Profiling of the plasma proteome across different stages of human heart failure

Supplementary information

Anna Egerstedt, John Berntsson, Maya Landenhed Smith, Olof Gidlöf, Roland Nilsson, Mark Benson, Quinn S. Wells, Selvi Celik, Carl Lejonberg, Laurie Farrell, Sumita Sinha, Dongxiao Shen, Jakob Lundgren, Göran Rådegran, Debby Ngo, Gunnar Engström, Qiong Yang, Thomas J. Wang, Robert E. Gerszten, J. Gustav Smith

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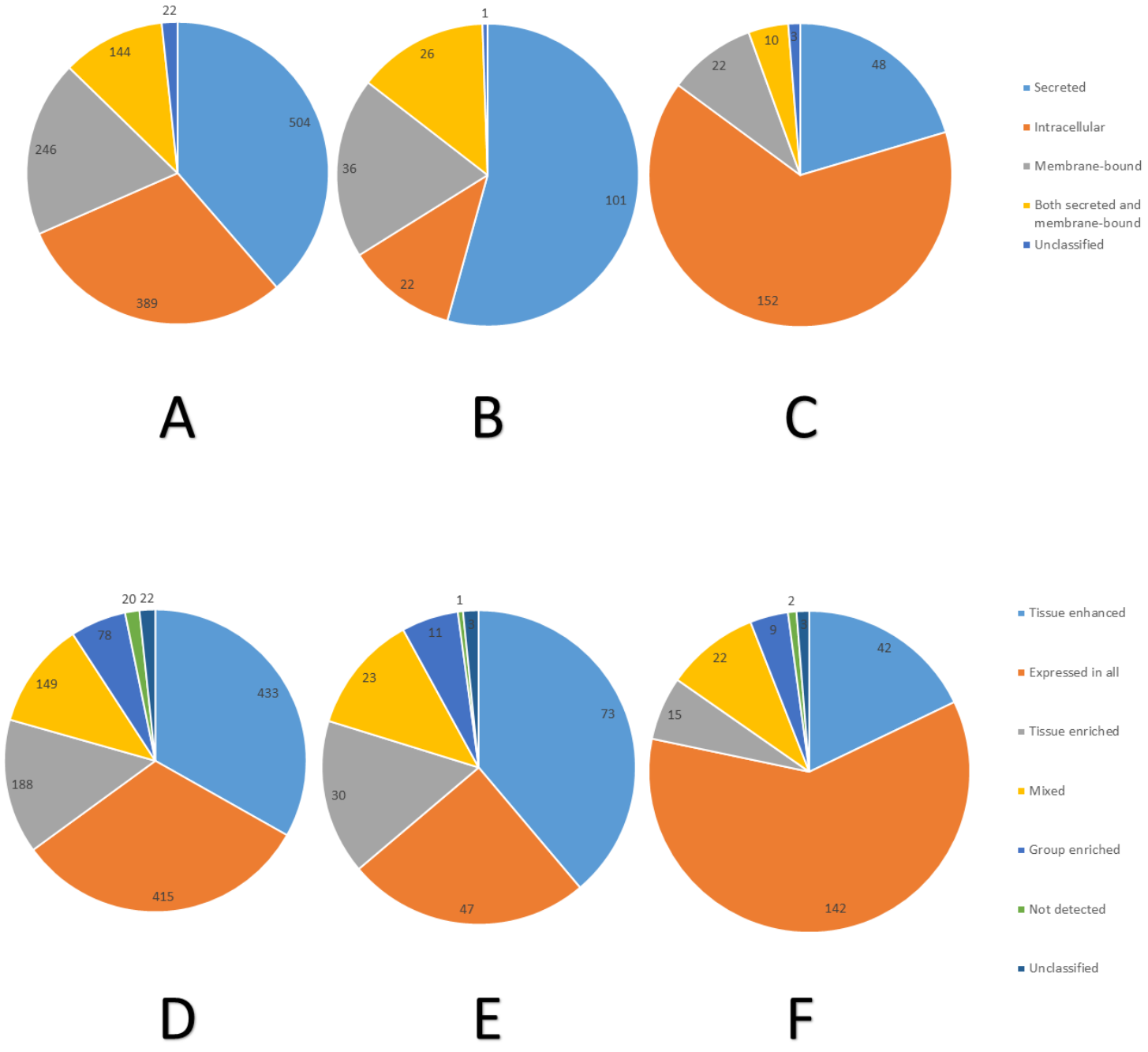
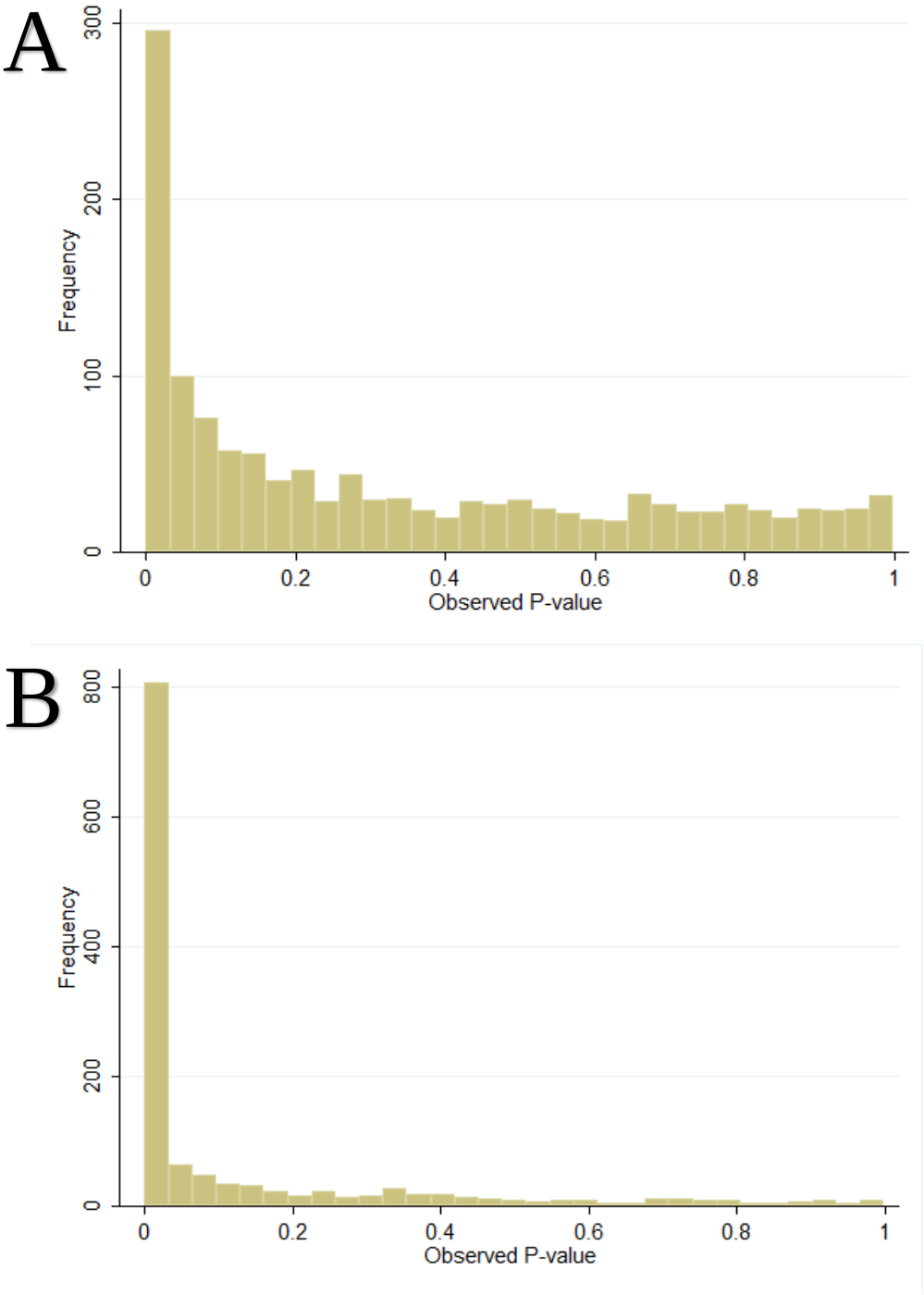
Supplementary Figure 1. Protein characteristics.



Distribution of protein classes (A-C) and tissue expression (D-F), among all 1,305 proteins assayed (A, D), the subset of 186 proteins higher in patients with HF (B,E), and among 235 proteins lower in patients with HF (C,F). Protein locations were computationally predicted based on structural features, as part of the Human Protein Atlas (HPA, https://www.proteinatlas.org/humanproteome/secretome, accessed sept 9, 2018). For membrane proteins, features indicating transmembrane regions included

