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Supplementary Table 1: APOL1 Sequences used to make Tet inducible HEK293 cells

Genotype	Sequence
G0	ATGGAGGGAGCTGCTTTGCTGAGAGTCTCTGTCCTCTGCATCTGGATG AGTGCACTTTTCCTTGGTGTGGGAGTGAGGGCAGAGGAAGCTGGAGC GAGGGTGCAACAAAACGTTCCAAGTGGGACAGATACTGGAGATCCTCA AAGTAAGCCCCTCGGTGACTGGGCTGCTGGCACCATGGACCCAGAGA GCAGTATCTTTATTGAGGATGCCATTAAGTATTTCAAGGAAAAAGTGAG CACACAGAATCTGCTACTCCTGCTGACTGATAATGAGGCCTGGAACGG ATTCGTGGCTGCTGCTGAACTGCCAGGAATGAGGCAGATGAGCTCC GTAAAGCTCTGGACAACCTTGCAAGACAAATGATCATGAAAGACAAAAA CTGGCACGATAAAGGCCAGCAGTACAGAACTGGTTTCTGAAAGAGTT TCCTCGGTTGAAAAGTGAGCTTGAGGATAACATAAGAAGGCTCCGTGC CCTTGCAGATGGGGTTCAGAAGGTCCACAAAGGCACCACCATCGCCAA TGTGGTGTCTGGCTCTCTCAGCATTTCTCTGGCATCCTGACCCTCGT CGGCATGGGTCTGGCACCCTTCACAGAGGGAGGCAGCCTTGTA CTCTT GGAACCTGGGATGGAGTTGGGAATCACAGCAGCTTTGACCGGGATTAC CAGTAGTACCATAGACTACGGAAAGAAGTGGTGGACACAAGCCCAAGC CCACGACCTGGTCATCAAAGCCTTGACAAATTGAAGGAGGTGAAGGA GTTTTTGGGTGAGAACATATCCAACCTTCTTTCTTAGCTGGCAATACTT ACCAACTCACACGAGGCATTGGGAAGGACATCCGTGCCCTCAGACGA GCCAGAGCCAATCTTCAGTCAGTACCGCATGCCTCAGCCTCACGCCCC CGGGTCACTGAGCCAATCTCAGCTGAAAGCGGTGAACAGGTGGAGAG AGTTAATGAACCCAGCATCCTGGAAATGAGCAGAGGAGTCAAGCTCAC GGATGTGGCCCCTGTAAGCTTCTTTCTTGTGCTGGATGTAGTCTACCTC GTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCAGAGACAGCT GAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACAT TCTCAACAATAATTATAAGATTCTGCAGGCGGACCAAGA ACTATGA
G1	ATGGAGGGAGCTGCTTTGCTGAGAGTCTCTGTCCTCTGCATCTGGATG AGTGCACTTTTCCTTGGTGTGGGAGTGAGGGCAGAGGAAGCTGGAGC GAGGGTGCAACAAAACGTTCCAAGTGGGACAGATACTGGAGATCCTCA AAGTAAGCCCCTCGGTGACTGGGCTGCTGGCACCATGGACCCAGAGA GCAGTATCTTTATTGAGGATGCCATTAAGTATTTCAAGGAAAAAGTGAG CACACAGAATCTGCTACTCCTGCTGACTGATAATGAGGCCTGGAACGG ATTCGTGGCTGCTGCTGAACTGCCAGGAATGAGGCAGATGAGCTCC GTAAAGCTCTGGACAACCTTGCAAGACAAATGATCATGAAAGACAAAAA CTGGCACGATAAAGGCCAGCAGTACAGAACTGGTTTCTGAAAGAGTT TCCTCGGTTGAAAAGTGAGCTTGAGGATAACATAAGAAGGCTCCGTGC CCTTGCAGATGGGGTTCAGAAGGTCCACAAAGGCACCACCATCGCCAA TGTGGTGTCTGGCTCTCTCAGCATTTCTCTGGCATCCTGACCCTCGT CGGCATGGGTCTGGCACCCTTCACAGAGGGAGGCAGCCTTGTA CTCTT GGAACCTGGGATGGAGTTGGGAATCACAGCAGCTTTGACCGGGATTAC CAGTAGTACCATAGACTACGGAAAGAAGTGGTGGACACAAGCCCAAGC CCACGACCTGGTCATCAAAGCCTTGACAAATTGAAGGAGGTGAAGGA GTTTTTGGGTGAGAACATATCCAACCTTCTTTCTTAGCTGGCAATACTT ACCAACTCACACGAGGCATTGGGAAGGACATCCGTGCCCTCAGACGA GCCAGAGCCAATCTTCAGTCAGTACCGCATGCCTCAGCCTCACGCCCC

