**Unique insert sequences from the prey isolated using the B1H system with each bait.** Bold letters indicate an overrepresented motif identified by MEME analysis. Italic letters indicate the inclusion of constant bases neighboring the randomized region to complete a binding site consistent with an overrepresented motif identified in the other sequences by MEME analysis. The E-value calculated by MEME for each identified motif is indicated. An asterisk following the sequence indicates that it is the reverse complement of the insert sequence. The absence of bold letters indicates a sequence that does not contain a binding site motif based on computational analysis. Numbers in parenthesis indicate how many times a sequence occurred among the analyzed clones.

**Zif268 sites** (Two step selection; E-value=3.4e-020)

 TTGAG**CGCGTGGGTGG**AT

CAACGT**TGTGTGGGCGT**G(2)

TTGCCC**CGCGTGGGCGT**C

 CA**TACGGAAGCGG**CGTGC**\***

TTGTTG**TGCGTAGGCGG**C

 AATCG**AGCGTGGGAGG**GG

ATCGAG**GGCGTGGGTGG**C

TGTAGT**TGCGTGGGAGG**G(2)

CTAACC**CGCGTGGGCGT**A

CGAGGA**TGCGGGGGTGT**G

TTGCCC**CACGTGGGCGT**G

CGATAA**TCCGGGGGCGT**A

 GAATG**TGTGTGGGCGG**CT

ATTTGT**AGCGTGGGCGA**T

GAGCGA**AGCGTAGGCGT**C

**PLAG1 sites** (Motif score = 2.278 calculated by Bioprospector). The Sequence logo for PLAG1 in Figure 2 is the reverse complement of the overrepresented motifs in these sequences. 18 of 21 sequences contain the identified PLAG1 motif.

 C**CCCCCGA**AAA**GGGCCCC**(6)

**CCCCCGA**TTTG**GGGCCTC**(3)

TT**CCCCATT**T**GGGCCCC**T(4)

AACA**CCCCTGTGGGCCCC**

 **CCCCCTA**AT**GCACCCC**AT

 **CCCCCGT**GG**AGGCCCC**AC(2)

 C**TACCTTT**TTC**TGGCCCC**(2)

AA**CCCCCTA**AG**GAGCCCC**(2)

 **CCCCCCT**CCT**AGGCCCC**C(2)

 **CCCCCTA**CCA**GGGCCTC**A(2)

 **CCCCCTT**GG**AGGCCAC**TA

CCC**CCCCCGTGGCCCCC**T

CCC**CACCCCTGGGCCCC**T

 **CCCCCTA**AC**GCGCCTC**CG

 A**CCCCCTT**AG**TGCCCCC**C

AC**CCCCATTTACCCCC**CC

GT**TCCCCTT**CC**CGGCCCC**

 **CCCCCTT**CAC**GGGCCCC**C

GTATCCCGGTGTATGCGA

GCGGAACCGGCTAGGAAT

GGGCCCCCAGAATTCCGG

**Zif268 sites** (One-step selection; E-value=3.0e-06)

 TGTAC**TGCGTGGGTGT**GG\*(2)

ATGCTTGTCGC**TACGTGG*GCGG***(2)

 CAGCC**TGCGTGGGAGG**TT**\***

TGGACAT**TGCGTAGGCGG**\*

 CCGCG**TGTGTGGGCGG**AA\*

 TTGCCC**CACGTGGGCGT**G\*

 CTCGCA**CGTGTGGGCGT**C**\***

 ACTATC**CACGTGGGCGT**G\*

#### ZnFp53 sites (E-value=1.7e-045). The Sequence logo for Znfp53 in Figure 2 is the reverse complement of the overrepresented motifs in these sequences

