Supporting information

Title

Therapeutic assessment of targeting ASNS combined with L-Asparaginase treatment in solid tumors and investigation of resistance mechanisms

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Figure S1

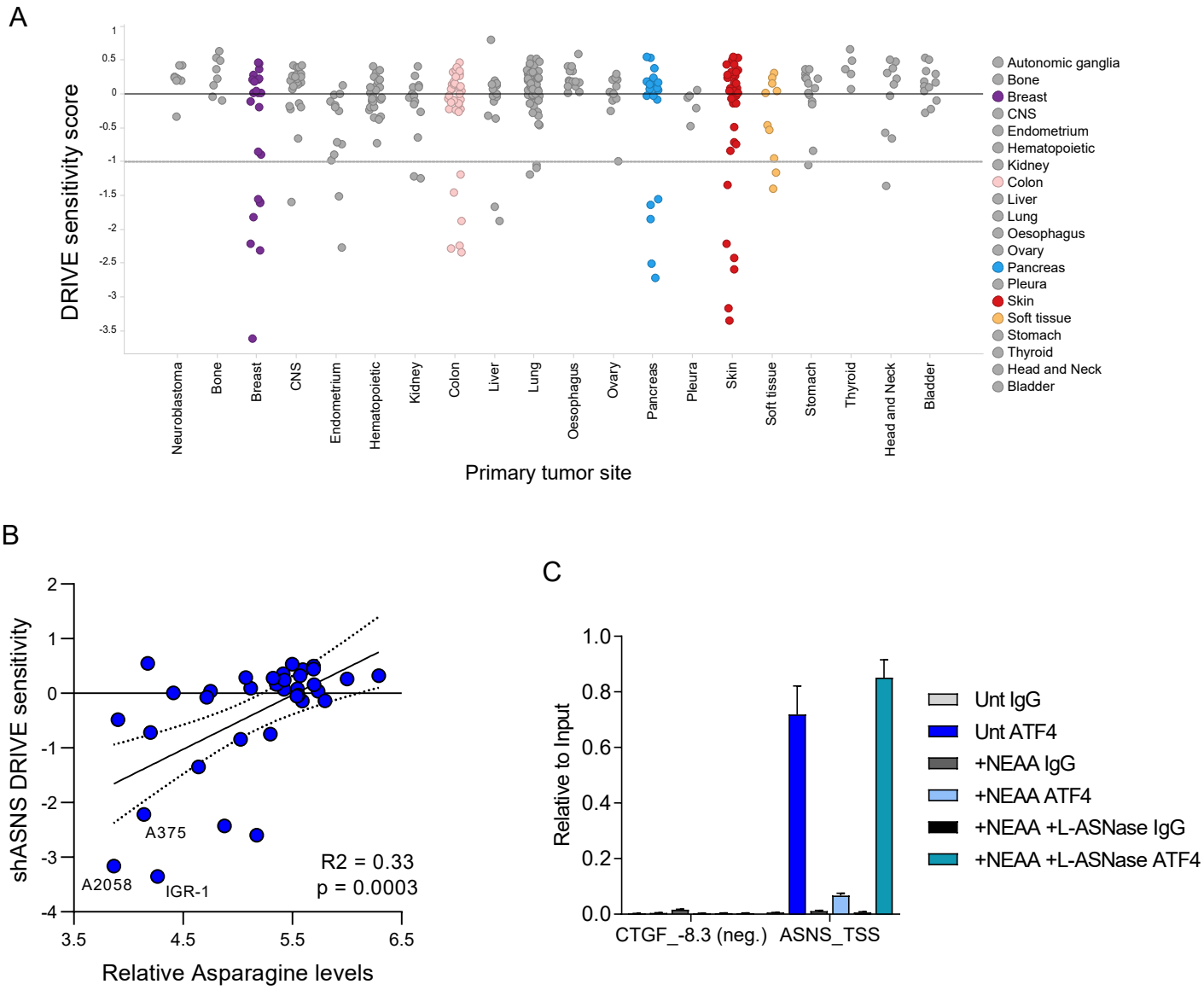


Figure S1. A) Sensitivity score from the DRIVE screen (y-axis) in the panel of cell lines tested binned by cells of origin (x-axis). Horizontal line at -1 represent a treshold of sensitivity as reported in (9). B) Correlation between increased sensitivity to shASNS in melanoma cells according to DRIVE dataset and increasing levels of Asparagine as reported in (7). C) ATF4 Chromatin immunoprecipitation assay on the ASNS promoter in A2058. Cells were grown either in basic media, supplemented with NEAA or by addition of L-ASNase. CTGF-8.3 is used as a negative control region.

