Supplemental Material

Efficient targeted mutagenesis in the monarch butterfly using Zinc Finger Nucleases

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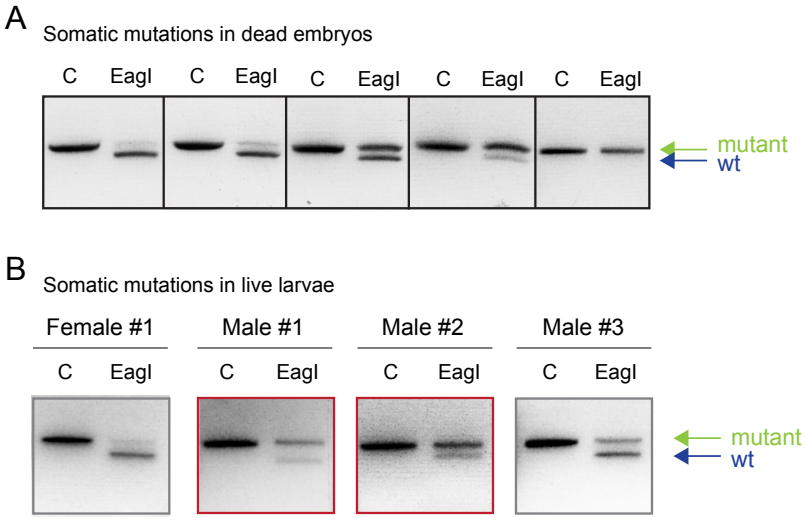
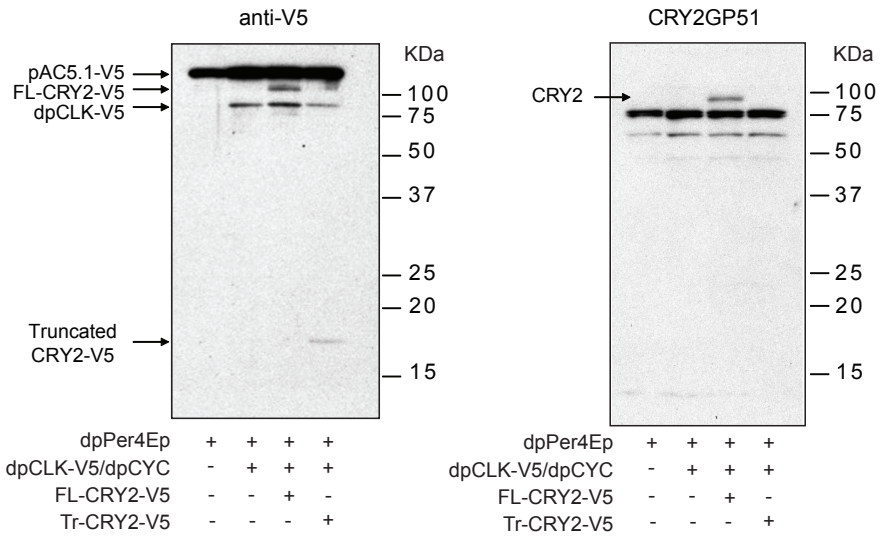
Figure S1: Level of targeted mutagenesis in somatic mosaics injected with 0.1 µg/µl ZFN encoding mRNAs targeting *cry2*.



