Inhibition of endoplasmic reticulum stress signaling rescues cytotoxicity of human *APOL1* risk variants in Drosophila.
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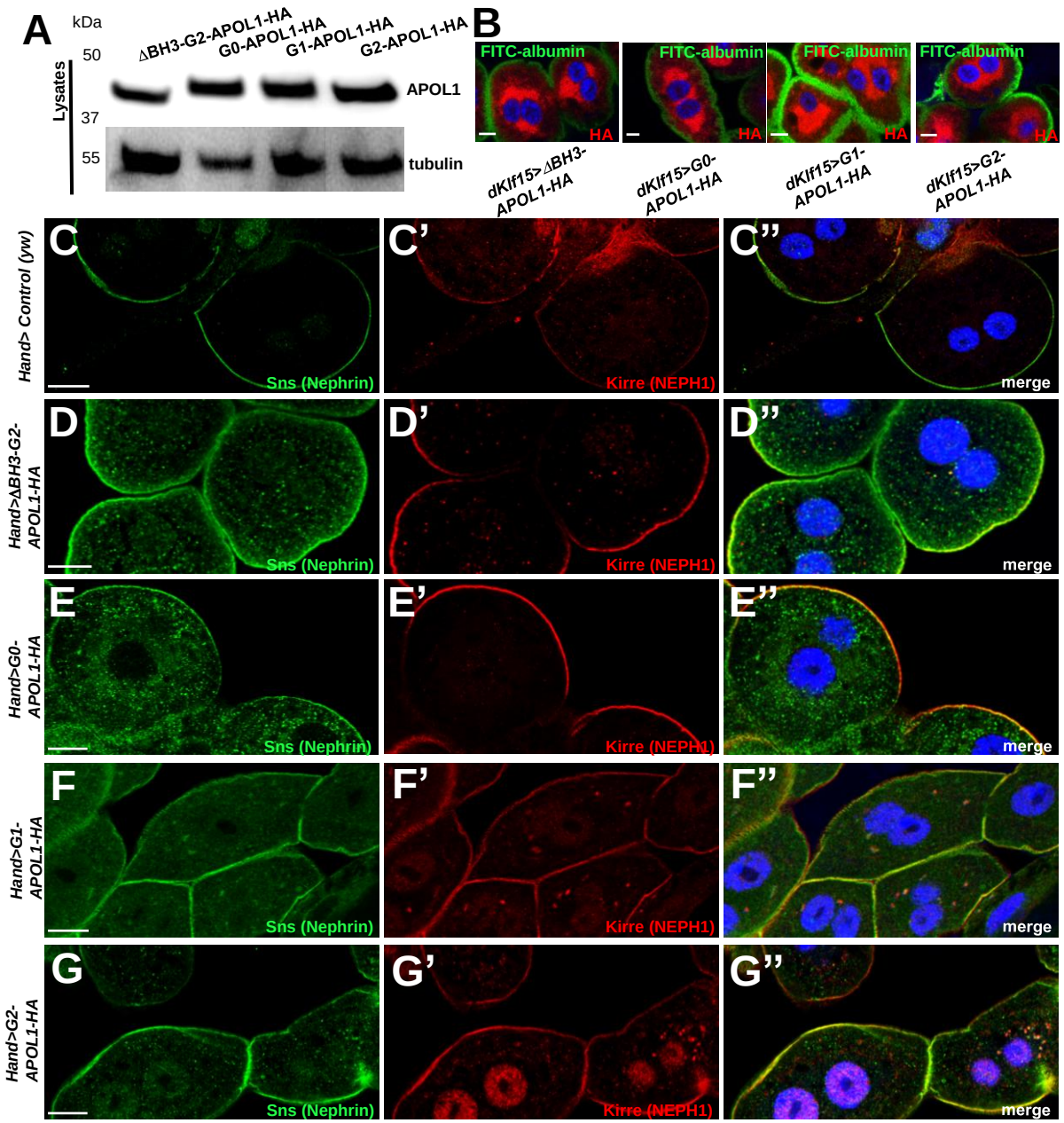


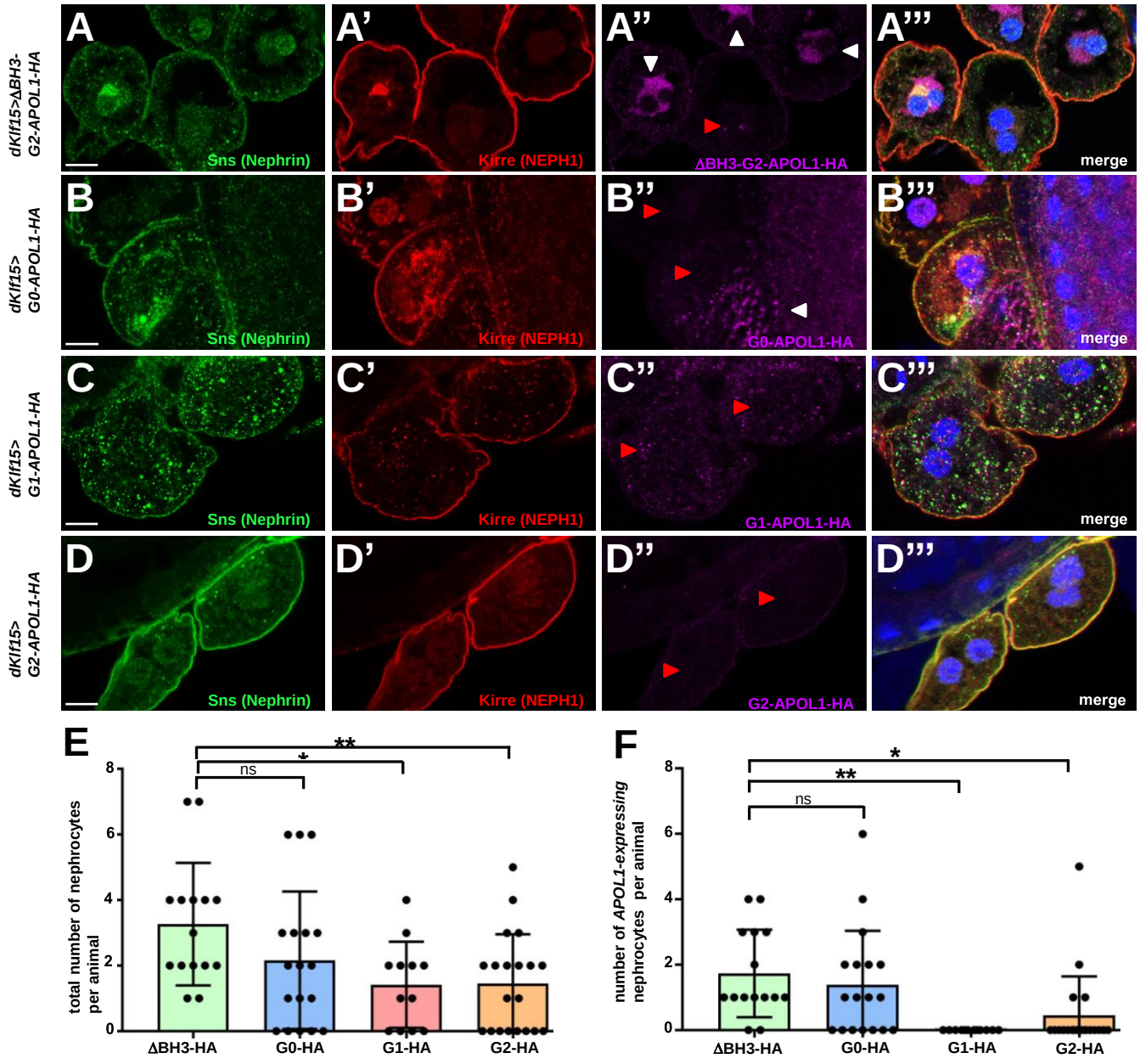
Supplemental Figure 1: *APOL1* variants are equally expressed in *Drosophila* and stainings of slit diaphragm upon *APOL1* variant expression.

