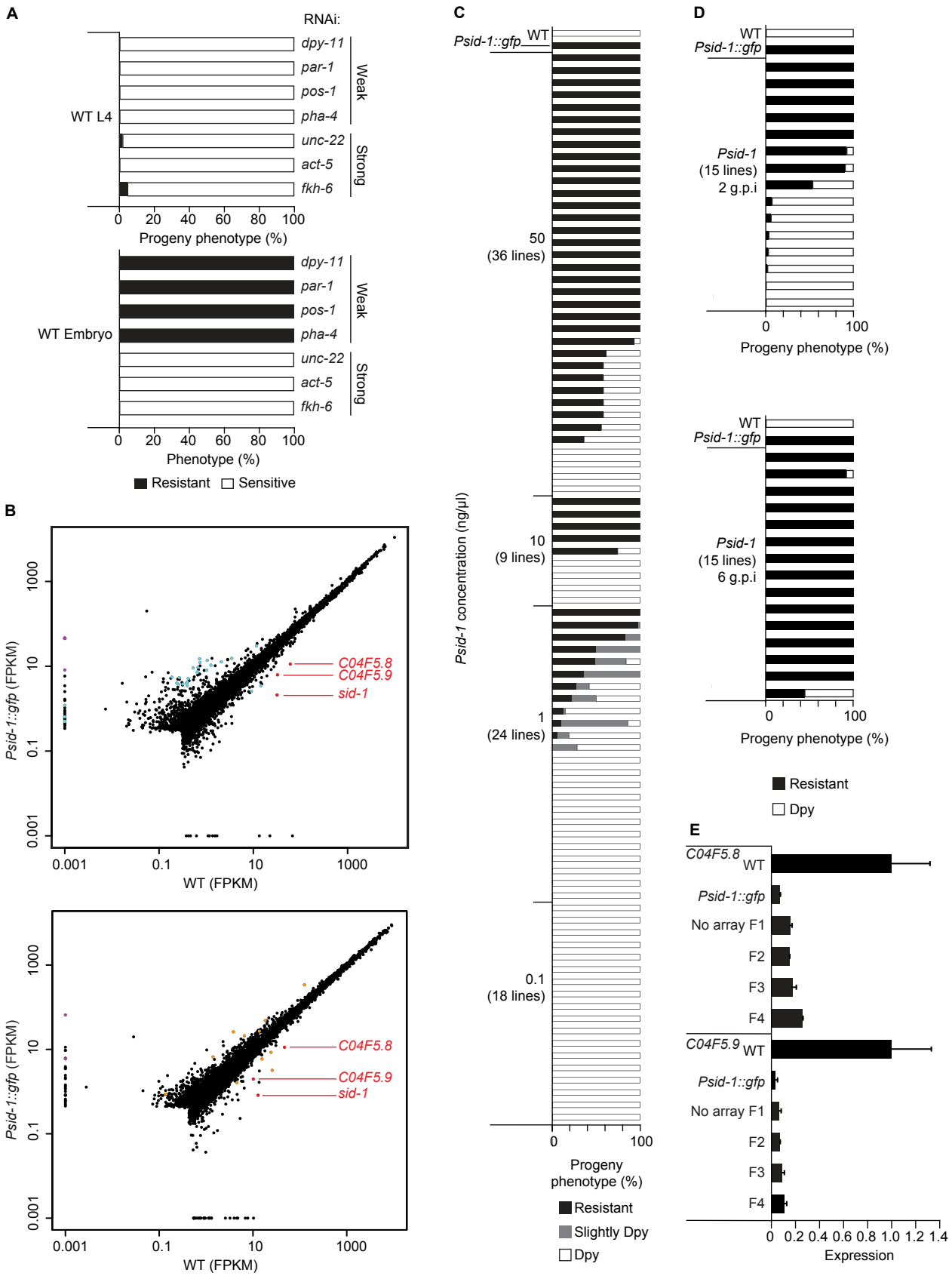


Figure S1. Related to Figure 1. (A) Phenotype of WT adults exposed to weak or strong RNAi foods as embryos (at least 50) or progeny of WT L4 worms (average of 91) placed on RNAi foods. **(B)** Gene expression (FPKM) for WT and *Psid-1::gfp* young adult mRNA-seq libraries that maintained strand-specific information (bottom, 2 replicates per strain) and two libraries that did not (top, 2 replicates per strain). Genes significantly (FDR < 0.05) downregulated (red) or upregulated (purple) in *Psid-1::gfp* in both experiments are highlighted. Genes differentially expressed in only one experiment are in blue and orange. The two genes upregulated in both experiments (*srbc-15, F56A4.3*) are unlikely to be biologically relevant to the phenotype of interest because both have many paralogs and reads aligning to *srbc-15* do not align to the whole gene, indicating that they are likely misaligned. Only genes tested for significance are shown. **(C)** Data used to generate Figure 1F. Each bar represents the RNAi sensitivity of the progeny of 3 L4 worms from each line fed *dpy-11* RNAi. Progeny were scored as adults. **(D)** Transgenic lines produced by injected *sid-1* promoter ("*Psid-1*", injected at 25-75ng/µl) scored on *dpy-11* RNAi two or six generations post injection (g.p.i.). Each bar represents the RNAi sensitivity of the adult progeny (average of 55 per line) of three L4 larvae from each independent line fed *dpy-11* RNAi. Line order is the same for both graphs. **(E)** Quantitative reverse transcription (qRT)-PCR analysis of *C04F5.8, C04F5.9* mRNA levels in single young adult wild-type, *Ex*[*Psid-1::gfp],* and non-array worms segregated from *Ex[Psid-1::gfp].* Expression is measured relative to *cpf-1* and wild-type expression is set to 1.0. Average of two worms and two technical replicates ± SD is shown.