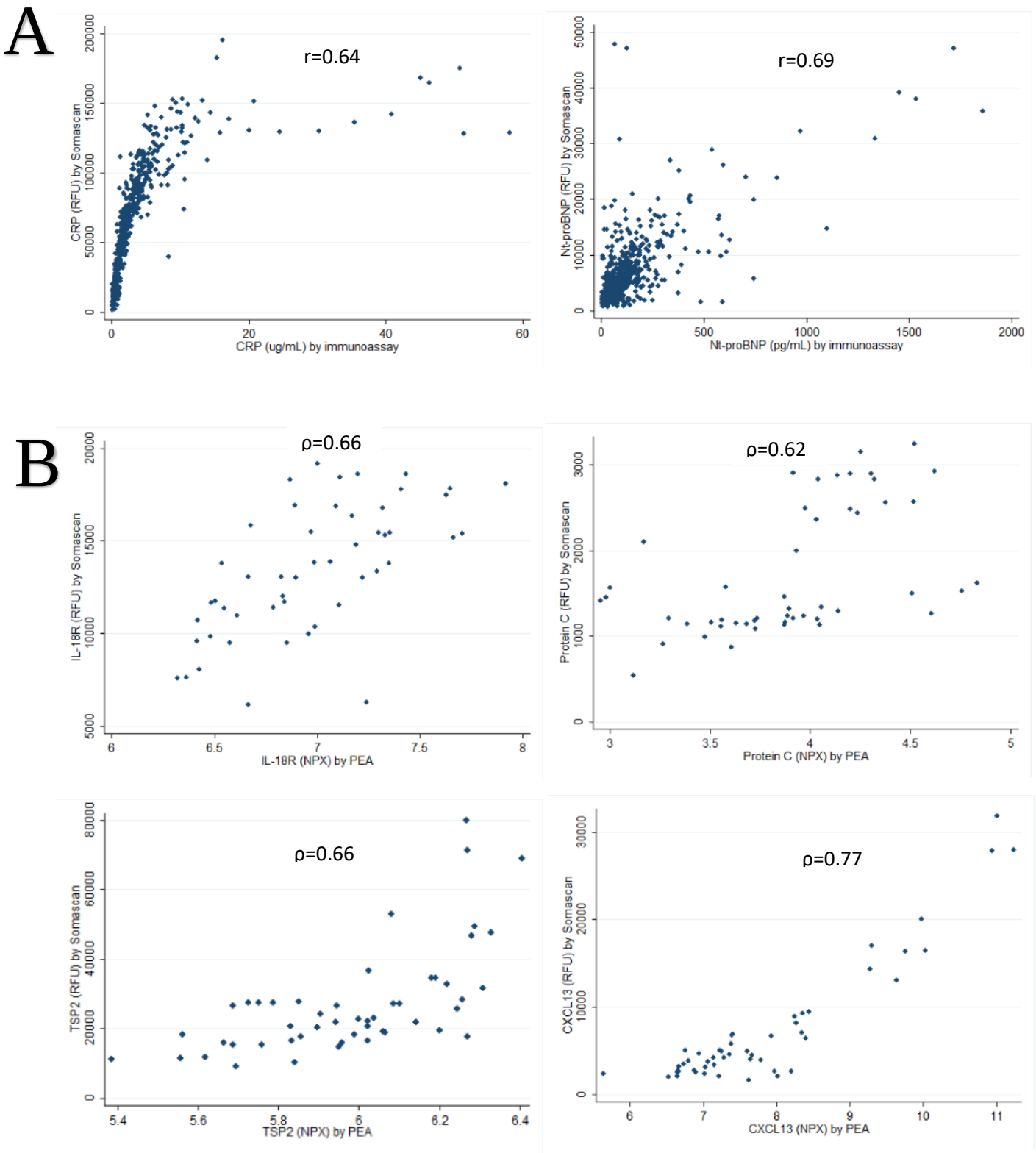
length, amino acid properties and hydrophobicity based on combination of seven algorithms. For secreted proteins, three algorithms for prediction of signal peptides were combined. Intracellular proteins were defined as lacking both transmembrane regions and signal peptides. A subset of proteins included both signal peptides and transmembrane regions and a small subset was unavailable in the HPA database. Information on tissue enrichment was also obtained from the HPA, based on RNA-sequencing of 64 cell lines and 37 tissues. Tissue expression was classified by the HPA into six categories: tissue enriched (expression in one tissue at least five-fold higher than all others), group enriched (five-fold higher in 2-7 tissues compared to all other), tissue enhanced (five-fold higher in one or more tissues compared to the mean of all tissues), expressed in all (≥1 transcript per million in all tissues), not detected (<1 transcript per million in all tissues), and mixed (detected in at least one tissue and not in the other categories).

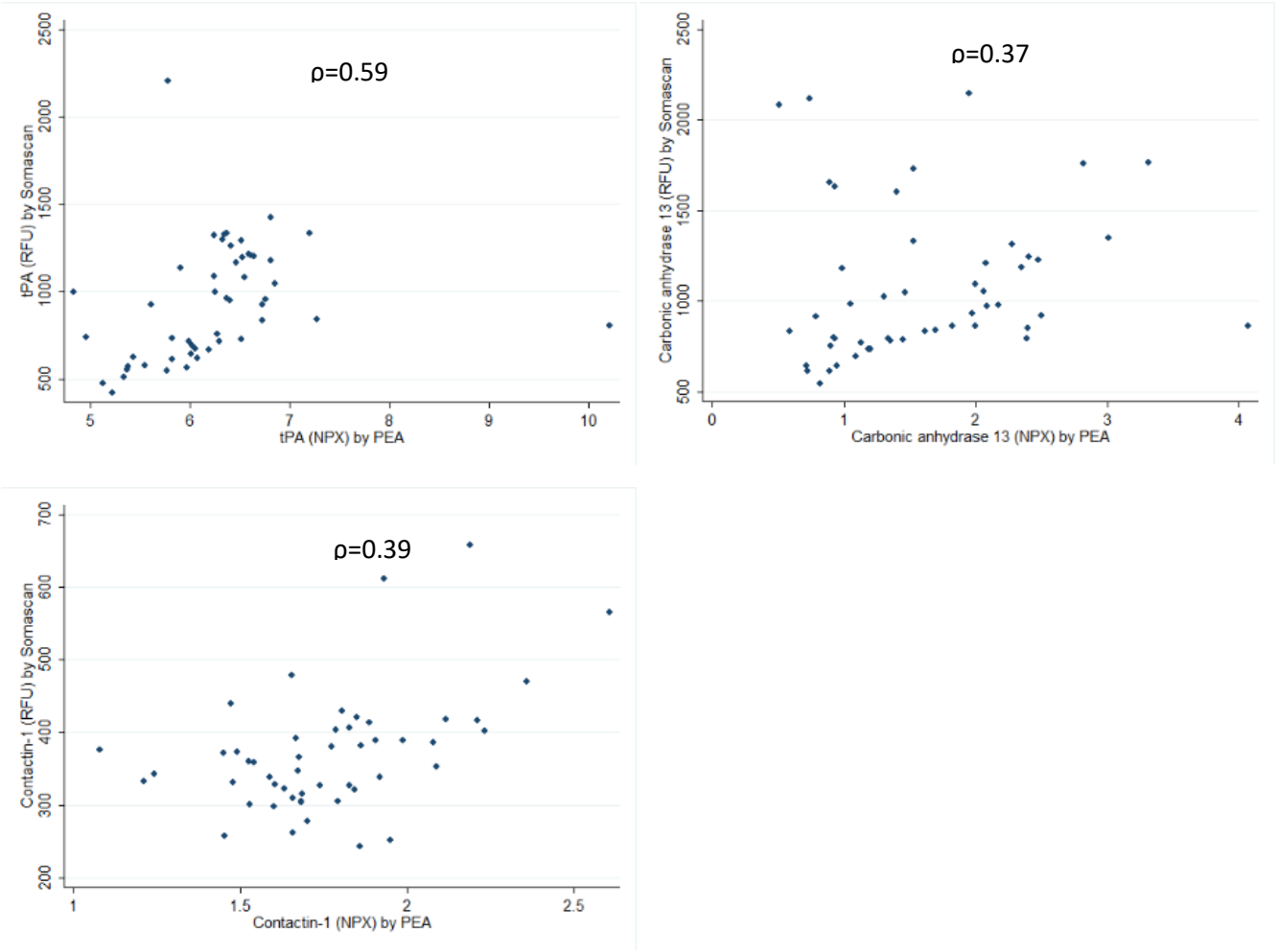
Supplementary Figure 2. P-value distribution for protein associations with heart failure.



Histogram showing distribution of p-values for association tests of 1,305 proteins with incident heart failure in a population-based cohort (panel a) and manifest heart failure (panel b). A marked excess of proteins with low p-values is observed with both but most markedly with manifest heart failure, consistent with a large number of association signals.

Supplementary Figure 3. Comparison of Somascan assay and immunoassays.

