# SUPPLEMENTARY MATERIAL

# p53 shapes genome-wide and cell type-specific changes in microRNA expression during the human DNA damage response

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Supplementary Tables 1 and 2

Supplementary Figure legends

Supplementary Figures S1-S4

**Supplementary Table 1, related to Figure 6. miRNA clustering and DDR-induced changes in expression.** ‘DDR’ signifies those miRNAs whose expression is significantly altered at 4 or 24 h after DNA damage using the statistical criteria noted in Methods, and ‘non-DDR’, miRNAs that do not exhibit such alterations.

| | DDR | non-DDR | total |
|---|---|---|---|
| clustered miRNAs | 53 | 92 | 145 |
| single miRNAs | 264 | 1122 | 1386 |
| total | 317 | 1214 | 1531 |

**Supplementary Table 2, related to Figure 5. Correlations between DDR miRNA expression and patient outcome in different cancer types.**

| Cluster | MicroRNA | Type of Cancer | Expression in poor outcome* | P value ** | GEO ID | Array ID |
|---|---|---|---|---|---|---|
| A | HSA-LET-7A* | prostate cancer | High | 0.000099 | GSE21036 | HSA-LET-7A* |
| | HSA-LET-7A* | breast cancer | Low | 0.02295 | GSE37405 | 17888 |
| | HSA-MIR-1 | prostate cancer | Low | 0.0000181 | GSE21036 | HSA-MIR-1 |
| | HSA-MIR-1 | breast cancer | Low | 0.0000505 | GSE37405 | 42573 |
| | HSA-MIR-33A | breast cancer | Low | 0.00058 | GSE37405 | 42716 |
| | HSA-MIR-33A | breast cancer | High | 0.0056 | GSE22216 | ILMN_3167691 |
| | HSA-MIR-33B | prostate cancer | High | 0.000002472 | GSE21036 | HSA-MIR-33B |
| | HSA-MIR-33B | breast cancer | High | 0.02439 | GSE22216 | ILMN_3166988 |
| | HSA-MIR-33B | breast cancer | Low | 0.047 | GSE37405 | 42715 |
| | HSA-MIR-34A | breast cancer | Low | 0.000899 | GSE37405 | 27217 |
| | HSA-MIR-34A | ovarian carcinoma | High | 0.049 | GSE27290 | 181 |
| | HSA-MIR-34A | hepatocellular carcinoma | High | 0.049 | GSE10694 | 135 |
| | HSA-MIR-99B | breast cancer | Low | 0.0007208 | GSE37405 | 11184 |
| | HSA-MIR-125A-5P | breast cancer | Low | 0.02208 | GSE37405 | 10928 |
| | HSA-MIR-139-3P | breast cancer | Low | 0.00378 | GSE37405 | 42451 |
| | HSA-MIR-143 | prostate cancer | Low | 0.0005368 | GSE21036 | HSA-MIR-143 |
| | HSA-MIR-143 | hepatocellular carcinoma | High | 0.00108 | GSE10694 | 187 |
| | HSA-MIR-143 | breast cancer | Low | 0.013226667 | GSE37405 | 13177 |
