Somatic mosaic *IDH1* or *IDH2* mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome

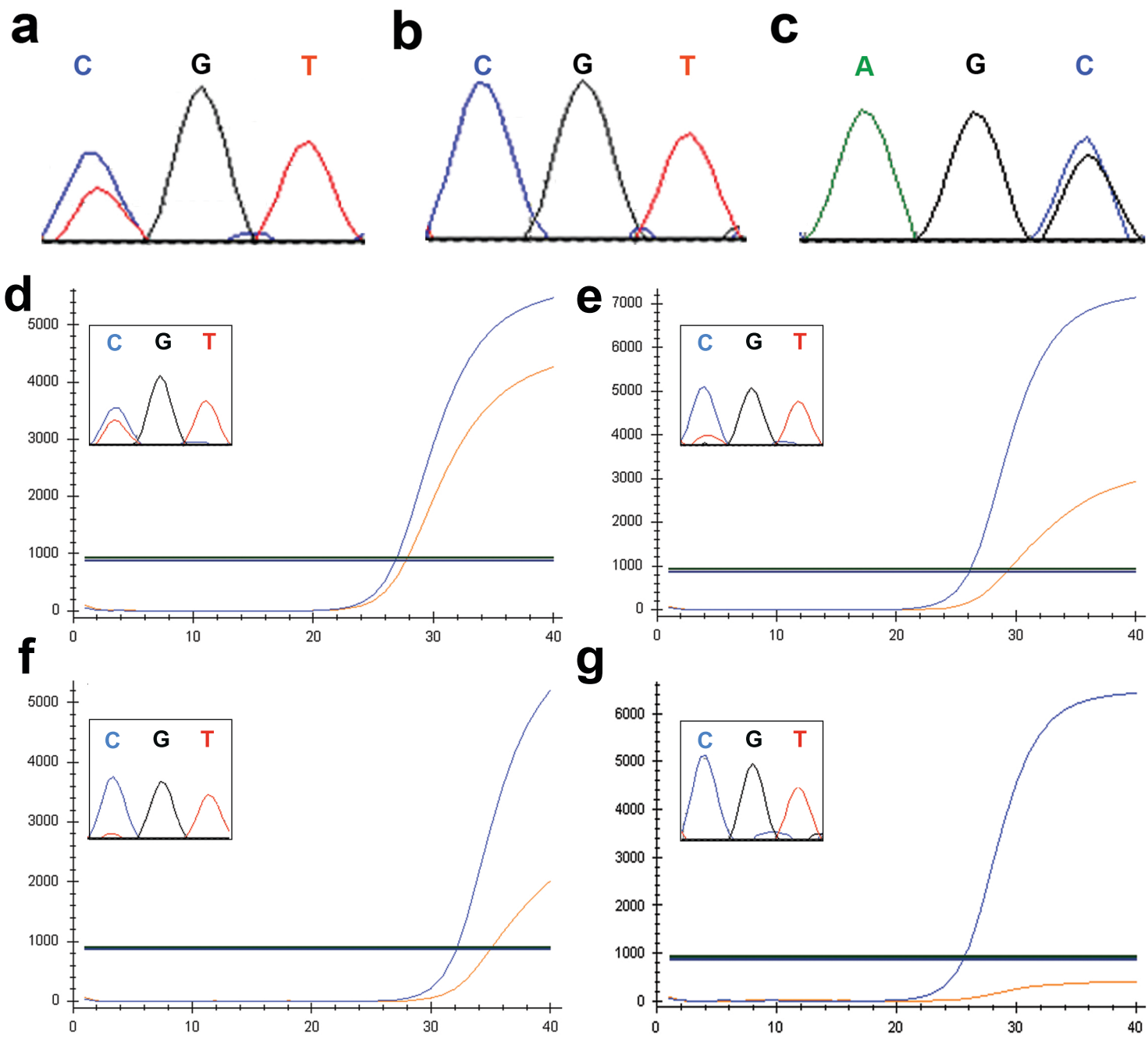
Twinkal C. Pansuriya[1], Ronald van Eijk[1], Pio d' Adamo[2], Marieke L. Kuijjer[1], Jan Oosting[1], Anne-Marie Cleton-Jansen[1], Sofie L.J. Verbeke[1,3], Daniëlle Meijer[1], Tom van Wezel[1], Karolin H. Nord[4], Luca Sangiorgi[5], Berkin Toker[6], Bernadette Liegl-Atzwanger[7], Mikel San-Julian[8], Raf Sciot[9], Nisha Limaye[10], Lars-Gunnar Kindblom[11], Soeren Daugaard[12], Catherine Godfraind[13], Laurence M. Boon[9, 14], Miikka Vikkula[9, 15], Kyle C. Kurek[16], Karoly Szuhai[17], Pim J. French[18] and Judith V.M.G. Bovée[1]

