Supplementary Information

**Che-1/miR-590-3p/TAZ axis sustains multiple myeloma disease.**

*Bruno et al.*

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## Supplementary Material and Methods

### Western blot analysis

Total cell extracts (TCEs) were prepared, and the proteins were resolved as previously described [10]. Following blocking step in 5% non-fat-dried milk in phosphate-buffered saline (PBS)-0.1% Tween, membranes were incubated with primary antibodies overnight at 4°C. After three washes in PBS - 0.1% Tween, membranes were incubated with the appropriate HRP-linked secondary antibodies (Bio-Rad Laboratories) for 45 min at room temperature, washed with PBS-0.1% Tween and analysed by chemi-luminescence (GE Healthcare Life Science). Images were acquired and quantified using Alliance Mini HD6 system by UVITEC Ltd., Cambridge, equipped with UVI1D Software (UVI- TEC, 14-630,275). Detailed information for all antibodies is provided in Supplementary Table S2.

### RNA-seq

Total RNA was extracted using Qiazol (Qiagen, IT), purified from DNA contamination through a DNase I (Qiagen, IT) digestion step and further enriched by Qiagen RNeasy columns for gene expression profiling (Qiagen, IT). Quantity and integrity of the extracted RNA were assessed by NanoDrop Spectrophotometer (NanoDrop Technologies, DE) and by Agilent 2100 Bioanalyzer (Agilent Technologies, CA), respectively. RNA libraries for sequencing were generated in triplicate using the same amount of RNA for each sample according to the Illumina Stranded Total RNA Prep kit with an initial ribosomal depletion step using Ribo-Zero Plus (Illumina, CA). The libraries were quantified by qPCR and sequenced in paired-end mode (2x100 bp) with NovaSeq 6000 (Illumina, CA). For each sample generated by the Illumina platform, a pre-process step for quality control was performed to assess sequence data quality and to discard low-quality reads.

**Computational methods**

RNA-seq analysis was run using the Kallisto v0.46.2 18 and Sleuth v0.30.019 recommended pipelines. Reads were quantified with the command kallisto quant -t 12 -b 30 and pseudoaligned to the reference genome GRCm38.96 downloaded from NCBI and successfully indexed using the command kallisto index. Differential analysis was performed using Sleuth pipeline deploying the full model.

**Statistical analysis**

Data are presented as mean of three independent experiments ± SD. Statistical analyses were performed using R software. Two-tailed Student's *t*-tests with Benjamini – Hochberg correction were performed to compare one parameter between two groups. For multiple group comparison, one-way ANOVA with Tukey HSD test was used. $P < 0.05$ was considered significant. Statistical significance is indicated by asterisks as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, ****$P < 0.001$, n.s. = not significant.

**CoMMpass dataset**

We downloaded the row gene counts matrix of the CoMMpass dataset (IA15 release) from the MMREF Research Gateway Portal (table name MMRF_CoMMpass_IA14a_E74GTF_Salmon_Gene_Counts). The patient cohort was filtered based on the availability of unique annotation of International Staging System (ISS) and transcriptome data and included 687 samples. Gene counts related to our selected cohort were normalized by following a multistep pipeline using edgeR functions (DGEList, estimateCommonDisp, estimateTaqwiseDisp, calcNormFactors). The matrix with normalized gene counts was expressed as transcripts per million (tpm). Gene of interest as TAZ, Che-1 and YAP were selected from the matrix and plotted by relative ISS score associated with each patient by utilizing ggpubr v.0.4.0 R package.

**Survival Analysis**

We carried out a survival analysis for defining the importance of TAZ in the progression of MM disease. The Overall Survival (OS) data were retrieved by downloading the table named MMRF_CoMMpass_IA15_STAND_ALONE_SURVIVAL.csv from the portal cited above. For this analysis, we removed the patients with OS greater than 65 months (n=56) from our cohort. Survival curves were obtained with the Kaplan-Meier product-limit method and compared with a log-rank test. Univariate Cox regression was computed to investigate the potential role of TAZ as a predictor of survival. The survival curve examined the patients with high level of TAZ expression versus those with low level. Patients with TAZ expression higher than its median expression in our whole cohort were included in the TAZ high group (n=312), and the other patients were collected into the TAZ low group (n=327). We used survfit and Surv functions in survival v.3.1.12 R package for the survival analysis. The Kaplan-Meier curve was generated by utilizing the function ggsurvplot of survminer v.0.4.819 R package and it had significance equal to log-rank p-value<0.001.

**Supplementary references**


1. Agnelli L, Mosca L, Fabris S, Lionetti M, Andronache A, Kwee I, et al. A SNP microarray and FISH-based procedure to detect allelic imbalances in multiple myeloma: an integrated genomics approach. reveals a wide gene dosage effect. GENES, CHROMOSOMES & CANCER 2009 Jul.;48(7):603-14.

2. Fulford A, Tapon N, Ribeiro PS. Upstairs, downstairs: spatial regulation of Hippo signalling. Review Curr Opin Cell Biol. 2018 Apr; 51:22-32.

