Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-23-685

Reviewer A

In this study, the authors conducted an assessment of the prognostic significance of dynamin-related protein 1 (DRP1 or DNM1L) in lung adenocarcinoma (LUAD) and its correlation with the immune tumor microenvironment (TME) by employing bioinformatic analyses based on publicly available datasets. The data revealed a significant increase in DNM1L expression in LUAD. Furthermore, DNM1L expression was found to be associated with survival outcomes and the level of infiltration by various immune cells in LUAD. Lastly, the authors validated the oncogenic role of DNM1L in LUAD cells through siRNA-mediated knockdown experiments.

Overall, this study offers valuable insights into the role of DNM1L in LUAD, with experimental results supporting the bioinformatics data. The manuscript is well-organized and well-written. However, several points require attention:

Specific comments:

1) In the “Methods” section, the authors should provide appropriate citations for "HPA," "Prognoscan," and the “KM plotter,” and offer more detailed information regarding the parameters and datasets used for the KM plotter analysis.

Reply 1: Thank you for the comment. We provide the appropriate citations for "HPA," "Prognoscan," and the “KM plotter" in the revised manuscript. In addition, the RNA-seq HTSeq counts and survival data used for the KM plotter analysis in this paper was acquired from the TCGA-LUAD repository. We split patients by auto select best cutoff. When this checkbox is selected, all possible cutoff values between the lower and upper quartiles of DNM1L expression are computed, and the best performing threshold is used as a cutoff. The results page will display the False Discovery Rate in addition to the p-value. We also add an appropriate citation in the revised manuscript.

Changes in the text: line 140-141, line 178-182.

2) In “cell proliferation assay”, “colony formation assay”, “wound healing assay”, and “transwell assay”, the authors should provide details about the quantitative analysis, including the name of the instrument and the software used, the number of replicates, and the number of fields/images utilized for quantification.

Reply 2: Thank you for the comment. For in vitro experiments, each experiment was repeated three times, and two-tailed Student t-test was used to determine the discrepancy between groups.

Changes in the text: line 292.

3) Concerning the results presented in Figure 4A and the corresponding text on page 11, line 347, the statistical tests and parameters used to define the DNM1L-associated genes are not specified. Additionally, the methods and tools used for functional enrichment analysis of differentially expressed genes (DEGs) related to DNM1L should be described. Furthermore, a summary of DNM1L-associated genes and DEGs in the form of supplementary table(s) would be beneficial.

Reply 3: Thank you for the comment. The GeneMANIA database defined the DNM1L-associated genes based on the comprehensive analysis of physical interactions, co-expression, predicted, co-localization, genetic interactions, pathway, and shared protein domains. Here, we provide the profile about the interactions of DNM1L and DNM1L-related genes in the database and uploaded as the supplemental material for your review, in which the statistical tests and parameters used to define the DNM1L-associated genes are shown.

The functional enrichment analysis of differentially expressed genes (DEGs) related to DNM1L was also provided as the supplemental material in GeneMANIA database.

4) For the results of immune cell correlation analysis (Figure 5, Table 1, and Table 2) and KM survival analysis (Figure 6), the tools and parameters employed for data analysis require clarification. Additional information is needed in the “Methods” and “Results” sections.

Reply 4: Thank you for the comment. Gene module in TIMER database allows users to select any gene of interest and visualize the correlation of its expression with immune infiltration level in diverse cancer types. Once your interested gene and immune infiltrates submitted, a heatmap with numbers will show the purity-adjusted spearman's rho across various cancer types. When you click your interested cell on the heatmap, a scatter plot will pop out to present the relationship between infiltrates estimation value and gene expression. We defined the correlation between immune cell infiltration and DNM1L expression based on the following standard. Positive correlation ($P < 0.05$, Pearson correlation coefficient > 0). Negative correlation($P < 0.05$, Pearson correlation coefficient < 0).

For the KM survival analysis, the RNA-seq HTSeq counts and survival data used for the KM plotter analysis in this paper was acquired from the TCGA-LUAD repository. We split patients by auto select best cutoff. When this checkbox is selected, all possible cutoff values between the lower and upper quartiles of DNM1L expression are

computed, and the best performing threshold is used as a cutoff. The results page will display the False Discovery Rate in addition to the p-value. We also add an appropriate citation in the revised manuscript.
Changes in the text: line 178-182, line 193-199.

5) About the results of gene knockdown experiments (Figure 7 and Figure 8), the label "siNC" is unclear. It should be clarified whether it refers to siControl, and if so, the label should be updated accordingly.
Reply 5: Thank you for the comment. The label “siNC” indicates siRNA negative control, and siNC is used as an abbreviation. We revised the description in figure legends.
Changes in the text: Figure legends 6 and 7, line 768-769, line 778-779.

