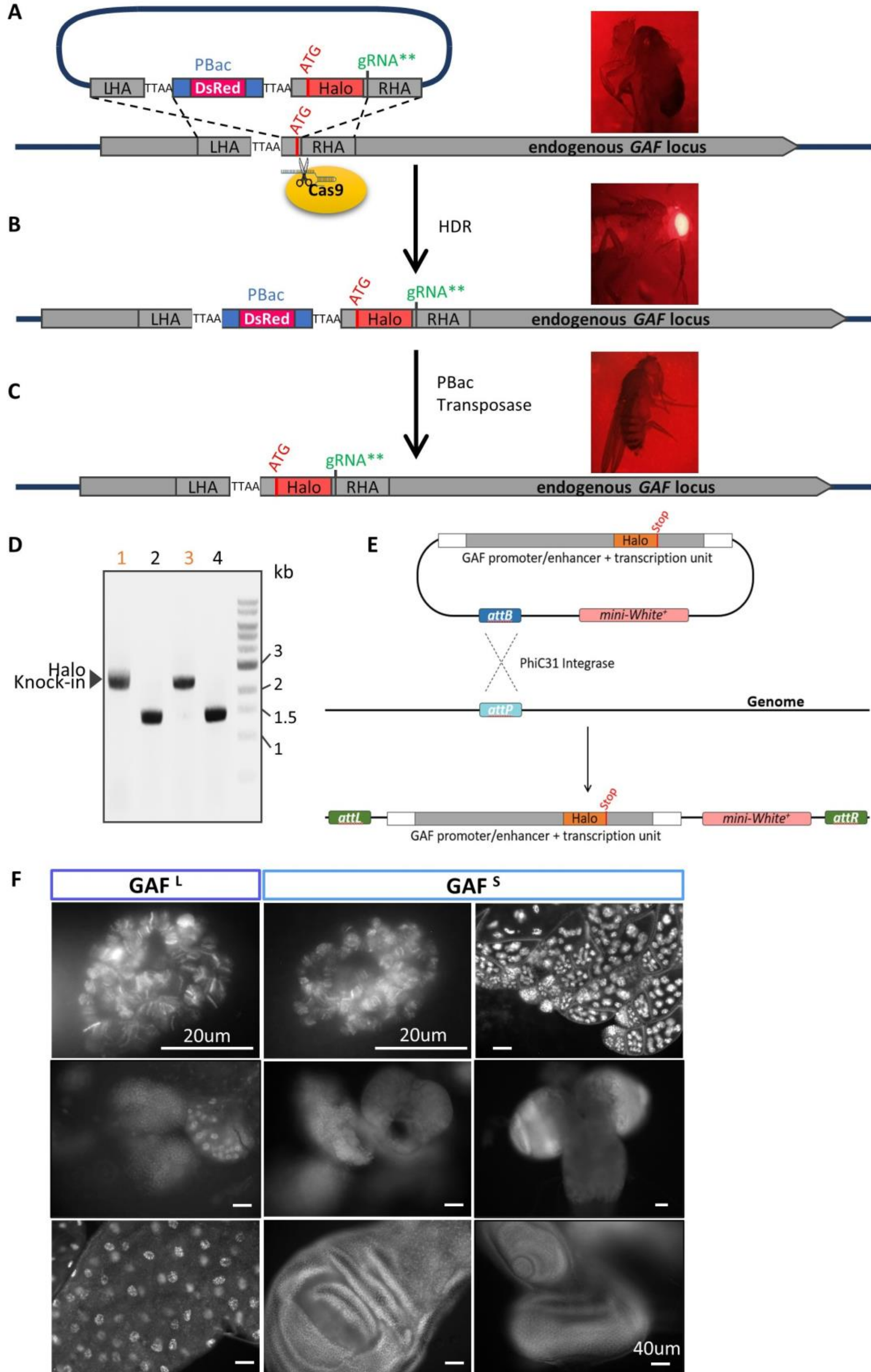
Supplementary information



Supplementary Fig. 1. Generation of N-terminal Halo-GAF knock-in fly strain and C-terminal GAF-Halo transgenic fly strains.

