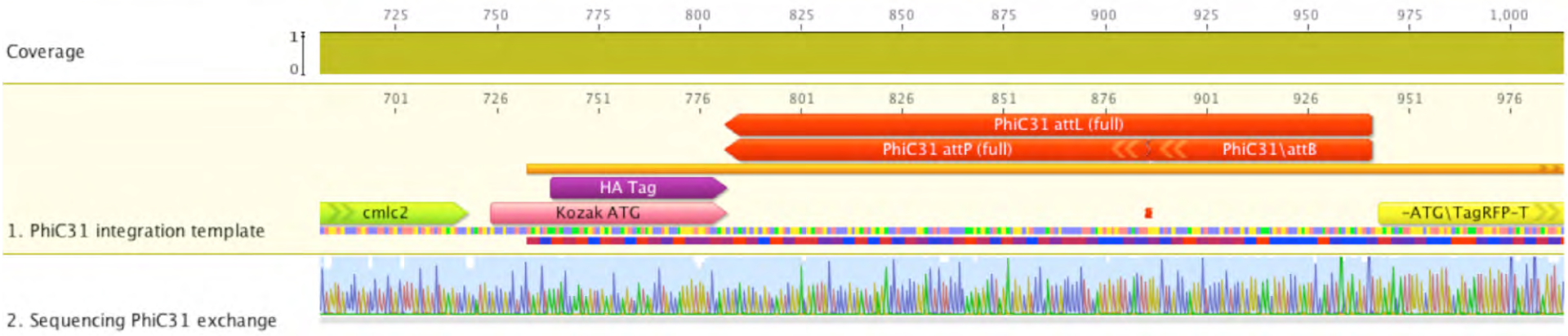

Figure S1 – Sequence verification of PhiC31 mediated vector targeting

The cmlc2 promoter (green arrow) drives the expression of an ORF (orange bar) that includes an HA tag (violet arrow), a full attL site (red arrows along with partial attP and attB sites) and a TagRFP-T fragment. Sequencing of injected docking site line fishes as well as germline transgenics verified PhiC31-mediated vector targeting and the maintenance of the ORF. Sequences were analyzed using Geneious software (Biomatters).

