

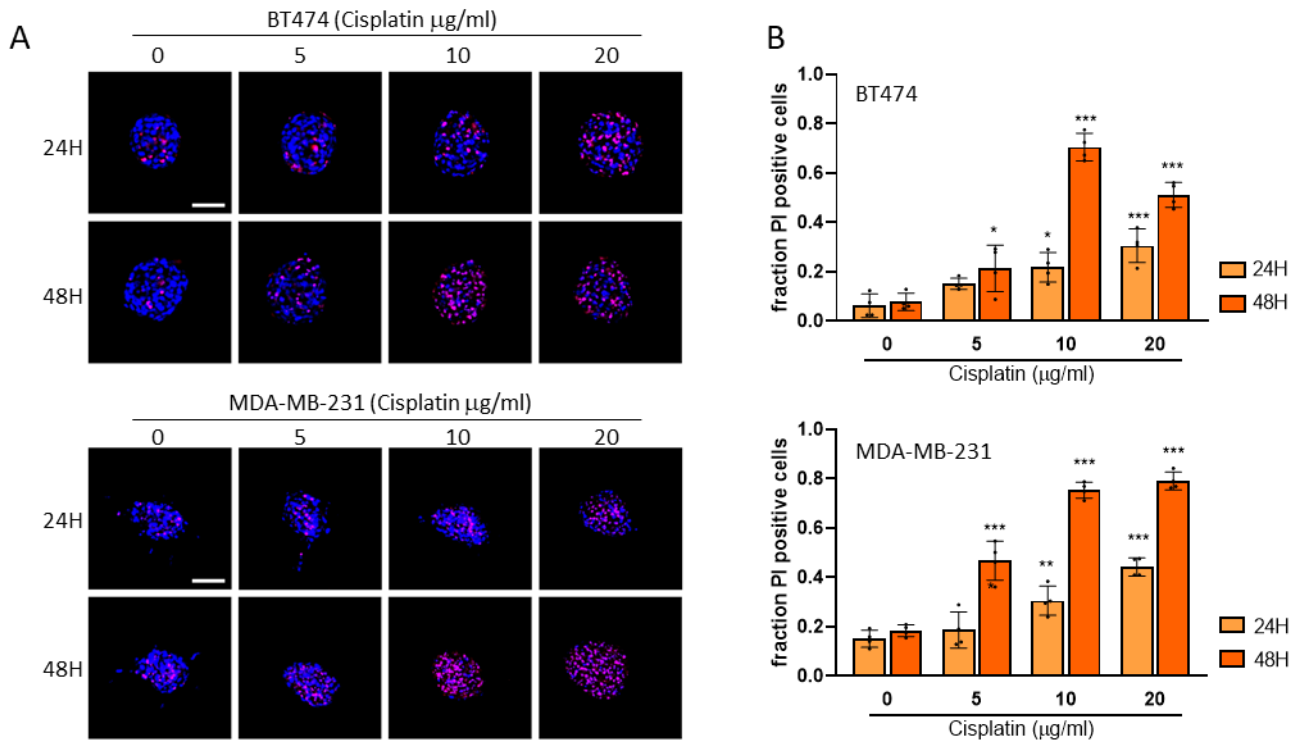
CD3-engaging bispecific antibodies trigger a paracrine regulated wave of T-cell recruitment for effective tumor killing

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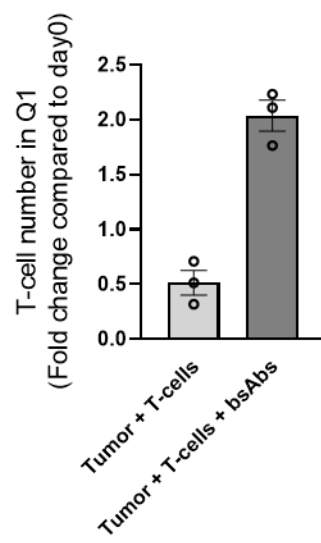
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Supplemental figures and figure legends

