Supplementary methods

Cell lines

Human cell lines were grown at 37°C, 5% CO_2. a) Human myeloma (HM)-CLs: AMO1, NCI-H929, SK-MM-1, U266, JJN3 and KMS-12-BM were purchased from DSMZ (Braunschweig, Germany). MM.1S, MM.1R and RPMI-8226 were purchased from ATCC (Manassas, VA, USA). AMO1 bortezomib-resistant (ABZB) and AMO1 carfilzomib-resistant (ACFZ) were kindly provided by Dr. Christoph Driessen (Eberhand Karls University, Tübingen, Germany). U266 melphalan-resistant (LR7) were kindly provided by Dr. Atanasio Pandiella (Universidad de Salamanca, Salamanca, Spain). KMS-11 were kindly provided by Dr. K.C. Anderson (Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA). KMS-26 were kindly provided by Dr. Giovanni Tonon (University of San Raffaele Scientific Institute, Milan, Italy). These cells were cultured in RPMI-1640 medium (Gibco® Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Lonza Group Ltd., Basel, Switzerland) and 1% penicillin/streptomycin (Gibco®, Life Technologies). IL-6 dependent cell lines INA-6 and XG-1, kindly provided by Dr. Renate Burger (University of Erlangen-Nuernberg, Erlangen, Germany), were cultured in the presence of 1 ng/mL rhIL-6 (R&D Systems, Minneapolis, MN). b) Mantle cell lymphoma (MCL)-CLs: Mino, Maver-1 and Jeko-1 (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). c) Diffuse large B cell lymphoma (DLBCL)-CLs: Oci-Ly-7, Pfeiffer and Toledo (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). d) Burkitt lymphoma (BL)-CLs: Sultan, P3HR1, Daudi and Raji (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). e) T cell lymphoma (TCL)-CLs: H9 and Jurkatt (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). f) Acute myeloid leukemia (AML)-CLs: THP-1, K562, HL-60 (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). g) Malignant pleural mesothelioma (MPM)-CLs: Mero-14, Mero-25, MPM-209, MPM-376 and IST-MES (kindly provided by Department of Pharmaceutical Sciences, University of

"Piemonte Orientale Amedeo Avogadro", Novara, Italy) were cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). h) Pancreatic cancer (PC)-CLs: Capan-1 and BxPC-3 (purchased from Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZLER), Brescia, Italy) were cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 20% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); Capan-2, AsPC-1, PANC-1 and MIA PaCa-2 (purchased from ATCC) were cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). i) Breast cancer (BC)-CLs: MCF7 (purchased from ATCC) were cultured in ATCC-formulated Eagle's Minimum Essential Medium, supplemented with 0.01 mg/ml human recombinant insulin, 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); MDA-231 (purchased from ATCC) were cultured in ATCC-formulated Leibovitz's L-15 Medium, supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); HCC1937 (purchased from ATCC) were cultured ATCC-formulated Leibovitz's L-15 Medium, supplemented with 15% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); SK-BR-3 (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). j) Non-small cell lung cancer (NSCLC)-CLs: A-549, LXF-289 (purchased from ATCC) were cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). k) Non-malignant (NM)-CLs: 293T (human embrionic kidney) and HS-5 (human stromal cells) were purchased from ATCC and cultured in DMEM (Dulbecco's modified Eagle's medium) (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); HK-2 (human kidney cells, cortex/proximal tubule) were purchased from ATCC and cultured in K-SFM (Keratinocyte Serum Free Medium) (Thermo Fisher Scientific, Waltham, MA, USA). supplemented in accordance with ATCC guide lines; THLE-2 (human liver cells) were purchased from ATCC and cultured in BEGM (Bronchial epithelial cell growth medium) (Lonza Group Ltd.) supplemented in accordance with ATCC guide lines; HCC-1143-Bl (human B cells) were

purchased from ATCC and cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). l) P493-6 were kindly provided by Dr. Dirk Eick (Max Planck Institute of Biochemistry, Helmholtz-Zentrum München, Germany and cultured in RPMI-1640 medium (Gibco® Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies). m) MYC-ER HMEC were kindly provided by Dr. Stephen J. Elledge (Howard Hughes Medical Institute, Harvard Medical School, Brigham & Women's Hospital, Boston, MA02115, USA) and cultured in MEGM (Mammary Epithelial Cell Growth Medium) (Lonza Group Ltd.) supplemented according to manufacturer's instructions.

Cells were periodically tested to exclude mycoplasma contamination. Cells were STR (short tandem repeats) authenticated.

Primary patient cells

Following informed consent approved by our University Hospital Ethical Committee, CD138+ cells were isolated from the BM aspirates of both MGUS and MM patients by Ficoll-Hypaque (Lonza Group, Basel, Switzerland) density gradient sedimentation, followed by antibody-mediated positive selection using anti-CD138 magnetic activated cell separation microbeads (Miltenyi Biotech, Gladbach, Germany). Purity of immunoselected cells was assessed by flow-cytometry analysis using a phycoerythrin-conjugated CD138 monoclonal antibody by standard procedures. For co-culture experiments: CD138+ cells from MGUS patients and from MM patients pt#1, pt#2 and pt#3 were seeded on HS-5 cells and cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies); CD138+ cells from MM patients pt#4, pt#5, pt#6, pt#7, pt#8, pt#9, pt#10 and pt#11 were cultured physically separated from HS-5 cells by means of Falcon Cell Culture Inserts (Corning, New York, NY, USA), according to manufacturer's instructions. CD138+ cells from MM patients pt#12 and pt#13 were cultured in RPMI-1640 medium (Gibco®, Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Lonza Group Ltd., Basel, Switzerland) and 1% penicillin/streptomycin (Gibco®, Life Technologies). Co-culture of HMCLs with hBMSCs was performed as previously reported[10].

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy adult donors, after informed consent approved by our University Hospital Ethical Committee. Cells were separated using Ficoll-hypaque method (Lonza Group Ltd.). PBMCs were cultured in RPMI-1640 medium (Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (Lonza Group Ltd.) and 1% penicillin/streptomycin (Gibco®, Life Technologies).

Antisense oligonucleotides, miRNA mimics/inhibitors and shRNAs

The following Long Non-Coding LNA gapmerRs were customly-designed and purchased from Exiqon (Vedbaek, Denmark):

IDS, Sequence and length of seven miR-17-92 LNA gapmeRs and three controls.

IDs	SEQUENCE 5'-3'	LENGHT (mer)
LNA gapmeR_02	ACATCGACACAATAA	15
LNA gapmeR_05	TCAGTAACAGGACAGT	16
LNA gapmeR_06 (MIR17PTi)	TACTTGCTTGGCTT	14
LNA gapmeR_10	ATGCAAAACTAACAGA	16
LNA gapmeR_12	GAAGGAAAATAGCAGGC	16
LNA gapmeR_15	AGCACTCAACATCAGC	16
LNA gapmeR_16	CGACAGGCCGAAGCT	15
Scr-NC (also known as Negative control A)	AACACGTCTATACGC	15
Lp-MIR17PTi	TACTTGCTTGGCTT	14
mix-MIR17PTi	TACTTGCTTGGCTT	14

Synthetic mimics and inhibitors for miR-17a, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a1 were purchased from Ambion (Applied Biosystems, CA, US).

The following MIR17HG shRNAs (Mission 3x Lac0 IPTG Inducible shRNAs) were customly-designed and purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA):

- *MIR17HG* shRNAs#1: tgggcttgaactgagatttaa
- *MIR17HG* shRNAs#2: tccaggcttatttgacttaaa
- *MIR17HG* shRNAs#3: tgggtgataaagtagatataa

A negative control (IPTG-inducible Non-targeting shRNAs) and a positive control (IPTG inducible TurboGFP shRNA) were also purchased from Sigma-Aldrich.

The following MIR17HG siRNAs (Lincode SMARTpool siRNA) were purchased from Dharmacon (Lafayette, Colorado):

- GCUUAGUGGGUAUGAGU (N-032556-09)
- CCGAAGAUGGUGGCGGCUA (N-032556-10)

- CACUUGAGACUUCAGAUUA (N-032556-11)
- GGCCUCCGGUCGUAGUAAA (N-032556-12)

Transient transfection of cells

Adherent cell lines: cells were transfected by Lipofectamine 2000 according to manufacturer instructions with 25 nM of LNA gapmeRs (Exiqon).

Suspension cell lines: cells were transfected (electroporation) by Neon Transfection System (Invitrogen, CA, US), (2 pulses at 1150, 30ms). LNA gapmeRs and miRNA inhibitors/mimics were used at 25nM. MIR17HG pooled siRNAs were used at 500nM. The transfection efficiency evaluated by flow-cytometric analysis relative to a FAM dye–labeled anti-miR–negative control reached 85% to 90%.

Transduction of cells

To generate cells stably expressing luciferase transgene, U266, AMO-1 and AMO-1/abzb cells were transduced with pLenti-III-PGK-Luc (ABM Inc., Richmond, BC, Canada) vector, following the manufacturer's instructions. To generate cells stably over-expressing miR-17-92 cluster, U266 were transduced with PMIRH17-92PA-1 lenti-vector (System Biosciences, Palo Alto, CA, USA). To generate cells stably expressing c-MYC, U266 were transduced with Precision LentiORF human MYC (GE Dharmacon, Lafayette, Colorado, USA).

CRISPR/CAS9-mediated genome editing

Genomic knock-out of BIM was achieved by transfection of AMO1 or U266^{MYC+} with CRISPR/CAS9 all-in-one vectors (pCLIP-ALL-hCMV-ZsGreen) (transOMIC technologies Inc., Huntsville, AL, USA). After two days, ZsGreen+ cells were sorted (BD FACSARIA III; BD Biosciences, Qume Drive San Jose, CA, USA) and single-cell cultured by limiting-dilution technique. Single-cell derived clones (two for each vector, TEVH-1110175 / TEVH-1177317 / TEVH-1244459) were selected and used for downstream experiments.

Gymnosis

Cells were seeded at low plating density in order to reach confluence on the final day of the experiments (day 6). Cell number at plating ranged from 0,5 to 2,5 x 10^3 in 96-well plates, from 2,5 to 10 x 10^4 in 12-well plates and from 1 to 3 x 10^5 in 6-well plates.

Survival assay

Cell viabilty was evaluated by Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies) and 7-AminoactinoMYCin (7-AAD) flow cytometry assays (BD biosciences), according to manufacturer's instructions. Flow cytometry analysis was performed either by FACS CANTO II (BD biosciences) or by Attune NxT Flow cytometer (Thermo Fisher Scientific).

Detection of apoptosis

Apoptosis was investigated by Annexin V/7-AAD flow cytometry assay (BD biosciences) and by electronic microscopy. Flow cytometry analysis was performed either by FACS CANTO II (BD biosciences) or by Attune NxT Flow cytometer (Thermo Fisher Scientific).

Cell cycle analysis

Analysis of cell cycle was performed by Propidium Iodide flow cytometry assay (BD biosciences), according to manufacturer's instructions. Flow cytometry analysis was performed either by FACS CANTO II (BD biosciences) or by Attune NxT Flow cytometer (Thermo Fisher Scientific).

