

## **Supplemental Material**

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

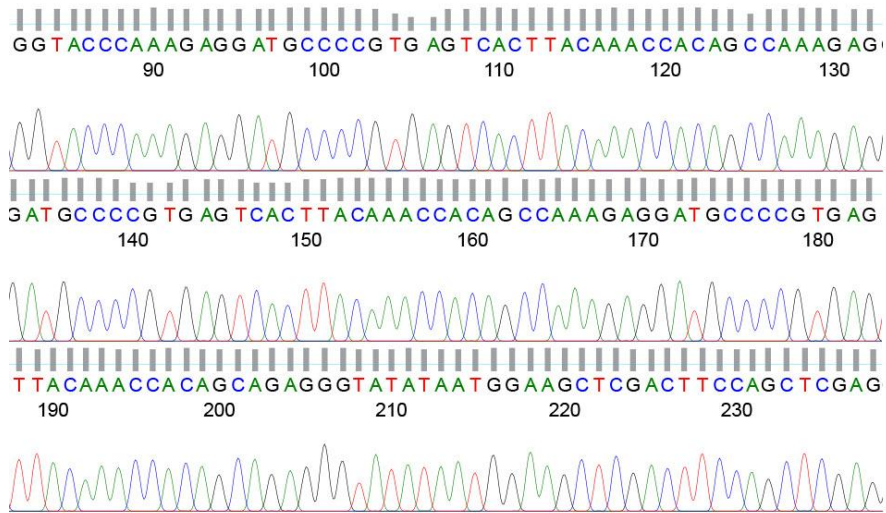
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<sup>1</sup> Department of Pathology, University of Cambridge, Tennis Court Road, CB2 1QP, UK.

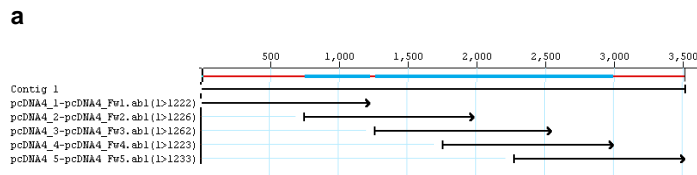
<sup>2</sup> Laboratory of Lymphocyte Signalling and Development, Babraham Institute, Cambridge, CB22 3AT, UK.

<sup>3</sup> CRUK Therapeutic Discovery Laboratories, Babraham Research Campus, CB22 3AT, UK.

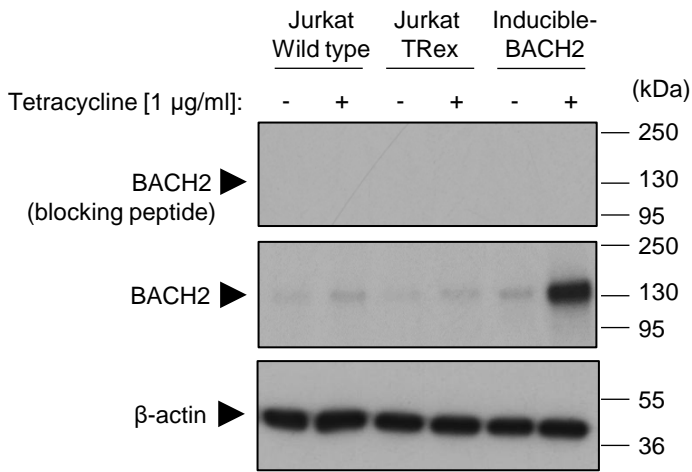
<sup>4</sup> Imaging Facility, Babraham Institute, Cambridge, CB22 3AT, UK.



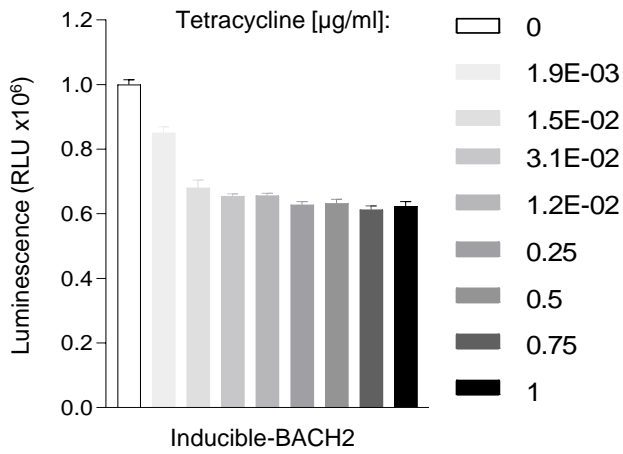
**Supplementary Figure 1. Sanger sequencing of insert region from pNL2.2 *Ifng* +18k reporter vector.** Chromatogram of the pNL2.2 *Ifng* +18k reporter vector insert containing a 3x concatenated sequence with a TPA-response element (TRE) embedded motif from the *Ifng* +18k enhancer region.



