SUPPLEMENTARY INFORMATION

A CRISPR screen identifies a pathway required for paraquat-induced cell death.

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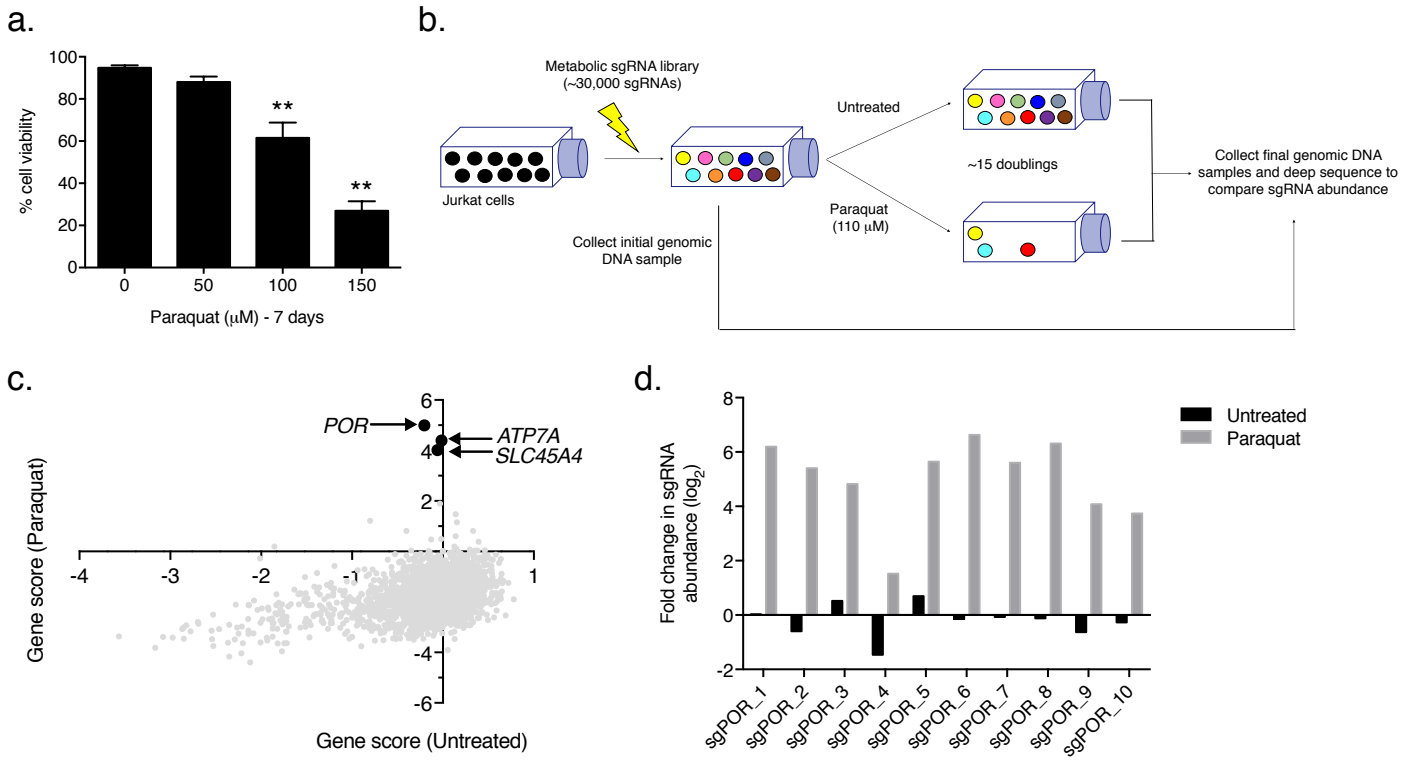
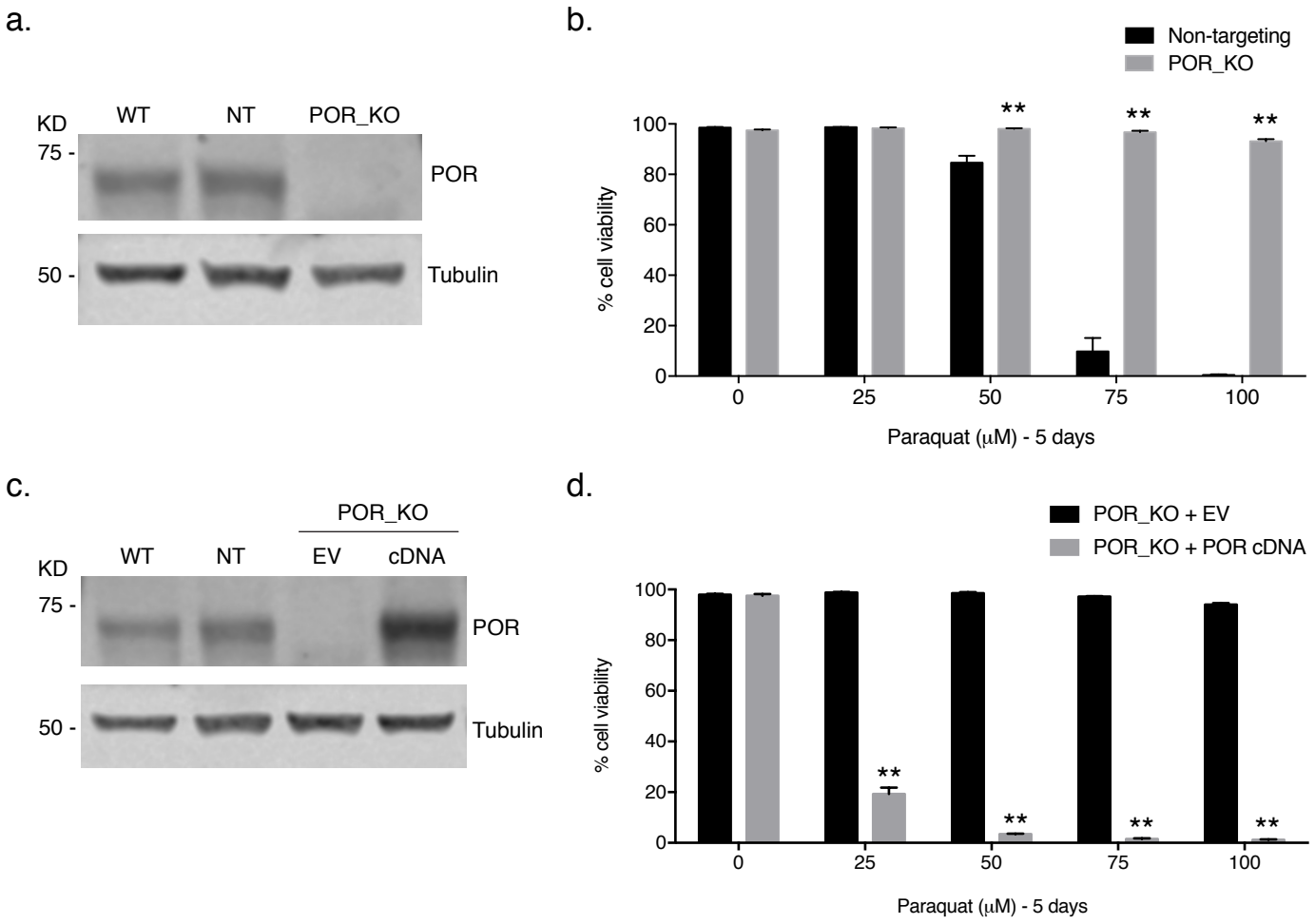
Supplementary Results



