# Supporting Information

## Nern et al. 10.1073/pnas.1111704108

### SI Methods

**Molecular Biology.** *Drosophila* codon-optimized recombinases and their cognate recognition sites were synthesized by DNA2.0, Inc. The coding sequences for the recombinases are shown in Fig. S1 for B3, KD, and B2 and Fig. S2 for R and FLP. The sequences of the stop cassettes used in this work for the B3, KD, B2, RS, and FLP stop cassettes are shown in Fig. S3.

To construct pBPhsFlp1 and pBPhsFlp2 sequences encoding yeast FLP were amplified from the pUAS-Flp vector (ref. 1; obtained from the Drosophila Genome Resource Center) to contain an optimal translation sequence and cloned as a 5′ KpnI–3′ Avr2 fragment into a modified pBDP (2) vector that contains the *Hsp70Bb* Heat Shock Promoter (−194 to +237) and 337-bp terminator (ref. 3; CG31359). Two variants of the FLP coding sequence were recovered; pBPhsFlp1 contains a D5 residue and pBPhsFlp2 a G5 residue. Sequences have been reported for the yeast Flp gene that encode either G5 or D5 (4, 5).

All recombinases used in the UAS vectors were synthesized to include a C-terminal PEST sequence; nuclear localization sequences were not added. The same recombinase genes (*Drosophila*-codon optimized and including the PEST sequence) and stop cassettes were used in flies, CHO cells, and mice. RFP was used as a reporter in the stop cassettes for B2, B3, KD, and R; GFP in the cassettes for Cre, Dre, and FLP.

The plasmids used for mouse in utero electroporation were based on an AAV virus backbone (6). The modular cassettes BlownOUT and KickedOUT were cloned into the BamHI site of the AAV-CAG_tdTomato vector (gift of Jinny Kim, Janelia Farm Research Campus) to generate CAG_BlownOUT_tdTomato and CAG_KickedOUT_tdTomato. AAV-syn-B3:PEST and AAV-syn-KD:PEST were constructed by substituting the *Drosophila*-codon optimized B3 and KD genes, respectively, for the GCaMP3 gene in the AAV-syn-GCaMP3 construct (7).

***Drosophila* Transgenics and Genetics.** Transgenic fly lines were generated as described (2, 8). For assaying recombinase activity, flies with appropriate stop-cassette reporter constructs inserted in attP40 and R31F10-GAL4 (in attP2) or elav-GAL4 (C155; ref. 9) were crossed to UAS recombinase flies (in attP2). To visualize the entire R31F10 pattern, a 10XUAS-mCD8::GFP reporter (pJFRC2 in attP40) was included in some cases. For testing 3XUAS recombinase constructs (in attP40), stop-cassette reporters in VK00005 and attP2 were used. For comparing the D5 and G5 FLP variants, low levels of FLP were expressed under hs-promoter control (using pBPhsFlp1 or pBPhsFlp2 in attP2) by raising flies at 25 °C without additional heat shock. To test for toxicity of recombinase expression, UAS recombinase drivers in attP2 (20XUAS) were crossed to elav-GAL4 (C155; ref. 9), tubP-GAL4 (10), or GMR-GAL4 (11).

**Imaging of Optic Lobes.** Optic lobes from 5- to 10-d adult flies, mounted in an appropriate orientation, were imaged on a Zeiss LSM 710 confocal microscope by using a 20× 0.8 NA objective. To aid quantification of medulla columns with stop-cassette excision, two methods were used to visualize all medulla columns: To assay KD, B2, B3, and R activity and potential cross-reactivity of these recombinases, the entire medulla pattern of R31F10 was revealed by using mCD8 GFP (pJFRC2-10XUAS-IVS-mCD8::GFP). In this case, samples were mounted in PBS and native GFP and RFP fluorescence were imaged directly after dissection. For all other experiments, mAb24B10 (12) staining of R7 and R8 photoreceptor neurons was used as an indirect marker for the positions of T1 neurons, which, like R7 and R8, are present once per medulla column. For these experiments, brains were dissected in PBS, fixed with 2% formaldehyde in PBS for 1 h, washed several times with PBT (PBS + 0.5% TX-100), blocked with PBT with 5% goat serum and incubated with rabbit anti-GFP (Invitrogen; 1:1,000 dilution) or rabbit anti-ds-Red (Clontech; 1:1,000 dilution) plus mAb24B10 (ref. 12; Developmental Studies Hybridoma Bank; 1:20 dilution) overnight at 4 °C. Secondary antibodies were donkey anti-mouse DyLight 649 (1:500; Jackson Immunoresearch) and donkey anti-rabbit DyLight 488 (1:500; Jackson Immunoresearch). Samples were mounted in Slowfade Gold (Invitrogen). Eye sections to assess recombinase toxicity were prepared as described (13).

