A

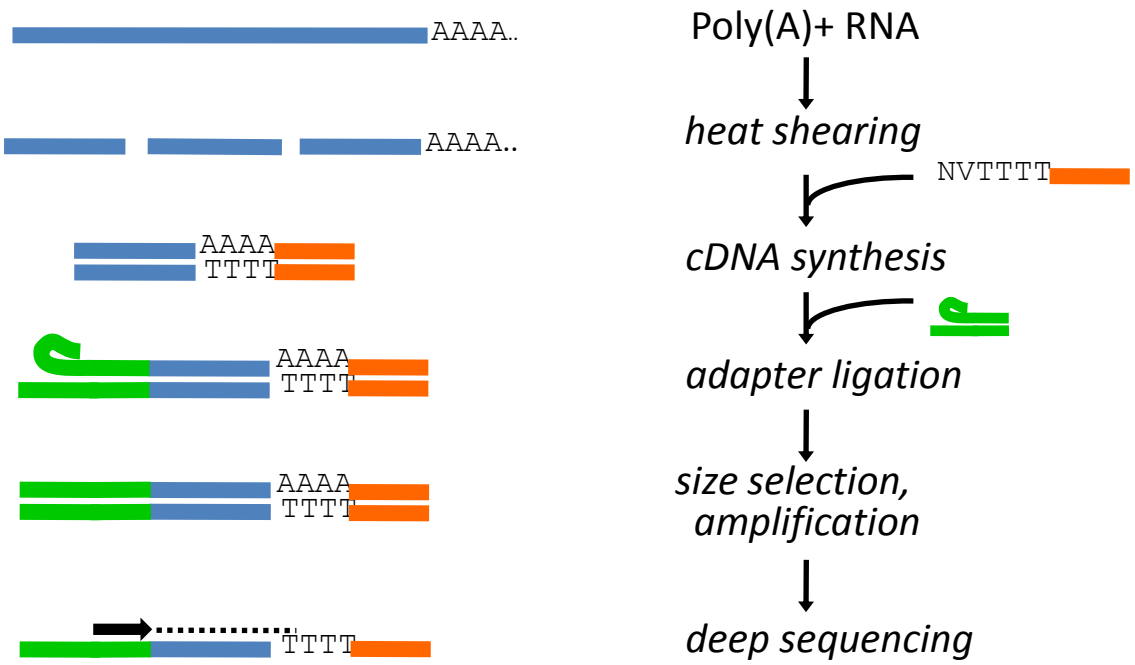
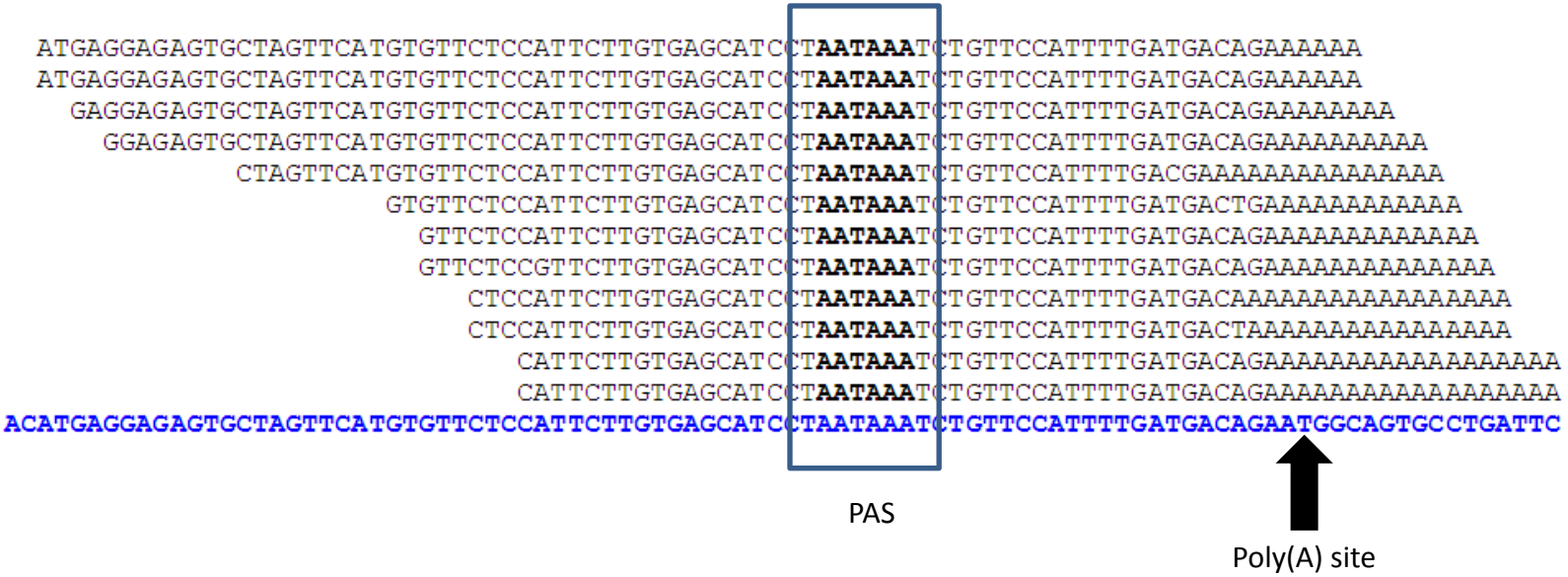

