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Supplemental Information

Headcase and Unkempt Regulate Tissue Growth and Cell Cycle Progression in Response to Nutrient Restriction

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Figure S1

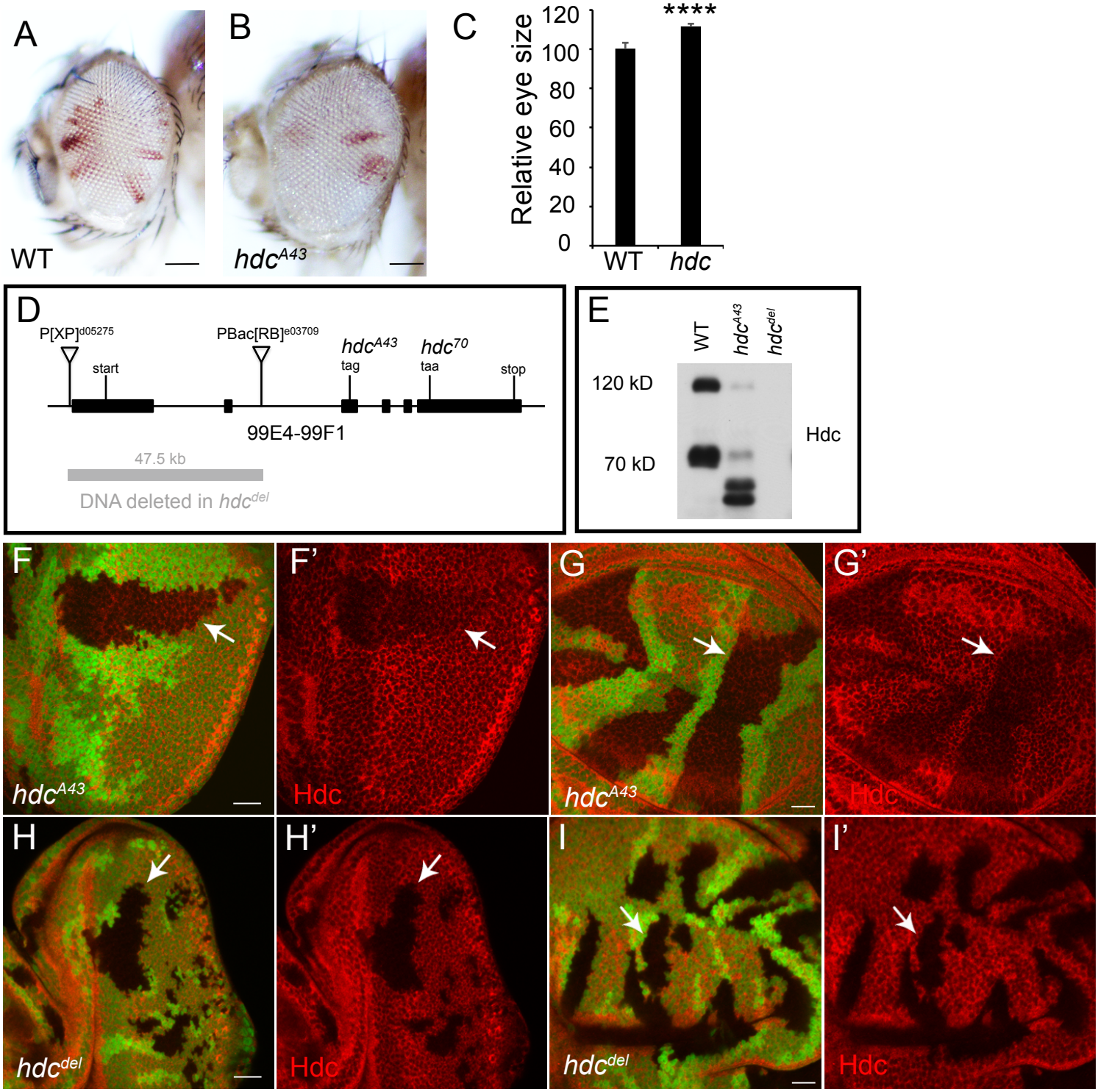

A
WT
B
hdcA43
C
Relative eye size
120
100
80
60
40
20
0
****
WT
hdc
D
P[XP]d05275
start
PBac[RB]e03709
hdcA43
tag
hdc70
taa
stop
99E4-99F1
47.5 kb
DNA deleted in hdcdel
E
WT
hdcA43
hdcdel
120 kD
70 kD
Hdc
F
hdcA43
F'
Hdc
G
hdcA43
G'
Hdc
H
hdcdel
H'
Hdc
I
hdcdel
I'
Hdc