(A) Immunoblotting after dissection of wing imaginal discs following acute *APOL1* variant induction confirms equal expression of APOL1 variants (shown through HA staining), tubulin serves as loading control. All flies were raised at 29 °C.

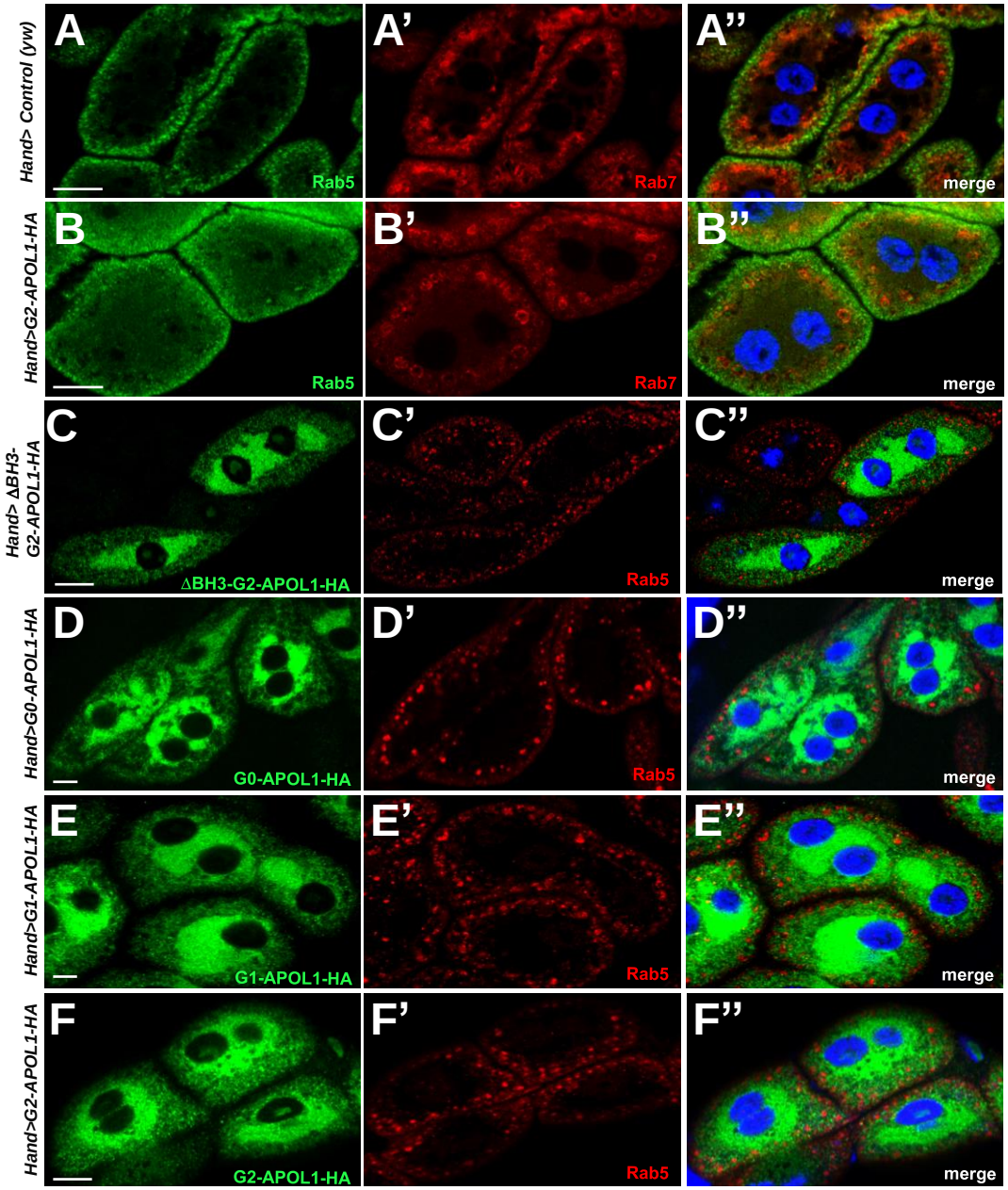
(B) *Drosophila* garland cell nephrocytes expressing the indicated *APOL1* variants under control of *dKlf15-GAL4* are exposed to the FITC-albumin assay followed by HA-staining. FITC-albumin uptake is increased for the risk variants under transgene expression at comparable levels. Scale bar represents 10 µm throughout the figure unless otherwise indicated.

(C-C'') Equatorial cross sections of control garland cell nephrocytes shows a regular staining pattern.

