

**Mutant and wild-type isocitrate dehydrogenase 1 share enhancing mechanisms involving
distinct tyrosine kinase cascades in cancer**

Dong Chen, et al.

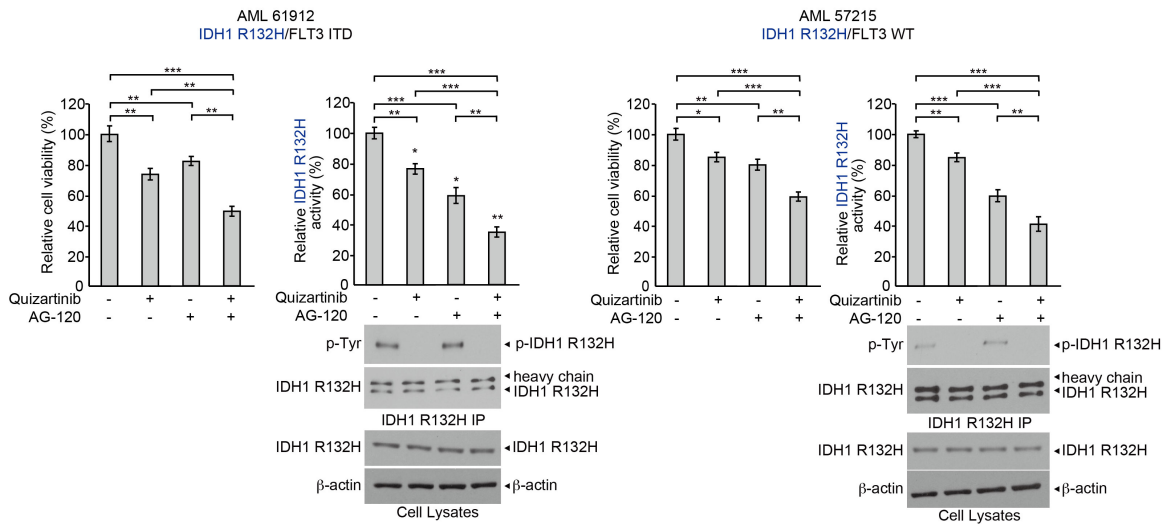
SUPPLEMENTARY DATA

Supplementary Figure S1-S7

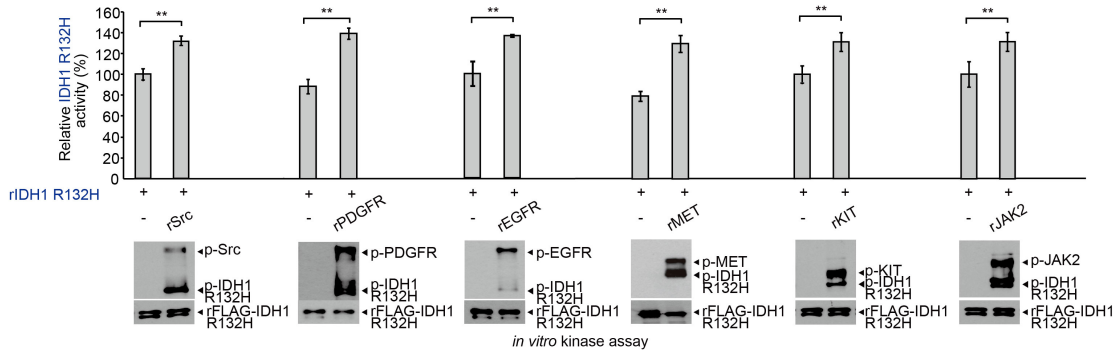
Supplementary Figure S1

A

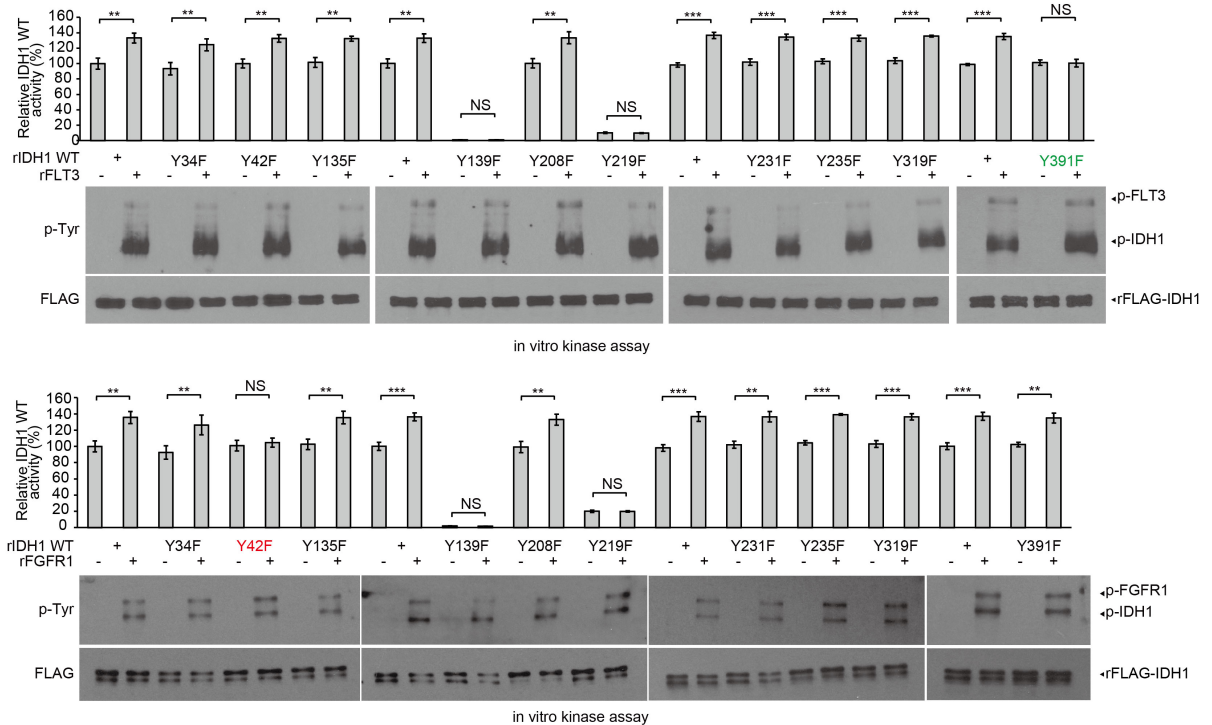
IDH1 mutant patient samples



B



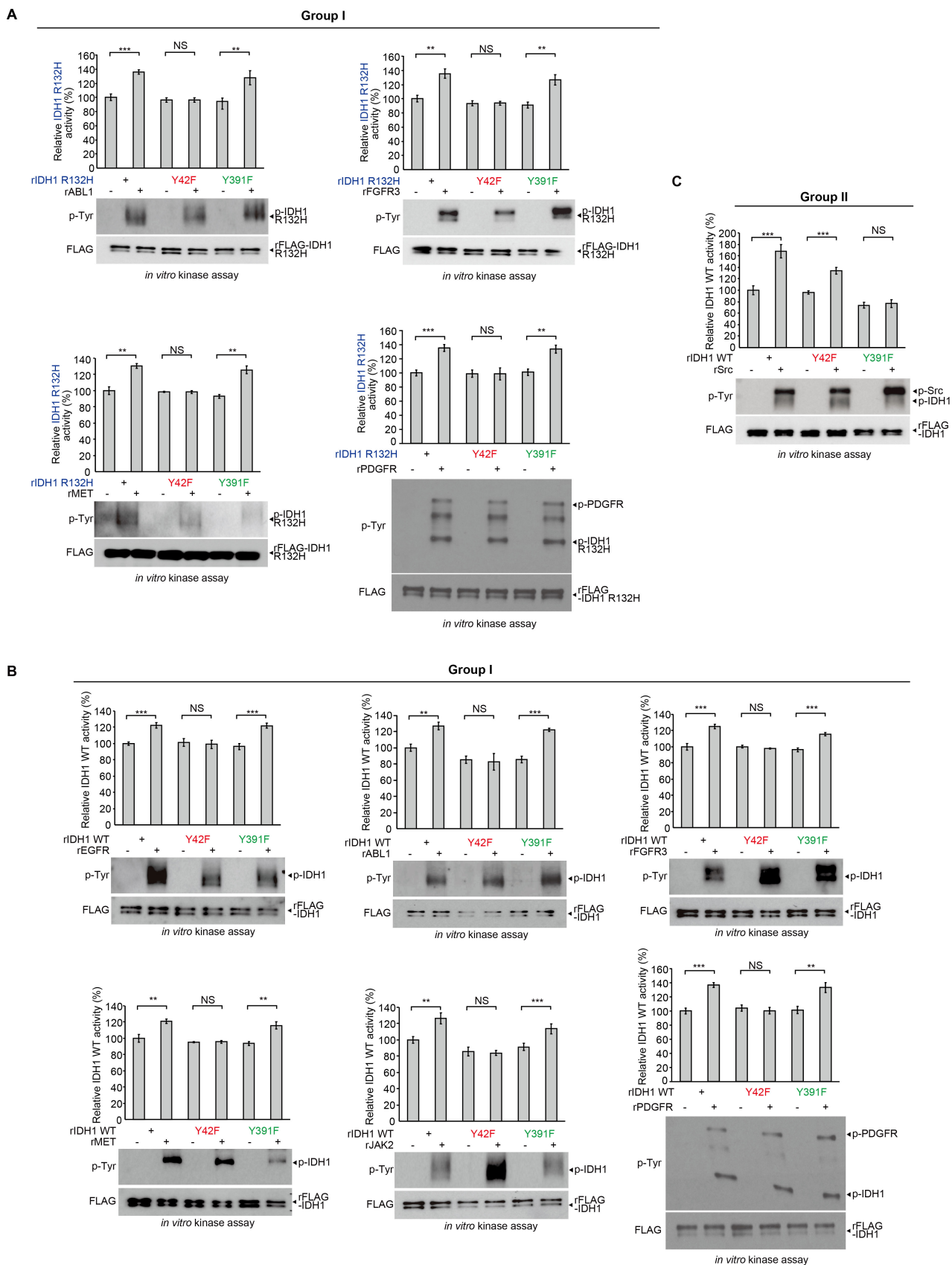
C



Supplementary Figure S1. FLT3 phosphorylates IDH1 R132H mutant in AML and diverse tyrosine kinases phosphorylate and activate WT IDH1. **A**, Human primary AML cells harvested from patient 61912 expressing IDH1 R132H/FLT3 ITD (*left 2 panels*) or from patient 57215 expressing IDH1 R132H/FLT3 WT (*right 2 panels*) were treated with FLT3 inhibitor quizartinib, IDH mutant inhibitor AG120, or the combination of quizartinib and AG120, followed by cell viability assay and IDH1 R132H enzyme activity assay (*upper panels*). Tyrosine phosphorylation (p-Tyr) of IDH1 R132H in each treated group was determined by Western blotting (*lower panels*). **B**, From *left to right*: Purified recombinant IDH1 R132H proteins were incubated with diverse recombinant active form of tyrosine kinases including rSrc, rPDGFR, rEGFR, rMET, rKIT and rJAK2 in an *in vitro* kinase assay. IDH1 R132H enzyme activity assay (*upper panels*) was assessed; tyrosine phosphorylation of IDH1 was detected by Western blotting (*lower panels*). **C**, Purified recombinant wild type IDH1 (IDH1 WT) and diverse IDH1 WT-YF mutants were incubated with diverse recombinant active form of tyrosine kinases rFLT3 (*upper 2 panels*) and rFGFR1 (*lower 2 panels*) in *in vitro* kinase assays. IDH1 WT enzyme activity assay (*upper*) was performed; tyrosine phosphorylation of IDH1 WT was detected by Western blotting (*lower*).

The error bars represent mean values \pm SD from three replicates of each sample (*: $0.01 < p < 0.05$; **: $0.01 < p < 0.001$; ***: $p < 0.001$; ns: not significant); Data are mean \pm SD; p values were obtained by a two-tailed Student's test.

