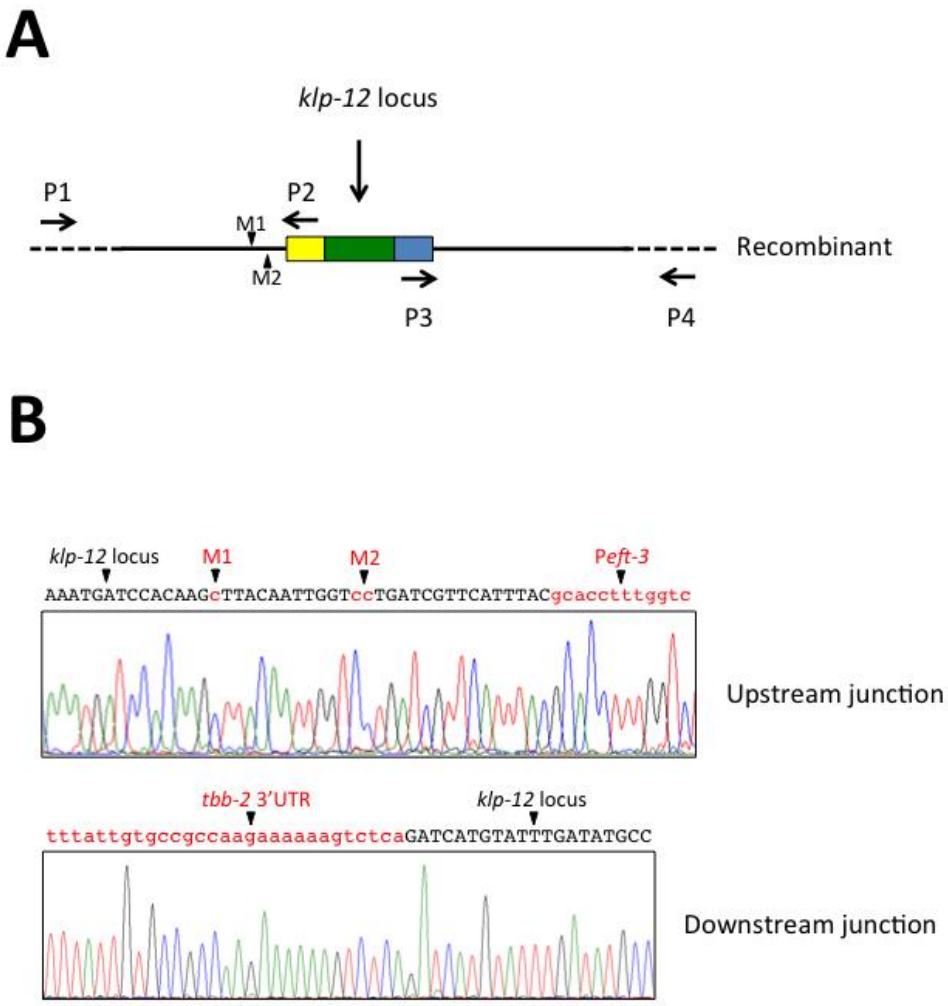

Figure S1 Confirmation of seamless recombination at the *klp-12* locus. Upper panel shows a sequence trace of the upstream recombinant junction. Endogenous upstream *klp-12* sequence is flanked by the M1 and M2 mutations described in Figure 2A, and the beginning of the *eft-3* promoter sequence. Bottom panel shows a sequence trace of the downstream recombinant junction, marking the end of the *tbb-2* 3'UTR followed again by endogenous *klp-12* sequence. All introduced sequences are labeled in red lower case letters.

