cancers

Supplementary materials: Genome-Wide Circular RNA Expression Patterns Reflect Resistance to Immunomodulatory Drugs in Multiple Myeloma Cells

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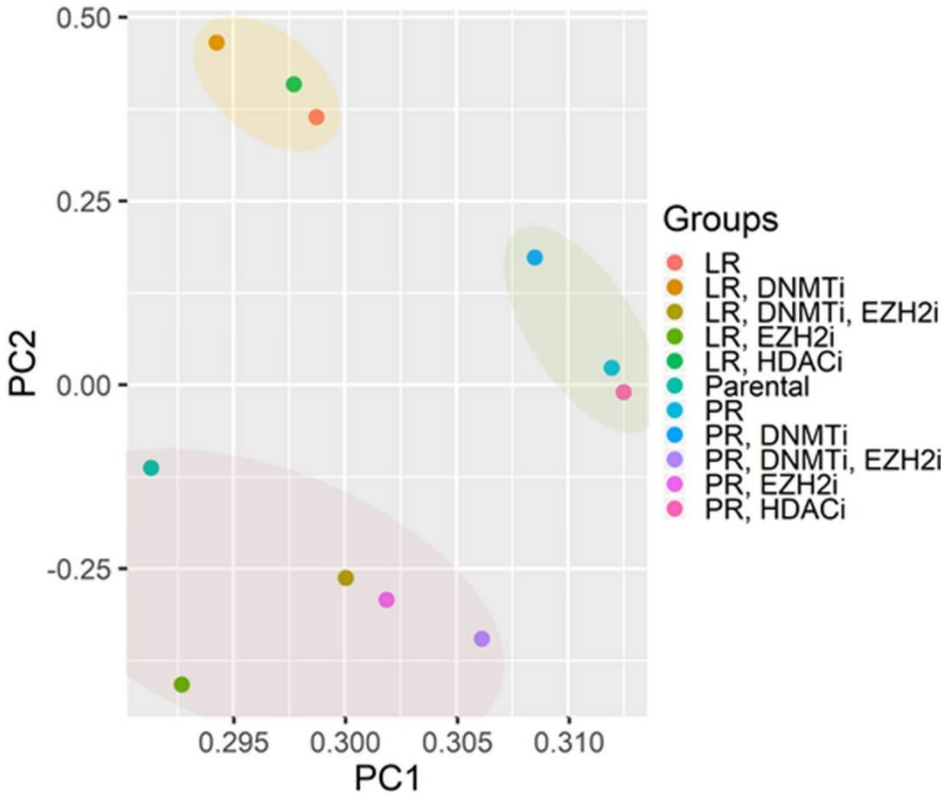

Figure S1. Principal component analysis (PCA) of all 6,368 circRNAs detected in the 11 samples (analyzed by RNA-Seq).

