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Supplemental Information

Inhibition of the Polyamine Synthesis Pathway Is Synthetically Lethal with Loss of Argininosuccinate Synthase 1

Matthew Locke, Essam Ghazaly, Marta O. Freitas, Mikaella Mitsinga, Laura Lattanzio, Cristiana Lo Nigro, Ai Nagano, Jun Wang, Claude Chelala, Peter Szlosarek, and Sarah A. Martin

SUPPLEMENTAL DATA

FIGURE S1. Methylation of the ASS1 gene sensitizes MPM cells to ADI-PEG20. Related to Figure 1.

(**A**) Methylation analysis across 7 CpG islands in the ASS1 promoter. Genomic DNA was extracted from the ASS1-ve Ju77, MSTO cells and the ASS1+ve, H28, H226 cells, and bisulphite converted. DNA was pyrosequenced across 7 CpG islands in the ASS1 promoter.

(**B**) The ASS1-methylated Ju77 and MSTO cells and the ASS1-proficient H226 and H28 MPM cells were treated with increasing concentrations of ADI-PEG20 (0, 1, 10, 100, 1000 & 10,000 ng/ml). After 4 days treatment, cell viability was measured using an ATP-based luminescence assay.

FIGURE S2. Metabolomics analysis of ASS1-deficient cells and patient plasma samples. Related to Figure 2 & 3.

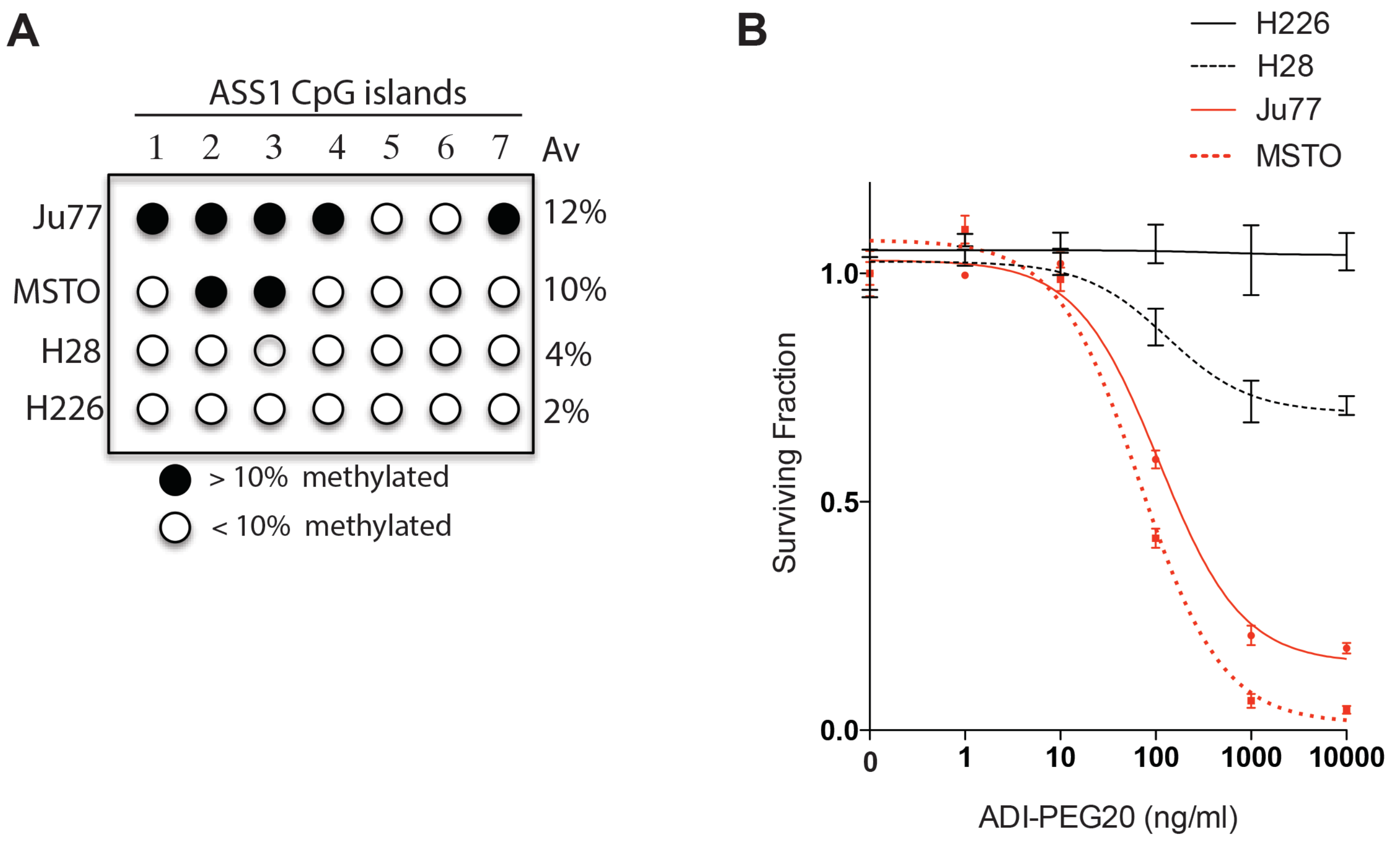
(A) Principle Component Analysis (PCA) score plot for ADI-PEG20-sensitive, Ju77S (JS) and the ADI-PEG20-resistant Ju77R (JR) MPM cells, extracted and analysed by UPLC-MS over 5 different days (D1-D5). PC1 = 41.7% and PC2 = 24.4%.