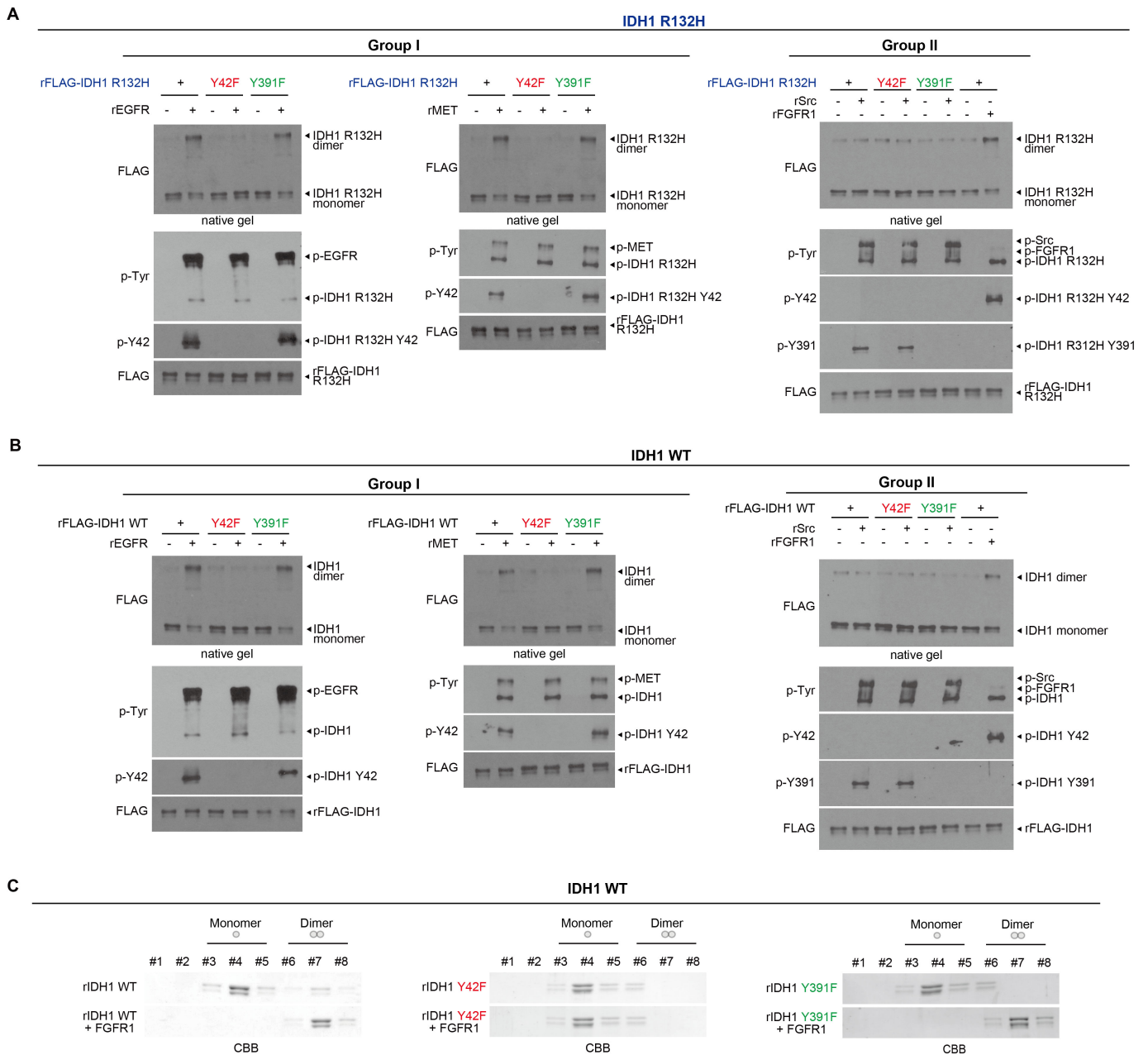
Supplementary Figure S2



Supplementary Figure S2. Distinct groups of tyrosine kinases preferentially phosphorylate and activate mutant and WT IDH1 at Y42 and Y391. **A**, Purified FLAG-IDH1 R132H, FLAG-IDH1 R132H Y42F and Y391F mutants were incubated with recombinant active Group I tyrosine kinases rABL1, rMET, rFGFR3, and rPDGFR, respectively, followed by IDH1 R132H enzyme activity assay (*upper* panels). Tyrosine phosphorylation of IDH1 R132H in each treated group was determined by Western blotting (*lower* panels). **B-C**, Purified FLAG-IDH1 WT, FLAG-IDH1 WT Y42F and Y391F mutants were incubated with recombinant active Group I tyrosine kinases rEGFR, rABL1, rFGFR3, rMET, rJAK2, and rPDGFR (**B**) and Group II tyrosine kinase rSrc (**C**), respectively, followed by IDH1 WT enzyme activity assay (*upper* panels). Tyrosine phosphorylation of IDH1 WT in each treated group was determined by Western blotting (*lower* panels).

The error bars represent mean values \pm SD from three replicates of each sample (**: $0.01 < p < 0.001$; ***: $p < 0.001$; ns: not significant); Data are mean \pm SD; p values were obtained by a two-tailed Student's test.

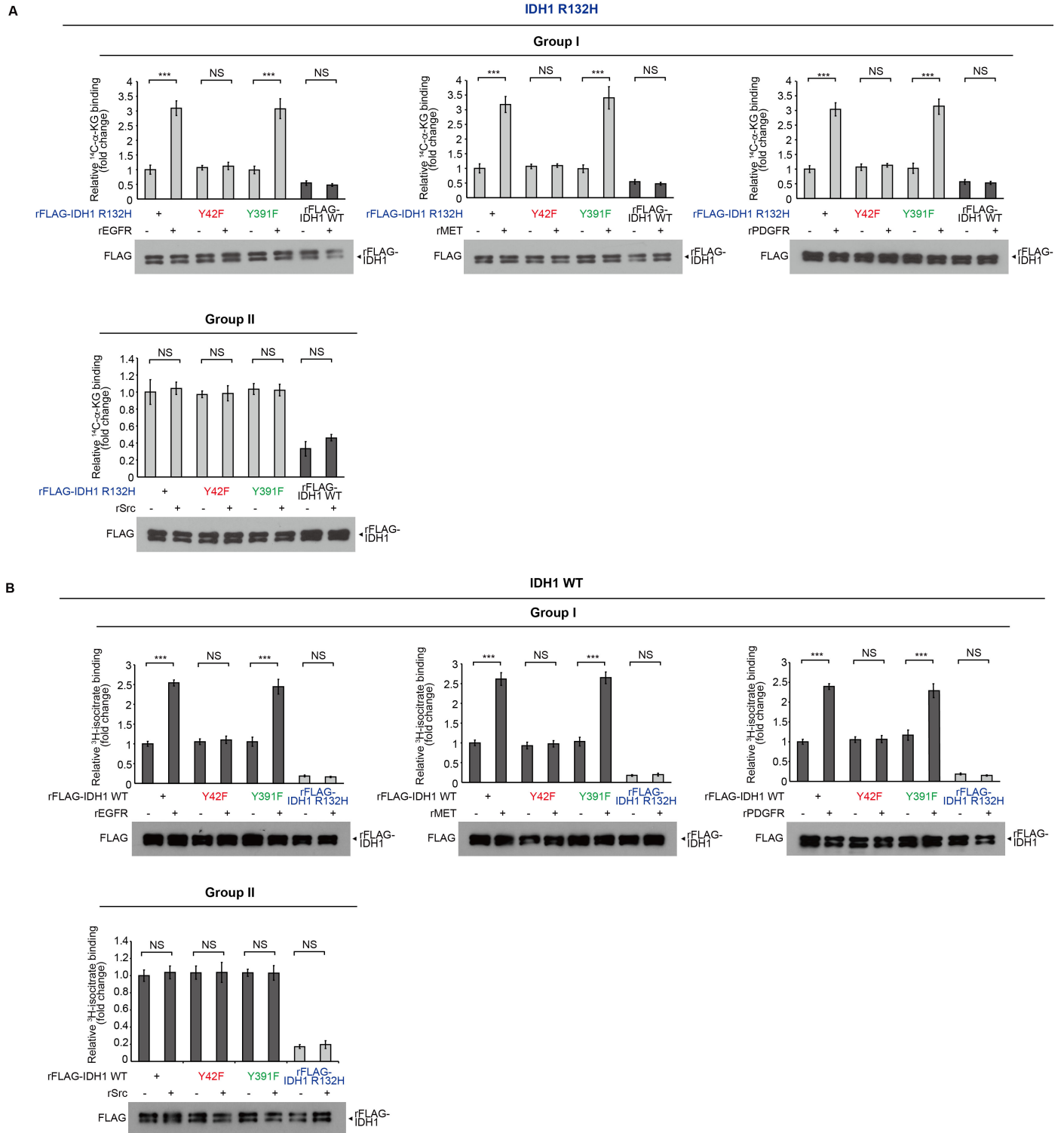
Supplementary Figure S3



Supplementary Figure S3. Y42 phosphorylation of WT and mutant IDH1 by Group I tyrosine kinases promotes protein dimerization. **A-B**, Purified FLAG-IDH1 R132H variants (**A**) or FLAG-IDH1 variants (**B**) were incubated with recombinant active Group I tyrosine kinases rEGFR and rMET, respectively (*left 2 panels*) or Group II tyrosine kinase rSrc (*right panel*). Dimeric and monomeric IDH1 R132H and WT proteins were determined by native PAGE and tyrosine phosphorylation of IDH1 R132H and WT in each treated group was detected by Western blotting. **C**, IDH1 WT (*left*), IDH1 WT Y42F (*middle*) and IDH1 WT Y391F (*right*) proteins were incubated with recombinant

active rFGFR1 prior to sucrose density ultracentrifugation. Collected fractions were applied to SDS PAGE, followed by CBB staining.

Supplementary Figure S4



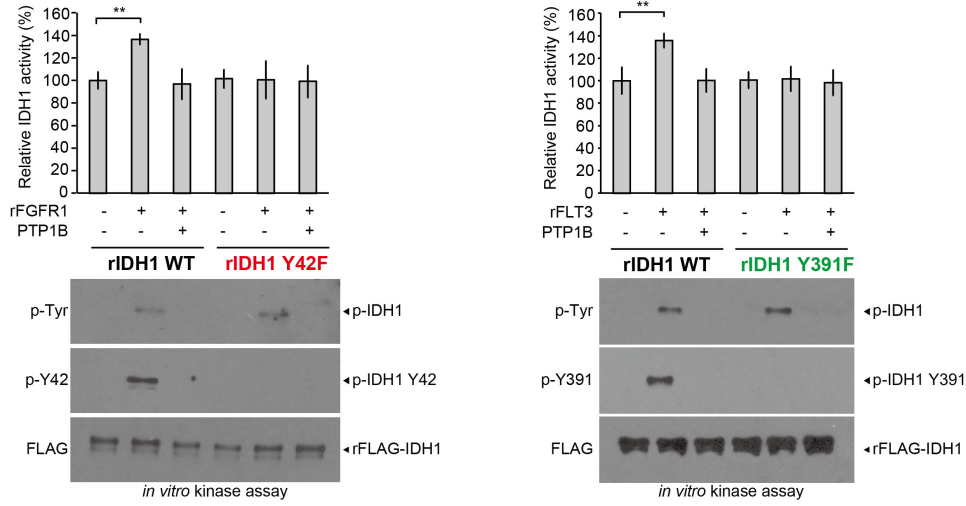
