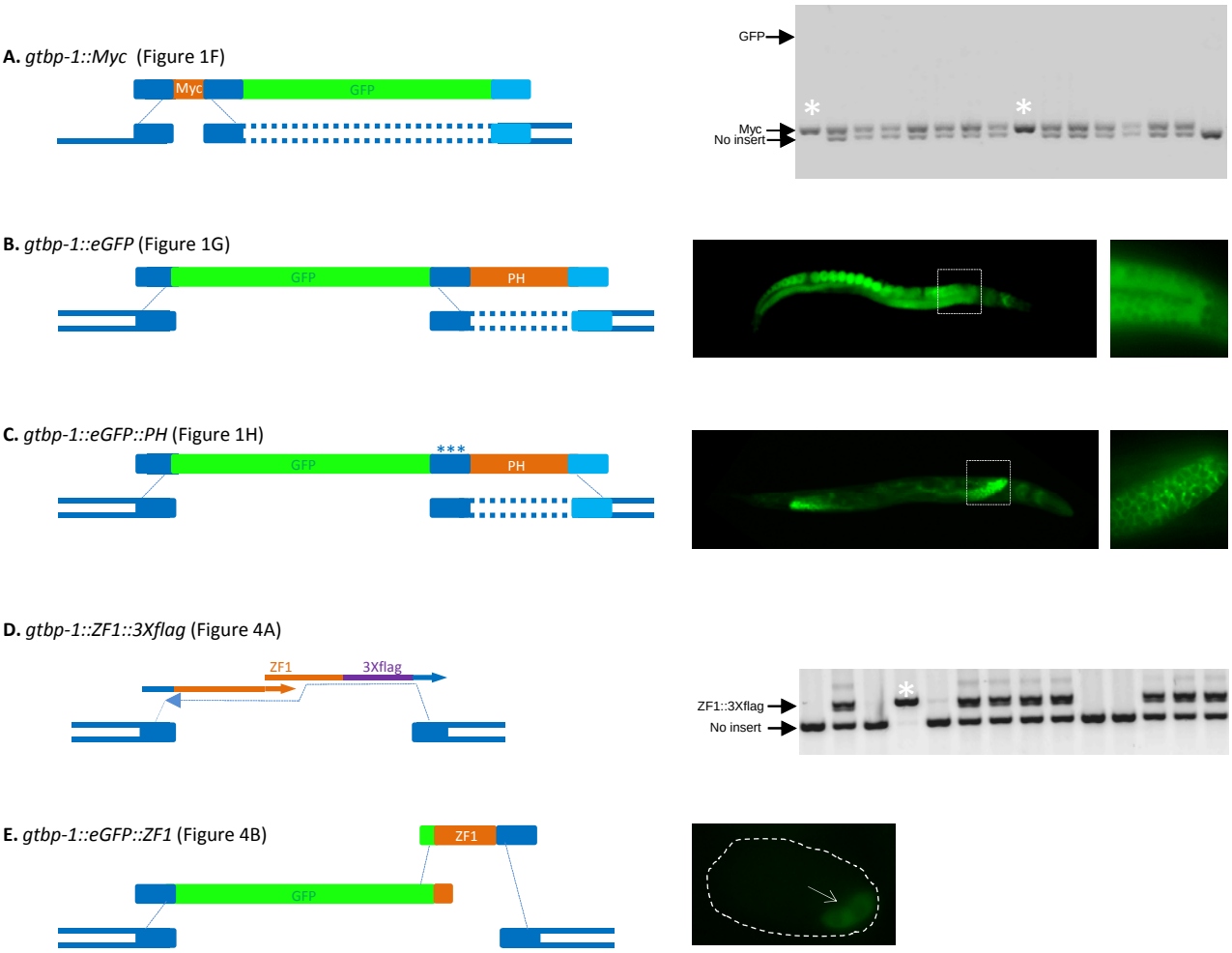


Supplementary Figure S1: Schematics of experiments from Figure 1 and Figure 4 with edit examples

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).

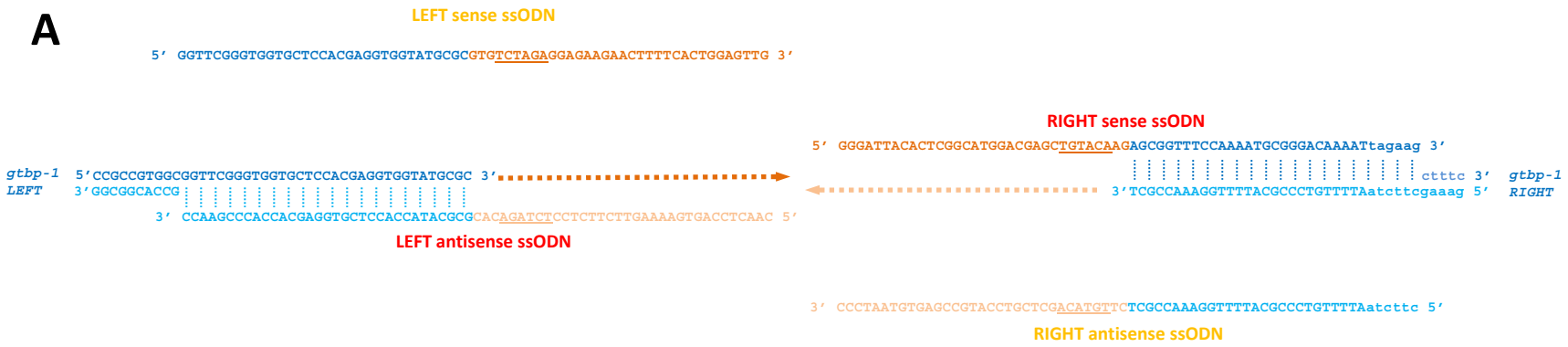
A. We identified edits based on GFP expression (not shown) and by PCR: the gel shows PCR amplification of the *gtbp-1* locus for a subset of F2 cohorts (Progenies of cloned single F1s). The lower band corresponds to the wild-type unedited allele and the upper band to the edited Myc insertion allele. 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.

