Supporting Information

Virus Mimicking Polymer Nanoparticles Targeting CD169^{+} Macrophages as Long-acting Nanocarriers for Combination Antiretrovirals

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Additional Methods Section

RPV and CAB release kinetics determination. The release data were fitted using zero- and first-order, Higuchi. Zero-order model is described with the equation: $Q_t = Q_0 + K_0t$, where Q_t is the amount of drug released at time t, Q_0 is the intimal amount of drug, and K_0 is the zero order release constant. In this model, the data are plotted as percentage of drug released *versus* time. First-order model is expressed with the equation: $\log Q_t = \log Q_0 – K_1t / 2.303$, where Q_t is the amount of drug released at time t, Q_0 is the intimal amount of drug, and K_1 is the first order release constant. In this model, the data are plotted as log of percentage of the drug remaining *versus* time. Higuchi model is described with the equation: $Q_t = K_Ht^{½}$, where Q_t is the amount of drug released at time t, and K_H is Higuchi dissolution constant. In this model, the data are plotted as percentage of the drug released *versus* square root of the time.

Table S1. Encapsulation efficiency and drug loading in DOPS PLA and GM3 PLGA NPs.

NP Type	Drug	Encapsulation Efficiency (%)	Drug Loading (%)
DOPS PLA NPs	RPV	38.1 ± 3.3 %	2.2 ± 0.1 %
DOPS PLA NPs	CAB	6.0 ± 0.7 %	15.6 ± 1.90 %
GM3 PLGA NPs	RPV	33.9 ± 1.2 %	1.1 ± 0.1 %
GM3 PLGA NPs	CAB	8.3 ± 0.7 %	14.6 ± 1.8 %

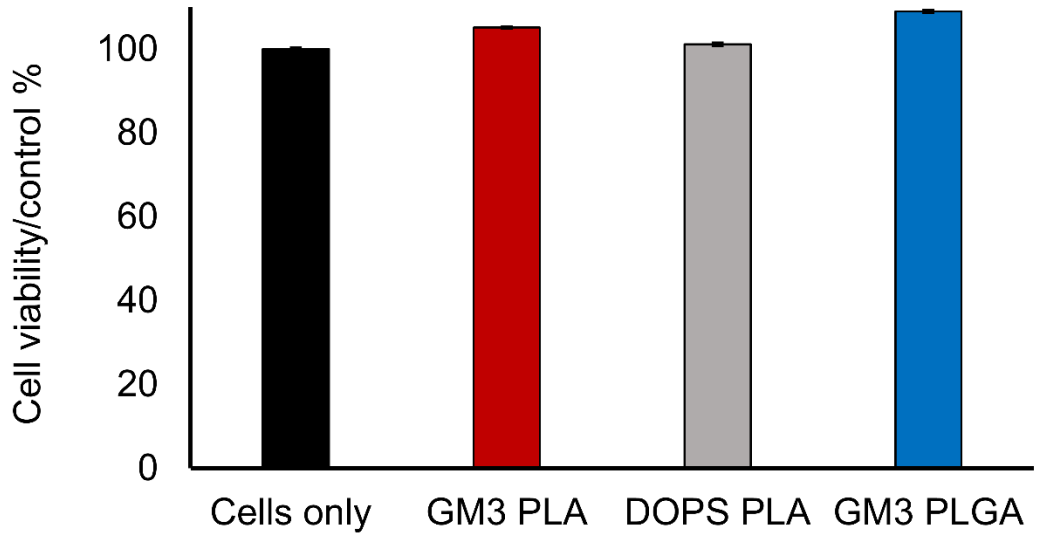

Figure S1. MTT cell viability assay of RPV and CAB loaded GM3 PLA, DOPS PLA, and GM3 PLGA NPs in $CD169^+$ MDMs. Cell viability was assessed after 5 days incubation following a 4 h NP incubation at a concentration of 1×10^{12} NPs/mL, corresponding to approximately 14 µM RPV and 148 µM CAB.

Table S2. IC_{50} values of different conditions based on RPV and CAB concentrations. Error bars represent standard error of the mean (SEM) of 3 replicates.

Conditions	IC_{50} (RPV)	Conditions	IC_{50} (CAB)
Free RPV	0.7 ± 0.3 nM	Free CAB	2.0 ± 0.3 nM
Free RPV + CAB	0.3 ± 0.1 nM	Free RPV + CAB	1.8 ± 0.1 nM
RPV + CAB GM3 PLA NPs	0.2 ± 0.04 nM	RPV + CAB GM3 PLA NPs	1.4 ± 0.3 nM
RPV + CAB GM3 PLGA NPs	0.1 ± 0.03 nM	RPV + CAB GM3 PLGA NPs	0.3 ± 0.1 nM
RPV + CAB DOPS PLA NPs	0.7 ± 0.3 nM	RPV + CAB DOPS PLA NPs	1.1 ± 0.1 nM

