Electronic Supplementary Material (ESI) for Materials Horizons.

Supporting Information

T cell force-responsive delivery of anticancer drugs using mesoporous silica microparticles

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Abbreviations

M mesoporous silica microparticle

RB rhodamine B

R6G rhodamine 6G-amide

GEM gemcitabine

M-RB rhodamine B-loaded mesoporous silica microparticle

M-GEM rhodamine B-loaded mesoporous silica microparticle

Ab anti-CD3/28 antibodies

Sol. **Ab** soluble anti-CD3/28 antibodies

IsoAb isotype antibody control

D DNA force sensor/gatekeeper

AbD anti-CD3/28 antibody-conjugated DNA force sensor/gatekeeper

IsoAbD isotype antibody-conjugated DNA force sensor/gatekeeper

AbD-M anti-CD3/28 antibody-conjugated DNA force sensor/gatekeeper-capped mesoporous silica microparticle

AbD-M-RB or **IsoAbD-M-RB** anti-CD3/28 antibody or isotype antibody-conjugated DNA force sensor/gatekeeper-capped mesoporous silica microparticle loaded with rhodamine B

AbD-M-R6G anti-CD3/28 antibody-conjugated DNA force sensor/gatekeeper-capped mesoporous silica microparticle covalently labeled with rhodamine 6G

AbD-M-GEM anti-CD3/28 antibody-conjugated DNA force sensor/capper-capped mesoporous silica microparticle loaded with gemcitabine

T activated Pmel $CD8^+$ T cell

Materials and methods

Chemicals and reagents

(3-isocyanatopropyl)triethoxysilane (ICPTES), mesoporous silica microparticle (MCM-48, particle size < 15 μm, pore size ~3 nm, surface area 1400-1600 m^2/g), $MgCl_2 \cdot 6H_2O$, 4′,6-diamidino-2-phenylindole dihydrochloride 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), Hoechst 33342, 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO), *N*-(8-Amino-3,6-dioxaoctyl) rhodamine 6G-amide bis(trifluoroacetate) (AmR6G), paraformaldehyde, deoxyribonuclease I (DNase I; from bovine pancreas) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). S1 nuclease was purchased from Promega (Madison, WI, USA). Rhodamine B (RB), 3-aminopropyl-trimethoxysilane (APTMS), acetonitrile (CH_3CN), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were obtained from Acros Organics (Fisher Scientific, Waltham, MA, USA). Neutravidin, EZ-Link™ NHS-Biotin, and agarose were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Gemcitabine (GEM) was purchased from MedChemExpress (Sollentuna, Sweden). Latrunculin A (LatA) (1 mM in DMSO) was purchased from Calbiochem (Merck, Darmstadt, Germany). Oligonucleotides 5'-(NH_2)-ACG GAG GCA CGA CAC-3' and 5'- (Biotin)-GTG TCG TGC CTC CGT-TTTTT-3' were ordered from IDT (Coralville, IA, USA). Human $gp100_{25-33}$ (hgp100) peptide was purchased from GenScript (Piscataway, NJ, USA). Recombinant mouse interleulin-2 (IL-2) and interleukin-7 (IL-7) were purchased from PeproTech (London, UK). Anti-mouse CD3 antibody (clone 17A2), anti-mouse CD28 antibody (clone 37.51) and isotype antibody (clone C1.18.4) were purchased from Bioxcell (West Lebanon, NH, USA). All products were used as received.

Animals and cell lines

All the mouse studies were approved by the Swiss authorities (Canton of Vaud, animal protocol ID 3206) and performed in accordance with EPFL CPG guidelines. Six- to eight-week-old female $Thy1.2^+$ C57Bl/6 (C57BL/6J) mice were purchased from Charles River Laboratories (Lyon, France). T cell receptor (TCR)-transgenic $Thy1.1^+$ pmel-1 (Pmel) mice (B6.Cg-*Thy1*^{a}/Cy Tg(TcraTcrb)8Rest/J) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained in the animal facility at EPFL. B16F10 murine melanoma cells and MC38 murine colon cancer cells were originally acquired from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific) supplemented with Fetal Bovine Serum (FBS) (Gibco, 10 v/v%) and penicillin/streptomycin (Gibco, 1 v/v%).

Double strand DNA force sensor/gatekeeper (D)

To generate double-strand DNA (dsDNA) force sensor/gatekeeper, a mixture of DNA anchor strand (5'-(NH_2)-ACG GAG GCA CGA CAC-3') and DNA capper strand (5'-(Biotin)-GTG TCG TGC CTC CGT-TTTTT-3') in 1:1 mole ratio (total concentration

50 μM) in hybridization buffer (20 mM Tris·HCl, 37.5 mM $MgCl_2$) was heated to 95 ℃ for 3 min and then cooled down to 25 ℃ slowly in order to achieve DNA hybridization.[1,2] The formation of dsDNA was validated with agarose gel electrophoresis (Figure S2). In brief, the agarose gel (2 w/v%) with 1× SYBR Safe DNA Gel Stain (Invitrogen, Thermo Fisher Scientific) was loaded with DNA samples, and run at 25 ℃ (110 V) for 40 min in Tris-acetate-EDTA (TAE) buffer (pH 6.5). In DNase I-treated groups, samples (20 μL) were mixed with DNase I solution (10 μL, 1.5 mg/mL in hybridization buffer) and incubated at 37 ℃ for 60 min before gel electrophoresis. In S1 nuclease-treated groups, samples (20 μL) were mixed with S1 nuclease solution (10 μL, 900 U/mL in hybridization buffer) and incubated at 25 ℃ for 20 min before gel electrophoresis. After electrophoresis, the gel was scanned with E-Gel imager (Invitrogen).

