SUPPLEMENTAL MATERIAL

Validation of modified aptamer-based measurements of APOM

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technology was used to discover the SOMAmer that can specifically bind APOM. The Dissociation Constant (Kd) value between SOMAmer (seq ID 10445-20) and its target protein, APOM, is 2.19 $x10^{-9}$ M. It was also confirmed that this SOMAmer could successfully pull down the recombinant APOM protein from buffer. Gel analysis of the buffer pulldown revealed 2 bands. The most intense band was the desired protein.

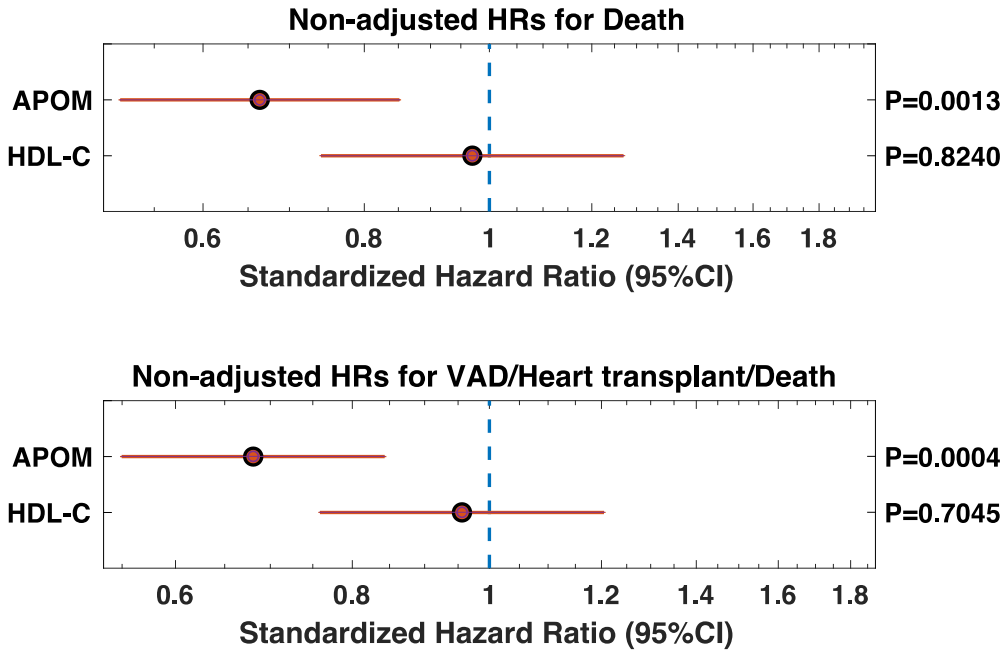
Emilsson V, et al.[29] applied the SomaScan® Assay by testing the serum samples from 5,457 Icelanders. The specificity validation of the SOMAmer reagent to the APOM protein was directly conducted using data dependent analysis (DDA) mass spectrometry. We have downloaded the peptides measured for the APOM SomaMER from the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) with the data identifier PXD008822:

EFPEVHLGQWYFI AGAAPTK
EEL ATFDPVDNIV FNMAAGSAPM QLHLR
M KDGLCVPR
KW IYHLTEGSTD LR
TEGRPDMK
TELFSSSCPG GIMLNETGQG YQR
FLLYNR
S PHPPEK
CVEEFK
SLTSCLDSK
AFLLTPR
NQEACELSNN
These peptides provide 78.2% coverage of human APOM. Blast search of these peptide sequences identifies one human protein, with an e-value of 2e-108. Based on these search output results, APOM is a very high confidence protein.