[1]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
[2]Institute for Maternal and Child Health - IRCCS "Burlo Garofolo" – Trieste, University of Trieste, Italy
[3]Department of Pathology, University Hospital Antwerp, Antwerp, Belgium
[4]Department of Clinical Genetics, Lund University Hospital, Lund, Sweden
[5]Department of Medical Genetics, Rizzoli Orthopedic Institute, Bologna, Italy
[6]Istanbul University Medical School, Istanbul, Turkey
[7]Institute of Pathology, Medical University, Graz, Austria
[8]Department of Orthopaedic Surgery and Traumatology, University Clinic of Navarra, Pamplona, Spain
[9]Department of Pathology, University of Leuven, Leuven, Belgium
[10]de Duve Institute, Université catholique de Louvain, Brussels, Belgium
[11]Department of Musculoskeletal Pathology, Royal Orthopaedic Hospital, Birmingham, United Kingdom
[12]Department of Pathology, University of Copenhagen, Copenhagen, Denmark
[13]Laboratory of Pathology, Cliniques universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium
[14]Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium
[15] Walloon Excellence in Lifesciences and Biotechnology (WELBIO), Université catholique de Louvain, Brussels, Belgium
[16]Department of Pathology, Children's Hospital Boston and Harvard Medical School, Boston, MA, USA
[17]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands
[18]Department of Neurology, Erasmus University Medical Center, Erasmus University Rotterdam, The Netherlands

Corresponding author

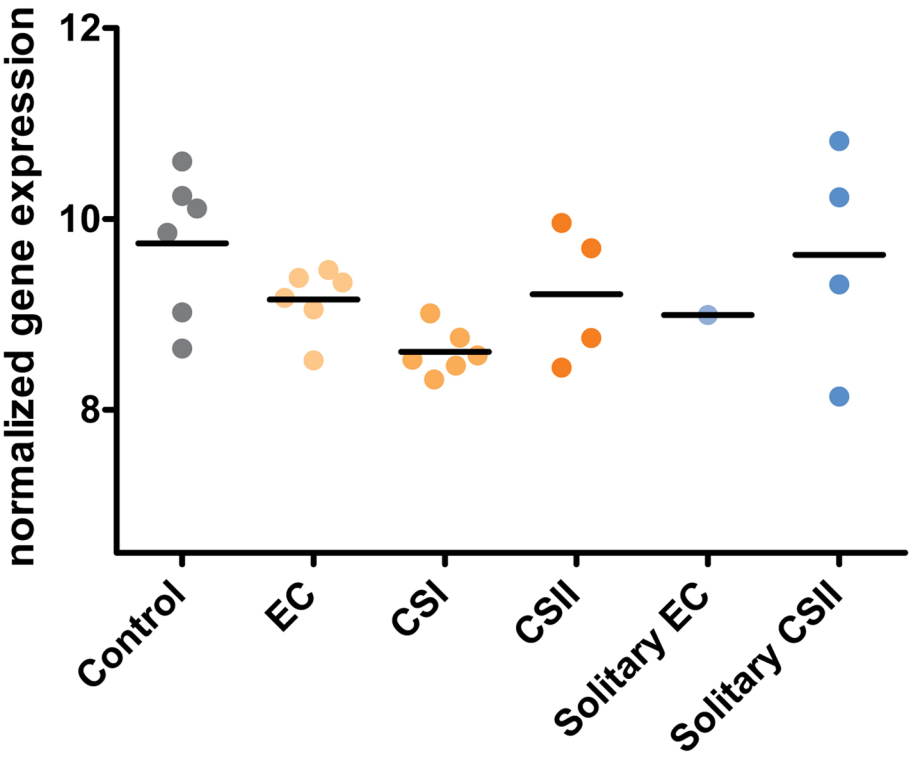
Judith V.M.G. Bovée, MD, PhD, Department of Pathology, Leiden University Medical Center, P.O. Box 9600, L1-Q, 2300 RC Leiden, The Netherlands. Tel: +31 71 5266617. Fax: +31 71 5266952. J.V.M.G.Bovée@lumc.nl