AAAA..
AAAA..
AAAA
TTTT
AAAA
TTTT
AAAA
TTTT
TTTT
Poly(A)+ RNA
heat shearing
NVTTTT
cDNA synthesis
adapter ligation
size selection,
amplification
deep sequencing

Figure S1

B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GAGGAGAGTGCTAGTTCATGTGTTCTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAAAAAAAA
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CTAGTTCATGTGTTCTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGACGAAAAAAAAAAAAAAA
GTGTTCTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACTGAAAAAAAAAAAA
GTTCTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAAAAAAAAAAAAA
GTTCTCCGTTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAAAAAAAAAAAAAA
CTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAAAAAAAAAAAAAAAAA
CTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACTAAAAAAAAAAAAAAAA
CATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAAAAAAAAAAAAAAAAA
CATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAAAAAAAAAAAAAAAAA
ACATGAGGAGAGTGCTAGTTCATGTGTTCTCCATTCTTGTGAGCATCCTAATAAATCTGTTCCATTTTGATGACAGAATGGCAGTGCCTGATTC
PAS
Poly(A) site

C

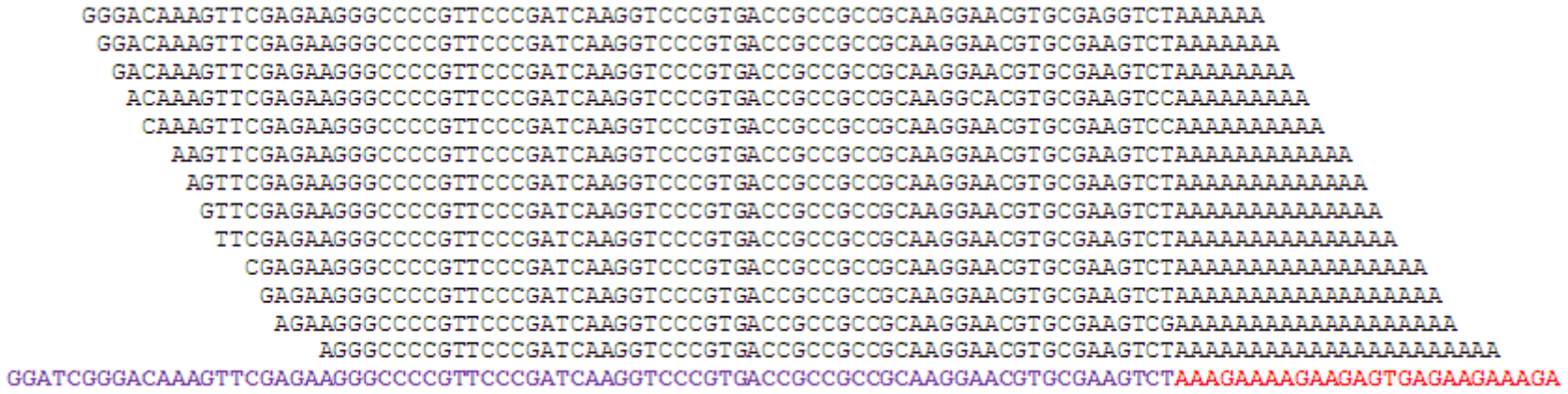
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Figure S1

