LETTER TO THE EDITOR

FOXM1 downregulation is a trigger of isatuximab-induced direct cell death in multiple myeloma cells with 1q+

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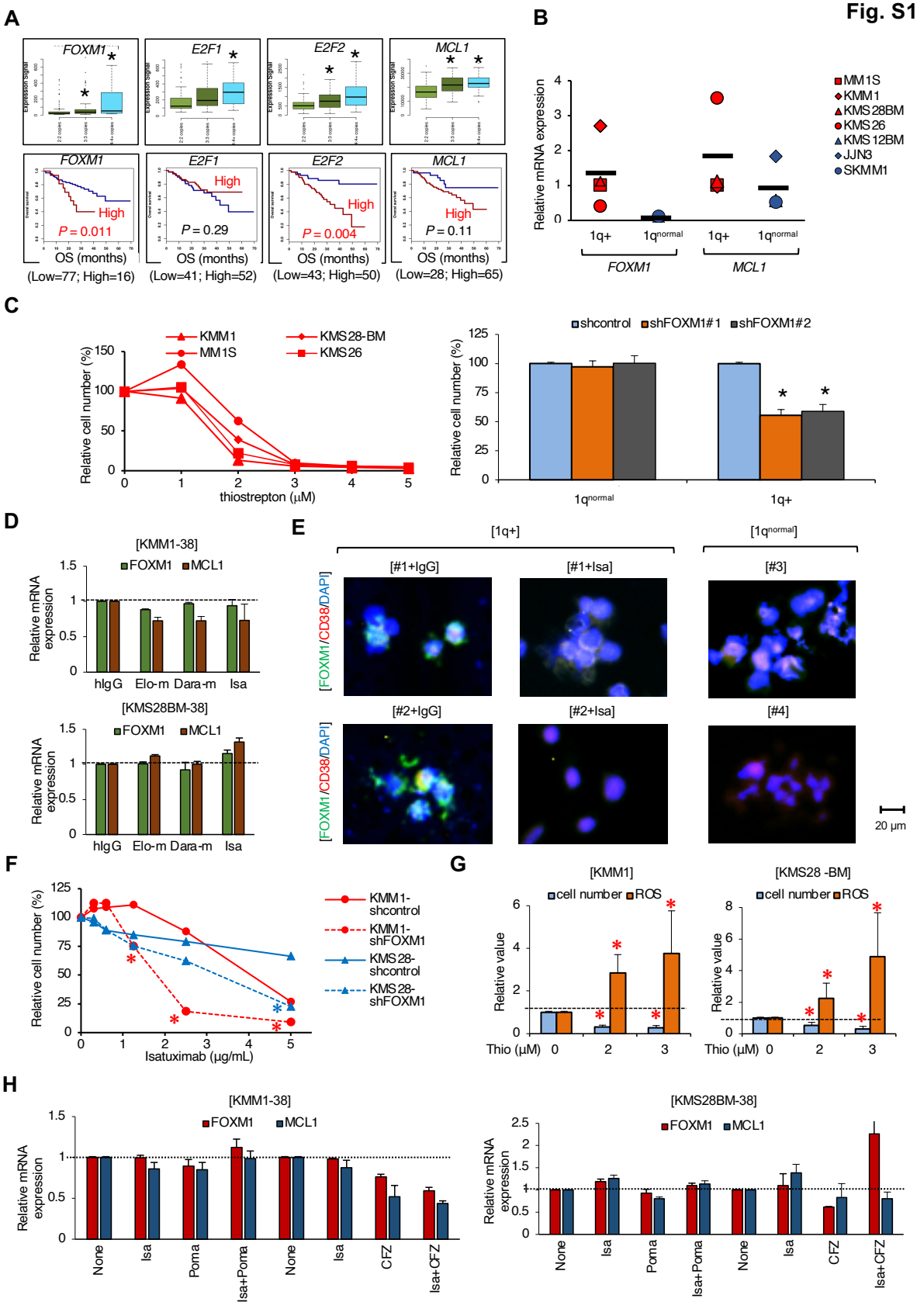
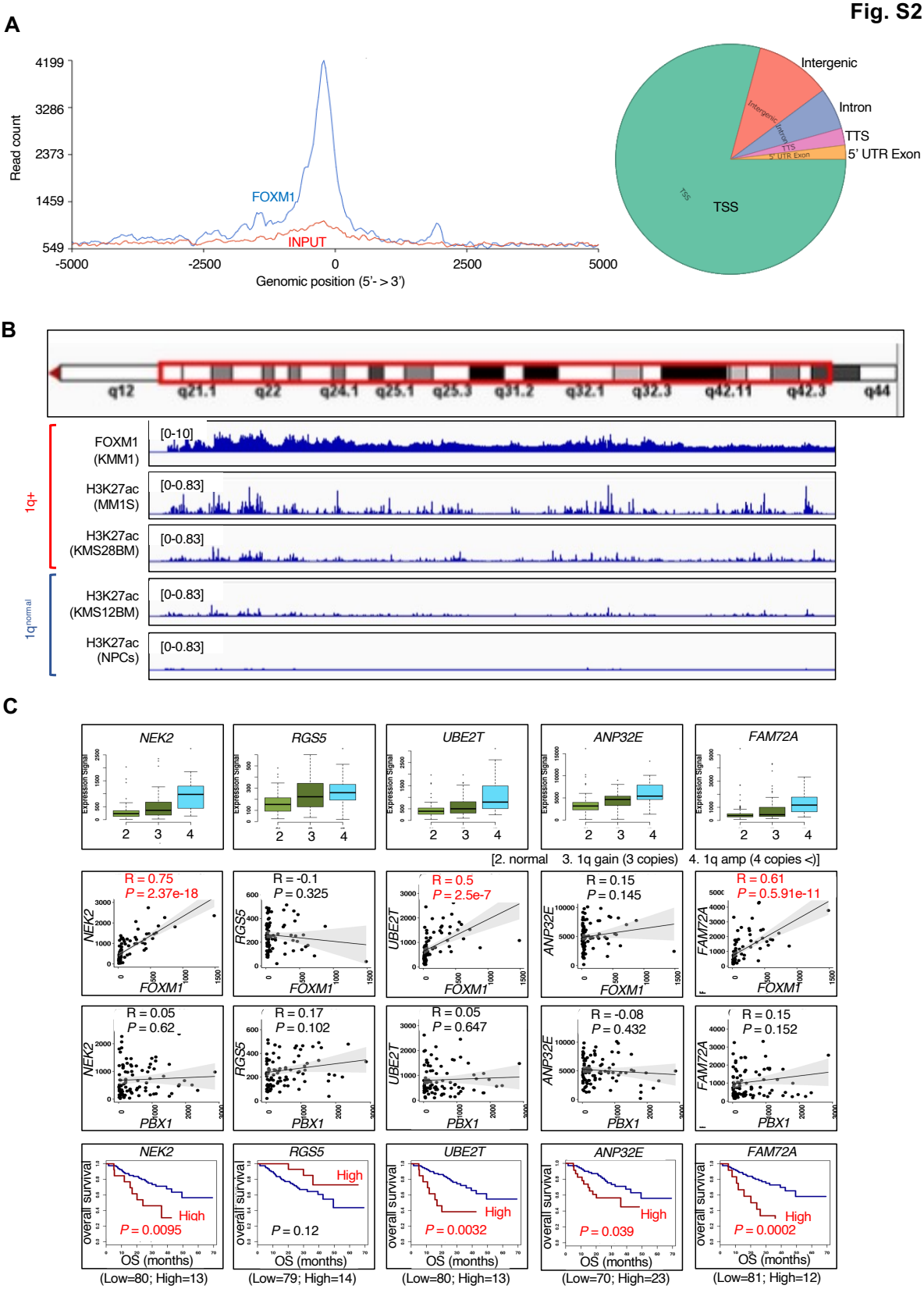
Fig. S1