Synergism quantification

Drug combination studies and their synergy quantification followed the Chou-Talalay method[40]. First, dose-effect curves were determined in AMO1 after six days of treatment for each drug, including dexamethasone, melphalan or bortezomib (Selleckchem.com). Then, different concentrations of each anti-MM agent were combined to MIR17PTi ranging 0.75-1.25µM. Combination indexes (CI) were calculated by CalcuSyn (BIOSOFT, Cambridge, UK).

Reverse transcription (RT) and quantitative real-time amplification (qRT-PCR)

RNA extraction, reverse transcription (RT) and quantitative real-time amplification (qRT-PCR) were performed as previously described[10]. Briefly, total RNA was extracted from cells with TRIzol® Reagent (Thermo Fisher Scientific), according to manufacturer's instructions. The integrity of total RNA was verified by nanodrop (Celbio Nanodrop Spectrophotometer nd-1000). For pri-mir-17-92 and mRNA dosage studies, oligo-dT-primed cDNA was obtained through the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) and then used as a template to quantify has-pri-mir-17-92 (pri-mir-17: Hs03295901; pri-mir-92a-1: Hs03302603), BIM (Hs00708019_s1), BZW2 (Hs00204063_m1), DUSP2

(Hs01091226_g1), CCNG2 (Hs00171119_m1), NAP1L1 (Hs00748775_s1), STAT3 (Hs00374280_m1), VDAC1 (Hs01631624_gH) and ARRDC3 (Hs00385845_m1). In all qRT-PCR experiments both taqman probes for pri-mir-17-92 were used, but only results with pri-mir-17 probe are shown. Normalization was performed with human GAPDH (Hs03929097_g1). Single-tube TaqMan miRNA assay (Thermo Fisher Scientific) was used to detect and quantify miR-17 (002308), miR-18a (002422), miR-19a (000395), miR-20a (000580), miR-19b (000396) and miR-92a-1 (000431), according to the manufacturer's instructions, by the use of ViiA7 RT reader (Thermo Fisher Scientific). Mature miRNAs expression was normalized on RNU44 (Thermo Fisher Scientific, assay Id: Hs03929097_g1). lncRNA MIR17HG-201 was detected by SYBR Green qRT-PCR using the following primers: Fw, 5' CTGCCTTGATAACATTTCATATGTGG; Rev, 5' CTTCCGGCTCGTATGTTGTGTGG.

Comparative real-time polymerase chain-reaction (RT-PCR) was performed in triplicate, including no-template controls. Relative expression was calculated using the comparative cross threshold (Ct) method.

Western blot analysis

Protein extraction and western blot analysis were performed as previously described[10]. Briefly, cells were lysed in lysis buffer containing 15mM Tris/HCl pH 7.5, 120mM NaCl, 25mM KCl, 1mM EDTA, 0.5% Triton 100, Halt Protease Inhibitor Single-Use cocktail (100X, Thermo Scientific). Whole cells lysates (~20 µg per lane) were separated using 4-12% Novex Bis-Tris SDS-acrylamide gels (Invitrogen), electro-transferred on Nitrocellulose membranes (Bio-Rad). After electrophoresis the nitrocellulose membranes were blocked and probed over-night with primary antibodies at 4 ^{0}C, then the membranes were washed 3 times in PBS-Tween and then incubated with a secondary antibody conjugated with horseradish peroxidase for 2 hours at room temperature. Chemiluminescence was detected using Western Blotting Luminol Reagent (sc-2048, Santa Cruz, Dallas, TX, USA). Signal intensity was quantified with the Quantity One Analyzing System (Bio-Rad).

Primary antibodies: BIM (#2933), Apaf-1 (#5088), Caspase 8 (#9746), Caspase-3 (#9665), Stat3 (#4904) were purchased from Cell Signalling Biotechnology. CCNG2 (ab54901) and BZW2 (ab96682) were purchased from Abcam (Cambridge, UK). GAPDH (sc-25778) and β-actin (ab96682) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

Confocal Microscopy

293T cells were treated with FAM-labeled MIR17PTi (2,5µM). After treatment, cells were washed with PBS1X, centrifuged onto glass slides, fixed in 4% paraformaldehyde in PBS for 12 minutes, and washed in PBS (3 times x 5 minutes). Cells were permeabilized (0,1 % Triton X-100 in PBS) for 15 minutes, washed in PBS (3 times x 5 minutes), and incubated for 1 hour with blocking buffer (1,5 % BSA in PBS). Cells were washed in PBS (3 times x 5 minutes) and mounted under coverslips with Prolong Antifade plus DAPI. Images were acquired at confocal microscopy TCS SP II Leica Microsystems.

Gene expression profiling

Gene expression profiles were obtained from pMM cells (pt#5, pt#6, pt#7, pt#9) and AMO1 after six days of gymnotic exposure to MIR17PTi or scr-NC. Cells were collected and used for total RNA (tRNA) extraction by RNeasy Mini kit (Qiagen, Hilden, Germany). A total of 300 ng RNA was used as starting material for preparing the hybridization target by using the GeneChip® WT PLUS Reagent Kit (Affymetrix Inc., Santa Clara, CA, USA). The integrity, quality and quantity of tRNA were assessed by the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). The amplification of cRNA, the clean-up and the fragmentation were performed according to the Affymetrix´s procedures. Microarray data was generated by GeneChip® Human Transcriptome 2.0 Array (Affymetrix Inc., Santa Clara, Ca). Arrays were scanned with an Affymetrix GeneChip Scanner 3000. Raw data produced by the Affymetrix Platform (i.e. CEL files) were first processed using Affymetrix Expression Console (EC). Pre-processing phase was performed according to Affymetrix guidelines and micro-CS software. Raw data were normalized using probe logarithmic intensity error (PLIER) algorithm coupled to quantile normalization. Annotation of data was also performed using Affymetrix Provided Libraries and EC version 1.4.1. Differential expression was assessed using a linear model method. *P*-values were adjusted for multiple testing using the Benjamini and Hochberg method. Tests were considered to be significant for adjusted $P<0.05$. Clustering and fold change (FC) analysis were done using the dChip software comparing relative gene expression of MIR17PTi *versus* scr-NC treated cells. For each pair of compared samples we calculated FC as follows: $FC=\log_2$ (MIR17PTi *versus* scr-NC). The gene lists were applied to gene set enrichment analysis (GSEA) or Ingenuity Pathway Analysis (IPA®, Ingenuity System, Redwood city, CA) software to reveal biological pathways modulated by MIR17PTi.

NOD SCID mice and *in vivo* model of human MM

Male CB-17 severe combined immunodeficient (SCID) mice (6- to 8-weeks old; Harlan Laboratories, Inc., Indianapolis) or female NOD/SCID-ɣ (NSG) mice (6- to 8-weeks old; Charles River, Burlington, MA, USA) were housed and monitored in our Animal Research Facility. All experimental procedures and protocols had been approved by the Institutional Ethical Committee (Magna Graecia University) and conducted according to protocols approved by the National Directorate of Veterinary Services (Italy). In accordance with institutional guidelines, mice were sacrificed when tumors reached one or two cm in diameter or in the event of paralysis or major compromise, to prevent unnecessary suffering. NOD/SCID mice were s.c. inoculated with $5x10^6$ NCI-H929 cells and treatments started when palpable tumors became detectable, approximately 3 weeks following injection of MM cells. NOD/SCID mice were also inoculated with $5x10^6$ luciferase gene-marked AMO1 (s.c.) or AMO1/ABZB (s.c.). Treatments were i.v. performed. Tumors were measured by electronic caliper and/or using IVIS LUMINA II Imaging System. The SCID-hu model was performed as previously described by our group[29].

Immunohistochemistry (IHC) and in situ hybridization (ISH)

These experiments were performed as recently described by our group[11].

Non-human primates study

These experiments were performed as recently described by our group[11].

Statistical Analysis

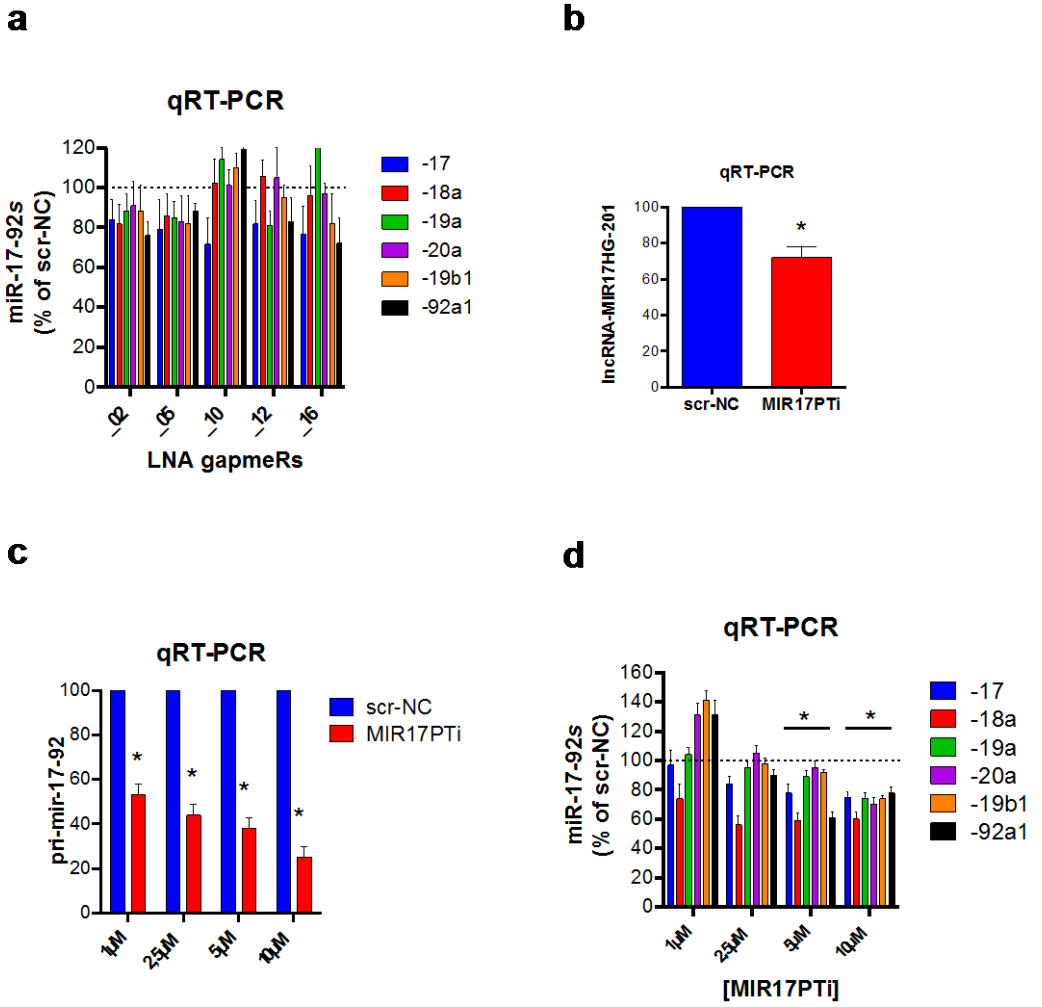
All in vitro experiments were repeated at least three times and performed in triplicate; a representative experiment was showed in figures. Statistical significances of differences were determined using Student's t test, with minimal level of significance specified as $P<0.05$. Statistical significance of the in vivo growth inhibition was determined using Student's t test. The minimal level of significance was specified as $P<0.05$. All statistical analyses were determined using GraphPad software (http://www.graphpad.com). Graphs were obtained using GraphPad software.

