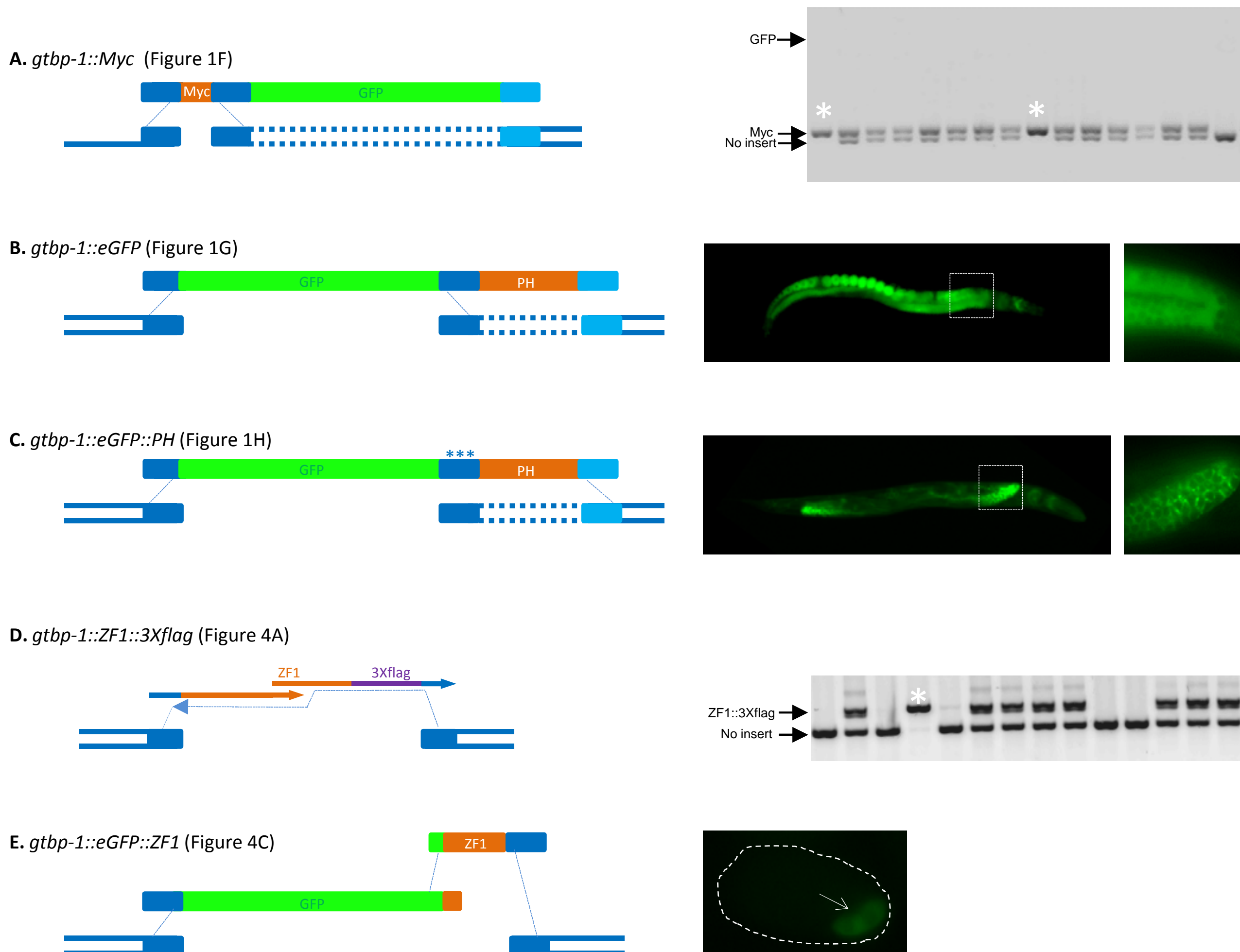


Supplementary Figure S1: Schematics of experiments from Figure 1 and Figure 4 with edit examples

Schematics on the left follow color schemes described in the main figures. Templates are shown on top and *gtbp*-1 locus is shown below. Lines between templates and *gtbp-1* locus represent strand invasion/template switching events inferred from the type of edits obtained in each experiment. Images on the right show method to identify edit (PCR amplification or GFP expression).

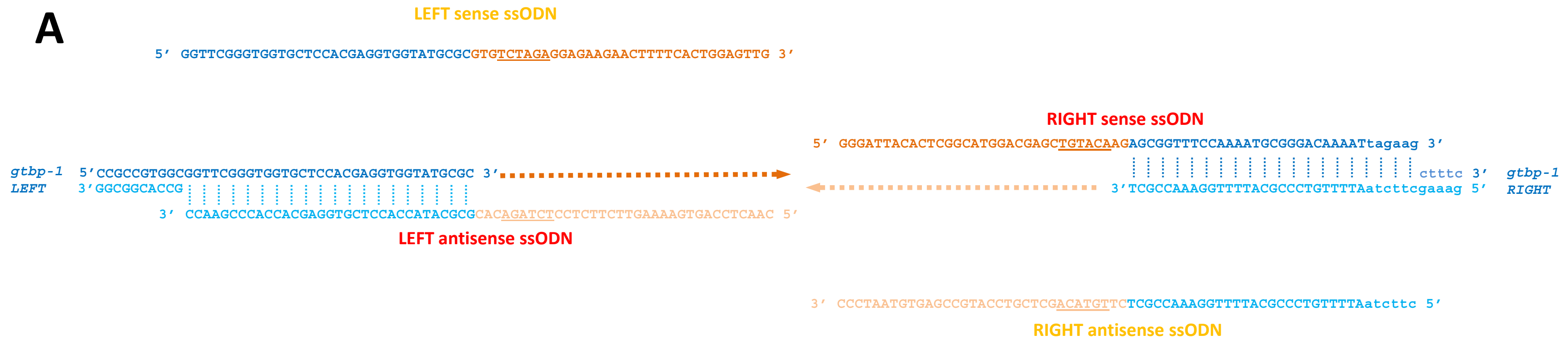
A. We identified edits based on GFP expression (not shown) and by PCR: the gel shows PCR amplification of the *gtbp-1* locus for a subset of F2 cohorts (Progenies of cloned single F1s). The lower band corresponds to the wild-type unedited allele and the upper band to the edited Myc insertion allele. Note that most edits are heterozygous, except for two, indicated by white asterisks. Heterozygous edits are most common since the injections were done in adult germlines that produce only oocytes, and the sperm were not exposed to the injection mix. Homozygous edits are obtained only in cases where the Cas9 complex (and possibly the repair template) perdures in eggs until after fertilization at which time paternal (sperm) DNA can be edited.

