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Identification of LAMC2 as a prognostic and predictive biomarker for determining response to gemcitabine-based therapy in pancreatic ductal adenocarcinoma

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Running title: LAMC2 as a predictive biomarker in PDAC

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ABSTRACT

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. While the extracellular matrix (ECM) components plays an integral role in PDAC pathogenesis and mediating chemoresistance, its role in predicting response to chemotherapy in PDAC patients remains unclear.

METHODS: We performed a systematic biomarker discovery by analyzing genomewide transcriptomic profiling data from 423 patients (GSE71729, GSE21501 and TCGA) for predicting overall survival (OS). This was subsequently validated in two independent clinical cohorts of 270 PDAC patients (training cohort; n=121 and validation cohort; n=149). In addition, we investigated EUS-FNA biopsy specimens from 51 PDAC patients with an unresectable cancer for predicting therapeutic response to gemcitabine-based therapy.

RESULTS: Following rigorous bioinformatic analysis, we identified LAMC2 to be a significant prognostic factor in all three PDAC datasets (GSE71729, HR=2.04, P=0.002; GSE21501, HR=2.17, P=0.031; TCGA, HR=2.57, P<0.001). High LAMC2 expression in PDAC patients associated with a significantly poor OS and relapse-free survival (RFS) in both training (P<0.001, P<0.001) and validation cohorts (P=0.001, P=0.003). More importantly, LAMC2 expression robustly identified PDAC patients with unresectable disease and those who responded to gemcitabine-based therapy (AUC= 0.79; 95%CI, 0.65-0.89). The univariate logistic regression analysis revealed that high LAMC2 expression was the only factor that predicted poor response to gemcitabine in PDAC patients (Odds Ratio [OR]=4.90; 95% CI, 1.45-16.6; P=0.011).

CONCLUSION: We conclude that LAMC2 is a novel prognostic and predictive biomarker for gemcitabine-based therapy in both adjuvant and palliative setting; which could have significant impact in precision and individualized treatment of PDAC patients.

Keywords: Extracellular matrix; LAMC2; pancreatic ductal adenocarcinoma; gemcitabine; predictive biomarker

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most challenging diseases because of the late diagnosis, high rates of disease recurrence, poor survival rates, and availability of limited therapeutic regimens [1, 2]. This issue is compounded further due to the continued rise in PDAC incidence, projecting it to become the second leading cause of cancer-related deaths by 2030 [3].

Gemcitabine based therapy remains the backbone and treatment of choice in PDAC patients – whether it be in a neoadjuvant, adjuvant or palliative treatment setting. Recent developments in gemcitabine-based combination therapies have shown to significantly improve the median and 5-year overall survival (OS) rates in both the resectable and unresectable PDAC patients [4-6]. Nonetheless, the overall prognosis for this malignancy still remains quite poor [7]. In the recent years, the FOLFIRINOX treatment (a combination of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) has led to an improvement of OS [8, 9]. However, currently there are no available validated biomarkers that can predict treatment response and facilitate selection of appropriate PDAC patient populations for such treatment regimens. Presently, such therapeutic decision-making and selection of patients with both local and metastatic PDAC primarily relies on patient's overall health and individual opinion of an oncologist. Several other molecular biomarkers have been proposed for their prognostic potential in PDAC [10-13]; however, their translation into the clinic has been challenging. Collectively, these data highlight the unmet clinical need for developing improved prognostic and predictive biomarkers that can help identify patients who have the highest likelihood of receiving therapeutic benefit from such chemotherapies and spare others from the toxicity and expense associated with these drugs.

The extracellular matrix (ECM) provides the critical scaffold for the tumor microenvironment, and is intimately involved in regulating PDAC progression [14, 15]. In addition, the ECM plays a pivotal role in mediating chemoresistance in cancer [16-19]. While accumulating evidence suggests that the ECM components may serve as potential diagnostic or prognostic biomarkers in PDAC [20-22], their role as biomarkers for predicting response to chemotherapy in PDAC patients have thus far not been explored.

We therefore performed a genome-wide systematic and comprehensive transcriptomic analysis to identify ECM-related molecular biomarkers involved in predicting prognosis and resistance to gemcitabine. We followed this initial discovery effort by validation of our findings in two independent clinical cohorts of surgical resected PDAC patients, as well as another independent cohort of patients with an unresectable disease who were treated with gemcitabine + nab-paclitaxel regimen. Through these comprehensive biomarker discovery and validation efforts, we successfully identified Laminin γ2 (LAMC2) as a novel biomarker for tumor prognosis and predicting response to gemcitabine-based therapy in PDAC patients.

MATERIALS AND METHODS

Study design and patient cohorts

For the systematic biomarker discovery phase, three publicly-available datasets (GSE21501, GSE71729 and the Cancer Genome Atlas [TCGA]) were analyzed to validate the expression of ECM-related genes in PDAC patients. The ECM associated genes were listed and defined as per the Gene Ontology (GO) database [23]. During the biomarker discovery phase, GSE21501 (n=102) and GSE71729 (n=145) datasets were downloaded from the GEO database directly (https://www.ncbi.nlm.nih.gov, accessed on July 17, 2019). In addition, normalized transcriptomic profiling data from the TCGA dataset for 178 PDAC patients was downloaded from the UCSC Xena Browser (https://xenabrowser.net, accessed on July 17, 2019), and used for an independent validation of the discovery cohort.

In the subsequent in-house validation phase, a total of 321 PDAC patients were analyzed. This included a training cohort of 121 patients enrolled at the Kumamoto University, a validation cohort of 149 patients seen at the Nara Medical University, Japan, and a cohort of 51 patients treated with chemotherapy and enrolled at the Tokushima University, Japan. None of the patients with surgical treatment received pre-operative cancer treatment, and all tumors were diagnosed as PDAC. The specimens from the patients with chemotherapy treatment were obtained by endoscopic ultrasound-fine needle aspiration (EUS-FNA), prior to initiation of treatment. All specimens were formalin-fixed paraffin-embedded (FFPE) tissues. The study workflow is summarized in **Supplementary Fig. S1**. The study was conducted in accordance with the Declaration of Helsinki. A written informed consent was obtained from all patients, and the study was approved by the institutional review boards of all participating institutions.

Total RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted using AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) assays were performed using the QuantStudio 6 Flex RT-PCR System (Applied Biosystems, Foster City, CA). The relative abundance of target transcripts was evaluated and normalized to the expression levels of beta-actin as an internal control using the 2-ΔCt method [24].

Statistical analysis

Statistical analyses were performed using Medcalc statistical software V.16.2.0 (Medcalc Software bvba, Ostend, Belgium), and GraphPad Prism V8.0 (GraphPad Software, San Diego, CA). Continuous variables were expressed as medians and were compared using a *t*-test or Mann Whitney U test. Categorical variables were compared using $\chi 2$ or Fisher's exact test. All P values were calculated using a two-sided test, and a $P<0.05$ was considered statistically significant. For time-to-event analyses, survival estimates were calculated using the Kaplan–Meier analysis, and the survival differences between groups were compared using the log-rank test.

RESULTS

Genomewide transcriptional profiling identified LAMC2 as a prognostic biomarker in patients with PDAC

Using the GO search engine and the search for 'extracellular matrix' keyword, we identified 852 genes associated with this biological process (**Supplementary Table S1**). We next analyzed the expression profiles of these genes in the GSE21501 and GSE71729 datasets, specifically in terms of their association with survival outcomes in PDAC patients. Following bioinformatic and biostatistical analysis, we identified a panel of 10 genes that were significantly associated with OS in PDAC patients. We next validated the performance of these genes in an independent cohort of patients within the TCGA dataset and LAMC2 was the only gene that emerged with a robust prognostic potential in PDAC. We observed that high expression of LAMC2 was the singular and significant prognostic factor in all three datasets (GSE71729; Hazard ratio [HR]=2.04; 95%CI, 1.30-3.19; P=0.002, GSE21501; HR=2.17; 95%CI, 1.08-4.38; P=0.031, TCGA; HR=2.57; 95%CI, 1.62-4.07; P<0.001, **Supplementary Fig. S2A-C**). Furthermore, LAMC2 expression was significantly higher in PDAC tissues compared to the normal mucosa (P<0.0001, **Supplementary Fig. S2D**).

High LAMC2 expression significantly associates with poorer outcome

Next, we assessed the clinical significance of LAMC2 expression in two independent PDAC patient cohorts (training cohort; n=121 and validation cohort; n=149). All patients were categorized into low- and high-risk groups based on the LAMC2 expression and by utilizing Youden's index-derived cutoff thresholds in the training cohort (**Fig. 1A**). To ensure clinical robustness of our findings, we used the same cut-off thresholds in the validation cohort. As illustrated in **Table 1**, other than the

tumor status in the training cohort (P=0.04), no significant differences were observed in the distribution of various clinicopathological variables between the LAMC2-high and low expression groups.

For evaluating the performance of LAMC2 expression into a clinically translatable prognostic assay, we first analyzed its relationship with the OS in patients within the training cohort. Interestingly, the median OS in LAMC2-high expression subgroup was 23.0 months vs. 32.1 months in PDAC patients with lower expression of this ECM-related gene (P<0.001; **Fig. 1B**). To further the prognostic potential of LAMC2, we next interrogated its relationship and cancer recurrence in the training cohort patients. In support of our earlier findings, the LAMC2 expression in patients with recurrence was significantly higher than those without recurrence (P=0.031; **Fig. 1C**). Moreover, Kaplan-Meier analysis for relapse-free survival (RFS) revealed that high LAMC2 levels in PDAC patients associated with a significantly poor RFS (P<0.001; **Fig. 1D**).

In accordance with our observations in the training cohort, high tumor LAMC2 expression was associated with poorer OS and RFS compared to the patients with low LAMC2 expression (P=0.001 and P=0.026, respectively; **Fig. 1E and F**).

High expression of LAMC2 is an independent prognostic risk factor in PDAC patients

When challenged on multivariate analysis in training cohorts (**Fig.2A**), patients with high LAMC2 expression (HR=2.02; 95% CI, 1.28-3.20; P=0.003), higher levels of CA19-9 (HR=1.68; 95% CI, 1.02-2.78; P=0.043), and those with LNM were associated with poor OS (HR=2.68; 95% CI, 1.59-4.50; P<0.001). Consistent with the training cohort results, in the multivariate analysis, high LAMC2 expression (HR=1.84; 95% CI, 1.24-2.74; P=0.003), CA19-9 status (HR=2.25; 95% CI, 1.36-3.70;

P=0.002), and LNM status (HR=2.02; 95% CI, 1.36-2.99; P<0.001) were the only clinicopathological factors that significantly associated with worse OS (**Fig.2B**).

To address a combination of LAMC2 expression together with other clinical factors, we stratified patients into three different groups: Group 1 included patients with low LAMC2 expression, low CA19-9 levels (<37U/ml), and absence of LNM, Group 2 patients included high LAMC2 expression and/or either high CA19-9 levels (≥37U/mL) nor LNM positivity and Group 3 patients were those who exhibited all three risk factors. The median OS was 95.0 months in group 1, 26.0 months in group 2 and 15.5 months in group 3 (P<0.001, **Fig. 2C**). Similarly, the median RFS was 68.4 months in group 1, 22.5 months in group 2 and 5.93 months in group 3 (P<0.001, **Supplementary Fig. S3A**). Likewise, we observed that patients in group 3 still exhibited significantly worse OS and RFS (P<0.001 and P=0.001, respectively) in the validation cohort (**Fig. 2D and Supplementary Fig. S3B**).