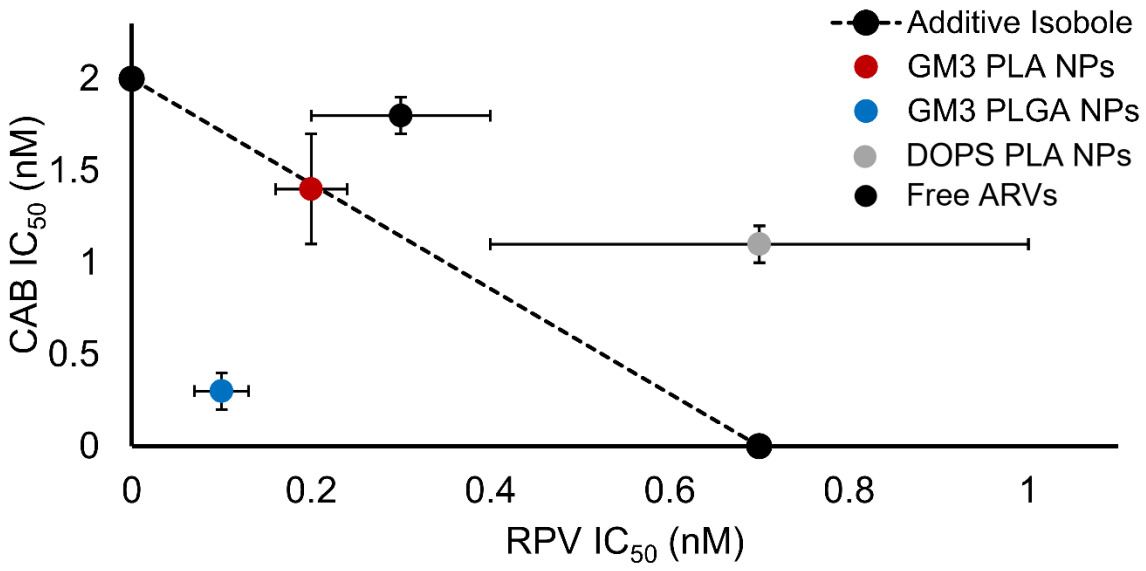

Figure S2. Isobologram analysis of RPV and CAB co-encapsulated GM3 PLA, GM3 PLGA, and DIOPS PLA NPs as well as free ARVs. An isobologram is spanned using the individual IC_{50} values of RPV (x-axis) and CAB (y-axis).[1-2] The IC_{50} values of the pure compounds define the x,y intercepts of a line formed by all IC_{50} values corresponding to simple additive interactions of the two compounds. This line is referred to as the "additive isobole". The additive isobole is shown using a dashed line. The localization of data points below and above the additive isobole demonstrate the synergism and antagonism, respectively. In the current case, the data show only small differences, that can be positive or negative relative to the additive isobole and lack a systematic trend.

An alternative strategy for quantifying drug synergism is based on the combination index (CI).[2] The CI is defined as: CI = a/A+b/B, where a (b) is the IC_{50} value for the combination of the two drugs based on the concentration of drug 1 (drug 2), and A (B) is the IC_{50} values of drug 1 (drug 2) alone. A CI equals to 1 is characteristic of simple additive interactions, whereas a CI value less than 1 indicates synergism.[2] We obtained a CI of 0.99 for GM3 PLA NPs, 0.29 for GM3 PLGA NPs, 1.55 for DOPS PLA NPs, and 1.33 for free ARVs.

Table S3. Percentages of RPV released at different temperatures.

Time	% of RPV Released @ 4 °C	% of RPV Released @ RT	% of RPV Released @ 37 °C
0 h	0.0	0.0	0.0
4 h	15.1 ± 5.8	15.4 ± 4.0	15.4 ± 1.5
8 h	16.9 ± 0.4	18.3 ± 3.2	19.6 ± 5.9
12 h	20.6 ± 1.6	24.8 ± 0.8	24.6 ± 3.0
Day 1	23.8 ± 2.8	30.1 ± 10.5	23.5 ± 3.8
Day 2	28.3 ± 3.4	31.4 ± 5.0	46.3 ± 5.1
Day 3	27.2 ± 3.8	35.7 ± 10.1	48.0 ± 3.8
Day 4	30.5 ± 7.3	29.8 ± 4.1	56.2 ± 1.9
Day 5	17.0 ± 4.0	30.0 ± 4.7	61.7 ± 3.8
Day 6	19.8 ± 2.5	32.2 ± 0.5	59.6 ± 1.9
Day 7	25.7 ± 3.6	23.7 ± 1.1	68.0 ± 6.2
Day 10	22.1 ± 0.6	31.0 ± 1.6	78.8 ± 4.7
Day 14	20.0 ± 4.1	32.6 ± 1.6	80.1 ± 2.8
Day 21	30.8 ± 6.3	39.1 ± 1.0	92.3 ± 1.8
Day 28	33.5 ± 3.5	29.6 ± 2.6	92.2 ± 0.6

Table S4. Percentages of CAB released at different temperatures.

Time	% of CAB Released @ 4 °C	% of CAB Released @ RT	% of CAB Released @ 37 °C
0 h	0.0	0.0	0.0
4 h	16.5 ± 6.6	14.5 ± 1.3	21.7 ± 2.6
8 h	18.9 ± 8.4	18.2 ± 4.6	44.5 ± 2.3
12 h	21.5 ± 2.0	29.6 ± 0.2	59.9 ± 2.6
Day 1	28.0 ± 3.2	49.1 ± 7.2	90.5 ± 0.9
Day 2	44.6 ± 8.5	74.3 ± 3.9	97.6 ± 0.3
Day 3	45.8 ± 3.8	85.6 ± 4.1	97.7 ± 0.2
Day 4	60.0 ± 10.3	94.8 ± 0.5	97.3 ± 0.1
Day 5	55.8 ± 8.0	96.2 ± 0.3	96.3 ± 1.2
Day 6	77.0 ± 1.8	97.5 ± 0.2	97.5 ± 0.1
Day 7	75.5 ± 4.7	97.0 ± 0.3	98.6 ± 0.1
Day 10	89.5 ± 2.4	98.3 ± 0.2	98.6 ± 0.2
Day 14	94.5 ± 1.3	97.5 ± 0.3	98.2 ± 0.0
Day 21	93.9 ±1.1	97.5 ± 0.2	98.7 ± 0.2
Day 28	96.6 ± 0.4	97.8 ± 0.2	98.6 ± 0.1

Table S5. Remaining RPV concentration in GM3 PLA NPs over the release period.

