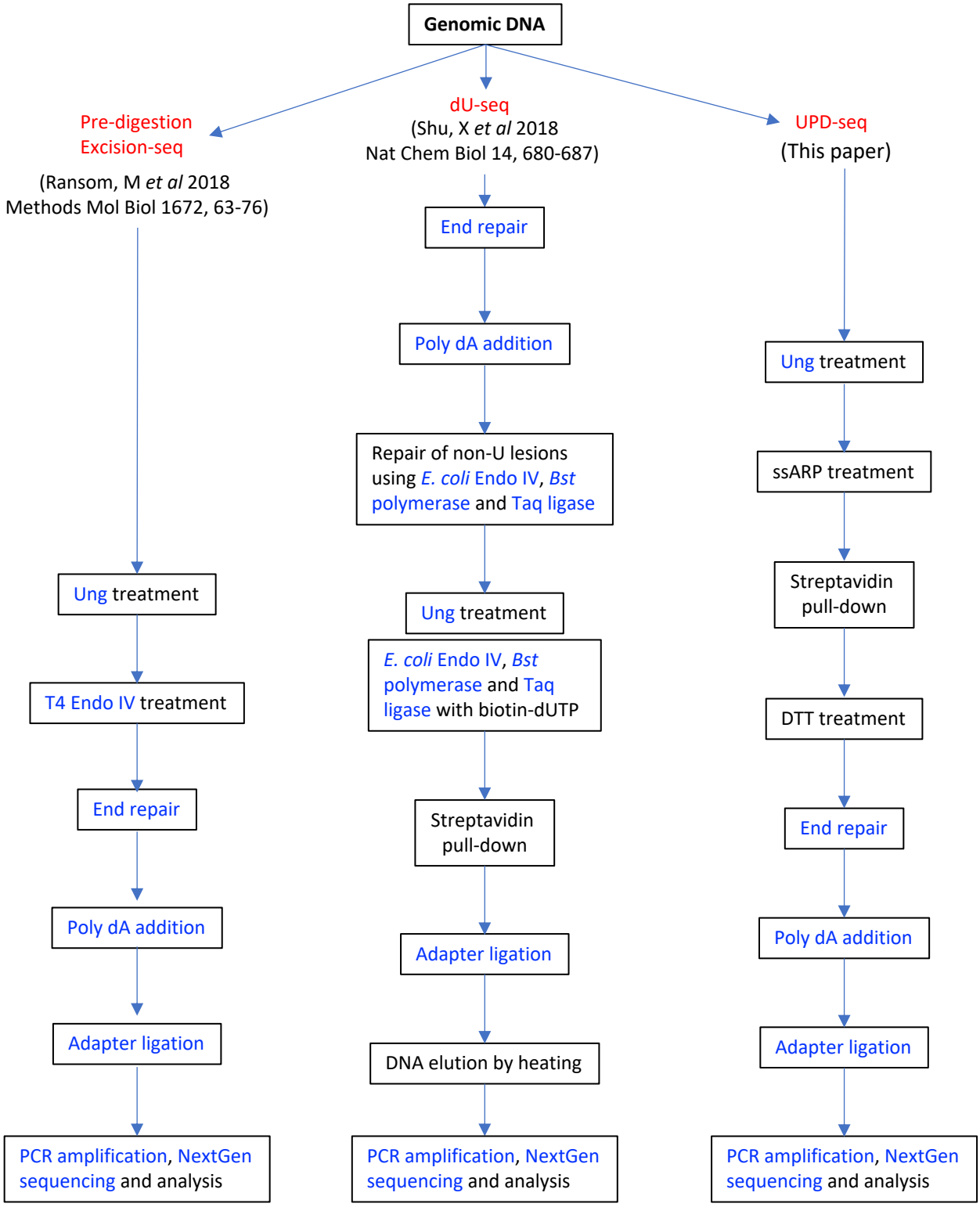
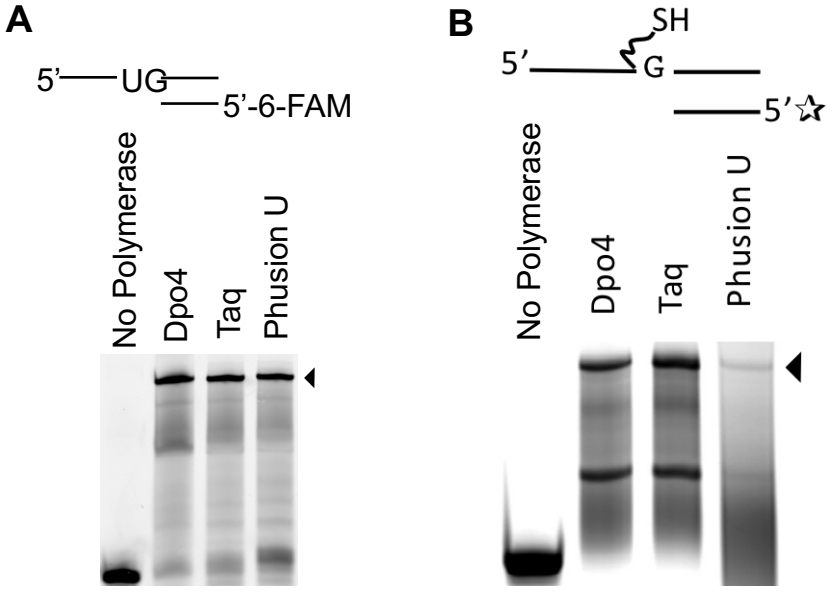


