

SUPPLEMENTAL MATERIAL

Sex-based differences in cardiovascular proteomic profiles and their associations with adverse outcome in patients with chronic heart failure

Running title: Sex differences in heart failure biomarkers

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Supplemental Methods

Proteomic analysis

Somalogic's previously described standard processes for normalization, calibration, and quality control (QC) were followed.[13] The normalization and calibration factors are deemed acceptable as follows: hybridization control, intraplate median signal normalization and plate scale factors are expected to be between 0.4 and 2.5; the distribution of QC sample ratios is expected to have 85% of individual SOMAmer reagents in the total array between 0.8 and 1.2. SOMAmers outside these ranges were not considered for the current study. Moreover, SOMAmers with non-human and/or not-validated targets were excluded from further analyses. When multiple SOMAmers were present for the same target protein, those with the highest binding affinity were used. Thus, 4210 out of the total 5,284 modified aptamers were included in the current analyses. Individual sample quality was judged by comparing normalized median signal relative to the external reference standard, with the acceptable normalization scaling range being 0.4 - 2.5. Data from 1,066 samples of patients with HFrEF passed quality-control criteria.

Statistical analyses

Sex-based differences in prognostic value of serially measured circulating proteins were evaluated using time-dependent Cox models for each of the individual proteins. Values of the circulating proteins were estimated and extracted for the moments at which the proteins were actually measured by linear mixed effects (LME) modeling. Protein levels were used as dependent variables. Sex, sampling time during follow-up, and an interaction term for sex and sampling time were used as independent variable. To allow the protein trajectories to differ between individuals at baseline and over time, an intercept and slope were included in the random-effects design matrix. If the model was not significantly improved, only a random intercept was retained. Non-linear evolutions were tested using natural cubic splines for both the fixed- and random-effects parts of the LME model. If the model was not significantly improved, a linear evolution was retained. Thereupon, the individual temporal trajectories of all circulating proteins were estimated by

the LME models described above and values of the circulating proteins as estimated by the models, for the moments at which the proteins were actually measured, were extracted. Subsequently, the estimated protein levels were standardized and entered into single protein time-dependent Cox models.

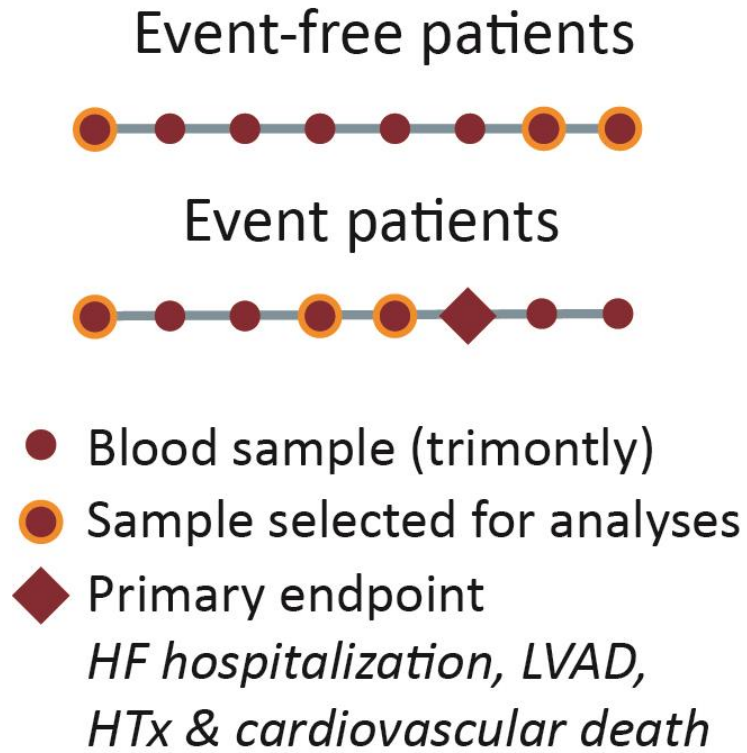
Supplemental Tables

Supplemental Table S1. Follow-up and study endpoints.