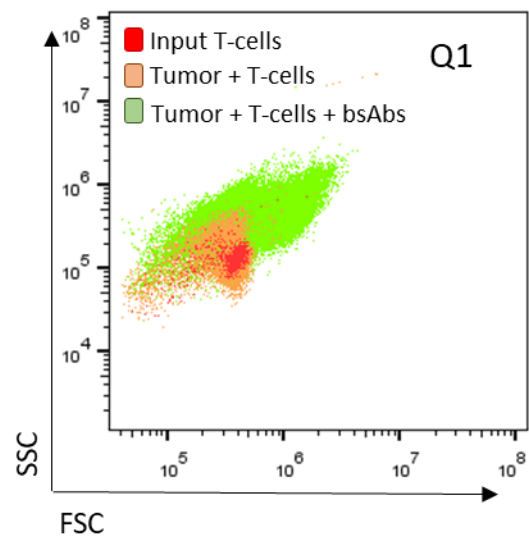


Supplementary Figure 1. Quantitative image-based analysis of viability of ECM embedded tumoroids. **(A)** Tumoroids were printed in collagen gels and exposed to a concentration range of cisplatin. Confocal image stacks were captured at 24 and 48 hours. Images show maximum projections. Bar = 100 μm . Blue, Hoechst; Red, PI. **(B)** Quantification of the image data as shown in A. Graphs show mean and SEM of 3 independent experiments, each performed in triplicate (3 individual wells each containing 1 collagen embedded BT474 or MDA-MB-231 tumoroid). Image quantification was performed on each individual z-section using the image analysis algorithm described in Fig 1B. P-value calculated using two-way ANOVA followed by Tukey's multiple comparisons test. *, $P < 0.05$; ***, $P < 0.001$, compared to untreated tumoroids.

