SUPPLEMENTAL MATERIALS

Supplemental Methods

Assay Validation for GDF-11 and GDF-8

We performed validation experiments across different techniques for GDF-11 and GDF-8 measurement in order to identify the most sensitive, specific and accurate method for use in our epidemiologic studies. We evaluated 5 leading available techniques for GDF-11 and/or GDF-8 quantitation involving immunoassays, somamer-binding assays and liquid chromatography tandem mass spectrometry (LC-MS/MS) developed for research or commercial use, whose methods have been previously detailed. These included the following: (1) GDF-8 enzyme-linked immunosorbent assay (ELISA) by R&D Systems (Minneapolis, MN),[1] with inter-assay coefficient of variation (CV) of 2.5%; (2) the GDF-11 immunoassay developed at Novartis (Basel, Switzerland) using R&D Systems antibodies and an electrochemiluminescence protocol by Meso Scale Diagnostics (MSD, Rockville, MD),[1] inter-assay CV of 8.0%; (3) two versions of the somamer (aptamer-based) binding assay (SomaLogic, Boulder, CO) tested in combination with a Luminex hybrid assay (magnetic bead protocol), namely, a first generation GDF8/11 somamer (Seq ID 2765-4), with interassay CV<6.0%,[2] and second generation somamers for GDF-11 (Seq ID 12060-28) and GDF-8 (Seq ID 14583-49) developed for improved GDF-11 and GDF-8 differentiation, each with inter-assay CVs of 3.0%; (4) an immunoplexed LC-MS/MS technique developed and tested by teams led by Nathan LeBrasseur, PhD (Mayo Clinic, Rochester, MN), with interassay CV's of 9.6-18.4% for GDF-11 and 4.3-20.9% for GDF-8[3,4]; and (5) an LC-MS/MS technique developed and tested by Shalender Bhasin, MD, and his group

(Brigham & Women's Hospital, Boston, MA), with interassay CVs of 8.7-12.0% and 12.0-15.1% for GDF-11 and GDF-8, respectively.[5]

Assay validation was performed in blinded fashion in serum specimens collected from 21 volunteers (median age [range]: 45 [22-66] years, male: 7 [33.3%]) at the Laboratory for Clinical Biochemistry Research at the University of Vermont (Burlington, VT). Measurements were performed in native (neat or unspiked) serum, as well as in serum spiked with recombinant GDF-11 and GDF-8 (R&D Systems) to achieve two concentration levels. Specifically, serum specimens from each volunteer were spiked to increase serum concentrations of GDF-11 by 1 ng/mL and 5 ng/mL, and GDF-8 by 4 ng/mL and 10 ng/mL. Specimens were allowed to equilibrate to achieve a mixture of forms (i.e., latent pro-complex, inactive latent complex, and active ligand).

For each of the 5 measurement methods evaluated, percent recovery of spiked recombinant protein at each of the two concentrations was calculated. The correlation between GDF-11 and GDF-8 levels in neat samples was determined by computing pairwise Pearson coefficients across the methods. Agreement between values obtained in neat and spiked specimens with different methods was evaluated with linear regression.

Methods for Bhasin LC-MS/MS Technique Selected for GDF-11 and GDF-8 Measurement

As reported previously,[5] serum samples were denatured, reduced, alkylated, and subjected to tryptic digestion following solid phase extraction. After further purification, the tryptic digests underwent LC-MS/MS, with isotope-labeled peptides unique to GDF-8 and GDF-11 added as internal standards. The standard curve was linear from 0 to 50 ng/mL for GDF-11 and from 0 to 100 ng/mL for GDF-8, with a lower limit of quantitation of 0.5 ng/mL for both

analytes. Inter-assay CVs for GDF-11 concentrations of 3.4, 7.4, 12.5, and 52.0 ng/mL were 8.7%, 13.0%, 14.2%, and 12.8%, respectively; inter-assay CVs at 8.7, 14.1, 17.3 and 51.1 ng/mL of GDF-8 were 15.1, 12.5, 16.4, and 12.0%, respectively. There was no detectable cross-reactivity for GDF-8 or IgG1 in the GDF-11 assay, nor for GDF-11 or IgG1 in the GDF-8 assay. The accuracy of the assay, determined as the percent recovery of spiked human plasma, ranged from 80 to 116% for GDF-11, and 81% to 111% for GDF-8.

Assay for Follistatin and FSTL-3

Follistatin and FSTL-3 were measured in serum using ELISA kits (R&D Systems, Minneapolis, MN). The manufacturer-reported detectable range for follistatin is ~ 250 - 16,000 pg/mL, with an inter-assay CV of 6.1%. The corresponding detectable range for FSTL-3 is ~313 – 24,622 pg/mL, with an inter-assay CV of 2.6%.