(D-G'') Equatorial cross sections of garland cell nephrocytes expressing ΔBH3-G2-*APOL1* (D-D'') and nephrocytes expressing G0-*APOL1* (E-E''), G1-*APOL1* (F-F''), or G2-*APOL1* (G-G''), using *Hand-GAL4* at 29 °C are shown. Cells are co-stained for the nephrin ortholog Sns (green) and the KIRREL/NEPH1 ortholog Kirre (red) revealing an unaffected staining pattern of slit diaphragm proteins for all indicated genotypes.



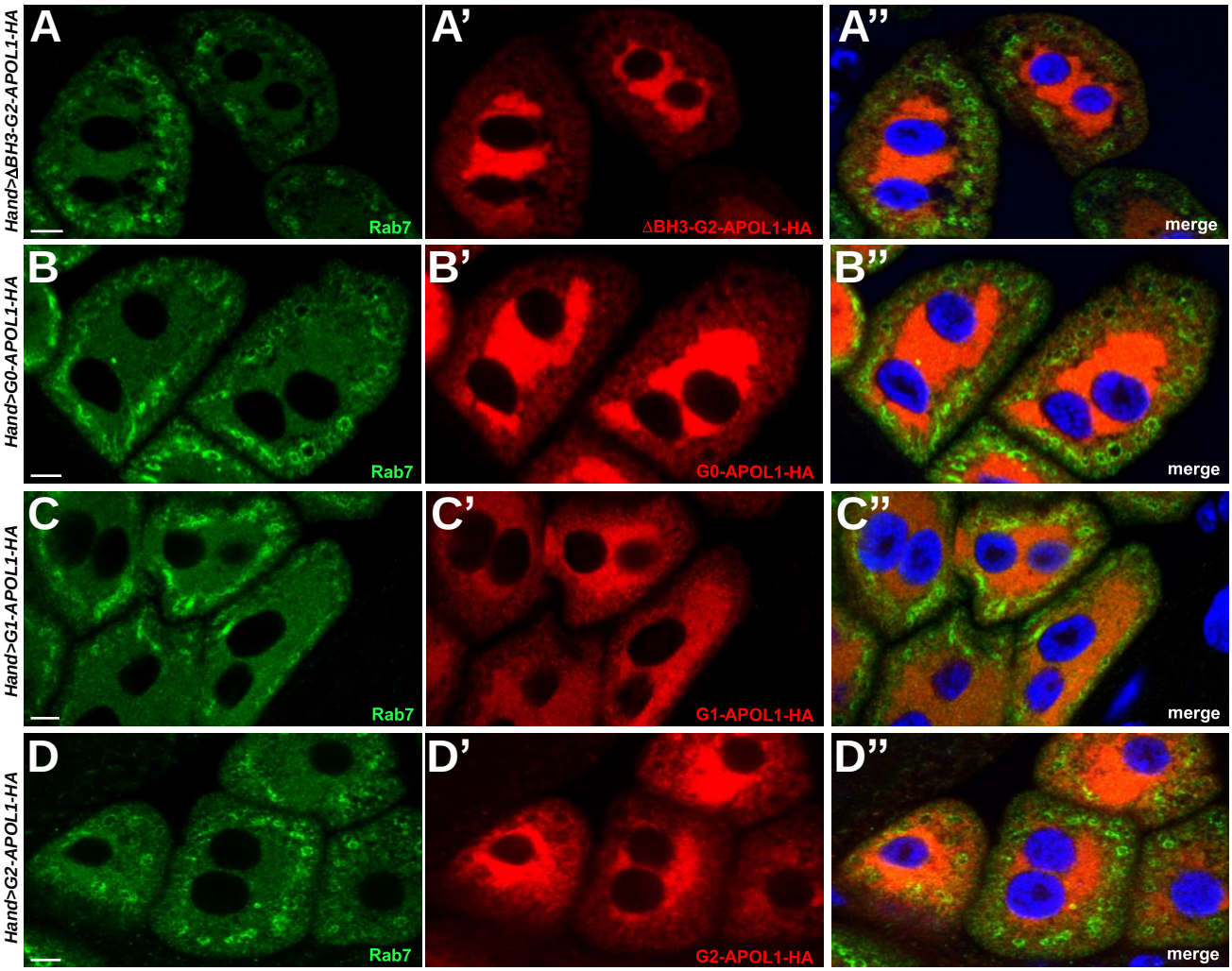
Supplemental Figure 2: Expression of *APOL1* variants via *dKlf15-GAL4* in adult nephrocytes.
(A-E) Adult garland cell nephrocytes dissected from animals within one day after eclosion are shown for the indicated genotypes. The slit diaphragm proteins Sns (nephrin) and Kirre (NEPH1) are stained together with anti-HA in far red (magenta). Most nephrocytes in animals expressing the control transgene *ΔBH3-G2-APOL1* under *dKlf15-GAL4* show transgene expression indicated by HA-staining (white arrow heads, A-A''). Only few and singular cells appear to downregulate most of the transgene expression (red arrow head). In nephrocytes expressing *G0-APOL1* much more non-expressing cells become detectable (read arrow heads), while few cells with lower expression are observed (white arrow head B-B''). Virtually no transgene expression is observed in nephrocytes when *G1-APOL1* (read arrow heads, C-C'') and *G2-APOL1* (red arrow heads, D-D'') are under control of *dKlf15-GAL4*. All flies were raised at 29°C. Scale bars represent 10 µm.
(E) Quantitation of data in (A-D) for the total number of nephrocytes per adult animal is shown (n= 12-20 animals per genotype. P>0.05 for *G0-APOL1,* P<0.05 for *G1-APOL1*, and P<0.01 for *G2-APOL1* compared to the control transgene *ΔBH3-G2-APOL1*.
(F) Quantitation of data in (A-D''') for the number of nephrocytes that exhibit transgene expression (HA-positivity) per adult animal is shown (n= 12-20 animals per genotype. P>0.05 for *G0-APOL1,* P<0.01 for *G1-APOL1*, and P<0.05 for *G2-APOL1* comparing to the control transgene *ΔBH3-G2-APOL1*.



