Expanded View Figures

Figure EV1. ITNKs derived from NuRD-subunit-deficient cells have similar characteristics to BCL11B-deleted ITNKs as compared with those of T cells.

A *k*-means clustering analysis of differentially expressed genes in RNA-seq transcriptomes revealed two distinct clusters of genes in purified $CD8^+NKp30^+$ subsets of T cells that were transduced with sg*BCL11B*, sg*MTA2*, sg*MBD2*, or sg*CHD4* and $CD8^+$ sgCtrl-transduced T cells. Relative enrichment of gene sets in the *k*-means cluster by hierarchical clustering heatmap analysis. Pathway enrichment (gene set and GO) was performed on cluster 1. Gene sets enriched in cluster 1 were binned into different categories and plotted.

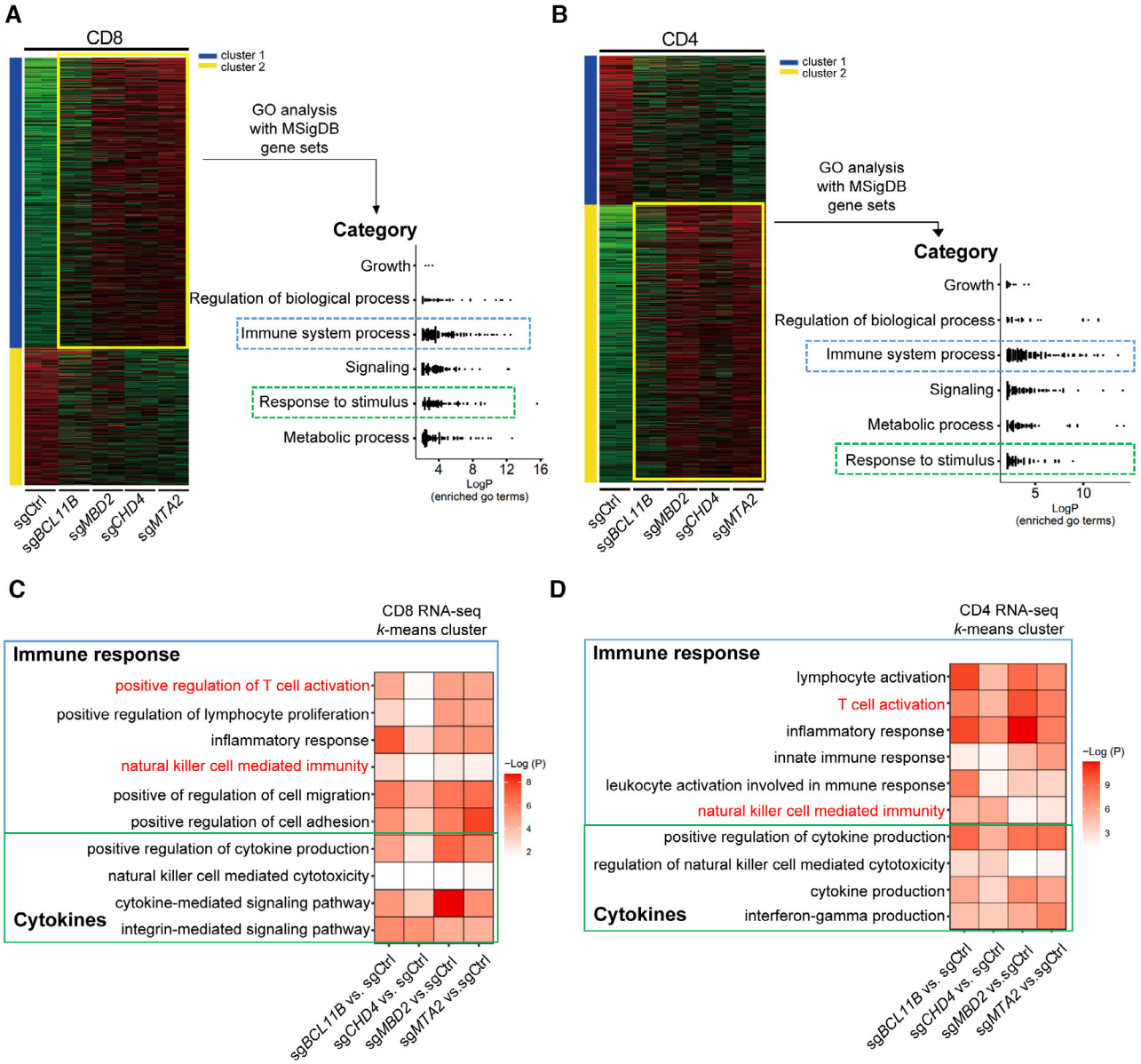
B *k*-means clustering of differentially expressed genes in purified $CD4^+NKp30^+$ subsets of T cells that were transduced with sg*BCL11B*, sg*MTA2*, sg*MBD2*, or sg*CHD4* and $CD4^+$ sgCtrl-transduced T cells. Gene sets enriched in cluster 2 were binned into different categories and plotted.