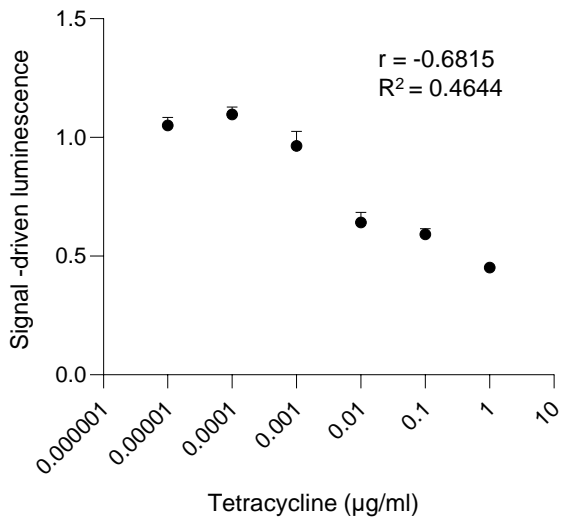
**Supplementary Figure 2. Sanger sequencing of insert region of pcDNA4-BACH2 inducible vector. a, and b, Sequence assembly of human *BACH2* cDNA subcloned into pcDNA4 vector data. a, Representation of primer walk strategy followed for insert sequencing. b, Chromatogram showing pcDNA4-BACH2 inducible vector insert sequence after data analysis.**



**Supplementary Figure 3. Specific induction of BACH2 protein expression upon tetracycline treatment of the BACH2 reporter line.** Western blot of total protein lysates with or without tetracycline pre-treatment. The specificity of the BACH2 signal was confirmed by pre-incubating the primary anti-BACH2 antibody with an anti-BACH2 blocking peptide prior to primary antibody staining.

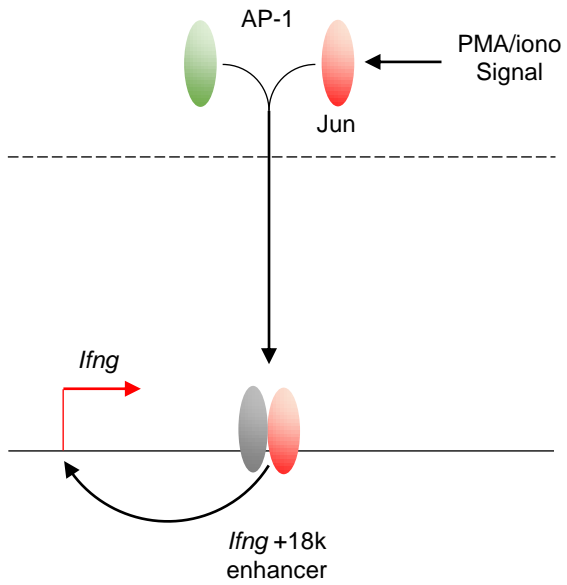


**Supplementary Figure 4. Signal-driven luciferase expression of inducible-BACH2 reporter cells pre-treated with titrated tetracycline doses.** Signal-driven luminescence of inducible-BACH2 reporter cells at given tetracycline concentrations following stimulation with PMA/ionomycin showing minimal luminescence at 1  $\mu\text{g/ml}$  tetracycline. Data show 4 culture replicates per condition. Bars and error represent mean (SD).