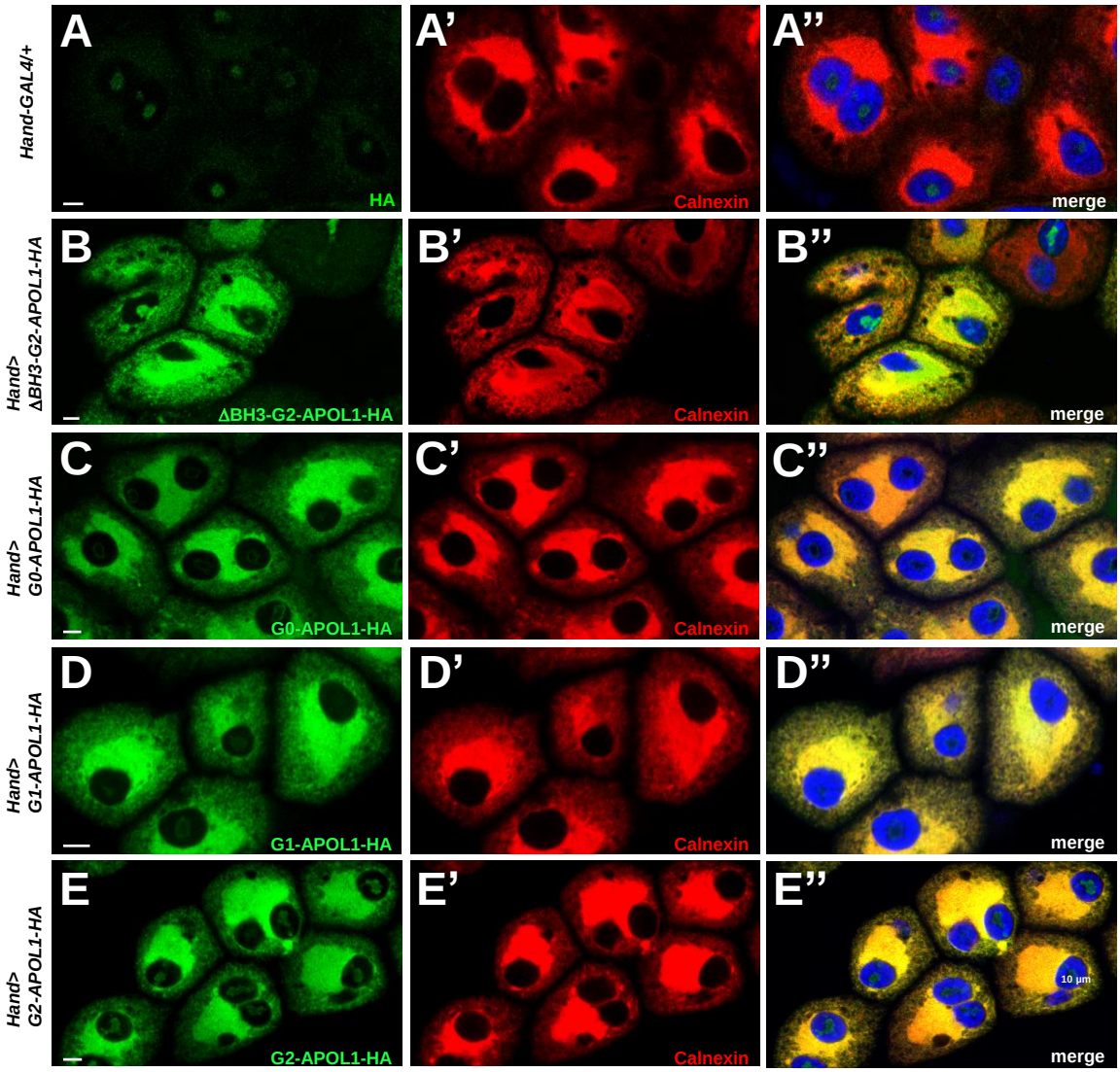
Supplemental Figure 3: Expression of G2-*APOL1* does not affect early/late endosomes and APOL1 transgenes do not overlap with Rab5.

(A-B'') Control garland cell nephrocytes (A-A'') stained for early endosomal marker Rab5 (green) and late endosomal marker Rab7 (red) show the typical distribution in two concentric rings with early endosomes closer to the cell surface. Nephrocytes expressing G2-*APOL1* show a regular staining pattern for endosomal markers Rab5 and Rab7 (B-B''). All flies are raised at 29°C. Scale bar represents 10 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.

(C-F'') Garland cell nephrocytes stained for the respective *APOL1* transgenes (HA-staining in green) and Rab5 (red) do not indicate any overlap for expression of ΔBH3-G0-A*POL1* (C-C''), G0-A*POL1* (D-D''), G1-A*POL1* (E-E'') and G2-A*POL1* (F-F'') with the early endosomal marker protein.