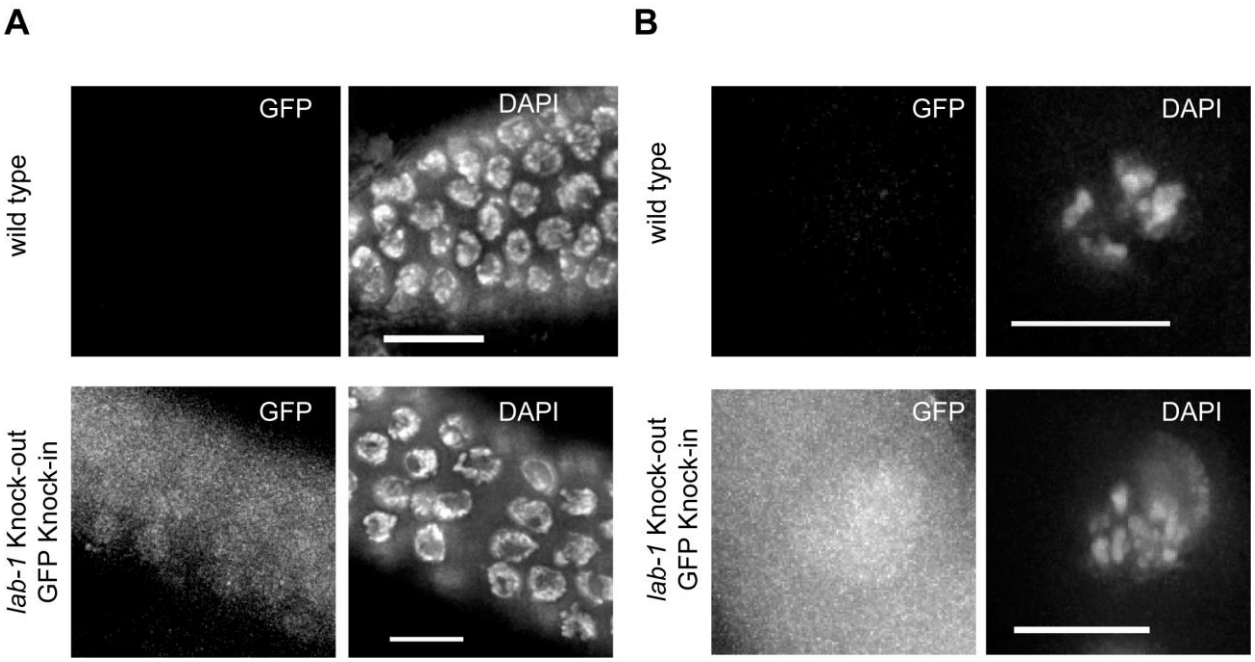

Figure S2 Immunostaining of germline nuclei with an anti-GFP antibody to examine the expression of the *Pbaf-1*::GFP transgene inserted into the *lab-1* locus. Shown are meiotic nuclei in early prophase I (transition zone and early pachytene) (A), and diakinesis (B), from whole mounted gonads of wild type and *lab-1* knockout GFP knock-in worms, co-stained with DAPI and anti-GFP. Bars, 10mm.

