Jurkat Reporter Cell Lines for Immunotherapy Drug Screening

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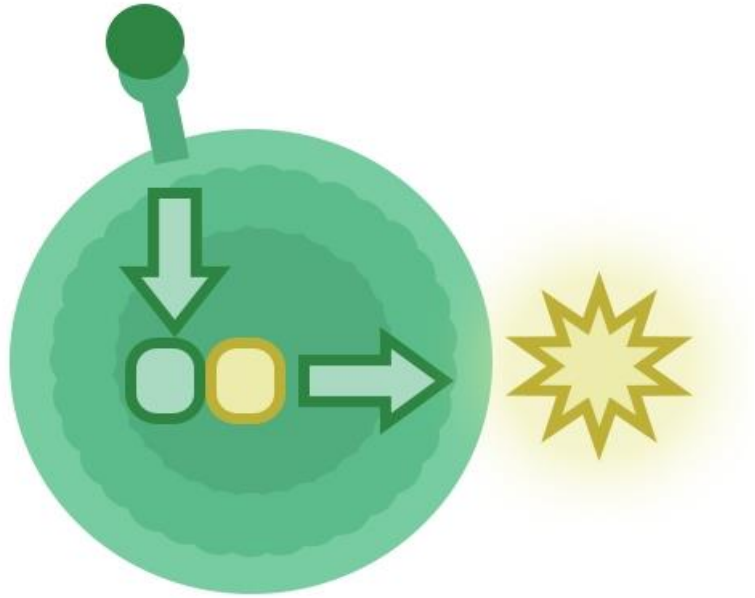
Reporter Cell Lines – Introduction

Reporter cell lines are stable cell lines expressing reporter genes, acting as useful tools for visualizing and tracking protein expression, screening for successfully transfected cells, gene expression regulation study, etc.

In general, a reporter vector includes a reporter gene, an upstream element such as a promoter or response element, and often a selectable marker to allow for stable cell line generation.