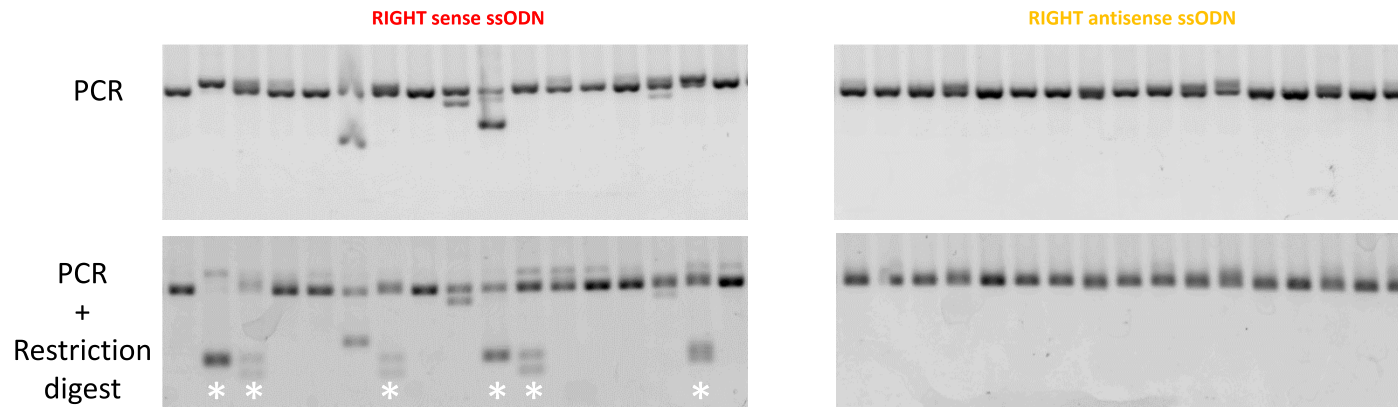
B. All edited hermaphrodites showed the characteristic cytoplasmic pattern of GTBP-1::GFP edits.

C. All edited hermaphrodites showed GFP localized to membranes consistent with the creation of a GTBP-1::GFP::PH domain fusion.

D. Edits were screened by PCR for the presence of the ZF1::3XFLAG insert. The gel shows PCR amplification of the *gtbp-1* locus for a subset of F2 cohorts. White asterisk denotes homozygous edit.

E. All edits showed the characteristic ZF1 degradation pattern, where protein is degraded in somatic, but not germline (arrow), blastomeres in embryos. Dotted line marks the embryo boundary.

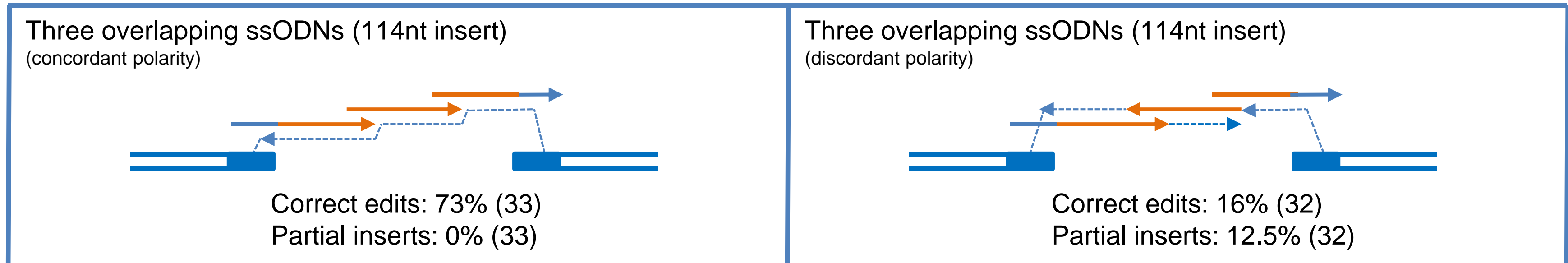




Supplementary Figure S2: Polarity requirements for donor ssODNs

A. Sequence of *gtbp-1* locus at DSB is indicated in blue (resected for clarity). ssODNs have homology arms (blue) corresponding to sequences on left or right side of the break, and unique sequence (brown) containing a restriction site (underlined). Only the antisense ssODN can anneal (parallel dotted lines) on the left side of the resected DSB, and only the sense ssODN can anneal on the right side of the resected DSB (labeled in red). Stippled arrows show DNA synthesis templated by the annealed ssODN.
B. Example of gels showing PCR amplification of the *gtbp-1* locus with (bottom panel) and without (top panel) restriction enzyme digestion after editing with the ssODNs with a right homology arm. Each well was loaded with DNA amplified from the progeny of one F1 *dpy-10*-edited worm. Only the sense ssODN yielded edits with the restriction site (white asterisks).

A



B

C

```
Right ssODN
GACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag

Middle ssODN
CGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAG

Left ssODN
GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATAC

Full-length insert
GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag

Partial insert
GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC--------------------GCGACTTTGTGATGCGTTCCGCCGTGAAGGACA--------------------------ATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
GGTTCGGGTGGTGCTCCACGAGGTGGT-------------------------------CACGAGGTGGAGGTTGTCCGTG-------------------------------TATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
GGTTCGGGTGGTGCTCCACGAGGTGGTATG--------------------------------AGGTGGT----------------------------------ATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
GGTTCGGGTGGTGCTCCACGAGG------------------------------CTTCCCATTGCACATCC---------------------------------ATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
```

Supplementary Figure S3: Template switching with ssODNs

A. Experimental set up as shown in Figure 2F and 2G.
B. Gels showing PCR amplification of representative edits for each experiment shown in A. + signs indicate full length inserts, - signs indicate partial inserts
C. Sequence of one full-length and 4 partial edits from experiment with discordant polarity ssODNs. All partial edits contain sequence from the right most ssODN. Sequence in black are non-homologous to locus or ssODN. The middle ssODN sequence is shown in the sense polarity for clarity.

