Inventory of supplemental items

Supplemental Experimental Procedures – provide more detailed information about experimental procedures used in the study.

Supplemental Figures – we provide 7 supplemental figures related to the main figures in the text.

Supplemental Tables - Supplemental Table 1 contains information on the efficiency of motor neuron induction using diverse media compositions; Supplemental Table 2 provides detailed statistical information about the number of independent experiments, number of cells examined, and relevant p values

Supplemental References

Supplemental Experimental Procedures

Differentiation of ES Cells

Experiments were performed using the following ES cell lines: HBG3 (harbors *Hb9-GFP* transgene) (Wichterle et al., 2002), HBR (*Hb9-RFP* transgene), and wild-type MM13. We generated HBR ES cell line by introducing *Hb9*::*RFP* transgene into the Olig2-GFP ES cell line (generously provided by Dr. Bennett Novitch). GFP expression driven by the Olig2 promoter in differentiated RFP^+ motor neurons is below the detection level settings for *Hb9-GFP* transgene in ES motor neurons. For RA/Hh differentiation (Wichterle et al., 2002), ES cells were plated at 50,000 cells/ml in ADFNK differentiation medium composed of Advanced D-MEM/F-12 (Invitrogen) : Neurobasal medium (Invitrogen) (1:1), 10% Knockout Serum Replacement (Invitrogen), 200 mM L-Glutamine (Invitrogen), 0.1 mM β-mercaptoethanol (Sigma), and Penicillin/Streptomycin (Pen/Strep) (Invitrogen). Additional medium components used in our studies include: Glasgow Minimum Essential Medium (GMEM) (Invitrogen), Dulbecco's Modified Eagle Medium (D-MEM) (Invitrogen), and F12 nutrient mixture (Invitrogen). Embryoid bodies (EBs) were split 1:4 on day 2 of differentiation and medium was supplemented with 1 μM all-trans retinoic acid (RA, Sigma) and Hh agonist: 0.5 μM SAG (Calbiochem) or 1 μM HhAg1.3 (Frank-Kamenetsky et al., 2002). For CV differentiation, ES cells were plated onto non-adherent tissue culture dishes in ADFNK medium (~20,000 cells/ml). EBs were split 1:4 on day 2 and medium was changed on days 1, 2 and 5 of differentiation. In addition, the following reagents were supplemented to the cultures: 10 nM-1 μM RA, 10 nM-1 μM Hh Agonist, 100 nM 61414 Hh receptor antagonist (Williams et al., 2003), 1 μg/ml recombinant mouse Dickkopf-1 (R&D Systems), 50-100 nM

FGF/VEGF Receptor Tyrosine Kinase Inhibitor (PD173074, Calbiochem) (Mohammadi et al., 1998), 100-625 ng/ml recombinant human bFGF (PeproTech), and 10 ng/ml recombinant rat GDNF (R&D Systems).

Dissociated Cultures of ES motor neurons

For immunocytochemical analysis, EBs were dissociated using Trypsin-EDTA (Invitrogen) on day 6 and plated at low density on poly-l-ornithine (Sigma; diluted in water) and mouse laminin (5 ng/ml final concentration in PBS; Invitrogen) pre-coated coverslips (Carolina Biological). Cells were plated and maintained in ADFNB culture medium [Advanced D-MEM/F12:Neurobasal (1:1), 1x B27 supplement (Invitrogen), 200 mM L-Glutamine, and 1x Pen/Strep]. Selected cultures were supplemented with GDNF (10 ng/ml). ES motor neuron cultures were fixed with 4% paraformaldehyde (PFA) for ~15 minutes at room temperature one day after plating (day 7) and processed for immunocytochemistry. For quantifications of ES motor neuron cultures, ~10 random fields were imaged using a confocal microscope LSM Zeiss Meta 510. Expression of transcription factors in individual cells was quantified and gated to GFP^+ ES motor neurons. For quantifications of motor neuron differentiation efficiency, embryoid bodies were dissociated and the total number of cells and Hb9-GFP^+ ES motor neurons determined using a tissue culture fluorescence microscope.

Immunocytochemistry

Immunocytochemistry on dissociated ES motor neurons and cryosectioned embryoid bodies was performed as described (Wichterle et al., 2002). In this study, we

used the following antibodies: goat anti-Meis1 (Santa Cruz Biotechnology, SCB), mouse anti-Lhx3 (Developmental Studies Hybridoma Bank, DSHB), mouse anti-Lhx1/2 (DSHB), mouse anti-Nkx2.2 (DSHB), goat anti-Hoxc6 (SCB), rabbit anti-Hoxd9 (SCB), rabbit anti-Otx2 (kindly provided by G. Corte), mouse anti-Isl1 (DSHB), mouse anti-NeuN (Chemicon), rabbit anti-dsRed (Clontech), rabbit anti-Brachyury (SCB), mouse anti-Sonic Hedgehog (DSHB), mouse anti-FoxA2 (DSHB), mouse anti-Pax7 (DSHB), mouse anti-Hb9 (DSHB), and guinea pig anti-Hb9 (kindly provided by the Project A.L.S. laboratory). In addition to the commercially available antibodies, we used following previously characterized polyclonal antibodies (Dasen et al., 2005; Liu et al., 2001; Novitch et al., 2003): mouse anti-Hoxc8, guinea pig anti-Olig2, guinea pig anti-Isl1/2, guinea pig anti-Hoxc6, guinea pig anti-Hoxa5, rabbit anti-Hoxc9, guinea pig anti-Hoxd10, rabbit and guinea pig anti-FoxP1, rabbit anti-Lhx3, rabbit anti-Pea3, and guinea pig anti-Scip.

Transplantation of ES motor neurons into Chick Neural Tube

Transplantation of ES motor neurons into the chick developing spinal cord was performed as described (Wichterle et al., 2002; Wichterle et al., 2009). To compare cervical to brachial and thoracic ES motor neurons, HBR ES cells were differentiated in the presence of RA/Hh and HBG3 ES cells were differentiated using CV condition. On day 5, Hb9-RFP and Hb9-GFP EBs were dissociated using Trypsin, mixed, and 5,000-10,000 cells were re-aggregated overnight in 50 µl hanging drops (Renoncourt et al., 1998). Cell aggregates were transplanted into HH stage 15-17 chick embryos at the brachial (somite levels ~15-20) or thoracic (somite levels ~20-25) level of the developing

spinal cord. Three days after transplantation, embryos were fixed with 4% PFA at 4ºC for ~1 hour and processed for immunohistochemistry. On average ~50-75% of chick embryos survive the transplantation manipulation. From these, ~50% of transplanted chick embryos contained successfully engrafted GFP^+ cells visible under the fluorescence dissecting microscope. ~700-1000 cells (containing ~100-300 ES motor neurons) were transplanted on average. ~5 serial sections containing 10-50 transplanted motor neurons could be recovered from each embryo. Thus approximately ~30-50% of ES motor neurons survive 3 days upon successful engraftment. Quantifications of axonal projections were performed by measuring areas of GFP and RFP fluorescence in the limb and the axial nerve branches using Image J software (NIH, http://rsbweb.nih.gov/ij/).

