Title: Prodrug Nanotherapy Demonstrate In Vivo Anticryptosporidial Efficacy in a Mouse Model of Chronic Cryptosporidium Infection

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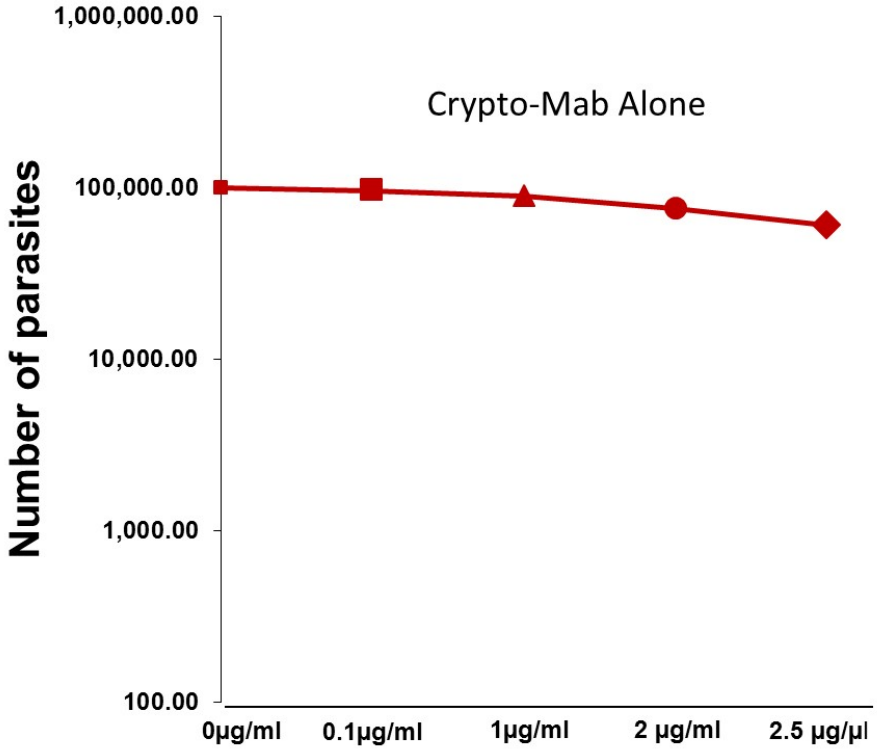

Fig S1. Effect of Mab on *C. parvum* growth. 5x10e5 parasites were used to infect HCT-8 cells and treated with MAb-GP25-200. After 24 hrs of infection number of parasites were determined by RT-PCR.