A
FOXM1
E2F1
E2F2
MCL1
High
P = 0.011
P = 0.29
P = 0.004
P = 0.11
OS (months)
(Low=77; High=16)
(Low=41; High=52)
(Low=43; High=50)
(Low=28; High=65)
B
Relative mRNA expression
MM1S
KMM1
KMS28BM
KMS26
KMS12BM
JJN3
SKMM1
1q+
1qnormal
FOXM1
MCL1
C
KMM1
MM1S
KMS28-BM
KMS26
Relative cell number (%)
thiostrepton (μM)
shcontrol
shFOXM1#1
shFOXM1#2
D
[KMM1-38]
[KMS28BM-38]
FOXM1
MCL1
Relative mRNA expression
hIgG
Elo-m
Dara-m
Isa
E
[1q+]
[1qnormal]
[#1+IgG]
[#1+Isa]
[#3]
[#2+IgG]
[#2+Isa]
[#4]
[FOXM1/CD38/DAPI]
20 μm
F
Relative cell number (%)
Isatuximab (μg/mL)
KMM1-shcontrol
KMM1-shFOXM1
KMS28-shcontrol
KMS28-shFOXM1
G
[KMM1]
[KMS28 -BM]
cell number
ROS
Relative value
Thio (μM)
H
[KMM1-38]
[KMS28BM-38]
FOXM1
MCL1
Relative mRNA expression
None
Isa
Poma
Isa+Poma
CFZ
Isa+CFZ