Data availability

The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information Files or are available from the corresponding authors on request.

Supplementary figures

Supplementary Figure 1



Supplementary Figure 1.

(**a**) qRT-PCR analysis of miR-17-92*s* expression in 293T two days after transfection with mir-17-92 LNA gapmeRs or scr-NC (25nM). The results are average expression levels after normalization with RNU44 and ΔΔCt calculation (expressed as percentage of scr-NC).

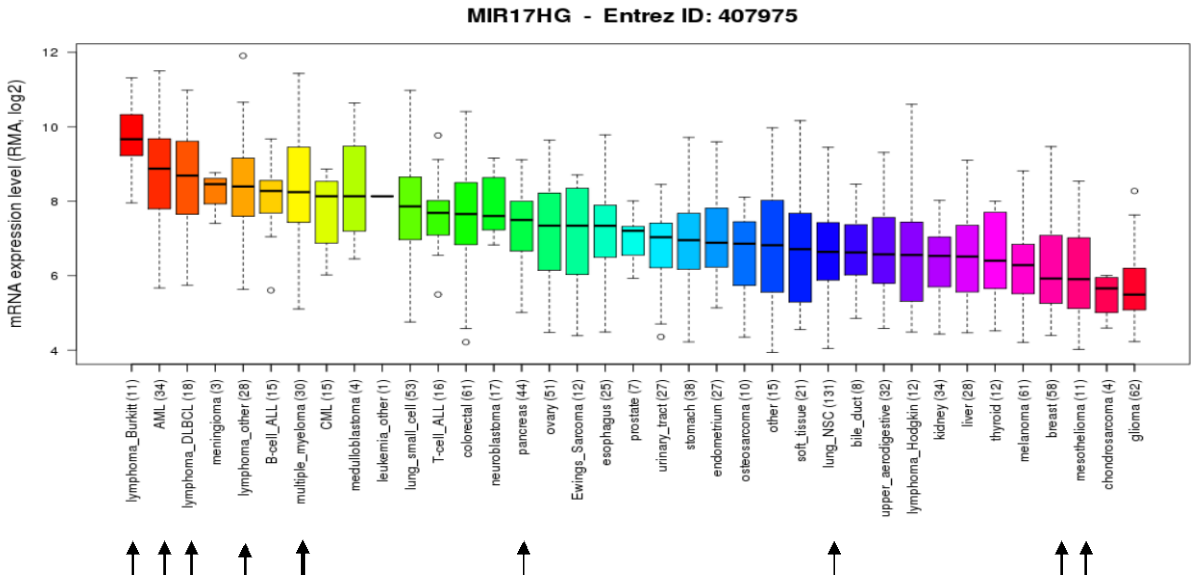
(**b**) qRT-PCR analyses of lncRNA MIR17HG-201 isoform in 293T two days after transfection with MIR17PTi or scr-NC (25nM). The results are average expression levels after normalization with GAPDH and ΔΔCt calculation (expressed as percentage of scr-NC).

(**c**) qRT-PCR analysis of pri-mir-17-92 in 293T after six days of exposure to indicated concentrations of MIR17PTi or scr-NC. The results are average expression levels after normalization with GAPDH and ΔΔCt calculations (expressed as percentage).

(**d**) qRT-PCR analysis of miR-17-92*s* in 293T after six days of exposure to indicated concentrations of MIR17PTi or scr-NC. The results are average expression levels after normalization with RNU44 and ΔΔCt calculations (expressed as percentage).

Data from 1 out of 3 independent experiments is shown in each panel. **indicates $p<0.05$*

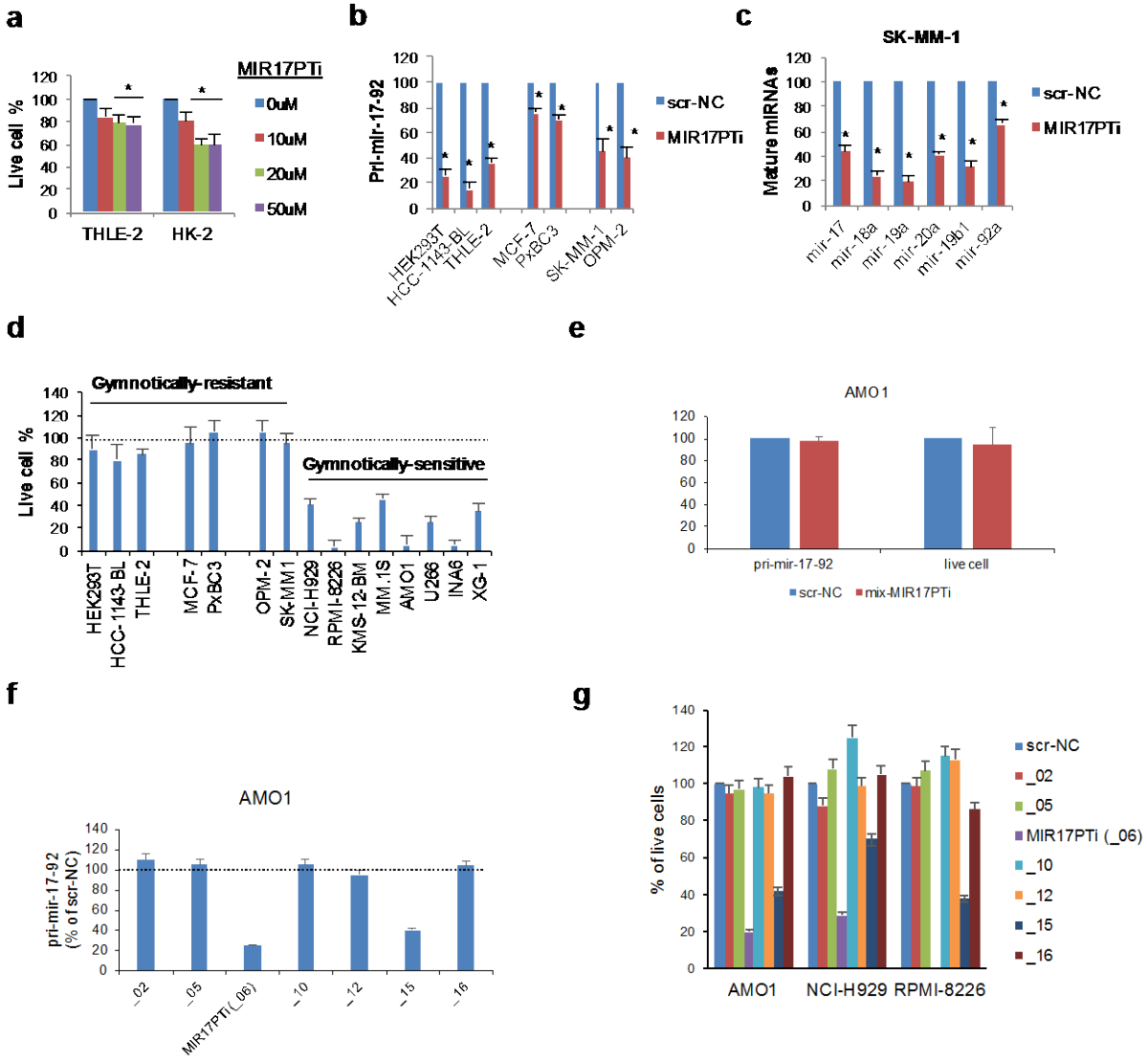
a



Supplementary Figure 2.

(**a**) *MIR17HG* expression at the CCLE. Arrows indicate CCLs used in Fig. 2a.

Supplementary Figure 3



Supplementary Figure 3.

(**a**) CCK-8 proliferation assay of two NM-CLs (THLE-2 and HK-2) exposed for six days to indicated concentrations of MIR17PTi.

(**b**) qRT-PCR analysis of pri-mir-17-92 in NM-CLs (n=3) and CCLs (n=4) exposed for six days to MIR17PTi (10μM) or equimolar scr-NC. Cell lines used in this panel were resistant to MIR17PTi (see Figure 2A and Supplementary Fig. S2A). The results shown are

average pri-mir-17-92 expression levels after normalization with GAPDH and ΔΔCt calculation (expressed as percentage).

(**c**) qRT-PCR analysis of miR-17-92*s* expression in SK-MM1 exposed for six days to MIR17PTi (10μM) or equimolar scr-NC. The results are average expression levels after normalization with RNU44 and ΔΔCt calculations (expressed as percentage).

(**d**) CCK-8 proliferation assay of NM-CLs (n=2) and CCLs (n=12) transfected with MIR17PTi (25nM) or equimolar scr-NC (two days after transfection).

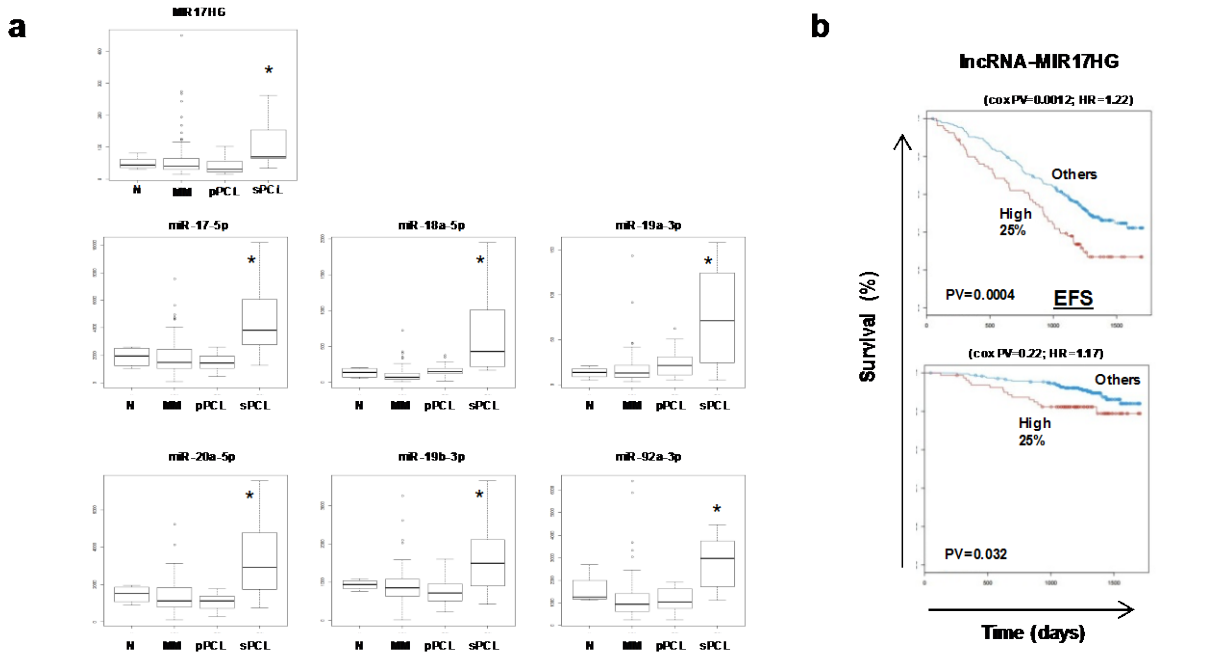
(**e**) Effects of mix-MIR17PTi on pri-mir-17-92 expression (left) and viability (right) after transfection of AMO1 cells (48h timepoint). The qRT-PCR results shown are average pri-mir-17-92 expression levels after normalization with GAPDH and ΔΔCt calculation (expressed as percentage). Viability was measured by CCK-8 assay.

