Engineered signaling reporter immune cell lines for monitoring immune activation and multidirectional interactions among cancer cells, immune cells, and tumor microenvironmental cells

Abstract #5225

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Abstract

While immunotherapies targeting T cells have achieved remarkable success, a significant subset of patients remain unresponsive or experience relapse, often attributed to the immunosuppressive nature of the tumor microenvironment. Emerging evidence suggests that other immune cell types, such as B cells and myeloid cells, play crucial roles in modulating the efficacy of cancer immunotherapies. However, there remains a clear lack of widely accessible immunological models capable of accurately representing the intricate multidirectional interactions among cancer cells, adaptive and innate immune cells, and other tumor microenvironmental cells. To address this gap, we engineered signaling reporter cell lines of T cell, B cell, or myeloid origin to express luciferase reporter protein in response to the activation of nuclear factor of activated T cells (NFAT) or nuclear factor kappa B (NF-κB) signaling pathway. The cell lines also endogenously express high level of T cell checkpoints (PD-1, TIGIT, and/or GITR) or myeloid checkpoints (SIRPα, Siglec-10, LILRB1, and/or B7-1) for additional application in immune checkpoint research. To validate luciferase expression, NFAT luciferase reporter T cell lines were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, while NF-κB luciferase reporter myeloid cell lines were activated with tumor necrosis factor alpha (TNF-α) or conditioned media from T cells. A B cell-derived NF-κB reporter cell line (BDCM-NF-κB-Luc2) with high basal luciferase activity was treated with an NF-κB signaling inhibitor. These reporter cells were subsequently evaluated in various co-culture conditions with primary immune cells, cancer cells, and/or other tumor microenvironmental cells to study immune cell crosstalk. Luciferase assays were performed for convenient quantification of luminescence intensity under all conditions. The results demonstrated that stimulations leading to NFAT or NF-κB signaling activation yielded dose-dependent increase in luciferase expression. In contrast, inhibition of the signaling led to dose-dependent decrease in luciferase expression. Furthermore, co-culture of reporter cell lines with various other immune and cancer cells significantly enhanced luminescence signals, with increases of up to 200-fold. In summary, these newly developed luciferase reporter immune cell lines provide a robust ex vivo model for evaluating cancer immunotherapies. These cell lines enable sensitive and reproducible monitoring of complex interactions among cancer cells, innate and adaptive immune cells, and other tumor microenvironmental cells, offering a valuable standard for the assessment of combinatorial immune responses in the tumor microenvironment.

