SUPPLEMENTARY DATA

Quantitative Analysis of TALE-DNA Interactions Suggests Polarity Effects

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Figure S1: Sequences of dTALEs used in this study.

A) N- and C-terminal truncations reported by several studies.

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this study
Mussolino et al. (1)                Miller et al. (2)
Sun et al. (3)                      PthXo1 structure (4)
Zhang et al. (5)                    dHax3 structure (6)

BamHI
254-this study
|287-Mussolino et al, Sun et al
||
MDPI
RPRRPSPARELLPGPQPDRVQPTADRGVSAPAGSPLDGLPARRTVSRTRL 201
                          207 Sun et al
PSPPAPSPAFSAGSFSDLLRPFDPSLLDTSLLDSMPAVGTPHTAAAPAEW 151
                                     N2-Zhang et al
                            Factor Xa| 111-this study
             153-Mussolino et al    || |        Δ152-Miller et al
                               |    || |        |135-Sun et al
DEAQSALRAADDPPPTVRVAVTAARPPRAKPAPRRRAAQPSDASPAAQVD 101
    N3-Zhang et al              N5-Zhang et al
    | 94 this study             |       N6-Zhang et al
    | |  125-Sun et al          |       |      N7-Zhang et al
    | |  |    PthXo1 Struct     |       |      |
    | |  |    |  N4-Zhang et al |       |      |
LRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALG 051
                          dHax3 Struct
                          |   N8-Zhang et al
                          |   |   50-Sun et al
                          |   |   |49-Mussolino et al
                          |   |
TVAVTYQHIITALPEATHEDIVGVGKQWSGARALEALLTDAGELRGPPLQ 001

LDTGQ…           --- TALE REPEATS ---       …SRPDP

                +28-Miller et al   47-Mussolino et al
                |  31-Sun et al    | Factor Xa
                |  |C4-Zhang et al | |   42 this study
                |  ||     Factor Xa| |   |
     17-Mussolino et al   |        | |   Factor Xa
     |          |  ||     |        | |   |
ALAALTNDHLVALACLGGRPAMDAVKKGLPHAPELIRRVNRRIGERTSHR 050
+63-Miller et al, Sun et al
 |C3-Zhang et al
VADYAQVVRVLEFFQCHSHPAYAFDEAMTQFGMSRNGLVQLFRRVGVTEL 100
     117-Sun et al
EARGGTLPPASQRWDRILQASGMKRAKPSPTSAQTPDQASLHAFADSLER 150
   163-Sun et al (imprecise due to low conservation with AvrXa10)
   |                         180 this study
   |                         |        200-Sun et al
DLDAPSPMHEGDQTRASSRKRSRSDRAVTGPSAQQAVEVRVPEQRDALHL 200
                   BamHI
                   231-Mussolino et al
