Supplemental figures, movies and data
to

Intracellular APOL1 risk variants cause cytotoxicity accompanied by energy depletion

by

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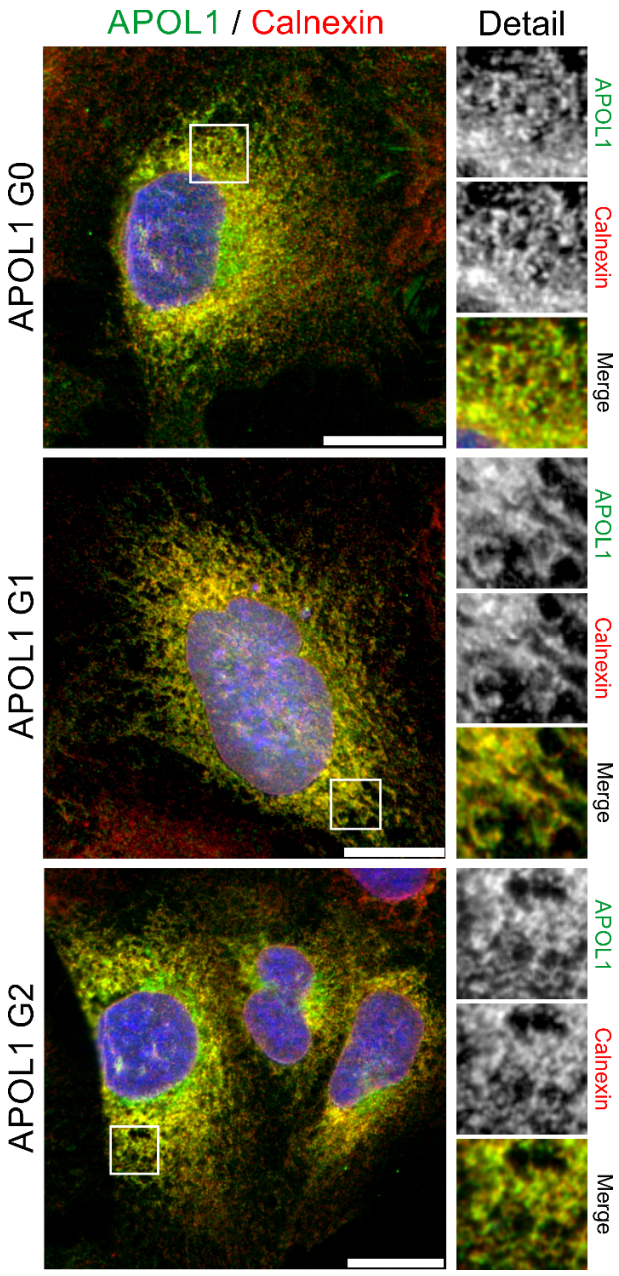
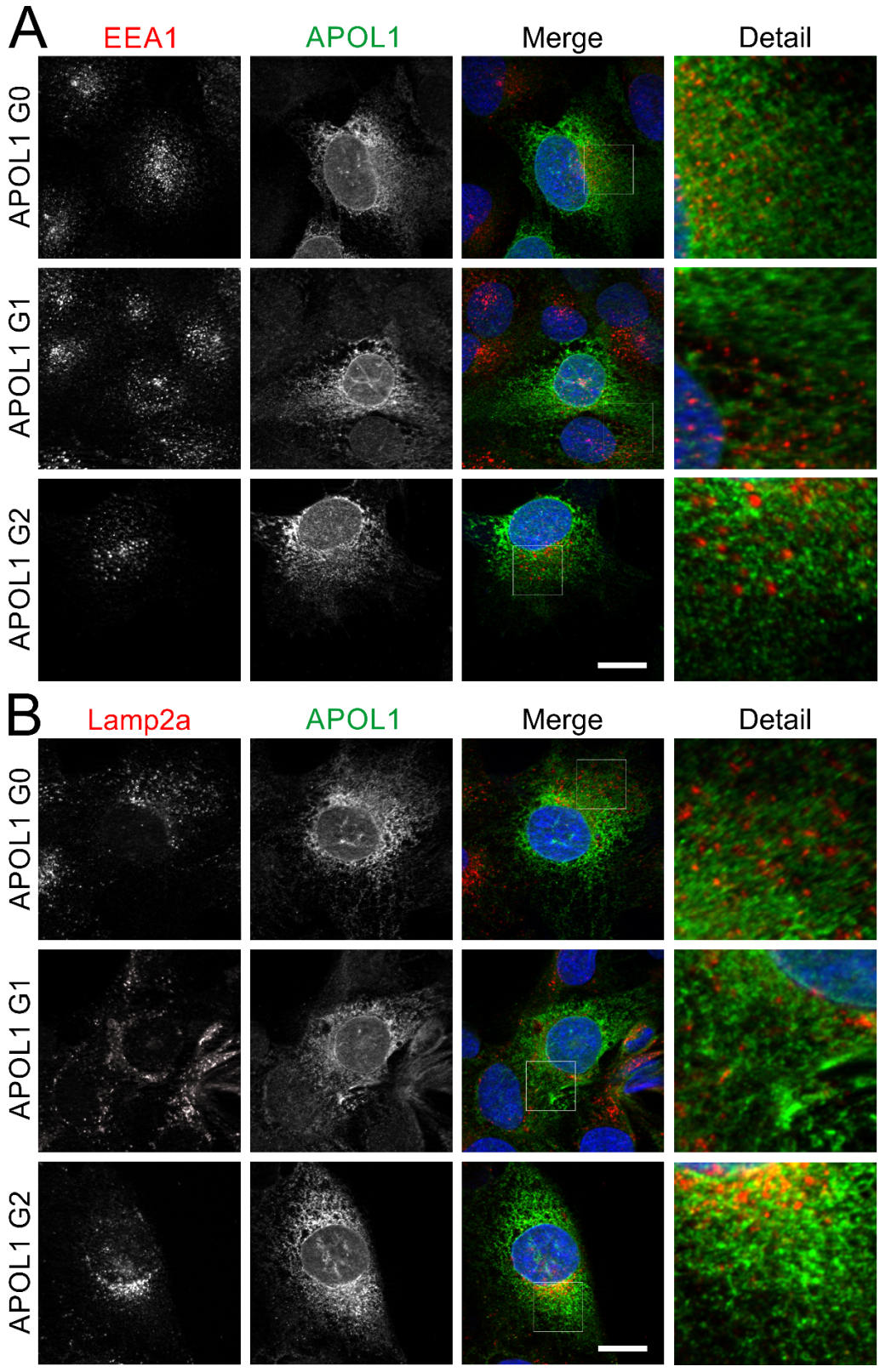
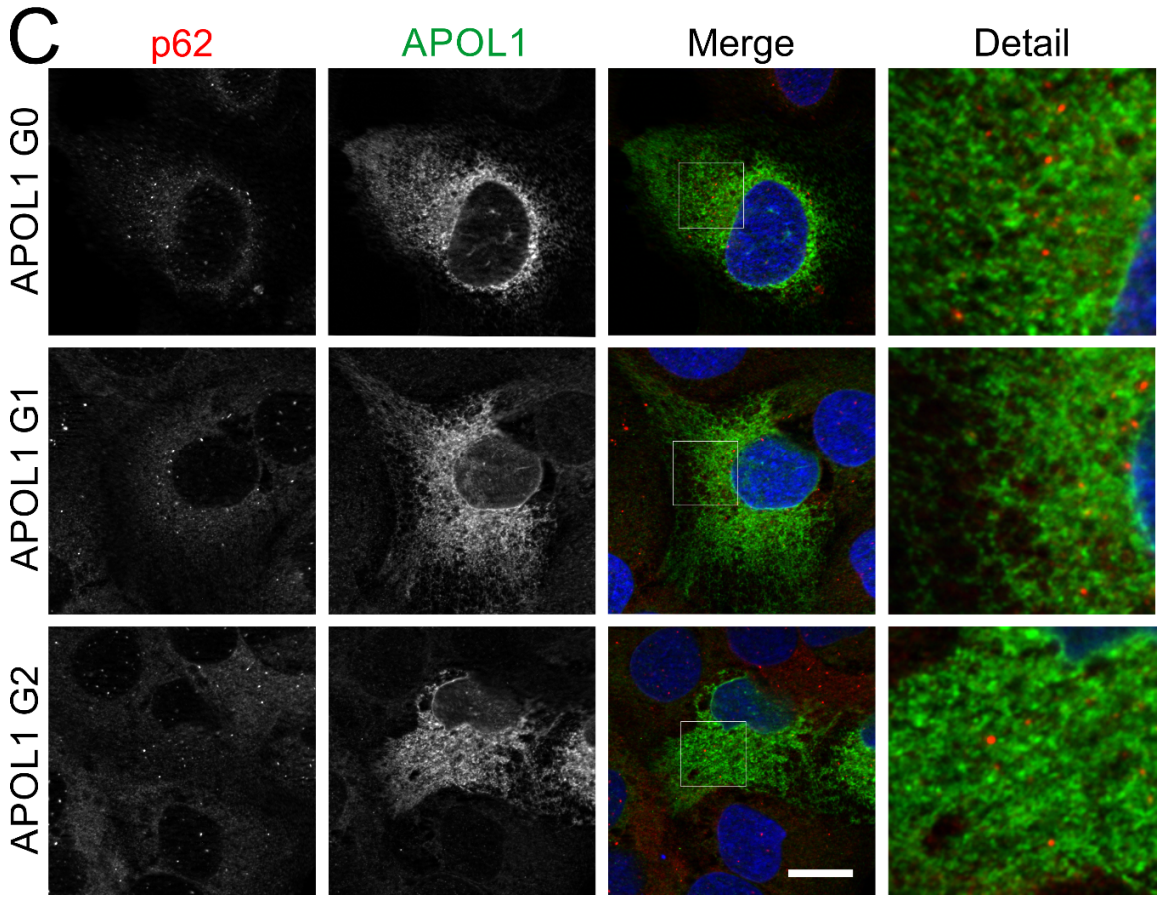


