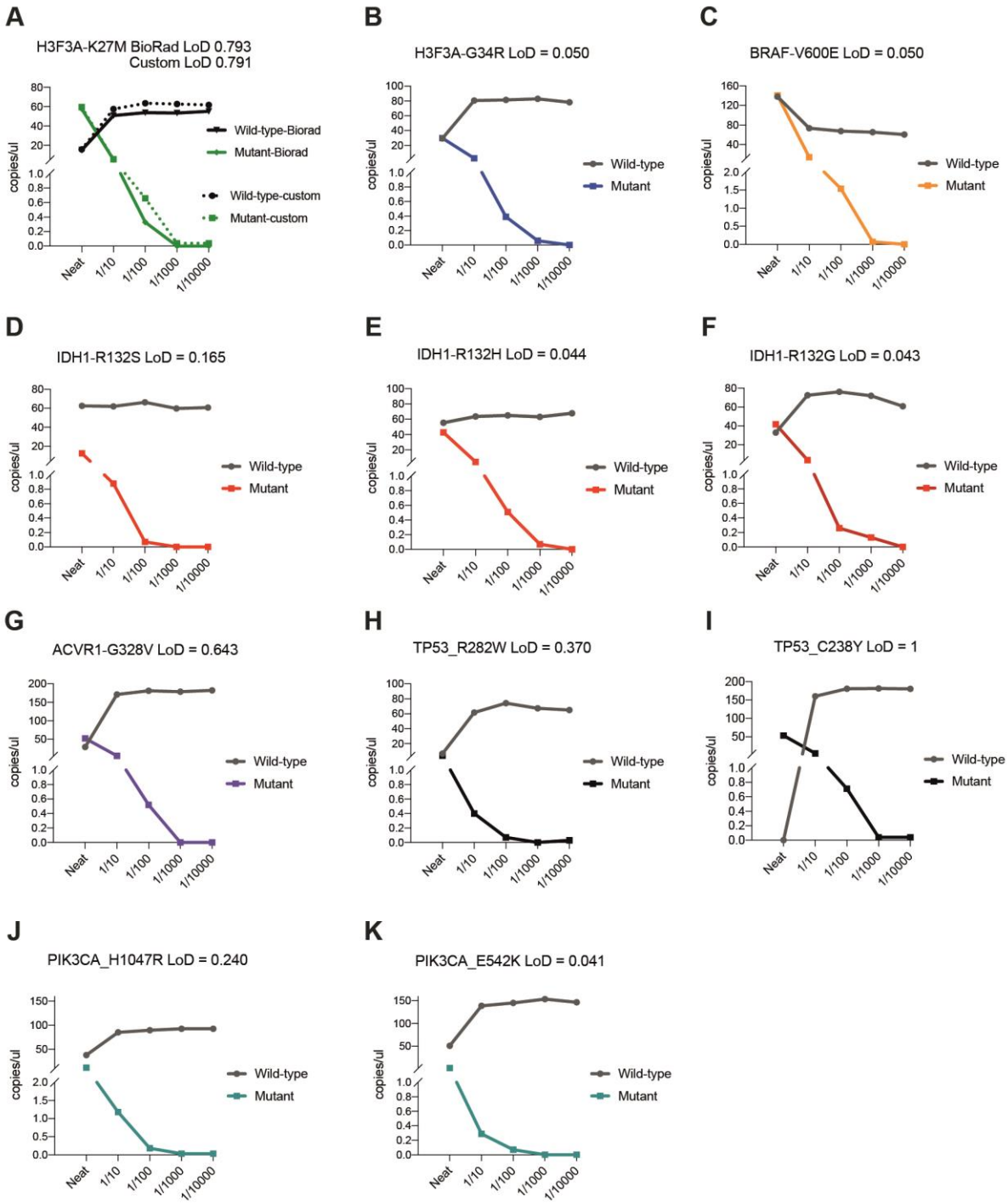
SUPPLEMENTARY FIGURE LEGENDS

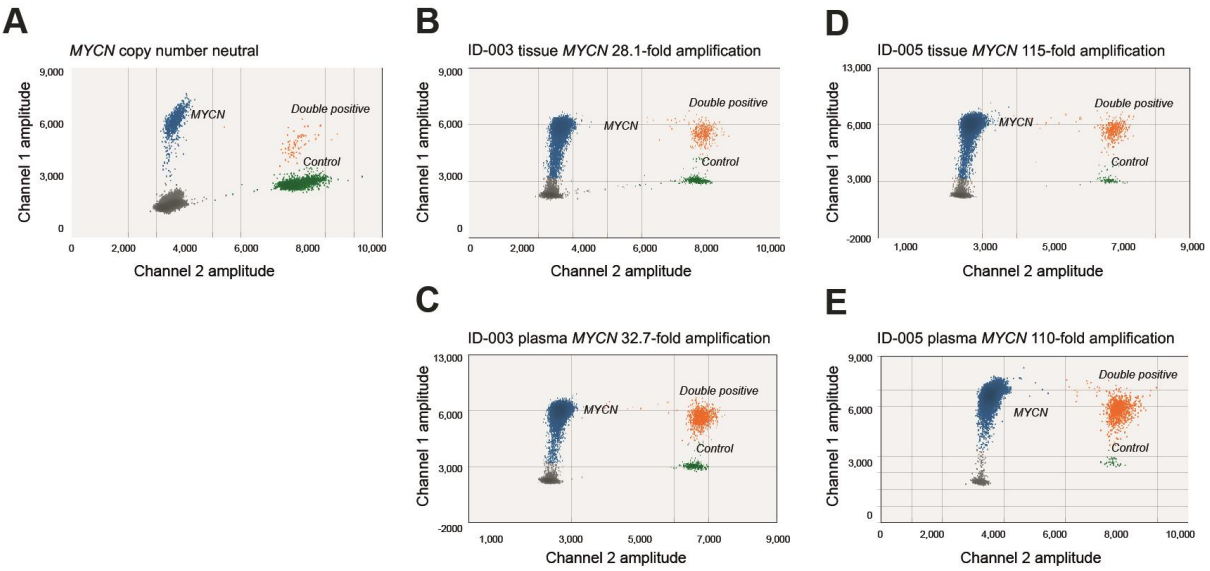
Supplementary Figure S1



Supplementary Figure S1 - *ddPCR assay limit of detection plots.* Linear dilutions of mutant DNA in a constant background of wild-type DNA are shown against number of copies/µl for

wild-type and mutant alleles. A total of 5 ng of total DNA was load in each PCR well. Samples were run in duplicates and merged data is shown. (A) *H3F3A*_K27M (green), (B) *H3F3A*_G34R (blue), (C) *BRAF*_V600E (gold), (D) *IDH1*_R132S (red), (E) *IDH1*_R132H (red), (F) *IDH1*_R132G (red), (G) *ACVR1*_G328V (purple), (H) *TP53*_R282W (grey), (I) *TP53*_C238Y (grey), (J) *PIK3CA*_H1074R (teal), (K) *PIK3CA*_E542K (teal).