Echocardiography

Methods for the 1994-95 CHS echocardiographic evaluation have been detailed previously.[6] Briefly, standardized echocardiography was performed with Toshiba SSH-160A machines, with images acquired on super-VHS tapes interpreted centrally. LV mass was calculated using a standard approach.[6] Indexation to height and weight was based on linear regression parameters generated in a healthy subset of the CHS population, and percent predicted LV mass calculated as detailed previously.[7]

Archived CHS echocardiograms were digitized in 2016-18, as previously reported.[8] Speckle-tracking strain analysis of the apical 4-chamber view was performed centrally using TomTec CPA, v4.5 (Unterschleiβheim, Germany).[8] Measures of interest included LV

longitudinal strain, LV early diastolic strain rate (LVESR) and left atrial (LA) reservoir strain. All strain measures were analyzed using their absolute values. We also examined medial e', measured by speckle-tracking analysis, and E/e'. Measurement of medial e' by speckle-tracking analysis has validated against conventional tissue Doppler imaging, but yields lower values.[9]

Supplemental References

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Supplemental Figure Legend

Supplemental Figure 1. Flow diagram of participant selection in CHS and Health ABC.

Supplemental Figure 2. Scatterplots of (A) GDF-11 and (B) GDF-8 concentrations obtained by different assay pairs in both unspiked and spiked serum specimens. Linear regression results are displayed in the graphs.

Supplemental Figure 3. Scatterplots comparing (A) GDF-11 vs. GDF-8 Somascan assays, and (B) GDF-8/11 vs. GDF-8 or GDF-11 Somascan assays. Protein concentrations were measured in unspiked and spiked specimens. Scatterplots are centered at zero.

Supplemental Figure 4. Associations of GDF-11, GDF-8, FST, FSTL-3 with Incident Heart Failure with Preserved (**A**) or Reduced (**B**) Ejection Fraction (CHS and Health ABC Combined).

*per doubling

Model 1. Unadjusted.

Model 2. Adjusted for age, sex, race and cohort.

Model 3. Adjusted for Model 2 covariates plus body mass index, systolic blood pressure, antihypertensive medication,diabetes, current smoking, heavy drinking, FEV_1, prevalent CHD, prevalent stroke, prevalent claudication, prevalent atrial fibrillation.

Model 4. Adjusted for Model 3 covariates plus $eGFR_{cys}$.

Additional adjustment for estrogen replacement therapy or C-reactive protein or urine albumin-creatinine ratio did not materially alter the results.

Supplemental Table 1. Concentrations of GDF-11 and GDF-8 in Native and Spiked Serum Specimens and Percent Recovery of Spiked Proteins Obtained with Different Measurement Techniques

	Protein Concentration (ng/mL)			Percent Recovery (%)	
Assay	**Native Serum**	**Spiked Serum 1***	**Spiked Serum 2†**	**Spiked Serum 1***	**Spiked Serum 2†**
Immunoassay					
GDF-11	1.81	2.83	5.81	101.46	80.0
GDF-8	4.00	8.34	14.51	108.4	105.1
Somamer					
GDF-8‡	6.56	8.98	18.38	60.5	118.2
Updated Somamer					
GDF-11	3.19	3.71	7.72	52.1	90.7
GDF-8	5.35	6.67	11.38	32.9	60.3
LC-MS/MS Bhasin					
GDF-11	3.46	4.45	9.39	98.1	118.5
GDF-8	9.87	14.15	20.67	106.9	108.0
LC-MS/MS LeBrasseur					
GDF-11	0.87	1.38	2.62	53.4	35.1
GDF-8	7.35	11.67	14.67	108.2	73.2

*Spiked to increase serum concentration of GDF-11 by 1 ng/mL and GDF-8 by 4 ng/mL.

†Spiked to increase serum concentration of GDF-11 by 5 ng/mL and GDF-8 by 1 ng/mL.

‡Assuming that all measured protein is GDF-8.

LC-MS/MS=Liquid chromatography tandem mass spectrometry.

Supplemental Table 2. Pearson Correlation Coefficients between Levels of GDF-11 and GDF-8 Determined in Native Specimens by Different Techniques

	Immunoassay Novartis GDF-11	**Updated Somamer GDF-11**	**LC-MS/MS Bhasin GDF-11**	**LC-MS/MS LeBrasseur GDF-11**
Immunoassay R&D GDF-8	r=-0.31, p=0.166	--	--	--
Updated Somamer GDF-8	--	r=0.88, p<0.001	--	--
LC-MS/MS Bhasin GDF-8	--	--	r=0.29, p=0.195	--
LC-MS/MS LeBrasseur GDF-8	--	--	--	r=0.67, p=0.003

LC-MS/MS=Liquid chromatography tandem mass spectrometry.

Supplemental Table 3. Characteristics of Participants Included and Excluded from the Study Sample in Each Cohort.

