Disparities in Lupus Outcomes: The Role of Environment and Genetics

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Disclosures

- Ashira Blazer: None

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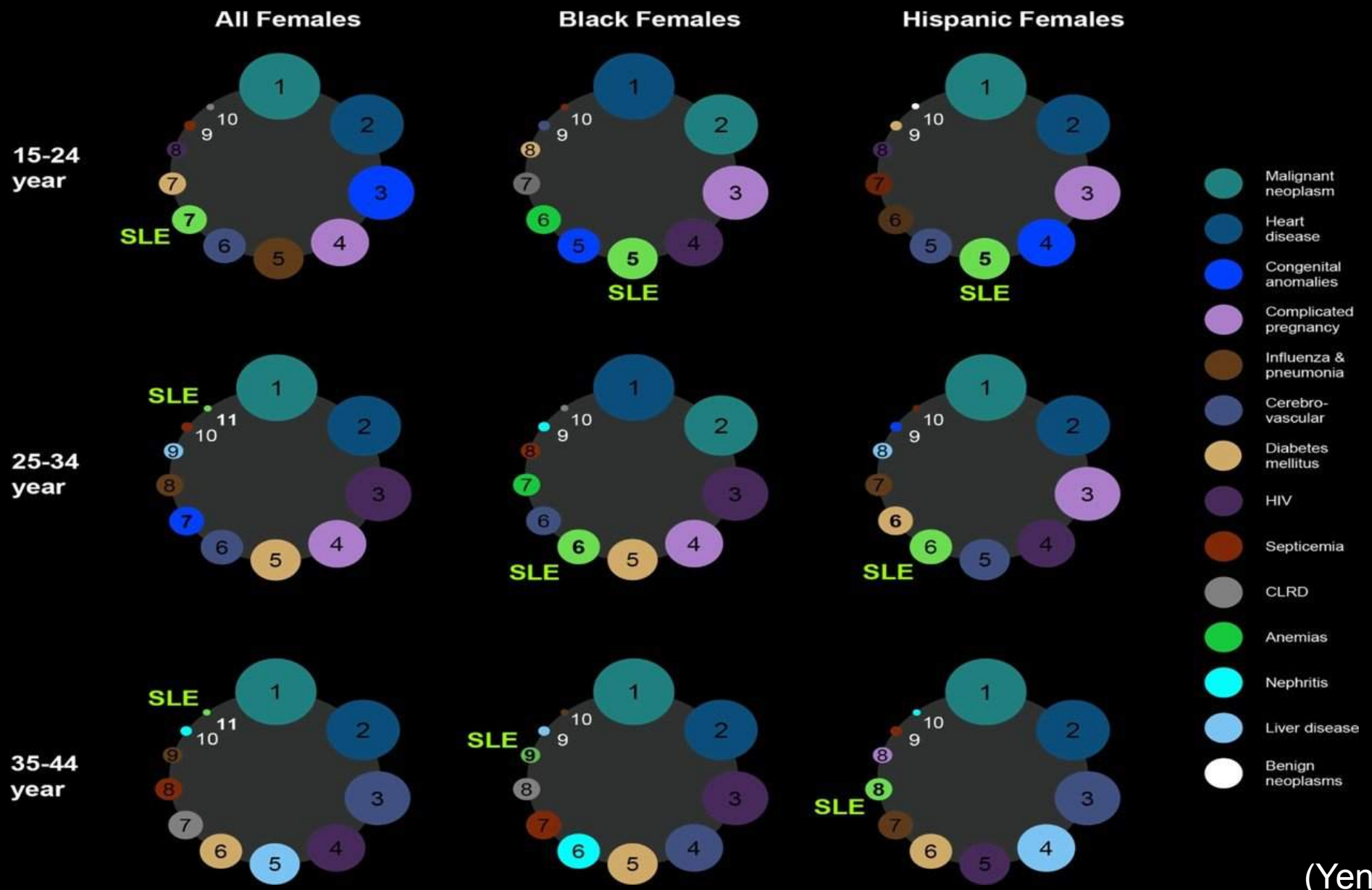
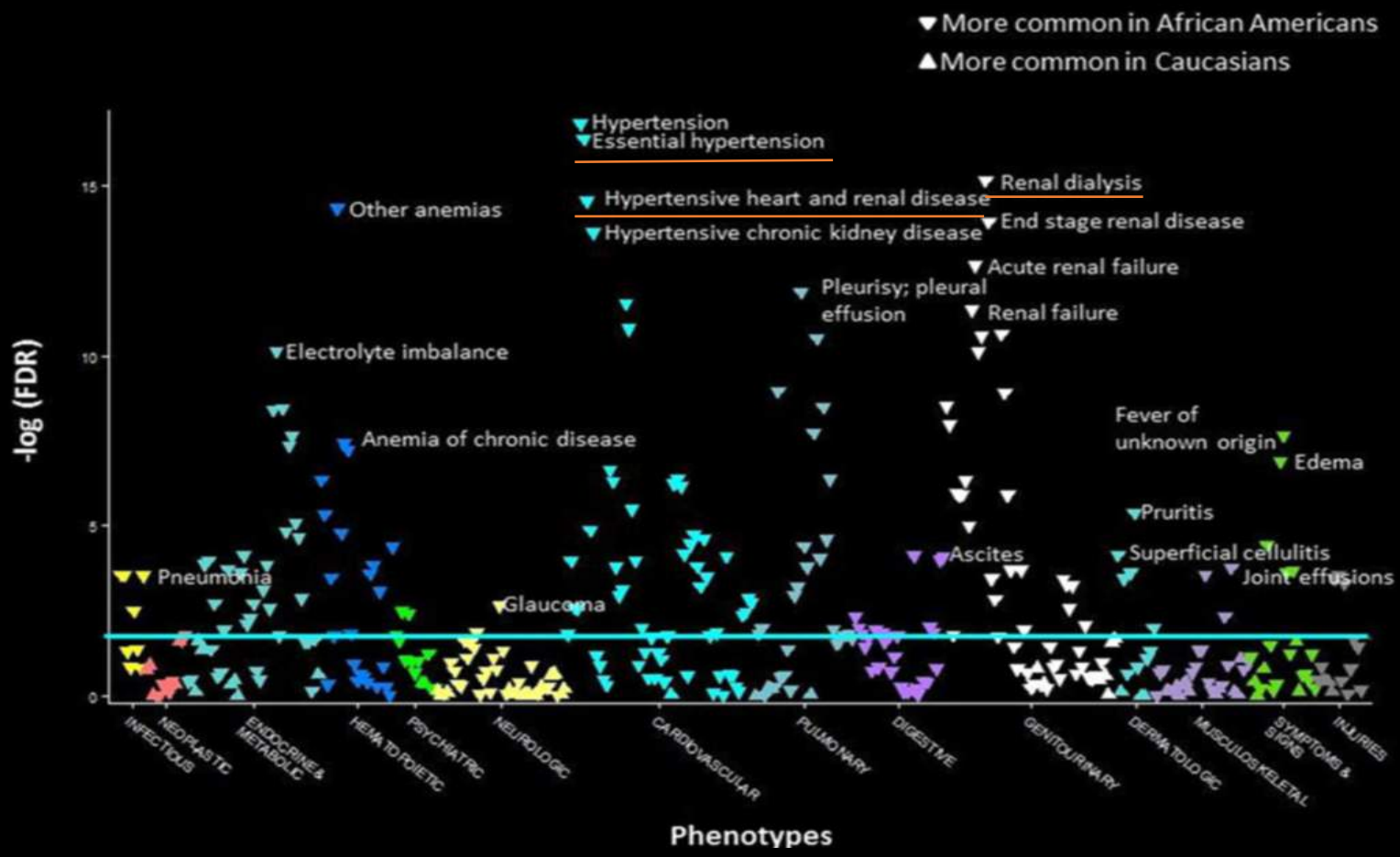
SLE is a Major Cause of Morbidity and Mortality Especially in Minority Women



(Yen and Singh 2018)

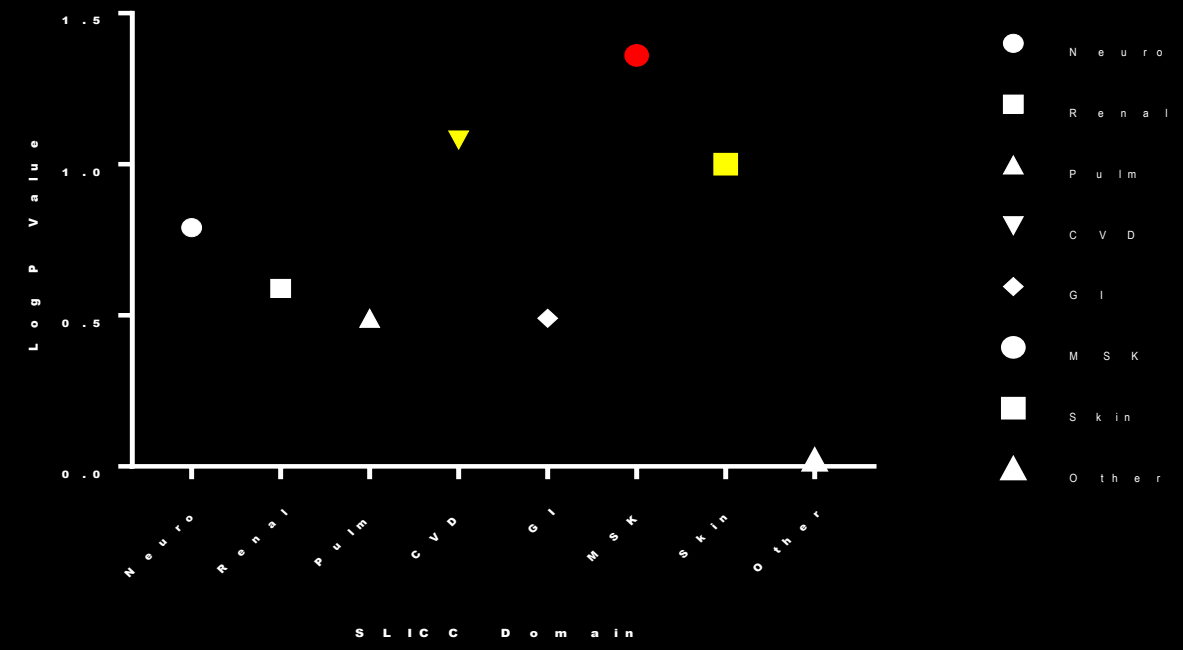
Phenome Wide Association Study: African American SLE Patients Exhibit Increased Burden of Comorbidities



	African American (n=270)	Caucasian (n=715)	P Value
Current age ± SD	44 ± 17	53 ± 17	P<0.001
Age at Diagnosis ± SD	35 ± 16	43 ± 17	P<0.001

(Barnado, Caroll, Crofford

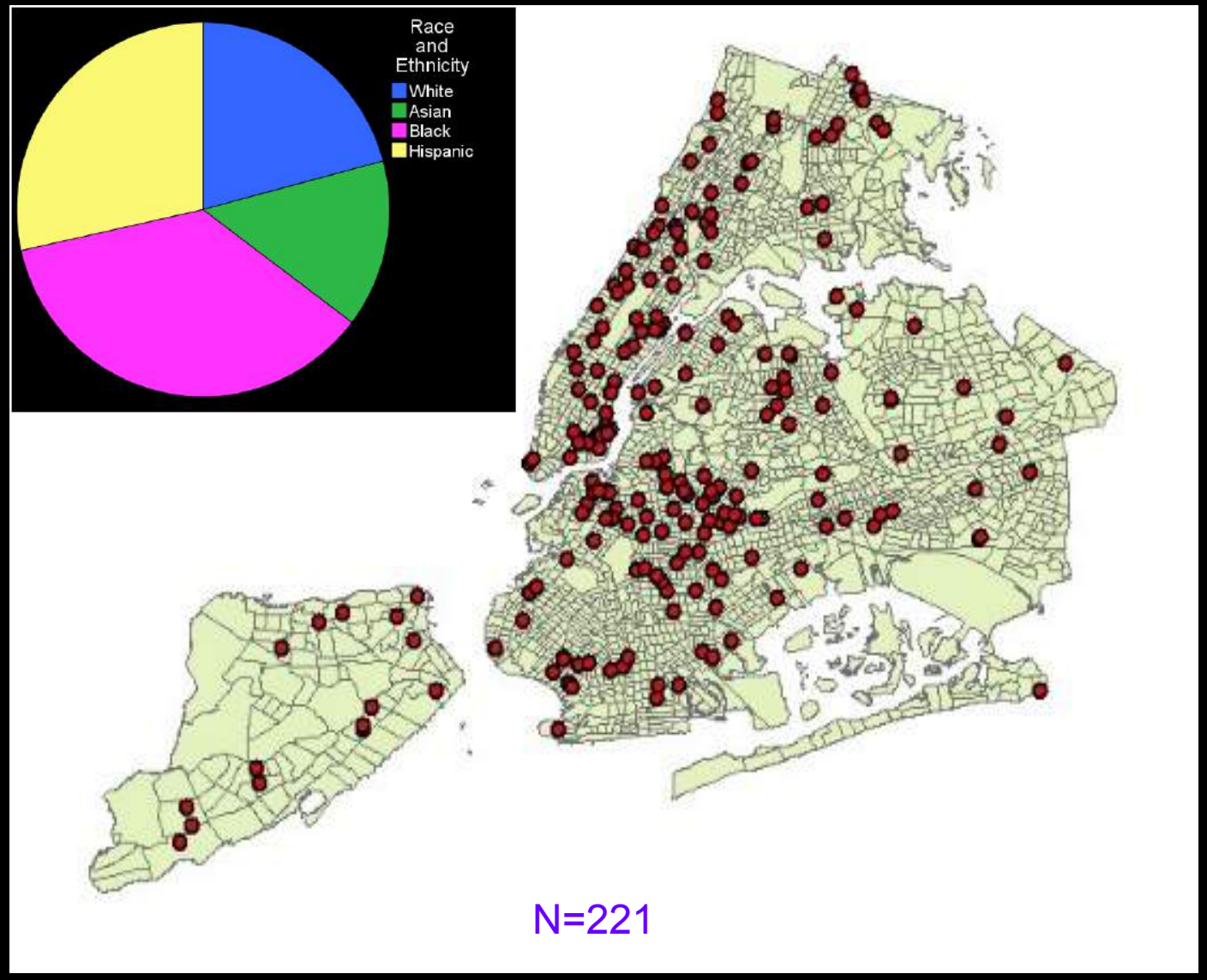
NYU SLE: Black and Hispanic Patients Exhibit Higher SLE SLICC Damage Index Within the First 5 Years of Diagnosis



Risk Factor	Unadjusted		Adjusted	
	HR	P Value	HR	P Value
Black or Hispanic Race	7.0	0.006	8.9	0.006
Age at SLICC	1.08	0.005	1.1	0.01

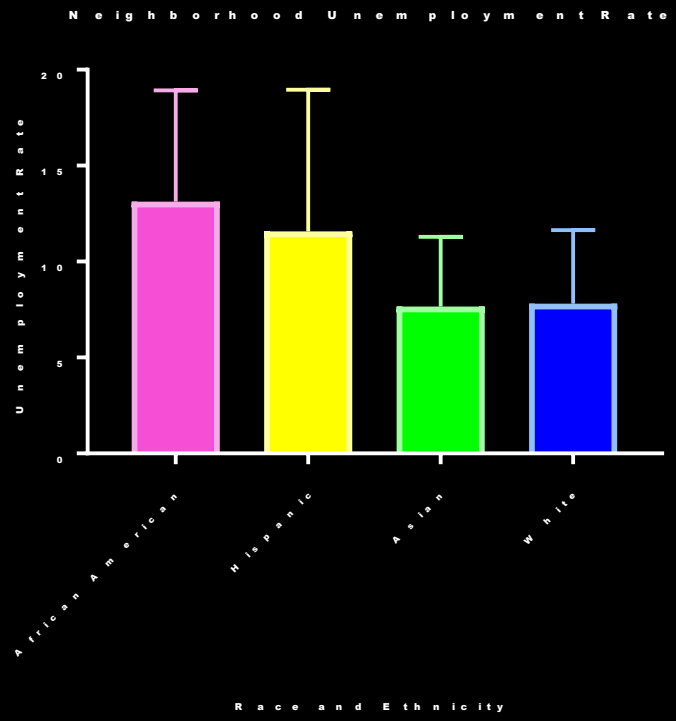
- A “non-zero” Damage index is independently associated with all cause mortality in SLE (Chambers, Allen et al. 2009)
- Renal and cardiovascular outcomes are among the strongest predictors of mortality (Danila, Pons-Estel et al. 2009)
- Compared to White or Asian race, Black and Hispanic SLE patients were more likely to have a “Non-Zero” Damage Index (HR 8.9) within the first 5 years of diagnosis
- Early Cardiovascular damage accrued in minority patients

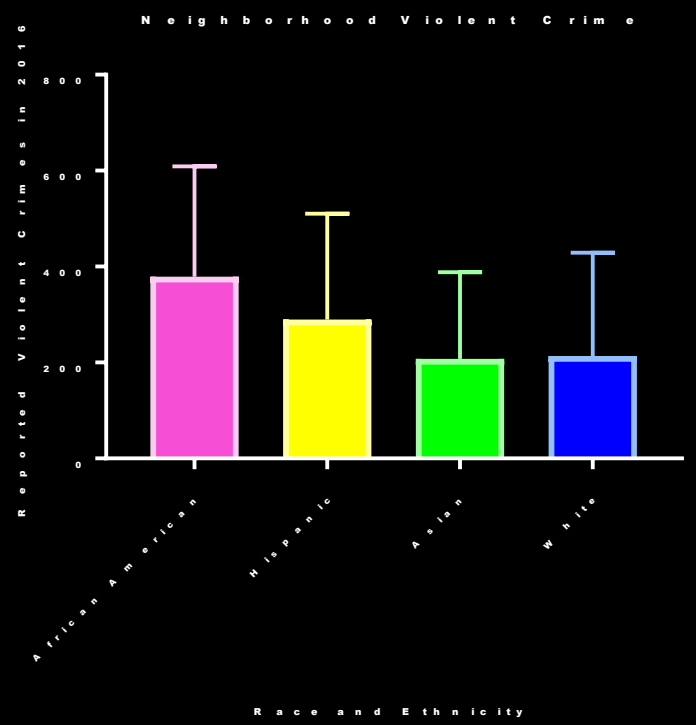
In a Single Academic Center, Are There Differences in Neighborhood Stress by Race and Ethnicity

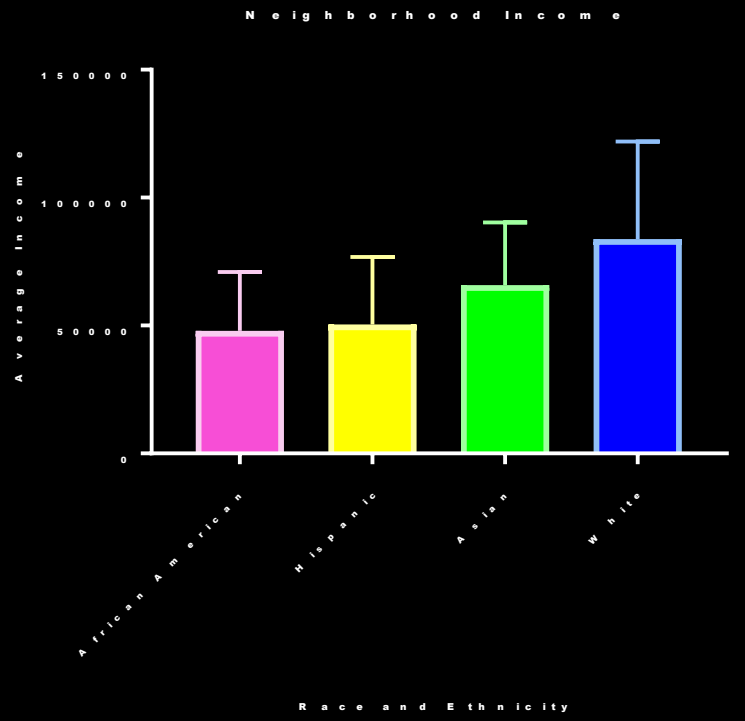


- SLE patients seen at 3 NYU hospital centers were recruited (n=221)
- Addresses were Geocoded by census track
- Publically available data (NYC open data, NYC.gov) were accessed
  - Violent Crimes
  - Income
  - Poverty
  - Unemployment
  - Average commute to work
  - Childhood educational attainment
  - Pharmacies
  - Greenspace