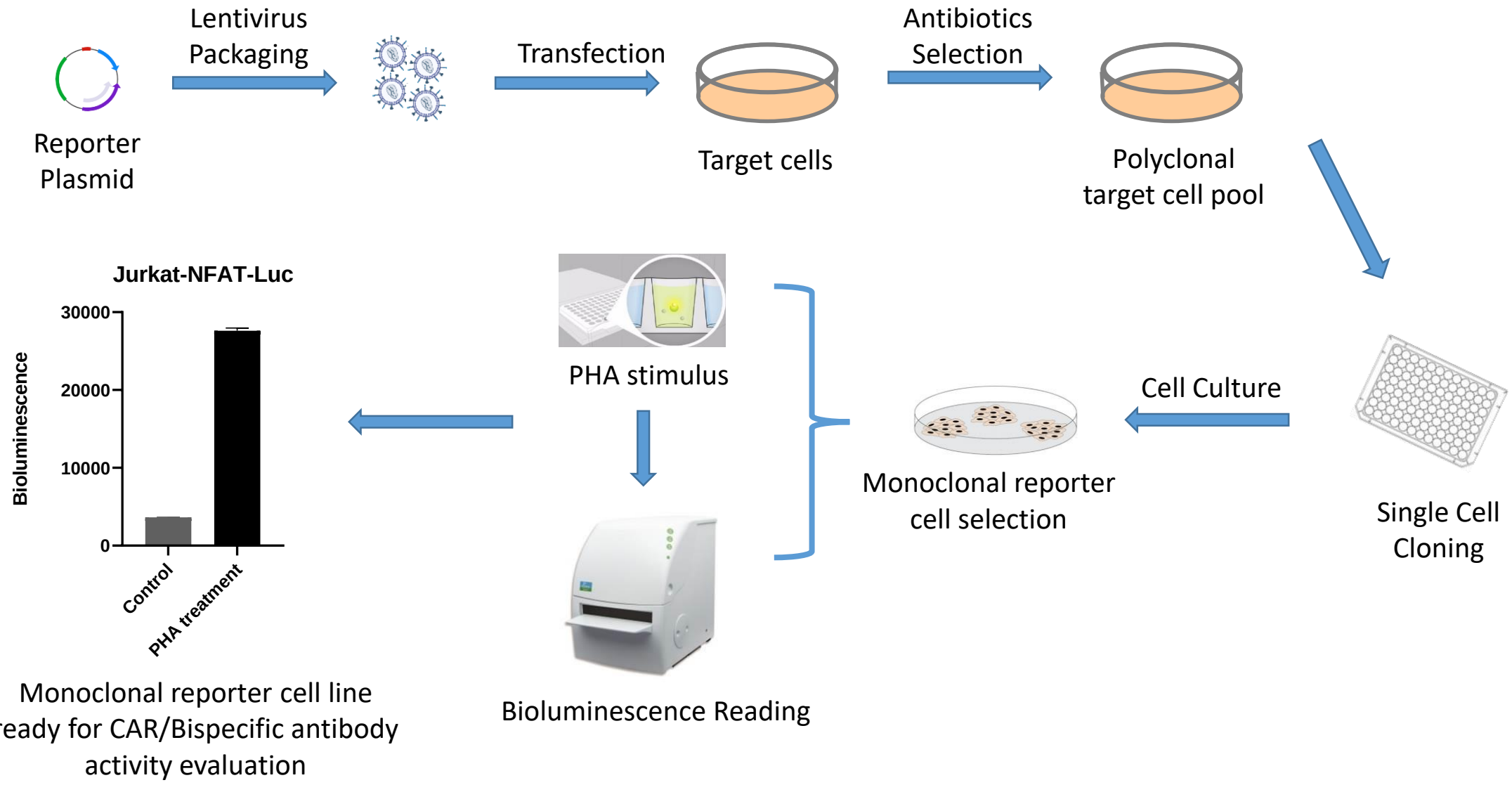


Reporter cells are grouped by cellular mechanism into these major categories:

- PRR reporter cells (TLRs, CLRs, NLRs, CDSs & SINTG, inflammasomes…)
- Transcription factor reporter cells (NF-κB, IRF, NFAT pathways)
- Cytokine reporter cells

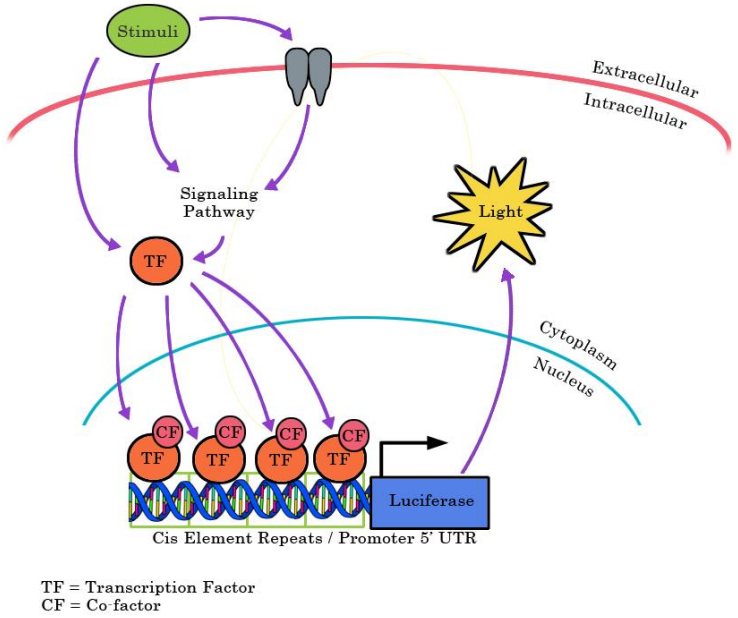
Construction and validation of reporter cell lines

Workflow of Jurkat-NFAT-Luc (JNL) cell line as example



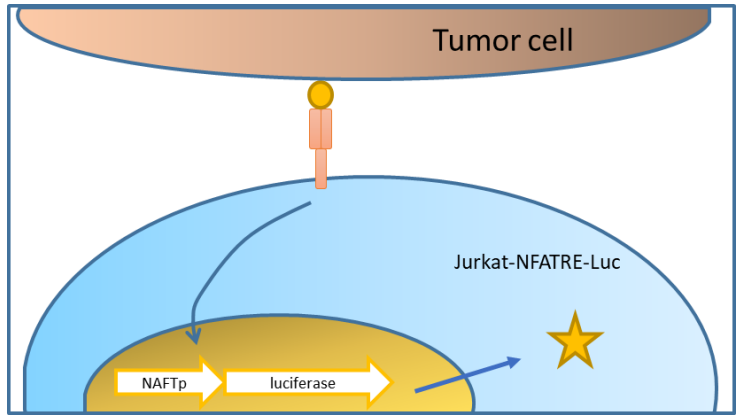
Reporter Cell Lines – CAR Activity Evaluation