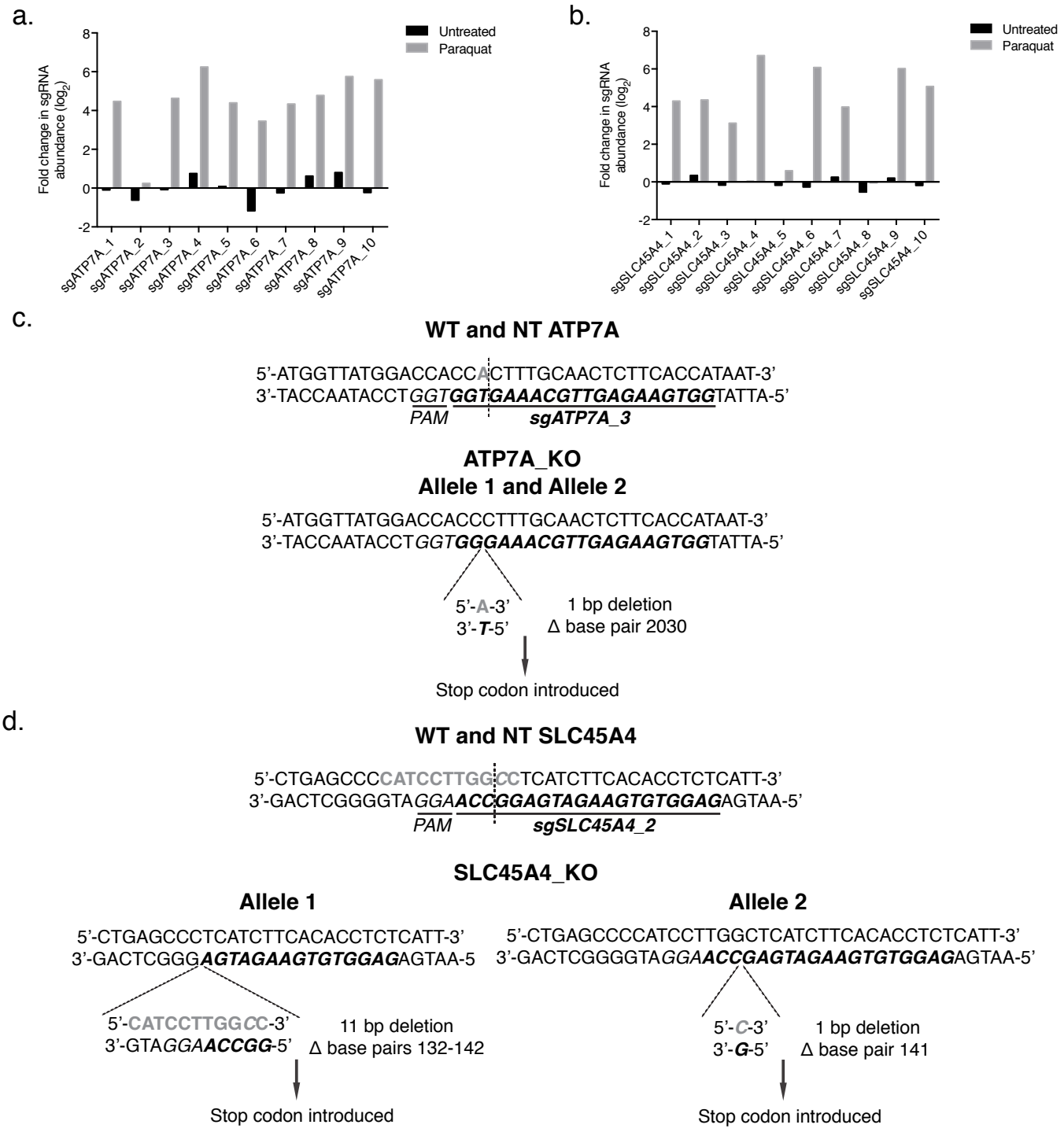
Supplementary Figure 1. Paraquat (PQ) positive selection CRISPR-based screen.

