Reviewer 2:

I have concerns regarding the stress response analysis. For instance, ATAC-seq was collected at all time points, but it is never shown that ATAC-seq changes in response to stress correspond with SMAC-seq changes (time point 0 concordance is shown in Supp Fig 15, but what we are interested in is whether the changes across time points are consistent). As well as a systematic analysis, the ATAC-seq time points should also be shown in genome browser plots.

We did generate ATAC-seq datasets for all time points, but we did not include them in the original figures because space there was limited and because ATAC-seq is generally highly concordant with SMAC-seq measurements, a point already established in the text prior to that, thus its inclusion would not have provided much additional important information. We have now added separate supplementary figures showing that changes in accessibility measured by SMAC-seq and by ATAC-seq are similar to each other for the genes shown in the main text figure.

Also regarding the stress response analysis, there is no systematic analysis of accessibility at HSF1 binding sites, despite the focus. Figure 5b shows accessibility of HSF1 TFBS 30 minutes after stress response, but what we are interested in are the *changes* in accessibility at these sites from 0 to 30 minutes, and from 30 to 60 minutes. Changes in accessibility are shown in individual genomic loci, but it is curious that a systematic analysis of accessibility changes are left out of Figure 5b. In general , I find it somewhat challenging to discern the appearance of a new NDR at time point 30 in TMA10, HSP26, and many of the other examples shown (Figure 5D and Supp Figs 52-65). A more systematic analysis of ATAC-seq and SMAC-seq changes at HSF1 TFBS is required.

We did not include such an analysis because different HSF1 sites exhibit their own temporal dynamics, while in the same time there are not that many of them in total. Thus it was better to examine them on a case-by-case basis.

The methylation error rate is of keen interest, especially for the m6a base which is novel and forms the bulk of SMAC-seq data. p.5 states ``we estimate the false positive rate of methylation base calling to be on the order of 20-25% for Tombo and around 10-15% for Nanopolish (Supp Figure 12)". While these plots do show general concordance, I do not understand how you are calculating an actual false positive rate from these plots (I could not find anything in the methods). If you have not performed your own naked DNA controls to estimate these rates yourselves, could you at least summarize estimates that have been done by others for Tombo false positive methylation rated for 5mC and 6mA.

We have generated naked DNA controls, the problem with those is that fully methylated DNA templates do not sequence well on the nanopore platform, so we did not focus much on them for that reason. Analysis of that data indicates a false positive rate of 18% (and additional, deeper sequenced controls have been included in the revised version of the text). The 20-25%

estimate is based on comparing the absolute occupancy of +1 nucleosomes estimated from dSMF data to that of the nanopore calls. It should also be noted that Nanopolish cannot be directly compared with Tombo as Nanopolish only calls CpG and GpC methylation, which is an easier problem than calling m6A.

Also regarding the stress response analysis, I think the quantitative change shown in Figure 5f is not reliable without an additional biological SMAC-seq replicate or locus-specific SMAC-seq. The quantitative difference could just be due to sampling variability.

Sampling variability is highly unlikely to explain the observed changes because we have sequenced to a depth of 100-200X at a read length of several kilobases. Biological replicates do in fact show the same general picture, but, as it often happens, the temporal dynamics of the time courses is not exactly the same, so only one of the two replicates is shown.

It is notable that the sequencing in this work produced reads with a median length of ~1.5kb, while a similar yeast approach in preprint (https://doi.org/10.1101/533158) and a similar approach in human cells (https://doi.org/10.1101/504993) produced reads of ~7kb and ~10kb median, respectively. This technology is rapidly evolving and so these differences are not surprising. But readers of this work will be interested in a discussion of whether these shorter read lengths are due to some inherent constraint or filtering necessary for your m6a analysis, or simply the result of using slightly older flow cell versions or instrumentation.

The read lengths depend on the details of the DNA isolation protocol and on which of the various ONT sequencing kits is used. With current methods of DNA isolation and size selection and with the ligation-based ONT sequencing kits we are routinely obtaining 10Kb mean read lengths in our practice.

Association between chromatin accessibility of TSS and TTS is compelling (Supp Fig 46). How much of this would be due to the fact that genes are close together in yeast and the TTS of one gene is almost immediately adjacent to the TSS of the another gene? If both genes transcriptionally active, this could lead to the observed association. Can you filter out any TTS which has a TSS nearby, or otherwise rule this out?

For this analysis, a minimal gene length requirement imposed on genes included in this analysis (>1kb). While the yeast genome is compact, 75% of intergenic regions are longer than 250bp.

For rDNA analysis, do any copies of the 5.8S unit contain polymorphisms near the Reb1 binding region? If so, are these associated with the differential chromatin state?

We are not aware of such polymorphisms.

On p.8, Figure 4 labels are wrong, jumping from 4c to 4f.

This has been corrected

For Figure 5d-e and Supp Figs 52-65, are the SMAC-seq reads ordered by (+) strand and then (-) strand, or are both strands clustered together. Please specify in figure or figure legend.

If there is no explicit separation of the two strands, then they are clustered together.

For Figure 5d, it is not clear whether the H4S47C was generated in the post-stress response cells or if it's from standard unsynchronized cells. If it's from the stress response experiment, please include in Figure 5a. If it's from a different source, please state this in figure legend and do not include it in the 60 minute plot.

We used publicly available chemical mapping data for this study.

If the ``naked DNA'' filter removes highly accessible regions like the rDNA arrays, how is one to apply this filter without losing important regions? What is your recommendation?

The best solution is to carefully execute experiments so that there are few dead cells in the input. Of note, this is also an important consideration for ATAC-seq experiments. We have observed very few such reads in subsequent experiments where we have controlled more stringently for cell viability.

Supp Fig 36-41. ATAC-seq has been shown to be able to footprint TF binding sites. Why are you not showing the ATAC-seq footprinting signal, especially since the SMAC-seq and DNase-seq do not always appear to be concordant?

We have extensively expanded the ATAC/DNAse/SMAC footprinting comparison in the revised text.

Supp Fig 60: 60 minute timepoint is missing for NMI plots. Maybe not enough reads?

This is a graphical issue and will be corrected for the final version of the supplement. We thank the reviewer for pointing this out.