SUPPLEMENTAL METHODS

Bone marrow and peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PB-MNCs) from healthy donors and MM patients, and BM mononuclear cells (BM-MNCs) from BM aspirates obtained from MM patients were isolated by Ficoll-Hypaque density-gradient centrifugation.

MM Cell lines and culture

The luciferase (LUC)-transduced MM cell lines UM9, RPMI-8226, U266 and MM1.S, as well as the $CD19^{+}$ Burkitt lymphoma cell line Raji, were cultured in RPMI 1640 (Invitrogen), supplemented with 10% HyClone FetalClone I serum (GE Healthcare Life Sciences) and antibiotics (100 units/mL penicillin, 100 µg/ml streptomycin; both Life Technologies). UM9 was obtained after prolonged *in vitro* culture of the BM aspirate of a MM patient. RPMI-8226, U226, Raji, and MM1.S were purchased from the American Tissue Culture Collection (ATCC). Monthly mycoplasma testing was performed using real-time PCR (Microbiome). Cell lines were authenticated by short-tandem repeat profiling carried out maximal 6 months before the most recent experiment. Cell lines were used for a time period no longer than 4 months.

Flow cytometry-based lysis assay using MM cell lines with PB-MNCs as effector cells.

The MM cell line RPMI-8226 was co-cultured with PB-MNCs from healthy donors at a ratio of 10:1 in 96-well U-bottom plates in the presence of control antibodies (4.0 µg/mL) or JNJ-7957 (0.00128-4.0 µg/mL) for 48 hours. The survival of MM cells, as well as T-cell activation and degranulation, were determined by flow cytometry as described above.

Bioluminescence imaging (BLI)-based lysis assay using LUC-transduced MM cell lines

LUC-transduced MM cell lines were cultured in the presence or absence of pooled BM stromal cells (BMSCs) obtained from newly diagnosed MM patients (n=12) for 16 hours prior to incubation with effector cells (freshly isolated PB-MNCs from healthy donors) at a ratio of 10:1, and serial dilutions of JNJ-7957 (0.00128–4.0 µg/mL) or control antibodies (4.0 µg/mL), in 96-well flat bottom plates (Greiner-Bio-One) for 48 hours. We used the HLA-DP4 restricted, minor histocompatibility antigen-specific $CD4^{+}$ T-cell clone 3AB11, as a control in the coculture experiments, since BMSCs have previously been shown to protect UM9 cells against this T-cell clone(1). In addition, the LUC^{+} RPMI-8226 MM cell line was incubated with effector cells (freshly isolated PB-MNCs from healthy donors) at ratios of 10:1, 3:1, 1:1, 1:3

and 1:10, and serial dilutions of JNJ-7957 (0.00128-4.0 μg/mL) or control antibodies (4.0 μg/mL) in 96-well flat bottom plates for 48 or 96 hours. The survival of LUC^{+}-MM cells was determined by BLI, 30 minutes after addition of the substrate luciferin (150 μg/mL; Promega). Lysis of MM cells was determined using the following formula: % lysis = 1- (mean BLI signal in the presence of effector cells and JNJ-7957 / mean BLI signal in the presence of effector cells in untreated wells) x100%.

To evaluate the effect of *in vivo* daratumumab treatment on the efficacy of JNJ-7957, the LUC-transduced MM cell line RPMI-8226 was incubated with PB-MNCs, obtained from RRMM patients before initiation of daratumumab monotherapy and during daratumumab monotherapy (ratio of 10:1) for 48 hours. Survival was determined by BLI assay, as described before.

Cytogenetic analysis

Cytogenetic abnormalities were assessed in purified MM cells by fluorescence in situ hybridization (FISH) and single nucleotide polymorphism (SNP) array. High-risk disease was defined by the presence of del(17p), del(1p), ampl(1q), t(4;14) or t(14;16)(2).

Soluble BCMA Assay

Soluble BCMA (sBCMA) was measured in cell culture supernatants using MSD GOLD™ 96-well Small Spot Streptavidin SECTOR plates (Meso Scale Diagnostics), according to the manufacturer's recommended protocol.

Granzyme B Assay

Granzyme B was measured in cell culture supernatants using MSD R-Plex Granzyme B assay plates (Meso Scale Diagnostics), according to the manufacturer's protocol.

Multiplex Cytokine Assay

Cytokines [interferon-gamma (IFN-γ), interleukin (IL)-2, IL-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF-α)] in the cell culture supernatants were analyzed using V-Plex proinflammatory Panel 1 Human Kit (Meso Scale Diagnostics), according to the manufacturer's protocol.

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. JNJ-7957 activity is dependent on the PB-MNC:MM cell ratio.

The LUC-transduced MM cell line RPMI-8226 was incubated with solvent control or JNJ-7957 (0.00128 – 4.0 µg/mL) and PB-MNCs obtained from healthy donors as effector cells at a ratio of 10:1, 3:1, 1:1, 1:3 and 1:10 for 48 (black bars) and 96 hours (grey bars). MM cell lysis was assessed using a BLI-based cytotoxicity assay, and was normalized to solvent control. Data represent mean MM cell lysis ± SEM of 3 independent experiments performed in duplicate.

Abbreviations: SEM, standard error of mean.

Figure S2. BMSCs inhibit T-cell-mediated lysis of the MM cell line UM9.