Background

Profiling of immune checkpoint expression in T cell lines and myeloid cell lines

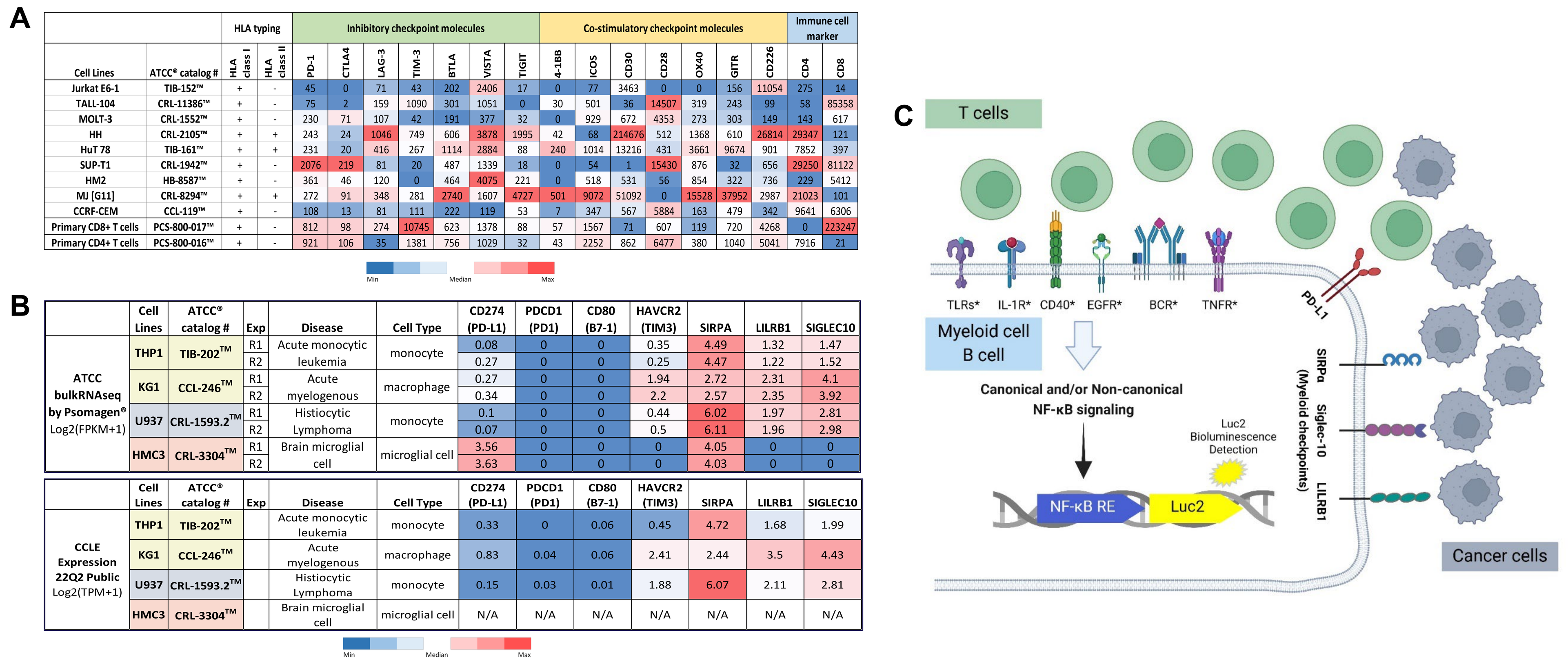

Figure 1: Protein profiling data of selected T cell lines and RNA-seq profiling data of selected myeloid cell lines for immune checkpoint molecule expression. (A) Immune checkpoint expression levels in T cell lines were profiled by flow cytometry. Table values represent median fluorescence intensity (MFI) of sample subtracted by isotype control MFI. Each column was color-coded separately to avoid cross comparison. (B) Immune checkpoint gene expression levels in myeloid cell lines were profiled by RNA sequencing (Psomagen) and the results were compared with Cancer Cell Line Encyclopedia (CCLE, Broad Institute) RNA-seq expression data. (C) Mechanism of action of the NFκB-Luc2 myeloid cell lines for myeloid checkpoint studies. Created with BioRender.com.

Results

NF-κB signaling pathway activation promotes luciferase expression in myeloid reporter cells