(**a**) Concentration-dependent effects of PQ on wildtype Jurkat cell viability. Mean ± SEM (n=4 independent experiments). ** indicates significance $p < 0.01$ compared to the 0 μM PQ condition. (**b**) Schematic of the PQ positive selection CRISPR-based screen. (**c**) A dot plot showing the post-screen median $\log_2$ fold change in the abundance of all single guide RNAs (sgRNAs) targeting a particular gene (termed gene score) in untreated versus PQ-treated (110 μM PQ) Jurkat cells. The gene scores for *POR, ATP7A, and SLC45A4* are indicated. Most genes, as well as non-targeting control sgRNAs, have a negative score after treatment with PQ. (**d**) Post-positive selection screen changes in the abundance of individual sgRNAs targeting the highest scoring gene POR in the presence or absence of PQ. (**e**) Representative western blot analysis of POR protein levels in wildtype (WT) Jurkat cells as well as clonal non-targeting (NT) and POR-null Jurkat cells (sgPOR_1 and sgPOR_8 from Supplementary Fig. 1d were used to generate the POR_KO1 and POR_KO2 clones, respectively). Tubulin was used as a loading control. (**f**) Representative western blot analysis of POR protein levels in two POR-null Jurkat clones stably transfected with either empty vector (EV) or human POR cDNA. The corresponding full, uncut gel images are shown in Supplementary Figure 8.



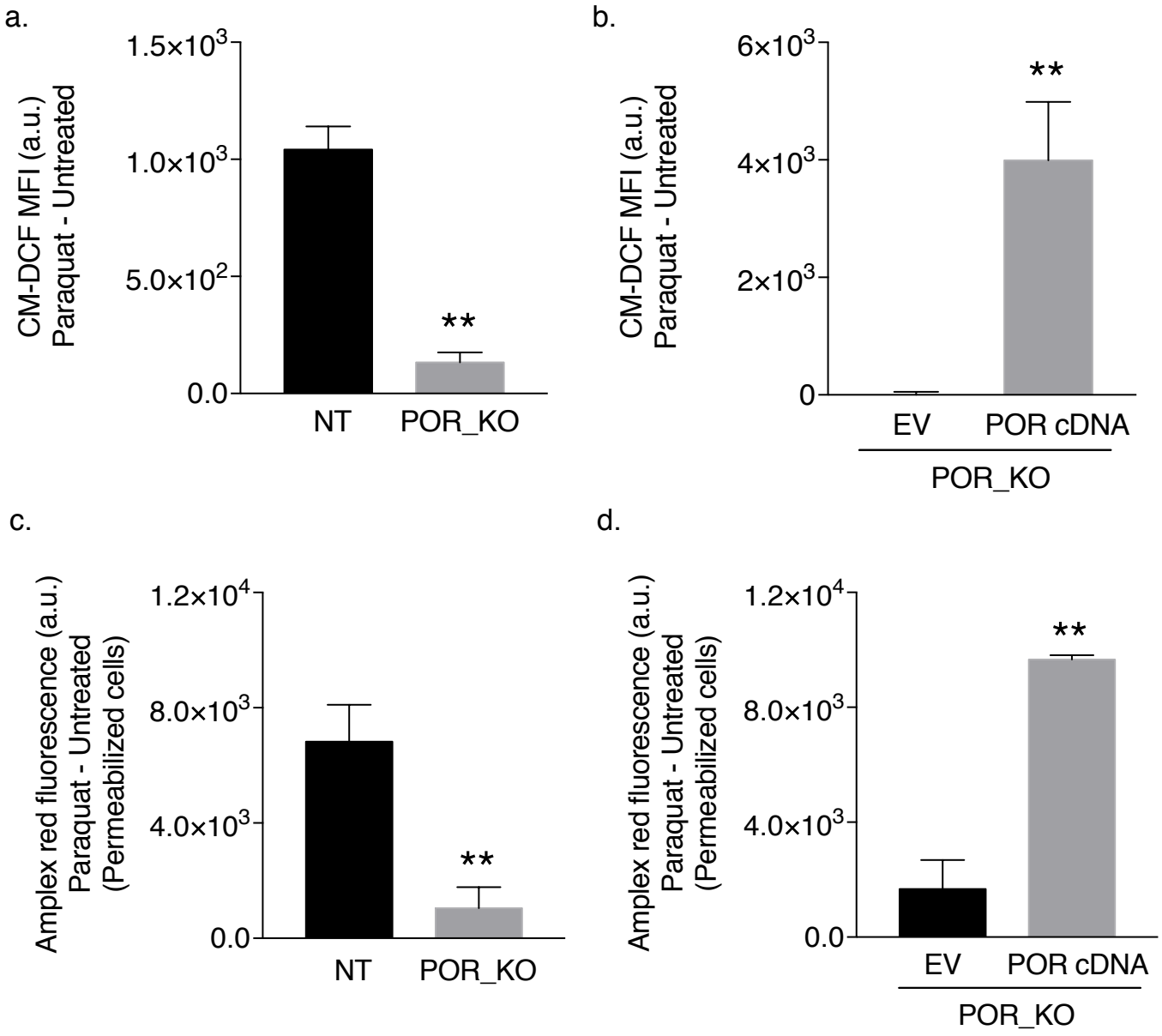
Supplementary Figure 2. Validation of the paraquat (PQ) positive selection CRISPR-based screen in A549 cells.

