Supplemental Data

Apolipoprotein L1 overexpression drives variant-independent cytotoxicity

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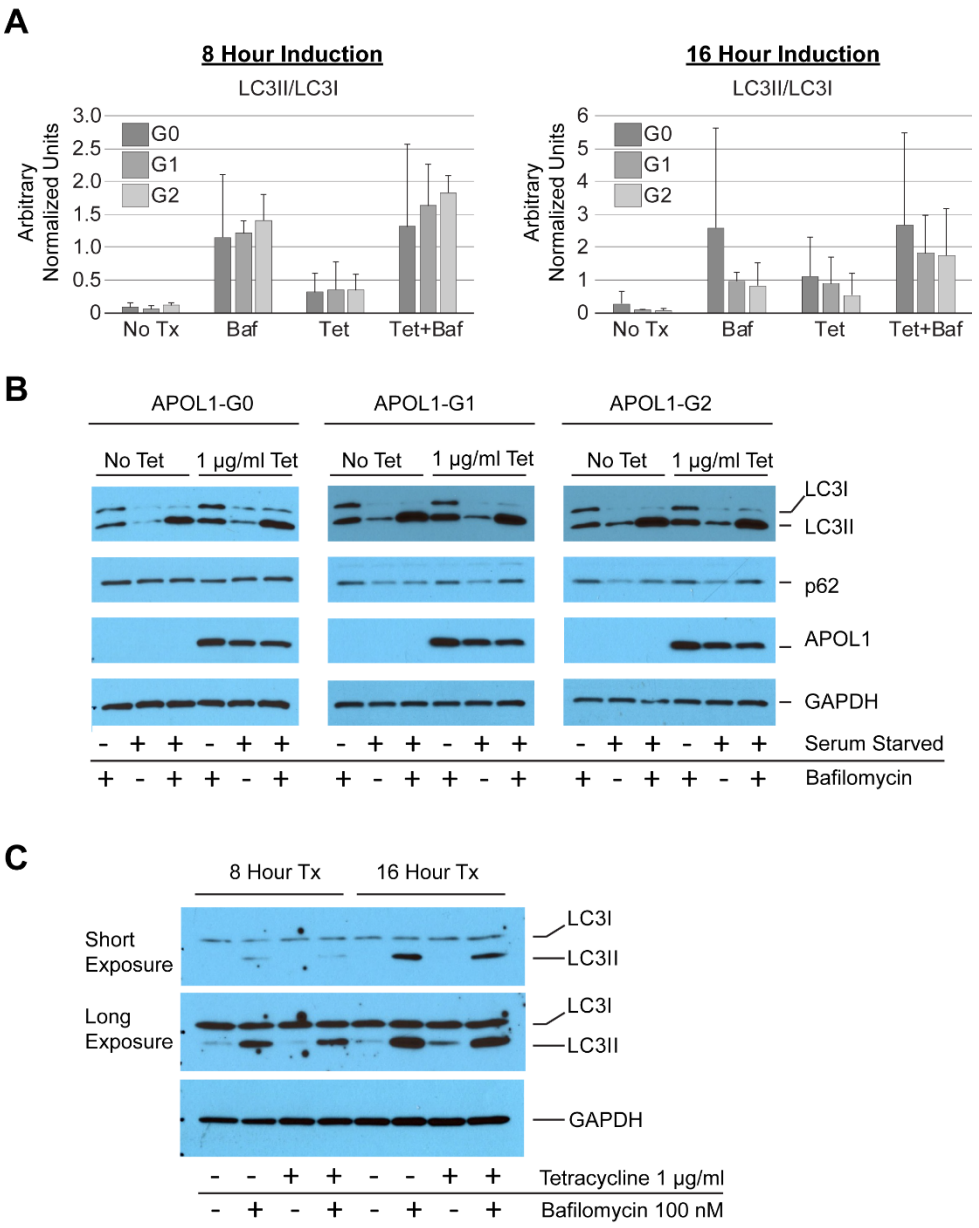
Supplemental Methods:

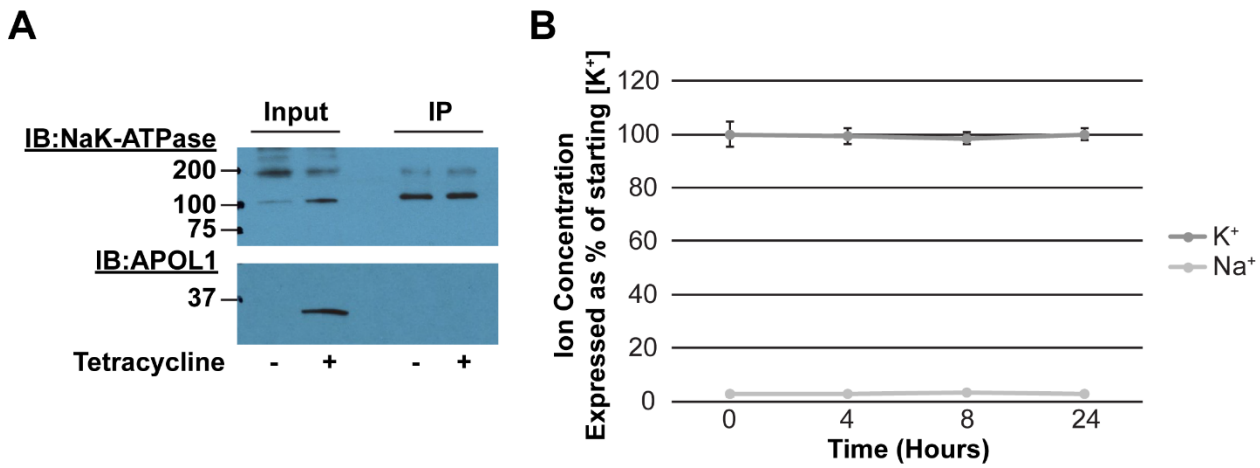
Measurement of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ was measured using the fluorescent indicator, fura-2, as previously described [1]. Briefly, cells were harvested and re-suspended in HBS (Hepes-buffered saline: (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 D-glucose, 15 HEPES, 0.1% bovine serum albumin, pH adjusted to 7.4 at 37°C with NaOH) containing 20 μM fura-2/AM. Following 30 min incubation at 37°C, the cell suspension was diluted ~10-fold with HBS, incubated for an additional 30 min, washed and resuspended in fresh HBS. Aliquots from this final suspension were subjected to centrifugation and washed twice immediately prior to fluorescence measurement. Fluorescence was recorded in a mechanically stirred cuvette using an AB2 spectrophotofluorometer. Excitation wavelength alternated between 340 and 380 nm every second, and fluorescence intensity was monitored at an emission wavelength of 510 nm. Calibration of the fura-2 associated with the cells was accomplished using Triton lysis in the presence of a saturating concentration of Ca^{2+} followed by addition of EGTA (pH 8.5). $[Ca^{2+}]_i$ was calculated by the equations of Grynkiewicz *et. al.* [2] using a K_d value for Ca^{2+} binding to fura-2 of 224 nM. All measurements were performed at 37°C.

References:

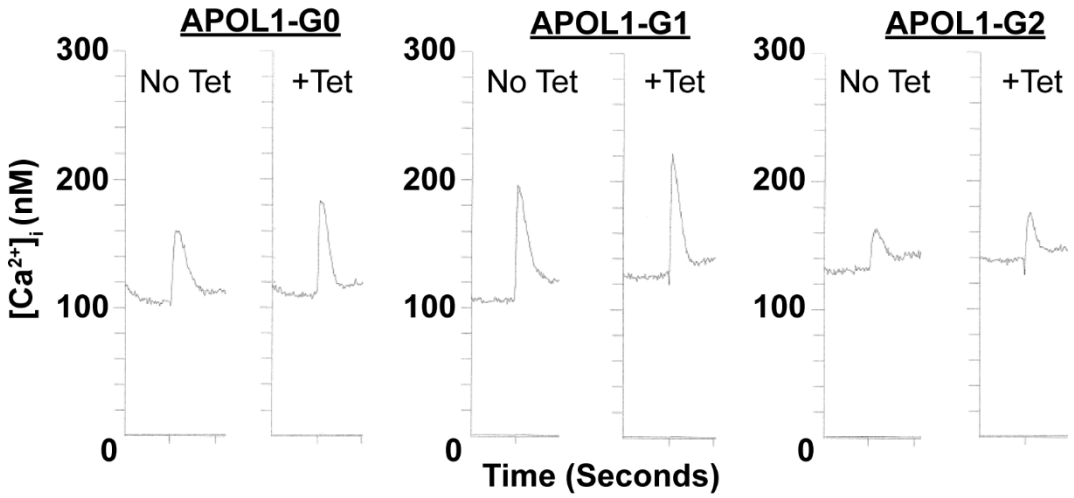
1. Schilling, WP, Rajan, L, Strobl-Jager, E: Characterization of the bradykinin-stimulated calcium influx pathway of cultured vascular endothelial cells. Saturability, selectivity, and kinetics. *The Journal of biological chemistry*, 264**:** 12838-12848, 1989.
2. Grynkiewicz, G, Poenie, M, Tsien, RY: A new generation of Ca2+ indicators with greatly improved fluorescence properties. *The Journal of biological chemistry*, 260**:** 3440-3450, 1985.



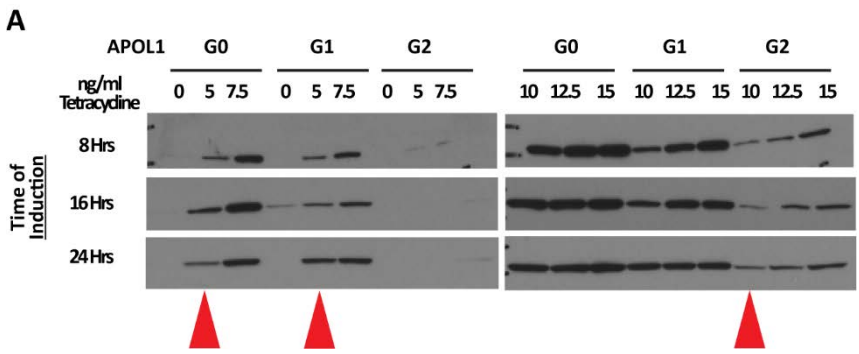
Supplementary Figure 1. ***APOL1 expression does not alter autophagy in serum fed or serum starved cells*.** The protein abundance of LC3II, LC3I, APOL1, p62 and GAPDH was assessed by immunoblotting (**A**) in bar graphs (mean ± SD) showing the expression of LC3II normalized to LC3I, stratified by *APOL1* genotype (G0, G1 and G2) after 8 or 16 hours tetracycline induction (1 µg/ml). Conditions include no treatment (Cont), treatment with bafilomycin A (Baf, 100 nM) alone for the last 3 hours to induce an autophagic block, treatment with tetracycline alone (Tet, 1 µg/ml), and treatment with tetracycline and bafilomycin A (Tet+Baf). (**B**) Stably transfected APOL1-G0, -G1, or –G2 293 cells were serum starved for 6 hours, induced with tetracycline [1 µg/ml] for 4 hours, and treated with bafilomycin A [100 nM] for 2 hours before collecting cells. Immunoblotting for LC3II, LC3I, p62, APOL1, and GAPDH, demonstrate that serum starvation effectively increases autophagic flux, but APOL1 has no effect upon autophagy. One of three representative gels from independent clones are shown for each genotype. Parental 293 cells (**C**) treated with tetracycline (1 µg/ml) for 8 or 16 hours, and/or Bafilomycin A (100 nM) for the last 2 hours did not demonstrate any changes in autophagic flux related to tetracycline treatment as assessed by immunoblotting for LC3II, LC3I, and GAPDH.