Primary study endpoint comprised the composite of:	Patients that reached endpoint during follow-up	Patients that reached primary study endpoint*
Re-hospitalization for acute or worsened HF	18 women and 72 men	18 women and 72 men
LVAD placement	2 women and 11 men	1 woman and 5 men
Heart transplantation	4 women and 13 men	2 women and 8 men
Cardiovascular death	6 women and 27 men	2 women and 6 men

*Only the first event was used for analysis in patients who reached multiple endpoints during follow-up (39 out of 114). Abbreviations: HF, heart failure.

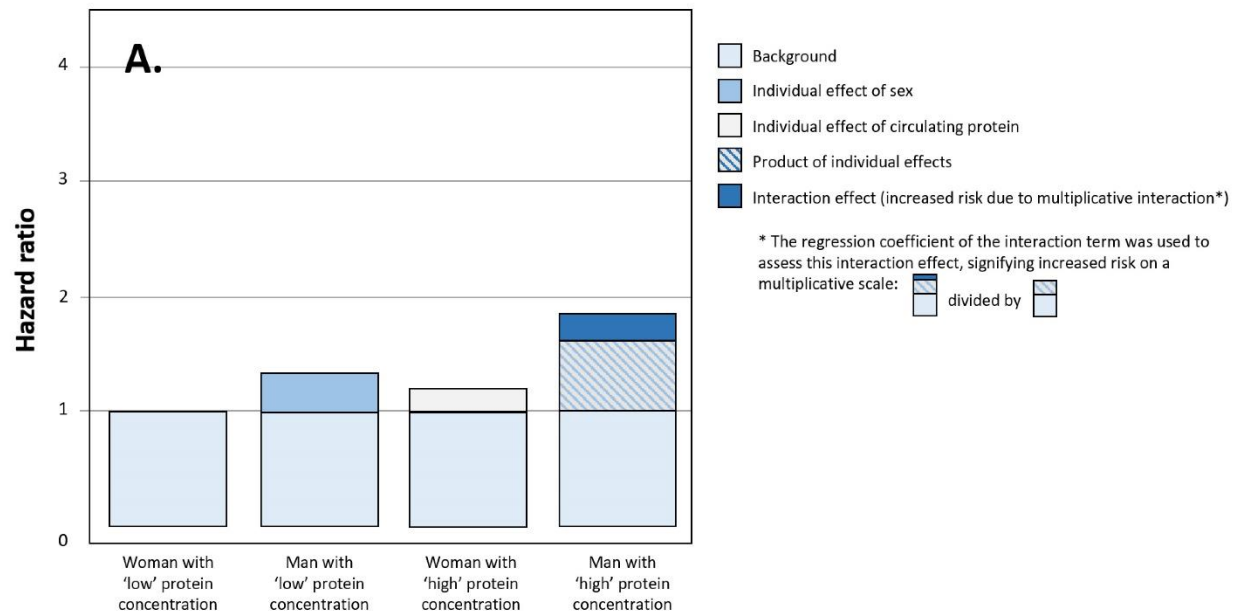
Supplemental Figures



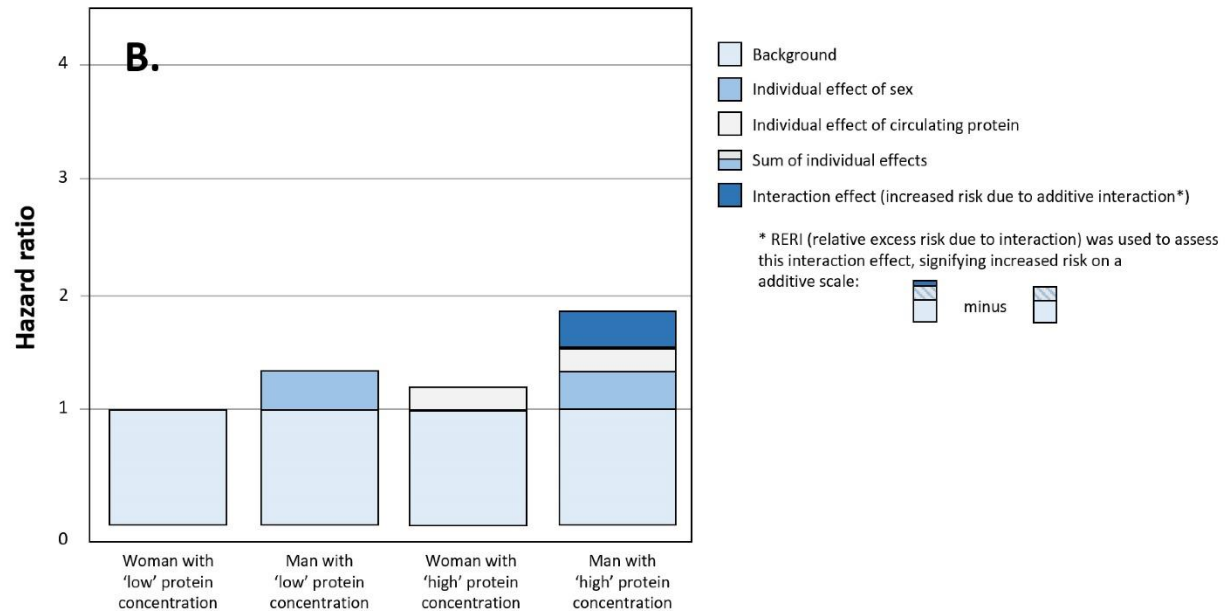
Supplemental Figure S1. Sample selection.