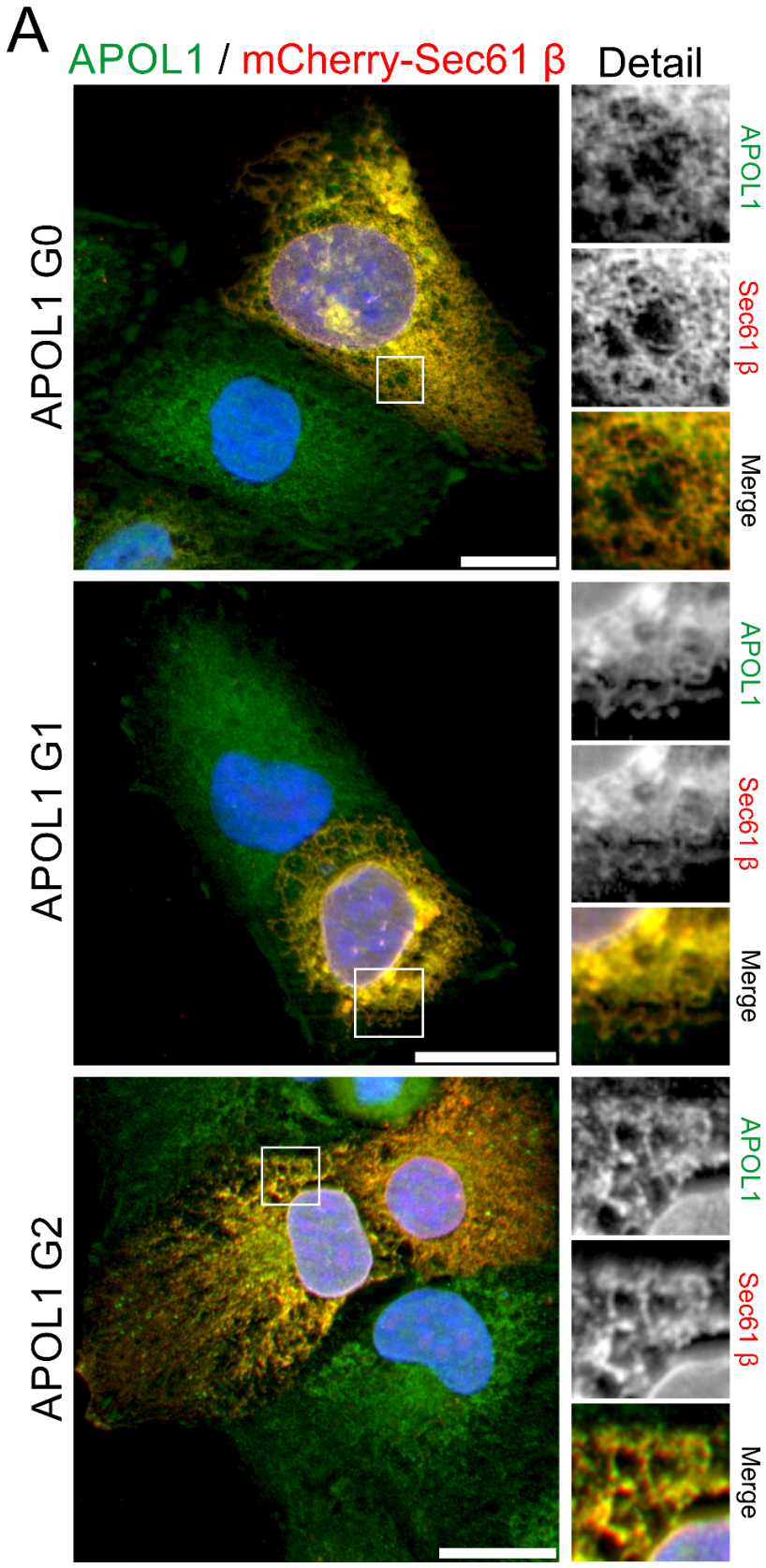
Suppl. Fig. SF1: *Full length APOL1 co-localizes with the ER resident protein Calnexin.*
PFA-fixed stable podocytes overexpressing untagged APOL1 G0 and RRVs (aa1-398, variant A) analyzed by immunofluorescence with antibodies against APOL1 (green) and the ER marker protein Calnexin (red) showed a strong presence of APOL1 at the ER (Dox induction 24 h). Scale bar and square length of details are 20 µm and 10 µm respectively.

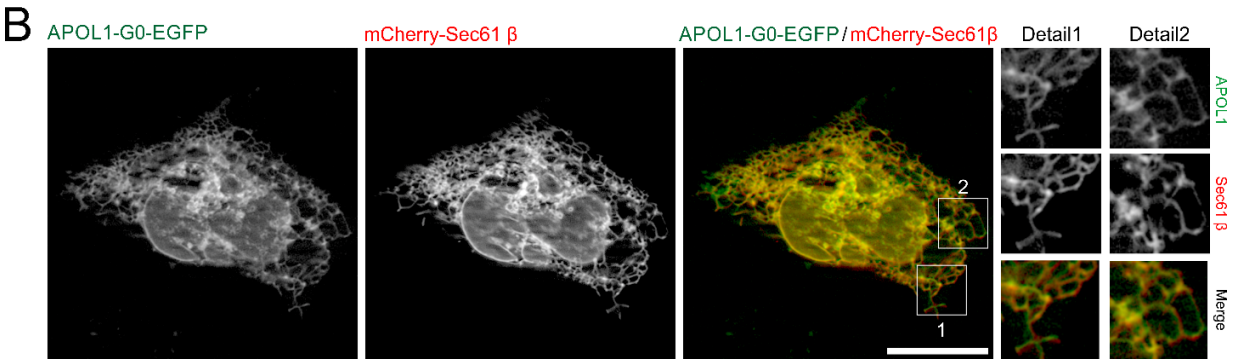

A
EEA1
APOL1
Merge
Detail
APOL1 G0
APOL1 G1
APOL1 G2
B
Lamp2a
APOL1
Merge
Detail
APOL1 G0
APOL1 G1
APOL1 G2



Suppl. Fig SF2: *Full length APOL1 is not detectable at endolysosomal or autophagosomal stuctures.*

PFA-fixed stable podocytes overexpressing untagged APOL1 G0 and RRVs (aa1-398, variant A) analyzed by immunofluorescence with antibodies against APOL1 (green) showed no or very low degree of co-localization with markers of early (EEA1, **A**) or late endosomes (Lamp2a, **B**) and the autophagy marker p62 (**C**, Dox induction 24 h). Scale bar and square length of details are 20 µm.

