Supplement

Somatic deletions of genes regulating MSH2 protein stability cause DNA mismatch repair deficiency and drug resistance in human leukemia cells.

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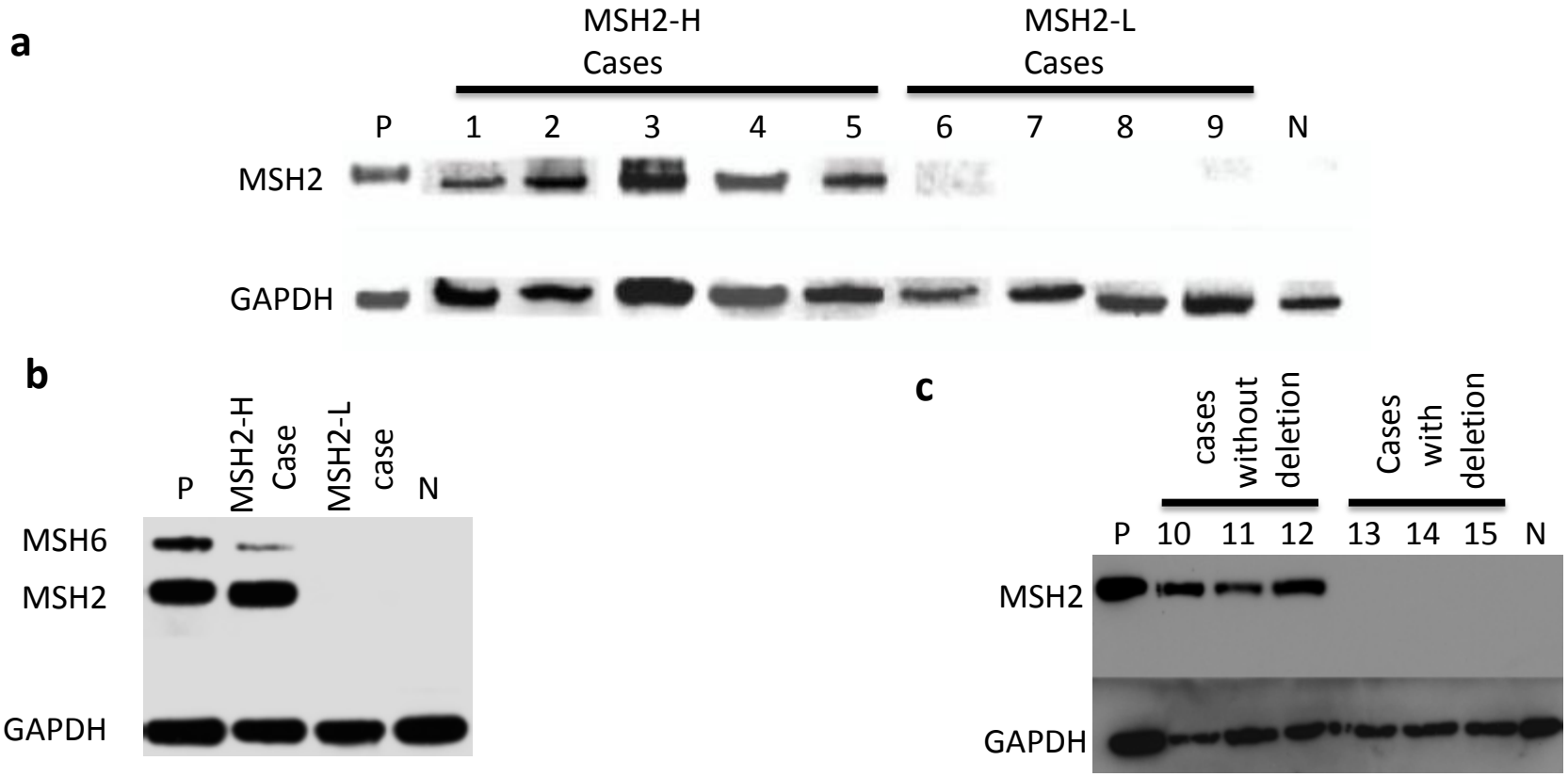
Supplement

Supplementary Figures and Tables

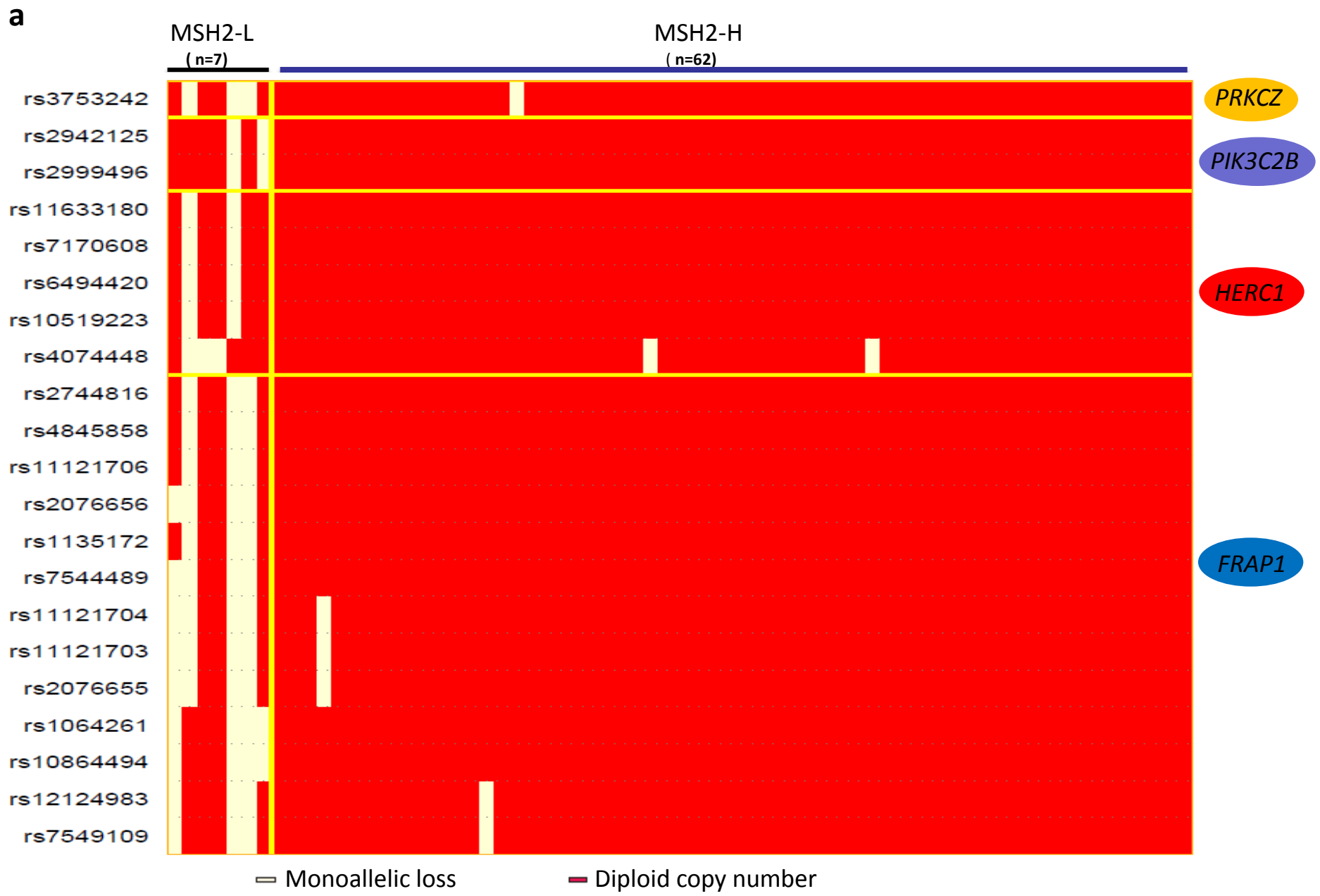
Supplementary Methods and Procedures



P= MSH2, MSH6 positive cell line (697 cell line)

N= MSH2, MSH6 negative cell line (NALM-6 cell line)

Supplementary Fig. 1. Representative western blots of MSH2-L and MSH2-H Patients. MSH2 protein levels were determined by western blot in primary human leukemia cells. (**a**) Patients from the discovery cohort with low and high MSH2 protein levels are shown with GAPDH as loading control. NALM-6 and 697 human leukemia cell lines were respectively used as MSH2 negative and MSH2 positive controls. (**b**) MSH6 protein levels were also determined by western blot in MSH2-H patients and MSH2-L patients documenting low MSH6 when MSH2 is low (representative patients from the discovery cohort are shown). (**c**) Representative western blots of leukemia cells with deletions and matched cases without deletions from the validation cohort. NALM-6 and 697 human leukemia cell lines were respectively used as MSH2 negative and MSH2 positive controls.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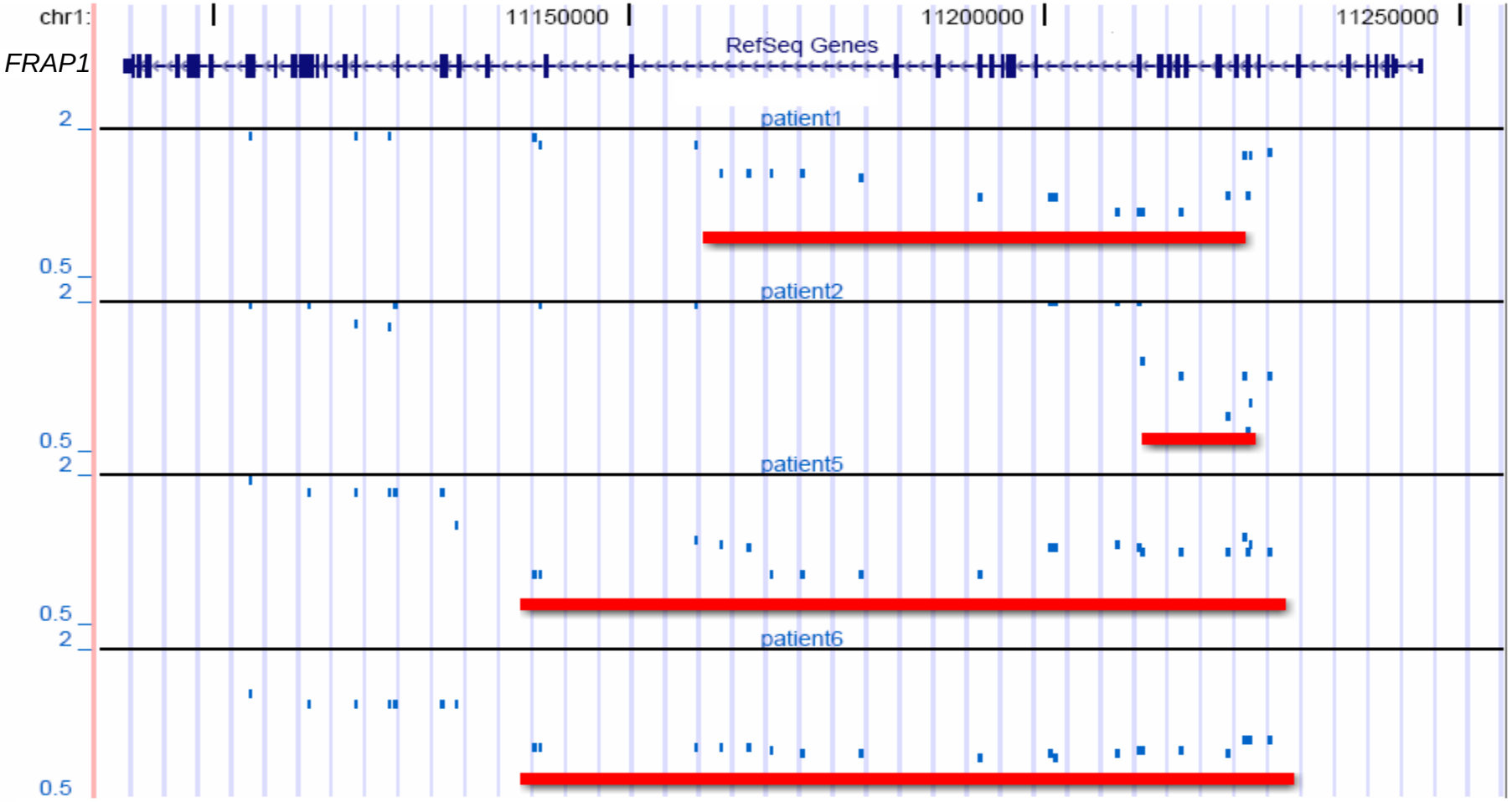
(n=7)
MSH2-H
(n=62)
rs3753242
rs2942125
rs2999496
rs11633180
rs7170608
rs6494420
rs10519223
rs4074448
rs2744816
rs4845858
rs11121706
rs2076656
rs1135172
rs7544489
rs11121704
rs11121703
rs2076655
rs1064261
rs10864494
rs12124983
rs7549109
PRKCZ
PIK3C2B
HERC1
FRAP1
Monoallelic loss
Diploid copy number