Figure S2

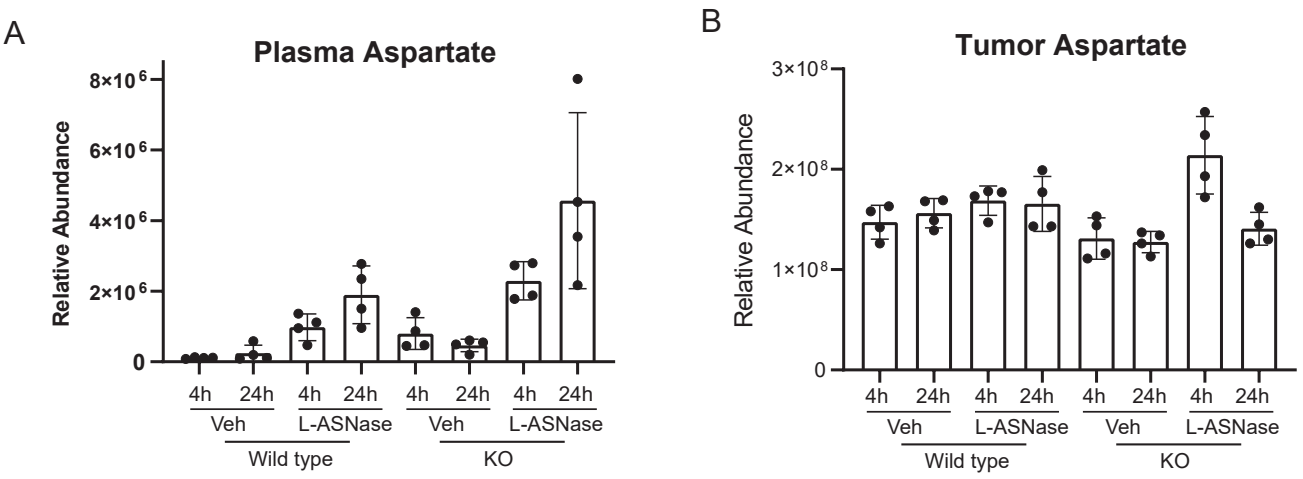
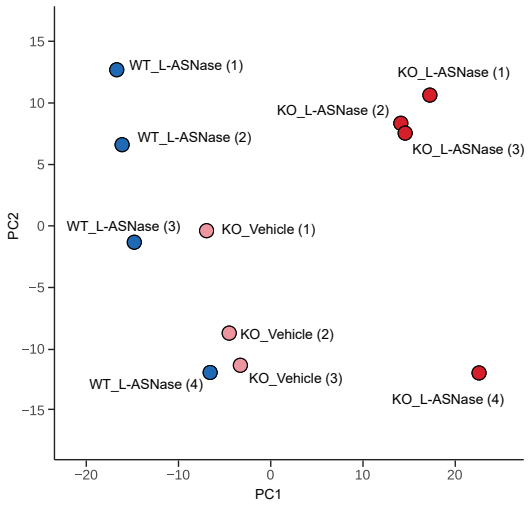


Figure S2. A-B) Relative abundance of Aspartate in plasma (A) and tumors (B) from mice of experiment in Figure 2A at 4h and 24hours after L-asparaginase injection at day 7.