Retrograde Labeling of Transplanted ES motor neurons

Retrograde labeling of motor neurons was performed as previously described (Dasen et al., 2005). Briefly, embryos were dissected three days post-transplantation, axial or limb GFP^+ nerve branches were cut under fluorescence dissection microscope and retrogradely labeled with 3000 MW lysine-fixable tetramethylrhodamine dextran (RhD, Molecular Probes). Embryos were incubated in an oxygenated bath containing DMEM (Chemicon) : F12 (Invitrogen) (1:1) medium supplemented with Pen/Strep at 37ºC for 3-5 hours, then fixed and processed for immunohistochemistry. FoxP1 and Lhx3 expression levels in individual retrogradely labeled transplanted (RhD^+ GFP^+) and endogenous (RhD^+) motor neurons were analyzed using MetaMorph software (Meta Imaging Series Software 7.1, Molecular Devices).

Cell Migration Quantifications

Cryosections of chick spinal cords harboring transplanted ES motor neurons were simultaneously labeled for Lhx3 and FoxP1. Z-stack images of 15-20 μm thick sections were acquired using LSM Zeiss Meta 510 confocal microscope. Relative medio-lateral position of endogenous and grafted motor neurons was measured (0 corresponds to the medial-most and 1 corresponds to the lateral-most endogenous motor neuron) using Image J software. Grafted motor neurons that failed to migrate from the transplantation site were not included in the analysis.

BrdU staining of day 5 ES motor neurons

Thymidine analog BrdU (10μM) was supplemented to culture media on day 5 of differentiation 4 hours prior to the administration of 1 μM RA. Cultures were dissociated on day 6 and plated on poly-l-ornithine and laminin pre-coated coverslips. Cells were immunostained with antibodies against Lhx1 and GFP, post-fixed with 4% PFA, rinsed with PBS, exposed to 2N HCl for 10 minutes at 37°C, rinsed with PBS, and immunostained with 1:200 diluted anti-BrdU, Alexa Fluor 647 conjugated antibody (Invitrogen).

Differentiation of CV ES motor neurons in the context of RA/Hh cells

Hb9-GFP ES cells were differentiated under CV condition and wild-type ES cells were differentiated using RA/Hh. EBs were dissociated into single cells on day 4 with Trypsin, mixed in 1:5 ratio (CV : RA/Hh), reaggregated in hanging drops and cultured

until day 7 in ADFNK medium. Aggregates were treated with 10ng/ml of GDNF on day 5. Aggregates were fixed on day 6 with 4% PFA, sectioned on a cryostat, and immunostained with antibodies against generic, columnar, and motor pool specific markers (see above).

Supplemental Figure Legends:

Supplemental Figure S1 related to Figure 1. Characterization of Endogenous Signals Contributing to the Rostrocaudal and Dorsoventral Patterning of Differentiating ES Cells.

A) Schematic representation of the principal extrinsic signals that control rostrocaudal patterning of the developing spinal cord. Below the spinal cord are depicted expression domains of Otx2, Hoxa5, Hoxc8, and Hoxd9 markers of forebrain/midbrain, cervical and rostral brachial, caudal brachial and rostral thoracic, and thoracic regions of the central nervous system, respectively.

B-E) Immunostained sections of day 6 embryoid bodies. To determine if ES cells differentiated under the CV condition (control, B) are patterned by endogenously acting signals and whether CV cultured ES cells can respond to exogenous factors, EBs were treated with 100 nM retinoic acid or 50 nM FGF receptor inhibitor (PD173074) on day 3 of differentiation, or 1 µg/ml of Wnt antagonist Dickkopf-1 (Dkk1) on day 2 of differentiation. Expression of Otx2 on day 5, and of Hoxa5, Hoxc8, Hoxd9, and neuronal marker NeuN on day 7, was examined. Blocking Wnt signaling with Dkk1 prevented the specification of spinal cells and motor neurons (absence of Hox expression and Hb9-GFP) and resulted in the appearance of $Otx2^{+}$ forebrain/midbrain progenitor cells (D).

Addition of 100 nM RA on day 3 of differentiation resulted in the generation of $Hoxa5^+$ ES motor neurons that lacked Hoxc8 and Hoxd9 (C) expression. Treatment with PD173074 prevented the generation of $Hoxc8^+$ and $Hoxd9^+$ ES motor neurons while induction of $Hoxa5^+$ ES motor neurons was not affected (E).

F) Significantly larger ($p<0.01$) fraction of $Hoxd9^+$ ES motor neurons were obtained after treatment of differentiating cells with FGF2 (100 ng/ml) on day 3. Results from three independent experiments (mean ± SEM).

G) Significantly larger ($p<0.001$) fraction of $Hoxd10^+$ ES motor neurons were obtained after treatment of differentiating cells with Gdf11 (10 ng/ml) on day 2. Results from three independent experiments (mean ± SEM).

H, I) To test if the induction of ES motor neurons depends on endogenous Sonic Hedgehog (Shh) signaling, embryoid bodies differentiated in CV condition were treated with 100 nM Shh antagonist (61414) on day 2 of differentiation and expression of Pax7, Olig2, Nkx2.2 (on day 5), and Hb9 (on day 7) was examined. Inhibition of endogenous Shh signaling resulted in the loss of Nkx2.2 and Olig2 expression and failure in ES motor neuron differentiation as indicated by the absence of $Hb9^+$ cells. Instead, the majority of cells acquired expression of dorsal spinal progenitor marker Pax7. These results suggest that ES motor neuron differentiation in the absence of exogenous factors is dependent on endogenous Shh signal induced in differentiating embryoid bodies.

