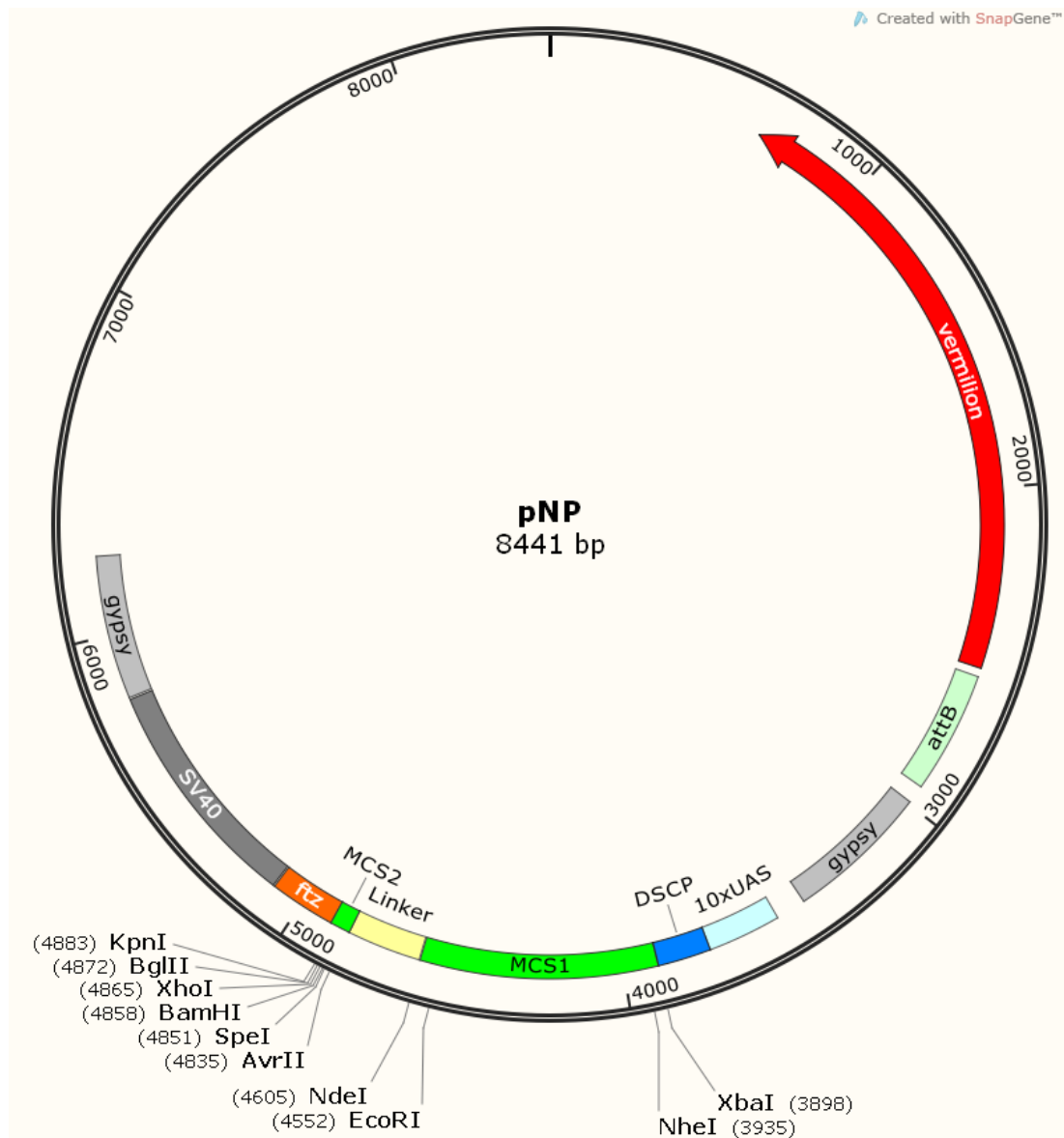


Supplementary Information

**An efficient and multiple target transgenic RNAi
technique