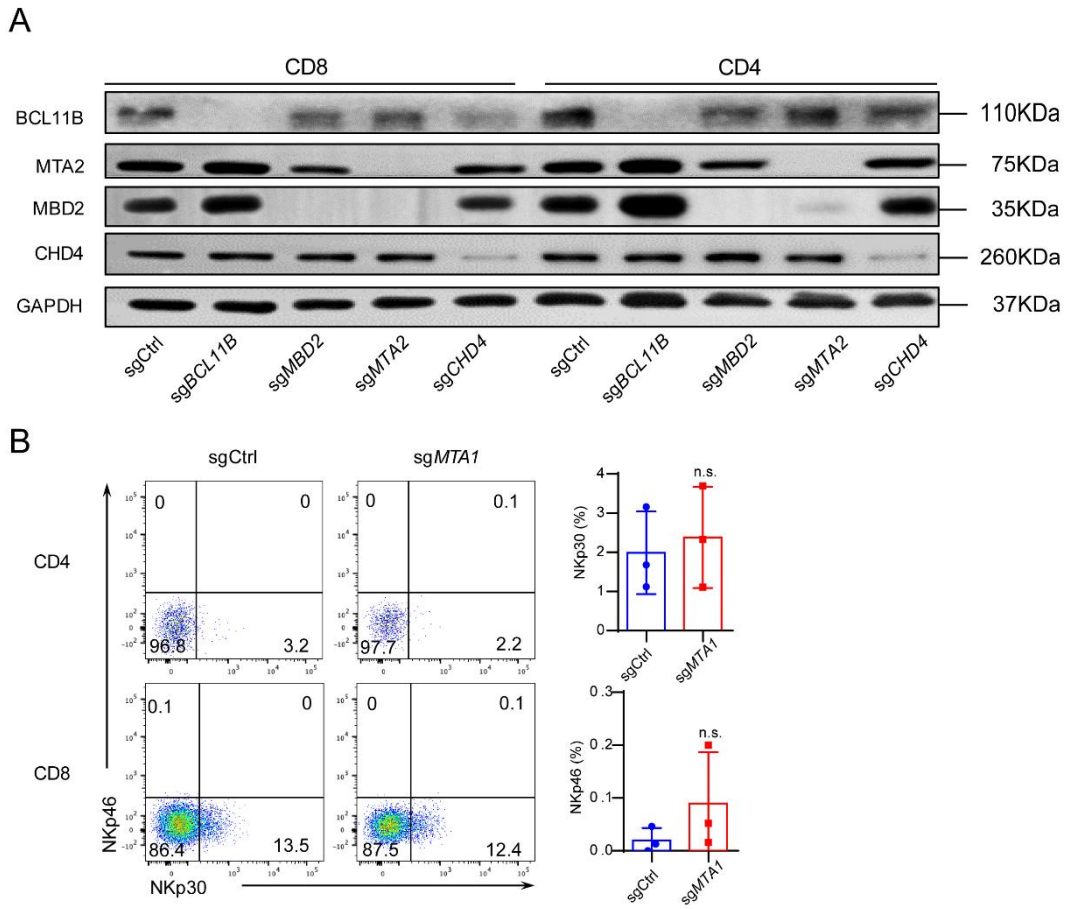
# Appendix

## Supplementary figures

## Appendix Figure S1

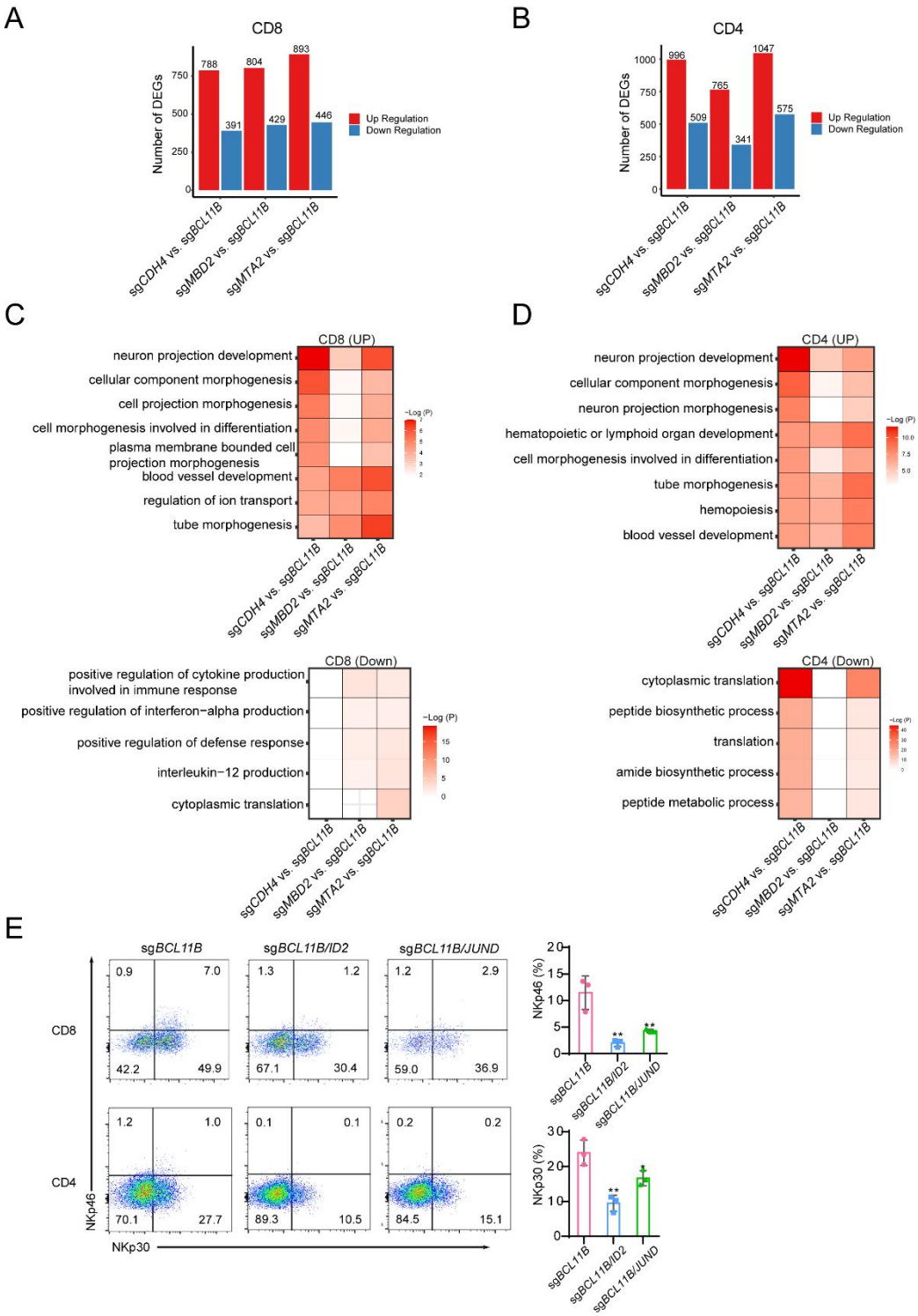



**Appendix Fig. S1. Inactivating BCL11B and NuRD subunits in human T cells.**

A. Representative western blot of BCL11B, MBD2, MTA2, and CHD4 protein levels in samples of purified $CD8^+$ or $CD4^+$ PBMC-derived T cells that were transduced with sgCtrl, sg*BCL11B*, sg*MBD2*, sg*MTA2*, and sg*CHD4*. The data represents the efficacy of the knockout experiment from Fig. 1B.

B. Representative flow cytometric detection of NKp30 and NKp46 in T cells transduced with sgCtrl and sg*MTA1*. Graph summarizing the $NKp46^+$ in $CD3^+CD8^+$ T cells and $NKp30^+$ in $CD3^+CD4^+$ T cells transduced with the indicated sgRNAs. Data were analyzed by two-tailed paired Student's t-test. Data represent mean ± SD (N=3 individual healthy donors).

