

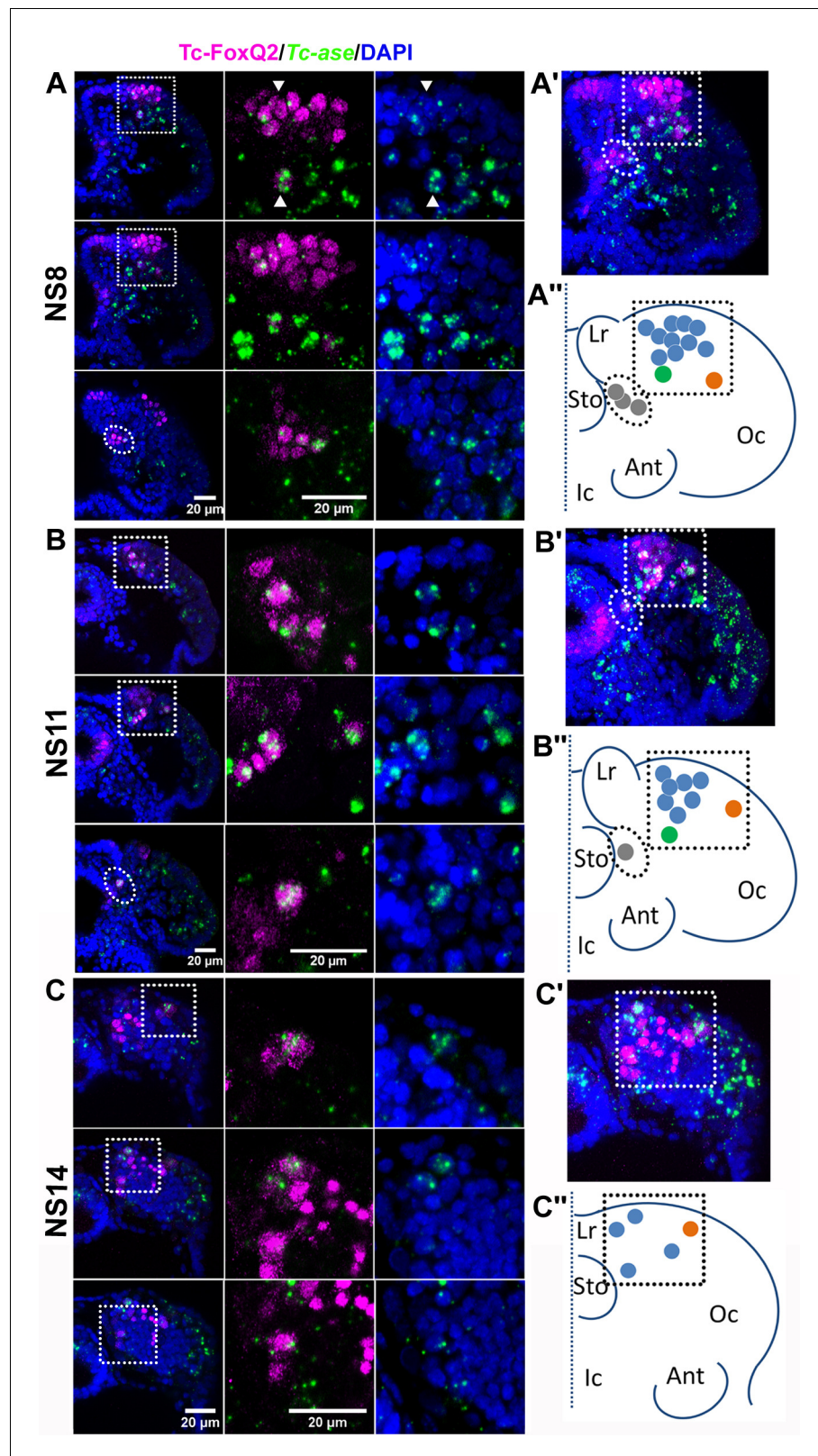


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## Figures and figure supplements

An ancestral apical brain region contributes to the central complex under the control of *foxQ2* in the beetle *Tribolium*

**Bicheng He et al**

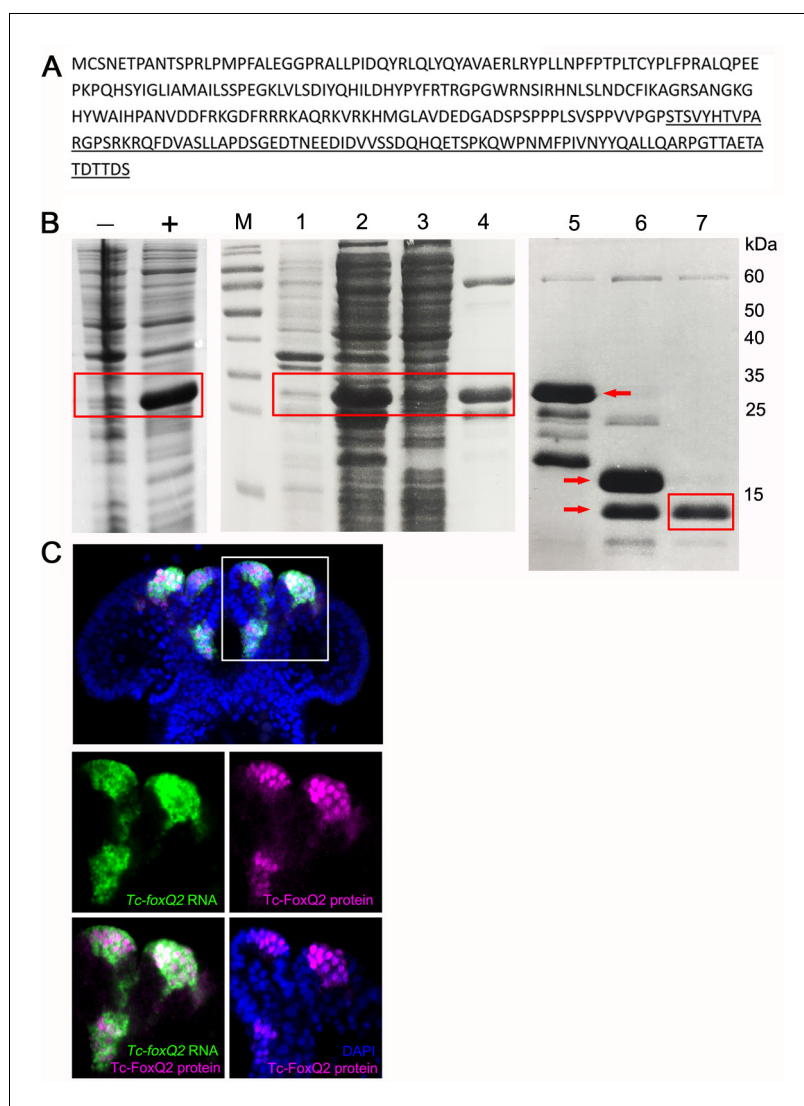


**Figure 1.** Tc-FoxQ2 positive neural progenitor cells. Tc-FoxQ2 protein is visualized by immunohistochemistry at different stages (magenta) while neural progenitor cells (NPCs) are marked by intronic *Tc-ase* whole mount in situ hybridization (green). Nuclei are visualized by DAPI (blue). Optical sections of right halves of stained heads are