Reviewer B

The study is well written and done. It gives the readers a good understanding DNM1L and its role in lung cancer. Whether it will come to use clinically is however uncertain.

For the section on clinical correlation, while KM curves have been presented, these are unadjusted curves. Results may change significantly if various factors are adjusted for in the multivariate model. Could this be done? If so, the results will have more meaning.
Reply: We genuinely appreciate your comment in evaluating our work. As you comment, we tried to perform univariate and multivariate regression analysis to evaluate the prognostic role in LUAD. The results showed that DNM1L expression is the independent prognostic factor and are shown in the following table. We perform the analysis using R package survival, rms in R studio (4.2.1 version).

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
DNM1L	530	1.298 (1.041 - 1.618)	0.020	1.274 (1.010 - 1.606)	0.041
Pathologic T stage	527				
T1	176	Reference		Reference	
T2	285	1.507 (1.059 -	0.023	1.154 (0.798 -	0.447

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
		2.146)		1.668)	
T3&T4	66	3.095 (1.967 - 4.868)	< 0.001	1.788 (1.045 - 3.060)	0.034
Pathologic N stage	514				
N0	345	Reference		Reference	
N1	96	2.293 (1.632 - 3.221)	< 0.001	1.764 (1.012 - 3.075)	0.045
N2&N3	73	2.993 (2.057 - 4.354)	< 0.001	1.455 (0.743 - 2.849)	0.274
Pathologic stage	522				
Stage I	292	Reference		Reference	
Stage II	123	2.341 (1.638 - 3.346)	< 0.001	1.308 (0.725 - 2.360)	0.372
Stage III&Stage IV	107	3.635 (2.574 - 5.132)	< 0.001	1.994 (0.980 - 4.057)	0.057
Gender	530				
Female	283	Reference			
Male	247	1.087 (0.816 - 1.448)	0.569		
Age	520				
<= 65	257	Reference			
> 65	263	1.216 (0.910 - 1.625)	0.186		

Reviewer C

1. The study relies on several databases, including UALCAN, HPA, GEPIA, PrognoScan, Kaplan-Meier plotter, TIMER2.0, STRING, and GeneMANIA. The reliability of the data from these sources may vary, and it's crucial to acknowledge the potential limitations or biases associated with each database.

Reply 1: Thank you for carefully reviewing my research and providing valuable feedback. We fully agree with your observations regarding the reliability and potential limitations of the databases we relied upon, including UALCAN, HPA, GEPIA, PrognoScan, Kaplan-Meier Plotter, TIMER2.0, STRING, and GeneMANIA. We are aware that web-based databases lack detailed information about data sources and methodologies, which is a significant limitation in my study. We discussed this situation in the limitation section in the revised text.

Nevertheless, to our knowledge, many data sources in public databases come from TCGA or GEO databases, which are uploaded by authors. We firmly believe that these databases have been established and maintained by relevant experts and are widely used in the field of bioinformatics data analysis, thus possessing a certain level of reliability. In future research, we will pay closer attention to and acknowledge these limitations while striving to seek more reliable data sources to support my findings.

Changes in text: line 519-524.

2. The study uses Student's t-test for prognostic analysis. Survival analysis typically involves more sophisticated statistical methods, such as Kaplan-Meier survival curves and Cox proportional hazards models. The simplicity of the statistical tests used may raise concerns about the robustness of the prognostic findings.

Reply 2: We genuinely appreciate your comment in evaluating our work. As you comment, to validate the robustness of the prognostic findings, we tried to perform univariate and multivariate regression analysis to evaluate the prognostic role in LUAD. The results showed that DNM1L expression is the independent prognostic factor and are shown in the following table. We perform the analysis based on the survival and RNA-seq data from the TCGA-LUAD cohort using R pachage survival, rms in R studio (4.2.1 version).

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
DNM1L	530	1.298 (1.041 - 1.618)	0.020	1.274 (1.010 - 1.606)	0.041

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Pathologic T stage	527				
T1	176	Reference		Reference	
T2	285	1.507 (1.059 - 2.146)	0.023	1.154 (0.798 - 1.668)	0.447
T3&T4	66	3.095 (1.967 - 4.868)	< 0.001	1.788 (1.045 - 3.060)	0.034
Pathologic N stage	514				
N0	345	Reference		Reference	
N1	96	2.293 (1.632 - 3.221)	< 0.001	1.764 (1.012 - 3.075)	0.045
N2&N3	73	2.993 (2.057 - 4.354)	< 0.001	1.455 (0.743 - 2.849)	0.274
Pathologic stage	522				
Stage I	292	Reference		Reference	
Stage II	123	2.341 (1.638 - 3.346)	< 0.001	1.308 (0.725 - 2.360)	0.372
Stage III&Stage IV	107	3.635 (2.574 - 5.132)	< 0.001	1.994 (0.980 - 4.057)	0.057
Gender	530				
Female	283	Reference			
Male	247	1.087 (0.816 - 1.448)	0.569		
Age	520				
<= 65	257	Reference			