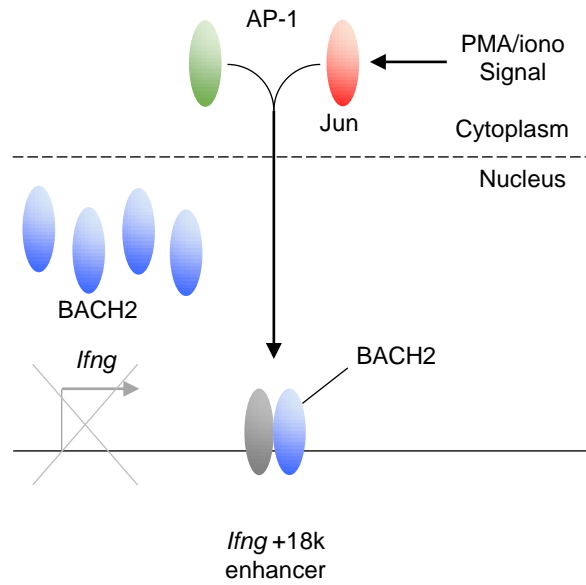


**Supplementary Figure 5. Association signal-driven luciferase expression and tetracycline dose in the BACH2 reporter cell line.** Signal-driven luminescence of inducible-BACH2 reporter cells at given tetracycline concentrations following stimulation with PMA/ionomycin. Data are representative of 2 independently repeated experiments with 3 culture replicates per condition. Bars and error represent mean (SD).

Low BACH2 expression (no tetracycline):



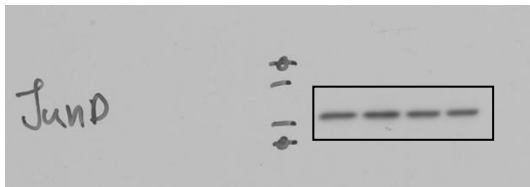
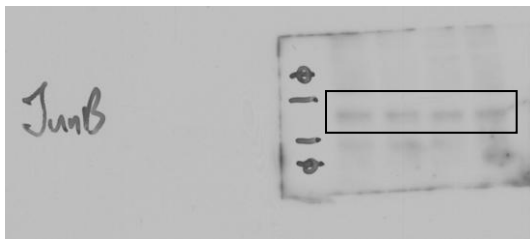
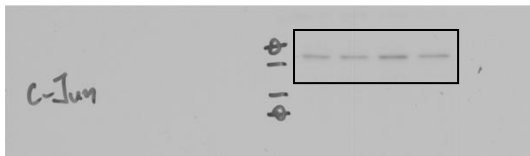
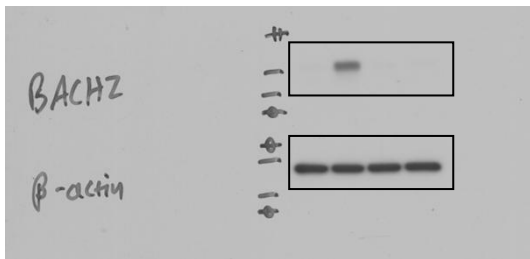
High BACH2 expression (+ tetracycline):



**Supplementary Figure 6. Schematic representation of the proposed function of BACH2 in the developed reporter assay.**

**Additional Figures:**

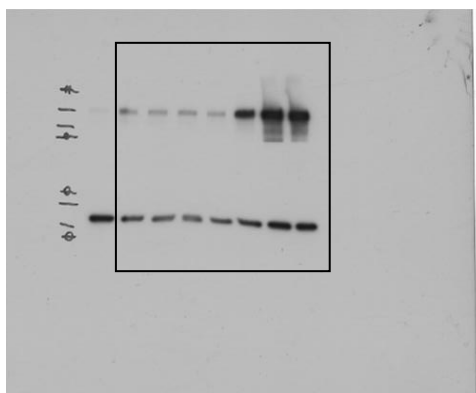
Full image from Fig. 2b:





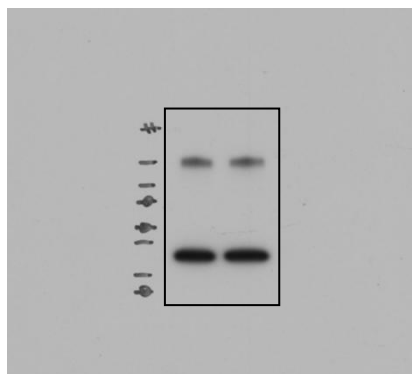
**Additional Figures:**

Full image from Fig. 3b:



**Additional Figures:**

Full image from Fig. 6c:



## Additional Figures:

Full image from Supplementary Figure 3:

