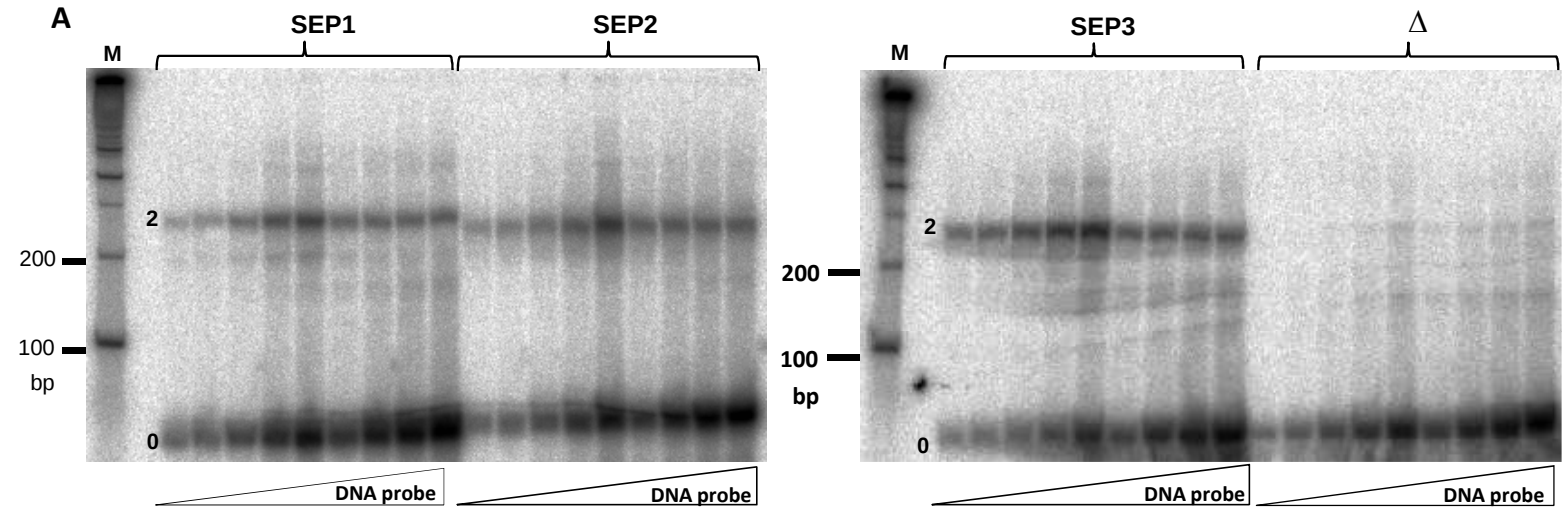
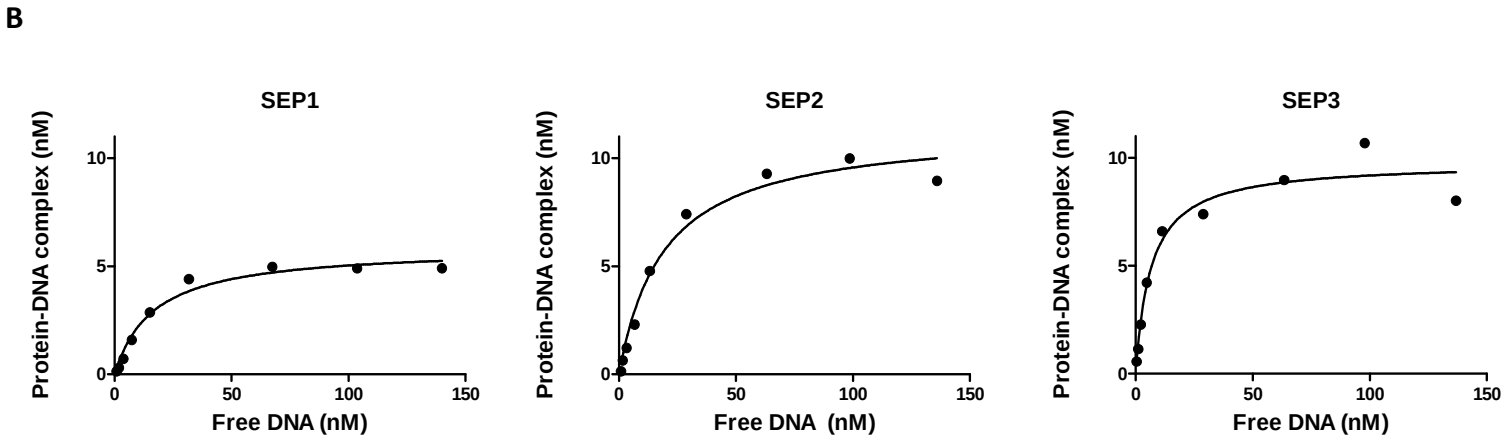
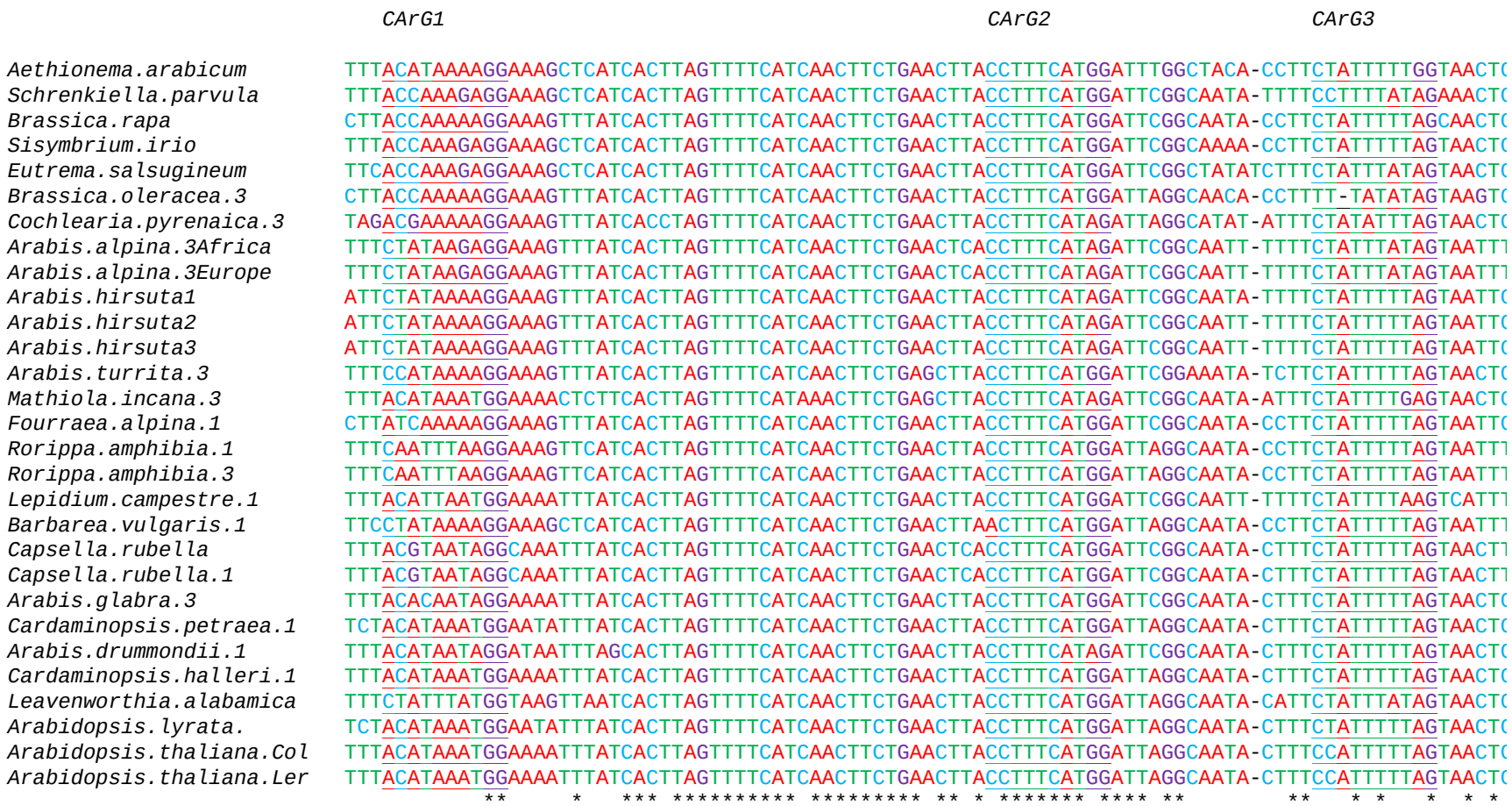
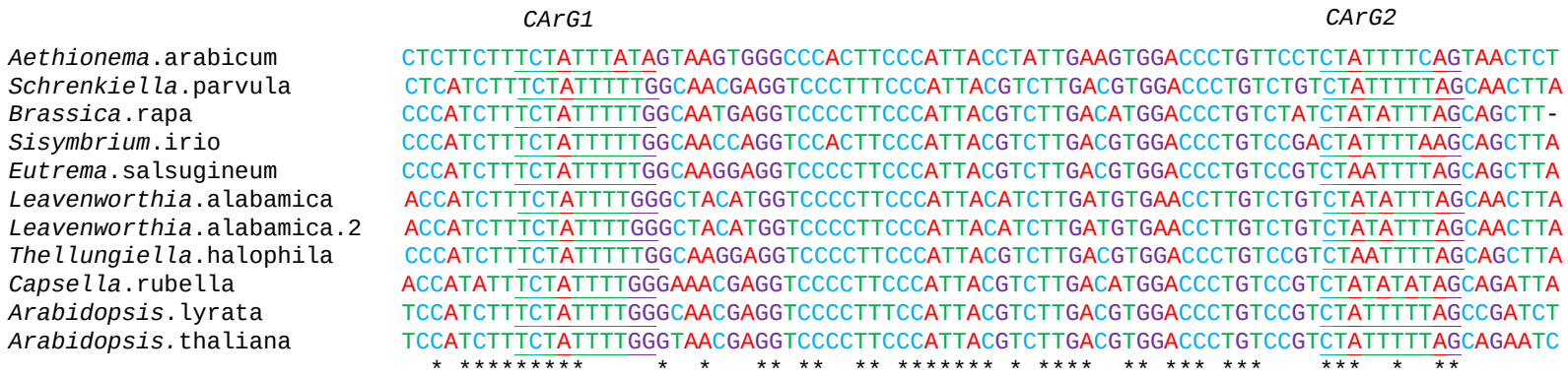




