

**Figure S1. Setup and tracking system.** (A) Scheme of the setup with an enlarged scheme of one of the wells. (B) Image of the wells. (C) Trajectory of each larva. (D) Strength of the peaks resulting from Hough transforms with different radii. The insets show the Hough transforms for 3 different radii. From this calculation we define  $rest = 58$  pixels. (E) Hough transform of the frame, using radii in a small interval around  $rest$  (in this case, in the interval between 47 and 71 pixels). (F) Same as E, but after removing the region that corresponds to the highest maximum. (G) Final result of the detection of wells. (H) Original image of two adjacent wells. (I) Image of the same wells, after a Gaussian filter. (J) Difference between the filtered image of the two wells, and the static background. The two larvae are clearly visible as regions with low values. (K) Difference between the filtered image of the two wells, and the dynamic background. Only the larva on the right is visible, because the larva on the left is not moving in this specific frame.

**Figure S2. Additional behavioral parameters.** (A-F) Correlation of six behavioral parameters to activity or radial index (in the case of circularity). (G-J) Correlation of the eight behavioral parameters between 5 dpf and 6 dpf. (K-M) The same as G-J but using six behavioral parameters between 7 dpf and 8 dpf. (N-P) Median of intra-individual variability (dotted lines) and inter-individual variability using six parameters during 5-8 dpf. (Q) Smoothed histogram of the coefficient of variation of the activity (left) and the radial index (right) showing the intra-individual variability (red) and inter-individual variability (blue) at 5 dpf. (R) Same as Q, for the group at 6 dpf. (S) Same as Q, for the group at 7 dpf. (T) Same as Q, for the group at 8 dpf.

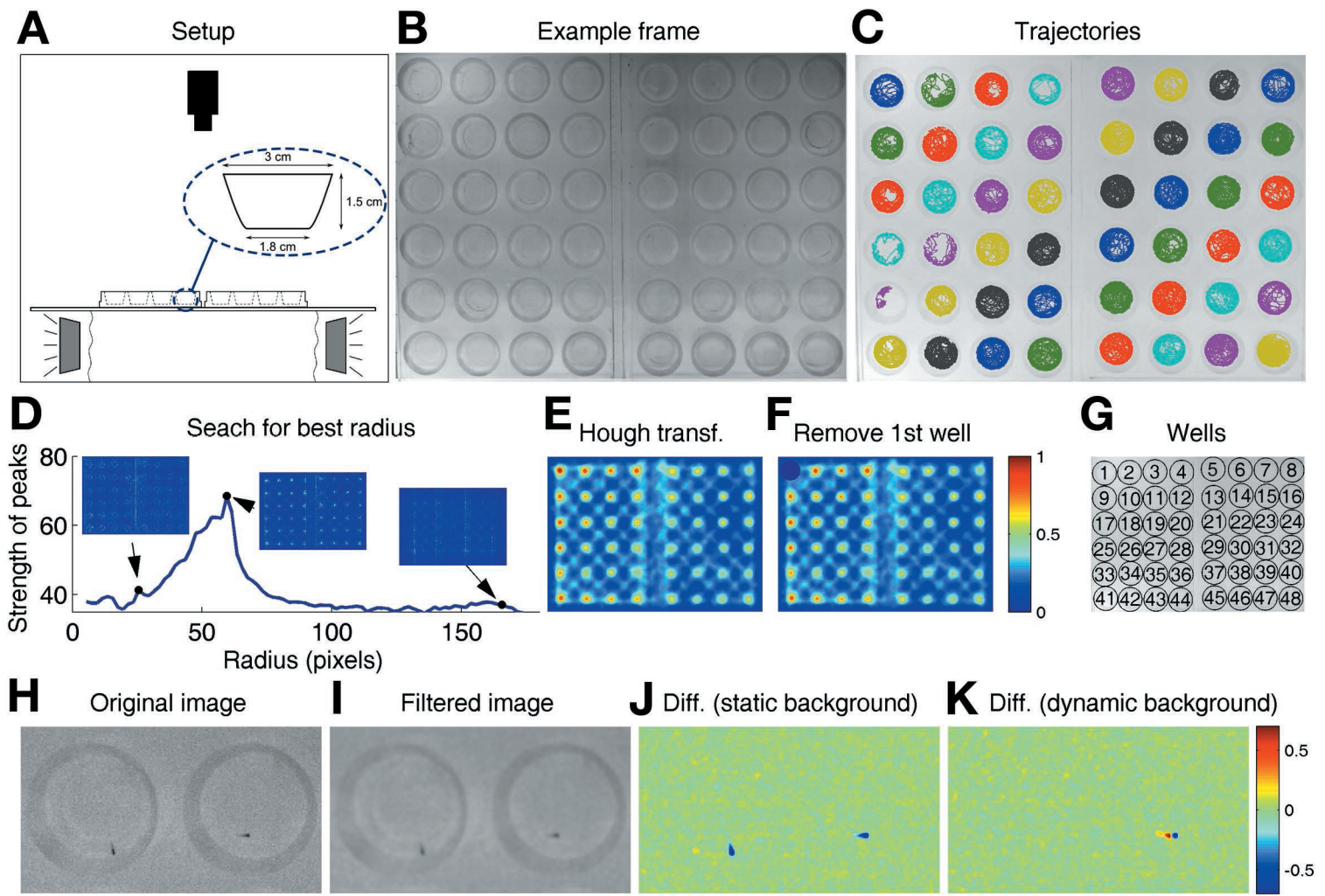
**Figure S3. Additional validation of activity and radial index.** (A) Changes in the behavior after 90° rotation of the plates (left) and after interchanging 24 larvae from external to internal wells (right). (B) Larval behavioral parameters (activity, blue; radial index, red) plotted against

