Motor pools organization depends on the combined function of N-cadherin and type II cadherins

Carola Dewitz¹,³, Xin Duan² and Niccolò Zampieri¹,³

¹Cluster of Excellence NeuroCure, Neuroscience Research Center, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
²Department of Ophthalmology and Physiology, Weill Institute for Neurosciences, University of California San Francisco, 10 Koret Way, San Francisco CA 94143, USA.
³Max-Delbrück-Center for Molecular Medicine Berlin-Buch, Robert-Rössle-Str. 10, 13125 Berlin, Germany.

Correspondence: niccolo.zampieri@mdc-berlin.de

Key Words: classical cadherins, motor neuron, adhesion, migration, neuronal development
Abstract
Type I and type II classical cadherins constitute a family of cell adhesion molecules expressed in complex combinatorial profiles in the nervous system, suggesting the hypothesis that a cadherin code implements specific adhesive recognition events that control the development of neural circuits. In the spinal cord, classical cadherins define at a molecular level the positional organization of motor neuron subtypes into discrete nuclear structures termed motor pools. However, the roles and contributions of different members of the family in defining motor neuron spatial organization are not clear yet. By combining mouse genetics with quantitative positional analysis, we found that motor neurons organization into pools depends on type II cadherins, nevertheless their function does not strictly reflect the predictions arising from binding specificities at a molecular level, but instead relies on N-cadherin, a type I cadherin whose elimination is required to reveal type II contributions.
**Introduction**

The precision with which neurons are connected during development underlies the function of the nervous system. Several processes, such as neurogenesis, migration, dendritic elaboration, axon guidance and synapse formation have to be coordinated to ensure the wiring of neural circuits. The mechanisms controlling these events often rely on expression of molecules promoting specific recognition and the cadherin superfamily of cell adhesion molecules has been implicated in most of these processes (Takeichi, 2007; Jontes, 2018). In particular, type II classical cadherins are expressed in the central nervous system in complex patterns often delineating discrete structures and circuits, suggesting important roles for neuronal organization and connectivity (Hirano and Takeichi, 2012).

The property of type II cadherins expression to highlight anatomical features of the nervous system is evident in the spinal cord, where motor neurons are organized into nuclear structures, termed motor pools, which are at the basis of muscolotopic organization of motor maps and the wiring of spinal circuits (Romanes, 1964; Sürmeli et al., 2011; Hinckley et al., 2015). Combinatorial expression of type II cadherins defines pools at a molecular level and manipulation of classical cadherin signaling perturbs segregation and clustering of motor neurons (Fig. S1A; Price et al., 2002; Demireva et al., 2011; Bello et al., 2012; Astick et al., 2014; Montague et al., 2017). The precise organization of motor pools in the lateral motor column (LMC) of the spinal cord is achieved by a two-step process: first, inside-out migration separates early- and late-born neurons in a two-layered structure on the medio-lateral axis, the medial and lateral divisions (Palmesino et al., 2010; Bello et al., 2012). A second, independent process, organizes pools within divisions on the dorso-ventral axis (Dewitz et al., 2018). Genetic analysis indicates that both processes are dependent on β- and γ-catenin adhesive function, but elimination of N-cadherin, a type I cadherin expressed by all motor neurons, selectively impairs the first phase without affecting relative organization of pools within divisions on the dorso-ventral axis (Dewitz et al., 2018). The effectors controlling the second phase are unknown and type II cadherins are obvious candidates because of their expression profiles and dependency on catenins activity (Nelson, 2008). However, elimination of individual type II cadherins does not perturb any aspect of motor neuron positioning (Demireva et al., 2011).

What is controlling the second phase of motor pool organization? What are the roles of type II cadherins? In order to address these questions, we combined mouse
genetics and quantitative positional analysis to investigate classical cadherins contributions. We found that type II cadherins control dorso-ventral organization of pools. Surprisingly, our data indicate that their function does not rely on the establishment of an adhesive code, but instead is only revealed in absence of N-cadherin, thus suggesting that cross talk between type I and type II cadherins is a crucial element orchestrating motor neuron positioning.

**Results and Discussion**

*Inactivation of a specificity group does not affect divisional organization*

Recent studies indicate that functional redundancy in subsets of type II cadherins is at the basis of adhesive recognition properties in the nervous system (Duan et al., 2014; Basu et al., 2017; Duan et al., 2018). At a molecular level, type II cadherins can be divided into three specificity groups according to their adhesive preferences (Brasch et al., 2018). Cadherin-8, -11 (cad); cad-6, -9, -10; cad-7, -12, -18, -20, -22 represent the groups displaying clear preference for heterophilic interactions according to their binding affinities. Functional compensation among members of a group could explain the lack of positioning phenotypes in single type II cadherin mutants. To test this hypothesis we eliminated all members of a specificity group by crossing cad-8 and cad-11 single mutant mice ($8/11^{-/-}$; Horikawa et al., 1999; Suzuki et al., 2007).

