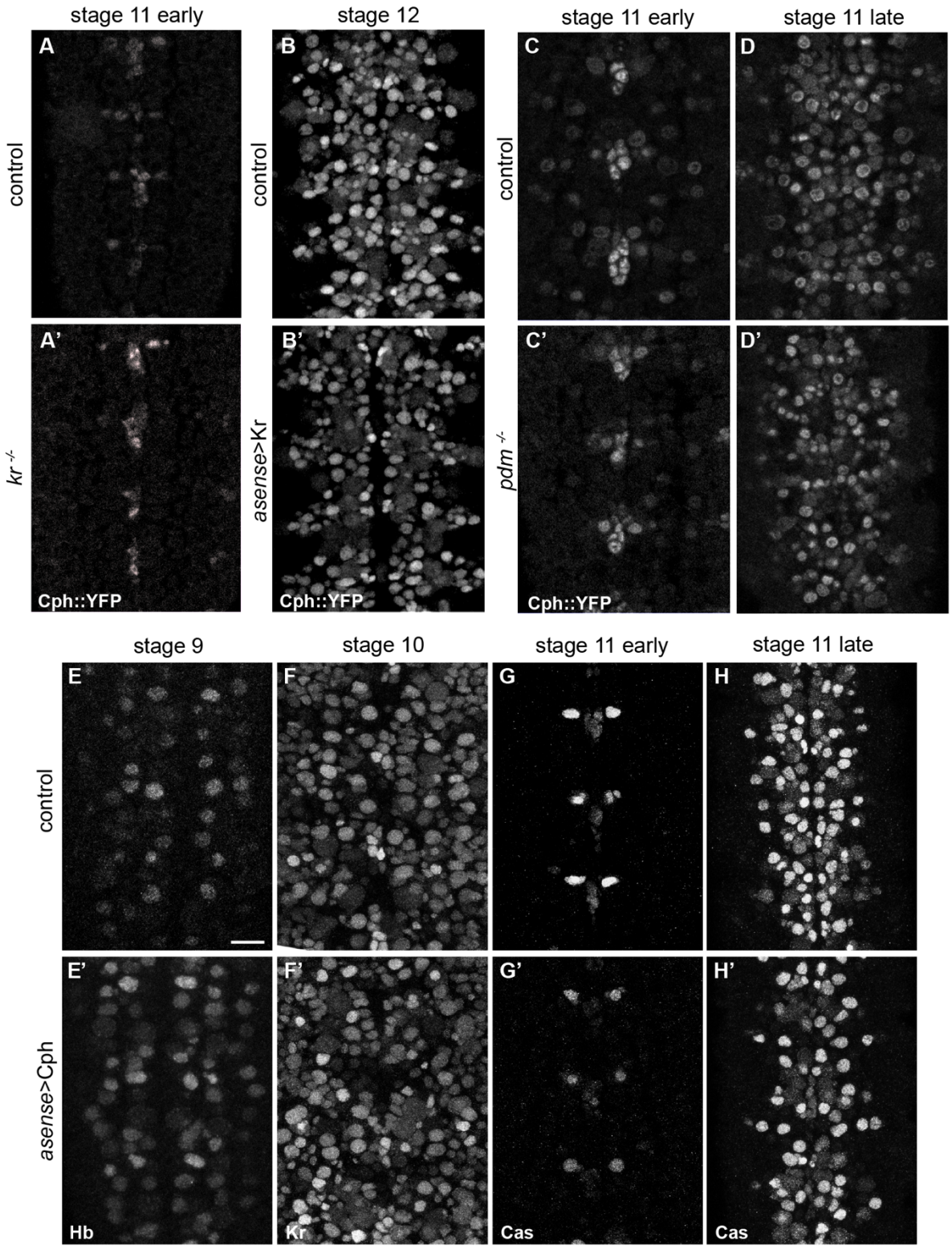

Figure 1

Fig. S1. Early misexpression of Cph does not interfere with temporal cascade progression.

(A-B') Neither loss of Kr function ($kr^{1}kr^{CD}$) nor misexpression of Kr impacts the timing of Cph. (C-D') Loss of *pdm* function (Df(2L)*Pdm*) does not impact Cph expression. Ventral view of the VNC of wild type embryos and embryos misexpressing *cph* (*asense*-GAL4 driving UAS-*cph*) shows no change to (E-E') Hb, (F-F') Kr and (G-H') Cas timing. Scale bars represent 20μm. For each condition, n=6 embryos were observed from 3 independent experiments.

