

Supplementary Information

1. Figure S1- Melted dsDNA donors promote homology directed repair
2. Figure S2- High doses of donor DNA reduce the number of Rollers
3. Figure S3- Precise genome editing using Cas12a nuclease and melted dsDNA donors
4. Figure S4- Quickly cooled donors are better repair templates than slowly cooled donors
5. Table S1- List of *C. elegans* strains
6. Table S2- Sequences of crRNAs
7. Table S3- Sequences of Oligos
8. File S1- Detailed editing protocol

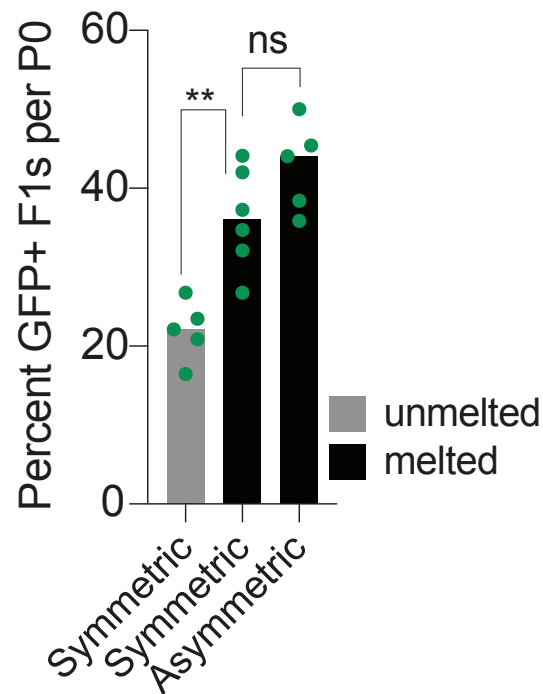


Figure S1: Melted dsDNA donors promote homology directed repair. HDR efficiencies at the *glh-1* locus using symmetric (unmelted or melted) and asymmetric donors (n=5 or 6 broods) without *rol-6* injection marker. Each data point (green) represents the percentage of animals expressing GFP among F1s scored per brood. Bars represent median. P-values (**, 0.0087 and ns, 0.1255) were determined by Mann Whitney test (unpaired, non-parametric, two-tailed)

glh-1

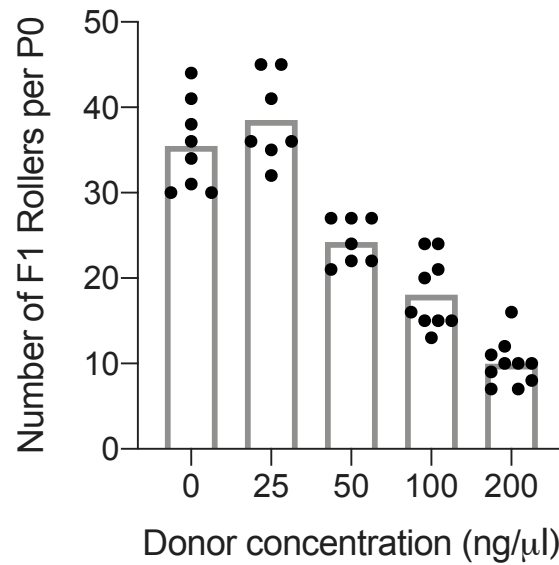
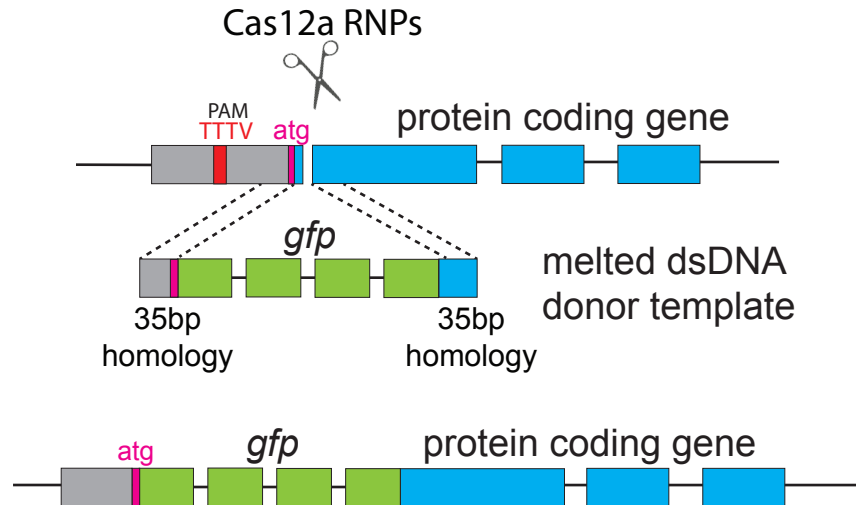