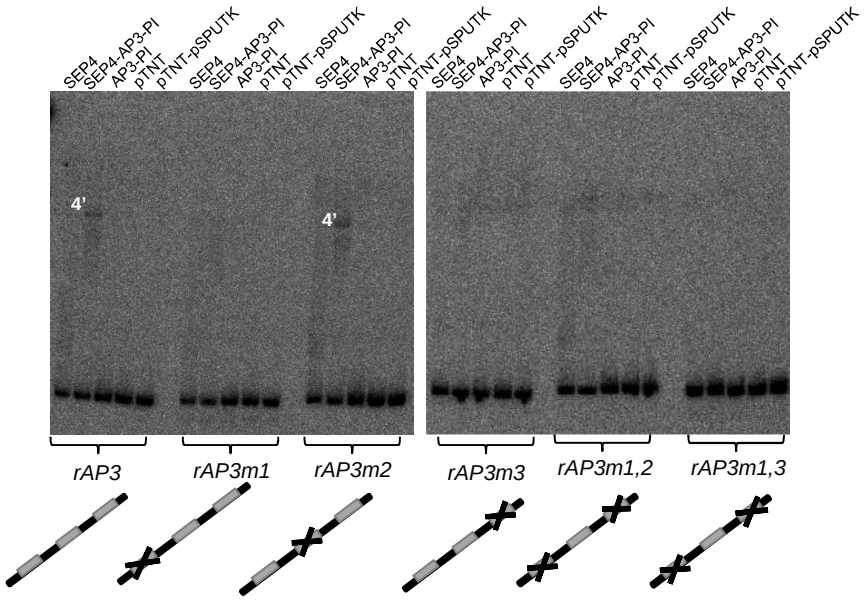
Supplementary Figure 1. (A) Gels demonstrating the binding of SEP1, SEP2 and SEP3 to a DNA probe having a single CArG-box. Increasing concentrations of DNA were incubated with a constant amount of protein. The *in vitro* translated protein used is marked over the gel. Δ represents a negative control in which the *in vitro* translation assay was programmed only by a pTNT vector not containing a cDNA insert. The volumes of *in vitro* translated protein solutions used were: 2µl of SEP1 solution, 4µl of SEP2 solution and 2µl of SEP3 solution. The various concentrations of the DNA probe used (with each protein) were: 1.05, 2.25, 4.50, 9.00, 18.00 nM (each prepared by mixing labelled with unlabeled DNA in a ratio of 1:50), 36.23, 72.45, 108.60, and 144.90 nM (each prepared by mixing labelled with unlabeled DNA in a ratio of 1:500) in the same order as seen on the gel. The numbers, 0 and 2 adjacent to the bands represent the respective number of protein molecules bound. (B) Concentration of protein-DNA complex formed as a function of the concentration of free DNA. Graphs were calculated as described in materials and methods.



