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Supplementary information

A genome-scale screen for synthetic drivers of T cell proliferation

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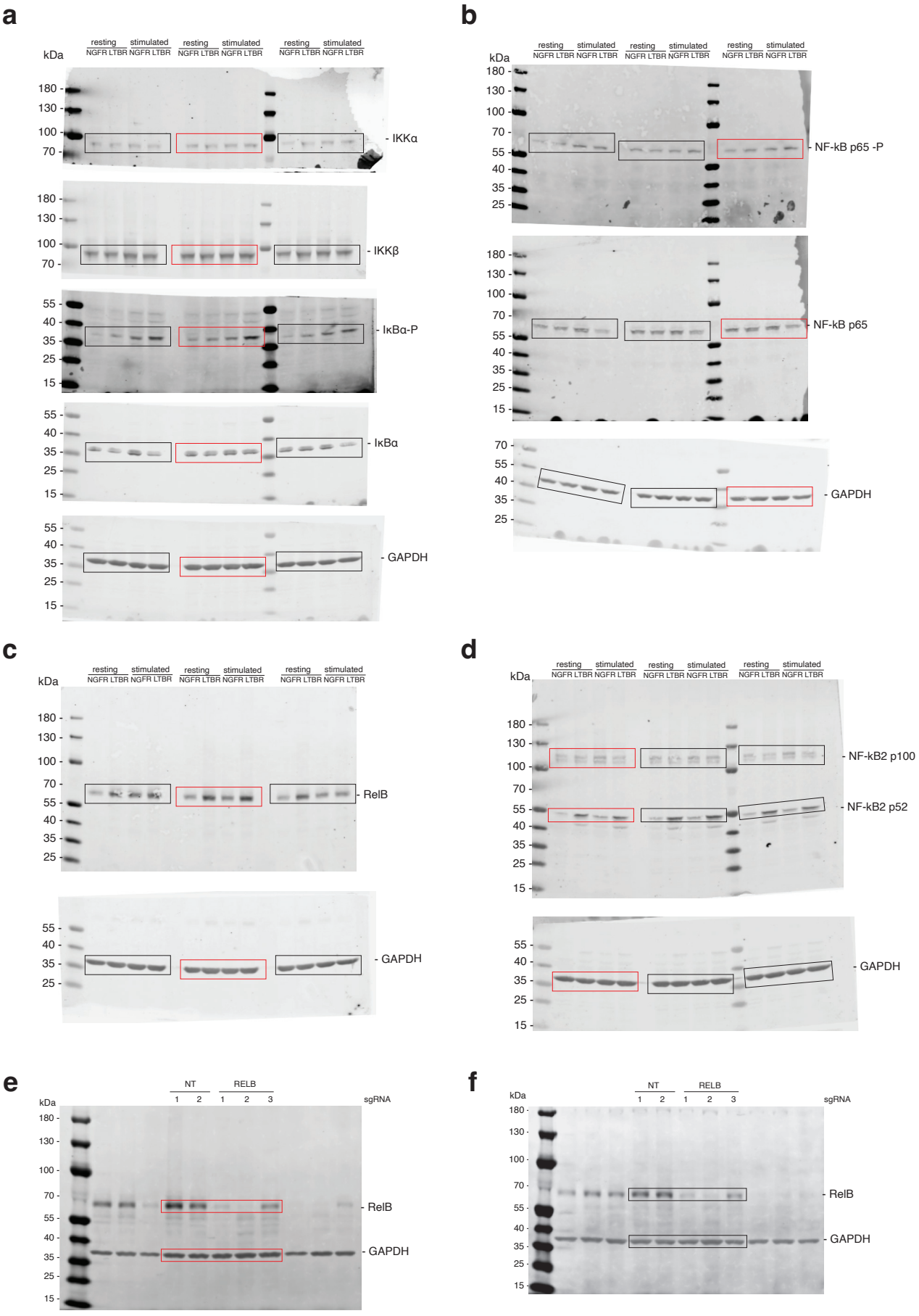