with low toxicity in *Drosophila***

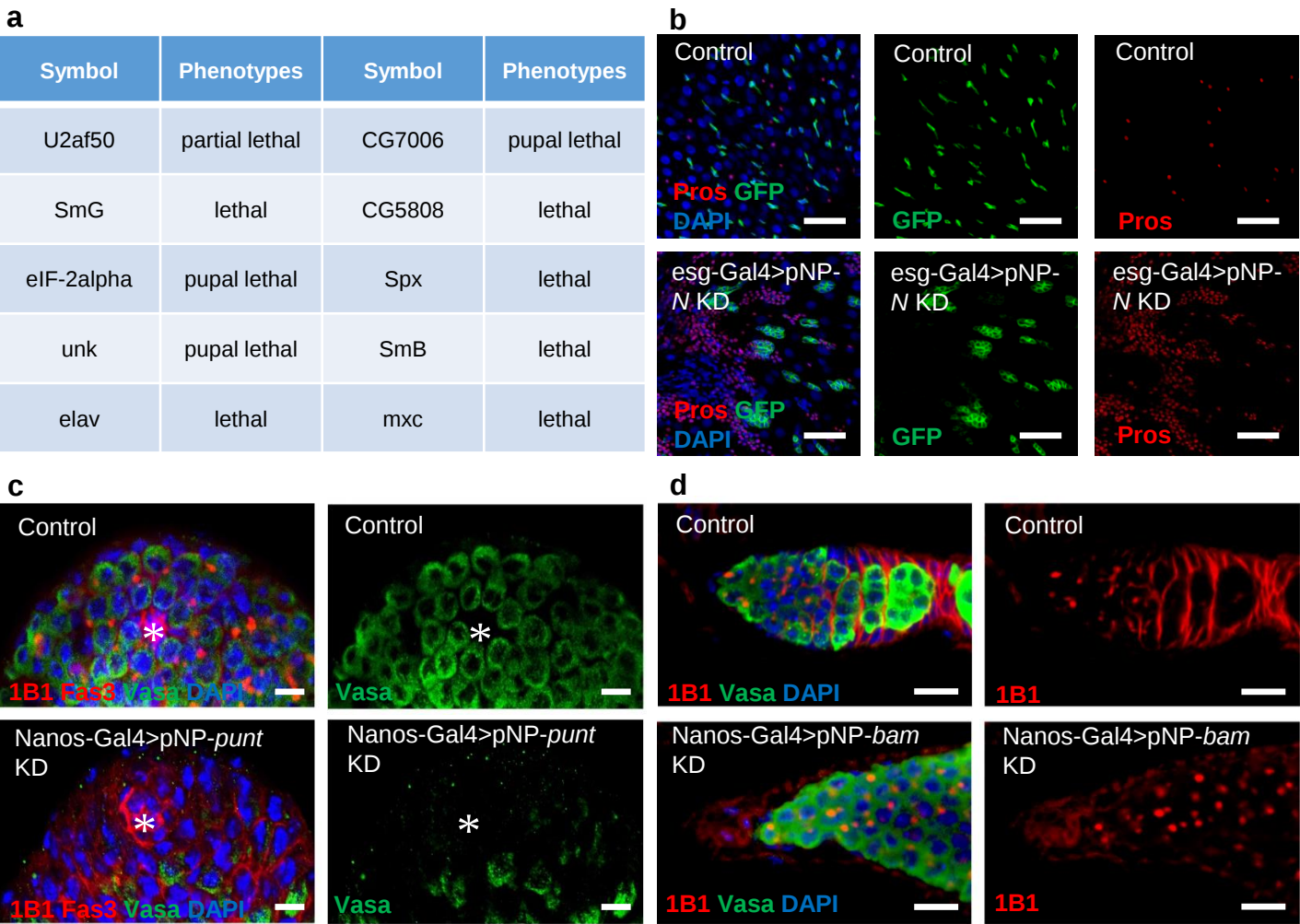
Qiao et. al.



Supplementary Figure 1

Map of pNP.

