Table S1 Primer and probe sets used in the NB5 assay

Purpose	Gene symbol	Gene name	Accession number	Primer sequence (5'–3')	Probe sequence (5'–3')[†]
qRT-PCR/ ddPCR	TH	Tyrosine hydroxylase	NM_199292	F: TCATCACCTGGTCACCAAGTTC	CCTGACCTGGACTTGGACCACCCG
				R: TGGAAGGCGATCTCAGCAATC	
	DDC	Dopa decarboxylase	NM_000790	F: TTAACGGGAGCCTTTAGACTGG	TGCCCAGTGGTATCTGCCAATGCC
				R: ACCACATTTTCAAAGAGCGAAATC	
	DCX	Doublecortin	NM_000555	F: TGAAGGGAAACCCATCAGCC	AGCCAAGAGCCCTGGTCCTATGCG
				R: GGAGGTTCCGTTTGCTGAGT	
	PHOX2B	Paired like homeobox 2B	NM_003924	F: CCGCAGTTCCTTACAAACTCTTC	CGGACCACGGCGGCCTCAACG
				R: GAGCTGGGCACTGGTGAAAG	
	CHGA	Chromogranin A	NM_001275	F: AAGGGGATACCGAGGTGATGA	CGACACACTTTCCAAGCCCAGCCC
				R: GGTCTTGGAGCTCCTTCAGTA	
	B2M	Beta-2-microglobulin	NM_004048	F: GAGTATGCCTGCCGTGTG	CCTCCATGATGCTGCTTACATGTCTC
				R: AATCCAAATGCGGCATCT	

[†], 5' labeled with fluorescein (FAM); 3', labeled with Black Hole Quencher 1 (BHQ1). NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); ddPCR, droplet digital polymerase chain reaction; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; F, forward; R, reverse.

Table S2 Comparison of NB5 assay results between PB and BM specimens

Method	Sample	Category description	N (%)
qRT-PCR	Peripheral blood	Positive/total[†]	6/22 (27.273)
	Bone marrow	Positive/total[†]	11/21 (52.381)

[†], number of assessments. NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); PB, peripheral blood; BM, bone marrow; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

Table S3 Bone marrow morphology and flow cytometric MRD NB5 assay data

Variables	Category	NB5 positive			NB5 negative		
		BM	Blood	Cerebrospinal fluid	BM	Blood	Cerebrospinal fluid
Bone marrow morphology	Positive	8	0	2	0	0	0
	Negative	22	0	0	59	0	0
MRD (flow cytometric)	Positive	15	0	0	0	0	0
	Negative	15	1	2	58	0	0

MRD, measurable residual disease; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; BM, bone marrow.

Table S4 ddPCR positive sample NB5 assay results

Gene	Positive	Negative	Total
CHGA	14 (87.500)	2 (12.500)	16 (100.000)
DCX	9 (56.250)	7 (43.750)	16 (100.000)
DDC	10 (62.500)	6 (37.500)	16 (100.000)
PHOX2B	12 (75.000)	4 (25.000)	16 (100.000)
TH	11 (68.750)	5 (31.250)	16 (100.000)
Total	57 (70.370)	24 (29.630)	81 (100.000)

Data are shown as n (%). ddPCR, droplet digital polymerase chain reaction; *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase.

Table S5 Analysis of Cox proportional hazards regression model under different variables

Variables	No. of patients	P value	HR	95% CI
Negative BM via flow cytometric MRD (positive *vs.* negative)	61	0.046	3.046	1.020 to 9.099
MRD (flow cytometric) (positive *vs.* negative)	70	0.519	0.694	0.229 to 2.104
NB5 ΔCt (positive *vs.* negative)	70	0.319	1.574	0.645 to 3.841
Bone marrow morphology (positive *vs.* negative)	70	0.492	0.644	0.184 to 2.259

BM, bone marrow; MRD, measurable residual disease; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; ΔCt, Δ threshold cycle; HR, hazard ratio; CI, confidence interval.