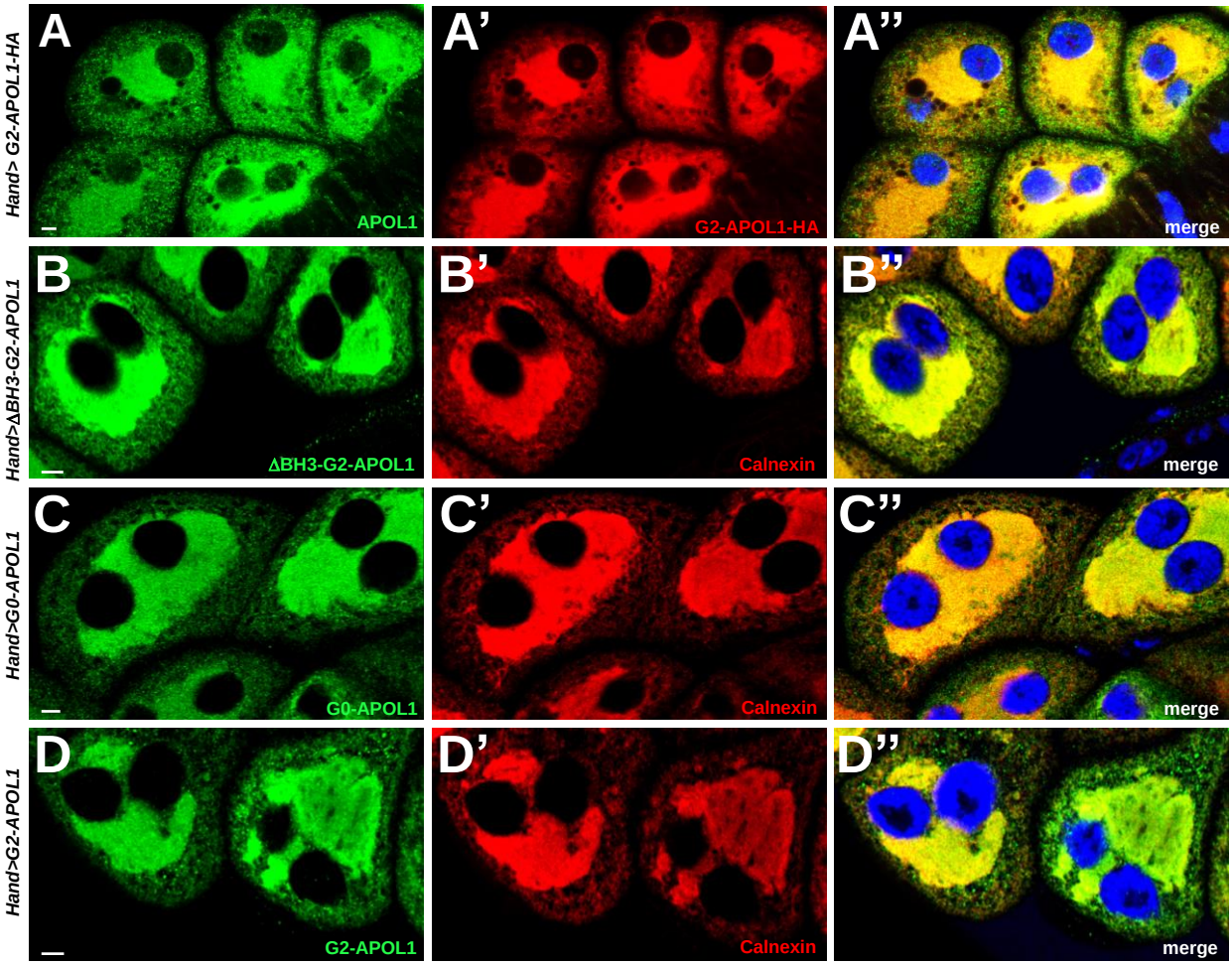
Supplemental Figure 4: APOL1 variants do not localize to late endosomes.
(A-D'') Garland cell nephrocytes stained for the respective *APOL1* transgene (HA-staining in red) and Rab7 (green) do not indicate any overlap for expression of ΔBH3-G2-A*POL1* (A-A''), G0-A*POL1* (B-B''), G1-A*POL1* (C-C''), and G2-A*POL1* (D-D'') with the late endosomal marker protein. All flies are raised at 29 °C. Scale bar represents 10 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.



Supplemental Figure 5: *APOL1* risk variants localize in the endoplasmic reticulum in *Drosophila* nephrocytes.

(A-A'') Confocal microscopy images showing equatorial cross sections of control garland cell nephrocytes that lack APOL1 transgene expression are shown. HA staining reveals a faint unspecific signal in an intra-nuclear position, while ER marker calnexin shows the typical ER pattern in a compact perinuclear signal. All animals were raised at 29 °C. Scale bar represents 10 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.

(B-E'') Shown are equatorial cross sections of garland cell nephrocytes expressing various *APOL1* transgenes under control of *Hand-GAL4*. Cells are co-stained for HA to visualize the tagged transgenes and the ER marker calnexin. Now that HA-tagged transgenes are expressed, the much stronger HA-specific signal becomes detectable. Confocal settings are identical to (A). All transgenes show complete co-localization with the ER marker which includes ΔBH3-G2-*APOL1* (B-B''), G0-*APOL1* (C-C''), G1-*APOL1* (D-D''), and G2-*APOL1* (E-E''). Thus, the *APOL1* variants studied here all seem to reside in the ER.

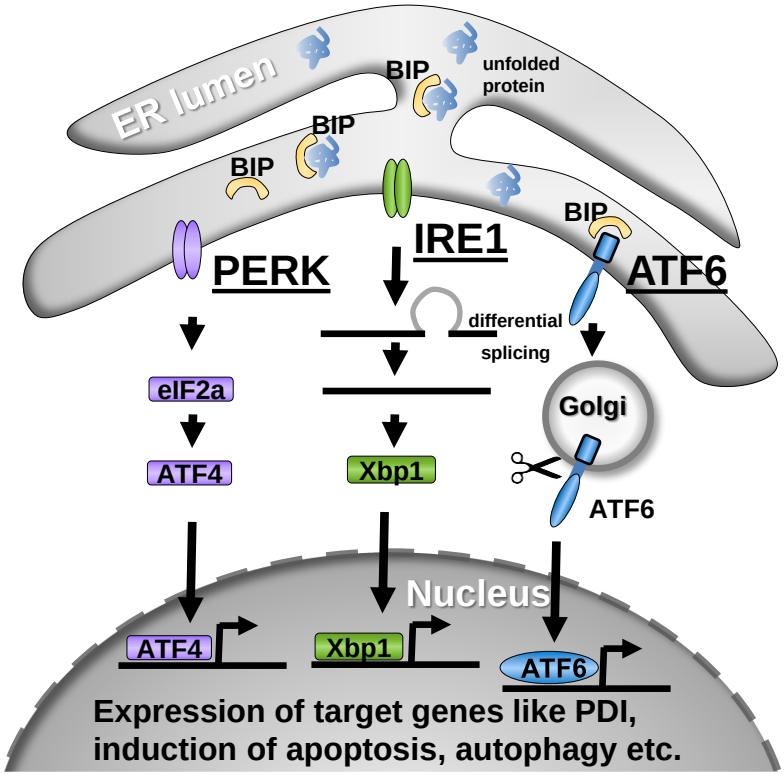


Supplemental Figure 6: Untagged *ΔBH3-APOL1*, *G0-APOL1* and *G2-APOL1* colocalize with the ER marker calnexin.