High LAMC2 expression predicts therapeutic response to gemcitabine-based therapy

We next analyzed the LAMC2 expression levels in the context of adjuvant chemotherapy. Intriguingly, high LAMC2 expression levels significantly associated with shorter median OS (P=0.018 and P=0.003, respectively) and RFS (P=0.023 and P=0.025, respectively) in the patients who were treated with gemcitabine based therapy in both cohorts (**Supplementary Fig. S4A-D**). On the other hand, LAMC2 expression in PDAC patients who received 5FU based adjuvant therapy did not associate significantly with OS and RFS (**Supplementary Fig. S4E-H**).

We next investigated an independent cohort of 51 PDAC patients with an unresectable cancer, who received gemcitabine and nab-paclitaxel regimen as an initial therapy. Patients were

classified as either responders (confirmed complete response [CR], partial response [PR], or stable disease [SD]) or non-responders (progressive disease [PD]) based on the best response evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 and were included in the waterfall plot (**Fig. 3A**). Among 51 patients, 24 patients exhibited the high LAMC2 expression, while 27 patients had the low expression (**Table 2**). Of note, the LAMC2 expression within the responder group was significantly lower than patients within the non-responder group (P<0.001, **Fig. 3B**). Within the responder group, 11 patients exhibited high LAMC2 expression (11/24; 45.8%) and 21 with low LAMC2 expression (21/27; 77.8%; P=0.023, **Fig. 3C**). More importantly, LAMC2 expression demonstrated robust identification of response in this cohort (AUC= 0.79; 95%CI, 0.65-0.89, **Fig. 3D**). When we analyzed the OS and progression free survival (PFS) of the patients, patients with high tumor LAMC2 expression had poorer OS and PFS vs. patients with low LAMC2 expression (P=0.031 and 0.040, respectively; **Fig. 3E and F**).

Next, the univariate logistic regression analysis revealed that higher levels of LAMC2 expression were the only factor that associated with a poor response to gemcitabine in the cohort (Odds ratio [OR]=4.90; 95% CI, 1.45-16.6; P=0.011; **Table 3**).

DISCUSSION

In the present study, using a comprehensive biomarker discovery approach, we initially identified LAMC2 that were significantly associated with poor OS in PDAC patients. Following rigorous training and validation, we validated LAMC2 to be the only gene that consistently exhibited prognostic significance across PDAC patient cohorts. Moreover, we noted that a risk-assessment model that combined high LAMC2 expression, high CA19-9 levels and presence of LNM was significantly superior in predicting the OS and RFS in PDAC patients. Finally, given its biological role as a ECM-related gene, we successfully identified that high expression of LAMC2 are predictive of therapeutic response to gemcitabine-based therapy in adjuvant and palliative settings.

We observed that high LAMC2 expression was significantly associated with poor OS and RFS in PDAC patients. While our results are in line with some of the previous reports [25, 26], the prior studies had several limitations, including inadequate sample size, lack of systematic and comprehensive biomarker discovery approach and lack of independent validation cohorts – all of which were addressed in our current article. Furthermore, we for the first time developed a PCR-based cut-off threshold to assess LAMC2 expression levels in a training cohort, which were successfully applied to an independent validation cohort. More importantly, the multivariate analysis revealed that high LAMC2 expression was an independent prognostic factor in PDAC patients – in large, independent, clinical cohorts.

Following a potentially curative surgery, approximately 80% of PDAC patients often develop metastasis mostly within the first 2 years after surgery [27]. Although adjuvant chemotherapy provides significant survival benefit in PDAC patients [4, 8], there is lack of

availability of predictive biomarkers that can guide therapeutic decision-making in individual PDAC patients. Several retrospective studies have investigated whether some nucleoside transporters involved in the uptake of gemcitabine could predict the response [12, 28-30], however, none of these studies have reached clinical significance. In our study, we deliberately focused on ECM-associated pathway and demonstrated that LAMC2 was significantly associated with poor prognosis – both in terms of OS and RFS, in patients who received gemcitabine based adjuvant therapy, while such an effect was not evident for 5FU based adjuvant therapy. Although further studies are required, the results of our study collectively highlight that LAMC2 expression might serve as a potentially attractive biomarker for predicting therapeutic response to gemcitabine chemotherapy in an adjuvant setting.

Thus far, no other biomarkers have reported predictive potential for gemcitabine and nab-paclitaxel therapy in unresectable PDAC patients. Von Hoff et al. demonstrated that secreted protein acidic and rich in cysteine (SPARC) was associated with improved OS in PDAC patients who received gemcitabine and nab-paclitaxel regimen [31]. However, a subsequent study failed to observe any significant associations between stromal SPARC levels and predictive efficacy [32]. Herein, we successfully demonstrated that LAMC2 expression is a robust predictive biomarker against gemcitabine therapy in a palliative setting.

We would like to acknowledge potential limitations of our work. First, this was a retrospective study with the potential inadvertent risk of bias. Hence, a prospective randomized clinical study in future could confirm our analysis before the translation of this biomarker into the clinic. Second, we did not analyze LAMC2 expression in unresectable PDAC patients who received FOLFIRINOX treatments; since such a patient cohort was not available to us at this time. Third,

although our cohorts are independent and large sample sizes, other studies are needed to confirm the utility of LAMC2 in PDAC. To overcome these limitations, a prospective randamised controlled study is required.

In conclusion, high LAMC2 expression emerged as a robust prognostic biomarker as it significantly correlated with poor OS and RFS in two large, independent cohorts of PDAC patients. More importantly, our results indicate that LAMC2 expression is a predictor of therapeutic response to gemcitabine resistance in PDAC patients. Collectively, our findings have important implications for the further prospective validation and development of LAMC2 as a prognostic and predictive biomarker for gemcitabine-based treatment in both adjuvant and palliative setting; hence, making a significant advance in precision and individualized treatment of patients suffering from this fatal malignancy.

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FIGURE LEGENDS

Figure 1. High LAMC2 expression associates with worse prognosis in PDAC patients in the training and validation cohort. (A) The distribution of LAMC2 expression in PDAC patients. (B) Kaplan-Meier curves for OS between PDAC patients with high (pink) and low (blue) LAMC2 expression in the training cohort. (C) Comparison of LAMC2 expression levels in PDAC patients with or without recurrence. (D) Kaplan-Meier curves for RFS between PDAC patients with high (pink) and low (blue) LAMC2 expression in the training cohort. Kaplan-Meier curves for (E) OS and (F) RFS in the validation cohort. *, P<0.05.

Figure 2. Validation of high LAMC2 expression for predicting poor prognosis in PDAC patients. Univariate and multivariate analysis in the (A) training and (B) validation cohort calculated by Cox regression model. Kaplan-Meier curves for OS among 3 Groups in the (C) training and (D) validation cohort. Lymph node metastasis, LNN; LNM positivity, LNP.

Figure 3. LAMC2 expression predicts therapeutic response to gemcitabine-based therapy. (A) Waterfall plots for predicting best tumor response in PDAC patients treated with gemcitabine and nab-paclitaxel as a primary treatment. (B) Comparison of LAMC2 expression levels in responders and non-responders in the primary chemotherapy cohort. (C) The proportion of responders and non-responders in the LAMC2-high and low patients. (D) ROC curves for the predicting therapeutic response to gemcitabine. Kaplan-Meier curves for (E) OS and (F) PFS in PDAC patients with high (pink) or low (blue) LAMC2 expression in the primary chemotherapy cohort. *, P<0.05; **P<0.001.

Table 1: Patient characteristics in the resectable cohort of PDAC patients within the training and validation cohorts

Characteristics	Training cohort				Validation cohort			
	Total	LAMC2 expression		*P*-value [a]	Total	LAMC2 expression		*P*-value [a]
	n = 121	Low (n = 87)	High (n = 34)		n = 149	Low (n = 90)	High (n = 59)	
Age, years				0.27				0.52
< 65, n (%)	44	29 (33.3)	15 (44.1)		37	24 (26.7)	13 (22.0)	
≥ 65, n (%)	77	58 (66.7)	19 (55.9)		112	66 (73.3)	46 (78.0)	
Gender				0.30				0.55
Male, n (%)	62	42 (48.3)	20 (58.8)		89	52 (57.8)	37 (62.7)	
Female, n (%)	59	45 (51.7)	14 (41.2)		60	38 (42.2)	22 (37.3)	
Tumor status				**0.04**[b]				0.41[b]
T1-2	16	15 (17.2)	1 (2.9)		15	11 (12.2)	4 (6.8)	
T3-4	105	72 (82.8)	33 (97.1)		134	79 (87.8)	55 (93.2)	
Nodal status				0.09[b]				0.86
N0	40	33 (37.9)	7 (20.6)		67	41 (45.6)	26 (44.1)	
N1	81	54 (62.1)	27 (79.4)		82	49 (54.4)	33 (55.9)	
UICC stage (ver. 7)				0.13				0.49
IA, IB	14	13 (14.9)	1 (2.9)		11	9 (10.0)	2 (3.4)	
IIA	25	20 (23.0)	5 (14.7)		54	31 (34.4)	23 (39.0)	
IIB	67	45 (51.7)	22 (64.7)		68	40 (44.4)	28 (47.5)	
III, IV	15	9 (10.4)	6 (17.7)		16	10 (11.2)	6 (10.1)	
CA19-9 (U/mL)				0.60				0.49
< 37, n (%)	40	30 (34.5)	10 (29.4)		40	26 (28.9)	14 (23.7)	
≥ 37, n (%)	81	57 (65.5)	24 (70.6)		109	64 (71.1)	45 (76.3)	
Tumor size (mm)				0.92				0.59
< 40, n (%)	93	69 (79.3)	24 (70.6)		120	73 (81.1)	47 (79.7)	
≥ 40, n (%)	27	18 (20.7)	9 (26.5)		16	11 (12.2)	5 (8.5)	
N/A	1		1 (2.9)		13	6 (6.7)	7 (11.8)	
Adjuvant therapy				0.59				0.25
Gemcitabine based	80	58 (66.7)	22 (64.7)		92	59 (65.6)	33 (55.9)	
Other	22	17 (19.5)	5 (14.7)		26	12 (13.3)	14 (23.7)	
none	19	12 (13.8)	7 (20.6)		28	17 (18.9)	11 (18.7)	
Unknown	0	0	0		3	2 (2.2)	1 (1.7)	

[a] Chi-square test

[b] Fisher's exact test

UICC, International Union Against Cancer; N/A, Not available

Table 2: Patients characteristics in PDAC patients with an unresectable disease

Characteristics	Total	LAMC2 expression		
	n = 51	Low (n = 27)	High (n = 24)	*P*-value [a]
Age, years				0.55
< 65, n (%)	17	10 (37.0)	7 (29.2)	
≥ 65, n (%)	34	17 (63.0)	17 (70.8)	
Gender				0.20
Male, n (%)	24	15 (55.6)	9 (37.5)	
Female, n (%)	27	12 (44.4)	15 (62.5)	
CA19-9 (U/mL)				0.43[b]
< 37, n (%)	7	5 (18.5)	2 (8.3)	
≥ 37, n (%)	44	22 (81.5)	22 (91.7)	
Tumor size (mm)				0.44
< 40, n (%)	22	13 (48.1)	9 (37.5)	
≥ 40, n (%)	29	14 (51.9)	15 (62.5)	
Locally or Metastasis				0.86
Locally advanced	9	5 (18.5)	4 (16.7)	
Distal metastasis	42	22 (81.5)	20 (83.3)	
Location				0.66
Head	16	8 (29.6)	8 (33.3)	
Body	26	13 (48.1)	13 (54.2)	
Tail	9	6 (22.3)	3 (12.5)	
site of metastasis				0.34
Liver	28	16 (66.7)	12 (50.0)	
Lung	8	5 (20.9)	3 (12.5)	
Peritoneum	5	1 (4.1)	4 (16.7)	
Lymph node	6	2 (8.3)	4 (16.7)	
Other	1	0 (0.0)	1 (4.1)	
No. of metastatic sites				0.46
0	10	5 (18.5)	5 (20.9)	
1	36	20 (74.1)	16 (66.7)	
2	3	2 (7.4)	1 (4.1)	
3	2	0 (0.0)	2 (8.3)	

[a] Chi-square test

[b] Fisher's exact test

Table 3: Univariate logistic regression analysis for LAMC2 as a predictive biomarker for therapeutic response in PDAC patients

Characteristics	**OR**	**95% CI**	***P*-value**
Age (≥65 vs. <65)	1.89	0.55 - 6.57	0.31
Gender (Female vs. Male)	2.26	0.71 - 7.19	0.17
Primary tumor location (Head vs. Other)	2.81	0.83 - 9.49	0.10
Locally Advanced vs. Metastatic	1.36	0.30 - 6.20	0.69
CA19-9 (≥37U/mL vs. <37U/mL)	4.56	0.51 - 41.1	0.18
LAMC2 status (High vs. Low)	4.90	1.45 - 16.6	**0.01**

OR, odds ratio; CI, confidence interval

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Overview of the study design.