 **ACGTGTCC**GTCAGACGGG

 A**GCGTGTCC**CTCAGTCCG

 TG**ACGTGGCC**TGTCACTC

GACGTGGCC**GGATGTCC**G

 AGC**TCGTGTCC**ATGATCA

GACGTGCCC**GGCTATCC**C

 G**ACATGTCC**ACGCCCTAG

 TAA**GCGTGTCC**GTAGCGG

 CCCCCGAC**ACGTGTCC**TA**\***

 G**GCGTGTCC**CGCCTGTTA

 AC**CCGTGTCC**AGCTTTAG

 CC**GCGTGTCC**GTAAAGGG

 G**TCGTGTCC**CCATGGTCG

 G**TCGTGTCC**TTGCACGGG

 AGT**GCGTGTCC**TGGTGCT

 A**GCGTGTCC**TGGTCTGCT

 G**ACGTGTCC**GGATTTCCC

 GGTG**ACGTGTCC**TGGGCA

 **GCGTGTCC**CGCAGCCTGT

 A**TCGTGTCC**GCTCACCTG

#### LAG-1 sites (E-value=1.4e-023). The Sequence logo for LAG-1 in Figure 3 is the reverse complement of the overrepresented motifs in these sequences. 12 of 17 sequences contain the identified LAG-1 motif.

TGTGAGA**ATTCCCACGGT**(2)

 GA**GTTCCCACGGT**CGACA\*(3)

 GGTCAC**TTTCCCACGGT**G(3)

TCAACTT**TTTCCCACTGA\***(3)

 CC**TTTCCCACGGT**TACCC\*(3)

AGTTGTT**TTTCTCACGGT**(2)

 CA**GTTCCCACGAT**TCCGC\*

 GAT**TTTCCCACGTG**TGGC\*

 CA**TTTCCCACGCT**TCAGA\*(2)

 TC**ATTCCCACGGT**AGCAG\*

 CTC**ATTCCCACGGT**CCAG\*

TCAACTT**TTTCCCACCTG\***

CTTCACGGCTCTGACCGG

GGTATGCAATCGATAGAG

GTCTGACCATGTCCTTCC

ATCCGATGTGTTGATCGC

CCCGCGGACACGAACGGT

**Dorsal sites** (E-value = 5.5e-058). The Sequence logo for Dorsal in Figure 2 is the reverse complement of the overrepresented motifs in these sequences. 32 of 35 sequences contain the identified Dorsal motif.

 CCACCACG**GGGAATTCCC**

AGGCACAGA**GGGAATTCC*C***

CCCACCACG**GGGAATTCC*C***(3)

CTGTGGACA**GGGATTTCC*C***

ATAGCCAGA**GGGATTTCC*C***

CCGGATTCT**GGGATTTCC*C***

TTTCGCGGG**GGGTTTTCC*C***

 ATCCGGCG**GGGAAAACCC**

 CGCGTACC**GGGAAAACCC**

GGTGCGTCT**GGGAAAACC*C***

TAATCACGG**GGGAAAACC*C***

GGTGCGTCT**GGGAAAACC*C***(2)

GGTGTGGCG**TGGAAAACC*C***

 CCACCGGA**CGGAATTCCC**

GCCATGTAC**CGGAATTCC*C***

TCGTATACT**GGGAAAAAC*C***

CGCCTGCGC**TGGAAAAAC*C***

ACCTCGCCT**GGAAAAACC*C***

 CGGTGCCT**TGGGATTCCC**

ACCTGCTCT**GGGAATTTC*C***(2)

CCTACCTCG**GAGAATTCC*C***

 CAGTGAAC**TGGGAAACCC**

CCTGGTAGC**CGGAAAAAC*C***

 CCCTAGC**GGGGTTTCCC**C

TGTTCACCC**GGGGTTTCC*C***

AACCTGGAG**CGGAAAAAC*C***(2)

ACTGCATCA**CGGGATTCC*C***(2)