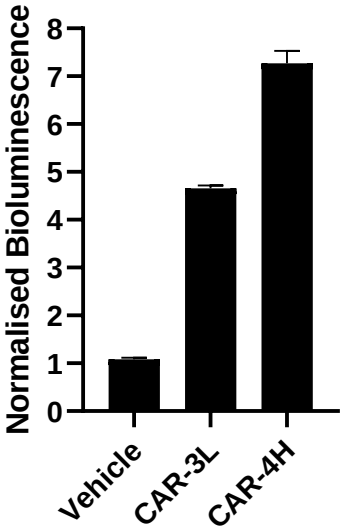
Transduction of NFAT-Luc report gene into Jurkat cell line for CAR activity evaluation



Cellular mechanism of NFAT-based reporter system: upon binding to extracellular stimuli through cell surface receptor on Jurkat cells, transcription factors are activated and translocated inside nucleus, where it activates luciferase expression for luminescence detection.

Cell-Cell
Activity

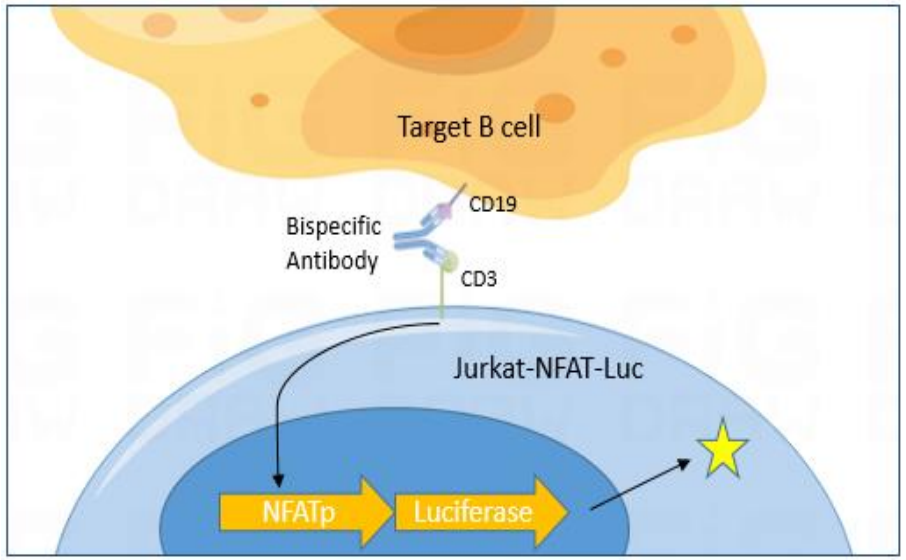




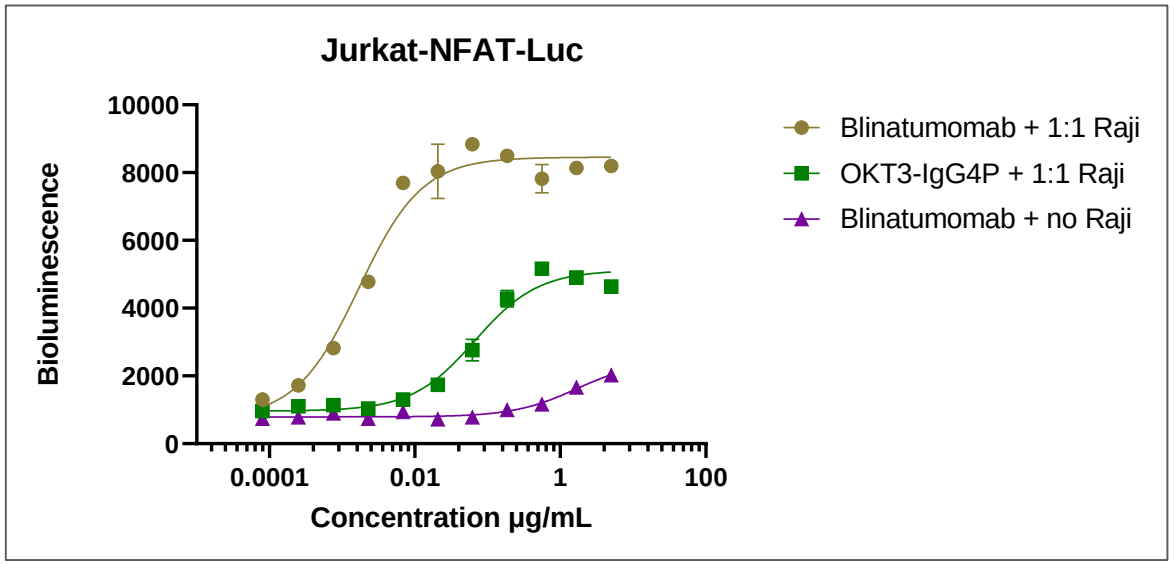
CAR activity evaluated by Jurkat-NFAT-Luc (JNL) reporter cell line: CAR-3L and CAR-4H were electroporated into JNL cell lines, followed by 4 hours co-culture