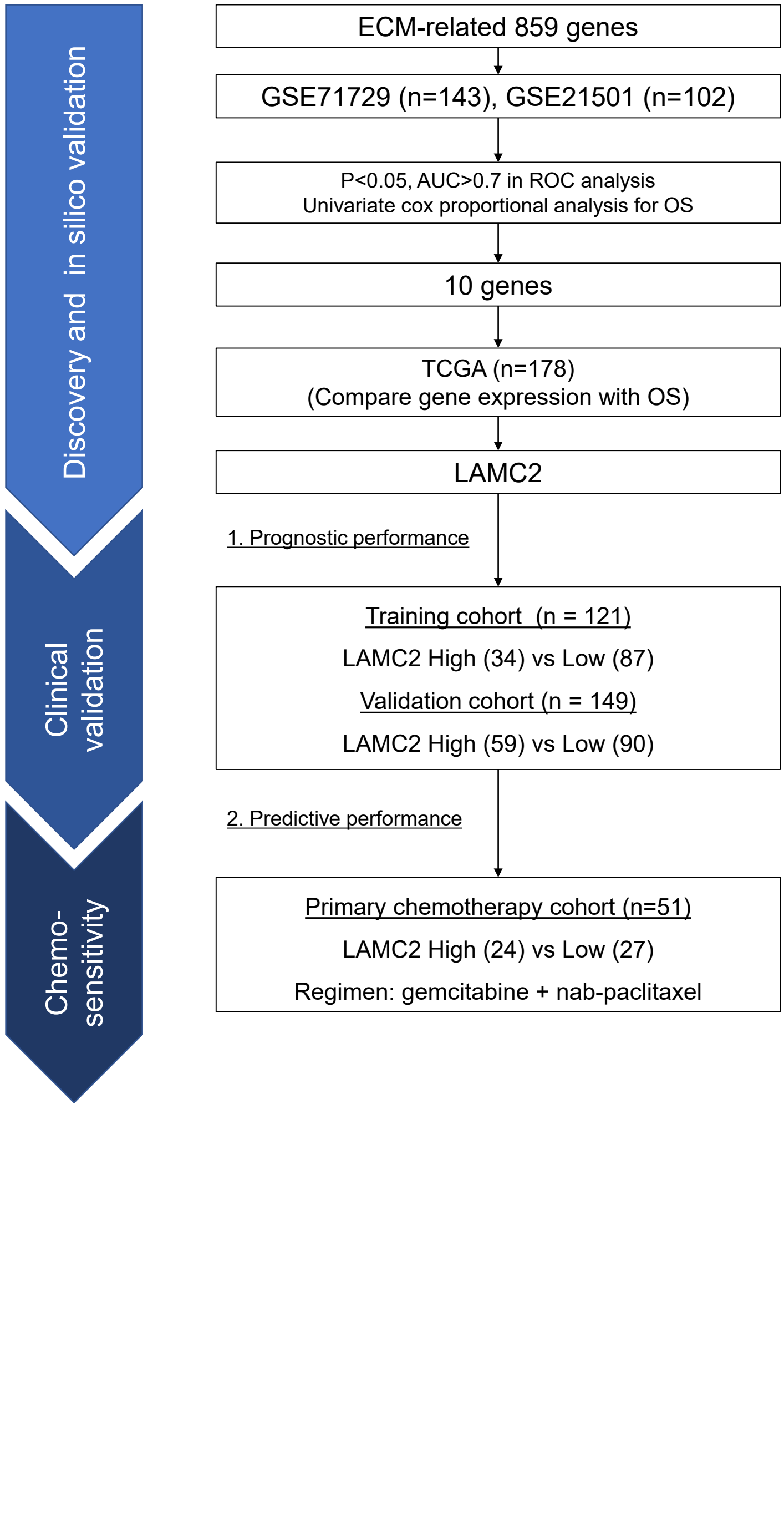
Supplementary Figure S2. The discovery phase identified LAMC2 expression and prognostic value for OS in the publicly available datasets. Kaplan-Meier curves for OS in PDAC patients based on LAMC2 expression in (A) GSE71729, (B) GSE21501, and (C) TCGA datasets. (D) The expression of LAMC2 in responders and non-responders in the GSE71729 dataset. ***, P<0.001.

Supplementary Figure S3. Univariate and multivariate analysis in the training cohort calculated by Cox regression model.

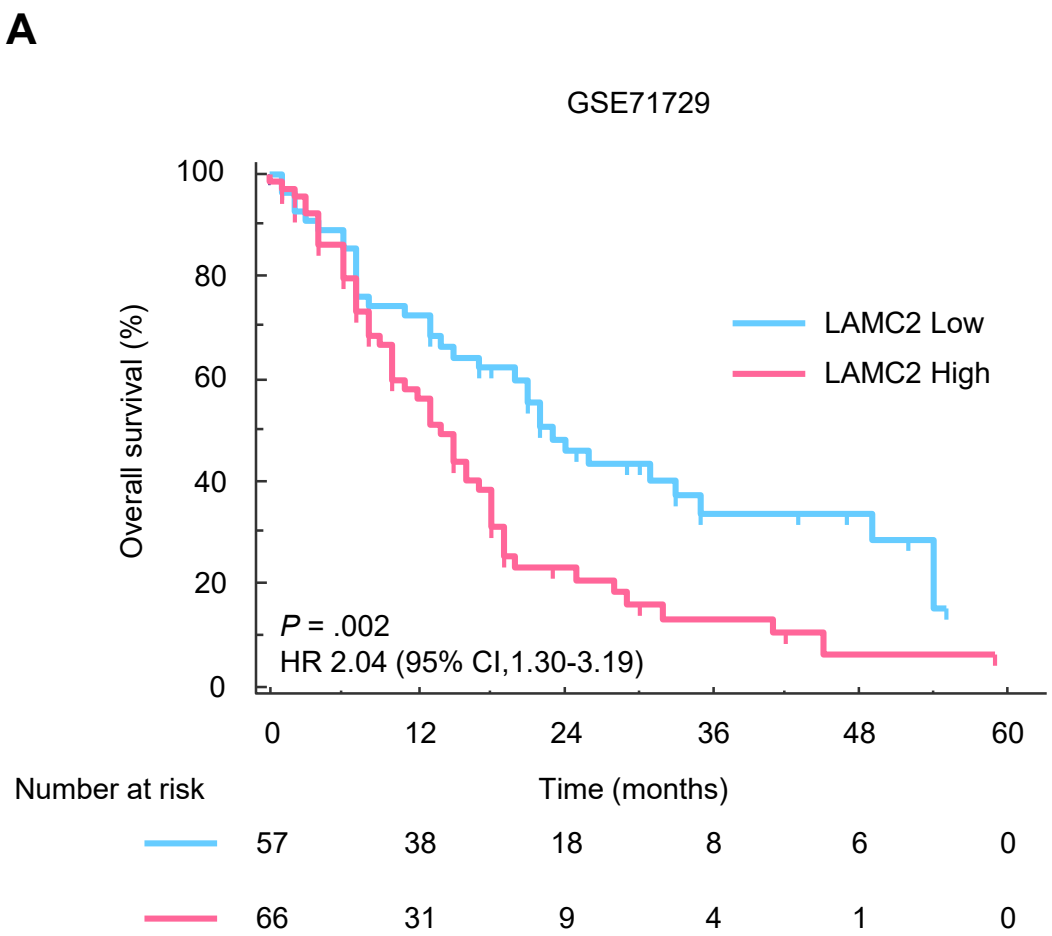
Supplementary Figure S4. A risk-assessment model that combines LAMC2 expression together with CA19-9 levels and lymph node metastasis status is a superior predictor of RFS in PDAC patients in the (A) training and (B) validation cohort. Lymph node metastasis, LNN; LNM positivity, LNP.

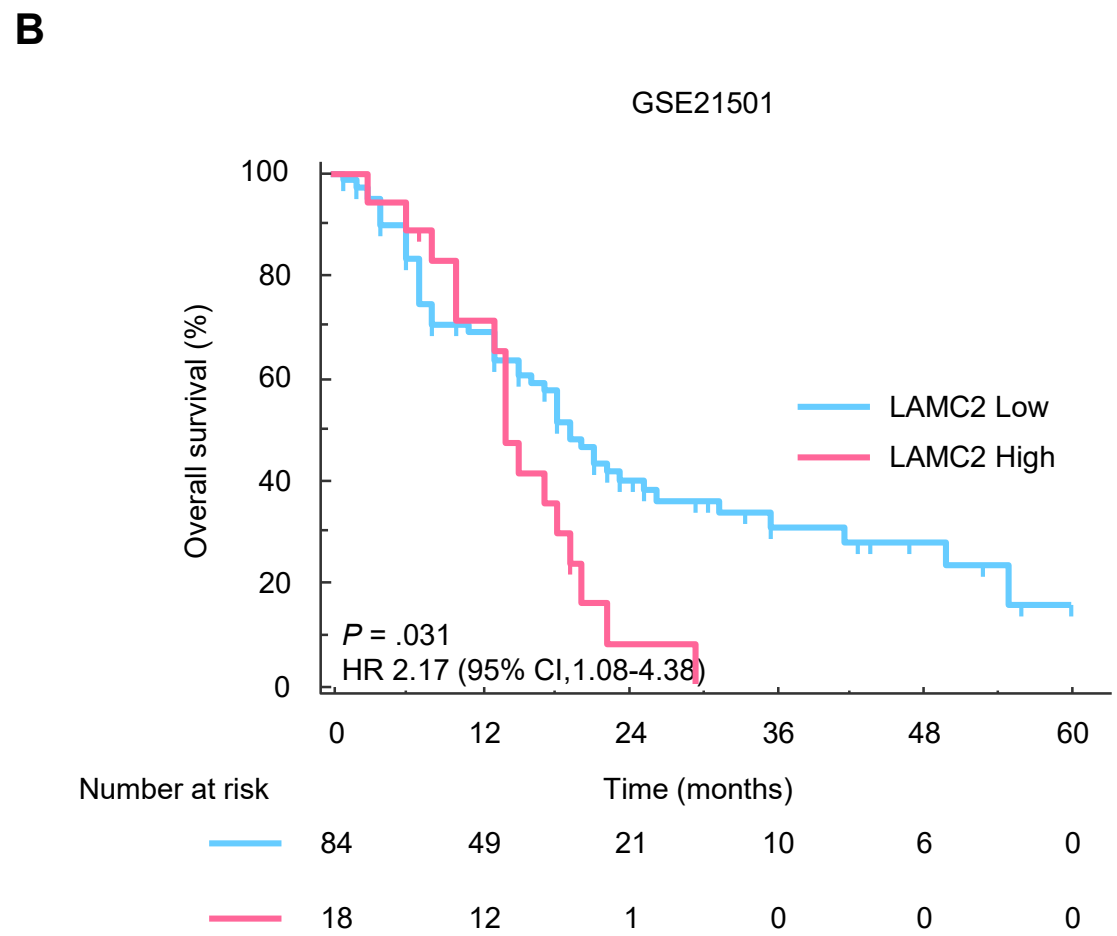
Supplementary Figure S5. LAMC2 expression predicts therapeutic response to gemcitabine response in PDAC patients treated in adjuvant setting. Kaplan-Meier curves for OS and RFS in PDAC patients for gemcitabine based adjuvant therapy in the training (A, C) and the validation cohort (B, D) or 5-FU based adjuvant therapy in the training (E, G) and the validation cohort (F, H).