Characteristics	**Health ABC**			**CHS**		
	Excluded	**Included**	**p**	**Excluded**	**Included**	**p**
	N=1,838	*N=1,237*		*N=3,336*	*N=1,506*	
Age, y	73.8 ± 2.9	73.4 ± 2.9	0.003	76.0 ± 5.0	76.9 ± 4.8	<0.001
Male, n (%)	862 (46.9)	629 (50.9)	0.032	601 (42.3)	617 (41.0)	0.458
White, n (%)	1,035 (56.3)	759 (61.4)	0.005	1032 (72.7)	1,442 (95.8)	<0.001
BMI, kg/m^2	27.5 ± 5.0	27.2 ± 4.6	0.044	26.9 ± 4.5	26.4 ± 4.3	0.002
Systolic BP, mm Hg	136 ± 22	135 ± 19	0.076	134 ± 21	132.6 ± 20.1	0.052
Antihypertensive medication, n (%)	1,009 (55.0)	663 (53.9)	0.528	1,829 (57.1)	761 (50.6)	<0.001
Diabetes, n (%)	317 (17.3)	210 (17.0)	0.84	641 (25.3)	228 (16.3)	<0.001
LDL cholesterol, mg/dL	121 ± 35	122 ± 35	0.615	127 ± 33	129 ± 33	0.135
HDL cholesterol, mg/dL	55 ± 17	53 ± 17	0.061	54 ± 15	53 ± 14	0.334
Triglycerides, mg/dL	137 ± 84	140 ± 80	0.412	143 ± 87	146 ± 85	0.412
Statin use, n (%)	226 (12.3)	169 (13.7)	0.255	190 (5.9)	112 (7.4)	0.049
Current smoker, n (%)	195 (10.6)	123 (10.0)	0.544	133 (9.5)	108 (7.2)	0.030
Heavy alcohol use, n (%)	83 (4.5)	45 (3.7)	0.233	98 (6.9)	146 (9.7)	0.006
Estrogen use (women), n (%)	207 (11.3)	142 (11.5)	0.832	123 (8.7)	156 (10.4)	0.117

Prevalent CHD, n (%)	417 (22.7)	244 (19.7)	0.050	288 (20.3)	361 (24.0)	0.016
Prevalent stroke, n (%)	182 (9.9)	115 (9.3)	0.577	80 (5.6)	87 (5.8)	0.868
Prevalent AF, n (%)	99 (5.4)	44 (3.6)	0.018	416 (12.5)	143 (9.5)	0.003
Prevalent claudication, n (%)	106 (5.8)	58 (4.7)	0.195	28 (2.0)	53 (3.5)	0.011
$eGFR_{cys}$, ml/min/1.73 m^2	71 ± 19	73.3 ± 18	0.002	68 ± 18	66 ± 17	0.052
Urine albumin/creatinine ratio	51 ± 205	36 ± 121	0.002	75 ± 370	53 ± 276	0.031
FEV_1, L	2.08 ± 0.64	2.21 ± 0.64	<0.001	1.95 ± 0.68	2.03 ± 0.64	<0.001
C-reactive protein, mg/L	3.3 ± 5.6	2.5 ± 3.1	<0.001	5.5 ± 9.1	4.6 ± 8.5	<0.001

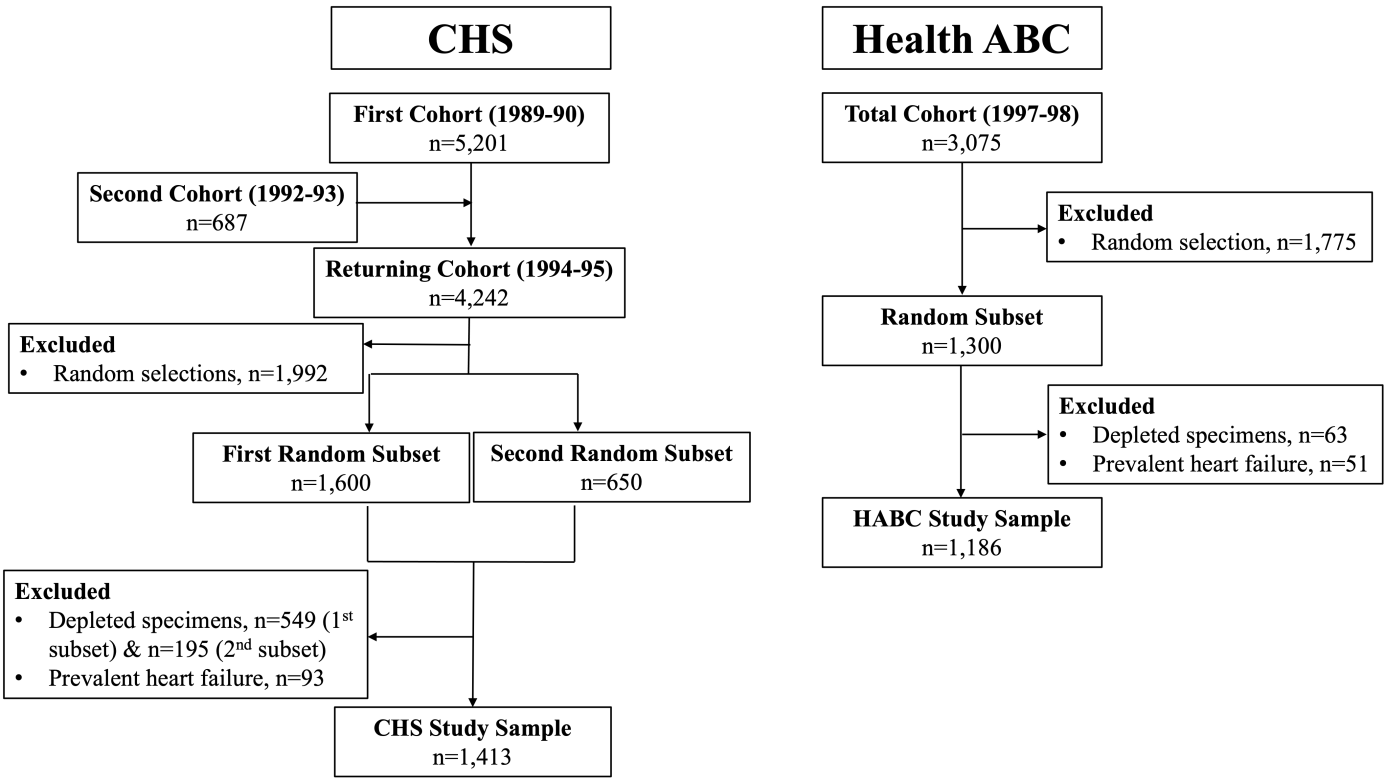
AF=Atrial fibrillation; BMI=Body mass index; BP=Blood pressure; CHD=Coronary heart disease; $eGFR_{cys}$=Estimated glomerular filtration rate based on cystatin C; FEV_1=Forced expiratory volume in 1 second.