$8/11^{-/-}$ mice are born at expected frequency and do not present any obvious phenotype. Analysis of motor neuron generation and subtype identities did not reveal any difference between $8/11^{-/-}$ and control mice (Fig. S1G). Next we studied divisional organization, transverse and longitudinal contour plots did not show any defect in positioning of medial (m) and lateral (l) LMC neurons (Fig. 1A-F). In addition, we did not observe changes either in motor neuron distribution or average position, both on the medio-lateral and dorso-ventral axis (Fig. 1G-M). Accordingly, we found that the positions of motor neurons sharing the same identity were highly correlated between control and $8/11^{-/-}$ embryos (Fig. 1N). Thus, joint elimination of cad-8, -11 does not affect divisional organization.

*$8/11$ and $6/9/10$ specificity groups are not required for pools organization*

It has been previously shown that motor neuron positioning is achieved following a two-step process. First, N-cadherin/catenin, with contributions from reelin
and afadin signaling, controls medio-lateral segregation of motor neuron divisions (Palmesino et al., 2010; Dewitz et al., 2018). In a second independent phase, whose effectors are unknown, motor neurons are organized into pools on the dorso-ventral axis (Dewitz et al., 2018). We asked whether joint elimination of cad-8 and cad-11 could have a selective role in dorso-ventral pool segregation and studied the positioning of dorsal (vasti, V, Er81+) and ventral motor neurons (hamstring, H, Nkx6.1+; rectus femoris/tensor fasciae latae, R/T, Nkx6.2+). Transverse contour analysis indicated that dorso-ventral organization was not affected in 8/11-/- embryos (Fig. 2A-D). Density, distribution and average position analyses confirmed that segregation of pools was preserved (Fig. 2G-I). In addition, consistent with the divisional data, we did not observe changes in pools positioning on the medio-lateral axis (Fig. S1B-E).

Functional redundancy in cad-6, -9, -10 has important functions in controlling neuronal interactions in the hippocampus and the retina (Basu et al., 2017; Duan et al., 2018). In order to expand our observation, we investigated the role of this group on motor neuron organization. Triple cad-6, -9, -10 mutant mice are viable and fertile (6/9/10-/-; Duan et al., 2018). We found that motor neurons were present at expected numbers with appropriate identities (Fig. S1G). Contour, average position, distribution and density analyses showed that organization of motor pools was similar in control, 8/11-/- and 6/9/10-/- embryos (Fig. 2E-I and S1D-F). As a consequence, coordinates of H, R/T and V neurons were highly correlated (Fig. 2J). These experiments show that elimination of two different specificity groups does not affect motor neuron organization.

**Joint elimination of type II cadherins with N-cadherin reveals type II contributions**

Since any attempt to disrupt motor neuron positioning by eliminating type II cadherins function in mice has failed, we reasoned that another factor might be involved in controlling their activities. According to previous results, such factor should be ubiquitously expressed in motor neurons and capable of interacting with catenin signaling. N-cadherin, a type I cadherin, fulfills these requirements and its conditional elimination from motor neurons has been shown to cause defects in divisional segregation without impairing dorso-ventral organization of pools (NΔMN, Dewitz et al., 2018). Thus, we combined elimination of cadherin-8 with N-cadherin conditional deletion in motor neurons (NΔMN8-/-). NΔMN8-/- mutants are lethal at late
embryonic stages. Generation of motor neurons was not affected in \( N^{\Delta MN} \) embryos (Fig. 1SG). We observed a migration defect resulting in post-mitotic motor neurons found in the progenitor area (Fig. S2B). The same phenotype, at lower penetrance, was observed in \( N^{\Delta MN} \) embryos (Fig. S2A-D). Next, we examined the radial glia scaffold at e13.5 in \( N^{\Delta MN} \) embryos and did not detect any obvious defect aside from local disruption at the progenitor zone (Fig. S2E and G). However, we cannot exclude the possibility that perturbations at earlier time-points may affect motor neuron migration. Since in \( N^{\Delta MN} \) embryos most of LMC neurons migrate out to the ventral horn, we next assessed divisional organization and observed extensive overlap in the positioning of LMCm and LMCi neurons (Fig. 3A-C). Distribution, average position and correlation analyses indicated that the defect is due to impaired medio-lateral migration of LMC neurons, with LMCi neurons settling in LMCm-like position (Fig. 3D, F and H). In addition, a ventralization in columnar location was observed (Fig. 3E and G). These phenotypes are reminiscent of the ones described for N-cadherin mutants, however distribution and average position analyses revealed that the LMC neurons medio-lateral migration phenotype in \( N^{\Delta MN} \) embryos was significantly more severe than in \( N^{\Delta MN} \), while the dorso-ventral positioning phenotype was indistinguishable (Fig. 3D-G; Dewitz et al., 2018). Thus, these data show that cadherin-8 contributes to medio-lateral divisional organization and elimination of N-cadherin is required to reveal its involvement.

