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RESEARCH ARTICLE

Figures and figure supplements

Steroid hormone induction of temporal gene expression in *Drosophila* brain neuroblasts generates neuronal and glial diversity

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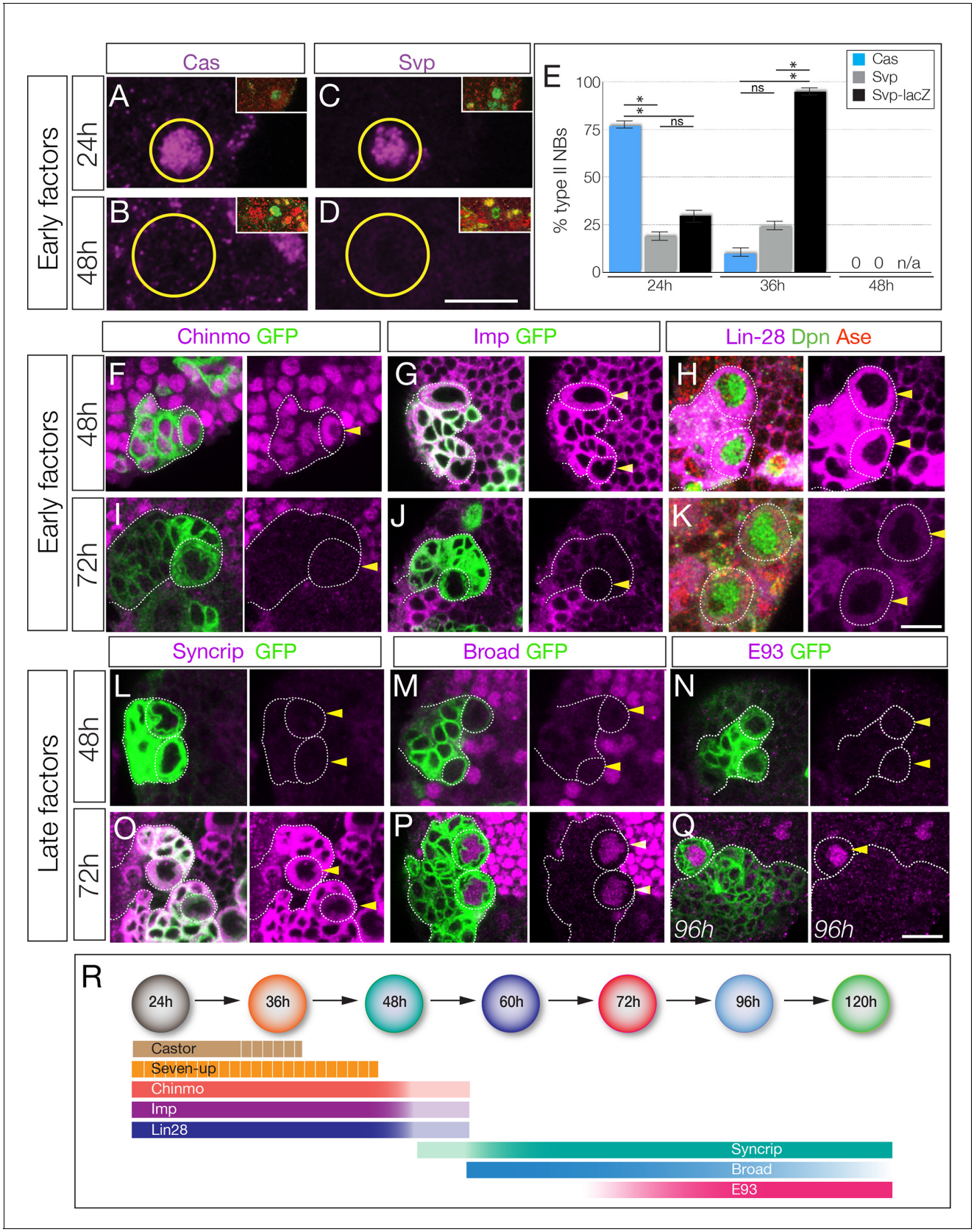

Figure 1. Identification of temporally expressed proteins in larval type II neuroblasts. (**A–E**) Cas and Svp are expressed from 24–36 hr (**A**,**C**,**E**) but not at 48 hr (**B**,**D**,**E**). Neuroblasts, outlined. (**F–K**) Early factors. Chinmo, Imp, and Lin-28:GFP (Lin-28) are detected in neuroblasts at 48 hr but not at 72 hr. (**L–Q**)

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Figure 1 continued

Late factors. Syncrip, Broad, and E93 are not detected in neuroblasts at 48 hr but are present at 72 hr or 96 hr. (**R**) Summary of temporal factor expression. Dashed bars indicate asynchronous expression during the indicated temporal window. Gradients indicate graded change in expression levels. In all panels, temporal factors are in magenta, and type II neuroblasts are identified by *wor-gal4 ase-gal80 UAS-mcd8:GFP* transgene expression (GFP, green, outlined) or as Dpn+ Ase- (green/red, respectively). Arrowhead, neuroblasts. For each panel n > 10 neuroblasts scored. Scale bar, 10 μm.
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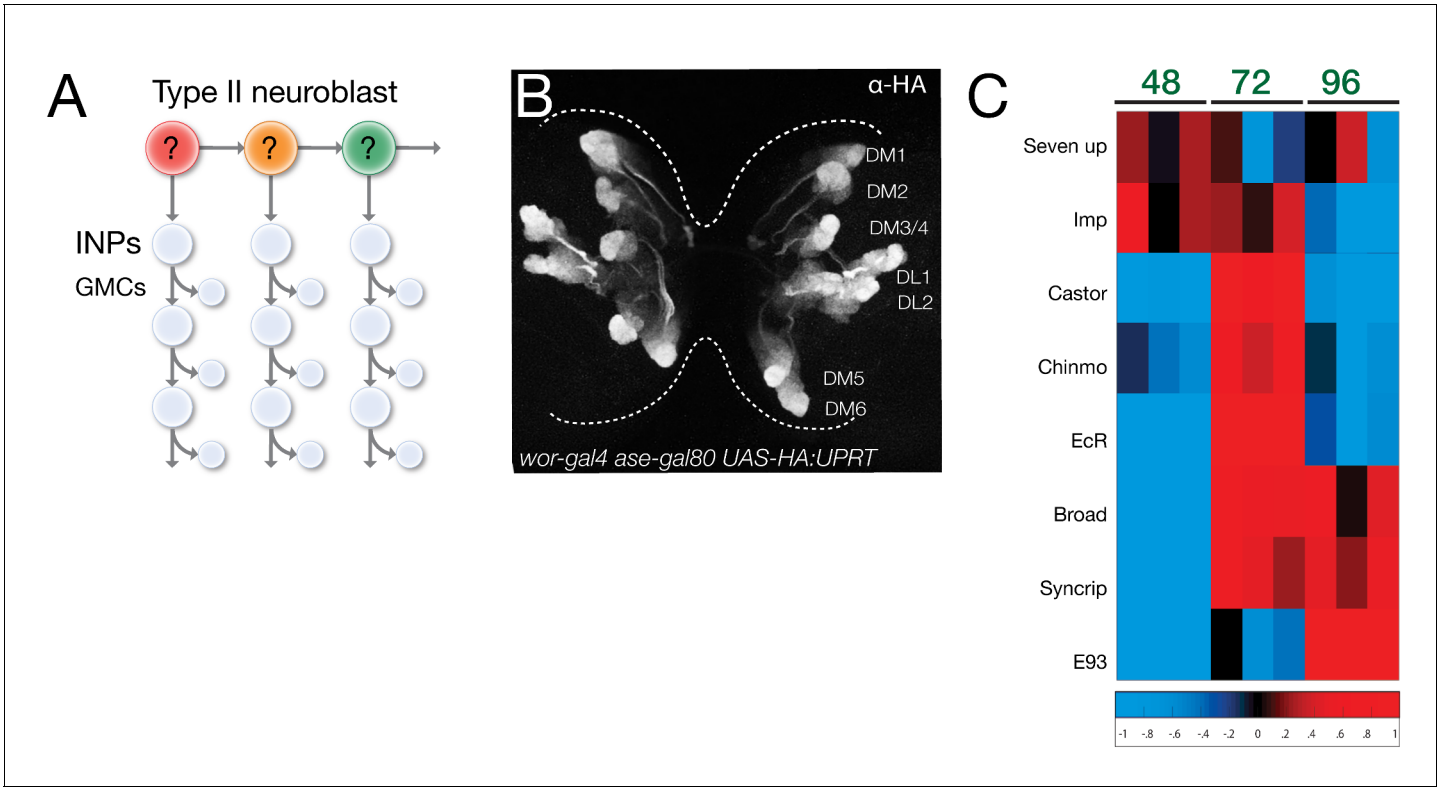

