Haptoglobin-2 variant increases susceptibility to the acute respiratory distress syndrome during sepsis

Supplemental Data

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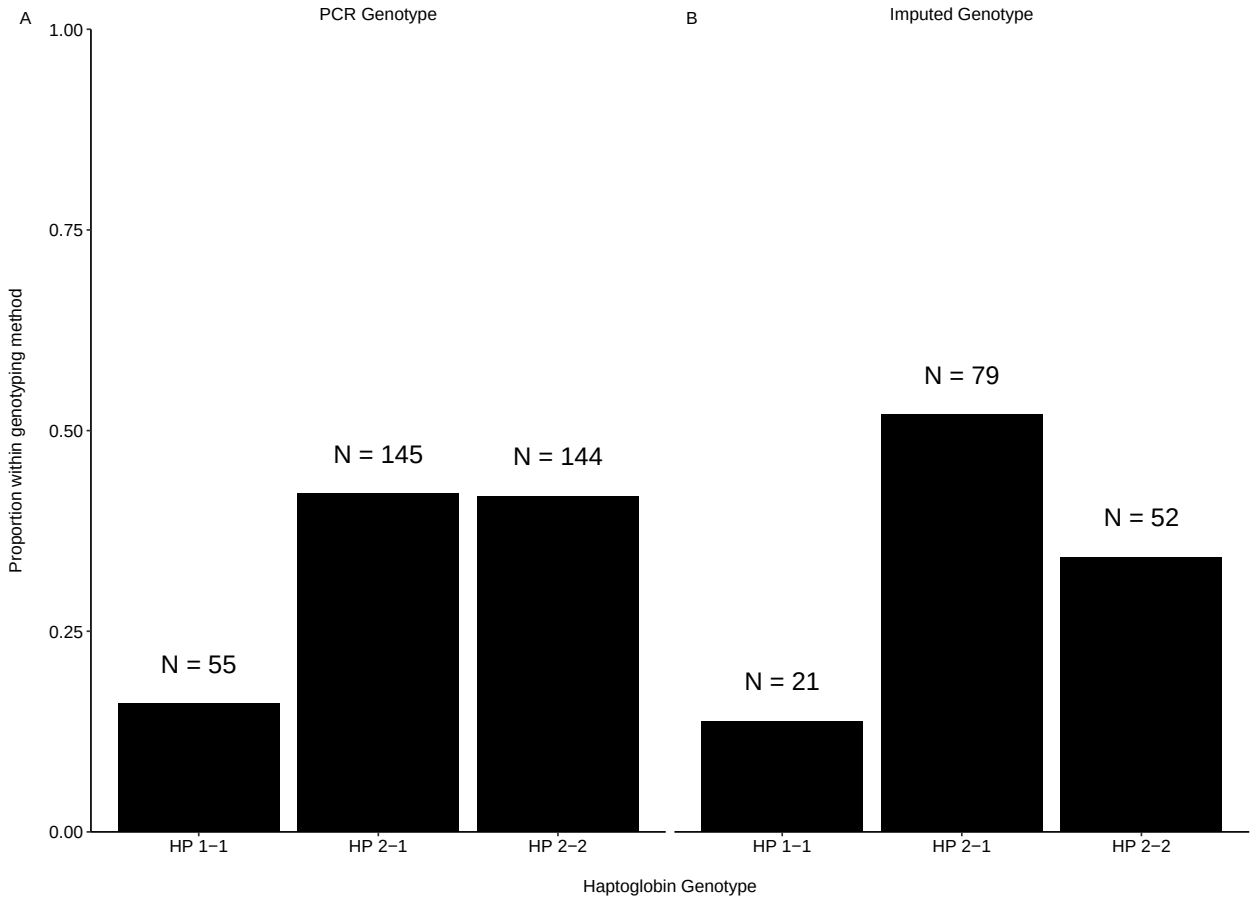
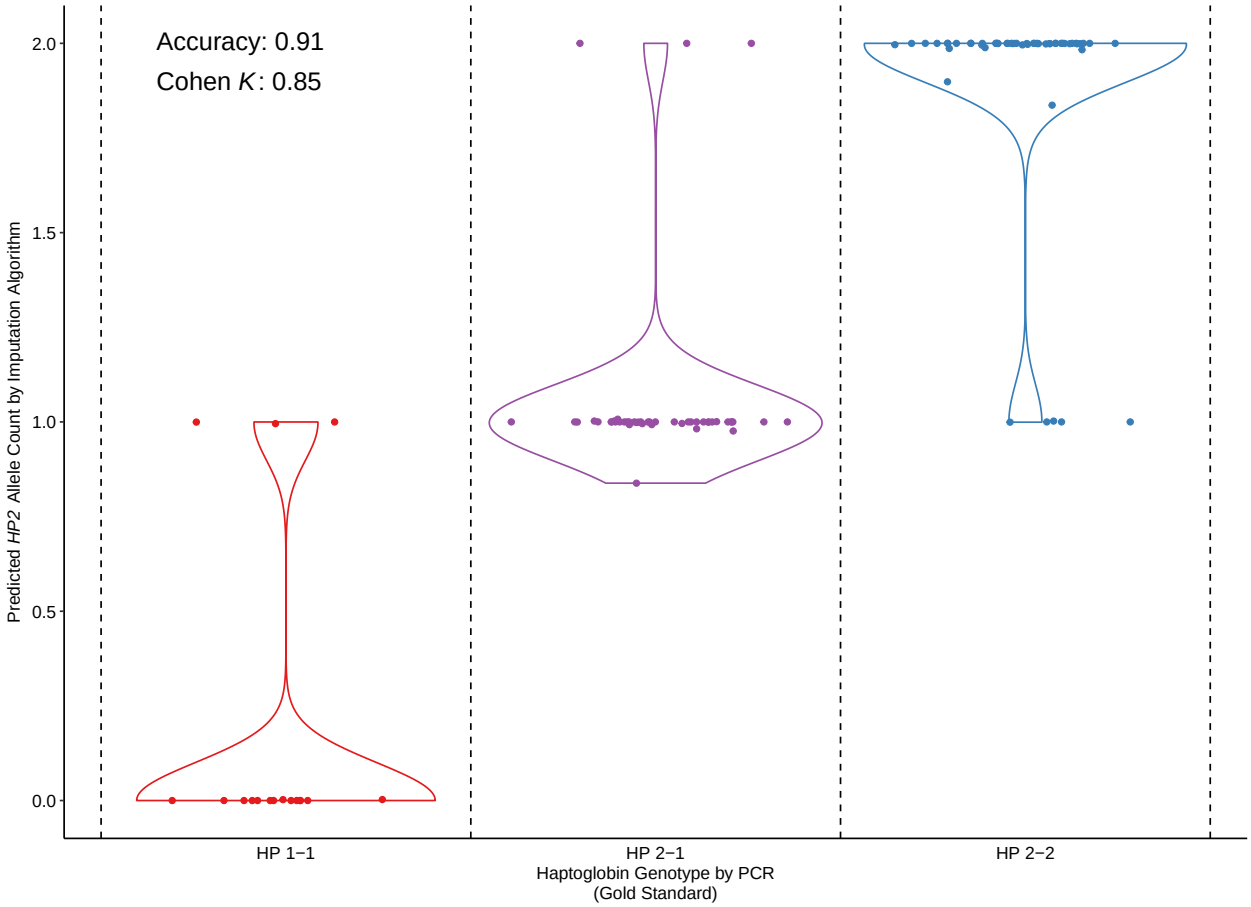
Supplemental Figure 1. Distribution of haptoglobin genotypes in study population stratified by genotyping method