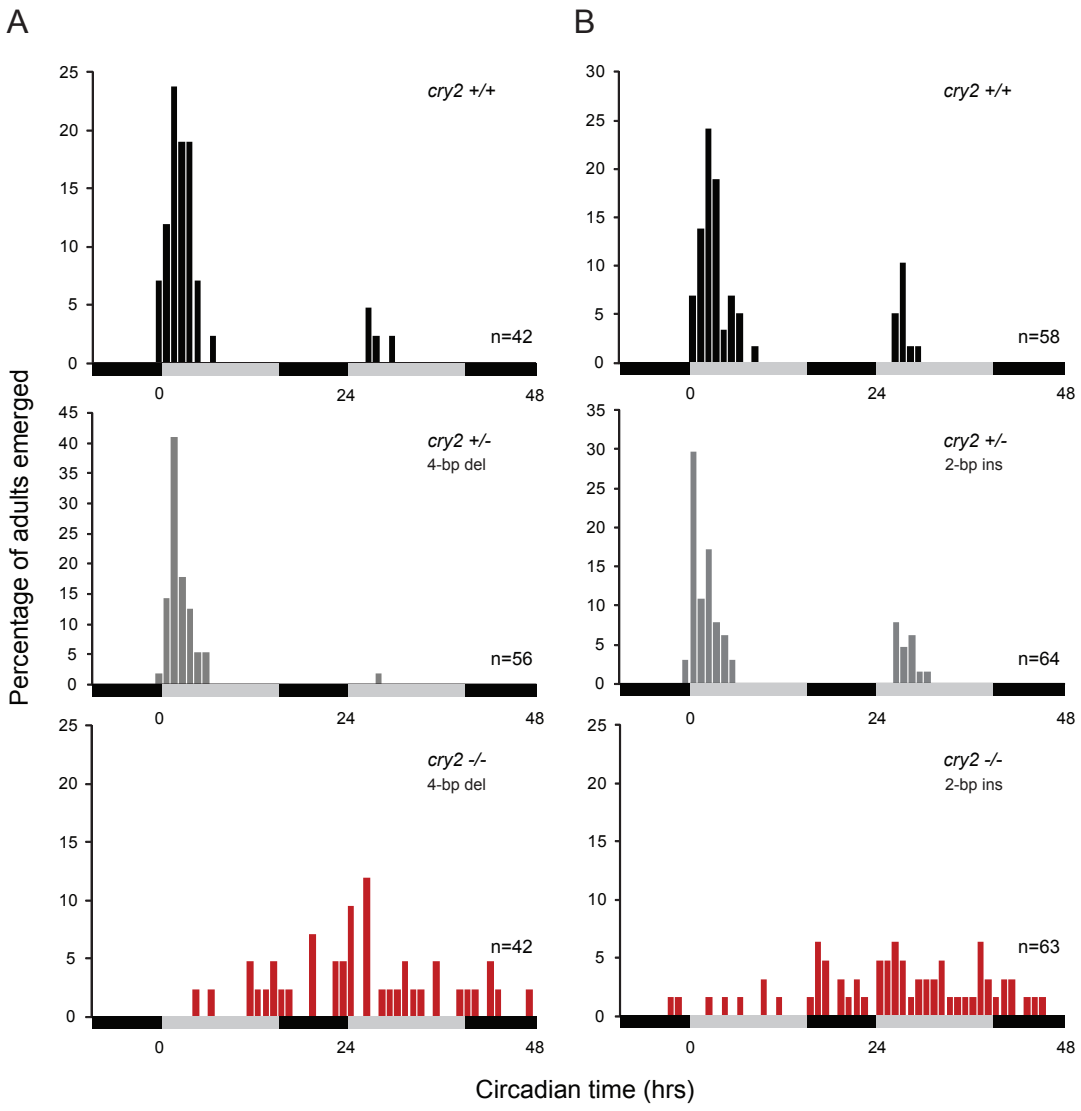
(*A*) Gels representing the levels of targeted mutagenesis observed in somatic cells of dead embryos (from a few percent to full targeting; left to right) after injection of ZFN mRNAs targeting *cry2* at 0.1µg/µl. For each panel, the undigested control (C) and the fragment subjected to the restriction enzyme (EagI) are shown. (*B*) Gel pictures representing the level of targeted mutagenesis in somatic cells of the four larvae injected with ZFN mRNAs targeting *cry2* at 0.1µg/µl and selected in Fig. 4A.

Figure S2. CRY2GP51 recognizes the N-terminus of the CRY2 protein.



Western blot of V5 epitope–tagged CRY2 protein expression probed by a V5 antibody (left panel) and a monarch-specific anti-CRY2 antibody (CRY2GP51) (Zhu et al. 2008) (right panel). The monarch *per* E-box enhancer luciferase reporter (dpPer4Ep; 50ng) was used in the presence (+) or absence (-) of dpCLK/dpCYC expression plasmids (50 ng each) and either the full-length CRY2 (FL-CRY2; 50ng) or truncated CRY2 (Tr-CRY2; 50 ng).

Figure S3. Profiles of adult eclosion during the first two days of constant darkness.



Profiles of wild-types (+/+, black), heterozygous mutants (+/-, gray) and CRY2 knockouts (-/-, red) siblings of the 4-bp deletion (*A*) and the 2-bp insertion mutant line (*B*), entrained throughout their larval and pupal stages in LD. Eclosion is binned at 1-hour intervals. Black horizontal bars, dark; gray horizontal bars, subjective day.

Table S1. Specification of the ZFNs designed to target monarch *cry2*.

	*cry2*_ZFNs
ZFN site	gACCCTGAGCCCTacggcCGGGTCCGGGACc
ZFN gap (bp)	5
5' ZFP site	AGGGCTCAGGGT
3' ZFP site	CGGGTCCGGGAC
5' ZFP F3 triplet	AGG
5' ZFP F2 triplet	GCT
5' ZFP F1 triplet	CAG
5' ZFP F0 triplet	GGT
3' ZFP F3 triplet	CGG
3' ZFP F2 triplet	GTC
3' ZFP F1 triplet	CGG
3' ZFP F0 triplet	GAC
5' ZFP AA seq	GTKPYKCPECGKSFSCAHHLTRHQRTHTGEKPYACPVESCDRRFSRSDNLLEHIRIH TGQKPFQCRICMRNFSVRSTLTRHIRTHTGEKPFACDICGRKFARSDHLTQHTKIHTG GS
3' ZFP AA Seq	GTKPYKCPECGKSFSLKGNLTRHQRTHTGEKPYACPVESCDRRFSRSDHLSDHIRIH TGQKPFQCRICMRNFSDRSALARHIRTHTGEKPFACDICGRKFARSDHLSDHTKIHTG GS
RFLP analysis	*Eag*I

References:

Zhu H, Sauman I, Yuan Q, Casselman A, Emery-Le M, Emery P, Reppert SM. 2008. Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. *PLoS Biol* **6**, e4.