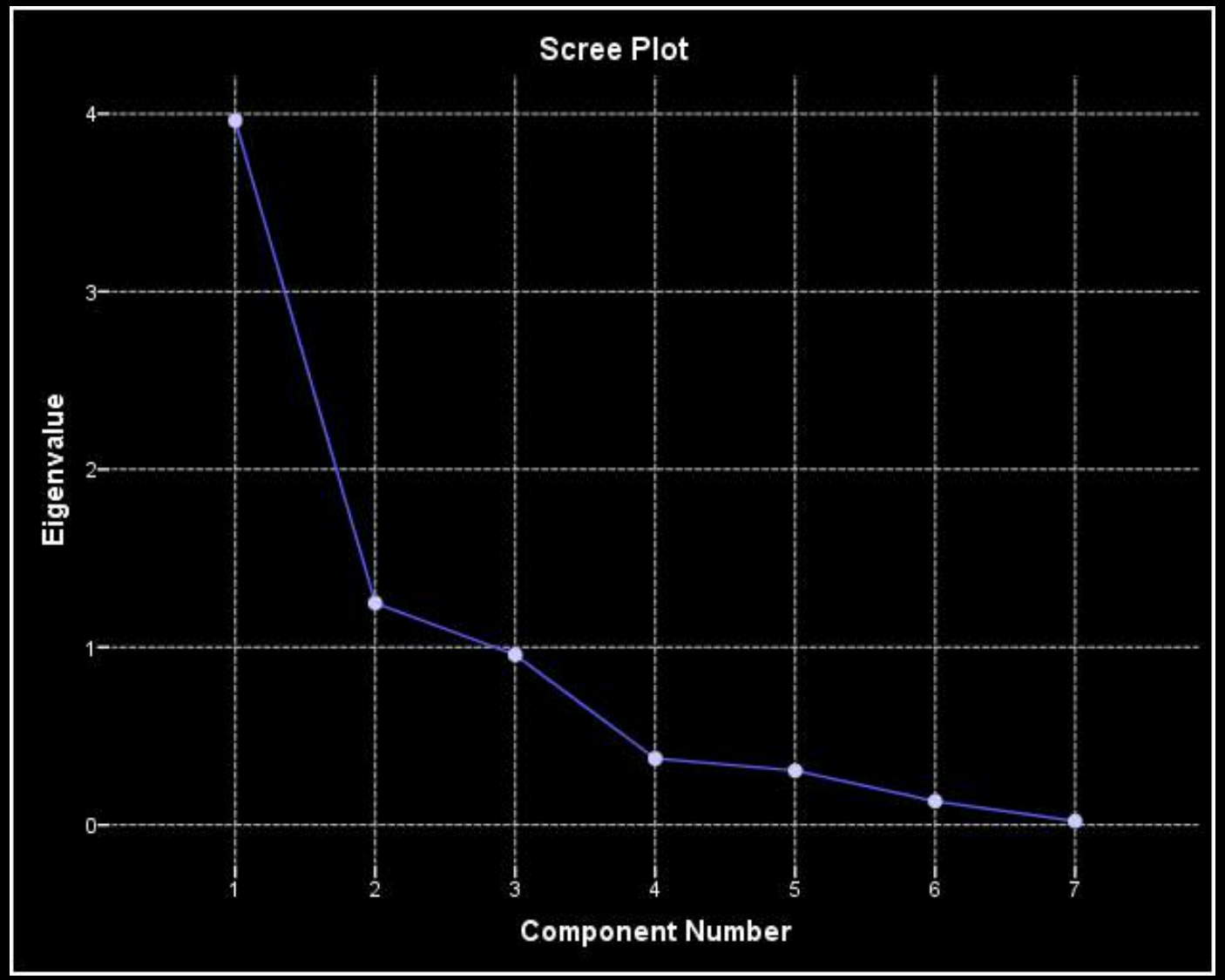
African American and Hispanic Neighborhoods Have Significantly Higher Violent Crime, Unemployment, and Lower Income





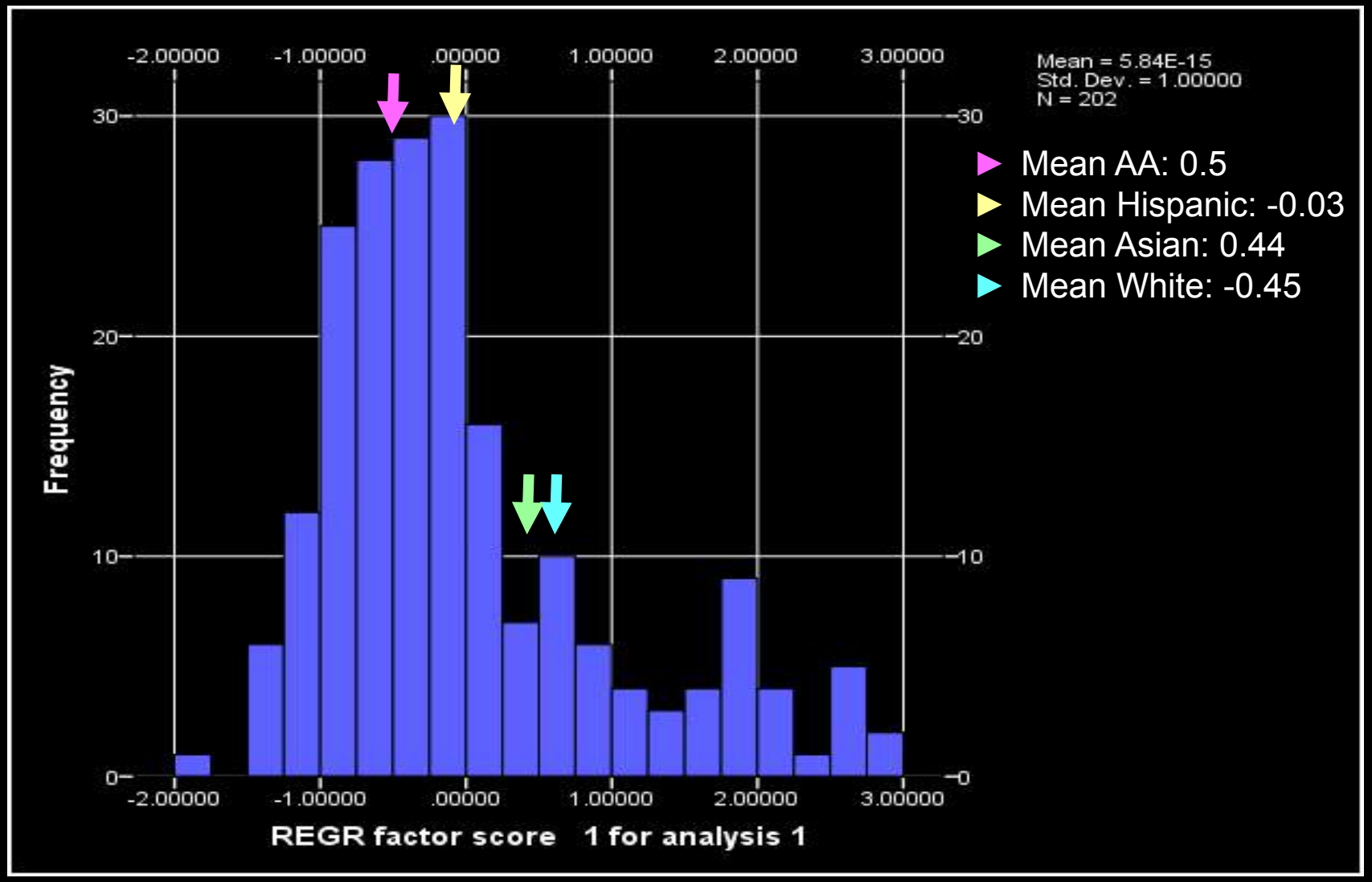


Principle Component Analysis of Neighborhood Factors Shows Greatest Neighborhood Variation in Income, Educational Attainment and Unemployment Rates



- Principal component 1 (57% of the variance)
  - Directly proportion to
    - Income
    - Childhood educational attainment
  - Inversely proportional to
    - Poverty
    - Unemployment
- Principal component 2 (18% of the variance)
  - Proportional to poverty
  - Inversely proportional to Distance traveled to work

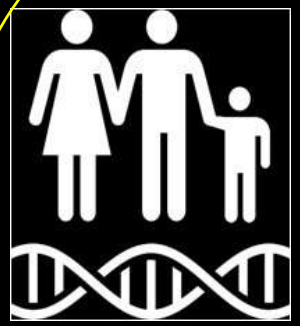
African American and Hispanic Patients Experience a Higher Degree of Neighborhood Stress



- Principal component 1
  - Directly proportion to
    - income
    - childhood educational attainment
  - Inversly proportional to
    - Poverty
    - Unemployment

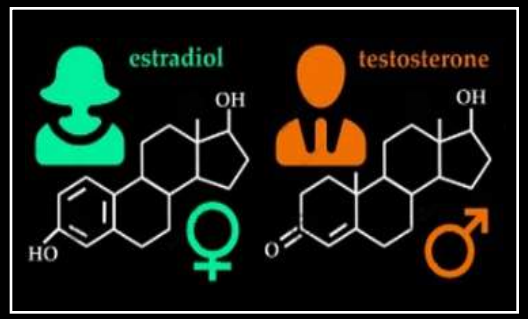
The Etiological Framework in SLE Health Disparities Reveals Multiple Causes

Pathophysiological mechanisms



Genetics

Environmental triggers



Hormones

Risk → Disease severity → Treatment Response → Mortality

Socioeconomic mechanisms

Income

Educational attainment

Occupation

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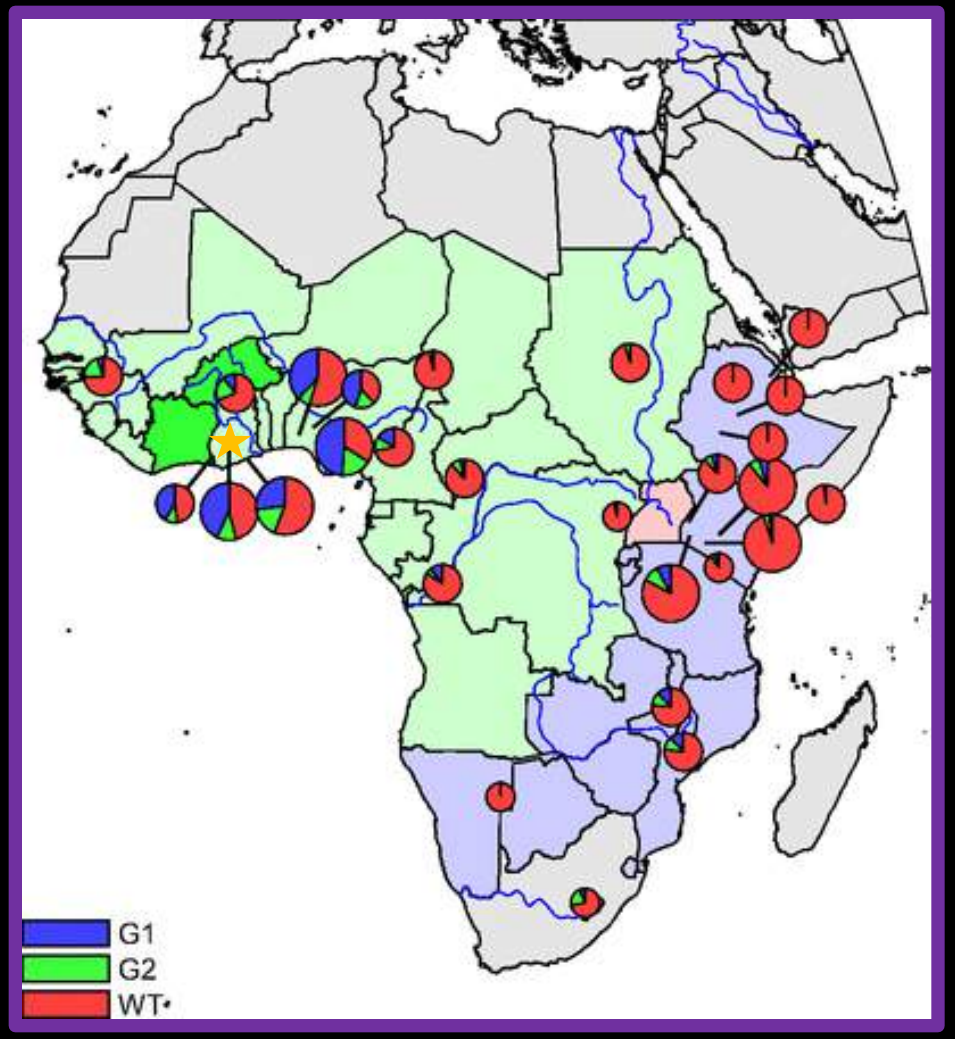
(Falasinnu T 2017)

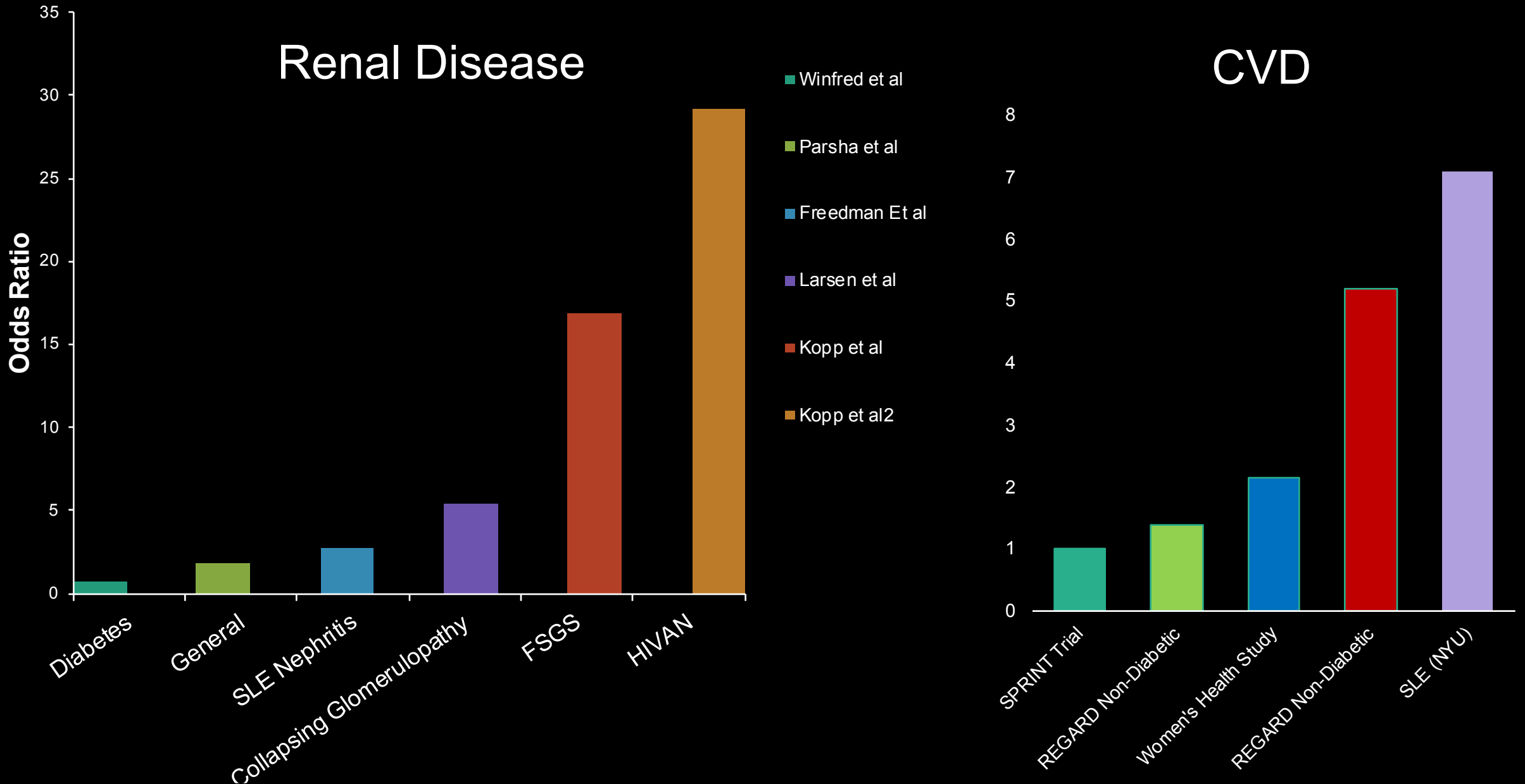
Apolipoprotein L1 (APOL1) Gene Mutations: High Allelic Frequencies in those of Recent West African Heritage



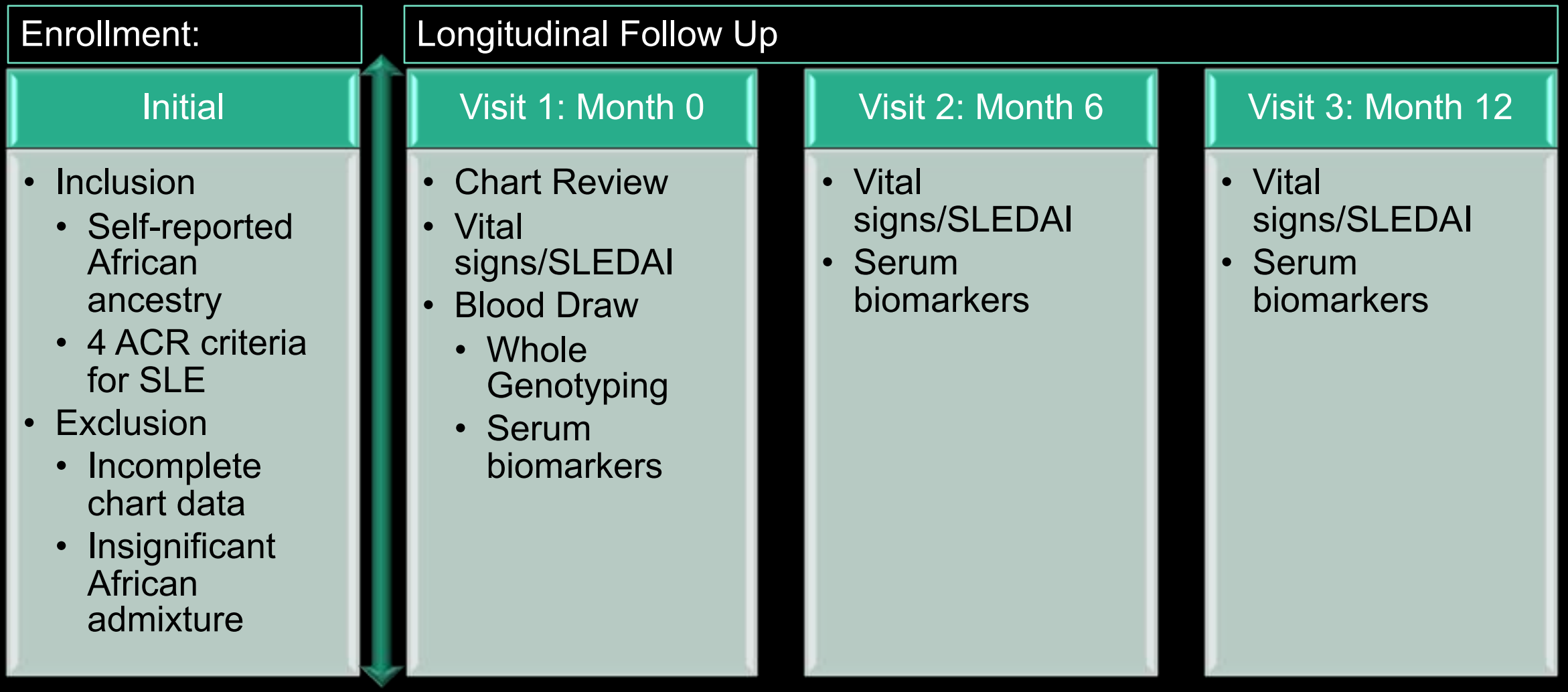
- These mutations offer resistance to African trypanosomiasis
- Evolutionary advantage comes with the risk of progressive renal and cardiovascular disease
- Allelic frequencies are high
  - Ghana
    - Asante: G1: 40.9% G2: 12.9%
    - Bulsa: G1: 11.4% G2: 21.4%
  - African Americans
    - New York: G1: 20.9% G2: 13.4%
    - Southern USA: G1: 19.7% G2: 13.4%

Limou et. al

APOL1 Polymorphism-Ascribed Renal and Cardiovascular Risk Differs Across Medical Comorbidity



Two Ancestrally African SLE Cohorts (NYC/Ghana) Were Followed Longitudinally



In Both Cohorts Demographics were Similar Across Genotypes

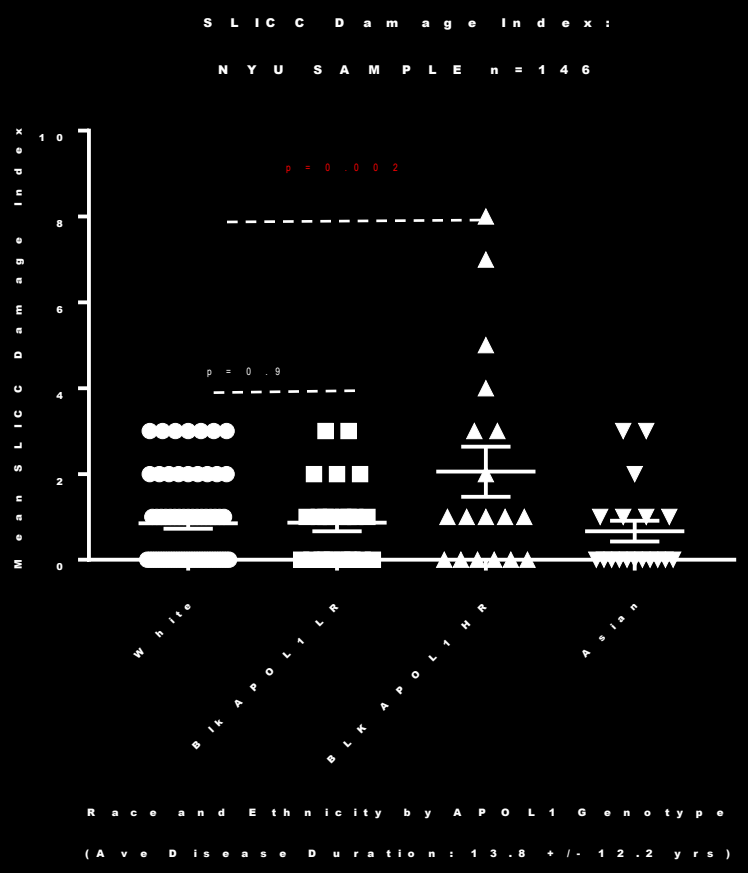
NYU	Genotype			P	Totals
	G0/G0 n=36	RV/G0 n=51	RV/RV n=13		
Age (yrs)	43.8 ± 14.3	41.3 ± 14.0	48.1 ± 13.9	NS	43.1 ± 14.1
Gender (% female)	88.9	88.2	92.3	NS	90.1
Smoking History (%)	25.0	21.6	23.1	NS	23.0
Diabetes (%)	5.5	3.9	7.6	NS	4.4
BMI	27.8 ± 8.9	29.6 ± 7.8	25.6 ± 3.6	NS	28.5 ± 7.9
A1C	5.2 ± 1.6	5.6 ± 0.6	6.1 ± 1.6	NS	5.6 ± 1.2
Dyslipidemia	68.4	65.7	77.8	NS	68.3