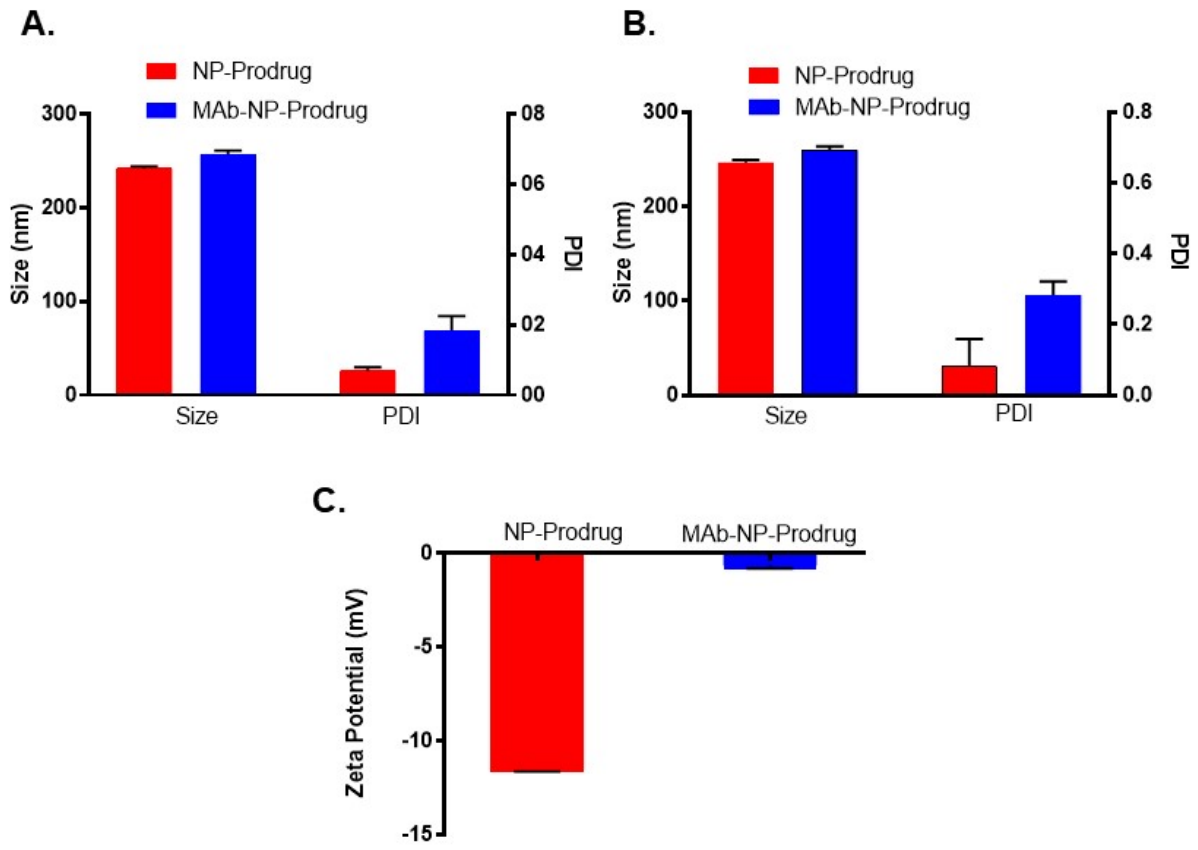

Fig S2: Physiochemical characterization **MAb** conjugated, **Prodrug** loaded PLGA nanoparticles **NPs** A) Particle size and PDI of NP-Prodrug and **MAb-NP-Prodrug** conjugated prodrug loaded PLGA NPs for cell culture study, B) Particle size and PDI of prodrug loaded PLGA **NP**s and **MAb-NP-Prodrug** for animal studies, C) Zeta potential of **NP-Prodrug** and **MAb-NP-Prodrug** measured by dynamic light scattering.