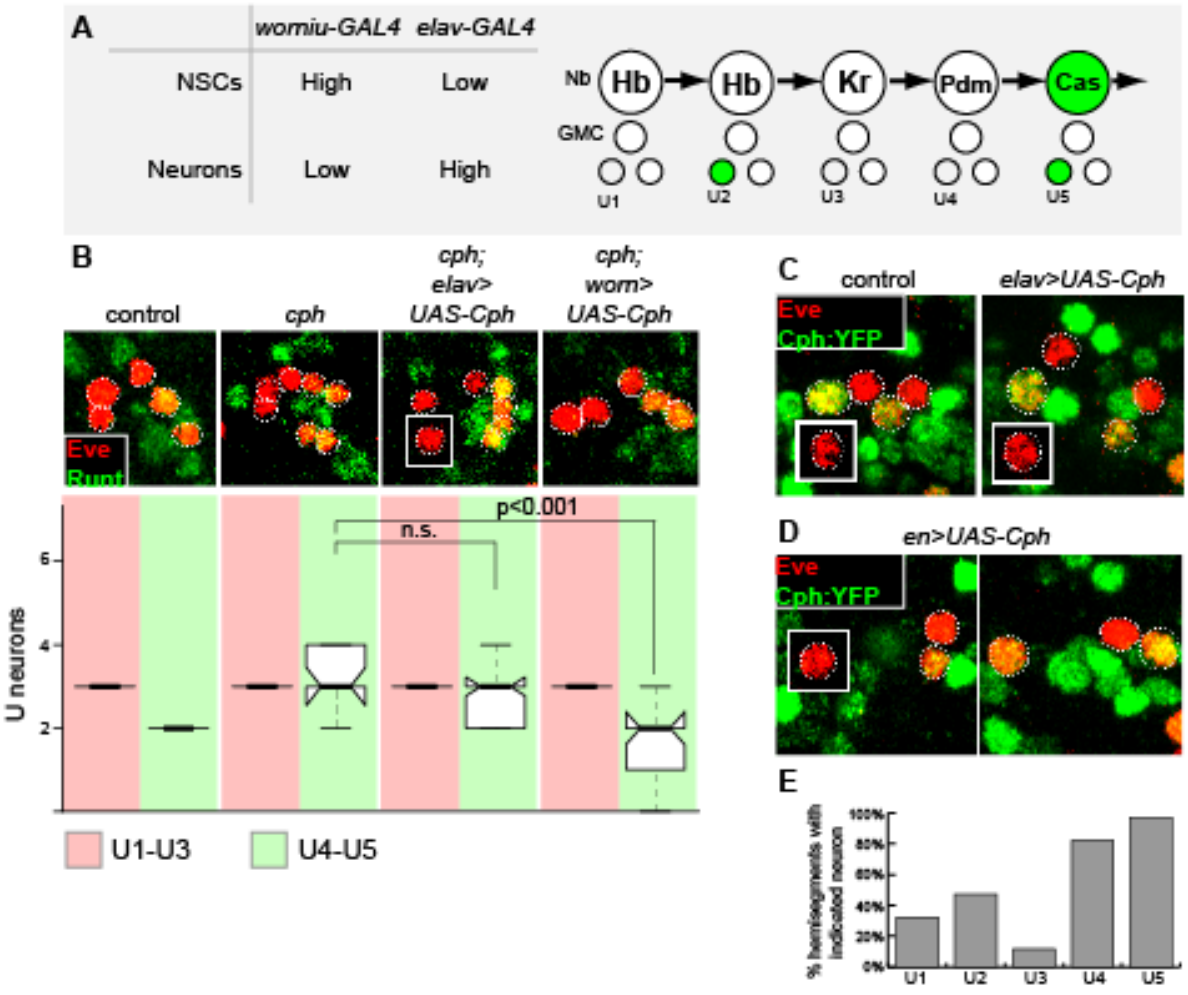

Figure 2

Fig. S2. Cph acts in neural stem cells to regulate temporal identity.

(A) Model of the first five divisions of the NB7-1 lineage. *worniu*-GAL4 and *elav*-GAL4 drive expression preferentially in the neural stem cell and in neurons, respectively. (B) Rescue analysis of *cph* U neuron phenotype. UAS-*cph* was driven in *cph* mutants using *worniu*-Gal4 or *elav*-Gal4. Whereas *worniu*-GAL4 rescued the U neuron phenotype nearly completely, *elav*-GAL4 had no effect. Error bars represent standard deviation. n=6 embryos from three independent experiments. (C) Analysis of U neurons misexpressing Cph . Cph::YFP is expressed from the endogenous promotor and is present in U2 and U5. Misexpression of Cph in neurons using *elav*-GAL4 does not alter the pattern of Cph::YFP expression. (D)

Misexpression of Cph using *engrailed*-GAL4 interferes with generation of U1, U2 and U3. Although *cph* is normally expressed in U2, misexpression of UAS-*cph* in the neural stem cell interferes with U2 generation. Loss of U1 versus U2 was not favoured in this misexpression experiment. (E) The percentage of hemisegments that have U1-U5 neurons. n=5 embryos.

