Supplemental Information

Supplemental Tables

Table S1. Conventional PCR primers used in SF1 and Scr 3C capture

NAME OF ELEMENT	OUTSIDE PRIMER FOR NESTED PCR (5'>3')		INSIDE PRIMER FOR NESTED PCR (5'>3')
SCR-P	CATCGGGGCTTAACGATTTTG		TTTATCGGTCTGTTTCGGGG
SF1	ACCCGAGCTGTCGGTTATTA		CTGAAATTTCGAAAGTATGAA
R1	TCGAGGGTTCCTTACCTCT		CGACGCAAGCACACAGA
R2	CAGGAGCTAAAAATGCGTTCA		TTCGCAACATTTTGGCCTG
R3	GAAAGGTTGCTCAGAACAGGG		CCGCACAAATTACATAATCACG
R4	CGACAAAAGACGCCAAGACA		ATGCGAATGGGAGAAATGCG
R5	TGAAAGGAGTTACTCGCAACT		TAATTTAGCAGGTCGGGCG
R6	CACGATCGATCGCCAATCAT		GCCAAACAGGTGTGTCTGG
R7	TGG CGC TCA AAA GCG TT		GGCCTGGCTTCGACTTTT
R8	AATGTCGTATCACACAGGCG		GGCAATCCGTCACTCACATT
R9	GCGACAAGTGTGTGCGTAG		GCGCTTTGCCTTTGGACAT
R10	GCCTTCTATTCGGGTATCTCT		ATAACCACTGCAGTCGGTTAG
R11	TGTCAGAAATCCACTCGAGC		TCCATCTCTCGATCCATCAA

Table S2. Primers for SF1-based 3C capture qPCR

SF1	CTGAAATTTCGAAAGTATGAA
FTZ-P/R-2-Q	TGATAATGGCCAAGAGACTT
FTZ-E/R-1-Q	ATGCACACTTGGAAAAAGTT
R1-Q	CAGTCCGAGAGTATCCTTTT
R2-Q	ATTTTGGCCTGACTAGGG
R3-Q	GTGCCGCACAAATTACATA
R4-Q	CTTTACTGGGGCGGATCA
R5-Q	TGAAACAATGATGAGGTTGA
R6-Q	TCCAGTCGATTAAAGTGAGC
R7-Qd	TAACGAACGATCAGCAATTA
R7-Qp	CTGGACGTGGATGGATAAT
R8-Q	ATGGTCAGGGGTGTAATATG
R9-Q	GAAAAGGAGGAACTGCACA
R10-Q	ATCGCTTTTTGGGAGTTCTA
R11-Qd	TATTTAAATCGTTCGGTTGC
R11-Qp	TACTTTACACGGGTTTTGTT
R12-Qp	TGGGTAACTCAAAACCACAT
R12-Qd	GACGAGGGATCAGGGAAT

Table S3. qPCR primers used in ftz promoter-based 3C capture

NAME OF ELEMENT	OUTSIDE PRIMER FOR NESTED PCR (5'>3')		INSIDE PRIMER FOR NESTED PCR (5'>3')
R3	GAAAGGTTGCTCAGAACAGGG		CCGCACAAATTACATAATCACG
R2	CAGGAGCTAAAAATGCGTTCA		TTCGCAACATTTTGGCCTG
R1	TCGAGGGTTCCTTACCTCT		CGACGCAAGCACACAGA
F1	CAAAAGAGGCAGCGCAAACA		AAGCCCAAAACACTCAGCTG
F3	TAATTGCCCGCGTATCCTTCG		CCCCTTCCTCCTTCCTTCGTT
F4	CATCATGCCCTTCCGTAGTCG		CGCCACACACTTTTCGTTACAAG
F5	GTCCTTGACTGCCATAAAGTCAG		AGTTTCAAGAAATTCTCTGTTCGCT
SF1	ACCCGAGCTGTCGGTTATTA		CTGAAATTTCGAAAGTATGAA
SCR-1	ACGAATGACCTATGCCATTTATCT		CAAAATCGATTTTGGGTGTGTCT
SCR-P	CATCGGGGCTTAACGATTTTG		TTTATCGGTCTGTTTCGGGG