Isocyanate-functionalized mesoporous silica microparticle (M) loaded with rhodamine B (RB) or gemcitabine (GEM)

Following a procedure in a previous study,[3] **M** (100 mg) and **RB** (10 mg) were suspended in CH_3CN (5 mL) with sonication. The suspension was shaken at 800 rpm (25 ℃) overnight. Afterward, ICPTES (125 μL) was added and the mixture was shaken at 800 rpm (25 ℃) for another 24 h. Finally, the product was obtained by washing with CH_3CN by centrifugation (6,000 g, 2 min) and drying at 50 ℃ for 3 h.

For **GEM** loading, **M** (20 mg) was suspended in a solution of GEM (40 mM) in dry DMF (400 μL) and the mixture was shaken at 800 rpm (25 ℃) overnight. Afterward, ICPTES (25 μL) was added and shaken at 800 rpm (25 ℃) for another 24 h. Finally, the products were obtained by washing with DMF using centrifugation (10,000 g, 3 min) and drying under vacuum at 25 ℃ for 5 h.

Biotinylated antibodies

NHS-Biotin (3 μL, 10 mM) solution in DMSO was added to anti-CD3, anti-CD28, or isotype antibody solution (2 mg/mL) in 100 μL of phosphate-buffered saline (PBS) (Gibco) at a final concentration of 300 μM of NHS-Biotin. After 30-min incubation on shaker at 25 ℃ (800 rpm), the biotinylated antibody solution was washed with PBS (500 μL) using Amicon® ultra centrifugal filter with 30-kDa MWCO (Merck) (8,000 g, 5 min, ×5). The final concentration of biotinylated antibodies was determined using a microvolume spectrophotometer (NanoDrop, Thermo Fisher Scientific). The biotinylated antibodies were stored at 4 ℃ before use.

Cellular force responsive M

Isocyanate-functionalized **M** loaded with **RB** or **GEM** were next used for assembly of a T cell force-responsive **M**s (Figure S3). To cap the surface of drug-loaded **M**, solution of **D** (100 μL, 25 μM) in hybridization buffer (20 mM Tris·HCl, 37.5 mM $MgCl_2$) was added to a suspension of drug-loaded **M** functionalized with isocyanate groups (1

mg/mL) in 700 μL of CH_3CN solution with **RB** (1 mM) or **GEM** (saturated) for fabrication of **D-M-RB** or **D-M-GEM**, respectively.[3] The mixture was stirred at 25 ℃ overnight and the resulting solid was isolated by centrifugation (6,000 g, 2 min) and washed with hybridization buffer to eliminate the residual drugs and the unbounded **D** to afford **D-M-RB** or **D-M-GEM**. Next, neutravidin solution in PBS (150 μL, 1 mg/mL) was mixed with a solution of **D-M-RB** or **D-M-GEM** (50 μL, 14 mg/mL) in hybridization buffer and the mixture was incubated at 25 ℃ for 5-min. The particles were next washed with hybridization buffer using centrifugation (6,000 g, 2 min, ×2), resuspended in 100 μL of hybridization buffer followed by addition of a solution (150 μL) of biotinylated anti-CD3 (0.5 mg/mL) + biotinylated anti-CD28 (0.05 mg/mL) (**Ab**) or biotinylated isotype antibody (0.55 mg/mL) (**IsoAb**) in hybridization buffer. After incubation at 25 ℃ for 5 min, the **AbD-M-RB**, **IsoAb-M-RB** or **AbD-M-GEM** was obtained by washing with hybridization buffer (6,000 g, 2 min).

Quantification of D in D-M

For DNA quantification, the amount of DNA in sample solutions (2 μL) containing **D-M** (1 mg/mL) or **M** (1 mg/mL) was quantified by a microvolume spectrophotometer (NanoDrop). The surface density of **D** on **D-M** was calculated by subtracting the background signal of **M**.

Quantification of Ab in AbD-M

The antibody amount on **AbD-M** was quantified using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Briefly, sample solutions (20 μL) containing **AbD-M** (4 mg/mL) before and after antibody conjugation were mixed with working solution (160 μL) in a flat bottom 96-well plate and shaken for 30 seconds followed by 30-min incubation at 37 ℃. After cooling down to 25 ℃, the absorbance at 562 nm was measured with a plate reader to quantify the amount of **Ab** (Varioskan LUX, Thermo Fisher Scientific). The surface density of **Ab** on **AbD-M** was calculated by subtracting the background signal of **D-M** (with neutravidin).

Characterizations of M

Isocyanate-functionalized **M** was sent to Institute of Chemical Sciences and Engineering at EPFL for elemental analysis to quantify the amount of isocyanate groups. The sizes and Zeta-potential of native **M**, **D-M** and **AbD-M** were characterized by dynamic light scattering (DLS) with a Zetasizer NanoZS (Malvern, Worcester, UK). For size measurement, experiments were performed at 25 ℃ using a He-Ne laser (633 nm) and non-invasive backscatter optics. For Zeta-potential measurement, the electrophoretic mobility measurements were performed at 25 ℃ using M3-PALS technology (Malvern). The scanning electron microscope (SEM) images of native **M** and **AbD-M** were obtained with the GeminiSEM 300 (Zeiss, Oberkochen, Germany).

Activation of Pmel CD8$^+$ T cells

Spleens collected from Pmel Thy1.1$^+$ mice were ground through a cell strainer (70 μm, Fisher Scientific, Pittsburgh, PA, USA), and red blood cells were lysed with ACK lysing buffer (Gibco, 2 mL per spleen) at 25 ℃ for 5 min. After washing twice with PBS, splenocytes were collected and cultured in complete RPMI 1640 medium supplemented with FBS (10 v/v%), HEPES (Gibco, 1 v/v%), penicillin/streptomycin (1 v/v%), and β-mercaptoethanol (Gibco, 0.1 v/v%) in the presence of hgp100 (1 μM), recombinant mouse IL-2 (10 ng/mL), and recombinant mouse IL-7 (2 ng/mL) for 3-days followed by Ficoll-Pague Plus (GE Healthcare, Chicago, IL, USA) gradient separation to eliminate dead cells. The activated Pmel CD8$^+$ T cells (> 95 % purity) were maintained in the medium containing recombinant mouse IL-2 (10 ng/mL) and IL-7 (10 ng/mL) and used between day 4 to 8 for both in vitro and in vivo studies.

