

Supplement Figure 1: ATRA treatment does not affect the viability of myeloma cell lines. MM.1S, OPM-2 and NCI-H929 cells were treated with ATRA for up to 72 hours. Cell viability was measured by flow cytometry and 7AAD staining (n=6). Bar diagrams show mean values +SD.

Supplement Figure 2: ATRA plus crenigacestat treatment enhance BCMA expression on myeloma cell lines. Bar diagram shows BCMA expression on OPM-2 cells (n=3) after treatment with 100 nM ATRA and/or 10 nM GSI crenigacestat for 72 hours. Bar diagram shows mean values +SD. P-values between indicated groups were calculated using unpaired t-test. *p<0.05, **p<0.01.

Supplement Figure 3: ATRA treatment leads to increased BCMA transcripts in OPM-2 myeloma cells. BCMA RNA levels in OPM-2 were analyzed by quantitative reverse transcription PCR (qRT-PCR) assay after incubation with increasing doses of ATRA for 48 hours (n=3). Bar diagram shows mean values +SD. P-values between indicated groups were calculated using unpaired t-test. *p<0.05.

Supplement Figure 4: ATRA treatment leads to enhanced BCMA expression on primary myeloma cells. Representative flow cytometric analysis of BCMA expression on primary myeloma cells that had been cultured in the absence or presence of ATRA at different concentrations for 72 hours. 7-AAD was used to exclude dead cells from analysis.

Supplement Figure 5: ATRA treatment does not impair viability of primary myeloma cells. Viability of primary myeloma cells with or without 72 hours of ATRA treatment was analyzed by flow cytometry and 7-AAD staining (n=5 biological replicates). Bar diagram shows mean values +SD.

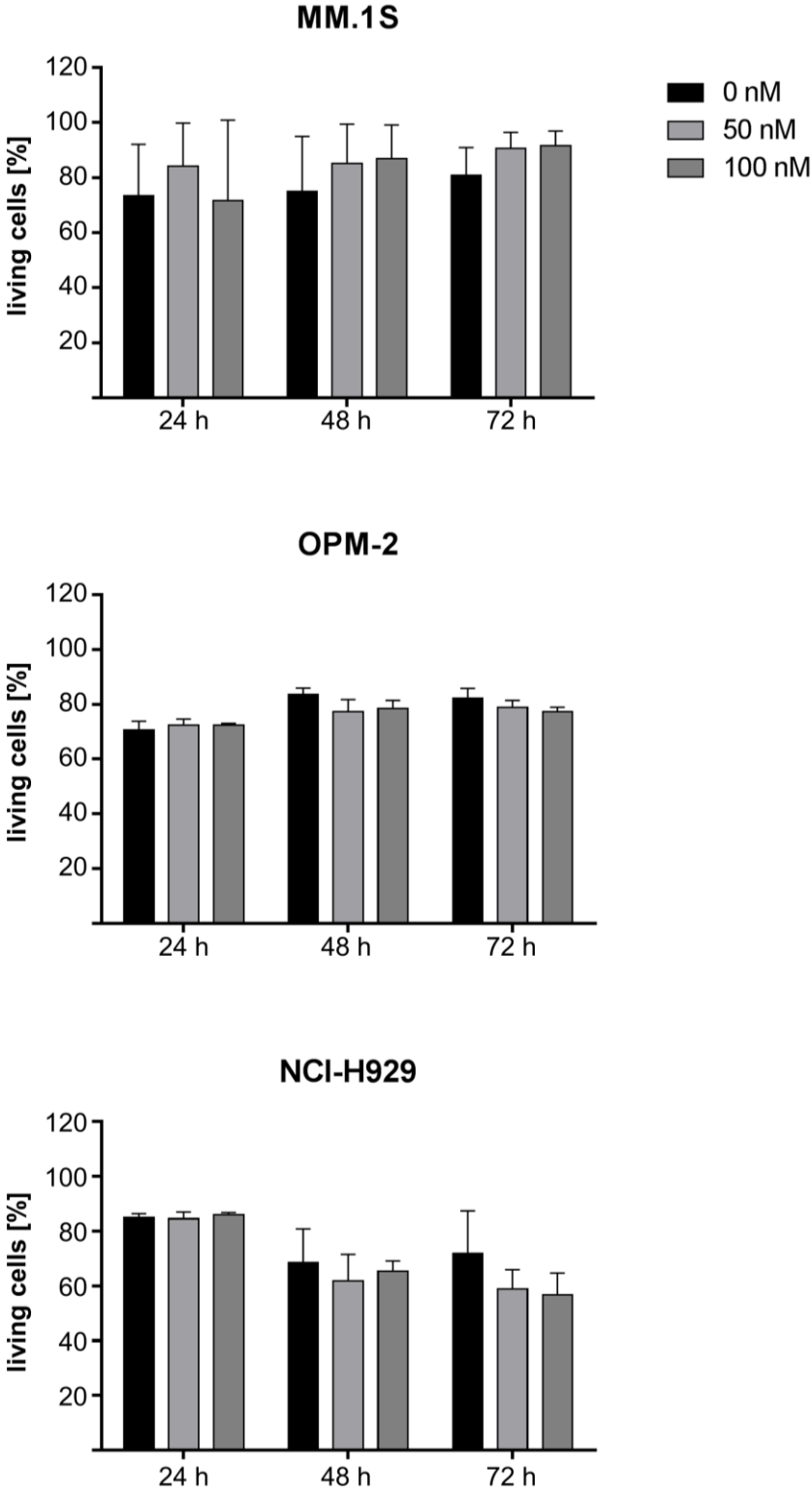
Supplement Figure 6: sBCMA does not impair BCMA CAR T cell functionality. CD8⁺ BCMA-CAR T-cells were co-cultured with MM.1S target cells in absence or presence of 150 ng/ml of soluble BCMA. After 4 hours, cytotoxicity was evaluated by bioluminescence-based assay. Diagram shows mean values +/-SD.

