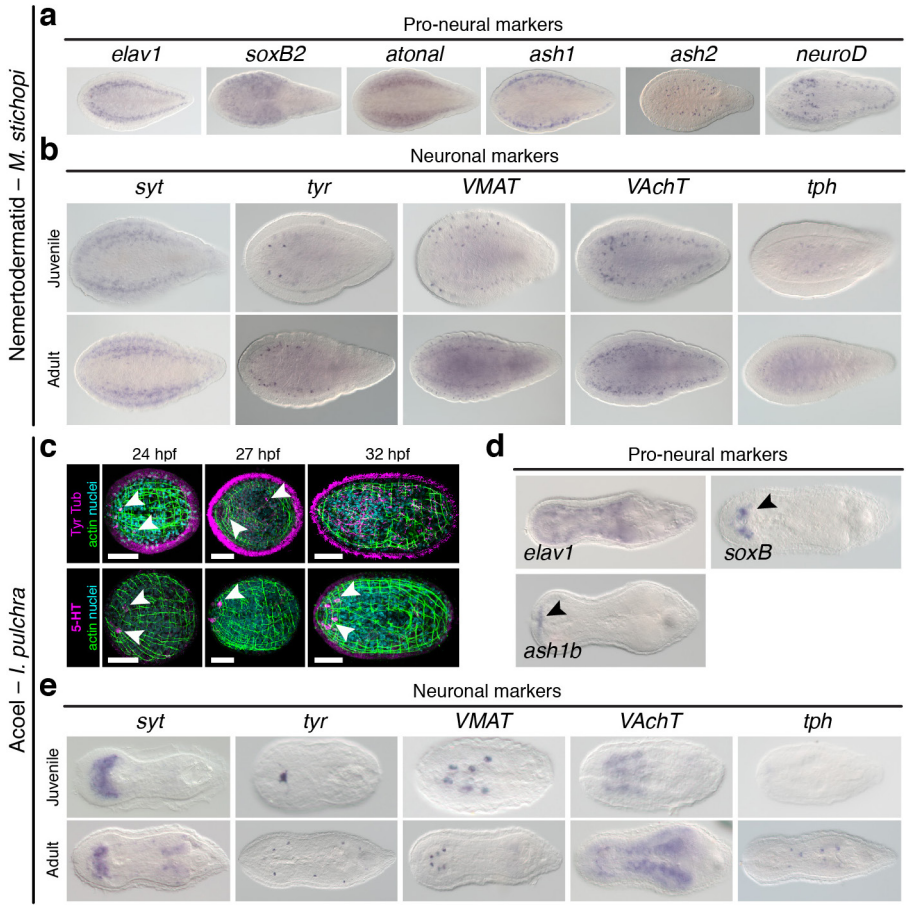

Extended Data Figure 5 | Expression of neuronal markers in Nemertodermatida and Acoela. a, In the nemertodermatid *M. stichopi*, the genes associated with neuronal fate commitment, *elav1*, *soxB2*, *ash1*, *ash2*, *atonal*, and *neuroD*, are detected along the dorsal nerve cords. **b**, Similarly, the neuronal markers *synaptotagmin* (*syt*), *tyrosine hydroxylase* (*tyr*), *vesicular monoamine transporter* (*VMAT*), *choline acetyltransferase* (*ChAT*), *vesicular acetylcholine transporter* (*VAchT*), and *tryptophan hydroxylase* (*tph*) are mostly expressed dorsally, along the dorsal nerve cords. **c**, Morphology of *I. pulchra* embryos stained against tyrosinated tubulin (Tyr Tub) and serotonin (5-HT), and counterstained with phallacidin (actin bundles) and DAPI (nuclei). The first tubulin-positive cells that resemble neurons appear anteriorly (arrowheads) at 24 h post-fertilization (hpf). By 32 h post-fertilization, the anterior and posterior lobes of the brain, as well as some neurite bundles, are visible. Similarly, the first serotonergic cells are detected at 24 h post-fertilization in the anterior end (arrowheads). **d**, In *I. pulchra*, the pro-neural marker *elav1* is broadly expressed, *soxB* is detected in the head region (arrowhead), and *ash1b* in the anterior tip (arrowhead). **e**, In *I. pulchra*, the neuronal marker *syt* is highly expressed in the anterior neuropile. The marker *tyr* is detected in the statocyst and isolated cells. *VMAT* is detected in isolated dorsal cell clusters in the juvenile that concentrate along the adult brain. *ChAT* and *VAchT* are expressed in the brain in juveniles and adults (gonadal staining in the adult is background). The gene *tph* is expressed in isolated ventral cells of the adult. All panels are dorsoventral views with anterior to the left. Scale bars, 50 μm in **c**.