Supplemental Table 4. Effect Modification of FSTL-3's Associations with Incident Heart Failure by Sex, Race, eGFRcys and Follistatin.

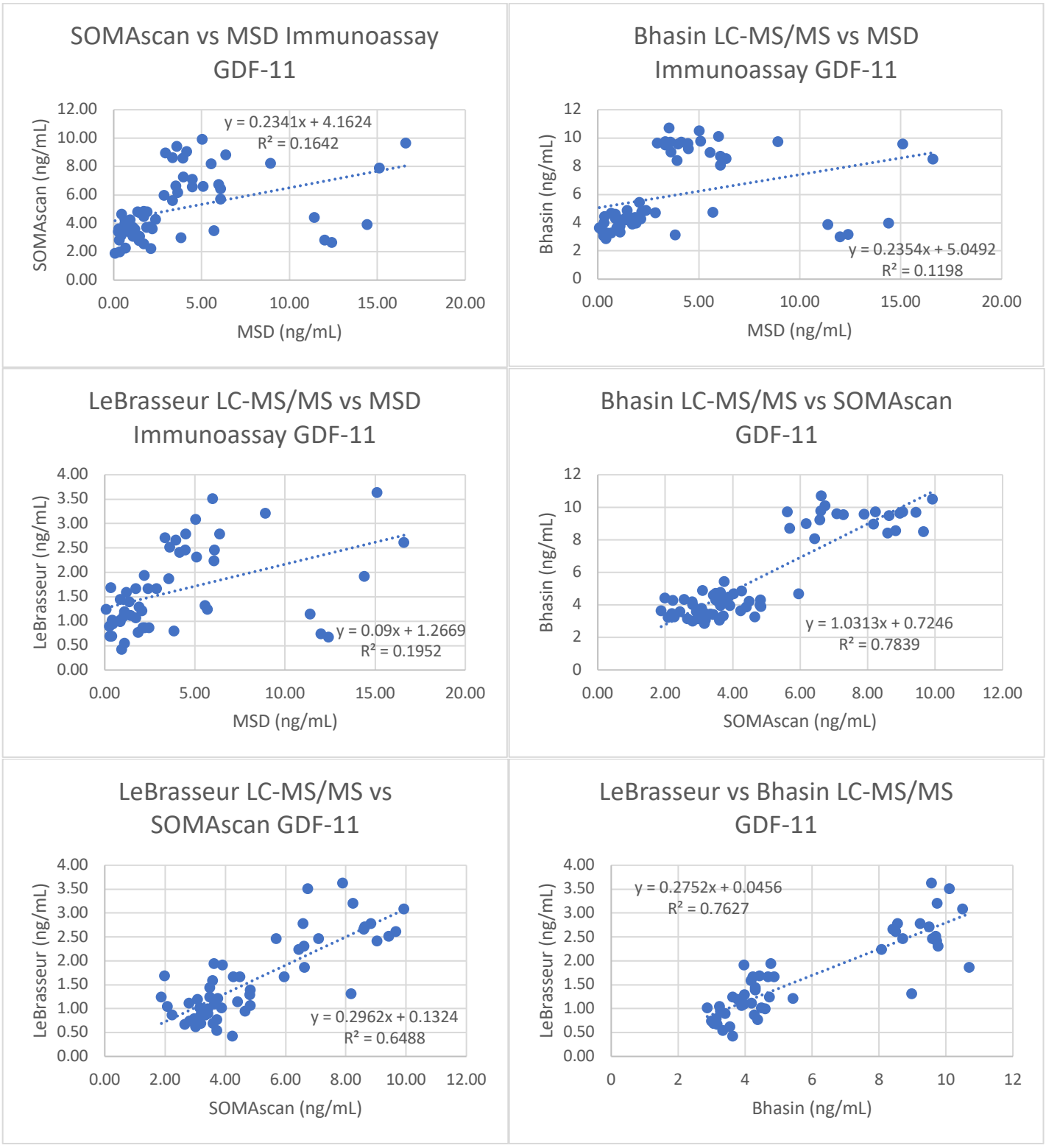
Variables	**Stratified Associations**				**$P_{interaction}$†**
	HR* (95% CI)	**P value**	**HR* (95% CI)**	**P value**	
Sex	***Men***		***Women***		0.025
FSTL-3	1.54 (0.97, 2.43	0.064	1.25 (0.85, 1.85)	0.256	
Race	***Black***		***White***		0.045
FSTL-3	1.83 (0.96, 3.47)	0.067	1.26 (0.90, 1.77)	0.181	
$eGFR_{cys}$	***<60 ml/min/1.73 m^2***		***≥60 ml/min/1.73 m^2***		0.031
FSTL-3	1.35 (0.82, 2.22)	0.241	1.28 (0.88, 2.87)	0.197	
Follistatin	***≥Median***		***<Median***		0.014
FSTL-3	1.69 (1.08, 2.64)	0.022	1.16 (0.76, 1.80)	0.491	
FSTL-3	***≥Median***		***<Median***		0.014
Follistatin	1.31 (1.09, 1.58)	0.004	0.96 (0.78, 1.18)	0.675	

*Per doubling of biomarker level.

