

Supplementary Material

Crosslinking of CD20 and CD38 by Drug-Free Macromolecular Therapeutics Enhances B Cell Apoptosis

M. Tommy Gambles^{1,2}, Jiahui Li^{1,2}, D. Christopher Radford^{1,4}, Douglas Sborov³, Paul Shami³, Jiyuan Yang^{1,2*}, Jindřich Kopeček^{1,2,4*}

¹ Center for Controlled Chemical Delivery, University of Utah, Salt Lake City, UT 84112, USA

² Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA

³ Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA

⁴ Department of Biomedical Engineering, University of Utah, Salt Lake City, Utah 84112, USA

* Correspondence: jindrich.kopecek@utah.edu; jiyuan.yang@utah.edu

Table of Contents

Figure S1. Fab'-MORF1 size exclusion chromatograms.

Figure S2. UV-Vis spectroscopy hybridization of nanoconjugates.

Figure S3. Size exclusion chromatograms of hybridized nanoconjugates.

Figure S4. CD20 and CD38 expression on Raji cells.

Figure S5. Single-target DFMT median effect plots.

Figure S6. Fab'_{DARA} + Fab'_{RTX} DFMT combination index experimental data.

Figure S7. Fab'_{DARA} + Fab'_{OBN} DFMT combination index experimental data.

Figure S8. Fab'_{ISA} + Fab'_{RTX} DFMT combination index experimental data.

Figure S9. Fab'_{ISA} + Fab'_{OBN} DFMT combination index experimental data.

Figure S10. Dual-target DFMT caspase 3 activation flow cytometry fluorescence histograms.

Figure S11. Dual-target DFMT Bax/Bcl-2 protein expression ratio.

Figure S12. Dual-target DFMT lysosomal enlargement inhibition using cathepsin B inhibitor.

Figure S13. Saline-treated mice weights and bone marrow immunostains.

Figure S14. Fab'_{RTX} DFMT single dose mice weights and bone marrow immunostains.

Figure S15. Fab'_{RTX} DFMT triple dose mice weights and bone marrow immunostains.

Figure S16. Fab'_{DARA} DFMT single dose mice weights and bone marrow immunostains.

Figure S17. Fab'_{RTX} DFMT triple dose mice weights and bone marrow immunostains.

Figure S18. Dual-target DFMT single dose mice weights and bone marrow immunostains.

Figure S19. Dual-target DFMT single dose mice weights and bone marrow immunostains.

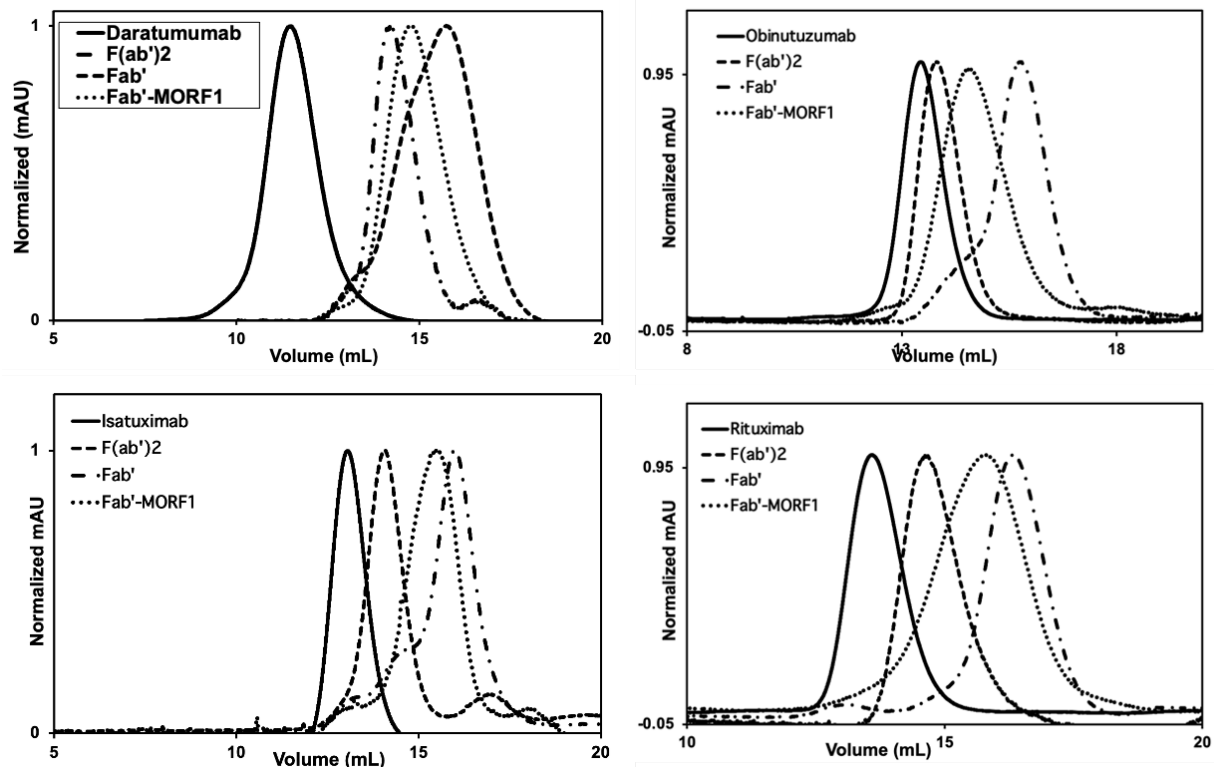


Figure S1. Fab'-MORF1 size exclusion chromatograms of intermediates and final products.

