

# All-trans retinoic acid works synergistically with the $\gamma$ -secretase inhibitor crenigacestat to augment BCMA on multiple myeloma and the efficacy of BCMA-CAR T cells

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**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.

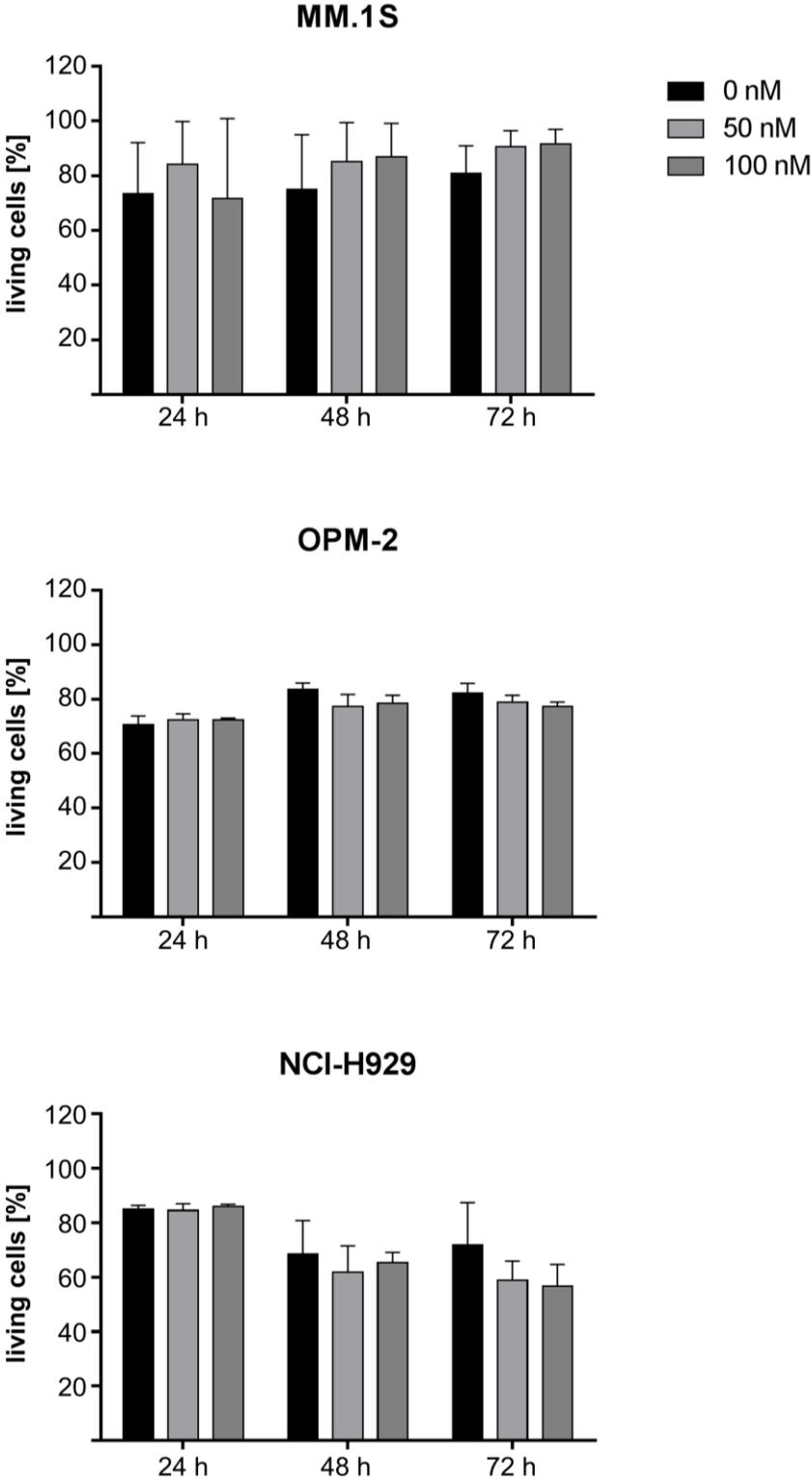
**Supplement Figure 6: sBCMA does not impair BCMA CAR T cell functionality.** CD8<sup>+</sup> BCMA-CAR T-cells were co-cultured with MM.1S target cells in absence or presence of 150 ng/ml of soluble BCMA. After 4 hours, cytotoxicity was evaluated by bioluminescence-based assay. Diagram shows mean values +/-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 1x10<sup>6</sup>/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.