A

5' sense ssODN

5' GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC--GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTG 3'

3' sense ssODN

5' GGGATTACACTCGGCATGGACGAGCTGTACAAG--AGCGGTTTCCAAAATGCGGGACAAAATtagaag 3'

gtbp-1

5'CCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCA---GFP---CTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAG AGCGGTTTCCAAAATGCGGGACAAAATtagaagctttc 3'

3'GGCGGCACCGCCAAGCCCACCACGAGGTGCTCCACCATACGCG CACTCATTTCCTCTTCTTGAAAAGTGACCTCAACAGGGT---GFP---GACGACCCTAATGTGAGCCGTACCTGCTCGACATGTTC TCGCCAAAGGTTTTACGCCCTGTTTTAatcttcgaaag 5'

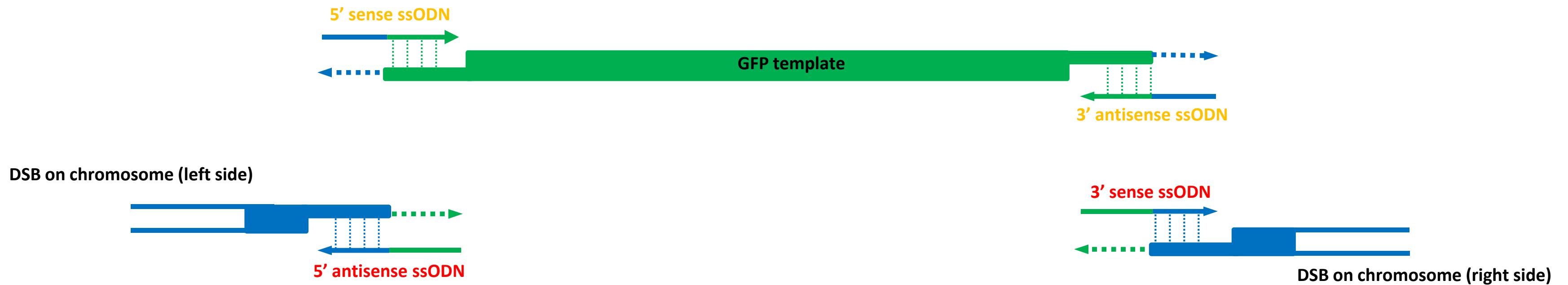
3' CCAAGCCCACCACGAGGTGCTCCACCATACGCG--CACTCATTTCCTCTTCTTGAAAAGTGACCTCAAC 5'

5' antisense ssODN

3' CCCTAATGTGAGCCGTACCTGCTCGACATGTTC--TCGCCAAAGGTTTTACGCCCTGTTTTAatcttc 5'

3' antisense ssODN

B



Supplementary Figure S4: Sequences of bridging ssODNs used to insert GFP at *gtbp-1* locus

A. Sequence from *gtbp-1* locus is blue and sequence from the GFP PCR repair template is green. crRNA sequence is underlined and PAM is double underlined in *gtbp*-1 locus. Bridging ssODNs that performed best in the experiments shown in Figure 3 are labeled in yellow.
B. Schematics illustrate how the ssODNs can pair with the resected GFP to add sequences homologous to the DSB (blue), or can pair with the resected DSB to add sequences homologous to GFP (bottom).

Supplementary Table S2: Sequences of crRNAs, repair templates and bridge ssODNs

locus	crRNA sequences (+PAM)	Note
[illegible] (5' crRNA, 3' crRNA)	GGCCTTAACCCAGAATAAGA(TGG)	5' crRNA (AP691): used in Figure 4-panel I together with 3' crRNA (AP728)
	CACGAGGTGGTATGCGCAG(CGG)	3' crRNA (AP728)
[illegible]	TCCCTCAAGATGAAGAAGGC(TGG)	3' crRNA (AP723): used in Figure 4-panel G
meg-3 deletion with hotspot	GGCAGGATGTCAGCCAGAGG(AGG)	Hotspot crRNA (AP872) in *meg-3* deletion: used in Figure 4-panel J
pgl-1	GGGGGTCGTGGTGGACGCGG(AGG)	3' crRNA (AP814): used in Figure 4-Table S1
lin-15B	ACCACCCAGCAACGAATAAG(TGG)	3' crRNA (AP701): used in Figure 4-Table S1
fbf-2	GGTAGTCACGGCGATGATTA(AGG)	3' crRNA (AP689): used in Figure 4-Table S1