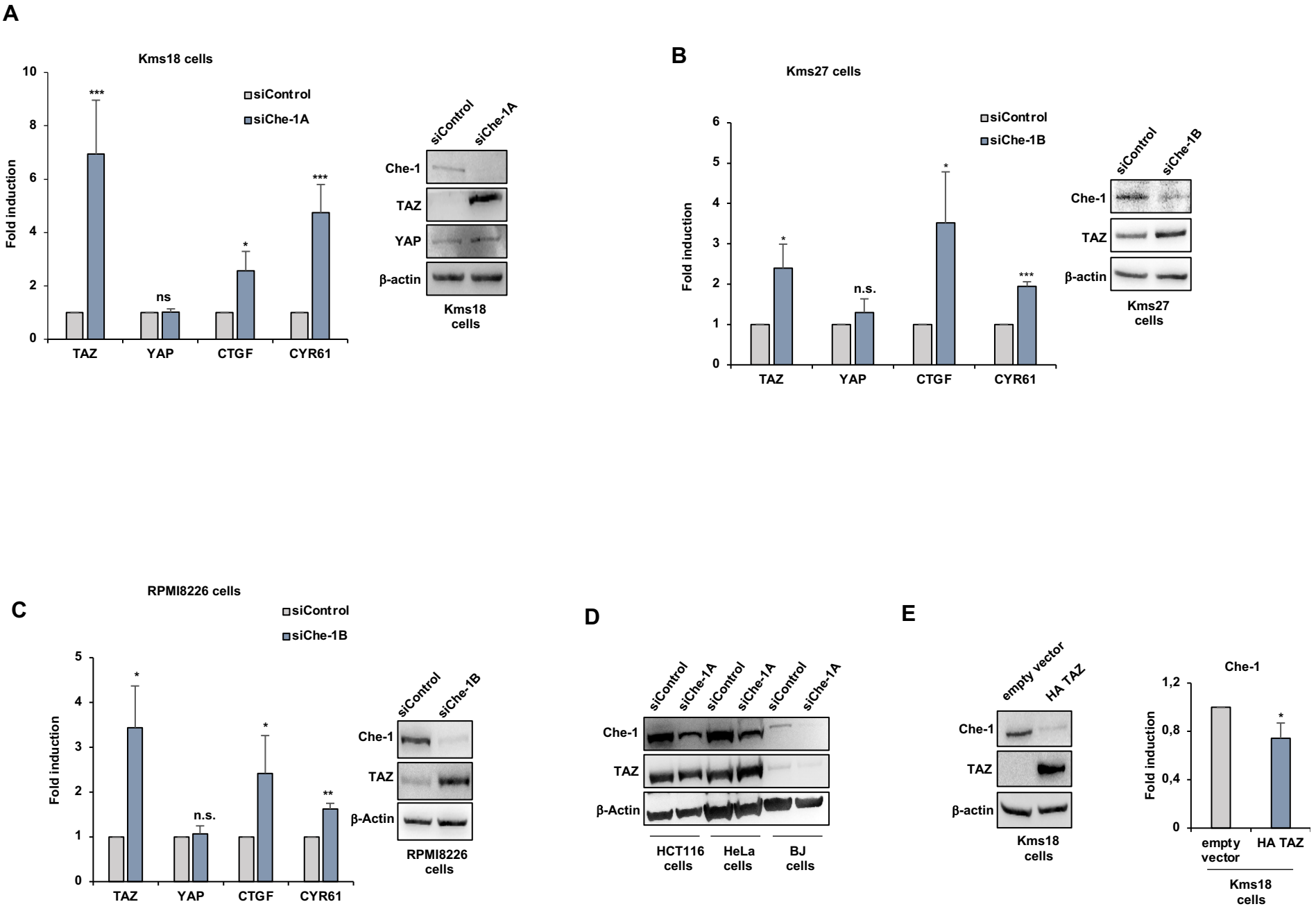
## Supplemental Table 1: Primer Index

| **RT-qPCR mRNA primers** | | |
|---|---|---|
| **Human Primers** | **Fwd/Rvs** | **Sequence 5'- 3'** |
| Actin | Fwd | GACAGGATGCAGAAGGAGATTACT |
| | Rvs | TGATCCACATCTGCTGGAAGGT |
| TAZ/WWTR1 | Fwd | GTCACCAACAGTAGCTCAGATC |
| | Rvs | AGTGATTACAGCCAGGTTAGAAAG |
| YAP | Fwd | CACAGCATGTTCGAGCTCAT |
| | Rvs | GATGCTGAGCTGTGGGTGTA |
| CTGF | Fwd | AGGAGTGGGTGTGTGACGA |
| | Rvs | CCAGGCAGTTGGCTCTAATC |
| CYR61 | Fwd | CCTTGTGGACAGCCAGTGTA |
| | Rvs | ACTTGGGCCGGTATTTCTTC |
| RUNX2 | Fwd | TGGTTACTGTCATGGCGGGTA |
| | Rvs | TCTCAGATCGTTGAACCTTGCTA |
| AXIN2 | Fwd | TACACTCCTTATTGGGCGATCA |
| | Rvs | TTGGCTACTCGTAAAGTTTTGGT |
| COL1A1 | Fwd | GAGGGCCAAGACGAAGACATC |
| | Rvs | CAGATCACGTCATCGCACAAC |
| EIF4H | Fwd | GCATAAGGAGTGTACGGCTAGT |
| | Rsv | CCATCGTATGTCAAGGCTTCC |
| Mycoplasm | Fwd | ACTCCTACGGGAGGCAGCAGTA |
| | Rvs | TCGACCATCTGTCACTCTGTTAAC |
| Epstein-Barr virus (EBV) | Fwd | GGAACCTGGTCATCCTTTGC |
| | Rvs | ACGTGCATGGACCGGTTAAT |
| miR590-3p | Fwd | TGAGCTTATTCATAAAAGTGC |
| | Rvs | TGCTGCATGTTTCAATCAGA |
| DEPTOR | Fwd | AGCTTTGCCACCGGCTTAT |
| | Rvs | GGCAGAAGGGACTGTCATGAG |