Supplementary Fig. S1
Comparison of methods for mapping genomic uracils

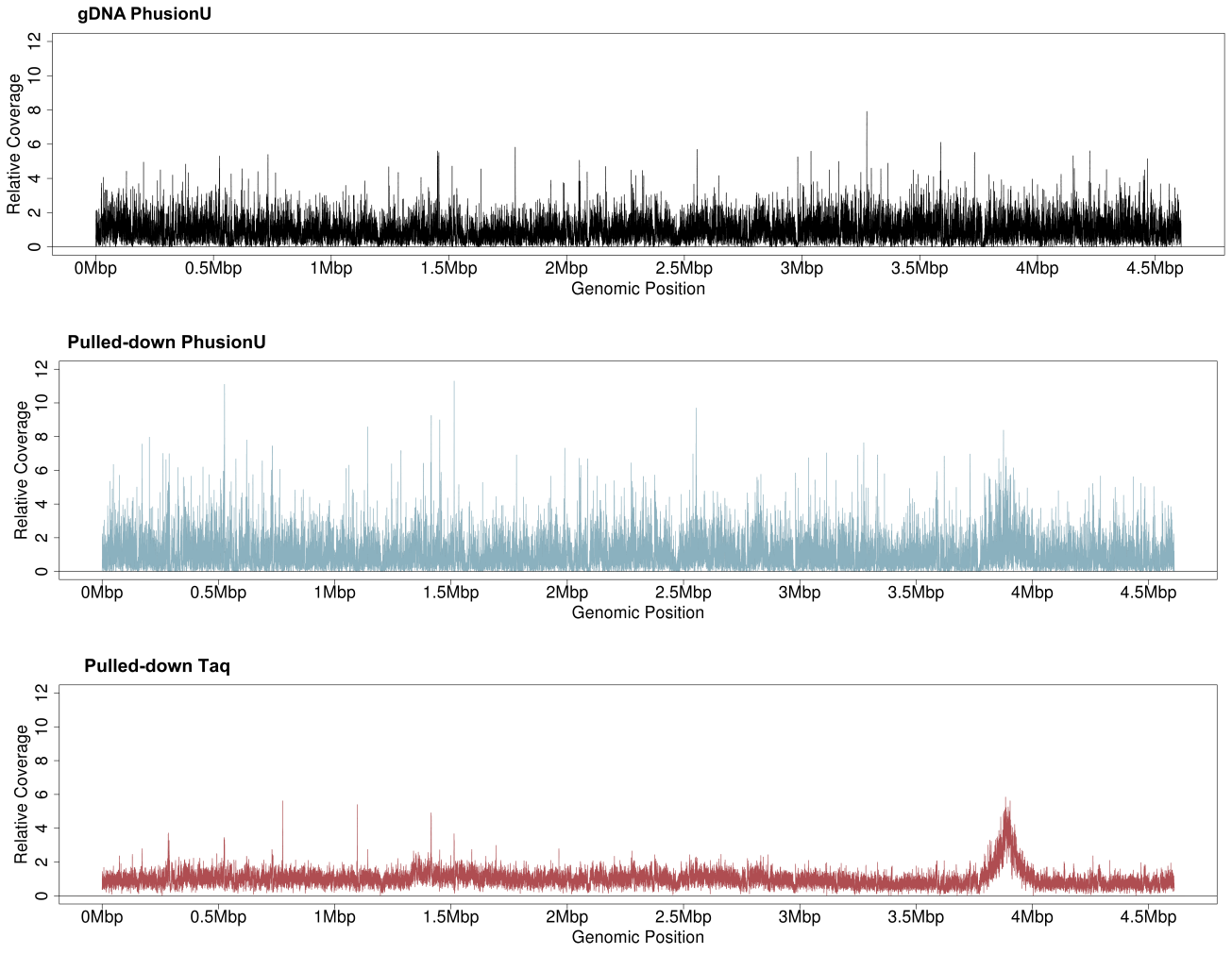
The biochemical steps in different techniques for mapping genomic uracils are outlined. Enzymatic manipulations are shown in blue.



Supplementary Fig. S2

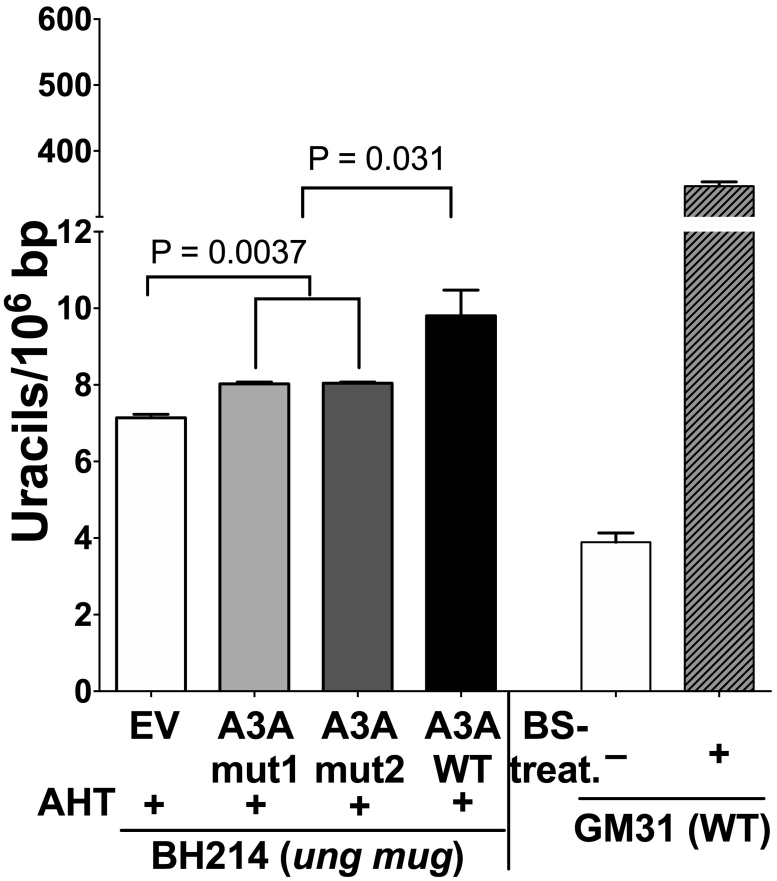
Bypass of uracils and ssARP scar by DNA polymerases

Primer extension with Dpo4, Taq and PhusionU polymerases across uracil (Part A) or the scar left behind after DTT treatment (Part B) in the template strand. The full-length product is indicated by a black triangle.