Figure S1. Isolation and characterization of *hdc* mutant alleles. Related to Figure 1.
(A-C) Adult eyes comprised predominantly of control cells (A) or hdc^{A43} cells (B). The genotypes are: *y w ey-flp; FRT 82B/FRT 82B P[w+], L(3)c1-R3* (A) and *y w ey-flp; FRT 82B* hdc^{A43} */FRT 82B P[w+], L(3)c1-R3* (B). Quantification of relative eye sizes (C). For each analysis (A and B), a total of 10 fly eyes (n=10) were used. The scale bars represent 0.1 mm.
(D) Genomic organization of the *hdc* gene. Also shown is the in frame stop codon (TAA) at *hdc* locus. The nonsense mutation (TAG) of hdc^{A43} allele and the 47kb genomic DNA deleted from the hdc^{del} allele are also indicated.
(E) Western blot from the extracts of third instar larvae with following genotypes: wild type (Left lane); hdc^{A43} / hdc^{A43} (Middle lane); hdc^{del} / hdc^{del} (Right lane).
(F-G') A third instar eye disc (F-F') or wing disc (G-G') containing hdc^{A43} mutant clones (marked by the absence of GFP), and stained for Hdc protein (red). Note the decreased, but still detectable, Hdc staining (F' and G') in hdc^{A43} mutant clones (arrows). The scale bars represent 20 μm.
(H-I') A third instar eye disc (H-H') or wing disc (I-I') containing hdc^{del} mutant clones (marked by the absence of GFP), and stained for Hdc protein (red). Note the complete absence of Hdc staining (H' and I') in hdc^{del} mutant clones (arrows). The scale bars represent 20 μm.
Data in (C) are represented as means ± SD; ****P<0.0001.