Table S4. Other primers used in for the Drosophila distance-frequency curve

CATCGCTTTTTCGTTTCGCAT
GCTTTACTCATGCCATTCTGTGT
GTTTGTTTACGGTTCGTAGCTGA
CGGGTGGCTCATAGTTCATAG
CCATTAATTTTCGCCGCCATTT
CCTGGCAAAAAGTTGAATGGGA
CCGTGTCCTTCTCAGCATTTT
GAAGTCGCGTTCGGCCAAATG
CTCTCGCTCTCGTGGTTTCCT
TCGAGGATGTGAGAGTACGGAT
ATTACTCAAGCATGGCACCCA
GGCAATCCCTTCTATACTTTCCTT
AATTTGGAATGTGTCCCGCTG
GGCTTAATGAGTGTAAGCCACA
GGTCCTAAGCAACTCCTGAAAA
CTGGGGGATACGAGTGTAAGA
CCCAGCTTAAGTGCTTAACCA
CCCGCTTATGAAATCGGCATA
TCATGGCCACGAAAATGTCCA
GTGGCATCGTTTTTGGTTGTTG
CTCACGTGTTTCTGACGTACG
ACGTACATATCTGGTTAAGC
CACCTAAATTGGGGTAACTG
TTAGAACCAAAAGGAGCTGGG
AGTTTGCCGGTAATGTTCT
TAAAGGCGCTGCTTTTGCAAG
TAAGCTGGAAAACTTTACCTCACC
GGATTAGTTTGAGTTCCGATCAGG
TTTCTCCATTTTCGATCCCGAG
GCCAATGATCTGAACGATGCG

ATGCCCGTCTGATGACGACAA
GTAAAAGCAAAGCCAGAATGCG
GGAAGATCAGTGTTATTGCGCT
TCCTGTGGCACTTTACTAGAACTT
GTGCATACAGGCGATTGAGAA
CAGCCGGGCAAACCGTCTATG
GATGTTGGCATCGGTGCTCCA
GGGAAAGCTTGATGCAACCAT
CAATTGCACAGAAAAAGTTTCGCT
CCCCGAACTGTGATAAACGGA
TCTTAGAGCATTCTGAATGAAGGA
AAATCGGGTTTTTCCTTGCCG
ACCTACACACTTGGTAGCATAATCA
CACGCGAAATGTCCGCTTACA
TGACTTCTTGGCAGCAATGCA
CGCCCTGGATCTGAAGACATA
ATCTGTAGGGTTGGCCGTATT
CCGTATATCTTGTTGCAGGCG
GATTTCGGAACCTTAGTACGTTTTCT

Table S5. PCR primers used for cloning STEs in enhancer-blocking transgenes

SF2B	TGCGGCCGCCCATCCTCTTGTGAGGCTGG
	TGCGGCCGCCTGATTGACGAATTGCGTGCG
R2	GAATTCGTCAATGTCAAAATCAGAC
	TTCTGTTTTTGCTTCCAGTGTG
R6	GCGGCCGCGAAATAAATGAATTCTGGT
	AGAATTCACCCGCCAATGTGT
R9	ATAAGAATATGCATCCCGCTCCTCGATTGTGTGAT
	ATAAGAATATGCATTCGATTTCGAGAGTCCGCTG
R10	GAATTCGAAGTCGAACTTTCGC
	TCAATTGTGCAAAAAAAGCCA
1.5 kb spacer	TTGCATTAAGAAGAGGTGACTG
	GCGGCCGCTCTAGATACCGAATTT
attB	CCAATGCATGTCGACGATGTAGGTCACG
	AACTGCAGTTCGGCTTGTCGACATGC

Supplemental Figure Legend

Figure S1.