PLSWRVKRPRTRIWGGLPDPGTPMAADLAASSTVMWEQDADPFAGAADDF 250
PAFNEEELAWLMELLPQ*
```

B) Construct sequences for bacterial expression for *in vitro* assays

Factor Xa site: IEGR^
TEV protease site: *ENLYFQ^S*
XhoI: CTCGAG
BamHI: GGATCC
StuI: AGGCCT
AatII: GACGTC
AgeI: ACCGGT
HindIII: AAGCTT
His6-purification tag: HHHHHH

Construct Schema:
MBP(not shown)-Factor Xa-*TEV*-XhoIBamHI-StuI-**TALE_repeats**-AatII-AgeI-His6-Stop-HindIII

AvrBs3$_{254-180}$ in pMal-TEV

ATCGAGGGAAGGCTC*GAAAATCTTTATTTTCAGTCT*CTCGAGGATCCCATTCGTTCGCGCACACCAAGTCCTGCCCG
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AvrBs3$_{111-42}$ in pMal-TEV

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GHHHHHH*KL

AvrBs3$_{94-42}$ in pMal-TEV

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II-NGp$_{111-42}$ in pMal-TEV

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CGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACACCCCAGCAGG
TGGTGGCCATCGCCAGCAATGGCGGCGGCAGGCCGGCGCTGGAGAGCATTGTTGCCCAGTTATCTCGCCCTGATCCG
GCGTTGGCCGCGTTGACCAACGACCACCTCGTCGCCTTGGCCTGCCTCGGCGGACGTCCTGCCATGGATGCAGTGAA
AAAGGGACTGCCGCACGCGCCGGAATTGATCAGAAGAGTCAATCGCCGTCCGGATCCTACCGGTCATCACCATCACC
ATCACTGAAAGCTT

IEGRL*ENLYFQS*LEMDPSDASPAAQVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHG
FTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQWSGARALEALLTVAGE
LRGPPLQ
LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLN
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQALLPVLCQAHG
LTPEQVVAIASNKGGKQALETVQALLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNIGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNKGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQALLPVLCQAHG
LTPQQVVAIASNGGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPAMDAVKKGLPHAPELIRRVNRRPDPT
GHHHHHH*KL

C) Construct sequences for eukaryotic expression for reporter assays

HAtag: YPYDVPDYA
NLS: PKKKRKV
XhoI: CTCGAG
StuI: AGGCCT
AatII: GACGTC
AgeI: ACCGGT
VP64: *DALDDFDLDMLDALDDFDLDMLDALDDFDLDMLDALDDFDLDML*
PstI: CTGCAG

Construct Schema:
HAtag-NLS-XhoI-StuI-**TALE_repeats**-AatII-AgeI-VP64-**Stop**-PstI

I-NGp$_{111-42}$ in pPGK-VP64

ATGTACCCATACGATGTCCCAGACTACGCGAATTCCCCGGGGATCCCAGGCATGGGGCCCAAAAAGAAACGCAAAGT
TGGGCGCCTCGAGATGGATCCCTCCGACGCTTCGCCGGCCGCGCAGGTGGATCTACGCACGCTCGGCTACAGTCAGC
AGCAGCAAGAGAAGATCAAACCGAAGGTGCGTTCGACAGTGGCGCAGCACCACGAGGCACTGGTGGGCCATGGGTTT
ACACACGCGCACATCGTTGCGCTCAGCCAACACCCGGCAGCGTTAGGGACCGTCGCTGTCACGTATCAGCACATAAT