with Raji tumor cells. Bioluminescence were normalized to corresponding JNL bioluminescence without Raji co-culture.

Reporter Cell Lines – Bispecific Antibody Evaluation

Transduction of NFAT-Luc report gene into Jurkat cell line for bispecific antibody evaluation



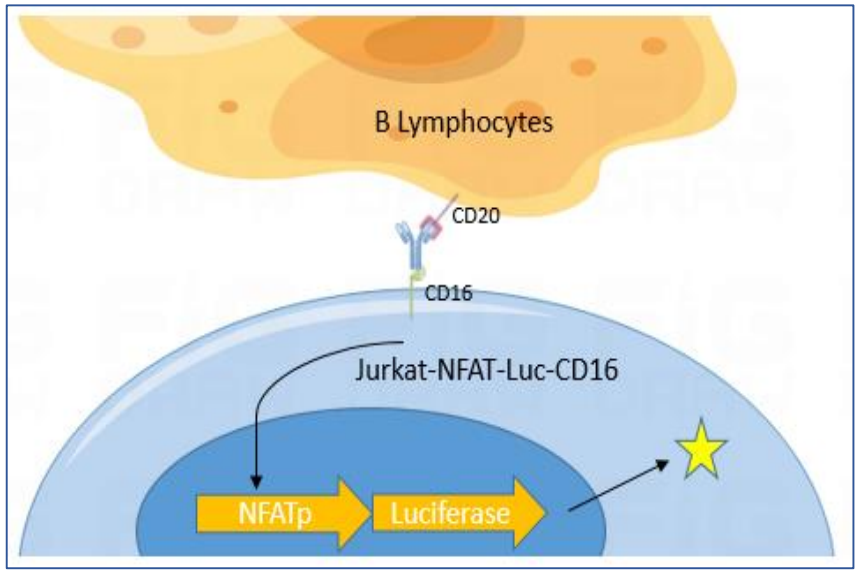
Cellular mechanism of NFAT-based reporter system: Bispecific antibody targets B cells through CD19 cell surface antigen, while simultaneously engaging Jurkat-NFAT-Luc T cells through CD3 antigen. Activation of Jurkat-NFAT-Luc cells leads to nuclear translocation of NFAT, promoting downstream luciferase expression for bioluminescence detection.



