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Automated protein-DNA interaction screening of *Drosophila* regulatory elements

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Note: Supplementary Tables 1 and 3 are available on the Nature Methods website.

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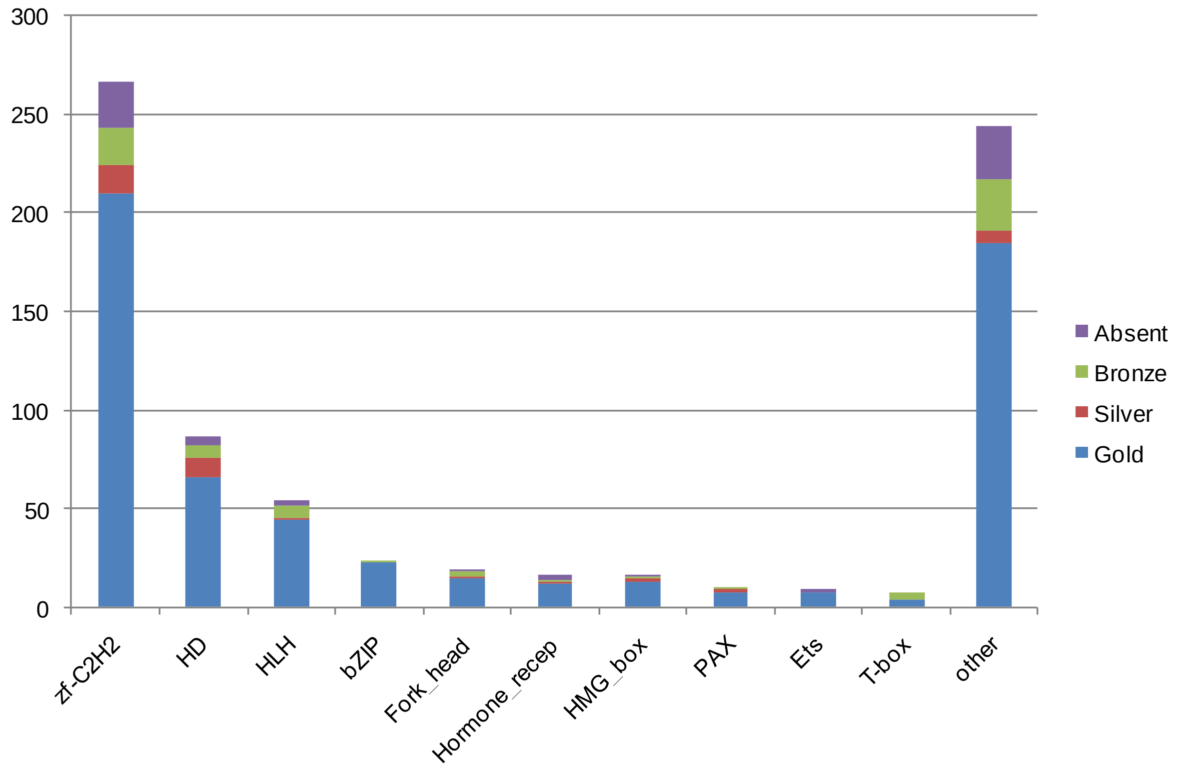
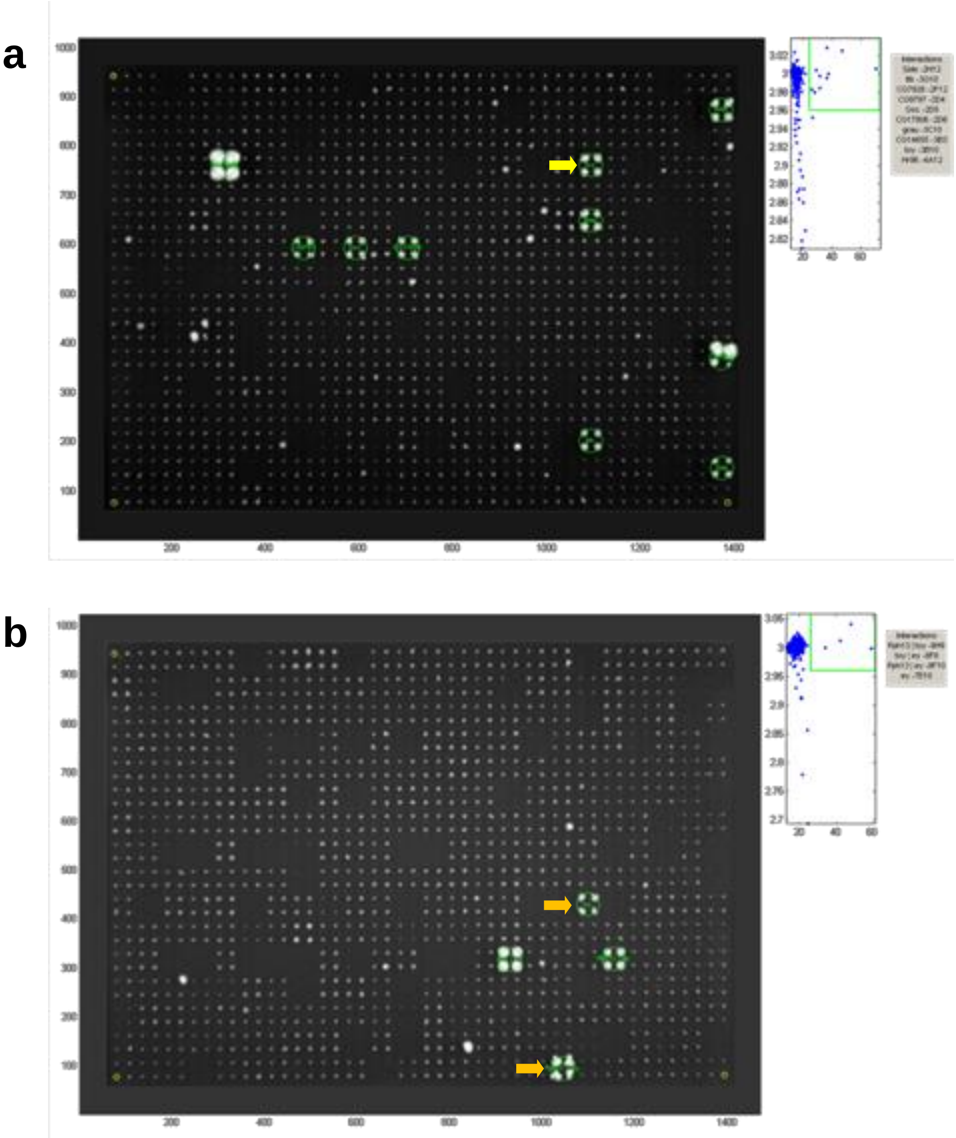
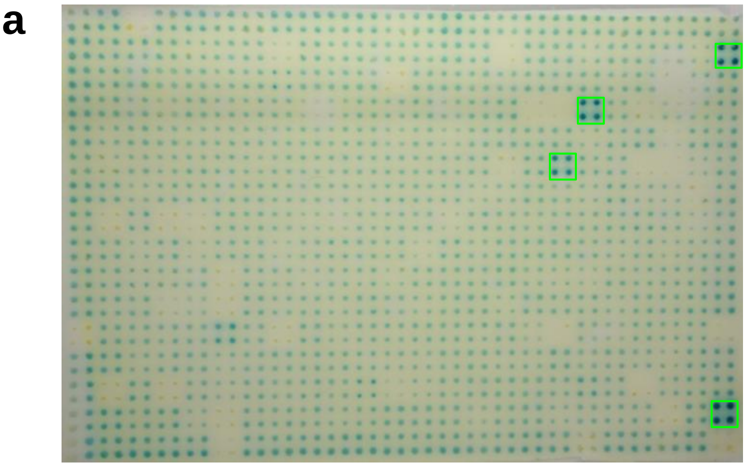
Supplementary Figures

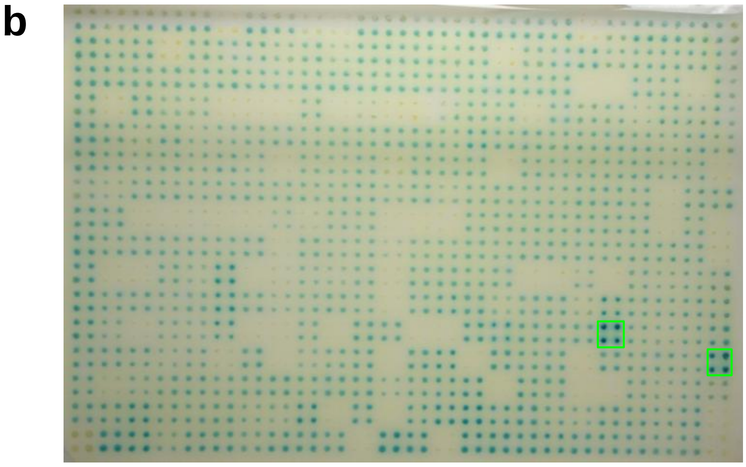


Supplementary Figure 1 ¦ Distribution of DNA-binding protein domains within the *Drosophila* TF repertoire and the quality of the obtained TF ORF clone: Gold (blue), silver (orange), bronze (green). The TFs for which no TF ORF clone was obtained are indicated in purple. For explanation about the quality scores, we refer to the **Online Methods** section.