J-L) To characterize the sources of Shh signaling, embryoid bodies were immunostained with antibodies against Shh, FoxA2, and Brachyury (Bra) on day 5. Endogenous Shh signaling centers expressed floor-plate/notochord marker FoxA2 (K,

L) and Shh (J). J and K are the same image triple labeled for GFP, FoxA2 and Shh. A subset of FoxA2$^+$ cells co-expressed notochord marker Brachyury (L) suggesting that floor plate and notochord-like signaling centers may contribute to the specification of ES motor neurons under CV conditions.

M) Model for CV differentiation. ES cells grown in the presence of FGF and absence of BMP signals differentiate to neural ectoderm (NE). Neuralized embryoid bodies can be caudalized with canonical Wnt signals to specify generic spinal progenitors (SP). Induction of cervical and brachial/thoracic ES motor neurons (MNs) is controlled by RA and FGF in combination with a ventralizing Shh signal.

Supplemental Figure S2 related to Figure 2. Columnar Identity of ES Motor Neurons.

A) To determine columnar identity of ES motor neurons, dissociated cultures differentiated under RA/Hh and CV conditions were immunostained for Lhx3 and FoxP1 on day 7. Majority of GFP$^+$ ES motor neurons (grey) generated in the presence of RA and Hh expressed Lhx3 but not FoxP1. In contrast, many ES motor neurons differentiated under CV conditions expressed FoxP1. FoxP1 and Lhx3 expressions were mutually exclusive in ES motor neurons.

B) When compared to RA/Hh conditions, CV differentiation of ES motor neurons resulted in a decrease in the number of Lhx3$^+$ ES motor neurons ($p<0.001$) and an increase in FoxP1$^+$ ES motor neurons ($p<0.001$). Results from three independent differentiation experiments (mean ± SEM).

Supplemental Figure S3 related to Figure 3. The Effect of Late Retinoic Acid Exposure on Rostrocaudal Identity of CV ES Motor Neurons.

A) Treatment of ES cells differentiating under CV conditions with RA on day 3 resulted in the loss of Hoxc8 expression in ES motor neurons. In contrast, day 4 RA treatment did not interfere with Hoxc8 expression in ES motor neurons.

B) Treatment of embryoid bodies with RA on day 3 led to a decrease in $Hoxc8^{+}$ ES motor neurons compared to control CV conditions ($p<0.001$). In contrast, RA treatment on day 4 or 5 resulted in a similar percentage of $Hoxc8^{+}$ ES motor neurons as in control conditions. Results from two independent experiments (mean ± SEM).

C) A subset of ES motor neurons derived under CV conditions expressed LMC marker retinaldehyde dehydrogenase 2 (RALDH2). $FoxP1^{+}$ and $Lhx3^{+}$ ES motor neurons are intermixed and failed to segregate into spatially distinct motor columns within embryoid bodies.

Supplemental Figure S4 related to Figure 4. Analysis of Motor Pool Identity of Caudal Brachial ES Motor Neurons.

A-C) To test whether expression of Pea3 or Scip depends on limb derived signals, we performed limb ablation in HH stage 17 developing chick embryos (A). This led to a loss of Pea3 expression on the ablated but not on the control side (B). On the contrary, Scip expression was not affected by the procedure (C).

D) As in vivo (Dasen et al., 2005), the majority of $Pea3^{+}$ ES motor neurons maintained Hoxc6 expression while $Scip^{+}$ were $Hoxc6^{-}$. $Pea3^{+}$ ES motor neurons did not express

Meis1. Pea3 expression could not be elicited by GDNF application to RA/Hh differentiated Isl1^{+} ES motor neurons of rostral cervical identity.

Supplemental Figure S5 related to Figure 6. Settling Preferences of Transplanted ES Motor Neurons in the Developing Spinal Cord.

A, B) CV differentiated ES motor neurons co-expressing Lhx1, FoxP1, and GFP after transplantation into brachial chick spinal cord. A and B Panels represent the same section triple labeled for Lhx1, FoxP1, and GFP.

C) GFP^{+} ES motor neurons derived under CV conditions retained Hoxc8 expression after transplantation.

D) ES motor neurons differentiated under CV conditions in the absence of GDNF do not express Pea3 five days after transplantation.

E) Pea3^{+} LMC neurons (FoxP1^{+}/GFP^{+}) were detected within grafts exposed to 10 ng/ml of GDNF 24 hours prior to transplantation.

F-K) CV generated GFP^{+} ES motor neurons were transplanted into the chick brachial spinal cord. We examined the positioning of Lhx3^{+} (F-H) and FoxP1^{+} (I-K) ES motor neurons after three days. Pairs of panels (F, I), (G, J), and (H, K) depict same sections triple labeled for FoxP1, Lhx3 and GFP. In each panel, the spinal cord boundary is outlined; lateral edge is to the right and midline to the left.

Supplemental Figure S6 related to Figure 7. Axonal Projection Analysis of Transplanted ES Motor Neurons.

A, B) Columnar axonal projections at brachial and cervical/thoracic spinal cord. At brachial level, LMC axons project to the limb muscles and MMC neurons axially (A).

HMC neurons that replace LMC neurons at cervical and thoracic levels project to body wall muscles (B).

C) Aggregates of RA/Hh induced RFP^+ and CV generated GFP^+ ES motor neurons were plated on laminin coated coverslips. Individual RFP^+ and GFP^+ axons are identifiable after 24 hours in culture.

D) RFP^+ motor neurons transplanted into the brachial chick spinal cord extend axons into the axial (A) and limb (L) nerve branches (same section as in Figure 7B).

E) Quantification revealed a consistent bias of RFP^+ axons towards the axial nerve branch over the limb nerve branch ($p=0.036$, see also Figure 7C). Results from six transplanted embryos (mean ± SEM).

F) Retrograde labeling of transplanted RA/Hh induced GFP^+ ES motor neurons.

G) Ventral horn of the spinal cord containing transplanted GFP^+ ES motor neurons (appear green), some of which were retrogradely labeled with RhD from the limb (appear yellow).

H-K) Retrogradely labeled GFP^+ ES motor neurons did not express FoxP1 (H, I) or Lhx3 (J, K). H, I and J, K are pairs of the same image triple labeled for FoxP1, RhD, and GFP (H, I) or Lhx3, RhD, and GFP (J, K).

Supplemental Figure S7. Specification of Motor Neuron Subtypes from Mouse ES Cells.

