Supplemental Material

For

Regulation of Sexual Dimorphism: Mutational and Chemogenetic Analysis of the Doublesex DM domain

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Figure S1. Single-site complementation of methyphosphonate modifications. (A) Sequences of unmodified *dsx*^{A} probe (a), top variant (b), and bottom variant (c). Central base pair is indicated by arrow in each sequence. Solid balls indicate sites of interference. (B) Top and bottom modifications impair binding of unmodified protein by about ten-fold. DNA sites: lane 1, control site; lane 2, top variant; and lanes 3 and 4, bottom variant. The concentration of active protein was approximately 12 nM in lane 1, 125 nM in lanes 2 and 3 and 250 nM in lane 4. Percentages of DNA probe shifted to the C1 and C2 forms are: lane 1 (C1 1% and C2 8%); lane 2 (6% and 5%); lane 3 (1% and 6%); and lane 4 (2% and 19%). Binding of the native domain to the top DNA variant is reduced by about 10-fold ($\Delta\Delta G \sim 1.3$ kcal/mole) whereas binding to the bottom DNA variant is reduced by between 5- and 10-fold ($\Delta\Delta G \sim 1.0$-1.3 kcal/mole). (C) GMSA gel showing binding of the K60Z domain to control DNA site, top variant, and bottom variant. The top and bottom modifications impair binding of variant domain by less than two-fold ($\Delta\Delta G < 0.4$ kcal/mole). Concentrations of K60Z domain (lanes 1-5) are: (1) 48 nM, (2) 96 nM, (3) 144 nM, (4) 240 nM, and (5) 500 nM. The series of protein concentrations in corresponding top and bottom studies (lanes 6-10 and 11-15, respectively) is the same. Percentages of DNA probe shifted to the C1 and C2 forms for the control site are as follows: lane 1 (C1 1% and C2 8%); lane 2 (4% and 33%); lane 3 (3% and 70%); lane 4 (non-detectable and 95%); and lane 5 (non-detectable and 96%). For the top DNA variant: lane 6 (C1 < 0.5% and C2 1%); lane 7 (1% and 15%); lane 8 (2% and 21%); lane 9 (2% and 76%); and lane 10 (1% and 83%). For the bottom DNA variant: lane 11 (C1 3% and C2 6%); lane 12 (5% and 15%); lane 13 (6% and 29%); lane 14 (6% and 37%); and lane 15 (4% and 77%).

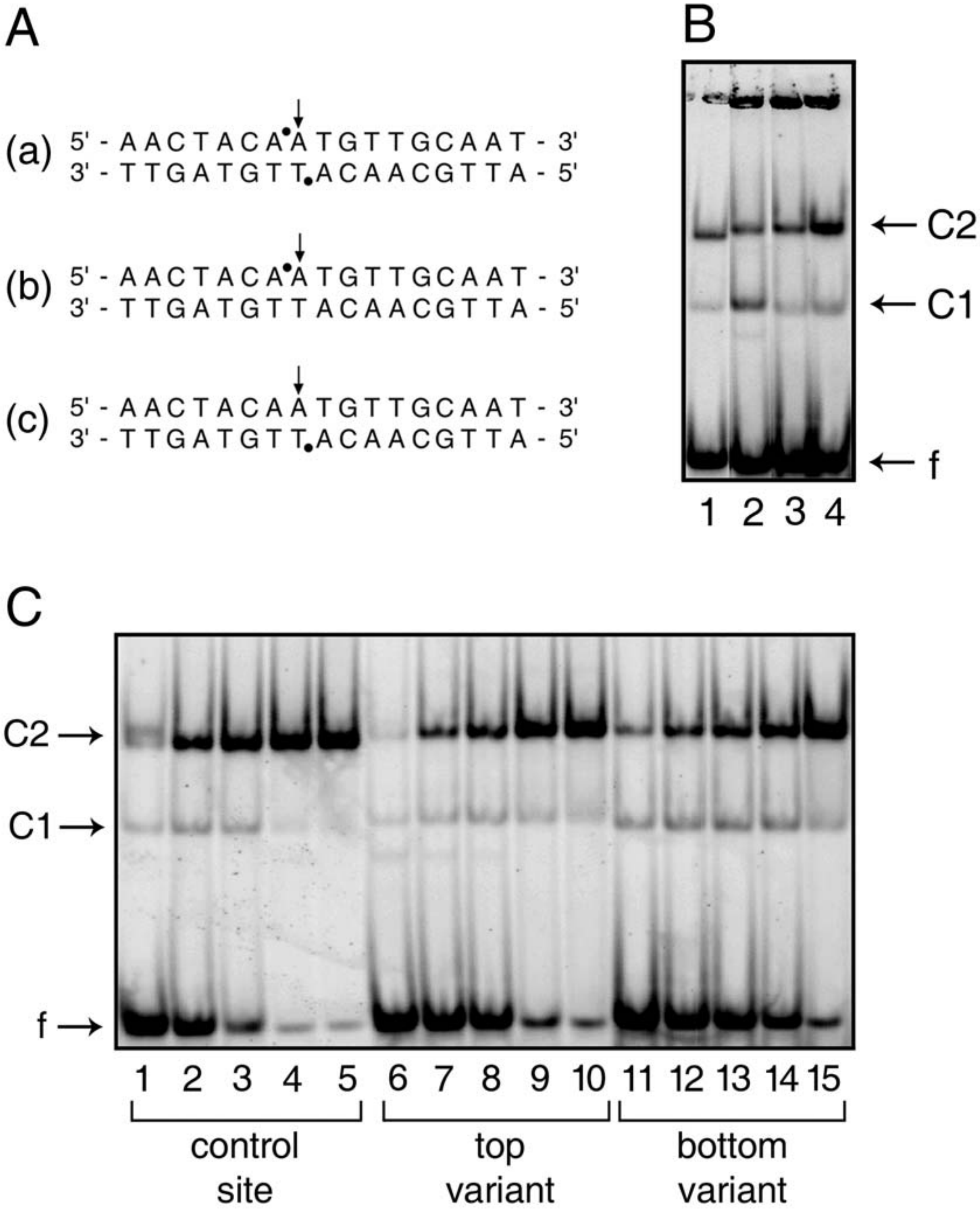
A
(a) 5' - AACTACAATGTTGCAAT - 3'
3' - TTGATGTTACAACGTTA - 5'
(b) 5' - AACTACAATGTTGCAAT - 3'
3' - TTGATGTTACAACGTTA - 5'
(c) 5' - AACTACAATGTTGCAAT - 3'
3' - TTGATGTTACAACGTTA - 5'
B
C2
C1
f
1 2 3 4
C
C2
C1
f
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
control site
top variant
bottom variant

Figure S1