CACGGCGTTGCCAGAGGCGACACACGAAGACATCGTTGGCGTCGGCAAACAGTGGTCCGGCGCACGCGCCCTGGAGG
CCTTGCTCACGGTGGCGGGAGAGTTGAGAGGTCCACCGTTACAGTTGGACACAGGCCAACTTCTCAAGATTGCAAAA
CGTGGCGGCGTGACCGCAGTGGAGGCAGTGCATGCATGGCGCAATGCACTGACGGGTGCCCCCCTGAACCTTACGCC
GCAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTGGCAAGCAGGCGCTGGAGACGGTGCAGCGGCTGCTTCCGGTGC
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ACGGTGCAGCGATTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAA
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CAGGTGGTGGCCATCGCCAGCAATGGCGGCGGCAAGCAGGCGCTGGAGACGGTGCAGGCGCTGTTGCCGGTGCTGTG
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TGCAGGCGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGAGCAGGTGGTGGCAATCGCCAGCAATGGC
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GCCCATGGCCTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATAAGGGTGGCAAGCAGGCGCTTGAGACGGTGCA
GCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGAGCAGGTGGTGGCCATCGCCAGCAATGGCGGTG
GCAAGCAGGCTCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGCCCATGGCCTGACCCCGGAGCAGGTG
GTGGCCATCGCCAGCCACGACGGGGGCAAGCAGGCGCTGGAGACGGTGCAGCGGCTGTTGCCGGTGCTGTGCCAGGC
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CGTCGCCTTGGCCTGCCTCGGCGGACGTCCTGCCATGGATGCAGTGAAAAAGGGACTGCCGCACGCGCCGGAATTGA
TCAGAAGAGTCAATCGCCGTCCGGATCCTACCGGTGCGGCCGCC*GACGCTTTGGATGACTTTGATTTGGACATGCTG*
GATGCTCTAGATGACTTCGACCTGGATATGCTGGACGCACTTGACGACTTTGACCTCGACATGCTAGACGCTCTGGA
*CGACTTCGATCTAGACATGCTC***TAA**GTCGACCTGCAG

MYPYDVPDYANSPGIPGMGPKKKRKVGRLEMDPSDASPAAQVDLRTLGYSQQQQEKIKPK
VRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVTYQHIITALPEATHEDIVGVGKQ
WSGARALEALLTVAGELRGPPLQ
LDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLN
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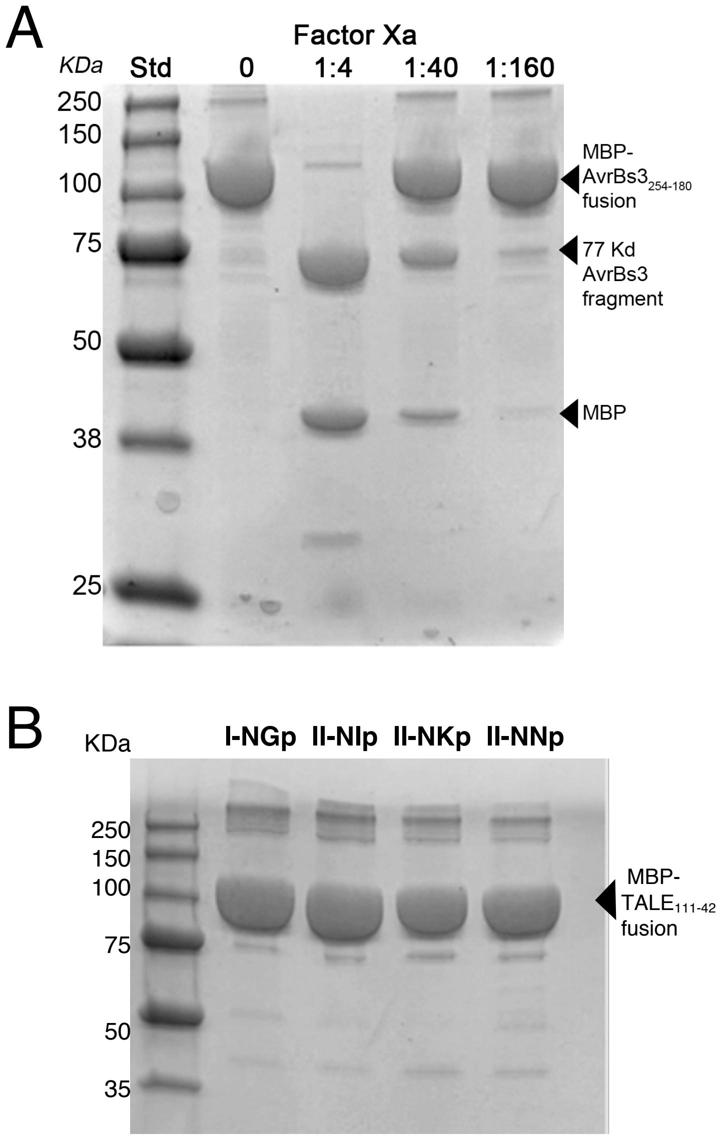
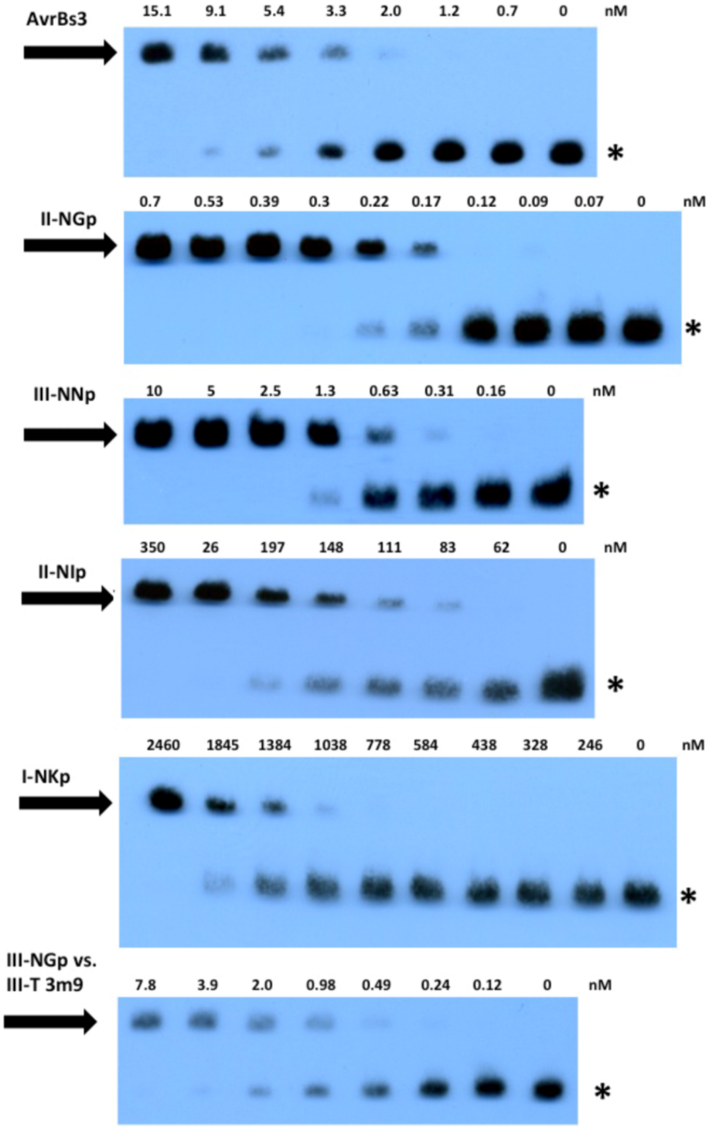
LTPEQVVAIASNIGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNKGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQALLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQALLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNIGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNKGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPQQVVAIASNGGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGRPALESIVAQ
LSRPDPALAALTNDHLVALACLGGRPAMDAVKKGLPHAPELIRRVNRRPDPTGAAA_DALD_
_DFDLDMLDALDDFDLDMLDALDDFDLDMLDALDDFDLDML_*VDLQ

Figure S2: Coomassie-stained protein gel of representative dTALEs.