**In Vivo Mouse Assays.** Embryonic day 16 timed-pregnant C57BL/6J mice (Charles River) were deeply anesthetized by using an isoflurane-oxygen mixture [2% (vol/vol) isoflurane in $O_2$]. The uterine horns were exposed and ≈1 μL of DNA solution was pressure-injected through a pulled glass capillary tube into the right lateral ventricle of each embryo. The DNA solution contained a mixture of plasmids in a 1:1 ratio at a final concentration of 2 μg/μL. The head of each embryo was placed between custom-made tweezer electrodes, with the positive plate contacting the right side of the head. Electroporation (14, 15) was achieved with five square pulses (duration 50 ms, frequency 1 Hz, 40 V). Electroporated mice were perfused with cold saline and 4% paraformaldehyde and fixed overnight. Analysis of tdTomato expression was performed in 50-μm-thick brain sections.


1. Duffy JB, Harrison DA, Perrimon N (1998) Identifying loci required for follicular patterning using directed mosaics. *Development* 125:2263–2271.
2. Pfeiffer BD, et al. (2008) Tools for neuroanatomy and neurogenetics in Drosophila. *Proc Natl Acad Sci USA* 105:9715–9720.
3. Ingolia TD, Craig EA, McCarthy BJ (1980) Sequence of three copies of the gene for the major Drosophila heat shock induced protein and their flanking regions. *Cell* 21: 669–679.
4. Hartley JL, Donelson JE (1980) Nucleotide sequence of the yeast plasmid. *Nature* 286: 860–865.
5. Babineau D, et al. (1985) The FLP protein of the 2-micron plasmid of yeast. Purification of the protein from Escherichia coli cells expressing the cloned FLP gene. *J Biol Chem* 260:12313–12319.
6. Petreanu L, Mao T, Sternson SM, Svoboda K (2009) The subcellular organization of neocortical excitatory connections. *Nature* 457:1142–1145.
7. Tian L, et al. (2009) Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nat Methods* 6:875–881.
8. Pfeiffer BD, et al. (2010) Refinement of tools for targeted gene expression in Drosophila. *Genetics* 186:735–755.
9. Lin DM, Fetter RD, Kopczynski C, Grenningloh G, Goodman CS (1994) Genetic analysis of Fasciclin II in Drosophila: Defasciculation, refasciculation, and altered fasciculation. *Neuron* 13:1055–1069.
10. Lee T, Luo L (1999) Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* 22:451–461.
11. Freeman M (1996) Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. *Cell* 87:651–660.
12. Zipursky SL, Venkatesh TR, Teplow DB, Benzer S (1984) Neuronal development in the Drosophila retina: Monoclonal antibodies as molecular probes. *Cell* 36:15–26.
13. Wolff T (2000) Histological Techniques for the Drosophila eye, Part II: Adult. *Drosophila protocols*, eds Sullivan W, Ashburner M, Hawley RS (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 229–243.
14. Petreanu L, Huber D, Sobczyk A, Svoboda K (2007) Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nat Neurosci* 10:663–668.
15. Kügler S, et al. (2001) Neuron-specific expression of therapeutic proteins: evaluation of different cellular promoters in recombinant adenoviral vectors. *Mol Cell Neurosci* 17:78–96.
16. Cavener DR, Ray SC (1991) Eukaryotic start and stop translation sites. *Nucleic Acids Res* 19:3185–3192.

17. Carswell S, Alwine JC (1989) Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol Cell Biol* 9:4248–4258.

18. Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401–415.