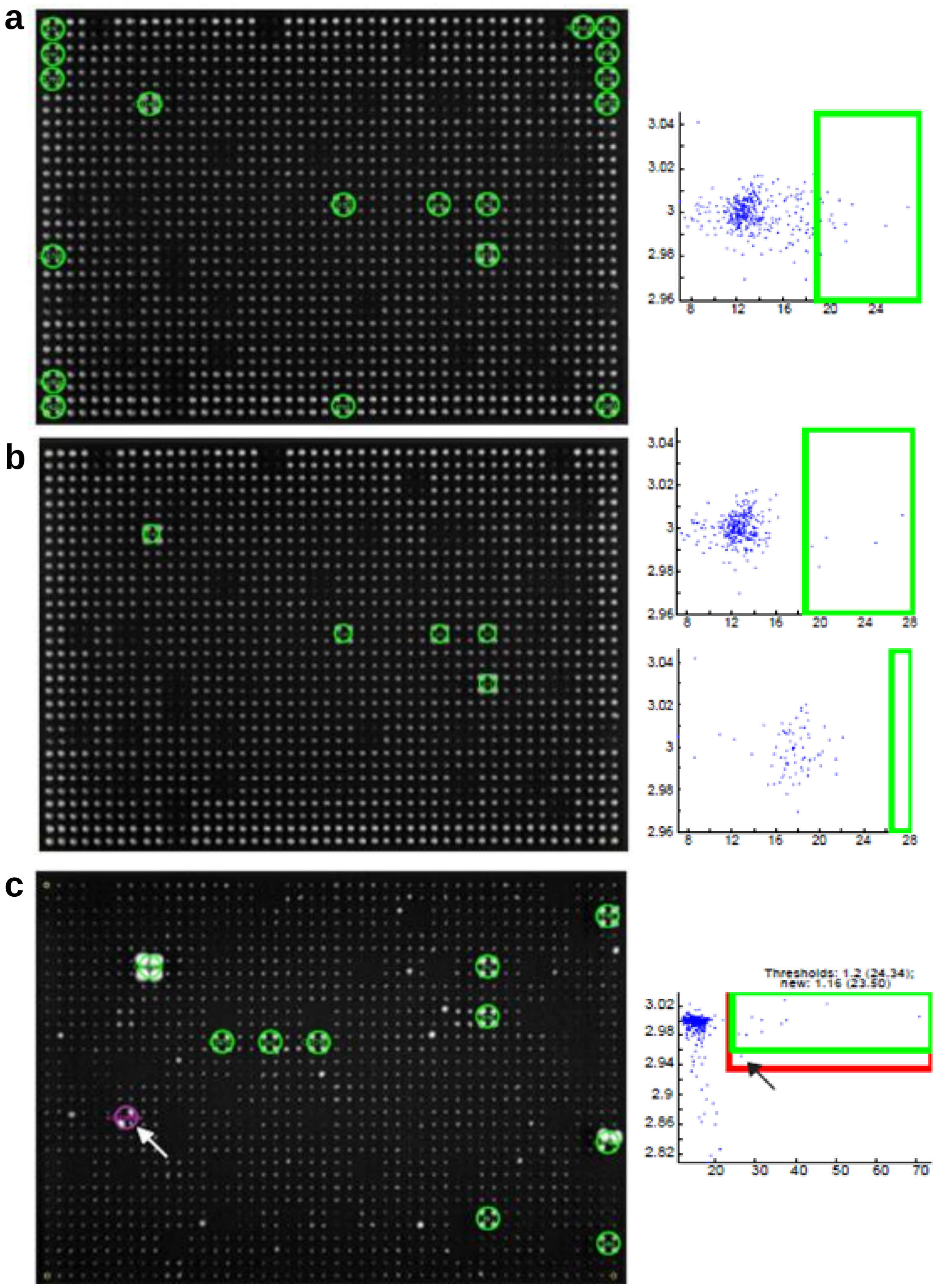


Supplementary Figure 2 ¦ Interactions observed with the *so10* bait upon growth on a selective plate containing 10 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The yellow and orange arrows point to yeast colonies containing respectively TOY and EY, the two positive control TFs.

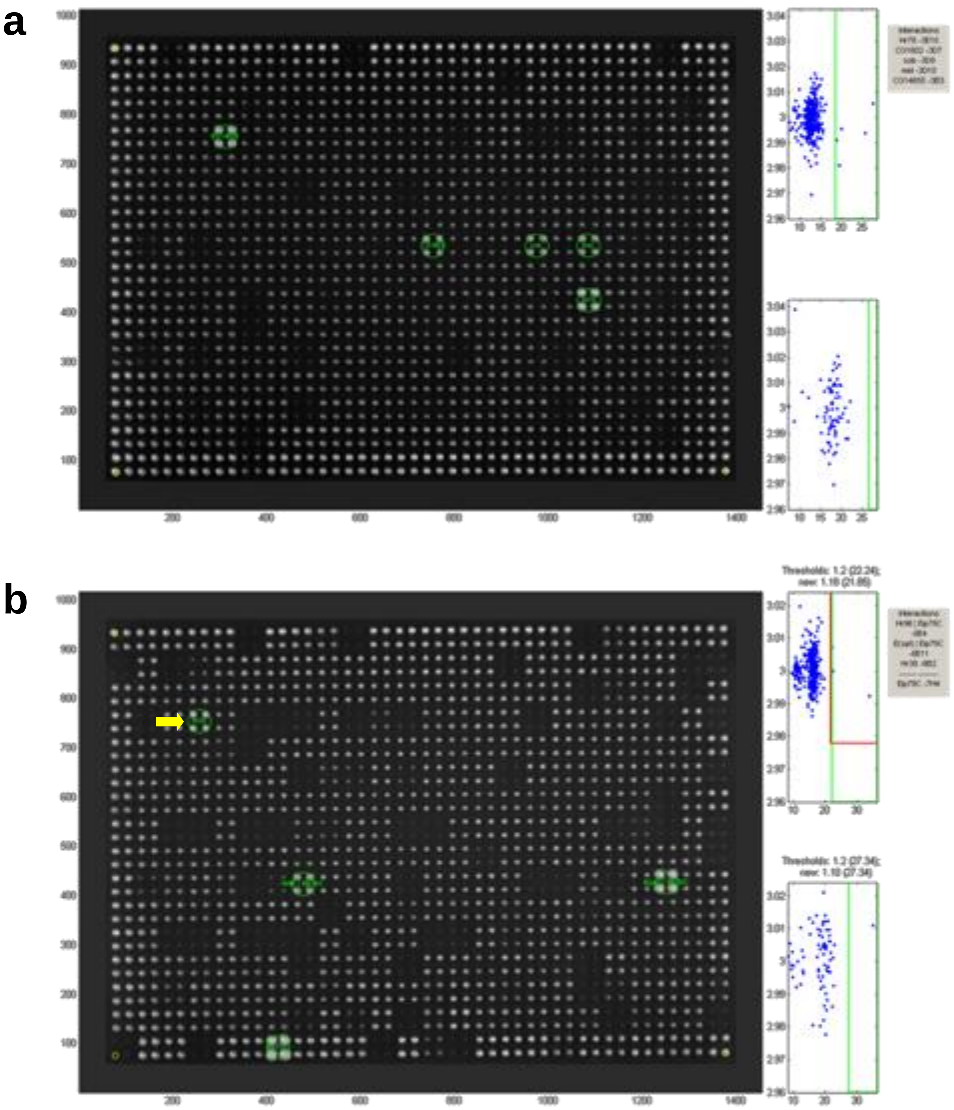




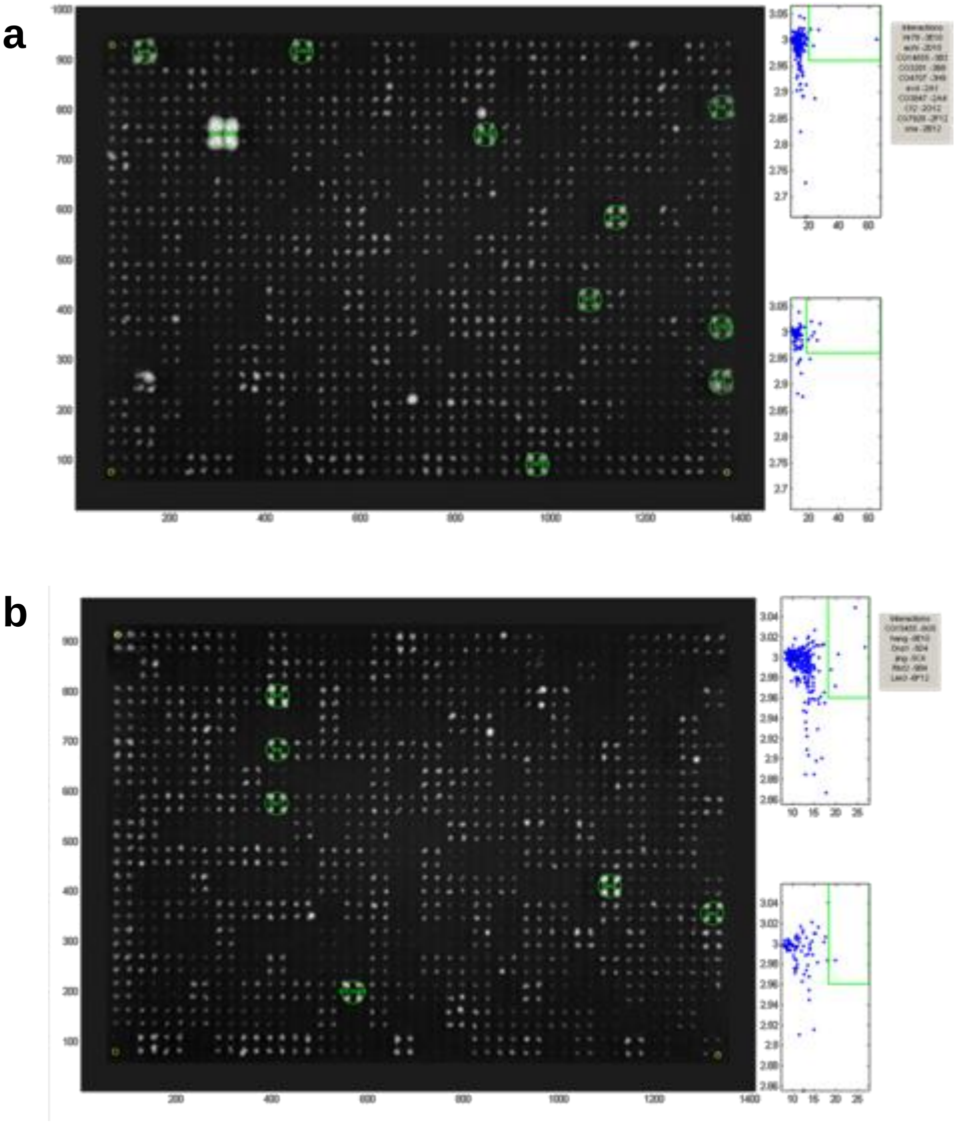
Supplementary Figure 3 ¦ Interactions observed with the *so10* bait using the *lacZ* reporter. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The green squares indicate yeast colonies that were considered positive for X-gal staining. The identities of the positive interactions are listed in **Supplementary Table 2**.



