Supporting Information

for

Electrostatic repulsion causes anticooperative DNA binding between tumor suppressor ETS transcription factors and JUN-FOS at composite DNA sites

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Supporting Information

Table S1. Equlibrium dissociation constants (K_D) for ETS proteins binding to *UPP* promoter DNA with and without JUN-FOS.

ETS[1]	DNA[1]	JUN-FOS[1]	K_D (nM)[2]	n
EHF	*UPP*	+	2,000 ± 1,000	2
EHF	*UPP*	-	56 ± 7	2
SPDEF	*UPP*	+	2,000 ± 800	2
SPDEF	*UPP*	-	119 ± 9	2
ETV1	*UPP*	+	700 ± 400	2
ETV1	*UPP*	-	400 ± 200	2
ETV4	*UPP*	+	700 ± 300	2
ETV4	*UPP*	-	340 ± 30	2
ERG	*UPP*	+	65 ± 2	2
ERG	*UPP*	-	310 ± 30	2
FLI1	*UPP*	+	20 ± 10	2
FLI1	*UPP*	-	500 ± 300	2

[1] Full-length ETS and JUN-FOS proteins were used in this experiment; see Table S8 for protein and DNA sequences.
[2] K_D values are listed as mean ± standard deviation.

Table S2. Equilibrium dissociation constants (K_D) for EHF and ERG binding to different DNA sequences.

ETS	DNA	K_D (nM)[1]	n	p[2]
EHF	SC1	1.0 ± 0.1	6	-
EHF	SC1 C2A	2.1 ± 0.5	6	4×10^{-6}
EHF	*COPS8*	2.4 ± 1.4	4	0.03
ERG	SC1	0.7 ± 0.2	4	-
ERG	SC1 C2A	11 ± 5	4	0.005
ERG	*COPS8*	17 ± 7	5	0.002

[1] K_D values are listed as mean ± standard deviation.
[2] p-values were calculated for EHF and ERG with comparison to SC1 DNA values.

Table S3. Peak coordinates for ERG-FLAG and EHF-FLAG occupied regions in RWPE1 cells.

See excel spreadsheet online for list of top 1000 ERG-FLAG and EHF-FLAG occupied regions in RWPE1 cells.

Table S4. RSAT analysis of ETS-AP1 spacing in top 1000 peaks of ERG and EHF ChIP datasets.

See excel spreadsheet online for list of RSAT matches from top 1000 ERG-FLAG and EHF-FLAG ChIP-Seq peaks to ETS-AP1 sites.

Table S5. Equilibrium dissociation constants (K_D) for EHF truncations binding to *COPS8* enhancer DNA with and without JUN-FOS.

EHF	DNA	JUN-FOS[1]	K_D (nM)[2]	n	p[3]
EHF	*COPS8*	-	3.7 ± 0.5	4	-
$EHF^{\Delta N183}$	*COPS8*	-	3.3 ± 0.5	4	0.3
$EHF^{\Delta N193}$	*COPS8*	-	3.2 ± 0.7	4	0.3
$EHF^{\Delta N203}$	*COPS8*	-	3.5 ± 0.6	4	0.7
EHF	*COPS8*	+	170 ± 80	4	-
$EHF^{\Delta N193}$	*COPS8*	+	130 ± 20	4	0.4
$EHF^{\Delta N183}$	*COPS8*	+	90 ± 20	4	0.1
$EHF^{\Delta N203}$	*COPS8*	+	20 ± 7	4	0.01

[1] $JUN^{\Delta N250\ \Delta C319}$- $FOS^{\Delta N131\ \Delta C203}$ proteins were used in this experiment.
[2] K_D values are listed as mean ± standard deviation.
[3] p-values were calculated for EHF truncations with comparison to full-length EHF.

Table S6. Equilibrium dissociation constants (K_D) for EHF$^{\Delta N193}$ mutants binding to *COPS8* enhancer DNA with and without JUN-FOS.

EHF$^{\Delta N193}$	DNA	JUN-FOS[1]	K_D (nM)[2]	n	p[3]
WT	*COPS8*	-	5 ± 2	6	-
K196E/K200E/K201E	*COPS8*	-	5 ± 4	4	0.7
K241E/S242P/A244E	*COPS8*	-	4 ± 3	4	0.6
K251E/K252Q	*COPS8*	-	4 ± 2		0.5
K272E	*COPS8*	-	4 ± 3	4	0.9
WT	*COPS8*	+	120 ± 40	6	-
K196E/K200E/K201E	*COPS8*	+	5 ± 3	4	2×10^{-4}
K241E/S242P/A244E	*COPS8*	+	140 ± 30	4	0.4
K251E/K252Q	*COPS8*	+	50 ± 10	4	0.007
K272E	*COPS8*	+	20 ± 10	4	7×10^{-4}

[1] JUN$^{\Delta N250\ \Delta C319}$- FOS$^{\Delta N131\ \Delta C203}$ proteins were used in this experiment.
[2] K_D values are listed as mean ± standard deviation.
[3] p-values were calculated for EHF truncations with comparison to full-length EHF.

Table S7. Equilibrium dissociation constants (K_D) for ETS truncations binding to *COPS8* enhancer DNA with and without JUN-FOS.

ETS[1]	DNA[1]	JUN-FOS[1]	K_D (nM)[2]	n
$ERF^{\Delta C126}$	*COPS8*	-	23 ± 2	2
$ERF^{\Delta C126}$	*COPS8*	+	6.5 ± 0.6	2
$GABPA^{\Delta N281}$	*COPS8*	-	3.7 ± 0.4	2
$GABPA^{\Delta N281}$	*COPS8*	+	3.5 ± 0.7	2
$ELF1^{\Delta N148\ \Delta C313}$	*COPS8*	-	8 ± 3	2
$ELF1^{\Delta N148\ \Delta C313}$	*COPS8*	+	50 ± 20	2
$ELK4^{\Delta C330}$	*COPS8*	-	1.9 ± 0.5	2
$ELK4^{\Delta C330}$	*COPS8*	+	1.0 ± 0.2	2

[1] $JUN^{\Delta N250\ \Delta C319}$- $FOS^{\Delta N131\ \Delta C203}$ proteins were used in this experiment.
[2] K_D values are listed as mean ± standard deviation.

Table S8. Protein and DNA sequences used in this study.

