Supplemental Materials

APOL1 Genetic Variants are Associated with Increased Risk of Coronary Atherosclerotic Plaque Rupture in African Americans

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Running title: *APOL1* Variants and Coronary Plaque Rupture.

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Supplemental Figures

Supplemental Figure I: Exemplary ROIs. Lumen areas are outlined in red, external elastic lamina areas are circled in yellow, and necrotic core areas are highlighted in green. **A-C**) Stable control human coronary arteries of carriers of the reference allele (**A**), 1 *APOL1* risk allele (**B**), and 2 *APOL1* risk alleles (**C**). **D-F**) Ruptured coronary arteries of carriers of the reference allele (**D**), 1 *APOL1* risk allele (**E**), and 2 *APOL1* risk alleles (**F**).

Supplemental Figure II: Immunofluorescent Staining for Apolipoprotein A1. Representative confocal microscopy images with staining against APOA1 (red) in stable (**A**) and in ruptured (**B**) human coronary artery plaques. APOA1-positive plaque area was similar regardless of *APOL1* genotype, both in severely narrowed stable plaques (**C**) and in ruptured plaques (**D**). **E**) APOA1-positive plaque area was positively correlated with APOL1-positive plaque area.

Supplemental Figure III: Assessment of Apoptosis. A+B) Representative confocal microscopy images with staining against cleaved caspase 3 (red), CD68 (green), and DAPI (blue) in stable **(A)** and in ruptured **(B)** human coronary plaques. The red boxed areas in the MOVAT-stained low-power image indicate the areas where confocal microscopy images were taken. Cleaved caspase 3-positivity co-localized primarily with CD68-positive cells. **C+D)** There were no differences in cleaved caspase 3-positive plaque areas among the three *APOL1* genotypes in stable **(C)** and in ruptured **(D)** plaques. Likewise, there were no differences in CD68-positivity, indicating macrophage infiltration. **E+F)** TUNEL staining in stable **(E)** and in ruptured **(F)** human coronary plaques. The microphotographs were obtained from the boxed areas in the MOVAT-stained low-power images, shown in **A+B)**. **G+H)** TUNEL-positive plaque areas were not significantly different in plaques from carriers of the reference allele versus carriers of one or two *APOL1* risk alleles.

Supplemental Tables

Supplemental Table I: Major Resources Table

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
CD68	Dako	M0814	1:200	20047711	https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/cd68-(concentrate)-76535
Apolipoprotein L1	Sigma	HPA018885	1:500	G117317	https://www.sigmaaldrich.com/catalog/product/sigma/hpa018885?lang=en&region=US
Apolipoprotein A1	Sigma	HPA046715	1:5000	A117438	https://www.sigmaaldrich.com/catalog/product/sigma/hpa046715?lang=en&region=US
Cleaved Caspase 3	Cell Signaling Technology	9664S	1:500	2	https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-mab/9664?site-search-type=Products&N=4294956287&Nt=+cleaved+caspase+3+%28d175%29&fromPage=plp

