nature cell biology

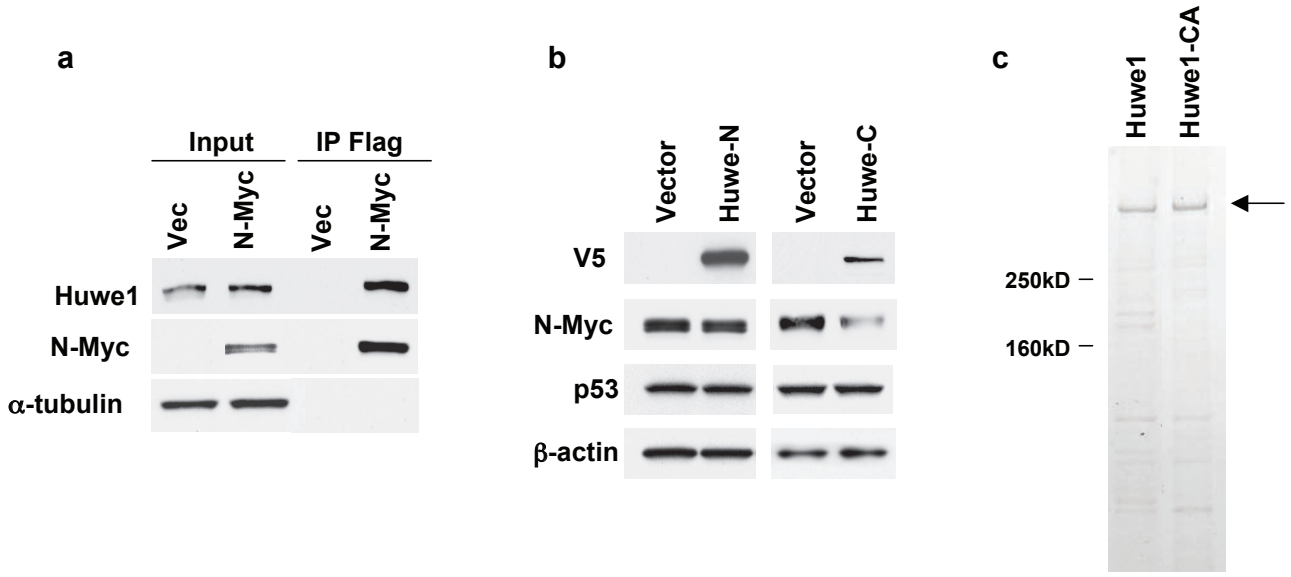


Figure S1 N-Myc binds Huwe1 *in vivo*. (**a**), Total extracts from SK-N-SH vector or stably expressing Flag-N-Myc were immunoprecipitated with Flag antibody. The levels of Huwe1 and N-Myc in the precipitates were determined by immunoblot. Input: 1/100 of total extracts used for each immunoprecipitation reaction. (**b**), Lysates from IMR32 transfected with plasmids expressing the V5-tagged N-terminus (1-1041), C-terminus (1041-4034) of Huwe1 or the empty vector. Endogenous levels of N-Myc and p53 were analyzed by immunoblot. The V5 antibody was used to detect exogenously expressed Huwe1. (**c**), Expression and purification of full-length Huwe1 from baculoviruses. Recombinant Huwe1 wild type and the catalytically inactive mutant Huwe1-CA were analyzed by SDS-PAGE and Coomassie staining. Recombinant proteins were used in ubiquitination and degradation assays.

