

Fig. S1. Expression of seven-up-lacZ, wingless-lacZ, ming-lacZ, huckebein-lacZ and unplugged-lacZ in neuroblasts of the tail region. Flat preparations (horizontal views; anterior to the top) of mid stage 11 (St11m), late stage 11 (St11l) or early stage 12 (St12e) embryos of the indicated genotype triple-stained against different combinations of molecular markers as illustrated. Location of the NBs is indicated; NBs which can be clearly identified by marker staining(s), position and/or delamination time point are highlighted in bold letters; identified NB daughter cells are surrounded by broken lines and highlighted in bold letters; segments are indicated on the right; ML, midline. (A) Most NBs transiently express seven-up (svp)-lacZ (Doe, 1992). Therefore, svp can serve as a marker to identify NBs during different delamination time points. The big lateral S1 NBs are all present in A8 and A9. Runt (Run) is expressed in a subset of only seven NBs (Dormand and Brand, 1998). Double labelling reveals that NB5-2 and NB5-3 are present in A8 and A9. There is also one svp/Run-positive NB in A10 (NB5-3). Note the absence of svp-positive NBs in the En stripe of A9. The NB7-1 in A9 does not express svp. (B) wingless (wg)-lacZ is expressed in all row 5 NBs (Doe, 1992). Double labelling with Run shows that both NB5-2 and NB5-3 are also present in A10. NB5-1 is missing in A9 and A10. In A9, all other row 5 NBs are present. (C) ming-lacZ is expressed in many NBs in the thorax and in anterior abdomen (Doe, 1992). However, in A9 and A10 ming becomes variably expressed in only few cells. (D) Staining for Run in huckebein (hkb)-lacZ (Doe, 1992) shows NB2-1 (Run negative) and NB2-2 (Run positive) to be present in A8 and A9. (E) We could also detect these two NBs in A10 and found that all *hkb*-positive NBs of row 4 (NB4-2, NB4-3, NB4-4, which are Gsb-d negative) and five (NB5-4, NB5-5, which are Gsb-d positive) are present in A8 and A9. NB2-4 is clearly missing in A9 and A10, but is present in A8. Note the hkb and Gsb-d-positive NB in A10 (NB5-4). (F) unplugged (unpg)-lacZexpression identifies NB4-1, NB5-3 and NB5-5 (Doe, 1992) in A8 and A9, and the MNB down to A10 (double labelling with En).