| MKK6 | Fwd | ACTGTCCATTCACTGTCACC |

| | | |
|---|---|---|
| | Rvs | AGCAACAGAGTCCACCAAG |
| IFIT2 | Fwd | GCGAAACAACTGCTCCATCT |
| | Rvs | CCAAGACATGCAAAGCCTCA |
| ICAM1 | Fwd | ATGCCCAGACATCTGTGTCC |
| | Rvs | GGGGTCTCTATGCCCAACAA |
| CXCL10 | Fwd | AGCAGAGGAACCTCCAGTCT |
| | Rvs | AGGTACTCCTTGAATGCCACT |
| **Mouse Primers** | **Fwd/Rvs** | **Sequence 5'- 3'** |
| TAZ/WWTR1 | Fwd | AACAGTAGCTCAGATCCTTTCCTC |
| | Rvs | CCGCTCTGCCTCATCACTTGGTC |
| YAP | Fwd | TGAGATCCCTGATGATGTACCAC |
| | Rvs | TGTTGTTGTCTGATCGTTGTGA |
| CTGF | Fwd | GGGCCTCTTCTGCGATTTC |
| | Rvs | ATCCAGGCAAGTGCATTGGTA |
| CYR61 | Fwd | CTGCGCTAAACAACTCAACGA |
| | Rvs | GCAGATCCCTTTCAGAGCGG |
| EIF4H | Fwd | AGTTCAGGGTGACATAGATGCT |
| | Rvs | GGGAATCCACCTCATCAAATTCT |
| | | |

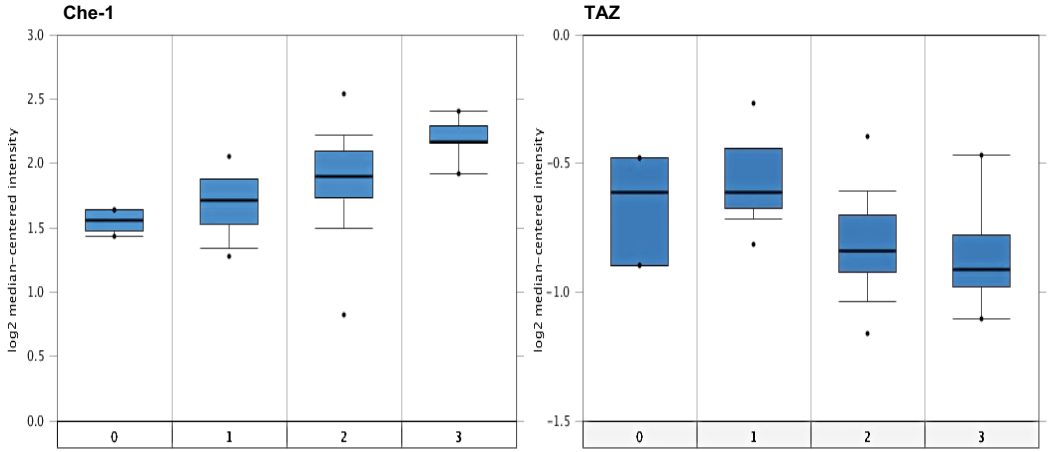
## Supplemental Table 2: Antibodies Index

| Antibody | Supplier | Reference |
|---|---|---|
| Rabbit Polyclonal anti-Che-1 | Millipore | Cat# ABC953 |
| Mouse Monoclonal anti-b-actin | Sigma Aldrich | Cat# A5441 |
| Rabbit TAZ/WWTR1 | Proteintech Group | 23306-I-AP |
| Rabbit YAP1 | Invitrogen | PA1-46189 |
| Rabbit Phospho-TAZ (Ser89) (E1X9C) | Cell Signaling | mAb #59971 |