C Representative gene sets enriched by Gene Ontology (GO) analysis of the *k*-means clusters 1 from (A). GO enrichment analysis was performed by R package clusterprofile (version 4.6.2). The was visualized as heatmaps generated by customed R scripts using ggplot2 (version 3.4.0).

D Representative gene sets enriched by GO term *k*-means clusters 2 from (B). GO enrichment analysis was performed by R package clusterprofile (version 4.6.2). The was visualized as heatmaps generated by customed R scripts using ggplot2 (version 3.4.0).

E GSEA enrichment plots for the indicated gene sets in the transcriptome of $CD4^+$ CTL (enrichment plot: NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY; Table EV1). The top portion of the plot shows the running enrichment score (RES) for the gene set as the analysis walks down the ranked list of genes and reflects the degree to which the gene set is overrepresented at the top or bottom of the ranked list of genes. The middle portion of the plot shows where the members of the gene set (indicated as black lines) appear in the ranked list of genes. The bottom portion of the plot shows the value of the ranking metric.

Source data are available online for this figure.



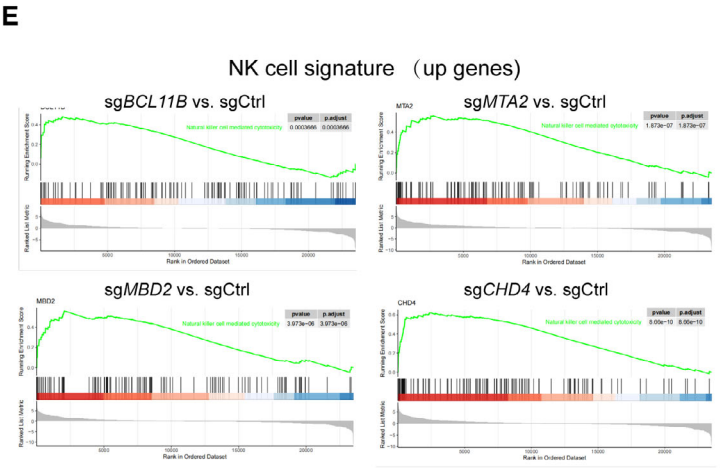


Figure EV1.

Figure EV2. ITNKs increase the H3K27Ac at TSS region of pluripotency gene sets.

