Immune Checkpoint Reporter Cell Lines Based on the Protein Profiling of ATCC® Cell Lines for Cancer Immunotherapy Drug Screening

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Abstract 6643

Abstract

The success of immune checkpoint inhibitors in the treatment of diverse types of cancers and their continued growth in the market have driven burgeoning interests in developing more drugs in this category. However, the intrinsic complexity of the immunological models and the variable drug responses among different cancer types have become the most prominent challenges.

To facilitate large-scale research projects and drug discovery of immune checkpoint inhibitors, we conducted a comprehensive protein profiling of ATCC®'s vast portfolio of human tumor and immune cell lines for several established and novel immune checkpoint molecules. Based on this protein profiling data, we generated immune checkpoint reporter cancer cell lines with high expression of endogenous immune checkpoint molecule ligands (PD-L1, CD155, and B7-H3). The reporter system contains a gamma interferon activation site (GAS)–response element upstream of the luciferase gene, preventing luciferase expression when the immune checkpoint molecule ligand binds to its corresponding receptor that suppresses T cell–mediated antitumor activity. In the presence of a relevant immune checkpoint inhibitor, a luciferase expression–based bioluminescent signal is produced, which can be readily detected and quantitated to evaluate the efficacy, potency, and dynamics of the inhibitor.

Our data showed that the bioluminescence in the reporter cancer cells increased approximately 100-250 fold in a dose-dependent manner in response to interferon gamma stimulation, which mimics the signaling from activated CD8+ cytotoxic T cells. The bioluminescence increased approximately 50-100 fold in response to CD8+ primary T cell–conditioned media stimulation. In particular, we observed an up to 5-fold increase in luciferase signal from our PD-L1 reporter cell line in response to co-culture with CD8+ primary T cells in the presence of an anti-PD-L1 blocking antibody in a dose-dependent manner. The luciferase expression and endogenous immune checkpoint molecule ligand expression were well maintained after the cell lines had reached >30 population doubling level. These results highlight the robustness and responsiveness of the reporter system for the assessment of T cell–mediated immune responses triggered by checkpoint inhibitors.

As compared to immune checkpoint assays that use an artificial checkpoint ligand overexpression system, these immune checkpoint reporter cancer cell lines yield exceptional in vitro and ex vivo assay sensitivity and reproducibility while simplifying the complex immunological model by providing physiologically relevant expression of immune checkpoint molecule ligands.

