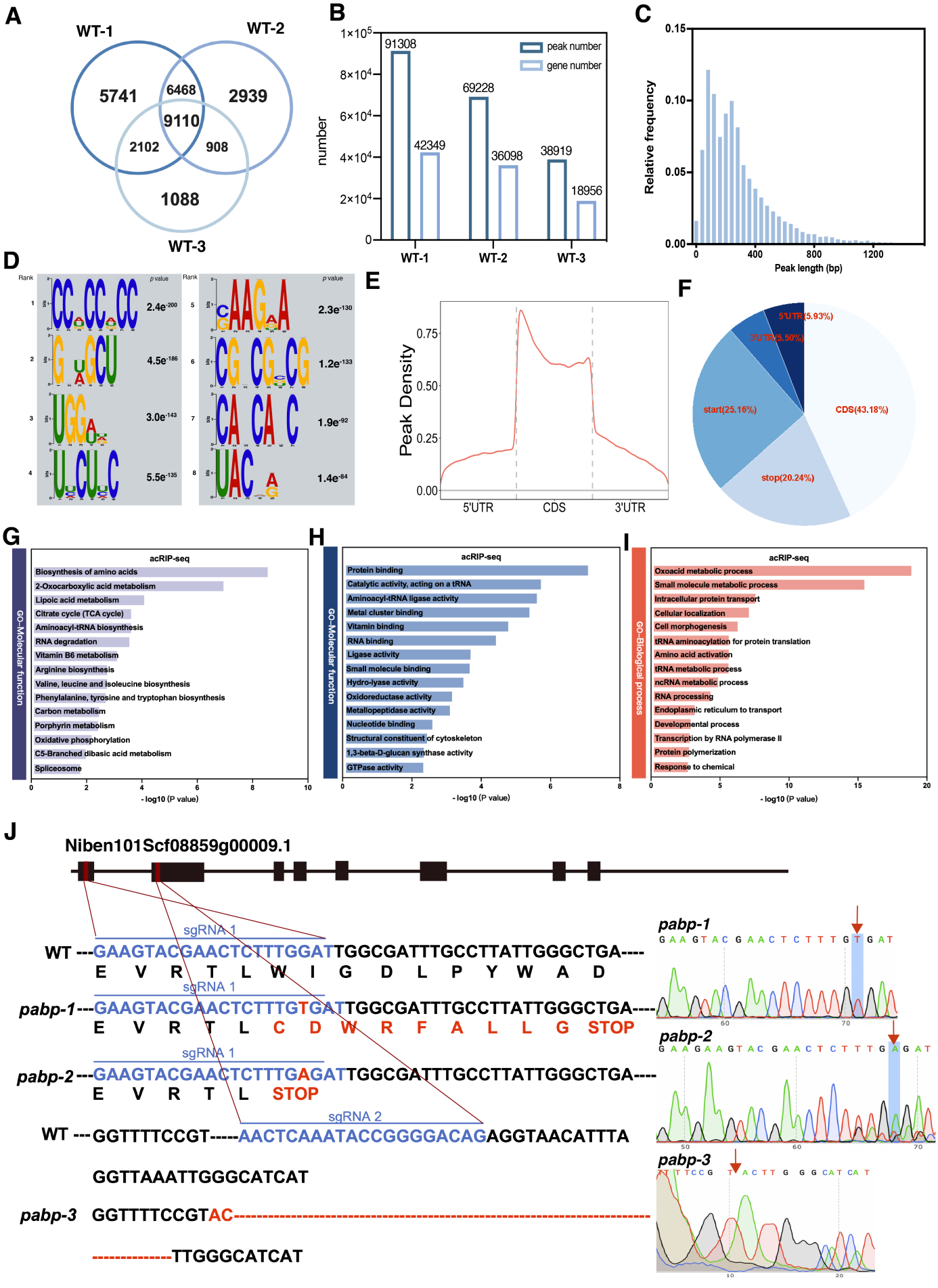
A
WT-1
WT-2
WT-3
5741
6468
2939
9110
2102
908
1088
B
number
peak number
gene number
91308
42349
69228
36098
38919
18956
WT-1
WT-2
WT-3
C
Relative frequency
Peak length (bp)
D
Rank
p value
2.4e-200
4.5e-186
3.0e-143
5.5e-135
2.3e-130
1.2e-133
1.9e-92
1.4e-84
E
Peak Density
5'UTR
CDS
3'UTR
F
5'UTR(5.93%)
3'UTR(5.50%)
start(25.16%)
CDS(43.18%)
stop(20.24%)
G
acRIP-seq
go-Molecular function
Biosynthesis of amino acids
2-Oxocarboxylic acid metabolism
Lipoic acid metabolism
Citrate cycle (TCA cycle)
Aminoacyl-tRNA biosynthesis
RNA degradation
Vitamin B6 metabolism
Arginine biosynthesis
Valine, leucine and isoleucine biosynthesis
Phenylalanine, tyrosine and tryptophan biosynthesis
Carbon metabolism
Porphyrin metabolism
Oxidative phosphorylation
C5-Branched dibasic acid metabolism
Spliceosome
- log10 (P value)
H
acRIP-seq
go-Molecular function
Protein binding
Catalytic activity, acting on a tRNA
Aminoacyl-tRNA ligase activity
Metal cluster binding
Vitamin binding
RNA binding
Ligase activity
Small molecule binding
Hydro-lyase activity
Oxidoreductase activity
Metallopeptidase activity
Nucleotide binding
Structural constituent of cytoskeleton
1,3-beta-D-glucan synthase activity
GTPase activity
- log10 (P value)
I
acRIP-seq
go-Biological process
Oxoacid metabolic process
Small molecule metabolic process
Intracellular protein transport
Cellular localization
Cell morphogenesis
tRNA aminoacylation for protein translation
Amino acid activation
tRNA metabolic process
ncRNA metabolic process
RNA processing
Endoplasmic reticulum to transport
Developmental process
Transcription by RNA polymerase II
Protein polymerization
Response to chemical
- log10 (P value)
J
Niben101Scf08859g00009.1
sgRNA 1
WT ---GAAGTACGAACTCTTTGGATTGGCGATTTGCCTTATTGGGCTGA----
E V R T L W I G D L P Y W A D
sgRNA 1
pabp-1---GAAGTACGAACTCTTTGTGATTGGCGATTTGCCTTATTGGGCTGA----
E V R T L C D W R F A L L G STOP
sgRNA 1
pabp-2---GAAGTACGAACTCTTTGAGATTGGCGATTTGCCTTATTGGGCTGA----
E V R T L STOP
sgRNA 2
WT ---GGTTTTCCGT-----AACTCAAATACCGGGGACAGAGGTAACATTTA
GGTTAAATTGGGCATCAT
pabp-3 GGTTTTCCGTAC----------
----------TTGGGCATCAT
pabp-1
pabp-2
pabp-3