Figure S2

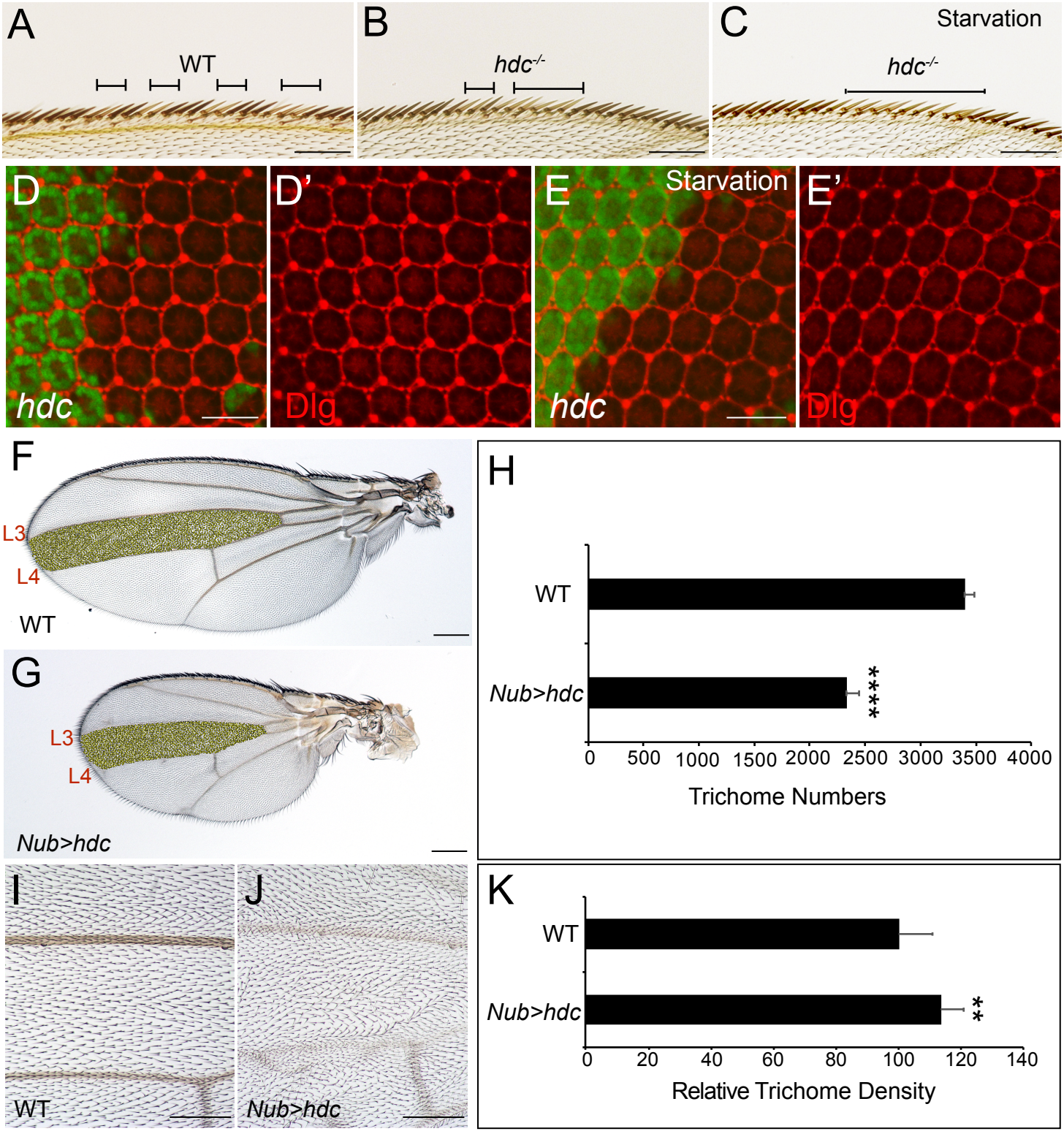

A
WT
B
hdc-/-
C
Starvation
hdc-/-
D
hdc
D'
Dlg
E
Starvation
hdc
E'
Dlg
F
L3
L4
WT
G
L3
L4
Nub>hdc
H
WT
Nub>hdc
****
0 500 1000 1500 2000 2500 3000 3500 4000
Trichome Numbers
I
WT
J
Nub>hdc
K
WT
Nub>hdc
**
0 20 40 60 80 100 120 140
Relative Trichome Density

Figure S2. Hdc regulates cell number. Related to Figure 3.
(A-C) Adult wing margins containing wild type (A), and *hdc* (B and C) mutant bristles (marked by y^-, indicated by lines above the wing margins). Note that *hdc* mutant bristles (yellow color) are indistinguishable in cell size from wild-type cells under both nutrient rich (B) and nutrient starvation conditions (C). The scale bars represent 0.1 mm.
(D–E') Mid-pupal retina containing *hdc* mutant clones marked by the lack of GFP, and stained for Discs-Large (red). Note loss of *hdc* does not cause any appreciable effect on cell size under both nutrient rich (D) and nutrient starvation (E) conditions. The scale bars represent 20 μm.
(F-H) Trichome numbers between L3 and L4 veins of a control wing (F) and a wing with Hdc overexpression (G) are quantified by Fijiwings (Dobens and Dobens, 2013). For each analysis (F and G), a total of 10 fly wings (n=10) were used. Note reduced trichome number in Hdc overexpression wings (H). The scale bars represent 0.2 mm.
(I-K) Close-up images of a control wing (I) and a wing with Hdc overexpression (J). Trichome densities are quantified (K). For each analysis (I and J), a total of 10 fly wings (n=10) were used.The scale bars represent 0.1 mm.
Data in (H) and (K) are represented as means ± SD; **P<0.01, ****P<0.0001.

