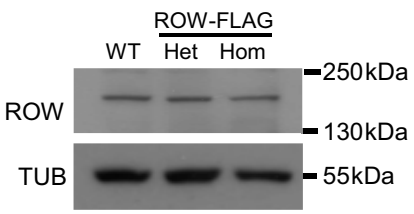
Expanded View Figures

A



B

Protein names	Control IBAQ	ROW-TAG IBAQ	Peptides number
HP1c	0	2.44E+08	9.3
HP1b	0	1.69E+08	10.7
ROW	0	7.93E+07	47.7
WOC	0	7.73E+07	62.3
Ctp	0	3.06E+07	2
Ubqn	0	2.54E+07	10.7
SsRbeta	0	3.90E+06	0.7
TM4SF	0	3.13E+06	1
CG16985	0	3.04E+06	1
BEAF-32	0	2.73E+06	2.7
Sgs3	0	2.62E+06	1
Rab1	0	2.05E+06	1.7
CathD	0	1.82E+06	0.7
CG9281	0	1.69E+06	1
Adh	0	1.26E+06	0.7
SdhC	0	1.15E+06	0.7
CG3192	0	1.01E+06	0.7
Trip1	0	8.78E+05	1.3
CG33303	0	7.57E+05	1
CG6126	0	6.71E+05	0.7
Lsp1gamma	0	6.42E+05	1.7
CG4045	0	6.20E+05	1
CG5973	0	5.00E+05	0.7
Rack1	0	4.79E+05	0.7
CG1737	0	4.50E+05	2.7
Whd	0	3.27E+05	1.3
gammaCop	0	2.68E+05	0.7
CG32638	0	2.40E+05	0.7
Chro	0	2.21E+05	1.7
Pst	0	2.15E+05	1
Tspo	0	2.14E+05	0.7
CASK	0	1.96E+05	0.7
T-cp1	0	1.90E+05	0.7
Foxo	0	1.48E+05	1
Flacc	0	1.33E+05	0.7
Hfp	0	1.30E+05	0.7
E(bx)	0	8.37E+04	0.7
Crol	0	4.16E+04	0.7

C

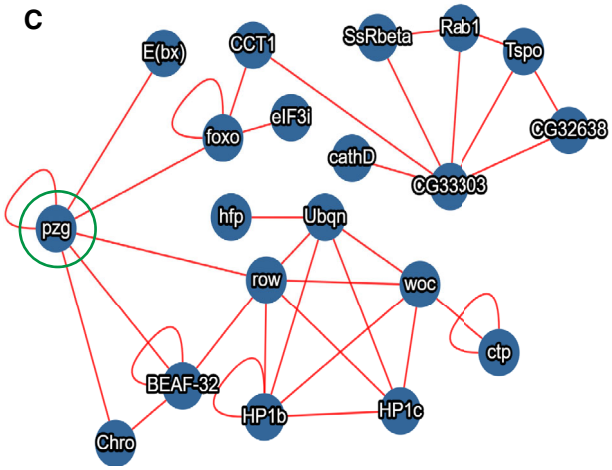


Figure EV1. *In vivo* interactome of ROW.

A Western blot for flies expressing endogenous FLAG-tagged ROW (ROW-TAG) with αROW antibody was used to verify that the tagging does not affect ROW protein levels. αTubulin (TUB) antibody was used as a reference. Het, Heterozygotes; Hom, Homozygotes for tagged ROW.

B Proteins co-purified with ROW in at least two of the three experiments that were also not detected in the control experiments. IBAQ (Accurate Label-Free Protein Quantitation) reflects the protein abundance in the sample. Peptide number is the number of Razor and unique peptides. The data are the mean of control (W^{1118}, $n = 3$) and ROW-FLAG flies ($n = 3$) samples.

C Protein–protein interactions that are supported by previous studies. The interactions are based on the molecular interaction search tool (MIST; Hu *et al*, 2018). The PZG (Z4) protein (circled in green) was not co-purified with ROW in our study but was found previously to interact with ROW (Guruharsha *et al*, 2011; Kessler *et al*, 2015) and may connect the two complexes we identified to interact with ROW.