b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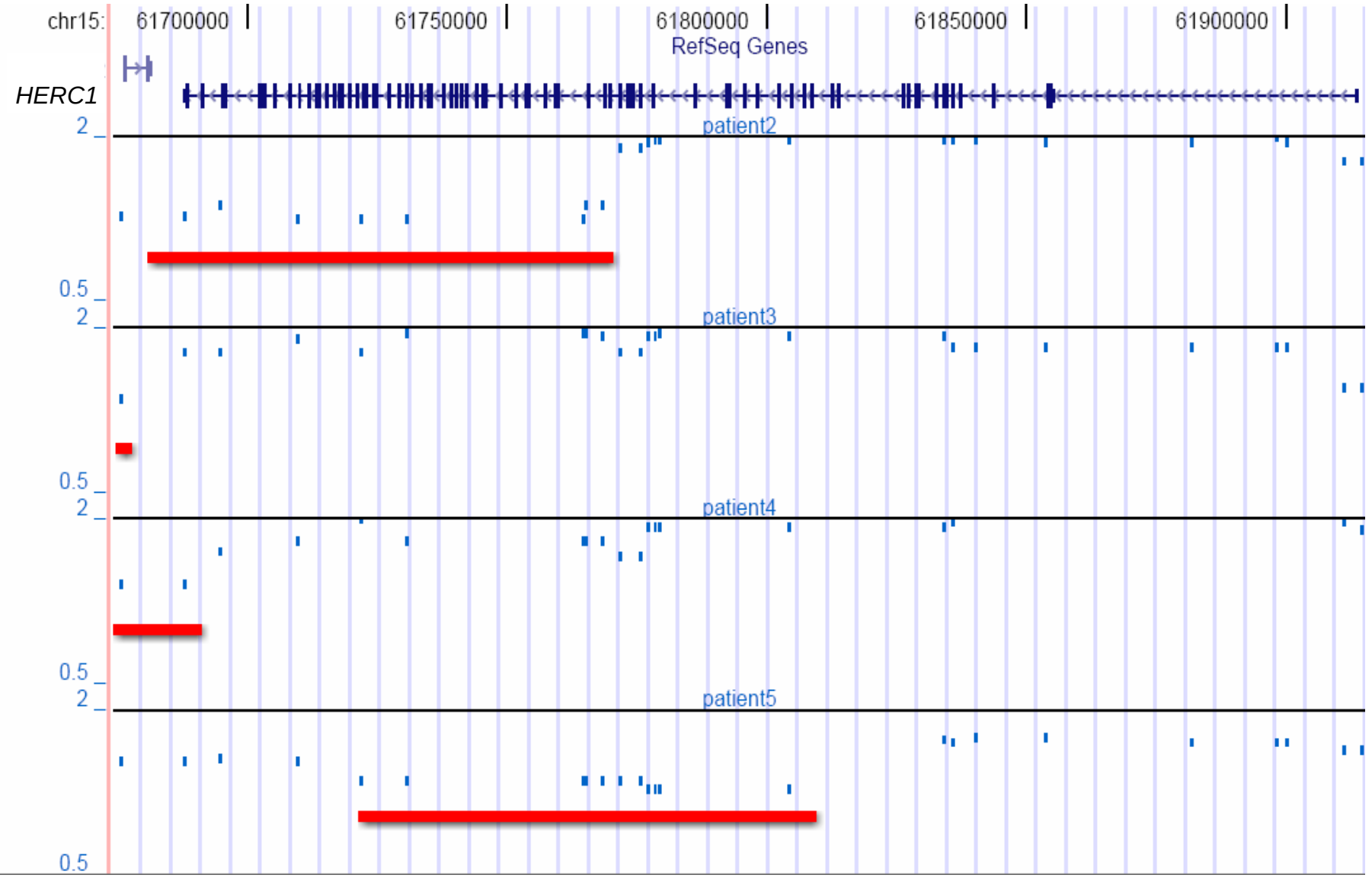
patient2
0.5
2
patient5
0.5
2
patient6
0.5

c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61900000
RefSeq Genes
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patient2
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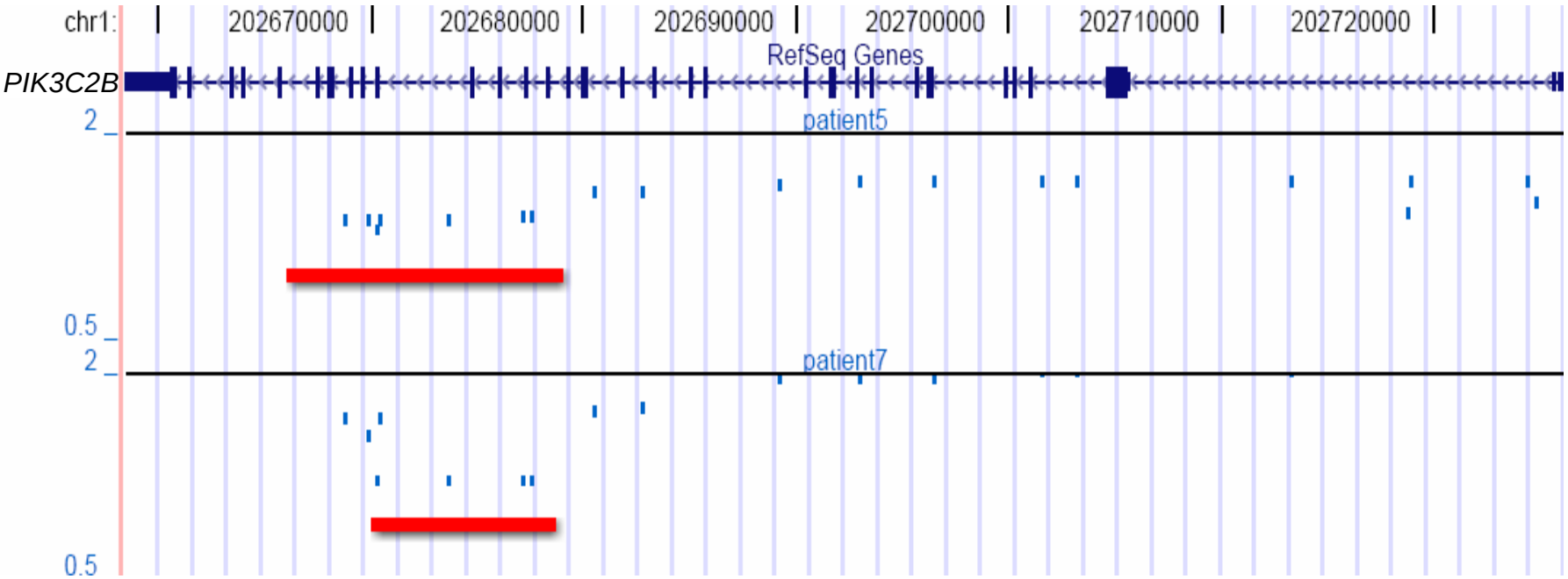
2
patient3
0.5
2
patient4
0.5
2
patient5
0.5

d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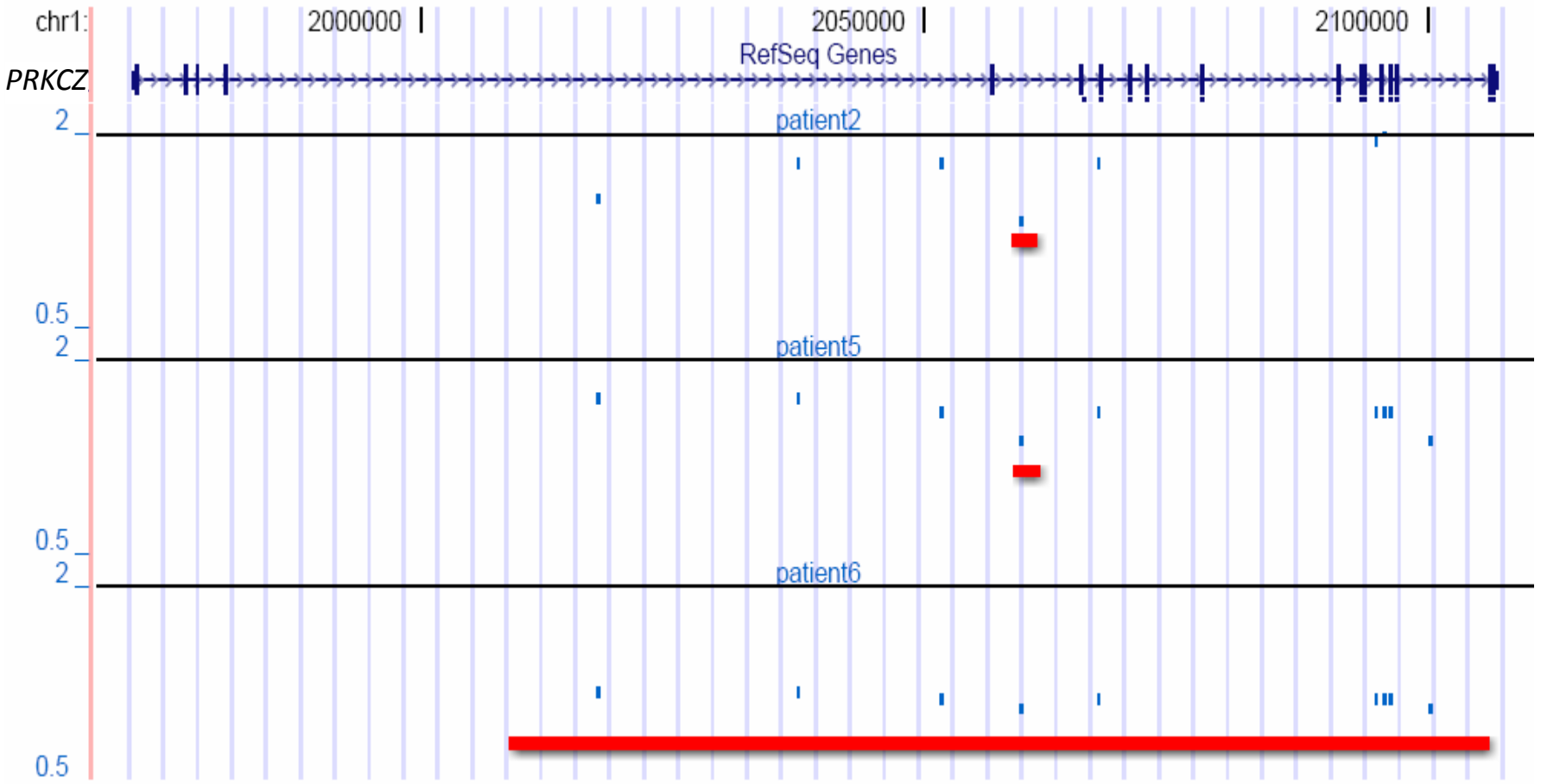
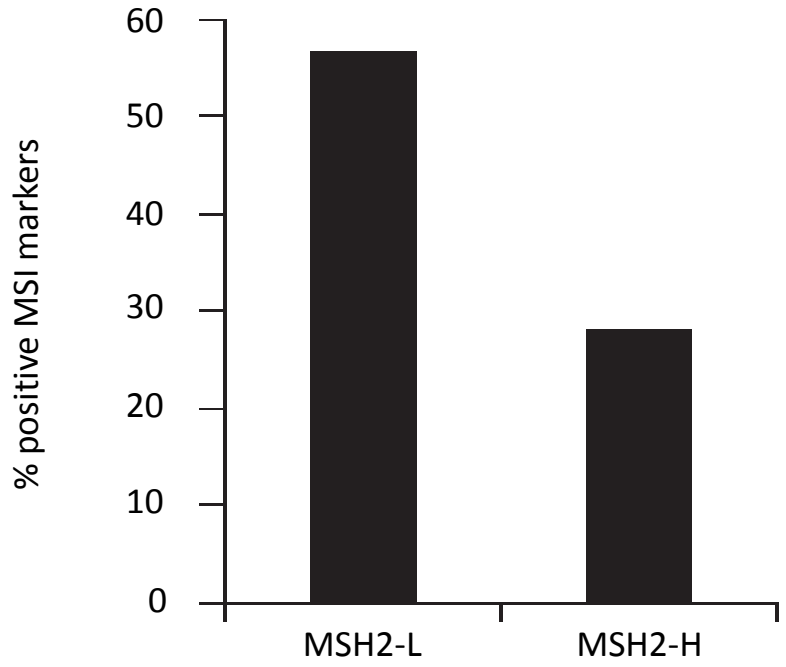
202710000
202720000
RefSeq Genes
PIK3C2B
2
patient5
0.5
2
patient7
0.5

e

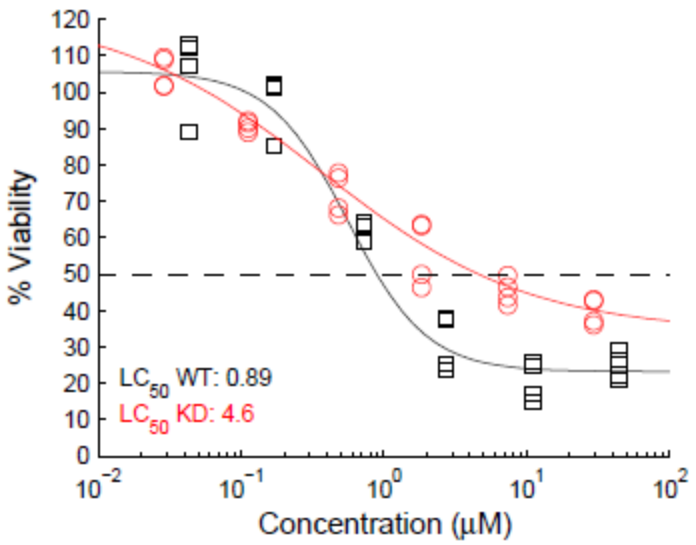


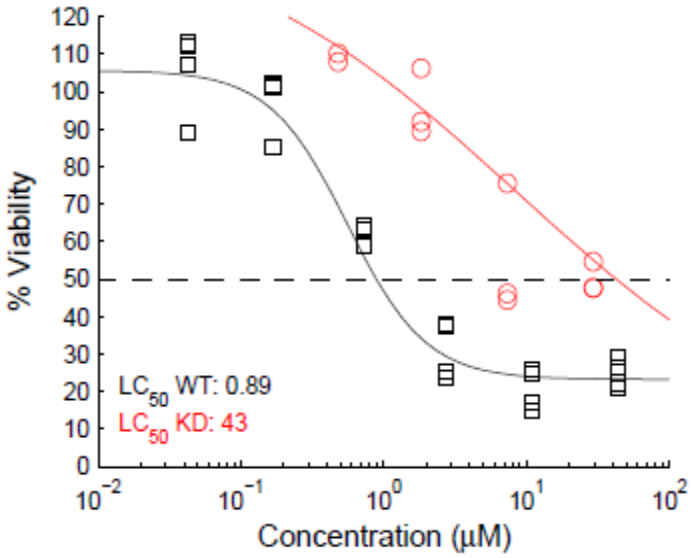
Supplementary Fig. 2. Gene copy analysis and location of deletions. (**a**) Gene copy number analysis of genes in the Pathway controlling MSH2 degradation depicting analysis of primary human leukemia cells with mono-allelic loss in yellow and diploid copy number in red. SNP array data were analyzed using dChip**.** (**b-d**) Location of deletions based on the SNP array data. Each light blue box is a SNP interrogated on the affy array and the horizontal black line represents two copies (labeled "2"). A copy number of < 1.4 was considered a deletion, and subsequently verified by RT-PCR.