The MM cell line UM9 was incubated with the $CD4^+$ T-cell clone 3AB11 at a ratio of 0.25:1, 0.5:1, 1:1 and 2:1 for 48 hours in the presence or absence of BMSCs. MM cell lysis was assessed using a BLI-based cytotoxicity assay. Data represent mean MM cell lysis ± SEM of 2 independent experiments performed in triplicate. *P*-values were calculated using an unpaired Student's t-test, $*P < 0.05$; $****P < 0.0001$.

Abbreviations: ns, not significant; SEM, standard error of mean.

Figure S3. JNJ-7957 does not affect absolute numbers of $CD4^+$ and $CD8^+$ T-cells.

BM-MNCs obtained from 11 NDMM, 21 daratumumab-naïve RRMM and 17 daratumumab-refractory RRMM patients were incubated with JNJ-7957 (0.0064 – 4.0 µg/mL) for 48 hours, after which the surviving T-cells, $CD4^+$ and $CD8^+$ T-cells were enumerated using flow cytometric analysis. Data represent mean ± SEM. All experiments were performed in duplicate.

Abbreviations: 3930, nullxnull control antibody; 7008, CD3xnull control antibody; BC3B4, BCMAxnull control antibody; SEM, standard error of mean.

Figure S4. JNJ-7957 does not induce NK-cell activation and degranulation.

JNJ-7957-mediated activation and degranulation of NK-cells, measured by cell surface expression of CD25 and CD107a, respectively, were assessed in BM-MNCs obtained from NDMM (black), daratumumab-naïve (blue) and daratumumab-refractory RRMM patient samples (red). Data represent mean ± SEM.

Abbreviations: 3930, nullxnull control antibody; 7008, CD3xnull control antibody; BC3B4, BCMAxnull control antibody; SEM, standard error of mean.

Figure S5. Effect of tumor characteristics on JNJ-7957 mediated MM cell lysis in patient subgroups.

(A) Baseline expression levels of BCMA and PD-L1 on MM cells (mean MFI ± SEM), and percentage of patients with high-risk cytogenetic abnormalities in the 3 different patient subgroups. *P*-values between the indicated groups were calculated using Mann-Whitney *U* test (BCMA and PD-L1) or Pearson Chi-Square test (cytogenetics), $*P < 0.05$. (B) Dose-response curves for JNJ-7957-mediated MM cell lysis in (i) NDMM patient samples, (ii) daratumumab-naïve RRMM samples, and (iii) daratumumab-refractory RRMM samples according to median BCMA or PD-L1 expression on primary MM cells, or presence of high-risk cytogenetic abnormalities. Data represent mean ± SEM. *P*-values were calculated using nonlinear regression analyses with BCMA and PD-L1 analyzed as continuous variables.

Abbreviations: dara, daratumumab; MFI, median fluorescence intensity; ns, not significant; SEM, standard error of mean.

Figure S6. Effect of T-cell composition on JNJ-7957 mediated MM cell lysis in patient subgroups.

(A) Baseline percentage of T-cells, Tregs, PD-1$^+$ T-cells and HLA-DR$^+$ T-cells, as well as E:T ratio and T-cell subsets in NDMM, daratumumab-naïve and daratumumab-refractory RRMM patient samples. Data represent mean ± SEM. *P*-values between the indicated groups were calculated using unpaired Student's t-test or Mann-Whitney *U* test, $**P < 0.01$. (B) Dose-response curves of JNJ-7957-mediated MM cell lysis in (i) NDMM patient samples, (ii) daratumumab-naïve RRMM samples, and (iii) daratumumab-refractory RRMM samples, according to the median percentage of T-cells, Tregs, PD-1$^+$ T-cells, HLA-DR$^+$ T-cells and naïve T-cells, as well as E:T ratio at baseline. Data represent mean ± SEM. *P*-values were calculated using nonlinear regression analyses with all immune characteristics analyzed as continuous variables.

Naïve T-cells were defined as CD45RA$^+$CD62L$^+$, effector memory (EM) T-cells as CD45RA$^-$CD62L$^-$, central memory (CM) T-cells as CD45RA$^-$CD62L$^+$; and terminally differentiated effector memory T-cells expressing CD45RA (TEMRA) as CD45RA$^+$CD62L$^-$.

Abbreviations: dara, daratumumab; HLA-DR, human leukocyte antigen – DR isotype; ns, not significant; SEM, standard error of mean.

Figure S7. Daratumumab pretreatment *in vivo* does not enhance JNJ-7957 mediated T-cell activation and degranulation.

(A) JNJ-7957-mediated activation (surface expression of CD25) and degranulation (surface expression of CD107a) of $CD4^+$ and $CD8^+$ T-cells were assessed using flow cytometry in sequential BM samples obtained from 8 RRMM patients, directly before the initiation of daratumumab treatment (black) and at the time of refractory disease during daratumumab treatment (blue; median duration of treatment 3 months, range 1 – 7). Data represent mean ± SEM, *P*-values were calculated using nonlinear regression analysis. (B) Similarly, JNJ-7957-mediated activation and degranulation of $CD4^+$ and $CD8^+$ T-cells were assessed by flow cytometry in sequential PB samples from 5 RRMM patients before (black) and during daratumumab treatment (blue; median duration of treatment 11 months, range 7 – 14 months). Data represent mean ± SEM, *P*-values were calculated using nonlinear regression analysis. Abbreviations: ns, not significant; SEM, standard error of mean.

Figure S8. Daratumumab abrogates the JNJ-7957-mediated increase in the frequency of $CD38^+$ T-cells, while T-cell numbers were not or only moderately affected.