D

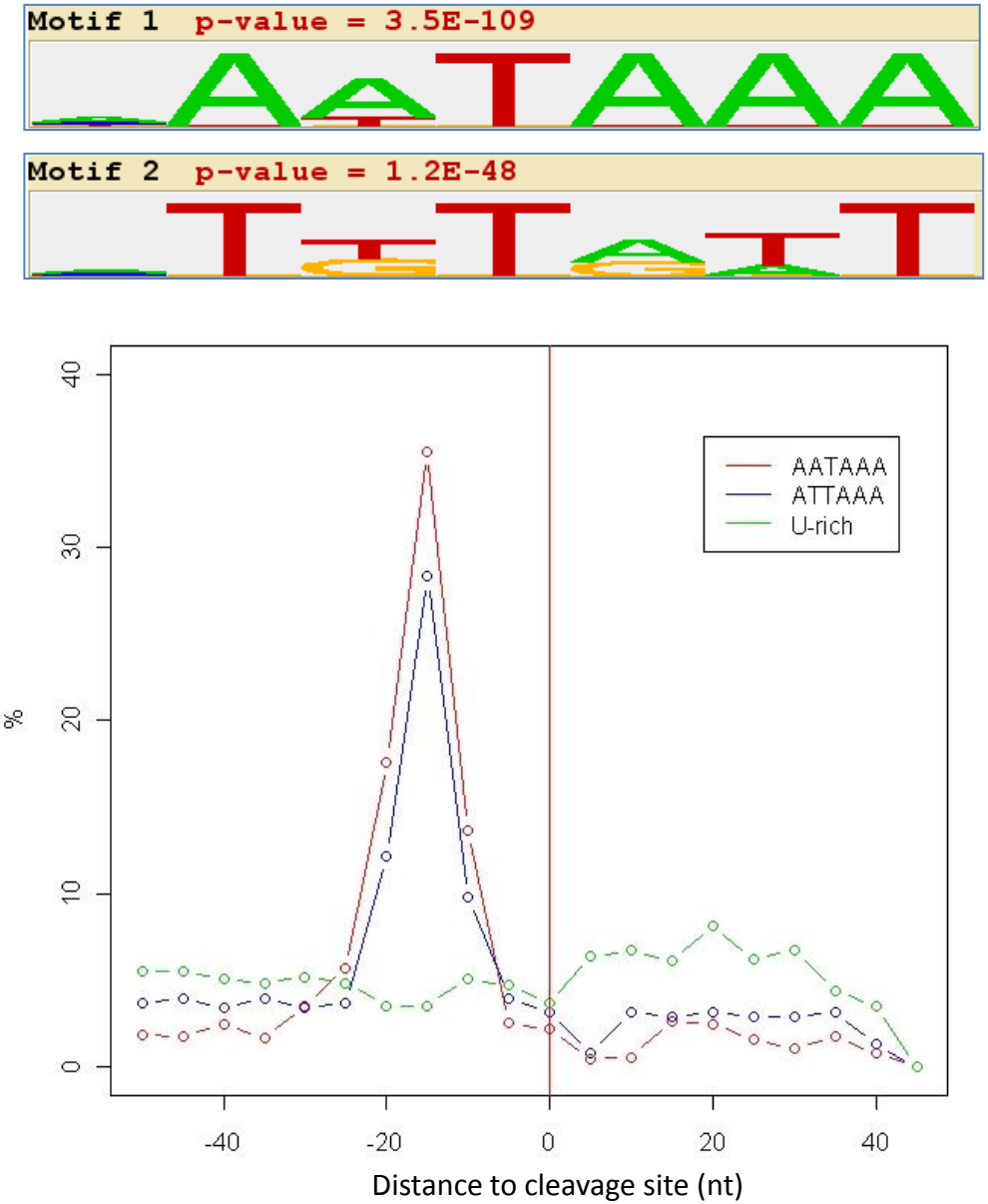