(**f**) qRT-PCR analysis of pri-mir-17-92 in AMO1 transfected with indicated miR-17-92 LNA gapmeRs (25nM) or equimolar scr-NC (two days after transfection).

(**g**) CCK-8 proliferation assay of AMO1 or NCI-H929 or RPMI-8226 transfected with indicated miR-17-92 LNA gapmeRs (25nM) or equimolar scr-NC (two days after transfection).

Data from 1 out of 3 independent experiments is shown in each panel. **indicates $p<0.05$*

Supplementary Figure 4

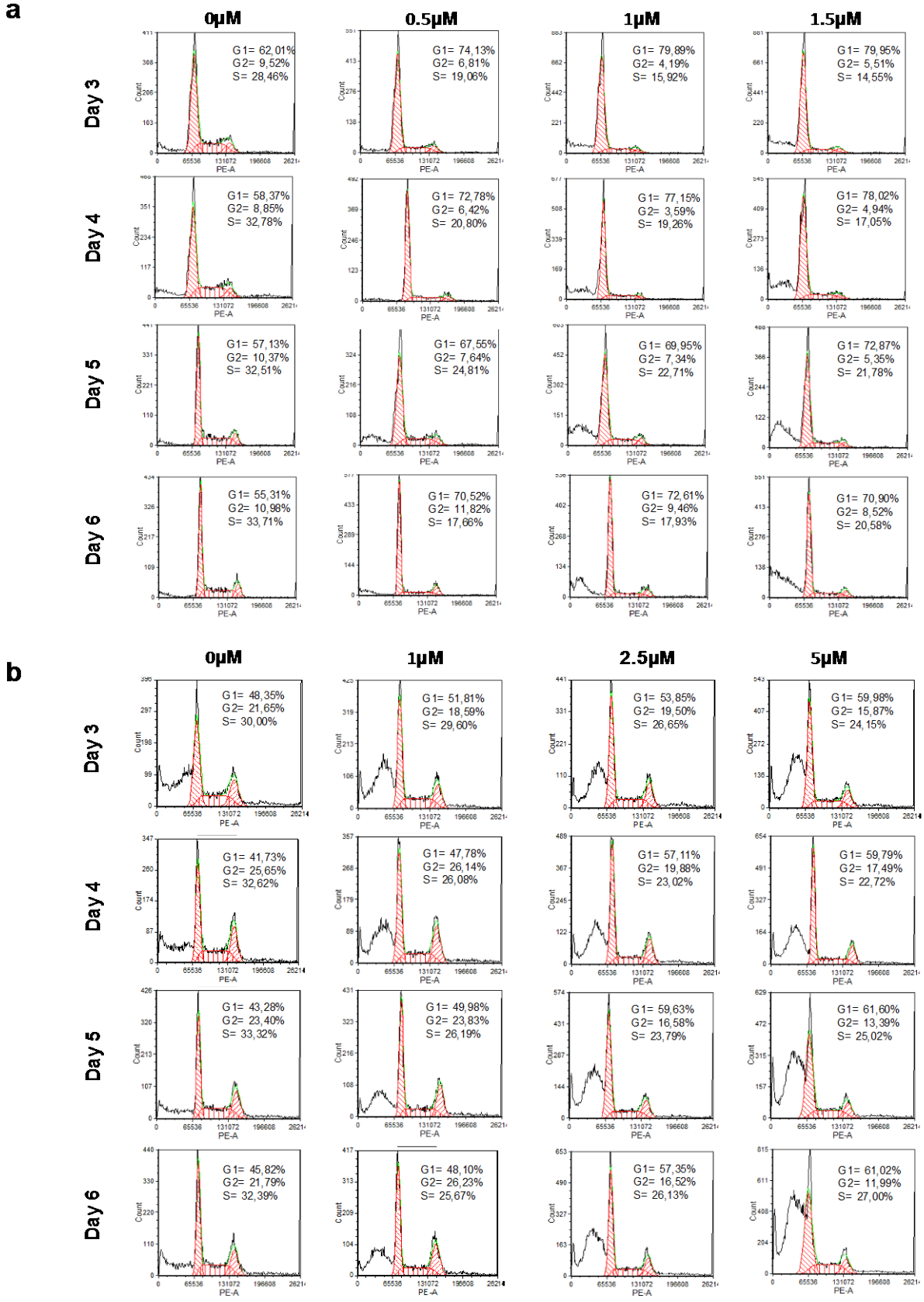


Supplementary Figure 4.

(**a**) Analysis of MIR17HG expression in CD138+ pMM cells (96 MM, 19 pPCL, 11 sPCL), as compared to normal donor-derived (N) CD138+ cells (n=4), from dataset series GSE70323 (GSE70319, MM; GSE73452, PCL) (upper panel); analysis of miR-17-92ss in CD138+ pMM cells (96 MM, 19 pPCL, 11 sPCL), as compared to normal donor-derived (N) CD138+ cells (n=4), from dataset series GSE70323 (GSE70254, MM; GSE73454, PCL).

(**b**) RNA-seq analysis of the prognostic significance (PFS and OS) of pri-mir-17-92 expression in 320 newly-diagnosed MM patients from IFM/DFCI 2009 clinical study (NCT0191060).

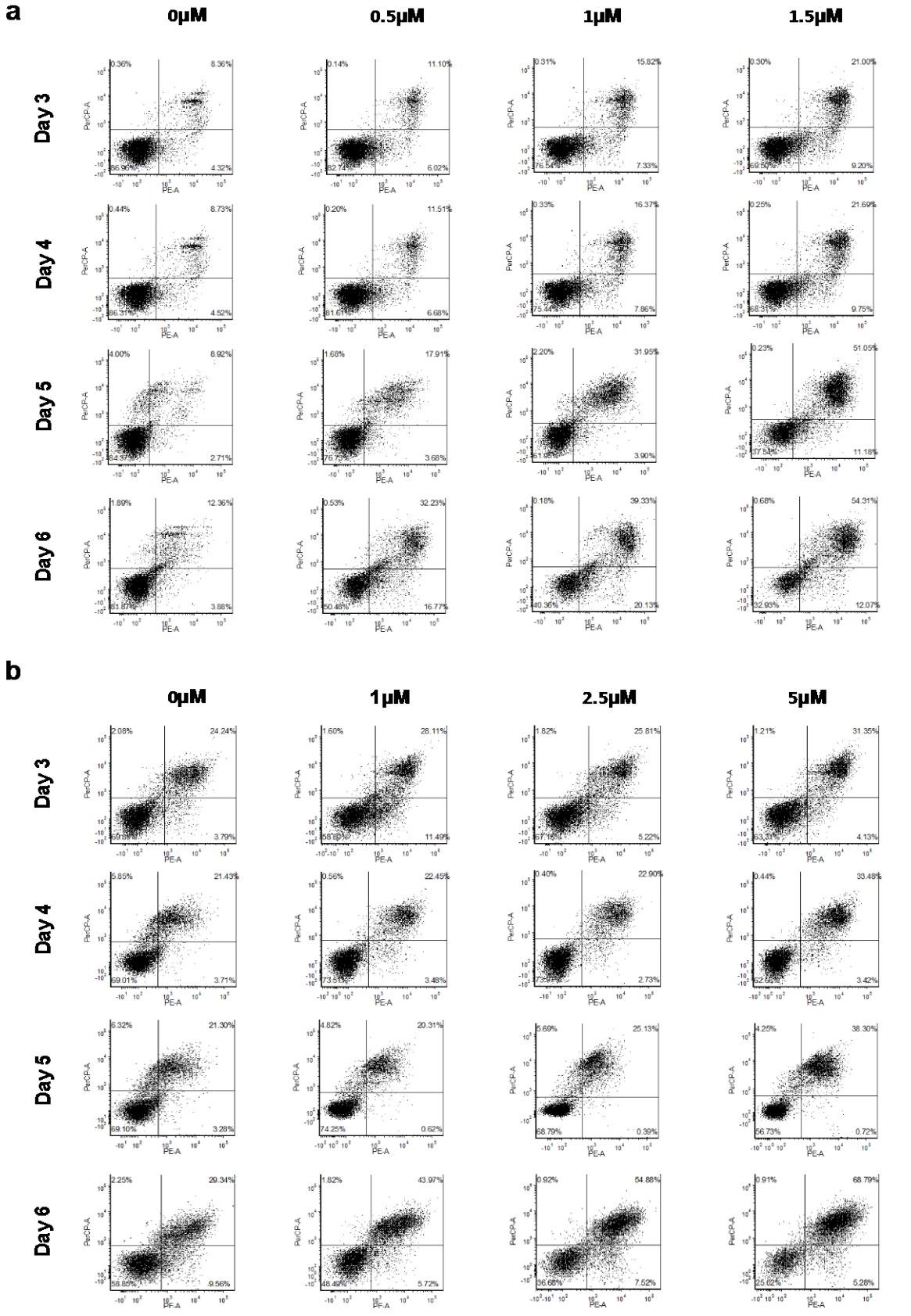
Supplementary Figure 5



Supplementary Figure 5.

(**a**-**b**) Cell cycle analysis of (A) AMO1 and (B) NCI-H929 exposed for different days (3-4-5-6) to indicated concentrations of MIR17PTi.