**B3 Recombinase**

CTCGAGAATCAAAATGAGCTCGTATATGGATCTTGTTGATGATGAACCAGCGACTTTGTACCATAAGTTCGTGGAGTGCT
TGAAAGCGGGCGAGAACTTCTGCGGAGACAAGCTGAGTGGAATTATTACCATGGCGATCCTTAAGGCAATCAAGGCGCTG
ACCGAAGTTAAAAAGACAACCTTTAACAAATATAAGACAACAATCAAGCAGGGCCTCCAGTATGACGTGGGTTCGTCGAC
TATCTCGTTTGTGTATCACTTGAAGGACTGTGATGAGCTGTCCAGGGGCTTGAGCGATGCCTTCGAGCCCTACAAATTCA
AAATTAAGTCGAATAAAGAGGCAACCTCGTTTAAGACTCTCTTTCGTGGCCCCTCGTTTGGCAGCCAGAAGAACTGGCGG
AAGAAAGAGGTGGACCGCGAGGTGGATAACTTGTTTCATAGCACCGAGACAGACGAATCGATTTTCAAATTCATCTTGAA
CACGTTGGATAGTATTGAGACACAAACGAACACGGATCGCCAAAAGACGGTGCTGACTTTCATCCTGTTGATGACATTTT
TCAACTGCTGTAGGAACAATGACCTGATGAACGTTGATCCCTCCACATTTAAGATTGTGAAAAACAAATTCGTCGGATAC
CTGCTGCAGGCTGAGGTCAAACAGACTAAGACACGCAAGTCGAGGAACATTTTCTTCTTTCCCATCCGCGAGAATCGATT
CGATCTGTTCCTGGCCTTGCACGATTTCTTCCGCACATGCCAGCCTACCCCAAAGTCGCGTCTTTCGGATCAAGTATCGG
AGCAGAAGTGGCAGCTTTTCCGAGATTCCATGGTCATTGATTACAACCGTTTCTTTAGGAAGTTTCCAGCTTCGCCTATT
TTCGCAATAAAGCACGGCCCCAAGTCCCATCTGGGCCGGCATCTGATGAACAGCTTTCTGCACAAGAATGAACTGGATTC
CTGGGCCAACTCCCTGGGCAACTGGAGCTCCTCCCAGAATCAACGCGAGTCCGGTGCGCGACTGGGCTACACCCACGGTG
GTCGAGATTTGCCACAGCCACTGTTCGGATTCCTGGCTGGCTACTGCGTTCGAAATGAAGAGGGCCACATCGTGGGCTTG
GGTCTGGAGAAGGACATTAATGATCTGTTTGACGGTATTATGGACCCACTTAATGAGAAGGAGGATACAGAGATTTGTGA
AAGCTACGGCGAGTGGGCCAAAATTGTGAGCAAGGATGTTCTGATATTTCTGAAGCGATATCATTCGAAGAACGCATGCC
GGCGATATCAGAACTCCACATTGTACGCACGTACGTTCCTTAAGACAGAGTCCGTCACCTTGAGCGGCTCCAAGGGAAGC
GAAGAGCCGAGCAGTCCCGTCAGGATTCCAATACTTAGTATGGGAAAAGCCTCCCCAAGCGAGGGCCGAAAGTTGCGTGC
CAGCGAACATGCCAACGATGATAATGAGATTGAGAAGATAGATTCGGATTCGTCGCAAAGCGAAGAGATACCTATCGAAA
TGTCCGACTCCGAGGACGAAACAACGGCAAGTAACATTAGCGGAATTTACCTGGACATGTCGAAAGCCAACTCCAACGTG
GTCTATTCCCCTCCGAGTCAGACTGGCCGTGCCGCCGGTGCCGGCCGTAAACGTGGCGTGGGTGGTCGCCGCACCGTTGA
GTCGAAGCGGCGGCGCGTCCTGGCACCAATTAACCGGGGATCCCATGGCTTCCCTCCAGAGGTGGAGGAGCAAGATGATG
GCACTCTCCCCATGAGCTGCGCTCAAGAGAGTGGCATGGATCGTCACCCCGCTGCTTGCGCCTCGGCTCGCATCAACGTG
TAATCTAGA

