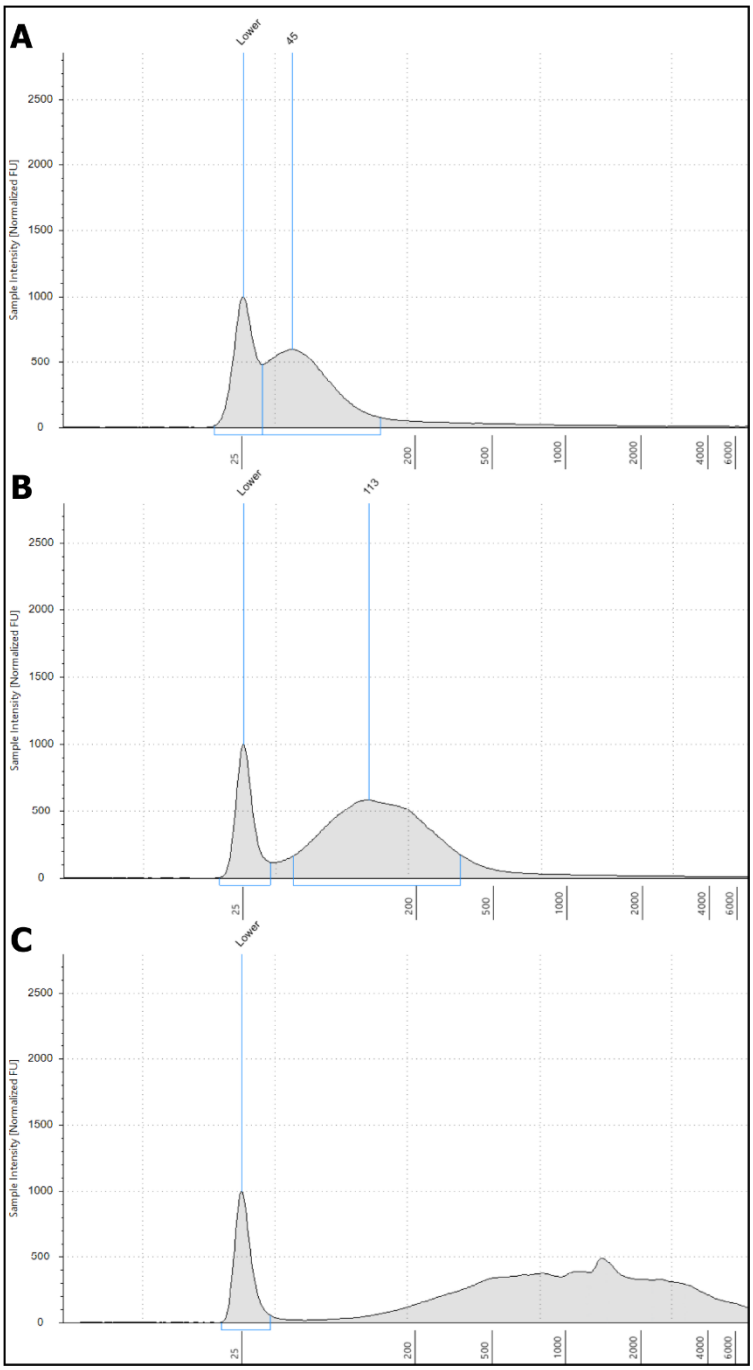
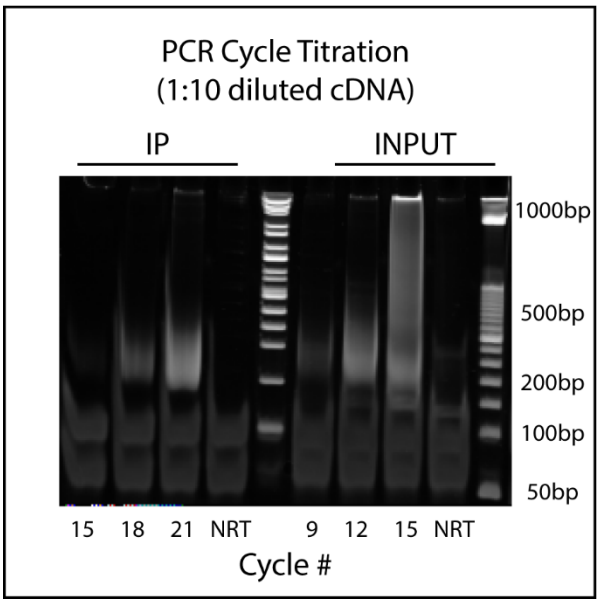
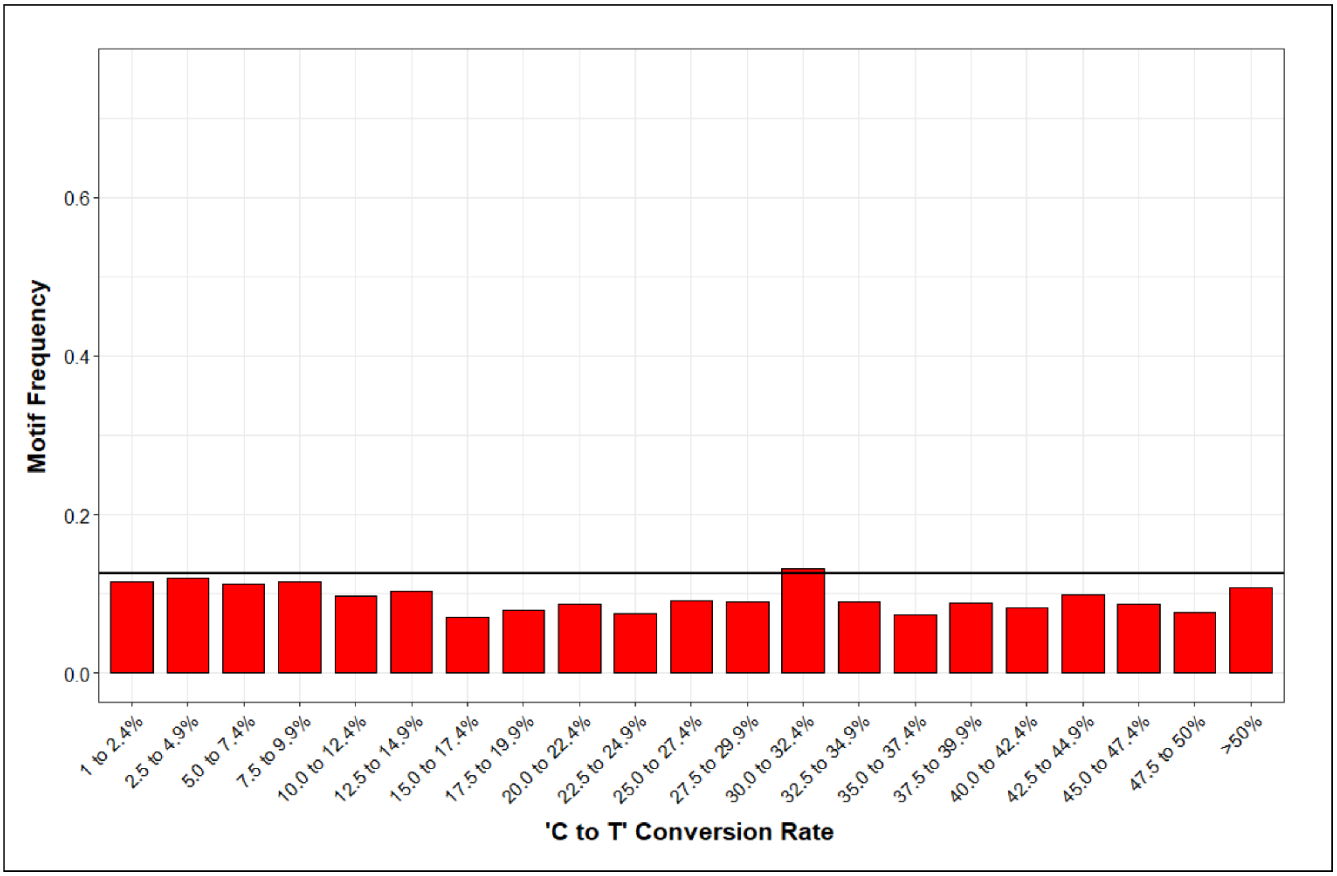
Supplemental Figures



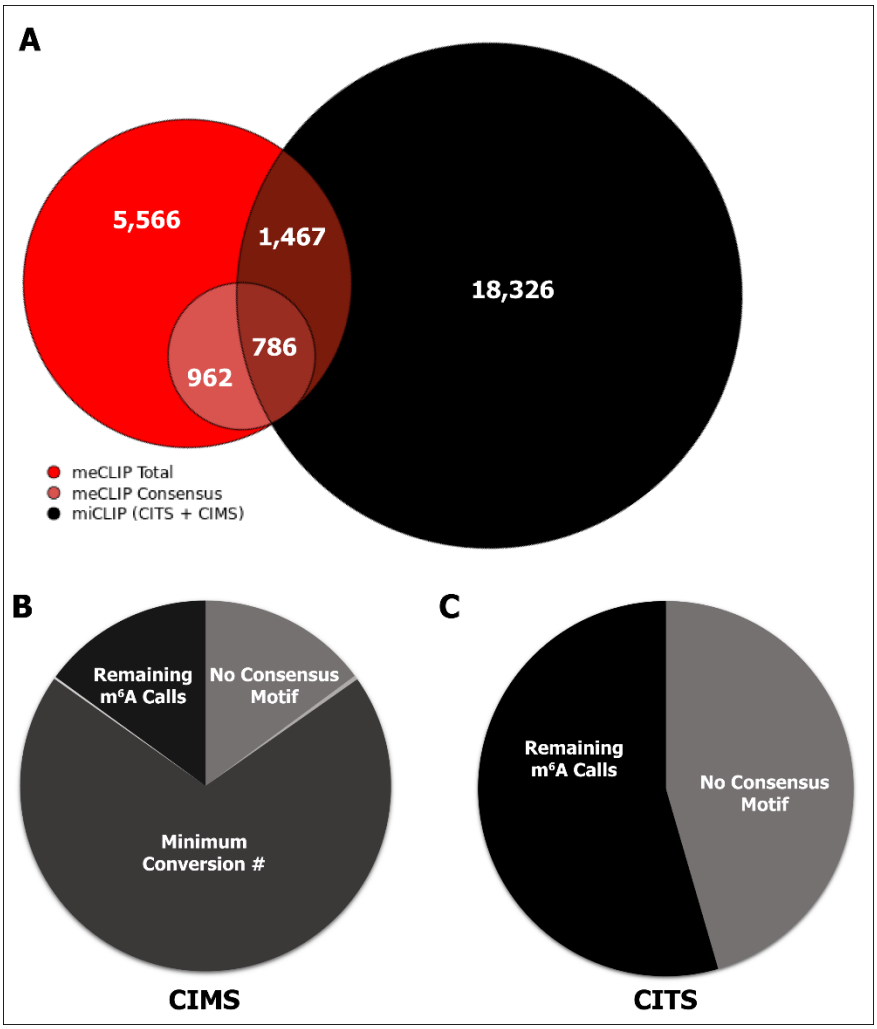
Supplemental Figure 1 – Agilent TapeStation 4200 results of RNA fragementation using High Sensitivity RNA ScreenTape. A small aliquot of total RNA (2µg) was fragemented for various durations at 70C and then a highly-diluted sample (~3ng/µL) was analyzed according to the manufacturer's protocol. A) Example of over-fragmented sample that is outside the recommended range of 100-200bp (9 min of fragmentation). B) Optimal fragmentation result (~3 min). C) Under fragmented sample (1 min with higher concentration); although minimal under-fragmenation will likely still yield acceptable libraries, more extreme scenarios such as the one depicted will result in large amplicons that are outside the recommended range of the sequencer. "Lower" refers to the lower size marker for the TapeStation instrument.