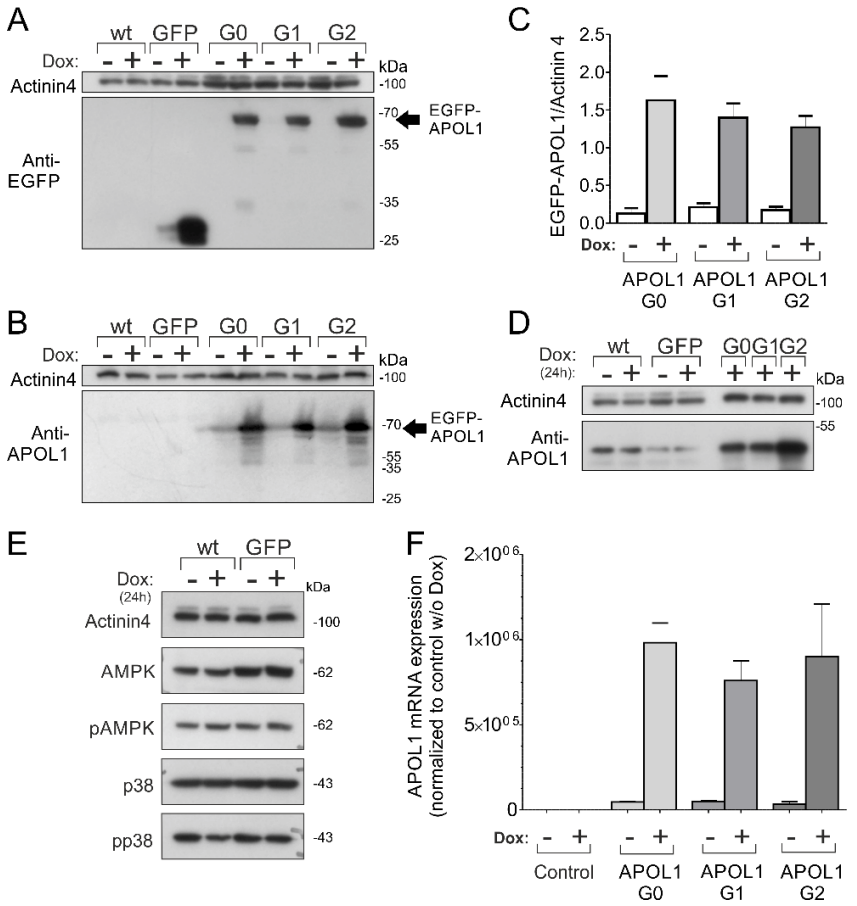

A
APOL1 / mCherry-Sec61 β
Detail
APOL1 G0
APOL1 G1
APOL1 G2
APOL1
Sec61 β
Merge
APOL1
Sec61 β
Merge
APOL1
Sec61 β
Merge



Suppl. Fig SF3: *Full length APOL1 co-localizes with the ER-resident protein Sec61 β.*

(A) PFA-fixed podocytes overexpressing untagged APOL1 G0 and RRVs (aa1-398, variant A), transiently transfected with a plasmid encoding mCherry-tagged Sec61β (red) analyzed by immunofluorescence with antibodies against APOL1 (green). The analysis confirmed the ER localization of all APOL1 variants. Dox induction 24 h. Scale bar and square length of details 10 and 20 µm respectively.

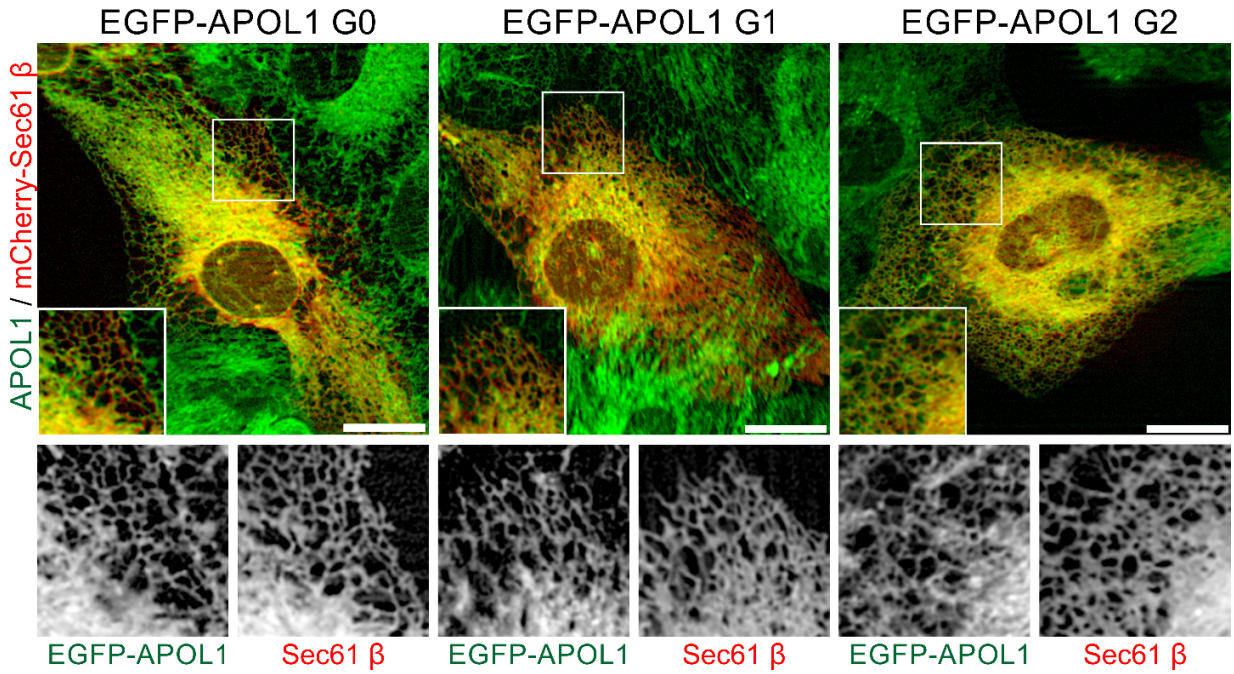
(B) Live cell imaging of podocytes transiently transfected with plasmids encoding C-terminally EGFP-tagged APOL1 G0 full length variant A (aa1-398) and mCherry tagged Sec61 β showed a strong co-localization of both proteins at the ER. Scale bar and square length of details 10 and 20 µm respectively.



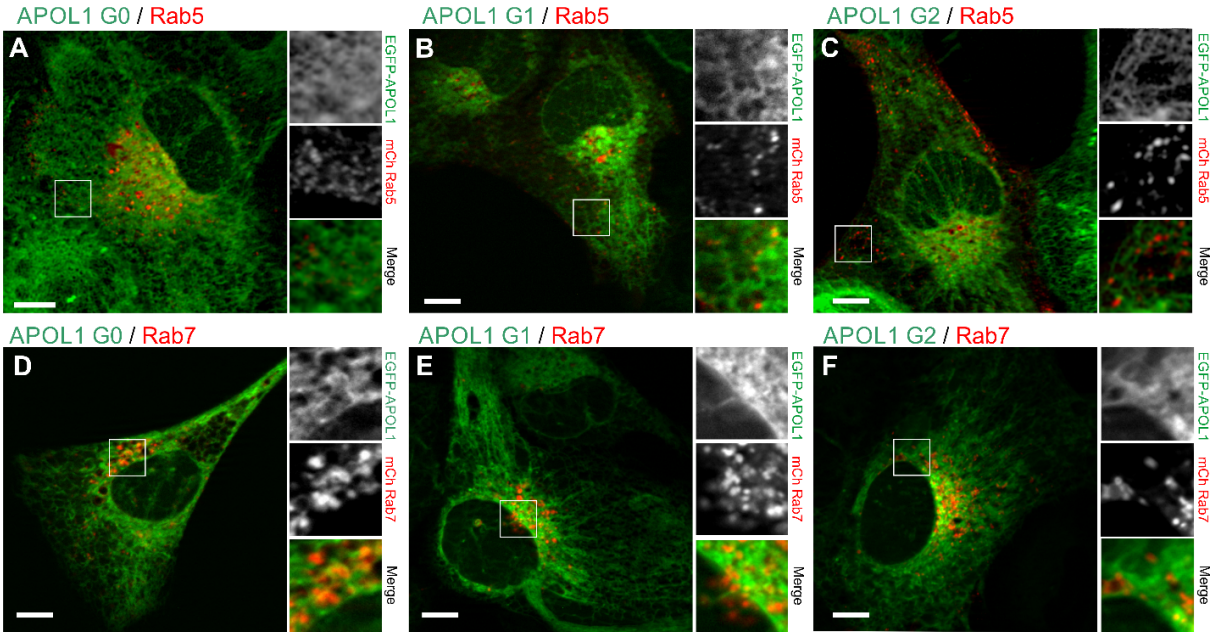
Suppl. Fig. SF4: *Doxycycline induction in stable HEK293T cells results in comparable expression levels of EGFP-APOL1 G0 and RRVs.*