Time	Remaining RPV (μg/mL) @ 4 °C	Remaining RPV (μg/mL) @ RT	Remaining RPV (μg/mL) @ 37 °C
0 h	19.5 ± 1.8	35.7 ± 4.3	27.6 ± 3.0
4 h	16.6 ± 2.9	30.2 ± 2.6	23.2 ± 2.2
8 h	16.2 ± 0.6	29.0 ± 3.6	21.7 ± 1.6
12 h	15.5 ± 1.7	23.6 ± 5.7	20.7 ± 2.2
Day 1	14.9 ± 1.3	24.9 ± 4.7	20.8 ± 1.4
Day 2	14.0 ± 0.9	24.5 ± 3.9	14.4 ± 0.7
Day 3	14.2 ± 1.7	22.5 ± 4.1	14.6 ± 2.5
Day 4	13.6 ± 1.2	24.9 ± 3.2	11.9 ± 1.0
Day 5	16.1 ± 1.6	25.0 ± 3.8	10.3 ± 0.8
Day 6	15.8 ± 1.9	25.6 ± 1.8	11.3 ± 1.6
Day 7	14.4 ± 0.9	29.2 ± 4.7	9.4 ± 2.4
Day 10	15.2 ± 2.3	24.5 ± 3.0	5.7 ± 1.4
Day 14	15.6 ± 3.4	24.1 ± 3.3	5.7 ± 1.3
Day 21	13.5 ± 2.2	21.6 ± 2.3	2.0 ± 0.4
Day 28	13.2 ± 1.9	25.2 ± 3.4	2.1 ± 0.2

Table S6. Remaining CAB concentration in GM3 PLA NPs over the release period.

Time	Remaining CAB (μg/mL) @ 4 °C	Remaining CAB (μg/mL) @ RT	Remaining CAB (μg/mL) @ 37 °C
0	474.1 ± 26.4	434.4 ± 14.1	497.5 ± 32.6
4 h	391.0 ± 9.6	371.1 ± 7.0	387.2 ± 12.9
8 h	384.7 ± 56.3	354.0 ± 13.6	276.4 ± 22.0
12 h	373.6 ± 29.6	221.0 ± 73.4	197.5 ± 7.3
Day 1	342.7 ± 29.6	223.3 ± 36.2	48.0 ± 7.7
Day 2	268.3 ± 50.3	110.8 ± 15.3	12.4 ± 2.6
Day 3	259.9 ± 31.2	61.2 ± 16.4	11.1 ± 0.6
Day 4	155.9 ± 57.6	22.4 ± 1.7	13.4 ± 0.9
Day 5	213.7 ± 44.2	16.6 ± 1.0	19.6 ± 7.2
Day 6	110.3±14.6	10.9 ± 0.4	12.6 ± 1.3
Day 7	118.6 ± 25.9	12.8 ± 1.3	7.1 ± 0.1
Day 10	48.7 ± 10.5	7.4 ± 0.7	7.4 ± 1.5
Day 14	18.2 ± 5.8	10.7 ± 1.3	9.1 ± 0.4
Day 21	22.4 ± 6.0	10.8 ± 0.6	6.5 ± 1.2
Day 28	15.8 ± 1.8	9.6 ± 0.6	6.8 ± 0.6