(**a**) Representative western blot analysis of POR protein levels in wildtype (WT) A549 cells as well as clonal non-targeting (NT) and POR-null A549 cells (sgPOR_6 from Supplementary Fig. 1d was used to generate the POR_KO A549 clone shown). (**b**) Non-targeting control and POR-null A549 cells were treated with the indicated concentrations of PQ for 5 days and live cell viability was assessed. (**c**) Representative western blot analysis of POR protein levels in POR-null A549 cells stably transfected with either empty vector (EV) or human POR cDNA. (**d**) EV- and POR cDNA-reconstituted POR-null A549 cells were treated with PQ for 5 days and live cell viability was determined. For Supplementary Figure 2a and 2c, the corresponding full, uncut gel images are shown in Supplementary Figure 9. For Supplementary Figure 2b and 2d, mean ± SEM (n=4 independent experiments) and ** indicates significance $p < 0.01$ compared to control cells.



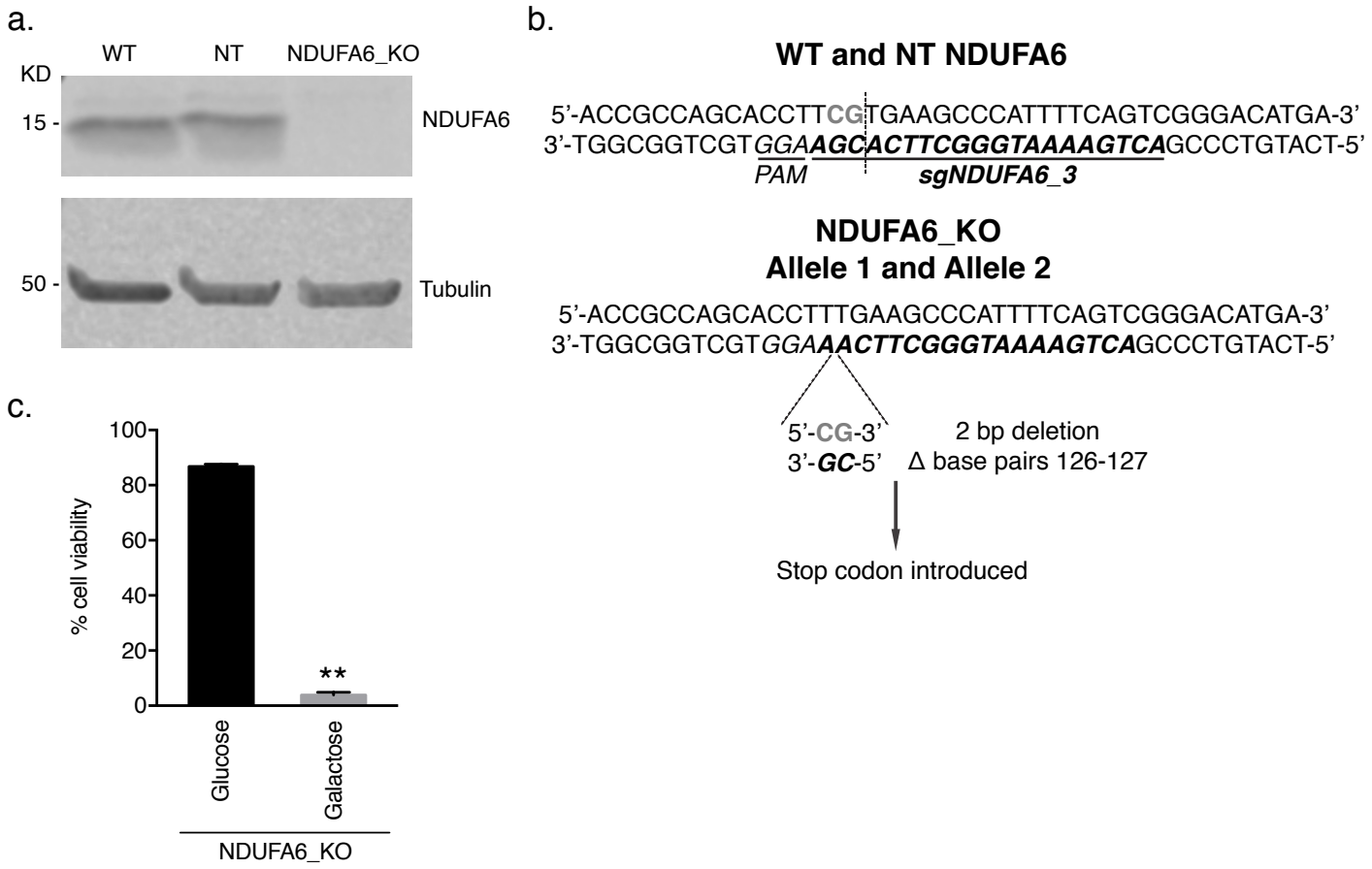
Supplementary Figure 3. Generation of ATP7A-null and SLC45A4-null Jurkat cells.

