Luciferase reporter cell lines with endogenous T cell or myeloid checkpoint expression enable monitoring of multi-directional crosstalk between immune and cancer cells in tumor microenvironment

Credible leads to Incredible

Abstract 1094

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Abstract

Background: Despite the huge success of immunotherapies targeting T cells, a substantial proportion of patients experience resistance or relapse due to the immunosuppressive nature of tumor microenvironment. Increasing number of studies have indicated that other immune cells, such as B cells and myeloid cells, also play a critical role in determining the overall efficacy of cancer immunotherapeutic. However, there is a definite shortage of broadly accessible immunological models effectively representing the complex multi-directional interaction amongst cancer cells, adaptive immune cells, and innate immune cells.

Methods: To address this need, we selected six human cell lines of T cell, B cell, or myeloid cell origin with high endogenous expression of T cell checkpoint (PD-1, TIGIT, and/or GITR) or myeloid checkpoint (SIRPα, Siglec-10, LILRB1, and/or B7-1) and engineered them to express luciferase reporter protein in response to nuclear factor of activated T cells gene (NFAT) or nuclear factor kappa B (NF-κB) signaling activation, respectively. To assess the robustness of luciferase expression, the established NFAT luciferase cell lines were activated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, and NF-κB luciferase cell lines were stimulated with tumor necrosis factor alpha (TNF-α) or T cell-conditioned media. One of the NF-κB cell lines of B cell origin with high basal luciferase expression (BDCM-NFκB-Luc2) was treated with a NF-κB signaling inhibitor to observe the change in luciferase expression. The reporter cells were then applied in various co-culture conditions with primary immune cells and/or cancer cells to evaluate the immune crosstalk among different cell types. All treatments and co-cultures were followed by a luciferase assay for convenient quantification of luciferase expression.

Results: The data showed that PMA and ionomycin activation increased luminescence intensity by 20-1,000 fold from NFAT reporter cell lines. TNF-α stimulation also increased luminescence intensity by 20-800 fold in a dose-dependent manner from all NF-κB reporter cell lines but from BDCM-NFκB-Luc2. Incubation with T cell–conditioned media resulted in 10-170-fold increase in luminescence intensity from all NF-κB reporter cell lines except for BDCM-NFκB-Luc2. Treatment of BDCM-NFκB-Luc2 cells with a NF-κB signaling inhibitor yielded 20-fold decrease in luminescence intensity. Further co-culture of the reporter cell lines with various immune cells and/or cancer cells significantly increased luminescence signal intensity by up to 200 fold.

Conclusions: These novel luciferase reporter cell lines with endogenous T cell or myeloid checkpoint expression offer an excellent ex vivo model for studying the complex interactions among cancer cells, innate immune cells, and adaptive immune cells in the tumor microenvironment.

Background

Immune checkpoint molecule expression profiling of T cell lines and myeloid cell lines