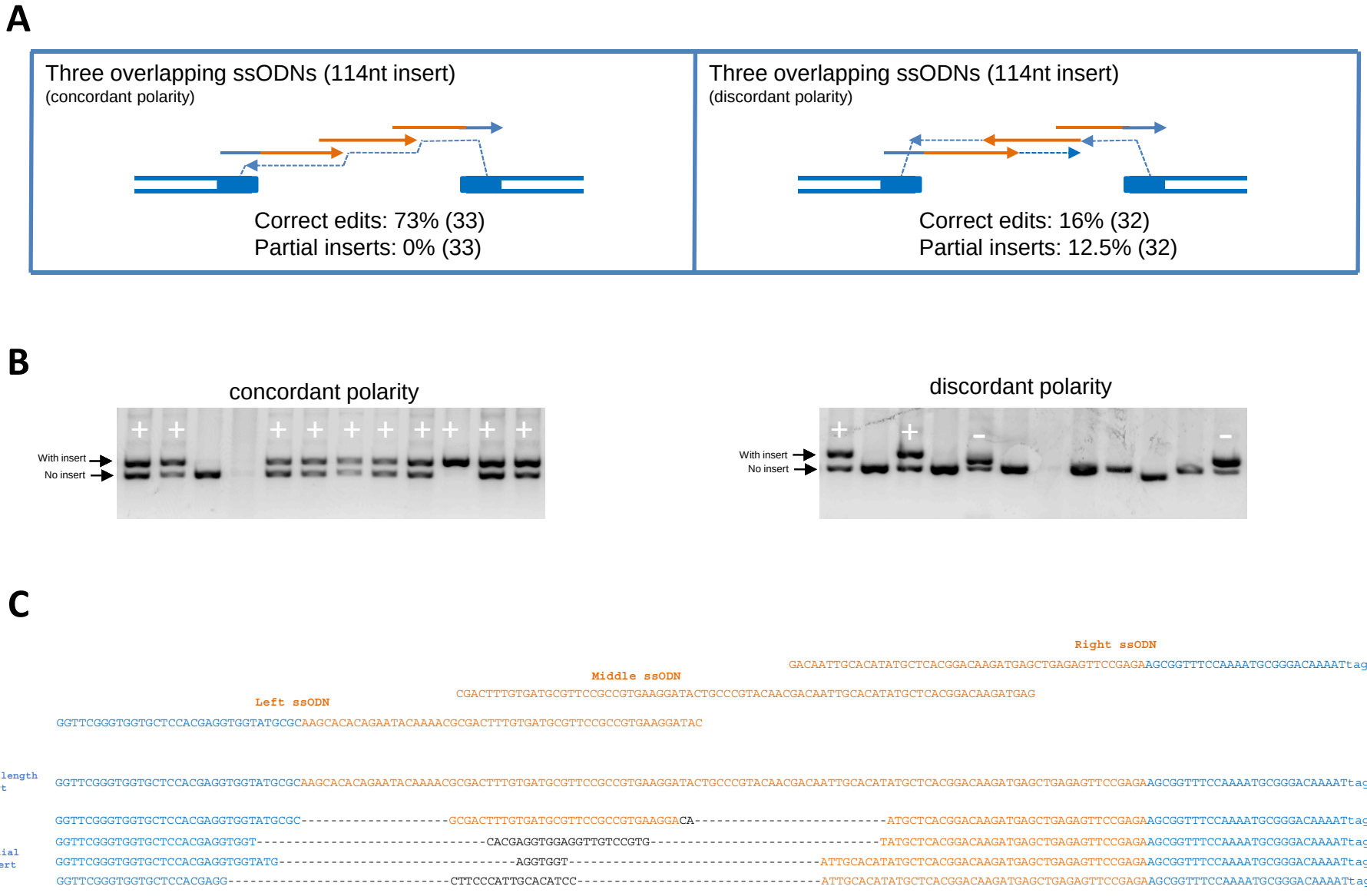
E. All edits showed the characteristic ZF1 degradation pattern, where protein is degraded in somatic, but not germline (arrow), blastomeres in embryos. Dotted line marks the embryo boundary.



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.

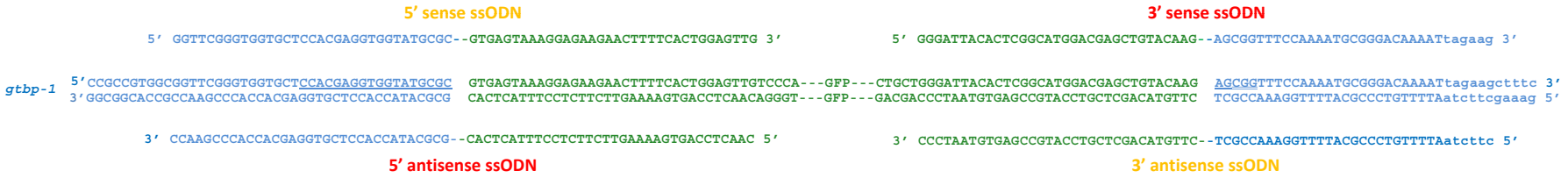
B. Example of gels showing PCR amplification of the *gtbp-1* locus with (bottom panel) and without (top panel) restriction enzyme digestion after editing with the ssODNs with a right homology arm. Each well was loaded with DNA amplified from the progeny of one F1 *dpy-10*-edited worm. Only the sense ssODN yielded edits with the restriction enzyme site (white asterisks).



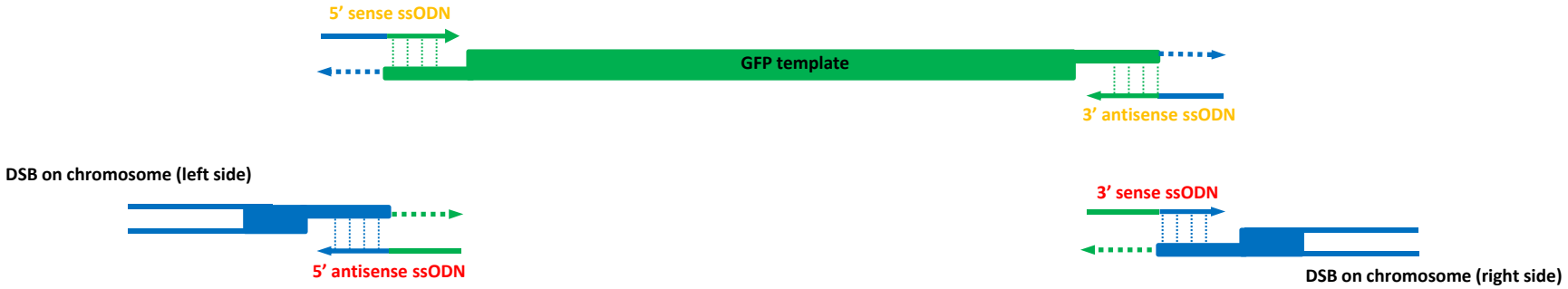
Supplementary Figure S3: Template switching with ssODNs

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 5 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.



B.