Figure 1 continued on next page

## Figure 1 continued

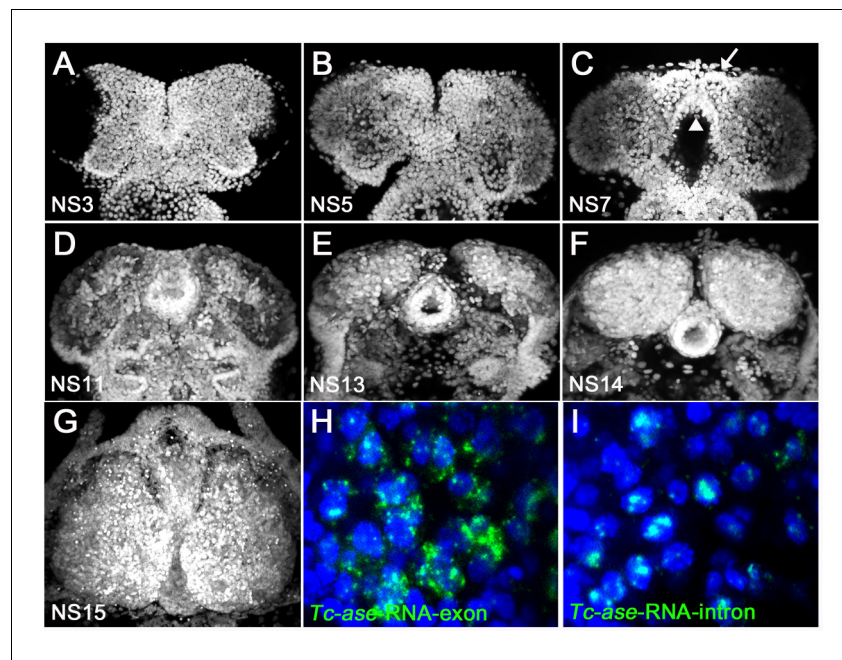
shown in the left column while respective close-ups are shown in second and third column (see hatched areas in left column in **(A, B and C)**). A projection of all optical sections is given in the right column (**(A', B' and C')**). The schemes represent the outline of right halves of the head lobes of flattened embryos. The dotted line represents the midline. This depiction is comparable to the one previously used for *Drosophila* neuroblast maps (**Urbach and Technau, 2003a**) (**(A'', B'' and C'')**). (**(A–A'')**) At NS8 about 15 Tc-FoxQ2 positive NPCs are found ( $n = 6$ ). By position, three groups are distinguished: A large anterior median group (blue in A'') with one neuroblast slightly separated posteriorly (green in A''), one single lateral NPC (orange in A'') and a group located closely to the midline (gray in A''). White arrowheads show two exemplary NPCs. (**(B–B'')**) At NS11 about 10 Tc-FoxQ2 positive NPCs are observed ( $n = 6$ ). (**(C–C'')**) At NS14, the number has decreased to 5–7 cells ( $n = 6$ ). The single lateral NPC remains distinguishable (orange in C''). Lr: labrum; Sto: stomodeum; Oc: ocular region; Ant: antenna; Ic: Intercalary region.  
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**Figure 1—figure supplement 1.** Generation of a Tc-FoxQ2 antibody. (A) Protein sequence of Tc-FoxQ2. The C-terminus containing 85 amino acids (underlined) has little homology to other proteins in *Tribolium* and was used for protein expression. (B) Coomassie-blue stained SDS-PAGE gel analysis of expression and purification of Tc-FoxQ2. (–) Before IPTG induction; (+) after IPTG induction. M, marker; lane 1, cell pellet; lane 2, supernatant; lane 3, flow through after  $\text{Ni}^{2+}$  chelate affinity chromatography; lane 4, eluted fractions by imidazole; lane 5, before SUMO protease digestion (red arrow); lane 6, after SUMO protease digestion, two bands are observed (red arrows): 6xHis-SUMO and Tc-FoxQ2; lane 7, flow through after re- $\text{Ni}^{2+}$  chelate affinity chromatography which contains Tc-FoxQ2. (C) Expression of *Tc-foxQ2* RNA (green) and Tc-FoxQ2 protein (magenta) in the embryo. *Tc-foxQ2* RNA is detected throughout the cytoplasm, while Tc-FoxQ2 protein is detected in the nuclei (blue). *Tc-foxQ2* RNA and Tc-FoxQ2 protein show a high overlap.

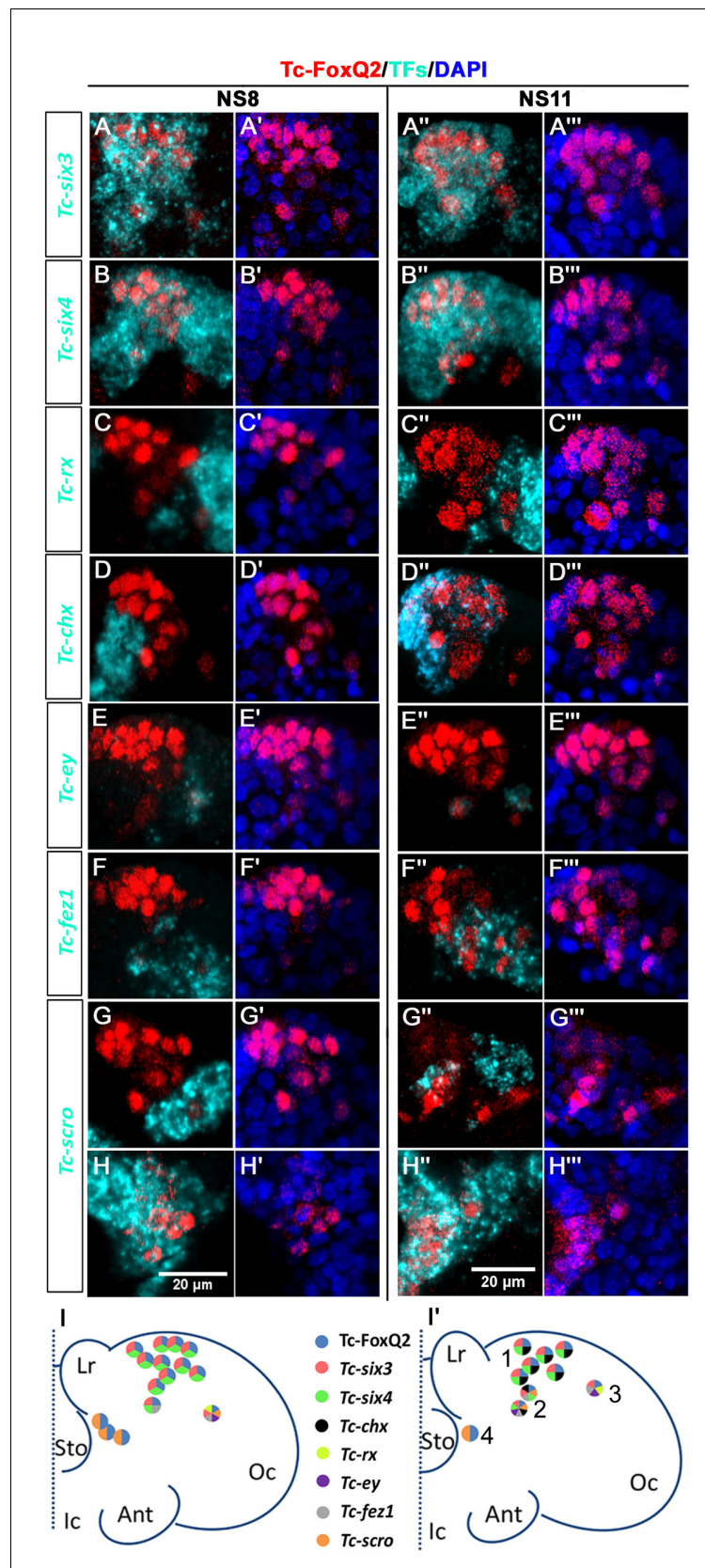
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**Figure 1—figure supplement 2.** Developmental staging and comparison of exonic versus intronic *Tc-asense* probes. In *Tribolium*, the established morphological staging system (Biffar and Stollewerk, 2014) with respect to early neurogenesis has 15 stages, termed NS1 to NS15. (A, B) From NS3 to NS5, the neuroectoderm is still a flat sheet of cells. (C) At NS7, the labral and stomodeal buds have formed (arrow, white arrowhead). (D) At NS11, the brain grows thicker and contains an increased number of cells. (E, F) From NS13 onwards, the brain hemispheres become an oval shape and grow. (G) At NS15, the brain hemisphere shows a pear-like shape. All planes are dorsal view. (H) *Tc-asense*-RNA exonic probe marks cytoplasm. (I) *Tc-asense*-RNA intronic probe marks only nuclei, which allows cell identification.