Figure 1—figure supplement 1. TU-tagging to identify temporally expressed genes in type II neuroblasts and their progeny. (**A**) Type II neuroblast lineage schematic, showing young or old neuroblast progeny markers. (**B**) Expression of HA:UPRT specifically in type II neuroblasts and their progeny. Scale bar, 50 μm. (**C**) Heat map of showing genes differentially expressed at least one developmental stage. Each column is an independent biological replicate; three per timepoint. Red, high expression; blue low expression. Note that elevated levels of Cas and Chinmo at 72 hr are likely due to the large number of neuroblast progeny expressing each gene; by 72 hr Cas and Chinmo proteins are undetectable in type II neuroblasts (***Figure 1***).
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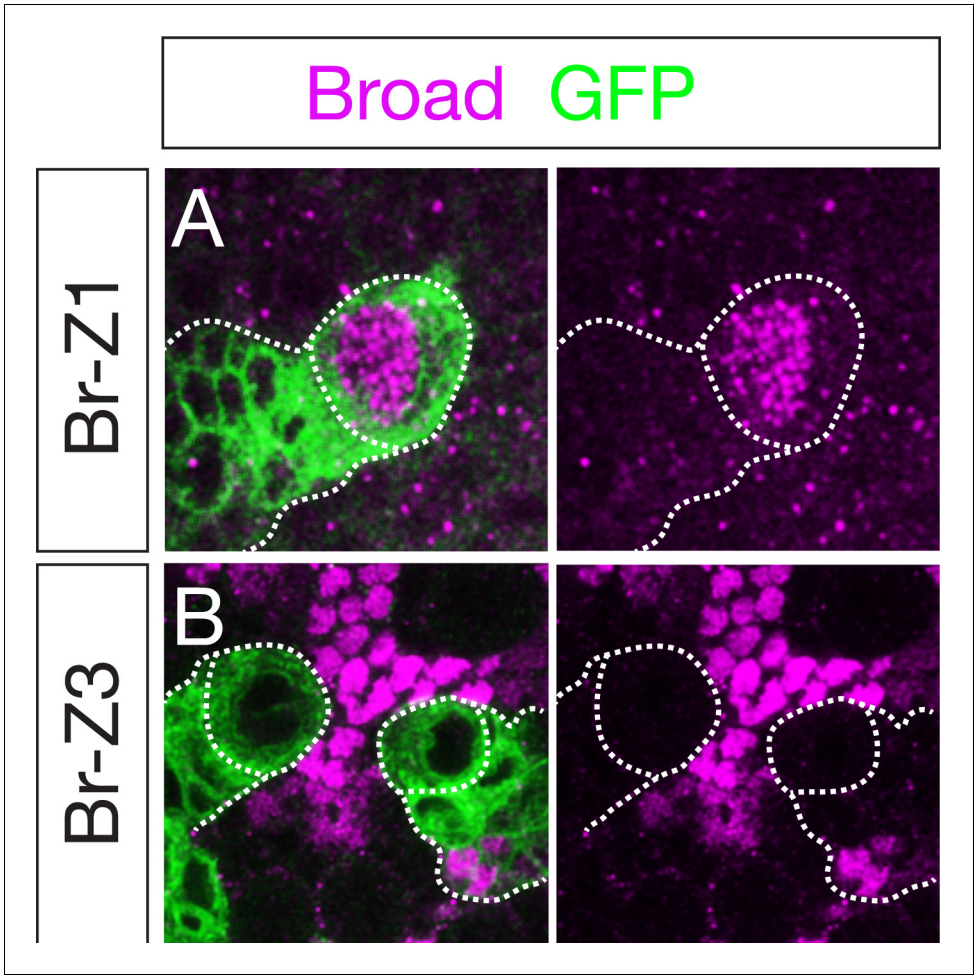

Figure 1—figure supplement 2. Broad-Z1 but not Broad-Z3 is expressed in type II neuroblasts. (**A**) Broad-Z1 (magenta) is detected in type II neuroblasts (dashed circle) at 96 hr. (**B**) Broad-Z3 (magenta) is not detected in type II neuroblasts (dashed circle) at 96 hr. In all panels, type II neuroblast lineages are marked by *wor-gal4 ase-gal80 UAS-cd8:GFP* expression (green).

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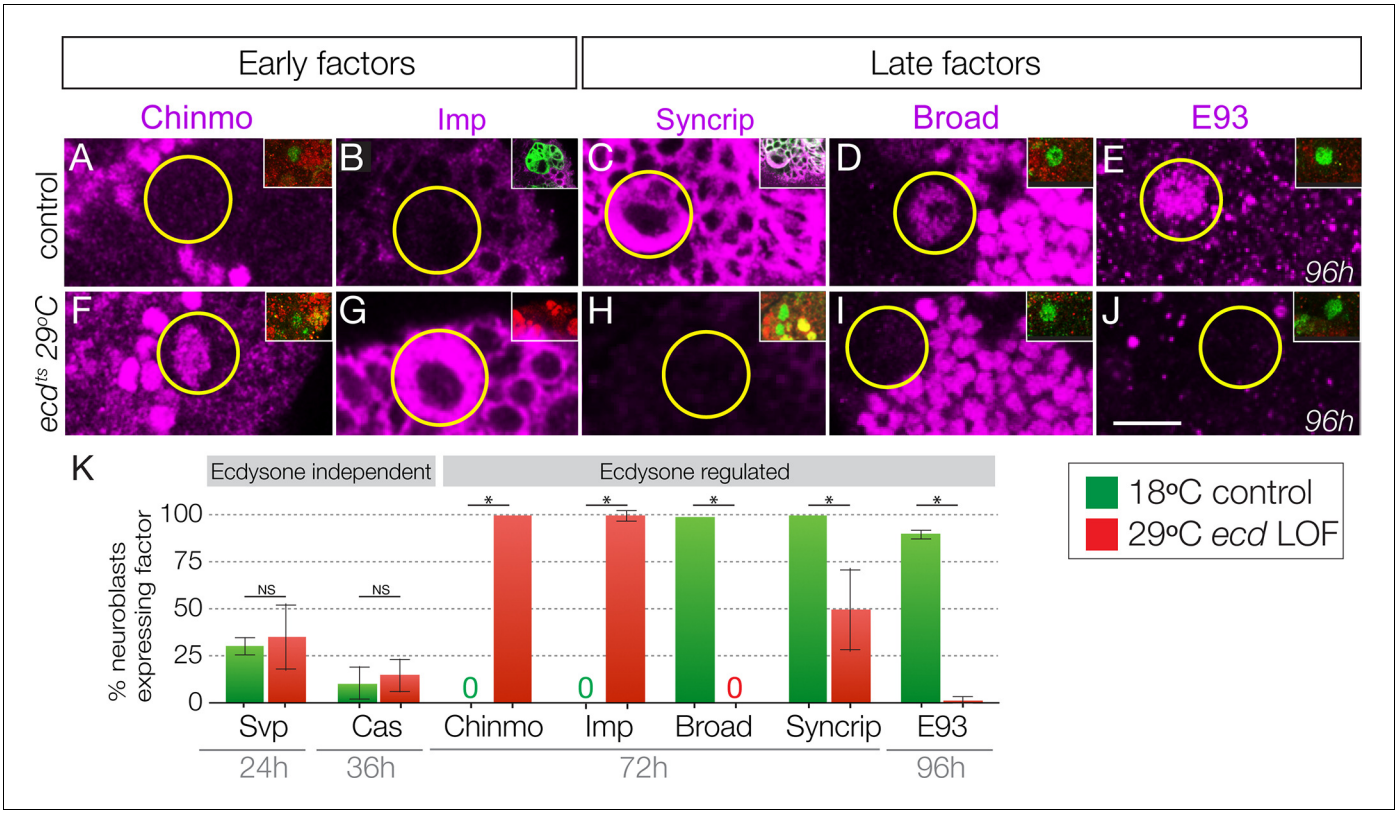

Figure 2. Ecdysone hormone is required for the early-to-late temporal factor transition. (**A–E**) Control *ecd*^{ts}/*deficiency* larvae at 18°C show normal temporal factor expression in type II neuroblasts (circled): the early factors Chinmo and Imp are off at 72 hr (**A–B**) and the late factors Syncrip, Broad, and E93 are on at 72 hr and 96 hr (**C–E**). (**F–J**) Loss of ecdysone in *ecd*^{ts} */deficiency* larvae at 29°C shows failure to down-regulate the early factors Chinmo and Imp (**F–G**) and failure to activate the late factors Syncrip, Broad and E93 (**H–J**) in type II neuroblasts (circled). (**K**) Quantification. n > 10 for each bar. Asterisk, p<0.003. In all panels, times are adjusted to the equivalent larval stage at 25°C, type II neuroblasts are identified as Dpn+ Ase- or large cells expressing *wor-gal4 ase-gal80 UAS-mcd8:GFP* (green in insets). Scale bar, 10 μm.

