nature

https://doi.org/10.1038/s41586-020-2475-6

Supplementary information

Mitochondrial ubiquinol oxidation is necessary for tumour growth

In the format provided by the authors and unedited

Inmaculada Martínez-Reyes, Luzivette Robles Cardona, Hyewon Kong, Karthik Vasan, Gregory S. McElroy, Marie Werner, Hermon Kihshen, Colleen R. Reczek, Samuel E. Weinberg, Peng Gao, Elizabeth M. Steinert, Raul Piseaux, G. R. Scott Budinger & Navdeep S. Chandel ✉

SUPPLEMENTARY INFORMATION

Mitochondrial ubiquinol oxidation is necessary for tumor growth

Inmaculada Martínez-Reyes[1], Luzivette Robles Cardona[1], Hyewon Kong[1], Karthik Vasan[1], Gregory S. McElroy[1], Marie Werner[1], Hermon Kihshen[1], Colleen R. Reczek[1], Samuel E. Weinberg[1], Peng Gao[2], Elizabeth M. Steinert[1], Raul Piseaux[1], G.R. Scott Budinger[1], and Navdeep S. Chandel[1,2]

[1]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611

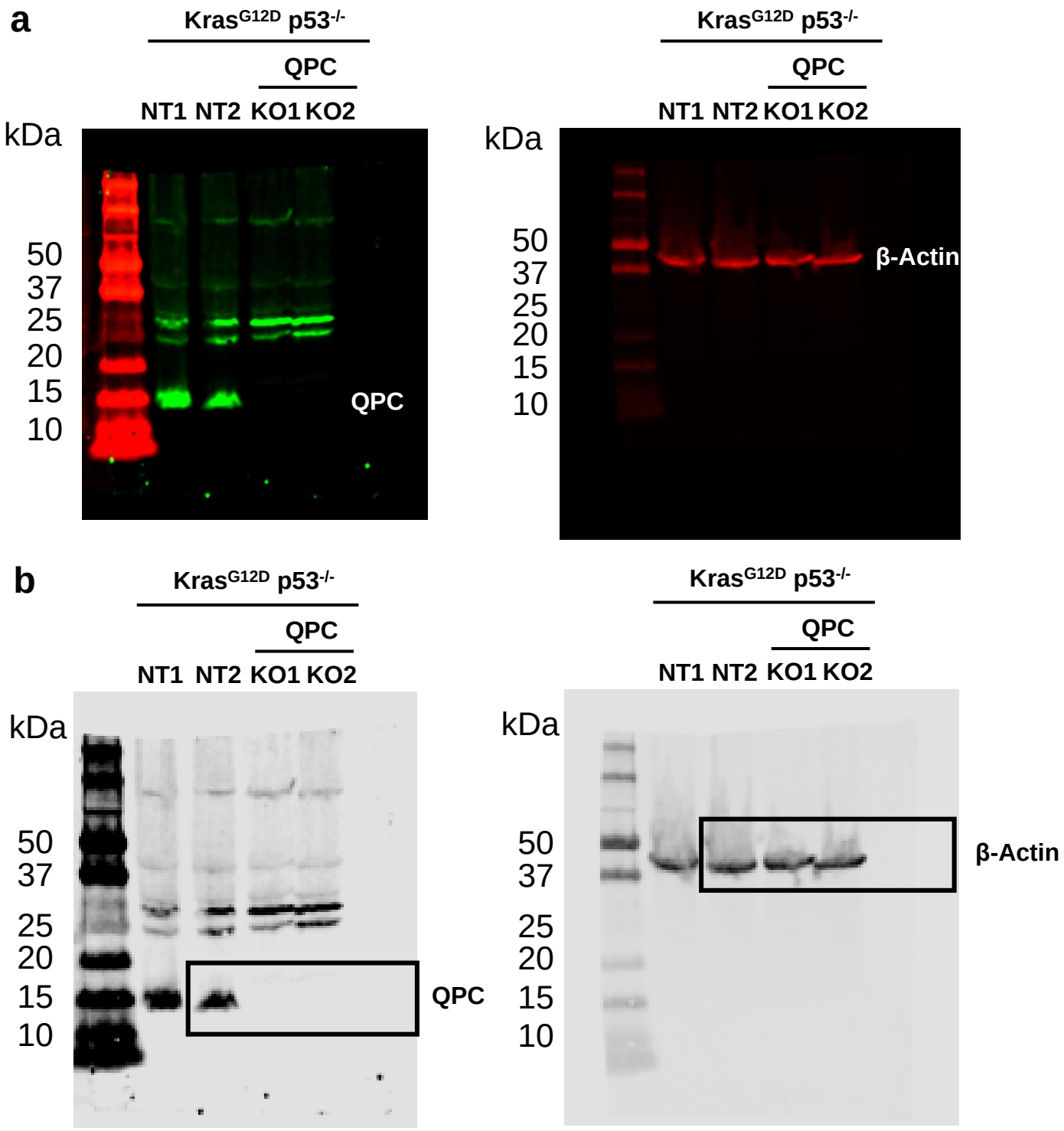
[2]Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL 60611