T cell force-responsive release of RB

Release buffer (200 μL/well, HBSS with 0.4 w/v% BSA and 10 mM HEPES) or Pmel T cell suspension (5×10^5/mL) in release buffer (200 μL/well) were firstly transferred to a 96-well plate (U bottom) followed by the addition of sample solutions (20 μL/well) containing **AbD-M-RB** (2.5 mg/mL), **M-RB** (2.5 mg/mL), **D-M-RB** (2.5 mg/mL) + DNase I, **IsoAbD-M-RB** (2.5 mg/mL), or **D-M-RB** (2.5 mg/mL) + sol. **Ab**. The plate was then centrifuged at 3000 rpm for 2 min to precipitate the particles and cells at bottom, and incubated at 37 ℃. At predetermined time points (0, 0.5, 1 and 1.5 h), supernatant of each well (50 μL) was retrieved, and release buffer (50 μL) was replenished in each well. Finally, the fluorescence of the supernatant was measured using a plate reader (Varioskan LUX, Ex 550 nm, Em 580 nm, acquisition time: 100 ms). Relative release was calculated by subtracting the background release of **RB** from **AbD-M-RB** alone.

To investigate the role of T cell force in control release, T cells were pre-treated with LatA (2 μM) in PBS at 37 ℃ for 10 min and washed with PBS before performing the release assay as abovementioned.

Confocal imaging of T cells cultured with M for internalization study

Isocyanate-functionalized **M** was first covalently labeled with **R6G** during the capping step. In brief, AmR6G (0.3 μL, 10 mg/mL in DMSO) was added to a suspension of isocyanate-functionalized **M** (700 μL, 1 mg/mL in CH_3CN) followed by addition of a solution of **D** (100 μL, 25 μM) in hybridization buffer. The mixture was stirred at 25 ℃ overnight and the resulting solid was isolated by centrifugation (6,000 g, 2 min) and washed with hybridization buffer to eliminate the residual AmR6G and the unbounded **D** to obtain **D-M-R6G**. Next, the anti-CD3/anti-CD28 antibody was conjugated to the **D-M-R6G** as abovementioned to obtain **AbD-M-R6G**.

To evaluate the potential internalization of **AbD-M** by T cells, a solution (20 μL) of

AbD-M-R6G (2.5 mg/mL) was added to a Pmel T cell suspension in release buffer (5×10^5/mL, 200 μL). After 2-h incubation at 37 ℃, T cells were stained with Hoechst 33342 (5 μg/mL) and DiO (5 μM) in PBS (200 μL) for 15 min at 25 ℃. After fixation with paraformaldehyde (2 wt%) in PBS (200 μL) for 10 min at 25 ℃, T cells were transferred onto a poly-L-lysine-coated coverslip and centrifuged at 1500 rpm for 2 min to settle down T cells on coverslip. The coverslip was then inverted on a drop of Fluoromount-G mounting medium (Invitrogen) on a glass slide, and each side of coverslip was sealed with nail polish. The confocal images were acquired using inverted microscope (IX83, Olympus, Tokyo, Japan) equipped with a spinning disk confocal scanner (CSU-W1, Visitron, Puchheim, Germany) and a 60×/1.42 UPLSAPO objective (Olympus).

Time-lapse fluorescence imaging of M loaded with RB for release study

A suspension (20 μL/well) of activated Pmel T cells (0.5×10^6/mL) in RPMI 1640 medium without Phenol Red (Gibco) was mixed with a suspension (50 μL/well) of **AbD-M-RB** (1 mg/mL) or **D-M-RB** (1 mg/mL) + sol. **Ab** in RPMI 1640 medium without Phenol Red followed by centrifugation at 3000 rpm for 2 min to settle down T cells and **M**s at the bottom of a 96-well plate. Time-lapse flurescence imaging was performed immediately using an EVOS™ M5000 inverted fluorescence microscope equipped with an onstage incubator for live cell imaging (Thermo Fisher Scientific). Time-lapse sequences were recorded with the integrated Invitrogen™ EVOS™ M5000 Software that allows autofocusing on the **AbD-M-RB** or **D-M-RB** in RFP channel (Ex 531/40 nm, Em 593/40 nm). Bright field and RFP channel images were acquired in sequence with a 10× 0.3 NA phase objective at 2-min intervals for 30 min. The acquired images were processed using Fiji.

Release of RB from D-M-RB under centrifugal force

To coat surface with neutravidin, a 96-well plate was first treated with NaOH solution (50 μL/well, 0.1 M) for 5 min. After NaOH solution was aspirated, and APTMS (20 μL/well) was applied for 3 min. The well plate was then thoroughly rinsed with de-ionized water. NHS-Biotin solution in PBS (50 μL/well, 100 μM) was added in each well followed by 2-h incubation at 25 ℃ and thorough rinsing with PBS. Next, neutravidin solution in PBS (50 μL/well, 50 μg/mL) was applied followed by overnight incubation at 4 ℃. The plate was washed with PBS for 3-times to remove free neutravidin.