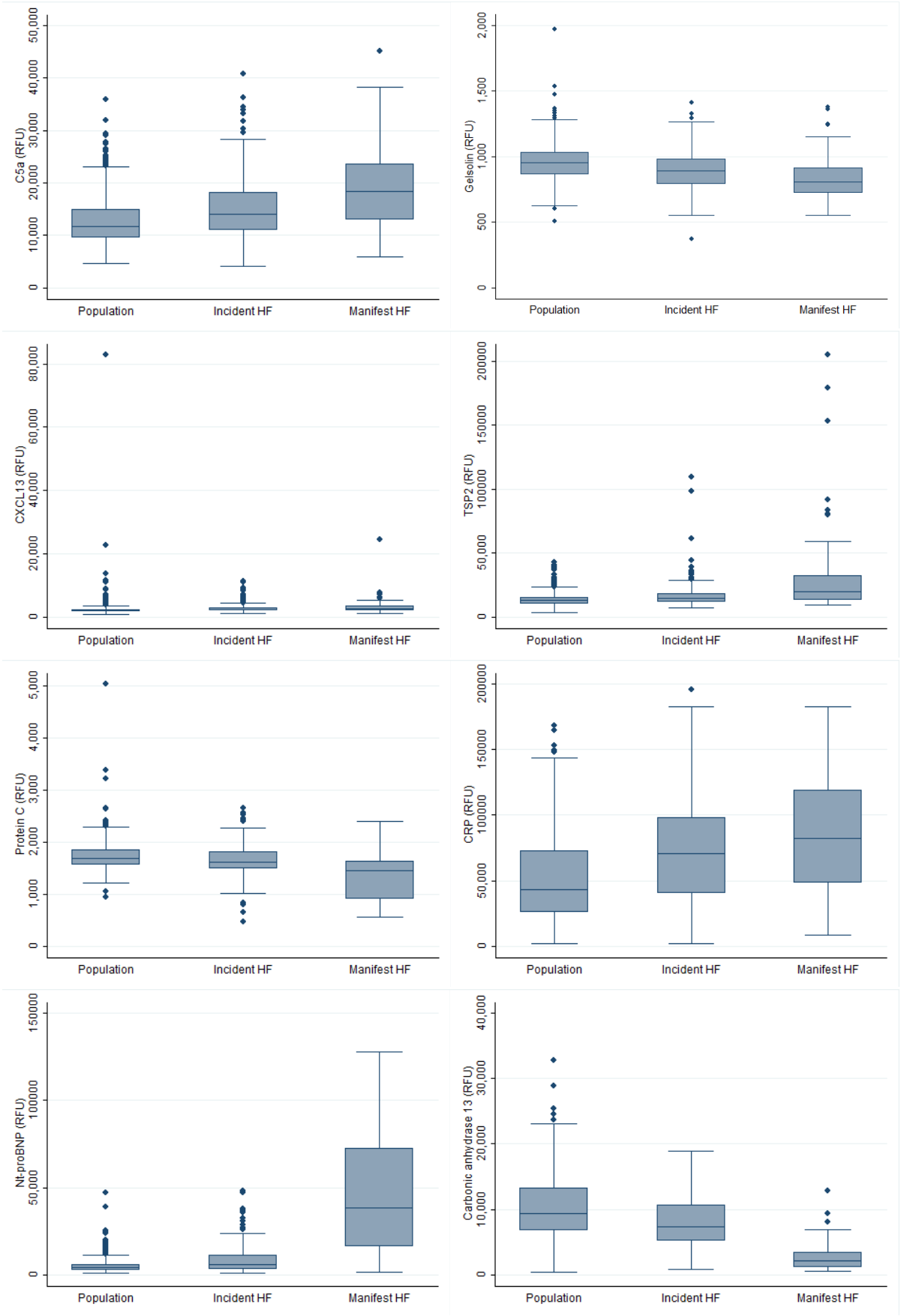


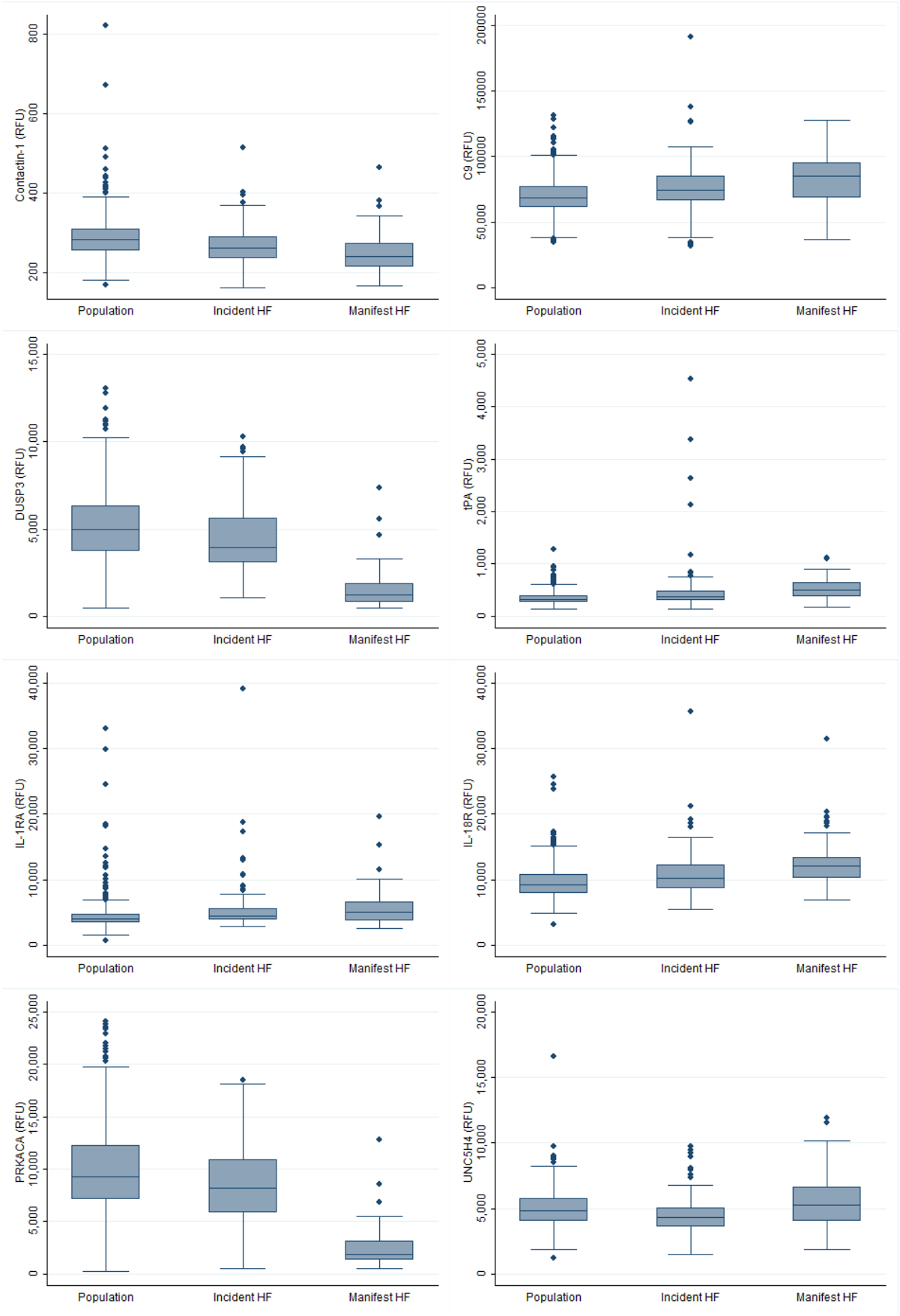


Panel A shows scatterplots for C-reactive protein (CRP) and N-terminal pro-B-type natriuretic peptide (Nt-proBNP) measured using Somascan (in relative fluorescent units, RFUs) or established immunoassays (in pg/mL) in the case-cohort sample from the MDCS. Pearson's correlation coefficients are presented (both $p<0.05$). CRP was measured by a high-sensitivity assay (Roche Diagnostics, Basel, Switzerland). Nt-proBNP was measured using the automated Dimension Vista Intelligent Lab System method (Siemens Healthcare Diagnostics Inc, Deerfield, Illinois, USA).

Panel B shows scatterplots for 7 proteins measured using Somascan or a proximity extension assay (PEA; Olink Proteomics, Uppsala, Sweden) in 50 samples: 10 samples from patients with myocardial infarction, and 4 samples (0, 10 min, 1 hour and 24 hours after) each from 10 patients with hypertrophic cardiomyopathy undergoing septal alcohol ablation. Spearman's rank correlation coefficients are presented (all $p<0.05$).

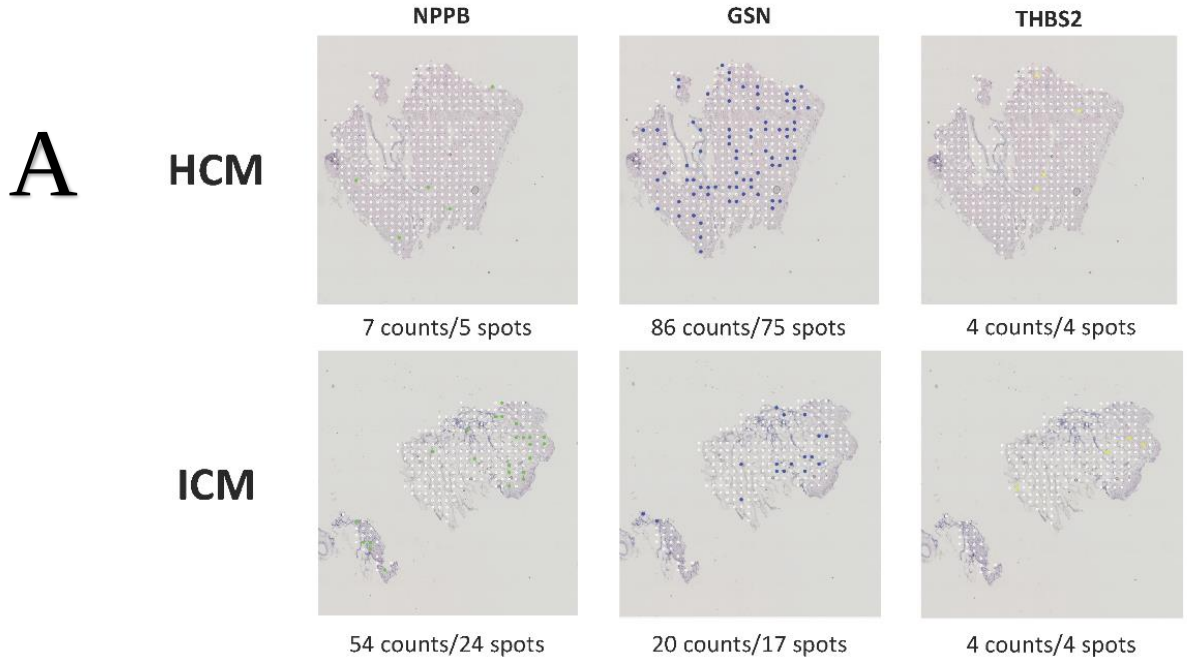
Supplementary Figure 4. Distribution of protein concentrations across study populations.

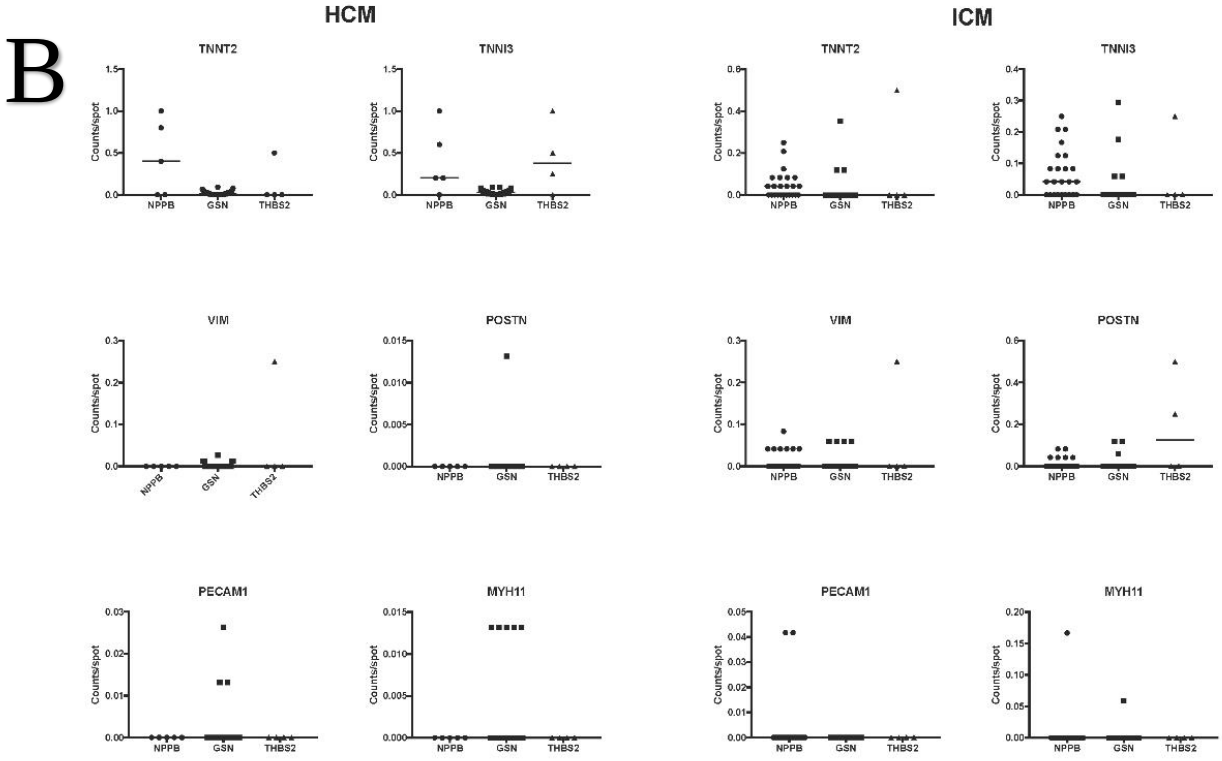




The box centre line indicates the median, box bounds represent 25th and 75th percentiles, and the whiskers are the most extreme values within 1.5 x the interquartile range from the nearer quartile.

Supplementary Figure 5: Spatial transcriptomic expression of cardiac proteins.