(A) BM-MNCs obtained from 8 NDMM, 10 daratumumab-naïve RRMM and 14 daratumumab-refractory RRMM patients were incubated with JNJ-7957 (0.0064 – 0.8 µg/mL) with or without daratumumab (10 µg/mL) for 48 hours, after which the frequency of $CD38^+$ T-cells was assessed by flow cytometric analysis. Data represent mean ± SEM. *P*-values were calculated using paired Student's *t*-test, *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$. (B) BM-MNCs obtained from the MM patients as described in (A) were incubated with JNJ-7957 (0.0064 – 0.8 µg/mL) with or without daratumumab (10 µg/mL) for 48 hours, after which surviving T-cells were enumerated using flow cytometric analysis. Lysis was calculated as described in materials and methods. Data represent mean ± SEM, all experiments were performed in duplicate. *P*-values were calculated using paired Student's *t*-test, *$P<0.05$; **$P<0.01$. Abbreviations: DARA, daratumumab; ns, not significant; SEM, standard error of mean.

Figure S9. Short-term co-treatment with daratumumab enhances JNJ-7957-mediated lysis of MM cell lines in an additive fashion. (A) MM cell lines were incubated with JNJ-7957 (0.0064 – 0.8 µg/mL) with or without daratumumab (10 µg/mL) for 48 hours, using healthy donor PB-MNC as effector cells at a ratio 10:1. MM cell lysis was determined by BLI-based cytotoxicity assay. Dots represent individual data, bars represent mean ± SEM.

The observed tumor cell lysis (O) in wells treated with both daratumumab and JNJ-7957 was compared to the expected lysis (E), which was calculated as described in material and methods. *P*-values between the indicated groups were calculated using a paired Student's t-test. (B) The LUC^{+} MM cell line UM9 was incubated with JNJ-7957 (0.00128 – 0.032 µg/mL), daratumumab (0.01 – 10 µg/mL), or a combination of JNJ-7957 and daratumumab for 96 hours, using healthy donor PB-MNC as effector cells at a ratio of 1:1. MM cell lysis was assessed using a BLI-based cytotoxicity assay. Dots represent individual data, bars represent mean ± SEM. The observed tumor cell lysis (O) in wells treated with both daratumumab and JNJ-7957 was compared to the expected lysis (E), which was calculated as described in material and methods. *P*-values between the indicated groups were calculated using a paired Student's t-test; $*P<0.05$.

Abbreviations: Dara, daratumumab; E, expected lysis; ns, not significant; O, observed lysis; SEM, standard error of mean.

Table S1. Patient characteristics

Parameter	NDMM n=11	RRMM patients, dara-naïve n = 21	RRMM patients, dara-refractory n=17	pPCL n=6
Median age, years (range)	66 (31 – 80)	66 (46 – 77)	68 (48 – 80)	65 (57-98)
Sex, male, n (%)	5 (45)	11 (52)	9 (53)	2 (33)
M-protein type				
- IgG, n (%)	5 (45)	15 (71)	13 (76)	2 (33)
- IgA, n (%)	0	1 (5)	2 (12)	0
- FLC only, n (%)	6 (55)	5 (24)	2 (12)	3 (50)
- Unknown	0	0	0	1 (17)
Cytogenetics, n (%)				
- High risk[a]	5 (45)	12 (57)	9 (53)	3 (50)
- Standard risk	5 (45)	7 (33)	5 (29)	1 (17)
- Not assessed	1 (9)	2 (10)	3 (18)	2 (33)
Previous lines of therapy, n (range)	0	3 (1 – 9)	6 (3 – 12)	0
Most recent treatment				
- No treatment	11 (100)	0	0	6 (100)
- PI based	0	2 (10)	0	0
- IMiD based	0	15 (71)	1 (6)[c]	0
- PI + IMiD	0	4 (19)	1 (6)[c]	0
- Daratumumab	0	0	15 (88)[c]	0
Lenalidomide	n.a.			n.a.
- exposed, n (%)		19 (90)[d]	17 (100)	
- refractory[b], n (%)		18 (86)	17 (100)	
Bortezomib	n.a.			n.a.
- exposed, n (%)		17 (81)[e]	16 (94)[f]	
- refractory[b], n (%)		10 (48)	11 (65)	
Pomalidomide refractory[b], n (%)	n.a.	13 (62)	10 (59)	n.a.
Carfilzomib refractory[b], n (%)	n.a.	4 (19)	4 (24)	n.a.
Daratumumab refractory[b], n (%)	n.a.	0	17 (100)	n.a.
Elotuzumab refractory[b], n (%)	n.a.	2 (10)	1 (6)	n.a.
Ixazomib refractory[b], n (%)	n.a.	1 (5)	1 (6)	n.a.

[a]High-risk disease was defined by the presence of del(17p), del(1p), ampl(1q), t(4;14) or t(14;16).

[b]Refractory disease is defined as progressive disease during therapy, no response (less than PR), or progressive disease within 60 days of stopping treatment, according to the International Uniform Response Criteria for Multiple Myeloma.

[c]BM aspirates were obtained immediately at the time of development of progressive disease during daratumumab monotherapy (n=15), while 2 BM samples were obtained 22 and 48 months after development of progression during daratumumab monotherapy, with 3 and 5 intervening lines of non-daratumumab-containing treatment, respectively.