Background

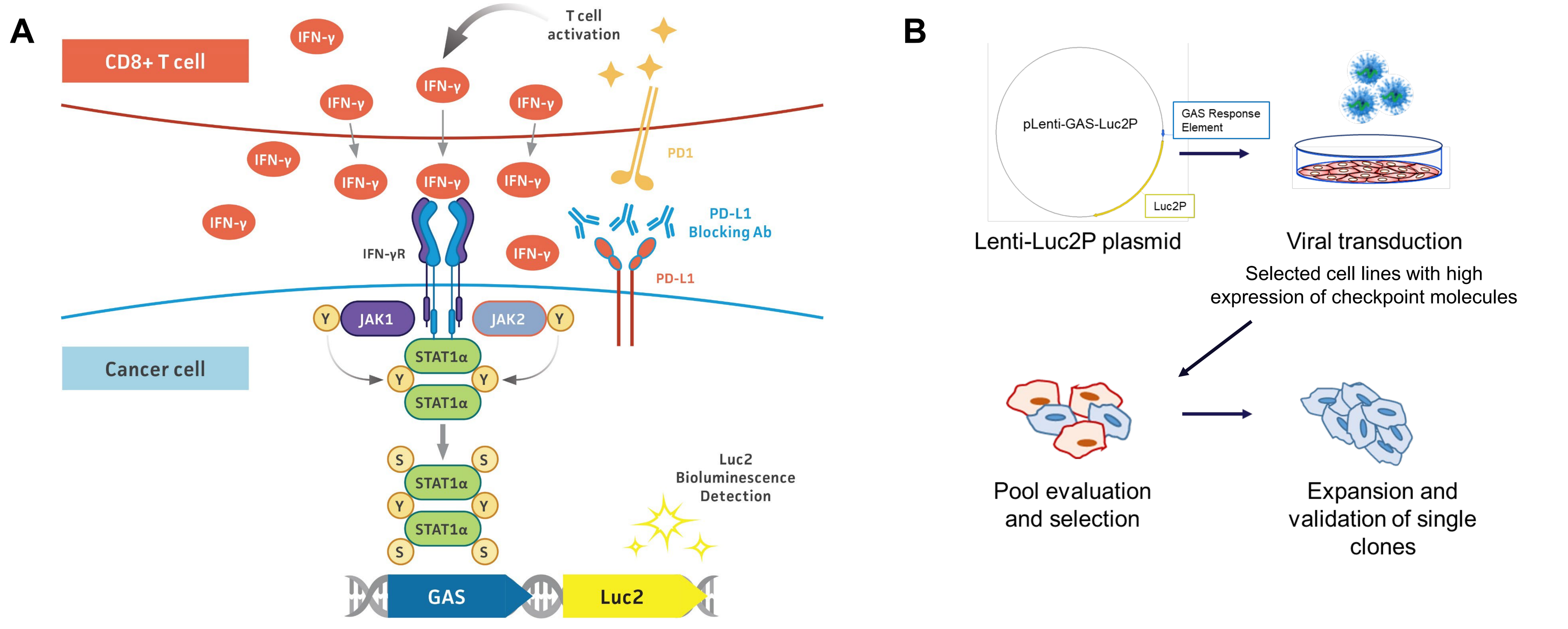
A
CD8+ T cell
T cell activation
IFN-γ
PD1
PD-L1 Blocking Ab
IFN-γR
PD-L1
JAK1
JAK2
Cancer cell
STAT1α
Luc2 Bioluminescence Detection
GAS
Luc2
B
pLenti-GAS-Luc2P
GAS Response Element
Luc2P
Lenti-Luc2P plasmid
Viral transduction
Selected cell lines with high expression of checkpoint molecules
Pool evaluation and selection
Expansion and validation of single clones

Figure 1: Schematics of immune checkpoint molecule-expressing GAS-Luc2 reporter system. (A) Disruption of immune checkpoint binding, such as PD-1/PD-L1 recognition, by a blocking antibody activates CD8+ T cells, which, in turn, release IFN-γ. IFN-γ activates JAK-STAT signaling in cancer reporter cells, promoting GAS-induced transcription of the luciferase gene, producing an easily detectable bioluminescence signal. Created with BioRender.com. (B) Selected cell lines with high endogenous expression of PD-L1, CD155, or B7-H3 were transduced with lentiviral-GAS-Luc2 plasmids in the presence of 50 µg/mL protamine sulfate (Sigma) for 24 hours. The cells were then enriched by puromycin selection and single cells were isolated by automatic cell sorting (Sony SH800). Expanded single cell clones were evaluated by IFN-γ stimulation. The clones that yielded the highest luciferase signal upon IFN-γ stimulation were selected for future experiments.

Results

Immune checkpoint protein profiling of cancer cell lines, T cell lines, and primary T cells

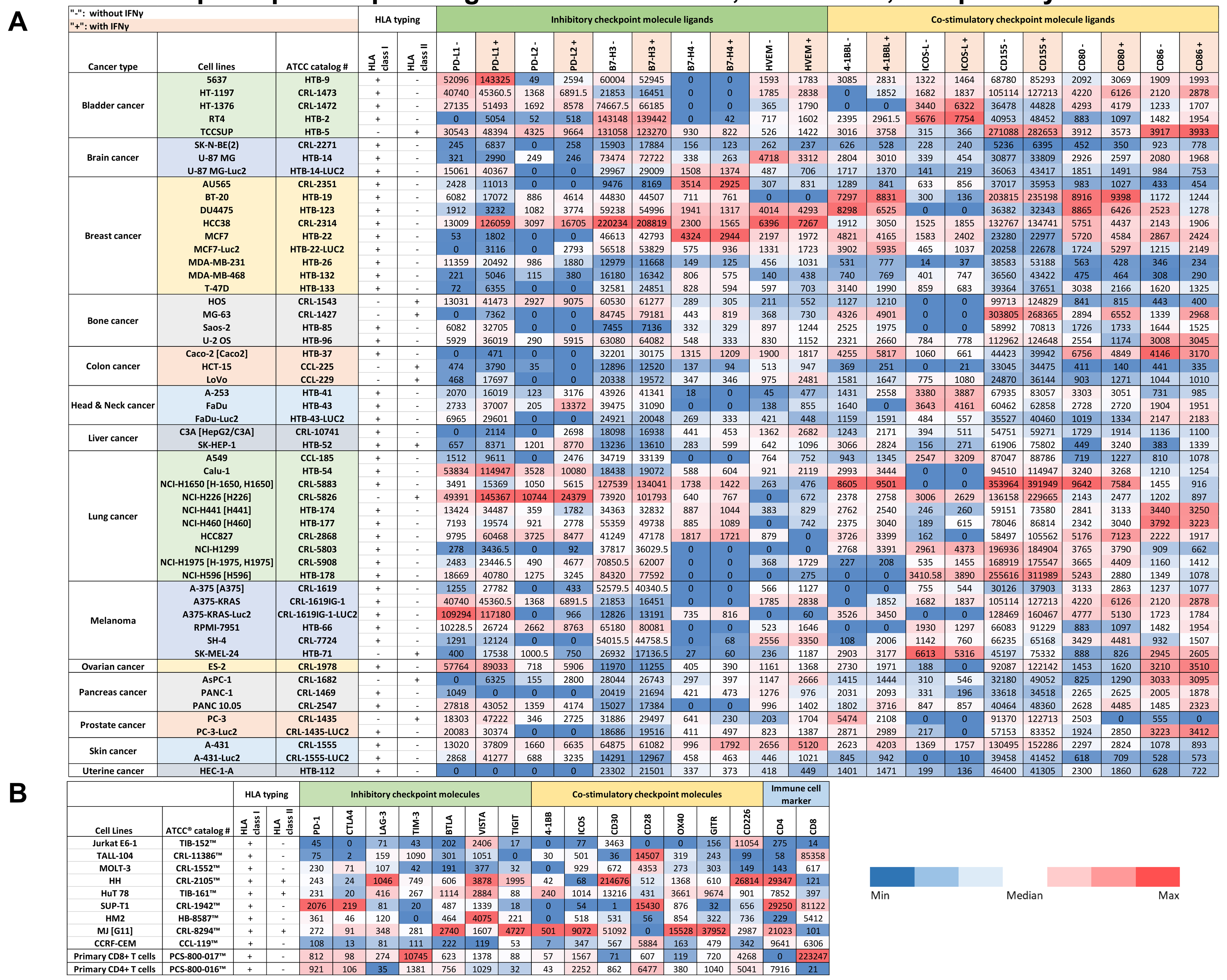

Figure 2: Heat maps based on protein profiling data of selected cancer cell lines, T cell lines, and primary T cells for immune checkpoint molecule expression by flow cytometry. (A) Immune checkpoint molecule ligand expression levels in cancer cell lines under basal (-) and 100 ng/mL IFN-γ stimulated (+) conditions were profiled. (B) Immune checkpoint molecule expression levels in T cell lines and primary T cells were profiled. HLA class was defined by either low expression (-) or high expression (+). Table values represent median fluorescence intensity sample values subtracted by isotype control MFI. Each column was color-coded separately to avoid cross comparison.