Allelic Frequencies: G0: 0.62 G1: 0.21 G2: 0.17

GH	Genotype			P	Totals
	G0/G0 n=39	RV/G0 n=51	RV/RV n=12		
Age (yrs)	31.4 ± 9.2	32.7 ± 8.7	32.1 ± 8.9	NS	32.1 ± 8.9
Gender (% female)	100	100	100	NS	100
Smoking History (%)	0.00	2.7	0.00	NS	1.4
Hypertension History	12.0	14.0	10.0	NS	13.0
BMI	28.4 ± 5.8	27.7 ± 5.2	25.9 ± 5.6	NS	27.8 ± 5.4
Diabetes	4.0	5.0	0.0	NS	4.0
Past Lipid Lowering	20.0	5.4	0	NS	9.7

Allelic Frequencies: G0: 0.63 G1: 0.24 G2: 0.13

NYU Cohort: APOL1 Variant Carriers Exhibited Accelerated Renal Disease, CVD, and Damage Accrual

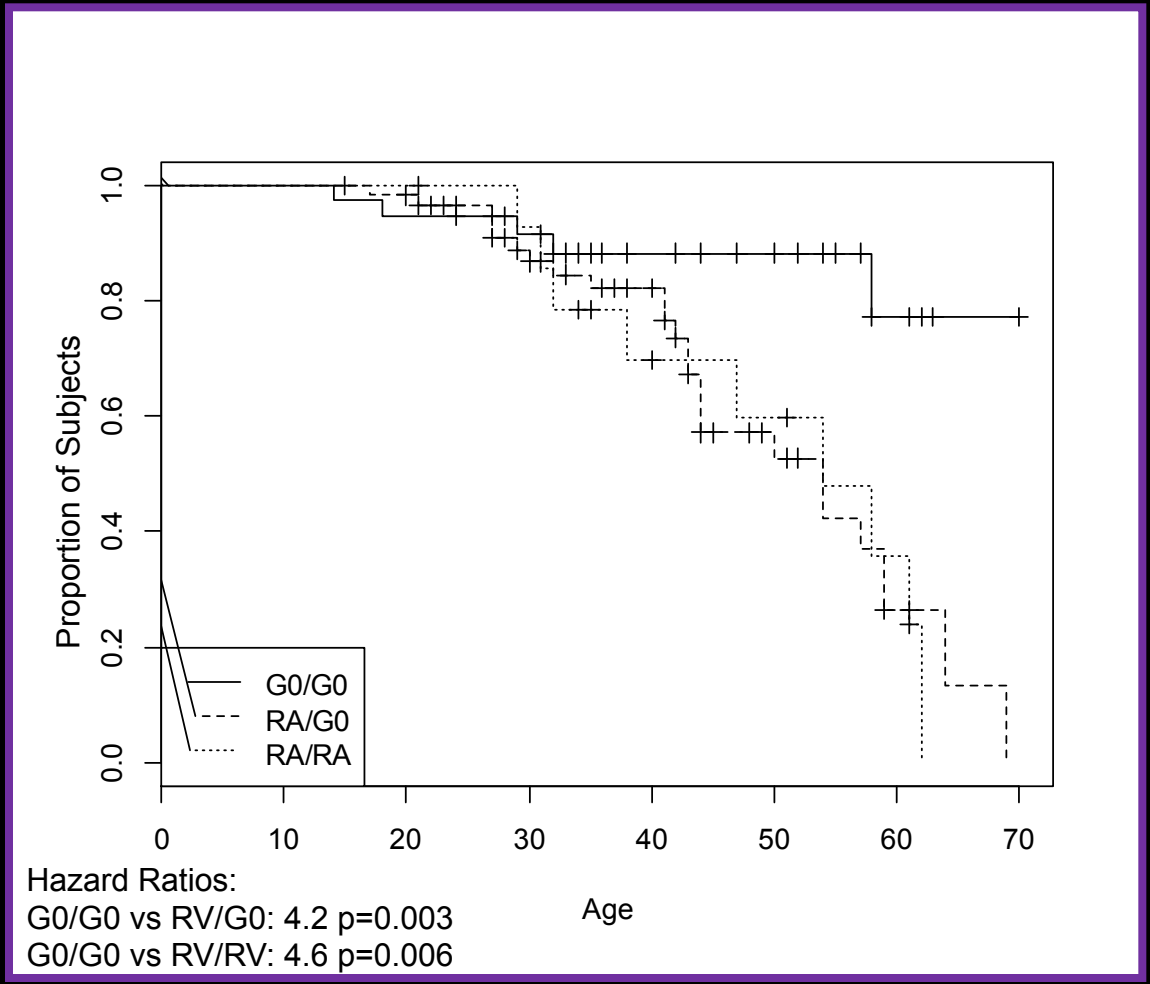


Multivariate Analysis: APOL1 Associates With CVD

Risk Factor	Odds Ratio	P value
1 or 2 APOL1 Allele Copies	7.9	0.006
ESRD	1.2	0.8
Smoking	5.3	0.008
12 Month Average Prednisone Dose >10mg	0.98	0.4
Body Mass Index	0.96	0.4
Hypertension	3.9	0.02

NYU Cohort: Variant Carriers Developed Atherosclerotic Disease at Earlier Ages

A. Time To Event Analysis



B.

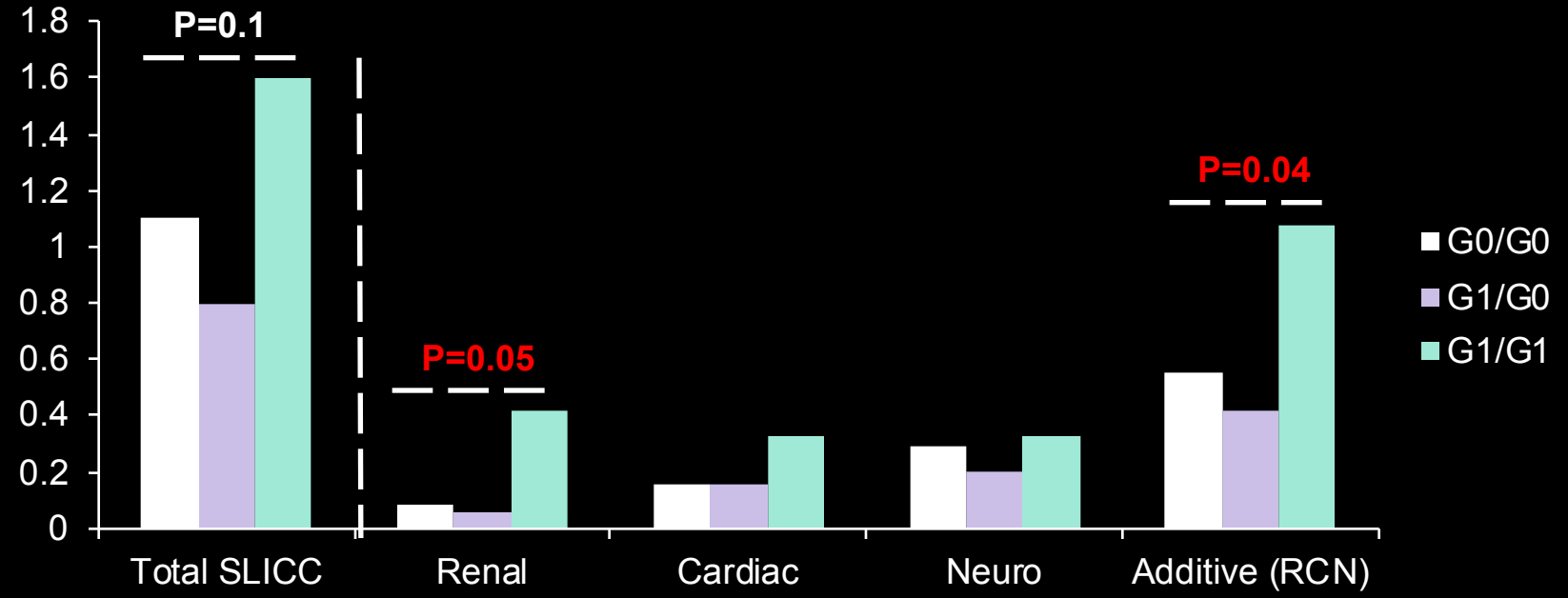
	Number at Risk by Decade				
	20	30	40	50	60
G0/G0	35	28	19	13	5
RV/G0	56	42	30	12	3
RV/RV	14	12	7	5	2

A) Time-to-event analysis for symptomatic atherosclerotic CVD as represented by Kaplan Meier Curves. The Y axis represents proportion of individuals free of the outcome, and the X axis represents subject age.

B) The number of individuals present at each decade time point.

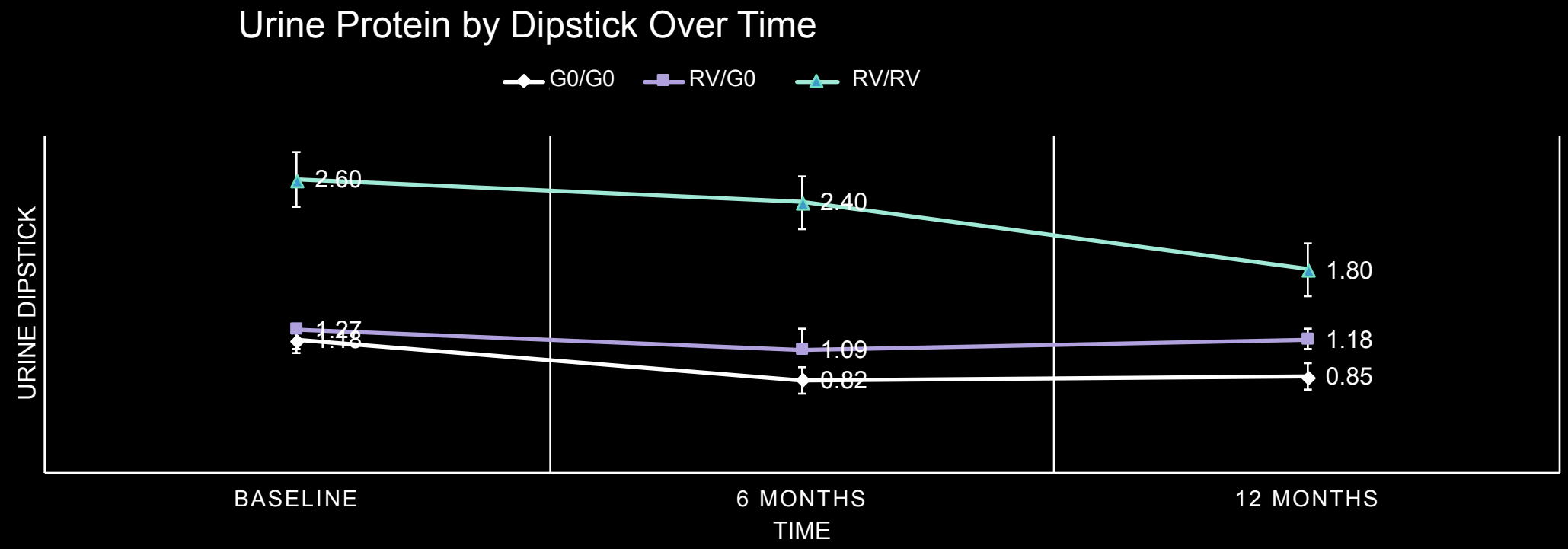
Blazer et. al

Ghanaian Cohort: APOL1 Variants Associate with Higher SLICC Damage Indexes



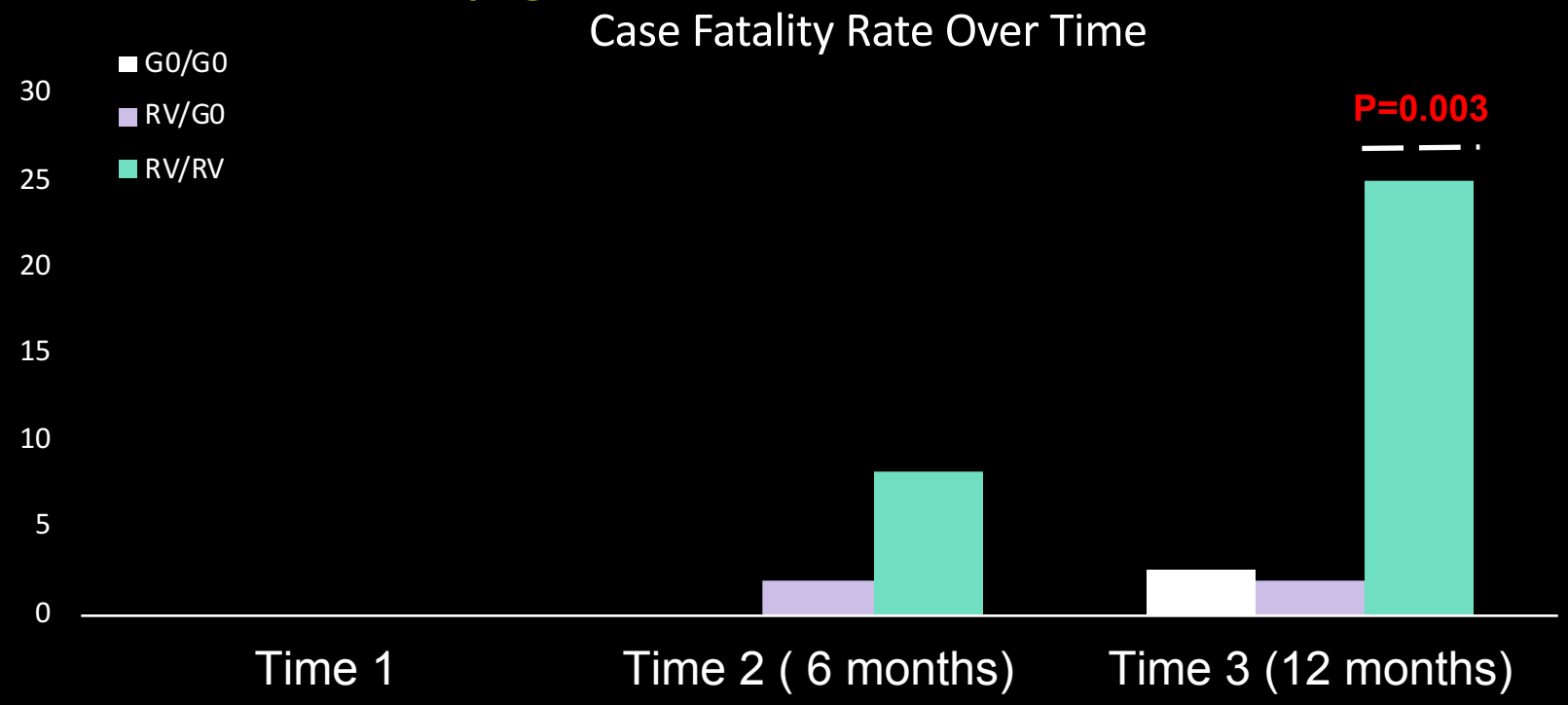
	Cataract	Optic Atrophy	Cognitive Impairment	Seizures	CVA	Neuropathy	Transverse Myelitis	eGFR<50%	Proteinuria>3.5	ESRD	Pulm HTN	Pulm Fibrosis	Pulm infarction	Angina	MI	Cardiomyopathy	Valve	Pericarditis	Claudication	Pulp loss	Significant tissue loss	Venous thrombosis	GI infarction Mesenteric Insufficiency	Chronic peritonitis	GI Stricture	Muscular Atrophy	erosive arthritis	Osteoporosis	AVN	Osteomyelitis	Scarring alopecia	Other scarring	Skin ulceration	Gonadal failure	Diabetes	Malignancy	Total SLICC
G0/G0	0	2.7	16.2	5.4	0	8.1	0	5.4	0	2.7	0	0	0	0	0	0	0	0	0	3	11	3	0	0	0	0	11	5	0	0	0	27	0	11	0	3	3	1.1
RV/G0	0	0	6	2	4	6	1	4	0	2	2	0	2	0	0	4	0	0	0	12	0	0	0	0	0	0	18	6	0	0	0	6	4	0	0	0	0	0.8
RV/RV	0	0	33	0	0	0	0	8.3	8.3	25	0	0	0	0	0	8	0	0	0	17	0	8	0	0	0	0	8	0	0	8	0	8	17	8	0	0	0	1.6
Total	0	1	14.7	4.5	1.8	6.4 2	1	4.5	1	4.5	1	0	1	0	0	3	0	0	0	8	4	2	0	0	0	0	15	6	0	0	0	15	5	6	0	1	1	1

APOL1 Variants Associate with Higher Urine Protein, Creatinine, and Blood Pressure



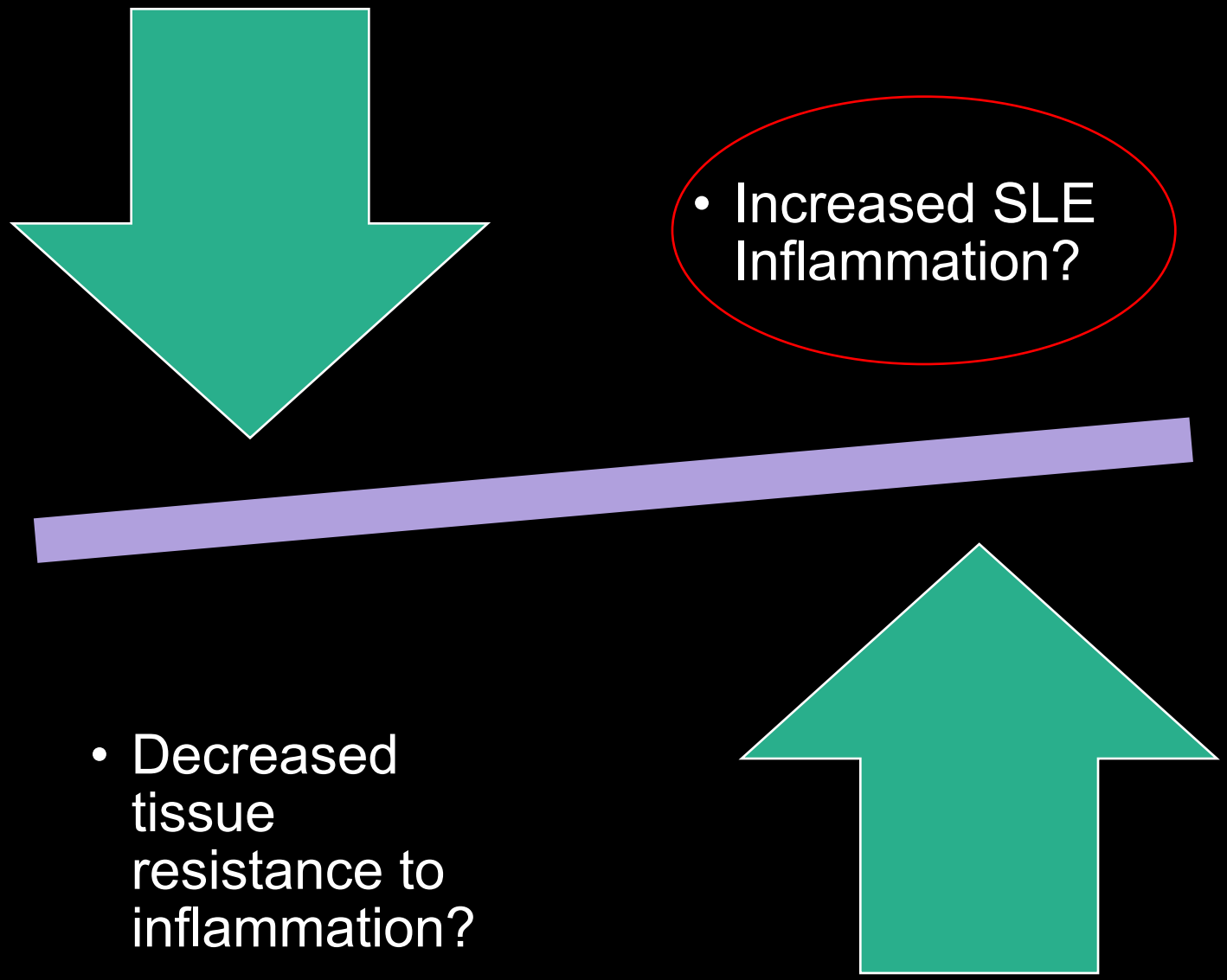
	G0/G0	RV/G0	RV/RV	P Value
Creatinine	0.9	1.06	2.49	0.03
Systolic BP	107±11	109 ± 18	121 ±20	0.01
Diastolic BP	72 ± 10	72± 11	83 ± 18	0.003

The SLE Case Fatality Rate Was Significantly Higher in APOL1 Variant Homozygous Patients

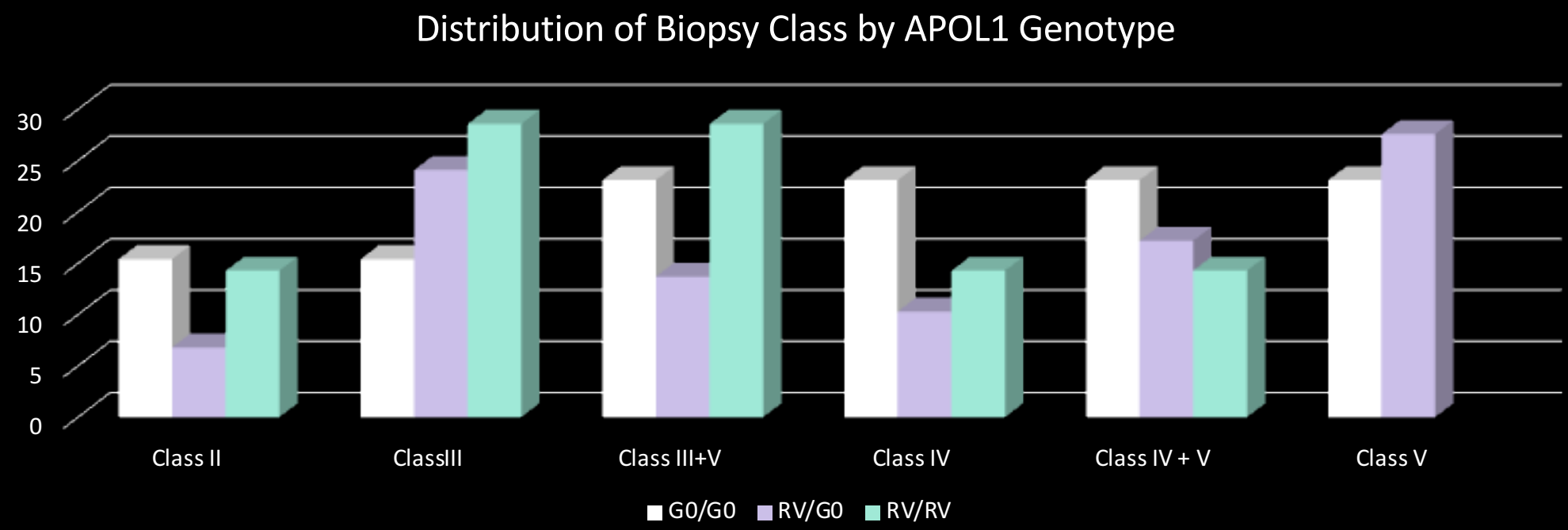


Causes of Death	G0/G0			• ESRD
	RV/G0		• Sepsis during pregnancy	
	RV/RV		• Heart Failure/Pulm edema	• ESRD • Unknown (renal failure at death

APOL1-Associated Damage Accrual: Increased Inflammation or Decreased Tissue Resistance?