**Supplementary Figure 1**

A



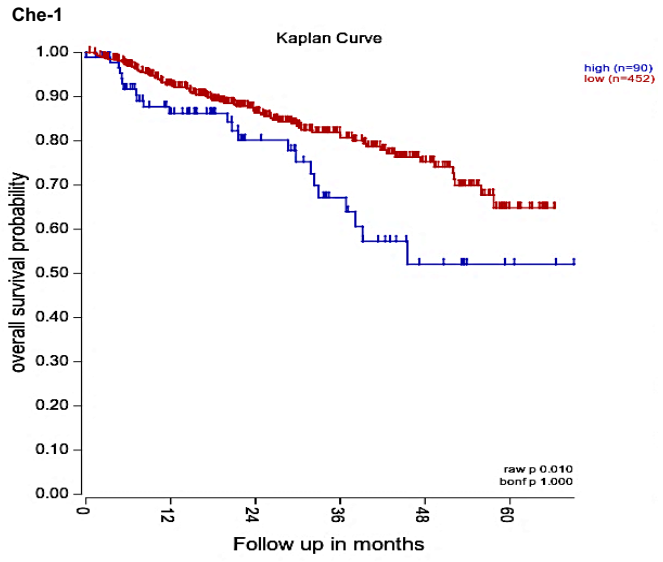

0= no value (5)

1= MGUS (11)

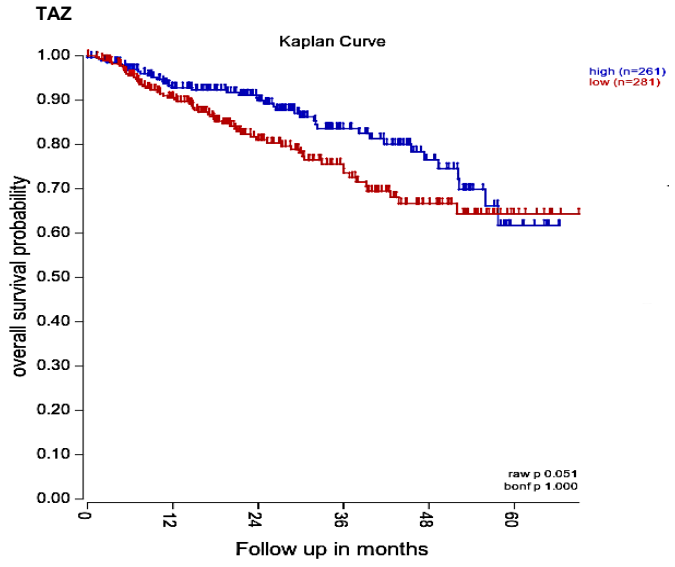
2= Multiple Myeloma (133)

3= Plasma Cell Leukemia (9)