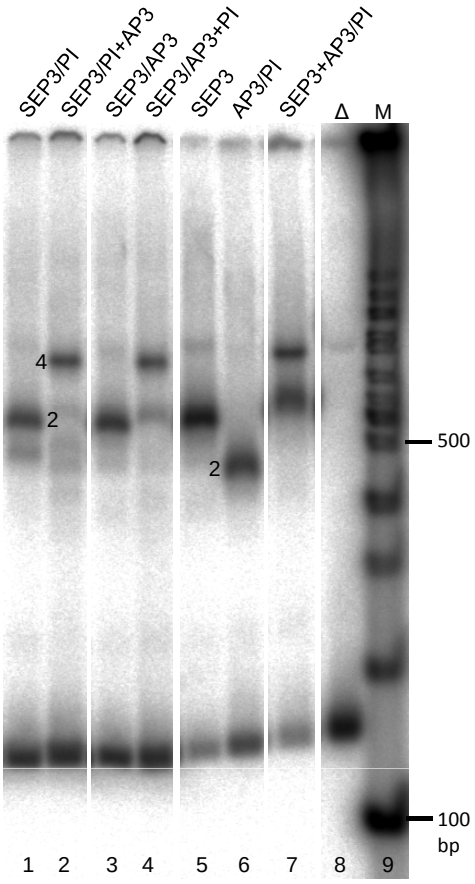
Supplementary Figure 2A. Alignment of the regulatory region of *AP3* from different *Brassicaceae* species. The regions where CArG-boxes are found in *Arabidopsis thaliana* are underlined. Identical positions across all the sequences are denoted by asterisks below the alignment.