Source data are available online for this figure.

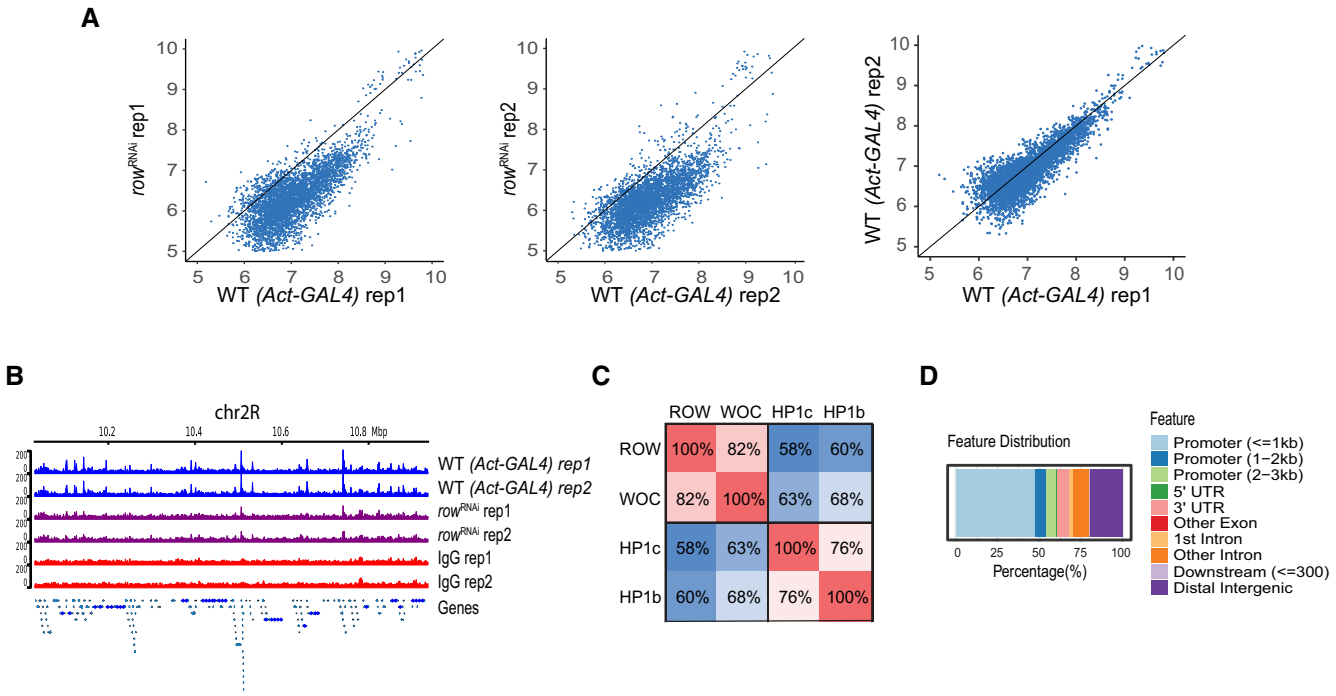


Figure EV2. ***In vivo*** **ChIP-seq results of ROW and its interactors.**

A Scatter plots of the ChIP-seq signal of ROW in *row*^{RNAi} flies compared with *Act-GAL4* flies (WT) and the results of the two biological replicates (rep1 and rep2) in WT flies against each other. The result shows that in *row*^{RNAi} flies, the signal is below the straight line with a slope of 1.

B ChIP-seq signals for ROW in a representative genomic region. The data are shown for *Act-GAL4* flies, *row*$^{RNAi\text{-}1}$ flies, and IgG ChIP-seq signals ($n = 2$ biological replicates for each).

C Percentage of overlap between the binding sites of ROW, WOC, HP1c, and HP1b.

D Genomic annotation of the shared binding sites between ROW, WOC, HP1c, and HP1b.