Supplement Figure 7: ATRA treatment does not increase shedding of sBCMA. sBCMA concentration in the supernatant of OPM-2 and NCI-H929 after incubation with increasing doses of ATRA was analyzed by ELISA. Cell lines were cultured at 1x10⁶/well (n=3 technical replicates). Bar diagrams show mean values +SD, P-values between indicated groups were calculated using 2way ANOVA. n.s. = not significant, *p<0.05, **p<0.01.

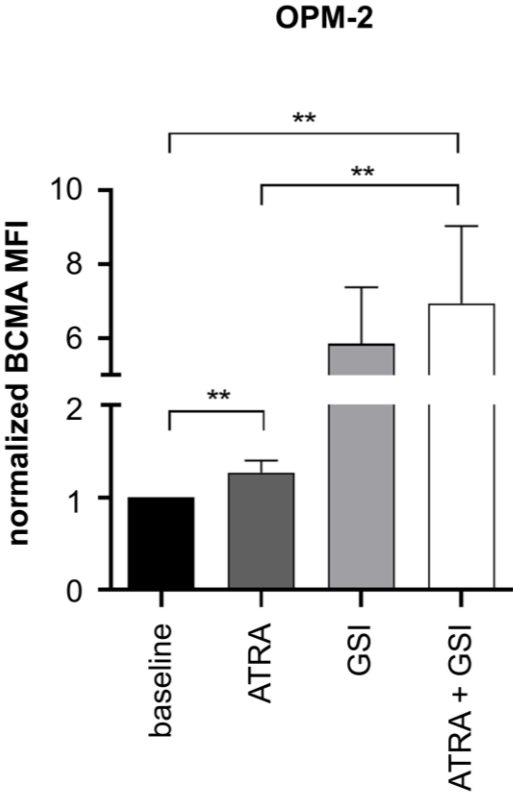
Supplement Figure 8: BCMA-CAR T-cells confer enhanced cytotoxicity against ATRA plus crenigacestat-treated OPM-2 cells *in vitro*. OPM-2 cells were incubated with 100 nM ATRA and/or 10 nM GSI for 72 hours or were left untreated. Cytolytic activity of CD8⁺ BCMA-CAR T-cells was determined in a bioluminescence-based assay after 4h of co-incubation with target cells. Assay was performed in triplicate wells with 5,000 target cells per well. Data are presented as mean values +SD (n=4 biological replicates). P-values between indicated groups were calculated using unpaired t-test. n.s. = not significant, *p<0.05.