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G2	ATGGAGGGAGCTGCTTTGCTGAGAGTCTCTGTCCTCTGCATCTGGATG AGTGCACTTTTCCTTGGTGTGGGAGTGAGGGCAGAGGAAGCTGGAGC GAGGGTGCAACAAAACGTTCCAAGTGGGACAGATACTGGAGATCCTCA AAGTAAGCCCCTCGGTGACTGGGCTGCTGGCACCATGGACCCAGAGA GCAGTATCTTTATTGAGGATGCCATTAAGTATTTCAAGGAAAAAGTGAG CACACAGAATCTGCTACTCCTGCTGACTGATAATGAGGCCTGGAACGG ATTCGTGGCTGCTGCTGAACTGCCCAGGAATGAGGCAGATGAGCTCC GTAAAGCTCTGGACAACCTTGCAAGACAAATGATCATGAAAGACAAAAA CTGGCACGATAAAGGCCAGCAGTACAGAAACTGGTTTCTGAAAGAGTT TCCTCGGTTGAAAAGTGAGCTTGAGGATAACATAAGAAGGCTCCGTGC CCTTGCAGATGGGGTTCAGAAGGTCCACAAAGGCACCACCATCGCCAA TGTGGTGTCTGGCTCTCTCAGCATTTCTCTGGCATCCTGACCCTCGT CGGCATGGGTCTGGCACCCCTTCACAGAGGGAGGCAGCCTTGTA CTCTT GGAACCTGGGATGGAGTTGGGAATCACAGCAGCTTTGACCGGGATTAC CAGTAGTACCATAGACTACGGAAAGAAGTGGTGGACACAAGCCCAAGC CCACGACCTGGTCATCAAAGCCTTGACAAATTGAAGGAGGTGAAGGA GTTTTTGGGTGAGAACATATCCAACCTTTCTTTCTTAGCTGGCAATACTT ACCAACTCACACGAGGCATTGGGAAGGACATCCGTGCCCTCAGACGA GCCAGAGCCAATCTTCAGTCAGTACCGCATGCCTCAGCCTCACGCCCC CGGGTCACTGAGCCAATCTCAGCTGAAAGCGGTGAACAGGTGGAGAG AGTTAATGAACCCAGCATCCTGGAAATGAGCAGAGGAGTCAAGCTCAC GGATGTGGCCCCTGTAAGCTTCTTTCTTGTGCTGGATGTAGTCTACCTC GTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCAGAGACAGCT GAGGAGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACAT TCTCAACAATAAGATTCTGCAGGCGGACCAAGAAGTATGA

Supplementary Table 2: List of Mitochondrial proteins pulled down after IP-APOL1 Mass Spectrometry.