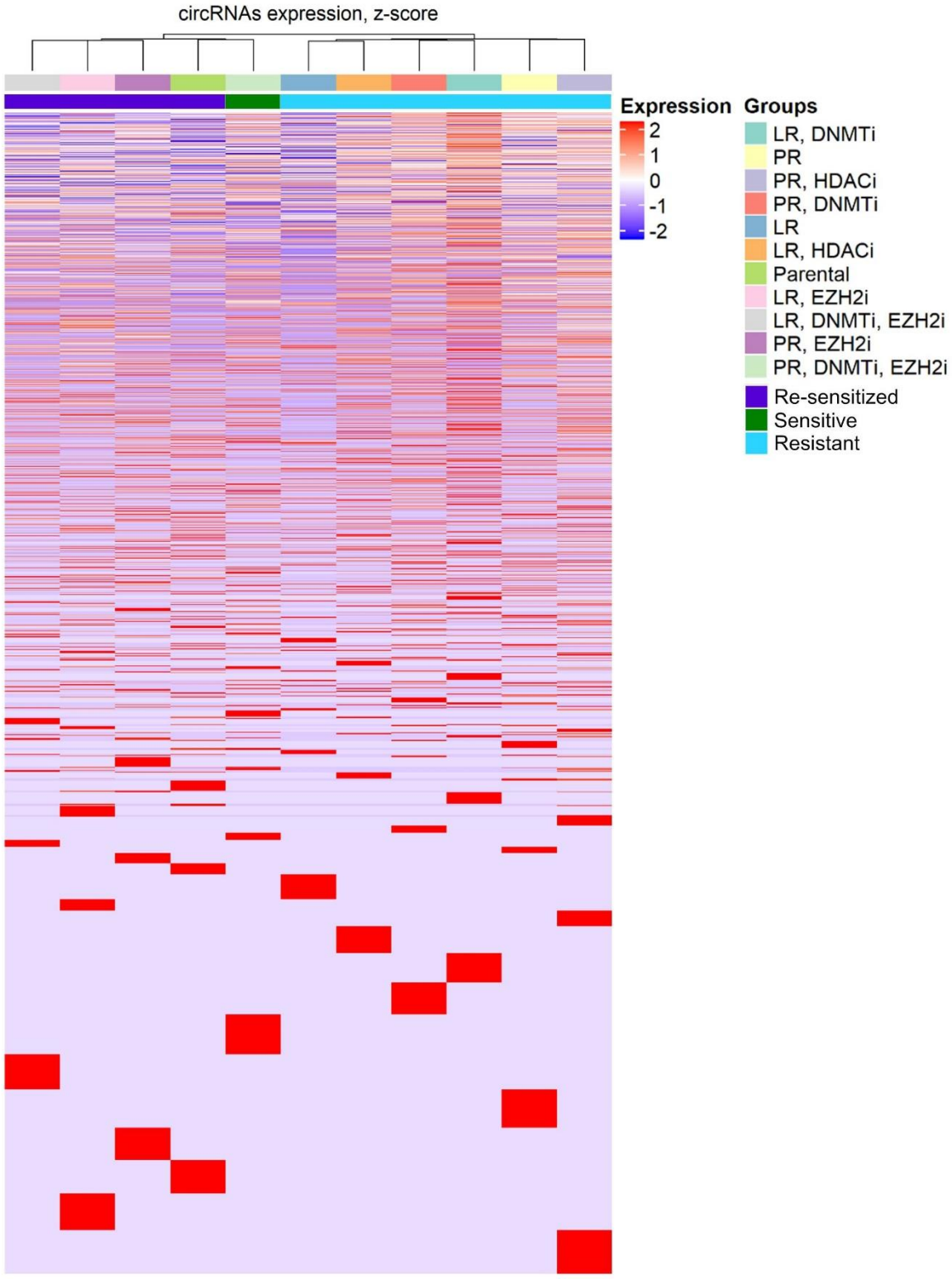

Figure S2. Heatmap showing expression and unsupervised clustering of all 6,368 circRNAs detected in the 11 samples (analyzed by RNA-Seq).