Next, we studied motor pools organization after joint elimination of N-cadherin and type II cadherins. In addition to \( N^{\Delta MN} \) embryos, we analyzed a double N-cad/cad-11 mutant (\( N^{\Delta MN} \)). Motor neuron generation was not affected in \( N^{\Delta MN} \) embryos and, as in \( N^{\Delta MN} \) embryos, a fraction of post-mitotic motor neurons failed to leave the progenitor zone (Fig. S1G and S2C-D). In both \( N^{\Delta MN} \) and \( N^{\Delta MN} \) embryos we observed extensive overlap in motor neurons distribution (Fig. 4A-D). Positional analysis confirmed the positioning defect observed at divisional level, with H and R/T neurons found in similar medio-lateral space (Fig. S3C-E). Next, we assessed relative pool positioning and observed that joint inactivation of N-cadherin and a type II cadherin resulted in a spatial reorganization of pools resembling the one found after elimination of \( \beta^-\gamma^-\)catenin (\( \beta^\gamma \); Fig. 4E). The distance between dorsal and ventral pools was dramatically reduced compared to control and almost undistinguishable from \( \beta^\gamma \) embryos (Fig. 4F).
Accordingly, the distribution of motor neurons on the dorso-ventral axis in \( N^{\Delta MN8/-} \) and \( N^{\Delta MN11/-} \) embryos was mostly overlapping as in \( \beta^\gamma_{\Delta MN} \) (Fig. 4G and S3A-B). In contrast, dorsal and ventral motor pools were mostly separated in \( N^{\Delta MN} \) embryos like in control embryos (Fig. 4E-G, S3A-B and S3F). Altogether these data indicate that type II cadherins coordinate motor neuron position first by contributing to inside-out migration, leading to divisional organization, and later as central effectors of dorso-ventral segregation of motor pools within divisions.

The importance of classical cadherins for the organization of motor pools has been evident since the first studies in chick and mouse embryos (Price et al., 2002; Demireva et al., 2011). Nevertheless, attempts to define the roles of type II cadherins have failed. There are evidences indicating that classical cadherins may work synergistically in defining adhesive recognition. Binding affinity analysis shows that type II cadherins can be divided into three specificity groups according to their binding preferences, where molecules belonging to the same group bind to each other in heterophilic manner, but discriminate molecules belonging to different groups (Brasch et al., 2018). Genetic experiments confirmed that redundant function of cad-6, -9, -10 is at the basis of specific neuronal interactions in the retina and hippocampus (Basu et al., 2017; Duan et al., 2018). These data indicate that elimination of multiple members of a group is necessary to reveal their contributions and support a model based on an adhesive code, where redundancy and combinatorial expression is used to generate developmental programs that are both specific and robust (Jontes, 2018). Surprisingly, our findings show that elimination of all the members of two out of three specificity groups has no effect on motor neuron spatial organization. A role for the remaining group cannot be excluded, however motor pools organization does not seem to follow a molecular logic based on a redundant adhesive code generated by combinatorial expression of type II cadherins.

Instead, our data show that the contributions of type II cadherins become evident only in the absence of N-cadherin. Almost identical phenotypes are observed by inactivation of N-cadherin with either cadherin-8 or -11, which did not show any defect when eliminated individually or in conjunction. Thus, cross talk between type II cadherins and N-cadherin appears to be a central feature for the control of motor neuron pools organization. The molecular and cellular bases for the emerging functions observed after concomitant elimination of N-cadherin and type II cadherins remain unclear. Structural and biophysical studies indicate that binding between type
I and type II cadherins is prohibited and there is no evidence of such functional interaction controlling cell adhesive recognition (Patel et al., 2006; Brasch et al., 2012). It has been suggested that differences in adhesive strength may be sufficient to implement developmental programs generating cellular patterns (Steinberg, 2007; Hassan and Hiesinger, 2015). In principle, varying cadherins expression profiles in motor neurons could promote differential adhesive properties to segregate pools. Indeed, it has been shown that changing levels of cadherins expression between otherwise identical cell populations is sufficient to promote segregation (Foty and Steinberg, 2013). Thus, N-cadherin, may serve to maintain a basal adhesive level among motor neurons necessary for type II cadherins to modulate relative adhesive strength in different pools. In the future, defining cell surface levels, as well as temporal dynamics of classical cadherins expression during development will be important to understand the principles controlling the spatial organization of motor neurons.