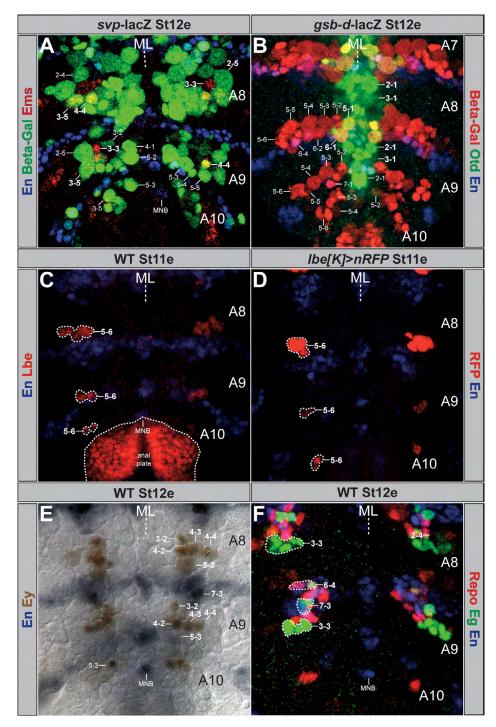


Fig. S2. Expression of Empty spiracles, Orthodenticle, Ladybird early, Eyeless and Reversed polarity in neuroblasts and their progeny of the tail region. Flat preparations (horizontal views; anterior to the top) of St11e or St12e embryos of the indicated genotype stained against different combinations of molecular markers as illustrated. Location of the NBs is indicated; NBs which can be clearly identified by marker staining(s), position and/or delamination time point are highlighted in bold letters; identified NB daughter cells are surrounded by broken lines and highlighted in bold letters; segments are indicated on the right; ML, midline. (A) The gap-gene Empty spiracles (Ems) is expressed in NB3-5, NB4-4 (and some of their progeny) and in NB3-3 (Hartmann et al., 2000). We identified these NBs down to A9. In A10, is only one Ems-positive NB, which is also positive for svp (NB3-5). (B) In the thorax, Orthodenticle (Otd) is expressed in four ventral NBs (NB2-1, NB3-1, NB5-1 and NB6-1; R.U., unpublished). All of them are found in A8. In A9, NB2-1 and NB3-1 can be identified, whereas NB5-1 and NB6-1 are missing. In A10, there is one Otd-positive NB (NB2-1), (C) Ladybird early (Lbe) is expressed in the newly formed NB5-6 and some of its daughter cells (De Graeve et al., 2004). We found these cells to be present down to A10, although their number is reduced in the most terminal segments. Note the expression in the anal plate (Jagla et al., 1997). (D) *lbe(K)-Gal4* driven RFP-expression corresponds to the pattern in C, except that expression in the anal plate is missing. (E) In the thorax, Eyeless (Ey) is expressed in a set of six different NBs per hemisegment (NBs 3-2, 4-2, 4-3, 4-4, 5-3, 7-3; R.U., unpublished). All of them are also present in A8, and all except NB7-3 are present in A9. Only one Ey-positive NB (NB5-3) is found in A10. (F) At St12e, the NB6-4-derived glial cells [co-expressing Eg. En and the glial-specific marker Reversed polarity (Repo)] are present in A8, but missing in A9. Please note that there is also one early-born glia cell (on either side) in A10 (derived from the LGB, NB5-6 or NB6-4).

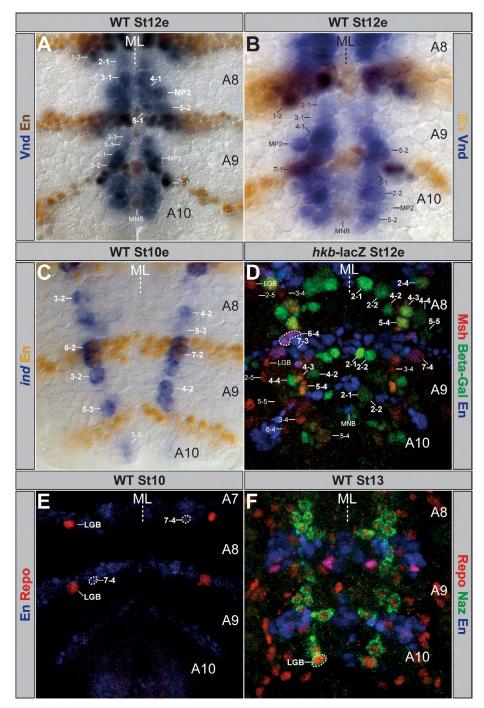
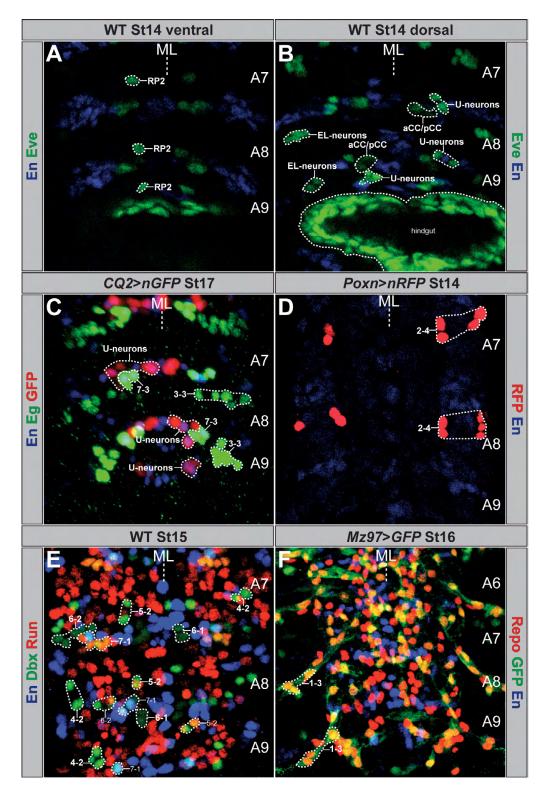


