SUPPLEMENTARY INFORMATION FOR

Enzyme Redesign Guided by Cancer-Derived *IDH1* Mutations

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SUPPLEMENTARY METHODS

Homology analysis. Structures were displayed in UCSF Chimera software version 1.5[24], using PDB 1T0L for *Hs*IDH1[18], 3TY3 for *Sp*HIDH[19], and 3ASJ for *Tt*HIDH[25]. Multiple Alignment using Fast Fourier Transform (MAFFT)[26] was used to align sequences using BLOSUM 30 matrix, 1.0 gap open penalty, and genafpair fourier transform settings. Alignments were drawn with ESPript[27], with secondary structure elements from *Hs*IDH1 (PBD 1T0L[18]) shown. GeneID was 49168486 for *Hs*IDH1, 28178832 for *Hs*IDH2, 2388955 for *Sp*HIDH, 731845 for *Sc*HIDH, 46199314 for *Tt*HIDH, 1052977 for *Sy*IDH, 209772816 for *Ec*IDH, 729813 for *Bs*IDH, 62897507 for *Hs*NAD-IDH, 238879624 for *Ca*HIDH, 320668365 for *Ec*TDH, 167033202 for *Pp*TDH, 66773874 for *Tt*IPMDH, 198283840 for *Tf*IPMDH, and 151943807 for *Sc*IPMDH.

Chemicals. NADH reduced dipotassium salt (purity ≥95%), 2-oxoadipic acid (≥95%), 2-oxoglutarate sodium salt (≥98%), 2-heptandioic acid (≥98%), and (+)-diacetyl-L-tartaric anhydride (≥97%) were from Sigma. A racemic mixture of *(R)*- and (S)-2-hydroxyadipate was generated by reacting 1 mg 2-oxoadipic acid with 1mg $NaBH_4$ in 200 µl of anhydrous methanol at 60˚C for 30 min and characterization matched literature data[6]. [3,3,4,4]-$^2H_4$-*(R/S)*-2-hydroxyglutarate (2HG-d4) was synthesized previously[21].

Expression and purification of HIDH mutants. The gene encoding *Sc*HIDH (*LYS12*, NC_001141.2) lacking a 3' STOP codon was amplified from an *S. cerevisiae* gDNA library. The gene encoding *Tt*HIDH (*TTC1012*, NC_005835.1) lacking a 3' STOP codon was PCR amplified from a *T. thermophilus* HB27 gDNA library. These fragments were cloned into pTrcHis2-TOPO, which appends a C-terminal 6x His tag (Invitrogen). Mutagenesis was performed using the QuikChange Site Directed mutagenesis kit (Agilent). Procedures for expression and purification of homoisocitrate dehydrogenases were adapted from published methods[10]. Expression constructs were transformed into BL21-DE3 E. coli (Stratagene). A single colony was inoculated into 5 ml of LB-Amp media starter cultures and grown by shaking (225 rpm at 37 ºC) for 6 h. 5 ml starter cultures were added to 45 ml of LB-Amp, shaken for 2 h, and shaken for an additional 2 h after induction with 1 mM IPTG. Pellets were harvested by centrifugation at 4500 x g at 4 °C and resuspended in 2 ml of buffer A (500 mM NaCl, 10 mM $MgCl_2$, 20 mM imidazole, 2 mM β-mercaptoethanol, 10 mM Tris, pH 7.5 supplemented with 1x EDTA-free Complete Mini protease inhibitor from Roche) on ice and sonicated for 6 cycles of 15 seconds each on ice on a Branson Sonifer 250 (Emerson). This crude lysate was cleared by centrifugation at 13,000 x g at 4 °C. Crude lysates were loaded on a Ni-NTA Spin Column (cat. No. 31014, Qiagen) that was preequilibrated with buffer A by spinning for 10 min at 300 x g at 4 °C. This was then washed with 10 column volumes of buffer A that was modified to contain 75 mM imidazole, 5% glycerol, and 0.1% Triton-X100 by spinning for 1 min at 900 x g. Eluates were obtained using buffer A that was modified to contain 500 mM imidazole. Eluates were brought to 10% glycerol and stored in aliquots at -80 °C for *Sc*HIDH and at 4 °C for *Tt*HIDH. Approximately 2.5 mg purified protein was obtained per g wet bacterial pellet. Purity was approximately 95% as assessed by SDS-PAGE stained with SilverQuest Silver Staining Kit (Life Technologies).