Western blot analysis **(A)** and quantification (C) of EGFP-APOL1 expressing HEK293T cells shows similar protein levels in case of G0, G1 or G2, analyzed with antibodies against EGFP. **(B)** Inducible expression can also be detected with antibodies against APOL1, confirming APOL1 antibody specificity. Significant amounts of EGFP-APOL1 are only detectable after Doxycycline treatment, and not detectable in HEK293T-wt cells (wt) or Dox inducible cells, expressing EGFP only (GFP). **(C)** Relative protein levels of EGFP-APOL1 expressing cells were quantified of three independent experiments. **(D)** Endogenous (required long exposure) and overexpressed APOL1 are detected at the same molecular mass, in HEK293T-wt cells (wt) or EGFP-expressing HEK293T cells (GFP) as well as Dox-induced full length APOL1 overexpressing podocytes (G0, G1, G2) and Doxycycline has no impact on endogenous APOL1 expression. **(E)** Dox treatment or EGFP expression does not influence AMPK and p38 activation in HEK293T-wt cells (wt) or Dox-inducible only EGFP-expressing HEK293T cells (GFP). **(F)** Relative mRNA levels of APOL1 in EGFP-APOL1 expressing HEK293T cells confirmed comparable expression levels of APOL1 G0 and RRVs, shown in western blot analyses (seen in A and C) also on mRNA level. APOL1 mRNA levels ($2^{-\Delta\Delta ct}$ values) were normalized to levels of HEK293T-wt cells (control) without Dox. Dox induction 24 h.

Suppl. movie: M1: AB8 podocytes expressing APOL1 variants G0, G1 or G2 were followed over time for 30 sec using TIRF microscopy. Zoomed areas of the cell peripheries are shown.



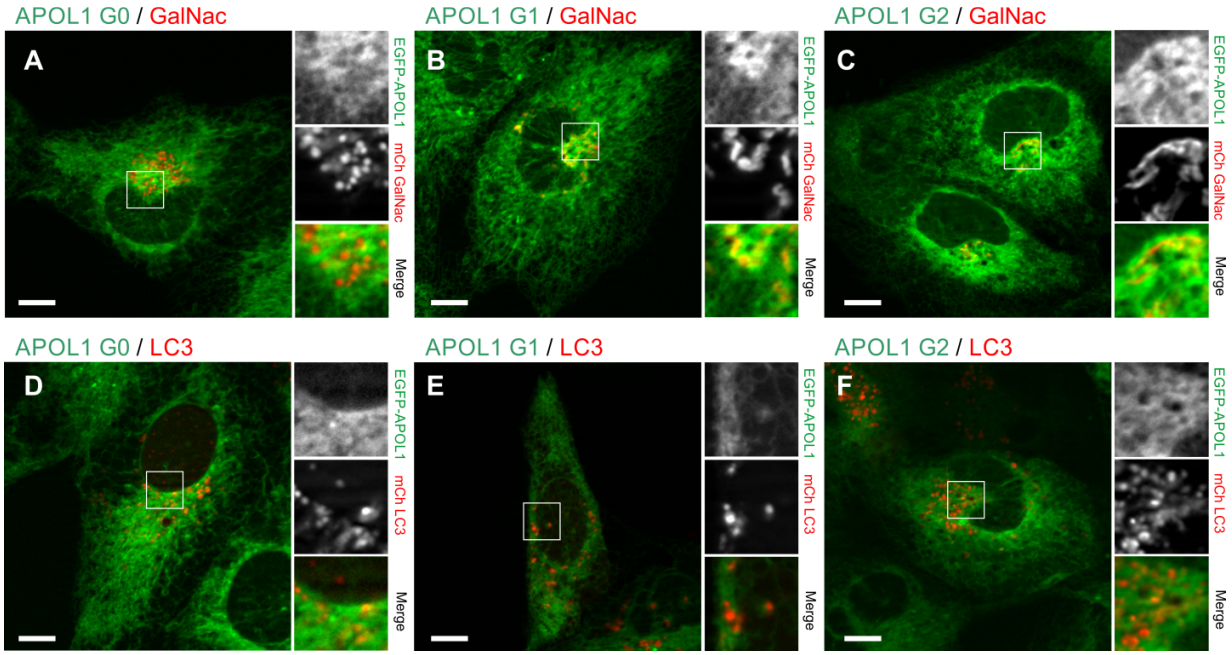
Suppl. Fig. SF5: *EGFP-APOL1 is targeted to the ER independently of the SP in podocytes.*
Live cell image analyses of EGFP-tagged APOL1 (green, Dox-induction 24 h) transiently transfected with a plasmid encoding mCherry-tagged Sec61 β (red) confirmed SP independent targeting of APOL1 to the ER. EGFP-APOL1 G0 and renal risk variants APOL1 G1 and G2 showed a similar intracellular distribution. Scale bar and square length of details are 20 µm and 10 µm respectively.



Suppl. Fig. SF6:

EGFP-APOL1 shows no or only minor co-localization with early or late endosomal marker proteins in podocytes.

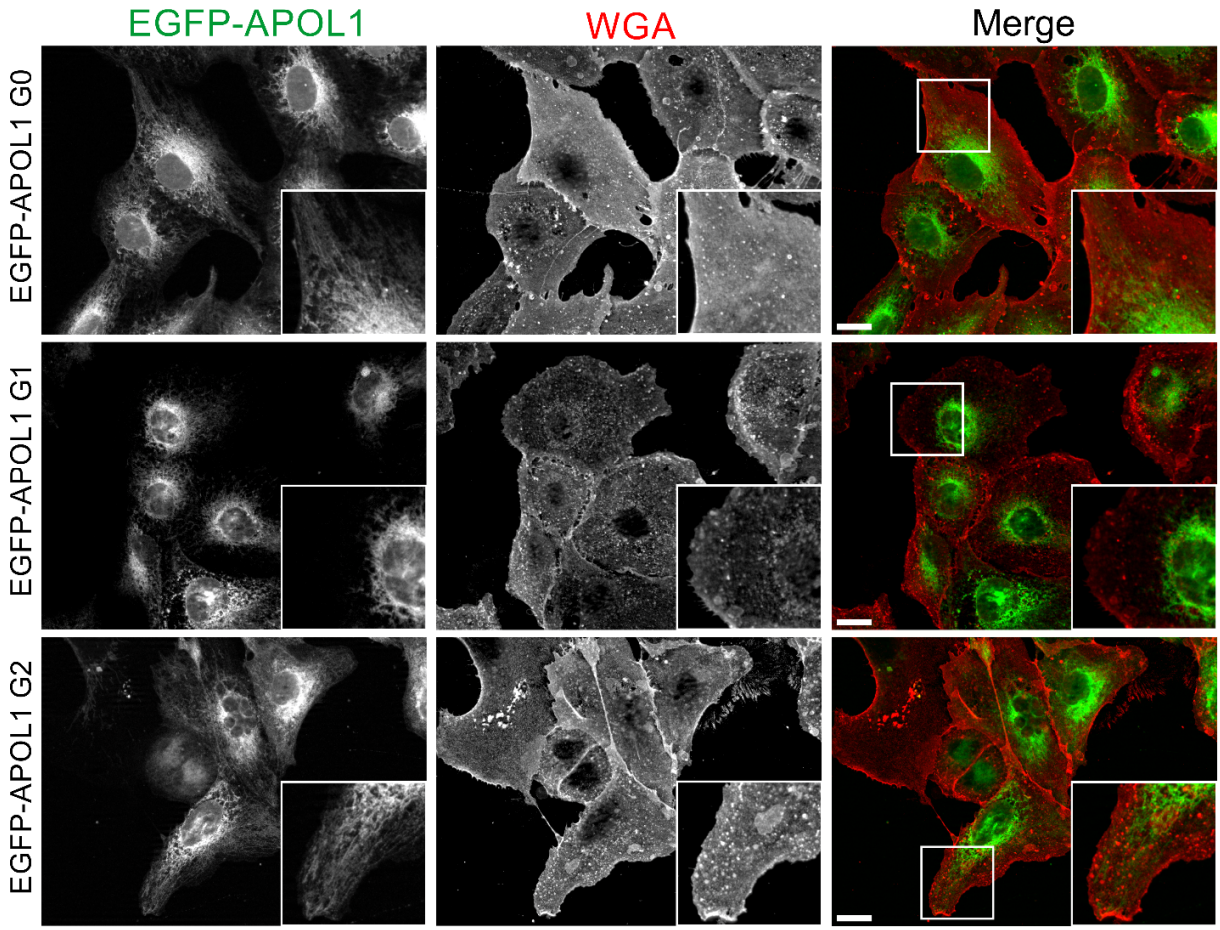
Live cell image analyses of EGFP-tagged APOL1 (green, Dox-induction 24 h) transiently transfected with plasmids encoding mCherry-tagged Rab5 (early) or Rab7 (late endosomal) showed no or only minor degree of co-localization with EGFP-APOL1. Scale bar and square length of details are 10 µm.