Supplementary Figure 6

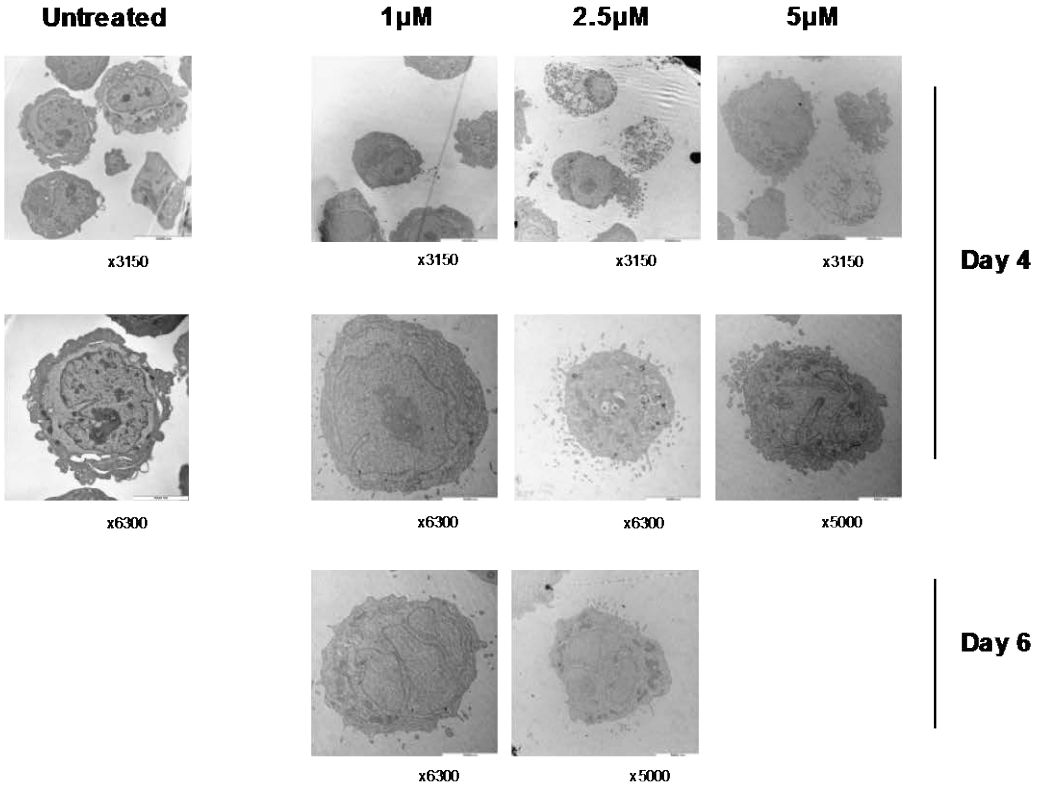


Supplementary Figure 6.

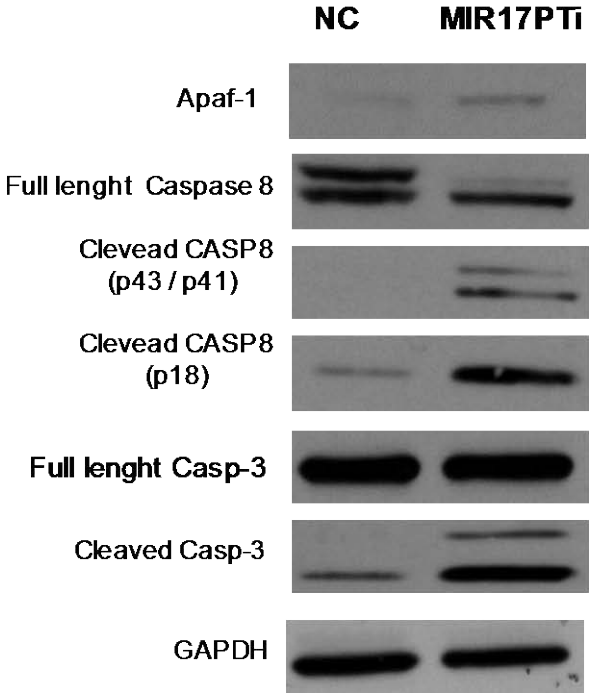
(**a**-**b**) Flow cytometry analysis of annexin V / 7-AAD stained (A) AMO1 and (B) NCI-H929 exposed for different days (3-4-5-6) to indicated concentrations of MIR17PTi.

Supplementary Figure 7

a



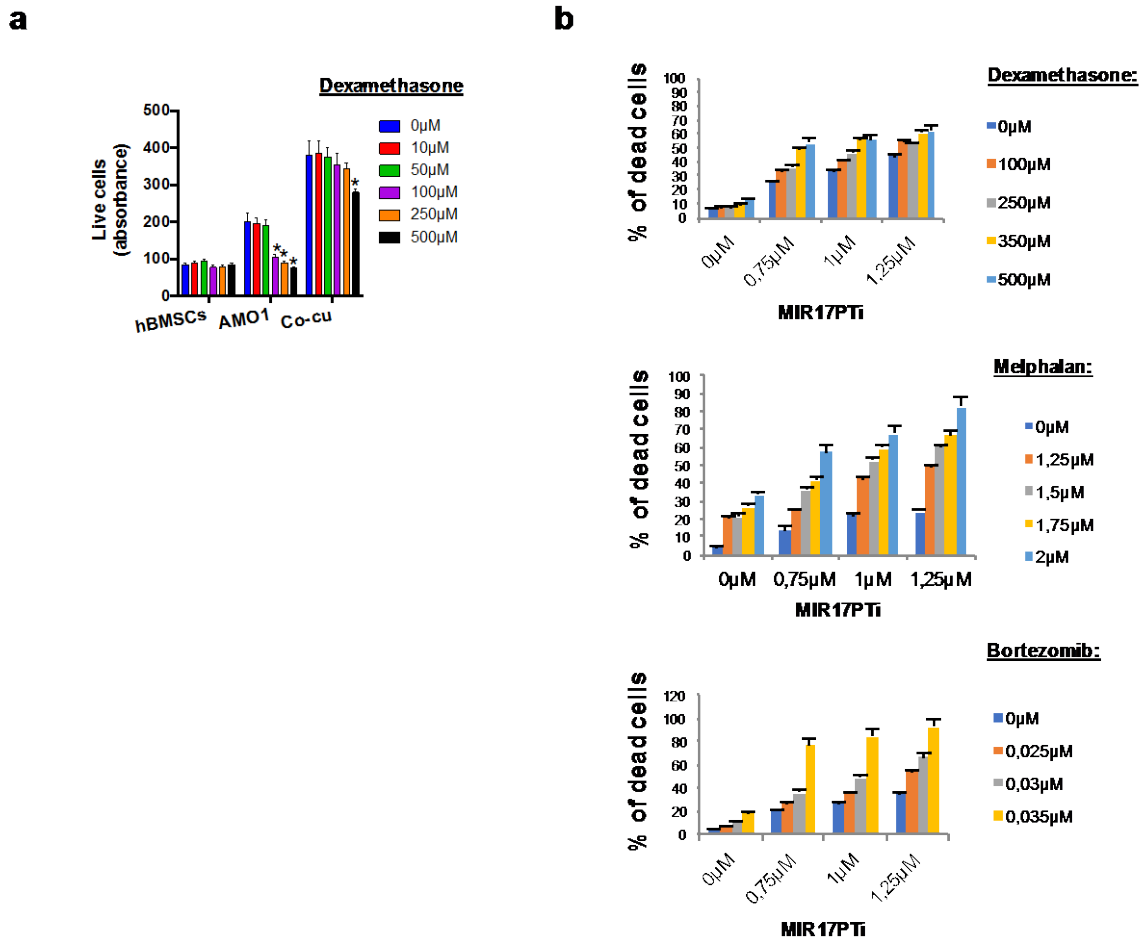
b



Supplementary Figure 7.

(**a**) Electron microscopy of NCI-H929 exposed for four or six days to indicated concentrations of MIR17PTi. Occurrence of apoptotic bodies is evident after treatment. (**b**) Western blot analysis of apoptosis-related proteins in lysates from NCI-H929 exposed for six days to MIR17PTi (2.5µM) or scr-NC (2.5µM). GAPDH was used as protein loading control.

Supplementary Figure 8



Supplementary Figure 8.

(**a**) CCK-8 assay of hBMSCs, AMO1 or AMO1 co-cultured with hBMSCs after six days of exposure to indicated concentrations of dexamethasone.

(**b**) Flow-cytometry analysis of 7-AAD stained AMO1 after six days of treatment with MIR17PTi (0-0.75-1-1.25 μM) and indicated concentrations of dexamethasone, melphalan or bortezomib. Data from 1 out of 3 independent experiments is shown in each panel.

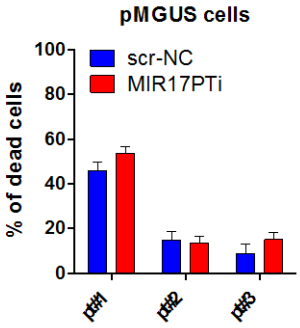
Data from 1 out of 3 independent experimens is shown in each panel. **indicates p<0.05*

Supplementary Figure 9

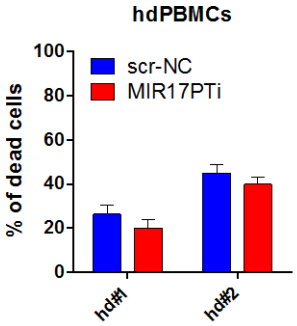
a

PATIENT ID	% of CD138+	Stage
MM#1	99	IM / ND
MM#2	70	IM / ND
MM#3	94	IM / ND
MM#4	98.5	IM / ND
MM#5	93	IM / Rel
MM#6	97	IM / ND
MM#7	90	IM / ND
MM#8	97	IM / ND
MM#9	98.1	IM / ND
MM#10	99.8	IM / Rel
MM#11	99.7	IM / ND
MM#12	95	EM / Rel
MM#13	95	EM / Rel
MGUS#1	77	IM
MGUS#2	52	IM
MGUS#3	60	IM

b



c



Supplementary figure 9.

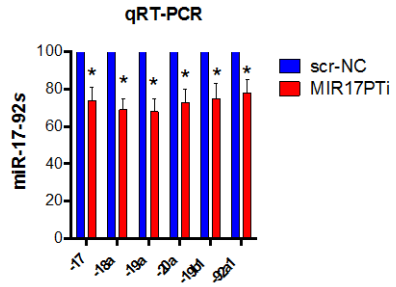
(**a**) Table showing the purity (% of CD138+ cells) and clinical stage (IM: intra-medullary disease; EM: extra-medullary disease; ND: newly diagnosis; Rel: relapsed disease) of immunoselected cells from MM or MGUS patients.

(**b**) Flow-cytometry analysis of 7-AAD stained CD138+ MGUS cells exposed for six days to MIR17PTi or scr-NC (2,5µM).

(**c**) Flow-cytometry analysis of 7-AAD stained healthy donor-PBMCs exposed for six days to MIR17PTi or scr-NC (10µM).

**indicates p<0.05*

a



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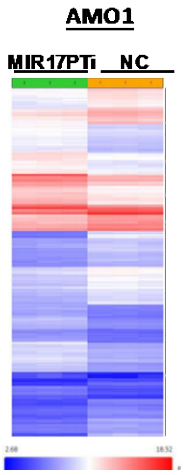

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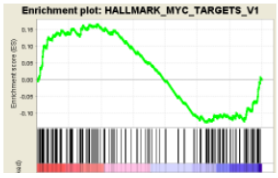


d

Dataset	Size	NES	FDR q-val
SCHUHMACHER_MYC_TARGETS_UP	74	2.27	0.04
DANG_MYC_TARGETS_UP	120	2.25	0.04
MENSEEN_MYC_TARGETS	48	2.15	0.04
DANG_REGULATED_BY_MYC_UP	66	1.69	0.06

Supplementary Figure 10.

(**a**) qRT-PCR analysis of miR-17-92*s* in pMM cells (n=3) exposed for six days to MIR17PTi (2,5µM) or equimolar scr-NC. The results shown are average miR-17-92*s* expression levels after normalization with RNU44 and ΔΔCt calculation (expressed as percentage).