Supplemental Table II: Study Population Characteristics per *APOL1* Risk Alleles

	Total Study Cohort, n=764	Reference Allele, n=312	1 *APOL1* Risk Allele, n=347	2 *APOL1* Risk Alleles, n=105	p-value* (un-adjusted)
Demographics					
Age (mean ± SD)	47.18 ± 12.75	47.57 ± 13.44	46.58 ± 12.62	48.02 ± 10.96	0.4662
Male Sex	524 (68.6%)	206 (66.0%)	239 (68.9%)	79 (75.2%)	0.2102
BMI, kg/m² (mean ± SD)	30.03 ± 8.36	30.51 ± 8.68	29.88 ± 8.11	29.07 ± 8.15	0.2825
Risk factors					
Hypertension	488 (63.9%)	193 (61.9%)	225 (64.8%)	70 (66.7%)	0.5931
Hyperlipidemia	120 (15.7%)	44 (14.1%)	58 (16.7%)	18 (17.1%)	0.5956
Diabetes Mellitus	117 (15.3%)	47 (15.1%)	50 (14.4%)	20 (19.0%)	0.5059
Smoking	65 (8.5%)	24 (7.7%)	33 (9.5%)	8 (7.6%)	0.6633
Kidney disease	60 (7.9%)	19 (6.1%)	29 (8.4%)	12 (11.4%)	0.1904
Dialysis	34 (4.5%)	8 (2.6%)	18 (5.2%)	8 (7.6%)	0.0629
History of drug abuse	120 (15.7%)	53 (17.0%)	53 (15.3%)	14 (13.3%)	0.6433
Serum Total Cholesterol (mg/dL)	201.91 ± 72.63 (n=179)	198.24 ± 61.65 (n=74)	207.93 ± 87.71 (n=81)	192.92 ± 41.74 (n=24)	0.5760
Serum HDL Cholesterol (mg/dL)	42.56 ± 1.92 (n=140)	44.76 ± 3.05 (n=60)	42.24 ± 2.85 (n=63)	36.00 ± 4.59 (n=17)	0.2253
Serum APOA1 (mg/dL)	138.1 ± 7.48 (n=48)	141.1 ± 14.62 (n=16)	140.2 ± 9.75 (n=27)	116.7 ± 16.74 (n=5)	0.6302
Serum Creatinine (mg/dL)	2.58 ± 1.88 (n=333)	2.60 ± 2.17 (n=136)	2.54 ± 1.74 (n=153)	2.62 ± 1.29 (n=44)	0.9478
Cause of Death					
Sudden Cardiac Death	590 (77.2%)	231 (74.0%)	272 (78.4%)	87 (82.9%)	0.1380
Coronary Death	293 (38.4%)	111 (35.6%)	138 (39.8%)	44 (41.9%)	0.3923
Intracoronary Thrombosis	185 (24.2%)	62 (19.9%)	96 (27.7%)	27 (25.7%)	0.0612
Rupture	*124 (16.2%)*	*39 (12.5%)*	*61 (17.6%)*	*24 (22.9%)*	0.0295
Erosion	*57 (7.5%)*	*22 (7.1%)*	*32 (9.2%)*	*3 (2.9%)*	0.0881
Calcified Nodules	*4 (0.5%)*	*1 (0.3%)*	*3 (0.9%)*	*0 (0.0%)*	NA
Non-thrombotic CAD	106 (13.9%)	47 (15.1%)	42 (12.1%)	17 (16.2%)	0.4166
Coronary Dissection	2 (0.3%)	2 (0.6%)	0 (0.0%)	0 (0.0%)	NA
Cardiac but Non-Coronary Death	297 (38.9%)	120 (38.5%)	134 (38.6%)	43 (41.0%)	0.8945
Sudden Non-Cardiac Death	174 (22.8%)	81 (26.0%)	75 (21.6%)	18 (17.1%)	0.1380
Degree of Atherosclerosis					
None (0 - 24% cross-sectional stenosis)	271 (35.5%)	110 (35.3%)	124 (35.7%)	37 (35.2%)	0.9904
Mild (25 – 50% cross-sectional stenosis)	74 (9.7%)	33 (10.5%)	30 (8.7%)	11 (10.5%)	0.6745
Moderate (50 – 74% cross-sectional stenosis)	122 (16.0%)	51 (16.3%)	58 (16.7%)	13 (12.4%)	0.5531
Severe (≥75% cross-sectional stenosis)	279 (38.9%)	118 (37.8%)	135 (38.9%)	44 (41.9%)	0.7589
Mean %cross-sectional luminal narrowing					
LAD (%)	39.81 ± 36.65	39.71 ± 36.55	40.01 ± 36.68	39.43 ± 37.19	0.9880

LCA (%)	30.24 ± 35.99	30.05 ± 35.39	31.01 ± 36.61	28.24 ± 35.90	0.7825
RCA (%)	34.87 ± 37.06	32.80 ± 36.12	36.23 ± 37.83	36.52 ± 37.32	0.4405
Number of vessels with any atherosclerosis					
0	278 (36.4%)	112 (35.9%)	127 (36.6%)	39 (37.1%)	0.9680
1	85 (11.1%)	38 (12.2%)	35 (10.1%)	12 (11.4%)	0.6910
2	78 (10.2%)	30 (9.6%)	37 (10.7%)	11 (10.5%)	0.9021
3	321 (42.0%)	131 (42.0%)	147 (42.4%)	43 (41.0%)	0.9675
NA	2 (0.3%)	1 (0.3%)	1 (0.3%)	0 (0.0%)	NA
Number of vessels with cross-sectional luminal narrowing ≥75% in histology					
0	467 (61.1%)	194 (62.2%)	212 (61.1%)	61 (58.1%)	0.7589
1	107 (14.0%)	48 (15.4%)	44 (12.7%)	15 (14.3%)	0.6048
2	114 (14.9%)	45 (14.4%)	54 (15.6%)	15 (14.3%)	0.9019
3	76 (9.9%)	25 (8.0%)	37 (10.7%)	14 (13.3%)	0.2410

* p values are calculated by comparison between carriers of the reference allele, carriers of 1 *APOL1* risk allele, and carriers of 2 *APOL1* risk alleles. CAD: coronary artery disease; LAD: left anterior descending coronary artery; LCA: left circumflex coronary artery; RCA: right coronary artery; LV: left ventricle; RV: right ventricle; MI: myocardial infarction; NA: not available.

Supplemental Table III: Kidney Pathology

	Reference Allele n=45	**2 *APOL1* Risk Alleles n=46**	**p-value**
Cortical Area (mm^2)	198.5 ± 73.86	183.8 ± 57.51	0.4541
Mean Glomerular Area (20 glomerula)	31,143 ± 9,659	30,791 ± 6,934	0.8429
Glomeruli	24.55 ± 4.99	23.40 ± 4.52	0.7472
Glomerular Density	2.17 ± 0.55	2.36 ± 0.49	0.0862
Sclerotic Glomeruli, n	45.11 ± 89.33	58.91 ± 95.02	0.0566
%Sclerotic Glomeruli	10.79 ± 20.59	15.68 ± 25.22	0.0491
Non-sclerotic Glomeruli, n	373.3 ± 196.8	361.5 ± 170.2	0.9314
%Interstitial Fibrosis and Tubular Atrophy	21.16 ± 22.47	25.76 ± 30.50	0.9050
Microcystic Dilation, n(%)	3 (6.67%)	9 (19.57%)	0.0690
Intimal Thickness LA mcm	55.14 ± 34.38	64.50 ± 50.93	0.6144