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

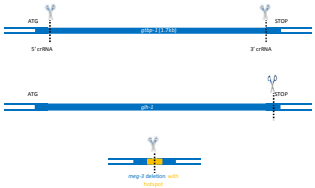
A. Sequence from *gtbp-1* locus is blue and sequence from the GFP PCR repair template is green. crRNA sequence is underlined and PAM is double underlined in *gtbp*-1 locus. Bridging ssODNs that performed best in the experiments shown in Figure 3 are labeled in yellow.
B. Schematics illustrate how the 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 S1: Expanded results from Figures and Tables

Figures/Tables where experiments are displayed	Edit	Strategy	Nb of repair templates / DNA: DNA junctions	Concentration PCR template (pmol/µl)	Size PCR template (bp)	Homology arms PCR template (bp)	Primers used for PCR template	Polarity bridge ssODN	Concentration bridge ssODN (pmol/µl)	Size bridge ssODN (nt)	Homology arms bridge ssODNs (nt)	Bridge ssODNs used	% edit (Roller)	# of precise edits / # of sequenced edits (Complete sequencing of the insertion + 60bp of surrounding genomic DNA, except if noted otherwise)
Figure 1	Panel A	*gtbp-1::eGFP*	1/2	0.48	780	32/33	645/646	na	na	na	na	na	71.7 (56/78)	nd
	Panel A (replicate)	*gtbp-1::eGFP*	1/2	0.48	780	32/33	645/646	na	na	na	na	na	76.4 (136/178)	nd
	Panel B	*gtbp-1::GFP*	1/2	0.48	982	58/59	636/637	na	na	na	na	na	46.7 (29/62)	nd
	Longer homology arms (1000bp)	*gtbp-1::GFP*	1/2	0.48	1064	98/101	527/528	na	na	na	na	na	33.3 (19/57)	nd
	3' homology arm containing 33bp non-homologous sequence at the end	*gtbp-1::GFP* with *3' homology arm containing 33bp non-homologous sequence at the end*	1	0.48	989	58/33 (+33 non-homologous)	636/1358	na	na	na	na	na	50 (28/56)	nd
	Panel C	*gtbp-1::GFP* with *5'/3' homology arms containing 33bp/33bp non-homologous sequences at the ends*	1/2	0.48	996	32 (+33 non-homologous) /33 (+33 non-homologous)	1357/1358	na	na	na	na	na	22 (13/59)	nd
	Panel D	*gtbp-1::eGFP* with *3' homology arm 27bp away from the cut*	1/2	0.48	783	32/36	645/1294	na	na	na	na	na	48.7 (57/117)	nd
	Panel E	*gtbp-1::eGFP* with *5'/3' homology arms 35bp/27bp away from the cut*	1/2	0.48	785	35/36	1375/1294	na	na	na	na	na	5 (5/99)	nd
	Panel F	*gtbp-1::Myc::gtbp-1(27bp)::eGFP (at Stop)*	1/2	0.47	843	35/36	1293/1295 (on 1292/1294)	na	na	na	na	na	85.2 (52/61) for Myc insertion alone, 0 (0/61) for eGFP insertion. 10/52 homozygous for Myc insertion, 1/52 heterozygous for Myc insertion but the other chromosome contains a small deletion, 1/61 has a homozygous small deletion	9/9
	Panel G	*gtbp-1::eGFP::gtbp-1(27bp)::PH (at Stop)*	1/2	0.47	1203	35/36	603/604	na	na	na	na	na	32.1 (37/115) for eGFP insertion alone, 0 (0/115) for eGFP::PH insertion	1/1
	Panel H	*gtbp-1::eGFP:: recoded gtbp-1(8/27bp, every 3bp)::PH (at Stop)*	1/2	0.47	1203	35/36	603/604	na	na	na	na	na	0 (0/76) for eGFP insertion alone, 34.2 (26/76) for eGFP::PH insertion	1/1
Figure 2	Panel A	*gtbp-1::*ssODN containing *Xba1* site and only homology to the *left side of the chromosome cut*	1/1	na	na	na	na	S	0.5	67	32/na	1407	0 (0/21 containing the restriction site)	nd
	Panel B	*gtbp-1::*ssODN containing *Xba1* site and only homology to the *left side of the chromosome cut*	1/1	na	na	na	na	AS	0.5	67	32/na	1408	40 (18/45 containing the restriction site)	nd
	Panel C	*gtbp-1::* ssODN containing *BsrGI* site and only homology to the *right side of the chromosome cut*	1/1	na	na	na	na	S	0.5	66	na/33	830	26.5 (13/49 containing the restriction site)	5/5 (perfect junction on the side of the homology arm and NHEJ on the other side)
	Panel D	*gtbp-1::* ssODN containing *BsrGI* site and only homology to the *right side of the chromosome cut*	1/1	na	na	na	na	AS	0.5	66	na/33	1016	0 (0/42 containing the restriction site)	nd
	Panel E	*gtbp-1::ZF1*	1/2	na	na	na	na	S	0.5	180	32/33	1378	76.4 (26/34 PCR bands of correct size for full-length insertion)	nd
	Panel F	*gtbp-1::ZF1*	3/4	na	na	na	na	S and S and S	0.5 and 0.5 and 0.5	87 and 78 and 81	32/33 and 33/33 and 33/33	1376 and 1365 and 1377	72.7 (24/33 PCR bands of correct size for full-length insertion). 1/24 homozygous	4/4
	Panel G	*gtbp-1::ZF1*	3/4	na	na	na	na	S and AS and S	0.5 and 0.5 and 0.5	87 and 78 and 81	32/33 and 33/33 and 33/33	1376 and 1363 and 1377	15.7 (3/19 PCR bands of correct size for full-length insertion), 10.5 (2/19 PCR bands of incorrect size for partial insertion)	See Supplementary Figure S3
	Panel G (replicate)	*gtbp-1::ZF1*	3/4	na	na	na	na	S and AS and S	0.5 and 0.5 and 0.5	87 and 78 and 81	32/33 and 33/33 and 33/33	1376 and 1363 and 1377	15.3 (2/13 PCR bands of correct size for full-length insertion), 15.3 (2/13 PCR bands of incorrect size for partial insertion)	See Supplementary Figure S3
