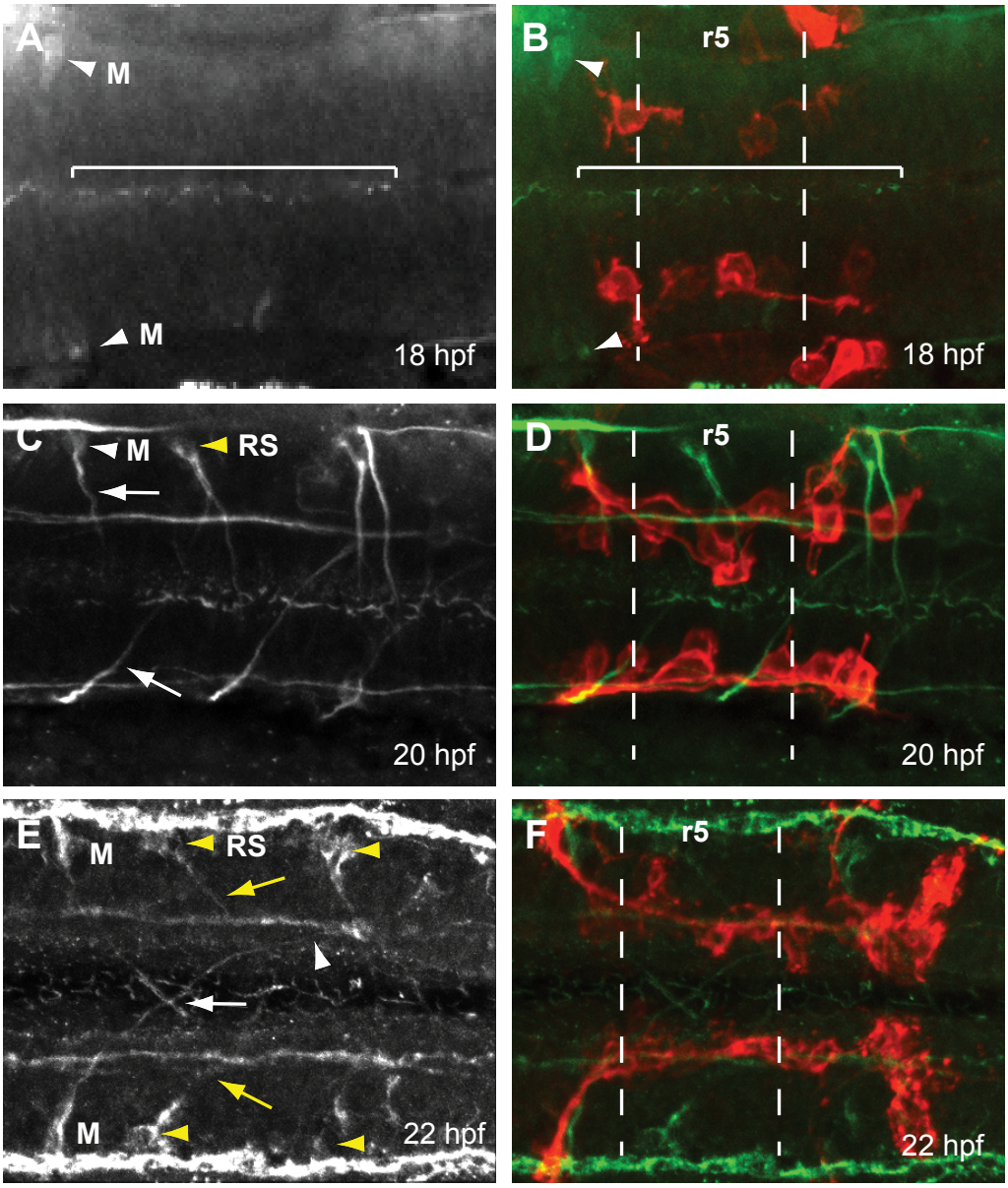



**Fig. S1**. **Early FBMN migration into r5 and r6 precedes both Mauthner and reticulospinal neuron axon projection into the hindbrain.** Maximum projection dorsal views of Tg(*zCREST*:membRFP) embryos immunostained with an acetylated tubulin antibody (A,C,E) and merged images (green; B,D,F). (**A**,**B**) At 18 hpf, Mauthner (M) neuron cell bodies are present in r4 (white arrowhead) but their axons are not yet present. Cilia in the lumen of the hindbrain (bracket) as well as stereocilia in the otic vesicle (not shown) are present at this stage, verifying antibody staining efficacy. (**C**,**D**) At 20 hpf, axons of the Mauthner neurons have begun to project towards the hindbrain midline (white arrow). Additionally, reticulospinal (RS) neuron cell bodies are present (yellow arrowhead) and have begun to project their axons toward the MLF. (**E**,**F**) At 22 hpf the axons of the Mauthner neurons have crossed at the midline in r5 (white arrow) and will soon contribute to the MLF axon tract (white arrowhead). RS neurons (yellow arrowhead) have sent out projections that have contributed to the MLF (yellow arrow).

