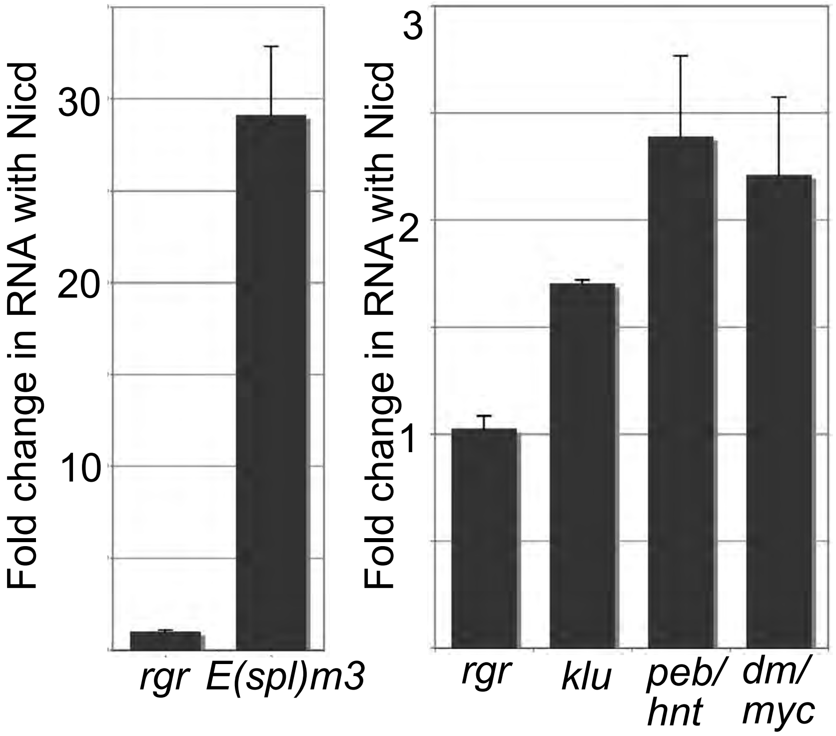

Fig. S1. Effects of transfected NICD on APG expression. Fold change in RNA levels of the indicated genes, detected by quantitative RT-PCR in populations of Kc cells transfected with NICD compared with empty vector. Based on measurements of transfection efficiencies, only 20-25% of the cells are likely to receive the NICD plasmid.