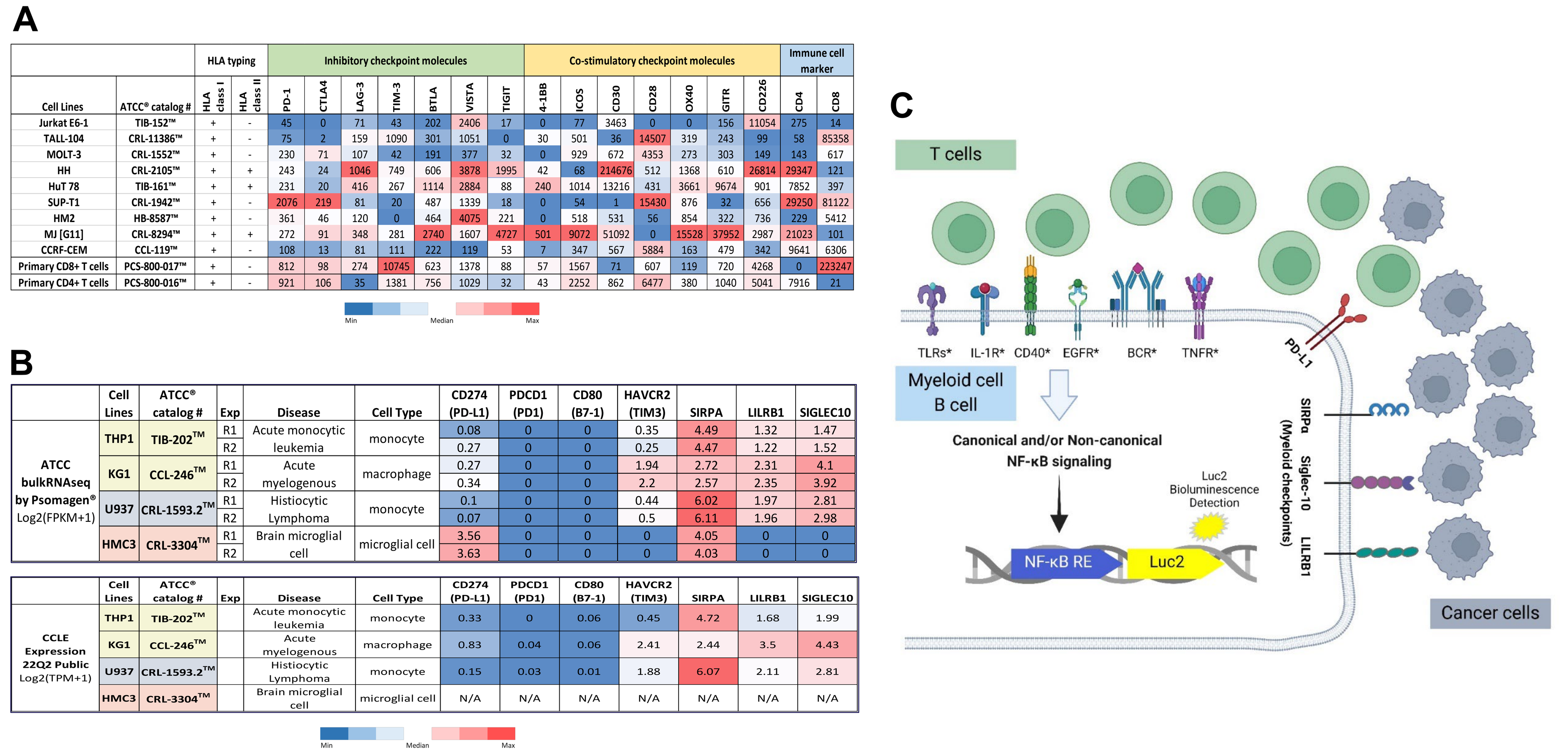

Figure 1: Heat maps based on protein profiling data of selected T cell lines and RNA-seq profiling data of selected myeloid cell lines for immune checkpoint molecule expression. (A) Immune checkpoint molecule expression levels in T cell lines were profiled by flow cytometry. Table values represent median 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 CCLE (Cancer Cell Line Encyclopedia, 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

Luciferase expression in myeloid reporter cells upon NF-κB signaling pathway activation

