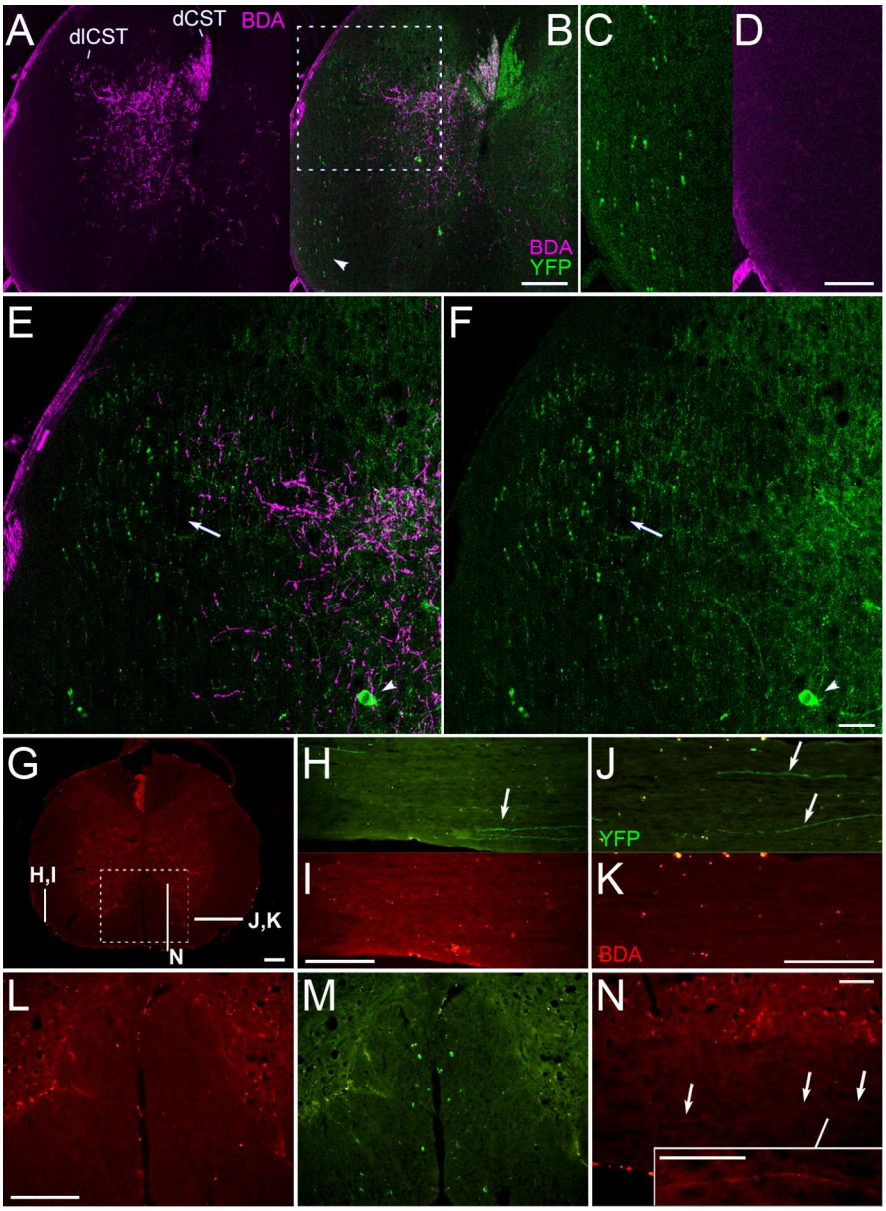
SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Version of Figure 1 with magenta-green confocal panels. YFP-labeled axons are in areas of the spinal cord that do not contain anterogradely-labeled CST axons. ***A***-***F***, Confocal images of a spinal cord cross section with amplified BDA signal. BDA labeled CST axons (***A***) are illustrated relative to YFP labeling (***B***) following BDA injections into the right sensorimotor cortex. The ventral lateral white matter (arrowhead, ***B***) is enlarged in ***C*** and ***D***; note the absence of BDA-labeled axons in ***D***. The dorsolateral column (boxed region in ***B***) is shown in higher magnification in ***E***-***F***; arrowheads indicate YFP-labeled neurons in the grey matter; arrows indicate the region of the rubrospinal tract, which overlaps with the dlCST. Note that some YFP-labeled axons in this region are distinctly thicker in appearance. ***G***, Cross section with mini-ruby BDA labeling and indicated locations of sections shown in ***H***-***N***. ***H***-***K***, Sections in ventral lateral white matter with YFP-labeled axons (arrows in ***H***,***J***) and as shown for BDA labeling (***I***,***K***); note the bilateral absence of BDA-labeled axons. ***L***-***M***, BDA (***L***) and YFP labeling (***M***) in the ventral column (boxed region from ***G***). ***N***, A BDA-labeled axon coursing longitudinally in the ventral column ipsilateral to the injected cortex, as seen in one of 14 mice. The inset in ***N*** has been further enhanced for contrast. dCST, dorsal corticospinal tract; dlCST, dorsolateral CST. Scale bars, 200 μm (***A***-***B, G***-***M***), 100 μm (***C***-***D, N*** and inset), 50 μm (***E***-***F***).