(A) Donor plasmid design for homology directed repair (HDR). HaloTag and a flexible linker (GGSGS, not shown) are placed downstream of the start codon ATG. A PBac transposon containing a DsRed cassette is inserted into a nearby genomic TTAA site adjacent to the gRNA target site in the coding region that is close to the start codon ATG. The TTAA site is duplicated so that both ends of the PBac transposon contain a TTAA sequence. Approximately 1 kb fragment downstream of the gRNA target site is cloned as the right homology arm (RHA), with silent mutations (gRNA**) introduced to destroy the gRNA PAM sequence in the donor plasmid. Similarly, a 1kb fragment upstream of the genomic TTAA site is cloned as the left homology arm (LHA).

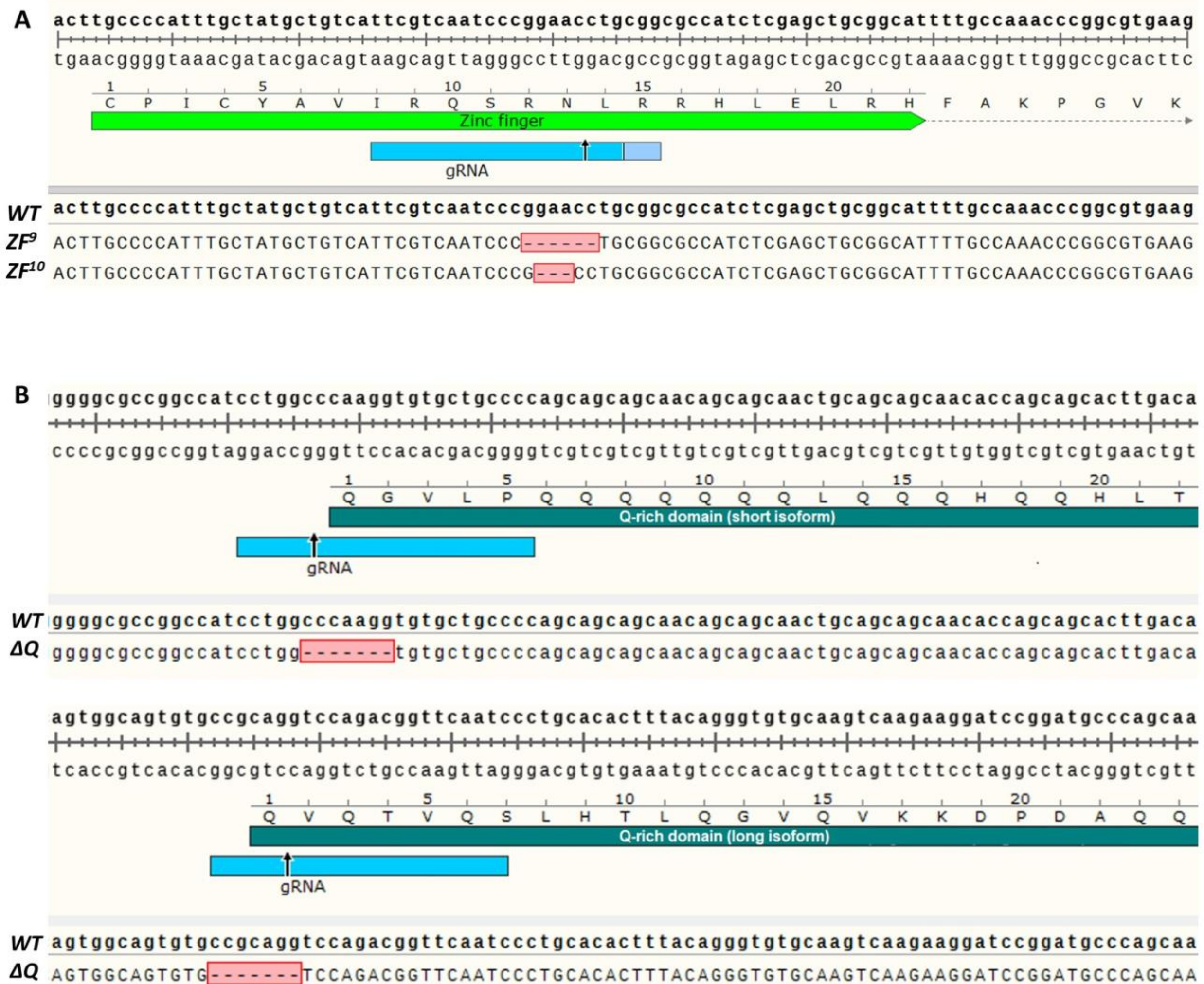
(B) LHA and RHA mediate HDR upon Cas9 cleavage, inserting HaloTag along with the DsRed cassette. Flies that have undergone HDR can be identified by eye DsRed fluorescence.