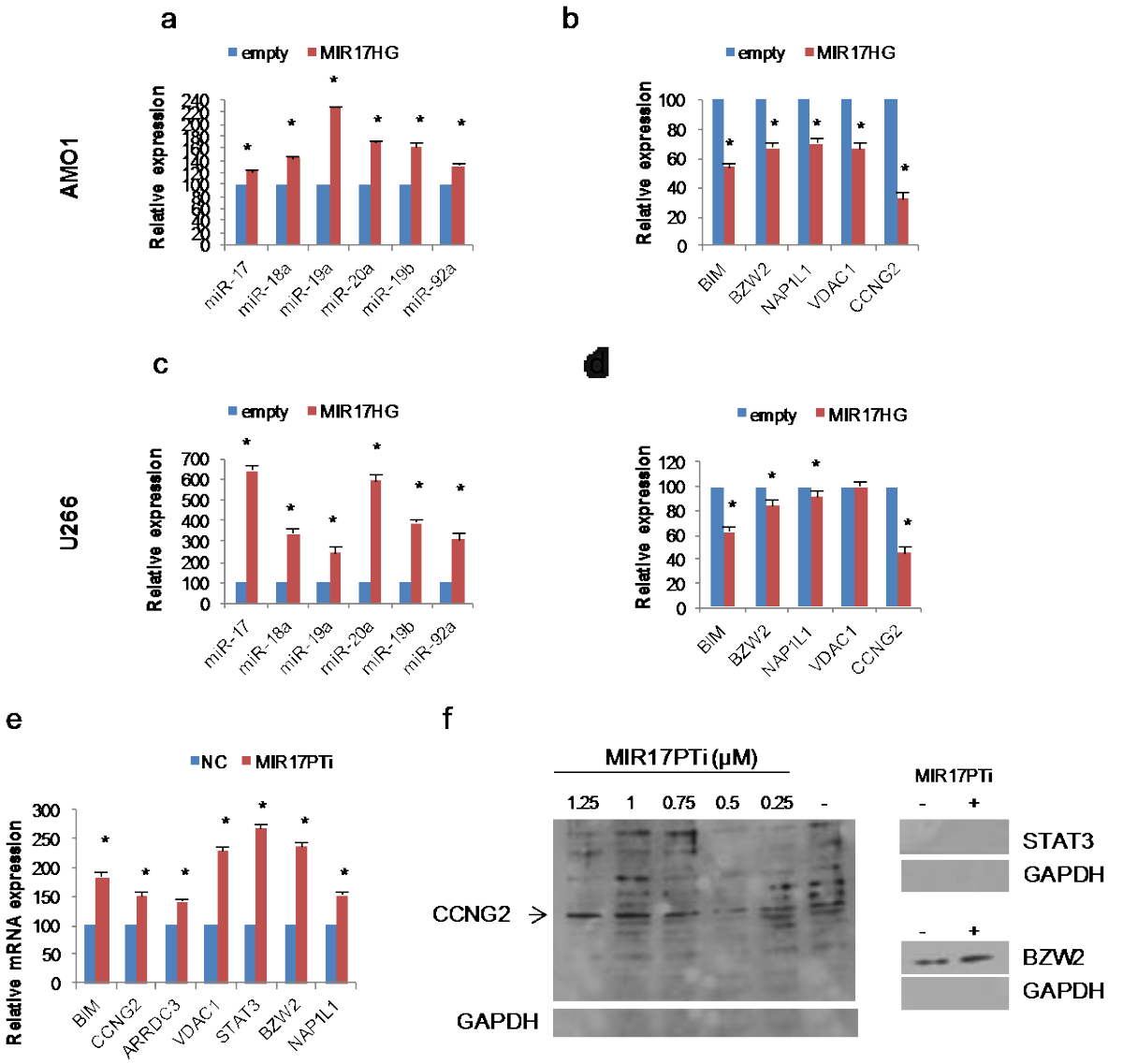
(**b**) Hierarchical clustering of AMO1 exposed for six days to MIR17PTi (1 µM) or equimolar scr-NC.

(**c**) Enrichment plot of the HALLMARK_MYC_TARGET_V1 in the positive phenotype (relative to AMO1 exposed to MIR17PTi).

(**d**) Table of gene sets, from the Hallmark collection, enriched of genes upregulated by MIR17PTi (positive phenotype) in AMO1 exposed to MIR17PTi (1µM) for six days.

Number of genes in each set (size), the normalized enrichment score (NES), and test of statistical significance (FDR q value) are highlighted.
**indicates $p<0.05$*

Supplementary Figure 11



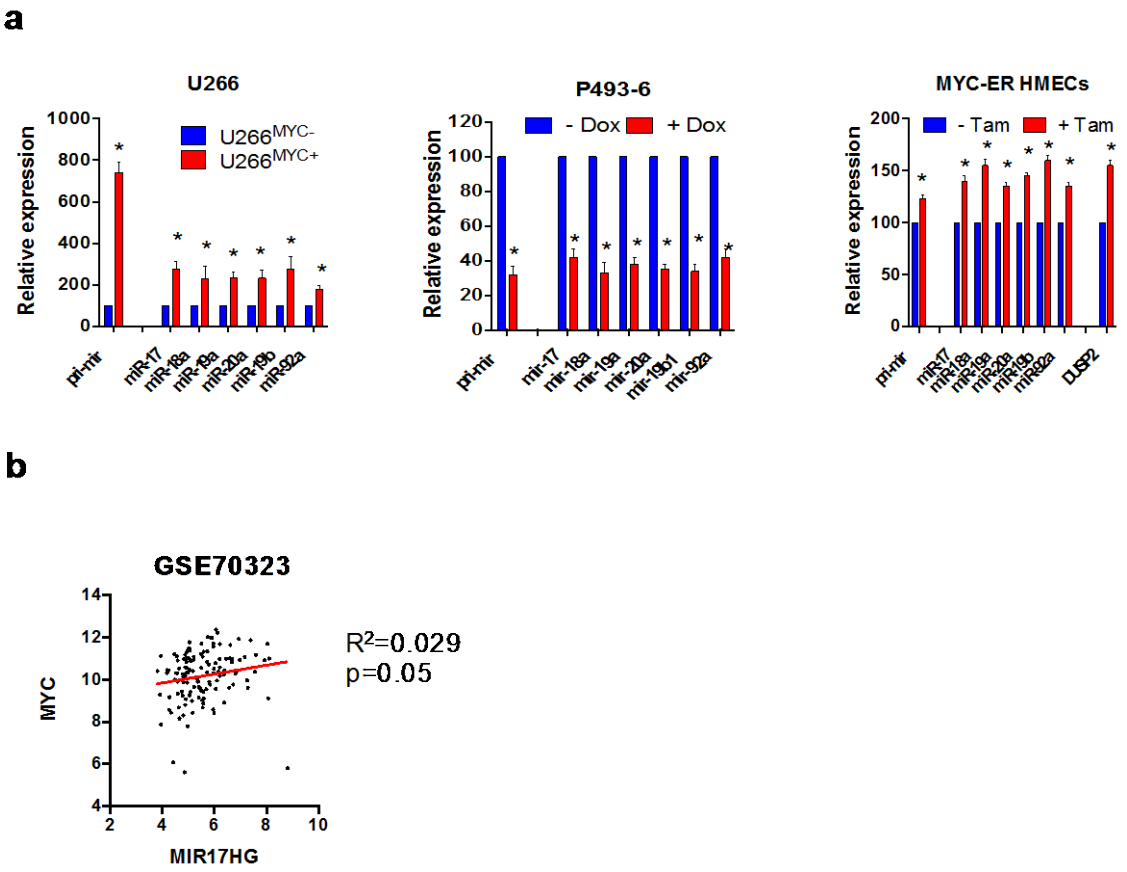
Supplementary Figure 11.

(**a**-**b**) qRT-PCR analysis of (A) miR-17-92*s* and (B) indicated mRNAs in AMO1 transduced with a miR-17-92*s* lentiviral vector.

(**c**-**d**) qRT-PCR analysis of (C) miR-17-92*s* and (D) indicated mRNAs in U266 transduced with a miR-17-92*s* lentiviral vector.

(**e**) qRT-PCR analysis of indicated mRNAs in AMO1 exposed to MIR17PTi (1µM) for six days. The results shown are average miRNA or mRNA expression levels after normalization with RNU44 or GAPDH and ΔΔCt calculation (expressed as percentage).
(**f**) Western blot analysis of CCNG2, BZW2 and STAT3 in lysates from AMO1 exposed to MIR17PTi for six days. GAPDH was used as protein loading control.
**indicates p<0.05*

Supplementary Figure 12

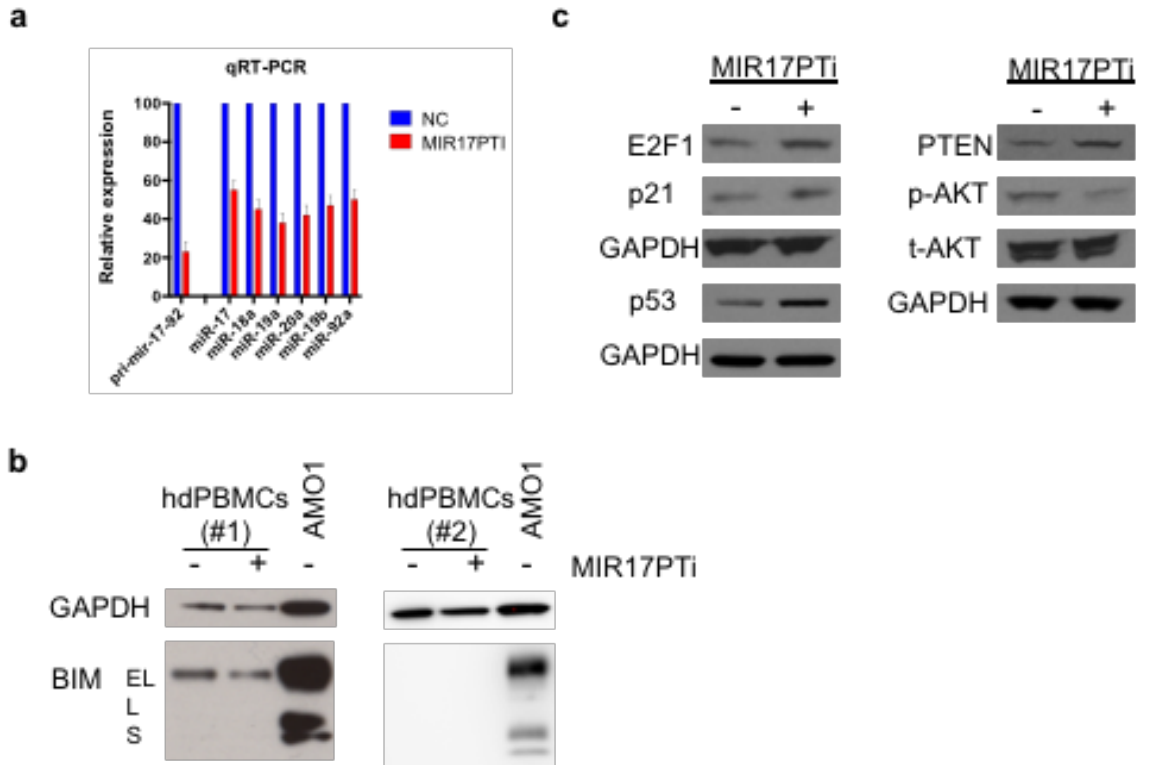


Supplementary Figure 12.