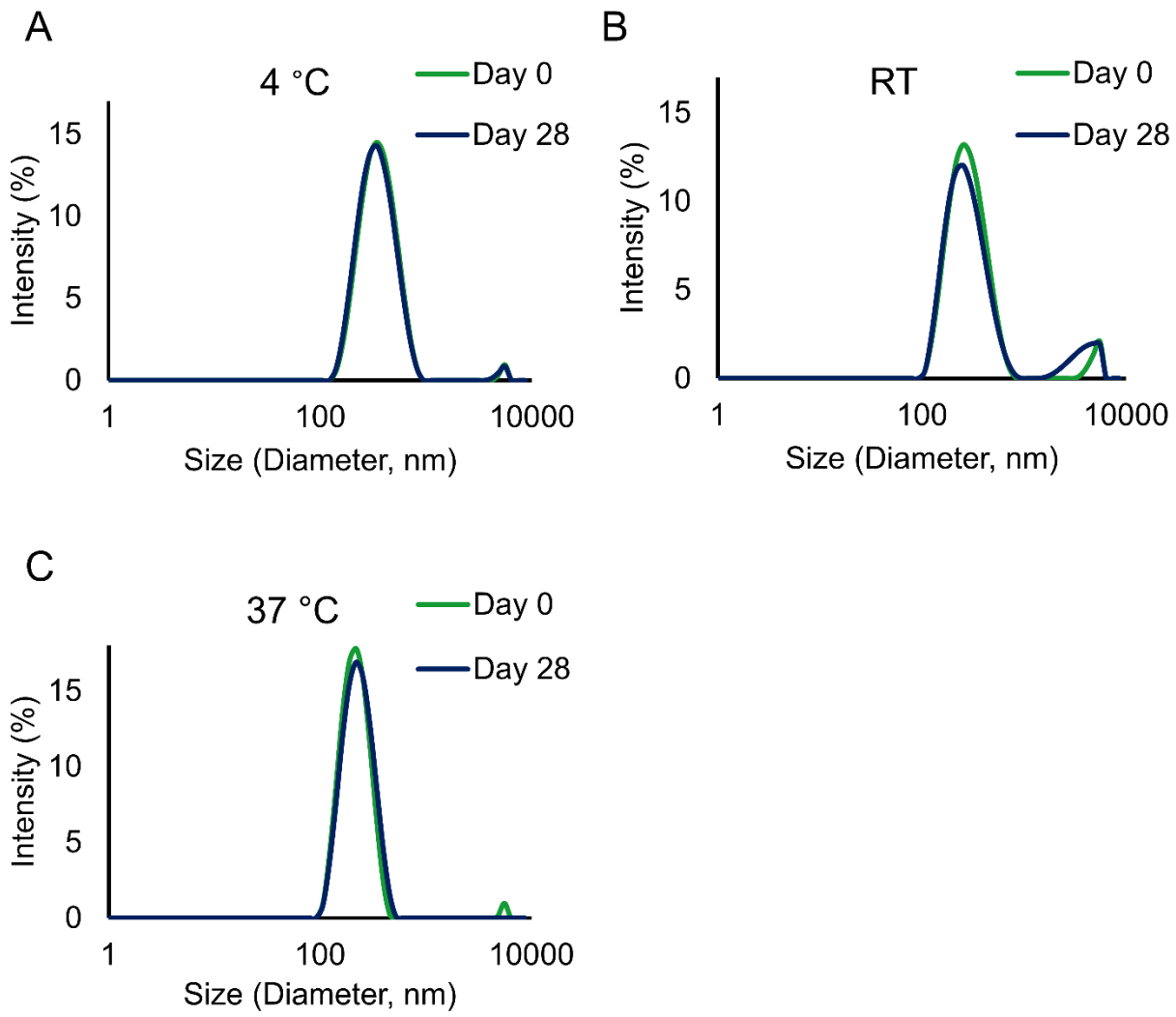

Figure S3. Hydrodynamic size of GM3 PLA NPs. **A**) Intensity statistics of size measurements of GM3 PLA NPs at 4 °C on day 0 and day 28. **B**) Intensity statistics of size measurements of GM3 PLA NPs at RT on day 0 and day 28. **C**) Intensity statistics of size measurements of GM3 PLA NPs at 37 °C on day 0 and day 28.

Table S7. Zero- and first-order, and Higuchi fits.

Model / Drug	Zero order	First order	Higuchi
RPV @ 4 °C	y = 0.43x + 13.87 R^2 = 0.934	y = -0.0023x + 1.94 R^2 = 0.9396	y = 3.11x + 8.81 R^2 = 0.9646
RPV @ RT	y = 0.73x + 13.32 R^2 = 0.9276	y = -0.0041x + 1.94 R^2 = 0.9377	y = 5.27x + 4.75 R^2 = 0.9591
RPV @ 37 °C	y = 0.34x + 19.63 R^2 = 0.8991	y = -0.0025x + 1.9121 R^2 = 0.917	y = 4.90x + 6.07 R^2 = 0.956
CAB @ 4 °C	y = 0.38x + 18.32 R^2 = 0.9153	y = -0.0027x + 1.92 R^2 = 0.9224	y = 5.05x + 5.14 R^2 = 0.9607
CAB @ RT	y = 1.79x + 6.38 R^2 = 0.9857	y = -0.0117x + 1.99 R^2 = 0.9871	y = 12.50x – 13.39 R^2 = 0.9669
CAB @ 37 °C	y = 4.77x + 3.84 R^2 = 0.9873	y = -0.0363x + 2.04 R^2 = 0.9997	y = 26.15x - 30.26 R^2 = 0.9986

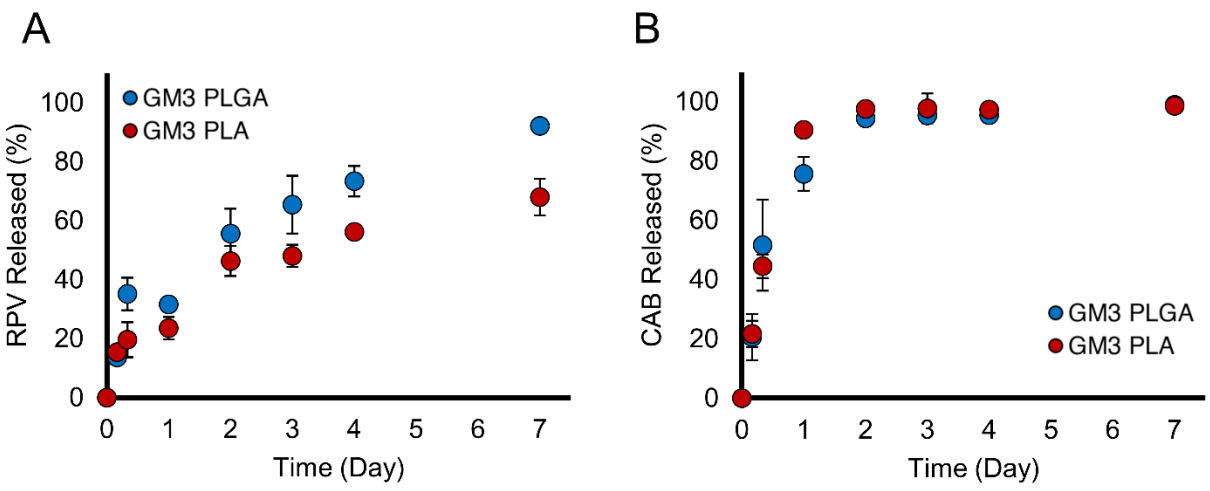

Figure S4. RPV and CAB release kinetics in GM3 PLA NPs *versus* GM3 PLGA NPs. **A)** Percentage of the RPV released at 37 °C in 1 × PBS over a time span of 7 days. **B)** Percentage of the CAB released at 37 °C in 1 × PBS over a time span of 7 days.