Supplementary Figure 1. Source gel images for western blot analyses.
(**a-d**) NF-κB signalling pathway. LTBR and tNGFR cells were stimulated for 24 hours with CD3/CD28 or left unstimulated. Bands marked in red (*a* - *d*) are shown in *Extended Data Figure 8b*. Bands marked in red and in black (*a* - *d*) are used for quantification shown in *Extended Data Figure 8c*. Blot shown in (*a*) was cut and the upper part (70 -180 kDa) was probed for IKKα, then stripped and reprobed for IKKβ while the lower part (15 - 55 kDa) was first probed for phospho-IκBα, stripped and probed for IκBα, and finally stripped and probed for GAPDH. Blot shown in (*b*) was first probed for phospho-NF-κB p65, stripped and probed for NF-κB, and then cut, stripped and probed for GAPDH. Blot shown in (*c*) was probed for RelB, then cut, stripped and reprobed for GAPDH. Blot shown in (*d*) was probed for NF-κB2 (p100/p52), then cut, stripped and reprobed for GAPDH.
(**e-f**) RelB knockout. LTBR (*e*) and tNGFR (*f*) cells were co-expressing non-targeting (NT) or RELB-targeting sgRNAs. Bands marked in red are shown in *Extended Data Figure 8n*. Bands shown in red and black were used for quantification in *Extended Data Figure 8o*. Unmarked lanes include experimental conditions that were not used.

Supplementary Table legends (see separate Excel file for Tables)

Supplementary Table 1. ORF barcode list. Correspondence of identified barcodes to genes.
Supplementary Table 2. Oligo sequences. Sequences of primers used in the study.
Supplementary Table 3. Antibodies and dyes. Reagents used for flow cytometry.
Supplementary Table 4. **ORF DESeq2**. Gene enrichment in the screen calculated on the ORF level, corresponding to *Extended Data Figure 1m*. DESeq2 was used for calculation of significance.
Supplementary Table 5. ORF RRA. Gene enrichment in the screen calculated on the barcode level, corresponding to *Figure 1c*. MaGeCK RRA was used for calculation of significance.
Supplementary Table 6. Validation data. Significance (p value), mean and FDR for proliferation, expression of CD25 and CD154, and cytokine (IL2, IFNG) secretion. Corresponding to *Figure 2b-e.*
Supplementary Table 7. Multiplex cytokines. Quantification of 48 cytokines secreted by resting or CD3/CD28 stimulated (24 h) CD8 T cells expressing a given ORF. Corresponding to *Extended Data Figure 3e*.
Supplementary Table 8. Top genes per cluster. Top 20 differentially expressed genes in each cluster, corresponding to *Figure 3d*. DESeq2 was used for calculation of significance.
Supplementary Table 9. DE genes CD8 LTBR stim. Differentially expressed genes, based on bulk RNA sequencing, between CD8 LTBR and CD8 tNGFR T-cells after 24 h stimulation with CD3/CD28. DESeq2 was used for calculation of significance.
Supplementary Table 10. DE genes CD8 LTBR rest. Differentially expressed genes, based on bulk RNA sequencing, between resting CD8 LTBR and CD8 tNGFR T-cells. DESeq2 was used for calculation of significance.
Supplementary Table 11. DE genes CD4 LTBR stim. Differentially expressed genes, based on bulk RNA sequencing, between CD4 LTBR and CD4 tNGFR T-cells after 24 h stimulation with CD3/CD28. DESeq2 was used for calculation of significance.
Supplementary Table 12. DE genes CD4 LTBR rest. Differentially expressed genes, based on bulk RNA sequencing, between resting CD4 LTBR and CD4 tNGFR T-cells. DESeq2 was used for calculation of significance.
Supplementary Table 13. ATAC CD8 LTBR stim. Differentially accessible chromatin regions, based on bulk ATAC sequencing, between CD8 LTBR and CD8 tNGFR T-cells after 24 h stimulation with CD3/CD28. DESeq2 was used for calculation of significance.
Supplementary Table 14. ATAC CD8 LTBR rest. Differentially accessible chromatin regions, based on bulk ATAC sequencing, between resting CD8 LTBR and CD8 tNGFR T-cells. DESeq2 was used for calculation of significance.
Supplementary Table 15. Statistics for Fig. 4. Exact p values for two-sided unpaired t-test.
Supplementary Table 16. sgRNA sequences. sgRNA sequences used in CRISPR knockout experiments.