Photo of PCR products after electrophoresis on 2% agarose gel and stained with ethidium bromide. Right: product from 3C capture between SF1 and R1-R11, followed by nested PCR. Middle and left: PCR product from positive control 3C using ligated genomic DNA after EcoRI digestion (GCL), and negative control 3C without crosslinking (NCL).

Figure S2

Relative frequency of 3C capture between SF1 and R-1, R1 and R2 in 4h-8h (orange bars) and 10h-14h (purple bars) embryos were quantitated by qPCR (for details see methods and Fig. 4 legend).

Figure S3

STEs correspond to developmentally regulated chromatin domain boundary in the *Scr-Antp* genomic region. Screen crops of H3K9Me3 and H3K4Me1 ChIP-seq profile in the *Scr-Antp* region from different embryonic stages (http://www.genome.gov/modENCODE. EcoRI sites are shown on top. Vertical shaded bars indicate CBEs in the regions (red, SF1; orange, R1, grey R2, R6 and R10).

Figure S4.

Binding profile of known insulator factors on STEs. Screen crop of ChIP-seq profile of several known insulator proteins at and near SF1 and STE sequences (http://www.genome.gov/modENCODE).

Figure S5.

NEE-blocking in labial thoracic tissues in R2 and R6 transgenic embryos.

Representative images of embryos from independent transgenic lines containing R2 or R6 element in the pFWNHZ transgene, stained with the anti-lacZ (**A-L**), or anti-lacZ and anti-Scr (**P-R**) RNA probes. Anterior region of embryos are shown in ventral lateral views, anterior to the left and dorsal up. **A-D**). Variation of NEE blocking levels in pFWNHW-R2 or R6 embryos (see **M**). **E-H**). Example of strong NEE-block in pFWHNZ-R2 embryos. **I-L**). Example of strong NEE-block in pFWHNZ-R6 embryos. **M**). Quantitation of NEE-blocking in the labial thoracic tissues (gap) in embryos from multiple pFWHNZ-R2 and pFWHNZ-R6 transgenic lines, stained with the lacZ probe (see methods for details). **N-Q**). Embryos from pFWHNZ-R6 lines double stained with anti-lacZ and anti-*Scr* probes.