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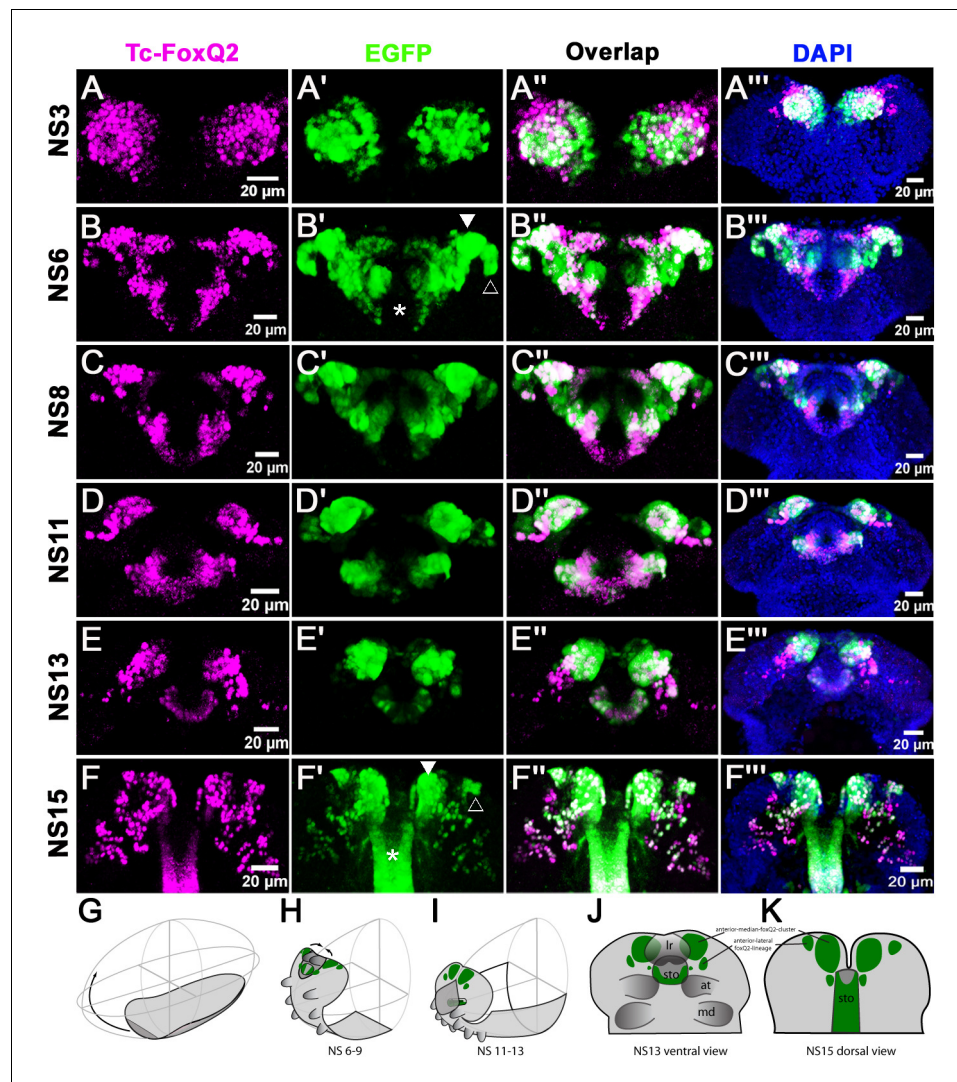


**Figure 2.** Neural progenitor cells co-express transcription factors. Tc-FoxQ2 is visualized by immunohistochemistry (red) while the other transcription factors (TFs) are marked by *fluorescent in situ hybridization* (cyan) at NS8 and NS11. Figure 2 continued on next page

## Figure 2 continued

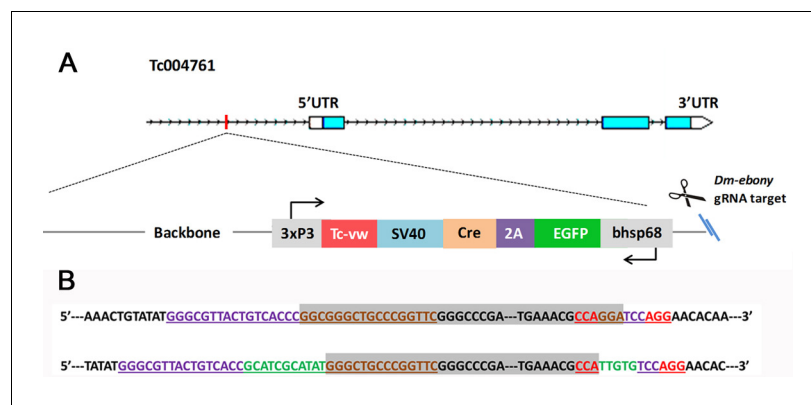
NS11. Nuclei are visualized by DAPI (blue). Close-ups of the right halves of embryonic heads are shown (A–H'') and schemes are given at the bottom (I and I'). (A–A'') Co-expression of *Tc-six3* and *Tc-FoxQ2*. (B–B'') Co-expression of *Tc-six4* and *Tc-FoxQ2*. (C–C'') Co-expression of *Tc-rx* and *Tc-FoxQ2*. (D–D'') Co-expression of *Tc-chx* and *Tc-FoxQ2*. (E–E'') Co-expression of *Tc-ey* and *Tc-FoxQ2*. (F–F'') Co-expression of *Tc-fez1* and *Tc-FoxQ2*. (G–H'') Co-expression of *Tc-scro* and *Tc-FoxQ2*. (I, I') Four different identities of NPCs are distinguished based on their position and co-expression. *P-fox-am* (1) is located in the anterior median neuroectoderm. The cells in this group co-express *Tc-FoxQ2* with *Tc-six3*, *Tc-six4* and later *Tc-chx*. *P-fox-amp* (2) is located posteriorly adjacent with additional expression of *Tc-scro*, *Tc-fez1* and in one of the cells also *Tc-ey*. *P-fox-l* (3) consists of one lateral cell which co-expresses *Tc-FoxQ2* with *Tc-six3*, *Tc-fez1*, *Tc-rx* and *Tc-ey*. *P-fox-v* (4) is located ventrally adjacent to the stomodeum, showing only co-expression of *Tc-FoxQ2* and *Tc-scro*. Same abbreviations as in **Figure 1**.

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**Figure 3.** *Tc-foxQ2* positive cells marked by antibody and the *foxQ2-5'-line*. The expression of EGFP (green) derived from the *foxQ2-5'-line* and Tc-FoxQ2 protein (magenta) correlate closely throughout embryogenesis. The morphology of the anterior neuroectoderm is visualized with DAPI staining (blue, right column). Shown are heads of embryos dissected out of the egg and flattened to reveal the staining within the neuroectoderm (A'''–C''') and the developing brain (D'''–F'''). Some differences between EGFP and Tc-FoxQ2 expression are observed, which may be due to either different dynamics of maturation and degradation of these proteins or to divergence of the enhancer trap signal from the endogenous expression. (A–A''') At NS3, *Tc-foxQ2* expression shows two bilateral domains within the anterior median region. (B–F''') Later, the expression domains split into a stomodeal (asterisk), a median (white arrowhead) and lateral domain (open arrowhead). At NS15, two clusters of cells are observed: The large *anterior-median-foxQ2-cluster* (white arrowhead in F') and a smaller *anterior-lateral-foxQ2-lineage* (open arrowhead in F'). (G–I) The general movements of the head tissue are shown from the germ rudiment (G) to an elongating (H) and a retracting stage (I). The approximate positions of the *Tc-foxQ2* marked cells underlying the head epidermis are shown. (J,K) Flat preparations of heads of stage NS13 (J) and NS15 (K) are shown with the approximate positions of the underlying *Tc-foxQ2* marked cells shown in green. (G–I) are redrawn from Posnien and Bucher (2010).