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
> 65	263	1.216 (0.910 - 1.625)	0.186		

3. The HPA dataset involves the evaluation of DNM1L protein expression in LUAD and healthy control tissues using IHC. The methodology of scoring based on staining intensity, fraction of stained cells, and subcellular localization is subjective and prone to inter-observer variability. A more standardized scoring system or automated quantification could enhance reliability.

Reply 3: Thank you for the comment. We agree. According to other reviewers' suggestions as well, we have included and elaborated further that information in the legends of the revised manuscript. We add the quantity of DNM1L staining in the revised text. In HPA, the quantity of DNM1L staining in lung tumor samples was above 75%, while the quantity of DNM1L staining in lung normal samples is between 25% and 75%. However, due to the limited number of DNM1L staining samples in the human protein profile, more specific data analysis cannot be conducted. In addition, omics analysis also increases the reliability of the conclusion.

Changes in the text: line 714-715.

4. The study uses the GEPIA database for correlation analysis of DNM1L with other genes. While this tool is valuable, the study should provide details on the statistical methods used and how multiple testing issues are addressed, considering the large number of genes being explored.

Reply 4: Thank you for the comment. In the Correlation Analysis pane of GEPIA database, we can compute the correlation of two genes or two signatures in multiple cancer types and tissues. We analyzed the correlation of DNM1L with other genes with spearman coefficient using the non-log scale for calculation and the log-scale axis for visualization.

Changes in the text: line 156-158.

5. The STRING and GeneMANIA databases are employed for PPI network analysis. While these tools provide valuable insights into potential interactions, the study should consider the limitations of predicting interactions based on databases, and experimental validation of key interactions would strengthen the findings.

Reply 5: Thank you for the comment. We appreciate your valuable feedback regarding our study. We fully acknowledge the importance of the STRING and GeneMANIA

databases in protein-protein interaction network analysis, as well as the limitations of predicting interactions based solely on databases. While experimental validation of interactions is crucial, our study aims to uncover the clinical role and potential mechanisms of DNM1L in LUAD. Given the involvement of DNM1L and its related genes in multiple molecular pathways, we agree that further experimental validation is essential. In future research, we intend to delve deeper into the mechanisms already identified to strengthen the reliability and scientific significance of our findings.

6. The use of specific cell lines for RNA interference experiments is mentioned. The study lacks information on the rationale for choosing these cell lines and whether they are representative of LUAD diversity. Details on the validation of knockdown efficiency and the specificity of the siRNAs would enhance the experimental rigor.
Reply 6: Thank you for the comment. The six LUAD cell lines we used in the preliminary stage are all commonly used in LUAD research. In order to explore the function of DNM1L, we screened out the cell lines with relatively high DNM1L expression for knockdown by RT-qPCR and western blot. In the pre-experiment, all three siRNAs can effectively knock down the DNM1L (Figure 6D,6E), so we use the mixture of three siRNAs to construct the DNM1L-knockdown cell lines, and the silence of DNM1L expression was confirmed by RT-qPCR and western blot.
Changes in the text: line 400-404.

7. More references on bioinformatics workflow should be added to attract a broader readership i.e., PMID: 36936815, PMID: 35851932.
Reply 7: Thank you for the suggestion. We cited these references in the revised manuscript.
Changes in the text: line 413-414.

8. The study performs various assays such as cell proliferation, colony formation, wound healing, and Transwell assays. While these experiments are important for functional characterization, the interpretation should be cautious, considering the complexity of cancer biology and the multifaceted roles of genes.
Reply 8: We greatly appreciate your concerns regarding the interpretation of our experimental results, especially considering the complexity of cancer biology and the multifaceted nature of gene interactions. As you said, proliferation, migration and invasion are important functional characterizations for cancer cells. In this study, our analyses showed that DNM1L correlates with immunity and prognosis. Then, we briefly validated the function of DNM1L. As for the specific regulatory mechanisms of DNM1L in cancer, deeper studies are still needed. We have carefully examined the relevant content in the article and made revisions to ensure a more cautious and rigorous

interpretation.

9. The statistical analysis uses Spearman correlation, Student t-test, and P values. However, the study lacks details on adjustments for multiple comparisons and whether the statistical tests meet assumptions. A more comprehensive statistical analysis plan should be provided.

