

Serum extracellular vesicle-derived circHIPK3 and circSMARCA5 are two novel diagnostic biomarkers for Glioblastoma Multiforme

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Background

- Glioblastoma multiforme (GBM) is the most frequent and deadly human brain cancer.
- Early diagnosis through non-invasive biomarkers may render GBM more easily treatable, improving the prognosis of this currently incurable disease.
- We propose the use of serum extracellular vesicle (sEV)-derived circular RNAs (circRNAs) as highly stable, minimally invasive biomarkers GBM diagnosis.

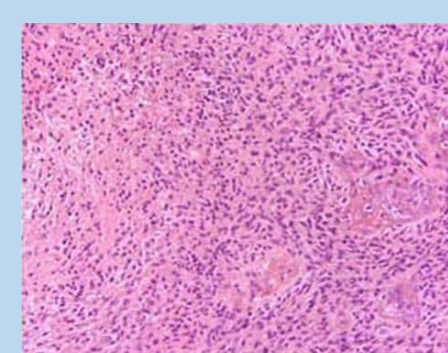


Fig. 1. Histopathologic slide demonstrating a GBM (<https://emedicine.medscape.com/article/283252-overview>)

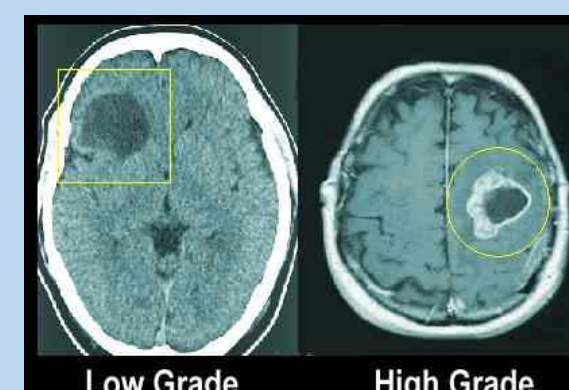


Fig 2: MRI scan of glioma and GBM (http://www.aboutcancer.com/mri_gbm.htm)

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Results

- CircSMARCA5 and circHIPK3 were significantly less abundant in sEVs from GBM patients with respect to UC (fold-change (FC) of -2.15 and -1.92, respectively) and GIII (FC of -1.75 and -1.4, respectively).
- Receiver operating characteristic curve (ROC) analysis, based on the expression of sEV-derived circSMARCA5 and circHIPK3, allowed to distinguish GBM from UC (area under the curve (AUC) 0.823 (0.667-0.979) and 0.855 (0.704 to 1.000), with a 95% confidence interval (CI), respectively).
- Multivariable ROC analysis, performed combining the expression of sEV-derived circSMARCA5 and circHIPK3 with preoperative neutrophil to lymphocyte (NLR), platelet to lymphocyte (PLR) and lymphocyte to monocyte (LMR) ratios, three known diagnostic and prognostic GBM markers, allowed to improve GBM diagnostic accuracy (AUC 0,901 (0,7912 to 1,000), 95% CI).