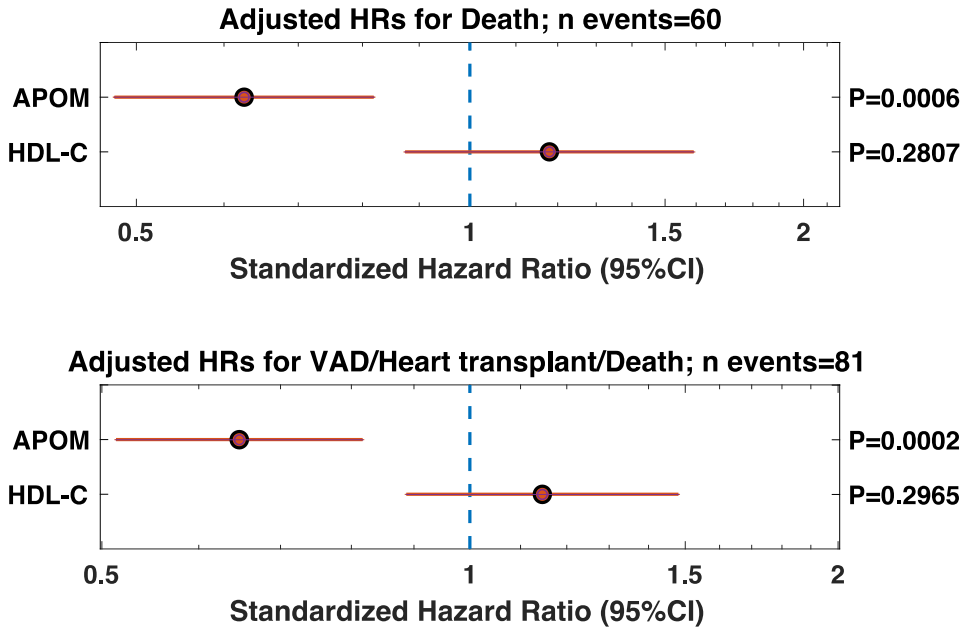
Furthermore, cis-acting protein SNPs were identified. Amongst them, SNP, rs115878542, was confirmed to correlate with APOM.In addition to this previous validation, we performed an analysis of the correlation between APOM measured by the SomaScan and APOM measured by ELISA in a subset of the PHFS (n=299). We found a linear relationship with a Pearson correlation coefficient of 0.73 (P<0.0001).

Online Figure 1. APOM, but not HDL-cholesterol, is associated with HF outcomes. Results of unadjusted analyses in non-adjusted analyses (A) and analyses in which APOM and HDL-C are adjusted for each other (B).

A.

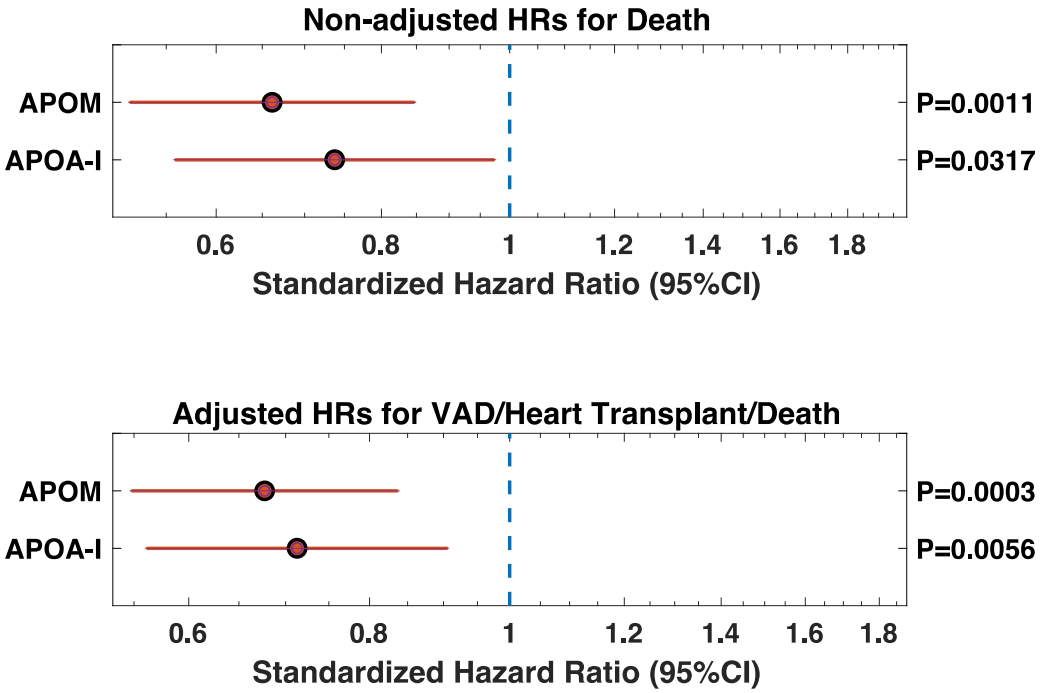


B.