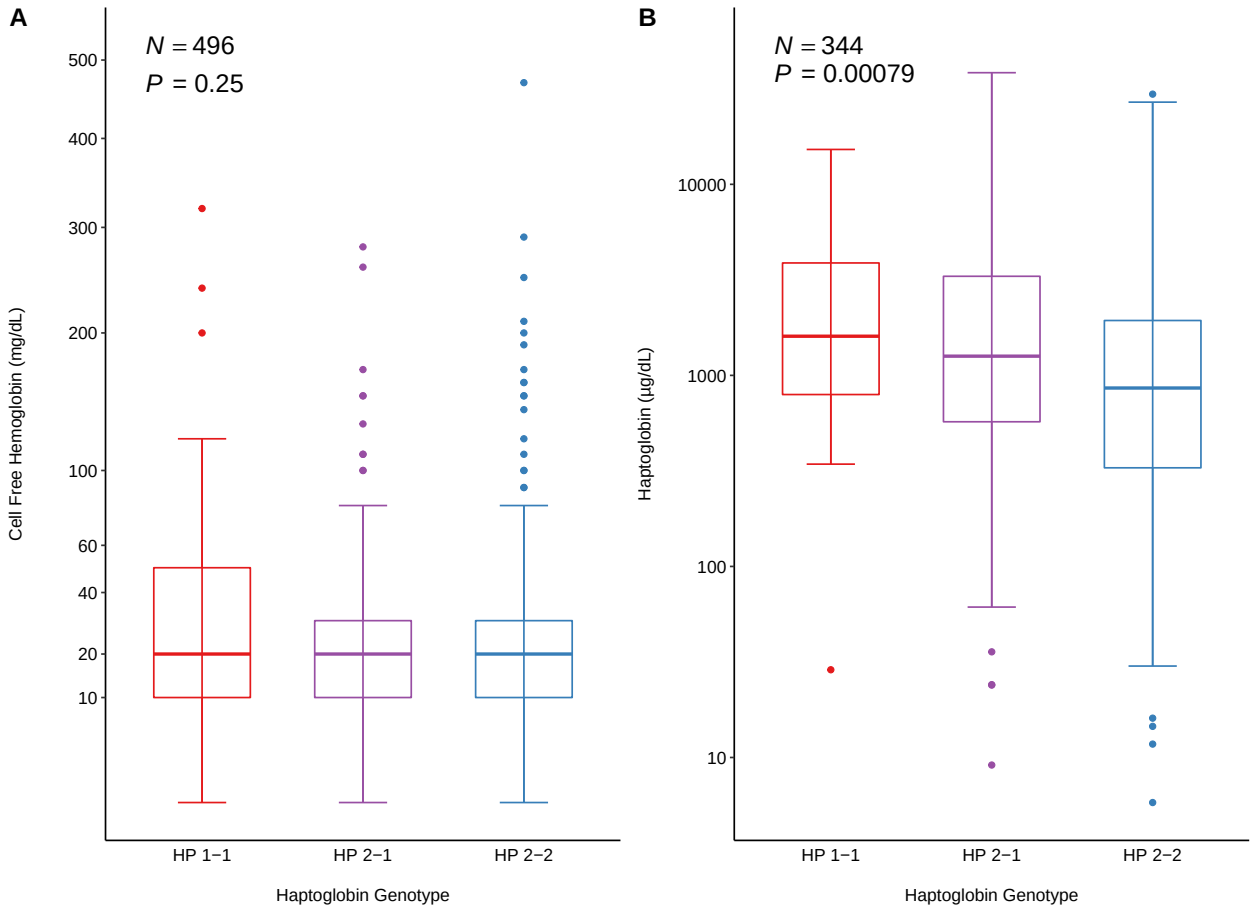
DNA samples were obtained from 496 critically ill adults with sepsis. (**A**) 344 patients underwent genotyping by real-time PCR, and (**B**) 152 by imputation from GWAS array data. Numbers over bars represent total number of patients with specified genotype, height of bars represent proportion of patients with the specified genotype within the genotyping group (PCR or Imputation). $P = 0.45$ for difference in *HP2* allele frequencies between PCR and imputed methods by 2-sample binomial proportion test.

Supplemental Figure 2. Imputed genotype accurately predicts PCR haptoglobin genotype



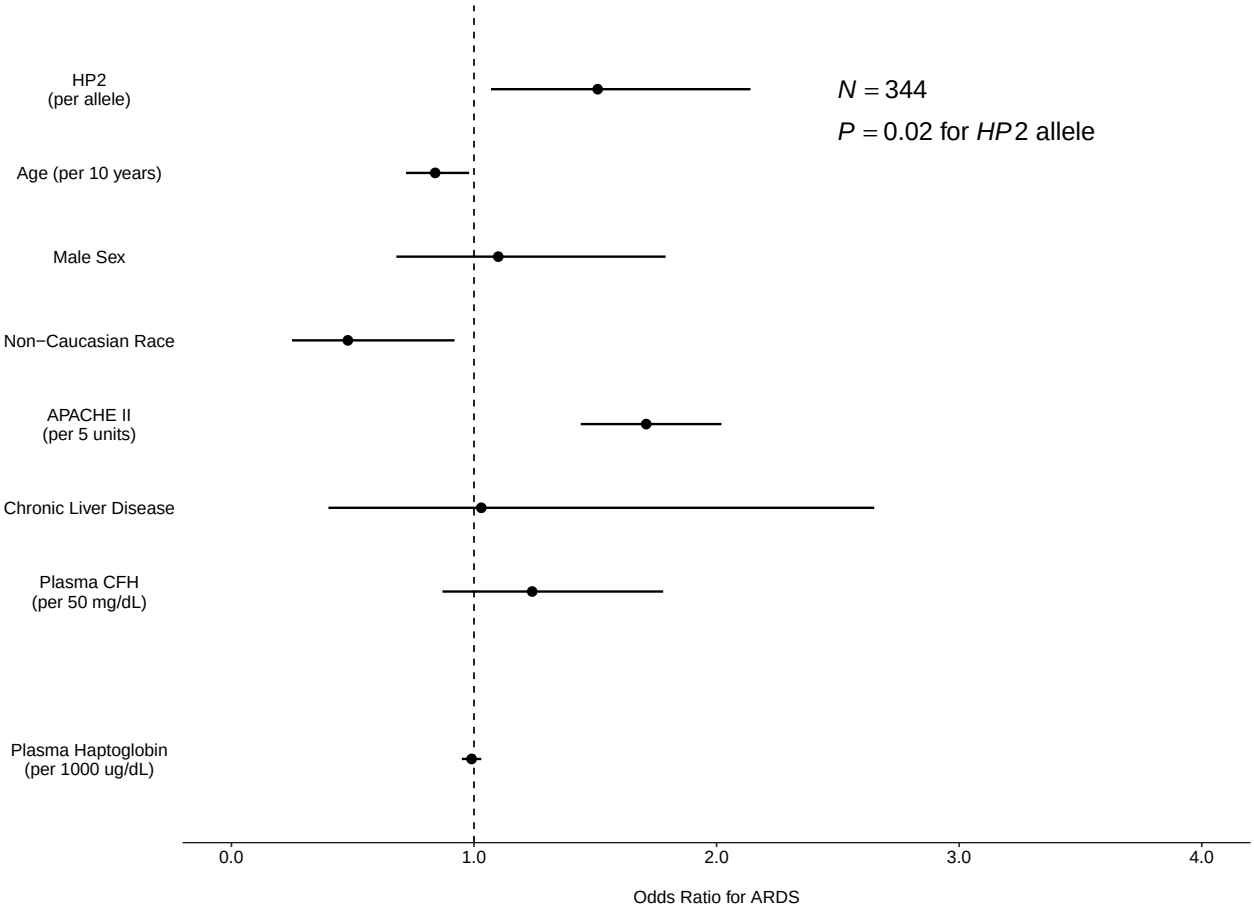
Comparison of the imputation algorithm versus PCR genotyping as the gold standard for determining haptoglobin (*HP*) genotype in patients where both techniques were performed ($N = 120$). Vertical axis indicates posterior-probability of *HP2* allele count from the imputation algorithm. Horizontal axis indicates PCR-determined *HP* genotype. Dots represent individual values. Violin plots indicate the probability density of predicted *HP2* allele count for each gold-standard *HP* genotype group. Balanced accuracy = 0.91 (95% CI: 0.84 - 0.95), Cohen's kappa coefficient = 0.85.

Supplemental Figure 3. Plasma cell-free hemoglobin and haptoglobin levels during sepsis by haptoglobin genotype



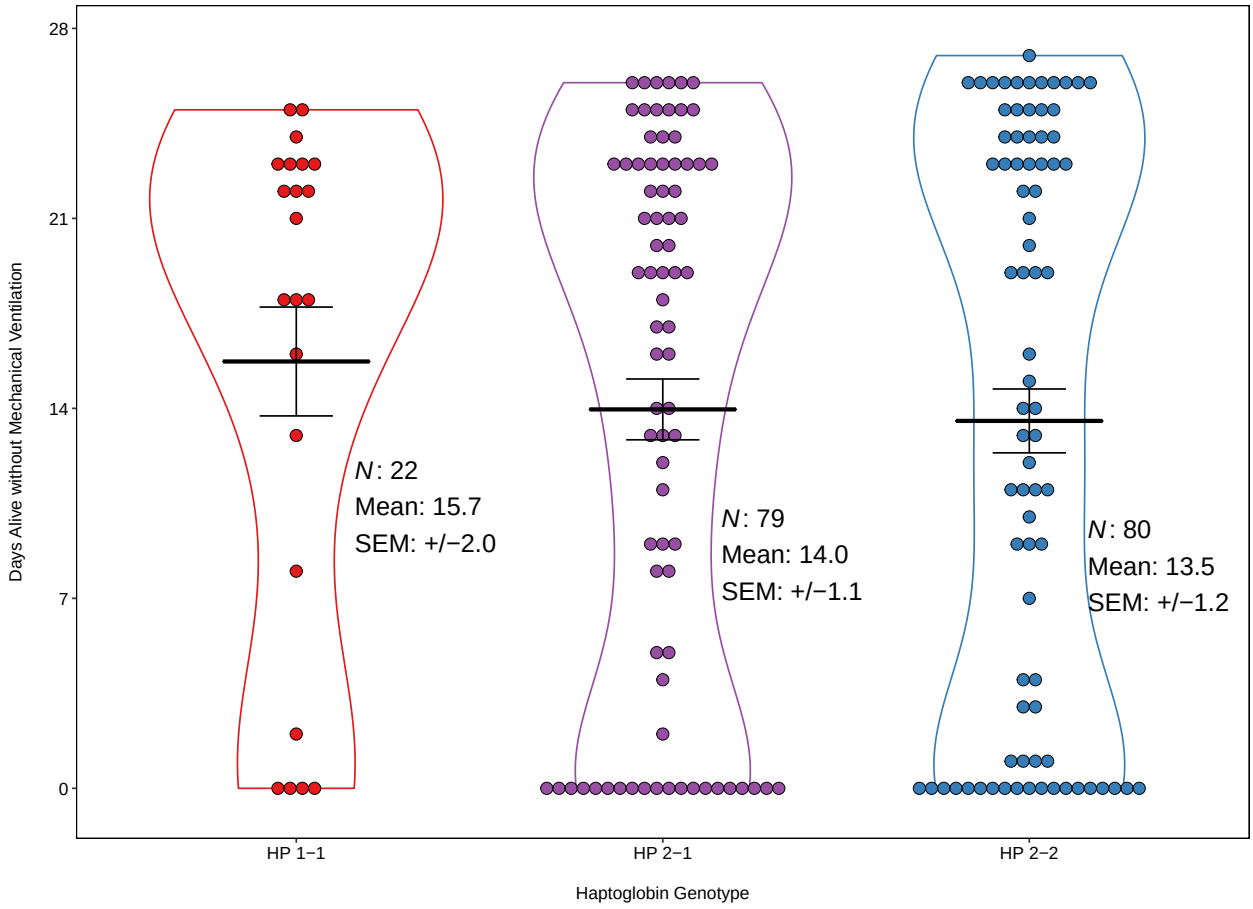
(**A**) Plasma CFH levels during sepsis were similar across all three genotype groups ($N = 496$). $P = 0.25$ by Kruskal-Wallis rank sum test. (**B**) Plasma haptoglobin levels by ELISA ($N = 344$ with samples available for assay) were highest in *HP1-1* patients and decreased with each additional *HP2* allele. $P = 0.0008$ by Kruskal-Wallis rank sum test. Thick horizontal bars represent the median, boxes represent the interquartile range (IQR, 25th and 75th percentiles), and whiskers represent the minimum and maximum values within 1.5 * IQR from the 25th and 75th percentiles. Dots represent outlier values.

Supplemental Figure 4. Haptoglobin 2 variant increases ARDS risk in septic adults when controlling for clinical factors and plasma haptoglobin



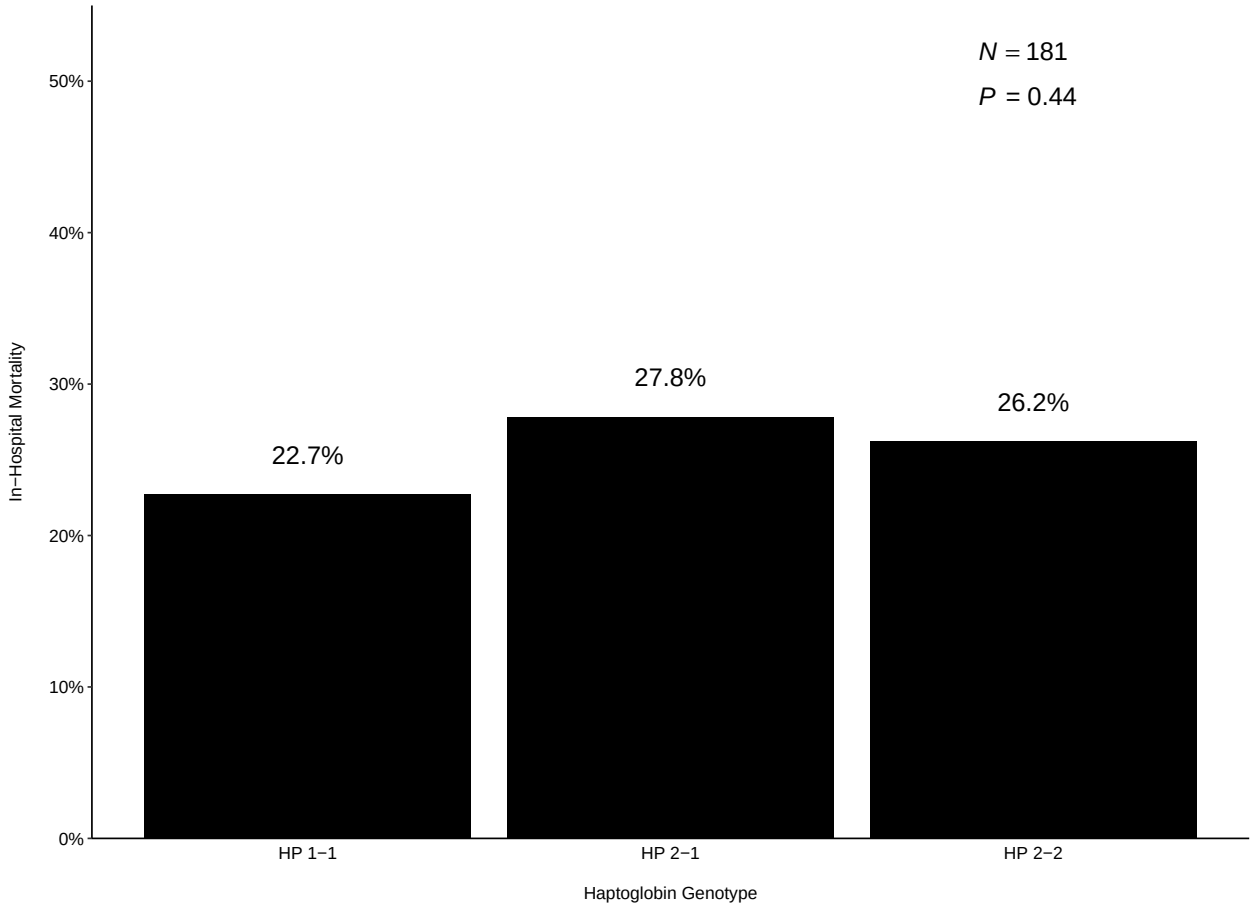
Multivariable logistic regression model for ARDS in patients with plasma haptoglobin levels available ($N = 344$). Dots represent point estimate odds ratios and horizontal lines represent 95% confidence intervals for each variable included in the model. Odds ratio > 1.0 indicates increased risk for ARDS, while odds ratio < 1.0 indicates decreased risk for ARDS. Black dashed vertical line indicates odds ratio of 1.0 (no change in ARDS risk). P = 0.020 for *HP2* variant. APACHE II – Acute physiology and chronic health evaluation II. *HP2* – Haptoglobin 2 variant. CFH – Cell-free hemoglobin.

Supplemental Figure 5. Ventilator-free days in ARDS patients by haptoglobin genotype



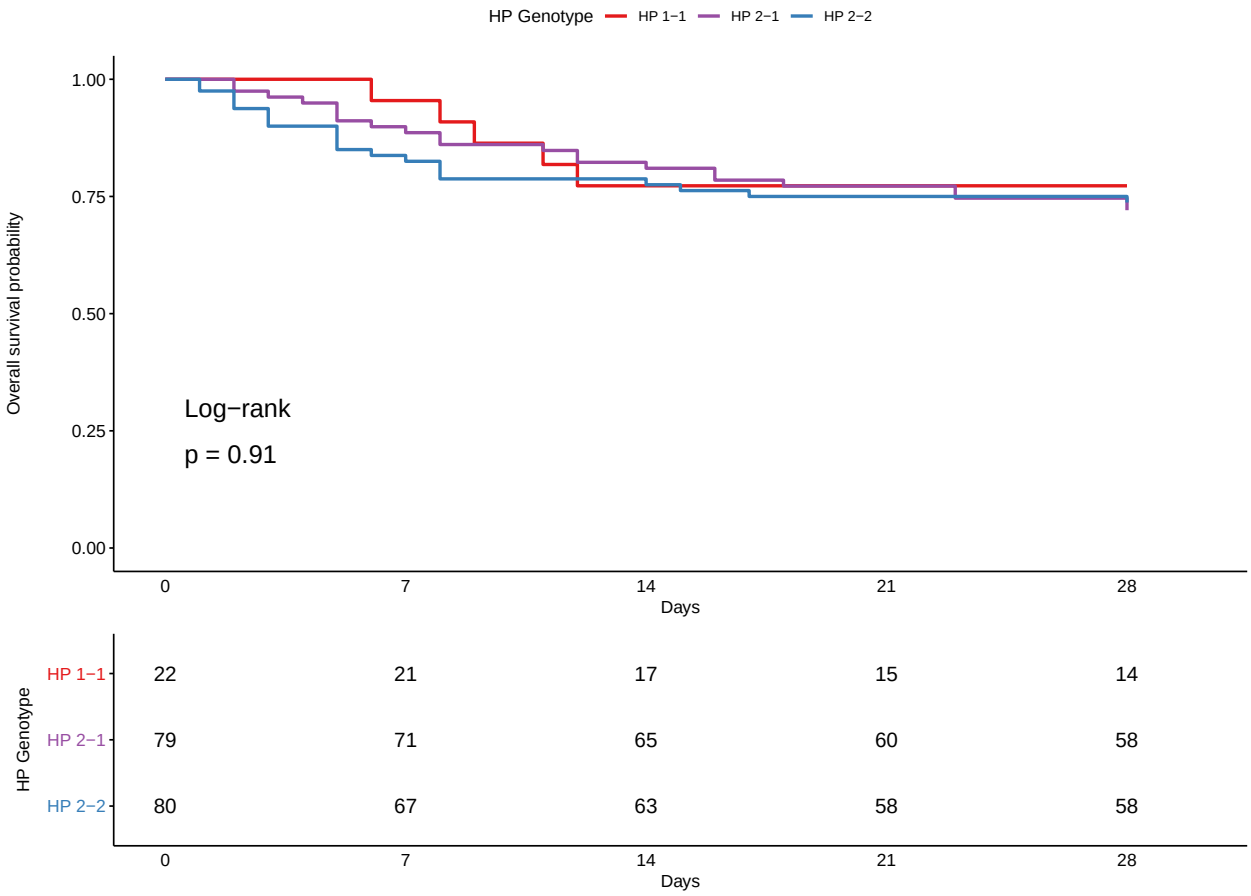
Vertical axis represents number of ventilator-free days (VFDs) in patients who experienced ARDS. VFDs were defined as defined as the number of days alive and not receiving mechanical ventilation from ICU day 1 to ICU day 28, with patients who died before day 28 assigned a value of zero. Dots represent individual values, violin plots represent probability density within each group. Thick horizontal bars represent groups means, and whiskers represent mean ± SEM. P = 0.89 by Kruskal-Wallis H test.