Experimental procedures

Mouse genetics

All experimental procedures were performed according to the policies of the Max Delbrück Center for Molecular Medicine and approved by the Landesamt für Gesundheit und Soziales and by the IACUC at UCSF. β-catβ (Brault et al., 2001), γ-catβ (Demireva et al., 2011), N-cadherinβ (Kostetskii et al., 2005), γ-catγγ (Ruiz et al., 1996), cad 6γγ; cad 9γγ; cad 10γγ (Duan et al., 2018), cad-8γγ (Suzuki et al., 2007), cad-11γγ (Horikawa et al., 1999) and olig2::Cre (Dessaud et al., 2007) mouse lines have been previously described.

Immunohistochemistry

Embryonic spinal cords were fixed with 4% paraformaldehyde on ice for 90 minutes, cryo-protected by equilibration with 30% sucrose over night at 4°C, frozen in OCT (Tissue-Tek) and sectioned at 16μm using a Leica cryostat. Immunohistochemistry was performed as previously described and images acquired on a Zeiss LSM 800 confocal microscope (Demireva et al., 2011). Antibodies used were previously described in Dasen et al., 2008; De Marco Garcia and Jessell, 2008; Agalliu et al., 2009.
**Motor neuron subtypes identification**

Motor neuron divisional subtypes were identified by the expression of homeobox transcription factors Isl1/2 (LMCm) and Hb9 (LMCl; Sockanathan and Jessell, 1998). Motor pools occupying different medio-lateral and dorso-ventral positions at lumbar spinal levels were identified by expression of homeobox and ETS transcription factors (De Marco Garcia and Jessell, 2008). The rectus femoris/tensor fasciae latae (R/T) complex was identified by expression of Nkx6.2; the hamstrings (H) complex was identified by expression of Nkx6.1 and the vasti (V) motor pool was identified by expression of Er81.

**Three-dimensional analysis of motor neuron subtypes positioning**

Motor neuron positions were acquired using the three-dimensional analysis as previously described (Dewitz et al., 2018). Briefly, motor neuron cell bodies were assigned Cartesian coordinates (x and y) using the imaging software Imaris (Bitplane) and expressed relative to the midpoint of the spinal cord midline (x,y = 0,0).

Coordinates were normalized to the size of a standardized spinal cord calculated by measuring average spinal cord size of mouse embryos at e13.5 (distance from the midline to the lateral edge = 365μm; distance from the midpoint of the midline to the ventral edge = 340μm). The z coordinates were obtained by tracking the order of histological sections. Datasets were aligned on the z axis by using the appearance of Isl1/2$^+$ motor neurons (for divisional analysis) or Nkx6.1$^+$ motor neurons (for pool analysis) as a starting point and analysis was performed for 512μm covering approximately the rostral half of the lumbar spinal cord. We analyzed at least n = 3 embryos for each genotype. See Table S1 for genotypes, number of embryos and number of sections analyzed per embryo.

**Statistical analysis**

Positional datasets were analyzed using custom scripts in "R project" (R Foundation for Statistical Computing, Vienna, Austria, 2005) as previously described (Dewitz et al., 2018).
Acknowledgements
We thank Liana Kosizki and Isabelle Werner for technical help and the Advanced Light Microscope facility at the MDC for assistance with image acquisition and analysis. We are grateful to Peter Robin Hiesinger for invaluable insights and discussions. Stephan Dietrich, Sofia Pimpinella and Sophie Skarlatou provided comments on the manuscript. X.D. was supported by Research to Prevent Blindness Award, E. Matilda Ziegler Foundation Grant, Klingenstein-Simons Neuroscience Fellowship and Whitehall Foundation Grant. C.D. and N.Z. were generously supported by the DFG (ZA 885/1-1 and EXC 257 NeuroCure).

Author contributions
C.D. performed experiments and data analysis. X.D. generated cadherin-6, -9 and -10 mouse models. C.D. and N.Z. devised the project, designed experiments and wrote the manuscript.

Declaration of interests
The authors declare no competing interests.
References


Palmesino, E., Rousso, D.L., Kao, T-J.J., Klar, A., Laufer, E., Uemura, O., Okamoto,
Figure 1. Elimination of cad-8, -11 does not perturb divisional organization.