the average light received by these 48 larvae. Min and max values are 0.1-0.5 and 0.3-0.7 for activity and radial index, respectively. **(C)** Average activity (left) and radial index (right) of a pool of different larvae recorded in the same wells for 15 experiments (>600 larvae) (top), compared to a single 48 larval population (bottom). **(D)** Activity (left) and radial index (right) plotted against the median size of the larva as detected by the tracking software. **(E)** Average activity (left) and radial index (right) of groups of ten larvae plotted against the gene expression fold change of *amigo1* normalized by *GAPDH* expression **(F)** Correlation between the behavior in different tests (Light Pulse, left; Mechanical Perturbation, center and Novel Tank with Light Preference, right) and our behavioral parameters in the usual conditions, using 16 larvae.

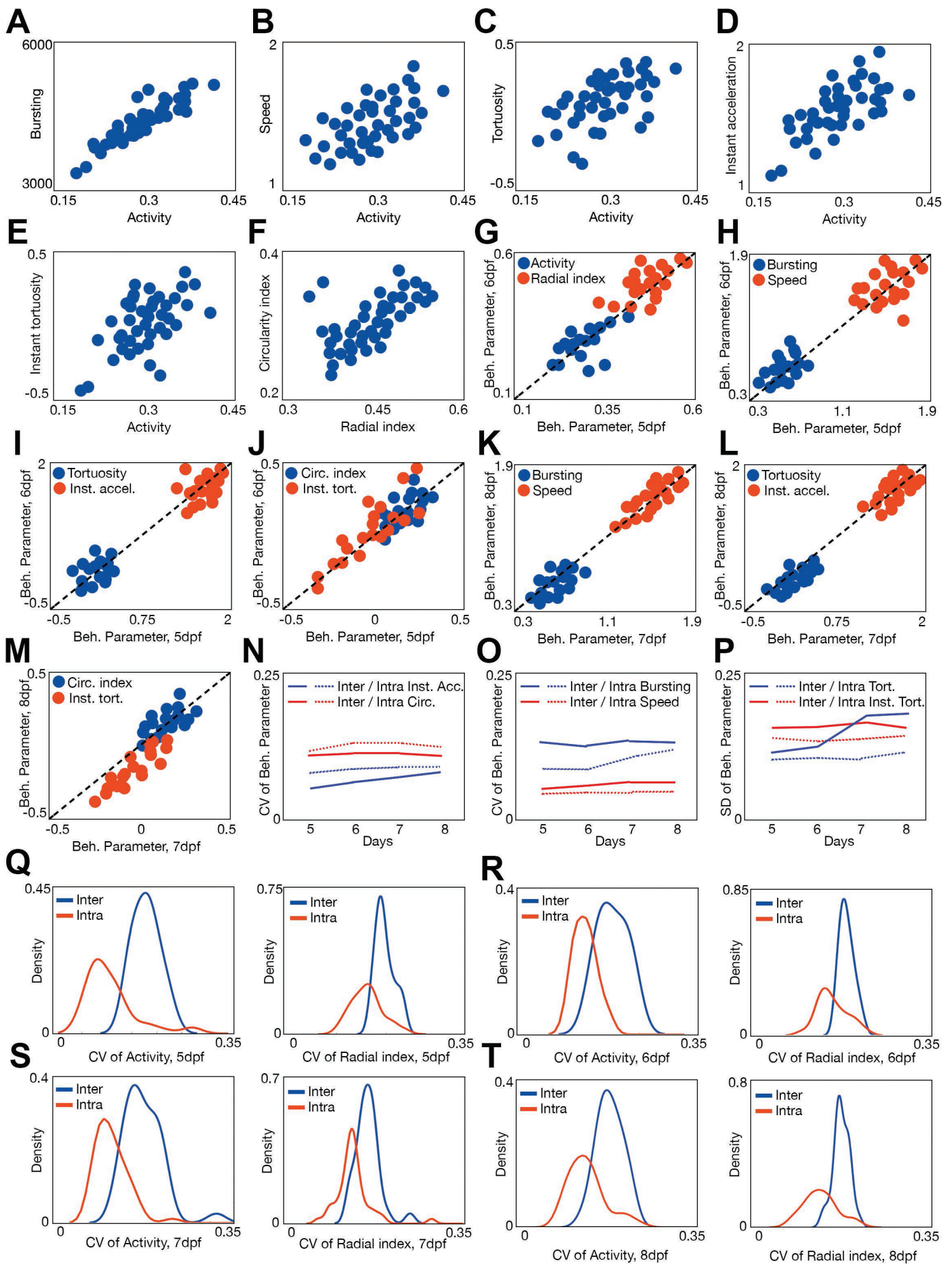
**Figure S4. Additional data for epigenetic treatments.** **(A)** Methylation levels comparing control (PBS) and AZA treatment. **(B)** ELISA results quantifying the levels of acetyl-H4 in the conditions shown in **Figure 3** and in groups of 20 fish from 5 to 9 dpf. **(C)** Probability density for 24 larvae 48h after treatment with NaBu only during the first 24h and then washed with PBS (PBS/PBS as control).

**Figure S5. Additional data for histone H4 acetylation and behavior.** **(A)** Relation between histone H4 acetylation levels and distance to average behavior: activity (left) and radial index (right). R coefficients are shown. **(B)** Same as **A**, but for H4K12 acetylation levels. **(C)** Differences in histone H3 (red) and H4 (blue) acetylation levels within extracts obtained in the same behavioral positions towards the average behavior of the population (left). Difference in histone H4 acetylation in clusters with different behavioral positions towards the average behavior of the population (right).

