nature portfolio

Peer Review File

Genomic and Transcriptomic Landscape of Human Gastrointestinal Stromal Tumors

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author): Expert in gastrointestinal cancers, molecular biology, and preclinical models

The study by Xie et al. provides a comprehensive genomic and transcriptomic analysis of a large number of gastrointestinal stromal tumors (GISTs), including early and advanced stage GISTs.
This paper is well-written and the data are very nicely presented.
There are many important insights, probably the most prominent ones are:
Firstly, the authors identify YLPM1 gene inactivation as a novel and frequent mutation of GISTs and provide functional data for YLPM1 being an important tumor suppressor using cell lines and xenografts. Their data reveal that this mutations is most prevalent in less aggressive GIST.
Furthermore, by using transcriptomic analyses, four subtypes of GISTs were postulated and related to clinical classifications, having implications for our understanding for the development, biological characteristics and even treatment of the subtypes.
This study improves the understanding of GIST biology. I have some remaining comments that should be addressed:

YLPM1 mutation found in less aggressive tumors: the authors interpretation is that this is an early mutation. If this was the case, one should still see the mutation in more aggressive one. My interpretation would rather be that the less aggressive ones are distinct from the aggressive ones.
Line 98: Why would identification of genomic and transcriptomic features of aggressive GISTs enable means to prevent cancer? Please clarify/adjust this sentence.
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Line 144: Please provide the primary source as reference for the tumor mutational burden of renal cell carcinoma and chronic lymphocytic leukemia.
Line 200: YLPM1 Mutation in 21 out of 86 patients: Figure 2a does not show this, please clarify.
Line 217/218: What could be the reason for a higher inactivation of YLPM1 on protein level compared to genomic level if WTS and DNA methylation cannot explain this observation? Is there a possible influence of the high occurrence of YLPM1 shallow deletion shown in Fig 2a bottom?
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Line 272, part Widespread Copy number Variations in GISTs: It would be interesting to see copy number alteration signatures in GISTs (Steele et al., Nature 2022).
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Figure 2: YLPM1 mutations in 11 samples according to black fields. However, on the left it was denoted that n=7. Please explain. Is 7 referring to the number of affected patients? Additionally, please explain observed shallow deletions in the main text of the manuscript.
Figure 7: The authors state the the subtypes might! Predict therapy response. I would be very careful

and revise the section and figure: Please clarify that this is just a hypothesis, figure 7K has a row called "therapy" – please re-name to make sure that this is not the actual therapy that the patients received, e.g. "potential conclusion regarding therapy" or so..

Reviewer #2 (Remarks to the Author): Early Career Researcher co-reviewer

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author): GIST clinical research, therapy, and immunotherapy

The article provides interesting and new information on the molecular subtypes on these rare tumors with possible therapeutic implications to be tested. It will be important to have a description of the survival of patients from the 4 subgroups in localized phase and first line advanced phase.

Reviewer #4 (Remarks to the Author): Expert in GIST and sarcoma genomics, functional genomics, and translational research

The study by Xie et al investigates a relatively large, clinically and molecularly heterogeneous GIST cohort by a combination of WES/WGS and WTS. One of their main findings is that increased genomic complexity (TMB, CNV burden) is positively associated with tumor size and mitotic count. The authors also investigate the association between chromothripsis and kataegis and aggressive GIST. Despite the very comprehensive analyses and platforms used, the study is confounded by the mixed bag of cases being investigated (low risk, high risk, metastases, TKI-treated, etc) with no real hypothesis to follow. Their main novel finding of YLPM1 mutations in GIST is similarly confounded by the discrepancies in their incidence in the various methods applied. The authors also try to address clonal evolution, a big topic in itself; however, the findings fall short as the authors only investigate 4 metastatic cases with multiple lesions, some being pretreated/resistant to TKI.
The paper is poorly written and requires editing for English language and syntax. Also there are lots of inconsistencies throughout the manuscript.
Summary – rephrase and describe simply what are the 2 clinical cohorts. It sounds like most patients (n= 105) are in fact advanced, 'lethal cancers', while only a minority are low risk, early-stage. The wording used is confusing, as it seems that the study is focusing on low risk GIST and mechanisms of tumor progression. Also, a more specific description of the cohort of GIST in which YLPM1 mutations is identified is required as well as its incidence and possible a very brief description of its function.
Intro – a number of sentences can be deleted or rephrased, as they have no meaning. For example: 'to study constraints to tumorigenic progression'; 'The opportunity to study less-aggressive lesions such as

low-risk GIST enables evaluations of the sequence of mutations accounting for oncogenic progression.'
Results: unclear how 68 matched normal samples from 105 patients?! Also the authors include in their genomic study both primary and metastatic samples at diagnosis.
NGS was performed in only 78 cases; WES (n=59) and WGS (n=19); did not include cell lines. Unclear why then the case denominator for cases tested for YLPM1 mutations is only 68 cases; YLPM1 mutations found in 7/68 (11%). The authors should add in the test (page 6) the number of additional cases that were tested by Sanger Seq; it remains unclear why the discrepancy in the incidence of YLPM1 mutations compared to NGS (24%, more than double). Moreover, unclear why by WB and IHC the loss of YLPM1 was detected in 48% and 47%, respectively, of GIST tested!

Reviewer #1 (Remarks to the Author): Expert in gastrointestinal cancers, molecular biology, and preclinical models

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This paper is well-written and the data are very nicely presented.

There are many important insights, probably the most prominent ones are:

Firstly, the authors identify YLPM1 gene inactivation as a novel and frequent mutation of GISTs and provide functional data for YLPM1 being an important tumor suppressor using cell lines and xenografts. Their data reveal that this mutation is most prevalent in less aggressive GIST.

Furthermore, by using transcriptomic analyses, four subtypes of GISTs were postulated and related to clinical classifications, having implications for our understanding for the development, biological characteristics and even treatment of the subtypes.

This study improves the understanding of GIST biology. I have some remaining comments that should be addressed:

We thank the reviewer for his/her appreciation of our work and its significance.

YLPM1 mutation found in less aggressive tumors: the authors interpretation is that this is an early mutation. If this was the case, one should still see the mutation in more aggressive one. My interpretation would rather be that the less aggressive ones are distinct from the aggressive ones.

We thank the reviewer for the comment, which allowed us to elaborate further. In our former manuscript, we assessed the inactivation frequency of YLPM1 at protein level by immunoblotting in 73 GISTs from 64 patients. YLPM1 protein loss was demonstrated in 31 of 64 (48%) patients **(Fig. 3b, Table S5)**. 32% (10 of 31) of the patients with loss of YLPM1 expression were classified as low-risk or intermediate-risk **(Fig. 3b and 3c).** Then, we performed immunohistochemistry to validate the frequency to which YLPM1 protein expression was lost. YLPM1 expression was negative in 47% (129/276) of GISTs on tissue microarray validation cohort, including 75 low or intermediate risk **(Fig. 3e)**, showing that YLPM1 protein loss could be an early event in GIST pathogenesis.