[3]Robert H. Lurie Cancer Center Metabolomics Core, Northwestern University Feinberg School of Medicine, Chicago, IL 60611

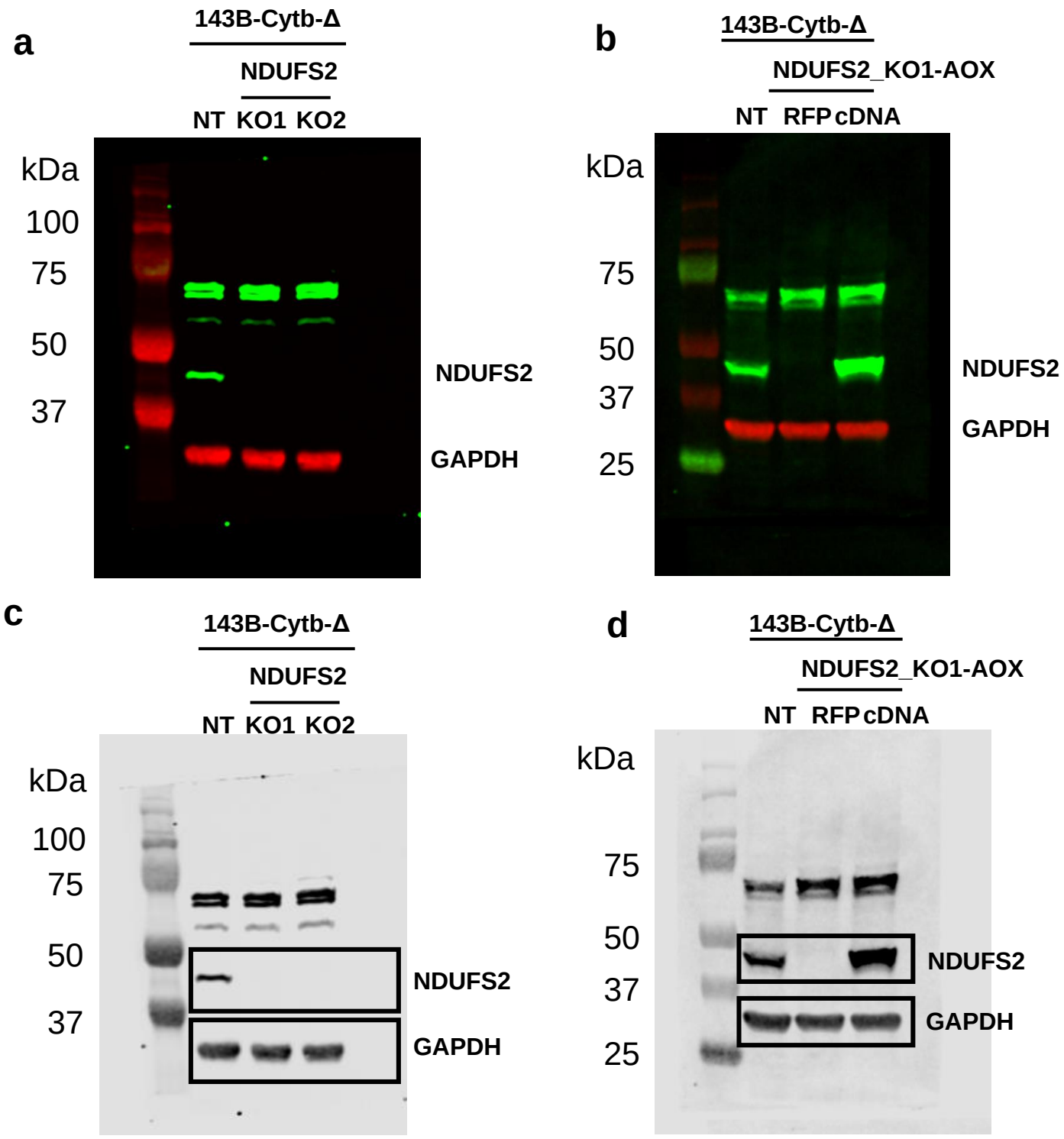
*Corresponding author:

Navdeep S. Chandel
Northwestern University
Feinberg School of Medicine
McGaw Pavilion, Rm. M-334 240
East Huron Street
Chicago, IL 60611
Phone: 312-503-2549
E-mail: nav@northwestern.edu

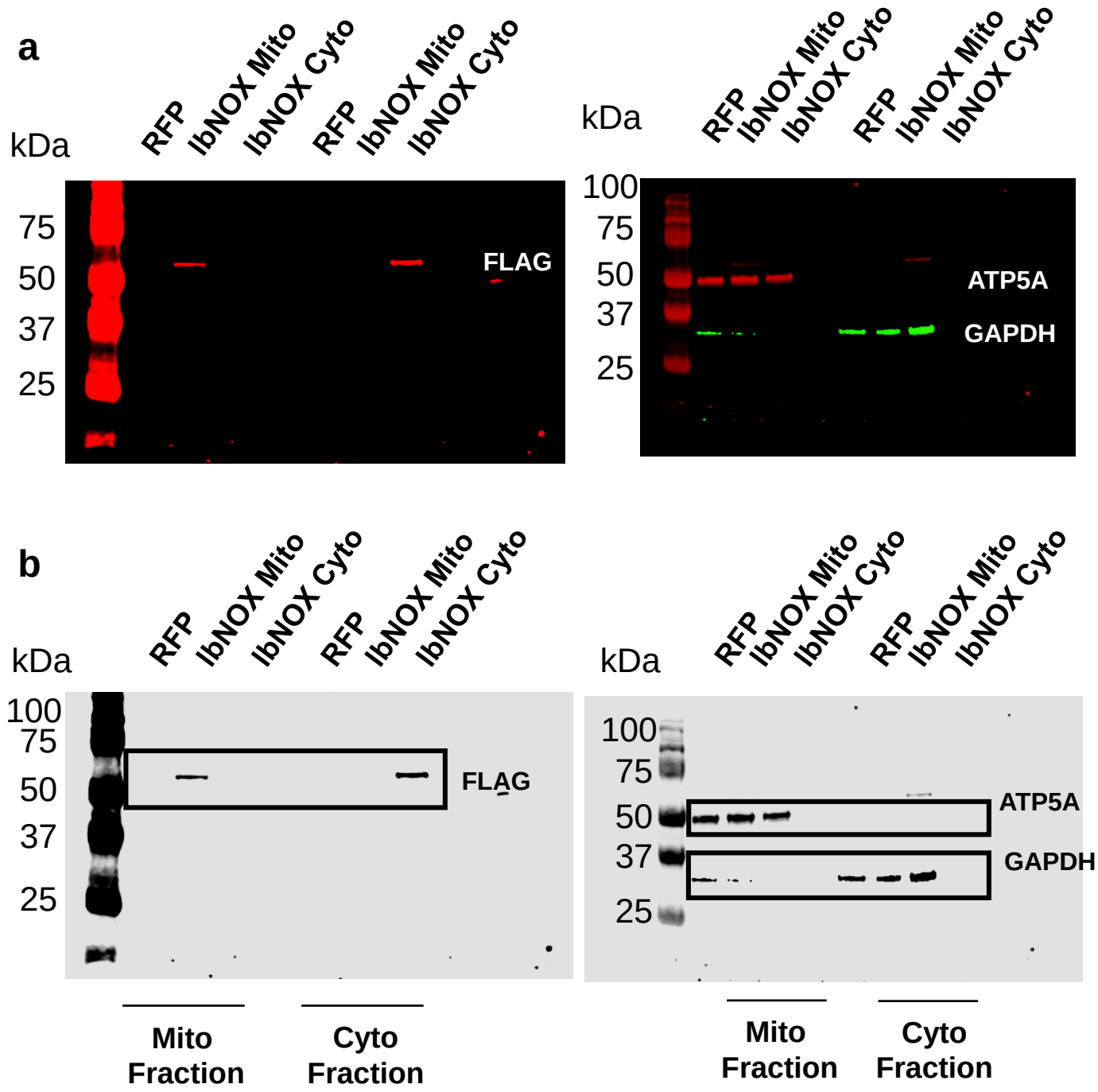
Supplementary Figures 1–6 show source data for western blots.
Supplementary Figure 7 shows the gating strategy for T-ALL experiments.
Supplementary Tables 1 shows the sequences of the sgRNAs used in this study.