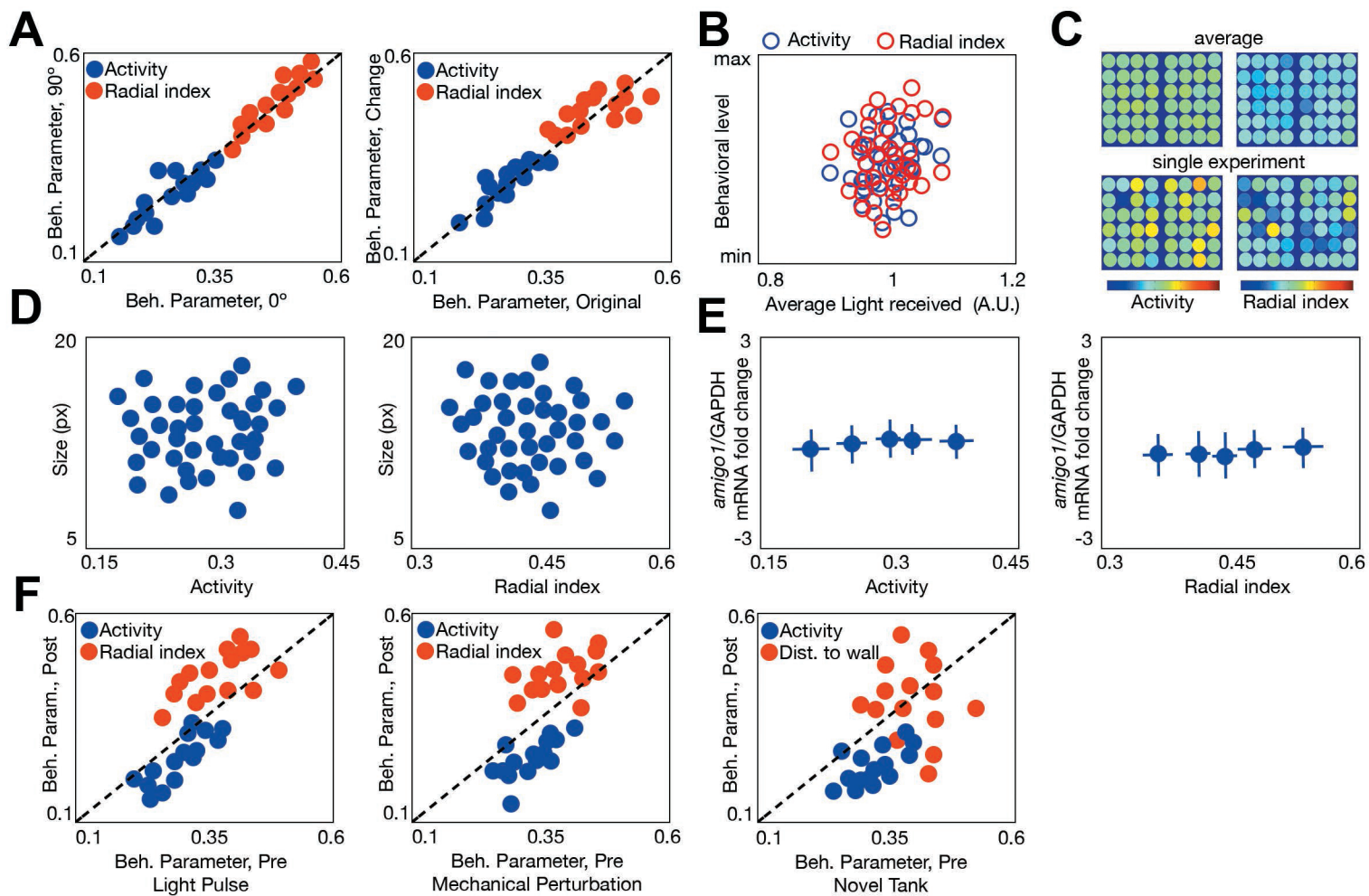
**Figure S6. Additional data for Histone H4 and YY1 acetylation.** (A) Plot showing acH4 normalized levels for each region in control samples (x-axis) versus random samples (y-axis), whose variability is not associated to behavioral differences (B) Histone H4 acetylation levels quantified in conventional ChIP as the fold change compared to non-bound fraction in eight selected regions in pools of 20 fish from 5 to 9 dpf. (C) Original blot from the **figure 6J** representing the acetyl-YY1 levels at different conditions.



