A PD-L1 reporter cell line based on the immune checkpoint protein profiling of ATCC cell lines facilitates cancer immunotherapy drug screening

Hyeyoun Chang, Brian Della Fera, Kevin Tyo, John Foulke, Luping Chen, Zhizhan Gu*, Fang Tian*

Immuno-Oncology Group, Cell Biology R&D, ATCC, Gaithersburg MD 20877

ATCC® Credible leads to Incredible™

Poster # 510

Abstract

The success of immune checkpoint inhibitors in the treatment of various 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 an immune checkpoint reporter cancer cell line with high expression of endogenous programmed death-ligand 1 (PD-L1), a highly validated target for immune checkpoint inhibitor therapeutics. The reporter system contains a gamma interferon activation site (GAS)-response element upstream of the luciferase gene, preventing luciferase expression when PD-L1 binds to programmed death-1 (PD-1) that suppresses T cell-mediated antitumor activity. In the presence of a PD-1/PD-L1 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 cell line increased approximately 250 folds in a dose-dependent manner in response to interferon gamma (IFN-γ) stimulation, which mimics the signaling from activated CD8+ cytotoxic T cells. The bioluminescence increased approximately 100 folds in response to CD8+ primary T cell-conditioned media stimulation, and up to 5 folds 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 PD-L1 expression were well maintained after the cell line 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 PD-1/PD-L1 checkpoint inhibitors. This PD-L1 immune checkpoint reporter cancer cell line yields exceptional in vitro, and ex vivo assay sensitivity and reproducibility, while simplifies the complex immunological model by providing physiologically relevant expression of PD-L1, in comparison to similar assays on the market with an artificial PD-L1 overexpression system.

Background

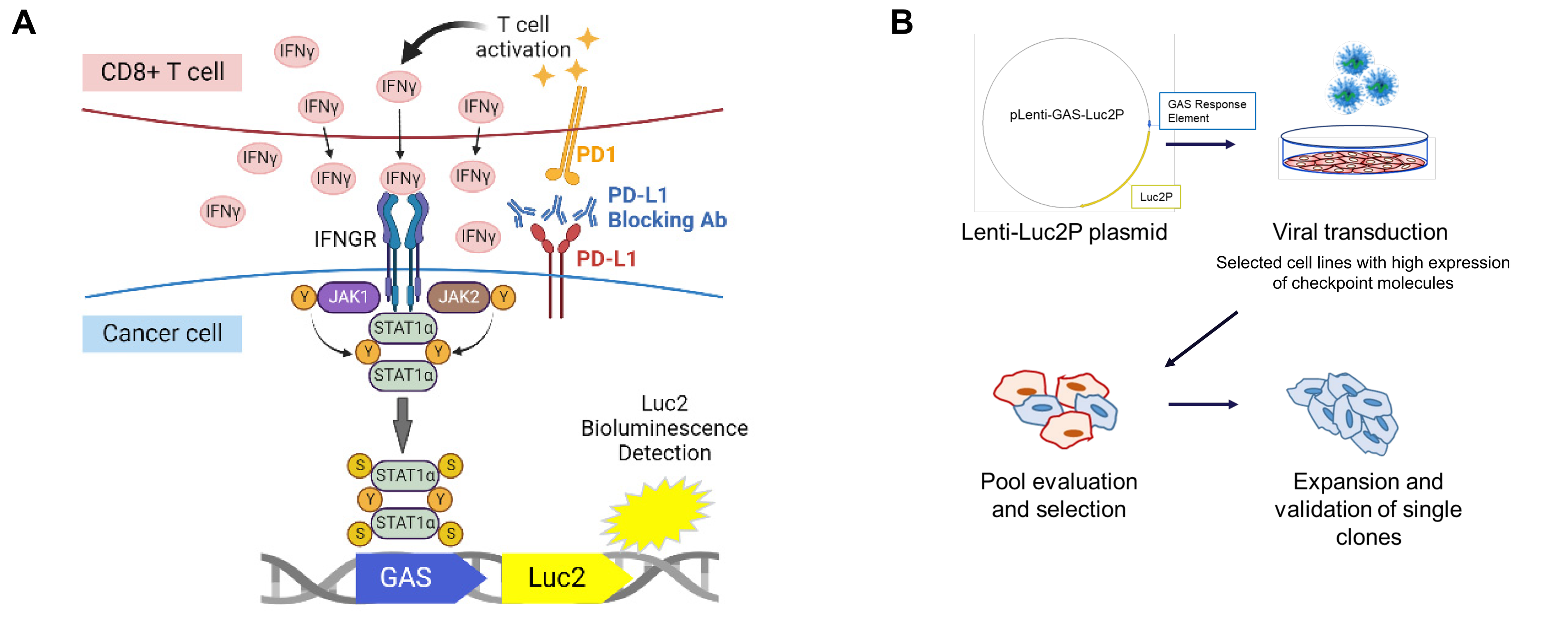
A
CD8+ T cell
T cell activation
IFNγ
PD1
PD-L1 Blocking Ab
PD-L1
IFNGR
JAK1
JAK2
Cancer cell
STAT1α
Luc2 Bioluminescence Detection
GAS
Luc2
B
pLenti-GAS-Luc2P
GAS Response Element
Lenti-Luc2P plasmid
Viral transduction
Selected cell lines with high expression of checkpoint molecules
Pool evaluation and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