| | HSA-MIR-148B | breast cancer | Low | 0.001024 | GSE37405 | 19585 |
| | HSA-MIR-184 | breast cancer | Low | 0.0002758 | GSE37405 | 10978 |
| | HSA-MIR-190 | breast cancer | Low | 0.04116 | GSE37405 | 27536 |
| | HSA-MIR-191 | lung cancer | High | 0.03416 | GSE16025 | BM10968 |
| | HSA-MIR-199B-5P | prostate cancer | Low | 0.00000912 | GSE21036 | HSA-MIR-199B-5P |
| | HSA-MIR-199B-5P | breast cancer | Low | 0.0384 | GSE37405 | 19591 |
| | HSA-MIR-215 | breast cancer | Low | 0.0003924 | GSE37405 | 11015 |
| | HSA-MIR-365 | breast cancer | Low | 0.0001 | GSE37405 | 11078 |
| | HSA-MIR-375 | breast cancer | Low | 0.02504 | GSE37405 | 42498 |
| | HSA-MIR-423-5P | breast cancer | Low | 0.000051 | GSE37405 | 27565 |
| | HSA-MIR-423-5P | prostate cancer | High | 0.006075 | GSE21036 | HSA-MIR-423-5P |
| | HSA-MIR-486-5P | breast cancer | Low | 0.00972 | GSE37405 | 32946 |
| | HSA-MIR-486-5P | prostate cancer | High | 0.0142 | GSE21036 | HSA-MIR-486-5P |
| | HSA-MIR-642 | breast cancer | Low | 0.000508 | GSE37405 | 42679 |
| | HSA-MIR-642 | breast cancer | Low | 0.00382 | GSE22216 | ILMN_3168055 |
| | HSA-MIR-671-3P | breast cancer | Low | 0.00975 | GSE37405 | 42525 |
| | HSA-MIR-942 | breast cancer | Low | 0.0145 | GSE37405 | 42608 |
| | HSA-MIR-1224-5P | nasopharyngeal carcinoma | Low | 0.01018 | GSE36682 | 946 |
| | HSA-MIR-1304 | nasopharyngeal carcinoma | Low | 0.00192 | GSE36682 | 458 |
| B | HSA-MIR-92A | prostate cancer | High | 0.01002 | GSE21036 | HSA-MIR-92A |
| | HSA-MIR-92A | breast cancer | Low | 0.0342 | GSE22216 | ILMN_3166986 |
| C | HSA-LET-7B* | breast cancer | Low | 0.01005 | GSE37405 | 42769 |
| | HSA-MIR-99A | breast cancer | Low | 0.034 | GSE22216 | ILMN_3168213 |
| D | HSA-MIR-125B-1* | nasopharyngeal carcinoma | Low | 0.0429 | GSE36682 | 16 |
| | HSA-MIR-125B-1* | breast cancer | Low | 0.000442 | GSE37405 | 17876 |
| | HSA-MIR-335 | nasopharyngeal carcinoma | High | 0.046 | GSE36682 | 68 |
| | HSA-MIR-484 | breast cancer | Low | 0.00029715 | GSE37405 | 42698 |
| | HSA-MIR-484 | prostate cancer | High | 0.00004109 | GSE21036 | HSA-MIR-484 |
| | HSA-MIR-550 | nasopharyngeal carcinoma | Low | 0.01494 | GSE36682 | 370 |
| | HSA-MIR-550 | breast cancer | Low | 0.00000048 | GSE37405 | 42914 |
| | HSA-MIR-1257 | nasopharyngeal carcinoma | Low | 0.0114 | GSE36682 | 953 |
| | HSA-MIR-1293 | nasopharyngeal carcinoma | Low | 0.0329 | GSE36682 | 711 |