Supplementary Fig. 1 Output of sequencing and hydrolysis probes assay



a, b) Example of Sanger sequencing results showing that R132C IDH1 mutation was present in enchondroma and absent in corresponding blood DNA of a patient with Ollier disease.
c) Example of Sanger sequencing results showing R172S IDH2 mutation in a single patient with Ollier disease. d-g) Relative Fluorescent Units (RFU) are plotted against the quantification cycle (Cq). The horizontal line at 950 RFU indicates the threshold level for allele calling. All samples show a positive signal for both wild type (blue) and the IDH1 c.395C>T, p.R132C mutant allele (orange). d) L1684 carrying the R132C IDH1 mutation on Sanger sequencing (inset) confirmed with hydrolysis probes assay. e) L1980 and f) L204 have minor IDH1 positive cell populations. The R132C IDH1 mutant allele is barely visible after Sanger sequencing (inset) but it clearly presents as mutant using hydrolysis probes assay. g) L172 is negative for the mutation, in Sanger sequencing (inset) as well as in hydrolysis probes assay.

Supplementary Fig. 2 Normalized expression levels of *DLX5*



EC: enchondroma, CS : chondrosarcoma

Supplementary Table 1. Clinical information of patients with Ollier disease and Maffucci syndrome

Patient ID	Sample ID	*IDH1* mutation	*IDH2* mutation	Gender	Age	Disease	Tumor	Tumor location
O1	L1083	R132C		male	48	Ollier	CSI	metacarpal
	L2218	R132C		male	49	Ollier	CSI	digit V
O2	L172[3,6]	no mutation	no mutation	male	40	Ollier	CSII	scapula
O3	L271	R132C		female	26	Ollier	CSI	distal femur
	L286 T	R132C		female	23	Ollier	CSII	femur
O4[1]	L149 T3	R132C		male	34	Ollier	CSI	unknown
	L204 II[2,3]	R132C		male	26	Ollier	CSI	femur
	L253 T3	R132C		male	26	Ollier	CSI	tibia
O5	L206	R132C		female	25	Ollier	EC	hand
O6	L816-1	no mutation	R172S	male	68	Ollier	CSIII	humerus
	L813 T	no mutation	R172S	male	68	Ollier	CSII	femur
O7[1]	L898[6]	no mutation	no mutation	male	18	Ollier	CSI	femur
O8[1]	L1251	R132H		male	15	Ollier	EC	hand
	L2220	R132H		male	14	Ollier	EC	digit
O9	L1974	R132C		male	48	Ollier	CSII	scapula
O10[1]	L1975	R132C		male	31	Ollier	CSII	femur
O11	L1976	R132C		male	41	Ollier	CSII	tibia
O12[1]	L1977	R132C		male	41	Ollier	CSI	tibia
	L1978	R132C		male	38	Ollier	EC	foot
	L1979	R132C		male	41	Ollier	CSI	tibia
	L3363	R132C		male	36	Ollier	EC	toe
O13	L1980[2]	R132C		female	63	Ollier	CSII	knee
O14	L1685	R132C		female	23	Ollier	CSI	pubic bone
O15	L1686	R132C		male	18	Ollier	EC	phalanx
	L1687	R132C		male	18	Ollier	CSI	phalanx
O16	L2386	R132H		female	13	Ollier	CSI	digit III
O17[1]	L2463	R132C		female	12	Ollier	EC	tibia
O18	L1629	R132C		male	36	Ollier	EC	unknown
	L1630	R132C		male	36	Ollier	CSI	iliac bone
O19	L2095	R132C		female	23	Ollier	EC	distal tibia

