Supplemental methods

Labeled RNA preparation

Purified RNA was synthesized by *in vitro* transcription (IVT) from a DNA template containing T7 promoter sequences. For labeled and unlabeled *unc-22* dsRNA, template DNA was prepared by PCR using primers with the T7 promoter sequence appended to the 5' end of both forward and reverse primers. For the Cy5- and 5EU-containing RNA in Fig. 4, the forward and reverse strands of the eventual dsRNA were prepared independently from template DNA containing only a single T7 promoter.

IVT was performed using an Ampliscribe T7 Flash kit (Epicentre) according to manufacturer instructions with the following modifications for labeled RNAs. For 5EU-labeled RNA, the 100 mM UTP in the reaction was substituted with an equal volume of a 1:1 mixture of UTP and 100mM 5-ethynyl-UTP (Jena Bioscience). 5EU-labeled RNA can also be synthesized using only 5-ethynyl-UTP and no unmodified UTP and behaves similarly in the experiments we performed. For Cy5-labeled RNA, instead of individually adding 0.9 μl of each NTP, a mixture of equal amounts of 100 mM ATP, CTP, and GTP was prepared, and 4.55 μl of this mixture was added to the reaction, followed by 1 μl of 100 mM UTP and 3 μl of 10 mM Cy5-UTP (Amersham). IVT reactions of labeled RNA were incubated at 37°C for 4.5 h, and then treated with DNase I at 37°C for 15 min. Synthesized RNA was purified using an RNeasy kit (Qiagen). Equal quantities of complementary strands of RNA were then annealed together by heating mixtures to 95°C for 2 min and then gradually lowering the temperature to 20°C at a rate of 0.1°C per second.

Immunohistochemistry

After injection of 5EU-labeled RNA, adults or isolated embryos were transferred to a poly-L-lysine coated slide and covered with 10 μl of 4% paraformaldehyde solution (PFA). A square cover glass was then placed on to the sample, and excess liquid removed by wicking to a tissue paper until the point of mechanical rupturing of eggshells or cuticles to provide access to the fixative. Samples were then left to fix for 15 min at room temperature before flash freezing in liquid nitrogen. Afterwards, the cover glass was removed with a razor blade to help remove cuticles and eggshells. Samples were then fixed in -20°C methanol for 15 min, and then briefly rinsed in PBS. The final preparation step before labeling was sample permeabilization in a 0.1% Tween-20 in phosphate buffered saline (PBS) solution for 15 min at room temperature.

Labeling of samples containing 5EU-RNA was performed with a Click-iT RNA Imaging kit (Invitrogen) according to manufacturer instructions, but reaction volumes reduced to 100 μl per slide. For adult worm samples, labeling was done with Alexa Fluor 594 azide (Thermo Fisher), but embryo samples typically substituted 2.66 μM biotin azide (Lumiprobe) for additional signal amplification. Click labeling reactions were incubated at room temperature for 30 min and then stopped with addition of stop solution from the kit, and then washed 3 times in PBS. Samples to be labeled only with Alexa fluor could be mounted in Vectashield with DAPI and sealed under a coverslip at this point, or further processed with additional labeling.

Embryo samples requiring signal amplification were further processed with the Alexa Fluor 594 Tyramide SuperBoost Kit with streptavidin (Invitrogen) according to manufacturer instructions, with the tyramide labeling reaction allowed to proceed for 8

minutes. After washing 3 times in PBS for 10 minutes each, slides were mounted and sealed, or additionally labeled by antibody staining.

Samples requiring antibody labeling were incubated at room temperature for 1 h with a rabbit polyclonal GFP antibody (Invitrogen) diluted 1:200 in a solution of 5% bovine serum albumin (BSA) in PBS with 0.1% Tween-20. After washing 3 times in PBS, samples were then incubated at room temperature for 1 h with an Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (Invitrogen). After washing 3 times in PBS, slides were mounted with Vectashield with DAPI and sealed.

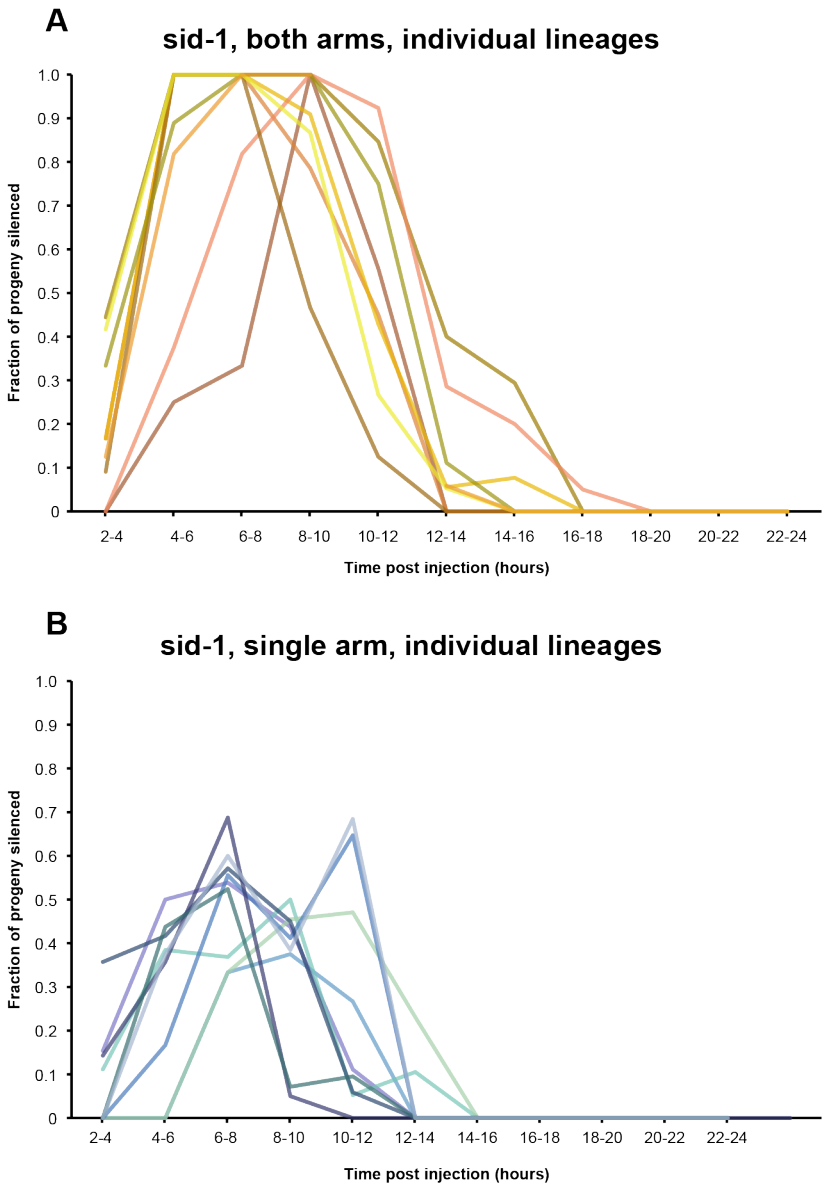

Figure S1. Individual lineages achieve maximum silencing with variable timing

Expanded data from Figure 1, panels 1A and 1C. Each panel in Figure 1 represents the average of two experiments, with the data corresponding to the progeny of each 10 injected animals in each duplicate. Here, the data from one of the replicates from panels 1A and 1C respectively are expanded in (A) and (B) to show the data from each individual injected animal as a separate line.