Supplementary Figure S2. Magenta-green version of Figure 4. Some brainstem neurons that project to the spinal cord express YFP. ***A***, Fluorescent Nissl-like labeling (NeuroTrace®) in the midbrain in a mouse that had FG injected into the spinal cord to retrogradely label supraspinal neurons. ***B***, FG and YFP labeling in the midbrain (same section as ***A***). ***C***-***F***, Merged FG and YFP imaging (***C***,***E***) and YFP imaging alone (***D***,***F***) in the red nucleus (***C***,***D***) and reticular formation (***E***,***F***). Note the FG-labeled rubrospinal and reticulospinal neurons that are YFP-positive (arrows). ***G***, Quantification of sampled YFP and FG co-labeled rubrospinal and reticulospinal neurons through 1.5 mm of midbrain in 3 mice. ***A***-***F*** are single plane confocal images. FG, Fluoro-Gold; Ret. formation, reticular formation. Scale bars, 200 μm (***A***-***B***), 100 μm (***C***-***F***).

Supplementary Figure S3. Version of Figure 5 with magenta-green confocal panels. Dense and faint YFP labeling impedes individual axon visualization and tracing. ***A-B***, Epifluorescent images at 20X magnification showing individual BDA-labeled CST axons in the white matter (arrowhead, ***A***) and grey matter (arrows, ***A***) and YFP labeling in the same section (***B***). Note the faint labeling of individual axons with YFP. ***C***-***E***, Confocal images at 40X magnification showing CST axons labeled with BDA from ***A*** (***C***, arrowhead, arrow and inset) are co-labeled with YFP (***D***, ***E***). ***F-H***, Confocal projections of one axon originating from the dCST and traced by experienced (X) and novice (+) axon tracers. Individuals were instructed to trace the axon from the arrowhead to the best of their ability within the stack of 40X confocal images, as visualized first by YFP and then BDA. Note that none of the YFP-based tracings extend beyond the grey matter interface (***F***), whereas all the BDA-based tracings extend into the grey matter (***H***). One novice tracer continued tracing to an incorrect axon segment under

visualization with BDA (+* in ***H***). dCST, dorsal corticospinal tract; gm, grey matter; wm, white matter. Scale bars, 100 μm (***A***-***B***), 50 μm (***C***-***H***), 10 μm (***C***-***E*** insets).



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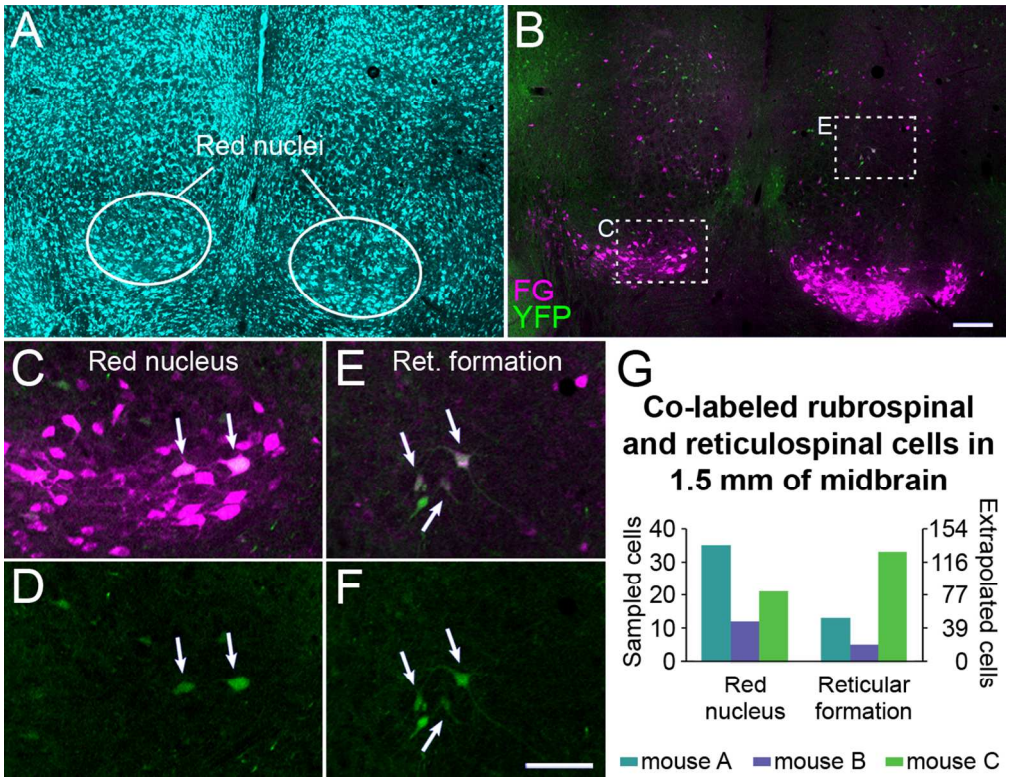