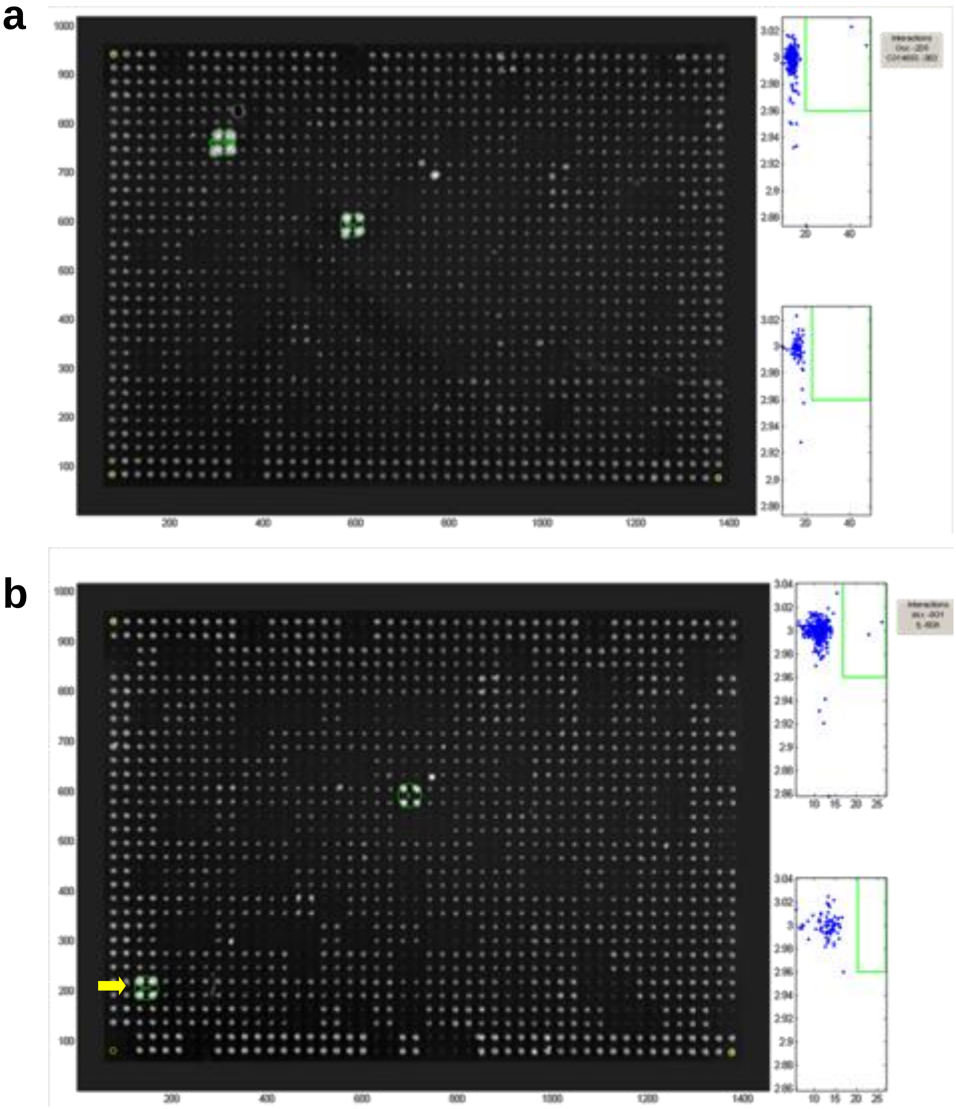
Supplementary Figure 4 ¦ Output of the TIDY program. Interactions identified by TIDY are indicated by a green circle on the analyzed plate image. The quadruplicated yeast colony patterns are detected by convoluting the image with a pattern of four bright spots on a dark background. The intensity value of the convoluted image is measured in the center of each quadrant. These values are plotted for each quadrant on the x-axis of a plot next to the picture. Greater values indicate stronger interactions. On the Y-axis of this plot, a uniformity coefficient is computed for each TF. A number close to 3 indicates little variation in intensity between the four colonies of a quadruplet whereas a number that deviates from 3 indicates heterogeneous growth of individual quadrant colonies. (**a**) Yeast colonies on the border of the plate often grow faster than those in the middle, which may lead to the erroneous detection of such border colonies as positives. (**b**) A separate background normalization for border colonies avoids this issue. (**c**) The default threshold can be adjusted to evaluate the detection stringency. Interactions (indicated with an arrow) that are detected when lowering either the intensity or uniformity threshold are indicated in magenta and are considered “weak”.



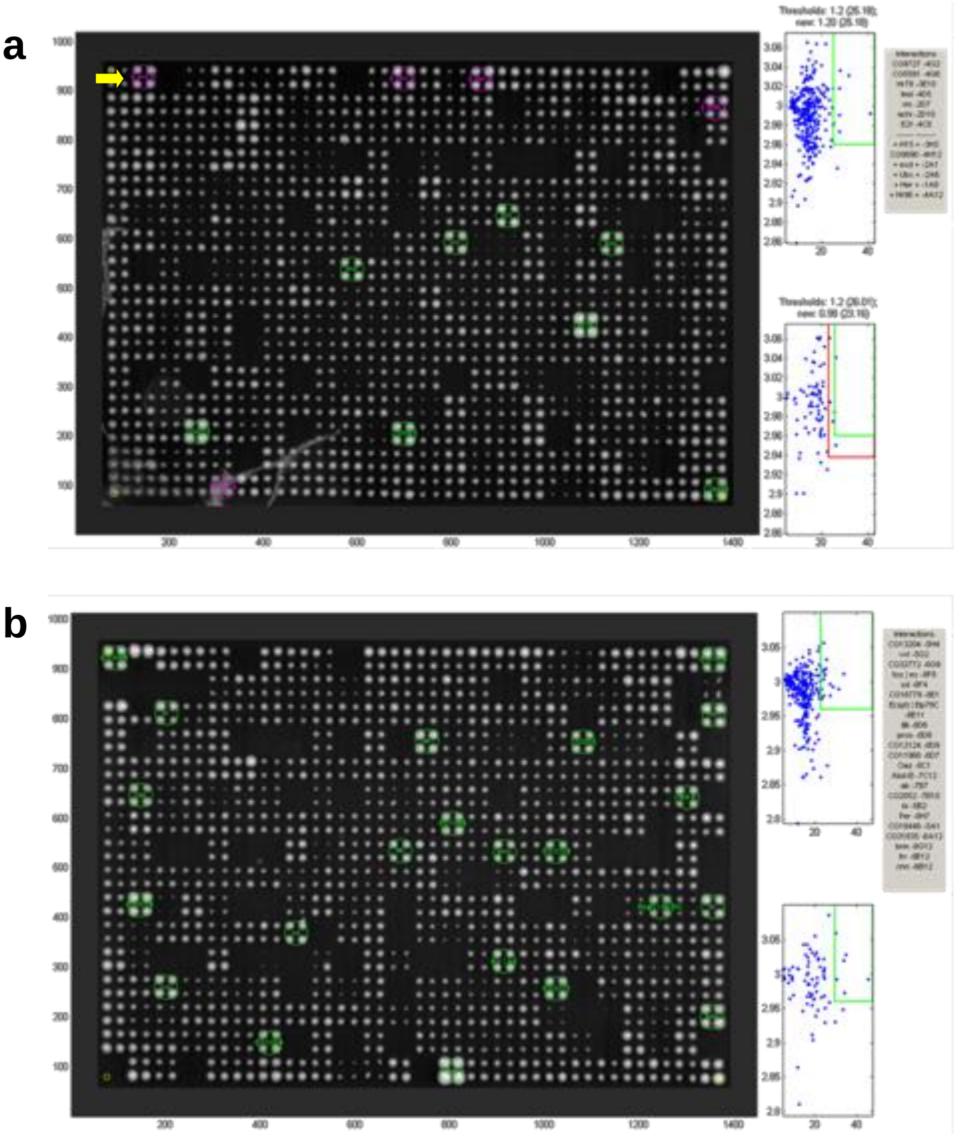
Supplementary Figure 5 ¦ Interactions observed with the *ng-EcRE* bait upon growth on a selective plate containing 20 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The yellow arrow points to yeast colonies containing Hr38, the positive control TF.



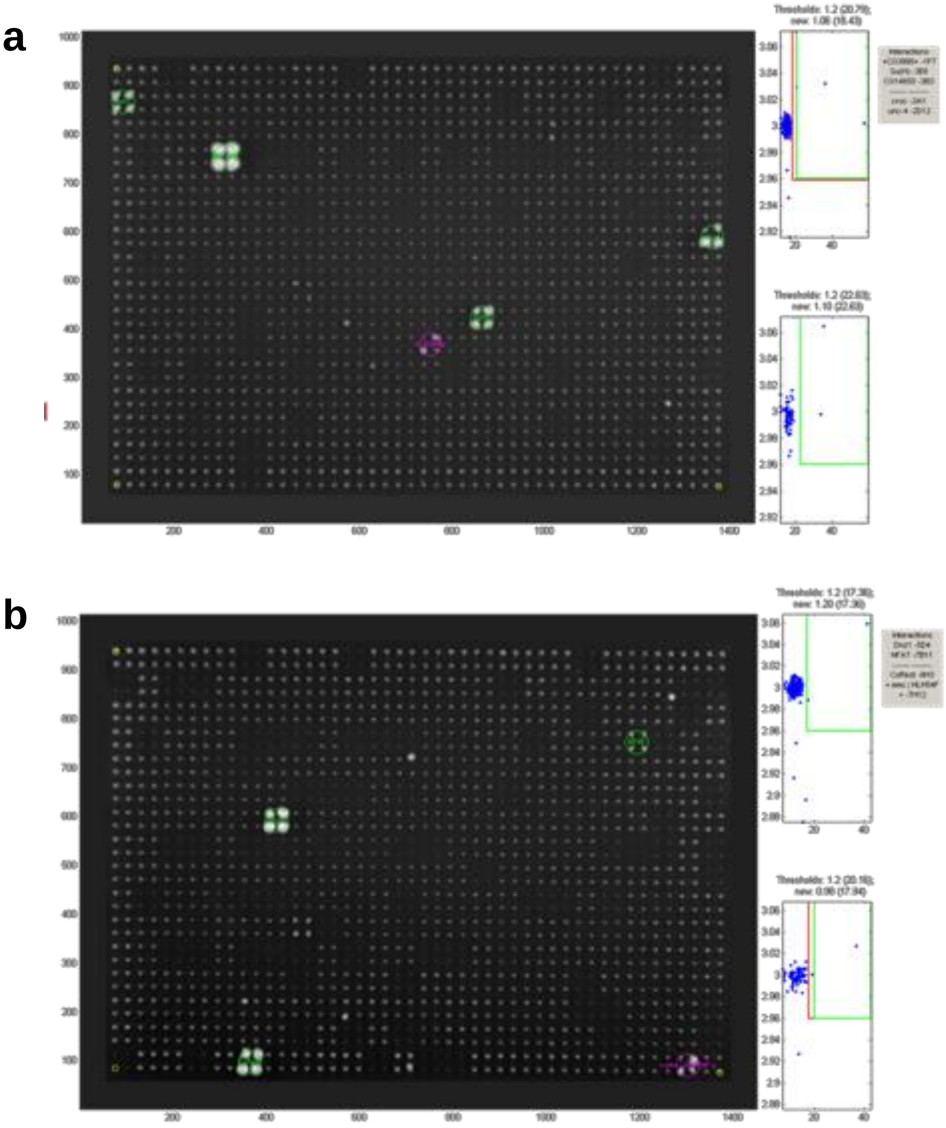
Supplementary Figure 6 ¦ Interactions observed with the *eve-stripe2* bait upon growth on a selective plate containing 40 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate.