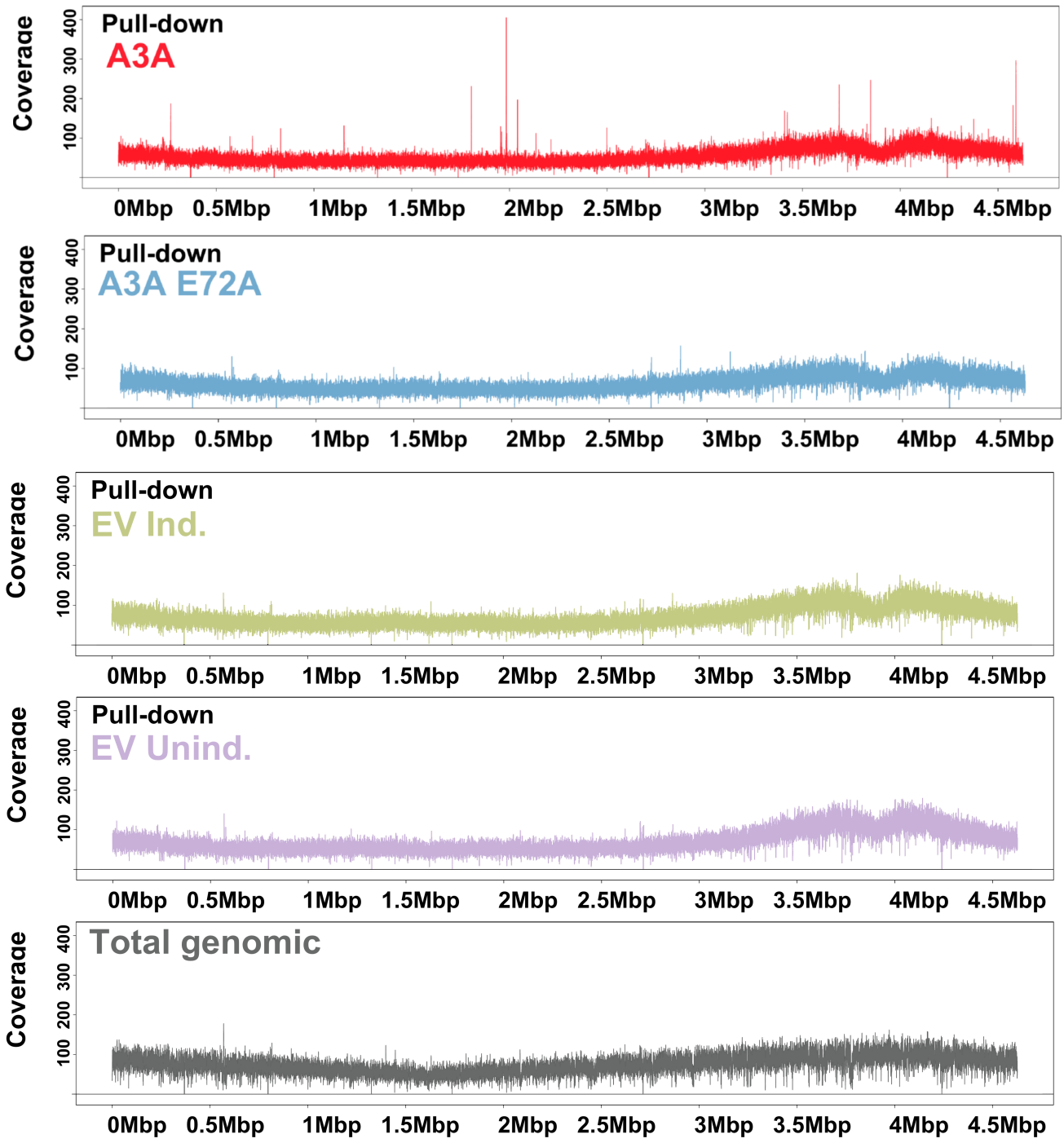
Supplementary Fig. S3

The tiled plots shown in Figure 1F are separated and shown one below the other.



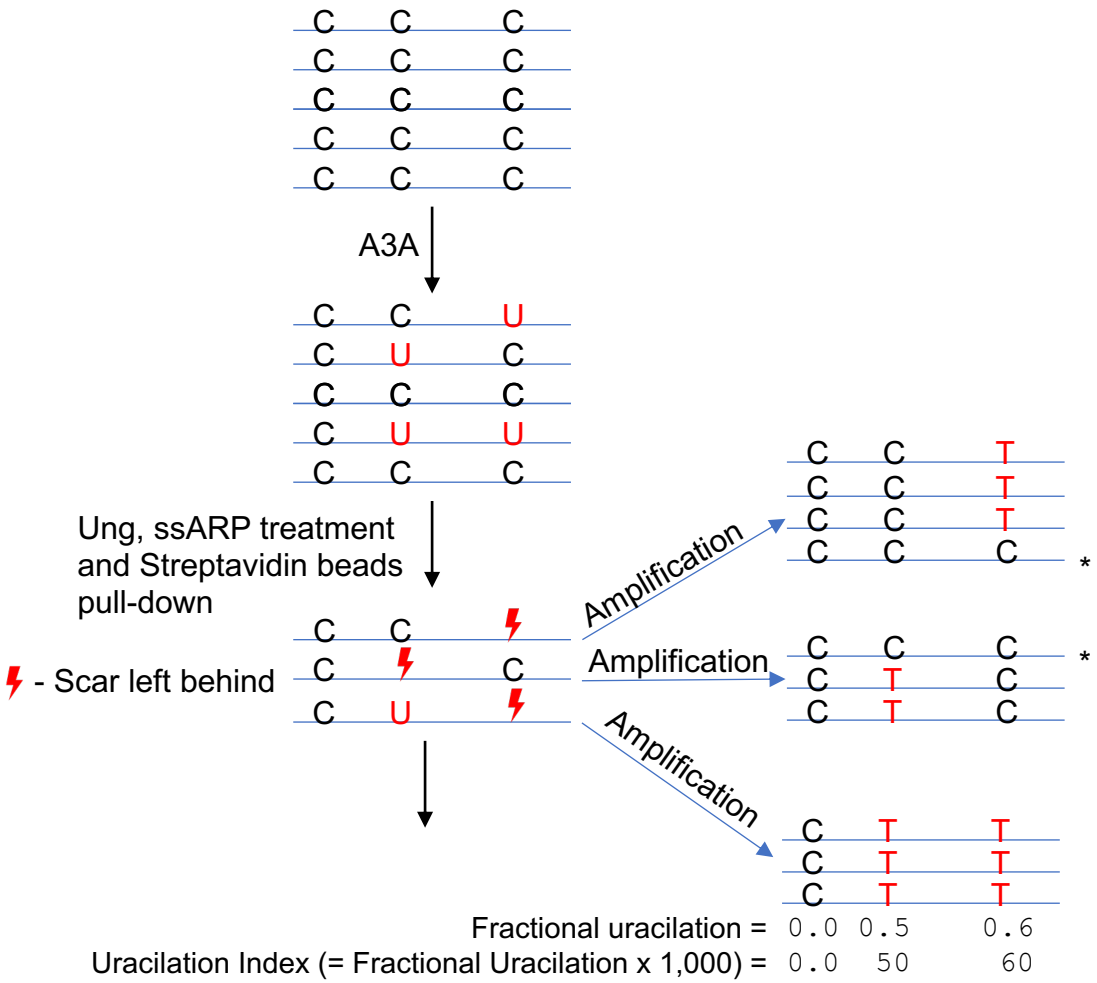
Supplementary Fig. S4

The uracil quantification assay was performed as described in the text. The A3A mut1 and A3A mut2 are uracil quantifications on DNA from independent cultures of BH214 cells expressing A3A-E72A mutant. EV- Empty vector. AHT- Anhydrotetracycline; BS-treat.- Bisulfite treatment. GM31 is DNA repair-proficient (*ung*$^{+}$ *mug*$^{+}$), while BH214 is repair-deficient (*ung mug*).