Name[1,2]	Sequence[3]
JUN	MGSSHHHHHHSSGLVPRGSHMTAKMETTFYDDALNASFLPSESGPYGY SNPKILKQSMTLNLADPVGSLKPHLRAKNSDLLTSPDVGLLKLASPELE RLIIQSSNGHITTTPTPTQFLCPKNVTDEQEGFAEGFVRALAELHSQNTLP SVTSAAQPVNGAGMVAPAVASVAGGSGSGGFSASLHSEPPVYANLSNF NPGALSSGGGAPSYGAAGLAFPAQPQQQQQPPHHLPQQMPVQHPRLQ ALKEEPQTVPEMPGETPPLSPIDMESQERIKAERKRMRNRIAASKCRKR KLERIARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMNHVNSG CQLMLTQQLQTF
JUN$^{\Delta N250\ \Delta C319}$	MGSSHHHHHHSSGLVPRGSHMQERIKAERKRMRNRIAASKCRKRKLER IARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVMNHVNSG
FOS	MMFSGFNADYEASSSRCSSASPAGDSLSYYHSPADSFSSMGSPVNAQDF CTDLAVSSANFIPTVTAISTSPDLQWLVQPALVSSVAPSQTRAPHPFGVP APSAGAYSRAGVVKTMTGGRAQSIGRRGKVEQLSPEEEEKRRIRRERN KMAAAKCRNRRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFI LAAHRPACKIPDDLGFPEEMSVASLDLTGGLPEVATPESEEAFTLPLLND PEPKPSVEPVKSISSMELKTEPFDDFLFPASSRPSGSETARSVPDMDLSGS FYAADWEPLHSGSLGMGPMATELEPLCTPVVTCTPSCTAYTSSFVFTYP EADSFPSCAAAHRKGSSSNEPSSDSLSSPTLLAL
FOS$^{\Delta N131\ \Delta C203}$	MGSSHHHHHHSSGLVPRGSHMQLSPEEEEKRRIRRERNKMAAAKCRN RRRELTDTLQAETDQLEDEKSALQTEIANLLKEKEKLEFILAAHRPA
EHF	MGSSHHHHHHSSGLVPRGSHMILEGGGVMNLNPGNNLLHQPPAWTDS YSTCNVSSGFFGGQWHEIHPQYWTKYQVWEWLQHLLDTNQLDANCIP FQEFDINGEHLCSMSLQEFTRAAGTAGQLLYSNLQHLKWNGQCSSDLF QSTHNVIVKTEQTEPSIMNTWKDENYLYDTNYGSTVDLLDSKTFCRAQI SMTTTSHLPVAESPDMKKEQDPPAKCHTKKHNPRGTHLWEFIRDILLNP DKNPGLIKWEDRSEGVFRFLKSEAVAQLWGKKKNNSSMTYEKLSRAM RYYYKREILERVDGRRLVYKFGKNARGWRENEN
EHF$^{\Delta N183}$	MGSSHHHHHHSSGLVPRGSHMESPDMKKEQDPPAKCHTKKHNPRGTH LWEFIRDILLNPDKNPGLIKWEDRSEGVFRFLKSEAVAQLWGKKKNNSS MTYEKLSRAMRYYYKREILERVDGRRLVYKFGKNARGWRENEN
EHF $^{\Delta N193}$	MGSSHHHHHHSSGLVPRGSHMPPAKCHTKKHNPRGTHLWEFIRDILLN PDKNPGLIKWEDRSEGVFRFLKSEAVAQLWGKKKNNSSMTYEKLSRA MRYYYKREILERVDGRRLVYKFGKNARGWRENEN
EHF$^{\Delta N193}$ K196E/ K200E/ K201E	MGSSHHHHHHSSGLVPRGSHMPPA**E**CHT**EE**HNPRGTHLWEFIRDILLN PDKNPGLIKWEDRSEGVFRFLKSEAVAQLWGKKKNNSSMTYEKLSRA MRYYYKREILERVDGRRLVYKFGKNARGWRENEN
EHF $^{\Delta N193}$ K241E/ S242P/ A244E	MGSSHHHHHHSSGLVPRGSHMPPAKCHTKKHNPRGTHLWEFIRDILLN PDKNPGLIKWEDRSEGVFRFL**EP**E**E**VAQLWGKKKNNSSMTYEKLSRA MRYYYKREILERVDGRRLVYKFGKNARGWRENEN

EHF $^{\Delta N193}$ K251E/K252Q

MGSSHHHHHHSSGLVPRGSHMPPAKCHTKKHNPRGTHLWEFIRDILLN
PDKNPGLIKWEDRSEGVFRFLKSEAVAQLWG**EQ**KNNSSMTYEKLSRA
MRYYYKREILERVDGRRLVYKFGKNARGWRENEN

EHF $^{\Delta N193}$ K272E

MGSSHHHHHHSSGLVPRGSHMPPAKCHTKKHNPRGTHLWEFIRDILLN
PDKNPGLIKWEDRSEGVFRFLKSEAVAQLWGKKKNNSSMTYEKLSRA
MRYYY**E**REILERVDGRRLVYKFGKNARGWRENEN

EHF $^{\Delta N203}$

MGSSHHHHHHSSGLVPRGSHMNPRGTHLWEFIRDILLNPDKNPGLIKW
EDRSEGVFRFLKSEAVAQLWGKKKNNSSMTYEKLSRAMRYYYKREIL
ERVDGRRLVYKFGKNARGWRENEN

ELF1$^{\Delta N148\ \Delta C313}$

MGSSHHHHHHSSGLVPRGSHMPEVMETQQVQEKYADSPGASSPEQPK
RKKGRKTKPPRPDSPATTPNISVKKKNKDGKGNTIYLWEFLLALLQDK
ATCPKYIKWTQREKGIFKLVDSKAVSRLWGKHKNKPDMNYETMGRAL
RYYYQRGILAKVEGQRLVYQFKEMPKDLIYINDEDPSSSIESSDP

ELK4$^{\Delta C330}$

MGSSHHHHHHSSGLVPRGSHMDSAITLWQFLLQLLQKPQNKHMICWT
SNDGQFKLLQAEEVARLWGIRKNKPNMNYDKLSRALRYYYVKNIIKK
VNGQKFVYKFVSYPEILNMDPMTVGRIEGDCESLNFSEVSSSSKDVENG
GKDKPPQPGAKTSSRNDYIHSGLYSSFTLNSLNSSNVKLFKLIKTENPAE
KLAEKKSPQEPTPSVIKFVTTPSKKPPVEPVAATISIGPSISPSSEETIQALE
TLVSPKLPSLEAPTSASNVMTAFATTPPISSIPPLQEPPRTPSPPLSSHPDID
TDIDSVASQPMELPENLSLEPKDQDSVLLEKDKVNNSSRSKKPKGLELA
PT