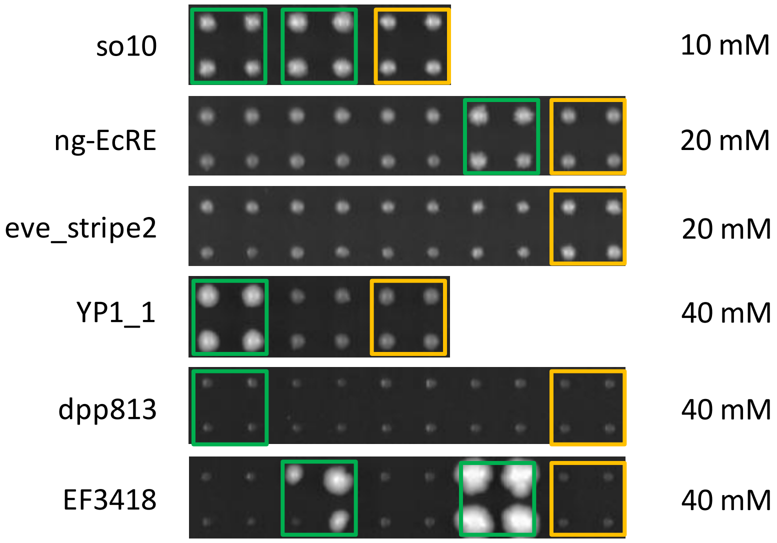
Supplementary Figure 7 ¦ Interactions observed with the *yp1-1* bait upon growth on a selective plate containing 20 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The yellow arrow points to yeast colonies containing DSX, the positive control TF.



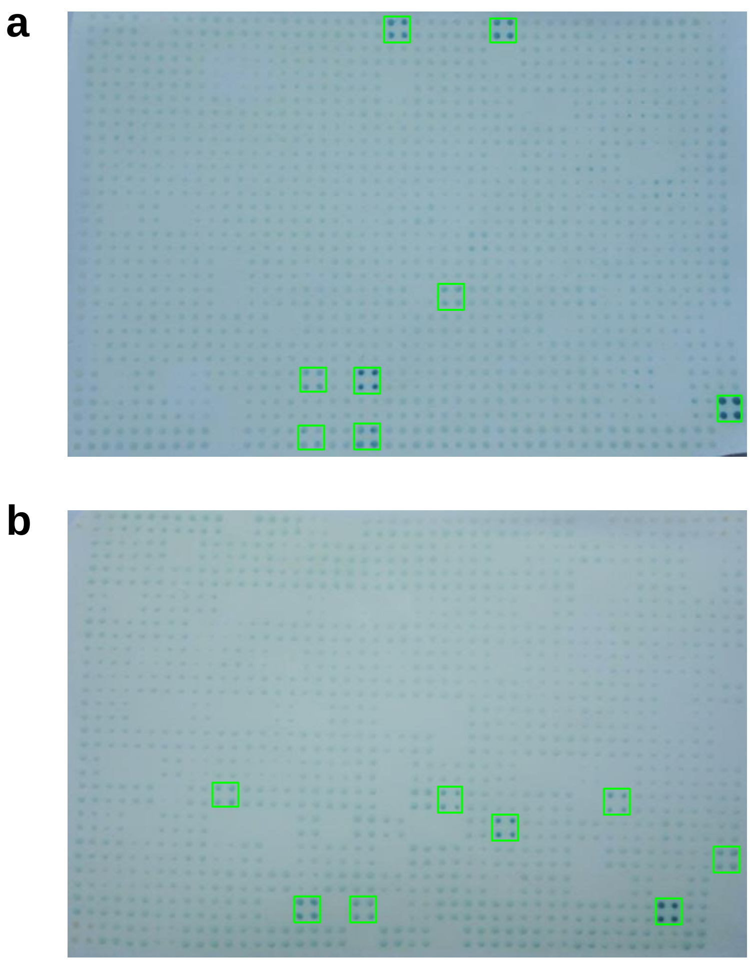
Supplementary Figure 8 ¦ Interactions observed with the *dpp813* bait upon growth on a selective plate containing 20 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The yellow arrow points to yeast colonies containing EXD, the positive control TF.



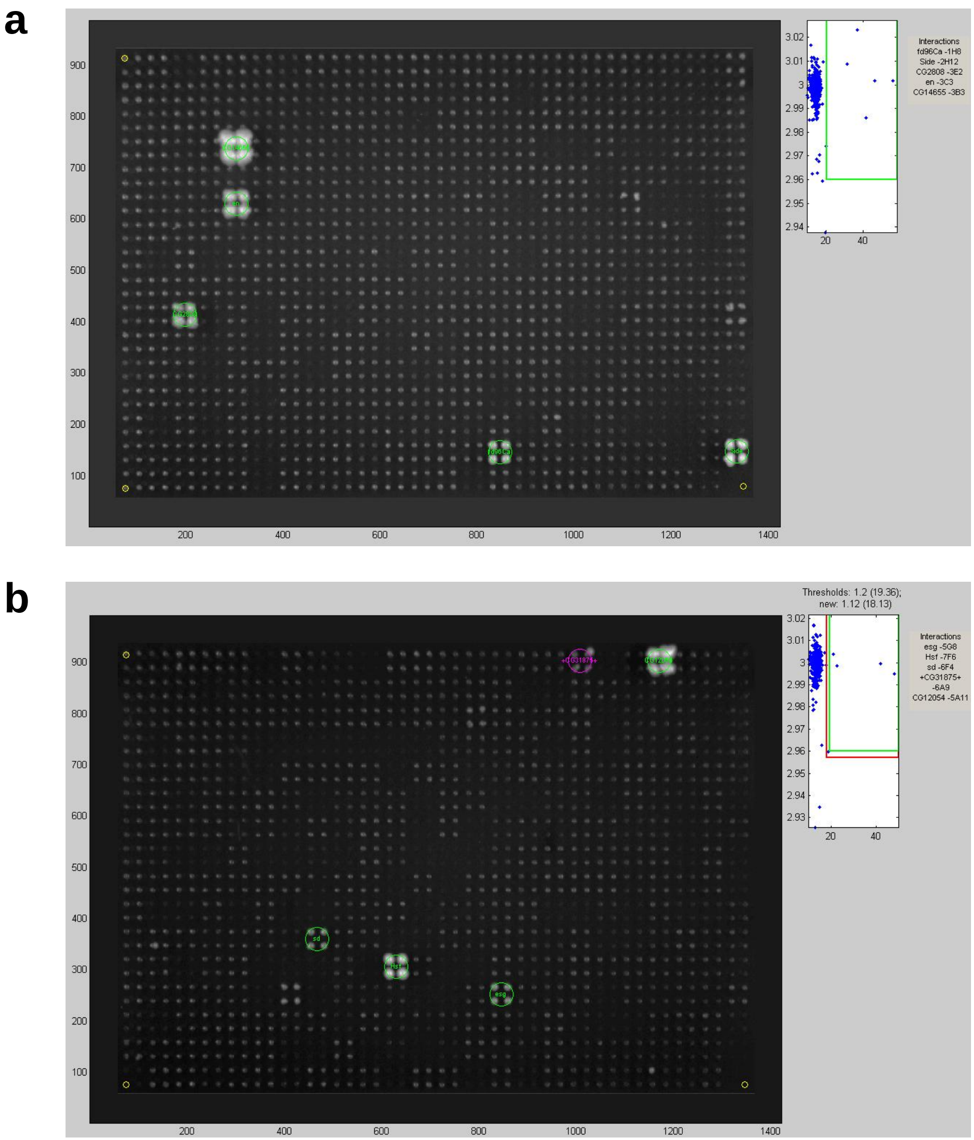
Supplementary Figure 9 ¦ Interactions observed with the *EF3418* bait upon growth on a selective plate containing 20 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate.



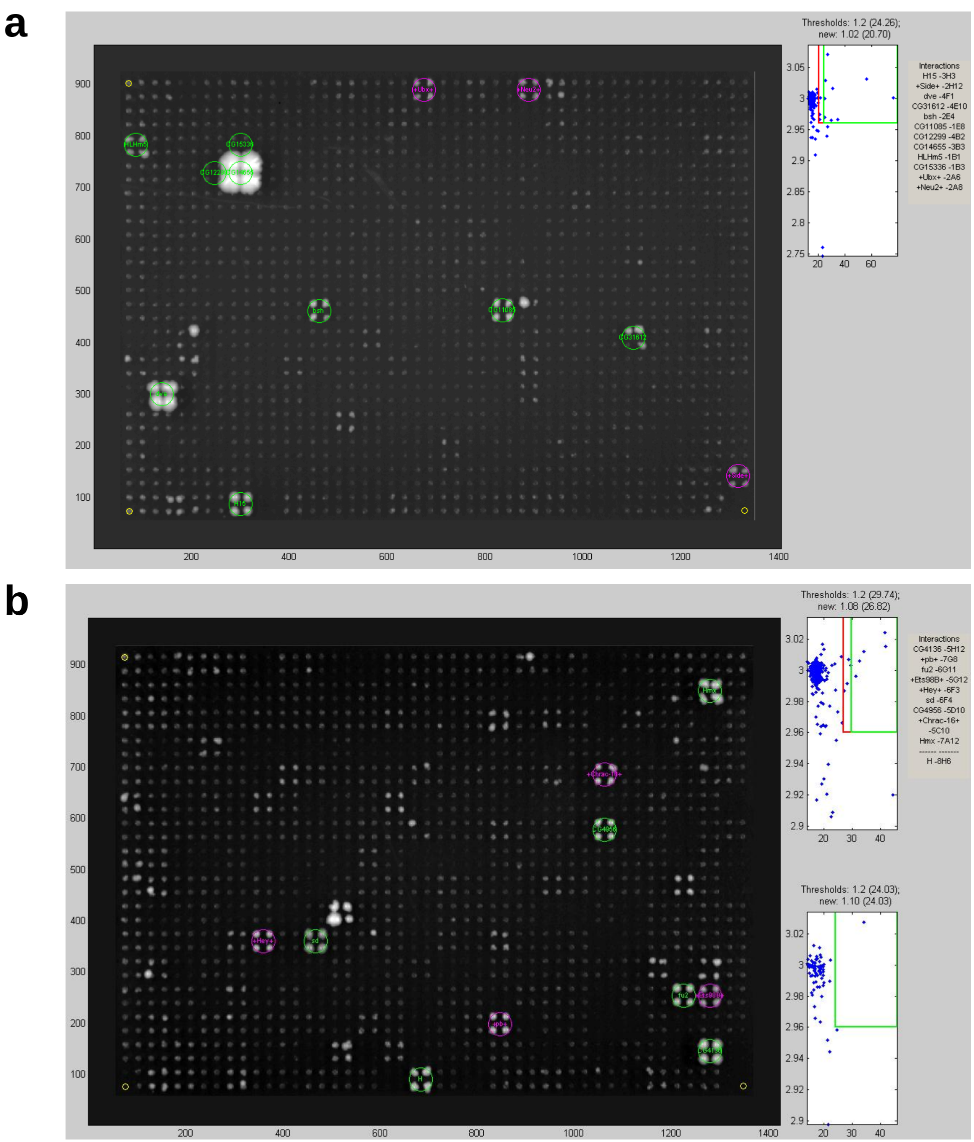
Supplementary Figure 10 ¦ Manual screen of positive control interactions. Negative controls (empty pAD-DEST vector) are indicated with yellow boxes. The green squares indicate yeast colonies that were considered positive. Bait names are indicated on the left. Tested TFs are listed in **Supplementary Table 4**. 3-AT concentrations of the selective plates are indicated on the right