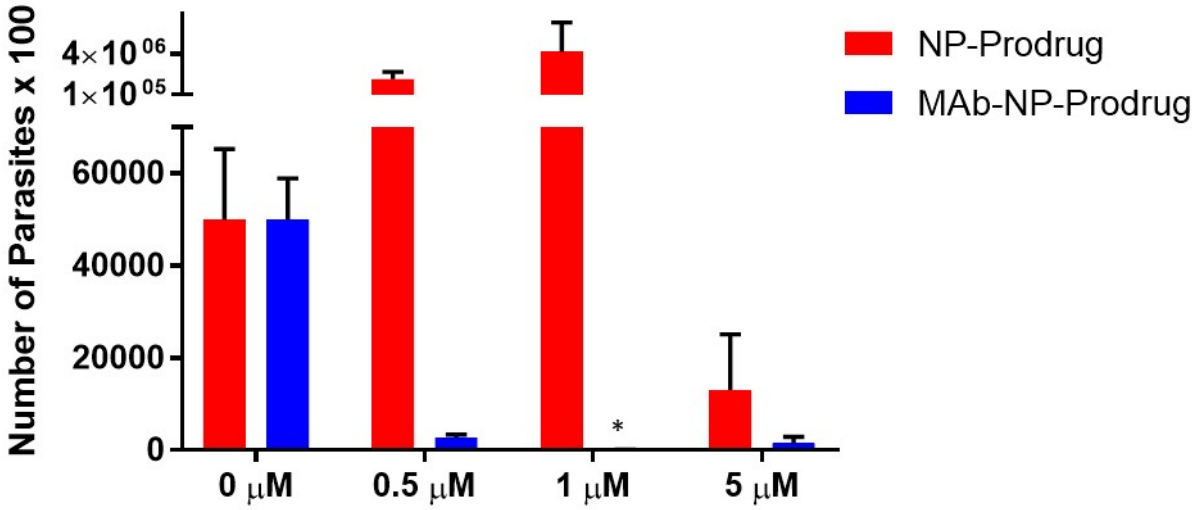

Fig S3: Anti-*Cryptosporidium* activity in cell culture for the **MAb-NP-Prodrug** and **NP-Prodrug**. Anticryptosporidial activity of **NP-Prodrug** (red) and **MAb-NP-Prodrug** (blue) was evaluated on RNA extracted from HCT-8 cells by RT-PCR. (*), below detection limit of experiment. A range of concentrations (0-5 μM) was used to evaluate the effect of drug on infected cells. The results are shown as the average of 3 independent experiments (bars), ± STD of the means are indicated with vertical bars.