Fig. S3. Expression of columnar genes and glial-specific genes in the terminal abdominal neuromeres. Flat preparations (horizontal views; anterior to the top) of St10, St12e or St13 embryos of the indicated genotype stained against different combinations of molecular markers as illustrated. Location of the NBs is indicated; NBs which can be clearly identified by marker staining(s), position and/or delamination time point are highlighted in bold letters; identified NB daughter cells are surrounded by broken lines and highlighted in bold letters; segments are indicated on the right; ML, midline. (A-D) Expression of dorso/ventral patterning (columnar) genes. (A) Ventral nervous system defective (Vnd) is expressed in the most ventral neuroectodermal column and in the NBs that delaminate from this region (Chu et al., 1998). In A8, Vnd expression is found in ventral NBs of which NB2-1, NB3-1, NB4-1, MP2, NB5-1 and NB5-2 are clearly identifiable. (B) In A9 and A10, some NBs are missing in the ventral region. (C) In more anterior segments of the trunk, intermediate neuroblasts defective (ind) is expressed for a short period in five different NBs (Weiss et al., 1998). All of them are present in A8 (NB3-2, NB4-2, NB5-3, NB6-2, NB7-2). In A9, NB6-2 and NB7-2 are clearly missing. Only one ind-expressing NB is found in A10 (NB5-3). (D) Muscle segment homeobox (Msh) is expressed in the lateral NBs of the VNC (Isshiki et al., 1997). In A8, we could clearly identify NB2-4, NB4-3 and NB5-4 by their co-expression of hkb and NB6-4 as well as NB7-4 by co-expression of En. NB4-3 and NB5-4 are also present in A9, whereas NB2-4, NB6-4 and NB7-4 are missing in this segment. In A10, three Msh-expressing NBs are found (NB3-4, NB5-4, NB6-4). (E) At St10, the early-born glial progeny of NB7-4 can be identified down to A8, but is missing in A9 and A10. The LGB can also be identified owing to its Repo expression (Xiong et al., 1994) down to A9. (F) Nevertheless, at St13 we also identified a longitudinal glia cell in A10. This cell is located in a typical dorsal position, and appears a bit distant from the longitudinal glia in A8 and A9. Beside Repo it expresses the marker Nazgul (Naz), which is specifically expressed in longitudinal glia (von Hilchen et al., 2010).



**Fig. S4. Expression of markers for characteristic progeny cells of specific neuroblasts.** Flat preparations (horizontal views; anterior to the top) of St14-St17 embryos of the indicated genotype stained against different combinations of molecular markers as illustrated. Identified daughter cells of specific NBs are surrounded by broken lines and highlighted in bold letters; segments are indicated on the right; ML, midline. (A,B) Even skipped (Eve) is expressed in NB4-2-derived RP2 (A) (Bossing et al., 1996), in NB1-1-derived aCC and pCC (Broadus et al., 1995), NB7-1-derived U-neurons (Bossing et al., 1996) and NB3-3-derived EL-neurons (B) (Schmidt et al., 1997). All of these characteristic daughter cells are present down to A9, but missing in A10. There is also Eve expression in the hindgut (Gorfinkiel et al., 1999). (C) *CQ2*-Gal4 drives expression in the NB7-1-derived U-neurons (Landgraf et al., 2003) and confirms their existence in A8 and A9 and absence in A10. (D) *Pox neuro (Poxn)*-Gal4 (Boll and Noll, 2002) drives expression in the NB2-4 lineage (Rogulja-Ortmann et al., 2008). We found this lineage to be present in A8, but missing in A9 and A10. (E) In more anterior segments, five different NBs (NB4-2, NB 5-2, NB6-1, NB6-2, NB7-1) have been shown to give rise to Dbx-expressing neurons (Lacin et al., 2009). We found all of these typical cells in A8, whereas in A9 the cells derived from NB6-1 and NB6-2 are missing. (F) *Mz97*-Gal4 drives expression in some peripheral glia cells, which derive from NB1-3 (von Hilchen et al., 2008). These cells are identifiable down to A9, but appear to be missing in A10.