ES cells differentiate into spinal progenitors (SP) in response to Wnt and FGF signals. Spinal progenitors can be further patterned along the rostrocaudal axis by RA and FGF signals. Motor neurons generated in the presence of RA acquire $Hoxa5^+$ cervical

identity while increasing the concentration of FGF drives the specification of Hoxc6$^+$ brachial and Hoxc9$^+$ thoracic identity. Correct rostrocaudal specification of ES motor neurons is demonstrated by their acquisition of columnar and motor pool identities that conform to their rostrocaudal identity. After transplantation into the chick spinal cord, ES derived MMC and LMC neurons settle in appropriate motor columns and execute correct axonal pathfinding choices towards axial and limb muscles, respectively.

Supplemental Tables

Supplemental Table 1. Comparison of basal media composition on the formation of embryoid bodies from ES cells (- refers to a failure to generate embryoid bodies; + refers to robust formation of embryoid bodies; +/- refers to relatively inefficient embryoid body formation), differentiation of Hb9-GFP expressing ES motor neurons (stated as a percentage of GFP$^+$ cells on day 7 of differentiation), and Hoxc8 expression status (+ reflects conditions in which Hoxc8$^+$ cells were detected on day 7 of differentiation). Reported are findings from two independent experiments.

Medium composition	embryoid bodies	% GFP$^+$ motor neurons	Hoxc8$^+$
Adv-DMEM/F12/Neurobasal/KSR (CV)	+	8.2	+
GMEM/KSR	+	0.7	-
DMEM/F12/KSR	+	0.0	-
Neurobasal/KSR	+/-	0.2	+
F12/Neurobasal/KSR	+/-	0.0	-
F12/KSR	-	n/a	n/a
DMEM/KSR	+/-	2.8	+
F12	-	n/a	n/a
DMEM/F12	-	n/a	n/a
Adv-DMEM/F12/Neurobasal	+/-	3.5	-

Supplemental Table 2.

The following tables summarize statistical analysis for results presented in the main and supplemental figures. The numbers of independent biological replicates (n), approximate number of examined cells, and p values are specified. All data were analyzed using a two-tail Student's t-test except for results contained in Figure 2 which were analyzed using one-way ANOVA and Bonferroni correction for multiple testing.

Figure 1B (% ES motor neurons RA/Hh vs. CV), n=3 independent experiments, ~1000 ES motor neurons

Hoxa5$^+$	**Hoxc6$^+$**	**Hoxc8$^+$**	**Hoxc9$^+$**
<0.001	0.098	<0.001	0.010

Figure 2C (FoxP1$^+$ ES motor neurons), n=3 independent experiments, ~200 Hox$^+$ ES motor neurons, Bonferroni corrected α=0.005.

		RA/Hh	**CV**	**CV**	**CV**
		%Hoxa5$^+$	**%Hoxa5$^+$**	**%Hoxc6$^+$**	**%Hoxc8$^+$**
CV	**%Hoxa5$^+$**	0.360			
CV	**%Hoxc6$^+$**	<0.001	<0.001		
CV	**%Hoxc8$^+$**	<0.001	<0.001	0.104	
CV	**%Hoxc9$^+$**	0.832	0.475	<0.001	<0.001

Figure 2C (Lhx3$^+$ ES motor neurons, n=3 independent experiments, ~200 Hox$^+$ ES motor neurons, Bonferroni corrected α=0.005.

		RA/Hh	**CV**	**CV**	**CV**
		%Hoxa5$^+$	**%Hoxa5$^+$**	**%Hoxc6$^+$**	**%Hoxc8$^+$**
CV	**%Hoxa5$^+$**	0.035			
CV	**%Hoxc6$^+$**	0.004	0.224		
CV	**%Hoxc8$^+$**	0.026	0.867	0.287	
CV	**%Hoxc9$^+$**	0.004	0.243	0.956	0.310

Figure 3B (control vs. day 5 RA), n=3 experiments, >300 FoxP1$^+$ ES motor neurons

% Lhx1$^+$ FoxP1$^+$ ES motor neurons
0.044

Figure 3F BrdU labeling (control v. day 5 RA) n=3 experiments, ~500 ES motor neurons

% Lhx1$^+$ BrdU$^-$ ES motor neurons
0.028

Figure 4G (GDNF presence vs. GDNF absence), n=3 experiments, >180 FoxP1$^+$ ES motor neurons

%Pea3$^+$ FoxP1$^+$	**%Scip$^+$ FoxP1$^+$**
<0.001	0.70

Figure 4H (Isl1$^+$ vs. Lhx1$^+$), n=3 experiments, ~200 Pea3$^+$ ES motor neurons

%Pea3$^+$ ES motor neurons
<0.001

Figure 5P (control vs. heterotopic), n=3 experiments, ~250 GFP$^+$ motor neurons

%FoxP1$^+$	**%Lhx3$^+$**
0.29	0.81

Figure 5Q (FoxP1$^+$ ES motor neurons; control vs. heterotopic) n=3 experiments, ~50 FoxP1$^+$ GFP$^+$ motor neurons

%Pea3$^+$	%Scip$^+$
0.40	0.44

Figure 6E (%RFP$^+$ vs. %GFP+ grafted ES motor neurons), n=3 embryos, >50 RFP and GFP ES motor neurons

FoxP1$^+$	Lhx3$^+$
0.004	0.084

Figure 6M (host vs. grafted motor neurons), n=4 embryos, ~50 host or grafted motor neurons per embryo

Lhx3$^+$	FoxP1$^+$
0.92	0.85

Figure 6M (Lhx3$^+$ vs. FoxP1$^+$ motor neurons), n=4 embryos, ~50 host or grafted motor neurons per embryo

host	grafted
<0.001	0.009

Figure 7C, S6E (limb vs. axial ES motor neuron axons), n = 6 embryos

%GFP$^+$	%RFP$^+$
0.011	0.036

Figure 7C, S6E (%RFP vs. %GFP ES motor neuron axons), n = 6 embryos

limb	axial
0.14	0.019

Figure 7P (grafted vs. host motor neurons), n=3 embryos per transplant/backfill experiments, >35 grafted and host RhD^+ motor neurons/embryo.

