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Supplementary Materials for

Regulatory encoding of quantitative variation in spatial activity of a *Drosophila* enhancer

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The PDF file includes:

Figs. S1 to S4
Tables S1 to S3
Legends for data files S1 to S4
Additional notes on *logRatios*

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/49/eabe2955/DC1)

Data files S1 to S4

Supplementary Materials

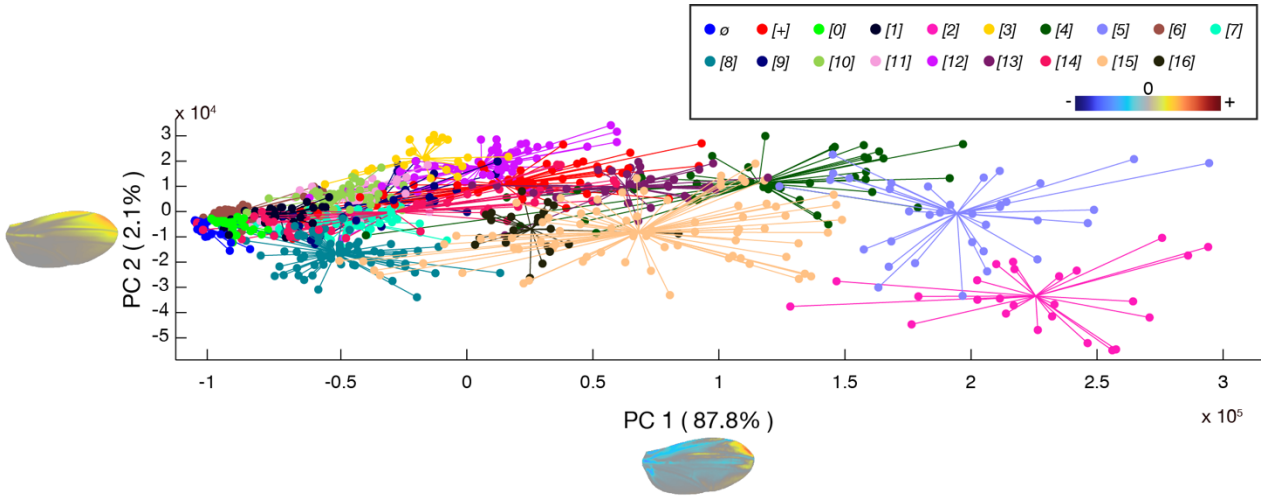

Figure S1. **First two axes of variation in a principal component analysis of all individual wings used to generate the average reporter expression of Figure 1**. Each wing is depicted by a colored dot, and each construct by a color. PC1 captures 87.8% of the variation and corresponds to overall changes in the activity of the *spot*^{196} CRE. PC2 captures 2.1% of the variation and appears to represent spatial difference in CRE activity between lines. The direction of variation along each principal component is represented on a wing with a colormap next to each axis.