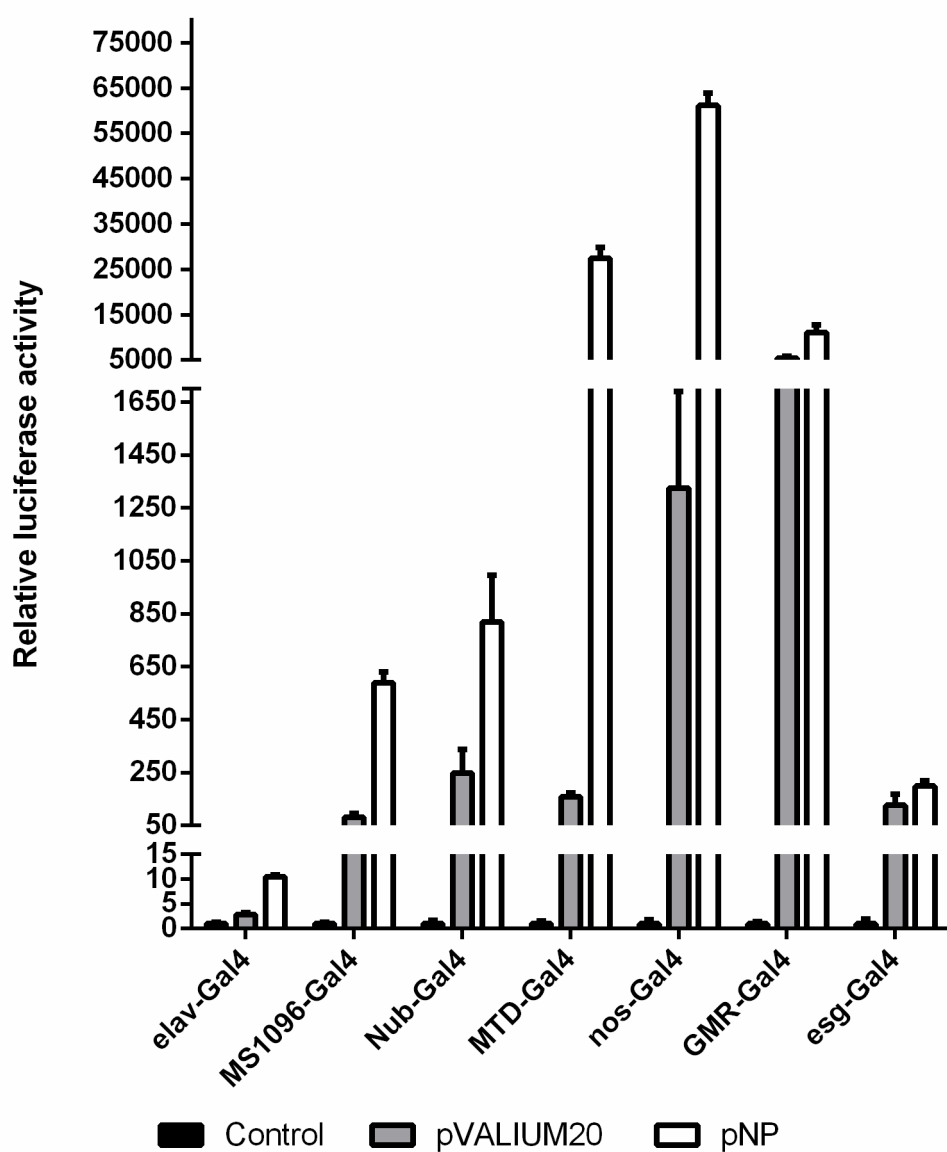
The pNP vector contains *vermillion* as a selectable marker, and an attB sequence to allow for phiC31 targeted integration at genomic attP landing sites. The *Drosophila* synthetic core promoter (DSCP) contains the TATA, Inr, MTE, and DPE sequence motifs. An intergenic linker between miR-2a-1 and miR-2b-2 allows precise processing and expression of multiple shRNAs. MCS1 allows a single shRNA to be cloned in both orientations, while with the help of MCS2 and the linker it could simultaneously generate multiple shRNAs once. The ftz intron, followed by the SV40 poly(A) tail, facilitates hairpin RNA expression, processing and export from the nucleus. The relevant sequences are flanked by two gypsy insulators to ensure stable transgenic expression.



Supplementary Figure 2

The pNP system works efficiently in the neurons, intestine, testis and ovarian stem cell system.

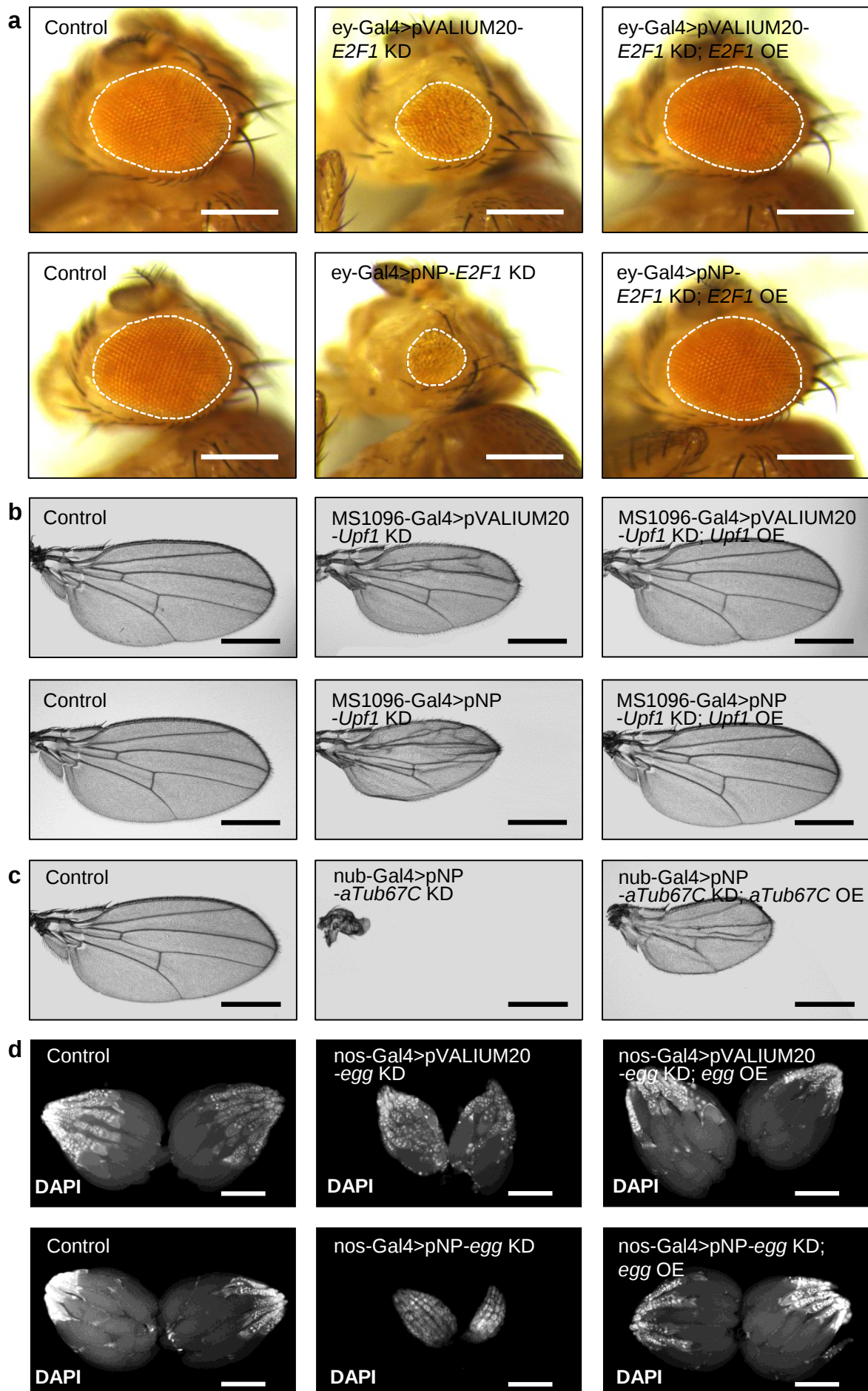
(a) Phenotypes of flies when target genes were knocked down in the neurons system driven by elav-Gal4. (b) Knockdown of *Notch* in the gut driven by esg-Gal4, UAS-GFP, tub-Gal80^{ts}/Cyo increased the number of intestinal stem cells (ISCs) and enteroendocrine cells (EE cells) compared with control. GFP (green) marks ISCs, Pros (red) marks EE cells, and DAPI (blue) marks nuclei. Scale bars, 20 μm. (c) The tip of a wildtype testis showing seven germline stem cells (GSCs) that contact the hub cells marked by asterisk, while the tips of *punt* knockdown testis showing no GSCs and germ cells close to the hub cells. Vasa (green) marks germ cells, Fasciclin III (red) marks the hub (asterisk), 1B1 (red) marks spectrosomes/fusomes, and DAPI (blue) marks nuclei. Scale bars, 10 μm. (d) Immunostaining of wild type ovary showing two GSCs in the germarium, while knockdown of *bam* increased the number of GSCs. Vasa (green) marks germ cells, 1B1 (red) marks spectrosomes/fusomes, and DAPI (blue) marks nuclei. Scale bars, 10 μm.