Since YLPM1 protein loss occurs in the low-risk GISTs, we hypothesize that loss of YLPM1 would be an essential event for GIST development, while the loss of protein may not be caused by genomic alterations. *YLPM1* truncated mutations are identified in 7 of 68 patients (10.3%), enriched in high-risk/metastatic GIST. Instead of the epigenetic regulation such as methylation of YLPM1 promoter that has not been detected in our study, one of the explanations is the post-transcriptional modification that influence the translational rate of YLPM1, since similar mechanism of other genes has been reported in GIST (1). Therefore, the protein synthesis pathway of YLPM1 could be dysregulated in parallel with GIST development, especially in early-stage. In our genomic landscape, we show that the tumor mutation burden increases from low-risk to aggressive GIST, suggesting that genomic instability increases during tumor progression, which makes genomic alteration become more prominent cause of YLPM1 protein loss, while in the early-stage, protein synthesis regulation could be the main cause. Similar situations have been found in other cancer types (2, 3).

We apologise that our manuscript was complicated and thank you for the opportunity to clarify here.

Response References:

(1) Xu K, Zhang Q, Chen M, Li B, Wang N, Li C, Gao Z, Zhang D, Yang L, Xu Z, Li X, Xu H. (2022). N6-methyladenosine modification regulates imatinib resistance of gastrointestinal stromal tumor by

enhancing the expression of multidrug transporter MRP1. Cancer Lett. 530:85-99.

(2) Wen YC, Lin YW, Chu CY, Yang YC, Yang SF, Liu YF, Hsiao M, Lee WJ, Chien MH. (2020). Melatonin-triggered post-transcriptional and post-translational modifications of ADAMTS1 coordinately retard tumorigenesis and metastasis of renal cell carcinoma. J Pineal Res. 69(2):e12668.

(3) Liu R, Zeng LW, Gong R, Yuan F, Shu HB, Li S. (2021). mTORC1 activity regulates post-translational modifications of glycine decarboxylase to modulate glycine metabolism and tumorigenesis. Nat Commun. 12(1):4227.

Line 98: Why would identification of genomic and transcriptomic features of aggressive GISTs enable means to prevent cancer? Please clarify/adjust this sentence.

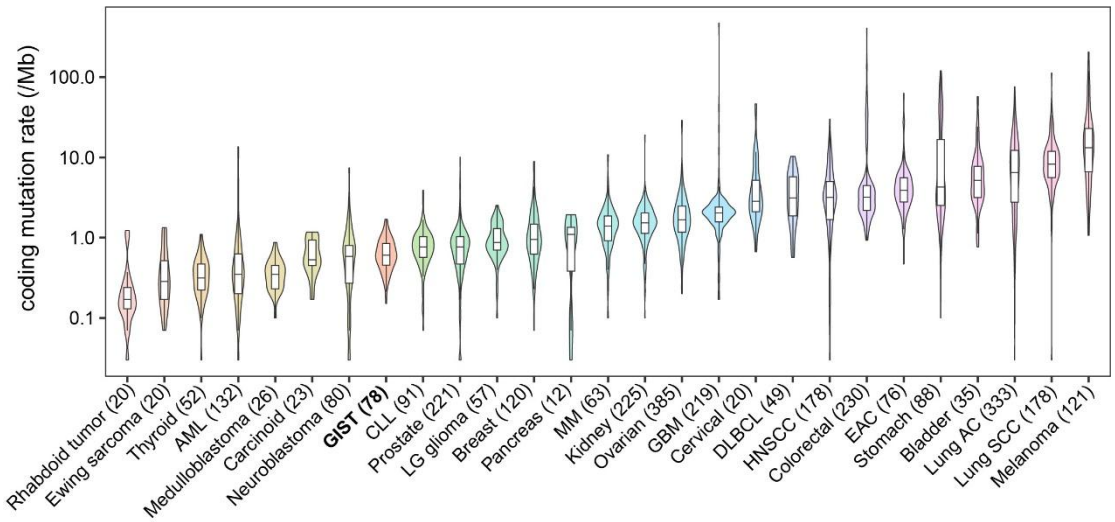
We thank the reviewer for the thoughtful comment. The sentence has been adjusted to "Deciphering the molecular changes contribute to the development of aggressive GIST may shine light on GIST biology and therapeutic strategies" (Lines 98-100).

Line 101: Please add the frequency of these gene mutations in GISTs.

We thank the reviewer for the excellent comment. The frequency of the gene mutations were added as follows (Lines 102-103):
More recently, using cytogenetic approaches and whole-exome sequencing (WES) in a small cohort of patients with GIST, we and others have reported recurrent somatic alterations of *DEPDC5* (17.5%), *DMD* (66%), *MAX* (32%), *SETD2* (11.2% in high-risk GISTs) and *SDH* (9.0%).

Line 144: Please provide the primary source as reference for the tumor mutational burden of renal cell carcinoma and chronic lymphocytic leukemia.

We thank this reviewer for the careful and thoughtful comment. In this study, we obtained the tumor mutational burden data from both Reference 17 (former manuscript) and Pan-Cancer Analysis of Whole Genomes (PCAWG) (1). The source data of the Reference 17 was analyzed by Lawrence *et al* (2). We initially utilized data from Reference 17 for comparing tumor mutational burdens (**Response Fig. 1**). Later, PCAWG published their pan-cancer mutation data. We found that our conclusion - low mutation burden in GIST - was indeed supported by both data from Reference 17 as well as the PCAWG data (**Response Fig. 1**). Then we updated the comparison with mutation burden data from PCAWG to keep up with the latest research achievement in the community. The data sources for PCAWG have also already been described in the legend of Figure S2 in the former manuscript. We have now removed the Reference 17 and added the PCAWG reference (1) in the revised manuscript (Line 147).



Response Fig. 1. Violin plot showing the comparison of mutational burdens between GIST and the other cancers. The data sources were obtained from Lawrence *et al.*

Response References:

(1) Alexandrov LB, Kim J, Haradhvala NJ, et al. The repertoire of mutational signatures in human cancer. Nature. 2020;578(7793):94-101. doi:10.1038/s41586-020-1943-3

(2) Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499(7457):214-218. doi:10.1038/nature12213

Line 200: YLPM1 Mutation in 21 out of 86 patients: Figure 2a does not show this, please clarify.