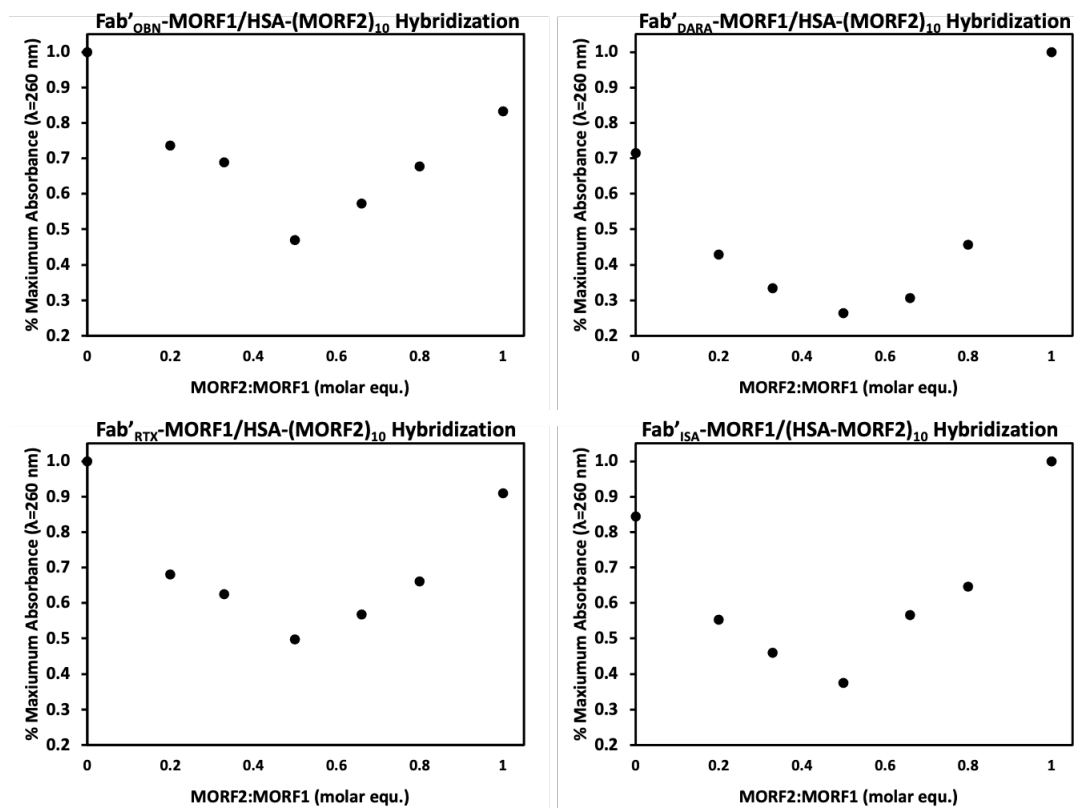


Figure S2. UV-Vis spectrophotometry observations of morpholino hybridization between Fab'-MORF1 nanoconjugates and HSA-(MORF2)₁₀. Absorbance at $\lambda = 260$ nm was monitored for base pair complementation with varying molar equivalences of MORF1:MORF2 in a PBS pH 7.4 solution $\epsilon_{\text{MORF1}} = 278,000 \text{ M}^{-1}\text{cm}^{-1}$; $\epsilon_{\text{MORF2}} = 252,120 \text{ M}^{-1}\text{cm}^{-1}$. Hybridization is indicated by a decrease in absorbance at this wavelength.

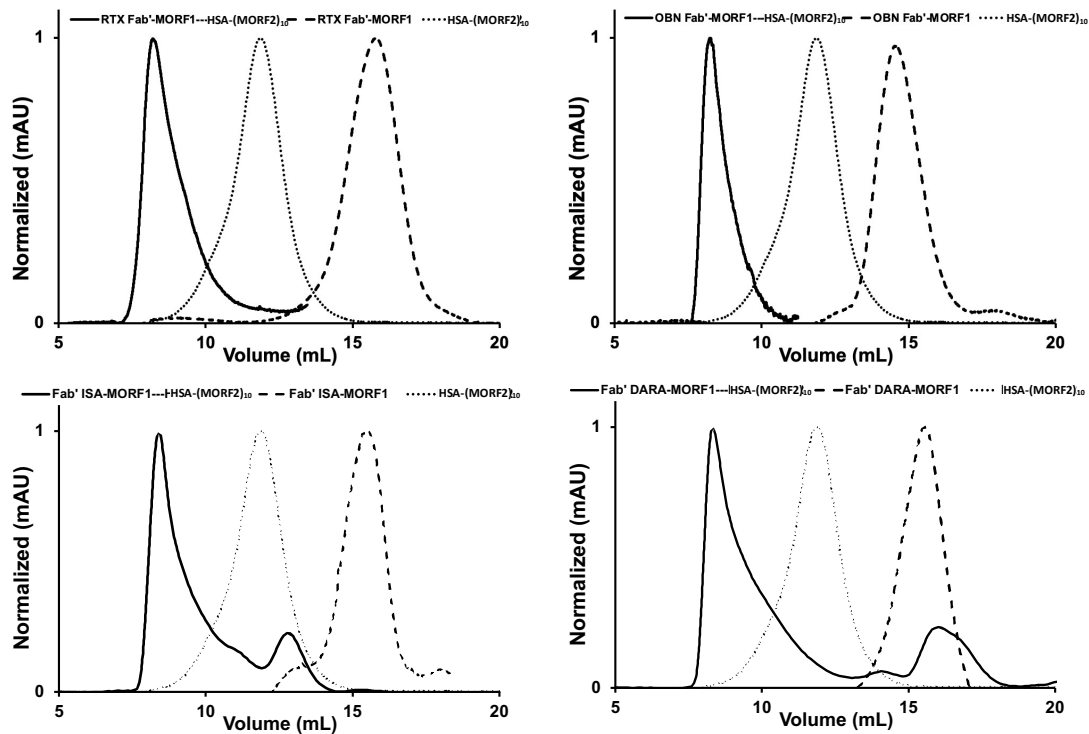


Figure S3. Fab'-MORF1 and HSA-(MORF2)₁₀ hybridization determined by SEC with a Superdex 200 10/300 GL column, PBS (pH 7.4) as eluant at 0.4 mL/min flow rate. A 1:1 MORF1:MORF2 molar equivalent solution of Fab'-MORF1 and HSA-(MORF2)₁₀ was premixed in PBS and allowed to hybridize for 10 min. Hybridized nanoconjugate (solid) was compared to Fab'-MORF1 (dashed) and HSA-(MORF2)₁₀ (dotted).

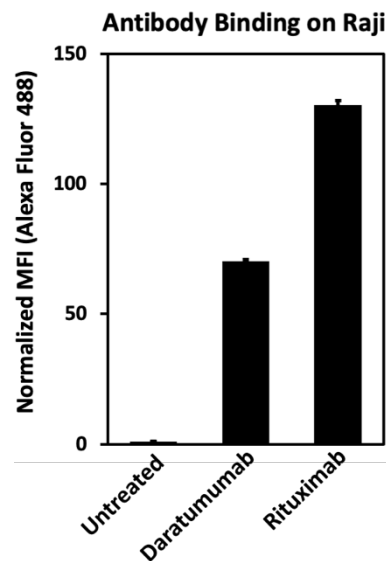
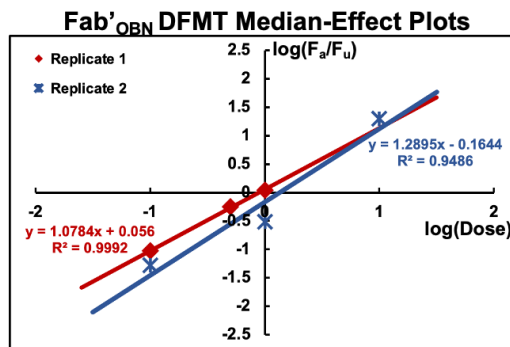
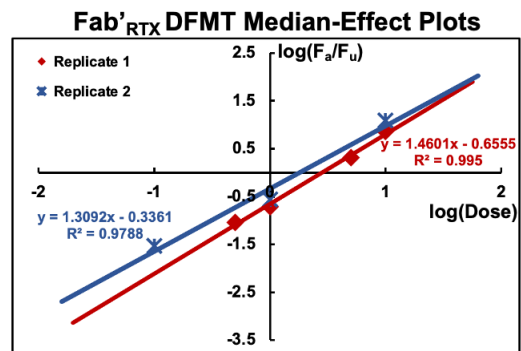


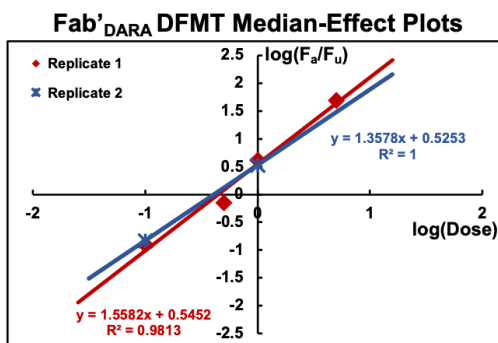
Figure S4. Fluorescence quantification of Rituximab mAb to Daratumumab mAb binding on Raji cells indicating about 2-fold higher CD20 expression than CD38 expression. Binding was measured by treating cells with mAb for 1 h at 4 °C followed by PBS wash followed by 1 h exposure to Fluor488 goat anti-human secondary antibody. Fluorescence of secondary antibody binding was quantified using flow cytometry as a mean fluorescent intensity and normalized to unstained cells. Higher CD20 expression would suggest better response to anti-CD20 DFMT systems on this cell type.