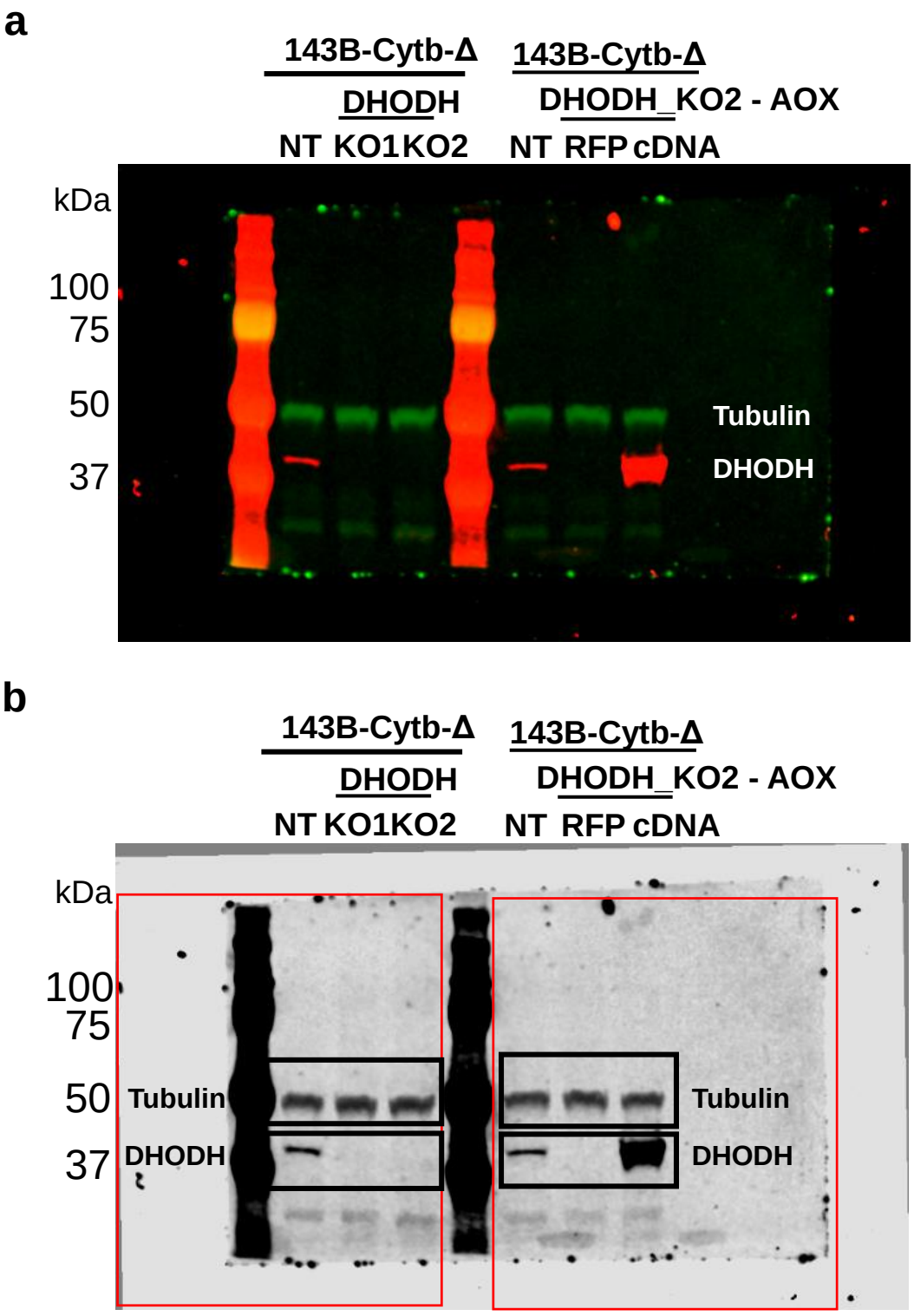
Supplementary Figure 1. Original western blot for Figure 1c. The full, uncut gel images of Figure 1c are shown. Control β-Actin was run on the same gel as loading control. The membrane was first blotted for QPC which was detected using IRDye 800CW goat anti-rabbit secondary antibody. The same membrane was then blotted for β-Actin using IRDye 680RD goat anti-mouse secondary. In **a and b** both 700 and 800 channels (left) or only channel 700 (right) are shown.