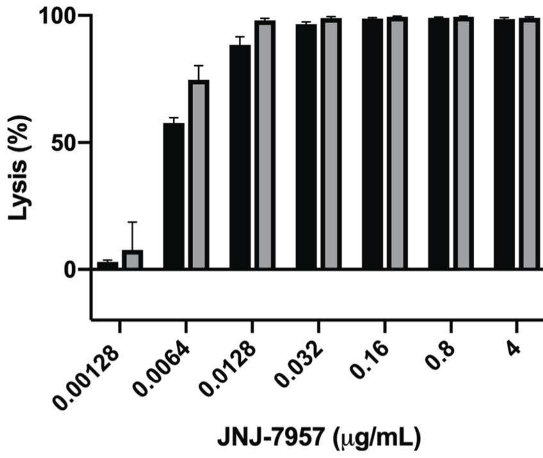
[d]Additionally, 1 out of 19 patients was lenalidomide intolerant;

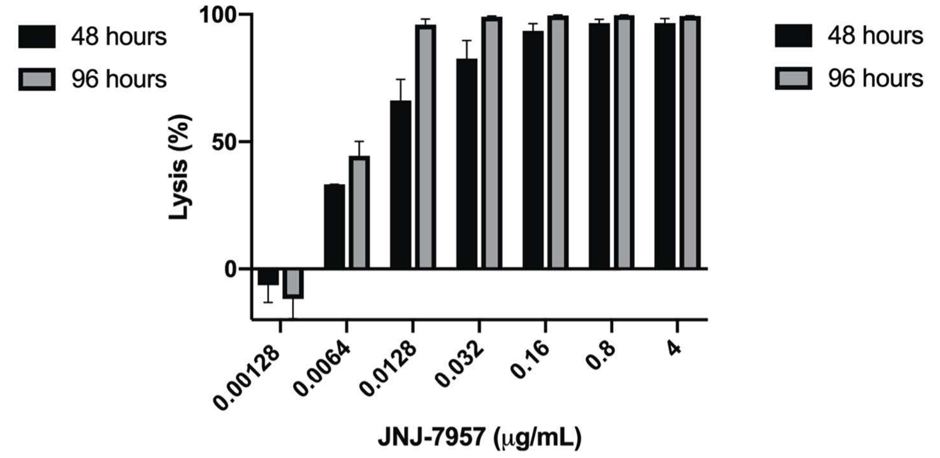
[e]Additionally, 4 out of 17 patients were bortezomib intolerant;

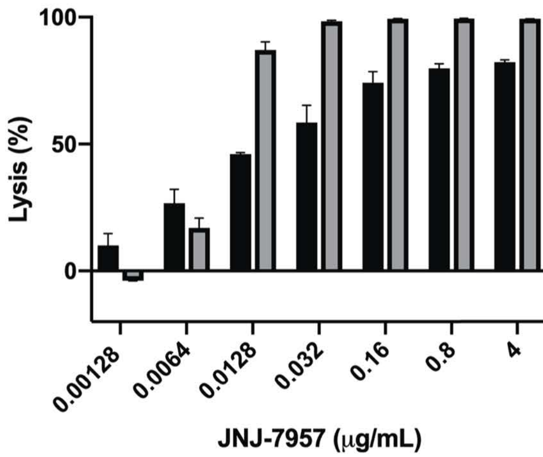
[f]Additionally, 3 out of 16 patients were bortezomib intolerant;

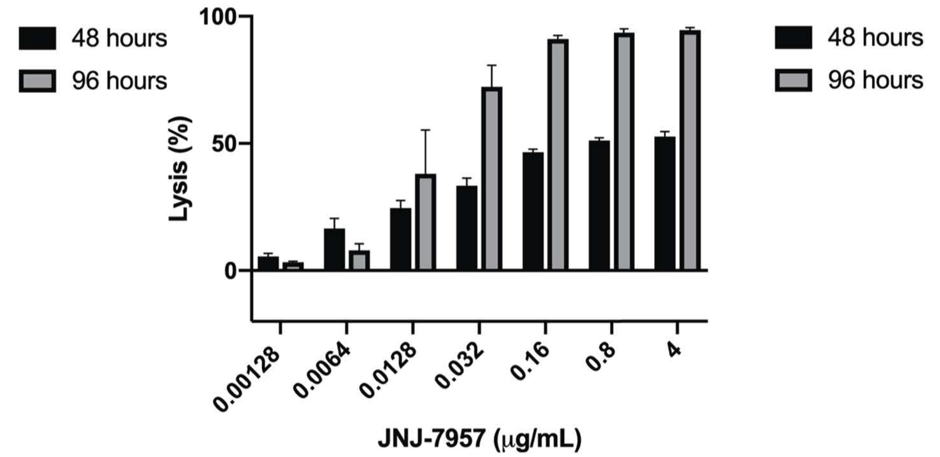
Abbreviations: ampl, amplification; dara, daratumumab; del, deletion; FLC, free light chain; IgA, immunoglobulin A; IgG, immunoglobulin G; n, number; t, translocation.

