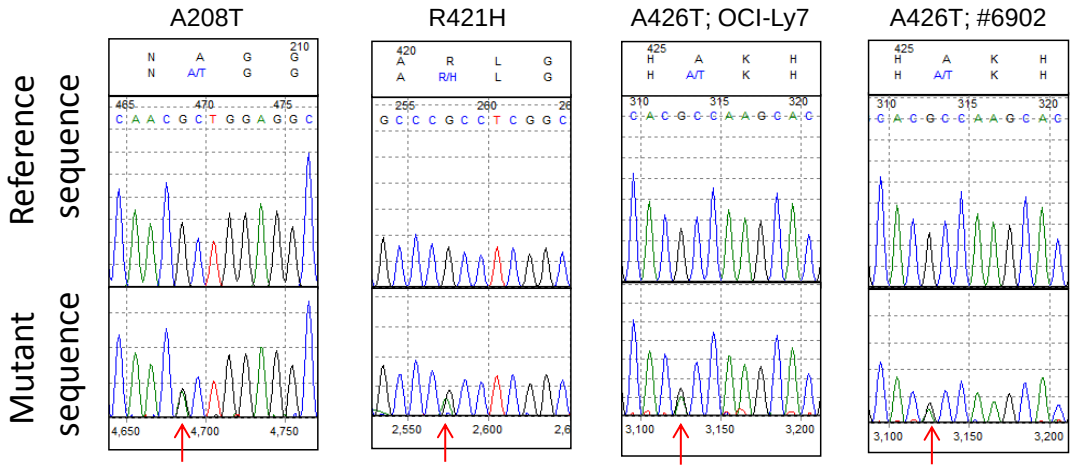
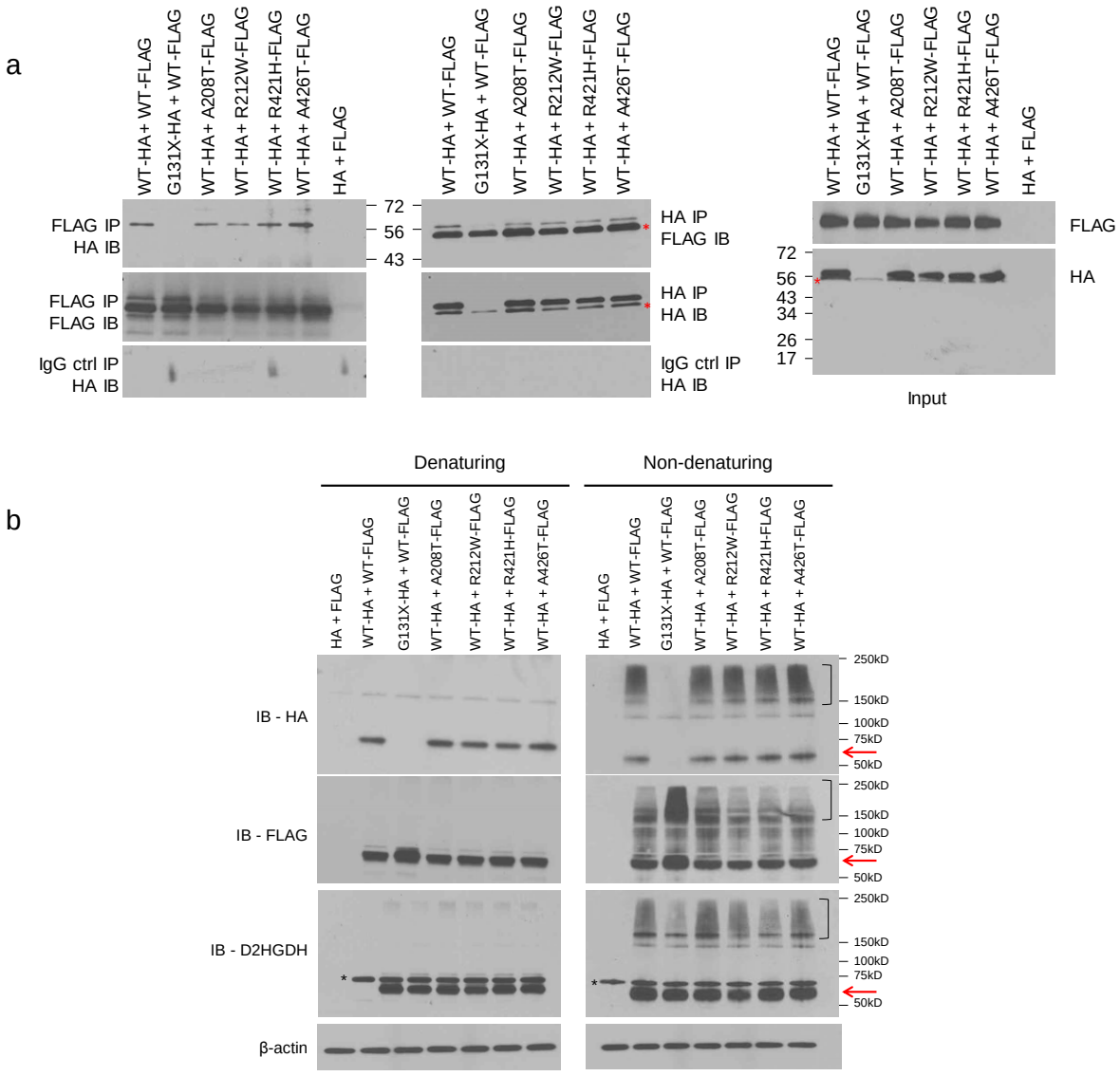
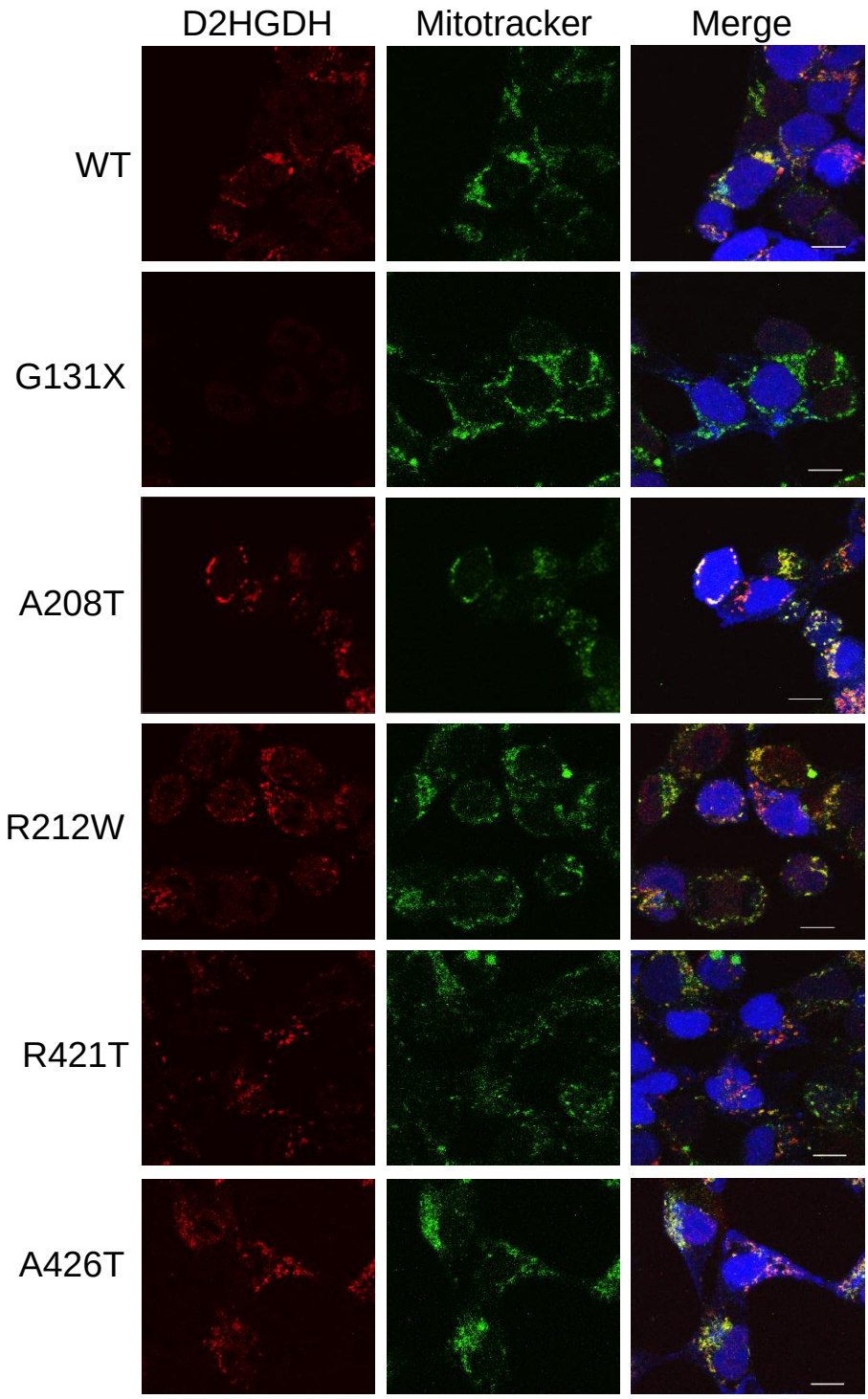


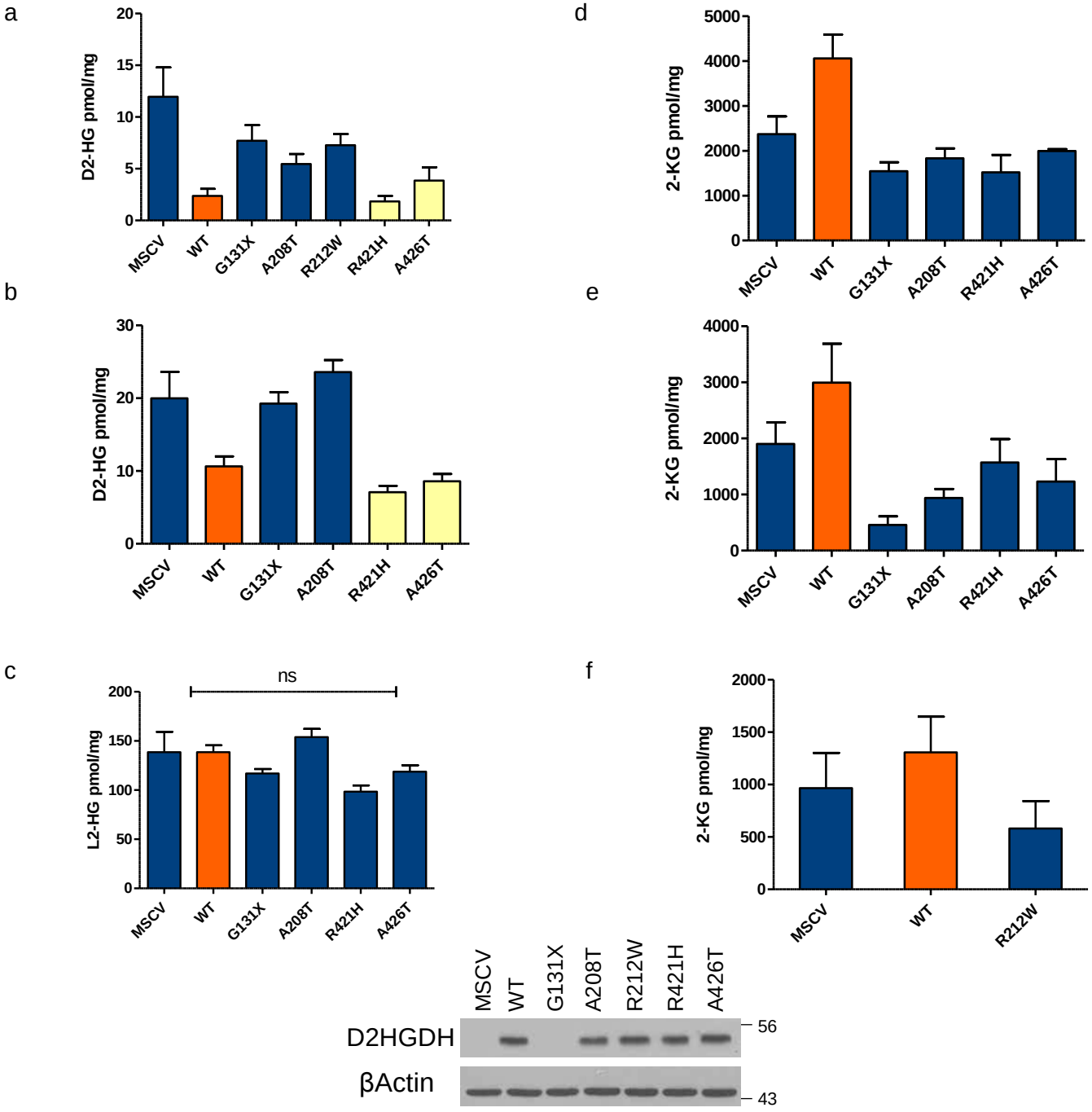
Supplementary Figure 1. Mutant and WT D2HGDH are co-expressed in DLBCL. Sequencing tracing of RT-PCR products representing the expression levels of three DLCBL-associated D2HGDH mutations indicate that the mutant and WT alleles are equally expressed. The frame-shift nature of the G131X prevented a clear display of the histogram for that variant. However, cloning and sequencing of those RT-PCR products showed a balanced representation of WT and mutant forms (4 mutant, 6 WT in 10 clones – data not shown). No mRNA was available from tumours harbouring the R212W mutation. The expression of A426T mutation is shown in both a cell line and in a primary DLBCL.



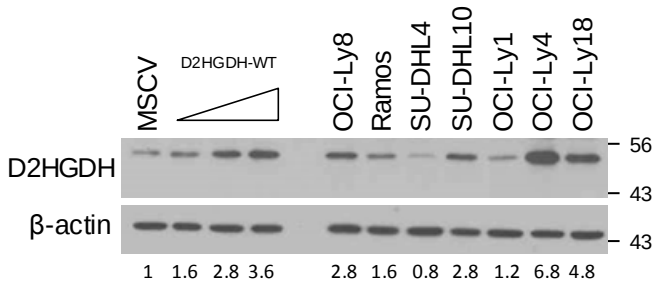
Supplementary Figure 2. D2HGDH is a self-interacting protein. **a)** FLAG- or HA-tagged D2HGDH WT and mutant constructs were co-transfected in HEK-293 cells (different combinations of WT and mutant proteins are listed on the top). Immunoprecipation (IP) of D2HGDH-FLAG pulled-down HA-D2HGDH (left panel - top). Likewise, IP of HA-D2HGDH, brought-down D2HGDH-FLAG (middle panel - top). These data indicate that D2HGDH self-complexes and that the DLBCL-associated mutations do not disrupt this binding. Note that the G131X truncating mutation probably results in an unstable protein (predicted size 17KD), which could not be detected with N-terminus tagging (HA-D2HGDH-G131X – see input blot). The HA-IP/IB yields a non-specific band indicated by an *. Co-transfection of empty-vectors, re-probing with FLAG or HA antibodies, and IP with control IgG (middle and lower panels, respectively) confirmed the specificity of these assays. Input protein is shown on the far right panel. **b)**. The D2HGDH self-association was further confirmed by analysing the proteins in denaturing vs. non-denaturing conditions. Again, FLAG- or HA-tagged D2HGDH WT and mutant constructs were co-transfected in HEK-293 cells (the different combinations of WT and mutant proteins are listed on the top). On the left, using three different antibodies and denaturing conditions D2HGDH expression is readily identified at its predicted size. On the right panels, non-denaturing gels revealed a larger product (indicated by brackets) detected every time D2HGDH is ectopically expressed (note its absence in the HA-G131X expressing cells, when IB with HA, and in all instances of empty HA and FLAG vectors co-expression). The D2HGDH self-interacting complex was detected between two D2HGDH-WT molecules (2nd lane from the left), as well as between D2HGDH-WT and any of the four missense D2HGDH mutants (A208T, R212W, R421H, A426T). At the moment we can not exclude the possibility that the self-assembly of D2HGDH includes more than two molecules. (i.e., homotrimer, homotetramer, etc.) or that other proteins are part of this complex (heterotrimer, etc.). *,non-specific band. Red arrow – D2HGDH at its predicted size.



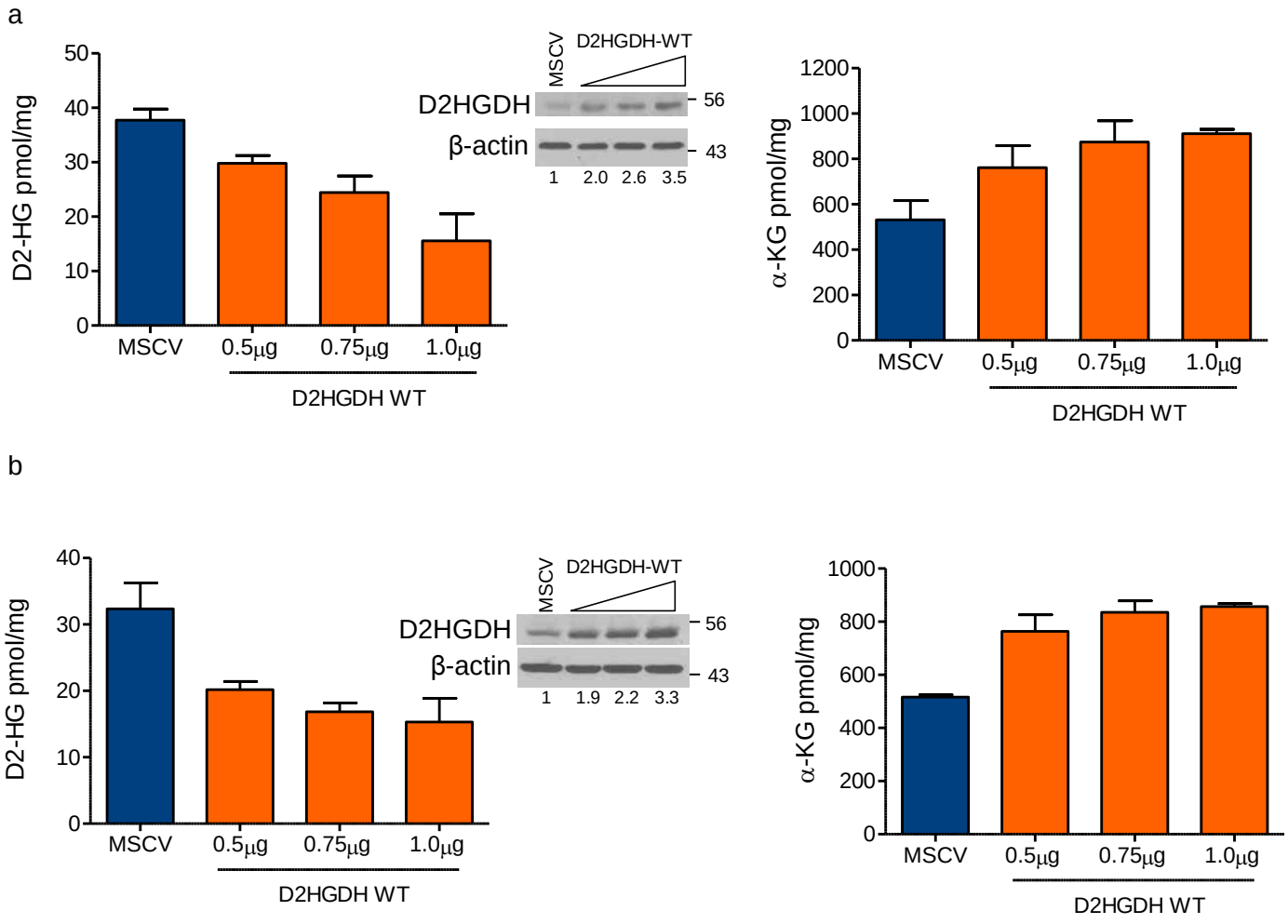
Supplementary Figure 3. D2HGDH mitochondrial localization by immunofluorescence. HEK-293 cells stably expressing WT or each D2HGDH mutant were co-stained with the mitochondrial probe MitoTracker (green), and with an anti-D2HGDH antibody (Cy3, red). D2HGDH-expressing cells are also GFP positive (pseudocolored in blue). Merged images indicate that, as expected, WT D2HGDH localizes to the mitochondria, and that the mutations analysed do not change this subcellular localization. As the D2HGDH antibody targets a C-terminus epitope, the truncated G131X mutant is not recognized and thus its subcellular distribution cannot be determined. Cells were imaged on an Olympus FV1000 confocal microscope, at 60x magnification (scale bar is 10µm).