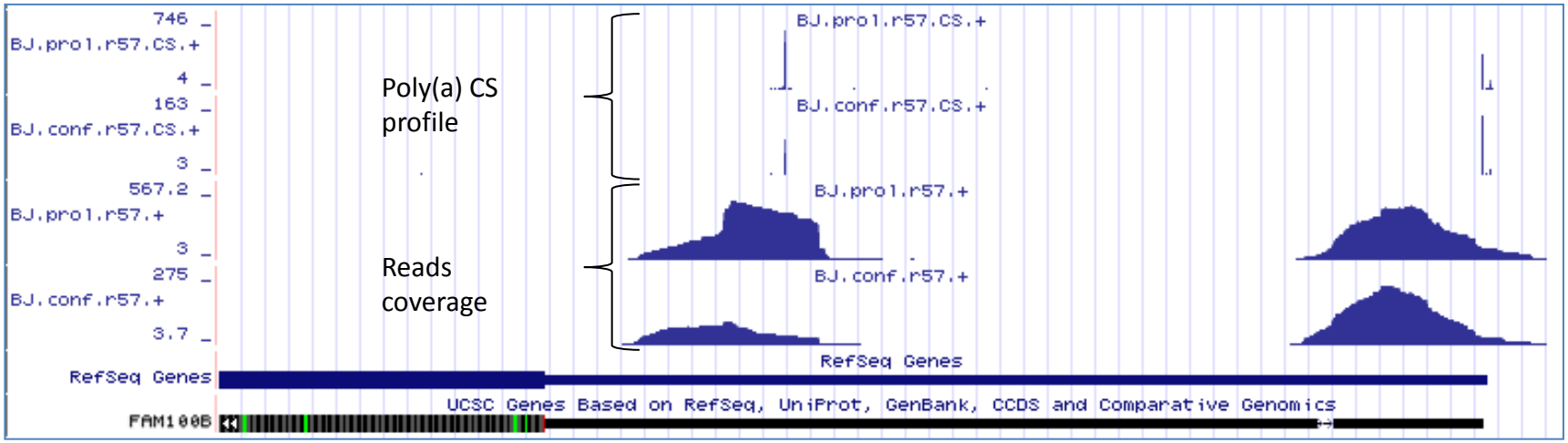

