

## SUPPLEMENTARY MATERIALS AND METHODS

### Construction of gRNA-expressing vectors

We chose two 20-nt sequences within the *pntP1*-specific exon 1, CAGCCATCCGCATCACCAAT (+) and ACGGGAGCGCGACTGCGAAC (-), which are 249 and 532 base pairs downstream of the start codon, as gRNA targets. For constructing gRNA-expressing vectors, we first amplified the U6 promoter from genomic DNA by PCR reactions using primers 5'-TTTTTTGCTCACCTGTGATT-3' (forward1) and 5'-CGACGTAAATTGAAAATAGGTC-3' (Reverse1). Then target-specific 20-nt spacer sequences followed by the first 24-nt of the common tracrRNA sequence were added downstream of U6 promoter by PCR reactions using primers 5'-TTTTTTGCTCACCTGTGATTGCTCCTACTCAAATACA-3'(forward2) and 5'-CTTGCTATTTCTAGCTCTAAAACATTGGTGATGCGGATGGCTGCGACGTTAAATTGAAAATAGGTC-3' (reverse2-1) or 5'-CTTGCTATTTCTAGCTCTAAAACACGGGAGCGCGACTGCGAACCGACGTTAAAATTGAAAATAGGTC-3' (reverse2-2). Finally, the remaining 56-nt tracrRNA sequence was added to the previous PCR products by PCR reactions using primers forward2 and primer 5'-AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTAACTTGCTATTTCTAGCTCTAAAAC-3' (reverse3). All PCR reactions were carried out using CloneAmp™ HiFi PCR Premix (Clontech, Mountain View, California ). Final PCR products were cloned into pJET1.2/blunt vector (ThermoFisher scientific, Pittsburgh, Pennsylvania). For detecting mutations in *pntP1* mutants, following primers were used for PCR amplification of genomic DNAs isolated from homozygous mutants and sequencing (Genewiz, South Plainfield, New Jersey): forward: 5'-ATGCCGCCCTCTGCGTTTTTA-3'; reverse: 5'-CTGTACCTGAACTAAAGAAGTTGACATCC-3'.

*btd-GAL4/+; UAS-mCD8-GFP/+; pntP1<sup>90</sup>*

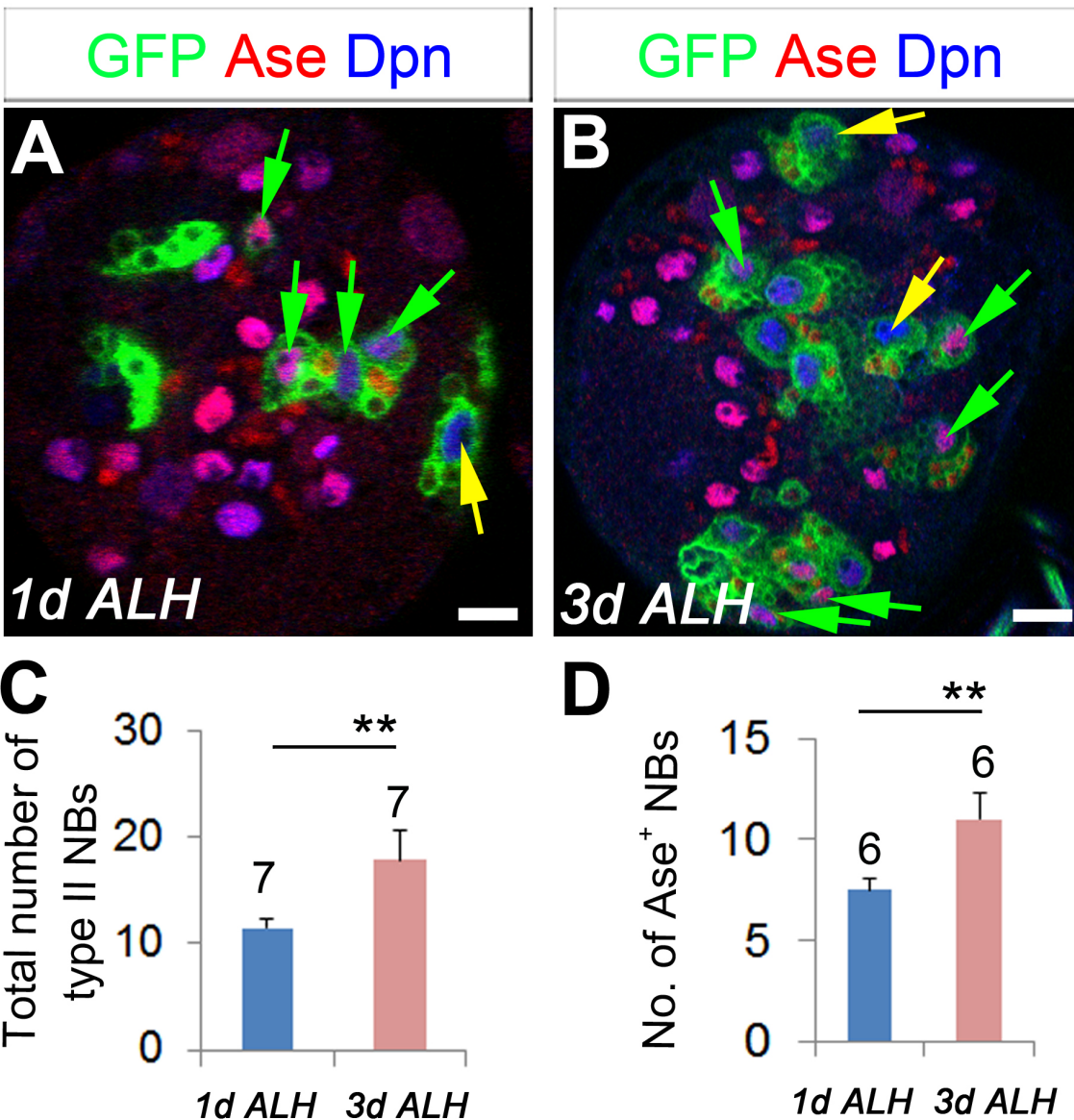


Figure S1. Reduction of Btd promotes ectopic Ase expression in *pntP1* mutant type II NBs. (A-B) *btd-GAL4/+; pntP1<sup>90</sup>* mutant larval brains at 1 day (A) and 3 days (B) ALH. Type II NB lineages are labeled with mCD8-GFP driven by *btd-GAL4*. Green arrows point to Ase<sup>+</sup> type II NBs and yellow arrows point to Ase<sup>-</sup> type II NBs. (C-D) Quantifications of the total number of type II NBs (C) and the Ase<sup>+</sup> type II NB number (D) in *btd-GAL4/+; pntP1<sup>90</sup>* mutant larval brains at 1 day and 3 days ALH. Numbers on top of each bar are sample sizes and individual bars represent mean  $\pm$  Stdev. \*\*,  $p < 0.01$  (student *t*-test). Scale bars: 20  $\mu$ m.