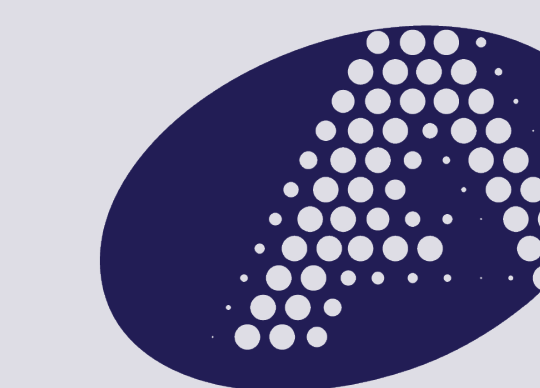


Gas-Luc2 reporter cell lines enable sensitive detection of interferon-gamma signaling for immune activation and CAR-T evaluation across 2-D and 3-D systems



ATCC®

Credible leads to Incredible®

Poster 6957

Hyeyoun Chang, PhD; John Foulke, MS; Luping Chen, BS; Meghan Sikes, MS; Catherine McManus, PhD; Fang Tian, PhD
ATCC®, Manassas, VA 20110

Abstract

Background: Interferon-gamma (IFN- γ) is a key cytokine regulating cellular immune activation and widely used as a functional biomarker in immunotherapy development. Standard ELISA method has limited sensitivity for early, low-level IFN- γ detection and does not effectively capture paracrine signaling in three-dimensional (3-D) models. To address these limitations, we developed IFN- γ -responsive luciferase reporter cell lines driven by a gamma-interferon activation site (GAS) promoter.

Methods: Three cancer cell lines with high endogenous expression of checkpoint ligands (PD-L1, CD155, B7-H3) were engineered with a GAS-Luc2 construct. An additional GAS-Luc2 reporter cell line was generated from THP-1 cells for monocyte- and macrophage-related applications. Reporter activity was evaluated after stimulation with recombinant IFN- γ , T cell-conditioned media, and co-culture with primary T or NK cells. Assays were performed in 2-D and 3-D systems and compared with IFN- γ ELISA. CAR-T cells were also evaluated using both methods.

Results: Reporter cells showed strong dose-dependent luciferase expression, with 100- to 250-fold increases after recombinant IFN- γ treatment and 50- to 100-fold induction with T-cell conditioned media. Co-cultures with primary immune cells produced 3- to 12-fold activation. Reporter signals remained robust in 3-D models and detected IFN- γ levels below the ELISA detection limit. In CAR-T assays, reporters identified early and low-level cytokine signaling not measurable by ELISA.

Conclusions: GAS-Luc2 reporter cell lines provide a sensitive, quantitative, and scalable platform for monitoring IFN- γ signaling. Their superior performance over ELISA, especially for early activation and 3-D applications, supports their utility in immunotherapy evaluation, immune checkpoint studies, and CAR-T functional assessment.

Results

GAS-Luc2 reporter cells respond to IFN- γ stimulation and T cell-derived cytokines