Motif 1 p-value = 3.5E-109
Motif 2 p-value = 1.2E-48
AATAAA
ATTAAA
U-rich
%
0
10
20
30
40
-40
-20
0
20
40
Distance to cleavage site (nt)

Figure S1

E



F

FAM100B	Proximal poly(A) site	Distal poly(A) site
BJ proliferation	1830	1811
BJ confluent	350	845

p= 1.88E-36

Figure S1

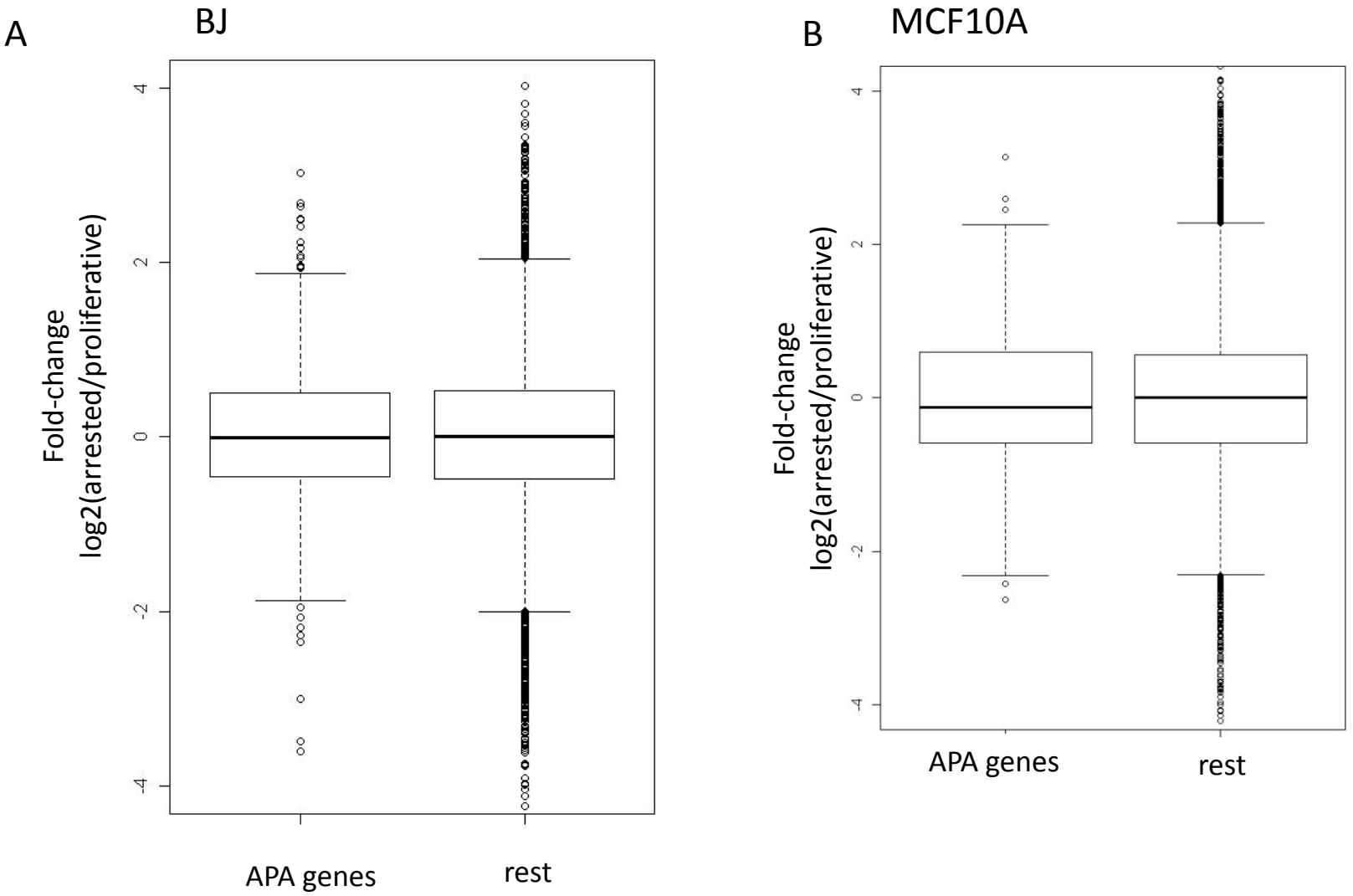
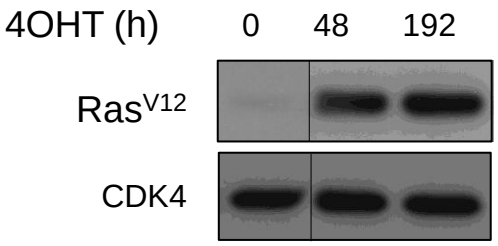

A
BJ
Fold-change
log2(arrested/proliferative)
4
2
0
-2
-4
APA genes
rest
B
MCF10A
Fold-change
log2(arrested/proliferative)
4
2
0
-2
-4
APA genes
rest

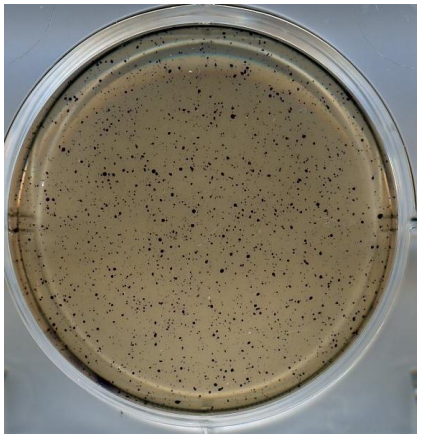
Figure S2

A.



B.