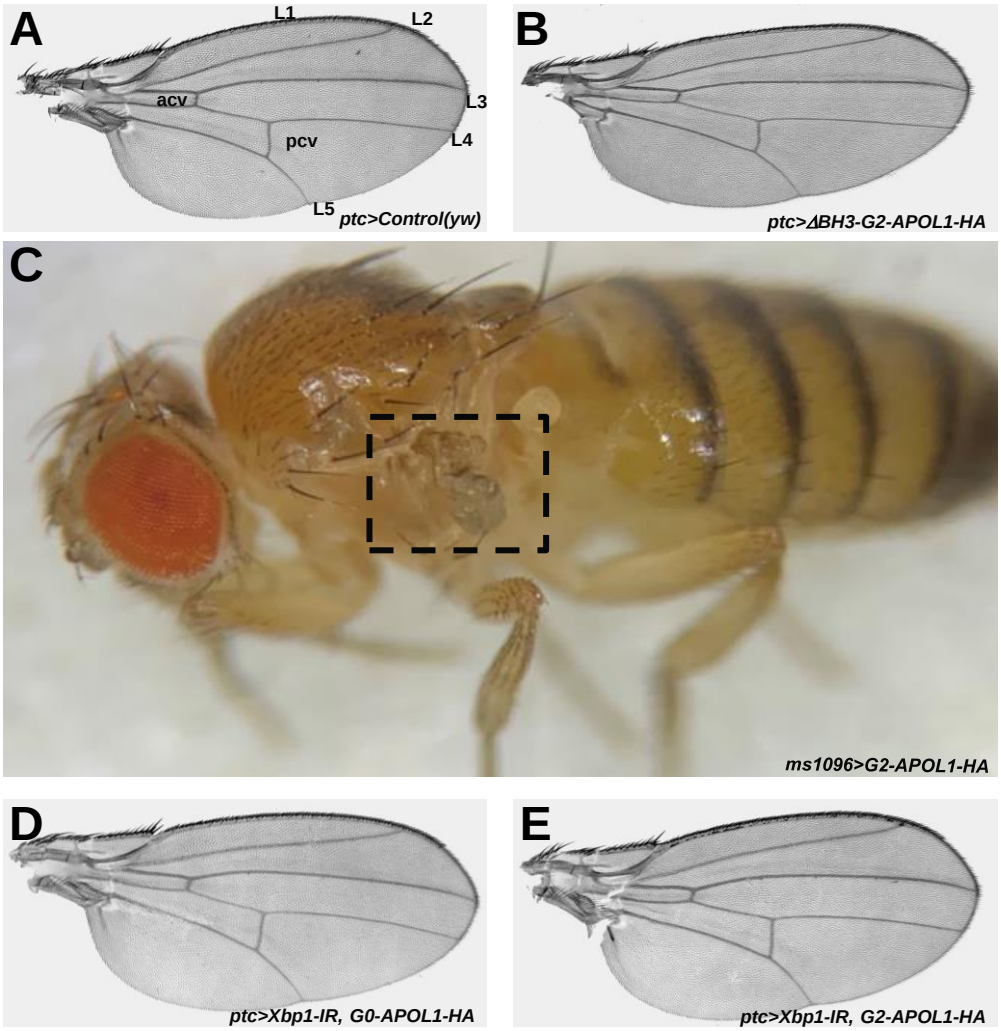
(A-A'') G2-*APOL1* expressing garland cell nephrocytes co-stained for human APOL1 protein (anti-APOL1 in green) and for the protein tag (HA-staining in red) confirm that tag and APOL1 staining render identical results. This excludes extensive cleavage of the c-terminal HA tag. All flies are raised at 29°C.

(B-B'') ΔBH3-G2-*APOL1* expressing garland cell nephrocytes co-stained for APOL1 (anti-APOL1 in green) and calnexin (in red) reveal complete co-localization of the transgene without tag with the ER marker.

(C-D'') *G0-APOL1* (C-C'') and G2-*APOL1* (D-D'') expressing garland cell nephrocytes co-stained for human APOL1 protein and the ER marker calnexin show colocalization for the no tag transgenes as well. Scale bar represents 10 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.



Supplemental Figure 7: Overview ER stress signaling.
Schematic showing a simplified overview of ER stress signaling. Bip protein is buffered by unfolded protein resulting in Bip-dissociation. Loss of Bip activates three independent sensor proteins (PERK, IRE1 and ATF6) that initiate downstream signaling.



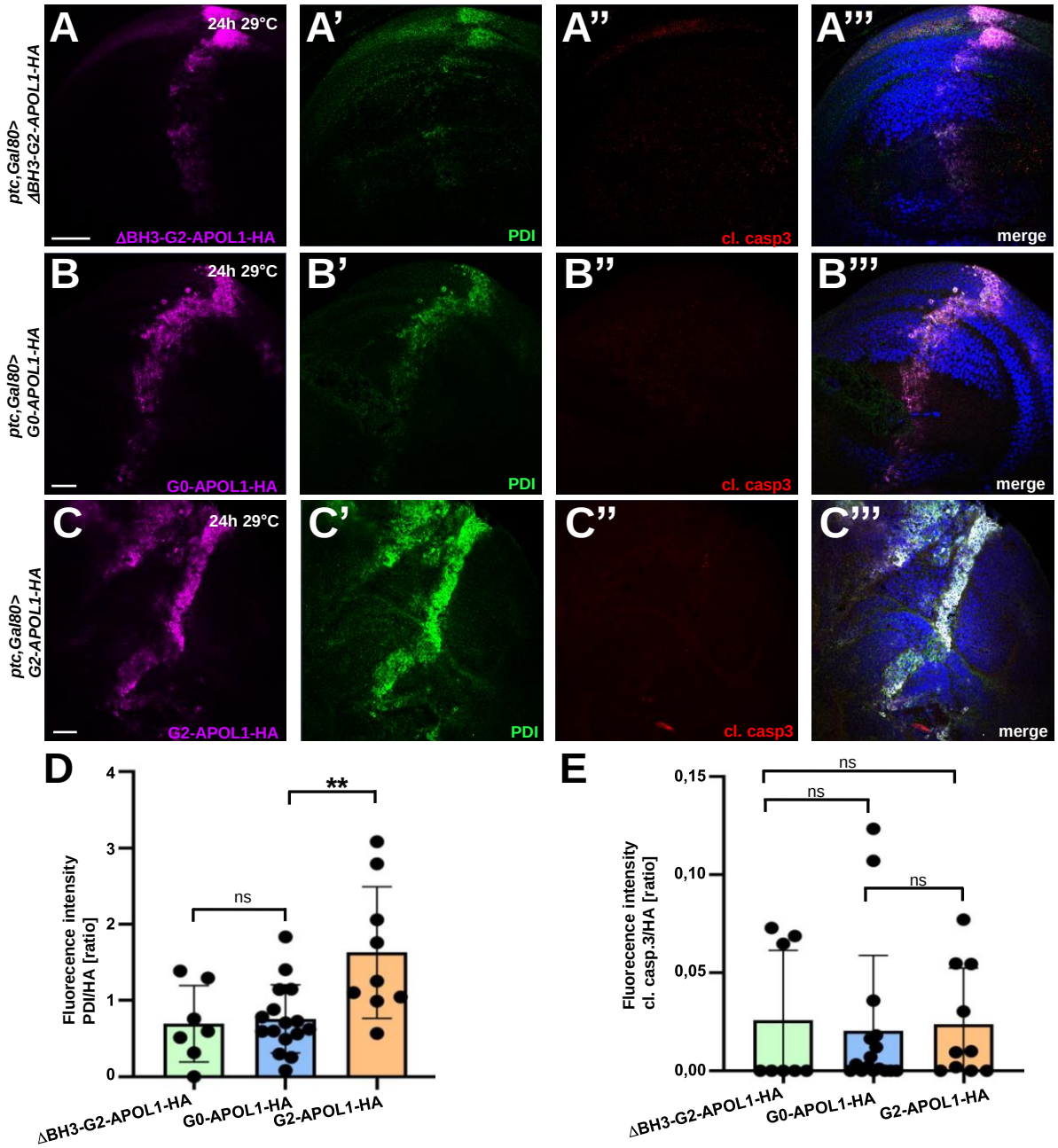
Supplemental Figure 8: Adult wing phenotypes upon expression of APOL1 variants.