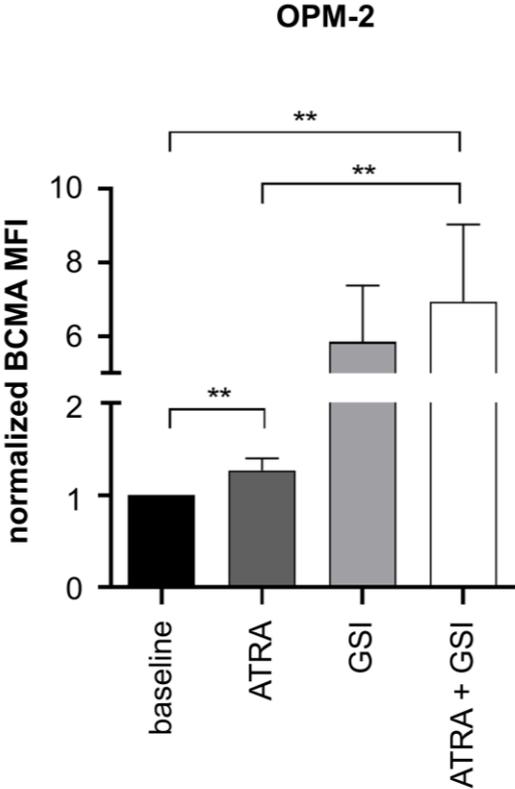
**Supplement Figure 8: BCMA-CAR T-cells confer enhanced cytotoxicity against ATRA plus crenigacestat-treated OPM-2 cells *in vitro*.** OPM-2 cells were incubated with 100 nM ATRA and/or 10 nM GSI for 72 hours or were left untreated. Cytolytic activity of CD8<sup>+</sup> BCMA-CAR T-cells was determined 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<sup>+</sup> 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