Supplementary Fig.S1. [A] Upper panels: The expression levels of *FOXM1*, *E2F1*, *E2F2*, and *MCL1* in MM cells with aberrant copy numbers of 1q. Lower panels: The prognostic significance of FOXM1, E2F2, E2F2, and MCL1 in the 1q+ MM patients. Kaplan-Meier curves showing the survival of MM patients with high (red) and low (blue) expression of each gene after total therapy 2 and 3 (TT2/3). *P* values were determined by the log-rank test. The GenomicScape tool (www.genomicscape.com) was used for the analysis. [B] Total RNAs were prepared from 1q+ MM cell lines (red) and $1q^{normal}$ MM cell lines (blue) and subjected to QPCR for *FOXM1* and *MCL1*. Data were quantified by the $2^{-\Delta\Delta Ct}$ method using *GAPDH* as the reference and are shown as fold changes against the value of MM.1S set at 1.0. We used TaqMan Fast Universal PCR Master Mix and Expression Assays (Hs01073586 for *FOXM1*, Hs06626047 for *MCL1*, and Hs01922876 for *GAPDH* (Thermo Fisher Scientific, MA, USA). [C] Left panel: KMM.1, KMS28BM, MM.1S, and KMS26 cells were treated with different concentrations of thiostreptone for 24 h. Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding untreated controls. Data are the means of multiple experiments (n > 3); S.D. was less than 10% and thus omitted. Right panel: The MM cell lines were transfected with shRNA targeting *FOXM1* (shFOXM1#1 and #2) or scrambled sequences (shcontrol) to 1q gain positive-KMM.1, KMS28-BM, or MM.1S cells (1q+), and 1q normal-KMS12BM, JJN3, or SK-MM1 cells ($1q^{normal}$). Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding sh-controls. Bars indicate the means of three MM cell lines. *P*-values were calculated by one-way ANOVA using a Student–Newman-Keuls multiple comparison test. The lentiviral short-hairpin RNA/short-interfering RNA (shRNA/siRNA) expression vector pLL3.7 was used for knockdown experiments. The siRNA sequences are as follows: #1; gaccactttccctacttta, #2; aagaagaaatcctggttaa. [D] KMM.1-38 and KMS28BM-38 cells were cultured in the absence or presence of 5 μg/mL human immunoglobulin (hIgG), elotuzumab-mimic (Elo-m), daratumumab-mimic (Dara-m), and isatuximab (Isa) for 24 h. Graphs show the expression levels of *FOXM1* and *MCL1* mRNAs relative to control IgG (values set at 1.0). The means ± S.D. (bars) of three independent experiments are shown. [E] CD138-positive cells were isolated from the bone marrow of MM patients and treated with 5 μg/ml hIgG or isatuximab (Isa) for 24 h. Cytospin specimens were prepared and stained with anti-FOXM1 antibody (green), and PE-conjugated anti-CD38 antibody (Red; BioLegend, CA, USA), followed by FITC-conjugated anti-rabbit IgG (Thermo Fisher Scientific). Nuclei were counterstained with DAPI (blue). Only merged images are shown. The copy number of chromosome 1q was detected by fluorescence in situ hybridization (FISH) using the CKS1B FISH probe; 1q gain was defined as having 3 copies of CKS1B, and 1q amp was