ERF$^{\Delta C126}$

MGSSHHHHHHSSGLVPRGSHMKTPADTGFAFPDWAYKPESSPGSRQIQ
LWHFILELLRKEEYQGVIAWQGDYGEFVIKDPDEVARLWGVRKCKPQ
MNYDKLSRALRYYYNKRILHKTKGKRFTYKFNFNKLVLVNYPFIDVGL
AGG

ERG

MGSSHHHHHHSSGLVPRGSHMASTIKEALSVVSEDQSLFECAYGTPHL
AKTEMTASSSSDYGQTSKMSPRVPQQDWLSQPPARVTIKMECNPSQVN
GSRNSPDECSVAKGGKMVGSPDTVGMNYGSYMEEKHMPPPNMTTNE
RRVIVPADPTLWSTDHVRQWLEWAVKEYGLPDVNILLFQNIDGKELCK
MTKDDFQRLTPSYNADILLSHLHYLRETPLPHLTSDDVDKALQNSPRLM
HARNTGGAAFIFPNTSVYPEATQRITTRPDLPYEPPRRSAWTGHGHPTP
QSKAAQPSPSTVPKTEDQRPQLDPYQILGPTSSRLANPGSGQIQLWQFLL
ELLSDSSNSSCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLS
RALRYYYDKNIMTKVHGKRYAYKFDFHGIAQALQPHPPESSLYKYPSD
LPYMGSYHAHPQKMNFVAPHPPALPVTSSSFFAAPNPYWNSPTGGIYPN
TRLPTSHMPSHLGTYY

ETV1	MGSSHHHHHHSSGLVPRGSHMDGFYDQQVPYMVTNSQRGRNCNEKPT NVRKRKFINRDLAHDSEELFQDLSQLQETWLAEAQVPDNDEQFVPDYQ AESLAFHGLPLKIKKEPHSPCSEISSACSQEQPFKFSYGEKCLYNVSAYD QKPQVGMRPSNPPTPSSTPVSPLHHASPNSTHTPKPDRAFPAHLPPSQSIP DSSYPMDHRFRRQLSEPCNSFPPLPTMPREGRPMYQRQMSEPNIPFPPQ GFKQEYHDPVYEHNTMVGSAASQSFPPPLMIKQEPRDFAYDSEVPSCHS IYMRQEGFLAHPSRTEGCMFEKGPRQFYDDTCVVPEKFDGDIKQEPGM YREGPTYQRRGSLQLWQFLVALLDDPSNSHFIAWTGRGMEFKLIEPEEV ARRWGIQKNRPAMNYDKLSRSLRYYYEKGIMQKVAGERYVYKFVCDP EALFSMAFPDNQRPLLKTDMERHINEEDTVPLSHFDESMAYMPEGGCC NPHPYNEGYVY
ETV4	MGSSHHHHHHSSGLVPRGSHMERRMKAGYLDQQVPYTFSSKSPGNGS LREALIGPLGKLMDPGSLPPLDSEDLFQDLSHFQETWLAEAQVPDSDEQ FVPDFHSENLAFHSPTTRIKKEPQSPRTDPALSCSRKPPLPYHHGEQCLY SSAYDPPRQIAIKSPAPGALGQSPLQPFPRAEQRNFLRSSGTSQPHPGHG YLGEHSSVFQQPLDICHSFTSQGGGREPLPAPYQHQLSEPCPPYPQQSFK QEYHDPLYEQAGQPAVDQGGVNGHRYPGAGVVIKQEQTDFAYDSDVT GCASMYLHTEGFSGPSPGDGAMGYGYEKPLRPFPDDVCVVPEKFEGDI KQEGVGAFREGPPYQRRGALQLWQFLVALLDDPTNAHFIAWTGRGME FKLIEPEEVARLWGIQKNRPAMNYDKLSRSLRYYYEKGIMQKVAGERY VYKFVCEPEALFSLAFPDNQRPALKAEFDRPVSEEDTVPLSHLDESPAY LPELAGPAQPFGPKGGYSY
FLI1	MGSSHHHHHHSSGLVPRGSHMDGTIKEALSVVSDDQSLFDSAYGAAA HLPKADMTASGSPDYGQPHKINPLPPQQEWINQPVRVNVKREYDHMN GSRESPVDCSVSKCNKLVGGGEANPMNYNSYMDEKNGPPPPNMTTNE RRVIVPADPTLWTQEHVRQWLEWAIKEYGLMEIDTSFFQNMDGKELCK MNKEDFLRATSAYNTEVLLSHLSYLRESSLLAYNTTSHTDQSSRLNVKE DPSYDSVRRGAWNNNMNSGLNKSPLLGGSQTMGKNTEQRPQPDPYQI LGPTSSRLANPGSGQIQLWQFLLELLSDSANASCITWEGTNGEFKMTDP DEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKF DFHGIAQALQPHPTETSMYKYPSDISYMPSYHAHQQKVNFVPSHPSSMP VTSSSFFGAASQYWTSPTAGIYPNPSVPRHPNTHVPSHLGSYY
GABPA$^{\Delta N281}$	MGSSHHHHHHSSGLVPRGSHMPTTIKVINSSAKAAKVQRAPRISGEDRS SPGNRTGNNGQIQLWQFLLELLTDKDARDCISWVGDEGEFKLNQPELV AQKWGQRKNKPTMNYEKLSRALRYYYDGDMICKVQGKRFVYKFVCD LKTLIGYSAAELNRLVTECEQKKLAKMQLHGIAQPVTAVALATASLQT EKDN
UPP	TAGGGGAAATGACTCATTCA
COPS8	TCGAAGAGAGGAAGTGACTCAGCCC
SC1	TCGACGGCCAAGCCGGAAGTGAGTGCC
SC1 C2A	TCGACGGCCAAGC**A**GGAAGTGAGTGCC

[1]Residues included in truncated proteins are indicated by superscript; proteins without superscript are full length.

[2]Gene promoter or enhancer DNA sequences are denoted in italic. SC1 refers to "selected clone 1", a consensus high-affinity ETS binding sequence (1).