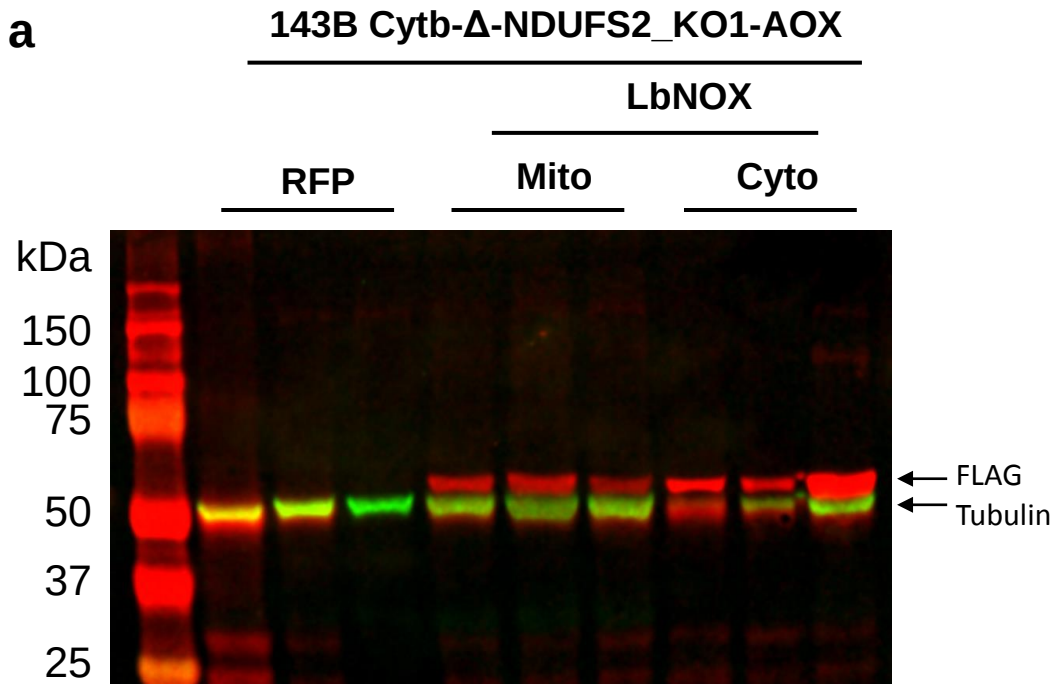
Supplementary Figure 2. Original western blot for Figure3a and Extended Figure 5c . The full, uncut gel images of Figure 3a (a) and Extended Figure 5c (b) are shown. Control GAPDH was run on the same gel as loading control. IRDye 800CW goat anti-rabbit secondary antibody was used to detect NDUFS2 while IRDye 680RD goat anti-mouse secondary was used to detect GAPDH. In figures **a-d** both 700 and 800 channels are shown.

