Supplementary Information

***Staphylococcus aureus* targets the purine salvage pathway to kill phagocytes**

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SI Materials and methods

Bacterial strains and growth media. Bacterial strains were grown in Luria Broth (LB, Becton Dickinson) or Tryptic Soy Broth (TSB, Becton Dickinson) supplemented with the appropriate antibiotics (ampicillin 100 μg/ml or kanamycin 50 μg/ml). All strains used in this study are listed in Supplementary Table S1.

Cell culture. HEK293FT cells were grown in DMEM medium (Gibco) supplemented 10% fetal bovine serum, 0.1 mM MEM non-essential amino acids, 6 mM L-glutamine, 1 mM sodium pyruvate, and 500 μg/ml geneticin. U937 cells were grown in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum. Cells were grown at 37°C under 5% CO_2. Mammalian cell lines used in this study are listed in Supplementary Table S2.

Lentivirus production. Lentiviral particles were harvested after 48-72 h infection with host cells using the Vira power kit (Thermo Fisher), and concentrated by using Lenti-X Concentrator (Clontech). Lentiviral particles were suspended in DMEM supplemented with 10% FBS, and 1% bovine serum albumin, and stored at -80°C.

Lentiviral transduction of U937 cells. U937 cells were transduced via spinfection in the presence of polybrene (Sigma, St. Louis, USA) at a multiplicity of infection (MOI) of approximately 0.3. Titers were determined by transducing U937 cells (1.0×10^6 cells/ml) grown in RPMI 1640 medium supplemented with 10% hi-FBS and 8 μg/ml polybrene with various volumes of lentiviral particles along with a non-virus containing control via spinfection (1,000 $\times g$ for 2 h at room temperature). Cell pellets were carefully suspended in RPMI 1640 medium containing 10% hi-FBS and incubated for 48 h at 37°C under 5% CO_2. Cells were centrifuged,

counted, and samples split in two wells with one well containing 2.5 μg/ml puromycin (Gibco). After 3 days, cells were counted, and the transduction efficiency was calculated as cell count from wells containing puromycin divided by cell count from wells without puromycin, and multiplied by 100.

CRISPR-Cas9 GeCKO screen. The human CRISPR-Cas9 GeCKO v2 library was a gift from Feng Zhang (1, 2) obtained via Addgene (Cambridge, MA, USA; Cat. Nr. 1000000048). The screen was performed as previously described (2). Briefly, the CRISPR-Cas9 GeCKO v2 plasmid library was amplified in *E. coli* Stbl3 cells. To produce lentiviral particles, the pooled CRISPR-Cas9 GeCKO v2 library from *E. coli* was transfected into HEK293FT cells. Next, 7.2×10^7 U937 cells were diluted in RPMI 1640 medium supplemented with 10% hi-FBS and 8 μg/ml polybrene to a final concentration of 1.0×10^6 cells/ml and transduced with lentiviral particles via spinfection. Cell pellets were suspended in fresh RPMI 1640 medium containing 10% hi-FBS, incubated for 48 h, and then selected with 2.5 μg/ml puromycin for 7 days to complete gene editing. For each reaction, puromycin-resistant U937 cells (5×10^7 cells) were intoxicated with dAdo (5 μM) and dCF (50 μM) or, as a control, were treated with dCF (50 μM) alone. During these procedures, cells were passaged every 3-4 days and continuously grown in the presence of dAdo/dCF or dCF alone. On day 28 post intoxication, the screen was terminated and the genomic DNA from 3.0×10^7 cells was isolated using the Blood & Cell Culture DNA Midi Kit (Quiagen, Hilden, Germany) and stored at -20°C.

CRISPR-Cas9-mediated gene disruption. LentiCRISPR v2 plasmids (1) containing individual sgRNAs for targeted gene disruption were purchased from Genscript (Piscataway, USA) (Supplementary Table S3), maintained in *E. coli* Stbl3 cells, and used to produce lentiviral

particles for transduction of U937 cells. sgRNAs used were: TTATCTTCAAGCCACTCCAG (*DCK*), ACAGCAGAGATGTCAAGCAG (*ADK*), and TGAACTGAGCAAGGACGCCC (*SLC29A1*). For simultaneous deletion of *ADK* and *DCK*, lentiCRISPR v2 plasmids containing *ADK* and *DCK* targeting sgRNAs were co-transduced into U937 cells. Following transduction, cells were selected with puromycin (2.5 μg/ml) for 7 days to complete gene editing. Single cells were isolated, and clonally expanded and genomic DNA was isolated with the DNeasy Blood & Tissue Kit (Quiagen, Hilden, Germany). Genomic regions targeted by the corresponding sgRNA and Cas9 were amplified by polymerase chain reaction (PCR) with primers listed in Supplementary Table S4, and cloned via the Zero Blunt™ TOPO™ PCR Cloning Kit (Thermo Fisher). Candidate plasmids from various *E. coli* clones were subjected to sequencing to confirm bi-allelic gene disruptions.