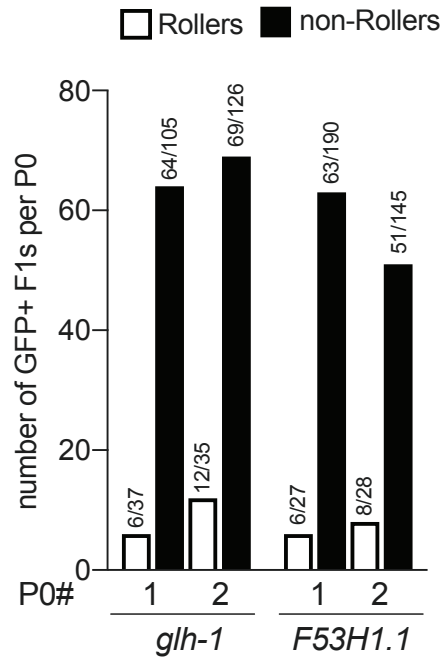
Figure S2. High doses of donor DNA reduce the number of Rollers. Injection mixes contain *Prf4::rol-6(su1006)*, Cas9 protein, crRNA targeting *glh-1* locus and *gfp::glh-1* dsDNA donor with ~35bp homology arms at indicated doses. Each dot represents the number of F1 rollers obtained per P0 animal and the bar represents mean; (n=7 to 10 broods per condition). dsDNA donors were not melted.

Cas12a based genome editing

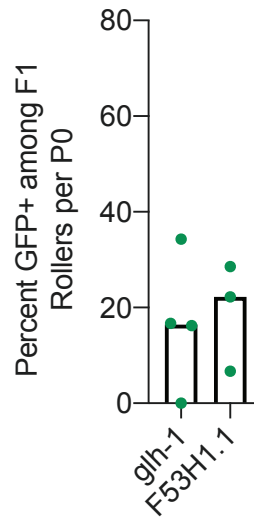
A



B



C



D

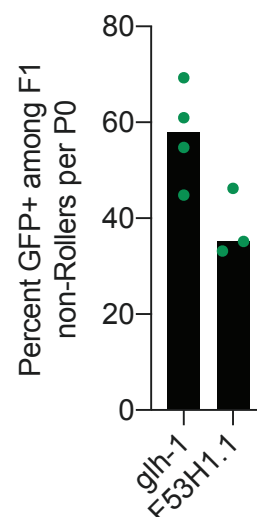


Figure S3. Precise genome editing using Cas12a nuclease and melted dsDNA donors. (A) Schematic representation of template dependent editing with Cas12a and melted dsDNA donor to insert *gfp* at the start codon (atg). Protospacer adjacent motif (PAM) for Cas12a system is TTTV, where V is A, C or G. HDR efficiencies at *glh-1* and *F53H1.1* loci are plotted as (B) number of GFP+ F1 animals among two representative broods, (C) percentage of GFP+ animals among F1 Rollers and (D) percentage of GFP+ animals among F1 non-Rollers; n= 3 or 4 broods. Number of GFP+ animals over number of animals scored are shown above the bars. Each data point represents the percentage of animals that are GFP+ among F1s scored in each cohort per brood and bars represent the median.