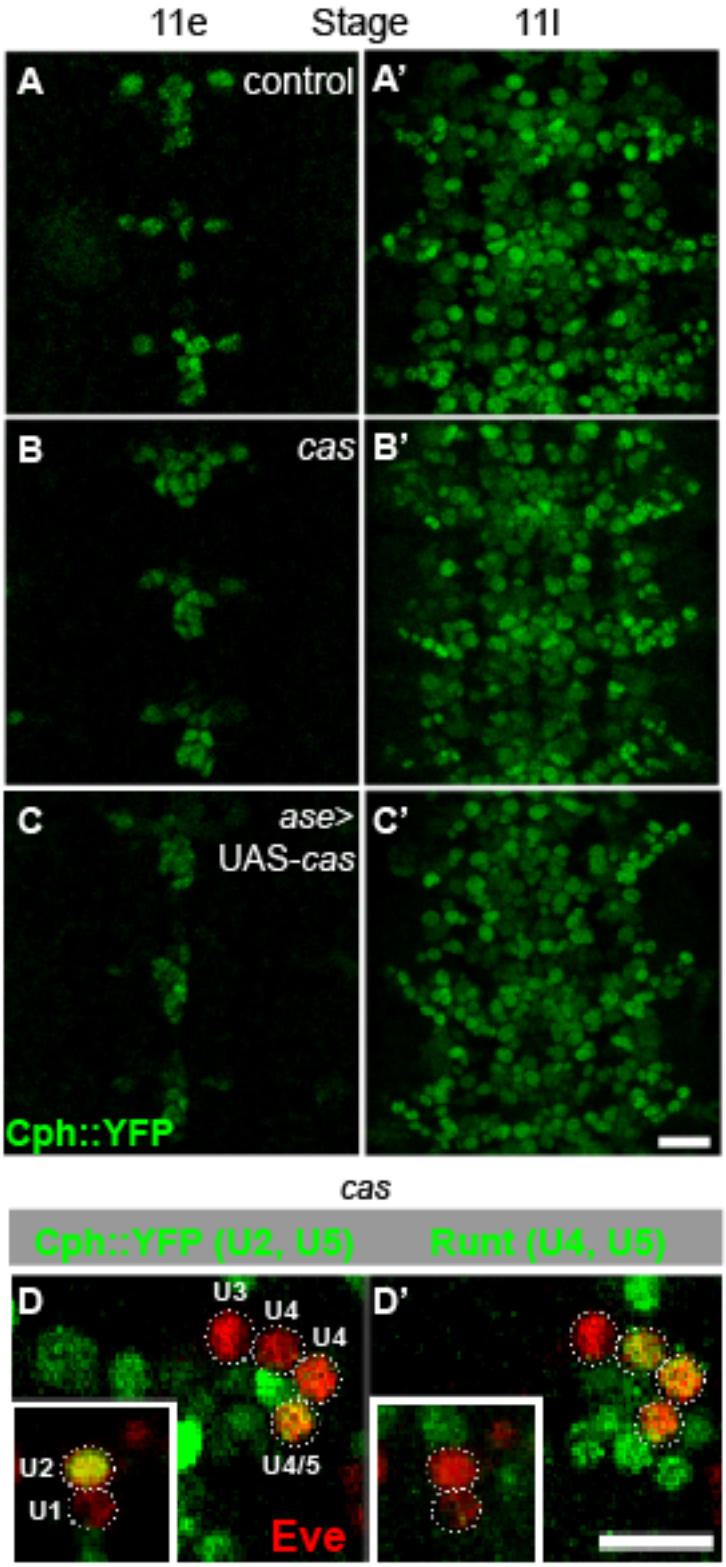

Figure 3

Fig. S3. Castor does not regulate *cph* expression.

(A-C') Ventral view of neural stem cell layer of embryos from early or late stage 11, stained for Cph::YFP expression (green). Ectopic Castor expression (B) was driven with *asense*-GAL4. (D-D') Maximum intensity projection of Eve positive (red) U neurons from individual hemisegments from stage 16 cas^{24} mutant embryos. Expression of Cph::YFP (D) and Runt (D') from the same hemisegment was used to identify individual U neuron subtypes. cas^{24} mutant hemisegments contain excess U4 neurons, but also contain a Cph and Runt positive U neuron that at least partially corresponds to U5 fate. Scale bar represents 20μm for A-C' and 10μm for D-D'. For each condition, n=6 embryos were observed from three independent experiments.