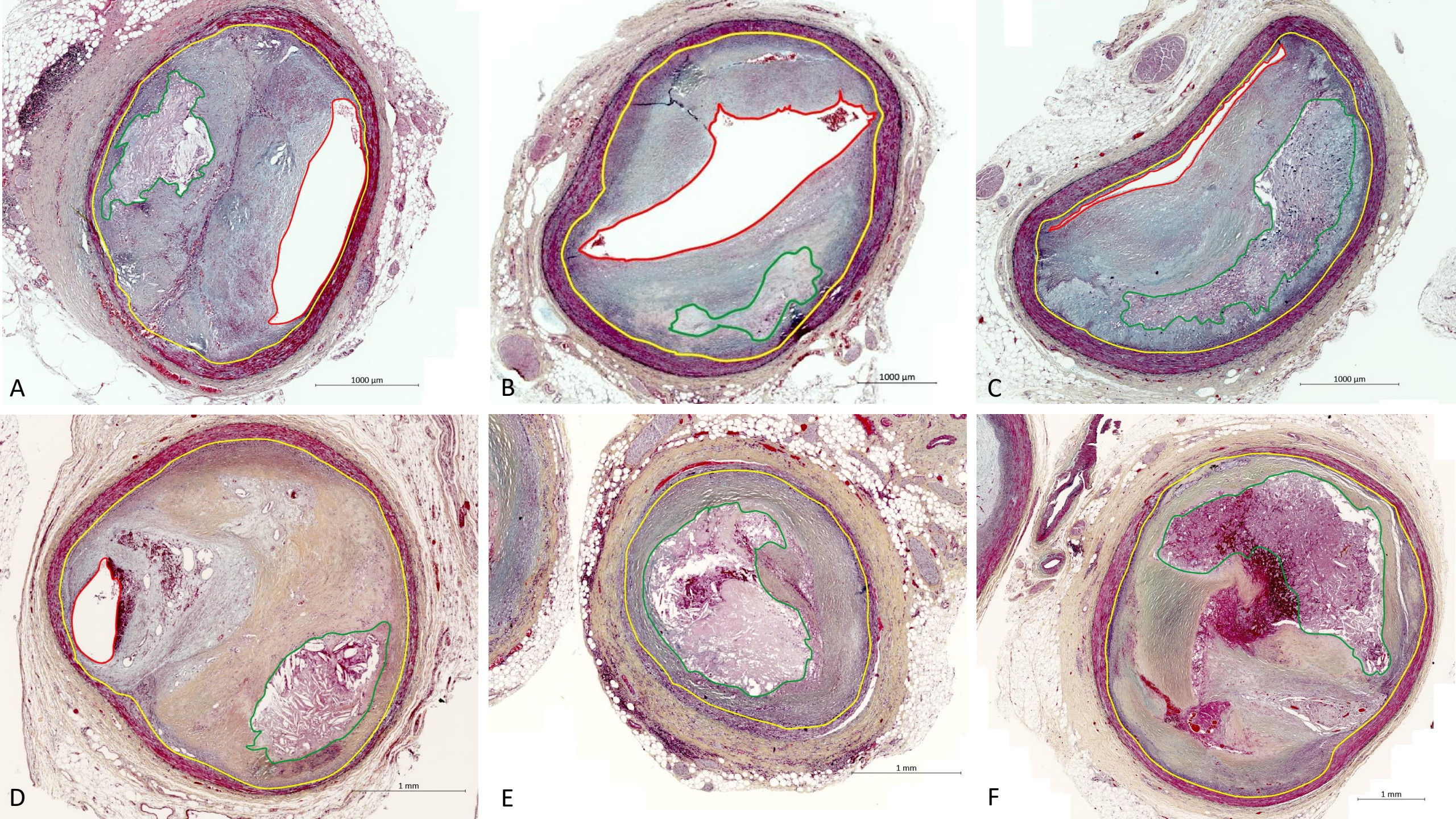
Supplemental Table IV: Causes of Death in Dialysis and Non-Dialysis Patients

	Total Study Cohort, n=764	Dialysis Patients, n=34	Non-Dialysis Patients, n=730	p-value (unadjusted)
Sudden Cardiac Death	590 (77.2%)	29 (85.3%)	561 (76.8%)	0.2511
Coronary Death	293 (38.4%)	12 (35.3%)	281 (38.5%)	0.7077
Intracoronary Thrombosis	185 (24.2%)	9 (26.5%)	176 (24.1%)	0.7534
Rupture	*124 (16.2%)*	*4 (11.8%)*	*120 (16.4%)*	*0.4700*
Erosion	*57 (7.5%)*	*3 (8.8%)*	*54 (7.4%)*	*0.7570*
Calcified Nodules	*4 (0.5%)*	*2 (5.9%)*	*2 (0.3%)*	*<0.0001*
Non-thrombotic CAD	106 (13.9%)	3 (8.8%)	103 (14.1%)	0.3834
Coronary Dissection	2 (0.3%)	0 (0.0%)	2 (0.3%)	NA
Cardiac but Non-Coronary Death	297 (38.9%)	17 (50.0%)	280 (38.4%)	0.1734
Sudden Non-Cardiac Death	174 (22.8%)	5 (14.7%)	169 (23.2%)	0.2511