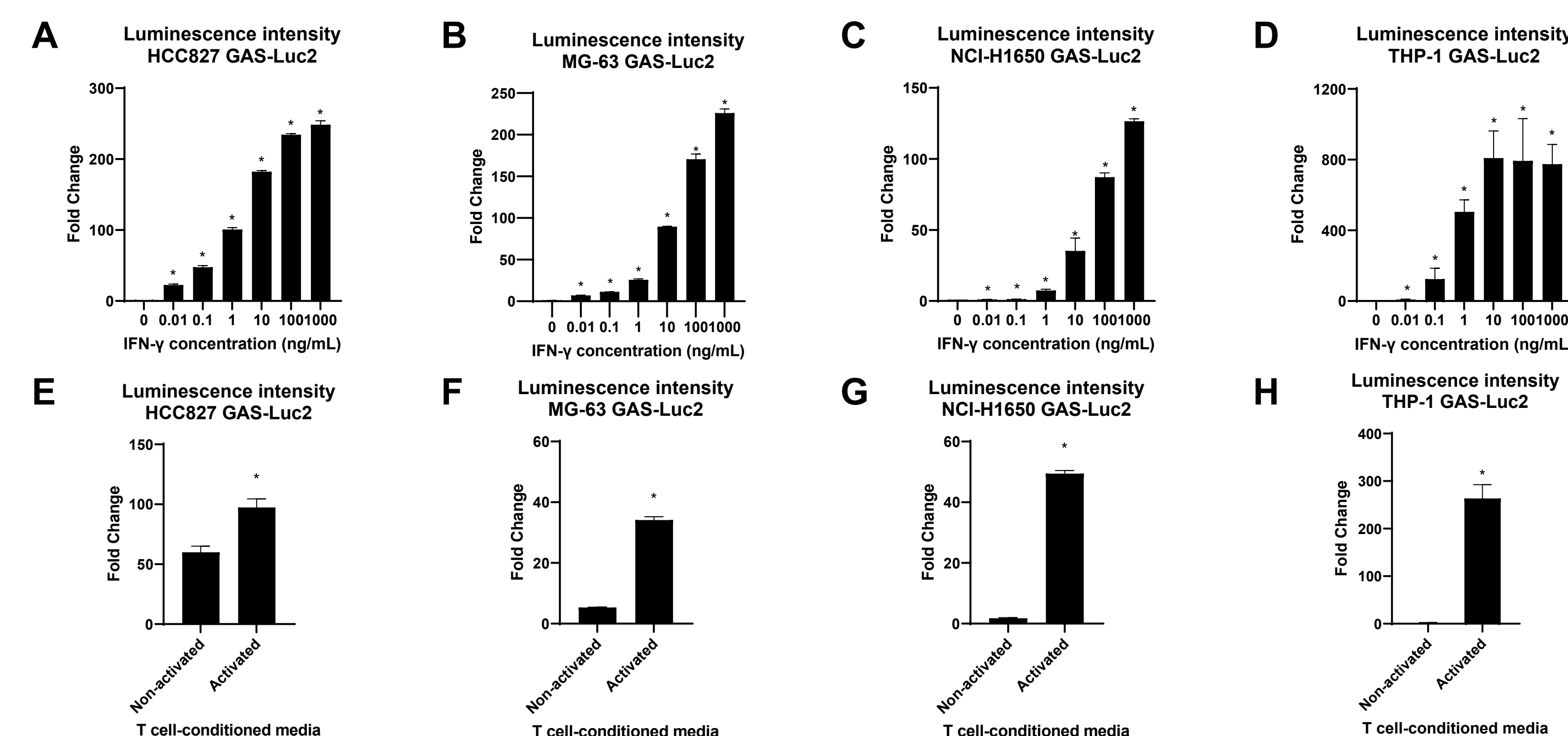


Figure 2: GAS-Luc2 reporter cells exhibit strong and dose-dependent activation in response to IFN- γ and T-cell-derived cytokines. (A-D) Reporter cell lines were stimulated overnight with IFN- γ . (A) HCC827 GAS-Luc2 (ATCC® CRL-2868-GAS-LUC2™), (B) MG-63 GAS-Luc2 (ATCC® CRL-1427-GAS-LUC2™), (C) NCI-H1650 GAS-Luc2 (ATCC® CRL-5883-GAS-LUC2™), and (D) THP-1 GAS-Luc2 (ATCC® TIB-202-GAS-LUC2™). (E-H) Reporter cells were treated with conditioned media from non-activated or activated human CD8+ T cells (activated three days with anti-CD2/CD3/CD28 beads). (E) HCC827 GAS-Luc2, (F) MG-63 GAS-Luc2, (G) NCI-H1650 GAS-Luc2, and (H) THP-1 GAS-Luc2. Luciferase activity was quantified using the Bright-Glo Luciferase Assay System (Promega) and measured on SpectraMax i3x (Molecular Devices). Data represent mean \pm SD (n=3). *P<0.05.