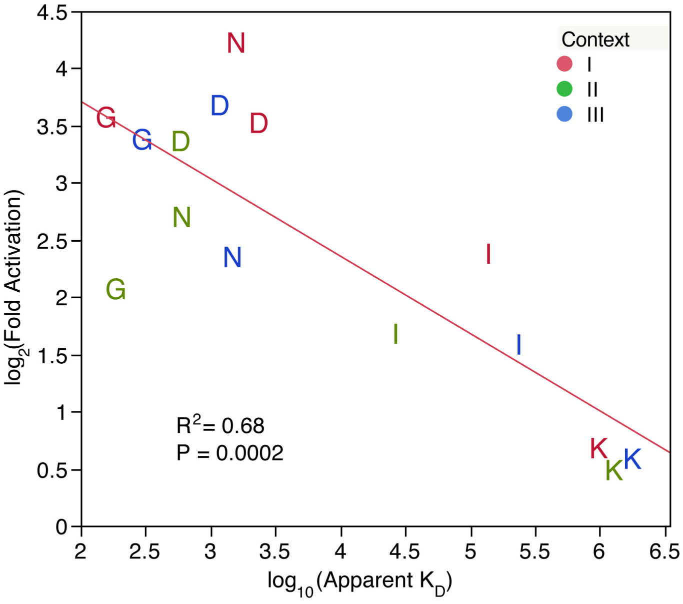
A) Coomassie-stained SDS PAGE gel shows purified **AvrBs3$_{254-180}$** after treatment with various concentrations of Factor Xa protease. B) Purified dTALE$_{111-42}$ proteins run at the expected 100 kilodalton size. Proteins shown are diluted 1:5 from stock. Typical protein yields were between 10 and 30 μM.

Figure S3. Representative EMSA data.



Sample data showing the EMSA dynamic range of 10^4. The TALEs used are shown and unless stated, the cognate DNA targets are used. The shifted TALE-DNA band is indicated with an arrow while the unshifted DNA is indicated with a “*”. The bottom panel shows representative data for a mixed site target used in polarity experiments. The sharpness of the transition (apparent nH = 3-4) is consistent over 4 logs of protein concentration, and therefore is not due to lack of equilibration or protein concentration-dependent aggregation. We do not know its origin.

Figure S4: Correlation of affinity with reporter gene activation.



The relationship between apparent K_D and fold activation of the reporter gene was not linear. The best fit model of these data was a log-log relationship. The correlation of $\log_2$(Fold Activation) versus $\log_{10}$(Apparent K_D (pM)) had an $R^2 = 0.68$ and a $P = 0.0002$. Host context is indicated by color, guest RVD by letter; G, N<u>G</u>; N, N<u>N</u>; D, H<u>D</u>; I, N<u>I</u>; K, N<u>K</u>.

Table S1: Primers used to create N- and C-terminal truncations.

EcoRI: GAATTC
5'SfiI: GGCCGCTAAGGCC
3’SfiI: GGCCAAGCTGGCC
XhoI: CTCGAG
BamHI: GGATCC
SmaI: CCCGGG
AgeI: ACCGGT

>PthXoI_5’111-f
5’-AATAGGAGGTGCACCGAATTCGTGGCCGCTAAGGCCCTCGAGATGGATCCTCCGACGCTTCGCCGGCCGCGCAGGT

>PthXoI_5’94-f
5’-AATAGGAGGTGCACCGAATTCGTGGCCGCTAAGGCCCTCGAGATGGATCCAGTCAGCAGCAGCAAGAGAAGATCAAACC

>PthXoI_5’end-r
5’-CGACGAGGTGGTCGTTGGTCAACG**CCCGGG**CTGTAACGGCGGACCTCTCAACTC

>PthXoI_3’end-f
5’-GAGTTGAGAGGTCCGCCGTTACAG**CCCGGG**CGTTGACCAACGACCACCTCGTCG

>PthXoI_3’42-r
5’-CAAGAAAGCTGGGTCGAATTCGGCCAAGCTGGCCTTACCGGTAGGATCCGGACGGCGATTGACTCTTCTGATCAATTC

Table S2: Primers used in EMSA assays.