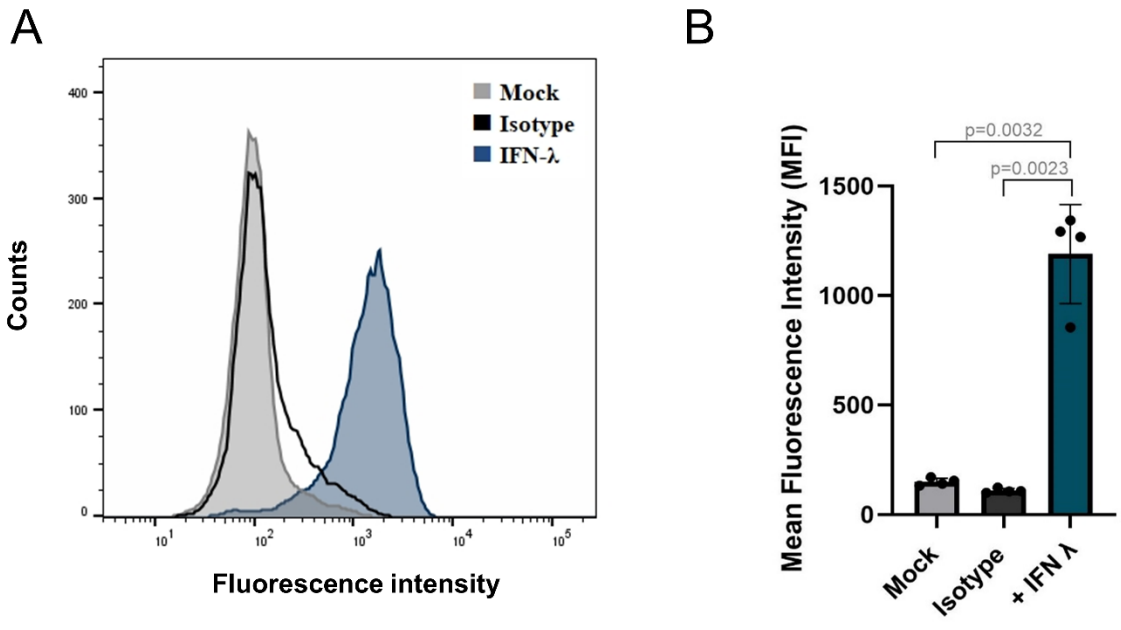

Figure S5. CD169 induction by IFN-λ in MDMs. **A)** Histogram representative of CD169 staining of MDMs, different conditions inducing mock, isotype, and IFN-λ treated are included. **B)** Mean fluorescence intensity (MFI) of CD169 in different conditions presented in a. MDMs were stimulated with IFN-λ (5ng/mL) for 48 h, and cell surface expression of CD169 was analyzed by FACS. Results are represented as mean ± SEM from 4 donors. Statistical analysis was assessed with a paired t-test.

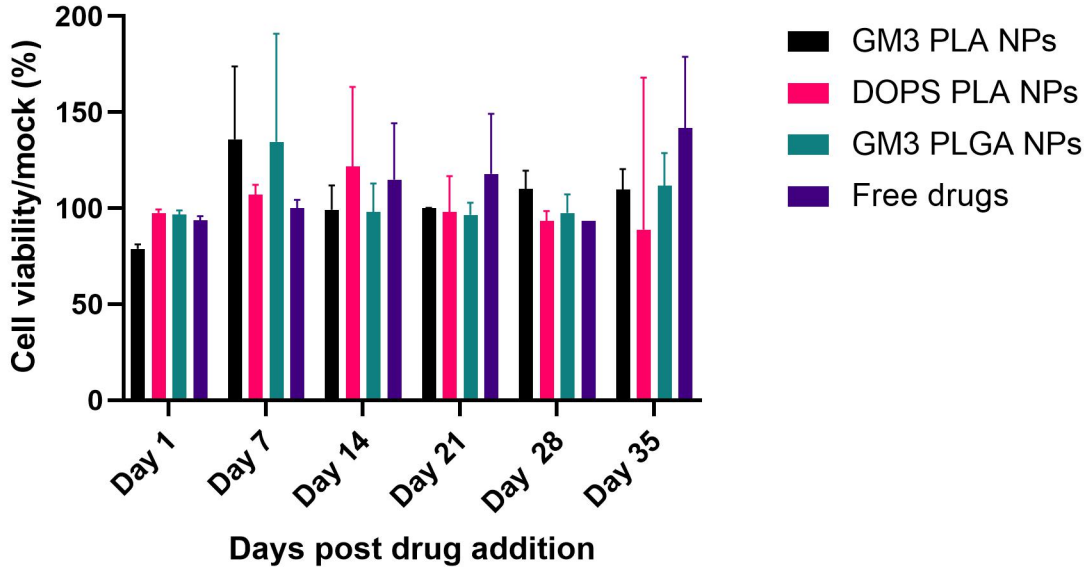

Figure S6. Cell viability assessed by MTT assay. Cell viability of CD169^{+} MDMs incubated with free ARVs or NPs at 1µM RPV equivalent concentration (CAB concentration varied between 6 to 33 µM) for 3 h. After NP treatment, cells were washed and cultured for up to 35 days. The data was normalized to the untreated cells. Results are shown as mean ± SEM from cells derived from 2 independent donors.