Immune checkpoint blockade enhances reporter activation in immune cell co-culture

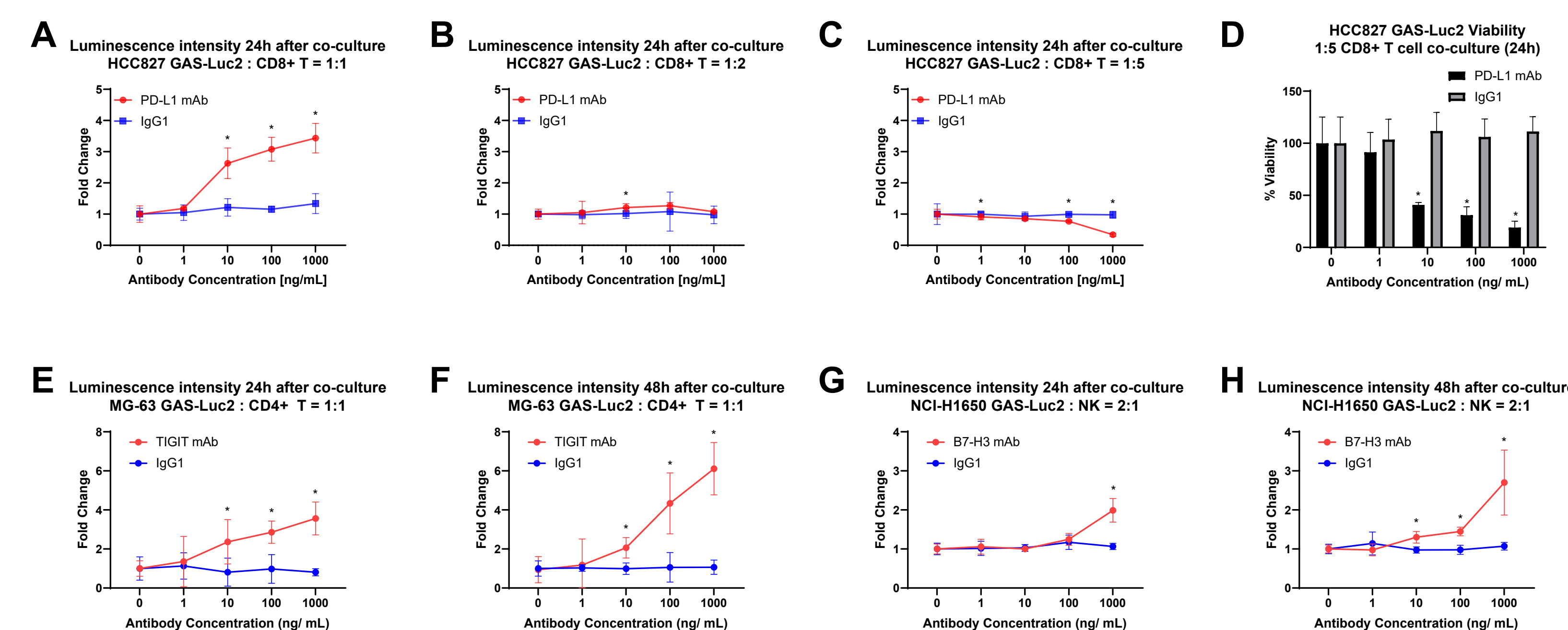


Figure 3: Immune checkpoint blockade enhances IFN- γ signaling detected by GAS-Luc2 reporter cells in immune cell co-cultures. (A-C) HCC827 GAS-Luc2 with CD8+ T cells (1:1, 1:2, or 1:5) \pm PD-L1 mAb. (D) HCC827 GAS-Luc2 viability under same conditions. (E-H) MG-63 GAS-Luc2 with CD4+ T cells (1:1) \pm TIGIT mAb for 24 or 48 h. (G-H) NCI-H1650 GAS-Luc2 with CD56+ NK cells (2:1) \pm B7-H3 ADCC mAb for 24 or 48 h. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luciferase activity was quantified using the Bright-Glo Luciferase Assay System (Promega) and measured on SpectraMax i3x (Molecular Devices). Data represent mean \pm SD (n=3). *P<0.05.