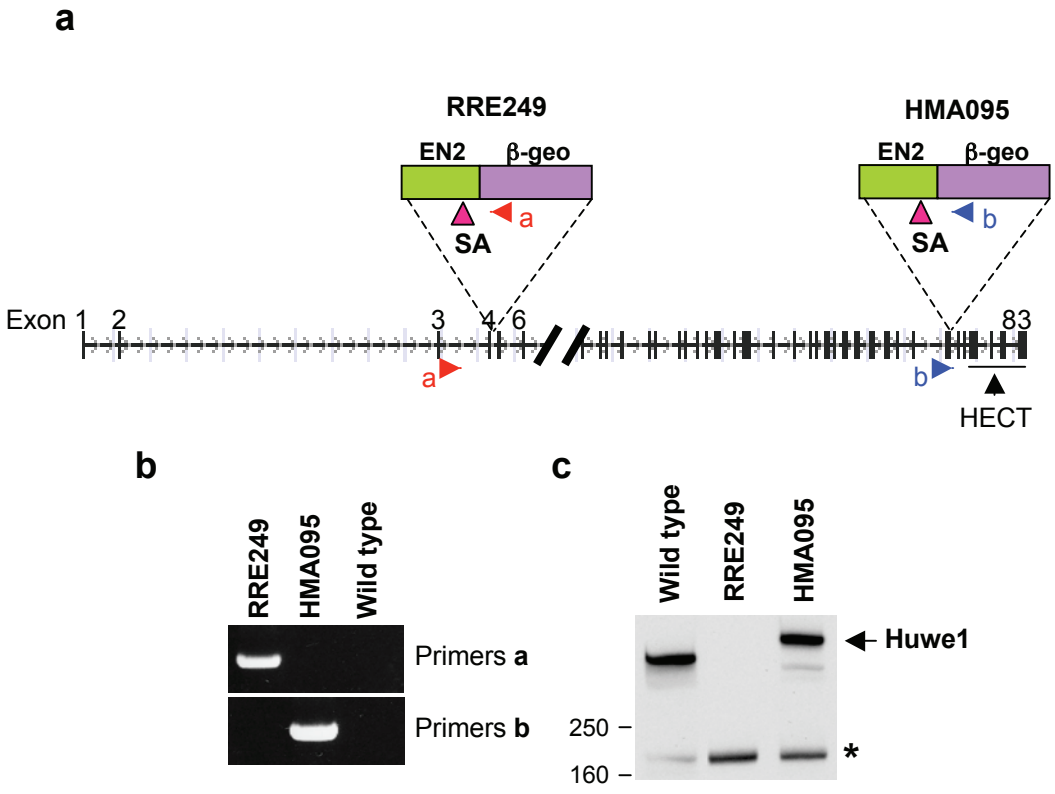


Figure S2 Structure and analysis of *Huwe1* trapped ES cell clones. **(a)**, Trap vector is inserted into intron 4 and intron 74 in clone RRE249 and clone HMA095, respectively. (**b)**, Trap vector insertion was confirmed by PCR using primer pair a for clone RRE249 (red triangles, product size: 3.1 Kb) and primer pair b for clone HMA095 (blue triangles; product size: 2.3Kb).Wild type ES cells are used as control. (**c)**, Immunoblot using Huwe1 antibody from wild type, RRE249 and HMA095 ES cells demonstrates the absence of Huwe1 protein in clone RRE249. Asterisk indicates a non-specific band.