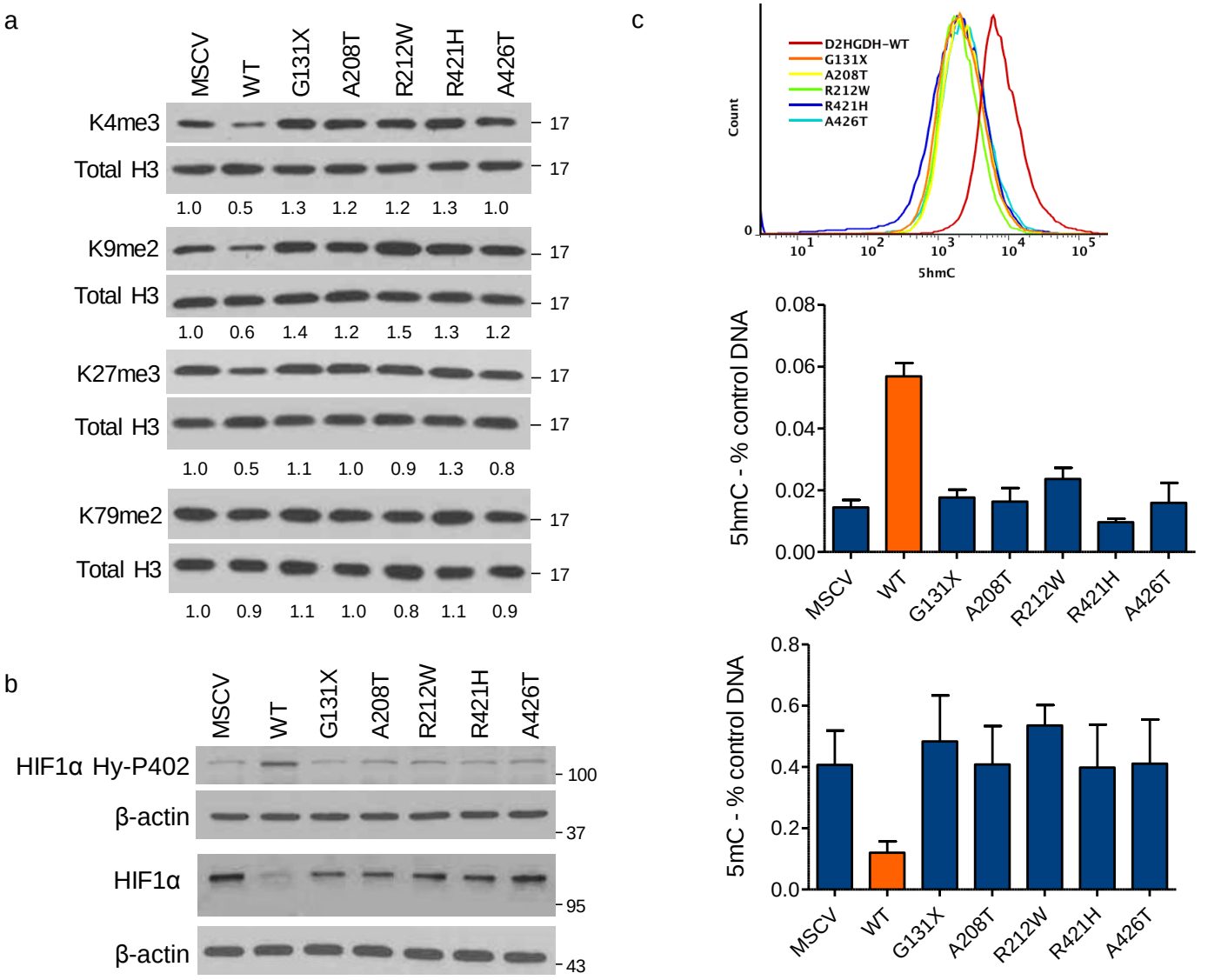
Supplementary Figure 4. HPLC-ESI-MS-based quantification of D2-HG and α-KG. Metabolites were extracted from HEK-293 cells stably expressing WT or mutant D2HGDH constructs. In **a)** and **b)** we show that expression of WT D2HGDH led to a decrease in D2-HG when compared to cells expressing the G131X, R208T, and R212W mutants (but not R421H or A426T) (in a, $p<0.0001$, in b, $p=0.0002$, ANOVA; in both $p<0.05$ Bonferroni's Multiple Comparison post-test). Data in a and b represent two biologically independent assays, performed with five and three replicates/sample type, respectively. In **c)**, we show that expression of D2HGDH (WT or mutant) does not alter the levels of L2-HG - assay performed with three replicates/sample type. In **d)**, **e)** and **f)**, we show that expression of D2HGDH-WT result in a significant accumulation of α-KG when compared to cells expressing an empty or any of the DLBCL-associated mutants (in c and d, $p< 0.0001$, in e $p=0.0112$, ANOVA; in all three assays $p<0.05$ Bonferroni's Multiple Comparison post-test). Data in d, e and f, represent three biologically independent assays, performed with four or five replicates/sample type. The data from panel a) and d) are shown in Figure 1a, depicting the relative abundance of D2-HG and α-KG in models of stable D2HGDH expression. Western blot (bottom) confirms the similar expression of WT and mutant D2HGDH – the antibody used is directed to the C-terminus region and would not detect the G131X truncated protein, which is likely to be unstable (see supplementary Figure 3). Metabolites quantifications were normalized by protein amount (mg).



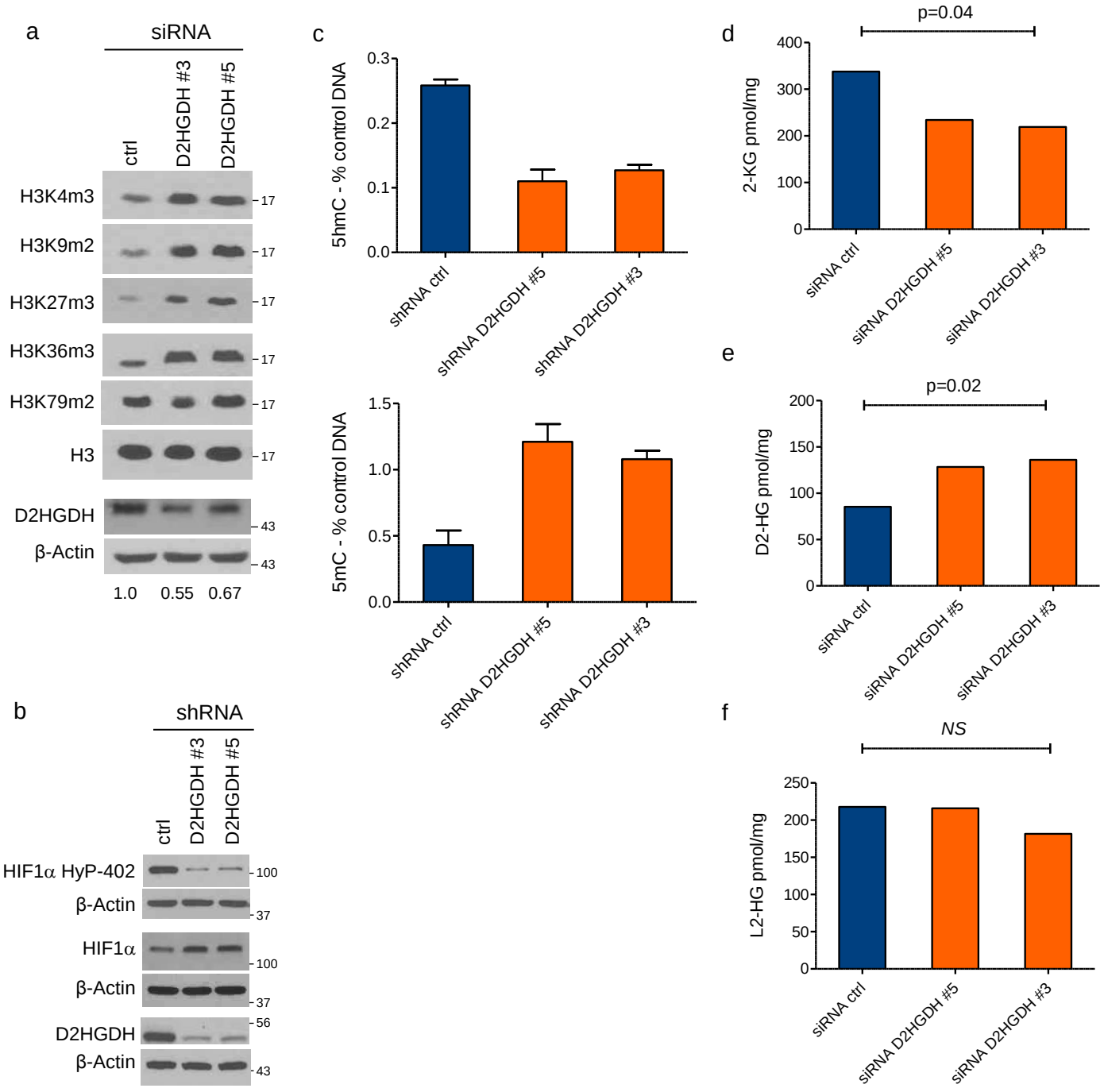
Supplementary Figure 5. D2HGDH expression levels in the transient transfection model are within endogenous expression range. Western blot analysis of D2HGDH in HEK-293 cells transiently transfected with an empty vector (MSCV, 1µg), or increasing amounts of a MSCV-D2HGDH construct (0.5µg, 0.75 µg and 1.0 µg) alongside a subset of the B cell lymphoma cell lines used in this study. This assay demonstrated that the expression level achieved in the transient transfection assay is within the range of endogenous D2HGDH expression found in B lymphoma cell lines. Densitometric quantification, normalized by β-actin, is show at the bottom.



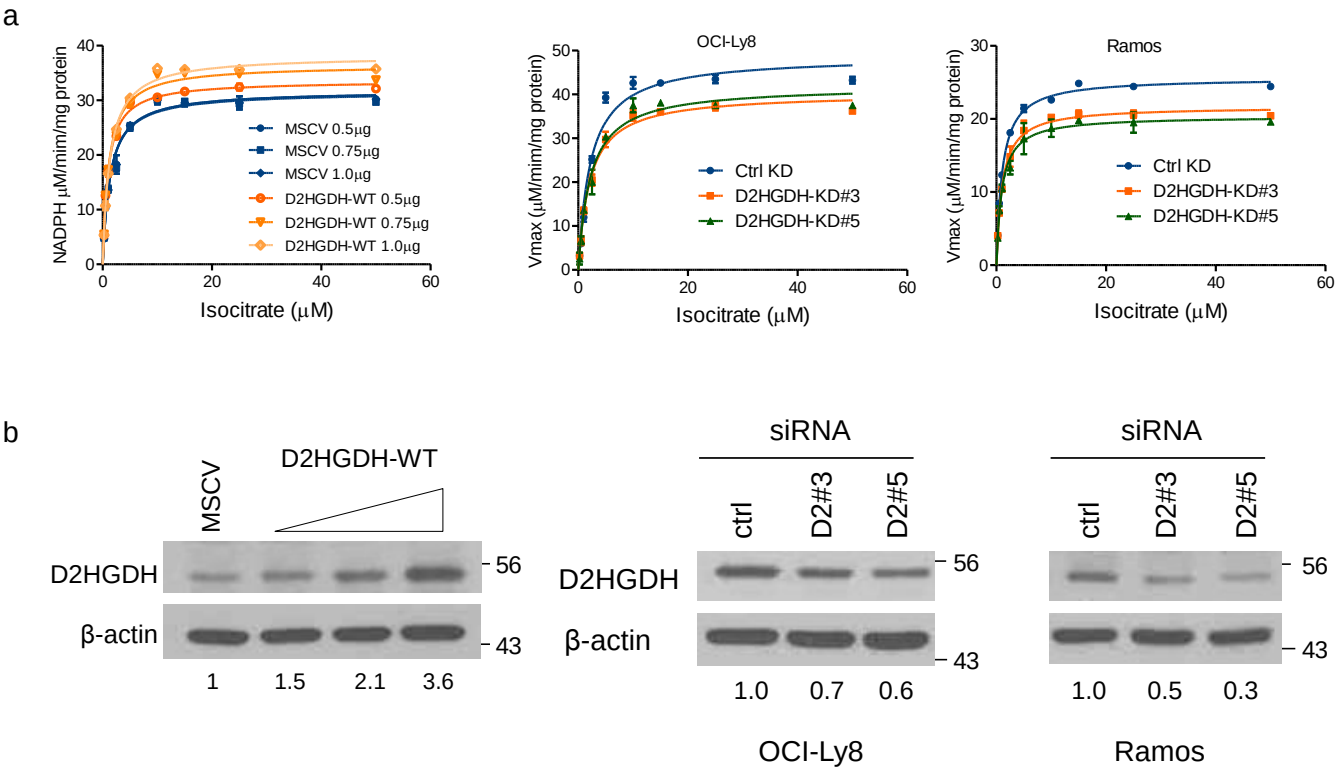
Supplementary Figure 6. HPLC-ESI-MS-based quantification of D2-HG and α-KG in cells transiently expressing wild-type D2HGDH. Metabolites were extracted from HEK-293 transiently expressing an empty vector or a WT D2HGDH construct. **a)** and **b)** represent two independent transient transfection assays, and in each instance the left panel depicts D2-HG level and the right panel, α-KG. Western blot examination confirmed the subtle increase in D2HGDH expression in this model, which resulted in a progressive, significant, decrease in D2-HG levels (left panels in **a)** and **b)**, p=0.0002 and p=0.0003, respectively – ANOVA). Concordantly, increasing D2HGDH expression significantly elevated the levels of α-KG in these cells (right panels, in **a)** and **b)**, p=0.0068 and p<0.0001, respectively – ANOVA). All data shown represent mean and SD of three replicates/sample type. The data from panel a) are shown in Figure 1d, which depicts the relative abundance of D2-HG and α-KG. Densitometric quantification, normalized by β-actin, is show at the bottom of the western blots.



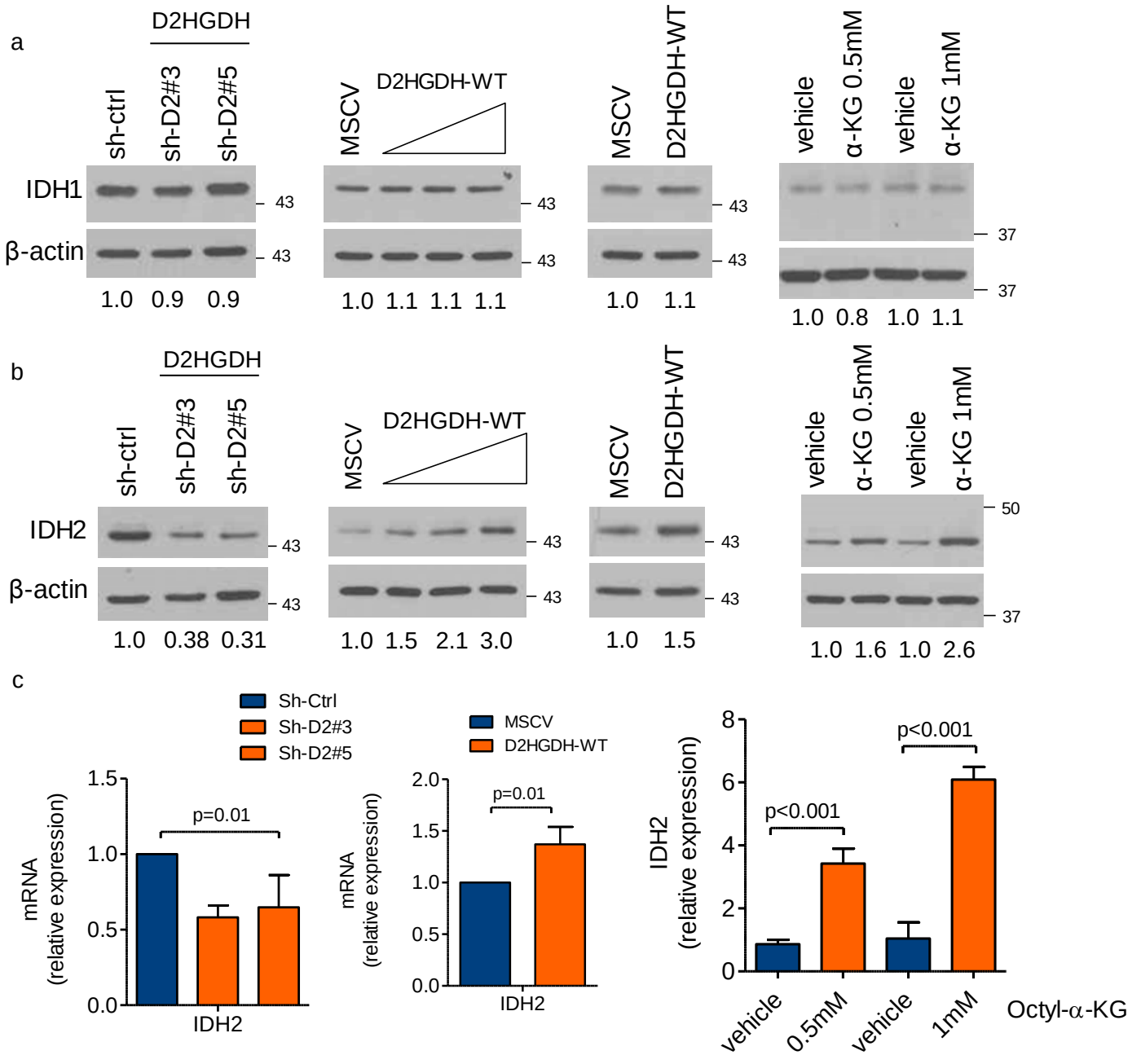
Supplementary Figure 7. Differential effects of WT and mutant D2HGDH towards α-KG-dependent dioxygenases a) Histone demethylases: the methylation of H3 lysine residues was determined by western blot in HEK-293 stably expressing WT or mutant D2HGDH; whereas the WT enzyme suppressed K4, K9, K27 methylation, expression of the mutant proteins had little or no effect, and mimicked the empty MSCV control cells. Note that the H3K79me2 level is not influenced by D2HGDH, as this lysine is not regulated by the Jumonji family of α-KG-dependent demethylases. Densitometric quantification, normalized by β-actin, is show at the bottom of each panel. **b) HIF1α prolyl-hydroxylases**: under hypoxia (1% O_2, 16h), the levels of HIF1α hydroxylation (Pro-402) were increased and total HIF1α markedly decreased in cells expressing WT but not any of the mutant D2HGDH enzymes. **c) TET enzymes**: Top panel: The levels of 5hmC were quantified by intracellular FACS and found to be lower in cells expressing mutant D2HGDH than in WT cells, as shown by the left-shift of D2HGDH-mutant expressing cells in the histogram. Mid panel: The quantification of 5hmC marks was also performed using a capture and detection antibody-based approach. Abundance of 5hmC marks was measured by absorbance, and reported as relative values normalized by a positive control, a polynucleotide containing 20% 5hmC. Cells expressing empty vector (MSCV) or any of the D2HGDH mutant enzymes had significantly lower levels of 5hmC- modified DNA than the D2HGDH WT cells ($p<0.001$, one-way ANOVA, $p<0.05$, Bonferroni's multiple comparison post-test). These data represent the mean and SD of an assay performed in triplicate, confirmed in a biological replicate. Bottom panel) The quantification of 5mC was performed as above; DNA methylation was significantly higher in mutant than in WT D2HGDH cells ($p<0.001$, one-way ANOVA, $p<0.05$, Bonferroni's multiple comparison post-test). The data show represent the combined values of three biological replicates, performed in triplicate or duplicate. The western blot data shown in a) and b) were confirmed in biological replicates at least three times, and all results independently validated with a transient transfection model (Figure 1).



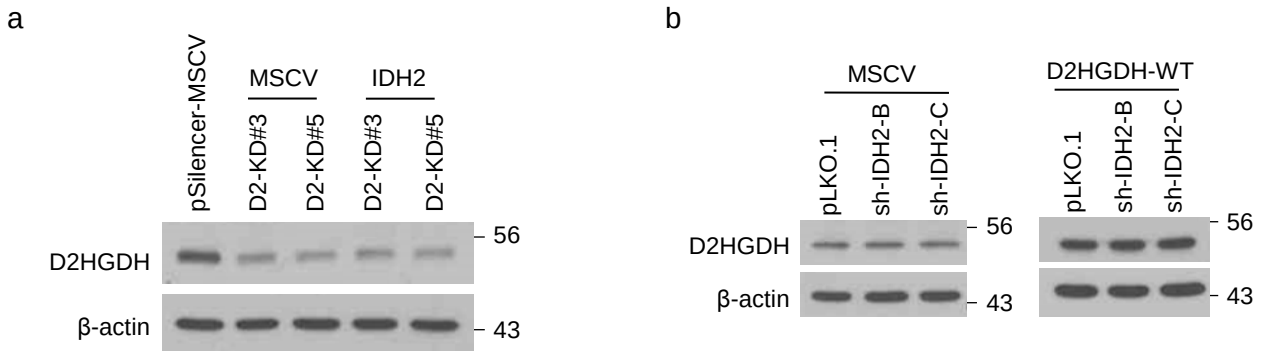
Supplementary Figure 8. Transient and stable knockdown (KD) of D2HGDH in HEK-293 cells. a) Transient transfection of two siRNA oligonucleotides directed at D2HGDH or a control siRNA led to increase in the methylation levels of H3K4, H3K9, H3K27 and H3K36; but not in H3K79 which is not regulated by α-KG-dependent HDMs. **b)** Upon exposure to hypoxic conditions for 16h, cells with stable shRNA-based KD of D2HGDH showed marked suppression of hydroxylated HIF1α with consequent accumulation of total HIF1α. Efficacy of the transient (siRNA) or stable (shRNA) KD of D2HGDH is shown by western blot (a and b). Note that the transient KD only partially suppresses D2HGDH expression (~60%). Densitometric quantifications are shown at the bottom. **c)** The levels of the DNA marks 5hmC and 5mC (top and bottom panels) were significantly lower and higher, respectively, in D2HGDH-KD cells than their controls ($p<0.0001$, ANOVA); **d)**, **e)** and **f)** show that the transient, partial, KD of D2HGDH significantly modify D2-HG ($p= 0.04$, ANOVA), α-KG ($p=0.02$, ANOVA), but not L2-HG levels ($p=0.4$, ANOVA), as determined by LC/MS. The 5hmC and 5mC measurements shown represent the mean and SD of five data points derived two independent assays. The LC/MS data shown is the mean of an experiment performed with two replicates; similar results were obtained in an independent assay performed with a single replicate.



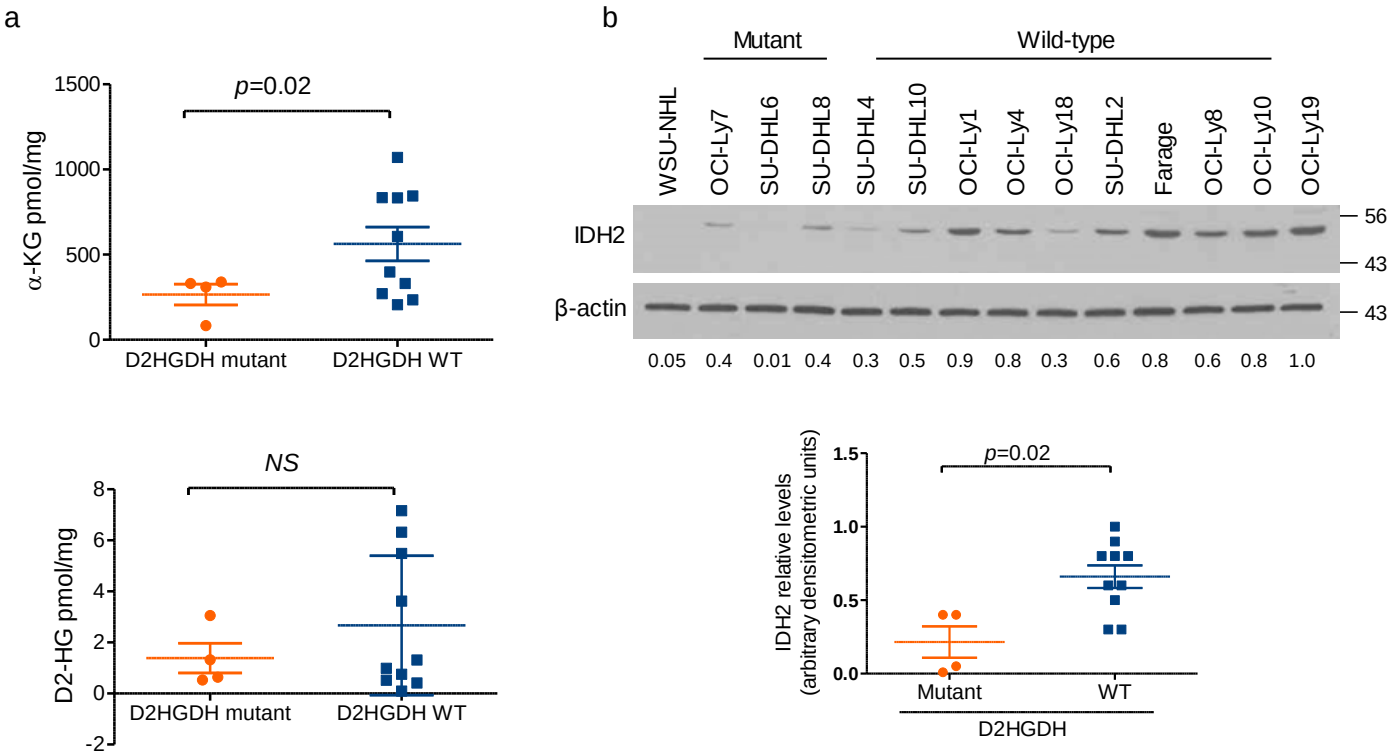
Supplementary Figure 9. D2HGDH expression and IDH activity a) Left - Transient expression of increasing amounts of WT D2HGDH significantly and progressively increased IDH activity in comparison to that of MSCV-expressing isogenic cells ($p<0.0001$ ANOVA, $p<0.05$ Bonferroni's Multiple Comparison post-test). Middle and Right panels - Transient knockdown of D2HGDH in the B cell lymphoma cell lines significantly decreased IDH activity in comparison to isogenic controls ($p<0.0001$; $p=0.0003$; ANOVA, for OCI-Ly8 and Ramos, respectively - $p<0.05$ Bonferroni's Multiple Comparison post-test). In **b)** western blot-based examination of D2HGDH expression in the proteins used for the IDH activity assay (shown in a) confirms its progressive elevation in the transient transfection of HEK-293 cells, and partial knockdown in the B cell lymphoma cell lines OCI-Ly8 and Ramos. Densitometric analysis is shown below the western blot.



