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Supplementary Materials for

Germline immortality relies on TRIM32-mediated turnover of a maternal mRNA activator in *C. elegans*

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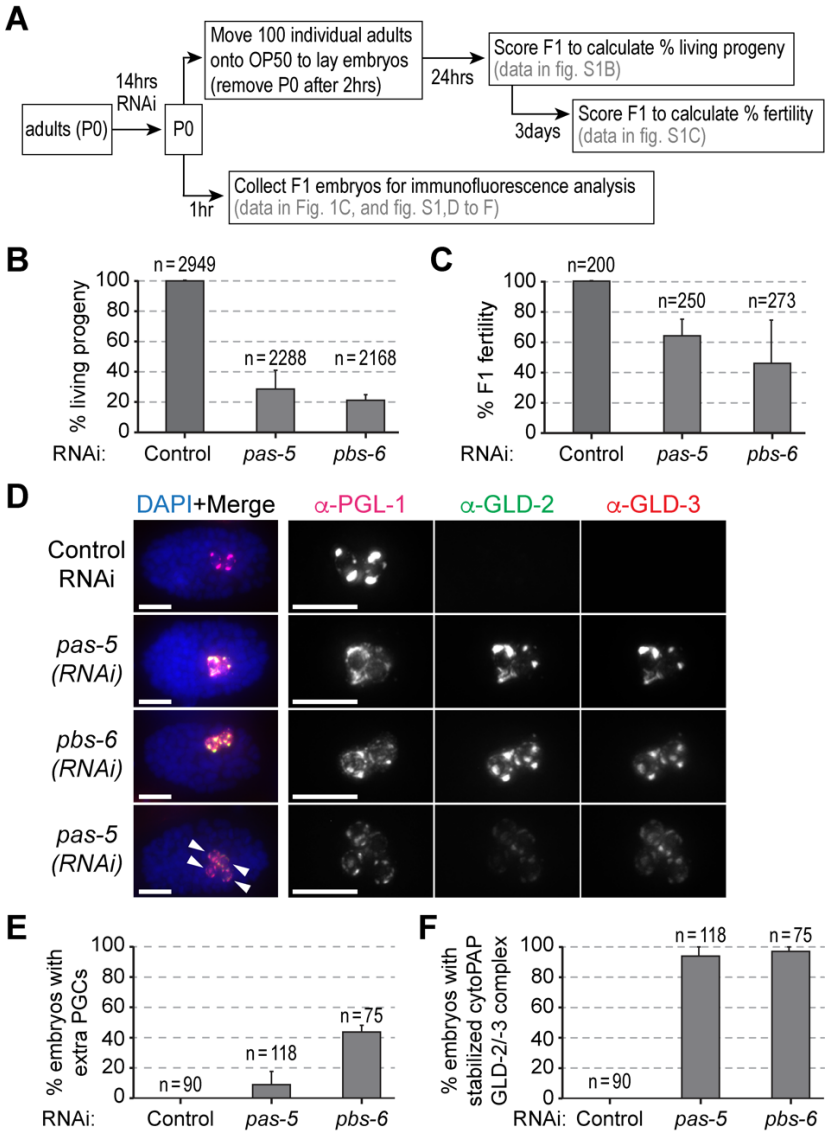

Fig. S1. Embryonic RNAi-knockdown regime to reduce proteasomal activity.

(**A**) Experimental workflow for partial knockdown of two proteasomal subunits in embryos. (B-F) All numbers (n) are the total of three independent RNAi feeding repetitions and all bar charts show mean (± Std. Dev.). (**B**) Bar graph showing the percentage of F1 embryos that hatched into larvae after feeding RNAi of P0 mothers. (**C**) Percentage fertility of living progeny (n) from (B), after reaching adulthood. F1 adults with no embryo in the uterus were scored as sterile, while those containing at least a single embryo in their uterus were scored as fertile. F1 animals that arrested as larvae (~68 % of *pas-5(RNAi)* and ~56 % of *pbs-6(RNAi)*) were not part of this sterility analyses. (**D**) Fluorescent images of ~100-cell stage wild-type embryos, treated with control, *pas-5*, or *pas-6* RNAi. Micrographs of individual protein channels are enlargements of the corresponding embryo to the left and focus on the PGCs; scale bar is 10 µm; arrowheads point at extra PGCs, a positive morphological marker of effective proteasomal knockdown (see main text for details). (**E** and **F**) Bar graph showing the percentage of embryos (n) displaying extra PGC phenotype and those displaying continued expression of GLD-2 and GLD-3 in PGCs after 15 hours of RNAi treatment.