The sample solution (50 μL/well) containing **D-M-RB** (1 mg/mL) was added to 96-well plates with or without neutravidin coating. The plate was centrifuged at 3000 rpm for 2 min to precipitate the particles at bottom. Afterward, release buffer (370 μL/well, 20 mM Tris·HCl, 37.5 mM $MgCl_2$) was carefully added on top of the sample solution. The well plate was then sealed using adhesive PCR plate seal (Thermo Fisher Scientific) to prevent liquid leakage. The well plate was placed upside down in centrifuge bucket. After centrifugation (100 g, 2 min), the well plate was unsealed and supernatant of each well (50 μL) was retrieved. Finally, the fluorescence of the supernatant was measured

using a plate reader (Varioskan LUX). Relative release rate was calculated by subtracting the background release of **RB** without centrifugation (0 g).

Killing assay of B16F10 cancer cells with AbD-M-GEM

B16F10 cells (4×10^4/well) were seeded at the bottom chamber of a transwell plate overnight. To the upper chamber, a solution (50 μL/well) of PBS buffer, activated Pmel T cells (1×10^5/well) only in RPMI 1640 medium with FBS (10 v/v%), or **AbD-M-GEM** (2 mg/mL) (or **D-M-GEM** [2 mg/mL] + sol. **Ab)** together with Pmel T cells in RPMI 1640 medium with FBS (10 v/v%) was added. After 2-h incubation at 37 ℃, the upper chamber was removed and the B16F10 cells in the bottom chamber were further cultured at 37 ℃ for another 24 h. Afterward, the bottom well was replaced with MTT solution in PBS (100 μL/well, 0.5 mg/mL) followed by 4-h incubation at 37 ℃. After that, MTT solution was replaced with 100 μL of DMSO and shaken at 500-rpm (25 ℃) for 10 min. Finally, the absorbance at 490 nm was measured with a plate reader (Varioskan LUX) to quantify the cell viability.

Toxicity of AbD-M-GEM against T cells

To evaluate potential toxicity of **AbD-M-GEM** against T cells, a solution (50 μL/well) of **AbD-M-GEM** (2 mg/mL) (or **D-M-GEM** [2 mg/mL] + sol. **Ab)** was added to activated Pmel T cells (100 μL, 1×10^5/well) in RPMI 1640 medium with FBS (10 v/v%). After 2-h incubation at 37 ℃, T cells were stained with DAPI and Alexa Fluor 488-conjugated Annexin V (Invitrogen) for flow cytometry analyses.

In vivo cancer prevention study

MC38 cancer cells (0.2×10^6) in PBS (100 μL) were mixed with a solution (100 μL) of PBS buffer, activated Pmel T cells alone (1×10^6) in PBS, or **AbD-M-GEM** (2 mg/mL) (or **D-M-GEM** [2 mg/mL] + sol. **Ab**) together with Pmel T cells (1×10^6) in PBS. The mixture was inoculated subcutaneously in the right flanks of Thy1.2$^+$ C57BL/6J mouse at day 0. Tumor area (product of measured orthogonal length and width) and body weight were measured every 2 days from day 5 post tumor inoculation. Mice were euthanized when the body weight loss was higher than 20 % of the pre-dosing weight or the tumor area reached 150 mm^2.

Statistical analyses

Statistical analyses were performed by using unpair t test in GraphPad Prism 8 software. Shown are mean ± s.e.m unless otherwise indicated. In all cases, two-tailed test with p values of less than 0.05 were considered significant.

References

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2 F. Chowdhury, I. T. S. Li, B. J. Leslie, S. Dołanay, R. Singh, X. Wang, J. Seong, S. H. Lee, S. Park, N. Wang and T. Ha, *Integr. Biol.*, 2015, **7**, 1265–1271.

3 L. Pascual, I. Baroja, E. Aznar, F. Sancenón, M. D. Marcos, J. R. Murguía, P. Amorós, K. Rurack and R. Martínez-Máñez, *Chem. Commun.*, 2015, **51**, 1414–1416.