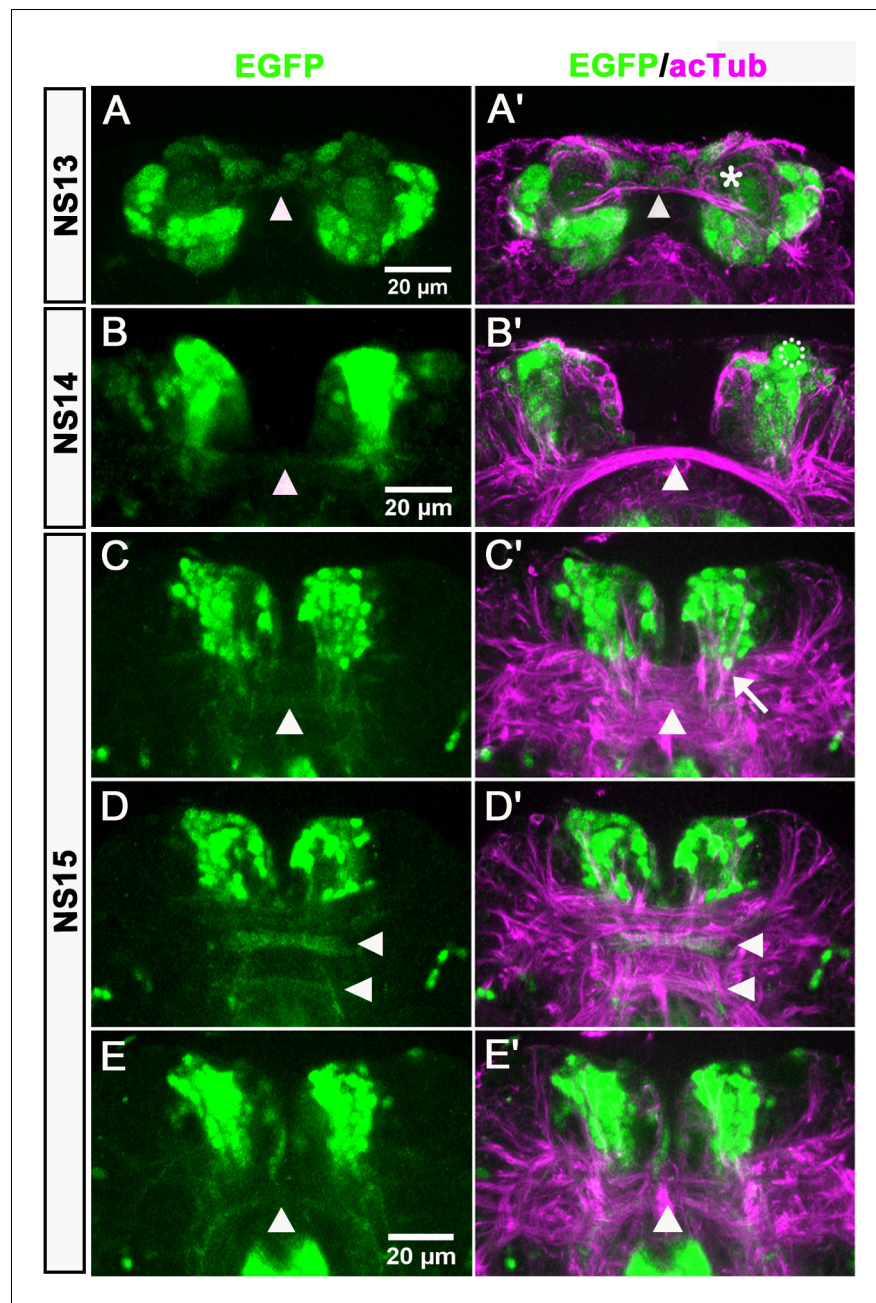
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**Figure 3—figure supplement 1.** Characterization of the *foxQ2-5'*-line. (A) The insertion (including the plasmid backbone) is about 6.8 kb in length and the bhsp68 promoter is located approximately ~700 bp upstream of the transcription start site. The EGFP transcription unit is oriented in the opposite direction to *Tc-foxQ2*. (B) The upper row is the predicted sequence of a perfect insertion without indels where gray indicates the left and right extremes of the 6.8 kb insert and the gRNA target sequence is underlined. The lower row is the actual sequencing results of this line. The insertion occurred at the predicted site (3 bp upstream of the PAM, AGG shown in red) and small insertions occurred at both integration sites (green). The purple bases represent the gRNA1 target sequence (targeting the genomic DNA) and the brown bases represent the gRNA-eb target sequence (targeting the plasmid for linearization). The genomic region and sequence of *Tc-foxQ2* (*Tc004761*) are viewed by the iBeetle genome browser (<http://ibeetle-base.uni-goettingen.de/gb2/gbrowse/tribolium/>). gRNA1 target sequence: 5' GGGCGT TACTGTCAACCCTCCAGG 3' gRNA-eb target sequence: 5' CCAGGAGGCGGGCTGCCCGGTTTC 3'.

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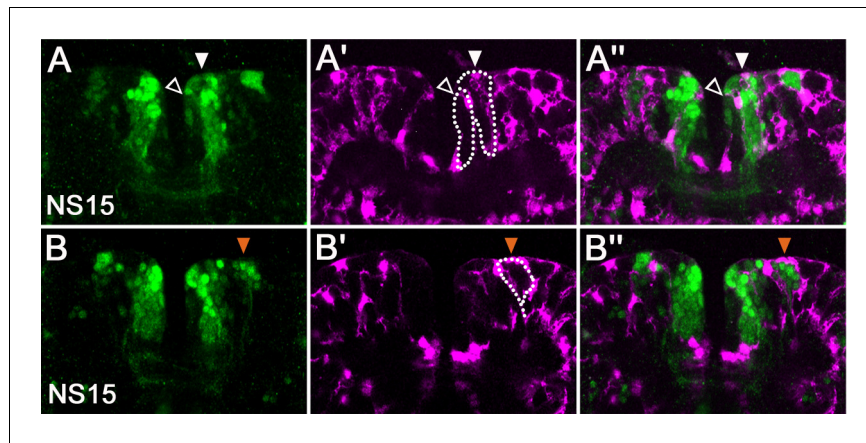