We are grateful for the comment and we are sorry for the wrong numbers. *YLPM1* truncated mutations were detected in 7 of 68 patients and deep deletions were detected in 2 of 68 patients. The errors were introduced from previous revision in other journal - we have removed the genomic data of the tumor samples without matched normal sample. We have modified the description in the manuscript as follows.

"Homozygous *YLPM1* mutations and deletions were identified in 9 of 68 (13%) patients (**Fig. 2a, S7a**)." (Lines 210-211)

We have also revised the Fig.S7a as follows.

	***YLPM1* aberrations**	**Normal *YLPM1***	**Total**	***YLPM1* aberrations frequency**
GISTs	9	59	68	13.24%
Non-GIST sarcomas (TCGA, Pan-Cancer Atlas)	8	243	253	3.16%
All TCGA PanCancer	364	10589	10953	3.32%

Line 217/218: What could be the reason for a higher inactivation of YLPM1 on protein level compared to genomic level if WTS and DNA methylation cannot explain this observation? Is there a possible influence of the high occurrence of YLPM1 shallow deletion shown in Fig 2a bottom?

We thank the reviewer for raising this important question. In addition to mutation, deletion and promoter hypermethylation, we have also conducted YLPM1 promoter mutation evaluations through Sanger sequencing

in paired tumor and normal samples. Unfortunately, no potential mutations in the promoter region have been identified, indicating that YLPM1 promoter mutations are not common in the regulation of YLPM1 expression in GISTs. We note that similar high frequency of DMD protein loss vs relatively low frequency of *DMD* genomic changes are also found in GIST, showing non-genomic inactivation mechanisms in GIST (1).

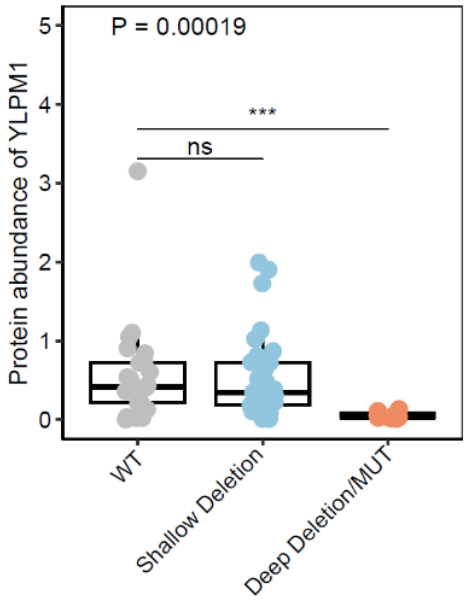
Since YLPM1 protein loss occurs in the low-risk GISTs, we hypothesize that loss of YLPM1 would be an essential event for GIST development, while the loss of protein may not be caused by genomic alterations. Instead of the epigenetic regulation such as methylation of YLPM1 promoter that has not been detected in our study, one of the explanations is the post-transcriptional modification that influence the translational rate of YLPM1, since similar mechanism of other gene has been reported in GIST (2). Also, targeting the synthesis of KIT protein which makes no effect on the mRNA level of KIT has been showed as a potential therapy strategy (3,4). Therefore, the protein synthesis pathway of YLPM1 could be dysregulated in parallel with GIST development, especially in early-stage. In our genomic landscape, we show that the tumor mutation burden increases from low-risk to advanced GIST, suggesting that genomic instability increases during tumor progression, which makes genomic alteration become more prominent cause of YLPM1 protein loss, while in the early-stage, protein synthesis regulation could be the main cause. Similar situations have been found in other cancer types (5, 6). More study is required in the future to clarify this question.

We have incorporated the above discussion, as follows (Lines 230-234):

"Similar high frequency of DMD protein loss versus relatively low frequency of *DMD* genomic changes are also found in GIST, showing non-genomic inactivation mechanisms in GIST. Whether non-genomic mechanisms, such as post-transcriptional modifications, lead to YLPM1 protein loss in the low-risk GISTs merits further investigation."

Re: a possible influence of the high occurrence of YLPM1 shallow deletion?

We share this wonderful comment and have preformed the association analyses with YLPM1 protein and genomic alterations. It looks *YLPM1* shallow deletions are not correlated with decreased YLPM1 protein expression (**Response Fig. 2**).



Response Fig. 2. Homozygous *YLPM1* deletions and mutations are correlated with decreased protein expression, whereas *YLPM1* shallow deletions are not.

Response References:

(4) Wang Y, Marino-Enriquez A, Bennett RR, Zhu M, Shen Y, Eilers G, Lee JC, Henze J, Fletcher BS, Gu Z, et al. (2014). Dystrophin is a tumor suppressor in human cancers with myogenic programs. Nat Genet. 46(6):601-6.

(5) Xu K, Zhang Q, Chen M, Li B, Wang N, Li C, Gao Z, Zhang D, Yang L, Xu Z, Li X, Xu H. (2022). N6-methyladenosine modification regulates imatinib resistance of gastrointestinal stromal tumor by enhancing the expression of multidrug transporter MRP1. Cancer Lett. 530:85-99.

(6) Klug LR, Bannon AE, Javidi-Sharifi N, Town A, Fleming WH, VanSlyke JK, Musil LS, Fletcher JA, Tyner JW, Heinrich MC. (2019). LMTK3 is essential for oncogenic KIT expression in KIT-mutant GIST and melanoma. Oncogene. 38(8):1200-1210.

(7) Lee DM, Sun A, Patil SS, Liu L, Rao AV, Trent PT, Ali AA, Liu C, Rausch JL, Presutti LD, Kaczorowski A, Schneider F, Amankulor NM, Shuda M, Duensing A. (2022). Targeting the translational machinery in gastrointestinal stromal tumors (GIST): a new therapeutic vulnerability. Sci Rep. 12(1):8275.

(8) Wen YC, Lin YW, Chu CY, Yang YC, Yang SF, Liu YF, Hsiao M, Lee WJ, Chien MH. (2020). Melatonin-triggered post-transcriptional and post-translational modifications of ADAMTS1 coordinately retard tumorigenesis and metastasis of renal cell carcinoma. J Pineal Res. 69(2):e12668.