Supplementary Fig. 3. Microsatellite instability (MSI) in primary ALL cells. By using the quasi-monomorphic consensus DNA markers NR24, BAT25, BAT26, NR21 and NR22, MSH2-L leukemia cells showed more positive MSI markers than the MSH2-H cells.

a


% Viability
Concentration (µM)
LC50 WT: 0.89
LC50 KD: 4.6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Concentration (µM)
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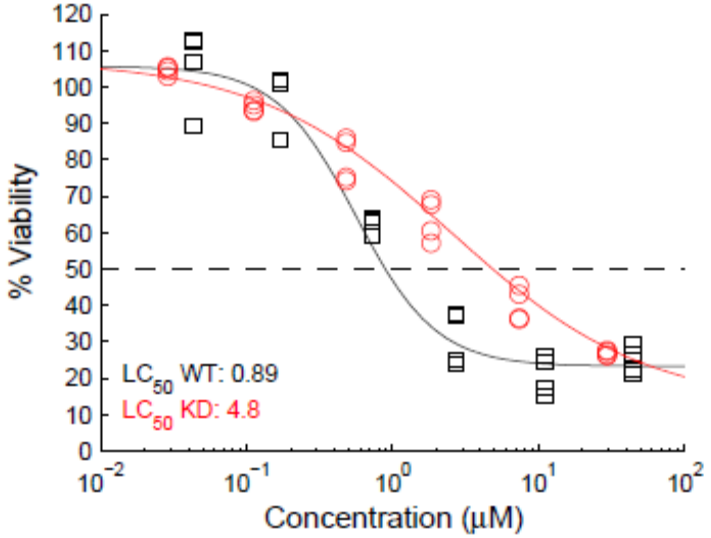
LC50 KD: 43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Concentration (µM)
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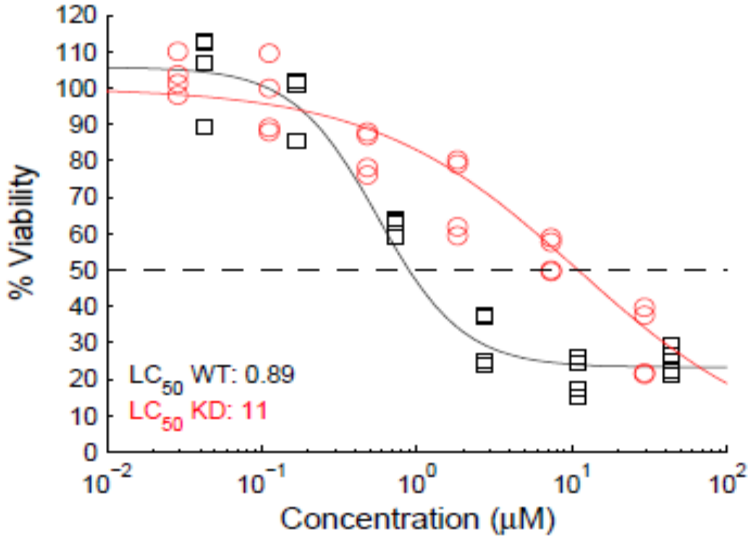
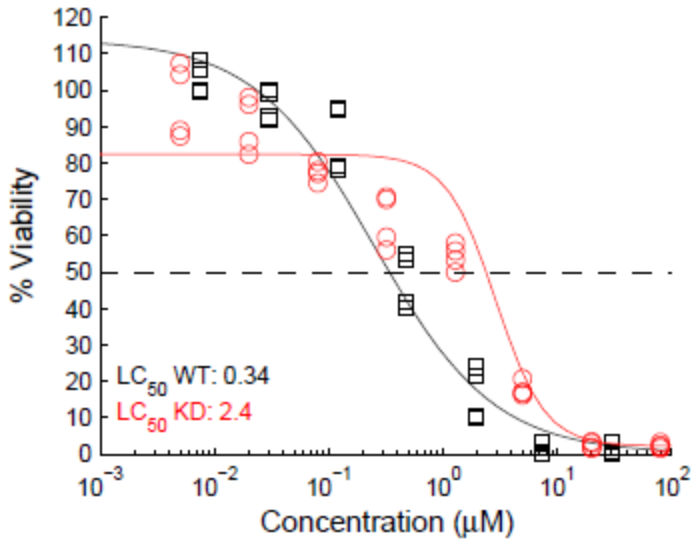
LC50 KD: 4.8


% Viability
Concentration (µM)
LC50 WT: 0.89
LC50 KD: 11

b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Concentration (µM)
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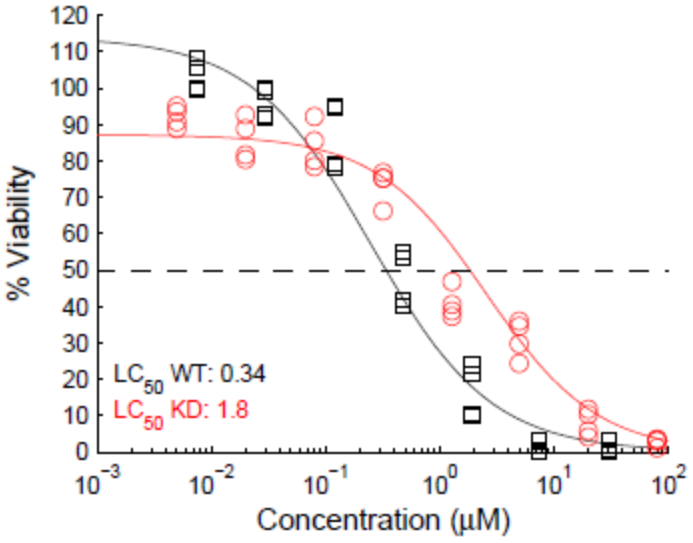
LC50 KD: 2.4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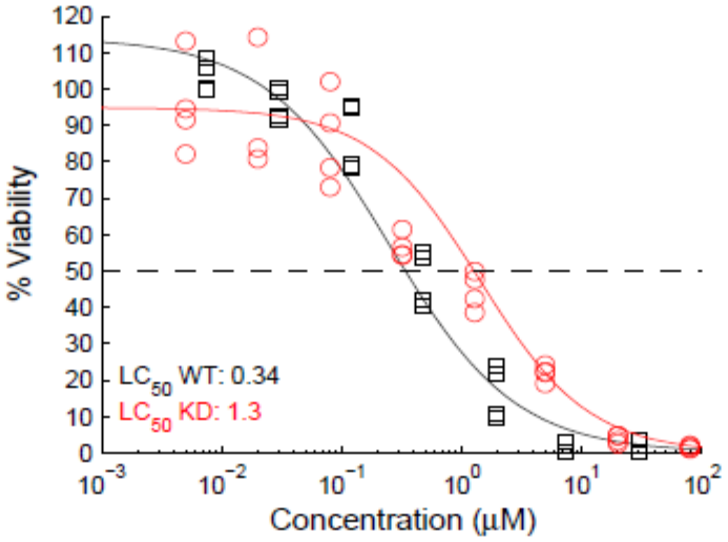
Concentration (µM)
LC50 WT: 0.34
LC50 KD: 1.8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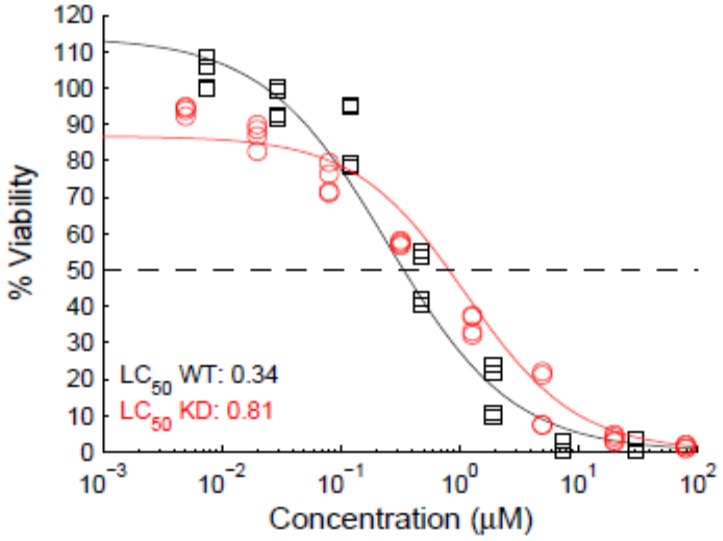
Concentration (µM)
LC50 WT: 0.34
LC50 KD: 1.3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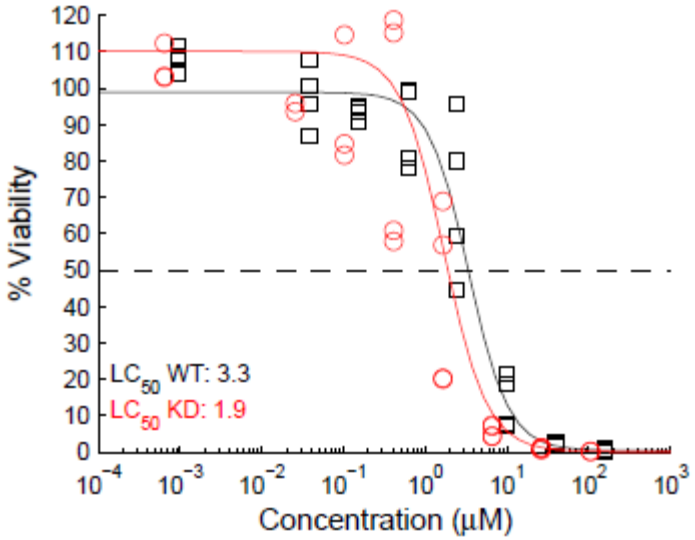
Concentration (µM)
LC50 WT: 0.34
LC50 KD: 0.81

c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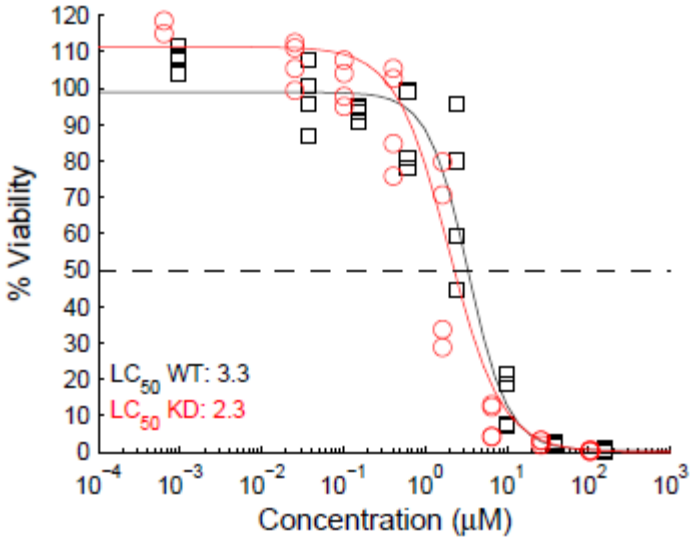
LC50 KD: 1.9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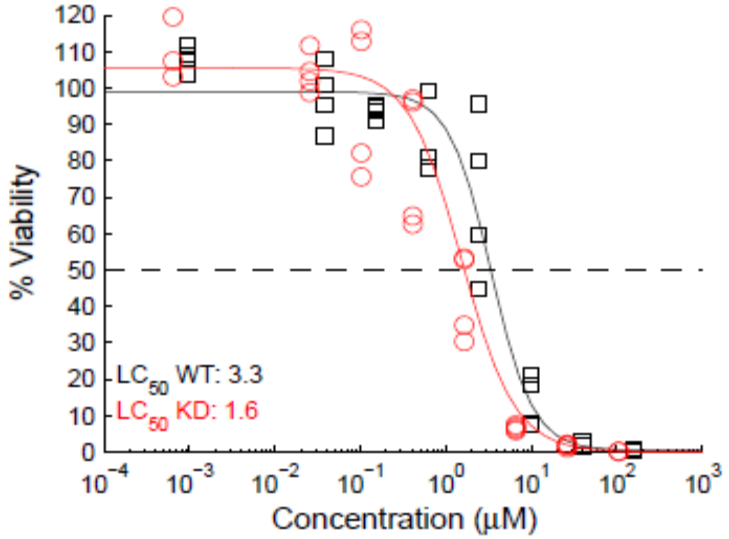
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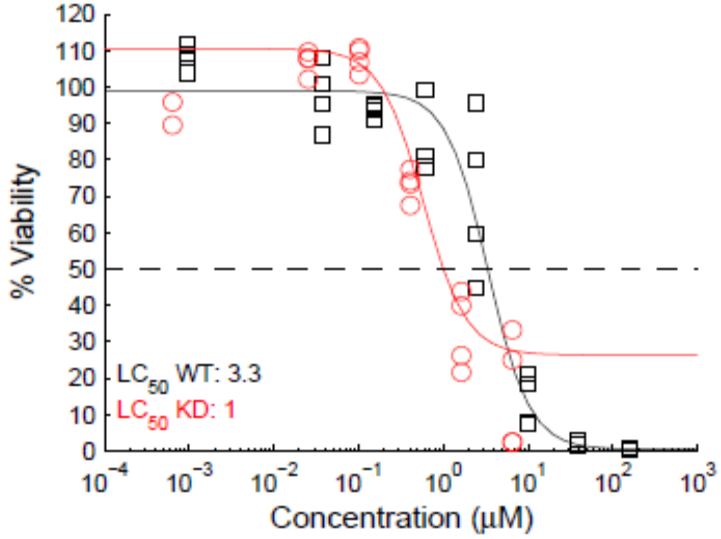
Concentration (µM)
LC50 WT: 3.3
LC50 KD: 1.6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Concentration (µM)
LC50 WT: 3.3
LC50 KD: 1