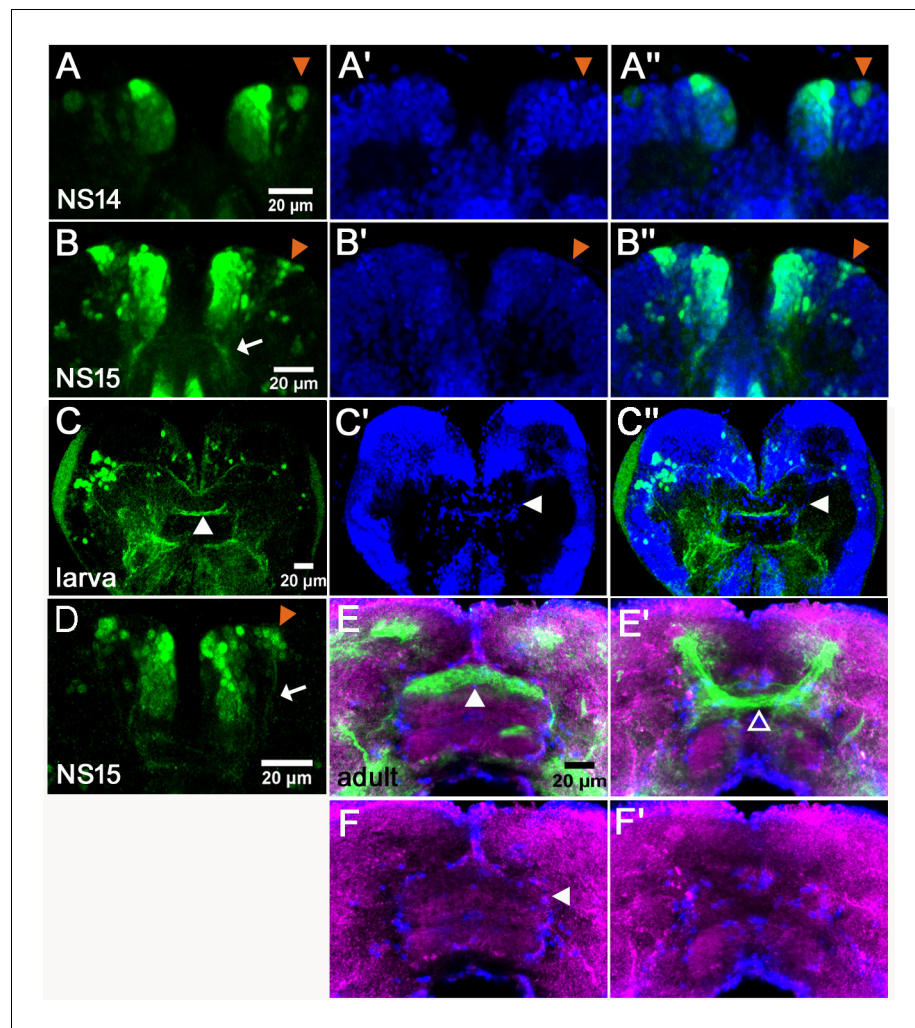
**Figure 4.** Anterior-median-foxQ2-cluster contributes to the central brain primordium. Double-immunohistochemistry visualizes the EGFP (green) derived from the *foxQ2-5'-line* and acTub (magenta) which marks axonal projections – neuraxis anterior is up. (A–A') At NS13, the first brain commissure marked by acTub appears (white arrowhead in A'). The cell bodies of the *anterior-median-foxQ2-cluster* are located around this commissure but do not yet project into it. A few weakly stained cells closely attached to the commissure are not Tc-FoxQ2 protein positive (asterisk in A'). (B–B') At NS14, projections within the brain commissure become visible but have not yet reached the midline (white arrowhead in B). One Tc-FoxQ2 positive NPC is recognized by its morphology and position (hatched circle in B'). (B) and (B') are not the same embryo but from the same developmental stage. (C–E) At NS15, at least three brain commissures are marked by the *anterior-median-foxQ2-cluster*: One in the circumesophageal commissure (white arrowhead in E), and two commissures within the central brain primordium (white arrowheads in D). The *anterior-median-foxQ2-cluster* produces more cells at this stage. (C'–E') acTub marked brain commissures expand into many fascicles and increase in size. 6–7 axon bundles emanating from the *anterior-median-foxQ2-cluster* separately join this midline brain primordium (one of them marked by an arrow in C').

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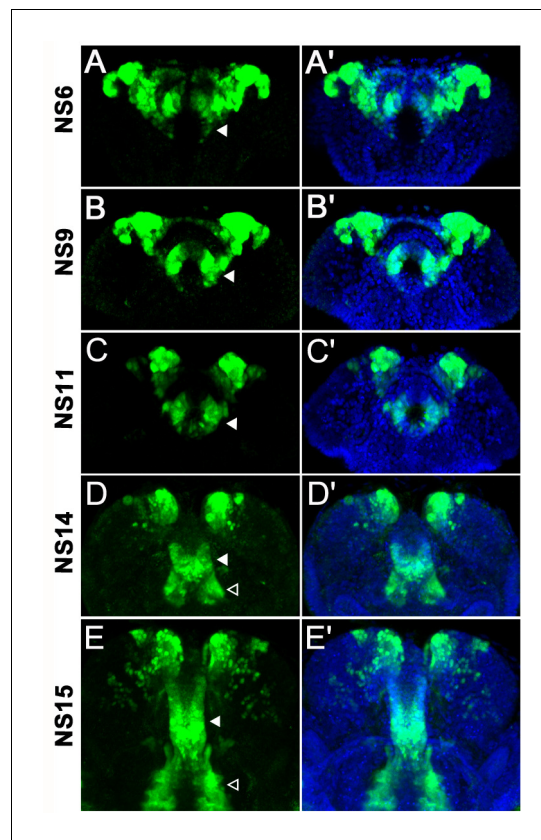


**Figure 4—figure supplement 1.** *Tc-foxQ2* cell clusters are surrounded by glial sheets. A cross of the *foxQ2-5'-line* (green) with the *glia-line* (Koniszewski et al., 2016; magenta) was imaged. (A–A'') Most of the cells of the *anterior-median-foxQ2-cluster* are within one glia sheet (white arrowheads, dashed line). The medial-most cells which show comparably weak EGFP signal are surround by another glia sheet (open arrowheads, dashed line). (B–B'') The *anterior-lateral-foxQ2-lineage* is surrounded by one glia sheet (orange arrowheads, dashed line). DOI: <https://doi.org/10.7554/eLife.49065.009>



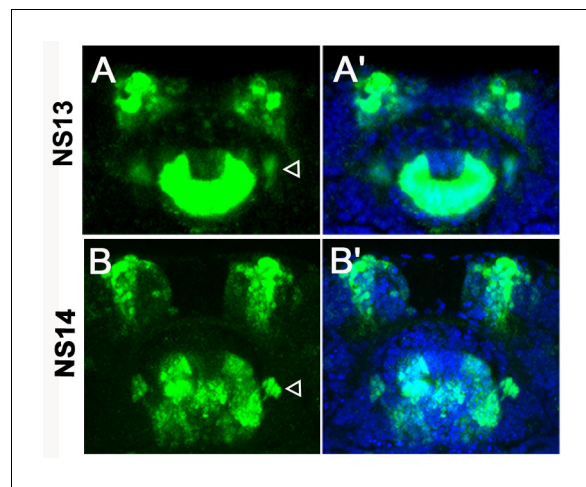
**Figure 5.** *Tc-foxQ2* positive cells project through the central brain primordium and contribute to the central complex. Immunohistochemistry visualizes EGFP (green) derived from the *foxQ2-5'-line*. Synapsin visualizes adult brain morphology (magenta in E,F) while nuclei are visualized by DAPI (blue in A'-C'). (A-A'') At NS14, the *anterior-lateral-foxQ2-cluster* consists of one NPC and a small number of progeny (orange arrowheads). (B-B'', D) At NS15, more cells are marked by EGFP (orange arrowheads) and their projections join a *Tc-foxQ2* positive axon bundle (white arrows in B, (D)). (C-C'') EGFP marked projections contribute to the central body in L5 larval brain, which is visualized by its surrounding glia cells (white arrowhead in C'). (E-F) EGFP marked projections contribute to the upper unit of the central body in the adult brain (white arrowhead in E) visualized by synapsin and surrounding glia (white arrowhead in F). (E'-F') Another fascicle projects across the midline directly posterior of the central body (open arrowhead).