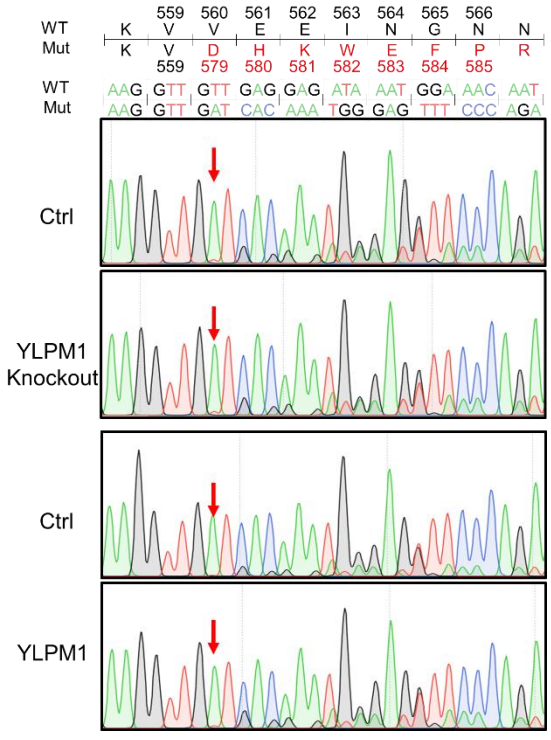
(9) Liu R, Zeng LW, Gong R, Yuan F, Shu HB, Li S. (2021). mTORC1 activity regulates post-translational modifications of glycine decarboxylase to modulate glycine metabolism and tumorigenesis. Nat Commun. 12(1):4227.

Line 222: Please provide evidence that gene KO causes reduced protein levels (e.g. Blot or staining for YLPM1 GIST-T1 cells).

We are sorry that the manuscript was complicated. Evidence that gene KO causes reduced protein level was shown with Western blotting in the Supplementary Information Fig. S11a (Note that former Fig. S10a are now Fig. S11a).

Line 231: please provide all data, if some data are not shown.

The data (not shown in the former manuscript) has been provided in the **NEW Fig S10g** (Line 247) (Note that former Fig. S9 are now Fig. S10). Thank you!

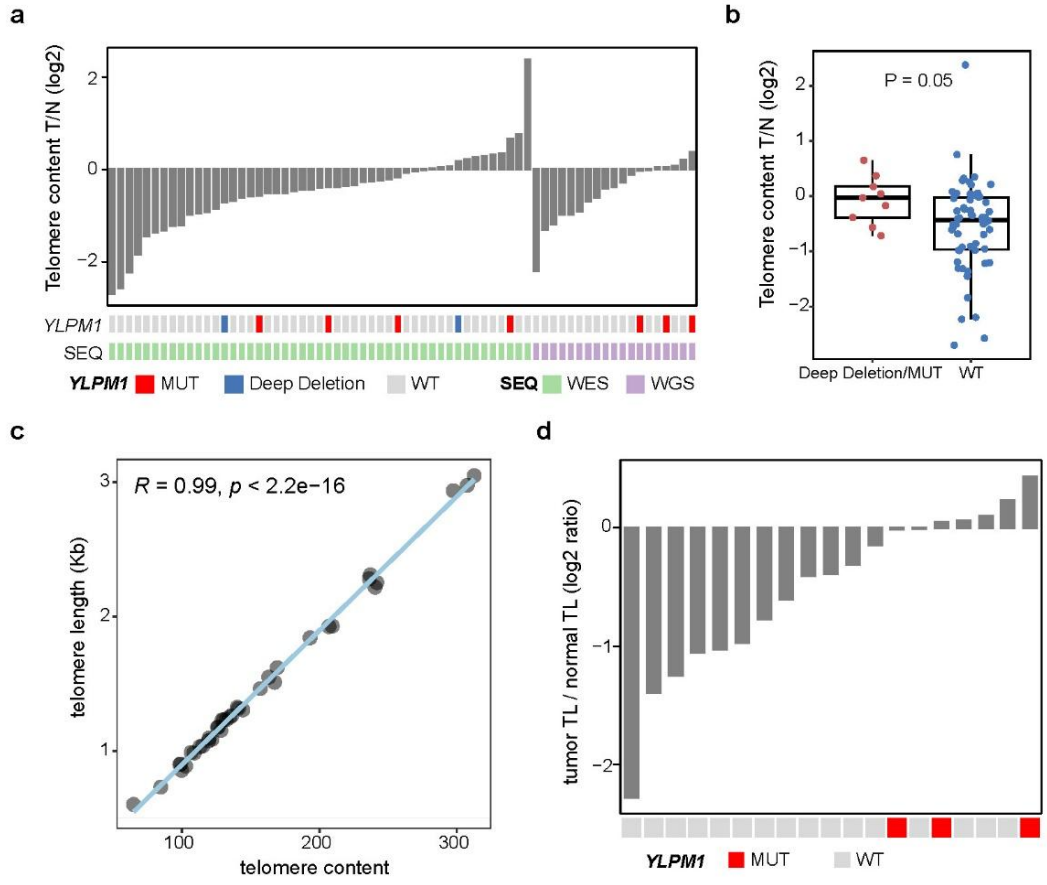


NEW Fig. S10g. Sanger sequencing confirms *KIT* exon 11 mutation (c.1678_1734del, p.Val560_Tyr578del) in YLPM1-KO tumor and YLPM1 overexpressed tumor.

Line 253: The precise way of acting of YLPM1 remains elusive. As YLPM1 is involved in telomere maintenance: did you check telomere content in YLPM1 WT and KO tumors? This should be at least discussed.

We thank this reviewer for raising this excellent point. This is a recommendation that we were very eager to explore, but which proved challenging to execute with the cell line models. These immortal cell lines were established by introducing telomerase into the primary cells. The elongation of telomeres increases the stability of chromosomes, making the cells immortal (1). We feel the telomere-modified cell models are not appropriate for the telomere-related study. However, we share the comment that the key question is whether YLPM1 is involved in telomere maintenance. Therefore, we performed NEW analyses. The telomere content difference between *YLPM1* mutated tumors and *YLPM1* WT samples has been calculated. The telomere content is quantified from 19 paired WGS-sequenced patients and 49 paired WES-sequenced patients using TelomereHunter. Patients harboring *YLPM1* mutations exhibit a higher ratio (tumor-vs-normal) of telomere content (**NEW Fig. S8a, b**). We have also performed telomere length (TL) analysis on 19 paired WGS-sequenced patients using Telseq (2) and observed a strong positive correlation between telomere length and telomere content (**NEW Fig. S8c**). Similarly, the TL ratio (tumor TL / normal TL) is higher in patients with *YLPM1* mutations (**NEW Fig. S8d**). These results suggested that genomic alteration of *YLPM1* is correlated with telomere length, in line with previous studies that YLPM1 is involved in telomere maintenance.

We have added the above information in the revised manuscript (**NEW Fig. S8**, Lines 211-212).



NEW Fig. S8. Genomic alteration of *YLPM1* is correlated with telomere length in human GISTs.
(a) Bar plot showing the log2 ratio of telomere content in tumors with *YLPM1* mutations.