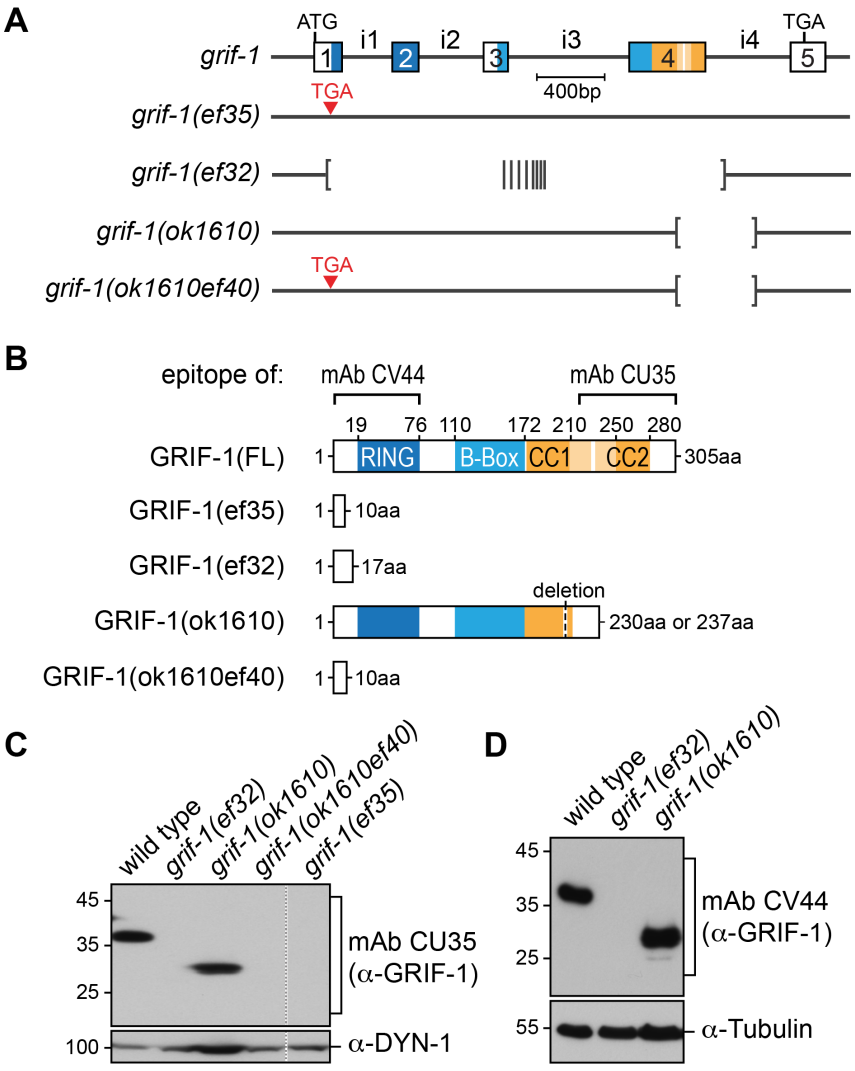

Fig. S2. Genomic organization, alleles and protein products of *grif-1* locus.

(**A** and **B**) Genomic locus of *grif-1*, spanning ~5.2 kilobase pairs (bp) on chromosome I, comprises 5 exons (boxes) and 4 introns (i). *ef35* and *ok1610ef40* alleles carry the identical premature stop codon (TGA) in wild-type background or the 1016bp-deleted *ok1610* allele. In *ef32*, a large portion of the *grif-1* locus is deleted and unrelated base pairs (bp) are inserted (stripes), leading in the corresponding transcript to a very early premature stop codon. (**B**) GRIF-1 protein domains (color code and label as in Fig. 2A) and theoretical protein lengths from wild-type and mutated/edited loci corresponding to (A). Roughly mapped epitopes of two monoclonal antibodies (mAb) are indicated. Note, *ok1610* produces two prevalent transcripts that emerge from an imprecise alternative activation of a cryptic splice site. Both template the production of an in frame deleted GRIF-1 protein (dashed bar), lacking either amino acids 205 until 273 or 199 until 273. (**C** and **D**) Immunodetection of non-synchronized embryonic extracts of ~8,000 - 10,000 embryos, representing a mixture of many different P-lineage stages. Protein blot probed for GRIF-1 and re-probed for loading control, Dynamin-1 (DYN-1) or alpha-tubulin. Note, in-frame deleted denatured GRIF-1(ok1610) proteins are not resolved but detected with both mAbs CU35 and CV44.