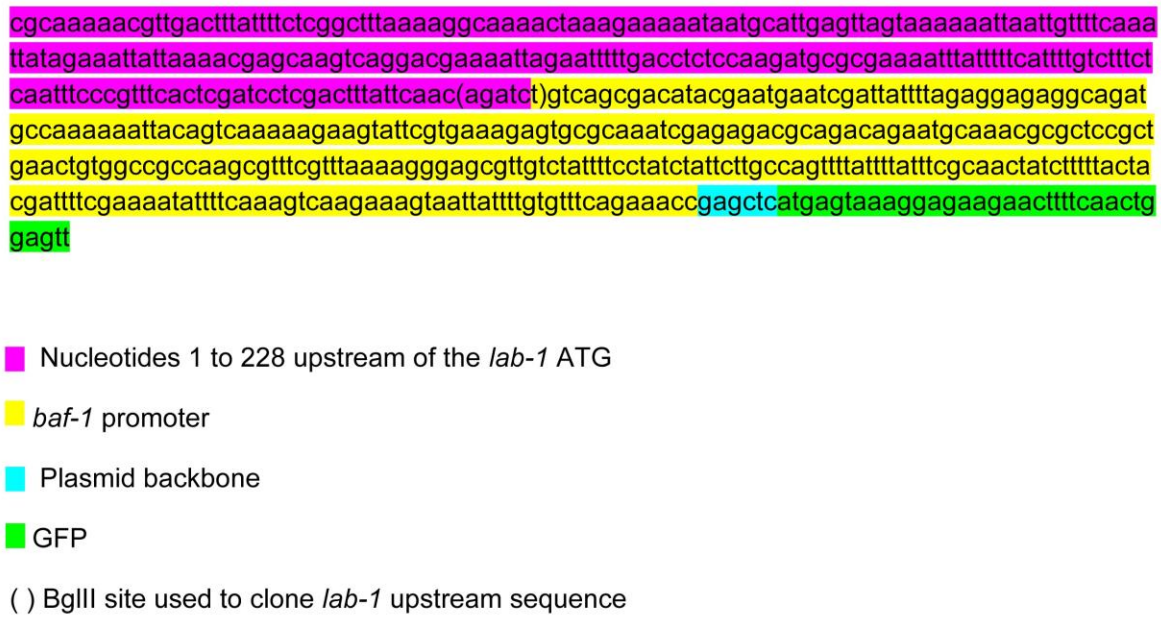

Figure S3 Confirmation of seamless recombination at the *lab-1* locus. Sequence resulting from the PCR product generated by using primers 1100 base pairs upstream and downstream of *lab-1.* Sequences from the *lab-1* upstream region (violet), *baf-1* promoter (yellow), plasmid backbone (light blue), and *gfp* (green) are marked. The site by which the upstream fragment was cloned is marked with brackets.

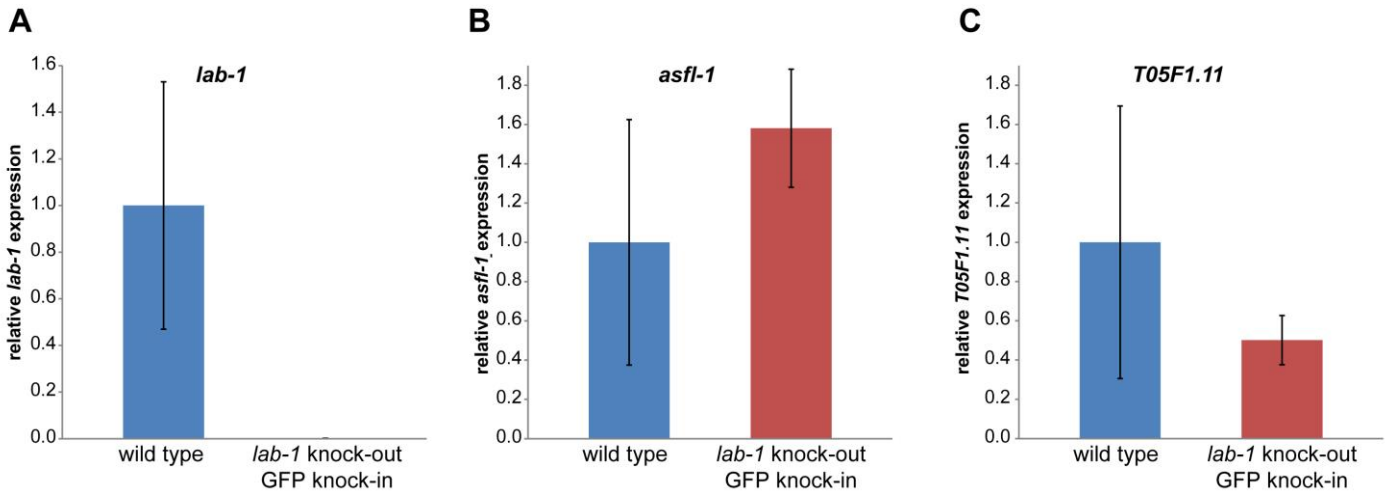

Figure S4 *lab-1* knock-out and *GFP* knock-in effect on the expression of flanking genes. RT-qPCR expression analysis of *lab-1* (A), *asfl-1* (B), and *T05F1.11* (C). Relative ± SEM values are presented. Sample values were normalized to *gpd-1* (GAPDH).

File S1

Supplementary Materials and Methods

Strains and maintenance. The N2 Bristol strain was used as the wild type background for all experiments. Worms were cultured at 20 and 25 °C under standard conditions as described in (Brenner 1974). The following strains were generated in this study: CV370 *lab-1(rj10[baf-1p::gfp])I,* JAC338 *klp-12(csb19[eft-3p::gfp::tbb-2 3'UTR]) IV.*