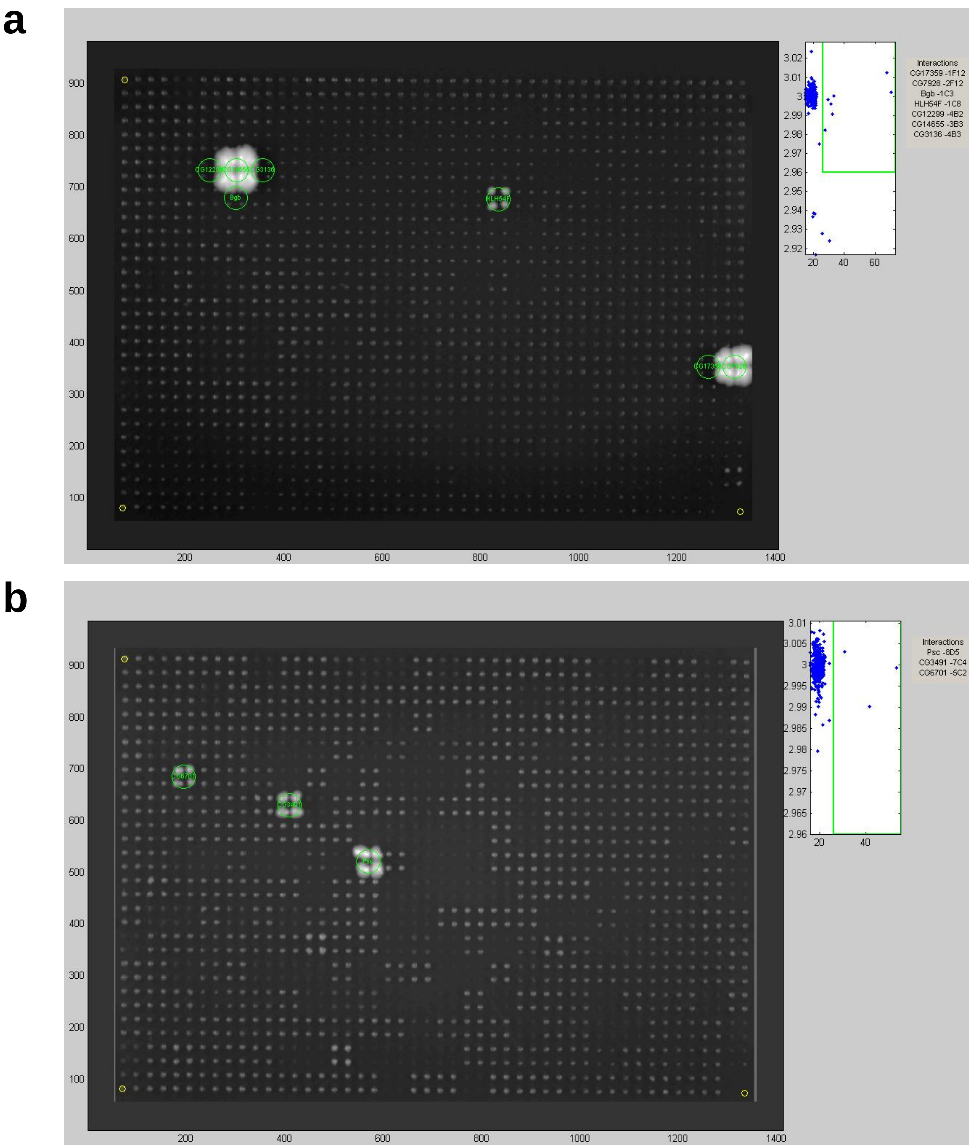
Supplementary Figure 11 ¦ Interactions observed with the *dpp813* bait using the *lacZ* reporter. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. The green squares indicate yeast colonies that were considered positive for X-gal staining. The identities of the positive interactions are listed in **Supplementary Table 2**.



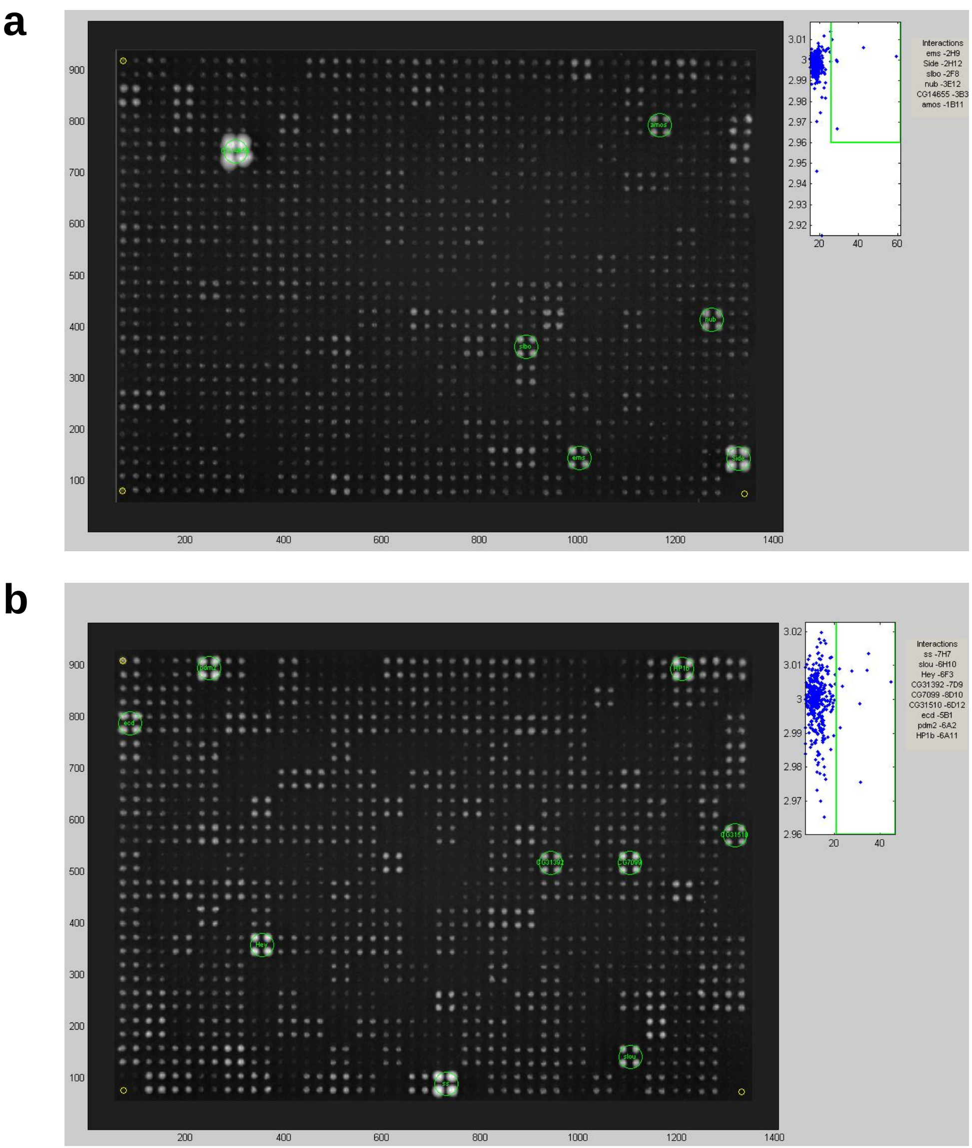
Supplementary Figure 12 ¦ Interactions observed with the *dpp813-Frag1* bait upon growth on a selective plate containing 20 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate.



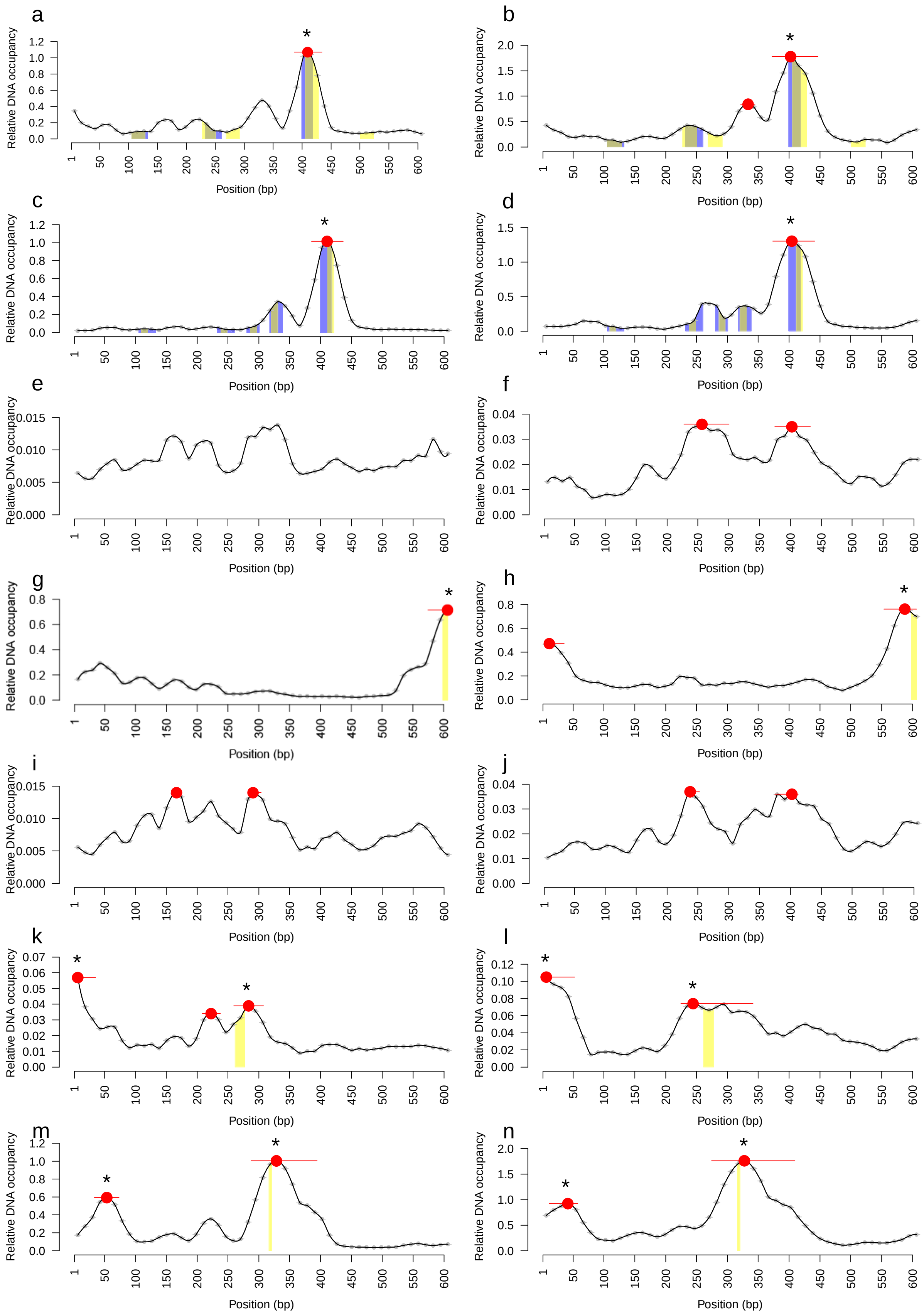
Supplementary Figure 13 ¦ Interactions observed with the *dpp813-Frag2* bait upon growth on a selective plate containing 10 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate. Interestingly, two out of three interactions found in both replicates and seven out of 30 in single screens (including both positive control interactions) were found at least once with the full-length *dpp813* element or the *dpp813-RC* element, indicating that this fragment is likely the major contributor of the observed protein-DNA interactions with the full-length *dpp813* element

