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Serum concentration of full-length- and carboxy-terminal fragments of endothelial lipase predicts future cardiovascular risks in patients with coronary artery disease

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Running title: A new ELISA system for EL predicts MACE

Abstract

Background: Endothelial lipase (EL), a regulator of plasma high-density lipoprotein cholesterol (HDL-C), is secreted as a 68-kDa mature glycoprotein, and then cleaved by proprotein convertases. However, the clinical significance of the circulating EL fragments remains unclear.

Objective: To analyze the impact of serum EL fragments on HDL-C levels and major adverse cardiovascular events (MACE).

Methods: Using novel monoclonal antibodies (RC3A6) against carboxy-terminal EL protein, we have established a new ELISA system, which can detect both full-length EL protein (full EL) and carboxy terminal truncated fragments (total EL) in serum. The previous sandwich ELISA detected only full EL. The full and total EL mass were measured in 556 patients with coronary artery disease (CAD). Among them, 272 patients who underwent coronary intervention were monitored for 2 years for MACE.

Results: There was a significant correlation between serum full- and total EL mass ($R = 0.45$, $P<0.0001$). However, the total EL mass showed a stronger inverse correlation with serum HDL-cholesterol concentration than the full EL mass ($R = -0.17$ vs. -0.02). Kaplan-Meier analysis documented an association of serum total EL mass and MACE (Log-rank $P = 0.037$). When an optimal cutoff value was set at 96.23 ng/mL, total EL mass was an independent prognostic factor for MACE in the Cox proportional hazard model (HR; 1.75, 95% CI; 1.10-2.79, $P = 0.018$).

Conclusion: Serum total EL mass could be a predictor for MACE in patients with CAD. This novel ELISA will be useful for further clarifying the impact of EL on HDL metabolism and atherosclerosis.

Key words: Lipase, endothelial; Enzyme-linked immunosorbent assay; High-density lipoprotein cholesterol; Lipoprotein; Phospholipase; Coronary artery disease

Introduction

Based on epidemiology, it has been believed for decades that low high-density lipoprotein cholesterol (HDL-C) is a risk factor for coronary artery disease (CAD) (1); thus global attention has focused on the potential of increasing HDL-C as a therapeutic strategy for CAD. However, recent clinical trials using HDL-C raising agents, such as CETP inhibitors or niacin, failed to demonstrate clinical benefits in cardiovascular outcomes (2,3). It remains unclear whether the high HDL-C merely represents the accompanying risks or HDL function. The discrepancy between the epidemiologic findings and the lack of beneficial effects with pharmaceutical intervention has been suggested to be the result of the functional quality of the HDL particle and/or its ability to promote reverse cholesterol transport (4,5).

Endothelial lipase (EL) is a member of the triacylglyceride lipase family, which regulates HDL metabolism (6-8). Previous studies have shown that the plasma EL mass or activity is inversely correlated with the HDL-C concentration in humans (9-11). Association-based human genetic studies have provided evidence that variation in the EL genomic LIPG locus is associated with differences in circulating HDL-C concentrations or CAD (12, 13). Recent studies, however, including a large number of subjects, showed an association between the LIPG single nucleotide polymorphisms (SNPs) and HDL-C concentration, but none with CAD (14). Thus, genetic variation in EL modulates the plasma HDL-C concentration, although the relationship with CAD remains controversial.

EL is synthesized mainly by vascular endothelial cells and secreted as a 68 kDa mature glycoprotein (6,7). It can be cleaved by proprotein convertases into 40- and

28 kDa fragments (15,16). Therefore, serum should contain several EL fragments of 28, 40, and 68 kDa. However, the clinical significance of these full-length or truncated fragments remains unclear. To obtain a better understanding of the association of EL and HDL metabolism and atherosclerosis, we aimed to investigate the impact of serum EL fragments on serum HDL-C levels and cardiovascular events.

Materials and Methods

Antibodies for EL ELISA

As shown in Figure 1 (left), we have previously generated a sandwich enzyme-linked immunosorbent assay (ELISA) system for mature EL protein using a pair of antibodies recognizing the amino (26A1)- and carboxy (48A1)-terminus (EL Full-Length Assay Kit, code # 27182, Immuno-Biological Laboratories Co. Ltd, Fujioka, Japan) (17).

For preferential detection of carboxy-terminus fragments, a novel monoclonal antibody (RC3A6) against recombinant EL protein was generated in rats. A new ELISA system was developed to recognize the carboxy-terminal EL fragments (EL C-Terminal Assay Kit, code #27263, Immuno-Biological Laboratories) (Figure 1, right). Thus, as shown in Figure 1, the amino (26A1)- and carboxy (48A1)-terminus ELISA can detect full-length EL (full EL), whereas the newly developed carboxy (RC3A6)-terminus ELISA detects both full-length and C-terminal truncated fragments (total EL).

Immunoblot and Immunoprecipitation-immunoblot analysis

We performed Immunoblot and Immunoprecipitation-immunoblot analysis as previously reported (18). In brief, HEK293 cells were transfected with human full-

length EL/pcDNA3.1(+), human N-terminus EL (21-330aa)/pcDNA3.1(+), or human C-terminus EL (331-500aa)/pcDNA3.1(+). For immunoblot, the conditioned medium was used for SDS-PAGE. For immunoprecipitation (IP)-immunoblot, human serum from healthy volunteers was used. Immunoaffinity gels with monoclonal antibodies (MoAbs) coupled to Affi-Gel 10 (Bio-Rad, Hercules, CA) were prepared according to the manufacturer's protocol. The samples were incubated with 50 μL of immunoaffinity gel for 60min at 37℃. After washing, the immunoaffinity gels were boiled in SDS-Tris buffer, and then separated by SDS-PAGE (5-20% gradient) and transferred to nitrocellulose membranes (GE Healthcare). Following blocking, the membranes were incubated with HRP-conjugated MoAbs (Fab') prepared according to standard procedures. EL signals were detected using ECL reagent (Takara Bio, Kusatsu, Japan).

Preparation of blood samples

The Kobe Cardiovascular Marker Investigation (CMI) registry, which is a single-center registry of patients referred to Kobe University Hospital with cardiovascular disease, was started in 2008 to identify blood-based biomarkers that have utility in predicting cardiovascular disease. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Review Committee at Kobe University (Japan) and was registered at the UMIN Clinical Trials Registry (identification number 000030297). Written informed consent was obtained from all patients prior to enrollment in the study.