A Bar chart indicating the number of peaks and the number of genes at the peak position in these omics-seq.
B Bar chart demonstrating the percentage of peaking with a TSS, TSS-promoter, transcription end site (TES), and other sites in these omics-seq.
C Tag density pileups of H3K27Ac peaks in $CD8^+$ T cells transduced with sg*BCL11B*, sg*MBD2*, or sg*CHD4* and $CD8^+$ sgCtrl-transduced T cells. Two individual healthy donors were used for the CUT&Tag assay.
D Tag density pileups of H3K27Ac peaks at the T-cell-activation gene set (see Table EV2) in $CD8^+$ T cells transduced with sg*BCL11B*, sg*MBD2*, or sg*CHD4* and $CD8^+$ sgCtrl-transduced T cells.
E Tag density pileups of H3K27Ac peaks at the natural-killer-cell-mediated-immunity gene set (see Table EV3) in $CD8^+$ T cells transduced with sg*BCL11B*, sg*MBD2*, or sg*CHD4* and $CD8^+$ sgCtrl-transduced T cells.
F Tag density pileups of H3K27Ac peaks at the oxidative-phosphorylation gene set (see Table EV4) in $CD8^+$ T cells transduced with sg*BCL11B*, sg*MBD2*, or sg*CHD4* and $CD8^+$ sgCtrl-transduced T cells.

Source data are available online for this figure.

