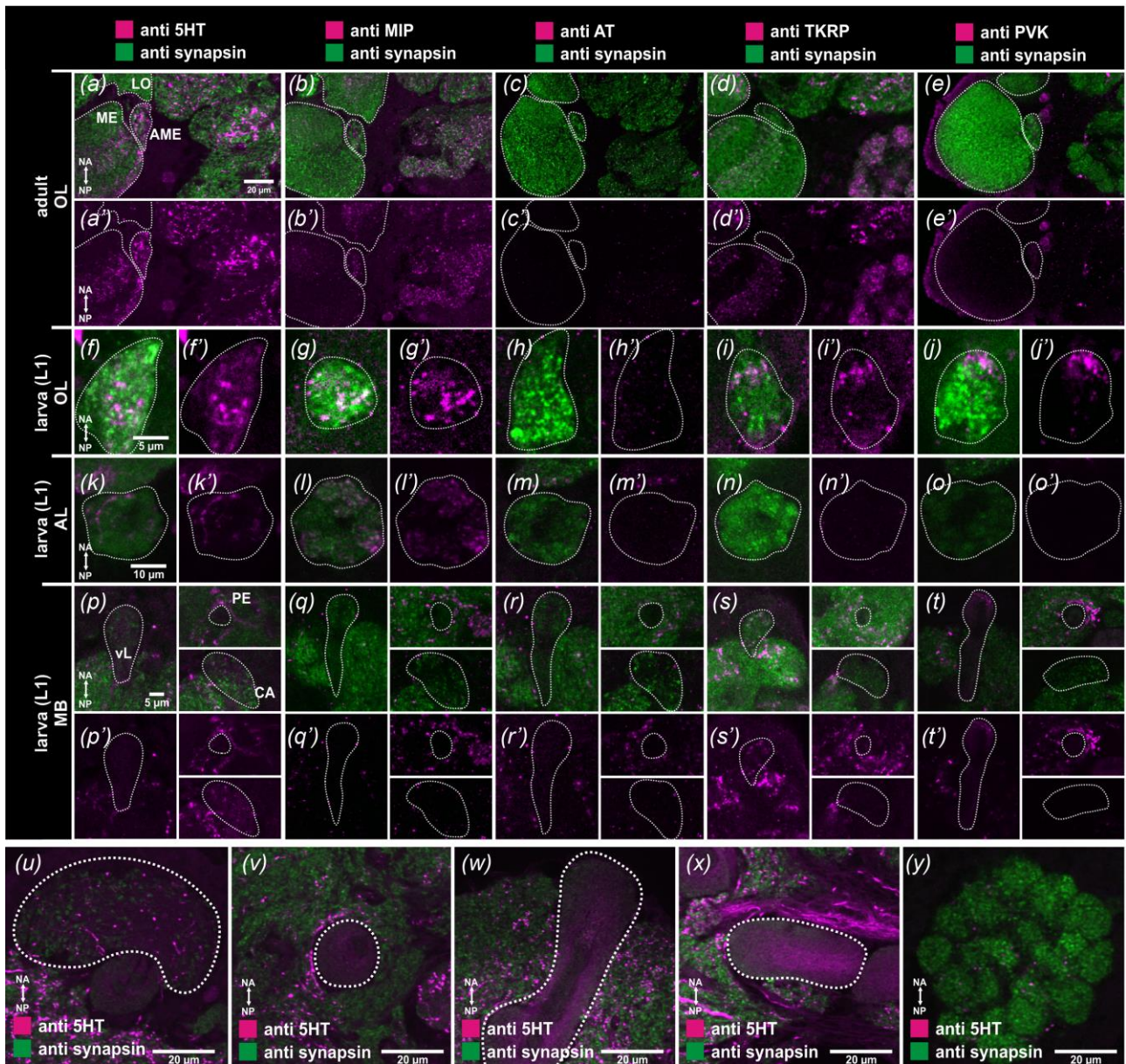
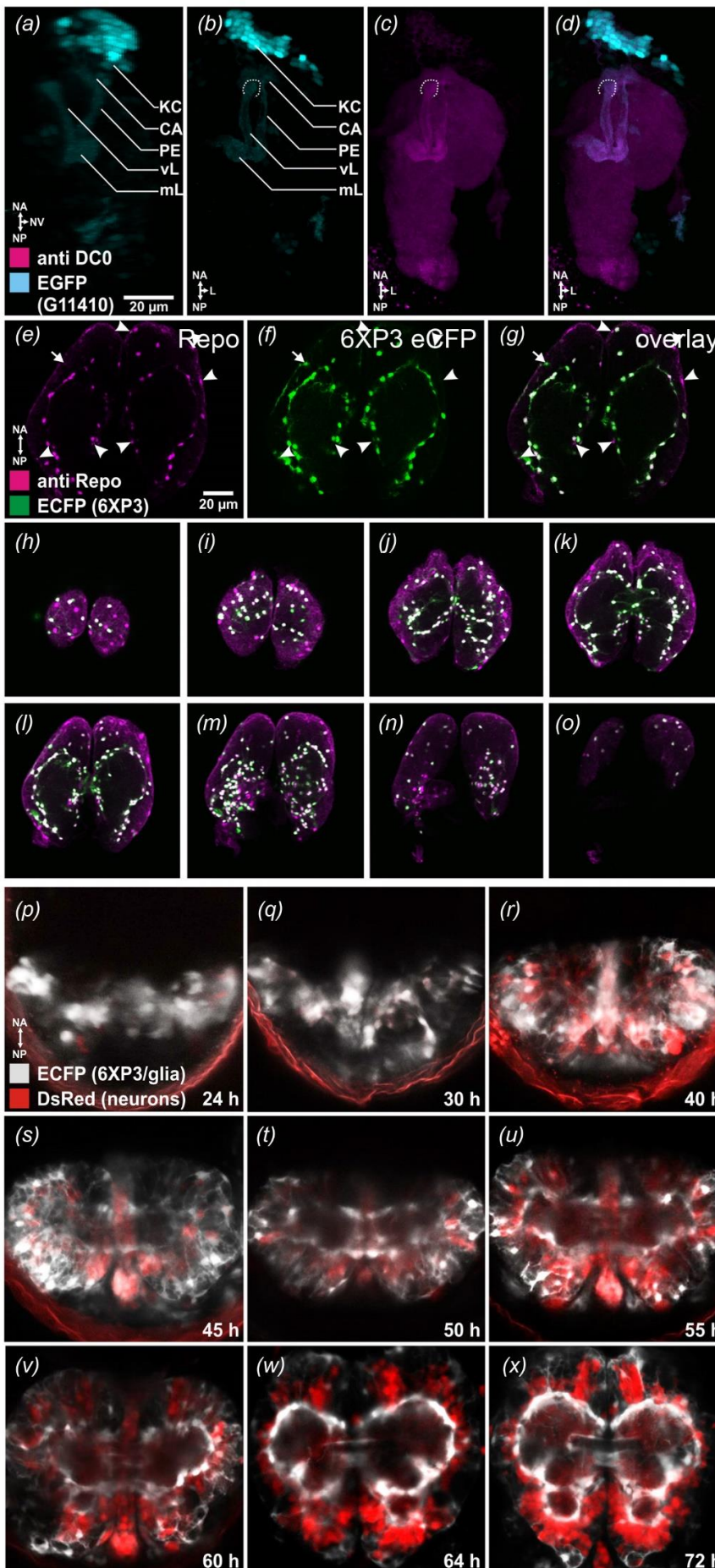