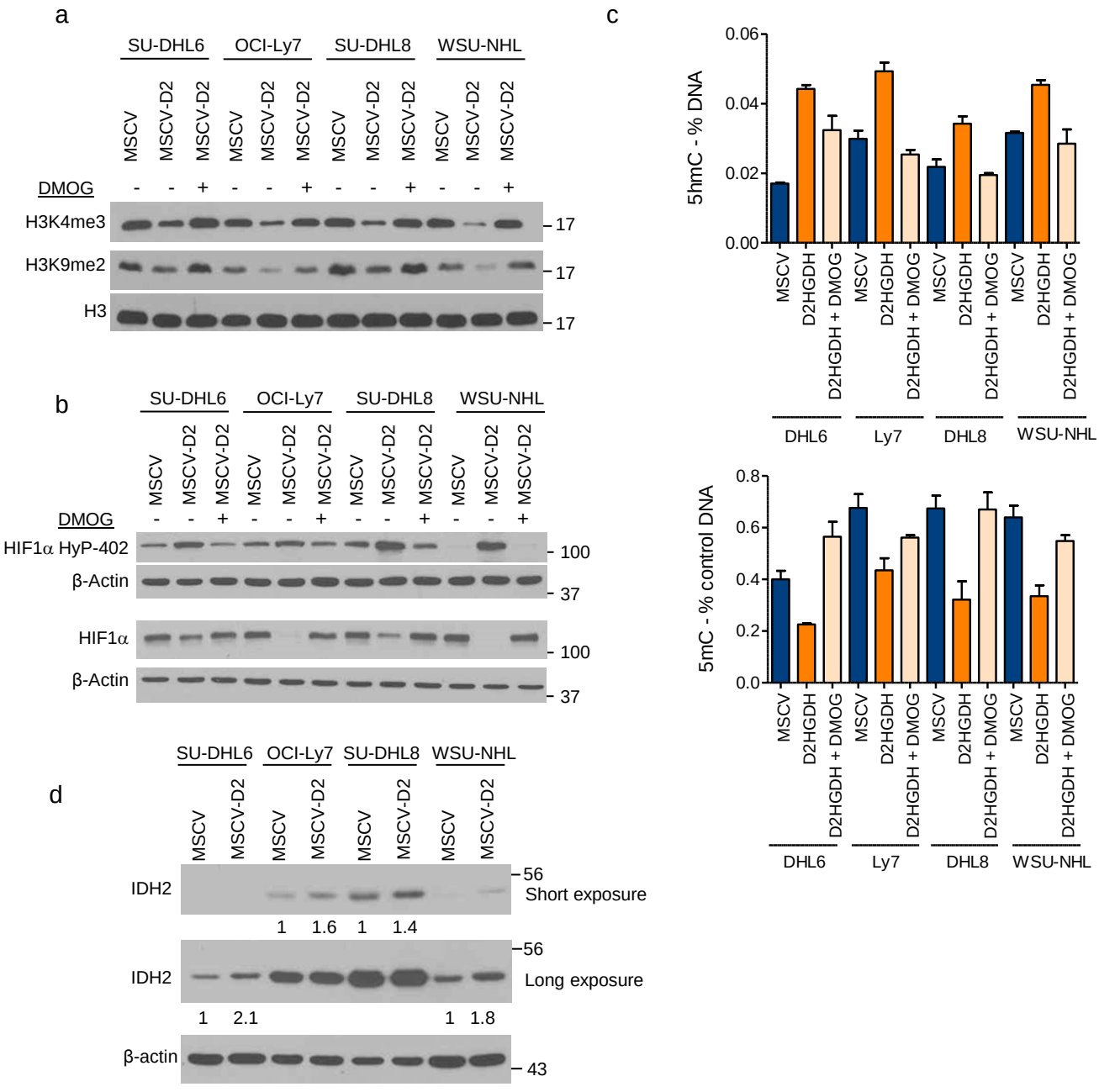
Supplementary Figure 10. IDH1 and IDH2 expression in models of gain and loss of D2HGDH and following exposure to synthetic cell-permeable α-KG. **a)** Western blots show that the expression of IDH1 is not modified by D2HGDH knockdown (left panel), its transient or stable ectopic expression (middle panels), or exposure to the cell-permeable octyl-α-KG (right panel). **b)** Western blot analysis show IDH2 downregulation in D2HGDH knockdown HEK-293 cells (left panel), and its upregulation in models of D2HGDH transient or stable expression (middle panels) or upon exposure to the octyl α-KG (right panel). Densitometric quantifications are shown at the bottom of the western blots. These assays were repeated two to four times. **c)** Left panel - real-time RT-PCR of HEK-293 cells with stable D2HGDH knockdown show a significant downregulation of IDH2 (p=0.01, ANOVA). Middle - quantification of IDH2 by real-time RT-PCR demonstrate a significantly higher IDH2 expression in D2HGDH-WT cells than in the empty-vector isogenic controls. (p=0.01, two-tailed Student's t-test). Right - quantification of IDH2 by real-time RT-PCR demonstrate a significantly higher IDH2 expression in HEK-293 cells exposed to synthetic α-KG than vehicle control (p<0.001, two-tailed Student's t-test). The data shown in **c)** (left and middle panels) represent mean and SD of three independent biological replicates (each assay performed in triplicate), displayed as relative expression (sh-ctrl vs. sh-D2HGDH, or MSCV vs. D2HGDH-WT); the data shown in the right panel is the mean -/+ SD of a representative assay performed in triplicate – two independent biological replicates were completed and yielded similar results. In all instances, *IDH2* expression was normalized to that of a housekeeping gene *TBP*, relative quantification achieved by calculating ΔΔCt, and expression defined as $2^{-\Delta\Delta Ct}$, where cells expressing control vector, or vehicle, represent the baseline.



Supplementary Figure 11. D2HGDH levels in models of IDH2 ectopic expression or knockdown. a) Western blot analysis shows that ectopic expression of IDH2 in models of D2HGDH knockdown (KD#3 and KD#5) does not change its expression – compare levels of D2HGDH in KD#3 and KD#5 MSCV-only cells vs. KD#3 and KD#5 in IDH2 cells. Expectedly, the D2HGDH levels are lower in KD#3 and KD#5 cells than in control pSilencer/MSCV cells. **b)** shRNA-mediated knockdown of IDH2 did not change D2HGDH expression levels in MSCV control cells (left panel) or in their isogenic counterparts expressing WT D2HGDH (right panel). Expectedly, the levels of D2HGDH are higher in the D2HGDH-WT cells than in MSCV controls.

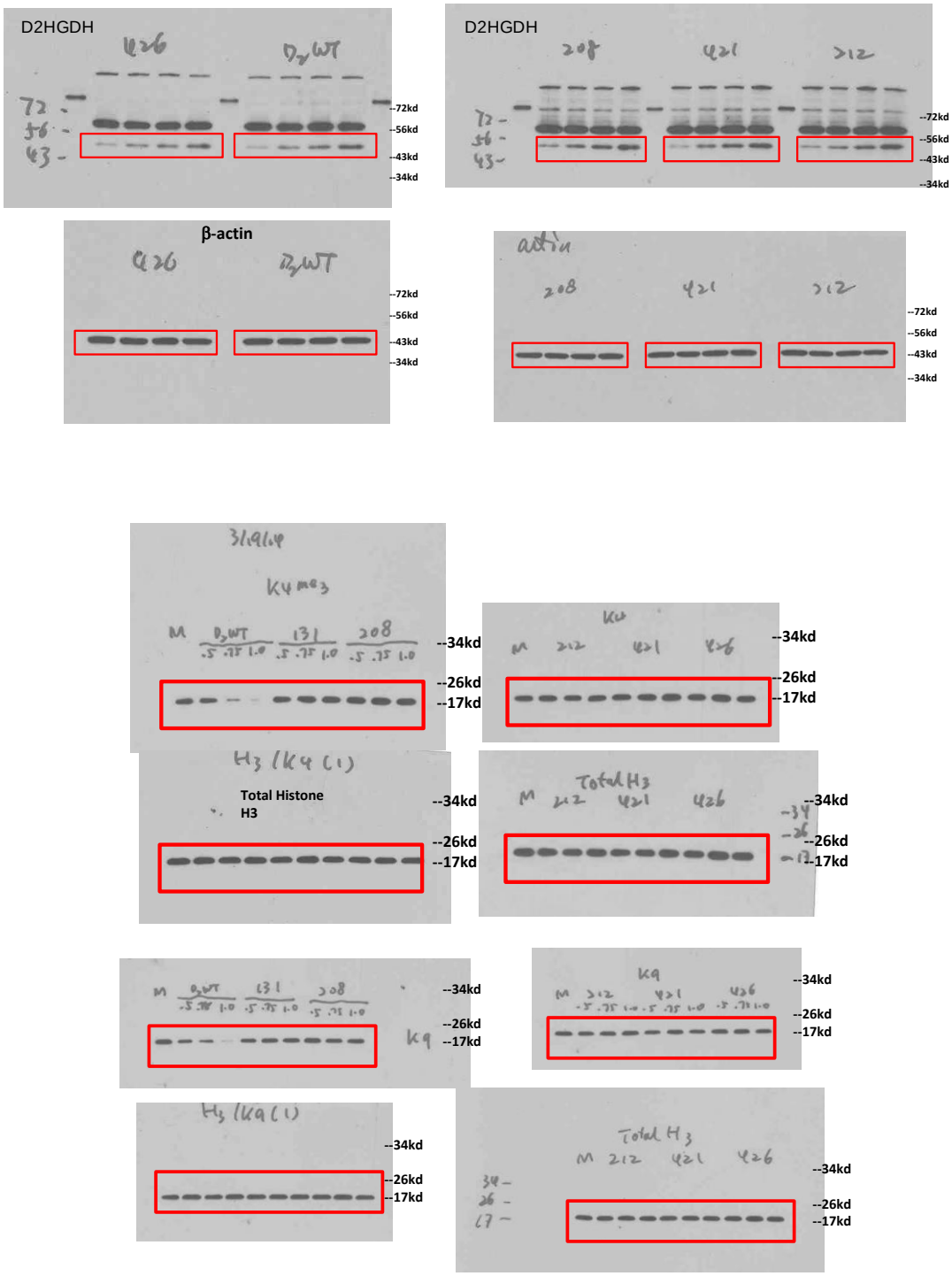


Supplementary Figure 12. **Interplay between D2HGDH, α-KG and IDH2 in DLBCL**. **a)** HPLC-ESI-MS-based quantification of α-KG and D2-HG in 14 parental DLBCL cell lines showed significantly lower levels of α-KG in D2HGDH mutant DLBCLs than in WT cells, but no significant difference in the abundance of D2-HG. For each cell line, the data shown represent the median value of three independent assays, each performed in triplicate (p=0.02, two-tailed Student's t-test). **b)** Western blot analysis of IDH2 in a set of 14 DLBCL cell lines, including four D2HGDH mutant and 10 wild-type. Densitometric quantification of IDH2 (normalized by β-actin) is shown below the blots and in graphic display with its statistical significance denoted (Mann-Whitney test). The metabolite quantification in a) was normalized by protein concentration.



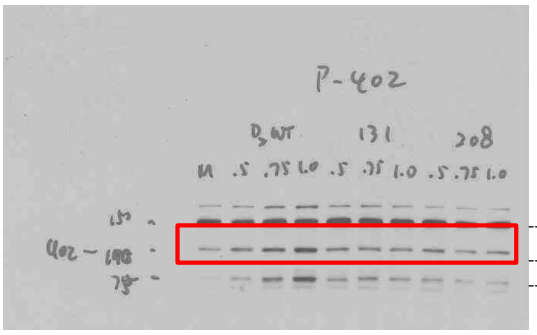
Supplementary Figure 13. Genetic modulation and functional analysis of D2HGDH-mutant DLBCL cell lines. Isogenic models of stable expression of an empty vector (MSCV) or WT D2HGDH were generated by retrovirus transduction in four independent D2HGDH-mutant DLBCL cell lines. **a)** Ectopic expression of D2HGDH decreased H3K4 and H3K9 methylation in all four models, an effect that was largely reversed by exposure to 1mM of dimethyloxalylglycine (DMOG), a competitive inhibitor of α-KG. **b)** The Hydroxylation of proline 402 in HIF1α-was elevated (top panel) and total HIF1α expression decreased (bottom panel) by expression of WT D2HGDH – in both instances the values were returned to baseline following exposure to DMOG. **c)** Quantification of 5hmC or 5mC show that ectopic expression of D2HGDH-WT in DLBCL mutant cells significantly elevated the abundance of 5hmC marks (top) ($p<0.0001$, ANOVA) and lowered total DNA methylation (5mC levels) (bottom) ($p<0.0001$, ANOVA). For both measurements, the effects of D2HGDH were largely abrogated by exposure to DMOG. The abundance of these modifications are reported as relative values normalized by a positive control, a polynucleotide containing 20% 5hmC or 50% 5mC. Experiments show in a) and b) were repeated twice; data in c) represent the mean and SD of a representative experiment (out of two independent assays) performed in triplicate. **d)** Stable ectopic expression of D2HGDH in the four D2HGDH-mutant DLBCL cell lines induced IDH2 expression. Densitometric quantification (normalized by β-actin) is shown at the bottom of the western blots

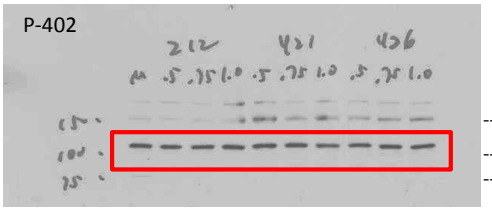
2b

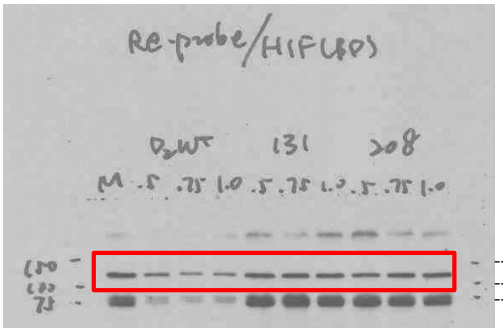


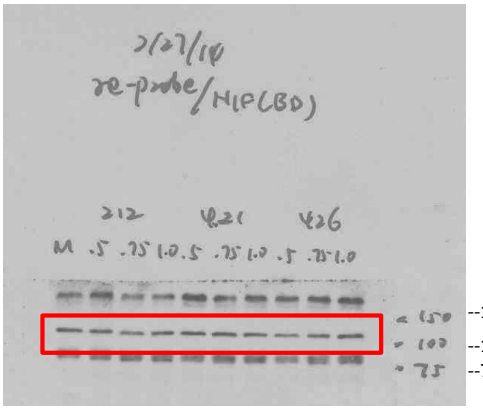
Supplementary Figure 14. Full scans of uncropped blots presented in the main paper. Red boxes indicate the cropped regions. Molecular weight markers are indicated in kDa.