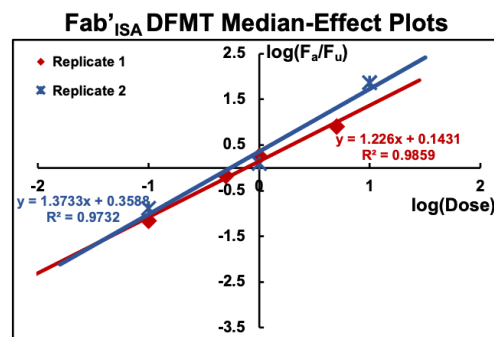
Fab' _{OBN} DFMT	D _m (nM)	m	r
Replicate 1	1.34	1.29	0.974
Replicate 2	0.887	1.08	0.999



Fab' _{RTX} DFMT	D _m (nM)	m	r
Replicate 1	1.81	1.31	0.989
Replicate 2	2.81	1.46	0.997



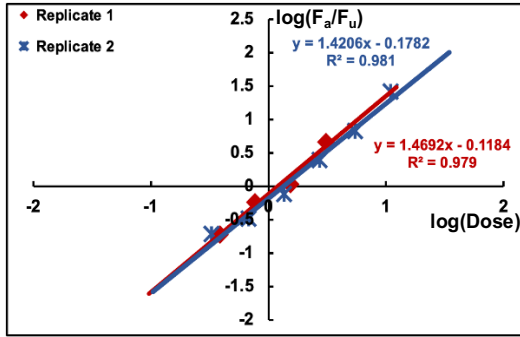
Fab' _{DARA} DFMT	D _m (nM)	m	r
Replicate 1	0.410	1.36	0.974
Replicate 2	0.447	1.56	0.991



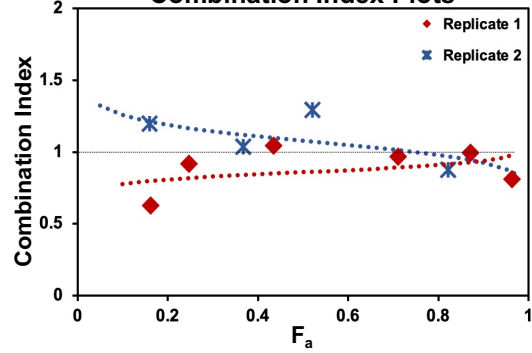
Fab' _{ISA} DFMT	D _m (nM)	m	r
Replicate 1	0.548	1.37	0.987
Replicate 2	0.764	1.23	0.993

Figure S5. Individual Fab' DFMT Median-Effect Plots, correlation data, slope and EC₅₀ values reported as D_m, or x-intercept. Two replicates per Fab' DFMT system were performed.

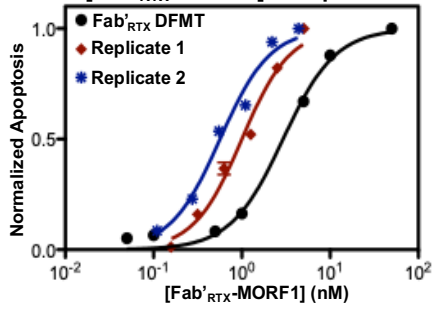
Fab'_{DARA} + Fab'_{RTX} DFMT Median-Effect Plots



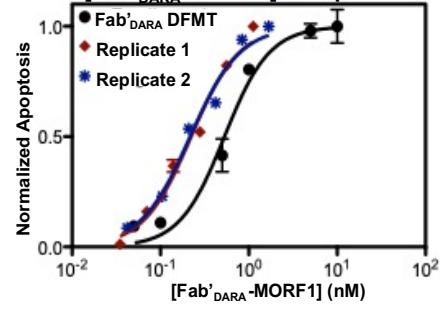
Fab'_{DARA} + Fab'_{RTX} DFMT Combination Index Plots



[Fab'_{RTX}-MORF1] Comparison



[Fab'_{DARA}-MORF1] Comparison

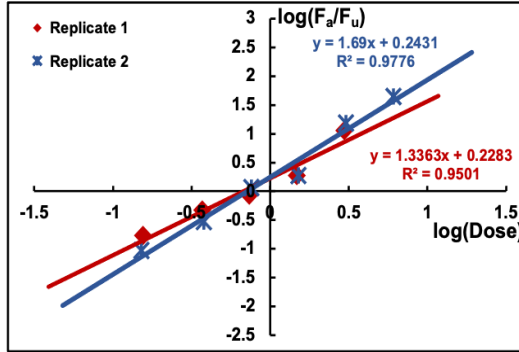


Treatment Group	Fab' _{DARA} -MORF1 (nM)	Fab' _{RTX} -MORF1 (nM)	Combined Dose (nM)
1	1.6 (4X EC ₅₀)	7.2 (4X EC ₅₀)	8.6
2	0.8 (2X EC ₅₀)	3.6 (2X EC ₅₀)	4.4
3	0.4 (1X EC ₅₀)	1.8 (1X EC ₅₀)	2.2
4	0.2 (1/2X EC ₅₀)	0.9 (1/2X EC ₅₀)	1.1
5	0.1 (1/4X EC ₅₀)	0.45 (1/4X EC ₅₀)	0.55
6	0.04 (1/10X EC ₅₀)	0.18 (1/10X EC ₅₀)	0.22

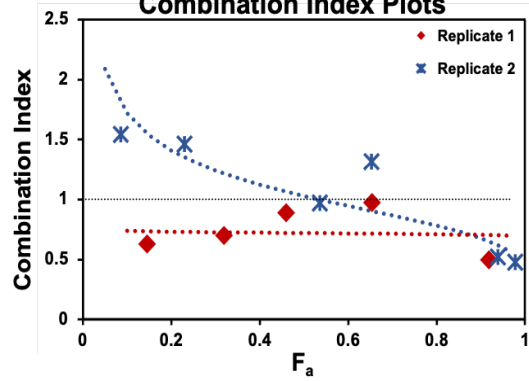
Fab' _{DARA} + Fab' _{RTX} DFMT	D _m (nM)	m	r	Combination Index Values		
				ED ₇₅	ED ₉₀	ED ₉₅
Replicate 1	1.20	1.47	0.989	0.999	0.926	0.880
Replicate 2	1.33	1.42	0.990	0.904	0.947	0.979

Figure S6. Fab'_{DARA} + Fab'_{RTX} DFMT combination index experimental data.