NYU Cohort: Variant Homozygotes Progressed to ESRD Despite More Reassuring Biopsies

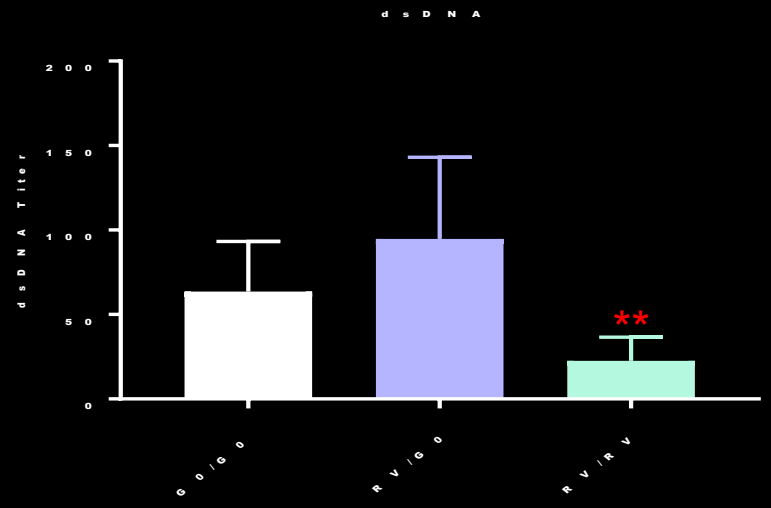


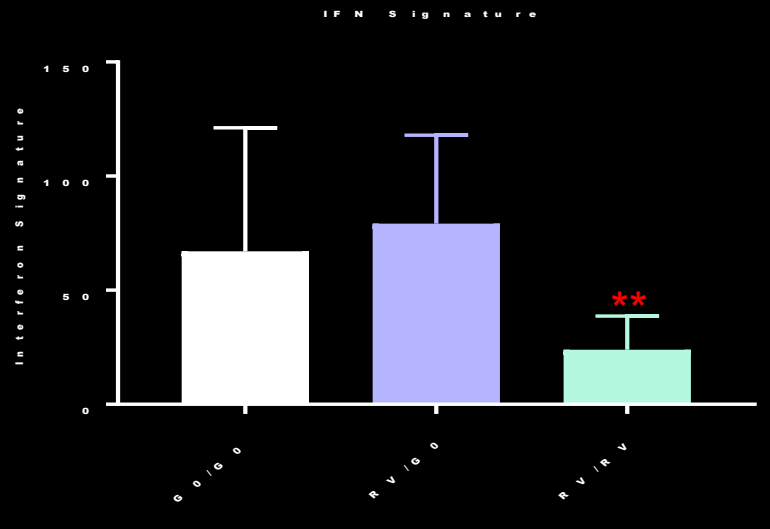
	Activity Index	Chronicity Index	% Sclerotic Glomeruli
G0/G0	5/24	3/12	29%
RV/G0	6/24	2/12	14%
RV/RV	1/24	1/12	8%
P value	0.04	0.01	0.06

	Creatinine	uPCR	% ESRD
G0/G0	1.2	2.5	18%
RV/G0	1.1	2.8	15%
RV/RV	2.2	4.1	43%
P value	0.01	0.04	**OR: 5.7 P=0.05**

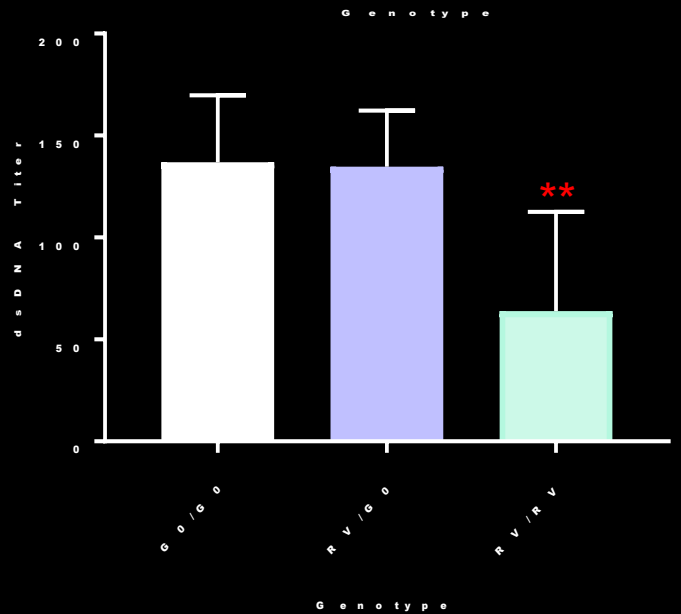
APOL1 Homozygotes Exhibit Less Serologic Activity

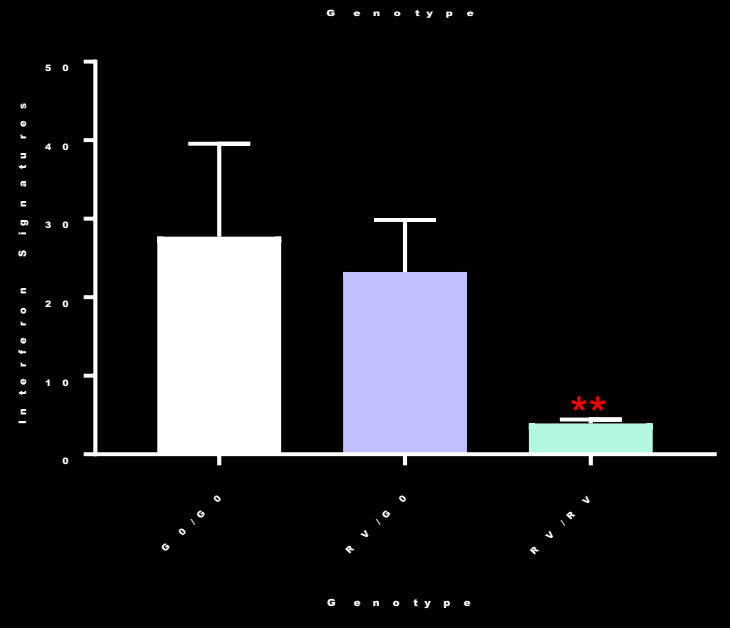
GH Cohort



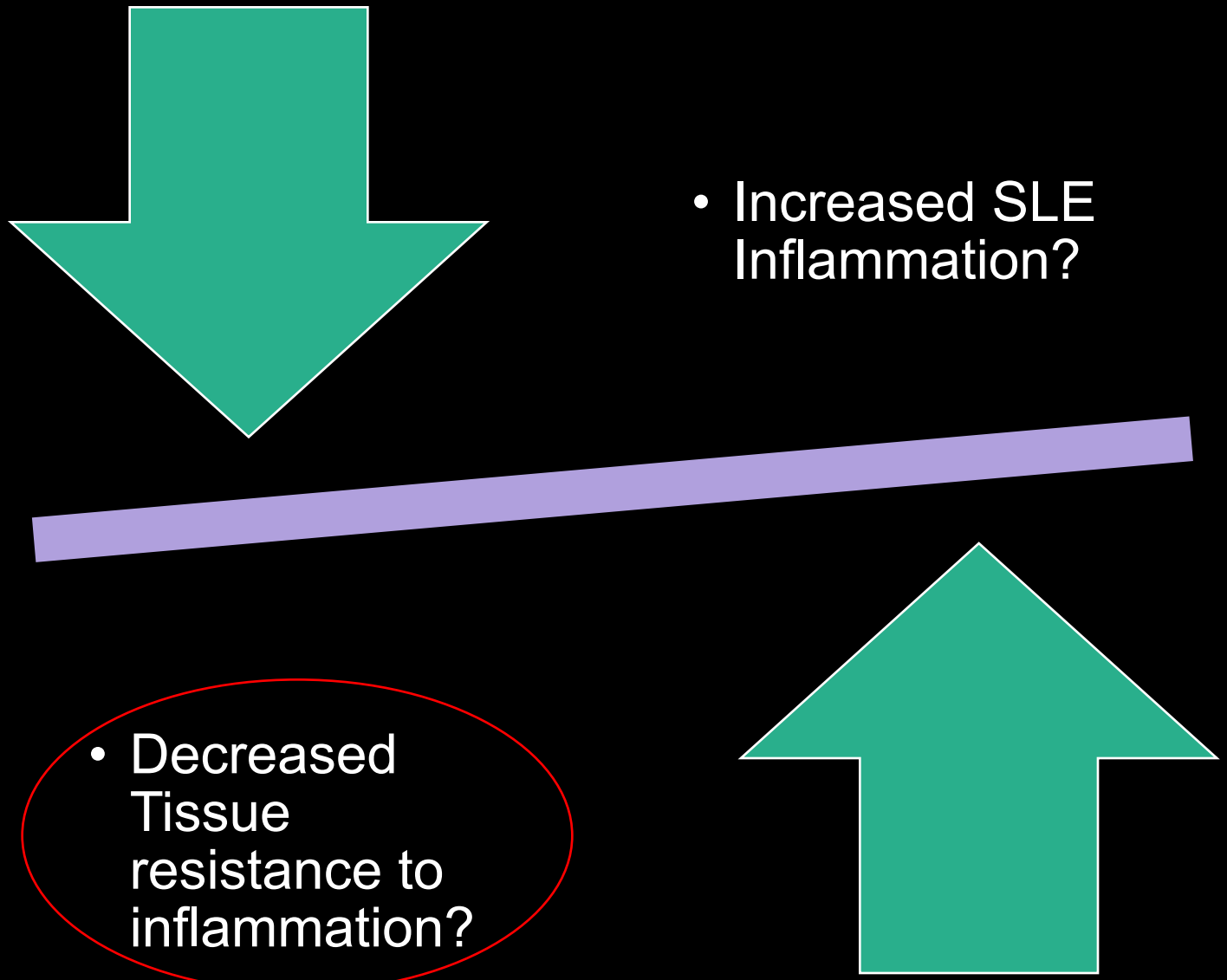


NYU Cohort





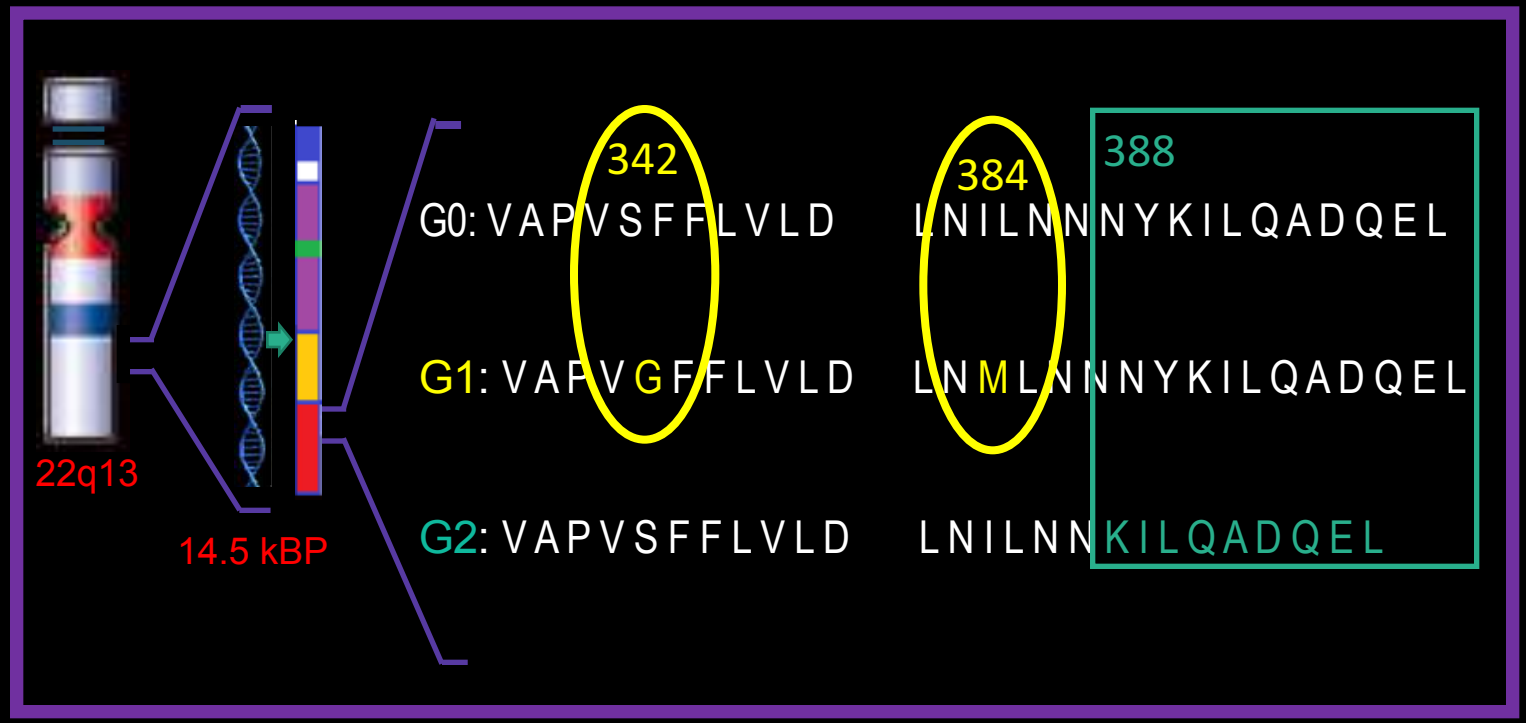
APOL1-Associated Damage Accrual: Increased Inflammation or Decreased Tissue Resistance?

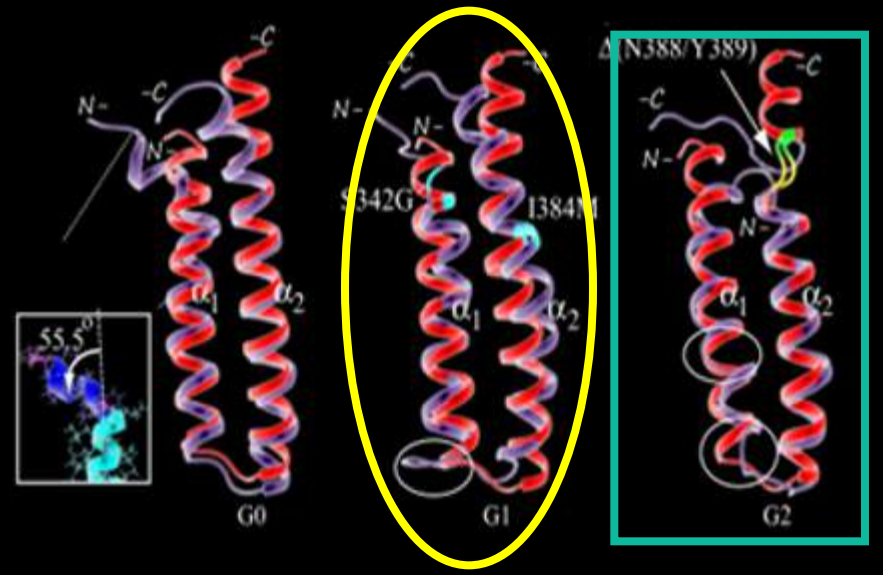


- Increased SLE Inflammation?
- Decreased Tissue resistance to inflammation?

APOL1 Variant Genes Encode Amino Acid Changes Which Reduce Protein Structural Stability

- APOL1: One member of a 6 gene family on chromosome 22.13
  - Ancestral G0 allelic frequency: 0.65
  - Variant G1: Two non-synonymous substitutions at position 342 and 384 allelic frequency: 0.22
  - Variant G2: Two amino acid deletion at position 388 allelic frequency: 0.13

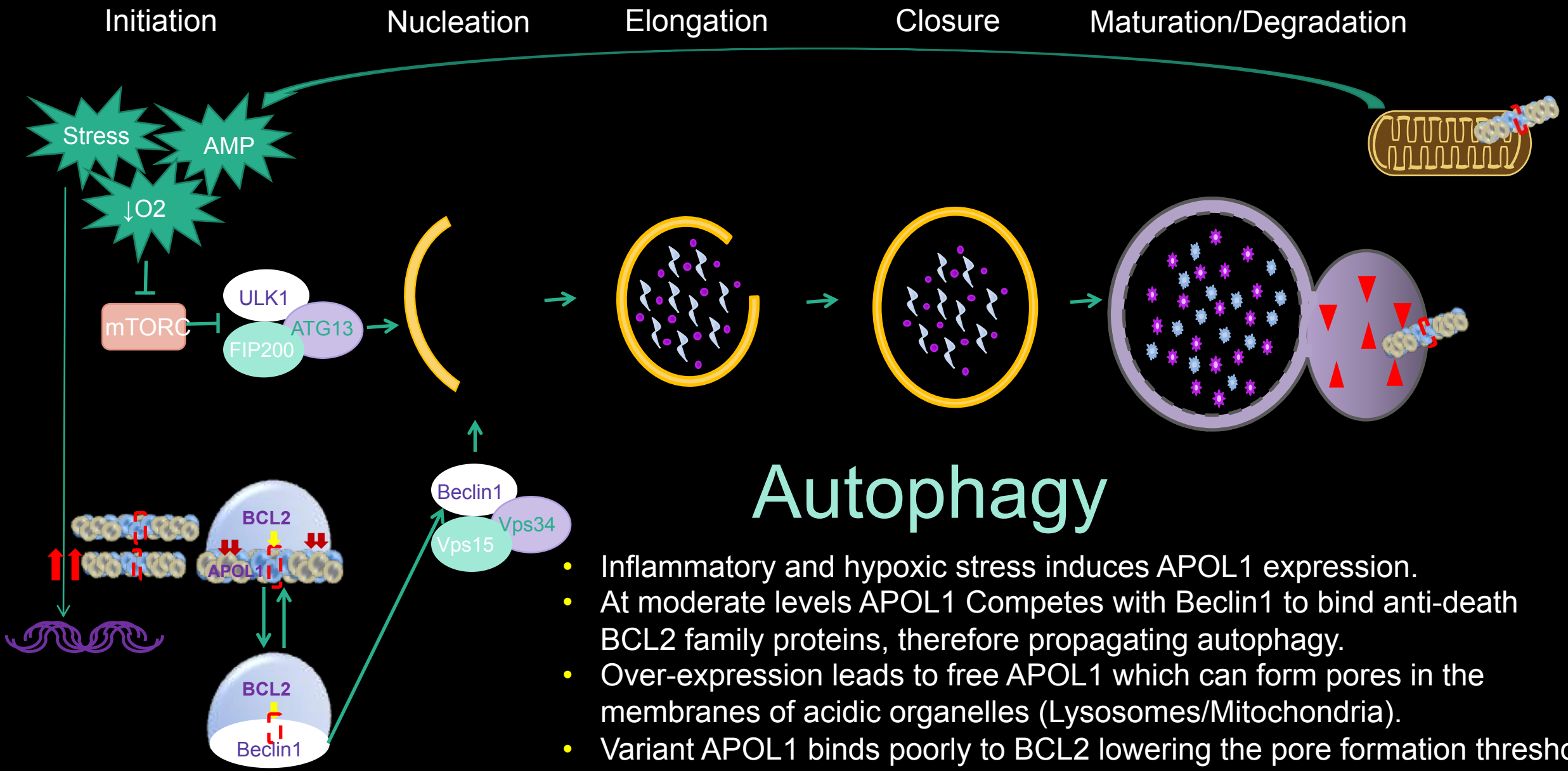




- Amino acid changes decrease APOL1 variant interaction efficiency with other proteins