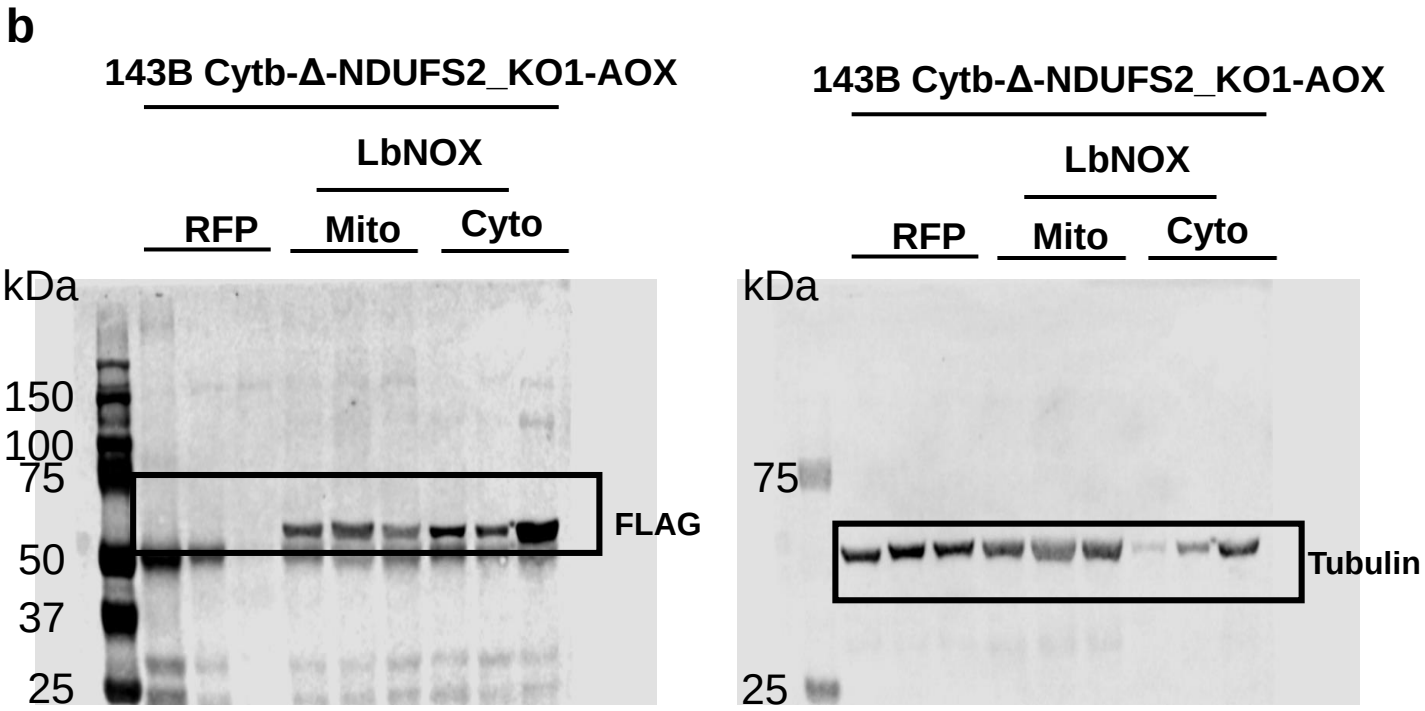


Supplementary Figure 3. Original western blot for Figure 4a. The full, uncut gel images of Figure 4a are shown. All proteins were blotted on the same gel. The membrane was first blotted for FLAG which was detected using IRDye 680RD goat anti-mouse secondary. The same membrane was then blotted for ATP5A and GAPDH. IRDye 800CW goat anti-rabbit secondary antibody was used to detect GAPDH while IRDye 680RD goat anti-mouse secondary was used to detect ATP5A. In figure b only channel 700 (left) or both 700 and 800 channels (right) are shown.

