

Introduction

The complexity and variability associated with genetically engineered immune cells such as chimeric antigen receptor (CAR)-expressing T (CAR T) cells necessitates ex vivo assays that are accurate and reproducible, as highlighted by the FDA, in its guidance for CAR T development.

Here, we present a whole-well live-cell imaging assay using the Agilent xCELLigence RTCA eSight for quantifying cell lysis of suspension targets in regular 96-well tissue culture plates to estimate CAR T cytotoxicity.

Methods

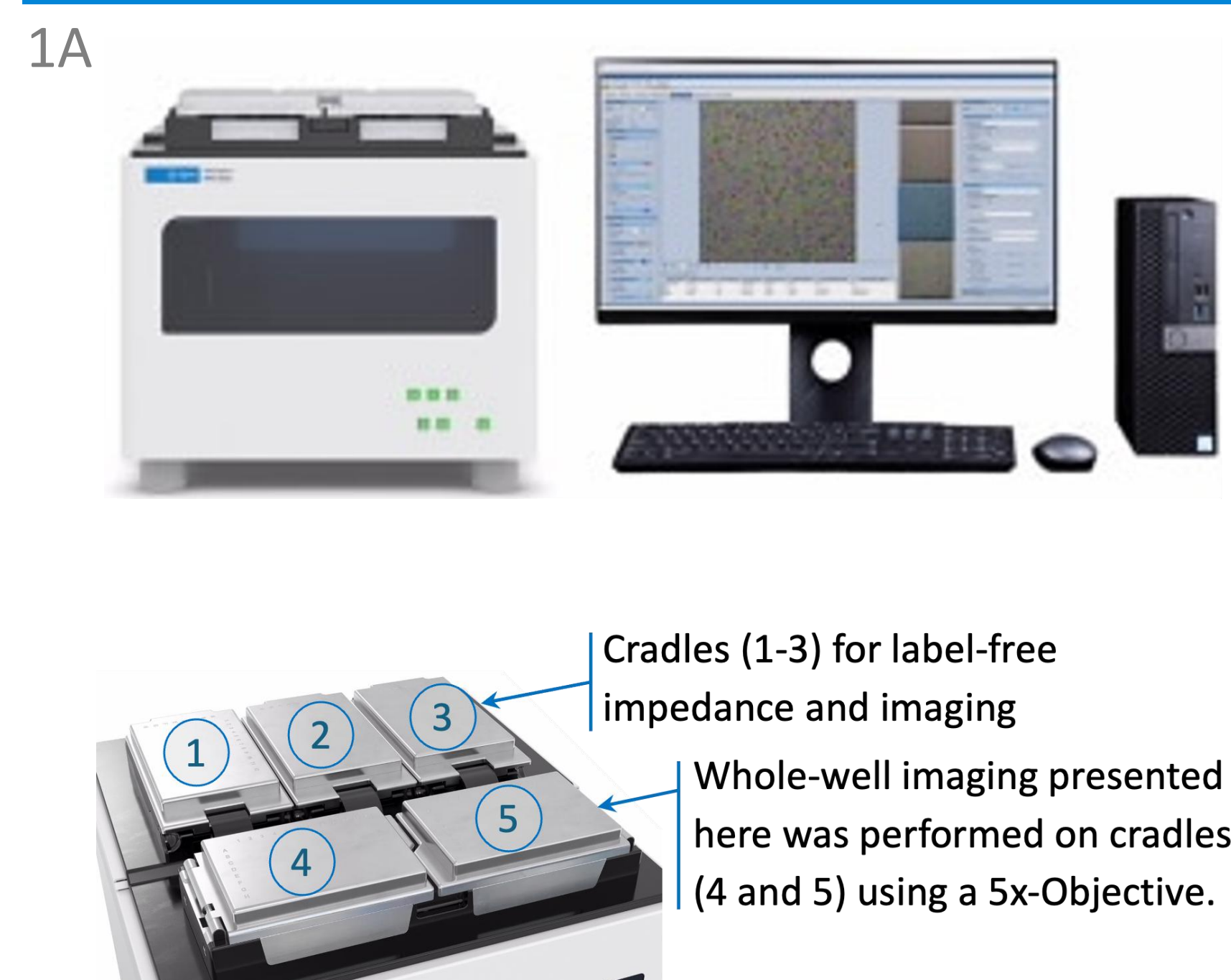
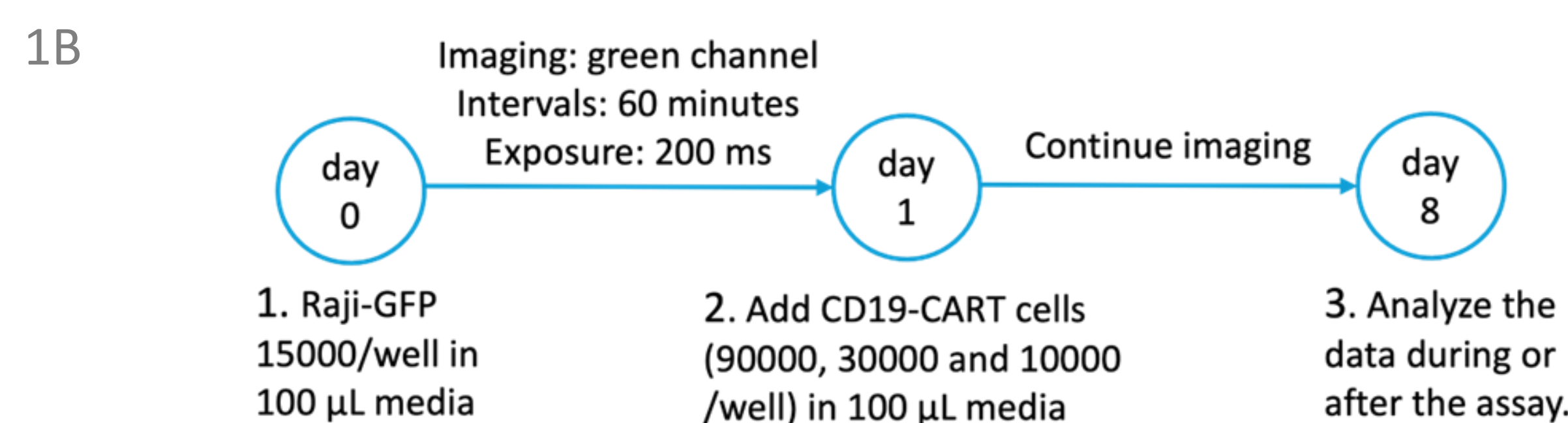