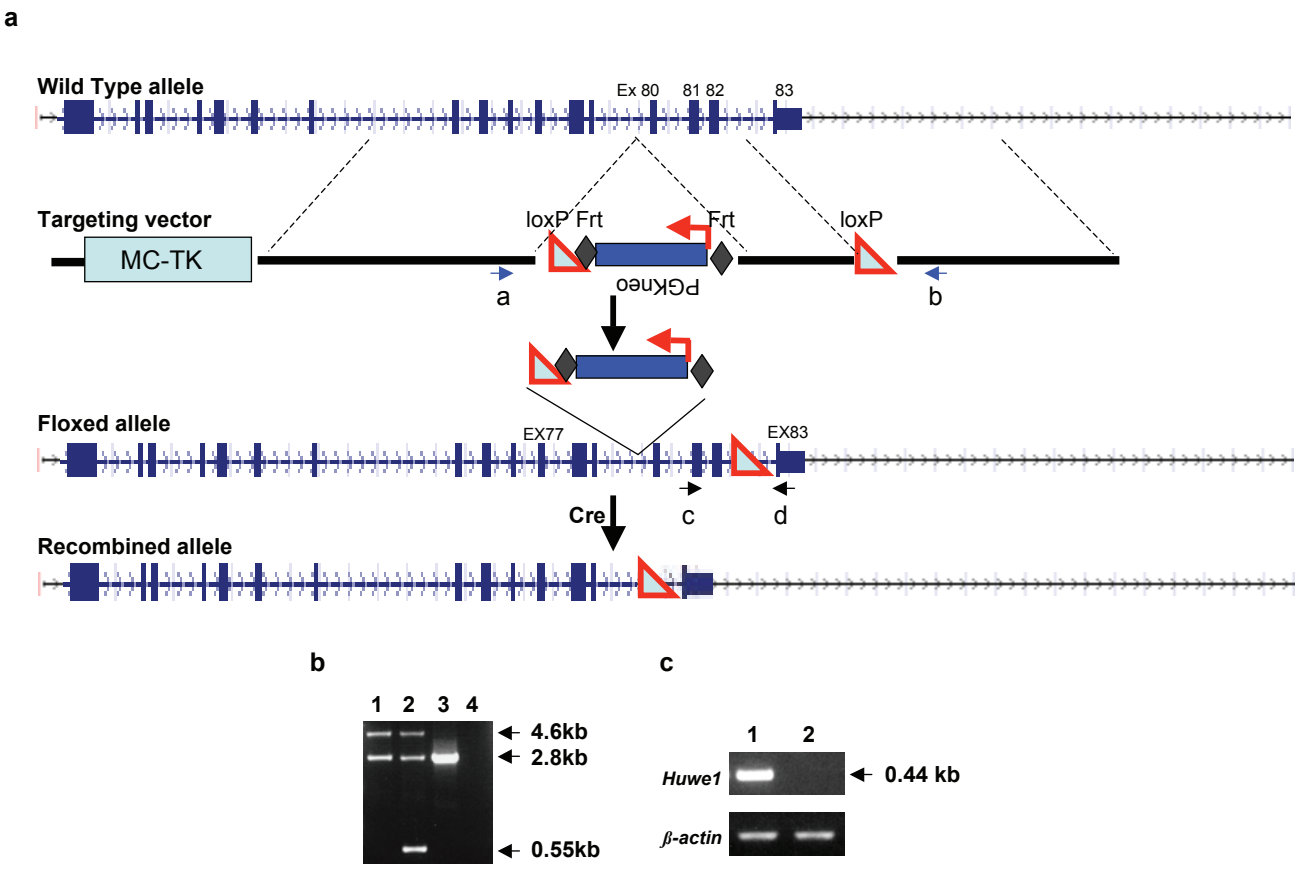


Figure S3 Generation of ES cells carrying a conditional mutant allele of *Huwe1* by gene targeting. **(a)**, Structure of the *Huwe1* genomic locus, targeting vector and targeted locus. The pGKNeo^{R} cassette is in the opposite transcriptional orientation to the *Huwe1* gene. Upon Cre-mediated recombination the region between exons 80 and 82 is deleted. Primer pairs used for PCR are indicated. **(b)**, Targeted cells were transfected with vector (lane 1) or plasmid encoding Cre recombinase (lane 2). Genomic PCR with primers a and b demonstrates that LoxP sites in targeted ES cells are functional. **(c)**, RT-PCR analysis of *Huwe1* mRNA in homologous recombined ES cells after infection with retroviruses expressing GFP (lane 1) or Cre recombinase (lane 2) using primer c and d (exon 81 and exon 83 respectively).

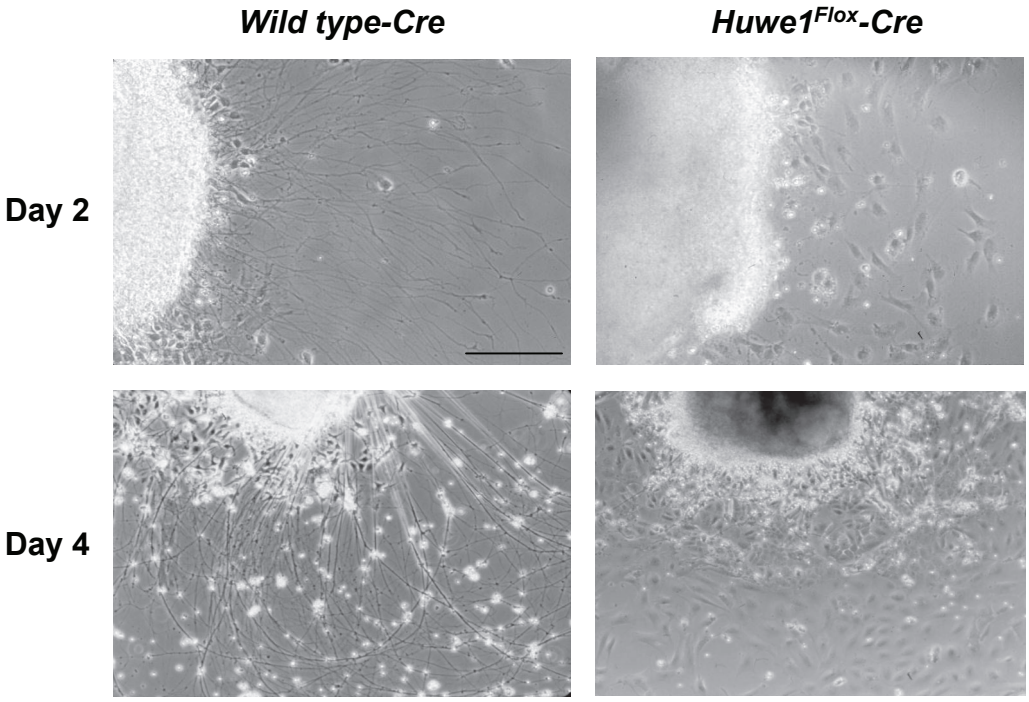


Figure S4 Expression of Cre recombinase in ES cells does not affect neuronal differentiation. Embryoid bodies derived from wild type and *Huwe1*^{Flox} ES cells that had been transduced with a Cre expressing retrovirus were allowed to adhere after being cultured in suspension for 8 days. Bright-field images were taken 2 and 4 days after re-plating. Scale bar is 150 μm.

