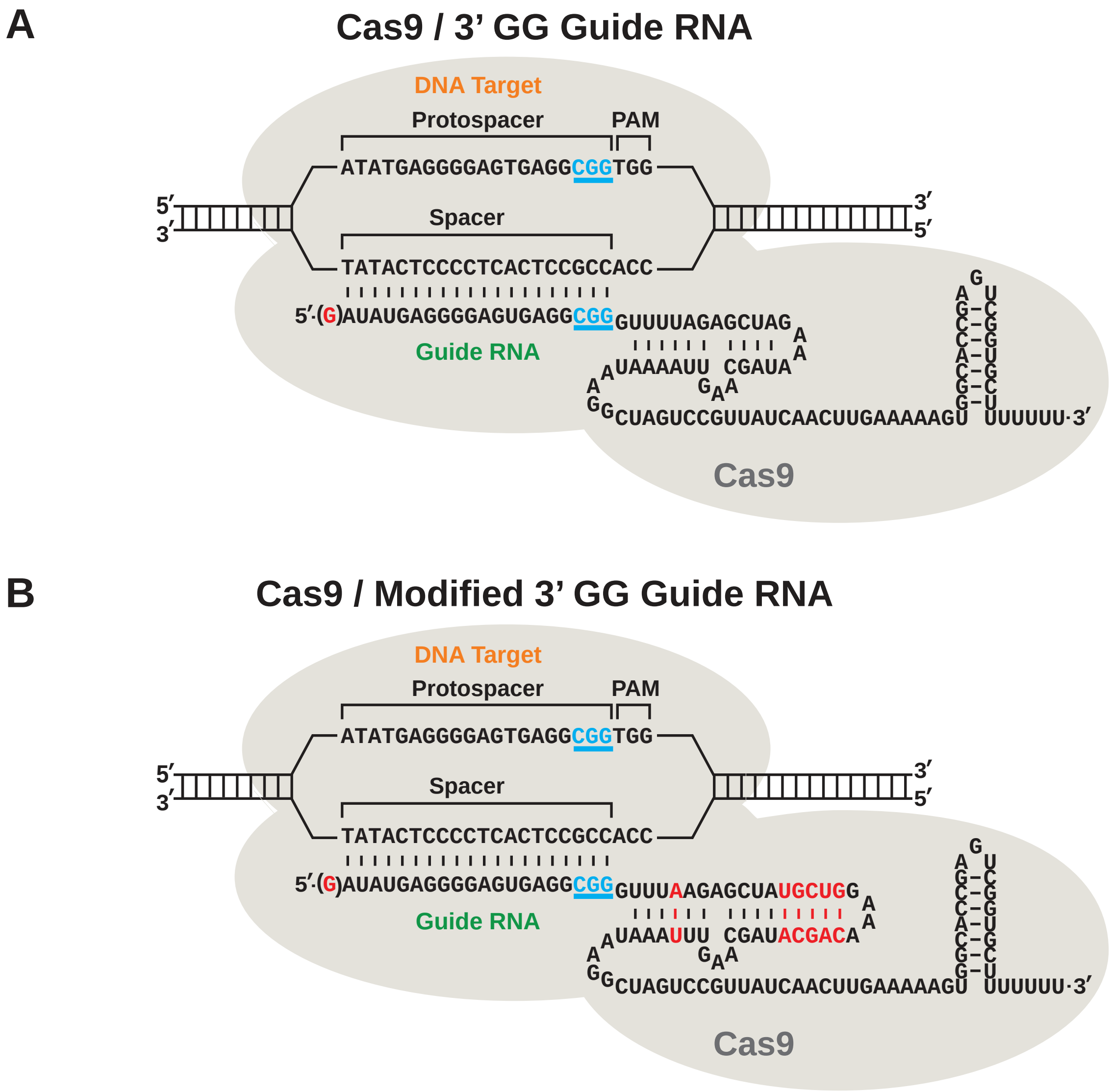

C

fox-1 guides	F_1s with transformation markers	Heterozygous F_1 mutants	Mutagenesis rate (%)
Guide (1)	38	11	29
Modified Guide (1)	94	23	25
Guide (2)	62	8	23
Modified Guide (2)	57	8	14
Guide (3)	94	20	21
Modified Guide (3)	76	18	23

Figure S1 Alternate guide RNA design with more extensive stem-loop structures does not enhance the Cas9 mutagenesis frequency achieved by 3' GG guide RNAs. (A-B) Shown are diagrams of two related guide RNAs bound in a complex with Cas9 to the same DNA target site. The 3' GG guide RNAs target Cas9 to sites with an NGG motif at the 3' end of the protospacer. The modified guide has the same target specificity as the 3' GG guide, but has a longer stem-loop structure, and an A-U flip that improved its efficacy in mammalian cell Cas9 experiments (Chen *et al.* 2013). (C) The addition of the longer stem-loop structure to three different 3' GG guide RNAs corresponding to three different targets in *fox-1* did not improve the frequency of mutagenesis directed by Cas9. Experiments were conducted as those presented in Figure 2.

