

Supplemental Figure Legends

Figure S1. Expression of SFKs and *Csk* in chick and mouse LMC motor neurons.

(A-K) Detection of mRNA in consecutive sections of the spinal cord. All chick sections (F, G) are HH st. 25/26 lumbar spinal cord; mouse sections are either e11.5 brachial (A-E, J, K) or e11.5 lumbar (H, I) spinal cord.

(A, B) Detection of *Lim1* and *Isl1* mRNA in the mouse spinal cord. *Lim1* (A) and *Isl1* (B) expression highlights lateral and medial LMC motor neurons, respectively.

(C, D, E) Detection of *Src* (H), *Fyn* (I), and *Csk* (J) mRNA in both medial and lateral mouse LMC neurons in brachial spinal cord.

(F, G) Detection of *Yes* (F) and *Lyn* (G) mRNA, in chick spinal cord. *Yes* and *Lyn* are expressed in ventricular zone but not in LMC.

(H, I, J, K) Detection of *Yes* and *Lyn* in mouse brachial (H, I) or lumbar (J, K) spinal cord. *Yes* and *Lyn* are expressed in the ventricular zone but not in LMC. *Yes* and *Lyn* are also expressed in the liver (inset of H, J).

Scale bar = 25 μm (A-E), 30 μm (F-K), or 70 μm (subset of H, I).

Figure S2. Characterization of *Csk* over-expression

(A-F) Detection of *Isl1* (blue), *FoxP1* (red), and GFP (green) protein in the LMC region of chick HH st. 28/29 embryos electroporated with *GFP* (A-C) or *Csk::GFP* (D-F) expression plasmids.

(G) Quantification of LMC motor neurons expressed as the average number of total (*FoxP1*⁺) LMC neurons per section (# *FoxP1*⁺/section).

(H, I) Quantification of total or electroporated medial (FoxP1⁺ Isl1⁺) and lateral (FoxP1⁺ Isl1⁻) LMC motor neurons in lumbar spinal cord expressed as the percentage of total motor neurons [FoxP1⁺ MNs (%)] (H) or electroporated motor neurons [GFP⁺ MNs (%)] (I).

(J-Q) Detection of EphA4 (red), EphB1 (blue), and GFP (green) proteins in the spinal cord and spinal nerves of chick HH st. 28/29 *Csk::GFP* electroporated embryos. The expression and localization of EphA4 in the LMC and spinal nerves and EphB1 in spinal nerves are not obviously changed compared with the contralateral side of the spinal cord. Statistical analysis: The average number of FoxP1⁺ motor neurons per section in *Csk::GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.054). Proportions of medial or lateral LMC neurons in *Csk::GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.540). Proportions of electroporated medial or lateral LMC neurons in *Csk::GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.895).

Number of embryos quantified: n=10 for *GFP* electroporated embryos, n=12 for *Csk* electroporated embryos; LMCm = medial LMC; LMCl = lateral LMC; SpN = spinal nerves; DRG = dorsal root gangion; error bars = standard deviation; n.s. = not significant.

Scale bar = 25 μ m (A-F) or 100 μ m (J-Q).

Figure S3. Characterization of the *e[Isl1]::GFP* medial LMC marker

(A-D) Detection of Isl1 (blue), FoxP1 (red), and GFP (green) protein in the LMC of chick HH st. 28/29 electroporated with *e[Isl1]::GFP* (A-B) or *Csk* and *e[Isl1]::GFP* (C-D) expression plasmids.

(E) Quantification of LMC motor neurons expressed as the average number of total (FoxP1⁺) LMC neurons per section (# FoxP1⁺/section).

(F, G) Quantification of total or electroporated medial (FoxP1⁺ Isl1⁺) and lateral (FoxP1⁺ Isl1⁻) LMC motor neurons in lumbar spinal cord expressed as the percentage of total motor neurons [FoxP1⁺ MNs (%)] (F) or electroporated motor neurons [GFP⁺ MNs (%)] (G).

Statistical analysis: The average number of FoxP1⁺ motor neurons per section in *e[Isl1]::GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.390). The average number of FoxP1⁺ motor neurons per section in *Csk* and *e[Isl1]::GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.831). Proportions of medial or lateral LMC neurons in *e[Isl1]::GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.186). Proportions of medial or lateral LMC neurons in *Csk* and *e[Isl1]::GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.519). Proportions of electroporated medial or lateral LMC neurons in *e[Isl1]::GFP* expressing embryos and those in *GFP* expressing embryos are significantly different using Student's unpaired t-test (p<0.001). Proportions of electroporated medial or lateral LMC neurons in *Csk* and

e[Isl1]::GFP expressing embryos and those in *GFP* expressing embryos are significantly different using Student's unpaired t-test ($p < 0.001$).

Number of embryos quantified: $n=5$ for *GFP* electroporated embryos, $n=6$ for *e[Isl1]::GFP* electroporated embryos, $n=6$ for *Csk* and *e[Isl1]::GFP* co-electroporated embryos; LMCm = medial LMC; LMCl = lateral LMC; error bars = standard deviation; n.s. = not significant; *** = $p < 0.001$.

Scale bar = 25 μm (A-D).

Figure S4. Characterization of *Src* mutant LMC motor neurons

(A-J) Detection of *Isl1* (blue), *FoxP1* (red), and *Src* protein in the LMC region and neurofilament (NF) in the forelimb of mouse e11.5 *Src* $+/+$ (A-E) or *Src* $-/-$ (F-J) embryos.