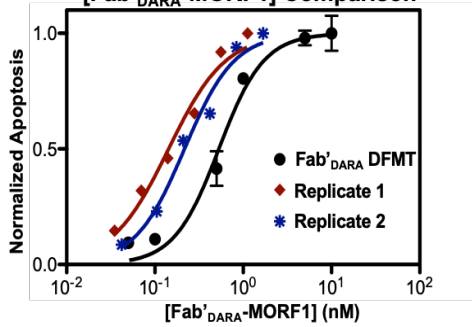
Fab'_{DARA} + Fab'_{OBN} DFMT Median-Effect Plots



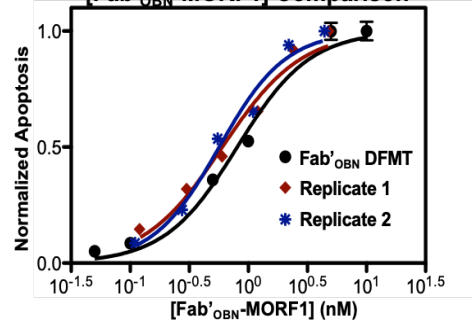
Fab'_{DARA} + Fab'_{RTX} DFMT Combination Index Plots



[Fab'_{DARA}-MORF1] Comparison



[Fab'_{OBN}-MORF1] Comparison

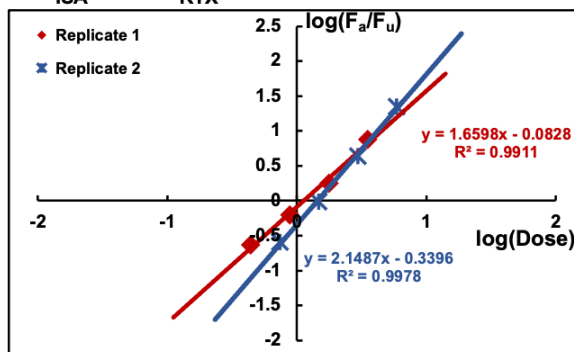


Treatment Group	Fab' _{DARA} -MORF1 (nM)	Fab' _{OBN} -MORF1 (nM)	Combined Dose (nM)
1	1.6 (4X EC ₅₀)	4.8 (4X EC ₅₀)	6.4
2	0.8 (2X EC ₅₀)	2.4 (2X EC ₅₀)	3.2
3	0.4 (1X EC ₅₀)	1.2 (1X EC ₅₀)	1.6
4	0.2 (1/2X EC ₅₀)	0.6 (1/2X EC ₅₀)	0.8
5	0.1 (1/4X EC ₅₀)	0.3 (1/4X EC ₅₀)	0.4
6	0.04 (1/10X EC ₅₀)	0.12 (1/10X EC ₅₀)	0.16

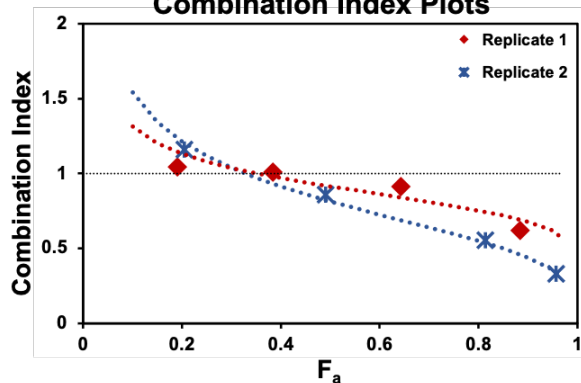
Fab' _{DARA} + Fab' _{OBN} DFMT	D _m (nM)	m	r	Combination Index Values		
				ED ₇₅	ED ₉₀	ED ₉₅
Replicate 1	0.675	1.34	0.975	0.711	0.704	0.699
Replicate 2	0.718	1.69	0.989	0.825	0.678	0.601

Figure S7. Fab'_{DARA} + Fab'_{OBN} DFMT combination index experimental data.

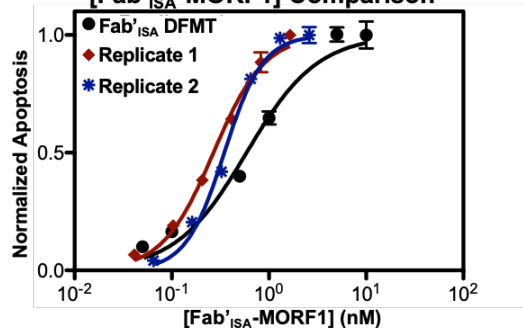
Fab'ISA + Fab'RTX DFMT Median-Effect Plots



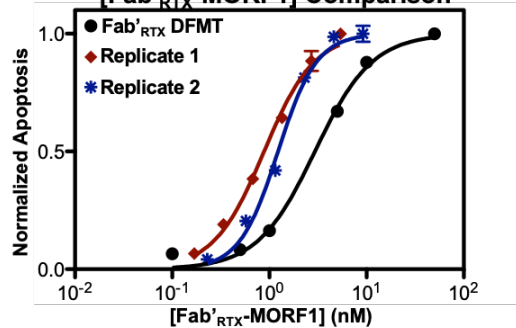
Fab'ISA + Fab'RTX DFMT Combination Index Plots



[Fab'ISA-MORF1] Comparison



[Fab'RTX-MORF1] Comparison

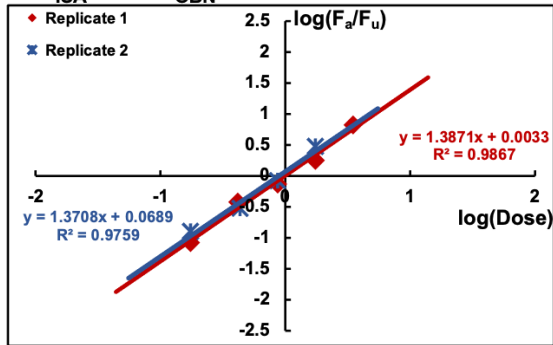


Treatment Group	Fab'ISA-MORF1 (nM)	Fab'RTX-MORF1 (nM)	Combined Dose (nM)
1	2.2 (4X EC ₅₀)	7.2 (4X EC ₅₀)	9.4
2	1.1 (2X EC ₅₀)	3.6 (2X EC ₅₀)	4.7
3	0.55 (1X EC ₅₀)	1.8 (1X EC ₅₀)	2.35
4	0.275 (1/2X EC ₅₀)	0.9 (1/2X EC ₅₀)	1.175
5	0.1375 (1/4X EC ₅₀)	0.45 (1/4X EC ₅₀)	0.5875
6	0.055 (1/10X EC ₅₀)	0.18 (1/10X EC ₅₀)	0.235

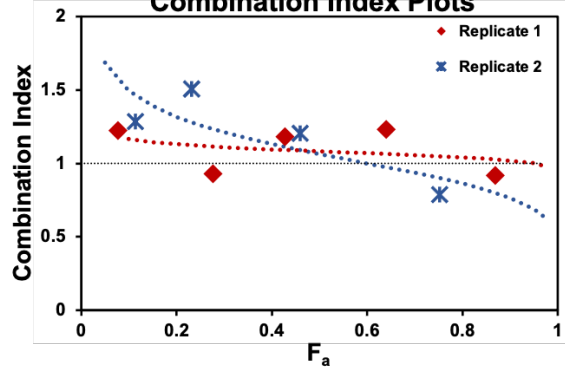
Fab'ISA + Fab'RTX DFMT	D _m (nM)	m	r	Combination Index Values		
				ED ₇₅	ED ₉₀	ED ₉₅
Replicate 1	1.12	1.66	0.996	0.768	0.655	0.588
Replicate 2	1.44	2.15	0.998	0.596	0.438	0.357

Figure S8. Fab'ISA + Fab'RTX DFMT combination index experimental data.