(A) Organization of Isl1/2\(^+\) LMCm and Hb9\(^+\) LMCl neurons at lumbar levels in e13.5 control (A) and 8/11\(^{-/-}\) (B) embryos.

(C and D) Transverse contour plots of LMCm (green) and LMCl (red) neurons in control (C) and 8/11\(^{-/-}\) (D) embryos.

(E and F) Coronal contour plots of LMCm (green) and LMCl (red) neurons in control (E) and 8/11\(^{-/-}\) (F) embryos.

(G and H) Medio-lateral density plots of LMCm (green) and LMCl (red) neurons in control (G) and 8/11\(^{-/-}\) (H) embryos.
(I) Average medio-lateral position of LMCm (green) and LMCl (red) neurons in control and $8/11^{-/}$ embryos (mean ± standard deviation, SD); differences not significant: control vs. $8/11^{-/}$ LMCm p=0.055; control vs. $8/11^{-/}$ LMCl p=0.132; t-test).

(J) Average dorso-ventral position of LMCm (green) and LMCl (red) neurons in control and $8/11^{-/}$ embryos (mean ± SD; differences not significant: control vs. $8/11^{-/}$ LMCm p=0.055; control vs. $8/11^{-/}$ LMCl p=0.052; t-test).

(K and L) Dorso-ventral density plots of LMCm (green) and LMCl (red) neurons in control (K) and $8/11^{-/}$ (L) embryos.

(M) Average medio-lateral and dorso-ventral positions of LMCm (green) and LMCl (red) neurons in control and $8/11^{-/}$ embryos (mean).

(N) Correlation analysis of LMC neurons coordinates in control and $8/11^{-/}$ embryos (control LMCm vs. $8/11^{-/}$ LMCm r > 0.8 and control LMCl vs. $8/11^{-/}$ LMCl r > 0.8).
Figure 2. Specificity groups cad-8, -11 and cad-6, -9, -10 are dispensable for pool organization.

(A and B) Organization of H (Nkx6.1⁺), R/T (Nkx6.2⁺), and V (Er81⁺) neurons in e13.5 control (A) and 8/11⁻/⁻ (B) embryos. Dashed line delimits motor neuron area.

(C and D) Contour density plots of H (green), R/T (red), and V (blue) neurons in control (C) and 8/11⁻/⁻ (D) embryos.

(E) Organization of H (Nkx6.1⁺), R/T (Nkx6.2⁺), and V (Er81⁺) neurons in e13.5 6/9/10⁻/⁻ embryos. Dashed line delimits motor neuron area.

(F) Contour density plot of H (green), R/T (red), and V (blue) neurons in 6/9/10⁻/⁻ embryos.
(G) Average dorso-ventral position of H (green), R/T (red), and V (blue) neurons in control, 8/11⁻/⁻ and 6/9/10⁻/⁻ embryos (mean ± SD; differences not significant for all comparisons between genotypes. V: control vs. 8/11⁻/⁻ p=0.762; control vs. 6/9/10⁻/⁻ p=0.966; 8/11⁻/⁻ vs. 6/9/10⁻/⁻ p=0.637. H: control vs. 8/11⁻/⁻ p=0.947, control vs. 6/9/10⁻/⁻ p=0.999; 8/11⁻/⁻ vs. 6/9/10⁻/⁻ p=0.942. R/T: control vs. 8/11⁻/⁻ p=0.733; control vs. 6/9/10⁻/⁻ p=0.769; 8/11⁻/⁻ vs. 6/9/10⁻/⁻ p=0.990; one-way ANOVA and post-hoc Tukey’s HSD test).

(H) Box-plots of dorso-ventral distributions of H (green), R/T (red), and V (blue) neurons in control, 8/11⁻/⁻ and 6/9/10⁻/⁻ embryos.

(I) Dorso-ventral density plots of H (green, ventral), R/T (red, ventral), and V (blue, dorsal) neurons in control, 8/11⁻/⁻ and 6/9/10⁻/⁻ embryos.

(J) Correlation analysis of H, R/T and V neurons coordinates in control, 8/11⁻/⁻ and 6/9/10⁻/⁻ embryos (for all three pool comparisons r>0.8). Scale bar indicates correlation values.
Figure 3. Joint elimination of N-cadherin and cadherin-8 perturbs divisional organization.

(A) Organization of Isl1/2+ LMCm and Hb9+ LMCI neurons at lumbar levels in e13.5 $\Delta^{MN8/-}$ embryos.