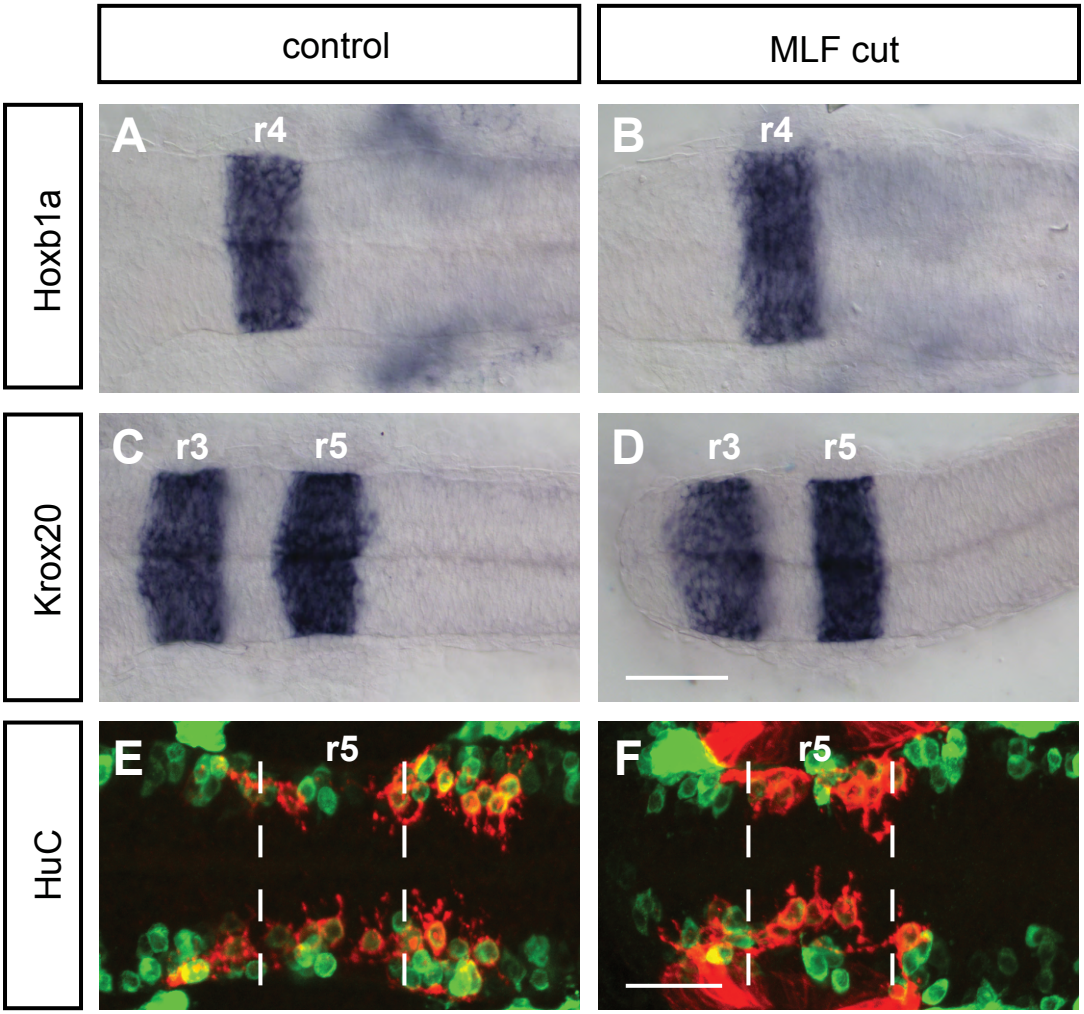



**Fig. S2. Blocking the MLF does not affect the organization of the hindbrain**. (A-D) *In situ* hybridization of anterior-posterior markers in control and MLF-blocked embryos at 24 hpf. (**A**,**B**) *hoxb1a* expression in r4 is unaffected in embryos lacking MLF axons (B) compared with controls (A). (**C**,**D**) *krox20* expression in r3 and r5 is unaffected in embryos lacking MLF axons (D) compared with controls (C). (**E**,**F**) HuC antibody staining in *zCREST1*:membRFP transgenic embryos at 24 hpf. (F) HuC-labeled neuron number (green) is not affected by blocking MLF