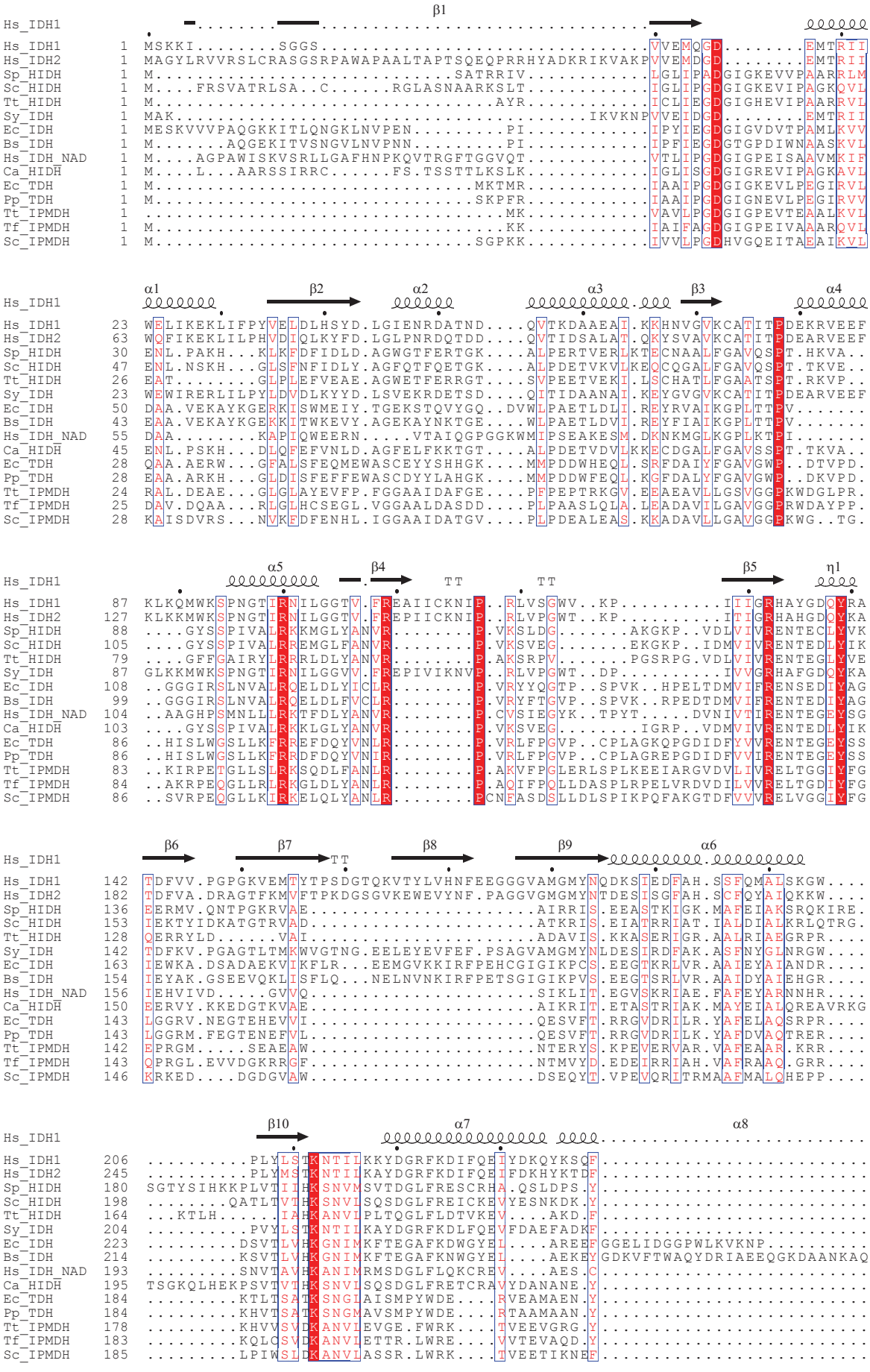
Activity measurement. Assays were performed with 40 ng/µl enzyme, 15 mM 2-oxoadipate, 100 µM NADH, 20 mM $MgCl_2$ and 100 mM HEPES, pH 7.3 in 10 or 20 µl in 384-well black microplates (788076, Greiner) unless otherwise specified. NADH was monitored by the decrease in fluorescence (ex. 340 nm, em. 450 nm) on a fluorescent plate reader. The levels of fluorescence were converted to NADH concentrations based on NADH standards. Reactions with high amounts of NADH (>300 µM) were monitored by the absorbance of NADH as indicated in the text, at 340 nm in a 40 µl reaction volume in a clear 96-well plate on the same plate reader. Reactions with *Sc*HIDH were carried out at 25 °C and reactions with *Tt*HIDH were carried out at 45 °C. Rates are initial rates from the first 10 minutes of reaction. For K_M determination, concentrations of substrate at least 5x higher than the K_M were used (for

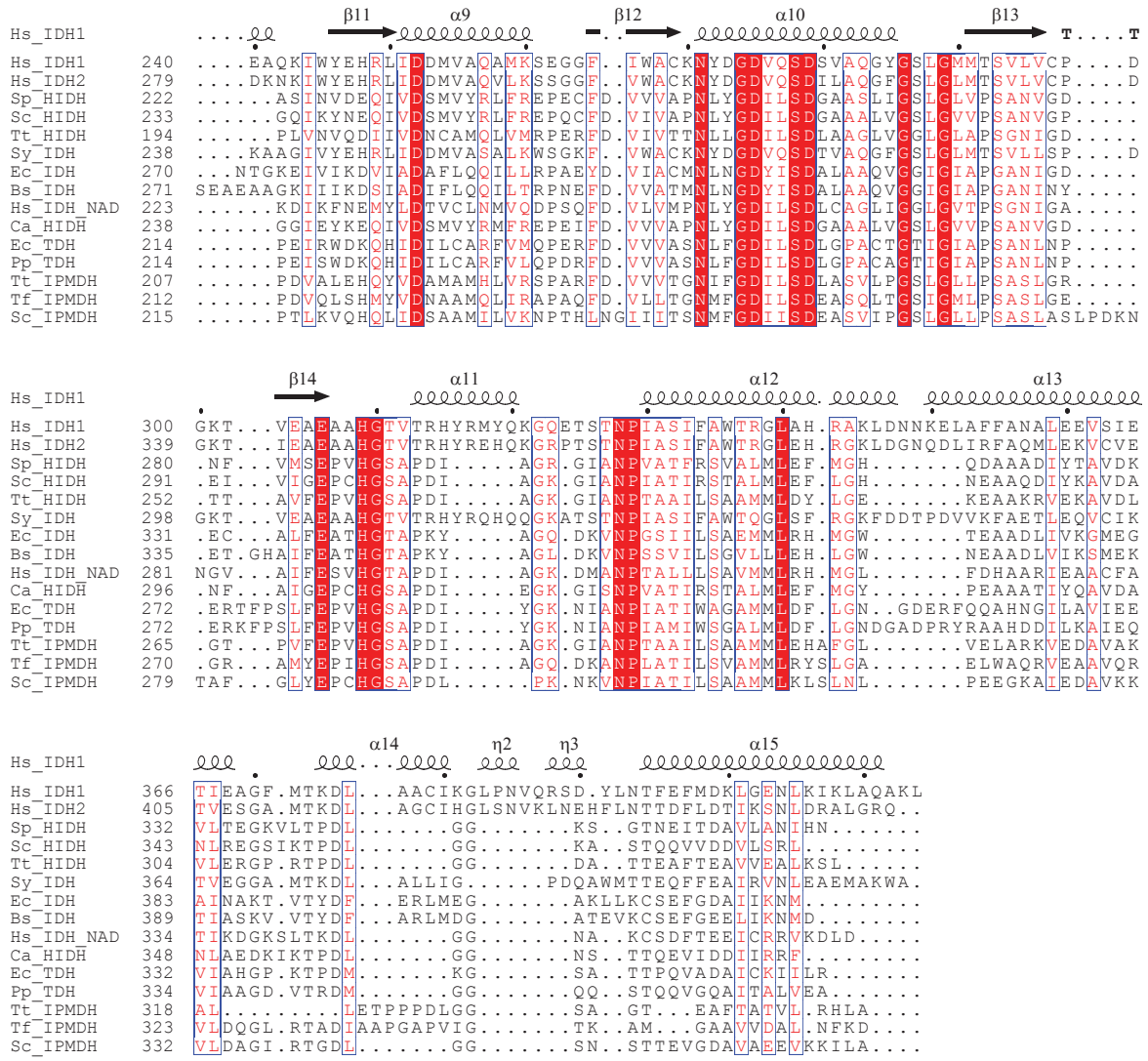
NADH, 500 μM; for 2-oxoadipate, 15 mM) while the substrate of interest was varied in concentration. K_M and V_{max} were estimated by fitting data to equation (1), where X is substrate concentration and Y is rate, using nonlinear regression.