**KD Recombinase**

CTCGAGAATCAAAATGAGTACTTTTGCAGAAGCAGCGCATCTTACACCGCATCAATGTGCGAACGAAATCAACGAGATTC
TGGAAAGTGATACATTCAACATTAATGCGAAAGAAATACGCAACAAGTTGGCTAGTCTCTTTTCCATTCTTACCATGCAA
TCGCTGTCCATCCGTAGGGAAATGAAGATTAACACGTATCGTAGCTACAAGTCCGCAATCGGAAAATCCCTGAGCTTTGA
CAAGGATGACAAGATTATCAAATTCACTGTACGGCTGAGGAAGACCGAGAGTCTGCAGAAAGACATTGAAAGCGCACTCC
CGTCCTACAAAGTCGTTGTTTCGCCATTCAAAAACCAGGAGGTGTCGCTTTTCGATCGATACGAGGAAACCCACAAGTAC
GATGCGTCCATGGTGGGCCTCCAATTTACGAACATTCTCAGCAAGGAGAAAGACATCTGGAAAATCGTTAGCCGAATCGC
TTGCTTCTTCGATCAGTCGTGCGTGACGACCACGAAGCGAGCGGAGTACCGCCTGCTTCTGCTGGGAGCCGTAGGTAACT
GTTGCCGTTATAGCGATCTCAAAAATCTGGACCCCCGGACCTTTGAGATTTACAACAATTCCTTCTTGGGACCAATCGTG
CGGGCCACGGTTACAGAGACAAAGAGTCGCACAGAGCGATATGTGAACTTTTACCCAGTGAATGGTGATTGCGATCTGTT
GATTTCCCTCTACGATTACCTCCGAGTTTGCTCCCCGATCGAGAAGACTGTGTCGAGCAATCGGCCGACAAACCAGACGC
ACCAGTTCCTCCCGGAATCCCTTGCGCGGACCTTCAGCCGCTTCCTGACCCAGCACGTTGACGAGCCAGTCTTCAAGATT
TGGAACGGCCCGAAGTCGCACTTCGGTCGGCACCTGATGGCTACCTTCTTGTCGCGCTCCGAAAAGGGAAAGTATGTTTC
CTCCCTGGGCAACTGGGCAGGTGACCGCGAAATCCAGAGCGCCGTCGCGCGCTCCCACTACTCGCATGGTTCCGTTACCG
TTGACGACCGGGTCTTTGCATTCATATCGGGATTTTACAAAGAAGCCCCGCTGGGTTCCGAGATTTATGTGTTGAAGGAC
CCCTCCAACAAGCCACTCAGCAGGGAGGAGCTGCTTGAGGAGGAGGGCAACTCGCTCGGAAGTCCCCCACTGAGTCCTCC
CTCCTCGCCCCGACTGGTGGCCCAGTCGTTCAGTGCACACCCGTCGCTGCAACTCTTCGAACAGTGGCACGGCATCATCA
GCGACGAGGTCCTGCAATTTATTGCGGAATATAGGCGCAAGCACGAGCTGCGGAGCCAGCGTACGGTGGTTGCAGGATCC
CATGGCTTCCCTCCAGAGGTGGAGGAGCAAGATGATGGCACTCTCCCCATGAGCTGCGCTCAAGAGAGTGGCATGGATCG
TCACCCCGCTGCTTGCGCCTCGGCTCGCATCAACGTGTAATCTAGA