Biotinylated forward primer	/5Biosg/CCTCTTCGCTATTACGCCAGC
Reverse primer	CACCCTGACTCGAGTACGATCGAACGTTC

For RVD affinity studies

I-A	CCTCTTCGCTATTACGCCAGC	TAAACAGATAAATAGACAA	GAACGTTCGATCGTACTCGAGTCAGGGTG
I-G	CCTCTTCGCTATTACGCCAGC	TGAGCGGGTGAGTGGGCGG	GAACGTTCGATCGTACTCGAGTCAGGGTG
I-C	CCTCTTCGCTATTACGCCAGC	TCACCCGCTCACTCGCCCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
I-T	CCTCTTCGCTATTACGCCAGC	TTATCTGTTTATTTGTCTT	GAACGTTCGATCGTACTCGAGTCAGGGTG
II-A	CCTCTTCGCTATTACGCCAGC	TAAATACAGAAACATAGAA	GAACGTTCGATCGTACTCGAGTCAGGGTG
II-G	CCTCTTCGCTATTACGCCAGC	TGAGTGCGGGAGCGTGGGG	GAACGTTCGATCGTACTCGAGTCAGGGTG
II-C	CCTCTTCGCTATTACGCCAGC	TCACTCCCGCACCCTCGCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
II-T	CCTCTTCGCTATTACGCCAGC	TTATTTCTGTATCTTTGTT	GAACGTTCGATCGTACTCGAGTCAGGGTG
III-A	CCTCTTCGCTATTACGCCAGC	TACAAAGACATAGAAATAA	GAACGTTCGATCGTACTCGAGTCAGGGTG
III-G	CCTCTTCGCTATTACGCCAGC	TGCGAGGGCGTGGGAGTGG	GAACGTTCGATCGTACTCGAGTCAGGGTG
III-C	CCTCTTCGCTATTACGCCAGC	TCCCACGCCCTCGCACTCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
III-T	CCTCTTCGCTATTACGCCAGC	TTCTATGTCTTTGTATTTT	GAACGTTCGATCGTACTCGAGTCAGGGTG

Zif268	CCTCTTCGCTATTACGCCAGC	GCGTGGGCGT	GAACGTTCGATCGTACTCGAGTCAGGGTG

For 5’T studies

Bs3	CCTCTTCGCTATTACGCCAGC	TATATAAACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5’A	CCTCTTCGCTATTACGCCAGC	AATATAAACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5’C	CCTCTTCGCTATTACGCCAGC	CATATAAACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5’G	CCTCTTCGCTATTACGCCAGC	GATATAAACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG

For polarity studies (B = C,G,T; D = A,G,T; H = A,C,T; V = A,C,G)

Bs3 variants

3m3	CCTCTTCGCTATTACGCCAGC	TATATAAACCTAACCAVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m6	CCTCTTCGCTATTACGCCAGC	TATATAAACCTAADDBVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m9	CCTCTTCGCTATTACGCCAGC	TATATAAACCVBBDDBVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m3	CCTCTTCGCTATTACGCCAGC	TBVBTAAACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m6	CCTCTTCGCTATTACGCCAGC	TBVBVBBACCTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m9	CCTCTTCGCTATTACGCCAGC	TBVBVBBBDDTAACCATCC	GAACGTTCGATCGTACTCGAGTCAGGGTG

III-C variants

3m3	CCTCTTCGCTATTACGCCAGC	TCCCACGCCCTCGCACVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m6	CCTCTTCGCTATTACGCCAGC	TCCCACGCCCTCGDBDVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m9	CCTCTTCGCTATTACGCCAGC	TCCCACGCCCVDHDBDVDD	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m3	CCTCTTCGCTATTACGCCAGC	TDDDACGCCCTCGCACTCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m6	CCTCTTCGCTATTACGCCAGC	TDDDBDHCCCTCGCACTCC	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m9	CCTCTTCGCTATTACGCCAGC	TDDDBDHDDDTCGCACTCC	GAACGTTCGATCGTACTCGAGTCAGGGTG

III-T variants

3m3	CCTCTTCGCTATTACGCCAGC	TTCTATGTCTTTGTATVVV	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m6	CCTCTTCGCTATTACGCCAGC	TTCTATGTCTTTGVBVVVV	GAACGTTCGATCGTACTCGAGTCAGGGTG
3m9	CCTCTTCGCTATTACGCCAGC	TTCTATGTCTVVHVBVVVV	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m3	CCTCTTCGCTATTACGCCAGC	TVDVATGTCTTTGTATTTT	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m6	CCTCTTCGCTATTACGCCAGC	TVDVBVHTCTTTGTATTTT	GAACGTTCGATCGTACTCGAGTCAGGGTG
5m9	CCTCTTCGCTATTACGCCAGC	TVDVBVHVDVTTGTATTTT	GAACGTTCGATCGTACTCGAGTCAGGGTG

Table S3: Primers for construction of ATF reporter plasmids.