Supplemental Table V: eQTL SNP rs136164 Genotypes

	Total Study Cohort, n=375	Homozygous Reference Allele, n=78	Heterozygous, n=190	Homozygous Alternative Allele, n=107	p-value (un-adjusted)
Demographics					
Age (mean ± SD)	46.82 ± 12.42	44.86 ± 10.75	47.55 ± 12.96	46.95 ± 12.54	0.3353
Male Sex	263 (70.1%)	54 (69.2%)	147 (77.4%)	62 (57.9%)	0.0021
BMI, kg/m² (mean ± SD)	30.46 ± 8.39	30.42 ± 7.44	29.98 ± 7.85	31.34 ± 9.84	0.6197
Risk factors					
Hypertension	254 (67.7%)	56 (71.8%)	126 (66.3%)	72 (67.3%)	0.6794
Hyperlipidemia	64 (17.1%)	14 (17.9%)	36 (18.9%)	14 (13.1%)	0.4239
Diabetes Mellitus	64 (17.1%)	9 (11.5%)	37 (19.5%)	18 (16.8%)	0.2914
Smoking	42 (11.2%)	6 (7.7%)	21 (11.1%)	15 (14.0%)	0.4017
Kidney disease	33 (8.8%)	7 (9.0%)	15 (7.9%)	11 (10.3%)	0.7830
Dialysis	18 (4.8%)	3 (3.8%)	6 (3.2%)	9 (8.4%)	0.1147
History of drug abuse	49 (13.1%)	14 (17.9%)	24 (12.6%)	11 (10.3%)	0.3013
Serum Cholesterol (mg/dL)	206.28 ± 77.05	208.75 ± 50.63 (n=12)	203.55 ± 80.90 (n=53)	212.05 ± 82.40 (n=20)	0.6143
Serum Creatinine (mg/dL)	2.59 ± 2.10	2.48 ± 1.26 (n=39)	2.12 ± 1.00 (n=78)	3.45 ± 3.36 (n=48)	0.0415
Cause of Death					
Sudden Cardiac Death	350 (93.3%)	75 (96.2%)	177 (93.2%)	98 (91.6%)	0.4653
Coronary Death	202 (53.9%)	44 (56.4%)	104 (54.7%)	54 (50.5%)	0.6844
Intracoronary Thrombosis	131 (34.9%)	30 (38.5%)	69 (36.3%)	32 (29.9%)	0.4114
Rupture	*85 (22.7%)*	*17 (21.8%)*	*48 (25.3%)*	*20 (18.7%)*	0.4212
Erosion	*42 (11.2%)*	*13 (16.7%)*	*18 (9.5%)*	*11 (10.3%)*	0.2227
Calcified Nodules	*4 (1.1%)*	*0 (0.0%)*	*3 (1.6%)*	*1 (0.9%)*	NA
Non-thrombotic CAD	70 (18.7%)	14 (17.9%)	34 (17.9%)	22 (20.6%)	0.8378
Coronary Dissection	1 (0.3%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	NA
Cardiac but Non-Coronary Death	148 (39.5%)	31 (39.7%)	73 (38.4%)	44 (41.1%)	0.8994
Sudden Non-Cardiac Death	25 (6.7%)	3 (3.8%)	13 (6.8%)	9 (8.4%)	0.4653
Degree of Atherosclerosis					
None (0 - 24% cross-sectional stenosis)	95 (25.3%)	23 (29.5%)	47 (24.7%)	25 (23.4%)	0.6168
Mild (25 – 50% cross-sectional stenosis)	33 (8.8%)	5 (6.4%)	20 (10.6%)	8 (7.5%)	0.4738
Moderate (50 – 74% cross-sectional stenosis)	47 (12.5%)	6 (7.7%)	19 (10.0%)	22 (20.6%)	0.0107
Severe (≥75% cross-sectional stenosis)	200 (53.5%)	44 (56.4%)	104 (54.7%)	52 (48.6%)	0.4938
Mean %cross-sectional luminal narrowing					
LAD (%)	49.16 ± 36.53	50.19 ± 37.83	49.05 ± 37.33	48.60 ± 12.55	0.8109
LCA (%)	39.57 ± 38.02	38.72 ± 37.94	39.34 ± 39.14	40.59 ± 36.35	0.9685
RCA (%)	43.31 ± 38.01	43.27 ± 39.03	44.03 ± 38.22	42.06 ± 37.19	0.2773
Number of vessels with any atherosclerosis					

