The highly patterned process of breathing is controlled by automatic respiratory centers in the brainstem, which signal to a specialized group of motor neurons that constitute the phrenic nerves (PNs). In mammals, the thoracic diaphragm adopts the function of the primary respiratory musculature. Faithful innervation by the PNs is therefore a prerequisite for the functionality of this highly specialized musculature. We utilized mouse lines in which either the binding site of Npn-1 for class 3 Semaphorins was mutated systemically (Npn-1<sup>Sema</sup>), or in which Npn-1 was removed selectively from motor neurons (Npn-1<sup>cond</sup>) to investigate the involvement of the Sema3-Npn1-signaling pathway during phrenic nerve targeting and fasciculation, as well as development of the diaphragm. Gene expression was validated by in situ hybridizations (ISH) at critical developmental stages. Wholemount immunohistochemistry (IHC) against neurofilament/synaptophysin on embryos or diaphragms in combination with a detailed Sholl analysis was carried out to quantify PN branching. The influence of ligands onto the migratory behavior of primary muscle progenitor cells (MPCs) was evaluated in a collagen matrix in vitro. Wholemount IHC revealed initial mild defasciculation of phrenic motor axons within the brachial plexus at E10.5. Interestingly, axons refasciculated and formed one distinct nerve before reaching the pleuroperitoneal fold at E11.5. During the development of costal diaphragm muscles, Sholl analysis revealed increased branching of PN axons, which persisted until the end of primary myogenesis at E16.5 and beyond. Additionally, significantly more axons extended into the central tendon region (CTR) when compared to littermate controls. Intriguingly, we observed formation of ectopic muscles (EM) patches within the CTR that were targeted by misprojecting axons in both mutant mouse lines. We therefore asked whether EM development was a direct result of manipulating Sema3A/Npn-1 signaling, or a secondary effect regarding interaction of ectopically migrating phrenic axons and MPCs. To elucidate the underlying mechanisms of ectopic recruitment of MPCs to the CTR, we focused on the Slit/Robo signaling pathway, which is employed by motor neurons during axon targeting and bundling, and was shown to be involved in targeted MPC migration at later developmental stages in Drosophila. Chemotaxis experiments revealed an attractive of Slit1/Slit2 onto primary MPCs. Thus, we postulate an influence of factors released by motor neuron growth cones on the migration properties of myoblasts during diaphragm development.