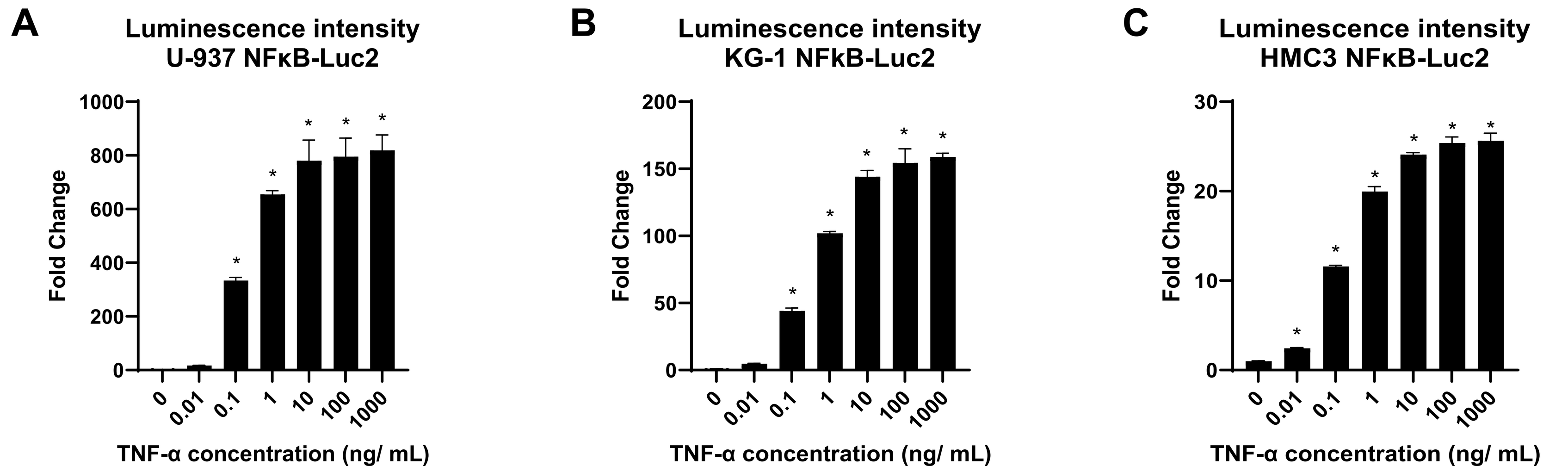

Figure 2: Activation of NF-κB signaling by TNF-α treatment induces luciferase expression in myeloid reporter cell lines. (A) U-937 NFκB-Luc2 cells with high endogenous SIRPα expression, (B) KG-1 NFκB-Luc2 cells with high endogenous siglec-10 expression, and (C) HMC3 NFκB-Luc2 cells with high endogenous PD-L1 and SIRPα expression were stimulated for 6 hours with varying concentrations (0.01-1,000 ng/ mL) of TNF-α. Luciferase expression was quantified by the 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 upon T cell-conditioned media stimulation