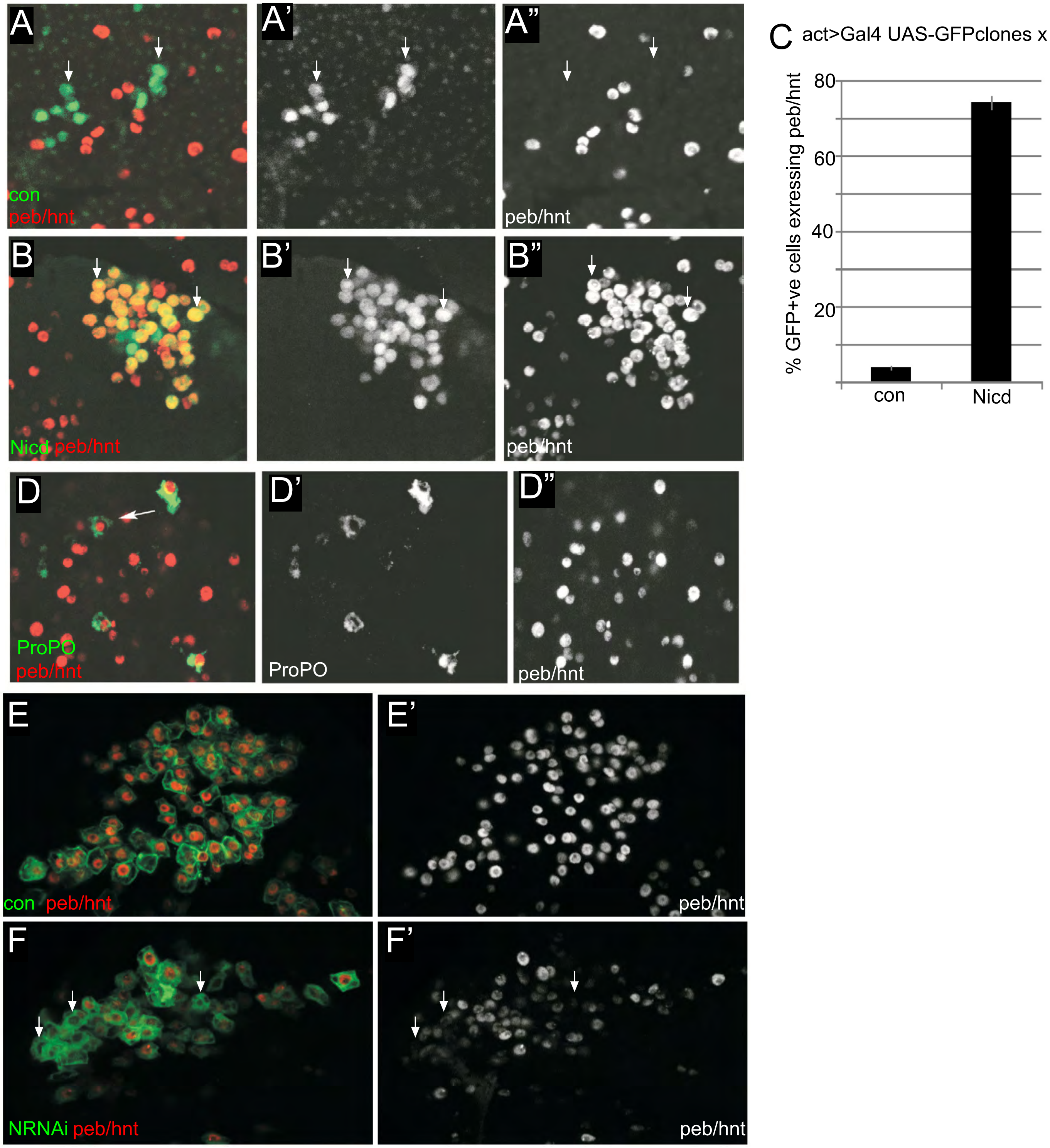

Fig. S2. Effects of NICD and NRNAi on Peb/Hnt expression and colocalization with ProPO in crystal cells. (A-C) Ectopic Peb/Hnt is detected in NICD-expressing clones. (**A**,**B**) Expression of Peb/Hnt (red, A,B; white, A″,B″) in lymph glands (LG) with (A) control (GFP only), (B) NICD-expressing clones [*hsFLP; act>stop>Gal4 UAS-GFP* combined with control (A) or *UAS-NICD* (B)]. (**C**) Percentages of GFP-labelled cells expressing Peb/Hnt were quantified in each genotype (15 control and 19 NICD LG). (**D-D**″) Hnt (red, D; white D″) is present in cells that express Pro-phenol oxidase (ProPO: D, green; white, D′) a marker of differentiated crystal cells (e.g. arrow in D). (**E**,**F**) Expression of *NRNAi* in crystal cell lineage (*lz-Gal4 UAS-GFP*) inhibits *peb/hnt* (red, E,F; white E″,F″) expression. White arrows indicate *lz-GFP* cell with reduced Peb/Hnt.