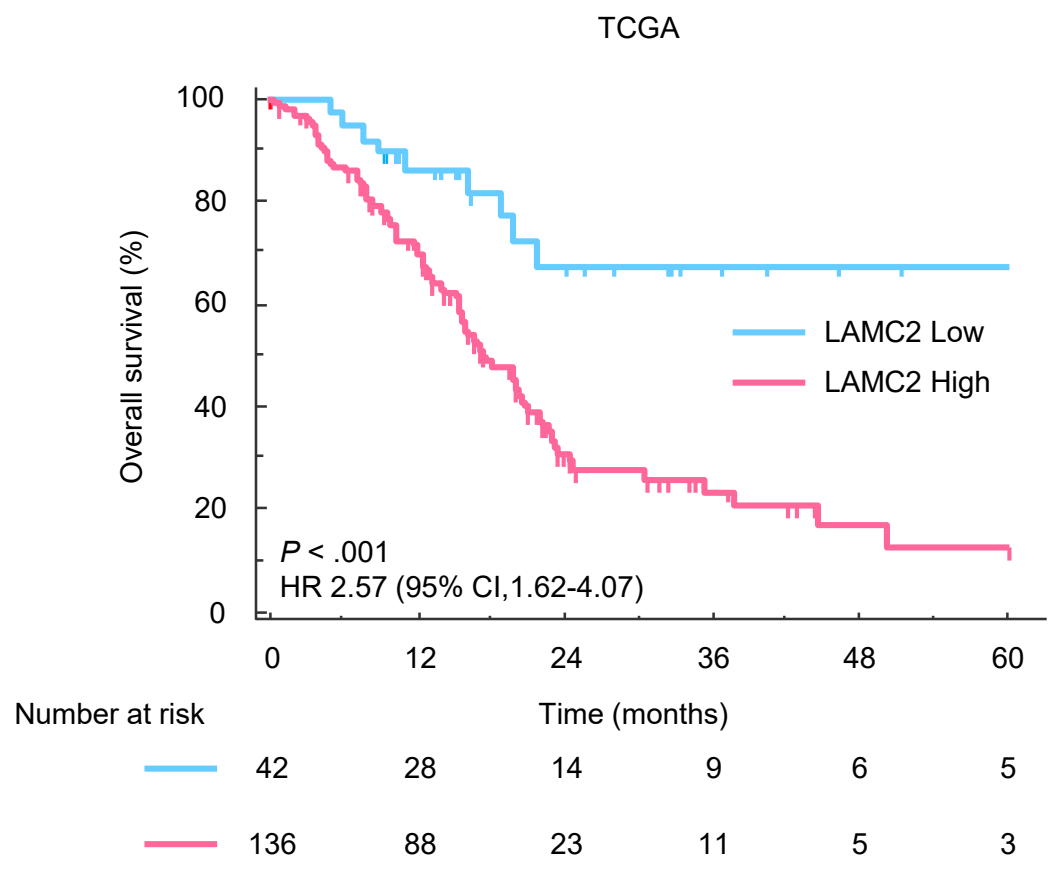
Supplementary Figure S1

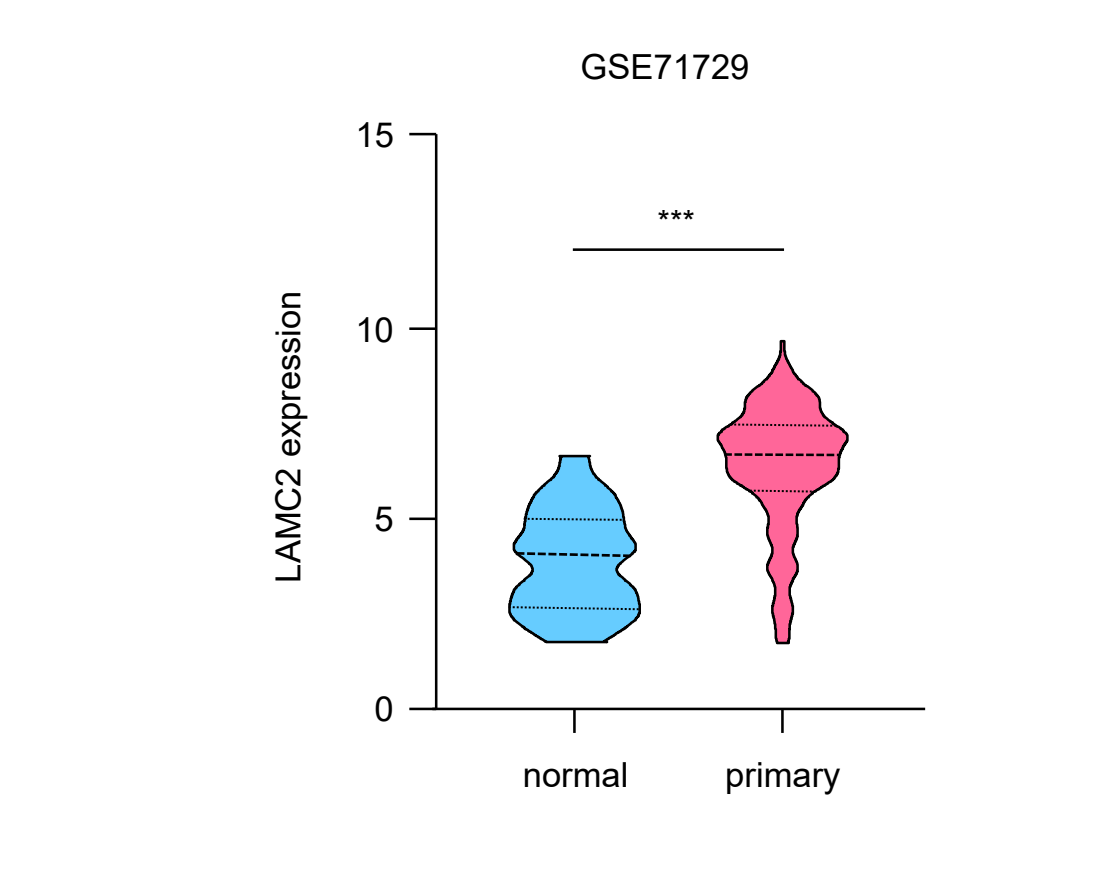


Supplementary Figure S2

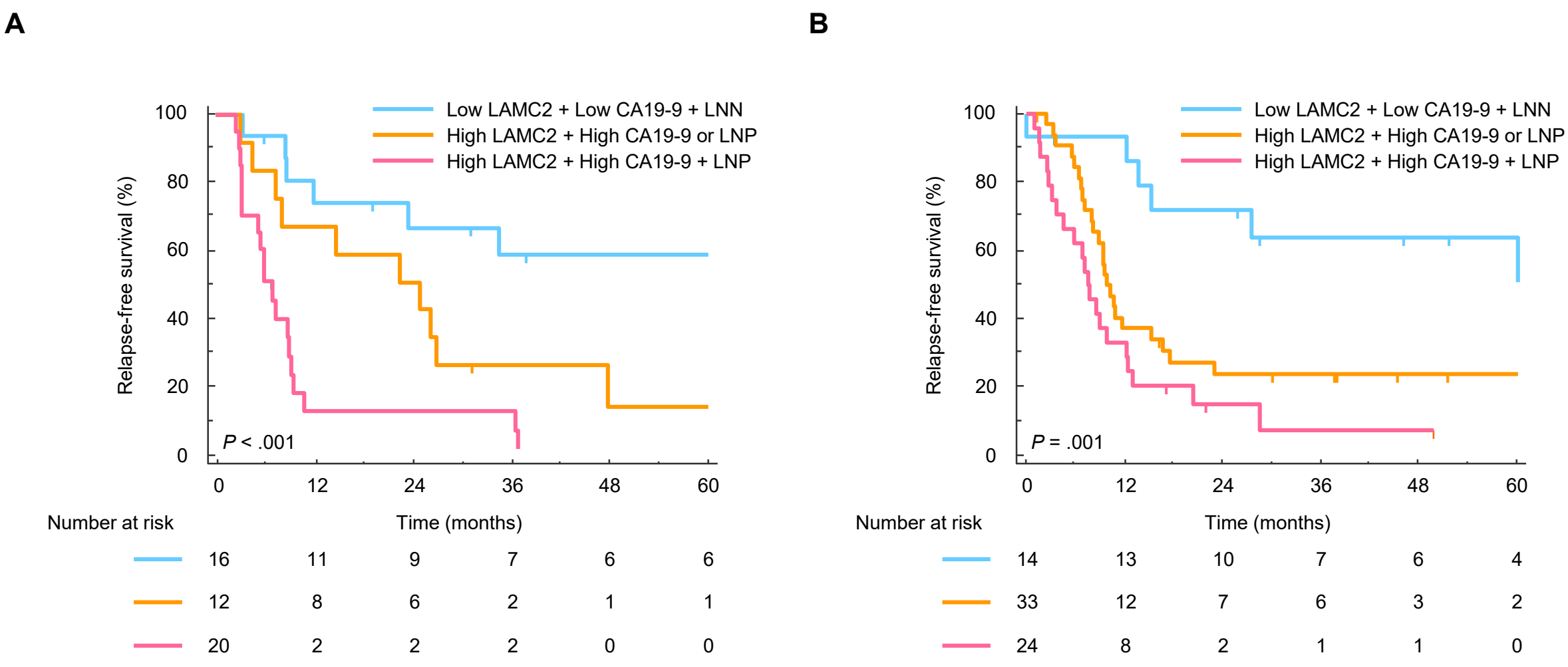




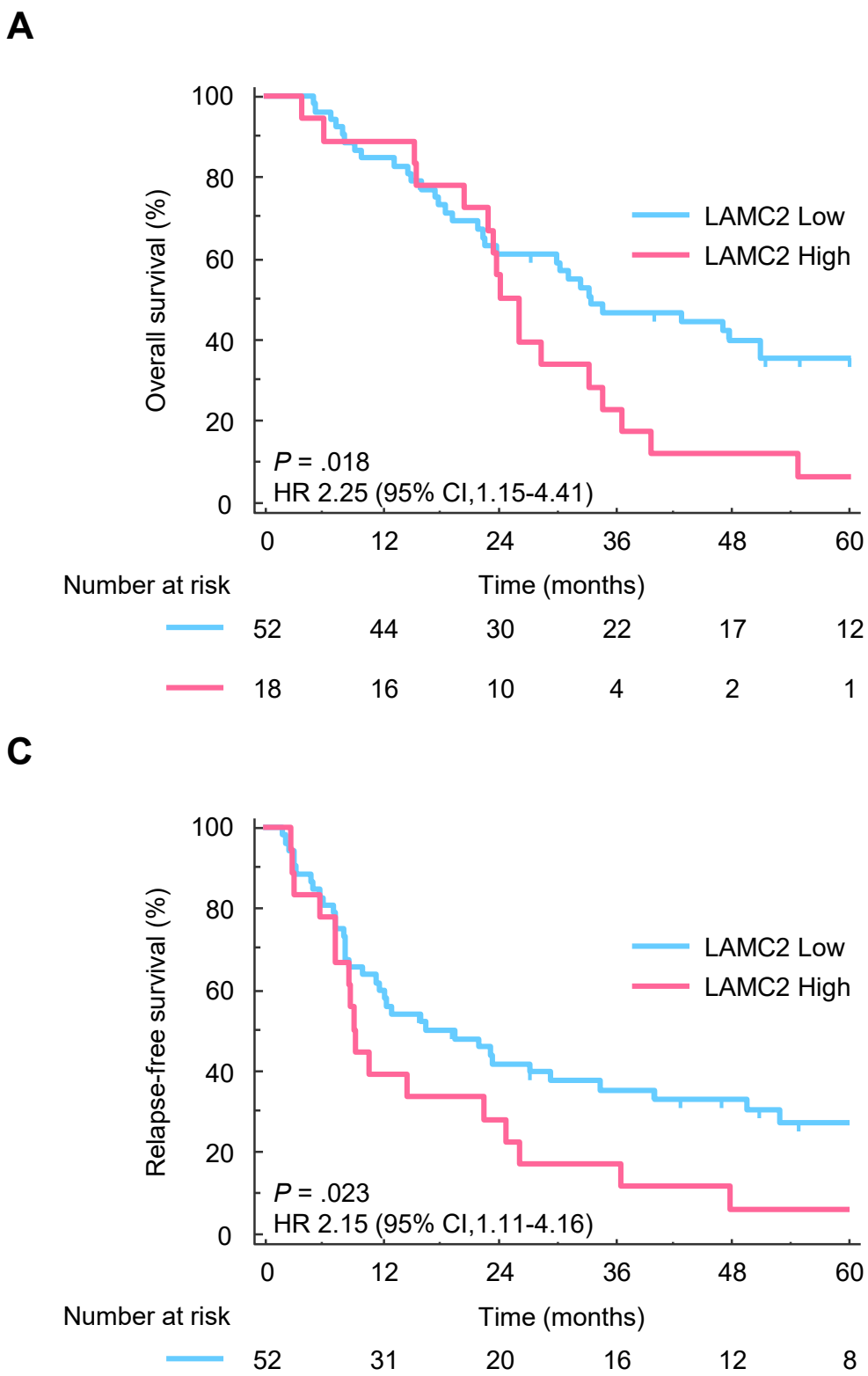


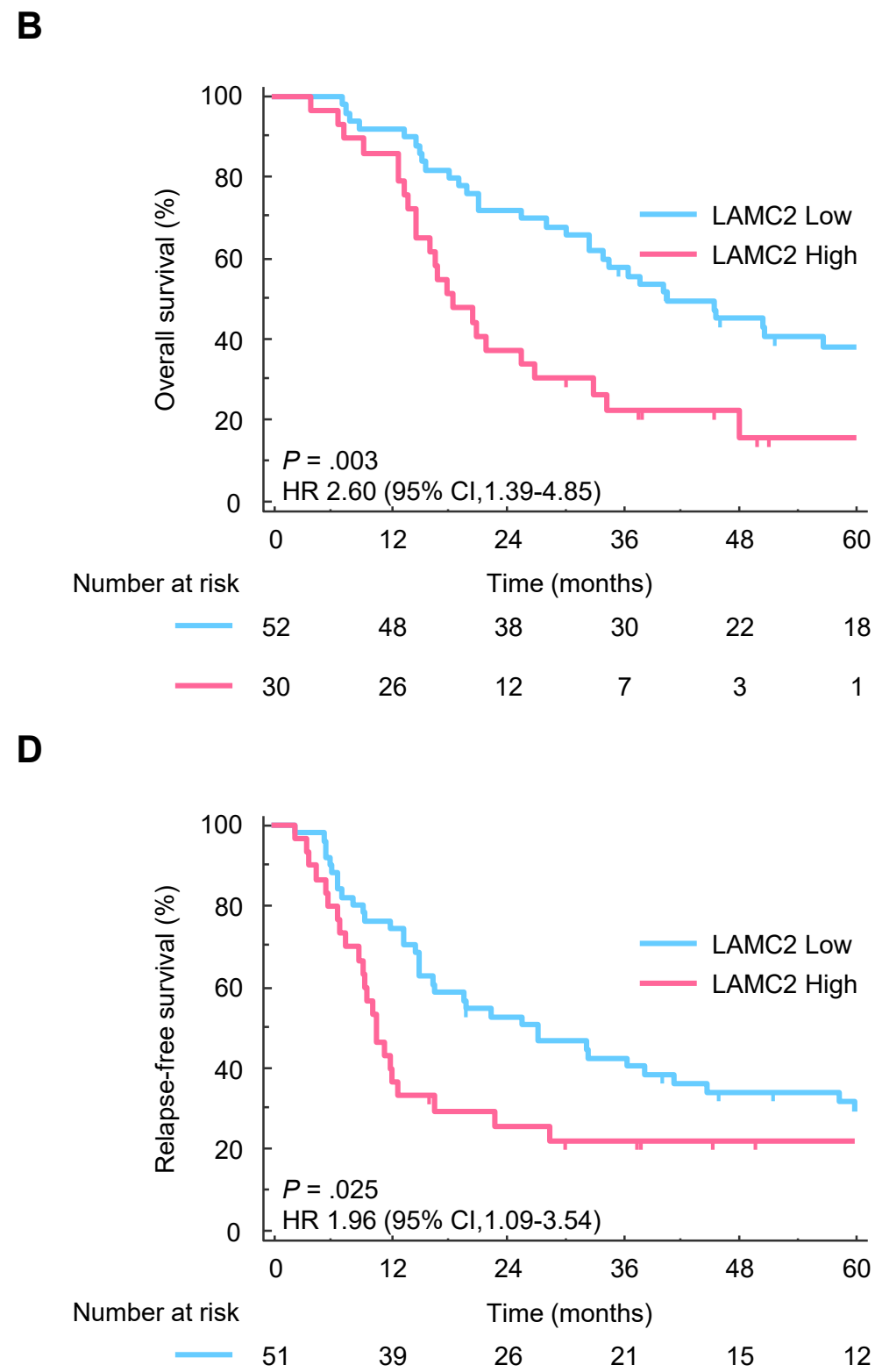


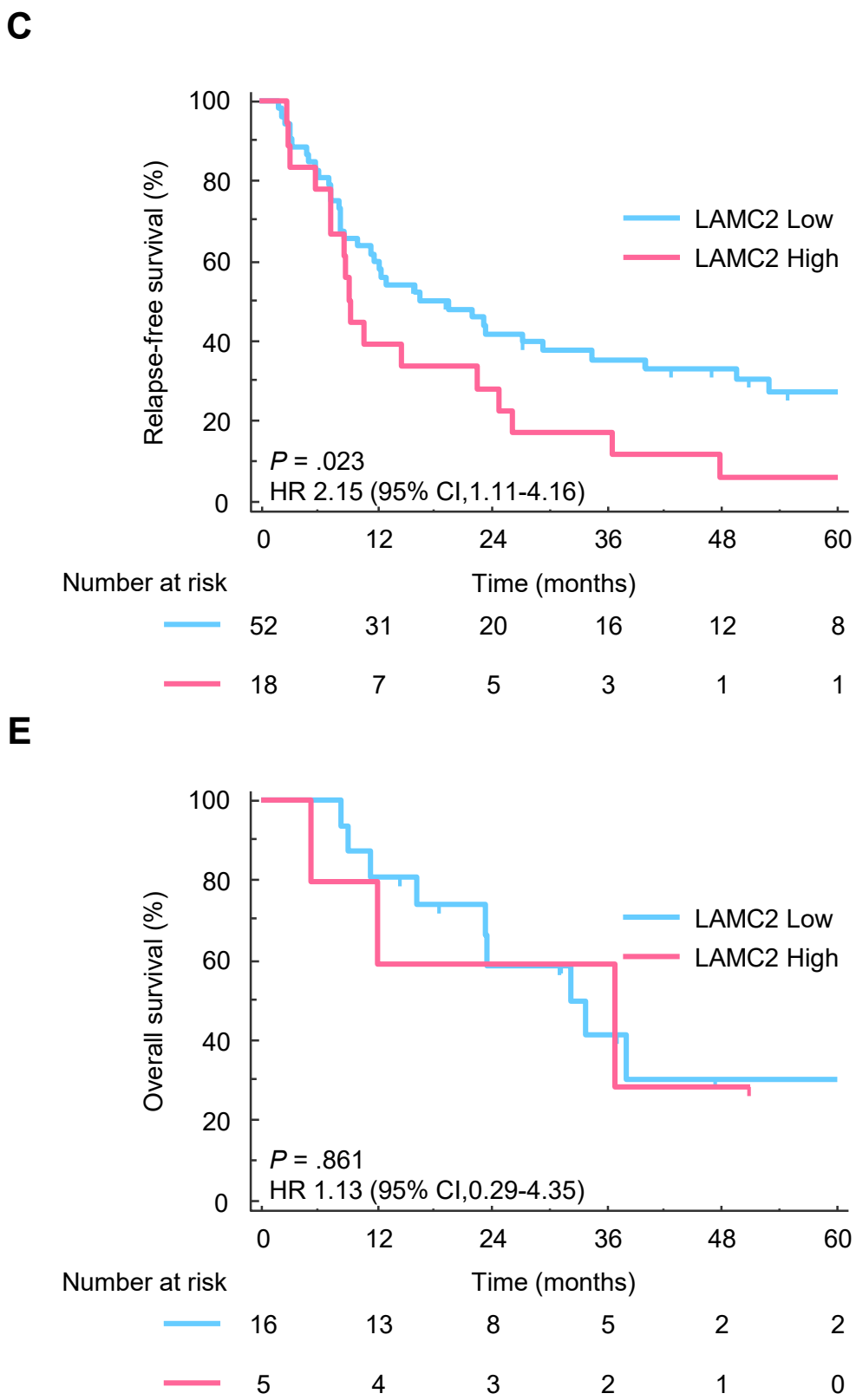
Supplementary Figure S3

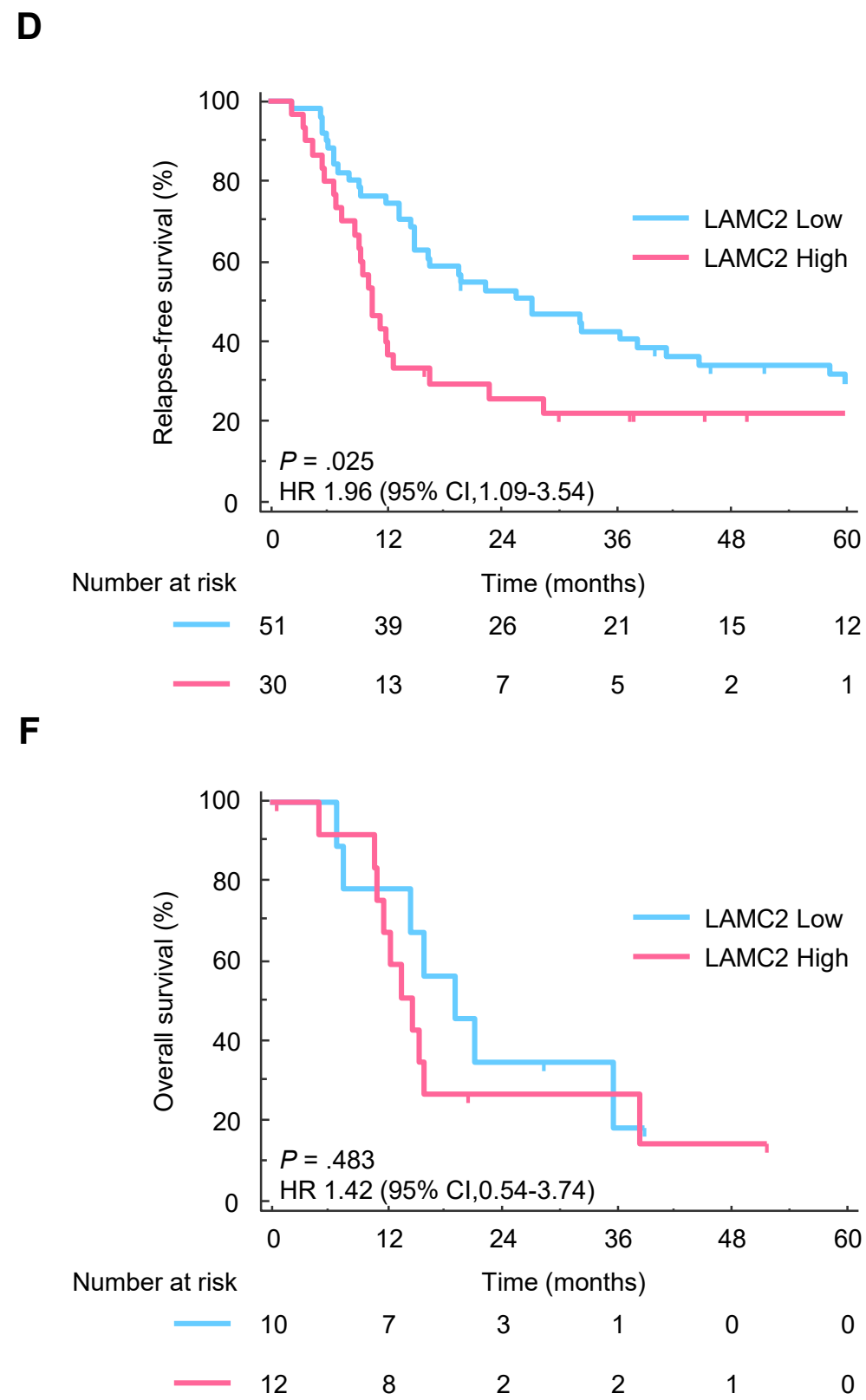


Supplementary Figure S4









Supplementary Table 1 Extracellular matrix related gene list

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A2M
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ACAN
ACHE
ACTB
ACTG1
ACVR1B
ACVR2B
ADAM10
ADAM11
ADAM12
ADAM15
ADAM19
ADAM8
ADAMDEC1
ADAMTS1
ADAMTS10
ADAMTS12
ADAMTS13
ADAMTS14
ADAMTS15
ADAMTS16
ADAMTS17
ADAMTS18
ADAMTS19
ADAMTS2
ADAMTS20
ADAMTS3
ADAMTS4
ADAMTS5
ADAMTS6
ADAMTS7
ADAMTS8
ADAMTS9
ADAMTSL1
ADAMTSL2
ADAMTSL3
ADAMTSL4
ADAMTSL5
ADGRA2
ADGRG1
ADGRG6
ADIPOQ
ADTRP

AEBP1
AGRN
AGT
AHSG
ALPL
AMBN
AMBP
AMELX
AMELY
AMTN
ANG
ANGPT1
ANGPTL2
ANGPTL4
ANGPTL6
ANOS1
ANTXR1
ANTXR2
ANXA1
ANXA11
ANXA2
ANXA2P2
ANXA4
ANXA5
ANXA6
ANXA7
ANXA8
APBB2
APCS
APLP1
APOA1
APOA4
APOC3
APOE
APOH
APP
ARHGAP9
ARHGDIA
ASPN
ATP7A
ATXN1L
AXL
AZGP1
B4GALT1
BCAM
BCAN
BCAR1

BCAR3
BCL3
BGN
BMP1
BMP4
BMP7
BSG
C17orf58
C1QA
C1QB
C1QC
C6orf15
CALR
CAPN1
CAPN10
CAPN11
CAPN12
CAPN13
CAPN14
CAPN15
CAPN2
CAPN3
CAPN5
CAPN6
CAPN7
CAPN8
CAPN9
CAPNS1
CAPNS2
CARMIL2
CASK
CASP3
CAST
CBLN1
CBLN4
CCBE1
CCDC80
CCN1
CCN2
CCN3
CCN6
CD151
CD180
CD248
CD34
CD4
CD44

CD47
CD55
CD6
CDH1
CDH13
CDH2
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CFP
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COLQ
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CPA6
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CPN2
CPZ
CREB3L1
CRELD1
CRISP3
CRISPLD2
CRTAC1
CRTAP
CSGALNACT1
CSPG4
CST3
CSTB
CTHRC1
CTRB1
CTRB2
CTSB
CTSC
CTSD

CTSF
CTSG
CTSH
CTSK
CTSL
CTSS
CTSV
CTSZ
CXCL12
DAG1
DAND5