## Appendix Figure S2




**Appendix Fig. S2. ITNKs derived from NuRD-subunit-deficient cells are different from BCL11B-deleted ITNKs.**

A. Bar chart indicating the numbers of up- and down-regulated genes from differentially expressed genes (DEGs) between NuRD-subunit-deficient $CD8^+$ ITNKs

and $CD8^+$ BCL11B-deficient ITNKs. Gene differential expression analysis was performed by R package DESeq2 (version 1.38.1). The number of differentially expressed genes was counted as visualized by customed R scripts using ggplot2 (version 3.4.0).

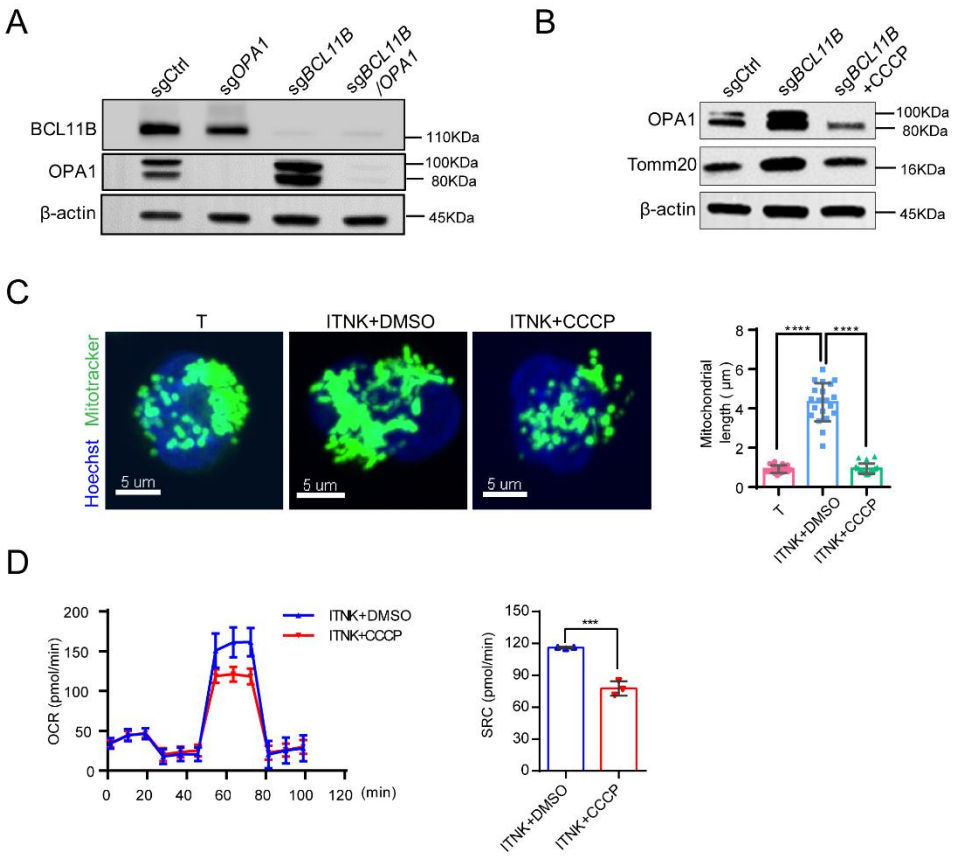
B. Bar chart indicating the numbers of up- and down-regulated genes from DEGs between NuRD-subunit-deficient $CD4^+$ ITNKs and $CD4^+$ BCL11B-deficient ITNKs.

C. Gene ontology analysis of up- and down-regulated genes from DEGs between NuRD-subunit-deficient $CD8^+$ ITNKs and BCL11B-deficient $CD8^+$ ITNKs. 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. Gene ontology analysis of up- and down-regulated genes from DEGs between NuRD-subunit-deficient $CD4^+$ ITNKs and BCL11B-deficient $CD4^+$ ITNKs.

E. Representative flow cytometric detection of NKp30 and NKp46 in PBMC-derived T cells transduced with sgBCL11B, sgBCL11B/ID2, and sgBCL11B/JUND. Graph summarizing the $NKp46^+$ in $CD3^+CD8^+$ T cells and $NKp30^+$ in $CD3^+CD4^+$ T cells transduced with the indicated sgRNAs. Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test. *$P \leq 0.05$ and **$P \leq 0.01$. Data represent mean ± SD (N=3 individual healthy donors).