Next generation sequencing (NGS), Sanger sequencing and bioinformatics. DNA samples obtained from the CRISPR-Cas9 GeCKO screen were used to prepare a sgRNA library with a two-step PCR protocol (1, 2). The first PCR was used to amplify the sgRNA containing cassette. Amplified products were subjected to a second PCR with a primer pair encoding a unique 8-bp barcode required for multiplexing along with a stagger sequence to increase library complexity, as described [http://genome-engineering.org/gecko; (1, 2)]. Products of the second PCR were purified, pooled, diluted and mixed with 10% PhiX, and sequenced with HiSeq2500 (Illumina).

The raw sequencing data were processed and analyzed using customized CRI CRISPR-Cas9 library screen pipelines. Briefly, sequencing reads were first de-multiplexed by using the barcode in the reverse primer, and processed by Cutadapt (3) to remove sequences from

beginning to sgRNA priming site primers. Trimmed reads were used to map sgRNA sequences to pooled GeCKO v2 libraries A and B. Read counts of sgRNA for each sample were quantified by MAGeCK v5.6.0 (4). Count data were filtered and normalized, and essential sgRNA and genes were ranked by MAGeCK (4).

DNA samples obtained from the CRISPR-Cas9 GeCKO screen were also used to analyze the distribution of individual sgRNAs via Sanger sequencing. Here, the sgRNA cassette was PCR-amplified from the dAdo-resistant U937 cell population, and cloned via the Zero Blunt™ TOPO™ PCR Cloning Kit (Thermo Fisher). 80 independent *E. coli*-derived plasmids containing the sgRNA cassette were sequenced, and used to identify genes targeted by the individual sgRNA. All sgRNA sequences recovered were mapped to pooled GeCKO v2 libraries A and B.

Immunoblotting. U937 cells were washed twice in ice-cold 1 × PBS, and lysed for 30 min in ice-cold lysis buffer (1 × PBS and 0.5 % Triton X-100) containing the protease inhibitor cocktail from Pierce. During this procedure, cells were sonicated 6 times (5 seconds pulse each) by using an ultrasonicator instrument. Cell lysates were centrifuged for 10 min at 15,000 ×*g* and 4°C. Supernatants were mixed with sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) loading buffer and boiled at 95°C for 10 min. Proteins were separated on 12% SDS-PAGE and transferred onto PVDF membranes for immunoblot analysis with the following rabbit primary antibodies: α-Adenosine kinase (α-ADK, PA5-27399, Thermo Fisher), α-deoxy-Cytidine kinase (α-DCK, PA5-21846, Thermo Fisher), α-hENT1 (PA5-11226, Thermo Fisher), and α-GAPDH (PA1-987, Thermo Fisher) which served as a loading control. Immunoreactive signals were revealed by using secondary antibody conjugated to horseradish peroxidase (Cell signaling, Danvers, MA, USA); horseradish peroxidase activity was detected with enhanced chemilumescent (ECL) substrate.

Cytotoxicity assays. dAdo-mediated cytotoxicity was analyzed as described elsewhere with minor modifications (5). Briefly, 2.0×10^5 U937 cells per well were seeded in a 24-well plate, and incubated for 24 h at 37°C under 5% CO_2 in RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 50 µM pentostatin (dCF), and 5 uM dAdo. Where indicated, recombinant NXPH1 (Novoprotein) and other small molecule inhibitors of DCK, ADK, hENT1 or p38 were added to the wells, namely deoxycytidine (Sigma), 5'-amino-5'-deoxyadenosine (Glentham Life Science), dipyridamole (Dipy., Sigma), or SB 203580 (Sigma). Dead cells were stained with Trypan Blue, and counted by using a microscope to calculate killing efficiency.