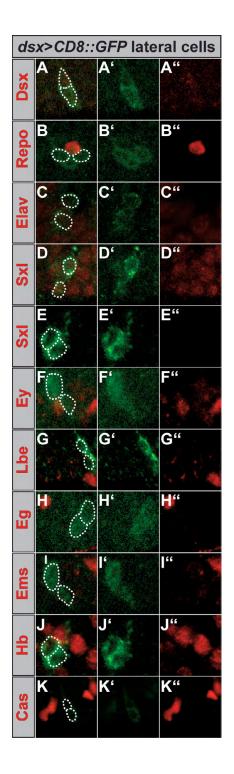
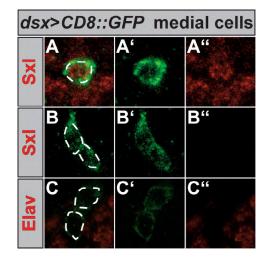


Fig. S5. Characterisation of *doublesex* (*dsx*)-Gal4-expressing lateral cells. (A-K")The lateral cells (one hemisegment) at St17 double-stained against GFP (dsx > CD8:: *GFP*; green) and molecular markers (red; as indicated); the first column shows a merge and indicates the *dsx*-positive cells by dashed lines; the second (') and the third (") columns show the separate channels.



**Fig. S6.** Characterisation of *doublesex*-Gal4-expressing medial cells. (A-C") Medial cells at St17 double-stained against GFP (dsx > CD8:: *GFP*; green) and molecular markers (red; as indicated); the first column shows a merge and indicates the dsx-positive cells by dashed lines; the second (') and the third (") columns show the separate channels.

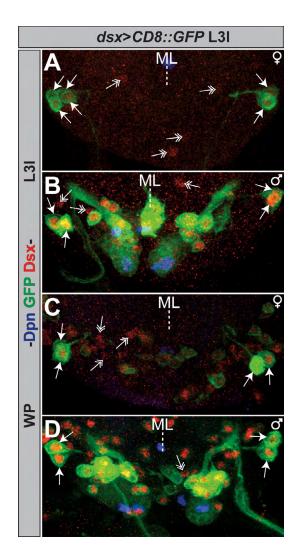


Fig. S7. Comparison of *doublesex*-Gal4 and Doublesex antibody expression in wandering larvae and white pupae. (A-D) Horizontal views of terminal neuromeres (anterior to the top) of dsx > CD8.: *GFP* females (A,C) and males (B,D) at late L3 (L31) or white pupal (WP) stages. All images are maximum projections. ML, midline. Initiator cells are marked by arrows. Some (predominantly dorsal) cells (marked by double-headed arrows) are detected by the antibody, but reveal no GFP expression (which appears with some delay).

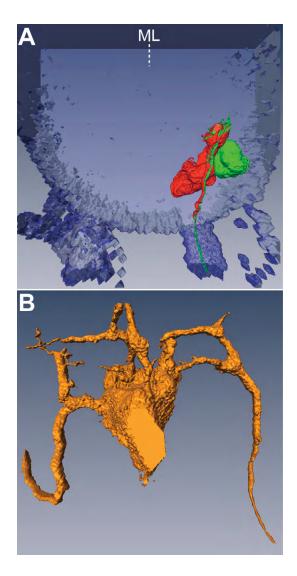


Fig. S8. 3D models of Flybow clones. 3D reconstructions generated by the Amira software. ML, midline. (A) Dorsal view of the two lateral clones shown in Fig. 5H. (B) Ventral view of the dorsally located midline clone shown in Fig. 5I.

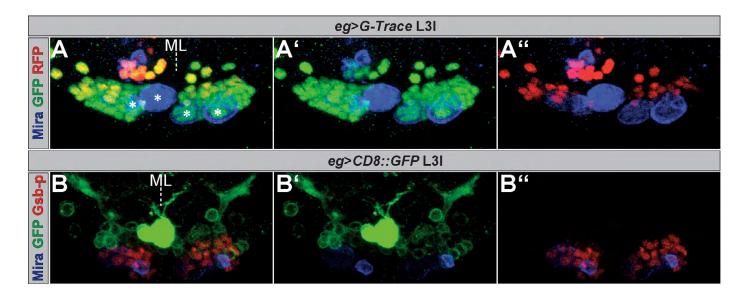


Fig. S9. Characterisation of male-specific lineages in wandering larvae. Horizontal views of terminal neuromeres (anterior to the top). ML, midline. (A) Maximum projection of eg > G-Trace L3l male (merge); sex-specific NBs are marked by asterisks. (B) Maximum projection of eg > CD8::*GFP* L3l male (merge). (A',B') Without red channel; (A'',B'') without green channel.