%FoxP1$^+$ limb	%Lhx3$^+$ limb	%FoxP1$^+$ axial	%Lhx3$^+$ axial
0.26	0.24	0.37	0.37

Figure 7P (%Lhx3$^+$ vs. %FoxP1$^+$ motor neurons), n=3 embryos, >35 grafted and host RhD^+ motor neurons/embryo

grafted (RhD limb)	host (RhD limb)	grafted (RhD axial)	host (RhD axial)
0.002	<0.001	<0.001	<0.001

Supplemental Figure S1F (Hoxd9$^+$ ES motor neurons) n=3 experiments, >1200 ES motor neurons

	RA/FGF/Hh	FGF	Gdf11
CV	0.31	<0.01	0.59

Supplemental Figure S1G (Hoxd10$^+$ ES motor neurons) n=3 experiments, >600 ES motor neurons

	RA/FGF/Hh	FGF	Gdf11
CV	0.96	0.49	<0.001

Supplemental Figure S2B (RA/Hh vs. CV), n=3 experiments, ~750 ES motor neurons

%FoxP1^{+}	**%Lhx3^{+}**
<0.001	<0.001

Supplemental Figure S3B (%Hoxc8^{+} ES motor neurons), n=2 experiments, ~100 ES motor neurons

control vs. day 3 RA	**control vs. day 4 RA**	**control vs. day 5 RA**
<0.001	0.18	0.76

Supplemental Figure S3D (%BrdU^{+} v. %BrdU^{-} ES motor neurons), n=3 experiments, ~500 ES motor neurons

BrdU^{+} v BrdU^{-} CV ES motor neurons
<0.001

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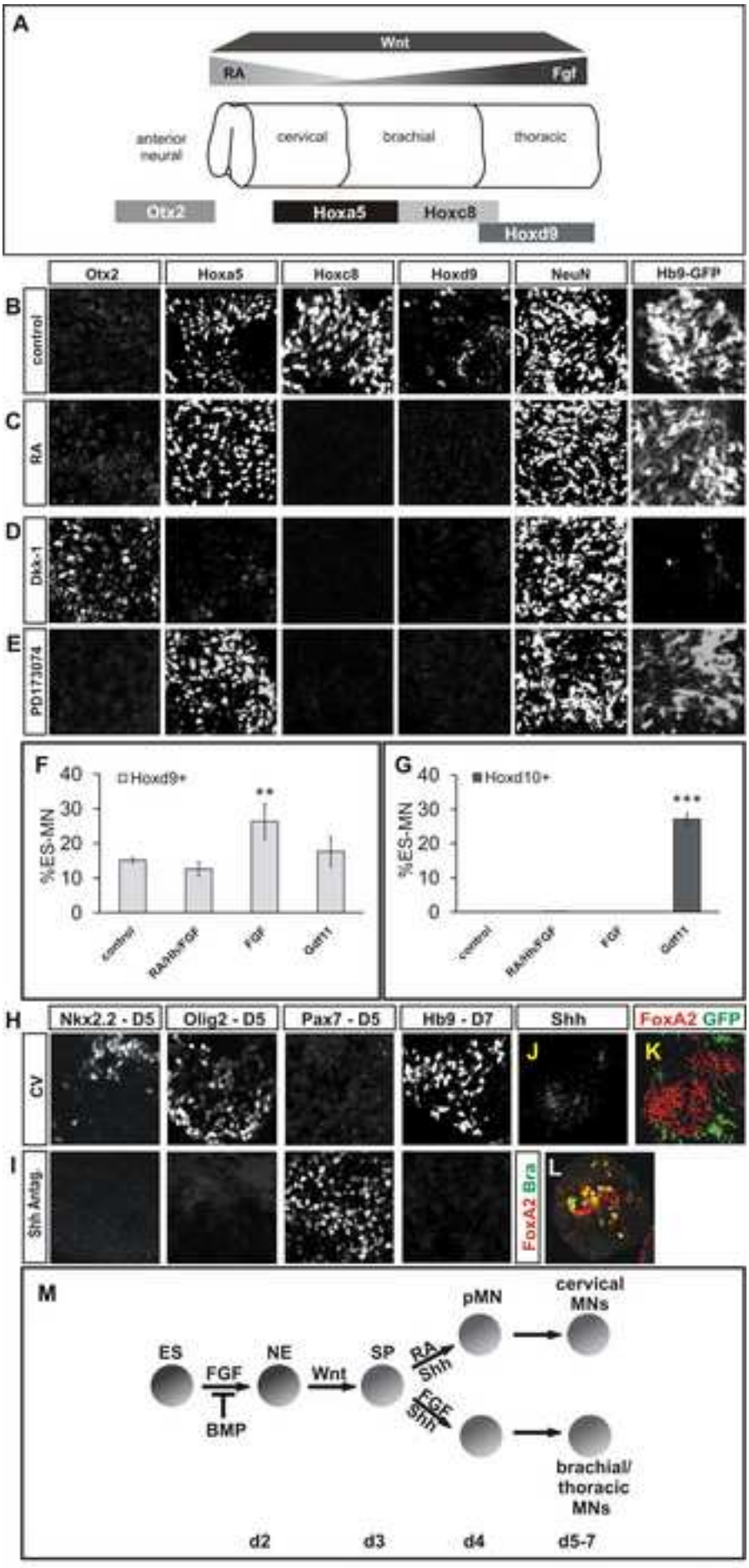
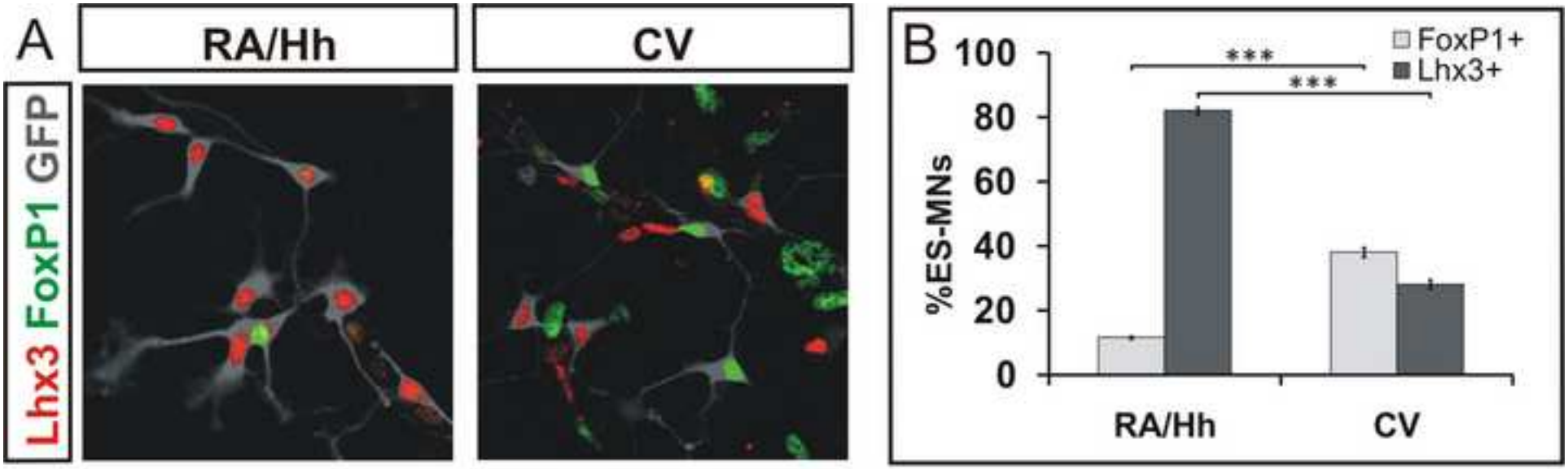
Supplemental Figure S1