Figures

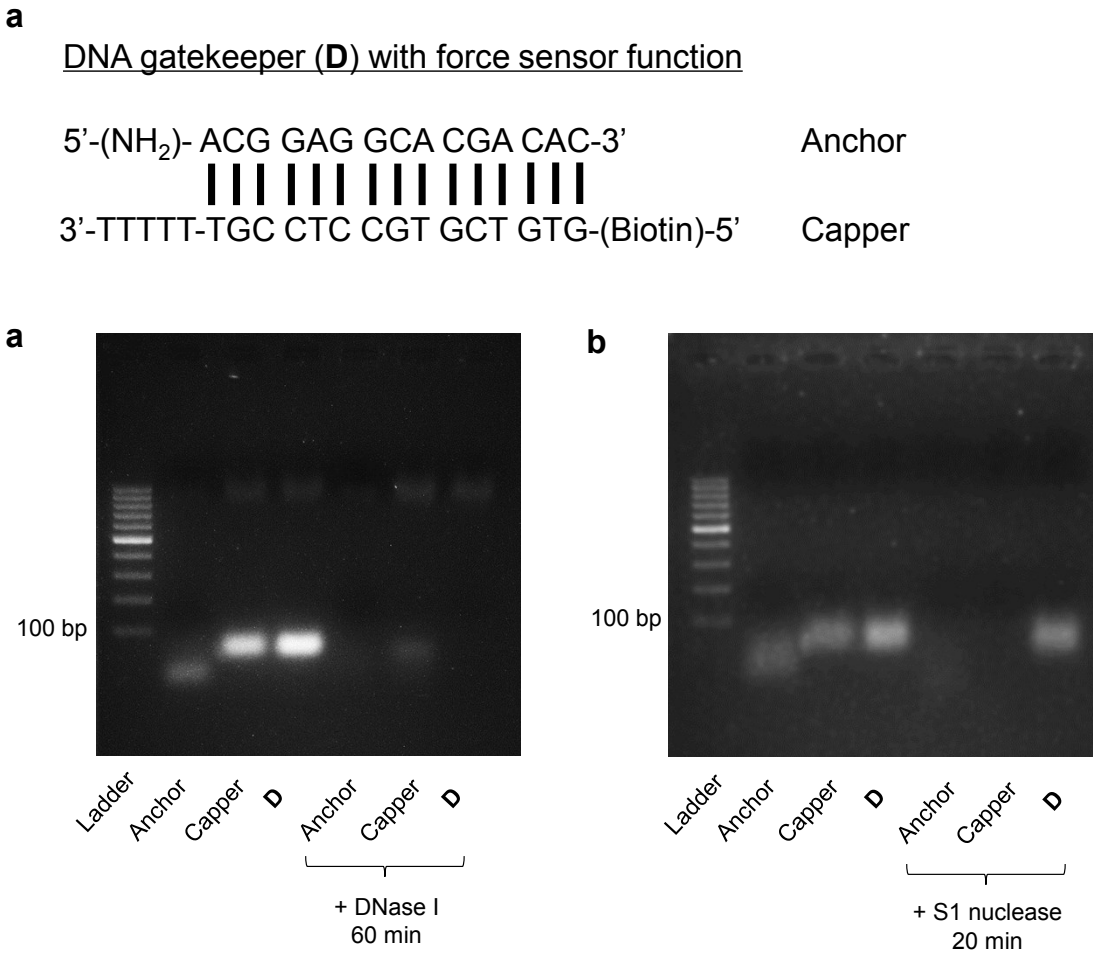
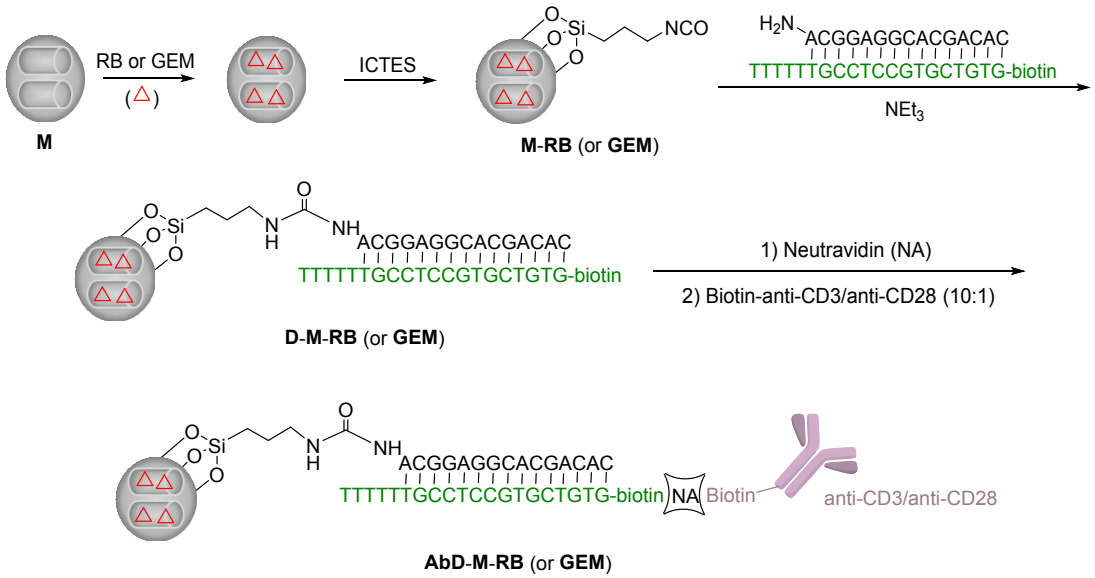


Figure S1. Design of a DNA gatekeeper (**D**) with force sensing function. (**a**) Sequence of **D** self-assembled from DNA anchor and capper. The 5' end of DNA anchor was modified with amino group for anchoring on mesoporous silica microparticle surface. For DNA capper, the 5' end was modified with biotin for conjugation with antibodies and the 3' end was extended with five repeating thymine nucleotides (T) for capping the nanochannels on mesoporous silica microparticle. A, deoxyadenosine; C, deoxycytidine; G, deoxyguanosine. (**b**, **c**) Gel electrophoresis of DNA capper, DNA anchor, and their self-assembled double-strand **D** in the absence or presence of DNase I (**b**) or S1 nuclease (**c**), an enzyme that specifically degrades single-strand nucleic acids.

Systems with drug loading



Systems without drug loading

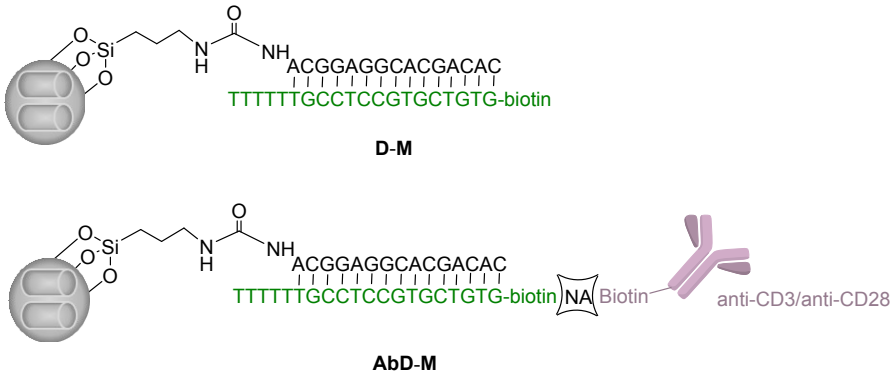


Figure S2. Schematic of the fabrication of T cell force-responsive mesoporous silica microparticles. ICTES, (3-Isocyanatopropyl)triethoxysilane; NEt_3, triethylamine.

