Supporting Information

Gosse and Baier 10.1073/pnas.0803202106

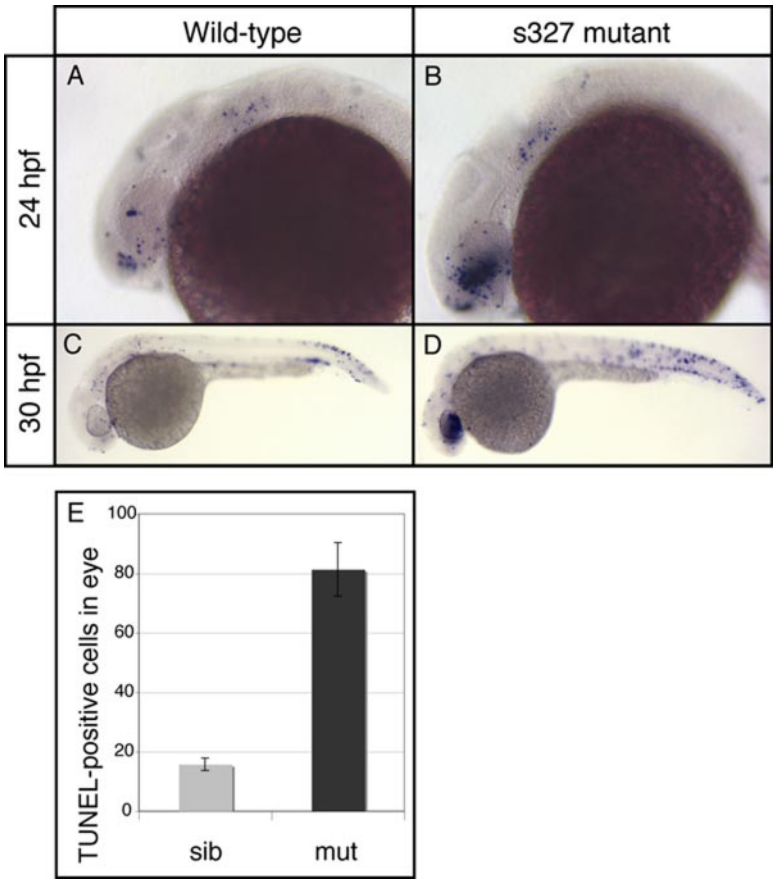

Fig. S1. Increased cell death in the eyes of *radar*^{s327} mutants. Lateral views of whole-mount TUNEL-stained embryos. (*A*) 24 hours post-fertilization (hpf) WT embryos have small numbers of TUNEL-positive cells throughout the head and trunk. (*B*) 24 hpf *radar*^{s327}mutant embryos have a large increase in TUNEL reactivity in the developing eye. (*C* and *D*) 30 hpf *radar*^{s327}mutant embryos have significantly more TUNEL-positive cells in the eyes than WT. The difference is less dramatic in the trunk. (*E*) Quantification of TUNEL staining in 30 hpf embryos. *radar*^{s327}mutants ($n = 6$) have significantly more TUNEL-positive cells in the eye than WT embryos ($n = 8$, *t* test, $P < 0.000001$).