Reply 6: Thank you for the comment. As pointed in question 1, We fully agree with your observations regarding the reliability and potential limitations of the databases I relied upon, including UALCAN, HPA, GEPIA, PrognoScan, Kaplan-Meier Plotter, TIMER2.0, STRING, and GeneMANIA. We are aware that web-based databases lack detailed information about data sources and methodologies, which is a significant limitation in my study. We have carefully examined the article and add the details on adjustments for statistical analysis in the revised text.

Changes in the text: line 156-158, line 178-182, line 193-199.

10. The study uses asterisks to denote significance levels (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). While this is a common practice, the study should explicitly state which tests and comparisons correspond to these significance levels to ensure transparency.

Reply 10: Thank you for the comment. We have carefully examined the relevant content in the article and made revisions to add these significance levels in the corresponding place.

Reviewer D

The manuscript entitled "Prognostic value and immune regulatory role of dynamin 1-like in lung adenocarcinoma" explored the role of DNM1L in tumor progression of LUAD and tried to evaluate the linical value and immune regulation of DNM1L in LUAD. In this manuscript, most of results are adapted from public databases. In general, the association among DNM1L expression, clinical variables, and poor survival outcomes was determined. The results actually showed the high expression of DNM1L might serve as a potential prognostic biomarker in LUAD. However, the analysis of infiltrating immune cells should be improved. The in vitro experiments are not sufficient to support the bioinformatic analysis, either. The current Manuscript should not be accepted.

1. Providing statistical results of DNM1L staining from Human Protein Atlas, especially tumor tissues. Such as Quantity, or intensity, and then draw a plot for this score parameter, rather than just providing single plot for tumor or normal tissue.

Reply 1: Thank you for the comment. According to other reviewers' suggestions as well, we have included and elaborated further that information in the legends of the revised manuscript. We add the quantity of DNM1L staining in the revised text. In HPA, the quantity of DNM1L staining in lung tumor samples was above 75%, while the quantity of DNM1L staining in lung normal samples is between 25% and 75%. However, due to the limited number of DNM1L staining samples in the human protein profile, more specific data analysis cannot be conducted. In addition, omics analysis also increases the reliability of the conclusion.
Changes in the text: line 714-715.

2. The correlation between DNM1L expression and clinical and molecular parameters was investigated in Figure 2. Some groups have small sample size, even less than 10. Therefore, the statistical analysis be relatively meaningless. It could be discussed in the "limitations" paragraph.
Reply 2: Thank you for the comment. We add the discussion about small sample size of the group in the correlation analysis between DNM1L expression and clinical and molecular parameters.
Changes in the text: line 519-524.

3. Figure 3. the color labels are not clear enough, especially the color label of 3A can be enlarged. In addition, which one 3B indicate the OS and PFS of LUAD? The authors should directly mark it on the figure, or clearly explain it in the figure legend.
Reply 3: Thank you very much for pointing this out. As shown in Figure 3, PFS and OS have been marked in the vertical axis. As you comment, we enlarged the color label of 3A in the revised Figure 3.

4. Kaplan-Meier plotter analysis is used in different figures, so which one is from pan cancer using RNA seq, or gene chip, must be described clearly in material and methods, and figure legend. In addition, it should be described how to divided high and low DNM1L expression groups in all survival analysis.
Reply 4: Thank you for the comment. For the KM survival analysis, the RNA-seq HTSeq counts and survival data used for the KM plotter analysis in this paper was acquired from the TCGA-LUAD repository. We split patients by auto select best cutoff. When this checkbox is selected, all possible cutoff values between the lower and upper quartiles of DNM1L expression are computed, and the best performing threshold is used as a cutoff. The results page will display the False Discovery Rate in addition to the p-value. We also add an appropriate citation in the revised manuscript.
Changes in the text: Line 193-199.

5. Although the authors tried to link the DNM1L expression with infiltration of immune cells, there are still many questions that need to be eludated.

(1) In the analysis of Figure 5, even if the P value reaches significance, the values presented by Rho(R) are weak correlation (0.2-0.39). Do such analysis results have the effect of being used as biomarkers? Can this still be used as a biomarker for LUAD infiltrating immune cell?

Reply 5 (1): Thank you for the comment. The correlation values presented in Figure 5 might indeed be weak, but statistical significance indicates that there is still a relationship between DNM1L expression and immune cell infiltration. We have carefully examined the relevant content in the article and made revisions to ensure a more cautious and rigorous interpretation. While these results may not be strong enough to serve as standalone biomarkers, they can contribute valuable information when combined with other markers or factors in a comprehensive analysis.

(2) Similar questions in Table 1 and Table 2, most of correlation between immune cell markers and DNM1L is weak or very weak.