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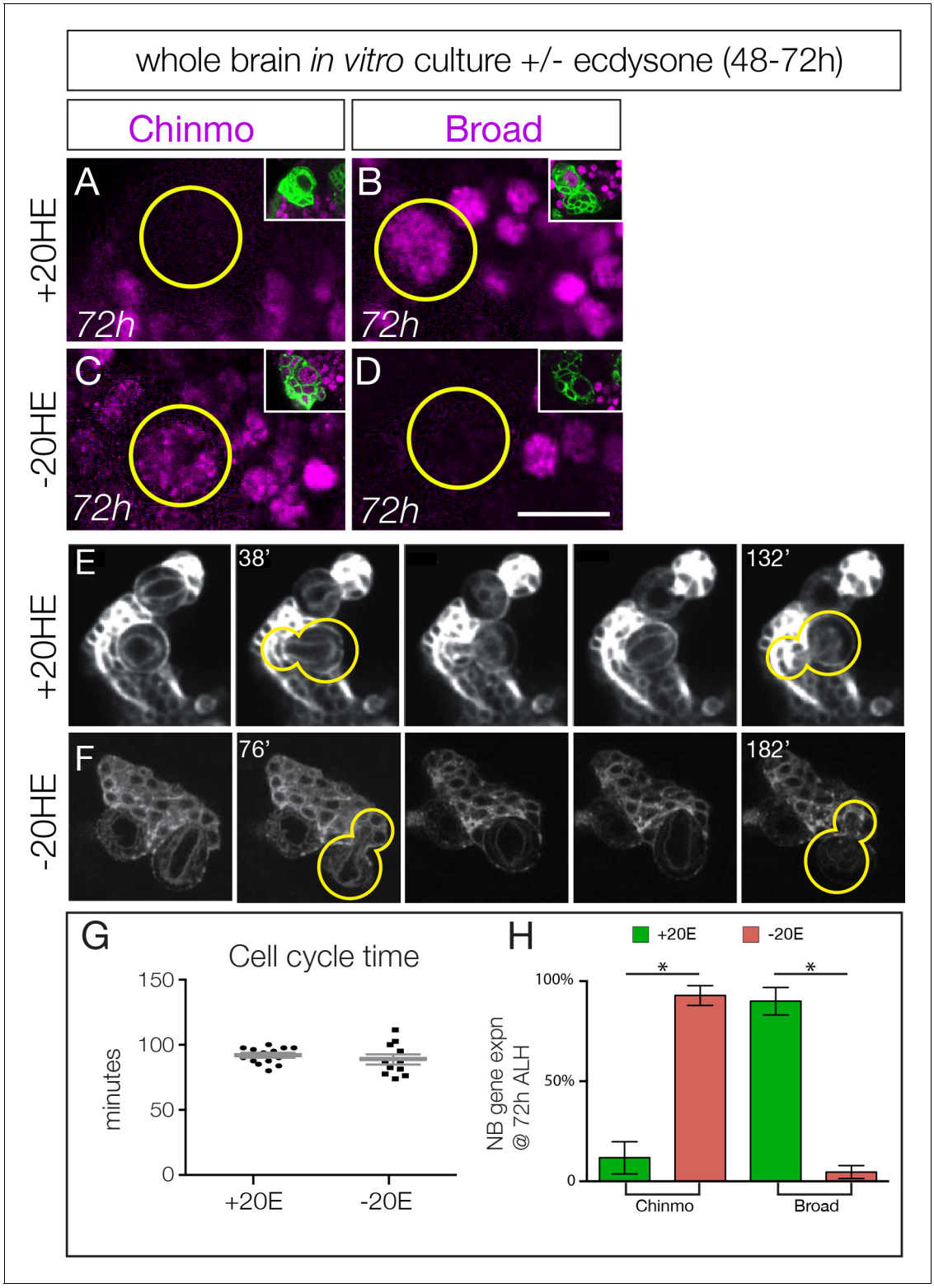

Figure 3. Ecdysone hormone activates neuroblast expression of Chinmo and Broad in isolated brain cultures. (**A–B**) Isolated larval brains cultured with added 20-hydroxy-ecdysone (+20 HE) from 48–72 hr show normal down-regulation of the early factor Chinmo (**A**) and activation of the late factor Broad (**B**). (**C–D**) Isolated larval brains cultured without added 20-hydroxy-ecdysone (−20HE) from 48–72 hr fail to down-regulate Chinmo (**C**) or activate Broad (**D**). (**E–F**) Live imaging of isolated larval brains from 48–72 hr cultured with ecdysone (+20 HE) or without ecdysone (−20HE). In each case two neuroblasts and their progeny were imaged; successive telophase stages are shown for one neuroblast (yellow outline); note that for both +20 HE and

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−20HE the cell cycle time is ~100 min (see timestamps). See ***Videos 1 and 2***. (**G**) Quantification of the experiment shown in **E–F**. (**H**) Quantification of the experiment shown in **A–D**. In all panels, type II neuroblasts are identified as large cells expressing *wor-gal4 ase-gal80 UAS- mcd8:GFP* (green in insets, **A–F**; white in **G–H**), n ≥ 10 per experiment. Asterisk, p<0.003. Scale bar, 10 μm.

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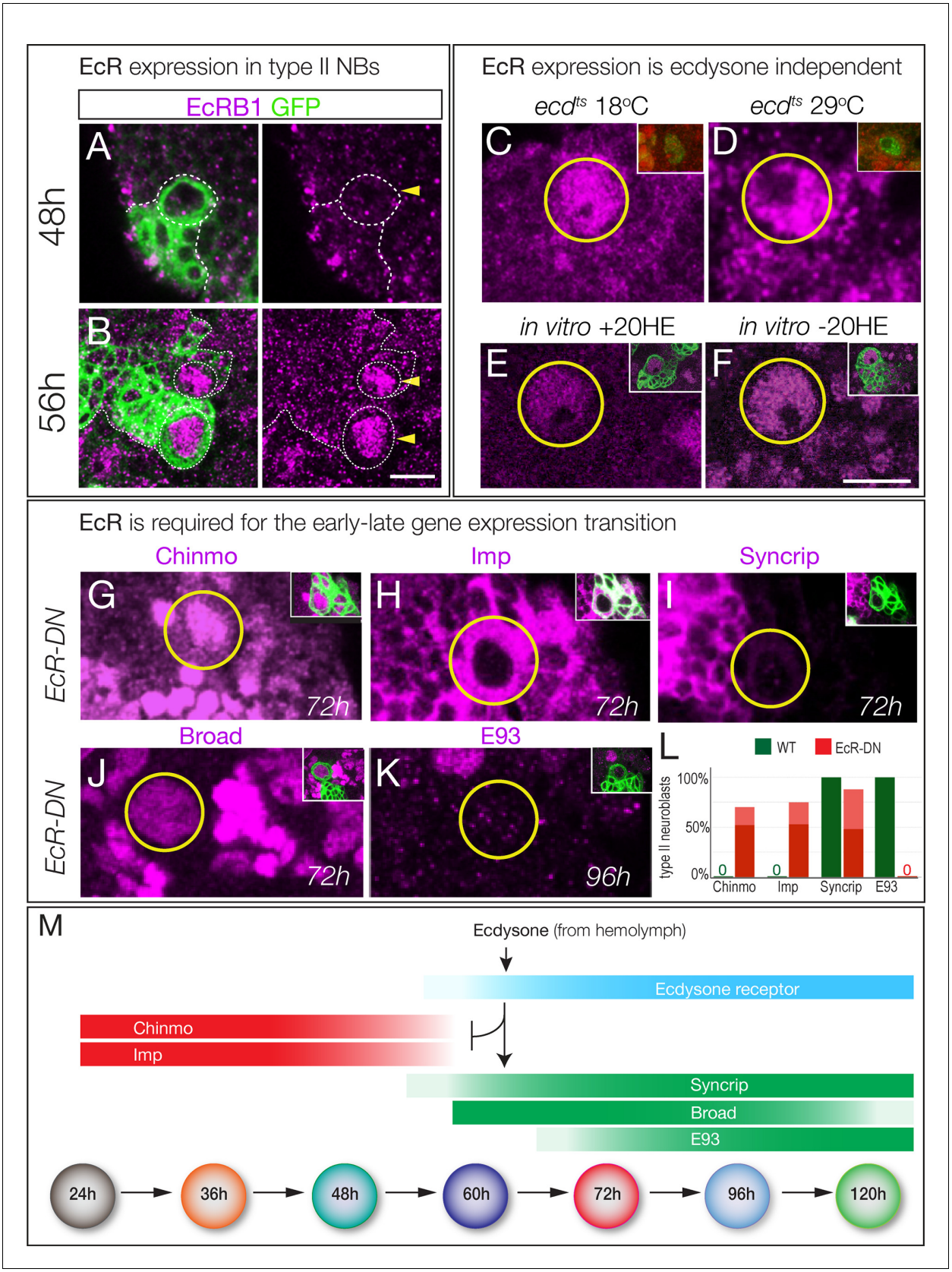