0	102 (27.2%)	23 (29.5%)	53 (27.9%)	26 (24.3%)	0.7022
1	37 (9.9%)	6 (7.7%)	20 (10.5%)	11 (10.3%)	0.7679
2	39 (10.4%)	10 (12.8%)	19 (10.0%)	10 (9.3%)	0.7223
3	197 (52.5%)	39 (50.0%)	98 (51.6%)	60 (56.1%)	0.6675
Number of vessels with cross-sectional luminal narrowing ≥75% in histology					
0	175 (46.7%)	34 (43.6%)	86 (45.3%)	55 (51.4%)	0.4939
1	67 (17.9%)	14 (17.9%)	33 (17.4%)	20 (18.7%)	0.9598
2	81 (21.6%)	19 (24.4%)	40 (21.1%)	22 (20.6%)	0.7975
3	52 (13.9%)	11 (14.1%)	31 (16.3%)	10 (9.3%)	0.2480
***APOL1* risk alleles**					
0	138 (36.8%)	33 (42.3%)	70 (36.8%)	35 (32.7%)	0.4092
1	181 (48.3%)	36 (46.2%)	92 (48.4%)	53 (49.5%)	0.9004
2	56 (14.9%)	9 (11.5%)	28 (14.7%)	19 (17.8%)	0.5003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1000 µm
B
1000 µm
C
1000 µm
D
1 mm
E
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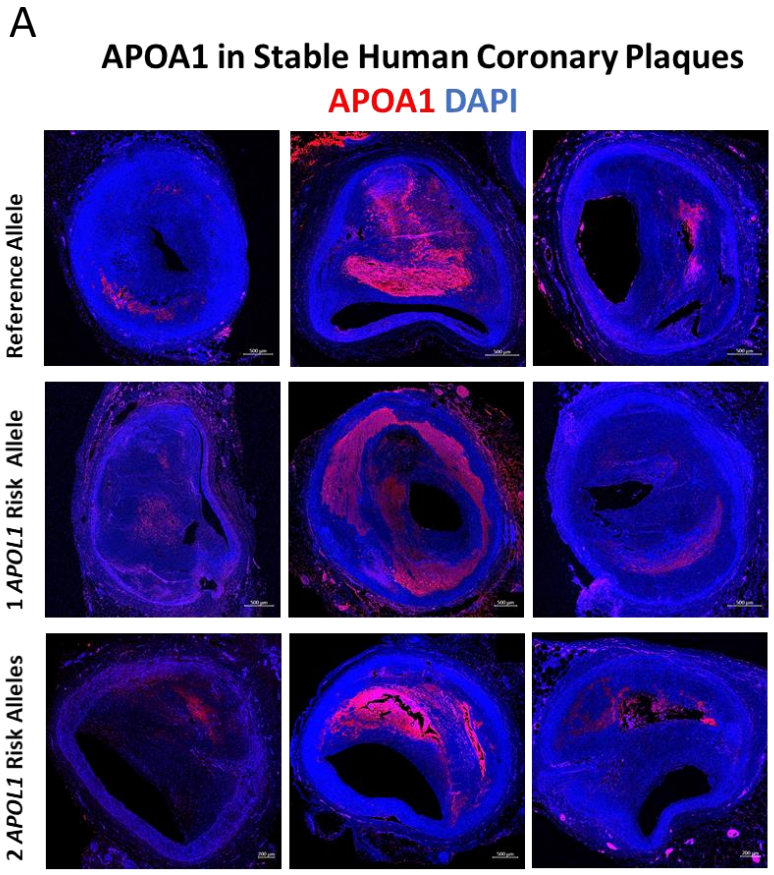
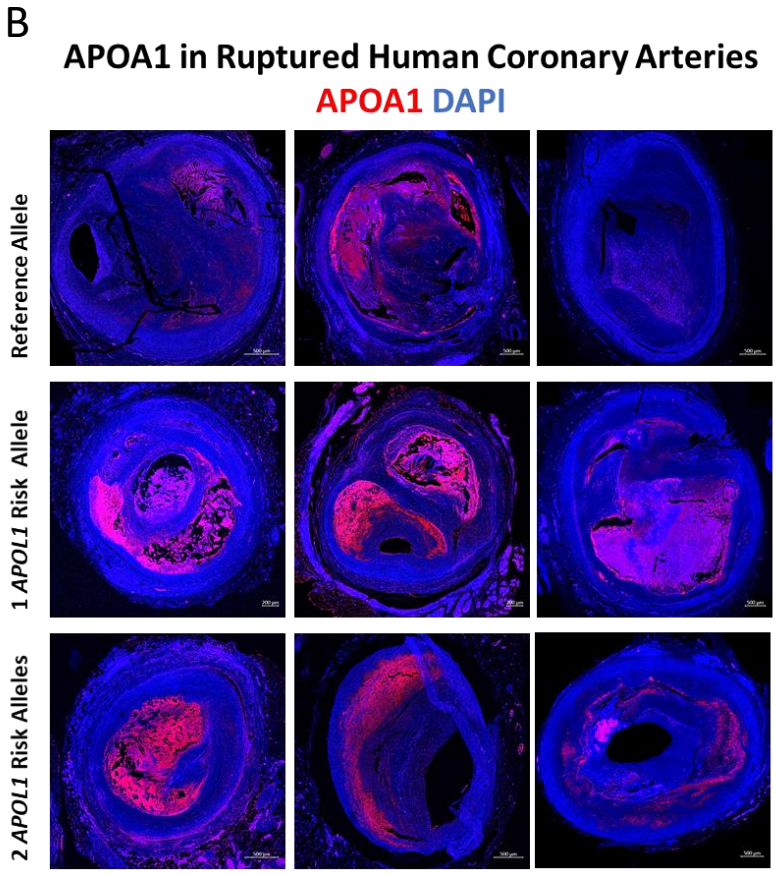
F
1 mm