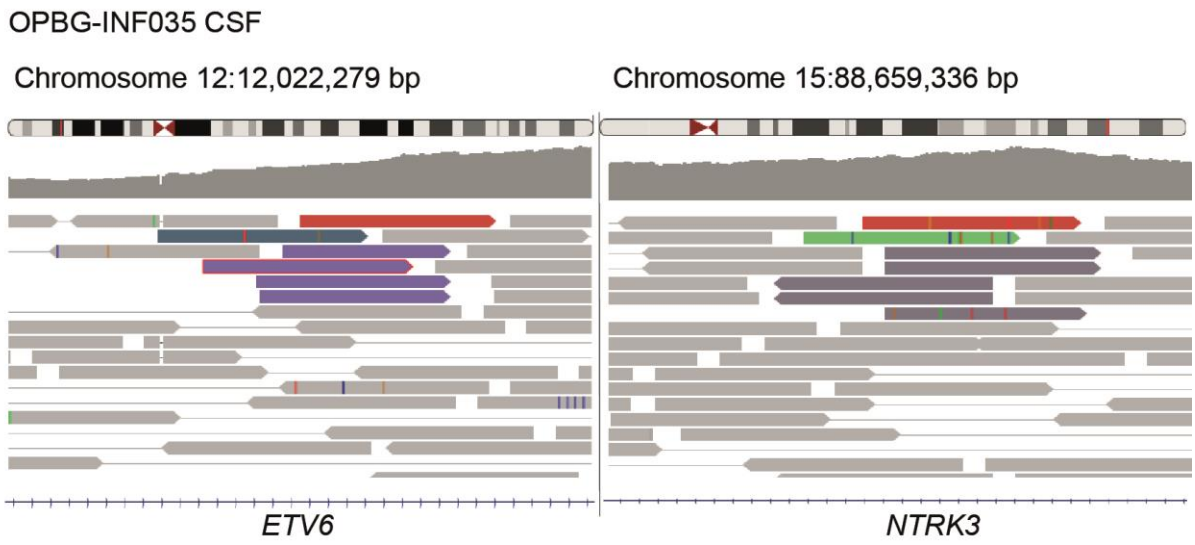
Supplementary Figure S2



Supplementary Figure S2 - *ddPCR assay validation.*

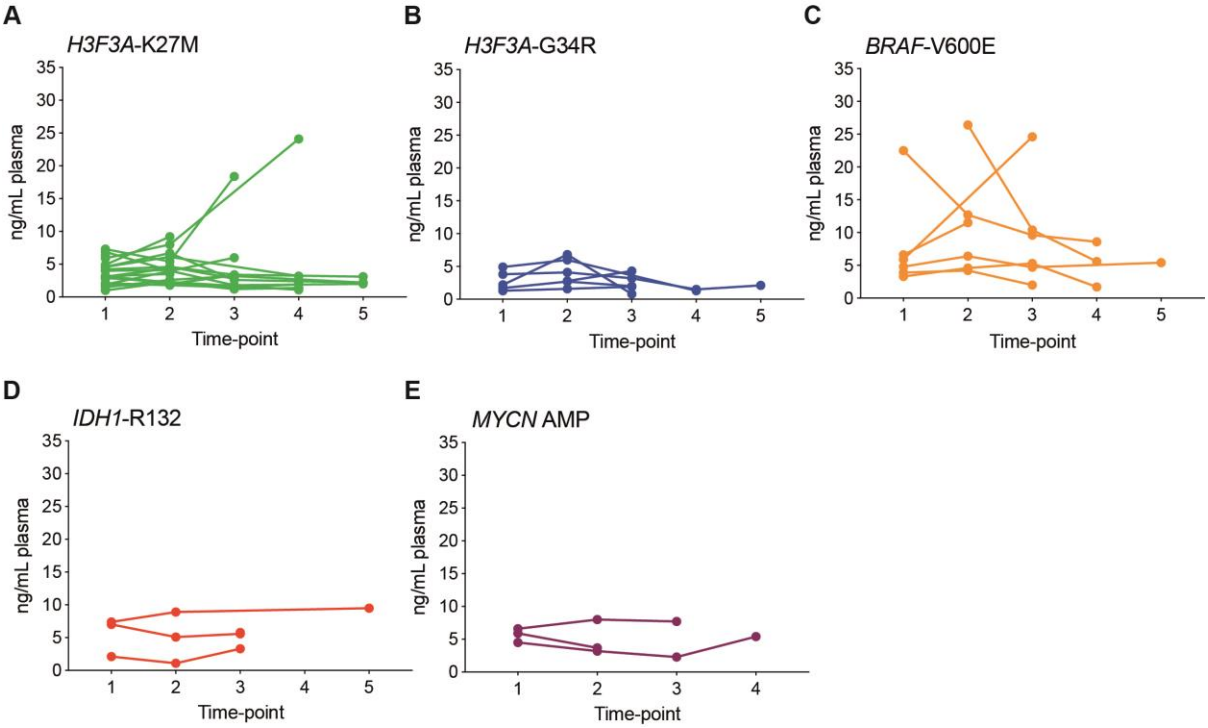
(A) Droplet digital PCR 2D amplitude plot of *MYCN* tested in a patient with copy neutral *MYCN*, and (B,D) tissue and (C,E) plasma from two positive control neuroblastoma patients with known *MYCN* amplification. *MYCN* droplets are shown in blue, droplets from a control region at chromosome 5p15.33 are shown in green, double positive droplets are shown in orange and empty droplets with no DNA are shown in grey.

Supplementary Figure S3



Supplementary Figure S3 – *Detection of ETV6:NTRK3 fusion in CSF.* Integrative Genomics Viewer IGV) snapshot of *ETV6* and *NTRK3* detected from ctDNA-CSF (OPBG_INF_035). Reads supporting the *ETV6:NTRK3* fusion are coloured in purple/grey.

Supplementary Figure S4



Supplementary Figure S4 – *Assessment of plasma cfDNA concentration over time in the HERBY cohort.* (A) cfDNA concentrations (y axis) plotted against time-point of sampling for HERBY patients with *H3F3A*-K27M mutations. (B) cfDNA concentrations (y axis) plotted against time-point of sampling for HERBY patients with *H3F3A*-G34R mutations. (C) cfDNA concentrations (y axis) plotted against time-point of sampling for HERBY patients with *BRAF*-V600E mutations. (D) cfDNA concentrations (y axis) plotted against time-point of sampling for HERBY patients with *IDH1*-R132 mutations. (E) cfDNA concentrations (y axis) plotted against time-point of sampling for HERBY patients with *MYCN* amplification.