Figure 4. Ecdysone receptor expression and function. (**A–B**) EcR-B1 is first detected at ~56 hr in most type II neuroblasts. (**C–F**) EcR-B1 expression is ecdysone-independent. (**C–D**) EcR-B1 is activated normally in *ecd*^{ts} mutants at both permissive (18˚C) and restrictive (29˚C) temperatures by 72 hr. (**E–F**)

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EcR-B1 expression is activated normally in isolated brains cultured from 48–72 hr with (**E**) or without (**F**) added 20-hydroxy-ecdysone (20HE). (**G–K**) Expression of an EcR dominant negative transgene in type II neuroblasts (*wor-gal4 ase-gal80 UAS-mcd8:GFP UAS-EcR^{DN}*) results in persistent expression of the early factors Chinmo and Imp (**G**,**H**) and failure to express the late factors Syncrip and E93 (**I**,**K**). Surprisingly, the late factor Broad is still expressed (**J**). (**L**) Quantification. Percent of type II neuroblasts expressing the indicated factors at the indicated levels (dark red, strong expression; light red, weak expression; dark green, strong expression). All data are from 72 hr larvae except E93 is from 96 hr larvae. (**M**) Summary: ecdysone signaling via EcR-B1 terminates expression of early factors and activates expression of late factors. In all panels, type II neuroblasts are identified by expression of *wor-gal4 ase-gal80 UAS-mcd8:GFP* (left panels or insets), developmental times are adjusted to the equivalent time at 25˚C. n > 10 for each experiment. Scale bar 10 μm.

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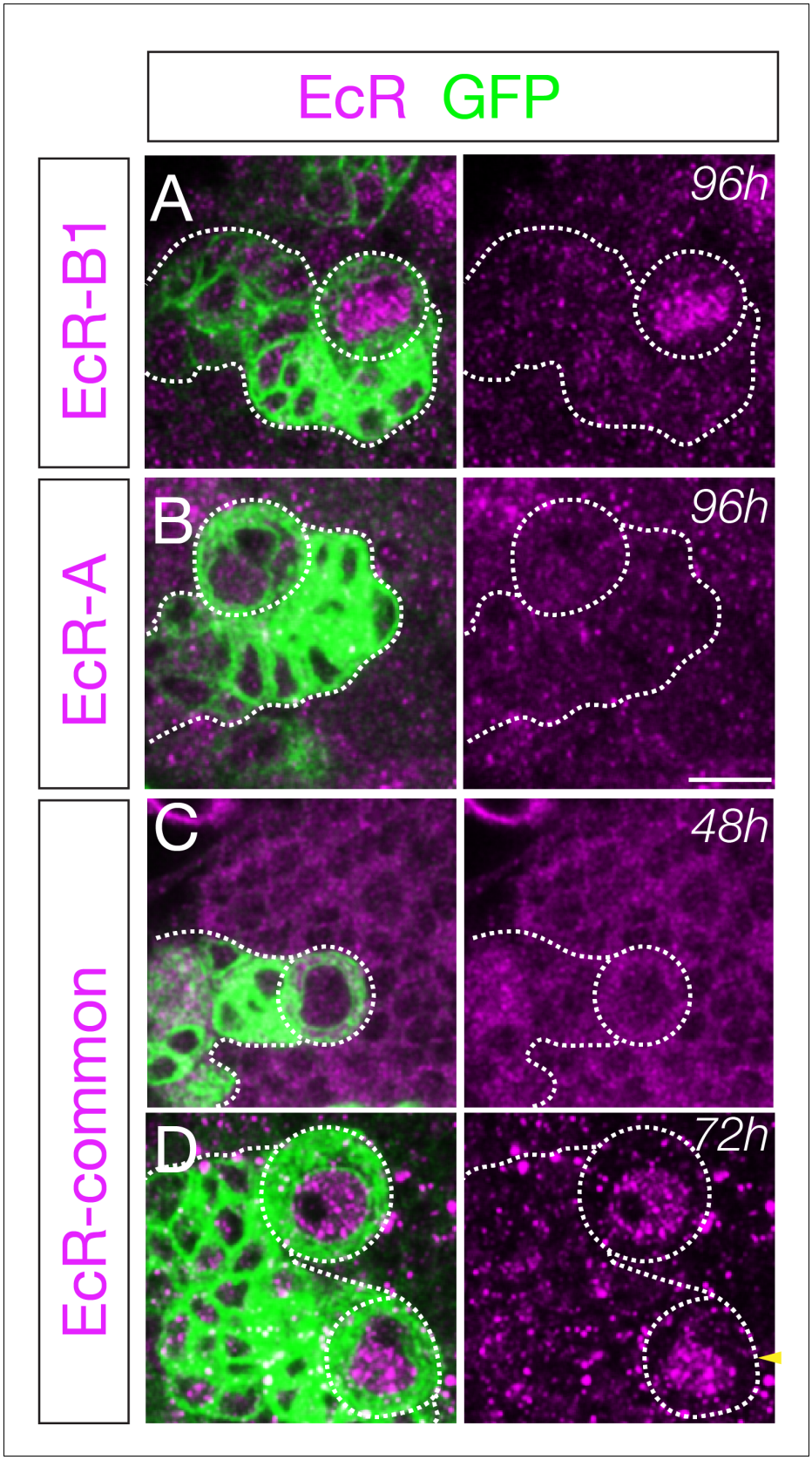

Figure 4—figure supplement 1. Ecdysone receptor isoform expression in type II neuroblasts. (**A**) EcR-B1 (magenta) is detected in type II neuroblasts (dashed circle) at 96 hr; n > 10. See also ***Figure 4A,B***. (**B**) EcR-A

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(magenta) is not detected in type II neuroblasts (dashed circle) at 96 hr; n > 10. (**C**) EcR-common (magenta) is not detected in type II neuroblasts (dashed circle) at 48 hr; n > 10. (**D**) EcR-common (magenta) is detected in type II neuroblasts (dashed circle) at 72 hr; n > 10. EcR-common detects all EcR isoforms. In all panels, type II neuroblast lineages are marked by *wor-gal4 ase-gal80 UAS-cd8:GFP* expression (green). Scale bar, 10 μm.