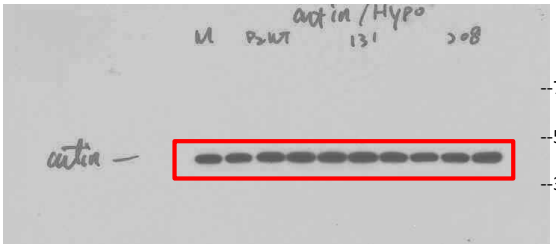
2b

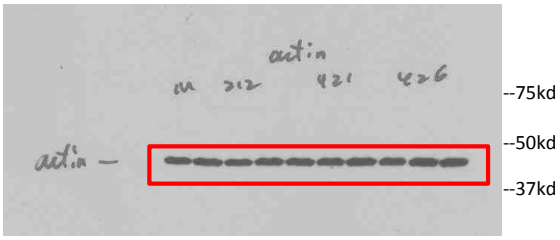


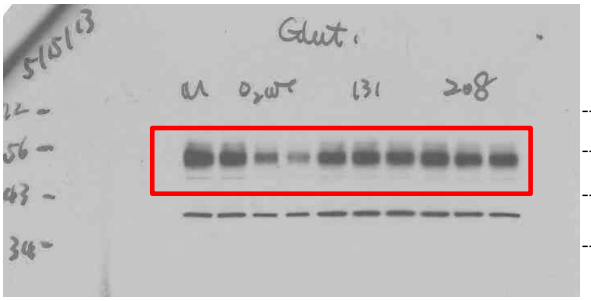


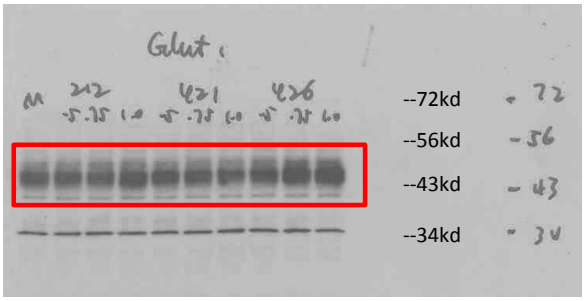




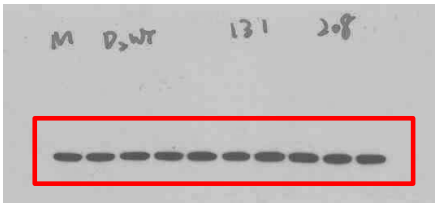




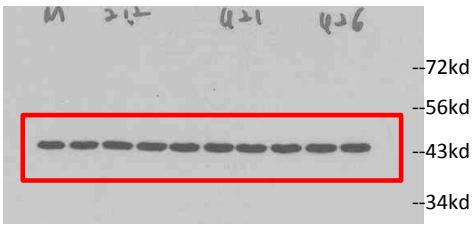




Actin

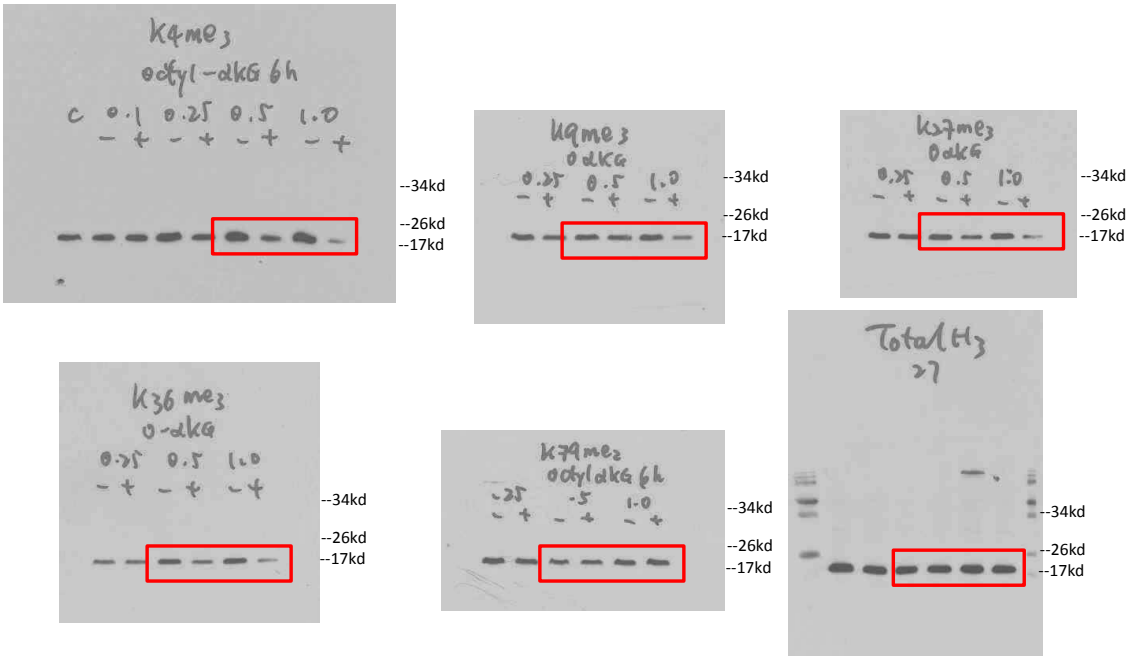


Actin

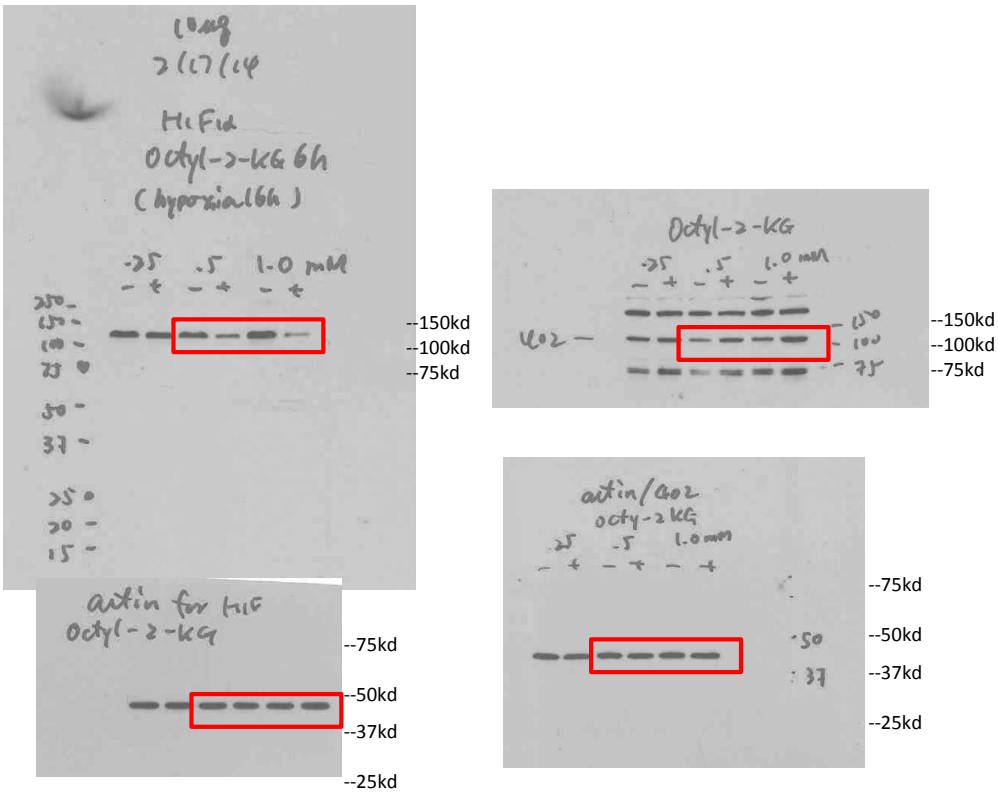


Supplementary Figure 14 - continued

3a

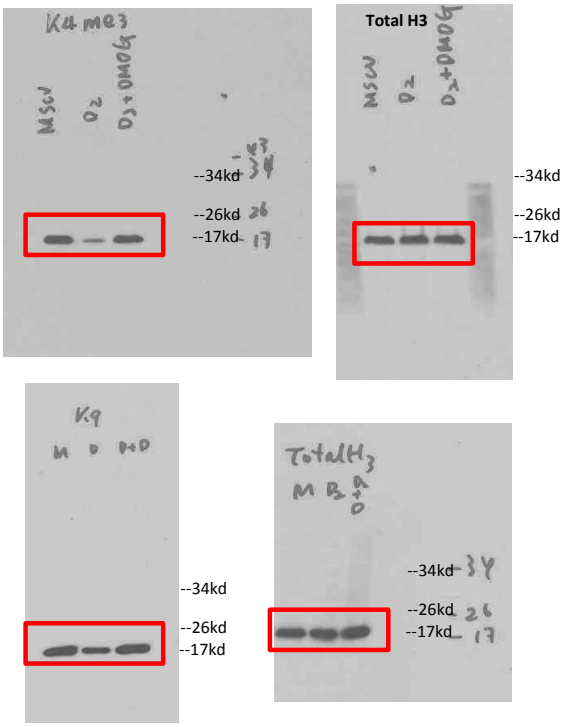


3b

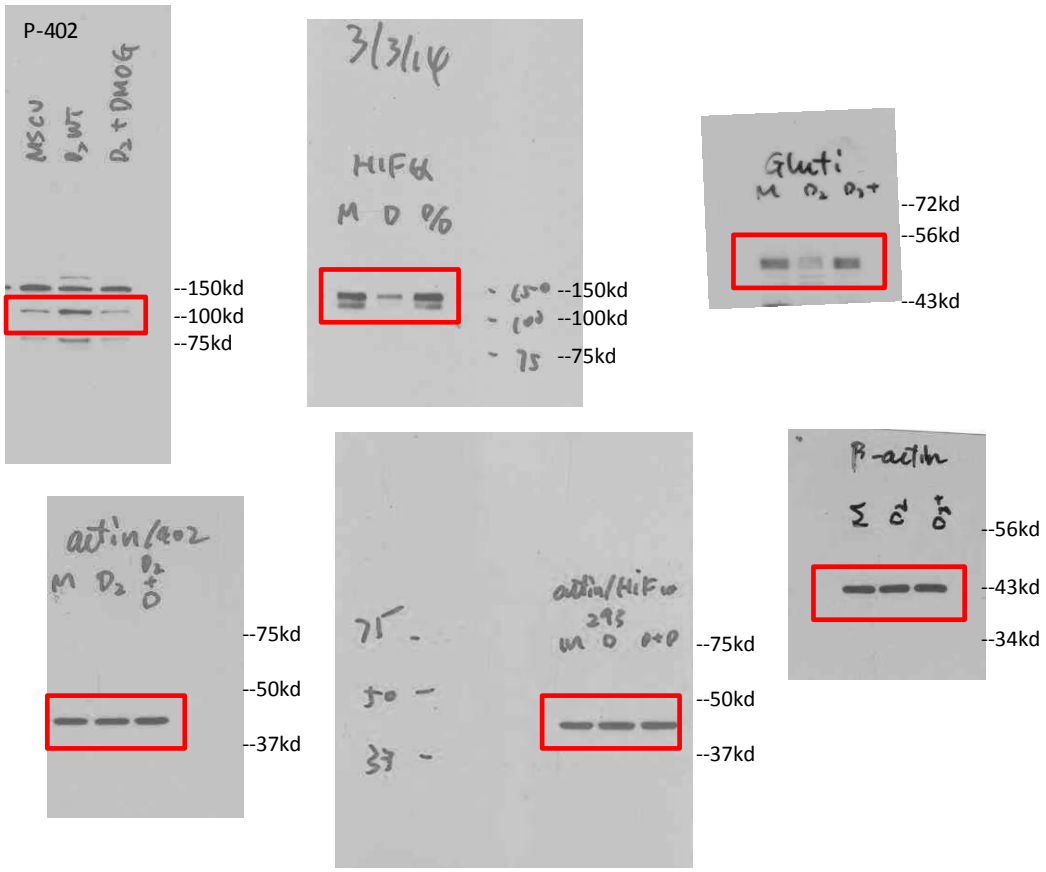


Supplementary Figure 14 - continued

3d

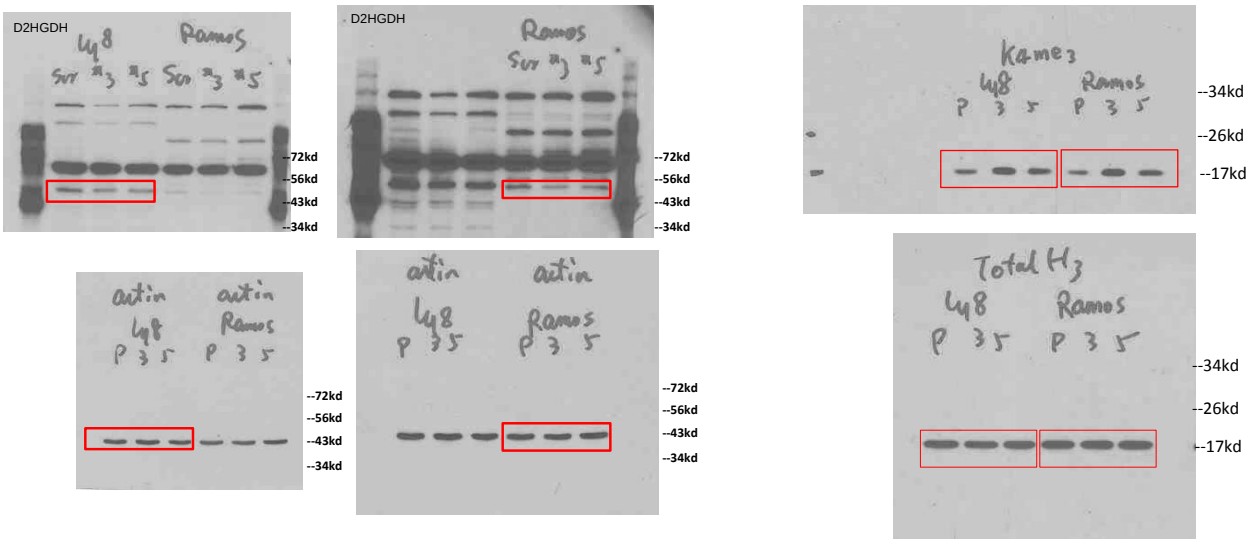


3e

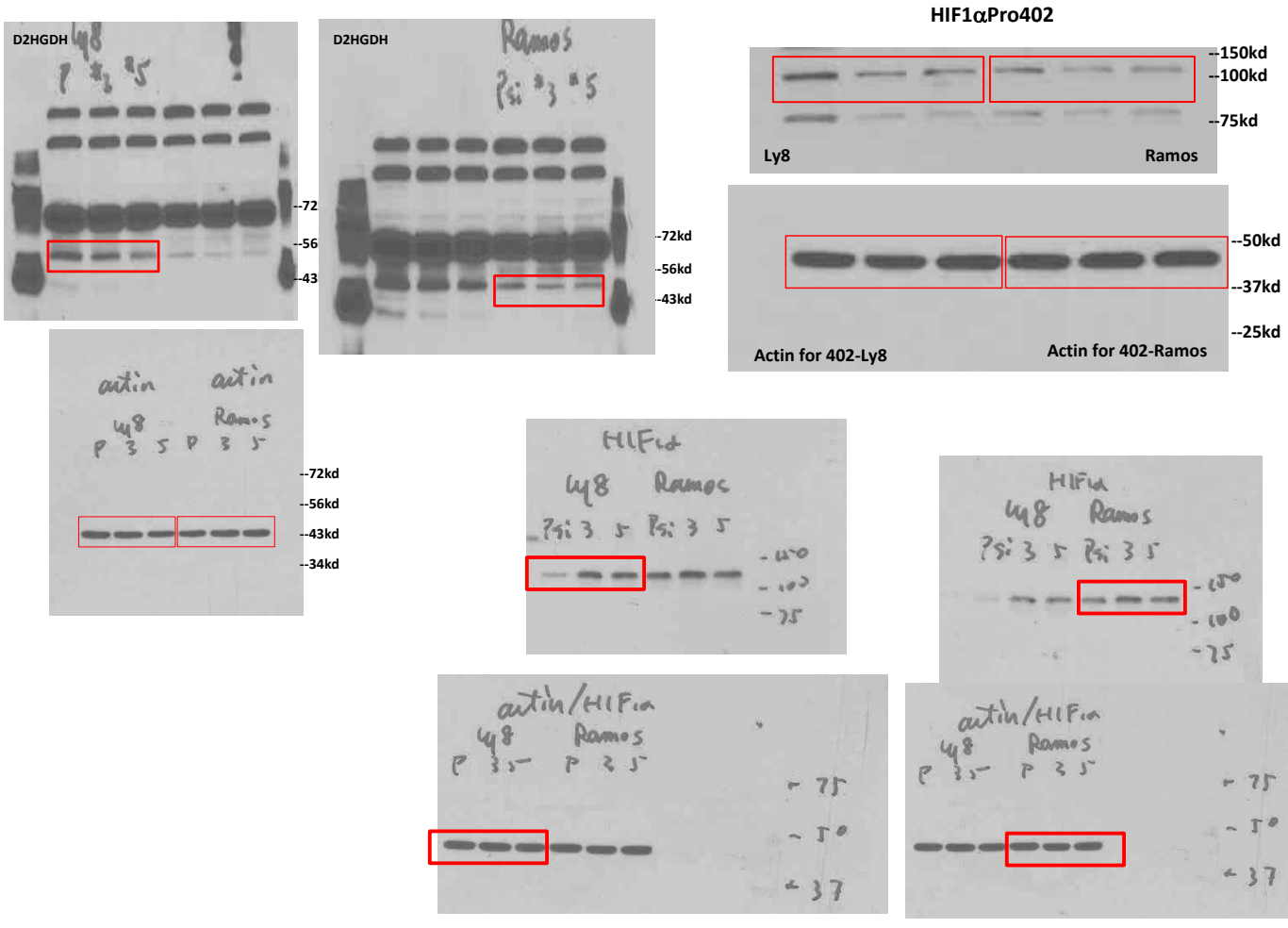


Supplementary Figure 14 - continued

4a

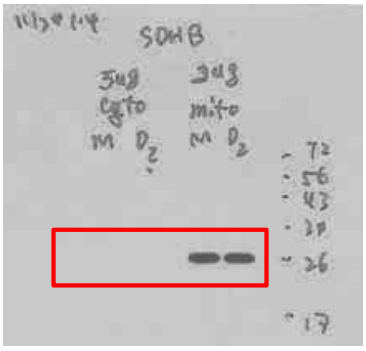


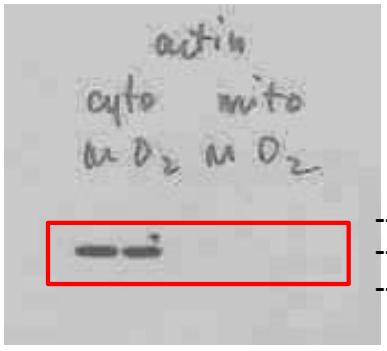
4b



Supplementary Figure 14 - continued

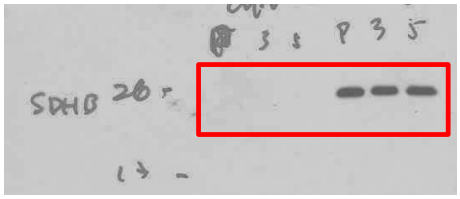
5a

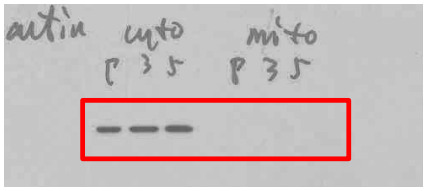




--56kd
--43kd
--34kd

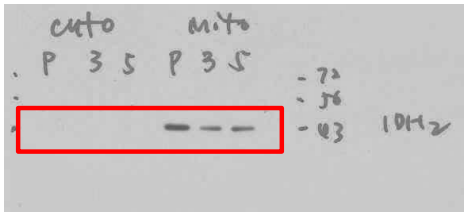
5b

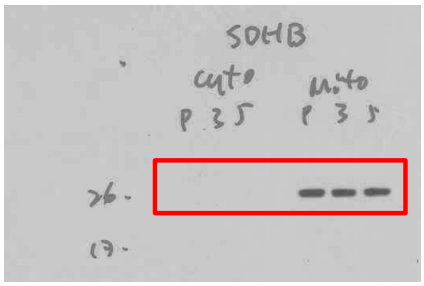


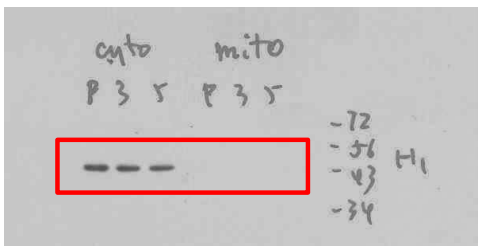


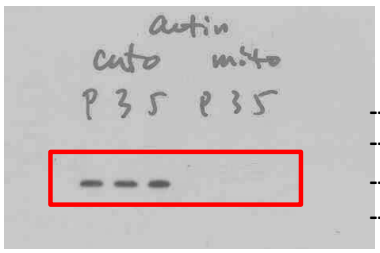
--56kd
--43kd
--34kd

6a



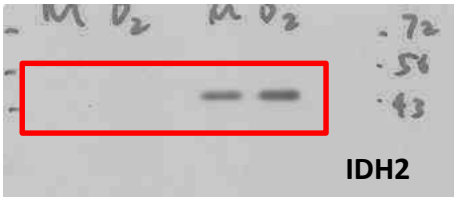


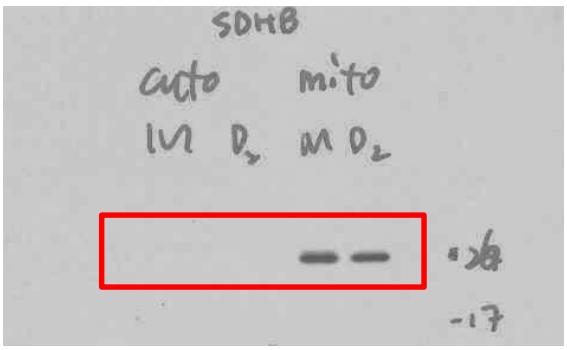


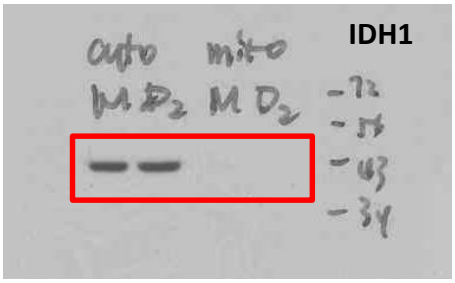


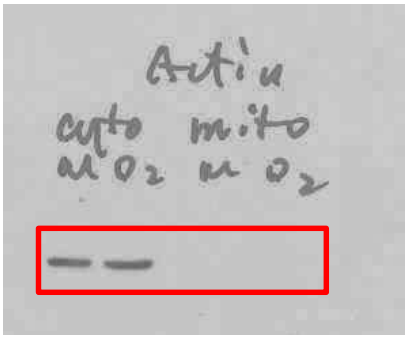
--72kd
--56kd
--43kd
--34kd

6a





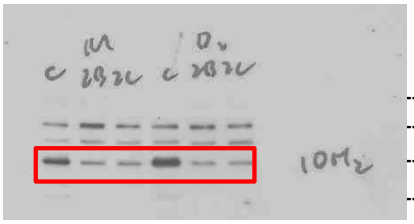


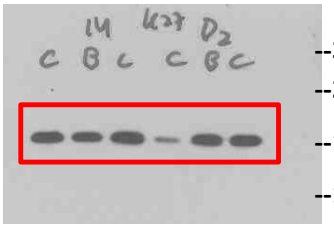


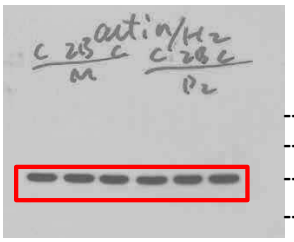
--72kd
--56kd
--43kd
--34kd

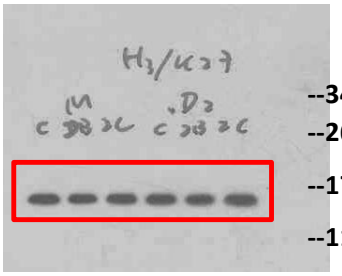
Supplementary Figure 14 - continued

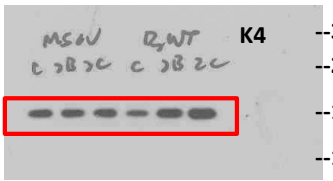
6b

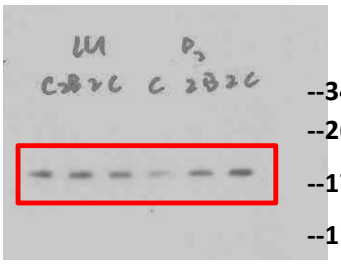


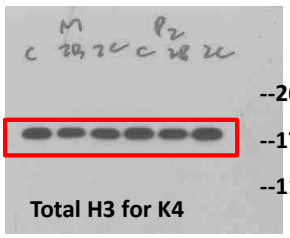


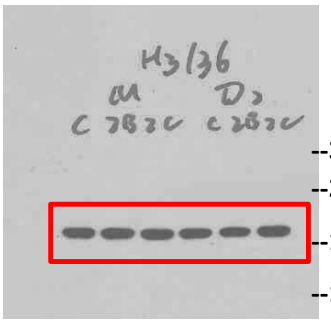


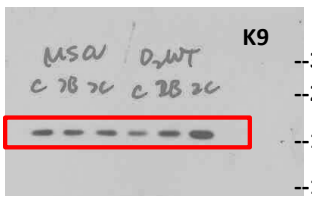


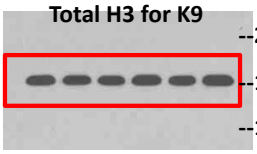


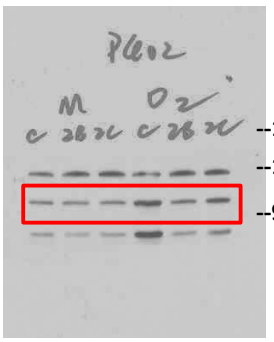


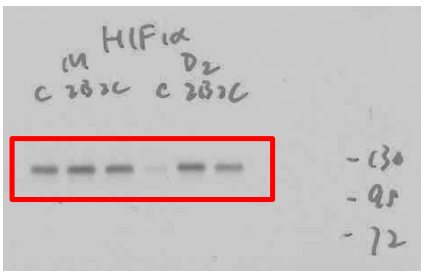


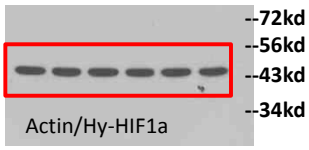


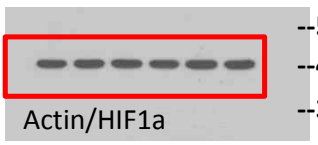






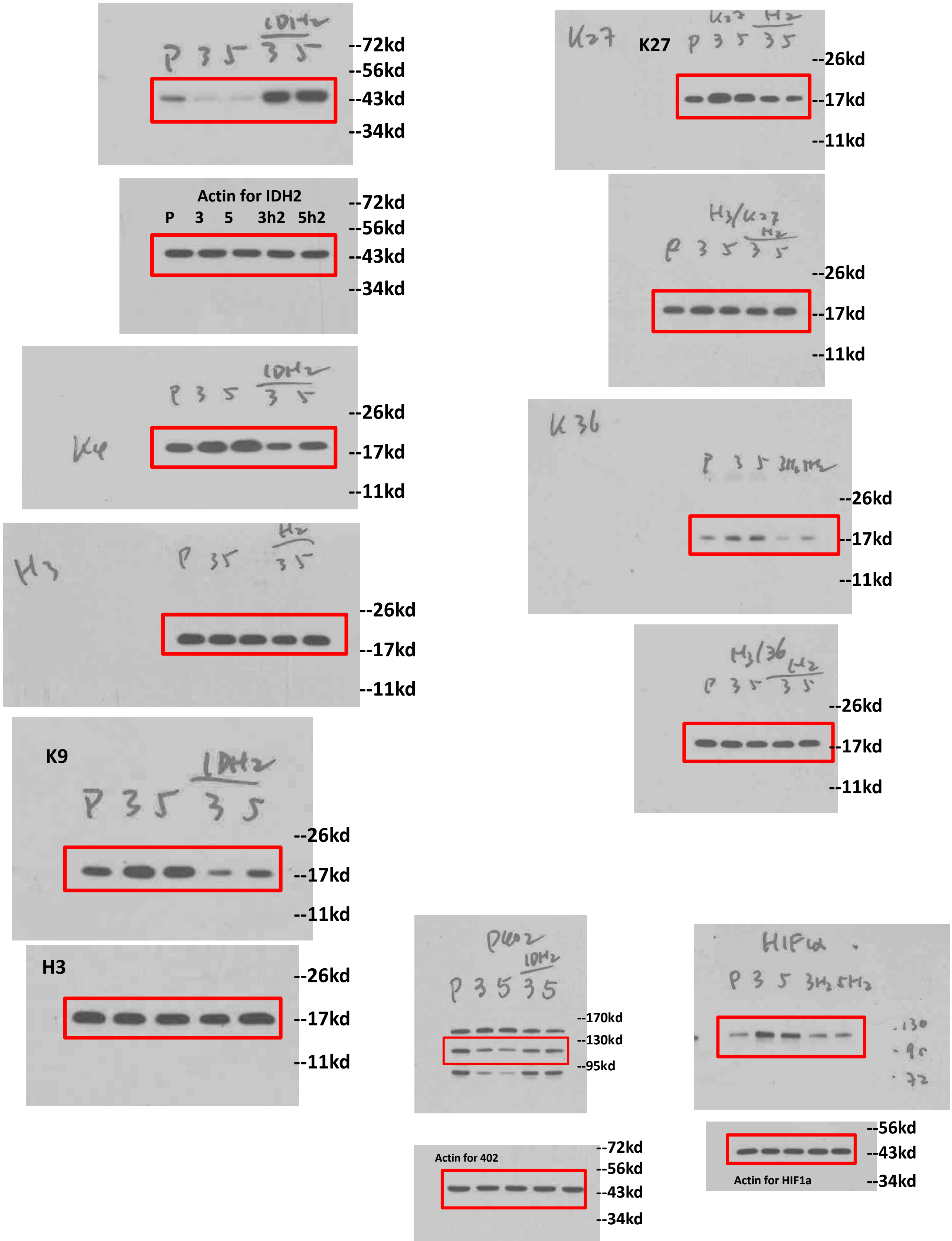






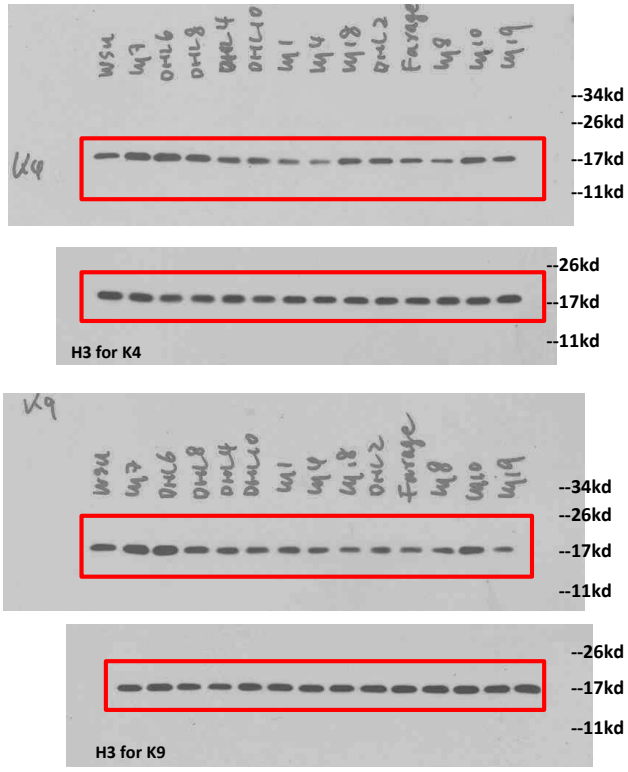
Supplementary Figure 14 - continued

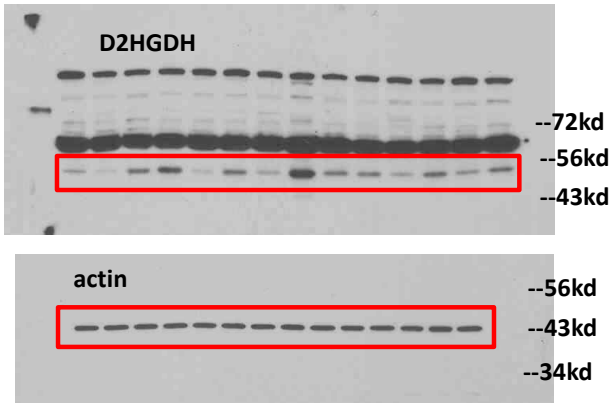
6d

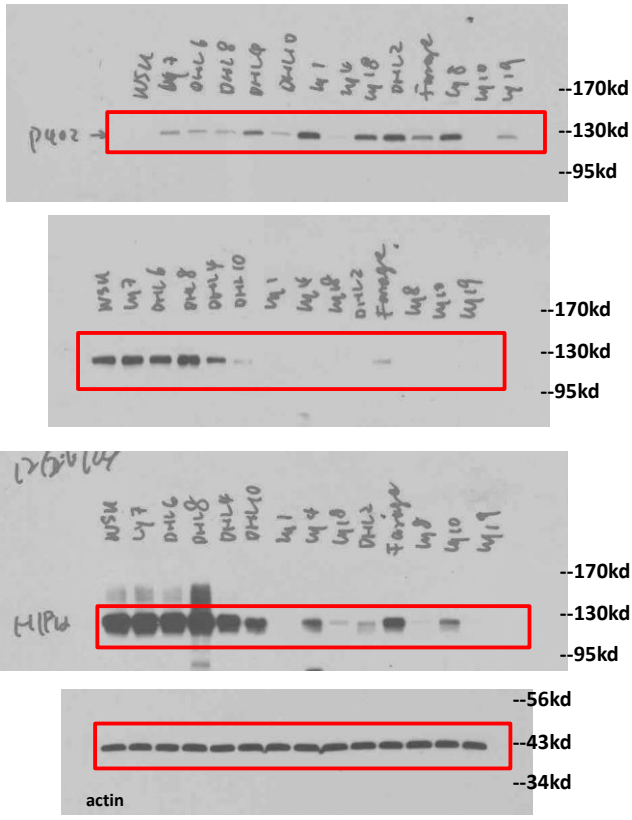


Supplementary Figure 14 - continued

7a

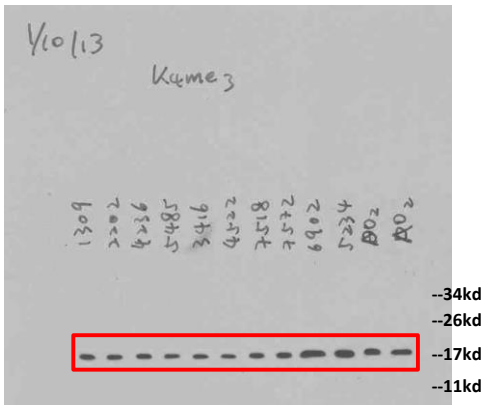


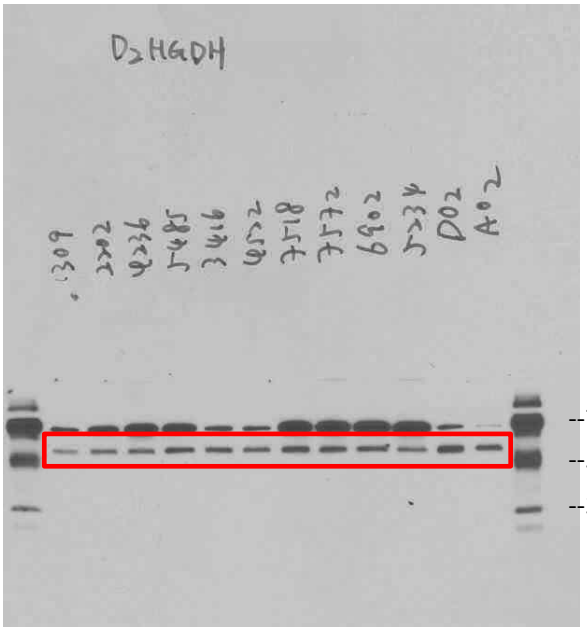


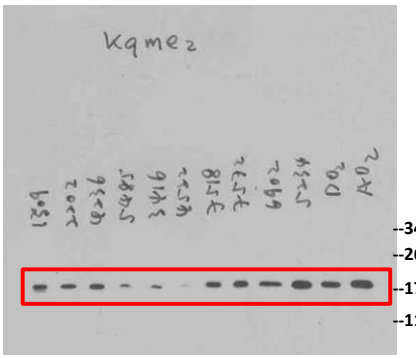


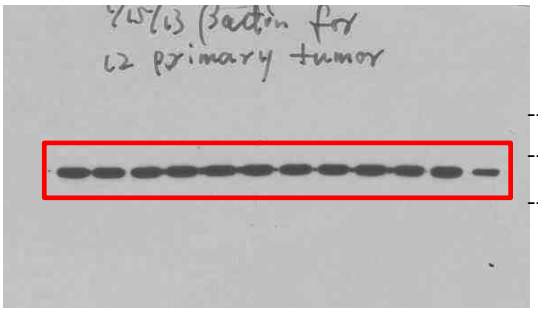
Supplementary Figure 14 - continued

7c









Supplementary Figure 14 - continued

Supplementary Table 1 - D2HGDH mutational status in DLBCL

Sample ID	Ex2	Ex3	Ex4	Ex5	Ex6	Ex7	Ex8	Ex9	Ex10
517	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Val c.1107T>CT; p.Asp369Asp	WT	c.1307-24A>G
559	c.292+28T>GT	WT	WT	WT	WT	WT	c.1063G>AG,p.Gly355Ser	WT	c.1307-24A>GA
642	c.292+28T>GT	c.293-18A>AG	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
865	c.292+28T>GT	c.293-18A>AG	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
1057	c.292+28T>GT	c.293-18A>AG	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1107T>CT; p.Asp369Asp	WT	c.1307-24A>G
1143	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
1309	c.292+28T>GT	WT	c.351-46G>AG;c.490+40T>CT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
1320	c.164G>AG,p.Arg55Gln c.292+28T>G	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Val c.1107T>CT; p.Asp369Asp	c.1306+68T>CT	c.1307-24A>GA
1326	c.43C>CG, p.Arg15Gly c.164G>AG,p.Arg55Gln	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	WT
1423	c.43C>CG, p.Arg15Gly c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
1479	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
1650	c.43C>CG, p.Arg15Gly c.292+28T>GT	c.293-18A>AG	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	WT
1750	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	WT	WT	c.1307-24A>G
2202	c.292+28T>GT	c.293-18A>AG	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA c.1377C>AC,p.Pro459Pro
2216	WT	WT	WT	WT	WT	WT	WT	WT	WT
2323	c.292+28T>GT	WT	WT	WT	c.685-9T>CT	WT	c.1306+68T>CT	WT	c.1307-24A>GA
2485	WT	WT	WT	WT	WT	WT	WT	WT	WT
2633	c.292+28T>GT	c.293-18A>AG	WT	WT	c.685-9T>C	WT	c.1082C>T p.Ala361Val	c.1272G>AG p.Pro424Pro	c.1307-24A>GA
2992	c.1-91 C>GC c.292+28T>GT	c.293-18A>AG	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	WT
3002	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>G c.1377C>AC,p.Pro459Pro
3271	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>AG	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1107T>CT; p.Asp369Asp	c.1306+68T>CT	c.1307-24A>G
3279	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA c.1377C>AC,p.Pro459Pro
3410	c.164G>AG,p.Arg55Gln	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Va	WT	WT
3416	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	c.490+40T>CT	WT	WT	WT	WT	WT	WT
3466	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
3522	WT	c.293-18A>AG	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
3533	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>GA c.1388A>TA,p.Cln463Va
3717	c.292+28T>GT	WT	WT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Va	WT	c.1307-24A>GA
3832	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	c.685-9T>CT	WT	c.1082C>T p.Ala361Val	WT	c.1307-24A>GA
4018	c.164G>AG,p.Arg55Gln	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>T p.Ala361Val	WT	WT
4021	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	c.1307-24A>G
4236	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1107T>CT; p.Asp369Asp	WT	c.1307-24A>GA
4350	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>T p.Ala361Val	WT	WT
4375	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
4450	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>G
4470	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	WT	WT
4494	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>T p.Ala361Val	WT	c.1307-24A>G
4522	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
4554	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
4756	c.1-91 C>GC c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA c.1377C>AC,p.Pro459Pro
5028	c.1-91 C>GC c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
5058	c.292+28T>GT	c.293-18A>AG	WT	WT	c.685-9T>CT	WT	c.1082C>CT p.Ala361Val	WT	c.1307-24A>GA
5081	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
5159	WT	WT	WT	WT	WT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
5160	c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Va	c.1306+68T>CT	c.1307-24A>G
5186	WT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
5190	c.164G>AG,p.Arg55Gln	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT c.774G>AG, p.Ser258Ser	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
5204	WT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
5234	c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1107T>CT; p.Asp369Asp	c.1276G>AG,p.Ala426Thr c.1306+68T>CT	WT
5278	WT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
5283	WT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
5364	c.164G>AG,p.Arg55Gln	WT	WT	WT	WT	WT	c.1064G>AG p.Gly355Ser	WT	c.1307-24A>GA
5370	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT; c.853+48G>AG	WT	c.1012G>AG p.Val338Ile	WT	WT
5485	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA c.1377C>AC,p.Pro459Pro
5536	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
5707	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	WT
5782	c.292+28T>GT	WT	WT	WT	WT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
5935	c.164G>AG,p.Arg55Gln	WT	WT	WT	WT	WT	WT	WT	WT
6290	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
6614	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>G
6697	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	WT	WT
6902	c.292+28T>GT	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	c.1276G>AG,p.Ala426Thr c.1306+68T>CT	c.1307-24A>G
6971	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	WT
7041	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>T p.Ala361Val	WT	c.1307-24A>GA