axons (F); however, the localization of these neurons is somewhat disorganized compared with controls (E). Dashed lines indicate r5 boundaries. Scale bars: 50 μm for A-D; 20 μm for E,F.

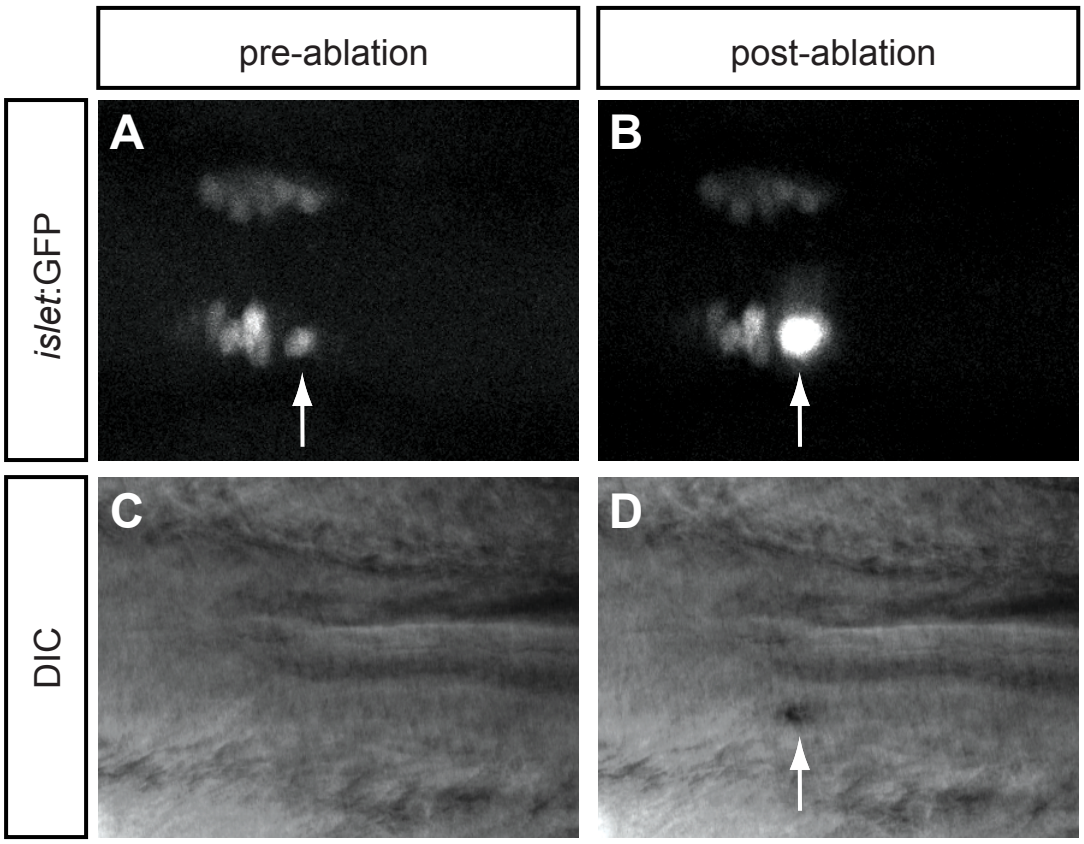



**Fig. 3. Laser ablation effectively kills the pioneer neuron.** (**A**,**B**) Dorsal views of a Tg(*islet*:GFP) embryo (A) before and (B) immediately after ablation of the pioneer neuron (arrow). (**C**,**D**) DIC imaging of the same embryo (C) before and (D) immediately after ablation of the pioneer neuron. (B,D) Arrows indicate that the pioneer neuron has indeed been killed by the ablation.