**B2 Recombinase**

CTCGAGAATCAAAATGTCGGAATTTAGTGAGTTGGTACGTATCTTGCCTTTGGATCAGGTTGCGGAGATCAAACGTATTC
TGAGCCGTGGCGATCCCATCCCACTCCAGCGCCTGGCTTCGCTGCTGACCATGGTGATTTTGACGGTGAACATGTCCAAA
AAGCGCAAATCGTCCCCTATAAAACTGTCGACCTTCACCAAGTACCGACGCAACGTGGCTAAGTCGTTGTACTACGATAT
GTCGTCCAAGACAGTGTTCTTTGAGTACCACTTGAAGAACACTCAGGACCTGCAGGAAGGCCTGGAGCAGGCCATCGCTC
CGTACAACTTCGTAGTGAAGGTGCATAAGAAACCCATAGACTGGCAGAAACAACTTAGTAGCGTCCACGAACGCAAGGCA
GGCCACCGTTCGATCCTGTCCAATAATGTGGGCGCCGAGATTTCCAAGCTGGCAGAGACCAAAGATAGCACTTGGTCGTT
CATCGAACGAACCATGGACCTGATCGAGGCTCGCACCCGTCAGCCCACCACCCGGGTTGCATATCGGTTTCTGCTGCAGC
TGACGTTCATGAACTGTTGCCGTGCGAATGACTTGAAGAACGCCGATCCCAGCACGTTCCAGATCATTGCTGATCCCCAC
CTGGGTCGTATCCTCCGCGCCTTTGTGCCCGAGACAAAGACCAGTATCGAGCGGTTTATCTACTTCTTCCCATGTAAAGG
CCGCTGCGATCCACTCCTTGCCTTGGACAGTTACCTGTTGTGGGTGGGCCCGGTGCCCAAGACGCAGACCACGGATGAAG
AGACGCAATACGACTATCAGCTGTTGCAAGATACACTCCTTATCAGCTACGATCGCTTCATAGCCAAAGAAAGTAAGGAA
AATATCTTCAAGATTCCCAACGGACCGAAGGCTCACCTGGGCCGCCACCTCATGGCCTCGTACCTTGGAAATAATTCGCT
TAAGTCGGAGGCTACGCTGTACGGTAACTGGTCGGTGGAACGGCAAGAGGGAGTTTCGAAAATGGCCGACAGCCGATACA
TGCACACCGTTAAGAAAAGCCCTCCTTCCTACCTCTTCGCCTTTTTGTCGGGTTATTACAAGAAGAGTAACCAAGGCGAA
TACGTGCTTGCAGAAACCCTCTACAACCCCTTGGATTACGATAAAACACTGCCAATAACTACCAATGAGAAGTTGATCTG
TCGCCGGTATGGTAAGAACGCGAAGGTGATCCCCAAAGATGCCCTGTTGTATCTGTACACCTATGCTCAGCAGAAGCGTA
AACAGCTTGCCGATCCGAATGAACAGAATCGCCTGTTTTCGAGCGAATCCCCGGCACACCCCTTCCTGACTCCCCAGTCG
ACGGGCAGCTCGACGCCGCTGACCTGGACCGCACCGAAGACGCTTTCCACCGGCCTGATGACACCGGGCGAAGAGGGATC
CCATGGCTTCCCTCCAGAGGTGGAGGAGCAAGATGATGGCACTCTCCCCATGAGCTGCGCTCAAGAGAGTGGCATGGATC
GTCACCCCGCTGCTTGCGCCTCGGCTCGCATCAACGTGTAATCTAGA

**Fig. S1.** The DNA sequences encoding recombinases B3, KD, and B2 are shown. The sequences have been codon optimized for *Drosophila*. The coding sequence of the recombinases are shown in black (the initiating ATG codon is underlined); a 7-bp translation initiation sequence (16), shown in blue, was included immediately upstream of the ORF. A C-terminal PEST sequence comprised of residues 422–461 from the mouse ornithine decarboxylase gene (see main text) was inserted as a C-terminal fusion (shown in red). Restriction enzyme recognition sequences used as linkers are shown in green.