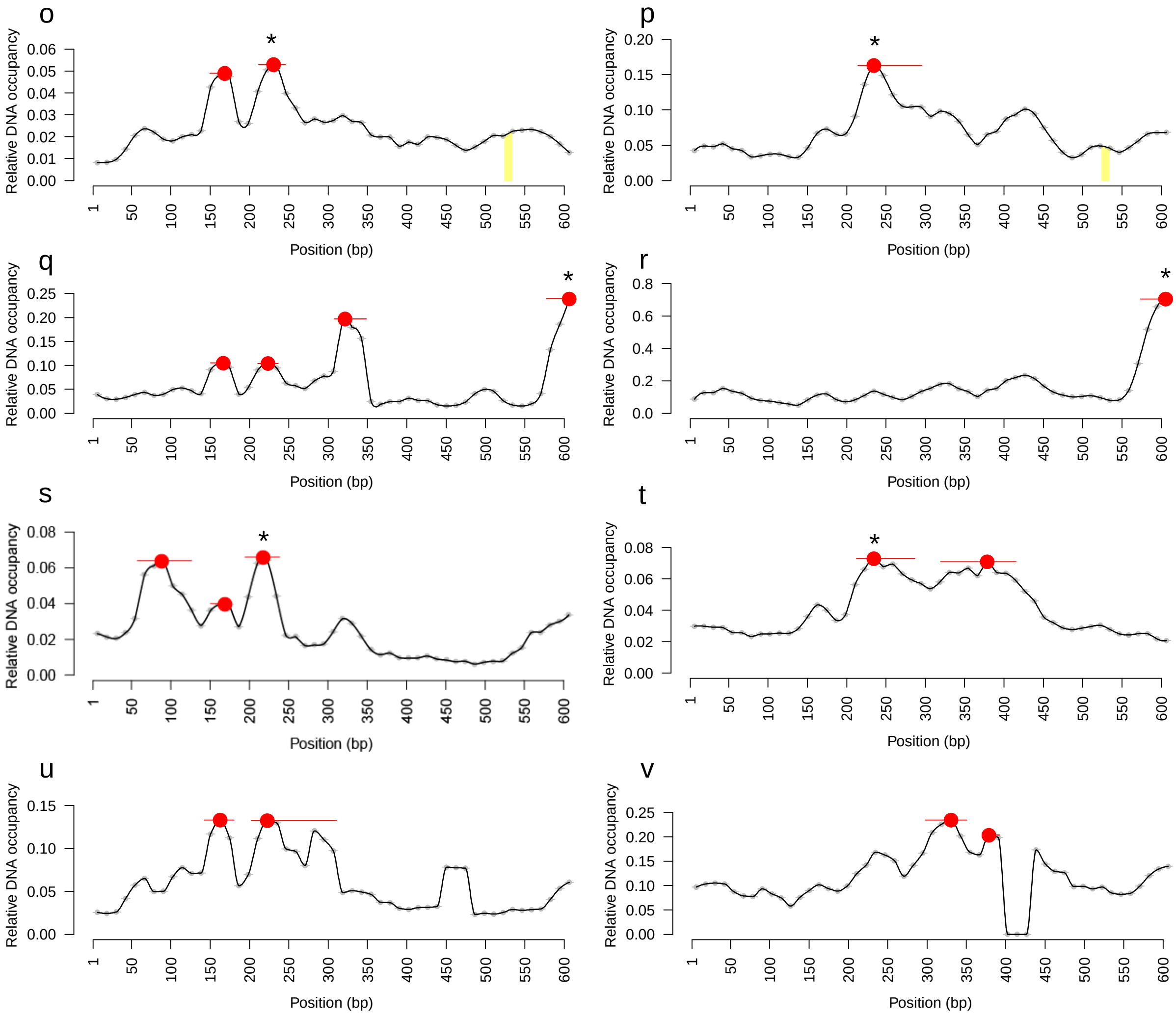


Supplementary Figure 14 ¦ Interactions observed with the *dpp813-Frag3* bait upon growth on a selective plate containing 10 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate.

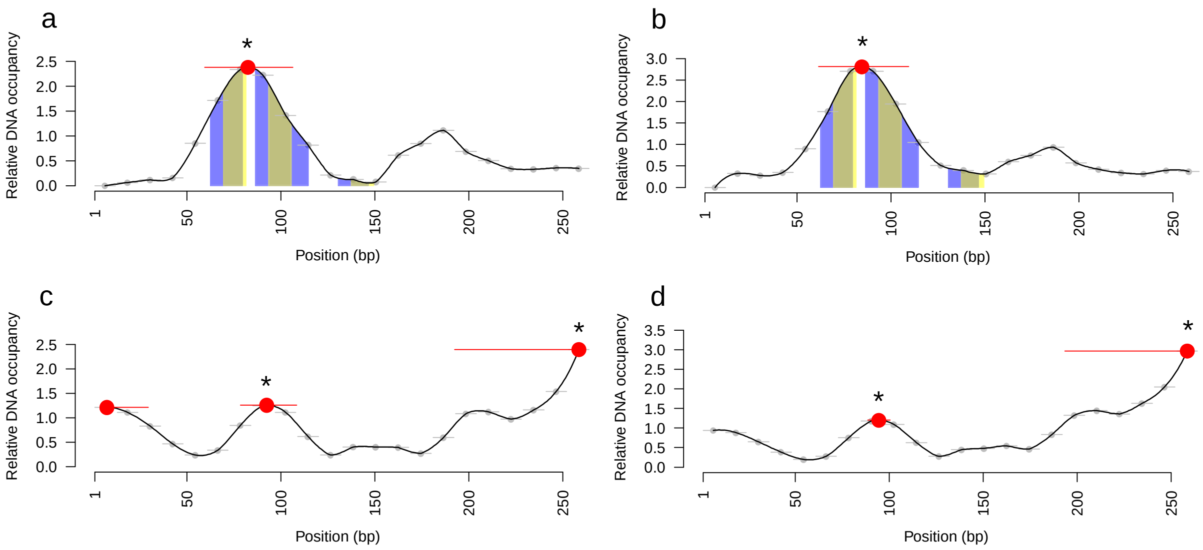


Supplementary Figure 15 ¦ Interactions observed with the *dpp813-RC* bait upon growth on a selective plate containing 10 mM 3-AT. One representative replicate is shown. (**a**) Interactions after transformation with the first 384-well TF plate. (**b**) Interactions after transformation with the second 384-well TF plate.

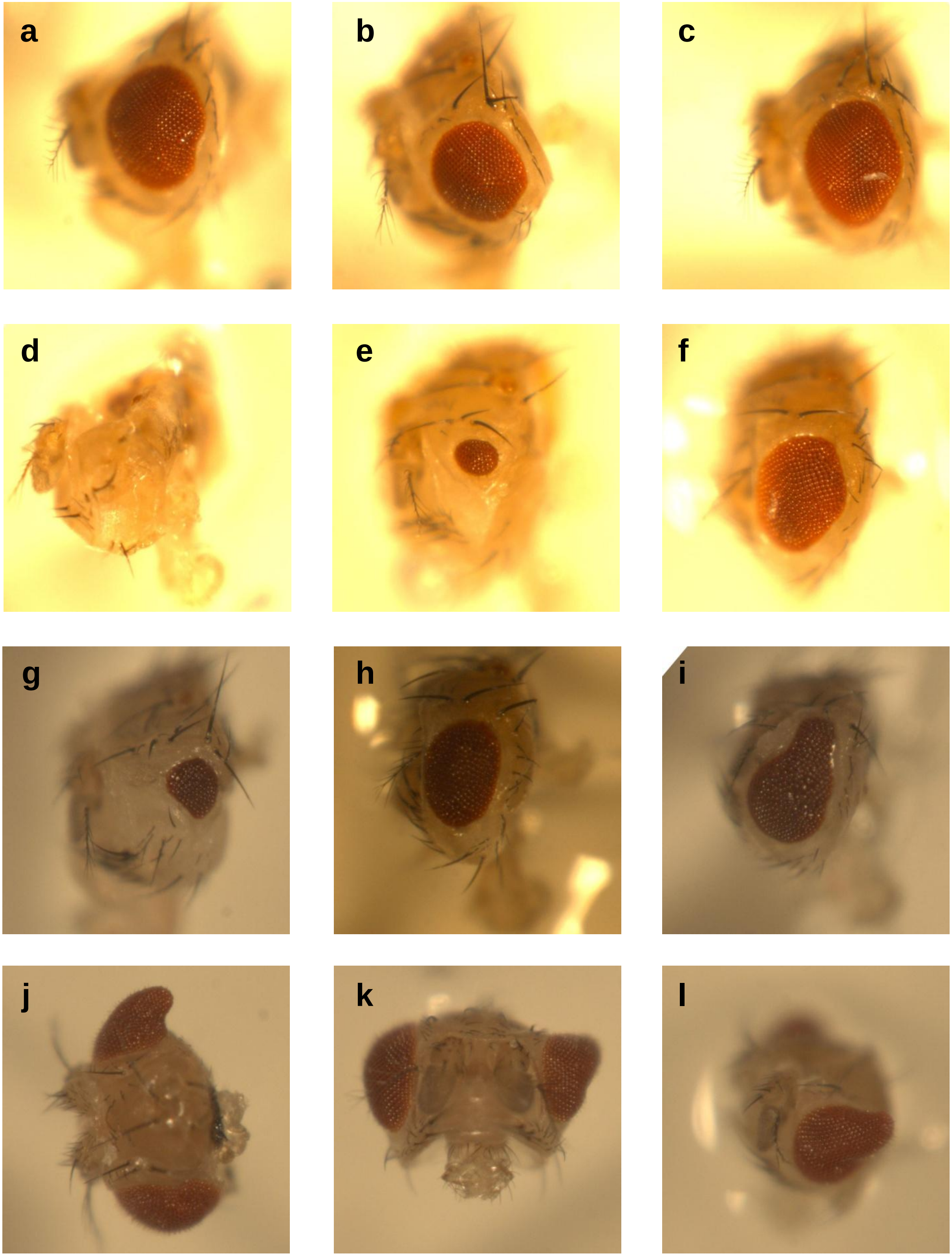

a
b
c
d
e
f
g
h
i
j
k
l
m
n
*
Relative DNA occupancy
Position (bp)
1
50
100
150
200
250
300
350
400
450
500
550
600
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.5
1.5
2.0
0.000
0.005
0.010
0.015
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.10
0.12