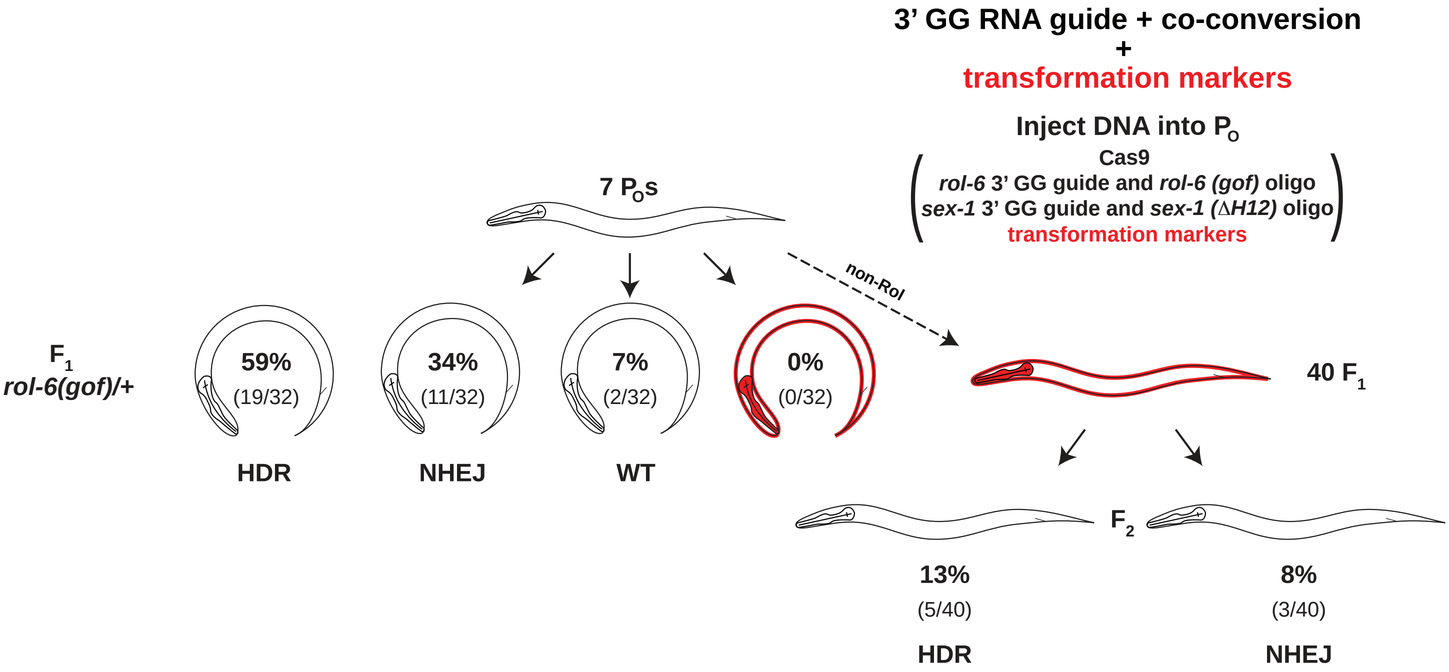

Figure S2 Comparison of the co-CRSPR / co-conversion scheme plus 3' GG guide RNA with the transformation marker scheme plus the 3' GG guide RNA. From 7 of the 61 P0 animals injected with Cas9, the *rol-6* and *sex-1* guides expressed from the R07E5.16 promoter, the *rol-6* and *sex-1* oligo repair templates, and the two DNA transformation markers *Pmyo-2::mCherry* and *Pmyo-3::mCherry,* we obtained Rol animals and red animals, with no overlap between the Rol and red animals. Of the Rol animals, 93% percent had a *sex-1* mutation: 59% from HDR repair and 34% from NHEJ repair. Of the red animals, 21% had a *sex-1* mutation: 13% from HDR repair and 8% from NHEJ repair. The reduced frequency of *sex-1* mutations among red animals (21% instead of 51%) in this experiment compared to that in Figure 2A is most likely due to the reduced concentration of *sex-1* guide RNA to make it equal that of the *rol-6* guide RNA. Thus, the easiest and most effective strategy to obtain mutations in loci of choice via HDR or NHEJ events is to combine our 3' GG guide RNA design with the co-CRISPR / co-conversion strategy.