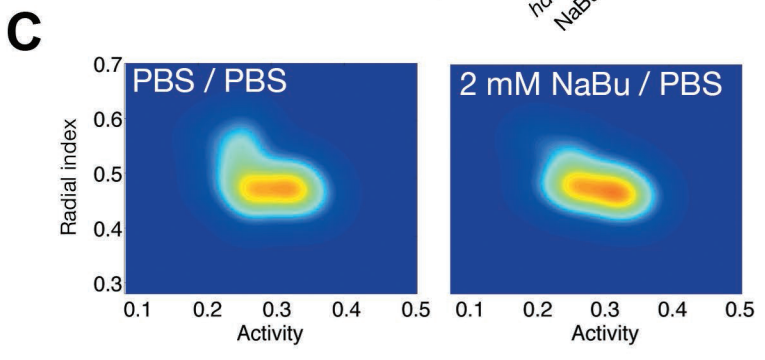
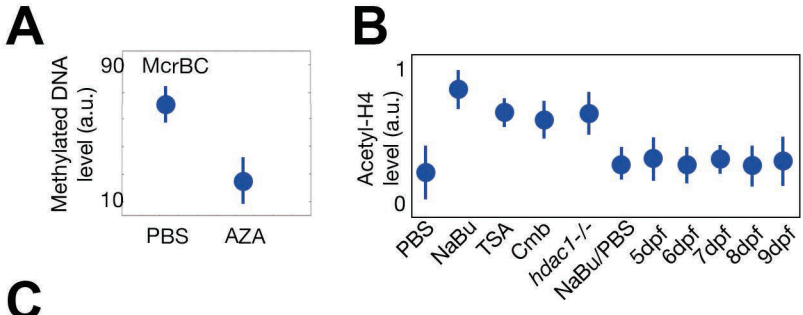
**Figure S1**



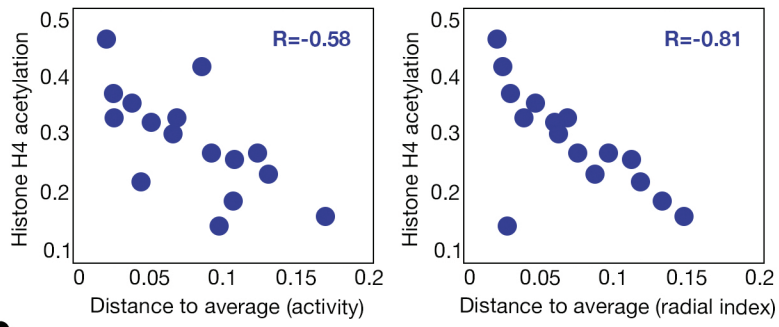
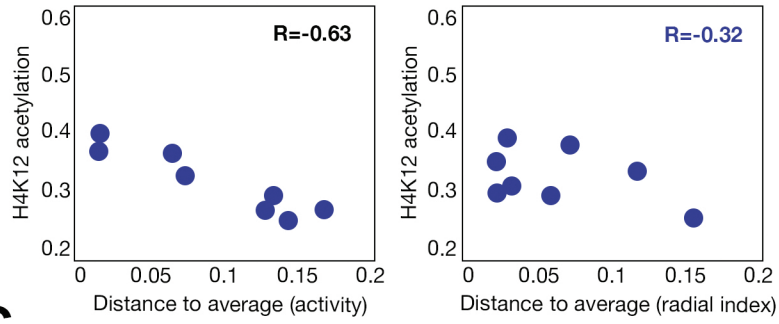
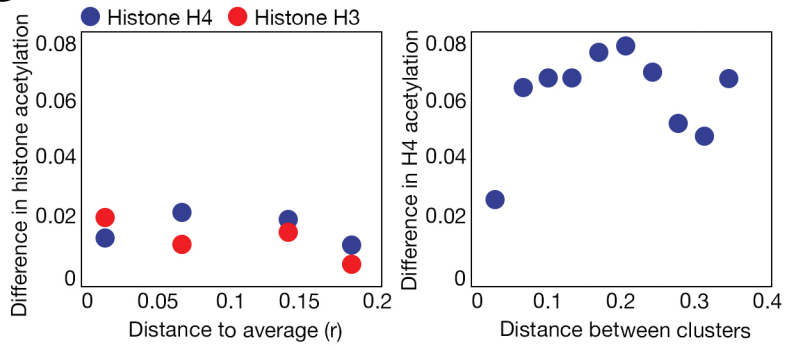
**Figure S2**