Supplemental Figure S1



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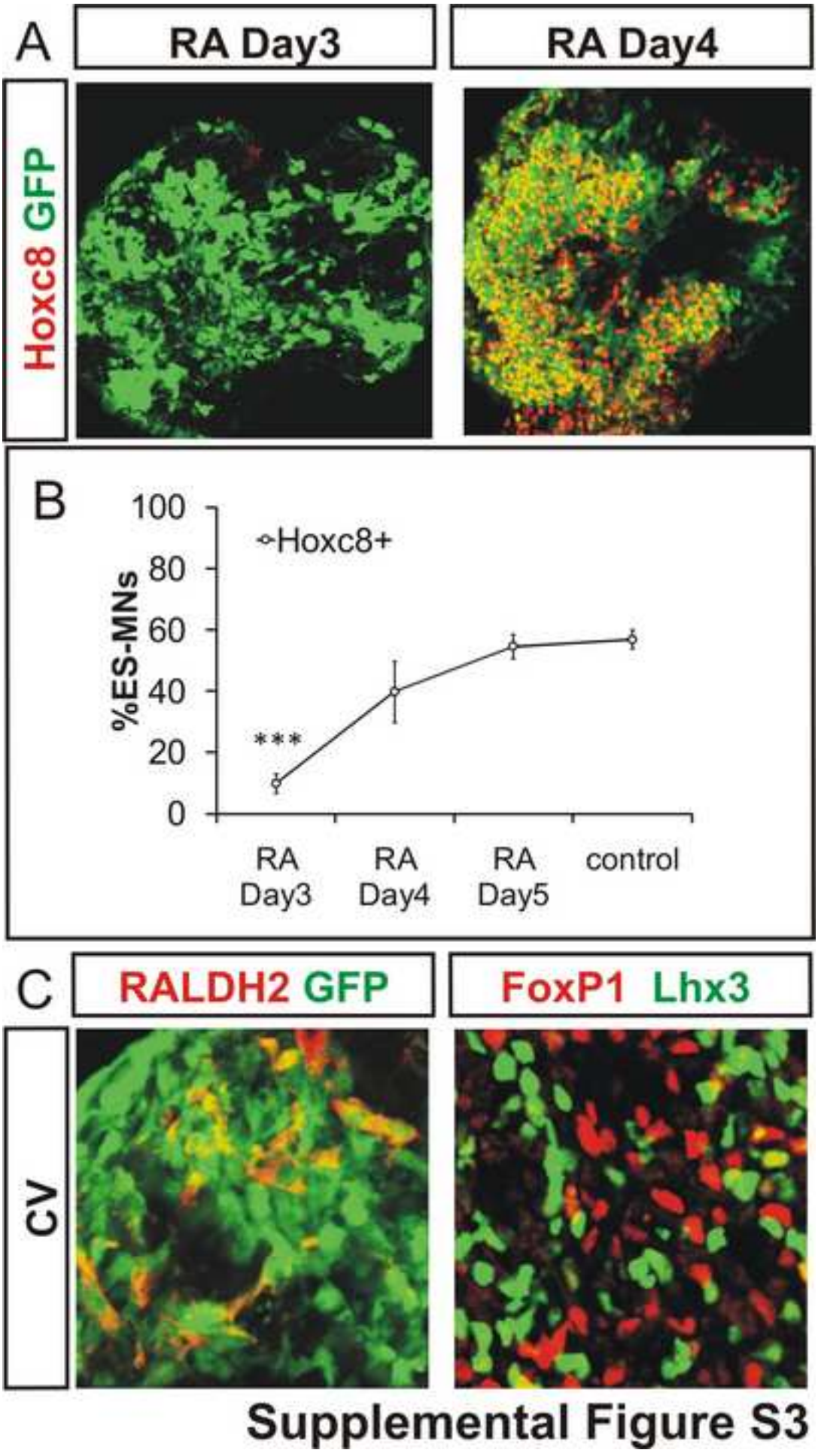