(**a**) Post-positive selection screen changes in the abundance of individual sgRNAs targeting the second highest scoring gene *ATP7A* in the presence or absence of PQ. (**b**) Post-positive selection screen changes in the abundance of individual sgRNAs targeting the third highest scoring gene *SLC45A4* in the presence or absence of PQ. (**c**) A portion of the wildtype (WT) and non-targeting (NT) ATP7A sequence is shown as well as the sequence of the mutant alleles of the ATP7A-null clone ATP7A_KO. CRISPR-mediated targeting of ATP7A with sgATP7A_3 (black bolded and italicized sequence; Supplementary Fig. 3a) generated a homozygous knockout. Both alleles contain the same single base pair (bp) deletion (shown in gray). This deletion causes a frameshift and introduces a stop codon just downstream, generating a truncated protein. (**d**) A portion of the wildtype (WT) and non-targeting (NT) SLC45A4 sequence is shown as well as the corresponding sequence of the mutant alleles of the SLC45A4-null clone SLC45A4_KO. CRISPR-mediated targeting of SLC45A4 with sgSLC45A4_2 (black bolded and italicized sequence; Supplementary Fig. 3b) generated a homozygous knockout. One allele contains an 11 base pair (bp) deletion (shown in gray) while the second allele contains a single bp deletion (shown in gray italics). Both deletions cause a frameshift and introduce a stop codon just downstream, generating a truncated protein. For Supplementary Figure 3c and 3d, the protospacer adjacent motif (PAM) sequence (black italics) and the Cas9 cut site (vertical black dashed line) are also shown.



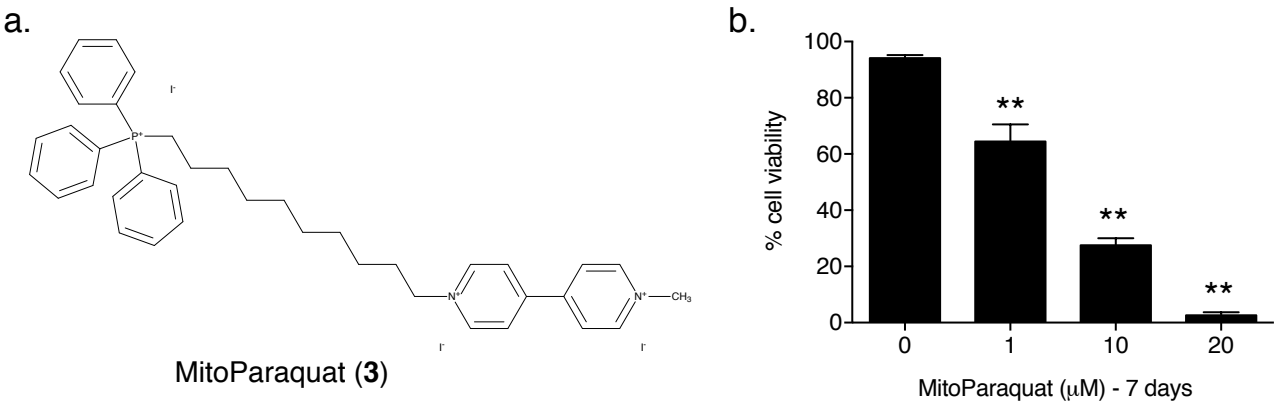
Supplementary Figure 4. POR is necessary for paraquat (PQ)-induced cytosolic ROS generation in A549 cells.

(**a**) Non-targeting (NT) control and clonal POR-null A549 cells were treated with 100 μM PQ for 48 hours and intracellular ROS levels were determined by flow cytometry and are presented as the mean fluorescence intensity (MFI) of CM-DCF. (**b**) Oxidation of CM-H2DCFDA was used to measure intracellular ROS in EV- and POR cDNA-reconstituted POR-null A549 cells treated with 25 μM PQ for 48 hours. (**c**) Amplex red was used to measure intracellular H_2O_2 production in saponin-permeabilized non-targeting (NT) control and clonal POR-null A549 cells treated with 100 μM PQ for 1 hour. (**d**) Intracellular H_2O_2 levels were measured by Amplex red in saponin-permeabilized clonal POR-null A549 cells reconstituted with either EV or POR cDNA following treatment with 100 μM PQ for 1 hour. Throughout Supplementary Figure 4, mean ± SEM (n=4 independent experiments) and ** indicates significance $p < 0.01$ compared to control cells. Data were background corrected to the mean value in untreated cells.