(**a**) qRT-PCR analysis of pri-mir-17-92 and miR-17-92*s* in: (left panel) U266^{MYC+} as compared to U266^{MYC-}; P493-6 cultured for two days with or without doxycycline (Dox); MYC-ER HMECs cultured for two days with or without tamoxifen (Tam). In this latter panel it is also shown the expression of DUSP2 as positive control of MYC activation. The results shown are average pri-mir-17-92 or DUSP2 or miR-17-92*s* expression levels after normalization with GAPDH (pri-mir-17-92 and DUSP2) or RNU44 (miR-17-92*s*), and ΔΔCt calculations (expressed as percentage).

(**b**) Integrative analysis of MYC and MIR17HG in CD138+ pMM cells (96 MM, 19 pPCL, 11 sPCL) from dataset series GSE70323 (GSE70319, MM; GSE73452, PCL).

Data from 1 out of 3 independent experiments is shown in each panel. **indicates p<0.05*



Supplementary Figure S13.

(**a**) qRT-PCR analysis of pri-mir-17-92 and miR-17-92*s* in healthy donor PBMCs (hdPBMCs; n=2) after 6 days of treatment with MIR17PTi (5 µM) or equimolar scr-NC. The results shown are average pri-mir-17-92 or miR-17-92*s* expression levels after normalization with GAPDH (pri-mir-17-92) or RNU44 (miR-17-92*s*), and ΔΔCt calculations (expressed as percentage).

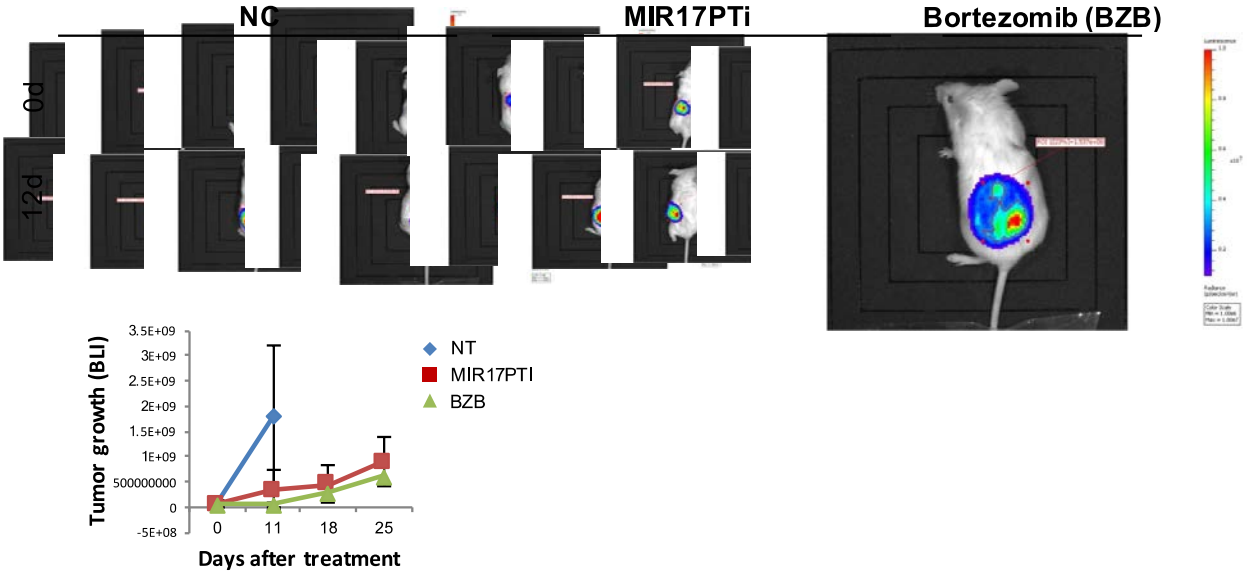
(**b**) Western blot analysis of BIM in lysates from hdPBMCs (n=2) after 6 days of treatment with MIR17PTi or scr-NC 5 µM or AMO1 (basal level). GAPDH was used as protein loading control.

(**c**) Western blot analysis of E2F1, p21, P53, PTEN, p-AKT and t-AKT in lysates from NCI-H929 exposed to MIR17PTi (2.5µM) for six days. GAPDH was used as protein loading control.

Supplementary Figure 14

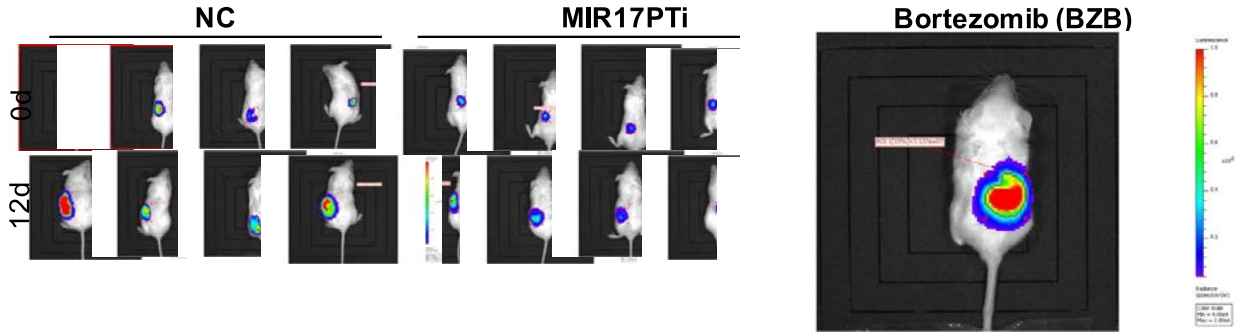
a

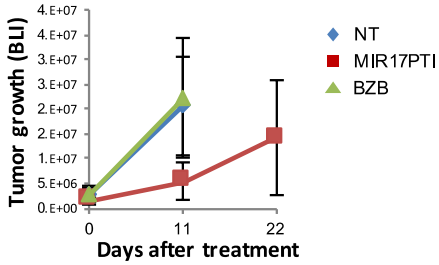
AMO1luc+



b

ABZBluc+

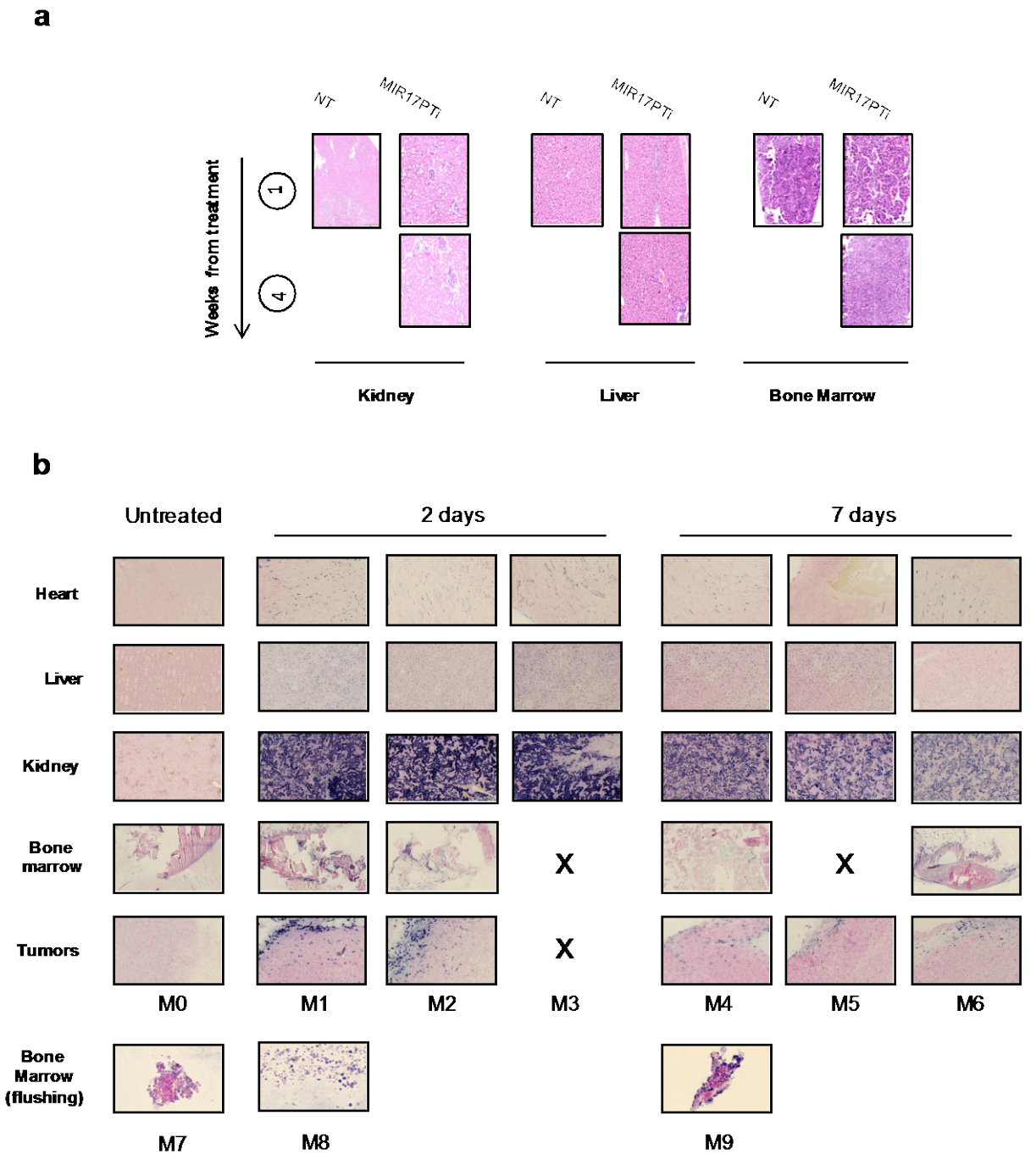




Supplementary Figure 14.

(**a**-**b**) IVIS images of NOD SCID mice s.c. xenografted with luciferase gene-marked (A) AMO1 or (B) ABZB. Tumor growth, as assessed by BLI analysis, is also reported.

Supplementary Figure 15



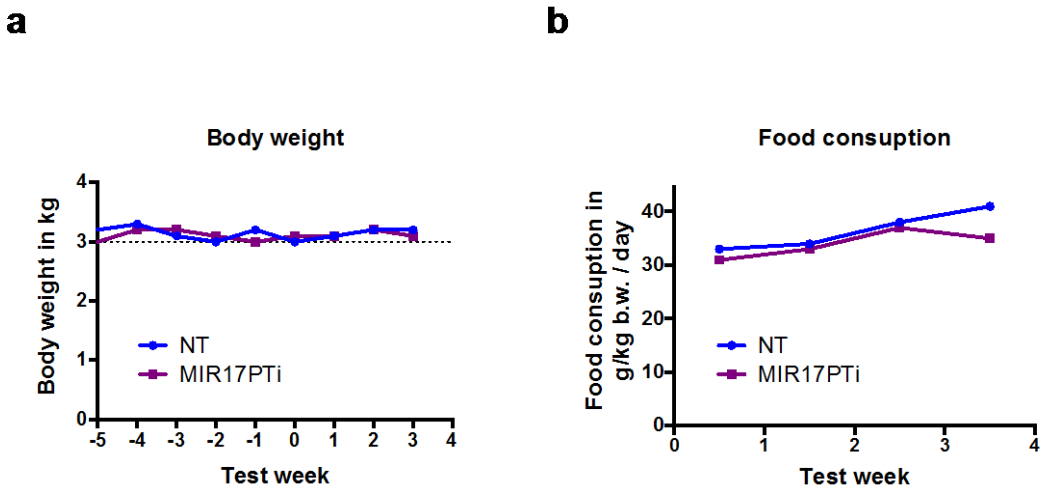
Supplementary Figure 15.