(A) Control wings show regularly formed *Drosophila* wings. Five longitudinal veins (L1-L5) and the anterior cross vein (acv) and posterior cross veins (pcv) are indicated.

(B) Expression of G0-*APOL1,* G1-*APOL1* and G2-*APOL1* by *ptc-GAL4* does not render adult offspring (not shown). In contrast, expression of ΔBH3-G2-*APOL1* by *ptc-GAL4* gives rise to adults that form normal wings (compare wing in (B) to wing in (A)). Flies are raised at 18°C.

(C) Expression of G2-*APOL1* with *1096-GAL4* results in adult animals that lack wings. Box highlights residual wing tissue. All flies are raised at 25°C.

(D-E) Attenuation of ER stress signaling by concomitant expression of *Xbp1*-RNAi using *ptc-GAL4* abolished lethality for expression of G0-*APOL1* (D) and G2-*APOL1* (E) with formation of phenotypically normal wings. *Ptc>Xbp1-RNAi, G1-APOL1* flies also developed phenotypically normal wings (data not shown). All flies are raised at 25°C.

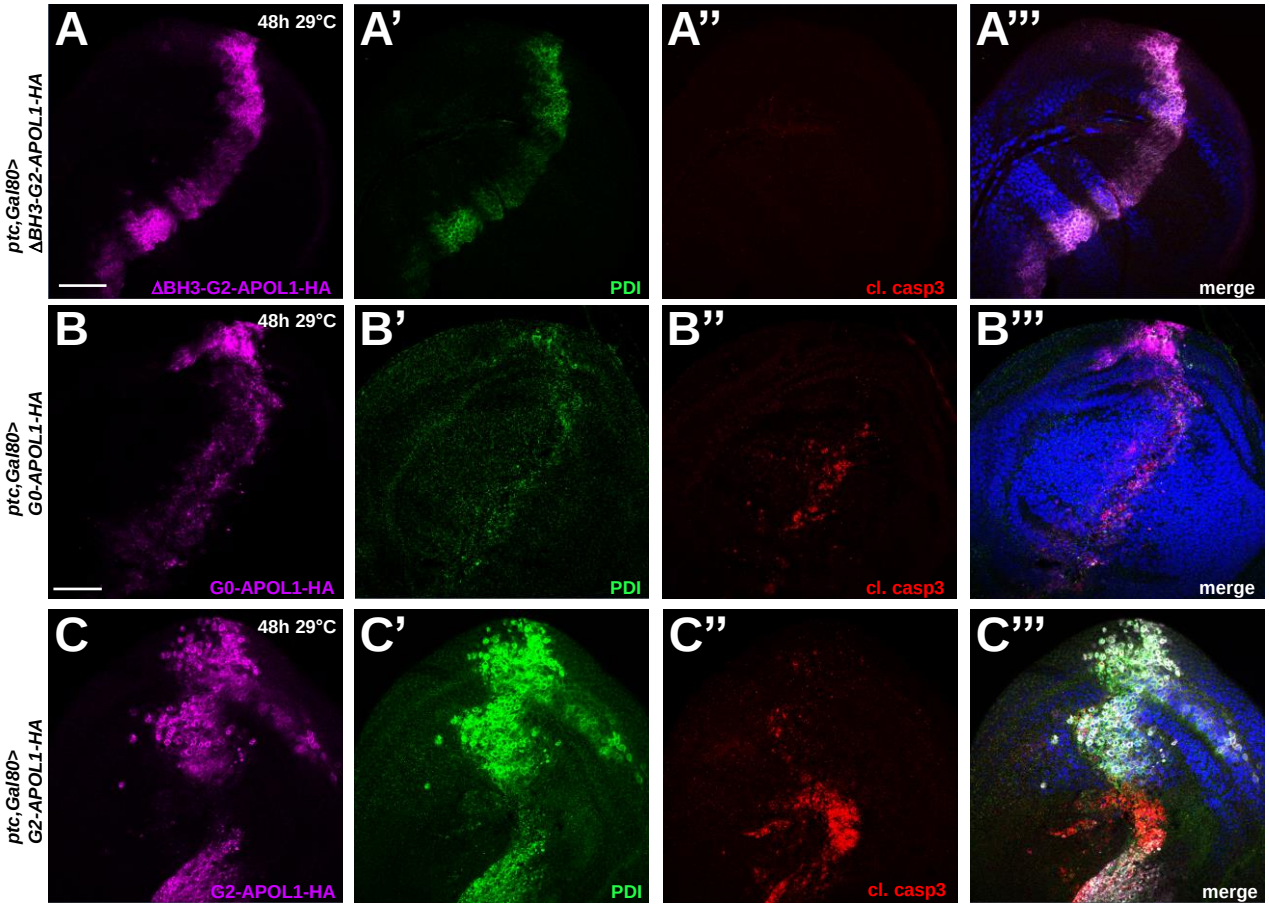


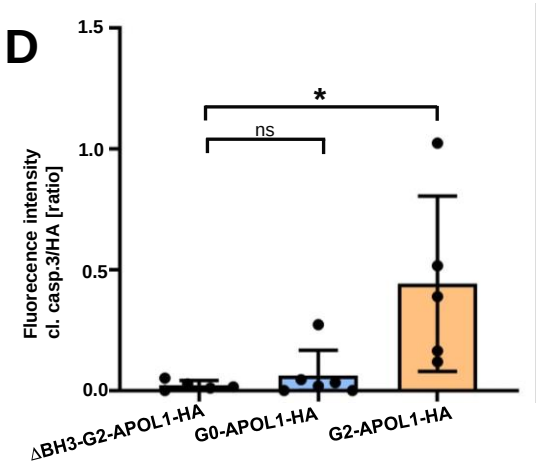
Supplemental Figure 9: G2-*APOL1*-dependent ER stress precedes apoptosis.