 TGG**TAGAATTCCC**ATGGT\*

TCAATCGGA**GGGGAAAAC*C***

ACCCAGCTT**GGGGAAATC*C***

TCGTCACGG**TGGTTTTCC*C***

CATCGGTTT**GGGGTTTCC*C***

GCCATGCTATCGGCCGCG

CACCCCCATCAGAATCCTC

CGAACCGTTAGCAGCTTAC

**Paired site (**E-value = 3.0e-028). 22 of 32 sequences contain the identified Paired motif.

 **AATTCGTCACGGT**TGATG

 **GATTCGTCACGCT**TGCTT

 TG**ATTTCGTTACGGT**CTG

 C**GATTAGTCACAGT**GCCA

 CT**AATTCGTTCCGCT**CCC(4)

 CC**GATTCGTTCCACT**ACC

 TT**AATTCTTTCCGCT**CCT

 CC**GATTCGTTCCTCT**ACC

 AC**AATTCGCTACGGT**GGT

 CC**GATTCGACCCAGT**CAC

 CC**GATTAGTCTCGGT**TCA

 CC**GCTTCGTCACGCT**ATG

 CT**GATTTGATACGCT**TCG

 CA**TATCCGTCACGGT**GTT

 A**AGAACGTCACGGT**TCAC

 CT**CGCTCGTCACGGT**TGG

 AC**AATTCTCTACTGT**GGT

 **CACTAGTTACTGT**ACCGC\*

 C**GTTTCTATAGGCT**GCGT\*

 A**AGAACGTCCCGTT**TCAC

 AT**AAATCTGACCGGT**GGG\*

 T**CATACGGCCCCTT**TCTC

GCGGTACAGTATGTCCCA(2)

TGAGTTATTATGGCGGGG

AGTGATTGTATGGTATAC

AACCTGTGCTAGAGTACC

AGTAAAGACCGTGCTGCC

CAAGCTGAGCCCAAAATG

TCTTATTTGTCAGTAGGA

CGGGCGGCTCGGATTAAT(2)

GGAATACTCTGAAAACAC

GCCCCGCTGCAGAATTAA

**Runt/Bgb sites** (Two step selection; E-value = 1.1e-067) The Sequence logo for Runt in Figure 2 is the reverse complement of the overrepresented motifs in these sequences.

# Sequences prior to counterselection

**TAGCCGCAG**ATTGACTGC

GATCTACAGGCGCTATGA

TCTCGGACGCAGAGTACG

AAACTGTCCCACCCCTAC

CACGCTATACTGCAACAC

ACATGTATACTTTGAGAG

AGCTACTCCTGGGAGAAT

ACTGGTATATTAGTGCGG

# Sequences after counterselection

 C**AAACCGCAA**TCCTCGCC(2)

 AAGTTCC**AAACCGCAA**GC

 GGTAGCC**TAACCGCAA**GT\*

 ACT**TAACCGCAA**AGGATG(2)

 AA**TAACCGCAA**ACTACCC(3)

 A**AAACCACAA**GCGCTCCT

 AT**AAACCGCAG**GCTCTAC

 CTCGAA**AAACCGCAG**CCT\*

 CTG**AAACCGCAG**TTTCGG

 T**AAACCGCAG**GTTCCAGC

 AAGC**TAACCGCAG**AAGTG

 C**TAACCGCAG**CGCTATTC

 ACCCCAG**TAACCACAA**GT\*

 CT**AAACCACAG**CTCCCCG

 CT**CAACCGCAA**GGACGAC

 ACC**CAACCACAA**AAGGTG

 ACCGCAA**AAGCCACAA**TC\*(2)

 AA**CGACCACAA**GTCCTTG

#### Odd sites

1.5 mM 3-AT (E-value=4.3e-031)