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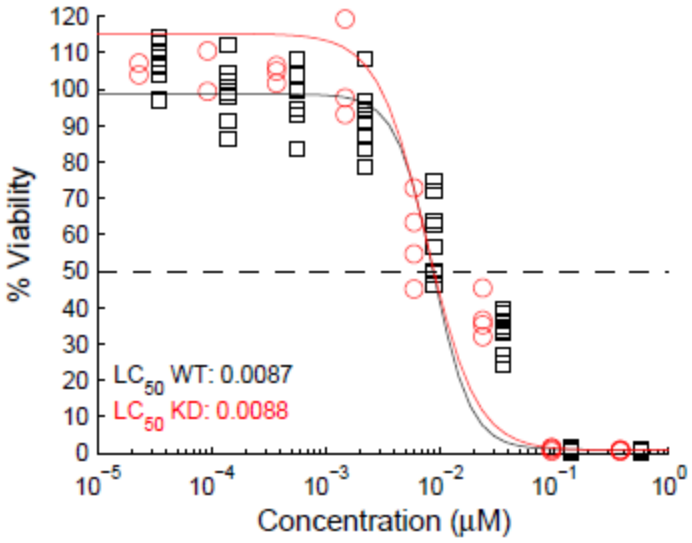
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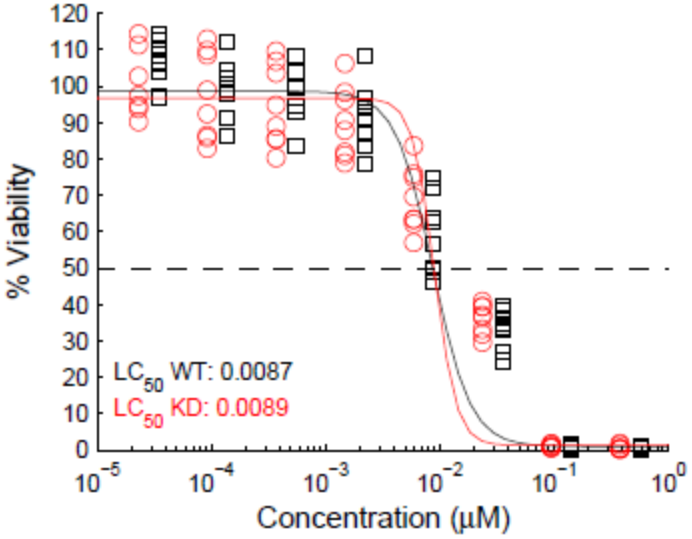
LC50 KD: 0.0088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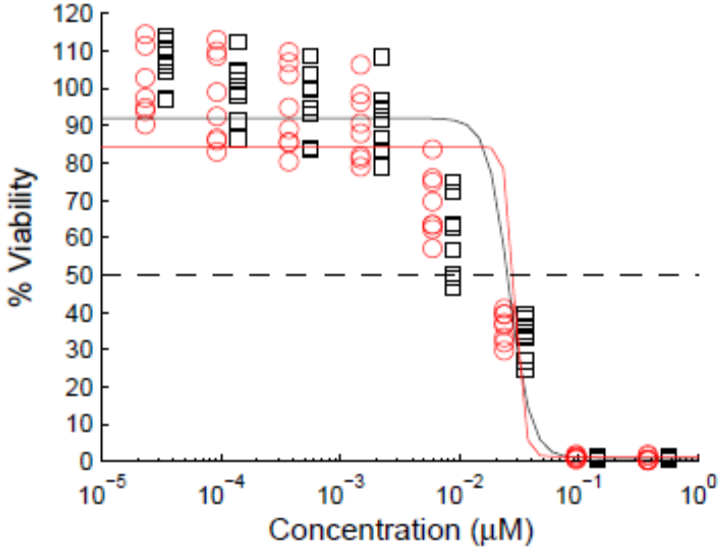
LC50 KD: 0.0089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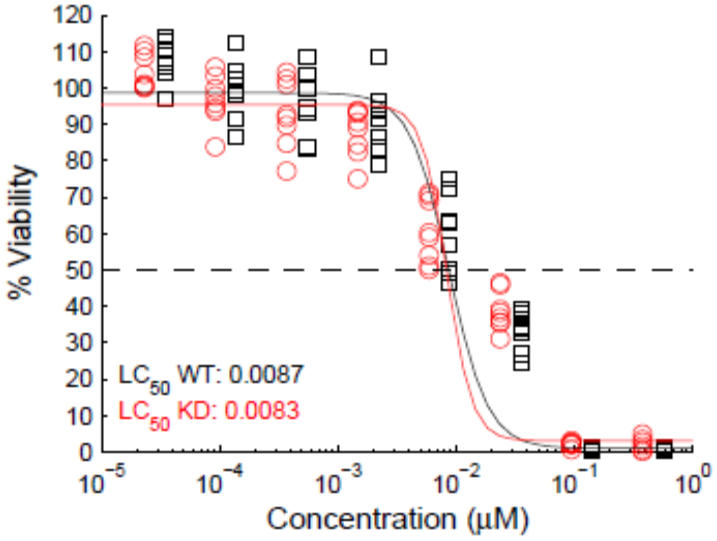
Concentration (µM)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Concentration (µM)
LC50 WT: 0.0087
LC50 KD: 0.0083

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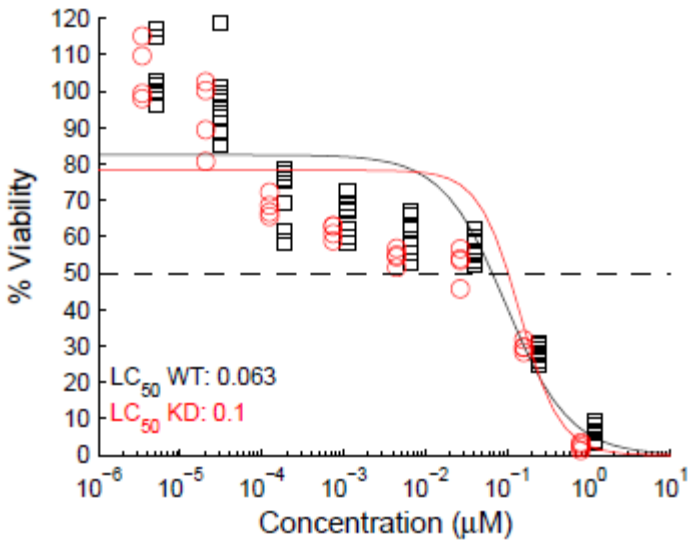
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Concentration (μM)
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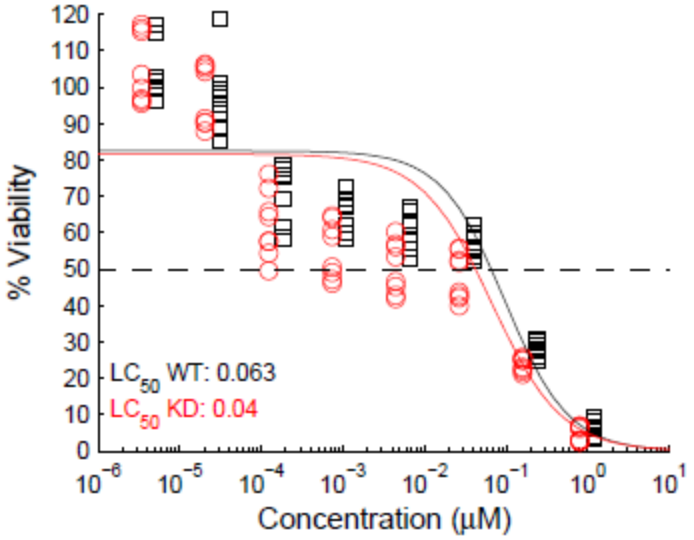
LC50 KD: 0.1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Concentration (μM)
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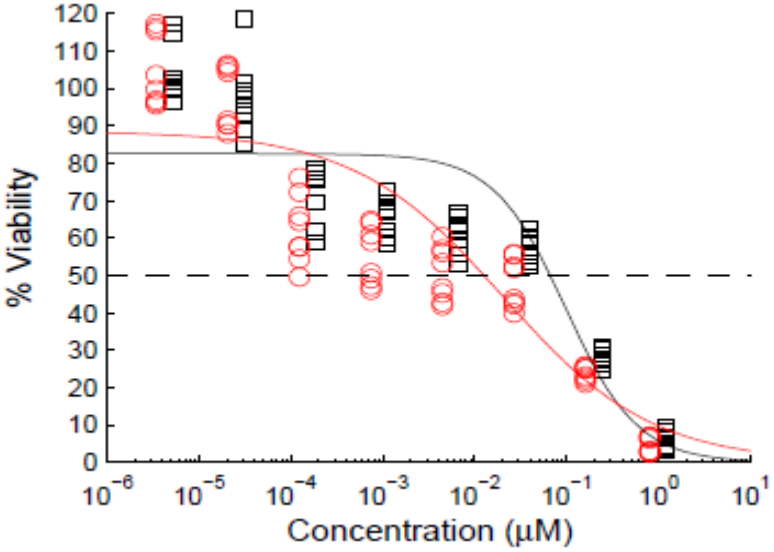
LC50 KD: 0.04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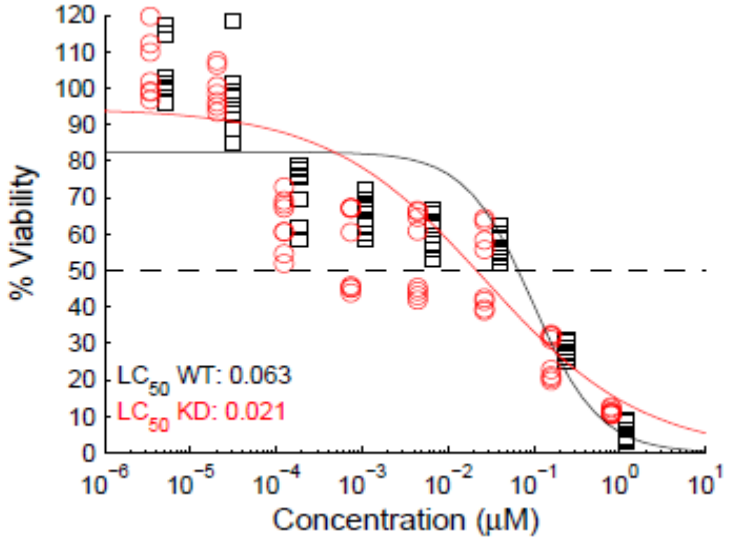
Concentration (μM)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Concentration (μM)
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LC50 KD: 0.021

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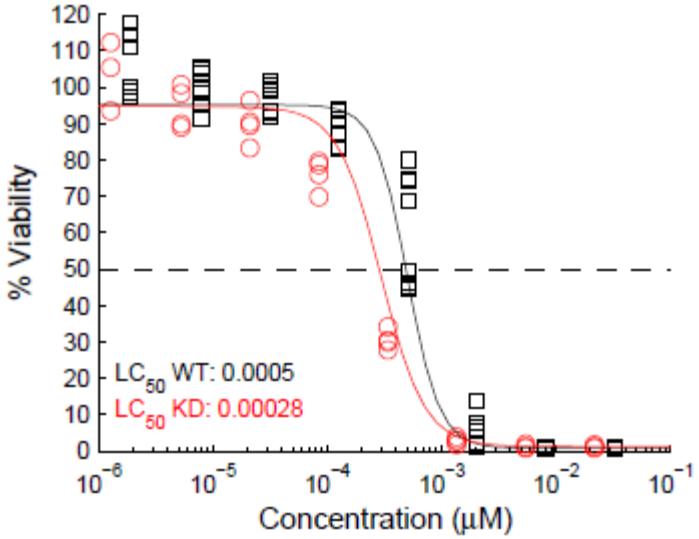
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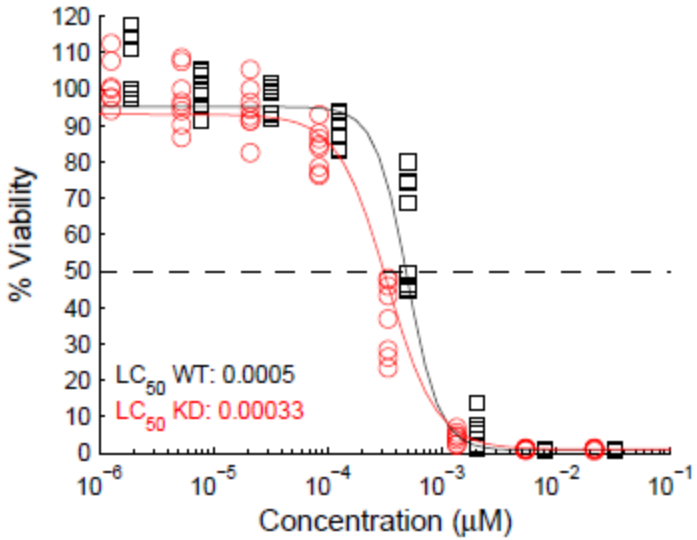
LC50 KD: 0.00028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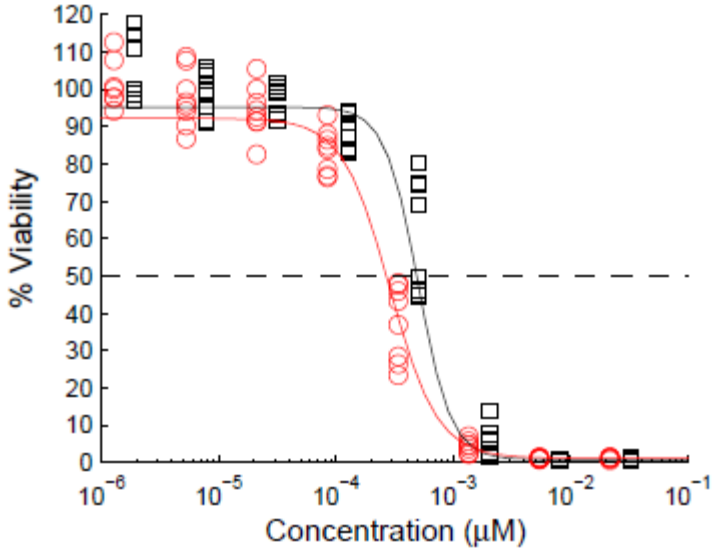
LC50 KD: 0.00033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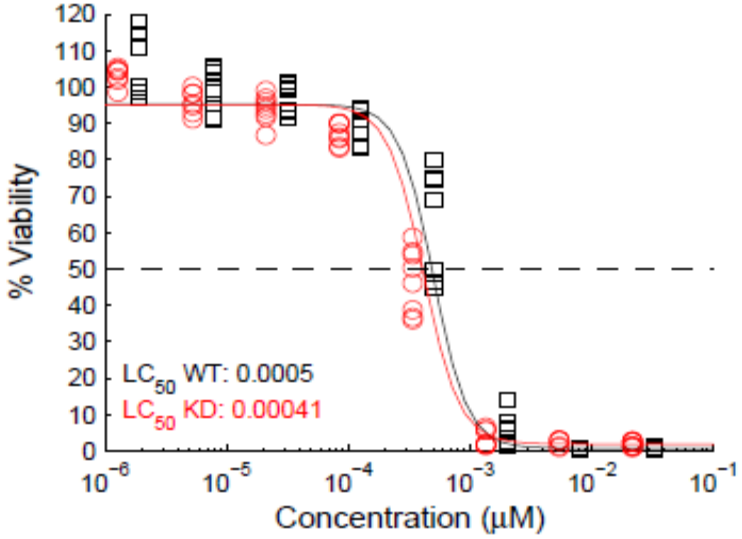
Concentration (µM)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LC50 KD: 0.00041