(**a**) H&E staining of kidney, liver and bone marrow from mice i.v. injected with MIR17PTi (2mg/kg) at days 1-4-8-15-22. Mice were sacrificed one or four weeks after last injection.

(**b**) Detection of MIR17PTi by ISH analysis of indicated organs or NCI-H929 retrieved xenografts (tumors). Mice were sacrificed two or seven days after single dose of MIR17PTi 2mg/kg.

Organs and tumor from untreated mouse is also shown.

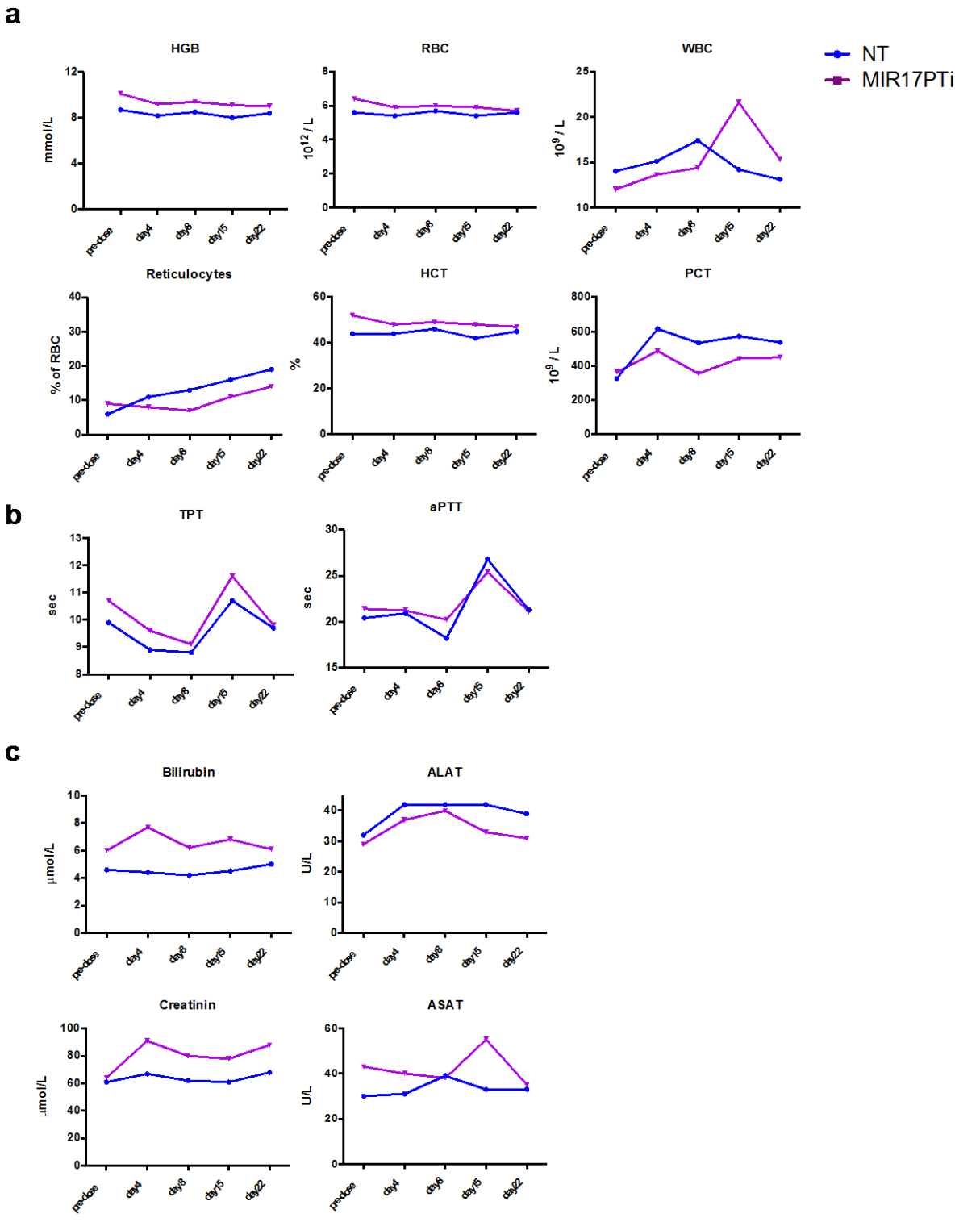
Supplementary Figure 16



Supplementary Figure 16.

(**a**) Body weight and (**b**) food consumption of Cynomolgus monkeys (n=2) i.v. injected with MIR17PTi (0.504mg/kg) or saline solution at days 1-4-8-15-22. Both parameters were evaluated once weekly for four weeks after first injection.

Supplementary Figure 17



Supplementary Figure 17.

(a) Hematology, (**b**) coagulation and (**c**) clinical biochemistry parameters in Cynomolgus monkeys (n=2) i.v. injected with MIR17PTi (0.504mg/kg) or saline solution at days 1-4-8-15-22. Sampling was performed before treatment (pre-dose) and at days 4-8-15 and 22.

Supplementary Table

Supplementary Table S1. miR-17-92*s* targets upregulated by MIR17PTi in sensitive pMM cells.

Gene symbol	Gene Family (according to IPA)	Targeting miRNA (predicted by miRcode)	Targeting miRNAs (validated by 3'UTR lucferase assay)	MYC-binding loci	MYC direct targeting	MYC direct targeting (validated in B cells)	Upregulated in AMO1 by MIR17PTi (p<0.05)	Upregulated in resistant pMM cells by MIR17PTi
AGPAT5	Enzyme	*-19 / -25*						
AKAP13	Other	*-17 / -18 / -19 / -25*		V[21]	V (neg)[21]	V[21]		
ANKRD28	Other	*-17 / -18 / -19 / -25*						
ATOX1	Transporter	*-17 / -18 / -19 / -25*		V[40]				
ATP2B1	Transporter	*-17 / -18 / -19 / -25*						
BCL2L11	Other	*-17 / -19 / -25*	V[41]	V[17]	V (pos)[17,26]	V[17]	V	
BICD2	Other	*-17 / -19 / -25*		V[21]	V (pos)[21]	V[21]	V	
BTG2	Transcription Regulator	*-17 / -18 / -25*						
BZW2	Translation regulator	*-17 / -19*		V[20,21]	V (pos)[20,21]	V[20,21]	V	
CDK13	Kinase	*-17 / -18 / -19*						
CHD4	Enzyme	*-17*					V	
COL4A3BP	Kinase	*-25*						
DDX42	Enzyme	*-18*						
DDX6	Enzyme	*-17 / -19 / -25*		V[21]				
DUSP2	Phosphatase	*-17*		V[21]	V (pos)[21]			
ESYT1	Other	*-19*						
GNAS	Enzyme	*-17 / -18 / -19 / -25*		V[21]	V (neg)[21]	V[21]		
GRK6	Kinase	*-19*						
H2AFV	Other	*-17 / -18*		V[21]				
HECA	Other	*-17*						
HP1BP3	Other	*-17 / -18 / -25*						
IGF2R	Transmembrane Receptor	*-18 / -19 / -25*						
ILF3	Trancription Regulator	*-19 / -25*						
IPO7	Transporter	*-17 / -25*		V[21]	V (pos)[21]	V[21]		
KIAA0226	Other	*-17 / -18*		V[21]				
KIAA1432	Other	*-17 / -18 / -19 / -25*						
MAML1	Transcription Regulator	*-18 / -19 / -25*						
MAN2A1	Enzyme	*-17 / -19 / -25*		V[21]			V	
MAT2A	Enzyme	*-17 / -19*		V[19]	V (pos)[19]	V[19]		
MBOAT2	Enzyme	*-17 / -19 / -25*					V	
MBTPS1	Peptidase	*-17*		V[21]				
MLXIP	Other	*-17 / -25*		V[42]	V (pos)[42]		V	
NAP1L1	Other	*-18 / -19 / -25*		V[20]	V (pos)[20]	V[20]	V	V

Gene symbol	Gene Family (according to IPA)	Targeting miRNAs (predicted by miRcode)	Targeting miRNAs (validated by 3' UTR luciferase assay)	MYC-binding loci	MYC direct targeting	MYC direct targeting (validated in B cells)	Upregulated in AMO1 by MIR17PTi	Upregulated in resistant pMM cells by MIR17PTi
NPTN	Other	*-19 / -25*						
NR4A2	Ligand Dependent Nuclear Receptor	*-17 / -19*						
OTUD1	Peptidase	*-17 / -19*						
PARP8	Other	*-18 / -19 / -25*					V	
PFN1	Other	*-19 / -25*		V[21]				
PHF1	Transcription regulator	*-17 / -25*						
PNRC1	Other	*-19*						
PTK2B	Kinase	*-17 / -18 / -19*		V[21]	V (neg)[21]	V[21]	V	
RAB30	Enzyme	*-17 / -19 / -25*						
RAF1	Kinase	*-17 / -19*						
RASSF3	Other	*-17 / -18 / -19 / -25*		V[21]				
RBM3	Other	*-18*						
RNF145	Other	*-17 / -18 / -19*					V	
SKIL	Transcription Regulator	*-17 / -19 / -25*		V[21]	V (neg)[21]	V[21]		
SLC31A2	Transporter	*-17 / -18 / -19*						
SLC5A6	Transporter	*-17 / -19*		V[19]	V (pos)[19]	V[19]		
SSFA2	Other	*-17 / -18 / -25*						
SYPL1	NA	*-18 / -19 / -25*						
TCEB3	Transcription regulator	*-17 / -19 / -25*		V[19]				
TGFBR2	Kinase	*-17 / -19 / -25*	V[43]					
TNPO2	Transporter	*-19*		V[21]				
TRAM2	Other	*-17 / -18 / -25*					V	
TRIM8	Other	*-17 / -18*		V[21]	V (neg)[21]	V[21]		
UBFD1	Other	*-17 / -19*		V[44]				
UBQLN4	Other	*-17*						
USP21	Peptidase	*-18 / -19 / -25*						
VDAC1	Ion channel	*-17*		V[21]	V (pos)[21]	V[21]	V	
VPS52	Other	*-18*						
WNK3	Kinase	*-17 / -18 / -19*		V[21]				
YAF2	Transcription regulator	*-17 / -18 / -19*		V[21]				
ZC3H7B	Other	*-17 / -18*						
ZDHHC18	NA	*-19*						

Supplementary References

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