Figure 3	Panel A	*gtbp-1::*eGFP	2/3	0.47 and 0.46	403 and 409	32/32 and 32/33	645/1014 and 1011/646	na	na	na	na	na	56.8 (129/227)	2/2
	Panel B	*gtbp-1::*eGFP	3/4	0.47 and 0.46	371 and 409	32/0 and 0/33	645/1012 and 1011/646	S	0.25	64	32/32	1013	55.4 (61/110)	3/3 (5'end junction not sequenced -chromosome / Nt half of eGFP)
	Panel C	*gtbp-1::*eGFP	2/3	0.44	747	0/33	831/646	S	0.5	67	32/34	829	60.5 (66/109)	2/2
	Panel D	*gtbp-1::*eGFP	2/3	0.44	747	0/33	831/646	S	0.5	199	98/100	1304	63.8 (60/94)	nd
	Panel E	*gtbp-1::*eGFP	2/3	0.44	747	0/33	831/646	S	0.5	31	34/16	1305	5.2 (3/57)	nd
	Panel F	*gtbp-1::*eGFP	2/3	0.44	747	0/33	831/646	AS	0.5	67	32/34	1015	46.4 (13/28)	1/1
	Panel G	*gtbp-1::*eGFP	2/3	0.45	746	32/0	645/832	S	0.5	66	33/33	830	43.3 (39/90)	2/2
	Panel H	*gtbp-1::*eGFP	2/3	0.45	746	32/0	645/832	AS	0.5	66	33/33	1016	70.2 (52/74)	nd
	Mix of S and AS bridging ssODNs	*gtbp-1::*eGFP	2/3 (mix of S and AS bridging ssODNs)	0.45	746	32/0	645/832	S and AS	0.25 and 0.25	66	33/33	830 and 1016	57.3 (132/230)	nd
	No bridging ssODN / homology arm	*gtbp-1::*eGFP	1/0	0.46	717	0/0	1017/1018	na	na	na	na	na	0 (0/157)	nd
Figure 4	Panel A	*gtbp-1::ZF1::* 3*Xflag*	2/3	na	na	na	na	S	0.5	142 and 137	32/33 and 33/33	1379 and 1380	62.8 (22/35 PCR bands of correct size for full-length insertion). 3/22 homozygous, 2/22 heterozygous but the other chromosome contains a small deletion, 1/35 has a heterozygous small deletion	2/2
	Panel B	*gtbp-1::eGFP::ZF1*	2/3	0.48 and 0.47	762 and 566	32/34 and 34/33	645/1272 and 1273/1274	na	na	na	na	na	52.3 (77/147)	5/6 (1/6 with one nucleotide substitution in ZF1 domain)
	Panel C	*gtbp-1::eGFP::* 4*flas::PH*	2/3	0.45 and 0.45	785 and 461	32/33 and 33/33	645/1263 and 1264/1265	na	na	na	na	na	34.2 (12/35), 1/35 incorrect (signal not on the membrane)	2/2
	One PCR fragment for 1572bp insert	*gtbp-1::*meGFP::*Linker::* 4*xNeon*	1/2	0.34	1638	32/33	645/1084	na	na	na	na	na	20.7 (16/77)	nd
	Panel D	*gtbp-1::*meGFP::*Linker::* 4*xNeon*	2/3	0.34 and 0.34	798 and 874	32/34 and 34/33	645/1096 and 1255/1084	na	na	na	na	na	25.6 (19/74)	1/1
	One PCR fragment for 1346bp insert	*gtbp-1::meGFP::Linker::* 4*xNeon::* 3*Xflag::* **tagBFP**	1/2	0.19	2412	32/33	645/1085	na	na	na	na	na	0 (0/112)	nd
	Two PCR fragments to assemble 2340bp insert	*gtbp-1::meGFP::Linker::* 4*xNeon::* 3*Xflag::* **tagBFP**	2/3	0.19 and 0.19	1204 and 1241	32/33 and 33/33	645/1153 and 1152/1085	na	na	na	na	na	5.4 (3/55)	1/1
	Panel E	*gtbp-1::meGFP::Linker::* 4*xNeon::* 3*Xflag::* **tagBFP**	3/4	0.18 and 0.20 and 0.19	798 and 875 and 807	32/34 and 34/34 and 34/33	645/1096 and 1255/1256 and 1099/1085	na	na	na	na	na	4.9 (6/122)	3/3
	As Figure 1-Panel A but lower concentration	*gtbp-1::eGFP*	1/2	0.13	780	32/33	645/646	na	na	na	na	na	9.4 (5/53)	nd
	One PCR fragment for 2340bp insert, lower concentration	*gtbp-1::meGFP::Linker::* 4*xNeon::* 3*Xflag::* **tagBFP**	1/2	0.13	2412	32/33	645/1085	na	na	na	na	na	0 (0/39)	nd
	Panel F	*gtbp-1::eGFP*	3/4	0.46	714	0/0	831/832	S and AS	1 and 1	67 and 66	32/34 and 33/33	829 and 1016	52.9 (71/134)	nd
	As Panel F but lower ssODNs concentration	*gtbp-1::eGFP*	3/4	0.46	714	0/0	831/832	S and AS	0.25 and 0.25	67 and 66	32/34 and 33/33	829 and 1016	50.9 (52/102)	7/8 (1/8 sequenced strain had two substitutions : one in the GFP and one in the homology arm bridging the GFP and the 3' bridge ssODN)
	As Panel F but with S and S bridging ssODNs	*gtbp-1::eGFP*	3/4	0.46	714	0/0	831/832	S and S	1 and 1	67 and 66	32/34 and 33/33	829 and 830	34.7 (33/95)	6/7 (1/7 sequenced strain had low GFP expression and contained a small indel in the GFP upstream of the 3' bridge ssODN)
	As Panel F but with AS and S bridging ssODNs	*gtbp-1::eGFP*	3/4	0.46	714	0/0	831/832	AS and S	1 and 1	67 and 66	32/34 and 33/33	1015 and 830	26.8 (11/41)	3/3
	Panel G	*gfh1::eGFP (at Stop, 6 bp away from the Cas9 cut)*	3/4	0.46	714	0/0	831/832	S and AS	0.5 and 0.5	75 and 75	35/34 and 33/62	1349 and 1350	24.2 (8/33)	nd
	Panel H	*gtbp-1::* 4*xNeon::* 3*Xflag*	2/3	0.48	840	32/0	715/1266	AS	0.49	131	32/33	1267	44.5 (78/175)	9/9
	Panel I	*gtbp-1* replacement with *meg-3 N‐terminal domain::*4*xNeon::* 4*flas*	3/4	0.31	1005	0/0	1392/1393	S and AS	0.14 and 0.14	70 and 142	33/37 and 38/35	1394 and 1395	52.7 (10/36 PCR bands of correct size for gene replacement)	3/3
	Panel J	Insertion of *mutated meg-3 coding sequence (122 mutations spread on 1.7 kb)* in a *meg-3 deletion strain* containing an *hotspot*	2/3	0.32 and 0.32	1053 and 823	34/41 and 41/37	1508/1507 and 1508/1509	na	na	na	na	na	6.3 (3/48 PCR bands of correct size for full-length insertion)	nd

