SUPPLEMENTAL MATERIAL

Micro(mi) RNA-34a targets protein phosphatase (PP)1γ to regulate DNA damage tolerance

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Supplemental figure legends.

Supplemental Table 1. Positions of miR-34a seed match sequences in the 3’UTR of PP1γ.

Figure S1. (A) Lysates of cells treated with PP1γ siRNA were used to validate the specificity of the anti-PP1γ antibody by western blot in comparison to Luc siRNA transfected controls. Ponceau staining was used as a loading control. (B) qRT-PCR of PP1γ and CDK6 mRNA expression. cDNA from CAL51 cells 48 h after exposure to 50 nM miR-34a mimic, or transfection with Luc siRNA was used as a substrate for the RT-PCR reactions. The plotted values show the mean ±SEM (one-way ANOVA with Dunett’s post-test; ***P<0.001) (n=2). (C) Representative qRT-PCR of miR-34a in cDNA prepared from CAL51 cells transfected with 50 nM or 75 nM miR-34a mimic. Luc siRNA was transfected as a negative control, as well as to equalize the molarity of miR-34a mimic transfection. Two different timepoints: 48 h and 72 h were studied (one-way ANOVA with Dunett’s post-test; ***P<0.001) (n=1; ±SD)

Figure S2. Damage-induced miR-34a expression is dependent on p53. (A) CAL51 cells were transfected with 30nM p53 deconvoluted siRNA (Qiagen), before extract preparation and Western blotting for p53 expression at the indicated times. (B) Experimental scheme: CAL51 cells were plated 24 h prior to siRNA transfection. The cells were transfected with 30nM p53 deconvoluted siRNA (Qiagen), irradiated at 3 Gy and harvested 72 h post irradiation. (C) qPCR analysis of miR-34a expression after exposure to 3 Gy IR, following treatment with control or p53 siRNA. p<0.01(“*) by one-way ANOVA with Bonferroni’s post-test. n=3, ± SEM. (D) p53 activation by Ser15 phosphorylation was determined 4h and 72h after IR in cells treated with control or p53 siRNA in the same experiment shown in panel (C).
Supplemental materials and methods.

Details of siRNA and miRNA mimic/inhibitor:

| siRNA/miRNA                  | Detail         | Manufacturer   |
|------------------------------|----------------|----------------|
| negative control siRNA       | Luciferase siRNA | Eurofins MWG Operon |
| negative control miRNA       | cel-miR-67     | Dharmacon      |
| miR-34a mimic                | hsa-miR-34a mimic | Dharmacon     |
| miR-34a inhibitor            | hsa-miR-34a inhibitor | Dharmacon |
| SMARTpool: ON-TARGET plus PP1γ | PP1γ pooled siRNA | Dharmacon |
| Set of 4: ON-TARGET plus TP63 siRNA | p63 deconvoluted siRNA | Dharmacon |
| Set of 4: ON-TARGET plus TP73 siRNA | p73 deconvoluted siRNA | Dharmacon |
| Hs_TP53_7 FlexiTube          | p53 deconvoluted siRNA | Qiagen |
| Hs_TP53_9 FlexiTube          | p53 deconvoluted siRNA | Qiagen |
| FlexiTube GeneSolution GS8626 for TP63 | p63 deconvoluted siRNA | Qiagen |
| FlexiTube GeneSolution GS7161 for TP73 | p73 deconvoluted siRNA | Qiagen |

Details of primers used for qRT-PCR experiments:

| Gene       | Primer direction | Sequence (5'->3') |
|------------|------------------|------------------|
| CDK6       | F/R              | TGCACAGTGTCTACGAGAAGAAGACAGA |
|            |                  | ACCTCGGAGAGCTGAACAGA |
| PPP1CC (PP1γ) | F/R          | TGGGTGGGAAAGGAGGTGTGA |
|            |                  | GACCCTCTCTACTCCAGAGCGCG |
| U6 snRNA   | F/R              | CGGCTGCGCACGACATATAAC |
|            |                  | TTCACGAAATTGCCTGTCAT |
| GAPDH      | F/R              | AGCCACATCGCTCAGCACAC |
|            |                  | GCCCAATACGACACATAC |
| miR-34a    | F/R              | TGGCAGTGTCTTAGCTGGTTGT |
| Universal reverse | F/R       | CGGAGCACAGAATTAATACGACTCAC |
| Gene   | Position of miRNA seed match sequence in 3'UTR | miR-34a and target seed match |
|--------|-----------------------------------------------|------------------------------|
| PPP1CC | 13-19                                        | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | PPP1CC 5'...NNAUUGCUUUGACACUGCCU... 3' |
| ANK3   | 34-40                                        | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        | 545-551                                      | ANK3 5'...GGAUCAUAAAGUUUUUGACUGCC... 3' |
|        | 1984-1991                                    | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | ANK3 5'...GGGCUACAAGGUACACUGCC... 3' |
| MYRIP  | 14-21                                        | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        | 28-34                                        | MYRIP 5'...NCAACAUUGGAUUCGACUGCC... 3' |
|        |                                               | MYRIP 5'...ACUGCCAGUGACCACUGCC... 3' |
| PKP4   | 694-701                                      | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | PKP4 5'...UCAUGGACACUUCAUCUGCCA... 3' |
| RALGPS2| 203-209                                      | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        | 249-255                                      | RALGPS2 5'...GGAUGUCAAACAAACUGCCA... 3' |
|        |                                               | RALGPS2 5'...UGACUCUGUGAGAACCCAUCUGCC... 3' |
| STRN3  | 57-64                                        | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | STRN3 5'...AAGAGGGUCUGCAUCACUGCCA... 3' |
| ARID4B | 272-278                                      | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | ARID4B 5'...AUGAGGCAUUUUCACUGCCA... 3' |
| JMJD1C | 482-488                                      | hsa-miR-34a 3' UGUUGGCAGAUUCUGUGACGGU 5' |
|        |                                               | JMJD1C 5'...CAUAUCAGAAAUUACUGCCA... 3' |
Supplemental Figure 1