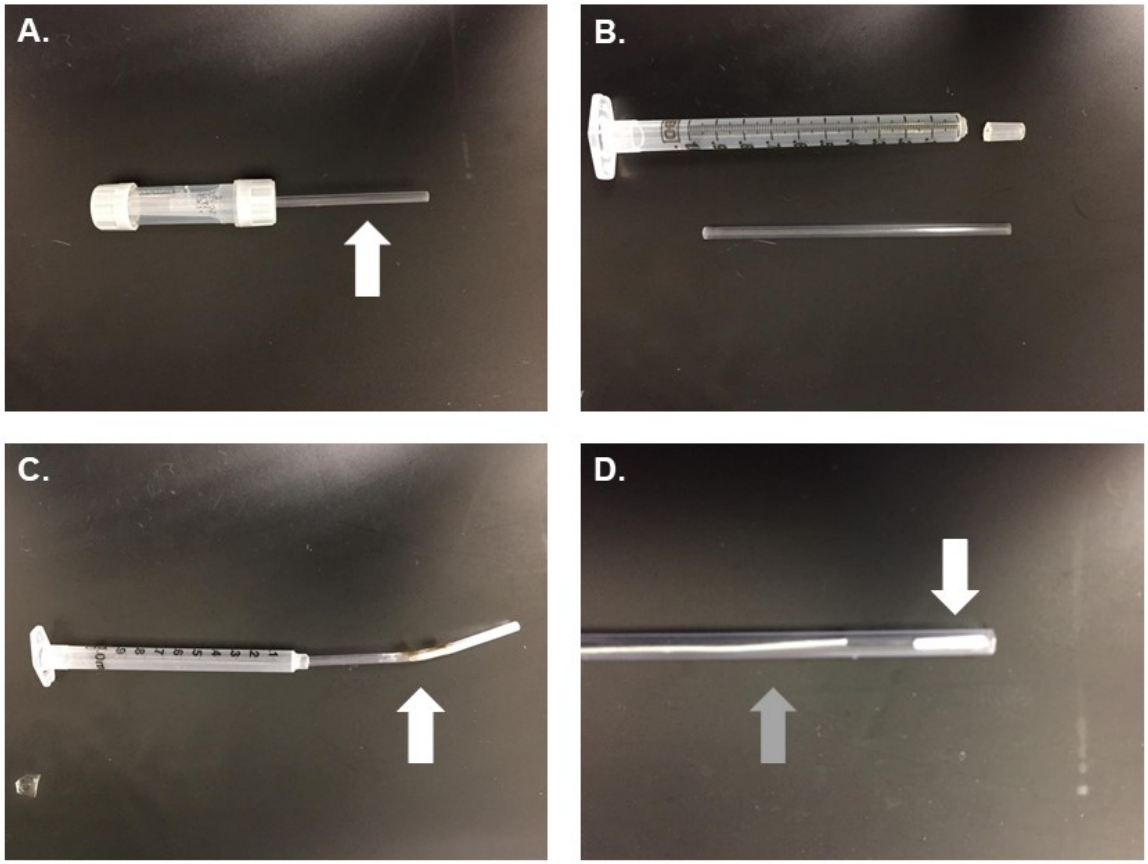

Fig S4. Construction of device used to deliver enteric coated capsules containing either **MAb-NP-Prodrug** or **NP** into SCID/Beige mouse stomach. A) Acrylic capillary (white arrow) is removed from a Microvette 200 Capillary Blood Collection Tube (Fisher Scientific) (Length including cap: 47.6 mm). B) The tip of a 1cc syringe is removed and the acrylic capillary is inserted into the syringe. C) The acrylic capillary is slightly bent (white arrow) by heating in boiling water for a few seconds. D) The enteric coated capsule containing either **MAb-NP-Prodrug** or **NP** is inserted into end of capillary (white arrow). Wire is inserted through syringe (gray arrow) to push capsule out of capillary once device enters the mouse stomach.

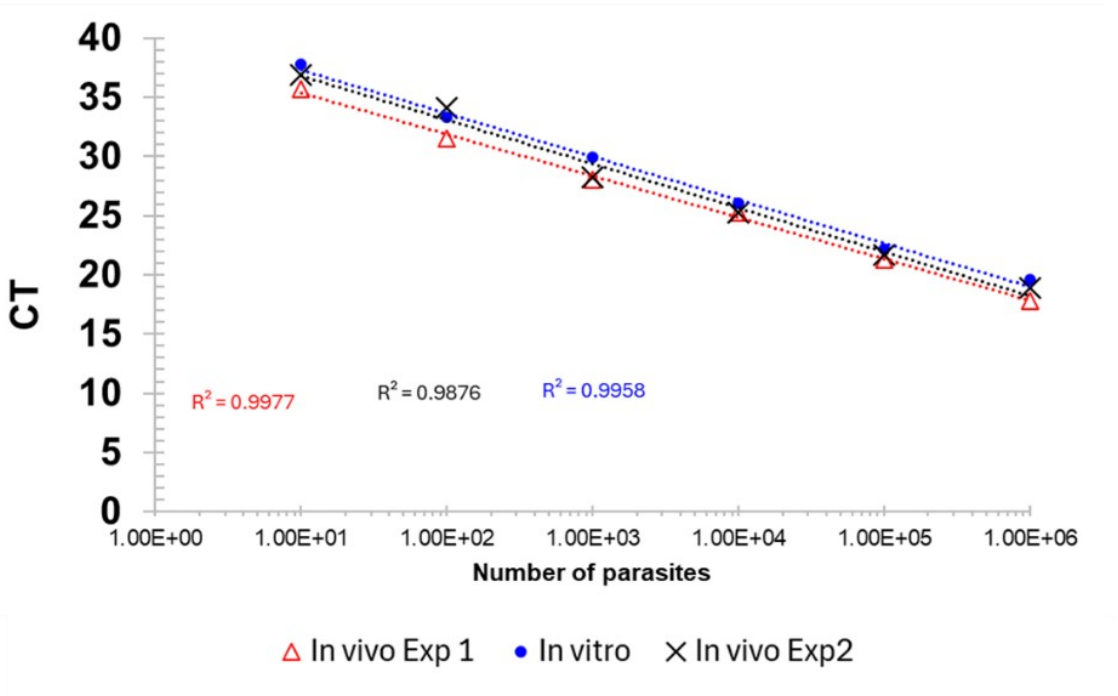

Fig S5. Standard curve for quantification of Cryptosporidium parasites by real-time PCR. The standard curve was obtained by amplification of *Cryptosporidium* DNA from $1x10^6$ to $1x10^0$. Standard curve (Sc) of mice experiment shown in figure 3 (red), Sc of In vitro experiment shown in figure 5 (blue), Sc of mice experiment shown in Figure 6 (black).