MCF10A
+4-OHT



MCF10A-
RAS^{G12V}ER
+4-OHT

Figure S3

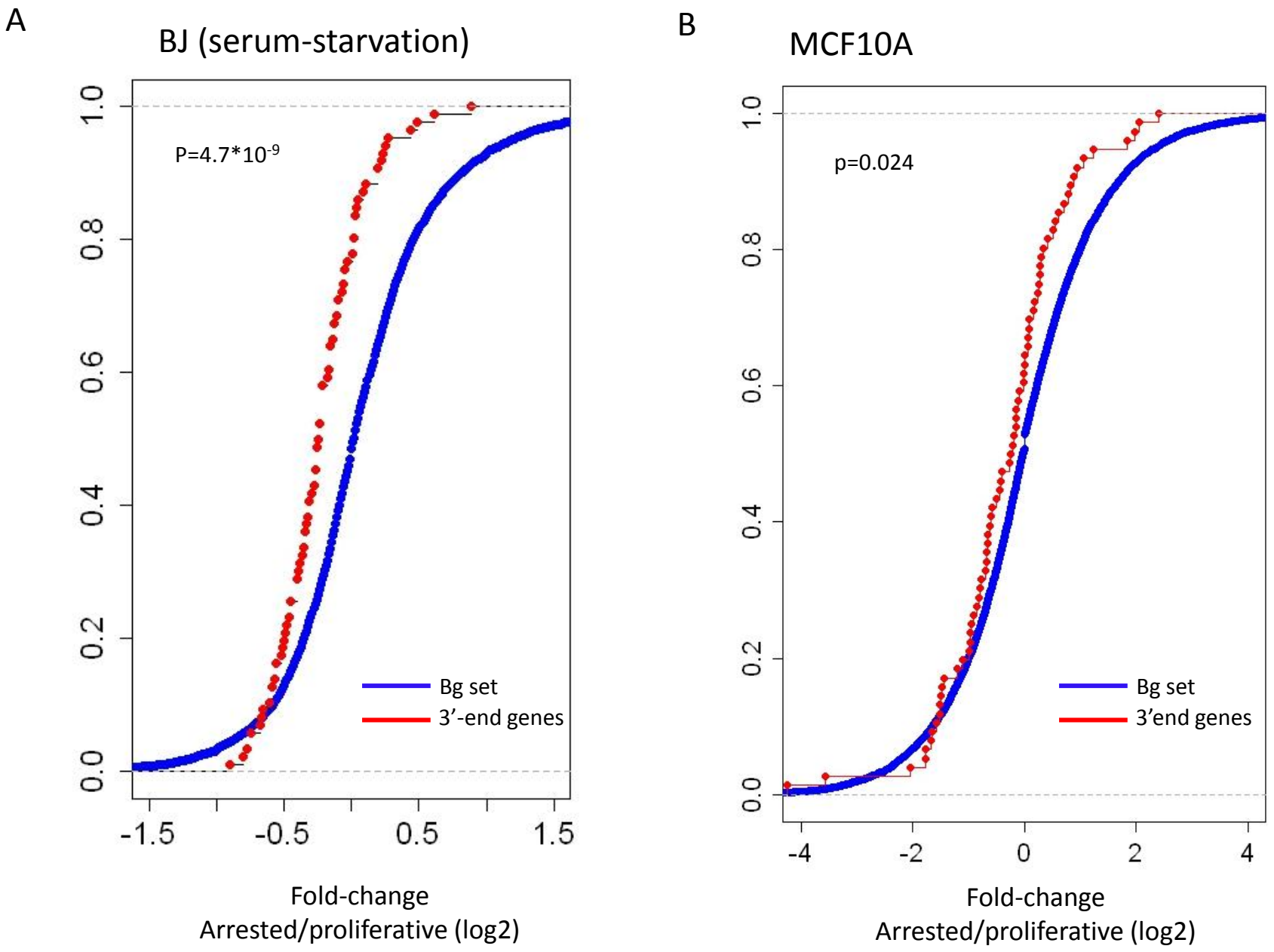

A
BJ (serum-starvation)
P=4.7*10-9
Bg set
3'-end genes
Fold-change
Arrested/proliferative (log2)
B
MCF10A
p=0.024
Bg set
3'end genes
Fold-change
Arrested/proliferative (log2)