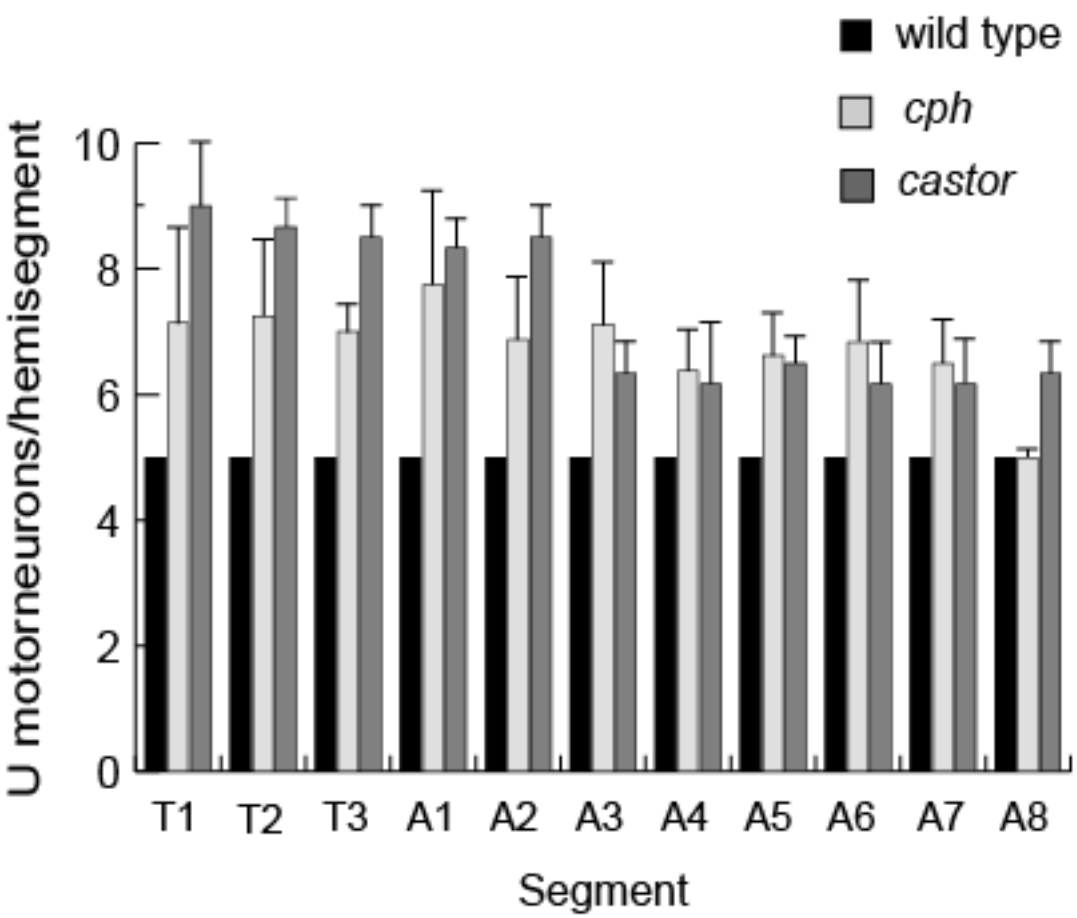

Figure 4

Fig. S4. The *cph* U neuron phenotype is not segment specific.

U neurons per hemisegment were scored based on segment identity. *cas*^{24} mutants display an excess U neuron phenotype that is more severe in abdominal segments. However, in *cph* mutants, the excess neuron phenotype did not depend on segment identity. Error bars show standard deviation, n=5 embryos per genotype from 3 independent experiments.

