Supporting Information
4 pages
Supplemental Figures S1-S3

Title:
Low pH facilitates heterodimerization of mutant isocitrate dehydrogenase IDH1-R132H and promotes production of 2 hydroxyglutarate

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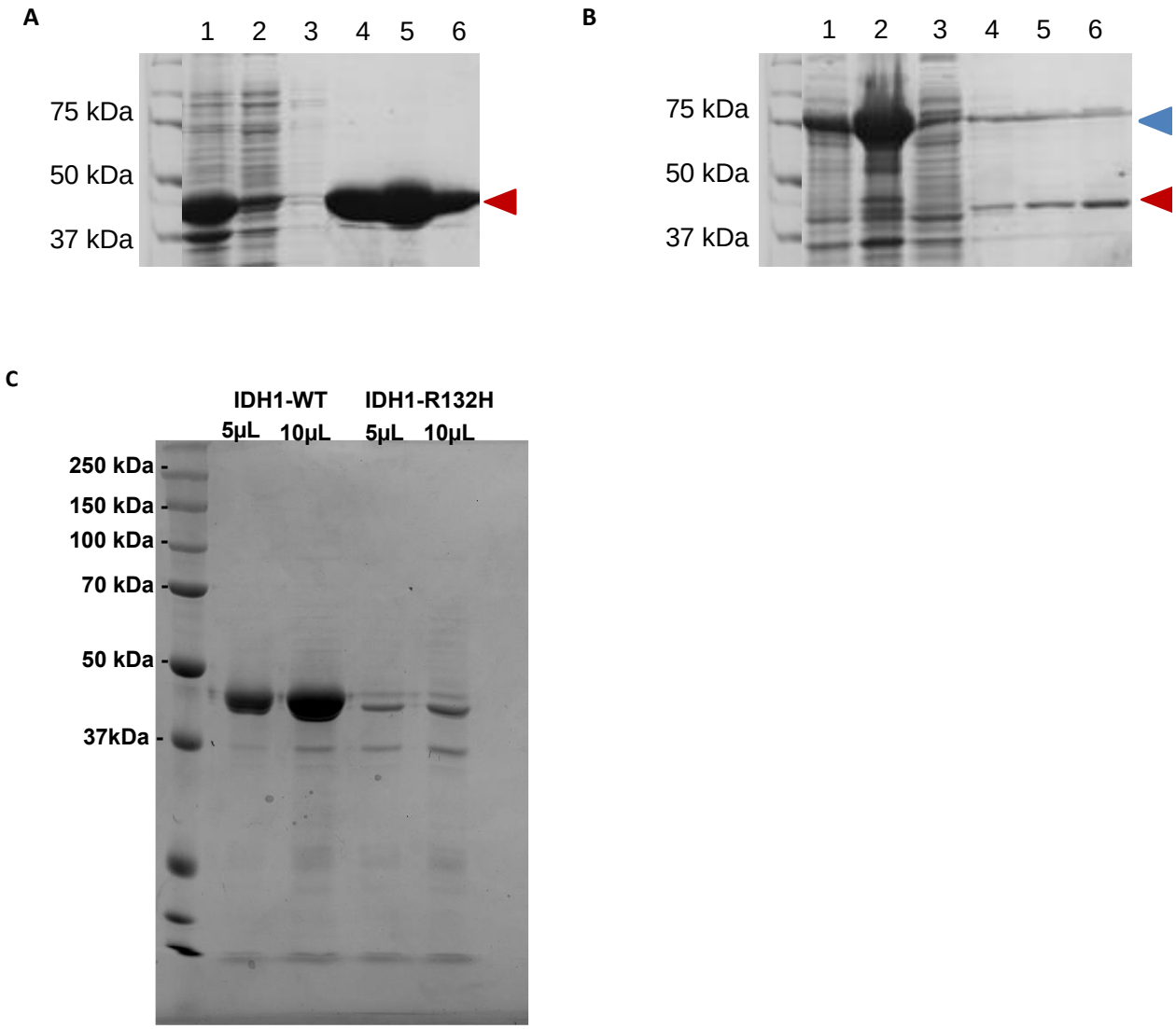

Figure S1. IDH1-WT and IDH1-R132H purified from E. Coli have similar purity levels. IDH1 protein was run on an SDS-PAGE electrophoresis gel (see methods). Two independent protein preparations are shown. A) 10% SDS-PAGE gel of purified IDH1-WT and B) IDH1-R132H proteins. 1: pellet, 2: flow through, 3: wash; 4-6: purified fractions. Red arrow is monomer. Blue arrow is a higher molecular weight protein that is occasionally co-eluted with IDH1-R132H, possibly a chaperone or Heat Shock Protein. C) Second representative preparation. IDH1-WT (5 µL, 10 µL) and IDH1-R132H (5 µL, 10 µL). All samples are pure, and IDH1-WT was determined to be more concentrated than IDH1-R132H. Note no higher-molecular weight band is seen in this preparation of IDH1-R132H.