(1) $Y=V_{max}*X/(K_m + X)$

LC-MS/MS. LC-MS/MS was based on the procedure described for 2-hydroxyglutarate[20, 21]. To 20 μL of reaction mix, 2 μL of 130 μg/mL of a racemic mixture of 2HG-d4 (internal standard) in water was added and the mixture dried by vacuum centrifuge (50 °C, 15 min). Dry residue was treated with 50 mg/mL freshly prepared diacetyl-L-tartaric anhydride in dichloromethane/glacial acetic acid (4/1 by volume) and heated (75 °C 30 min). After drying (50 °C, 15 min) the residue was dissolved in 100 μL LC mobile phase A (see below) for analysis. An Agilent 1200 series HPLC (Santa Clara, CA) was used for liquid chromatography (LC) and a Sciex/Applied Biosystems API 3200 QTrap (Carlsbad, CA) was used for triple quadrupole mass spectrometry (MS/MS). Mobile phase A: water, 3% acetonitrile, 280 μL ammonium hydroxide (~25%), pH adjusted to 3.6 by formic acid (~98%). Mobile phase B: methanol. Analytical column: Kinetex C_{18}, 150×4.6 mm, 2.6 μm, and SafeGuard C_{18} 4×3 mm guard-column from Phenomenex (Torrance, CA). Column temperature: 45 °C. Elution gradient at 1 mL/min flow rate: 0–1 min 0% B, 1–2 min 0-100% B, 2–3.5 min 100%B, 3.5–4 min 100-0% B, 4–10 min 0% B. Injection volume: 10 μL. For initial product characterization, Q1/Q3 (*m/z*) transitions monitored were predicted based on the known fragmentation pattern of 2HG-d4[20, 21] as shown in the mass fragmentation diagrams in **Supplementary Fig. 4**: 421.3/205.1, 421.3/187.0, 377.0/161.2, 377.0/143.2, 367.0/151.0, 367.0/132.0 for homoisocitrate transitions 1 and 2, 2-hydroxyadipate transitions 1 and 2, and 2HG-d4 transitions 1 and 2, respectively. For enantiospecific 2-hydroxyadipate quantification, Q1/Q3 (*m/z*) transitions monitored: 377/161 and 367/151 for 2-hydroxyadipate and 2HG-d4. To calibrate, 0, 1.6, 8, 40, 200, and 1000 μg/ml 2-hydroxyadipate reacted with diacetyl-L-tartaric anhydride was analyzed in reaction buffer. *(R)* and *(S)* 2-hydroxyadipate enantiomers were discriminated based on time of elution from the HPLC column, using the relative elution time for *(R)*-2HG-d4 compared to the racemic mixture of 2HG-d4[21]. Standard samples were analyzed alongside experimental samples and accuracy acceptance criteria was 85% for each standard. The lower limit of quantification was taken as the concentration of the lowest standard.

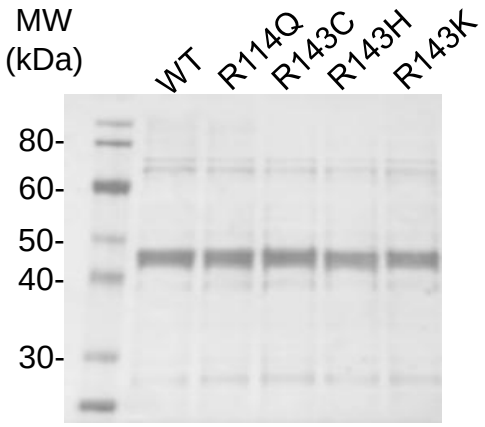
Statistical analysis. Activity measurements and LC-MS/MS analyses are representative of experiments were performed in triplicate unless otherwise specified. Data points in activity measurement plots represent mean ± s.d. from n=2 reactions unless otherwise specified.