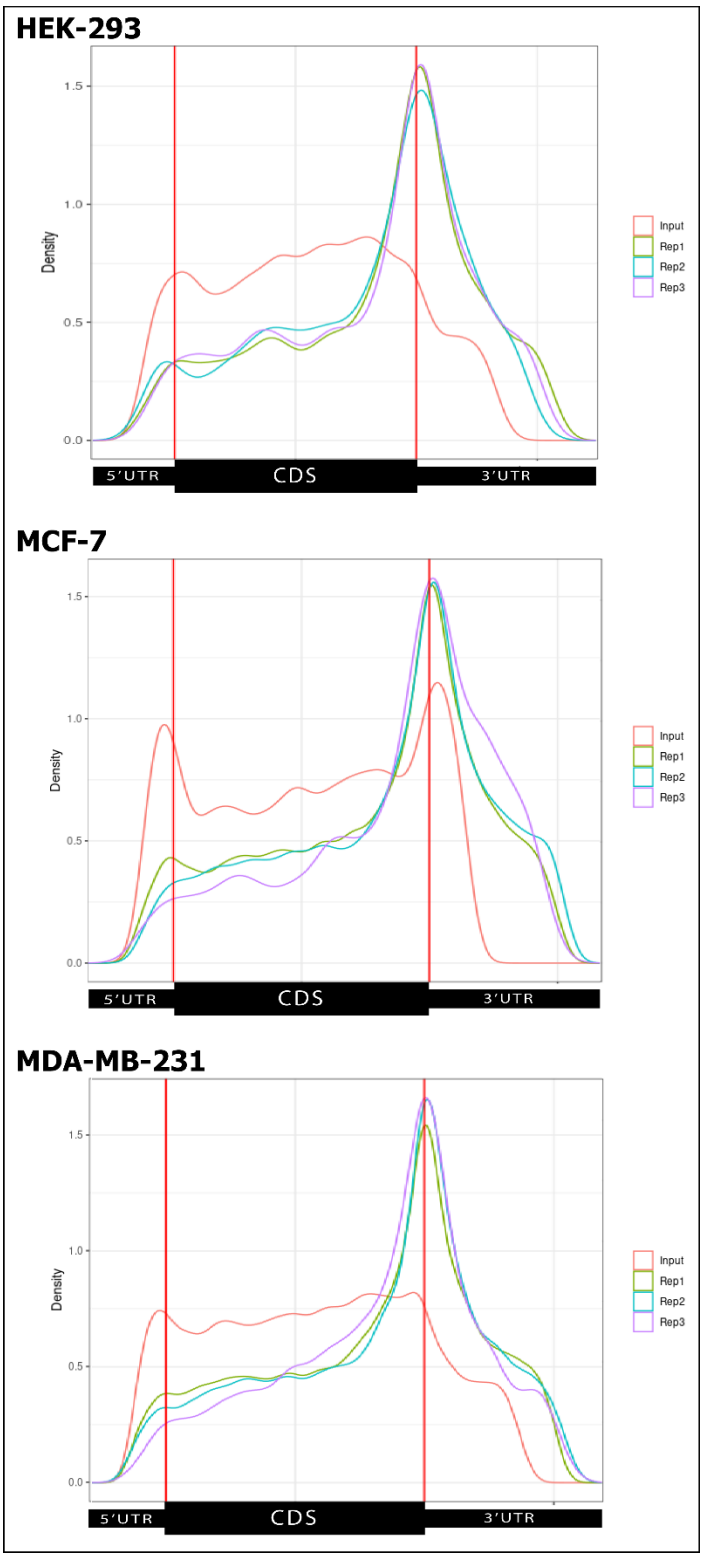
Supplemental Figure 2 – Example of PCR cycle titration used to optimize the final library amplification. Based on qPCR results (Cq) obtained using a diluted sample of cDNA (1:10), the sample is amplified with three different cycles (Cq-3, Cq, Cq+3) and run on a polyacrylamide gel for visualization (i.e. the Cq obtained from qPCR for the diluted IP sample was 18, so the PCR cycle titrations were 15, 18, and 21). 'NRT' is a 'no reverse transcriptase' control. Optimal amplification typically results when the smear representing the fragment (~175-300bp) is barely visible (here 18 and 10 cycles were used for the IP and INPUT samples, respectively). Primer dimers are seen ~50-100bp. The chosen cycle is adjusted for the full undiluted library (subtract 3 cycles) and used for the final amplification.