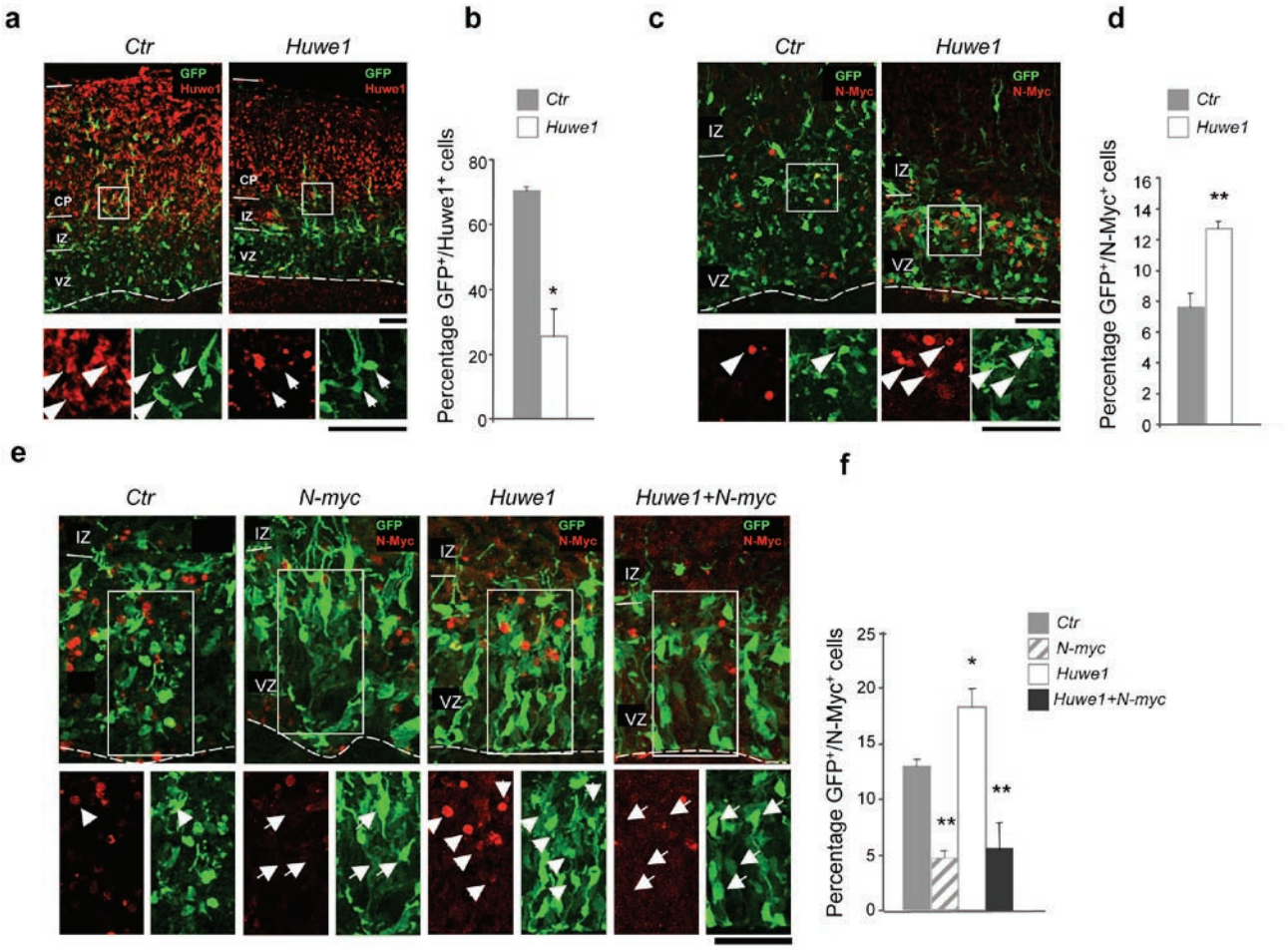


Figure S5 Expression of N-Myc protein is increased in neural progenitors following Huwe1 loss. **(a)**, *Control* and *Huwe1* siRNA were electroporated *ex vivo* into E13.5 mouse cortices. Organotypic slice culture was performed for 3 days. Cortical slices were double labeled with Huwe1 (red) and GFP (green) to identify transfected cells. Scale bars are 50 μm. **b**, Quantification of GFP-positive/Huwe1-positive cells. (**c**), Cortical slices were double labeled with N-Myc (red) and GFP (green). (**d**), Quantification of GFP-positive/N-Myc-positive cells. (**e**) *Control*, *N-myc*, *Huwe1*, and *N-myc* plus *Huwe1* siRNA were electroporated into E13.5 mouse cortices followed by organotypic slice culture for 1.5 days. Cortical slices were double labeled with N-Myc (red) and GFP (green). Scale bars are 50 μm. (**f**), Quantification of GFP-positive/N-Myc-positive cells. Results are mean±SEM ($n = 3$ from two independent experiments ;*, $P < 0.05$ **, $P < 0.01$ Student's *t*-test). Arrowheads indicate GFP-positive/Cy3-positive cells; arrows indicate GFP-positive/Cy3-negative cells.

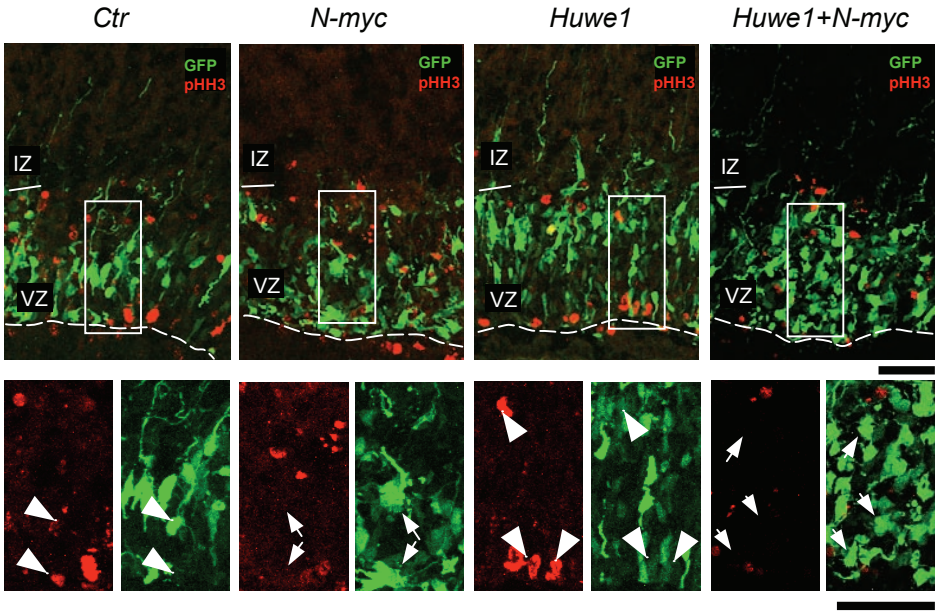


Figure S6 Analysis of mitotic activity in embryonic cortices following silencing of *Huwe1* and *N-myc*. *Ex vivo* electroporation of *Control*, *N-myc*, *Huwe1*, and *N-myc* plus *Huwe1* siRNA into E13.5 mouse cortices followed by organotypic slice culture for 1.5 days. Cortical slices were double labeled with pHH3 (red) and GFP (green) to identify transfected cells. Arrowheads indicate GFP-positive/Cy3-positive cells; arrows indicate GFP-positive/Cy3-negative cells. Scale bars are 50 μm.