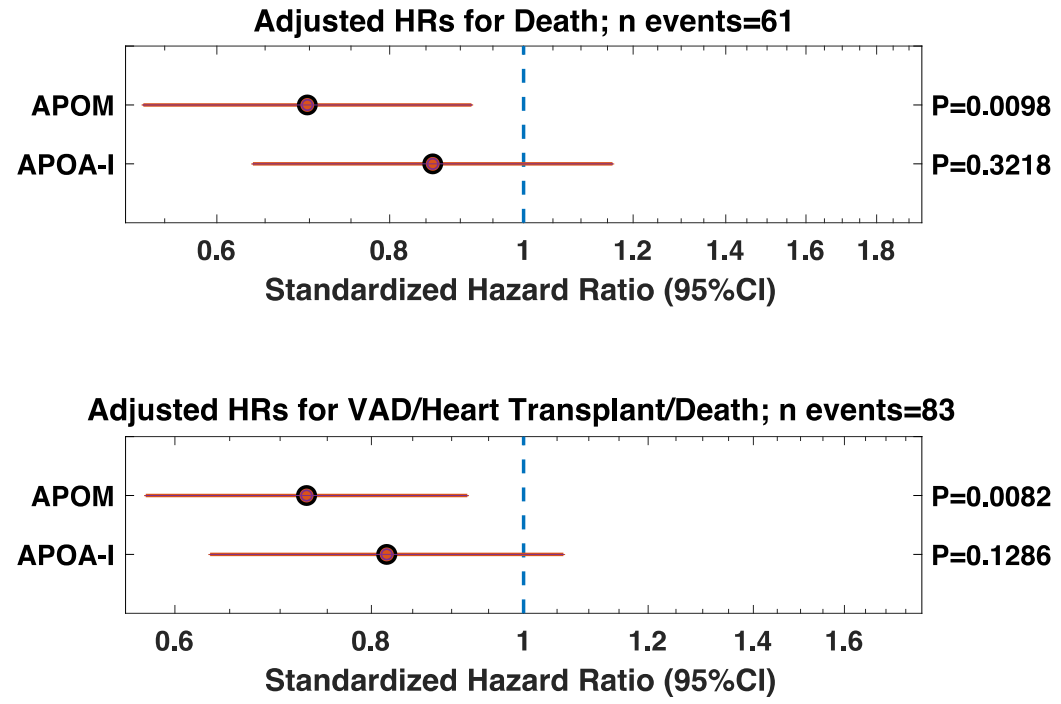


Online Figure 2. Associations between APOM measured by ELISA vs. APOA-I (measured by immunonephelometry) and various endpoints in non-adjusted analyses (A) and analyses adjusted for each other (B).

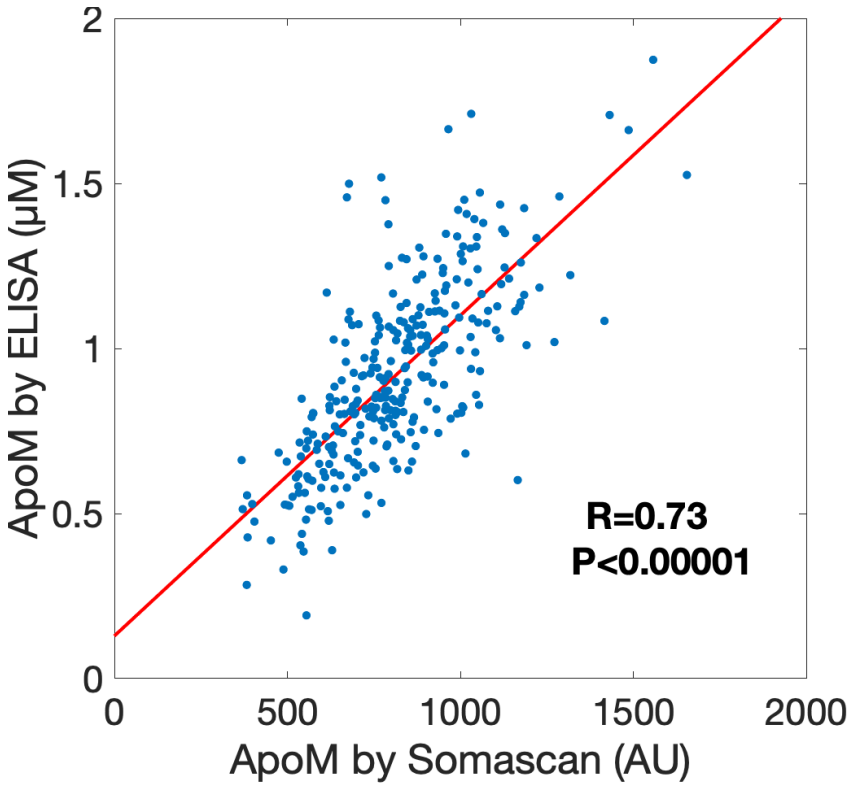
A.