Figure 1. Whole-well cytotoxicity assays using Agilent xCELLigence RTCA eSight.

(1A) xCELLigence RTCA Sight is kept in a CO₂ incubator and managed using the RTCA eSight software running on the control unit. This assay uses the immunotherapy module. **(1B)** Experiment outline.



Cell culture: Raji-GFP cells stably expressing nuclear-localized green fluorescent protein (GFP) were generated by transducing the parental Raji cells (ATCC) with eLenti Green (Agilent Technologies) and performing dilution cloning on xCELLigence RTCA eSight. Cytotoxicity assays were performed in RPMI media (ATCC) supplemented with 10% FBS (Avantor) used for maintenance of Raji cells. CD19 CAR T cells were produced using the Lonza Cocoon platform using T cells kindly provided by Lonza. Briefly, T cells were thawed and rested overnight. On day zero, T cells were activated in a Cocoon Cassette with TransAct activator (Miltenyi Biotec). On day one, activation medium was refreshed with expansion medium (X-VIVO 15, 5% human serum, 200 U/mL IL2), and CAR transduction was performed at a multiplicity of infection (MOI) equal to 0.25 in the expansion medium. On day two, transduction was stopped by replacing the media with expansion media. The media was recirculated daily and exchanged every one to two days until cell harvest on day 10. T cells stored in liquid nitrogen in CytoStorCS10 (StemCell Technologies) were thawed on d0 of the assay and rested for a day in ImmunoCult-XF T Cell Expansion Medium (StemCell). Percent cytotoxicity, specific cytotoxicity, Area under the Curve (AUC, 0 to 100 hours) and time to 50% cytotoxicity (KT50) was generated on RTCA software. Prism (Graphpad) was used for ANOVA analysis.