Table S1. Characterization drug loading for *Cryptosporidium*-MAb-PLGA-Prodrug (**MAb-NP-Prodrug**)

NPs	EE %	DL %
Batch 1	52.4 ± 5.0	7.1 ± 0.8
Batch 2	54.7 ± 5.9	12.2 ± 1.3
Batch 3	59.7 ± 6.7	10.8 ± 1.3

Table S2 IC_{50} (μM) for *Ch*TS-DHFR [a]

Compound	*Ch*TS IC_{50}	*Ch*DHFR IC_{50}
906[b]	0.38 ± 0.04	0.049 ±0.005
906-ester	> 100	30.0 ± 2.5

[a] SD from triplicate measurement. [b] Results from reference [1, 2]

Table S3 Parasite Number Reduction with Duodenal Catheter Dosing of Drug (per 25 mg stool)

	Day 0	Day 8	Day 15
Mouse Treated with PBS			
1	3900	7207	3008
2	1078	5623	4190
3	16590	433	21900
4	25700	50	17899
Mouse Treated with **MAb-NP-Prodrug**			
1	1908	1	1
2	6409	1	3656
3	21090	7	1008
4	14007	1	13222
5	4290	909	1

Table S4 Parasite Number Reduction Oral Dosing of Drug (per 25 mg stool)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 8	Day 10	Day 12	Day 15	Day 16
Mouse Treated with Enteric Coated Capsules with **NP**											
1	85151	64275	10844.05	20665.02	317149.8	86703.64	11255.58	40104.3	0.9	30794.31	33566.06
2	97661.1	57598.8	74151	70621.48	24382.05	0.9	6165.06	99146.09	73530.69	36626.27	0.9
3	8208.103	78151	0.9	0.9	54583.43	0.9	6255.73	6842.40	0.9	2671.243	278915.1
4	71151	0.9	62522.59	35879.25	65490.18	28727.83	7612.02	0.9	54517.2	0.9	14806.09
5	58344	5699.7	79151	209101.4	76062.14	9537.71	0.9	58225.54	3870.68	0.9	19288.59
6	84443.23	75151	0.9	46318.65	48166.86	28028.37	46318.65	18683.79	4327.33	11394.34	25280.31
7	65151	84542.2	0.9	115763.2	41261.48	0.9	29870.14	14667.31	26301.09	17795.85	39071.04
Mouse Treated with Enteric Coated Capsules with **MAb-NP-Prodrug**											
1	75151	124591.1	0.9	0.9	148916.6	0.9	4460.088	67912.03	0.9	9581	2956.98
2	0.9	16171.38	76151	0.9	99279.5	0.9	10940.36	0.9	0.9	0.9	15029.65
3	42091.51	72151	0.9	23867.52	47935.4	21491.59	0.9	0.9	0.9	0.9	0.9
4	63151	31149.26	8329.17	15456.8	73564.26	18809.41	7116.84	70345.25	28152.38	22446.05	11625.88
5	116455	136700.7	70151	0.9	0.9	0.9	0.9	52790.16	19668.35	1156.72	0.9
6	84007.4	75051	0.9	0.9	0.9	33851.53	0.9	45868.93	0.9	20947.63	0.9
7	77451	0.9	18115.32	58930.26	25454.72	0.9	0.9	35471.64	20837.31	0.9	0.9

References for Supplemental Information

[1] Kumar, V. P., Ciscernos, J. A., Frey, K. M., Castellanos-Gonzalez, A., Wang, Y., Gangjee, A., White, A. C., Jorgensen, W. L., and Anderson, K. S. (2014) Structural studies provide clues for analog design of specific inhibitors of Cryptosporidium hominis Thymidylate Synthase-Dihydrofolate Reductase, *Bioorg Med Chem Lett 24*, 4158-4161.

[2] Mukerjee, A., Iyidogan, P., Castellanos-Gonzalez, A., Cisneros, J. A., Czyzyk, D., Ranjan, A. P., Jorgensen, W. L., White, A. C., Jr., Vishwanatha, J. K., and Anderson, K. S. (2015) A nanotherapy strategy significantly enhances anticryptosporidial activity of an inhibitor of bifunctional thymidylate synthase-dihydrofolate reductase from Cryptosporidium, *Bioorg Med Chem Lett 25*, 2065-2067.