Plasmids. The pUC57 *klp-12* sgRNA and *Peft-3::*cas9 plasmids were described in (Friedland *et al.* 2013). The *lab-1* sgRNA plasmid was constructed by replacing the *unc-119* sgRNA sequence with a sequence corresponding to 310-330 bp downstream of the *lab-1* ATG genomic sequence as described (Friedland *et al.* 2013). To create the *klp-12 Peft-3*::GFP donor template repair vector, upstream and downstream 1.5 kb *klp-12* homology arms were amplified from N2 genomic DNA using the primers klp-12 F and klp-12 PAM mut HIII R and primers klp-12 ds F and klp-12 R, respectively (see supplementary Table 1 for a complete list of primers). We amplified the *eft-3* promoter using our *Peft-3*::Cas9-SV40 NLS vector (Friedland *et al.* 2013) as a template with primers klp-12 PAM *P*eft-3 F and *P*eft-3 GFP R. The *tbb-2* 3'UTR was also amplified from our *Peft-3*::Cas9-SV40 NLS vector using the primers *tbb-2* UTR F and *tbb-2* UTR klp-12 R. The GFP transgene was amplified from the pBALU1 vector (a kind gift from Oliver Hobert) in two fragments using the primers GFP F and intron R and intron GFP F and GFP *tbb-2* UTR R. The PCR products corresponding to the *eft-3* promoter, both GFP amplicons, and the *tbb-2* 3'UTR, were sequentially stitched together by overlapping PCR to create one seamless product. Finally, the upstream and downstream *klp-12* homology arms, and the GFP transgene fragment, were all cloned into the pUC57 vector using Gibson Assembly (Gibson *et al.* 2009).

The pAD010 vector was a kind gift of Yosef Gruenbaum and was described in (Bank *et al.* 2011). In brief, the pEGFP1 vector (Clontech Laboratories) was used to clone the *baf-1* promoter (286 bp upstream to the ATG) between the BglII and SacI sites, the *unc-119* rescue fragment between the NotI and HpaI sites, and GFP between the SacI and SacII sites. The *lab-1* donor vector was constructed by cloning 1020 bp upstream and 1029 bp downstream of the *lab-1* coding sequence into the pAD010 vector by using the BglII and SacII sites, respectively.

RT-qPCR. RNA was extracted from 20 worms in triplicate using TRIzol (Invitrogen). RT-PCR was performed using the SuperScript III First Strand Synthesis System (Invitrogen). Quantitative PCR was done using the SsoFast EvaGreen Supermix (Biorad). Values were normalized to *gpd-1* expression.

DNA microinjection. Plasmid DNA was microinjected into the germline as described in (Kadandale *et al.* 2009). Injection solutions in experiment A (Table 1) were prepared to contain a final concentration of 150 ng/µl with pCFJ104 (P*myo-3*::mCherry, obtained through Addgene through the kind gift of E. Jorgensen) as a co-injection marker at 5 ng/µl, the donor vector at 50 ng/µl, the sgRNA vector at 45 ng/µl, and the *Peft-3*Cas9-SV40 NLS*tbb-2* 3'UTR at 50 ng/µl. In experiment B and C (Table 1) a total of 500 ng/µl solution was used with pCFJ104 and pCFJ90 (P*myo-2*::mCherry, also obtained from Addgene through E. Jorgensen) as co-injection markers at 25 ng/µl and 2.5 ng/µl, respectively, the donor vector at 92.5 ng/µl, the sgRNA vector at 180 ng/µl, and the *Peft-3*Cas9-SV40 NLS*tbb-2* 3'UTR at 200 ng/µl.

Screening for recombinant worms. F_1 animals were screened for fluorescence using a Zeiss Axiozoom microscope, and candidates were isolated, allowed to lay eggs, and then lysed in 5 µl of single worm lysis buffer (10 mM Tris pH 8.0, 50 mM KCl, 2.5 mM MgCl2, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin and 100 µg/ml proteinase K), placed in -80 °C for 10 minutes, followed by a 1 h incubation at 60 °C, and then 15 minutes at 95 °C to inactivate the proteinase K. The recombinant fragment was amplified by using one primer within the insertion and one on the genomic sequence flanking the donor vector sequence.

Phusion high fidelity polymerase (Thermo Scientific) was used as recommended by the manufacturer, using all 5 µl of worm lysate as a template (see Supplementary Table 1 for a list of all primers used for PCR amplification and genotyping).

To monitor inheritance of the homologous recombination engineered mutation, we repeated the PCR reaction for the F2 progeny of the F1s that showed the recombinant-specific PCR fragment, and then sequenced a PCR amplicon that was generated by primers annealing to sites outside the donor vector.