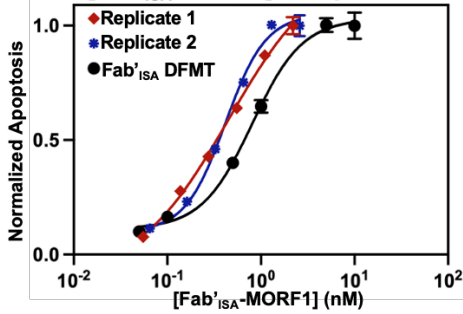
Fab'ISA + Fab'OBN DFMT Median-Effect Plots



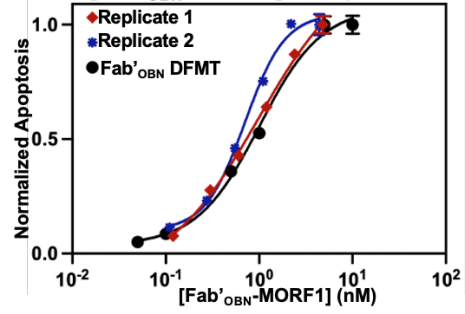
Fab'ISA + Fab'OBN DFMT Combination Index Plots



[Fab'ISA-MORF1] Comparison



[Fab'OBN-MORF1] Comparison



Treatment Group	Fab'ISA-MORF1 (nM)	Fab'OBN-MORF1 (nM)	Combined Dose (nM)
1	2.2 (4X EC ₅₀)	4.8 (4X EC ₅₀)	7
2	1.1 (2X EC ₅₀)	2.4 (2X EC ₅₀)	3.5
3	0.55 (1X EC ₅₀)	1.2 (1X EC ₅₀)	1.75
4	0.275 (1/2X EC ₅₀)	0.6 (1/2X EC ₅₀)	0.875
5	0.1375 (1/4X EC ₅₀)	0.3 (1/4X EC ₅₀)	0.4375
6	0.055 (1/10X EC ₅₀)	0.12 (1/10X EC ₅₀)	0.175

Fab'ISA + Fab'OBN DFMT	D _m (nM)	m	r	Combination Index Values		
				ED ₇₅	ED ₉₀	ED ₉₅
Replicate 1	0.995	1.39	0.993	1.04	1.01	0.991
Replicate 2	0.891	1.37	0.989	0.901	0.767	0.688

Figure S9. Fab'ISA + Fab'OBN DFMT combination index experimental data.

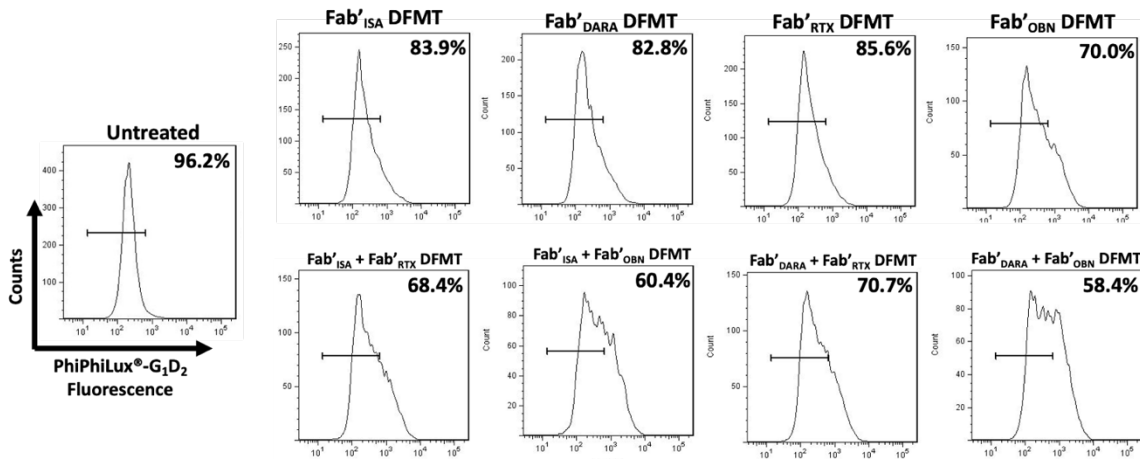


Figure S10. Caspase 3 activation experiment histograms from flow cytometry. Corresponds with bar graph data presented in Figure 5C.

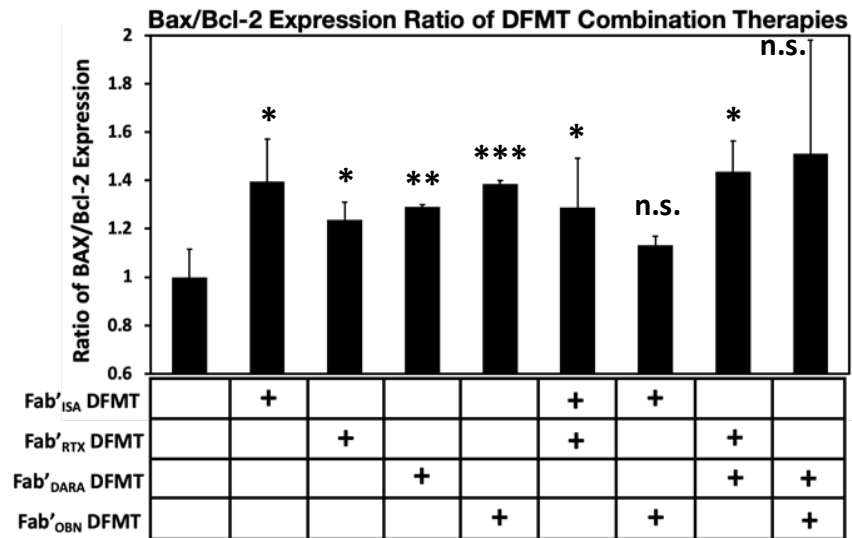


Figure S11. Bcl-2 and Bax protein expression presented as a ratio of Bax / Bcl-2 of Raji cells treated with various Fab' DFMT systems. Bcl-2 and Bax protein expression was quantified using immunostaining of the two proteins and fluorescence quantified using flow cytometry. Higher Bax-to-Bcl-2 ratio indicates a proapoptotic state in the treated cells. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. not significant by One-Way ANOVA and Tukey test.