*Expression levels in tumour specimen from patients with poor outcome.

**All the listed miRNAs have $p < 0.05$ with the Chi square test. Corrected p values by false discovery rate (FDR) are given.

## Supplementary Figure Legends

**Supplementary Figure 1, related to Figure 1. Data analysis pipeline and outline results.** **(A)** Flowchart of the data analysis pipeline for the discovery of DDR-regulated miRNAs**.** Blue boxes represent the steps in the data analysis pipeline. White boxes represent the inputs and outputs. **(B)** The up-regulated miRNAs after IR are categorised into 4 conditions; MCF10A two replicates, MCF10A-1 (red) and MCF10A-2 (blue), HCT116 two replicates, HCT116-1 (orange) and HCT116-2 (green) using a Venn-diagram. Twenty-three miRNAs (numbers in black) belong to at least 2 conditions are selected as the most robustly induced miRNAs. **(C)** The down-regulated miRNAs after IR are categorised into 4 conditions with a Venn diagram similar manor with **B**. The ten miRNAs satisfy 2 conditions as the robustly decreased miRNA after DNA damage.

**Supplementary Figure 2, related to Figure 2. Analysis of DDR miRNAs by qRT-PCR and concordance with next-generation sequencing.**

The $\log_2$ values (M values) of the fold changes at 4 and 24hour after DNA damage, which were measured by the next generation sequencing (NGS) and Q-RT-PCR (qRT-PCR) in 20 miRNAs with the two cell lines are colour coded in each cell. The concordance between the sequencing and qRT-PCR are determined if it is true (T) or failed (F) individually by the cell lines.

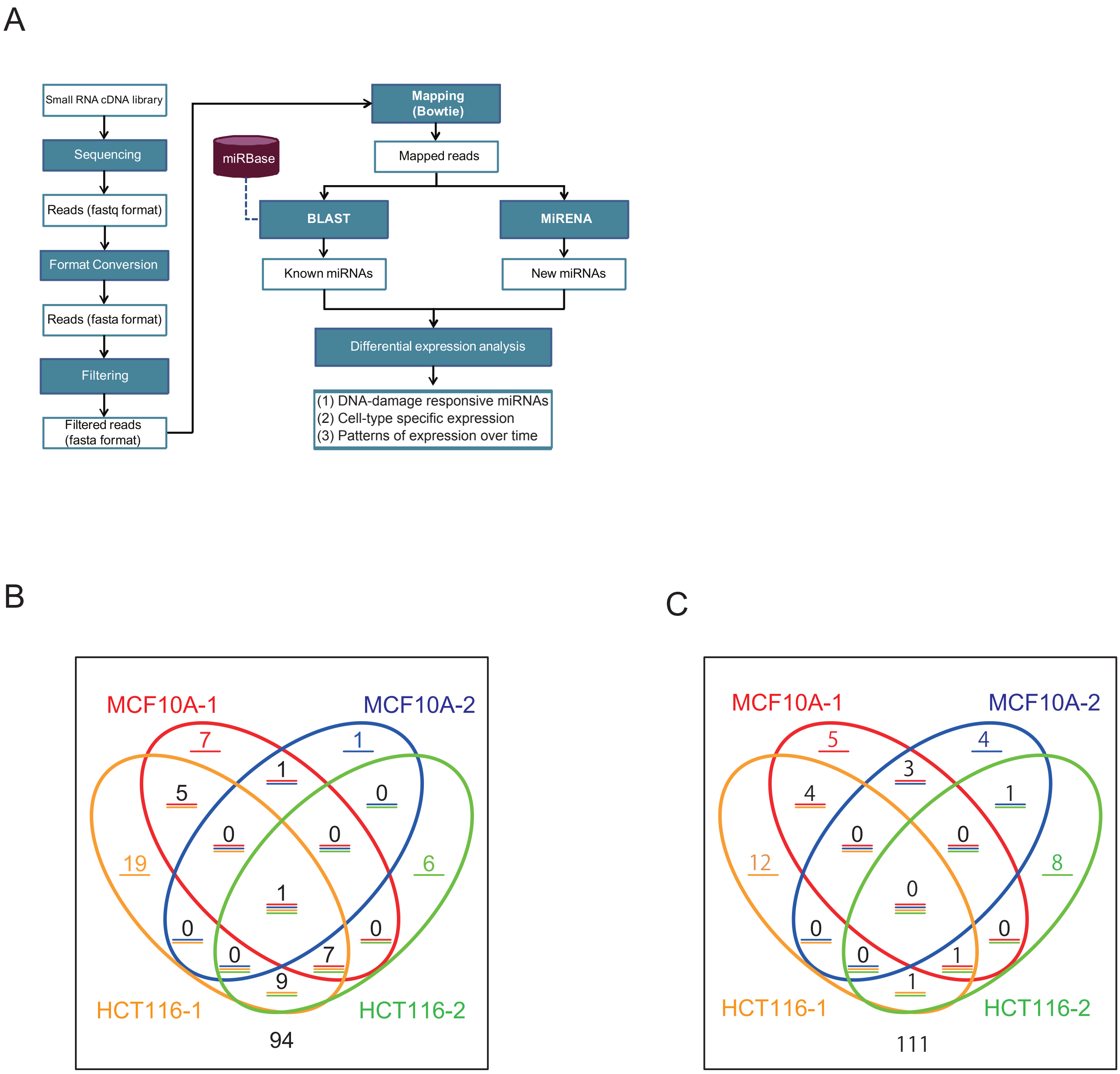
NA represents the data are not applicable.