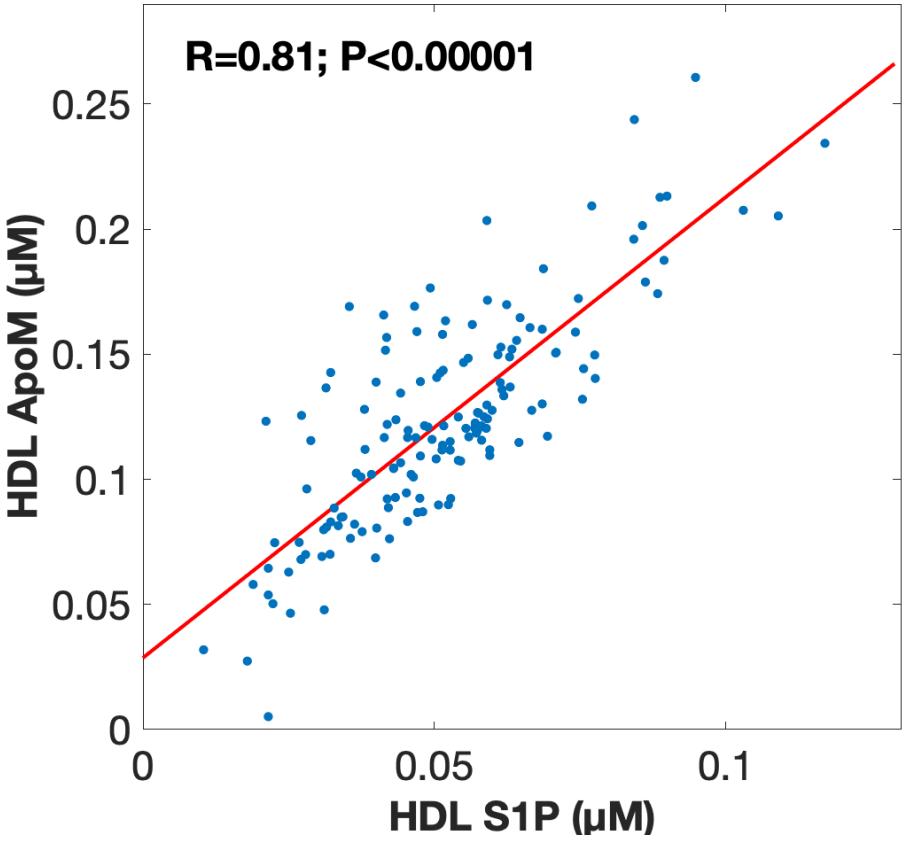
B.



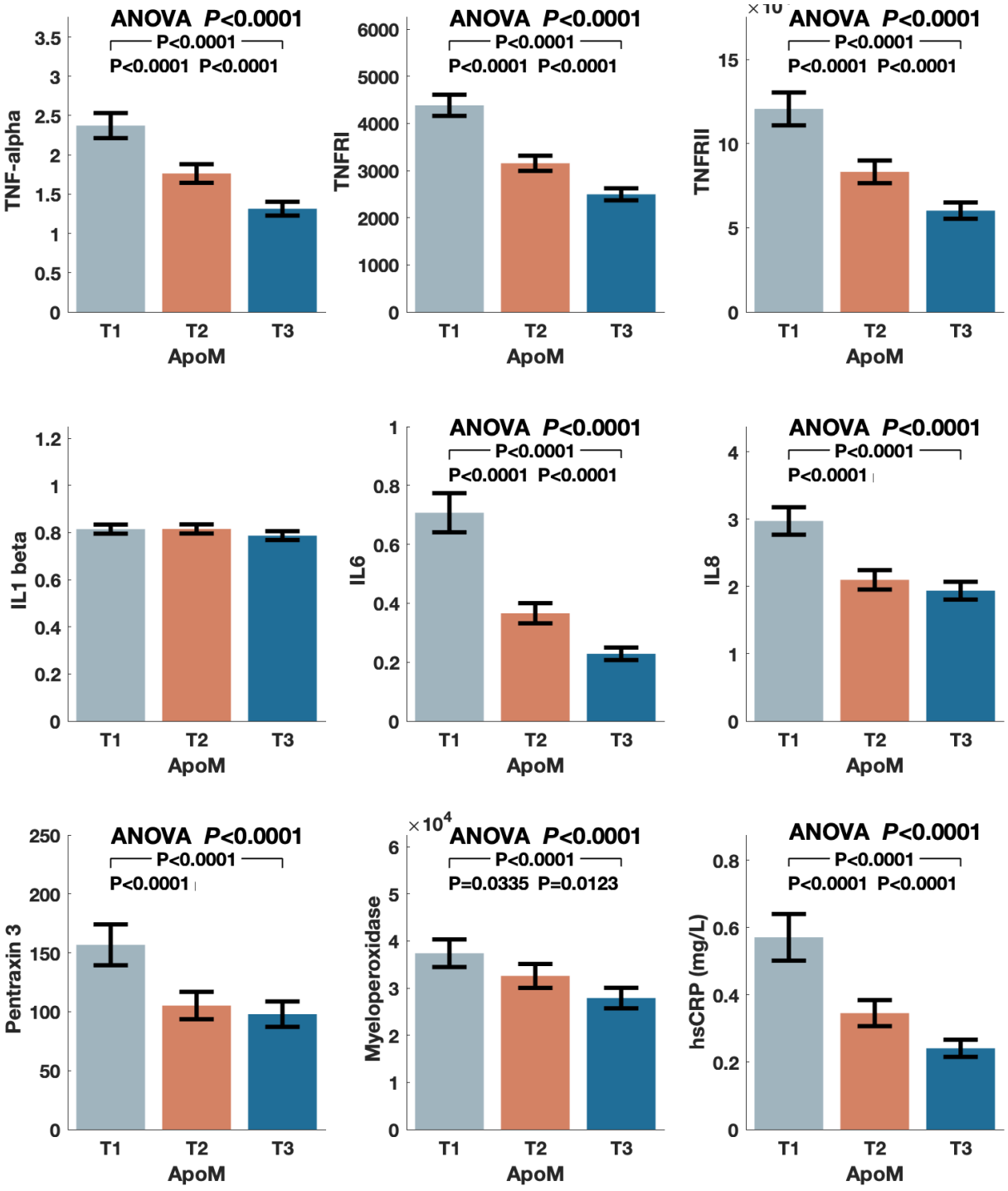
Online Figure 3.