Experimental variant	Strategy	PCR repair templates	Bridge ssODNs (sense sequence, 5' to 3')
Figure 1-Panel A		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair template contains homology arms)
Figure 1-Panel B		GCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagctttccgttctcctttttccttcttg	na (PCR repair template contains homology arms)
Figure 1-Table S1		GGCGGCGGATTCCGAGGAGGACGTGGAGGTTTCCAACCACGCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGC TCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagctttccgttctcctttttccttcttgtaagaattgcacatccattagattctgtacctatccttctca	na (PCR repair template contains homology arms)
Figure 1-Table S1		GCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagagtgataaatgcaaaatcctttcaagcattccc	na (PCR repair template contains homology arms)
Figure 1-Panel C		CATGATATCGATTACAAGGATGACGATGACAAGGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagagtgataaatgcaaaatcctttcaagcattccc	na (PCR repair template contains homology arms)
Figure 1-Panel D		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---tagaagctttccgttctcctttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel E		GTTTCCAACCACGCGGTGGACAATTCTTCCGCCGT---eGFP---tagaagctttccgttctcctttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel F		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---Myc---AGCGGTTTCCAAAATGCGGGACAAAAT---eGFP--- tagaagctttccgttctcctttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel G		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---AGCGGTTTCCAAAATGCGGGACAAAAT---PH--- tagaagctttccgttctcctttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel H		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---AGCGG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*C---PH- --tagaagctttccgttctcctttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 2-Panel A		na	GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCGTG*TCTAGA* GGAGAAGAACTTTTCACTGGAGTTG
Figure 2-Panel C		na	GGGATTACACTCGGCATGGACGAGC*TGTACA* AGAGCGGTTTCCAAAATGCGGGACAAAATtagaag
Figure 2-Panel E		na	GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACAT ATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
Figure 2-Panel F		na	GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATAC and CGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAG and GACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAAGCGGTTTCCAAAATGCGGGACAAAATtagaag
Figure 3-Panel A		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---first half of eGFP and second half of eGFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair template contains homology arms)
Figure 3-Panel B		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---first half of eGFP and second half of eGFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	GACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCG
Figure 3-Panel C		---eGFP---AGCGGTTTCCAAAATGCGGGACAAAATtagaag	GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTG
Figure 3-Panel D		---eGFP---AGCGGTTTCCAAAATGCGGGACAAAATtagaag	GGCGGCGGATTCCGAGGAGGACGTGGAGGTTTCCAACCACGCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCGTGAGTAAAGG AGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTG
Figure 3-Panel E		---eGFP---AGCGGTTTCCAAAATGCGGGACAAAATtagaag	CGAGGTGGTATGCGCGTGAGTAAAGGAGAAG
Figure 3-Panel G		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---	GGGATTACACTCGGCATGGACGAGCTGTACAAGAGCGGTTTCCAAAATGCGGGACAAAATtagaag
Figure 3-Table S1		C---eGFP---GA (in frame if PCR repair template inserted at Cas9 cut)	na
Figure 4-Panel A		na	GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACAT ATGCTCACGGACAAGATGAGCTGAGAGTTCand CATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAGGCGGTT TCCAAAATGCGGGACAAAATtagaag
Figure 4-Panel B		na	CCAGTGTACCAACTCAAGTCCCTCAAGA*CGA*GGAAGG*ATGGAGAACCTCTACTTCCAAGGATACGTAGCCGGCGAACAAAAACTGATATCAGAAGAGGATCTGTGCCC GGGGGATCGGTGGAGCTCCACCGGTGGCGGCCGCTCTAGAACTAGTGACand CTCCACCGGTGGCGGCCGCTCTAGAACTAGTGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAGgaaaaccgaccaattga tagtgtttcgcatttattaatgc
Figure 4-Panel C		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP::22nt of ZF1 and 19nt of eGFP::ZF1--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Panel D		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP::ollas and ollas::PH--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Table S1		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker::mNeon--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair template contains homology arms)
Figure 4-Panel E		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker and Linker::mNeon--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Table S1		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker::mNeon::3Xflag::tagRFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair template contains homology arms)
Figure 4-Table S1		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker::first half of mNeon and second half of mNeon::3Xflag::tagRFP---AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Panel F		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker and Linker::mNeon::3Xflag and 3Xflag::tagRFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Panel H		---eGFP---	GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCG*AAG*TGG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*CGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtagaagctttccgttctcctttttccttcttgtaag
Figure 4-Table S1 (as Panel H but with mutated PAM)		---eGFP---	GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCG*AAGC*TG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*CGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtagaagctttccgttctcctttttccttcttgtaag
Figure 4-Panel I		---eGFP---	CCAGTGTACCAACTCAAGTCCCTCAAGA*CGA*GGAAGG*ATGGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtagaaaaccgaccaattgatagtgtttcgcatttattaatgc
Figure 4-Table S1 (*pgl-1*)		---eGFP---	GTGGACGTGGTGGTTACGGGGGTCGTGG*AGGACG*TGG*TGG*CTT*GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtaaactccaactattgaatgtttaatttgtttttaag
Figure 4-Table S1 (*lin-15B*)		---eGFP---	TAAGCTGCCAACCCCCACACCACCCAGCAACGAAGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtaagtggaaatattttcatccaccgttcctgattg
Figure 4-Table S1 (*fbf-2*)		---eGFP---	CAAATTTTCTTCTTCCGGTAGTCACGGCGATGATGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGand GGGATTACACTCGGCATGGACGAGCTGTACAAGtaaggtggaactttctcaccataaatctcatcc
Figure 4-Panel J		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC--mNeon	CGGACGTAATGGGAATGGACGAACTCTACAAGGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAGGCGGTTTCCAAA ATGCGGGACAAAATtagaag
Figure 4-Panel K		---*meg-3* Nt domain---	aaATGCAGATAACCTCGGCCTTAACCCAGAATAAGATGAGTTCCTCAAAACCTTACCCAAGCGGTCTGCCand CAAATGACGATGAATTAACTGACGATGTGTTCATAGAGGGAAGTTTGTCCAGAGCAGAGGATCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAAGTgaagcgg tttccaaaatgcgggacaaaattagaagc
Figure 4-Panel L		TCAAAACCTTACCCAAGGGCAGGATGTCAGCCAG---first half of mutated *meg-3* and second half of mutated *meg-3* ---aggagggatcctaattgtaccaatttatatctattac	na (PCR repair templates are overlapping and contain homology arms)

Schematics as in Figures: dark and light blue (proximal/distal homology arms flanking the Cas9 site and mutated *meg-3* coding sequence), green (GFP), orange (mNeonGreen and hotspot - new Cas9 recognition site), red (tagRFP), purple (3Xflag, ollas, additional non-homologous bases or linker), brown (RE, ZF1, PH, Myc tag, Tev site or *meg-3* Nt domains), stars (recoded sequence). crRNA sequence (underlined) and PAM (double underline). Coding sequence in uppercase and non-coding in lowercase.