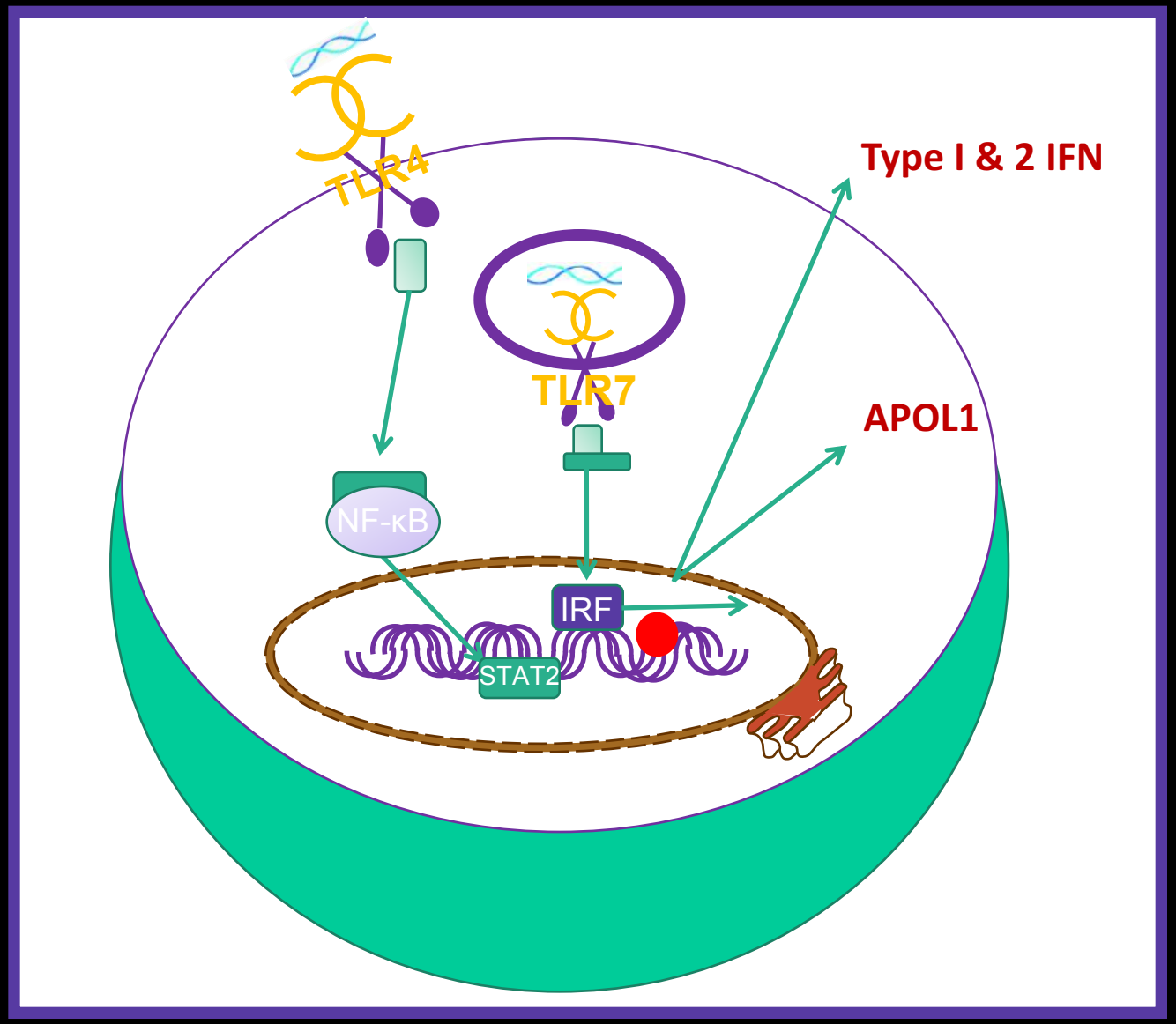
(Grams, Rebholz et al. 2016)(Sharma, Friedman et al. 2016)

Toxicity Model: APOL1 Variants May Upset the Autophagy/Pore Formation Balance (Literature Summation Transfection Models)



- Inflammatory and hypoxic stress induces APOL1 expression.
- At moderate levels APOL1 Competes with Beclin1 to bind anti-death BCL2 family proteins, therefore propagating autophagy.
- Over-expression leads to free APOL1 which can form pores in the membranes of acidic organelles (Lysosomes/Mitochondria).
- Variant APOL1 binds poorly to BCL2 lowering the pore formation threshold

Study Rationale: SLE Inflammatory Pathways Increase APOL1 Expression and Burden of Toxic Protein in Variant Carrying Endothelium



- Toll Like Receptor 4/7 Ligation
  - Down stream TLR4 effectors bind the APOL1 Promoter
  - Interferon Regulatory Factor, enhances both interferon and APOL1 transcription
- Inflammatory Cytokines
  - Types I and II Interferons
  - TNF-α

(Nichols et al. 2015) (Blazer et al. 2017)

Conceptual Model: SLE-Related Stimuli Increase Intracellular APOL1 Expression Causing Injury in Variant Carrying Cells

- Autophagy/Cell maintenance
- Normal Metabolism

Inflammatory Stimulus:
- TLR Ligand
- IFN Gamma

Reference Allele Cell

Risk Variant Cell

↑*APOL1* Expression

Risk Variant Cell

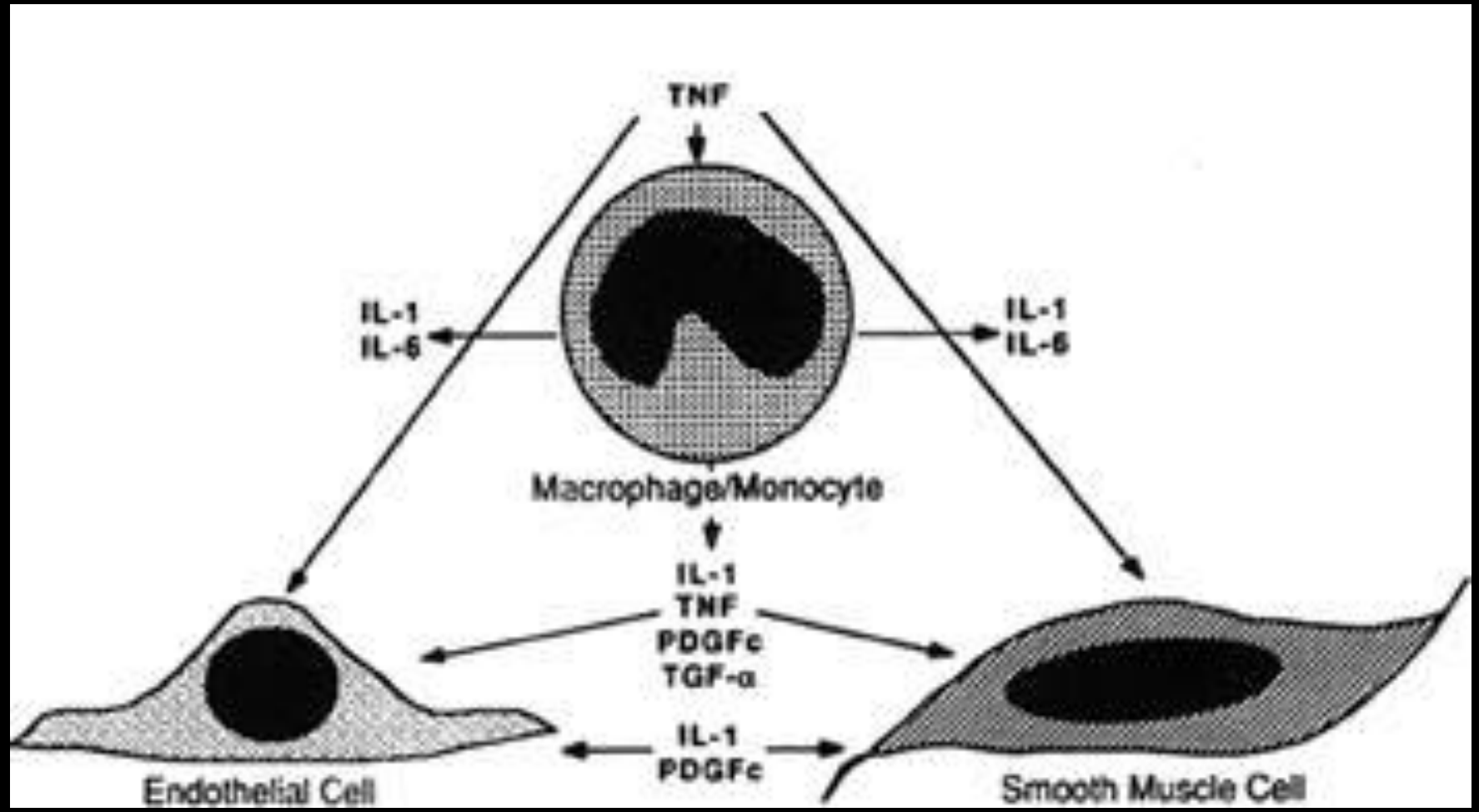
- Autophagy initiation
- Pore formation
  - Lysosome:
    - ↓Autophagic flux
    - ↓ Mitophagy
  - Mitochondria respiration defects

Risk Variant Cell

- Functional Consequences?

Atherosclerosis Risk

APOL1 is Expressed in Vascular-Relevant Cell Types: Endothelial Cells and Mononuclear Cells. How Might Variants Influence Their Behavior?



Study Question:

What are the functional consequences of IFN-induced APOL1 over expression in human umbilical vein endothelial cells (HUVECs)?

Approach:

- Establish HUVEC cell cultures representing each genotype
- Confirm APOL1 expression response with cytokine treatment using qPCR and immunoblot
- Functional read outs:
  - Lysosome integrity
  - Autophagosome accumulation
  - Mitochondrial morphology

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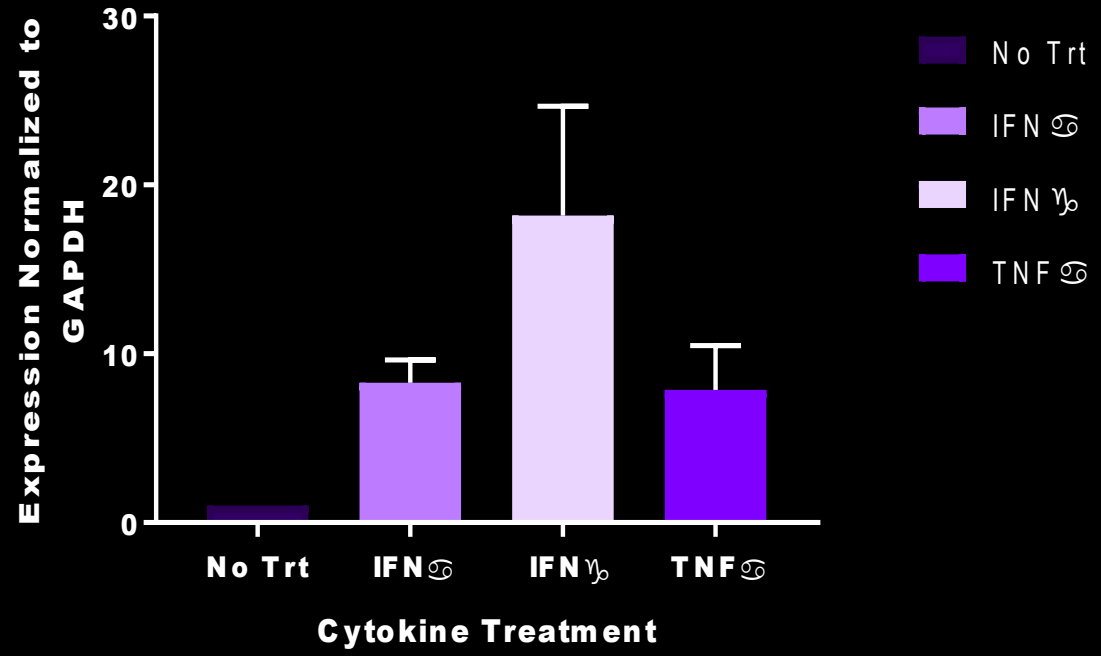
SLE-Relevant Inflammatory Stimuli Increase Endothelial Cell APOL1 Expression Across Genotypes

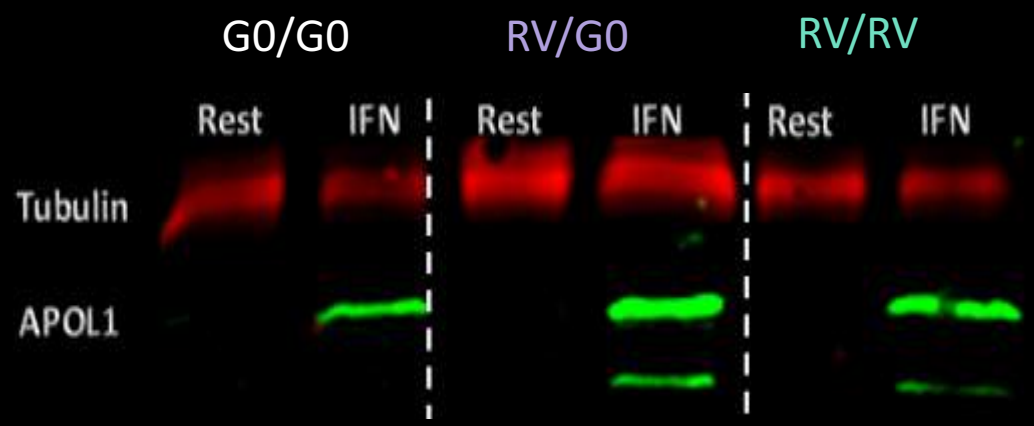
APOL1 Messenger RNA: qPCR



IFNα, IFNγ, and TNFα induced APOL1 messenger RNA: means= 8.29, 18.2, 7.8 respectively

**IFNγ> other cytokines: (F=6.1; p=0.036)

APOL1 Protein: Immunoblot



Protein control: tubulin (red) APOL1 protein: (green)

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In vivo Correlate APOL1 is Expressed in the Vascular Microenvironment

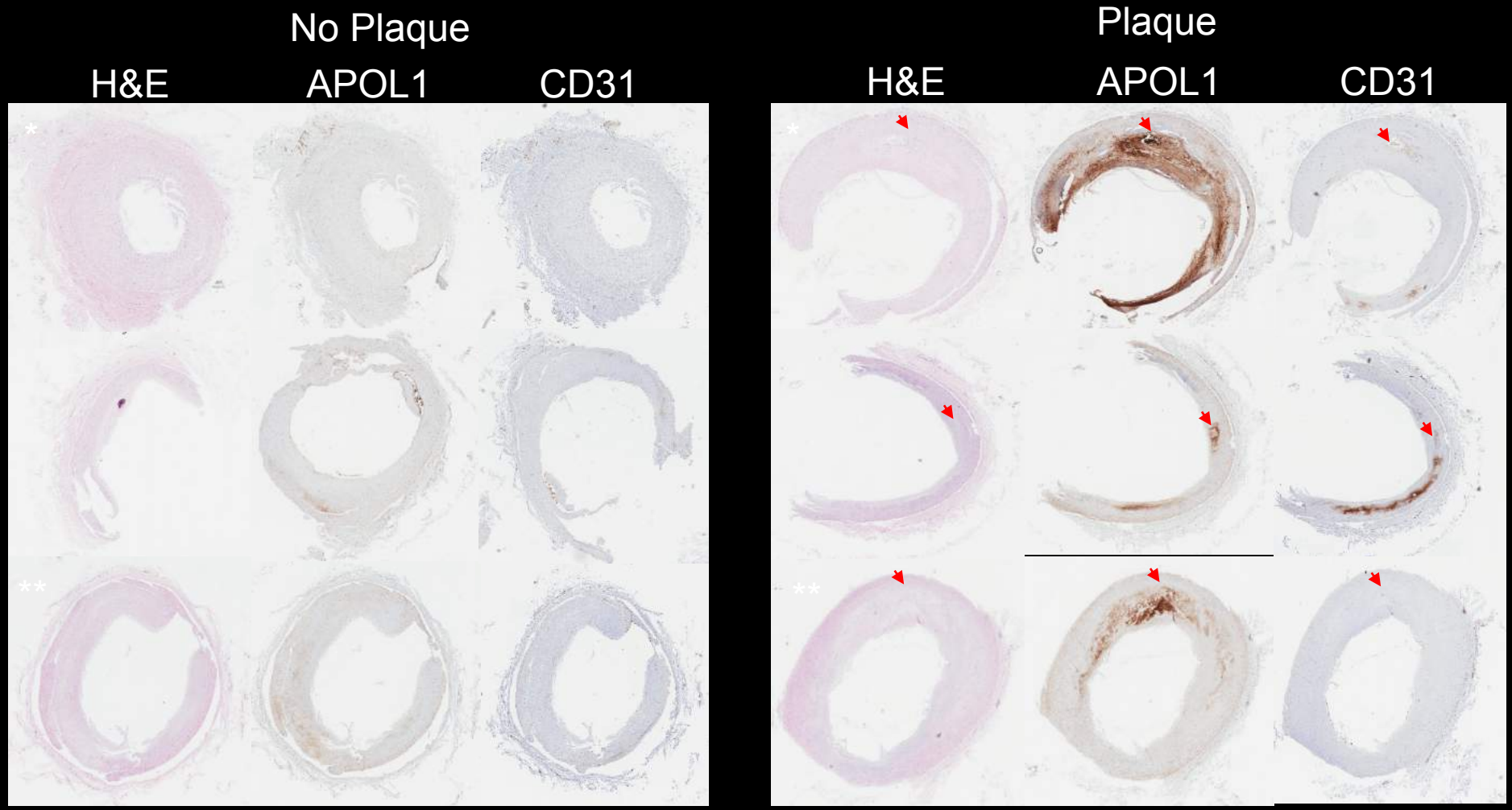
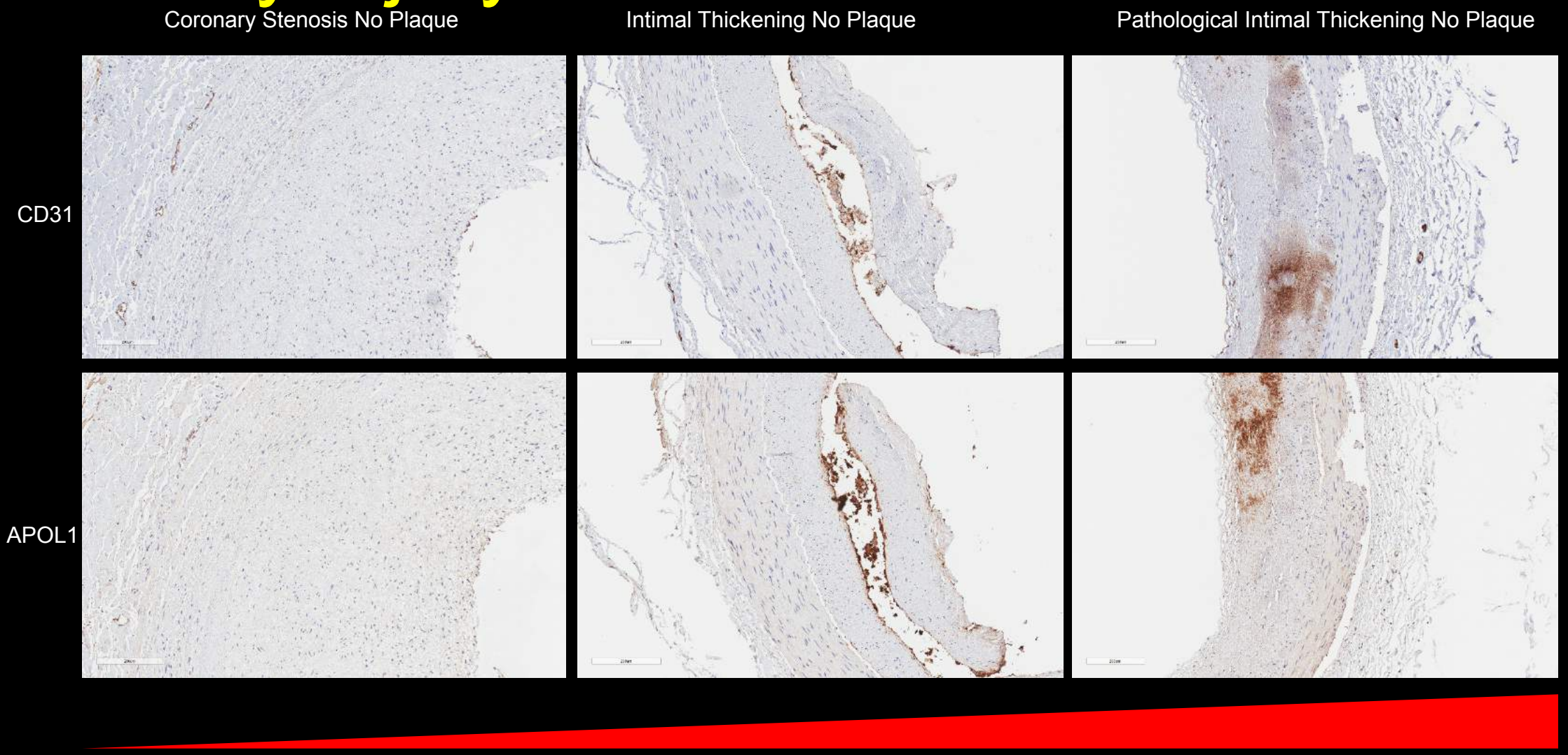


Figure 1: Axial sections of coronary arteries (explanted human hearts, magnification 1X). Sections denoted by * and ** represent samples taken from the same donor at a plaque free and plaque containing sites