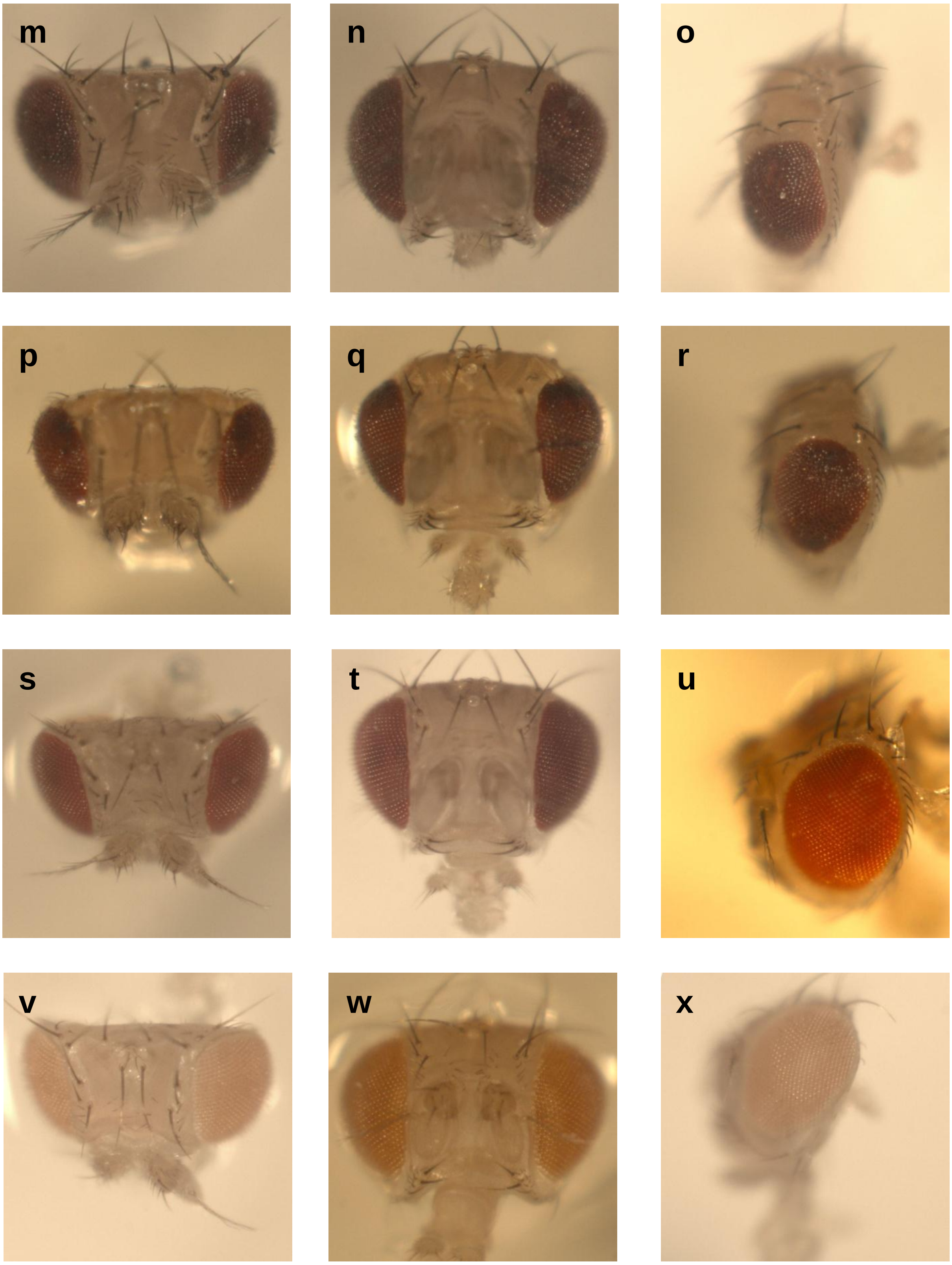


Supplementary Figure 16 ¦ MARE analysis of *so10* for recognition by Y1H-identified TFs in two replicates. For clarity, replicates that were represented in the main manuscript are recapitulated here. Bound DNA levels normalized over surface-immobilized protein amounts are plotted for each 12 bp nucleotide stretch and as an interpolated curve. Significant peaks are indicated with a red line, peak maxima are indicated with a red dot. Peaks found in both replicates are indicated with an asterisk. Where available, DNase I footprinting data and PWM-based binding site predictions are indicated with blue and yellow bars respectively. Overlapping DNase I footprinting data and PWM-based binding site predictions are indicated with grey bars. (**a-b**) EY. (**c-d**) TOY. (**e-f**) CG9797 (**g-h**) TTK. (**i-j**) CG17806. (**k-l**) CG7928. (**m-n**) GSC. (**o-p**) SIDE. (**q-r**) HR39. (**s-t**) GRAU. (**u-v**) HR96.



Supplementary Figure 17 ¦ MARE analysis of *yp1-1* for recognition by Y1H-identified TFs in two replicates. For clarity, replicates that were represented in the main manuscript are recapitulated here. Bound DNA levels normalized over surface-immobilized protein amounts are plotted for each 12 bp nucleotide stretch and as an interpolated curve. Significant peaks are indicated with a red line, peak maxima are indicated with a red dot. Peaks found in both replicates are indicated with an asterisk. Where available, DNase I footprinting data and PWM-based binding site predictions are indicated with blue and yellow bars respectively. Overlapping DNase I footprinting data and PWM-based binding site predictions are indicated with grey bars. (**a-b**) DSX. (**c-d**) TJ.


a
b
c
d
e
f
g
h
i
j
k
l



Supplementary Figure 18 ¦ Eye phenotypes observed after RNAi-mediated knock-down of TFs identified in Y1H. For lateral views, anterior is to the left, dorsal is to the top. (**a-c**) OK107>*ey*-RNAi$_{\text{TRiP}}$, lateral view. (**d-f**) *so10*>*ey*-RNAi$_{\text{TRiP}}$, lateral view. (**g-i**)

OK107>CG9797-RNAi$_{\mathrm{TRiP}}$, lateral view. (**j-l**) *so10>CG9797*-RNAi$_{\mathrm{VDRC}}$; (**j**) Dorsal view, (**k**) Frontal view (**l**) lateral view. (**m-o**) *OK107>ttk*-RNAi$_{\mathrm{VDRC}}$; (**m**) Dorsal view, (**n**) Frontal view (**o**) lateral view. (**p-r**) *so10>ttk*-RNAi$_{\mathrm{VDRC}}$; (**p**) Dorsal view, (**q**) Frontal view (**r**) lateral view. (**s-u**) *OK107>gfp*; (**s**) Dorsal view, (**t**) Frontal view (**u**) lateral view. (**v-x**) *so10>gfp*; (**v**) Dorsal view, (**w**) Frontal view (**x**) lateral view.

Supplementary Table 2: Observed interactions in two independent screens

🟩 Strong interaction	🟪 Weak interaction	**LacZ positives found in both HIS3 screens**

so10			
Screen 1	**Screen 2**	**overlap**	**LacZ**
🟩 CG9797	🟩 CG9797	CG9797	**ey**
🟩 CG17806	🟩 CG17806	CG17806	**Hr39**
🟩 CG7928	🟩 CG7928	CG7928	**Hr96**
🟩 ey	🟩 ey	ey	**Side**
🟩 gce	🟩 grau	grau	**toy**
🟩 grau	🟩 Gsc	Gsc	CG4496
🟩 Gsc	🟩 Hr39	Hr39	
🟪 Hr39	🟩 Hr96	Hr96	
🟩 Hr96	🟩 Side	Side	
🟩 lz	🟩 toy	toy	
🟩 Side	🟩 ttk	ttk	
🟩 toy			
🟩 ttk			

ng-EcRE		
Screen 1	**Screen 2**	**Overlap**
🟩 CG1602	🟩 btd	CG1602
🟩 chn	🟪 CG1602	Eip78C
🟩 CrebA	🟪 CG17568	Hr38
🟩 Eip78C	🟩 Eip78C	Hr78
🟩 Hr38	🟩 Hr38	mid
🟩 Hr78	🟩 Hr78	sob
🟩 mid	🟩 mid	
🟩 sob	🟩 sob	

eve_stripe2		
Screen 1	**Screen 2**	**Overlap**
40mM	40mM	
🟩 CG11456	🟩 achi	CG7928
🟩 CG32830	🟩 Cf2	exd
🟩 CG7928	🟩 CG15455	Hr78
🟪 CG9890	🟩 CG3281	sna
🟩 dimm	🟩 CG3847	
🟪 exd	🟩 CG4707	
🟩 Hr78	🟩 CG7928	
🟩 sd	🟩 Dnz1	
🟩 sna	🟩 exd	
🟪 sug	🟩 hang	
🟪 Trf	🟩 Hr78	

unc-4	jing	
	Lim3	
	Rbf2	
	sna	

yp1-1

Screen 1	**Screen 2**	**Overlap**
dsx	dsx	dsx
sd	Gsc	tj
tj	tj	

dpp813

Screen 1	**Screen 2**	**Overlap**	**LacZ**
ab	ac	E(spl)	**ubx**
Abd-B	CG11676	E2f	**ey**
achi	CG13424	Eip78C	**toy**
bi	CG16801	ubx	Neu2
CG11966	CG18011	ey	toe
CG12124	CG31612	toy	CG11696
CG13204	CG6276		Dek
CG16779	CG8359		CG13123
CG18446	CG8478		side
CG2052	D		hey
CG31835	Dif		lbe
CG32772	drm		gcm
CG5591	E(spl)		CG4496
CG9727	E2f		eyg
CG9890	Eip78C		opa
chn	ey		pnt
E(spl)	kn		
E2f	l(1)sc		
Eip78C	Neu2		
exd	peb		
ey	Rel		
H15	retn		
Her	sim		
Hr78	Su(var)2-10		
Hr96	sv		
lilli	tgo		
lmd	toy		
Oaz	trh		
pros	Ubx		
sd	wor		
toy			
trr			
Ubx			
vis			
vvl			

