A cell based bioluminescence assay reveals dose dependent and contextual repression of AP 1 driven gene expression by BACH2

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Whereas e f ector CD4$^+$ and CD8$^+$ T cells promote immune activation and can drive clearance of infections and cancer, CD4$^+$ regulatory T (T_{reg}) cells suppress their function, contributing to both immune homeostasis and cancer immunosuppression. The transcription factor BACH2 functions as a pervasive regulator of T cell di f erentiation, promoting development of CD4$^+$ T_{reg} cells and suppressing the e f ector functions of multiple e f ector T cell ($T_{e f}$) lineages. Here, we report the development of a stable cell based bioluminescence assay of the transcription factor activity of BACH2. Tetracycline inducible BACH2 expression resulted in suppression of phorbol 12 myristate 13 acetate (PMA)/ ionomycin driven activation of a luciferase reporter containing BACH2/AP 1 target sequences from the mouse *Ifng* +18k enhancer. BACH2 expression repressed the luciferase signal in a dose dependent manner but this activity was abolished at high levels of AP 1 signalling, suggesting contextual regulation of AP 1 driven gene expression by BACH2. Finally, using the reporter assay developed, we f nd that the histone deacetylase 3 (HDAC3) selective inhibitor, RGFP966, inhibits BACH2 mediated repression of signal driven luciferase expression. In addition to enabling mechanistic studies, this cell based reporter may enable identi f cation of small molecule agonists or antagonists of BACH2 function for drug development.

CD8$^+$ and CD4$^+$ conventional T (T_{conv}) cells drive immune activation and promote clearance of infections and cancer. However, their function can also provoke autoimmune and allergic in ammation. e immune system therefore employs a variety of suppressive mechanisms, known as immunoregulatory mechanisms, which act both intrinsically within T_{conv} cells and extrinsically to restrain excessive T cell activation. Immunoregulatory mechanisms also suppress bene cial anti-tumour T cell responses to drive deleterious immunosuppression in cancer. Important among extrinsic immunoregulatory mechanisms is the activity of CD4$^+$ regulatory T (T_{reg}) cells which limit T_{conv} cell function and promote immune homeostasis and tumour immunosuppression[1–6]. Immunoregulatory mechanisms are therefore important targets for the development of new therapies aimed at treating in ammatory diseases, disorders of excessive immunopathology and cancer.

Discussion

repression is itself regulated by the strength of activating signals that cells receive. is is consistent with a requirement for T cells expressing high levels of BACH2 to nevertheless be able to di erentiate into e ector cells in the presence of strong TCR and in ammatory signalling. Indeed, a number of regulatory pathways are known to a ect the post-translational stability, localisation and function of BACH2 and an opportunity to further interrogate their role in a reductionist system is provided by this assay. However, such investigations would need

Gibco), 0.25 µg/ml of amphotericin B (15290026, Gibco) and 10 µg/ml of blasticidin (R21001, Invitrogen) and maintained a er selection in half the concentration of the indicated selection antibiotics at 37 °C with 5% CO_2.

Sanger sequencing and data analysis. Inserts regions of the constructed vectors pNL2.2 *Ifng* + 18 k reporter and pcDNA.4/BACH2-inducible were con rmed using Sanger sequencing. Primers were designed for sequencing of pNL2.2 *Ifng* + 18k reporter vector as follows: Fw: '5–TCGATAGTACTAACATACGC–3' and Rv: '5–GTTGTAGCCGGCTGTCTGTCG–3'. A primer walk strategy was followed to verify the pcDNA.4/BACH2-inducible vector insert and involved designing ve di erent forward and reverse primer sequences as follows: Fw1: '5–CGCAAATGGGCGGTAGGCGTG–3'; Fw2: '5–ACGATGGATTCAGAGACGGC–3'; Fw3: '5–CTTAAGGTCTCTGTTCAGC–3'; Fw4: '5–AATCAAAGTCTGCCCTCG–3'; Fw5: '5–AATTTAGAATGTGAAATCCG–3'; and Rv1: '5–TAGAAGGCACATCGAGG–3'; Rv2: '5–TTTCTCACACACCAATTTGC–3'; Rv3: '5–GAATAGGAAGAGCAGGAGC–3'; Rv4: '5–TCCACACTTTTCGTTATGC–3'; Rv5: '5–TCATCCTCCTCCTCTCCTGC–3'. Sequencing data were analysed using FinchTv 1.4.0 so ware (Geospiza) and ChromasPro 2.1.9 so ware (Technelysium) for pNL2.2 *Ifng* + 18k reporter and pcDNA4/BACH2 inducible-vector respectively. Images of the con rmed insert sequences were merged a er data analysis with Adobe Photoshop CS6 so ware (Adobe Creative Suite 6 Master Collection).

Luciferase assay. Clonally derived cell lines were treated with or without tetracycline (T8032, Sigma-Aldrich) for 18 h. Subsequently, cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (P1585,