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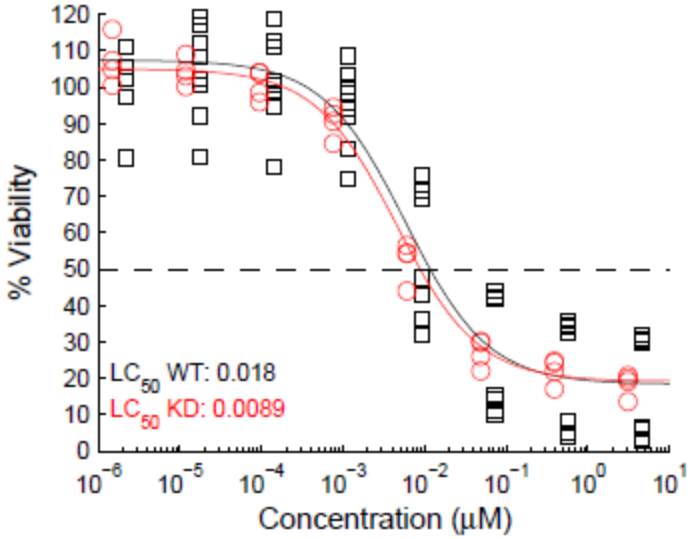
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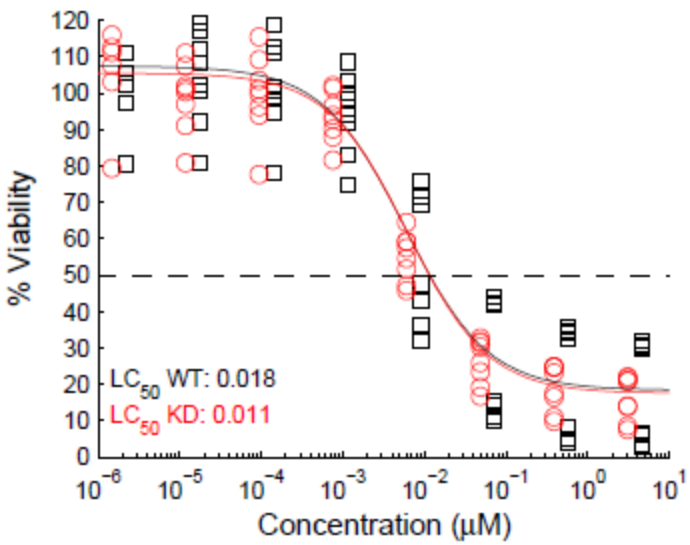
Concentration (μM)
LC50 WT: 0.018
LC50 KD: 0.0089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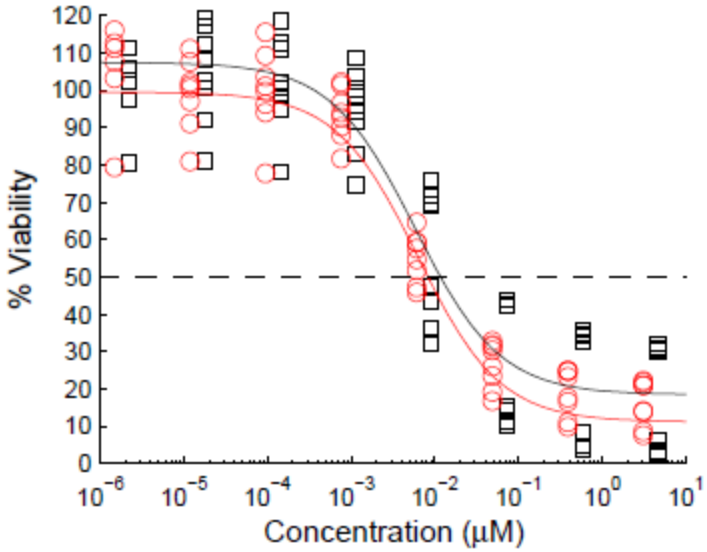
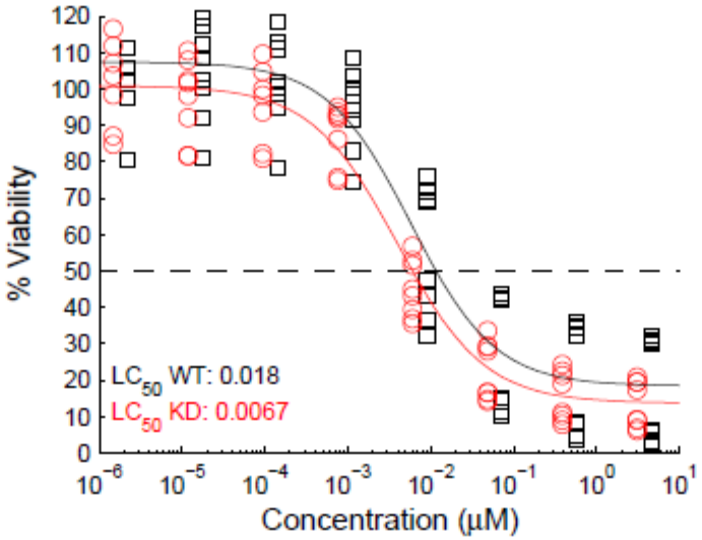
Concentration (μM)
LC50 WT: 0.018
LC50 KD: 0.011


% Viability
Concentration (μM)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Concentration (μM)
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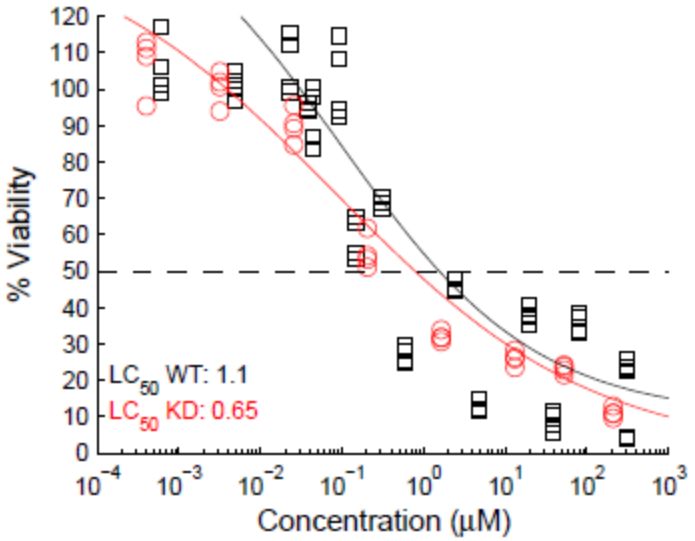
LC50 KD: 0.0067

h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Concentration (µM)
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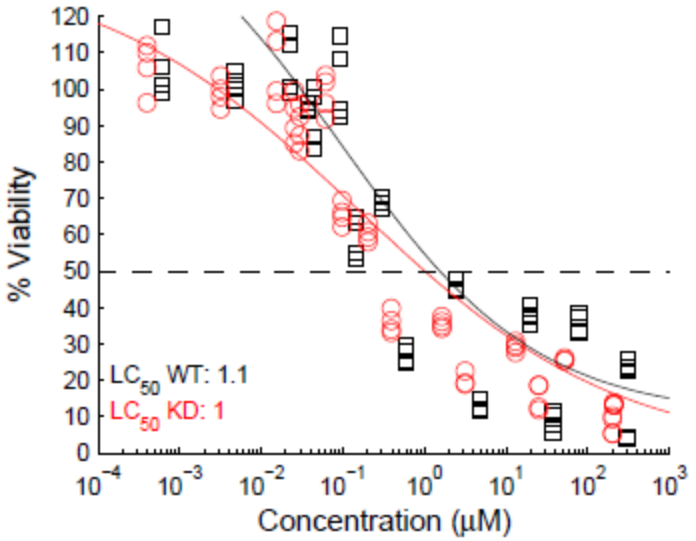
LC50 KD: 0.65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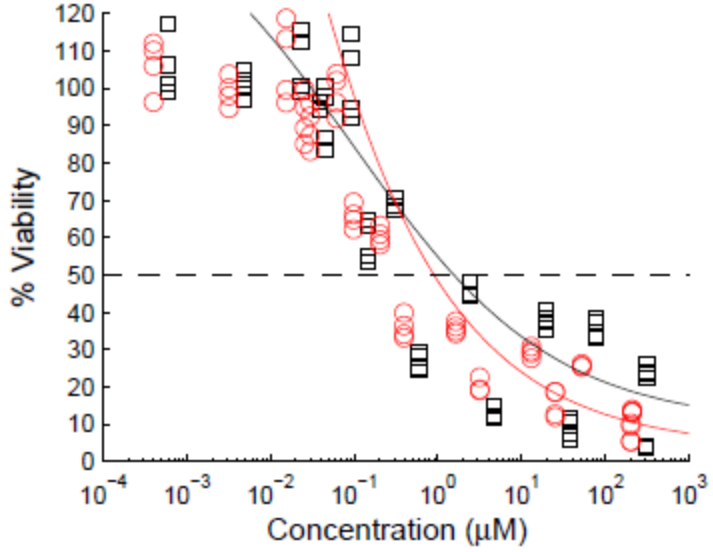
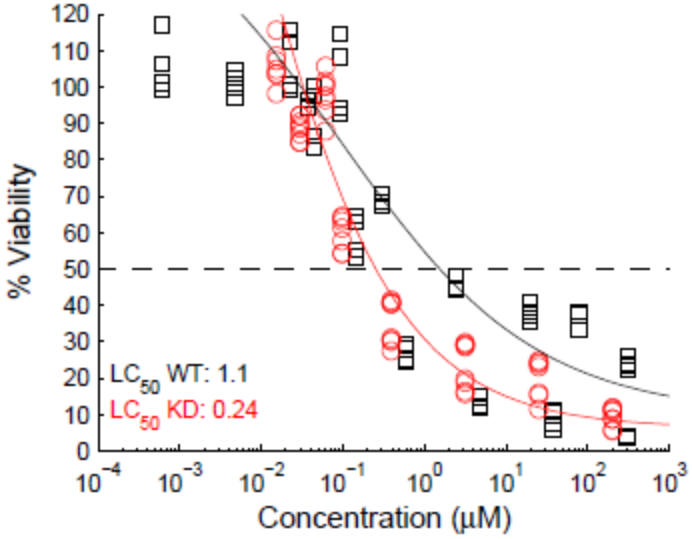
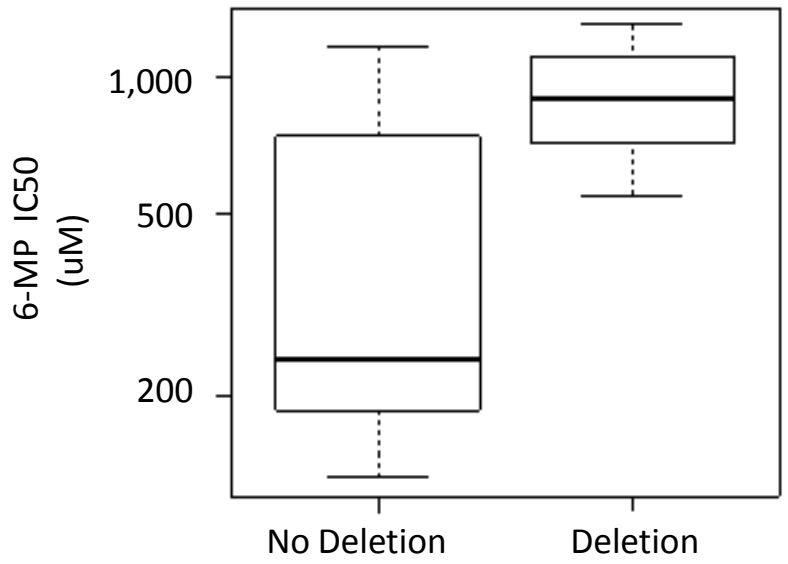
LC50 KD: 1

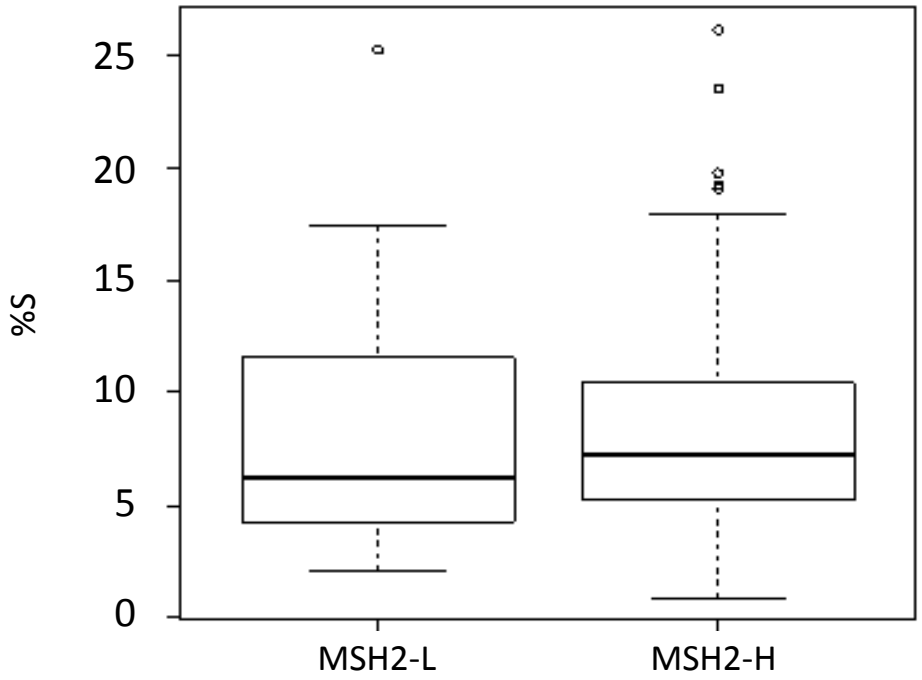

% Viability
Concentration (µM)


% Viability
Concentration (µM)
LC50 WT: 1.1
LC50 KD: 0.24

Supplementary Fig. 4. Effects of knockdowns on drug sensitivity. CCRF-CEM cells in which *FRAP1 (*top left*)*, *HERC1* (top right), *PIK3C2B* (bottom left) or *PRKCZ* (bottom right) were knocked-down and were treated for 72 hours in the presence of increasing concentrations of 6-MP (**a**), 6-TG (**b**), melphalan (**c**), daunorubicin (**d**), L-asparaginase (**e**), vincristine (**f**), dexamethasone (**g**) and prednisolone (**h**). Cell viability was determined by the MTT assay and expressed relative to untreated control cell cultures.

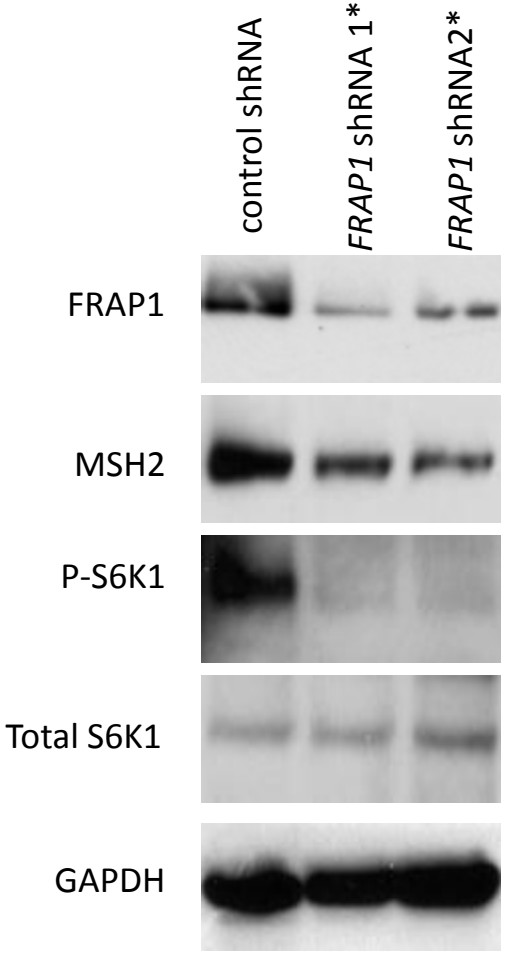


Supplementary Fig. 5. Sensitivity of primary ALL cells to mercaptopurine (6-MP). Primary ALL cells isolated from patients without (8 patients) or with (4 patients) deletions of *PIK3C2B*, *HERC1*, *FRAP1* or *PRKCZ* were tested for sensitivity to 6-MP using the MTT assay . The eight patients without deletions were matched to the cases with deletions based on ALL lineage, molecular subtype and patient race. The horizontal lines in each boxplot indicate the mean IC50 values for each group and the bottom and top of the box depict the 25^{th} and 75^{th} percentile.



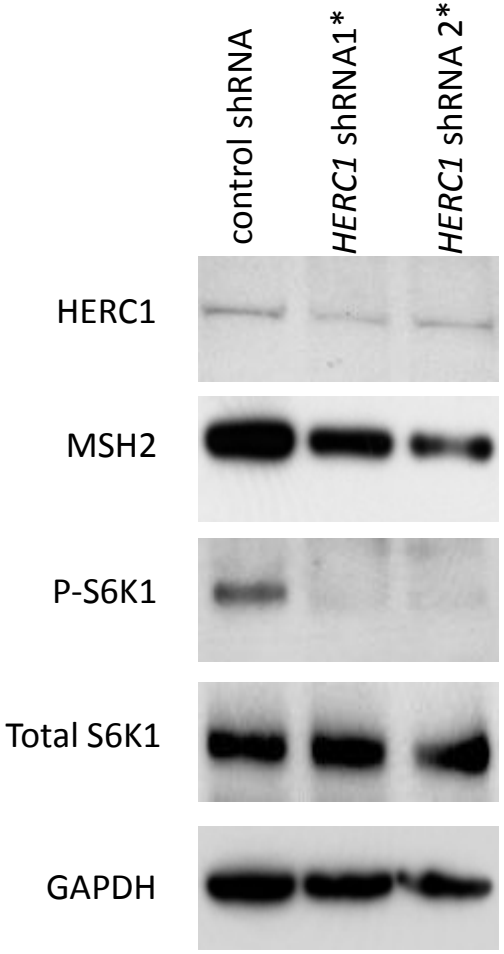
Supplementary Fig. 6. Percentage of cells in S phase at diagnosis in the MSH2-L and MSH2-H patients (69 patients in the discovery cohort with 62 MSH2-H and 7 MSH2-L and 21 patients in the validation cohort with 15 MSH2-H and 6 MSH2-L). Wilcoxon rank-sum test indicated no significant difference between the two groups. (*P = 0.79*)

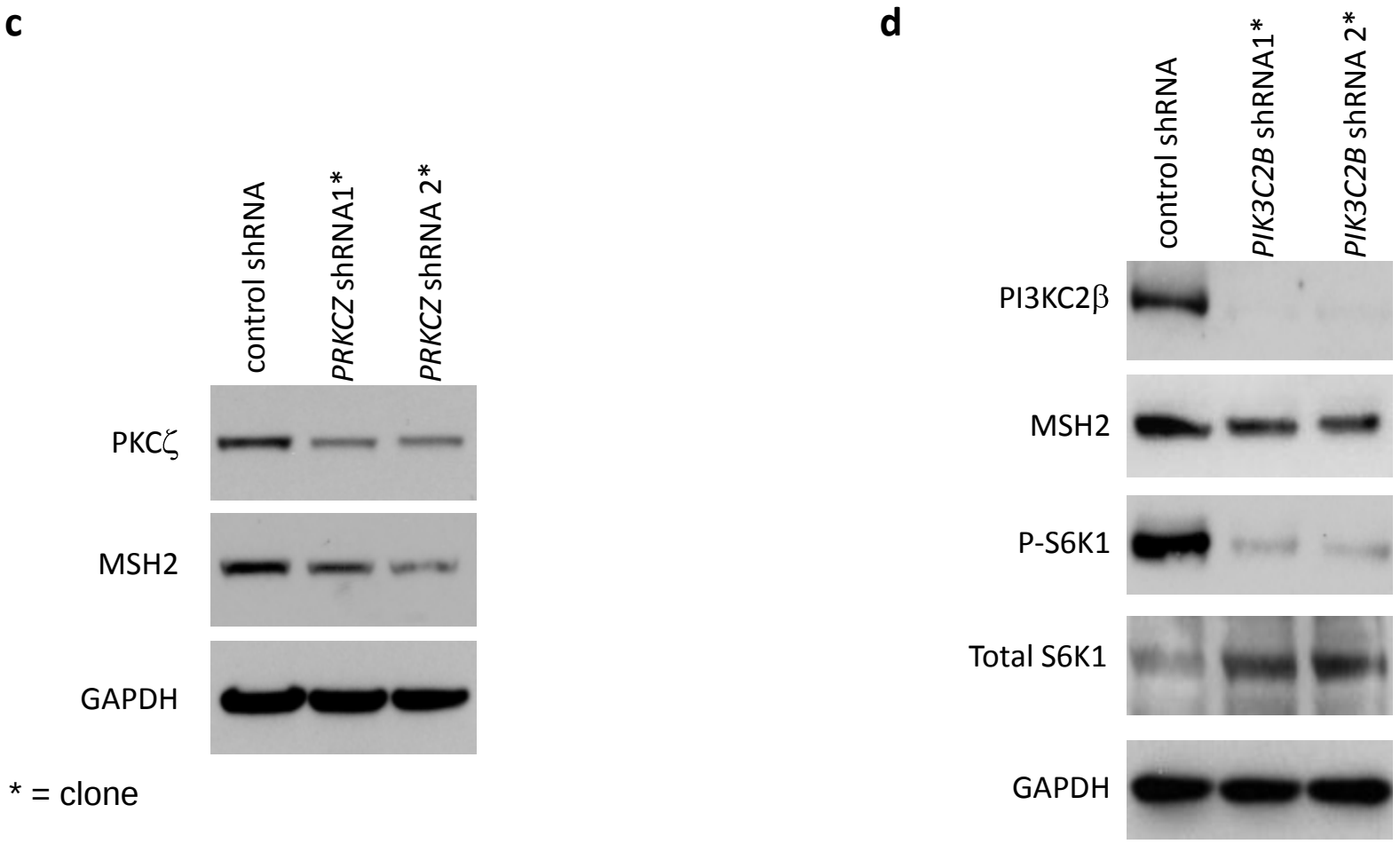
a


control shRNA
FRAP1 shRNA 1*
FRAP1 shRNA2*
FRAP1
MSH2
P-S6K1
Total S6K1
GAPDH

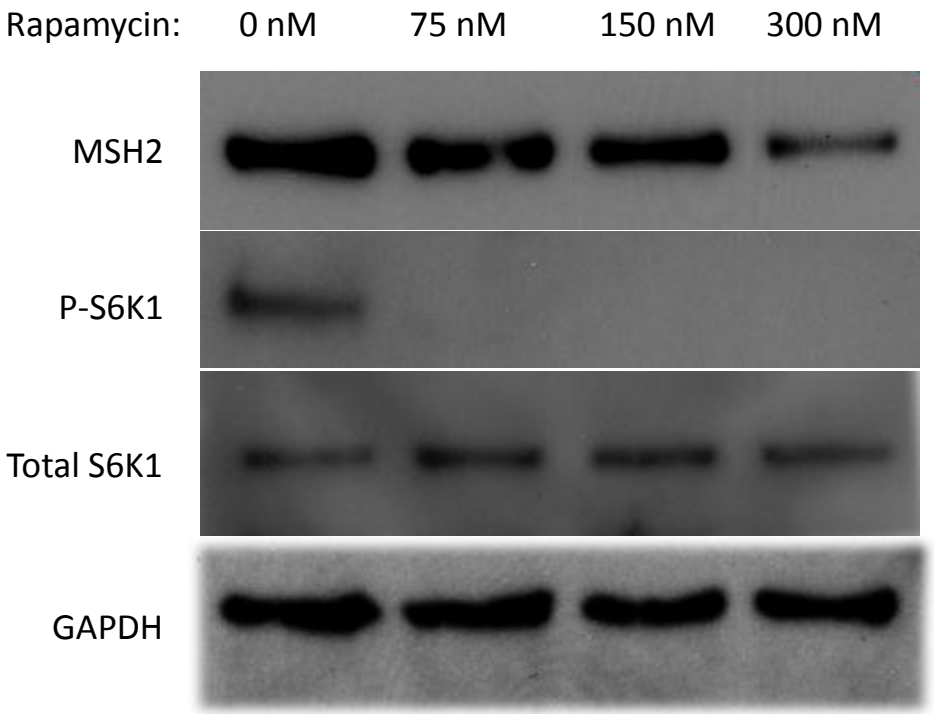
* = clone

b

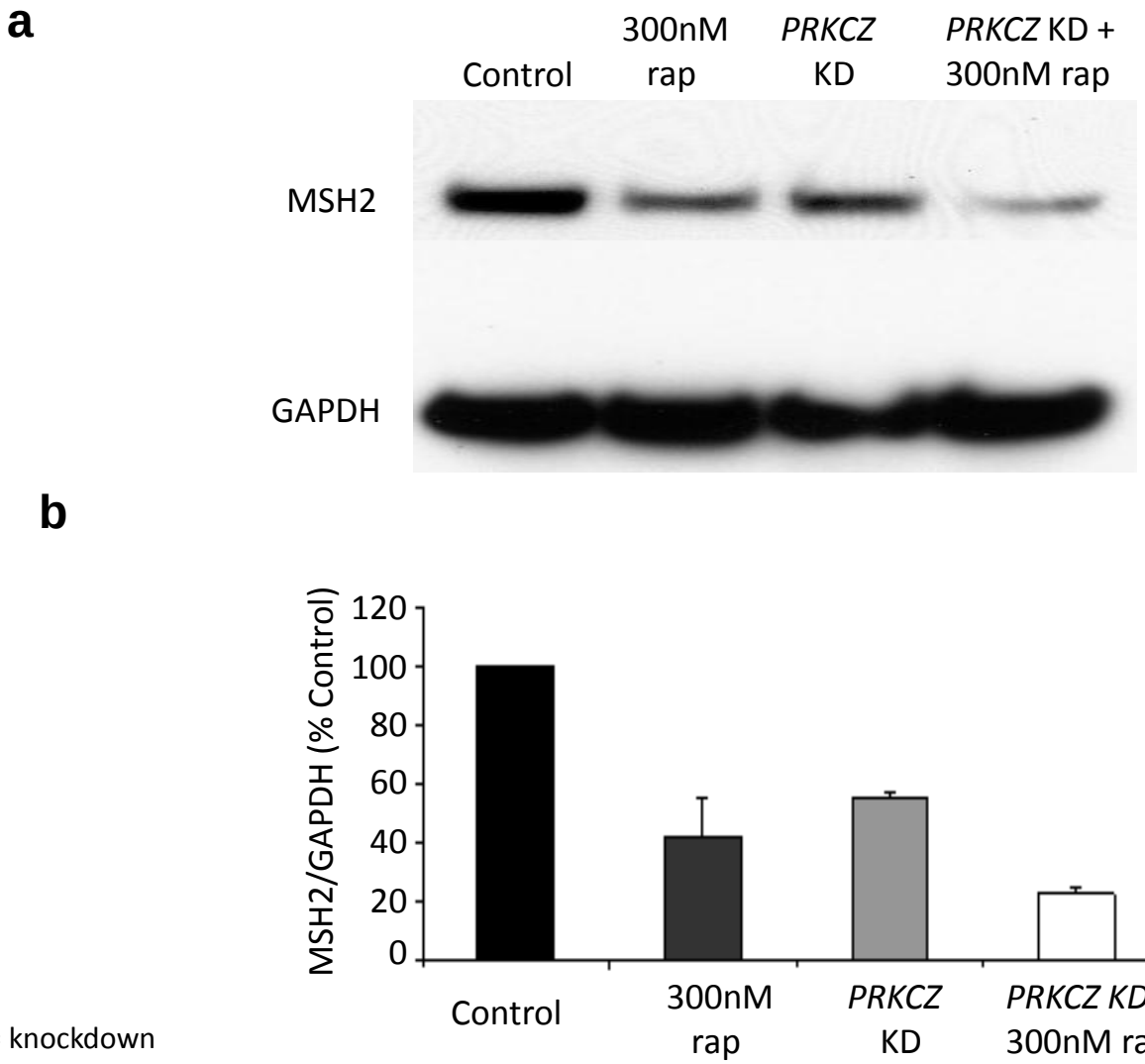

control shRNA
HERC1 shRNA1*
HERC1 shRNA 2*
HERC1
MSH2
P-S6K1
Total S6K1
GAPDH



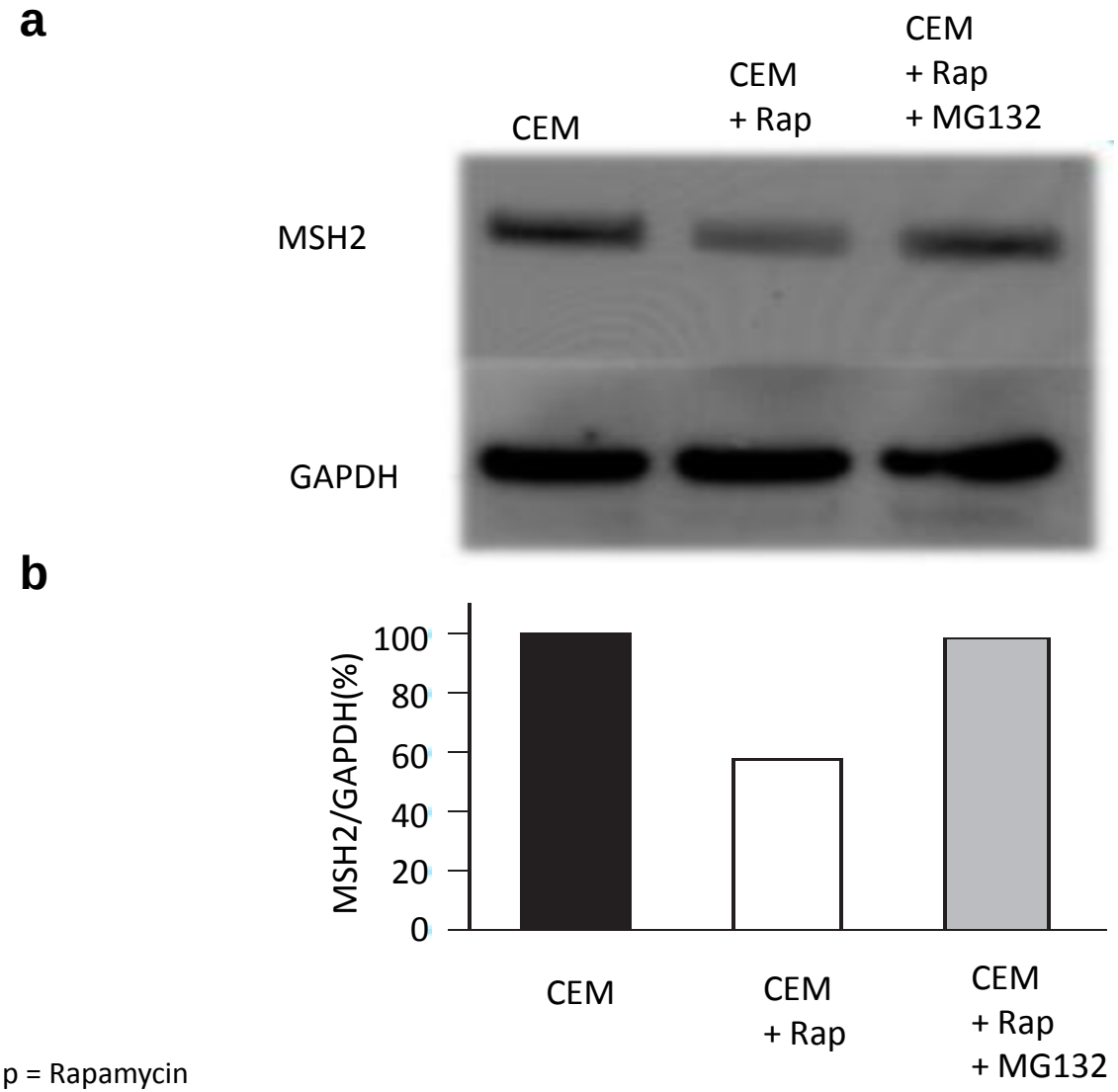
Supplementary Fig. 7. Knockdown of *FRAP1*, *HERC1*, *PIK3C2B* and *PRKCZ* by two different shRNA clones for every gene. *FRAP1* (***a***), *HERC1* (***b***), *PRKCZ* (**c**) and *PIK3C2B* (**d**) genes were each knocked down by two different shRNAs (sequences of the clones are provided in supplementary Methods). The protein signals were determined by western blot.



Supplementary Fig. 8. Rapamycin treatment of human B cell precursor leukemia. Pre-B ALL human cell line (697) were treated with increasing concentrations of rapamycin. MSH2 and GAPDH protein levels were determined by western blot. Phosphorylation of P70S6 kinase (Thr389) was measured by a phospho-specific antibody.



Supplementary Fig. 9. Rapamycin treatment after knockdown of *PRKCZ* gene (equivalent to double knockdown). Human leukemia cells (CCRF-CEM) were treated with 300nM of rapamycin, with or without transduction of shRNA against *PRKCZ*. (**a**) MSH2 and GAPDH protein levels were determined by western blot. (**b**) The level of MSH2 protein level was quantified and normalized to GAPDH.



Supplementary Fig. 10. Enhanced degradation of MSH2 after FRAP1 inhibition is blocked by proteasome inhibitor MG132. Human leukemia cells (CEM) were treated or not with 300nM of rapamycin, with or without 10nm of MG132. MSH2 and GAPDH protein levels were determined by western blot and the MSH2/GAPDH ratio is depicted in the bottom panel).

Table 1. Confirmation of gene deletions in ALL cells by RT-PCR.

	1	2	4	5	6	7	Control(Not deleted)
FRAP1/mTOR	**0.40**	**0.68**	0.72	**0.37**	**0.30**	0.84	1.32
HERC1	0.72	**0.50**	**0.62**	**0.43**	0.89	1.79	1.87
PIK3C2B	0.71	1.17	1.01	**0.59**	0.94	**0.65**	1.11
PRKCZ	0.72	1.08	1.13	**0.64**	**0.69**	1.13	1.08

Bold represents deletions. Cutoffs of 0.7 and 0.3 were used to identify hemizygous and homozygous deletions respectively.

Table 2. Clinical features of patients assayed for MSI Genotyping.

Patient	MSH2 status	Gender	Race	Age	Risk	Lineage
1	MSH2-L	Male	Black	8.4	Standard	T
2	MSH2-L	Male	Black	7.8	High	T
3	MSH2-H	Male	Black	15.2	Standard	T
4	MSH2-L	Male	White	5.7	Standard	B
5	MSH2-H	Male	White	6.2	Low	B
6	MSH2-L	Male	White	5.4	Low	B
7	MSH2-H	Male	White	4.2	Standard	B
8	MSH2-L	Male	Black	11.1	Standard	B
9	MSH2-H	Male	Black	11.1	Standard	B
10	MSH2-L	Female	Black	11.6	Low	B
11	MSH2-H	Female	Black	12.9	Low	B

Table 3. Microsatellite instability results.

Patient	MSH2 status	Bat25	Bat26	NR21	NR22	NR24	positive/total loci
1	MSH2-L	no	no	yes	yes	no	2/5
2	MSH2-L	no	no	yes	no	yes	2/5
3	MSH2-H	no	no	yes	yes	no	2/5
4	MSH2-L	yes	yes	yes	yes	yes	5/5
5	MSH2-H	yes	no	no	no	yes	2/5
6	MSH2-L	yes	no	yes	no	no	2/5
7	MSH2-H	no	no	no	no	no	0/5
8	MSH2-L	yes	yes	yes	yes	yes	5/5
9	MSH2-H	yes	no	no	no	no	1/5
10	MSH2-L	yes	no	no	no	no	1/5
11	MSH2-H	yes	no	no	yes	no	2/5

Table 4. Sensitivity of human leukemia cells (CEM) after shRNA knockdown of each gene listed. Control = CEM with control vector (SHC002V).

The drug concentration required for 50% cytotoxicity (IC50) was measured by the MTT assay.

Drug	Knockdown	IC_{50}	p-value	Percent of control
6-MP	Control	0.89		
	FRAP1	4.85	0.012	545
	HERC1	42.52	<0.00001	4777
	PIK3C2B	4.85	<0.00001	545
	PRKCZ	10.97	<0.00001	1233
6-TG	Control	0.34		
	FRAP1	2.39	<0.00001	703
	HERC1	1.84	<0.00001	541
	PIK3C2B	1.26	<0.00001	371
	PRKCZ	0.81	<0.00001	238
Melphalan	Control	3.35		
	FRAP1	2.39	<0.00001	56
	HERC1	1.84	<0.00001	68
	PIK3C2B	1.65	<0.00001	49
	PRKCZ	1.00	<0.00001	30

Table 5. Comparison of clinical and biological features in MSH2-L and MSH2-H cases of ALL in both cohorts.

group	MSH2	number	mean	max	median	range	Exact Wilcoxon rank test p value
AGE at Diagnosis	MSH2-H	81	6.4	18.0	4.9500	16.5	0.3408
	MSH2-L	16	7.2	16.4	6.2	13.8	
WBC at Diagnosis ($x\ 10^9/L$)	MSH2 -H	81	66.2	392.0	32.3	391.2	0.0046
	MSH2-L	16	30.8	309.0	6.05	306.8	

Clinical Features			MSH2		Exact Chi-square Tests
Loci	Clinical Features	Total Number of Patients in Each Groups	MSH2-L (%)	MSH2-H (%)	Two-sides P Values
SEX	Male	62 (63.9)	12 (19.4)	50 (80.6)	0.3999
	Female	34 (35.4)	4 (11.8)	30 (88.2)	
RACE	White	74 (76.3)	10 (13.5)	64 (86.5)	0.2015
	Black	18 (18.6)	5 (27.8)	13 (72.2)	
	Other	5 (5.2)	1 (20.0)	4 (80.0)	
ALL Lineage	B-lineage	78 (80.4)	13 (16.7)	65 (83.3)	1.0000
	T lineage	19 (19.6)	3 (15.8)	16 (84.2)	
B lineage ALL subtype	BCRABL	2 (2.6)	0 (0.0)	2 (100.0)	0.9768
	E2APBX, t(1;19)	5 (6.4)	1 (20.0)	4 (80.0)	
	TELAML1	23 (29.5)	3 (13.0)	20 (87.0)	
	Hyperdiploidy 50 or more	17 (21.8)	3 (17.6)	14 (82.4)	
	B others	31 (39.7)	6 (19.4)	25 (80.6)	

Table 6. Copy number changes (deletions) of *FRAP1*, *HERC1*, *PRKCZ* and *PIK3C2B* in patients with sporadic colon cancer.
In the colorectal dataset, the analysis of 104 cases and corresponding germline DNA using the Affy 250k STY SNP Ship showed that 14 of 104 cases (13.5%) had deletions of one or more of these four genes (4 *FRAP1* only, 7*HERC1* only, 1*FRAP1*+*PRKCZ*, 1 *FRAP1*+*PRKCZ*+*HERC1* and 0 *PIK3C2B*). Three were focal deletions and 11 were large chromosomal deletions. Analysis was performed on the publicy available dataset (http://research3.dfci.harvard.edu/cdk8colon/index.php?dir=Firestein%20et%20al/)

Genes	Chromosome	Patient with focal deletion	Patient with large deletion
HERC1	Chr15	3 cases: PRAWN_p_Sty26_Mapping 250K_Sty_D08_95216 SIDES_p_Sty16_Mapping 250K_Sty_D03_76242 WHELK_p_Sty27_Mapping 250K_Sty_E12_97010	5 cases: CHAMS_p_Sty31_(CO124089)_Mapping 250K_Sty_G02_112344 BARRE_p_Sty10_Mapping 250K_Sty_H06_56526 SIDES_p_Sty16_Mapping 250K_Sty_B09_76334 WHELK_p_Sty27_Mapping 250K_Sty_E10_96978 WHELK_p_Sty27_Mapping 250K_Sty_H12_97016
FRAP1	Chr1	0 case	6 cases: CHAMS_p_Sty31_(CO124089)_Mapping 250K_Sty_A06_112396 CHAMS_p_Sty31_(CO124089)_Mapping 250K_Sty_E07_112420 BARRE_p_Sty10_Mapping 250K_Sty_A06_56512 BARRE_p_Sty10_Mapping 250K_Sty_G02_56460 KNACK_p_Sty28_(C0-106345)_Mapping 250K_Sty_C07_104664 SPICA_p_Sty32_(CO-166838)_Mapping 250K_Sty_A05_121568
PRKCZ	Chr1	0 case	2 cases: CHAMS_p_Sty31_(CO124089)_Mapping 250K_Sty_E07_112420 CHAMS_p_Sty31_(CO124089)_Mapping 250K_Sty_A06_112396
PIK3C2B	Chr1	0 case	0 case

Table 7. Copy number changes (deletions) of *FRAP1*, *HERC1*, *PRKCZ* and *PIK3C2B* in adult ALL patients.

The analysis of 45 adult ALL samples using the Affy 500k chip showed deletions of one or more of these 4 genes in 7 of 45 cases (16%). One had deletions of *FRAP1*, *HERC1*, *PRKCZ* and *PIK3C2B*, three had deletion of two genes (*FRAP1+HERC1*) and three had deletions of only one gene (2 *HERC1*, 1 *FRAP1*) Analysis was performed on the publicy available dataset (GSE9611)

Genes	Chromosome	Patient with focal deletion	Patient with large deletion
HERC1	Chr15	2 cases GSM243089 GSM243099	4 cases GSM243067 GSM243079 GSM243086 GSM243091
FRAP1	Chr1	1 case GSM243089	4 cases GSM243066 GSM243067 GSM243086 GSM243091
PRKCZ	Chr1	0 case	1 cases GSM243091
PIK3C2B	Chr1	0 case	1 cases GSM243091

Table 8. Primers and Fluorescent Markers used for the MSI genotyping.

Name	Gene	Genbank number	Length and Location of the repeat	Fluorescent marker	Primer sequence 5' to 3'	Average PCR product size bp
BAT-26	hMSH2	U41210	26(A) Intron 5	FAM	tgactacttttgacttcagcc aaccattcaacatttttaaccc[a]	120
BAT-25	c-kit	L04143	25(T) Intron 16	Cy3	tcgcctccaagaatgtaagt[a] tctgcattttaactatggctc	124
NR-21	SLC7A8	XM_033393	21(T) 5' UTR	HEX	taaatgtatgtctcccctgg attcctactccgcattcaca[a]	103
NR-22	Transm-embrane Precursor protein B5	L38961	22(T) 3' UTR	FAM	gaggcttgtcaaggacataa aattcggatgccatccagtt[a]	142
NR-24	Zinc finger2 (ZNF-2)	X60152	24(T) 3' UTR	HEX	ccattgctgaattttacctc attgtgccattgcattgcattccaa[a]	132

[a]Fluorescent primer

Supplementary Methods

Western blot analysis. Western blot analysis was performed as previously described[1]. Briefly, $1x10^6$ leukemia cells were collected from the bone marrow of each patient before treatment. The human pre-B leukemia cell lines 697 and NALM-6 (German Collection of Microorganisms and Cell Cultures [DSMZ], Braunschweig, Germany) were used as MSH2 positive and negative controls, respectively. Cell lysates from ~1 million ALL cells were separated by electrophoresis on a 12% SDS-polyacrylamide gel, and the proteins were then electroblotted onto a Hybond P PVDF membrane. Protein expression was analyzed using the following antibodies: antibody to human MSH2 (Ab-2; Oncogene Science, Cambridge, MA), and antibody to GAPDH (Chemicon, Temecula, CA). Bands were visualized and quantified by PhosphorImager with the ImageQuaNT® software system (GE Healthcare Life Sciences), using blue fluorescence/chemifluorescence at 488 nm excitation. The amount of MSH2 protein in each leukemia sample was quantified based on the ratio of MSH2 protein signal to GAPDH signal from the same sample, expressed as relative units (RU). The estimated limit of detection of MSH2 protein was ~2ng/1 million ALL cells, corresponding to < ~6 RU%.

Validation Cohort. To validate our findings in an independent cohort of patients with ALL, we performed the same CNA analysis of leukemia cell DNA from an additional 170 patients treated on the St Jude Total XV Protocol, to identify cases in which one or more of these 4 genes (*FRAP1*, *HERC1*, *PIK3C2B*, *PRKCZ*) were deleted. We then performed western blot analysis to determine MSH2 protein levels in all cases with deletions for whom a sufficient quantity of ALL

cells was available for analysis, and in cases that did not have evidence of these gene deletions, matched (2:1) for ALL lineage, ploidy and molecular subtype.

Quantitative RT-PCR. Primers and probes (see below) for genomic quantitative PCR were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA) and Taqman® RNase P primers and probes (Applied Biosystem, Foster City, CA) were used for amplification control. 50 ng of leukemic blast DNA were amplified using a 7900HT Real-Time PCR system (Applied Biosystems, Foster City, CA) using the 7900 universal cycling conditions: 50 ^{o}C for 2 minutes, followed by 95 ^{o}C for 10 minutes, then 40 cycles of 95 ^{o}C for 1 minute and 60 ^{o}C for 1 minute. Standard curves for each gene were performed using normal human DNA. Assays were performed in duplicate. Copy number values were normalized by dividing the value obtained for every gene by the paired value obtained for RNase P for each sample. Cutoffs of 0.7 and 0.3 were used to identify hemizygous and homozygous deletions respectively[2].