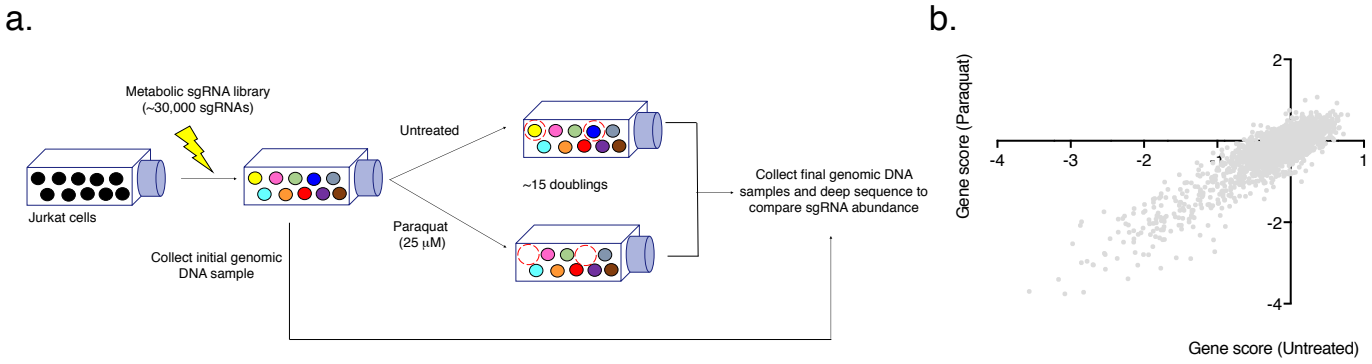
Supplementary Figure 5. Generation of NDUFA6-null Jurkat cells.

(**a**) Representative western blot analysis of NDUFA6 protein levels in wildtype (WT) Jurkat cells as well as clonal non-targeting (NT) and NDUFA6-null Jurkat cells (NDUFA6_KO clone). Tubulin was used as a loading control. Note, NDUFA6 is a subunit of mitochondrial complex I. The corresponding full, uncut gel image is shown in Supplementary Figure 10. (**b**) A portion of the wildtype (WT) and non-targeting (NT) NDUFA6 sequence is shown as well as the corresponding sequence of the mutant alleles of the NDUFA6-null clone NDUFA6_KO. CRISPR-mediated targeting of NDUFA6 with sgNDUFA6_3 (black bolded and italicized sequence) generated a homozygous knockout. Both alleles contain the same two base pair (bp) deletion (shown in gray). This deletion causes a frameshift and introduces a stop codon just downstream, generating a truncated protein. The protospacer adjacent motif (PAM) sequence (black italics) and the Cas9 cut site (vertical black dashed line) are also shown. (**c**) Clonal NDUFA6-null Jurkat cells (NDUFA6_KO) were cultured in media containing either 11.1 mM glucose or 11.1 mM galactose supplemented with 1 mM methyl pyruvate for 4 days and live cell viability was assessed. Mean ± SEM (n=4 independent experiments) and ** indicates significance $p < 0.01$ compared to control cells.



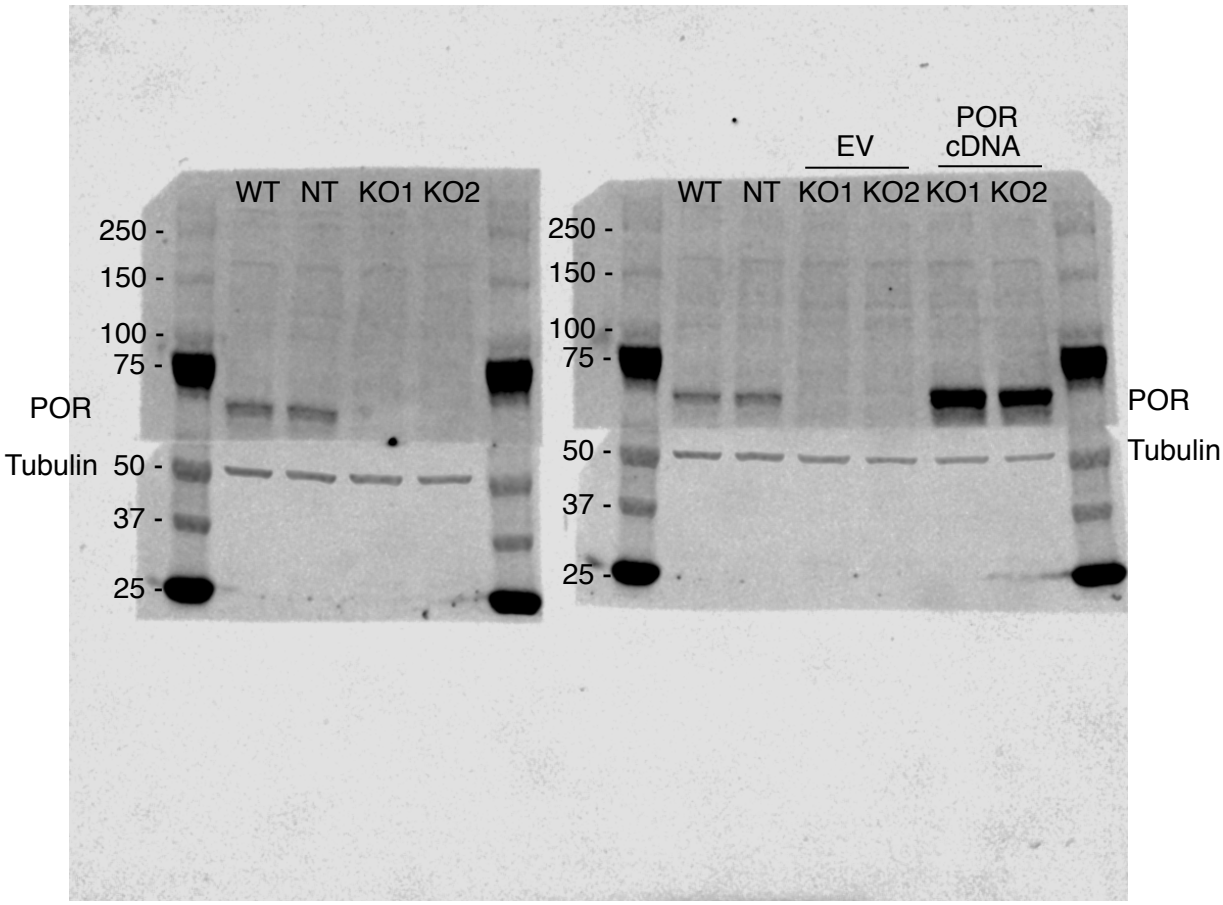
Supplementary Figure 6. Mitochondrial-targeted paraquat (MitoPQ) induces cell death.