From the Kobe CMI registry, we enrolled a total of 556 patients with CAD, who were hospitalized at Kobe University Hospital, Kobe, Japan, from July 2011 to March

2014. Among them, we enrolled 272 consecutive patients who underwent coronary intervention for stable angina, unstable angina and myocardial infarction and monitored them for 2 years for major adverse cardiovascular events (MACE, a composite of cardiac death, myocardial infarction, revascularization, hospitalization due to heart failure, ischemic stroke, other cardiac events). Clinical outcome data were obtained by reviewing outpatient records or telephone interviews. The definition of hypertension, diabetes mellitus, and dyslipidemia were described as previously reported (19).

Whole blood was obtained in the morning after overnight fasting on the day of coronary intervention with written informed consent. Because previous studies and our pilot experiments (Suppl. Fig. S2) have shown that EL mass is similar between pre- and post-heparin plasma, blood was collected without administration of heparin (9, 17). The sera were immediately separated by centrifugation and kept frozen at -80 ºC until assay. Serum concentrations of HDL-C, LDL-C, and TG were measured enzymatically using standard assays.

Statistical analysis

All statistical analysis was done using Stata 13.1 (Stata, TX, USA) and GraphPad Prism software version 6.0 (GraphPad Software). $P < 0.05$ was considered statistically significant. Spearman correlation coefficient analysis was used to assess associations between measured parameters. Continuous data were expressed as mean ± standard deviation (SD) and compared using Student's t-test or Mann-Whitney's nonparametric test as appropriate. Categorical values were presented as frequency (%) and tested

using Chi-squared test. Kaplan-Meier analysis and log-rank test were performed to estimate the incidence of MACE and to compare the difference between groups, respectively. Univariate Cox proportional hazards regression was used to analyze factors in association with CAD. All variables in the univariate analysis were enrolled into multivariate analysis.

Results

Identification of C-terminus EL in human serum

We newly generated 2 monoclonal antibodies recognizing the carboxy terminus (RC3A6) or amino terminus (RN121A1) of EL. Both of them showed a highly specific reactivity with the recombinant EL proteins derived from HEK293 cells transfected with full length, N-terminal, or C-terminal EL expression vectors (Fig. 2, Suppl. Fig. S1). Intriguingly, immunoprecipitation-immunoblot analysis provided a strong signal for 28-kDa C-terminus EL fragment, to our knowledge, whose existence in human serum has never been shown by immunoblot analysis (Fig. 2).

Establishment of the ELISA for carboxy terminal EL

The new antibody (RC3A6) with a highly specific reactivity with the carboxy terminal EL protein was used for establishment of the new sandwich ELISA system (Fig. 1). The standard dose-response curve for the total EL ELISA system exhibited a linear shape when plotted on a log/log scale over a range from 0.13 to 8.00 ng/mL and the linearity was excellent (R^2= 0.999, Suppl. Fig. S3A). The new ELISA system worked equally well for analysis of both serum and EDTA plasma samples (Suppl. Fig. S3B and S3C). The

cross-reactivity of this ELISA against human LPL and HTGL was <0.1%. Imprecision was determined with three supplemented QC controls (high, middle, and low). The intra-assay imprecision exhibited coefficients of variation (CVs) of 3.2% in the high, 3.0% in the middle, and 3.7% in the low controls (Suppl. Table 1). Additionally, the inter-assay results for the CVs were 2.7, 2.7, and 3.1% in the high, middle, and low controls, respectively. Thus, we considered the ELISA system to be reliable from the standpoint of imprecision. The recoveries were 89.7~94.0% for human serum samples at 50 times dilution, 90.3~94.1% for human EDTA plasma (Suppl. Table 2). The assay limit of quantification was calculated to be 0.01 ng/mL using NCCLS protocols.

Impact of serum EL mass on HDL-C levels and cardiovascular events

The median of total EL mass in the CAD patients was 82.12 ng/mL (interquartile range, 64.08-98.93). The distribution was skewed to the left (Fig. 3A), as was the case with full EL mass (17). There was a significant correlation between serum full- and total EL mass ($R = 0.45$, $P<0.0001$, Fig. 3B). Interestingly, the concentration of total EL mass was much higher than that of previously reported full EL mass (17), probably reflecting the abundant cleaved fragments in the serum. The total EL mass showed a significant inverse correlation with serum HDL-C concentration compared to the full EL mass ($R = -0.17$ vs. $R = -0.02$, Fig. 4A, 4B).

As shown in Figure 5, both total- and full EL mass were significantly higher in patients with MACE compared to those without MACE. Hence, we next examined the

impact of EL mass on the prognosis of CAD. We divided the 272 patients who underwent percutaneous coronary intervention (PCI) into two groups according to the median of the serum concentration of total EL mass and observed MACE in the two groups for 2 years. MACE occurred in a total of 78 cases (cardiac death [n=8], myocardial infarction [n=2], revascularization [n=56], hospitalization due to heart failure [n=5], ischemic stroke [n=6], other cardiac events [n=1]). Upon EL analysis, 31 and 47 cases identified in the low and high total EL mass group, respectively. The characteristics of these two groups are shown in Table 1. The high total EL group was significantly younger and had higher BMI than those in low total EL group (Table 1). Regarding conventional risk factors of CAD, there were significant differences in total-cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, and triglyceride levels between the two groups.

Kaplan-Meier survival analysis demonstrated that the event-free rate of MACE at 2 years after revascularization was 77.2% in low total EL mass group and 65.4% in high total EL mass group (Log-rank P = 0.0371), while full EL mass was not significantly correlated with MACE (Fig. 6). With respect to total EL mass, we found more significant difference in the event-free rate of MACE at 1 year than at 2 years (Suppl. Fig. S4). We could not confirm the statistical significance between the low and high groups of total EL mass by univariate and multivariate Cox proportional hazards regression analysis. Therefore, we set the optimal cutoff value at 96.23 ng/mL and compared the impact of total EL mass on MACE. As shown in Table 2, total EL mass was an independent prognostic factor for MACE in the Cox proportional hazard model (HR; 1.75, 95% CI; 1.10-2.79, P = 0.018).

Discussion

The newly established ELISA system detected both full-length and carboxy-terminal EL, referred to as “total EL” in this study (Fig. 1). The median concentration of total EL mass was approximately 400-fold higher than that of full EL measured by the previously reported ELISA system (17), probably due to the long stability of the carboxy-terminal fragment in serum. Notably the previously reported pre-heparin EL levels by the polyclonal assay are similar to total EL shown in the current assay (10), which implies that the circulating EL mass may mainly consist of C-terminus fragments. Also, the total EL mass exhibited a stronger inverse association with serum HDL-C levels than full EL mass. This finding suggests that total EL may more precisely reflect the cumulative amount of EL secreted from endothelial cells than full EL, leading to the significant inverse correlation between HDL-C and total EL mass. The high sensitivity of the new ELISA system was expected to improve the prognostic usefulness for MACE, and in fact, we found that the total EL mass could be used as a predictor of MACE. Although the reasons underlying the association of total EL mass and MACE remain unclear, it is reasonable to speculate that the total EL may reflect cumulative impact of EL on the vessel wall and HDL metabolism, in contrast to the putative action of full EL at the time of sample collection.