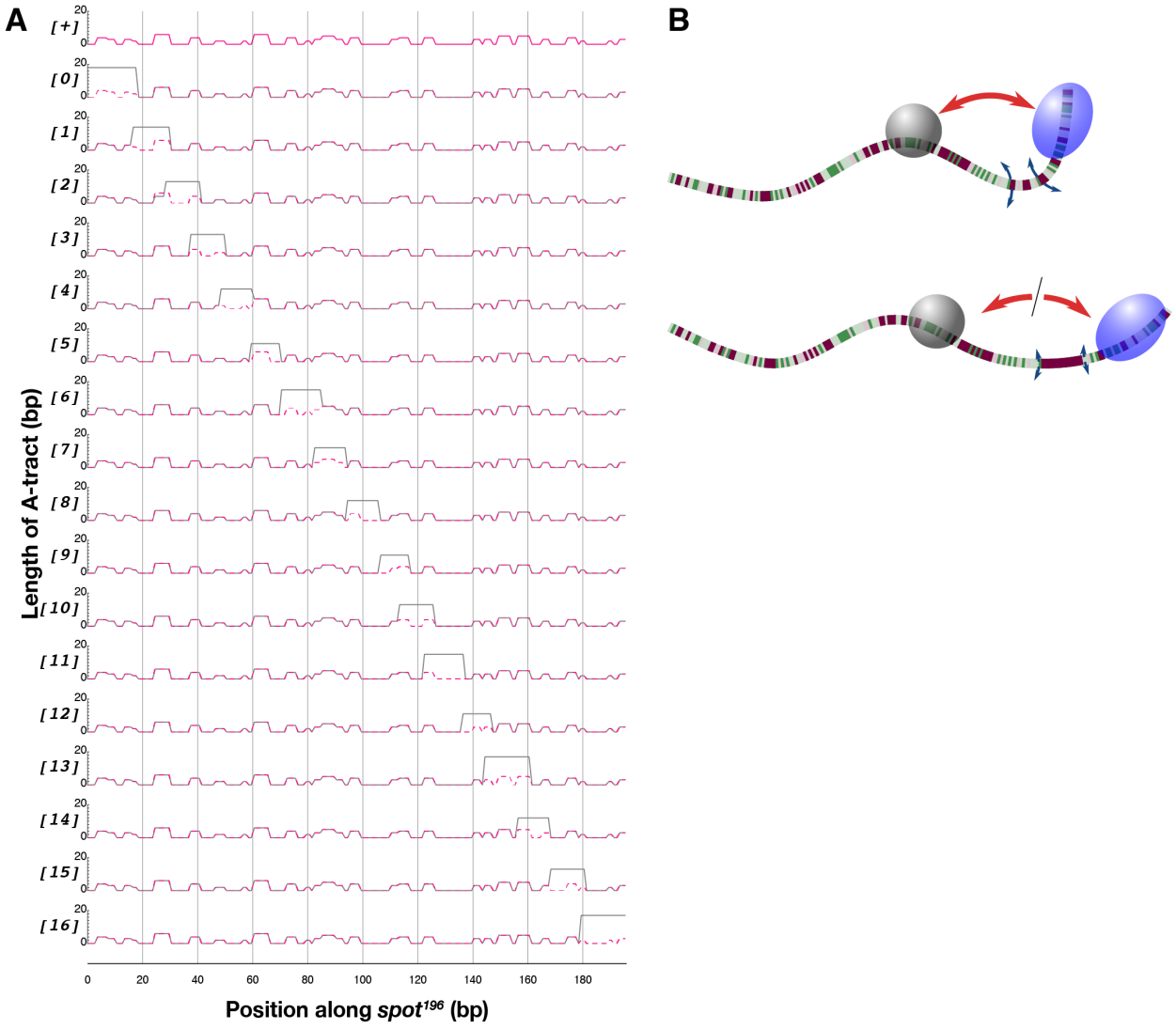

Figure S2. Local rigidity along the wild-type and mutant *spot*^{196}. (**A**) Each graph is a plot of the length of the longest consecutive A_nT_n sequence that a base pair participates in, a proxy for sequence rigidity at this position. The first graph on top is the wild type (*[+]*) alone. The remaining graphs show plots for each mutant (*[0]*, …, *[16]*) with a solid black line, compared to the wild type represented with a dotted magenta line. (**B**) Schematics illustrating the hypothetical consequence of local DNA rigidity (caused by an A-tract) on TF interactions. A flexible linker between two TFBSs would favor interactions between 2 bound TFs, while a stiffer linker of the same length would limit, or prevent these interactions.