Suppl. Fig. SF7:

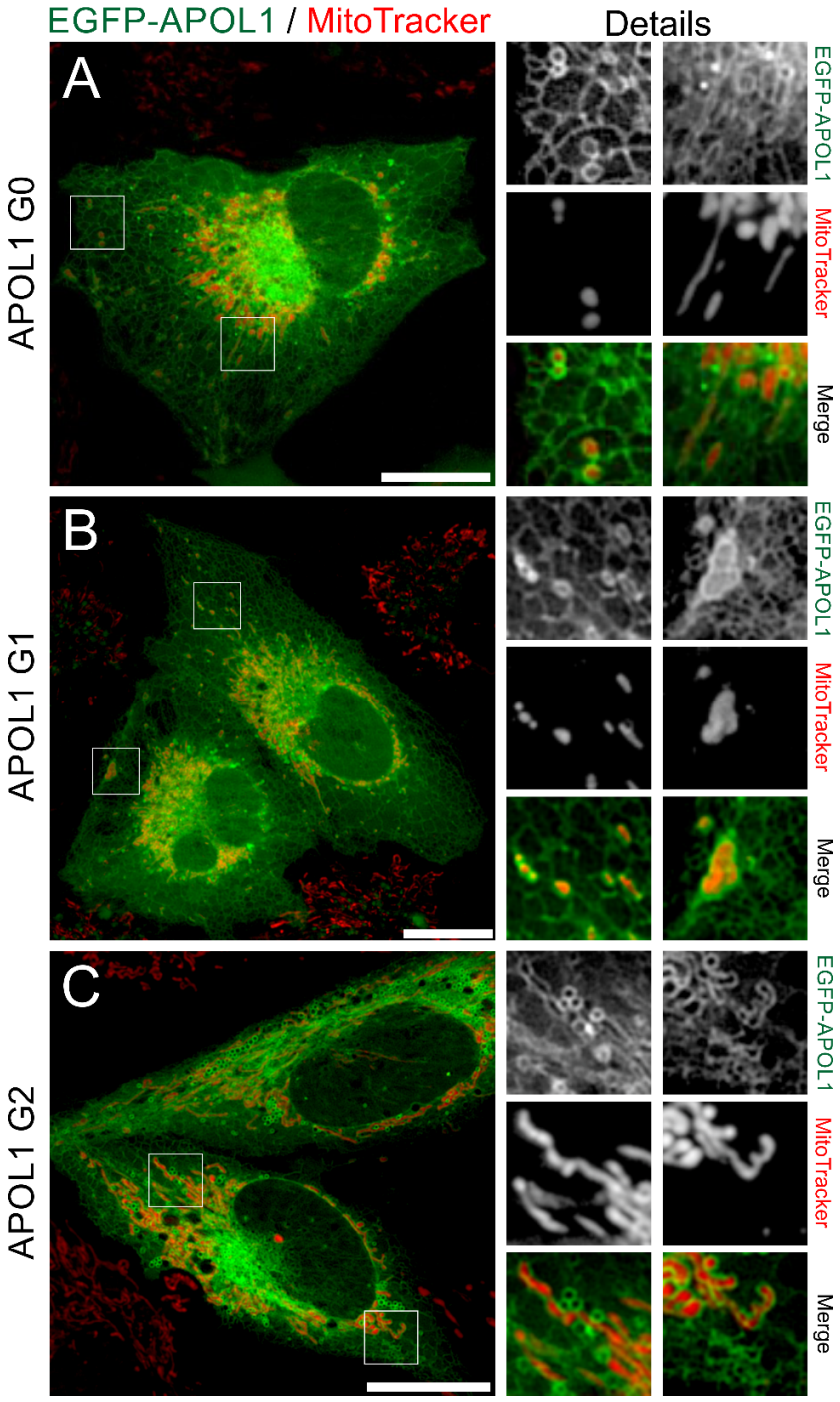
EGFP-APOL1 shows no or only minor co-localization with autophagosomal markers or markers of the Golgi-apparatus in podocytes.

Live cell image analyses of EGFP-tagged APOL1 (green, Dox-induction 24 h) transiently transfected with plasmids encoding mCherry-tagged GalNac or LC3 (red), showed no or only minor degree of co-localization with these markers for the *trans*-Golgi-apparatus (GalNac) or autophagosomes (LC3). Scale bar and square length of details are 10 µm.

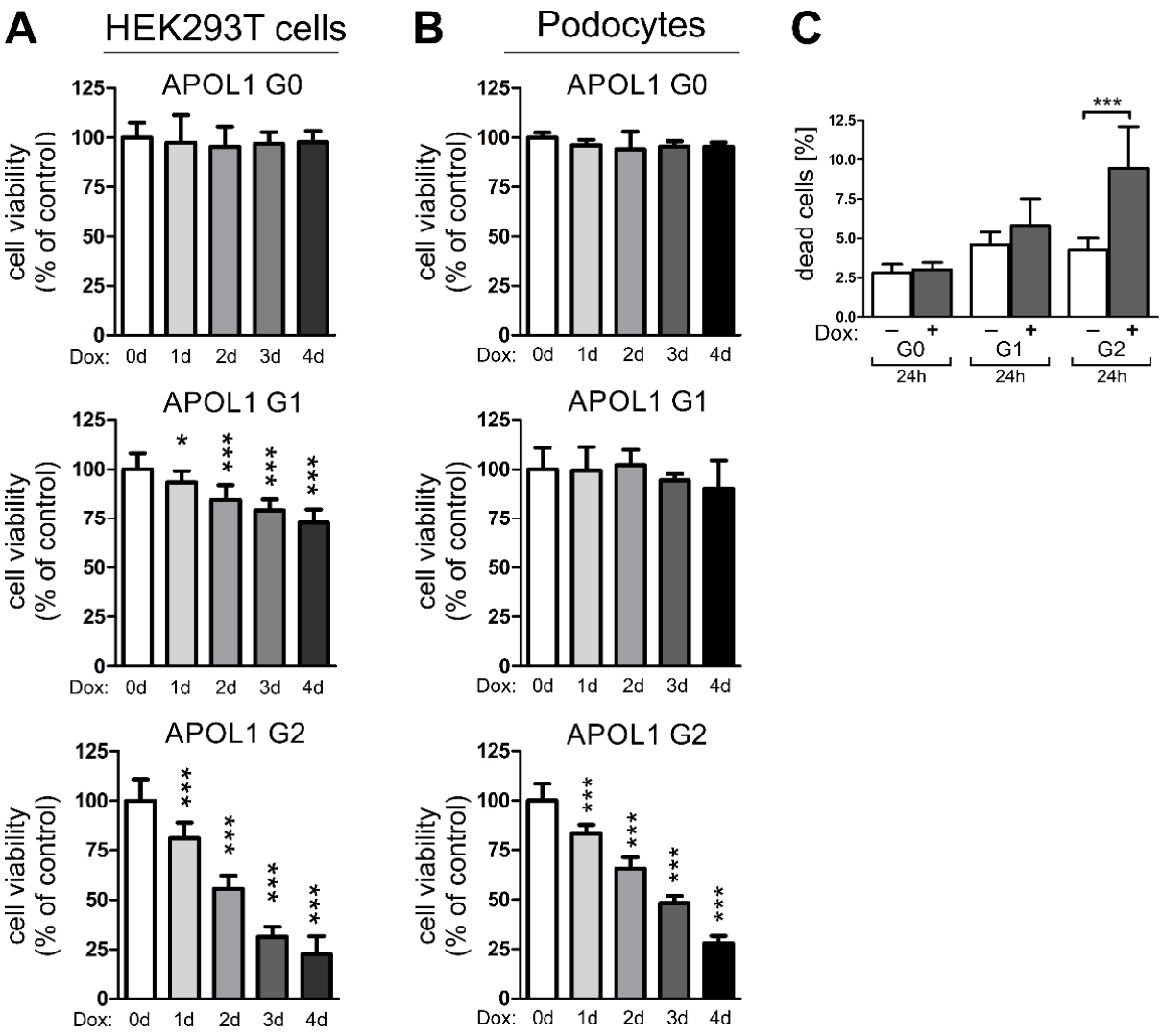


Suppl. Fig. SF8: *APOL1 did not localize at the PM.*

10 min before fixation with paraformaldehyde EGFP-APOL1 expressing podocytes, lacking the SP were incubated with Alexa633-conjugated WGA (wheat germ agglutinin) which specifically marks the PM due to its binding to sialic acid or N-acetylglucosamine containing surface proteins. We observed no co-localization of EGFP-APOL1 G0 and RRVs with WGA (red). (Dox+ 24 h) Scale bar and square length of details are 20 µm and 50 µm respectively.