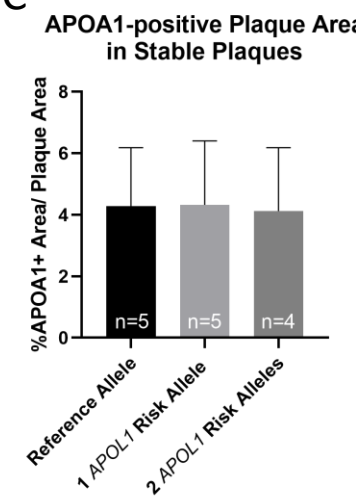
A


APOA1 in Stable Human Coronary Plaques
APOA1 DAPI
Reference Allele
1 APOL1 Risk Allele
2 APOL1 Risk Alleles

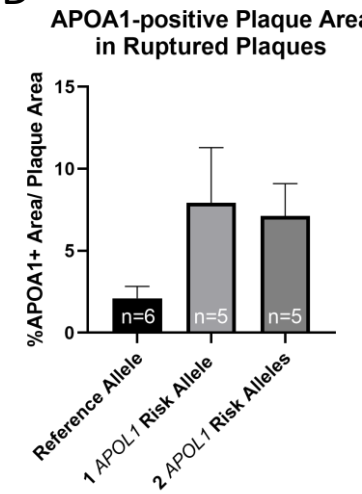
B


APOA1 in Ruptured Human Coronary Arteries
APOA1 DAPI
Reference Allele
1 APOL1 Risk Allele
2 APOL1 Risk Alleles

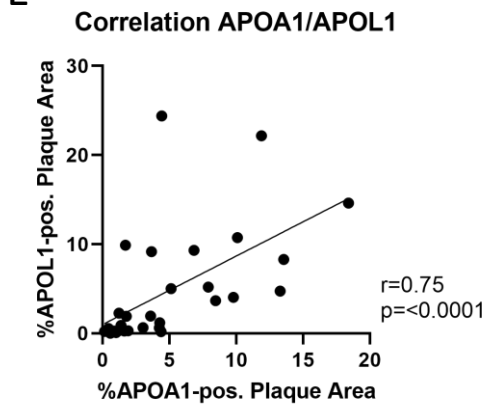
C


APOA1-positive Plaque Area in Stable Plaques
%APOA1+ Area/ Plaque Area
0
2
4
6
8
n=5
n=5
n=4
Reference Allele
1 APOL1 Risk Allele
2 APOL1 Risk Alleles

D

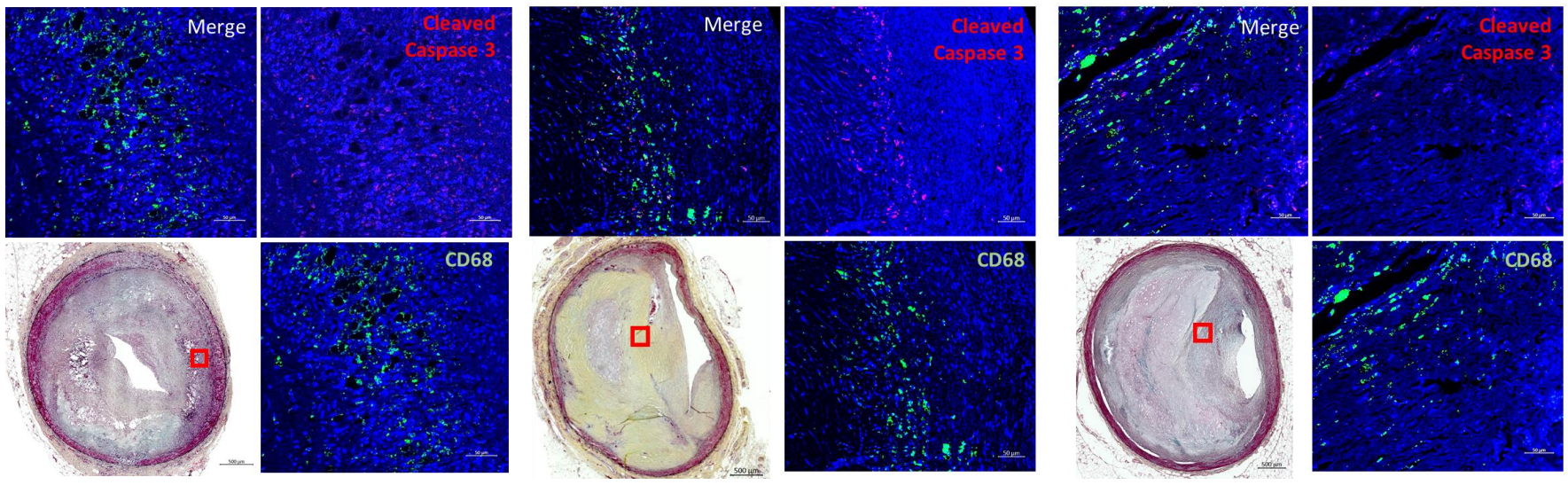

APOA1-positive Plaque Area in Ruptured Plaques
%APOA1+ Area/ Plaque Area
0
5
10
15
n=6
n=5
n=5
Reference Allele
1 APOL1 Risk Allele
2 APOL1 Risk Alleles

E


Correlation APOA1/APOL1
%APOL1-pos. Plaque Area
0
10
20
30
0
5
10
15
20
%APOA1-pos. Plaque Area
r=0.75
p=<0.0001