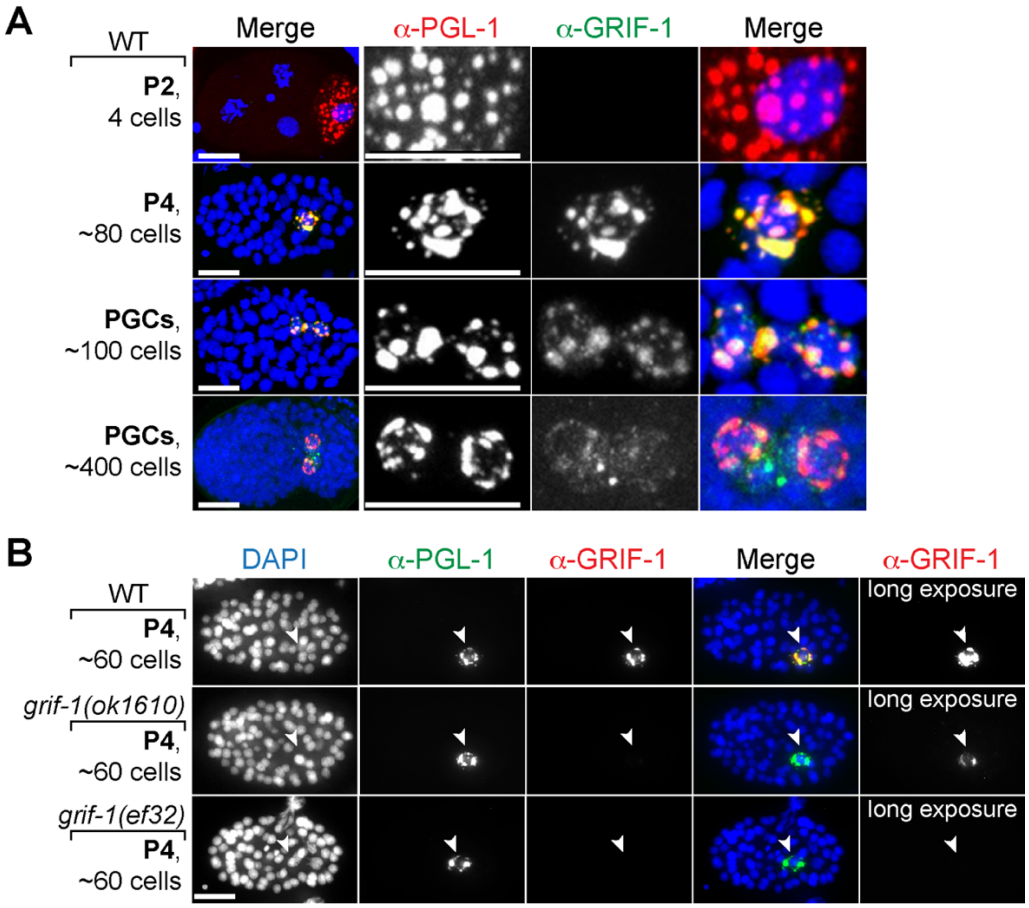

Fig. S3. Expression profile of wild-type GRIF-1 and GRIF-1(ok1610).

(**A** to **B**) Fluorescent images of fixed embryos at different developmental embryonic stages (n>100, each stage) stained for DNA (DAPI) and indicated proteins in given genetic backgrounds. Merge, all channels. Scale bars, 10 μm. (**A)** In wild type, GRIF-1 protein redistributes from initially concentrated in perinuclear P granules in P4 to bright non-PGL-positive cytosolic foci in late PGCs. Confocal microscope-generated full embryo images to the left, zoom in on germ cells, enlargements to right. The blow up of PGCs in embryos of ~400 cell stage (bottom row) was rotated by 90 degrees in clockwise direction. (**B**) Widefield images comparing two *grif-1* alleles to wild type when using mAb CU35. A long exposure of the GRIF-1 channel does not reveal a signal in the null mutant *ef32* but shows expression of CC2-deleted GRIF-1 protein of *grif-1(ok1610)* in the P4 germ cell precursor.

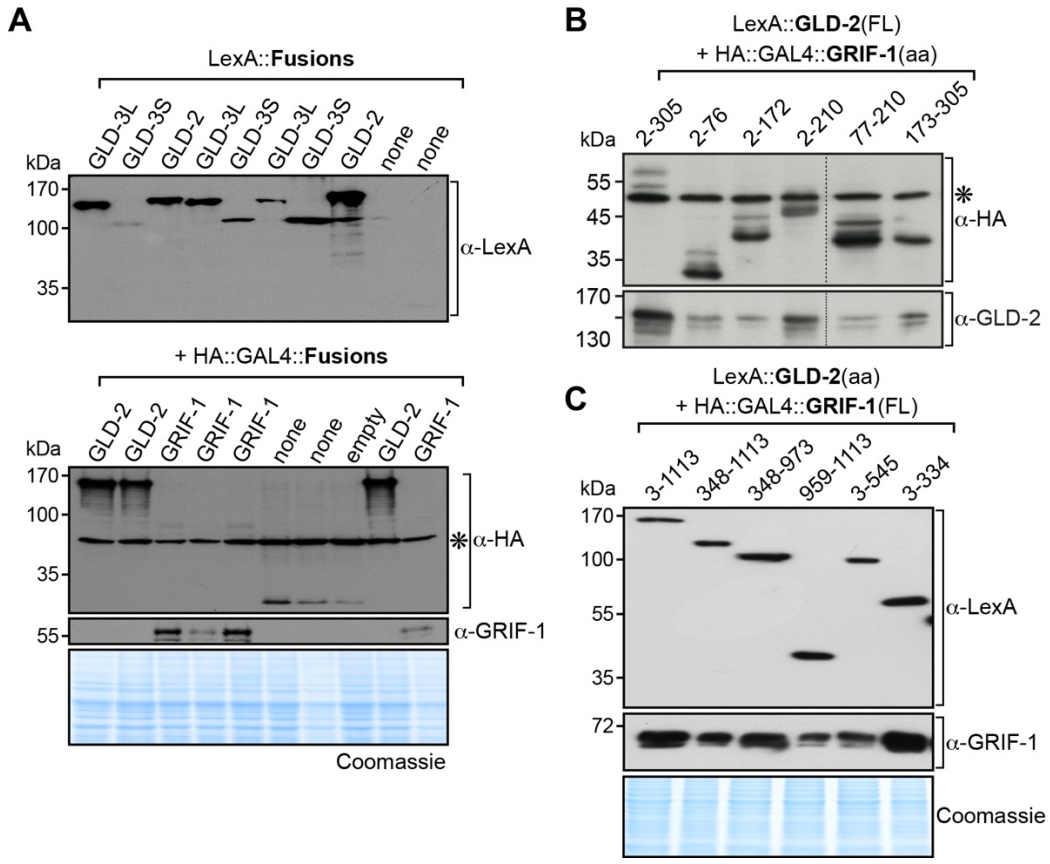

Fig. S4. Expression levels of fusion proteins used in yeast 2-hybrid experiments.