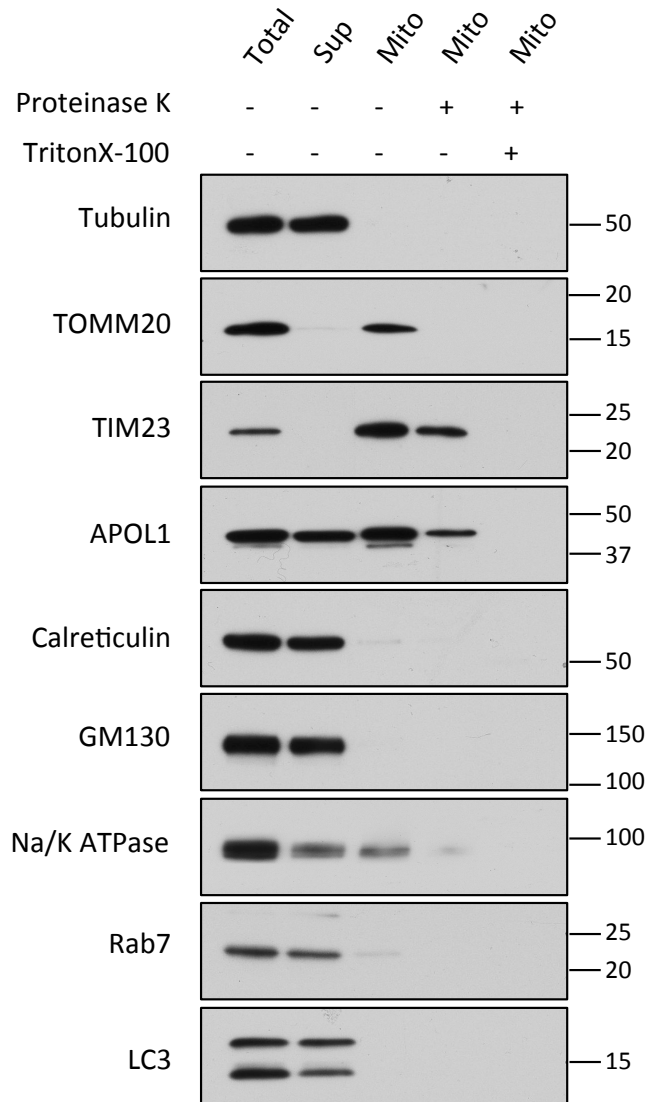
Gene Symbol	Total Peptides (after subtracting background)		
	G0	G1	G2
APOL1	466	322	281
ABAT	5	3	1
ABCF2	4	3	6
ACADSB	5	7	16
ACAT1	1	4	5
ACOT9	3	5	8
ACSL1	10	7	9
ACSL3	6	9	13
AFG3L2	9	4	17
AGPS	2	1	7
AIFM1	12	11	27
ALDH18A1	0	0	8
ALDH1B1	8	17	17
ALDH7A1	4	5	0
ARAF	8	5	8
ARMCX3	0	3	5
ATAD3A	29	38	62
ATAD3B	2	5	8
ATP5A1	51	51	62
ATP5B	56	72	40
ATP5C1	9	16	12
ATP5F1	5	3	1
ATP5O	3	5	7
BSG	1	9	3
C1QBP	5	3	4
CDK1	11	18	23
CHPF	16	10	17
CLPP	2	0	5
CLPX	6	3	7
CLU	12	15	36
CPNE3	0	5	2
CS	6	7	5
DBT	11	8	6
DDX3X	1	4	8
DHX30	0	4	6

DLAT	4	5	11
DLD	2	9	8
DLST	8	8	4
DNAJA1	13	7	12
DNAJA3	9	13	11
ECH1	2	9	4
ETFA	4	9	7
GARS	4	3	6
GCDH	4	3	6
GLUD1	24	16	16
GSTP1	5	5	3
HADHA	17	29	20
HADHB	6	6	7
HAX1	5	7	11
HK1	3	1	8
HSD17B10	4	10	7
HSP90AB1	6	10	5
HSPA1A	16	10	11
HSPA9	41	49	37
HSPD1	36	49	54
IARS2	1	5	1
IDE	8	7	21
IDH2	7	2	10
IDH3A	7	5	11
IDH3B	4	8	9
ILF3	5	3	6
IMMT	2	5	18
KARS	1	5	5
LRPPRC	11	16	13
MAOA	4	0	5
MCCC2	2	5	4
MDH2	6	18	16
MMAB	0	3	6
MRPL4	1	6	6
MRPS22	5	7	7
MRPS27	1	3	5
MTHFD1	19	16	13
MTHFD1L	10	8	12
MTHFD2	0	5	4
MTOR	5	2	3
NDUFA4	7	7	22