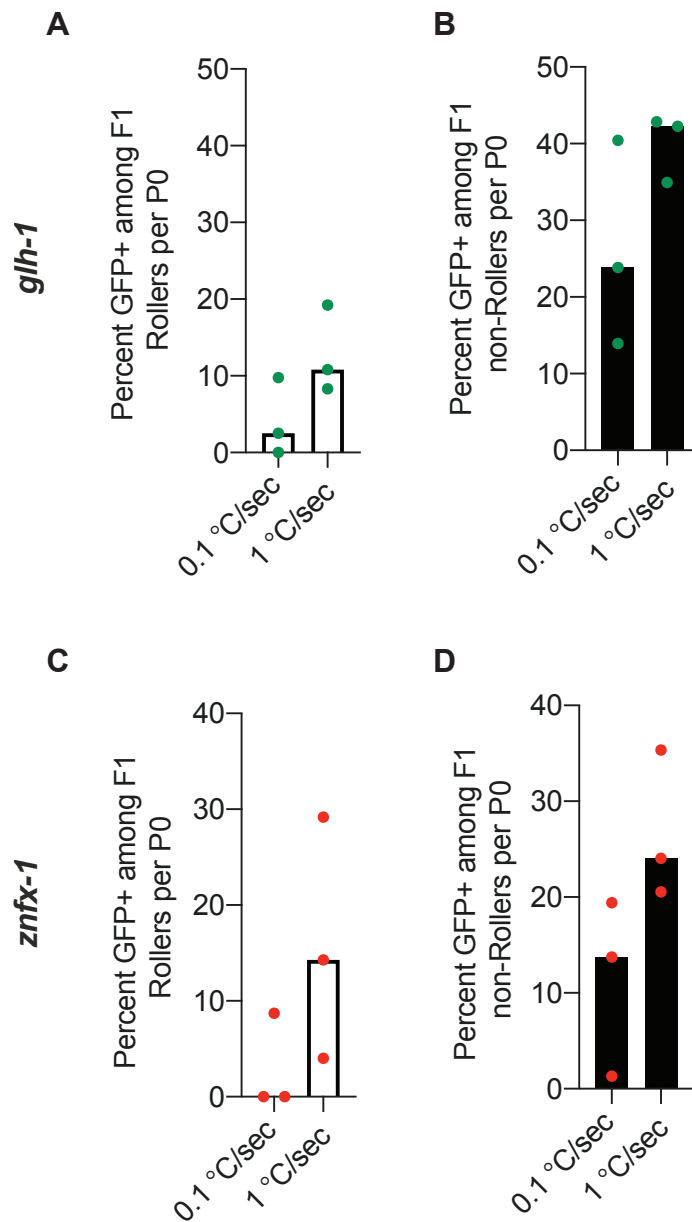


Figure S4. Quickly cooled donors act as better repair templates than slowly cooled donors. *gfp* (green dots) insertion efficiencies at *glh-1* loci are plotted for (A) Rollers and (B) non-Rollers using slow (0.1 °C/sec) and quick (1 °C/sec) cooled donors as percentage. (C and D) *mCherry* (red dots) insertion efficiencies at *znfx-1* locus. Each data point represents an F1 brood and bars represent median. Thermal cycler program for slow cooling: 95 °C - 2:00 min; 85 °C - 1:00 min; 75 °C - 1:00 min, 65 °C - 1:00 min, 55 °C - 1:00 min, 45 °C - 1:00 min, 35 °C - 1:00 min, 25 °C - 1:00 min, 4°C- hold. Ramp down rate: 0.1 °C/sec. P-value = 0.2 for panels A, B, C and 0.1 for panel D (Mann-witney test, two-tailed). See **File S1** for quick cooling conditions.