Fig S1

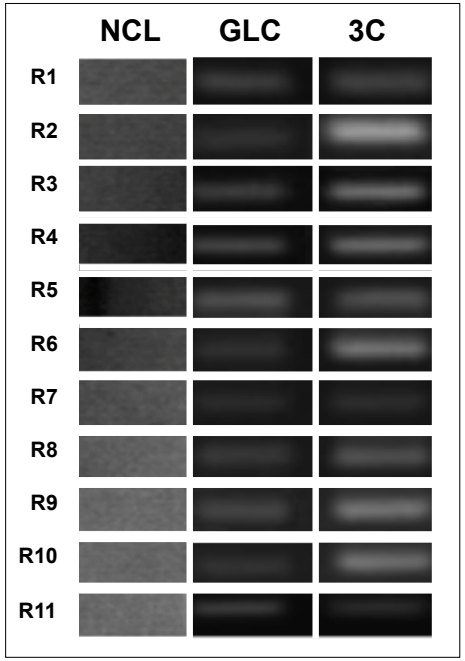


Fig S2

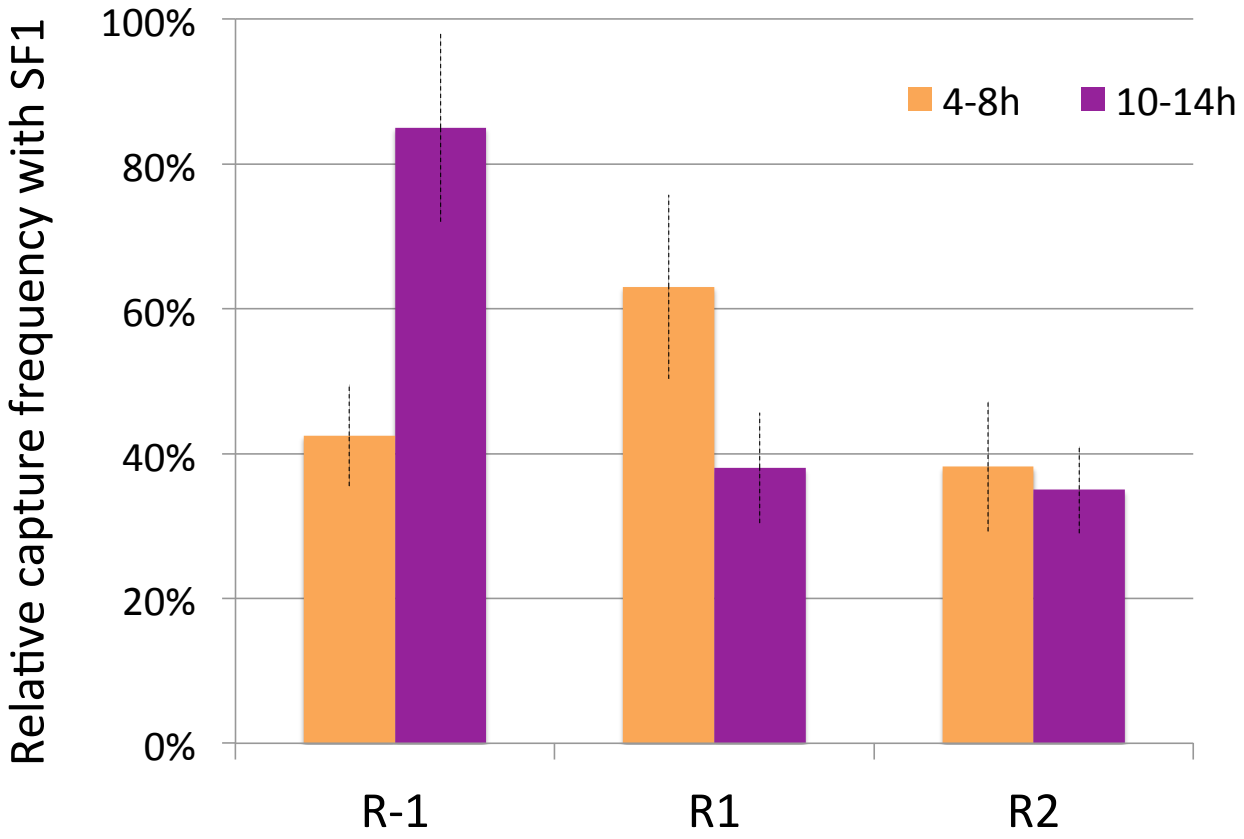

Relative capture frequency with SF1
100%
80%
60%
40%
20%
0%
4-8h
10-14h
R-1
R1
R2