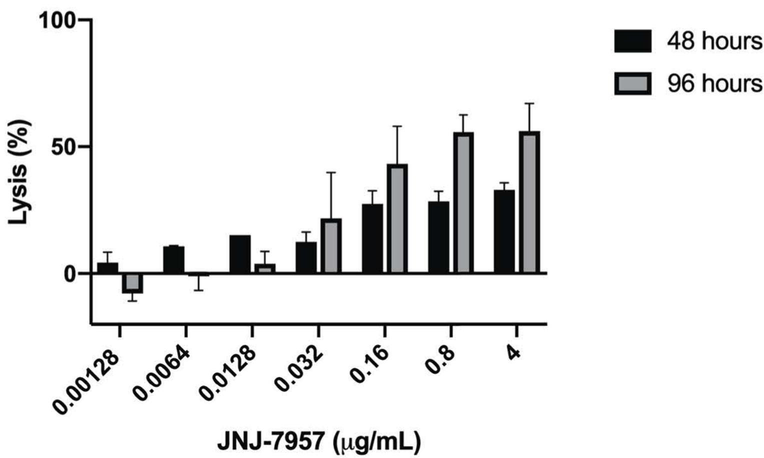
Figure S1











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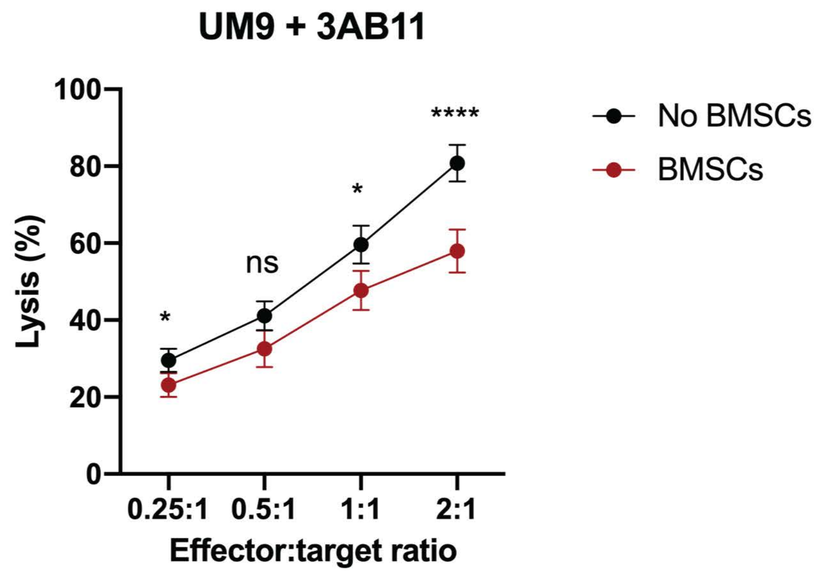

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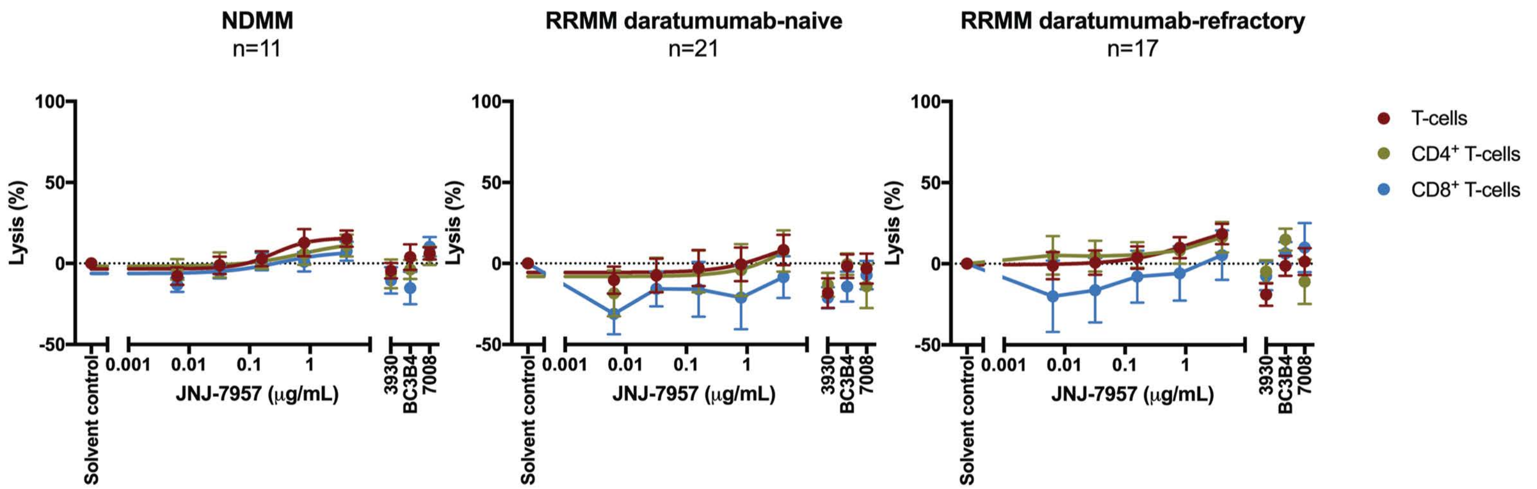

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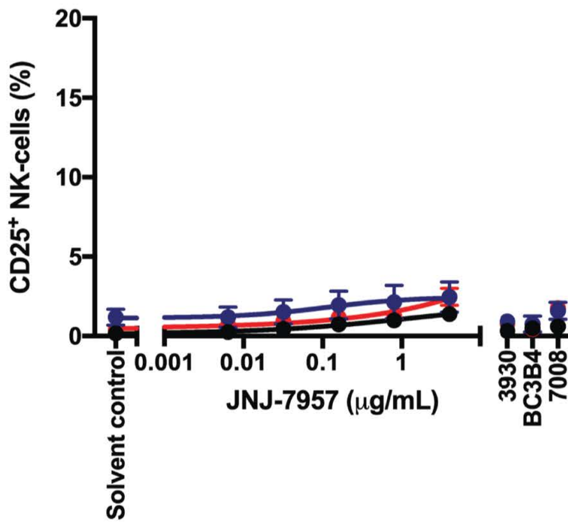

