

## **RNA** molecules control repair of human DNA in cancer cells

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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  $\pm$  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 yH2AX 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 yH2AX was performed under the same conditions but without overexpression of scaRNA2. f Immunostaining of yH2AX 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  $\pm$  SD, n = 3) whose nuclei contained > 10 yH2AX foci, \*\*p

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