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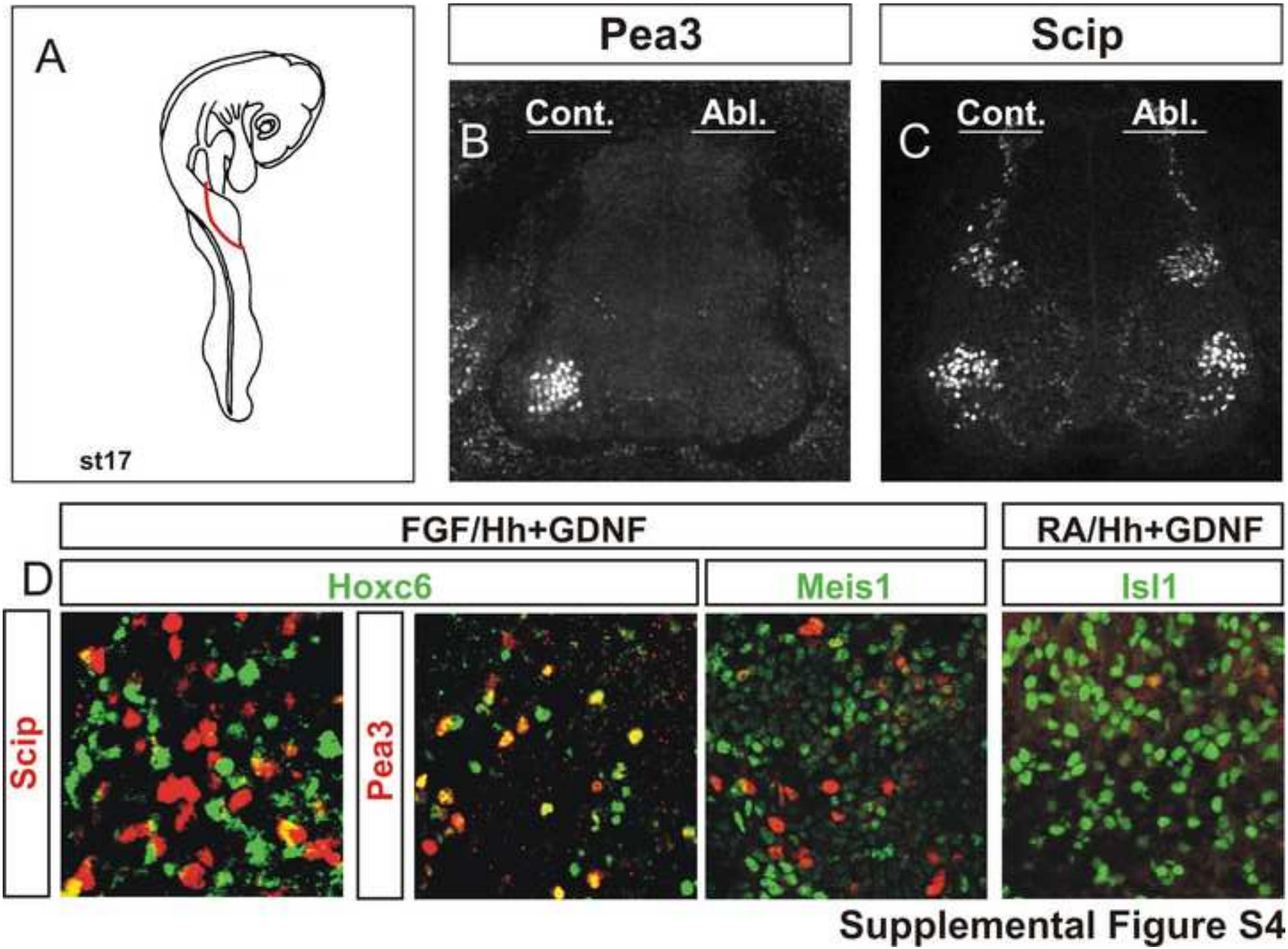

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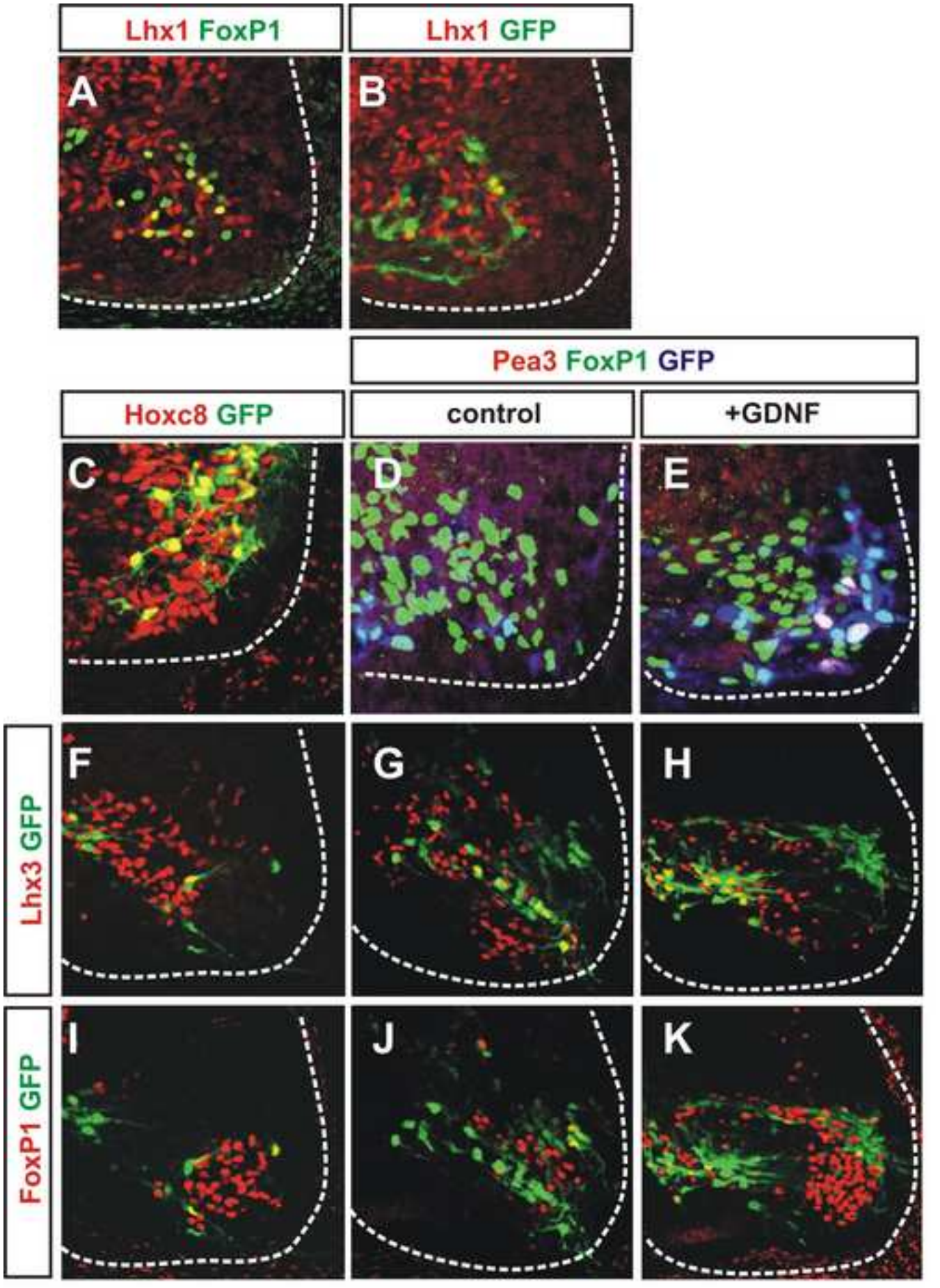