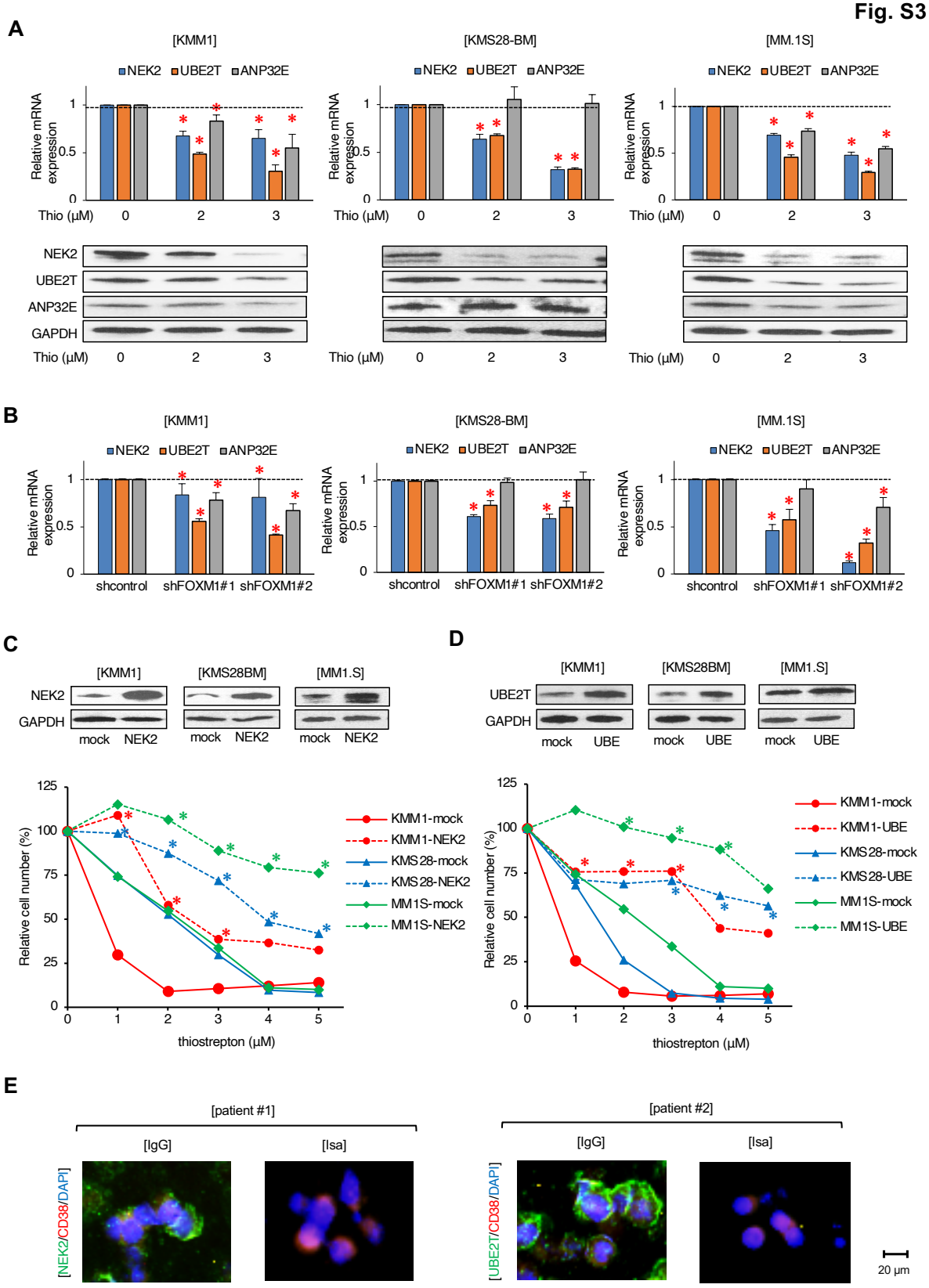
defined as having ≥4 copies of CKS1B. Patient #1 and #2 with ≥4 copies of chromosome 1q (1q+), and #3 and #4 with 2 copies of chromosome 1q ($1q^{normal}$). The FISH analyses were outsourced to SRL Inc. [F] KMM.1-38 and KMS28BM-38 cells were transduced with shFOXM1(#1) or shcontrol lentiviral vector and cultured with different concentrations of isatuximab (Isa) for 24 h. Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding untreated cells. Data are the means of multiple independent experiments (n > 3). S.D. was less than 10% and thus omitted; **P* < 0.05 by one-way ANOVA with Student-Newman-Keuls multiple comparison test. [G] KMM.1 and KMS28-BM cells were treated with different concentrations of the FOXM1 inhibitor thiostreptone (Thio) for 24 h. Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding untreated controls (blue bars). ROS levels were assessed using the ROS assay kit and the data is shown relative to that of the untreated controls (orange bars). Data are the means ± S.D. (bars) of multiple independent experiments (n = 3); **P* < 0.05 by one-way ANOVA with Student-Newman-Keuls multiple comparison test. [H] KMM.1-38 and KMS28BM-38 cells were treated with vehicle alone (None), 2.5 μg/ml isatuximab (Isa), 2 μM pomalidomide (Poma), 2 nM carfilzomib (CFZ), or their combinations (Isa+Poma or Isa+CFZ) for 24 h. The graphs show the expression levels of *FOXM1* and *MCL1* in the indicated groups. Data were quantified by the $2^{-\Delta\Delta Ct}$ method using *GAPDH* as the reference and are shown as fold changes against untreated control. The means ± S.D. (bars) of multiple independent experiments (n = 3) are shown.