7089	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1104+39C>TC	c.1306+68T>C	c.1307-24A>G
7119	c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile c.1082C>TC p.Ala361Val c.1107T>CT; p.Asp369Asp	c.1306+68T>C	c.1307-24A>G
7295	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
7518	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
7572	c.292+28T>GT	c.293-18A>GA	c.490+40T>C	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G c.1377C>AC,p.Pro459Pro
7924	c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile	c.1306+68T>C	c.1307-24A>G
7970	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	WT
7987	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	WT
A1	c.164G>AG p.Arg55Gln, c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
A2	c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	c.634C>TC p.Arg212Trp	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
A3	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
A4	c.292+28T>GT	WT	WT	WT	WT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
A5	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
A6	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>C	WT	c.1012G>A p.Val338Ile	WT	c.1307-24A>G
B1	c.292+28T>G	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA, c.1377C>AC p.Pro459Pro
B2	c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G, c.1377C>AC p.Pro459Pro
B3	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>C	WT	WT	WT	c.1307-24A>GA
B4	WT	WT	WT	WT	WT	WT	WT	WT	WT
B5	WT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA
B6	WT	WT	WT	WT	WT	WT	WT	WT	WT
C1	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
C2	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
C3	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
C4	WT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
C5	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
C6	c.164G>AG p.Arg55Gln, c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA
D1	c.292+28T>GT	WT	WT	WT	WT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
D2	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	c.634C>TC p.Arg212Trp	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
D3	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile,c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
D4	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>G
D5	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>CT	WT	c.1012G>A p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
D6	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
E1	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	c.1307-24A>GA
E2	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-23A>TA, c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
E3	c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
E4	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	WT	WT	c.1307-24A>G
E5	WT	WT	WT	WT	WT	WT	WT	WT	WT
E6	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-23A>TA, c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
F1	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	c.1307-24A>GA
F2	c.164G>AG p.Arg55Gln, c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
F3	c.292+28T>G	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1038C>TC p.Asn346Asn, c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
F4	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	WT	WT	c.1307-24A>G
F5	c.164G>AG p.Arg55Gln, c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
F6	Fw not good and there is no reverse	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>G
G1	c.164G>A p.Arg55Gln, c.292+28T>G	c.293-18A>G	c.490+40T>C	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile; c.1107T>CT,p.Asp369Asp	c.1306+68T>C	c.1307-24A>G
G2	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
G3	c.164G>AG p.Arg55Gln, c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
G4	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1012G>AG p.Val338Ile,c.1082C>TC p.Ala361Val	c.1306+68T>C	c.1307-24A>G
G5	c.164G>AG p.Arg55Gln, c.292+28T>G	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA
G6	c.292+28T>G	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>G
H1	c.164G>AG p.Arg55Gln, c.292+28T>G	WT	WT	WT	WT	WT	WT	WT	WT
H2	c.164G>AG p.Arg55Gln, c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA, c.1377C>AC p.Pro459Pro
H3	c.164G>AG p.Arg55Gln, c.292+28T>G	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	WT	c.1307-24A>GA
H4	c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
H5	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
H6	NA	WT	c.490+40T>CT	WT	c.685-9T>CT	WT	c.1012G>AG p.Val338Ile	na	WT
DHL10	c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA
DHL16	WT	WT	WT	WT	WT	WT	WT	WT	WT
DHL2	c.164G>AG,p.Arg55Gln	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1012G>GA p.Val338Ile	WT	c.1307-24A>GA
DHL4	WT	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	WT	WT
DHL5	c.292+28T>GT	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>G
DHL6	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	c.1262G>AG, p.Arg421His	c.1307-24A>GA
DHL7	WT	WT	WT	WT	WT	WT	WT	WT	c.1307-24A>GA
DHL8	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	c.622G>AG, p.Ala208Thr	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
Farage	WT	WT	WT	WT	WT	WT	WT	WT	WT
HBL-1	c.292+28T>G	c.293-18A>G	WT	WT	c.685-9T>C	WT	c.1082C>TC p.Ala361Val	WT	WT
HT	WT	WT	WT	WT	WT	WT	WT	WT	WT
K1106P	c.164G>AG,p.Arg55Gln	WT	WT	WT	WT	WT	WT	WT	WT
K422	c.164G>AG,p.Arg55Gln	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
Ly1	c.164G>AG,p.Arg55Gln	WT	c.490+40T>CT	WT	WT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
Ly10	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>G
Ly18	WT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	WT	WT	WT
Ly19	WT	WT	WT	WT	WT	WT	WT	WT	WT
Ly3	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>GA	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>G
Ly4	WT	WT	WT	WT	WT	WT	WT	WT	WT
Ly7	c.1-91 C>GC c.292+28T>GT	c.293-18A>G	c.490+40T>C	WT	c.685-9T>C	WT	c.1012G>A p.Val338Ile c.1107T>CT; p.Asp369Asp	c.1276G>AG,p.Ala426Thr c.1306+68T>CT	c.1307-24A>G
Ly8	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	c.1307-24A>GA
NU-DHL1	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	WT	WT
NU-DUL1	c.164G>AG,p.Arg55Gln	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1012G>A p.Val338Ile	c.1306+68T>CT	c.1307-24A>GA
Pfeiffer	WT	WT	c.490+40T>CT	WT	WT	WT	WT	WT	WT
RC-K8	c.292+28T>GT	c.293-18A>GA	WT	WT	c.685-9T>CT	WT	c.1082C>TC p.Ala361Val	c.1306+68T>CT	c.1307-24A>GA
Toledo	c.164G>AG,p.Arg55Gln c.292+28T>GT	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>CT	WT	WT	c.1306+68T>CT	c.1307-24A>GA
U2932	c.164G>AG,p.Arg55Gln c.292+28T>G	c.293-18A>G	c.490+40T>CT	WT	c.685-9T>C	WT	c.1012G>A p.Val338Ile	c.1306+68T>CT	c.1307-24A>G
USC-DHL1	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	WT	WT	WT	WT	WT	WT	WT
WSU-NHL	c.164G>AG,p.Arg55Gln c.292+28T>GT	WT	c.392delG; Gly131fsX21	WT	WT	WT	c.1064G>AG p.Gly355Ser	WT	c.1307-24A>GA

rs62191976: c.1-91 C>GC= 2.7% (4%)
rs4675887: c.43C>CG, p.Arg15Gly= 2% (3.8%)
rs77940364: c.164G>AG p.Arg55Gln= 46.6% (21.7%)
rs62191977: c.292+28T>GT= 77% (73%)

rs4073889: c.293-18A>GA=61.4% (5TMP-ESP-2-24261803: c.351-46G>AG= 0.6% (0.02 - 0.04)
rs145731647: c.293-23A>TA=1.3% (1rs78147778: c.490+40T>CT= 39.1% (22%)

rs4234097: c.685-9T>CT= 68.2% (52.7%)
TMP-ESP-2-242684213: c.774G>AG, p.Ser258Ser= 0.6% (0.01
rs180688843: c.853+48G>AG= 0.6% (0.001%)

rs1106639: c.1012G>AG p.Val338Ile= 31% (21%)
rs139931290: c.1063G>AG,p.Gly355Ser= 0.6% (0.001%)
rs1105273:c.1082C>TC p.Ala361Val= 28.3% (21.4%)
rs141343442: c.1107T>CT,p.Asp369Asp= 6% (2.8%)

rs139611034: c.1262G>A, p.Arg421His= 0.6% (0.01
rs756901: c.1307-24A>GA= 67.5% (62.1%)
TMP-ESP-2-242695395: c.1272G>AG p.Pro424Pro= rs143940595: c.1377C>AC p.Pro459Pro= 6% (0.01%)
rs145578303: c.1276G>AG,p.Ala426Thr= 2% (<0.01%)
rs62192024: c.1306+68T>CT= 37.1% (33.7%)