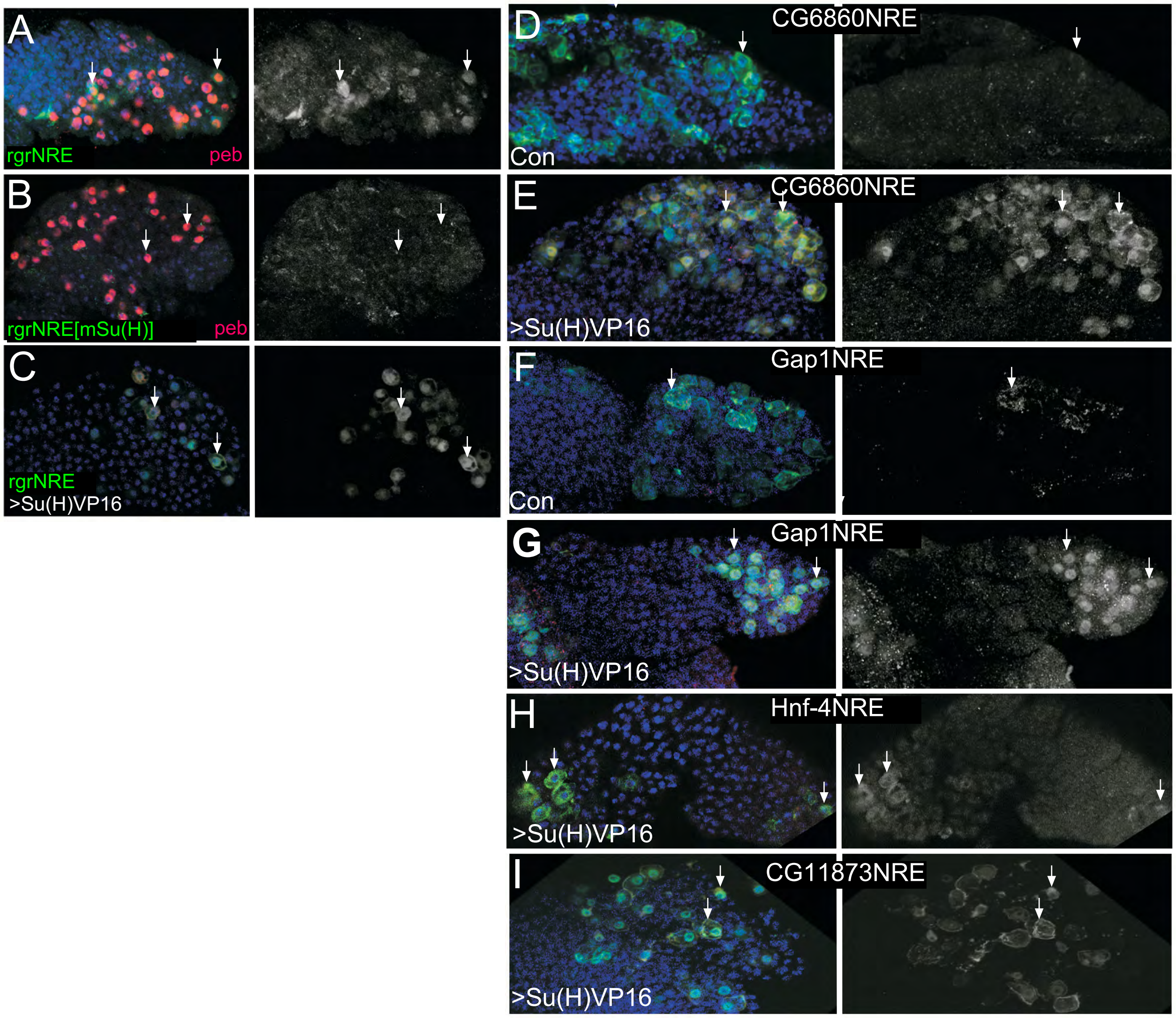

Fig. S3. Response of NRE reporters to Su(H)VP16. (**A-C**) Expression from *rgrNRE* (green, left column; single column grey scale right), either unmutated (A,C,) or with Su(H) motifs mutated: [mSu(H)] in wild type (A,B) or Su(H)VP16 overexpressing (C) tissue. Co-staining shows Peb/Hnt (A,B) or *lz-Gal4* (C) to mark crystal cell lineage. (**D-I**) Expression of the indicated reporters (red, left column; single channel grey scale, right column) in crystal cell lineage (*lz-Gal4 UAS-GFP*; green) in the absence (con; D and F) or presence of Su(H)VP16 (E,G-I).