[3]ETS and AP1 DNA binding sites are underlined, mutations to protein and DNA sequences are emboldened.

Table S9. qPCR primer sequences used in this study.

Genomic coordinates	Gene	Fwd/Rev	Primer Sequence
chr8:131918646-131919405	ADCY8	Fwd	ATGCTGAAACTGGCCCAGAA
chr8:131918646-131919405	ADCY8	Rev	TCCAGGGTGGTAGAGAGACG
chr5:94417054-94417894	MCTP1	Fwd	CGGCAAAAGAGGCTGTTCAC
chr5:94417054-94417894	MCTP1	Rev	TCATTCCAAGTCGCTGCTGT
chr19:11205375-11206235	LDLR	Fwd	CCCTCGTACAGAGGTAGGGA
chr19:11205375-11206235	LDLR	Rev	ATCACCCCACTAGGTGACCG
chr6:136931277-136931996	MAP3K5	Fwd	CTCAGTGGTTGCGTTGCCTA
chr6:136931277-136931996	MAP3K5	Rev	GGTTGACTCCCCTACTCCAC
chr9:73053231-73053934	KLF9	Fwd	GCTGGGAAGGAAGGTTCTGG
chr9:73053231-73053934	KLF9	Rev	AAAGACCCACATGGCTTGCT
chr20:7837265-7837937	PLCB1	Fwd	ATGGCTGTCTAACTTTGCCCC
chr20:7837265-7837937	PLCB1	Rev	AGTTCAAGACGATTGCCAGGT
chr12:31901844-31902653	AMN1	Fwd	TGTGGATTGGTTGGGGGATG
chr12:31901844-31902653	AMN1	Rev	ACCGTGACTGCAACAAAGGA
chr12:109231955-109232747	SSH1	Fwd	GAGGGCCTCTCACATACACG
chr12:109231955-109232747	SSH1	Rev	GTGGTCTCCGAGCAGGAAAA
chr6:148762145-148763173	SASH1	Fwd	GAGTGAACGGGCTGTAGCTT
chr6:148762145-148763173	SASH1	Rev	TTCTGAGGGAACACCTGAGC
chr16:55786521-55787940	CES1P2	Fwd	TGGGGTGGGAAAGTTGTAGC
chr16:55786521-55787940	CES1P2	Rev	TGGACTGTACCTTCCCCGAT
chr13:114102955-114104117	ADPRHL1	Fwd	TGTGCTCACTCTAGCAGCAG
chr13:114102955-114104117	ADPRHL1	Rev	AAAGTCCTTCACCCCACGAG
chr17:41465400-41467142	LINC00910	Fwd	AGAGACCTGTAGGCGGAGAG
chr17:41465400-41467142	LINC00910	Rev	CCCACTGGTGACGACGTAAA
chr1: 1724266- 1724486	CDK11A	Fwd	CGCAGTTTCTTTTGGAGTCCTG
chr1: 1724266- 1724486	CDK11A	Rev	TCGGAACTCACCCCTACGGG
chr7:6424763-6425534	RAC1	Fwd	GGGCCTGTAATCTGCCTTG
chr7:6424763-6425534	RAC1	Rev	GAGCCCTTCTCTCTGCTGTG
chr7:31532974-31534069	CCDC129	Fwd	TCTGAAAGTGGGGACTTGGC
chr7:31532974-31534069	CCDC129	Rev	ACTCATCTGGGAGTCTGGGG
chr16:15684488-15685337	MARF1	Fwd	GCCTCTGGTACTTCCGCTAAG
chr16:15684488-15685337	MARF1	Rev	GAAGGTGGCCCTGGTAATCT
chr5:134475533-134477670	c5orf66	Fwd	AGGCAGGGACTAGCTCTGAA
chr5:134475533-134477670	c5orf66	Rev	CGAGGCTGATGCTCAGAGAG
chr20:45988666-45989914	ZMYND8	Fwd	CTGCATCCCGAGATAGCCTG
chr20:45988666-45989914	ZMYND8	Rev	TCCATTTCGCGTAACGGTCA