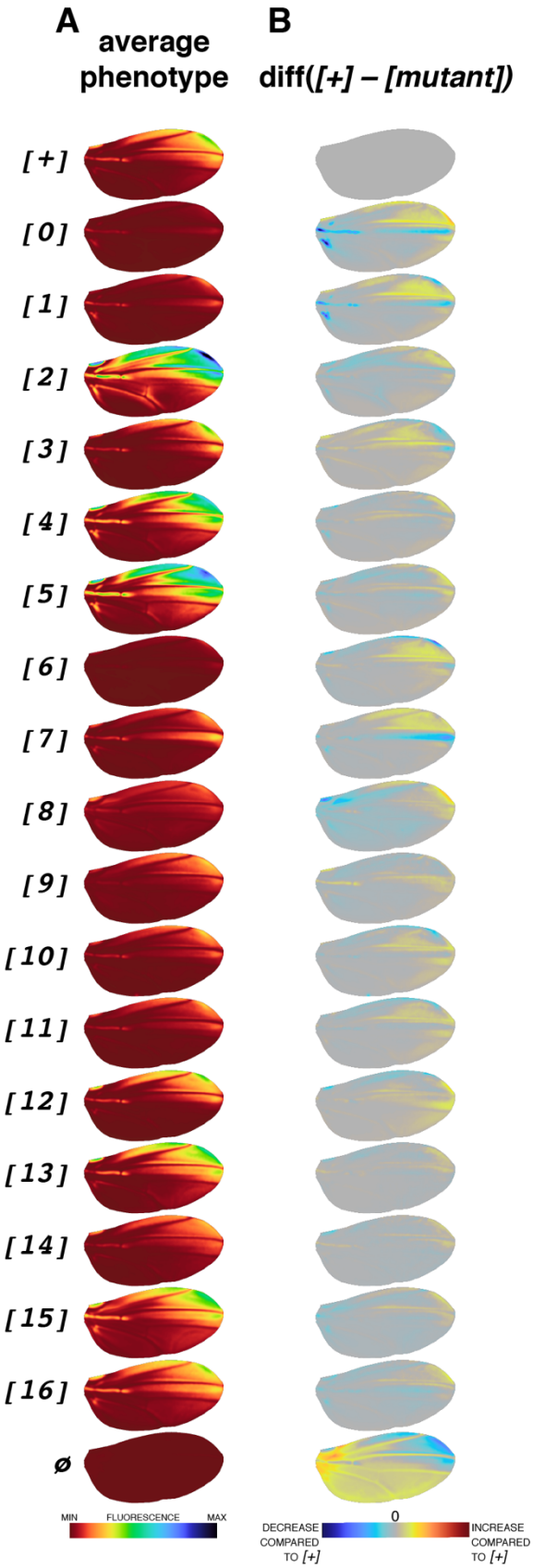
A
average phenotype
B
diff([+] – [mutant])
[+]
[0]
[1]
[2]
[3]
[4]
[5]
[6]
[7]
[8]
[9]
[10]
[11]
[12]
[13]
[14]
[15]
[16]
ø
MIN
FLUORESCENCE
MAX
0
DECREASE COMPARED TO [+]
INCREASE COMPARED TO [+]

Figure S3. **Pattern changes between wild-type and mutant *spot*^{196} constructs**. (**A**) Average phenotypes reproduced from Figure 1B. (**B**) difference images (*[+] – [mutant]*) for intensity values of each pixel of registered wing images) highlight changes in the distribution of the enhancer activity across the wing. Note that this operation introduces a visual bias towards changes in region of high expression, contrasting with *logRatio* images of Figure 2.