Figure S4

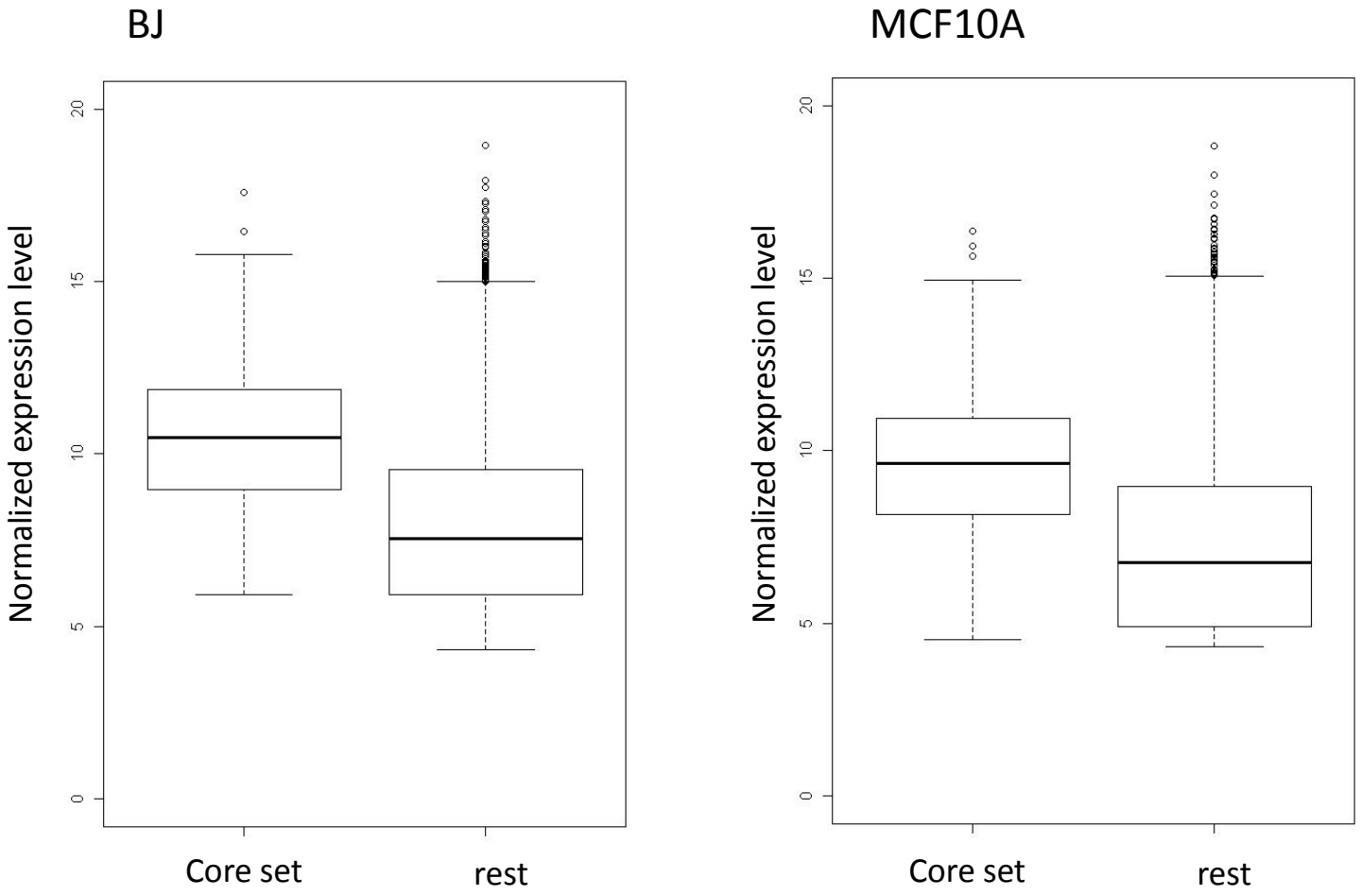

BJ
MCF10A
Normalized expression level
Normalized expression level
0
5
10
15
20
Core set
rest
Core set
rest

Figure S5

Supplemental Figure Legends

Figure S1. A. Schematic description of the 3'-Seq method. **B.** An example for reads which span a poly(A) site and therefore allow its mapping with a nucleotide resolution. The canonical PAS upstream the cleavage site is indicated as well. The transcript's reference sequence is colored in blue below the aligned sequenced reads. **C.** An example for reads which stem from internal priming of the poly-dT primers to A-rich region within a transcript. Such reads were filtered out to avoid false calls of poly(A) sites. **D.** Enriched motifs identified on the set of CSs which mapped to introns, and their location distribution with respect to the mapped sites. **E.** An example for read coverage and poly(A) cleavage site profiles. Two poly(A) CSs were identified for the transcript shown here (FAM100B). **F.** Testing for shifts in usage of 3'UTR poly(A) sites between the examined conditions was done using chi-square test. The example shows the decrease in the usage of FAM100B proximal poly(A) site relative to the usage of the distal one in the transition of BJ cells from proliferative to confluent state.

Figure S2. No significant effect on gene expression levels was detected for the 3'UTR lengthening associated with proliferation arrest in either BJ (left) or MCF10A (right) cells. In both cases, fold-change distribution calculated for the set of transcripts that underwent 3'UTR lengthening did not significantly differ from the distribution calculated for the background set containing all the other transcripts in the dataset.