(**a**) Structure of mitochondrial-targeted paraquat (MitoParaquat; MitoPQ). (**b**) Concentration-dependent effects of MitoPQ on non-targeting control Jurkat cell viability. Mean ± SEM (n=4 independent experiments) and ** indicates significance $p < 0.01$ compared to control cells.



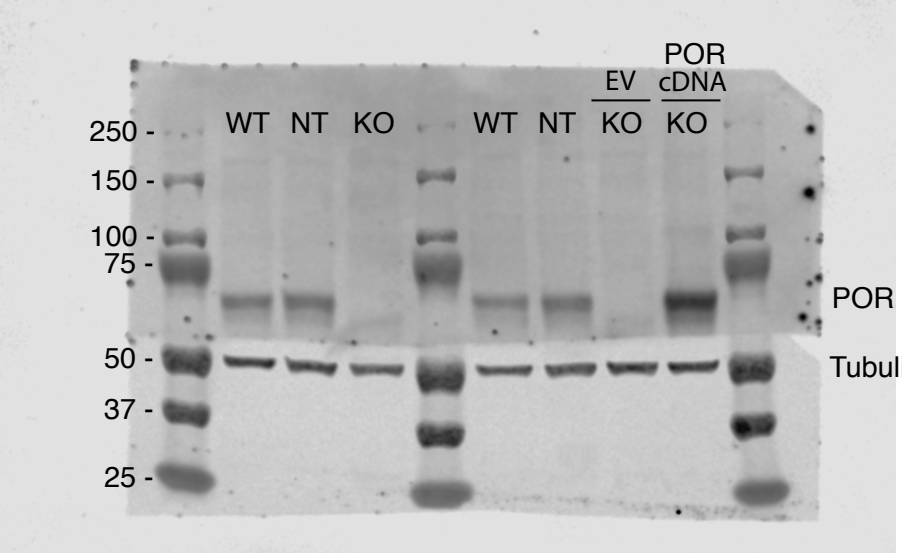
Supplementary Figure 7. Paraquat (PQ) negative selection CRISPR-based screen.

(**a**) Schematic of the PQ negative selection CRISPR-based screen. (**b**) A dot plot showing the post-screen median $\log_2$ fold change in the abundance of all sgRNAs targeting a particular gene (termed gene score) in untreated versus PQ-treated (25 μM PQ) Jurkat cells.



