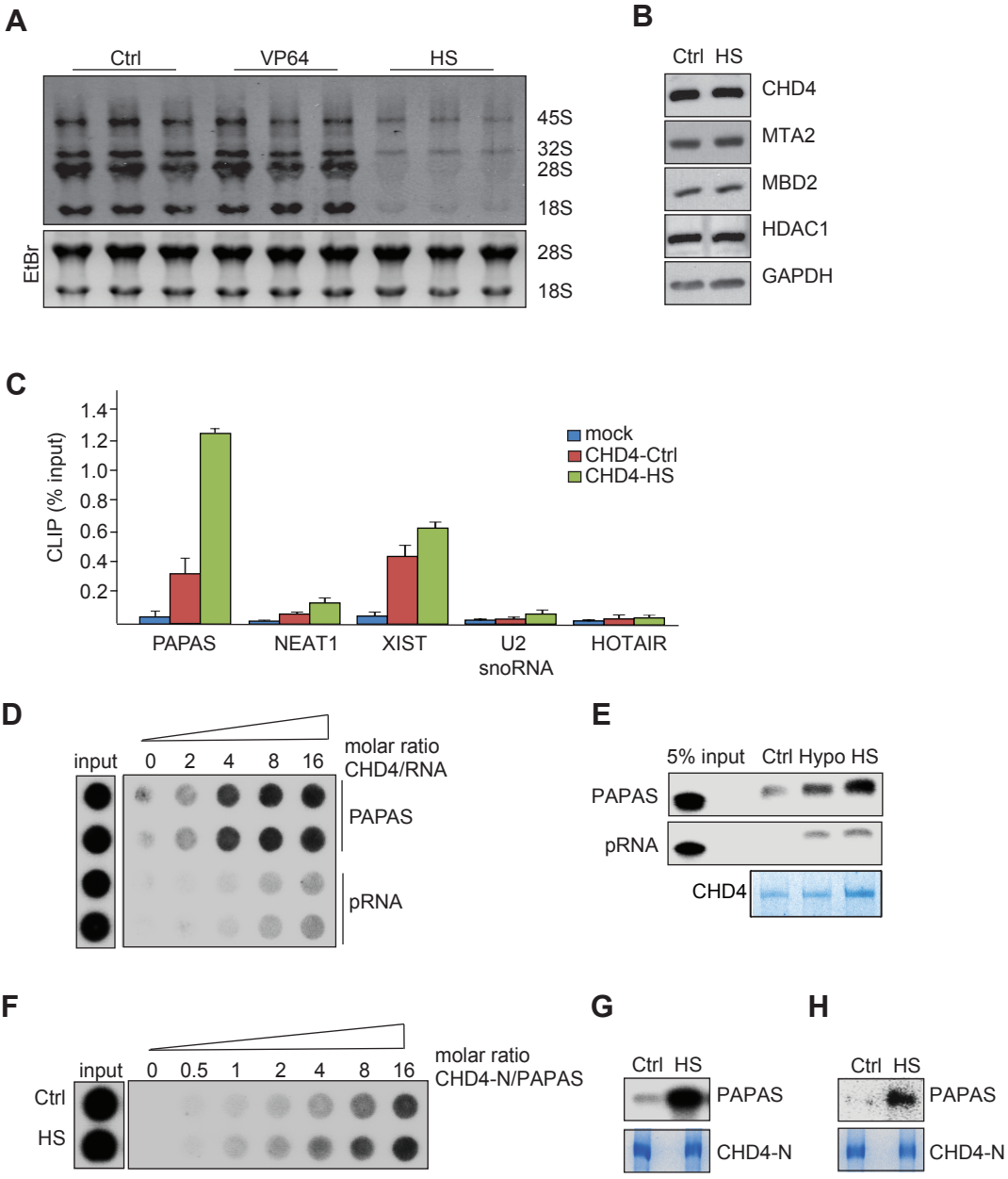
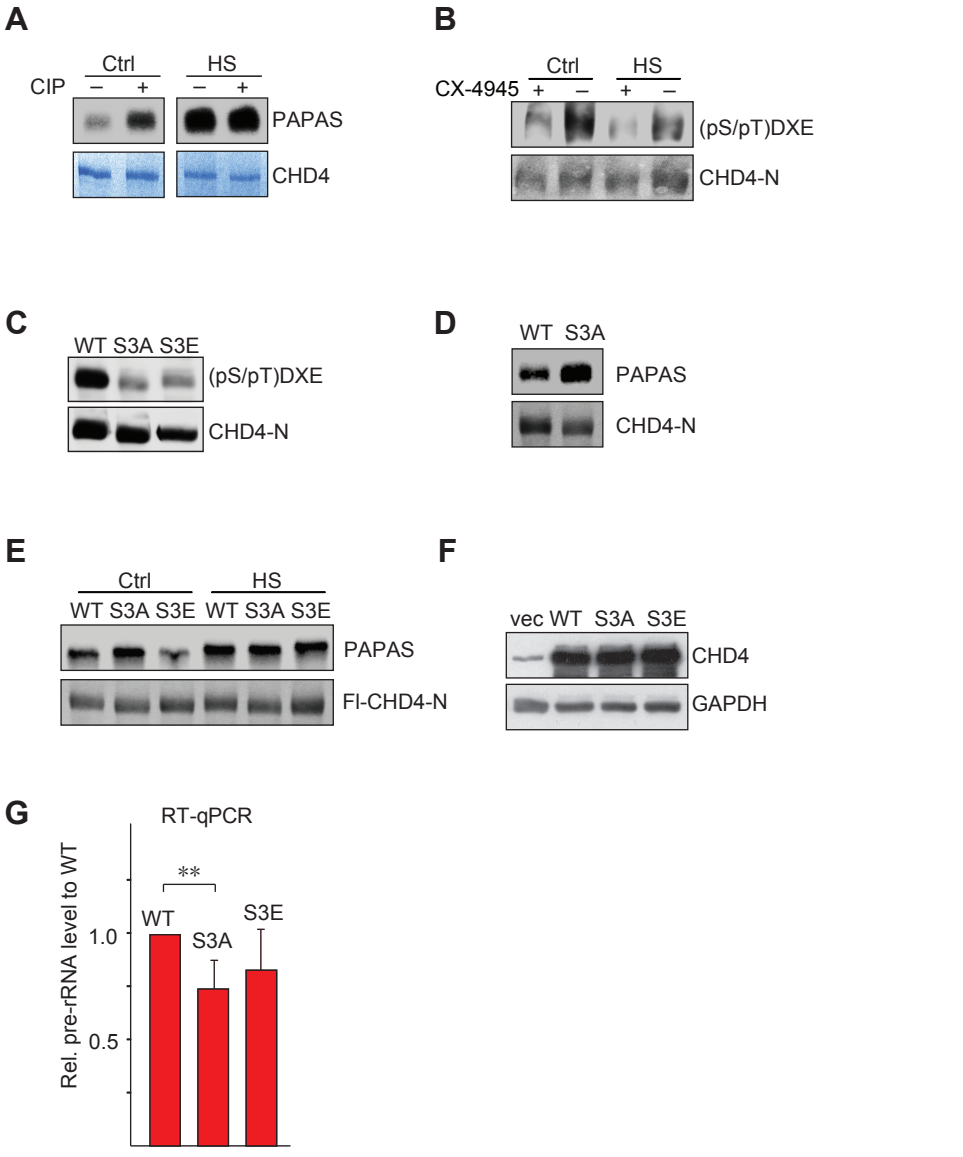