Reporter cells detect immune activation in 2-D and 3-D culture systems

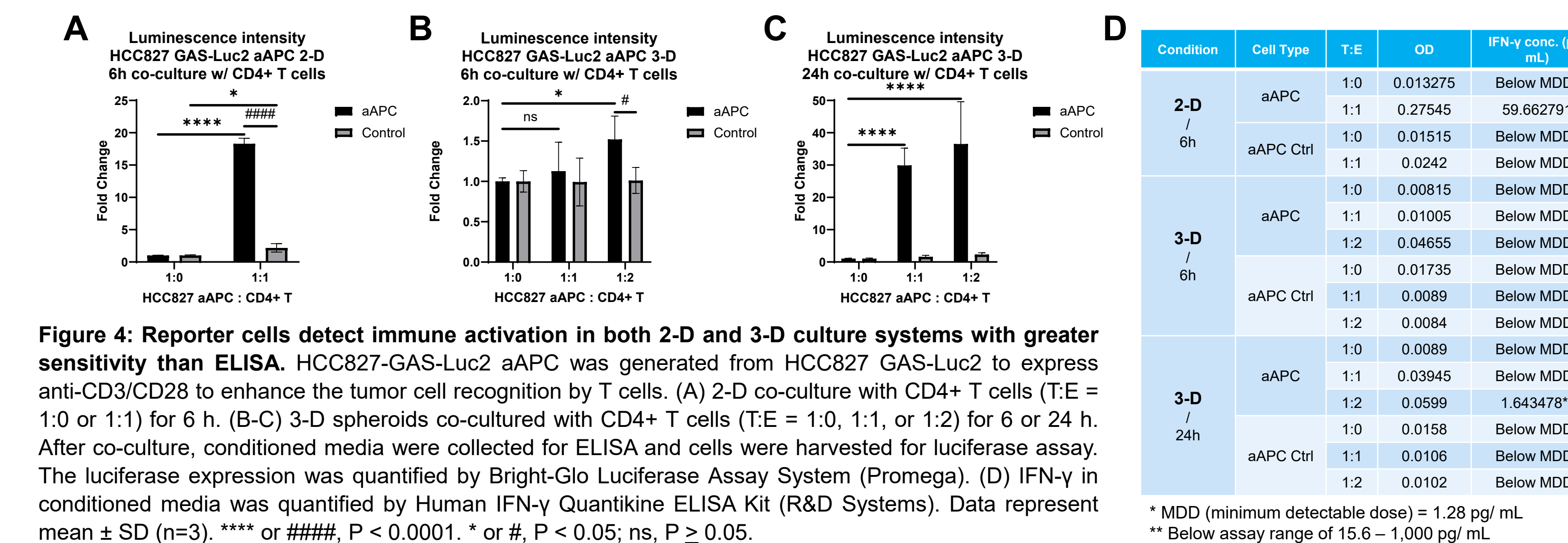


Figure 4: Reporter cells detect immune activation in both 2-D and 3-D culture systems with greater sensitivity than ELISA. HCC827-GAS-Luc2 aAPC was generated from HCC827 GAS-Luc2 to express anti-CD3/CD28 to enhance the tumor cell recognition by T cells. (A) 2-D co-culture with CD4+ T cells (T:E = 1:0 or 1:1) for 6 h. (B-C) 3-D spheroids co-cultured with CD4+ T cells (T:E = 1:0, 1:1, or 1:2) for 6 or 24 h. After co-culture, conditioned media were collected for ELISA and cells were harvested for luciferase assay. The luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). (D) IFN- γ in conditioned media was quantified by Human IFN- γ Quantikine ELISA Kit (R&D Systems). Data represent mean \pm SD (n=3). **** or #####, P < 0.0001. * or #, P < 0.05; ns, P \geq 0.05.