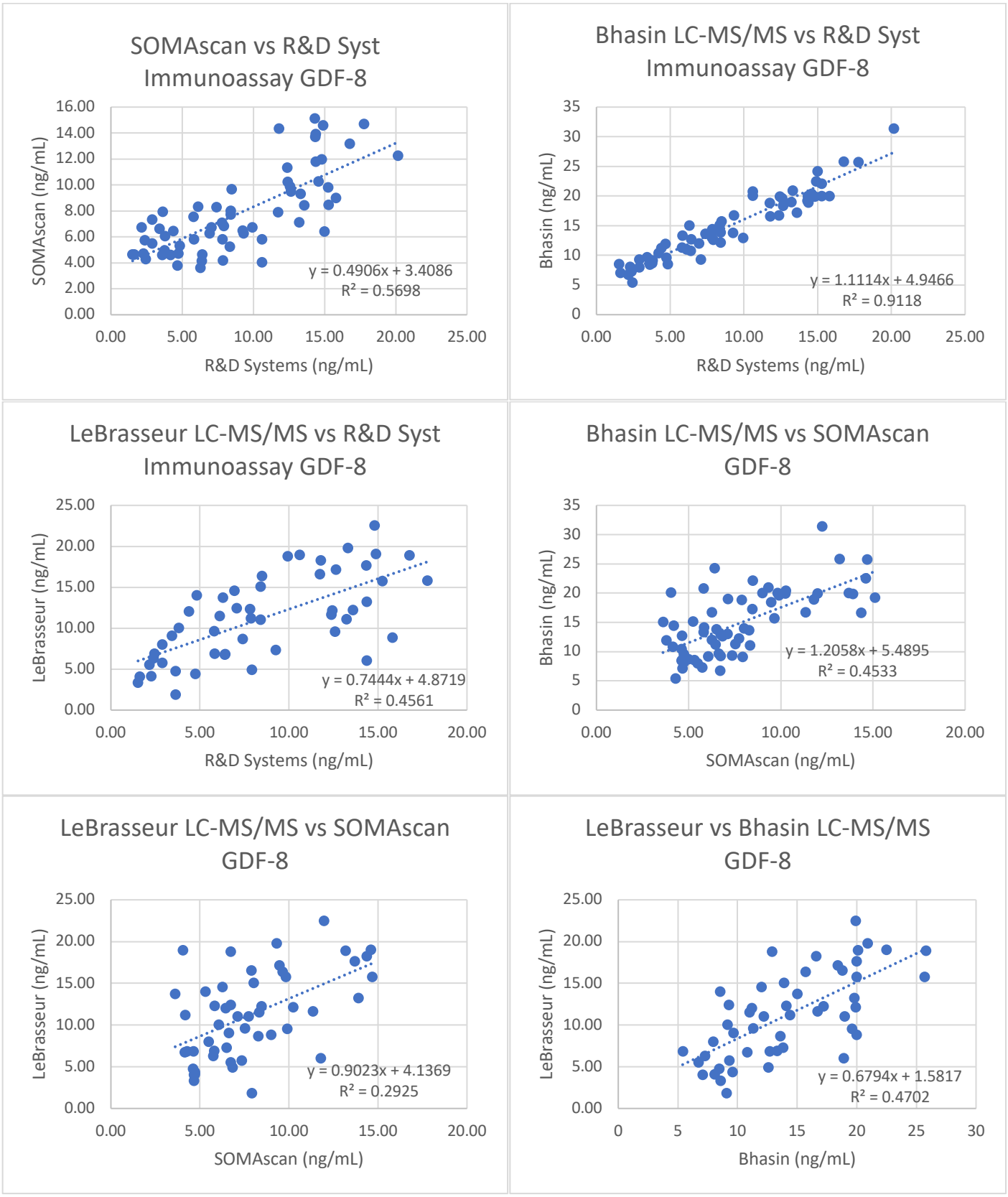
†For continuous by categorical variables (sex and race), or continuous by continuous variables (follistatin, FSTL-3 and $eGFR_{cys}$).