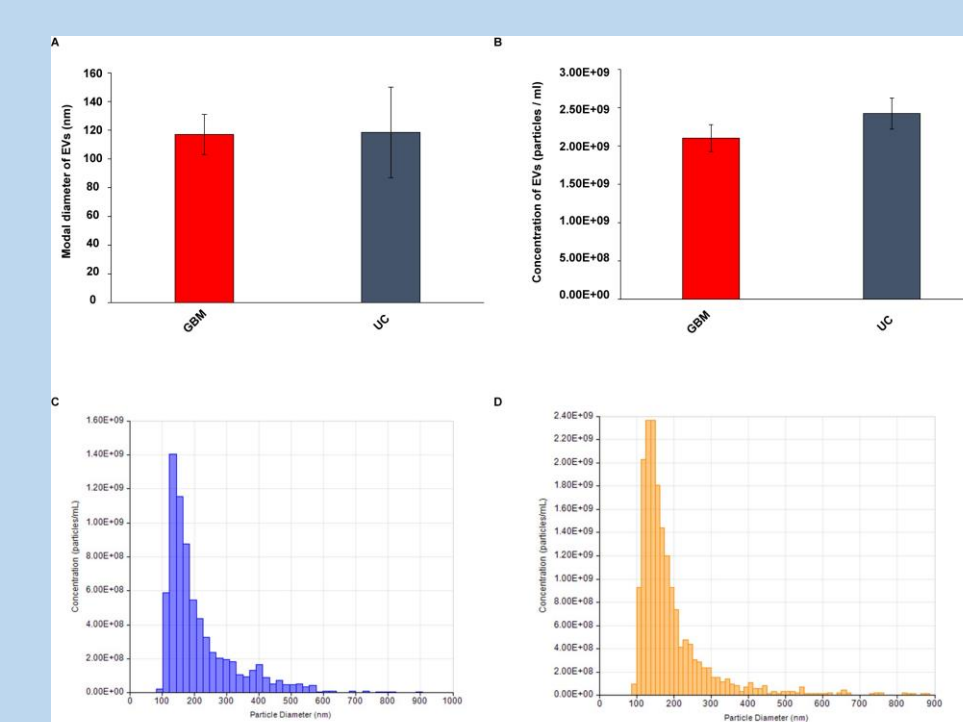


Figure 3. Bar graphs showing the modal diameter (nm) and concentration (particles / ml) of EVs isolated from UC (C) and GBM (D)

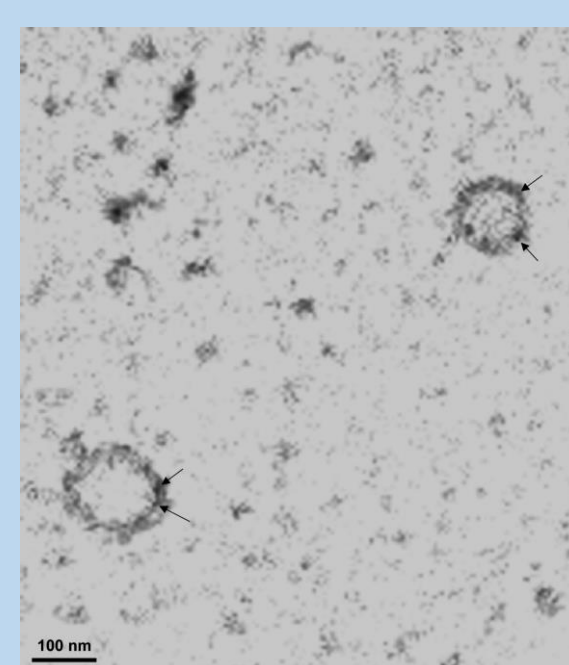


Figure 4. Transmission electron microscopy (TEM) of isolated sEVs positive to the tetraspanin CD81

circBase ID	exonBase ID	Gene symbol	Median expression in serum EVs from healthy individuals (RPM)	Differential expression (glioma tissue vs normal brain parenchyma)
hsa_circ_0001445	exo_circ_000006	SMARCA5	16237.253	Downregulated (PMID: 29415469; 30736462)
hsa_circ_0000284	exo_circ_000027	HIPK3	3193.315	Upregulated (PMID: 30057315)
hsa_circ_0001009	exo_circ_000142	FANCL	1448.15	Downregulated (PMID: 26873924)
hsa_circ_0001730	exo_circ_000064	EPH4	503.7355	Upregulated (PMID: 29921068)
hsa_circ_0003496	exo_circ_000134	UBAP2	375.249	Upregulated (PMID: 29920451)
hsa_circ_0122319	exo_circ_000449	PLOD2	339.669	Upregulated (PMID: 26873924)
hsa_circ_0008386	exo_circ_000476	LRRFIP2	309.193	Downregulated (PMID: 29920451)
hsa_circ_0000915	exo_circ_000422	FKBP8	225.519	Downregulated (PMID: 26873924)
hsa_circ_0074371	exo_circ_000452	ARHGAP26	174.8015	Upregulated (PMID: 30388059)
hsa_circ_0001819	exo_circ_001051	UBR5	159.979	Upregulated (PMID: 29920451)
hsa_circ_0000199	exo_circ_000834	AKT3	105.7865	Downregulated (PMID: 26873924)

Table 1. Candidate DE circRNAs in gliomas and their expression in sEVs from healthy individuals according to exonBase (<http://www.exonbase.org/exonBase/tolIndex>) (RPM = reads per million)

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Methods

- EVs were isolated by size exclusion chromatography from sera of 23 GBM and 5 grade 3 glioma (GIII) patients, and 10 unaffected controls (UC).
- The expression of two candidate circRNAs (circSMARCA5 and circHIPK3) was assayed by droplet digital PCR (ddPCR).