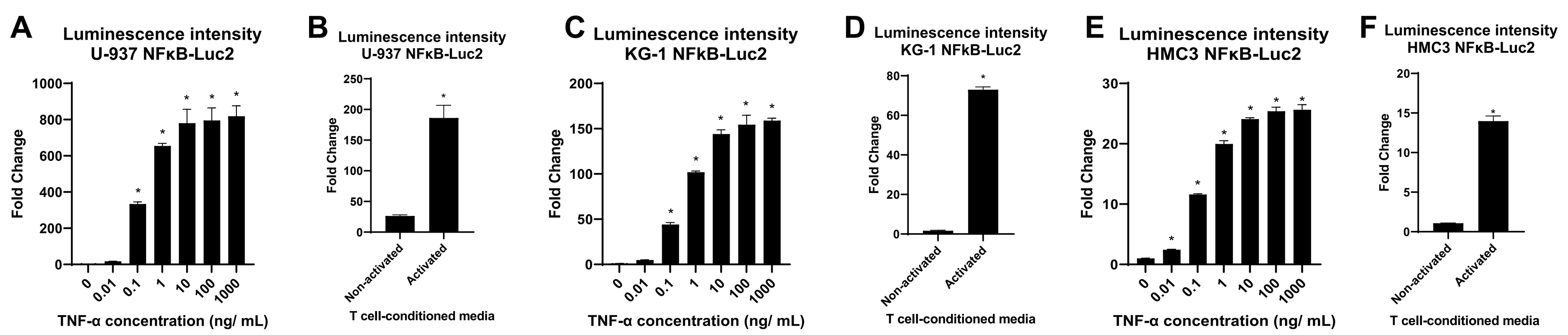

Figure 2: Activation of NF-κB signaling induces luciferase expression in myeloid reporter cell lines. (A-B) U937 NFκB-Luc2 cell line with high endogenous SIRPα expression was stimulated for 6 hours with (A) TNF-α or (B) T cell–conditioned media. (C-D) KG-1 NFκB-Luc2 cell line with high endogenous siglec-10 expression was stimulated for 6 hours with (C) TNF-α or (D) T cell–conditioned media. (E-F) HMC3 NFκB-Luc2 cell line with high endogenous PD-L1 and SIRPα expression was stimulated for 6 hours with (E) TNF-α or (F) T cell–conditioned media. For the conditioned media stimulation, the cells were administered with the conditioned media collected from non-activated or activated human primary CD4+ helper T cells. The activated conditioned media were harvested 3 days post-activation with anti-CD2/CD3/CD28 beads. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

NF-κB signaling pathway inhibition decreases luciferase expression in B reporter cells