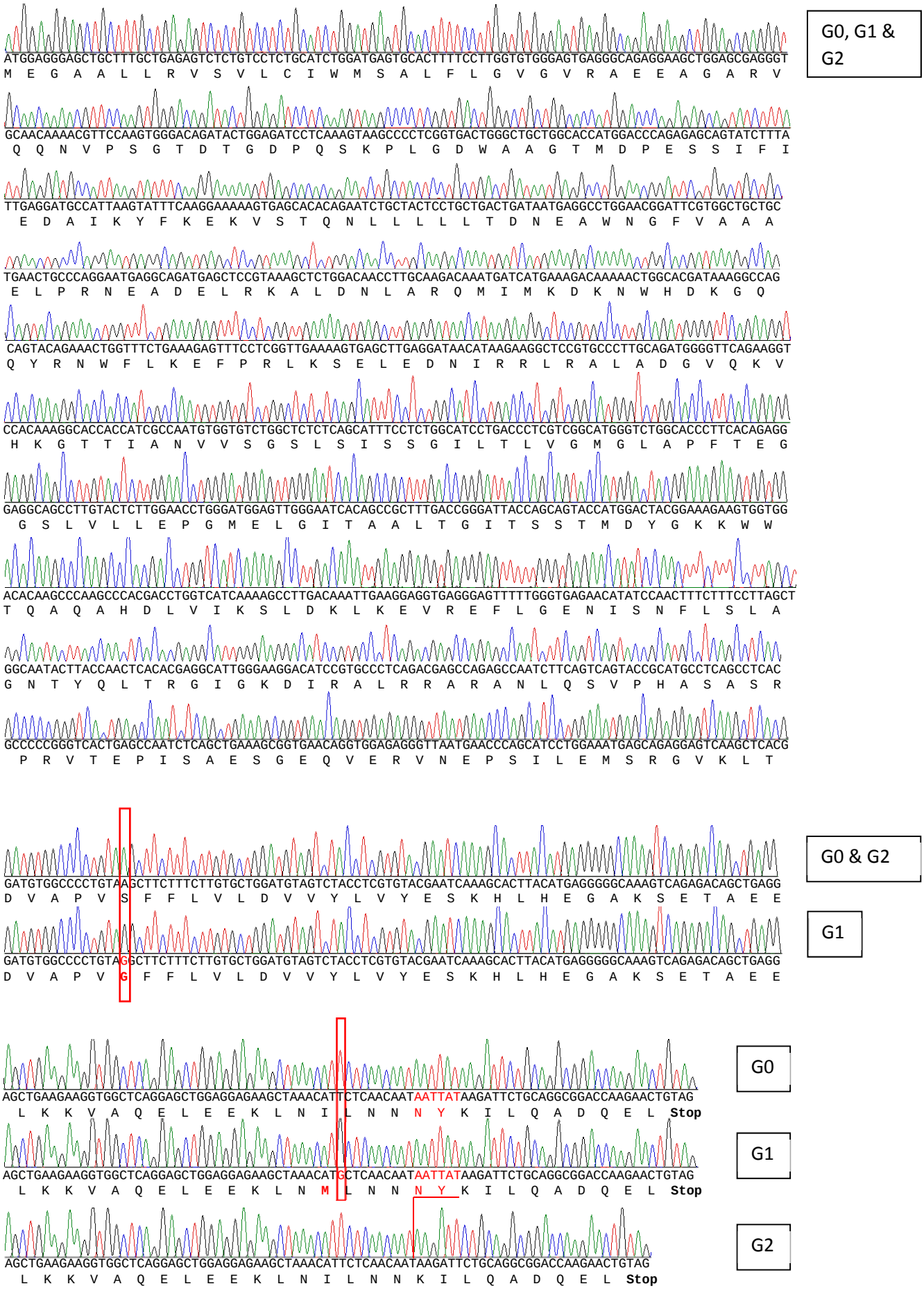
Supplementary Figure 2. ***Induced expression of APOL1 with a deletion of the carboxy-terminus (amino acids 339-398) does not localize to the plasma membrane and does not affect cellular sodium and potassium content.*** (**A**) Clones with a C-terminal deletion of APOL1 (Δ339-398) were cultured without tetracycline or with tetracycline [1 µg/ml] for 24 hours. Cell surface proteins were biotinylated and cells were collected in lysis buffer. APOL1 was detectable in the input lysates of tetracycline-stimulated but not unstimulated cells (left panel). Biotinylated APOL1 was not identified in immunoprecipitates (IP) plasma cell membrane proteins in tetracycline-treated cells; Na^+-K^+-ATPase was present in the immunoprecipitates (IP) of unstimulated and stimulated cells (right panel). (**B**) Cell-associated K^+ and Na^+ content were measured in APOL1Δ339-398 cells without (Time 0) or with tetracycline [1 µg/ml] for 4, 8, and 24 hours. Measurements are normalized to mean cellular K^+ content at time zero (mean ± SD, n=3).