A

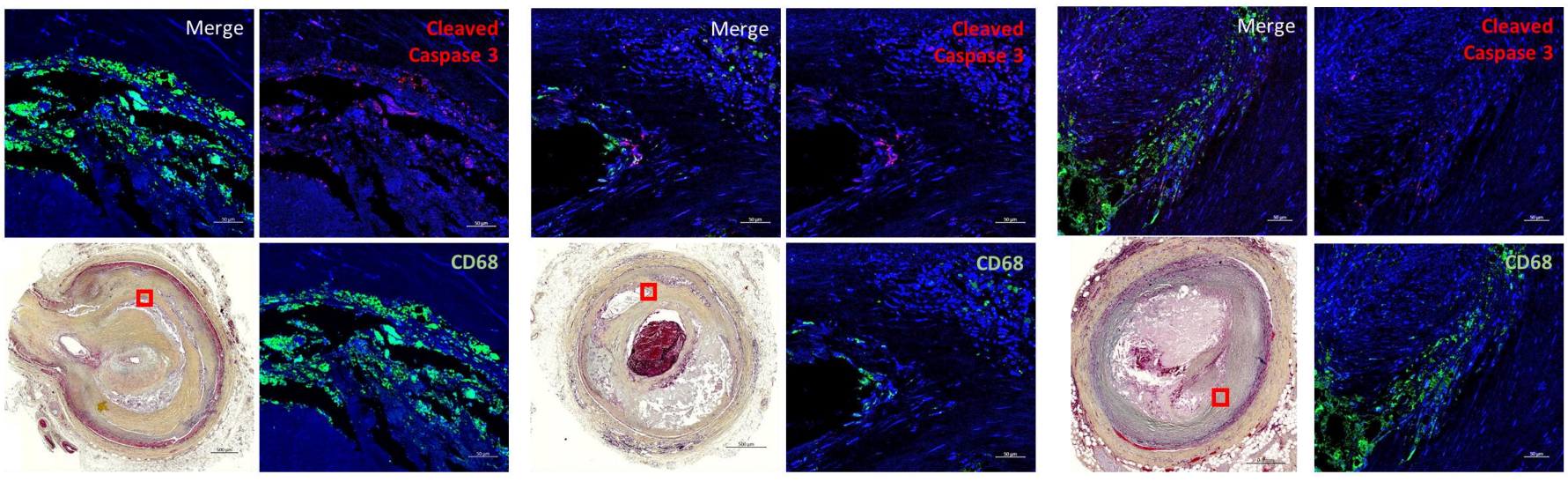
Human Coronary Stable Plaques



Reference Allele | 1 *APOL1* Risk Allele | 2 *APOL1* Risk Alleles

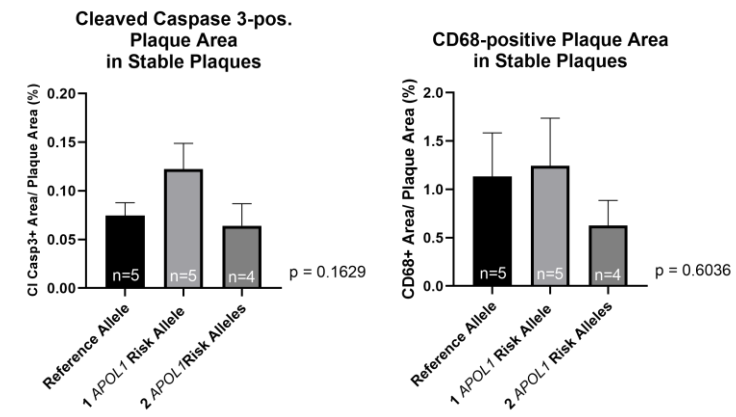
B

Human Coronary Ruptured Plaques

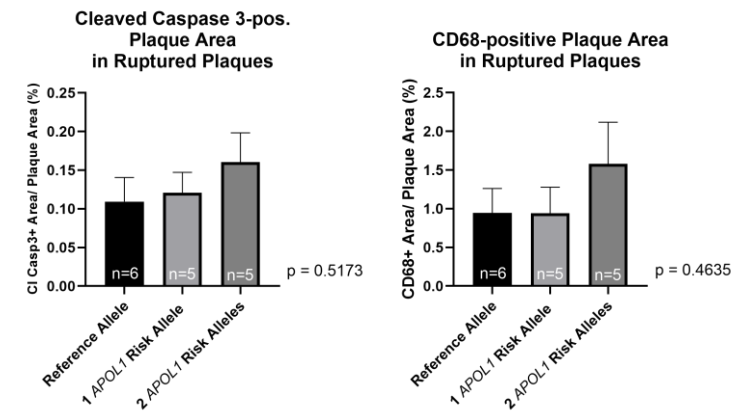


Reference Allele | 1 *APOL1* Risk Allele | 2 *APOL1* Risk Alleles

