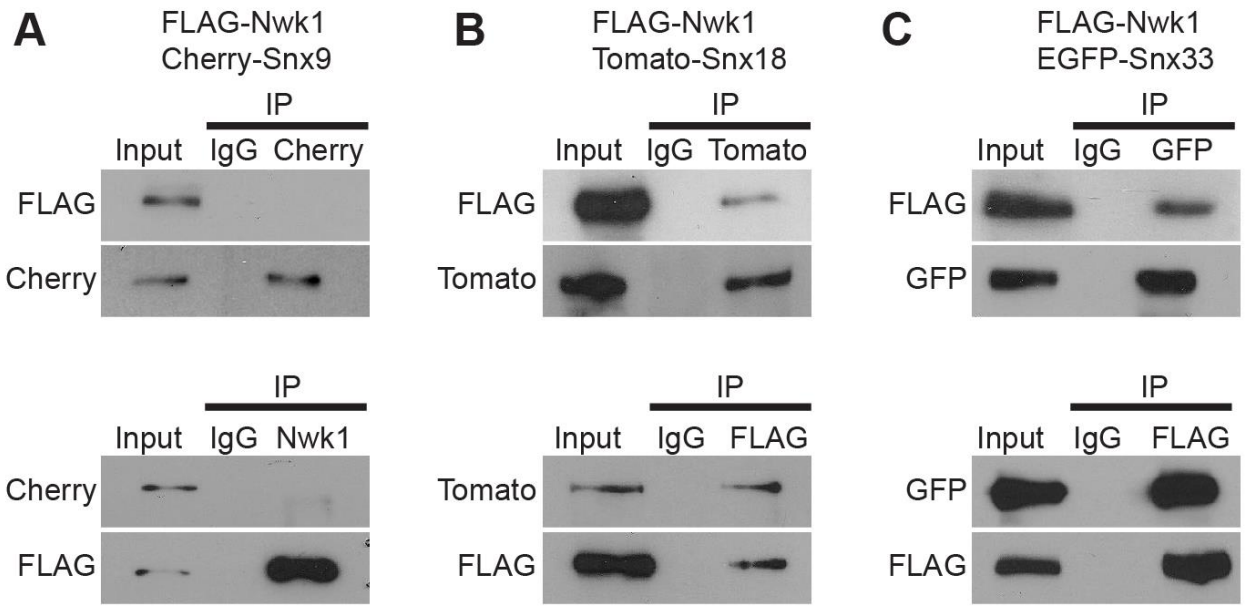

Figure S1. Nwk1 binds Snxs 18 and 33, but not Snx9. (A-C) Coimmunoprecipation experiments between Nwk1 and BAR-SH3 Sorting nexins. COS-7 cells were transfected with FLAG-Nwk1 and pmCherry-Snx9 (A), Tomato-Snx18 (B) or EGFP-Snx33 (C). Immunoprecipitation was performed with FLAG antibody (upper panel) and confirmed via reciprocal immunoprecipitation experiments using antibodies for the appropriate fluorescent tag (lower panel). Input lanes contain lysate equal to 1/5 of the amount used for the pull-down assays. IP indicates the antibody used for immunoprecipitation.

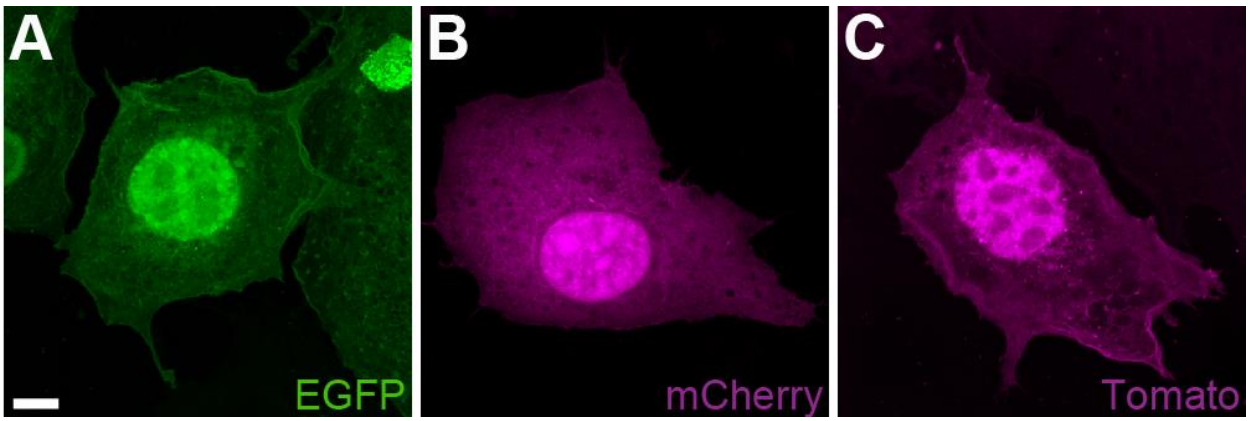

Figure S2. COS-7 controls. (A-C) COS-7 cells transfected with vectors encoding only the following tags: EGFP (A), mCherry (B), tdTomato (C). Scale bar = 10 µm.