Supplementary Figure 2B. Alignment of the regulatory region of *SEP3* from different *Brassicaceae* species. The regions where CArG-boxes are found in *SEP3* of *Arabidopsis thaliana* are underlined. Identical positions across all the sequences are denoted by asterisks below the alignment.



Supplementary Figure 3. Binding of SEP4 to CArG-boxes derived from the regulatory region of *AP3*. DNA probes used are denoted below the gel. The volume of each *in vitro* translated protein solution loaded was 5 µl. If more than one protein is denoted, co-translations were used. Δ-Δ' represents a negative control in which the *in vitro* translation assay was programmed by pTNT and pSPUTK vectors not containing a cDNA insert. 4' represents a potential heterotetrameric protein-DNA complex.



Supplementary Figure 4: AP3 or PI alone cannot form tetrameric complexes with SEP3. In this EMSA, a probe carrying only one CArG-box was used. SEP3 alone does barely constitute a homotetramer on this probe but heterotetramers of SEP3, AP3 and PI are readily formed, as demonstrated previously (21). When SEP3 is incubated alone or in combination with only AP3 or PI, only dimeric protein-DNA complexes are formed. However, when SEP3, AP3 and PI are present together, a complex of low electrophoretic mobility indicative of tetramer formation is reconstituted.

Lane and band labelling is as in Figure 1. Volumes of the *in vitro* translated protein solution added to the binding reaction were 2 µl in lanes 1, 3, 5 and 6; 2 µl +2 µl in lanes 2, 4, 7 (individual *in vitro* translations separated by '+' as noted above the gel) and 4µl in lane 8.