Supplemental Table S1: List of *C. elegans* strains

Genotype	Strain Name
F53H1.1(ne4815[gfp::F53H1.1]) IV	WM703
glh-1(ne4816[gfp::glh-1]) I	WM704
csr-1(ne4483[flagx3::linker::tev] IV	WM705
znfx-1(ne4817[mcherry::znfx-1]) II	WM706
hrde-1(ne4818[gfp::hrde-1]) III	WM707
csr-1(ne4819[flagx3::linker::gfp::tev::csr-1]) IV	WM708

Supplemental Table S2: Sequences of crRNAs

ID#	Target locus	Sequence (5'→3')	Notes
CMG-18	<i>hrde-1</i>	CATAATTTTGTCTGAGCAAGT	To insert N-terminal tag
CMG-25	<i>csr-1</i>	AAGATGTTTCAGGGCAAGTCT	To insert N-terminal tag
CMG-33	<i>Flagx3::linker::tev</i>	TATAAAGACGATGACGATAA	To insert gfp at csr-1 locus
CMG-34	<i>glh-1</i>	TTTTCTGCGAAAATGTCTGA	To insert N-terminal tag
CMG-35	<i>glh-1</i>	TGCGAAAATGTCTGATGGTTG	To insert N-terminal tag; (A.s. Cpf1)
CMG-77	<i>F53H1.1</i>	TTCCAGTTTTCGATGGGTCG	To insert N-terminal tag
CMG-79	<i>F53H1.1(A.s. Cpf1)</i>	CAGTTTTTCGATGGGTCGCGGC	To insert N-terminal tag
CMG-88	<i>znfx-1</i>	AGGTTTCTGACCATTGAATA	To insert N-terminal tag

Supplemental Table S3: Sequences of oligos

ID#	Sequence (5'→3')	Notes
cmo-KG475F	ATTTTCTGGAAAAATCTTAA	<i>gfp::glh-1</i> donor
cmo-KG476R	TTAGCAGCACTTTCGCTATC	
cmo-KG830F	/SP9/TCGTTTCATCGTTTCTTATTTCAGTCAAACATGTCCGGAGGGAGTGGA	<i>gfp::hrde-1</i> donor
cmo-KG831R	/SP9/GTTGGAAGACGAACCTCCCATAAATTTTGTCTGAGCAAGTCTGCAGAACCTCCGCCACC	
cmo-KG832F	/SP9/ATCCAAAAATCCCCAATTTTTTCCAGTTTTCGATGTCCGGAGGGAGTGGA	<i>gfp::F53H1.1</i> donor
cmo-KG833R	/SP9/ACGCTTTCGTTTGTGCTCTTTGTGCTCGCCGCGACCAGAACCTCCGCCACC	
cmo-T1193F	CTTGTTTCAGACCAATTCGCCAACCGTATTCAATGGTCTCAAAGGGTGAAGAAGA	<i>mCherry::znfx-1</i> donor
cmo-T1194R	GGCGGCGGGAGCCCTGGGGGGGCGAGGTTTCTGACCTTATACAATTCATCCATGC	
cmo17648	AATCTCAATCAGGACGGTAAAG	<i>hrde-1</i> indel detection
cmo17649	GAACCTCTAGGCATAATGTGTA	
cmo-JG55F	ACATAAAACGATAAATCGGC	<i>F53H1.1</i> indel detection
cmo-JG56R	TTCCGTGACTCTTCCATTTT	
cmo-KG825R	CGCCGTTTACTCTCTTT	
cmo17659	CGATTGGAAGTAGAGGTTCT	<i>gfp::csr-1</i> donor
cmo17660	ATCATGATATTGACTATAAA	
cmd-25	gaactatactttttcaggacttaactctgacatgGATTACAAAGACCATGATGGTGACTATAAGGATCATGATA TTGACTATAAAGACGATGACGATAAGGGTGGCGGAGAGAACCTCTACTTCCAATCGaaCca AaaAcaGaaCccTagGctAgcActAaacatcttcgggcttgagctctctgagcgaacgat	ssODN donor to knock-in flagx3::linker::tev at <i>csr-1</i> locus