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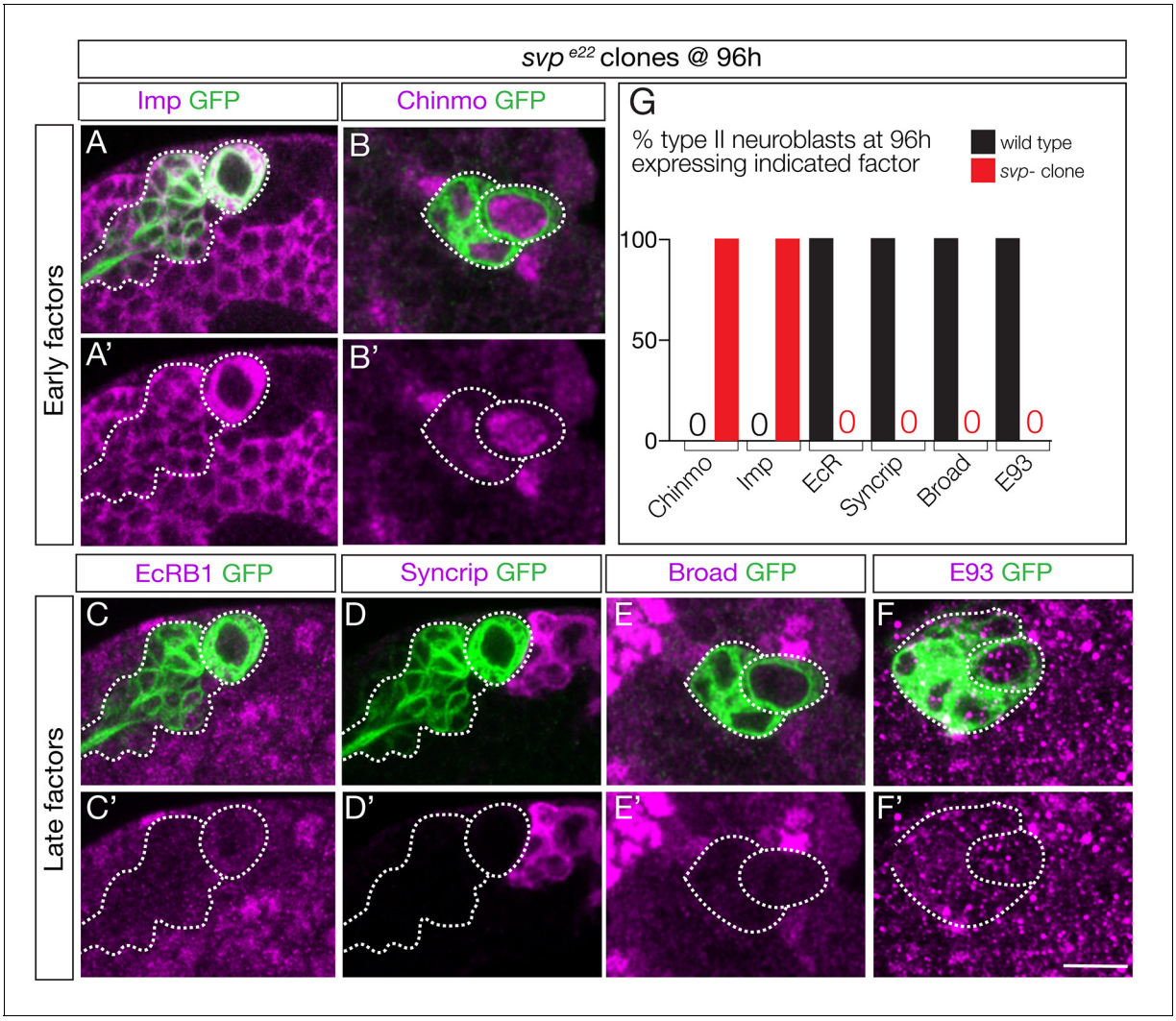

Figure 5. Seven-up activates expression of the Ecdysone receptor in type II neuroblasts. (**A–F**) *svp* mutant MARCM clones (GFP+, green and outlined) induced at 0–4 hr and assayed at 96 hr for the indicated factors. (**G**) Quantification (red, *svp* mutant clone; black, wild type *UAS-FLP actin-FRT-stop-FRT-gal4; wor-gal4 ase-gal80; UAS-mCD8: GFP*). 100% of mutant type II neuroblasts fail down-regulate the early factors Imp and Chinmo, and fail to activate the late factors Syncrip, EcR-B1, E93 and Broad. Number of *svp* mutant clones scored: Imp n = 11, Chinmo n = 4, Syncrip n = 19, EcR-B1 n = 11, E93 n = 2, Broad n = 4. Number of wild type neuroblasts scored n > 10 for each marker. In all panels, type II neuroblasts are identified by expression of *wor-gal4 ase-gal80 UAS-mcd8:GFP* (outlined). Scale bar 10 μm.

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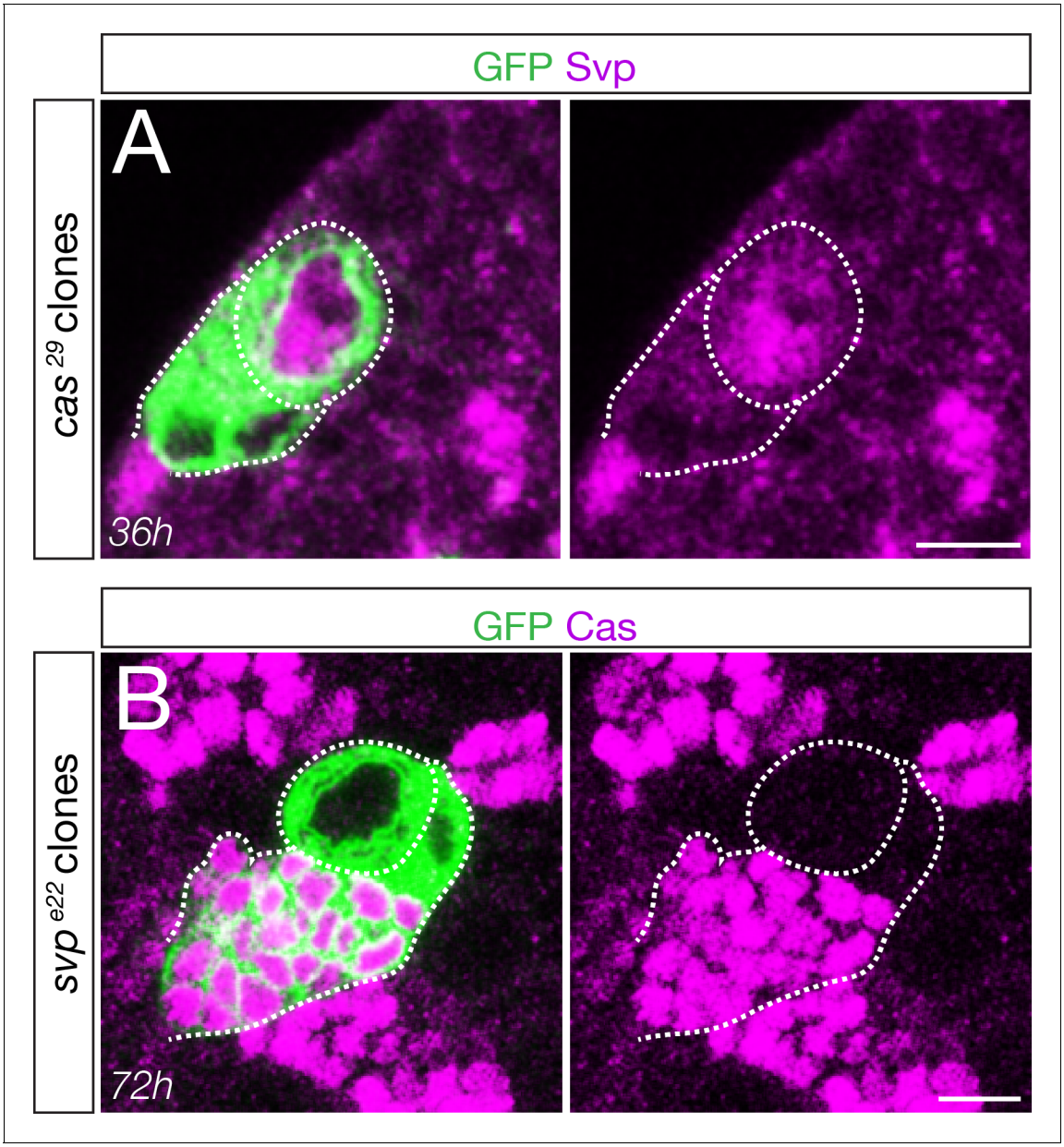