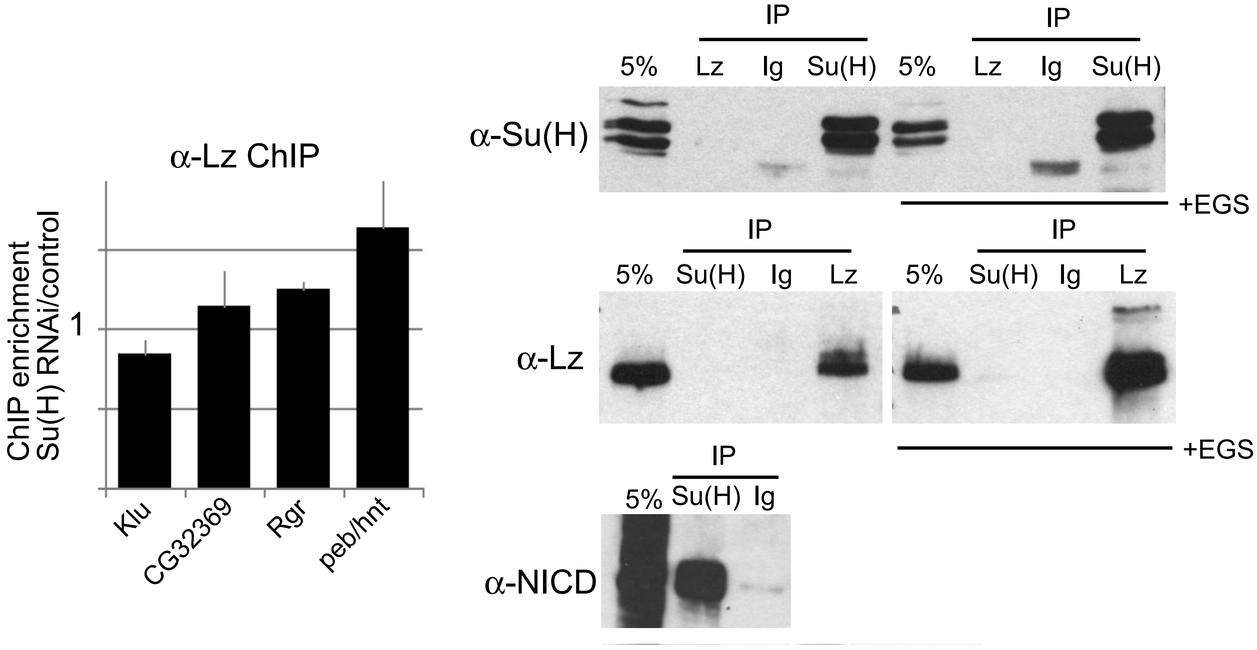

Fig. S4. Relationship of Su(H) and Lz recruitment. (**A**) Binding of Lz to the indicated enhancers is not inhibited in Kc cells treated with *Su(H)* RNAi. Graph shows the ratio of fragment enrichment in Lz ChIP from *Su(H)RNAi*-treated compared with control-treated cells. Su(H) mRNA levels were reduced to 23% of control levels. (**B**) Western blots of immunoprecipitations performed with the antibodies indicated above the lanes (Ig, indicates Goat anti_Ig control) were probed to detect Su(H), Lz and NICD as indicated. 5% indicates total proteins, 5% of input. EGS indicates proteins were crosslinked with EGS prior to immunoprecipitation. Upper panel, Su(H) does not co-immunoprecipitate with Lz; middle panel, Lz does not co-immunoprecipitate with Su(H); bottom panel, NICD co-immunoprecipitates with Su(H).