To analyze dAdo-mediated cytotoxicity of U937-derived macrophages, 4.0×10^5 U937 cells per well were seeded in a 24-well plate, and incubated for 48 h at 37°C under 5% CO_2 in RPMI1640 medium containing 10% heat-inactivated fetal bovine serum and 160 nM phorbol 12-myristate 13-acetate (PMA). Differentiated cells were washed once, and further incubated in normal growth media lacking PMA for 24 h. Media were replaced by normal growth media containing 50 µM pentostatin (dCF), and 10 uM dAdo as indicated. Cells were further incubated for 24 h, detached using Trypsin-EDTA solution, and viability quantified with Trypan Blue staining.

Cytotoxicity mediated by *S. aureus*-derived dAdo was analyzed by incubating *S. aureus* strains in the presence of Thymus DNA, and subsequent incubation with U937 cells. Briefly, *S. aureus* Newman or mutant strains were grown overnight in TSB, diluted 1:100 in RPMI 1640 medium, and grown at 37°C to 5.0×10^7 colony forming units (CFU)/ml. Next, 6.0×10^7 CFU were incubated in RPMI 1640 containing 28 µg/ml Thymus DNA (Sigma) for 3 h at 37°C. Controls

were equally processed, and lacked bacteria or DNA, or included *S. aureus* mutants that cannot generate dAdo precursors (*nuc*) or cannot produce dAdo (*adsA*). Bacteria were removed by centrifugation, culture supernatants filter-sterilized and incubated with 2.0×10^5 U937 wild-type or mutant cell lines per well, or with 4.0×10^5 U937-derived macrophages (24-well plate) in the presence of 50 µM dCF. Cells were incubated for 24 h at 37°C under 5% CO_2, and killing efficiency was quantified with Trypan Blue staining.

Analysis of caspase-3 activity. Caspase-3 activity was determined using the colorimetric caspase-3 detection kit (Sigma). Briefly, wild-type and mutant U937 cells were incubated for 24 h at 37°C under 5% CO_2 with dCF (50 µM) and dAdo (5 and 10 µM for U937 cells and U937-derived macrophages, respectively). Cells (1.0×10^7) were washed once in PBS and lysed on ice for 20 min in lysis buffer (Sigma kit). Lysates were centrifuged (18,000 ×*g* for 10 min at 4°C) and supernatants incubated with acetyl-DEVD-pNA, a substrate of caspase-3. The capase-3 inhibitor Ac-DEVD-CHO was used in control experiments (Sigma kit). Caspase-3 activity was measured in µmol of pNA released per min per ml of cell lysate.

Deoxyadenosine uptake studies. Uptake of deoxyadenosine into U937 cells was performed as described previously with minor modifications (6). Briefly, 1.5×10^6 U937 cells were suspended in 400 µl growth medium and seeded into 24-well plates. 1 µCi [^{3}H]deoxyadenosine was added (Moravek Biochemical; 38.1 Ci/mmol) and cells incubated for 1 h at 37°C under 5% CO_2. In control experiments, cells were treated with [^{3}H]deoxyadenosine and 10 µM dipyridamole to block hENT1. Cells were washed twice with cold PBS and lysed by the addition of 100 µl 1 M NaOH for 5 min at room temperature. Samples were neutralized by the addition of 100 µl 1 M HCl and 187.5 µl of this sample was added to 4.8 ml scintillation

cocktail (Ultima Gold™ LSC Cocktail, Sigma). Radioactivity in cell pellets was quantified by liquid scintillation counting (LSC). The remaining 12.5 µl of neutralized samples were used to determine the protein content via the biconchoninic acid (BCA) assay (Pierce). dAdo uptake levels are reported as pmol/mg protein.

Quantification of nucleotides. In brief, 1.5×10^6 U937 cells were suspended in 400 µl growth medium and seeded into 24-well plates in the presence of 0.5 µCi [^{14}C]deoxyadenosine (American Radiolabeled Chemical; 48.8 mCi/mmol) and dCF (50 µM) for 4 h at 37°C under 5% CO_2. Cells were washed twice with cold PBS, and treated with 500 µl ice-cold 60% methanol to extract cellular nucleosides and nucleotides (7). Samples were vigorously vortexed for 20 sec and incubated at 95°C for 3 min. Aliquots (25 µl) were removed to determine the protein concentration via the BCA method. The remaining sample was centrifuged to remove cell debris and insoluble material for 5 min at 16,000 $\times g$. Supernatants containing soluble nucleosides and nucleotides were removed, dried, suspended in 15 µl deionized water and applied (3 µl) to TLC plates (SIL G, Macherey-Nagel). The migratory positions of dAdo, dAMP, AMP, dADP and dATP were identified with non-radiolabeled standards that were visualized under UV light at 254 nm. TLC sheets were developed in the ascending direction at room temperature by using a water/isopropanol/ammonium bicarbonate mixture (25%:75%:0.2 M). Chromatograms were developed by autoradiography. Individual dATP, dADP, and dAMP spots were excised from TLC plates and radioactivity quantified by LSC.