Fig S3

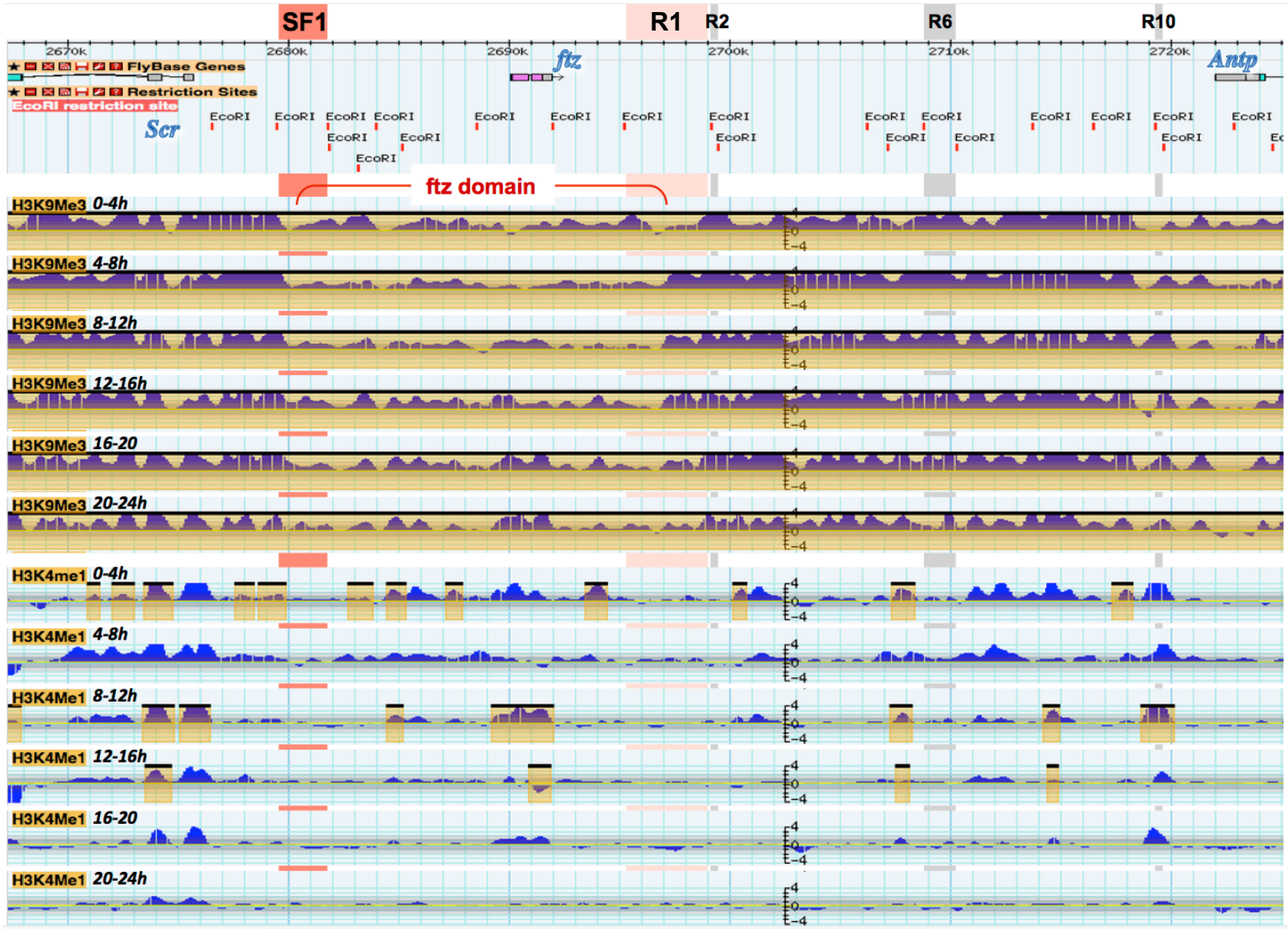

SF1
R1
R2
R6
R10
2670k
2680k
2690k
2700k
2710k
2720k
ftz
Antp
FlyBase Genes
Restriction Sites
EcoRI restriction site
Scr
EcoRI
ftz domain
H3K9Me3 0-4h
H3K9Me3 4-8h
H3K9Me3 8-12h
H3K9Me3 12-16h
H3K9Me3 16-20
H3K9Me3 20-24h
H3K4me1 0-4h
H3K4Me1 4-8h
H3K4Me1 8-12h
H3K4Me1 12-16h
H3K4Me1 16-20
H3K4Me1 20-24h