Primers and Probes for Real-Time PCR. Primers and probes used were: *FRAP1* Taqman Forward (5'-TCCAGACCCTGATCCAAACC-3'), *FRAP1* Taqman Reverse (5'-CCTTTACCTGTGCCAATTCTCCTA-3'), *FRAP1* Taqman probe (5'-AGGTGTGATCAATAATGTCCTGGCA-3'), *HERC1* Taqman Forward (5'-GACATGTGTTTGGTGTAATAAAAAAGG-3'), *HERC1* Taqman Reverse (5'-TGCTTCTTGGTAACATTCCATACG-3'), *HERC1* Taqman probe (5'-TGCCATCATTGCCACTTGTAGCCAAA-3'), *PIK3C2B* Taqman Forward (5'-CCGACATAATGACAACATCATGCT-3'), *PIK3C2B* Taqman Reverse (5'-CCAGGAAGCGGCCAAAAT-3'), *PIK3C2B* Taqman probe (5'-ACCACTGGTCACATGTTCCACATT-3'), *PRKCZ* Taqman Forward (5'-

TTGGTTAATTATCAGCCCAGGAAT-3'), *PRKCZ* Taqman Reverse (5'-CCACCTGCCGTTTACTGTGA-3'), *PRKCZ* Taqman probe TGGGTAGGAATCCAGGCTGCTG-3')

mRNA gene expression analysis. Total RNA was extracted from a minimum of $5x10^6$ leukemia cells or normal leukocytes and purified in a solution of phenol-chloroform-isoamyl alcohol (25:24:1). RNA integrity was assessed as previously described[3]. RNA was hybridized to U133A GeneChip oligonucleotide microarrays, per the manufacturer's protocol (Affymetrix, Santa Clara, CA). These arrays contain 22,283 probe sets that include approximately 13,021 well-characterized human genes.

***MSH2* cDNA sequencing.** mRNA from leukemia cells of 3 MSH2-low and 3 MSH2-high patients was isolated and the *MSH2* cDNA was produced by reverse transcription and amplified by PCR. PCR products containing the entire coding region (3058 bp, from 36 bases 5' of translation start, to 217 bases 3' of termination) were cloned and sequenced using Big Dye® Terminator (v3.1) chemistry on an Applied Biosystems 3730XL DNA Analyzer. Eight clones from each patient were sequenced using 6 overlapping primer pairs. Every base was successfully sequenced in at least 5 clones from each patient. One published 3' non-coding SNP in MSH2 (rs17225053) was found in 2 of the 6 patient samples in which the MSH2 cDNA was sequenced (1 MSH2-L and 1 MSH2-H case), serving as an internal control for detection of DNA variations. Based on cDNA sequencing, with validation by sequencing of amplified DNA from the same leukemia cells, there were no sequence variants detected in the MSH2 coding DNA of any of the 6 cases. Because *MSH2* mRNA levels were not lower in the MSH2-L cases, we did not assess the methylation status of

MSH2 in these cases.

Genotyping. Genomic DNA and RNA were extracted from leukemia cells by using TRI Reagent® (Molecular Research Center Inc, Cincinnati, OH). The 3' untranslated region (UTR) of MSH2 was amplified using 10 ng of genomic DNA and 1x AmpliTaqGold master mix (Applied Biosystems, Foster City, CA) with PCR conditions of initial denaturation at 95 °C for 10 min, 35 cycles of 95 °C for 15 s and 60 ^{o}C for 1 min, and a final extension at 72 °C for 7 min. The following primers were used for *MSH2* 3' UTR amplification and sequencing: MSH2E17f and MSH2E17r (see sequences below). 400 ng of total RNA isolated from ALL cells was reverse-transcribed using the SuperScript III™ First-Strand Synthesis System (Invitrogen). The *PRKCZ* cDNA coding region was amplified using two sets of primers: PRKCZ-1f and PRKCZ-1r; or PRKCZ-2f and PRKCZ-2r. PCR amplification was performed according to the manufacturer's protocol using GC-RICH PCR system with an initial denaturation step at 95 ^{o}C for 3 min, followed by 40 cycles at an annealing temperature of 55 ^{o}C. *PRKCZ* mRNA transcripts variants were detected using a nested PCR method. First round PCR was performed using primers PRKCZ-1f and PRKCZ-4r with 40 amplification cycles at an annealing temperature of 55 ^{o}C. Second round PCR was performed using primers PRKCZ-3f and PRKCZ-3r with 40 cycles of amplification at an annealing temperature of 59 ^{o}C. Primer sequences are listed below.

Genotyping. The primers used for the genotyping were:

MSH2E17f (5'-ATATTGGGTGGGCTAATGTGGG-3'), MSH2E17r (5'-TCACTCGCAATGATCTCCATGG-5'), PRKCZ-1f (5'-GGAGTTCCGCGGAGTTGA-3'), PRKCZ-1r (5'-AAGTTGAGGGAGATGCAGAT-3'),

PRKCZ-2f (5'-AGTGGTGAAGAAAGAGCTGGTG-3'), PRKCZ-2r (5'-GCATATGCGGTGGTTAAGGAT-3'), PRKCZ-3f (5'-GGACATCTTCATCACCAGCGT-3'), PRKCZ-3r (5'-GCATATGCGGTGGTTAAGGAT-3'), PRKCZ-4r (5'-CTTGGAAACCGCATGACAG-3')

Gene deletions in adult ALL and colorectal cancer. To assess the frequency of gene deletions in adult ALL, Affy 500k CEL files were downloaded from the NCBI GEO site (GSE9611). For colorectal cancer, Affy 250K STY CEL files were downloaded from the author's website: http://research3.dfci.harvard.edu/cdk8colon/index.php?dir=Firestein%20et%20)al/.[4]
We used dChip with default settings to perform copy number analysis .

Antibodies. Primary antibodies were purchased for GAPDH, Phospho-PKCZ (Thr410), and PI3KC2β (16L9) (Santa Cruz Biotechnology), PKCζ (Biosource), AKT and Phospho-AKT (Ser 473), P70S6 kinase and Phospho-P70S6 Kinase (Thr389), and mTOR (Cell Signaling Technology). HERC1 antibodies 363 and 410 were provided by Rosa, J. L and were described previously[5]. Polyclonal rabbit antibody to mouse IgG horseradish peroxidase, polyclonal Swine antibody to rabbit IgG horseradish peroxidase and polyclonal rabbit Antibody to Goat IgG horseradish peroxidase were purchased from Dako.

In vitro sensitivity (MTT) assay. We determined in vitro drug sensitivity of primary ALL cells and of CEM cell lines (wild-type or each knockdown cell line) for 6-mercaptopurine, 6-thioguanine, melphalan, daunorubicin, L-asparaginase, vincristine, dexamethasone and prednisolone using a modification of the MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazoliumbromide) assay

as we have described previously[6]. Briefly, cells in mid-log phase were exposed to serial dilutions of the different drugs and analyzed after three days of incubation. The concentration of drug required for 50% cell kill (IC50) was used as the measure of relative sensitivity to each medication.

MSH2 protein stability. CCRF-CEM cells were seeded in culture medium, and cycloheximide (Sigma-aldrich) dissolved in DMSO was added to the cells to a final concentration of 5μg ml^{-1}. Cells were harvested at different time points (0–48h) after cycloheximide treatment and washed with PBS. Lysates were prepared in SDS cell lysis buffer. The samples were fractionated in 4–12% Nupage Bis-Tris gels and immunoblotted with MSH2 and GAPDH antibodies. Levels of MSH2 were quantified by densitometry and the optical density normalized to GAPDH signal was plotted. Each of the cases (control, rapamycin treatment and knockdowns) was normalized to its own y-intercept.

Immunoprecipitation and detection of Ubiquitinated MSH2. CCRF-CEM cells were treated or not with 300 nM of rapamycin for 48h. 24h before harvesting, 10 nM of MG132 was added to the cell culture. Cell lysis and immunoprecipitation were performed using the co-immunoprecipitation kit from Pierce. Mouse Antibody to human MSH2 (Ab-2; Oncogene Science, Cambridge, MA) and mouse antibody to IgG1 were covalently coupled onto an amine-reactive resin. After elution ubiquitinated MSH2 proteins were resolved by SDS-PAGE, transferred (transfert buffer: 25mM Tris, 190mM glycine, 20% methanol, pH adjusted to 8.0 and 0.05% SDS) to PVDF membranes. Blots were incubated with mouse antibody to ubiquitin

from BD Pharmingen.

PP2A assay. The PP2A assay was performed using the malachite green-based PP2A immunoprecipitation phosphatase assay kit (US Biological), following the manufacturer's protocol. Cells were incubated in an extraction buffer containing 20 mM imidazole-HCl, 2 mM EDTA, 2 mM EGTA, pH 7.0 with protease inhibitors and without phosphatase inhibitors. The cells were sonicated, and PP2A immunoprecipitations were performed using 100 ug of proteins from the supernatants, 4 μg of antibody to PP2A -catalytic subunit antibody and 25 μl of protein A-agarose beads. After 2 hours incubation at 4 ^{o}C with constant rocking, beads were washed. The immunoprecipitates were resuspended in 20 μl of Ser/Thr assay buffer. The reaction was initiated by the addition of 60 μl of phosphopeptide substrate (K-R-p-T-I-R-R). After incubation for 10 min at 30 ^{o}C in a shaking incubator, the reaction mixture was centrifuged briefly and 25 μl of the supernatant were transferred to a 96-well microtiter plate. The reaction was terminated by the addition of 100 μl of malachite green phosphate detection solution for 10–15 min at room temperature, and free phosphate was quantified by measuring the absorbance at 650 nm with a microplate reader. The phosphatase activity was calculated using a phosphate standard curve. Values are means ± SD of three determinations.

In vitro MMR Assay. MMR activity was analyzed using an *M13mp2* lacZ α -complementation method as described previously[7]. The double-stranded M13mp2 heteroduplex DNA contains a nick in the (-) strand and a 2 base loop in the lacZ α -complementation gene. The (+) strand encodes a colorless plaque phenotype, whereas the (-) strand encodes a blue plaque

phenotype. The repair was directed to the (-) strand of *M13mp2* by the presence of a nick. Introduction of this heteroduplex DNA into an Escherichia coli strain defective in mismatch repair yields M13 plaques on host indicator plates that are blue, colorless, or a mixture of the two. The repair reactions (25 μl) contained 30 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfomic acid (pH 7.8), 7 mM $MgCl_2$, 200 μM each of CTP, GTP, 4 mM ATP, 100 μM each of dATP, dGTP, dTTP, and dCTP, 40 mM creatine phosphate, 100 mg ml^{-1} creatine phosphokinase, 15 mm sodium phosphate, 5 ng of purified heteroduplex DNA, and 50 μg of cellular extract. After incubation for 30 min at 37 ^{o}C, the DNA was introduced into E. coli NR9162 (mutS strain) by electroporation. The cells were plated to score plaque colors. As the nick directs repair to the (-) strand, the (+) phenotype increases and the (-) phenotype decreases, producing more colorless plaques in this instance. The repair efficiency was expressed in percentage, as 100 x (ratio of mixed colonies in the control group – the ratio of mixed colonies in repaired group) / (ratio of mixed colonies in the control group). The rescue experiments were performed by adding 0.2 μg of MutSα in 25 μl of reaction. Each experiment was repeated three times, and data represent the mean ± S.D. of three separate determinations

MutSα protein purification. Human MutSα was purified in the Hsieh lab (NIH, NIDDK) from a baculovirus overexpression system essentially as described for the corresponding mouse MutSα protein[8]. ORFs derived from human cDNA clones encoding MSH2 and MSH6 (Open Biosystems Thermo Scientific #4110354 and #3629489, respectively) were cloned into pFB1 (Invitrogen), and baculovirus stocks were produced in Sf9 cells (Kinnakeet Biotechnology). High

Five cells (Invitrogen) were co-infected at an MOI of 10 and lysates prepared 48–72 hours post-infection. Recombinant hMutSα was purified by sequential passage over a 6-ml Resource Q column, a 5-ml Heparin HiTrap column and Hi-Prep 16/60 Sephacryl S-300 gel filtration (GE Healthcare). Protein concentrations were estimated using the Bio-Rad Bradford protein assay with bovine serum albumin as standard and are expressed as molar concentrations of heterodimeric protein.

Sequences of shRNA used for the knockdown of *FRAP1*, *HERC1*, *PIK3C2B* and *PRKCZ* genes.

For the knockdown the sequences of the shRNAs were:

CCGGGCATGGAAGAATACACCTGTACTCGAGTACAGGTGTATTCTTCCATGCTTTTTG and CCGGCCGCTAGTAGGGAGGTTTATTCTCGAGAATAAACCTCCCTACTAGCGGTTTTTG for *FRAP1/MTOR*; CCGGGCCCAGAATATCACTGTCCTTCTCGAGAAGGACAGTGATATTCTGGGCTTTTT and CCGGGCCATTGAATATCGACTTCATCTCGAGATGAAGTCGATATTCAATGGCTTTTT for *HERC1*; CCGGCCTCTTGTCTTCCCTACTTTACTCGAGTAAAGTAGGGAAGACAAGAGGTTTTT and CCGGCCTCCACTGTAGACTTGCTTACTCGAGTAAGCAAGTCTACAGTGGAGGTTTTT for *PIK3C2B*; CCGGCATGAAAGTGGTGAAGAAAGACTCGAGTCTTTCTTCACCACTTTCATGTTTTT and CCGGACCTAATCAGAGTCATCGGGCCTCGAGGCCCGATGACTCTGATTAGGTTTTTT for *PRKCZ*.

Microsatellite Instability Analysis. Genomic DNA from leukemia cells from 6 MSH2-L patients and from 5 MSH2-H patients (matched for ALL subtype) was amplified using quasi-monomorphic mononucleotide markers[9,10]. The 5 mononucleotide repeats were co-amplified in

1 multiplex PCR (primers, see Supplementary Table 8) using the True Allele PCR Premix kit from ABI PRISM. The pentaplex PCR was performed as described below:

1-Denaturation at 94 ^{o}C for five minutes

2-Denaturation at 94 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 30 seconds Extension at 72 ^{o}C for 30 seconds 35 cycles

3-Extension at 72 ^{o}C for 7 minutes

Separation and detection of PCR products were performed on a 3730xL DNA Analyser (Applied Biosystems) following the manufacturer's protocol. GeneMapper 4.0 was used to calculate the size of each fluorescent PCR product.

Supplementary References

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