Supplementary Figure 3. ***Cytosolic free calcium and responsiveness to purinergic stimulation was unchanged by expression of APOL1-G0, -G1, and –G2.*** Cytosolic free calcium levels ($[Ca^{2+}]_i$) measured with fura-2 in cell suspensions was comparable before and after tetracycline treatment [1 µg/ml] to induce APOL1-G0, -G1, and –G2 expression. Tetracycline treatment also did not alter the $[Ca^{2+}]_i$ response of the APOL1-G0, -G1, or –G2 cell lines to purinergic receptor stimulation with ATP. Traces from one of four representative experiments are shown for each genotype before and after tetracycline treatment.



Supplementary Figure 4. ***APOL1 expression across genotypes in response to increasing tetracycline doses over time***. One clone from each APOL1 genotype were chosen to determine the dose of tetracycline that led to matched APOL1 expression. Each clone was grown in culture and treated with increasing doses of tetracycline (0, 5, 7.5, 10, 12.5 and 15 ng/ml) for increasing amounts of time (8, 16, and 24 hours). Western blotting for APOL1 of cell lysates demonstrated variability of APOL1 expression in response to increasing tetracycline doses across genotypes. At 24 hours (red arrowheads), APOL1 expression was similar when APOL1-G0 cells were treated with 5 ng/ml tetracycline, APOL1-G1 cells were treated with 5 ng/ml tetracycline and APOL1-G2 cells were treated with 10 ng/ml tetracycline.