Supplemental Figure 6. In-hospital mortality in ARDS patient by haptoglobin genotype



There was no significant difference in in-hospital mortality between each *HP* genotype in patients who developed ARDS. Height of bars and numbers over bars indicate proportion of patients with in-hospital death for each group. $P = 0.44$ by Cochran-Armitage test for increasing risk ordered by number of *HP2* alleles.

Supplemental Figure 7. Survival curves for ARDS Patients by haptglobin genotype



Survival curves showing 28-day survival among ARDS patients by *HP* genotype. Table shows number of patients at risk at each time point. No difference was observed between HP genotypes. $P = 0.91$ by Mantel-Cox log-rank test.

Supplemental Tables

Supplemental Table 1. Performance characteristics of haptoglobin genotype imputation

	Predicted Genotype			
	HP 1-1	*HP 2-1*	*HP 2-2*	*Total*
PCR Genotype				
HP1-1	14	3	0	17
HP2-1	0	43	3	46
HP2-2	0	5	50	55
Total	14	51	53	
Statistic				
Sensitivity	0.82	0.93	0.91	
Specificity	1.00	0.89	0.95	
Positive Predictive Value	1.00	0.84	0.94	
Negative Predictive Value	0.97	0.96	0.92	
Balanced Accuracy	0.91	0.91	0.93	
F1 Score	0.90	0.89	0.93	

Supplemental Table 2. Multivariable logistic regression model in septic ICU patients including plasma haptoglobin levels

	Odds Ratio	95% Confidence Intervals	P
HP2 Allele Count	1.51	[1.07; 2.14]	0.02
Age (per 10 years)	0.84	[0.72; 0.98]	0.027
Male Sex	1.10	[0.68; 1.79]	0.7
Non-Caucasian Race	0.48	[0.25; 0.92]	0.028
APACHE II (per 5 units)	1.71	[1.44; 2.02]	5.5e-10
Chronic Liver Disease	1.03	[0.40; 2.65]	0.94
Plasma CFH (per 50 mg/dl)	1.24	[0.87; 1.78]	0.24
Plasma Haptoglobin (per 1000 ug/dl)	0.99	[0.95; 1.03]	0.61

Number of subjects: 344

APACHE: Acute Physiology and Chronic Health Evaluation score. CFH: Circulating free hemoglobin. *HP2*: Haptoglobin-2 variant.