Sequencing chromatograms and statistical analysis. Sequencing chromatograms were generated with DNASTAR (DNASTAR software, Inc., Madison WI, USA, Version 12.0.0). Statistical analysis was performed with GraphPad Prism (GraphPad Software, Inc., La Jolla USA, Version

5.04). Statistically significant differences were calculated by using statistical methods as indicated. *P* values ≤ 0.05 were considered significant.

***SI* Tables**

Table S1 – Bacterial strains used in this study

Bacterial strain	**Descriptions**	**Reference**
E. coli TOP10	One Shot® TOP10 chemically competent *E. coli*	Invitrogen
E. coli Stbl3	Host strain used for plentiCRISPR v2 constructs	Thermo Fisher
S. aureus Newman	wildtype, clinical isolate	(8)
S. aureus Newman *nuc*	Newman *nuc::ermB*	(5)
S. aureus Newman *adsA*	Newman *adsA::ermB*	(5)

Table S2 – Mammalian cell lines used in this study

Cell line	**Descriptions**	**Reference**
U937 wild type	U937 cell line, ATCC® CRL-1593.2™	ATCC
U937 *DCK*$^{-/-}$	U937, bi-allelic deletion in *DCK*	This study
U937 *ADK*$^{-/-}$	U937, bi-allelic deletion in *ADK*	This study
U937 *SLC29A1*$^{-/-}$	U937, bi-allelic deletion in *SLC29A1*	This study
U937 *ADK*$^{-/-}$ *DCK*$^{-/-}$	U937, bi-allelic deletion in *ADK/DCK*	This study
HEK293FT	293FT cell line	Thermo Fisher

Table S3 –Plasmids used in this study

Plasmid	**Descriptions**	**Reference**
plentiCRISPRv2	Cas9/gRNA expression plasmid, 3rd generation system	(1)
plentiCRISPRv2-*DCK*-gRNA5	contains gRNA target sequence TTATCTTCAAGCCACTCCAG targeting *DCK*	Genscript, USA
plentiCRISPRv2-*ADK*-gRNA6	contains gRNA target sequence ACAGCAGAGATGTCAAGCAG targeting *ADK*	Genscript, USA
plentiCRISPRv2-*SLC29A1*-gRNA4	contains gRNA target sequence TGAACTGAGCAAGGACGCCC targeting *SLC29A1*	Genscript, USA
pCR™Blunt II-TOPO®	Plasmid used for cloning and sequencing	Thermo Fisher
ViraPower™ Lentiviral Packaging Mix	contains three packaging plasmids pLP1, pLP2, and pLP/VSVG	Thermo Fisher

Table S4 – Oligonucleotides used to confirm gene disruption in U937 KO cell lines

Primer	Sequence	Application	Reference
DCK-up	ACTTAATGGGGTCCTCACTCCGTATTTTGA	cloning and genotyping	This study
DCK-dn	TATTTCTTGATATGGACCTCCATCAACATTGCCAT	cloning and genotyping	This study
ADK-up	ATTCTCATTGGTGTGAGATGGTGTCTCATTG	cloning and genotyping	This study
ADK-dn	GGAAACAGCAGAGCTTCATATCCTCTGAAT	cloning and genotyping	This study
SLC29A1-up	ATACAAAGCTGTCTGGCTTATCTTCTTCATG	cloning and genotyping	This study
SLC29A1-dn	CAATTAATGAGCACGATCTTGATCATGGTGAT	cloning and genotyping	This study