(B) Two component orthogonal partial least squares discriminant analysis (OPLS-DA) plot showing sample clustering according to ADI-PEG20 treatment in plasma samples obtained from week 6 and 7 of treatment cycles *versus* pre-treatment samples. There was a clear separation based on ADI-PEG20 treatment with reproducible metabolomic changes.

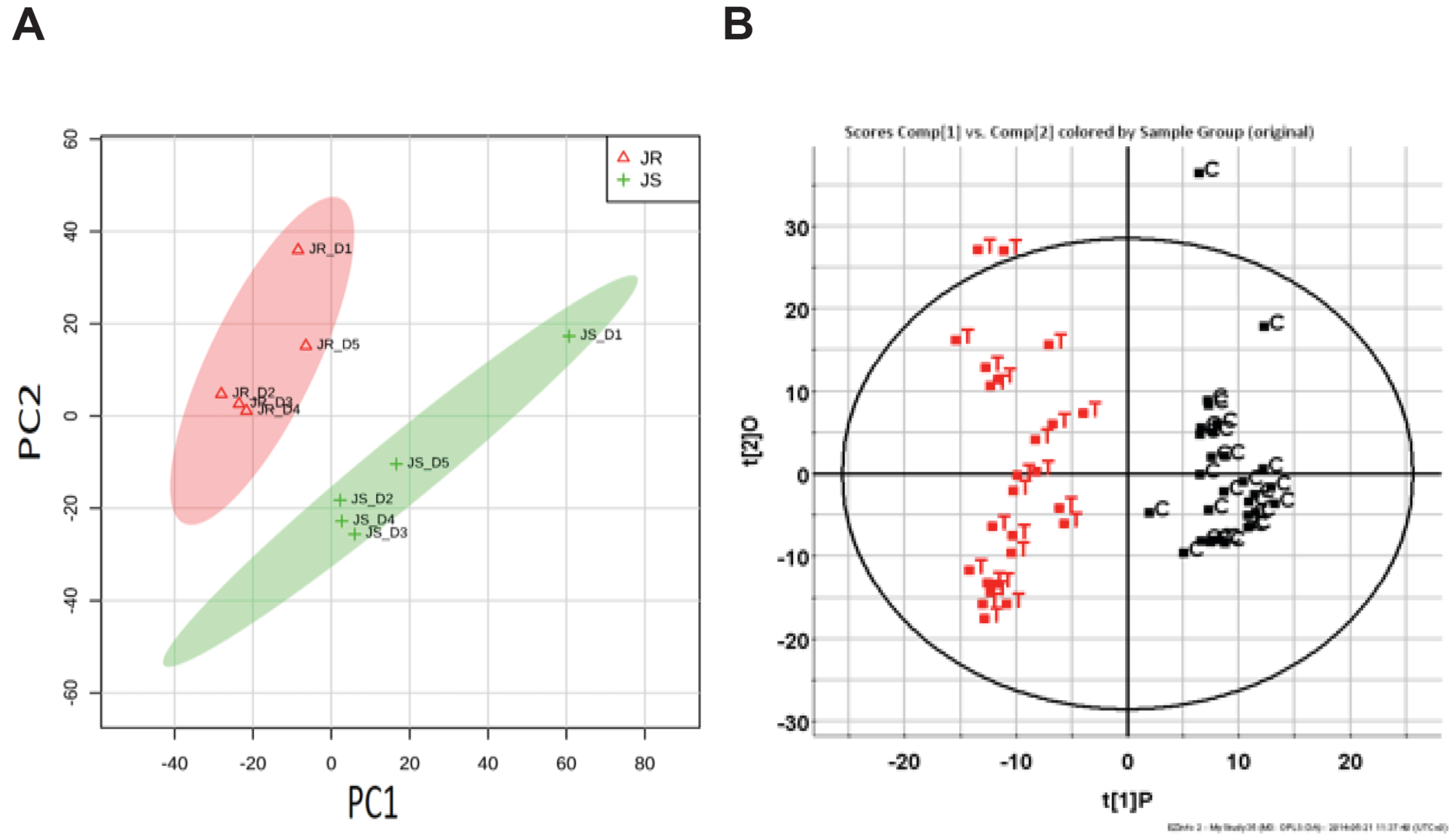
FIGURE S3. Gene Expression analysis of the Ju77S and Ju77R cells. Related to Figure 4.

Gene expression heat map from microarray analysis of the Ju77S and Ju77R cells.
Individual RNA replicates are represented as columns.

SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3