β1
Hs_IDH1 1 MSKKI.....SGGS.....VVEMQGD.....EMTRII
Hs_IDH2 1 MAGYLRVVRSLCRASGSRPAWAPAALTAPTSQEQPRRHYADKRIKVAKPVVEMDGD.....EMTRII
Sp_HIDH 1 M.....SATRRIV.....LGLIPADGIGKEVVPAARRLM
Sc_HIDH 1 M....FRSVATRLSA..C.....RGLASNAARKSLT.....IGLIPGDGIGKEVIPAGKQVL
Tt_HIDH 1 M.....AYR.....ICLIEGDGIGHEVIPAARRVL
Sy_IDH 1 MAK.....IKVKNPVVEIDGD.....EMTRII
Ec_IDH 1 MESKVVVPAQGKKITLQNGKLNVPEN.....PI.....IPYIEGDGIGVDVTPAMLKVV
Bs_IDH 1 M.....AQGEKITVSNGVLNVPNN.....PI.....IPFIEGDGTGPDIWNAASKVL
Hs_IDH_NAD 1 M....AGPAWISKVSRLLGAFHNPKQVTRGFTGGVQT.....VTLIPGDGIGPEISAAVMKIF
Ca_HIDH 1 M....L...AARSSIRRC.....FS.TSSTTLKSLK.....IGLISGDGIGREVIPAGKAVL
Ec_TDH 1 M.....MKTMR.....IAAIPGDGIGKEVLPEGIRVL
Pp_TDH 1 M.....SKPFR.....IAAIPGDGIGNEVLPEGIRVV
Tt_IPMDH 1 .....MK.....VAVLPGDGIGPEVTEAALKVL
Tf_IPMDH 1 M.....KK.....IAIFAGDGIGPEIVAAARQVL
Sc_IPMDH 1 M.....SGPKK.....IVVLPGDHVGQEITAEAIKVL
α1 β2 α2 α3 β3 α4
Hs_IDH1 23 WELIKEKLIFPYVELDLHSYD.LGIENRDATND....QVTKDAAEAI.KKHNVGVKCATITPDEKRVEEF
Hs_IDH2 63 WQFIKEKLILPHVDIQLKYFD.LGLPNRDQTDD....QVTIDSALAT.QKYSVAVKCATITPDEARVEEF
Sp_HIDH 30 ENL.PAKH...KLKFDFIDLD.AGWGTFERTGK....ALPERTVERLKTECNAALFGAVQSPT.HKVA..
Sc_HIDH 47 ENL.NSKH...GLSFNFIDLY.AGFQTFQETGK....ALPDETVKVLKEQCQGALFGAVQSPT.TKVE..
Tt_HIDH 26 EAT.....GLPLEFVEAE.AGWETFERRGT....SVPEETVEKI.LSCHATLFGAATSPT.RKVP..
Sy_IDH 23 WEWIRERLILPYLDVDLKYYD.LSVEKRDETSD....QITIDAANAI.KEYGVGVKCATITPDEARVEEF
Ec_IDH 50 DAA.VEKAYKGERKISWMEIY.TGEKSTQVYGQ..DVWLPAETLDLI.REYRVAIKGPLTTPV.....
Bs_IDH 43 EAA.VEKAYKGEKKITWKEVY.AGEKAYNKTGE....WLPAETLDVI.REYFIAIKGPLTTPV.....
Hs_IDH_NAD 55 DAA.....KAPIQWEERN.....VTAIQGPGGKWMIPSEAKESM.DKNKMGLKGPLKTPI.....
Ca_HIDH 45 ENL.PSKH...DLQFEFVNLD.AGFELFKKTGT....ALPDETVDVLKKECDGALFGAVSSPT.TKVA..
Ec_TDH 28 QAA.AERW...GFALSFEQMEWASCEYYSHHGK....MMPDDWHEQL.SRFDAIYFGAVGWP..DTVPD.
Pp_TDH 28 EAA.ARKH...GLDISFEFFEWASCDYYLAHGK....MMPDDWFEQL.KGFDALYFGAVGWP..DKVPD.
Tt_IPMDH 24 RAL.DEAE...GLGLAYEVFP.FGGAAIDAFGE....PFPEPTRKGV.EEAEAVLLGSVGGPKWDGLPR.
Tf_IPMDH 25 DAV.DQAA...RLGLHCSEGL.VGGAALDASDD....PLPAASLQLA.LEADAVILGAVGGPRWDAYPP.
Sc_IPMDH 28 KAISDVRS...NVKFDFENHL.IGGAAIDATGV....PLPDEALEAS.KKADAVLLGAVGGPKWG..TG.
α5 β4 TT TT β5 η1
Hs_IDH1 87 KLKQMWKSPNGTIRNILGGTV.FREAIICKNIP..RLVSGWV..KP.....IIIGRHAYGDQYRA
Hs_IDH2 127 KLKKMWKSPNGTIRNILGGTV.FREPIICKNIP..RLVPGWT..KP.....ITIGRHAHGDQYKA
Sp_HIDH 88 ....GYSSPIVALRKKMGLYANVR.....P.VKSLDG.....AKGKP..VDLVIVRENTECLYVK
Sc_HIDH 105 ....GYSSPIVALRREMGLFANVR.....P.VKSVEG.....EKGKP..IDMVIVRENTEDLYIK
Tt_HIDH 79 ....GFFGAIRYLRRRLDLYANVR.....P.AKSRPV.....PGSRPG.VDLVIVRENTEGLYVE
Sy_IDH 87 GLKKMWKSPNGTIRNILGGVV.FREPIVIKNVP..RLVPGWT..DP.....IVVGRHAFGDQYKA
Ec_IDH 108 ..GGGIRSLNVAIRQELDLYICLR.....P.VRYYQGTP..SPVK..HPELTDMVIFRENSEDIYAG