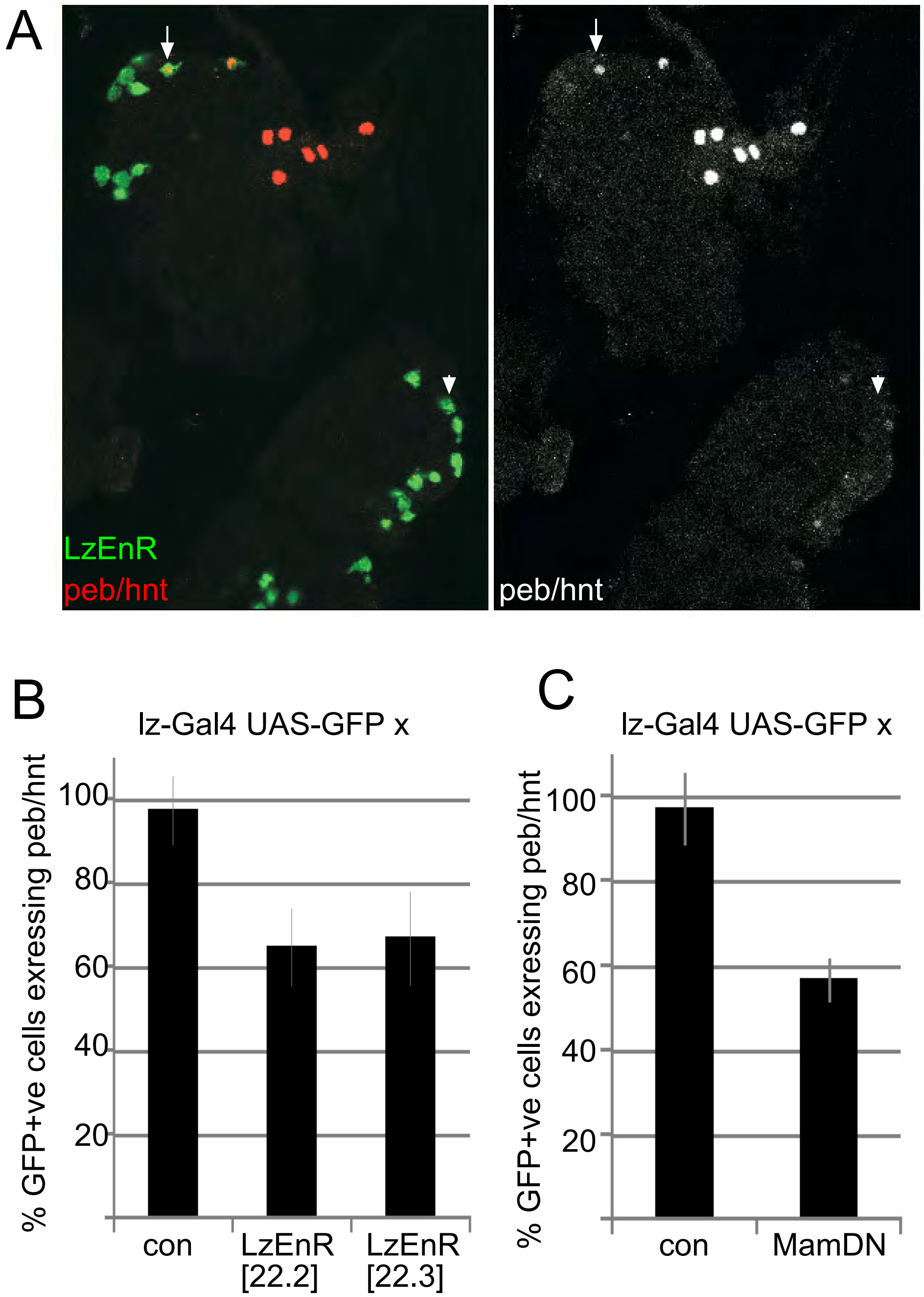

Fig. S5. Effect of LzEnR on Peb/Hnt expression. (**A**) Expression of LzEnR in crystal cell lineage (*lz-Gal4 UAS-GFP*) inhibits Peb/Hnt (red; left, single channel white) expression. White arrow indicates *lz>GFP* cell with residual Peb/Hnt; white arrowhead indicates *lz>GFP* cell with no detectable Peb/Hnt. Asterisk indicates patch of peb/hnt expressing cells of different origin. (**B**) Percentages of *lz>GFP*-labelled cells expressing Peb/Hnt were quantified in each genotype (over six LG per genotype). (**C**) Effects of MamDN in the same experiment are shown for comparison.