## Appendix Figure S3




**Appendix Fig. S3. Suppressing mitochondrial fusion inhibits the reprogramming of T cells into ITNKs and the antitumor effects of ITNKs.**

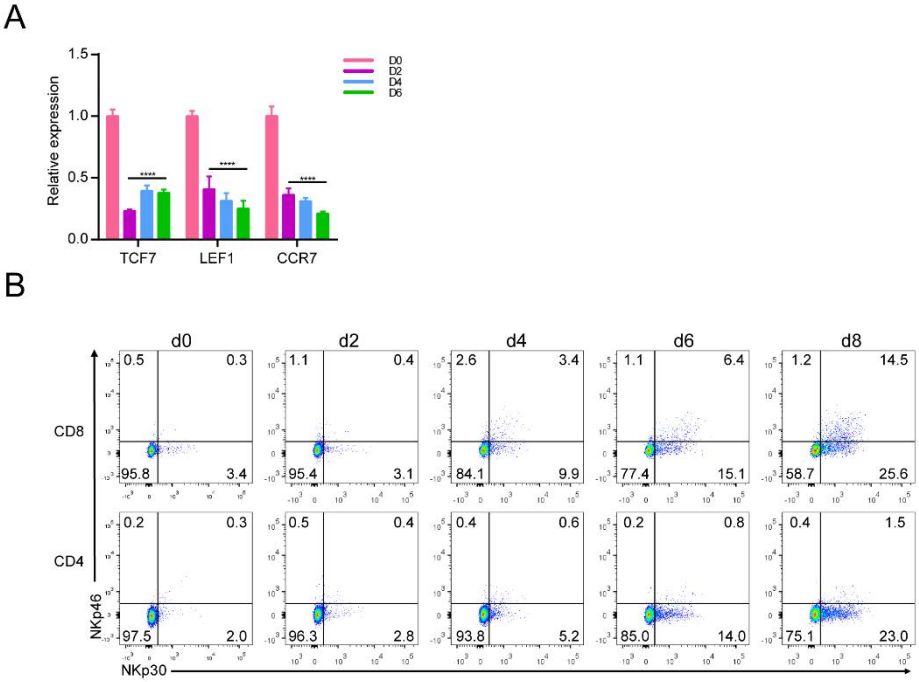
A. Representative western blots of BCL11B and OPA1 protein levels in samples of T cells transduced with sgCtrl, sg*OPA1*, sg*BCL11B*, or the combination of sg*BCL11B* and sg*OPA1*.

B. CCCP (5 μM, mitochondrial fission inducer) was added to a culture of sg*BCL11B*-transduced human T cells derived from PBMCs 24 hours after electroporation. OPA1 protein, Tomm20, and β-actin were detected by western blot assay.

C. Confocal microscopy images showing T cells, ITNK cells, and ITNK cells treated with CCCP in which the mitochondria (MitoTracker, green) and nuclei (Hoechst; blue) are stained. Scale bars: 5 μm. Relative lengths of the mitochondria, as analyzed by confocal microscopy, are shown. The images were digitized using ImageJ software. Each dot represents the mean relative length of the mitochondria in a sample. ****P≤0.0001, one-way ANOVA with Tukey's multiple comparisons test.

D. OXPHOS (OCR: O2 consumption rate) assays were performed in ITNKs and ITNKs treated with CCCP. The maximum OCR values were achieved after FCCP uncoupling (maximum respiration). The curves represent the mean ± SD (N=5 individual healthy donors). ***P ≤ 0.001, two-tailed paired Student's t-test.

## Appendix Figure S4




**Appendix Fig. S4. Related genes express at multiple time points during reprogramming.**

A. Time course of T cell-related genes expression by RT-qPCR. The results represent mean ± SD (N=4 individual healthy donors); ****P≤0.0001, two-way ANOVA with Dunnett's multiple comparisons test.

B. T cells transduced sgBCL11B (from (Fig. 6A)) were cultured in vitro. The makers (NKp30 and NKp46) of $CD4^+$ and $CD8^+$ ITNKs were detected using flow cytometry at multiple timepoints.