R Recombinase

CTCGAGAATCAAAATGCAGCTTACCAAGGACACTGAGATTTCCACTATAAACCGACAGATGTCCGACTTCTCCGAACTGA
GTCAGATTCTCCCCCTGCACCAGATCAGCAAGATTAAGGACATTCTGGAGAACGAGAACCCACTGCCGAAGGAAAAGCTC
GCCAGCCACCTGACTATGATTATTCTTATGGCTAACCTGGCATCGCAAAAGCGTAAAGATGTGCCGGTTAAGCGCTCGAC
ATTCCTTAAGTATCAGAGGTCCATCAGCAAGACGCTCCAATACGATTCCTCGACAAAAACAGTCTCGTTCGAGTACCACC
TTAAGGACCCGAGCAAATTGATCAAGGGCCTTGAGGACGTTGTGAGTCCGTACCGTTTTGTCGTGGGTGTGCATGAGAAG
CCGGACGATGTGATGAGCCATCTTAGCGCCGTACACATGCGTAAGGAGGCAGGCCGCAAGCGTGATCTCGGCAACAAGAT
CAATGACGAGATAACGAAAATCGCGGAGACCCAGGAGACGATCTGGGGATTTGTCGGAAAGACCATGGATCTCATTGAAG
CGCGGACTACACGGCCAACCACAAAAGCTGCCTACAATCTGCTCCTGCAAGCCACTTTCATGAACTGCTGTCGCGCCGAT
GACCTTAAAAACACAGACATCAAGACATTTGAGGTCATTCCGGACAAGCACTTGGGACGGATGCTCCGAGCGTTTGTCCC
GGAGACGAAGACAGGCACCCGCTTTGTGTACTTCTTTCCTTGCAAAGGACGGTGCGATCCGCTGCTGGCTCTGGACAGTT
ACCTGCAGTGGACTGATCCGATCCCTAAAACACGCACCACGGATGAGGATGCCCGCTATGACTACCAACTGTTGCGTAAC
TCGTTGCTGGGTAGCTATGATGGTTTCATTAGTAAGCAGTCCGACGAATCGATATTCAAGATTCCCAATGGTCCGAAGGC
GCACCTGGGTCGCCACGTGACGGCATCCTATTTGAGCAACAATGAGATGGACAAGGAGGCAACATTGTATGGAAACTGGT
CGGCAGCCCGCGAAGAAGGTGTCAGCAGGGTCGCTAAAGCGCGCTACATGCATACCATTGAGAAGTCGCCTCCAAGCTAC
CTTTTTGCTTTTCTGTCCGGATTTTACAACATTACCGCCGAGCGAGCGTGCGAACTGGTGGACCCAAACTCCAACCCCTG
TGAGCAAGACAAGAATATACCAATGATAAGCGATATAGAGACACTTATGGCACGTTACGGAAAGAATGCAGAAATCATCC
CGATGGATGTGCTGGTCTTCTTGAGCTCGTACGCAAGGTTTAAGAACAACGAGGGTAAGGAATATAAGCTGCAAGCTCGG
TCGAGCCGCGGAGTGCCAGATTTTCCAGATAACGGACGAACAGCGCTCTATAACGCCCTGACTGCGGCCCATGTTAAGCG
CAGGAAAATCTCGATAGTCGTAGGACGTTCCATCGACACCTCGGGATCCCATGGCTTCCCTCCAGAGGTGGAGGAGCAAG
ATGATGGCACTCTCCCCATGAGCTGCGCTCAAGAGAGTGGCATGGATCGTCACCCCGCTGCTTGCGCCTCGGCTCGCATC
AACGTGTAATCTAGA

FLP Recombinase

CTCGAGAATCAAAATGCCGCAGTTTGATATCCTCTGCAAGACCCCACCAAAGGTGTTGGTGCGTCAATTCGTGGAGCGAT
TTGAGAGGCCGTCGGGTGAGAAGATCGCCCTGTGCGCTGCCGAGTTGACCTATTTGTGTTGGATGATCACTCATAATGGC
ACCGCGATTAAGCGCGCTACCTTTATGAGCTATAACACTATCATTAGCAATTCCCTGTCCTTTGACATAGTAAACAAGTC
CCTGCAGTTTAAATACAAGACTCAGAAGGCCACTATATTGGAGGCTTCGCTGAAAAAGTTGATCCCGGCATGGGAGTTCA
CGATCATCCCATACTACGGTCAGAAACACCAGAGCGATATTACCGATATTGTAAGCAGCCTCCAGCTGCAGTTTGAGTCC
AGCGAAGAGGCTGATAAGGGTAATAGTCACAGCAAAAAGATGCTGAAGGCACTGCTGTCCGAGGGCGAAAGCATCTGGGA
GATTACTGAGAAAATCCTGAACTCGTTTGAGTACACCAGCCGATTCACCAAGACGAAGACCCTGTACCAGTTCCTCTTTT
TGGCAACCTTCATCAATTGTGGTCGCTTCAGTGACATCAAAAACGTGGACCCTAAATCGTTCAAGCTGGTGCAGAATAAG
TACCTGGGTGTTATCATCCAGTGCTTGGTTACCGAGACTAAGACCTCCGTGTCCAGGCACATTTATTTCTTCTCCGCAAG
GGGTCGCATCGATCCGCTGGTATACTTGGATGAGTTCCTTCGGAATAGTGAACCAGTCTTGAAGCGCGTGAACAGGACGG
GCAATTCCAGTAGCAACAAGCAAGAGTACCAGCTGCTGAAGGATAATCTTGTTCGGTCGTACAACAAAGCCTTGAAGAAA
AATGCGCCATACTCGATCTTTGCAATCAAGAATGGTCCCAAGAGTCATATTGGCCGGCACCTGATGACCAGTTTCTTGTC
CATGAAGGGTCTGACTGAGCTCACAAACGTCGTTGGCAACTGGTCGGACAAGCGAGCGAGTGCCGTCGCCCGCACGACAT
ACACCCACCAGATCACGGCCATACCAGATCACTATTTCGCGCTGGTGTCGCGTTATTATGCCTATGATCCCATCAGCAAG
GAGATGATCGCGCTGAAGGACGAAACCAATCCAATCGAGGAGTGGCAGCATATCGAGCAACTTAAGGGAAGCGCTGAGGG
TAGCATCCGTTACCCCGCCTGGAACGGCATCATCAGCCAGGAGGTTCTGGATTACCTGAGCTCCTACATCAATCGCCGTA
TTGGATCCCATGGCTTCCCTCCAGAGGTGGAGGAGCAAGATGATGGCACTCTCCCCATGAGCTGCGCTCAAGAGAGTGGC
ATGGATCGTCACCCCGCTGCTTGCGCCTCGGCTCGCATCAACGTGTAATCTAGA