Reply 5 (2): Thank you for the comment. The weak or very weak correlations observed in Table 1 and Table 2 imply that DNM1L alone may not be a robust marker for immune cell infiltration. However, it is crucial to consider that immune cell markers and DNM1L might interact in complex ways within the tumor microenvironment. Additional investigations are needed to understand the potential interplay and identify more reliable markers.

(3) The survival analysis trends of most enriched and decreased cell types are the similar in Figure 6, only the results of 6I/6J and 6O/6P are opposite. Does this result mean that regardless of the number of infiltrating immune cells, it does not significantly affect the expression of DNM1L and the survival of LUAD patients? including CD8+ T cell. Moreover, the survival results are not completely consistent with the results seen in Figure 5.

Therefore, the current analysis did not support DNM1L can be a marker for infiltating immune cells, even a marker for immunotherapy.

Reply 5 (3): Thank you for the comment. We restrict analysis based on cellular content in Kaplan-Meier Plotter database. When we explored the enriched and decreased type of each immune cell, we overlooked the state of the other cells. Considering the complexity of the tumor microenvironment and the interactions between immune cells and cells, the result required further validation. Therefore, we delete this content to enhance rigorism of the paper. This does not affect the other conclusions of the article. However, it is important to note that scientific research often involves iterative processes, and these initial findings provide valuable insights for future investigations.

6. The issues of in vitro experiments.

(1) Five LUAD cell lines were selected. What are the differences between the cells? Why are there obvious differences in the expression of DNM1L? What are the characteristics of these five cell lines? For example, are there any mutations? Or isolated from different stages of LUAD? The authors did not provide any statements to explain it.

Reply 6 (1): We would like to express our gratitude for your valuable feedback on our manuscript. We use multiple LUAD cell lines in an effort to capture the diversity within lung adenocarcinoma. The six LUAD cell lines used in the preliminary stage are all commonly used in LUAD research. In order to explore the function of DNM1L, we screened out the cell lines with relatively high DNM1L expression for knockdown by RT-qPCR and western blot.

(2) In Figure 4, the results indicate the DNM1L may involve in biological processes, including microbody, peroxisome organization, regulation of mitochondrial fission, mitochondrial fission, and regulation of mitochondrion organization. However, the author did not perform any experiments to validate these pathways in this study. In contrast, no evidence suggests the DNM1L affect cell proliferation and cell migration, but the experiments were performed. Why?

Reply 6 (2): Thank you for the comment. The previous study showed DNM1L as a gene associated with mitochondrial fission, and its role in mitochondrial dynamics has been extensively described. However, the specific involvement of DNM1L in the context of lung adenocarcinoma remains to be fully elucidated. Our hypothesis was that DNM1L may impact the biological functions of proliferation, migration, and invasion in lung adenocarcinoma through its influence on mitochondrial fission. Therefore, we conducted experiments to explore this potential link.

While it is true that no direct evidence was found to suggest the effect of DNM1L on cell proliferation and migration in our study, we believe that investigating these aspects is crucial for understanding the malignant characteristics of lung adenocarcinoma and their impact on patient prognosis. Our objective was to explore the potential impact of DNM1L on the aggressive traits of lung adenocarcinoma by focusing on its role in mitochondrial dynamics. In this study, our analyses showed that DNM1L correlates with immunity and prognosis. Proliferation, migration and invasion are important functional characterizations for cancer. So, we briefly validated the effect of DNM1L on LUAD cell phenotype. As for the specific regulatory mechanisms of DNM1L in cancer, deeper studies are still needed.

7. In the western blot of 7D and 7G. Double bands of DNM1L were observed, but only has one was detected the 7I . What is the difference? Protein degradation? Antibody specificity issues? Please improve this issue.

Reply 7: Thank you for the comment. The antibody for DNM1L is purchased from

Proteintech, Wuhan, China with 1:6000 dilution. Theoretically, the band of DNM1L should be a single band, while there are double bands, we speculate that it should be due to compression gel of the PAGE. The compression gel is not tight enough, resulting in double bands.

8. In Figure 8C. The conloy is still too small to count. It may indicate the cell can be collected at later time points. In addition, the statistical results should be provided.
Reply 8: Thank you for the comment. In this study, we set an experimental period of 7 days for colony formation assay. At the end of the experiment, the number of cells per clone was already satisfied to be greater than 50. It is just that the PC-9 cells are relatively small and it looks like the clones are not big. The statistical results are presented in Figure 7C.

Reviewer E

Submitted manuscript titled "Prognostic value and immune regulatory role of dynamin1-like in lung 2 adenocarcinoma", investigated the role of DNM1L in LUAD. The article was generally well written, there are some clinically important findings.