Results

Cytolysis of Raji B cells increased with CD19 CAR T numbers

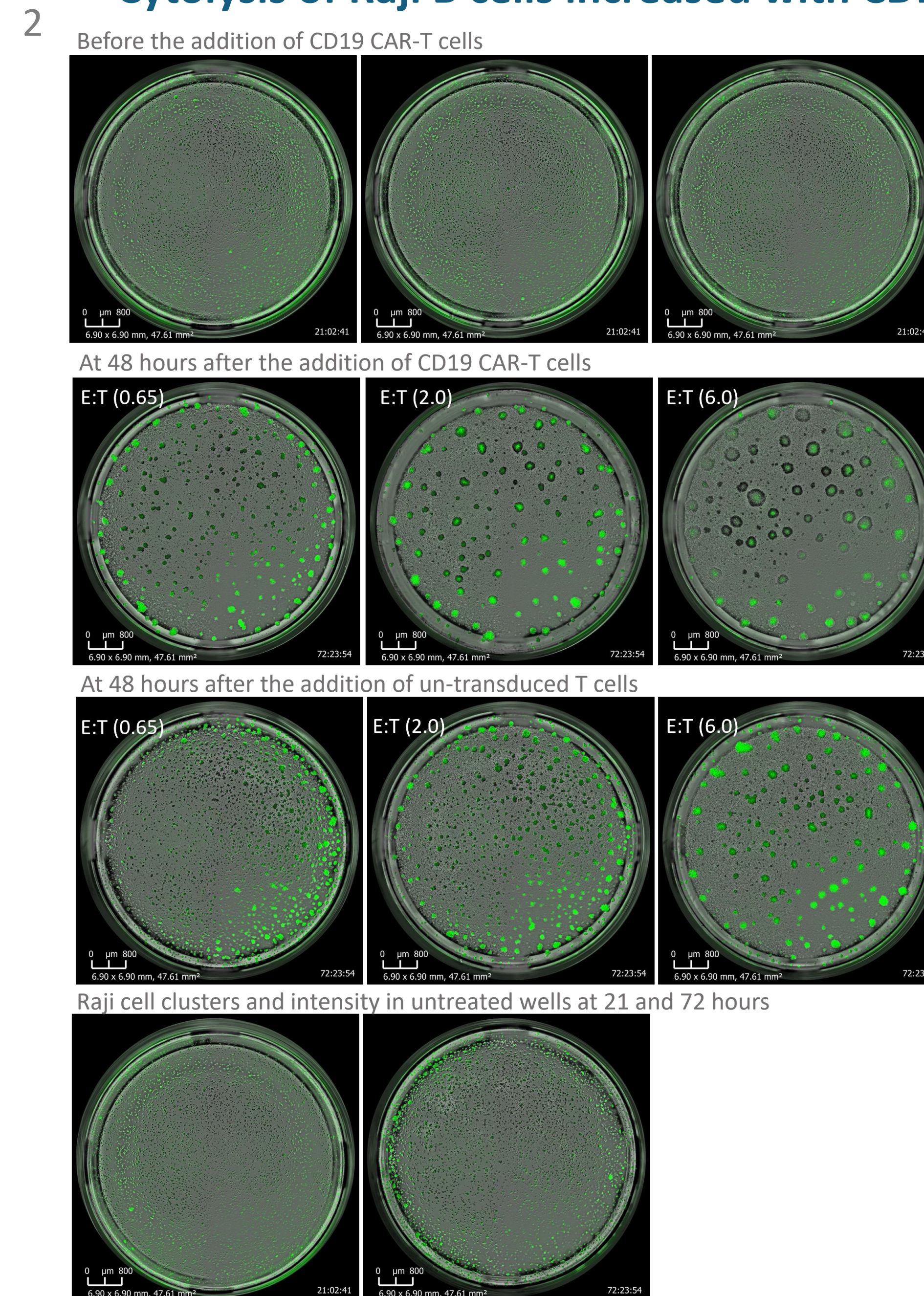


Figure 2. Representative images showing changes in GFP signal from target cells.

Distribution of Raji cells before the addition of T cells at ~ 21 hours (**top row**) and at 48 hours after coculture with CD19 CAR T (**second row**) or nontransduced T cells (**third row**).

Raji cells form larger clusters in the presence of T cells compared to untreated wells (**last row**).