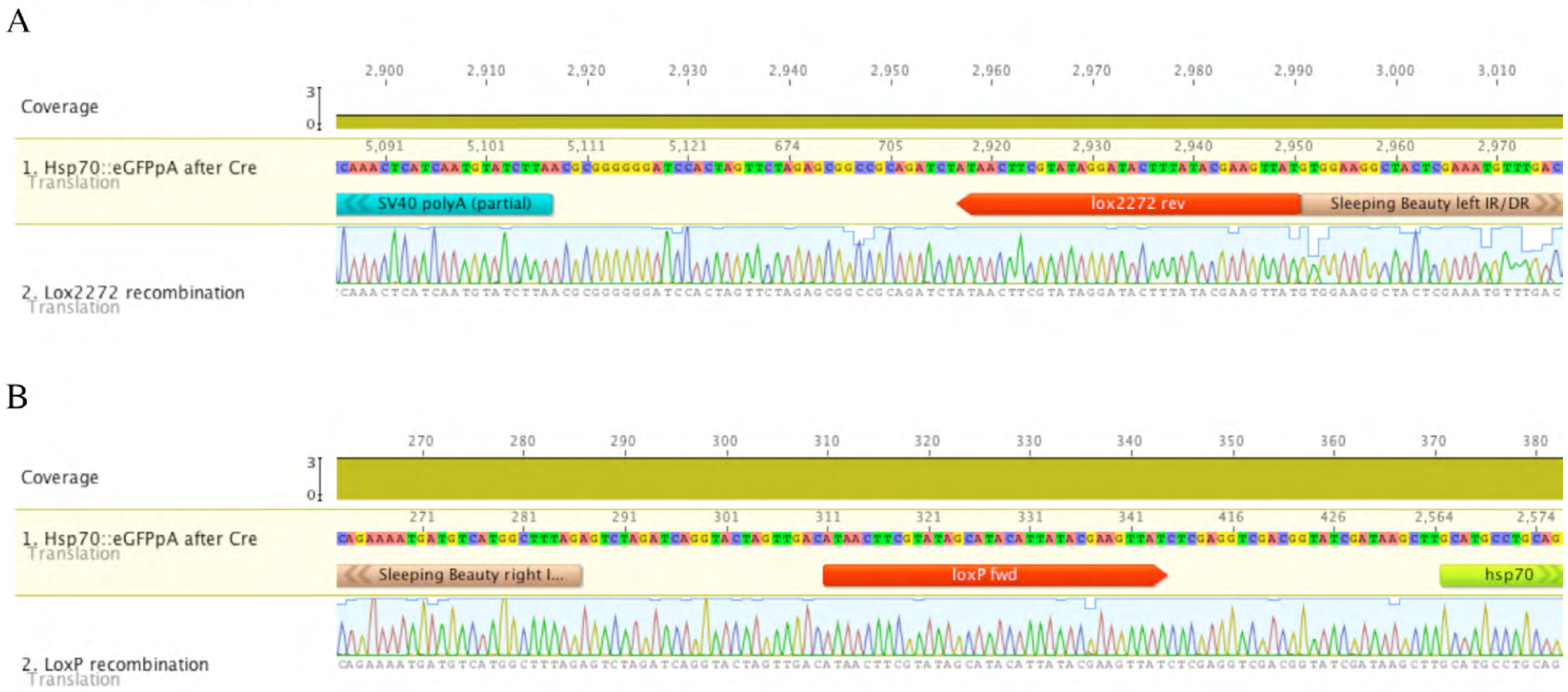

Figure S2 – Sequence verification of Cre-mediated locus cleanup

Genomic DNA of targeted embryos injected with CreNLS was analyzed by a genotyping PCR and subsequent sequencing. Sequencing verifies Cre-mediated locus cleanup over the Lox2272 (A) as well as LoxP (B) sites.