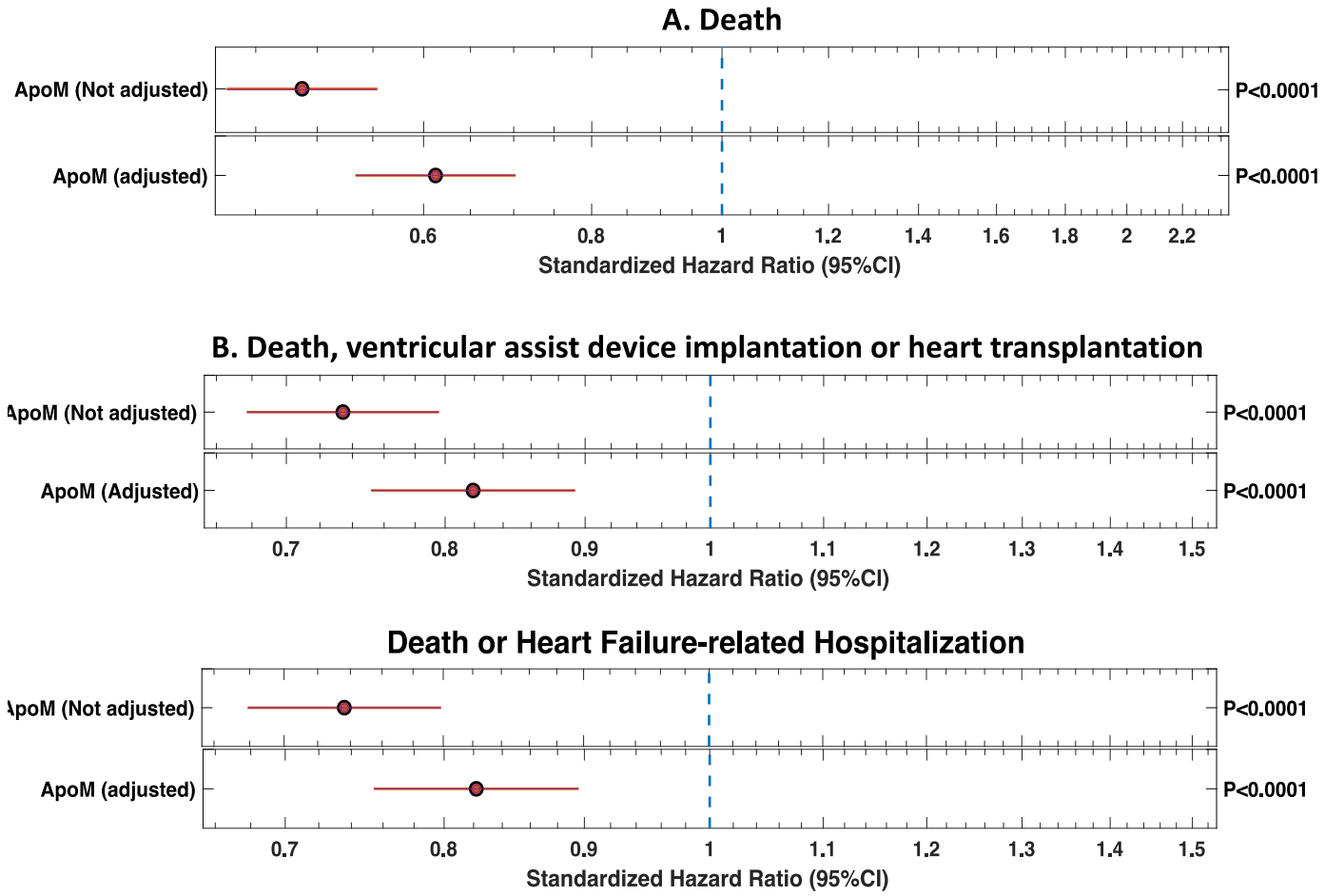
Online Figure 4. Correlation of HDL-associated APOM and S1P (PHFS participants). HDL was isolated from PHFS patients with subsequent ELISA for APOM and S1P determination by liquid chromatography–mass spectrometry