A

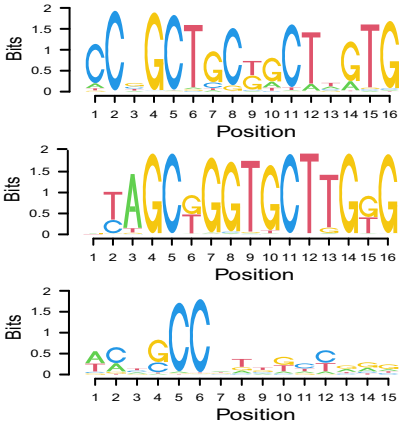
ROW Sequence	E-value
TWWWTATTTWTWWTWTWWWTW	1.50E-254
GAATTAAATAT	3.90E-115
ATATWTATWATWRT	1.80E-114
AAATRTW	8.40E-82
TTAATTA	6.50E-81

WOC Sequence	E-value
WTWTATTTATWWWWTTWTTTW	1.30E-172
GAATTAAATAT	9.90E-69
ATATWTATWATWRT	1.10E-65
AATAAW	6.20E-44
AAATAT	1.10E-43

HP1c Sequence	E-value
TWWWTWTWTTTWTTWWTTTWTTTTWTTTTT	2.20E-175
TAAWAATA	5.00E-21
GAATTAAATAT	5.20E-19
ATATWTATWATWRT	4.40E-17
MATAAAAAAAA	9.00E-16

HP1b Sequence	E-value
AAAAAAWAAAAHAAAAAAAWAAAWAAAA	1.30E-146
WAAATATW	1.50E-21
ATATWTATWATWRT	8.30E-15
GAATTAAATAT	6.10E-09
TAAWAATA	1.10E-08

B



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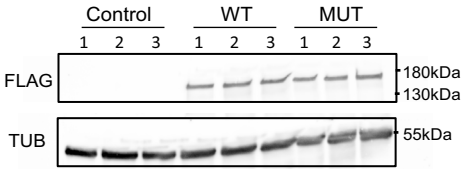

Figure EV3. ROW and its interactors bind to AT-rich sequences.

A Top five centrally enriched sequences in ROW-, WOC-, HP1b-, and HP1c-binding sites in fly heads based on MEME-ChIP tool.

B Predicted DNA sequences for ROW- Cys2His2 zinc fingers under different models.

C Validation of the expression of ROW-FLAG tagged proteins in S2 cells transfected with WT, or AT-hook mutant ROW-FLAG tagged plasmids (MUT) by western blot. Control are cells not transfected with ROW-FLAG tagged plasmids. Western blot was performed with αFlag tag and αTubulin (TUB) antibodies.

A

ROW Sequences	E-value
TTWTTWWWWTWWWTT	1.2E-114
ATATWTATWATWRT	2.3E-112
GAATTAAATAT	9.9E-95
AHTADTTA	3.2E-87
WTAATT	4.1E-83

BEAF-32 Sequences	E-value
NNAMCTATCGATADH	2.4e-839
AACTATCG	9.0e-821
VHTATCGATADB	5.3e-688
TATCGATA	4.9e-585
WNWTATCGATAR	1.1e-564