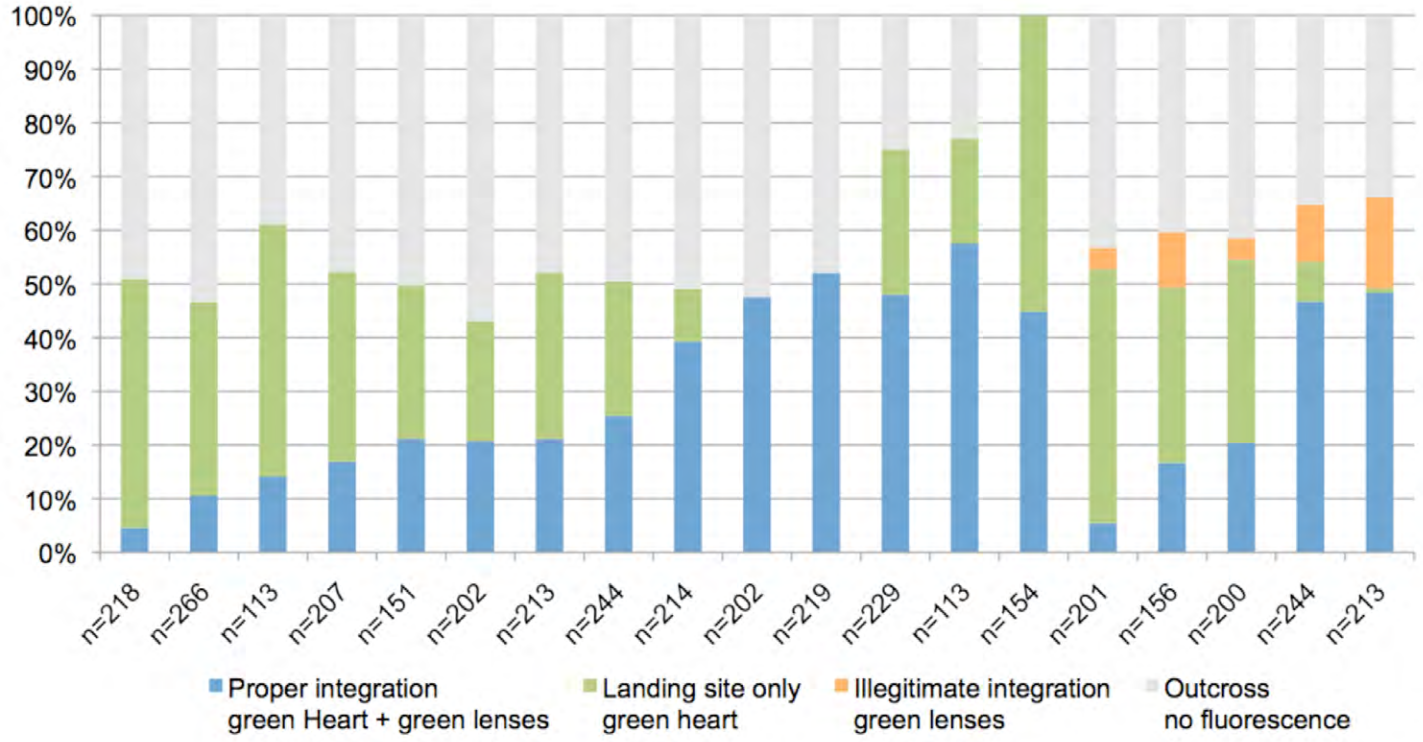

Figure S3 - Germline transmission rates for all positive founders

From a pool of founders carrying unmapped landing sites, 19 out of 26 (65%) of preselected fish transmitted the targeting vector through the germline. The transmission was scored in an outcross against wildtype (Cab), hence the maximum achievable transmission rate of a single genomic landing site is 50%. 3 fish yielded higher germline transmission, indicating the presence of multiple non-linked genomic landing sites. Illegitimate integrations of targeting vector outside of the landing site (orange) was identified by the presence of transgene-specific expression features (EGFP in lens) without co-occurring targeting sensor and/or landing site features (EGFP/TagRFP-T in heart muscle). Illegitimate integrations were restricted to 5 fish and in every case occurred only in a subset of targeted offspring.

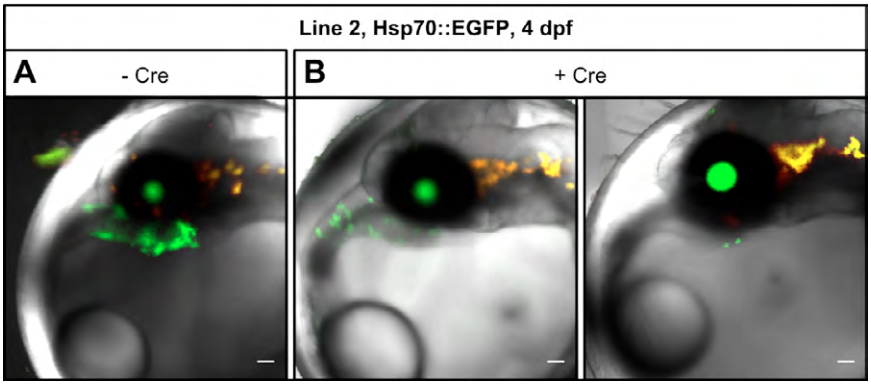

Figure S4 – Cre-mediated locus cleanup in line 2

(A) A lateral view of a fish transgenic for the hsp70::EGFP insert. Strong EGFP expression is detectable in the lens and the heart. As described in the main text, the heart-specific expression originates from an interaction between the cmlc2 promotor of the landing site and the insert-specific hsp70 promotor.
(B) Embryos from (A) were injected with CreNLS mRNA at the one-cell stage. Cre-mediated locus cleanup in the injected generation can be visualized by highly mosaic EGFP reporter gene expression in the heart. These fish submit the "cleaned" locus via the germline at high frequencies (100%, n=2)

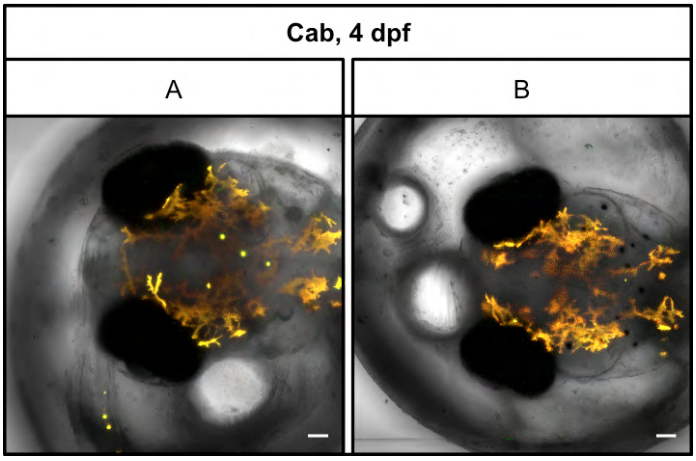

Figure S5 – Wildtype Cab strain imaged with Nikon AZ100

The wildtype Cab strain develops autofluorescent pigment cells. Autofluorescence of these cells can be excited with the 488nm laser and the emission is detected with the green as well as the red channel. Thereby, proper EGFP expression can be distinguished from the pigment cells.