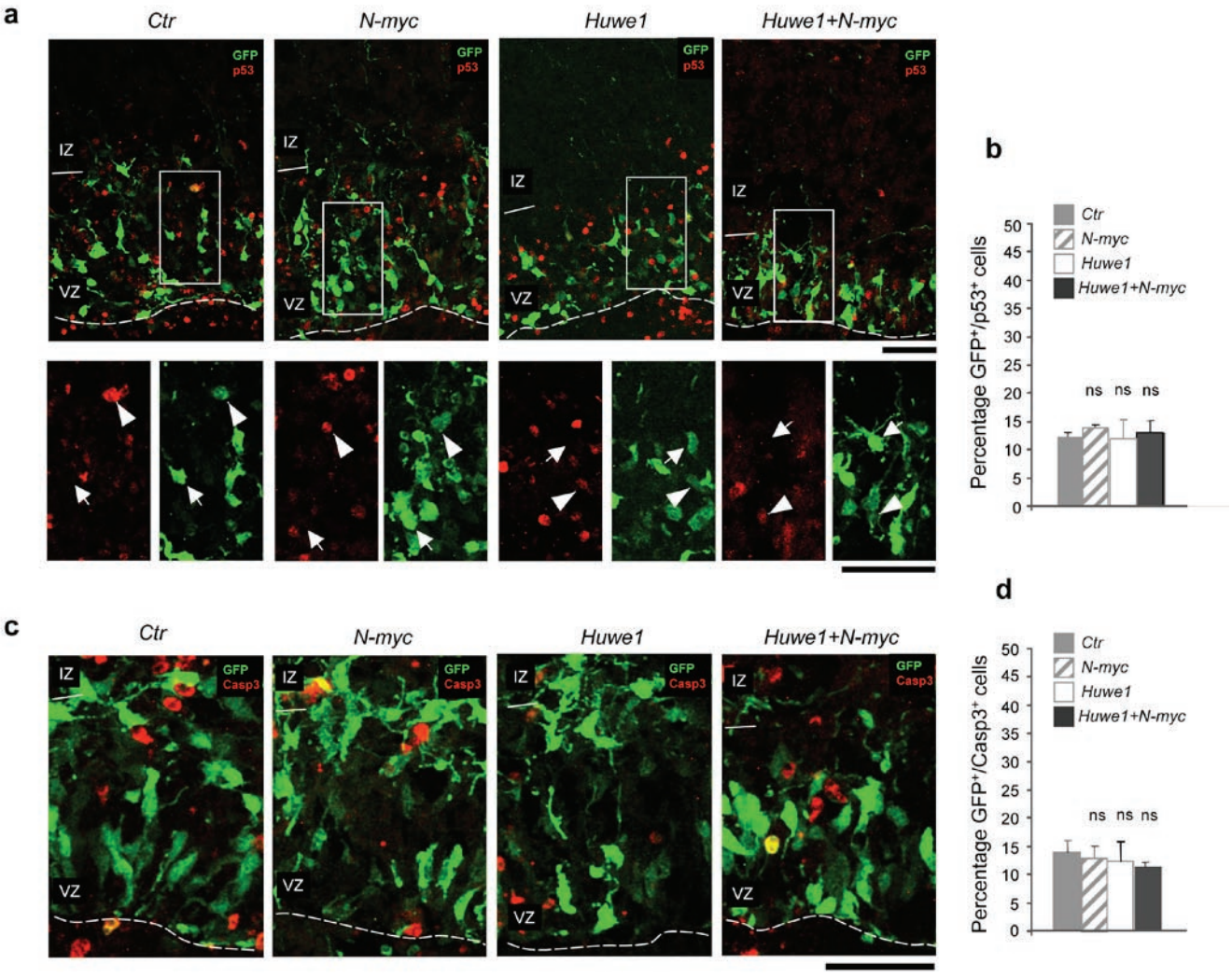


Figure S7 Silencing of *Huwe1* in neural progenitors does not affect p53 expression or apoptotic cell death. (**a**), *Ex vivo* electroporation of *Control*, *N-myc*, *Huwe1*, and *N-myc* plus *Huwe1* siRNA into E13.5 mouse cortices followed by organotypic slice culture for 1.5 days. Cortical slices were double labeled with p53 (red) and GFP (green) to identify transfected cells. (**b**), Quantification of GFP-positive/p53-positive cells. (**c**), Cortical slices were double labeled with Caspase-3 (red) and GFP (green). (**d**), Quantification of GFP-positive/Caspase-3-positive cells. Arrowheads indicate GFP-positive/Cy3-positive cells; arrows indicate GFP-positive/Cy3-negative cells. Results are mean±SEM ($n = 3$ from two independent experiments). Scale bars are 50 μm.