**Fig. S2.** The DNA sequences encoding recombinases R and FLP are shown. The FLP sequence encodes an aspartic acid residue at position 5 (D5). The sequences have been codon optimized for *Drosophila*. Color coding is as in Fig. S1.

A: Blown-OUT stop cassette

AGATCTGGTTGCTTAAGAATAAGTAATTCTTAAGCAACCTAACGTAAGCTAGCTAGACCG
GTGTCGACTAAAGCCAAATAGAAAATTATTCAGTTCCTGGCTTAAGTTTTTAAAAGTGAT
ATTATTTATTTGGTTGTAACCAACCAAAAGAATGTAAATAACTAATACATAATTATGTTA
GTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTACTTGGTTAATAAATAGAAT
ATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGTGAGTTTGCTTAAAAACTCG
TTTAGGTTTGTCCTCCCGAAATTATTTATTTAAATGCGATGGAGAGTTGGCGCCGAATCG
AAAACTTTACGCGCTTAAAAGCACGAGTTGGCATCCCTAACGCGTAGGATCTTTGTGAAG
GAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCT
AAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGT
GTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAAT
GAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGAC
TCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCT
TCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTT
GCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATAT
TTGATGTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTT
ACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAAT
TGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCAC
AAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCAT
CAATGTATCTTATCATGTCTGGATCACTAGTGATCTGGCCGGGGTTGCTTAAGAATAAGT
AATTCTTAAGCAACCCTCGAG

B: KD recombination site used in the Kicked-OUT stop cassette
AAACGATATCAGACATTTGTCTGATAATGCTTCATTATCAGACAAATGTCTGATATCGTTT

C: B2 recombination site used in the Baild-OUT stop cassette
GAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTC

D: RS recombination site used in the Rpd-OUT stop cassette
TTGATGAAAGAATAACGTATTCTTTCATCAA

E: FRT recombination site used in the Flpd-OUT stop cassette
GAAGTTCCTATACTTTCTAGAGAATAGGAACTTC

**Fig. S3.** Stop cassettes. (*A*) The sequence of the Blown-OUT stop cassette is shown. The stop cassettes used for each recombinase differed only by the sequence of the recombinase target site, shown here for B3 in the green boxes, that flank the transcriptional terminators. Note that recombination sites for B3 and the other recombinases are themselves inverted repeats separated by a spacer that determines the directionality of the recombination reaction. The same tandem transcriptional terminators, which are derived for the *Drosophila* Hsp70Bb gene (ref. 3; shown in blue) and the early SV40 transcription unit (refs. 17 and 18; shown in red) were used in all stop cassettes. The sequence of the target sites used for the other recombinases are shown in *B*–*E*; references for these target site sequences can be found in the main text.