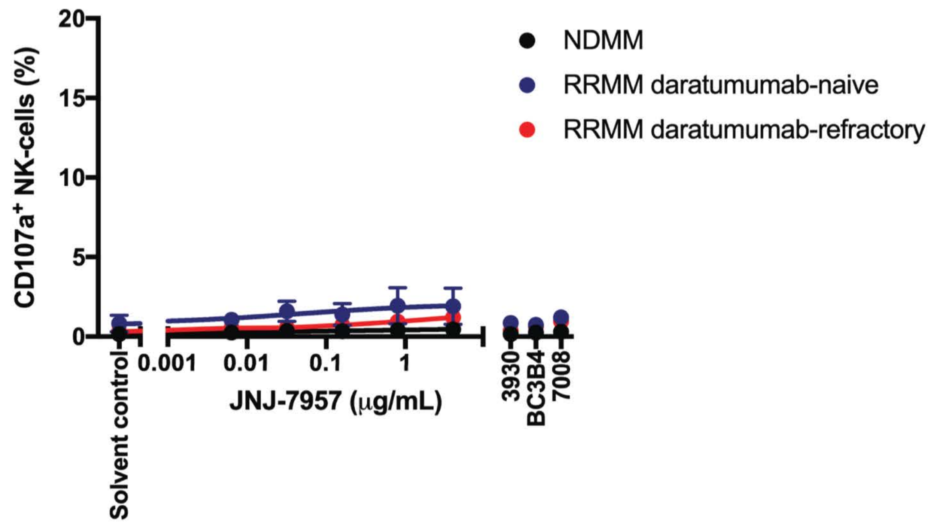

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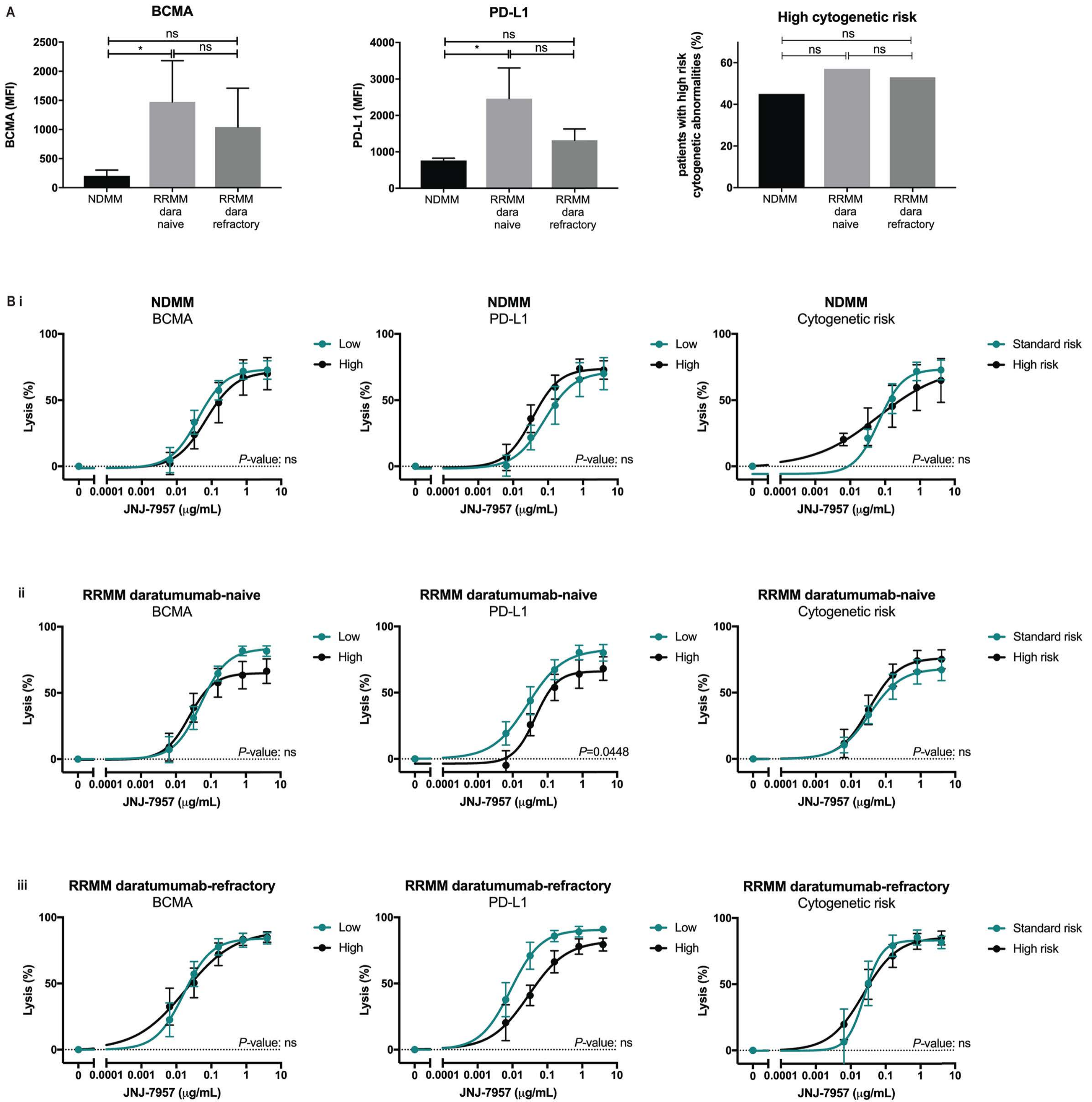

