General comments

The manuscript by Tenen et al. reports the discovery of "SPEARs", a novel class of non-coding RNAs that are claimed to be expressed in early S phase upstream of the promoters of genes, and to play a role in the deposition of acetylated H2A.Z onto newly synthesized chromatin by physically bringing together H2A.Z and the Tip60 HAT. However, the experiments and analyses presented in support of these claims are nowhere near sufficiently convincing. In addition, the manuscript is overall not well put together. Therefore, I cannot recommend it for publication.

Specific comments

1. The research on SPEARs is not motivated and explained well at all. There is really no direct logical connection between the previously described by the same group "DNMT1-sinteracting RNAs (DiRs)" and the existence of SPEARs (in fact, after reading this manuscript, one cannot help but very much doubt the existence and validity of the DiRs too). This leads directly to the first major issue with the study itself:

2. There is really no proper definition of SPEARs presented. A cartoon is shown of a transcript called "*UpTr*" that is supposedly upstream of *CEPBA*, but there is no information presented in the figures on how exactly that transcript was identified, what its boundaries are, etc. Some qRT-PCR results are shown in Figure 1A, but one cannot even find what the numbers "1", "2", "3", and "4" correspond to in the legend for Figure 1A, and it is not at all clear where the RT-PCR probes are located and what exactly they are measuring. Then we get another leap in logic, from the "*UpTr*" RNA (which hasn't even been properly established to exist as an entity on its own) to there being many other such "SPEARs" playing a role in the "reassembly of chromatin" in early S-phase. This does not really follow at all.

3. The authors then carry out nascent RNA-seq upon release from S-phase and identify thousands of SPEARs. It should be obvious that one cannot claim anything about such transcripts playing a specific role in S-phase without having profiled them all throughout the cell cycle, yet there is no data to be seen about the other phases of the cell cycle.

4. There is also no proper definition of SPEARs presented. Are these upstream transcripts in the same direction as the protein coding gene they are associated with? Or are they predominantly in the other orientation? The "*UpTr*" RNA is shown to be in the same direction as *CEBPA*, but how can we know this is true for all claimed SPEARs? There is no analysis presented to distinguish these possibilities, but this is highly relevant because divergent transcription is a well-established phenomenon in mammalian cells. If they are all in convergent orientation, then where do they originate from? And why haven't they been noticed until now in the dozens of studies profiling nascent transcripts in mammalian cells published over the last now more than a decade? Figure 1C shows correlation between the expression level of the protein coding gene and the level of SPEARs, which does not at all instill confidence in SPEARs not being one of the already known quickly degraded byproducts of transcription without a distinct function on its own.

   This same issue confounds the interpretation of the experiments involving chemical inhibition of transcription presented later in the paper.

   Finally, what is the length distribution of the SPEARs? Again, no in-depth analysis is presented. It is said that:

   > "The sequences of five SPEARs identified by nasRNA-Seq (Supplementary Data #1) and corresponding to gene loci *c-MYC*, *PU.1*, *MYB*, *CEBPA*, and *CTCF* were verified by primer extension and 53 RACE (Figures S1G and S1H).

   However, Figures S1G and S1H only show the raw sequence of the claimed SPEAR, and only for the *MYC* locus, not for all the others.

5. The authors carry out H2A.Z, acH2A.Z and Tip60 RIP-seq and claim they pull down a lot of SPEARs. However, given the failure to properly define SPEARs, and the absence of the needed companion experiments throughout the cell cycle, this is very far from conclusive evidence in support of their claims.

6. The motif analysis is also very far from convincing. First, it is not even explained that RNA motifs are being searched for, one has to look in the STAR methods to find that out. Be that as it may, if the identified RM9A motif is of such key importance, one would have liked to see biochemical dissection of more than one SPEAR containing it, rather than just the *MYC* one, and *in vivo* analysis of the importance of these motifs (e.g. through CRISPR-mediated inactivation of the motif) would be needed, but this is missing from the current manuscript.

7. There are numerous sloppily written sentences and phrases throughout the manuscript. Some examples:

   - On p. 5: "close proximity of SPEARs to TSS" should be "close proximity of SPEARs to TSSs"
   - On p. 5: "possibilities of a link between SPEARs and H2A.Z/acH2A.Z was" should be "possibilities of a link between SPEARs and H2A.Z/acH2A.Z were"
   - On p. 7: what is "Broad HMM" referring to? One can guess these are ChromHMM states, and one can also find it in the STAR methods, but one should not have to guess on such things or go the STAR methods. And even in the STAR methods, no citation is provided to any of the Ernst et al. papers on the subject.
   - It is not clear why the word "loci" has to be italicized all throughout the manuscript
   - Also, "*PU.1*" is not the proper name of the gene, it a name often used for the transcription factor encoded by the *SPI1* gene.
   - On p. 4: " it remains unexplained how the balance between the acetylated and unmodified forms is inherited and preserved.". Presumably here "inheritance" after cell division is meant, but the way the sentences is written, it could also mean across generations (which would be wrong).
   - Etc.

   Also, on numerous occasions way too much technical information is presented in the main text, while in the same time, as discussed above, critically important details that are very much needed to understand what actually was done and whether it supports what is claimed are nowhere to be seen.