EF3418		
Screen 1	**Screen 2**	**Overlap**
40 mM	40 mM	
CG3995	CG4854	croc
unc-4	ac	NFAT
croc	TFAM	
Su(H)	bsh	
Dnz1	croc	
NFAT	CG3726	
emc	pita	
HLH54F	Lhr	
	CG18446	
	cbt	
	sd	
	E5	
	NFAT	
	Mnf	
	CG1244	

Supplementary Table 4: manual screening of reported interactions

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so10	reported interacting TF	HT Y1H	manual Y1H
	ey	ok	ok
	toy	ok	ok

ng-EcRE	reported interacting TF	HT Y1H	manual Y1H
	Usp		
	EcR		
	Hr39		
	Hr38	ok	ok

eve_stripe2	reported interacting TF	HT Y1H	manual Y1H
	bcd		
	hb		
	Kr		
	gt		

yp1_1	reported interacting TF	HT Y1H	manual Y1H
	dsx	ok	ok
	Aef1		

dpp813	reported interacting TF	HT Y1H	manual Y1H
2A6	Ubx	ok	ok
3H10	bin		
1F10	abd-A		
2A1	exd	1/2	

EF3418	reported interacting TF	HT Y1H	manual Y1H
8G6	tin		
3H10	bin		ok
2A9	bap		
1B8	mef2		ok

Supplementary Table 5: *dpp813-Frag1-3* and *dpp813-RC* interactions

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🟩 Strong interaction

🟪 Weak interaction

interaction found in full-length dpp813

interaction found in full-length dpp813-RC

dpp813-Frag1

Screen 1	**Screen 2**	**Overlap**
🟩 fd96Ca	🟩 fd96Ca	fd96Ca
🟩 Side	🟩 Side	Side
🟩 CG2808	🟩 CG7928	**sd**
🟩 en	🟩 CG11971	CG31875
🟩 esg	🟩 Jra	
🟩 Hsf	🟩 **Her**	
🟩 **sd**	🟩 CG15073	
🟪 CG31875	🟩 fd59A	
🟩 CG12054	🟩 **sd**	
🟩	🟩 Doc1	
🟩	🟩 CG31875	
🟩		
🟩		
🟩		

dpp813-Frag2

Screen 1	**Screen 2**	**Overlap**
🟩 Side	🟩 **H15**	Side
🟩 slbo	🟪 Side	**Neu2**
🟩 Oli	🟩 dve	ets98B
🟩 **exd**	🟩 **CG31612**	
🟩 **Neu2**	🟩 bsh	
🟩 sug	🟩 CG11085	
🟩 Rpd3	🟩 HLHm5	
🟩 CG10543	🟪 **Ubx**	
🟩 CG1620	🟪 **Neu2**	
🟩 CG6792	🟩 H	
🟪 CG15455	🟩 CG4136	
🟩 Ets98B	🟪 pb	
🟩 CG12236	🟩 fu2	
🟩 CG17287	🟪 Ets98B	
🟩 CG8314	🟪 Hey	
🟩 Mes4	🟩 **sd**	
🟩 CG18764	🟩 CG4956	
🟩 Alh	🟪 Chrac-16	
	🟩 Hmx	

dpp813-Frag3

Screen 1	**Screen 2**	**Overlap**
CG7928	cg	Psc
HLH54F	Side	CG6701
Psc	CG14710	
CG3491	Z4	
CG6701	pnt	
	dsf	
	CG4496	
	CG34422	
	Psc	
	CG6701	

dpp813-RC

Screen 1	**Screen 2**	**Overlap**
ems	cg	Side
Side	Side	
slbo	caup	
nub	Dr	
amos	maf-S	
ss	CG9705	
slou	pnt	
Hey	Hand	
CG31392		
CG7099		
CG31510		
ecd		
pdm2		
HP1b		

Supplementary Table 6: Effect of RNAi-mediated TF knock-down on eye development

UAS-RNAi line				**GAL4 driver line**	**Referred to in main text as**	**eye phenotype**
stock #	**stock center**	**target gene**				
110498	VDRC	CG9797	x	*OK107*	*OK107* >*CG9797* -RNAi$_{VDRC}$	/
104319	VDRC	Gsc	x	*OK107*		/
101592	VDRC	CG17806	x	*OK107*		/
35290	VDRC	CG7928	x	*OK107*		/
3066	VDRC	side	x	*OK107*		/
110353	VDRC	toy	x	*OK107*	*OK107* >*toy*- RNAi$_{VDRC}$	/
104196	VDRC	grau	x	*OK107*		/
101980	VDRC	ttk	x	*OK107*	*OK107* >*ttk*- RNAi$_{VDRC}$	rough eye
10958	VDRC	Hr96	x	*OK107*		/
106628	VDRC	ey	x	*OK107*	*OK107* >*ey*- RNAi$_{VDRC}$	/
60100	VDRC	/	x	*OK107*	*OK107* >attP	/
32858	TRiP	CG9797	x	*OK107*	*OK107* >*CG9797*- RNAi$_{TRiP}$	rough eye, reduced eye field
31956	TRiP	CG17806	x	*OK107*		/
33679	TRiP	toy	x	*OK107*	*OK107* >*toy*- RNAi$_{TRiP}$	/
32486	TRiP	ey	x	*OK107*	*OK107* >*ey*- RNAi$_{TRiP}$	rough eye, reduced eye field
27992	TRiP	Hr96	x	*OK107*		/
33624	TRiP	Hr39	x	*OK107*		/
26315	TRiP	ttk	x	*OK107*	*OK107* >*ttk*- RNAi$_{TRiP}$	/
5130	Bloomington	mCD8::GFP (expression)	x	*OK107*	*OK107* >mCD8::GFP	/

110498	VDRC	CG9797	x	*so10-GAL4*	*so10>CG9797-RNAiVDRC*	reduced eye field, outgrowth of eye
104319	VDRC	Gsc	x	*so10-GAL4*		/
101592	VDRC	CG17806	x	*so10-GAL4*		/
35290	VDRC	CG7928	x	*so10-GAL4*		slight deformation of eye edges, low penetrance
3066	VDRC	side	x	*so10-GAL4*		/
110353	VDRC	toy	x	*so10-GAL4*	*so10>toy-RNAiVDRC*	/

104196	VDRC	grau	x	*so10-GAL4*		/
101980	VDRC	ttk	x	*so10-GAL4*	*so10>ttk-RNAiVDRC*	rough eye
10958	VDRC	Hr96	x	*so10-GAL4*		/
106628	VDRC	ey	x	*so10-GAL4*	*so10>ey-RNAiVDRC*	/
60100	VDRC	/	x	*so10-GAL4*	*so10>attP*	/
32858	TRiP	CG9797	x	*so10-GAL4*	*so10>CG9797-RNAiTRiP*	lethal?
31956	TRiP	CG17806	x	*so10-GAL4*		/
33679	TRiP	toy	x	*so10-GAL4*	*so10>toy-RNAiTRiP*	/
32486	TRiP	ey	x	*so10-GAL4*	*so10>ey-RNAiTRiP*	rough eye
27992	TRiP	Hr96	x	*so10-GAL4*		/
33624	TRiP	Hr39	x	*so10-GAL4*		/
26315	TRiP	ttk	x	*so10-GAL4*	*so10>ttk-RNAiTRiP*	/
5130	Bloomington	mCD8::GFP (expression)	x	*so10-GAL4*	*so10>mCD8::GFP*	/

Supplementary Data: PWMs used for binding site predictions

```
AC  Ey
XX
ID  Ey
XX
PO        A         C         G         T
1         18        167       34        137
2         223       46        152       200
3         19        242       83        298
4         23        174       263       182
5         21        27        444       150
6         57        12        63        509
7         47        25        567       3
8         20        167       20        438
9         1         133       379       132
10        13        540       3         89
11        409       3         231       2
12        33        102       173       336
13        1         59        14        568
14        8         285       337       15
15        325       0         300       18
16        217       36        197       193
17        8         66        546       23
18        7         305       12        318
19        28        5         595       15
20        27        13        153       446
21        227       12        358       38
22        464       71        45        58
23        360       53        167       61
24        187       93        200       158
25        124       113       242       160
XX
//
AC  Toy
XX
ID  Toy
XX
PO    A     C     G     T
1         0         5         0         0
2         1         4         0         0
3         2         2         1         0
4         1         4         0         0
5         0         0         0         5
6         0         5         0         0
7         5         0         0         0
8         0         3         0         2
9         0         1         1         3
10        0         3         2         0
11        3         0         2         0
12        0         1         1         3
XX
//
AC  Ttk
XX
ID  Ttk
XX
PO        A         C         G         T
1         29        6         12        3
2         7         1         1         40
```

```
3           1           0           9           40
4           34          0           17          0
5           0           0           0           52
6           0           52          0           0
7           0           52          0           0
8           0           1           0           50
9           1           5           22          22
10          2           9           12          5
XX
//
AC  CG7928
XX
ID  CG7928
XX
PO          A           C           G           T
1           30          302         42          70
2           455         0           0           3
3           0           0           449         9
4           0           1           456         1
5           0           0           454         4
6           214         185         14          45
7           16          44          6           392
8           96          15          338         9
9           35          272         145         6
10          274         84          72          28
11          135         108         129         86
12          248         60          93          57
13          266         122         41          25
14          209         95          89          61
15          7           5           2           5
16          2           11          0           6
17          3           8           5           3
18          3           14          0           0
XX
//
AC  Gsc
XX
ID  Gsc
XX
PO          A           C           G           T
1           0           0           0           22
2           22          0           0           0
3           22          0           0           0
4           0           0           0           22
5           0           22          0           0
6           0           14          2           6
XX
//
AC  Side
XX
ID  Side
XX
PO          A           C           G           T
1           12          0           6           5
2           17          3           3           0
3           21          2           0           0
4           16          6           1           0
5           0           23          0           0
6           23          0           0           0
7           0           10          9           4
```

```
8       5       18      0       0
9       1       1       0       21
10      0       0       23      0
11      0       5       2       15
XX
//
AC  Dsx
XX
ID  Dsx
XX
PO         A   C      G      T
1          0   3      0      0
2          0   0      0      3
3          2   0      1      0
4          0   3      0      0
5          2   0      0      1
6          3   0      0      0
7          2   0      0      1
8          0   0      3      0
9          0   0      0      3
10         0   1      1      1
11         2   0      1      0
12         0   1      0      2
13         1   1      0      1
14         3   0      0      0
XX
//
```