Reporter assay enables rapid functional evaluation of CAR-T cells

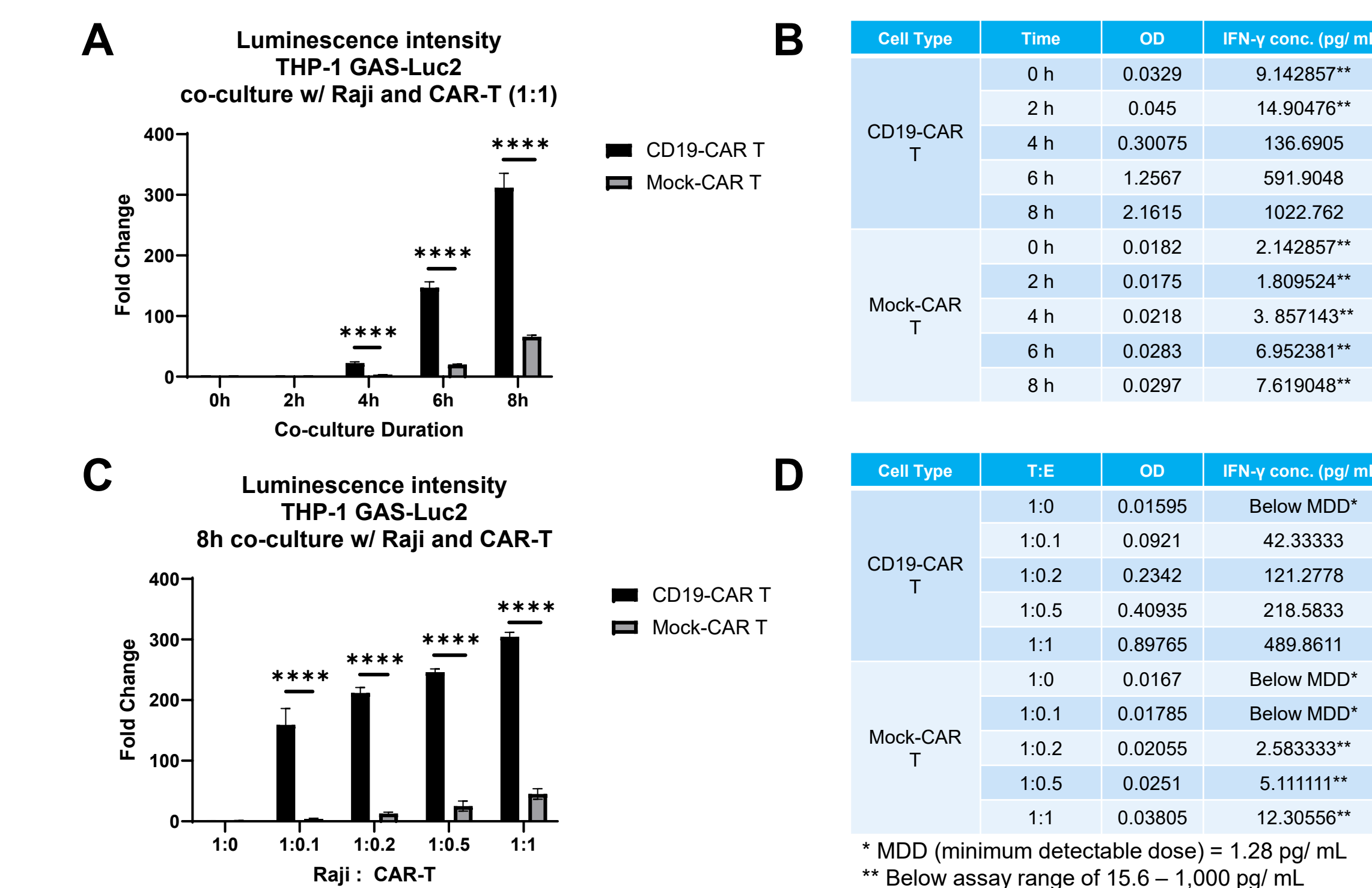


Figure 5: GAS-Luc2 reporter cells enable rapid and sensitive functional evaluation of CAR-T cell activation. Raji cells were co-cultured with CD19-CAR or mock-CAR T cells (ProMab) \pm THP-1 GAS-Luc2. (A-B) T:E = 1:1 for 0, 2, 4, 6, or 8 h. (C-D) T:E = 1:0, 1:0.1, 1:0.2, 1:0.5, or 1:1 for 8 h. The activation of CAR-T was measured by (A,C) luciferase assay and (B,D) ELISA. CAR-T activation was measured by luciferase assay and IFN- γ ELISA. Data represent mean \pm SD (n=3). ****, P < 0.0001.

Conclusion

- GAS-Luc2 reporter cells provide a sensitive method to detect IFN- γ signaling associated with immune activation.
- Reporter activation occurs in response to cytokine stimulation, immune cell co-culture, and checkpoint blockade.
- Compared to ELISA, reporter assays detect IFN- γ signaling at lower cytokine concentrations and earlier time points.
- Reporter cells function in both 2-D and 3-D culture systems, enabling evaluation in physiologically relevant models.
- The platform supports rapid functional testing of CAR-T therapies and other immunotherapeutic modalities.