Schematics (not to scale) show the *gtbp-1*, *gfh-1* or *meg-3* locus and recombineered DNAs for each experiment: dark and light blue (proximal/distal homology arms flanking the Cas9 site and mutated*meg-3* coding sequence), green (GFP), orange (mNeonGreen, hotspot), red (tagRFP), purple (3Xflag, sfGa, additional non-homologous sequence or linker), brown (4*, ZF1, PH, Myc tag or meg-3 Nt domains), stars (recoded sequence). Bridging ssODNs are represented with one line with arrow at 3' end (Sense: S; AntiSense: AS). Dotted lines are used to connect contiguous sequences. In Figure 4-panel I, the light colored dotted lines represent deleted genomic sequences between the two Cas9 sites used for editing in this experiment. Homology arms are the length of the shared sequence between the genomic sequence surrounding the Cas9 cut and the repair template or between repair templates (PCR template and ssODN). % edition correspond to the number of roller worms (co-CRISPR) expressing the fluorescent protein (green signal or green+red signals) in the germline. % edition for Figure 1-panel F, Figure 2 and Figure 4-panel A/I/J correspond to the number of roller worms positive by PCR or RE genotyping . Repair template and bridge ssODN concentration were calculated using these equivalences: 1µg of 1bp dsDNA = 1626pmol and 1µg of 1nt ssDNA = 3250.4pmol. na: non-applicable, bp: base pairs, nt: nucleotides, nd: non-determined.

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

locus	crRNA sequences (+PAM)	Note
	GGCCTTAACCCAGAATAAGA(TGG)	5' crRNA (AP691): used in Figure 4-panel I together with 3' crRNA (AP728)
	CACGAGGTGGTATGCGCAG(CGG)	3' crRNA (AP728)
	TCCCTCAAGATGAAGAAGGC(TGG)	3' crRNA (AP723): used in Figure 4-panel G
	GGCAGGATGTCAGCCAGAGG(AGG)	Hotspot crRNA (AP872) in *meg-3* deletion: used in Figure 4-panel J