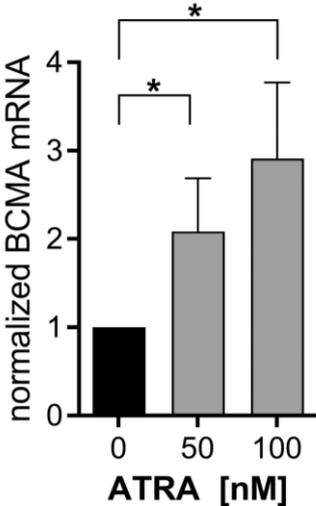
Supplement Figure 1



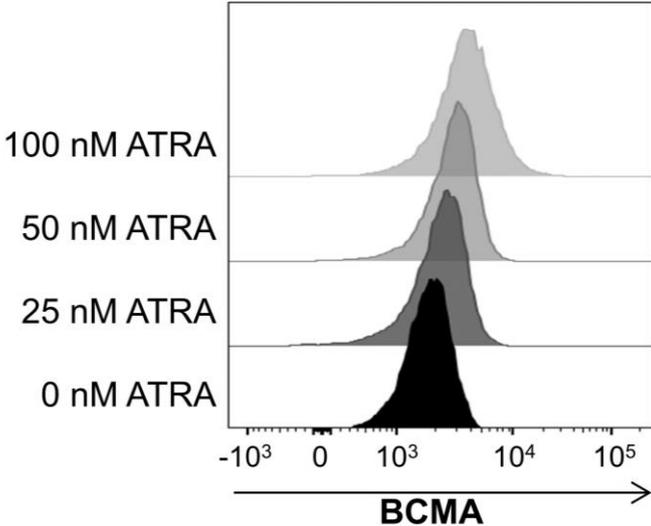
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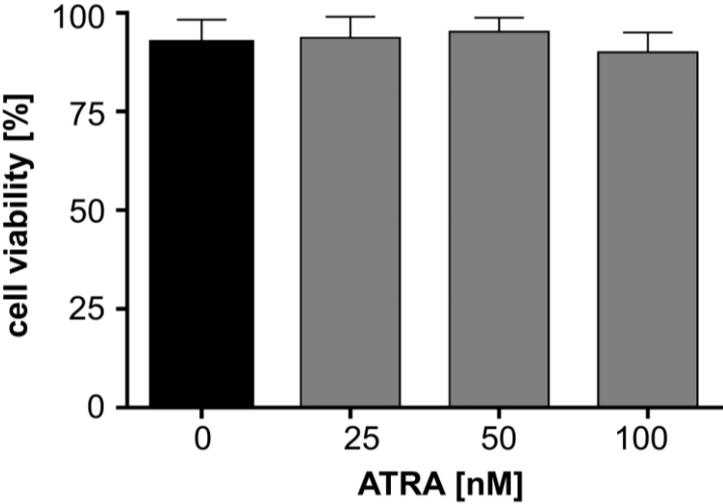
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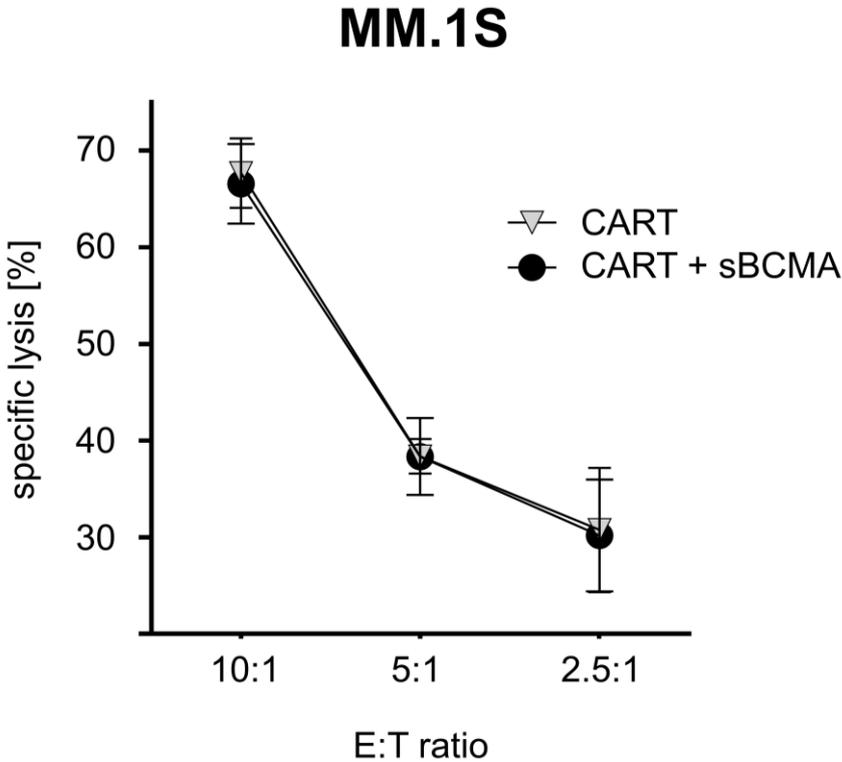
**Supplement Figure 4**