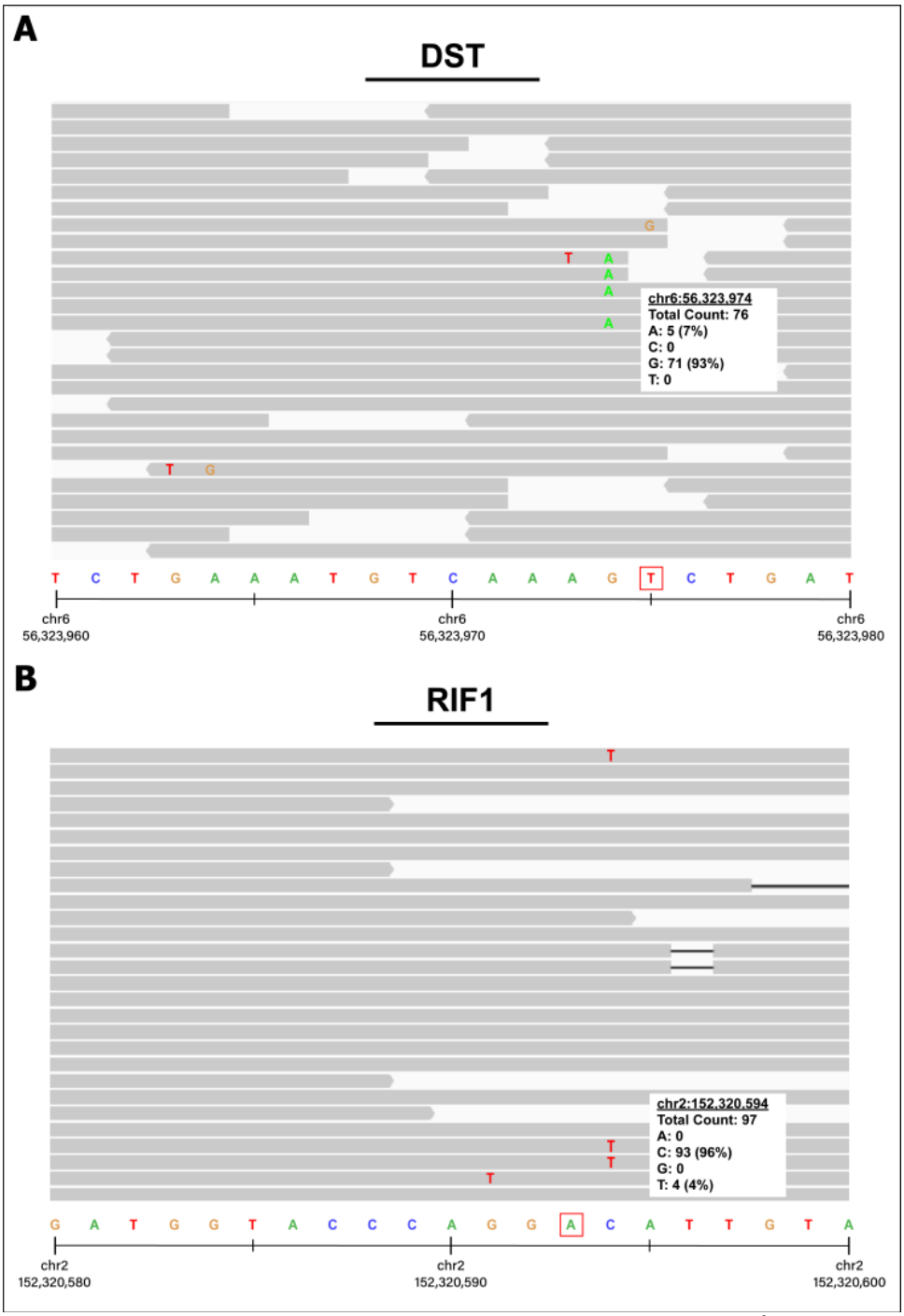
Supplemental Figure 3 - Analysis depicting the occurrence of the 'RAC' consensus motif relative to C-to-T mutations in the input sample (compare to Figure 3). Black line at 12.5% represents the random chance of having 'RA' upstream of the C. As expected, without the anti-m^{6}A antibody specific mutations, the occurrence of the motif within each frequency interval is right at 12.5%.