Supplementary Figure 3

The luciferase levels were significantly higher in pNP system than the pVALIUM20 system driven by different Gal4 lines.

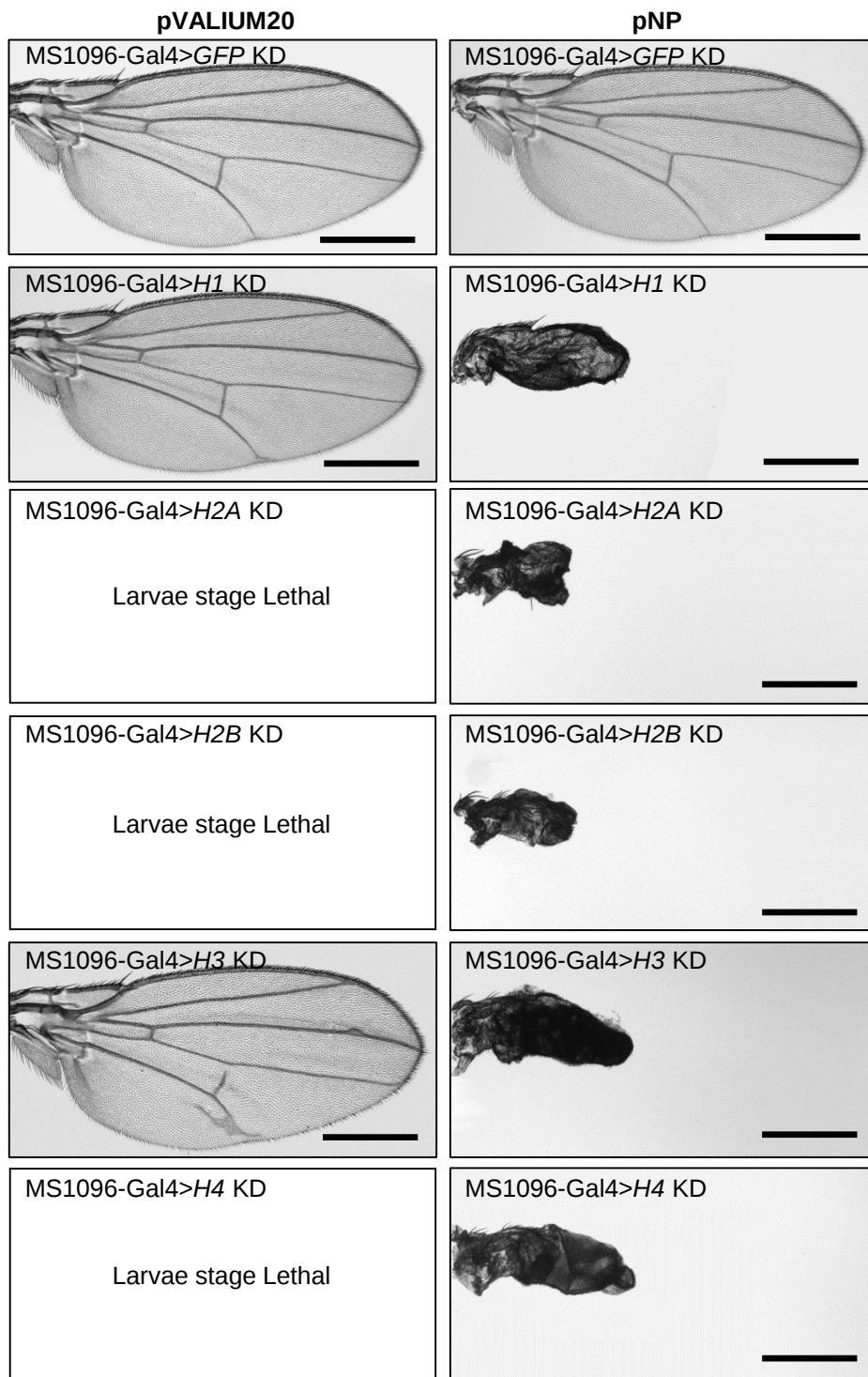
Relative luciferase expression levels at different tissues of pVALIUM20-*luciferase* and pNP-*luciferase* flies with different Gal4 drivers (n=5, mean±s.d.).