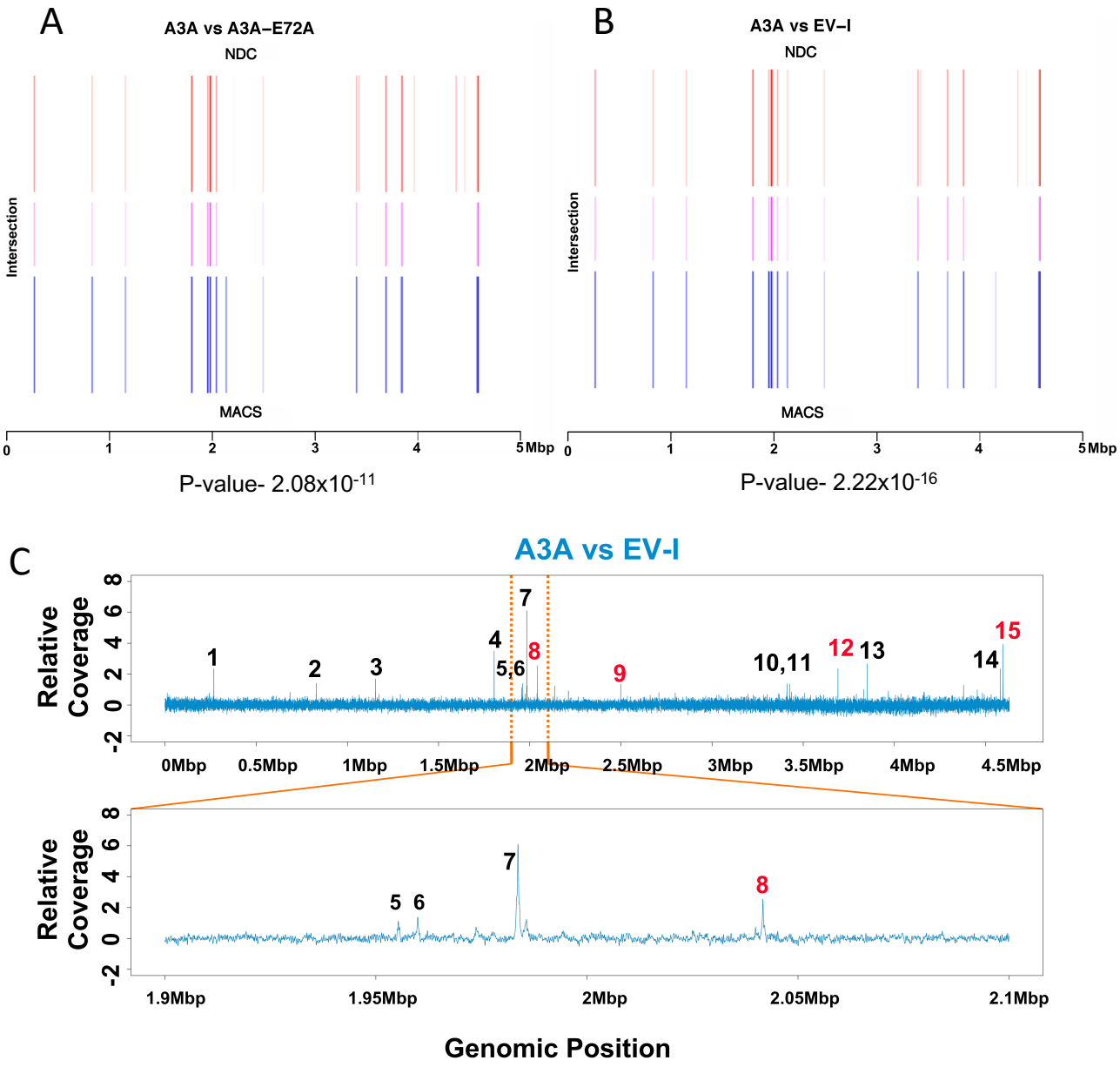
Supplementary Fig. S5

The tiled plots shown in Figure 2C are separated and shown one below the other.



Supplementary Fig. S6
Definition of Uracilation Index

Uracilation index is defined in the context of UPD-seq. A region in the *E. coli* genome with three cytosines that may be deaminated by A3A is shown in five independent cells. A3A converts cytosines in three of the molecules to uracils. Ung/ssARP/Streptavidin treatment pulls down only these three molecules and DTT treatment releases them from the streptavidin beads leaving behind a chemical scar. One of the uracils is not excised by Ung and is left intact. Amplification of the pull-down DNA inserts A across U and (in most cases) the scar, except when the polymerase inserts a guanine across a scar. The latter event restores the original cytosine.. The uracilation index is defined as fractional uracilation at any site among all the sequenced molecules times 1,000.