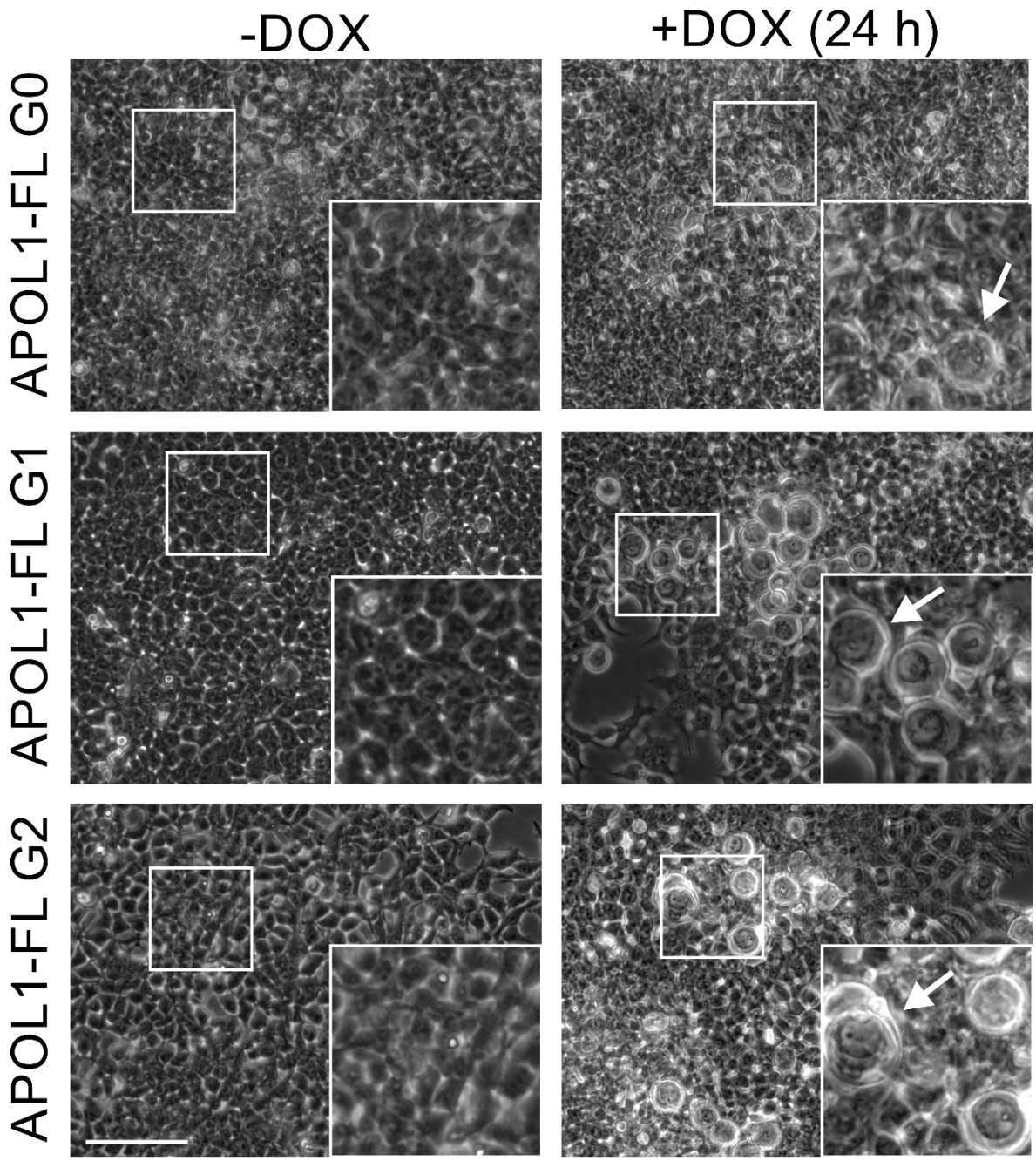


Suppl. Fig. SF9: *APOL1 membrane recognition is conserved during evolution.*
(A-C) Life cell image analyses of non-human renal CV-1 cells expressing EGFP-tagged APOL1 (green, 24 h after transfection) in combination with MitoTracker (red) confirmed ER and partial mitochondrial targeting of APOL1. Again, EGFP-APOL1 G0 (A) and renal risk variants APOL1 G1 (B) and G2 (C) showed a similar intracellular distribution. Scale bar and square length of details are 20 µm.

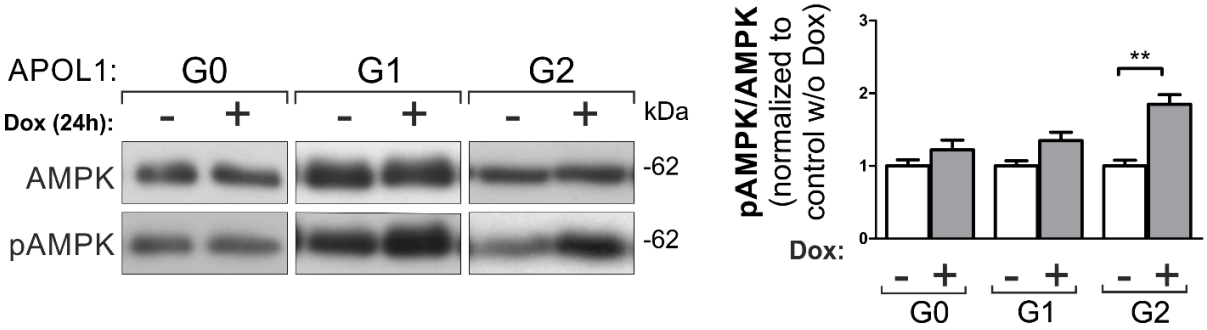


Suppl. Fig. SF10: *Overexpression of EGFP-APOL1 renal risk variants show reduced cell viability and increased cytotoxicity.*

(A/B) Decreased cell viability mediated by APOL1 renal risk variant expression was further confirmed by an alternative method for stable Dox-inducible HEK293T cells and podocytes *via* a MTT assay. **(C)** Decreased cell viability is accompanied by an increased number of dead cells especially for variant G2 expressing cells. Cytotoxicity was measured with Cytotox-Glo assay in HEK293T cells in two independent experiments.

