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RNAseq of primary human T cells overexpressing BATF3

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Andrea Daniel: This protocol was adapted from Sean McCutcheon's work in the Gersbach lab at Duke University.

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We use this protocol and it's working

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Abstract

This is a protocol describes methods for performing RNAseq using human CD8+ T cells overexpressing BATF3 or a GFP control.

Transfections for high-titer lentiviral production

- 1 Plate 1.2×10^6 or 7×10^6 HEK293T cells in a 6 well plate or 10 cm dish in the afternoon with 2 mL or 12 mL of complete opti-MEM (Opti-MEMTM I Reduced Serum Medium supplemented with 1x Glutamax, 5% FBS, 1 mM Sodium Pyruvate, and 1x MEM Non-Essential Amino Acids).
- 2 The next morning, transfect HEK293T cells with 0.5 µg pMD2.G, 1.5 µg psPAX2, and 0.5 µg transgene for 6 well plates or 3.25 µg pMD2.G, 9.75 µg psPAX2, and 4.3 µg transgene for 10 cm dishes using Lipofectamine 3000.
- 3 Exchanged media 6 hours after transfection and collect and pool lentiviral supernatant at 24 hours and 48 hours after transfection.

Primary human CD8⁺ T cell cultures

- 4 Isolated CD8⁺ T cells from individual donors were obtained directly from vials purchased from StemCell Technologies.
- 5 Culture T cells were in PRIME-XV T cell Expansion XSFM (FujiFilm) supplemented with 5% human platelet lysate (Compass Biomed), 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. All media were supplemented with 100 U ml⁻¹ human IL-2 (Peprotech).

Transduction of primary human CD8⁺ T cells

- 6 Centrifuged lentiviral supernatant at 600g for 10 min to remove cellular debris.
- 7 Concentrate lentivirus to 50–100× the initial concentration using Lenti-X Concentrator (Takara Bio).
- 8 Transduce T cells at 5–10% v/v of concentrated lentivirus at 24 h post-activation. For dual transduction experiments, T cells were serially transduced at 24 h and 48 h.

RNAseq

- 9 T cells overexpressing either BATF3 or GFP were cultured for 10 days post transduction.



- 10 RNA was isolated using Norgen's Total RNA Purification Plus Kit and submitted to Azenta (formerly Genewiz) for standard RNA-seq with polyA selection.
- 11 Reads were first trimmed using Trimmomatic⁷² v0.32 to remove adapters and then aligned to GRCh38 using STAR v2.4.1a aligner.
- 12 Gene counts were obtained with featureCounts⁷³ from the subread package (version 1.4.6-p4) using the comprehensive gene annotation in Gencode v22.
- 13 Differential expression analysis was determined with DESeq2 (ref. ⁶⁸) where gene counts are fitted into a negative binomial generalized linear model and a Wald test determines significant DEGs.
- 14 DESeq2 results of RNA-seq analyses with BATF3 OE and ZNF217 or GATA3 KO are presented in Supplementary Tables 4 and 7, respectively.
- 15 Upregulated and downregulated DEGs were input into EnrichR's GO Biological Processes 2021 database⁷¹ for functional annotation.