Online Figure 5. Comparison of inflammatory biomarkers measured with independent methods (Luminex assay) and high-sensitivity CRP (Abbott standard clinical assay), across tertiles of APOM, with T1 being the lowest tertile and T3 being the highest.



Online Figure 6. Attenuation of the relationship between APOM and death (A) and death, ventricular assist device implantation or heart transplantation (B). For each endpoint, Standardized hazard ratios for APOM in an unadjusted model and in a model adjusted for TNF-alpha, TNF-RI, TNF-RII, IL-1beta, IL-6, IL-8, pentraxin3, myeloperoxidase and high-sensitivity C-reactive protein are shown.



Online Table 1. General Characteristics of Penn Heart Failure Study Participants (*n*=2170)

General Characteristic	Mean±SD, median (IQR) or count (%)
Age (years)	57.8 (47.6,66.1)
Male sex	1435 (66.13%)
Race/Ethnicity	
Caucasian	1580 (75.82%)
African American	460 (22.07%)
Other	86 (4.4 %)
BMI, (kg/m^2)	28.8 (25.1,33.9)
Systolic BP (mmHg)	112 (100,128)
Diastolic BP (mmHg)	70 (62,78)
Ischemic etiology	662 (30.72%)
History of PCI	468 (21.57%)
History of CABG	392 (18.06%)
Current smoking	196 (9.03%)
Diabetes	622 (28.66%)
Atrial fibrillation or flutter	781 (35.99%)
History of pacemaker	137 (6.31%)
History of ICD	456 (21.01%)
History of Biventricular pacer	33 (1.52%)
Serum creatinine	1.1 (0.93,1.49)
LV EF (%)	30 (20,45)
EF Category	
Reduced EF	1705 (82.45%)
Recovered EF	203 (9.82%)
Preserved EF	160 (7.74%)
NYHA Class	
NYHA 1	374 (17.36%)
NYHA 2	968 (44.94%)
NYHA 3	685 (31.80%)
NYHA 4	127 (5.90%)
BNP (pg/mL)	163 (47,551)
Medication Use	
Beta Blocker	1923 (88.62%)
Aspirin	1234 (56.87%)
ACEI/ARBs	1856 (85.53%)
Hydralazine	183 (8.43%)
Organic Nitrates	343 (15.81%)
Digoxin	774 (35.67%)
Loop diuretic	1524 (70.23%)
MRA	739 (34.06%)
Statin	1127 (51.94%)

CCBs	200 (9.22%)
APOM (ELISA, μM), n=304	0.92±0.28
APOM (AU), n=2170	804 (665,962)

APOM=apolipoprotein M; ARB=angiotensin receptor blocker; ACE: angiotensin converting enzyme; CCB: calcium channel blocker; BNP = b-type natriuretic peptide; ICD=Implanted Cardioverter Defibrillator; LV EF= left ventricular ejection fraction; BMI=body mass index; NYHA=New York Heart Association; MRA=mineralocorticoid receptor antagonist.

Online Table 2. Association between APOM, measured by ELISA, and outcomes in PHFS.