(**A** to **C**) Immunoblots of yeast extracts probed with indicated antibodies to determine and compare fusion protein expression levels: DNA-binding domain fusions to GLD-2 derivatives (anti-LexA or anti-GLD-2) and GAL4 activation domain fusions to GRIF-1 derivatives (anti-HA or mAb CU35). The lowest panels in A and C are images of SDS-PAGE gels stained with Coomassie, containing the very same total protein extracts used in immunoblot analysis to demonstrate equal loading. All proteins were successfully detected, albeit to variable levels. Asterisk (*), unrelated background signal. (**A**) Protein extracts of colonies used in Fig. 3A. (**B**) Corresponds to colonies used in Fig. 3C. (**C**) Extracts of colonies used in Fig. 3E.

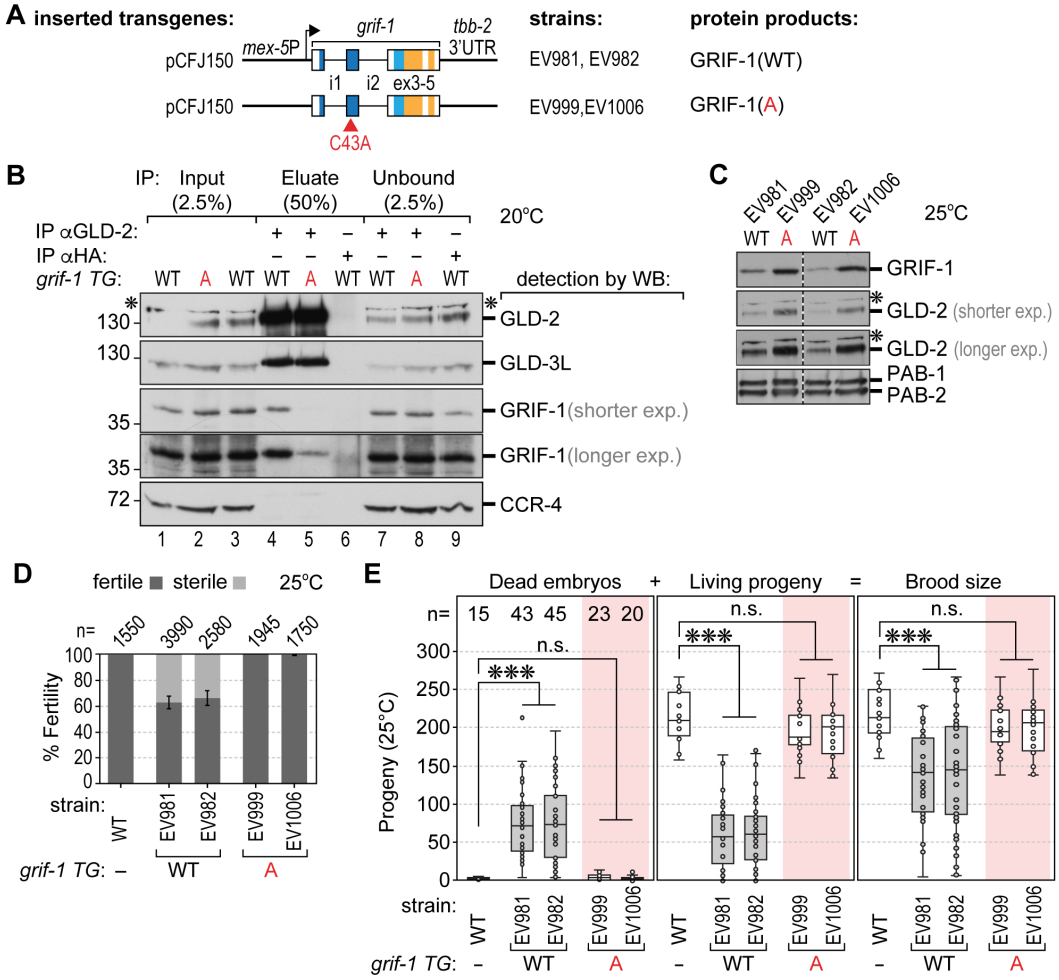

Fig. S5. Effects of a RING finger mutant on GLD-2 interaction and germline development.
(**A**) Cartoon display of strains harboring chromosome II-integrated *grif-1* transgenes (TG), ectopically expressing either wild-type (WT) or cysteine-to-alanine mutated (A) RING-finger GRIF-1 proteins during germline development (via *mex-5* promoter and unregulated *tbb-2* 3'UTR sequences). Two independent transgenic lines per genotype (EV numbers) generated per *Mos1*-mediated Single Copy Insertion (MosSCI). (**B**) Western blot detection of indicated proteins after specific (anti-GLD-2) and control (anti-HA) immunoprecipitation (IP) in which extracts from transgenic young adults reared at 20°C were used, which ectopically expressed either GRIF-1(WT) (strain EV981) or GRIF-1(C43A) (strain EV999). Note RING finger mutated GRIF-1(A) is equally expressed to GRIF-1(WT) but compromised in GLD-2 interaction. Lanes (1 and 3) and (7 and 9) are from the same extract. (**C**) Western blots of *grif-1* transgenic strains reared at 25°C indicate GLD-2 abundance changes (50 adults/lane); PAB-1 and PAB-2 are unaffected, serving also as loading controls. Asterisk (*), unspecific background signal. (**D**) A fraction of ectopically expressing wild-type but not RING mutant *grif-1* TG F1 adults (n) became sterile at 25°C, lacking differentiated gametes and showing signs of severe underproliferation. Bar charts combines data from >3 independent replicates each strain, mean (± Std. Dev.). (**E**) Depending on a wild-type RING finger, non-sterile *grif-1* TG+ animals show partial infertility and produce dead embryos. Box plot of wild-type (WT) or transgenic (TG) homozygote mothers (n) grown at 25°C. Student's t-test: ***= P<0.001, n.s. = not significant.

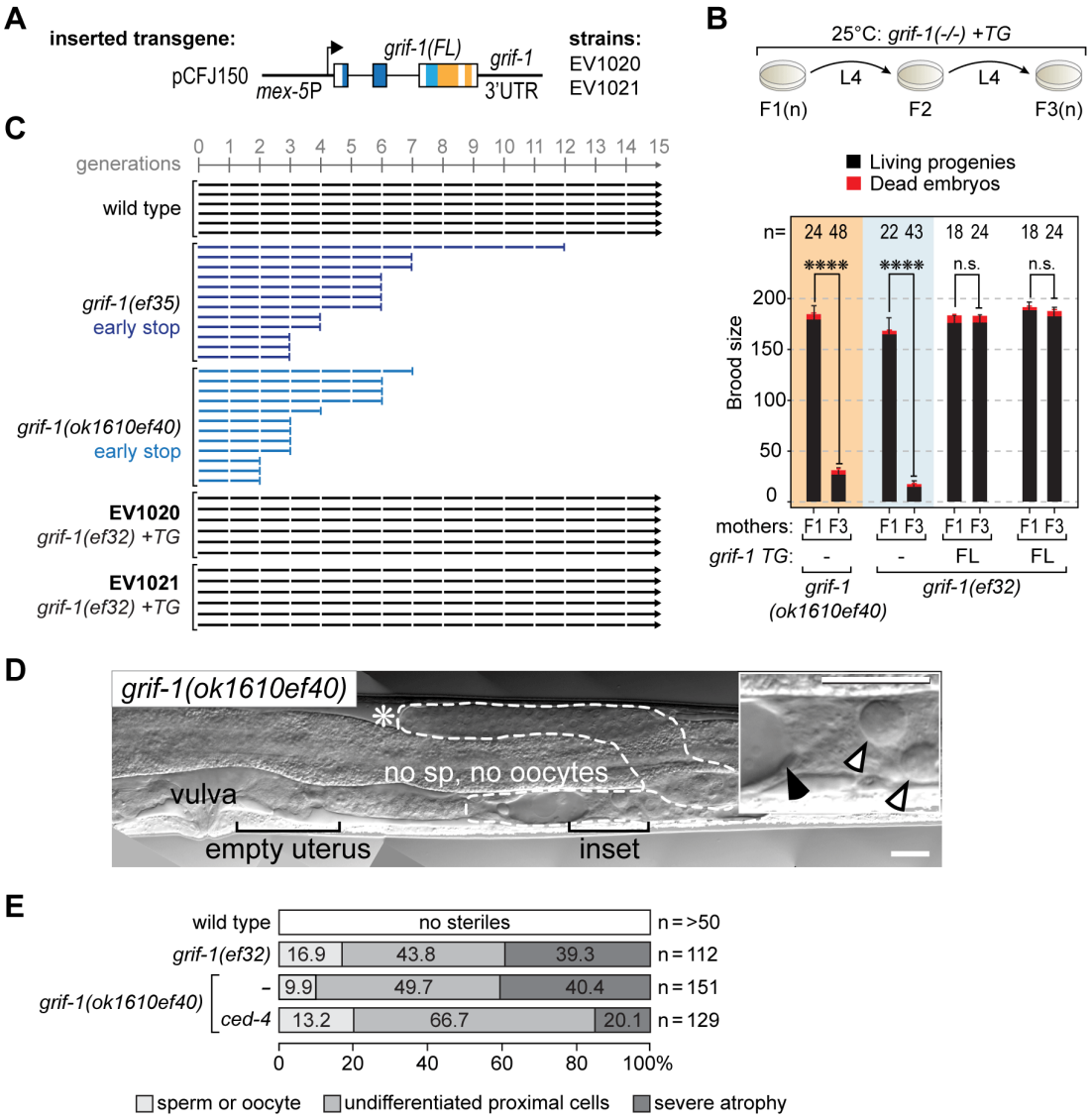

Fig. S6. Transgenerational defects are specific to loss of *grif-1* activity and partially independent of *ced-4* caspase-mediated cell death.

(**A**) Cartoon of the chromosome II-integrated *grif-1* transgene (TG), expressing full-length GRIF-1, transcribed by the germ cell-specific *mex-5* promoter and post-transcriptionally regulated by *grif-1's* own 3'UTR. Two independent lines were generated and characterized as individual strains (EV number). (**B**) Brood size analysis of F1 and F3 mothers (n), displayed separately as hatched animals (living progeny) and the number of dead embryos of rescued *grif-1(ef32)* mutants for comparison. *grif-1* alleles display a similar drop in fertility at 25°C, which can be rescued by the wild-type *grif-1* transgene (strain EV1020 to the left and EV1021 to the right). Student's t-test: ****= P<0.0001, n.s. = not significant. (**C**) Graphical depiction (corresponding to Fig. 5B) showing transgenerational fertility of wild type, transgenerational sterility of *grif-1* mutant alleles, and regained fertility upon *grif-1* transgene (TG) complementation. Each horizontal line represents six mothers of independently maintained plates (worm line). *grif-1* mutant animals become completely sterile at early but varying generations and cannot be further maintained (vertical lines), while *grif-1* mutant animals rescued by *grif-1*

transgenes or wild type continued to be fertile for many generations beyond the indicated ones (arrows). (**D**) Nomarski images of *grif-1(ok1610ef40)* displaying germ cell corpses (empty arrowheads) and germline atrophy (filled arrowhead) in the gonad (white dashed line); no differentiated gametes (sp, sperm) are present in this gonad. Distal end of gonad indicated by asterisks (*). Scale bars, 20 μm. (**E**) Sterile *grif-1* animals display three major germ cell phenotypes in their gonads. Blocking CED-4 caspase-mediated germ cell apoptosis fails to prevent severe atrophy, neither at the bend nor in the proximal region of the gonad.

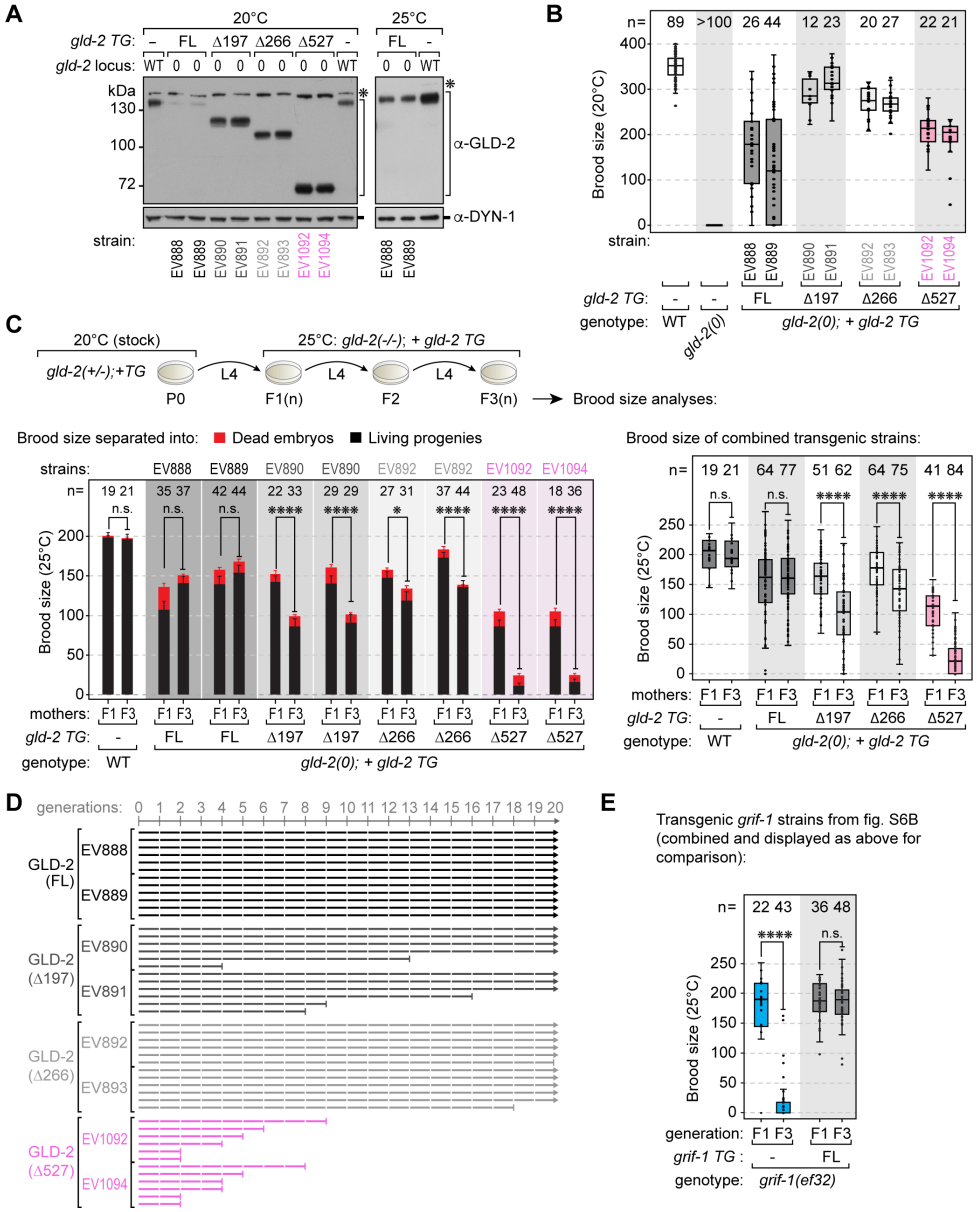

Fig. S7. *gld-2* transgenes lacking the entire GRIF-1 interaction domain rescue *gld-2(0)* sterility but induce transgenerational defects at 25°C.

(A to D) Two strains of two independent integration events were analyzed. (**A**) Immunoblots of wild-type (WT) and *gld-2* transgenic (TG) strains expressing N-terminally truncated (Δ) GLD-2 variants at 20˚C and 25˚C to compare protein expression amounts. Asterisk (*) denotes an unspecific background signal of anti-GLD-2 antibody. Full-length (FL) GLD-2 transgene expresses weaker at 20˚C and better at 25˚C, compared to endogenous protein in wild type. Dynamin (DYN-1), loading control. (**B** and **C**) Box plots and bar charts show brood size of analyzed animals (n). (**B**) Homozygote F1 mothers grown at 20°C; each left item of two transgenic strains corresponds to the strains also used in Fig. 6. (**C**) Brood size of F1 and F3 animals analyzed two generations apart at 25°C. Bars charts show mean (± Std. Dev.) and display each strain individually. Box plot to the right displays combined transgenic stains of equal genotype.