(K) Quantification of LMC motor neurons expressed as the average number of total (*FoxP1*⁺) LMC neurons per section (# *FoxP1*⁺/section).

(L) Quantification of medial (*FoxP1*⁺ *Isl1*⁺) and lateral (*FoxP1*⁺ *Isl1*⁻) LMC motor neurons in brachial spinal cord expressed as the percentage of total motor neurons [*FoxP1*⁺ MNs (%)].

(M-V) Detection of *Isl1* (blue) and *EphA4* (red) protein and *EphB2* mRNA in the spinal cord of mouse e11.5 *Src* $+/+$ (M-Q) and *Src* $-/-$ (R-V) embryos. The expression and localization of *EphA4* and *EphB1* in the LMC are not obviously changed compared with wild-type littermates.

Statistical analysis: The average number of *FoxP1*⁺ motor neurons per section in *Src* $-/-$ embryos and that in *Src* $+/+$ embryos are not significantly different using Student's

unpaired t-test ($p=0.749$). Proportions of medial or lateral LMC neurons in *Src* $-/-$ expressing embryos and those in *Src* $+/+$ embryos are not significantly different using Student's unpaired t-test ($p=0.300$).

Number of embryos quantified: $n=5$ for *Src* $+/+$ embryos, $n=4$ for *Src* $-/-$ embryos;

LMCm = medial LMC; LMC_l = lateral LMC; error bars = standard deviation; n.s. = not significant.

Scale bar = 25 μm (A-D, F-I), 75 μm (E, J), or 15 μm (M-V).

Figure S5. Characterization of *Src*^{Y527F} and *Src*DN over-expression

(A-F) Detection of Isl1 (blue), FoxP1 (red), GFP (green), and Src protein in the LMC region of chick HH st. 28/29 embryos electroporated with *Src*^{Y527F} and *GFP* (A-C) or *Src*DN and *GFP* (D-F) expression plasmids. Over-expression of Src was shown in the electroporated side of spinal cord (C, F) compared with that in the contralateral side of spinal cord (insets of C, F).

(G) Quantification of LMC motor neurons expressed as the average numbers of total (FoxP1⁺) LMC neurons per section (# FoxP1⁺/section).

(H, I) Quantification of total or electroporated medial (FoxP1⁺ Isl1⁺) and lateral (FoxP1⁺ Isl1⁻) LMC motor neurons in lumbar spinal cord expressed as the percentage of total motor neurons [FoxP1⁺ MNs (%)] (H) or electroporated motor neurons [GFP⁺ MNs (%)] (I).

Statistical analysis: The average number of FoxP1⁺ motor neurons per section in *Src*^{Y527F} and *GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.371$). The average number of FoxP1⁺ motor

neurons per section in *SrcDN* and *GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.273$).

Proportions of medial or lateral LMC neurons in *SrcY527F* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.231$). Proportions of medial or lateral LMC neurons in *SrcDN* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.533$). Proportions of electroporated medial or lateral LMC neurons in *SrcY527F* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.417$). Proportions of electroporated medial or lateral LMC neurons in *SrcDN* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test ($p=0.196$).

Number of embryos quantified: $n=5$ for *GFP* expressing embryos, $n=6$ for *SrcY527F* and *GFP* expressing embryos, $n=5$ for *SrcDN* and *GFP* expressing embryos; LMC_m = medial LMC; LMC_l = lateral LMC; error bars = standard deviation; n.s. = not significant.

Scale bar = 25 μm (A-F), 67 μm (insets of C, F).

Figure S6. Characterization of EphA4Y602E over-expression

(A-F) Detection of Isl1 (blue), FoxP1 (red), GFP (green), and EphAs in the LMC region of chick HH st. 28/29 electroporated with *EphA4* and *GFP* (A-C) or *EphA4Y602E* and *GFP* (D-F) expression plasmids. Ephrin-A5-Fc overlays demonstrated the over-expression of EphAs in the electroporated side of spinal cord (C, F) compared with that in the contralateral side of spinal cord (insets of C, F).

(G) Quantification of LMC motor neurons expressed as the average numbers of total (FoxP1⁺) LMC neurons per section (# FoxP1⁺/section).

(H, I) Quantification of total or electroporated medial (FoxP1⁺ Isl1⁺) and lateral (FoxP1⁺ Isl1⁻) LMC motor neurons in lumbar spinal cord expressed as the percentage of total motor neurons [FoxP1⁺ MNs (%)] (H) or electroporated motor neurons [GFP⁺ MNs (%)] (I).

Statistical analysis: The average number of FoxP1⁺ motor neurons per section in *EphA4* and *GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.309). The average number of FoxP1⁺ motor neurons per section in *EphA4Y602* and *GFP* expressing embryos and that in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.710). Proportions of medial or lateral LMC neurons in *EphA4* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.265). Proportions of medial or lateral LMC neurons in *EphA4Y602* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.543). Proportions of electroporated medial or lateral LMC neurons in *EphA4* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.451). Proportions of electroporated medial or lateral LMC neurons in *EphA4Y602* and *GFP* expressing embryos and those in *GFP* expressing embryos are not significantly different using Student's unpaired t-test (p=0.711).

Number of embryos quantified: n=5 for *GFP* expressing embryos, n=5 for *EphA4* and *GFP* expressing embryos, n=4 for *EphA4Y602* and *GFP* expressing embryos; LMCm = medial LMC; LMC1 = lateral LMC; error bars = standard deviation; n.s. = not significant. Scale bar = 25 μ m (A-F), 67 μ m (insets of C, F).