Model	Standardized Hazard Ratio	*P* value
APOM measured by ELISA (*n*=297)		
All-cause death (*NE*=91)		
Non-adjusted	0.63 (0.51-0.76)	<0.0001
Adjusted for MAGGIC risk score	0.71 (0.56-0.90)	0.0044
Adjusted for MAGGIC risk score plus BNP	0.73 (0.57-0.93)	0.0107
Death / VAD / Heart Transplant (*NE*=126)		
Non-adjusted	0.67 (0.57-0.79)	<0.0001
Adjusted for MAGGIC risk score	0.77 (0.63-0.94)	0.0110
Adjusted for MAGGIC risk score plus BNP	0.79 (0.65-0.97)	0.0274

Online Table 3. Formal interaction analysis between APOM levels at baseline and ischemic vs. non-ischemic etiology as predictors of outcomes. The numbers shown are the P value for the interaction terms.

	Death, VAD or heart transplant	Death or HF-related hospitalization	Death
APOM by SomaScan	0.1064	0.1826	0.0820
APOM by ELISA	0.5606	0.5522	0.7292

Online Table 4. Relationship between APOM and outcomes, in analyses stratified according to ischemic vs. non-ischemic etiology.

Ischemic (n=688)	**HR**	**95%CI, LB**	**95%CI, UB**	***P* value**
Death, VAD or heart transplant	0.82	0.73	0.92	0.0006
Death or HF-related hospitalization	0.81	0.72	0.91	0.0003
Death	0.63	0.53	0.74	<0.0001
Non-ischemic (n=1542)				
Death, VAD or heart transplant	0.71	0.65	0.78	<0.0001
Death or HF-related hospitalization	0.72	0.66	0.79	<0.0001
Death	0.49	0.42	0.57	<0.0001

Online Table 5. General Characteristics of the Validation Cohort Study Participants in the Washington University Heart Failure Registry

General Characteristic	Mean±SD, median (IQR) or count (%)
Age (years)	53.1 (51 to 55.2)
Male sex	100 (57.8)
Race/Ethnicity	
Caucasian	127 (73.4)
African American	46 (26.6)
Other	---
BMI, (kg/m^2)	31 (29.8 to 32.2)
Systolic BP (mmHg)	117 (114 to 119)
Diastolic BP (mmHg)	71.6 (69.9 to 73.3)
Ischemic etiology	41 (23.7)
History of PCI	32 (18.50%)
History of CABG	19 (10.98%)
Current smoking	19 (10.98%)
Diabetes	39 (22.54%)
Atrial fibrillation or flutter	56 (32.37%)
History of pacemaker	13 (7.51%)
History of ICD	56 (32.37%)
History of Biventricular pacer	29 (16.76%)
Serum creatinine	1.15 (1.08 to 1.22)
LV EF (%)	39.9 (37.5 to 42.3)
EF Category	
Reduced EF	111 (64.16%)
Preserved EF	62 (35.84%)
NYHA Class	
NYHA 1	24 (13.9)
NYHA 2	96 (55.5)
NYHA 3	40 (23.1)
NYHA 4	13 (7.5)
Medication Use	
Beta Blocker	149 (86.13%)
Aspirin	112 (64.74%)
ACEI	115 (66.47%)
ARBs	33 (19.08%)
Hydralazine	31 (17.92%)
Organic Nitrates	48 (27.75%)

Digoxin	54 (31.21%)
Loop diuretic	137 (79.19%)
MRA	69 (39.88%)
Statin	95 (54.91%)
CCBs	25 (14.45%)

Values represent the mean ± standard deviation, median (interquartile range) or count (percentage) as appropriate.

ARB=angiotensin receptor blocker; ACE: angiotensin converting enzyme; CCB: calcium channel blocker; BNP = b-type natriuretic peptide; ICD=Implanted Cardioverter Defibrillator; LV EF= left ventricular ejection fraction; BMI=body mass index; NYHA=New York Heart Association; MRA=mineralocorticoid receptor antagonist.

Online Table 6. Hazard Ratios for death and death/heart failure admission per standard deviation increase in APOM in the 2 validation cohorts