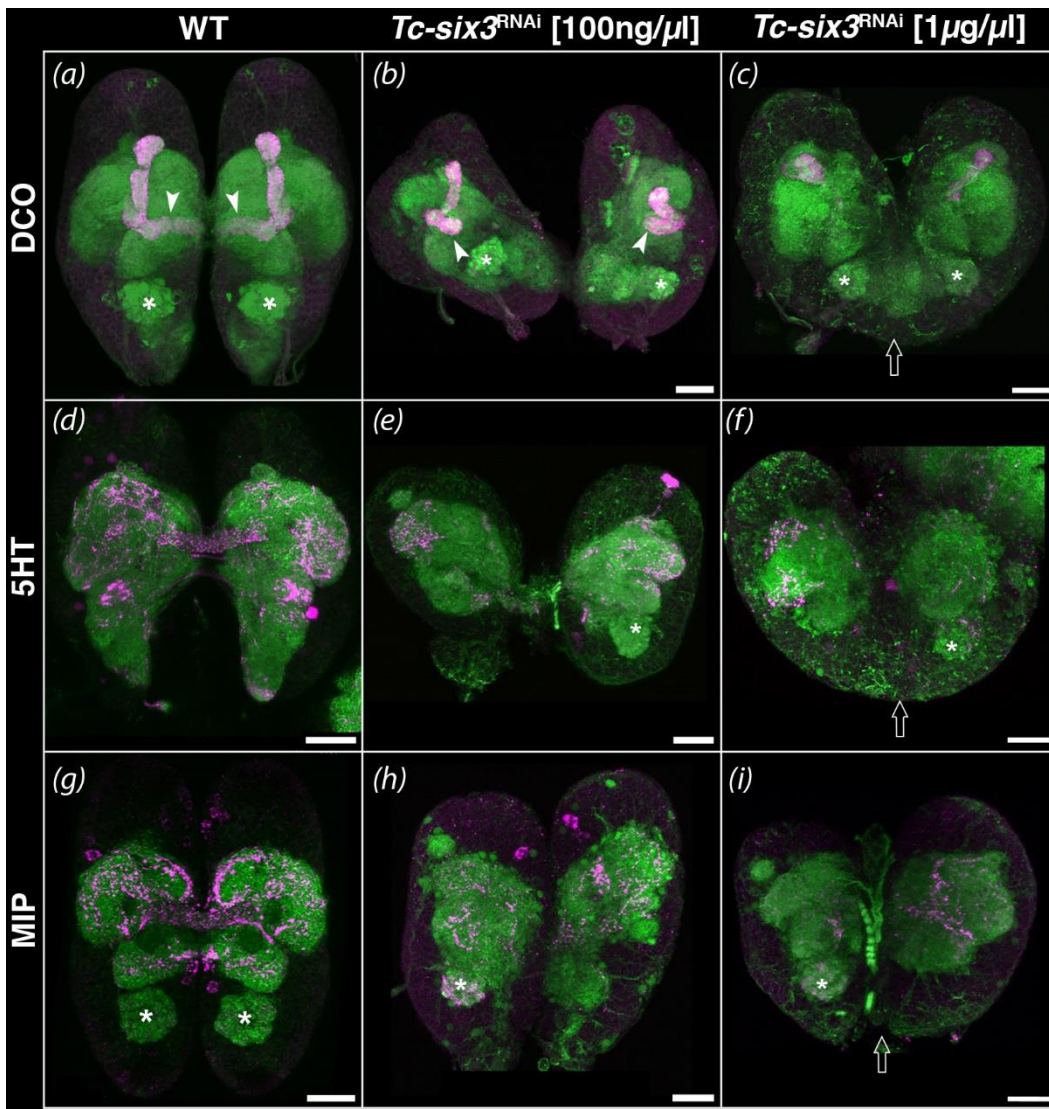
Supplementary Figures:



Supplementary figure S1 Comparison of brain areas between adults and first instar larvae of *Tribolium*

Immunostainings of adult and first instar larval (L1) brains of *Tribolium* of three of the four main neuropils: (a-j and a'-j') optic lobes (OL); (k-o and k'-o') antennal lobes (AL) and (p-t and p'-t') mushroom body (MB). An antibody against synapsin (green) was used in combination with one additional antibody against a neuromediator (5HT: serotonin, MIP: Myoinhibitory Peptide, AT: Allatotropin, TKRP: Tachykinin related peptide, PVK: Periviscerokinin) (magenta). AME: accessory medulla, CA: calyx, LO: lobula, ME: medulla, mL: median lobe, PE: pedunculus, and vL: ventral lobe. Scale bars, orientation bars (NA: neuraxis anterior and NP: neuraxis posterior), labeling of the neuropils and scale bars in the left column of the line applies for all pictures of the line. (u-y) Immunostainings of neuropils from the adult animal against 5HT and synapsin of the CA (u), the PE (v), the vL (w), the median lobe (x), and the AL (y).





Supplementary Figure S3 Analysis of the *Tc-six3* phenotype by immunohistochemistry

(a,d,g) L1 wildtype brains stained for DCO (a), serotonin (5HT) (d), and myoinhibitory protein (MIP) (g). (b,c,e,f,h,i) Brains of late *Tc-six3* RNAi knock-down embryos. Anterior is up and phalloidin staining is shown in green in all panels. In mild RNAi treatments (100 ng/μl), the mushroom bodies are still present but the median lobes do not touch at the midline (compare location of arrowheads in (a,b)). The CBU is marked by serotonin and MIP, but is absent in RNAi embryos (e,h). With higher RNAi concentrations (1 μg/μl), the phenotype gets stronger and the midline of the brain becomes more severely affected (open arrow in (c,f,i)). Scale bars depict 20 μm.