Fig. S2


A
Read count
4199
3286
2373
1459
549
FOXM1
INPUT
-5000
-2500
0
2500
5000
Genomic position (5'- > 3')
Intergenic
Intron
TTS
5' UTR Exon
TSS
B
q12
q21.1
q22
q24.1
q25.1
q25.3
q31.2
q32.1
q32.3
q42.11
q42.3
q44
1q+
1q normal
FOXM1 (KMM1)
H3K27ac (MM1S)
H3K27ac (KMS28BM)
H3K27ac (KMS12BM)
H3K27ac (NPCs)
[0-10]
[0-0.83]
C
NEK2
RGS5
UBE2T
ANP32E
FAM72A
Expression Signal
2 3 4
[2. normal 3. 1q gain (3 copies) 4. 1q amp (4 copies <)]
R = 0.75
P = 2.37e-18
R = -0.1
P = 0.325
R = 0.5
P = 2.5e-7
R = 0.15
P = 0.145
R = 0.61
P = 0.5.91e-11
FOXM1
R = 0.05
P = 0.62
R = 0.17
P = 0.102
R = 0.05
P = 0.647
R = -0.08
P = 0.432
R = 0.15
P = 0.152
PBX1
overall survival
High
P = 0.0095
P = 0.12
P = 0.0032
P = 0.039
P = 0.0002
OS (months)
(Low=80; High=13)
(Low=79; High=14)
(Low=80; High=13)
(Low=70; High=23)
(Low=81; High=12)

Supplementary Fig.S2. [A] Left panel: Average plot of FOXM1 ChIP-seq reads over all transcription start sites (TSSs) ± 5,000 bp. Right panel: Pie chart showing gene section breakdown. [B] Visualization of the binding of FOXM1 and acetylated histone H3K27 (H3K27ac) on chromosome 1q region on the ChIP-Atlas platform. The ChIP-seq data of the following cell types was used: MM.1S (SRX20095564), KMS28BM (SRX7798959), KMS12BM (SRX7798957), and normal plasma cells isolated from healthy volunteers (NPCs) (SRX7798977). [C] Upper panel: The expression of possible target genes of FOXM1 (*NEK2*, RGS5, *UBE2T*, *AN32E*, and *FAM72A*) in MM cells with aberrant copy numbers of 1q. Middle panel: Correlation between the expression of FOXM1-target genes (y-axis) and the indicated genes (x-axis). Lower panel: The Kaplan-Meier curves indicate the survival of 1q+ MM patients with high (red) and low (blue) expression of FOXM1-target genes after TT2/3. *P* values were determined by a log-rank test. The GenomicScape tool (www.genomicscape.com) was used for the analyses.