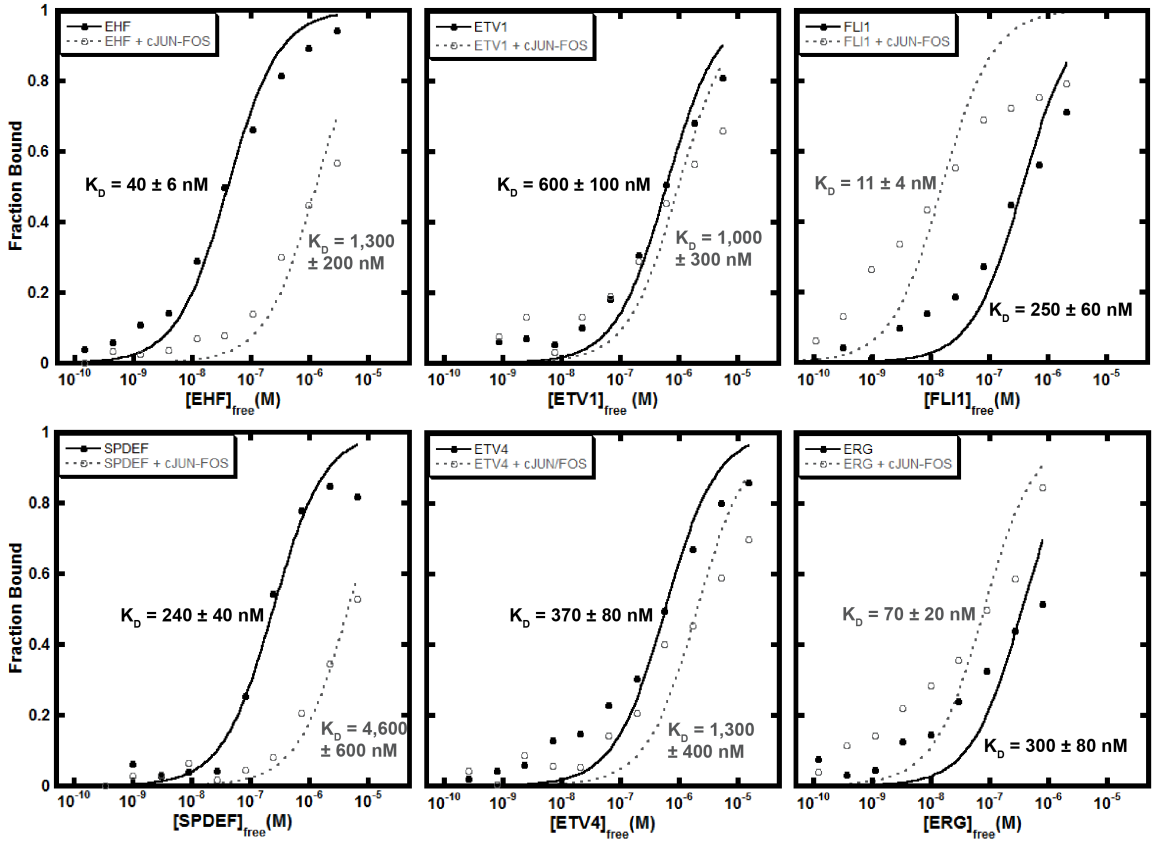


Figure S1. JUN-FOS differentially influences the DNA binding of ETS factors. Binding isotherms for ETS factors binding to *UPP* promoter DNA in the absence (black) or presence of JUN-FOS (gray). Each data point is the mean from two replicates. See Methods for details.

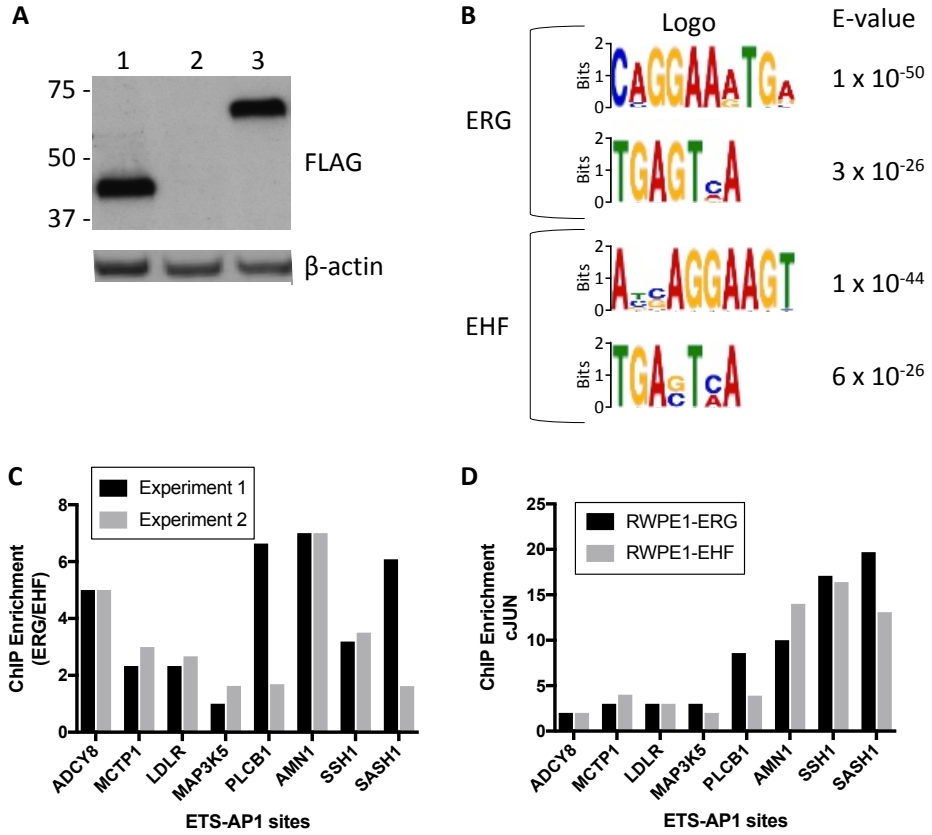


Figure S2. ETS and AP1 sites are overrepresented at ERG- and EHF- bound genomic regions. *A*, Western blot of lysates from cells infected with EHF-FLAG (lane 1), empty control (lane 2), and ERG-FLAG (lane 3) retroviruses. FLAG-tagged ERG and EHF were expressed at similar levels upon retroviral expression in RWPE1 cells. *B*, Full AP1 DNA-binding sequences (TGANTCA) were overrepresented in both ERG-FLAG and EHF-FLAG ChIP datasets, as determined by MEME (2). Note that despite the overrepresentation of AP1 DNA-binding sequences in both datasets, the closely spaced ETS-AP1 composite sites were more frequently observed for ERG than for EHF (Fig. 3B). *C*, Ratio of ERG and EHF enrichment at the eight ETS-AP1 sites in Figure 3C. Replicate qPCRs from two experiments are shown for each region. D, Comparison of JUN enrichment at ETS-AP1 sites in RWPE1 cells expressing ERG-FLAG (black) and EHF-FLAG (gray). JUN enrichment was similar at most sites but depleted at PLCB1 and SASH1 ETS-AP1 sites in RWPE1 cells expressing EHF-FLAG. Two to three independent biological replicates provided similar patterns, but different maximum levels of enrichment. A representative experiment is shown.

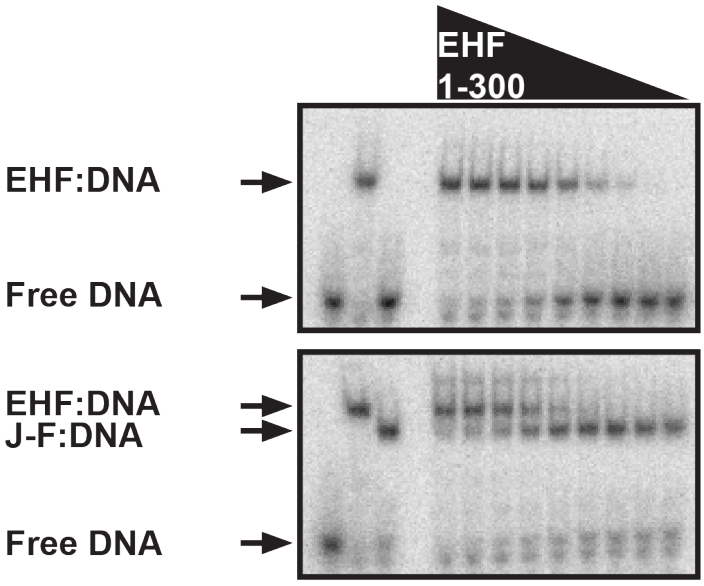


Figure S3. The minimal DNA-binding domains of JUN and FOS are sufficient for anticooperative DNA binding with EHF. *COPS8* enhancer DNA was titrated with EHF by itself (top) and with near saturating amounts of JUN-FOS DNA-binding domains ($JUN^{\Delta N250\ \Delta C319}$ and $FOS^{\Delta N131\ \Delta C203}$) (bottom). Similar levels of anticooperative binding that were observed with JUN-FOS DNA-binding domains as with full-length JUN-FOS (Fig. 1). Therefore, JUN-FOS DNA-binding domains are sufficient for anticooperative DNA binding with EHF.