C

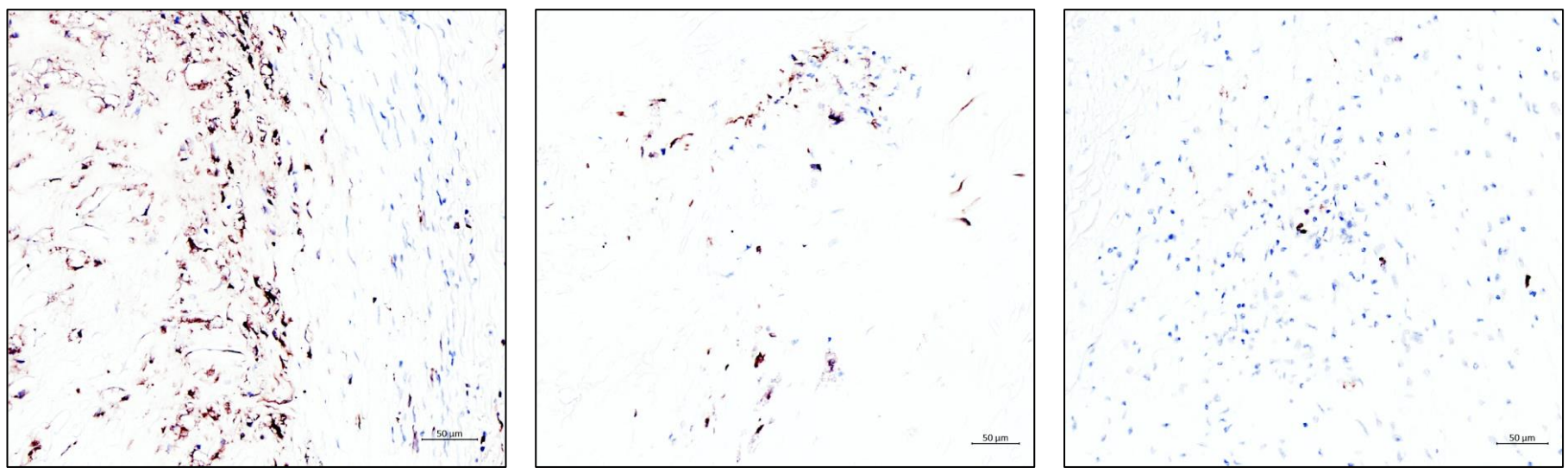


D



E

Human Coronary Stable Plaques



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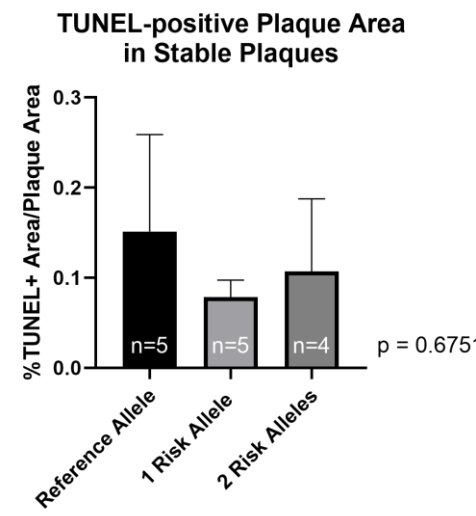
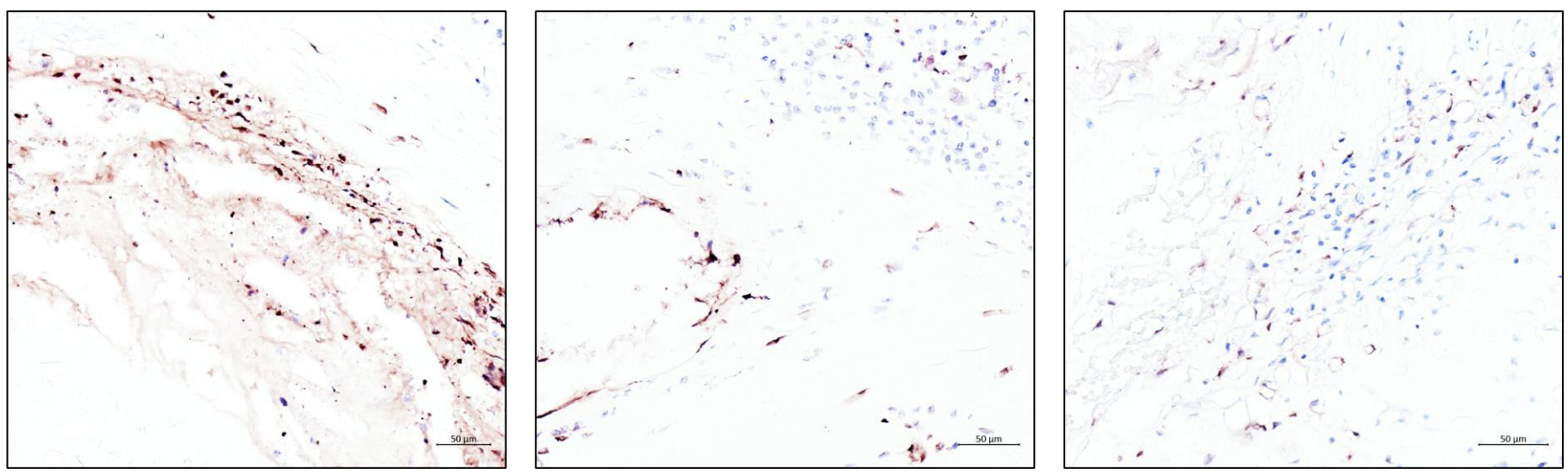


F

Human Coronary Ruptured Plaques



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