Approximately 2 ng of a DNA-probe with the sequence 5'-CTCGA *G*GTCG GAAAT TTAAT TATAT T<u>CCAA ATAAG G</u>AAAG TATGG AACGTT CGACGG TATCGA TAAGC TTGAT ATATG TTCAT CATAA AAATA TAATTC TCCTT GCCGT TTTAT ATCGA ATTCC TGCAG CCCGGG GGATC CACTA GTTCT AGA-3' was used. The CArG-box is underlined.

Supplementary Table 1: Primer pairs used for the amplification and cloning of the SEP cDNAs into pTNT vector. Restriction sites are italicized.

cDNA	Primer pairs used for cloning: forward, reverse (5'-3')
SEPALLATA1	5'-AATGAT*CTCGAG*CCATGGGAAGAGGAAGAGTAGAGCTG-3', 5'-ACTTAT*GTCGAC*TCAGAGCATCCACCCCG-3'
SEPALLATA2	5'-TTATAT*CTCGAG*CCATGGGAAGAGGAAGAGTAGA-3', 5'-TACATA*GTCGAC*TCACAGCATCCAGCCAGGGAT-3'
SEPALLATA4	5'-AGACTA*CTCGAG*AGCCATGGGAAGAGGGAAAGTTGAG-3', 5'-CGAA*TCTAGA*GTATTGTTTTCAGACCATCCATCC-3'

Supplementary Table 2: DNA probe sequences used in the study. The restriction sites used to excise the DNA probes from the vector are italicized. CArG-boxes are underlined and mutated CArG-boxes are underlined with broken lines.

DNA probe	Cloned in	Recognition sites used for excision from plasmid	Sequence of the DNA probe used, 5'-3'
DNA probes used to check specific binding by the SEP proteins (used in Figure 1)			
DNA probe with 2 CArG boxes spaced by 6 helical turns	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTCGACGGTATCGATAAGCTTGATGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTATCGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*-3'
DNA probe with 1 CArG box	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGATAAAACGGCAAGGAGAATTATATTTTTATGATGAACATATCGACGGTATCGATAAGCTTGATGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTATCGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*-3'
DNA probe with 0 CArG box	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGATAAAACGGCAAGGAGAATTATATTTTTATGATGAACATATCGACGGTATCGATAAGCTTGATATAAAACGGCAAGGAGAATTATATTTTTATGATGAACATATATCGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*-3'
DNA probes used for the stereospecific DNA binding and cooperative DNA binding assays (used in Figure 2 and 3)			
CArG-boxes spaced by 1 helical turn	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGACCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*- 3'
CArG-boxes spaced by 1.5 helical turns	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAATTCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*- 3'
CArG-boxes spaced by 2 helical turns	pBluescript SK II+	*XhoI, XbaI*	5'-*CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAGTAATATTCCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*- 3'

CArG-boxes spaced by 2.5 helical turns	pBluescript SK II+	*XhoI, XbaI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGTTA TATTCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*- 3'
CArG-boxes spaced by 3 helical turns	pBluescript SK II+	*XhoI, XbaI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAA TTAATTATATTCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCCACTAGT*TCTAGA*- 3'
CArG-boxes spaced by 3.5 helical turns	pBluescript SK II+	*XhoI, SpeI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAA CGAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCC*ACTAGT*- 3'
CArG-boxes spaced by 4 helical turns	pBluescript SK II+	*XhoI, SpeI*	*5'-* *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAA CGTTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTGAATTCCTGCAGCCCGGGGGATCC*ACTAGT*- 3'
CArG-boxes spaced by 4.5 helical turns	pBluescript SK II+	*XhoI, SpeI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCAAATTTAATTATATTCCAAATAAGGAAAGTATGGAAC GTTCGAGATGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTGAATTCCTGCAGCCCGGGGGATCC*ACT AGT*- 3'
CArG-boxes spaced by 5 helical turns	pBluescript SK II+	*XhoI, SpeI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCTTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTC GACGGCTTGATGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAAGAATTCCTGCAGCCCGGGGGATCC*ACTA GT*- 3'
CArG-boxes spaced by 5.5 helical turns	pBluescript SK II+	*XhoI, SpeI*	5'- *CTCGAG*GTCGACGGTATCGATAAGCTTGATATCGAATTCAATTATATTCCAAATAAGGAAAGTATGGAACGTTCGA CGGTATAGCTTGATGAAATTTAATTATATTCCAAATAAGGAAAGTATGGGAATTCCTGCAGCCCGGGGGATCC*ACT AGT*- 3'
DNA probe used for the saturation binding assay (used in Supplementary Figure 1)			
Single CArG box used in saturation binding assay	-	-	5'- AATTCGAAATTTAATTATATTCCAAATAAGGAAAGTATGGAACGTTG- 3' 3'-GCTTTAAATTAATATAAGGTTTATTCCTTTCATACCTTGCAACTTAA-5'