(B) Transverse contour plots of LMCm (green) and LMCI (red) neurons in $\Delta^{MN8/-}$ embryos.

(C) Coronal contour plots of LMCm (green) and LMCI (red) neurons in $\Delta^{MN8/-}$ embryos.

(D) Medio-lateral density plots of LMCm (green) and LMCI (red) neurons in control, $\Delta^{MN}$ and $\Delta^{MN8/-}$ embryos.
(E) Dorso-ventral density plots of LMCm (green) and LMCl (red) neurons in control, \(N^{AMN}\) and \(N^{AMN8^{-/-}}\) embryos.

(F) Average medio-lateral position of LMCm (green) and LMCl (red) neurons in control, \(N^{AMN}\) and \(N^{AMN8^{-/-}}\) embryos (mean ± SD; for LMCm neurons: control vs. \(N^{AMN}\) p=0.258; control vs. \(N^{AMN8^{-/-}}\) p=0.091; \(N^{AMN}\) vs. \(N^{AMN8^{-/-}}\) *p<0.05. For LMCl neurons: control vs. \(N^{AMN}\) p=0.156; control vs. \(N^{AMN8^{-/-}}\) **p<0.01; \(N^{AMN}\) vs. \(N^{AMN8^{-/-}}\) *p<0.05; one-way ANOVA and post hoc Tukey’s HSD test).

(G) Average dorso-ventral position of LMCm (green) and LMCl (red) neurons in control, \(N^{AMN}\) and \(N^{AMN8^{-/-}}\) embryos (mean ± SD; for LMCm neurons: control vs. \(N^{AMN}\) and \(N^{AMN8^{-/-}}\) ***p<0.001; \(N^{AMN}\) vs. \(N^{AMN8^{-/-}}\) p=0.254. For LMCl neurons: control vs. \(N^{AMN}\) and \(N^{AMN8^{-/-}}\) ***p<0.001; \(N^{AMN}\) vs. \(N^{AMN8^{-/-}}\) p=0.332; one-way ANOVA and post hoc Tukey’s HSD test).

(H) Correlation analysis of medio-lateral LMC neurons coordinates in control and \(N^{AMN8^{-/-}}\) embryos.
Figure 4. Joint elimination of N-cadherin and type II cadherins disrupts pools dorso-ventral organization.

(A) Organization of H (Nkx6.1\(^+\)), R/T (Nkx6.2\(^+\)), and V (Er81\(^+\)) neurons in e13.5 \(N^{MM}g^{-}\) embryos. Dashed line delimits motor neuron area.
(B) Transverse contour plots of H (green, ventral/medial), R/T (red, ventral/lateral), and V (blue, dorsal) neurons in N\textsuperscript{ΔMN}8\textsuperscript{-/-} embryos.

(C) Organization of H (Nkx6.1\textsuperscript{+}), R/T (Nkx6.2\textsuperscript{+}), and V (Er81\textsuperscript{+}) neurons in e13.5 N\textsuperscript{ΔMN}11\textsuperscript{-/-} embryos. Dashed line delimits motor neuron area.

(D) Transverse contour plots of H (green, ventral/medial), R/T (red, ventral/lateral), and V (blue, dorsal) neurons in N\textsuperscript{ΔMN}11\textsuperscript{-/-} embryos.

(E) Average medio-lateral and dorso-ventral positions of H (green), R/T (red), and V (blue) neurons in ventral horn of control, N\textsuperscript{ΔMN}, N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-}, and βγ\textsuperscript{ΔMN} embryos.

(F) Average distance between V-H and V-R/T neurons in control, N\textsuperscript{ΔMN}, N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-}, and βγ\textsuperscript{ΔMN} embryos (mean ± SD; for V-H: control vs. N\textsuperscript{ΔMN} p=0.747; control vs. N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-} and βγ\textsuperscript{ΔMN} ***p<0.001; N\textsuperscript{ΔMN} vs. N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-} and βγ\textsuperscript{ΔMN} *p<0.05; N\textsuperscript{ΔMN}8\textsuperscript{-/-} vs. N\textsuperscript{ΔMN}11\textsuperscript{-/-} p>0.999; N\textsuperscript{ΔMN}8\textsuperscript{-/-} vs. βγ\textsuperscript{ΔMN} p>0.999; N\textsuperscript{ΔMN}11\textsuperscript{-/-} vs. βγ\textsuperscript{ΔMN} p=0.999. For V-R/T: control vs. N\textsuperscript{ΔMN}, N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-}, βγ\textsuperscript{ΔMN} ***p<0.001; N\textsuperscript{ΔMN} vs. N\textsuperscript{ΔMN}8\textsuperscript{-/-} p=0.294; N\textsuperscript{ΔMN} vs. N\textsuperscript{ΔMN}11\textsuperscript{-/-} *p<0.05; N\textsuperscript{ΔMN} vs. βγ\textsuperscript{ΔMN} **p<0.01; N\textsuperscript{ΔMN}8\textsuperscript{-/-} vs. N\textsuperscript{ΔMN}11\textsuperscript{-/-} p=0.449; N\textsuperscript{ΔMN}8\textsuperscript{-/-} vs. βγ\textsuperscript{ΔMN} p=0.246; N\textsuperscript{ΔMN}11\textsuperscript{-/-} vs. βγ\textsuperscript{ΔMN} p=0.969; one-way ANOVA and post hoc Tukey’s HSD test).