Figure 5—figure supplement 1. Cas is not required to activate Svp expression, and Svp is not required to terminate Cas expression. *cas* mutant clones induced during embryogenesis show normal Seven-up expression at 36 hr. *svp* mutant clones induced during 0–4 hr show normal loss of Cas expression at 72 hr. In all panels, type II neuroblast lineages are marked by *wor-gal4 ase-gal80 UAS-cd8:GFP* expression (green). Scale bar, 10 μm.
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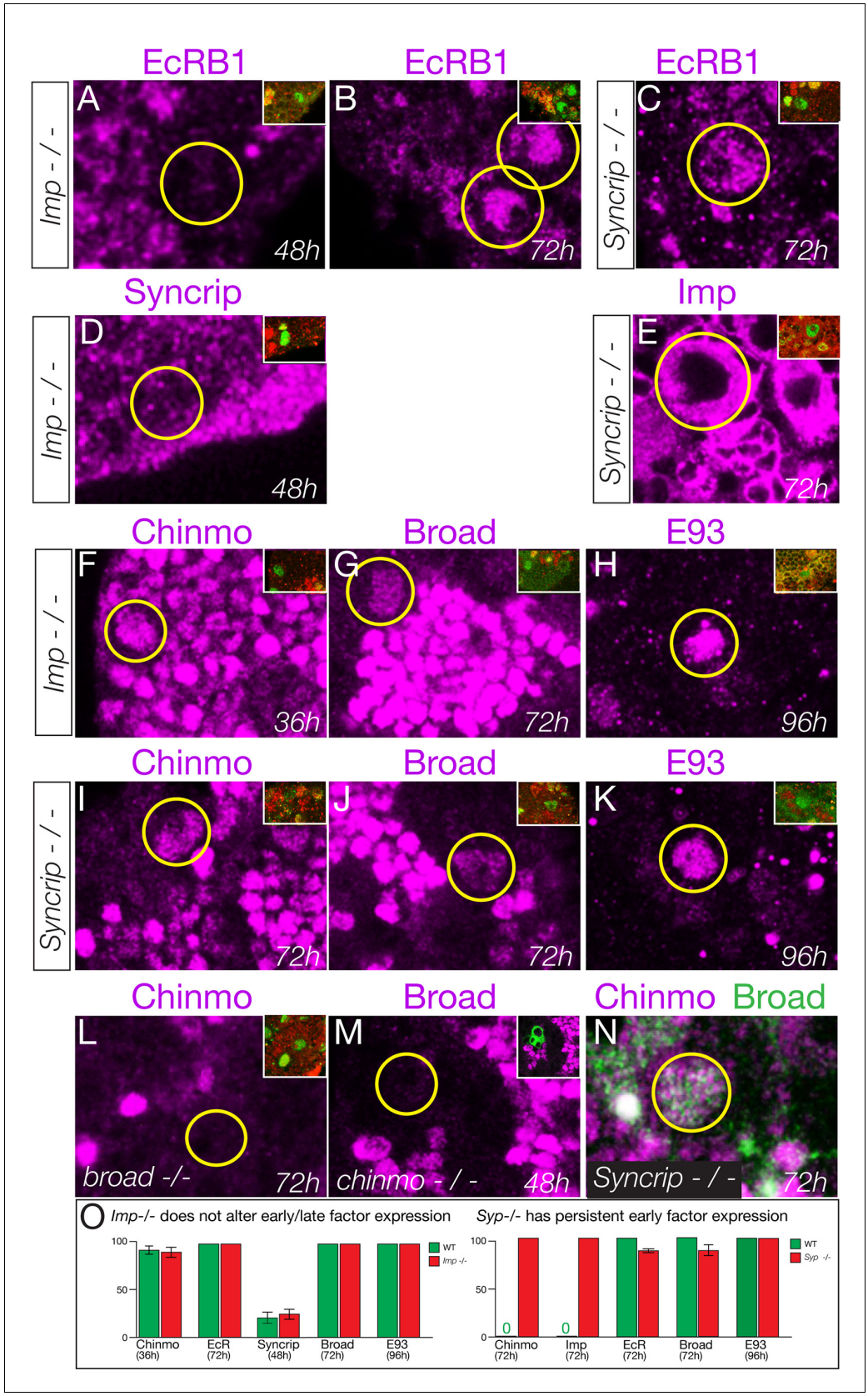

Figure 6. Syncrip and Imp function downstream of the Ecdysone receptor in type II neuroblasts. (**A–C**) *Imp* mutants (*imp*^{G0072} / *imp*^{G0072}) and *Syncrip* mutants (*Syncrip* ^{f03775}/ *deficiency*) show normal expression of EcR in type II neuroblasts: off at 48 hr and on at 72 hr. (**D**) *Imp* mutants (*Imp*^{G0072} /

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Imp^{G0072}) do not precociously upregulate Syncrip in type II neuroblasts at 48 hr. (**E**) *Syncrip* mutants (*Syncrip* ^{f03775}/ *deficiency*) show prolonged expression of Imp in type II neuroblasts at 72 hr. (**F–H**) *Imp* mutants (*Imp*^{G0072} / *Imp*^{G0072}) show normal temporal expression of Chinmo, Broad, or E93 in type II neuroblasts. (**I–K**) *Syncrip* mutants (*Syncrip* ^{f03775}/ *deficiency*) show prolonged expression of Chinmo but normal expression of the late factors Broad and E93 in type II neuroblasts. (**L**) *broad* mutants (*broad*^{npr3}/ *broad*^{npr3}) have normal Chinmo expression: absent from 72 hr type II neuroblasts. (**M**) *chinmo* mutants (*chinmo*^{1} mutant clones) have normal Broad expression: absent from 48 hr type II neuroblasts. (**N**) *Syncrip* mutants (*Syncrip*^{f03775}/ *deficiency*) have type II neuroblasts that abnormally co-express Chinmo and Broad at 72 hr. (**O**) Quantification. Percent of type II neuroblasts expressing the indicated factors at the indicated timepoints. Insets identify the pictured type II neuroblast (green) based on its expression of Dpn (green) not Ase (red) or by expression of *wor-gal4 ase-gal80 UAS-mcd8:GFP* (green). n > 5 for all panels. Scale bar, 10 μm.

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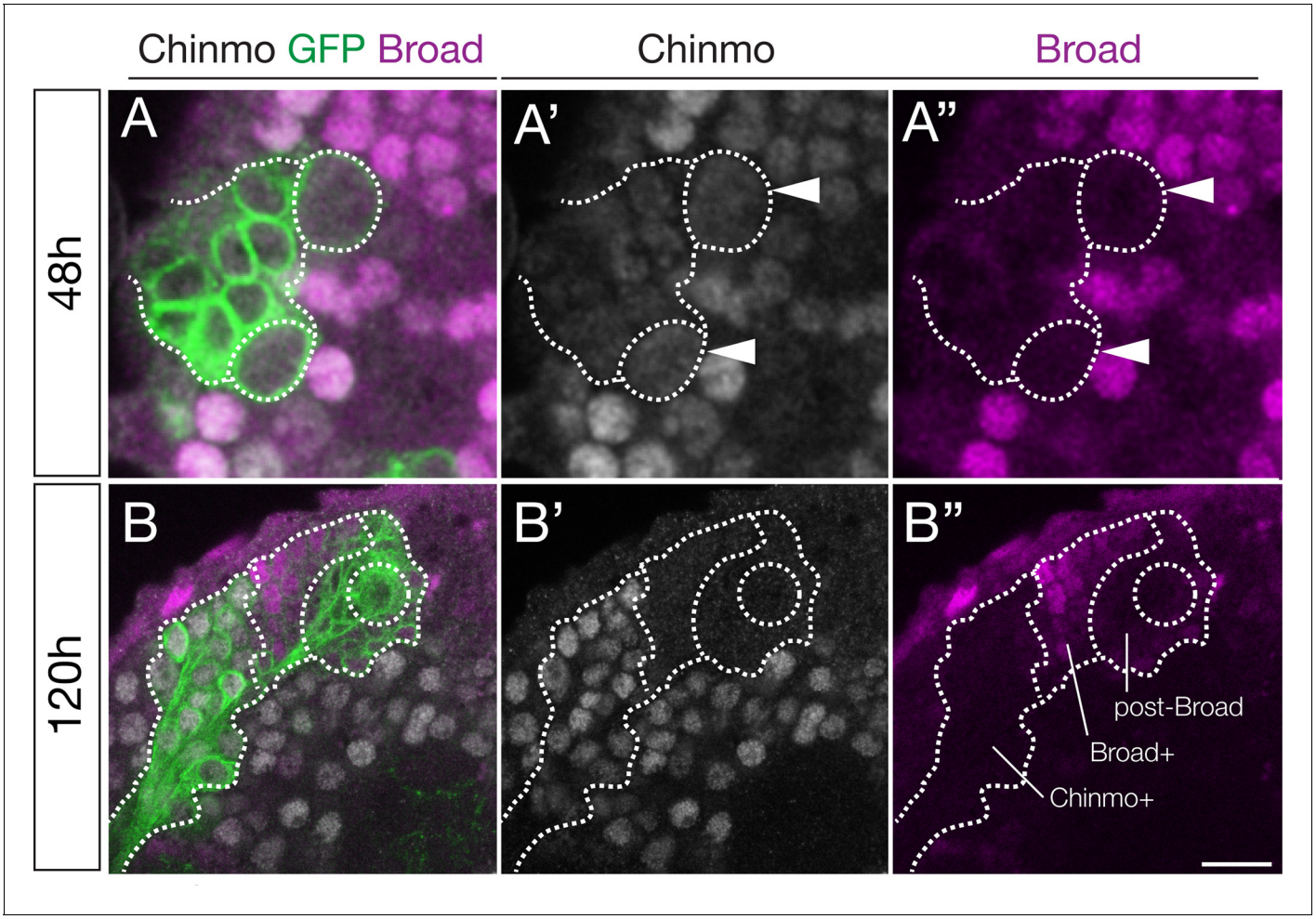

Figure 6—figure supplement 1. Chinmo and Broad have mutually exclusive expression in neuroblasts and neurons. (**A**) Chinmo is expressed in type II neuroblasts (dashed circle) and adjacent neuronal progeny at 48 hr, whereas Broad is not detected. (**B**) Broad is expressed in type II neuroblasts (dashed circle) and adjacent neuronal progeny at 120 hr, whereas Chinmo is not detected in the neuroblast but only in the earlier-born neurons furthest away from the parental neuroblast. In all panels, type II neuroblast lineages are marked by *wor-gal4 ase-gal80 UAS-cd8:GFP* expression (green). Scale bar, 10 μm.