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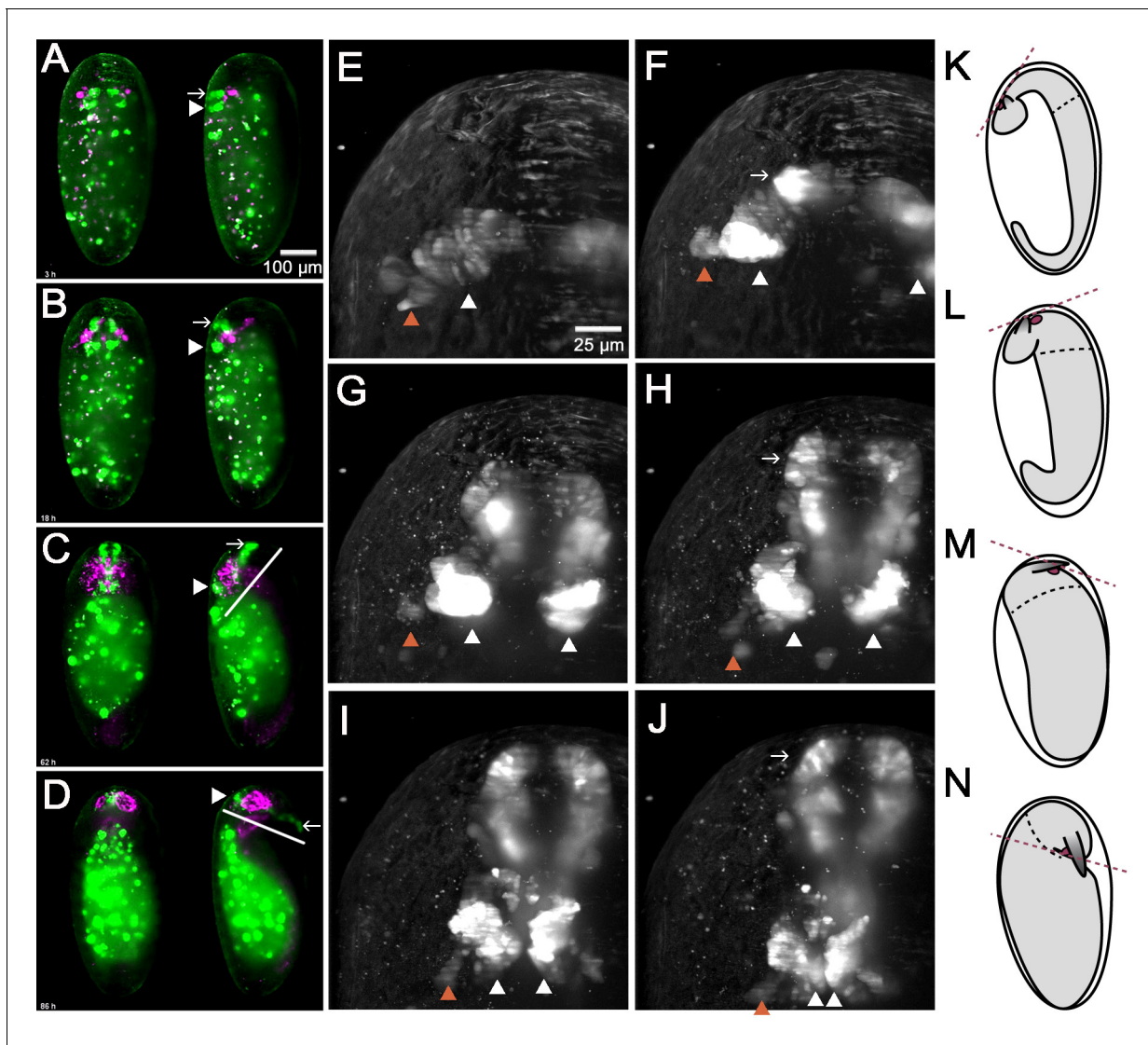
**Figure 5—figure supplement 1.** *foxQ2-5'-line* marks cells of the stomodeum and the lateral parts of the labrum. Immunohistochemistry visualizes the EGFP (green) expressed by the *foxQ2-5'-line*. The outline is visualized with DAPI staining (blue). The expression of *Tc-foxQ2* marked by EGFP from NS6 to NS15 is shown. (A–C') The stomodeum is located posteriorly in anterior median region and cells of it except for the dorsal roof are marked (white arrowheads). (D–E') At NS14 and NS15, additional marked epidermal cells in the lateral parts of the labrum are observed (open arrowheads).

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**Figure 5—figure supplement 2.** The *foxQ2-5'-line* marks cells lateral of the stomodeum. Immunohistochemistry visualizes the EGFP (green) derived from the *foxQ2-5'-line*. The morphology of the brain is visualized with DAPI staining (blue). (A–A') At NS13, several cells lateral to the stomodeum are observed (open arrowhead). (B–B') At NS14, this group of cells project into the posterior circumesophageal commissure (open arrowhead).

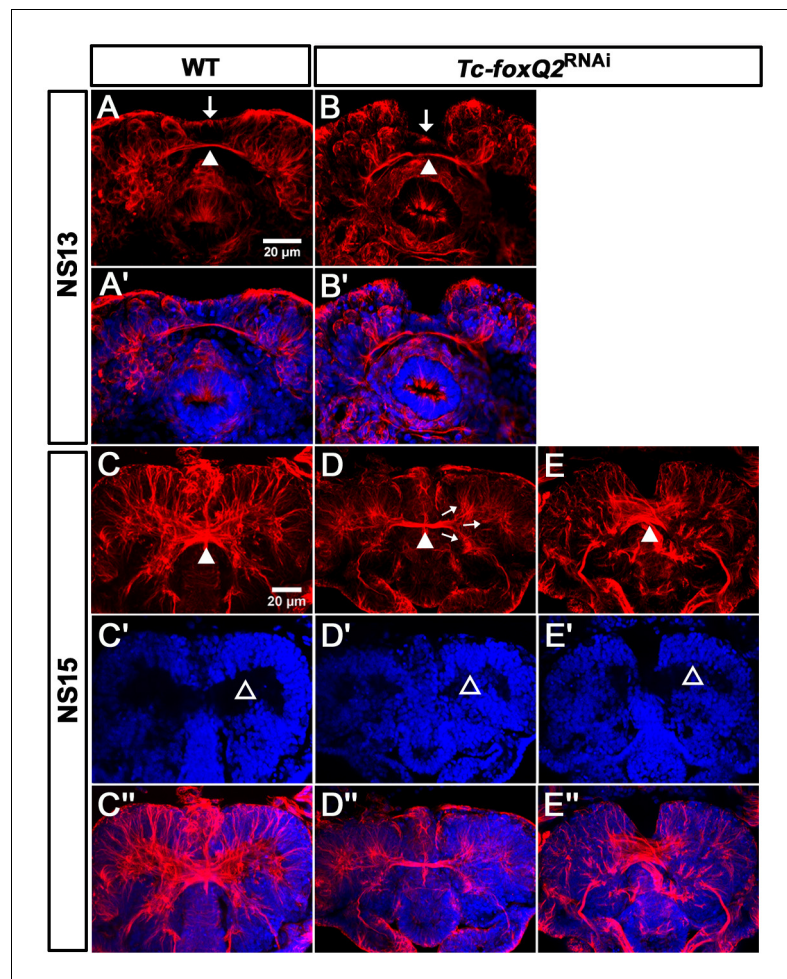
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**Figure 6.** In vivo imaging reveals morphogenetic movements during brain development. A cross of the *foxQ2-5'-line* (green) with the AGOC #6 glia reporter line (magenta) was imaged. (A–D) Shown are dorsal views (left specimen) and lateral views with dorsal to the left (right specimen) (A) EGFP signals in brain and stomodeum start out at the dorsal side (white arrowhead and white arrow, respectively). (B–C) Later, the stomodeum becomes elongated and bends away towards the ventral side. (C–D) An overall ventral bending of the head and brain follows, where the relative positions within the head remain similar (compare white lines in C and D). (E–J) Shown is one specimen from the dorsal side with anterior to the top. At the same time, the bilateral *Tc-foxQ2* positive cell clusters approach each other towards the midline (white arrowheads). Both the *anterior-median-foxQ2-cluster* (white arrowheads in E–J) and the *anterior-lateral-foxQ2-cluster* (orange arrowheads in E–J) can be distinguished throughout development. Note that a small group of marked cells detach from the cluster and fuse at the midline. However, these cells are not *Tc-FoxQ2* positive and are, hence, not further considered. (K–N) The overall morphogenetic movements of embryos are depicted schematically with the embryo highlighted in gray. Marked are the stomodeum (red circle) and the antennae. The black hatched line marks the boundary between thorax and head segments while the red hatched line indicates the plane to which the mouth opens. The embryos are redrawn from **Strobl and Stelzer (2014)** and do not exactly match the stages shown in A–D.