DCN
DDR1
DDR2
DEFA1
DEFA1; DEFA1B
DGCR6
DLG1
DMBT1
DMD
DMP1
DNAJB6
DPP4
DPT
DSPP
DST
DUOX1
DUOX2
DYM
ECM1
ECM2
EDIL3
EFEMP1
EFEMP2
EFNA5
EGFL6
EGFL7
EGFLAM
ELANE
ELF3
ELFN1
ELFN2
ELN
EMCN
EMID1
EMILIN1
EMILIN2

EMILIN3
ENAM
ENG
ENTPD2
EPHA1
EPN3
EPYC
ERBIN
ERCC2
ERO1A
ERO1B
ETS1
EXOC8
EYS
F11R
F12
F13A1
F2
F3
F7
F9
FAP
FBLIM1
FBLN1
FBLN2
FBLN5
FBLN7
FBN1
FBN2
FBN3
FCN1
FER
FERMT1
FERMT2
FGA
FGB
FGF1
FGF10
FGF2
FGF20
FGF9
FGFBP1
FGFBP3
FGFR1
FGFR2
FGFR4
FGG

FGL2
FLG
FLNA
FLNC
FLOT1
FLRT1
FLRT2
FLRT3
FLT4
FMOD
FN1
FOXF1
FOXF2
FRAS1
FREM1
FREM2
FREM3
FSCN1
FURIN
FZD4
GAS6
GDF10
GDF15
GFOD2
GH1
GLDN
GLG1
GOLGA7B
GOLM1
GP1BA
GPC1
GPC2
GPC3
GPC4
GPC5
GPC6
GPLD1
GPM6B
GREM1
HAPLN1
HAPLN2
HAPLN3
HAPLN4
HAS1
HAS2
HAS3
HDGF

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HMCN2
HNRNPM
HPSE
HPSE2
HPX
HRG
HRNR
HSD17B12
HSP90B1
HSPG2
HTRA1
HTRA3
I6L893
IBSP
ICAM1
ICAM2
ICAM3
ICAM4
ICAM5
IFNA2
IGF1R
IGFALS
IGFBP7
IHH
IL6
IL7
ILK
IMPG1
IMPG2
INHBE
ITGA1
ITGA10
ITGA11
ITGA2
ITGA2B
ITGA3
ITGA4
ITGA5
ITGA6
ITGA7
ITGA8
ITGA9
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ITGAE
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JAM3
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KERA
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KLK4
KLK5
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LAMB3
LAMB4
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LAMC2
LAMC3
LCP1
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LEFTY2

LGALS1
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LGALS3BP
LGALS4
LGALS8
LGALS9
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LIMS2
LINGO1
LINGO2
LINGO3
LINGO4
LMAN1
LMAN1L
LOX
LOXL1
LOXL2
LOXL4
LPL
LPP
LRIG1
LRIG2
LRIG3
LRP1
LRP2
LRRC15
LRRC17
LRRC24
LRRC32
LRRC3B
LRRC3C
LRRN1
LRRN2
LRRN3
LRRTM1
LRRTM3
LRRTM4
LTBP1
LTBP2
LTBP3
LTBP4
LUM
MADCAM1
MAMDC2
MARCO
MARCOL
MATN1

MATN2
MATN3
MATN4
MBL2
MDK
MEGF9
MELTF