Supplementary Table 2 - Copy number at the D2HGDH locus

	Q-PCR Upstream #1		Q-PCR Upstream #2		MLPA Ex2		Q-PCR Ex3		Q-PCR Ex4		Q-PCR Ex5		MLPA Ex6		MLPA Ex7		Q-PCR Ex8		MLPA Ex9		MLPA Ex10	
Sample ID	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev	Mean	Std-dev
A01	0.78	n/a	0.80	n/a	0.85	0.18	0.88	n/a	1.21	n/a	1.02	n/a	0.92	0.16	1.10	0.69	1.05	n/a	1.81	1.02	0.94	0.55
B01	0.80	0.11	0.77	0.08	1.44	0.16	0.76	0.07	1.06	0.12	1.08	0.11	1.50	0.21	1.50	0.14	0.97	0.40	1.22	0.24	1.00	0.31
C01	1.01	0.32	0.80	0.15	0.85	0.16	0.99	0.29	0.77	0.20	0.71	0.13	1.00	0.20	0.86	0.14	0.94	0.22	1.01	0.24	0.89	0.31
D01	0.94	0.15	0.84	0.09	0.75	0.08	1.16	0.07	0.86	0.12	0.89	0.07	0.79	0.16	1.08	0.58	1.13	0.20	1.84	0.85	1.29	0.20
E01	1.07	0.30	0.76	0.05	1.00	0.16	0.99	0.27	0.82	0.01	1.13	0.13	1.15	0.20	1.03	0.14	1.42	0.04	0.95	0.24	1.02	0.31
F01	1.16	0.05	0.68	0.03	1.00	0.16	0.86	0.14	0.79	0.03	0.90	0.26	0.97	0.20	0.94	0.14	1.31	0.16	0.86	0.24	1.14	0.31
A02	1.08	0.27	0.85	0.11	0.99	0.06	1.09	0.35	1.14	0.15	1.06	0.14	1.06	0.15	0.73	0.58	1.19	0.20	0.68	0.84	1.02	0.18
D02	1.00	0.21	0.46	0.11	1.00	0.16	1.51	0.01	0.69	0.23	0.95	0.33	1.05	0.20	0.92	0.14	0.85	n/a	0.83	0.24	0.98	0.31
H02	1.04	0.21	0.70	0.07	0.97	0.16	1.08	0.04	0.80	0.04	0.86	0.27	0.95	0.20	0.96	0.14	1.14	0.24	0.85	0.24	0.96	0.31
B03	0.96	0.00	0.84	0.01	1.12	0.02	0.97	0.06	1.00	0.03	0.82	0.01	1.15	0.03	0.85	0.02	1.09	n/a	1.28	0.08	1.05	0.19
D03	0.91	0.09	0.67	n/a	0.86	0.03	0.85	0.07	0.72	0.05	0.75	0.05	1.21	0.05	0.99	0.04	0.84	0.06	1.38	0.09	0.99	0.20
E03	1.21	0.16	0.95	0.10	1.12	0.05	1.20	0.11	0.82	0.08	0.88	0.48	1.36	0.21	0.91	0.48	1.10	0.10	0.88	0.85	0.97	0.31
F03	1.11	0.08	0.89	0.11	1.12	0.07	1.25	n/a	0.94	0.10	1.00	0.08	1.46	0.22	0.99	0.49	1.29	0.10	0.98	0.85	0.96	0.31
A04	1.17	0.09	0.93	0.07	1.52	0.22	1.16	0.13	0.80	0.07	0.94	0.09	1.15	0.18	1.59	0.70	1.15	0.09	2.08	1.03	1.02	0.55
C04	0.86	0.13	0.71	0.03	0.78	0.03	0.79	0.12	1.05	0.11	0.81	0.28	1.34	0.17	0.87	0.48	1.10	n/a	1.05	0.69	1.16	0.21
D04	1.08	0.15	0.84	0.10	1.09	0.11	1.27	0.83	1.56	1.33	0.90	0.11	1.25	0.23	0.75	0.24	1.29	0.19	0.89	0.45	1.03	0.03
G04	0.97	0.14	0.69	0.03	1.14	0.03	1.21	0.02	0.91	0.29	0.82	n/a	1.14	0.17	0.76	0.48	0.83	0.01	0.76	0.69	1.02	0.21
F04	1.63	0.34	0.93	0.20	0.99	0.10	1.38	0.29	1.18	0.29	1.03	0.22	1.26	0.14	0.84	0.42	1.08	0.29	0.95	0.63	1.10	0.21
H04	0.98	0.10	0.96	0.06	0.75	0.04	0.92	0.14	0.70	0.13	0.92	0.06	1.06	0.06	0.85	0.04	0.91	0.08	1.28	0.10	0.92	0.20
A05	1.02	0.02	1.05	0.03	0.99	0.05	1.09	0.10	1.43	0.41	1.08	0.02	1.34	0.07	1.08	0.06	1.10	0.01	1.60	0.12	1.02	0.20
B05	1.26	0.29	1.13	n/a	1.40	0.11	1.01	0.23	0.74	0.78	0.90	0.02	1.27	0.23	0.75	0.24	0.84	0.05	0.81	0.45	1.19	0.03
C05	0.99	0.04	0.80	0.04	0.90	0.12	1.25	0.16	0.96	0.09	0.96	0.05	0.84	0.23	1.16	0.24	1.26	0.06	1.70	0.46	0.85	0.05
D05	1.55	0.13	1.03	0.10	0.90	0.30	1.74	0.23	0.91	0.11	0.97	0.09	1.00	0.14	0.86	0.34	1.10	0.13	0.65	0.73	0.90	0.03
F05	0.92	n/a	0.71	0.09	0.89	0.11	1.13	0.11	0.91	0.18	0.88	0.04	1.38	0.24	0.82	0.24	1.18	0.03	1.00	0.46	0.88	0.04
G05	1.03	0.19	0.80	0.05	0.92	0.02	1.35	0.17	0.99	0.11	1.06	0.09	1.07	0.03	0.93	0.02	1.24	0.10	1.21	0.08	1.11	0.18
H05	0.82	0.19	0.75	0.03	1.01	0.11	1.25	0.16	1.23	0.25	1.18	0.04	1.32	0.24	0.90	0.24	1.52	0.13	1.11	0.46	1.08	0.05
SU-DHL4	0.99	0.08	0.79	0.07	1.04	0.03	1.30	0.07	1.03	0.03	1.01	0.07	1.04	0.12	1.02	0.09	1.09	0.04	1.12	0.06	0.90	0.03
SU-DHL5	0.87	0.18	0.82	0.01	1.04	0.03	1.17	0.04	1.05	0.02	0.84	0.15	0.92	0.12	0.91	0.09	1.00	0.16	0.98	0.06	0.93	0.03
SU-DHL7	0.93	0.08	0.85	0.01	1.43	0.08	1.05	0.01	1.03	0.12	0.97	0.03	1.26	0.14	1.31	0.11	1.09	0.05	1.29	0.09	1.21	0.07
SU-DHL8	1.06	0.06	1.01	0.03	1.06	0.12	0.74	0.05	0.80	0.02	0.80	0.05	0.72	0.15	2.54	0.30	0.88	0.02	3.26	0.37	1.29	0.15
SU-DHL9	0.94	0.06	0.89	0.04	1.05	0.04	1.09	0.15	1.50	0.12	1.33	0.06	1.02	0.20	0.74	0.48	1.39	0.06	0.67	0.85	1.00	0.31
SU-DHL10	0.86	0.06	0.86	0.03	1.01	0.61	0.96	0.23	1.56	0.19	1.32	0.05	1.09	0.55	1.14	0.58	1.37	0.07	1.05	0.50	1.08	0.47
SU-DHL-16	0.99	0.07	1.00	0.06	1.18	0.04	1.22	0.16	1.31	0.12	1.09	0.07	1.35	0.20	0.99	0.48	1.34	0.13	0.81	0.85	0.92	0.31
Farage	1.01	0.09	1.05	0.19	0.88	0.10	1.06	0.21	1.59	0.13	1.42	0.11	0.68	0.14	1.66	0.43	1.48	0.12	1.85	0.64	1.23	0.22
HBL1	0.83	0.05	0.83	0.03	1.36	0.06	1.15	0.27	1.02	0.02	1.03	0.04	1.11	0.13	1.18	0.10	1.03	0.04	1.16	0.08	0.94	0.04
HT	0.90	0.10	0.76	0.05	1.01	0.05	0.92	0.10	1.00	0.04	1.01	0.06	1.00	0.13	1.03	0.10	1.02	0.05	1.11	0.08	1.04	0.06
Karpas 422	0.89	0.08	0.83	0.05	1.01	0.01	1.28	0.19	1.20	0.06	1.20	0.07	0.99	0.12	0.99	0.08	1.12	0.05	1.01	0.06	0.88	0.01
Ly1	0.87	0.03	0.81	0.00	1.03	0.02	1.13	0.03	1.08	0.13	1.07	0.02	1.09	0.12	1.17	0.09	1.04	0.02	1.09	0.06	0.73	0.02
Ly7	0.87	0.12	0.85	0.02	1.01	0.04	0.83	0.21	0.75	0.01	0.82	0.03	0.88	0.13	0.90	0.09	0.84	0.01	0.95	0.07	0.89	0.04
Ly8	0.87	0.10	1.33	1.13	1.36	0.14	1.01	0.13	0.97	0.06	0.91	0.11	0.84	0.15	2.93	0.31	0.95	0.06	3.11	0.33	0.80	0.08
Ly10	1.03	0.31	0.87	0.11	1.19	0.02	1.03	0.15	1.09	0.17	1.01	0.11	0.70	0.14	2.83	0.27	0.94	0.10	2.99	0.28	0.90	0.08
Ly18	0.70	0.12	0.81	0.07	1.02	0.02	1.26	0.21	0.97	0.14	1.05	0.08	0.93	0.12	0.98	0.09	0.98	0.08	1.05	0.06	1.09	0.02
Ly19	0.81	0.02	0.94	0.02	0.77	0.02	1.27	0.08	0.95	0.30	1.09	0.02	0.78	0.12	0.75	0.09	0.95	0.03	0.85	0.06	1.02	0.03
Pfeiffer	0.93	0.13	0.89	0.05	1.00	0.01	0.96	0.21	1.09	0.14	1.10	0.07	1.03	0.12	1.00	0.08	1.14	0.06	1.08	0.06	1.01	0.01
Toledo	0.95	0.03	1.12	0.48	1.00	0.02	1.05	0.07	0.97	0.07	0.95	0.23	1.09	0.03	1.04	0.02	1.05	0.34	1.14	0.08	1.13	0.18
U2932	0.52	0.07	0.33	n/a	0.50	0.02	0.78	0.16	0.45	0.05	0.41	n/a	0.51	0.03	0.51	0.02	0.54	0.07	0.62	0.08	0.53	0.18
WSU-NHL	1.13	0.24	1.01	0.27	0.89	0.11	1.30	0.30	0.95	0.16	1.10	0.20	1.06	0.23	0.76	0.24	1.12	0.49	0.70	0.46	0.97	0.04
USC-DHL1	1.08	0.14	1.12	0.01	0.99	0.02	1.04	0.27	0.91	0.06	0.91	0.07	1.17	0.03	1.06	0.02	0.88	0.02	1.16	0.08	0.91	0.18
NU-DUL1	0.73	n/a	1.06	n/a	1.00	0.61	1.52	n/a	n/a	n/a	1.40	n/a	1.01	0.55	1.03	0.58	1.26	n/a	0.98	0.50	0.90	0.48
NU-DH-1	1.78	0.36	1.12	0.23	1.09	0.02	2.42	0.71	1.08	0.33	1.08	0.23	1.15	0.03	1.11	0.02	1.38	0.29	1.17	0.08	1.19	0.18

The cell line U2932 showed a pattern of putative single copy loss across the entire D2HDGH locus and the upstream region (4 Mbp from D2HGDH's transcription start site).
This finding was in agreement with an earlier report on the karyotype of this cell line (Leukemia and Lymphoma, 2002; 43: 2179 -2189).
The threshold for positive calls (copy number loss) was 0.7 or, when only one exon appeared to be targeted, 0.65.

Supplementary Table 3 - α-KG abundance in multiple D2HGDH models in [U-^{13}C]-glutamine labeling assay

Sample ID	unspiked % enriched	spiked % enriched	fold dilution	nmoles in solution	nmoles of α-KG/mg of protein	Relative abundance
MSCV	0.6031	0.3972	1.5184	48.2273	0.1901	0.46
D2HGDH -WT	0.5584	0.4568	1.2224	112.4016	0.4107	1.00
D2HGDH - R212W	0.5947	0.4429	1.3427	72.9414	0.3004	0.73
D2HGDH -A426T	0.5761	0.3829	1.5046	49.5471	0.1938	0.47
4h						
MSCV	0.6205	0.4651	1.3341	74.8230	0.2872	0.75
D2HGDH -WT	0.6045	0.4952	1.2207	113.2662	0.3850	1.00
D2HGDH - R212W	0.6112	0.4219	1.4487	55.7184	0.1898	0.49
D2HGDH -A426T	0.5941	0.3878	1.5320	46.9947	0.1809	0.47
8h						

Supplementary Table 4 – Summary of IDH V_{max} and K_m in multiple D2HGDH genetic models

Stable expression – gain-of-function

Cell ID	Total cell lysate - HEK-293	
	V_{max} (mean ± SD)	K_m (mean ± SD)
MSCV (n=3)	29.7 (±0.51)	0.69 (±0.06)
D2HGDH – WT (n=3)	33.9 (±0.74)	0.56 (±0.06)
D2HGDH – G131X (n=3)	26.9 (±0.55)	0.76 (±0.08)
D2HGDH – A208T (n=3)	27.4 (±0.50)	0.82 (±0.07)
D2HGDH – R212W (n=3)	29.8 (±0.57)	0.78 (±0.07)
D2HGDH – R421H (n=3)	26.1 (±0.50)	0.66 (±0.06)
D2HGDH – A426T (n=3)	29.2 (±0.58)	0.62 (±0.06)

Stable expression – loss-of-function

Cell ID	Total cell lysate – HEK-293 KD	
	V_{max} (mean ± SD)	K_m (mean ± SD)
si-control (n=3)	30.4 (±0.38)	0.57 (±0.03)
si-D2HGDH#3 (n=3)	25.0 (±0.29)	0.59 (±0.03)
si-D2HGDH#5 (n=3)	25.9 (±0.28)	0.52 (±0.03)

Transient expression – gain-of-function

Cell ID	Total cell lysate HEK-293 – transient transfection	
	V_{max} (mean ± SD)	K_m (mean ± SD)
MSCV 0.5µg (n=3)	31.7 (±0.53)	1.33 (±0.10)
MSCV 0.75µg (n=3)	31.5 (±0.56)	1.31 (±0.11)
MSCV 1.0µg (n=3)	31.7 (±0.53)	1.33 (±0.10)
D2 – WT 0.5µg (n=3)	33.5 (±0.34)	0.96 (±0.04)
D2 – WT 0.75µg (n=3)	36.4 (±0.49)	1.12 (±0.07)
D2 – WT 1.0µg (n=3)	38.1 (±0.39)	1.29 (±0.06)

Transient expression – loss-of-function

Cell ID	Total cell lysate – OCY-Ly8 KD	
	V_{max} (mean ± SD)	K_m (mean ± SD)
si-control (n=3)	48.7 (±1.29)	2.22 (±0.25)
si-D2HGDH#3 (n=3)	40.2 (±0.69)	2.08 (±0.15)
si-D2HGDH#5 (n=3)	41.9 (±0.93)	2.20 (±0.21)

Transient expression – loss-of-function

Cell ID	Total cell lysate – Ramos KD	
	V_{max} (mean ± SD)	K_m (mean ± SD)
si-control (n=3)	25.6 (±0.20)	1.06 (±0.04)
si-D2HGDH#3 (n=3)	21.6 (±0.25)	1.05 (±0.06)
si-D2HGDH#5 (n=3)	19.5 (±0.24)	0.89 (±0.05)

Stable expression – loss-of-function

	Subcellular fractions – HEK-293			
Cell ID	Mitochondria		Cytosol	
	V_{max} (mean ± SD)	K_m (mean ± SD)	V_{max} (mean ± SD)	K_m (mean ± SD)
si-control (n=3)	84.9 (±1.42)	1.01 (±0.07)	17.9 (±0.23)	0.67 (±0.04)
si-D2HGDH#3 (n=3)	55.3 (±1.20)	1.10 (±0.10)	17.6 (±0.24)	0.72 (±0.04)
si-D2HGDH#5 (n=3)	54.4 (±0.89)	0.88 (±0.06)	17.5 (±0.21)	0.80(±0.04)

Transient expression – gain-of-function

	Subcellular fractions – HEK-293			
Cell ID	Mitochondria		Cytosol	
	V_{max} (mean ± SD)	K_m (mean ± SD)	V_{max} (mean ± SD)	K_m (mean ± SD)
MSCV (n=3)	58.8 (±1.18)	1.01 (±0.09)	17.6 (±0.26)	0.83 (±0.05)
D2HGDH – WT (n=3)	84.3 (±1.66)	1.06 (±0.09)	17.7 (±0.26)	0.91 (±0.06)

Supplementary Table 5. IDH activity assay in total cell lysates

Stable expression

MSCV	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0027	5.71	29.14	0.0022	4.65	30.02	0.0018	3.81	29.88
0.5	0.0067	14.17		0.0065	13.75		0.0064	13.54	
1	0.0087	18.40	Km	0.0085	17.98	Km	0.0096	20.31	Km
2.5	0.0107	22.63	0.65	0.0114	24.12	0.74	0.0112	23.69	0.69
5	0.0120	25.39		0.0118	24.96		0.0111	23.48	
10	0.0135	28.56		0.0142	30.04		0.0140	29.62	
15	0.0130	27.50		0.0135	28.56		0.0140	29.62	
25	0.0133	28.14		0.0135	28.56		0.0135	28.56	
50	0.0135	28.56		0.0141	29.83		0.0135	28.56	

D2HGDH WT	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0029	6.48	33.34	0.0025	5.58	34.01	0.0035	7.82	34.56
0.5	0.0072	16.08		0.0075	16.75		0.0085	18.98	
1	0.0097	21.66	Km	0.0094	20.99	Km	0.0137	30.59	Km
2.5	0.0126	28.14	0.61	0.0124	27.69	0.67	0.0124	27.69	0.43
5	0.0125	27.91		0.0126	28.14		0.0130	29.03	
10	0.0144	32.15		0.0145	32.38		0.0152	33.94	
15	0.0145	32.38		0.0144	32.15		0.0150	33.49	
25	0.0144	32.15		0.0145	32.38		0.0155	34.61	
50	0.0145	32.38		0.0155	34.61		0.0152	33.94	

D2HGDH G131X	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0014	2.96	27.04	0.0022	4.65	27.04	0.0025	5.29	26.72
0.5	0.0030	6.35		0.0040	8.46		0.0062	13.12	
1	0.0084	17.77	Km	0.0080	16.92	Km	0.0084	17.77	Km
2.5	0.0107	22.63	0.88	0.0099	20.94	0.83	0.0107	22.63	0.6
5	0.0107	22.63		0.0109	23.06		0.0105	22.21	
10	0.0117	24.75		0.0122	25.81		0.0122	25.81	
15	0.0120	25.39		0.0122	25.81		0.0122	25.81	
25	0.0122	25.81		0.0120	25.39		0.0121	25.60	
50	0.0120	25.39		0.0122	25.81		0.0125	26.44	

D2HGDH A208T	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0020	5.02	27.5	0.0018	4.52	27.59	0.0022	5.53	27.45
0.5	0.0040	10.05		0.0045	11.30		0.0055	13.82	
1	0.0058	14.57	Km	0.0063	15.83	Km	0.0070	17.58	Km
2.5	0.0071	17.84	0.98	0.0073	18.34	0.87	0.0077	19.34	0.63
5	0.0092	23.11		0.0090	22.61		0.0103	25.87	
10	0.0100	25.12		0.0106	26.63		0.0111	27.88	
15	0.0111	27.88		0.0105	26.38		0.0100	25.12	
25	0.0100	25.12		0.0105	26.38		0.0106	26.63	
50	0.0107	26.88		0.0108	27.13		0.0105	26.38	

D2HGDH R212W	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0018	3.36	30.05	0.0025	4.67	29.94	0.0014	2.62	29.6
0.5	0.0068	12.71		0.0058	10.84		0.0079	14.77	
1	0.0096	17.95	Km	0.0096	17.95	Km	0.0104	19.44	Km
2.5	0.0126	23.55	0.79	0.0123	22.99	0.87	0.0123	22.99	0.7
5	0.0138	25.80		0.0120	22.43		0.0130	24.30	
10	0.0155	28.98		0.0152	28.42		0.0151	28.23	
15	0.0155	28.98		0.0155	28.98		0.0152	28.42	
25	0.0150	28.04		0.0156	29.16		0.0154	28.79	
50	0.0153	28.60		0.0156	29.16		0.0155	28.98	

D2HGDH R421H	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0020	4.02	26.24	0.0022	4.42	25.74	0.0025	5.02	26.35
0.5	0.0060	12.06		0.0068	13.67		0.0052	10.45	
1	0.0080	16.08	Km	0.0091	18.29	Km	0.0098	19.69	Km
2.5	0.0097	19.49	0.74	0.0096	19.29	0.58	0.0091	18.29	0.68
5	0.0111	22.31		0.0111	22.31		0.0111	22.31	
10	0.0123	24.72		0.0126	25.32		0.0130	26.13	
15	0.0130	26.13		0.0125	25.12		0.0125	25.12	
25	0.0126	25.32		0.0125	25.12		0.0130	26.13	
50	0.0125	25.12		0.0124	24.92		0.0126	25.32	

D2HGDH A426T	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
0.25	0.0025	6.70	27.88	0.0021	5.63	29.36	0.0015	4.02	30.66
0.5	0.0063	16.88		0.0042	11.25		0.0048	12.86	
1	0.0073	19.56	Km	0.0066	17.68	Km	0.0079	21.17	Km
2.5	0.0087	23.31	0.46	0.0090	24.12	0.72	0.0087	23.31	0.69
5	0.0098	26.26		0.0100	26.80		0.0105	28.14	
10	0.0099	26.53		0.0101	27.06		0.0114	30.55	
15	0.0099	26.53		0.0111	29.74		0.0105	28.14	
25	0.0105	28.14		0.0105	28.14		0.0111	29.74	
50	0.0099	26.53		0.0098	26.26		0.0106	28.40	

Enzyme kinectis calculated using the Michaelis-Menten equation

Transient Transfection

MSCV (0.5ug)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0012	4.95	32.56	0.0012	4.95	31.8	0.0013	5.36	Vmax
0.5	0.0026	10.72		0.0027	11.13		0.0026	10.72	30.89
1	0.0032	13.19	Km	0.0035	14.43	Km	0.0036	14.84	
2.5	0.0041	16.90	1.51	0.0041	16.90	1.34	0.0043	17.73	Km
5	0.0060	24.73		0.0060	24.73		0.0065	26.80	1.13
10	0.0073	30.09	protein	0.0072	29.68	protein	0.0074	30.51	
15	0.0074	30.51	39ug	0.0073	30.09	39ug	0.0070	28.86	protein
25	0.0075	30.92		0.0074	30.51		0.0070	28.86	39ug
50	0.0074	30.51		0.0073	30.09		0.0070	28.86	

D2HGDH WT(0.5ug)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0013	5.23	33.58	0.0013	5.23	33.71	0.0014	5.63	Vmax
0.5	0.0031	12.46		0.0032	12.86		0.0031	12.46	33.43
1	0.0040	16.08	Km	0.0042	16.88	Km	0.0045	18.09	
2.5	0.0058	23.31	1.02	0.0058	23.31	0.96	0.0059	23.71	Km
5	0.0071	28.54		0.0075	30.14		0.0075	30.14	0.9
10	0.0077	30.95	protein	0.0076	30.55	protein	0.0075	30.14	
15	0.0079	31.75	40ug	0.0078	31.35	40ug	0.0079	31.75	protein
25	0.0081	32.56		0.0082	32.96		0.0079	31.75	40ug
50	0.0079	31.75		0.0080	32.15		0.0081	32.56	

MSCV(0.75)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0012	4.95	31.87	0.0011	4.53	31.66	0.0012	4.95	Vmax
0.5	0.0026	10.72		0.0026	10.72		0.0025	10.31	31.09
1	0.0033	13.60	Km	0.0032	13.19	Km	0.0035	14.43	
2.5	0.0042	17.31	1.37	0.0042	17.31	1.36	0.0045	18.55	Km
5	0.0060	24.73		0.0063	25.97		0.0060	24.73	1.22
10	0.0074	30.51	Protein	0.0073	30.09	protein	0.0072	29.68	
15	0.0073	30.09	39ug	0.0072	29.68	39ug	0.0072	29.68	protein
25	0.0073	30.09		0.0070	28.86		0.0071	29.27	39ug
50	0.0072	29.68		0.0073	30.09		0.0071	29.27	

D2HGDH WT(0.75ug)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0015	5.36	36.83	0.0015	5.36	36.28	0.0016	5.72	Vmax
0.5	0.0033	11.79		0.0035	12.50		0.0038	13.58	36.24
1	0.0047	16.79	Km	0.0049	17.51	Km	0.0049	17.51	
2.5	0.0065	23.22	1.243	0.0064	22.87	1.12	0.0069	24.65	Km
5	0.0079	28.22		0.0084	30.01		0.0085	30.37	1.01
10	0.0098	35.01	protein	0.0097	34.66	protein	0.0097	34.66	
15	0.0099	35.37	45ug	0.0097	34.66	45ug	0.0098	35.01	protein
25	0.0099	35.37		0.0096	34.30		0.0098	35.01	45ug
50	0.0095	33.94		0.0095	33.94		0.0093	33.23	