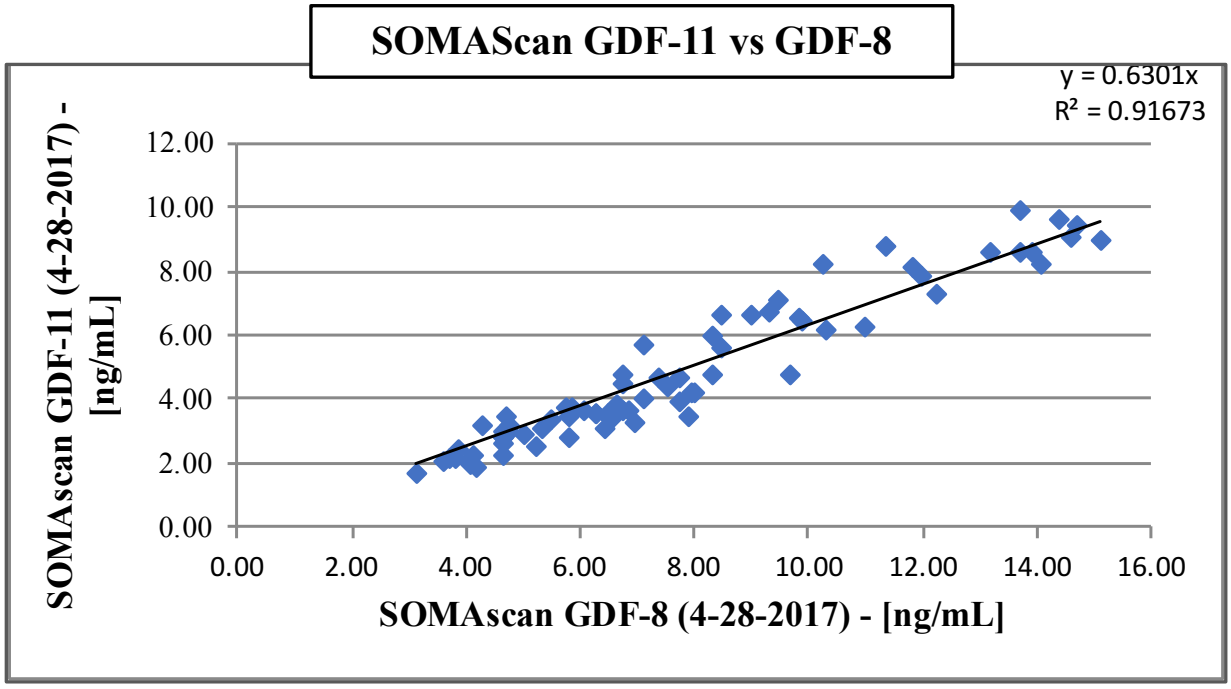
Supplemental Figure 1



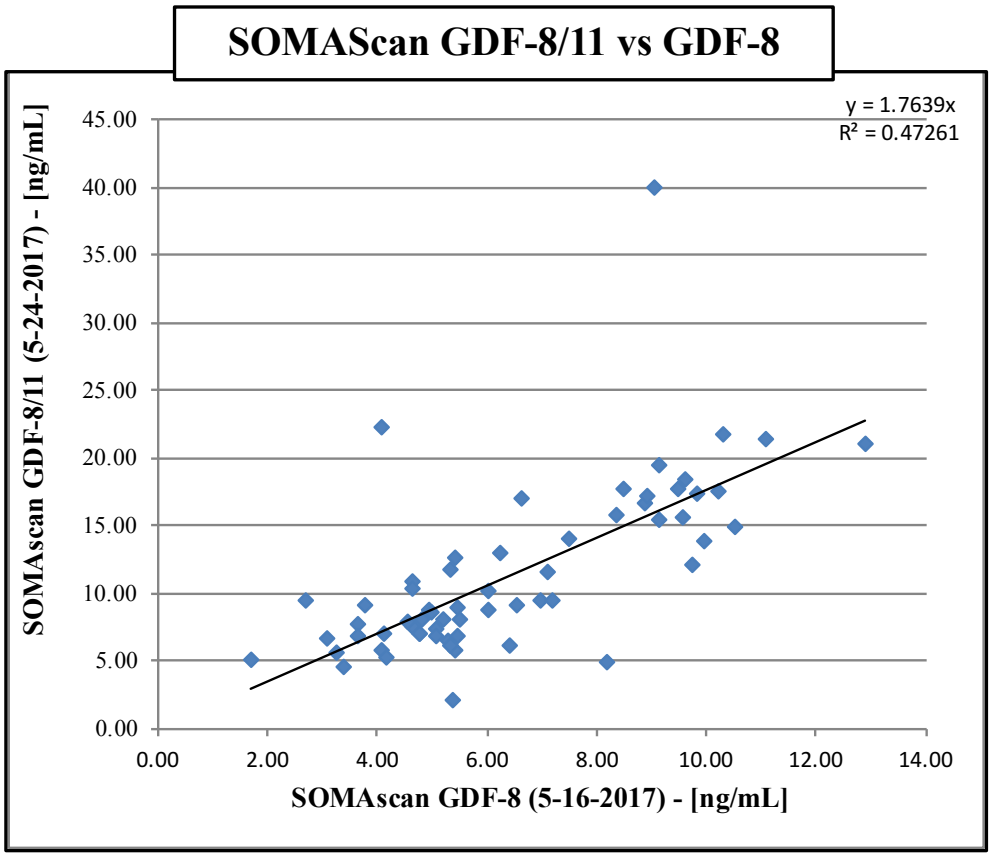
Supplemental Figure 2A