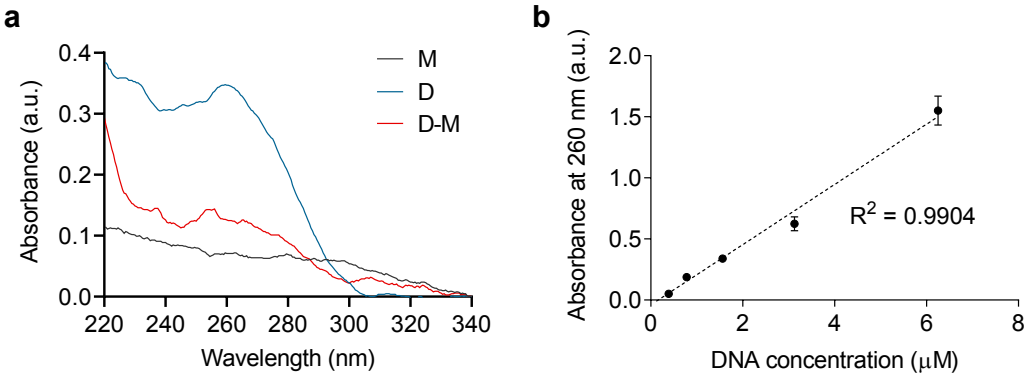


Figure S3. (**a**) UV-vis spectra of native mesoporous silica microparticle (**M**), DNA force sensor (**D**), and DNA-capped mesoporous silica microparticle (**D-M**). (**b**) A calibration curve for **D** in hybridization buffer (20 mM Tris·HCl, 37.5 mM $MgCl_2$). Shown are mean ± s.d. The plot is simple linear regression of the data points.

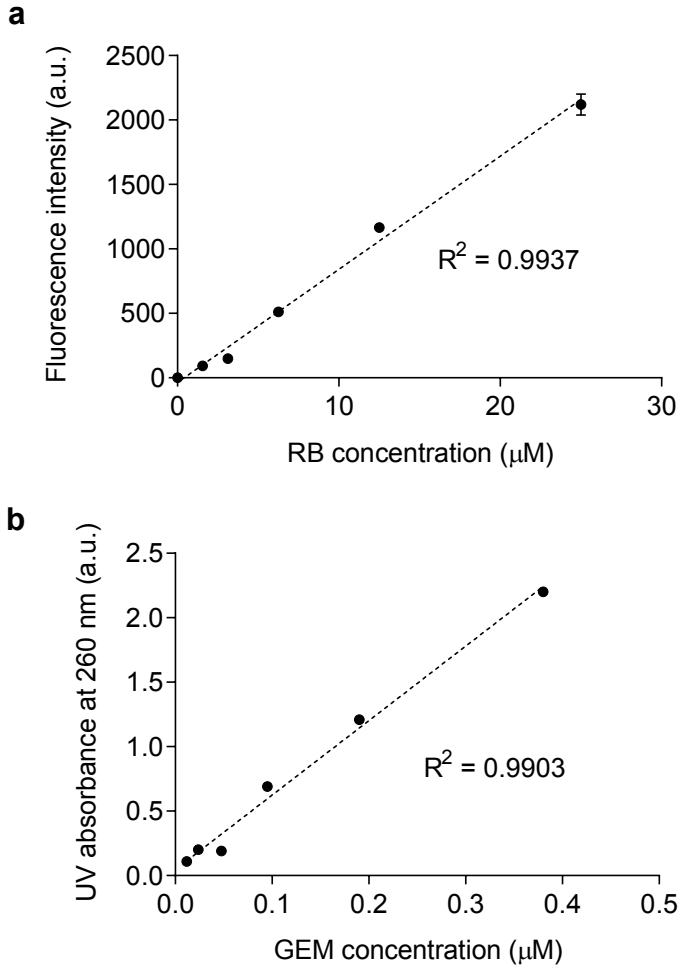


Figure S4. Calibration curves for rhodamine B (**RB**) (**a**) and gemcitabine (**GEM**) (**b**) in Hank's balanced salt solution (HBSS). Shown are mean ± s.d. The plot is simple linear regression of the data points.