(b) Box plot showing the log2 ratio of telomere content in tumors with *YLPM1* mutations. P value is determined by Wilcoxon rank-sum test.
(c) Scatter plot showing the correlation between telomere content and telomere length detected on WGS data.
(d) Bar plot showing the log2 ratio of telomere length in tumors with *YLPM1* mutations based on WGS data.

Response references:
(1) Maqsood MI, Matin MM, Bahrami AR, Ghasroldasht MM. Immortality of cell lines: challenges and advantages of establishment. *Cell Biol Int*. 2013;37(10):1038-1045. doi:10.1002/cbin.10137
(2) Ding Z, Mangino M, Aviv A, Spector T, Durbin R; UK10K Consortium. Estimating telomere length from whole genome sequence data. Nucleic Acids Res. 2014;42(9):e75. doi:10.1093/nar/gku181

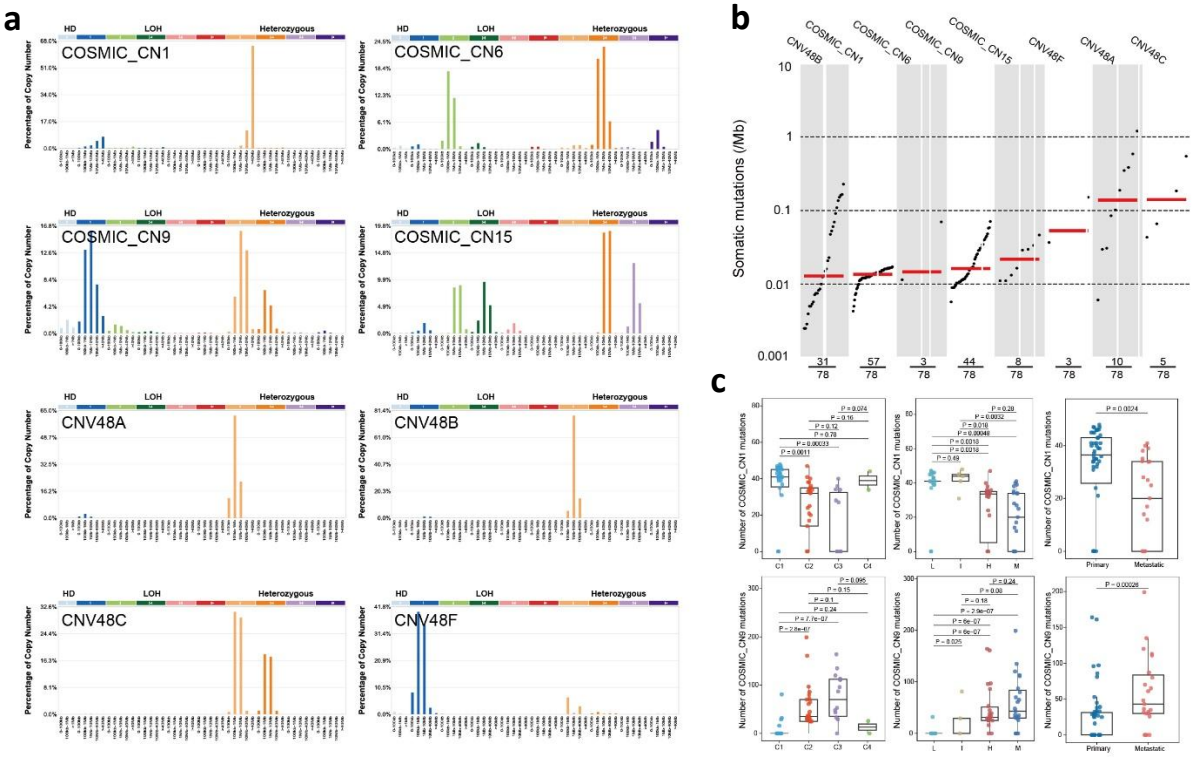
Line 272, part Widespread Copy number Variations in GISTs: It would be interesting to see copy number alteration signatures in GISTs (Steele et al., Nature 2022).

We fully agree with the constructive comments. Therefore, we utilized SigProfilerMatrixGenerator to generate a matrix for copy number variations (CNVs) and employed SigProfilerExtractor to extract the CNV signatures. Herein, a total of 8 CNV signatures were identified, among which 4 were novel (**NEW Table S7, NEW Fig. S13**). The CNV48A, CNV48B, and COSMIC_CN1 are characterized by heterozygous segments with a total copy number (TCN) of 2 and sizes ranging from 100Kb to 1Mb, 1-10Mb, and >40Mb, respectively. COSMIC_CN6 consists of LOH segments ranging from 100K-10Mb with a TCN of 2, as well as heterozygous segments ranging from 100K-10Mb with TCNs of 3-4. COSMIC_CN9 is identified as a signature of chromosomal instability on a diploid background. COSMIC_CN15 is a chromosomal LOH signature with chromosomal or arm-scale losses before twice-genome-doubled. CNV48C is a signature of chromosomal instability on a diploid background. CNV48F consists of 100KB-10Mb LOH segments with TCN of 1. The presence of COSMIC_CN1 and COSMIC_CN9 is observed in >50% of cases, indicating their extensive involvement in GISTs (**NEW Fig. S13**). To investigate the enrichment of CNV signatures in our molecular subtypes and clinicopathologic classification associations, we performed correlation analyses between signature intensity and molecular subtypes and clinicopathologic information. Specifically, COSMIC_CN9 was enriched in the C2 and C3 subtypes, exhibiting a strong association with metastatic GIST (**NEW Fig. S13**). Conversely, COSMIC_CN1 demonstrated an opposing pattern to COSMIC_CN9, consistent with normal diploid characteristics (**NEW Fig. S13**)

We have included the CNV signatures in the revised manuscript (**NEW Fig. S13**; **NEW Table S7**; Lines 290-293, 477-482; Supplemental Information MATERIALS AND METHODS, Lines 123-127)

NEW Table S7. Decomposition of de novo CNV signatures to reference signatures.

De novo extracted	Global NMF Signatures	L1 Error %	L2 Error %	KL Divergence	Cosine Similarity	Correlation
Signature 48-A	Signature 48-A	0	0	0	1	1
Signature 48-B	Signature 48-B	0	0	0	1	1
Signature 48-C	Signature 48-C	0	0	0	1	1
Signature 48-D	COSMIC_CN1 (100%)	53.63	39.14	0.33	0.92	0.92
Signature 48-E	COSMIC_CN6 (30.10%), COSMIC_CN9 (31.70%), COSMIC_CN15 (38.20%)	49.05	37.45	0.34	0.93	0.90
Signature 48-F	Signature 48-F	0	0	0	1	1



NEW Figure S13. CNV signatures in GISTs.
(a) Total profiles of CNV signatures in 78 GISTs form 68 patients.
(b) Mutation burden of CNV signatures. Mutation burden per megabase of the CNV signatures was sorted by median (red line) with each dot representing one tumor and the number of tumors with signature indicated below.
(c) The correlation of CNV signature intensities with subtypes and clinicopathologic classifications. Boxplots showing the number of COSMIC_CN1/CN9 mutations in mRNA subtypes and clinicopathologic classifications, respectively. P values are determined by Wilcoxon rank-sum test. L, low-risk; I, intermediate-risk; H, high-risk and M, metastatic.