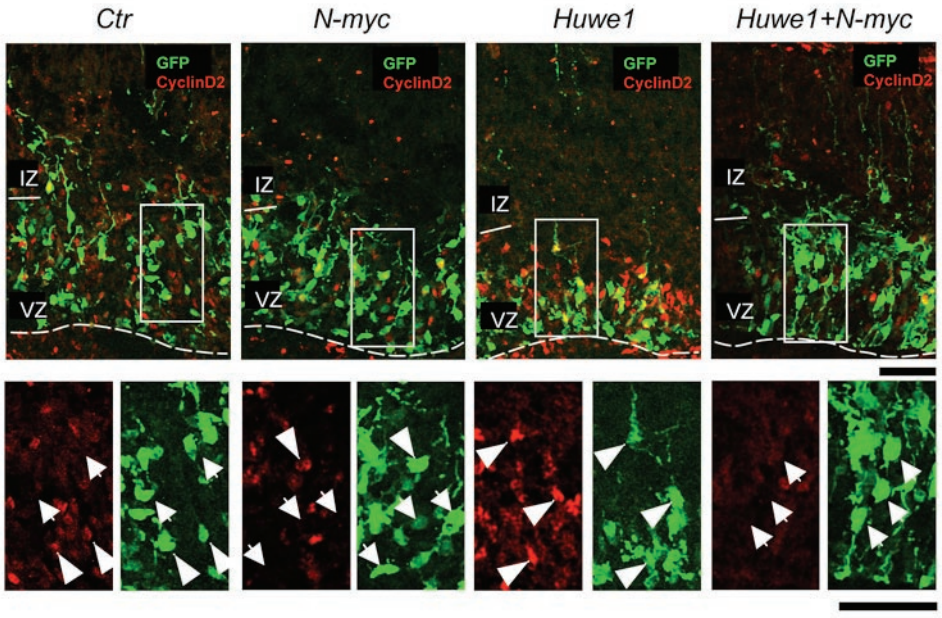


Figure S8 Analysis of expression of cyclin D2 in embryonic cortices following silencing of *Huwe1* and *N-myc*. *Ex vivo* electroporation of *Control*, *N-myc*, *Huwe1*, and *N-myc* plus *Huwe1* siRNA into E13.5 mouse cortices followed by organotypic slice culture for 1.5 days. Cortical slices were double labeled with Cyclin D2 (red) and GFP (green) to identify transfected cells. Arrowheads indicate GFP-positive/Cy3-positive cells; arrows indicate GFP-positive/Cy3-negative cells. Scale bars are 50 μm.