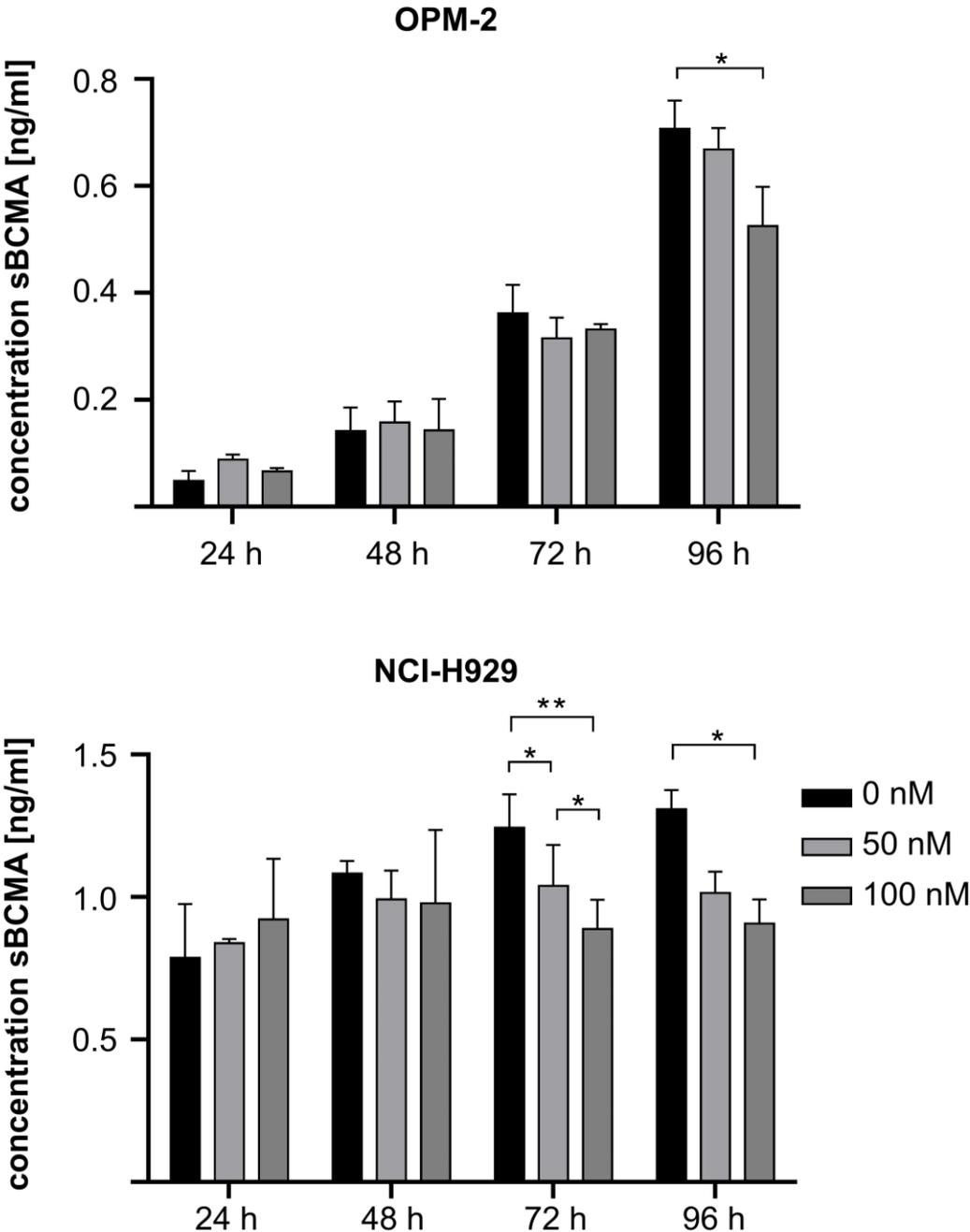
**Supplement Figure 5**



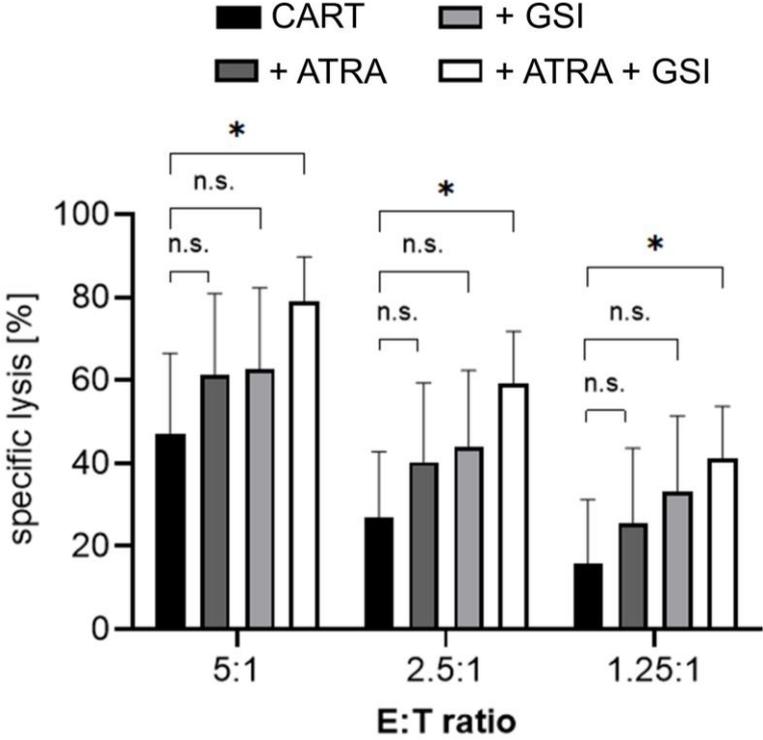
Supplement Figure 6



Supplement Figure 7



Supplement Figure 8



Supplement Figure 9