APOL1 Expression Increases with Increasing Coronary Injury



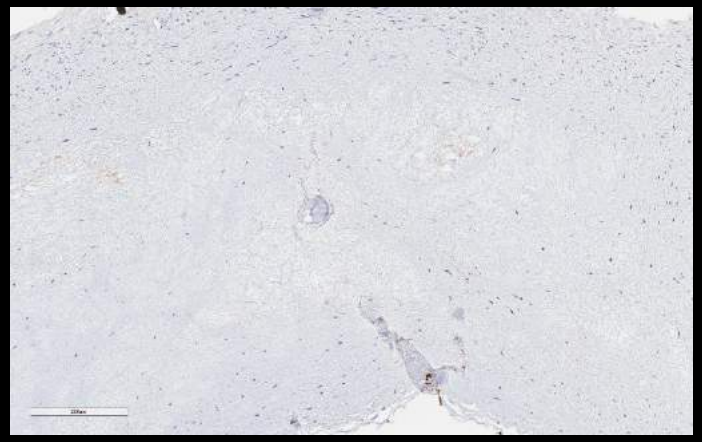
APOL1 Expression Increases with Increasing Coronary Injury

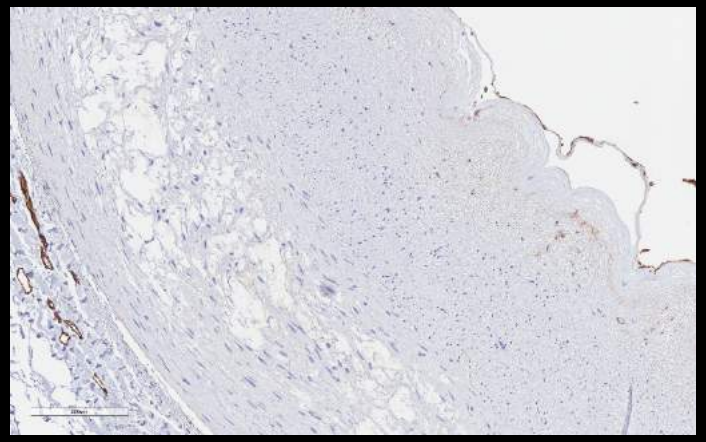
Cholesterol Clefts

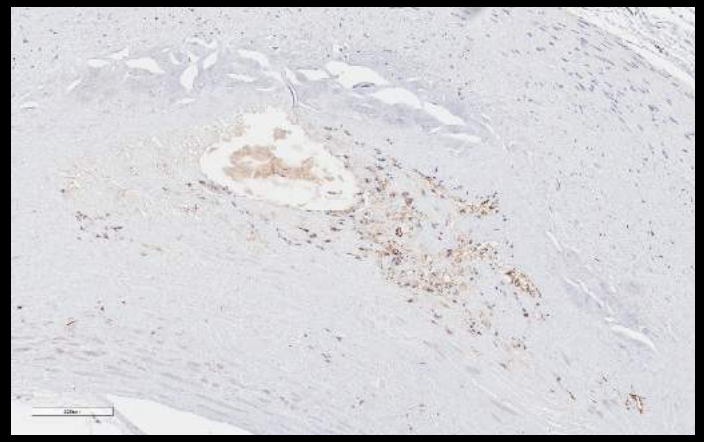
Fibroatherometous Plaque: Fibrous Cap, Necrotic Core, Foam Cells, and Extracellular Cholesterol

Advanced, Necrotic, Atherosclerotic Plaque

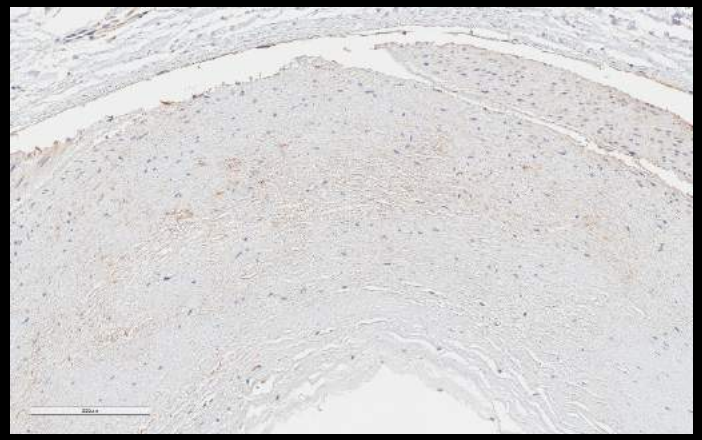
CD31

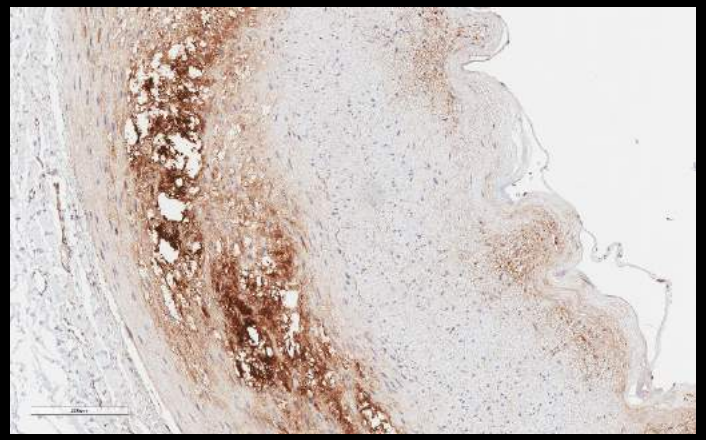


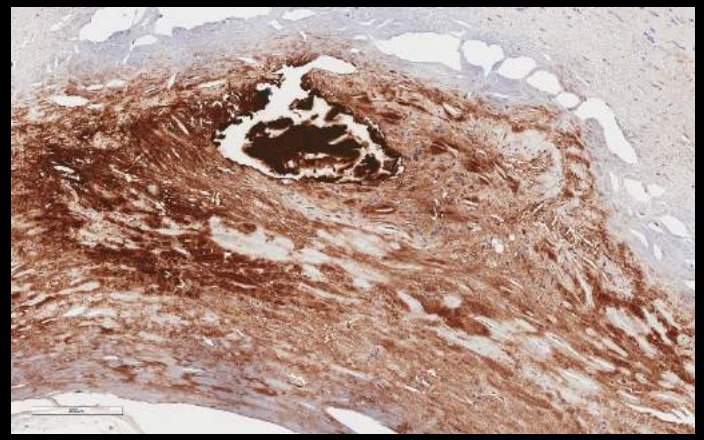




APOL1

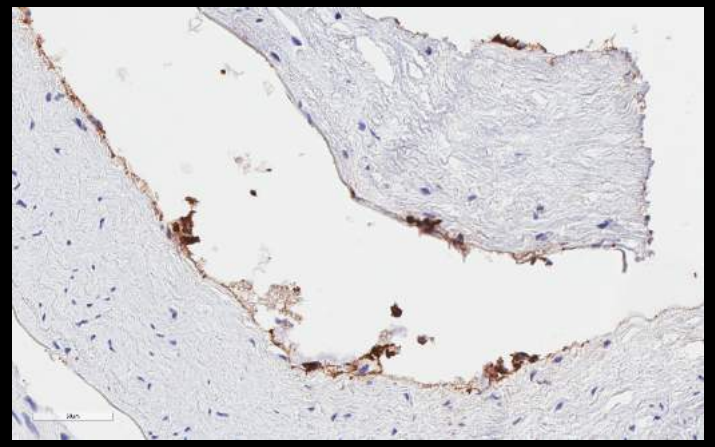
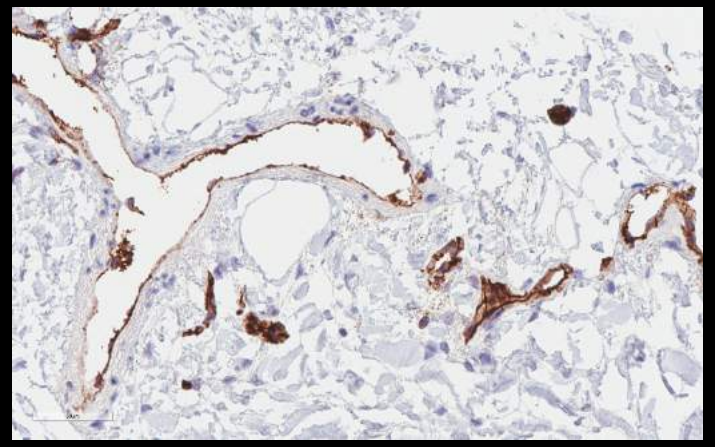
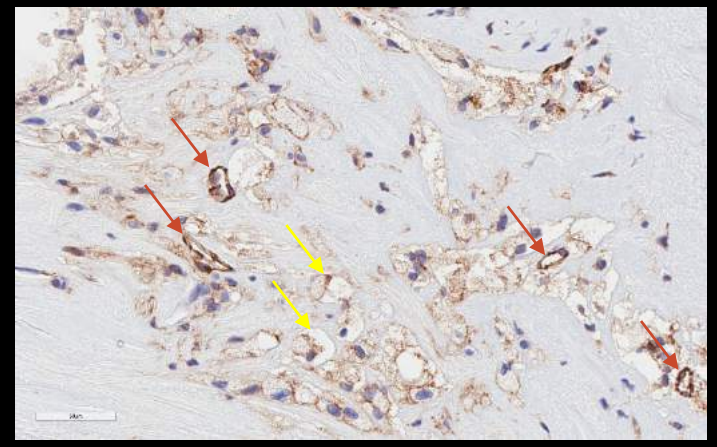
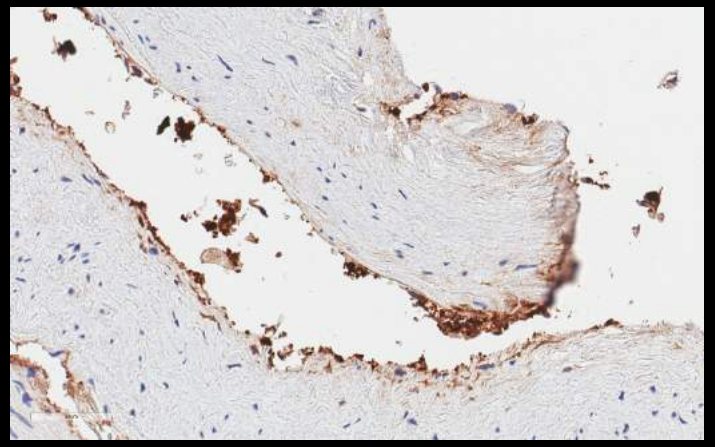
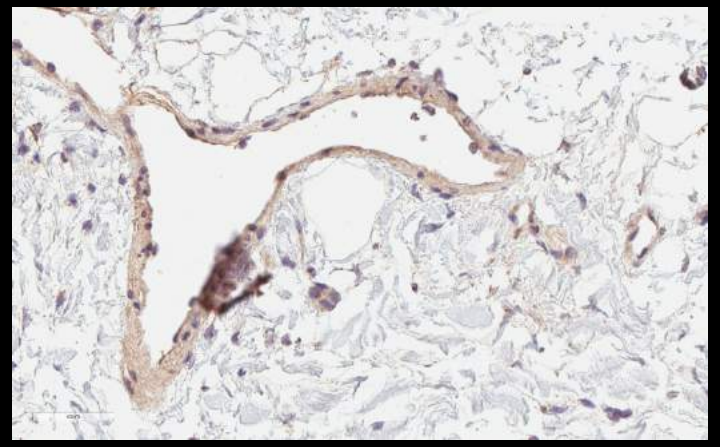
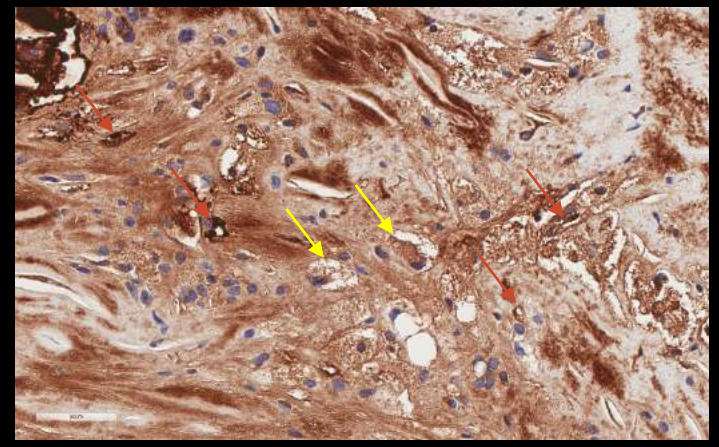




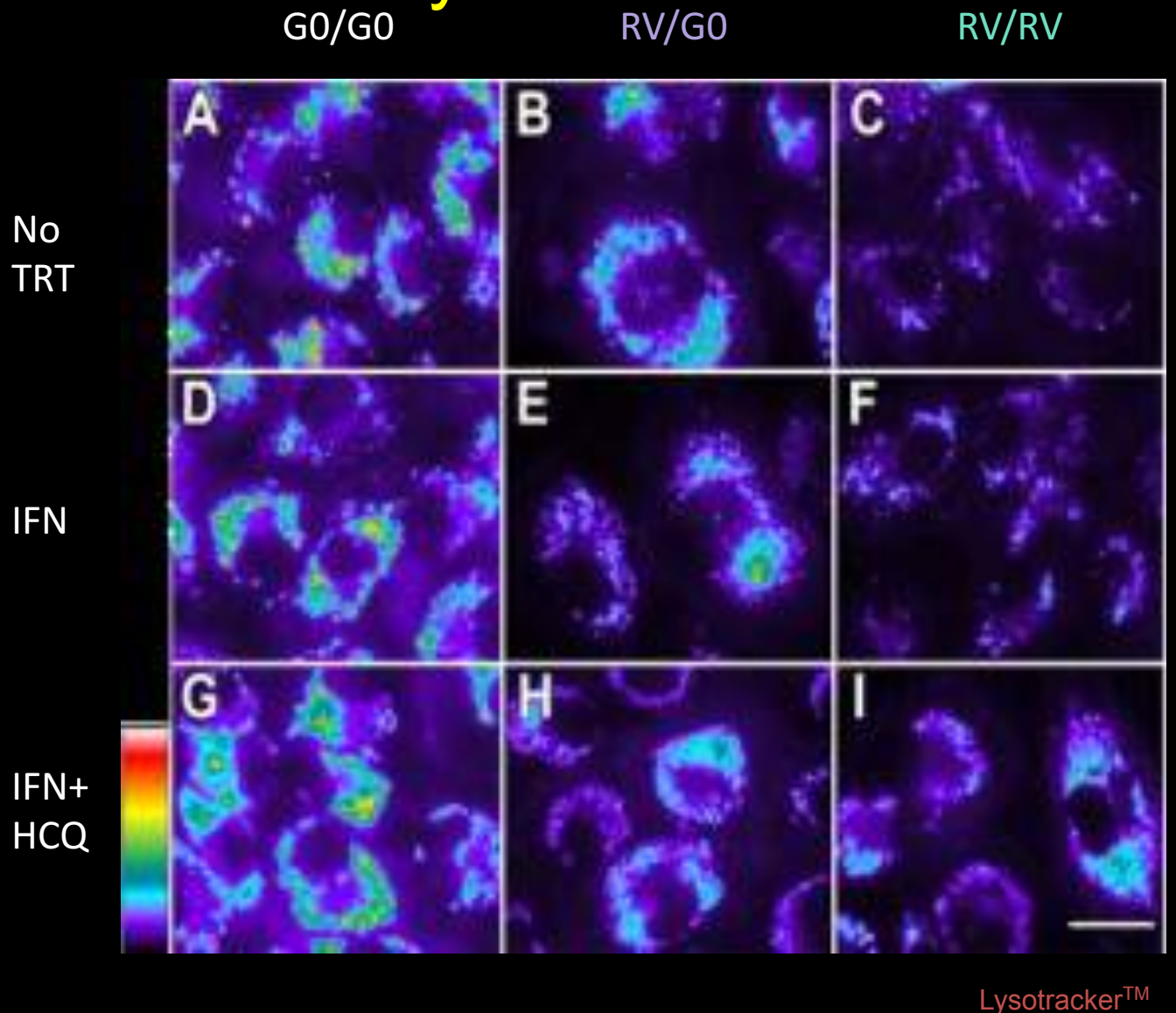


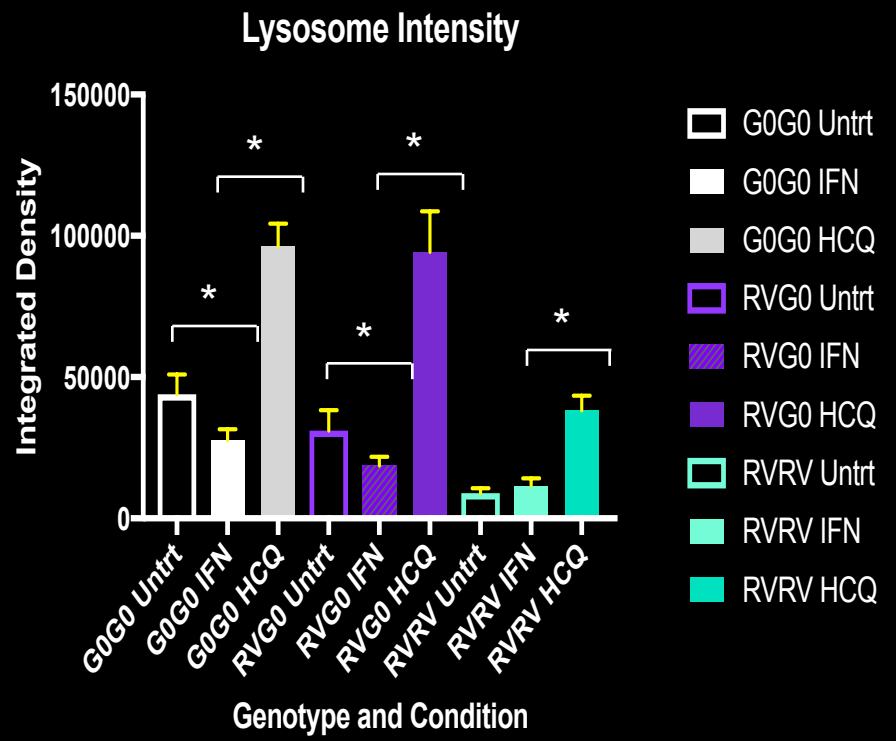
Pathological Diagnosis

APOL1 Staining Can Be Seen in Coronary Endothelial Cells and Invading Macrophages

	Coronary Artery Lumen	Vaso-Vasorum	Necrotic Core (Neovascularization)
CD31			
APOL1			

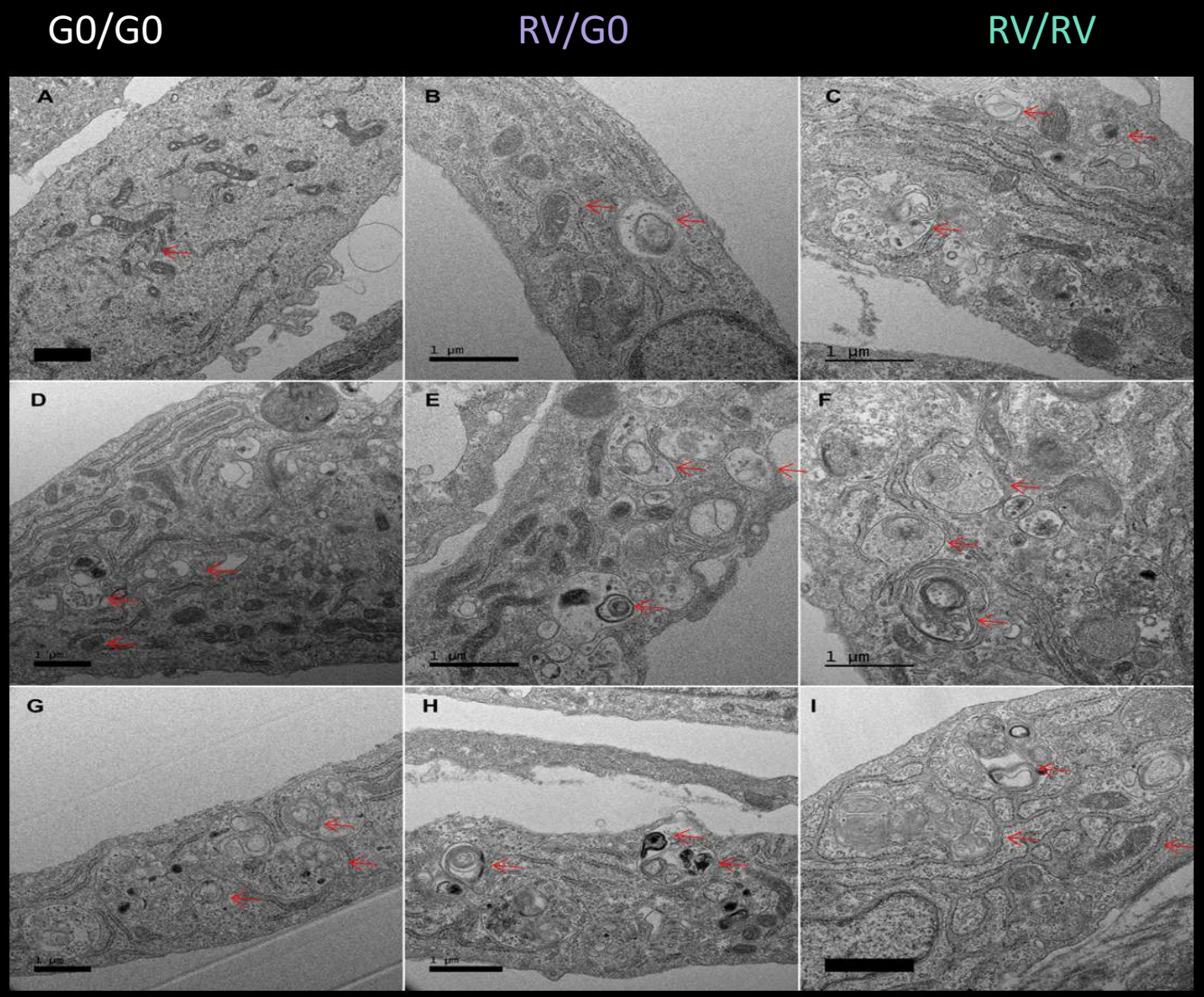
APOL1 Variant Carrying Endothelial Cells Exhibit Decreased Lysosomal Membrane Integrity