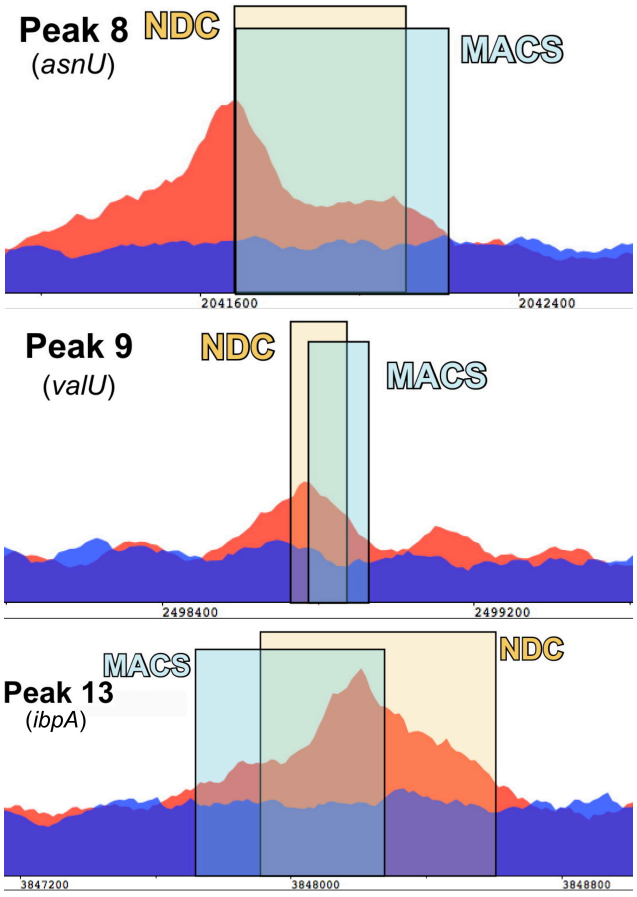


Supplementary Fig S7

Comparison of NDC and MACS generated peaks

R Package GenometriCorr was used to compare the peaks detected by different algorithms (NDC vs MACS). Top panel (red) shows the peaks detected using NDC , lower panel (blue) shows the peaks detected using MACS and the middle panel (pink) shows the intersection of these two sets of peaks. P-values for relative distances was calculated using Kolmogorov-Smirnov test and are shown below each plot. Peaks generated by comparing A3A with (**A**) A3A-E72A and (**B**) empty vector-induced as control libraries.

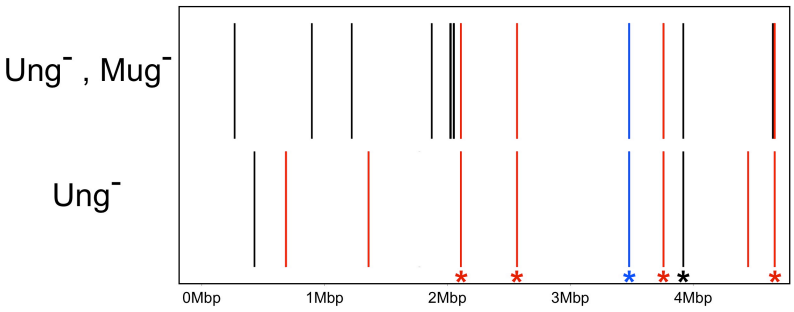
(**C**) Similar to Fig. 4A, NDC plot comparing A3A with empty vector-induced



Supplementary Fig. S8

Comparison of individual peaks generated by NDC and MACS

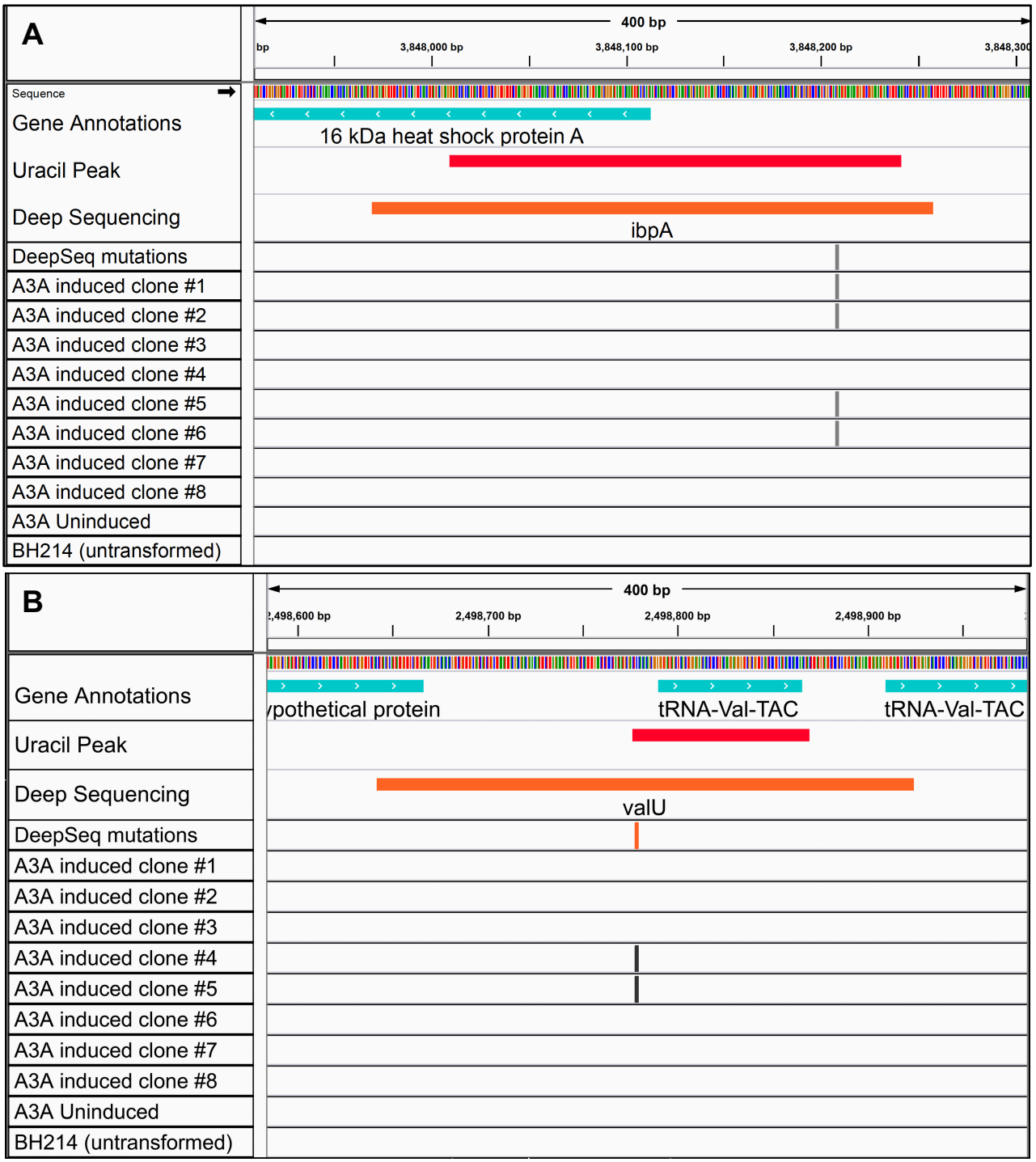
Depth of coverage plots of UPD-Seq libraries A3A (red) and A3A-E72A (blue) around peak 8 , peak 9 and peak 13. Blue and yellow boxes show the genomic regions that were detected as a peak using NDC algorithm or MACS software.