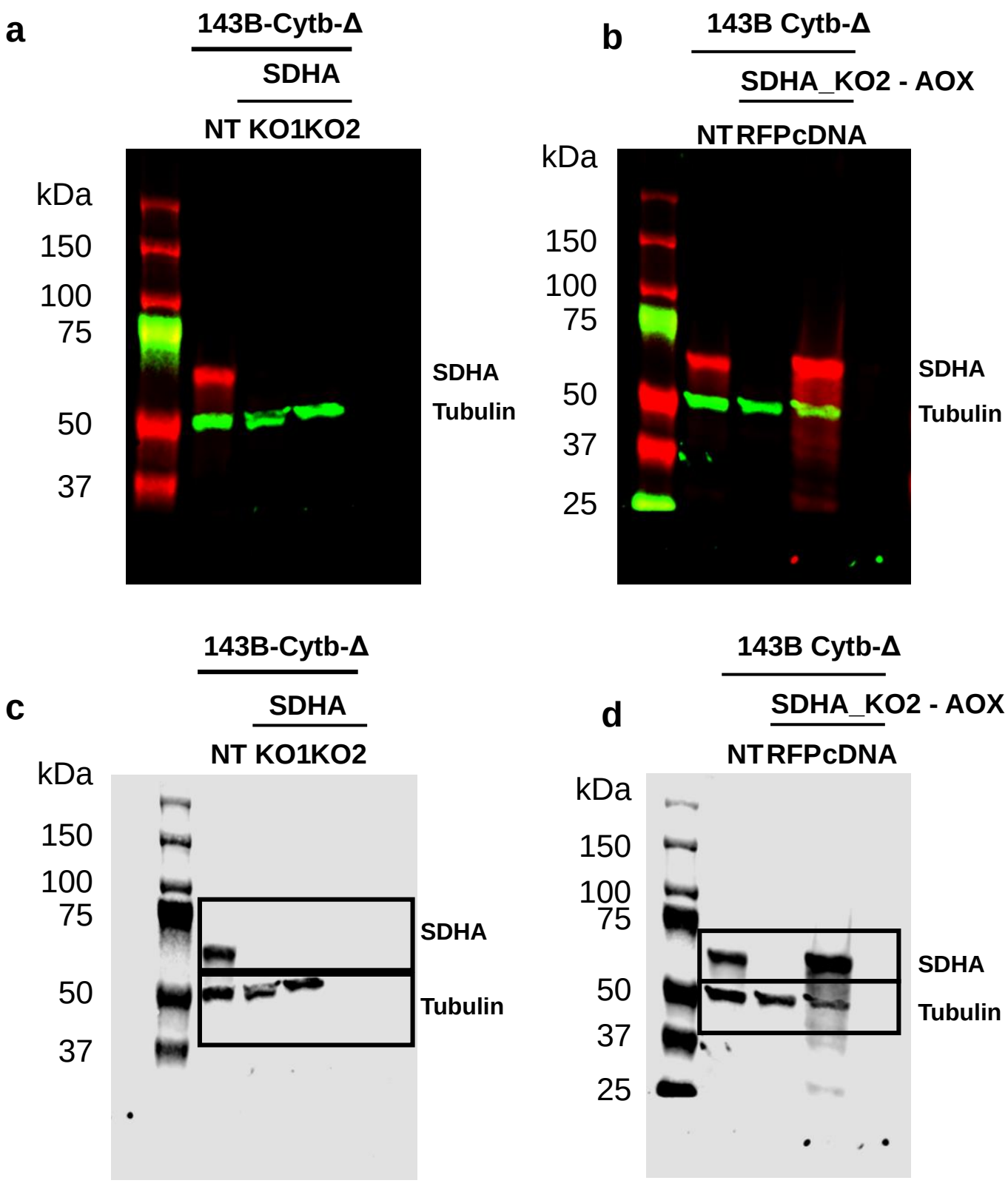


Supplementary Figure 4. Original western blot for Extended Figure 4b and Extended Figure 4f. a and b, The full, uncut gel images of Extended Figure 4b and Extended Figure 4f are shown. Control Tubulin was run on the same gel as loading control. IRDye 800CW goat anti-rabbit secondary antibody was used to detect Tubulin while IRDye 680RD goat anti-mouse secondary was used to detect DHODH. Both 700 and 800 channels are shown.