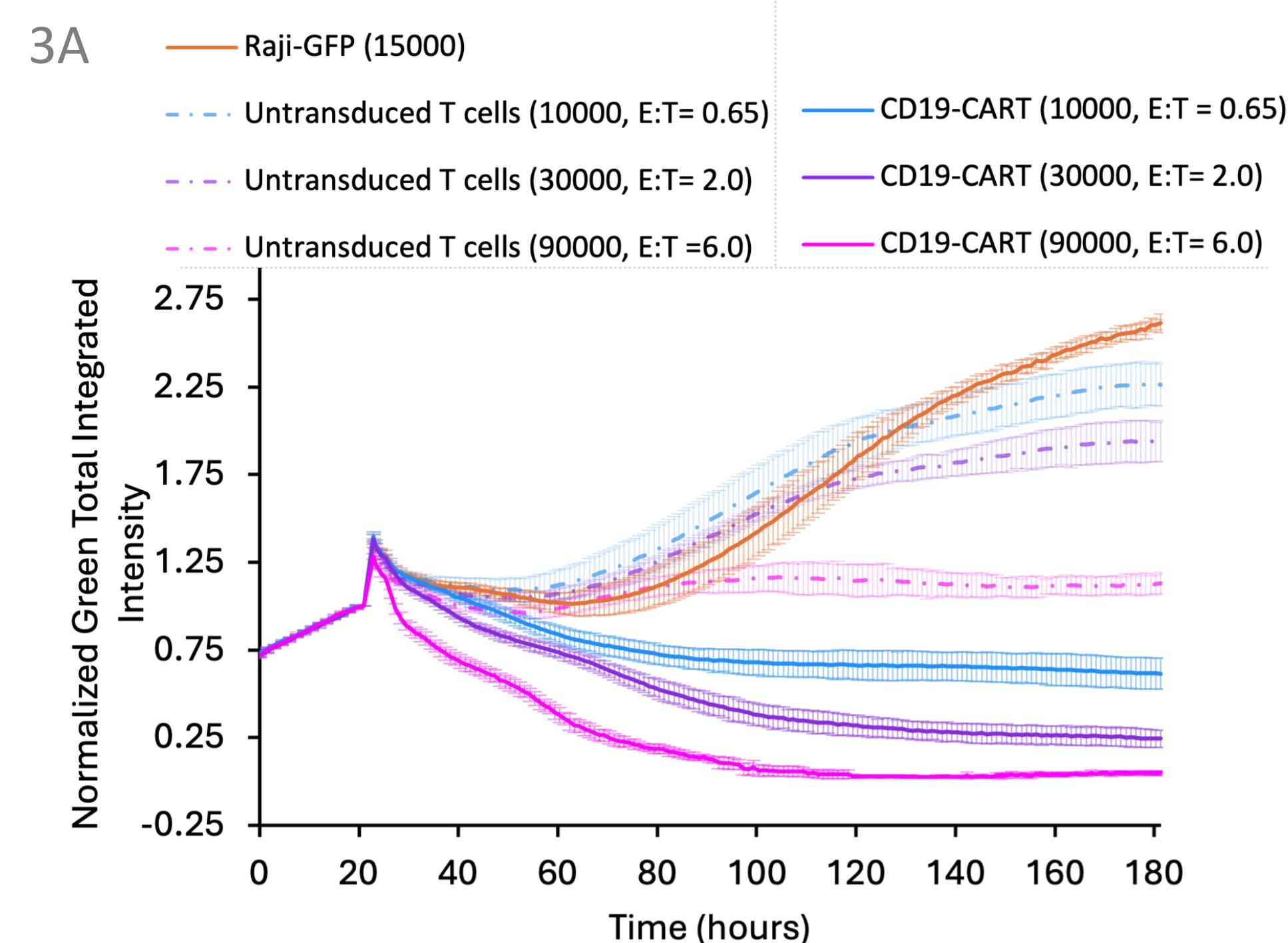


Figure 3. CD19 CAR T cytotoxicity was monitored in real time by measuring the loss of GFP signal from target cells. (3A) Total integrated green fluorescence intensity and **(3B)** the AUC for CAR T treated wells after normalization at the timepoint before T cell addition (21 hours).