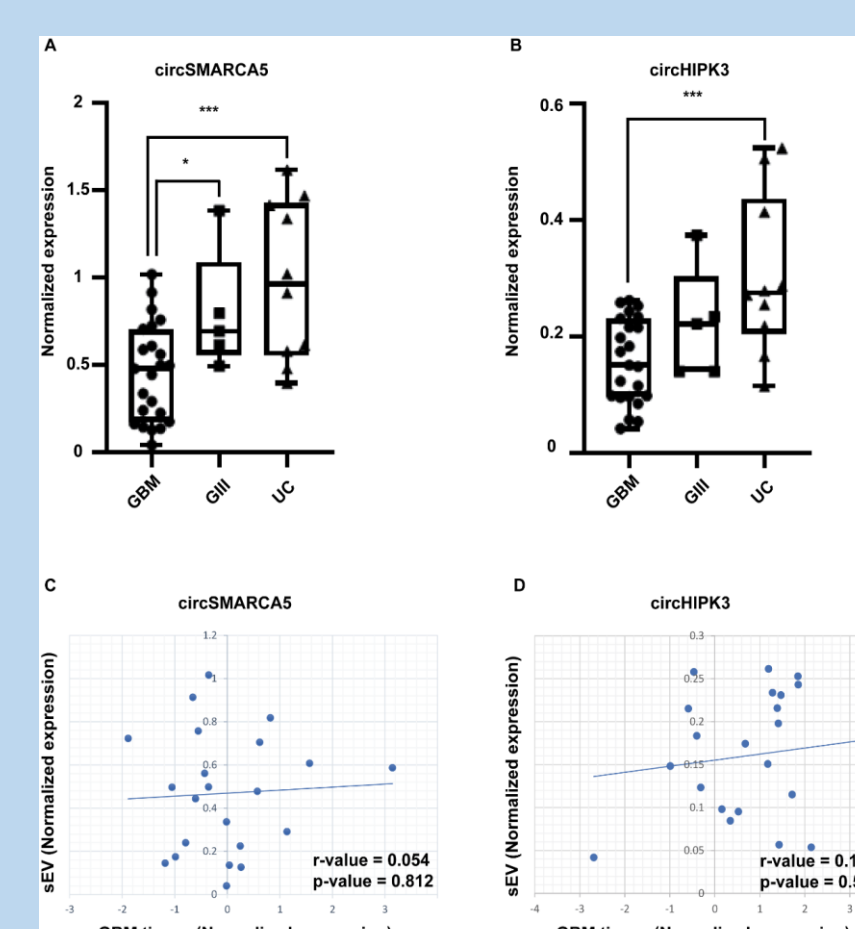