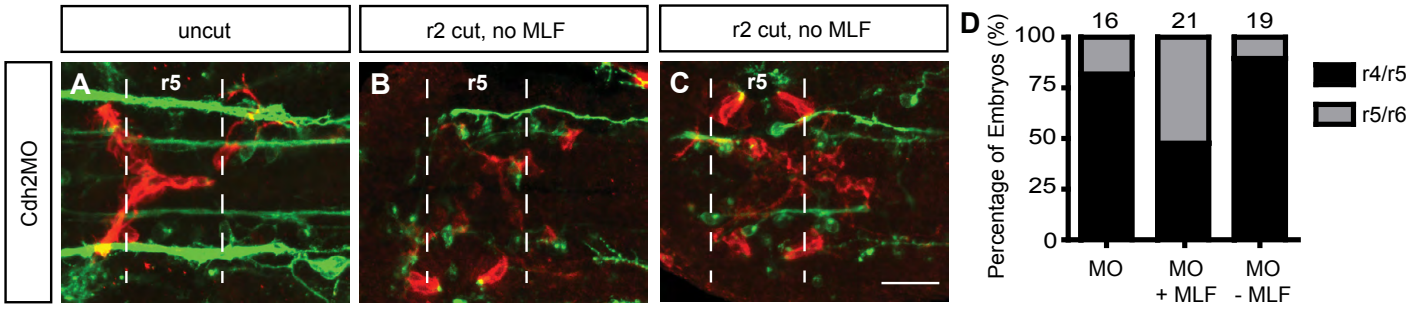
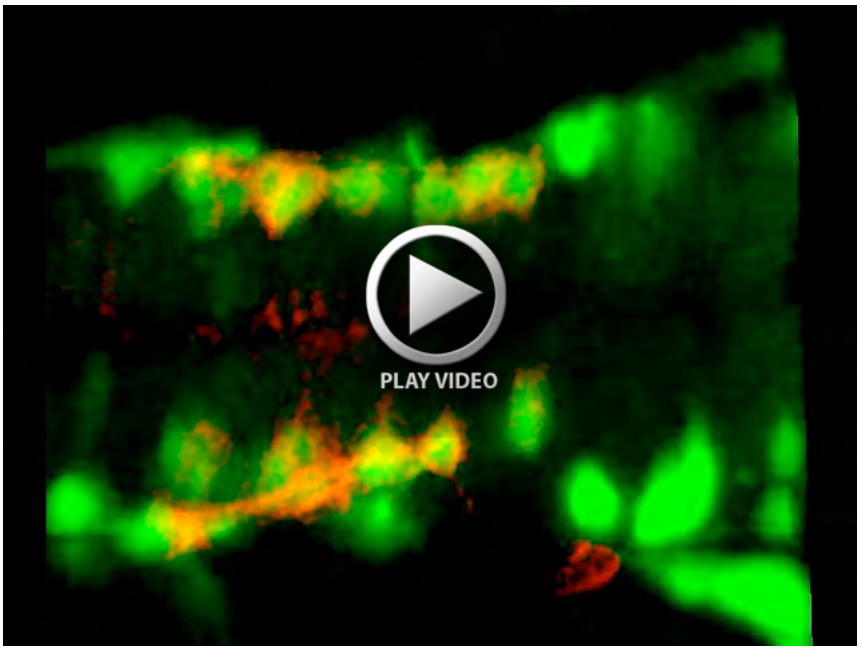
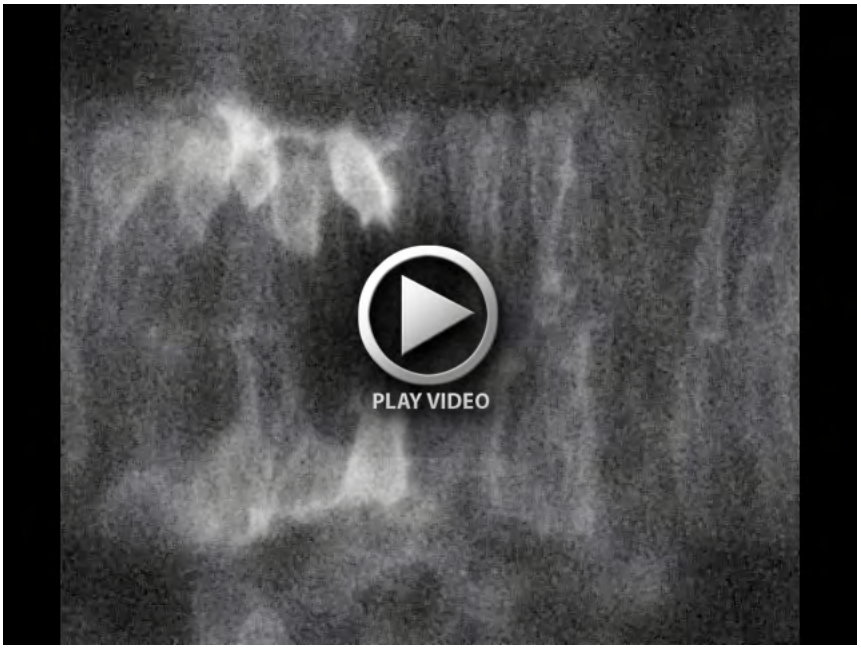