***SI* Figures and Legends**

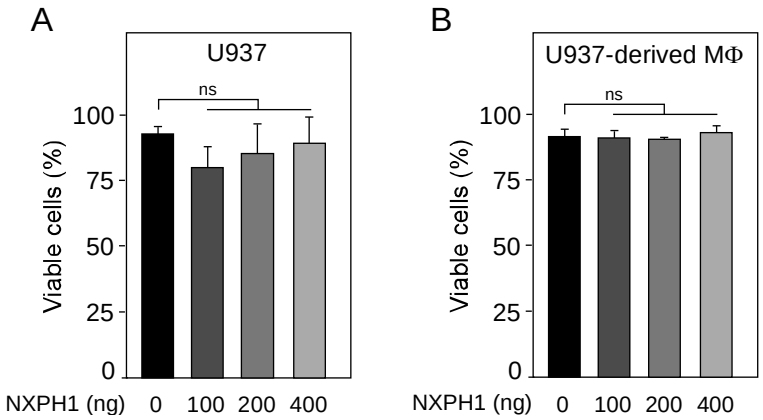

Figure S1 – Human Neurexophilin 1 does not affect the viability of U937 cells and macrophages. Survival of U937 (*A*) or U937-derived macrophages (*B*) treated with recombinant Neurexophilin at various concentrations (0-400 ng). Cell viability was analyzed after 24 h. Statistical analyses were performed by one-way ANOVA with Bonferroni's multiple comparison test; ns: not significant.

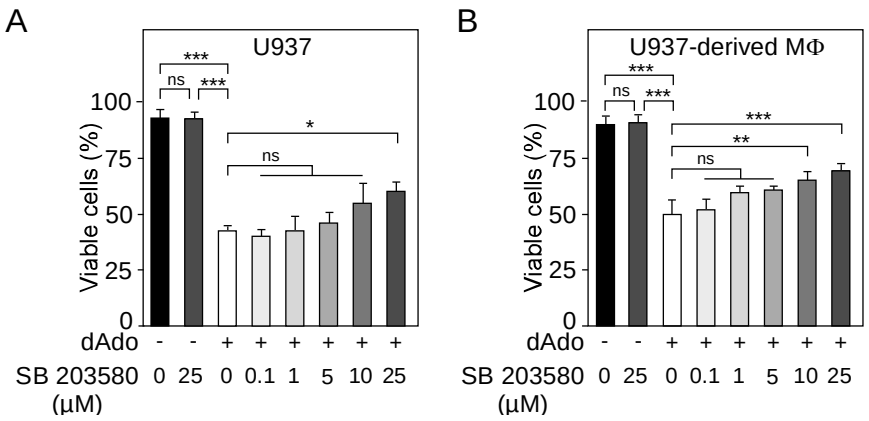

Figure S2 – Disruption of the TAO kinase 1 pathway by p38 inhibition reduces deoxyadenosine-induced cell death in human macrophages. Survival of U937 (*A*) or U937-derived macrophages (*B*) treated with dAdo/dCF and increasing concentrations of SB 203580, an inhibitor of p38, 50 μM dCF alone or dCF with 25 μM SB 203580. Cell viability was analyzed after 24 h. Data are the mean (±SD) of three independent determinations. Statistical analyses were performed by one-way ANOVA with Bonferroni's multiple comparison test; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