Supplement Figure 9: Patient-derived BCMA-CAR T-cells confer enhanced cytotoxicity against ATRA-treated MM.1S cells. MM.1S cells were incubated with 50 nM ATRA for 72 hours or were left untreated. Cytolytic activity of MM patient-derived CD8⁺ BCMA-CAR T-cells was determined in a bioluminescence-based assay after 4h of co-incubation with target cells. Data are presented as mean values +SD of triplicate wells. P-values between indicated groups were calculated using unpaired t-test. *p<0.05, **p<0.01

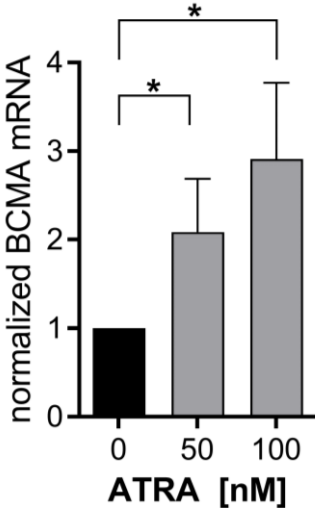
Supplement Figure 1



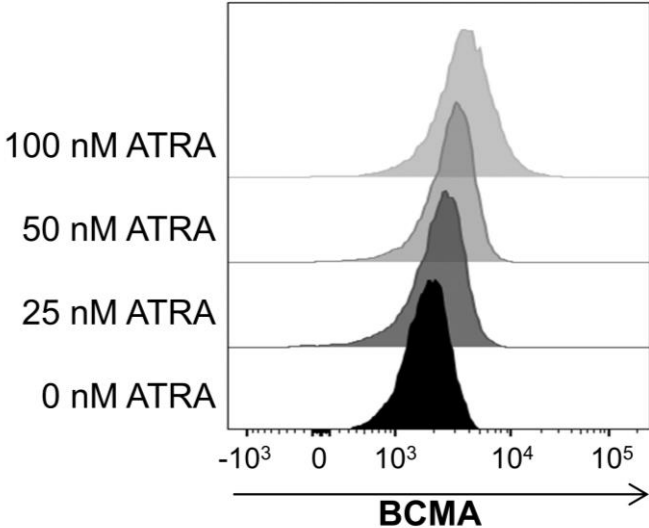
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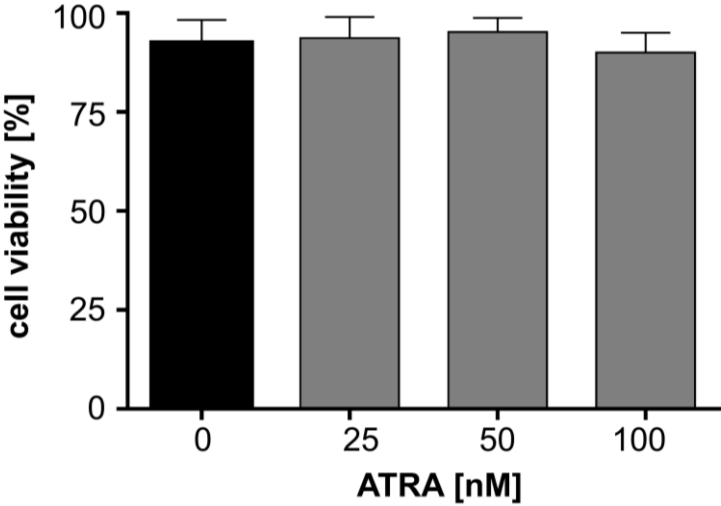