**Supplementary Figure 3, related to Figure 9. qRT-PCR analysis of TP53-regulated miRNAs.**

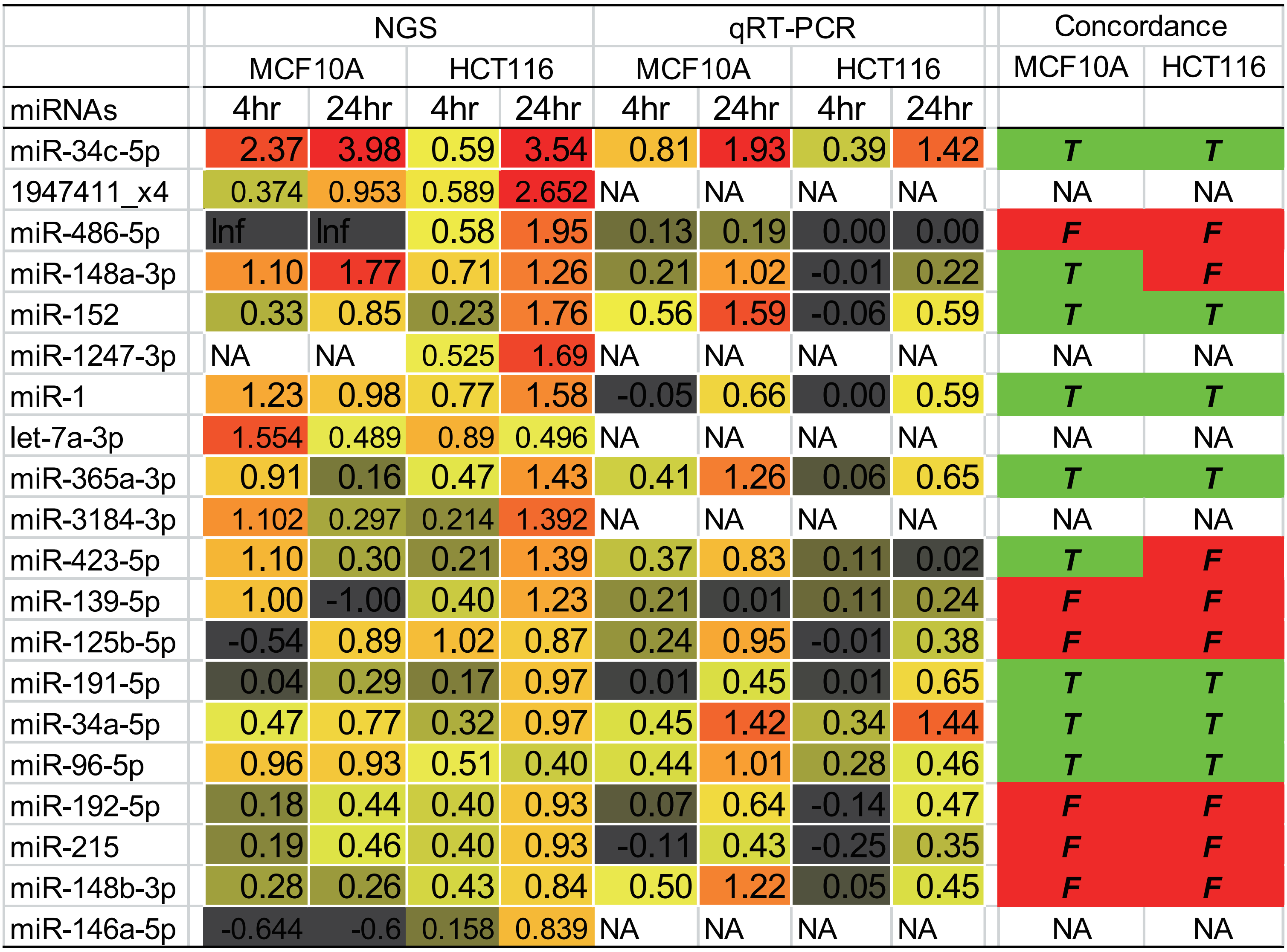
miR-34 group miRNA, miR-34a and miR-34c are measured in HCT116 and HCT116*TP53-/-* cells at 0, 4, and 24hour time points. For the normalization of the mount of each template, the cDNA which synthesised from the same amount of total RNA between the targets are used. In addition, RNU48, a small nucleolar RNA, is offered as an internal control to confirm the equal amount of total RNAs are applied in each qRT-PCR reaction. Y-axis depicts the relative miRNA levels, which normalized to 0 hour time point in each cell line and target miRNA. The error bar represents standard deviation (S.D.) from the experiments in triplicate.