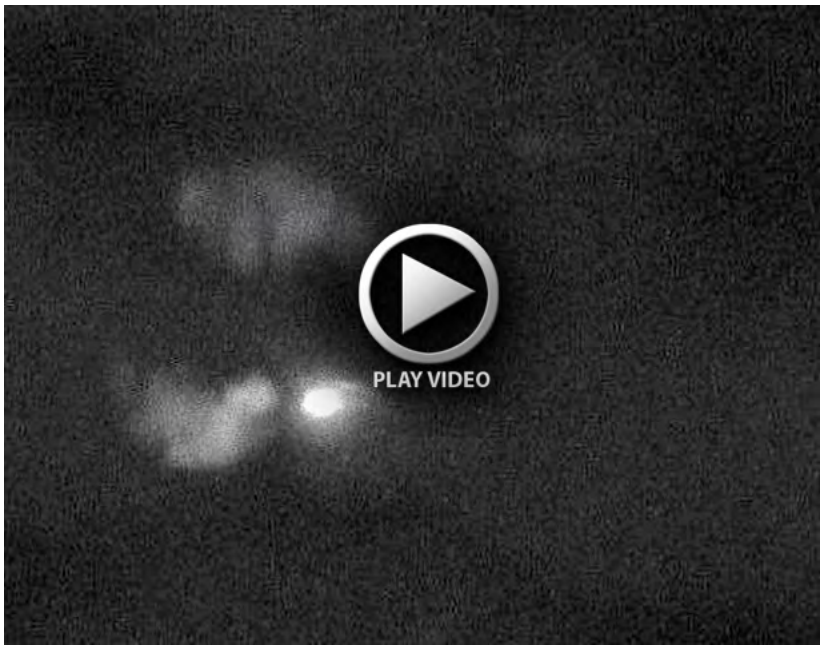

**Fig. S4. Loss of both Cdh2 and MLF axons in the hindbrain blocks FBMN migration.** (A-C) Maximum projection dorsal views of FBMNs (red) and the MLF (green) in 24 hpf Cdh2-depleted Tg(*zCREST1*:membRFP) zebrafish embryos stained with zn-12 antibody. In all treatments, FBMNs coalesce in the midline; however, FBMNs never migrate along either the LLF or projections of the reticulospinal neurons. Broken lines highlight r5 boundaries. (**A**) Uncut control Cdh2-depleted embryos at 24 hpf show typical Cdh2-depletion phenotype of FBMN migration into r5 and coalescence of neurons in the midline. In a smaller proportion of Cdh2-depleted embryos, FBMNs still coalesce at the midline but are able to migrate into r6. (**B**) In embryos lacking both Cdh2 and the MLF, the majority of embryos show FBMNs that migrate only into r5. (**C**) In a smaller number of embryos lacking both Cdh2 and the MLF, FBMNs migrate into r6. (**D**) Percentage of embryos affected by Cdh2 depletion and severing manipulations. Cdh2 depletion alone (MO) results in 82% of embryos exhibiting stalled FBMN migration in r5 and 18% with FBMNs migrating into r6. In Cdh2-depleted embryos in which the embryos were severed incompletely so the MLF is still present (MO +MLF), 48% of embryos show stalled migration in r5, whereas 52% of embryos show FBMN migration reaching r6. In embryos depleted of both Cdh2 and the MLF (MO –MLF), 89% result in FBMNs stalling in r5 and 11% result in FBMNs reaching r6. The number of embryos scored is indicated. Scale bar: 20 µm.