Figure S3

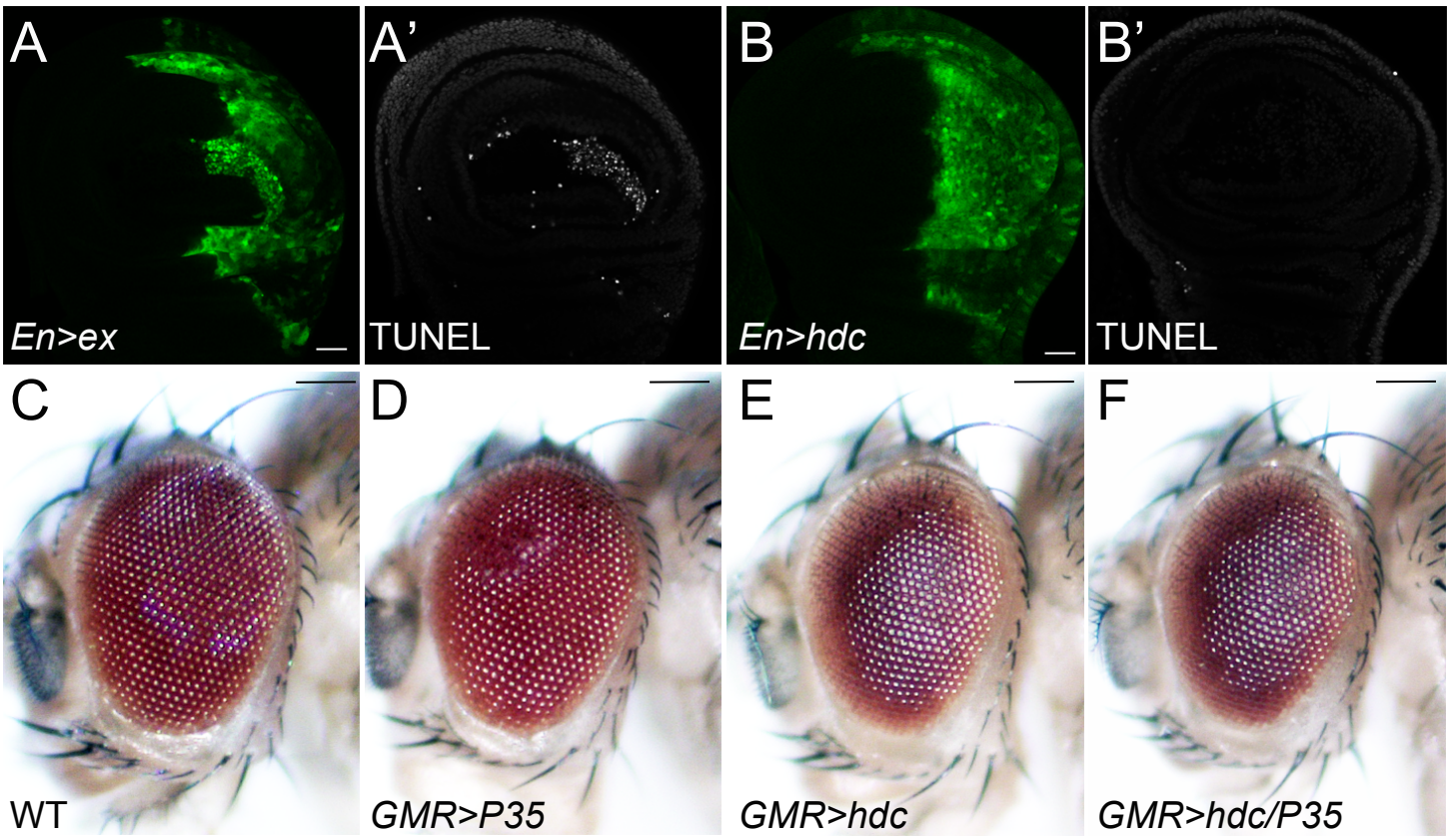

A
En>ex
A'
TUNEL
B
En>hdc
B'
TUNEL
C
WT
D
GMR>P35
E
GMR>hdc
F
GMR>hdc/P35

Figure S3. Hdc does not regulate cell death. Related to Figure 3.
(A-B) TUNEL staining of third instar wing discs containing *expanded* (A) or *hdc* (B) overexpressing clones (GFP-positive). Note the ectopic cell death in *ex*-overexpressing (A'), but not *hdc*-overexpressing clones (B'). The scale bars represent 20 μm.
(C-F) Images of compound eyes from the following genotypes: (C) *GMR-gal4*, (D) *GMR-gal4; UAS-P35*, (E) *GMR-gal4; UAS-hdc*, and (F) *GMR-gal4; UAS-hdc/UAS-P35*. The scale bars represent 0.1 mm