**Supplementary Figure 4, related to Figure 7. Correlations between patient survival and the expression levels of DDR miRNAs under TP53 regulation in tumour samples.**

TP53 transcription factor networks of the DDR miRNAs were selected from the analysis shown in Figure 7. Four miRNAs (miR-34a, -34c, -192 amd -215) are regulated by the TP53 transcription factor; of these, miR-34a and miR-215 showed a correlation with patient survival. The Kaplan-Meier survival analysis was performed in cancer patients according to the expression levels of miR-34a (**A**, **B**, **C**) or miR-215 (**D**) in tumour samples. The survival curve in green represents the patients with lower expression of DDR miRNAs, whereas the curve in red shows those with higher expression. The X-axis represents the duration of survival after diagnosis. The Y-axis shows the probability of patient survival. **A**, Breast cancer, p value (corrected by FDR)=0.000899 (GEO dataset ID: GSE37405). **B**, Ovarian carcinoma, p value (corrected by FDR)=0.049 (GEO dataset ID: GSE27290). **C**, Hepatocellular carcinoma, p value (corrected by FDR)=0.049 (GEO dataset ID: GSE10694). **D**, Breast cancer, p value (corrected by FDR)= 0.0003924 (GEO dataset ID: GSE37405).

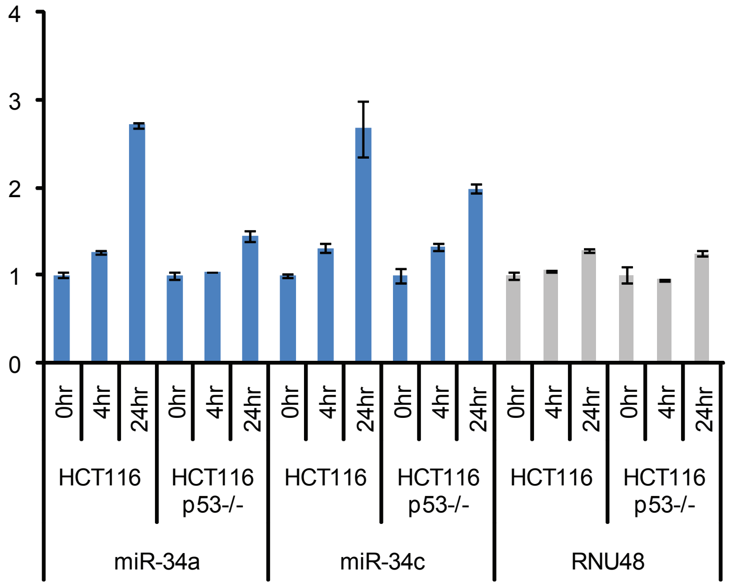


Supplementary Figure 1. Hattori et al

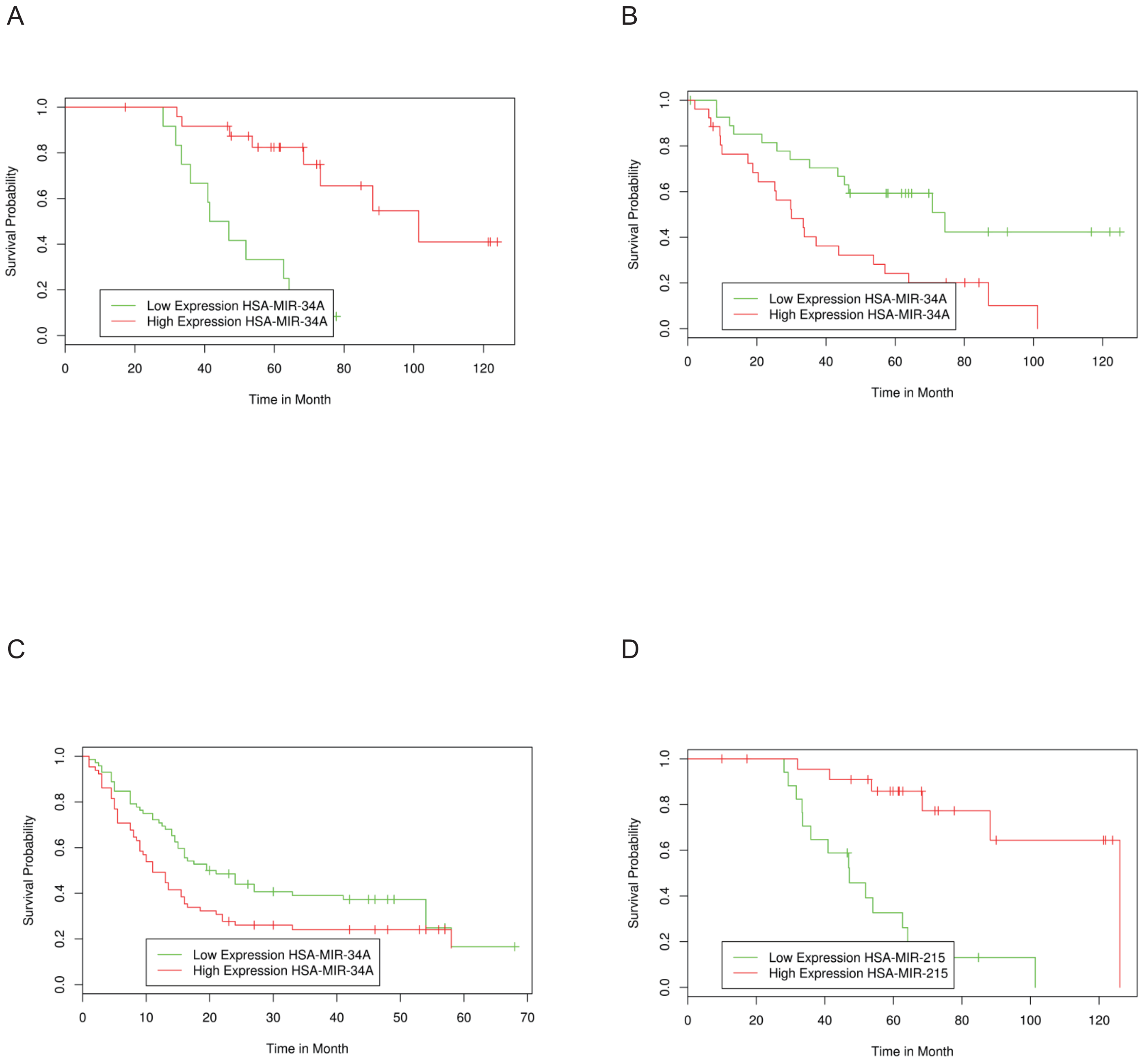


| | NGS | | | | qRT-PCR | | | | Concordance | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MCF10A | | HCT116 | | MCF10A | | HCT116 | | MCF10A | HCT116 |
| miRNAs | 4hr | 24hr | 4hr | 24hr | 4hr | 24hr | 4hr | 24hr | | |
| miR-34c-5p | 2.37 | 3.98 | 0.59 | 3.54 | 0.81 | 1.93 | 0.39 | 1.42 | ***T*** | ***T*** |
| 1947411_x4 | 0.374 | 0.953 | 0.589 | 2.652 | NA | NA | NA | NA | NA | NA |
| miR-486-5p | Inf | Inf | 0.58 | 1.95 | 0.13 | 0.19 | 0.00 | 0.00 | ***F*** | ***F*** |
| miR-148a-3p | 1.10 | 1.77 | 0.71 | 1.26 | 0.21 | 1.02 | -0.01 | 0.22 | ***T*** | ***F*** |
| miR-152 | 0.33 | 0.85 | 0.23 | 1.76 | 0.56 | 1.59 | -0.06 | 0.59 | ***T*** | ***T*** |
| miR-1247-3p | NA | NA | 0.525 | 1.69 | NA | NA | NA | NA | NA | NA |
| miR-1 | 1.23 | 0.98 | 0.77 | 1.58 | -0.05 | 0.66 | 0.00 | 0.59 | ***T*** | ***T*** |
| let-7a-3p | 1.554 | 0.489 | 0.89 | 0.496 | NA | NA | NA | NA | NA | NA |