XhoI: CTCGAG
NotI: GCGGCCGC

For RVD studies

>pGL3-control-Not-F
5′- GAGGAGGCGGCCGCAATAAAATATCTTTATTTTC

>PGL3-I-A-R
5′- CTCCTCCTCGAGTTGTCTATTTATCTGTTTACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-I-G-R
5′- CTCCTCCTCGAGCCGCCCACTCACCCGCTCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-I-C-R
5′- CTCCTCCTCGAGGGGGCGAGTGAGCGGGTGACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-I-T-R
5′- CTCCTCCTCGAGAAGACAAATAAACAGATAACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-II-A-R
5′- CTCCTCCTCGAGTTCTATGTTTCTGTATTTACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-II-G-R
5′- CTCCTCCTCGAGCCCCACGCTCCCGCACTCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-II-C-R
5′- CTCCTCCTCGAGGGCGAGGGTGCGGGAGTGACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-II-T-R
5′- CTCCTCCTCGAGAACAAAGATACAGAAATAACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-A-R
5′- CTCCTCCTCGAGTTATTTCTATGTCTTTGTACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-G-R
5′- CTCCTCCTCGAGCCACTCCCACGCCCTCGCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-C-R
5′- CTCCTCCTCGAGGGAGTGCGAGGGCGTGGGACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-R
5′- CTCCTCCTCGAGAAAATACAAAGACATAGAACCCGGGCTAGCACGCGTAAGAGCTC -3′

For AvrBs3 variant studies

>PGL3-Bs3-wt-R
5′- CTCCTCCTCGAGGGATGGTTAGGTTTATATACCCGGGCTAGCACGCGTAAGAGCTC -3′

>pGL3-Bs3-UPA-R
5′- CTCCTCCTCGAGAGAGGGTTAGGTTTATATACCCGGGCTAGCACGCGTAAGAGCTC -3′

For AvrBs3 5′T studies

>PGL3-Bs3-5A-R
5′- CTCCTCCTCGAGGGATGGTTAGGTTTATATTCCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-5C-R
5′- CTCCTCCTCGAGGGATGGTTAGGTTTATATGCCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-5G-R
5′- CTCCTCCTCGAGGGATGGTTAGGTTTATATCCCCGGGCTAGCACGCGTAAGAGCTC -3′

For polarity studies

>PGL3-Bs3-3m3-R
5′- CTCCTCCTCGAGCCCTGGTTAGGTTTATATACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-3m6-R
5′- CTCCTCCTCGAGCCCCCCTTAGGTTTATATACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-3m9-R
5′- CTCCTCCTCGAGCCCCCCAACGGTTTATATACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-5m3-R
5′- CTCCTCCTCGAGGGATGGTTAGGTTTAACCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-5m6-R
5′- CTCCTCCTCGAGGGATGGTTAGGTAACACCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-Bs3-5m9-R
