Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

A High-Throughput SAMDI-Mass Spectrometry Assay for Isocitrate Dehydrogenase 1

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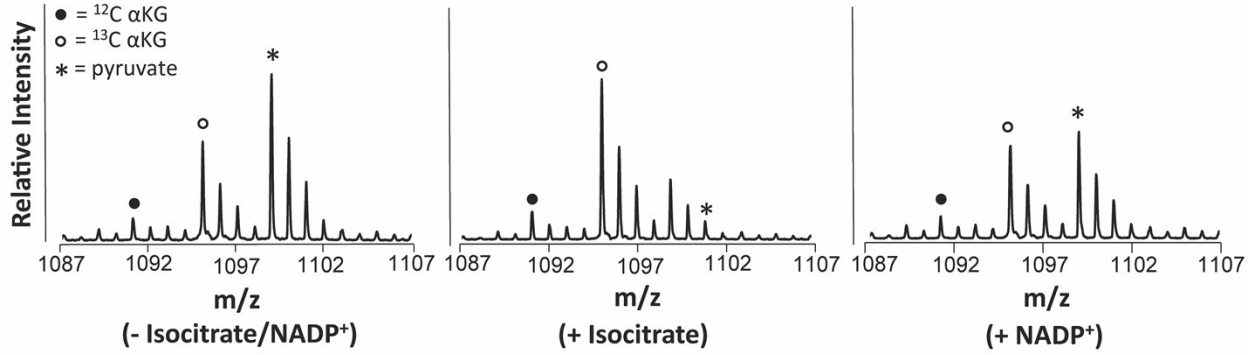

Figure S1. Representative SAMDI-MS spectra of untreated lysates (left), lysates treated with 5 mM isocitrate at 37 °C for 2 hours (center) and lysates treated with 1 mM $NADP^+$ for 2 hours at 37 °C (right) displaying ^{12}C αKG capture at m/z=1091 (sodium adduct of α-cleavage product), ^{13}C αKG capture at m/z=1095 (sodium adduct of α-cleavage product) and pyruvate capture at m/z=1099 (sodium adduct). Untreated lysates gave an αKG concentration value of 410 µM ± 42 µM, isocitrate-treated lysates gave an αKG concentration value of 317 µM ± 33 µM and $NADP^+$-treated lysates gave an αKG concentration value of 450 µM ± 36 µM (mean ± standard deviation with and with n=3 biological replicates per condition). As indicated by the comparably low αKG concentrations measured in all 3 groups, neither isocitrate nor $NADP^+$ independently increases αKG production. However, the spectra of both the isocitrate-treated lysates and the $NADP^+$-treated lysates display a decrease in the amount of immobilized pyruvate compared to the spectra of the untreated lysates (isocitrate produces a greater degree of decrease in immobilized pyruvate than $NADP^+$). This result suggests that the decrease in pyruvate level observed upon addition of isocitrate and $NADP^+$ to the lysate is not solely a product of excess αKG outcompeting pyruvate for reaction with the hydrazide sites. Rather, it seems that treatment with IDH1's substrate or cofactor produces a shift in pyruvate metabolism that ultimately lowers its concentration in the lysate.

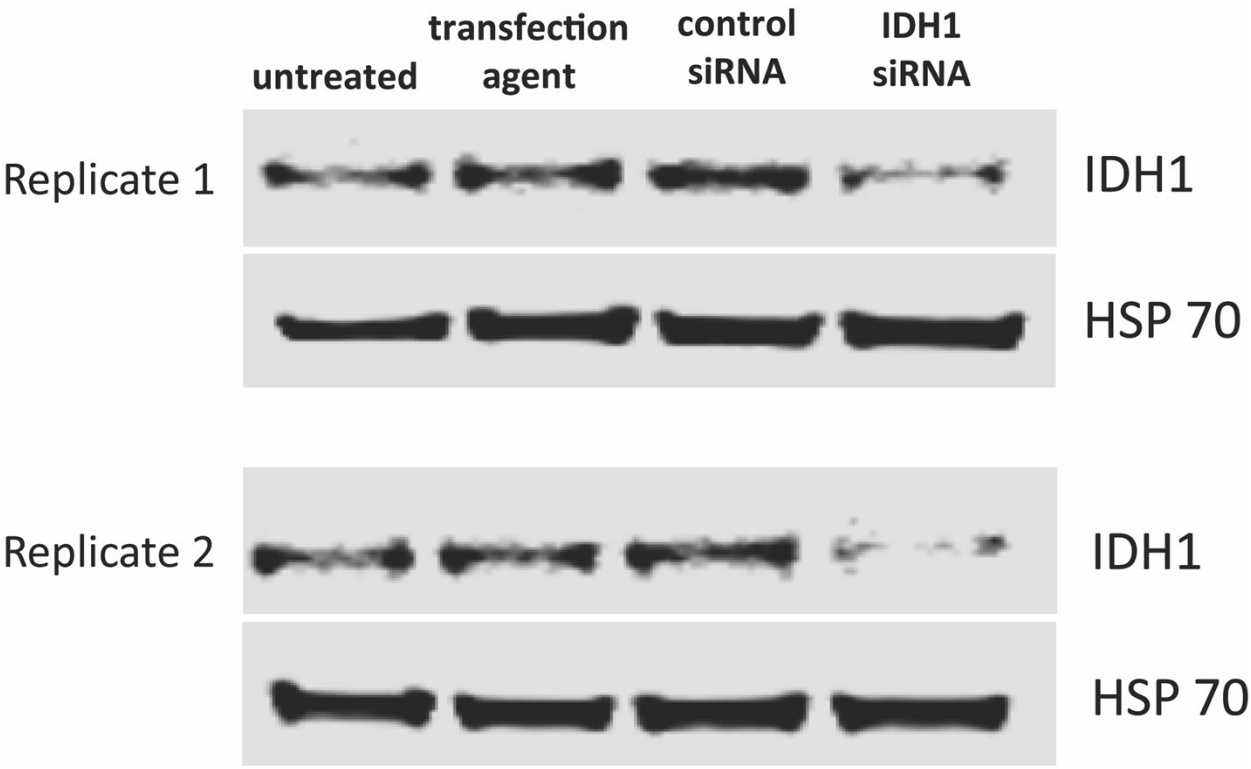

Figure S2. Image of western blot gel featuring bands for IDH1 and HSP 70 loading control for 2 experimental replicates.