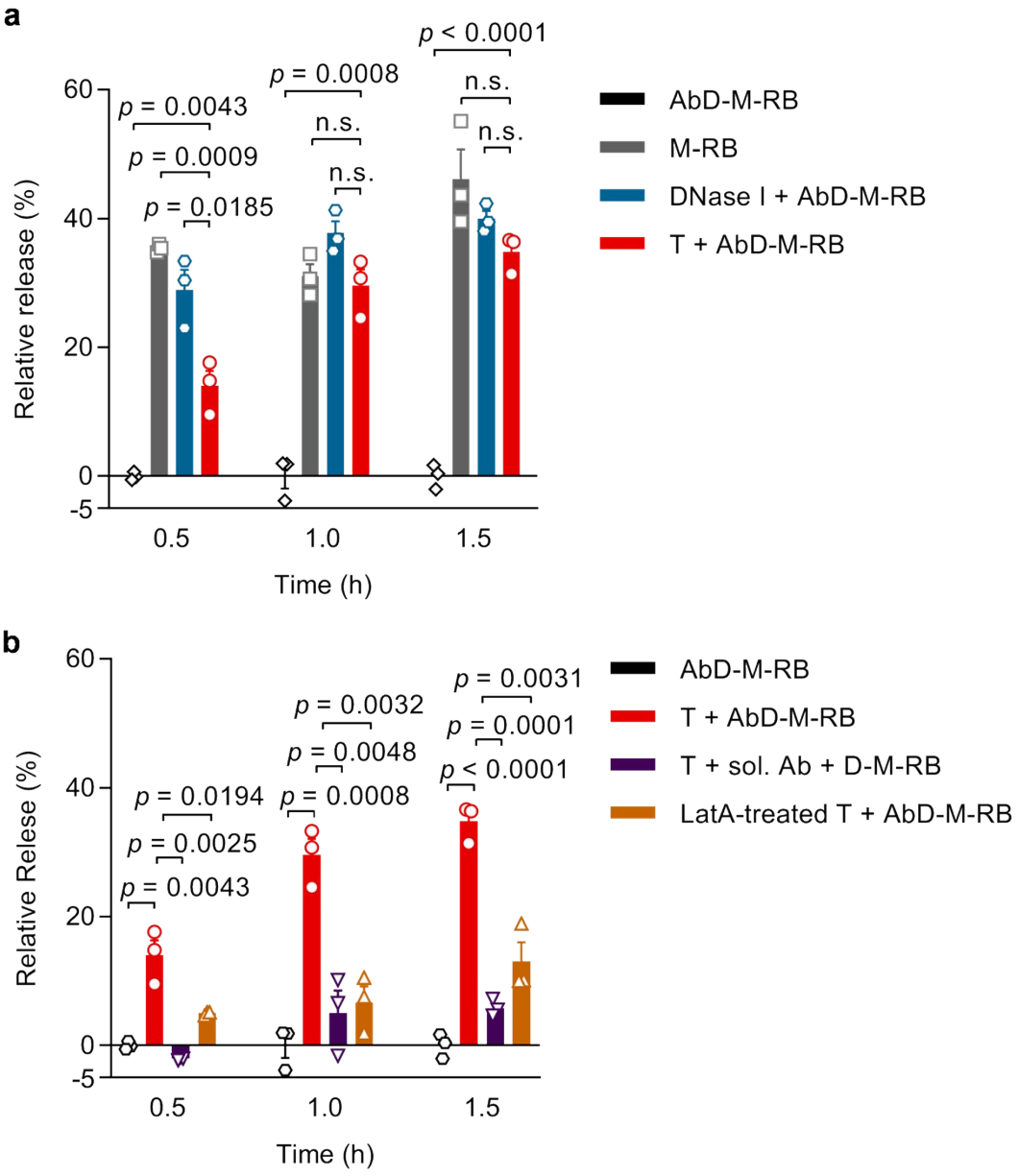


Figure S5. Release kinetics of rhodamine B (**RB**) from indicated systems at 37 ℃ over 1.5 h (n=3). DNase I, deoxyribonuclease I; T, T cells; LatA, latrunculin A.

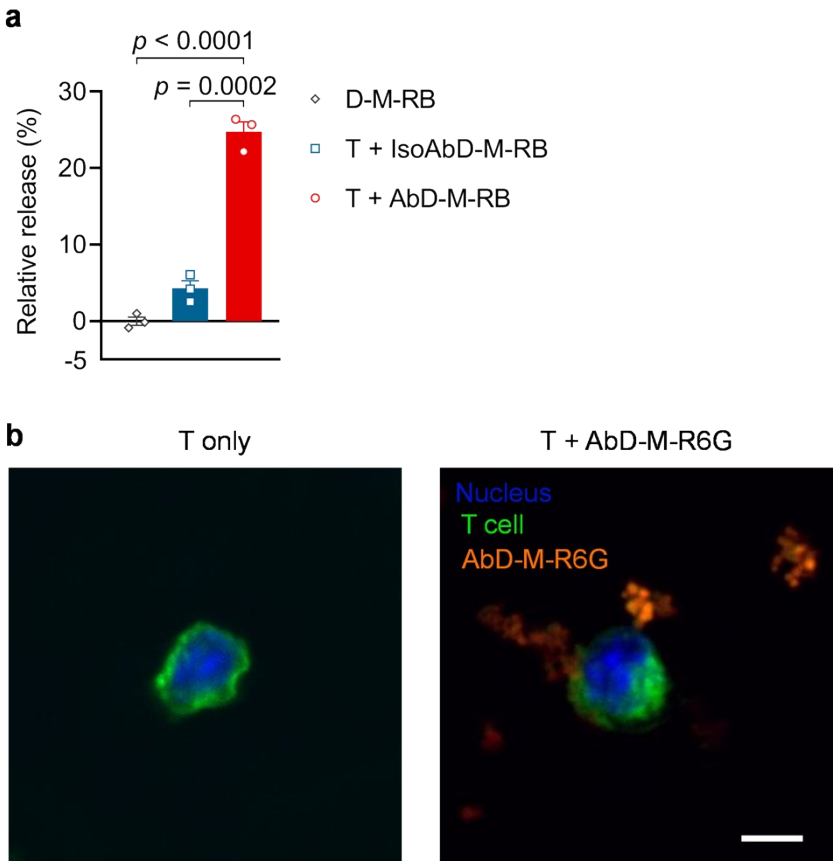


Figure S6. (**a**) Relative release profiles of rhodamine B (**RB**) from indicated systems after 1.5-h incubation at 37 ℃ (n = 3). (**b**) Representative confocal images of T cells (**T**) only or **T** after 2-h co-culture with rhodamine 6G (**R6G**)-labeled **AbD-M** (**AbD-M-R6G**) at 37 ℃. The nucleus and plasma membrane of T cells were stained with Hoechst 33342 (blue) and DiO (green), respectively. **AbD-M-R6G** was shown in red. Scale bar, 5 μm. Statistical analyses were performed by using unpair t test and data are mean ± s.e.m.

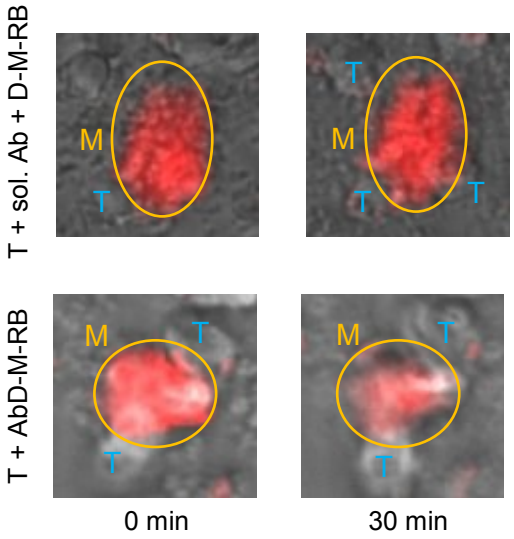


Figure S7. T cells (**T**) triggered the release of a model drug, rhodamine B (**RB**), from cellular force-responsive mesoporous silica microparticle system upon close contact. The fluorescence images of **AbD-M-RB** and **D-M-RB** (+ sol. **Ab)** in close contact with Pmel $CD8^+$ T cells upon 0- or 30-min incubation time. Yellow circles indicate the microparticles (**M**).