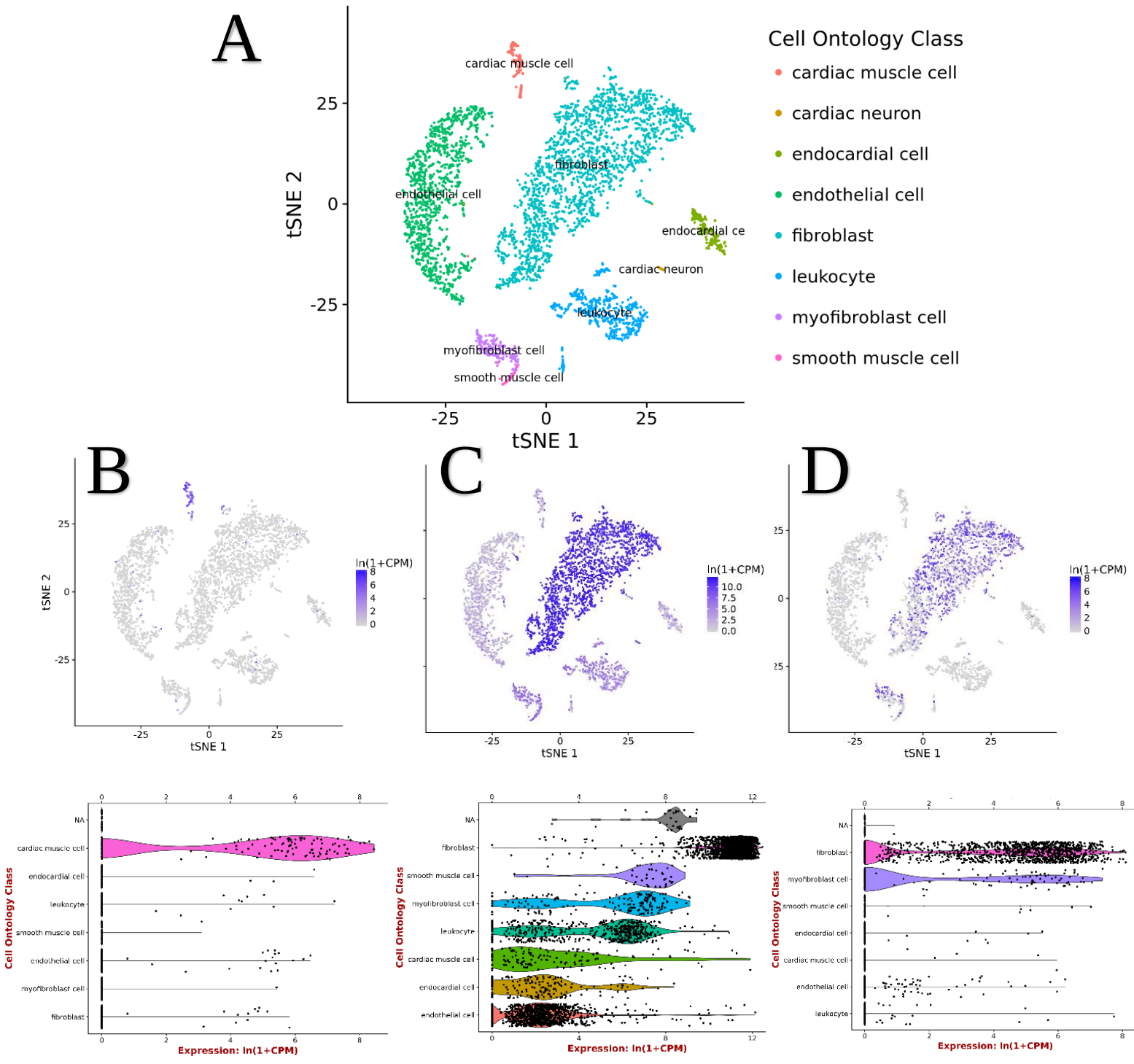




Panel (A) Myocardial expression across spatially distinct spots on tissue sections for Nt-proBNP (*NPPB*), thrombospondin-2 (*THBS2*) and gelsolin (*GSN*), for which evidence of cardiac origin was observed, in two patients with heart failure of different etiologies: ischemic (ICM) and hypertrophic

(HCM) cardiomyopathy. Panel (B) Coexpression was tested for spots expressing at least one count of each transcript with markers specific for cardiomyocytes (*TNNT2*, *TNNI3*), fibroblasts (*VIM*, *POSTN*), endothelial cells (*PECAM1*) and vascular smooth muscle cells (*MYH11*). Expression of IL18R, and presence of leukocytes (*PTPRC* [CD45]), was below the level of detection for all spots and was not included.

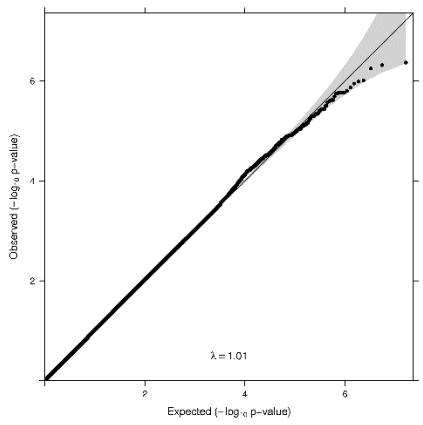
Supplementary Figure 6: Cell-type specific expression of cardiac proteins in murine hearts.



Single-cell full length transcript analysis of mouse hearts generated by SMART-Seq2 RNA-sequence libraries prepared from individually fluorescence-activated cell sorter (FACS) sorted cells within the Tabula Muris project. (https://tabula-muris.ds.czbiohub.org/, retrieved on March 18, 2019) Panel A shows cell clustering for murine hearts based on t-distributed stochastic neighbor embedding (tSNE) in two dimensions. The upper panels for each of *NPPB* (B), *GSN* (C) and *THBS2* (D) indicate

expression of each transcript in blue, while the lower panels show violin plots with distribution of gene expression across cell types, expressed in transformed counts per million (CPM).

Supplementary Figure 7: QQ plots from genome-wide association studies of identified proteins.



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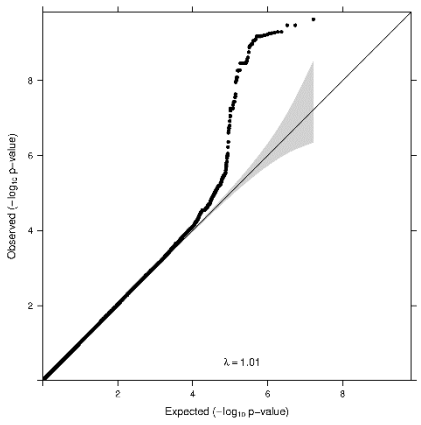

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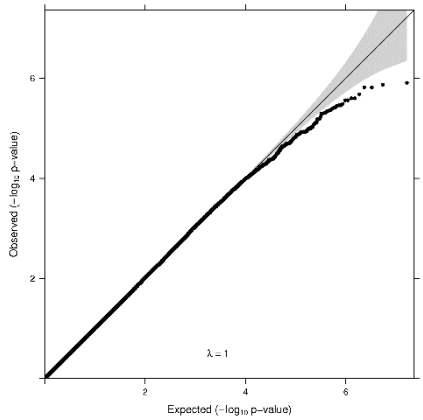

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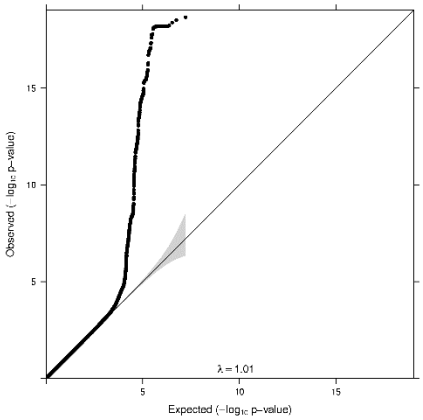
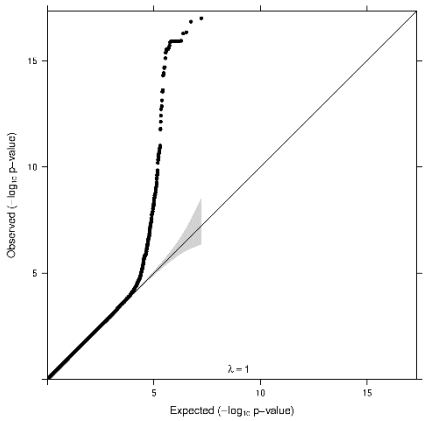


Carbonic anhydrase XIII



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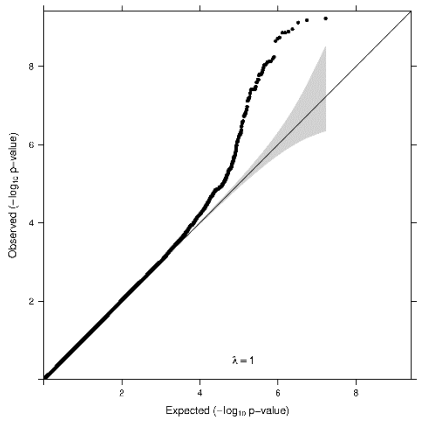

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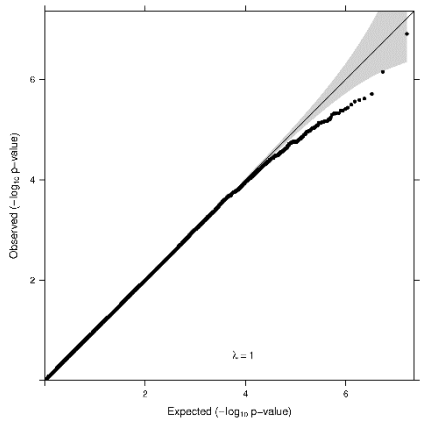

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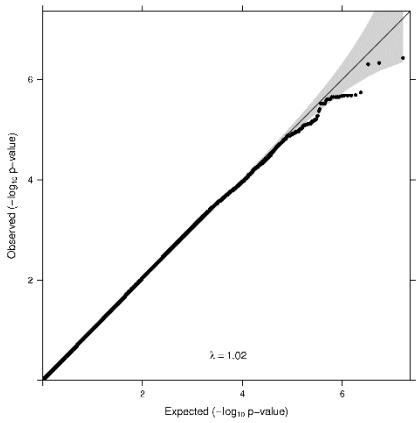

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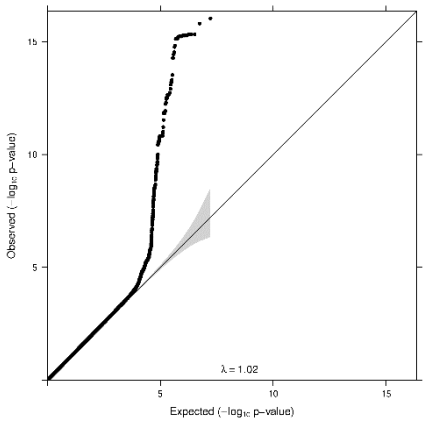

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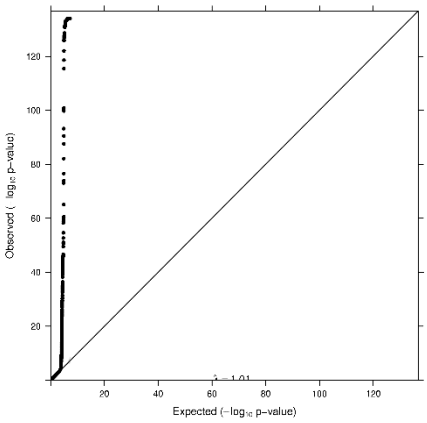