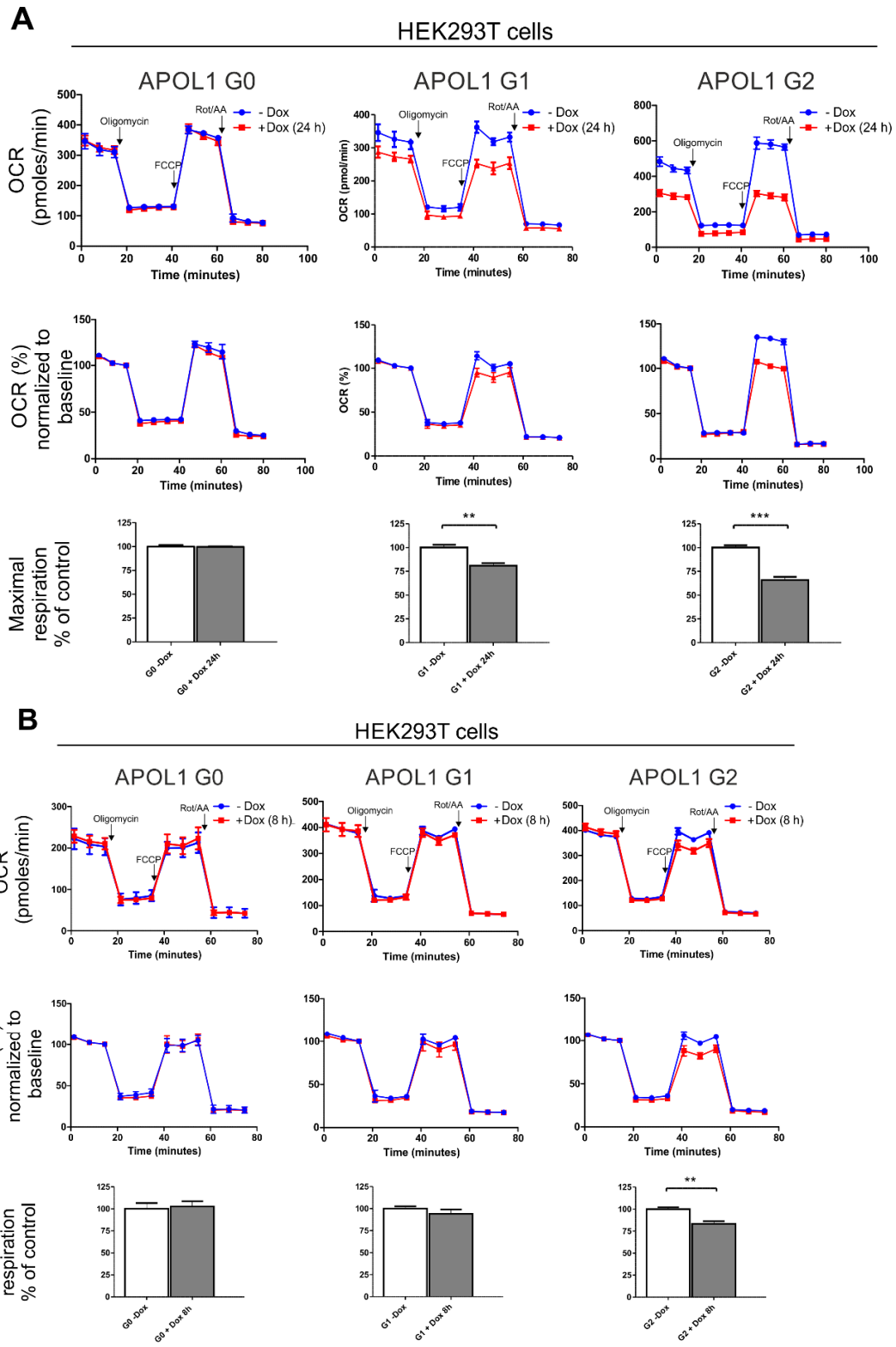


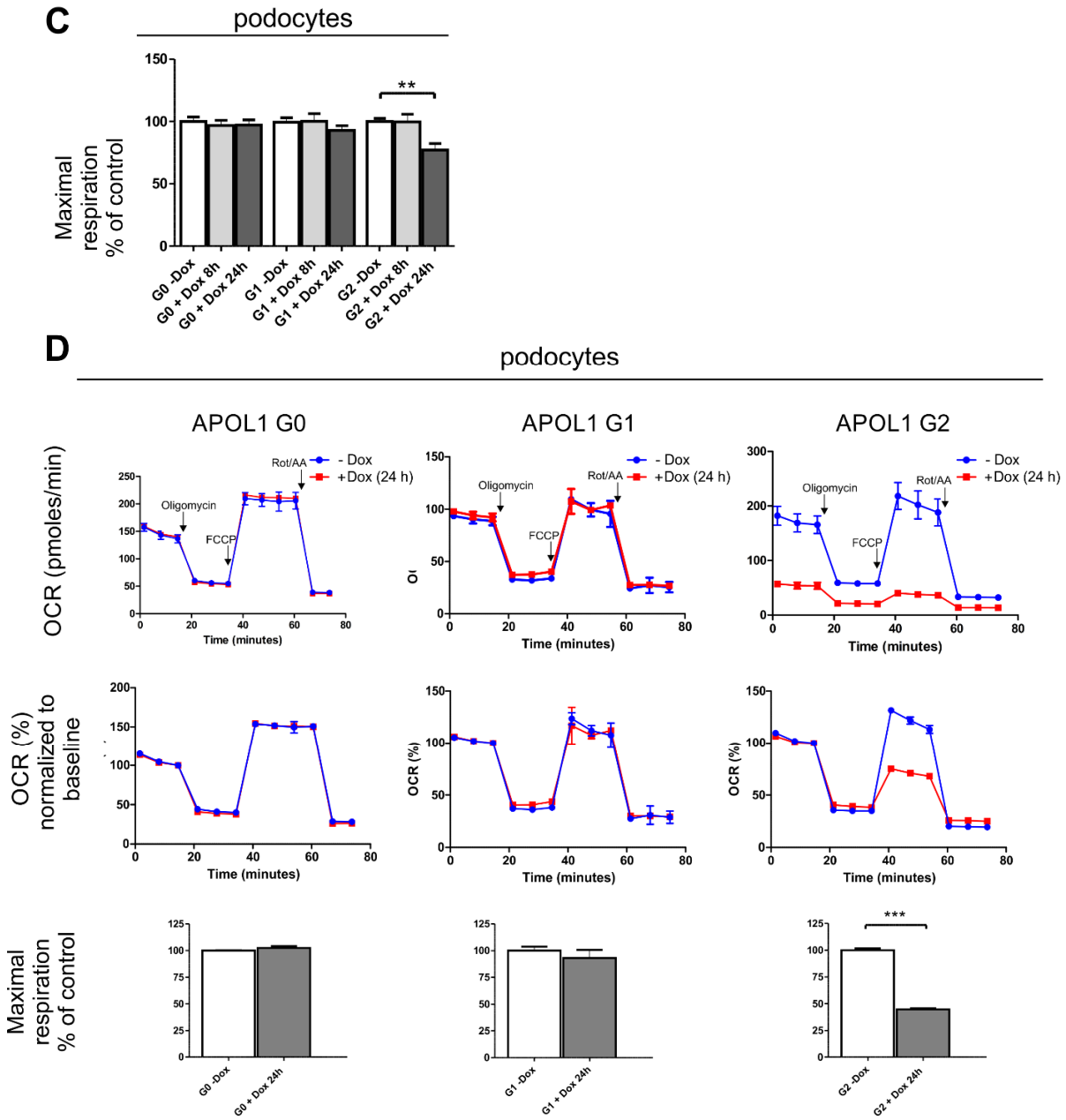
Suppl. Fig. SF11: *Overexpression of EGFP-APOL1 renal risk variants mediate cellular swelling.*
Transmitted light bright field images of HEK293T cells overexpressing full length (FL) APOL1 renal risk variants (Dox induction 24 h) showed increasing amounts of cells with swollen morphology compared to variant G0. Arrows point to swollen cells. Scale bar: 100 µm.