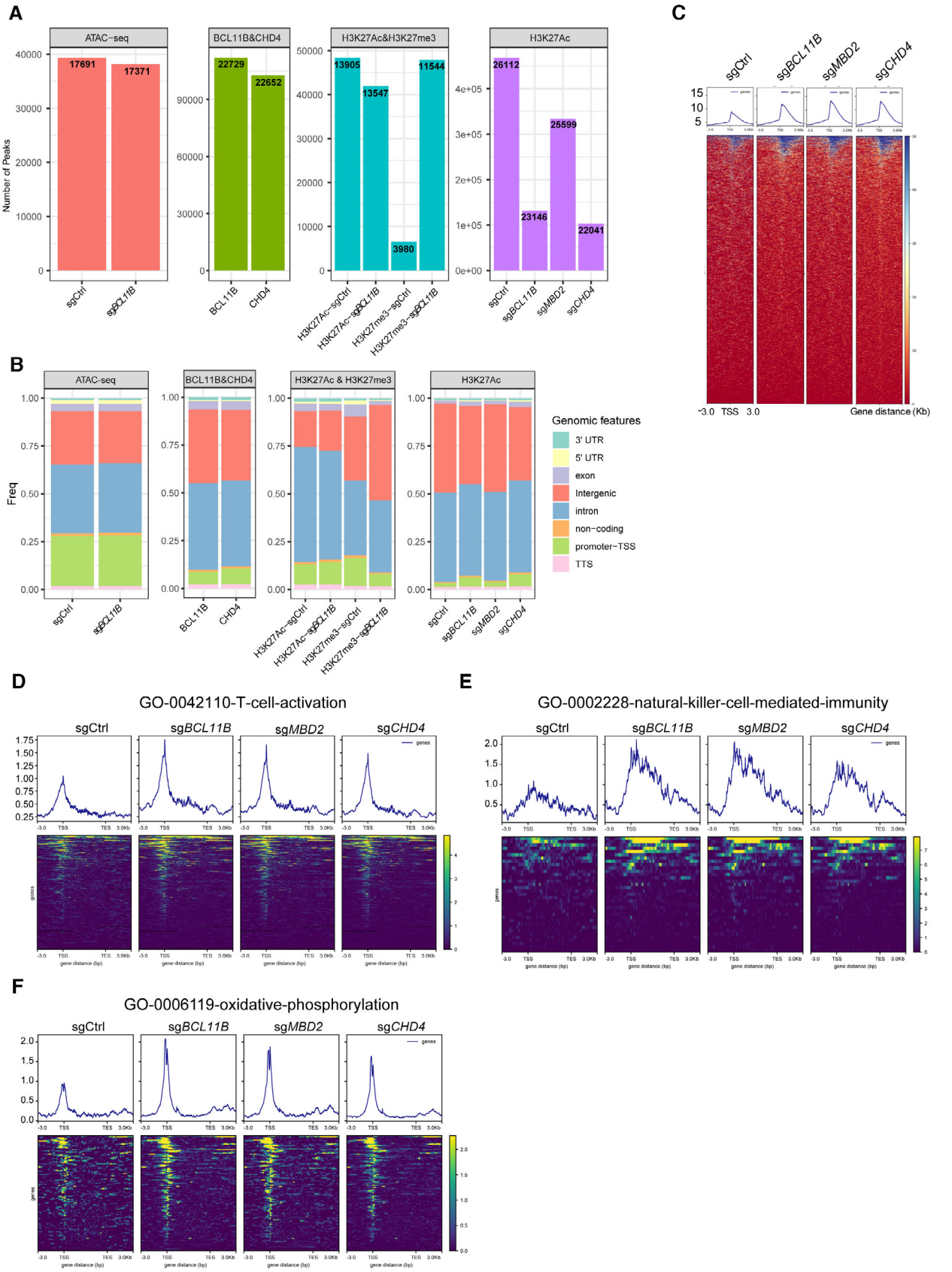


Figure EV2.

Figure EV3. Mitochondrial dynamics-related protein expression in ITNKs.

A Schematic diagram of BCL11B binding sits in the TSS region of OPA1 and OPA1 qPCR primer design strategies. OPA1 qPCR Primers 1 and 2 were designed in the range of 500 bp downstream of the OPA1 TSS; primers 3 and 4 were designed in the range of 500–1,500 bp; and primers 5 and 6 were designed in the nearby control region from 1,500 to 3,000 bp.

B The heatmap shows the upregulation of OPA1 and transcripts associated with TCA (tricarboxylic acid) and FAO (fatty acid oxidation) processes in ITNKs ($N = 3$ individual donors). Cutoff: absolute $\log_2$ (fold change) ≥ 1; adjusted P value ≤ 0.05.

C Relative mRNA levels of BCL11B in samples of human T cells, ITNKs ($CD3^+NKp46^+$), and NK cells ($CD3^-CD56^+$) based on quantitative RT-PCR.

D Western blot analysis of BCL11B levels in samples of human T cells, ITNKs ($CD3^+NKp46^+$), and NK cells ($CD3^-CD56^+$). Graph summarizing the relative protein levels of BCL11B is shown in the right panel.

E Western blot analysis of mitochondrial fusion (MFN1, MFN2) and fission (DRP1, $DRP1^{pS616}$) protein levels in samples of PBMC-derived T cells, ITNKs ($CD3^+NKp46^+$), and NK cells ($CD3^-CD56^+$). A graph summarizing the relative protein levels of MFN1, MFN2, DRP1, and $DRP1^{pS616}$ in ITNKs compared to T cells is shown in the right panel.

F T cells transduced with sgCtrl and sg*BCL11B* and NK cells enriched from PBMC from the same donor were cultured for 10 days *in vitro* and collected for FACS sorting. Representative flow cytometry analysis of purified T cells ($CD3^+NKp30^-$), ITNKs ($CD3^+NKp46^+$), and NK cells ($CD3^-CD56^+$).

G Representative western blot of OPA1 in T cells with different OPA1 isoforms overexpression. β-actin was used as a control. Isoform 1 is the long form and its mutant S1 only produces a single long form OPA1. Isoform 5 is the short form of OPA1.

H Mitochondrial morphology of T cells with different OPA1 isoform overexpression in which the mitochondria (MitoTracker; red) and nuclei (Hoechst; blue) are stained. Scale bars: 5 μm. Each dot represents the mean relative length of the 20 mitochondria per replicate.

I Representative western blot of OPA1 in T cells with M1 treatment. β-actin was used as a control.

J Mitochondrial morphology of T cells with M1 treatment in which the mitochondria (MitoTracker; red) and nuclei (Hoechst; blue) are stained. Each dot represents the mean relative length of the 20 mitochondria per replicate. Scale bars: 5 μm.

Data information: In (C–E, H, and J), data are presented as mean ± SD, $N = 3$ individual healthy donors. *$P \leq 0.05$, **$P \leq 0.01$ and ****$P \leq 0.0001$, two-tailed paired Student's *t*-test (C–E and J) or one-way ANOVA with Tukey's multiple comparisons test (E).
Source data are available online for this figure.