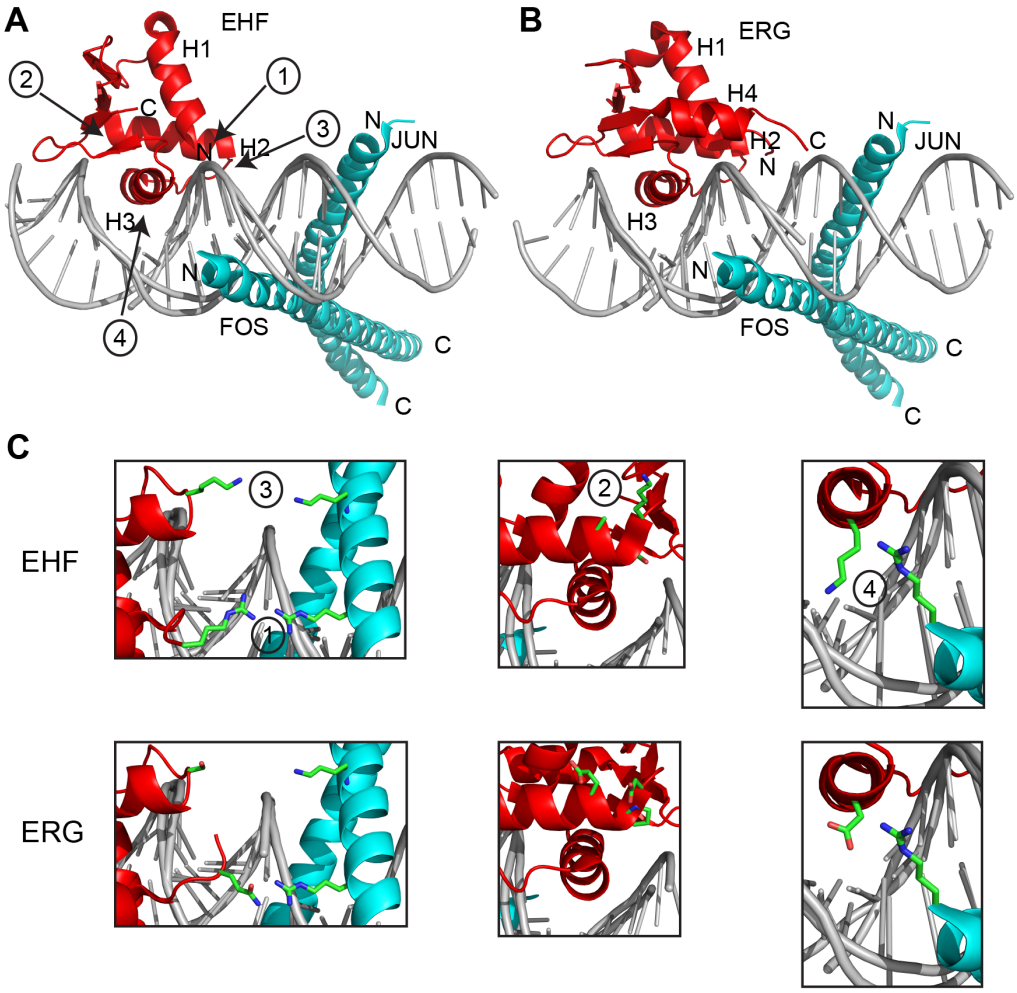


Figure S4. EHF and ERG have distinct JUN-FOS interfaces. Model of EHF, *A*, and ERG, *B,* binding to an ETS-AP1 composite DNA sequence with JUN-FOS. Models are oriented as in Fig. 5A,B, and proteins are shown in cartoon format. *C*, Model of EHF (top) and ERG (bottom) zoomed in on regions of EHF that were mutated in Fig. 5C-D. Positively charged residues in EHF along the JUN-FOS interface are shown in stick format, as well as the corresponding residues in ERG.

Figure S5. Alignment of ETS domain and flanking sequences for human ETS factors. Sequences corresponding to the ETS domain and flanking regions (up to 60 residues on each side or until the native amino- and caboxy-terminus) were aligned using Clustal Omega (3). The secondary structure of the conserved ETS domain is illustrated above the sequences; α-helices are represented as cylinders and β-strands are represented as arrows. Black arrows underneath the sequences refer to amino acids that were mutated in EHF that contribute to anticooperative DNA binding with JUN-FOS (Fig. 5C). High density of positive residues at the amino terminus of the SPI, ELF1/2/4, and EHF subfamilies are boxed in blue. SPI1 was previously observed to bind to DNA in an anticooperative fashion with JUN-FOS (4), and we show that EHF and ELF1 behave similarly in this manuscript. Therefore, we suggest that ETS factors that bind to DNA anticooperatively with JUN-FOS possess a positive interface composed of residues from the amino terminal region of the ETS domain, the loop between α-helices H2 and H3, and the carboxy-terminus of α-helix H3.