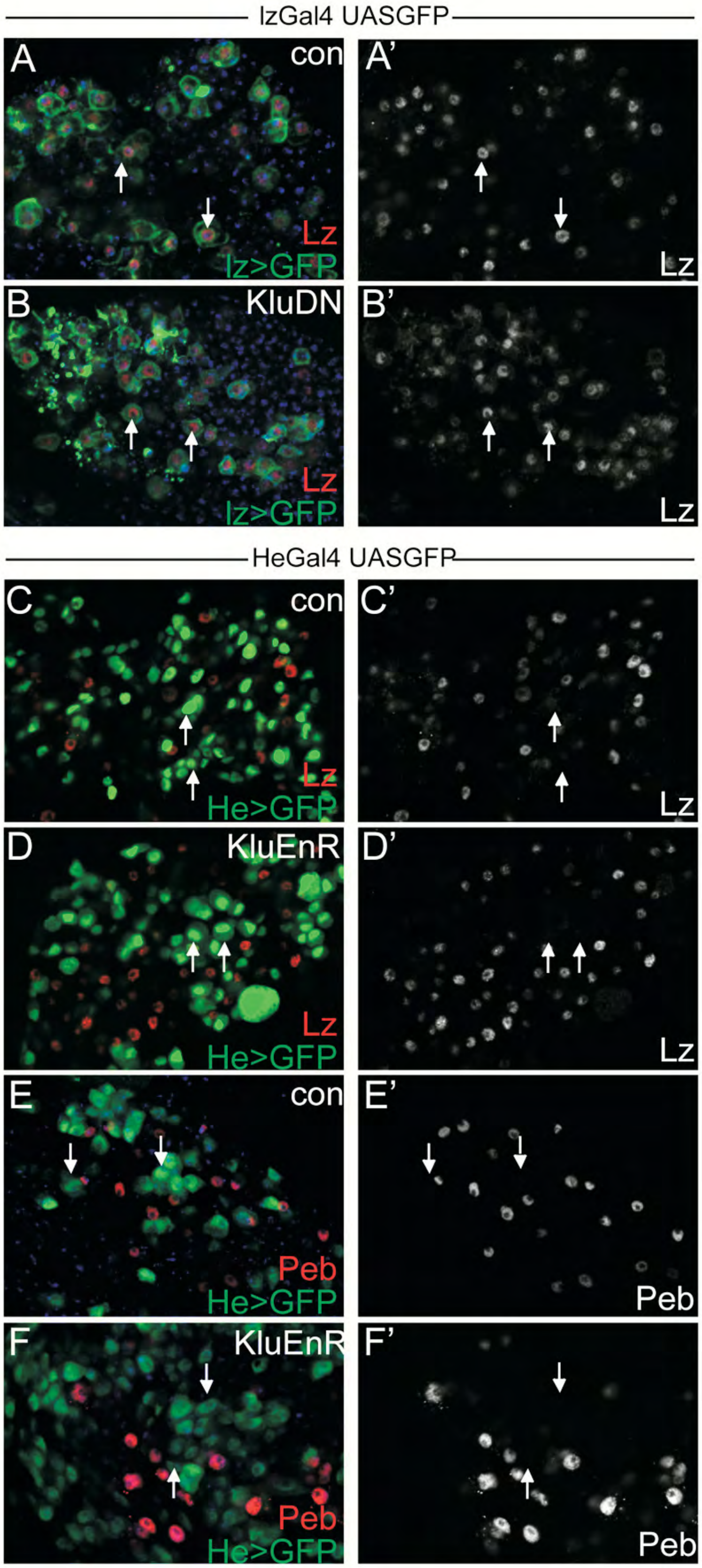

Fig. S6. Effects of KluDN on Lz and Peb/Hnt expression. (**A-D**) Lz (red) expression in control (con, A,C) or in lymph glands expressing KluDN (B; green *lz>GFP*) or KluEnR (D; green *He>GFP*). KluDN does not perturb Lz expression (arrows, B,B′; compare with A,A′) and KluEnR is not sufficient to cause ectopic Lz in the He>GFP cells (arrows, D,D′; compare with C,C′). (**E**,**F**) Peb/Hnt expression in wild-type or in lymph glands expressing KluEnR (F; green, He>GFP). No ectopic Peb/Hnt is detected in the KluEnR-expressing cells (arrows in F,F′, compare with E,E′).