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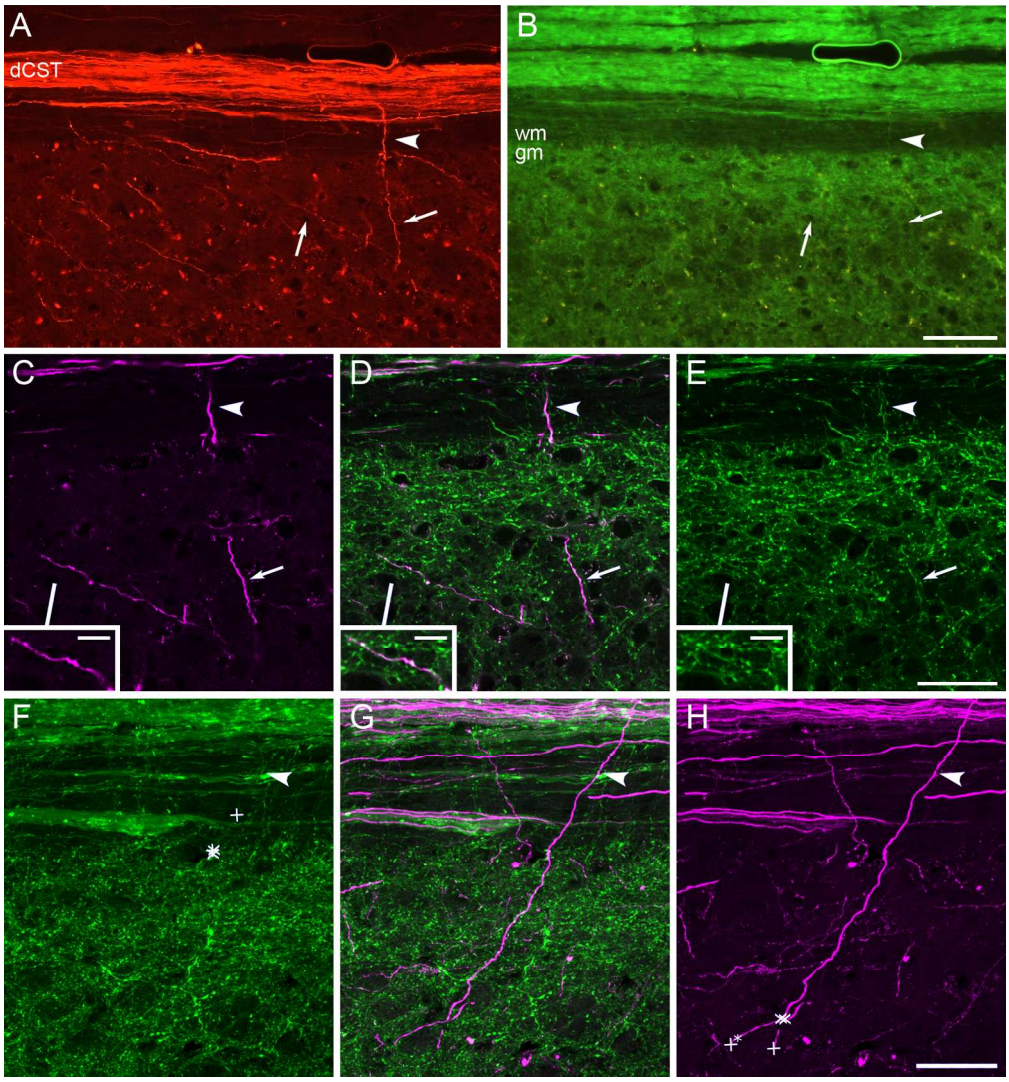

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