Table S1 Summary of current and published editing frequencies using 3’ GG and non-GG guides

Our Guide Efficiencies

GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)	Publication
lir-2	3' GG	GGCTGATTTTCGCAGTTCGG (GGG)	GG	72	This study
Y62E10A.17	3' GG	CGCACCGATGCTCTCCGAGG (AGG)	GG	57	This study
sex-1	3' GG (1)	GGATGAGAATCTGACAAAGG (TGG)	GG	54	This study
cpsf-2	3' GG	CACTTTCAATTTGATAATGG (AGG)	GG	52	This study
sex-1	3' GG (2)	AACATTTCCACAACGAGAGG (AGG)	GG	51	This study
fox-1	3' GG (1)	ATATGAGGGGAGTGAGGCGG (TGG)	GG	29	This study
fox-1	3' GG (3)	ATTACAGTGAAGTACAGCGG (AGG)	GG	21	This study
fox-1	3' GG (2)	AATATCGTTTACCAAAACGG (GGG)	GG	13	This study
xol-1	3' GG	AGCGATTTCTGGCGATTGGG (GGG)	GG	10	This study
			median:	**51**	

Non-GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)	Publication
sex-1	3' GG-shift (1)	AACGGATGAGAATCTGACAA (AGG)	AA	21	This study
fox-1	3' GG-shift (1)	CATTTGATATGAGGGGAGTG (AGG)	TG	20	This study
Y62E10A.17	3' GG-shift	ATACGCACCGATGCTCTCCG (AGG)	CG	14	This study
sex-1	3' GG-shift (2)	TGGAACATTTCCACAACGAG (AGG)	AG	8	This study
lir-2	3' GG-shift	CTCGGCTGATTTTCGCAGTT (CGG)	TT	1	This study
cpsf-2	3' GG-shift	AAACACTTTCAATTTGATAA (TGG)	AA	0	This study
fox-1	3' GG-shift (2)	TTGAATATCGTTTACCAAAA (CGG)	AA	0	This study
fox-1	3' GG-shift (3)	ACAATTACAGTGAAGTACAG (CGG)	AG	0	This study
xol-1	3' GG-shift	TCTAGCGATTTCTGGCGATT (GGG)	TT	0	This study
cpsf-2	3' non-GG (1)	GTGGTTGGGATGAGCGATTC (GGG)	TC	0	This study
lir-2	3' non-GG (1)	AATCAGCCGAGATGTAAGTT (TGG)	TT	0	This study
lir-2	3' non-GG (2)	TTGACTCGTTCCATTTCAGC (TGG)	GC	0	This study
sex-1	3' non-GG (1)	AAACCTGCCTCCTCTCGTTG (TGG)	TG	0	This study
			median:	**0**	

Published Guide Efficiencies

GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)	Publication
klp-12		GATCCACAAGTTACAATTGG (TGG)	GG	80.3	Friedland *et al.* 2013
vet-2		GTTGGATCATAGGATACCGG (TGG)	GG	38	Kim *et al.* 2014
			median:	**59**	

Non-GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)	Publication
C35E7.6		GGGCACCATACCGAGTGATG (GGG)	TG	100	Kim *et al.* 2014
lon-2		GGGAAACTATACCCTCACTG (TGG)	TG	30	Kim *et al.* 2014
pie-1	a	GGCTCAGATTGACGAGGCGC (CGG)	GC	24	Kim *et al.* 2014
lin-5		GGAGCTTACTGAGACTCTTC (GGG)	TC	20.8	Waaijers *et al.* 2013
avr-14	(2)	GATTGGAGAGTTAGACCACG (TGG)	CG	20	Kim *et al.* 2014
Y61A9LA.1		GGATGGATGTGTAGTCAATT (CGG)	TT	18.1	Friedland *et al.* 2013
pie-1	b	GCTGAGAGAAGAATCCATCG (GGG)	CG	15	Kim *et al.* 2014
avr-14	(1)	GAATATTGAAAGACTATGAT (TGG)	AT	10	Kim *et al.* 2014
unc-4	(1)	GTTATCGTCATCCGGTGACG (TGG)	CG	10	Kim *et al.* 2014
dpy-11		GCAAGGATCTTCAAAAAGCA (TGG)	CA	10	Waaijers *et al.* 2013
pie-1	c	GGACAAAGAGAGGGGGTGAG (TGG)	AG	7.5	Kim *et al.* 2014
unc-22	(2)	GAACCCGTTGCCGAATACAC (AGG)	AC	5	Kim *et al.* 2014
pie-1	d	GTTGAGTGCAGCCATTTGCT (CGG)	CT	5	Kim *et al.* 2014
unc-119		GTTATAGCCTGTTCGGTTAC (CGG)	AC	4.9	Waaijers *et al.* 2013
unc-119		GAATTTTCTGAAATTAAAGA (CGG)	GA	3.7	Friedland *et al.* 2013
rol-1		GGAGGTTGACTCCAATACTA (AGG)	TA	1.4	Waaijers *et al.* 2013
dpy-13		GGACATTGACACTAAAATCA (AGG)	CA	0.5	Friedland *et al.* 2013
dpy-11	(2)	GCAAGGATCTTCAAAAAGCA (CGG)	CA	0.4	Kim *et al.* 2014
ben-1	(5)	GGGAGAAAGTGATTTGCAGT (TGG)	GT	0	Kim *et al.* 2014
ben-1	(3)	GGATATCACTTCCCAGAACT (TGG)	CT	0	Kim *et al.* 2014
bli-2	(2)	GATGGACGGGATGGTAGAGA (TGG)	GA	0	Kim *et al.* 2014
bli-2	(1)	GGATTTGCTGCTACTGAATC (CGG)	TC	0	Kim *et al.* 2014
dpy-5	(2)	GTCGGATTCGGCGCTGCATG (CGG)	TG	0	Kim *et al.* 2014
dpy-5	(3)	GGTTTCCTGGAGCTCCGGCT (GGG)	CT	0	Kim *et al.* 2014
dpy-11	(4)	GATGCTTGTAGTCTGGAACT (GGG)	CT	0	Kim *et al.* 2014
unc-22	(9)	GCCTTTGCTTCGATTTTCTT (TGG)	TT	0	Kim *et al.* 2014
unc-32	(1)	GATAGGAAGCATCAGATTGA (AGG)	GA	0	Kim *et al.* 2014
unc-32	(2)	GTTGCTGAACTGGGAGAGCT (CGG)	CT	0	Kim *et al.* 2014
			median:	**4.3**	