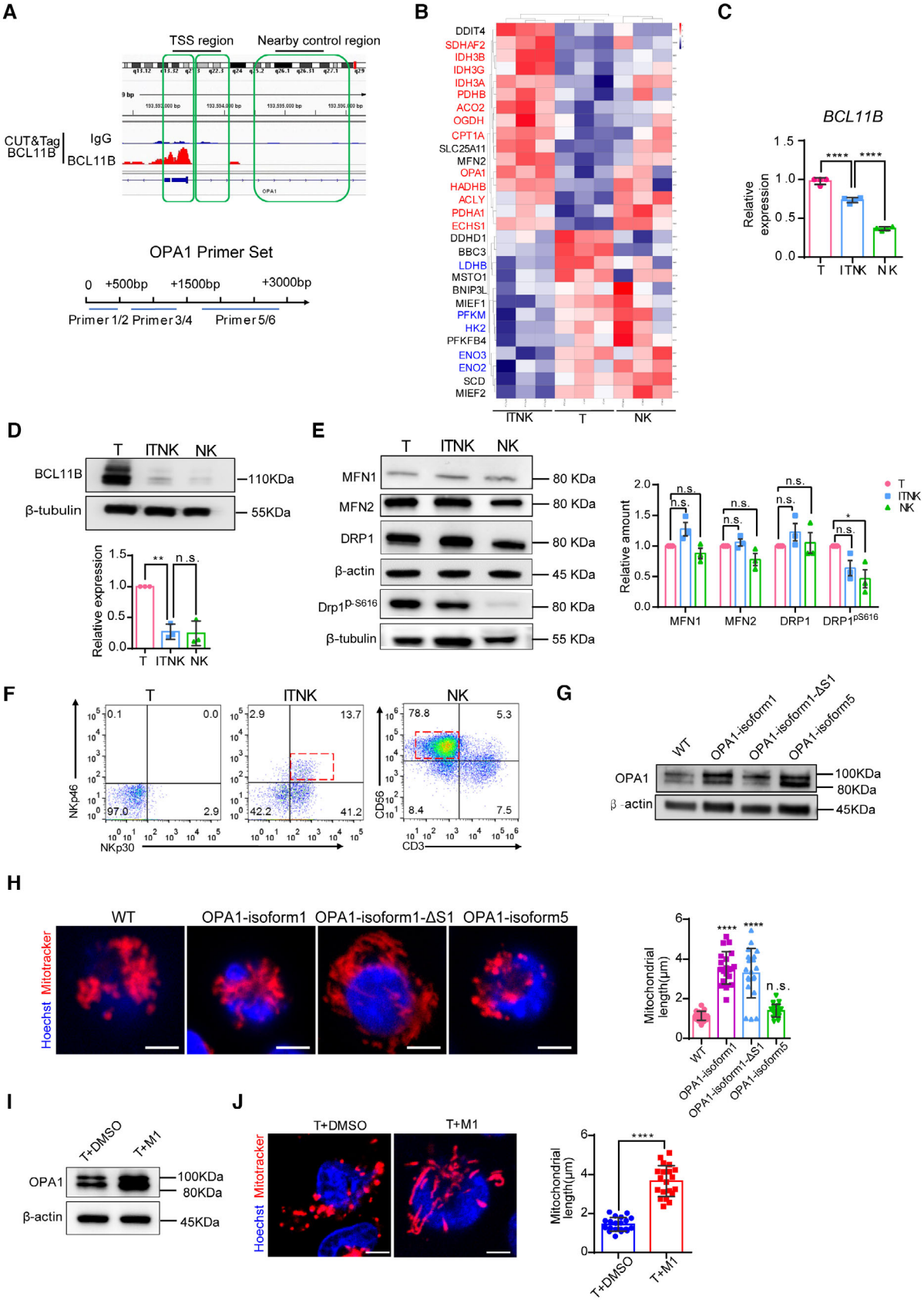


Figure EV3.