Supplementary Figure 4

The phenotypes produced by pNP system and pVALIUM20 system can be significantly rescued by co-expression of target genes.

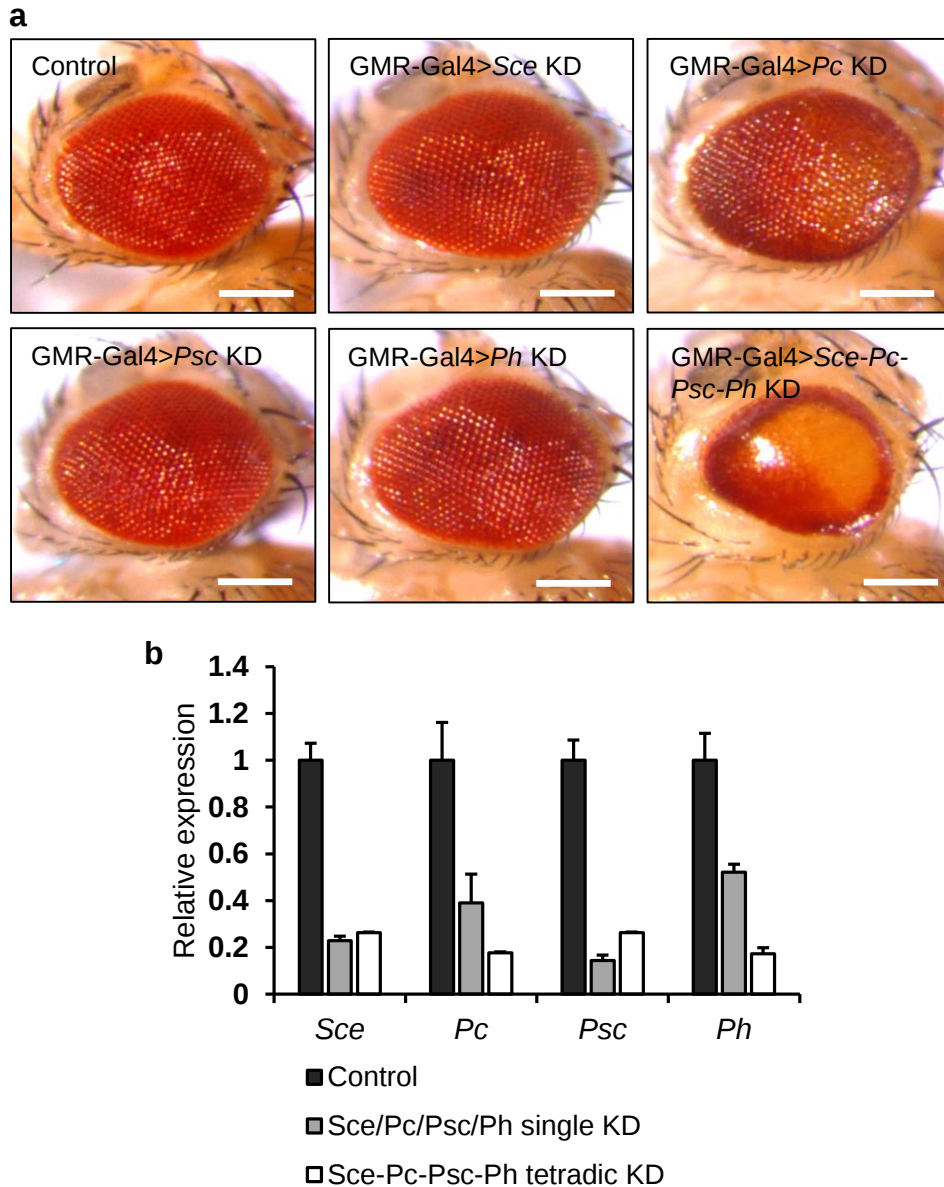
(a) Knockdown of *E2F1* in the eye using the pVALIUM20 system or the pNP system with the same shRNA driven by ey-Gal4 can be rescued by *E2F1* overexpression, respectively. Scale bars, 200 μm . (b) RNAi of *Upf1* by the pVALIUM20 system or the pNP system in the wing using MS1096-Gal4 can be rescued by *Upf1* overexpression, respectively. Scale bars, 500 μm . (c) Targeting of *aTub67C* with the pNP system driven by Nub-Gal4 can be rescued by *aTub67C* overexpression. We did not performed this rescue assay in pVALIUM20- *aTub67C* flies, as it showed no phenotype driven by Nub-Gal4. Scale bars, 500 μm . (d) Knockdown of *egg* in the germline controlled by nos-Gal4 can be rescued by *egg* overexpression. Scale bars, 300 μm .