G0, G1 & G2
ATGGAGGGAGCTGCTTTGCTGAGAGTCTCTGTCCTCTGCATCTGGATGAGTGCACTTTTCCTTGGTGTGGGAGTGAGGGCAGAGGAAGCTGGAGCGAGGGT
M E G A A L L R V S V L C I W M S A L F L G V G V R A E E A G A R V
GCAACAAAACGTTCCAAGTGGGACAGATACTGGAGATCCTCAAAGTAAGCCCCTCGGTGACTGGGCTGCTGGCACCATGGACCCAGAGAGCAGTATCTTTA
Q Q N V P S G T D T G D P Q S K P L G D W A A G T M D P E S S I F I
TTGAGGATGCCATTAAGTATTTCAAGGAAAAAGTGAGCACACAGAATCTGCTACTCCTGCTGACTGATAATGAGGCCTGGAACGGATTCGTGGCTGCTGC
E D A I K Y F K E K V S T Q N L L L L L T D N E A W N G F V A A A
TGAACTGCCCAGGAATGAGGCAGATGAGCTCCGTAAAGCTCTGGACAACCTTGCAAGACAAATGATCATGAAAGACAAAAACTGGCACGATAAAGGCCAG
E L P R N E A D E L R K A L D N L A R Q M I M K D K N W H D K G Q
CAGTACAGAAACTGGTTTCTGAAAGAGTTTCCTCGGTTGAAAAGTGAGCTTGAGGATAACATAAGAAGGCTCCGTGCCCTTGCAGATGGGGTTCAGAAGGT
Q Y R N W F L K E F P R L K S E L E D N I R R L R A L A D G V Q K V
CCACAAAGGCACCACCATCGCCAATGTGGTGTCTGGCTCTCTCAGCATTTCCTCTGGCATCCTGACCCTCGTCGGCATGGGTCTGGCACCCTTCACAGAGG
H K G T T I A N V V S G S L S I S S G I L T L V G M G L A P F T E G
GAGGCAGCCTTGTACTCTTGGAACCTGGGATGGAGTTGGGAATCACAGCCGCTTTGACCGGGATTACCAGCAGTACCATGGACTACGGAAAGAAGTGGTGG
G S L V L L E P G M E L G I T A A L T G I T S S T M D Y G K K W W
ACACAAGCCCAAGCCCACGACCTGGTCATCAAAAGCCTTGACAAATTGAAGGAGGTGAGGGAGTTTTTGGGTGAGAACATATCCAACTTTCTTTCCTTAGCT
T Q A Q A H D L V I K S L D K L K E V R E F L G E N I S N F L S L A
GGCAATACTTACCAACTCACACGAGGCATTGGGAAGGACATCCGTGCCCTCAGACGAGCCAGAGCCAATCTTCAGTCAGTACCGCATGCCTCAGCCTCAC
G N T Y Q L T R G I G K D I R A L R R A R A N L Q S V P H A S A S R
GCCCCCGGGTCACTGAGCCAATCTCAGCTGAAAGCGGTGAACAGGTGGAGAGGGTTAATGAACCCAGCATCCTGGAAATGAGCAGAGGAGTCAAGCTCACG
P R V T E P I S A E S G E Q V E R V N E P S I L E M S R G V K L T
G0 & G2
GATGTGGCCCCTGTAAGCTTCTTTCTTGTGCTGGATGTAGTCTACCTCGTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGG
D V A P V S F F L V L D V V Y L V Y E S K H L H E G A K S E T A E E
G1
GATGTGGCCCCTGTAGGCTTCTTTCTTGTGCTGGATGTAGTCTACCTCGTGTACGAATCAAAGCACTTACATGAGGGGGCAAAGTCAGAGACAGCTGAGG
D V A P V G F F L V L D V V Y L V Y E S K H L H E G A K S E T A E E
G0
AGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTCTCAACAATAATTATAAGATTCTGCAGGCGGACCAAGAACTGTAG
L K K V A Q E L E E K L N I L N N N Y K I L Q A D Q E L Stop
G1
AGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATGCTCAACAATAATTATAAGATTCTGCAGGCGGACCAAGAACTGTAG
L K K V A Q E L E E K L N M L N N N Y K I L Q A D Q E L Stop
G2
AGCTGAAGAAGGTGGCTCAGGAGCTGGAGGAGAAGCTAAACATTCTCAACAATAAGATTCTGCAGGCGGACCAAGAACTGTAG
L K K V A Q E L E E K L N I L N N K I L Q A D Q E L Stop

Supplementary Figure 5. ***APOL1-G0 corresponds to the reference sequence of the transcript variant 1, encoding a 398-amino acid protein, NM 003661.3.*** APOL1 cDNA was amplified from all clones with primers specific to the vectors utilized to generate the stable cell lines and these amplicons were directly sequenced with overlapping APOL1 primers. APOL1-G1 sequence diverged from the APOL1-G0 sequence only at the sites of the two single nucleotide polymorphisms (SNP's), which make up the G1 haplotype, rs73885319 (S342G) and rs60910145 (I384M), APOL1-G1 nucleotides and amino acids in red text with red boxes showing position in the chromatograms. APOL1-G2 sequence diverged from APOL1-G0 sequence only at the site of the six base pair deletion, rs71785313, deleting amino acids 388-389, asparagine (N) and tyrosine (Y). The nucleotides and amino acids comprising the G2 haplotype (rs71785313) are represented in red text in the APOL1-G0 and –G1 chromatograms, underlined in red with a red line indicating the position of this deletion in the APOL1-G2 chromatogram. The first ten lines of chromatographic tracings are common to all three APOL1 genotypes and show the chromatogram above the sequence of nucleotides1-1008 and translation of amino acids 1-336. The next two chromatographic lines show nucleotides 1009-1108, and amino acids 337-370. The top chromatogram represents APOL1-G0 and –G2 which are identical in this region of APOL1 and the bottom chromatogram shows rs73885319 (S342G), the first part of G1. The next three chromatographic lines show nucleotides 1109-1197, and amino acids 371-398, and the chromatograms show APOL1-G0 on top, -G1 in the middle and –G2 on the bottom.