1. In page 8, the expression of DNM1L in cancer and healthy tissues was evaluated using the TIMER2.0 database. As far as I know, TIMER2.0 database is a tool used for analyzing immune cell distribution. The expression of DNM1L was analyzed via UALCAN or HPA, wasn't it.
Reply 1: Thank you for the comment. As you comment, TIMER2.0 database is a tool used for analyzing immune cell distribution. However, in the previous version of TIMER2.0 database, DiffExp module allows users to study the differential expression between tumor and adjacent normal tissues for any gene of interest across all TCGA tumors. Distributions of gene expression levels are displayed using box plots, with statistical significance of differential expression evaluated using the Wilcoxon test. Users can identify genes that are up- or down- regulated in the tumors compared to normal tissues for each cancer type, as displayed in gray columns when normal data are available.

2. The labeling of tumor and normal is missing in FIG1A. The figure FIG1A has too many tumor types, making it difficult to interpret. It would be better to include only tumor types that are statistically significant.
Reply 2: Thank you for your valuable feedback on our manuscript. We have carefully considered your comments and would like to provide a detailed response addressing

the points you raised. It is important to note that the data we utilized for analysis was sourced from a pan-cancer dataset, which is derived from the same sequencing platform. Consequently, segregating individual cancer types for separate analysis poses challenges due to the potential variation in sequencing platforms and could compromise the rigor of our analysis. Nonetheless, we recognize the significance of presenting statistically meaningful tumor types and will make efforts to refine Figure 1A by excluding non-significant tumor types to improve its clarity without compromising the integrity of the data.

3. Author need to describe the reason for analyzing the correlation between DNM1L and the p53/Rb-related pathway and the MYC/MYCN pathway on page 10.

Reply 3: Thank you for the comment.

The correlation analysis between DNM1L and the p53/Rb-related pathway as well as the MYC/MYCN pathway was conducted to explore potential molecular interactions and regulatory mechanisms that may underlie the observed variations in DNM1L expression in the context of lung adenocarcinoma (LUAD).

p53/Rb-Related Pathway: The p53 and Rb pathways are critical in regulating cell cycle progression, DNA repair, and apoptosis. Dysregulation of these pathways is commonly associated with cancer development and progression. Examining the correlation between DNM1L and components of the p53/Rb-related pathway could provide insights into the potential role of DNM1L in LUAD pathogenesis and its relationship with key tumor suppressor mechanisms.

MYC/MYCN Pathway: The MYC family of proteins, including MYC and MYCN, are known oncogenes that play crucial roles in cell proliferation, metabolism, and tumorigenesis. These pathways are frequently dysregulated in various cancers, including LUAD. Analyzing the correlation between DNM1L and the MYC/MYCN pathway could shed light on the involvement of DNM1L in modulating cellular processes linked to tumor growth and aggressiveness in LUAD.

4. In FIG3B, the authors conducted overall survival analysis on 300 patients and progression-free survival analysis on 504 patients. Is the difference in the number of patients due to a difference in the cohorts analyzed in the Kaplan-Meier plotter? Is there a specific reason to have different study cohorts? If a specific cohort was chosen for analysis among the patients in the Kaplan-Meier plotter, the reason for this selection needs to be explained. What is the Affy ID used for DNM1L analysis in the Kaplan-Meier plotter?

Reply 4: Thank you for the comment. We performed the survival analysis based on the RNA-seq HTSeq counts and survival data from 26 different tumor types (including TCGA-LUAD cohort) acquired from the TCGA repository in Kaplan-Meier plotter.

Secondly, the varying patient numbers in the OS and PFS analyses stem from the incomplete nature of some patients' information. Specifically, some patients had data available only for OS and lacked PFS data, leading to the differing patient counts in the two analyses.
For the Affy ID, in the analysis, we used mRNA RNA-seq data not the gene chip data. We entered the name of the gene (DNM1L) and analyzed it.

5. Furthermore, it is essential to clearly define the criteria for 'low' and 'high' levels of DNM1L in the survival analysis conducted in FIG3.
Reply 5: Thank you for the comment. Here, we split patients by auto select best cutoff. When this checkbox is selected, all possible cutoff values between the lower and upper quartiles of DNM1L expression are computed, and the best performing threshold is used as a cutoff. The results page will display the False Discovery Rate in addition to the p-value. We also add an appropriate citation in the revised manuscript.
Changes in the text: line 193-199.

6. The gene names in FIG4A are not clear, so improvement is essential.
Reply 6: Thank you for the comment. We improved the pixel of FIG4A to make it clearer.