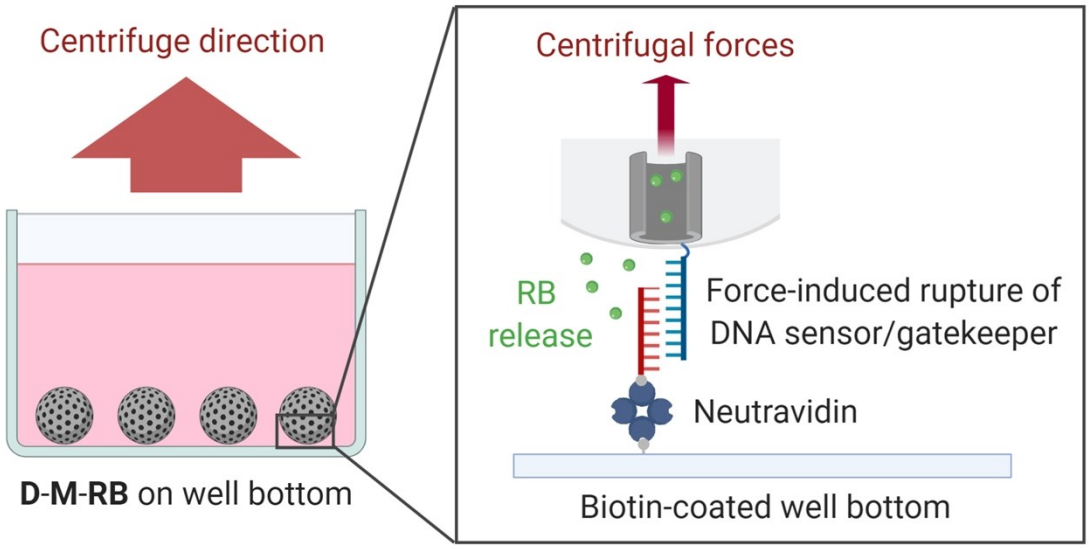


Figure S8. Schematic of an in vitro release assay of **D-M-RB** using centrifugal forces.

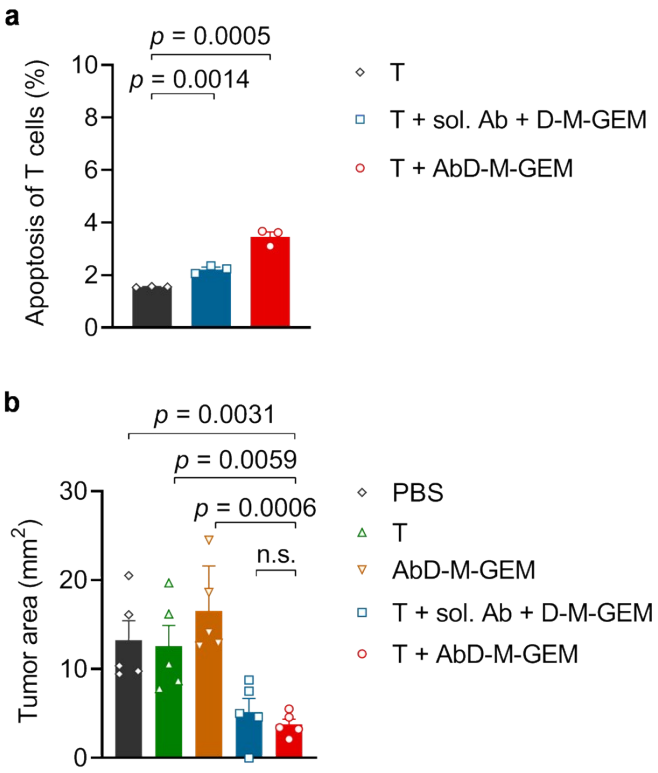


Figure S9. (**a**) Percentage of apoptotic Pmel T cells after 2-h co-culture with the indicated conditions (n = 3). (**b**) Tumor areas at day 5 post inoculation of MC38 cancer cells mixed with indicated reagents (n = 5 mice per group). Statistical analyses were performed by using unpair t test and data are mean ± s.e.m.

Table S1. Elemental analysis of bare mesoporous silica microparticle (**M**) and isocyanate-functionalized **M** (**M-NCO**). Data are mean ± s.d.

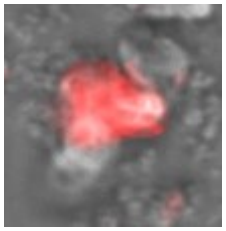
	N (wt%)	C (wt%)	H (wt%)
M	0	0	0.55 ± 0.06
M-NCO	1.48 ± 0.02	7.16 ± 0.05	1.04 ± 0.01

Table S2. Loading capacity of rhodamine B (**RB**) or gemcitabine (**GEM**) in **M**. Data

are mean ± s.d.

	RB (μmol/g **M**)	GEM (μmol/g **M**)
M-RB	157.7 ± 0.4	/
M-GEM	/	670 ± 7.7

Movie S1. The video showing the fluorescence decay in **AbD-M-RB** (**RB** shown in red) co-incubated with T cells over a period of 30-min.



Movie S2. The video showing the fluorescence decay in **D-M-RB** (**RB** shown in red color) co-incubated with T cells and sol. **Ab** over a period of 30-min.