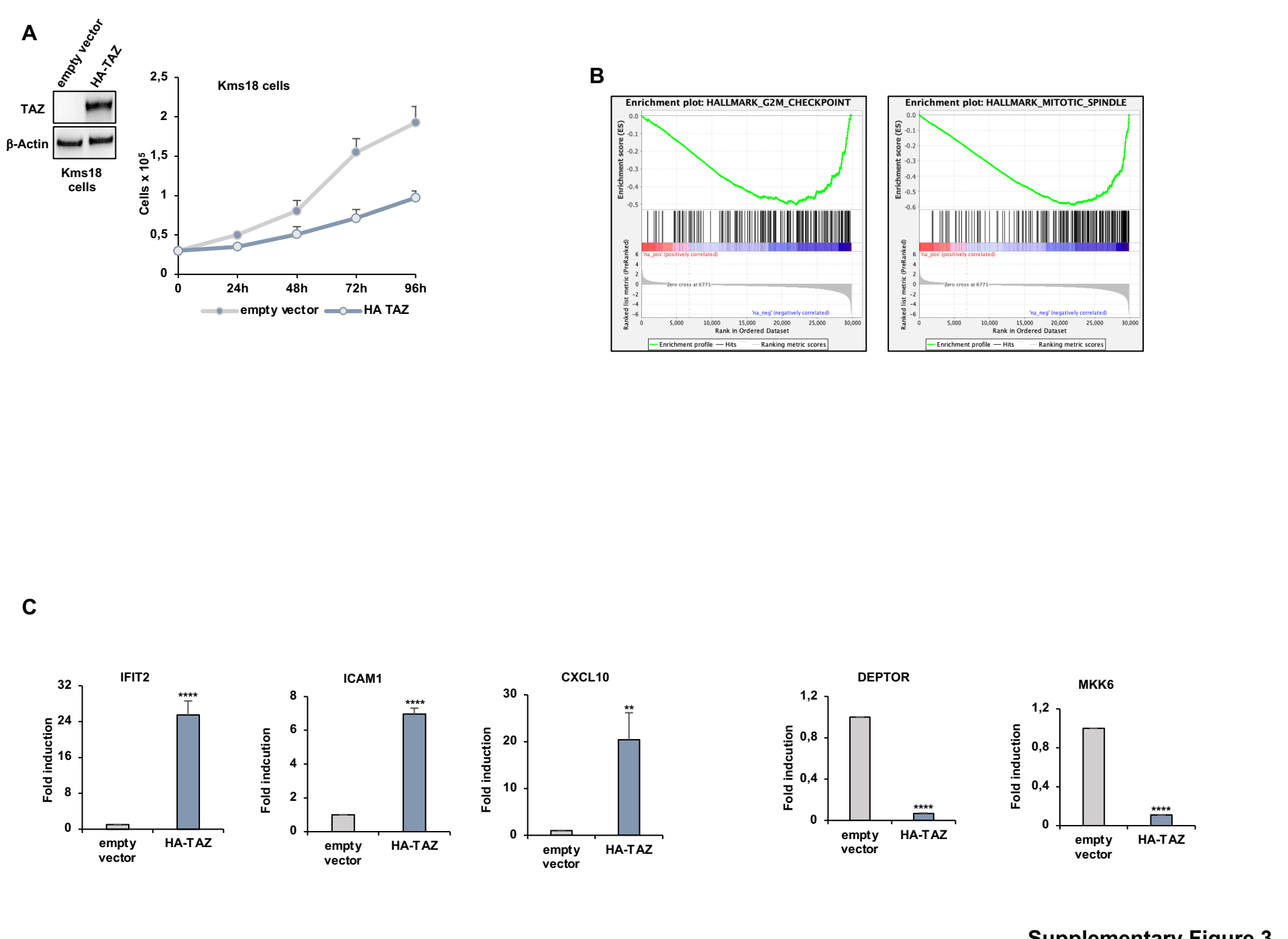
B



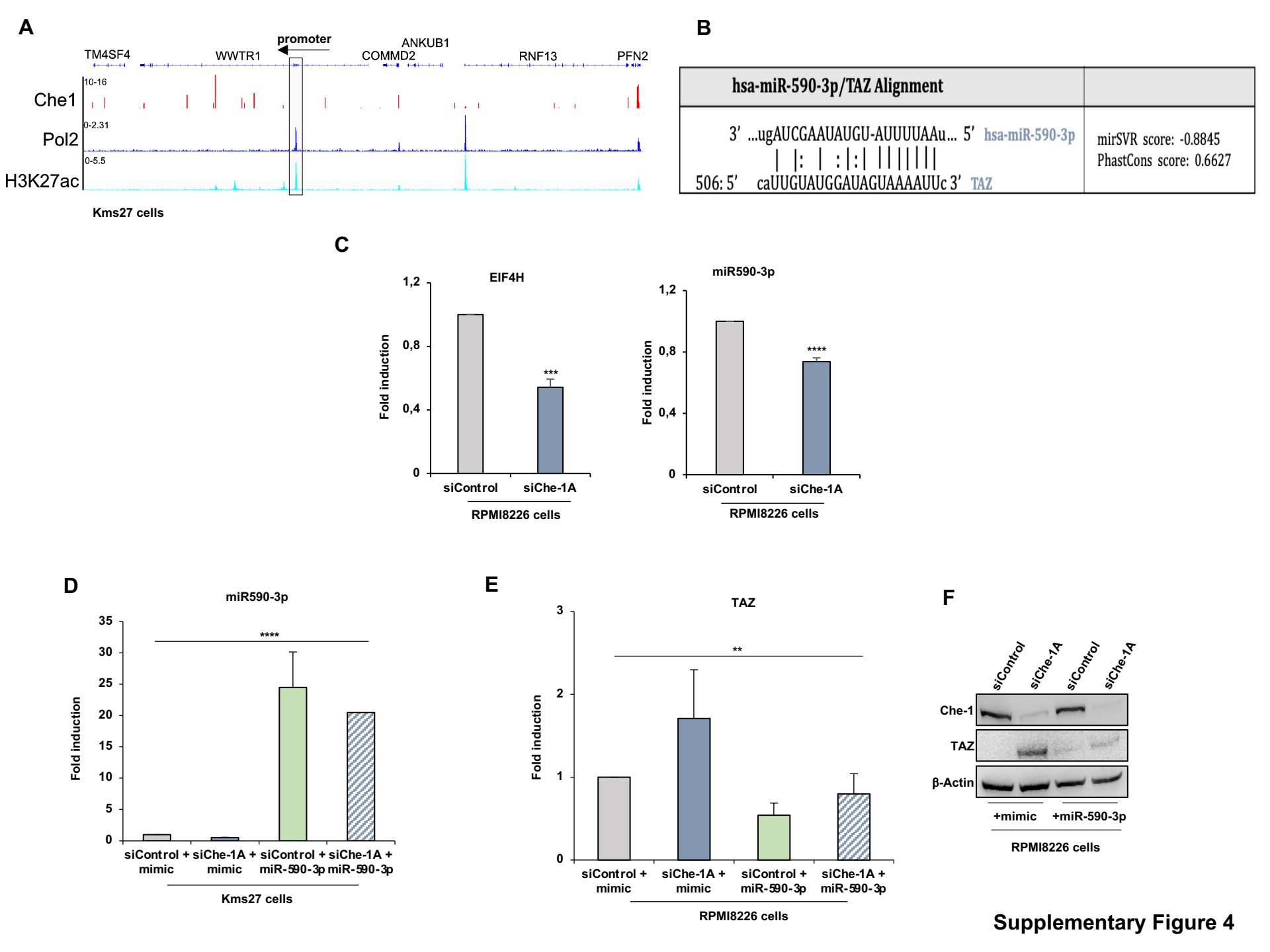

C



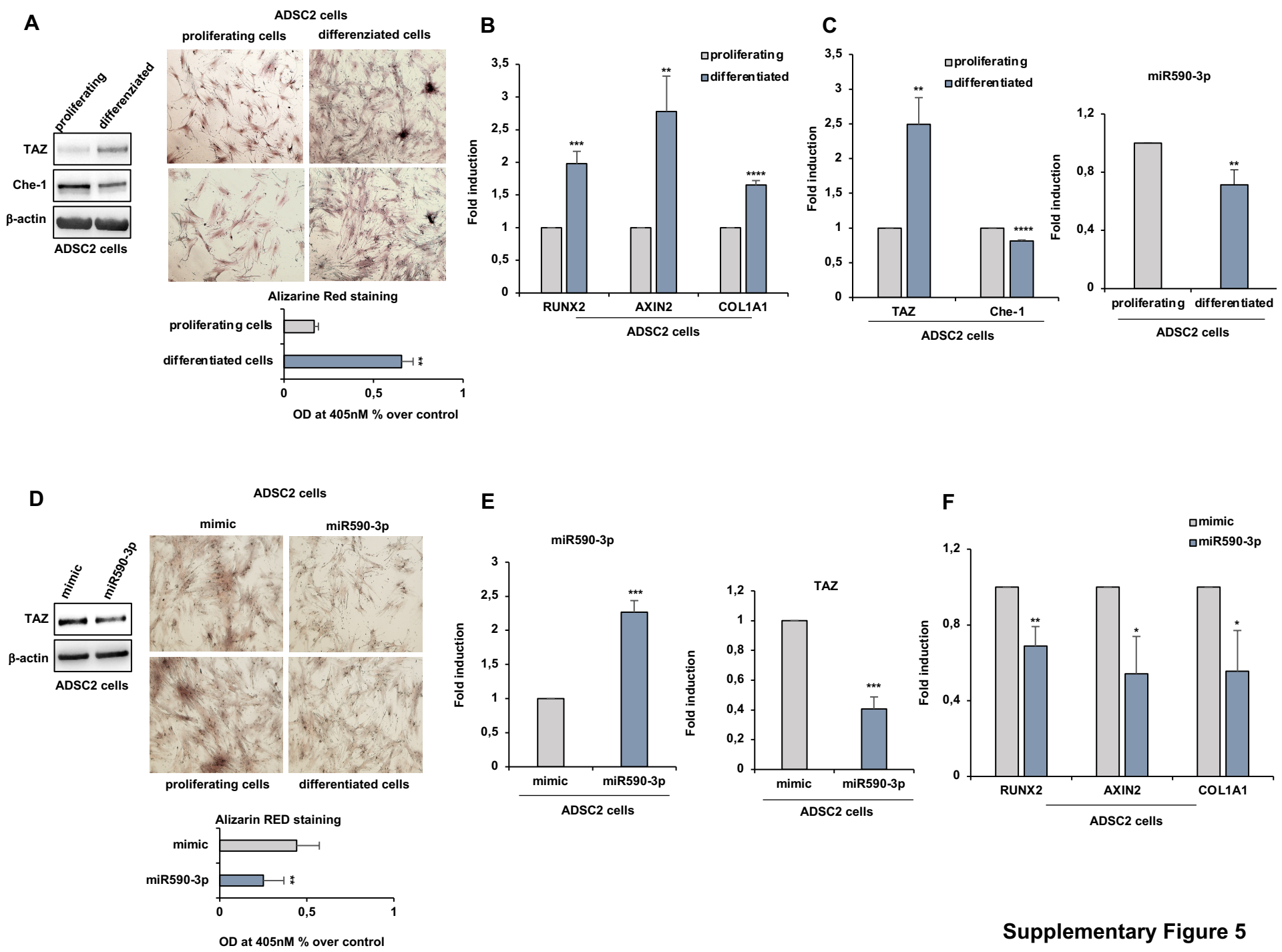

**Supplementary Figure 2**



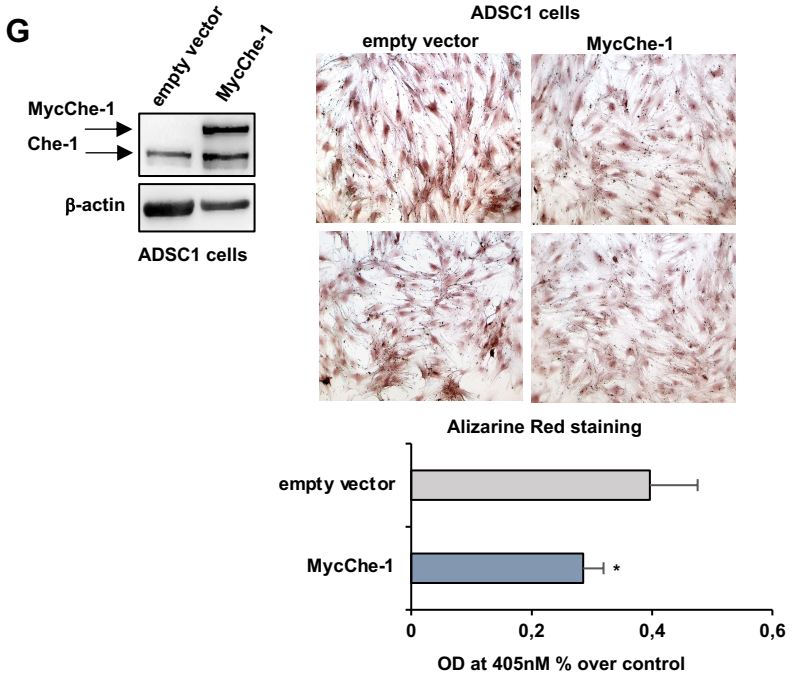

**Supplementary Figure 3**

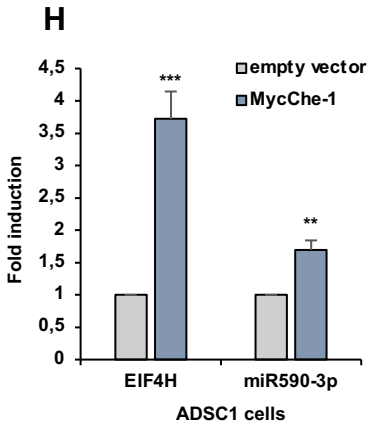


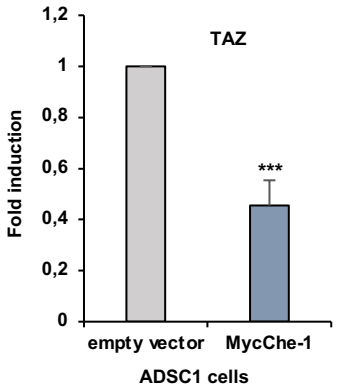

**Supplementary Figure 4**

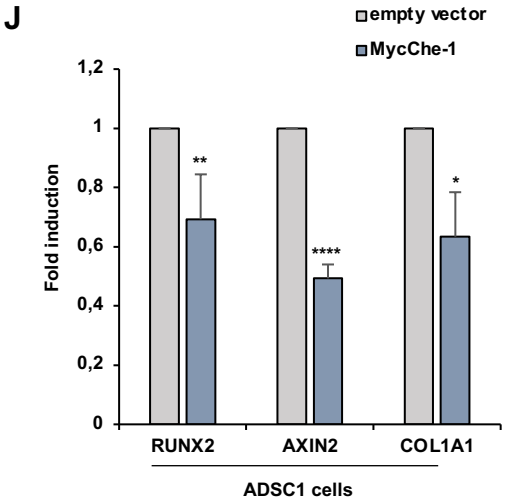



**Supplementary Figure 5**




**Supplementary Figure 5**

**Supplementary Figures Legend**

**Supplementary Figure S1 related to Figure 1**. **Che-1 regulates TAZ expression in MM in *vivo* and in *vitro*.** (**A**) Kms18 Multiple Myeloma (MM) cells transiently transfected with siChe-1A or siControl. **Left**: Quantitative real time PCR (RT-qPCR) analysis for the indicated genes. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. *P < 0.01, ***P < 0.005, n.s.= not significant. **Right**: Western Blot (WB) analysis of total cell extracts (TCEs) with the indicated antibodies (Abs) of Kms18 MM cells transfected as described in **Left**. **(B and C)** RT-qPCR for the indicated genes (**left**) and WB analysis with specific Abs (**right**) of Kms27 (**B**) and RPMI8226 (**C**) MM cell lines transiently transfected with another sequence of siRNA targeting Che-1 (Che-1B). Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. *P < 0.05, **P < 0.01, ***P<0.005, n.s.= not significant. (**D**) WB analysis of TCEs from different cell lines transfected with siControl or siChe-1A and incubated with the indicated Abs. (**E**) WB analysis for specific antibodies (**left**) and RT-qPCR (**right**) for indicated genes of Kms18 after overexpression of TAZ. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. *P < 0.01.