O20[5]	L2590[2]	R132H		female	36	Ollier	CSI	metatarsal I
	L2761	no mutation	no mutation	female	37	Ollier	CSI	tibia
O21	L2098[3]	no mutation	no mutation	female	15	Ollier	CSII	humerus
O22	L2099	R132C		female	49	Ollier	CSI	prox humerus
O23	L2100	no mutation	no mutation	male	27	Ollier	EC	femur
O24	L2103a	R132C		male	39	Ollier	EC	phalanx
	L2103b	R132C		male	39	Ollier	CSI	distal phalanx
O25	L2104a	R132C		male	36	Ollier	CSIII	prox tibia
O26[1]	L2221	R132C		female	42	Ollier	CSI	distal femur
O27[1]	L1513	R132C		female	23	Ollier	CSI	distal femur
O28[1]	L1490	R132H		female	12	Ollier	EC	phalanx
O29	L2640	R132C		female	34	Ollier	EC	phalanx
	L2641	R132C		female	34	Ollier	EC	thumb
O30	L2205	R132C		male	6	Ollier	EC	illum
O31	L1683	R132C		unknown	29	Ollier	CSI	metacarpal
O32[1]	L2280	R132C		female	24	Ollier	CSI	left acromion
O33	L2513[3]	no mutation	no mutation	male	33	Ollier	CSI	pelvis
O34	L2746	R132C		female	58	Ollier	CSI	digit II
O35[1]	L3325A	R132H		male	6	Ollier	EC	hand
O36[1]	L3362A	R132C		female	6	Ollier	EC	proximal tibia
	L3362C	R132C		female	6	Ollier	EC	left distal femur
O37	OLR30 [4]	R132C		male	8	Ollier	EC	right leg
O38	S-03-3802[2,4]	R132H		female	16	Ollier	EC	hand
O39	S-05-4941[4]	R132H		male	13	Ollier	EC	hand
O40	S-05-6625[4]	R132C		female	11	Ollier	EC	hand
O41	S-08-3234[4]	R132H		female	4	Ollier	EC	hand
O42	S-08-7943[4]	no mutation	no mutation	male	12	Ollier	EC	hand
O43	S-08-9181[4]	no mutation	no mutation	female	12	Ollier	EC	hand
M1	L1684	R132C		female	37	Maffucci	EC	phalanx
M2	L2097b	R132C		female	19	Maffucci	EC	toe
	L2097a	R132C		female	19	Maffucci	EC	prox tibia
M3	L2102	R132C		male	29	Maffucci	CSII	distal femur
M4	MAF100[2]	R132C		male	birth	Maffucci	CSI	hand
M5	MAF200	R132C		female	4	Maffucci	SCH	right hand

M6	MAF210[3]	no mutation	no mutation	female	-	Maffucci	SCH	-
M7	MAF230	R132C		female	2	Maffucci	SCH	right foot
M8	MAF250[3]	no mutation	no mutation	female	3	Maffucci	EC	right hand
M9	S08-0010959 4A[4]	no mutation	no mutation	male	15	Maffucci	SCH	digit
	S08-0010959 5A[4]	no mutation	no mutation	male	15	Maffucci	SCH	forearm
M10	S08-0007382[2,4]	R132C		male	9	Maffucci	SCH	hand
M11	S05-0006227[2,4]	R132C		male	10	Maffucci	SCH	digit
M12	S97-0002538 1A[4]	R132C		female	30	Maffucci	SCH	foot
	S97-0002538 3A[4]	R132C		female	30	Maffucci	SCH	first web space
M13	S03-0001121[4]	R132C		female	23	Maffucci	EC	digit
	S97-0004447 4B[2,4]	R132C		female	17	Maffucci	SCH	lower back

All patients were diagnosed as having Ollier disease or Maffucci syndrome based on the radiographical features and/or presence of more than two cartilaginous tumors (Ollier disease) in combination with hemangioma (Maffucci syndrome). [1] indicates DNA from normal tissue was also tested, [2] indicates cases negative in Sanger sequencing but positive in hydrolysis probes assay, [3] indicates cases used for sequencing of all exons of *IDH1* and *IDH2*, [4] indicates DNA was isolated from paraffin embedded tissue, [5]indicates patient with multiple tumors in which one tumor showed clearly mutation in the sequencing and another tumor was negative for the mutation. DNA was unavailable to perform hydrolysis probe assay. [6]Two chondrosarcomas of patients with Ollier disease were negative at the mutation analysis, while other tumors of the same patients demonstrated positive cells at R132H IDH1 immunohistochemistry, suggesting that the percentage of patients carrying mutations in *IDH1* or *IDH2* is even higher than we report in this study. EC: enchondroma, CS: chondrosarcoma, SCH: spindle cell hemangioma.

Supplementary Table 2. Tiling Array design

Gene	Number of probes
IDH1	312
IDH2	208
PTHLH	338
PTPN11	413
PTH1R	104
EXT1	833
EXT2	334
ACP5	93

Supplementary Table 4. Differentially expressed genes between tumors with and without *IDH1* or *IDH2* mutations at Sanger sequencing