Table S6 Contribution of different signatures to the AUC

Signature	AUC	95% CI	P value	Change from DDC (%)
DCX	0.733	0.604 to 0.863	0.002	–
DDC	0.679	0.554 to 0.805	0.017	−7.367
PHOX2B	0.679	0.546 to 0.812	0.017	−7.367
CHGA	0.649	0.512 to 0.786	0.047	−11.501
TH	0.649	0.510 to 0.788	0.047	−11.501
NB5	0.710	0.591 to 0.829	0.005	−3.111
NB5 minus *CHGA*	0.714	0.596 to 0.833	0.004	−2.592
NB5 minus *DDC*	0.712	0.595 to 0.833	0.004	−2.851
NB5 minus *DCX*	0.685	0.562 to 0.807	0.014	−6.589
NB5 minus *PHOX2B*	0.704	0.584 to 0.823	0.007	−4.011
TH/DCX	0.710	0.584 to 0.836	0.005	−3.111
PHOX2B/TH/DDC	0.693	0.571 to 0.815	0.010	−5.430
PHOX2B/TH/DCX	0.719	0.601 to 0.836	0.004	−1.937
PHOX2B/TH	0.698	0.578 to 0.818	0.008	−4.789

AUC, area under curve; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *CHGA*, chromogranin A; *TH*, tyrosine hydroxylase; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); CI, confidence interval.