Figure 1. Schematics of PD-L1-expressing GAS-Luc2 reporter system. (A) Disruption of PD-1/PD-L1 recognition by PD-L1 blocking antibody activates CD8+ T cells, which, in turn, release IFN-γ. IFN-γ binding activates JAK-STAT signaling in cancer cells, promoting GAS-induced transcription of Luciferase gene, producing easily detectable bioluminescence signal. Created with BioRender.com. (B) Selected cell lines with high endogenous expression of PD-L1 were transfected with lentiviral-GAS-Luc2 plasmids using TransfeX™ transfection reagent (ATCC). 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 clone that yielded the highest luciferase signal upon IFN-γ stimulation was selected for future experiments.

Results

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

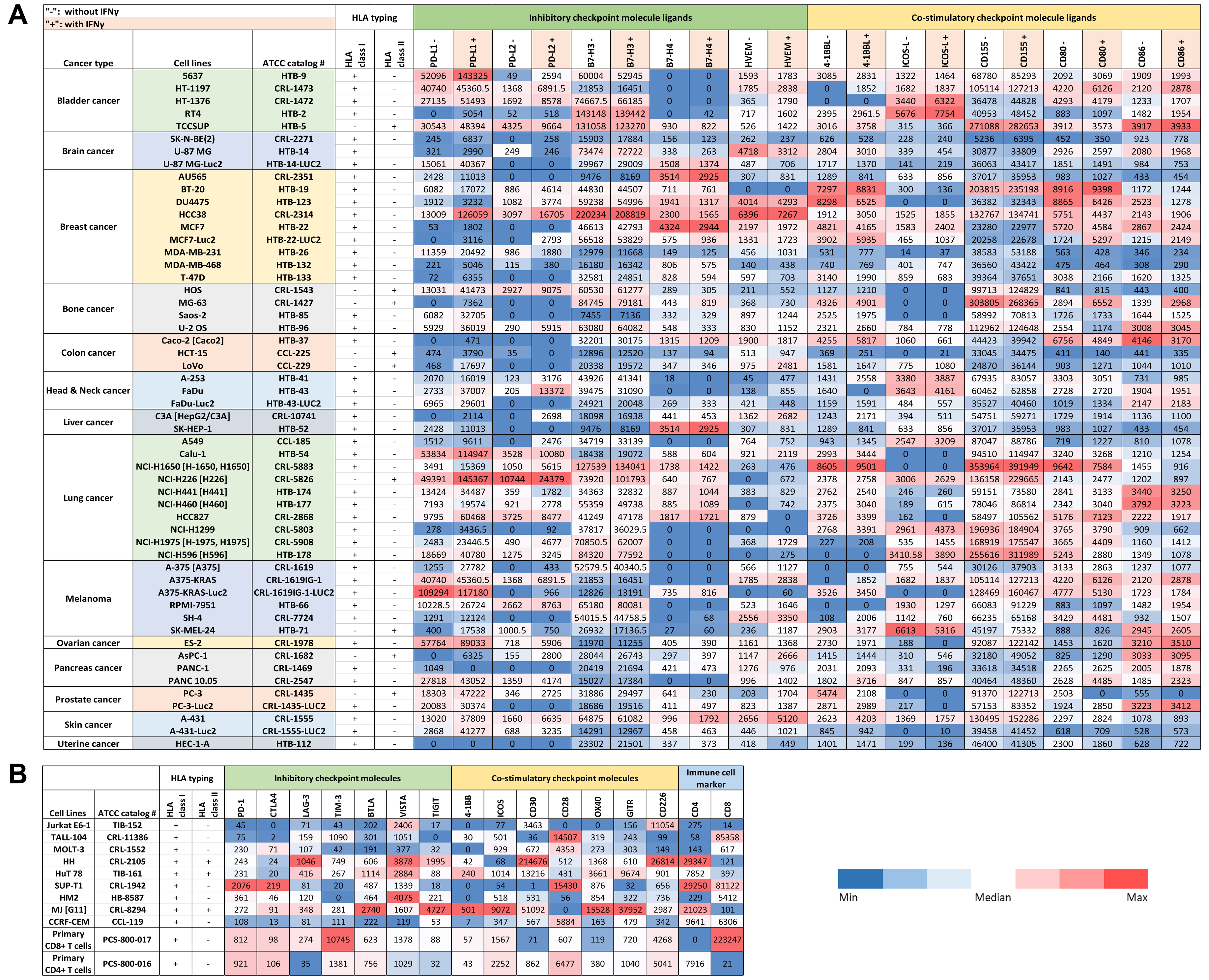

Figure 2. Heat maps based on protein profiling data of selected cancer cell lines, T cell lines, and primary T cells for immune checkpoint molecule expression by flow cytometry. (A) Immune checkpoint molecule ligand expression levels in cancer cell lines under basal (-) and 100 ng/ mL IFN-γ stimulated (+) 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