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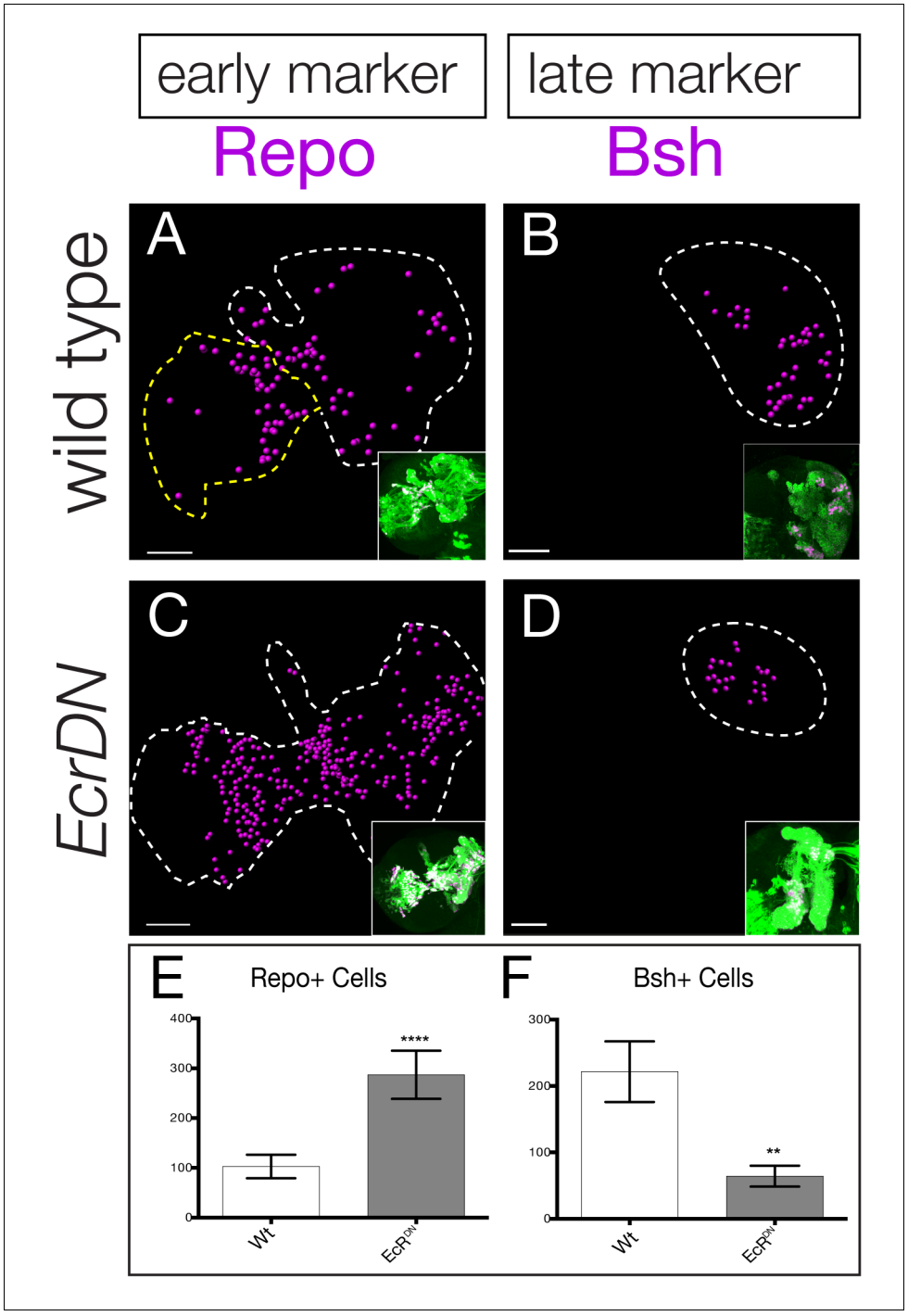

Figure 7. Late temporal transcription factors specify neuronal and glial identity. (**A**,**B**) Wild type or (**C**,**D**) *EcR*^{DN} brains at 0 hr after puparium formation. The inset shows GFP+ cells permanently marking the type II neuroblast lineage (*wor-gal4 ase-gal80 UAS-FLP actin-FRT-stop-FRT-gal4 UAS-mCD8:GFP*; green) which is circled with dashed lines in the main figure. Repo+ or Bsh+ nuclei located within the volume of the type II progeny were identified using Imaris and represented as magenta spheres. This provides the optimal way to visualize cell numbers within the three dimensional GFP+ volume. (**E**,**F**) Quantification. **p<0.01, ****p<0.0001.

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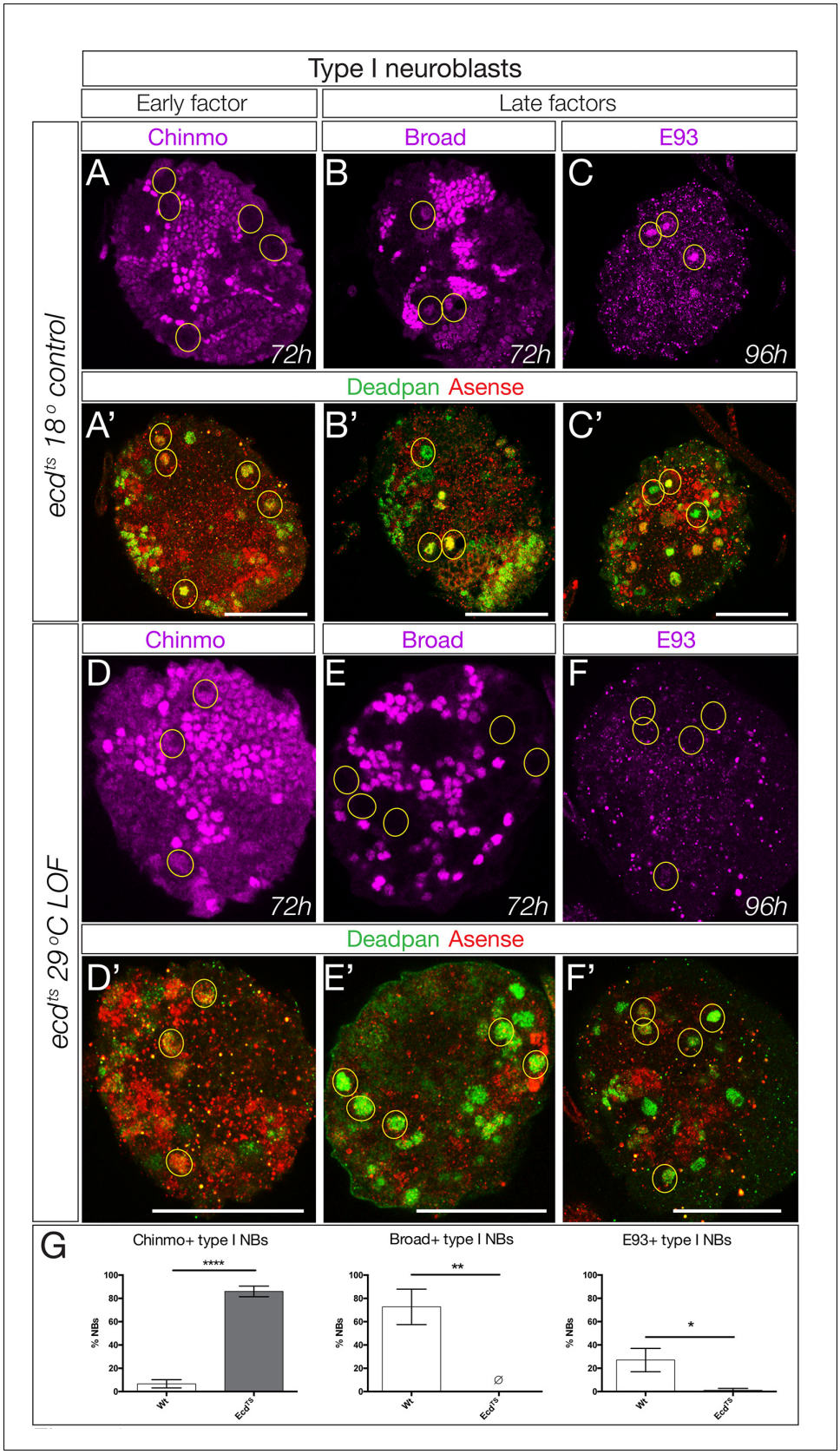

Figure 8. Ecdysone is required for early to late temporal factor transition in type I neuroblasts. (**A–C**) Control brains (*ecd-ts/deficiency* at 18˚C) at the indicated timepoint. (**A–C**) Normal down-regulation of the early factor

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Chinmo and activation of the late factors Broad and E93. (**E–G**) Experimental brains with reduced ecdysone (*ecd-ts/deficiency* at 29°C) at the indicated timepoint. (**E–G**) Abnormal prolonged expression of the early factor Chinmo and failure to activation of the late factors Broad and E93. (**D**,**H**) Quantification. n = 6 brain lobes; *p<0.03, **p<0.03; ****p<0.0001. In all panels, central brain type I neuroblasts are identified as Dpn+Ase+ (subset outlined), and times are adjusted to the equivalent larval stage at 25°C as described in the Materials and methods. Note that ecd^{ts}*/deficiency* brains are smaller than control brains primarily due to severe loss of the optic lobe. Scale bar, 50 μm.