For the current investigation, all baseline blood samples were selected. Additionally, the last two samples drawn before the occurrence of the primary endpoint (PE), or the last two samples that were available before censoring for patients who remained endpoint-free, were selected.

Example multiplicative interaction



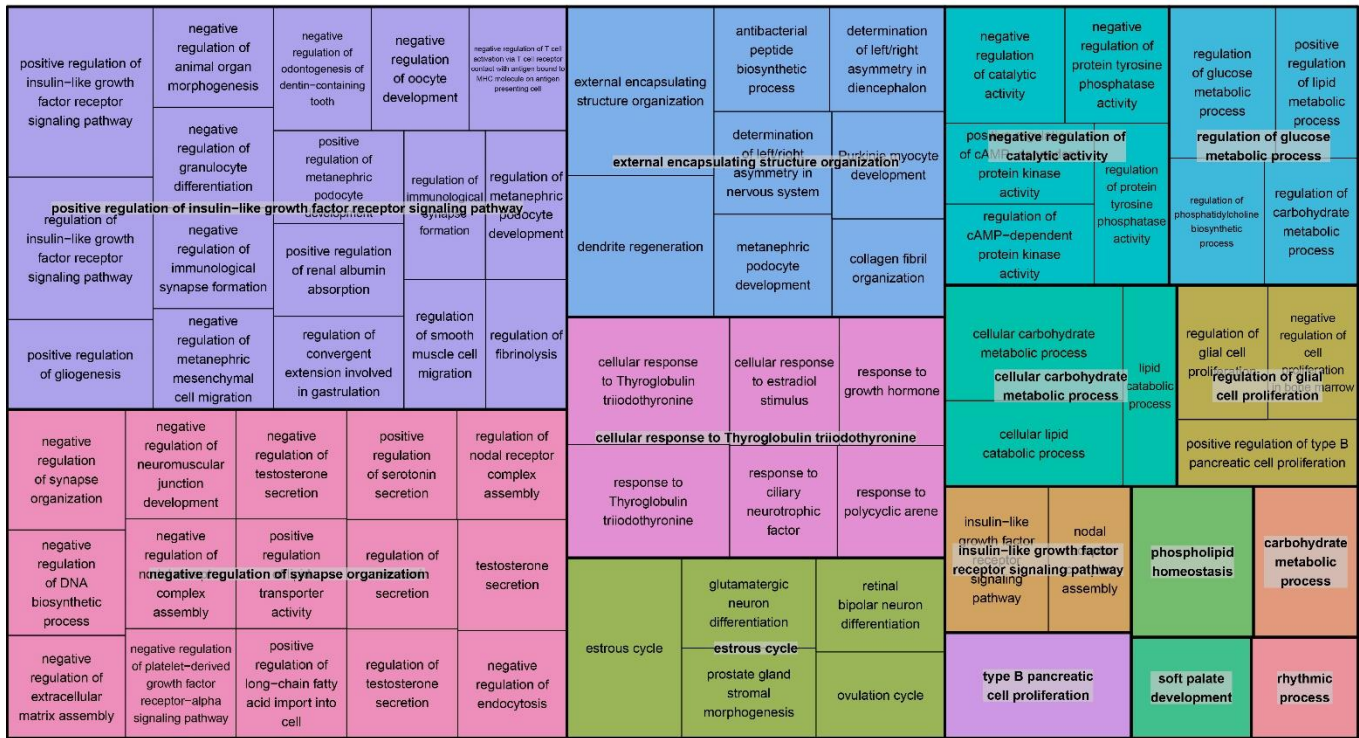
Example additive interaction



Supplemental Figure S2. Examples of interaction on the multiplicative and additive scale.

Interaction on the multiplicative scale (A) signifies that the combined effect of sex and protein level is larger [or smaller] than the product of the individual effects, and was assessed using the regression coefficient of the interaction term. Interaction on the additive scale (B) signifies that the combined effect of sex and protein level is larger [or smaller] than the sum of the individual effects, and was assessed using relative excess risk due to interaction (RERI).

A. Women



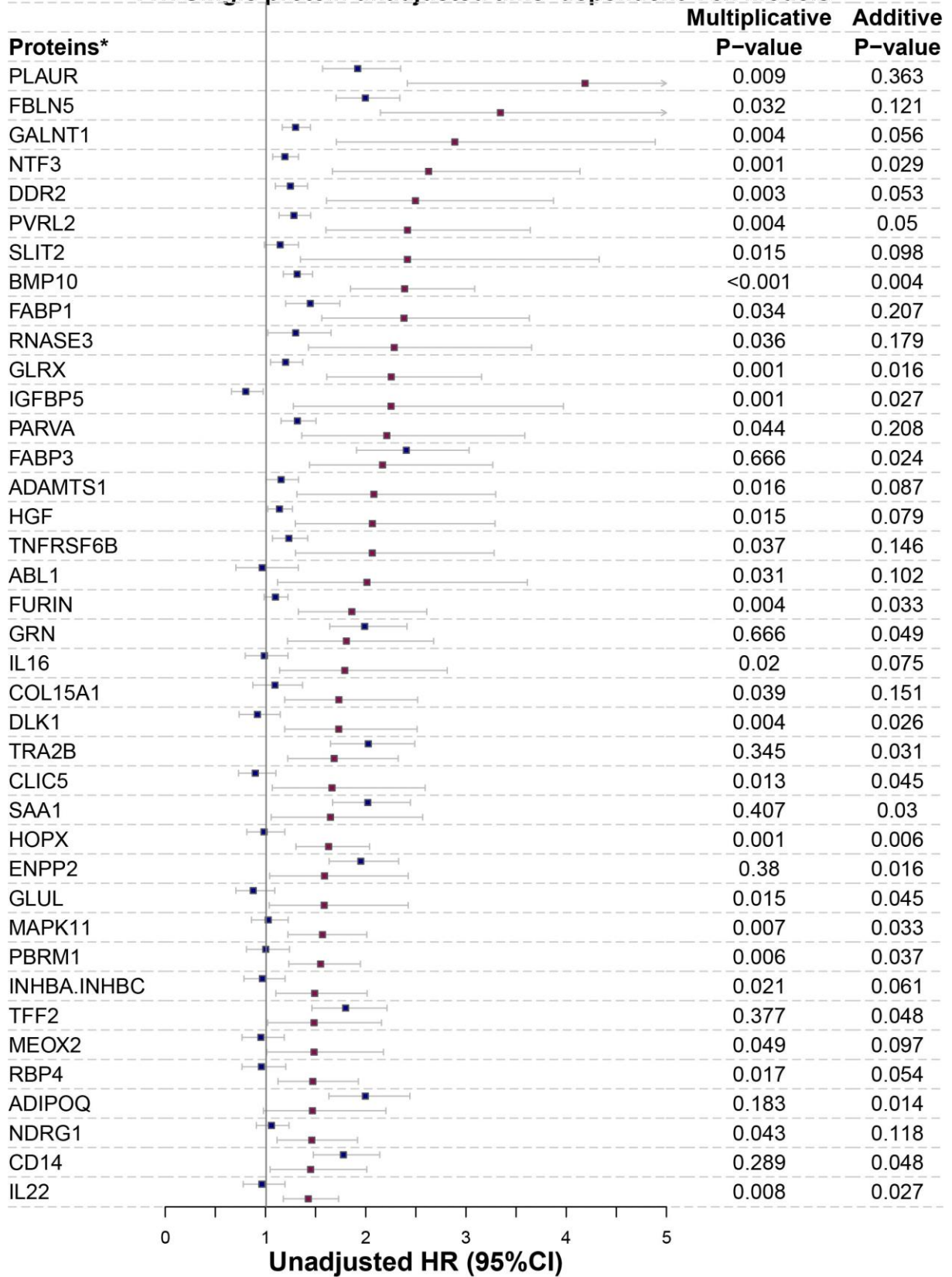
B. Men



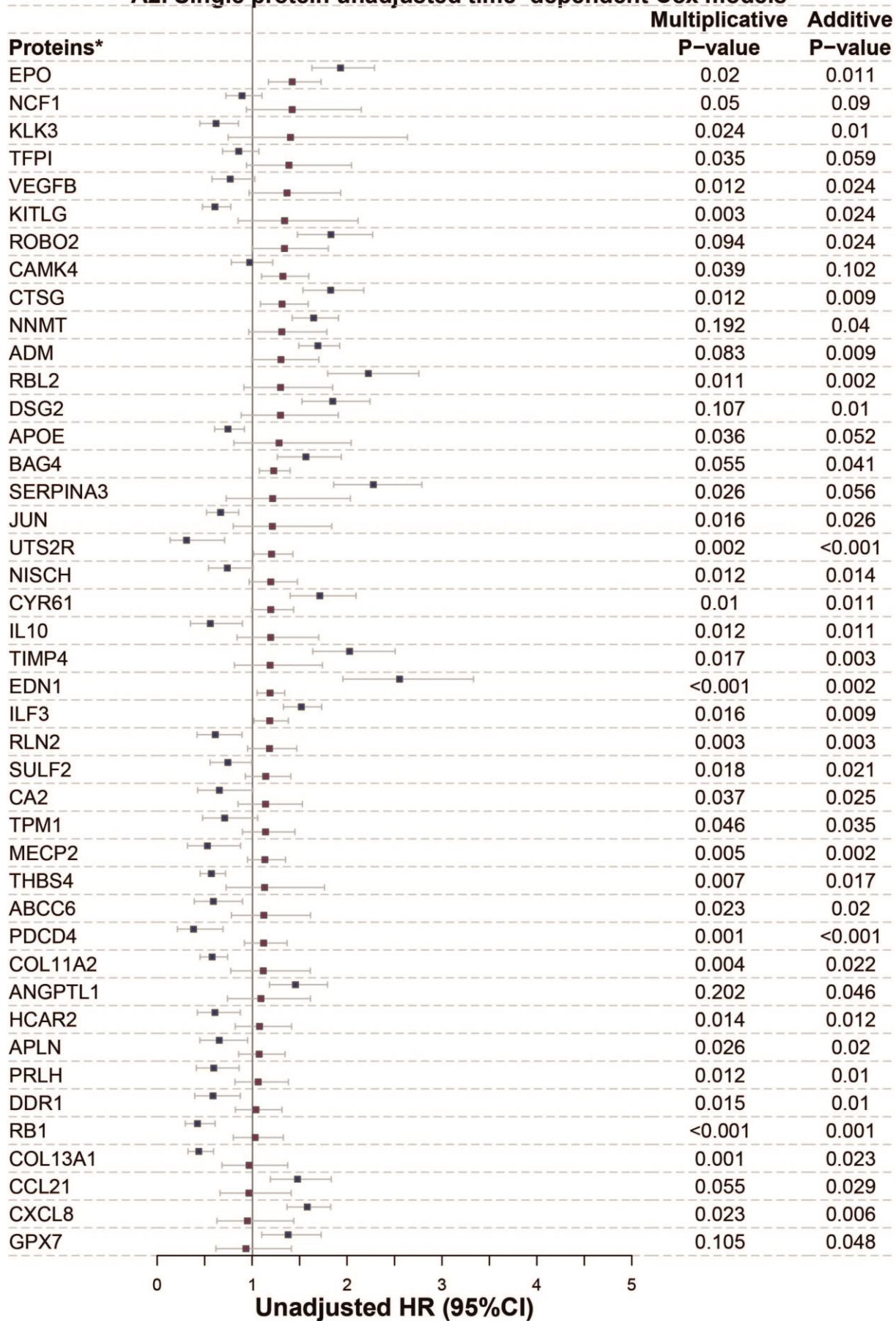
Supplemental Figure S3. Visualization of gene-enrichment analysis.

Treemap of GO biological processes associated with the circulating protein profile of women (A) and men (B), generated using REVIGO [38], a visualization tool that groups closely related GO terms together based on network analysis. Groups of closely related GO terms are plotted together in the same color. Size of the GO term blocks is proportional to the $-\log_{10}(\text{p-value})$ of their association with the sex-specific protein profile. Not all significant biological processes are represented as REVIGO removes some redundant terms that are very similar to other terms.

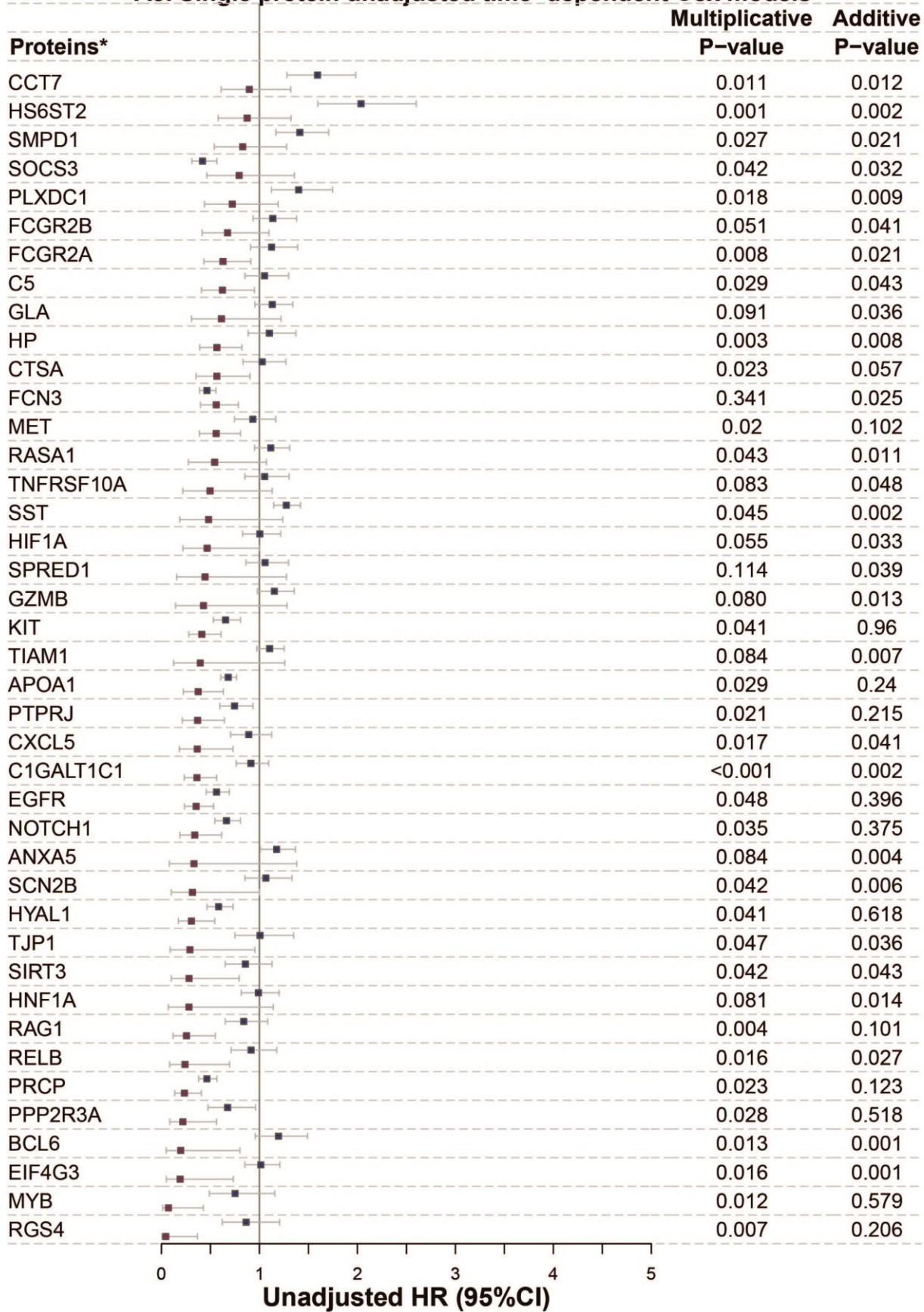
A1. Single protein unadjusted time-dependent Cox models