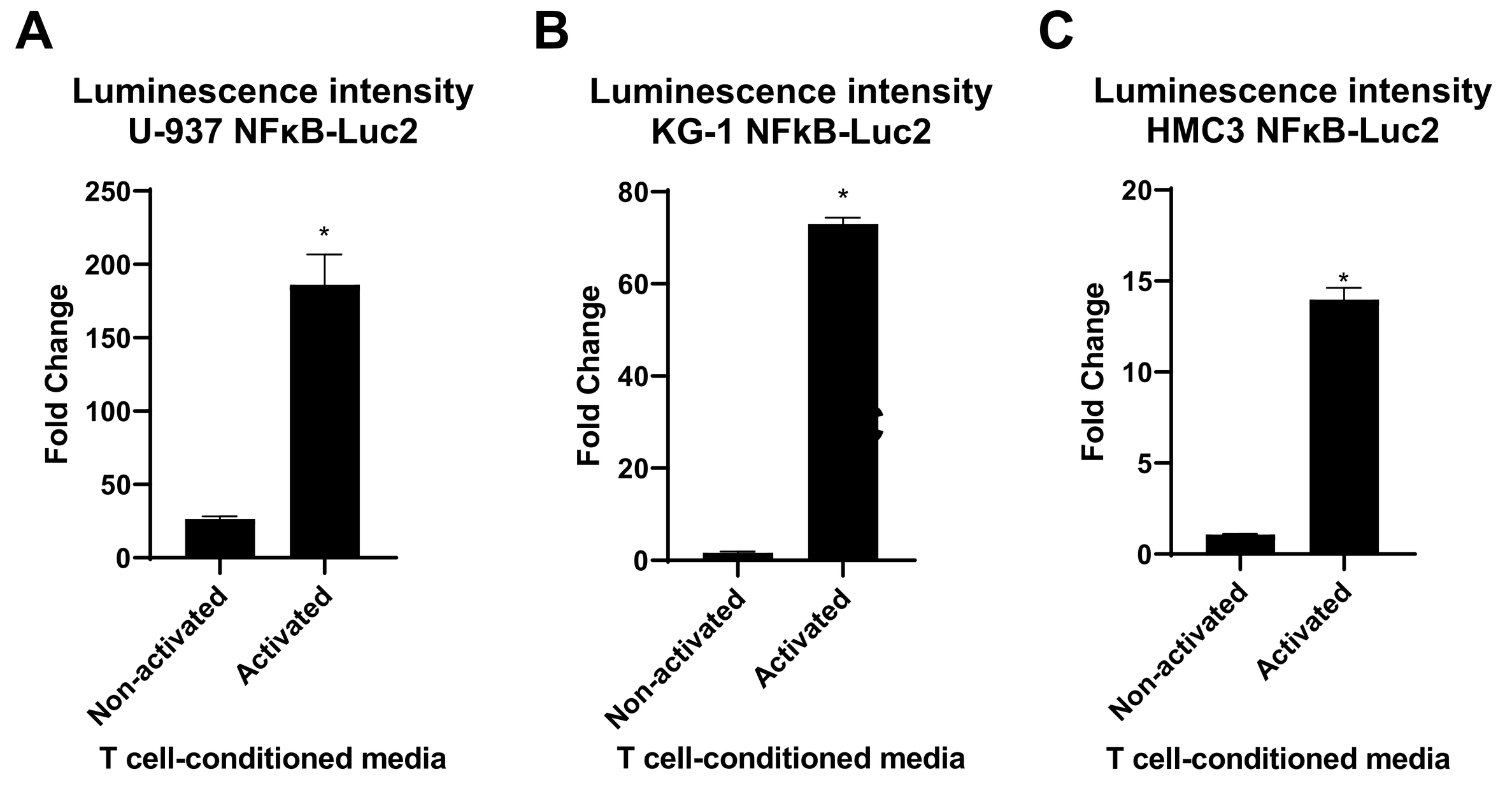

Figure 3: Activation of NF-κB signaling by T cell–conditioned media induces luciferase expression in myeloid reporter cell lines. (A) U-937 NFκB-Luc2 cells, (B) KG-1 NFκB-Luc2 cells, and (C) HMC3 NFκB-Luc2 cells were stimulated for 6 hours with non-activated or activated T cell–conditioned media. The 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 of the T cells 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.

Luciferase expression in B reporter cells upon NF-κB signaling pathway inhibition