Since its discovery, EL has been known as a determinant of plasma HDL-C concentrations. However, the role of EL in atherogenesis was inconsistent. In animal models, some reports have suggested that EL may act as a pro-atherogenic molecule (20,21), while others imply an anti-atherogenic role (22) or a neutral effect

(23). In human studies, several studies suggest a relationship between elevated EL and atherosclerosis; the plasma EL mass or activity in CAD patients is increased compared to that in non-CAD patients (9-11, 17). Also, plasma EL levels are associated with subclinical atherosclerosis and proinflammatory cytokines as well as C-reactive protein (10, 17, 24, 25). Recent human genetic studies documented an association of EL SNP with plasma HDL-C levels, but not with CAD (14), although the plasma EL mass or activity was not evaluated in these studies. Further investigations of other SNPs regulating plasma HDL-C levels did not reveal an impact on coronary artery disease when the effect of LDL-C or triglycerides was co-evaluated (26). Based on these findings, it is currently considered that HDL-C merely represents some existing coronary risks in individuals. Given the highly-regulated expression of EL in vivo (10, 21, 24), however, we speculated that the effect of EL on blood vessels should be also evaluated by means of its mass and/or activity. Moreover, since EL is upregulated by inflammatory stimuli, correlation between circulating EL fragments and MACE could reflect an inflammatory state promoting both phenomena. That is to say, total EL may be a biomarker for inflammation, and the relation to MACE may not be related to its phospholipase activity. In this case, we speculate that total EL assay as an inflammatory biomarker could explain for the impact on MACE.

On the other hand, the HDL particle has a variety of anti-atherosclerotic properties including cholesterol efflux activity; the functional quality of HDL has an impact on CAD events and is expected as a therapeutic target (27-29). EL has been reported to regulate not only the plasma HDL-C level but also HDL composition and function (30,31). In particular, the HDL phospholipid is a regulator of HDL function

(32,33), and EL has a phospholipase A1 activity against phospholipids in the HDL particle. Furthermore, EL expression is markedly increased by a variety of inflammatory stimuli both in vitro and in vivo, and may represent the local or systemic inflammatory status (10, 21, 24, 31, 34). Given the potential impact of inflammation on HDL modification, EL may play a role in the genesis of dysfunctional HDL. Thus, the new ELISA system may help to expand our understanding of EL on atherosclerotic vascular disease in terms of HDL function. Also, this total EL ELISA system could facilitate identification of genetic EL deficiency in humans, which has not been reported to date.

In this study, we measured serum EL mass without heparin administration because we again found that total EL mass was similar in pre- and post-heparin samples (Suppl. Fig. S2). Taken together with previous results (9-11), it was confirmed that the measurement of EL mass could be routinely done by using pre-heparin samples.

There are some limitations of our study. First, this study could not clarified the reason why total EL showed more significant correlation with MACE than full length EL despite undergoing cleavage and inactivation. Given a large amount of the protein of C-terminus EL in serum as shown in Figure 2 and its high concentration in the ELISA system, C-terminus EL might remain longer in serum because of the delayed clearance and reflect the cumulative secretion more sensitively than full-length EL. Second, we can not exclude the possibility that total EL partly reflects the inflammation in patients with CAD. As mentioned above, since it is well known that EL is upregulated under different kinds of inflammatory conditions, the systemic inflammation due to CAD might

affect the EL production or catabolism. Finally, we collected the blood samples from patients with some different pathologies including stable angina, unstable angina, and myocardial infarction, which seem likely to be heterogenous populations. Further studies are required to address these issues.

Conclusion

We have demonstrated that total EL mass could be a predictor for MACE in patients with CAD. This novel ELISA system may be useful for further clarifying the impact of EL on HDL quantity and quality. Finally, it would provide a novel clue in the confliction between classic epidemiology, association-based genetics, and pharmacologic intervention, regarding the role of this classic, negative risk factor for CAD.

Author Contributions

All authors contributed substantially to the conception, writing, and critical review of the article. Additionally, K. Miyashita developed monoclonal antibodies. K. Mori collected human serum samples and clinical data. M. Nagao, R. Toh, and Y. Irino were involved in acquisition, analysis and interpretation of data with the statistical analysis.

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None.

Declaration of interest

None.

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Figure legends

Figure 1. Establishment of total EL ELISA system

For specific detection of full-length endothelial lipase (EL), we utilized a sandwich ELISA using a pair of antibodies recognizing the amino (26A1)- and carboxy (48A1)-terminus. For detection of carboxy-terminal fragments, we established an ELISA using a monoclonal antibody (RC3A6) recognizing the carboxy-terminus. This C-terminal ELISA can detect both full-length and carboxy-terminal truncated fragments (total EL).

Figure 2. Detection of C-terminus EL in human serum.

HEK293 cells were transfected with human full-length EL/pcDNA3.1(+) (indicated as "Full"), human N-terminus EL(21-330aa)/pcDNA3.1(+) (indicated as "N"), or human C-terminus EL(331-500aa)/pcDNA3.1(+) (indicated as "C"). The samples from the conditioned medium (left; "Recombinant EL") and immunoprecipitated samples (right; "IP") were separated by SDS-PAGE (5–20% gradient) under reducing condition. Following electrophoresis, the samples were subjected to immunoblot using anti C-terminus EL antibody (RC3A6). N-term; RN121A1 affinity gel IP, C-term; RC3A6 affinity gel IP, Nega; negative control.

Figure 3. Frequency of total EL and correlation with full EL.

(A) Histogram shows the skewed distribution of serum total EL mass (n = 556). (B) The serum total EL mass was well correlated with serum full EL mass in all patients.

Figure 4. Correlation of serum HDL-C and full- or total EL mass

Correlation of serum full- (A) and total EL (B) mass with serum HDL-C concentrations. Serum total EL mass was inversely correlated with serum HDL-C levels (n=548).

Figure 5. Serum EL mass in patients with MACE was higher than in those without MACE.

Serum full (A)- and total EL mass (B) in patients without MACE (no MACE) vs. with MACE (n=194 vs. n=78 in each group). *P < 0.05, by Mann-Whitney U test. Values are mean ± SD.