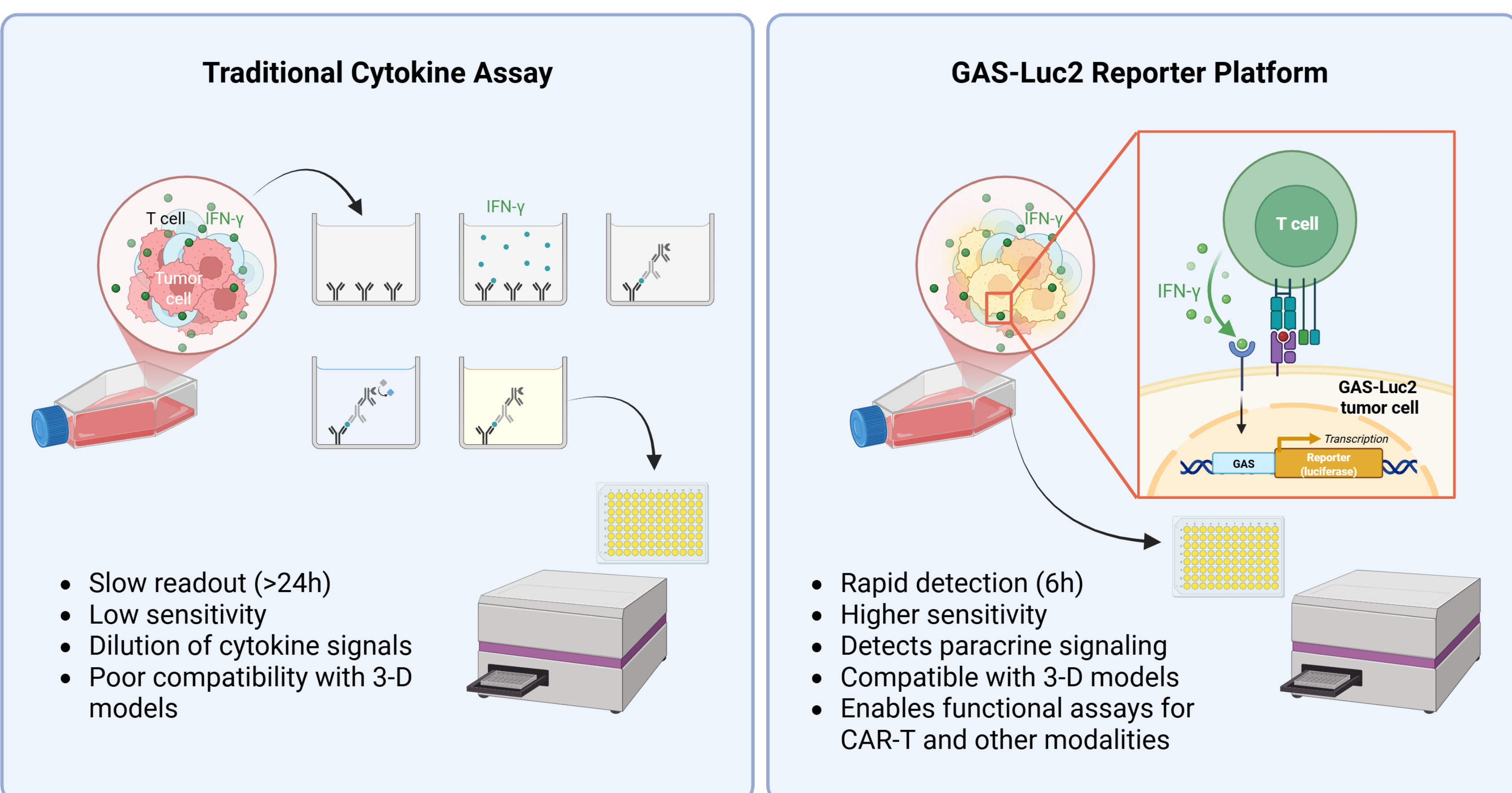


Figure 1: Schematic comparison of conventional ELISA-based cytokine detection and the GAS-Luc2 reporter platform. ELISA measures secreted IFN- γ from conditioned media, requiring longer incubation and showing limited sensitivity. In contrast, GAS-Luc2 reporter cells convert IFN- γ receptor activation into a luminescent signal via JAK/STAT-mediated luciferase expression, enabling rapid, sensitive, detection of immune activation. This system captures paracrine signaling in co-culture and is compatible with both 2-D and 3-D models, supporting applications in immunotherapy and CAR-T evaluation. Image created with BioRender.com