Supplementary Fig. S9

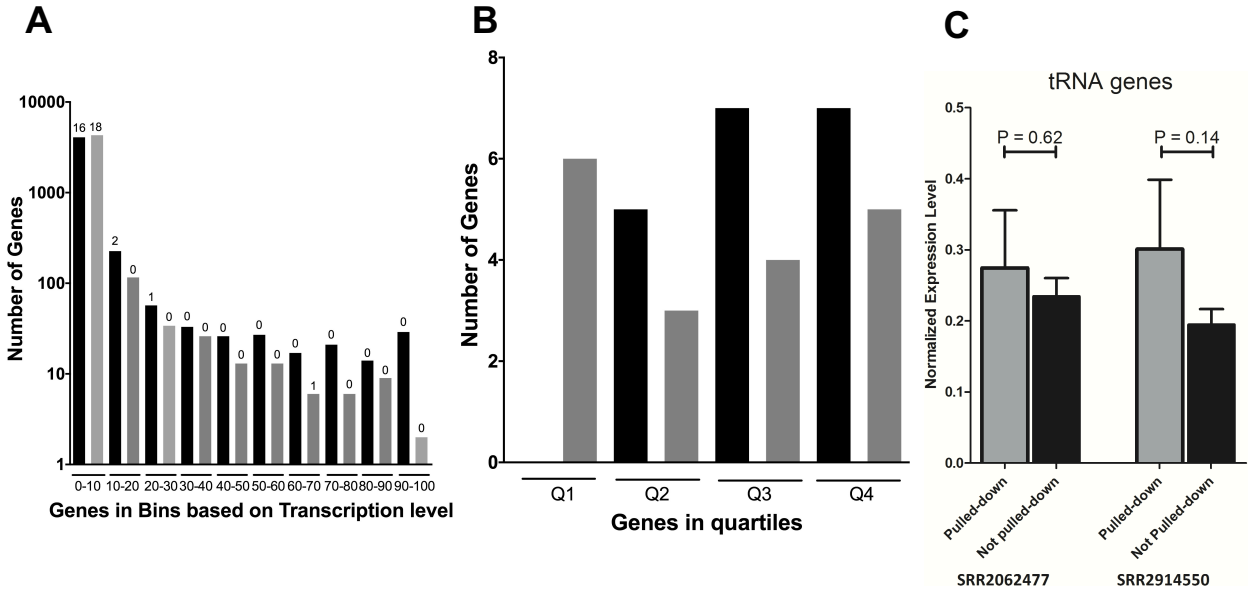
Comparison of uracilated peaks in BH214 and BH212 genomes

BH214 is *ung*⁻ *mug*⁻, while BH212 is *ung*⁻ *mug*⁺
Red is a tRNA gene, blue is an rRNA gene and black is a CDS
Uracilated peaks common in the two strains are identified by asterisks (*)



Supplementary Fig. S10

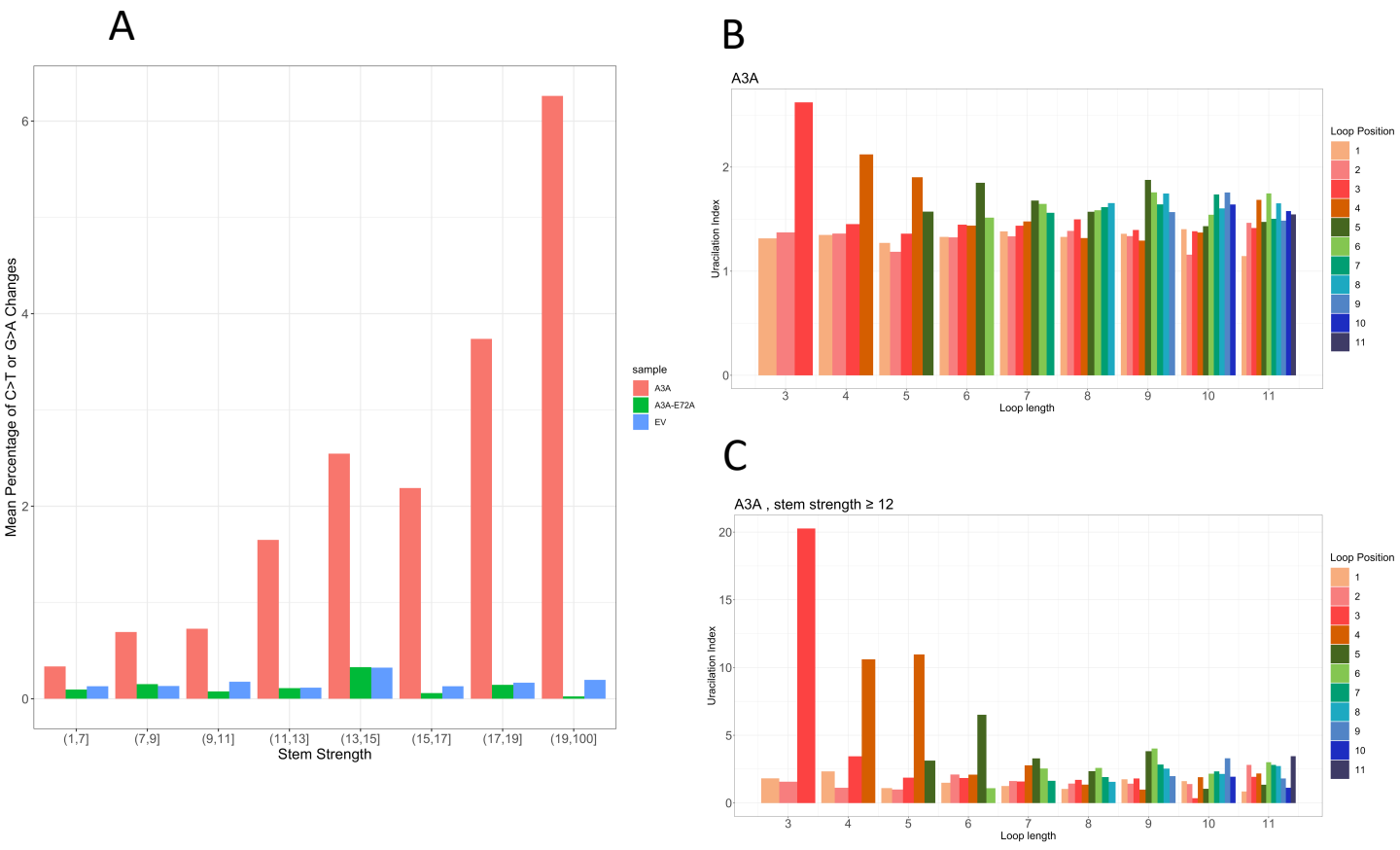
IGV visualization of 400 bp region around *ibpA* and *valU* genes. Successive lines show the span of the uracilation peak (red), the deep sequenced region (orange) and the position of the C to T changes in uracilation (orange) and mutations in whole genome sequence of eight independent clones expressing A3A (black), one clone with no induction of A3A and one clone without A3A.