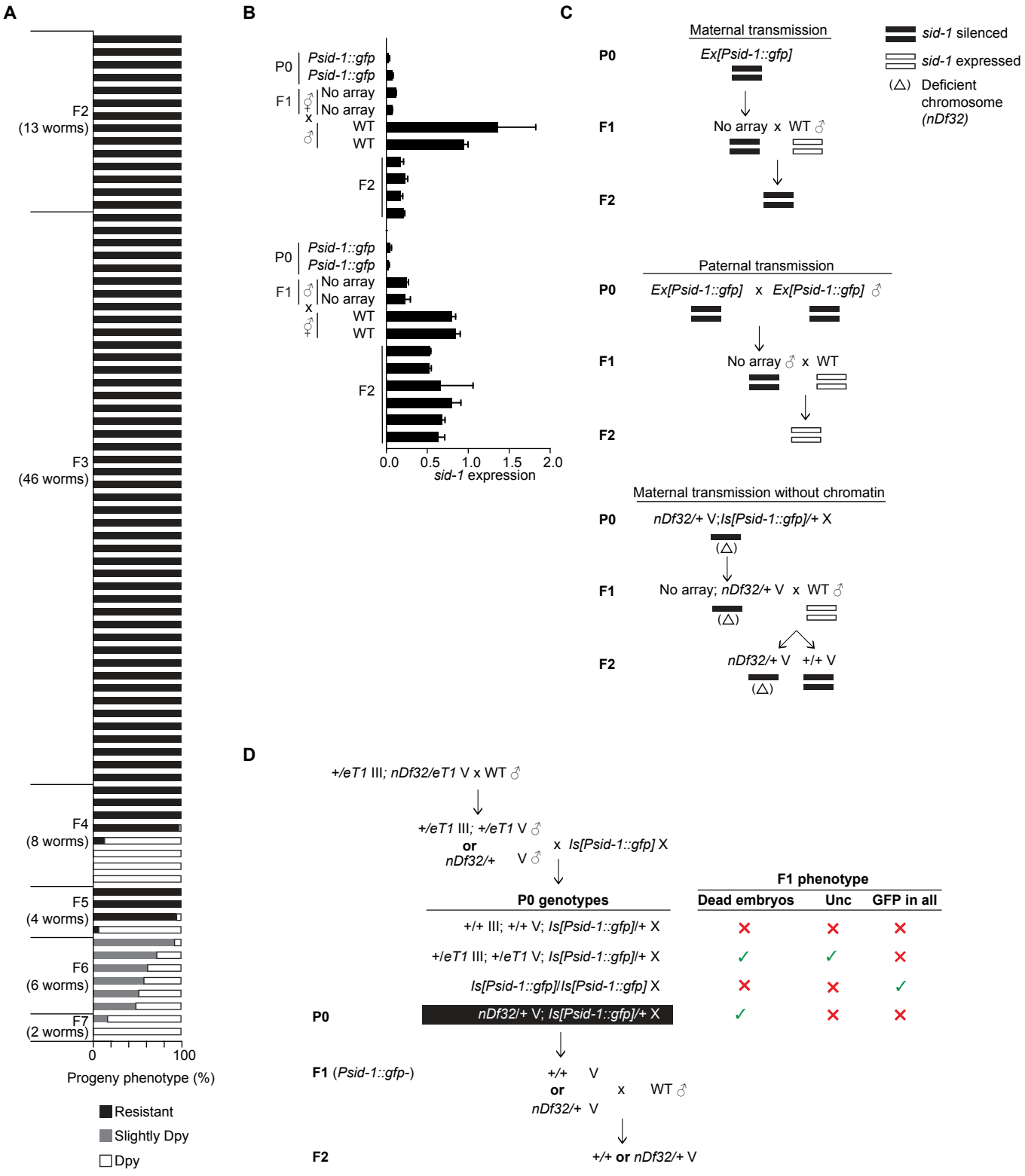