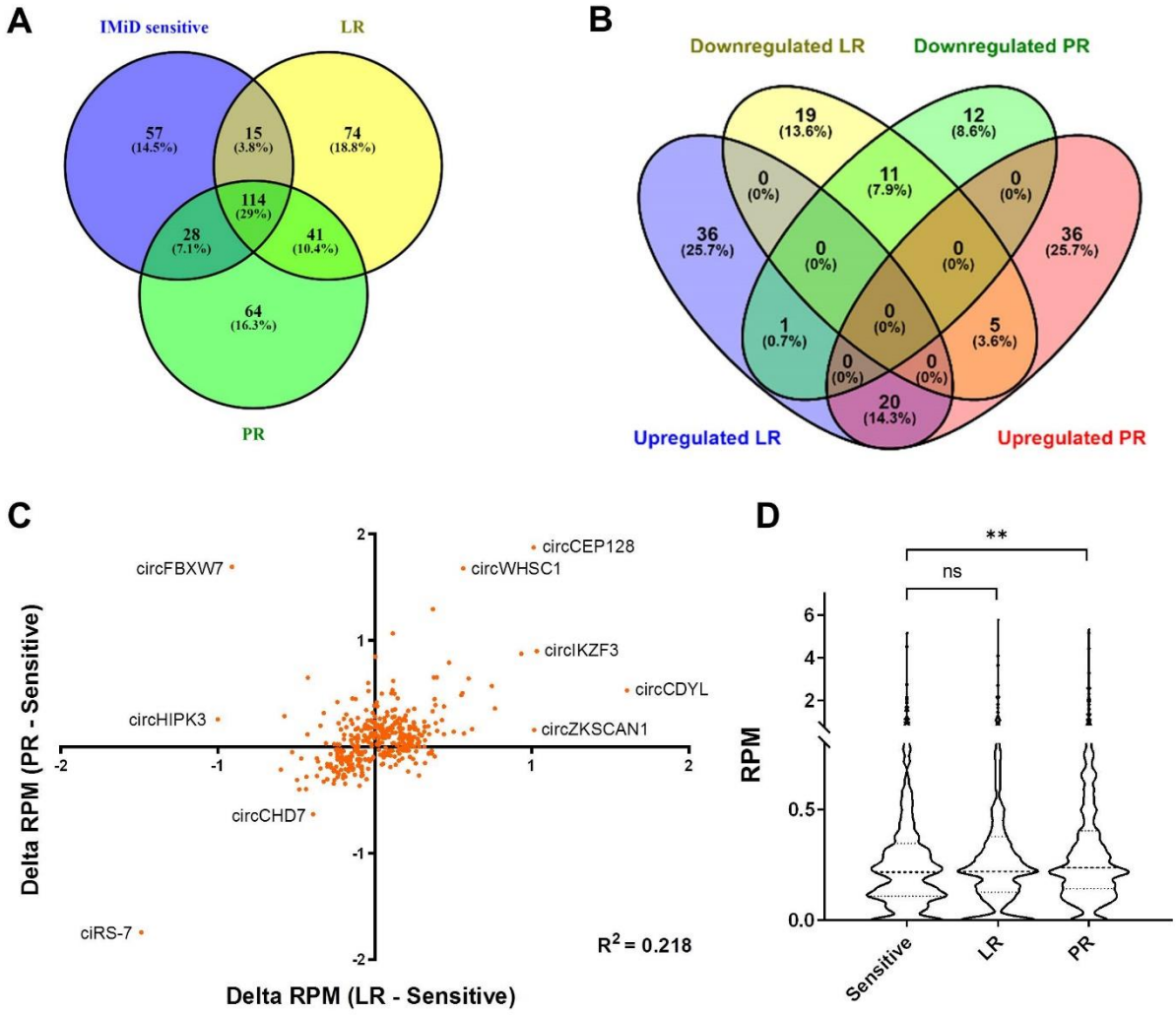

Figure S3. Comparison of circRNA expression changes in LR vs PR cells. **(A)** Venn diagram illustrating the overlap of high abundance circRNAs (with RPM>0.2) detected in parental (IMiD sensitive), LR and PR cells. **(B)** Venn diagram illustrating the overlap between up- and downregulated circRNAs in LR and PR. **(C)** Scatter plot showing the up- and downregulated circRNAs in LR and PR plotted against each other, with corresponding R^2 value. **(D)** Violin plot showing all circRNAs expressed in parental (sensitive), LR and PR cells, in RPM, **p<0.01 (Mann-Whitney test). LR, lenalidomide-resistant, PR, pomalidomide-resistant.