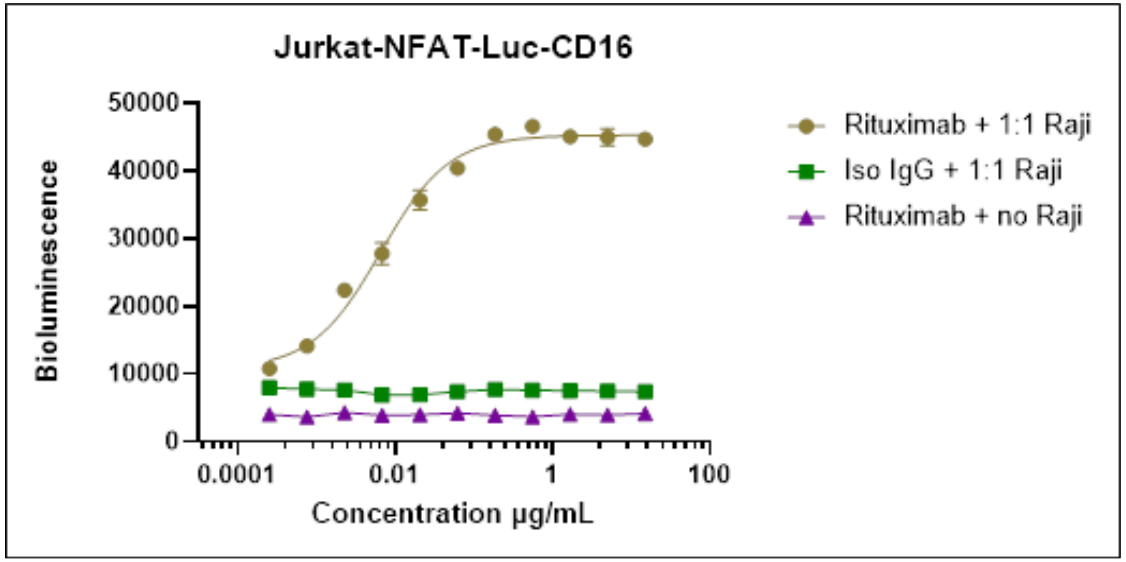
Evaluation of CD19xCD3 targeting Blinatumomab by Jurkat-NFAT-Luc reporter cell line:
Blinatumomab were cultured with reporter cells, followed by 4 hours co-culture with or without 1:1 Raji tumor cells. CD3-targeting monoclonal antibody (OKT3-IgG4P) showed partial activation on Jurkat reporter cells.

Reporter Cell Lines – Monoclonal Antibody Evaluation

Transduction of target report gene into Jurkat-NFAT-Luc cell line for monoclonal antibody evaluation

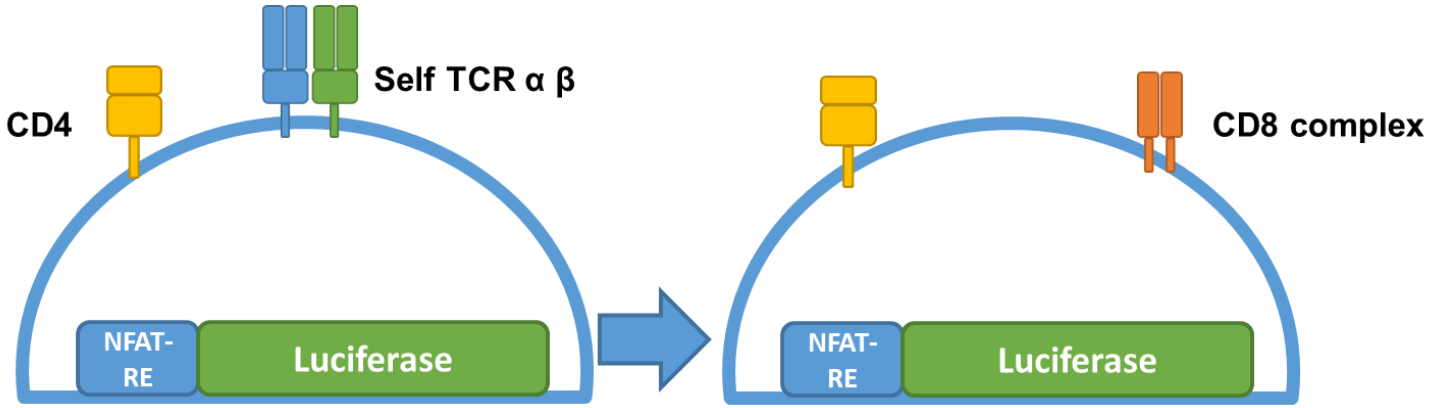


Cellular mechanism of CD16-NFAT-based reporter system: Rituximab Fab region targets B-lymphocytes through cell surface expressing CD20 antigen, while its Fc region activates CD16 on Jurkat-NFAT-Luc reporter cells. Upon activation, nuclear translocation of NFAT promotes downstream luciferase expression for bioluminescence detection.