DNA probes derived from the regulatory region of the SEP proteins' targets (used in Figures 4 and 5)			
rAP3	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTCAGTTTACATAAATGGAAAATTTATCACTTAGTTTTCATCAACTTCTGA ACTTACCTTTCATGGATTAGGCAATACTTTCCATTTTTAGTAACTCAATCTT*TCTAGA*- 3'
rAP3m1	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTCAGTTTATATAAATTTAAAATTTATCACTTAGTTTTCATCAACTTCTGA ACTTACCTTTCATGGATTAGGCAATACTTTCCATTTTTAGTAACTCAATCTT*TCTAGA*- 3'
rAP3m2	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTCAGTTTACATAAATGGAAAATTTATCACTTAGTTTTCATCAACTTCTGA ACTTATTTTTCATAAATTAGGCAATACTTTCCATTTTTAGTAACTCAATCTT*TCTAGA*- 3'
rAP3m3	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTTCAGTTTACATAAATGGAAAATTTATCACTTAGTTTTCATCAACTTCTG AACTTACCTTTCATGGATTAGGCAATACTTTTTATTTTTAATAACTCAAATCTTT*CTAGA*- 3'
rAP3m1,2	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTCAGTTTATATAAATTTAAAATTTATCACTTAGTTTTCATCAACTTCTGA ACTTATTTTTCATAAATTAGGCAATACTTTCCATTTTTAGTAACTCAATCTT*TCTAGA*- 3'
rAP3m1,3	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATTCAGTTTATATAAATTTAAAATTTATCACTTAGTTTTCATCAACTTCTGA ACTTACCTTTCATGGATTAGGCAATACTTTTTATTTTTAATAACTCAATCTT*TCTAGA*- 3'
rSEP3	pJET1.2.	*XhoI, XbaI*	5'- *CTCGAG*TTTTTCAGCAAGATAAACTCCATCTTTCTATTTTGGGTAACGAGGTCCCCTTCCCATTACGTCT TGACGTGGACCCTGTCCGTCTATTTTTAGCAGAATCACTAGTATCTT*TCTAGA*- 3'

Supplementary Table 3: K_{dim} and k_{coop} values used in equations (14) and (17) to calculate protein concentrations necessary to half-occupy two CArG-boxes simultaneously that are spaced by 2 or 6 helical turns and are located on naked or nucleosomal DNA. For a SEP dimer binding to CArG I on nucleosomal DNA an energy penalty of 20.92 kJ/mol was introduced.

	Naked DNA						**Nucleosomal DNA**					
			CArG I and II spaced by 2 turns		CArG I and II spaced by 6 turns				CArG I and II spaced by 2 turns		CArG I and II spaced by 6 turns	
	K_{dimN} (CArGI) ($\cdot 10^6$ M^{-1})	K_{dimN} (CArGII) ($\cdot 10^6$ M^{-1})	k_{coop2}	Protein concentration necessary to half-occupy both CArG-boxes simultaneously (nM)	k_{coop6}	Protein concentration necessary to half-occupy both CArG-boxes simultaneously (nM)	K_{dimH} (CArGI) ($\cdot 10^6$ M^{-1})	K_{dimN} (CArGII) ($\cdot 10^6$ M^{-1})	k_{coop2}	Protein concentration necessary to half-occupy both CArG-boxes simultaneously (nM)	k_{coop6}	Protein concentration necessary to half-occupy both CArG-boxes simultaneously (nM)
SEP1	52	52	155	1.7	≥200	≤ 1.5	0.005	52	155	1,300	≥200	≤ 1000
SEP2	68	68	n.d.	n.d.	≥200	≤ 1.1	0.007	68	n.d.	n.d.	≥200	≤ 750
SEP3	150	150	1.1	15	39	1.3	0.015	150	1.1	60,600	39	1,700