RNA molecules control repair of human DNA in cancer cells

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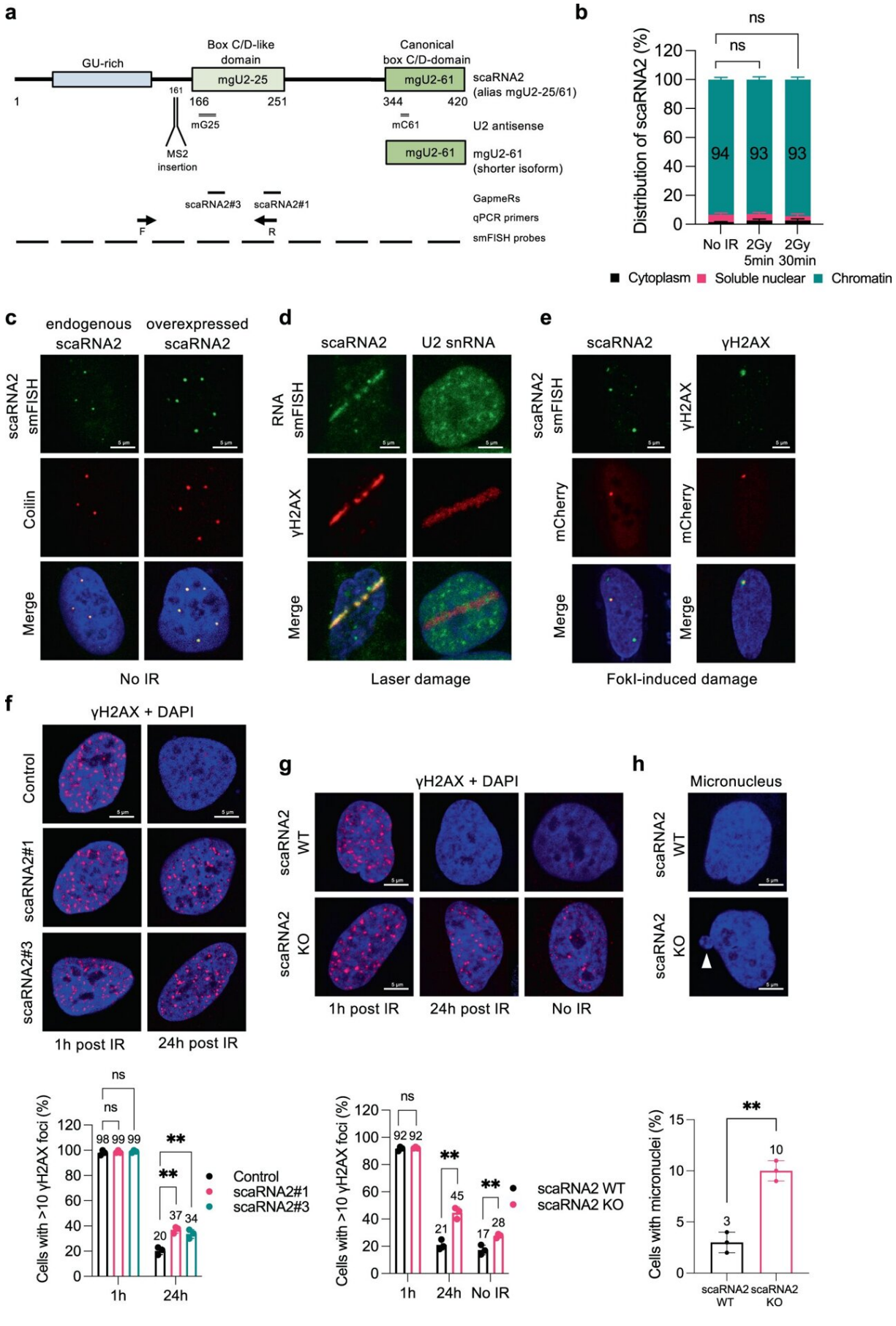
a
GU-rich
Box C/D-like domain
Canonical box C/D-domain
mgU2-25
mgU2-61
scaRNA2 (alias mgU2-25/61)
1
161
166
251
344
420
MS2 insertion
mG25
mC61
U2 antisense
mgU2-61
mgU2-61 (shorter isoform)
scaRNA2#3
scaRNA2#1
GapmeRs
F
R
qPCR primers
smFISH probes
b
ns
ns
Distribution of scaRNA2 (%)
120
100
80
60
40
20
0
94
93
93
No IR
2Gy 5min
2Gy 30min
Cytoplasm
Soluble nuclear
Chromatin
c
endogenous scaRNA2
overexpressed scaRNA2
scaRNA2 smFISH
Coilin
Merge
No IR
d
scaRNA2
U2 snRNA
RNA smFISH
γH2AX
Merge
Laser damage
e
scaRNA2
γH2AX
scaRNA2 smFISH
mCherry
Merge
FokI-induced damage
f
γH2AX + DAPI
Control
scaRNA2#1
scaRNA2#3
1h post IR
24h post IR
g
γH2AX + DAPI
scaRNA2 WT
scaRNA2 KO
1h post IR
24h post IR
No IR
h
Micronucleus
scaRNA2 WT
scaRNA2 KO
ns
ns
**
**
Cells with >10 γH2AX foci (%)
120
100
80
60
40
20
0
98 99 99
20
37
34
1h
24h
Control
scaRNA2#1
scaRNA2#3
ns
ns
**
**
**
92 92
21
45
17
28
1h
24h
No IR
scaRNA2 WT
scaRNA2 KO
**
Cells with micronuclei (%)
15
10
5
0
3
10
scaRNA2 WT
scaRNA2 KO

Fig. 1: scaRNA2 localizes at DNA lesions and loss of this RNA leads to accumulation of DNA double-strand breaks and genomic instability. a Schematic structure of scaRNA2, illustrating the GU-rich region, C/D domains and U2 snRNA antisense regions. Binding sites for smFISH probes, qPCR primers, GapmeRs and the MS2 loop insertion site are also shown. b Sub-cellular distribution of scaRNA2 in untreated or irradiated U2OS cells as determined by qPCR. The values shown are means ± SD, n = 3. Unless otherwise indicated, all n = 3 refer to three biologically independent experiments. Ns (not significant) as determined by one-way ANOVA and two-sided Dunnett's multiple comparisons test. c smRNA FISH of scaRNA2 and immunostaining of the Cajal body marker coilin in U2OS cells expressing scaRNA2 endogenously or overexpressed for 24 h (n = 3). Nuclei were stained with DAPI in all immunofluorescence experiments. d smRNA FISH of scaRNA2 and immunostaining of the DNA damage marker γH2AX in laser micro-irradiated (5 min recovery) U2OS cells overexpressing scaRNA2 for 24 h (n = 3). smRNA FISH for U2 snRNA was performed under the same conditions but without overexpression of scaRNA2. e smFISH of scaRNA2 in U2OS FokI cells transfected with a scaRNA2 plasmid for 24 h and treated with Shield and 4-OHT for an additional 4 h (n = 3). Immunostaining of γH2AX was performed under the same conditions but without overexpression of scaRNA2. f Immunostaining of γH2AX in irradiated (2 Gy, 1 or 24 h recovery) U2OS cells depleted or not of scaRNA2 for 48 h. The graph below shows the percentage of 100–200 cells (means ± SD, n = 3) whose nuclei contained > 10 γH2AX foci, **p