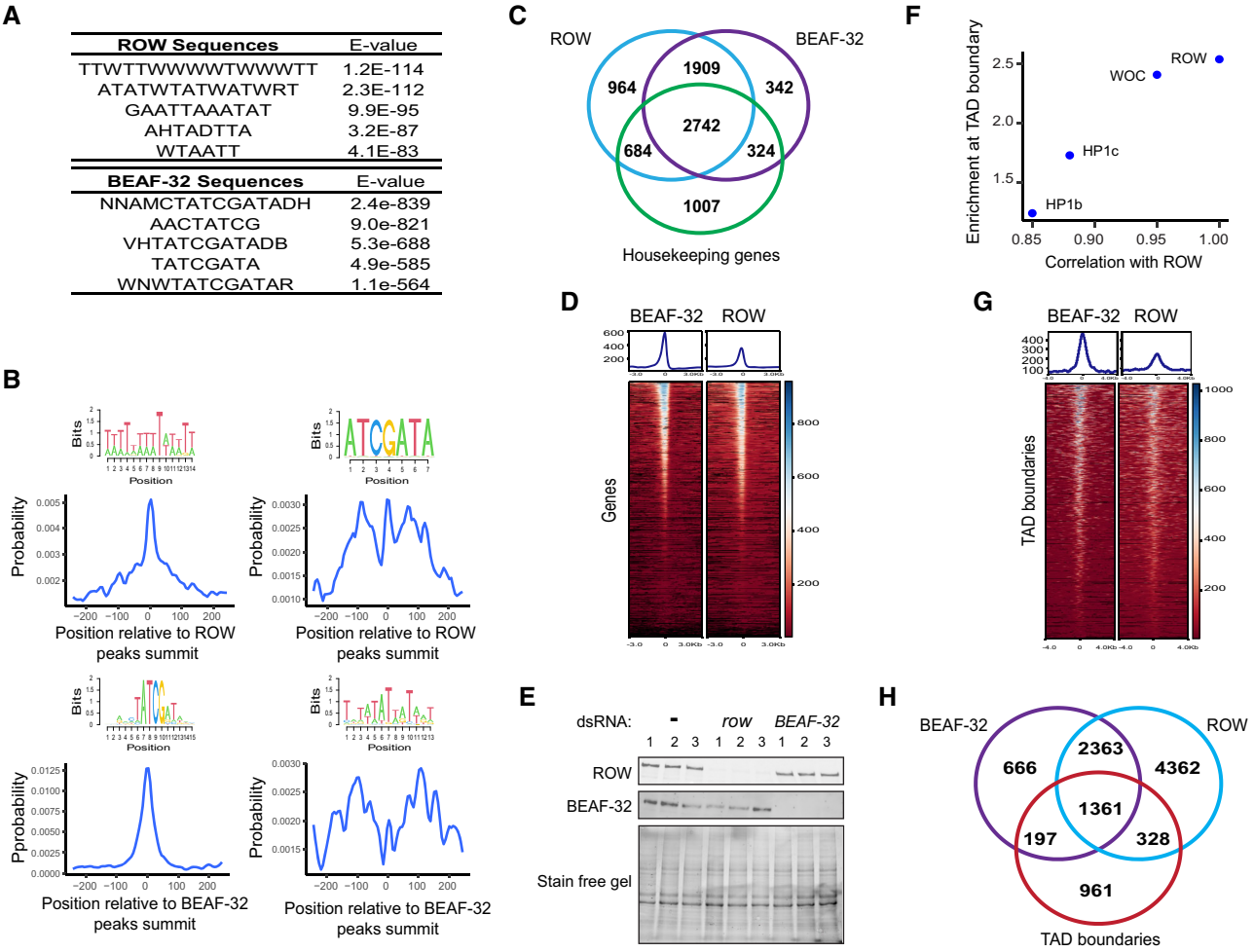

Figure EV4. Genomic distribution of ROW and BEAF-32.

A Top five centrally enriched sequences in ROW- and BEAF-32-binding sites in S2 cells based on MEME-ChIP tool.

B Central enrichment of AT-rich sequences and BEAF-32 consensus motifs (logo) in ROW-binding regions (upper figures) and BEAF-32-binding regions (lower figures) in S2 cells. The plots show the probability of having the motif relative to ROW or BEAF-32 ChIP-seq peak's summits (calculated with centriMo).

C Overlap between the genes bound by ROW, BEAF-32, and housekeeping genes.

D Heatmap of ROW and BEAF-32 ChIP-Seq signals in S2 cells ± 3 kb around TSS (average of $n = 3$ biological replicates).

E Protein levels of ROW and BEAF-32 in nontreated S2 cells (–) and cells treated with dsRNA against *row* or *BEAF-32*. The stain-free gel was used as a loading control.

F Enrichment at TAD boundaries of ROW, WOC, HP1c, and HP1b ($\widehat{\beta}$) as a function of the correlation of the ChIP-Seq signals in fly heads of the proteins with ROW.

G Heatmap of ROW and BEAF-32 ChIP-Seq signals in S2 cells ± 4 kb around TAD boundaries (average of $n = 3$ biological replicates).