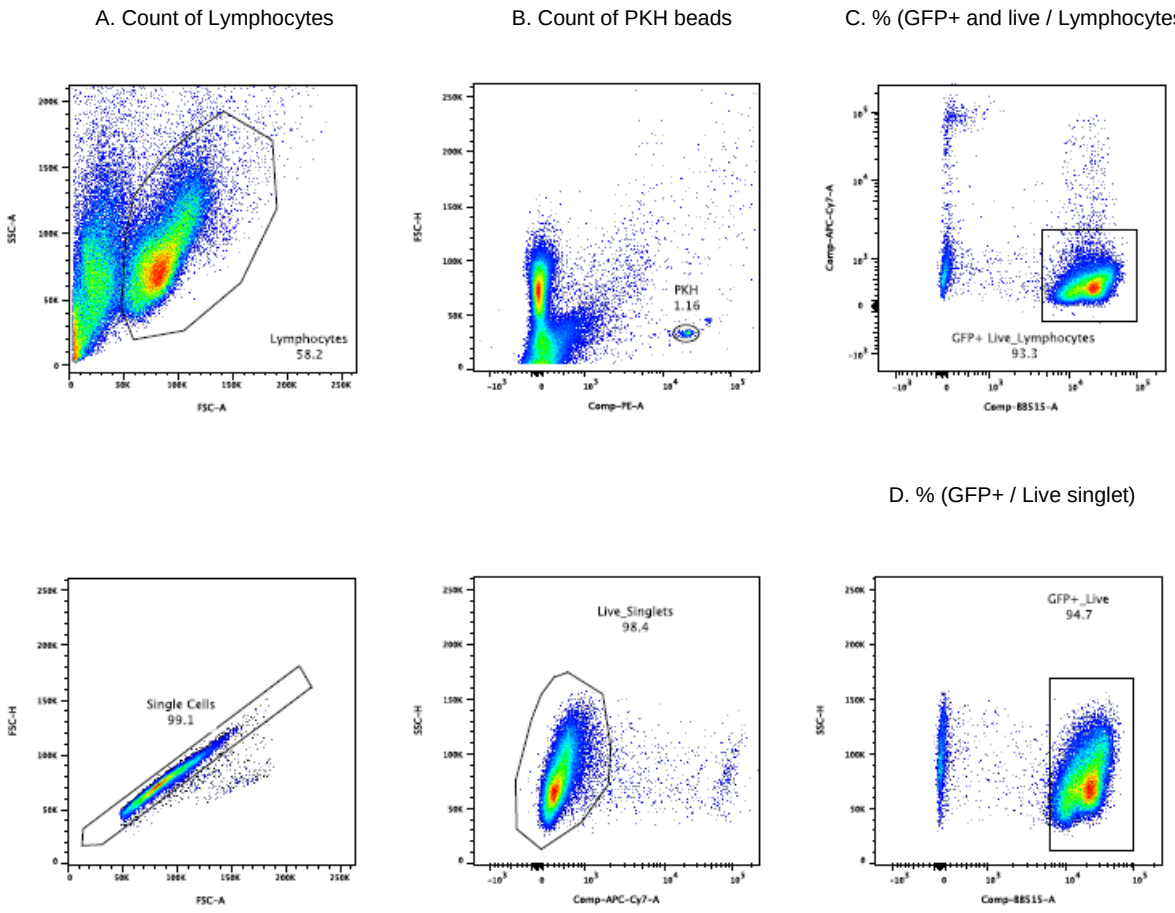




Supplementary Figure 5. Original western blots for Extended Figure 8g. The full, uncut gel images of Extended Figure 8g (a) are shown. Control Tubulin was run on the same gel as loading control. IRDye 800CW goat anti-rabbit secondary antibody was used to detect Tubulin while IRDye 680RD goat anti-mouse secondary was used to detect FLAG. In figure b only channel 700 (left) or 800 (right) is shown.



Supplementary Figure 6. Original western blots for Extended Figure 10b and Extended Figure 11a. The full, uncut gel images of Extended Figure 10b (a) and Extended Figure 11a (b) are shown. Control Tubulin was run on the same gel as loading control. IRDye 800CW goat anti-rabbit secondary antibody was used to detect Tubulin while IRDye 680RD goat anti-mouse secondary was used to detect SDHA. In figure a-d both 700 and 800 channels are shown.



Supplementary Figure 7. T-ALL Gating Strategy. Representative flow plots from the bone marrow cells of a QPC-WT recipient. To determine the number of GFP+ cells, the flow plots in the upper row of the figure were used. The number was calculated using the following equation: (A / B) x PKH bead concentration (beads/ml) x total volume of cell suspension (ml) x C. To determine the percentage of GFP+ cells, the flow plots in the lower row of the figure were used. The percentage was calculated as D.

sgRNA ID	Strand	Sequence
Human-sgNDUFS_1	–	GGTCACTCACCATTCCAAGG
Human-sgNDUFS2_2	+	CTGCAGCCGGAGTAAGATGG
Human-sgSDHA_1	–	GCCCATCACCTCGACCACGG
Human-sgDHODH_1	–	ATAGAAACGCTCATCTCCCG
Human-sgDHODH_2	+	GCTGCAGGATTTGACAAGCA
Human- sgNon-targeting	NA	GTAGCGAACGTGTCCGGCGT
Mouse-sgQPC_1	–	GATCCCTACAGCGTTTGTAG
Mouse- sgNon-targeting	NA	GCGAGGTATTCGGCTCCGCG

Supplementary Table 1. sgRNA sequences.