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Results

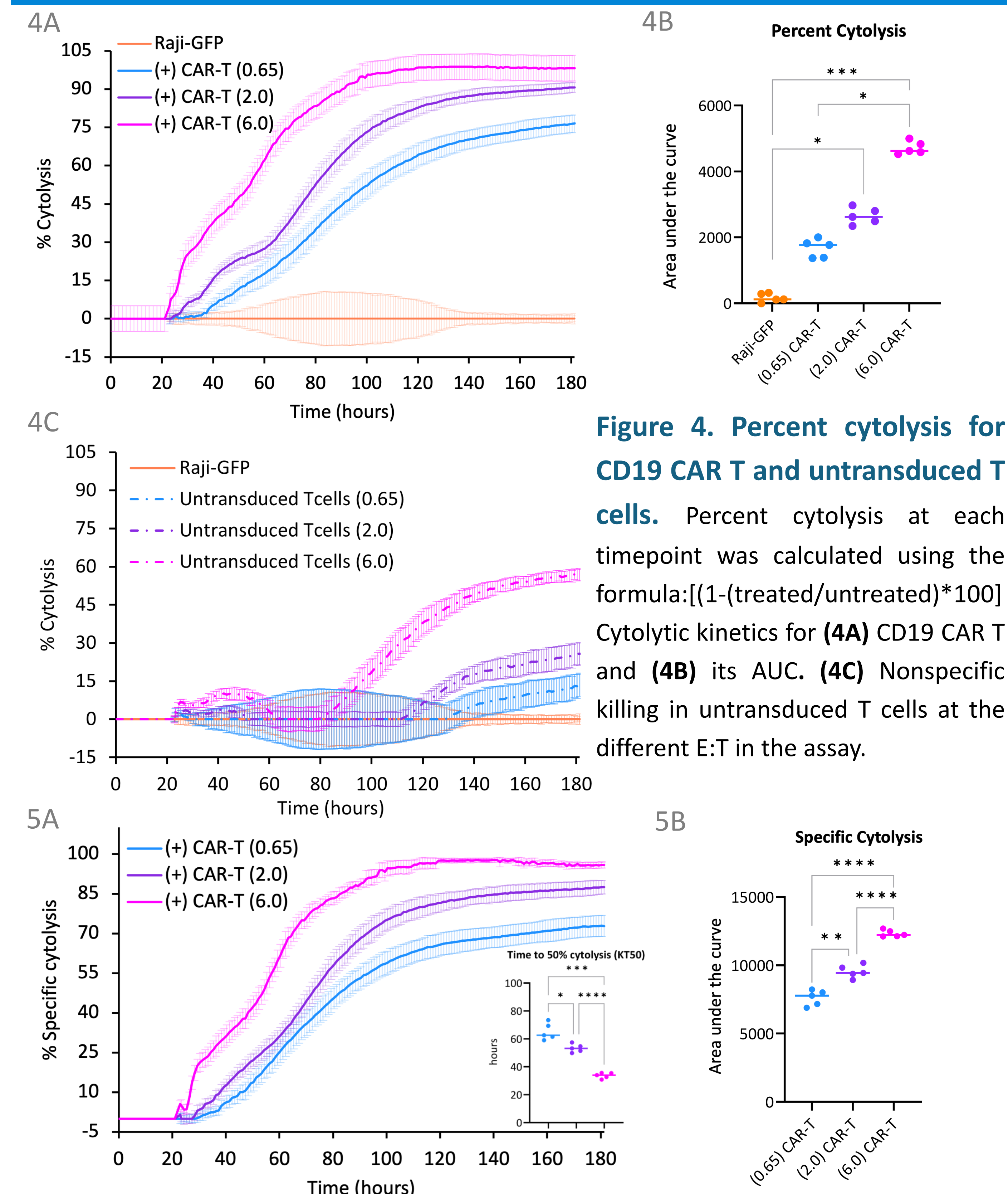


Figure 4. Percent cytotoxicity for CD19 CAR T and untransduced T cells. Percent cytotoxicity at each timepoint was calculated using the formula: [(1-(treated/untreated))*100] Cytolytic kinetics for **(4A)** CD19 CAR T and **(4B)** its AUC. **(4C)** Nonspecific killing in untransduced T cells at the different E:T in the assay.

Conclusions

- Immune cell-mediated cytotoxicity against suspension tumor cells was monitored and measured using real-time whole-well live cell imaging on the Agilent xCELLigence RTCA eSight system with its 5x objective.
- The cytolytic potency of CD19 CAR T cells against Raji-GFP cells was assessed by quantifying the loss of total green fluorescence intensity from the cells over time on regular 96-well tissue culture plates.
- The percentage of cytotoxicity and the onset of killing, derived from live-cell imaging, demonstrated a strong dependence on the number of effector cells.