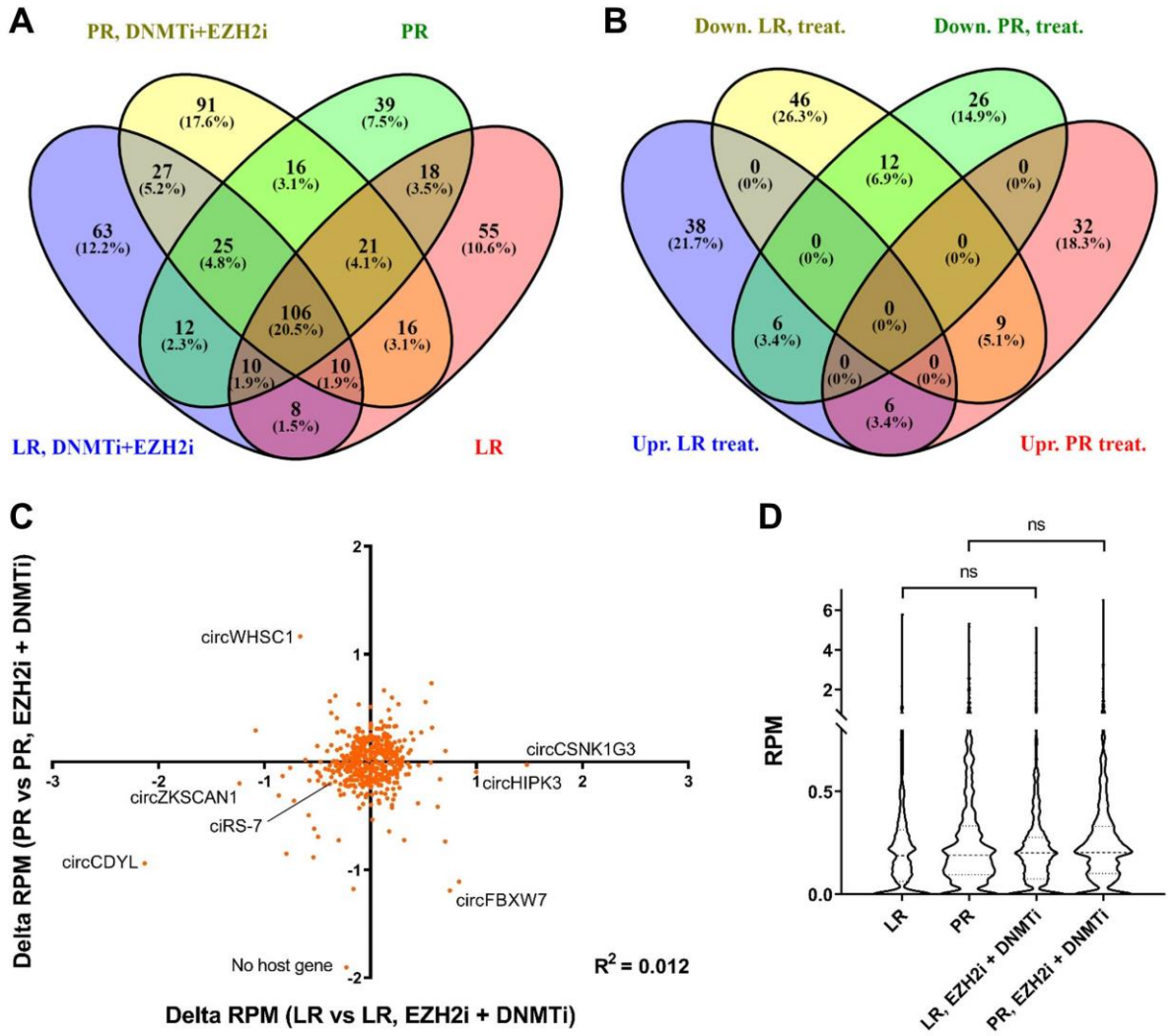

Figure S4. CircRNA expression changes upon combined DNMT and EZH2 inhibition. **(A+B)** Venn diagrams illustrating the overlap between circRNAs expressed in LR, LR–treat, PR and PR–treat **(A)** and the up- and downregulated circRNAs in LR-treat and PR-treat **(B)**. **(C)** Scatter plot showing the up- and downregulated circRNAs in LR-treat and PR-treat plotted against each other. **(D)** Violin plot showing the overall expression of circRNAs in RPM. LR, lenalidomide-resistant, PR, pomalidomide-resistant, Down., downregulated, Upr., upregulated, LR-treat, LR cells treated with EZH2i and DNMTi. PR-treat, PR cells treated with EZH2i and DNMTi.

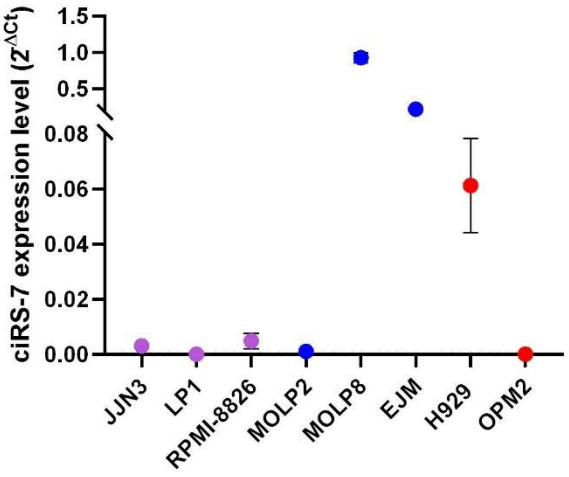

Figure S5. ciRS-7 expression in eight MM cell lines (purple=primary resistant cell lines, blue=partially resistant cell lines, red=sensitive cell lines). Relative ciRS-7 expression (RT-qPCR), error bars reflect technical triplicates.