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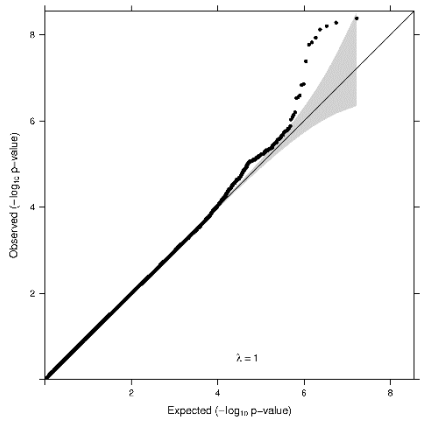

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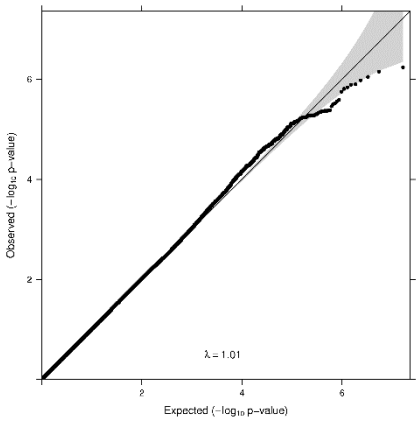

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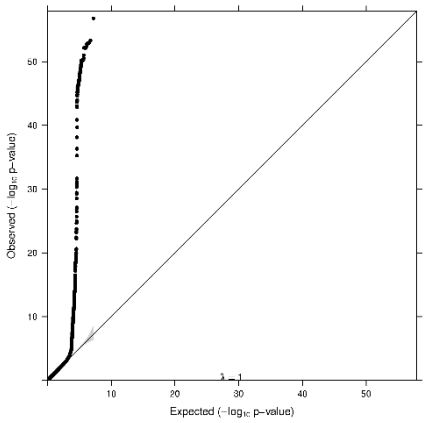
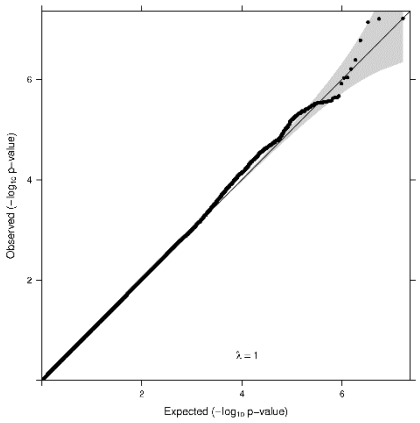


Protein C



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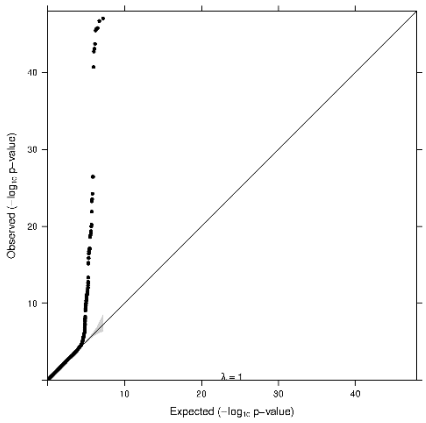

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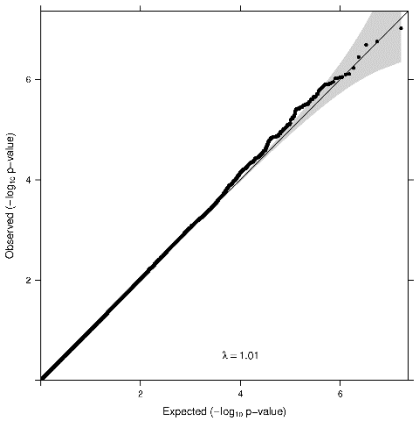

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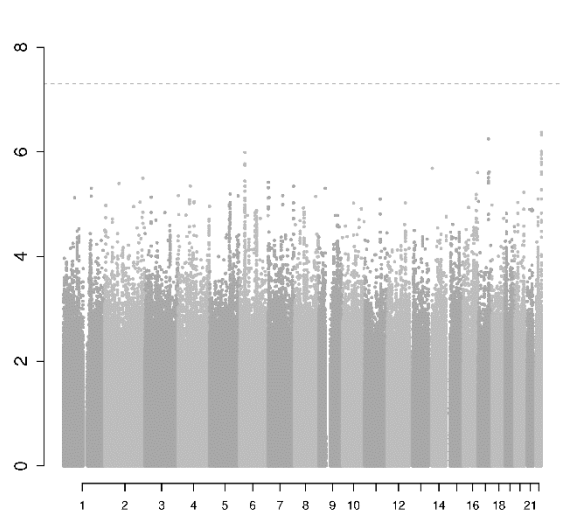
Supplementary Figure 8: Manhattan plots from genome-wide association studies of identified proteins.



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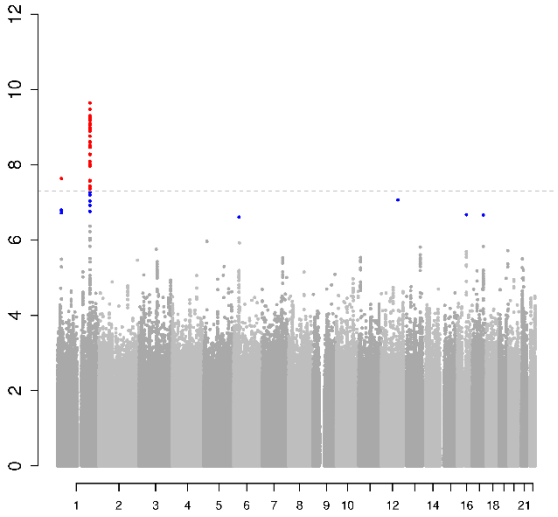

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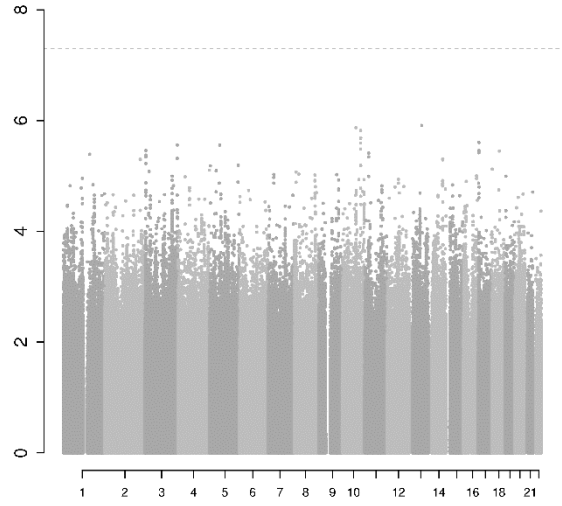

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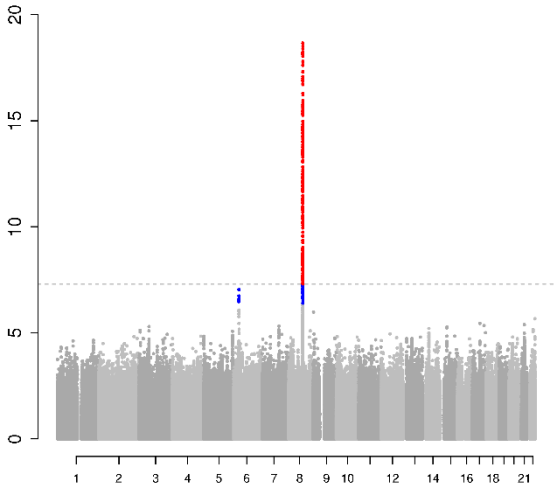
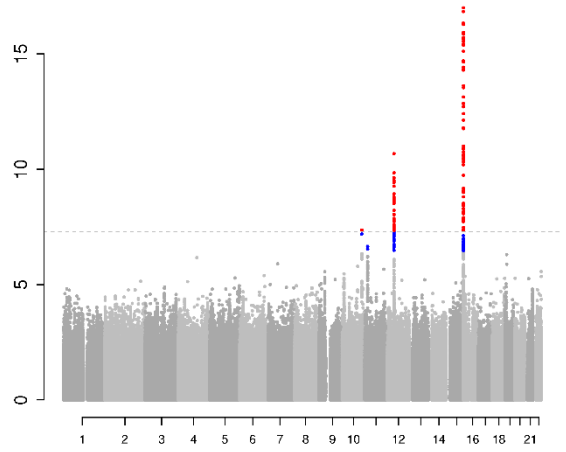


Carbonic anhydrase XIII



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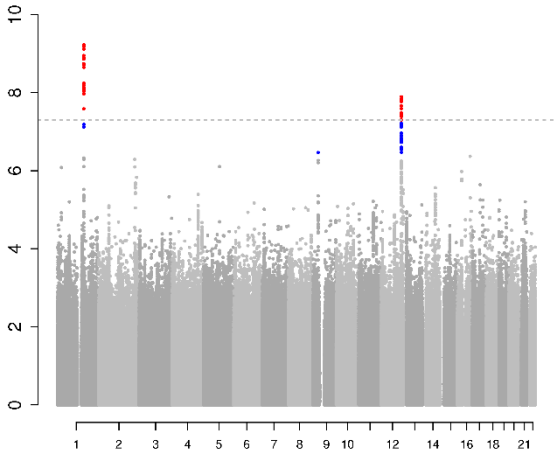

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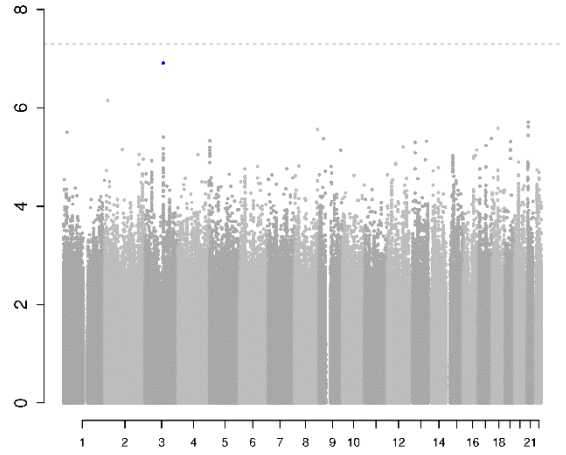

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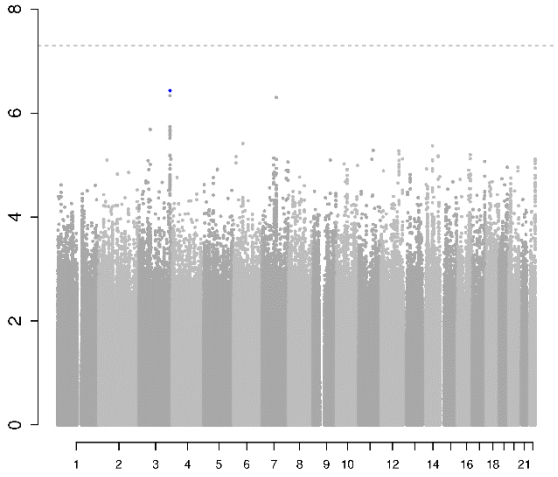