Supplementary Fig. S11

Relationship between transcription and preferential targeting by A3A

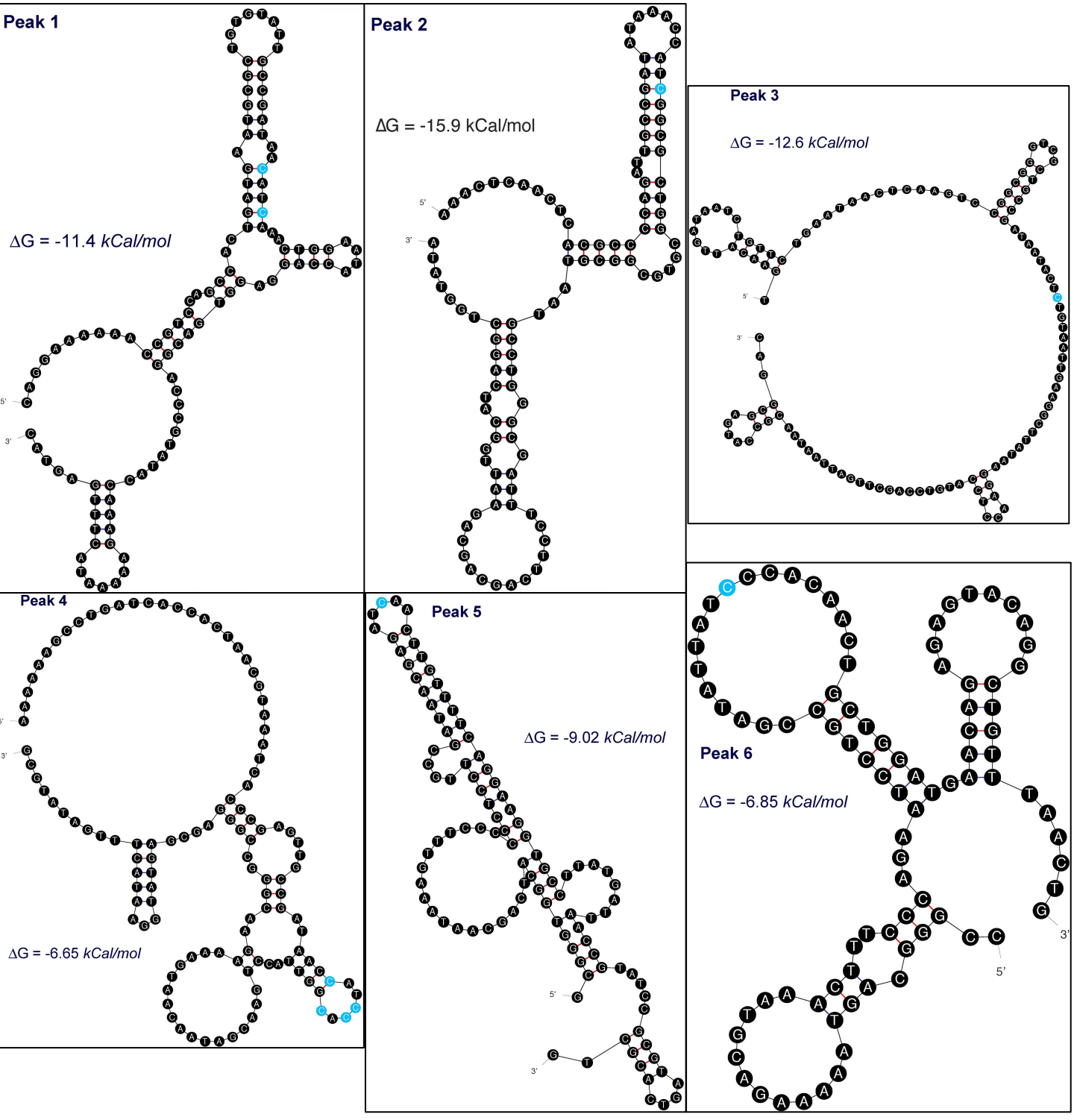
(A) All *E. coli* genes are divided into 10 bins based on level of transcription. Bars represent the number of genes found in each bin. Number of genes detected in the pull-down experiments are shown above each bar. Most of the pull-down genes are in the 10th percentile of genes based on their transcription levels. The two datasets used were SRR2062477 and SRR2914550.
(B) *E. coli* genes were sorted based on transcription levels and divided into quartiles (equal numbers). Number of pulled-down genes in each quartile are shown with bars.
(C) Comparison of transcription levels tRNA genes that were pulled down from BH214 and BH212 genomic DNAs with those that were not pulled down.