Supplementary Figure 5

The pNP system could efficiently modulate high expression genes.

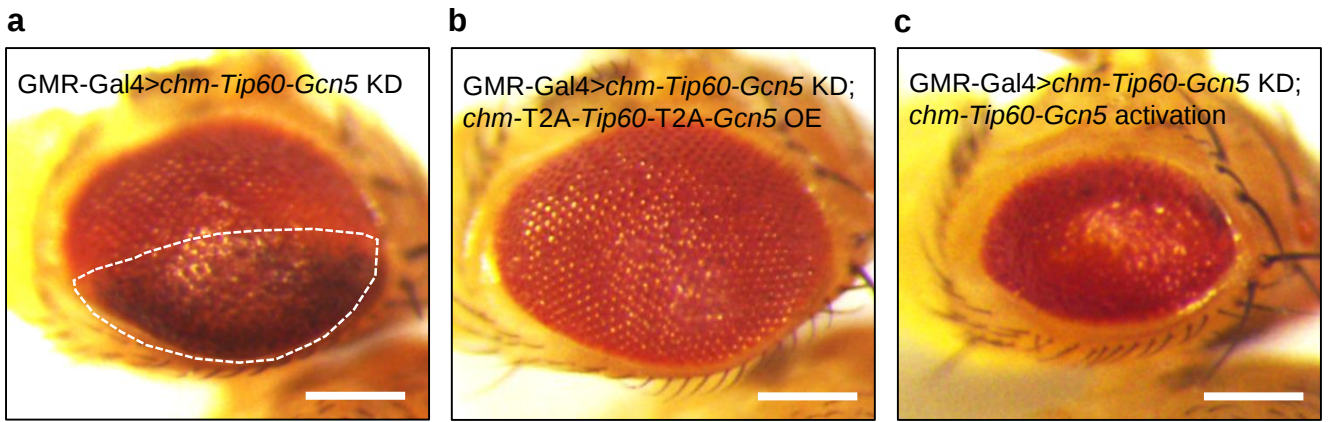
When driven by MS1096-Gal4, knockdown of all the histones using the pNP system generated a phenotype with severe wing developmental defects. Using the pVALIUM20 system, depletion of H1 and H3 produced an almost wild type phenotype, while depletion of H2A, H2B or H4 caused lethality. Scale bars, 500 μ m.



Supplementary Figure 6

Simultaneous knockdown of four subunits of PRC1 generated severe eye defects using the pNP system.