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--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagctttccgttctccttttttccttcttg	na (PCR repair template contains homology arms)
Figure 1-Table S1		GGCGGCGGATTCCGAGGAGGACGTGGAGGTTTCCAACCACGCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGT GCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagctttccgttctccttttttccttcttgtaagaatgcacatccattagattctgtacctatccttctc a	na (PCR repair template contains homology arms)
Figure 1-Table S1		GCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagagtgataaatgcaaaatcctttcaagcattcccc	na (PCR repair template contains homology arms)
Figure 1-Panel C		CATGATATCGATTACAAGGATGACGATGACAAGGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---GFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaagagtgataaatgcaaaatcctttcaagcattcccc	na (PCR repair template contains homology arms)
Figure 1-Panel D		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---tagaagctttccgttctccttttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel E		GTTTCCAACCACGCGGTGGACAATTCTTCCGCCGT---eGFP---tagaagctttccgttctccttttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel F		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---Myc---AGCGGTTTCCAAAATGCGGGACAAAAT---eGFP--- tagaagctttccgttctccttttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel G		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---AGCGGTTTCCAAAATGCGGGACAAAAT---PH--- tagaagctttccgttctccttttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 1-Panel H		GGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP---AGCGG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*C--- PH---tagaagctttccgttctccttttttccttcttgtaag	na (PCR repair template contains homology arms)
Figure 2-Panel A		na	ggttcgggtggtgctccacgaggtggtatgcgcgtgtgtgaga ggagaagaacttttcactggagttg
Figure 2-Panel C		na	gggattacactcggcatggacgagctatacca agagcggtttccaaaatgcgggacaaaattagaag
Figure 2-Panel E		na	ggttcgggtggtgctccacgaggtggtatgcgcaagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgagctgagagtt ccgagaagcggtttccaaaatgcgggacaaaattagaag
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	GGCGGCGGATTCCGAGGAGGACGTGGAGGTTTCCAACCACGCGGTGGACAATTCTTCCGCCGTGGCGGTTCGGGTGGTGCTCCACGAGGTGGTATGCGCTGAGTAAA GGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTG
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	ggttcgggtggtgctccacgaggtggtatgcgcaagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgagctgagagtt c and catatgctcacggacaagatgagctgagagttccgagagactacaaagaccatgacggtgattataaagatcatgatatcgattacaaggatgacgatgacaagagcggtttccaaaatgcgggacaaaattagaag
Figure 4-Panel B		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---eGFP::22nt of ZF1 and 19nt of eGFP::ZF1--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Panel C		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 D		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 E		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---meGFP::Linker and Linker::mNeon::3Xflag and 3Xflag::tagRFP--- AGCGGTTTCCAAAATGCGGGACAAAATtagaag	na (PCR repair templates are overlapping and contain homology arms)
Figure 4-Panel G		---eGFP---	CCAGTGTACCAACTCAAGTCCCTCAAGA*CGA*GGAAGG*ATGGGTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTG and GGGATTACACTCGGCATGGACGAGCTGTACAAGtagaaaccgaccaattgatagtgttcgcatttattaatgc
Figure 4-Panel H		GGTTCGGGTGGTGCTCCACGAGGTGGTATGCGC---mNeon	CGGACGTAATGGGAATGGACGAACTCTACAAG::3Xflag(GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG)::AG CGGTTTCCAAAATGCGGGACAAAATtagaag
Figure 4-Panel I		---*meg-3* Nt domain---	aaATGCAGATAACCTCGGCCTTAACCCAGAATAAGATGAGTTCCTCAAAACCTTACCCAAGCGGTCTGCC and CAAATGACGATGAATTAACTGACGATGTGTTCATAGAC::hotspot(GGGAAGTTTGTCCAGAGCAGAGGA)::ollas(TCCGGATTCGCCAACGAGCTCGGACCACGTCTCAT GGGAAAG)tga::cagcggtttccaaaatgcgggacaaaattagaagc
Figure 4-Panel J		TCAAAACCTTACCCAAGGGCAGGATGTCAGCCAG---first half of mutated *meg-3* and second half of mutated *meg-3*---agggagggatccctaattgtaccaatttatatctattac	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 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
eGFP	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAA CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAG
GFP (with 3 introns)	AGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGgtaagtttaaacatatatatactaactaa ctgattatttaaattttcagCCAACACTTGTCACTACTCTTGTTATGGTGTTCAATGCTTCTCGAGATACCCAGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTaagtttaaacagttcggtactaacta atacatatttaaattttcaggtGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGACGAACTATACAAA
eGFP::recoded * *gtbp-1* ::PH	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAA CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGCGG*CTT*TCA*GAA*CGC*CGG*TCA*GAA*CCACGGGCTCCAGGATGACCCGGACCTTCAGGCCCTTCTGAAGGGCAGCCAGCTTCTGAAGGTGAAGTCCAGCTCGTGGCGTAGGGA GCTTCTACAAGCTACAGGAGGACTGCAAGACCATCTGGCAGGAATCTCGAAAGGTCATGAGGTCCCCGGAGTCGCAGCTGTTCTCCATCGAGGACATTCAGGAGGTACGGATGGGACACCGCACAGAAGGCCTGGAGAAGTTTGCCCGAGACATCCCCGAGGATCGATGCTTCTCCATTGTCTTCAAGGACCAGCGCAACACCC ACCTCATTGCCCCATCACCAGCTGACGCTCAGCACTGGGTGCAGGGCCTGCGCAAGATCATCCACCACTCCGGCTCCATGGACCAGCGGCAGAAG
eGFP::ZF1 domain	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAA CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGAAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGA
eGFP::ollas::PH domain	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAA CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGTCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAGCACGGGCTCCAGGCATCTTCGAGGCTGAAGTCCAGCTCGTGGCGTAGGGA AGGGAACGCTTCTACAAGCTACAGGAGGACTGCAAGACCATCTGGCAGGAATCTCGAAAGGTCATGAGGTCCCCGGAGTCGCAGCTGTTCTCCATCGAGGACATTCAGGAGGTACGGATGGGACACCGCACAGAAGGCCTGGAGAAGTTTGCCCGAGACATCCCCGAGGATCGATGCTTCTCCATTGTCTTCAAGGACCAGCGCAACACCC CACCCTAGACCTCATTGCCCCATCACCAGCTGACGCTCAGCACTGGGTGCAGGGCCTGCGCAAGATCATCCACCACTCCGGCTCCATGGACCAGCGGCAGAAG
meGFP::Linker::mNeonGreen	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTAAGCTTTCGAAA CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAGTCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAGCACGGGCTCCAGGCATCTTCGAGGCTGAAGTCCAGCTCGTGGCGTAGGGA AACGGTGTCGATTTCGACATGGTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAG gtaagtttaaacatatatatactaactaaccctgattatttaaattttcag GGGGACCTCCAATTTTCCCCATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATG CCATTTCAAGCGGCCATGGTAGACGGAAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAGTCAAAGGAACTGGATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTACC AAAAAGACGTACCCAAATGACAAG gtaagtttaaacagttcggtactaactaaccatacatatttaaattttcag ACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGACGAGCTACACGTTCGCCAAGCCCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACC GCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGGAATGGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG
meGFP::Linker::mNeonGreen (with 2 introns)::3Xflag::tagRFP	GTGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTCTGACCTACGGCGTGCAG TTCAGCCGGTACCCCGACCACATGAAGCAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACAC ATTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTAAGCTTTCGAAAG CCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACTCGGCATGGACGAGCTGTACAAG TGCCCGGGGGATCGGTGGAGCTCCACCGGTGGCGGCCGCTCTAGAACTAGT GTCTCCAAAGGGGAGGAAGACAACATGGCGTCCCTCCAGCGACGCACGAACTCCATATCTTTGGGTCCA AACGGTGTCGATTTCGACATGGTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAG gtaagtttaaacatatatatactaactaaccctgattatttaaattttcag GGGGACCTCCAATTTTCCCCATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATG CCATTTCAAGCGGCCATGGTAGACGGAAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAGTCAAAGGAACTGGATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTACC AAAAAGACGTACCCAAATGACAAG gtaagtttaaacagttcggtactaactaaccatacatatttaaattttcag ACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGACGAGCTACACGTTCGCCAAGCCCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACC GCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGGAATGGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG GTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACAT AGGGCACCGTGAACAACCACCACTCAAATGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTTGACATCCTGGCTACCAGCTTCATGTACGGCAGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTTCC GAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGTCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCAATACCGAGATGCTGTACCCCGCTGACGGCGGC TGGAAGGCAGAAGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAATTTCAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAAGAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACT CGACCTCCCTAGCAAACTGGGGCACAAACTTAAT
mNeonGreen (with 2 introns)::3Xflag	GTCTCCAAAGGGGAGGAAGACAACATGGCGTCCCTCCAGCGACGCACGAACTCCATATCTTTGGGTCCATAAACGGTGTCGATTTCGACATGGTAGGACAAGGGACCGGAAATCCAAATGATGGATATGAGGAGCTAAACCTCAAGAGTACCAAG gtaagtttaaacatatatatactaactaaccctgattatttaaattttcag GGGGACCTCC/ TTTCCCCATGGATACTCGTCCCCCATATCGGGTACGGATTCCATCAATACCTACCATATCCAGATGGGATGTCCCCATTTCAAGCGGCCATGGTAGACGGAAGTGGATACCAAGTACATCGTACGATGCAATTTGAGGACGGGGCCTCCCTAACCGTCAACTATCGTTACACGTACGAGGGAAGTCACATCAAAGGAGAGGCGCAAG AAAGGAACTGGATTTCCAGCCGATGGACCAGTCATGACGAATTCCCTAACCGCCGCCGATTGGTGTCGTAGTAAAAAGACGTACCCAAATGACAAG gtaagtttaaacagttcggtactaactaaccatacatatttaaattttcag ACCATAATCTCCACGTTCAAGTGGTCCTATACCACCGGGAACGGAAAGCGGTACCGTAGTACGGCCCGGAC CGTACACGTTCGCCAAGCCCATGGCCGCTAATTACCTGAAGAATCAGCCAATGTATGTCTTCCGTAAAACCGAGCTAAAGCACTCCAAAACCGAGCTAAACTTCAAGGAATGGCAAAAGGCGTTCACGGACGTAATGGGAATGGACGAACTCTACAAG GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTAC GGATGACGATGACAAG
meg-3 Nt domain (with endogenous intron)::hotspot::ollas	ATGAGTTCCTCAAAACCTTACCCAAGCGGTCTGCCAAACTCGAGAAGAAAAAGAGGAG gtgagagttttttgcatcaattttcattcaatttgtaaattcagGTCGCCGCTCAAGTAGTCGTAGCAATCAGGAAAGTGCATCCAACAACATGGAACACCAAATCACTCTTGACGAATTGTTCAATCCGATCGCAAAACAAC CAGTGCTCAGTCAACAAGCCGTGAATATGGTGCAAAATCGGGGATATCTCATCACGGATCCGTATCATTCAACGGGAATACATTCATGAATGGCCAACAGCTGAATCACTCGATGACTCGACATGGTCGAGTATTCAATCAGTCGATGCATGCAGCTCAAGGAAACGGCAGCAACGCCTTTAACAGTATT CTCCTACGGCCCCCGTCTTCTCTGCAGACTTCCGCCGCAACTTGCAAACACGCAATAGCTCCAGCTGGTATGAACGAAGATTCCCAGTATCCACAGATCAGGACGCAGCTTCAGCAATCAAACACAAGACGTAGTAGAAGTCGTCAGAATGGACAACATGGCCTAAGCTTCTCGGATGGTTCGAACAATTA GGTCACGCTGGGAATAAGTCATTCAGTGTGAGTTCCGTTCCCGTTGGGTTCCAGAAGCAAGAAAATAATTCTAAGAAACTGCGACAAACCAATGTTCACCAGCAATGTCTTGGAAATAAAAGCTTCAACGCTCAAGCTGGAGTTCATGGACACGCTTTTAAAAAAGGCCATAAGGACAATAAAAATGCAT CTGGAAAGGAGGTTATCAATTCTAGTCTTGCAAAAGCACGATGCTATTAAGTCGCGGAATTTGAACCAGAGCTTCTCTGGATTTCCTACACATGAAACCTCATCGATGAAAAATCAACAACAGAAATCAAGAAACGACAGAAAAAAATCACGTGGTAGCAGCAACTTCCAGGATCGTACTTACTTCAAC ACAAATGACGATGAATTAACTGACGATGTGTTCATAGAC GGGAAGTTTGTCCAGAGCAGAGGA TCCGGATTCGCCAACGAGCTCGGACCACGTCTCATGGGAAAGtga
mutated *meg-3* coding sequence (122 mutations spread on 1.7 kb) (with endogenous introns)	ACGGTCTGCCAAACGCTAGAAGAAAAAGAGGAG gtgagagttttttgcatcaattttcattcaatttgtaaattcagGTCGCCGCGCAGCTGCTCGTGCCAATCAGGAAGCTGCAGCCAACAACATGGAACACCAAATCACTCTTGACGAATTGTTCAATCCGATCGCAAAACAAGACGCTGCTCAGGCAACAGCCCGTG ATATGGTGCAAAAGCGGGGATAGCTCATCACGGAGCCGTAGCATTCAACGGGAATACATTCATGAATGGCCAACAGCTGAATCACGCTATGACTCGACATGGTCGAGTATTCAATCAGGCCATGCATGCAGCTCAAGGAAACGGCGCCAACGCCTTTAACGCTATTCCTCCTACGGCCCCCGTCTTCGCT CAGACTTCCGCCGCAACTTGCAAACACGCAATGCCGCCGCCTGGTATGAACGAAGATTCCCAGTAGCCACAGATCAGGACGCAGCTTCAGCAAGCAAACACAAGACGTGCTAGAGCTCGTCAGAATGGACAACATGGCCTAGCCTTCGCGGATGGTGCGAACAATTATGGTCACGCTGGGAATAAGGCA TCGCTGTGGCTGCCGTTCCCGTTGGGTTCCAGAAGCAAGAAAATAATGCTAAGAAACTGCGACAAACCAATGTTCACCAGCAATGTCTTGGAAATAAAGCCTTCAACGCTCAAGCTGGAGTTCATGGACACGCTTTTAAAAAAGGCCATAAGGACAATAAAAATGCAGCTGGAAAGGAGGTTATCAATT TGCTCTTGTCCAAAAGCACGATGCTATTAAGGCTCGGAATTTGAACCAGGCCTCGCTGGATTTCCTACACATGAAACCGCAGCTATGAAAAATCAACAACAGAAAGCAAGAAACGACAGAAAAAAAGCACGTGGTGCCGCCAACTTCCAGGATCGTACTTACTTCAACACAAATGACGATGAATTAACT ACGATGTGTTCATAGACGATGCCATGGATGCTGCCCGTGGTCGGAGAGCTCGAGCAGTCACTAAGAAACTTCAACAAGCGACTTATGCAAAGCAGAATGCAGGCGCTAAACAATTGACTGAAAAATGCAAAGCTGCTGAAGAAGCGGCTAAACGTAACCTGGTAGCCAATGTATTCGCCAAGGACGGC ACTGAACTCGCCATTGAACAGTTATTGGAAATTGTCGCCATGAAGATCGGACAACAGATTCACTTGCCTGCCGCCGCTCATGGAGAATGCGCCAATTTGAATCGGACATTACCAGCAGCTGATCTAAATTGCGCAATTGGTGAAGACTTTGACGCCGCTTTTGTGGATGCTAACAACCAAACCCTACCAGT GGCTCTTCCCAAGAAAACTGCACTGGCGATCAAGCGCCGTGGAGCTGCTCGTGCTGCCGCCCGTCTGGCCGCTCTTGATGTGACACTGGAAACTGTTGAAGAAGCAGGAAACCCACGCCAGCTCCACGCCGGCTGCTCCTCCAAAAGCGGCTAACCGTAACCTGGTAGCCAATGTATTCGCCAAGGACGGC GAGGAAATGAGACGTTTACTTCATGGGGACCCTGAGATGCCGAAAGCTGCAAATCGTGCTGCCGCAAG gtatgtagtgaaatagtaaaatctttagtaaaaatttaaatttcagCAAGGATCAGATCAATCGGAACAATGTGGATGTAAAGAGAAACACCTGCCGGGAAGTTTGTCCAGAGCAGAGGATCCGGATTCGCC ACGAGCTCGGACCACGTCTCATGGGAAAGtga
Myc	GAACAAAAACTGATATCAGAAGAGGATCTG
ZF1 domain	AAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGA
ZF1 domain::3Xflag	AAGCACACAGAATACAAAACGCGACTTTGTGATGCGTTCCGCCGTGAAGGATACTGCCCGTACAACGACAATTGCACATATGCTCACGGACAAGATGAGCTGAGAGTTCCGAGA GACTACAAAGACCATGACGGTGATTATAAAGATCATGATATCGATTACAAGGATGACGATGACAAG