Figure 6. Serum total EL mass was significantly correlated with MACE.

Kaplan-Meier curves for event-free rate of MACE at 2 years after revascularization between high- and low full EL (A) and total EL mass (B) groups. The high total EL mass, but not high full EL mass, was significantly associated with MACE (P=0.0371).

Supplemental Figure S1. Detection of N-terminus EL in human serum.

Immunoblot and immunoprecipitation-immunoblot analysis were performed with the same methodology and samples as Figure 2. Immunoblot was conducted using anti N-terminus EL antibody (RN121A1).

Supplemental Figure S2. The association between pre and post heparin total EL mass.

Total EL mass was measured by the new ELISA system in pre and post heparin samples in patients with CAD. There was no significant difference between pre and post EL mass.

Supplemental Figure S3. Characteristic of the hEL ELISA system.

(A) Calibration curve for calculation of total EL concentrations. (B) Dilution linearity of serum. (C) Dilution linearity of EDTA-Plasma.

Supplemental Figure S4. The correlation of serum total EL mass and full EL mass with MACE at 1 year after revascularization.

Kaplan-Meier curves for event-free rate of MACE at 1 year after revascularization between high- and low full EL (A) and total EL mass (B) groups. The high total EL mass was more significantly associated with MACE at 1 year than 2 years (P=0.0192).

Table 1. Baseline Characteristics

	Total EL, low (n = 136)	Total EL, high (n = 136)	P value
Age (years)	68.9±9.8	66.5±10.6	*0.047
Gender (male, %)	83.8	79.4	0.348
BMI (kg/m^2)	24.2±3.1	24.5±3.5	*0.004
Current smoking (%)	14.9	22.8	0.081
Hypertension (%)	82.4	83.0	0.978
Diabetes mellitus (%)	51.5	58.5	0.247
Dyslipidemia (%)	80.7	87.5	0.128
SBP (mmHg)	127.0±16.2	128.5±15.6	0.442
DBP (mmHg)	66.0±9.8	67.5±9.7	0.198
HbA1c (%)	6.4±1.1	6.5±1.1	0.766
Glucose (mg/dL)	106.3±25.0	111.8±34.0	0.140
Total-C (mg/dL)	166.4±34.5	174.9±34.5	*0.048
LDL-C (mg/dL)	93.4±28.8	101.8±27.8	*0.017
HDL-C (mg/dL)	47.5±14.6	42.0±11.3	*0.001
Triglyceride (mg/dL)	128.9±68.2	157.3±88.3	*0.004
Medications (%)			
Aspirin	83.1	90.0	0.111
Thienopyridine	54.4	55.9	0.807
Statin	62.5	89.7	0.801
ACEI/ARB	58.1	53.7	0.223

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, Total-C: total cholesterol, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, ACEI/ARB; angiotensin converting enzyme inhibitor/ angiotensin II receptor Blocker.

Table 2. Cox proportional hazards regression analysis.

	Univariate Analysis			Multivariate analysis		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Total EL >96.23 (ng/mL)	1.77	1.14 - 2.77	0.014	1.75	1.10-2.79	0.018
Age	1.00	0.98 - 1.02	0.776	-	-	-
Gender	1.06	0.58 - 1.93	0.845	-	-	-
BMI	0.99	0.93 - 1.06	0.868	-	-	-
Smoking	1.63	0.98 - 2.71	0.059	-	-	-
Hypertension	1.51	0.78 - 2.94	0.224	-	-	-
DM	1.54	0.97 - 2.44	0.069	-	-	-
DL	0.78	0.45 - 1.38	0.398	-	-	-

HR: hazard ratio, CI: confidence interval, BMI: body mass index, DM: diabetes mellitus, DL: dyslipidemia.

Suppl. Table 1. Intra- and inter-assay imprecision

Intra-assay imprecision

QC	Measured values, ng/mL	SD, ng/mL	CV, %	n
High	4.15	0.13	3.2	24
Middle	1.15	0.03	3.0	24
Low	0.38	0.01	3.7	24

Inter-assay imprecision

QC	Measured values, ng/mL	SD, ng/mL	CV, %	n
High	4.30	0.12	2.7%	9
Middle	1.20	0.30	2.7%	9
Low	0.41	0.01	3.1%	9

QC, quality controls; SD, standard deviation; CV, coefficient of variation.

Suppl. Table 2. Recovery validation

Sample	Theoretical value, ng/mL	Measured values, ng/mL	%
Human serum	5.30	4.98	94.0%
	4.30	4.00	93.0%
	3.80	3.41	89.7%
Human plasma	4.89	4.60	94.1%
	3.89	3.58	91.9%
	3.39	3.06	90.3%