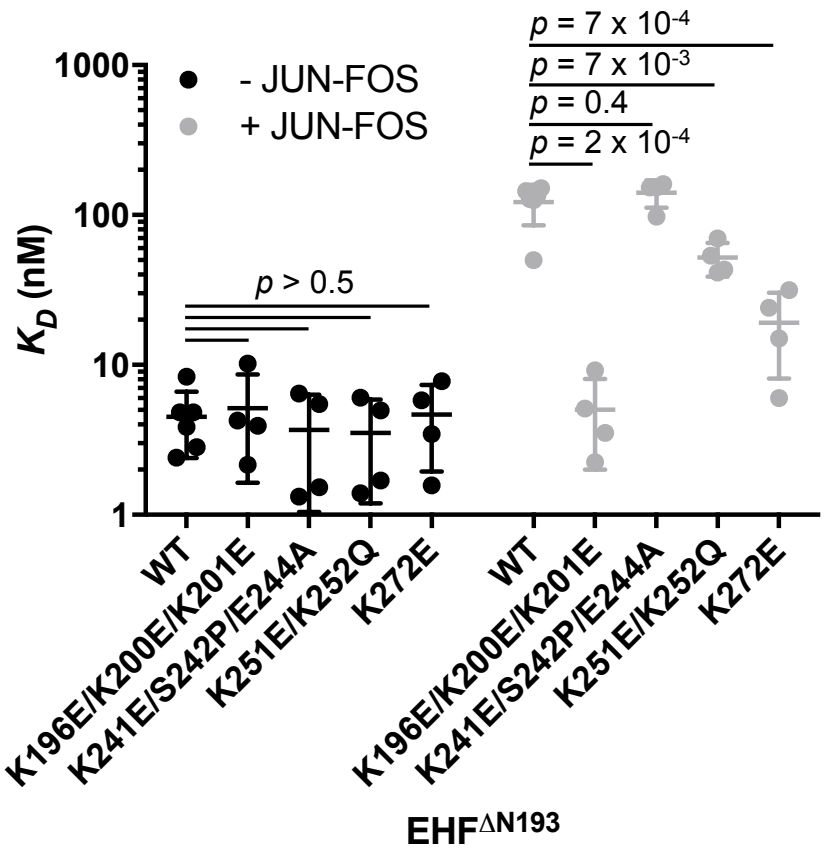


Figure S6. **Positive residues on the JUN-FOS interface of EHF mediate anticooperative DNA binding.** EMSAs were performed for $EHF^{\Delta N193}$ wildtype (WT) and indicated mutants alone (black), and with near-saturating amounts of $JUN^{\Delta N250\ \Delta C319}$-$FOS^{\Delta N131\ \Delta C203}$ (gray). Each closed circle represents the K_D for EHF from a single experiment; at least four replicate experiments were performed with each EHF protein. Bars represent the mean and standard deviation for each dataset, and *p* values comparing EHF proteins are displayed. Mean, standard deviation, number of replicates, and *p* values are listed in Table S6. Representative EMSAs for each version of EHF with $JUN^{\Delta N250\ \Delta C319}$-$FOS^{\Delta N131\ \Delta C203}$ are shown in Figure 5D. The mutation of positive residues on the JUN-FOS interface (K196E/K200E/K201E, K251E/K252Q, and K272E; see Figure 5A and Figure S4 for residue locations) enhances EHF binding to DNA with JUN-FOS, but does not alter EHF binding to DNA alone.

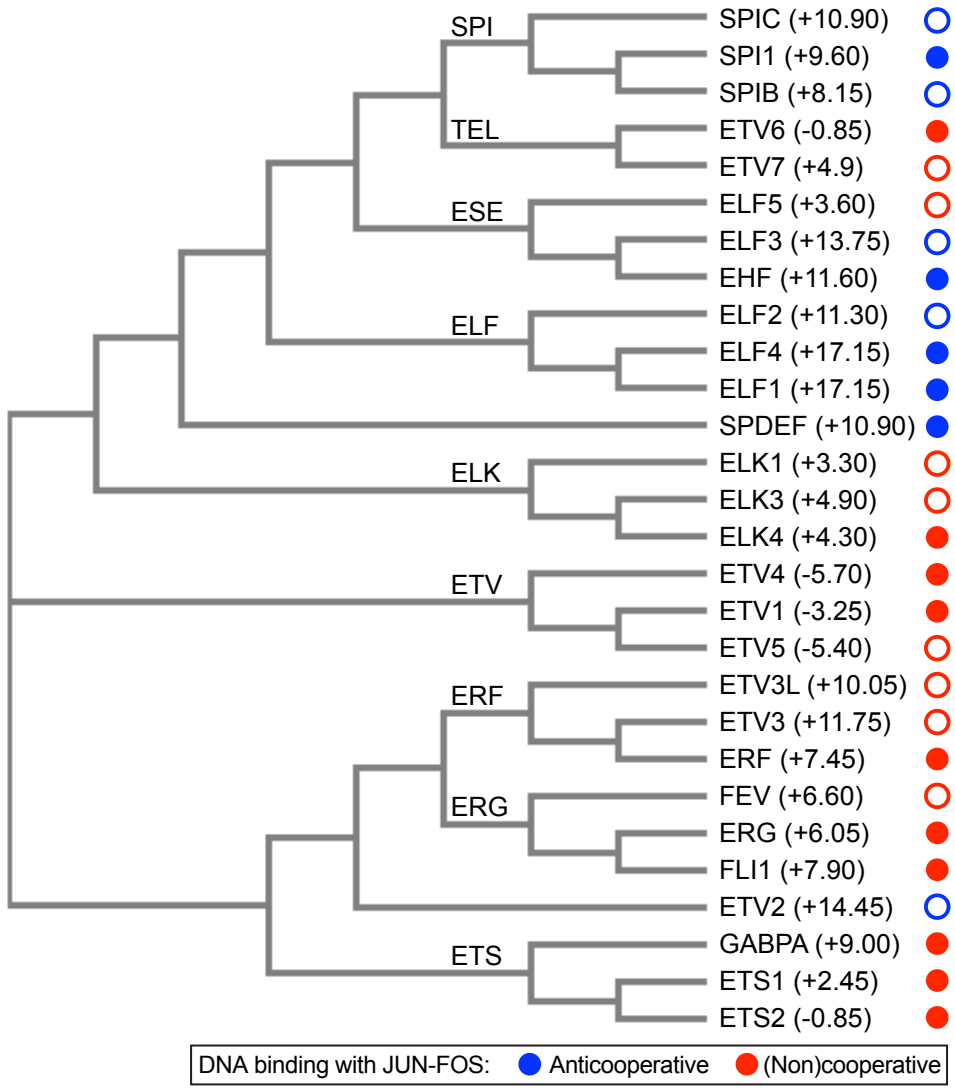
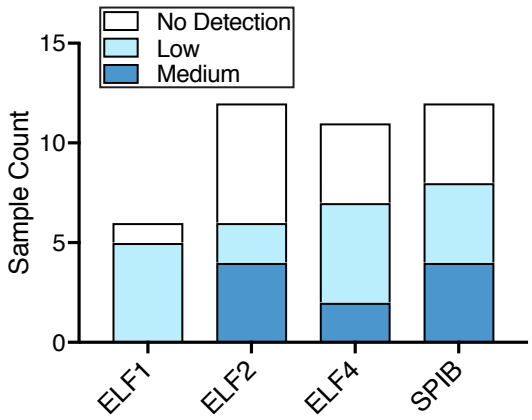