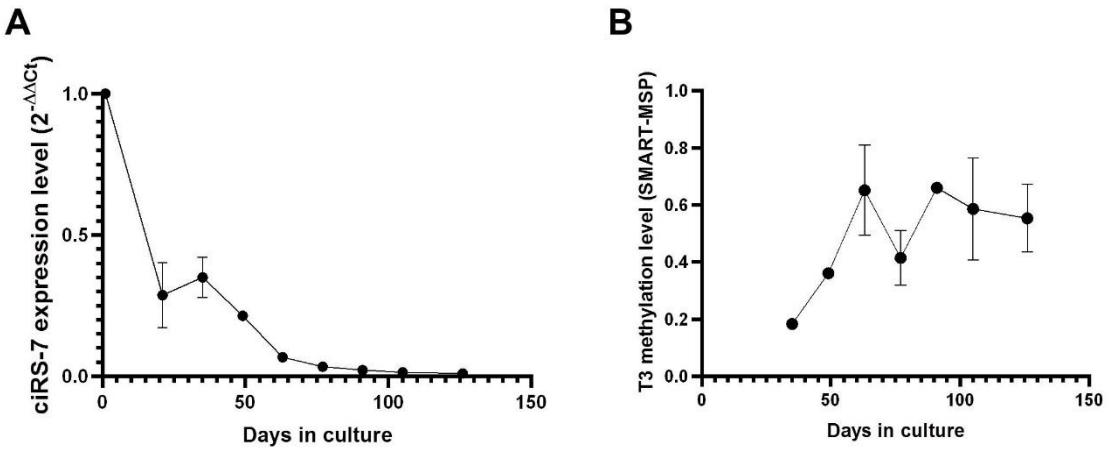

Figure S6. Loss of ciRS-7 expression over time in parental cells. **(A)** Relative ciRS-7 expression (RT-qPCR) of NCI-H929 cells growing in culture. Error bars reflect technical triplicates. **(B)** Relative methylation level (SMART-MSP) of T3 in NCI-H929 cells growing in culture. Error bars reflect technical triplicates.