Figure S3

A

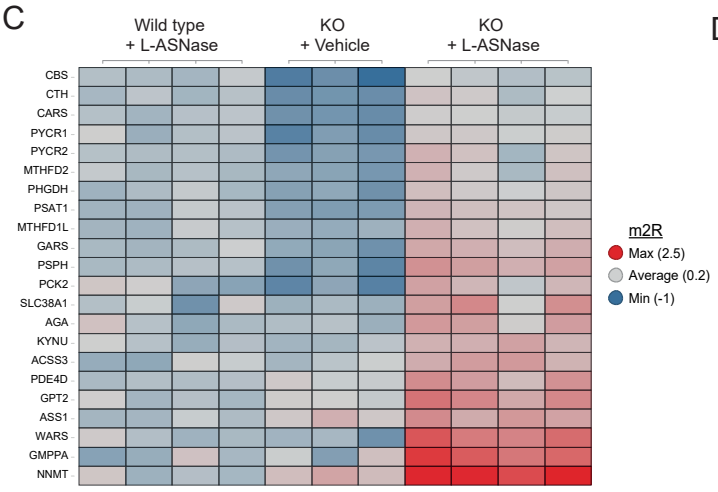


B

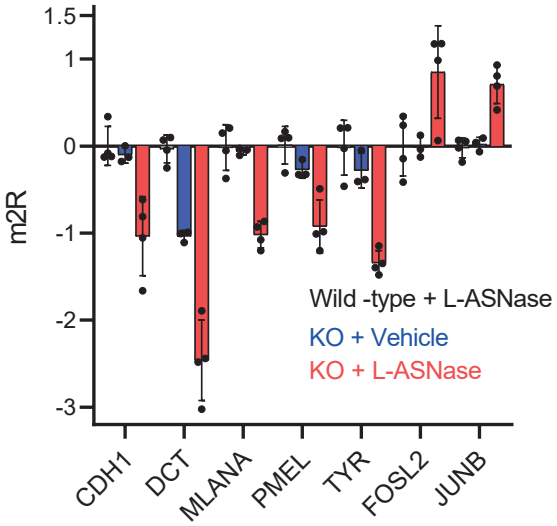
GO term	Biological process (Down)	p-value
GO:0042438	melanin biosynthetic process	2.57E-05
GO:0022617	extracellular matrix disassembly	4.08E-04
GO:0035726	common myeloid progenitor cell proliferation	4.29E-04
GO:0006096	glycolytic process	5.01E-04
GO:0055114	oxidation-reduction process	0.0015101
GO:0030318	melanocyte differentiation	0.0037379
GO:0019433	triglyceride catabolic process	0.0058152
GO:0001558	regulation of cell growth	0.0059132

GO term	Biological process (Up)	p-value
GO:0006564	L-serine biosynthetic process	1.66E-04
GO:0008652	cellular amino acid biosynthetic process	3.43E-04
GO:0001649	osteoblast differentiation	0.0022649
GO:0019343	cysteine biosynthetic process via cystathionine	0.0158172
GO:0042127	regulation of cell proliferation	0.0168527
GO:0006418	tRNA aminoacylation for protein translation	0.019015
GO:0030198	extracellular matrix organization	0.0203665
GO:0070814	hydrogen sulfide biosynthetic process	0.0210345
GO:0019344	cysteine biosynthetic process	0.0210345
GO:0019346	transsulfuration	0.0262244
GO:0055129	L-proline biosynthetic process	0.0262244

C



D



E

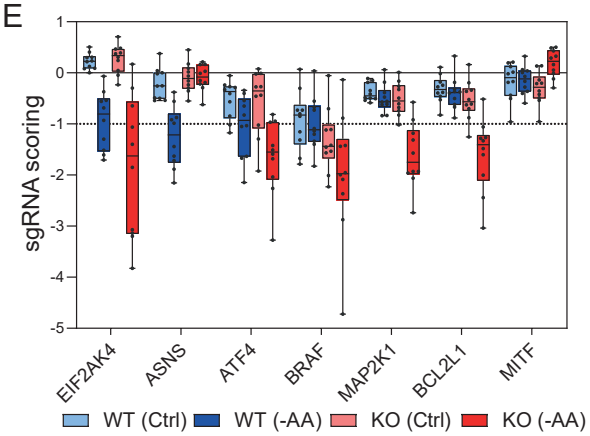


Figure S3. A) PCA plot of the quantitative proteomics data from the analyzed A2058 tumors. Samples are color coded by genotype and treatment. B) Gene Ontology enrichment for proteins differentially expressed in WT and KO tumors treated with L-Asparaginase. C) Heatmap of the proteomics data for differentially expressed proteins involved in metabolic pathways. D) Histogram plot of the proteomics data for proteins involved in melanocyte differentiation (CDH1-TYR) and MAPK (FOSL2 and JUNB). E) sgRNA representation (Y-axis) for the individual sgRNAs targeting hits identified in the CRISPR screen from figure 4A and 4B.