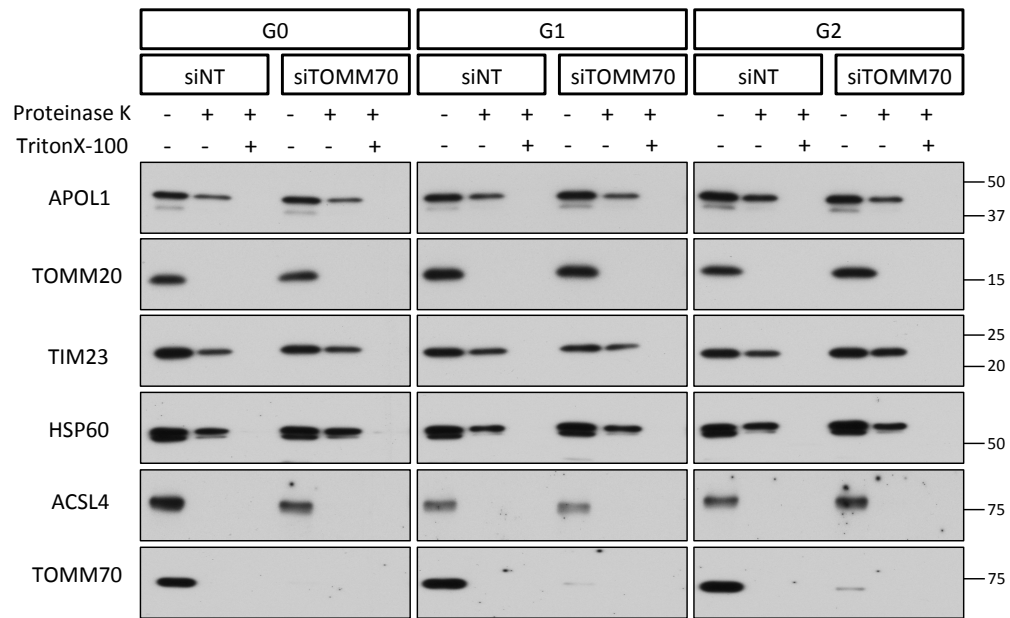
NDUFS2	5	3	0
OAT	19	11	26
OPA1	1	6	11
P4HA1	6	6	12
PARP1	13	0	12
PDHB	11	12	15
PK3	2	7	5
PGAM5	2	7	10
PHB	13	18	12
PHB2	8	24	16
PKM	33	37	32
PNPT1	2	0	5
PON2	3	6	4
PPP2R1A	7	10	14
PRDX3	6	12	13
PTRF	2	0	6
QARS	0	12	10
RARS2	3	2	5
RPS3	44	54	60
SARS2	2	11	6
SDHA	4	0	5
SFXN1	11	26	28
SFXN2	0	6	6
SFXN3	3	4	6
SHMT2	3	6	3
SLC25A1	2	6	12
SLC25A11	9	11	16
SLC25A12	3	6	17
SLC25A13	11	9	15
SLC25A3	28	20	27
SLC25A4	17	25	29
SLC25A5	14	25	17
SLC25A6	5	6	5
SQRDL	4	3	9
SSBP1	0	5	0
STOM	3	51	16
STOML2	5	4	14
SUCLG2	2	5	1
TARS2	0	1	7
TIMM50	5	20	18
TRAP1	4	6	9

TUFM	25	33	52
UBA1	13	13	17
UBA52	52	33	31
VDAC1	1	7	4
VDAC2	10	8	13
VRK2	5	2	3
YME1L1	6	8	7
YWHAB	12	17	12
YWHAQ	9	9	6
YWHAZ	6	12	5

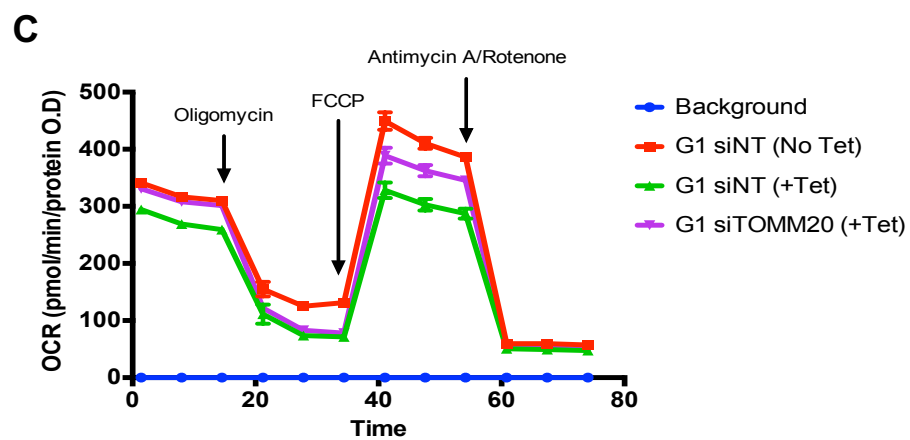
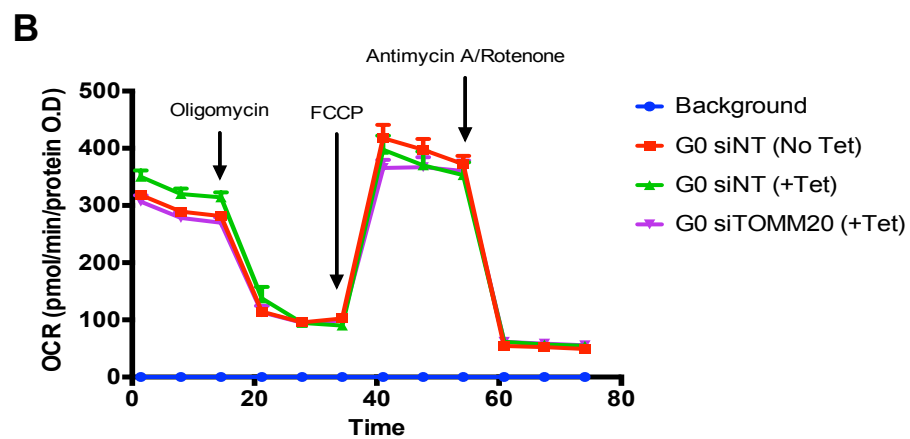
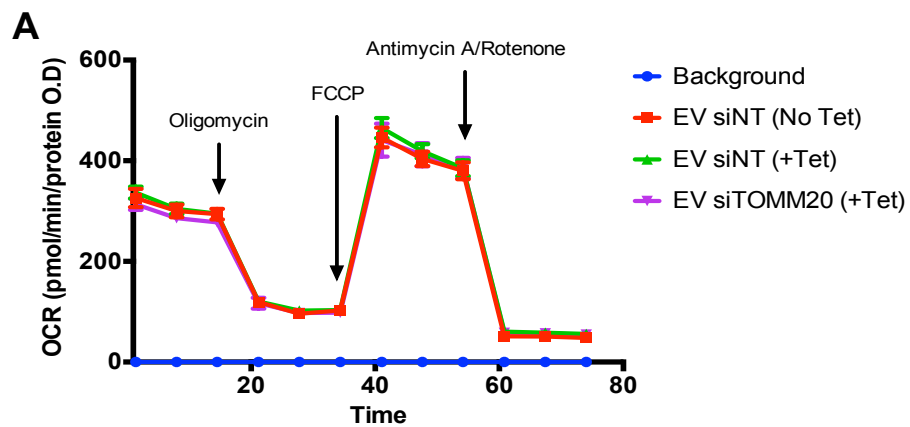
Supplementary Figures:

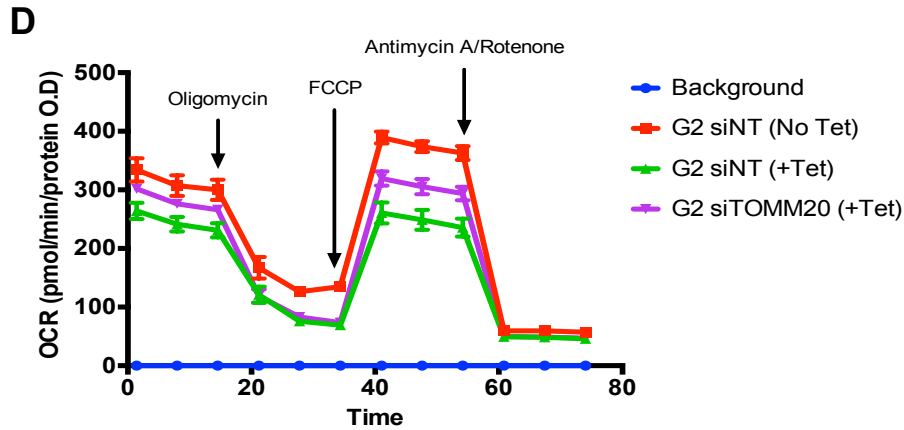


Supplementary Figure 1: Western blot analysis of organelle markers in the cytosolic and mitochondrial-enriched fractions. HEK293 cells stably transfected with APOL1 G0 were induced with tetracycline for 6 hours and then subjected to differential centrifugation. The mitochondria-enriched fraction was subjected to proteinase K digestions. Each cell fraction was tested for presence of markers for cytosol (Tubulin), OMM (TOMM20), IMM (TIMM23), ER (Calreticulin), Golgi (GM130), plasma membrane (Na/K ATPase), endosome (Rab7) and autophagosome (LC3).

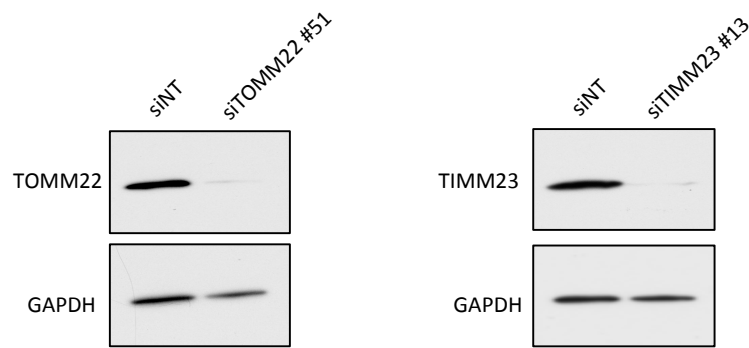


Supplementary Figure 2: APOL1 translocation to mitochondrial matrix is independent of OMM carrier pathway protein TOMM70. TOMM70 knockdown does not affect mitochondrial APOL1. Neither non-target siRNA (siNT) nor TOMM70 siRNA (siTOMM70) sensitize APOL1 to Proteinase K digestion.