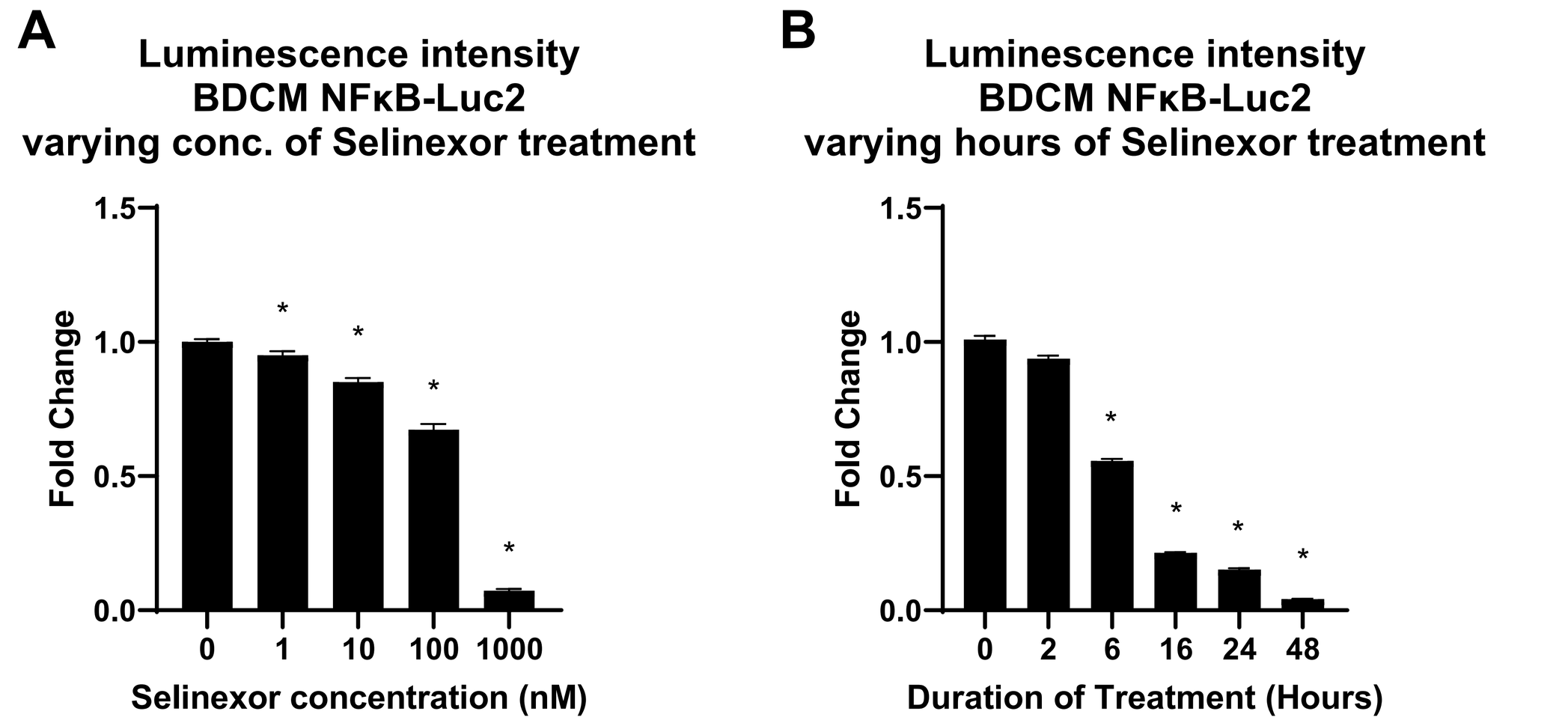

Figure 3: Inhibition of the NF-κB signaling pathway by Selinexor treatment results in a decrease in luciferase expression in the B reporter cell line. BDCM NFκB-Luc2 cells that endogenously express high levels of checkpoint molecules LILRB1 and B7-1 were incubated with (A) various concentrations of Selinexor for 24 hours or (B) 1 μM Selinexor for different lengths of time. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *P < 0.05.

NFAT signaling pathway activation yields luciferase expression in T reporter cells