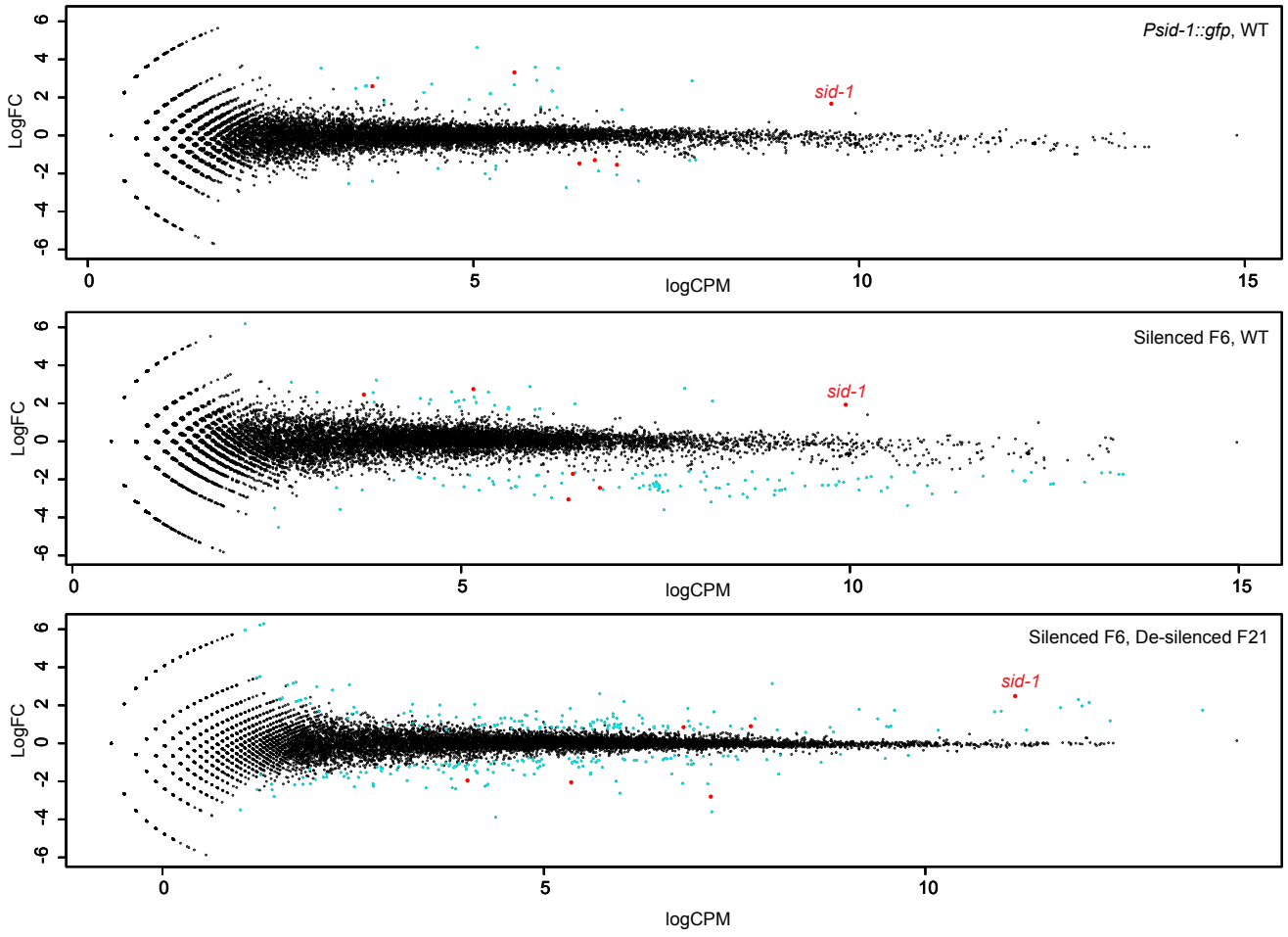