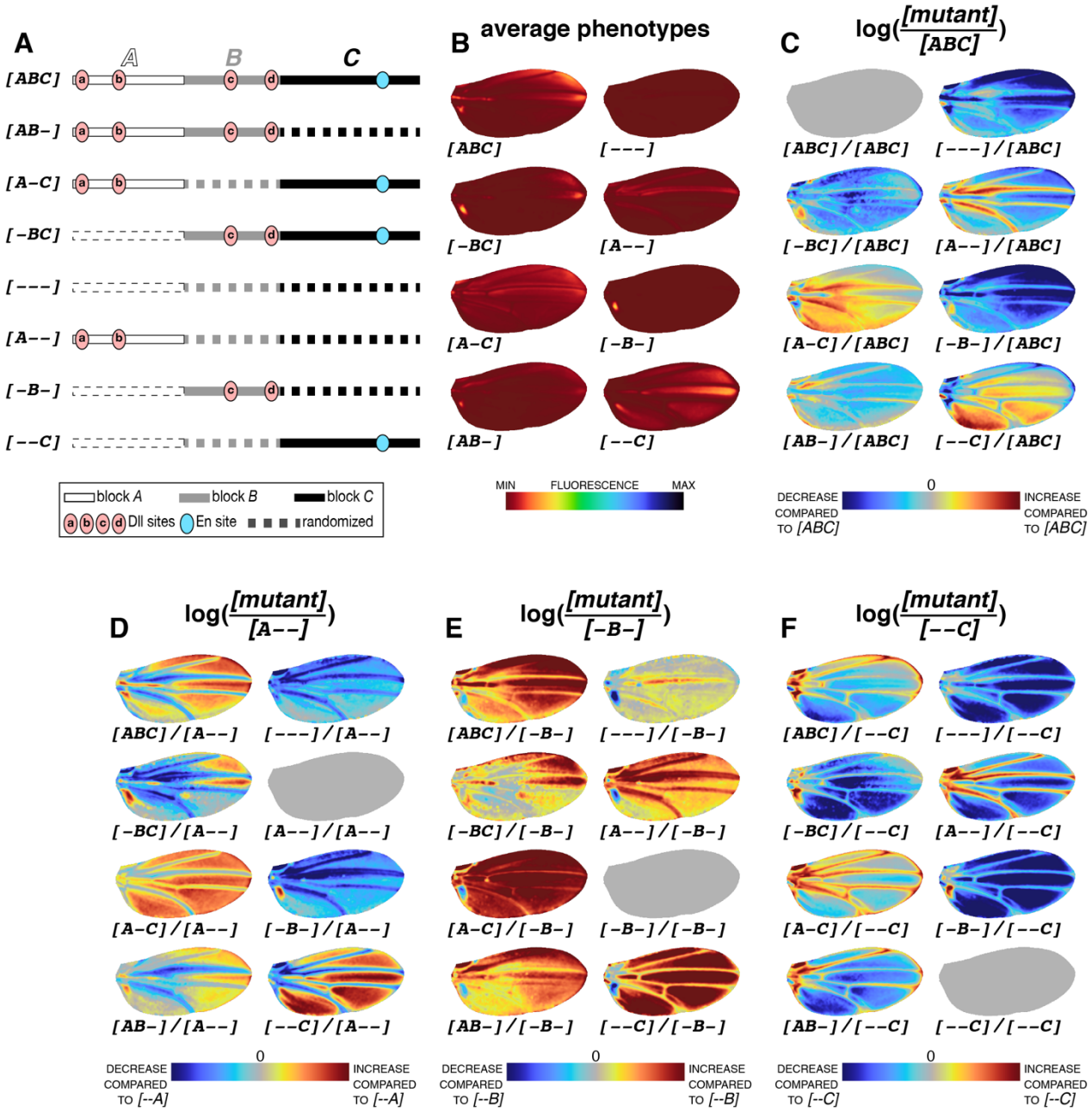

Figure S4. ***logRatio* of all block constructs.** (**A**) Schematics of block constructs repeated from Figure 3A for legibility. (**B**) Average phenotypes of constructs shown in (**A**), repeated from Figure 3B for legibility. Colormap of average phenotypes normalized for all constructs of the block series, including block permutations of Figure 4B. (**C**) Average phenotypes in (**B**) compared to the average phenotype of the wild type *[ABC]* (*logRatio*). (**D**) Average phenotypes in (**B**) compared to the average phenotype of *[A--]* (*logRatio*). (**E**) Average phenotypes in (**B**) compared to the average phenotype of *[-B-]* (*logRatio*). (**F**) Average phenotypes in (**B**) compared to the average phenotype of *[--C]* (*logRatio*). Colormaps in (**C**)-(**F**) indicate an increase or a decrease of activity compared to the reference (denominator).

Table S1. Sequences of *spot*^{196} enhancer variants.