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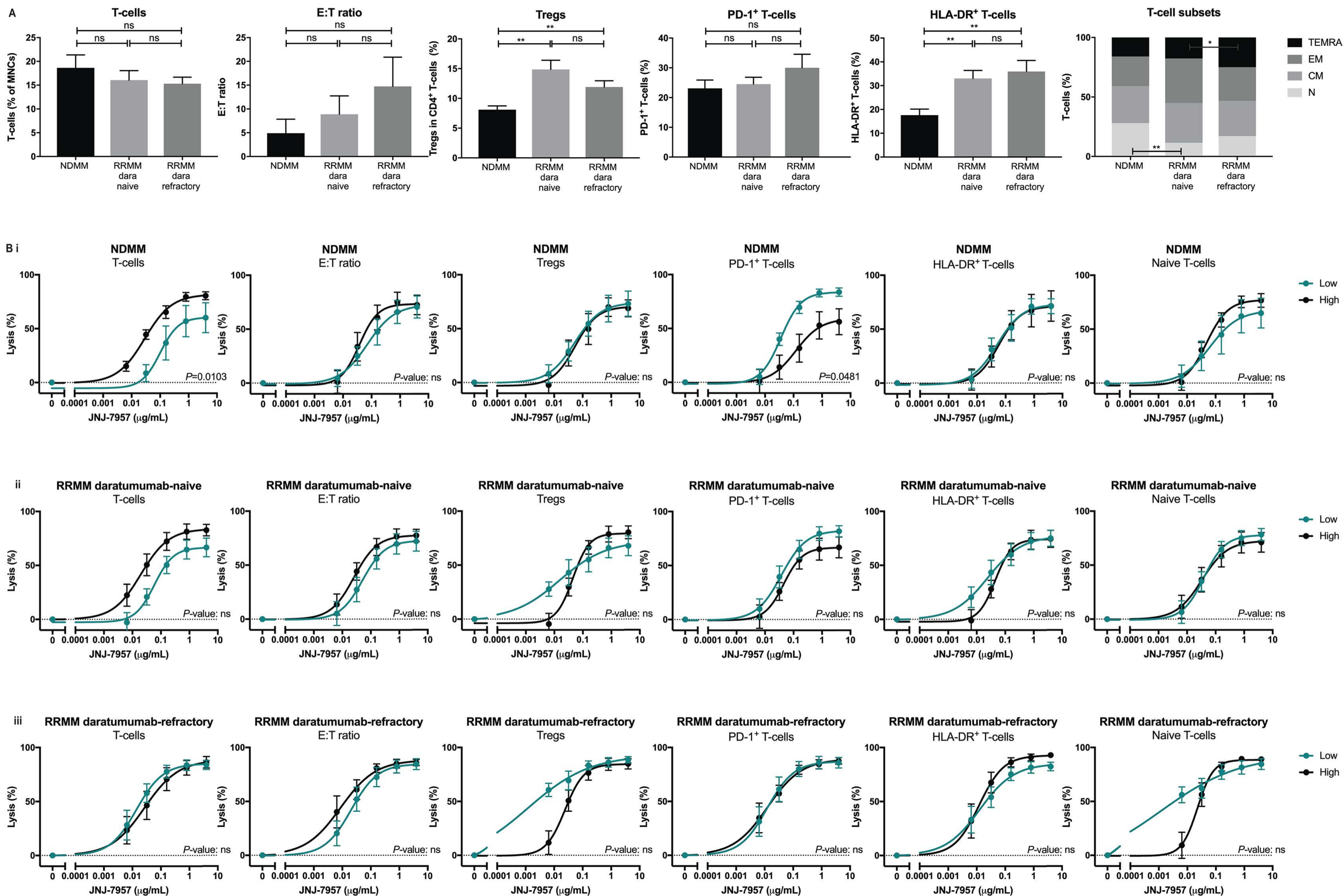