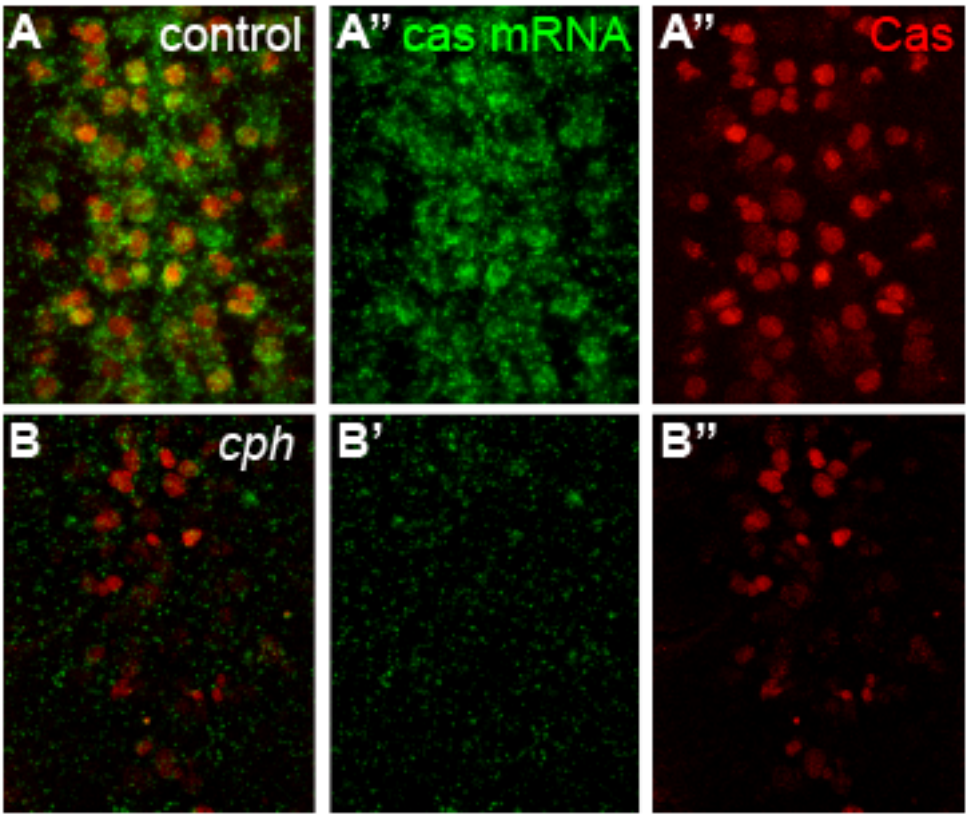

Figure 5

Fig. S5. Cph **regulates Cas transcription.**

Wild type (A) or *cph* (B) stage 12 embryos. *in situ* hybridisation against *castor* mRNA (green) and Castor antibody staining (red). Ventral view of the neural stem cell layer.