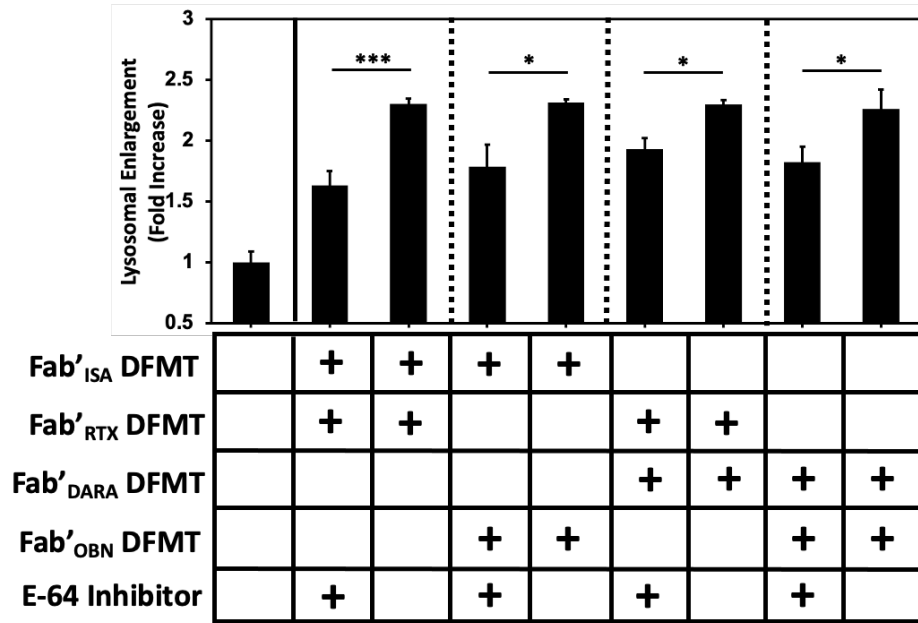


Figure S12. Lysosomal enlargement inhibition of dual-target DFMT treated Raji cells with or without co-treatment with E-64 cysteine proteinase inhibitor. Corresponds to histograms presented in main text Figure 7D. *** $p < 0.001$, * $p < 0.05$ by One-Way ANOVA and Tukey test.

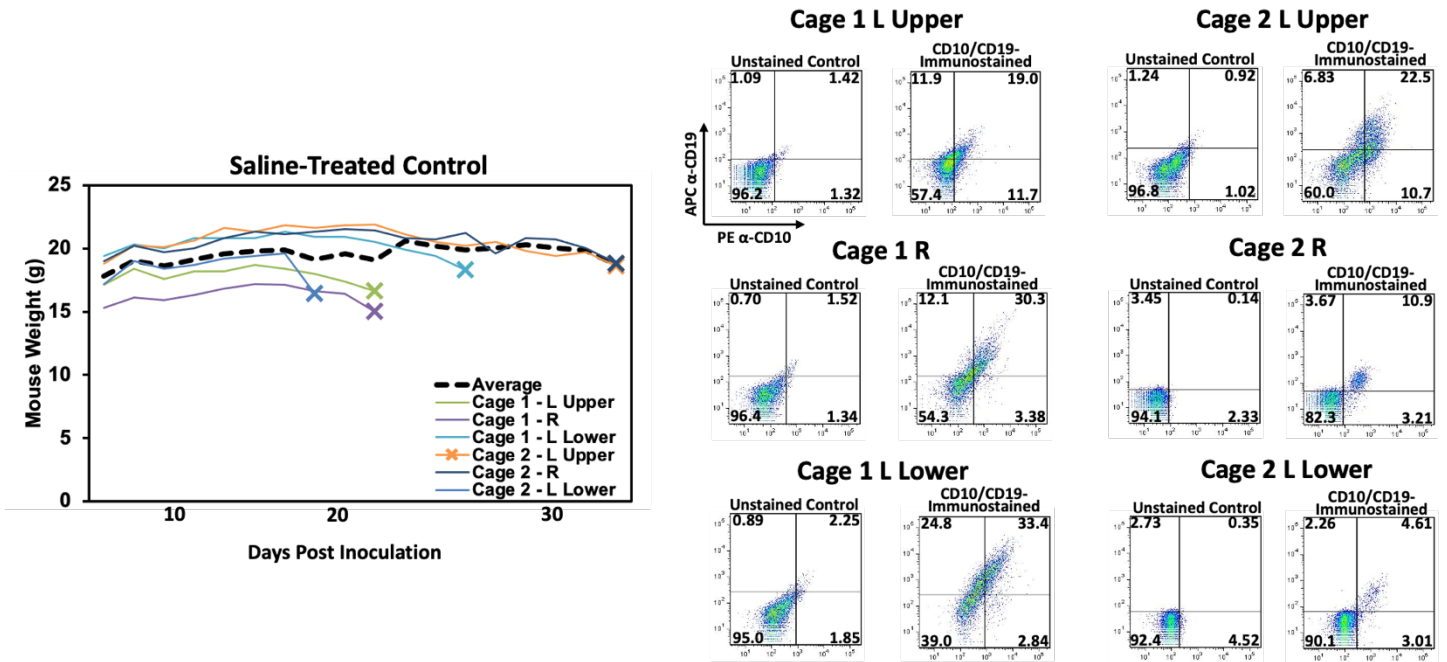


Figure S13. In vivo dual-target DFMT. Saline-treated mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies.

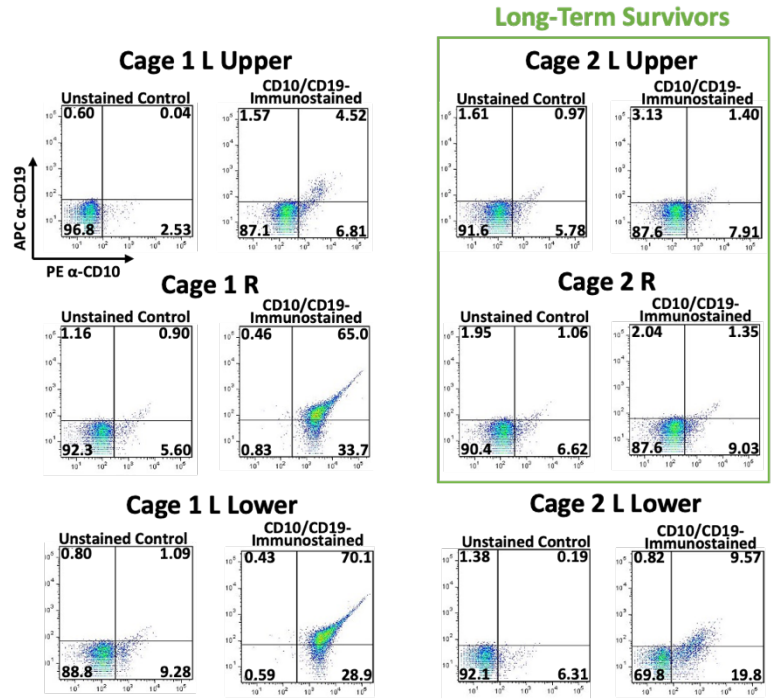
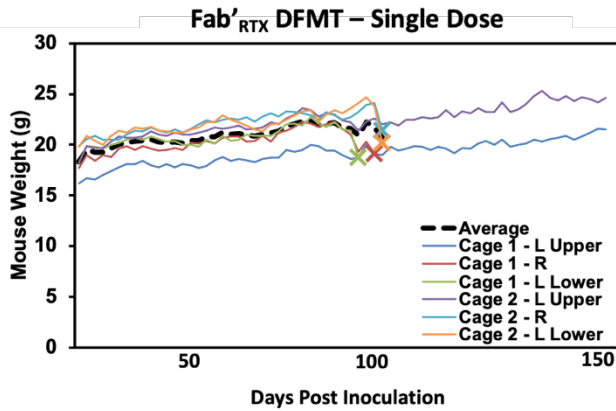


Figure S14. In vivo dual-target DFMT. Fab'RTX DFMT single dose mouse cohort. Body weights and bone marrow immunostaining with human α -CD10 and human α -CD19 antibodies. Long-term surviving mice indicated with green box.