Figure EV4. Mitochondrial morphology and functions of ITNKs.

A Confocal microscopy images showing purified $CD8^+$ T cells and $CD8^+NKp30^+$ ITNKs in which the mitochondria (MitoTracker; green) and nuclei (Hoechst; blue) are stained. Each dot represents the mean relative length of the 24 mitochondria per replicate. Scale bars: 5 μm.

B Confocal microscopy images showing purified $CD4^+$ T cells and $CD4^+NKp30^+$ ITNKs in which the mitochondria (MitoTracker; green) and nuclei (Hoechst; blue) are stained. Each dot represents the mean relative length of the 24 mitochondria per replicate. Scale bars: 5 μm.

C ITNKs that were derived from T cells transduced with sg*BCL11B*, sg*MTA2*, sg*MBD2*, or sg*CHD4* or sgCtrl-transduced T cells were cultured for 10 days for observation of mitochondrial morphology. Confocal microscopy images are shown; mitochondria (MitoTracker; green); nuclei (Hoechst; blue), and NKp30 (purple) are stained. Scale bars: 5 μm.

D $CD4^+NKp30^+$ and $CD8^+NKp30^+$ ITNKs derived from sg*BCL11B*-transduced T cells were purified and assessed the mitochondrial functions by FACS. $CD8^+$ T cells and $CD8^+NKp30^+$ ITNKs (left) and $CD4^+$ T cells and $CD4^+NKp30^+$ ITNKs (right) were stained with MitoTracker green and analyzed by flow cytometry.

E $CD8^+$ T cells and $CD8^+NKp30^+$ ITNKs (left) and $CD4^+$ T cells and $CD4^+NKp30^+$ ITNKs (right) were stained with TMRM and analyzed by flow cytometry.

F $CD8^+$ T cells and $CD8^+NKp30^+$ ITNKs (left) and $CD4^+$ T cells and $CD4^+NKp30^+$ ITNKs (right) were stained with MitoSOX and analyzed by flow cytometry.

Data information: In (A, B, and D–F), data are presented as mean ± SD, $N = 3$ (A and B) or 6 (D–F) individual healthy donors. *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$, two-tailed paired Student's *t*-test.
Source data are available online for this figure.