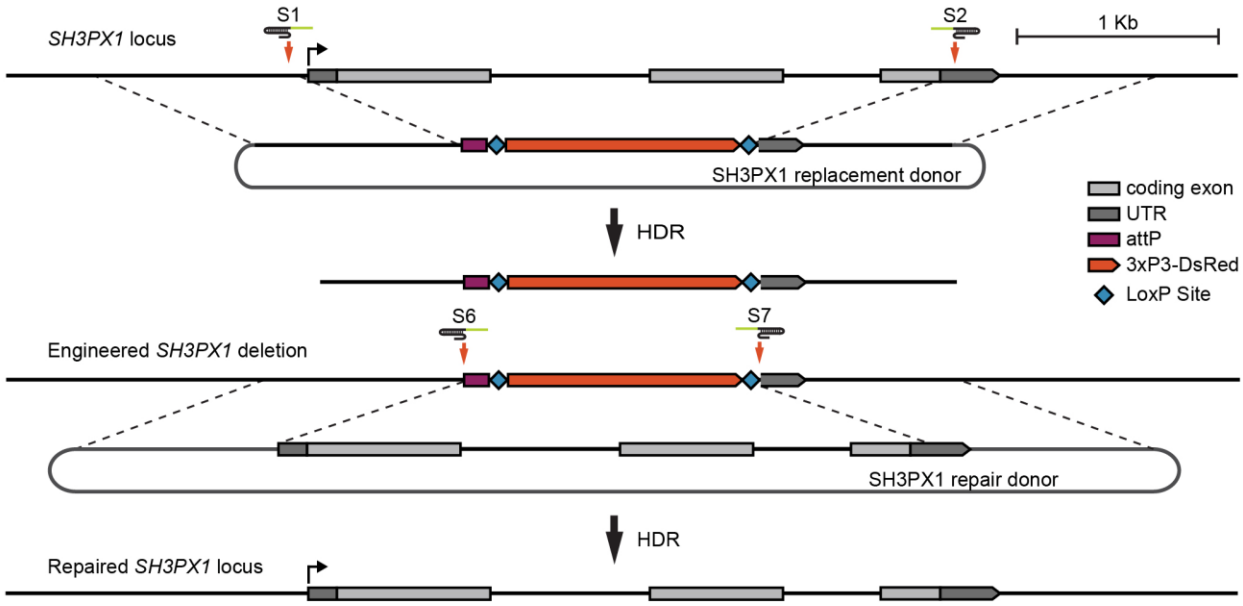

Figure S3. CRISPR-mediated generation of *SH3PX1* alleles. Schematic of the HDR strategy used to replace the *SH3PX1* coding sequence with a 3xP3-DsRed marker. The S1 and S2 target sites were used to generate $SH3PX1^{10A}$; the S1 target site was used for $SH3PX1^{C1}$. $SH3PX1^{repair}$ was generated using a similar HDR strategy and the S6 and S7 target sites to restore the deleted *SH3PX1* locus to wild type.

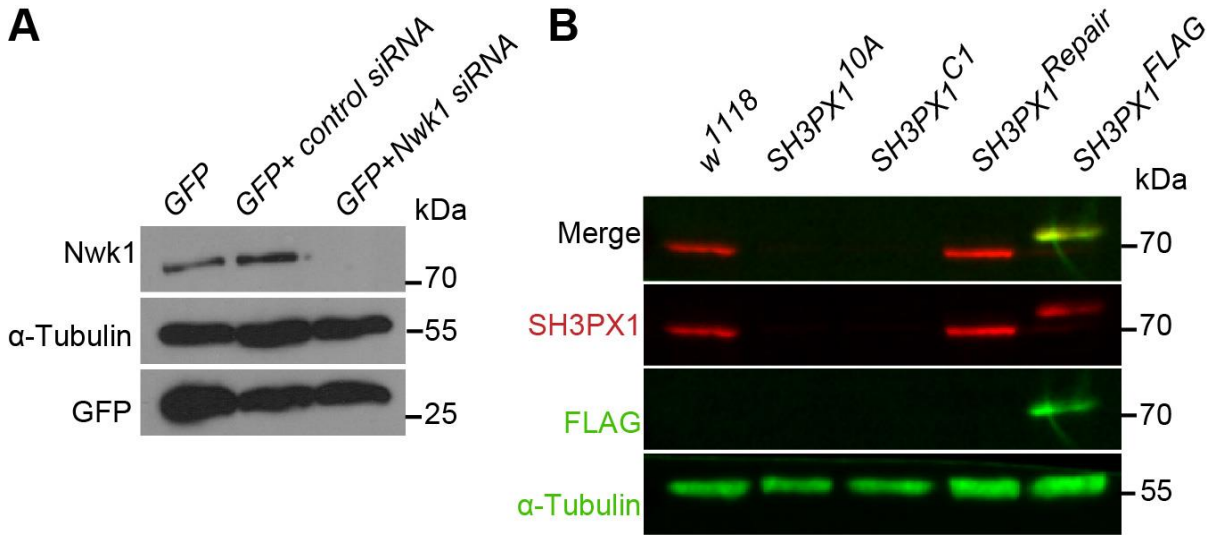

Figure S4. Confirmation of CRISPR alleles and antibody specificity. (A) Western blot analysis of CAD lysates following transfection with control siRNA and Nwk1 siRNA (SMART pool, Dharmacon) to verify the specificity of Nwk1 antibody. A GFP expression vector was used as a control for transfection efficiency. α-tubulin was used as a loading control. (B) Western blot analysis of lysates prepared from adult flies. SH3PX1 protein expression was detected with rabbit anti-SH3PX1 antibody. The equivalent one fly lysate was loaded in each lane. α-tubulin was used as loading control.

Table S1.

gRNA	Target sequence + PAM
S1	GAATTCTACACTTGGAA | CAGAGG
S2	GGAGCACAAAGAAACCG | CACCGG
S4	AGGTCATCTTGGTGCTC | TCCTGG
S5	GAACCAGCCCAAATTAC | CCTTGG
S6	CCCAGTTGGGGCACTAC | CAGAGG
S7	ATGCTATACGAAGTTAT | CACCGG

Sequences of guide RNAs (gRNAs) used to generate SH3PX1 alleles. Cleavage sites are indicated by a line. The PAM sequences, which are not included in the gRNAs, are underlined.