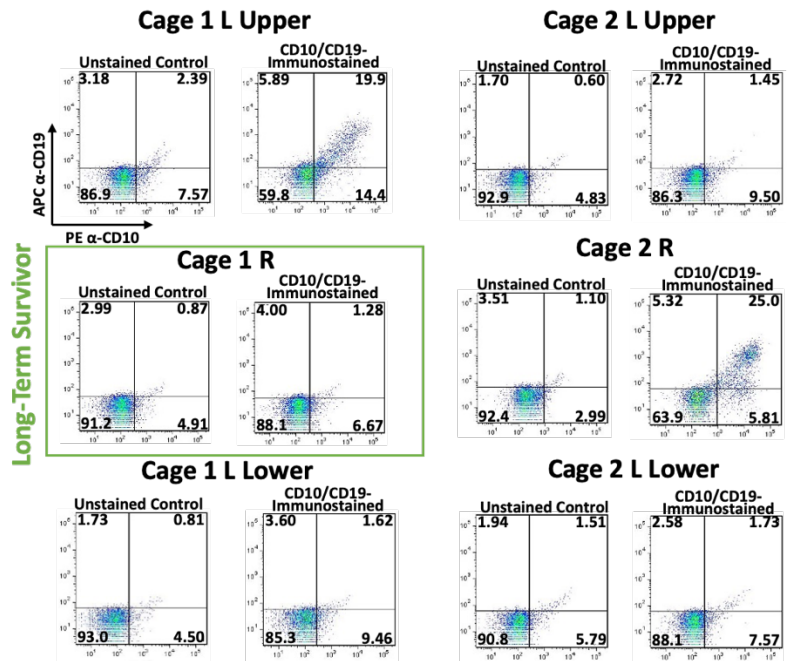
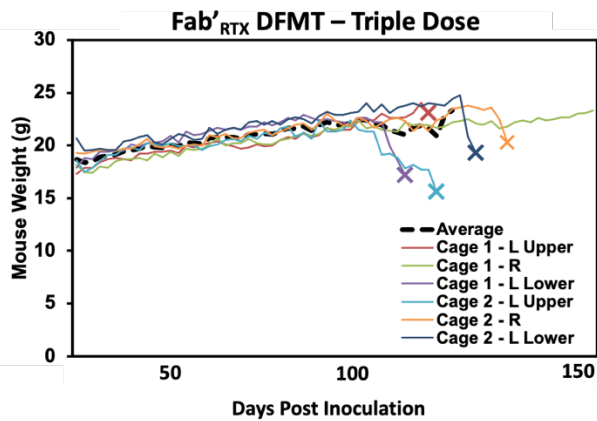


Figure S15. In vivo dual-target DFMT. Fab'RTX DFMT triple dose mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies. Long-term surviving mice indicated with green box.

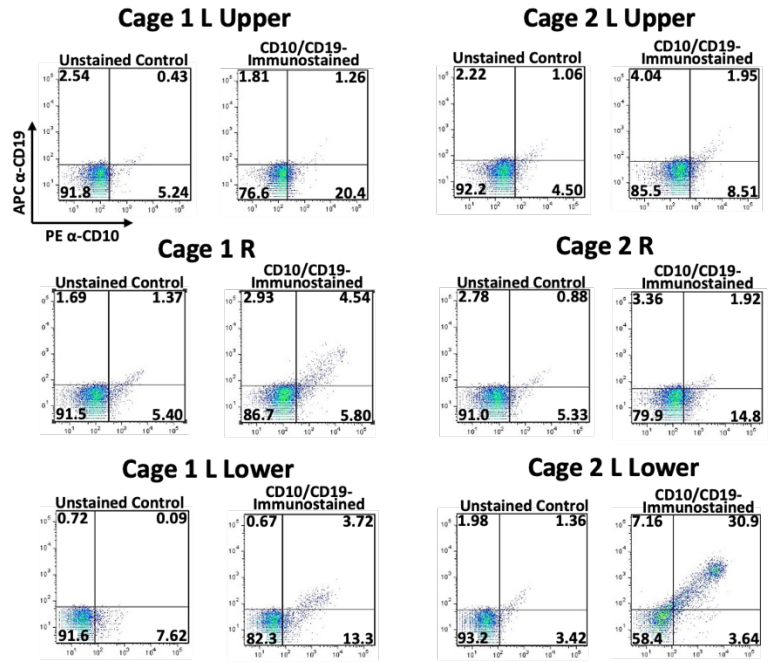
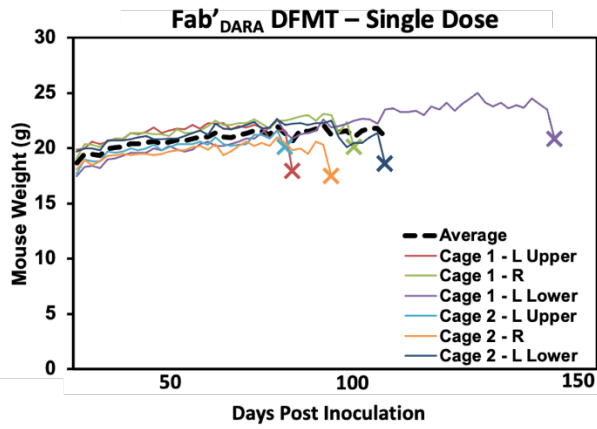


Figure S16. In vivo dual-target DFMT. Fab_{DARA} DFMT single dose mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies.