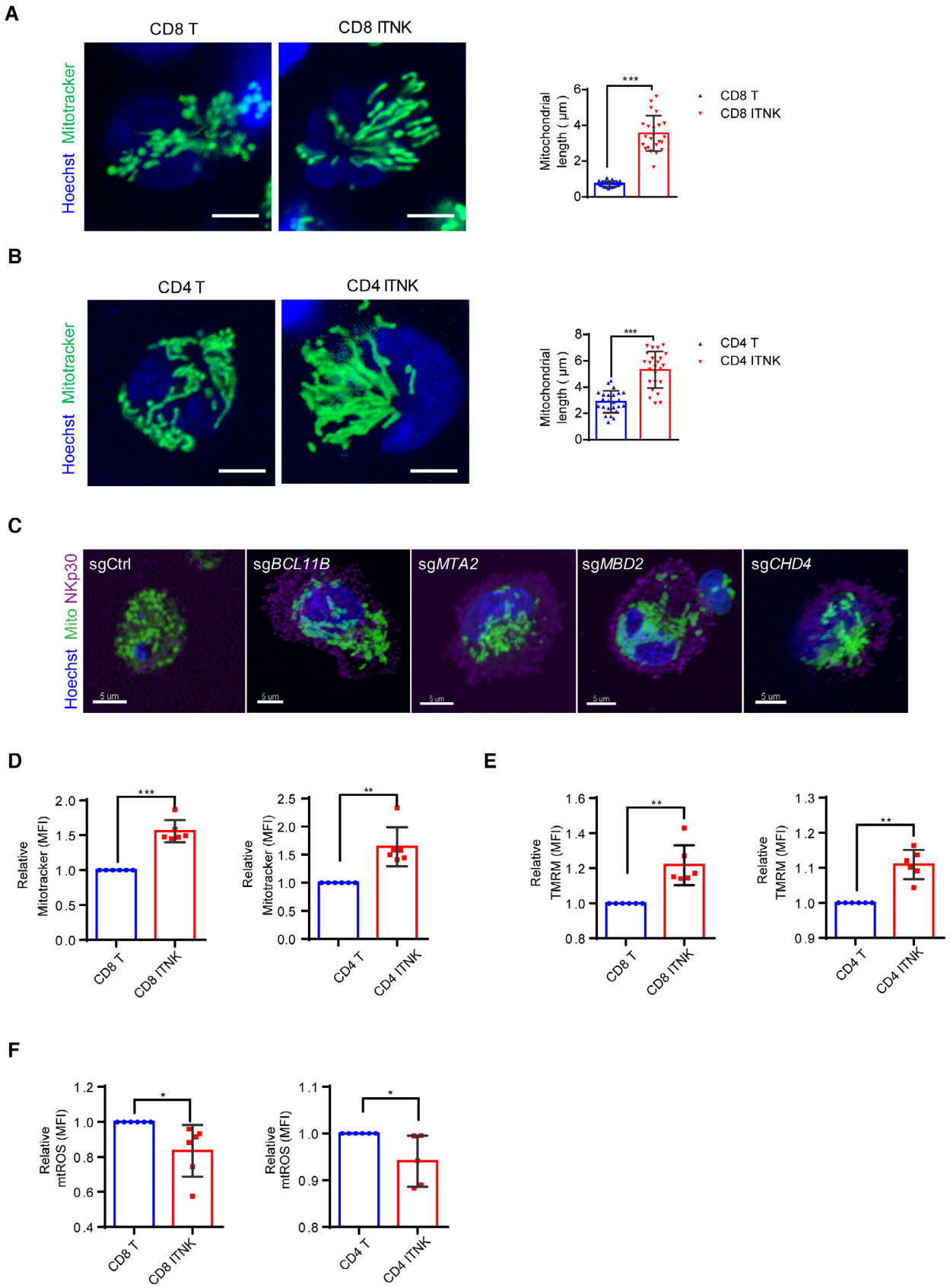


Figure EV4.

Figure EV5. Acetate restored the reprogramming efficacy and function of ITNKs.

A Relative acetyl-CoA levels in $CD8^+$ (left) and $CD4^+$ (right) ITNKs on Day 10 that were reprogrammed from T cells transduced with sg*BCL11B* and sgCtrl-transduced T cells.

B Relative α-KG levels in $CD4^+$ (left) and $CD8^+$ (right) ITNKs on Day 10 that were reprogrammed from T cells transduced with sg*BCL11B* and sgCtrl-transduced T cells.

C Graph summarizing the percentages of $NKp30^+$ in CD4 T cells and CD8 T cells transduced with sg*BC11B* or the combination of sgB*CL11B* and sg*OPA1* and treated with or without acetate (from Fig 5B).

D Cytokine secretion profiles of T cells and ITNKs from Fig 5C that were incubated with HepG2 cells at an E:T ratio of 1:1 for 24 h. The supernatants were then harvested, and the concentrations of the indicated cytokines were measured by a multiplex immunoassay.

E Relative mRNA levels of *ZBTB16*, *NCR1*, *NCR2*, *NCR3*, *IFNγ*, *CSF2*, and *GZMB* in T cells and ITNKs from Fig 5A, housekeeping genes (*EEF2*, *B2M*, *MYH9*, and *TUBB*) as negative controls, measured by quantitative RT-PCR.

Data information: In (A–E), data are presented as mean ± SD, $N = 2$ (B) or 3 (A and C–E) individual healthy donors. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ and ****$P \leq 0.0001$, two-tailed paired Student's *t*-test (A) or one-way ANOVA with Tukey's multiple comparisons test (C–E).
Source data are available online for this figure.