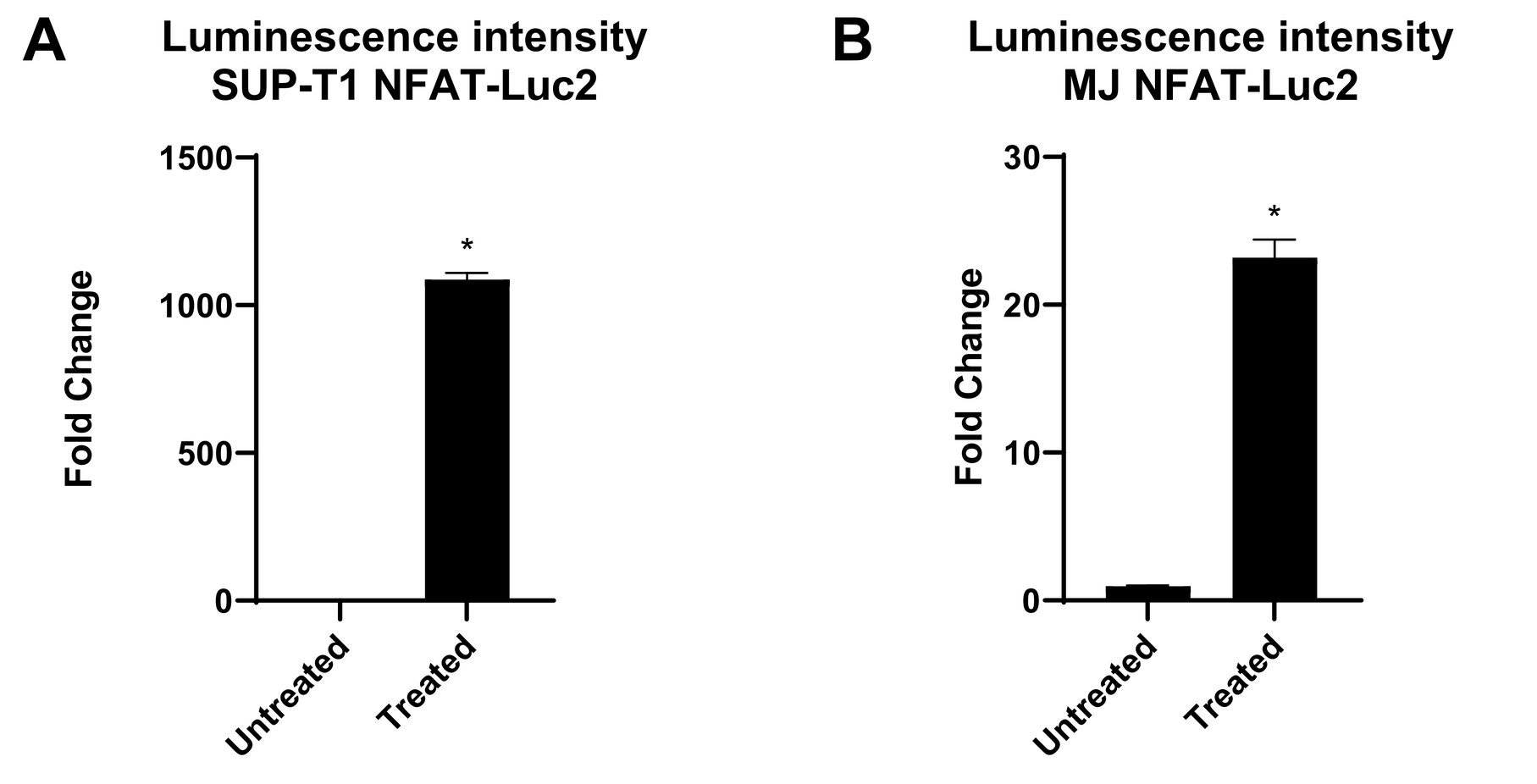

Figure 4: Activation of the NFAT signaling pathway leads to an increase in luciferase expression in T reporter cell lines. (A) The SUP-T1 NFAT-Luc2 cell line with high endogenous expression of PD-1 and (B) MJ NFAT-Luc2 cell line with high endogenous expression of TIGIT and GITR were stimulated for 6 hours with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 10 ug/mL ionomycin. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *, P < 0.05.

Key References

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Luciferase expression in myeloid reporter cells upon co-culture with primary T cells

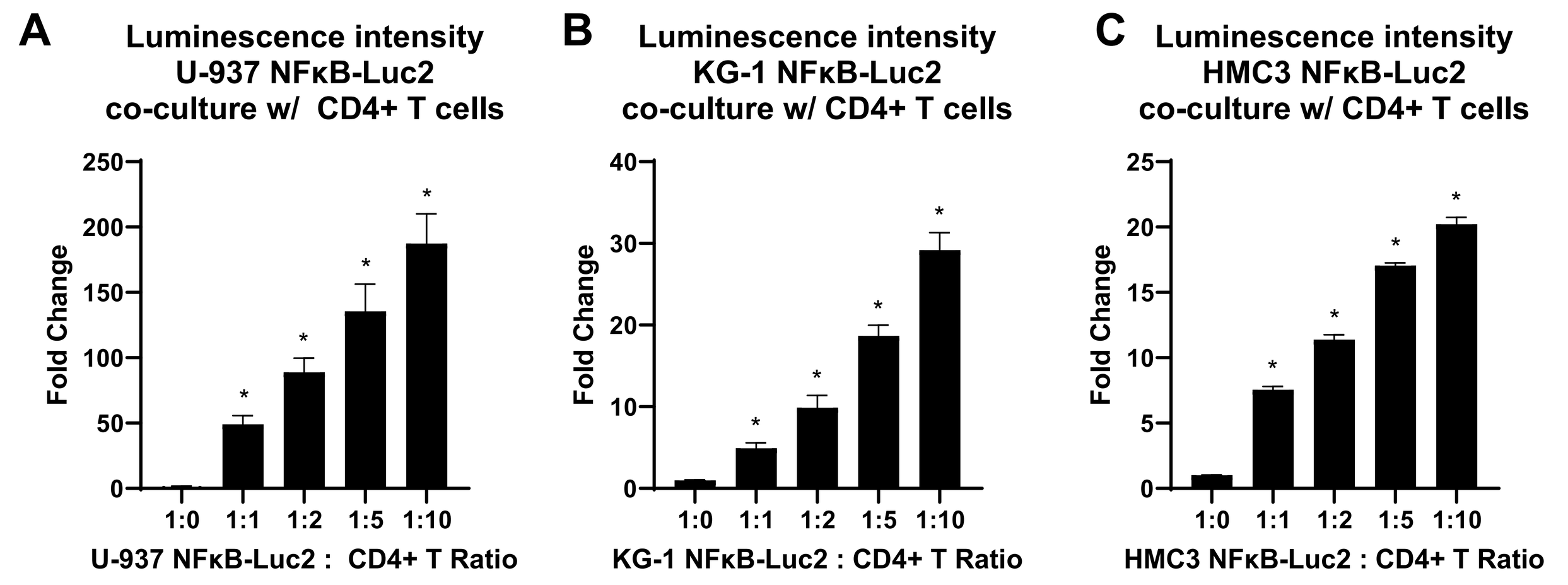
Luminescence intensity U-937 NFκB-Luc2 co-culture w/ CD4+ T cells
Luminescence intensity KG-1 NFκB-Luc2 co-culture w/ CD4+ T cells
Luminescence intensity HMC3 NFκB-Luc2 co-culture w/ CD4+ T cells
U-937 NFκB-Luc2 : CD4+ T Ratio
KG-1 NFκB-Luc2 : CD4+ T Ratio
HMC3 NFκB-Luc2 : CD4+ T Ratio
Fold Change