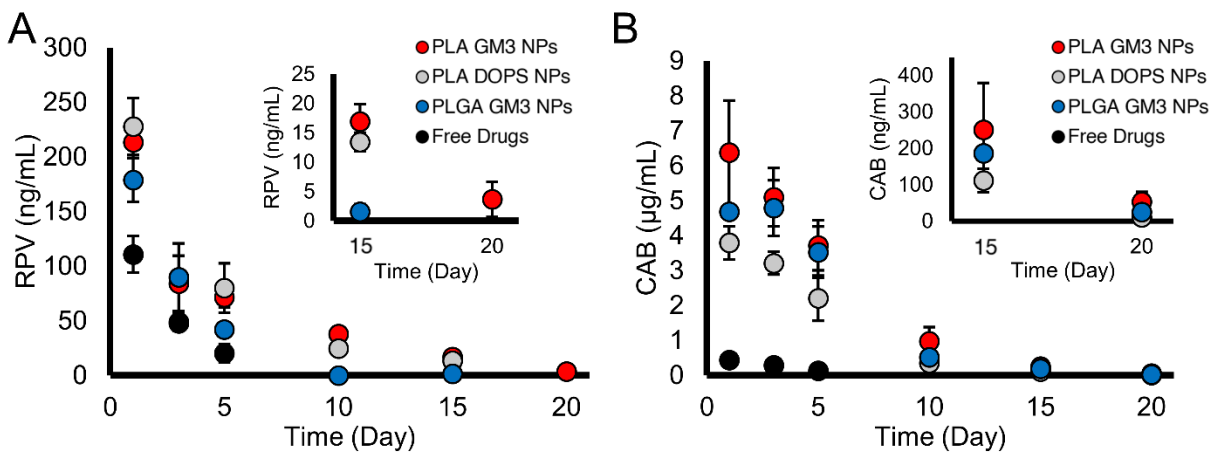

Figure S7. Quantification of RPV and CAB released from CD169^{+} MDMs into the medium. **A**) RPV concentration in the medium released from CD169^{+} MDMs over 20 days, the inset shows the zoomed in view of released RPV after day 15 for better visualization of different NP types. **B**) CAB concentration in the medium released from CD169^{+} MDMs over 20 days, the inset shows the zoomed in view of released CAB after day 15 for better visualization of different NP types.

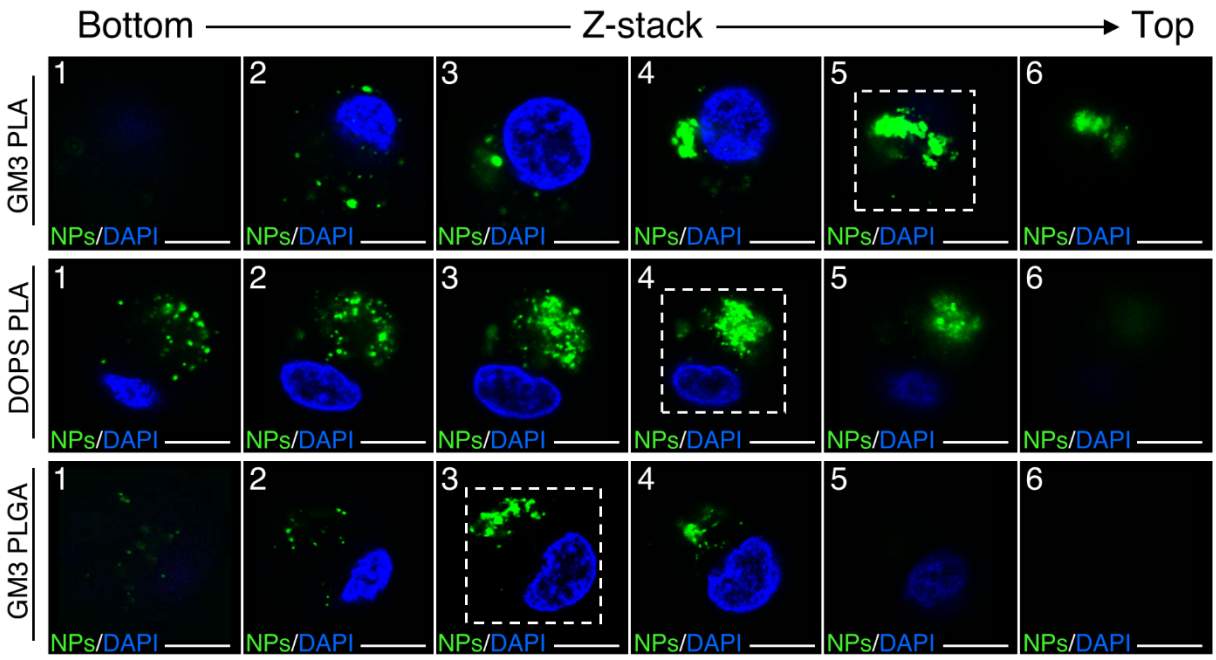

Figure S8. Localization of GM3 PLA, DOPS PLA, and GM3 PLGA NPs in $CD169^+$ MDMs. Confocal z-stack images of $CD169^+$ MDMs 1 day post NP treatment. The dashed square is used to demonstrate the section with the highest NP-contained area. Z-stack images were collected at 3 μm steps. Scale bar = 10 μm.