- wild type *[+]* or *[ABC]*

>*spot*^{196} *[+]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

- single mutants *[0]* to *[16]*

>*spot*^{196} *[0]*

AAAAAAAAAAAAAAAAAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAG
ATCTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTA
AAACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCG
CCTAATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[1]*

TCTAATTATTCCGTTTAAAAAAAAAAAATTCTGAGCTAAAACTCGCTTATGGAGAGA
TCTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[2]*

TCTAATTATTCCGTTTAAGGACGCAATTAAAAAAAAAAAAACTCGCTTATGGAGAGA
TCTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[3]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAAAAAAAAAATGGAGAGA
TCTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[4]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTAAAAAAAAAA
AATAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[5]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CAAAAAAAAAAAAGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[6]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCAAAAAAAAAAAAAAATAAATTAATCGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC

TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [7]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAAAAAAAAAAAAGAATTCCCCGCTGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [8]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCAAAAAAAAAAAAAGGCTATTAA
AACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [9]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTAAAAAAAAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [10]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
AAAAAAAAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [11]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAAAAAAAAAAATCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [12]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGAAAAAAAAAAATGTAAATTGCAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [13]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAAAAAAAAAAAAAAATTGCTCAATCCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*$^{196\ [14]}$
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAAAAAAAAAACCGCC
TAATTGATGTGCGCCCATGCAAT

>*spot*[196] *[15]*
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATAAAAA
AAAAAAATGTGCGCCCATGCAAT

>*spot*[196] *[16]*
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGAAAAAAAAAAAAAAAA

- Permutations of blocks

> *spot*[196] *[ACB]*
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCC
GCCTAATTGATGTGCGCCCATGCAATTTTCCCCGCTTTTGGCTTGAATAAATTAATCG
AATTCCCCGCTGGCTATTAAAA

>*spot*[196] *[BAC]*
TTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAAATCTA
ATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGATCTAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*[196] *[BCA]*
TTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAAACACA
CAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCTAATTG
ATGTGCGCCCATGCAATTCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAA
CTCGCTTATGGAGAGATCTAAA

>*spot*[196] *[CBA]*
CACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCTA
ATTGATGTGCGCCCATGCAATTTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTC
CCCGCTGGCTATTAAAATCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAA
CTCGCTTATGGAGAGATCTAAA

- Randomized blocks

>*spot*[196] *[A--]*
TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATCCGAATTTTTTCTTGTCCGACTAGAAACGACTAATTTAGCCGTACCACATGT
TGTCGACTCAGAAACATTATTCCCATTTACGCGTAAGCAAAAAATGCGTCCTTATCGA
ACTTACACTCGCCTGCGTTGGT

>*spot*[196] *[-B-]*
ATAATATTGCATCTCATTGTGGTGCTAGATAATCATCTAGGCTAAATCCAAAACTGTT
GCATGTTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
AGTCGACTCAGAAACATTATTCCCATTTACGCGTAAGCAAAAAATGCGTCCTTATCG
AACTTACACTCGCCTGCGTTGGT

>*spot*^{196} *[--C]*

ATAATATTGCATCTCATTGTGGTGCTAGATAATCATCTAGGCTAAATCCAAAACTGTT
GCATGTCCGAATTTTTTCTTGTCCGACTAGAAACGACTAATTTAGCCGTACCACATGT
TCACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[AB-]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
AGTCGACTCAGAAACATTATTCCCATTTACGCGTAAGCAAAAAATGCGTCCTTATCG
AACTTACACTCGCCTGCGTTGGT

>*spot*^{196} *[A-C]*

TCTAATTATTCCGTTTAAGGACGCAATTTTCTGAGCTAAAACTCGCTTATGGAGAGAT
CTAAATCCGAATTTTTTCTTGTCCGACTAGAAACGACTAATTTAGCCGTACCACATGT
TCACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[-BC]*

ATAATATTGCATCTCATTGTGGTGCTAGATAATCATCTAGGCTAAATCCAAAACTGTT
GCATGTTTCCCCGCTTTTGGCTTGAATAAATTAATCGAATTCCCCGCTGGCTATTAAA
ACACACAAAAGGCGCTCTCGTCTGTTTCAATGTAAATTGCAAATTGCTCAATCCGCCT
AATTGATGTGCGCCCATGCAAT