Bs_IDH 99 ..GGGIRSLNVALRQELDLFVCLR.....P.VRYFTGVP..SPVK..RPEDTDMVIFRENTEDIYAG
Hs_IDH_NAD 104 ..AAGHPSMNLLLRKTFDLYANVR.....P.CVSIEGYK..TPYT.....DVNIVTIRENTEGEYSG
Ca_HIDH 103 ....GYSSPIVALRKKLGLYANVR.....P.VKSVEG.....IGRP..VDMVIVRENTEDLYIK
Ec_TDH 86 ..HISLWGSLLKFRREFDQYVNLR.....P.VRLFPGVP..CPLAGKQPGDIDFYVVRENTEGEYSS
Pp_TDH 86 ..HISLWGSLLKFRREFDQYVNIR.....P.VRLFPGVP..CPLAGREPGDIDFVVIRENTEGEYSS
Tt_IPMDH 83 ..KIRPETGLLSIRKSQDLFANLR.....P.AKVFPGLERLSPLKEEIARGVDVLIVRELTGGIYFG
Tf_IPMDH 84 ..AKRPEQGLLRIRKGLDLYANLR.....P.AQIFPQLLDASPLRPELVRDVDILVVRELTGDIYFG
Sc_IPMDH 86 ..SVRPEQGLLKIRKELQLYANLR.....PCNFASDSLLDLSPIKPQFAKGTDFVVVRELVGGIYFG
β6 β7 TT β8 β9 α6
Hs_IDH1 142 TDFVV.PGPGKVEMTYTPSDGTQKVTYLVHNFEEGGGVAMGMYNQDKSIEDFAH.SSFQMALSKGW....
Hs_IDH2 182 TDFVA.DRAGTFKMVFTPKDGSGVKEWEVYNF.PAGGVGMGMYNTDESISGFAH.SCFQYAIQKKW....
Sp_HIDH 136 EERMV.QNTPGKRVAE.....AIRRIS.EEASTKIGK.MAFEIAKSRQKIRE.
Sc_HIDH 153 IEKTYIDKATGTRVAD.....ATKRIS.EIATRRIAT.IALDIALKRLQTRG.
Tt_HIDH 128 QERRYLD.....VAI.....ADAVIS.KKASERIGR.AALRIAEGRPR....
Sy_IDH 142 TDFKV.PGAGTLTMKWVGTNG.EELEYEVFEF.PSAGVAMGMYNLDESIRDFAK.ASFNYGLNRGW....
Ec_IDH 163 IEWKA.DSADAEKVIKFLR...EEMGVKKIRFPEHCGIGIKPCS.EEGTKRLVR.AAIEYAIANDR....
Bs_IDH 154 IEYAK.GSEEVQKLISFLQ...NELNVNKIRFPETSGIGIKPVS.EEGTSRLVR.AAIDYAIEHGR....
Hs_IDH_NAD 156 IEHVIVD.....GVVQ.....SIKLIT.EGVSKRIAE.FAFEYARNNHR....
Ca_HIDH 150 EERVY.KKEDGTKVAE.....AIKRIT.ETASTRIAK.MAYEIALQREAVRKG
Ec_TDH 143 LGGRV.NEGTEHEVVI.....QESVFT.RRGVDRILR.YAFELAQSRPR....
Pp_TDH 143 LGGRM.FEGTENEFVL.....QESVFT.RRGVDRILK.YAFDVAQTRER....
Tt_IPMDH 142 EPRGM.....SEAEAW.....NTERYS.KPEVERVAR.VAFEAAR.KRR....
Tf_IPMDH 143 QPRGL.EVVDGKRRGF.....NTMVYD.EDEIRRIAH.VAFRAAQ.GRR....
Sc_IPMDH 146 KRKED....DGDGVAW.....DSEQYT.VPEVQRITRMAAFMALQHEPP....
β10 α7 α8
Hs_IDH1 206 .....PLYLSTKNTIIKKYDGRFKDIFQEIYDKQYKSQF.....
Hs_IDH2 245 .....PLYMSTKNTIIKAYDGRFKDIFQEIFDKHYKTDF.....
Sp_HIDH 180 SGTYSIHKKPLVTIIHKSNVMSVTDGLFRESCRHA.QSLDPS.Y.....
Sc_HIDH 198 .....QATLTVTHKSNVISQSDGLFREICKEVYESNKDK.Y.....
Tt_HIDH 164 ...KTLH.....IAHKANVIPLTQGLFLDTVKEV....AKD.F.....
Sy_IDH 204 .....PVYLSTKNTIIKAYDGRFKDLFQEVFDAEFADKF.....
Ec_IDH 223 .....DSVTLVHKGNIMKFTEGAFKDWGYELAREEFGGELIDGGPWLKVKNP.....
Bs_IDH 214 .....KSVTLVHKGNIMKFTEGAFKNWGYELAEKEYGDKVFTWAQYDRIAEEQGKDAANKAQ
Hs_IDH_NAD 193 .....SNVTAVHKANIMRMSDGLFLQKCREV....AES.C.....
Ca_HIDH 195 TSGKQLHEKPSVTVTHKSNVISQSDGLFRETCRAVYDANANE.Y.....
Ec_TDH 184 .....KTLTSATKSNGIAISMPYWDE....RVEAMAEN.Y.....
Pp_TDH 184 .....KHVTSATKSNGMAVSMPYWDE....RTAAMAAN.Y.....
Tt_IPMDH 178 .....KHVVSVDKANVIEVGE.FWRK....TVEEVGRG.Y.....
Tf_IPMDH 183 .....KQLGSVDKANVIETTR.LWRE....VVTEVAQD.Y.....
Sc_IPMDH 185 .....LPIWSIDKANVIASSR.LWRK....TVEETIKNEF.....