MSCV(1.0)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0012	4.95	32.39	0.0011	4.53	31.82	0.0011	4.53	Vmax
0.5	0.0025	10.31		0.0025	10.31		0.0026	10.72	31.13
1	0.0032	13.19	Km	0.0033	13.60	Km	0.0034	14.02	
2.5	0.0041	16.90	1.49	0.0049	20.20	1.260	0.0043	17.73	km
5	0.0061	25.15		0.0061	25.15		0.0063	25.97	1.26
10	0.0073	30.09	protein	0.0075	30.92	protein	0.0071	29.27	
15	0.0073	30.09	39ug	0.0072	29.68	39ug	0.0070	28.86	protein
25	0.0075	30.92		0.0070	28.86		0.0070	28.86	39ug
50	0.0073	30.09		0.0074	30.51		0.0073	30.09	

D2HGDH WT(1.0ug)	#1			#2			#3		
d-iso(uM)	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	
0.25	0.0017	5.26	38.2	0.0018	5.57	38.49	0.0017	5.26	Vmax
0.5	0.0039	12.06		0.0030	9.28		0.0035	10.82	37.79
1	0.0056	17.31	Km	0.0052	16.08	Km	0.0053	16.39	
2.5	0.0079	24.42	1.23	0.0080	24.73	1.38	0.0081	25.04	Km
5	0.0098	30.30		0.0099	30.61		0.0098	30.30	1.26
10	0.0117	36.17	protein	0.0115	35.56	protein	0.0116	35.86	
15	0.0117	36.17	52ug	0.0116	35.86	52ug	0.0113	34.94	protein
25	0.0115	35.56		0.0117	36.17		0.0114	35.25	52ug
50	0.0117	36.17		0.0115	35.56		0.0115	35.56	

Enzyme kinectis calculated using the Michaelis-Menten equation

Supplementary Table 5 (continued)

Transient knockdown

OCI-Ly8 KD

		#1			#2			#3		
siRNA ctrl		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0009	3.36	48.00	0.0008	2.99	49.91	0.0009	3.36	48.21
	0.25	0.0017	6.36		0.0015	5.61		0.0016	5.98	
	0.5	0.0031	11.59	Km	0.0030	11.22	Km	0.0035	13.09	Km
	1	0.0065	24.30	2.24	0.0068	25.42	2.31	0.0069	25.80	2.13
	2.5	0.0105	39.26		0.0108	40.38		0.0102	38.14	
	5	0.0110	41.13		0.0117	43.74		0.0115	43.00	
	10	0.0113	42.25		0.0115	43.00		0.0114	42.62	
	15	0.0114	42.62		0.0119	44.49		0.0116	43.37	
	25	0.0115	43.00		0.0118	44.12		0.0114	42.62	
	50									

		#1			#2			#3		
D2HGDH siRNA #3		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0005	2.44	40.61	0.0008	3.90	40.78	0.0007	3.41	39.5
	0.25	0.0013	6.33		0.0012	5.85		0.0016	7.79	
	0.5	0.0029	14.13	Km	0.0028	13.64	Km	0.0026	12.67	Km
	1	0.0041	19.97	2.25	0.0040	19.49	2.2	0.0045	21.92	1.84
	2.5	0.0058	28.26		0.0060	29.23		0.0065	31.67	
	5	0.0070	34.10		0.0075	36.54		0.0072	35.08	
	10	0.0074	36.05		0.0075	36.54		0.0073	35.56	
	15	0.0079	38.49		0.0076	37.03		0.0075	36.54	
	25	0.0075	36.54		0.0075	36.54		0.0073	35.56	
	50									

		#1			#2			#3		
D2HGDH siRNA #5		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0003	1.79	41.6	0.0003	1.79	42.65	0.0007	4.17	41.57
	0.25	0.0011	6.55		0.0010	5.95		0.0013	7.74	
	0.5	0.0022	13.10	Km	0.0024	14.29	Km	0.0023	13.70	Km
	1	0.0039	23.22	2.1	0.0032	19.05	2.35	0.0030	17.86	2.17
	2.5	0.0050	29.77		0.0051	30.37		0.0052	30.96	
	5	0.0060	35.73		0.0065	38.70		0.0064	38.11	
	10	0.0065	38.70		0.0063	37.51		0.0064	38.11	
	15	0.0063	37.51		0.0064	38.11		0.0062	36.92	
	25	0.0063	37.51		0.0064	38.11		0.0062	36.92	
	50									

Ramos KD

		#1			2			#3		
siRNA ctrl		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0012	3.86	25.4	0.0013	4.18	25.92	0.0013	4.18	25.49
	0.25	0.0026	8.36		0.0027	8.68		0.0026	8.36	
	0.5	0.0039	12.54	Km	0.0038	12.22	Km	0.0038	12.22	Km
	1	0.0056	18.01	1.04	0.0057	18.33	1.06	0.0056	18.01	1.07
	2.5	0.0067	21.54		0.0068	21.86		0.0065	20.90	
	5	0.0070	22.51		0.0071	22.83		0.0070	22.51	
	10	0.0076	24.44		0.0078	25.08		0.0078	25.08	
	15	0.0076	24.44		0.0077	24.76		0.0075	24.12	
	25	0.0075	24.12		0.0077	24.76		0.0076	24.44	
	50									

		#1			2			#3		
D2HGDH siRNA #3		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0012	4.02	21.76	0.0012	4.02	21.88	0.0012	4.02	21.45
	0.25	0.0021	7.03		0.0022	7.37		0.0021	7.03	
	0.5	0.0030	10.05	Km	0.0032	10.72	Km	0.0031	10.38	Km
	1	0.0043	14.40	1.07	0.0049	16.41	0.97	0.0041	13.73	1.12
	2.5	0.0059	19.76		0.0055	18.42		0.0051	17.08	
	5	0.0060	20.10		0.0060	20.10		0.0061	20.43	
	10	0.0060	20.10		0.0063	21.10		0.0062	20.77	
	15	0.0063	21.10		0.0062	20.77		0.0060	20.10	
	25	0.0060	20.10		0.0062	20.77		0.0061	20.43	
	50									

		#1			2			#3		
D2HGDH siRNA #5		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0012	3.94	19.25	0.0011	3.61	19.68	0.0011	3.61	19.68
	0.25	0.0024	7.87		0.0024	7.87		0.0025	7.87	
	0.5	0.0032	10.50	Km	0.0033	10.83	Km	0.0033	10.83	Km
	1	0.0044	14.44	0.81	0.0039	12.80	0.93	0.0039	12.80	0.93
	2.5	0.0051	16.73		0.0049	16.08		0.0049	16.08	
	5	0.0055	18.05		0.0055	18.05		0.0056	18.05	
	10	0.0055	18.05		0.006	19.69		0.0059	19.69	
	15	0.0054	17.72		0.0057	18.70		0.0062	18.70	
	25	0.0060	19.69		0.0059	19.36		0.0060	19.36	
	50									

Stable knockdown

HEK-293

		#1			2			#3		
siRNA ctrl		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso(uM)	0.0027	8.68	30.58	0.0028	9.00	30.85	0.0025	8.04	30.09
	0.25	0.0037	11.90		0.0039	12.54		0.0048	15.43	
	0.5	0.0061	19.61	Km	0.0068	21.86	Km	0.0070	22.51	Km
	1	0.0069	22.19	0.66	0.0070	22.51	0.56	0.0071	22.83	0.49
	2	0.0083	26.69		0.0088	28.30		0.0084	27.01	
	4	0.0085	27.33		0.0093	29.90		0.0085	27.33	
	5	0.0093	29.90		0.0094	30.23		0.0089	28.62	
	10	0.0090	28.94		0.0092	29.58		0.0091	29.26	
	15	0.0091	29.26		0.0089	28.62		0.0090	28.94	
	25	0.0092	29.58		0.0090	28.94		0.0093	29.90	
	50									

		#1			2			#3		
D2HGDH siRNA #3		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso	0.0020	6.43	25.26	0.0022	7.07	24.63	0.0020	6.43	25.43
	0.25	0.0032	10.29		0.0035	11.25		0.0044	14.15	
	0.5	0.0050	16.08	Km	0.0053	17.04	Km	0.0053	17.04	Km
	1	0.0059	18.97	0.67	0.0056	18.01	0.59	0.0059	18.97	0.51
	2	0.0066	21.22		0.0063	20.26		0.0072	23.15	
	4	0.0068	21.86		0.0065	20.90		0.0073	23.47	
	5	0.0076	24.44		0.0074	23.79		0.0078	25.08	
	10	0.0075	24.12		0.0075	24.12		0.0073	23.47	
	15	0.0076	24.44		0.0075	24.12		0.0078	25.08	
	25	0.0077	24.76		0.0078	25.08		0.0077	24.76	
	50									

		#1			2			#3		
D2HGDH siRNA #5		A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax	A340nm/min	umol/min/mg	Vmax
	d-iso	0.0023	7.40	26	0.0022	7.07	25.91	0.0024	7.72	25.85
	0.25	0.0036	11.58		0.0036	11.58		0.0041	13.18	
	0.5	0.0055	17.68	Km	0.0061	19.61	Km	0.0058	18.65	Km
	1	0.0067	21.54	0.53	0.0057	18.33	0.56	0.0062	19.94	0.49
	2	0.0074	23.79		0.0069	22.19		0.0075	24.12	
	4	0.0075	24.12		0.0073	23.47		0.0070	22.51	
	5	0.0076	24.44		0.0078	25.08		0.0078	25.08	
	10	0.0077	24.76		0.0078	25.08		0.0076	24.44	
	15	0.0078	25.08		0.0079	25.40		0.0079	25.40	
	25	0.0078	25.08		0.0079	25.40		0.0080	25.72	
	50									

Enzyme kinectis calculated using the Michaelis-Menten equation

Supplementary Table 6. IDH activiity assay in subcellular fractions

Cytosolic fraction

MSCV	A340nm/min			umol NADPH/min/mg			mean	SD		
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg			
0.25	0.0014	0.0013	0.0013	4.33	4.02	4.02	4.12	0.18		
0.5	0.0020	0.0021	0.0019	6.18	6.49	5.87	6.18	0.31	Best fit values	Std. Error
1	0.0035	0.0034	0.0033	10.82	10.51	10.20	10.51	0.31	Vmax	
2.5	0.0040	0.0039	0.0039	12.37	12.06	12.06	12.16	0.18	17.65	0.26
5	0.0049	0.0048	0.0050	15.15	14.84	15.46	15.15	0.31	Km	
10	0.0054	0.0057	0.0055	16.70	17.62	17.00	17.11	0.47	0	0.0585
25	0.0053	0.0054	0.0058	16.39	16.70	17.93	17.00	0.82		
50	0.0053	0.0055	0.0057	16.44	17.06	17.68	17.06	0.62		

D2HGDH WT	A340nm/min			umol NADPH/min/mg			mean	SD		
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg			
0.25	0.0013	0.0014	0.0013	3.88	4.18	3.88	3.98	0.17		
0.5	0.0019	0.0020	0.0020	5.68	5.98	5.98	5.88	0.17	Best fit values	Std. Error
1	0.0034	0.0035	0.0032	10.16	10.46	9.56	10.06	0.46	Vmax	
2.5	0.0039	0.0040	0.0040	11.65	11.95	11.95	11.85	0.17	17.77	0.27
5	0.0050	0.0050	0.0052	14.94	14.94	15.54	15.14	0.35	Km	
10	0.0058	0.0057	0.0059	17.33	17.03	17.63	17.33	0.30	0.9171	0.0641
25	0.0057	0.0058	0.0056	17.03	17.33	16.73	17.03	0.30		
50	0.0057	0.0058	0.0055	17.09	17.39	16.49	16.99	0.46		

Mitochondrial fraction

MSCV	A340nm/min			umol NADPH/min/mg			mean	SD		
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg			
0.25	0.0004	0.0003	0.0004	11.69	8.77	11.69	10.72	1.69		
0.5	0.0006	0.0007	0.0006	17.54	20.46	17.54	18.51	1.69	Best fit values	Std. Error
1	0.0011	0.0010	0.0012	32.15	29.23	35.08	32.15	2.92	Vmax	
2.5	0.0013	0.0014	0.0013	38.00	40.92	38.00	38.97	1.69	58.8	1.18
5	0.0017	0.0016	0.0016	49.69	46.77	46.77	47.74	1.69	Km	
10	0.0019	0.0020	0.0020	55.54	58.46	58.46	57.49	1.69	1.018	0.0932
25	0.0019	0.0019	0.0020	55.54	55.54	58.46	56.51	1.69		
50	0.0021	0.0018	0.0018	61.58	52.79	52.79	55.72	5.08		

D2HGDH WT	A340nm/min			umol NADPH/min/mg			mean	SD		
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg			
0.25	0.0005	0.0006	0.0005	14.62	17.54	14.62	15.59	1.69		
0.5	0.0008	0.0009	0.0008	23.38	26.31	23.38	24.36	1.69	Best fit values	Std. Error
1	0.0016	0.0015	0.0015	46.77	43.85	43.85	44.82	1.69	Vmax	
2.5	0.0019	0.0017	0.0019	55.54	49.69	55.54	53.59	3.38	84.3	1.66
5	0.0025	0.0024	0.0025	73.08	70.15	73.08	72.10	1.69	Km	
10	0.0028	0.0029	0.0028	81.85	84.77	81.85	82.82	1.69	1.063	0.0948
25	0.0027	0.0028	0.0027	78.92	81.85	78.92	79.90	1.69		
50	0.0027	0.0027	0.0026	79.18	79.18	76.25	78.20	1.69		

Enzyme kinectis calculated using the Michaelis-Menten equation

Supplementary Table 6 (continuation)

Cytosolic fraction

siRNA ctrl	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0018	0.0017	0.0016	5.10	4.82	4.54	4.82	0.28	
0.5	0.0025	0.0024	0.0025	7.09	6.81	7.09	6.99	0.16	Best fit va Std. Error
1	0.0042	0.0041	0.0041	11.91	11.63	11.63	11.72	0.16	Vmax
2.5	0.0048	0.0047	0.0046	13.61	13.33	13.04	13.33	0.28	17.99 0.24
5	0.0058	0.0057	0.0056	16.45	16.16	15.88	16.16	0.28	
10	0.0063	0.0062	0.0061	17.86	17.58	17.30	17.58	0.28	Km
25	0.0061	0.0062	0.0060	17.30	17.58	17.01	17.30	0.28	0.6772 0.044
50	0.0059	0.0064	0.0060	16.78	18.21	17.07	17.35	0.75	

D2HGDH siRNA #3	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0015	0.0016	0.0014	4.54	4.84	4.24	4.54	0.30	Best fit va Std. Error
0.5	0.0021	0.0021	0.0023	6.36	6.36	6.96	6.56	0.35	Vmax
1	0.0037	0.0038	0.0036	11.20	11.51	10.90	11.20	0.30	17.66 0.24
2.5	0.0043	0.0044	0.0042	13.02	12.72	12.72	12.82	0.17	Km
5	0.0052	0.0053	0.0051	15.74	16.05	15.44	15.74	0.30	0.7263 0.048
10	0.0056	0.0057	0.0055	16.96	17.26	16.65	16.96	0.30	
25	0.0057	0.0059	0.0055	17.26	17.86	16.65	17.26	0.61	
50	0.0055	0.0058	0.0054	16.71	17.62	16.40	16.91	0.63	

D2HGDH siRNA #5	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0013	0.0012	0.0012	4.25	3.92	3.92	4.03	0.19	Best fit va Std. Error
0.5	0.0018	0.0019	0.0018	5.88	6.21	5.88	5.99	0.19	Vmax
1	0.0032	0.0033	0.0031	10.46	10.78	10.13	10.46	0.33	17.57 0.21
2.5	0.0039	0.0041	0.0038	12.74	13.40	12.42	12.85	0.50	Km
5	0.0048	0.0047	0.0048	15.69	15.36	15.69	15.58	0.19	0.8098 0.046
10	0.0052	0.0051	0.0052	16.99	16.67	16.99	16.88	0.19	
25	0.0052	0.0051	0.005	16.99	16.34	16.34	16.56	0.38	
50	0.0051	0.0052	0.0052	16.72	17.05	17.05	16.94	0.19	

Mitochondrial fraction

siRNA ctrl	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0007	0.0006	0.0007	17.29	14.82	17.29	16.46	1.43	
0.5	0.0009	0.0010	0.0010	22.23	24.70	24.70	23.87	1.43	Best fit va Std. Error
1	0.0019	0.0019	0.0017	46.92	46.92	41.98	45.28	2.85	Vmax
2.5	0.0024	0.0025	0.0023	59.27	61.74	56.80	59.27	2.47	84.92 1.42
5	0.0030	0.0029	0.0028	74.09	71.62	69.15	71.62	2.47	
10	0.0033	0.0035	0.0032	81.50	86.44	79.03	82.32	3.77	Km
25	0.0034	0.0032	0.0032	83.97	79.03	79.03	80.67	2.85	1.012 0.07732
50	0.0031	0.0033	0.0032	76.80	81.76	79.28	79.28	2.48	

D2HGDH siRNA #3	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0003	0.0004	0.0002	9.74	12.99	6.50	9.74	3.25	Best fit va Std. Error
0.5	0.0005	0.0005	0.0004	16.24	16.24	12.99	15.16	1.88	Vmax
1	0.0009	0.0009	0.0008	29.23	29.23	25.98	28.15	1.88	55.35 1.20
2.5	0.0012	0.0011	0.0011	38.97	35.73	35.73	36.81	1.88	Km
5	0.0014	0.0015	0.0014	45.47	48.72	45.47	46.55	1.88	1.105 0.108
10	0.0016	0.0018	0.0015	51.97	58.46	48.72	53.05	4.96	
25	0.0017	0.0016	0.0015	55.21	51.97	48.72	51.97	3.25	
50	0.0016	0.0017	0.0015	52.13	55.39	48.88	52.13	3.26	

D2HGDH siRNA #5	A340nm/min			umol NADPH/min/mg			mean	SD	
D-isocitrate(uM)	#1	#2	#3	#1	#2	#3	umolNADPH/min/mg		
0.25	0.0005	0.0004	0.0005	14.59	11.67	14.59	13.62	1.68	Best fit va Std. Error
0.5	0.0007	0.0006	0.0006	20.42	17.51	17.51	18.48	1.68	Vmax
1	0.0011	0.0010	0.0011	32.10	29.18	32.10	31.12	1.68	54.4 0.89
2.5	0.0013	0.0012	0.0013	37.93	35.01	37.93	36.96	1.68	Km
5	0.0016	0.0015	0.0016	46.69	43.77	46.69	45.71	1.68	0.8854 0.068
10	0.0018	0.0017	0.0018	52.52	49.60	52.52	51.55	1.68	
25	0.0019	0.0018	0.0017	55.44	49.60	49.60	51.55	3.37	
50	0.0019	0.0018	0.0019	55.62	52.69	55.62	54.64	1.69	

Enzyme kinectis calculated using the Michaelis-Menten equation

Supplementary Table 7 - Mutational status of chromatin modifiers in DLBCL cell lines

Cell line	D2HGDH	MLL2	MLL3	MLL4	MLL5	CREBBP	EP300	EZH2
WSU-NHL	Mutant	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	Mutant[1-3]	WT[1-3]	WT[1-3]
OCI-Ly7	Mutant	WT[1-3]	Mutant[2]	WT[2]	WT[2]	WT[1-3,5]	WT[1-3,5]	WT[1-3,5]
SU-DHL6	Mutant	Mutant[1,3,5]	WT[1,3,5]	WT[1,3,5]	WT[1,3,5]	WT[5]; Mutant[1,3]	Mutant[1,3,5,7]	Mutant[1,3,5,6]
SU-DHL8	Mutant	Mutant[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	Mutant[1,3]	Mutant[1,3,7]	WT[1,3,7]
SU-DHL4	WT	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1,3] Del[7]	WT[2,7], Mutant[1,3]
SU-DHL10	WT	Mutant [1,3]	WT[1,3]	WT[1,3]	WT[1,3]	Mutant[1,3]	Mutant[1,3,7]	WT[1,3,7]
OCI-Ly1	WT	Mutant[1,3,5]	WT[1-3,5,6]	WT[1-3,5,6]	WT[1-3,5,6]	Mutant[1,3] WT[2,5]	WT[1-6]	Mutant[1-3,5,6]
OCI-Ly4	WT	Mutant[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]
OCI-Ly18	WT	Mutant[1,3]	WT[1]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]
SU-DHL2	WT	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	Del[1,3]	WT[1,3]
Farage	WT	WT[2]	WT[2]	WT[2]	WT[2]	WT[2]	WT[2]	WT[2]
OCI-Ly8	WT	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	Mutant[1,3] WT[2]	WT[1,3,4]	WT[1-3]
OCI-Ly10	WT	WT[1-3]	WT[1-4]	WT[1-4]	WT[1-4]	WT[1-4]	WT[1,3,4] M[7]	WT[1-4,7]
OCI-Ly19	WT	WT[2,5]	WT[2,5]	WT[2,5]	WT[2,5]	WT[2,5]	WT[2,5]	WT[2,5,7]

The cell lines analyzed for the histone and DNA methylation, HIF hydroxylation and alpha-KG or D2-HG levels (Figure 7 and Supplementary Figure 12) are listed above. The remaining cell lines sequenced for D2HGDH (Supplementary Table1) are listed below.

MLL2 and MLL3 encode histone methyltransferase that methylates H3K4; MLL2 and MLL3 mutations are likely loss-of-function thus leading to lower methylation of H3K4, yet cell lines mutant for both D2HGDH and MLL2 or MLL3 display high H3K4me3 levels.