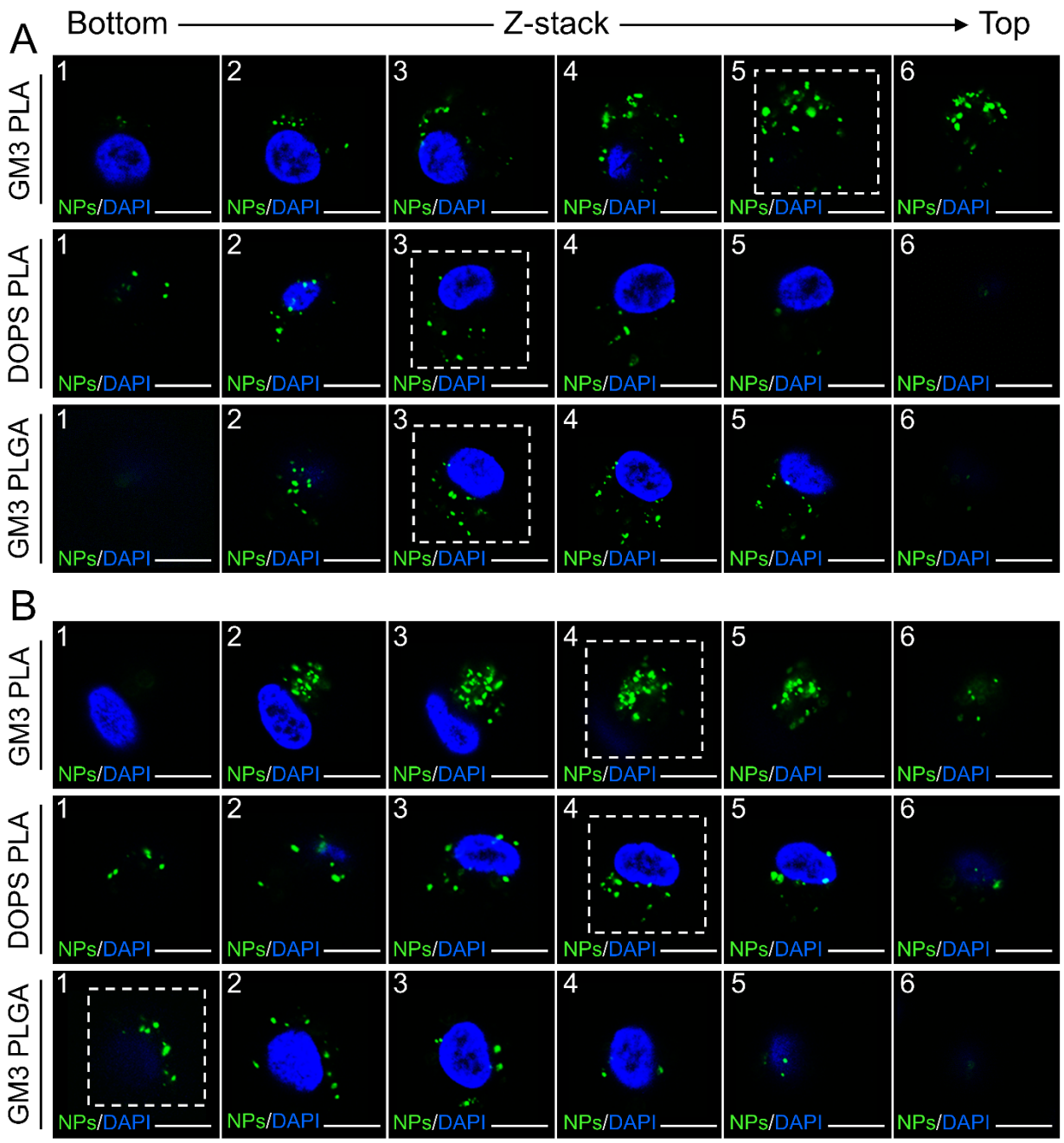

Figure S9. Localization of GM3 PLA, DOPS PLA, and GM3 PLGA NPs in CD169^{+} MDMs. **A**) Confocal z-stack images of CD169^{+} MDMs 15 days post NP treatment. **B**) Confocal z-stack images of CD169^{+} MDMs 20 days post NP treatment. The dashed square is used to demonstrate the section with the highest NP-contained area. Z-stack images were collected at 3 μm steps. Scale bar = 10 μm.

Table S8. Manders' colocalization coefficients (MCCs) quantification for GM3 PLA NPs.

Day Post NP Treatments	Day 1		Day 5		Day 10	
MCCs	M_1	M_2	M_1	M_2	M_1	M_2
Marker: CD169	0.92 ± 0.05	0.53 ± 0.13	0.71 ± 0.12	0.39 ± 0.22	0.73 ± 0.22	0.28 ± 0.13
Marker: CD9	0.83 ± 0.14	0.05 ± 0.03	0.66 ± 0.19	0.03 ± 0.02	0.61 ± 0.18	0.01 ± 0.01
Marker: LAMP-1	0.27 ± 0.12	0.06 ± 0.09	0.06 ± 0.06	0.002 ± 0.002	0.04 ± 0.04	0.004 ± 0.004
Day Post NP Treatments	Day 15		Day 20		Day 25	
MCCs	M_1	M_2	M_1	M_2	M_1	M_2
Marker: CD169	0.56 ± 0.17	0.35 ± 0.23	0.42 ± 0.29	0.34 ± 0.26	0.18 ± 0.13	0.16 ± 0.22
Marker: CD9	0.56 ± 0.26	0.01 ± 0.01	0.44 ± 0.15	0.02 ± 0.01	0.16 ± 0.16	0.003± 0.003
Marker: LAMP-1	0.20 ± 0.13	0.02 ± 0.01	0.16 ± 0.16	0.23 ± 0.39	0.07 ± 0.08	0.01 ± 0.01

M_1 describes the overlap of NPs with the respective markers (CD169 or CD9 or LAMP-1) signal, and M_2 quantifies the overlap of the marker (CD169 or CD9 or LAMP-1) signal with the NP signal. M_1 and M_2 values were calculated for 10 cells per staining and NP types, in total 180 cells.