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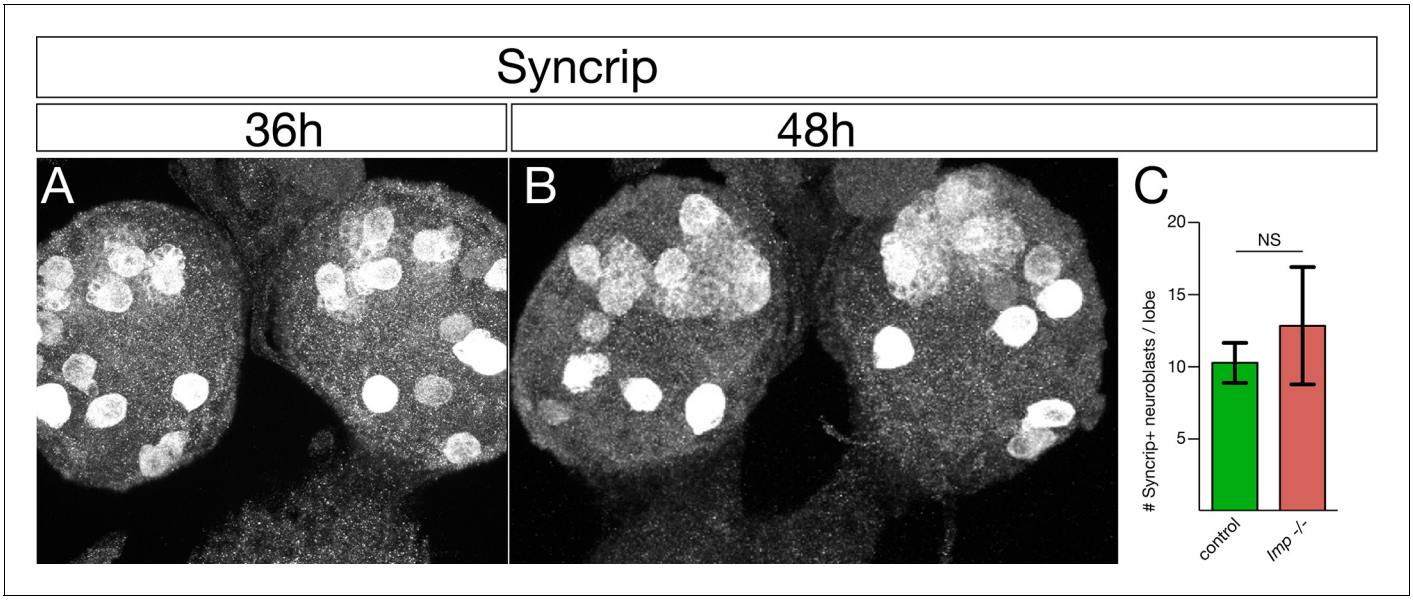

Figure 8—figure supplement 1. Syncrip is expressed in a subset of central brain neuroblasts at 36 hr and 48 hr. (**A**,**B**) Syncrip is detected in ~10 central brain neuroblasts at 36 hr and 48 hr. Maximum intensity projection of entire brain lobes. Anterior up. (**C**) Quantification.
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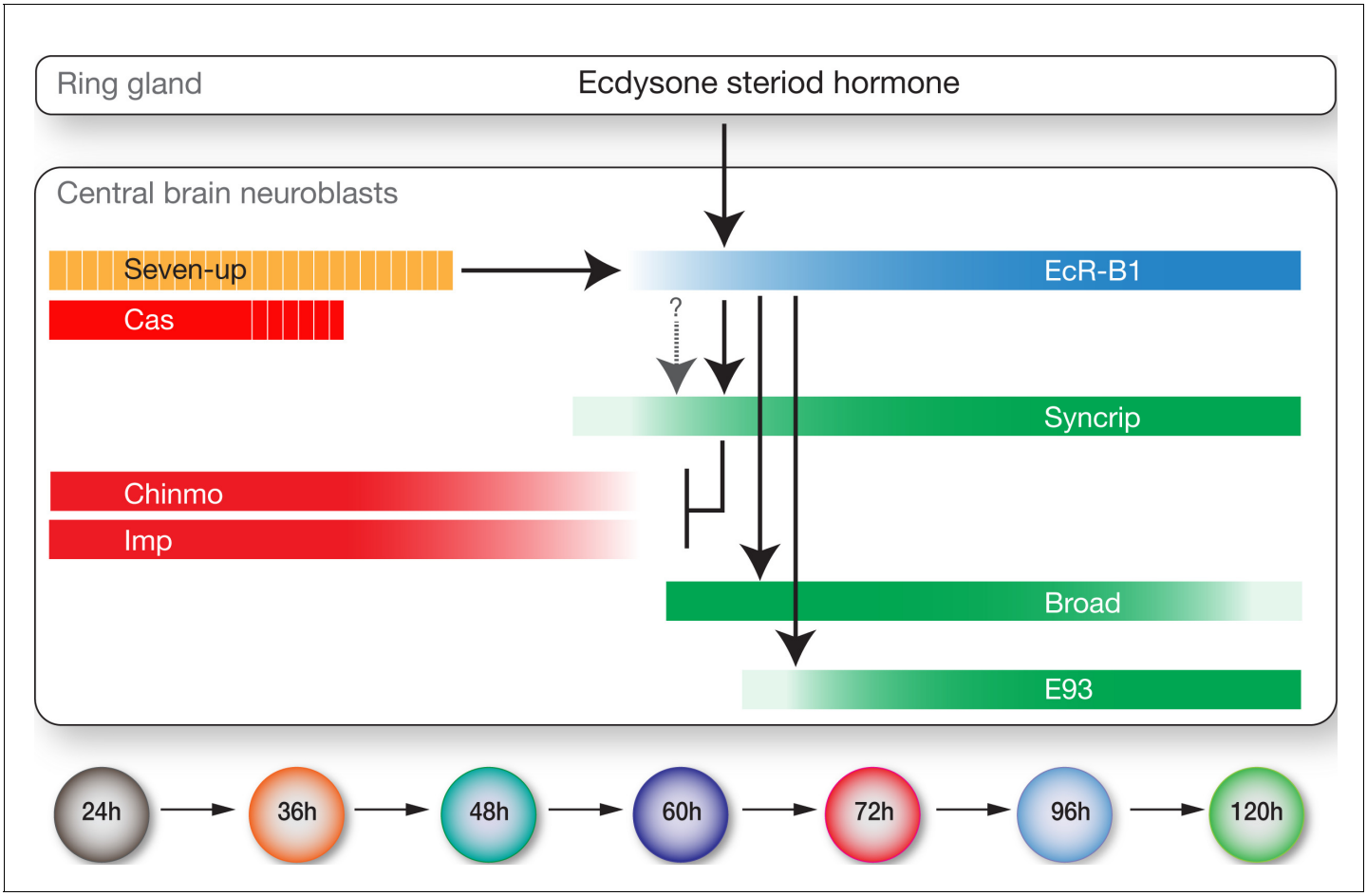

Figure 9. Model showing hormonal regulation of early to late temporal transitions in central brain larval neuroblasts. Summary of regulatory interactions driving larval neuroblast early-to-late temporal factor expression. Arrows indicate positive regulation; 'T' indicates negative regulation; dashed bars indicate asynchronous expression during the indicated temporal window; gradients indicate graded change in expression levels.
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