Fig. S3


A
[KMM1]
[KMS28-BM]
[MM.1S]
NEK2 UBE2T ANP32E
Relative mRNA expression
Thio (μM) 0 2 3
NEK2
UBE2T
ANP32E
GAPDH
B
shcontrol shFOXM1#1 shFOXM1#2
C
[KMM1] [KMS28BM] [MM1.S]
mock NEK2
Relative cell number (%)
thiostrepton (μM)
KMM1-mock
KMM1-NEK2
KMS28-mock
KMS28-NEK2
MM1S-mock
MM1S-NEK2
D
UBE2T
mock UBE
KMM1-UBE
KMS28-UBE
MM1S-UBE
E
[patient #1]
[patient #2]
[IgG] [Isa]
[NEK2/CD38/DAPI]
[UBE2T/CD38/DAPI]
20 μm

Supplementary Fig.S3.

[A] KMM.1, KMS28BM, and MM.1S cells were treated with different concentrations of thiostreptone (Thio) for 24 h. Upper panel: QPCR results showing the expression levels of candidate FOXM1-target genes (*NEK2*, *UBE2T*, and *ANP32E*). Data were quantified by the $2^{-\Delta\Delta Ct}$ method using *GAPDH* as the reference and are shown as fold changes against untreated control. Data are the means ± S.D. (bars) of multiple independent experiments (n = 3). **P* < 0.05 by one-way ANOVA with a Student-Newman-Keuls multiple comparison test. Lower panel: Immunoblot showing the expression of NEK2, UBE2T, ANP32E, and GAPDH (loading control) in the indicated groups. [B] KMM.1, KMS28BM, and MM.1S cells were transduced with shFOXM1#1 and #2 or shcontrol. The expression levels of candidate FOXM1-target genes (*NEK2*, *UBE2T*, and *ANP32E*) are shown. Data were quantified by the $2^{-\Delta\Delta Ct}$ method using *GAPDH* as the reference and are shown as the fold changes against the values of shcontrol. Data are the means ± S.D. (bars) of multiple independent experiments (n = 3). **P* <0.05 by one-way ANOVA with Student-Newman-Keuls multiple comparison test. [C] KMM.1, KMS28BM, and MM.1S cells were transduced with either an empty lentiviral vector (mock) or NEK2-overexpressing vector (NEK2). Upper panel: Immunoblot showing expression of NEK2 and GAPDH (loading control) in the stable transformants. Lower panel: Each transformant was treated with different concentrations of thiostrepton at 24 h. Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding untreated controls. Data are the means of multiple experiments (n > 3); S.D. was less than 10% and thus omitted. **P* < 0.05 by one-way ANOVA with Student-Newman-Keuls multiple comparison test. [D] KMM.1, KMS28BM, and MM.1S cells were transduced with either an empty lentiviral vector (mock) or UBE2T-overexpressing vector (UBE). Upper panel: Immunoblot showing expression of UBE2T (UBE) and GAPDH (loading control) in the stable transformants. Lower panel: Each transformant was treated with different concentrations of thiostrepton at 24 h. Cell proliferation was assessed by the MTT reduction assay and the data is shown relative to that of the corresponding untreated controls. Data are the means of multiple experiments (n > 3); S.D. was less than 10% and thus omitted. **P* < 0.05 by one-way ANOVA with Student-Newman-Keuls multiple comparison test. [E] CD138-positive cells were isolated from the bone marrow of MM patients with 1q+ (patient #1 and #2) and treated with 5 μg/ml hIgG or isatuximab (Isa) for 24 h. Cytospin specimens were prepared and stained with anti-NEK2 antibody or anti-UBE2T antibody (green), and PE-conjugated anti-CD38 antibody (Red), followed by FITC-conjugated anti-rabbit IgG. Nuclei were counterstained with DAPI (blue). Only merged images are shown.