Line 457: The T-Cell-mediated tumor cell killing assays is missing in the methods. Please add.

We thank the reviewer and the T-Cell-mediated tumor cell killing assay was included as follows (Supplemental Information MATERIALS AND METHODS, Lines 414-420)

T-Cell-mediated tumor cell killing assay
To analyze T cell-mediated tumor cell killing, human T cells were activated by culturing human PBMC in ImmunoCult-XF T cell expansion medium (10981, Stemcell) with ImmunoCult human CD3/CD28 T cell activator (10971, Stemcell) and IL-2 (10 ng/mL, 78036, Stemcell) for 7 days. Then adhered GIST-CN16 or GIST-T1 cells were co-cultured with activated human T cells at a ratio of 1:5 or 1:10 for 72 h. T cells and cell debris were washed with PBS, and living cells were measured by Cell Counting Kit-8 (HY-K0301, MedChemExpress) according to the manufacturer's instructions [40].

Line 817: How many samples were included in this analysis? Please provide n.

We thank this reviewer for the careful comment. We have added the number of samples for each mRNA subtype in the figure legend and revised the sentence "mRNA-based clustering results. Heatmap was generated

with 520 differentially expressed genes among 4 subtypes" to "Consensus clustering results of GISTs (n=106) based on the RNA expression. Heatmap shows 520 differentially expressed genes among 4 subtypes. The number of tumors for C1, C2, C3, and C4 subtype is 51, 30, 18, and 7, respectively" (Lines 876- 878).

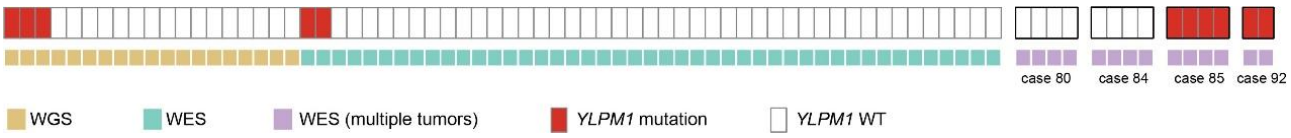
Figure 2: YLPM1 mutations in 11 samples according to black fields. However, on the left it was denoted that n=7. Please explain. Is 7 referring to the number of affected patients? Additionally, please explain observed shallow deletions in the main text of the manuscript.

We thank this review for the extremely careful comment. Yes, the number on the left side of the Fig 2a represents the number of affected patients, showing in the figure legend as follows:

"The mutation frequency of each gene is shown as a bar plot on the left with the number of affected patients labeled in parentheses."
YLPM1 mutations were detected in 11 GIST samples from 7 patients (**Response Fig. 3)**.

YLPM1 copy number variations (CNVs) were detected in 42 of 68 (61%) patients (Fig 2a), including shallow deletions in 40 of 68 (59%) patients and deep deletions in 2 of 68 (3%) patients. Heterozygous deletion of chromosome 14q is one of the most frequent genomic events in GISTs, as reported previously (1). Human *YLPM1* locates in 14q24. Heterozygous deletion of chromosome 14q likely counts for the frequent shallow deletions.

The above information has been incorporated in the main text (Lines 205-210)



Response Fig. 3. Integrated plot the *YLMP1* mutations in 78 GIST samples from 68 patients.

Response Reference:
(1) El-Rifai W, Sarlomo-Rikala M, Andersson LC, Knuutila S, Miettinen M. DNA sequence copy number changes in gastrointestinal stromal tumors: tumor progression and prognostic significance. Cancer Res. 2000 Jul 15;60(14):3899-903. PMID: 10919666.

Figure 7: The authors state the the subtypes might! Predict therapy response. I would be very careful and revise the section and figure: Please clarify that this is just a hypothesis, figure 7K has a row called "therapy" – please re-name to make sure that this is not the actual therapy that the patients received, e.g. "potential conclusion regarding therapy" or so.

We fully agree with the constructive comments. We have revised the main text and the Fig. 7k as follows:
"Combined with genomic variations, expression profiles, immune characteristics, and clinical information, we summarized the key features for the 4 mRNA subtypes and proposed hypothesis regarding treatment strategy (Fig. 7k)" (Lines 505-506)

"Our hypothesis regarding subtype-specific treatment strategies were mainly based on analyses of genomic and transcriptomic data and experimental study; prospectively well-designed clinical trials should be added before we translate our results into clinical practice." (Discussion, Lines 564-566)

We have also changed the "therapy" to "potential conclusion regarding therapy" in the Fig. 7k.

Reviewer #2 (Remarks to the Author): Early Career Researcher co-reviewer

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank this reviewer for taking the time and effort to review the manuscript, which helped us to improve the quality of the manuscript.

Reviewer #3 (Remarks to the Author): GIST clinical research, therapy, and immunotherapy

The article provides interesting and new information on the molecular subtypes on these rare tumors with possible therapeutic implications to be tested. It will be important to have a description of the survival of patients from the 4 subgroups in localized phase and first line advanced phase.

We thank this reviewer for taking the time and effort to review the manuscript, which helped us to improve the quality of the manuscript. This is a recommendation that we were very eager to explore, but which proved challenging to execute. The management of GIST was revolutionized by the introduction of imatinib, which has become the standard first line treatment for metastatic GIST. GIST patients with *KIT* or *PDGFRA* mutations sensitive to the tyrosine kinase inhibitor (TKI) imatinib that are at high risk of relapse have improved survival with adjuvant TKI treatment. In advanced disease, median overall survival has improved to >70 months since the introduction of TKIs. Therefore，GIST patients enrolled in this study (all are within recent 6 years) have a favorable prognosis and it is not surprising that there is no difference between localized phase and first line advanced phase. However, in the coming years, we will continue to collect the survival information of the patients from the 4 subgroups to identify the prognosis.