Extended Data Fig. 6 Analysis of ac^{4}C targets in *N. benthamiana*.

a. Determine of the extent of overlap between ac^{4}C-modified genes across three biological replicates of *N. benthamiana* transcripts.

b. The barplot is presented of the number of ac^{4}C peaks and associated genes across the three biological replicates of *N. benthamiana* transcripts.

c. The barplot is presented of the length distribution of ac^{4}C peaks in associated genes.

d. Top 8 motifs identified from ac^{4}C-modified transcripts by acRIP-seq.

e. The metaplot is presented of the distribution of ac^{4}C-modified transcripts. The relative position of UTRs and CDS is shown at the bottom.

f. Proportional distribution of ac^{4}C-modified transcripts across different functional regions of genes.

g. KEGG pathway analysis of ac^{4}C-modified transcripts.

h. GO enrichment analysis of biological processes (BP) associated with ac^{4}C-modified transcripts.

i. GO enrichment analysis of molecular functions (MF) associated with ac^{4}C-modified transcripts.

j. The positions of the two sgRNA target sites (T1 and T2) for CRISPR/Cas9 editing *PABP* (Niben101Scf08859g00009.1) (upper panel). Black rectangles indicate exons, red rectangles indicate sgRNA target sites. DNA sequences of three independent *N. benthamiana PABP* mutant lines (left panel). In the *pabp-1* mutant, an additional thymine was inserted at the sgRNA-1 target site, resulting in a frameshift mutation and the generation of a premature stop codon. The *pabp-2* mutant has an additional adenine was inserted at the sgRNA-1 target site, resulting in a frameshift mutation and the generation of a premature stop codon. The *nbnat10-3* mutant exhibits a large deletion at the sgRNA-2 site. Sanger sequencing chromatograms of CRISPR/Cas9-targeted DNA regions in *pabp* knockout lines (*pabp-1, pabp-2, pabp-3*). The red arrows indicate the mutation sites (right panel).