Figure 1

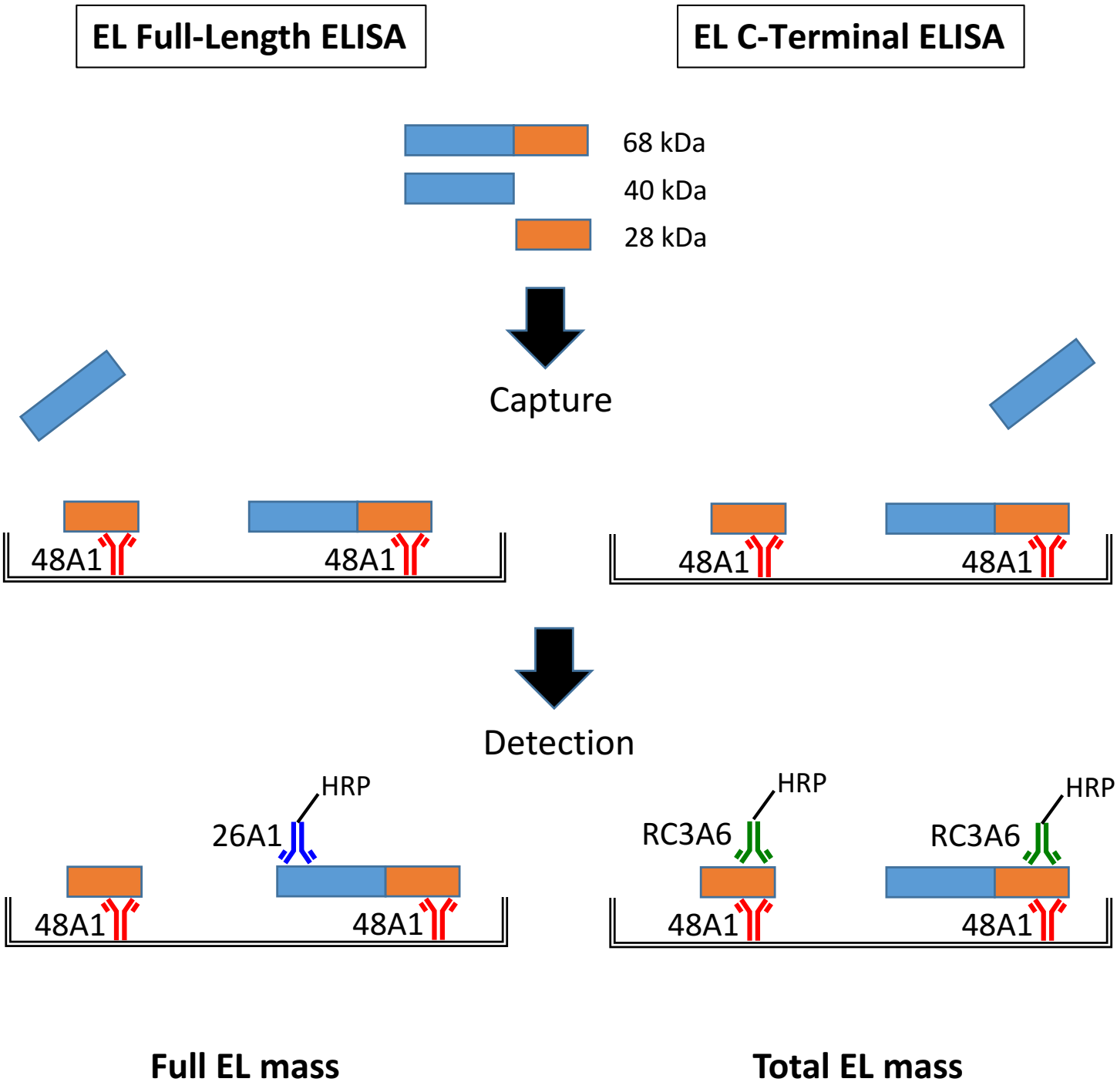
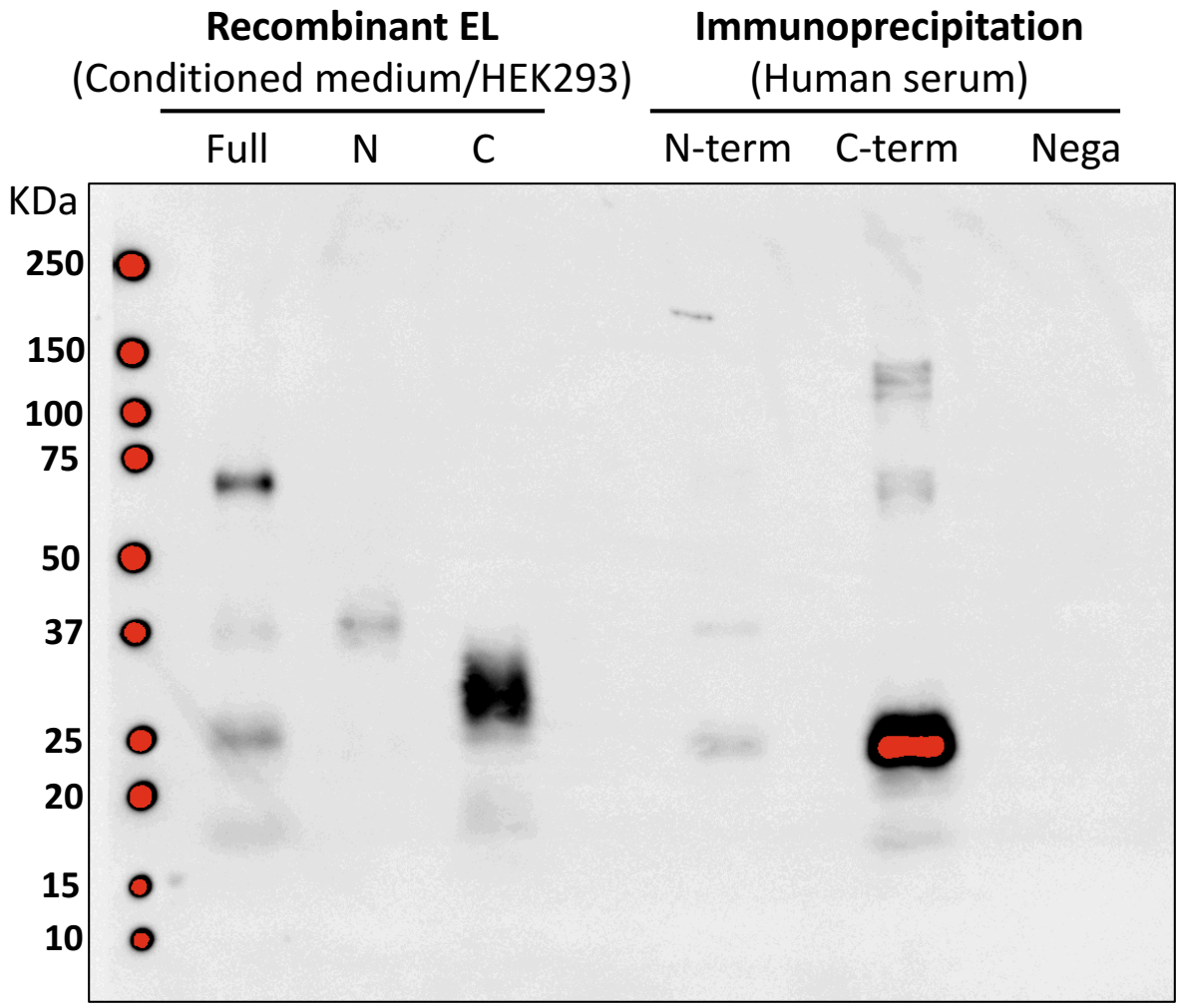

EL Full-Length ELISA
EL C-Terminal ELISA
68 kDa
40 kDa
28 kDa
Capture
48A1
48A1
48A1
48A1
Detection
HRP
26A1
48A1
48A1
HRP
RC3A6
48A1
HRP
RC3A6
48A1
Full EL mass
Total EL mass

Figure 2



Immunoblot; Anti C-terminus EL Antibody (RC3A6)