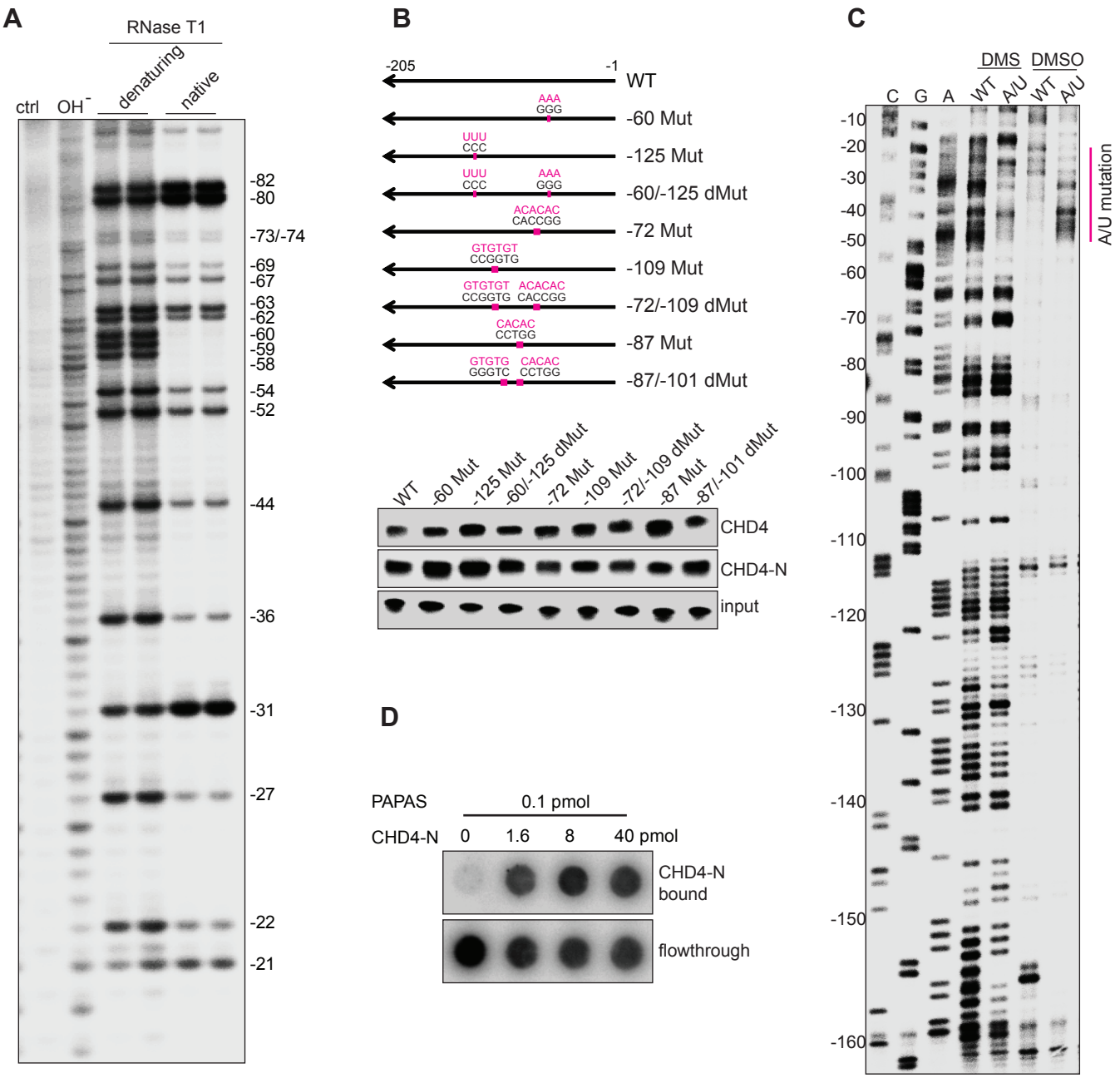