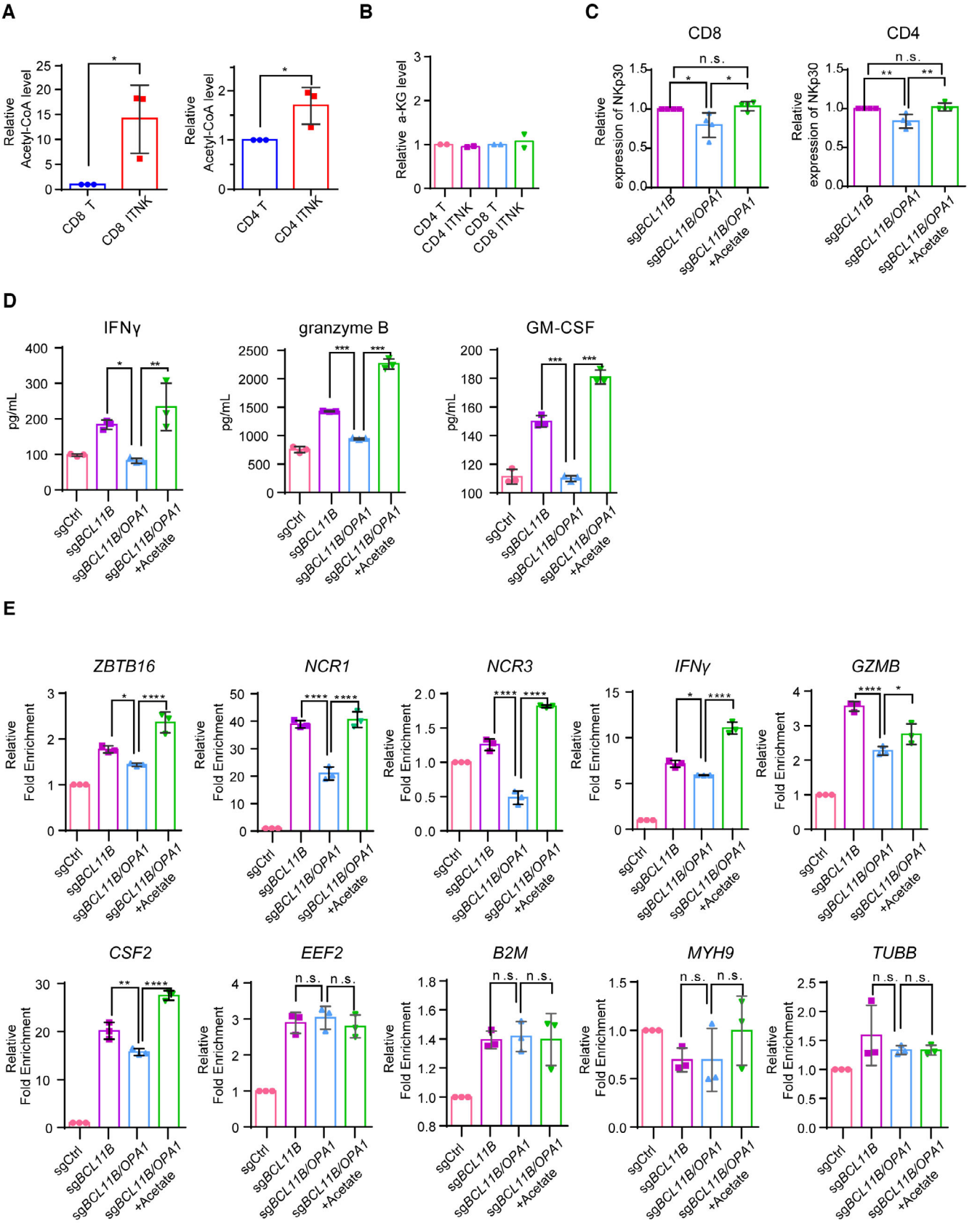


Figure EV5.