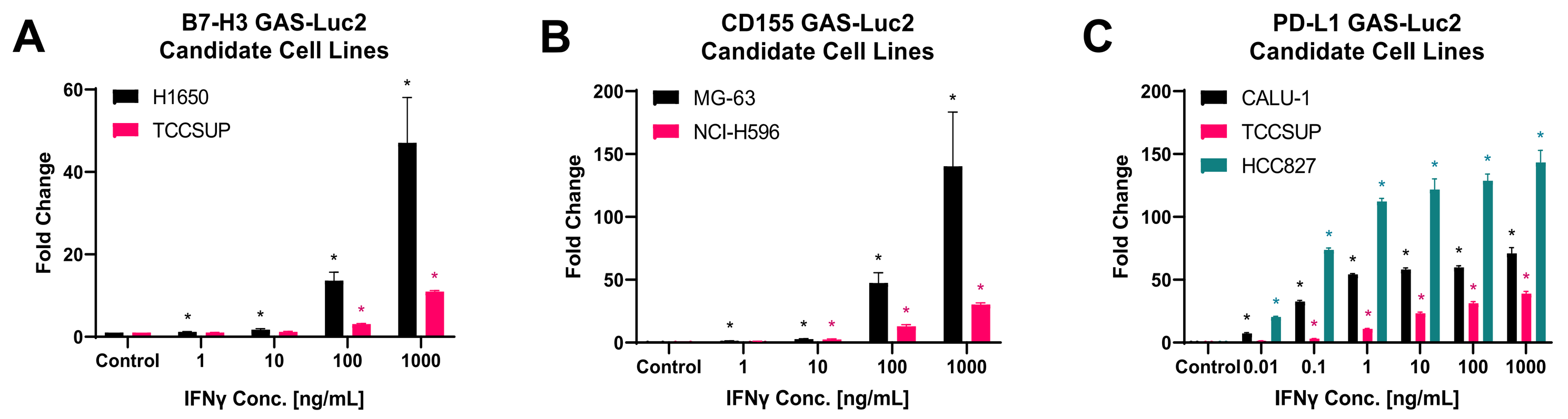

Figure 3. IFN-γ stimulation of GAS-Luc2 reporter candidate cell lines. Candidate cell lines were selected for GAS-Luc2 modification based on high expression of selected immune checkpoint markers and were assessed via IFN-γ cytokine stimulation assay following viral transduction. GAS-Luc2 modified cells that endogenously expressed high (A) B7-H3, (B) CD155, or (C) PD-L1 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

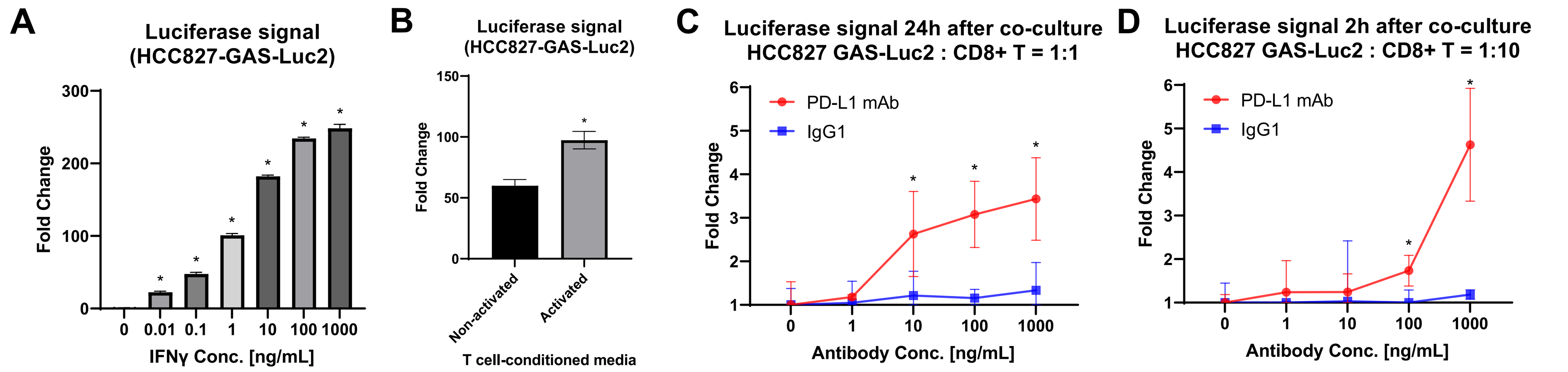
A
Luciferase signal (HCC827-GAS-Luc2)
IFNγ Conc. [ng/mL]
B
Luciferase signal (HCC827-GAS-Luc2)
Non-activated
Activated
T cell-conditioned media
C
Luciferase signal 24h after co-culture HCC827 GAS-Luc2 : CD8+ T = 1:1
D
Luciferase signal 2h after co-culture HCC827 GAS-Luc2 : CD8+ T = 1:10
PD-L1 mAb
IgG1
Antibody Conc. [ng/mL]
Fold Change

Figure 4. Evaluation of monoclonal HCC827 GAS-Luc2 cell line as a PD-L1 checkpoint reporter. (A) IFN-γ stimulation assays were conducted on the selected monoclonal HCC827 GAS-Luc2 cell line by using IFN-γ concentrations ranging 0.01-1,000 ng/ mL. (B) HCC827 GAS-Luc2 cells were administered with either non-activated or CD3/CD28 beads activated primary human CD8+ T-cell conditioned media. (C-D) HCC827 GAS-Luc2 cells were co-cultured with 1:1 (C) and 1:10 (D) ratio of CD8+ T cells for 24 and 2 hours, respectively. Different concentrations of PD-L1 monoclonal antibodies were added to block the PD-L1 checkpoint ligand. N=3 in all experiments. *, P < 0.05.