Fig S4

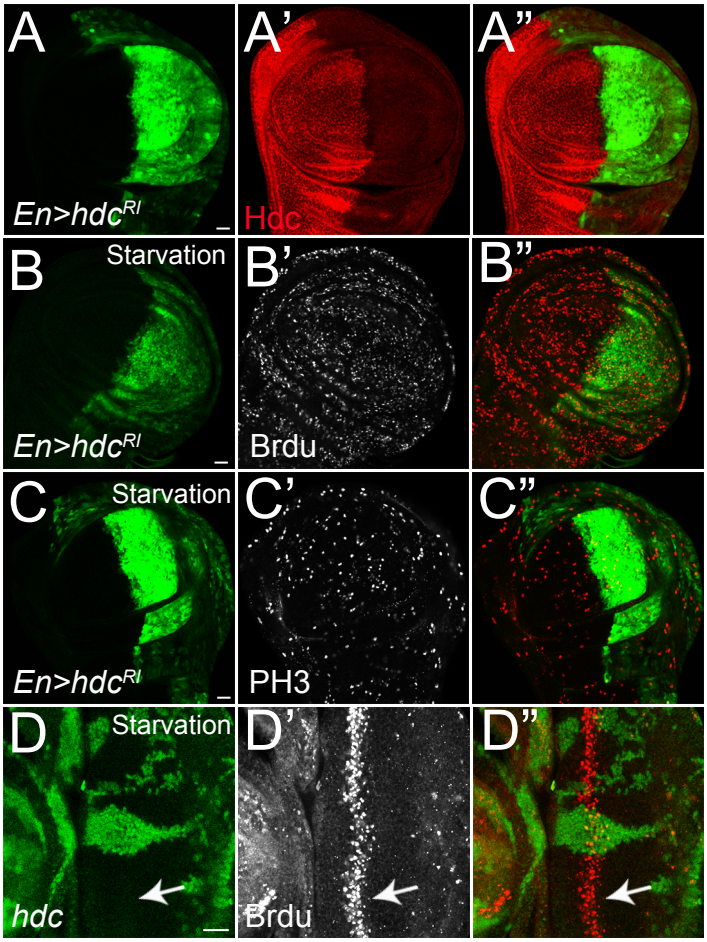
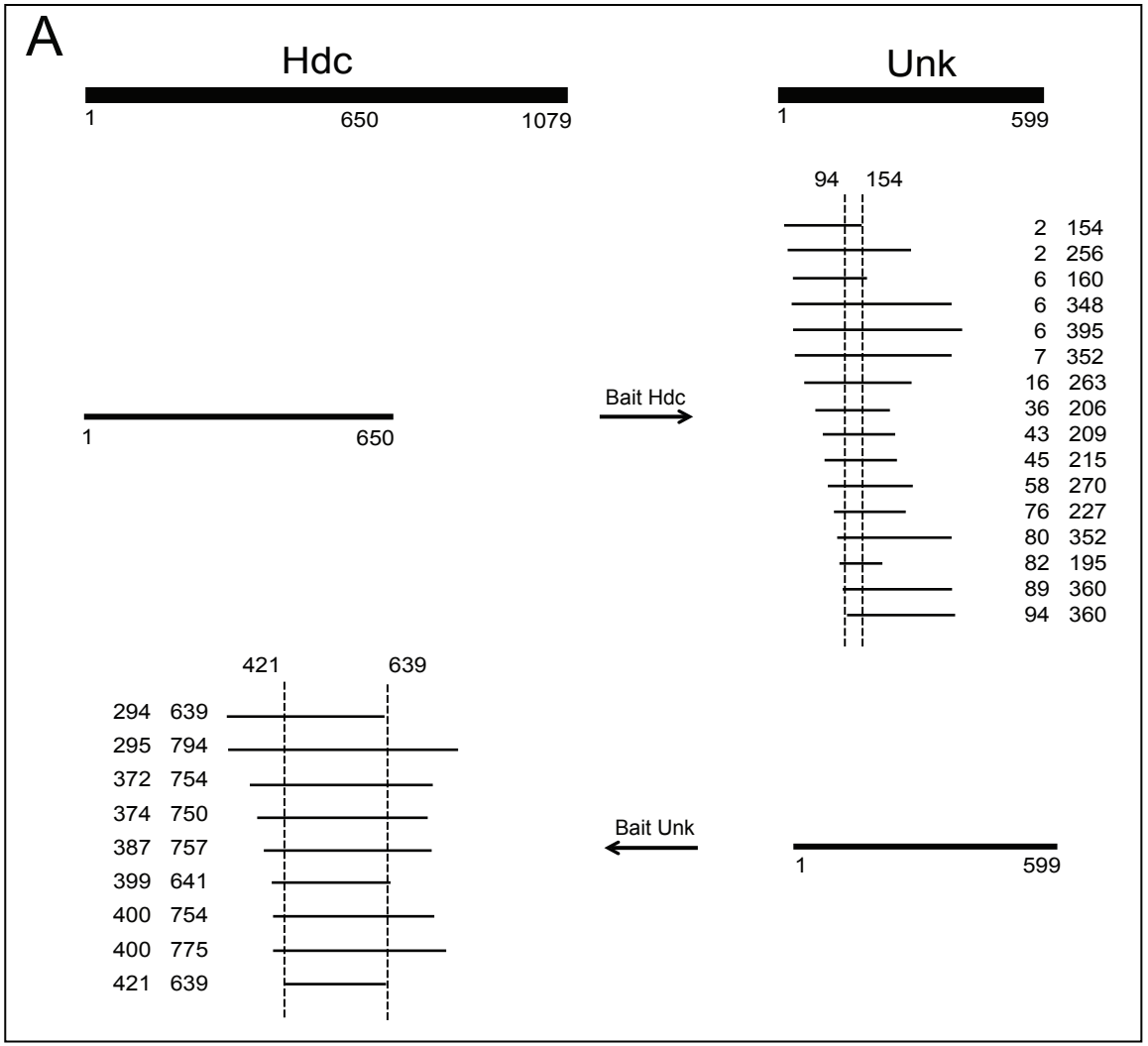
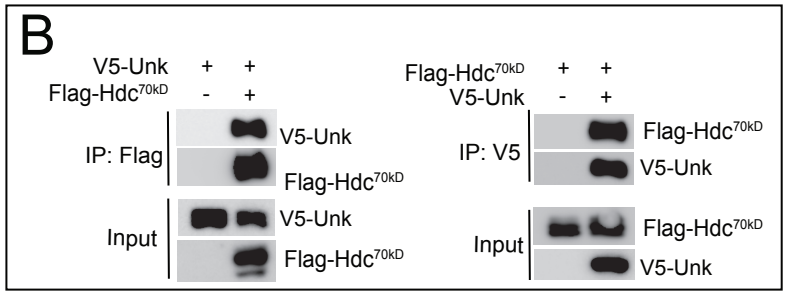
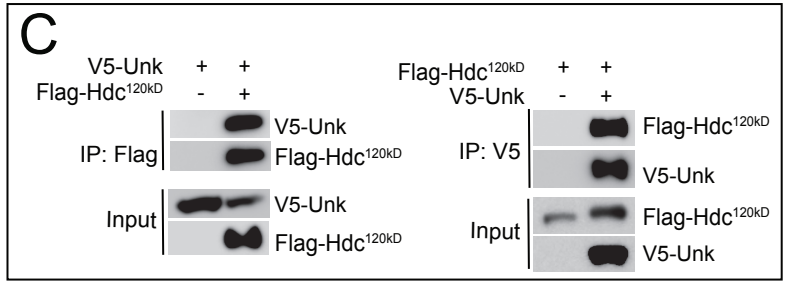