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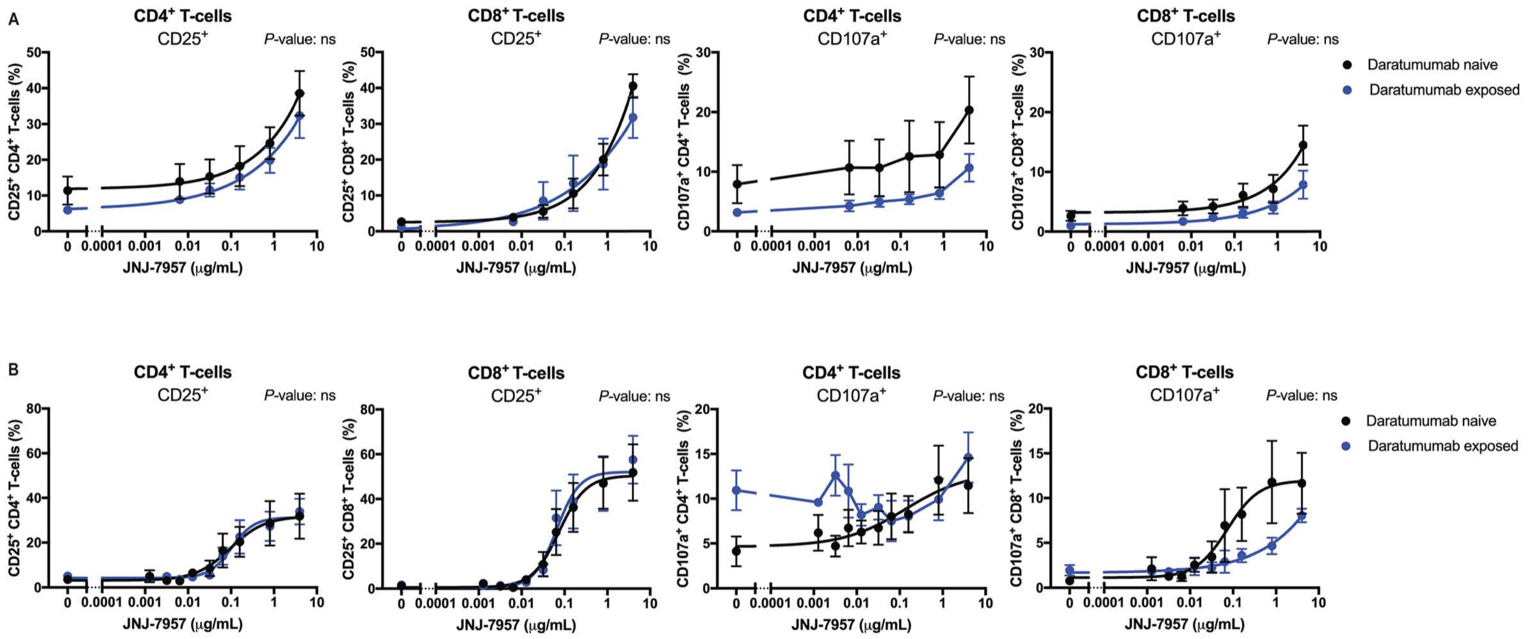

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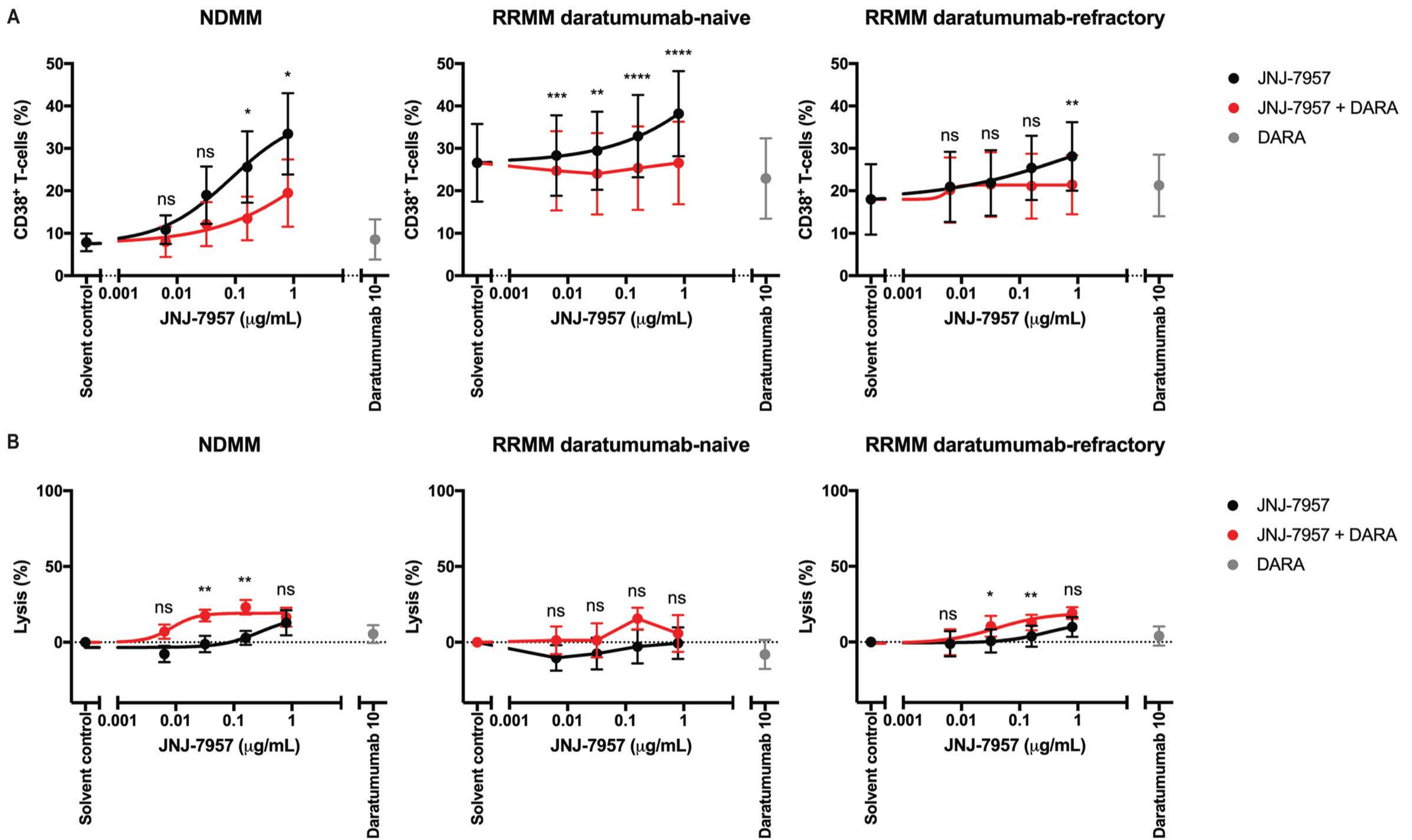

Figure S9