5′- CTCCTCCTCGAGGGATGGTTACCAAACACCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-3m3-R
5′- CTCCTCCTCGAGCCCATACAAAGACATAGAACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-3m6-R
5′- CTCCTCCTCGAGCCCCACCAAAGACATAGAACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-3m9-R
5′- CTCCTCCTCGAGCCCCACGCCAGACATAGAACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-5m3-R
5′- CTCCTCCTCGAGAAAATACAAAGACATCCCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-5m6-R
5′- CTCCTCCTCGAGAAAATACAAAGAGCACCCACCCGGGCTAGCACGCGTAAGAGCTC -3′

>PGL3-III-T-5m9-R
5′- CTCCTCCTCGAGAAAATACAACCCGCACCCACCCGGGCTAGCACGCGTAAGAGCTC -3′

Alignment

>PGL3-*Bs3*-UPA-R	TATATAAACCTAACCCTCT
>PGL3-*Bs3*-wt-R	TATATAAACCTAACCATCC
>PGL3-*Bs3*-5A-R	AATATAAACCTAACCATCC
>PGL3-*Bs3*-5C-R	CATATAAACCTAACCATCC

>PGL3-*Bs3*-5G-R	GATATAAACCTAACCATCC

>PGL3-I-A-R	TAAACAGATAAATAGACAA
>PGL3-I-G-R	TGAGCGGGTGAGTGGGCGG
>PGL3-I-C-R	TCACCCGCTCACTCGCCCC
>PGL3-I-T-R	TTATCTGTTTATTTGTCTT
>PGL3-II-A-R	TAAATACAGAAACATAGAA
>PGL3-II-G-R	TGAGTGCGGGAGCGTGGGG
>PGL3-II-C-R	TCACTCCCGCACCCTCGCC
>PGL3-II-T-R	TTATTTCTGTATCTTTGTT
>PGL3-III-A-R	TACAAAGACATAGAAATAA
>PGL3-III-G-R	TGCGAGGGCGTGGGAGTGG
>PGL3-III-C-R	TCCCACGCCCTCGCACTCC
>PGL3-III-T-R	TTCTATGTCTTTGTATTTT

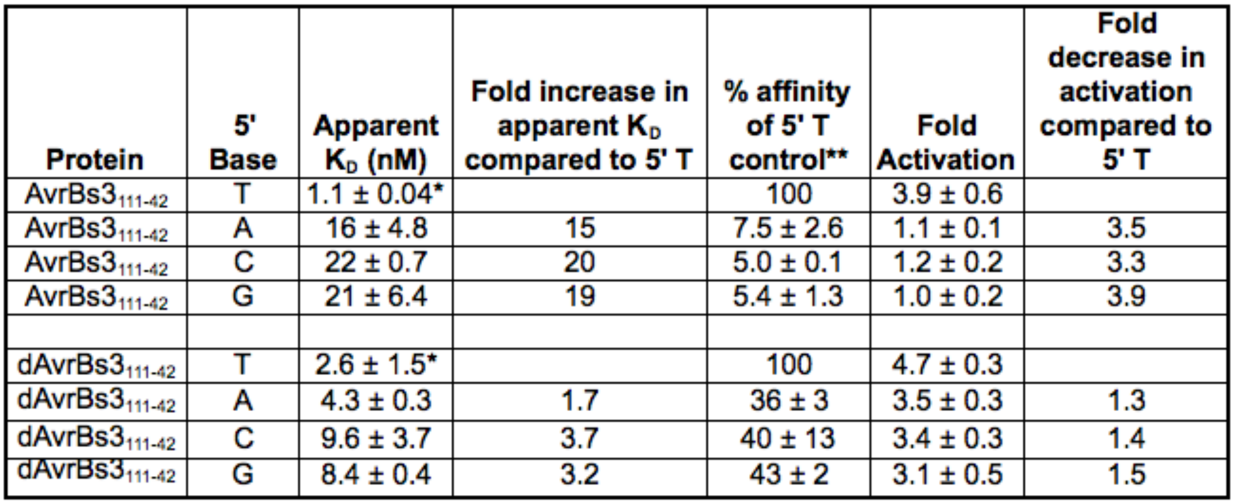
>PGL3-*Bs3*-wt-R	TATATAAACCTAACCATCC
>PGL3-*Bs3*-3m3-R	TATATAAACCTAACCAGGG
>PGL3-*Bs3*-3m6-R	TATATAAACCTAAGGGGGG
>PGL3-*Bs3*-3m9-R	TATATAAACCGTTGGGGGG
>PGL3-*Bs3*-5m3-R	TGGTTAAACCTAACCATCC
>PGL3-*Bs3*-5m6-R	TGGTGTTACCTAACCATCC
>PGL3-*Bs3*-5m9-R	TGGTGTTTGGTAACCATCC

>PGL3-III-T-wt-R	TTCTATGTCTTTGTATTTT
>PGL3-III-T-3m3-R	TTCTATGTCTTTGTATGGG
>PGL3-III-T-3m6-R	TTCTATGTCTTTGGTGGGG
>PGL3-III-T-3m9-R	TTCTATGTCTGGCGTGGGG
>PGL3-III-T-5m3-R	TGGGATGTCTTTGTATTTT
>PGL3-III-T-5m6-R	TGGGTGCTCTTTGTATTTT
>PGL3-III-T-5m9-R	TGGGTGCGGGTTGTATTTT

Table S4: EMSA and ATF data for G/A specificity of the NN RVD.

dTALE	Target Site	Apparent K_D (nM)	Fold increase in apparent K_D over G guest	Fold Activation	Fold decrease in activation over G guest
I-NNp	I-G	1.3 ± 0.3		5.1 ± 0.4	
I-NNp	I-A	64 ± 5	49	0.86 ± 0.17	6
II-NNp	II-G	1.3 ± 0.3		19 ± 2	
II-NNp	II-A	53 ± 11	41	2.4 ± 0.1	8
III-NNp	III-G	0.6 ± 0.2		6.5 ± 0.9	
III-NNp	III-A	10 ± 4	17	0.53 ± 0.03	12

Table S5: EMSA and ATF data probing the requirement of a 5'T.