Table S2 Comparison of observed versus predicted guide RNA editing efficiency using the Doench *et al.* 2014 algorithm

GG Guides

Target Gene	Guide RNA	Protospacer Sequence (PAM)	sgRNA Bases 19,20	Mutagenesis Rate (%)	sgRNA Score (Doench *et al*. 2014)
lir-2	3' GG	GGCTGATTTTCGCAGTTCGG (GGG)	GG	72	no value
Y62E10A.17	3' GG	CGCACCGATGCTCTCCGAGG (AGG)	GG	57	0.041
sex-1	3' GG (1)	GGATGAGAATCTGACAAAGG (TGG)	GG	54	0.198
cpsf-2	3' GG	CACTTTCAATTTGATAATGG (AGG)	GG	52	0.063
sex-1	3' GG (2)	AACATTTCCACAACGAGAGG (AGG)	GG	51	0.439
fox-1	3' GG (1)	ATATGAGGGGAGTGAGGCGG (TGG)	GG	29	0.178
fox-1	3' GG (3)	ATTACAGTGAAGTACAGCGG (AGG)	GG	21	0.749
fox-1	3' GG (2)	AATATCGTTTACCAAAACGG (GGG)	GG	13	0.422
xol-1	3' GG	AGCGATTTCTGGCGATTGGG (GGG)	GG	10	0.277

Non-GG Guides

sex-1	3' GG-shift (1)	AACGGATGAGAATCTGACAA (AGG)	AA	21	0.282
fox-1	3' GG-shift (1)	CATTTGATATGAGGGGAGTG (AGG)	TG	20	0.063
Y62E10A.17	3' GG-shift	ATACGCACCGATGCTCTCCG (AGG)	CG	14	0.887
sex-1	3' GG-shift (2)	TGGAACATTTCCACAACGAG (AGG)	AG	8	0.355
lir-2	3' GG-shift	CTCGGCTGATTTTCGCAGTT (CGG)	TT	1	no value
cpsf-2	3' GG-shift	AAACACTTTCAATTTGATAA (TGG)	AA	0	0.026
fox-1	3' GG-shift (2)	TTGAATATCGTTTACCAAAA (CGG)	AA	0	0.106
fox-1	3' GG-shift (3)	ACAATTACAGTGAAGTACAG (CGG)	AG	0	0.669
xol-1	3' GG-shift	TCTAGCGATTTCTGGCGATT (GGG)	TT	0	0.010
cpsf-2	3' non-GG (1)	GTGGTTGGGATGAGCGATTC (GGG)	TC	0	0.005
lir-2	3' non-GG (1)	AATCAGCCGAGATGTAAGTT (TGG)	TT	0	0.045
lir-2	3' non-GG (2)	TTGACTCGTTCCATTTCAGC (TGG)	GC	0	0.108
sex-1	3' non-GG (1)	AAACCTGCCTCCTCTCGTTG (TGG)	TG	0	0.156

This table provides a comparison of our observed editing frequencies using 3' GG and non-GG guides compared to the scores we derived using the algorithm from Doench *et al.* (2014) that predicts guide editing frequencies based on experiments in mammalian cells. See website: http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design. The presence of the 3' GG motif in the protospacer is a better predictor of editing outcome than the algorithm. A score of 1 by this algorithm indicates a highly efficient guide.