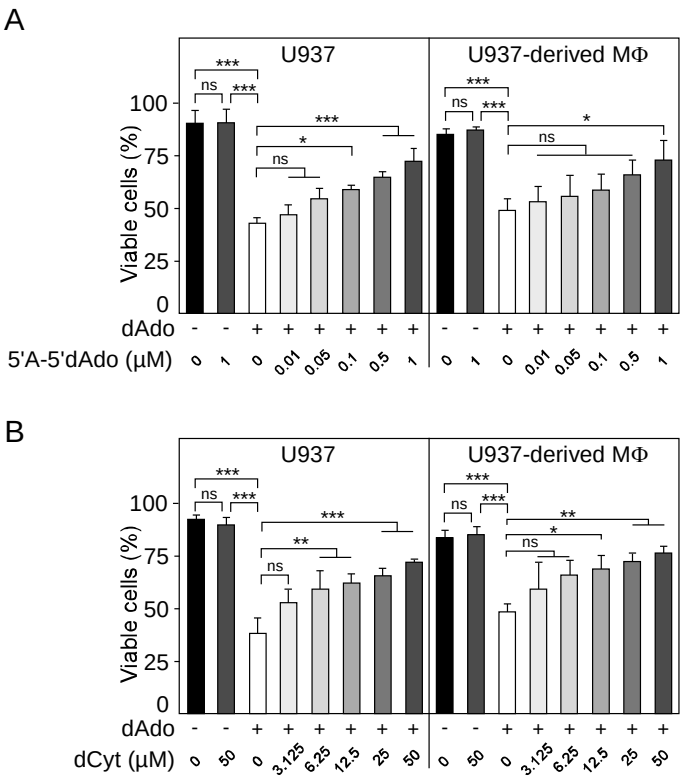

Figure S3 – Inhibition of purine salvage pathway kinases prevents deoxyadenosine-induced cell death in human macrophages. Survival of U937 cells or U937-derived macrophages treated with dAdo/dCF and increasing concentrations of 5'-amino-5'-deoxyadenosine (an inhibitor of ADK, *A*), deoxycytidine (a competing substrate of DCK, *B*) or 50 μM dCF alone or dCF with either 1 μM 5'-amino-5'-deoxyadenosine or 50 μM deoxycytidine. Cell viability was analyzed after 24 h. Data are the mean (±SD) of three independent determinations. Statistical analyses were performed by one-way ANOVA with Bonferroni's multiple comparison test; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

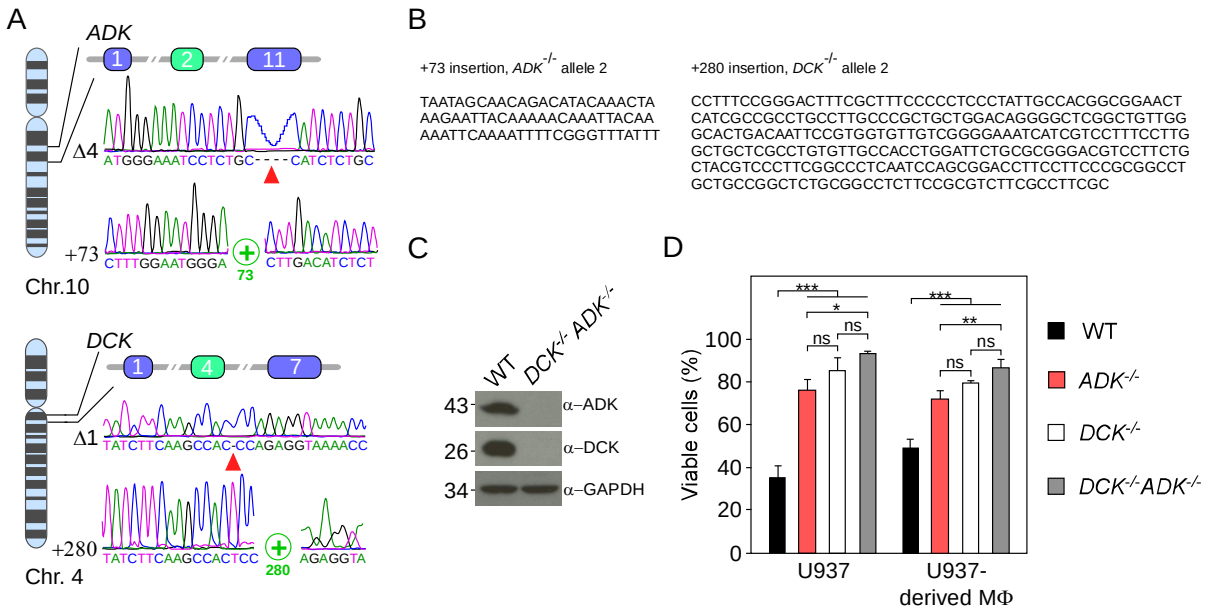

Figure S4 – Simultaneous inactivation of *ADK* and *DCK* prevents deoxyadenosine-induced cell death in human macrophages. (*A*) Diagram illustrating the position of *ADK* on chromosome 10 and exons 1, 2 and 11 of *ADK* mRNA as well as the position of *DCK* on chromosome 4 and exons 1, 4 and 7 of *DCK* mRNA. Sequencing results for mutated *ADK* exon 2 alleles or *DCK* exon 4 alleles cloned from *DCK*$^{-/-}$ *ADK*$^{-/-}$ cells (green boxes). *(B)* Insertion sequences identified in one allele of each *ADK,* or *DCK* in the U937 *DCK*$^{-/-}$ *ADK*$^{-/-}$ cell line. (*C*) Immunoblotting of lysates from wild-type (WT) U937 cells and their *DCK*$^{-/-}$ *ADK*$^{-/-}$ variant with ADK-, DCK- and GAPDH-specific antibodies. Numbers to the left of blots indicate the migration of molecular weight markers in kDa. (*D*) Survival of undifferentiated or differentiated U937 macrophages and their *DCK*$^{-/-}$ *ADK*$^{-/-}$ variants after 24 h dAdo treatment. Data are the mean (±SD) of three independent determinations. Statistical analyses were performed by one-way ANOVA with Bonferroni's multiple comparison test; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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