

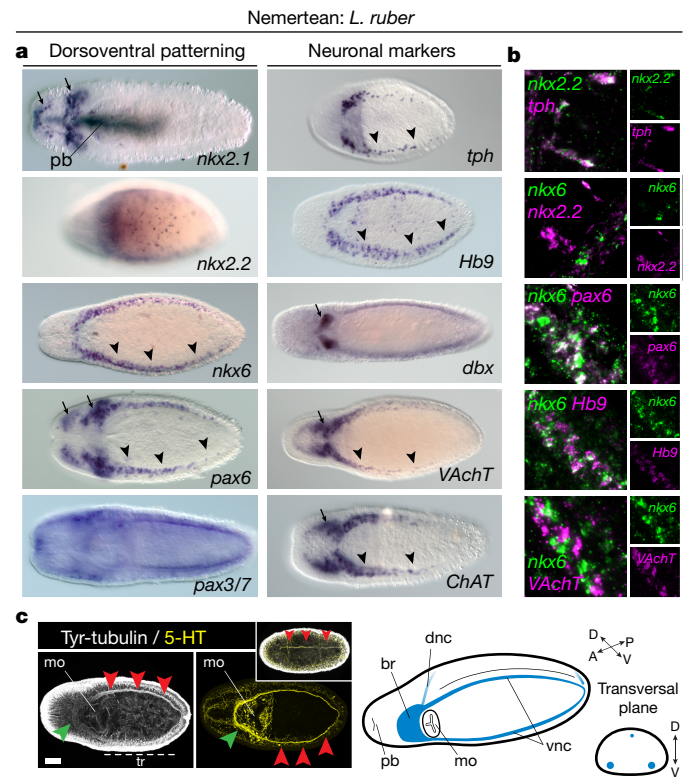
**Figure 3 | Dorsoventral patterning in Brachiopoda.** A, Transcription factors *nkx2.2* and *nkx6* are in the trunk midline (arrowheads), posterior tip (arrows), gut, and apical cells (*nkx6*); *pax3/7* is expressed laterally (arrowheads) and in the apical lobe (arrow); *msx* is in the mantle and ventral pedicle. B, There is an *nkx2.2*<sup>+</sup>/*nkx6*<sup>+</sup> medioventral region, and a more lateral *nkx6*<sup>+</sup>/*pax6*<sup>+</sup>/*pax3/7*<sup>+</sup> anterior trunk domain. C, *T. transversa* larval CNS (green arrowheads indicate the neuropile in a, and the trunk serotonergic condensation in b; red arrowheads mark the VNCs; pink arrowheads indicate the innervation of the chaetae). D, Only *tph* is expressed in the trunk (arrows and arrowheads indicate expression areas). E, Transcription factors *nkx2.2* and *nkx6* are in the trunk ventral midline (arrowheads), apical lobe (arrows), and gut; *pax3/7* is in the mesoderm (arrows), and in two ventrolateral trunk domains (arrowheads); *msx* is in the trunk, shell epithelium (arrowhead), and mesoderm (arrows). F, *N. anomala* larval CNS (green arrowhead indicates the neuropile; red arrowheads in a mark the VNCs; red arrowheads in b indicate the innervation of the chaetae). Abbreviations: ao, apical organ; bp, blastopore; ch, chaetae; mo, mouth; np, neuropile; vnc, ventral nerve cord. Scale bars, 50  $\mu$ m.

neuroanatomies suggests that the dorsoventral patterning of the nerve cords also evolved after the Xenacoelomorpha–Nephrozoa split.

### Dorsoventral patterning in Brachiopoda

To investigate the conservation of the dorsoventral nerve cord patterning in Nephrozoa, we focused on Spiralia<sup>29</sup>, one of the three major nephrozoan clades. Although some lineages have a medially condensed VNC (that is, Annelida), a main pair of lateral VNCs is widespread and probably homologous in Spiralia<sup>5</sup>. We first studied the brachiopod *Terebratalia transversa*, in which we identified staggered expression of dorsoventral transcription factors in the anterior ventral midline of the larval trunk. At this stage, *nkx2.1* (ref. 30) and *pax6* (ref. 31) are expressed in the apical lobe, albeit *pax6* expression projects slightly into the mantle lobe. However, there is a medial *nkx2.2*<sup>+</sup>/*nkx6*<sup>+</sup> domain, a more lateral *nkx6*<sup>+</sup>/*pax6*<sup>+</sup>/*pax3/7*<sup>+</sup> region, and a broad, dorsolateral *msx*<sup>+</sup> area in the anterior ventral ectoderm of the larval 'trunk' (that is, mantle and pedicle lobes) (Fig. 3A, B and Extended Data Fig. 7a). Additionally, a narrow line of cells below the apical–mantle boundary crossing the ventral midline expresses *pax3/7* (Fig. 3A, B and Extended Data Fig. 7a). These expression domains disappear in the highly modified adult body (Extended Data Fig. 7a–c). The staggered expression of dorsoventral transcription factors in the ventral anterior ectoderm of the trunk only partly correlates with the larval neuroanatomy, which consists of an anterior condensation and a medial accumulation of serotonergic cells on the ventral side, from which pairs of neurites innervate the chaetae and posterior end (Fig. 3C). The dorsoventral transcription factors do not co-express with most neuronal markers<sup>12</sup>, which are mostly expressed in the anterior region (Fig. 3A, D and Extended Data Fig. 7a, d). Only two *tph*<sup>+</sup> clusters in the medial serotonergic condensation of the larval trunk co-localize with the *nkx2.2*<sup>+</sup>/*nkx6*<sup>+</sup> medial domain. Therefore, the brachiopod *T. transversa* resembles vertebrates, arthropods, and *P. dumerilii* in the presence of a ventral serotonergic *nkx2.2*<sup>+</sup>/*nkx6*<sup>+</sup> area<sup>4,9,12,32</sup>, as well as in the *nkx6*, *pax6*, *pax3/7*, and *msx* dorsolateral domains, which are, however, not apparently connected to any neural trunk structure.

The staggered ectodermal expression of dorsoventral transcription factors in the anteroventral trunk of *T. transversa* is largely conserved



**Figure 4 | Dorsoventral patterning in Nemertea.** a, Transcription factors *nkx2.1* and *nkx2.2* are in the head (arrows), proboscis (*nkx2.1*), and trunk cells (*nkx2.2*); *nkx6* and *pax6* are in the head (arrows) and VNCs (arrowheads); *pax3/7* is broadly expressed. Neuronal markers are in the brain (arrows) and VNCs (arrowheads). b, In the VNCs, *nkx2.2*<sup>+</sup> cells express *tph*, but not *nkx6*; *nkx6*<sup>+</sup> cells express *pax3/7* and *Hb9*, but not *VAChT*. c, *L. ruber* CNS (green arrowheads indicate the brain; red arrowheads mark the VNCs and the dorsal neurite in the upper inset). Abbreviations: br, brain; dnc, dorsal nerve cord; mo, mouth; tr, trunk; pb, proboscis; vnc, ventral nerve cord. Scale bar, 100  $\mu$ m.