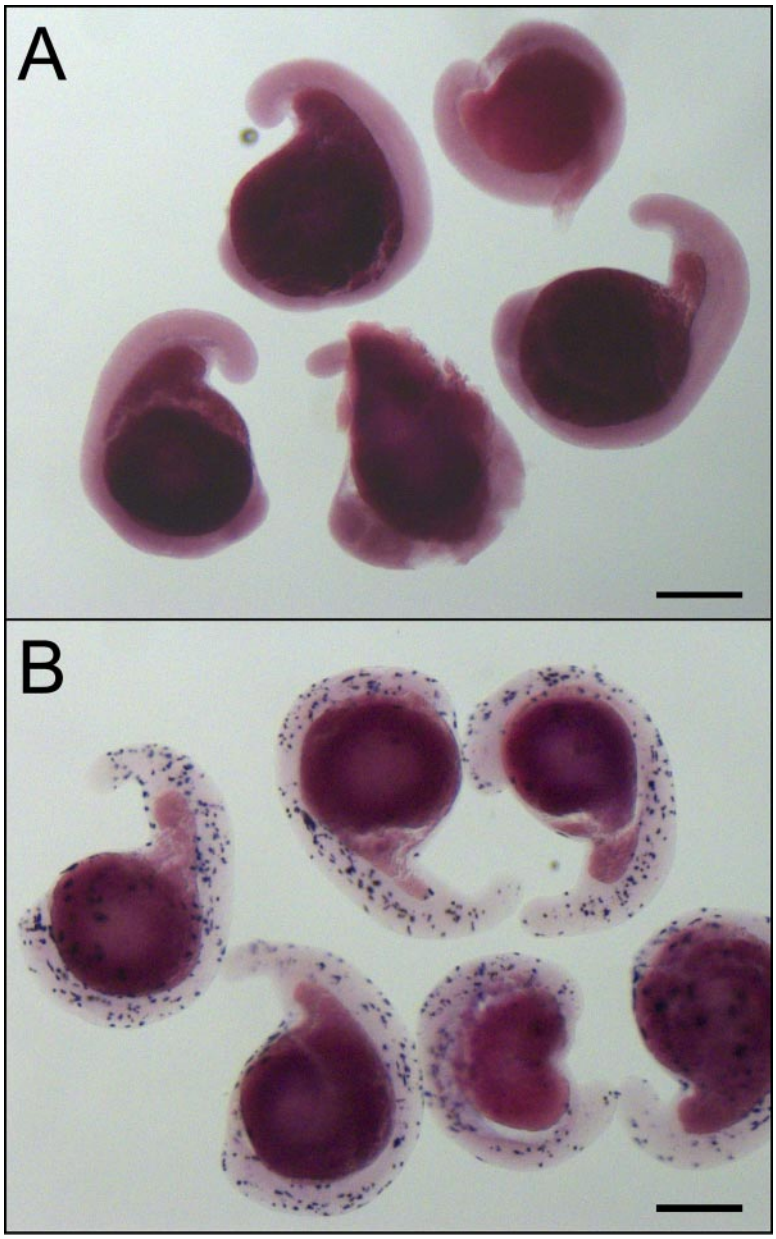

Fig. S2. In situ hybridzation reveals transient overexpression of *radar*. (*A*) Uninjected 18 somite embryos, heatshocked at 12–14 somites show no specific pattern of expression after 30 min of color reaction. (*B*) Embryos injected with 25 ng/μl *hsp70:radar*^{WT}, heat-shocked at 12–14 somites show intense staining after 10 min of color reaction, with broad mosaic expression, often, but not always, including the eye. (Scale bars, 100 μm.)

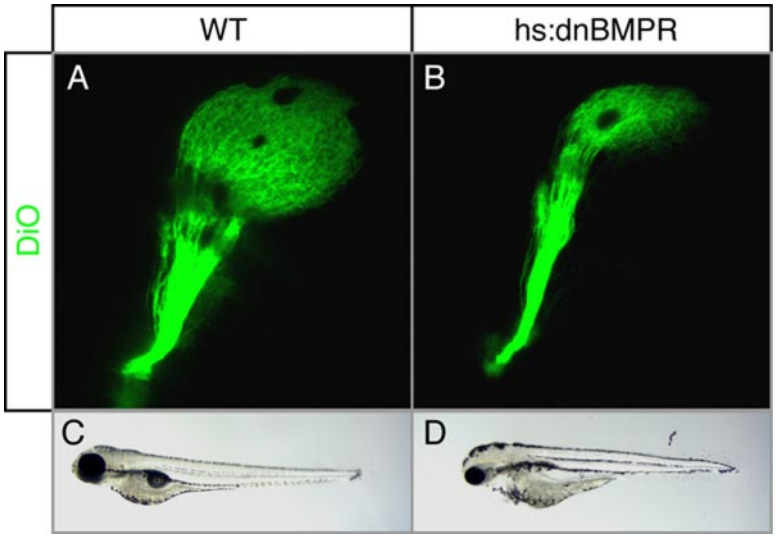

Fig. S3. Dominant-negative inhibition of Bmp/Gdf signaling in the eye. (*A* and *B*) Lateral view of DiO-filled retinotectal projections in 5 days post-fertilization (dpf) larvae heatshocked at 12 somites. In controls (*A*), the entire tectum is innervated. In *hs:dnBMPR* transgenic animals (*B*) only dorsal tectum is innervated, similar to *radar*^{s327}. (*C* and *D*) Brightfield lateral view of 4 dpf larvae. Control larvae (*C*) show normal eye size, whereas *hs:dnBMPR* larvae (*D*) have smaller eyes similar to *radar*^{s327}.