A
Ctrl
VP64
HS
45S
32S
28S
18S
EtBr
28S
18S
B
Ctrl HS
CHD4
MTA2
MBD2
HDAC1
GAPDH
C
CLIP (% input)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
mock
CHD4-Ctrl
CHD4-HS
PAPAS
NEAT1
XIST
U2 snoRNA
HOTAIR
D
input
0 2 4 8 16
molar ratio CHD4/RNA
PAPAS
pRNA
E
5% input
Ctrl Hypo HS
PAPAS
pRNA
CHD4
F
input
0 0.5 1 2 4 8 16
molar ratio CHD4-N/PAPAS
Ctrl
HS
G
Ctrl HS
PAPAS
CHD4-N
H
Ctrl HS
PAPAS
CHD4-N

Zhao_Fig.S2

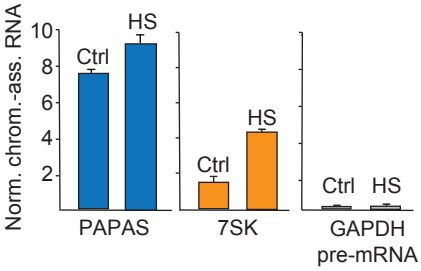

A
Ctrl
HS
CIP
– + – +
PAPAS
CHD4
B
Ctrl
HS
CX-4945 + – + –
(pS/pT)DXE
CHD4-N
C
WT S3A S3E
(pS/pT)DXE
CHD4-N
D
WT S3A
PAPAS
CHD4-N
E
Ctrl
HS
WT S3A S3E WT S3A S3E
PAPAS
Fl-CHD4-N
F
vec WT S3A S3E
CHD4
GAPDH
G
RT-qPCR
**
WT
S3A
S3E
1.0
0.5
Rel. pre-rRNA level to WT

Zhao_Fig.S3


A
RNase T1
denaturing
native
ctrl
OH-
-82
-80
-73/-74
-69
-67
-63
-62
-60
-59
-58
-54
-52
-44
-36
-31
-27
-22
-21
B
-205
-1
WT
AAA
GGG
-60 Mut
UUU
CCC
-125 Mut
-60/-125 dMut
ACACAC
CACCGG
-72 Mut
GTGTGT
CCGGTG
-109 Mut
-72/-109 dMut
CACAC
CCTGG
-87 Mut
GTGTG
GGGTC
CCTGG
-87/-101 dMut
WT
-60 Mut
-125 Mut
-60/-125 dMut
-72 Mut
-109 Mut
-72/-109 dMut
-87 Mut
-87/-101 dMut
CHD4
CHD4-N
input
C
DMS
DMSO
C
G
A
WT
A/U
WT
A/U
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
A/U mutation
D
PAPAS
0.1 pmol
CHD4-N
0
1.6
8
40 pmol
CHD4-N
bound
flowthrough