Supplementary Table S3: Inserted sequences

Name	Sequence
Myc	GAACAAAAACTGATATCAGAAGAGGATCTG
ZF1 domain	AAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGA
Tev::Linker::Myc::Linker::3Xflag	GAGAACCTCTACTTCCAAGGATACGTAGCCGGCGAACAAAAACTGATATCAGAAGAGGATCTGTGCCCGGGGGATCGGTGGAGCTCCACCGGTGGCGGCCGCTCTAGAACTAGTGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG
ZF1 domain::3Xflag	AAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGAGACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG
eGFP	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTT CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAA AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAG
GFP (with 3 introns)	AGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGgtaagtttaaacatatatatactaactaaccctgatt aaattttcagCCAACACTTGTCACTACTTTCTGTTATGGTGTTCAATGCTTCTCGAGATACCCAGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTaagtttaaacagttcggtactaactaaccatacatatttaaatt ggtGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTgtaagtttaaacatgattttactaactaatctgattta ttcagAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTG TTACACATGGCATGGACGAACTATACAAA
eGFP::recoded * *gtbp-1* ::PH	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTT CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAA AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGAGCGG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*CCACGGGCTCCAGGATGACCCGGACCTTCAGGCCCTTCTGAAGGGCAGCCAGCTTCTGAAGGTGAAGTCCAGCTCGTGGCGTAGGGAACGCTTCTACAAGCTACAGG ACTGCAAGACCATCTGGCAGGAATCTCGAAAGGTCATGAGGTCCCCGGAGTCGCAGCTGTTCTCCATCGAGGACATTCAGGAGGTACGGATGGGACACCGCACAGAAGGCCTGGAGAAGTTTGCCCGAGACATCCCCGAGGATCGATGCTTCTCCATTGTCTTCAAGGACCAGCGCAACACCCTAGACCTCATTGCCCCATCACCAGCTGA TCAGCACTGGGTGCAGGGCCTGCGCAAGATCATCCACCACTCCGGCTCCATGGACCAGCGGCAGAAG
eGFP::ZF1 domain	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTT CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAA AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGA
eGFP::ollas::PH domain	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTT CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAA AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGTCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAAGCACGGGCTCCAGGATGACCCGGACCTTCAGGCCCTTCTGAAGGGCAGCCAGCTTCTGAAGGTGAAGTCCAGCTCGTGGCGTAGGGAACGCTTCTACAAG CAGGAGGACTGCAAGACCATCTGGCAGGAATCTCGAAAGGTCATGAGGTCCCCGGAGTCGCAGCTGTTCTCCATCGAGGACATTCAGGAGGTACGGATGGGACACCGCACAGAAGGCCTGGAGAAGTTTGCCCGAGACATCCCCGAGGATCGATGCTTCTCCATTGTCTTCAAGGACCAGCGCAACACCCTAGACCTCATTGCCCCATCAC GCTGACGCTCAGCACTGGGTGCAGGGCCTGCGCAAGATCATCCACCACTCCGGCTCCATGGACCAGCGGCAGAAG
meGFP::Linker::mNeonGreen	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTT CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTAAGCTTTCGAAAGATCCCAACGAAAA AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGTGCCCGGGGGATCGGTGGAGCTCCACCGGTGGCGGCCGCTCTAGAACTAGTGTCTCCAAAGGGGAGGAAGACAACATGGCGTCCCTCCCAGCGACGCACGAACTCCATATCTTTGGGTCCATAAACGGTGTCGATTTCGAC GTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAGgtaagtttaaacatatatatactaactaaccctgattatttaaattttcagGGGGACCTCCAATTTTCCCCATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATGTCCCCATTTCAAGCGGCCATGGTAGAC AAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAGTCAAAGGAACTGGATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTAGTAAAAAGACGTACCCAAATGACAAGgtaagt acagttcggtactaactaaccatacatatttaaattttcagACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGACGACGTACACGTTCGCCAAGCCCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACCGAGCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGG GGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG
meGFP::Linker::mNeonGreen (with 2 introns)::3Xflag::tagRFP	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAGTGCTTCAG CCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATA AACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTAAGCTTTCGAAAGATCCCAACGAAAAGAG AGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAG TGCCCGGGGGATCGGTGGAGCTCCACCGGTGGCGGCCGCTCTAGAACTAGTGTCTCCAAAGGGGAGGAAGACAACATGGCGTCCCTCCCAGCGACGCACGAACTCCATATCTTTGGGTCCATAAACGGTGTCGATTTCGACA GTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAG gtaagtttaaacatatatatactaactaaccctgattatttaaattttcagGGGGACCTCCAATTTTCCCCATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATGTCCCCATTTCAAGCGGCCATGGTAGACG AAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAGTCAAAGGAACTGGATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTAGTAAAAAGACGTACCCAAATGACAAG gtaagt acagttcggtactaactaaccatacatatttaaattttcagACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGACGACGTACACGTTCGCCAAGCCCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACCGAGCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGGA GGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG GTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAATGCACATCCGAG CGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTTGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTTCCTGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGT CTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGTCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCAATACCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAAGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCT AATTTCAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAAGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTAAT
mNeonGreen (with 2 introns)::3Xflag	GTCTCCAAAGGGGAGGAAGACAACATGGCGTCCCTCCCAGCGACGCACGAACTCCATATCTTTGGGTCCATAAACGGTGTCGATTTCGACATGGTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAG gtaagtttaaacatatatatactaactaaccctgattatttaaattttcagGGGGACCTCCAATTTTC ATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATGTCCCCATTTCAAGCGGCCATGGTAGACGGAAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAGTCAAAGGAACTGG ATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTAGTAAAAAGACGTACCCAAATGACAAG gtaagtttaaacagttcggtactaactaaccatacatatttaaattttcagACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGACGACGTACACGTTCGCCAA CCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACCGAGCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGGAATGGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG
meg-3 Nt domain (with endogenous intron)::hotspot::ollas	ATGAGTTCCTCAAAACCTTACCCAAGCGGTCTGCCAAACTCGAGAAGAAAAAGAGGAGgtgagagttttttgcatcaatttttcattcaatttgtaaattcag GTCGCCGCTCAAGTAGTCGTAGCAATCAGGAAAGTGCATCCAACAACATGGAACACCAAATCACTCTTGACGAATTGTTCAATCCGATCGCAAAACAAGACAGT CAGTCAACAAGCCGTGAATATGGTGCAAAATCGGGGATATCTCATCACGGATCCGTATCATTCAACGGGAATACATTCATGAATGGCCAACAGCTGAATCACTCGATGACTCGACATGGTCGAGTATTCAATCAGTCGATGCATGCAGCTCAAGGAAACGGCAGCAACGCCTTTAACAGTATTCCTCCTACGGCC GTCTTCTCTGCAGACTTCCGCCGCAACTTGCAAACACGCAATAGCTCCAGCTGGTATGAACGAAGATTCCCAGTATCCACAGATCAGGACGACGTTCAGCAATCAAACACAAGACGTAGTAGAAGTCGTCAGAATGGACAACATGGCCTAAGCTTCTCGGATGGTTCGAACAATTATGGTCACGCTGGGAATAAG TTCAGTGTGAGTTCCGTTCCCGTTGGGTTCCAGAAGCAAGAAAATAATTCTAAGAAACTGCGACAAACCAATGTTCACCAGCAATGTCTTGGAAATAAAAGCTTCAACGCTCAAGCTGGAGTTCATGGACACGCTTTTAAAAAAGGCCATAAGGACAATAAAAATGCATCTGGAAAGGAGGTTATCAATTCTAGT TCCAAAAGCACGATGCTATTAAGTCGCGGAATTTGAACCAGAGCTTCTCTGGATTTCCTACACATGAAACCTCATCGATGAAAAATCAACAACAGAAATCAAGAAACGACAGAAAAAAATCACGTGGTAGCAGCAACTTCCAGGATCGTACTTACTTCAACACAAATGACGATGAATTAACTGACGATGTGTTCAT CGGGAAGTTTGTCCAGAGCAGAGGATCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAAGtga
mutated *meg-3* coding sequence (122 mutations spread on 1.8 kb) (with endogenous introns)	ACGGTCTGCCAAACGCTAGAAGAAAAAGAGGAGgtgagagttttttgcatcaatttttcattcaatttgtaaattcag GTCGCCGCGCAGCTGCTCGTGCCAATCAGGAAGCTGCAGCCAACAACATGGAACACCAAATCACTCTTGACGAATTGTTCAATCCGATCGCAAAACAAGACGCTGCTCAGGCAACAGCCCGTGAATAT TGCAAAAGCGGGGATAGCTCATCACGGAGCCGTAGCATTCAACGGGAATACATTCATGAATGGCCAACAGCTGAATCACGCTATGACTCGACATGGTCGAGTATTCAATCAGGCCATGCATGCAGCTCAAGGAAACGGCGCCAACGCCTTTAACGCTATTCCTCCTACGGCCCCCGTCTTCGCTGCAGACTTCCG CAACTTGCAAACACGCAATGCCGCCGCCTGGTATGAACGAAGATTCCCAGTAGCCACAGATCAGGACGACGTTCAGCAAGCAAACACAAGACGTGCTAGAGCTCGTCAGAATGGACAACATGGCCTAGCCTTCGCGGATGGTGCGAACAATTATGGTCACGCTGGGAATAAGGCATTCGCTGTGGCTGCCGTT GTTGGGTTCCAGAAGCAAGAAAATAATGCTAAGAAACTGCGACAAACCAATGTTCACCAGCAATGTCTTGGAAATAAAGCCTTCAACGCTCAAGCTGGAGTTCATGGACACGCTTTTAAAAAAGGCCATAAGGACAATAAAAATGCAGCTGGAAAGGAGGTTATCAATGCTGCTCTTGTCCAAAAGCACGATGC AAGGCTCGGAATTTGAACCAGGCCTTCGCTGGATTTCCTACACATGAAACCGCAGCTATGAAAAATCAACAACAGAAAGCAAGAAACGACAGAAAAAAAGCACGTGGTGCCGCCAACTTCCAGGATCGTACTTACTTCAACACAAATGACGATGAATTAACTGACGATGTGTTCATAGACGATGCCATGGATGC CCGTGGTCGGAGAGCTCGAGCAGTCACTAAGAAACTTCAACAAGCGACTTATGCAAAGCAGAATGCAGGCGCTAAACAATTGACTGAAAAATGCAAAGCTGCTGAAGAAGCGGCTAAACGTAACCTGGTAGCCAATGTATTCGCCAAGGACGGCACTGAACTCGCCATTGAACAGTTATTGGAAATTGTCGCC AAGATCGGACAACAGATTCACTTGCCTGCCGCCGCTCATGGAGAATGCGCCAATTTGAATCGGACATTACCAGCAGCTGATCTAAATTGCGCAATTGGTGAAGACTTTGACGCCGCTTTTGTGGATGCTAACAACCAAACCCTACCAGTGGCTCTTCCCAAGAAAACTGCACTGGCGATCAAGCGCCGTGGAGCT CGTGCTGCCGCCCGTCTGGCCGCTCTTGATGTGACACTGGAAACTGTTGAAGAAGACGAGGAACCCACGCCAGCTCCACAGCCGGCTGCTCCTCCAAAGATCGCAAGAAGAAAGTGGACAGGAACTTTTGATGCCAACGTTGAGGAAATGAGACGTTTACTTCATGGGGACCCTGAGATGCCGAAAGCTGCAA GTGCTGCCGCAAGgtatgtagtgaaatagtaaaatctttagtaaaaatttaaatttcag CAAGGATCAGATCAATCGGAACAATGTGGATGTAAAGAGAACACCTGCCGGGAAGTTTGTCCAGAGCAGAGGATCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAAGtga

Supplementary Table S4: Primers and ssODN sequences

Name	Sens	Description	Sequence (5' to 3')
Bridge and repair ssODNs			
829	S	Bridging 5'end eGFP with *gtbp-1*	ggttcgggtggtgctccacgaggtggtatgcggtgagtaaaggagaagaacttttcactggagttg
830	S	Bridging 3'end eGFP (contain *BsrG1* site) with *gtbp-1*	gggattacactcggcatggacgagc*tgtaca* agagcggtttccaaaatgcgggacaaaattagaag
1013	S	Bridging first and second halfs of eGFP together	gacgggaactacaagacacgtgctgaagtcaagtttgaaggtgatacccttgttaatagaatcg
1015	AS	as 829 but inversed polarity	caactccagtgaaaagttcttctcctttactcaccgcataccacctcgtggagcaccacccgaacc
1016	AS	as 830 but inversed polarity	cttctaattttgtcccgcattttggaaaccgctt*tgtaca* gctcgtccatgccgagtgtaatccc
1267	AS	Bridging 3'end mNeonGreen with *gtbp-1* and inserting a 3Xflag tag	cttctaattttgtcccgcattttggaaaccgcttttgtcatcgtcatccttgtaatcgatatcatgatctttataatcaccgtcatggtttgtagtccttgtagagttcgtccattcccattacgtccg
1349	S	Bridging 5'end eGFP with *glh-1* (at Stop, 6bp away from the Cas9 cut)	ccagtgtaccaactcaagtccctcaagacgaggaaggatggtgagtaaaggagaagaacttttcactggagttg
1350	AS	Bridging 3'end eGFP with *glh-1* (at Stop)	gcattaataaatgcgaaacactatcaattggtcggttttctacttgtacagctcgtccatgccgagtgtaatccc
1363	AS	as 1365 but inversed polarity	ctcatcttgtccgtgagcatatgtgcaattgtcgttgtacgggcagtatccttcacggcggaacgcatcacaaagtcg
1365	S	Partial ZF1	cgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgag
1376	S	Partial ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatac
1377	S	Partial ZF1 with *gtbp-1* homology arm	gacaattgcacatatgctcacggacaagatgagctgagagttccgagaagcggtttccaaaatgcgggacaaaattagaag
1378	S	ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgagctgagagttccgagagcggtttccaaaatgcgggacaaaattagaag
1379	S	Partial ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgagctgagagttc
1380	S	Partial ZF1 and 3Xflag with *gtbp-1* homology arm	catatgctcacggacaagatgagctgagagttccgagagactacaaagaccatgacggtgattataaagatcatgatatcgattacaaggatgacgatgacaagagcggtttccaaaatgcgggacaaaattagaag
1394	S	Bridging 5'end of *meg-3* Nt domain with 5' end of *gtbp-1*	aaatgcagataacctcggccttaacccagaataagatgagttcctcaaaaccttacccaagcggtctgcc
1395	AS	Bridging 3'end of *meg-3* Nt domain with 3'end of *gtbp-1* and inserting a ollas tag and hotspot	gcttctaattttgtcccgcattttggaaaccgctgcactttcccatgagacgtggtccgagctcgttggcgaatccggacctctgctctggacaaacttcccgtctatgaacacatcgtcagttaattcatcgtcatttg
1407	S	Left *gtbp-1* homology arm with *Xba1* site	ggttcgggtggtgctccacgaggtggtatgcggtg*tctaga* ggagaagaacttttcactggagttg
1408	AS	as 1407 but inversed polarity	caactccagtgaaaagttcttctcc*tctaga* caccgcataccacctcgtggagcaccacccgaacc
1462	R	Tev::Linker::Myc and partial Linker::3Xflag with *glh-1* (at Stop, 6bp away from the Cas9 cut) homology arm	gtcactagttctagagcggccgccaccggtggagctccaccgatcccccgggcaagatcctcttctgatatcagtttttgttgccggctacgtatccttggaagtagaggttctcccatccttcctcgtcttgagggacttgagttggtacactgg
1463	R	Partial Linker::3Xflag with *glh-1* (at Stop) homology arm	gcattaataaatgcgaaacactatcaattggtcggttttctacttgtcatcgtcatccttgtaatcgatatcatgatctttataatcaccgtcatggtctttgtagtcactagttctagagcggccgccaccggtggag
1465	S	Bridging 5'end eGFP with *lin-15B* (at Stop)	taagctgccaacccccacaccacccagcaacgaagtgagtaaaggagaagaacttttcactggagttg
1466	AS	Bridging 3'end eGFP with *lin-15B* (at Stop)	caatcaggaacggtggatgaaaatatttccacttacttgtacagctcgtccatgccgagtgtaatccc
1475	S	Bridging 5'end eGFP with *gtbp-1* (at Stop, 27bp away from the Cas9 cut, recoded)	ggcggttcgggtggtgctccacgaggtggtatgcgaagtggctttcagaacgccggtcagaagtgagtaaaggagaagaacttttcactggagttg
1476	AS	Bridging 3'end eGFP with *gtbp-1* (at Stop)	cttacaagaaggaaaaaggagaacggaaagcttctacttgtacagctcgtccatgccgagtgtaatccc
1489	S	Bridging 5'end eGFP with *pgl-1* (at Stop, 10bp away from the Cas9 cut)	gtggacgtggtggttacgggggtcgtggaggacgtggtggcttgtgagtaaaggagaagaacttttcactggagttg
1490	AS	Bridging 3'end eGFP with *pgl-1* (at Stop)	cttaaaaaacaaattaaacattcaatagttggagtttacttgtacagctcgtccatgccgagtgtaatccc
1493	S	Bridging 5'end eGFP with *fbf-2* (at Stop)	caaattttcttcttccggtagtcacggcgatgatgtgagtaaaggagaagaacttttcactggagttg
1494	AS	Bridging 3'end eGFP with *fbf-2* (at Stop)	ggatgagatttatggtgagaaagttccaccttacttgtacagctcgtccatgccgagtgtaatccc
1521	S	Bridging 5'end eGFP with *gtbp-1* (at Stop, 27bp away from the Cas9 cut, recoded + PAM mutation)	ggcggttcgggtggtgctccacgaggtggtatgcgaagctgctttcagaacgccggtcagaagtgagtaaaggagaagaacttttcactggagttg
PCR primers for linear repair templates			
436	F	*gtbp-1* ~60 bp homology arm amplification (plasmid AP554-1)	gcggtggacaattcttccgccg
437	R	*gtbp-1* ~60 bp homology arm amplification (plasmid AP554-1)	caagaaggaaaaaggagaacgg
527	F	*gtbp-1* ~100 bp homology arm amplification (plasmid AP553-1)	ggcggcggattccgaggaggac
528	R	*gtbp-1* ~100 bp homology arm amplification (plasmid AP553-1)	tgagaaggataggtacagaatc
603	F	*gtbp-1* homology arm amplification (plasmid 1297-1 and 1298-1)	ggcggttcgggtggtgctccac
604	R	*gtbp-1* homology arm amplification (plasmid 1297-1 and 1298-1)	cttacaagaaggaaaaaggag
645	F	eGFP/meGFP amplification with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcggtgagtaaaggagaagaac
646	R	eGFP amplification with *gtbp-1* homology arm	cttctaattttgtcccgcattttggaaaccgctttgtacagctcgtccatgcc
715	F	mNeonGreen amplification with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcggtctccaaaggggaggaagac
831	F	eGFP amplification without homology arm	gtgagtaaaggagaagaac
832	R	eGFP amplification without homology arm	cttgtacagctcgtccatg
1011	F	Second half of eGFP amplification	gtttgaaggtgatacccttg
1012	R	First half of eGFP amplification	ttgacttcagcacgtgtcttg
1014	R	First half of eGFP amplification with overlapping sequence with the second half	cgattctattaacaagggtatc
1017	F	eGFP amplification without homology arm but with extranucleotide in order to be in frame if PCR repair template inserted at Cas9 cut	cgtgagtaaaggagaagaac
1018	R	eGFP amplification without homology arm but with extranucleotide in order to be in frame if PCR repair template inserted at Cas9 cut	tccttgtacagctcgtccatg
1084	R	mNeonGreen amplification with *gtbp-1* homology arm	cttctaattttgtcccgcattttggaaaccgctcttgtagagttcgtccattc
1085	R	tagRFP amplification with *gtbp-1* homology arm	cttctaattttgtcccgcattttggaaaccgctattaagtttgtgccccag
1096	R	meGFP::Linker amplification	actagttctagagcggccg
1099	F	3Xflag::tagRFP amplification	gactacaaagaccatgacgg
1152	F	second half of mNeonGreen (with 1 intron)::3Xflag::tagRFP amplification	ccgtcaactatcgttacac
1153	R	meGFP::Linker:: first half of mNeonGreen (with 1 intron) amplification	gacttccctcgtacgtg
1255	F	Linker::mNeonGreen amplification	gagctccaccggtgg
1256	R	mNeonGreen::3Xflag amplification	gatctttataatcaccgtcatggtc
1263	R	eGFP amplification with ollas homology arm	ccatgagacgtggtccgagctcgttggcgaatccggacttgtacagctcgtccatg
1264	F	PH amplification with ollas homology arm	gattcgccaacgagctcggaccacgtctcatgggaaagcacgggctccaggatgacc
1265	R	PH amplification with *gtbp-1* homology arm	cttctaattttgtcccgcattttggaaaccgctttctgccgctggtccatg
1266	R	mNeonGreen amplication without homology arm	cttgtagagttcgtccattc
1272	R	eGFP amplification with ZF1 homology arm	gtattctgtgtgcttcttgtacagctcgtccatg
1273	F	ZF1 amplification with eGFP homology arm	catggacgagctgtacaagaagcacacagaatacaaaacgc
1274	R	ZF1 amplification with *gtbp-1* homology arm	cttctaattttgtcccgcattttggaaaccgcttctcggaactctcagctcatcttg
1292	F	eGFP amplification (1st PCR round) with *gtbp-1(27bp)* and partial Myc	aaaaactgatatcagaagaggatctgagcggtttccaaaatgcgggacaaaatgtgagtaaaggagaagaac
1293	F	Partial Myc amplification (2nd PCR round) with *gtbp-1* homology arm	ggcggttcgggtggtgctccacgaggtggtatgcggaacaaaaactgatatcagaag
1294	R	eGFP amplification (1st PCR round) with *gtbp-1* homology arm	cttacaagaaggaaaaaggagaacggaaagcttctacttgtacagctcgtccatgcc
1294	R	eGFP amplification with *gtbp-1* homology arm away from the cut	cttacaagaaggaaaaaggagaacggaaagcttctacttgtacagctcgtccatgcc
1295	R	*gtbp-1* homology arm amplification (2nd PCR round)	cttacaagaaggaaaaaggagaacggaaag
1357	F	Additional non-homologous bases (partial 3xFlag tag) and *gtbp-1* homology arm amplification (plasmid 554-1)	catgatatcgattacaaggatgacgatgacaagggttcgggtggtgctccac
1358	R	Additional non-homologous bases (partial *tbb-2* 3'UTR) and *gtbp-1* homology arm amplification (plasmid 554-1)	gggaatgcttgaaaggattttgcatttatcactcttctaattttgtcccgc
1375	F	eGFP amplification with *gtbp-1* homology arm away from the cut	gtttccaaccacgcggtggacaattcttccgccggtgagtaaaggagaagaac
1392	F	*meg-3* Nt domain amplification without homology arm	atgagttcctcaaaaccttaccc
1393	R	*meg-3* Nt domain amplification without homology arm	gtctatgaacacatcgtcagtta
1506	F	Mutated *meg-3* coding sequence amplification with *meg-3 deletion::hotspot* homology arm	tcaaaaccttacccaagggcaggatgtcagccagacggtctgccaaacgctagaagaaaaagaggaggtg
1507	R	First half of mutated *meg-3* coding sequence amplification	tgctcgagctctccgaccac
1508	F	Second half of mutated *meg-3* coding sequence amplification	acgatgccatggatgctgcc
1509	R	Mutated *meg-3* coding sequence amplification with *meg-3 deletion::hotspot* homology arm	gtaatagatataaattggtacaattaggatccctccttcactttcccatgagacgtggtccgagctcgttggcgaatccggatcctctgctctggacaaacttcccggcaggtgttctctttacatccacattgttccgattg
PCR primers for genotyping			
321	F	*gtbp-1* genotyping (3' end cut)	agtaaaagccattttcgaagcgg
322	R	*gtbp-1* genotyping (3' end cut)	gataggatgtgaaaatgggatgc
390	F	*gtbp-1* genotyping (gene replacement)	gtgcctgcgaatcaaactgg
391	R	*gtbp-1* genotyping (gene replacement)	gatgcgggctgattggattg
402	F	*gtbp-1* edits sequencing (3' end cut)	ccactcaaactggatgcattcg
408	F	*gtbp-1* edits sequencing (gene replacement)	ctttcgaatttcgcgcgttcgg
412	R	*gtbp-1* edits sequencing (3' end cut)	gatctactaaaacgctacacgc
738	F	*lin-15B* genotyping	tcgaagacggagaaattgtcgg
739	R	*lin-15B* genotyping	agtgtatgataagcaagatagg
836	F	*glh-1* genotyping	gacaacattgatgactatatcc
837	R	*glh-1* genotyping	gattagctgagagagcaatgtg
1510	F	*meg-3* genotyping	cattcttgttgcgtggtccc
1511	R	*meg-3* genotyping	tcacaaggagaagcgagttg
1535	F	*fbf-2* genotyping	tgaactccaatcttcgggacac
1536	R	*fbf-2* genotyping	tgtatggaagcaggaagctctc