Figure 3

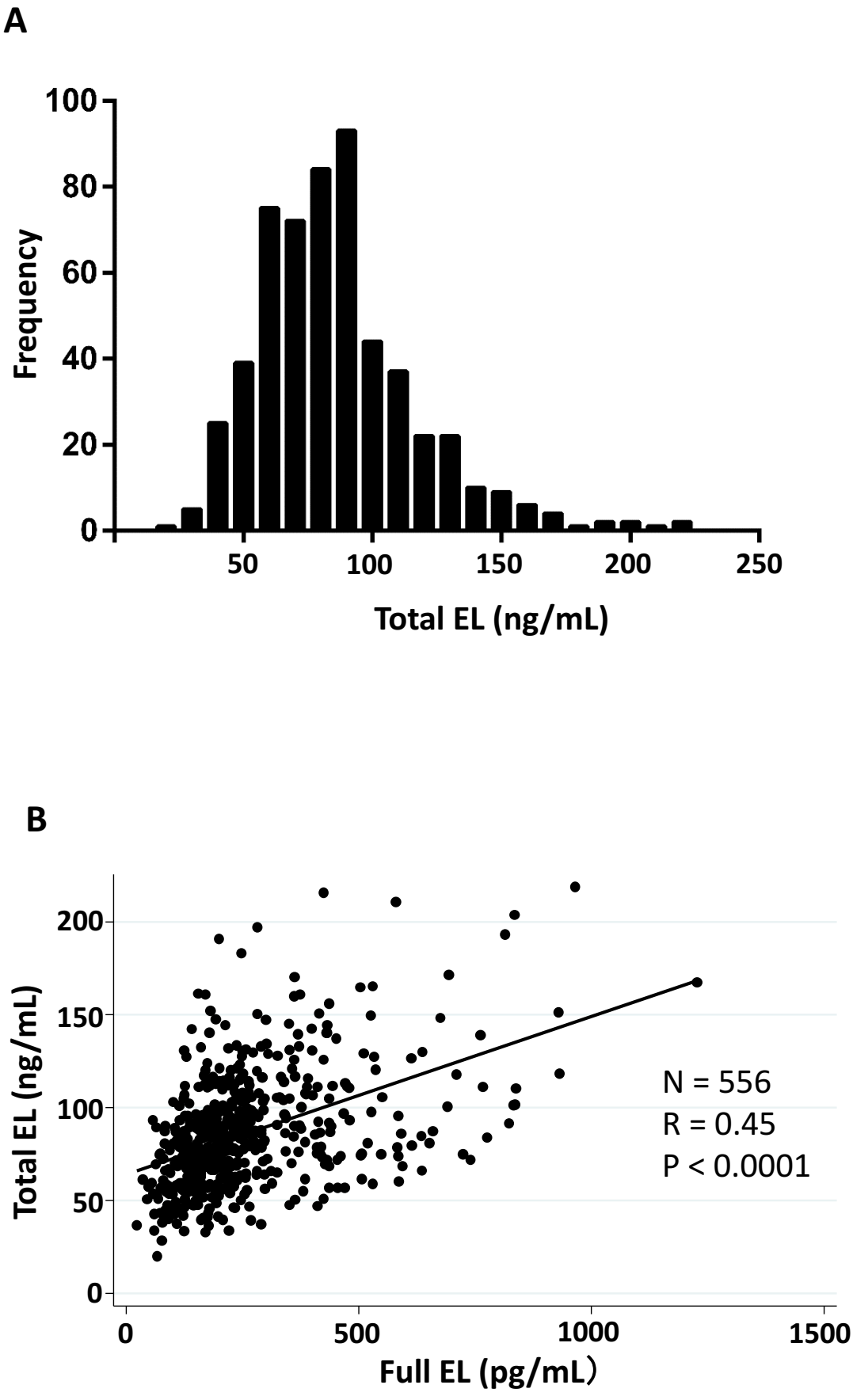
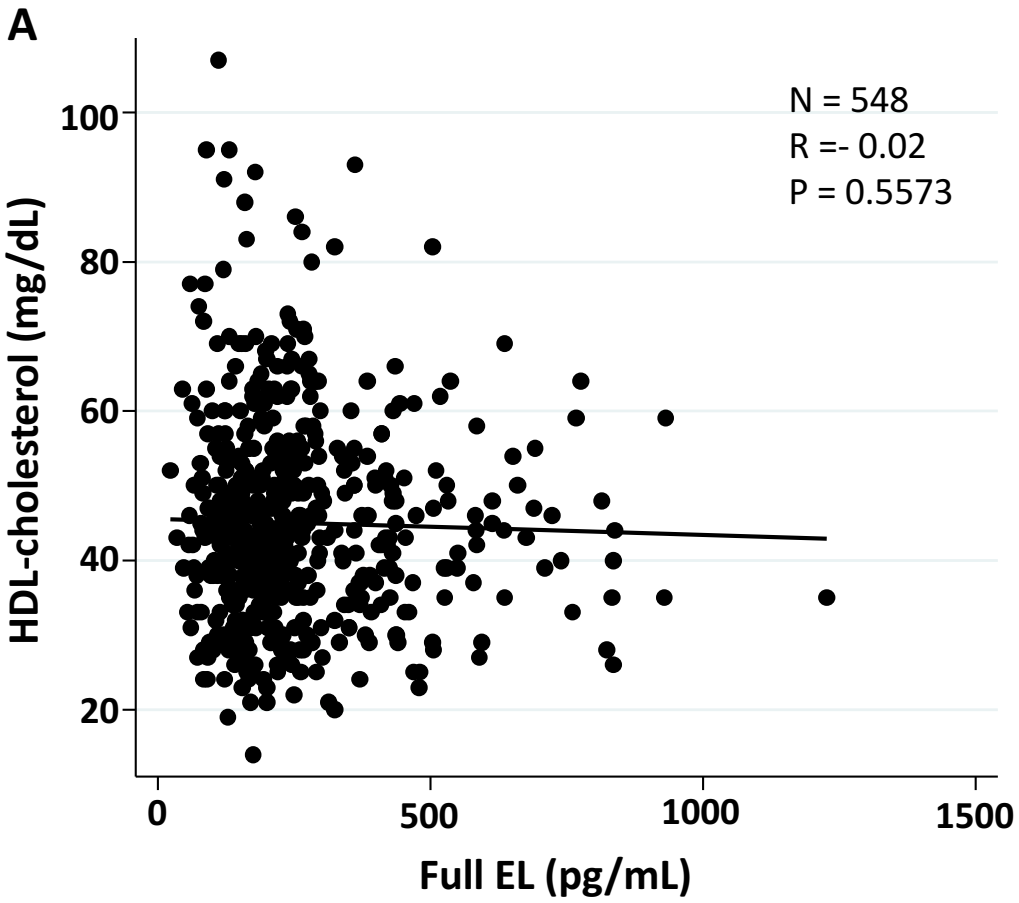