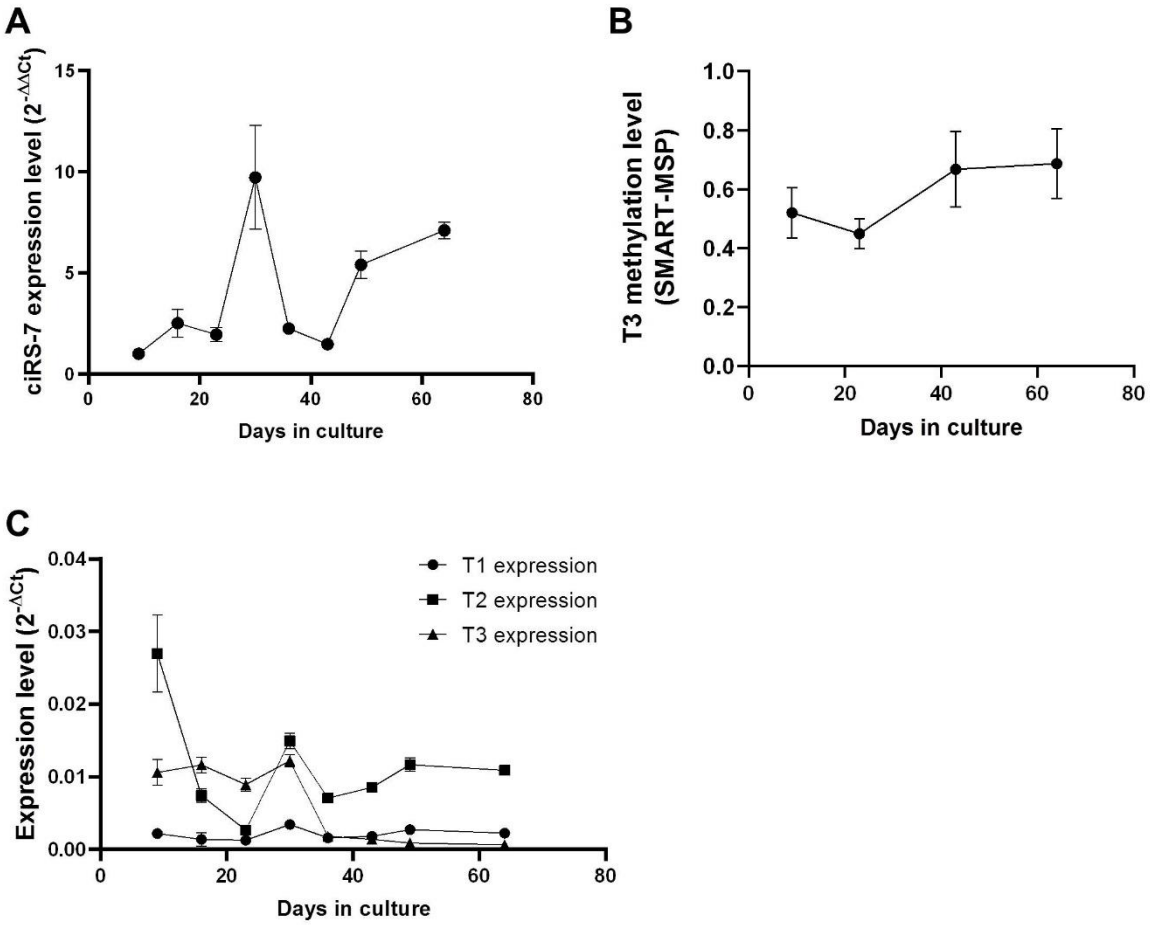

Figure S7. ciRS-7 expression over time in MOLP8 cells. **(A)** ciRS-7 expression (analyzed by RT-qPCR) in MOLP8 cells growing in culture. Error bars reflect technical triplicates. **(B)** T1, T2 and T3 expression (analyzed by RT-qPCR) in MOLP8 cells growing in culture. Error bars reflect technical triplicates. **(C)** Relative methylation level (SMART-MSP) of T3 in MOLP8 cells growing in culture. Error bars reflect technical triplicates.

Table S1. sisiRNA and siRNA sequences used for ciRS-7 knockdown.

Target	Sequence
ciRS-7 back-splice junction	AAAACCCUGGAUAUUGCAGAC
ciRS-7 internal sequence	UUGGAAGACACAAGUAGGCGCU [1]
circIKZF3 back-splice junction 1	UCCGCACUUGCAAUGAAUUUC[dT][dT]
circIKZF3 back-splice junction 2	GCACUUGCAAUGAAUUUCUGA[dT][dT]

[1] The underlined nucleotides are locked nucleic acids (LNAs) that increase the hybridization properties of the sisiRNA.

Table S2. Primers for RT-qPCR.

Target gene/transcript	Forward primer	Reverse primer
LINC00632 (T1)	5′GACATGGAATTCCTGGATCA	5′GTCACTAGGTGGCAAACGTG
LINC00632 (T2)	5′ CCGTCCCTTGCTGATTGTAT	5′ GTCACTAGGTGGCAAACGTG
LINC00632 (T3)	5′GAGGCGGTTAAGGAGAGGAG	5′TCAGGAGTCAAGGTCAGGCTA
ciRS-7	5′ACGTCTCCAGTGTGCTGA	5′CTTGACACAGGTGCCATC
Linear IKZF3	5′ ACATTTCTTCAGAGCACTGACC	5′ TCTGCTTTGATGTGTCTTGCCT
Circular IKZF3	5′GAAAAAGCTCAATGCCTCAGAAATTC	5′ TCTGCTTTGATGTGTCTTGCCT
SF3A1	5′TCCATCCGTGAGAAGCAGAGC	5′TCTGGATCTCCTCCTCACCG
PUM1	5′CATGCCAGGTTATCCGGTGT	5′GCGCCTGCATTCACTACAAG

Table S3. Methylation-specific primers used for SMART-MSP.

Target gene/transcript	Forward primer	Reverse primer
LINC00632 (T3)	5′ gtgg**cg**agtTaggagT**cg**ac [1]	5′ tctAAaAaAacc**cg**cccctA**cg** [1]
Alu control	5′ GGTTAGGTATAGTG GTTTATATTTGTAATTTTAGTA	5′ ATTAACTAAACTAATCTTA AACTCCTAACCTCA

[1] For the LINC00632 (T3) primer set, upper-case T indicates that the base was C before sodium bisulfite conversion and CG sites are indicated in bold.

Table S4. Methylation-independent primers for amplification of bisulfite-treated DNA and subsequent Sanger sequencing.

Target gene/transcript	Forward primer	Reverse primer
LINC00632 (T3)	5′ggTataTtgTaaTtaggtTagaagtgaT [1]	5′ gagtTTtTTTTagagaggggagg [1]

[1] Upper-case T indicates that the base was C before sodium bisulfite conversion.