Fig. S4

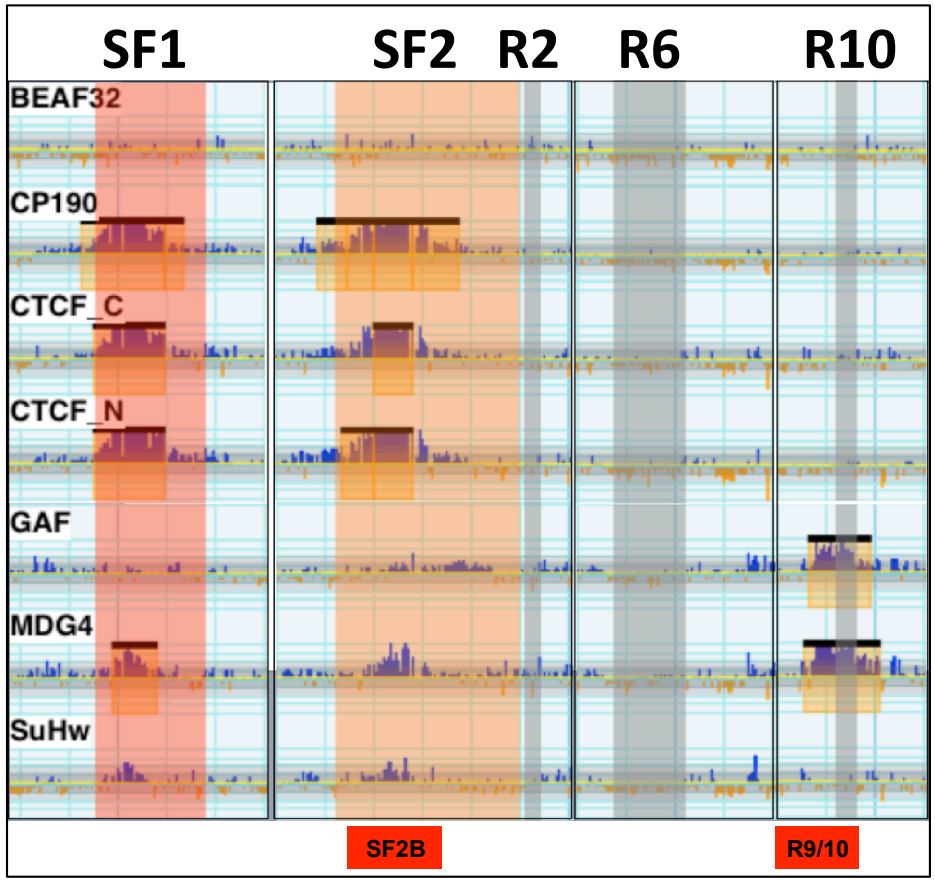
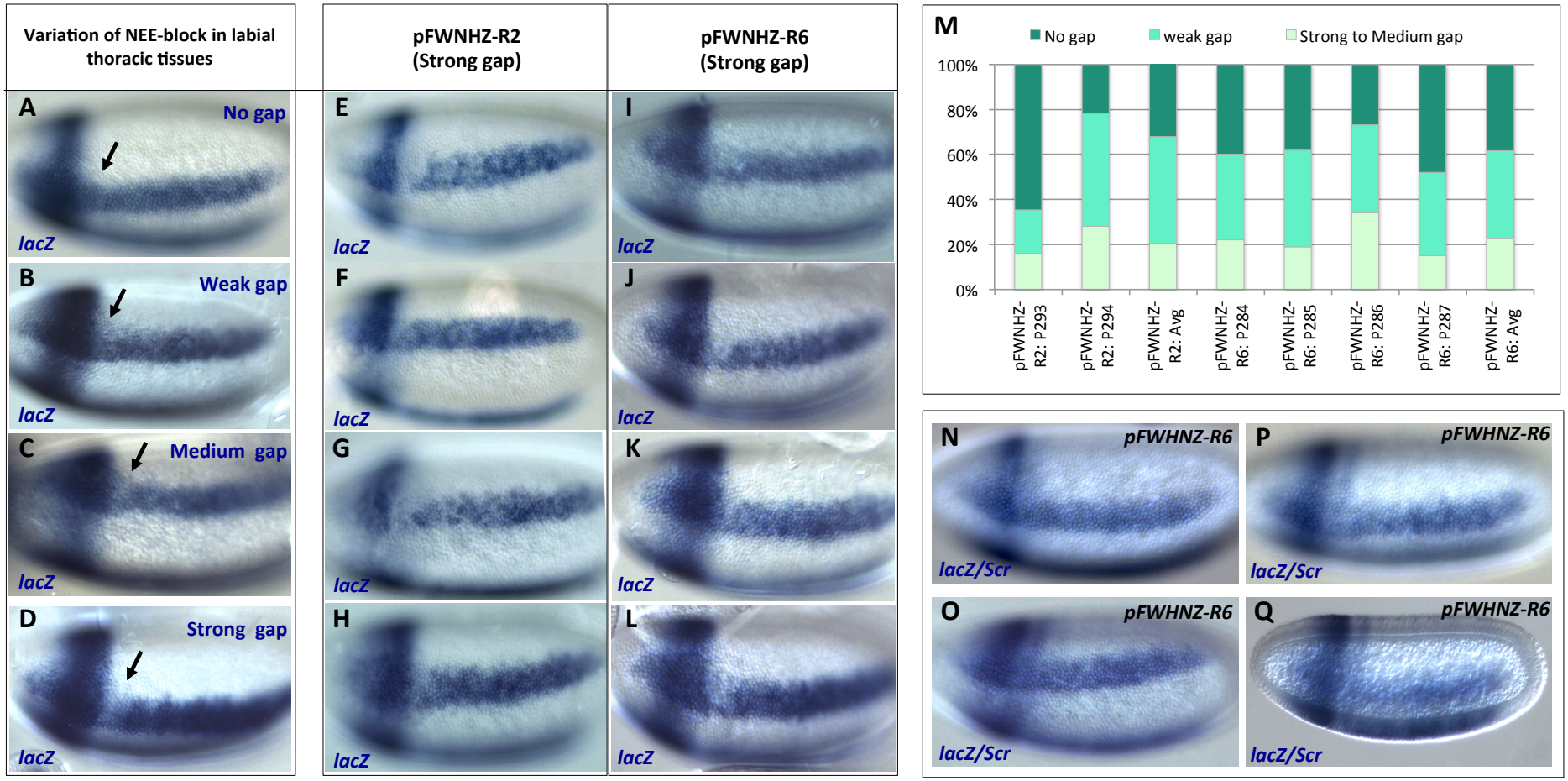

SF1
SF2
R2
R6
R10
BEAF32
CP190
CTCF_C
CTCF_N
GAF
MDG4
SuHw
SF2B
R9/10

Fig. S5


Variation of NEE-block in labial thoracic tissues
pFWNHZ-R2 (Strong gap)
pFWNHZ-R6 (Strong gap)
A
No gap
lacZ
B
Weak gap
lacZ
C
Medium gap
lacZ
D
Strong gap
lacZ
E
lacZ
F
lacZ
G
lacZ
H
lacZ
I
lacZ
J
lacZ
K
lacZ
L
lacZ
M
No gap
weak gap
Strong to Medium gap
100%
80%
60%
40%
20%
0%
pFWNHZ-R2: P293
pFWNHZ-R2: P294
pFWNHZ-R2: Avg
pFWNHZ-R6: P284
pFWNHZ-R6: P285
pFWNHZ-R6: P286
pFWNHZ-R6: P287
pFWNHZ-R6: Avg
N
pFWHNZ-R6
lacZ/Scr
P
pFWHNZ-R6
lacZ/Scr
O
pFWHNZ-R6
lacZ/Scr
Q
pFWHNZ-R6
lacZ/Scr