Comparison of HCC827 GAS-Luc2 cell line and HCC827 parental cell line

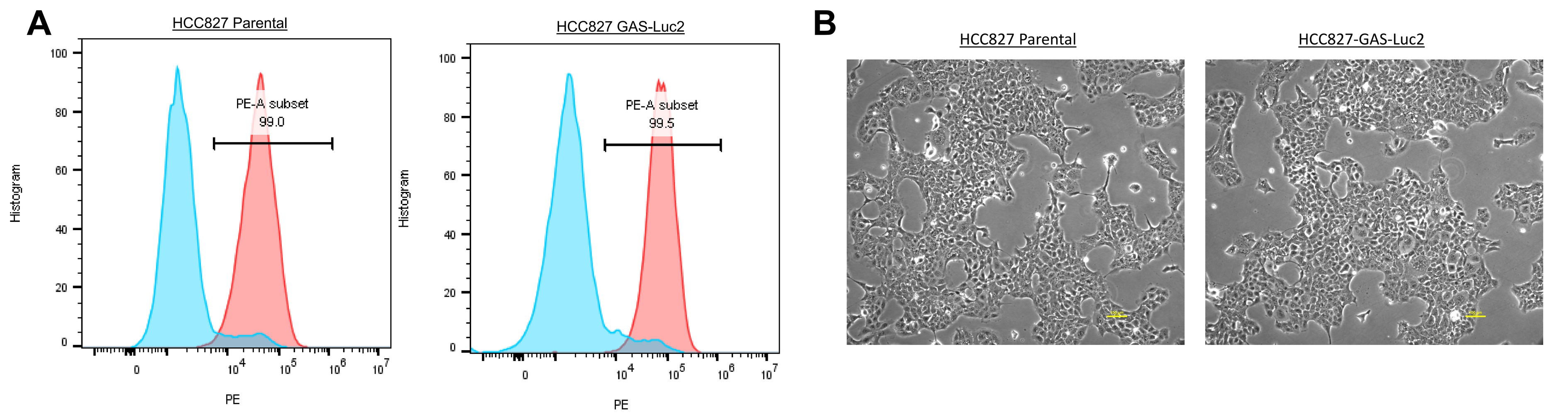

Figure 5. Comparison between HCC827 GAS-Luc2 and HCC827 parental cell lines in PD-L1 biomarker expression and cell morphology. (A) Flow cytometry analysis was performed to confirm the high expression levels of PD-L1 (pink) on both GAS-Luc2 and parental cell lines compared to isotype controls (blue). The cells were tested at >30 PDL (population doubling level). (B) The cells were observed via Nikon Eclipse TE300 inverted microscope for morphological comparison. Size bar represents 100 µm.

Conclusion

- The expansive immune checkpoint protein profiling of cancer cell lines, T cell lines, and primary T cells provides a unique and effective approach in checkpoint assay development for studying checkpoint molecule interactions and screening biologics as cancer immunotherapy treatments.
- Based on the protein profiling data, HCC827 GAS-Luc2 reporter cell line was developed for the convenient detection of PD-L1 blockade-induced signaling that results in T cell activation and transcription of the luciferase reporter gene.
- The stable and robust luciferase signal produced by the reporter cell line upon stimulation enables reliable measurement of the potency and stability of immune checkpoint inhibitors.
- Endogenously expressed high level of PD-L1 in HCC827 GAS-Luc2 reporter cell line delivers physiological relevance to the checkpoint assay.

References

- Kubli SP, et al (2021) Beyond immune checkpoint blockade: emerging immunological strategies. *Nat Rev Drug Discov* 20, 899-919
- Versteven M, et al. (2018) A versatile T cell-based assay to assess therapeutic antigen-specific PD-1-targeted approaches. *Oncotarget* 9, 27797-27808
- Dall'Olio FG, et al. (2022) Tumour burden and efficacy of immune-checkpoint inhibitors. *Nat Rev Clin Oncol* 19, 75-90

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** SalesRep@atcc.org **Web:** www.atcc.org