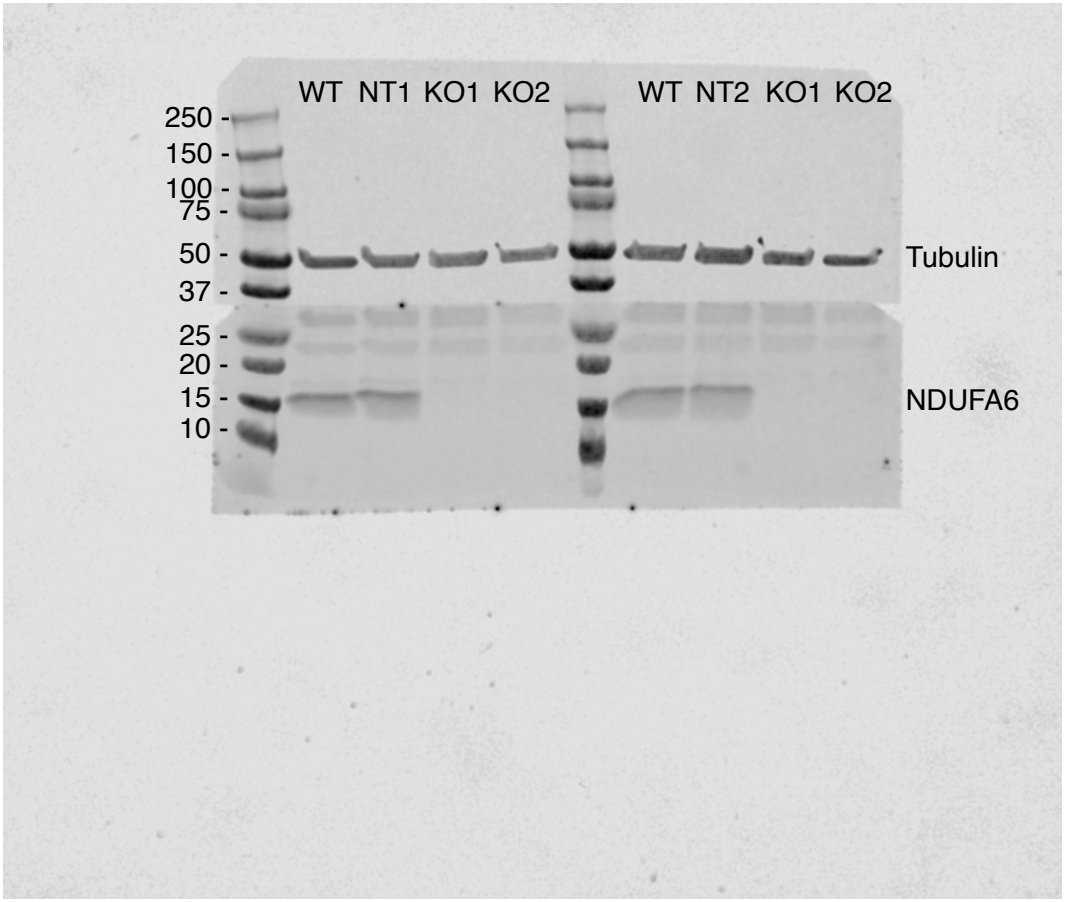
Supplementary Figure 8. Original western blot for Supplementary Figure 1e and 1f.

The full, uncut gel images of Supplementary Figure 1e (left) and Supplementary Figure 1f (right) are shown.



Supplementary Figure 9. Original western blot for Supplementary Figure 2a and 2c.

The full, uncut gel images of Supplementary Figure 2a (left) and Supplementary Figure 2c (right) are shown.



Supplementary Figure 10. Original western blot for Supplementary Figure 5a.

The full, uncut gel image of Supplementary Figure 5a (left) is shown. Note, NT1 and KO1 as labeled here are the clonal NT and NDUFA6_KO Jurkat cell lines, respectively, used throughout this study.

sgRNA ID	Strand	Sequence
sgPOR_1	–	GCTGCTCTCTCTGACAGAGG
sgPOR_6	+	CAACATGGGAGACTCCCACG
sgPOR_8	–	TGCCTCGCATCCCGTAGCGG
sgATP7A_3	–	GGTGAAGAGTTGCAAAGTGG
sgSLC45A4_2	+	GAGGTGTGAAGATGAGGCCA
sgNDUFA6_3	+	ACTGAAAATGGGCTTCACGA
sgNon-targeting	NA	GTAGCGAACGTGTCCGGCGT

sgRNA Oligonucleotide ID	Sequence
sgPOR_1 F	5'–caccgGCTGCTCTCTCTGACAGAGG
sgPOR_1 R	5'–aaacCCTCTGTCAGAGAGAGCAGCc
sg POR_6 F	5'– caccgCAACATGGGAGACTCCCACG
sg POR_6 R	5'– aaacCGTGGGAGTCTCCCATGTTGc
sgPOR_8 F	5'– caccgTGCCTCGCATCCCGTAGCGG
sgPOR_8 R	5'– aaacCCGCTACGGGATGCGAGGCAc
sgATP7A_3 F	5'– caccgGGTGAAGAGTTGCAAAGTGG
sgATP7A_3 R	5'– aaacCCACTTTGCAACTCTTCACCc
sgSLC45A4_2 F	5'– caccgGAGGTGTGAAGATGAGGCCA
sgSLC45A4_2 R	5'– aaacTGGCCTCATCTTCACACCTCc
sgNDUFA6_3 F	5'– caccgACTGAAAATGGGCTTCACGA
sgNDUFA6_3 R	5'– aaacTCGTGAAGCCCATTTTCAGTc
sgNon-targeting F	5'– caccgGTAGCGAACGTGTCCGGCGT
sgNon-targeting R	5'– aaacACGCCGGACACGTTCGCTACc

Supplementary Table 1. sgRNA and sgRNA oligonucleotide sequences.

Oligonucleotide ID	Sequence
XhoI-POR F	5'– TAAGCACTCGAGGCCACCATGATCAACATGGGAGACTCCCACGTG
XhoI-POR R	5'– TAAGCACTCGAGCTAGCTCCACACGTCCAGGGAGTAG
sgATP7A_3 F	5'– TCTGTATTCCTGTAATGGGGCT
sgATP7A_3 R	5'– AGCTGAAAATAAACCTTGCCTG
sgSLC45A4_2 F	5'– AGTCACCGTCCTGCTCTTGT
sgSLC45A4_2 R	5'– TGCCCTCAAGGCAAAAACTA
sgNDUFA6_3 F	5'– TAGCCAATGGGAAAAGACATTC
sgNDUFA6_3 R	5'– CCACGTAAGCCGCTACCTC

Supplementary Table 2. Oligonucleotide sequences.