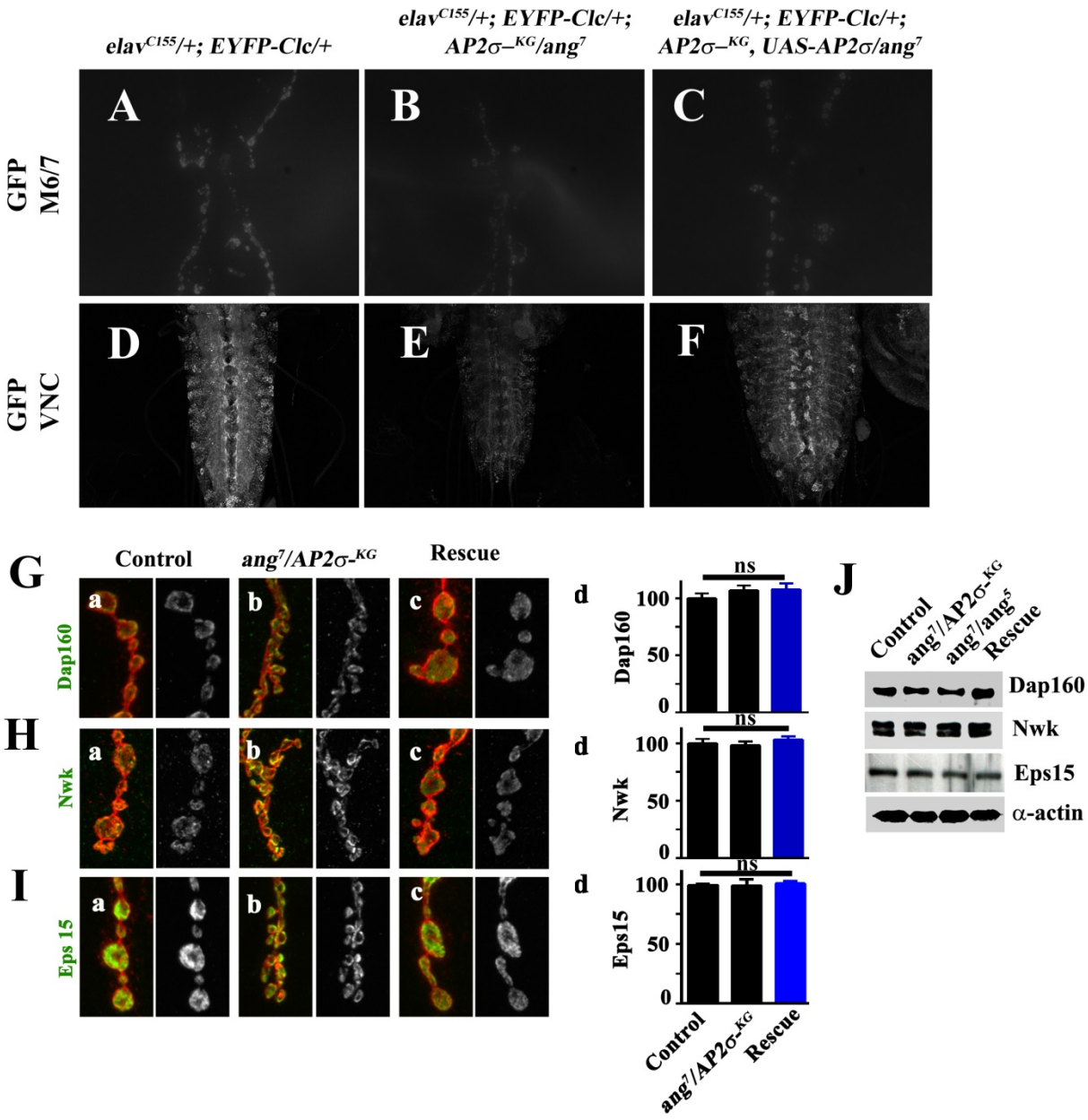
elav^C155/+; EYFP-Clc/+
elav^C155/+; EYFP-Clc/+; AP2σ–^KG/ang^7
elav^C155/+; EYFP-Clc/+; AP2σ–^KG, UAS-AP2σ/ang^7
GFP M6/7
GFP VNC
A
B
C
D
E
F
G
H
I
Control
ang^7/AP2σ-^KG
Rescue
Dap160
Nwk
Eps 15
a
b
c
d
ns
100
50
0
Dap160
Nwk
Eps15
Control
ang^7/AP2σ-^KG
Rescue
J
Control
ang^7/AP2σ-^KG
ang^7/ang^5
Rescue
Dap160
Nwk
Eps15
α-actin

Figure S3

Figure S3. Reduced AP2 levels affect Clathrin stability

(**A-C**) Representative images of third instar larval NMJ synapses from control (*elav^{C155}*/+; *EYFP-Clc*/+), heteroallelic *σ$_2$-adaptin* mutant expressing EYFP-Clc in neurons (*elav^{C155}*/+; *EYFP-Clc*/+; *angur^{7}/AP2σ^{KG02457}*) and transgene rescued NMJ synapses coexpressing EYFP-Clc (*elav^{C155}*/+;*EYFP-Clc*/+;*AP2σ^{KG02457},UAS-AP2σ/angur^{7}*).

(**D-F**) Representative images of ventral nerve cord of third instar larvae from control (*elav^{C155}*/+; *EYFP-Clc*/+), heteroallelic *σ$_2$-adaptin* mutant expressing EYFP-Clc in neurons (*elav^{C155}*/+; *EYFP-Clc*/+; *angur^{7}/AP2σ^{KG02457}*) and transgene rescued NMJ synapses coexpressing EYFP-Clc (*elav^{C155}*/+;*EYFP-Clc*/+; *AP2σ^{KG02457},UAS-AP2σ/angur^{7}*).

(**G-I**) Representative images of third instar larval NMJ synapses from (a) control, (b) heteroallelic *σ$_2$-adaptin* mutant (*angur^{7}/AP2σ^{KG02457}*) and (c) rescued animals (*actin5C/+; angur^{7}/UAS-AP2σ, AP2σ^{KG02457}*) colabelled with HRP (red) and other synaptic proteins (green), (G) Dap160; (H) Nwk and (I) Eps15. The histograms in right (d) show quantification of these synaptic proteins in the boutons, expressed as percentage of control levels. The fluorescence intensity of at least 50 individual boutons was measured and the background subtracted for the quantification. The fluorescence intensity values of all genotypes are represented in Table S3.

Error bars represent standard error of the mean (SEM). Statistical analysis based on one-way ANOVA followed by post-hoc Tukey's multiple comparison test.

(**J**) Western blot analysis of various synaptic proteins in larval brain of wild-type, heteroallelic *σ$_2$-adaptin* mutants (*angur^{7}/AP2σ^{KG02457}* and *angur^{7}/angur^{5}*) and rescued animals (*actin5C/+; angur^{7}/UAS-AP2σ, AP2σ^{KG02457}*). Level of α-actin was used as loading control.