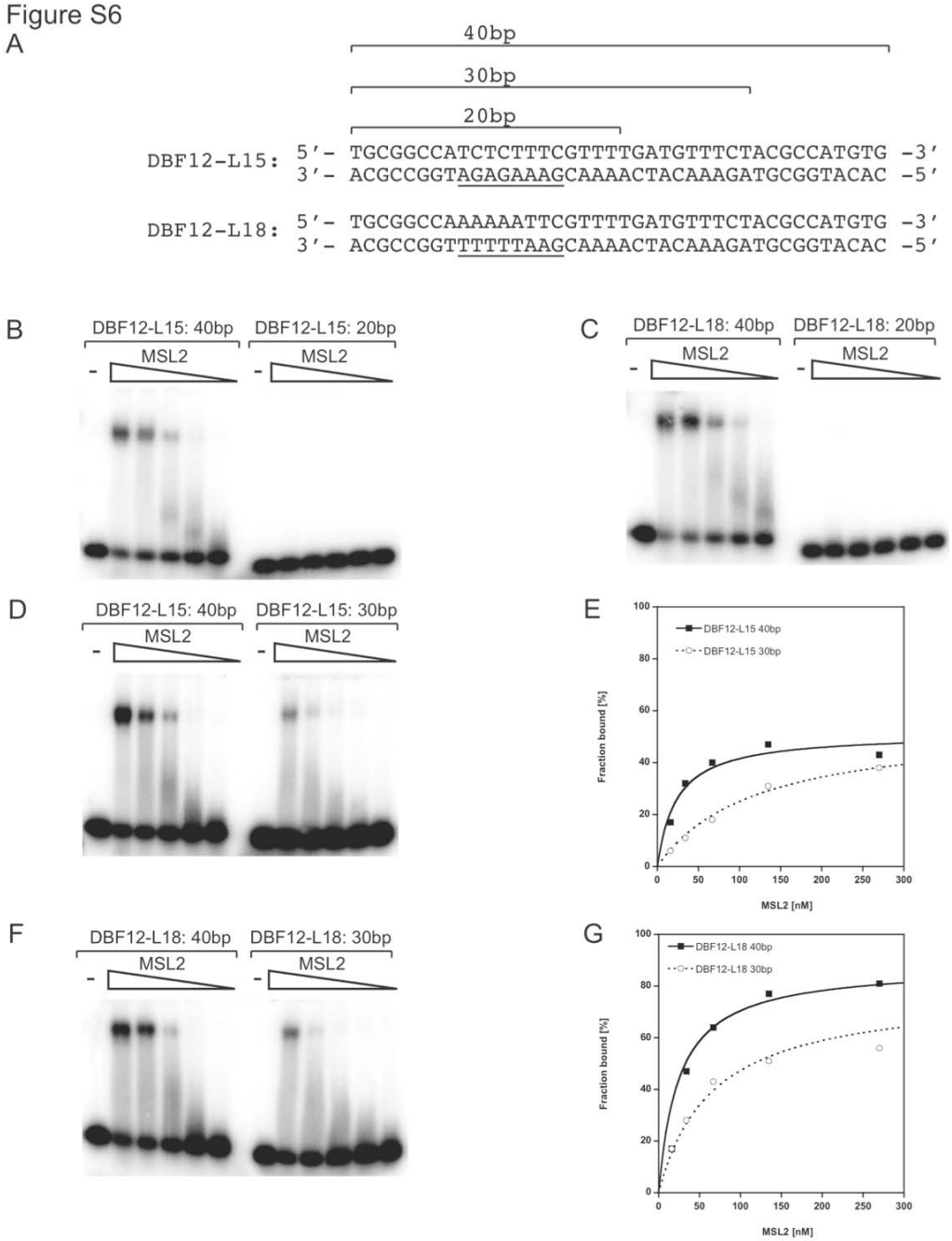

Figure S6: Binding of MSL2 to different DNA fragments *in vitro*. (A) Sequences of the DNA fragments used in EMSA. The GA-repeat of DBF12-L15 and the mutated sequence of DBF12-L18 are underlined. (B)-(G) Increasing concentrations of MSL2 were assayed as in Fig. S2.

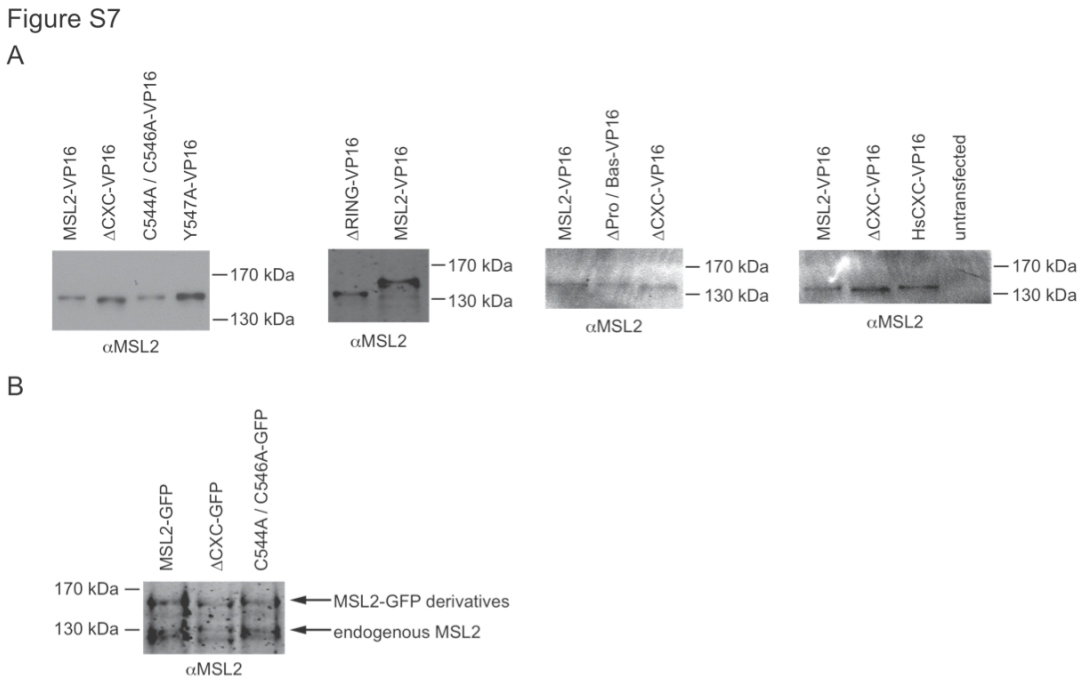

Figure S7: Western blots showing expression of different MSL2 derivatives in SL2 cells. (A) Transiently transfected SL2 cells were harvested, lysed and proteins precipitated using TCA. Precipitated proteins from roughly 2.5 x 10^5 cells were subjected to western blot analysis. (B) Stable SL2 cell lines were harvested, lysed and roughly 5 x 10^4 cells were subjected to western blot analysis. A rabbit anti-MSL2 antibody was used to detect the endogenous and the different MSL2-GFP derivatives in all blots.