Figure S3. RAS^{G12V} induction and cellular transformation. A. MCF10A-RAS^{G12V}-ER cells were cultured in the presence or absence of 4-OHT for the indicated time periods, harvested and Immunoblot analysis was performed to detect RAS-ER and the control CDK4. **B.** MCF-10A and MCF10A-RAS^{G12V}-ER cells were plated in soft agar plates containing 4-OHT. Pictures were taken after 2 weeks.

Figure S4. Down-regulation of 3'-end processing genes upon serum starvation in BJ (A) and MCF10A (B) cells. This figure is the same as Figure 6A, but here calculation was done for the transition of BJ and MCF10A cells from proliferative to arrested state, induced by serum depletion. Here, for BJ cells (proliferating and serum-starved), gene expression levels were measured using rna-seq.

Figure S5. The statistical test applied for detection of shifts in poly(A) site usage is inherently less sensitive to changes in lowly expressed transcripts. Accordingly, the detected events were biased towards highly expressed genes.

Table S1. Summary of numbers of sequenced and uniquely mapped reads in the 3'-Seq experiments

	Reads length	Number of reads	Number of mapped reads	Number of uniquely mapped reads
BJ proliferation	35	27,416,302	24,064,349	10,730,927
BJ confluent	35	25,413,161	21,218,021	9,621,471
BJ RAS-transformed	35	26,165,194	22,670,994	11,180,489
BJ proliferation	100	75,157,030	41,705,981	29,582,142
BJ confluent	100	70,515,553	37,238,235	26,762,307
MCF10A proliferation	98	70,248,024	36,492,416	24,247,097
MCF10A serum-starved	98	49,911,326	20,170,825	13,592,273
MCF10A RAS-transformed (0 days)	98	64,782,691	28,640,391	18,560,756
MCF10A RAS-transformed (2 days)	98	55,089,264	25,921,230	16,952,246
MCF10A RAS-transformed (8 days)	98	65,208,661	27,490,250	20,911,698

Table S2

A. BJ cells

Enriched GO categories among genes down-regulated in the transition of BJ cells from proliferation to confluent state.

GO Term	Description	P-value
GO:0007049	cell cycle	3.27E-51
GO:0000279	M phase	3.36E-33
GO:0006260	DNA replication	3.57E-18
GO:0051726	regulation of cell cycle	6.73E-17
GO:0000075	cell cycle checkpoint	3.10E-14
GO:0006281	DNA repair	3.93E-12
GO:0006310	DNA recombination	5.08E-12
GO:0031055	chromatin remodeling at centromere	1.87E-11
GO:0034728	nucleosome organization	1.59E-10

Enriched GO categories among genes up-regulated in the transition of BJ cells from proliferation to confluent state.

GO Term	Description	P-value
GO:0007166	cell surface receptor linked signaling pathway	1.48E-08
GO:0032502	developmental process	2.90E-08
GO:0006695	cholesterol biosynthetic process	1.14E-07
GO:0009653	anatomical structure morphogenesis	1.26E-07
GO:0007155	cell adhesion	5.46E-07

B. MCF10A cells

Enriched GO categories among genes down-regulated in the transition of MCF10A cells from proliferation to serum-starved state.

GO Term	Description	P-value
GO:0007049	cell cycle	1.63E-18
GO:0007067	mitosis	3.86E-14
GO:0006260	DNA replication	5.41E-10
GO:0000075	cell cycle checkpoint	9.42E-08
GO:0009058	biosynthetic process	1.24E-07
GO:0000082	G1/S transition of mitotic cell cycle	2.96E-07
GO:0006364	rRNA processing	5.80E-07

Enriched GO categories among genes up-regulated in the transition of MCF10A cells from proliferation to serum-starved state.

GO Term	Description	P-value
GO:0007155	cell adhesion	3.52E-08
GO:0032502	developmental process	1.70E-07
GO:0033275	actin-myosin filament sliding	2.28E-07
GO:0048856	anatomical structure development	4.34E-07
GO:0006695	cholesterol biosynthetic process	1.08E-06
GO:0009888	tissue development	1.52E-06
GO:0030334	regulation of cell migration	3.33E-06