Immunostaining, Imaging and Microscopy. Whole mount preparation of dissected gonads, DAPI staining and LAB-1 immunostaining, were carried out as in (Colaiacovo *et al.* 2003; Saito *et al.* 2009; Tzur *et al.* 2012). Primary antibodies were used at the following dilutions: rabbit α-LAB-1, 1:500; and chicken α-GFP, 1:500 (Abcam). Secondary antibodies used were Cy3 anti-rabbit and FITC anti-chicken (both from Jackson Immunoresearch) each at 1:1000. Immunofluorescence and GFP images were collected at 0.2 µm and 1 µm increments, respectively, with an IX-70 microscope (Olympus) and a cooled CCD camera (model CH350; Roper Scientific) controlled by the DeltaVision system (Applied Precision). Images were subjected to deconvolution analysis using the SoftWorx 3.0 program (Applied Precision) as in (Nabeshima *et al.* 2005).

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Table S1 List of oligonucleotide primers used in this study

klp-12 **GFP transgene donor vector cloning and recombinant screening primers**	
klp-12 F	CGACGTTGTAAAACGACGGCCAGTGAATTCGATTCACACAACTTGCGAAAATTCTTG
klp-12 PAM mut HIII R	GTAAATGAACGATCAGGACCAATTGTAAGCTTGTGGATCA
klp-12 PAM Peft-3 F	CAATTGGTCCTGATCGTTCATTTACGCACCTTTGGTCTTTTATTGTCAACT
Peft-3 GFP R (P2)	GTGAAAAGTTCTTCTCCTTTACTCATTAAGCCTGCTTTTTTGTACAAACTTGTGAG
GFP F	ATGAGTAAAGGAGAAGAACTTTTCAC
intron R	GTACCGAACTGTTTAAACTTACGTG
intron GFP F	CACGTAAGTTTAAACAGTTCGGTACTAACTAACCATACATATTTAAATTTTCAGGTGCTG
GFP tbb-2 UTR R	GGGAATGCTTGAAAGGATTTTGCATTTATCCTATTTGTATAGTTCATCCATGCCATG
tbb-2 UTR F (P3)	GATAAATGCAAAATCCTTTCAAGCATTCC
tbb-2 UTR klp-12 R	GAGTAGGCATATCAAATACATGATCTGAGACTTTTTTCTTGGCGGCACA
klp-12 ds F	GATCATGTATTTGATATGCCTACTC
klp-12 R	AACAGCTATGACCATGATTACGCCAAGCTTGAAGACGTGTCAATTTCGAATCAC
klp-12 us outside HR F (P1)	GAGCGAAAAGTGTCGGTTATTTACG
klp-12 ds outside HR R (P4)	CATCAGTGTTCGGCTGAAATGTGATAG
Cloning ***lab-1*** **upstream region**	
lab-1-974UPSF-bglII	TTCTGAAGATCTGGAATGGACTGTCATTAGAC
lab1-2-UPSR-bglII	TGGCTCAGATCTGTTGAATAAAGTCGAGGATC
Cloning ***lab-1*** **downstream region**	
lab-1-24F-sacII	TAATCGCCGCGGTCAAACTCAAAAACGCTGTG
lab-1-840DWSR-SacII	CGCCGACCGCGGCAAGCTACTTGGTGACAATG
Creating ***lab-1*** **sgRNA**	
lab-1 mgRNA-F-Cor	GATCTGGGTGCCCGATGAGTGTTTTAGAGCTAGAGCTAGAAATAGC
lab-1 mgRNA-R	ACTCATCGGGCACCCAGATCAAACATTTAGATTTGCAATTCA
Detecting ***lab-1*** **knock-out and** ***gfp*** **knock-in**	
CC01F	CTGCAGCGCAAAATAATTCA

bafP-R	TTTGGCATCTGCCTCTCCTC
Amplifying and sequencing *lab-1* locus outside the donor vector homology region	
lab1+1100-R	GCATTGGTTAATCACTGGAA
CC01F	CTGCAGCGCAAAATAATTCA
gfp-N-R	GTGCCCATTAACATCACCAT

RT-qPCR

gpd-1F	ACTCGTCCATTTTCGATGCT
gpd-1R	TCGACAACACGGTTCGAGTA
lab-Ex34-RT-F	CCAACCTCAGGAATCTGTGTCTT
lab-405-RT-R	CCTCGGATGTATCGGAATCC
asfl-ex34-RT-R	TCATCATCGTCCTCTTCCTCC
asfl-532-RT-F	CCATCATCATGCAATGGCAT
T05F-Ex34-R	TCCCAAGTTGCAATTTCAATAATC
T05F-454-F	GCTCATGATGAAATTCGCTACAA