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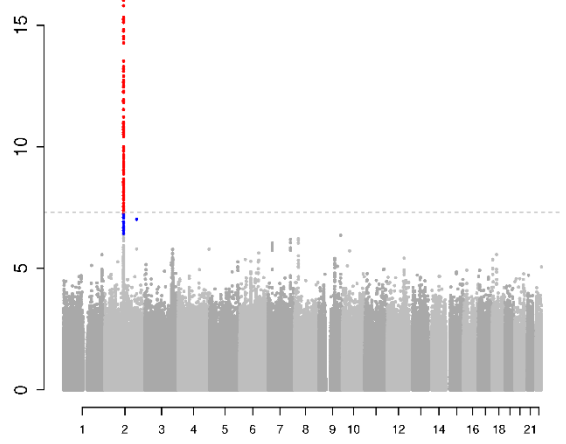

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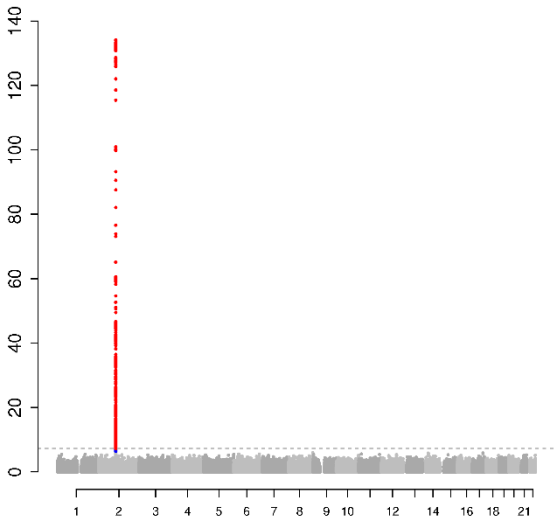

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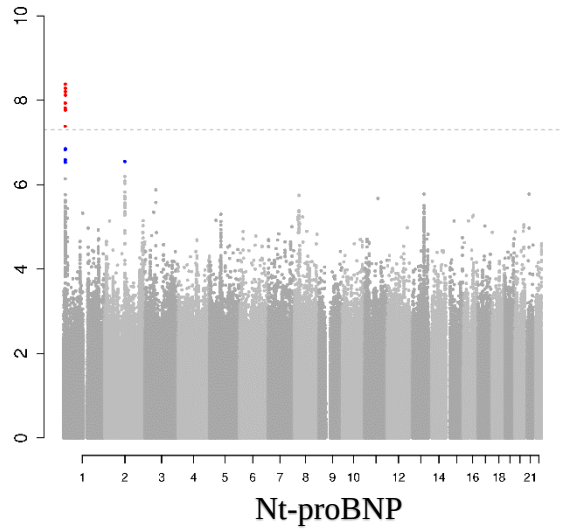
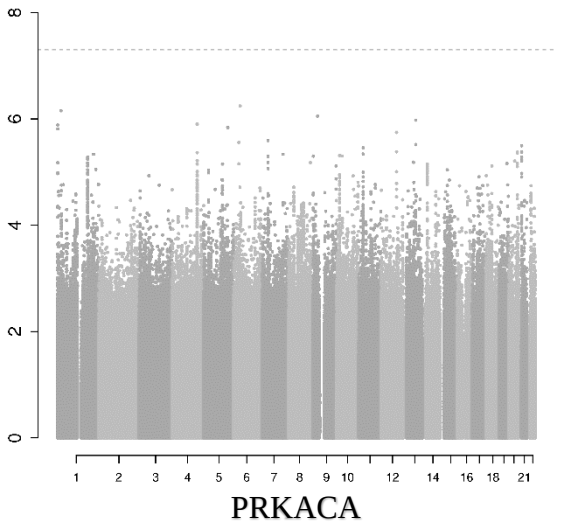


Nt-proBNP



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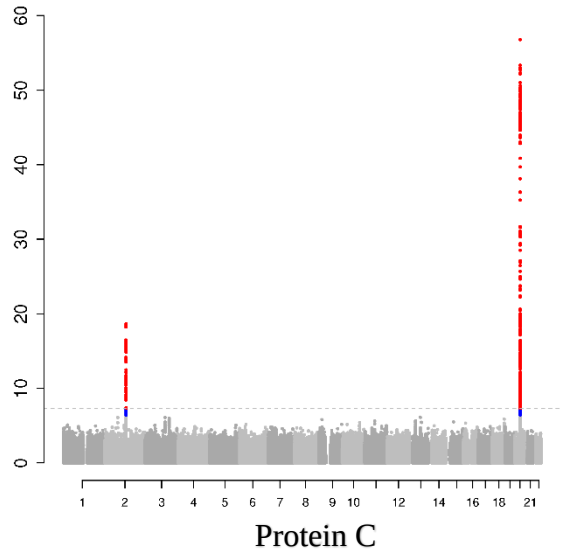
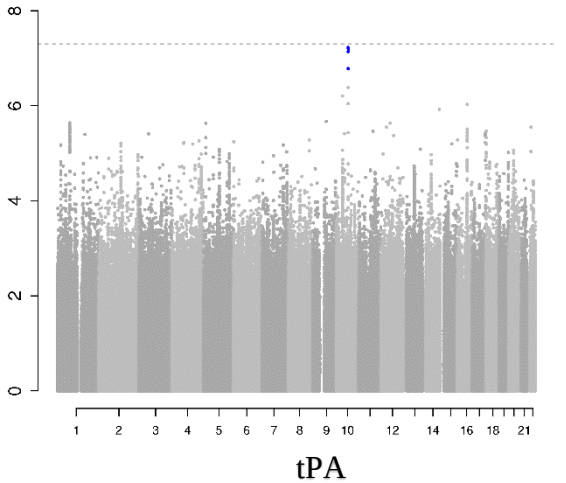


Protein C



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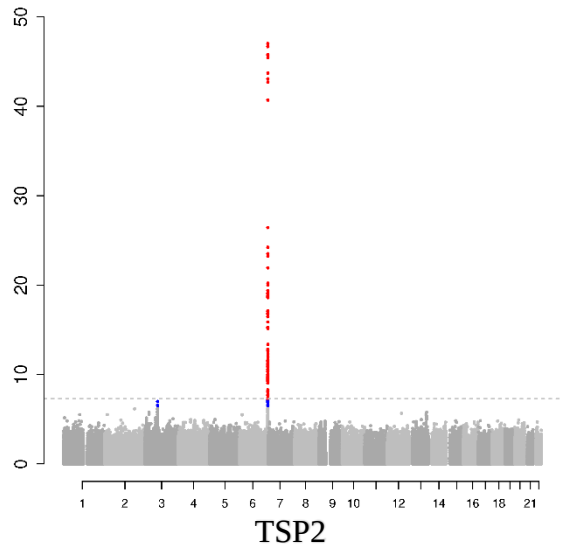

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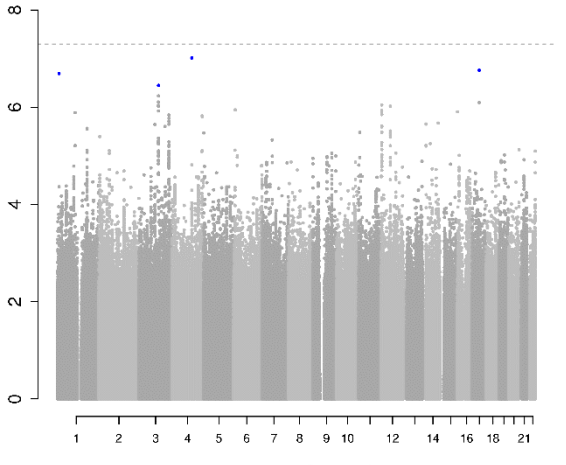

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Supplementary table 1. Comparison of risk factor estimates for incident HF in the full population-based cohort and the nested case-cohort sample.

Covariate	Population cohort		Nested case-cohort	
	Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**
Age, per year	1.12	1.09-1.15	1.17	1.11-1.24
Male sex	1.83	1.37-2.45	1.96	1.18-3.24
Systolic blood pressure, per 10 mm Hg	1.05	0.96-1.15	1.04	0.86-1.27
Diastolic blood pressure, per 10 mm Hg	1.00	0.82-1.20	1.18	0.77-1.82
Antihypertensive medications	1.96	1.46-2.62	1.93	1.16-3.21
History of diabetes	2.50	1.68-3.70	2.46	0.96-6.30
Body mass index	1.10	1.07-1.13	1.15	1.08-1.21
LDL cholesterol	0.96	0.84-1.10	0.78	0.59-1.03
HDL cholesterol	0.75	0.49-1.14	0.82	0.42-1.62
Current smoking	1.80	1.34-2.41	1.88	1.13-3.14
History of myocardial infarction	2.26	1.38-3.70	1.04	0.34-3.22
Nt-proBNP, standardized	1.63	1.43-1.86	1.96	1.18-3.24

Estimates of relative risk for incident HF with individual risk factors in Prentice-weighted Cox proportional hazards regression in the nested subcohort were similar to unweighted estimates from the full population-based cohort study (Malmö Diet and Cancer Cardiovascular Cohort, n=6,103), with the exception of lack of effect of a history of myocardial infarction. The number of subjects with a history of myocardial infarction was however low in the nested case-cohort set, and overlap of confidence intervals was observed between the two cohorts.

Supplementary table 2. Baseline characteristics of clinical heart failure cohorts.

	HF outpatients *(n=84)*	**Heart transplant recipients** *(n=30)*
Heart rate, (bpm)	68 (60.75-81.25)	77 (71-88)
LVEF, (%)	30 (25-40)	20 (10-24)
Medications, (n [%])		
ACE inhibitor	66 (78.57%)	13 (43.44%)
ARB	20 (23.80%)	14 (46.67%)
Betablocker	76 (90.48%)	28 (93.33%)
MRA	48 (57.14%)	24 (80%)
Loop diuretic	65 (77.38%)	29 (96.67%)
CRT, (n [%])	10 (11.90%)	8 (26.67%)
ICD, (n [%])	22 (26.19%)	24 (80%)
Etiology, (n [%])		
Ischemic	28 (33.33%)	3 (10%)
DCM	33 (39.29%)	20 (66.67%)
Tachy-CM	4 (4.76%)	-
HCM	3 (3.57%)	3 (10%)
Myocarditis	2 (2.38%)	1 (3%)
PPCM	2 (2.38%)	0
Other	12 (14.29%)	3 (10%)
NYHA class, (n [%])		
I	22 (26.19%)	1 (3%)
II	37 (44.05%)	0
III	24 (28.57%)	27 (87%)
IV	-	3 (10%)

Median and interquartile range are presented for heart rate and LVEF. ACE, Angiotensin Converting Enzyme. ARB, Angiotensin II Receptor Blocker. CRT, cardiac resynchronization therapy. DCM, Dilated Cardiomyopathy. HCM, Hypertrophic Cardiomyopathy. HFMP, outpatient-based Heart Failure Management Program. ICD, intracardiac defibrillator. LVEF, left ventricular ejection fraction. MRA, mineralcorticoid receptor antagonists. NYHA, New York Heart Associaton. PPCM, Peripartum Cardiomyopathy. Tachy-CM, Tachycardia-induced cardiomyopathy.