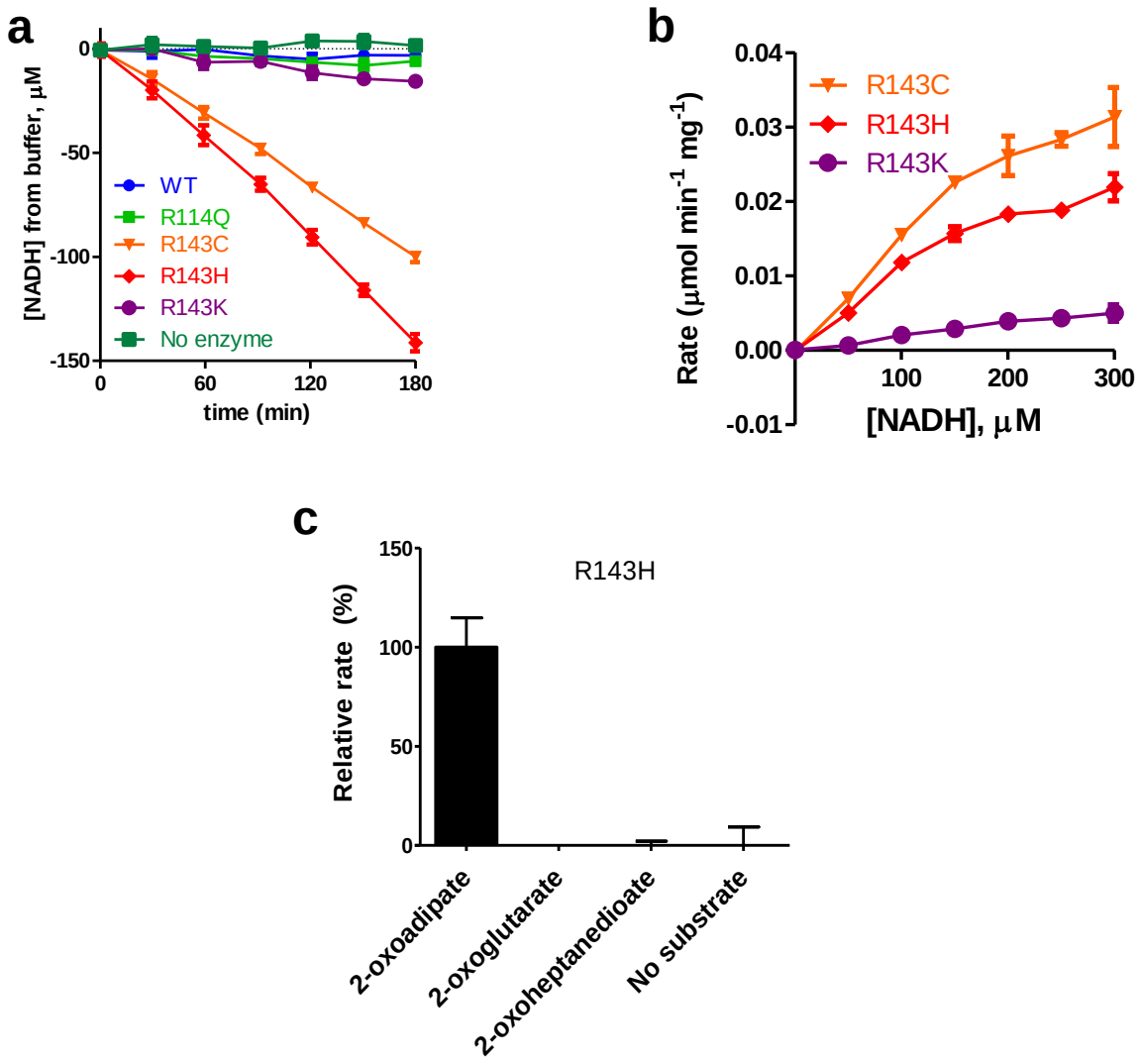
Supplementary Figure 1. Alignment of β-hydroxyacid oxidative decarboxylases.