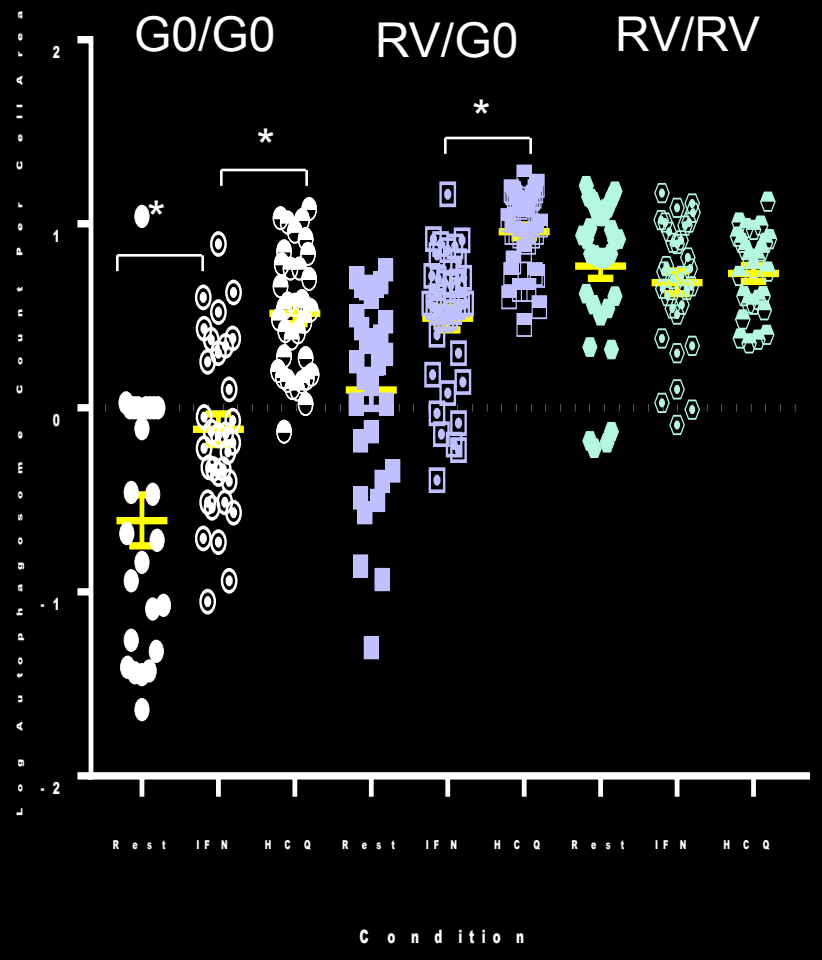




Blazer et. al

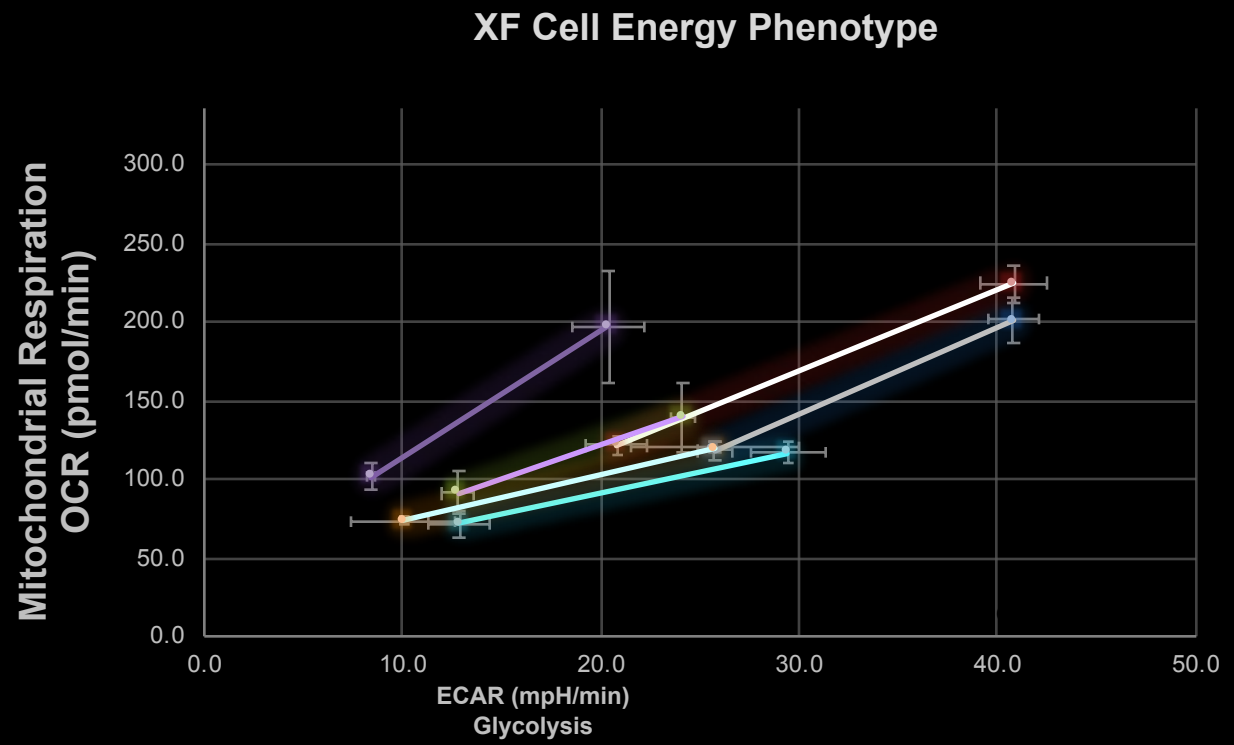
Variant Carrying Endothelium is Deficient in Autophagy—a Cellular Maintenance Process





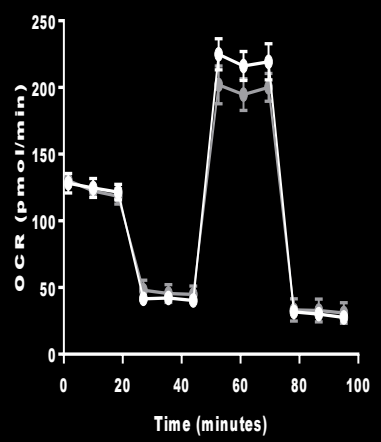
Autophagosomes: Green fluorescent Puncta

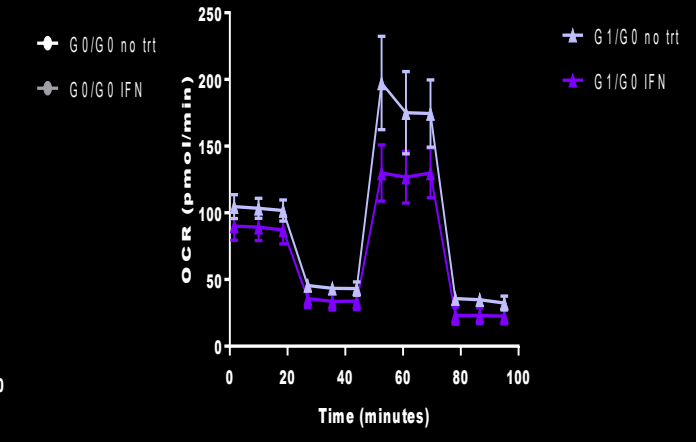
Representative Images: APOL1 Variant-Carrying HUVECs are Energetically Senescent Exhibiting Lower Overall and Maximum OCR

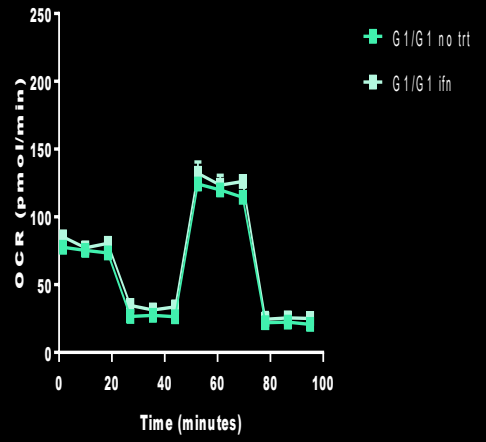


Mitochondrial respiration profiles by genotype and treatment condition:

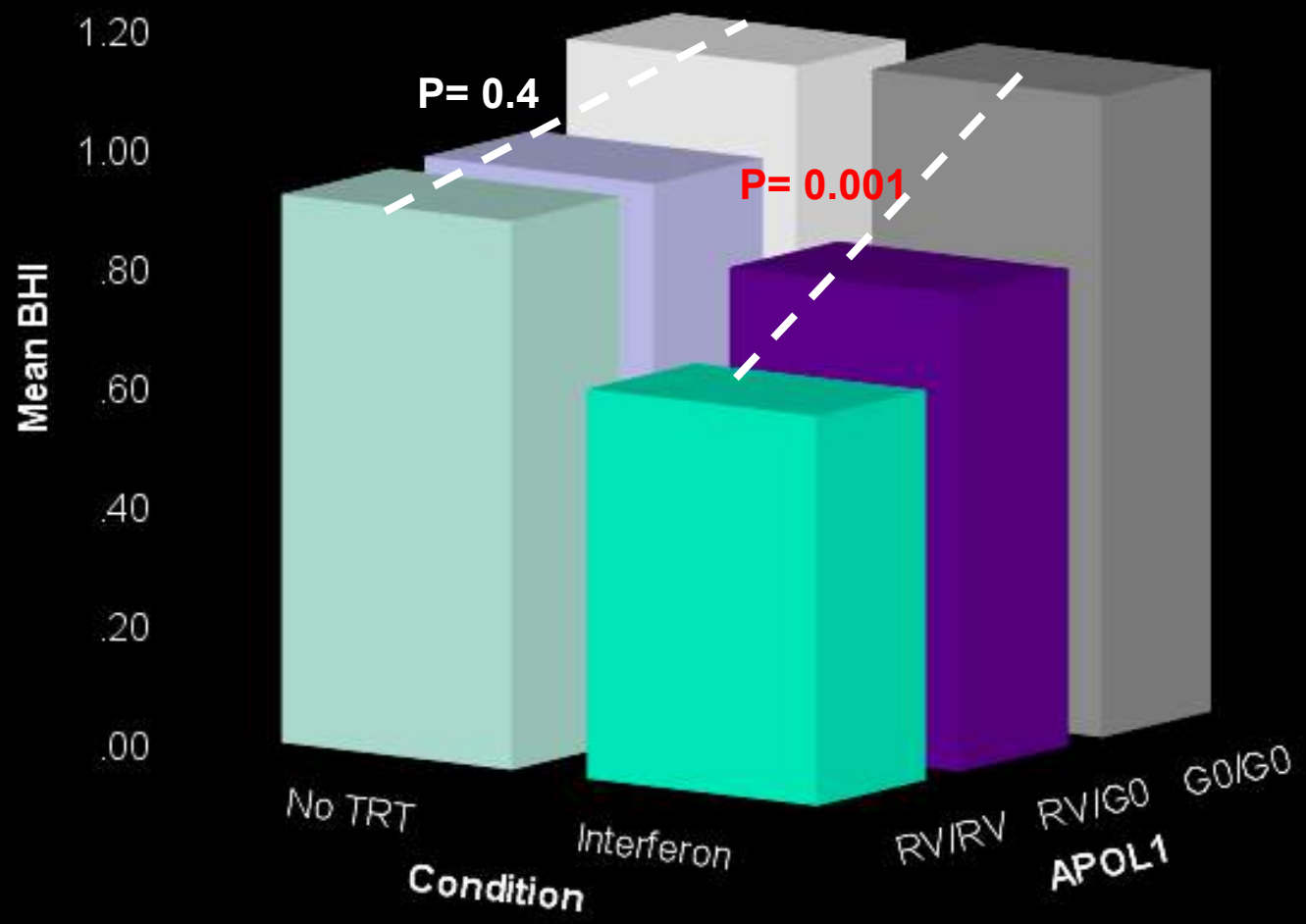
- White: G0/G0
- Purple: RV/G0
- Green RV/RV







Interferon Lowers BHI More Significantly in Variant Carrying Endothelial Cells



- Bioenergetic Health Index (BHI): Validated measure of mitochondrial health
- Untreated HUVECs: No difference in BHI (p=0.4)
- Interferon-treated RV/G0 and RV/RV HUVECs significantly dropped BHI (p=0.001)

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Healthy Mitochondria Must Be Maintain Membrane Potential to Undergo Fusion and Escape Mitophagy

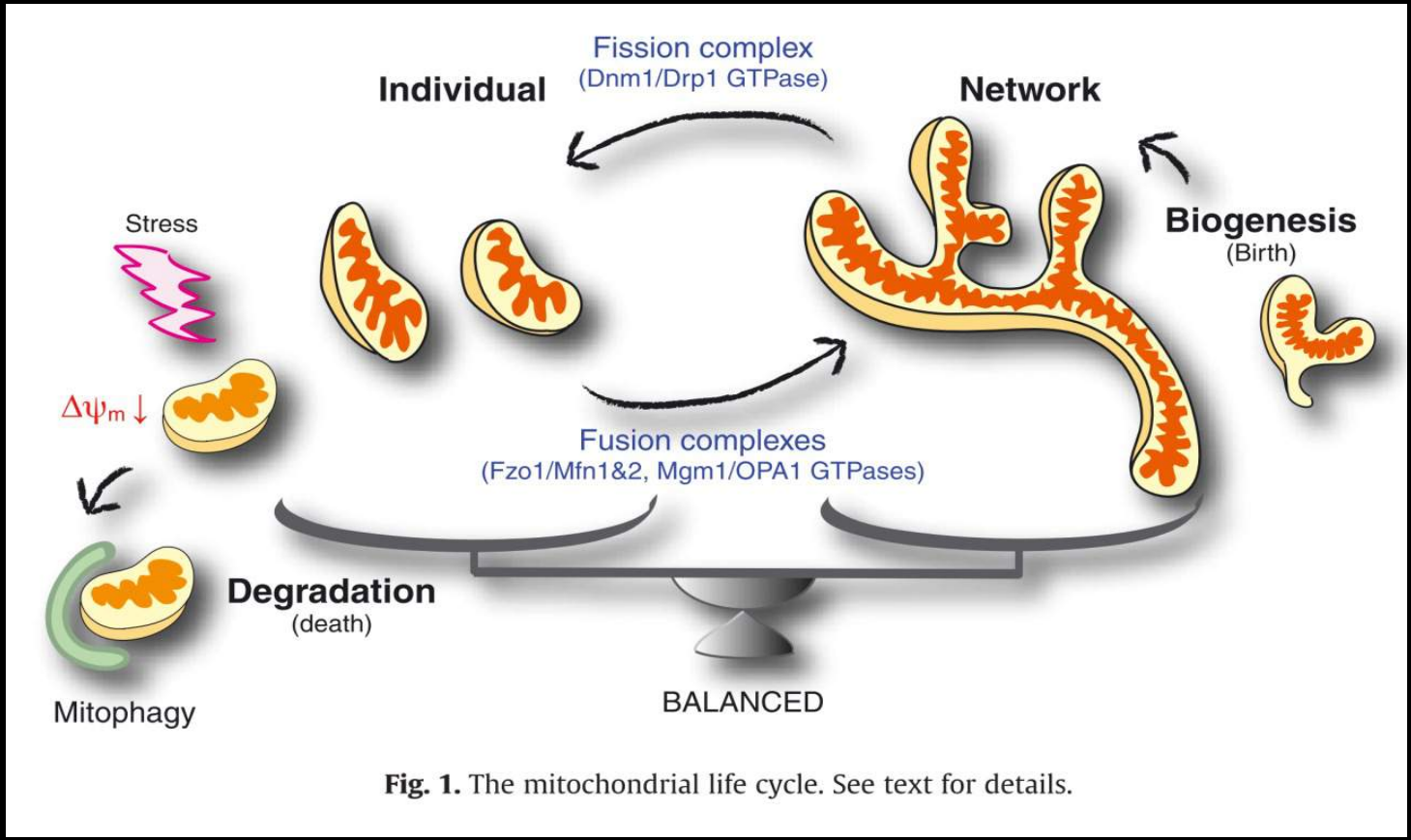

Fig. 1. The mitochondrial life cycle. See text for details.

Healthy mitochondria escape degredation by mitophagy (anaogous to autophagy), by fusing to form networks. This process requires adequate membrane potential. (Okamoto et al)