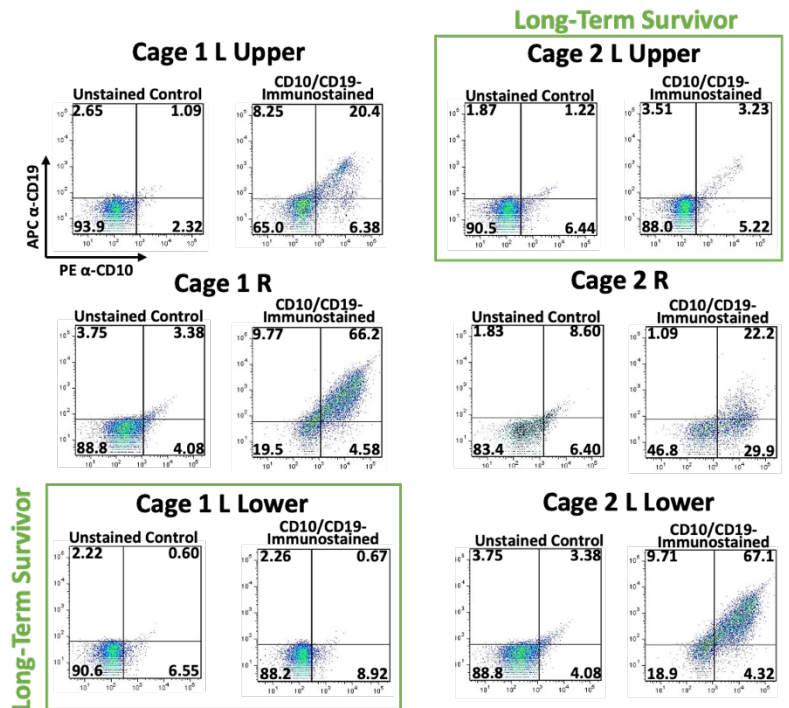
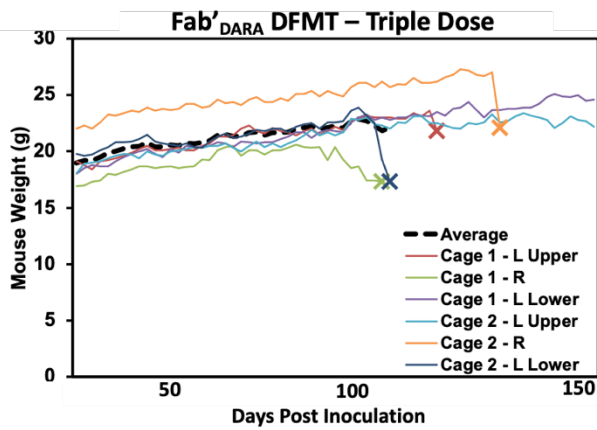


Figure S17. In vivo dual-target DFMT. Fab_{DARA} DFMT triple dose mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies. Long-term surviving mice indicated with green boxes.

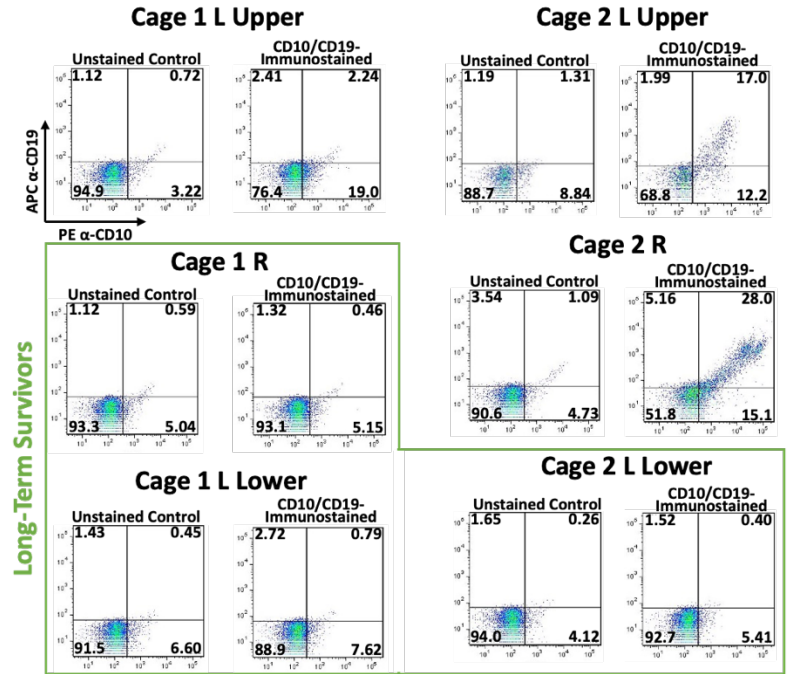
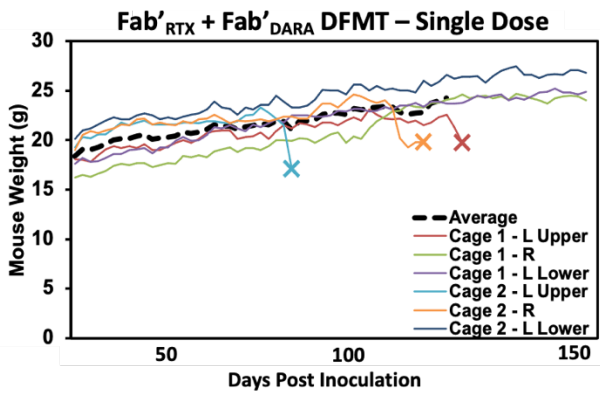


Figure S18. In vivo dual-target DFMT. Dual-target DFMT single dose mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies. Long-term surviving mice indicated with green box.

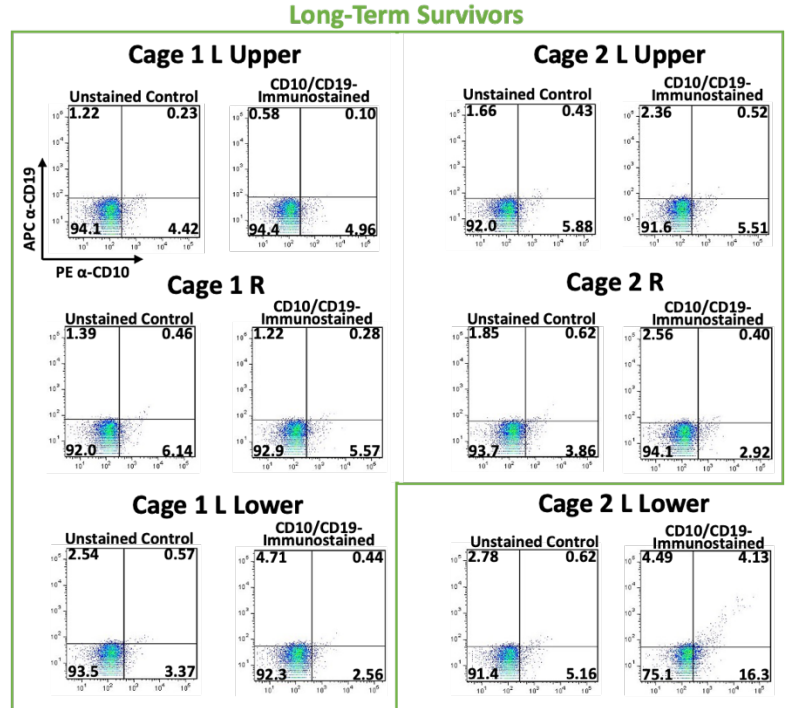
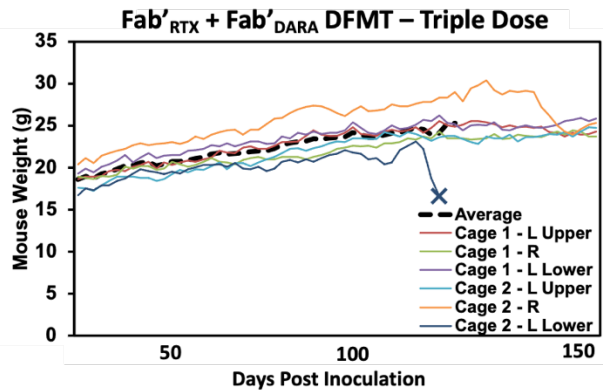


Figure S19. In vivo dual-target DFMT. Dual-target DFMT triple dose mouse cohort. Body weights and bone marrow immunostaining for human α -CD10 and human α -CD19 antibodies. Long-term surviving mice indicated with green box.