Figure S7. Diversification of charge in ETS factor evolution. A dendrogram of ETS domain and flanking sequences (up to sixty residues on each side of the ETS domain) shows the degree of relatedness for 28 human ETS factors. Subfamilies are named on the left, following previous nomenclature (5), and individual ETS factors are named on the right. The number indicates the net charge of the ETS domain and flanking sequences. Circles indicate DNA binding of each ETS factor with JUN-FOS. Blue circles indicate anticooperative binding with JUN-FOS, and red circles indicate noncooperative or cooperative binding with JUN-FOS. Closed circles are based on experimental evidence in this study or previously (4). Open circles are predictions based on sequence alignment (Fig. S5). The dendogram illustrates a large extent of clustering between ETS factors that bind to DNA anticooperatively with JUN-FOS at the top, and those that bind to DNA noncooperatively or cooperatively at the bottom.

A



B

Cohort	# ELF1 deletion / cohort size (%)	Reference
Michigan	11 / 61 (18%)	(Grasso et al. 2012)
TCGA	74 / 492 (15%)	cBioPortal website
TCGA 2015	45 / 333 (14%)	(Cancer Genome Atlas Research 2015)
Broad/Cornell	5 / 56 (8.9%)	(Baca et al. 2013)
FHCRC	10 / 136 (7.4%)	(Kumar et al. 2016)
SU2C	9 / 150 (6.0%)	(Robinson et al. 2015)
MSKCC	3 / 103 (2.9%)	(Taylor et al. 2010)

Figure S8. The ELF1 gene is frequently deleted in prostate cancer patients. *A*, Count of prostate cancer samples displaying relative protein levels of ELF1, ELF2, ELF4, and SPIB from the Protein Atlas (6,7). All of these proteins are present at a "medium" level in normal prostate samples; therefore, "low" or "no detection" represents a reduction in protein levels in cancer samples. *B*, Compilation of prostate cancer studies that found deletion of the ELF1 gene in greater than one percent of tested patients. Data was compiled from cBioPortal (8,9) using previously published prostate cancer studies (10-15).

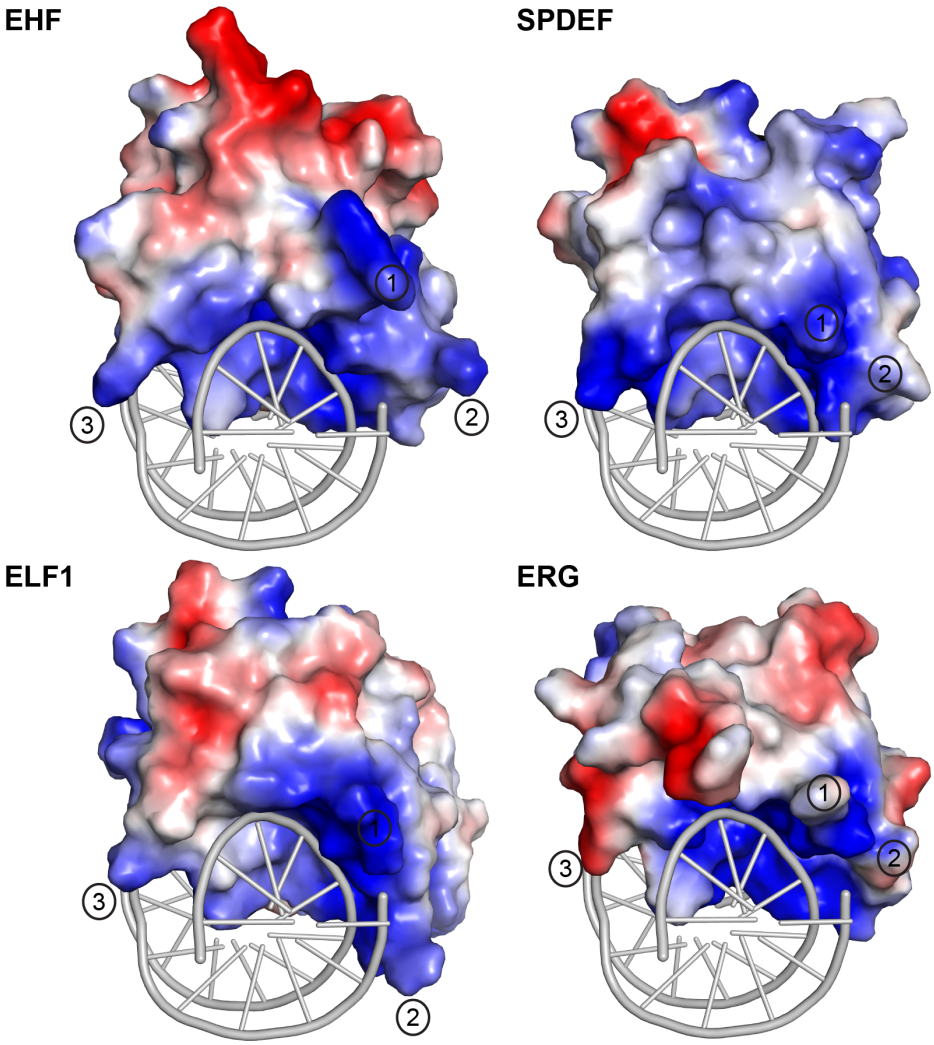


Figure S9. Positively charged JUN-FOS interface on tumor suppressor ETS factors prevents simultaneous DNA binding at composite ETS-AP1 sites. Structures of EHF (homology model based on PDB entry 3JTG), SPDEF (1YO5), ELF1 (homology model based on 3JTG), and ERG (4IRI) bound to DNA. Images are oriented from the perspective of JUN-FOS, so the proximal region near DNA is the contact surface for JUN-FOS. Regions that are mutated in EHF in Figure 5 are notated; (1) N-terminal of ETS domain, (2) H2-H3 loop, (3) C-terminus of H3. The positively charged residues in all of these regions for EHF, SPDEF, and ELF1 are necessary for anticooperative DNA binding with JUN-FOS. In contrast, the relative lack of positive charges in these regions allows ERG to simultaneously bind to DNA with JUN-FOS. Note that all ETS factors possess a conserved basic DNA-binding interface. Thus, a basic interface that is variably present in ETS factors is able to selectively control transcription factor partnerships.

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