Evaluation of luciferase-expressing cell lines

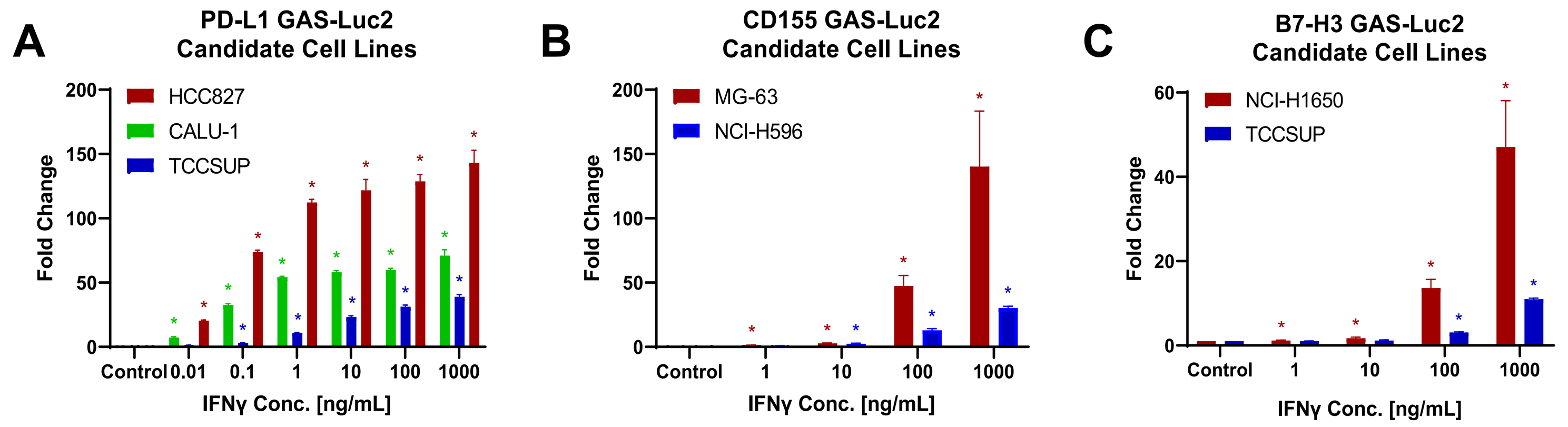

Figure 3: IFN-γ stimulation of GAS-Luc2 reporter candidate cell lines. Candidate cell lines were selected for GAS-Luc2 modification based on high expression of selected immune checkpoint markers and were assessed via IFN-γ cytokine stimulation assay following viral transduction. GAS-Luc2 modified cells that endogenously expressed a high level of (A) PD-L1, (B) CD155, or (C) B7-H3 checkpoint molecules were administered IFN-γ of different concentrations. Multi-clone pool cells demonstrating the highest luciferase expression, shown here as fold increase of relative luminescence units (RLUs) relative to untreated controls were selected for further study. N=3 in all experiments. *, P < 0.05.