A
Frequency
100
80
60
40
20
0
50
100
150
200
250
Total EL (ng/mL)
B
Total EL (ng/mL)
200
150
100
50
0
0
500
1000
1500
Full EL (pg/mL)
N = 556
R = 0.45
P < 0.0001

Figure 4


A
N = 548
R =- 0.02
P = 0.5573
HDL-cholesterol (mg/dL)
100
80
60
40
20
0
500
1000
1500
Full EL (pg/mL)

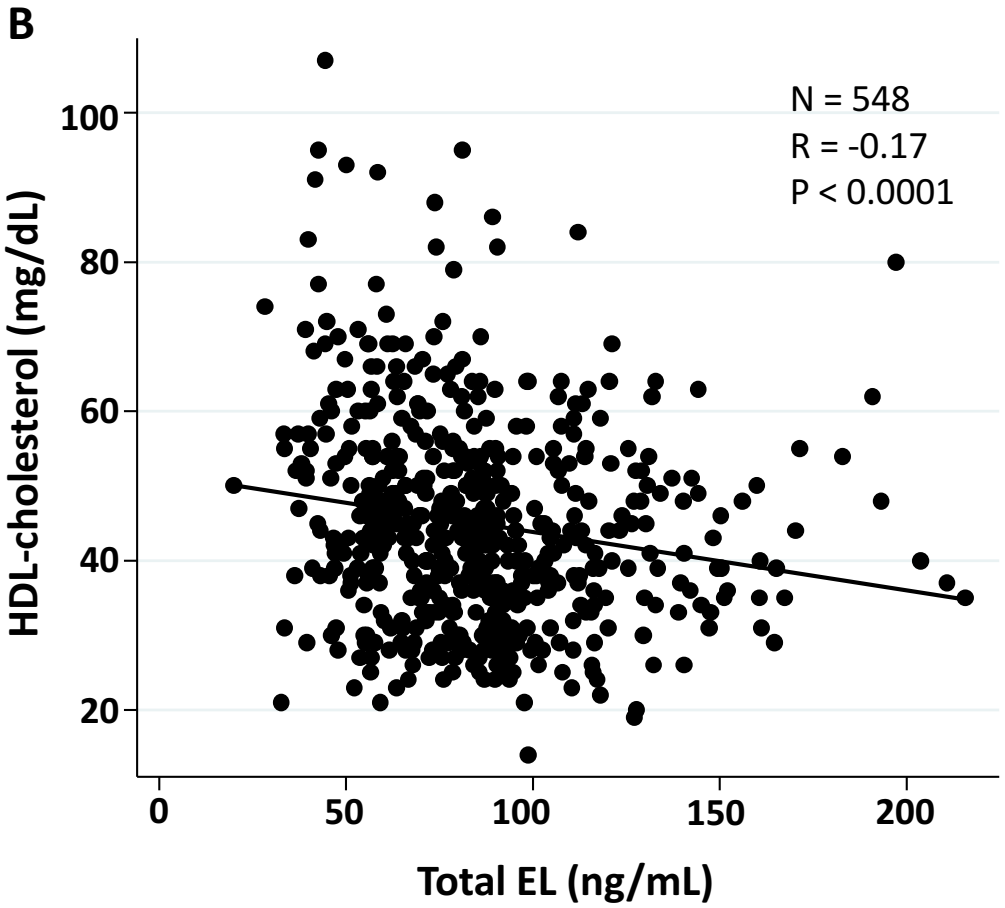

B
N = 548
R = -0.17
P < 0.0001
HDL-cholesterol (mg/dL)
100
80
60
40
20
0
50
100
150
200
Total EL (ng/mL)