Figure 5: Activation or inhibition of NF-κB signaling upon co-culture with primary CD4+ T cells or cancer cells results in a change in luciferase expression in myeloid reporter cell lines. (A) U-937 NFκB-Luc2 cells, (B) KG-1 NFκB-Luc2 cells, and (C) HMC3 NFκB-Luc2 cells were co-cultured with human primary CD4+ helper T cells for 6 hours. The ratios of myeloid reporter cells to CD4+ T cells were 1:0, 1:1, 1:2, 1:5, and 1:10. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. *P < 0.05.

Luciferase expression in myeloid reporter cells in 3-D co-culture with diverse cancer cell lines

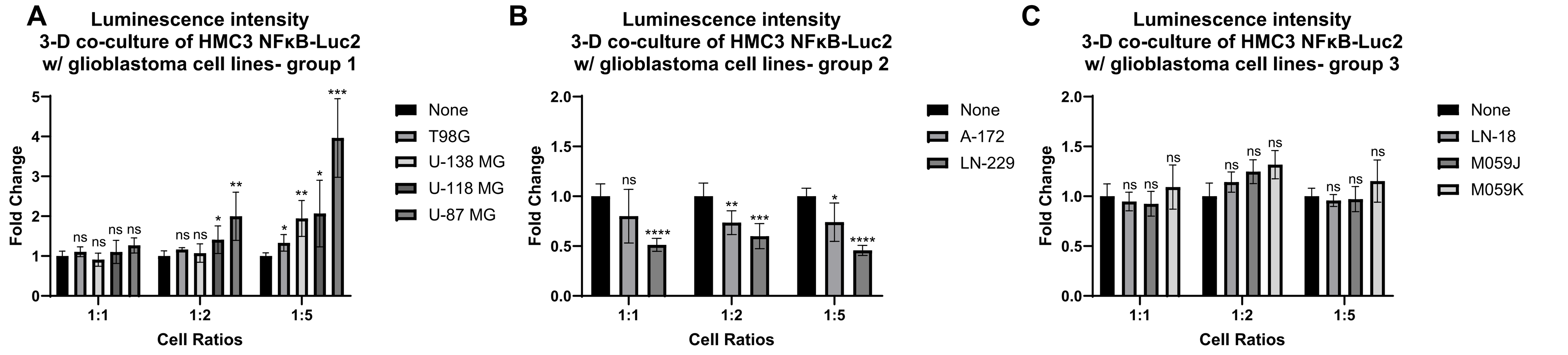
Luminescence intensity 3-D co-culture of HMC3 NFκB-Luc2 w/ glioblastoma cell lines- group 1
Luminescence intensity 3-D co-culture of HMC3 NFκB-Luc2 w/ glioblastoma cell lines- group 2
Luminescence intensity 3-D co-culture of HMC3 NFκB-Luc2 w/ glioblastoma cell lines- group 3
None
T98G
U-138 MG
U-118 MG
U-87 MG
A-172
LN-229
LN-18
M059J
M059K
Cell Ratios
Fold Change