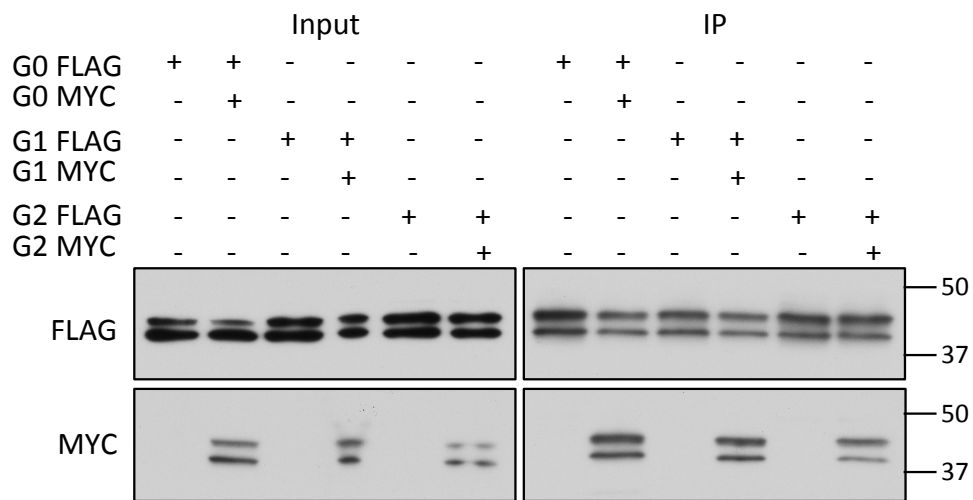




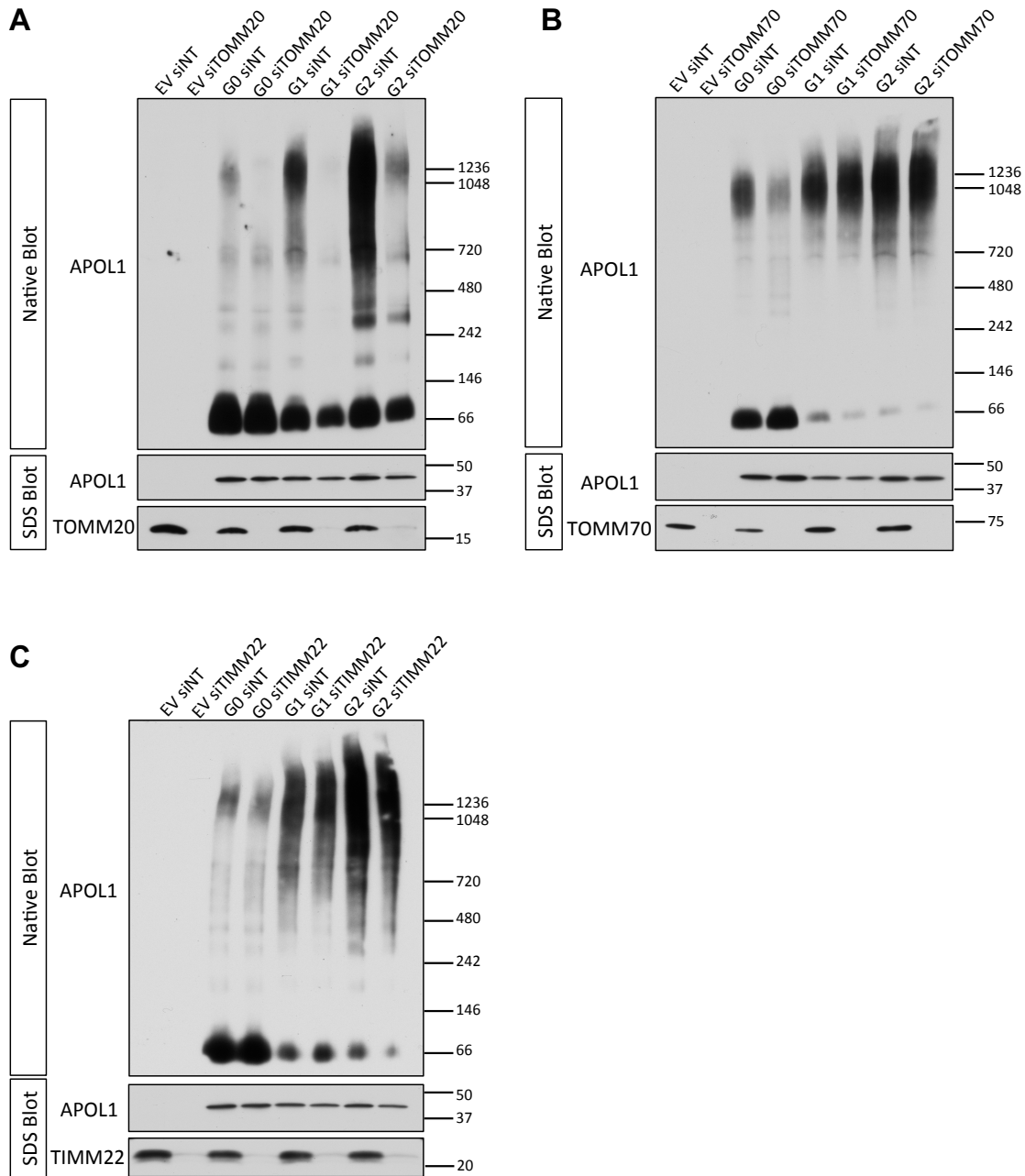
Supplementary Figure 3: TOMM20 knockdown partially rescues APOL1 RV-mediated mitochondrial dysfunction. (A-D) Seahorse oxygen consumption profile for HEK293 cells with or without tetracycline induction and TOMM20 knockdown, in EV, G0, G1, and G2 cells respectively, demonstrating basal respiratory rate, ATP production, maximal respiratory capacity and non-mitochondrial respiration rates. We validated previously published data showing that expression of G1 or G2 impairs mitochondrial function, as seen in baseline oxygen consumption rate (prior to oligomycin addition) and max OCR (post-FCCP until Antimycin A/Rotenone addition). TOMM20 knockdown completely rescued baseline OCR in G1, partially restored baseline OCR in G2, and partially rescued maximal OCR in both G1 and G2. Seahorse data is from a single representative run. The experiment was repeated 3 independent times.