Table S1.

	LM-PCR primer
5'-us-Hsp92II	GGATTTGCTGGTGCAGTACAGGCCTTAAGAGGGACTACATG
5’-ls	PO4-TAGTCCCTCTTAAGGCCT-NH2
3'-us-Hsp92II	GTAATACGACTCACTATAGGGCTCCGCTTAAGGGACTACATG
3’-ls	PO4-TAGTCCCTTAAGCGGAG-NH2
LM_SB-left2	GATGTCCTAACTGACTTGCCAAA
LM_SB-left1	TGAAAAACGAGTTTTAATGACTC
LM_SB-right1	CTCGAAATGTTTGACCCAAGT
LM_SB-right2	AAAGGCAATGCTACCAAATACT
oLM-5rev	GGATTTGCTGGTGCAGTACAG
oLM-5rev-nest	AGTACAGGCCTTAAGAGGGA
oLM-3rev	GTAATACGACTCACTATAGGGC
oLM-3rev-nest	AGGGCTCCGCTTAAGGGAC
	Verification of Cre cleanup
Cretest_SB_F	GGACATCTACTTTGTGCATGACACA
Cretest_Hsp70_R	ACCAAGCGACACCCCTGAAGGA
Cretest_SV40pA_F	GTGGTTTGTCCAAACTCATC
Cretest_SB_R	TCACATTCCCAGTGGGTCAGAAGT
	Genotyping
PhiC31_cmlc2-F2	CCAGTGACCCAGGAGCCCAGACC
PhiC31_tagRFPt-R1	TGCCCTCGTAGGGCTTGCCT
PhiC31_eGFP-R3	AGCTTGCCGGTGGTGCAGATG
Dock_Cmlc2_inner	TCGGGGTTTGCCTGGATTGTGT
Chr16_R	AAGCGTTCAGTTGCTTTAACGGTCA
Chr18_R	ACGCTGTGAGCAGGGAGGAGT
Scaffold_R	TGGACAGATGATGGTGTTGCATGGT

Table S1. Important oligos used in this study

Table S2

Site	Sequence
attP	GCTTCACGTTTTCCCAGGTCAGAAGCGGTTTTCGGGAGTAGT GCCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCG TAGGGTCGCCGACATGACACAAGGGGTTGTGACCGGGGTGG ACACGTACGCGGGTGCTTACGACCGTCAGTCGCGCGAGCG
attB	GTCGACGATGTAGGTCACGGTCTCGAAGCCGCGGTGCGGGT GCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACC TCACCCATCTGGTCCATCATGATGAACGGGTCGAGGTGGCG GTAGTTGATCCCGGCGAACGCGCGGCGCACCGGGAAGCCCT CGCCCTCGAAACCGCTGGGCGCGGTGGTCACGGTGAGCAC GGGACGTGCGACGGCGTCGGCGGGTGCGGATACGCGGGGC AGCGTCAGCGGGTTCTCGACGGTCACGGCGGG
attB mut	GTCGACGATGTAGGTCACGGTCTCGAAGCCGCGGTGCGGGT GCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCACC TC**T**CCCATCTGGTCCATCATGATGAACGGGTCGAGGTGGCG GTAGTTGATCCCGGCGAACGCGCGGCGCACCGGGAAGCCCT CGCCCTCGAAACCGCTGGGCGCGGTGGTCACGGTGAGCAC GGGACGTGCGACGGCGTCGGCGGGTGCGGATACGCGGGGC AGCGTCAGCGGGTTCTCGACGGTCACGGCGGG

Table S2 – Sequences of used PhiC31 att sites
The attB mut site is used when complementing the EGFP reporter gene with regulatory DNA. The mutation is underlined (A->T) and deletes an in-frame stop codon after proper recombination. The mutation is outside of the core attB sequence and we observed the same efficiencies with attB mut as with wildtype attB.