SUPPLEMENTARY TABLES

ddPCR assay	NGS VAF	ddPCR VAF	Dilution	LoD (%)	Mutant droplets	Mutant copies/ul	WT copies/ul
H3F3A-K27M-BioRad	81	79.3	1/100	**0.793**	10	0.33	53.22
H3F3A-K27M-custom	81	79.1	1/100	**0.791**	17	0.66	63.63
*H3F3A*_G34R-BioRad	48	50.3	1/1000	**0.050**	2	0.07	50.30
*BRAF*_V600E-custom	49	50.4	1/1000	**0.050**	2	0.07	65.30
IDH1-R132S-custom	13	16.5	1/100	**0.165**	2	0.07	60.60
IDH1-R132H-custom	40	43.6	1/1000	**0.044**	2	0.07	63.20
IDH1-R132G-custom	45	43.1	1/1000	**0.043**	4	0.13	72.00
TP53-C238Y-custom	99.3	100	1/100	**0.993**	19	0.71	180.61
TP53-R282W-custom	38.6	37.0	1/100	**0.370**	2	0.07	74.30
ACVR1-R328V-custom	54	64.3	1/100	**0.643**	14	0.52	181.14
PIK3CA-E542K-custom	5	4.1	1/100	**0.041**	2	0.07	145.10
PIK3CA-H1047R-custom	30	24.0	1/100	**0.240**	5	0.03	92.60

Supplementary Table 1 - *ddPCR assay limit of detection results.*

Samples were run in duplicate and merged data is provided. Shown are the mutations assessed by ddPCR, the biological source material, variant allele frequency (VAF), sample dilution and limit of detection (LoD), number of mutant droplets as well as number of copies of mutant and wild-type per µl.

Assay	FW	RV	WT-probe	Dye	Mutant-probe	Dye
H3F3A-K27M	GGTAAAGCACCCAGGAAG	CAAGAGAGACTTTGTCCC	TC+GC+A+A+GA+GT+GC	HEX	TC+GC+A+**T**+GA+GTGC	FAM
BRAF-V600E	CATGAAGACCTCACAGTAAAAATAGGTGAT	TGGGACCCACTCCATCGA	CTAGCTACAGTGAAATC	VIC	TAGCTACAGAGAAATC	FAM
ACVR1-G328V	GCTAGTGGTCTTGCACATTTGC	CTCTTTAAATCTCGATGGGCAATGG	ACCCAAGGGAAACCA	VIC	ACCCAAGTGAAACCA	FAM
IDH1-R132G	CTTGTGAGTGGATGGGTAAAACCTA	CACATTATTGCCAACATGACTTACTTGAT	AAGCATGACGACCTATG	VIC	AAGCATGACCACCTATG	FAM
IDH1-R132H	CTTGTGAGTGGATGGGTAAAACCTA	CCAACATGACTTACTTGATCCCCATA	CATCATAGGTCGTCATGC	VIC	ATCATAGGTCATCATGC	FAM
IDH1-R132S	CTTGTGAGTGGATGGGTAAAACCTA	CACATTATTGCCAACATGACTTACTTGAT	CATAAGCATGACGACCTAT	VIC	CCATAAGCATGACTACCTAT	FAM
TP53-C238Y	TGGCTCTGACTGTACCACCAT	GATGGGCCTCCGGTTCAT	ACAACTACATGTGTAACAGT	VIC	ACAACTACATGTATAACAGT	FAM
TP53-R282W	GCTTTGAGGTGCGTGTTTGTG	CTTTCTTGCGGAGATTCTCTTCCT	TGCGCCGGTCTCT	VIC	TGCGCCAGTCTCT	FAM
PIK3CA-E542K	GGGAAAATGACAAAGAACAGCTCAA	GCACTTACCTGTGACTCCATAGAAA	CCTCTCTCTGAAATCA	VIC	CCTCTCTCTAAAATCA	FAM
PIK3CA-H1047R	GCAAGAGGCTTTGGAGTATTTCATG	GCTGTTTAATTGTGTGGAAGATCCAA	CCACCATGATGTGCATC	VIC	CACCATGACGTGCATC	FAM

Supplementary Table S2 - *ddPCR primers.*

Sequences for assays used to detect mutations in *H3F3A* (K27M), *BRAF* (V600E), *ACVR1* (G328V), *IDH1* (R132G, R132H and R132S), *TP53* (C238Y and R282W) and *PIK3CA* (E542K and H1047R). “+” denotes locked nucleic acid bases.