Reviewer #4 (Remarks to the Author): Expert in GIST and sarcoma genomics, functional genomics, and translational research

The study by Xie et al investigates a relatively large, clinically and molecularly heterogeneous GIST cohort by a combination of WES/WGS and WTS. One of their main findings is that increased genomic complexity (TMB, CNV burden) is positively associated with tumor size and mitotic count. The authors also investigate the association between chromothripsis and kataegis and aggressive GIST. Despite the very comprehensive analyses and platforms used, the study is confounded by the mixed bag of cases being investigated (low risk, high risk, metastases, TKI-treated, etc) with no real hypothesis to follow. Their main novel finding of YLPM1 mutations in GIST is similarly confounded by the discrepancies in their incidence in the various methods applied. The authors also try to address clonal evolution, a big topic in itself; however, the findings fall short as the authors only investigate 4 metastatic cases with multiple lesions, some being pretreated/resistant to TKI. The paper is poorly written and requires editing for English language and syntax. Also there are lots of inconsistencies throughout the manuscript.

We would like to thank this reviewer for taking the time and effort to review the manuscript. We sincerely appreciate all valuable comments and suggestions, which helped us to improve the quality of the manuscript.

Summary – rephrase and describe simply what are the 2 clinical cohorts. It sounds like most patients (n= 105) are in fact advanced, 'lethal cancers', while only a minority are low risk, early-stage. The wording used is confusing, as it seems that the study is focusing on low risk GIST and mechanisms of tumor progression. Also, a more specific description of the cohort of GIST in which YLPM1 mutations is identified is required as well as its incidence and possible a very brief description of its function.

We thank the reviewer for the thoughtful comments. The 117 GIST samples (from 105 patients) include 31 low-risk, 18 intermediate-risk, 29 high-risk, 34 metastatic. Note that the remaining 5 GISTs with preoperative neoadjuvant TKI therapy cannot be classified as pretreatment impacts mitotic count.

We have made the modifications according to the constructive comments as follows.

"We comprehensively describe the genomic and transcriptomic landscape of a cohort of 117 GISTs including 31 low-risk, 18 intermediate-risk, 29 high-risk and 34 metastatic and 5 neoadjuvant GISTs from 105 patients" (Lines 73-74)

"Despite the paucity of mutations, recurrent inactivating *YLPM1* mutations are identified (10.3%, 7 of 68 patients), enriched in high-risk/metastatic GIST and functional study further demonstrates *YLPM1* inactivation promotes GIST proliferation, growth and oxidative phosphorylation" (Lines 77-81)

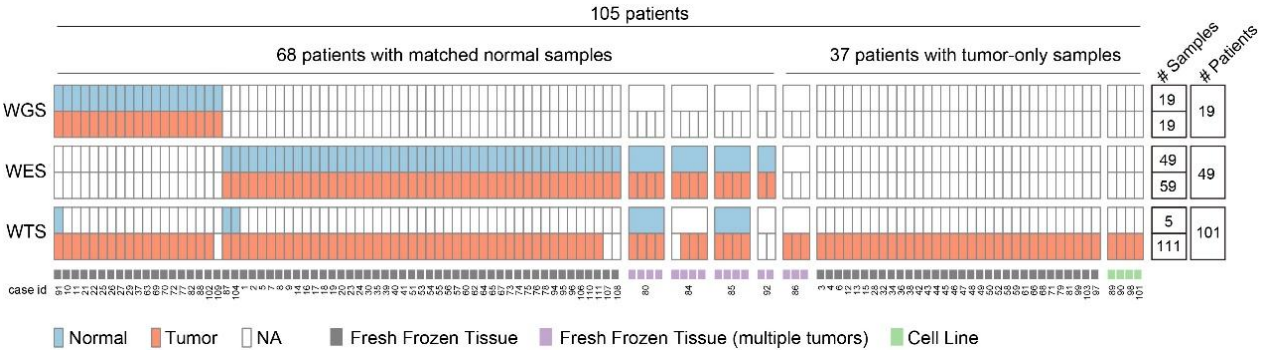
Intro – a number of sentences can be deleted or rephrased, as they have no meaning. For example: 'to study constraints to tumorigenic progression'; 'The opportunity to study less-aggressive lesions such as low-risk GIST enables evaluations of the sequence of mutations accounting for oncogenic progression.'

We fully agree with the comments and the following sentences have been deleted.
"Therefore, GISTs provide an ideal model by which to study constraints to tumorigenic progression[4,6]. The opportunity to study less-aggressive lesions such as low-risk GIST enables evaluations of the sequence of mutations accounting for oncogenic progression[7,8]."

Results: unclear how 68 matched normal samples from 105 patients?!

We thank the reviewer for the careful review. In this study, 68 patients (pts) with matched normal samples were analyzed using WGS (19 pts, 19 GISTs) or WES (49 pts, 59 GISTs). Among these 68 pts, 94% pts (64 out of

68) were also analyzed using whole-transcriptome sequencing (WTS). The remaining 37 pts without matched normal samples were only analyzed using WTS (**Revised Fig. S1b**). Therefore, out of 105 patients, only 68 patients had matched normal samples. To make the information easy to follow, we have revised the Fig. S1b.



Revised Fig. S1b. Composition of three GIST next generation sequencing cohorts in this study. The number of patients and tumor samples per cohort is labeled and indicated.

Also the authors include in their genomic study both primary and metastatic samples at diagnosis.

We are grateful for the comment. We respectfully guess this reviewer comments on case #92. 92-1T is a primary gastric GIST (tumor was resected at 59 yrs). 92-2T is a subsequent metastasis, diagnosed one year later (tumor was resected at 60 yrs, **Table S1**). Hence, case 92 had longitudinal lesions in the natural history of the GIST (Lines 366-367). Thank you for the opportunity to clarify here!

NGS was performed in only 78 cases; WES (n=59) and WGS (n=19); did not include cell lines.

We thank the reviewer for raising this point. WES or WGS was not performed in the 4 GIST cell lines. In this study, we focus on the somatic genomic aberrations. The mutations in GIST without normal samples should be viewed with caution. Unfortunately, we do not have the matched normal samples from the cell lines and we have no evidence the mutations are somatic. Therefore, the GIST cell lines were not included in the WES or WGS.

Unclear why then the case denominator for cases tested for YLPM1 mutations is only 68 cases; YLPM1 mutations found in 7/68 (11%). The authors should add in the test (page 6) the number of additional cases that were tested by Sanger Seq; it remains unclear why the discrepancy in the incidence of YLPM1 mutations compared to NGS (24%, more than double).