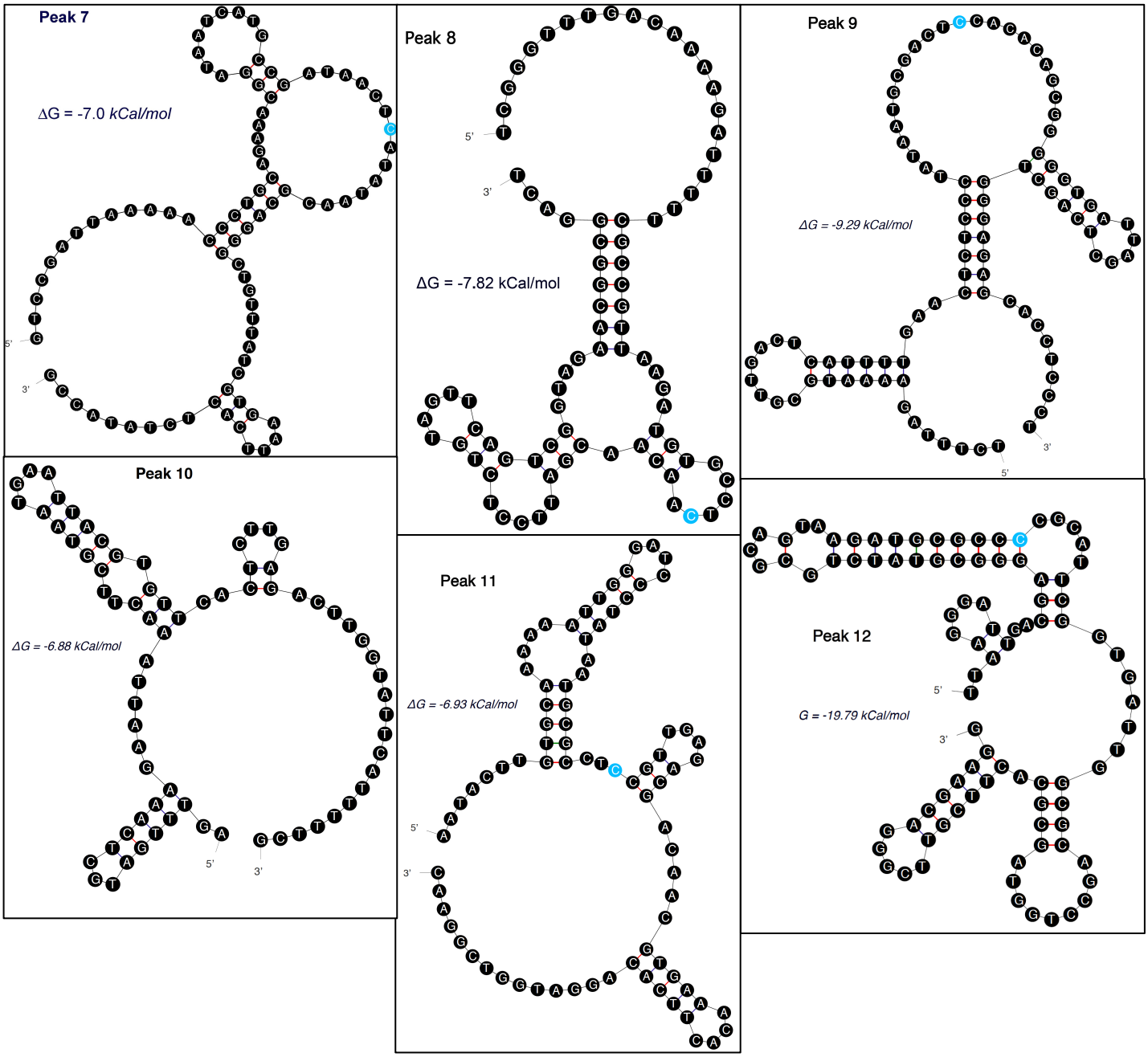
Supplementary Fig. S12

A. Uracilation Index of predicted hairpins in 3 UPD-seq libraries grouped by a range of hairpin stem strengths (strength >1 through 7, >7 through 9 etc)

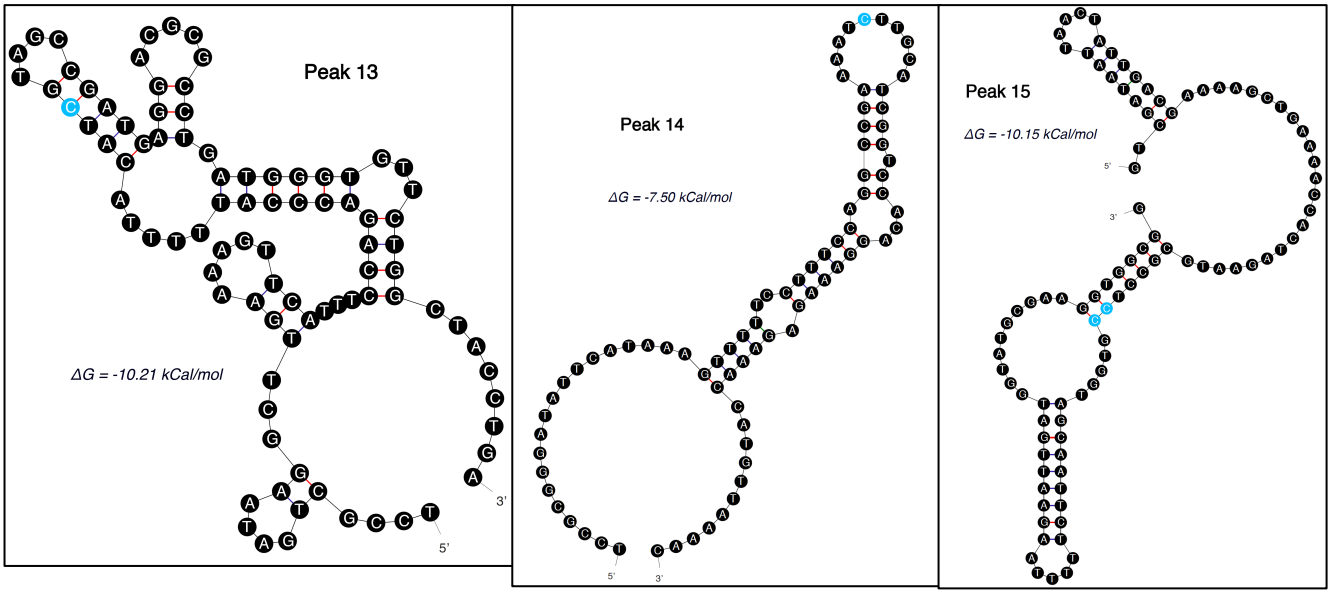
B and C. Uracilation Index in the A3A UPD-seq library grouped by hairpin loop length and the position of the cytosine within the loop, for all TC dinucleotides (**B**) and TC dinucleotides with a hairpin stem strength ≥ 12 (**C**).



Supplementary Fig. S13
Part 1



Supplementary Fig. S13
Part 2



Supplementary Fig. S13
Part 3

Secondary structures of DNA sequences within uracilation peaks predicted by Mfold using the default parameters. The white bases on blue background (C) are most frequently read as T in UPD-seq.