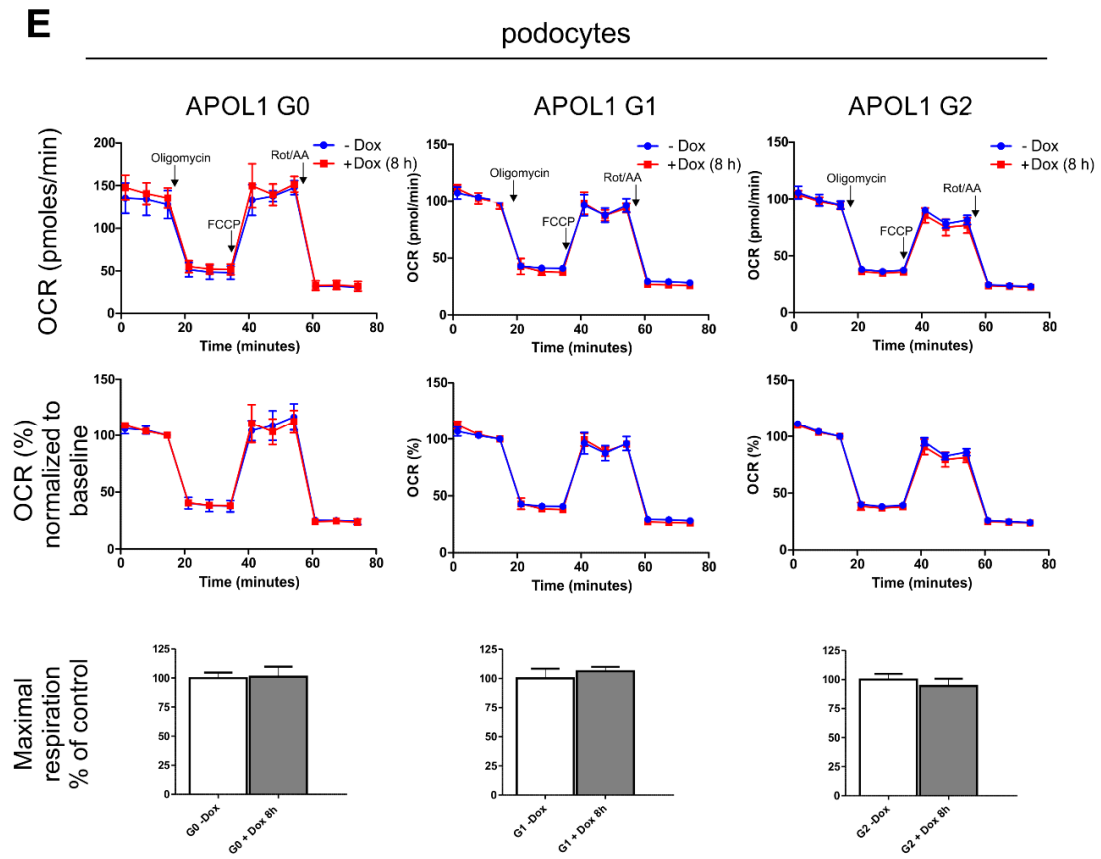


Suppl. Fig. SF12: *APOL1 G2 expression mediates activation of AMPK in podocytes.*

Western blot analysis (left) and quantification (right) of EGFP-APOL1 variant expressing podocytes shows increased pAMPK levels (pAMPK/AMPK ratio) in case of variant G2 expression (Dox induction 24 h).


A
HEK293T cells
APOL1 G0
APOL1 G1
APOL1 G2
OCR (pmoles/min)
OCR (pmol/min)
Oligomycin
FCCP
Rot/AA
- Dox
+Dox (24 h)
Time (minutes)
OCR (%) normalized to baseline
OCR (%)
Maximal respiration % of control
**
***
G0 -Dox
G0 + Dox 24h
G1 -Dox
G1 + Dox 24h
G2 -Dox
G2 + Dox 24h
B
HEK293T cells
APOL1 G0
APOL1 G1
APOL1 G2
+Dox (8 h)
G0 -Dox
G0 + Dox 8h
G1 -Dox
G1 + Dox 8h
G2 -Dox
G2 + Dox 8h


C
podocytes
Maximal respiration % of control
150
100
50
0
**
G0 -Dox
G0 + Dox 8h
G0 + Dox 24h
G1 -Dox
G1 + Dox 8h
G1 + Dox 24h
G2 -Dox
G2 + Dox 8h
G2 + Dox 24h
D
podocytes
APOL1 G0
APOL1 G1
APOL1 G2
OCR (pmoles/min)
Oligomycin
FCCP
Rot/AA
- Dox
+Dox (24 h)
Time (minutes)
OCR (%) normalized to baseline
OCR (%)
Maximal respiration % of control
***
G0 -Dox
G0 + Dox 24h
G1 -Dox
G1 + Dox 24h
G2 -Dox
G2 + Dox 24h



Suppl. Fig. SF13 *APOL1 renal risk variant expression results in reduced mitochondrial respiration in HEK293T cells and podocytes.*

Metabolic measurements (OCR; oxygen consumption rate) of EGFP-APOL1 overexpressing HEK293T cells **(A/B)** and podocytes **(C-E)**, measured with Mito Stress Test Kit using a Seahorse extracellular flux analyzer (Agilent Technologies).

Representative Seahorse measurement curves of HEK293T cells expressing APOL1 G0, G1 or G2 after 24 h **(A)** or 8 h of Dox induction **(B)** (upper panel). Basal respiration was set at 100% (middle panel, OCR normalized to baseline). Baseline corrected values were normalized to non-induced control (Dox-) and changes in the maximal respiration rate were shown (lower panel, maximal respiration % of control). HEK293T cells overexpressing EGFP-APOL1 G1 and G2 show significantly reduced maximal mitochondrial respiration rates in contrast to APOL1 G0, 24 h after Dox induction (A). Variant G2 expressing cells even showed a reduced mitochondrial respiration rates after 8h of Dox induction (B). **(C)** Summary of maximal respiration levels over all applied experiments (N≥6). Baseline corrected values were normalized to non-induced control (Dox-)

In case of EGFP-APOL1 expressing podocytes, only variant G2 expressing cells showed reduced maximal respiration rates 24 h after Dox induction **(C/D)**. After 8h Dox induction **(E)** the decrease in mitochondrial respiration was not yet detectable in the podocyte cell system. Oligomycin: 2 µM; FCCP: 0.5 µM; AntimycinA (AA) and Rotenone (Rot): 0.5 µM.

Suppl. Table ST1: Constructs used in the study

No	Construct name	intention	Insert	cloned by	Backbone
1	pEGFP-APOL1 G0$_{aa1-398}$	Expression of EGFP-fusion	APOL1 aa1-398; APOL1 G0, variant A, with signal peptide*	-	pEGFP-N1
2	pENTR-APOL1 G0 $_{aa1-398}$	cloning	APOL1 aa1-398; APOL1 G0, variant A	AscI /PacI	pENTR-EGFP
3	pENTR-APOL1 G1 $_{aa1-398}$	cloning	APOL1 aa1-398; APOL1 G1, variant A	AscI /PacI	pENTR-EGFP
4	pENTR-APOL1 G2 $_{aa1-398}$	cloning	APOL1 a1-398; APOL1 G2, variant A	AscI /PacI	pENTR-EGFP
5	pENTR-EGFP-APOL1 G0 $_{aa28-398}$	cloning	APOL1 aa28-398; APOL1 G0, variant A, without signal peptide	AscI /PacI	pENTR-EGFP
6	pENTR-EGFP-APOL1 G1 $_{aa28-398}$	cloning	APOL1 aa28-398; APOL1 G1, variant A, without signal peptide	AscI /PacI	pENTR-EGFP
7	pENTR-EGFP-APOL1 G2 $_{aa28-398}$	cloning	APOL1 aa28-398; APOL1 G2, variant A, without signal peptide	AscI /PacI	pENTR-EGFP
8	pInd21P- APOL1 G0 $_{aa1-398}$	Dox-induced expression of untagged APOL1 G0	APOL1 aa1-398; APOL1 G0, variant A	via LR Clonase	pInd21P**
9	pInd21P- APOL1 G1 $_{aa1-398}$	Dox-induced expression of untagged APOL1 G1	APOL1 aa1-398; APOL1 G1, variant A	via LR Clonase	pInd21P
10	pInd21P-APOL1 G2 $_{aa1-398}$	Dox-induced expression of untagged APOL1 G2	APOL1 aa1-398; APOL1 G2, variant A	via LR Clonase	pInd21P
11	pInd21P-EGFP-APOL1 G0 $_{aa28-398}$	Dox-induced expression of EGFP-fusion protein	APOL1 aa28-398; APOL1 G0, variant A, without signal peptide	via LR Clonase	pInd21P
12	pInd21P-EGFP-APOL1 G1 $_{aa28-398}$	Dox-induced expression of EGFP-fusion protein	APOL1 aa28-398; APOL1 G1, variant A, without signal peptide	via LR Clonase	pInd21P
13	pInd21P-EGFP-APOL1 G2 $_{aa28-398}$	Dox-induced expression of EGFP-fusion protein	APOL1 aa28-398; APOL1 G2, variant A, without signal peptide	via LR Clonase	pInd21P
14	pENTR-EGFP	cloning	EGFP	via AscI/PacI	pInd21P
15	pInd21P-EGFP-	Dox-induced expression of EGFP	EGFP	via LR Clonase	pInd21P

*) Numbering of amino acids (aa) base on the uniprot sequences given with UniProtKB - O14791 (APOL1_HUMAN, variant A)
**) pInd21P (pINDUCER21-Puro) was established from the pINDUCER21 backbone by replacing the GFP cDNA insert by a Puromycin resistance gene. Details are given in Meerbrey et al., 2011 PNAS and Schulze et al., 2014 MCP.