 ***G*CTACTGGT**CTTAGGATCT

 CT**GCTACTGTA**ATGGTAAC

 ***G*CTACCGGT**TCCTTACAGA

 ***G*CTACCGGA**AGTCATCGTA

 **GGATCTGTT**TGTTCGGTG

 GA**GCTACTGTC**TTGCGGG

 ***G*CTACTGCT**TAGTCTTGGT

 ***G*CTACCGGA**CTAGTTACCC

 A**GCTTCTGAA**ATTCCAGA

 **GCTACTGGA**CCCACCTAG

 ***G*CTACTGGC**GGGGCAACTC

 ***G*CTACTGGA**CTGTTGTCGC

 **GCTACCGAT**GCTCACTCC

 GA**GGATCCGGA**AGCAACG

GGTCCTTCCGT**GCTACTG*GC***

 **GCTACTGGT**GTCCTTAGT

 ***G*CTACTTGT**CACGGTCCGA

 T**GCTACCGAG**CATGAGCG

 ***G*CTACTGCA**AGCGGTGCGTA

**ACTACCGTT**TAGTTGGCG

 G**ACTACTGGA**AGCCTGGT

 C**GCTTCCGGA**AACTACCG

 G**GGATCCGTT**GGAGCGAT

2.5 mM 3-AT (E-value=9.5e-43)

 **GCTACCGTT**TTACCCGGG

 ***G*CTACTGGT**CTTAGGATCT(2)

 T**GCTACTGTG**TAATGCGC

 T**GCTACTGGA**AGTTGGTC

 C**GCTACTGTT**GTGTGTGG

 **GCTTCTGTA**TTGTTGCGA

TG**GCTACTGTC**TGCCACC

TT**GCTACTGTA**CCCGGGG

 **GCTACCGTG**TGACTCGGA

 **GCTACTGTA**GGCGAACAA

 **GCTACCGTA**GACGCTCGG

 **GCTACCGTG**TGTACCGCA

 A**GCTACCGGA**GCCCTAAG

 **GCTATCTGG**TGGAAGTTG

 **GCTACCGGA**AGTTCCTAG

 **GCTACCGTT**ATGTCGTCG

 ***G*CTACTGAT**TCCCCCTTTA

 ***G*CTACTGAT**TGCGGAAGCA

 **GCTTCCGGA**AAAGGCGTG

 **GCTACTGGT**GCTGCACCC

 G**GCTACTGGT**AACAGAGT

GG**GCTACCGGG**GGAATTC

 ***G*CTACCGTG**AAGTTGTTGT

 **GCTACCGGA**CGGTCCCTC

GGATCCGTATGAGTGCCTC

5 mM 3-AT (E-value=3.2e-049)

 ***G*CTACCGGT**TCCTTACAGA

 C**GCTACCGAA**TTTAAGGG

 **GCTACCGTA**TACTTTCCA

GC**GCTACTGTT**TGGGGTG

 ***G*CTACTGTT**TTCTGTCACC

 **GCTACTGGT**AAAACCAGT

 ***G*CTACTGTT**GCGTGCAAGG

 T**GCTACTGGA**CAAAATCG

 T**GCTACTGTT**CACTCGGG(3)

 T**GCTACTGGA**AGTTGGTC

 T**GCTACTGGA**AGGGGTGA

 A**GCTACTGTA**TCAGTGGT(3)

 ***G*CTACTGTT**TACGCATGGG

 T**GCTACCGTA**GCGACGAG

 ***G*CTACTGGT**CTTAGGATCT

 T**GCTACTGTG**CAATGCGG

 T**GCTACTGTG**GATGAGCG

 T**GCTACTGTG**TAATGCGC(3)

CG**GCTACTGTG**CAGCTAG

 **GCTACTGTG**CAGTTGCTG

CG**GCTACTGTA**TACGTCC

GT**GCTACTGTA**AGCTCAG

GT**GCTACCGTT**TGTTCGG

GGACTAGGCTGTCTACCC

GACACATCCTCGGCGGCT

GGCCTAGGGAAAGTCTGT

GCCCCGAGGAGCATAAAG