| miR-365a-3p | 0.91 | 0.16 | 0.47 | 1.43 | 0.41 | 1.26 | 0.06 | 0.65 | ***T*** | ***T*** |
| miR-3184-3p | 1.102 | 0.297 | 0.214 | 1.392 | NA | NA | NA | NA | NA | NA |
| miR-423-5p | 1.10 | 0.30 | 0.21 | 1.39 | 0.37 | 0.83 | 0.11 | 0.02 | ***T*** | ***F*** |
| miR-139-5p | 1.00 | -1.00 | 0.40 | 1.23 | 0.21 | 0.01 | 0.11 | 0.24 | ***F*** | ***F*** |
| miR-125b-5p | -0.54 | 0.89 | 1.02 | 0.87 | 0.24 | 0.95 | -0.01 | 0.38 | ***F*** | ***F*** |
| miR-191-5p | 0.04 | 0.29 | 0.17 | 0.97 | 0.01 | 0.45 | 0.01 | 0.65 | ***T*** | ***T*** |
| miR-34a-5p | 0.47 | 0.77 | 0.32 | 0.97 | 0.45 | 1.42 | 0.34 | 1.44 | ***T*** | ***T*** |
| miR-96-5p | 0.96 | 0.93 | 0.51 | 0.40 | 0.44 | 1.01 | 0.28 | 0.46 | ***T*** | ***T*** |
| miR-192-5p | 0.18 | 0.44 | 0.40 | 0.93 | 0.07 | 0.64 | -0.14 | 0.47 | ***F*** | ***F*** |
| miR-215 | 0.19 | 0.46 | 0.40 | 0.93 | -0.11 | 0.43 | -0.25 | 0.35 | ***F*** | ***F*** |
| miR-148b-3p | 0.28 | 0.26 | 0.43 | 0.84 | 0.50 | 1.22 | 0.05 | 0.45 | ***F*** | ***F*** |
| miR-146a-5p | -0.644 | -0.6 | 0.158 | 0.839 | NA | NA | NA | NA | NA | NA |

Supplementary Figure S2. Hattori et al



Supplementary Figure S3. Hattori et al



Supplementary Figure S4. Hattori et al