A
En>hdc^RI
A'
Hdc
A"
B
Starvation
En>hdc^RI
B'
Brdu
B"
C
Starvation
En>hdc^RI
C'
PH3
C"
D
Starvation
hdc
D'
Brdu
D"

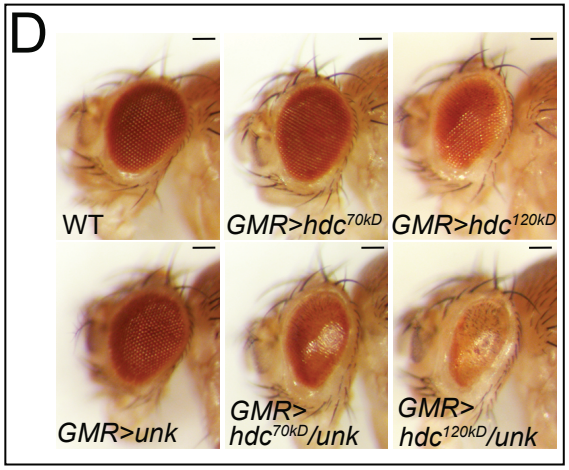
Figure S4. Hdc regulates cell cycle progression. Related to Figure 3.
(A-C') Third instar wing discs containing hdc^{RNAi}-overexpressing clones (GFP-positive) were stained for Hdc (A'), Brdu (B'), and PH3 (C'). Note the mild decreased staining of Brdu in hdc^{RNAi}-overexpressing clones. The scale bars represent 20 μm.
(D-D") A third instar eye disc containing *hdc* mutant clones (GFP-negative) raised under nutrient starvation conditions were stained for Brdu (D'). Note Brdu staining in *hdc* mutant cells are more anterior than wild-type control cells in the SMW (D', arrow). The scale bar represents 20 μm.