MEP1B
MEPE
MERTK
MET
MFAP1
MFAP2
MFAP4
MFAP5
MFGE8
MGAT5
MGP
MKLN1
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MMP8
MMP9
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MRC2
MSANTD3-TMEFF1

MST1
MST1R
MUC15
MUC17
MUC2
MUC3A
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MUC6
MXRA5
MXRA7
MYF5
MYOC
NAV2
NBL1
NCAM1
NCAN
NCSTN
NDNF
NDP
NF1
NFKB2
NID1
NID2
NOTCH1
NOX1
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NPNT
NPPA
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NRAP
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NTN3
NTN4
NTN5
NYX
OC90
OGN
OLFML2A
OMD
OPTC
ORM1
ORM2
OTOA
OTOL1
P3H1

P3H2
PALLD
PARVA
PARVB
PCOLCE
PCSK6
PDGFA
PDGFB
PDGFD
PDGFRA
PDPN
PECAM1
PF4
PHEX
PHOSPHO1
PI3
PIK3CA
PKM
PLG
PLGLA
PLGLB1; PLGLB2
PLOD3
PLSCR1
PMEPA1
PODN
PODNL1
POMT1
POSTN
PPIB
PRDM5
PRDX4
PRELP
PRG2
PRG3
PRG4
PRKCE
PRSS1
PRSS2
PRSS36
PRTN3
PSAP
PSEN1
PTK2
PTN
PTPRZ1
PTX3
PXDN

PXN
PZP
QSOX1
RACK1
RARRES2
RB1
RBP3
RCC2
RECK
RELL2
RELN
RGCC
RPSA
RPTN
RRP1B
RTBDN
RTN4RL1
RTN4RL2
S100A10
S100A4
S100A6
S100A7
S100A8
S100A9
SBSPON
SCARA3
SCUBE1
SCUBE3
SDC2
SDC3
SEMA3B
SEMA3E
SEMA7A
SERAC1
SERPINA1
SERPINA3
SERPINA5
SERPINB1
SERPINB12
SERPINB5
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SERPING1
SERPINH1
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SFTPD
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SLPI
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SMAD4
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SMOC2
SMPD3
SNORC
SNTA1
SOD3
SORBS3
SORL1
SORT1
SOST
SOX9
SPACA3
SPARC
SPARCL1
SPINK5
SPINT1
SPOCK1
SPOCK2
SPOCK3
SPON1
SPON2
SPP1

SPP2
SRC
SRPX
SRPX2
SSC5D
SSPN
ST14
ST7
STAB2
STATH
SULF1
TCF15
TECTA
TECTB
TEK
TFF3
TFIP11
TFPI2
TGFB1
TGFB1I1
TGFB2
TGFB3
TGFBI
TGFBR1
TGFBR2
TGFBR3
TGM2
TGM4
THBS1
THBS2
THBS3
THBS4
THSD4
TIMP1
TIMP2
TIMP3
TIMP4
TINAG
TINAGL1
TLL1
TLL2
TLR3
TMEFF1
TMEFF2
TMEM150B
TMEM38B
TMPRSS6