Supplementary Figure S4. Dimeric mutant and WT IDH1 contribute to the binding ability to its substrates α KG and isocitrate, respectively. **A**, Purified FLAG-IDH1 R132H variants were incubated

with recombinant active Group I tyrosine kinases rEGFR, rMET and rPDGFR, respectively (*upper* 3 panels) or Group II tyrosine kinase rSrc (*lower* panel), followed by ^{14}C - αKG binding assays. Purified IDH1-WT protein was used as a negative control. **B**, Purified FLAG-IDH1 variants were incubated with recombinant active Group I tyrosine kinases rEGFR, rMET and rPDGFR, respectively (*upper* 3 panels) or Group II tyrosine kinase rSrc (*lower* panel), followed by ^3H -isocitrate binding assays. Purified IDH1-R132H protein was used as a negative control.

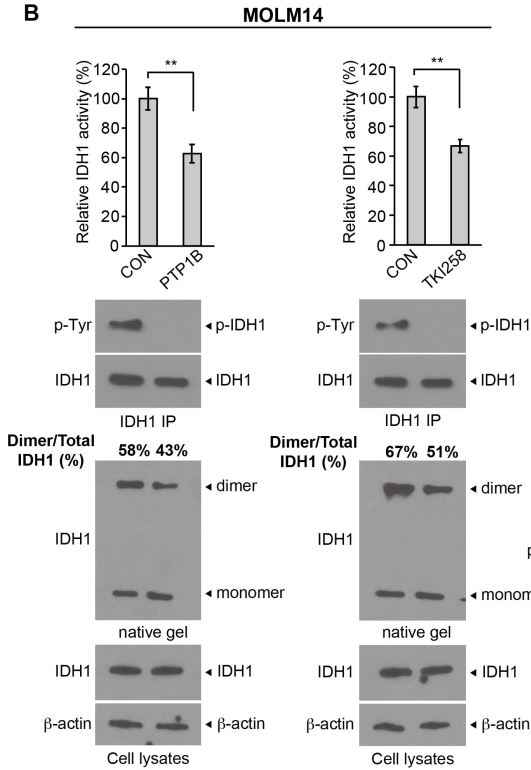
The error bars represent mean values \pm SD from three replicates of each sample (***: $p < 0.001$; ns: not significant); Data are mean \pm SD; p values were obtained by a two-tailed Student's test.

Supplementary Figure S5

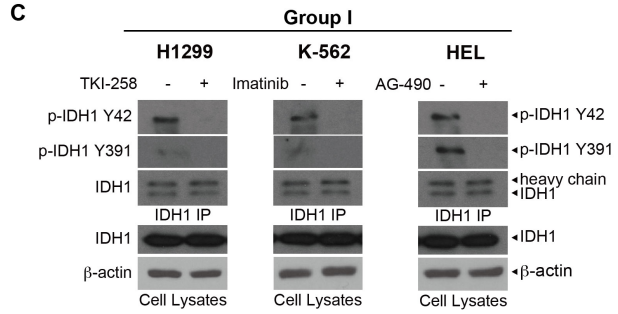
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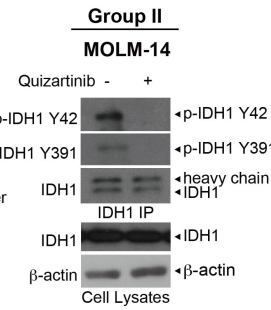
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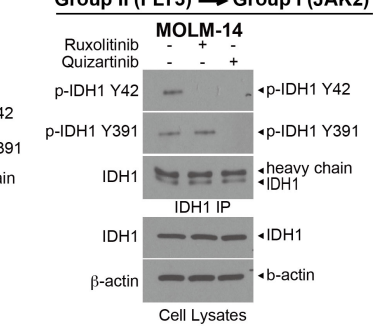
C



D



E

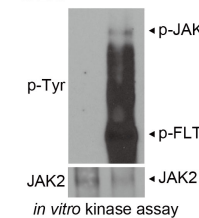


F

Group II (FLT3) → Group I (JAK2)

JAK2 IP pre-treated with PTP1B

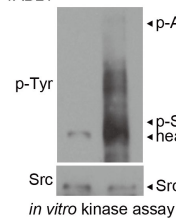
JAK2 IP	+	+
rFLT3	-	+



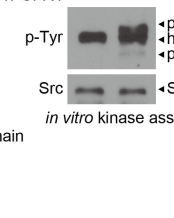
Group I → Group II (Src)

Src IP pre-treated with PTP1B

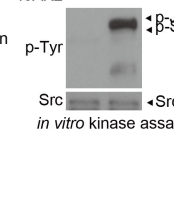
Src IP	+	+
rABL1	-	+



Src IP	+	+
rFGFR1	-	+



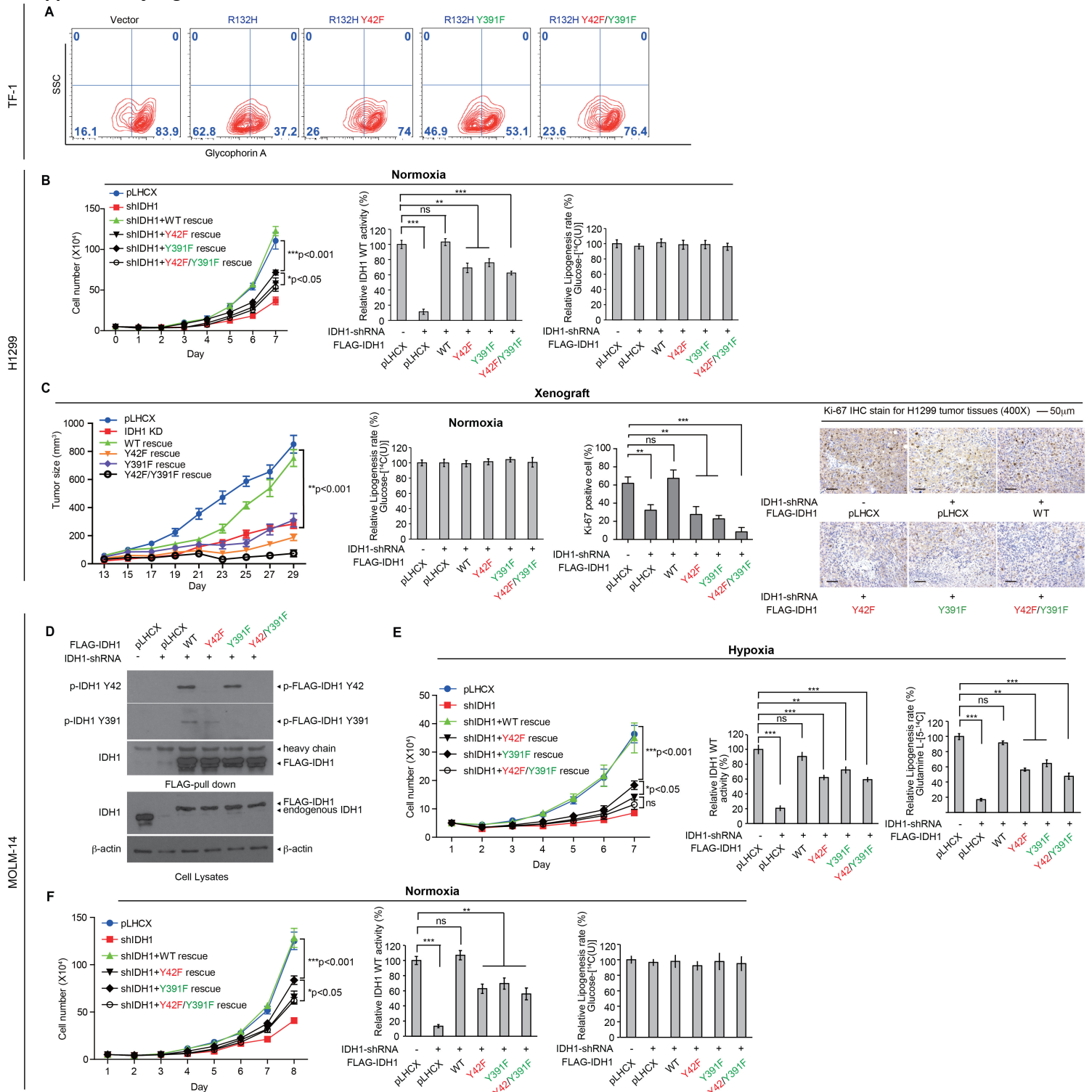
Src IP	+	+
rJAK2	-	+



Supplementary Figure S5. WT and mutant IDH1 are activated by distinct oncogenic tyrosine kinase cascades. **A**, Beads-bound purified IDH1 WT and IDH1 WT-Y42F (*left panels*), or IDH1 WT and IDH1 WT-Y391F proteins (*right panels*) pre-treated with or without FGFR1 or FLT3, were treated with or without Tyr phosphatase PTP1B. The proteins were eluted from beads and applied to IDH1 WT enzyme activity assay (*upper panels*) and Western blotting to detect pan tyrosine phosphorylation and tyrosine phosphorylation of Y42 or Y391 of IDH1 protein, respectively (*lower panels*). **B**, MOLM-14 cell lysates were treated with or without PTP1B (*left panels*) or TKI-258 (*right panels*), followed by endogenous IDH1 protein immunoprecipitation and IDH1 WT enzyme activity assay (*upper panels*). Pan tyrosine phosphorylation of IDH1 protein was detected by Western blotting. Dimeric and monomeric IDH1 levels were measured by Western blotting using native PAGE (*lower panels*). **C-D**, H1299, K562, HEL, and MOLM-14 cells were treated with tyrosine kinase inhibitors including TKI-258 (FGFR1), imatinib (ABL1), AG490 (JAK2), and quizartinib (FLT3), respectively, followed by endogenous IDH1 immunoprecipitation. Tyrosine phosphorylation at Y42 and Y391 of IDH1 was detected by Western blotting. **E**, MOLM-14 cells were treated with tyrosine kinase inhibitors ruxolitinib and quizartinib, respectively, followed by endogenous IDH1 immunoprecipitation. Tyrosine phosphorylation at Y42 and Y391 of IDH1 was detected by Western blotting. **F**, JAK2 immunoprecipitated from 293T cells and pre-treated with PTP1B was incubated with purified rFLT3 in an *in vitro* kinase assay. Phosphorylation of JAK2 by recombinant active form of rFLT3 was detected by Western blotting (*left*). Src immunoprecipitated from 293T cells and pre-treated with PTP1B was incubated with purified recombinant active form of rABL1, rFGFR1 or rJAK2 in an *in vitro* kinase assay. Phosphorylation of Src was detected by Western blotting (*right 3 panels*).

The error bars represent mean values \pm SD from three replicates of each sample (**: $0.01 < p < 0.001$); Data are mean \pm SD; p values were obtained by a two-tailed Student's test.