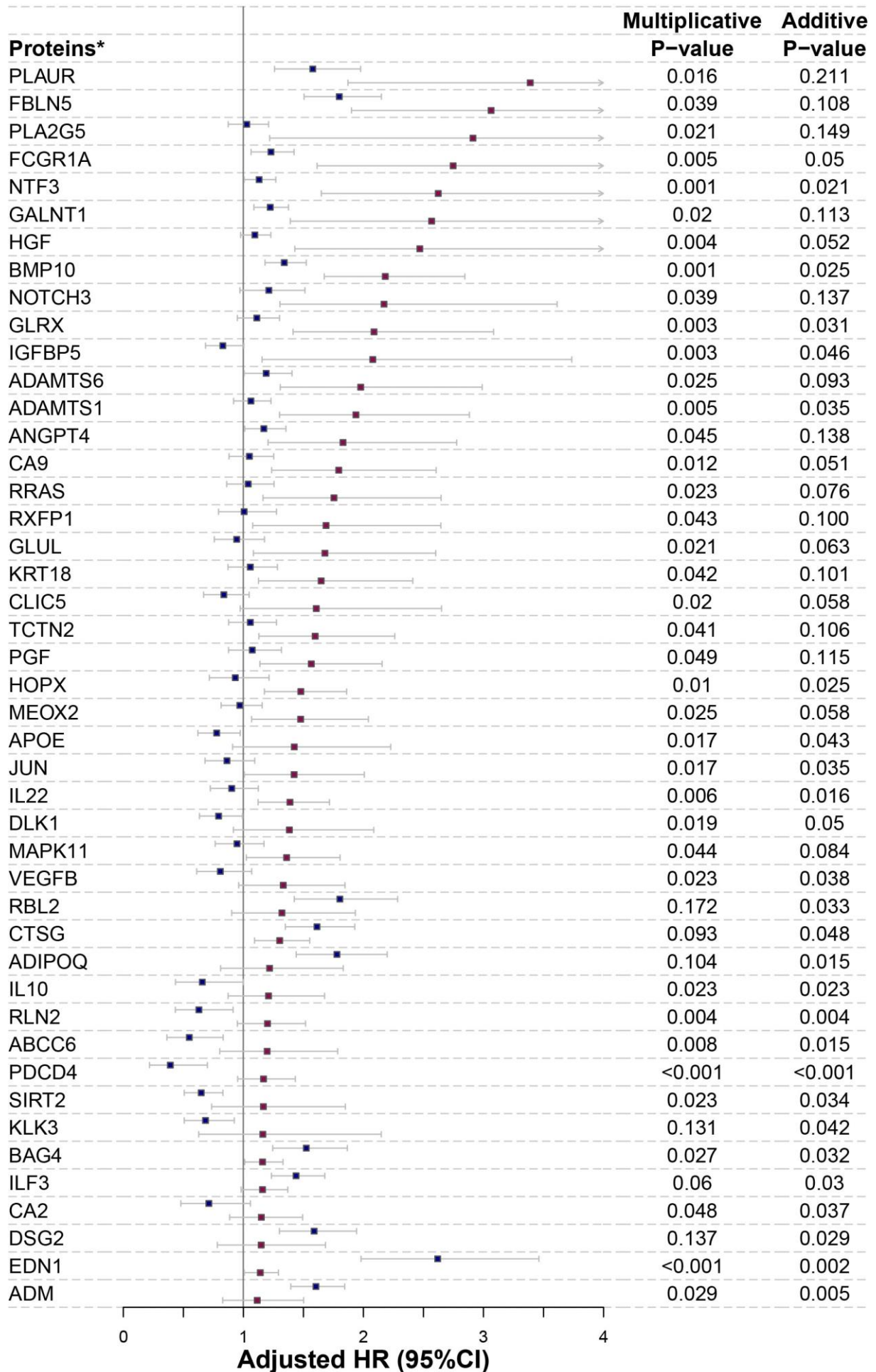
A2. Single protein unadjusted time-dependent Cox models