Figure 6: 3-D co-culture of HMC3 NFκB-Luc2 reporter cell line with various glioblastoma cell lines for observing the effect of different cancer cells on the NF-κB signaling of the reporter cells. (A-C) HMC3 NFκB-Luc2 cells were co-cultured with nine individual glioblastoma cell lines in 3-D spheroids for 48 hours in varying cell ratios. (A) Co-culture with glioblastoma cell lines T98G, U-138 MG, U-118 MG, or U-87 MG induced an increase in luciferase expression in HMC3 NFκB-Luc2 cells. (B) Co-culture with glioblastoma cell lines A-172 or LN-229 induced a decrease in luciferase expression in HMC3 NFκB-Luc2 cells. (C) Co-culture with glioblastoma cell lines LN-18, M059J, or M059K did not induce any statistically significance change in luciferase expression in HMC3 NFκB-Luc2 cells. The ratios of the myeloid reporter cells to glioblastoma cells were 1:1, 1:2, and 1:5. Luciferase expression was quantified by Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). N=3 in all experiments. ****, P < 0.0001. ns, P ≥ 0.05.

Conclusion

- Utilizing RNA-seq profiling data for myeloid and B cell lines and protein profiling data for T cell lines for various immune checkpoint molecules, we developed three myeloid reporter cell lines, U-937 NFκB-Luc2 (ATCC® CRL-1593.2-NFkB-LUC2™), KG-1 NFκB-Luc2 (ATCC® CCL-246-NFkB-LUC2™), and HMC3 NFκB-Luc2 (ATCC® CRL-3304-NFkB-LUC2™), which have high endogenous expression of SIRPα, siglec-10, and PD-L1/SIRPα, respectively; one B reporter cell line, BDCM NFκB-Luc2 (ATCC® CRL-2740-NFkB-LUC2™), which has high endogenous expression of LILRB1 and B7-1; and two T reporter cell lines, SUP-T1 NFAT-Luc2 (ATCC® CRL-1942-NFAT-LUC2™) and MJ NFAT-Luc2 (ATCC® CRL-8294-NFAT-LUC2™), which have high endogenous expression of PD-1 and TIGIT/GITR, respectively.
- Engineered with NF-κB or NFAT response element upstream of luciferase gene, these immune reporter cell lines produce robust, sensitive, and reproducible luciferase expression upon NF-κB signaling activation in myeloid and B cells or NFAT signaling activation in T cells. They enable reliable quantification of the respective immune signaling activation, providing a useful tool for quantifying immune cell-mediated pro-inflammatory responses from these reporter cells.
- While preserving physiological relevance and stable expression of the immune checkpoint molecules owing to the endogenous expression, these immune reporter cell lines effectively eliminate the donor variability issue commonly associated with using primary immune cell models, offering an excellent alternative to the conventional models.
- The co-culture experiments engaging myeloid reporter cells with T cells and cancer cells reveal the potential of these novel immune reporter cell lines in studying more intricate multi-directional interactions among cancer cells, innate and adaptive immune cells, and other tumor microenvironmental cells, which is crucial in understanding the complex immune contexture in tumor microenvironment.

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