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*	ggttccggatggtgctccacgaggtggtatgcgcgtgagtaaaggagaagaacttttcactggagttg
830	S	Bridging 3'end eGFP (contain *BsrGI* site) with *gtbp-1*	gggattacactcggcatggacgagcgtatacaagcggtttccaaaatgcgggacaaaattagaag
1013	S	Bridging first and second halfs of eGFP together	gacggaactacaagacacgtgctgaagtcaagtttgaaggtgataccctttgttaatagaatcg
1015	AS	as 829 but inversed polarity	caactccagtgaaaagttcttctccttttactcagcgcatacaccatcgtggagcaccacccgaacc
1016	AS	as 830 but inversed polarity	cttctaatttttgtcccgcattttggaaaccgctctgtatacg gctcgtccatgccgagtgtaatccc
1267	AS	Bridging 3'end mNeonGreen with *gtbp-1* and inserting a 3XFlag tag	cttctaatttttgtcccgcattttggaaaccgctcttgtcatcgtcatccttgtaatcgatatcatgatcttttataatcaccgtcatggtctttgtagtccttgtagagtt cgtccattcccattacgtccg
1349	S	Bridging 5'end eGFP with *glh-1*	ccagtgtaccaactcaagtcccttcaagacgaggaaggatgggtgagtaaaggagaagaacttttcactggagttg
1350	AS	Bridging 3'end eGFP with *glh-1*	gcattaataaatgcgaaacactatcaattggtcggttttctacttgtacagctcgtccatgccgagtgtaatccc
1363	AS	as 1365 but inversed polarity	ctcatcttgtccgtgagcatatgtgcaattgtcgttgtacgggcagtatccttcacggcggaacgcatcacaaagtcg
1365	S	Partial ZF1	cgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgacaattgcacatatgctcacggacaagatgag
1376	S	Partial ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcaagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatac
1377	S	Partial ZF1 with *gtbp-1* homology arm	gacaattgcacatatgctcacggacaagatgagctgagagttccgagaagcggtttccaaaatgcgggacaaaattagaag
1378	S	ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcaagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgac aattgcacatatgctcacggacaagatgagctgagagttccgagaagcggtttccaaaatgcgggacaaaattagaag
1379	S	Partial ZF1 with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcaagcacacagaatacaaaacgcgactttgtgatgcgttccgccgtgaaggatactgcccgtacaacgac aattgcacatatgctcacggacaagatgagctgagagttc
1380	S	Partial ZF1 and 3Xflag with *gtbp-1* homology arm	catatgctcacggacaagatgagctgagagttccgagagactacaaagaccatgacggtgatatataaggatcatgatatcgattacaaggatgacgatgac aagagcggtttccaaaatgcgggacaaaattagaag
1394	S	Bridging 5'end of *meg-3* Nt domain with 5' end of *gtbp-1*	aaatgcagataacctcggccttaacccagaataagatgagttcctcaaaccttacccaagcggctgcc
1395	AS	Bridging 3'end of *meg-3* Nt domain with 3'end of *gtbp-1* and inserting a ollas tag and hotspot	gcttctaatttttgtcccgcattttggaaaccgctgtcacttttcccatgagacgtggtccgagctcgttggcgaatccggatccctctgctctggacaaactccgtc tatgaacacatcgtcagttaattcatcgtcatttg
1407	S	Left *gtbp-1* homology arm with *Xba1* site	ggttcgggtggtgctccacgaggtggtatgcgcgtgtctaga ggagaagaacttttcactggagttg
1408	AS	as 1407 but inversed polarity	caactccagtgaaaagttcttctccttctaga cacgcgcatacacctcgtggagcaccacccgaacc
PCR primers for linear repair templates			
436	F	*gtbp-1* ~60 bp homology arm amplification (plasmid AP554-1)	gcggtggacaatttcttccgccg
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)	ggcggcggatttccgaggaggac
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)	ggcggtttcgggtggtgctccac
604	R	*gtbp-1* homology arm amplification (plasmid 1297-1 and 1298-1)	cttacaagaaggaaaaaggag
645	F	eGFP/meGFP amplification with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcgtgagtaaaggagaagaac
646	R	eGFP amplification with *gtbp-1* homology arm	cttctaatttttgtcccgcattttggaaaccgctcttgtacagctcgtccatgcc
715	F	mNeonGreen amplification with *gtbp-1* homology arm	ggttcgggtggtgctccacgaggtggtatgcgcgtcttcccaaaggggaggaagaac
831	F	eGFP amplification without homology arm	gtgagtaaaggagaagaac
832	R	eGFP amplification without homology arm	cttgtacagctcgtccatg
1011	F	Second half of eGFP amplification	gtttgaaggtgataccctttg
1012	R	First half of eGFP amplification	ttgacttcagcacgtgtcttg
1014	R	First half of eGFP amplification with overlapping sequence with the second half	cgattctatttaacaaggggtatc
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	cttctaatttttgtcccgcattttggaaaccgctcttgtagagttcgtccattc
1085	R	tagRFP amplification with *gtbp-1* homology arm	cttctaatttttgtcccgcattttggaaaccgctattaagtttgtgccccag
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	gacttcccctcgtacgtg
1255	F	Linker::mNeonGreen amplification	gagctccaccggtgg
1256	R	mNeonGreen::3Xflag amplification	gatctttataatcaccgtcatggtc
1263	R	eGFP amplification with ollas homology arm	ccatgagacgtggtccgagctcgttggcgaatccggaacttgtacagctcgtccatg
1264	F	PH amplification with ollas homology arm	gattcgccaacgagctcggaccacgtctcatgggaaagcacgggctccaggatgacc
1265	R	PH amplification with *gtbp-1* homology arm	cttctaatttttgtcccgcattttggaaaccgctcttctgcccgctggtccatg
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	cttctaatttttgtcccgcattttggaaaccgcttctcggaactctcagctcatcttg
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	ggcggttcgggtggtgctccacgaggtggtatgcgcgaaacaaaactgatatcagaag
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)	catgatatcgattacaaggatgacgatgacaaggtttcgggtggtgctccac
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	gtttccaaccacgcggtggacaattcttccgccgtgtgagtaaaggagaagaac
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	tcaaaaccttacccaaggcaggaatgtcagccagacggtctgccaaacgctagaagaaaaagaggaggtg
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	gtaatagatataaattggtacaattaggatcccctcctcacttttcccatgagacgtggtccgagctcgttggcgaatccggatcctctgctctggacaaactccc ggcaggtgttctctttacatccacatgttccgattg
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
1510	F	*meg-3* genotyping	cattcttgttgcgtggtccc
1511	R	*meg-3* genotyping	tcacaaggagaagcgagttg

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	*gtbp-1::eGFP::ZF1*
Figure 4-Panel C	*gtbp-1::eGFP::ollas::PH*
Figure 4-Panel D	*gtbp-1:: meGFP::Linker::mNeon*
Figure 4-Panel E	*gtbp-1:: meGFP::Linker::mNeon::3Xflag::tagRFP*
Figure 4-Panel G	*glh-1::eGFP*
Figure 4-Panel H	*gtbp-1::mNeon::3Xflag*
Figure 4-Panel I	*gtbp-1 replacement with meg-3 Nt domain::hotspot::ollas*
Figure 4-Panel J	*mutated meg-3 coding sequence (122 mutations spread on 1.7 kb)*