Graphical display of experimental setup for better orientation above. (**D**) Transgenerational fertility of all generated *gld-2* transgenic animals. Each top strain data is also shown in Fig. 6. (**E**) Brood size of rescued *grif-1(ef32)* mutants for comparison with E. Data is identical to fig. S6B but strains were combined. Student's t-test: *= P<0.05, ****= P<0.0001, n.s. = not significant.

Table S1. List of strains used in this study and methods by which they were generated.

Strain	Genotype	Methods applied	Source
RB1413	*Y51F10.2(ok1610) I.*	EMS mutagenesis	CGC
EV647	*grif-1(ok1610) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	Crossing	This study
EV802	*efIs134[Cbr-unc-119(+) + Pmex-5::PEST::GFP::H2B::grif-1 short 3'UTR] II*	MosSCI	This study
EV803	*efIs135[Cbr-unc-119(+) + Pmex-5::PEST::GFP::H2B::grif-1 short 3'UTR] II*	MosSCI	This study
EV902	*grif-1(ef32) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	CRISPR/Cas9 and Crossing	This study
EV899	*grif-1(ef35) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	CRISPR/Cas9 and Crossing	This study
EV896	*grif-1(ok1610ef40) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	CRISPR/Cas9 and Crossing	This study
EV866	*grif-1(ef36) I.*	CRISPR/Cas9	This study
SS104	*glp-4(bn2ts) I.*	EMS mutagenesis	CGC
JK3743	*fog-1(q785) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	EMS mutagenesis	CGC
EV761	*gld-2(q497) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	JK3026 back-crossed	(37)
MT2550	*unc-79(e1068) ced-4(n1162) III.*	N/A	CGC
EV971	*grif-1(ok1610ef40) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III); ced-4(n1162) III/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III).*	Crossing	This study
EV981	*efSi207[Cbr-unc-119(+) + Pmex-5::grif-1 genomic::tbb-2 3'UTR] II; unc-119(ed3) III.*	MosSCI	This study
EV982	*efSi208[Cbr-unc-119(+) + Pmex-5::grif-1 genomic::tbb-2 3'UTR] II; unc-119(ed3) III.*	MosSCI	This study
EV999	*efSi217[Cbr-unc-119(+) + Pmex-5::grif-1 C43A genomic::tbb-2 3'UTR] II; unc-119(ed3) III.*	MosSCI	This study
EV1006	*efSi218[Cbr-unc-119(+) + Pmex-5::grif-1 C43A genomic::tbb-2 3'UTR] II; unc-119(ed3) III.*	MosSCI	This study
EV886, EV887	*gld-2(q497) I; efSi158[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa1-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV888	*gld-2(q497) I; efSi160[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa198-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV889	*gld-2(q497) I; efSi161[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa198-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV890, EV891	*gld-2(q497) I; efSi162[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa267-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV1092	*gld-2(q497) I; efSi224[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa528-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV1094	*gld-2(q497) I; efSi228[Cbr-unc-119(+) + Pmex-5::intronized GLD-2 aa528-1113::gld-2 3'UTR] II.*	MosSCI, Crossing	This study
EV1020	*grif-1(ef32) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III); efSi215[Cbr-unc-119(+) + Pmex-5::grif-1 genomic::grif-1 3'UTR] II.*	MosSCI, Crossing	This study
EV1021	*grif-1(ef32) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III); efSi216[Cbr-unc-119(+) + Pmex-5::grif-1 genomic::grif-1 3'UTR] II.*	MosSCI, Crossing	This study

Table S2. List of primers used in this study.