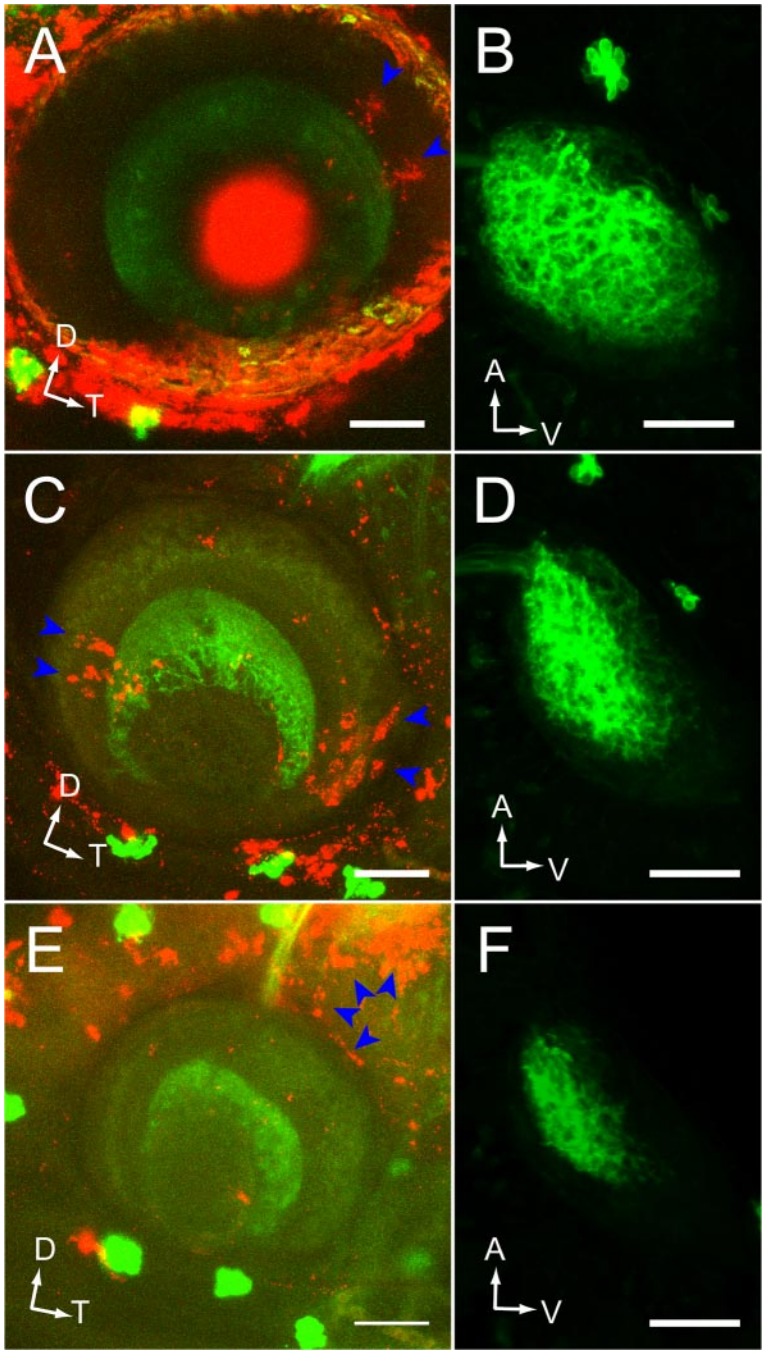

Fig. S4. Donor-derived clone location and position-dependent rescue of the retinotectal projection in WT-> *radar*^{s327} chimeras. (*A*, *C*, and *E*) Lateral confocal projection of the eye. Donor-derived clones were labeled with rhodamine-dextran (blue arrowheads). GFP was only expressed in RGCs of the host. Dorsal up, temporal to the right. (Scale bars, 50 μm.) (*B*, *D*, and *F*) Corresponding dorsal confocal projections of tectum. Anterior up, ventral to the right. (Scale bars, 50 μm.) (*A* and *B*) Donor-derived clones in dorsal eye drive substantial rescue of ventral tectum innervation. (*C* and *D*) Donor-derived clones in the ventral eye fail to rescue ventral tectum innervation. (*E* and *F*) Donor-derived clones outside of the eye do not rescue ventral tectum innervation.

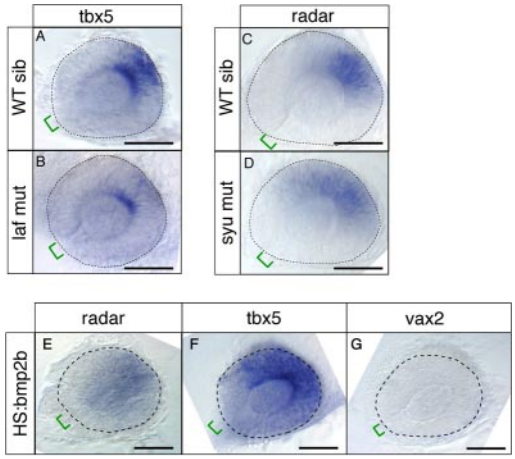

Fig. S5. Additional analysis of Radar signaling and regulation. (*A*–*G*) Dissected eyes following whole-mount in situ hybridizations on 26-somite embryos with the antisense probes indicated. (Scale bars, 50 μm.) (*A*) WT eyes express *tbx5* in the dorsal retina, opposite of the optic fissure. (*B*) *lost-a-fin* (*laf*) mutants, lacking functional Alk8 receptor, have reduced expression of *tbx5*. (*C*) In WT eyes *radar* expression is restricted to a narrow patch opposite of the optic fissure. (*D*) In *sonic you* (*syu*) mutants, in which Shh is disrupted, *radar* expression is slightly expanded to the ventral retina. (*E*–*G*) *bmp2b* overexpression mirrors the effects of *bmp4* overexpression. *hsp70*:*bmp2b* transgenic fish were induced at 12–14 somites to overexpress *bmp2b*. *radar* expression is weakly expanded, *tbx5* expression is strongly up-regulated, *vax2* expression is absent from the retina.