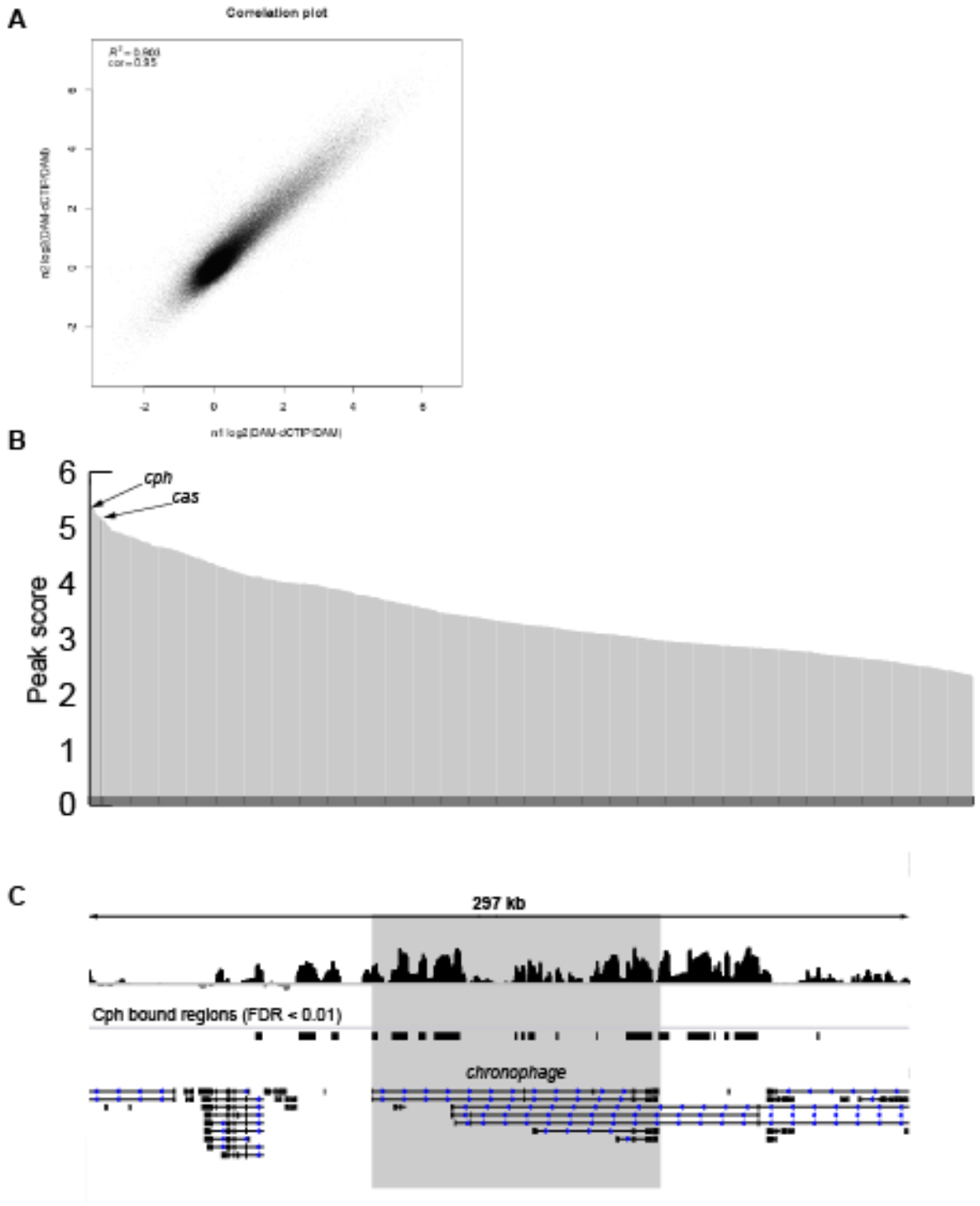

Figure 6

Fig. S6. Targeted DamID to detect Cph genomic binding sites.

(A) Correlation plot of two biological replicates of Cph Targeted DamID experiments. (B) Rank order chart of all 1624 target genes bound by Cph. The *cph* peak score ranked 2/1624 and *cas* ranked 20/1624. (C) Binding profile of the *cph* locus.

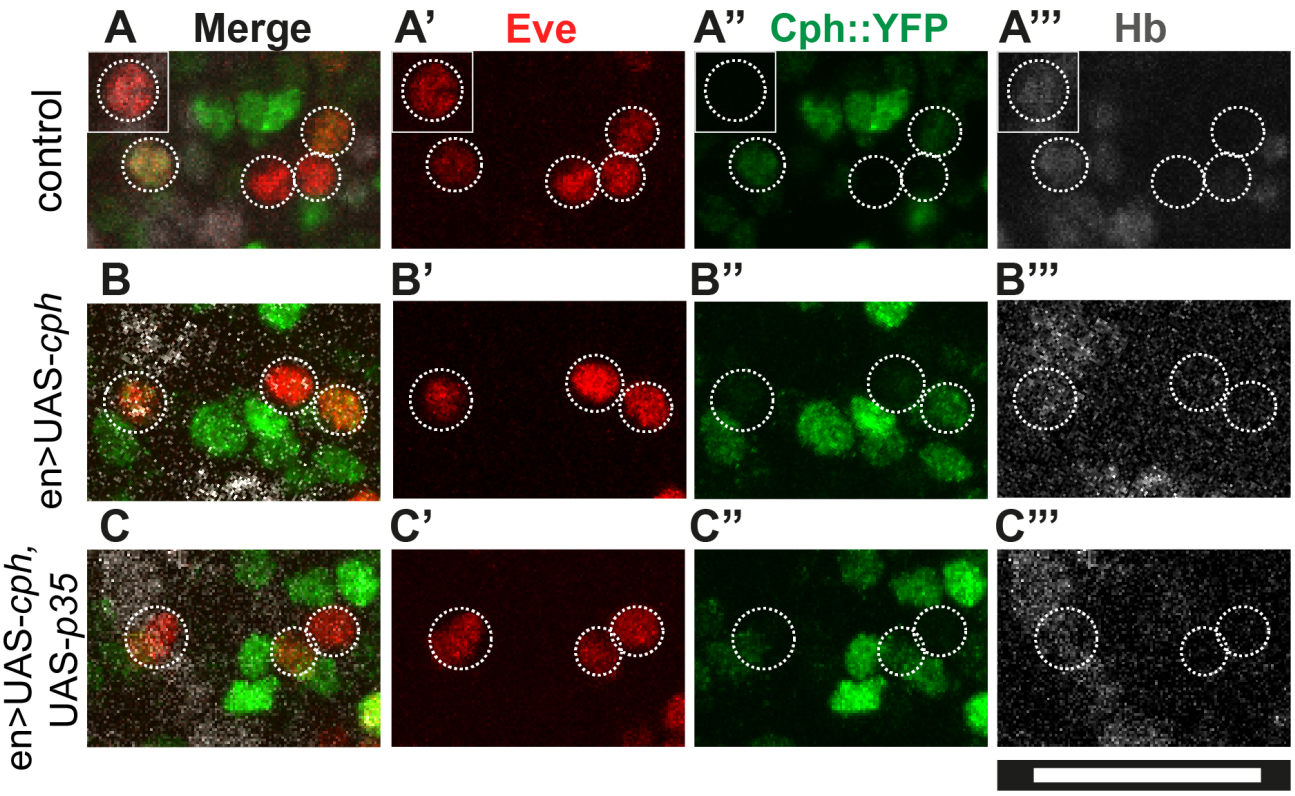

Fig. S7. Preventing apoptosis does not rescue the loss of U1-U3 neurons upon Cph misexpression.