Supplementary Table 3: Association with proteins from established pathways.

Protein	Manifest heart failure		Incident heart failure	
	OR	*P-value*	*HR*	*P-value*
Neurohormones				
Angiotensinogen	0.45	$5x10^{-5}$	1.03	0.76
Nt-ProBNP	29.68	$3x10^{-15}$	1.85	$4x10^{-7}$
Renin	6.36	$1x10^{-12}$	1.05	0.69
Inflammation				
CRP	2.05	$2x10^{-4}$	1.86	$3x10^{-7}$
TNFα	1.29	0.01	1.13	0.04
Myocyte stress				
ST2	2.50	$3x10^{-7}$	1.41	0.002
Troponin T	3.20	$1.72.10^{-10}$	1.33	0.002
Matrix remodeling				
Galectin-3	5.15	$8x10^{-10}$	1.18	0.11
Metalloproteinases				
MMP1	1.11	0.38	0.90	0.26
MMP2	2.19	$1x10^{-7}$	0.98	0.85
MMP3	1.20	0.11	0.89	0.27
MMP7	13.86	$8x10^{-8}$	1.34	0.02
MMP8	1.20	0.21	0.85	0.19
MMP9	3.35	$1x10^{-16}$	1.13	0.23
MMP10	1.53	0.009	1.00	0.97
MMP12	1.52	0.003	1.36	0.004
MMP13	1.38	$3x10^{-4}$	1.03	0.81
MMP14	1.36	0.007	0.96	0.72
MMP16	1.10	0.24	1.00	0.97
MMP17	2.40	0.003	1.07	0.33
MMP inhibitors				
TIMP1	0.97	0.74	1.03	0.78
TIMP2	2.01	$1x10^{-4}$	0.86	0.27
TIMP3	0.52	$8x10^{-4}$	0.92	0.35

Results are presented as odds ratios (OR) or hazard ratios (HR) with p-values.

Supplementary Table 4. Association with manifest heart failure for proteins identified for heart failure development.

Protein	**Nested population cohort** (n=583)	**Incident HF** (n=185)	**Manifest HF outpatients** (n=84)	**P-value**	**Protein class**	**Subcellular location**	**Tissue enrichment**
Vascular messengers							
Nt-proBNP	4,089 (2,351-5,981)	5,615 (3,281-11,537)	38,630 (16,352-72,782)	<0.001	Secreted		Tissue enriched
Matrix remodelling							
TSP2	12,930 (10,462-15,771)	14,592 (11,800-18,667)	20,051 (13,371-32,780)	<0.001	Secreted	Nuclear speckles, plasma membrane, cytosol	Tissue enhanced
Immune system							
CXCL13	2,011 (1,672-2,479)	2,302 (1,967-2,996)	2,599 (2,078-3,438)	<0.001	Secreted		Group enriched
CRP	43,186 (25,712-73,110)	70,995 (40,625-98,303)	82,280 (48,078-118,930)	<0.001	Secreted		Group enriched
IL-1R antagonist protein	3,966 (3,441-4,811)	4,506 (3,833-5,580)	4,982 (3,739-6,660)	<0.001	Secreted		Tissue enhanced
IL-18 receptor 1	9,274 (7,976-10,867)	10,225 (8,658-12,315)	12,062 (10,254-13,408)	<0.001	Secreted, Membrane	Mitochondria	Mixed
Complement system							
C5a	11,621 (9,491-14,956)	14,075 (11,003-18,282)	18,478 (12,977-23,682)	<0.001	Secreted		Tissue enriched
C9	68,658 (61,101-76,994)	74,440 (66,080-84,930)	85,160 (68,345-95,255)	<0.001	Secreted	Plasma membrane, cytosol	Tissue enriched
Coagulation system							
Protein C	1,685 (1,561-1,854)	1,619 (1,486-1,811)	1,451 (900-1,640)	<0.001	Secreted		Tissue enriched

tPA	320 (259-400)	379 (297-488)	499 (370-639)	<0.001	Secreted	Actin filaments	Tissue enriched
Intracellular or membrane-bound							
Gelsolin	954 (866-1,035)	890 (790-984)	806 (722-913)	<0.001	Secreted	Actin filaments	Expressed in all
Carbonic anhydrase 13	9,336 (6,732-13,319)	7,277 (5,129-10,709)	2,126 (1,150-3,468)	<0.001	Intracellular	Nucleus, nucleoli, vesicles	Mixed
Contactin-1	282 (254-310)	261 (236-291)	240 (213-273)	<0.001	Secreted		Tissue enhanced
DUSP3	4,956 (3,735-6,350)	3,930 (3,084-5,600)	1,227 (811-1,861)	<0.001	Intracellular	Nucleoplasm, cytosol	Expressed in all
PRKACA	9,280 (7,099-12,312)	8,222 (5,863-10,889)	1,846 (1,354-3,100)	<0.001	Intracellular	Cytosol	Expressed in all
Netrin receptor UNC5D	4,816 (4,002-5,762)	4,320 (3,615-5,050)	5,274 (4,042-6,651)	0.08	Membrane		Tissue enhanced

Values in relative fluorescent units from the general population cohort and cases with manifest heart failure presented as median and interquartile range. P-values for difference between subjects from the general population and manifest heart failure were computed using linear regression of log-transformed concentrations adjusted for age and sex. Information on protein class (intracellular, membrane-bound, secreted, unclassified) and subcellular location (cytosol, nucleus, vesicles, Golgi apparatus, etc) was obtained from the Human Protein Atlas (HPA, https://www.proteinatlas.org, accessed sept 9, 2018). Information on tissue enrichment was also obtained from the HPA, based on RNA-sequencing of 64 cell lines and 37 tissues. Tissue expression was classified by the HPA into six categories: tissue enriched (expression in one tissue at least five-fold higher than all others), group enriched (five-fold higher in 2-7 tissues compared to all other), tissue enhanced (five-fold higher in one or more tissues compared to the mean of all tissues), expressed in all (≥1 transcript per million in all tissues), not detected (<1 transcript per million in all tissues), and mixed (detected in at least one tissue and not in the other categories).

Supplementary table 5. Correlation of proteins associated with heart failure development.

	C5a	Gelsolin	CXCL13	TSP2	Protein C	CRP	Nt-proBNP	CA13	Contactin-1	C9	DUSP3	tPA	IL-1RA	IL-18R1	PRKACA	Netrin receptor UNC5D
C5a	1.00															
Gelsolin	**-0.43**	1.00														
CXCL13	0.06	-0.15	1.00													
TSP2	0.12	-0.15	0.29	1.00												
Protein C	0.06	-0.04	-0.20	**-0.31**	1.00											
CRP	**0.58**	**-0.41**	0.17	0.20	-0.09	1.00										
Nt-proBNP	-0.12	0.14	0.21	**0.30**	-0.20	0.01	1.00									
CA13	-0.20	0.24	-0.14	-0.24	0.21	-0.22	-0.08	1.00								
Contactin-1	**-0.40**	**0.38**	-0.10	-0.03	0.006	**-0.36**	0.10	0.18	1.00							
C9	**0.33**	-0.19	0.14	0.05	-0.16	**0.34**	0.14	-0.06	-0.23	1.00						
DUSP3	-0.28	0.21	-0.18	-0.27	0.19	-0.25	-0.10	**0.82**	0.12	-0.10	1.00					
tPA	0.03	-0.15	0.11	0.18	-0.10	0.20	0.10	-0.24	-0.01	-0.19	-0.22	1.00				
IL-1RA	0.20	-0.17	0.12	0.28	-0.17	0.29	0.03	-0.13	-0.19	0.008	-0.14	0.14	1.00			
IL-18 receptor 1	0.20	-0.19	0.24	**0.48**	-0.23	0.22	0.14	**-0.32**	-0.09	0.0007	**-0.34**	0.19	0.29	1.00		
PRKACA	-0.27	**0.31**	-0.21	-0.20	0.12	-0.22	-0.07	**0.75**	0.18	-0.13	**0.74**	-0.17	-0.10	-0.28	1.00	
Netrin receptor UNC5D	-0.19	0.28	0.02	0.10	-0.10	-0.23	0.26	-0.007	0.18	0.02	-0.07	-0.14	0.003	0.12	0.02	1.00

Pairwise correlation of log-transformed proteins associated with heart failure, expressed as Pearson's correlation coefficients. Coefficients >0.3 have been indicated in bold. All correlation coefficients >0.07 were had $p<0.05$. CA13, Carbonic anhydrase 13. IL-1RA, IL-1R antagonist protein.

Supplementary Table 6: Gene set enrichment analysis for manfiest heart failure.

Gene Set	Genes, total (K)	Genes, overlapping (k)	k/K	FDR q-value	Description
HALLMARK_PI3K_AKT_MTOR_SIGNALING	105	26	0.2476	$6x10^{-28}$	Genes up-regulated by activation of the PI3K/AKT/mTOR pathway.
HALLMARK_COMPLEMENT	200	29	0.1450	$1x10^{-24}$	Genes encoding components of the complement system, which is part of the innate immune system.
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	200	29	0.1450	$1x10^{-24}$	Genes defining epithelial-mesenchymal transition, as in wound healing, fibrosis and metastasis.
HALLMARK_COAGULATION	138	23	0.1667	$5x10^{-21}$	Genes encoding components of blood coagulation system; also up-regulated in platelets.
HALLMARK_IL6_JAK_STAT3_SIGNALING	87	19	0.2184	$7x10^{-20}$	Genes up-regulated by IL6 [GeneID=3569] via STAT3 [GeneID=6774], e.g., during acute phase response.
HALLMARK_ALLOGRAFT_REJECTION	200	25	0.1250	$8x10^{-20}$	Genes up-regulated during transplant rejection.
HALLMARK_INFLAMMATORY_RESPONSE	200	20	0.1000	$4x10^{-14}$	Genes defining inflammatory response.
HALLMARK_ANGIOGENESIS	36	11	0.3056	$1x10^{-13}$	Genes up-regulated during formation of blood vessels (angiogenesis).
HALLMARK_HYPOXIA	200	19	0.0950	$3x10^{-13}$	Genes up-regulated in response to low oxygen levels (hypoxia).
HALLMARK_TNFA_SIGNALING_VIA_NFKB	200	19	0.0950	$3x10^{-13}$	Genes regulated by NF-kB in response to TNF [GeneID=7124].
HALLMARK_APICAL_JUNCTION	200	17	0.0850	$3x10^{-11}$	Genes encoding components of apical junction complex.
HALLMARK_GLYCOLYSIS	200	17	0.0850	$3x10^{-11}$	Genes encoding proteins involved in glycolysis and gluconeogenesis.
HALLMARK_MYC_TARGETS_V1	200	17	0.0850	$3x10^{-11}$	A subgroup of genes regulated by MYC - version 1 (v1).
HALLMARK_XENOBIOTIC_METABOLISM	200	17	0.0850	$3x10^{-11}$	Genes encoding proteins involved in processing of drugs and other xenobiotics.
HALLMARK_INTERFERON_GAMMA_RESPONSE	200	16	0.0800	$3x10^{-10}$	Genes up-regulated in response to IFNG [GeneID=3458].
HALLMARK_KRAS_SIGNALING_UP	200	16	0.0800	$3x10^{-10}$	Genes up-regulated by KRAS activation.
HALLMARK_MTORC1_SIGNALING	200	16	0.0800	$3x10^{-10}$	Genes up-regulated through activation of mTORC1 complex.
HALLMARK_APICAL_SURFACE	44	9	0.2045	$7x10^{-10}$	Genes encoding proteins over-represented on the apical surface of epithelial cells, e.g., important for cell polarity (apical area).
HALLMARK_FATTY_ACID_METABOLISM	158	14	0.0886	$9x10^{-10}$	Genes encoding proteins involved in metabolism of fatty acids.