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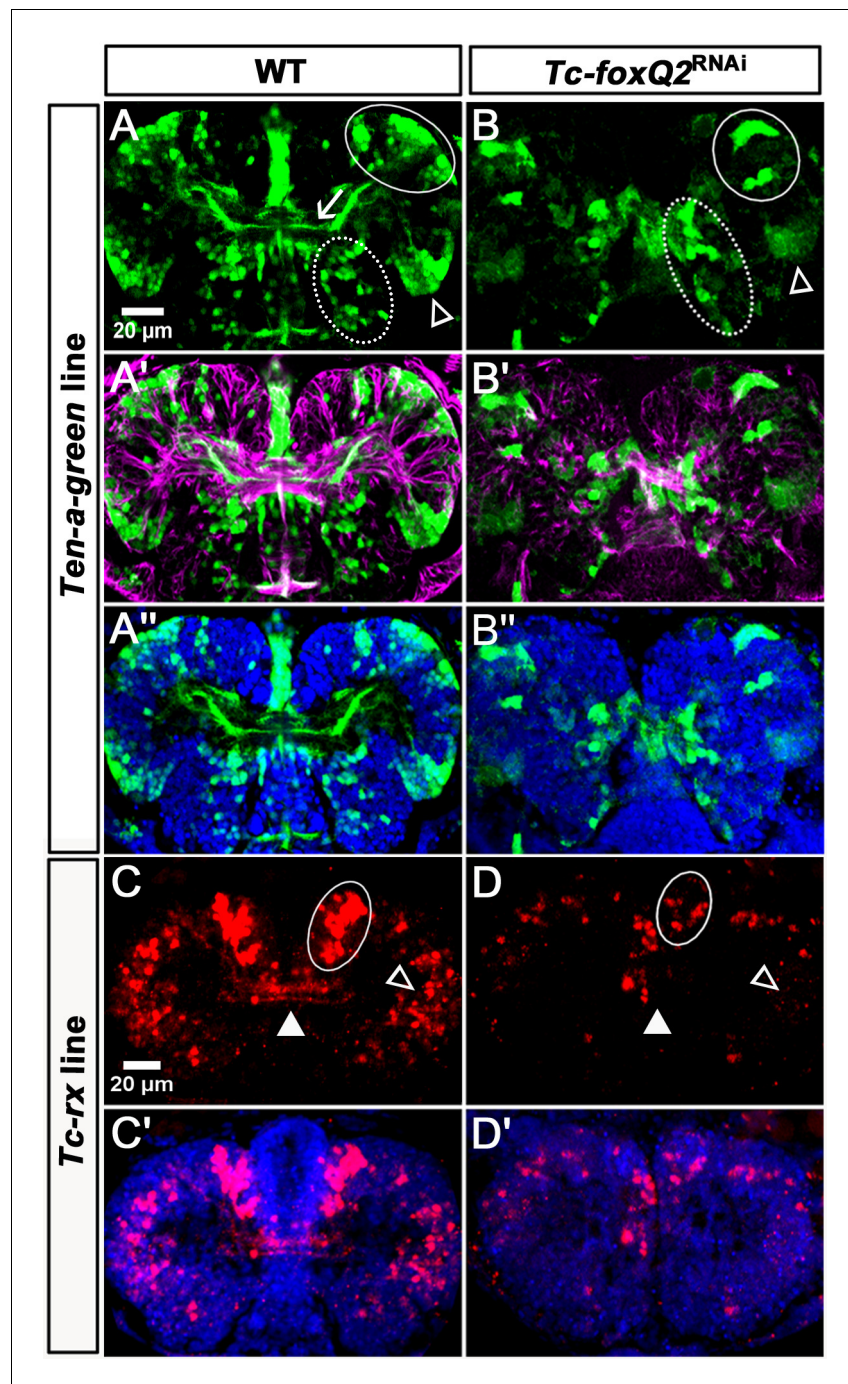




**Figure 7.** Loss of *Tc-foxQ2* function leads to arrest of development of brain midline structures in the embryo. Axonal projections are marked by acTub (red) and cell bodies are visualized by DAPI (blue). (A, A') In WT, the primary brain commissure forms at NS13 (white arrowhead in A). (B, B') In RNAi embryos, the primary brain commissure is slightly irregular (white arrowhead in B). The anterior epidermal aberrations reflect the loss of the labrum (compare white arrows in A and B; *Kitzmann et al., 2017*). (C–C'') In WT NS15 embryo, the central brain primordium increases in size and contains more fascicles, some of which form chiasmata at the midline (white arrowhead in C). (D–D'') In strong phenotypes, the primary commissure remains detectable along with three main branches (arrows in D). However, the structures do not expand and commissure splitting does not occur. At the same time, the brain neuropil volume is strongly reduced (compare black space between the cell bodies in C' and D', open arrowheads (arrows in D)). (E–E''). Weak phenotypes show some degree of splitting of the brain commissure but axonal projections are disarranged (white arrowhead in E).