	Washington University HF Registry (n=173)			
	All-Cause Death (*NE* = 21)		Death / LVAD / Heart Transplantation (*NE* = 29)	
Model	**Standardized HR (95%CI)**	***P* value**	**Standardized HR (95%CI)**	***P* value**
Non-adjusted	0.57 (0.41-0.80)	0.0011	0.60 (0.41-0.87)	0.0077
Adjusted for MAGGIC RS	0.59 (0.41-0.86)	0.0066	0.63 (0.43-0.94)	0.024
Adjusted for MAGGIC RS and NT-ProBNP	0.58 (0.40-0.84)	0.0042	0.64 (0.43-0.93)	0.0213
	TOPCAT (n=218)			
	All-Cause Death (*NE* = 48)		Death / HF Admission (*NE* = 77)	
Model	**Standardized HR (95%CI)**	***P* value**	**Standardized HR (95%CI)**	***P* value**
Non-adjusted	0.76 (0.58-0.99)	0.0419	0.65 (0.51-0.82)	0.0002
Adjusted for MAGGIC RS	0.75 (0.58-0.98)	0.0368	0.64 (0.51-0.81)	0.0002
Adjusted for MAGGIC RS and NT-ProBNP	0.74 (0.57-0.97)	0.0312	0.63 (0.50-0.80)	0.0001

***n*=total number of participants included in the analysis.**

***NE*=number of events**

Online Table 7. General Characteristics of Participants in the TOPCAT trial

Included in this study (*n*=218).

General Characteristic	Mean±SD, median (IQR) or count (%)
Age (years)	*71.2 (69.9 to 72.5)*
Male sex	122 (55.96%)
Race/Ethnicity	
Caucasian	188 (86.24%)
African American	26 (11.93%)
Asian	1 (0.46%)
Other	3 (1.38%)
BMI, (kg/m^2)	33.1 (32.1 to 34.2)
Systolic BP (mmHg)	124 (121 to 126)
Diastolic BP (mmHg)	68.5 (67 to 70)
History of PCI	56 (25.69%)
History of CABG	58 (26.61%)
History of smoking	127 (61.95%)
Diabetes	103 (47.25%)
Atrial fibrillation	108 (49.54%)
History of pacemaker	27 (12.39%)
eGFR (ml/min/1.73 m^2)	61.7 (59 to 64.4)
NYHA Class	
NYHA 1-2	135 (61.9%)
NYHA 3-4	83 (38.07%)
Medication Use	
Beta Blocker	181 (83.03%)
Aspirin	135 (61.93%)
ACEI	101 (46.33%)
ARBs	68 (31.19%)
Organic Nitrates	46 (21.10%)
Diuretic	198 (90.83%)
Statin	161 (73.85%)
CCBs	85 (38.99%)

Values represent the mean ± standard deviation, median (interquartile range) or count (percentage) as appropriate. ARB=angiotensin receptor blocker; ACE: angiotensin converting enzyme; CCB: calcium channel blocker; BMI=body mass index; NYHA=New York Heart Association; eGFR=estimated glomerular filtration rate.

Online Table 8. Top canonical pathways associated with APOM

Canonical Pathway	*-log(p-value)*	*Ratio*	*z-score*
Acute Phase Response Signaling	8.72	0.712	-2.25
Coagulation System	6.33	0.9	-0.192
LXR/RXR Activation	5.48	0.701	1.457
Huntington's Disease Signaling	4.29	0.653	-1.265
FXR/RXR Activation	4.29	0.684	
Leptin Signaling in Obesity	3.88	0.8	-1.698
Complement System	3.64	0.793	0.728
EGF Signaling	3.24	0.743	-1.177
Role of Tissue Factor in Cancer	2.94	0.636	
Clathrin-mediated Endocytosis Signaling	2.77	0.602	
Axonal Guidance Signaling	2.67	0.551	
Gα12/13 Signaling	2.66	0.645	-1.897
IGF-1 Signaling	2.66	0.645	-2.043
Leukocyte Extravasation Signaling	2.62	0.609	-2.449
Extrinsic Prothrombin Activation Pathway	2.59	0.857	0
tRNA Charging	2.59	0.857	-2.309
Intrinsic Prothrombin Activation Pathway	2.46	0.694	-0.209
14-3-3-mediated Signaling	2.4	0.643	-1.715
UVB-Induced MAPK Signaling	2.4	0.71	-1.706
Renin-Angiotensin Signaling	2.34	0.633	-2.333
Dendritic Cell Maturation	2.33	0.596	-2.496
RhoGDI Signaling	2.32	0.629	1.48
eNOS Signaling	2.31	0.647	-0.557
Renal Cell Carcinoma Signaling	2.25	0.659	-1.279
Colorectal Cancer Metastasis Signaling	2.23	0.579	-2.433
VEGF Signaling	2.22	0.632	-1.061
IL-3 Signaling	2.22	0.652	-2.191
Aldosterone Signaling in Epithelial Cells	2.19	0.627	-1.807
Insulin Receptor Signaling	2.19	0.627	-2.535
Growth Hormone Signaling	2.17	0.667	-1.043

Supplemental Excel File

Title: Proteome-wide regression against APOM