The methylation of H3K4m3 was higher in OCI-Ly7, SU-DHL6 and SU-DHL8, than in SU-DHL10, OCI-Ly1, OCI-Ly4 or OCI- Ly18 and thus appear to be driven by D2HGDH

Cell line	D2HGDH	MLL2	MLL3	MLL4	MLL5	CREBBP	EP300	EZH2
HBL-1	WT	WT[1,3,4]	WT[1,3] M[4]	WT[1,3,4]	M[4]	WT[1,3] M[4]	WT[1,3,4]	WT[1,3,4]
HT	WT	WT[2]	WT[2]	WT[2]	WT[2]	WT[2]	WT[2]	WT[2,7]
K1106P	WT	NA	NA	NA	NA	NA	NA	NA
K422	WT	WT[2,6] M[5]	WT[2,5,6]	WT[2,5,6]	WT[2,5,6]	WT[2] M[5]	M[5]	M[2,5,6]
OCI-Ly3	WT	WT[1-4]	WT[1-4]	M[4]	WT[1-4]	WT[1-4]	WT[1-4]	WT[1-4]
NU-DHL1	WT	M[5]	WT [5]	WT [5]	WT [5]	M[5]	WT [5]	WT[5,7]
NU-DUL1	WT	M[5]	WT [5]	WT [5]	WT [5]	WT [5]	WT [5]	WT[5,7]
Pfeiffer	WT	WT[2] M[5]	WT[2]	WT[2]	WT[2]	M[2]	M[7]	WT[2,7]
RC-K8	WT	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	Del[1,3]	WT[1-3]
SU-DHL5	WT	WT[1,3]	WT[1,3]	WT[1,3]	WT[1,3]	Del[1,3]	WT[1,3] Del[7]	Del[1,3,7]
SU-DHL7	WT	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[2] M[1,3]	WT[1-3]	WT[1-3]
SU-DHL16	WT	NA	NA	NA	NA	NA	NA	NA
Toledo	WT	M[5]	WT[2] M[7]	WT[2,5]	WT[2,5]	WT[2,5]	M[7]	WT[2,5,7]
U2932	WT	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]	WT[1-3]
USC-DHL1	WT	NA	NA	NA	NA	NA	NA	NA

Supplementary Table 8 - Mutational status of chromatin modifiers in DLBCL

Tumor ID	D2HGDH	MLL2 (% variant allele)	MLL3 (% variant allele)	MLL4	MLL5	EZH2 (% variant allele)	EP300 (% variant allele)	CREBBP (% variant allele)	TET2
2202	WT	c.C11968T:p.Q3990X (25%); c.C6520T:p.Q2174X (37%)	WT	WT	WT	WT	c.C5597T:p.P1866L (34%)	WT	WT
3416	WT	WT	c.C5636T:p.P1879L (20%)	WT	WT	WT	WT	WT	WT
4236	WT	WT	WT	WT	WT	WT	WT	WT	WT
4522	WT	WT	WT	WT	WT	WT	WT	WT	WT
5234	A426T	WT	WT	WT	WT	WT	WT	WT	WT
5485	WT	c.A15176T:p.H5059L (82%)	WT	WT	WT	c.A1769T:p.Y641F (38%)	c.T4197G:p.D1399E (39%)	c.C4378T:p.R1460X (78%)	WT
6902	A426T	WT	WT	WT	WT	WT	WT	WT	WT
7518	WT	c.10982delG:p.G3661fs (67%)	WT	WT	WT	c.C1907T:p.A687V (17%)	WT	WT	WT
7572	WT	WT	WT	WT	WT	WT	c.4057delT:p.F1353fs (40%)	WT	WT

Supplementary Table 9 - D2HGDH mutational status in cancer

Tumor Type	Mutation - aa modification	Frequency*	Somatic Status	References
Glioma (GBM)	M153T, S178G, S197T, N443S	<1%	confirmed somatic	TCGA (COSU329, COSU545) and Nature Genetics 2013;45(10):1141-9
Endometrioid carcinoma	R107Q, V142I, A170V, R215Q, A420T	1.2%	somatic or unknown	TCGA (COSU419)
Colon Rectal Cancer	G98S, S197G#, D403N, L505M	4.7% (3/70 cases)	#cell line confirmed somatic	Nature 2012;488:660-4
Lung adenocarcinoma/squamous cel	Q129H, S149F, G201W, K252N, T390K, G438C, A455T	<1%	somatic or unknown	TCGA (COSU417/418); Cell 2012;150(6):1107-20; Nature Genetics 2012, 44(10):1104-10 and :1111-8
Melanoma	P141L, R215*, F336L, P424L, E447K, E475K	1.7%	confirmed somatic	TCGA (COSU540)
Stomach - adenocarcinoma	G193R, A373T, D413L	<1%	confirmed somatic	TCGA (COSU413/)
Bladder	A446T, G477A,	<1%	confirmed somatic	TCGA (COSU541/581)
Burkitt Lymphoma	C172Y	<1%	unknown	Nature 2012;490:116-20

Green font - same residue was reoprted mutated in D-2-hydroxyglutaric aciduria (D2-HGA
Red font - same mutation found in D2-HGA patients
When it was investigated, all mutations were found to be heterozygou

*To be more stringent, all SNPs - rare or frequent - were removed from this analysis, even when the variant was described in COSMIC as a somatic ever

Supplementary Table 10 – Primers sequence

D2HGDH – PCR and sequencing
D-HGex2F 5′-TGCTTCTGCAAGCGTGTTTC-3′
D-HGex2R 5′-TTTGAAGCCTCCACGGGAAG-3′
D-HGex3F 5′-GAGTGACCACTTGCCTCATC-3′
D-HGex3R 5′-AACCAAGATGTCATCGGCTG-3′
D-HGex4F 5′-GCAGGGTAATCAGGATTTGG-3′
D-HGex4R 5′-GCCCTAACTCATTCACCCAC-3′
D-HGex5F 5′-GTTCCTTCTGGGTGGCTTG-3′
D-HGex5R 5′-ATGAGAGCCGTGAGAGGAC-3′
D-HGex6F 5′-GTCCATCCTTCAGCCTCTTG-3′
D-HGex6R 5′-CTTCCTCACACCAACAGTG-3′
D-HGex7F 5′-TGTTTGTTGCAGTGCCAGTC-3′
D-HGex7R 5′-TGTGTCCAGACGTGCAGAAG-3′
D-HGex8F 5′-TCTTGGCCACGAAAGATCAG-3′
D-HGex8R 5′-CTGTCTAGGCTGCACCAATG-3′
D-HGex9F 5′-ATACAGAACATGCTGCTGCC-3′
D-HGex9R 5′-GATATGCTAAGCCAGAGACC-3′
D-HGex10F 5′-ATCTTGGGAGGGGCTGTTG-3′
D-HGex10R 5′-TTGGCAGCAGCAGGAGTG-3′

L2HGDH – PCR and sequencing
L-HGex1F 5′-AAGGCGCGCCACTTCATTG-3′
L-HGex1R 5′-CGGGACAGGGAAATACGAAC-3′
L-HGex2F 5′-TGCATGTGAAGTTTGGCGAG-3′
L-HGex2R 5′-CACTGACATTCAGCATGAAAG-3′
L-HGex4F 5′-CTCCTTTGGGTCATACAATAG-3′
L-HGex4R 5′-CTGTGACAGGATTATCTAACTG-3′
L-HGex5F 5′-TAGCAGCAAGAAAAGCTTGG-3′
L-HGex5R 5′-ATGGAGGGCTGACTATATTC-3′
L-HGex6F 5′-GGTGCAATCATAGTAATGAC-3′
L-HGex6R 5′-ACTTAAAATACAGCCCTGTG-3′
L-HGex7F 5′-CCCTCTTGACCTATTCTAC-3′
L-HGex7R 5′-CATCTCCTTTATGACCACC-3′
L-HGex8F 5′-TGCATGAGAAGAAAGTGTTTTATG-3′
L-HGex8R 5′-CCAATCACAAATATGGGGATTTAC-3′
L-HGex9F 5′-GCCTAGATTTTTGTGATGAC -3′
L-HGex9R 5′-GTATTTACACTCCTTATCCC -3′
L-HGex10F 5′-CGCTGACTTGTAAAGTATCC-3′
L-HGex10R 5′-TGCAGTGGTTATCTTTGACC-3′

IDH1/IDH2 – PCR and sequencing
IDH1F 5′-GAGCTCTATATGCCATCACTGC-3′
IDH1R 5′-CAAGTTGGAAATTTCTGGGC-3′
IDH2F 5′-ATTCTGGTTGAAAGATGGCG-3′
IDH2R 5′-ACAAGAGGATGGCTAGGCG-3′

D2HGDH – cloning, mutagenesis, RT-PCR
D2F-BglII gatagatctAAGGAGGAGCCCGAGGTC
D2R-XhoI cactcgagAGCAGCAGGAGTGGCCGTCAG
D2-131F 5′GAACCTGGCCGTGAACCCACAGGGGGxCAACACAGGCATGGTG 3′
D2-131R 5′CACCATGCCTGTGTTGxCCCCCTGTGGGTTCACGGCCAGGTTC 3′
D2-208F 5′GGGGGAAACGTGGCAACCAACACTGGAGGCCTGCGGTTTCTTC 3′
D2-208R 5′GAAGAAACCGCAGGCCTCCAGTGTTGGTTGCCACGTTTCCCCC 3′
D2-212F 5′ CAACCAACGCTGGAGGCCTGTGGTTTCTTCGATATGGCTCA 3′
D2-212R 5′ TGAGCCATATCGAAGAAACCACAGGCCTCCAGCGTTGGTTG 3′
D2-421F 5′CGTGACTGACCTGCGCGCCCACCTCGGCCCGCACGCCAAGC 3′
D2-421R 5′GCTTGGCGTGCGGGCCGAGGTGGGCGCGCAGGTCAGTCACG 3′
D2-426F 5′ GCCCGCCTCGGCCCGCACACCAAGCACGTGGTGGGCTATGG 3′
D2-426R 5′ CCATAGCCCACCACGTGCTTGGTGTGCGGGCCGAGGCGGGC 3′
D2F-Rtime 5′ GATATGGCTCACTGCATGGGAC 3′ (exon 5)
D2R-Rtime 5′ GTCTGCAGAACCTCAGCAAAGC 3′ (exon 7)

D2HGDH – siRNA
D2HGDH shRNA #3 target sequence: CTGTCATGAATGTCCAGTA
5′- GATCCTGTCATGAATGTCCAGTAATTCAAGAGATTACTGGACATTCATGACAGAA - 3′
5′ - AGCTTTCTGTCATGAATGTCCAGTAATCTCTTGAATTACTGGACATTCATGACAG - 3′
D2HGDH shRNA #5 target sequence: CCCAGCTGGGAAGACAGTT
5′ - GATCCCCAGCTGGGAAGACAGTTATTCAAGAGATAACTGTCTTCCCAGCTGGCAA - 3′
5′ - AGCTTTGCCAGCTGGGAAGACAGTTATCTCTTGAATAACTGTCTTCCCAGCTGGG - 3′

D2HGDH – tagged constructs
D2HGDH cloning into pHM6 – n-terminus HA tag:
D2HAFw-HindIII – ataagcttgCTGCCCCGTCGGCCTCT
D2HARv-NotI - aagcggccgcgaGGCCTGGCTGGGCA

D2HGDH cloning into p3xFLAG-CMV – c-terminus FLAG tag:
D2FlagFw-HindIII - ataagcttGAGCCCGAGGTCTCCGTC
D2FlagRv-BamHI - ttggatccGGCCTGGCTGGGCAGCGT

IDH2 - cloning and RT-PCR oligos
IDH2F-XhoI - tactcgagTCTCCAGCTTGGGATG
IDH2R-EcoRI - gagaattcCTCCACTGCAGCCATG
IDH2-QPCR-Fw 5′ AGCTGGATGGGAACCAAGAC 3′ (exon 9)
IDH2-QPCR-Rv 5′ CTCGTTCAGCTTCACATTGC 3′ (exon 11)

Supplementary Table 11. Antibodies validation

Antibody	Commercial source	Validation
D2HGDH	Proteintech, #13895-1-AP	Antibodypedia
H3K4me3	Cell Signaling Technologies #9751	*Nature* 483, 474-78 (2012).
H3K9me2	Cell Signaling Technologies #9753	*Nature*, 464:306-10 (2010)
H3K27me3	Cell Signaling Technologies #9733	*Nature*, 464:306-10 (2010)
H3K36me3	Cell Signaling Technologies #4909	*Nature*, 469:231-05 (2011)
H3K79me2	Cell Signaling Technologies #9757	*Nature* 483, 474-78 (2012)
Total histone H3	Cell Signaling Technologies #4499	J Immunol 186:3986-96 (2011).
HIF1α	BD Biosciences #61095	1degreebio.org
HIF1α-hydroxyproline (Pro402)	Millipore #07-1585	J. Biol. Chem., 286: 13041-51 (2011)
GLUT1	Novus Biologicals # NB300-666	Antibodypedia
IDH1	Cell Signaling Technology #8137	Science 324:261-5 (2009)
IDH2	Abcam, #ab55271	*Nature* 483, 474-78 (2012)
β-actin	Sigma-Aldrich, #A2228	1degreebio.org

Supplementary Table 12 - Metabolites quantification

D2HGDH stable expression cell model

D2-HG pmol/mg of protein - assay#1, 5 replicates

MSCV	8.431277	9.934035	15.45281	13.72072	12.26284
D2HGDH - WT	1.502575	1.806834	2.834707	2.913745	2.851159
D2HGDH - G131X	8.921748	9.508083	7.696046	6.301116	6.145552
D2HGDH - A208T	4.66838	5.456726	6.028038	6.740521	4.368945
D2HGDH - R212W	5.536191	7.573859	6.988648	8.26961	7.989582
D2HGDH - R421H	1.335891	2.469225	2.337813	1.663264	1.389835
D2HGDH - A426T	2.344331	3.82037	2.875458	5.230389	5.053128

D2-HG pmol/mg of protein - assay#2, 3 replicates

MSCV	26.34733	19.78871	13.83083
D2HGDH - WT	9.383159	13.36428	9.20061
D2HGDH - G131X	17.01825	18.52593	22.26802
D2HGDH - A208T	23.99778	26.22417	20.56295
D2HGDH - R421H	5.51374	8.497204	7.246866
D2HGDH - A426T	7.529353	10.59393	7.630245

L2-HG pmol/mg of protein, 3 replicates

MSCV	179.3523	113.1284	123.4135
D2HGDH - WT	141.5041	148.9788	125.1581
D2HGDH - G131X	125.0785	116.308	109.4466
D2HGDH - A208T	167.5777	138.9724	155.4484
D2HGDH - R421H	111.0916	91.20035	92.75989
D2HGDH - A426T	120.1405	129.3849	106.5934

2-KG pmol/mg of protein, assay#1, 4 replicates

MSCV	1971.995	2196.937	2416.924	2896.858
D2HGDH - WT	4719.369	4166.875	3449.139	3894.311
D2HGDH - G131X	1431.407	1766.205	1333.068	1651.335
D2HGDH - A208T	1843.989	2075.87	1531.019	1865.886
D2HGDH - R421H	1466.916	1622.627	1958.838	1030.705
D2HGDH - A426T	2050.196	1964.029	1959.822	2005.751

2-KG pmol/mg of protein, assay#2, 4 replicates

MSCV	1855.615	1558.616	1744.538	2453.218
D2HGDH - WT	3999.018	2828.462	2733.508	2423.464
D2HGDH - G131X	238.939	539.663	480.198	578.263
D2HGDH - A208T	734.603	1043.636	887.809	1088.109
D2HGDH - R421H	1420.497	2164.322	1502.578	1198.894
D2HGDH - A426T	1830.906	1006.023	1047.18	1040.221

2-KG pmol/mg of protein, assay#3, 5 replicates

MSCV	1025.726	768.7465	776.5064	727.4821	1530.187
D2HGDH - WT	1581.188	763.424	1491.919	1513.157	1188.635
D2HGDH - R212W	740.7486	357.0364	924.6938	297.2062	583.04

D2HGDH transient expression cell model

D2-HG pmol/mg of protein - assay#1, 3 replicates

MSCV	36.64416	36.4722	40.08173
D2HGDH - WT 0.5ug	29.68443	27.44684	32.32854
D2HGDH - WT 0.75ug	22.03808	23.38029	27.88385
D2HGDH - WT 1.0ug	14.61641	20.90789	11.17389

D2-HG pmol/mg of protein - assay#2, 3 replicates

MSCV	31.75372	28.76147	36.49974
D2HGDH - WT 0.5ug	19.03621	21.51516	19.99729
D2HGDH - WT 0.75ug	16.49129	15.69138	18.33014
D2HGDH - WT 1.0ug	12.7291	19.3764	13.86678

2-KG pmol/mg of protein - assay#1, 3 replicates

MSCV	375.6534	546.3336	671.2131
D2HGDH - WT 0.5ug	848.2435	655.7721	779.4605
D2HGDH - WT 0.75ug	803.1845	839.1634	980.6399
D2HGDH - WT 1.0ug	930.7704	911.0911	893.8879

2-KG pmol/mg of protein - assay#2, 3 replicates

MSCV	521.091	507.3479	521.134
D2HGDH - WT 0.5ug	820.3983	774.3329	696.7507
D2HGDH - WT 0.75ug	814.7245	885.1288	805.5704
D2HGDH - WT 1.0ug	845.1482	860.8818	865.4253

Supplementary Table 12 (continuation)

D2HGDH transient siRNA-KD model

D2-HG pmol/mg of protein - assay#1, 2 replicates

si-RNA ctrl	89.93771	80.97425
si-RNA D2#5	114.5639	142.3544
si-RNA D2#3	134.5945	137.5139

2-KG pmol/mg of protein - assay#1, 2 replicates

si-RNA ctrl	313.5986	362.0601
si-RNA D2#5	238.3543	229.6885
si-RNA D2#3	206.4567	231.8885

L2-HG pmol/mg of protein - assay#1, 2 replicates

si-RNA ctrl	227.8028	207.9399
si-RNA D2#5	208.3678	223.5751
si-RNA D2#3	152.5984	210.5491

DLBCL cell lines - D2HGDH WT

D2-HG pmol/mg of protein, 3 biological replicates (each representing the mean of technical triplicates)

OCI-Ly1	11.94	4.19	5.49
OCI-Ly4	0.08	0.52	0.59
OCI-Ly18	1.56	1.21	1.31
SU-DHL4	5.99	0.99	0.97
SU-DHL10	18.40	3.62	0.26
SU-DHL2	0.02	0.52	0.10
Farage	0.20	0.62	0.41
OCI-Ly8	16.41	1.12	6.32
OCI-Ly10	0.25	0.75	5.56
OCI-Ly19	8.25	1.28	7.16

2-KG pmol/mg of protein, 3 biological replicates (each representing the mean of technical triplicates)

OCI-Ly1	264.38	1509.22	398.72
OCI-Ly4	186.97	302.15	234.85
OCI-Ly18	699.86	1232.28	1070.77
SU-DHL4	67.70	737.33	607.24
SU-DHL10	267.42	2265.06	843.83
SU-DHL2	139.60	270.09	8952.50
Farage	98.63	331.14	572.42
OCI-Ly8	81.27	834.09	4894.93
OCI-Ly10	65.09	205.27	448.81
OCI-Ly19	1024.32	708.69	832.61

DLBCL cell lines - D2HGDH mutant

D2-HG pmol/mg of protein, 3 biological replicates (each representing the mean of technical triplicates)

WSU-NHL	9.84	0.53	0.40
SU-DHL6	7.54	1.32	1.18
SU-DHL8	12.39	0.51	0.64
OCI-Ly7	0	0.69	5.40

2-KG pmol/mg of protein, 3 biological replicates (each representing the mean of technical triplicates)

WSU-NHL	143.47	330.17	803.20
SU-DHL6	39.79	82.87	585.57
SU-DHL8	171.76	309.72	783.20
OCI-Ly7	339.31	1175.34	102.27

Supplementary Note 1:

No somatic variants were reported for D2HGDH in four published sequencing studies on DLBCL, accounting for over 200 patients. Below, we detail the potential reasons for these negative findings.

In Pasqualucci et al[1] only 6 DLBCLs (normal and tumour DNA pairs) were examined by whole-exome sequencing. Subsequently, the authors perform targeted re-sequencing of 56 genes (D2HGDH was NOT one of them) in 48 to 105 additional DLBCL biopsies. In that report, the size of the initial cohort, coupled with the low frequency of DH2HGDH mutations and the selection of a restricted list of candidate gene set for the subsequent analysis, likely explain the absence of D2HGDH variants in their DLBCL collection.

Zhang et al[2] defined their set of significantly mutated genes based on a discovery cohort of 34 paired DLBCL samples. According to their criteria, genes not previously associated with cancer were required to be mutated in at least two samples for inclusion in their list of significant candidates, which would eliminate genes with mutation frequencies lower than 6% (thus including D2HGDH which we found to be mutant in 3.3% of the primary samples or 5.8% primary samples + cell lines). In Zhang et al, the subsequent validation set was only screened for the genes identified in the smaller discovery cohort and thus would not have revealed D2HGDH mutations. Of note, Zhang et al. explored in great detail the degree of overlap between 'DLBCL genes' identified by theirs and the other studies discussed above; they found a surprisingly low concordance among the four studies at all mutation frequency levels, but especially for the genes at the lower end of the frequency spectrum. Remarkably, they noted that 30% of the genes mutated in at least 10% of the cases in their study were not detected by any of the remaining three studies and concluded that genetic heterogeneity in the disease

might contribute to the observed patterns of disparate mutations. The report on the 21 lymphoma cell lines studied by Zhang et al[2] is limited to the "322 DLBCL genes" that the authors identified in the initial 34 paired-samples cohort, and that does not include D2HGDH

The design of the study by Morin et al[5] was powered to pick up mutations at frequencies of 2.8% and higher, which would theoretically be sensitive enough to detect D2HGDH mutations at our estimated frequency of 5.4% (8 mutations in 148 samples – 4 in 120 primary tumors – 3.3%; 4 in 28 DLBCL cell lines 14%). However, in that study coding variants present in the dbSNP were removed at the first step of the analysis. If we apply these criteria to our cohort, 2 variants found in 4 of our primary biopsies (R421H and A426T), would have been excluded, leaving a residual frequency of D2HGDH mutations of 2.7%, which would fall within the margin of error of Morin et al.'s detection. Morin et al. also sequenced 10 DLBCL cell lines, and of those, the only ones that we report as having D2HGDH mutation are SU-DHL-6 and OCI-Ly7. Coincidently, these are exactly the cell lines with the R421H and A426T variants reported in dbSNP, which again would be removed from their analysis. We should indicate here that not only we demonstrated that these variants render D2HGDH inactive, but their frequency in non-malignant tissues (i.e., reference databases) is markedly lower than that of our DLBCL cohort. Finally, as referred to before, the A426T allele has been found to segregate with familial cases of D-2-hydroxyglutaric aciduria, further highlighting its biological relevance.

Lohr et al[8] studied a cohort of 49 normal and tumour DNA pairs. Although they applied algorithms to recognize significantly mutated genes at low frequency, their sample size would put them right at the limit of detection for D2HGDH variants (3.3% based on 4 D2HGDH mutants in 120 primary tumours in our cohort). In other words, all things being equal, they could find ~ 1.6 tumours mutant for D2HGDH in 49 samples. Importantly, the authors recognize that some mutations that may be functionally relevant would not meet statistical significance based

on their algorithms. In fact, by expanding the scale of their genomic analysis and applying novel statistical definitions, the same group recognized that current genomic studies have not yet achieved saturation for cancer gene discovery[9].

Supplementary References:

1 Pasqualucci, L. *et al.* Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet* **43**, 830-837, doi:10.1038/ng.892 (2011).

2 Zhang, J. *et al.* Genetic heterogeneity of diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A* **110**, 1398-1403, doi:10.1073/pnas.1205299110 (2013).

3 Pasqualucci, L. *et al.* Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* **471**, 189-195, doi:10.1038/nature09730 (2011).

4 Ngo, V. N. *et al.* Oncogenically active MYD88 mutations in human lymphoma. *Nature* **470**, 115-119 (2011).

5 Morin, R. D. *et al.* Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature* **476**, 298-303 (2011).

6 Morin, R. D. *et al.* Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* **42**, 181-185, doi:10.1038/ng.518 (2010).

7 Barretina, J. *et al.* The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603-607, doi:10.1038/nature11003 (2012).

8 Lohr, J. G. *et al.* Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci U S A* **109**, 3879-3884, doi:10.1073/pnas.1121343109 (2012).

9 Lawrence, M. S. *et al.* Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* **505**, 495-501, doi:10.1038/nature12912 (2014).