7. The GeneMANIA database is a multifunctional web tool. On page 11, it is essential to describe in detail the specific method used to select the five genes related to DNM1L.
Reply 7: Thank you for the comment. The GeneMANIA database defined the DNM1L-associated genes based on the comprehensive analysis of physical interactions, co-expression, predicted, co-localization, genetic interactions, pathway, and shared protein domains. However, the statistical tests and parameters in detail are invisible in GeneMANIA database. We are aware that web-based databases lack detailed information about data sources and methodologies, which is a significant limitation in my study.
Nevertheless, we firmly believe that these databases have been established and maintained by relevant experts and are widely used in the field of bioinformatics data analysis, thus possessing a certain level of reliability. In future research, we will pay closer attention to and acknowledge these limitations while striving to seek more reliable data sources to support my findings.

8. The tool used to analyze the results in Figure 5 is missing from both the results section and Figure 5.
Reply 8: Thank you for the comment. We performed the Spearman correlation analysis to evaluate the correlation between DNM1L expression and infiltration levels of

immune cell subsets using ggplot2 R package in R software (4.2.1 version). We add the relevant content in the revised text.
Changes in the text: line 376-379.

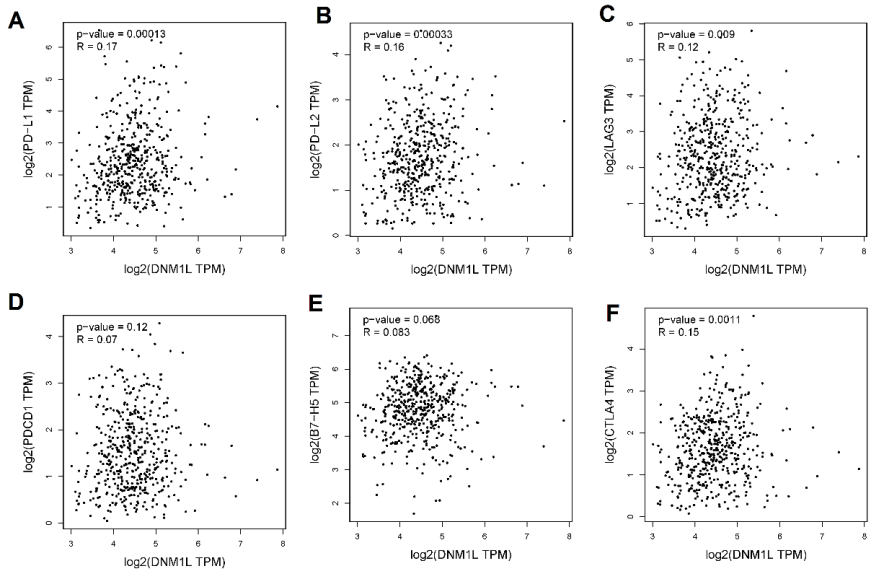
9. A revalidation of the correlation between immune marker sets and DNM1L expression using CIBERSORTx is necessary.
Reply 9: Thank you for the suggestion. As you mentioned, a revalidation of the correlation between immune marker sets and DNM1L expression using CIBERSORTx is necessary. However, we have encountered an issue as there is currently no R package version available for CIBERSORTx, and our analyses need to be conducted online. Unfortunately, the website is currently inaccessible to us.

To address this challenge, we are actively exploring alternative solutions to validate the correlation between immune marker sets and DNM1L expression. We plan to utilize other publicly available tools or methods for similar analyses and ensure the accuracy and reliability of the results.

10. The expression of DNM1L has been reported to be positively correlated with CD8+ T cells. However, CD8+ T cells are effector T cells, which are typically associated with better prognosis. What could be the reason for the poor prognosis despite the high levels of CD8+ T cells in the DNM1L high group?
Reply 10: We performed the Spearman correlation analysis to evaluate the correlation between DNM1L expression and infiltration levels of immune cell subsets using ggplot2 R package in R software (4.2.1 version). The results showed that DNM1L expression was significantly positively associated with the infiltration levels of T helper (Th) cells, especially Th2 cells, while, negatively associated with the infiltration levels of B cells, CD8 T cells, dendritic cells, and mast cells ($P<0.05$).
We restrict analysis based on cellular content in Kaplan-Meier Plotter database. When we explored the enriched and decreased type of each immune cell, we overlooked the state of the other cells. Considering the complexity of the tumor microenvironment and the interactions between immune cells and cells, the result required further validation. Therefore, we delete this content to enhance reliability. This does not affect the other conclusions of the article.
Based on the current analysis, it is true that the evidence does not support DNM1L as a stand-alone marker for immune cell infiltration or immunotherapy response in LUAD. We rechecked the text and rephrased certain sentences with caution However, it is important to note that scientific research often involves iterative processes, and these initial findings provide valuable insights for future investigations.