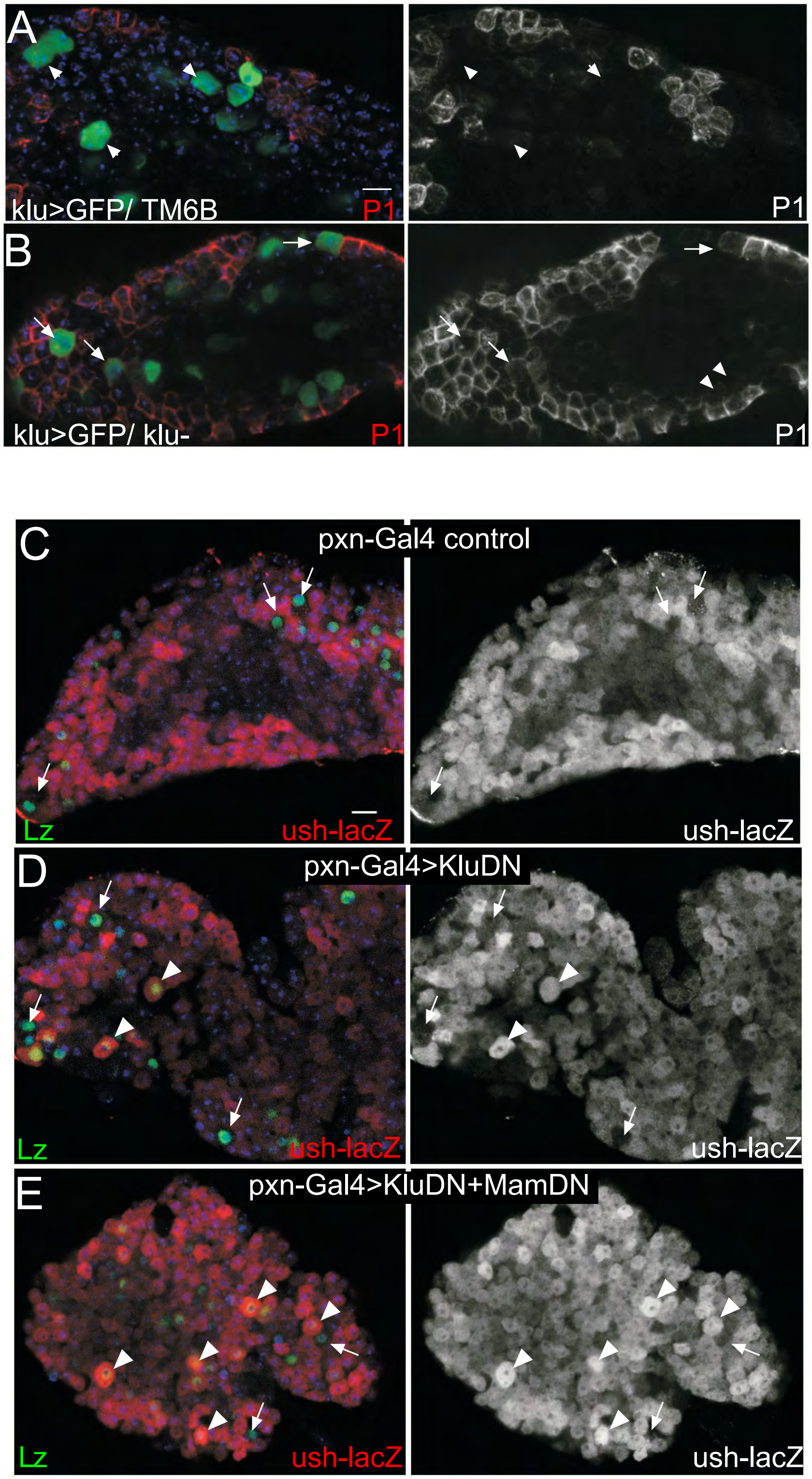

Fig. S7. Phenotype of Klu mutants and effects of KluDN on ush expression. (**A**,**B**) P1 (red; single channel, white) is not normally detected in *klu>GFP*-expressing cells (green, A) but is ectopically expressed in the *klu* mutant (*klu>GFP/ klu*). Arrowheads indicate *klu>GFP* cells with no P1 detectable; arrows indicate mutant *klu>GFP* cells with P1 present. (**C-E**) Heterogeneous expression of *ush-lacZ* (red, single channel white) in wild-type (con; E) and in glands broadly (*pxnGal4* expressing KluDN (D) or a combination of MamDN and KluDN (E). Crystal cell lineage (Lz, green) generally has a low levels of *ush-lacZ* (e.g. arrows) in wild type (D) Expression of KluDN gives rise to some Lz$^+$ cells that exhibit elevated *ush-lacZ* (e.g. arrowheads). (E) The proportion of Lz+ cells with elevated *ush-lacZ* is further increased by the combined expression of MamDN and KluDN (e.g. arrowheads, E).