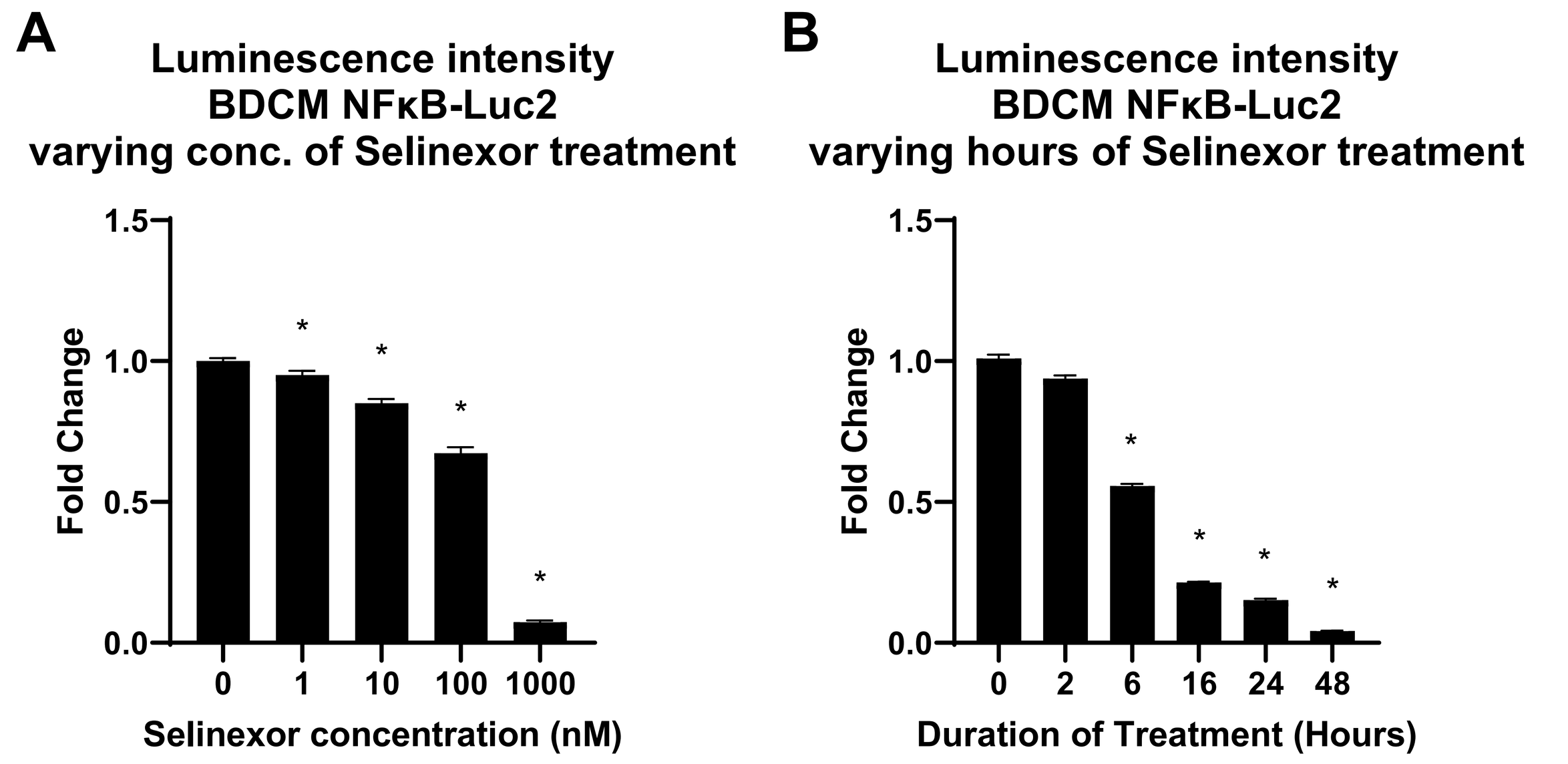

Figure 4: Inhibition of the NF-κB signaling pathway by Selinexor treatment results in a decrease in luciferase expression in the B reporter cell line. BDCM NFκB-Luc2 cells that endogenously express high levels of checkpoint molecules LILRB1 and B7-1 were incubated with (A) various concentrations of Selinexor 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.