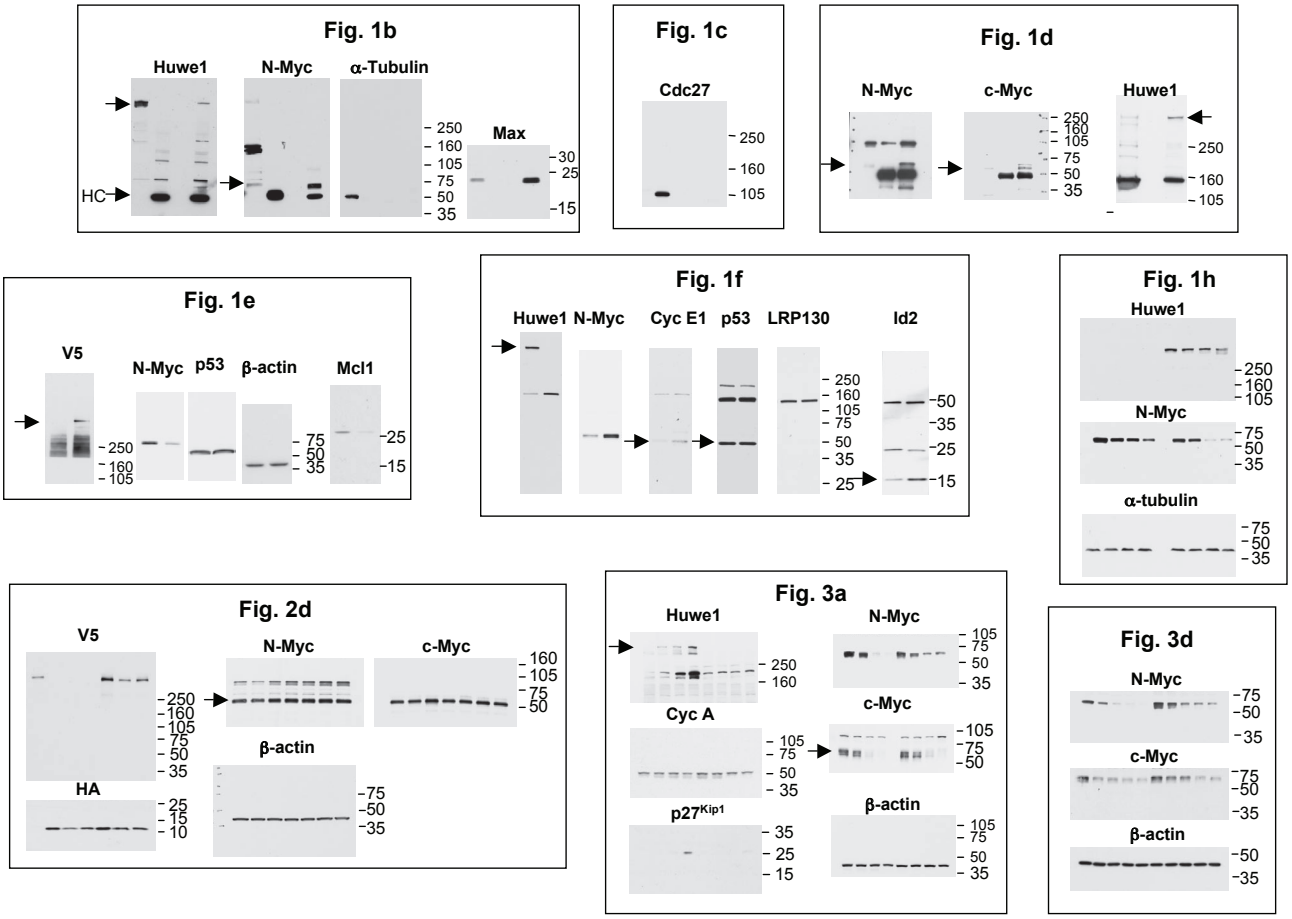
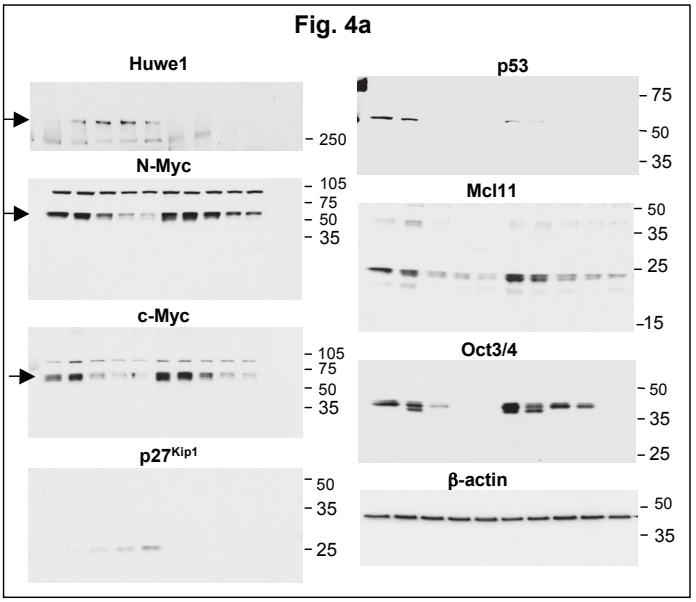
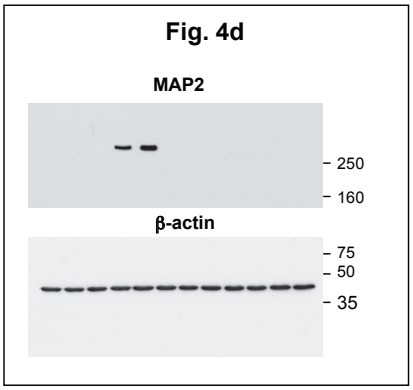
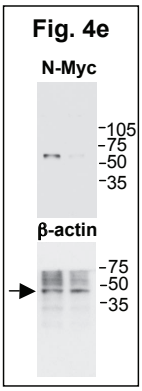
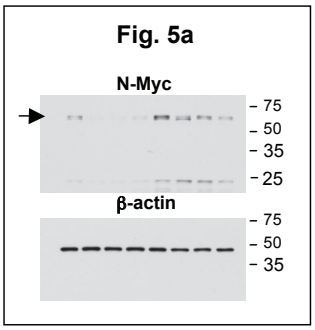


Figure S9 Full scans of key western blot data shown in Figure 1b-d, f, h; Figure 2d; Figure 3a, d; Figure 4a, d-e; Figure 5a-b. The molecular weight is indicated at the right-hand side. Arrows indicate the analyzed protein.









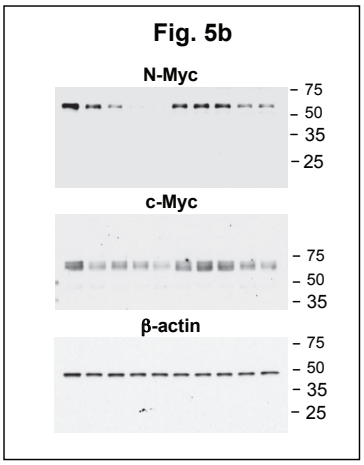


Figure S9 continued