(G) Dorso-ventral density plots of H (green, ventral) and V (blue, dorsal) neurons in control, N\textsuperscript{ΔMN}, N\textsuperscript{ΔMN}8\textsuperscript{-/-}, N\textsuperscript{ΔMN}11\textsuperscript{-/-} and βγ\textsuperscript{ΔMN} embryos.
Figure S1. **Medio-lateral pool organization is preserved after elimination of type II cadherins specificity group.**

(A) Schematic showing classical cadherins expression by motor pools at lumbar levels 2-3.

(B and C) Medio-lateral density plots of H (green, medial) and R/T (red, lateral) neurons in control (B) and 8/11\(^{-/-}\) (C) embryos.

(D) Box-plots of medio-lateral distributions of H (green, medial) and R/T (red, lateral) neurons in control, 8/11\(^{-/-}\) and 6/9/10\(^{-/-}\) embryos.

(E) Average medio-lateral position of H (green, medial) and R/T (red, lateral) neurons in control, 8/11\(^{-/-}\) and 6/9/10\(^{-/-}\) embryos (mean ± SD; differences not significant for H neurons: control vs. 8/11\(^{-/-}\) p=0.250; control vs. 6/9/10\(^{-/-}\) p=0.176; 8/11\(^{-/-}\) vs. 6/9/10\(^{-/-}\) p=0.997; for R/T neurons: control vs. 8/11\(^{-/-}\) p=0.998; control vs. 6/9/10\(^{-/-}\) p=0.05; 8/11\(^{-/-}\) vs. 6/9/10\(^{-/-}\) p=0.077; one-way ANOVA and post hoc Tukey’s HSD test).

(F) Medio-lateral density plot of H (green, medial) and R/T (red, lateral) neurons in 6/9/10\(^{-/-}\) embryos.

(G) Average number of motor neurons in e13.5 lumbar spinal cords of control, 8/11\(^{-/-}\), 6/9/10\(^{-/-}\), \(N^\text{MN}\), \(N^\text{MN}8^{-/-}\), \(N^\text{MN}11^{-/-}\) and \(\beta\gamma^\text{MN}\) embryos (motor neurons/100\(\mu\)m, mean ± SD; all differences between genotypes not significant p>0.05; one-way ANOVA and post hoc Tukey’s HSD test).
Figure S2. Motor neuron migration arrest at the progenitor zone after joint elimination of N-cadherin and a type II cadherin.

(A-C) Motor neurons at the progenitor zone in $N^{AMN}$ (A), $N^{AMN8^{-/-}}$ (B) and $N^{AMN11^{-/-}}$ (C) e13.5 embryos.

(D) Number of motor neurons found in the ventral horn expressed as percentage of total number in $N^{AMN}$, $N^{AMN8^{-/-}}$ and $N^{AMN11^{-/-}}$ embryos (mean ± SD; $N^{AMN}$ vs. $N^{AMN8^{-/-}}$ **p<0.01; $N^{AMN}$ vs. $N^{AMN11^{-/-}}$ ***p<0.001; $N^{AMN8^{-/-}}$ vs. $N^{AMN11^{-/-}}$ p=0.112; one-way ANOVA and post hoc Tukey’s HSD test).

(E-H) Radial glia organization assessed by nestin immunostaining in control (E), $N^{AMN}$ (F), $N^{AMN8^{-/-}}$ (G) and $N^{AMN11^{-/-}}$ (H) e13.5 embryos. Magnification of the progenitor zone (PZ) and ventral horn (VH) areas on the side.
Figure S3. Motor pools organization in cadherin/catenin mutant mice.

(A) Transverse contour plots of V (blue, dorsal) and R/T (red, ventral) neurons in control, NΔMN, NΔMN8−/−, NΔMN11−/− and βγΔMN embryos.