Alignment of NADP$^+$-dependent IDHs HsIDH1 and HsIDH2 that are mutated in human cancer with other β-hydroxyacid oxidative decarboxylases that act on *(R)*-hydroxyacid substrates, including an ancestral NADP$^+$-dependent IDH from the proteobacteria *S. yanoikuyae* (Sy_IDH); NAD$^+$-dependent IDHs from *H. sapiens*, *E. coli*, and *B. subtilis*; HIDHs from *S. cerevisiae, S. pombe, C. albicans*, and *T. thermophilus*; isopropylmalate dehydrogenases (IPMDHs) from *T. thermophilus, T. ferridoxans*, and *S. cerevisiae*; and tartrate dehydrogenases (TDHs) from *E. coli* and *P. putida*. Secondary structure elements from *Hs*IDH1 are shown.



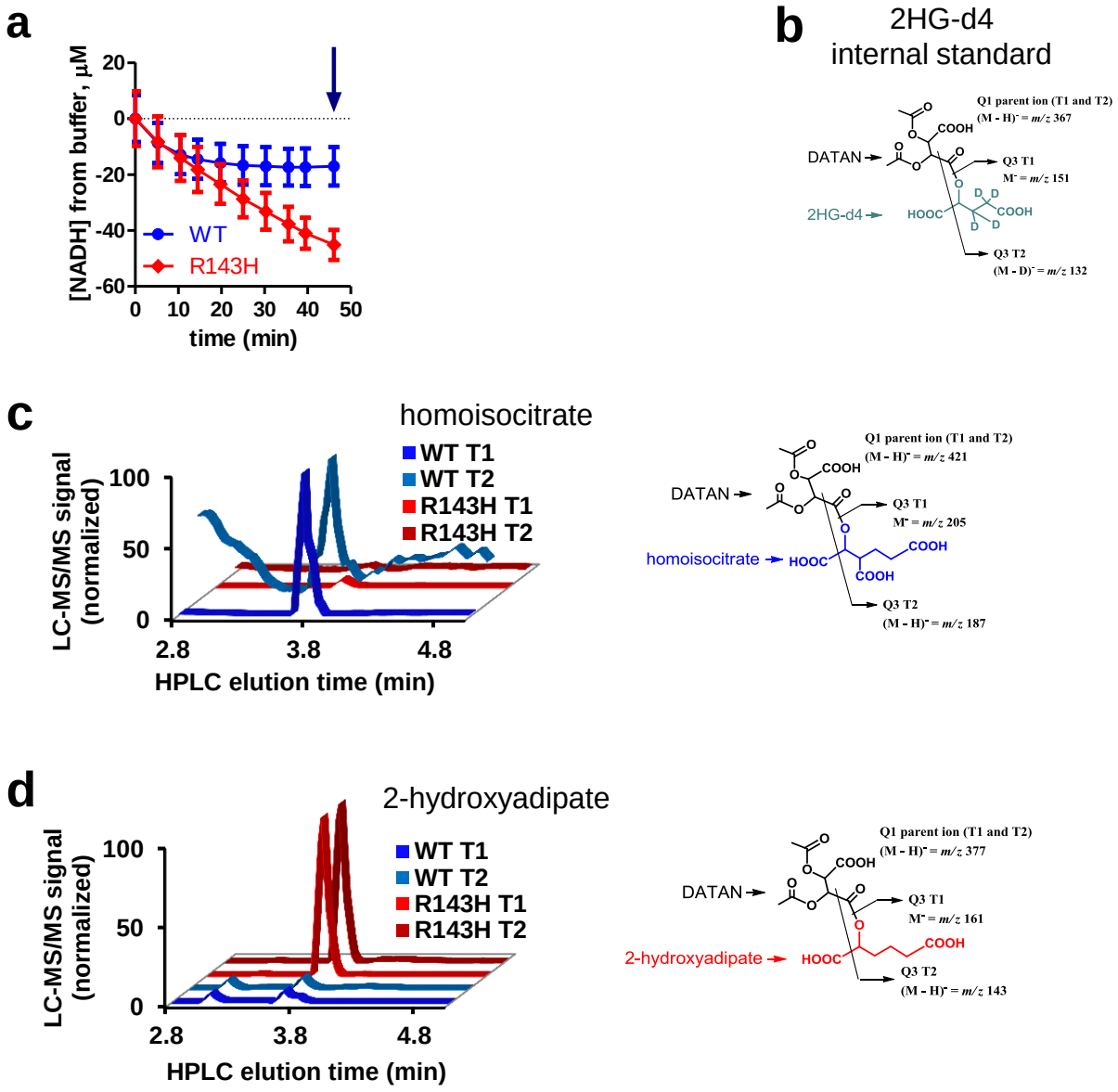
Supplementary Figure 2. Purification of *Sc*HIDH mutants.

SDS-PAGE with silver stain of purified *Sc*HIDH mutants. 15 μg of each purified protein were loaded.



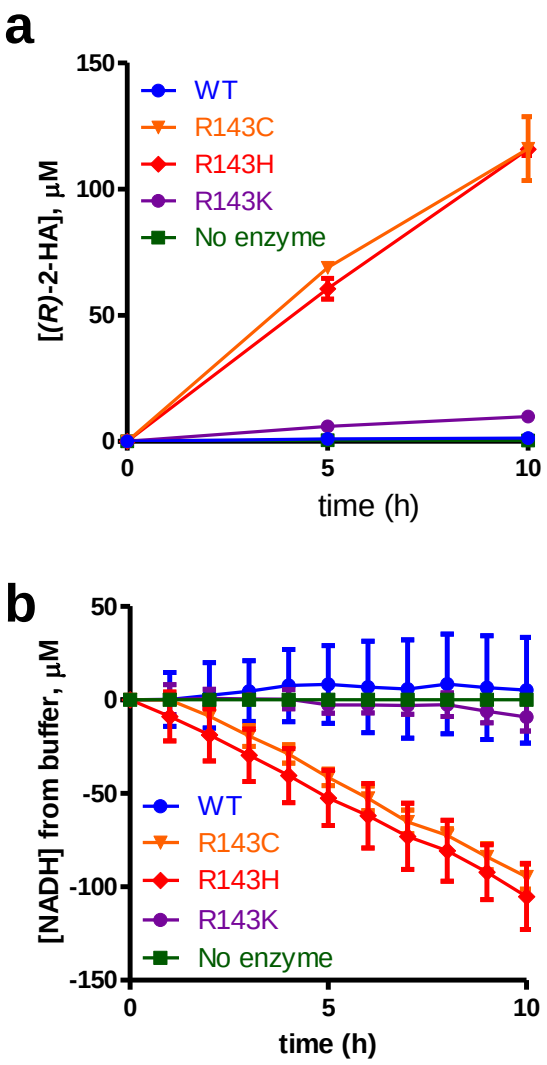
Supplementary Figure 3. Rate analysis of *Sc*HIDH mutants.

(**a**) Decrease in NADH over time in reactions containing 10 mM 2-oxoadipate, 300 µM NADH, and the indicated *Sc*HIDH mutant. (**b**) Initial rate of NADH decrease in reactions containing the indicated *Sc*HIDH mutant and 0-300 µM NADH. (**c**) Initial rate of NADH decrease in reactions containing *Sc*HIDH-R143H and 15 mM of either 2-oxoadipate, 2-oxoglutarate, or 2-oxoheptanedioate. Reactions contained 40 ng/µl of the indicated purified enzyme, 15 mM 2-oxoadipate, 100 mM HEPES, pH 7.3, 20 mM MgCl2, and 100 µM NADH unless otherwise specified. Rates are expressed either in µmol NADH min^{-1} mg^{-1} enzyme, or in arbitrary relative rate of NADH decrease per unit time per unit of enzyme mass. Data are mean ± s.d. (n=2) and are representative of three independent experiments.