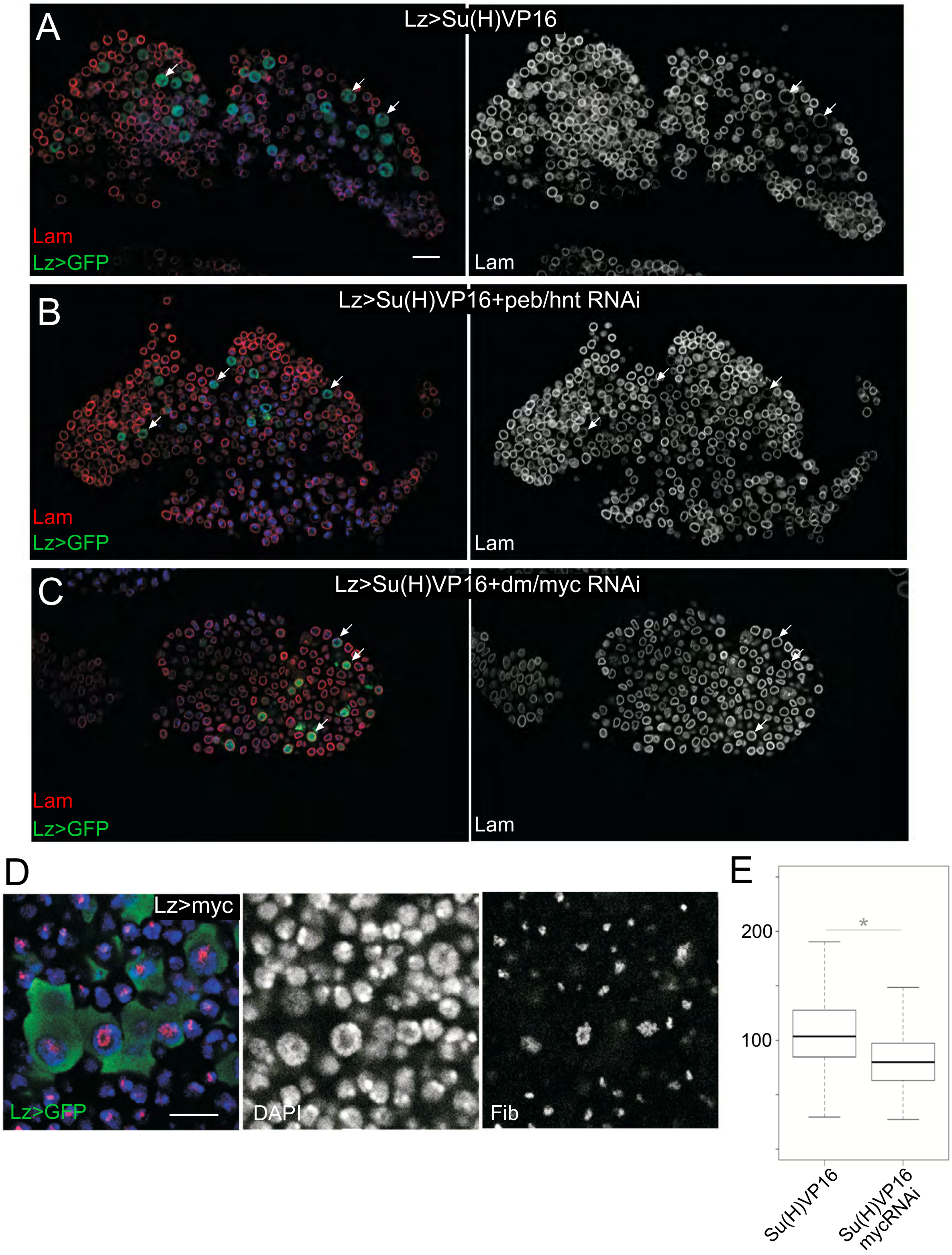

Fig. S8. Effects of Peb/Hnt and Myc on nuclear dimensions. (**A-C**) Expression of Su(H)VP16 (with *lz-Gal4 UAS-GFP*, green) causes significant enlargement of nuclei (A, e.g. arrows) that is suppressed by co-expression of RNAi targeting *peb/hnt* (B, e.g. arrows) or *dm/myc* (C, e.g. arrows). Nuclear Lamin (Lam red, white single channel) marks the nuclear periphery and DAPI (blue) stains DNA. (**D**) Co-expression of Myc with *lz-Gal4 UAS-GFP*(green) causes significant enlargement of nuclei, based on DAPI staining (e.g. arrows), and an increase in Fibrillarin (e.g. arrows). (**E**) A measurable increase in nuclei (DAPI) size obtained by expressing Su(H)VP16 (with *lz-Gal4*) is suppressed by co-expressing RNAi targeting *myc*. RNAi targeting *white* was used as control. Asterisk indicates that results were significantly different, *P*<2.2e-16, using the two-sample Kolmogorov-Smirnov test. Over 300 cells were scored for each genotype.

Table S1. Assigned peak genes in Kc cells. Genes significantly upregulated in Kc cells located within 10 kb of a region bound by Su(H) in ChIP. Table is ranked by AvgM, the average difference between expression levels after normalization (log2).

Table S2. Sequences of primers and co-ordinates used for cloning NREs

Klu2 chr3L :10998098..10999097
TAGGGTACCGGGCGTCCGGTAAATAGAAT
TAGTCTAGAGCTCCTATCCGATCACAACC

Gap1 chr3L :9840780..9841785
TAGGGTACCAACGTTTCGTCGTAGTCC
TAGTCTAGAGCTGCACTGAATCAATTTGC

Hnf4 chr2L :8696454..8697554
TAGGGTACCTCCGTCTGGCCTCTTAATTG
TAGACGCGTAACGGGCGAATAAATTGTGA

CG6860 chr2L :17379175..17380141
TAGGGTACCCGTGCCCACTGTAAGCAAT
TAGTCTAGATACCGGCAAACAAAACAACA

CG32369 chr3L :7785645..7786648
TAGGGTACCGGCAAAAAGGCAGAAATCAA
TAGTCTAGATAACTTTTATGGCCCGCTGA

CG17119 chr3R :18882020..18883218
TAGGGTACCGTCCTGCTGGCATCTTCTTC
TAGTCTAGAACTTGTAGCCATCGGCAGTC

CG11873 chr3R :24911888..24912888
TAGACGCGTCTTTCCCCCTCCAGACAAAT
TAGGGTACCCAAACACACGGACACAAAGG

rgr chr2R :4494945..4495937
TAGTCTAGAGGGCACTCTCATTCCAGGTA
TAGGCGGCCGCGAAGGTGTTCCACTTACCG

Myc1 chrX :3273788..3276869
TAGTCTAGAAAGATGTATCCACGCCCAAG
TAGGGTACCTAAGCGAACGAAGCCAAGAT

Myc2 chrX :3256693..3257724
TAGGCGGCCGCCCCGGAATGTCTGACTTTGT
TAGGGTACCACCACTGGAACCACTGGAAC

Myc3 chrX :3245789..3247157
TAGGCGGCCGCGCCAGTTTCCTTTTGGCATA
TAGGGTACCCCACAGCAAGGGTCGTATTT

Peb1 chrX :4471690..4473743
TAGTCTAGACGACAGAGCAGAGCAGTCAG
TAGGGTACCTCTCGAGTAAATGGGGATCG

Peb2 chrX :4497689..4498737
TAGTCTAGAAAGCTCATCCGACTGATTGG
TAGGGTACCAAGCGTCTCCGTTTCAAAAA

Peb3 chrX :4505487..4506671
TAGTCTAGACCCCGCTTTTGATCAACTAA
TAGGGTACCACTTCGCTCGAAATCGTTGT