Figure S2. Related to Figure 2. (A) Data used to generate Figure 2B. Each bar represents the RNAi sensitivity of the adult progeny of a single L4 worm fed *dpy-11* RNAi. **(B)** Data in Figure 2A, with wild-type expression values not normalized to 1.0. **(C)** Summary of Figure 2 results. **(D)** *nDf32*/+ V; *Is[Psid-1::gfp]*/+ X P0 worms (referred to as "P0" for consistency with Figure 2) are generated from described cross. Note that *+/eT1* III; *dpy-11(e224) Df32/eT1* V worms are referred to as *+/eT1* III; *nDf32/eT1* V. The genotype of P0 worms is determined by the phenotype of F1 progeny. Dead embryos result from non-complementary inheritance of *eT1* or inheritance of two *nDf32* chromosomes. The chromosome III breakpoint of the reciprocal translocation *eT1* disrupts the *unc-36* locus, thus worms that inherit both sets of translocated chromosomes are uncoordinated. F1 progeny that all contain *Psid-1::gfp* are self progeny. F1 progeny from *nDf32*/+ V; *Is[Psid-1::gfp]*/+ X P0 worms that do not contain the array are singled and crossed to wild-type males (marked by *Is[Podr-1::rfp]*). Cross progeny F2 are placed on *dpy-11* RNAi food as L4 larvae and their progeny scored as adults. The genotype of F2 worms is determined by the presence of dead F3 embryos from *nDf32*/+ V F2s. Only F2s from the *nDf32*/+ V x WT ♂ F1 cross were considered in the analysis (determined by the presence of *nDf32*/+ V F2s).

A

	Reverse 22G read locations			Forward 22G read locations		
	5' independent	5' dependent	5' dependent / 5' independent	5' independent	5' dependent	5' dependent / 5' independent
sid-1 coding	288	12	4.2%	1	0	N/A
sid-1 promoter	61	11	18.0%	37	4	10.8%

B



C

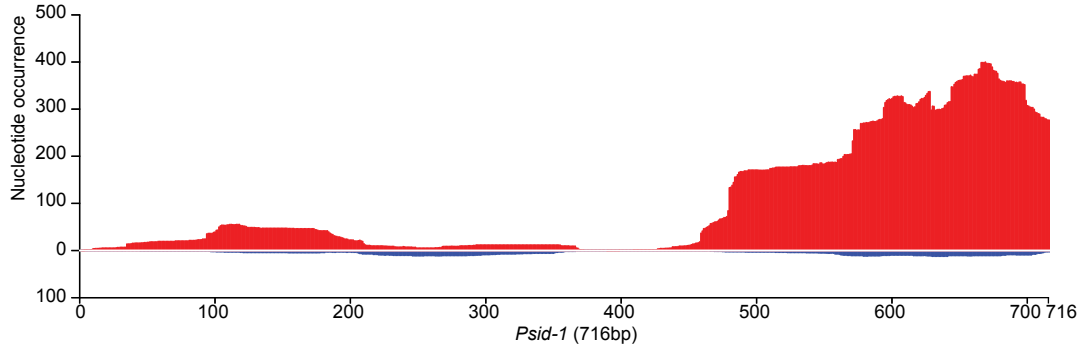


Figure S3. Related to Figure 3. (A) Number of locations at which 22G small RNA reads are found along *sid-1* promoter and coding region in small RNA-seq libraries prepared with (5' independent) or without (5' dependent) polyphosphatase treatment. **(B)** Log_2 counts per million (CPM) vs. Log_2 fold change (FC) for pairs of small RNA-seq libraries. Genes with significant ($p<0.05$) differences in aligned small RNAs in blue. The six genes (including *sid-1*) with significant differences in small RNAs shared in all three comparisons in red. **(C)** RNA-seq reads from two stranded *Psid-1::gfp* libraries were aligned to *Psid-1*. Reads per nucleotide were averaged between the two libraries. Only reads that were 75 or more nucleotides and aligned perfectly to *Psid-1* were included in this analysis. Forward reads (aligning to same strand as *sid-1* RNA) are in red, reverse reads are in blue. No reads from libraries prepared from wild-type worms aligned to the *Psid-1* region.