Luciferase expression upon JAK-STAT signaling pathway activation

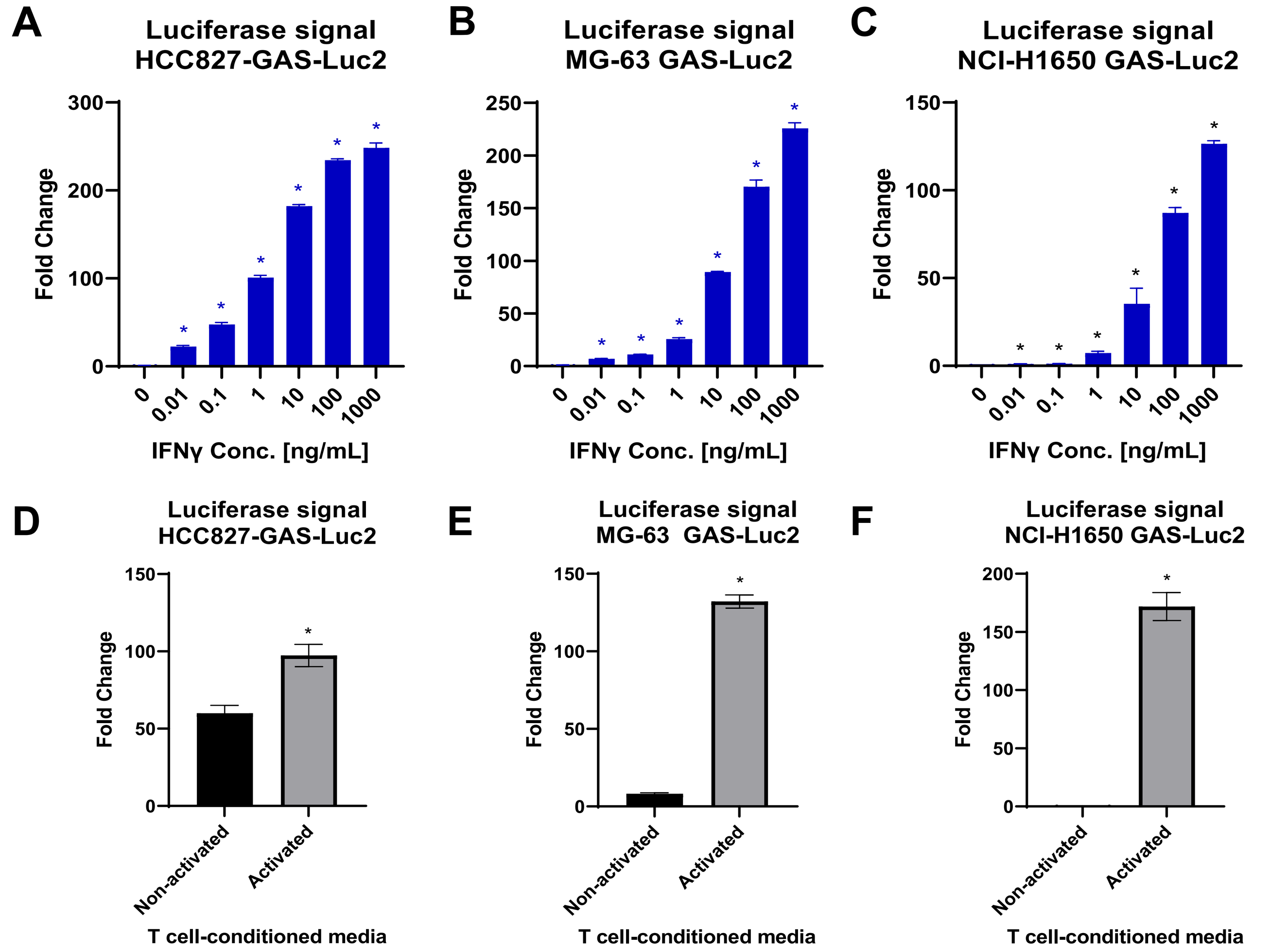
A
Luciferase signal HCC827-GAS-Luc2
B
Luciferase signal MG-63 GAS-Luc2
C
Luciferase signal NCI-H1650 GAS-Luc2
Fold Change
IFNγ Conc. [ng/mL]
D
Luciferase signal HCC827-GAS-Luc2
E
Luciferase signal MG-63 GAS-Luc2
F
Luciferase signal NCI-H1650 GAS-Luc2
Non-activated
Activated
T cell-conditioned media

Figure 4: Evaluation of monoclonal GAS-Luc2 cell lines as immune checkpoint reporters. (A-C) IFN-γ stimulation assays were conducted on the selected (A) monoclonal HCC827 GAS-Luc2 cell line with high endogenous PD-L1 expression, (B) monoclonal MG-63 GAS-Luc2 cell line with high endogenous CD155 expression, and (C) NCI-H1650 GAS-Luc2 cell line with high endogenous B7-H3 expression. The cells were treated overnight with IFN-γ at the concentrations ranging 0.01-1,000 ng/mL. (D-F) The same monoclonal immune checkpoint reporter cancer cell lines were administered with either non-activated or activated human primary CD8+ cytotoxic T-cell conditioned media and incubated overnight. The activated T cells were pre-treated with anti-CD2/CD3/CD28 beads. N=3 in all experiments. *, P < 0.05.

