COMPLETE METHODS

DNA extraction
DNA was extracted from FFPE samples using the QIAamp® DNA FFPE Tissue Kit (Qiagen, Valencia, CA). The DNA concentration of each sample was determined using the Nanodrop™ 8000 (Thermo Scientific, Wilmington, DE).

Genotyping
In order to use the least amount of DNA for genotyping we designed a nested multiplex PCR assay to amplify the two regions of the APOL1 that contain the rs73885319 SNP and the 6 bp indel (rs71785313). The primer sequences for rs73885319 were: 5' TGA ACC CAG CAT CCT GGA AA 3' and 5' TTG CCC CCT CAT GTA AGT GC 3'. The primer sequences for rs71785313 are: 5' GCT GAG GAG CTG AAG AAG GT 3' and 5' GGC ATA TCT CTC CTG GTG GC 3'. Amplification was performed with a mix of each primer at 0.5 uM, 2X Phusion® High Fidelity Polymerase (New England Biolabs Inc., Ipswich, MA) and 1-10 ng DNA in a 5 ul reaction mix. The PCR was performed on a 384 well Veriti™ Thermal Cycler (Life Technologies, Carlsbad, CA) using the program 98°C 30 sec, 35 cycles of 98°C 10 sec, 68°C 30 sec, 72°C 10 sec, 72°C 5 min and 4°C hold. Samples were diluted 1:60,000 in water prior to genotyping. Genotyping was performed on the ViiA™ 7 Real-Time PCR System in 5 ul reaction in 384 well plates using the 2X genotyping master mix (Life Technologies) and 20X genotyping primer/probes designed by Kopp, et al and synthesized by Life Technologies. On each plate; 10 each of 1/1, 1/2 and 2/2 positive allele samples and 10 no template controls (NTC) were genotyped with the samples. Each run was checked for accuracy of calls in the controls and samples.

Statistical Methods
Genotyping data was analyzed in the ViiA7 sequence detection software v2.0. Amplification plots were assessed for correct genotyping calls. There was > 95% genotype call rate. Clinical and genotype data for all SNPs were entered into a database in SPSS (Statistical Package for Social Sciences) Software Version 20.0 for Windows (Gorinchem, The Netherlands) and analyzed for genetic association. The allele G of rs73885319 and deletions (D) of rs71785313 were considered detrimental and their combination represented 2 risk alleles (G2), whereas the presence of a single risk allele was coded as 1 (either G or D; G1) and their absence was coded as 0 (G0). Case-control genotype associations were assessed by χ^2 analyses. Association with sub-phenotypes, such as ISN/RPS class, was explored similarly. ANOVA was used for comparing means of continuous variables such as age, chronicity and activity indices. We considered nominal p values <0.05 as significant for all tests.