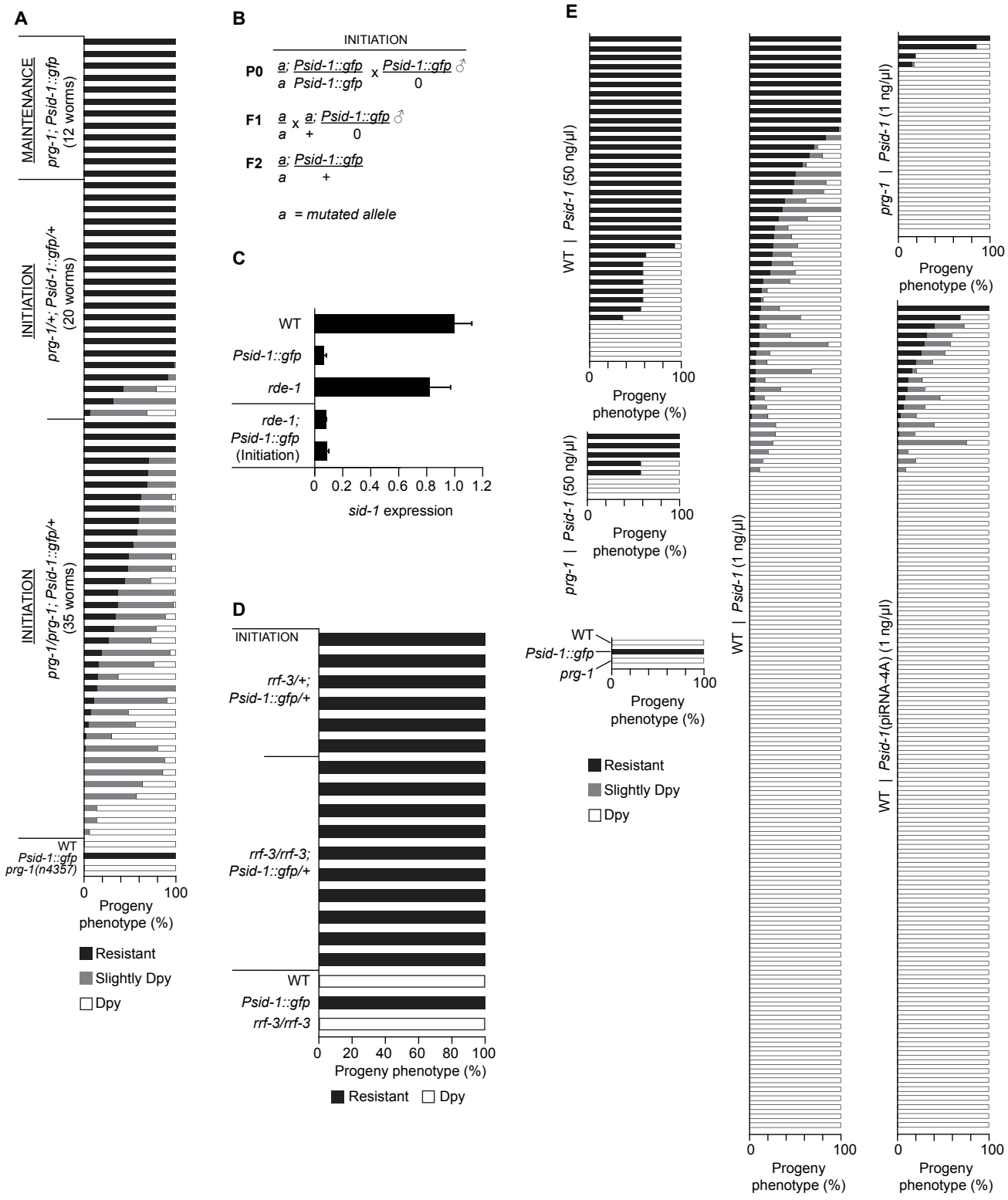


Figure S4. Related to Figure 4. (A) Data used to generate Figure 4C. Each bar represents the RNAi sensitivity of the adult progeny of a single L4 worm fed *dpy-11* RNAi. **(B)** Genetic cross used to test requirement for *rde-1* and *rrf-3* in initiation of silencing. **(C)** qRT-PCR analysis of *sid-1* mRNA levels in two *rde-1; Psid-1::gfp* lines made via the initiation cross and controls. Expression is measured relative to *gpd-2/3* and wild-type expression is set to 1.0. Average ± SD of two technical replicates. **(D)** Feeding RNAi sensitivity of progeny of singled F2 *rrf-3/+; Psid-1::gfp/+* or *rrf-3/rrf-3; Psid-1::gfp/+* animals and controls produced by the initiation cross fed *dpy-11* RNAi as L4 larvae. >200 progeny were scored per parent. **(E)** Data used to generate Figure 4E. Each bar represents the RNAi sensitivity of the adult progeny of three L4 worms from each independent line fed *dpy-11* RNAi. See Table S2 for strains and alleles.

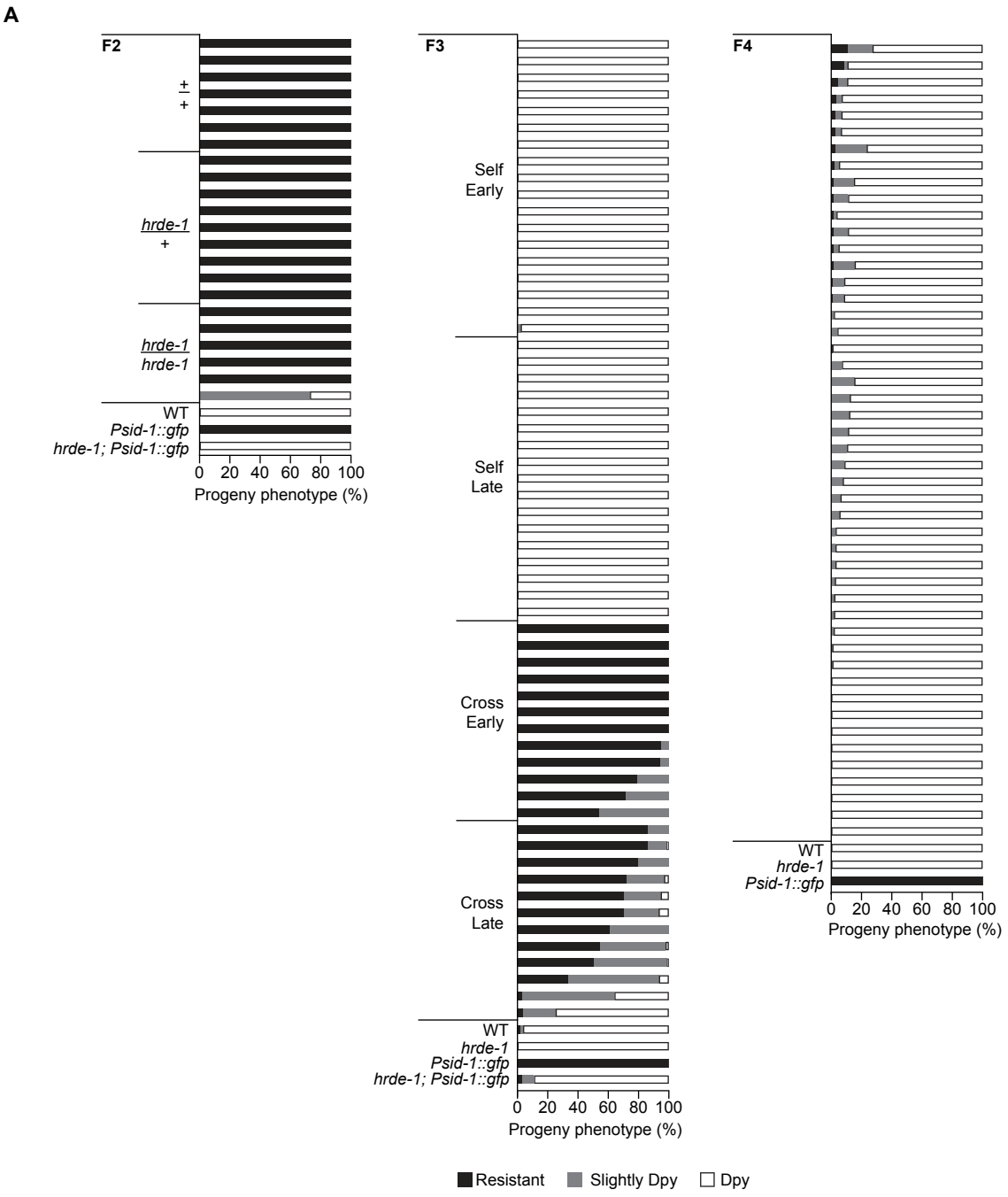


Figure S5. Related to Figure 5. (A) Data used to generate Figure 5. Each bar represents the RNAi sensitivity of the adult progeny of a single L4 worm fed *dpy-11* RNAi. **(B)** *sid-1* and *C04F5.9* transcription start sites (TSS) defined in Saito et al., 2013.

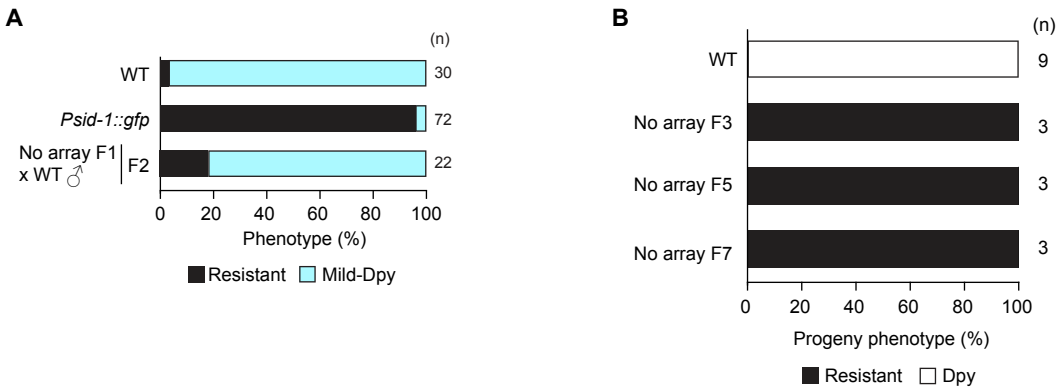


Figure S6. Related to Figure 6. (A) The RNAi sensitivity of (n) F2 cross progeny from described cross and controls placed on *dpy-11* RNAi food as embryos and scored as adults. **(B)** Feeding RNAi sensitivity of progeny of non-array worms of given generation, segregated from *Ex[Psid-1::gfp]* and fed *dpy-11* RNAi as (n) L4 larvae. >200 worms were scored per generation. Concurrent with experiment in Figure 6C.

Table S1. Comparison of heritable silencing in *C. elegans.*

		sid-1	***oma-1***	***ceh-13***	***gfp***	***gfp* (RNAe)**	**piRNA sensor**
	Reference:	1	2	3	3, 4, 5	6	7
	Initiating signal	Psid-1 multi-copy array	dsRNA	dsRNA	dsRNA	piRNA	piRNA, piRNA sensor transgene
Silenced locus	**Endogenous**	x	x	x			
	Transgene				x	x	x
	Stability	F13	F4	"Indefinite"	>F80	∞	>F12
Requires selection?	**Yes**		x	x	x		
	No	x				x	x
Transmission	**Oocyte**	x	x	x	x	x	x
	Sperm		x	ND	ND	x	ND
Tissue expression	**Germline**	x	x		x	x	x
	Soma	x		x			

1. This paper
2. Alcazar et al., 2008
3. Vastenhouw et al., 2006
4. Ashe et al., 2012
5. Buckley et al., 2012
6. Shirayama et al., 2012
7. Sapetschnig et al., 2015

ND: Not determined.

Table S2. Related to STAR Methods. Strains used in this study.

Strain	Genotype	Reference
HC125	*qtIs6[Psid-1::NLS::gfp::unc-54 3' UTR] X* Referred to as "*qtIs6[Psid-1::gfp]*" below	Winston et al., 2002
HC83	*qtEx6[Psid-1::NLS::gfp::unc-54 3' UTR, pRF4]*	Winston et al., 2002
WM27	*rde-1(ne219) V*	Tabara et al., 1999
HC971	*rde-1(ne219) V; qtIs6[Psid-1::gfp] X*	This paper
HC1011	*rde-4(ne301) III*	Tabara et al., 1999 and this paper
HC1012	*rde-4(ne301) III; qtIs6[Psid-1::gfp] X*	This paper
GR1373	*eri-1(mg366) IV*	Kennedy et al., 2004
HC1004	*eri-1(mg366) IV; qtIs6[Psid-1::gfp] X*	This paper
HC888	*rrf-3(pk2042) II*	Sijen et al., 2001 and this paper
HC999	*rrf-3(pk2042) II; qtIs6[Psid-1::gfp] X*	This paper
YY186	*nrde-2(gg91) II*	Guang et al., 2010
HC972	*nrde-2(gg91) II; qtIs6[Psid-1::gfp] X*	This paper
HC981	*nrde-3(gg66) X*	Guang et al., 2008 and this paper
HC979	*nrde-3(gg66); qtIs6[Psid-1::gfp] X*	This paper
YY538	*hrde-1(tm1200) III*	Buckley et al., 2012
HC995	*hrde-1(tm1200) III; qtIs6[Psid-1::gfp] X*	This paper
HC1001	*mut-2(ne298) I*	Tabara et al, 1999 and this paper
HC1000	*mut-2(ne298) I; qtIs6[Psid-1::gfp] X*	This paper
HC196	*sid-1(qt9) V*	Winston et al., 2002
CB4037	*glp-1(e2141) III*	Priess et al., 1987
HC1040	*glp-1(e2141) III; qtIs6[Psid-1::gfp] X*	This paper
HC1038	*set-32(ok1457) I; set-25(n5021) III*	This paper
HC1039	*set-32(ok1457) I; set-25(n5021) III; qtIs6[Psid-1::gfp] X*	This paper
SX922	*prg-1(n4357) I*	Bagijn et al., 2012
PY2417	*oyIs44[Podr-1::rfp] V*	Lanjuin et al., 2003
MT2583	*dpy-11(e224) nDf32 V/eT1(III;V)*	Park and Horvitz, 1986
HC992	*prg-1(n4357) I; qtIs6[Psid-1::gfp] X*	This paper
	Ex[Psid-1, pHC183 (myo3::dsRed2)]	This paper
	prg-1(n4357) I; Ex[Psid-1, pHC183 (myo3::dsRed2)]	This paper
	Ex[Psid-1(piRNA-4A), pHC183 (myo3::dsRed2)]	This paper

Table S3. Related to STAR Methods. Primers used for qRT-PCR and cloning injection constructs.

qRT-PCR primers (5' → 3')	
***sid-1* mRNA**	CGAAGGCTAAACTTTGTGGAGC
	GAGTAGCAGGCATGGCTTG
***gpd-2/3* mRNA**	CTCTGGAGCCGACTATGTC
	CGTACTTCTCGTGGTTGACTC
***gpd-2/3* mRNA (single worm only)**	GGAGGAGCCAAGAAGGTCATC
	CGTACTTCTCGTGGTTGACTC
***C04F5.9* mRNA**	GACACGAAAATGAATAGTTGTCGG
	GTCAGTTGATTACGATGAACGGG
***C04F5.8* mRNA**	CTCGGACTATGCTGCTCTC
	GATTATCCTTGAAGACGTGGGC
***cpf-1* mRNA**	CGATGAAAACGTTGTCGGAAG
	CATATGCCTGAGCTGTTTCAATG
***sid-1* intron 4**	GCCAATTTCAGTCTATGCGGG
	CGACACAAGCTCTATAGTAGCC
***sid-1* intron 6**	GCTCACTTGTCATTTGGGGG
	GGCAAAACGGGAAATTACCG
***cpf-1* intron 5**	GCGTCGAAGAGTGTTTCTAAAAAAATC
	CATTATGATATTCTTACTTCGCTCTCG
Cloning primers (5' → 3')	
Psid-1	GGTCATGAGAGGGTCGAGAG
	GGAAAAATGAGGAGTTTTAATTTC
Psid-1(piRNA-4A)	Site directed mutagenesis: AAATTTTCAGCTTAATATAAGTATTAAATTCATAAAAAAAATCAGAAGAAAAACAG
	ATGAATTTAATACTTATATTAAGCTGAAAATTTTTTAAAAGATATATAGAGGATG
	Amplification: GGTCATGAGAGGGTCGAGAG
	GGAAAAATGAGGAGTTTTAATTTC