(A-C''') Shown are wing discs expressing indicated *APOL1* transgenes with *ptc-GAL4* for 24 h following disinhibition of *GAL80^{ts}* through temperature shift to 29°C. Short-term expression of ΔBH3-G2-*APOL1* (A-A''') and G0-*APOL1* (B-B''') are accompanied by a mild and insignificant upregulation of PDI without detection of cleaved caspase-3. The short-term expression of G2-*APOL1* (C-C''') entails much stronger induction of ER stress as indicated by PDI expression levels, but also without induction of apoptosis. This indicates that ER stress induction specifically by the risk variant occurs before appearance of apoptotic cells. Scale bar represents 50 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.

(D) Quantitation of data in (A-C''') for fluorescence intensity of PDI staining in ratio to the expression strength indicated by the HA signal (n=7-17 per genotype, P>0.05 for G0-*APOL1*, and P<0.01 for G2-*APOL1*).

(E) Quantitation of data in (A-C''') for fluorescence intensity of cleaved caspase-3 staining in ratio to the expression strength indicated by the HA signal shows no apoptosis induction for either genotype in this setting (n=8-16, P>0.05 for G0-*APOL1*, and P>0.05 for G2-*APOL1*).





Supplemental Figure 10: G2-APOL1 induces apoptosis after expression for 48 h.
(A-C''') Shown are wing discs expressing *APOL1* transgenes with *ptc-GAL4* for 48 h following disinhibition of *GAL80^{ts}* through temperature shift to 29°C. The prolonged expression of G2-*APOL1* (C-C''') entails much stronger induction of ER stress as indicated by PDI expression levels than control variant ΔBH3-G2-*APOL1* (A-A''') and the wild type G0-APOL1 (B-B'''). Wing cells that stain cleaved caspase-3 are detected for *G2-APOL1* (C'') but not ΔBH3-G2-*APOL1* (A'') and G0-APOL1 (B''). This indicates that intense ER stress induction for 48 h is sufficient to trigger cell death. Scale bar represents 50 µm and nuclei are marked by Hoechst 33342 in blue here and throughout the figure.
(D) Quantitation of data in (A-C''') for fluorescence intensity of cleaved caspase-3 staining in ratio to the expression strength indicated by the HA signal shows significant apoptosis induction for G2-APOL1 in this setting (n=5-6, P>0.05 for G0-*APOL1*, and P<0.05 for G2-*APOL1*).

flystocks	obtained from	used
Xbp1-RNAi	Bloomington Drosophila Stock Center BDSC #36755	expresses dsRNA for RNAi of *Xbp1*
bip-RNAi	Vienna Drosophila RNAi Center VDRC #14882	expresses dsRNA for RNAi of *bip*
EGFP-RNAi	BDSC #41553	as control RNAi
prospero-GAL4	gift from Chris Doe	to control expression in garland cell nephrocytes
Hand-GAL4	kindly provided by A. Paululat via H. Jasper	to control expression in garland cell nephrocytes
dKIF15-Gal4	kindly provided by P. Hartley	to control expression in garland cell nephrocytes
ptc-GAL4	from N. Perrimon	for wing expression
1096-GAL4	from G. Pyrowolakis	for wing expression
Xbp1-EGFP	BDSC #39719	as a reporter for *Xbp1*-dependent ER stress
Gal80 on III	BDSC #7018	express a transgene temperature-dependently
yellow white (yw)	from M. Köttgen	as wildtype control

Supplemental Table 1: Fly stocks.

Construct	direction	Primer sequence
G0-*APOL1* -HA	forward	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCatggagggagctgctttgct
	reverse	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGCATAGTCCGGGACGTCATAGGGATAGAAGCCTGCcagttcttggtccgcctgc
G1-*APOL1* -HA	forward	5'-CTCAACAATAATTATAAGATTCTGCAG
	reverse	5'-TACAGGGGCCACATCCGTGA
	forward	5'-TCACGGATGTGGCCCCTGTAGGCTTCTTTCTTGTGCTGGATGTAG
	reverse	5'-CTGCAGAATCTTATAATTATTGTTGAGCATGTTTAGCTTCTCCTCCAGC
G2-*APOL1* -HA	forward	5'-AGCTAAACATTCTCAACAATAAGATTCTGCAGGCGGAC
	reverse	5'-GTCCGCCTGCAGAATCTTATTGTTGAGAATGTTTAGCT
ΔBH3-G2-*APOL1* -HA	forward	5'-GTAAGCTTGAGGATAACATAAGAAGGAAGGTCCACAAAGGCACCAC
	reverse	5'-GTGGTGCCTTTGTGGACCTTCCTTCTTATGTTATCCTCAAGCTTAC
G0/G2/ΔBH3-*APOL1* no tag	forward	5'-GTACAAAAAAGCAGGCTTCACC
	reverse	5'-CTTTGTACAAGAAAGCTGGGTCCTACAGTTCTTGGTCCGCCTGC

Supplemental Table 2: Primer sequences.

primary antibodies	obtained	1st Antibody dilution rate
rabbit anti-sns	gift from S. Abmayr	1:200
guinea pig anti-Kirre	gift from S. Abmayr	1:200
rat anti-HA	1186742300, Merck/Roche	IF: 1:200; WB: 1:500
rabbit anti-Rab5	ab18211, abcam	1:200
rabbit anti-APOL1	HPA018885, Sigma	1:200
rabbit anti-cleaved caspase-3	9661, Cell Signaling Technology	1:200
mouse anti-Rab7	Rab7, DSHB	1:200
mouse anti-calnexin	Cnx99A 6-2-1, DSHB	1:200
mouse anti-PDI	ADI-SPA-891-D, Enzo	1:200
mouse anti-beta-tubulin	E7, DSHB	1:500

Supplemental Table 3: Antibodies.