Fig S5


A
Hdc
1
650
1079
Unk
1
599
94
154
2 154
2 256
6 160
6 348
6 395
7 352
16 263
36 206
43 209
45 215
58 270
76 227
80 352
82 195
89 360
94 360
Bait Hdc
1
650
421
639
294 639
295 794
372 754
374 750
387 757
399 641
400 754
400 775
421 639
Bait Unk
1
599


B
V5-Unk + +
Flag-Hdc70kD - +
IP: Flag
V5-Unk
Flag-Hdc70kD
Input
V5-Unk
Flag-Hdc70kD
Flag-Hdc70kD + +
V5-Unk - +
IP: V5
Flag-Hdc70kD
V5-Unk
Input
Flag-Hdc70kD
V5-Unk


C
V5-Unk + +
Flag-Hdc120kD - +
IP: Flag
V5-Unk
Flag-Hdc120kD
Input
V5-Unk
Flag-Hdc120kD
Flag-Hdc120kD + +
V5-Unk - +
IP: V5
Flag-Hdc120kD
V5-Unk
Input
Flag-Hdc120kD
V5-Unk


D
WT
GMR>hdc70kD
GMR>hdc120kD
GMR>unk
GMR> hdc70kD/unk
GMR> hdc120kD/unk

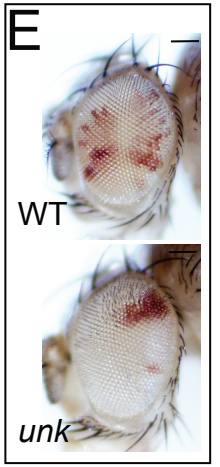
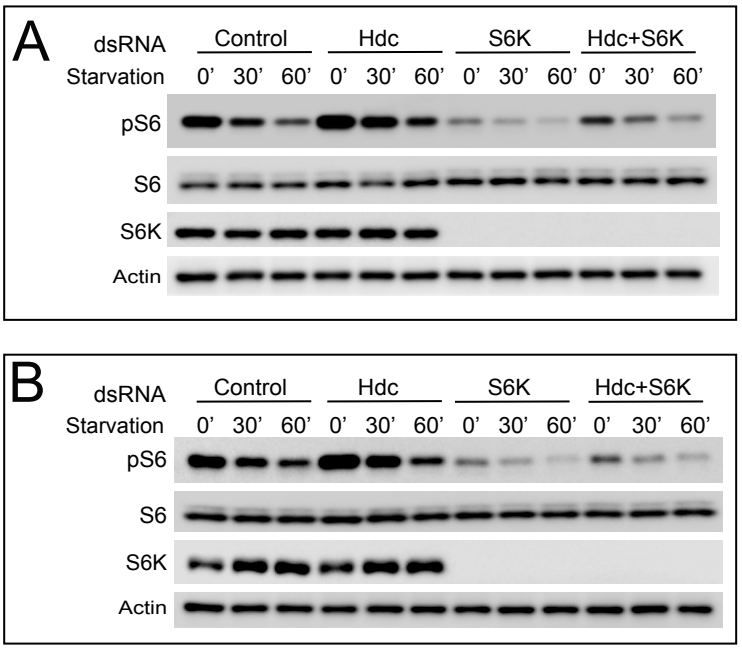

E
WT
unk

Figure S5. Hdc interacts with Unk. Related to Figure 4.
(A) Unbiased yeast two-hybrid screens identify Hdc and Unk as interacting proteins. Schematics of the Bait and the interacting preys from each screen are shown. Also shown are the minimal binding regions based on overlapping prey sequences.
(B-C) Physical association between Unk and Hdc 70kD (B) form or 120kD form (C). Immunoprecipitates of S2 cell lysate expressing the indicated combination of FLAG-Hdc and V5-Unk constructs were probed with the indicated antibodies.
(D) Images of adult eyes from the indicated genotypes. The scale bars represent 0.1 mm.
(E) Adult eyes comprised predominantly of control cells (up) or unk^{ex24} cells (B). The genotypes are: *y w ey-flp; FRT 82B/FRT 82B P[w+], L(3)c1-R3* (up) and *y w ey-flp; FRT 82B* unk^{ex24} */FRT 82B P[w+], L(3)c1-R3* (bottom). The scale bars represent 0.1 mm.

Figure S6


A
dsRNA
Control
Hdc
S6K
Hdc+S6K
Starvation
0’ 30’ 60’ 0’ 30’ 60’ 0’ 30’ 60’ 0’ 30’ 60’
pS6
S6
S6K
Actin

B
dsRNA
Control
Hdc
S6K
Hdc+S6K
Starvation
0’ 30’ 60’ 0’ 30’ 60’ 0’ 30’ 60’ 0’ 30’ 60’
pS6
S6
S6K
Actin

Figure S6. Hdc and Unk regulate pS6 partially bypass S6K. Related to Figure 5.
(A-B) S2R+ cells were incubated with dsRNA of GFP, *hdc*, or *unk* in combination with dsRNA of S6K for three days and treated with PBS starvation for indicated time before western blot. Total cell lysates were probed with indicated antibodies. Note that RNAi of *hdc* or *unk* partially rescued S6 phosphorylation decreases caused by S6K RNAi knock-down.