(A-A'") Control embryos have U1-U5 neurons, of which U1-2 are Hb-positive. U2 and U5 express Cph::YFP. (B-B'") Misexpression of Cph (using *engrailed*-GAL4) results in a loss of U1-U3 neurons (U1/U2 are alternately repressed). (C-C'") Co-expression of UAS-Cph and UAS-p35 (with *engrailed*-GAL4) does not rescue the loss of U1-U3 neurons. Scale bars represent 20μm. n=5 embryos per genotype from 3 independent experiments.

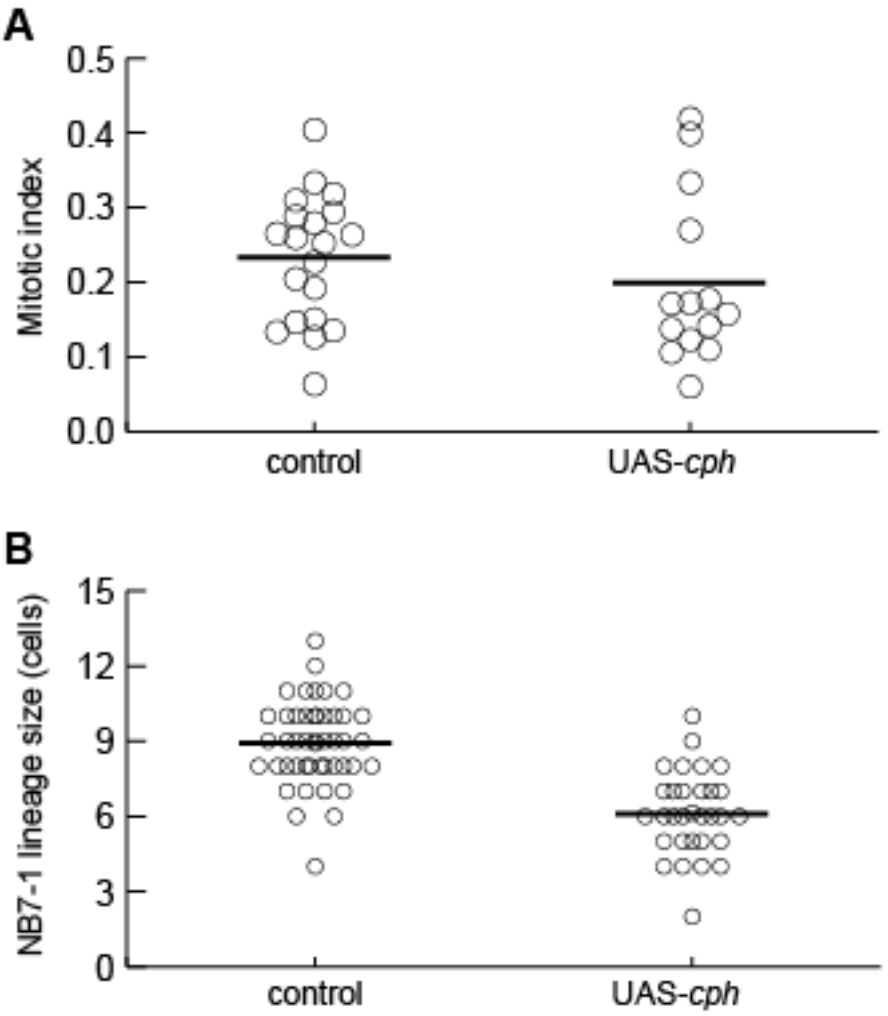

Figure 7

Fig. S8. Misexpression of Cph has variable affects on the NB7-1 lineage. (A) Mitotic index was scored in stage 9 and 10 NSCs, identified by Dpn and phospho-Histone H3 staining. Each data point represents an individual embryo obtained from 3 independent experiments. Approximately 100 NSCs were scored per embryo. No significant difference was observed between control embryos and *cph* misexpression embryos (*asense*-GAL4 was used to drive UAS-*cph*). (B) NB7-1 lineage size at stage 12 was scored using a NB7-1 lineage tracer (NB7-1 split GAL4). Lineage size after stage 12 was not scored as the expression of the lineage tracer became inconsistent. ($p<0.001$; Welch's t-test)