Figure S1 Summary of patient and sample characteristics information. ΔCt, Δ threshold cycle; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; CR, complete remission; SD, stable disease; PR, partial remission; VGPR, very good partial remission. MRD, measurable residual disease; ddPCR, droplet digital polymerase chain reaction.
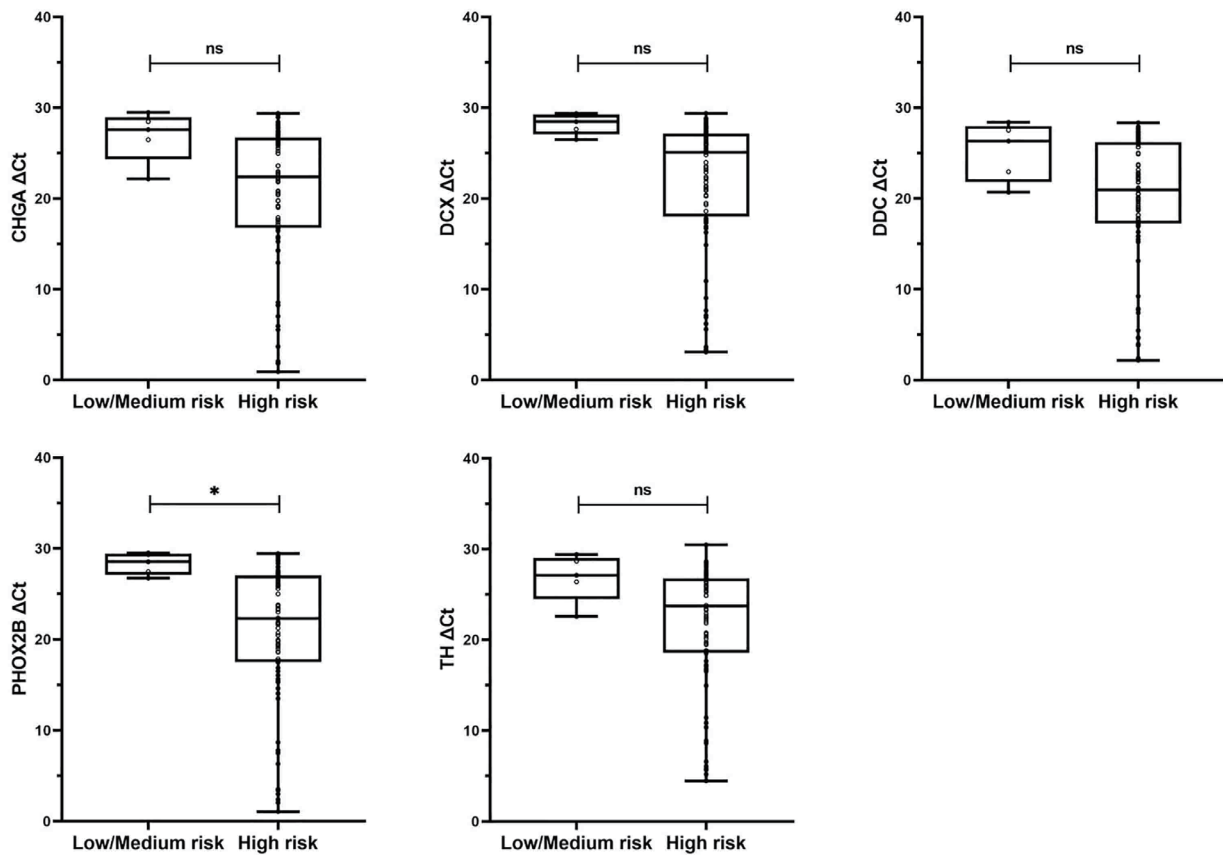
Figure S2 Level of NB5 ΔCt in BM in the different risk groups. n=5 and n=84 in the low/medium risk and high risk, respectively. *, P<0.05. ns, no significant difference; ΔCt, Δ threshold cycle; *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; NB5, five neuroblastoma genes (*CHGA*, *DCX*, *DDC*, *PHOX2B*, and *TH*); BM, bone marrow.
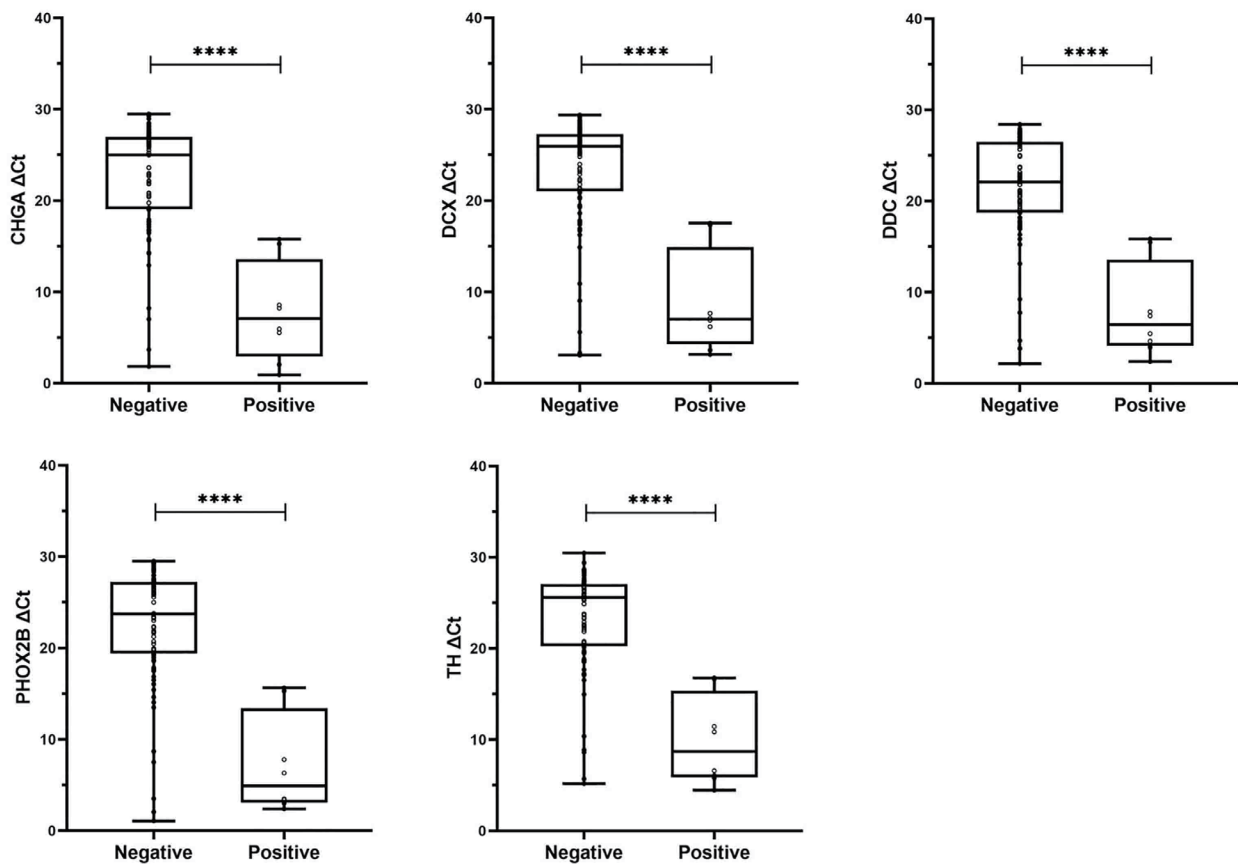
Figure S3 Level of NB5 ΔCt in BM in the different bone marrow morphology groups. n=81 and n=8 in the bone marrow morphology negative group and positive group, respectively. ****, P<0.0001. ΔCt, Δ threshold cycle; *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B*, and *TH*); BM, bone marrow.

