**Response to Reviewers**

We thank the reviewers and editors for their thoughtful and constructive comments, which have significantly helped improve the clarity, rigor, and impact of our manuscript. Below, we provide a detailed point-by-point response to each comment. Reviewer comments are shown in **bold**, followed by our responses in plain text. Line numbers refer to the revised manuscript unless otherwise indicated.

**Reviewer 1**

**1. The reference cited as number 4 (line 38, page 2) does not appear to include RNA-seq data.**  
Thank you for noting this. We have replaced reference 4 with a more suitable publication that includes RNA-seq data and supports the relevant statement. The corrected sentence and citation appear on page 2, line 40.

**2. The analytical pipeline described on page 3 (starting line 16)... extension to the TCGA dataset is questionable.**  
We acknowledge the reviewer’s concern regarding non-purity of TCGA tumor samples. Our goal was to corroborate CCLE findings in an independent RNA sequencing data set, and in the revised manuscript we have chosen to examine transcript and gene level expression from the Tsoi et al data set of 53 melanoma cell lines. In addition, we have updated the CCLE analysis by using latest available (2024q4) CCLE data. We find that

**3. The initial filtering criterion for transcript inclusion... appears too lenient.**  
We agree. To improve biological relevance, we have implemented a stricter criterion requiring transcripts to have >10 counts in at least 25% of CCLE melanoma samples. This change is described in the Methods (page 3, line 9) and applied in all relevant figures and supplementary data.

**4. Figure 1A... difficult to interpret... red-labeled points appear to the right of the diagonal and below the 0.5 threshold.**  
Thank you for this feedback. We have revised Figure 1A to more clearly depict discordant transcripts. We have plotted Pearson and Spearman correlations separately (Figure 1A and Supplemental Figure 1A), showing that all discordant transcripts are left of the diagonal and above the 0.5 threshold. We also improved axis scaling and updated the figure legends for clarity.

**5. In Figure 2A, more than 50% of discordant transcripts lack detectable MITF binding peaks...**  
We now clarify in the text that not all discordant transcripts show direct ChIP-seq evidence of MITF binding. These may reflect indirect regulation of some transcripts or that some transcripts are markers of MITFhigh vs MITFlow cells states rather than direct MITF targets. We expanded the Discussion (page 8, lines 10–20) to address this limitation and suggest experimental strategies to validate candidate targets.

**6. The biological impact of transcripts representing <20% of total expression is questionable...**  
We acknowledge that low-abundance transcripts may have limited biological impact. We now include absolute expression values (baseMean) for all transcripts in Supplementary Table 1 and discuss expression levels explicitly in both Results (page 5) and Discussion (page 9). We caution against overinterpreting weakly expressed transcripts and propose them as candidates for future validation.

**7. Supp Table 1 must include additional information: expression level and correlation with MITF-M.**  
Supplementary Table 1 has been updated to fully annotate each transcript, include baseMean expression values in CCLE and Tsoi data sets, Pearson and Spearman correlation coefficients with MITF-M, presence of ChIP peak in proximal promoter, and effect (from published RNA-seq data sets) of MITF knockdown and overexpression.

**8. Abscissa and/or ordinate of Figure 1C, Supp 2B, Supp3 are illegible.**  
We have regenerated these figures with improved resolution and enlarged axis text. All figures are now legible in PDF format.

**Reviewer 2**

**1. CCLE vs TCGA handling unclear... different inclusion thresholds...**  
We thank the reviewer for highlighting this. Our goal in using TCGA data set was to provide independent validation of CCLE findings. We have removed the TCGA analysis and replaced (as described above) with data from a second data melanoma cell line data set. This update is described in the revised Methods (page 3, lines 8–12) and addressed in Supplementary Figure 1.

**2. Definition of an expressed transcript is too lax.**  
As above, we now use a more stringent threshold: >10 counts in ≥25% of CCLE melanoma samples.

**3. The 20% cutoff rationale is not clearly justified.**  
We appreciate this point. We have utilized a statistical threshold analysis and threshold (FDR≤0.05) to compare transcript and gene level correlations (Diedenhoffen and Musch, 2016, PMID: 2583500

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**4. The Webster ChIP-seq dataset is not the only one available... consider other sources.**  
We thank the reviewer for suggesting additional datasets. now expanded our analysis to include MITF ChIP-seq data from Laurette et al. (2015), Louphrasitthiphol et al. (2020), and Dilshat et al. (2021) and the recent CUT&RUN dataset from Chang et al. (2025). Comparative results are summarized in Supplementary Figure 5 and discussed on page 6, lines 2–12.

**5. Verification of MITF regulation of the discordant ABR transcript... need for experimental validation.**  
We agree that functional validation is critical. Our reanalysis of CCLE and TSOI data sets, utilizing a statistical threshold for discordance, provides an updated list of “discordant” transcripts. We chose two discordant transcripts (from genes PEX10 and METTL9 that are highly expressed in melanoma cell lines. We utilized qPCR analysis to demonstrate that for two “discordant” transcripts their expression after siMITF is markedely decreased while gene level expression is only modestly decreased supporting MITF-dependent regulation of these novel discordantly regulated transcripts (Figure 1C, Supplemental Figure 1C).

**Minor Comments:**

**– Reference 4 does not include gene expression data.**  
Corrected as noted above.

**– Figure 2 should include range of observed values.**  
We now include the full range (min to max) of MITF binding peak counts in Figure 2 and clarify this in the legend. Additionally, we analyzed whether peak number correlates with expression (see Supplementary Figure 7) and note the lack of strong association in Results (page 5, lines 22–25).

**Conclusion**

We greatly appreciate the reviewers’ insightful comments, which have led to substantial improvements in both analysis and presentation. We believe that the revised manuscript addresses all concerns and offers a clearer and more robust evaluation of transcript-specific regulation by MITF.