>*spot*^{196} *[---]*

ATAATATTGCATCTCATTGTGGTGCTAGATAATCATCTAGGCTAAATCCAAAACTGTT
GCATGTCCGAATTTTTTCTTGTCCGACTAGAAACGACTAATTTAGCCGTACCACATGT
TGTCGACTCAGAAACATTATTCCCATTTACGCGTAAGCAAAAAATGCGTCCTTATCGA
ACTTACACTCGCCTGCGTTGGT

genotype	number of individuals
ø	38
[+]	49
[0]	27
[1]	31
[2]	25
[3]	22
[4]	38
[5]	35
[6]	51
[7]	60
[8]	67
[9]	27
[10]	46
[11]	33
[12]	61
[13]	39
[14]	44
[15]	77
[16]	23
WT-[ABC]	61
[-BC]	32
[A-C]	49
[AB-]	24
[A--]	33
[-B-]	35
[--C]	32
[---]	37
[ACB]	39
[BAC]	34
[BCA]	37
[CBA]	34

Table S2. Number of individuals analyzed for each construct in this study.

	regulatory potential (sufficiency)	**necessity**
[A--]	A is sufficient for vein expression	
[-B-]	B is sufficient for alula expression	
[--C]	C is sufficient for wing blade expression	
[AB-]		C is necessary for high levels in the spot
[A-C]	A is sufficient to repress wing blade expression (outside of spot region)	B is necessary for alula expression B is necessary for full spot levels
[-BC]	B is sufficient to repress wing blade expression (outside of spot region)	A is necessary for full spot levels

Table S3. Analysis of necessity and sufficiency of each block.

Data file S1. Scores for the PCA shown in Figure S1.

Data file S2. **Significance of difference in activity between pairs of groups, using the first 6 principal components.**

Data file S3. Significance of the difference in average expression levels among constructs of the first mutant series (*[0]-[16]*).

Data file S4. Significance of difference in average expression levels among constructs of the second mutant series (blocks).

Additional notes on *logRatios*.

Using average phenotypes to evaluate the effect of the mutations we introduced is useful but limited. Indeed, the differences we observe are visually driven by changes in regions of the wing with elevated enhancer activity. It is then difficult to appreciate whether a mutation affects enhancer activity locally or uniformly across the wing. Differential gene expression is generally represented using log ratios (see reference *(30)* in main text), which measure the fold changes in expression level of a gene relative to a reference (*e.g.*, the expression of the same gene under different conditions). We applied this principle to our image data to visually compare the activity of different constructs across the wing. Classical log ratio translates here to the log of the pixel-wise ratio between two average phenotypes at every pixel (hereafter noted *logRatio*). *logRatio* images of mutants *vs.* wild type are of particular interest to decipher the regulatory logic, because they reveal in which proportion a mutant affects the enhancer activity across the wing. Compared to absolute difference, *logRatio* are not driven by regions with high levels of expression, but by regions with a large fold change, irrespective of the wild-type activity pattern. In a theoretical case where the enhancer activity depends directly and linearly on a given TF concentration, the *logRatio* image reflects logically the spatial distribution of this particular TF. This is also the case if this integration of this TF information is only modulated by uniformly distributed TFs. The underlying logic is straightforward: in this theoretical case, a sequence mutation breaking the interaction between the DNA and the TF will have a significant effect on the phenotype. The intensity of the local phenotypic effect (relatively to the wild-type levels) will depend on the local intensity of the TF-DNA interaction across the wing, and therefore on the

local concentration of the TF. Logically, this interaction is not happening where the TF is absent, with no effect on the phenotype. For any situation departing from these ideal conditions, the resemblance between the *logRatio* and the TF distribution is compromised. For instance, when a TF is locally repressed by another, *logRatio* will correspond to the net loss of spatial information integration, including the loss of this repression. The *logRatio* of a mutant affecting a known TFBS for which the corresponding TF distribution is known therefore informs us on its contribution in the regulatory logic of the enhancer, and how linearly this integration happens. Moreover, even without additional knowledge on the regulatory logic and TF spatial variation, the variety of *logRatio* patterns suggests the action of different spatial inputs integrated by the enhancer.