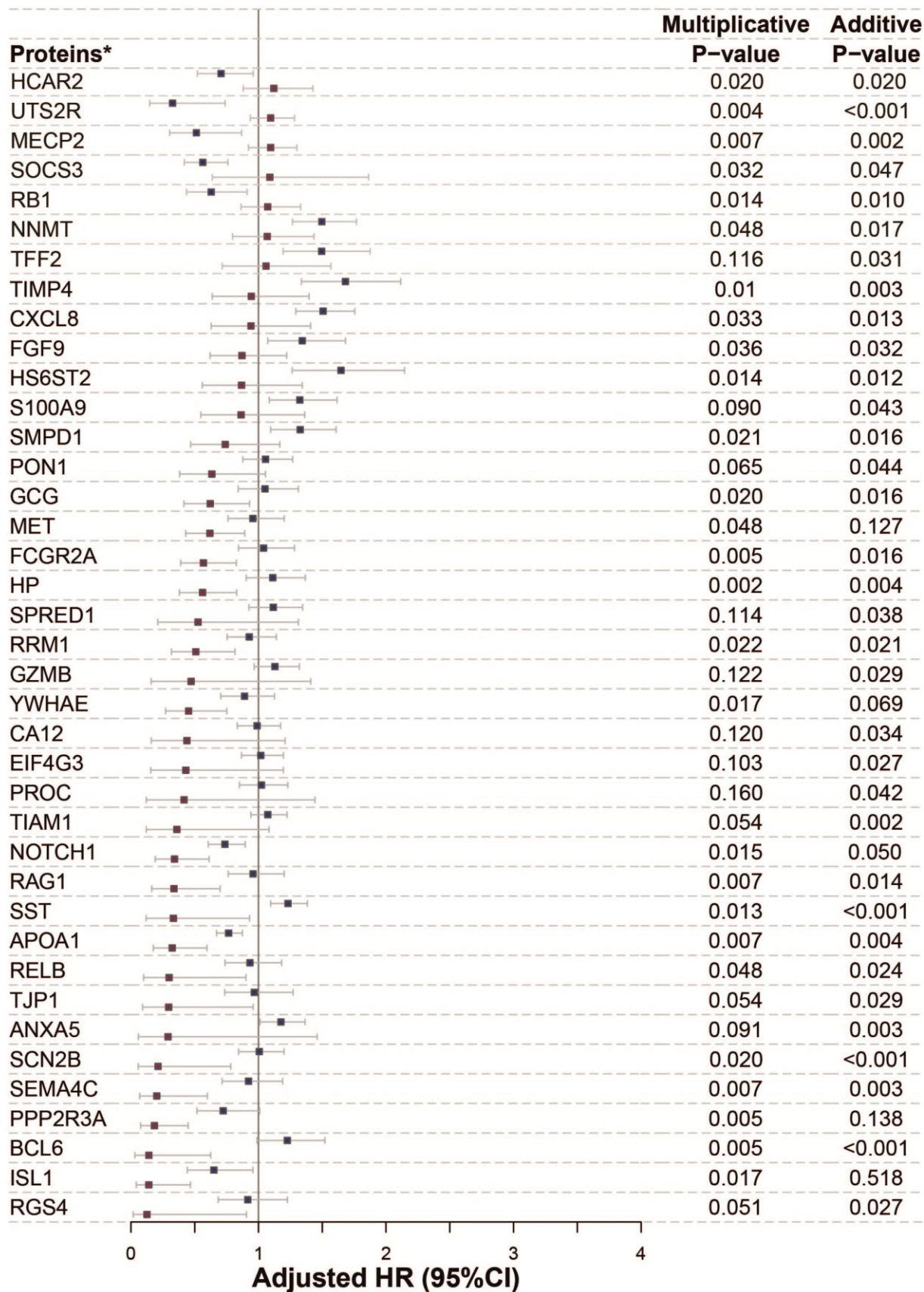
A3. Single protein unadjusted time-dependent Cox models



B1. Single protein adjusted time-dependent Cox models



B2. Single protein adjusted time-dependent Cox models



Supplemental Figure S4. Sex-based differences in predictive value of serially measured proteins (without multiple testing correction).

* Proteins are depicted by their HUGO Gene Nomenclature Committee (HGNC) gene symbol.