TNC
TNF
TNFAIP6
TNFRSF11B
TNFRSF1A
TNFRSF1B
TNN
TNR
TNXB
TPSAB1
TPSB2
TRIL
TTR
TUFT1
TYRO3
UCMA
USH2A
VASN
VASP
VCAM1
VCAN
VEGFA
VEGFB
VIT
VLDLR
VTN
VWA1
VWA2
VWC2
VWF
WASHC1
WISP1
WISP2
WNT1
WNT10A
WNT10B
WNT11
WNT16
WNT2
WNT2B
WNT3
WNT3A
WNT4
WNT5A
WNT5B
WNT6
WNT7A

WNT7B
WNT8A
WNT8B
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ZP2
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Figure 1

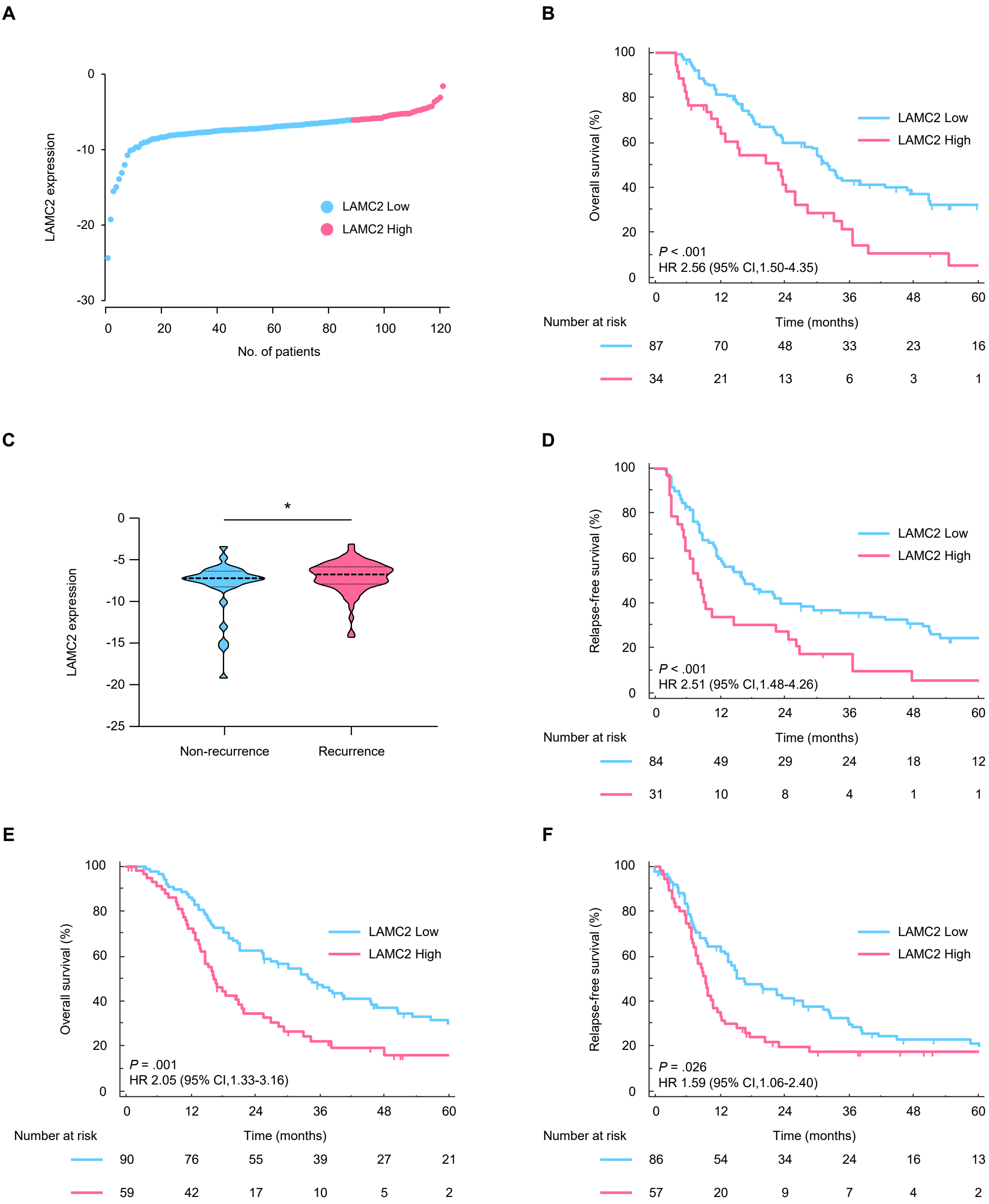
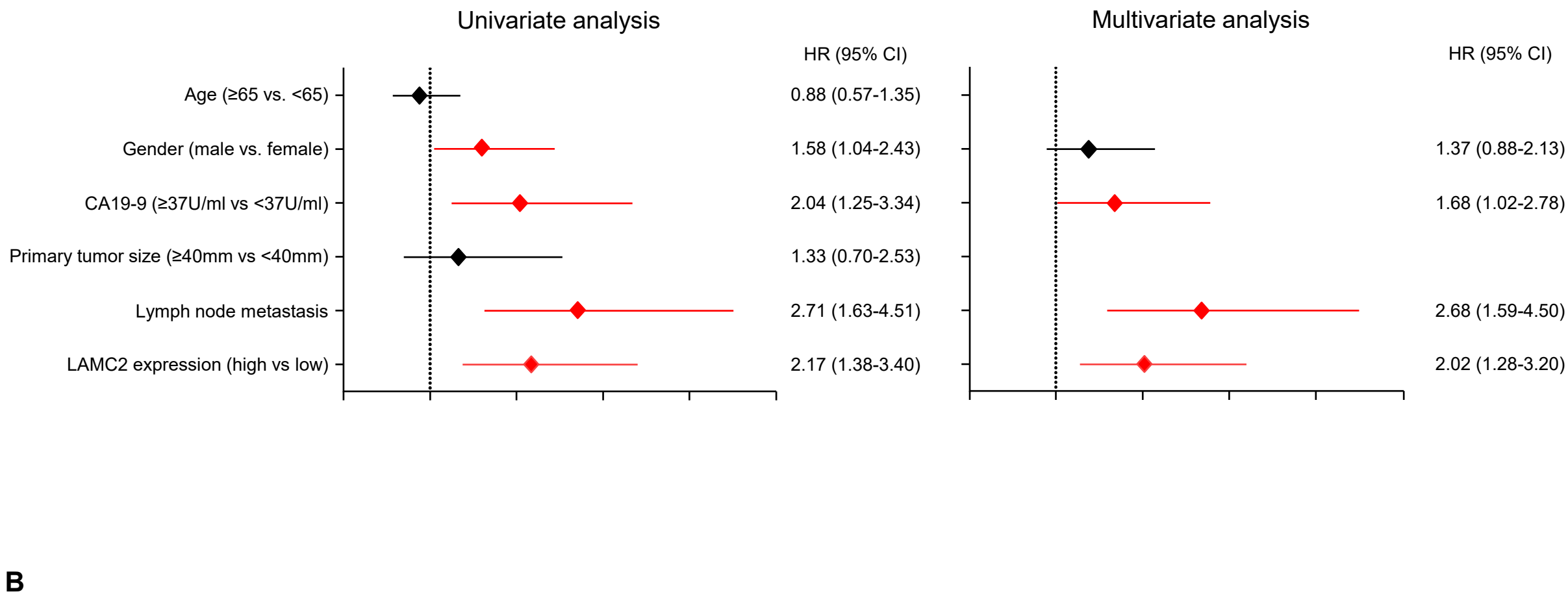
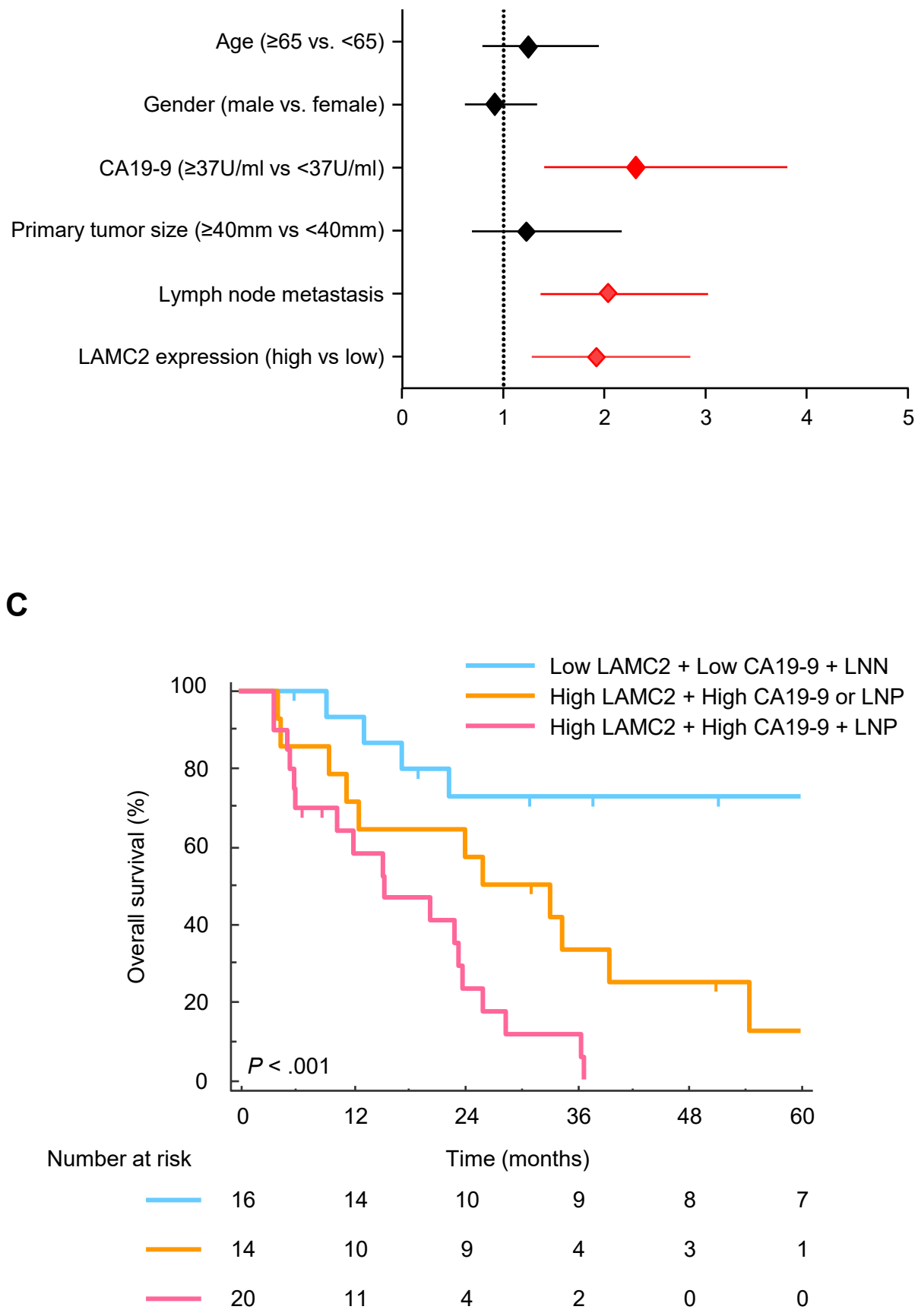