(B) Transverse contour plots of V (blue, dorsal) and H (green, ventral) neurons in control, NΔMN, NΔMN8−/−, NΔMN11−/− and βγΔMN embryos.

(C) Transverse contour plots of H (green, medial) and R/T (red, lateral) neurons in control, NΔMN, NΔMN8−/−, NΔMN11−/− and βγΔMN embryos.

(D) Coronal contour plots of H (green, medial) and R/T (red, lateral) neurons in NΔMN8−/− and NΔMN11−/− embryos.

(E) Average medio-lateral position of H (green, medial) and R/T (red, lateral) neurons in control, NΔMN, NΔMN8−/− and NΔMN11−/− embryos (mean ± SD; for H neurons: control vs. NΔMN p=0.999; control vs. NΔMN8−/− p=0.810; control vs. NΔMN11−/− p=0.455; NΔMN vs. NΔMN8−/− p=0.868; NΔMN vs. NΔMN11−/− p=0.384; NΔMN8−/− vs. NΔMN11−/− p=0.126. For R/T neurons: control vs. NΔMN **p<0.01; control vs. NΔMN8−/− and NΔMN11−/− ***p<0.001; NΔMN vs. NΔMN8−/− p=0.212; NΔMN vs. NΔMN11−/− **p<0.01; NΔMN8−/− vs. NΔMN11−/− *p<0.05; one-way ANOVA and post hoc Tukey’s HSD test).

(F) Correlation analysis of V neurons dorso-ventral coordinates in NΔMN, NΔMN8−/−, and NΔMN11−/− embryos. Scale bar indicates correlation values (NΔMN8−/− vs. NΔMN11−/− r=0.94; NΔMN vs. NΔMN8−/− r=0.34; NΔMN vs. NΔMN8−/− r=0.58).
<table>
<thead>
<tr>
<th>Experiment</th>
<th>MN Subtype</th>
<th>Genotype</th>
<th>Embryo ID</th>
<th># of Sections/Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>“control”</td>
<td>Divisions</td>
<td>afadin fl/+</td>
<td>#6</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#8</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#25</td>
<td>30</td>
</tr>
<tr>
<td>“control”</td>
<td>Pools</td>
<td>afadin fl/+</td>
<td>#6</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#43</td>
<td>30</td>
</tr>
<tr>
<td>“8/11-/-”</td>
<td>Division</td>
<td>cadherin-8 -/-;</td>
<td>#4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-11 -/-</td>
<td>#7</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#2</td>
<td>27</td>
</tr>
<tr>
<td>“8/11-/-”</td>
<td>Pools</td>
<td>cadherin-8 -/-;</td>
<td>#2</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-11 -/-</td>
<td>#5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#6</td>
<td>24</td>
</tr>
<tr>
<td>“6/9/10-/-”</td>
<td>Pools</td>
<td>cadherin-6 -/-;</td>
<td>#1</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-9 -/-;</td>
<td>#4</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-10 -/-</td>
<td>#6</td>
<td>17</td>
</tr>
<tr>
<td>“NΔMN”</td>
<td>Divisions</td>
<td>N-cadherin fl/-;</td>
<td>#68</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#72</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#73</td>
<td>31</td>
</tr>
<tr>
<td>“NΔMN”</td>
<td>Pools</td>
<td>N-cadherin fl/-;</td>
<td>#6</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#10</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#66</td>
<td>31</td>
</tr>
<tr>
<td>“βγΔMN”</td>
<td>Divisions</td>
<td>β-catenin fl/fl;</td>
<td>#9</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ-catenin fl/-;</td>
<td>#2</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#13</td>
<td>30</td>
</tr>
<tr>
<td>“βγΔMN”</td>
<td>Pools</td>
<td>β-catenin fl/fl;</td>
<td>#2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ-catenin fl/-;</td>
<td>#3</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#4</td>
<td>28</td>
</tr>
<tr>
<td>“NΔMN8-/-”</td>
<td>Divisions</td>
<td>N-cadherin fl/-;</td>
<td>#40</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-8 -/-;</td>
<td>#58</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#60</td>
<td>31</td>
</tr>
<tr>
<td>“NΔMN8-/-”</td>
<td>Pools</td>
<td>N-cadherin fl/-;</td>
<td>#5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-8 -/-;</td>
<td>#64</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#110</td>
<td>26</td>
</tr>
<tr>
<td>“NΔMN11-/-”</td>
<td>Pools</td>
<td>N-cadherin fl/-;</td>
<td>#4</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cadherin-11 -/-;</td>
<td>#5</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olig2::Cre +/-</td>
<td>#7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>#8</td>
<td>19</td>
</tr>
</tbody>
</table>