Supplementary Figure 4: siRNA Validation blots for knockdown efficiencies not shown in main figures. Immunoblots for Tomm22 and Timm23 48h after siRNA transfection in Empty Vector cells.



Supplementary Figure 5: APOL1 can bind to other APOL1 polypeptides. HEK293 cells were co-transfected with Myc-tagged and FLAG-tagged APOL1 constructs, pulled down with FLAG beads and immunoblotted for Myc and FLAG.

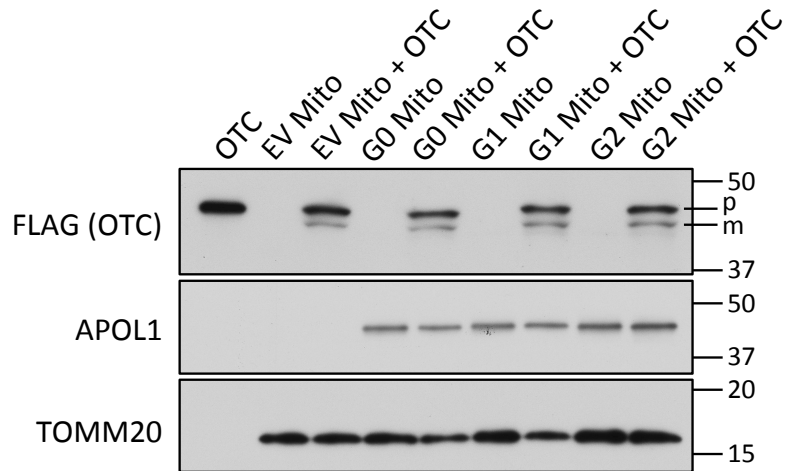


Supplementary Figure 6: TOMM20 knockdown reduces APOL1 oligomerization while TOMM70 and TIMM22 knockdown do not prevent APOL1 oligomerization.

