Supplementary Information

Tuning Antibody Presentation to Enhance T cell Activation for Downstream Cytotoxicity

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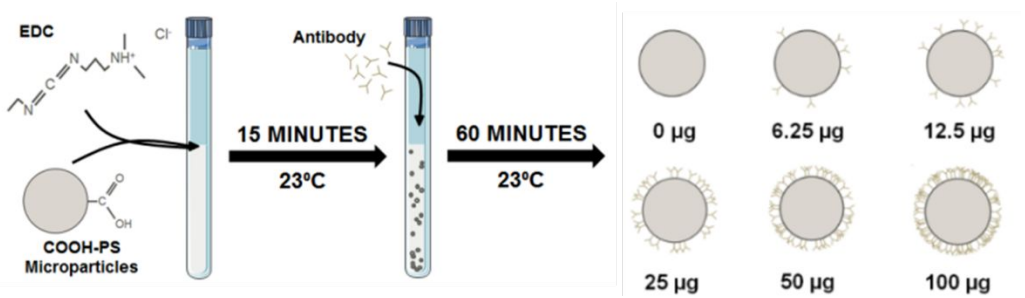
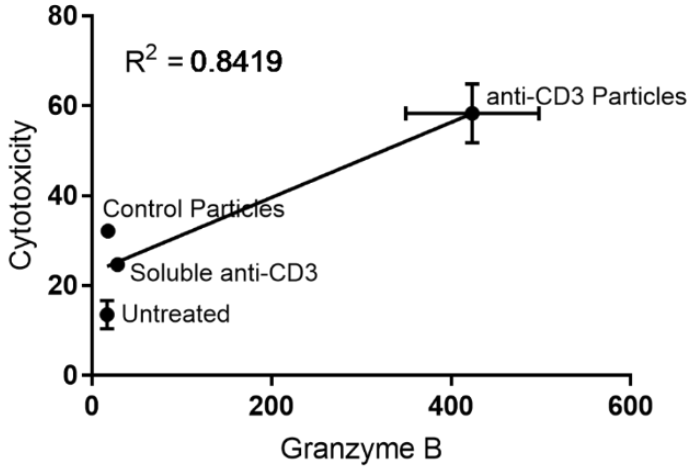
Description of Supporting Information

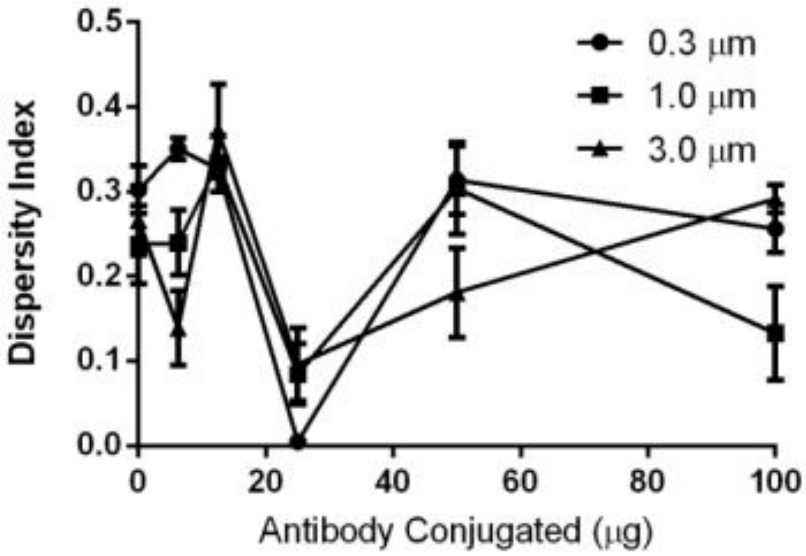
Description of the particle functionalization process. Correlation between cytotoxicity in HEYA8 cells and secreted granzyme B. Dispersity index of anti-CD3 particles analyzed via (Dynamic Light Scattering) DLS. Correlation of mean fluorescent intensity and granzyme b secretion in anti-CD3 particles labeled with F(ab)2-specific and Fc-specific fluorescent secondary antibodies. Percentage of particle surface functionalized with antibody at each size for each conjugation amount. Variable identification for experimental designs for the proximity of immune cells to cancer cells.



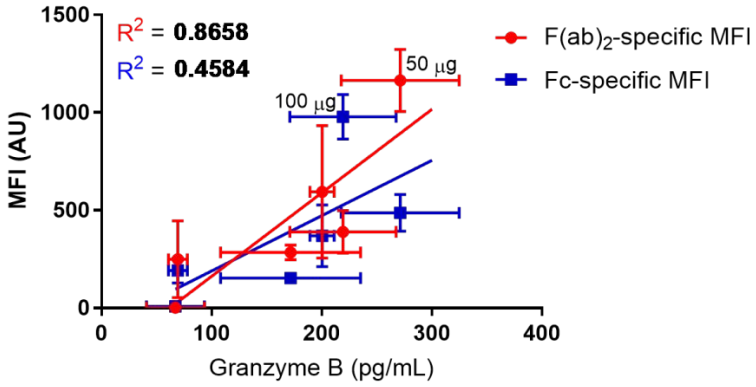
Supplementary Figure S1. Particle functionalization process. Anti-CD3 antibody functionalization of three sizes of carboxylated polystyrene particles at varying labeling densities using EDC.



Supplementary Figure S2. Correlation between cytotoxicity in HEYA8 cells and secreted GrB. Results demonstrates positive correlation.



Supplementary Figure S3. Anti-CD3 particles characterization. Anti-CD3 particles were analyzed using DLS to calculate the size heterogeneity of the particle population. Data are represented as mean ± SEM, n=10..



Supplementary Figure S4. Anti-CD3 particles elicit T cell granzyme b secretion. Anti-CD3 particles labeled with F(ab)2-specific or Fc-specific fluorescent secondary antibodies were measured for intensity. Data are mean ± SEM, n=6; *p<0.05.

Table S1. Surface Saturation. The percentage of particle surface functionalized with antibody at each size was calculated for each conjugation amount.

Conjugated Antibody (µg)	Surface Saturation (%)
0	0
6.25	10
12.5	21
25	41
50	83
100	>100

Table S2. Variable identification for experimental designs. Each experimental design is described by the identified variables – continuous dosing and the proximity of immune cells to cancer cells.

	Conditioned Media	Transwell	Direct Co-Culture
Continuous Dosing	✘	✓	✓
Cell Proximity	✘	✘	✓