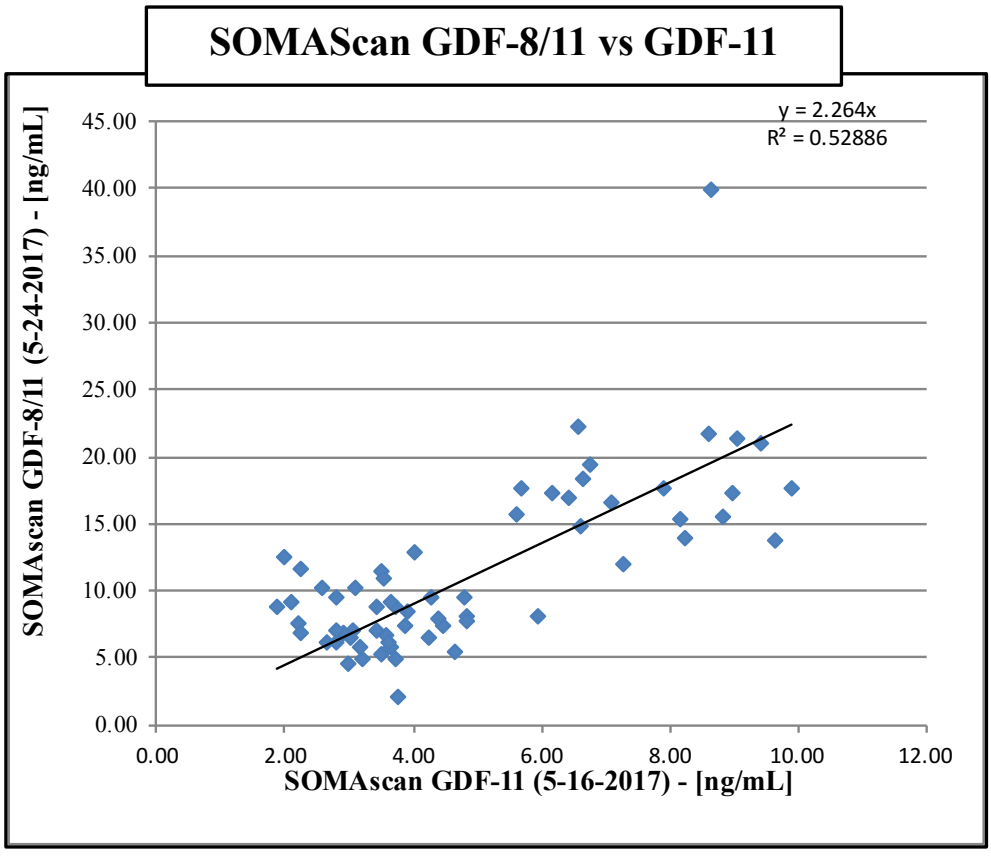


Supplemental Figure 2B

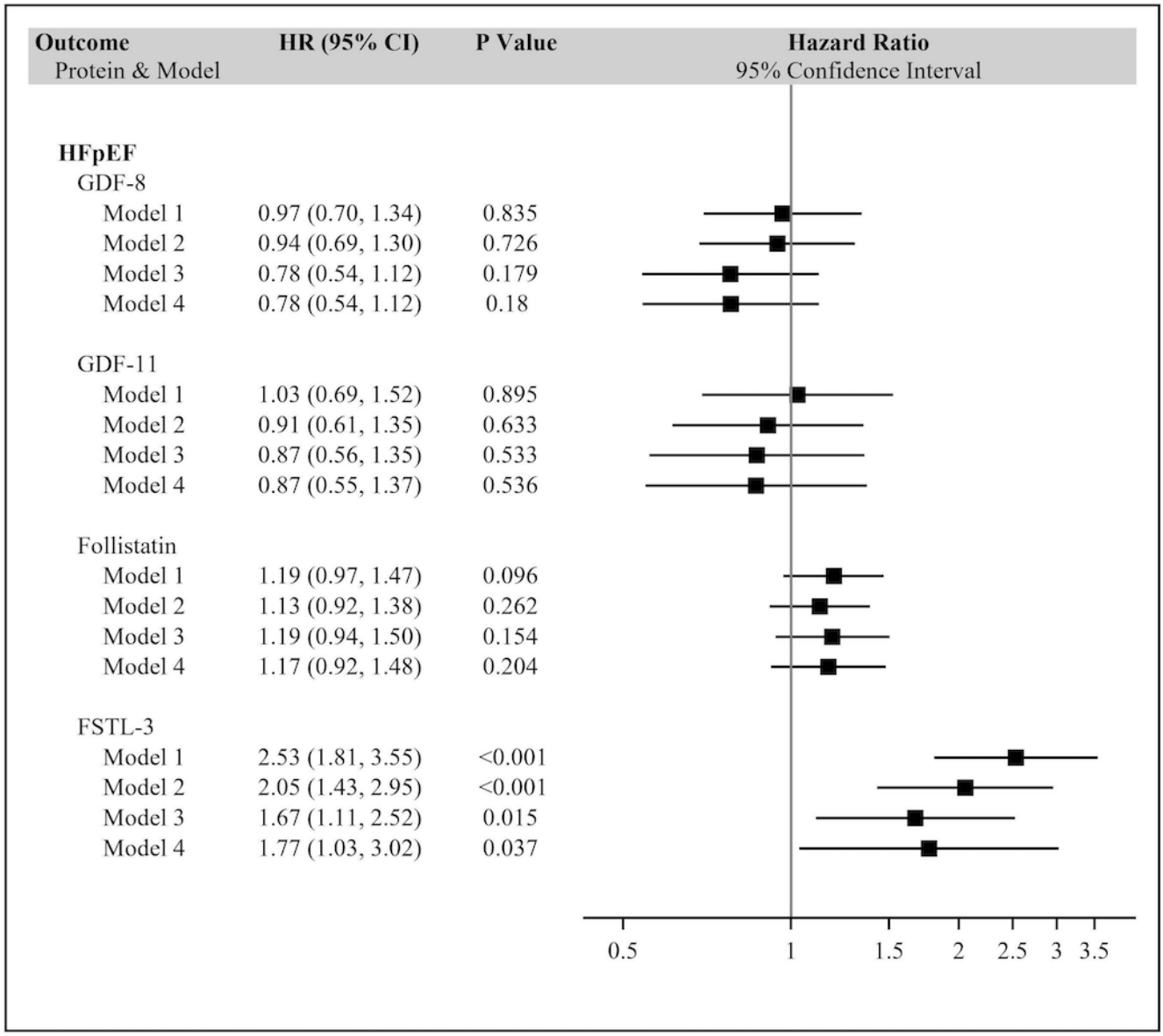


Supplemental Figure 3A