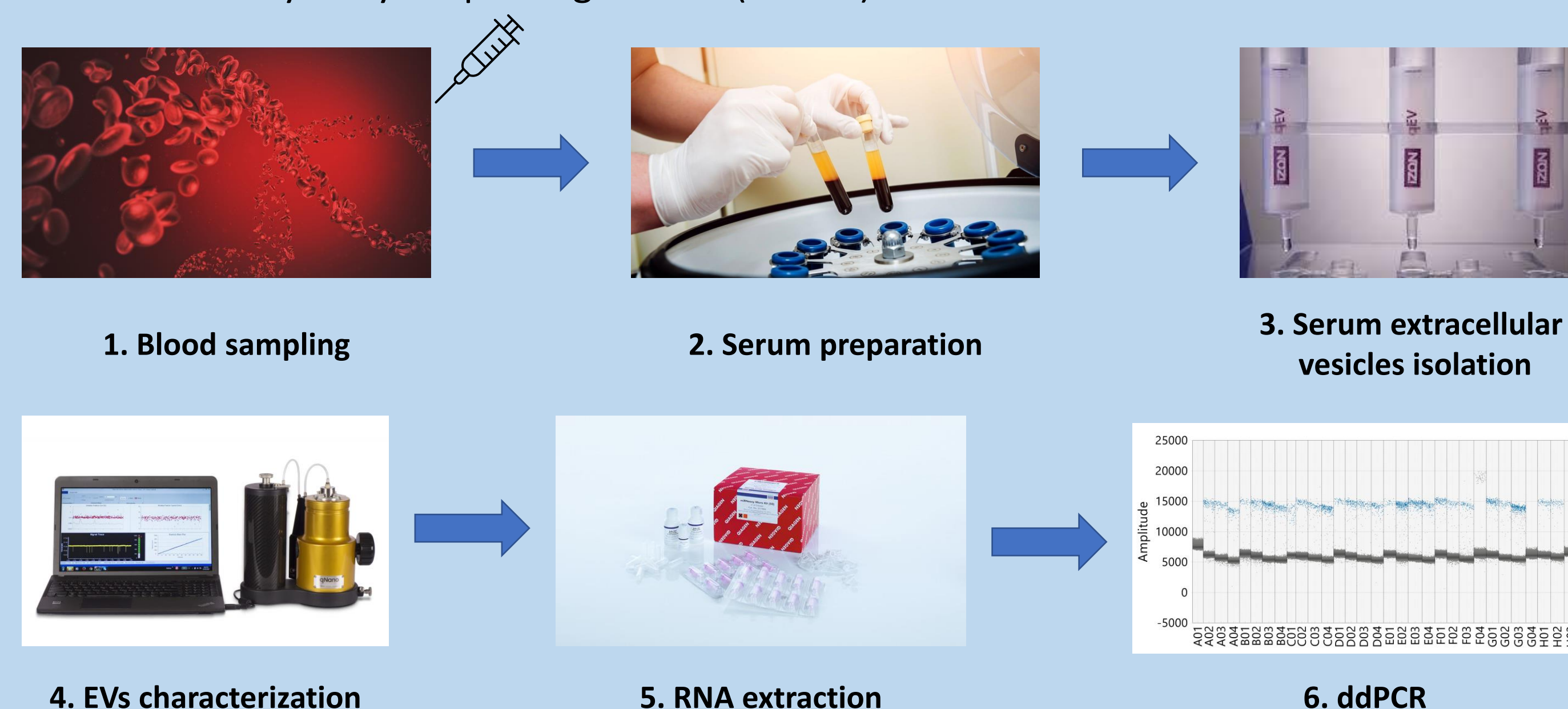