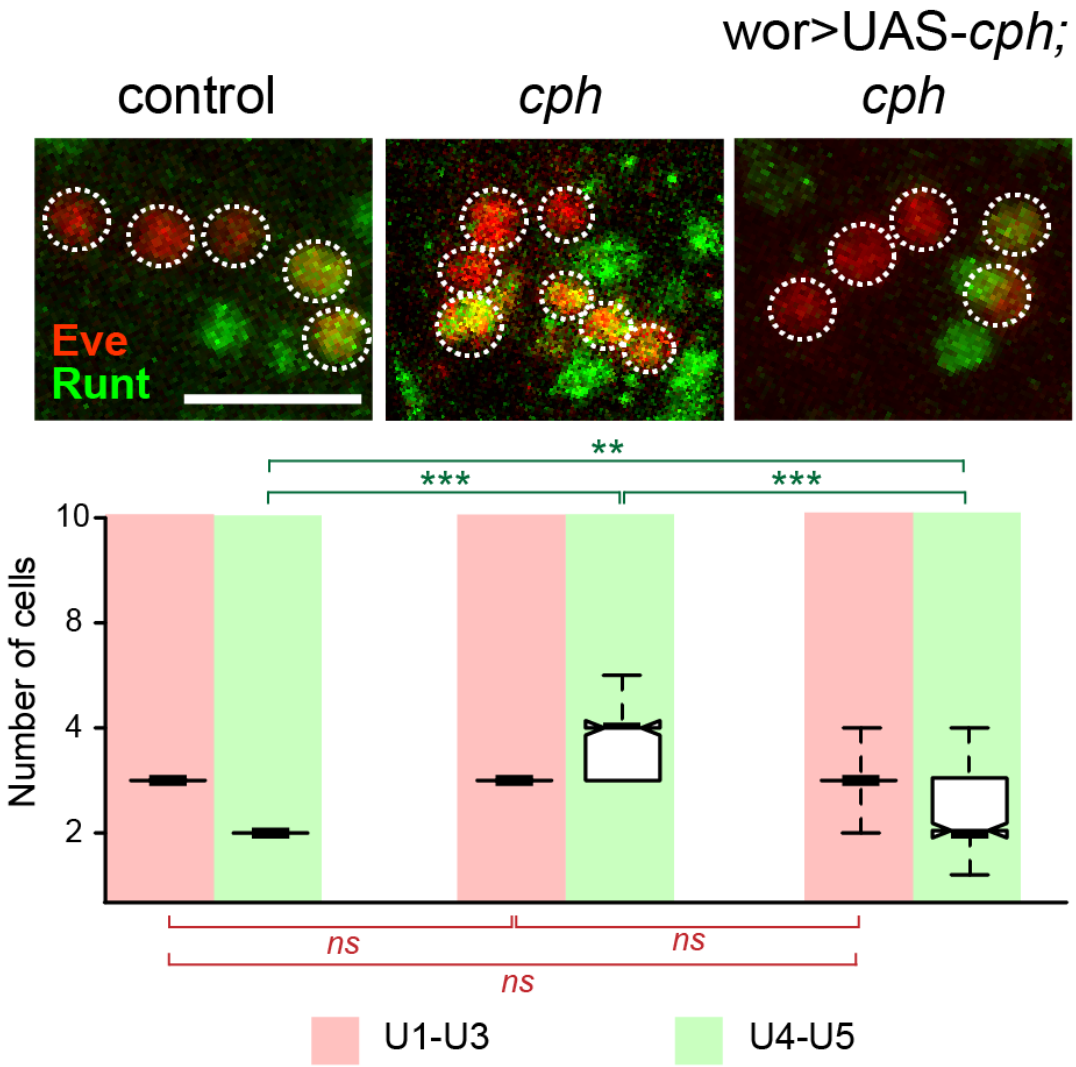

Fig. S9. UAS-Cph rescues the *cph* mutant.

Using *wor*-GAL4 to drive expression of UAS-Cph in a *cph* mutant rescues the number of U4-U5 neurons in the NB 7-1 lineage. In *cph* mutants there is an excess number of U4-U5 neurons but this number is reduced when Cph is expressed in NSCs. Scale bar represents 20µm; n= 6 embryos from 3 independent experiments. Mann-Whitney Test was used to test for statistical significance between the experimental condition and control (***, $p<0.001$; **, $p<0.01$). Plots represent median values (middle bars) and first to third interquartile range (boxes); whiskers indicate maximum and minimum values.