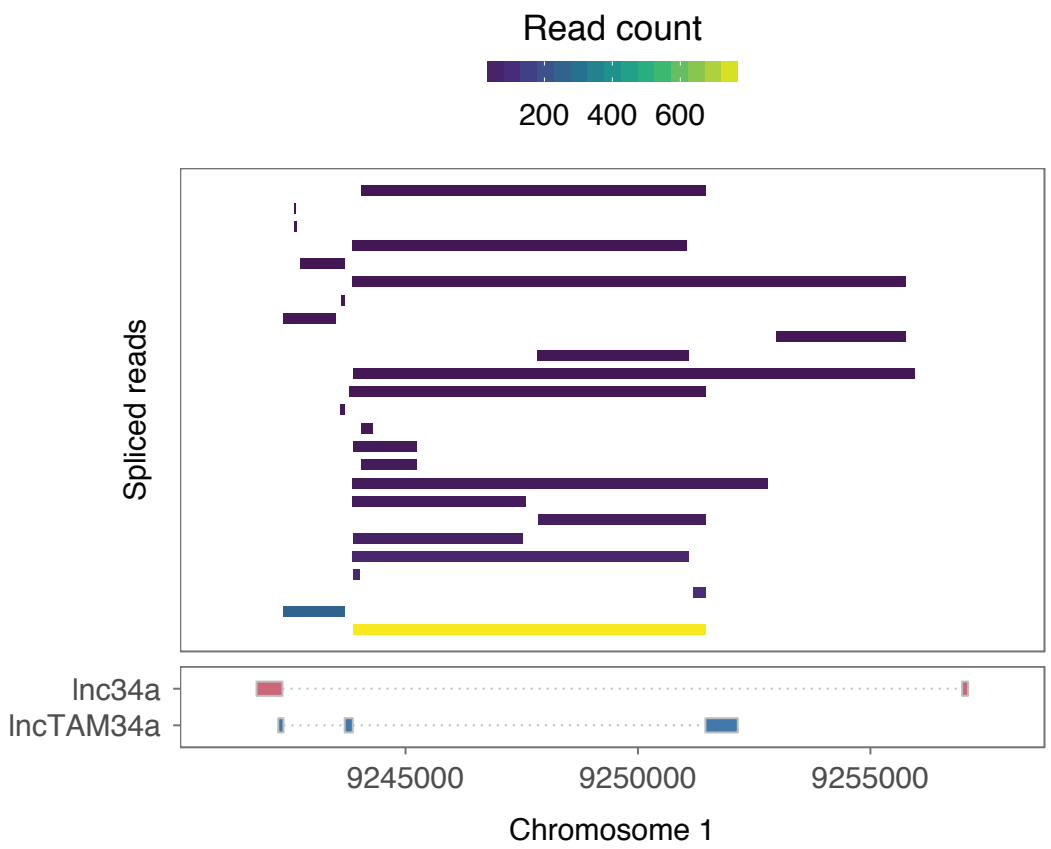

Supplementary Figure 7: All available whole cell (i.e. non-fractionated) spliced read data originating from the Cold Spring Harbor Lab in the ENCODE project for 38 cell lines was downloaded from the UCSC genome browser. Of these cell lines, 36 had spliced reads mapping to the plus strand of chromosome 1 and in the region between the Lnc34a start (9241796) and transcription termination (9257102) site (note that lncTAM34a resides totally within this region). Splice junctions from the following cell lines were included in the final figure: A549, Ag04450, Bj, CD20, CD34 mobilized, Gm12878, H1hesc, Haoaf, Haoec, Hch, Helas3, Hepg2, Hfdpc, Hmec, Hmepc, Hmscat, Hmscbm, Hmscuc, Hob, Hpcpl, Hpiepc, Hsavec, Hsmm, Huvec, Hvmf, Hwp, Imr90, Mcf7, Monocd14, Nhdf, Nhek, Nhemfm2, Nhemm2, Nhlf, Skmc, and Sknsh. All splice junctions were included in the figure and coloured according to the number of reads corresponding to each (top panel). In cases where the exact same read was detected multiple times the read count was summed and represented as one read in the figure. lncTAM34a and Lnc34a transcripts are represented for reference (bottom panel).

In order to detect Lnc34a expression in a manner that is not dependant on 5'-capping, we proceeded to examine spliced RNA sequencing reads from 36 cell lines, taking advantage of the fact that Lnc34a has an exon which is not present in any annotated or PCR cloned lncTAM34a isoforms. These results indicate that, although splice junctions corresponding to the annotated lncTAM34a transcript and multiple isoforms found via PCR cloning were detected, the data give no support for the presence of the splice junction between the first and second exon of Lnc34a. In summary, these results indicate that Lnc34a is unlikely to represent the same asRNA transcript as lncTAM34a and that its expression may be confined to CCSCs.

In addition, there are several other lines of evidence indicating that the asRNA described in our paper is distinct from Lnc34a. We noted several relevant comments in the public review that was published in conjunction with the work by Wang et al. The authors mention, and provide data, indicating that Lnc34a expression is not changed upon ectopic expression of TP53. In contrast, lncTAM34a is strongly regulated by TP53 as the evidence shows in our, as well as, others findings (Léveillé 2015, Rashi-Elkeles 2014, Hünten 2015, Ashouri 2016, Kim 2017). Furthermore, Wang et al. also mention in the public review that Lnc34a has a low expression level in HCT116 cells