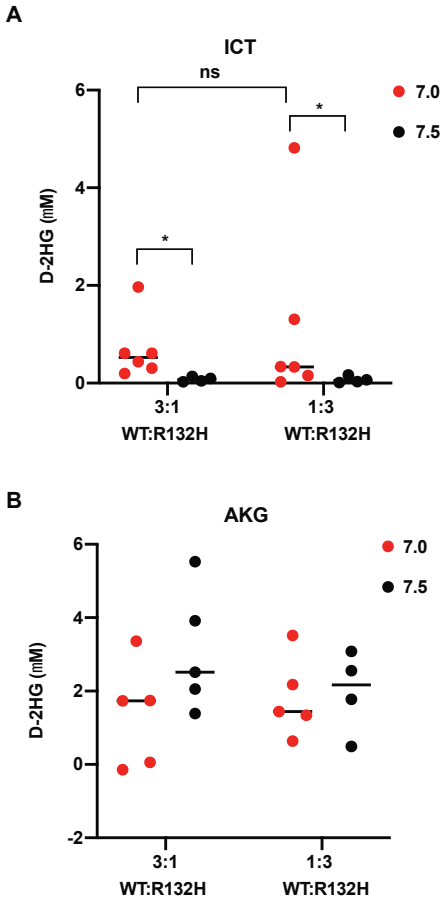

Figure S2. D-2HG production is pH sensitive with WT:R132H heterodimer even when excess IDH1-WT or IDH1-R132H are present in the reaction mixtures. Colormetric analysis of enzymatic reactions prepared with mixtures of IDH1-WT and IDH1-R132H. For 3:1 mixtures 3.75 μM of IDH1-WT and 1.25 μM of IDH1-R132H was used. For 1:3 mixtures 1.25 μM of IDH1-WT and 3.75 μM of IDH1-R132H was used. Under these conditions, maximal heterodimer formation is consistent across these two conditions, while the 3:1 mixture has excess IDH1-WT and the 1:3 mixture has excess IDH1-R132H. A) Oxidation reactions were prepared with 8 mM ICT, 200 μM $NADP^+$, and enzyme. B) Reduction reactions were prepared with 8 mM AKG, 500 μM NADPH. Shown are scatterplots from 5 experimental replicates from 2 protein preparations (each result is average of 2 technical replicates), medians shown. Significance determined by ratio paired t-test, two-tailed, (*$p<0.01$).

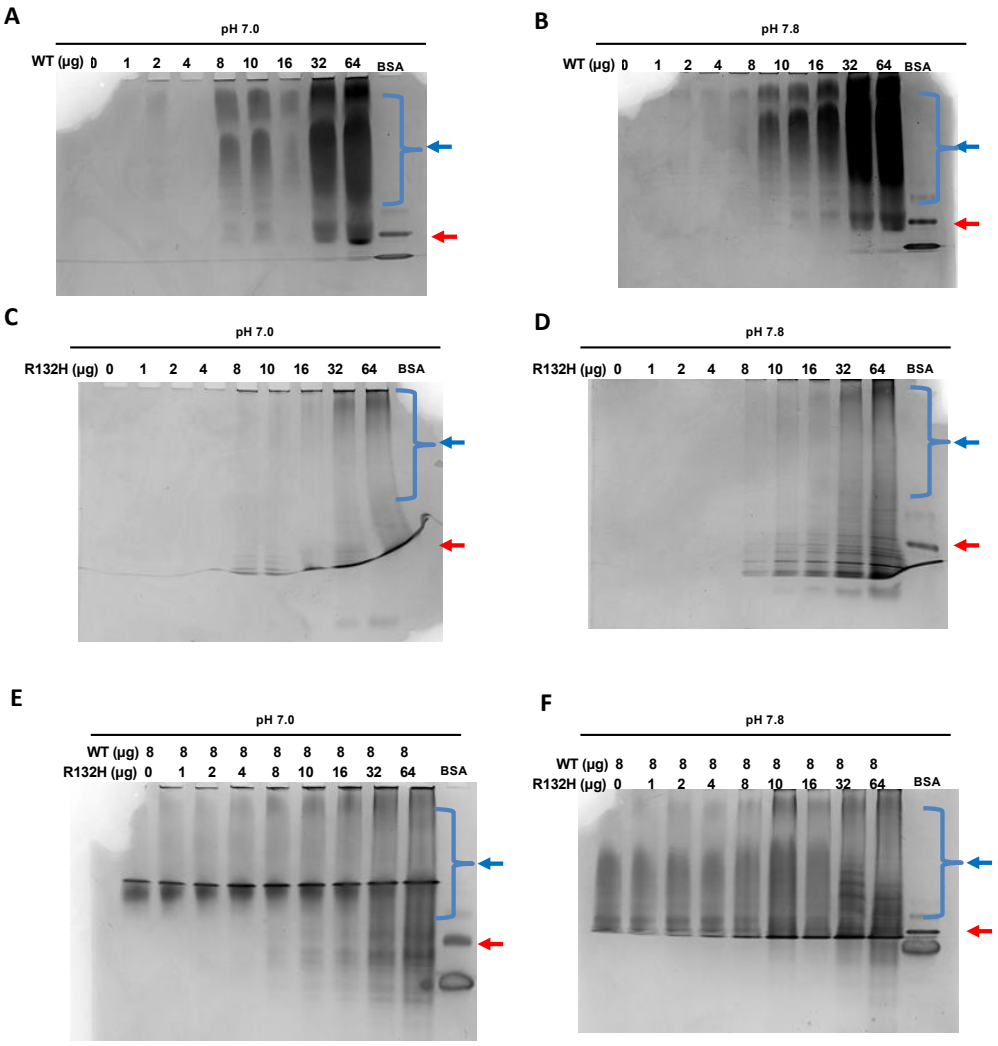

Figure S3. Representative blots from quantification in Figure 5. IDH1 protein was run on native gels at two buffer pH values (see methods) and varying protein concentrations. Indicated on the gels are IDH1 monomers (~40 kDa, red arrows), dimers (~80 kDa, blue arrows/brackets). Formation of IDH1-WT homodimer at A) pH 7.0 and B) pH 7.8 is pH-insensitive. Formation of IDH1-R132H homodimer at C) pH 7.0 and D) pH 7.8 is minimal and pH-insensitive. Formation of WT:R132H heterodimer at E) pH 7.0 and F) pH 7.8 is increased at low pH.