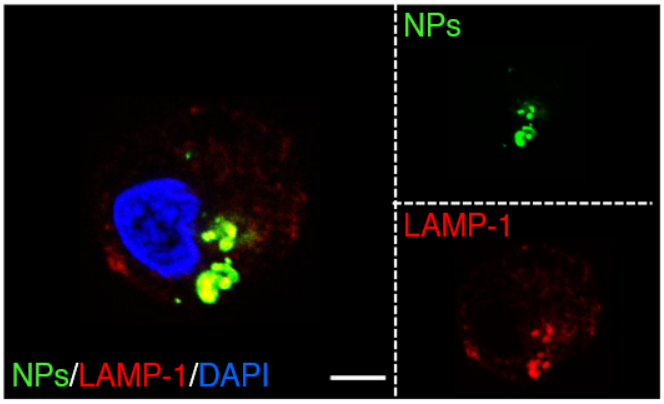

Figure S10. Optical colocalization of DOPS PLA NPs and LAMP-1 in $CD169^+$ MDMs. Single confocal sections of $CD169^+$ MDMs incubated with DOPS PLA NPs and stained for LAMP-1 on day 1. Scale bar = 5 µm. MCCs (M_1 and M_2) values were calculated for 19 cells (4 independent donors), and the average of M_1 is 0.94 ± 0.03 and M_2 is 0.10 ± 0.04. M_1 describes the overlap of NP with the LAMP-1 signal, and M_2 quantifies the overlap of the LAMP-1 with the NP signal.

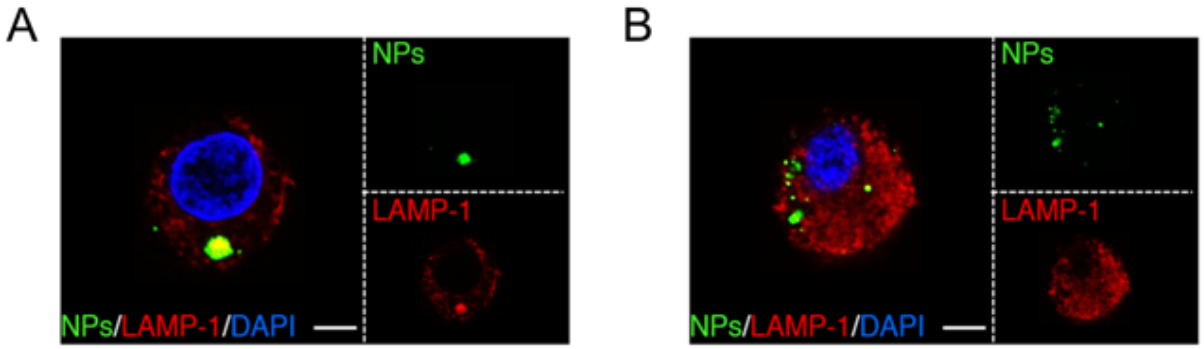

Figure S11. Optical colocalization of GM3 PLGA NPs and LAMP-1 in $CD169^+$ MDMs. Single confocal sections of $CD169^+$ MDMs incubated with DOPS PLA NPs and stained for LAMP-1 on day 1. A, B were acquired from two different donors. A shows a robust colocalization while the colocalization is weaker for B. Scale bar = 5 μm.

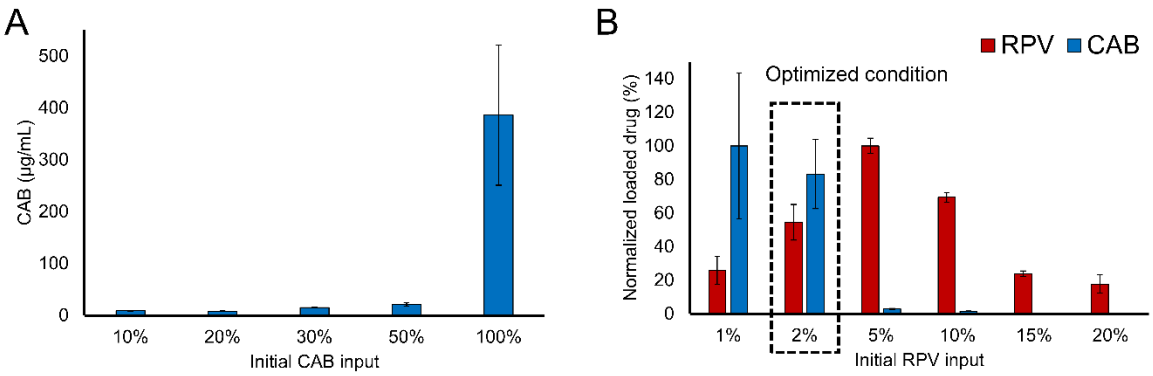

Figure S12. Optimized co-loading of RPV and CAB. **A**) CAB concentration in polymer NPs as a function of initial CAB input, the percentage is relative to polymer weight, the highest average loading of CAB obtained using a constant 1:1 weight ratio of CAB : polymer. **B**) By keeping the CAB : polymer ratio constant, the initial RPV input was changed and optimized co-loading of RPV and CAB achieved using 2% of the polymer weight. Further increase in the RPV input concentration resulted in a strong decrease of CAB loading.

References

1. Tallarida, R. J., Quantitative methods for assessing drug synergism. *Genes & cancer* **2011,** *2* (11), 1003-1008.

2. Foucquier, J.; Guedj, M., Analysis of drug combinations: current methodological landscape. *Pharmacology research & perspectives* **2015,** *3* (3), e00149.