Supplementary Figure S6

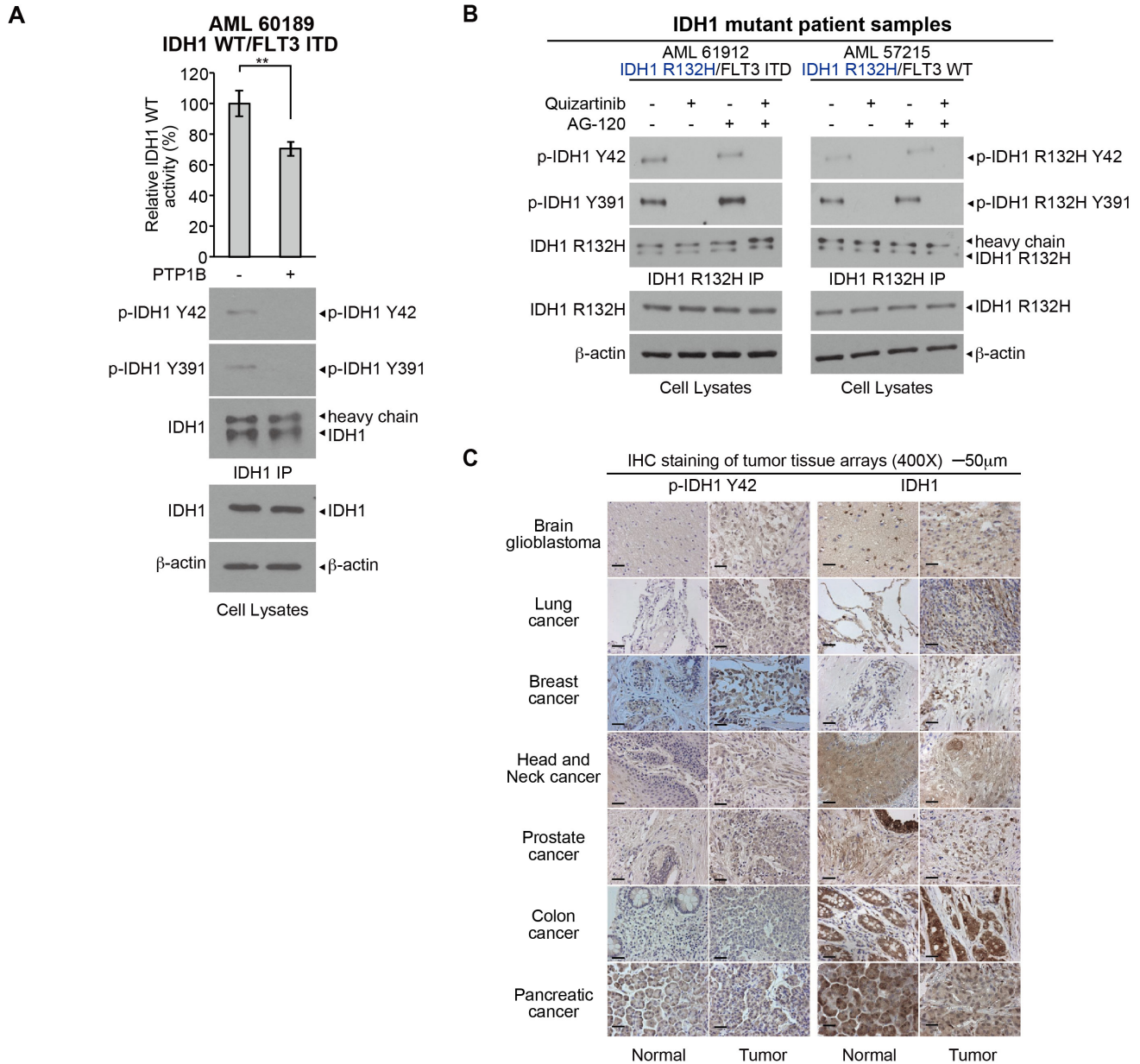


Supplementary Figure S6. Abolishment of tyrosine phosphorylation in IDH1 mutant and WT attenuates TF-1 cell proliferation potential in the absence of cytokine and cell proliferation and tumor growth potential of H1299 cells under normoxia, respectively. **A**, TF-1 stable cell lines expressing

IDH1 R132H and phospho-deficient variants were stained with glycophorin A, followed by flow cytometry analysis. **B**, Cell proliferation rate (*left*), IDH1 WT enzyme activity (*middle*) and lipogenesis rate (*right*) were determined in H1299 IDH1-knockdown cells with stable “rescue” expression of diverse FLAG-IDH1 variants under normoxia. **C**, Tumor growth curve (**C**, *left*), lipogenesis rate (**C**, *middle left*), Ki-67 positive cells (**C**, *middle right*) and representative of Ki-67 IHC images (**C**, *right*) from xenograft tumors derived from H1299 IDH1-knockdown cells with stable rescue expression of FLAG-IDH1 variants in a xenograft mouse model. **D**, Generation of MOLM-14 cells with stable knockdown of endogenous human IDH1 and stable “rescue” expression of FLAG-IDH1 WT, Y42F, Y391F or Y42F/Y391F. Rescued IDH1 protein levels were detected by Western blotting. **E**, Cell proliferation assay (*left*), IDH1 WT enzyme activity assay (*middle*) and lipogenesis rate using labeled glutamine (Glutamine L-[5-¹⁴C]) as a carbon source (*right*) were performed using MOLM-14 stable rescue cells under hypoxic conditions. **F**, Cell proliferation rate (*left*), IDH1 WT enzyme activity (*middle*) and lipogenesis rate (*right*) were determined in MOLM-14 IDH1-knockdown cells with stable “rescue” expression of diverse FLAG-IDH1 variants under normoxia.

The error bars represent mean values \pm SD from three replicates of each sample (*: 0.01<p<0.05; **: 0.01<p<0.001; ***: p<0.001; ns: not significant); Data are mean \pm SD, except \pm SEM for tumor growth; p values were obtained by a two-tailed Student’s test except for a two-way ANOVA test for cell proliferation assay and tumor growth curve.

Supplementary Figure S7



Supplementary Figure 7. FLT3 WT and ITD mutant activate WT and mutant IDH1 through direct phosphorylation of Y391 and indirect phosphorylation of Y42 in primary AML cells. **A**, Endogenous IDH1 protein was immunoprecipitated from primary leukemia cells with IDH1 WT/FLT3-ITD from an AML patient, followed by IDH1 WT enzyme activity assay (*upper* panel). Tyrosine phosphorylation of Y42 and Y391 of IDH1 was detected by Western blotting. **B**, Primary leukemia cells from representative AML patients expressing IDH1 R132H/FLT3 ITD (*left*) and IDH1 R132H/FLT3 WT (*right*) were treated with quizartinib and/or AG-120, followed by IDH1 R132H protein immunoprecipitation. Y42 and Y391 tyrosine phosphorylation of IDH1 was detected by

Western blotting. C, Representative IHC staining images of IDH1 Y42 phosphorylation and IDH1 protein in human normal or primary tumor tissues in diverse human tumor tissue arrays. The error bars represent mean values \pm SD from three replicates of each sample (**: $0.01 < p < 0.001$); Data are mean \pm SD; p values were obtained by a two-tailed Student's test.