

Supplementary information, Figure S1

Figure S1 Serotonergic RST axons descend through the ventral spinal cord from E12.5 through E16.5. Immunostaining with anti-5-HT was applied to whole mounts and coronal sections to label specifically the serotonergic RST axons in the developing spinal cord. (**A**) Serotonergic RST axons originated from the E12.5 CRN. Pioneer axons of descending serotonergic RST began projecting into the spinal cord at E12.5. Scale bar: 1 000 μ m. (**B**) Descending serotonergic RST axons projected into

the upper cervical segment of the E12.5 spinal cord, through the ventral and ventral lateral funiculus. Scale bar: 200 μ m. (C) Descending serotonergic RST axons reached the thoracic spinal cord at E13.5. Scale bar: 1 000 μ m. (D) Descending serotonergic RST axons were localized in the ventral and ventral lateral funiculus of E13.5 thoracic spinal cords. Scale bar: 200 μ m. (E) Descending serotonergic RST axons projected into the lumbar spinal cord at E14.5. Scale bar: 1 000 μ m. (F) Descending serotonergic RST axons were localized in the ventral and ventral lateral funiculus of E14.5 upper lumbar spinal cords. Scale bar: 200 μ m. (G) Descending serotonergic RST axons projected to the sacral spinal cord at E16.5. Scale bar: 1 000 μ m. (H) Major bundles of descending serotonergic RST axons remained localized in the ventral and ventral lateral funiculus of E16.5 lumbar spinal cords. Scale bar: 200 μ m. Rectangles outline the brainstem region containing serotonergic CRN. White arrowheads point to descending serotonergic RST axons.