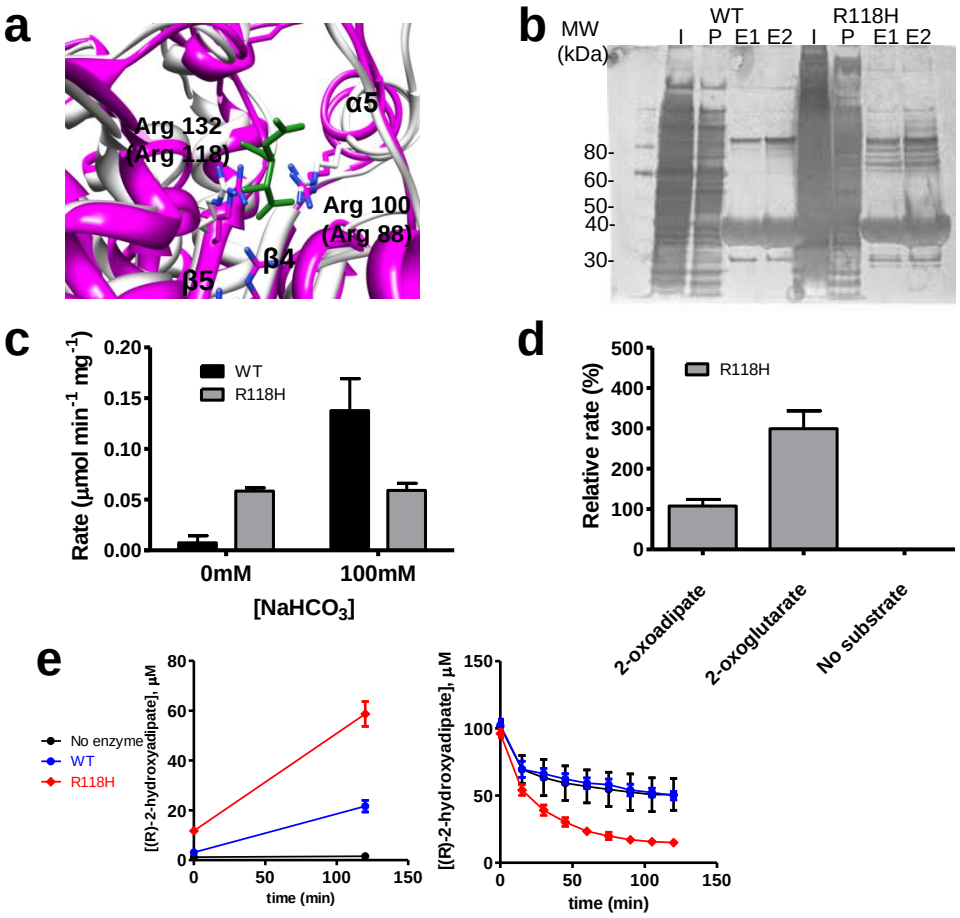
Supplementary Figure 4. LC-MS/MS analysis of *Sc*HIDH reactions.

(**a**) NADH consumption was monitored in reactions containing 50 mM HEPES, pH 7.3, 5 mM $MgCl_2$, 50 mM $NaHCO_3$, 5 mM 2-oxoadipate, 100 µM NADH. After 45 min (arrow), [3,3,4,4]-$^2H_4$-2-hydroxyglutarate (2HG-d4) internal standard was added and reactions were derivatized with diacetyl-L-tartaric anhydride (DATAN) and subjected to LC-MS/MS. (**b**) Fragmentation pattern for 2HG-d4, which was used as an internal standard to normalize the ion counts between different reactions. T1: Q1/Q3 (*m/z*) = 367.0/151.0, T2: Q1/Q3 (*m/z*) = 367.0/132.0. **(c)** Transitions (denoted T1 and T2) corresponding to homoisocitrate. T1: Q1/Q3 (*m/z*) = 421.3/205.1, T2: Q1/Q3 (*m/z*) = 421.3/187.0 as shown by the mass fragmentation diagram on the right. (**d**) Transitions corresponding to 2-hydroxyadipate. T1: Q1/Q3 (*m/z*) = 377.0/161.2, T2: Q1/Q3 (*m/z*) = 377.0/143.2 as shown in the mass fragmentation diagram on the right. Results are representative of two independent experiments. Data in (**a**) are mean ± s.d. from two independent experiments.



Supplementary Figure 5. *Sc*HIDH mutants stochiometrically produce *(R)*-2-hydroxyadipate.

(**a**) *(R)*-2-hydroxyadipate concentration as quantified by LC-MS/MS for reactions initially containing 40 ng/µl of the indicated *Sc*HIDH mutant, 2 mM NADH, 2 mM 2-oxoadipate, 20 mM $MgCl_2$, and 500 mM HEPES. (**b**) NADH concentration as assessed by absorbance at 340 nm for the same reactions. Data points are mean ± s.d. from n=3 independent experiments.



Supplementary Figure 6. R118H mutation enhances *Tt*HIDH *(R)*-2-hydroxyadipate dehydrogenase activity.

(**a**) Superimposition of three-dimensional structure of the active site for *Tt*HIDH[25] (*white*) onto *Hs*IDH1[18] (*pink*; complex with isocitrate in *green*). *Hs*IDH1 residues Arg100 and Arg132 are shown and the corresponding residues for *Tt*HIDH are shown in parentheses. (**b**) SDS-PAGE with silver stain of purified *Tt*HIDH mutants. I, lysates induced to express protein; P, pass-through of lysate onto Ni-NTA column; E1, purified eluate 1; E2, purified eluate 2. (**c**) Addition of CO_2 to the reaction as 100 mM $NaHCO_3$ stimulates NADH consumption in the presence of *Tt*HIDH-WT, but has no effect on NADH consumption in the presence of *Tt*HIDH-R118H. (**d**) Relative rate of NADH consumption for reactions performed with either no substrate, 15 mM 2-oxoglutarate (5-carbons), or 15 mM 2-oxoadipate (6 carbons). (**e**) Concentration of *(R)*-2-hydroxyadipate and decrease in NADH from buffer for reactions containing *Tt*HIDH-WT or *Tt*HIDH-R118H. All reactions were performed at 45°C with 15 mM of 2-oxoadipate, 100 μM NADH, and 500 mM HEPES, pH 7.3, 20 mM $MgCl_2$ unless otherwise specified. Data are mean ± s.d. from n=2 reactions and representative of three independent experiments for (**c**) and (**d**), and from n=3 reactions and representative of two independent experiments for (**e**).

Supplementary References

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