H Overlap of the binding sites of ROW and BEAF-32 with TAD boundaries.

Source data are available online for this figure.

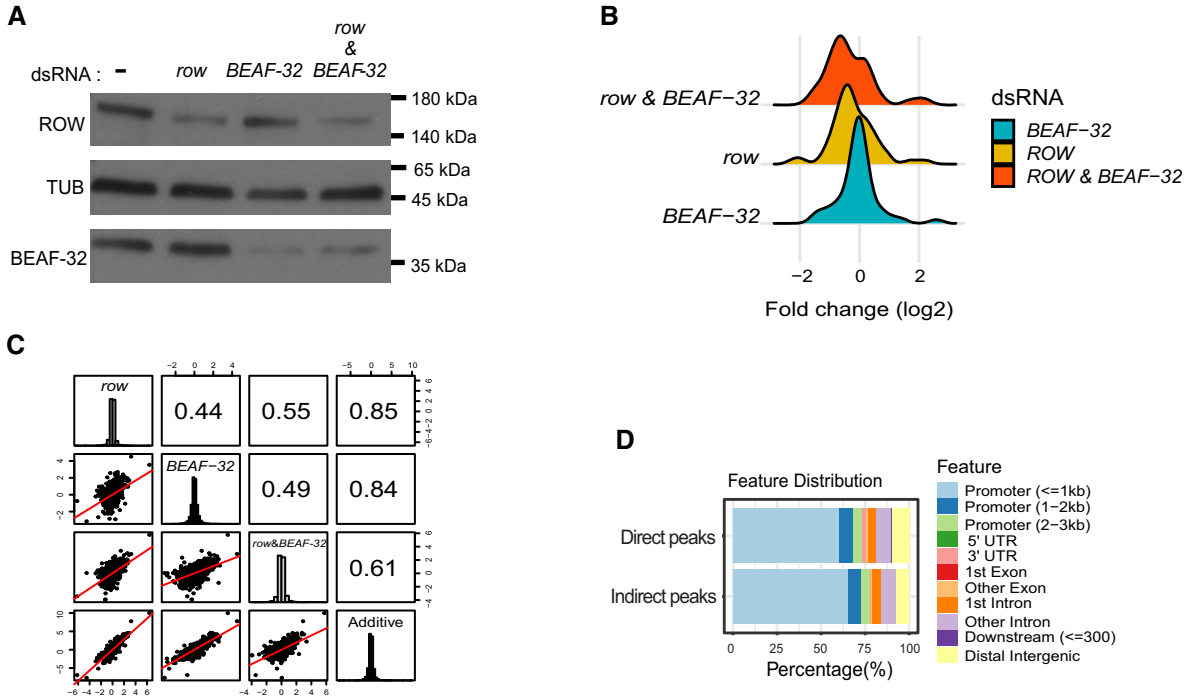

Figure EV5. Differential expression analysis in S2 cells.

A Protein levels of ROW and BEAF-32 in nontreated S2 cells (–) and cells treated with dsRNA against *row*, *BEAF-32*, or both. αTubulin (TUB) was used as a loading control.

B Density plot of the gene expression fold changes (log2) in cells treated with row^{dsRNA}, $BEAF\text{-}32^{dsRNA}$, and row^{dsRNA} & $BEAF\text{-}32^{dsRNA}$, shown for significantly differentially expressed genes.

C Pairwise correlation between the gene expression fold changes (log2) in cells treated with row^{dsRNA}, $BEAF\text{-}32^{dsRNA}$, row^{dsRNA} and $BEAF\text{-}32^{dsRNA}$, and the additive model (sum of fold change in the separate knockdowns of *row* and *BEAF-32*). Fold changes were calculated relative to control cells (using edgeR). In the boxes of the upper triangle are the Pearson correlation coefficients. In the diagonal boxes are histograms showing the distributions of the fold changes. On the lower triangle, boxes are bivariate scatter plots with linear regression lines.

D Genomic annotation of direct and indirect peaks of ROW shows that most are gene promoters.

Source data are available online for this figure.