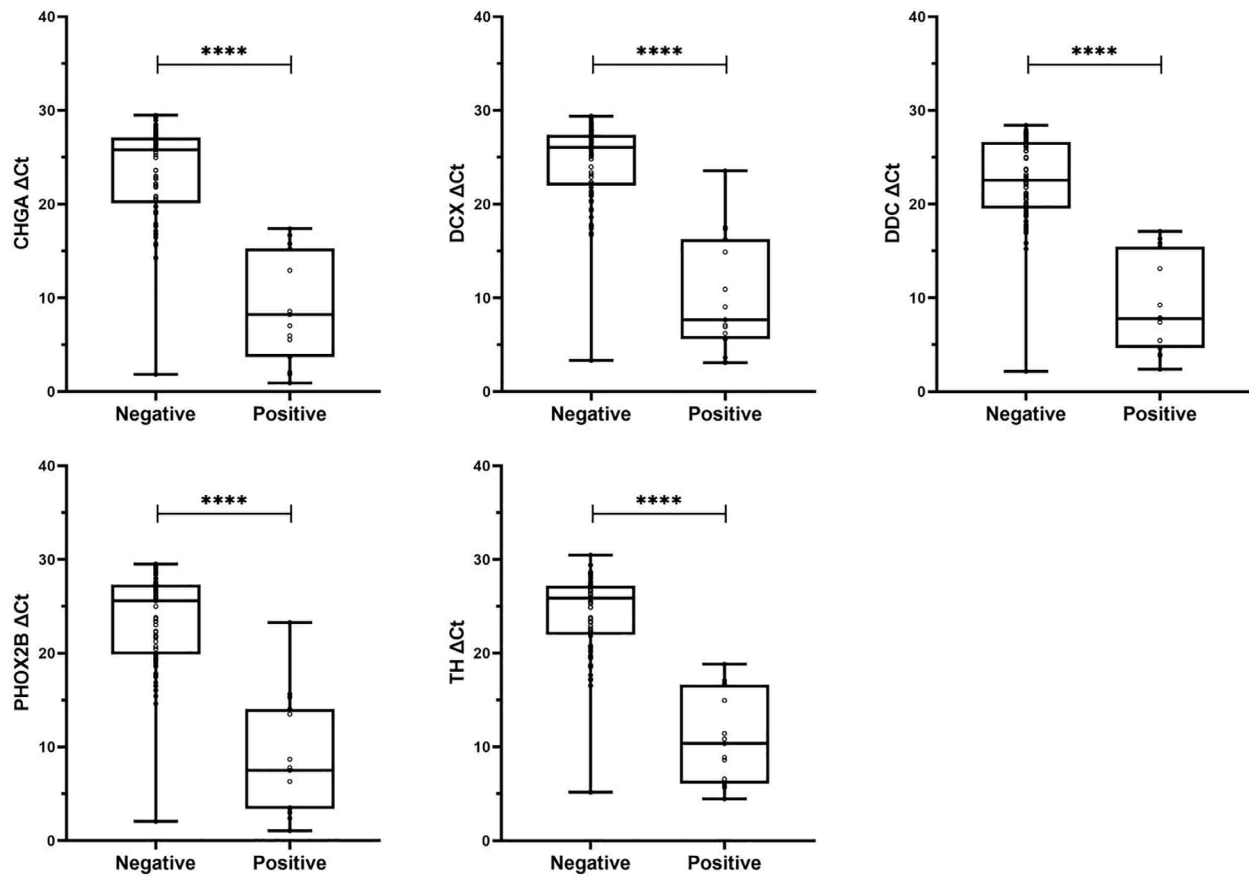**Figure S4** Level of NB5 ΔCt in the BM in different flow cytometric MRD groups. n=73 and n=15 in the flow cytometric MRD negative group and positive group, respectively. ****, P<0.0001. ΔCt, Δ threshold cycle; *CHGA*, chromogranin A; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *TH*, tyrosine hydroxylase; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B,* and *TH*); BM, bone marrow; MRD, measurable residual disease.
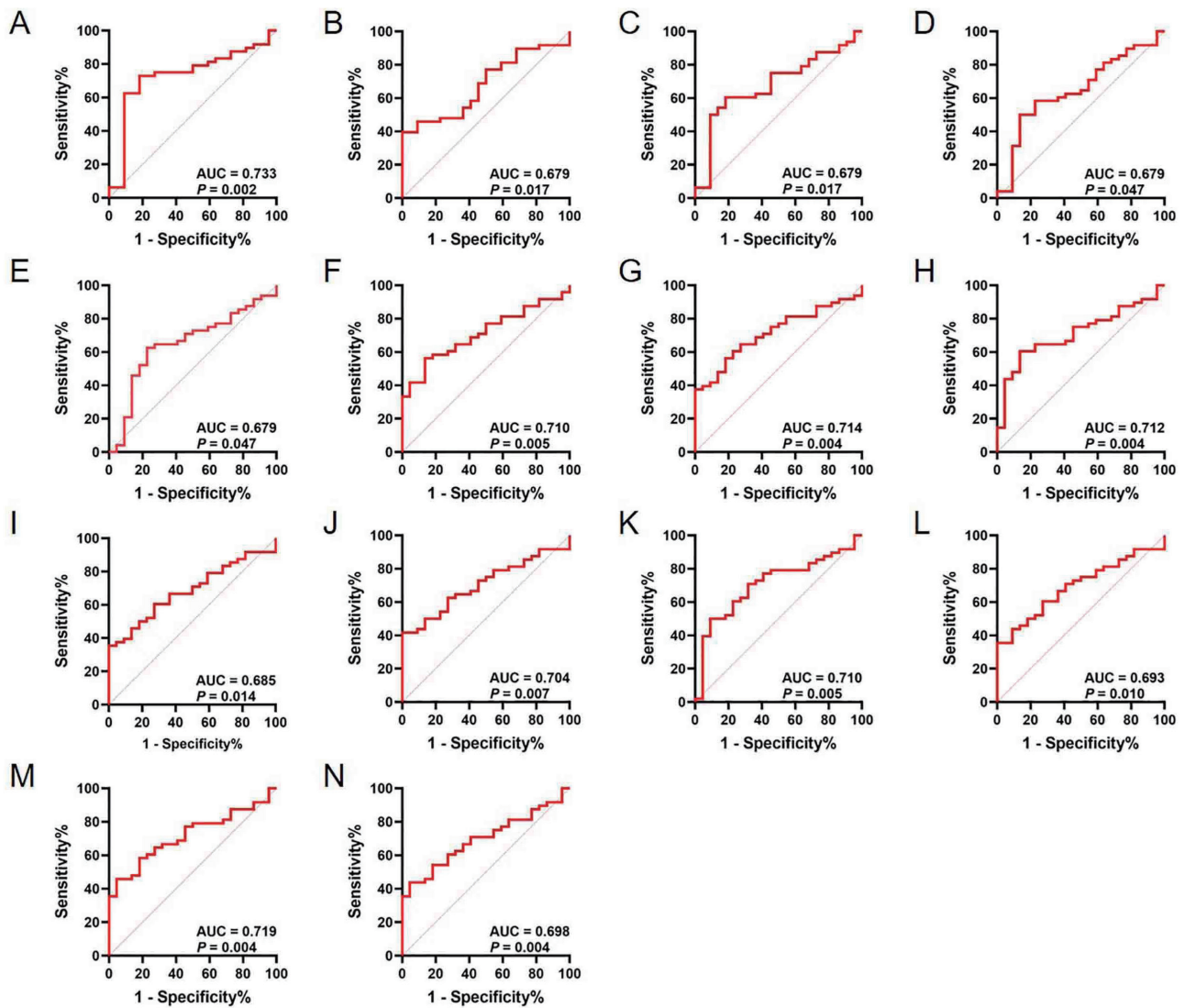
Figure S5 ROC analysis for assays based on different combinations of genes. (A) *DCX*; (B) *DDC*; (C) *PHOX2B*; (D) *CHGA*; (E) *TH*; (F) NB5; (G) NB5 minus *CHGA*; (H) NB5 minus *DDC*; (I) NB5 minus *DCX*; (J) NB5 minus *PHOX2B*; (K) *TH/DCX*; (L) *PHOX2B/TH/DDC*; (M) *PHOX2B/TH/DCX*; (N) *PHOX2B/TH*. AUC, area under the curve; ROC, receiver operating characteristic; *DCX*, doublecortin; *DDC*, dopa decarboxylase; *PHOX2B*, paired-like homeobox 2b; *CHGA*, chromogranin A; *TH*, tyrosine hydroxylase; NB5, five neuroblastoma genes (*CHGA, DCX, DDC, PHOX2B,* and *TH*).