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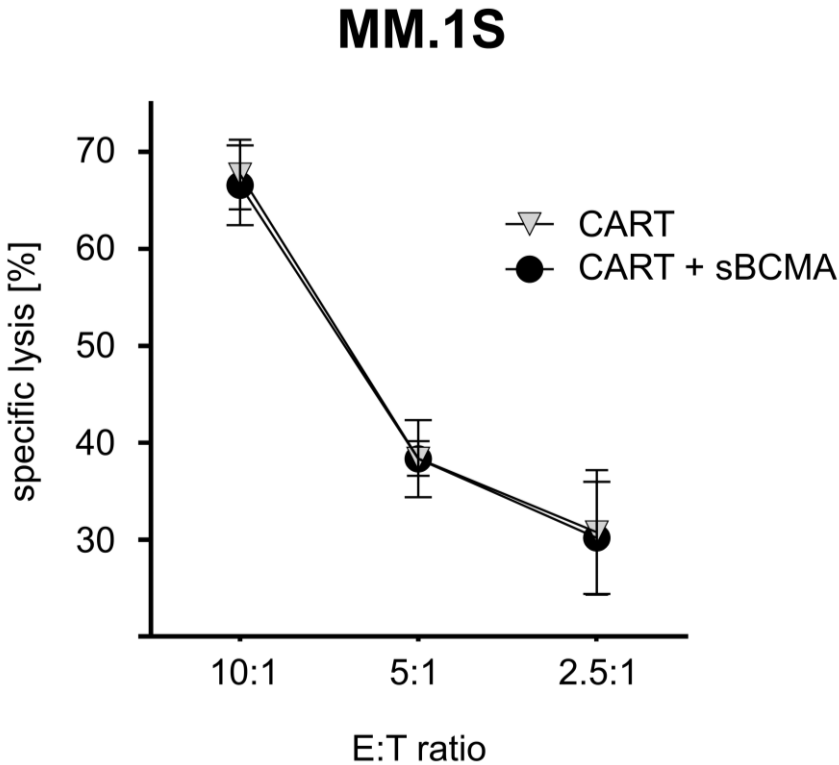
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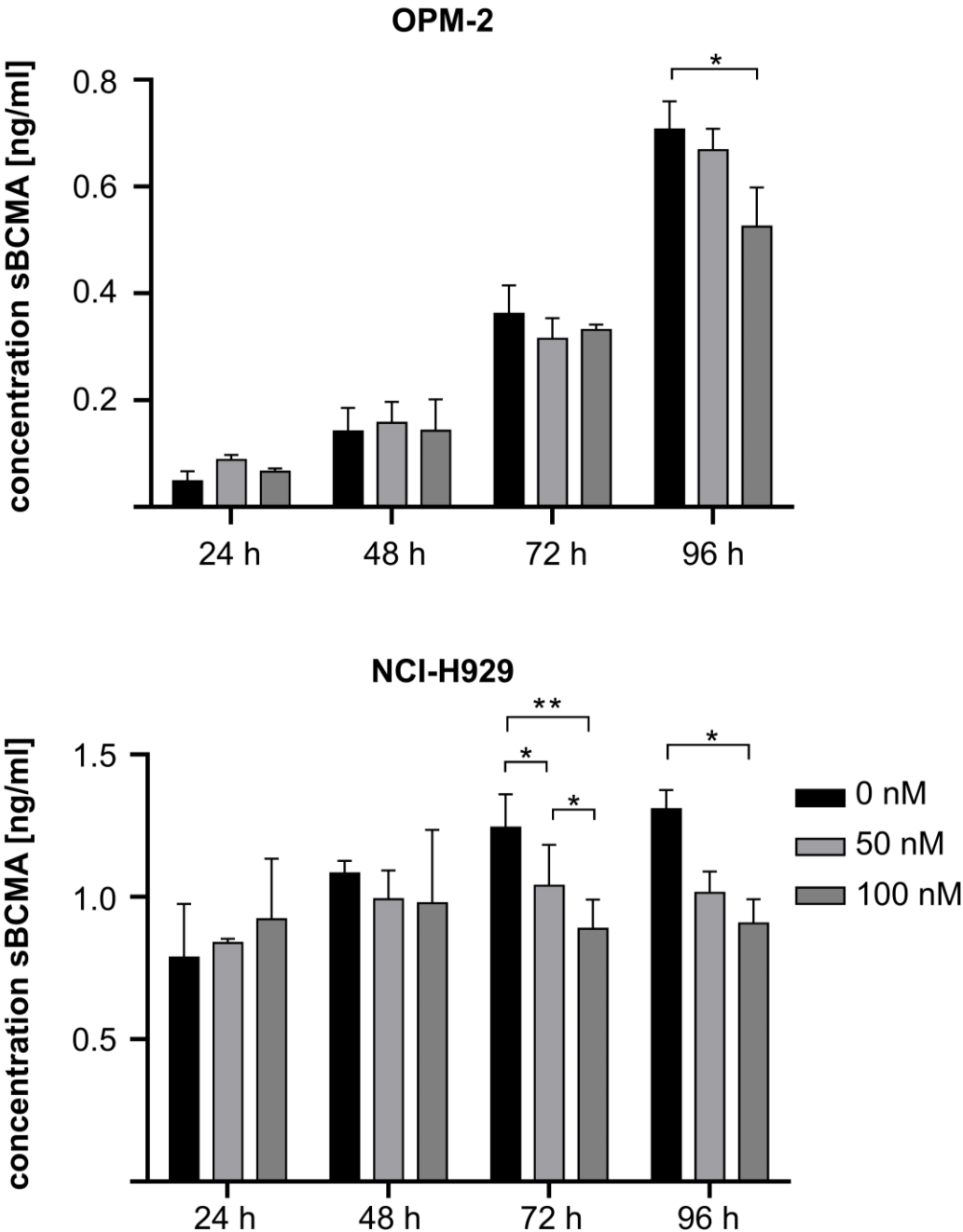
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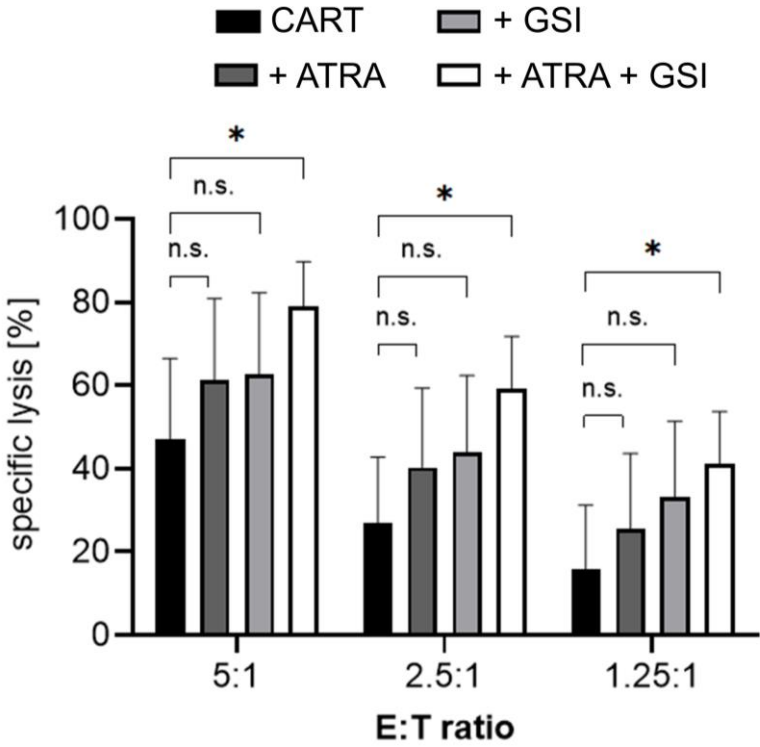
Supplement Figure 6



Supplement Figure 7



Supplement Figure 8



Supplement Figure 9