Gene or allele	CE#[1]	Sequence (5'-to-3' direction)	Vector	For/ Rev[2]	Inner/ outer[3]
Used for genotyping					
grif-1(ef35)	5499	CTAGGCCTAATCTCAATTTTAACTG	N/A	For	N/A
grif-1(ef35)	5191	ACGGGTGTAAGATCGGAGTG	N/A	Rev	N/A
grif-1(ef36)	5198	TTGAGTCTAATGAAGCAATCCG	N/A	For	N/A
grif-1(ef36)	4554	CTGATTCTTGGGGGCTCTTTTAGAACAAATTG	N/A	Rev	N/A
grif-1(ef38)	5499	CTAGGCCTAATCTCAATTTTAACTG	N/A	For	N/A
grif-1(ef38)	5191	ACGGGTGTAAGATCGGAGTG	N/A	Rev	N/A
grif-1(ef32)	6548	GGAAGCTTCTTAGTGGCTTATACGTC	N/A	For	N/A
grif-1(ef32)	6550	GCCTATCTACCTGCCTACAAGGCC	N/A	Rev	N/A
grif-1(ok1610)	4571	TTCTGATCCTCCCCCTTCTT	N/A	For	outer
grif-1(ok1610)	4572	AAAACTGGCGGTATTGATGC	N/A	Rev	outer
grif-1(ok1610)	4567	ATGCTCATCCCACTGAACTC	N/A	For	inner
grif-1(ok1610)	4568	AAATAGTAGGCGACGGAGTTG	N/A	Rev	inner
gld-2(q497)	1956	AAGTCATCTTGCCGAGCTGT	N/A	For	outer
gld-2(q497)	46	TGGACGAACCTCGAGTGCAGCCCTTTC	N/A	Rev	outer
gld-2(q497)	72	CTTCTTTCGGATCCACTCTCTAGGATGG	N/A	For	inner
gld-2(q497)	47	CGAATCGAGATGGCCTCGAGATCATAATTG	N/A	Rev	inner
ced-3(n717)	1016	TCTTGGATTCTGTCGACGGAG	N/A	For	N/A
ced-3(n717)	1017	TGTCTGAAATCCACAAGCGACC	N/A	Rev	N/A
ced-4(n1162)	1019	TCCACGACTTTGAACCACGTG	N/A	For	N/A
ced-4(n1162)	1020	ACTGATTTTCCGGATCCAGCTC	N/A	Rev	N/A
fog-1(q785)	5281	GCGGATTTTTAAATTTTTCG	N/A	For	N/A
fog-1(q785)	5282	AAACTTCGGAACGAGAAATG	N/A	Rev	N/A
MosSCI	3376	CCAGCTTTCTTGTACAAAGTGG	N/A	For	outer
MosSCI	2640	CGTGTTCTCCCATTCTTCAC	N/A	Rev	outer
MosSCI	2634	ATCGGGAGGCGAACCTAACTG	N/A	For	inner
MosSCI	4314	ATAATTCACTGGCCGTCGTTTTACA	N/A	Rev	inner
Used to generate RNAi clones					
grif-1	4249	atatccatggctTACAGTTTCAAAAGCAGCACCGA	pL4440	For	N/A
grif-1	4250	atatctcgagAAATTGGTGCTCAAAATAGTAGGC	pL4440	Rev	N/A
lgg-1	5010	cgatgaattcgagctccaccgCGAATCAAAATGAAGTGGGC	pL4440	For	N/A
lgg-1	5011	cctcgaggtcgacggtatcgTGATTTTCTGGGAGGGG	pL4440	Rev	N/A
Used to generate MosSCI constructs (Gateway)					
grif-1 3'UTR reporter	5065	ggggacagctttcttgtacaaagtggCACCATTCGACAACTCCG	pDONR221	For	N/A
grif-1 3'UTR reporter	5066	ggggacaactttgtataataaagttgCGATCTCAATTTCACTTCCG	pDONR221	Rev	N/A
grif-1(WT) ectopic expression	5059	ggggacaagtttgtacaaaaaagcaggctggATGTACAGTTTCAAAAGCAGCACC	pDONR221	For	N/A

grif-1(WT) ectopic expression	5072	ggggaccactttgtacaagaaagctgggtgTCAAAAT TGGTGCTCAAAATAGTAGG	pDONR221	Rev	N/A
grif-1(C43A) ectopic exp.	5731	GCCGAGAGATGCATCGGTTTGCTCG	pDONR221	For	N/A
grif-1(C43A) ectopic exp.	5733	GACCGTGTGGGCACATTGCAG		Rev	N/A
gld-2 aa1-1113 (FL)	5478	ggggacaagtttgtacaaaaaagcaggctggATGGTT ATGGCTCAACAGCAGAAAAATG	pDONR221	For	N/A
gld-2 aa1-1113 (FL)	5477	ggggaccactttgtacaagaaagctgggtgTCATTGA GATACATTTGATGATGCCATC	pDONR221	Rev	N/A
gld-2 aa198-1113	5473	ggggacaagtttgtacaaaaaagcaggctggATGAAT CATGATCCTAAAATTCATTTGTAC	pDONR221	For	N/A
gld-2 aa198-1113	5477	ggggaccactttgtacaagaaagctgggtgTCATTGA GATACATTTGATGATGCCATC	pDONR221	Rev	N/A
gld-2 aa267-1113	5474	ggggacaagtttgtacaaaaaagcaggctggATGCCA GCCTCAATAGAGCTCC	pDONR221	For	N/A
gld-2 aa267-1113	5477	ggggaccactttgtacaagaaagctgggtgTCATTGA GATACATTTGATGATGCCATC	pDONR221	Rev	N/A
gld-2 aa528-1113	5801	ggggacaagtttgtacaaaaaagcaggctggGGATTC GCATCGCCATCTCCAC	pDONR221	For	N/A
gld-2 aa528-1113	5477	ggggaccactttgtacaagaaagctgggtgTCATTGA GATACATTTGATGATGCCATC	pDONR221	Rev	N/A

[1]) lab identifier number
[2]) PCR primers forward (For) or reverse (Rev) direction
[3]) nested PCR primers

Primer sequences with upper case anneal 100% with target sequences. 5' lower case sequences are overhangs that were used either for Gateway assembly or to introduce restriction enzyme site for subsequent cloning. Primers were synthesized and HPLC purified by metabion GmbH (Planegg/Steinkirchen).

Table S3. Identity of sgRNAs and repair templates used to modify *grif-1* locus.

protospacer (Sequence 5'->3')	Single stranded repair template (Sequence 5'->3')	Type of modification	*grif-1* alleles generated
CE04921 gctaccataggcaccacgag)	CE04922(dpy10_HR) (acttcaatacggcaagatgagaatgactggaaac cgtaccgcatgcggtgcctatggtagcggagcttc acatggcttcagaccaac)	*dpy-10* co-CRISPR	N/A
CE04770 (gctgatataagtcttcacgt)	CE05505(grif_D/Estop) (caaaagcagcaccgacgtgaagacttatatcagc acaccgtgaagcttcgtgccagatatgtcttgagcc atttggttagtgagtttttg)	Stop codon (exon 1)	*ef35, ok1610ef40*
CE04770 and CE04771 (gctgatataagtcttcacgt), (gcgacggagttgtcgaatgg)	None	Deletion (that introduces a premature stop codon)	*ef32*

Desired genomic modifications are colored in red.
Repair templates were synthesized and HPLC purified by biomers.net GmbH (Ulm).