Protein	5' Base	Apparent K_D (nM)	Fold increase in apparent K_D compared to 5' T	% affinity of 5' T control**	Fold Activation	Fold decrease in activation compared to 5' T
$\text{AvrBs3}_{111\text{-}42}$	T	1.1 ± 0.04*		100	3.9 ± 0.6	
$\text{AvrBs3}_{111\text{-}42}$	A	16 ± 4.8	15	7.5 ± 2.6	1.1 ± 0.1	3.5
$\text{AvrBs3}_{111\text{-}42}$	C	22 ± 0.7	20	5.0 ± 0.1	1.2 ± 0.2	3.3
$\text{AvrBs3}_{111\text{-}42}$	G	21 ± 6.4	19	5.4 ± 1.3	1.0 ± 0.2	3.9
$\text{dAvrBs3}_{111\text{-}42}$	T	2.6 ± 1.5*		100	4.7 ± 0.3	
$\text{dAvrBs3}_{111\text{-}42}$	A	4.3 ± 0.3	1.7	36 ± 3	3.5 ± 0.3	1.3
$\text{dAvrBs3}_{111\text{-}42}$	C	9.6 ± 3.7	3.7	40 ± 13	3.4 ± 0.3	1.4
$\text{dAvrBs3}_{111\text{-}42}$	G	8.4 ± 0.4	3.2	43 ± 2	3.1 ± 0.5	1.5

* These values of **$\text{AvrBs3}_{111\text{-}42}$** and **$\text{dAvrBs3}_{111\text{-}42}$** binding to the *Bs3* targets represent the average of two independent EMSA experiments performed on the same days as other individual determinations in the table. The values differ from that in Table 1, but were included in the computation of the Table 1 value.

** Percentage affinities of 5' A, C and G were calculated using the values for **$\text{AvrBs3}_{111\text{-}42}$** or **$\text{dAvrBs3}_{111\text{-}42}$** binding to *Bs3* (5' T) targets, determined on the same day, as a reference.

Table S6: EMSA and ATF data for polarity effects.

Protein	Target	Apparent K_D (nM)	% wild type K_A	Fold Activation
III-HDp	wt	0.6 ± 0.1	100	n.d.
III-HDp	5m3	35 ± 3.7	1.7 ± 0.3	n.d.
III-HDp	5m6	530 ± 134	0.12 ± 0.02	n.d.
III-HDp	5m9	>1650	<0.04	n.d.
III-HDp	3m3	1.7 ± 1.0	44 ± 21	n.d.
III-HDp	3m6	39 ± 28	2.4 ± 1.9	n.d.
III-HDp	3m9	68 ± 23	0.91 ± 0.16	n.d.
III-NGp	wt	0.3 ± 0.1	100	4.3 ± 0.45
III-NGp	5m3	4.2 ± 0.7	6.5 ± 2.9	0.35 ± 0.09
III-NGp	5m6	100 ± 10	0.27 ± 0.13	0.18 ± 0.02
III-NGp	5m9	320 ± 76	0.08 ± 0.03	0.14 ± 0.04
III-NGp	3m3	0.3 ± 0.1	95 ± 8.8	3.1 ± 1.4
III-NGp	3m6	0.5 ± 0.2	49 ± 11	5.3 ± 1.4
III-NGp	3m9	2.5 ± 1.1	12 ± 6	1.6 ± 0.33
AvrBs3$_{111-42}$	wt	5.4 ± 2.6	100	3.9 ± 0.32
AvrBs3$_{111-42}$	5m3	115 ± 11	3.4 ± 1.2	1.2 ± 0.07
AvrBs3$_{111-42}$	5m6	>1500	<0.25	1.1 ± 0.15
AvrBs3$_{111-42}$	5m9	>1500	<0.25	1.2 ± 0.17
AvrBs3$_{111-42}$	3m3	140 ± 78	4.4 ± 2.1	1.3 ± 0.03
AvrBs3$_{111-42}$	3m6	490 ± 48	0.77 ± 0.18	1.0 ± 0.22
AvrBs3$_{111-42}$	3m9	970 ± 64	0.58 ± 0.29	1.1 ± 0.08
dAvrBs3$_{111-42}$	wt	3.2 ± 0.7	100	4.7 ± 0.35
dAvrBs3$_{111-42}$	5m3	1100 ± 85	0.29 ± 0.04	0.52 ± 0.05
dAvrBs3$_{111-42}$	5m6	>1500	<0.22	0.55 ± 0.04
dAvrBs3$_{111-42}$	5m9	>1500	<0.22	0.60 ± 0.04
dAvrBs3$_{111-42}$	3m3	46 ± 12	7.4 ± 2.7	1.4 ± 0.06
dAvrBs3$_{111-42}$	3m6	120 ± 23	2.7 ± 0.59	1.2 ± 0.15
dAvrBs3$_{111-42}$	3m9	93 ± 5.3	3.5 ± 0.66	1.0 ± 0.08
n.d., not done				

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