Supplemental data.

Preparation of dsRNA coated particles. dsRNAs were synthesized as described in Ref. (Baker and Macagno, 2000). RNAs were complementary to contiguous cDNA sequences spanning nucleotides 1-981 for leech *netrin* (GenBank accession number AF101029) and nucleotides 1-930 for HmLAR2 (GenBank accession number AF017083). The DNA templates used for RNA synthesis were products of PCR reactions. Each primer contained the T7 promoter on its 5' end (forward primer: gCgTAATACgACTCACTATAgggTgTTTCTCAgTCgTTTgCATggTTCgA; reverse primer:gCgTAATACgACTCACTATAgggCTTTgCTCgAATCTTCgTgATCCgCAT) The templates were purified using crossed phenol/chloroform extraction and ethanol precipitation. The RNA products were dissolved in water or 10mM Tris [pH 7.5]. dsRNA was immobilized to S08ri gold particles (Seashell Technology, LLC; average diameter 1.6 μm) as described in the manufacturers protocol. Samples were prepared using 10 μg dsRNA per 10 mg of gold carrier particles, washed once with cold isopropanol, sonicated briefly in a bath sonicator and dried onto glass slides. The coated gold particles were then scraped from the glass slides using a cover slip and loaded into a tygon tubing line with a spatula. The number of dsRNA molecules on a particle was estimated based on the size of the molecules and surface area of the particles as ~15,000.

Preparation of DNA-coated particles. Modified EGFP-C3 plasmids (Clontech) were used to express fluorescent proteins (Baker, in press). Briefly, the CMV promoter was replaced with a leech cytoplasmic actin promoter Act1 (Accession number DQ333328). F-actin fusion proteins were created by expressing the actin coding region of Act1 in the multiple cloning region of a modified EGFP-C3 plasmid. Miniprep plasmid DNA was phenol and chloroform extracted, ethanol precipitated, and resuspended in dH_20.

S08d gold particles (Seashell Technology, LLC; average diameter 1.6 μm) were coated as described in the manufacturers protocol. Samples were prepared using 10 μg plasmid DNA per 1 mg of gold carrier particles, washed once with ethanol, sonicated briefly in bath sonicator and dried onto glass slides.

Preparation of dye-coated particles. The preparation followed the procedures developed by Gan et al. (Gan et al., 2000) Three mg of dye, DiI or DiO (Molecular Probes, catalog numbers D-282 and D-275), were dissolved in 100 μL methylene chloride. About 10 mg of SO8 gold particles (Seashell Technology, LLC; average diameter 1.6 μm) were spread evenly onto a glass slide, and a dye solution was dispensed onto them. After the solvent evaporated, the particles were resuspended in 0.5 mL of distilled water and sonicated for 5 min to prevent the formation of clusters. The sonicated solution was dried onto a clean glass slide.

Animal Preparation. Embryos of *H. medicinalis* (E8-12) (Fernandez and Stent, 1982) were anaesthetized in a solution of 8% ethanol in sterile artificial pond water and placed ventral side up in a groove carved in a piece of silicone (Sylgard 184, Dow Corning, Midland, MI). Immediately before shooting particles, the ventral surface of the embryos was uncovered by removing part of the solution. After bombardment with particles, the embryos were transferred to artificial pond water with no ethanol and kept in a darkened chamber at room temperature until processed for imaging.

In Situ Hybridization and Immunocytochemistry on whole-mount embryos. To assay for *netrin* mRNA, digoxigenin-labeled leech netrin riboprobes were hybridized to whole leech embryos, using the procedures developed by Nardelli-Haefliger and Shankland (1992) (Nardellhaefliger and Shankland, 1992). A full-length netrin riboprobe was synthesized with digoxygenin labeled nucleotides using T7 RNA polymerase. The template for the in vitro transcription reaction was a full length netrin cDNA (GenBank accession number AF101029) created using PCR (forward primer: CgTCgACATTCCTCgACATATTCT; reverse primer with T7 promoter: TAATACgACTCACTATggggATATTTTCTTCCTTTgCATTT). The expression pattern of netrin was the same as observed in a previous study (Gan et al., 1999). AntiGFP immunocytochemistry was performed on whole-mount embryos using anti-GFP mAb 3E6 and an Alexa 488-conjugated goat anti-mouse IgG (both from Molecular Probes) using established methods (Baker et al., 2000) Nuclei were stained using Hoechst 33258.

Staining and transfecting Neuro 2A cells in culture. Neuro 2A cells, a mouse neuroblastoma cell line (ATCC; CCL-131), were maintained in Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum at 37° C/5% CO2. The cells were transferred into 35 mm tissue culture dishes at densities sufficient to achieve 85-95% confluence at the time of the biolistic delivery assays. The DNA plasmid encoding GFP was obtained from Aldevron (Fargo, ND). Red fluorescent lipophilic dye (DiI) was obtained from, Molecular Probes (catalog numbers D-282). The protocols for coating the gold particles (Seashell Technology, LLC; average diameter 1.6 μm) with plasmid DNA and lipophilic dye were the same as described above.

Microscopy. Brightfield images were acquired with a digital camera (Nikon, Coolpix 4500) attached to a Laborlux 12 (Leitz Wetzlar) microscope, using a 25X/0.6W objective. Fluorescent imaging was performed using a Nipkow Spinning disk confocal scan head (QLC100, VisiTech) with a Krypton/Argon laser attached to a Nikon Eclipse E600 microscope with a 40x/0.80W NikonFluor DIC M objective and a Cooke Sensicam QE CCD camera, and with a Leica SP2-AOBS scanning confocal microscope with a 20X objective lens. Cells in culture were imaged under a fluorescence microscope, Olympus BX40, equipped with 10X and 50X objectives, and a color digital camera, Optronics MacroFire.

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