F and R : Forward and Reverse primers for PCR amplification. S and AS: Sens and AntiSens polarity for ssODNs.

Supplementary Table S5: Plasmids and new strains

Plasmid name	Backbone	Insert
AP553-1	pUC19	gtbp-1 (~500 bp flanking sequence)::GFP (with 3 introns)::gtbp-1 (flanking sequence)
AP554-1	pUC19	gtbp-1 (~60bp flanking sequence)::GFP (with 3 introns)::gtbp-1 (flanking sequence)
AP625-1	Paix *et al.* , 2015	eGFP
AP763-1	pUC19	Pleckstrin Homology Domain From Phospholipase C Delta (Green *et al.* , 2011)
AP931-8N	pUC19	TEV::meGFP::Linker::mNeonGreen (with 2 introns)::3Xflag::tagRFP
AP1297-1	pUC19	gtbp-1 (flanking sequence)::eGFP::gtbp-1 (27bp)::PH::gtbp-1 (flanking sequence)
AP1298-1	pUC19	gtbp-1 (flanking sequence)::eGFP::recoded gtbp-1(8/27bp, every 3bp)::PH::gtbp-1 (flanking sequence)

Plasmid containing the *pie-1* ZF1 domain was a gift from Chih-Yung Lee, Seydoux lab

Experimental variant	CRISPR alleles
Figure 1-Panel F	*gtbp-1::Myc*
Figure 1-Panel H	*gtbp-1::eGFP::recoded gtbp-1(8/27bp, every 3bp)::PH (at Stop)*
Figure 2-Panel F	*gtbp-1::ZF1*
Figure 4-Panel A	*gtbp-1::ZF1::3Xflag*
Figure 4-Panel B	*glh-1::Tev::Linker::Myc::Linker::3Xflag (at Stop)*
Figure 4-Panel C	*gtbp-1::eGFP::ZF1*
Figure 4-Panel D	*gtbp-1::eGFP::ollas::PH*
Figure 4-Panel E	*gtbp-1:: meGFP::Linker::mNeon*
Figure 4-Panel F	*gtbp-1:: meGFP::Linker::mNeon::3Xflag::tagRFP*
Figure 4-Panel H	*gtbp-1::eGFP (at Stop)*
Figure 4-Panel I	*glh-1::eGFP (at Stop)*
Figure 4-Table S1	*pgl-1::eGFP (at Stop)*
Figure 4-Table S1	*lin-15B::eGFP (at Stop)*
Figure 4-Table S1	*fbf-2::eGFP (at Stop)*
Figure 4-Panel J	*gtbp-1::mNeon::3Xflag*
Figure 4-Panel K	*gtbp-1 replacement with meg-3 Nt domain::hotspot::ollas*
Figure 4-Panel L	*mutated meg-3 coding sequence (122 mutations spread on 1.8 kb)*