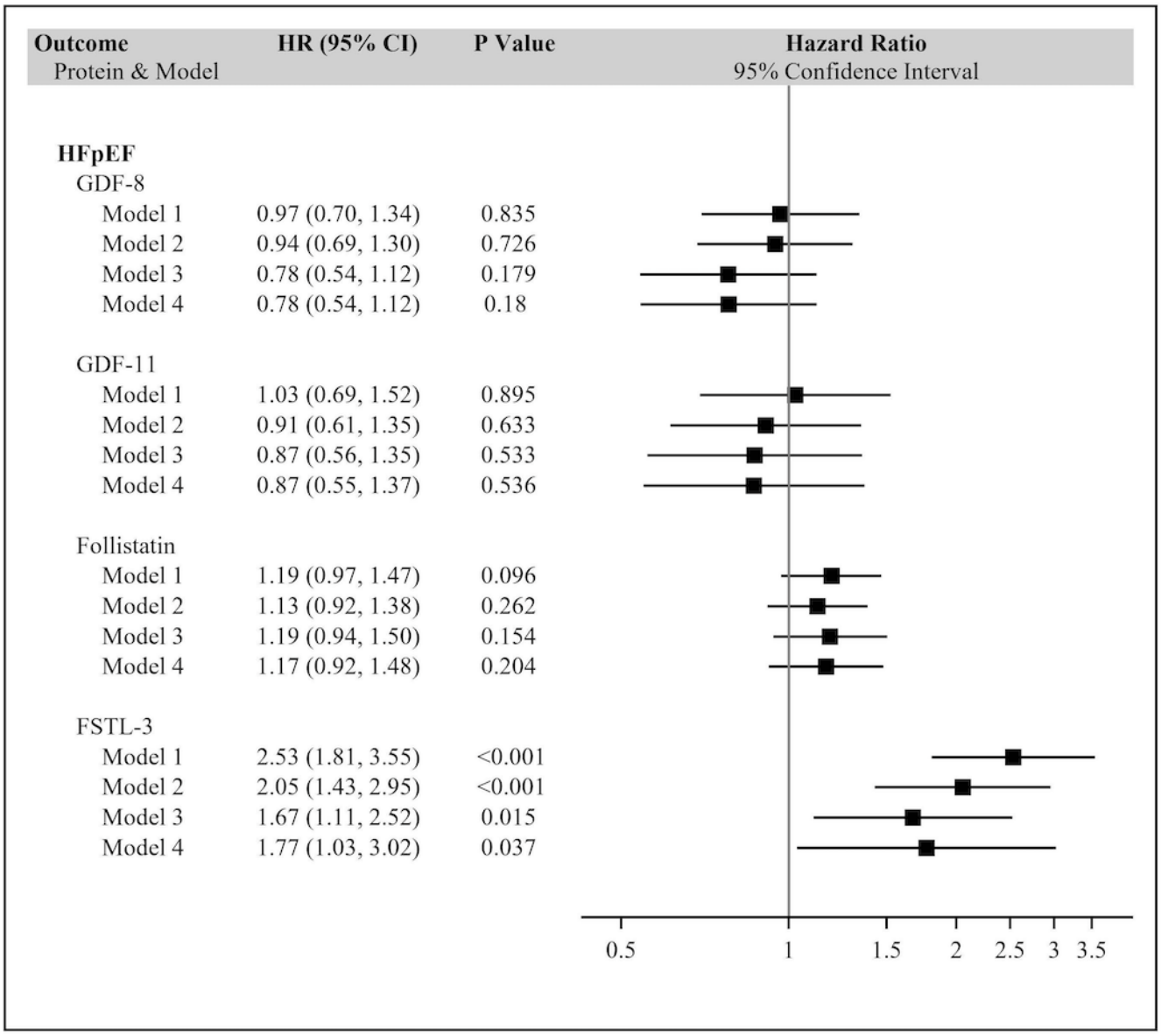




Supplemental Figure 3B



Supplemental Figure 4A



Supplemental Figure 4B