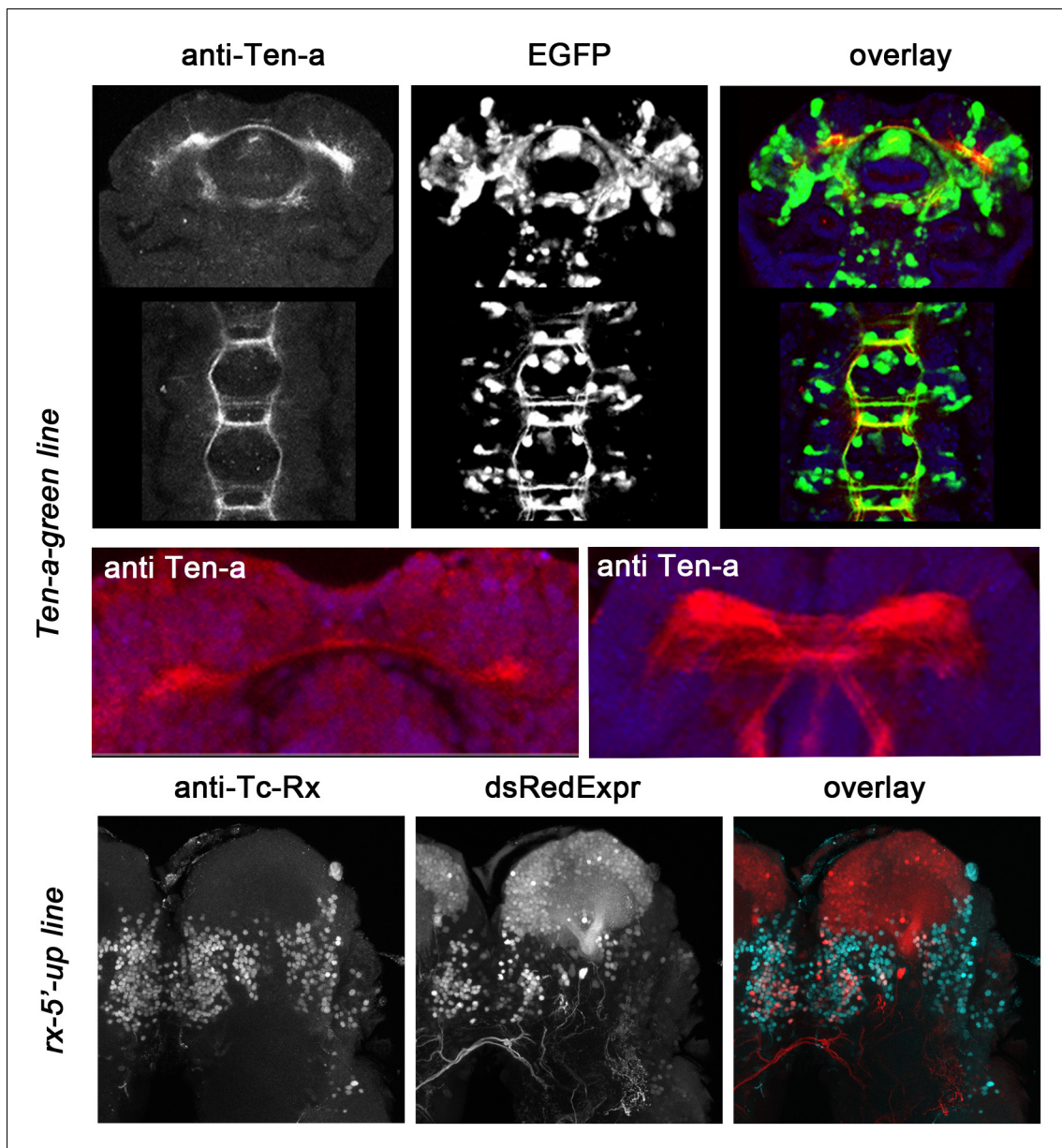
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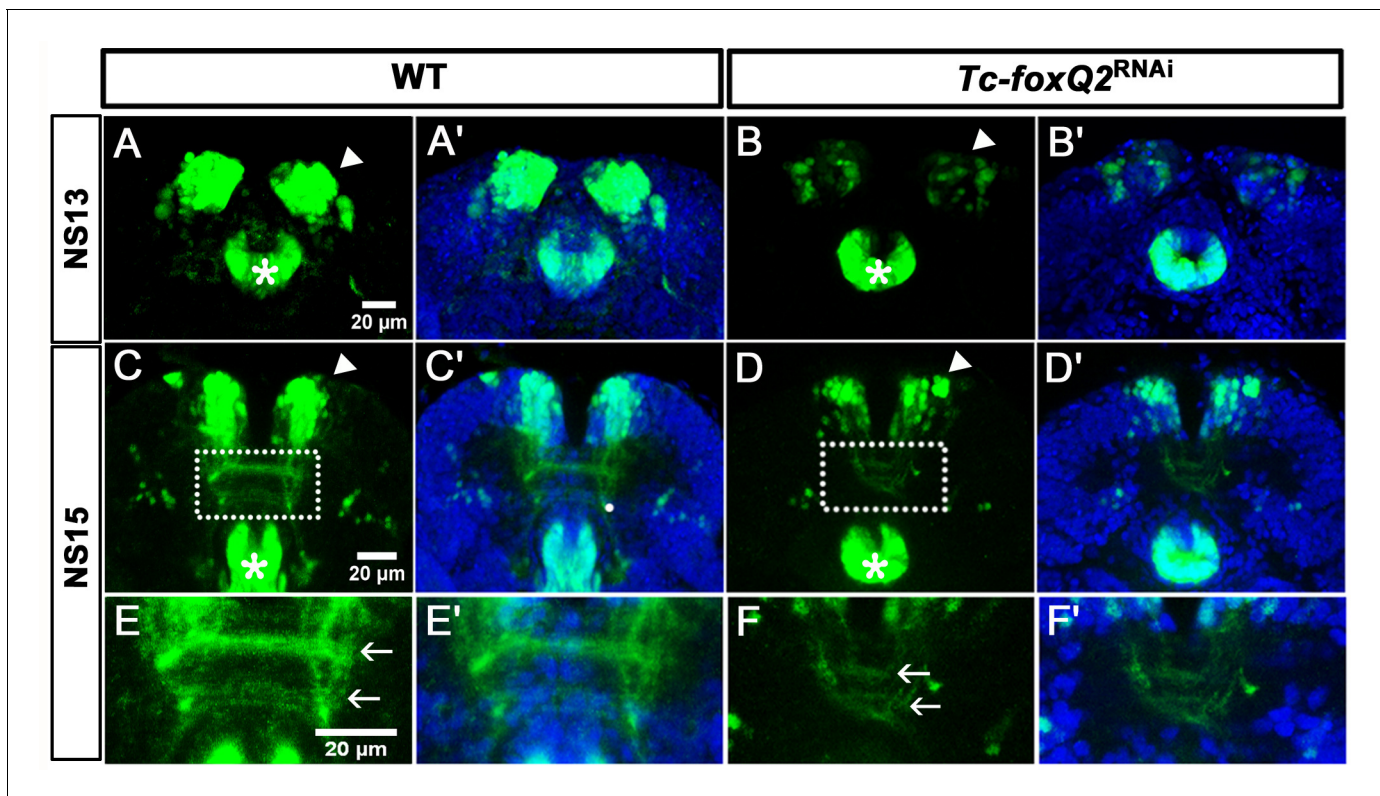
**Figure 8.** Loss of *Tc-foxQ2* function in novel imaging lines confirms the midline phenotype. (A–A'') In WT *Ten-a-green* embryos, three groups of cells are marked by EGFP: An anterior group (white circle), a posterior-lateral group (open arrowhead) and a posterior-medial group (dashed circle). The central brain primordium is marked with *Ten-a* positive fascicles projecting across the midline (white arrow in A). (B–B'') In *Tc-foxQ2* RNAi, the *Ten-a* positive projections and the number of the marked cells is reduced ( $n = 4$ ). (C–C') In WT *Tc-rx-5'-up* line, the anterior median group of cells marked by DsRed project into the central brain (white circle and white arrowhead). (D–D') In *Tc-foxQ2* RNAi, the cell number in the anterior median group is strongly reduced ( $n = 6$ ; white circle) and the marked brain commissures are absent (white arrowhead). The peripheral cells are reduced in number as well ( $n = 6$ ; compare open arrowheads in C,D).

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**Figure 8—figure supplement 1.** Characterization of imaging lines. Shown are antibody stainings (left column), signal from transgenic reporter lines (medial column) and overlay (right column). Upper panels: Co-localization of reporter protein with endogenous Ten-a protein in the *Ten-a-line*. Middle panel: anti Ten-a staining in *Tribolium* embryo marks the first midline crossing fascicle (left panel). Later, it marks several midline crossing fascicles of the central brain (right panel). Lower panels: Partial co-localization of reporter protein with endogenous protein in the *rx-5'-up line*.

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**Figure 9.** RNAi in the *foxQ2*-5'-line indicates self-regulation. (A–B') At NS13, *Tc-foxQ2* RNAi shows the strongly reduced number of marked cells in the anterior-median-*foxQ2*-cluster ( $n = 4$ ; white arrowheads) while the signal in the stomodeum appears to be unaffected (stars). (C–F') In *Tc-foxQ2* RNAi, the number of marked cells decreased significantly ( $n = 4$ ; white arrowhead). The fascicles are reduced and follow an abnormal rounded path instead of the straight line in WT (compare white arrows in E and F). Legends figure supplements.

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