Probe ID	Target ID	logFC	adj.P.Val
6280168	*SERPINA3*	3.24	0.04267
4220431	*EXT1*	-0.73	0.03714
4210750	*STARD7*	-0.73	0.04397
2690541	*C18ORF10*	-0.57	0.04310
5050608	*TIMM23*	-0.49	0.03737
7320386	*TTL*	-0.42	0.04267
780021	*OPN3*	-0.37	0.04293
7380709	*YWHAB*	-0.35	0.02844
7040600	*ARSB*	-0.35	0.04267
2060112	*CCNYL1*	-0.29	0.02035
4480341	*DHCR24*	-0.29	0.03737
6270148	*AK5*	-0.29	0.02711
1410398	*CCNYL1*	-0.29	0.04152
6380193	*DLX3*	-0.27	0.02844
70270	*MGC39900*	-0.27	0.03099
4200070	*MGC39900*	-0.27	0.03313
6100390	*CD276*	-0.24	0.04267
6480333	*TCIRG1*	-0.23	0.04901
2810022	*C1ORF163*	-0.21	0.03174
5290358	*CPT1A*	-0.21	0.04293
3840750	*15E1.2*	-0.20	0.04267
4570242	*LARGE*	-0.19	0.03455
1050278	*SRD5A1*	-0.19	0.04293
6840753	*SPTLC2*	-0.18	0.03737
1570064	*KIAA1522*	-0.18	0.03737
6900309	*ARSB*	-0.18	0.04901
3440451	*ADAMTS7*	-0.17	0.04293
2450202	*KIF3C*	-0.17	0.00149
4920382	*VAC14*	-0.16	0.03737
360463	*SRR*	-0.16	0.03737
4180376	*PI4KII*	-0.14	0.04267
1450451	*DOPEY2*	-0.11	0.04293
1580397	*ISCA2*	-0.11	0.02844
2000020	*CAMKK2*	-0.11	0.03737
3170102	*C12ORF49*	-0.11	0.02844
10440	*MARS2*	-0.10	0.03778

Supplementary Table 5. Primers used for Sanger sequencing

Gene	Direction	Exon	Tissue type	Primer sequence (5' to 3')
IDH1	Forward	4	Frozen	TGTAAAACGACGGCCAGTCCATCACTGCAGTTGTAGGTT
IDH1	Reverse	4	Frozen	CAGGAAACAGCTATGACCCACATACAAGTTGGAAATTTCTGG
IDH1	Forward	4	Paraffin	TGTAAAACGACGGCCAGTCGGTCTTCAGAGAAGCCATT
IDH1	Reverse	4	Paraffin	CAGGAAACAGCTATGACCGCCAACATGACTTACTTGATCC
IDH1	Forward	2	Frozen	TGTAAAACGACGGCCAGTGGGCTGTCTGGCAGGTACTA
IDH1	Reverse	2	Frozen	CAGGAAACAGCTATGACCTGTTGGAATTCGTTGTTGGA
IDH1	Forward	3	Frozen	TGTAAAACGACGGCCAGTACCGCGTGTGAAACATAACA
IDH1	Reverse	3	Frozen	CAGGAAACAGCTATGACCGTTTGCTACACGGAGGGGTA
IDH1	Forward	5	Frozen	TGTAAAACGACGGCCAGTTCCTTACAATTCCTGCTAGGG
IDH1	Reverse	5	Frozen	CAGGAAACAGCTATGACCTTGTGCCTTTATTCATGCCA
IDH1	Forward	6	Frozen	TGTAAAACGACGGCCAGTTGGTGGGTGATTTTAGCCTT
IDH1	Reverse	6	Frozen	CAGGAAACAGCTATGACCTGGTTTTGTTTCACTCCTGCT
IDH1	Forward	7	Frozen	TGTAAAACGACGGCCAGTTGTTTGGGACAAGCAGATGA
IDH1	Reverse	7	Frozen	CAGGAAACAGCTATGACCCAAAACTCCCCTTCCCAAAT
IDH1	Forward	8	Frozen	TGTAAAACGACGGCCAGTTGCTCTTCATGCAGTTGGAC
IDH1	Reverse	8	Frozen	CAGGAAACAGCTATGACCTGCACACAAAACACTGAGCA
IDH1	Forward	9	Frozen	TGTAAAACGACGGCCAGTCCATGCCATGAAAATGTGTT
IDH1	Reverse	9	Frozen	CAGGAAACAGCTATGACCGATGCTCTGAGCCCAGTGAG
IDH1	Forward	10	Frozen	TGTAAAACGACGGCCAGTGGACTTTACCACTACCTGCTACC
IDH1	Reverse	10	Frozen	CAGGAAACAGCTATGACCTGGCCTGAGCTAGTTTGATCT
IDH2	Forward	4	Frozen	TGTAAAACGACGGCCAGTTTGTTGCTTGGGGTTCAAAT
IDH2	Reverse	4	Frozen	CAGGAAACAGCTATGACCCTGCAGAGACAAGAGGATGG
IDH2	Forward	4	Paraffin	TGTAAAACGACGGCCAGTAACATCCACGCCTAGTCC
IDH2	Reverse	4	Paraffin	CAGGAAACAGCTATGACCCAGTGGATCCCCTCTCCAC
IDH2	Forward	1	Frozen	TGTAAAACGACGGCCAGTCTCGTTCGCTCTCCAGCTT
IDH2	Reverse	1	Frozen	CAGGAAACAGCTATGACCGCCACCGTCCCTCAAGTC
IDH2	Forward	2	Frozen	TGTAAAACGACGGCCAGTATGATGCGCTGTGTGTCC
IDH2	Reverse	2	Frozen	CAGGAAACAGCTATGACCGGGACAGAACAATCCCTGG
IDH2	Forward	3	Frozen	TGTAAAACGACGGCCAGTGTCCCTGAGTCACTGGGGT
IDH2	Reverse	3	Frozen	CAGGAAACAGCTATGACCCCTGTGACCCTCCCTGG
IDH2	Forward	5	Frozen	TGTAAAACGACGGCCAGTAGCTCCTCGCCTAGCCAT
IDH2	Reverse	5	Frozen	CAGGAAACAGCTATGACCTGAAGAGACAAGCTGGGAGA
IDH2	Forward	6	Frozen	TGTAAAACGACGGCCAGTCCAGGCTAGGGCACCAC
IDH2	Reverse	6	Frozen	CAGGAAACAGCTATGACCGGGAAGAAAGGCCACAGAGT
IDH2	Forward	7	Frozen	TGTAAAACGACGGCCAGTCCTCTCCCCATAACAGACCTT
IDH2	Reverse	7	Frozen	CAGGAAACAGCTATGACCAGAAGACCAACAGTCCACCC
IDH2	Forward	8	Frozen	TGTAAAACGACGGCCAGTAGGCCCTGAGAGAAAGGCT
IDH2	Reverse	8	Frozen	CAGGAAACAGCTATGACCGGTAGAGGGGCATTGTGAGG