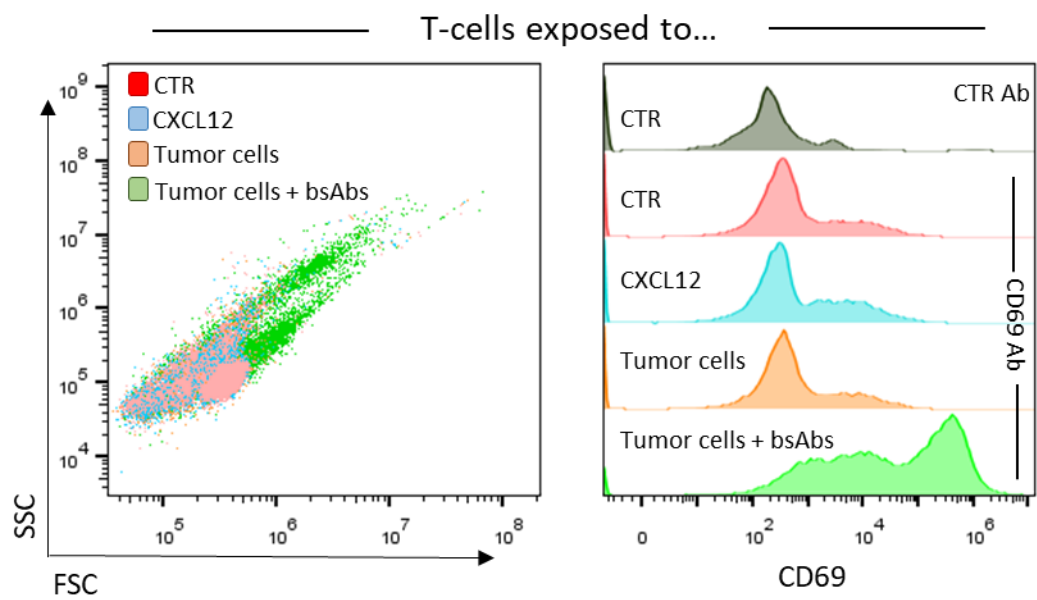
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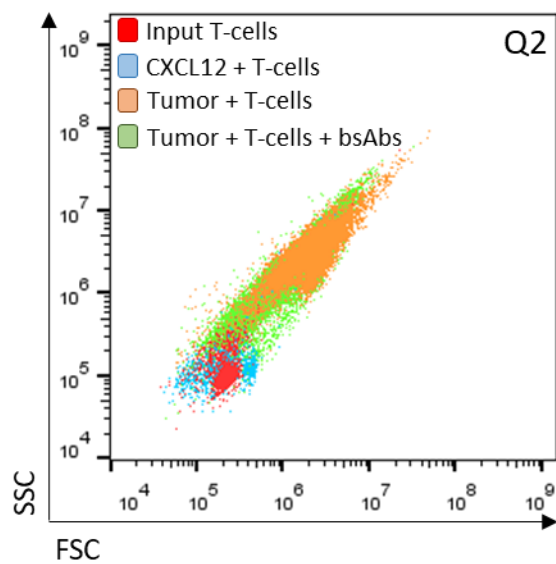
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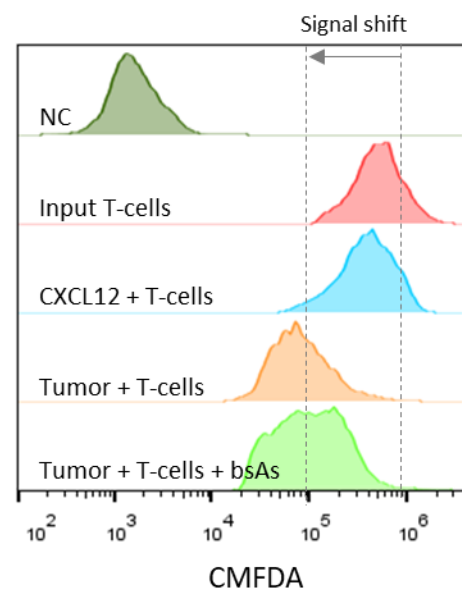
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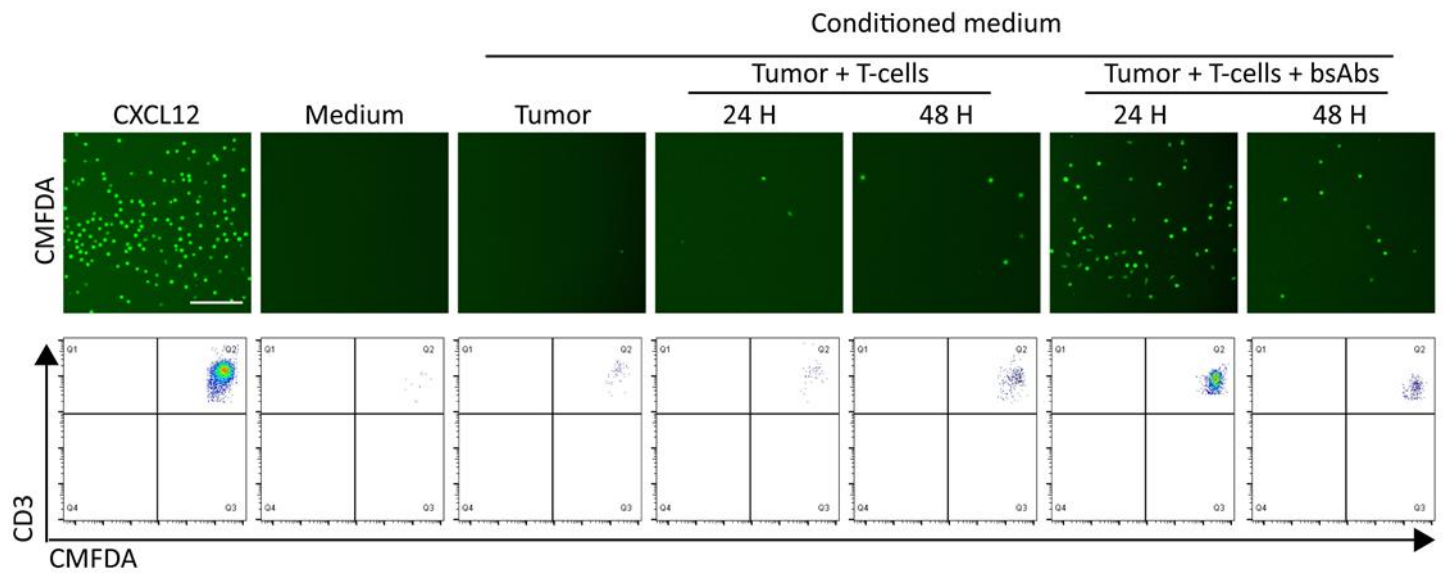
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Supplementary Figure 2. Signs of proliferation and activation of T-cells in the presence of bsAbs. **(A)** The number of T-cells in Q1 (unlabeled T-cells co-cultured with tumor cells in the bottom chamber in absence or presence of CD3_{wt}xHER2₁₆₉ bsAbs; see Fig 7C), normalized to day 0. Mean and SEM from 3 biological replicates are shown. **(B)** Plot showing forward scatter (FSC) versus side scatter (SSC) of T-cells in Q1 of Fig 7C. Input T-cells were collected before being added to the lower compartment. **(C)** FSC/SSC plot (left) and CD69 staining (right) for T-cells exposed to the indicated stimuli. **(D)** Plot showing FSC versus SSC of CMFDA-labeled T-cells from Q2 (labeled T-cells recruited from the upper chamber; see Fig 7C). Input T-cells were collected after CMFDA labeling and before being added to the upper chamber. **(E)** CMFDA intensity of T-cells from Q2. NC, T-cells not labeled with CMFDA.



Supplementary Figure 3. Transwell assay for T-cell migration in the presence of CXCL12, control medium, or conditioned media obtained from BT474 cells alone or BT474 cells cultured in the presence of T-cells with or without CD3_{wt}xHER2₁₆₉ bsAbs. Top row shows green fluorescence channel images taken in the lower chamber after 48h (CMFDA-labeled T-cells migrated from the upper to lower compartment). Bar = 100 μ m. Bottom row shows plots with CMFDA versus CD3 labeling of the migrated T-cells acquired from the lower chamber.