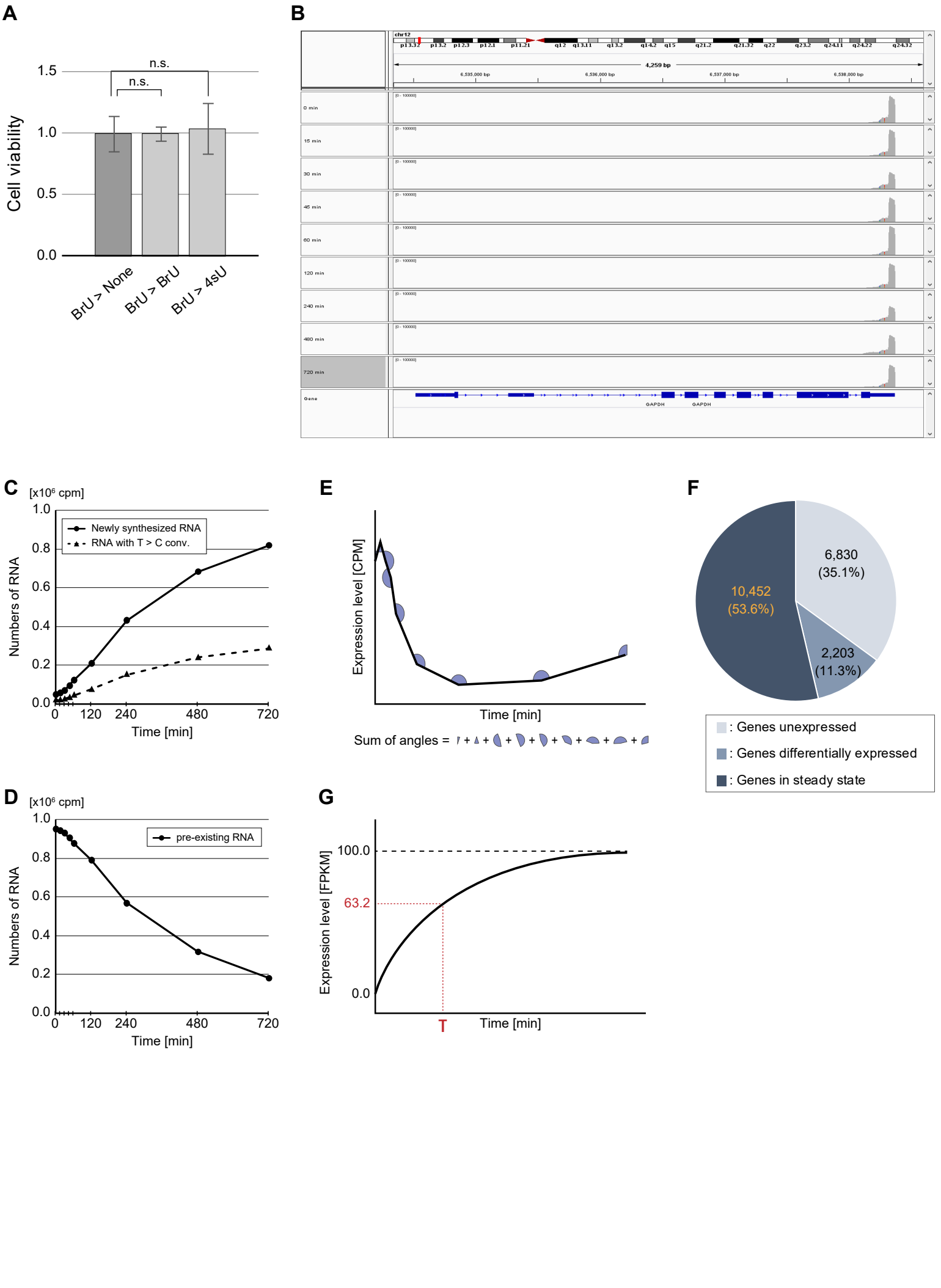
A
n.s.
n.s.
1.5
1.0
0.5
0.0
Cell viability
BrU > None
BrU > BrU
BrU > 4sU
B
chr12
4,259 bp
0 min
15 min
30 min
45 min
60 min
120 min
240 min
480 min
720 min
Gene
GAPDH
GAPDH
C
[x10⁶ cpm]
Newly synthesized RNA
RNA with T > C conv.
Numbers of RNA
Time [min]
D
[x10⁶ cpm]
pre-existing RNA
Numbers of RNA
Time [min]
E
Expression level [CPM]
Time [min]
Sum of angles =
F
6,830
(35.1%)
10,452
(53.6%)
2,203
(11.3%)
: Genes unexpressed
: Genes differentially expressed
: Genes in steady state
G
Expression level [FPKM]
100.0
63.2
0.0
T
Time [min]

Supplemental Figure S1. Examination of labeling conditions with BrU and 4sU

(**A**) The viability of HeLa cells cultured with medium containing uridine analogs was assessed by Cell Counting Kit assays. The cells were cultured with medium containing BrU for 12 h, and then the medium was changed to that containing BrU (BrU > BrU) or 4sU (BrU > 4sU) as well as that without uridine analogs (BrU > None). Results are expressed as the mean ± SD (n = 4). (**B**) A representative example of the alignment of reads sequenced using QuantSeq. The 3′UTRs of IP-RNAs and Alkyl-RNAs were sequenced using QuantSeq, poly(A)-dependent sequencing. (**C**) Estimation of newly synthesized RNAs from sequencing of Alkyl-RNAs. The proportion of newly synthesized RNAs incorporating 4sU was calculated by fitting a time series of RNAs including T > C conversions to a logarithmic curve (see Methods). The amounts of newly synthesized RNAs at individual time points were estimated by divided the RNAs including T > C conversions by the proportion of newly synthesized RNAs incorporating 4sU. Solid and dashed lines indicate the time series of estimated newly synthesized RNAs and RNAs with T > C conversions, respectively. (**D**) Estimation of pre-existing RNAs from the sequencing of IP-RNAs. Since the total amount of intracellular RNAs can be assumed to be constant during culture, the amount of pre-existing RNAs at each time point was estimated by subtracting the amount of estimated newly synthesized RNAs from a constant (1 million). (**E**) Definition of the trend of gene expression. To remove the genes differentially expressed after 4sU labeling, a filter testing constant trends of expression was designed (see Methods). The sum of the angles was defined as the sum of the angles formed by the lines connecting certain time points and neighboring ones, within a time series of total read numbers for each gene calculated from Alkyl-RNAs. (**F**) Distribution of gene expression state. Among the total of 19,485 annotated genes, 35.1%, 11.3%, and 53.6% were identified as those not expressed, differentially expressed, and in a steady state, respectively. (G) Definition of time constant (τ). The τ is defined as the time when the change of expression level reached $1-e^{-1}$ ($\approx 63.2\%$) of the final value.