Co-culture of HCC827 GAS-Luc2 cell line with CD8+ cytotoxic T cells

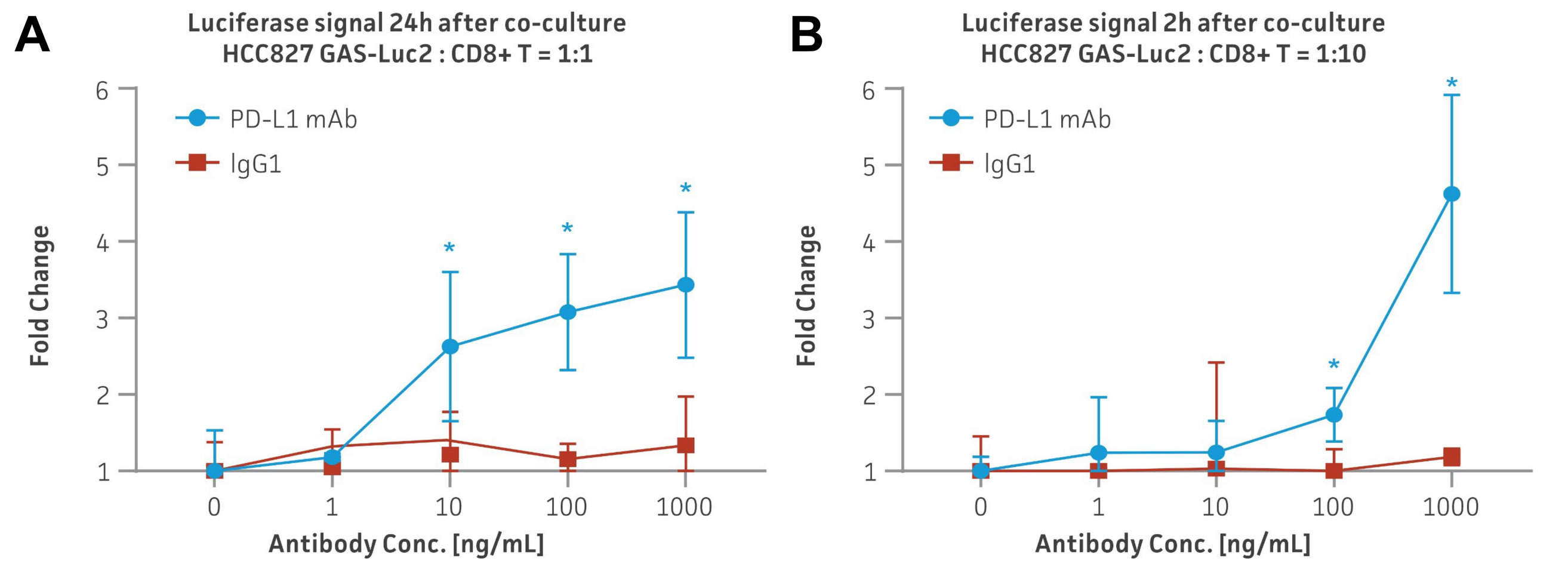

Figure 5: Application of the HCC827 GAS-Luc2 cell line in a co-culture assay using CD8+ cytotoxic T cells. HCC827 GAS-Luc2 cells were co-cultured with a (A) 1:1 or (B) 1:10 ratio of human primary CD8+ cytotoxic T cells for 24 and 2 hours, respectively. Different concentrations of PD-L1 monoclonal antibodies were added to block the PD-L1 checkpoint protein binding. N=3 in all experiments. *, P < 0.05.

Conclusion

- Despite the recent success of immune checkpoint inhibitors as cancer treatments, the built-in complexity of the immunological models and the variable drug responses among different cancer types are currently the most conspicuous challenges in this area of immuno-oncology.
- We conducted an extensive protein profiling of cancer cell lines, T cell lines, and primary T cells for numerous immune checkpoint molecules and their ligands to identify the cell lines with high endogenous expressions of these molecules.
- Subsequently, we developed robust, responsive, and reproducible reporter cell lines for the assessment of T cell and other immune/ tumor microenvironmental cell-mediated immune responses triggered by PD-L1, CD155, or B7-H3 checkpoint inhibitors.
- While maintaining physiological relevance and stable expression of the checkpoint ligand owing to the endogenous expression, these reporter cell lines provide high signal sensitivity and reproducibility, effectively eliminating the donor variability issue commonly experienced by using primary cell models.

References

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