**Supplementary Figure S2 related to Figure 2. Low TAZ expression is correlated with poor survival of MM patients.** (**A**) Data from Agnelli et al. (1) provided by the Oncomine database and reanalyzed to show expression levels of Che-1 and TAZ in normal bone marrow, MGUS, MM and plasma cell leukemia (n=158). The associated P value is shown above. Box and whisker plot show the upper and lower quartiles (25-75%) with a line at the median, whiskers extend from the 10th to the 90th percentile, and dots correspond to the minimal and maximal values. (**B and C**) Kaplan-Meier survival curve for Che-1 and TAZ expression in the Hanamura et al. cohort of MM patients (n = 542).

**Supplementary Figure S3 related to Figure 3. TAZ overexpression inhibits MM cell proliferation.** (**A**) WB of TCEs (**left**) and cell proliferation analysis (**right**) of Kms18 MM cells ($1x10^5$) at indicated points after TAZ overexpression. Error bars represent the standard error of three different experiments. **P < 0.01, ***P < 0.005. (**B**) GSEA analysis of Kms27 MM cells after overexpression of TAZ. (**C**) RT-qPCR of Kms27 overexpressing TAZ and analyzed for the indicated genes. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. **P < 0.01, ****P < 0.001.

**Supplementary Figure S4 related to Figure 4. miR-590-3p expression Che-1-dependent affects TAZ activity in MM.** (**A**) Profiles at a fixed scale of Che-1 ChIP-seq *(Red)* H3k27ac *(Light Blue)* and RNA Pol II *(Blue)* on KMS27 cells at the genomic locus of the TAZ (WWTR1) gene. (**B**) Sequence alignment between miR-590-3p and human TAZ. (**C**) RT-qPCR for the endogenous expression of EIF4H (**left**) and miR-590-3p (**right**) in RPMI8226 MM cells depleted or not for Che-1A. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. ***P < 0.005, ****P < 0.001. (**D**) RT-qPCR for ectopic expression of miR-590-3p in Kms27 MM cells. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. ****P < 0.001. (**E**) RPMI8226 MM cells transiently transfected with siControl, siChe-1A, miR-590-3p or its mimic where indicated. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. **P < 0.01. (**F**) WB of TCEs from RPMI8226 MM cell line transfected as in **E** and analyzed for the indicate Abs.

**Supplementary Figure S5 related to Figure 6. miR590-3p negatively influences osteogenic differentiation in primary mesenchymal cell lines.** (**A**) **Left**: WB analysis of TCEs of ADSC2 mesenchymal cell line induced or not for osteogenic differentiation and analyzed for specific antibodies. **Right**: Two representative images of Alizarine Red S staining from ADSC2 mesenchymal

cell line induced or not as reported in **A**. **Bottom**: Bar Plot represented the measure of absorbance of Alizarine Red stain extraction at 405nM. Data is the mean of three different experiments. **P<0.01. (**B**) Evaluation of the expression of the osteogenic differentiation markers *RUNX2, AXIN2 and COL1A1* of ADSC2 mesenchymal cell line induced or not as in **A**. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. **P < 0.01, ***P < 0.005, ****P < 0.001. (C) RT-qPCR of the expression of TAZ, Che-1 (**left**) and miR590-3p (**right**) after the induction of the osteogenic program as reported in **A**. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. **P < 0.01, ****P < 0.001 (D) **Left**: WB analysis of TAZ expression in ADSC2 mesenchymal cell line overexpressing ectopic miR590-3p and subjected to osteogenic differentiation. **Right**: Two representative Alizarine Red S staining images of ADSC2 mesenchymal cell line treated as in **D**. **Bottom**: Bar Plot represented the measure of absorbance of Alizarine Red stain extraction at 405nM. Error bars represent the standard error of three different experiments. **P<0.01. (**E**) RT-qPCR analysis for miR590-3p ectopic expression (**left**) and TAZ (**right**) in ADSC2 mesenchymal cell line induced or not as in **D**. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. ***P < 0.005. (**F**) Evaluation of the expression of the indicated genes of ADSC2 mesenchymal cell treated as in **D.** Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. *P < 0.05, **P < 0.01, ***P < 0.005. (**G**) **Left**: WB analysis of TCEs of ADSC1 mesenchymal cell line subjected to osteogenic differentiation and transiently transfected with Myc Che-1 or empty vector and analyzed for the indicated Abs. **Right**: Two representatives Alizarin Red S staining images from ADSC1 mesenchymal cell treated as in **G. Bottom**: Bar Plot represented the measure of absorbance of stain extraction at 405nM. Error bars represent the standard error of three different experiments. *P<0.05. (**H and I**) RT-qPCR analysis of the indicated genes and for miR590-3p ectopic expression from ADSC1 mesenchymal cells transfected as in **G.** Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. **P < 0.01, ***P<0.005. (**J**) RT-qPCR of RUNX2, AXIN2 and

COL1A1 expression in ADSC1 mesenchymal cells treated as in **G**. Values were normalized to Actin expression. Error bars represent the standard error of three different experiments. *P < 0.05, **P < 0.01, ****P < 0.001.