Figure S7

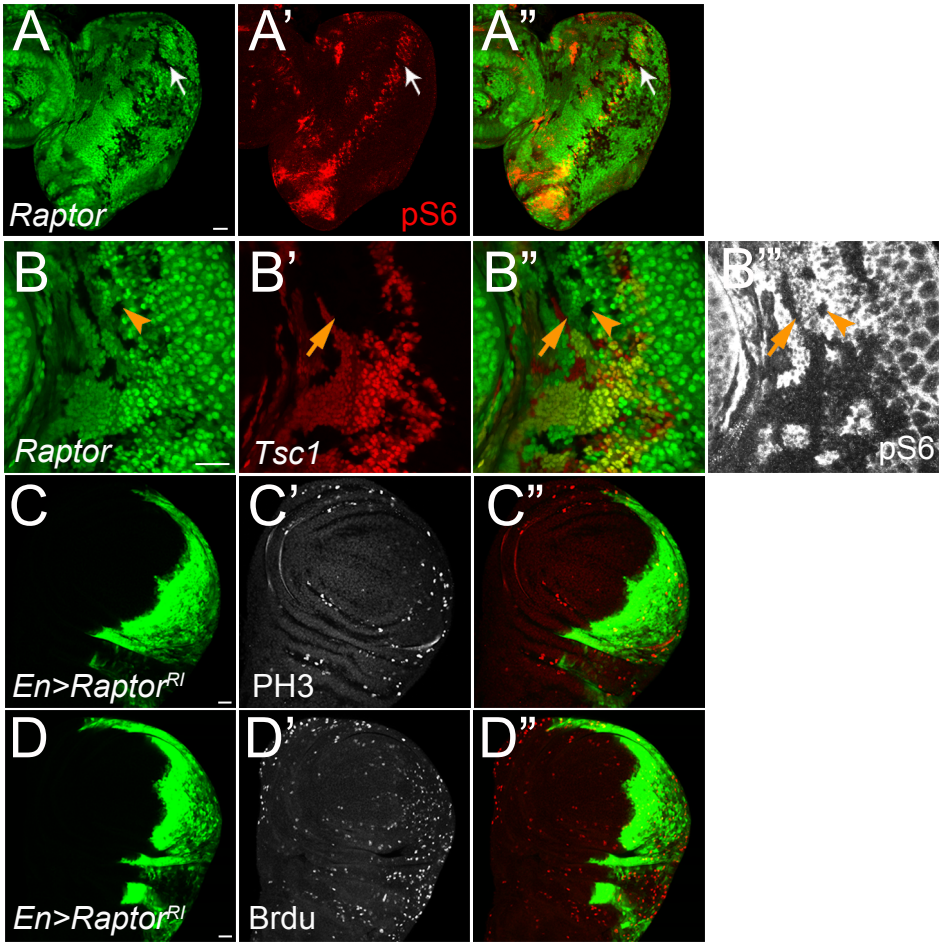

A
A'
A"
Raptor
pS6
B
B'
B"
B'"
Raptor
Tsc1
pS6
C
C'
C"
En>Raptor^RI
PH3
D
D'
D"
En>Raptor^RI
Brdu

Figure S7. Raptor regulates pS6 and cell cycle progression. Related to Figure 6.
(A-A") A third instar eye disc containing *raptor* mutant clones (A, GFP negative) was stained with pS6 antibody (A', red). Note the pS6 staining is abolished in *raptor* mutant clones (A', arrow). The scale bar represents 20 μm.
(B-B"') Eye discs containing mutant clones of the indicated genotypes were stained with pS6 antibody (white). Mutant clones of *raptor* were marked by loss of GFP, and mutant clones of *tsc1* were marked by loss of RFP. Note the pS6 staining in *tsc1* mutant clones was completely suppressed with loss of *raptor* (arrowheads, black areas in the merged channel). The scale bar represents 20 μm.
(C-D') Third instar wing discs containing $raptor^{RNAi}$-overexpressing clones (GFP-positive) were stained for PH3 (C') and Brdu (D'). Note the increased staining of PH3 and Brdu in $raptor^{RNAi}$-overexpressing clones. The scale bars represent 20 μm.