(C) By crossing to a fly strain expressing PBac transposase, the DsRed cassette can be removed, as indicated by loss of fluorescence, leaving only one TTAA sequence, thereby allowing scarless HaloTag knock-in with a removable selection marker. Arrows indicate positions of the primers used for validating HaloTag insertion.

(D) PCR validation of HaloTag knock-in after DsRed cassette removal (lane 1 and 3). Halo-GAF homozygous flies are viable, showing only 1 band ~900 bp larger than flies without HaloTag knock-in (lane 2 and 4).

(E) Strategy used to generate transgenic fly strains GAF^L-Halo, GAF^S-Halo, Halo-H2B and HSF-Halo. An ~15 kb fragment containing the Trl transcription unit and ~1kb upstream and downstream regions was cloned, and HaloTag ORF was inserted upstream of the stop codons for GAF^L or GAF^S, respectively. Thus, each of the two transgenic flies express a Halo-tagged GAFL or GAFS isoform and another isoform (non-tagged), under native Trl promoter control.

(F) Tissue-specific expression of transgenic GAF^L-Halo and GAF^S-Halo. Shown are major expressing larval tissues. GAF^L-Halo (top to bottom): salivary gland, lymph gland, intestine; GAF^S-Halo (top to bottom, then left to right): salivary gland, lymph gland, wing disc, ovary, brain, eye-antenna disc.



Supplementary Fig. 2. Deletions of genomic DNA in Halo-GAF *ZF*^{9}, *ZF*^{10}, and *ΔQ*.

(A) A genomic DNA fragment of the GAF-encoding gene *Trl*, shown at the top with amino acid sequence of the zinc finger. The location of gRNA sequence used for CRISPR editing is shown in the middle. Alignment of *WT* and *ZF*^{9}, *ZF*^{10} sequences is shown at the bottom. Arrow, Cas9 cut site.

(B) Two genomic DNA fragments of the GAF-encoding gene *Trl*, shown with amino acid sequence of N-termini of the Q-rich domains (short and long isoforms). The locations of gRNA sequences used for CRISPR editing are shown below each fragment, along with alignments of *WT* and *ΔQ* sequences. Arrow, Cas9 cut site

Supplementary Table 1. gRNA sequences for CRISPR gene editing

Strain	*gRNA sequence* (*PAM sequence in red*)	*Silent mutations in donor plasmid*
Halo-GAF knock-in	GCTGGTGCCGTAATCGCCCCAGG	GCTGGTGCCGTAATCACCCCAAG
ΔPOZ	GTACCGCGGAGAGGTGAGCGTGG	no mutation needed because the gRNA sequence is targeted for deletion and not present in the donor plasmid
ZF^{9} and ZF^{10}	TTCGTCAATCCCGGAACCTGCGG	N/A
ΔQ short isoform	GGGGCAGCACACCTTGGGCCAGG	N/A
ΔQ long isoform	GATTGAACCGTCTGGACCTGCGG	N/A

Supplementary Video 1. Fast-tracking movie of Halo-GAF

A fast-tracking movie of Halo-GAF labeled with 1nM JF554. Movie was acquired with 10 ms camera integration time for single molecule tracking after 10-30 s of initial nuclear glow.

Supplementary Video 2. Slow-tracking movie of GAF^{L}-Halo

A low-tracking movie of GAF^{L}-Halo labeled with 0.05 nM JF552 (and 50 nM nonfluorescent JF700 blocker). Images acquired at 500 ms exposure time to motion blur diffusing molecules and selectively observe chromatin-bound molecules. Movie frames are placed on a 3D-axis of time and x, y coordinates to display identified trajectories. Tracking parameters are adjusted to avoid identification of blurred molecules.