IDH2	Forward	9	Frozen	TGTAAAACGACGGCCAGTGCTCTTGATCTCCCTGCAAC
IDH2	Reverse	9	Frozen	CAGGAAACAGCTATGACCGGACCCAGAGCCTGTCCT
IDH2	Forward	10	Frozen	TGTAAAACGACGGCCAGTGCACAGATGGGGTCTCATTC
IDH2	Reverse	10	Frozen	CAGGAAACAGCTATGACCAGGGTCTGCCTACCACCC
PTH1R	Forward	4	Frozen	CCTGTCTGCCGGAATGG
PTH1R	Reverse	4	Frozen	TGATTGAAGTCATAAATGTAGTCCG
PTH1R	Forward	5	Frozen	TTGGAGCTAGGGGTTCAGTG
PTH1R	Reverse	5	Frozen	GTAGTTGGCCCACGTCCTGT
PTH1R	Forward	9	Frozen	ATCCACATGCACCTGTTCCT
PTH1R	Reverse	9	Frozen	GGCAGAGGGGTACTCACGTA
GNAS	Forward	8	Frozen	TGTAAAACGACGGCCAGTTCGGTTGGCTTTGGTGAGATCCAT
GNAS	Reverse	8	Frozen	CAGGAAACAGCTATGACCTGACTTTGTCCACCTGGAACTTGGT

Supplementary Table 6. Probes used in hydrolysis probe assays for *IDH1*

Name	Direction	Sequence	Dye	Remark
R132C *IDH1*	Forward	CTTGTGAGTGGATGGGTAAAACCTA	-	-
R132H *IDH1*	Forward	CTTGTGAGTGGATGGGTAAAACCTA	-	-
R132C *IDH1*	Reverse	CACATTATTGCCAACATGACTTACTTGAT	-	-
R132H *IDH1*	Reverse	CCAACATGACTTACTTGATCCCCATA	-	-
R132C *IDH1*_V	-	AAGCATGACGACCTATG	VIC	Reporter 1
R132H *IDH1*_V	-	CATCATAGGTCGTCATGC	VIC	Reporter 1
R132C *IDH1*_M	-	AAGCATGACAACCTATG	FAM	Reporter 2
R132H *IDH1*_M	-	ATCATAGGTCATCATGC	FAM	Reporter 2