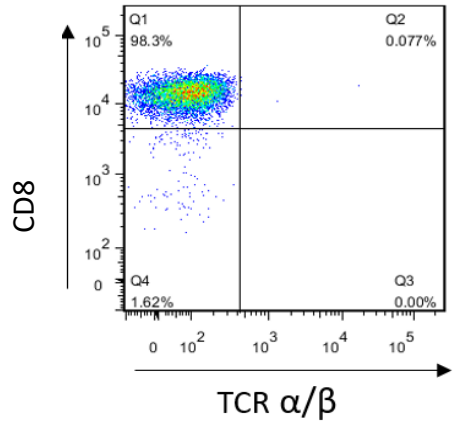


Evaluation of CD16-targeting Rituximab by Jurkat-NFAT-Luc-CD16 reporter cell line:
Rituximab were added in CD16-expressing Jurkat reporter cells, followed by 3 hours co-culture with or without 1:1 Raji B-lymphocytes. In the absence of Raji cells, Rituximab showed little activation on Jurkat reporter cells. Isotype control antibody showed little background stimulation.

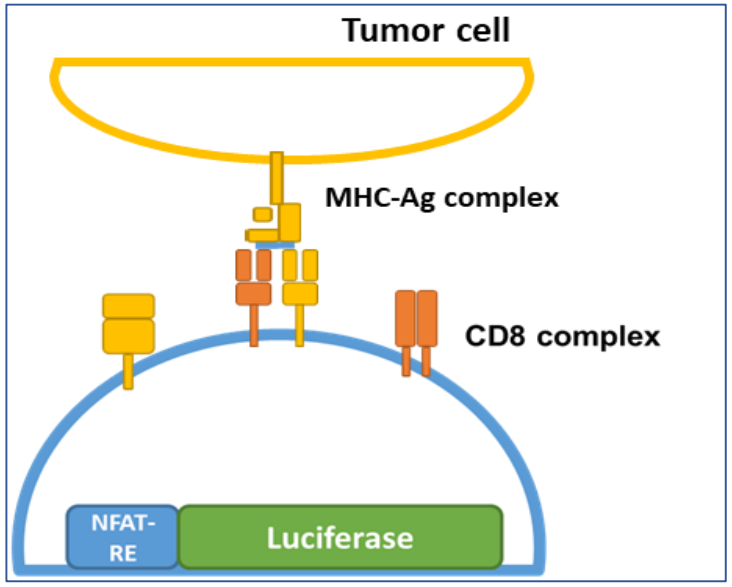
Reporter Cell Lines – TCR Activity Evaluation

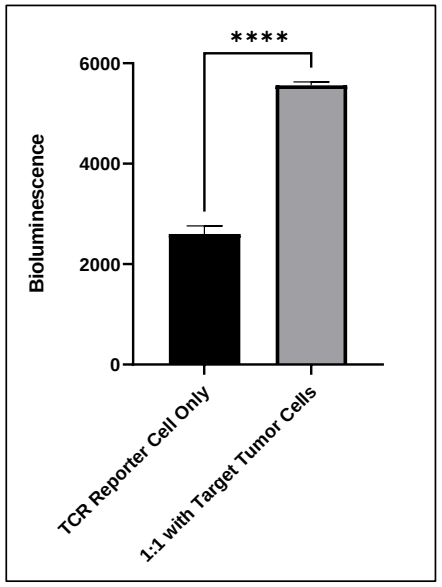


Endogenous TCR α/β knockout and CD8 overexpression in Jurkat-NFAT-Luc cells



Flow cytometry validation





TCR activity evaluated by CD8+ TCR -/- Jurkat-NFAT-Luc reporter cell line:
After overnight transduction with NY-ESO-1 TCR, CD8+ TCR -/- Jurkat-NFAT-Luc reporter cells were co-cultured with or without 1:1 A375 tumor cells for 4 hours. Bioluminescence reading showed around 2-fold difference (N=3, *p*-value < 0.0001 by unpaired t-test).

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