A
LAMC2 expression
0
-10
-20
-30
0
20
40
60
80
100
120
No. of patients
LAMC2 Low
LAMC2 High
B
Overall survival (%)
100
80
60
40
20
0
P < .001
HR 2.56 (95% CI,1.50-4.35)
0
12
24
36
48
60
Time (months)
Number at risk
87
70
48
33
23
16
34
21
13
6
3
1
C
LAMC2 expression
0
-5
-10
-15
-20
-25
*
Non-recurrence
Recurrence
D
Relapse-free survival (%)
100
80
60
40
20
0
P < .001
HR 2.51 (95% CI,1.48-4.26)
0
12
24
36
48
60
Time (months)
Number at risk
84
49
29
24
18
12
31
10
8
4
1
1
E
Overall survival (%)
100
80
60
40
20
0
P = .001
HR 2.05 (95% CI,1.33-3.16)
0
12
24
36
48
60
Time (months)
Number at risk
90
76
55
39
27
21
59
42
17
10
5
2
F
Relapse-free survival (%)
100
80
60
40
20
0
P = .026
HR 1.59 (95% CI,1.06-2.40)
0
12
24
36
48
60
Time (months)
Number at risk
86
54
34
24
16
13
57
20
9
7
4
2

Figure 2


A
Univariate analysis
HR (95% CI)
Age (≥65 vs. <65)
0.88 (0.57-1.35)
Gender (male vs. female)
1.58 (1.04-2.43)
CA19-9 (≥37U/ml vs <37U/ml)
2.04 (1.25-3.34)
Primary tumor size (≥40mm vs <40mm)
1.33 (0.70-2.53)
Lymph node metastasis
2.71 (1.63-4.51)
LAMC2 expression (high vs low)
2.17 (1.38-3.40)
0
1
2
3
4
5
Multivariate analysis
HR (95% CI)
1.37 (0.88-2.13)
1.68 (1.02-2.78)
2.68 (1.59-4.50)
2.02 (1.28-3.20)
B
Univariate analysis
HR (95% CI)
Age (≥65 vs. <65)
1.25 (0.80-1.95)
Gender (male vs. female)
0.91 (0.62-1.34)
CA19-9 (≥37U/ml vs <37U/ml)
2.31 (1.40-3.80)
Primary tumor size (≥40mm vs <40mm)
1.23 (0.70-2.17)
Lymph node metastasis
2.04 (1.38-3.03)
LAMC2 expression (high vs low)
1.92 (1.29-2.85)
0
1
2
3
4
5
Multivariate analysis
HR (95% CI)
2.25 (1.36-3.70)
2.02 (1.36-2.99)
1.84 (1.24-2.74)


C
Low LAMC2 + Low CA19-9 + LNN
High LAMC2 + High CA19-9 or LNP
High LAMC2 + High CA19-9 + LNP
Overall survival (%)
100
80
60
40
20
0
P < .001
0
12
24
36
48
60
Time (months)
Number at risk
16 14 10 9 8 7
14 10 9 4 3 1
20 11 4 2 0 0

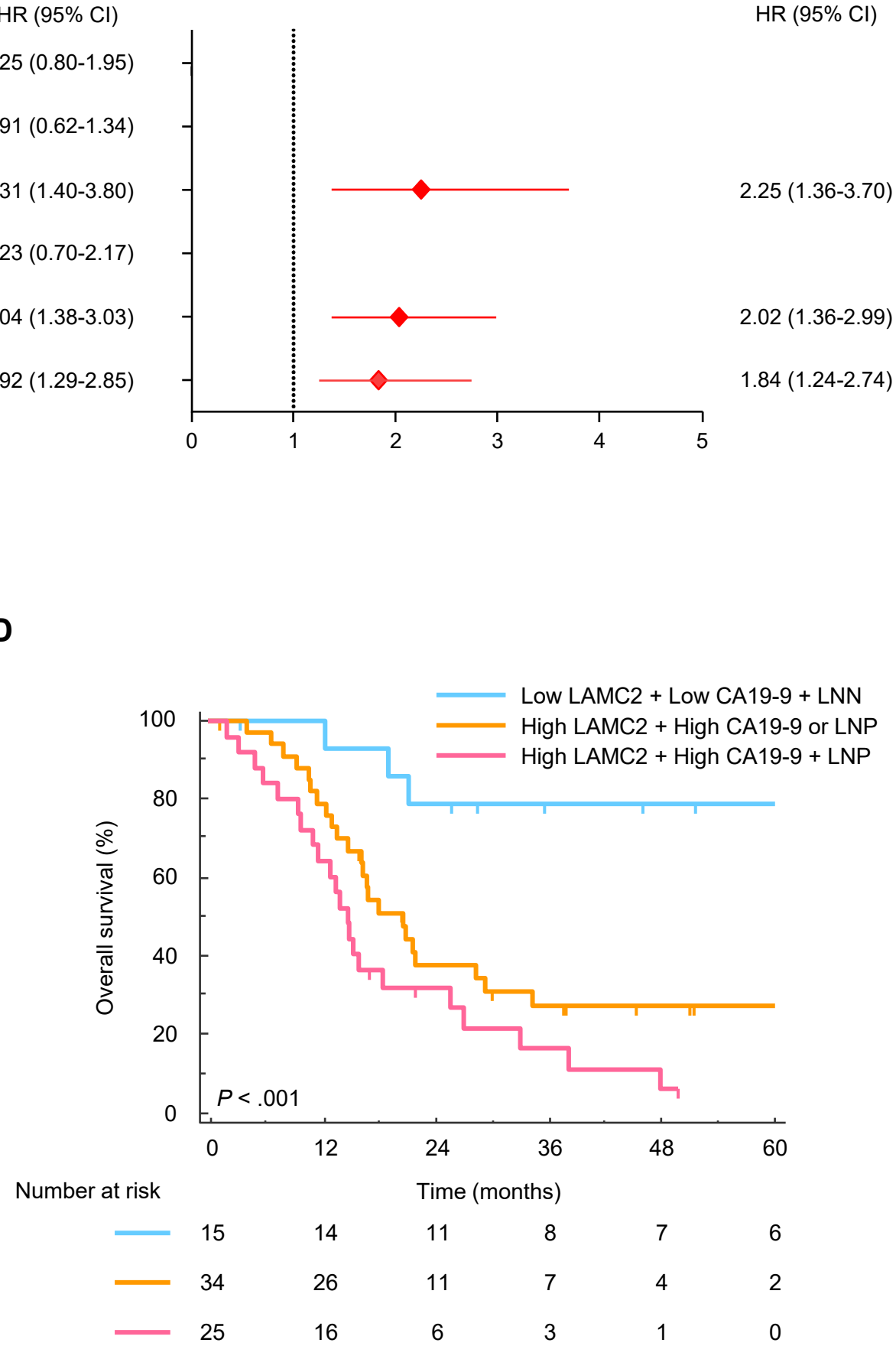
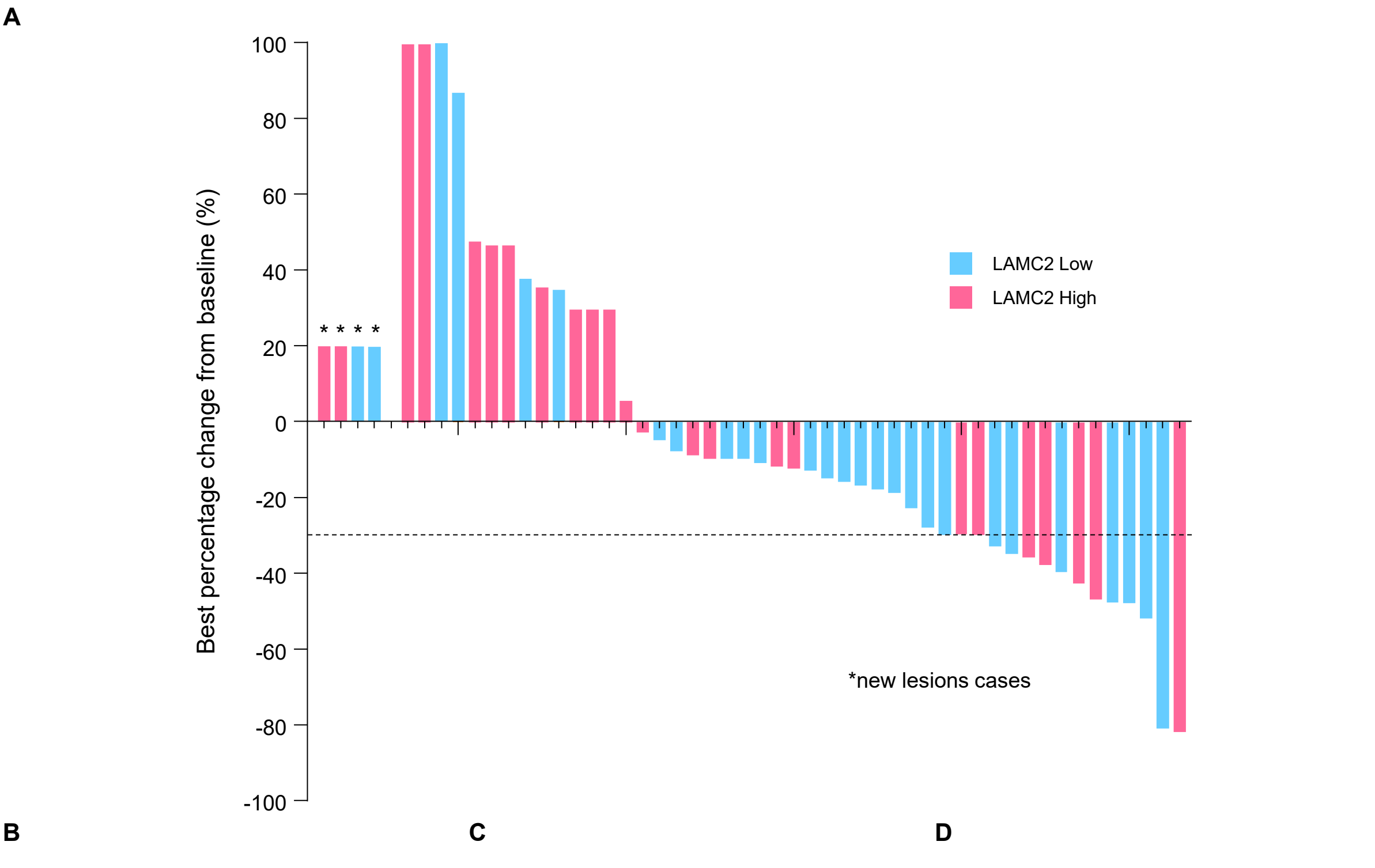

D
Low LAMC2 + Low CA19-9 + LNN
High LAMC2 + High CA19-9 or LNP
High LAMC2 + High CA19-9 + LNP
Overall survival (%)
100
80
60
40
20
0
P < .001
0
12
24
36
48
60
Time (months)
Number at risk
15 14 11 8 7 6
34 26 11 7 4 2
25 16 6 3 1 0

Figure 3


A
Best percentage change from baseline (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
* * * *
LAMC2 Low
LAMC2 High
*new lesions cases
B
C
D
0
-5
-10
-15
***
LAMC2 expression
Responder
Non-responder
*
100
80
60
40
20
0
Percent of patients (%)
High
Low
LAMC2 expression
Responder
Non-responder
100
80
60
40
20
0
Sensitivity
AUC = 0.79
95% CI: 0.65-0.89
0
20
40
60
80
100
100-Specificity
E
F
Overall survival (%)
LAMC2 Low
LAMC2 High
P = .031
HR 2.10 (95% CI,1.07-4.12)
0
6
12
18
24
30
Time (months)
Number at risk
27 22 9 6 3 3
23 13 8 3 1 0
Progression-free survival (%)
LAMC2 Low
LAMC2 High
P = .040
HR 1.88 (95% CI,1.03-3.43)
0
6
12
18
24
Time (months)
Number at risk
27 17 5 2 1
24 9 3 1 0