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

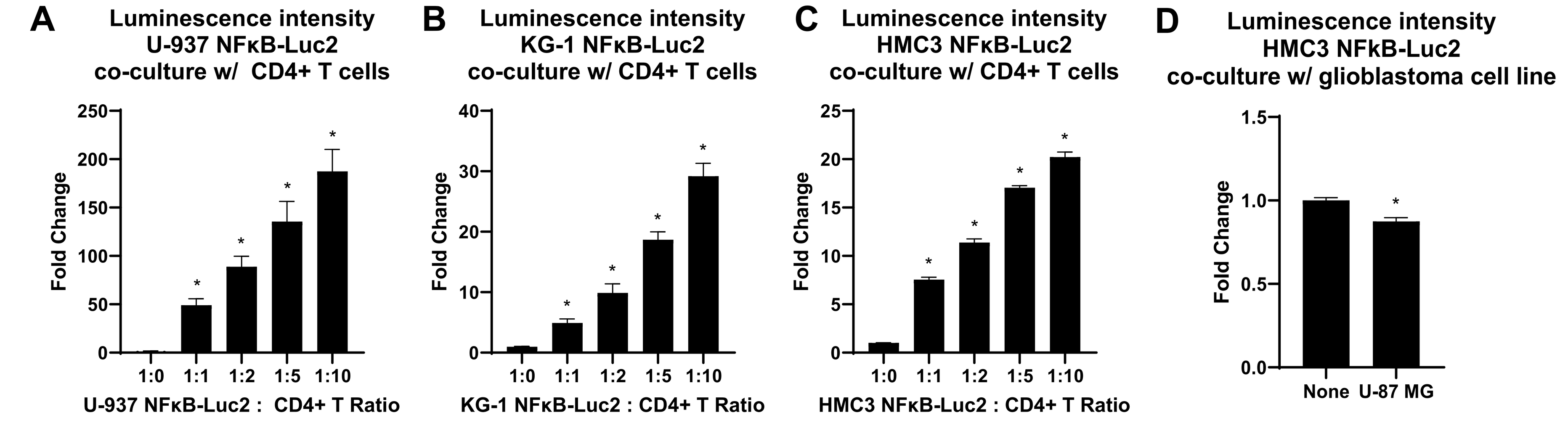
A
Luminescence intensity
U-937 NFκB-Luc2
co-culture w/ CD4+ T cells
B
Luminescence intensity
KG-1 NFκB-Luc2
co-culture w/ CD4+ T cells
C
Luminescence intensity
HMC3 NFκB-Luc2
co-culture w/ CD4+ T cells
D
Luminescence intensity
HMC3 NFκB-Luc2
co-culture w/ glioblastoma cell line
Fold Change
U-937 NFκB-Luc2 : CD4+ T Ratio
KG-1 NFκB-Luc2 : CD4+ T Ratio
HMC3 NFκB-Luc2 : CD4+ T Ratio
None
U-87 MG

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. (D) HMC3 NFκB-Luc2 cells were co-cultured with the human glioblastoma cell line, U-87 MG, for 6 hours at a 1:1 ratio of myeloid reporter cells to glioblastoma cells. 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 T reporter cell lines upon NFAT signaling pathway activation