Supplemental Figure 4 – Venn diagram of m^{6}A sites called in HEK-293 cells using meCLIP experimental approach and analysis pipeline compared to the m^{6}A sites identified in the original miCLIP paper (Linder et al. 2015). A) A consensus set of 8,781 unique m^{6}As was generated from our three replicates ('meCLIP Total') and then compared to the 20,579 unique m^{6}As reported from the combined CITS + CIMS analysis of miCLIP ('miCLIP (CITS + CIMS'). A subset of 1,748 consensus m^{6}As that were identified in at least 2 of our replicates ('meCLIP Consensus') were also compared to the miCLIP set, with 786 (~45%) overlap. B) Pie chart depicting the number of m^{6}As identified via the CIMS component of miCLIP that would be removed if they were subjected to our filtering thresholds (>2 conversion events, ≥2.5% conversion rate, occurrence within the 'RAC' motif, and removal of residues overlapping with calls from input samples). The total number of remaining of m^{6}As is 1,430. C) Similar to panel B, using the CITS-determined sites from miCLIP. The number of remaining m^{6}As after removing sites that did occur in the consensus motif is 6,567.



Supplemental Figure 5 – Metagene plots of the m6A distribution for the three cell lines analyzed with meCLIP. The m6As from each replicate are plotted relative to their corresponding input sample for comparison (the input plot is generated from a combined set of unique C-to-T conversions occurring within the 'RAC' motif that were identified from the input samples of each replicate). The plots are segregated (red lines) into sections corresponding to regions of the transcript (5'UTR, CDS, and 3'UTR) as indicated by the bottom black boxes.



Supplemental Figure 6 - Genome browser snapshots of the m^{6}A residues identified in DST (A) and RIF1 (B) that were validated via meRIP (Figure 5). Red box surrounding the 'A' or 'T' in the colored sequence of nucleotides indicates the m^{6}A site, with the C-to-T mutations at the downstream nucleotide depicted in the reads (grey bars) aligning to that loci (white box depicts the number of each nucleotide at that position).

Supplemental Table 1. Summary of the percent of m^6A residues removed from the final call list for each meCLIP experiment due to the input filter. The list of input residues used for filtering was a combined list of residues identified from all the input samples for that cell line.

Experiment	% Removed From IP
HEK-293 (*Rep #1*)	2.79%
HEK-293 (*Rep #2*)	4.34%
HEK-293 (*Rep #3*)	3.72%
MCF-7 (*Rep #1*)	4.44%
MCF-7 (*Rep #2*)	7.14%
MCF-7 (*Rep #3*)	7.07%
MDA-MB-231 (*Rep #1*)	9.17%
MDA-MB-231 (*Rep #2*)	11.05%
MDA-MB-231 (*Rep #3*)	11.28%