HALLMARK_APOPTOSIS	161	14	0.0870	$1x10^{-9}$	Genes mediating programmed cell death (apoptosis) by activation of caspases.
HALLMARK_IL2_STAT5_SIGNALING	200	15	0.0750	$2x10^{-9}$	Genes up-regulated by STAT5 in response to IL2 stimulation.
HALLMARK_UV_RESPONSE_UP	158	13	0.0823	$8x10^{-9}$	Genes up-regulated in response to ultraviolet (UV) radiation.
HALLMARK_ADIPOGENESIS	200	12	0.0600	$1x10^{-6}$	Genes up-regulated during adipocyte differentiation (adipogenesis).
HALLMARK_MYOGENESIS	200	12	0.0600	$1x10^{-6}$	Genes involved in development of skeletal muscle (myogenesis).
HALLMARK_P53_PATHWAY	200	11	0.0550	$7x10^{-6}$	Genes involved in p53 pathways and networks.
HALLMARK_UV_RESPONSE_DN	144	9	0.0625	$2x10^{-5}$	Genes down-regulated in response to ultraviolet (UV) radiation.
HALLMARK_ESTROGEN_RESPONSE_LATE	200	10	0.0500	$4x10^{-5}$	Genes defining late response to estrogen.
HALLMARK_G2M_CHECKPOINT	200	9	0.0450	$2x10^{-4}$	Genes involved in the G2/M checkpoint, as in progression through the cell division cycle.
HALLMARK_ANDROGEN_RESPONSE	101	6	0.0594	$7x10^{-4}$	Genes defining response to androgens.
HALLMARK_ESTROGEN_RESPONSE_EARLY	200	8	0.0400	0.001	Genes defining early response to estrogen.
HALLMARK_TGF_BETA_SIGNALING	54	4	0.0741	0.003	Genes up-regulated in response to TGFB1 [GeneID=7040].
HALLMARK_PROTEIN_SECRETION	96	5	0.0521	0.003	Genes involved in protein secretion pathway.
HALLMARK_INTERFERON_ALPHA_RESPONSE	97	5	0.0515	0.003	Genes up-regulated in response to alpha interferon proteins.
HALLMARK_HEME_METABOLISM	200	7	0.0350	0.004	Genes involved in metabolism of heme (a cofactor consisting of iron and porphyrin) and erythroblast differentiation.
HALLMARK_NOTCH_SIGNALING	32	3	0.0938	0.005	Genes up-regulated by activation of Notch signaling.
HALLMARK_HEDGEHOG_SIGNALING	36	3	0.0833	0.006	Genes up-regulated by activation of hedgehog signaling.
HALLMARK_PANCREAS_BETA_CELLS	40	3	0.0750	0.008	Genes specifically up-regulated in pancreatic beta cells.
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	49	3	0.0612	0.01	Genes up-regulated by reactive oxigen species (ROS).
HALLMARK_KRAS_SIGNALING_DN	200	6	0.0300	0.01	Genes down-regulated by KRAS activation.
HALLMARK_MYC_TARGETS_V2	58	3	0.0517	0.02	A subgroup of genes regulated by MYC - version 2 (v2).
HALLMARK_CHOLESTEROL_HOMEOSTASIS	74	3	0.0405	0.04	Genes involved in cholesterol homeostasis.
HALLMARK_E2F_TARGETS	200	5	0.0250	0.05	Genes encoding cell cycle related targets of E2F transcription factors.
HALLMARK_MITOTIC_SPINDLE	200	5	0.0250	0.05	Genes important for mitotic spindle assembly.

Pathways enriched (false discovery rate [FDR] <0.05) among proteins associated with manifest heart failure in gene-set enrichment analysis (GSEA) of hallmark pathways from the Molecular Signatures Database.

Supplementary Table 7. Evidence of cardiac origin.

Protein	Coronary sinus	Femoral vein	Ratio	Cardiac expression
Vascular messengers				
Nt-proBNP	-	-	-	G, Hg, N/A, M
Matrix remodelling				
TSP2	19,903 (10,844-29,252)	18,922 (10,248-30,996)	1.05	G, Hg, Hi, M
Immune system				
CXCL13	6,486 (2,973-9,651)	7,348 (2,849-9,515)	0.88	-, -, -, -
CRP	36,630 (34,278-40,330)	37,878 (36,402-39,364)	0.97	-, -, -, M
IL-1R antagonist protein	-	-	-	-, -, -, M
IL-18 receptor 1	11,827 (8,650-13,962)	11,552 (8,422-12,671)	1.02	G, Hg, Hi, M
Complement system				
C5a	14,576 (10,762-15,988)	12,855 (10,175-18,259)	1.13	N/A, M
C9	73,155 (63,848-102,885)	82,635 (64,692-100,912)	0.89	-, -, -, M
Coagulation system				
Protein C	1,734 (1,646-2,083)	1,747 (1,650-2,029)	0.99	-, -, -, M
tPA	843 (646-920)	792 (682-905)	1.06	-, -, -, M
Intracellular or membrane-bound				
Gelsolin	795 (777-882)	768 (747-900)	1.04	G, Hg, -, M
Carbonic anhydrase 13	881 (689-1,055)	939 (868-1,030)	0.94	G, Hg, Hi, M
Contactin-1	311 (277-352)	335 (278-258)	0.93	-, -, -, M
DUSP3	560 (480-900)	622 (555-768)	0.90	G, Hg, N/A, M
PRKACA	1,346 (1,241-1,463)	1,530 (1,154-1,796)	0.88	G, Hg, Hi, M
Netrin receptor UNC5D	5,068 (4,667-7,901)	4,616 (4,072-7,944)	1.10	-, -, N/A, -

Samples obtained from patients with heart failure due to hypertrophic cardiomyopathy with outflow obstruction before underoing alcohol septal ablation. Abundance expressed in relative fluorescent units from coronary sinus and femoral vein plasma presented as median (25th-75th percentile). Paired measures from the two sampling sites were compared with the Wilcoxon signed-rank test. All proteins except Nt-proBNP and IL-1RA were available on the older version of the aptamer-based proteomic assay used in this cohort. For cardiac expression, ‘G‘ indicates cardiac gene expression in GTEx, ‘Hg’ cardiac gene expression in the Human Protein Atlas, ‘Hi’ cardiac protein expression according to immunohistochemistry in the Human Protein Atlas, and ‘M’ cardiac protein expression according to mass spectrometry. N/A indicates that the protein was not available in the immunohistochemistry data, and for C5a that is was also not available in the gene expression datasets due to the post-translational processing necessary for generation of this protein.

Supplementary Table 8: Results from genome-wide association analyses.

Protein	Location	Locus	Position	SNP	Beta	SE	P-value	cA1	A2	CAF	
Nt-proBNP	cis	1p36	11,915,467	rs198379	0.22	0.03	$1x10^{-13}$	T	C	0.60	*
TSP2	Cis	6q27	169,624,770	rs74507247	0.83	0.06	$2x10^{-47}$	A	G	0.11	
CXCL13	-	-		-	-	-	-	-	-	-	
CRP	cis	1q23	159,693,605	rs2211320	-0.23	0.04	$6x10^{-10}$	A	G	0.34	
IL-1RAP	cis	2q13	113,834,434	rs12468224	0.32	0.04	$9x10^{-17}$	G	C	0.44	
IL-18R1	cis	2q12	102,991,181	rs6731154	0.87	0.04	$9x10^{-135}$	C	T	0.27	
C5a	cis	9q33	123,778,148	rs10116271	0.16	0.03	$3x10^{-10}$	T	C	0.51	*
	trans	1q31	196,664,793	rs485632	0.25	0.04	$2x10^{-10}$	T	G	0.40	
C9	cis	5p13	39,327,888	rs62358361	-1.62	0.26	$4x10^{-10}$	T	G	0.01	
Protein C	cis	2q14	128,165,478	rs61185143	-0.35	0.04	$2x10^{-19}$	T	C	0.43	
	trans	20q11	33,784,021	rs11906148	0.90	0.06	$2x10^{-57}$	A	C	0.10	
tPA	cis	8p11	42,008,698	rs182065212	0.57	0.11	1x10-7	C	G	0.98	*
Gelsolin	cis	9q33	124,103,182	rs41273436	-0.80	0.13	2x10-9	T	C	0.99	*
Carbonic anhydrase 13	cis	8q21	86,139,240	rs6987314	-0.66	0.07	$2x10^{-19}$	A	G	0.08	
Contactin-1	cis	12q12	41,186,595	rs11178396	0.25	0.04	$2x10^{-11}$	C	A	0.34	
	trans	16p13	420,907	rs540	0.31	0.04	$1x10^{-17}$	T	C	0.42	
DUSP3	-	-	-	-	-	-	-	-	-	-	
PRKACA	-	-	-	-	-	-	-	-	-	-	
UNC5D	cis	8p12	35,035,535	rs6468309	-0.15	0.03	1x10-6	A	G	0.54	*

Results from genome-wide association analyses in the Malmö Diet and Cancer Study of 16 proteins associated with incident heart failure. Results from the index SNP, based on lowest p-value, at each locus. Beta coefficients with standard errors (SE) and p-value from general linear models adjusted for age and sex, as implemented in the R package GWAF. Coded allele (cA1), non-coded allele (A2) and coded allele frequency (CAF) are also presented. Positions refer to NCBI 37. * Asterisk indicates loci that were discovered in a second stages focused on discovery of cis-regulatory variants for the 7 proteins for which such variants were not discovered in the Malmö Diet and Cancer study. This analysis was conducted in the combination of Malmö Diet and Cancer results with those from a previously published analysis of the Framingham Heart Study (Benson M et al, Circulation 2018;137:1158-72). The number of SNPs tested in this analysis was smaller and a more permissive significance treshold was used.