We thank this reviewer for the constructive comment. We performed WES in 49 patients and WGS in 19 patients, for a total of 68 patients. *YLPM1* truncated mutations were identified in 7 out of 68 patients (10.3%). *YLPM1* mutations were further confirmed by Sanger sequencing in the WES/WGS cohort. To make the information clear, we have modified the statement as follows (Lines 199-200):

"Somatic homozygous *YLPM1* mutations (SNVs and indels) were confirmed by Sanger sequencing in the WES/WGS cohort **(Fig. S6a)"**

In addition to *YLPM1* truncated mutations, homozygous deletions were detected in 2 of 68 patients (same cohort as WES/WGS). Therefore, *YLPM1* aberrations frequency is 13.24% (**Fig.S7a**). Please note that we have revised the Fig.S7a in response to Reviewer #1. Please see our responses (*Line 200: YLPM1 Mutation in 21 out of 86 patients: Figure 2a does not show this, please clarify*) to Reviewer #1 for details.

Moreover, unclear why by WB and IHC the loss of YLPM1 was detected in 48% and 47%, respectively, of GIST tested!

In the former manuscript, we assessed the inactivation frequency of YLPM1 at protein level by WB in 73 GISTs from 64 patients. YLPM1 protein loss was demonstrated in 31 of 64 (48%) patients **(Fig. 3b, Table S5)**. Then, we performed IHC to validate the frequency to which YLPM1 protein expression was lost. YLPM1 expression was negative in 47% (129/276) of GISTs on tissue microarray validation cohort.

This expert reviewer raised a key question: What could be the explanation for a higher inactivation of YLPM1 on protein level (~47%) compared to genomic level (~13%)? In our initial manuscript to *Nature Communications*, we have tested whether the promoter hypermethylation leads to *YLPM1* inactivation. WTS data and DNA methylation studies indicated that dysregulation of DNA methylation was not common in the regulation of YLPM1 expression in GISTs. In addition to mutation, deletion and promoter hypermethylation, during the past months, we have also conducted YLPM1 promoter mutation evaluations through Sanger sequencing in paired tumor and normal samples. Unfortunately, no potential mutations in the promoter region have been identified, indicating that YLPM1 promoter mutations are not common in the regulation of YLPM1 expression in GISTs. We note that similar high frequency of DMD protein loss versus relatively low frequency of *DMD* genomic changes are also found in GIST, showing non-genomic inactivation mechanisms in GIST (1).

Since YLPM1 protein loss occurs in the low-risk GISTs, we hypothesize that loss of YLPM1 would be an essential event for GIST development, while the loss of protein may not be caused by genomic alterations. Instead of the epigenetic regulation such as methylation of YLPM1 promoter that has not been detected in our study, one of the explanations is the post-transcriptional modification that influence the translational rate of YLPM1, since similar mechanism of other genes has been reported in GIST (2). Also, targeting the synthesis of KIT protein which makes no effect on the mRNA level of KIT has been showed as a potential therapy strategy (3,4). Therefore, the protein synthesis pathway of YLPM1 could be dysregulated in parallel with GIST development, especially in early-stage. In our genomic landscape, we show that the tumor mutation burden increases from low-risk to advanced GIST, suggesting that genomic instability increases during tumor progression, which makes genomic alteration become more prominent cause of YLPM1 protein loss, while in the early-stage, protein synthesis regulation could be the main cause. Similar situations have been found in other cancer types (5, 6). More study is required in the future to clarify this question.

We have incorporated the above discussion, as follows (Lines 230-234):

"Similar high frequency of DMD protein loss versus relatively low frequency of *DMD* genomic changes are also found in GIST, showing non-genomic inactivation mechanisms in GIST. Whether non-genomic mechanisms, such as post-transcriptional modifications, lead to YLPM1 protein loss in the low-risk GISTs merits further investigation."

Response References:

(1) Wang Y, Marino-Enriquez A, Bennett RR, Zhu M, Shen Y, Eilers G, Lee JC, Henze J, Fletcher BS, Gu Z, et al. (2014). Dystrophin is a tumor suppressor in human cancers with myogenic programs. Nat Genet. 46(6):601-6.

(2) Xu K, Zhang Q, Chen M, Li B, Wang N, Li C, Gao Z, Zhang D, Yang L, Xu Z, Li X, Xu H. (2022). N6-methyladenosine modification regulates imatinib resistance of gastrointestinal stromal tumor by enhancing the expression of multidrug transporter MRP1. Cancer Lett. 530:85-99.

(3) Klug LR, Bannon AE, Javidi-Sharifi N, Town A, Fleming WH, VanSlyke JK, Musil LS, Fletcher JA, Tyner JW, Heinrich MC. (2019). LMTK3 is essential for oncogenic KIT expression in KIT-mutant GIST and melanoma. Oncogene. 38(8):1200-1210.

(4) Lee DM, Sun A, Patil SS, Liu L, Rao AV, Trent PT, Ali AA, Liu C, Rausch JL, Presutti LD, Kaczorowski A, Schneider F, Amankulor NM, Shuda M, Duensing A. (2022). Targeting the translational machinery in

gastrointestinal stromal tumors (GIST): a new therapeutic vulnerability. Sci Rep. 12(1):8275.

(5) Wen YC, Lin YW, Chu CY, Yang YC, Yang SF, Liu YF, Hsiao M, Lee WJ, Chien MH. (2020). Melatonin-triggered post-transcriptional and post-translational modifications of ADAMTS1 coordinately retard tumorigenesis and metastasis of renal cell carcinoma. J Pineal Res. 69(2):e12668.

(6) Liu R, Zeng LW, Gong R, Yuan F, Shu HB, Li S. (2021). mTORC1 activity regulates post-translational modifications of glycine decarboxylase to modulate glycine metabolism and tumorigenesis. Nat Commun. 12(1):4227.

We would be delighted to respond to any additional criticisms that might arise in re-review of the manuscript.

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We are most grateful for this opportunity to resubmit our revised manuscript.

With best wishes and many thanks,

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REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have addressed all of our points. I dont have any additional points. Congratulations on this important contribution!

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author):

Adequate responses to the different questions.

Reviewer #4 (Remarks to the Author):

the authors tried their best to address my questions and comments.
it still remains a very complicated and confusing study, not easy to read or to follow.

Reviewer #4 (Remarks to the Author):

the authors tried their best to address my questions and comments.
it still remains a very complicated and confusing study, not easy to read or to follow.

We agree with the comments. This manuscript is complicated, providing huge amount of data (7 Main Figures, 22 Supplemental Figures and 14 Supplementary Datasets). We have revised the manuscript to make it concise and clear during this final revision stage, especially in the following sections.

- Figure legends for Fig 1 and 7 (lines 1258-1276, 1361-1385);
- Results (lines 453-473)

In our view, this study will be useful as a resource for the GIST research community. We hope this expert reviewer find the manuscript is in a good shape for publication with the insights and criticisms from our four reviewers and editors. Thank you!