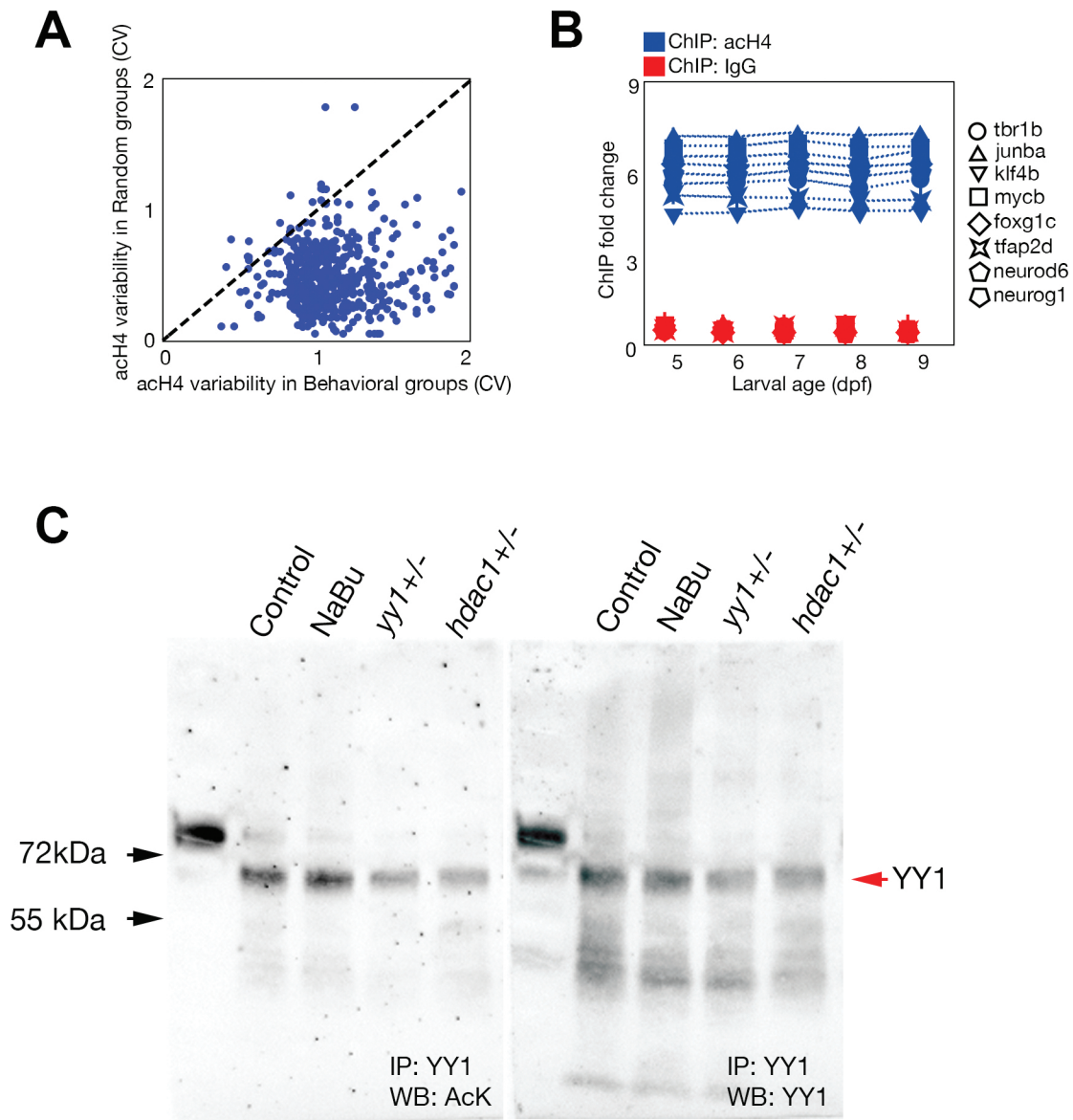
**Figure S3**



**Figure S4**

**A****B****C**





**Figure S6**