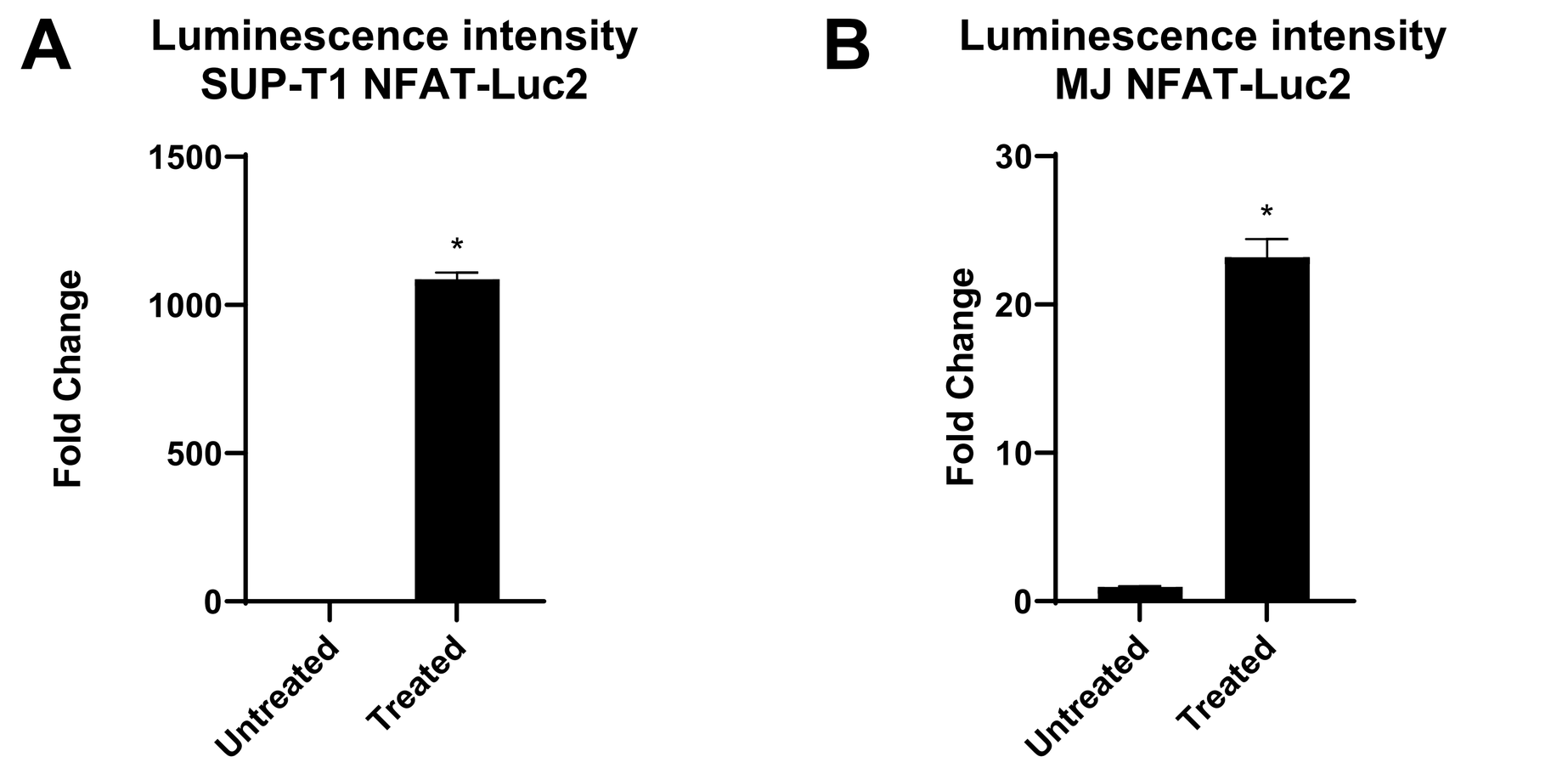

Figure 6: Activation of the NFAT signaling pathway leads to an increase in luciferase expression in T reporter cell lines. (A) The SUP-T1 NFAT-Luc2 cell line with high endogenous expression of PD-1 and (B) MJ NFAT-Luc2 cell line with high endogenous expression of TIGIT and GITR were stimulated for 6 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.

Conclusion

- Based on RNA-seq profiling data for myeloid and B cell lines and protein profiling data for T cell lines for numerous immune checkpoint molecules, we developed the 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; the B reporter cell line BDCM NFκB-Luc2 (ATCC® CRL-2740-NFkB-LUC2™), which has high endogenous expression of LILRB1 and B7-1; and the 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.
- 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, allowing reliable quantification of immune cell-mediated pro-inflammatory responses triggered by relevant immune checkpoint inhibition.
- While maintaining 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 experienced by using primary immune cell models, offering an excellent alternative to the conventional models.
- The co-culture experiments of the myeloid reporter cell lines with primary T cells or cancer cells reveal the potential of these novel immune reporter cell lines in studying more intricate multi-directional interactions among cancer cells, innate immune cells, and adaptive immune cells, which is crucial in understanding the complex immune contexture in tumor microenvironment.

Key References

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