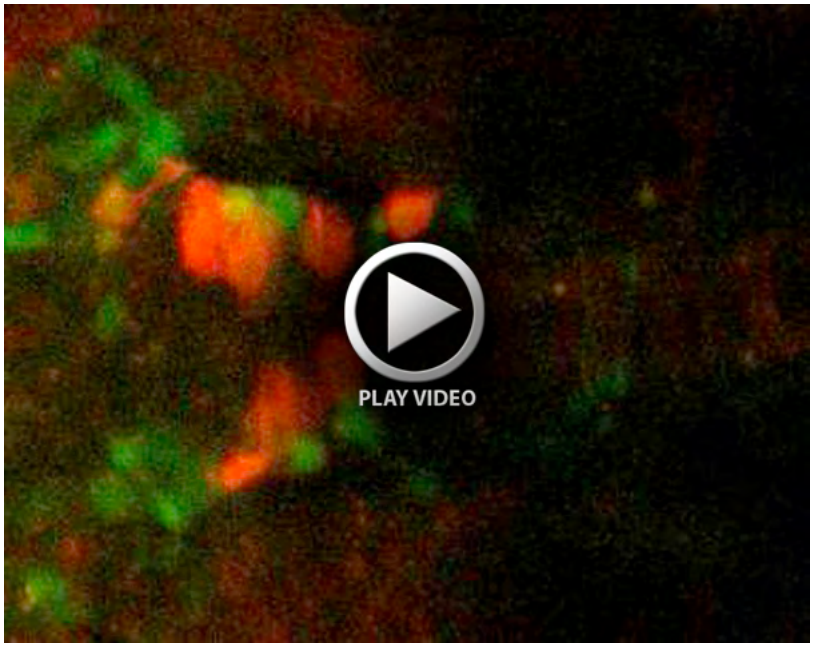

**Movie 1. FBMN migration into r5/r6 occurs prior to the presence of the MLF.** Time-lapse movie of a *zCREST1*:membRFP/*HuC*-GFP double transgenic embryo from 18-20 hpf. *Z*-stacks were taken at 5-minute intervals. FBMNs (red) migrate from r4 into r5 before the first MLF axon (green) enters the r4-r6 hindbrain region.




**Movie 2. The first FBMN to migrate navigates the environment.** Time-lapse movie of a *zCREST1*:membRFP transgenic embryo from 18-20 hpf. *Z*-stacks were taken at 5-minute intervals. The first FBMN to migrate proceeds ahead of the following FBMNs, leaves behind a trailing axon and sends out many protrusions in the direction of migration.




**Movie 3. Ablation of the pioneer neuron blocks FBMN migration into r5 but does not affect FBMN motility.** Time-lapse movie of a *zCREST1*:membRFP transgenic embryo taken from 19-20 hpf after ablation of the pioneer neuron. *Z*-stacks were taken at 5-minute intervals. Ablation of the pioneer neuron blocks FBMN migration into r5; however, FBMNs are still motile. The detritus of the ablated cell was followed in all eight pioneer ablation time-lapse movies obtained and it was always excluded from the neuroepithelium, either at the lateral edge or into the ventricle (as observed in this movie).




**Movie 4. Cdh2 depletion affects FBMN migration, but not pioneer migration.** Time-lapse movie of a *zCREST1*:membRFP/*HuC*-GFP double transgenic embryo injected with Cdh2MO taken from 18-22 hpf. *Z*-stacks were taken at 5-minute intervals. FBMNs (red) migrate from r4 into r5 before the first MLF axon (green) enters the r4-r6 hindbrain region. The top FBMN chain shows the migration of most FBMNs stalls in r5, whereas a pioneer neuron and a second FBMN escape into r6. Bottom FBMN chain shows Cdh2-depleted FBMN migration into the r5 midline.