11. The relationship between DNM1L expression and immune checkpoint is important, so it is necessary to add the correlation between DNM1L expression and the expression of PD-L1, PD-L2, PD-1, LAG3, VISTA, and CTLA4.

Reply 11: Thank you for the suggestion. As you comment, we add the correlation between DNM1L expression and the expression of PD-L1, PD-L2, PD-1, LAG3, VISTA, and CTLA4 based on the Correlation Analysis pan in GEPIA database. We showed the results in supplemental Figure 2.

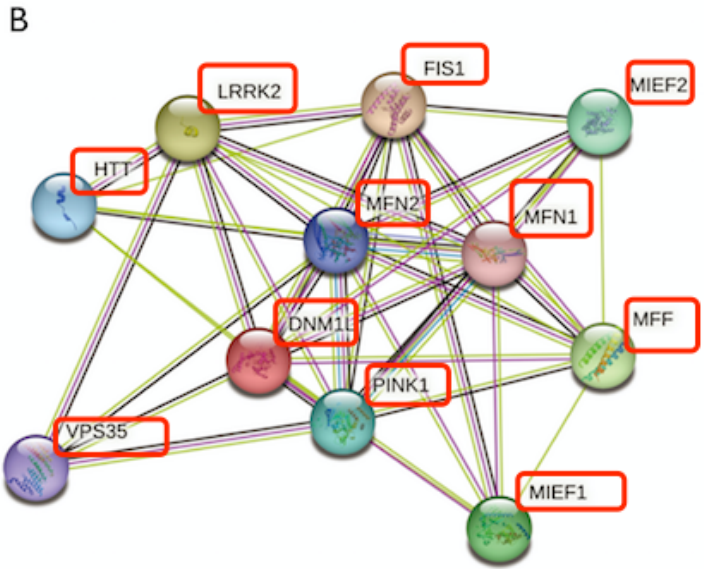


Reviewer F

1. Figure 4B

There are 11 proteins in figure, but it is “10” in the main text. Please check and revise.

367 *DNM1L* gene, its associated PPI network was examined using STRING. The top 10
368 proteins identified in the *DNM1L* network were shown (*Figure 4B*). Among these, the
369 five genes with the highest degree of centrality were mitochondrial fission 1 (*FIS1*)



Response: Thank you. We check and revised the digit in the text.

2. Figure 7C

Please indicate the staining method and observation method of figure 7C in figure legend.

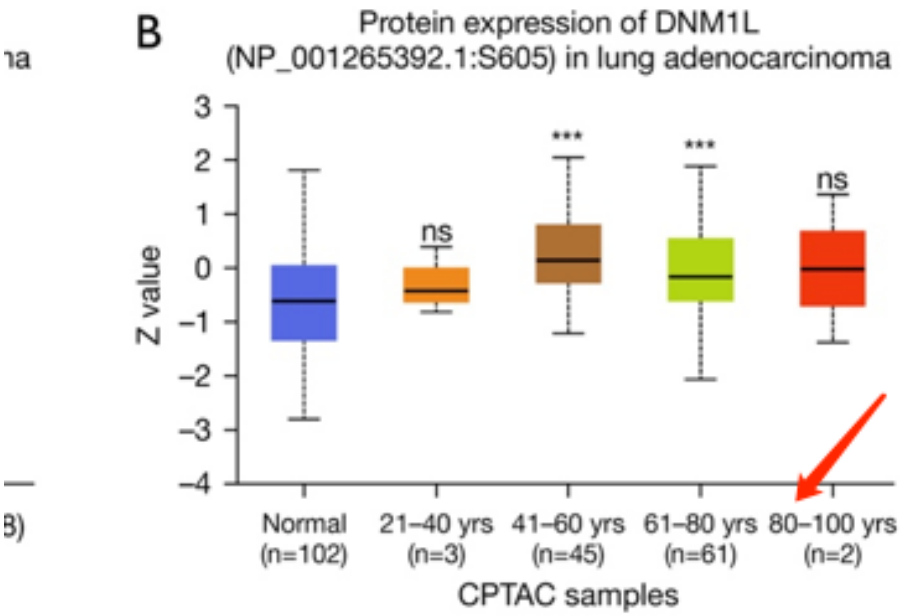
Response: Thank you. Colonies were stained with crystal violet and imaged on the digital camera (Nikon, Japan). The colony numbers were counted using ImageJ. We add it in figure 7 legend.

3. Please indicate the full term of "FIS1, MFF" in figure S1 legend.

Response: Thank you. We indicate the full term of "FIS1, MFF" in figure S1 legend.

4. Figure 2B

Should the pointed "80" be "81"? Please check and revise.



Response: Thank you. We checked and revised to 81-100 yrs.