Figure 5

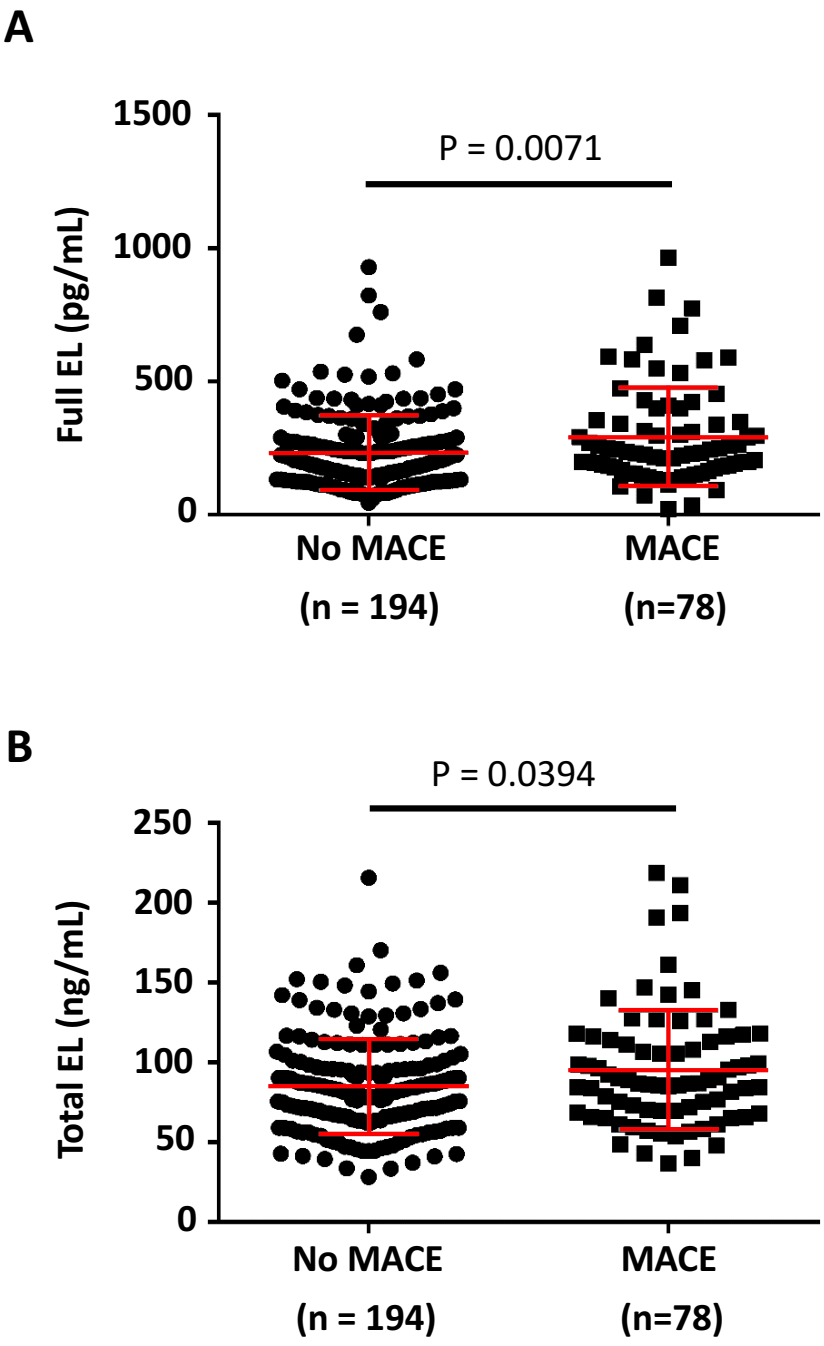
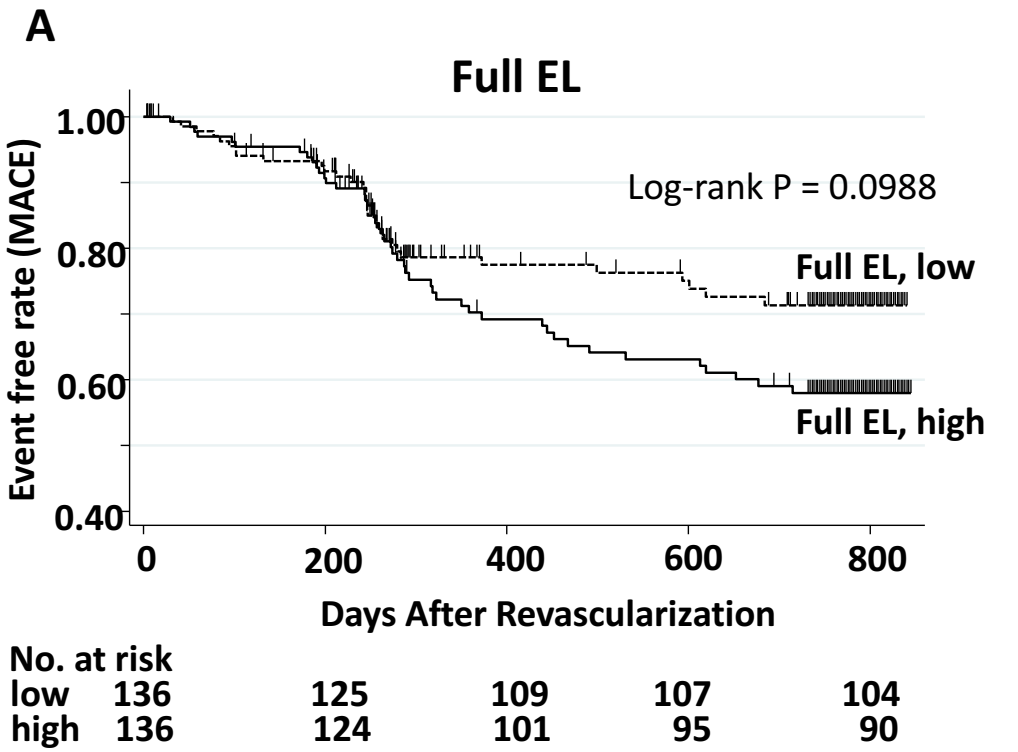
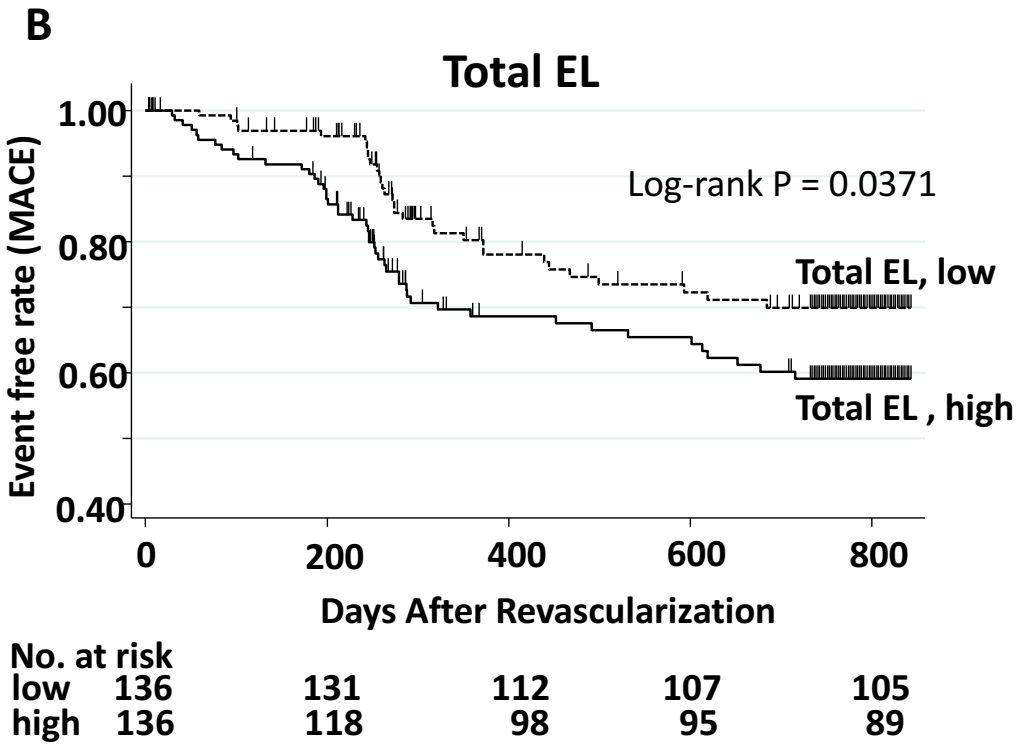

A
1500
1000
500
0
Full EL (pg/mL)
P = 0.0071
No MACE
(n = 194)
MACE
(n=78)
B
250
200
150
100
50
0
Total EL (ng/mL)
P = 0.0394
No MACE
(n = 194)
MACE
(n=78)

Figure 6


A
Full EL
1.00
0.80
0.60
0.40
Event free rate (MACE)
Log-rank P = 0.0988
Full EL, low
Full EL, high
0
200
400
600
800
Days After Revascularization
No. at risk
low 136 125 109 107 104
high 136 124 101 95 90


B
Total EL
1.00
0.80
0.60
0.40
Event free rate (MACE)
Log-rank P = 0.0371
Total EL, low
Total EL , high
0
200
400
600
800
Days After Revascularization
No. at risk
low 136 131 112 107 105
high 136 118 98 95 89