

Supplementary Materials

Table S1. Oligonucleotide sequences used to generate NADSYN1 deficient A549 and HEK293T cells

NADSYN1 #1	Forward: GCAAATTGCCCTCGAAGTCC Reverse: GGACTTCGAGGGCAATTTC
NADSYN1 #2	Forward: GCAGGACATCATCTGCGACGT Reverse: ACGTCGCAGATGATGTCCTGC

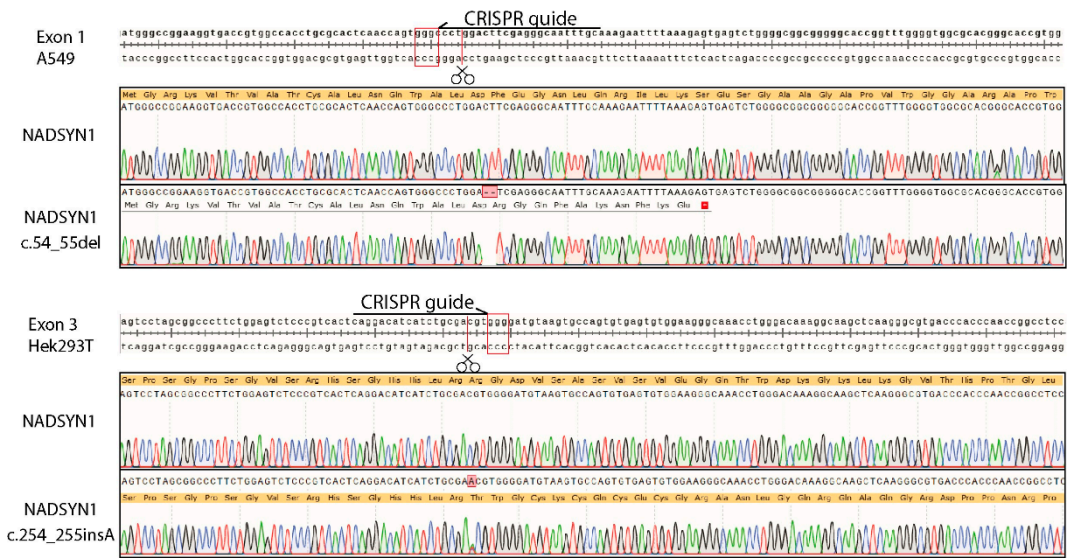
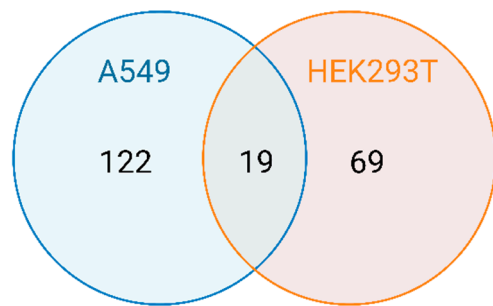


Figure S1. Sequences derived from genomic DNA of wild type and NADSYN1 deficient A549 and HEK293T cells.

Table S2. Primer sequences

NADSYN1 #1	Forward: gacctgtgtcttctgtctcc Reverse: cctgacctcaggtgacttcc
NADSYN1 #2	Forward: gctgtctattggaatcacct Reverse: ttacggggctgagataggaa



Metabolite	A549	HEK293T
1D-Myo-inositol 1,4-bisphosphate	Increased	Increased
2-Amino-3-carboxymuconic acid semialdehyde	Decreased	Decreased
2-Hexenoylcarnitine	Decreased	Decreased

D-Glyceraldehyde 3-phosphate	Increased	Increased
DOPA sulfate	Increased	Increased
D-Sedoheptulose 7-phosphate	Increased	Increased
Fructose 6-phosphate	Increased	Increased
Galactitol	Increased	Increased
Gluconolactone	Decreased	Decreased
Guanosine	Increased	Increased
L-Homocysteine sulfonic acid	Increased	Increased
Oxoglutaric acid	Decreased	Decreased
Prostaglandin E2	Decreased	Decreased
Sedoheptulose 1,7-bisphosphate	Increased	Increased
Ureidosuccinic acid	Decreased	Decreased
Uridine 2',3'-cyclic phosphate	Increased	Increased
(S)-5-Diphosphomevalonic acid	Decreased	Increased
Deoxyuridine	Decreased	Increased
Inosine	Increased	Decreased

**Figure S2.** Venn diagram depicting the overlap between significantly (based on  $FC > 2$  and  $P < 0.05$ ) altered metabolites between A549 and HEK293T cells as a consequence of NADSYN1-/- dependent NAD<sup>+</sup> deficiency. The 19 overlapping metabolites are represented in the table with an additional column indicating whether a metabolite is increased or decreased in A549 and HEK293T cells as a consequence of NADSYN1-/- dependent NAD<sup>+</sup> deficiency. Adapted from “Venn Diagram with Organs (Layout)”, by BioRender.com (2023). Retrieved from <https://app.biorender.com/biorender-templates>.