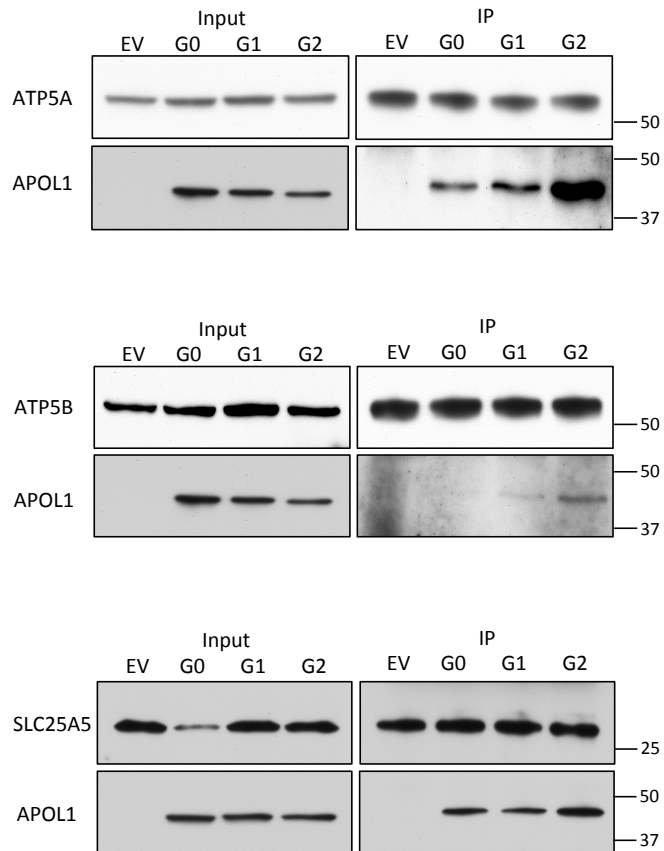
A. Native PAGE blot of APOL1 and SDS-PAGE blots of APOL1 and TOMM20 after TOMM20 knockdown followed by 50 ng/ml tetracycline induction for 15 h. TOMM20 knockdown reduced APOL1 RV oligomerization and APOL1 RV-induced cell death (see Fig 2C).

B. Native PAGE blot of APOL1 and SDS-PAGE blots of APOL1 and TOMM70 after TOMM70 knockdown followed by 50 ng/ml tetracycline induction for 15h. TOMM70 knockdown did not affect APOL1 RV oligomerization or APOL1-induced cell death (see Fig 2C).

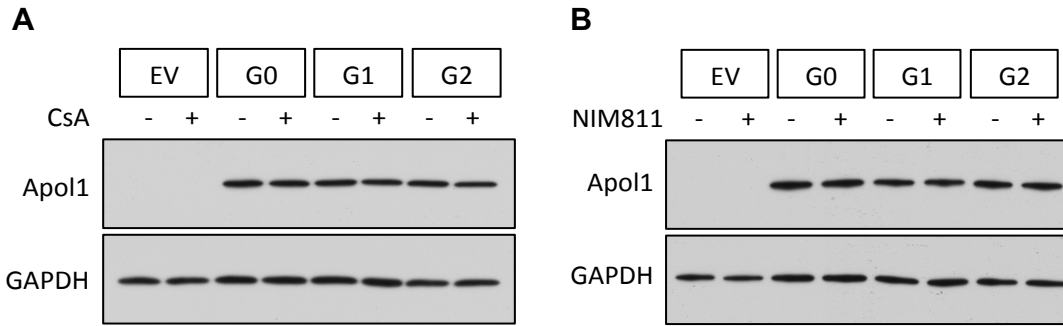
C. Native PAGE blot of APOL1 and SDS-PAGE blots of APOL1 and TIMM22 after TIMM22 knockdown followed by 50 ng/ml tetracycline induction for 15h. TIMM22 knockdown does not affect APOL1 RV oligomerization or APOL1-induced cell death (see Fig 2D).



Supplementary Figure 7: G1 and G2 do not inhibit global import of newly synthesized protein into the mitochondria. pOTC Immunoblots after import assay performed with in-vitro translated pOTC and mitochondria isolated from EV, G0, G1 and G2 cells 6h after 50 ng/ml tetracycline induction. Precursor OTC is marked “p” and the imported, mature, post-signal peptide cleavage OTC is marked “m”.



Supplementary Figure 8: APOL1 interacts with multiple mPTP components. Anti-APOL1 Immunoblots after immunoprecipitate pull downs performed with antibodies against ATP5A, ATP5B and SLC25A5 (ANT2) showing that APOL1 can bind ATP5A, ATP5B and SLC25A5. IP was performed using whole cell lysates. Risk variants demonstrate stronger binding in most cases. Top rows show immunoblot for protein being pulled down; bottom row shows APOL1 using anti-APOL1 antibody.



Supplementary Figure 9: Treatment with CsA or with NIM811 treatment does not affect APOL1 protein levels.

A. APOL1 and GAPDH immunoblots of cells pretreated with DMSO or 1 μ M CsA followed by 50ng/ml Tet for 15h.

B. APOL1 and GAPDH immunoblots of cells pretreated with DMSO or 1 μ M NIM811 followed by 50ng/ml Tet for 15h.