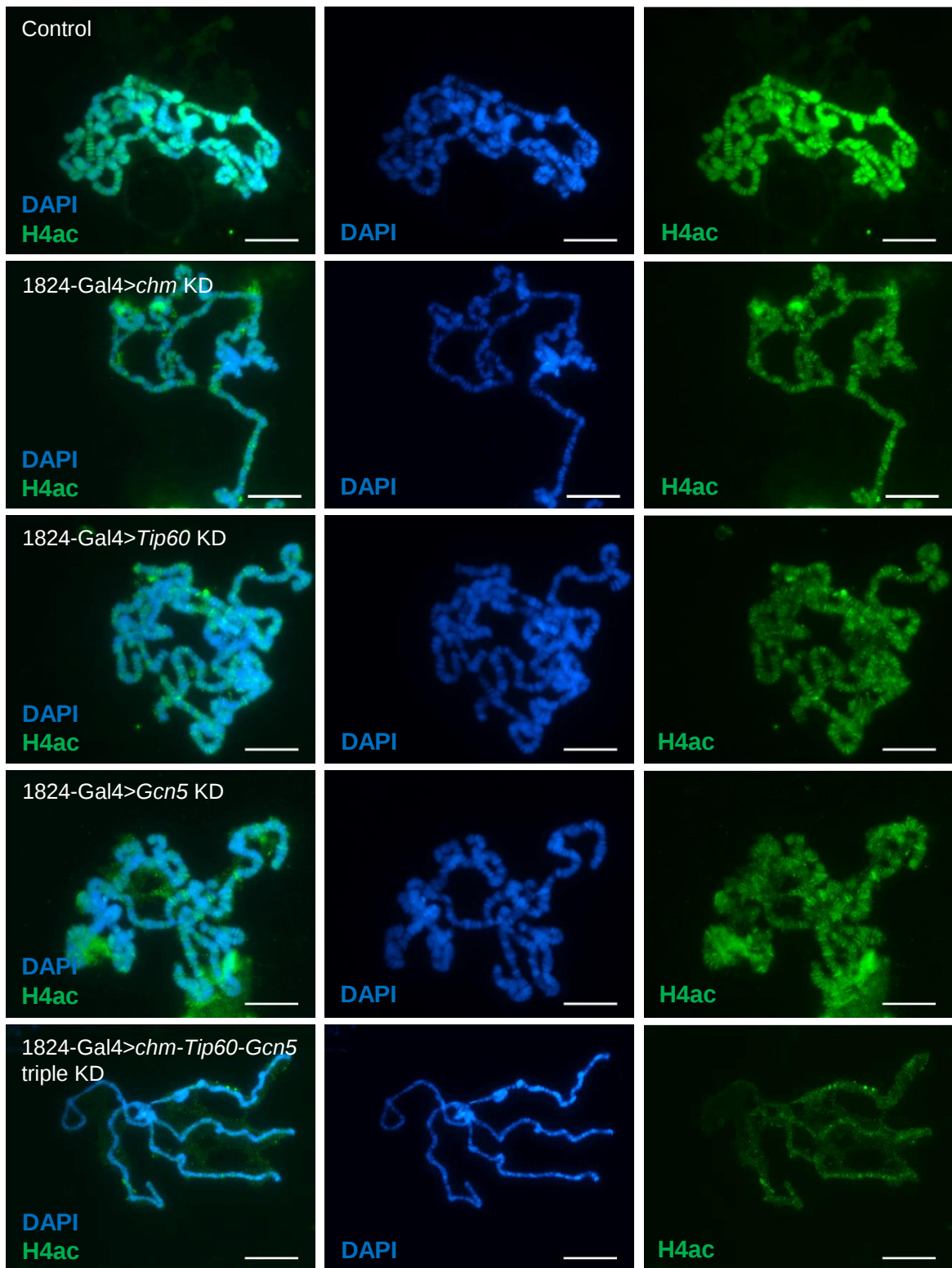
(a) Simultaneous knockdown of four subunits of PRC1 driven by GMR-Gal4 showed severe phenotypes: loss of pigmentation, fused ommatidia and small eyes, while knock down of one or two subunits of PRC1 exhibited no obvious phenotype. Scale bars, 200 μ m. (b) qRT-PCR results show that the efficiency of single-gene knock down and tetradic knock down for *Sce*, *Pc*, *Psc*, *Ph* was comparable ($n=3$, mean $\pm$ s.d.).



Supplementary Figure 7

Overexpression of *chm-T2A-Tip60-T2A-Gcn5* or activation of these HATs simultaneously can significantly rescue the eye defect phenotype in *chm-Tip60-Gcn5* KD flies.

Simultaneous knockdown of *chm-Tip60-Gcn5* using the pNP system driven by GMR-Gal4 produced severe eye defects (a), which can be rescued by overexpression of *chm-T2A-Tip60-T2A-Gcn5* (b) and the activation of *chm-Tip60-Gcn5* simultaneously (c). Scale bars, 200 μ m.



Supplementary Figure 8

Synergistic effects of HATs in histone acetylation.

Compared with control, single gene knockdown of *chm*, *Tip60*, *Gcn5* slightly decreased the global H4 acetylation level, while triple gene knockdown of *chm-Tip60-Gcn5* induced much less global H4 acetylation driven by 1824-Gal4. DNA was visualized with DAPI (blue) and H4ac was stained with rabbit polyclonal anti-acetyl-histone H4 antibody (green). Scale bars, 20 μ m.

Supplementary Note 1: Sequence of pNP vector

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