Figure 5. CircSMARCA5 and circHIPK3 were significantly less abundant in sEVs from GBM patients with respect to UC and GIII

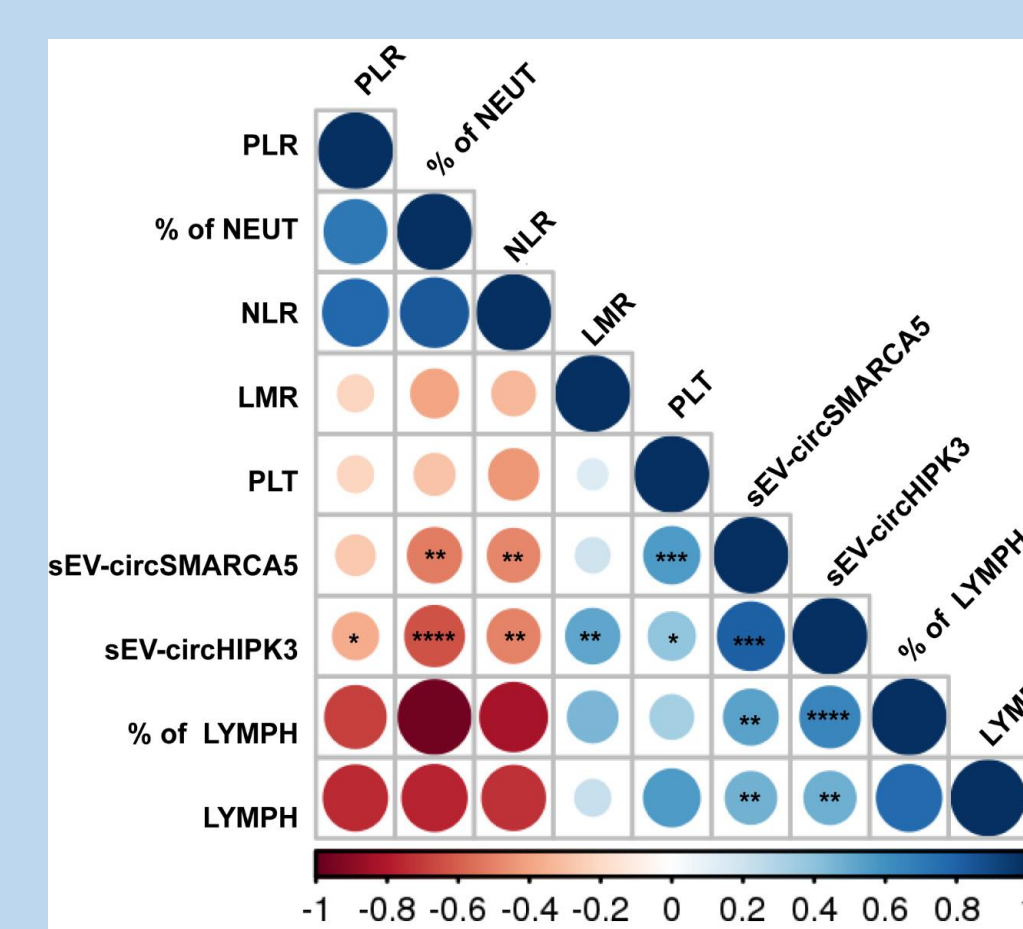


Figure 6. Corrologram showing correlations between the expression of sEV-derived circSMARCA5 and circHIPK3 and hematological data

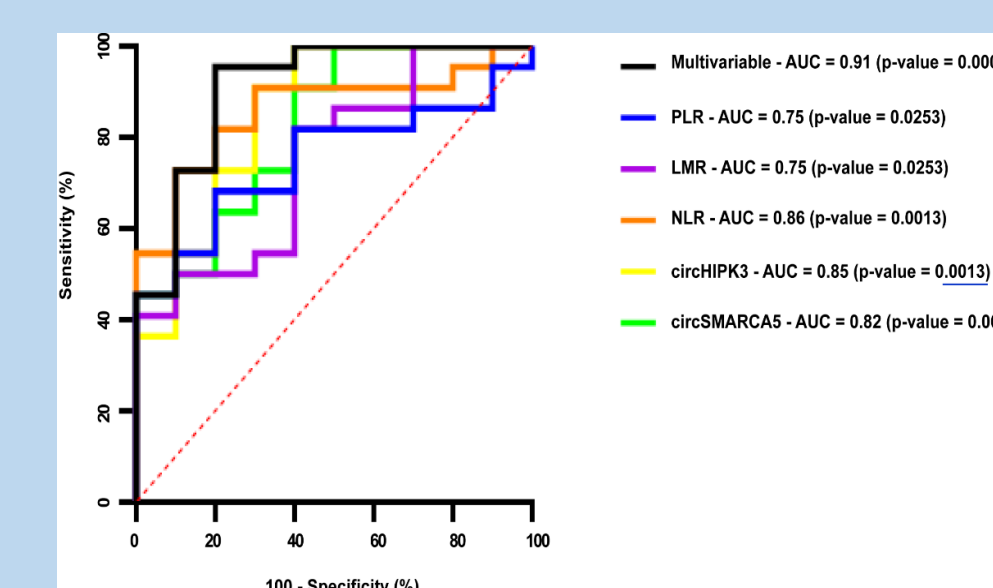


Figure 7. Univariable and multivariable receiver operating characteristic (ROC) curve analysis

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Conclusions

- Our data convincingly suggest sEV-derived circSMARCA5 and circHIPK3 as new good diagnostic GBM biomarkers, especially when combined with preoperative NLR, PLR and LMR data.

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