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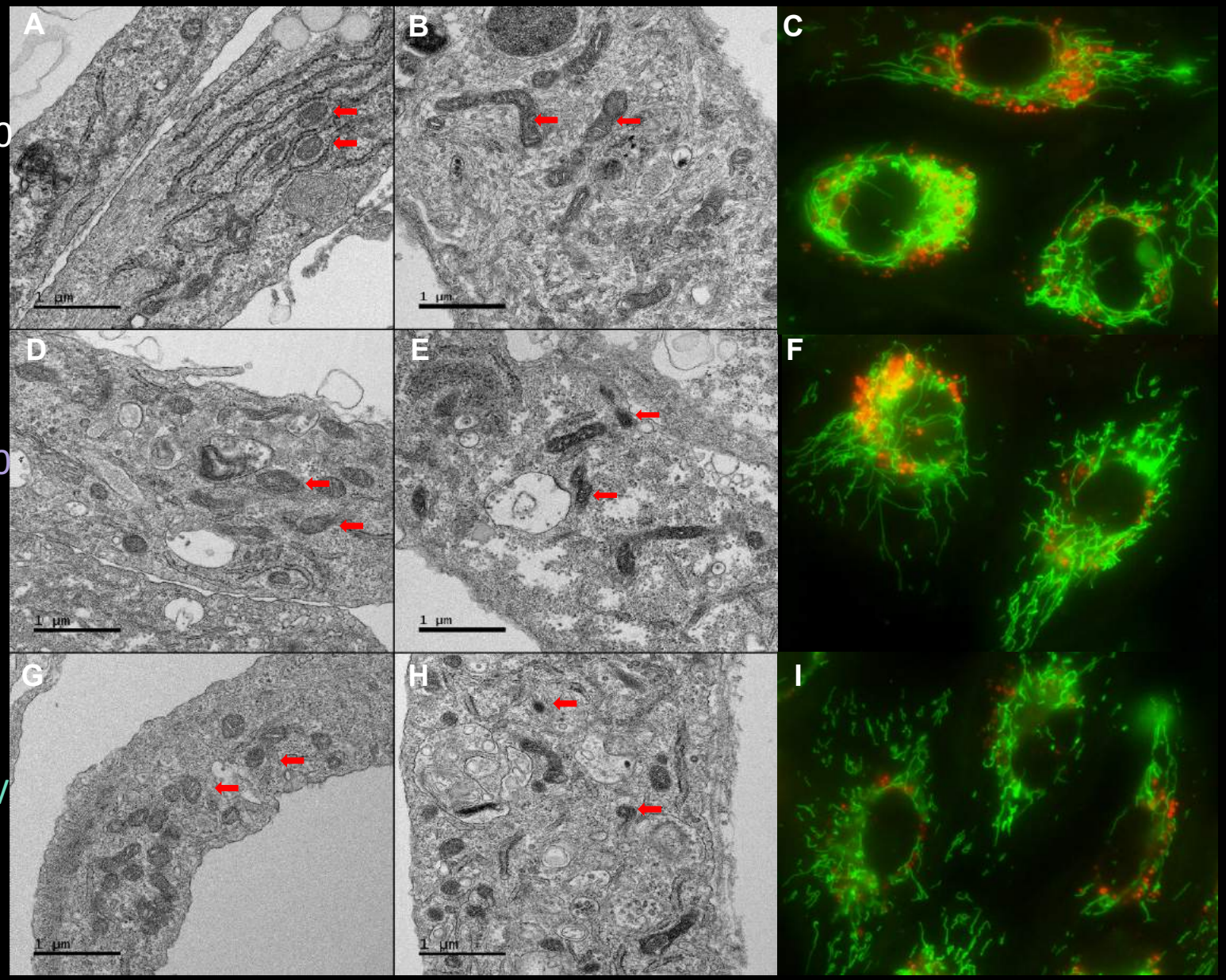
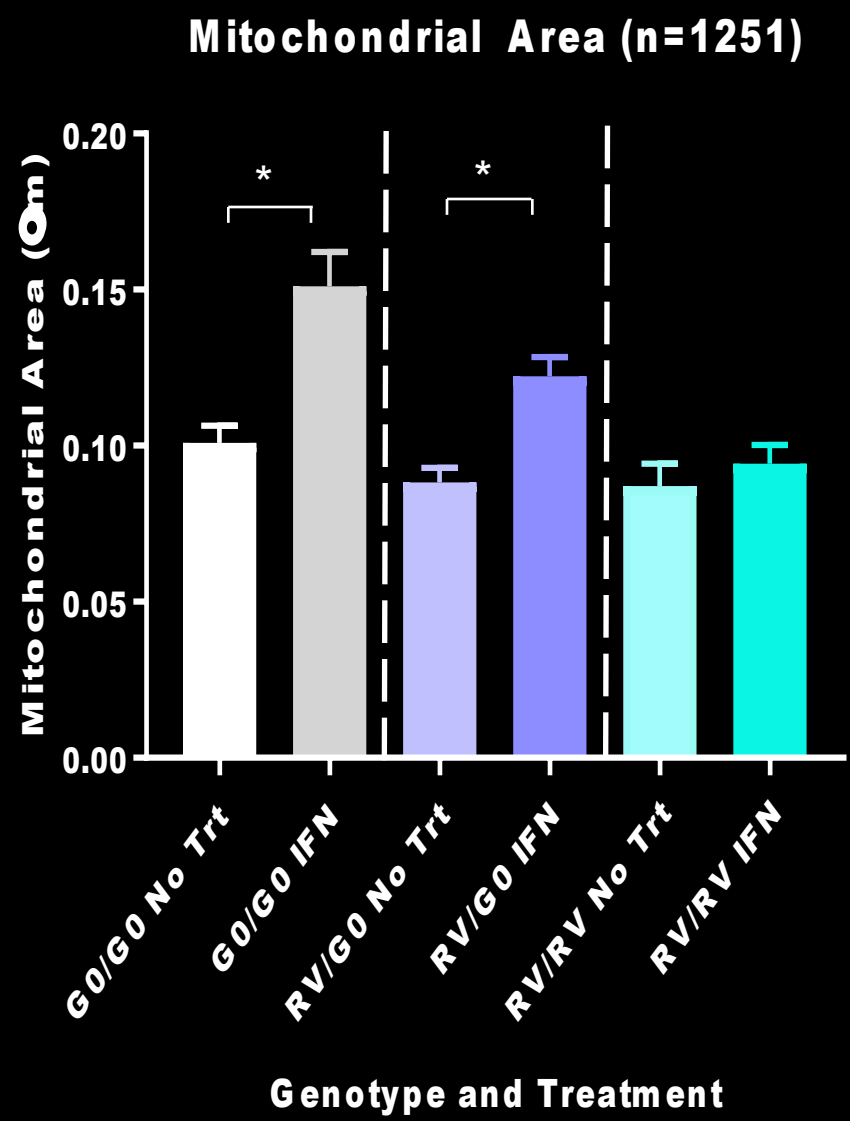
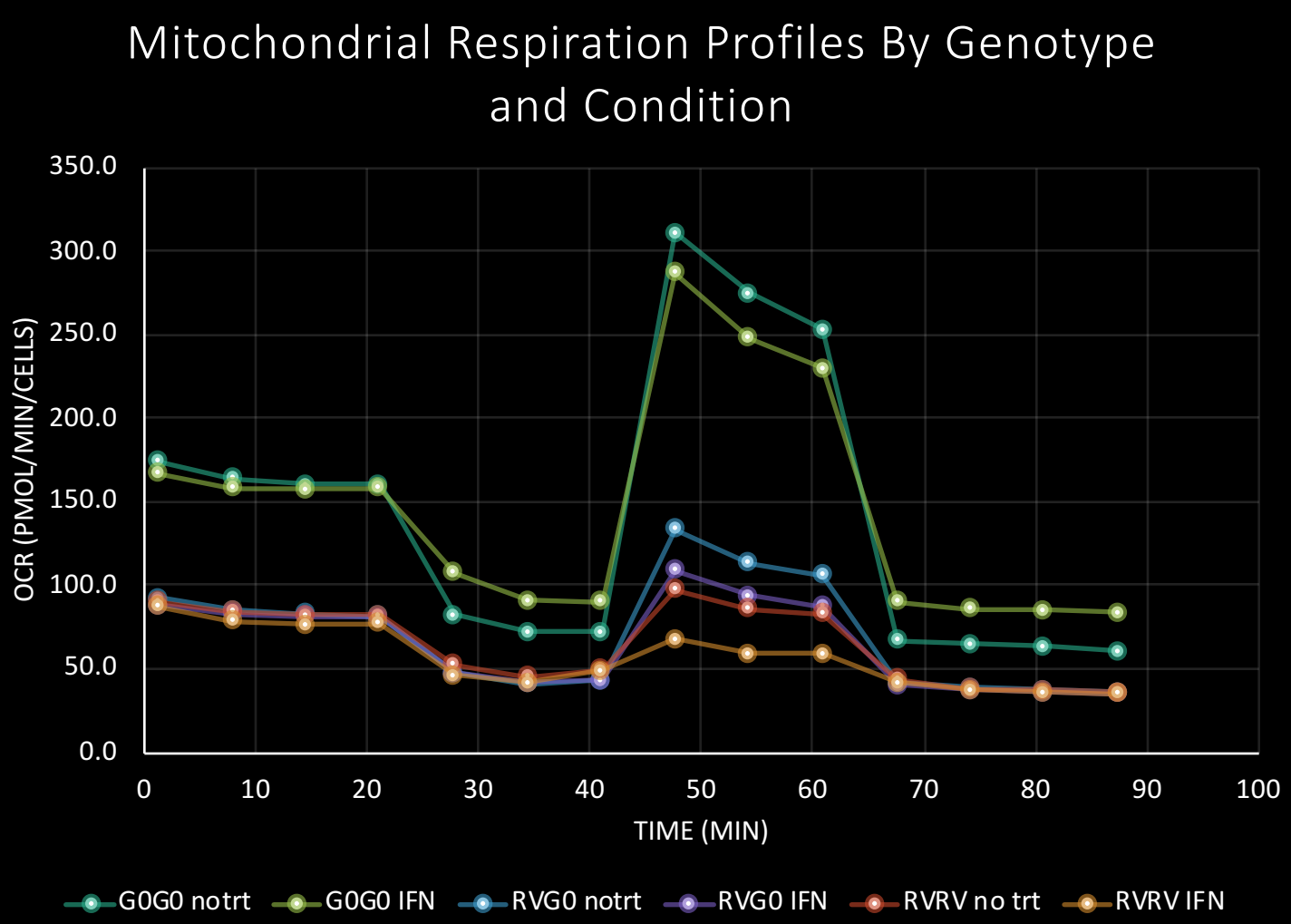
RV Homozygotes Exhibit IFN Dependent Mitochondrial Defects

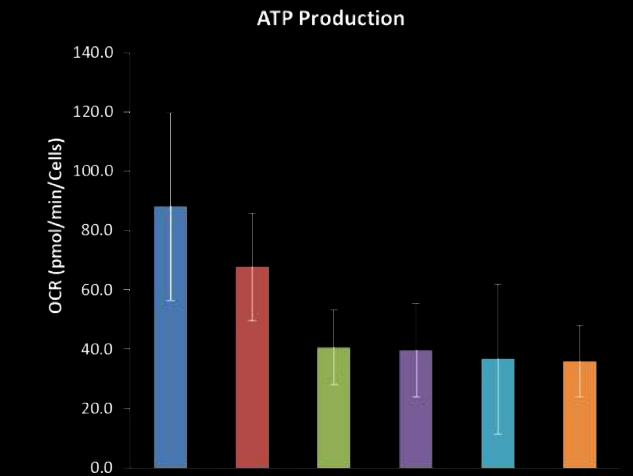


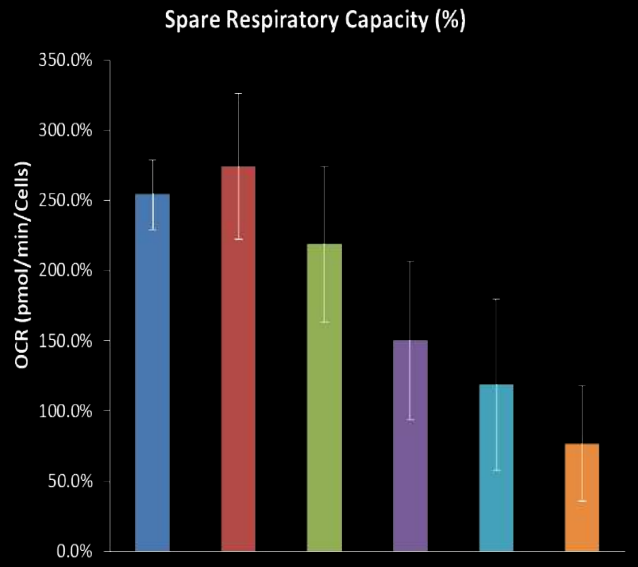


Macrophage Read Outs

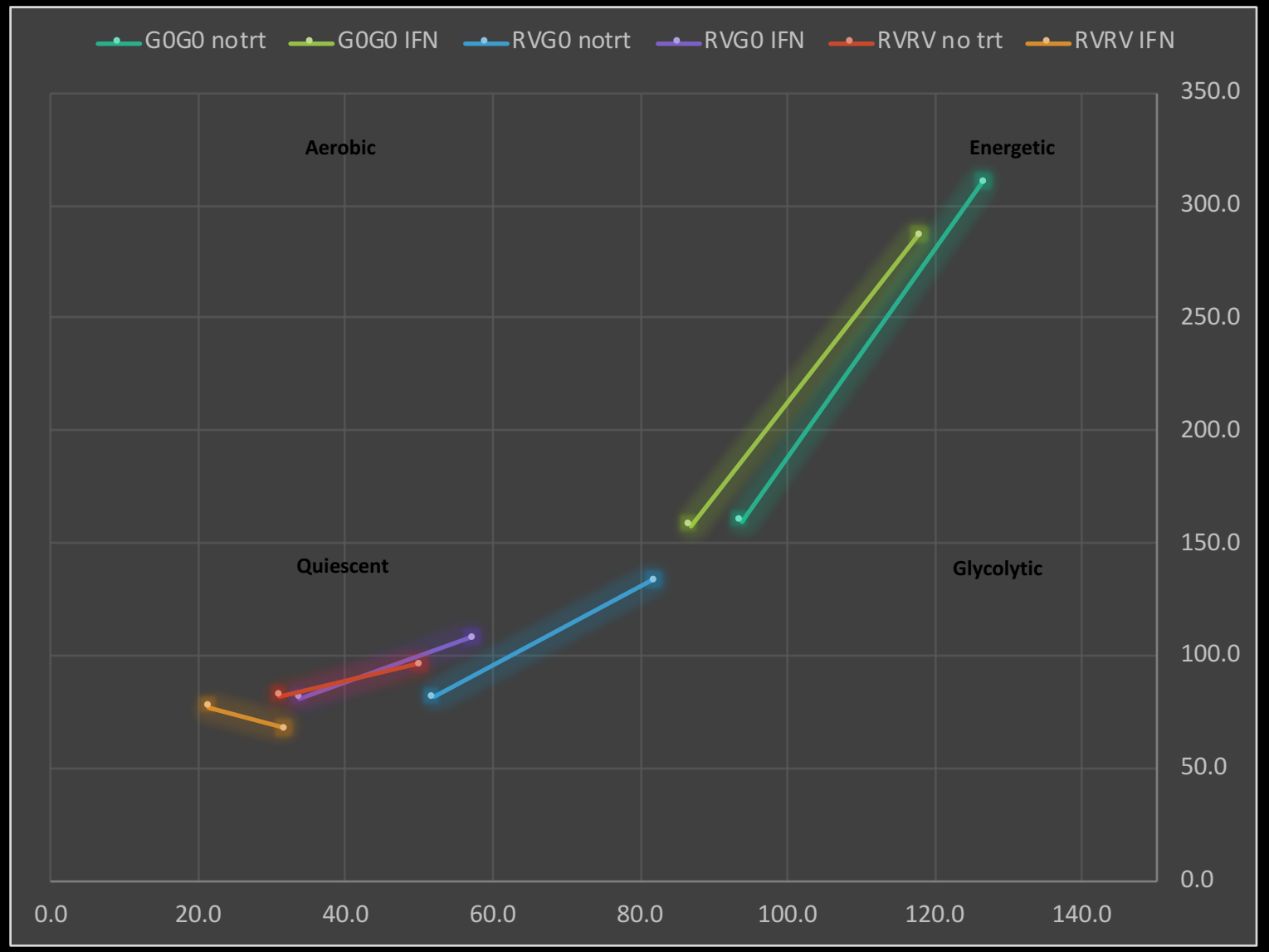
APOL1 Variant Carrying Macrophages Exhibit Impaired Mitochondrial Respiration



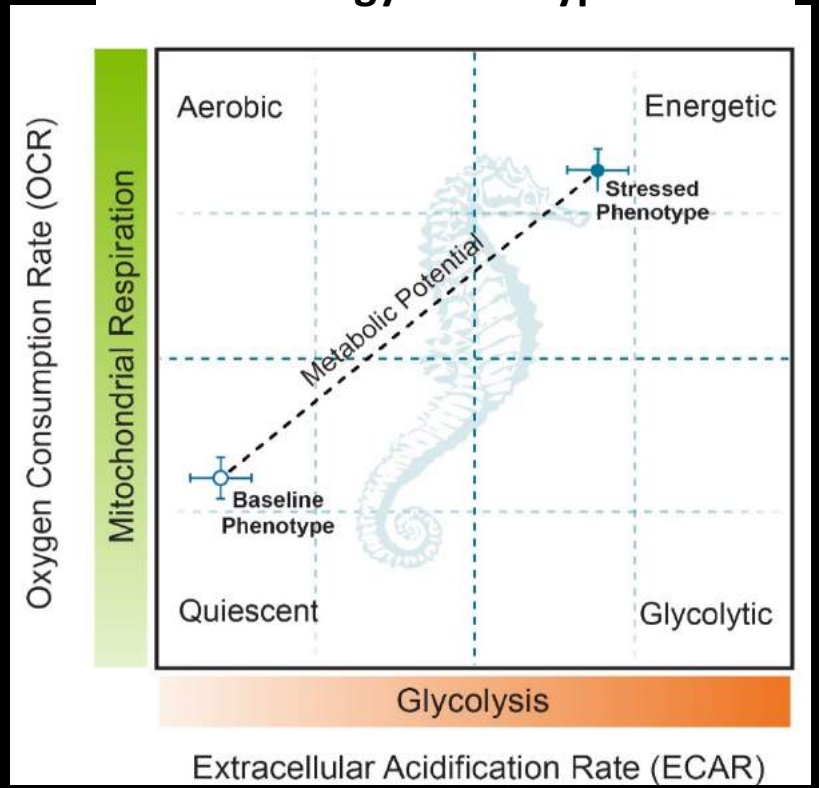




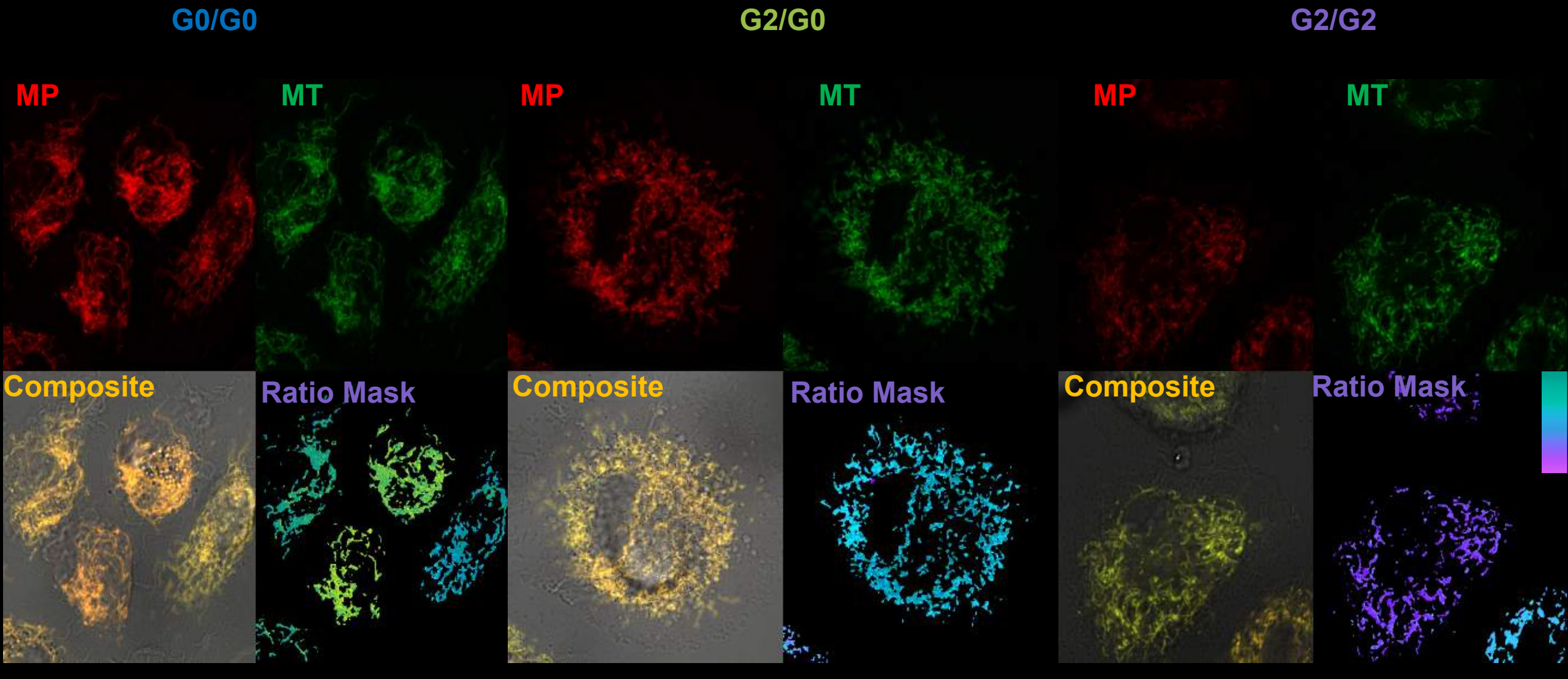
Variant-Carrying Macrophages Exhibit Less Metabolic Potential



XF Cell Energy Phenotype Profile



Confirmatory Immunoflourescence: Variant-Carrying Macrophages Exhibit Fewer Polarized Mitochondria as Measured by MitoProbe to MitoTracker Ratios



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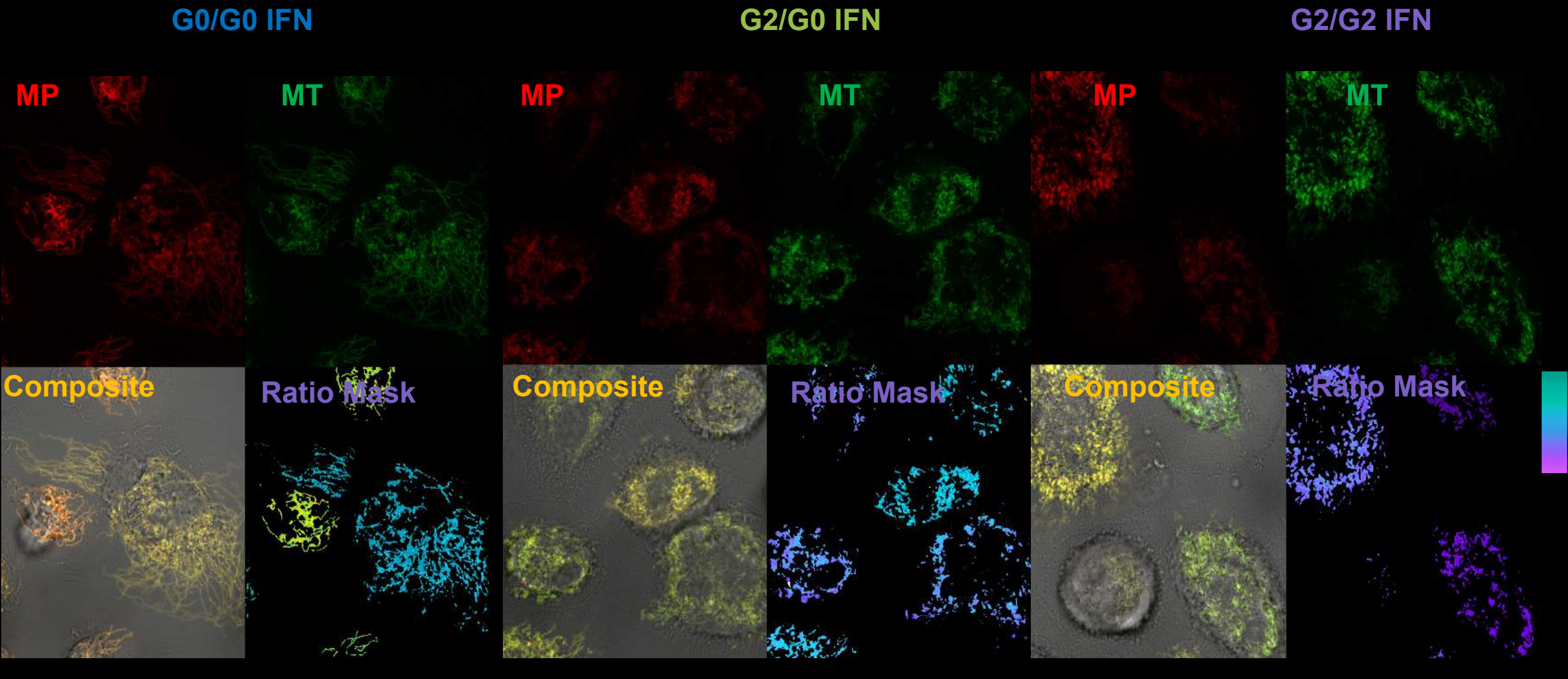

Translational implications

- Disparities in Cardiovascular outcomes—particularly in autoimmune disease—may be in part due to a genetic propensity towards endothelial injury
- APOL1 variant alleles are common in African Americans and may prime carriers for tissue injury
- The influence of APOL1 on tissue injury appears to be out of proportion to inflammatory insult
- Wider APOL1 genetic allele testing may aid in risk stratification

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Rheumatology Research Foundation

Advancing Treatment | Finding Cures

Colton Center for Autoimmunity

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Innate and TH17 Serum Cytokine Levels by SLEDAI and APOL1 Genotype