Zhao_Fig.S4

A


Norm. chrom.-ass. RNA
10
8
6
4
2
Ctrl
HS
Ctrl
HS
Ctrl
HS
PAPAS
7SK
GAPDH
pre-mRNA

Supplemental Figures

Supplemental Fig. S1.

(A) Pre-rRNA synthesis in metabolically labeled control, heat-shocked and and dCas9-VP64 transfected cells. NIH3T3 cells were labeled for 2 h with ^{3}H-uridine and pre-rRNA was visualized by gel electrophoresis and fluorography.

(B) Western blot showing the levels of CHD4, MTA2, MBD2, HDAC1 and GAPDH in untreated or heat-shocked cells.

(C) CLIP assay monitoring the interaction of Fl-CHD4 with different control RNAs in untreated or heat-shocked HEK293 cells. Data were normalized to input (n=3).

(D) Filter binding assay monitoring the interaction of 100 pmoles of radiolabeled PAPAS or pRNA with serial dilutions of Fl-CHD4 purified from heat-shocked HEK293T cells. Membrane-bound CHD4/PAPAS was monitored by PhosphorImaging.

(E) Pull-down assay showing the interaction of radiolabeled PAPAS or pRNA with Fl-CHD4 purified from untreated, hypotonic stress and heat-shocked cells. The Coomassie brilliant blue stain shows the amounts of bead-bound Fl-CHD4.

(F) Filter binding assay monitoring the interaction of 100 pmoles of radiolabeled PAPAS with serial dilutions of Fl-CHD4-N purified from untreated or heat-shocked cells. Membrane-bound CHD4/PAPAS complexes were monitored by PhosphorImaging.

(G) Pull-down assay showing the interaction of radiolabeled PAPAS with Fl-CHD4-N purified from untreated or heat-shocked cells.

(H) Northwestern blot showing binding of radiolabeled PAPAS (-1/-205) to Fl-CHD4-N purified from untreated or heat-shocked cells. Bound RNA was detected by PhosphorImaging.

Supplemental Fig. S2.

(A) Pull-down assay showing the interaction of radiolabeled PAPAS with CIP-treated Fl-CHD4 purified from untreated or heat-shocked cells.

(B) Western blot with an anti-phospho-CK2 antibody showing decreased phosphorylation of CHD4-N in heat-shocked HEK293T cells exposed to CX-4945 (5 μM, 24 h).

(C) Western blot with anti-phospho-CK2 substrate antibody showing reduced phosphorylation of Fl-CHD4-N/S3A and Fl-CHD4-N/S2E.

(D) Northwestern blot showing PAPAS binding to Fl-CHD4-N/WT and Fl-CHD4/S3A. The Ponceau stain below shows the amount of bound Fl-CHD4-N and Fl-CHD4-C.

(E) Pull-down experiment showing binding of PAPAS to Fl-CHD4-N/WT, Fl-CHD4/S3A and Fl-CHD4/S3E isolated from untreated or heat-shocked cells.

(F) Western blot showing the levels of overexpressed wildtype and mutant CHD4.

(G) RT-qPCR of pre-rRNA in HEK293T cells expressing Fl-CHD4-N/WT, Fl-CHD4-N/S3A and Fl-CHD4-N/S3E. Data are normalized to WT (n=3).

Bars represent mean values ± SD.

Supplemental Fig. S3.

(A) RNase T1 digestion profile of native and denatured PAPAS (-1/-205)

(B) Pull-down assay monitoring binding of Fl-CHD4 or Fl-CHD4-N to radiolabeled wildtype and mutant PAPAS (**Supplemental Table S2**). Mutated sequences are marked in red.

(C) DMS/SHAPE-RT probing comparing the secondary structure of PAPAS-WT and PAPAS-A/U.

(D) Filter binding assay monitoring binding of radiolabeled PAPAS (-1/-205) to Fl-CHD4-N.

Supplemental Fig. S4.

(A) RT-qPCR comparing the levels of chromatin-bound PAPAS (-36/-135), 7SK and GAPDH pre-mRNA from control (Ctrl) and heat-shocked (HS) NIH3T3 cells. Values are normalized to the respective cellular RNA values (n=3).

Data are presented as mean ± SEM.