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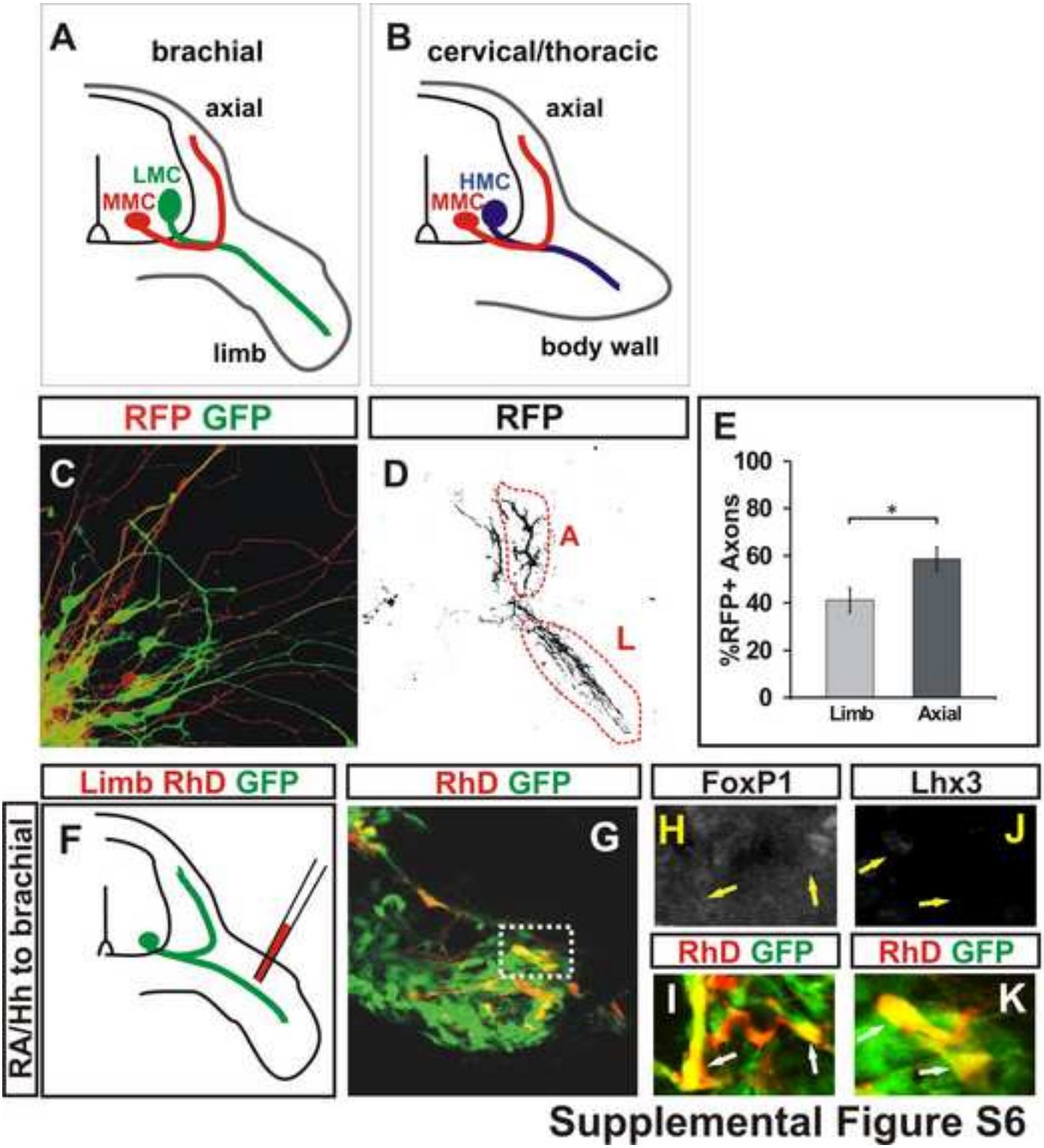
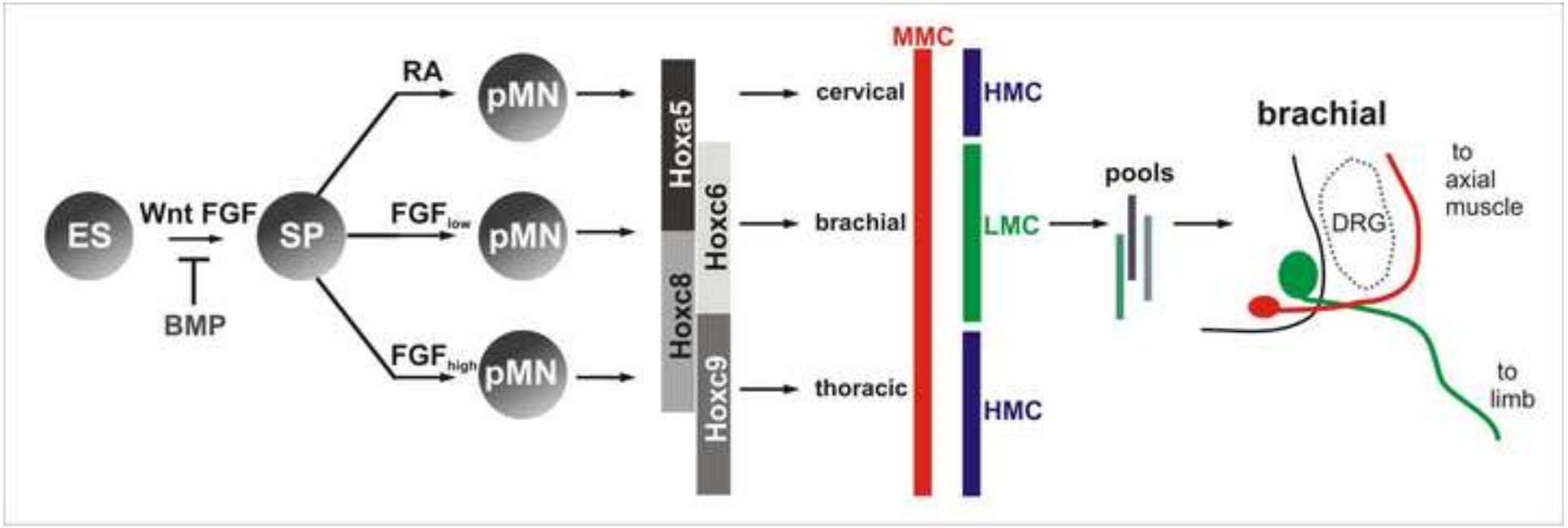


Supplemental Figure S6



Supplemental Figure S7