

TRIM28 selective nanobody reduces glioblastoma stem cell invasion

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TABLES

Table S1: List of TaqMan® assays used for RT-qPCR analysis (Thermo Fisher Scientific, USA).

Probe name	Gene name	assay ID	assay type	Cat.number
GAPDH	GAPDH	Hs99999905_m1	FAM-MGB, S(250rxns)	4331182
HPRT1	HPRT1	Hs02800695_m1	FAM-MGB, XS(75rxns)	4453320
TRIM28	TRIM28	Hs00232212_m1	FAM-MGB, XS(75rxns)	4448892
COL1A2	COL1A2	Hs01028956_m1	FAM-MGB, XS(75rxns)	4331182
COL1A	COL1A	Hs00164004_m1	FAM-MGB, XS(75rxns)	4331182
TGFBI	TGFBI	Hs00998133_m1	FAM-MGB, XS(75rxns)	4331182
THBS1	THBS1	Hs00962908_m1	FAM-MGB, XS(75rxns)	4331182
DAB2	DAB2	Hs01120074_m1	FAM-MGB, XS(75rxns)	4331182
S100A4	S100A4	Hs00243202_m1	FAM-MGB, XS(75rxns)	4331182
P2RX7	P2RX7	Hs00175721_m1	FAM-MGB, XS(75rxns)	4331182
STMN4	STMN4	Hs00229288_m1	FAM-MGB, XS(75rxns)	4331182
SOX10	SOX10	Hs00366918_m1	FAM-MGB, XS(75rxns)	4331182
HER3	ERBB3	Hs00176538_m1	FAM-MGB, XS(75rxns)	4331182
ACSBG1	ACSBG1	Hs00209500_m1	FAM-MGB, XS(75rxns)	4331182
KCNF1	KCNF1	Hs00266908_s1	FAM-MGB, XS(75rxns)	4331182
ID1	ID1	Hs03676575_s1	FAM-MGB, XS(75rxns)	4453320
SOX2	SOX2	Hs01053049_s1	FAM-MGB, XS(75rxns)	4453320
CD44	CD44	Hs00174139_m1	FAM-MGB, S(250rxns)	4331182
OLIG2	OLIG2	Hs00377820_m1	FAM-MGB, XS(75rxns)	4331182

Table S2: Characterisation of GB sample subtypes based on expression of the 15 genes.

Sample	Subtype	COLIA 2								ACSBG 1						
		COL1A	TGFB1	THBS1	DAB2	S100A4	P2RX7	STMN4	SOX10	ERBB3	KCNF1	OLIG2	NOTCH	NF-KB		
s001	CL	0,0107430,037266	2,11509	0,0390420,7604530,5504670,910709	0,54942	0,2535180,164841	1,6422657,522426	1,840483	1,398655	1,044742						
s002	CL	0,0224970,081461	1,2246710,063549	1,4223150,928091	0,937	0,8068890,2002760,304841	2,4364956,4991482,079257	1,87655	0,804075							
s003	CL	0,030444	0,06344	1,2279040,3862130,578327	1,0475430,4812170,3053060,0116550,048439	3,86452	20,13352	1,745498	1,6082180,973727							
s004	CL	0,7027521,4666381,8281780,170579	1,45851	1,5111080,4960750,1444970,0150170,0544855,1867152,2853760,626281	2,4901120,952573											
s005	CL	0,0167120,030106	1,1964950,040828	0,28123	1,2001770,2405010,0371460,0011870,008603	2,968765	15,214240,3720590,7721490,633171									
s006	CL	0,0177040,028778	1,4955440,024088	1,604888	1,900744	2,15123	2,446527	1,3263970,263375	2,919774	1,545422	5,842775	3,960549	1,391409			
s007	mix	0,876903	1,77348	1,4517370,681141	1,3165310,870173	0,38851	0,5728740,1000160,0589730,456403	0,91292	NA	0,85655	1,937338					
s008	mix	0,0269650,1066240,7349120,090394	1,5066250,8867890,4192850,1080250,0268520,0284920,544383	2,298997	0,17622	0,455887	1,050222									
s009	PN	0,0135690,025849	0,92241	0,2360471,2496941,262799	1,5157414,1670970,0591740,131645	1,62002	1,267204	40,86526	5,56947	1,702867						
s010	CL	0,00773	0,059795	1,7229490,1266740,9455030,664747	1,4634610,9485590,2463360,085554	2,1928447,011375	1,430553	3,337731	2,684161							
s011	CL	0,0020460,0115930,9921690,015331	1,0287520,6965690,5302030,6418380,030463	0,14358	2,45679	6,957769	2,784954	2,206164	1,554648							
s012	mix	0,034775	0,01097	0,5020310,0227020,2145020,3742880,763597	3,2220490,5509350,8188090,6424570,569106	1,864008	2,1712680,818807									
s013	mix	0,005647	NA	0,4965430,005452	0,13519	0,206847	1,5007	1,206942	1,328509	10,280740,9340380,0836090,3450650,690321	1,466832					
s014	CL	0,1207810,297379	1,2521420,525453	2,9368470,7844450,985969	NA	0,01017	0,430233	1,722773	2,8211660,407732	0,74736	1,732473					
s015	mix	0,1446140,1217220,6535080,1166340,488642	1,0061930,1118750,009543	NA	0,016811	1,156279	0,40288	0,252031	0,5576990,834504							
s016	PN	0,0106220,004869	3,6963730,009845	1,6885230,752452	2,549226	14,418296,100389	1,453963	4,859812	3,311124	5,994735	3,075015	2,317953				
s017	CL	0,0354570,153516	1,8549530,083594	2,798036	2,9817410,7923810,2358940,1526770,131058	2,474198	3,187105	1,381073	2,367617	2,13069						
s018	CL	0,0623380,345172	1,285005	0,16959	1,568448	0,83846	0,706737	1,474376	0,86961	0,229355	3,621238	3,9642120,415488	1,006912	1,569175		
s019	CL	0,1243250,064741	2,0933450,058946	1,948841	2,531989	1,190443	1,156693	0,00436	0,91481	3,001199	3,404616	5,88396	1,784224	1,976168		
s020	PN	0,0049220,003056	2,8422880,004971	3,9946080,088696	2,242342	4,99102	0,887788	1,646237	3,558184	2,023709	17,34632	4,262166	2,908399			
s021	mix	0,0198650,0075690,886218	0,05875	0,2389850,7245280,9033310,688064	1,1874970,856964	1,268091	2,512177	3,29844	1,642758	0,854799						
s022	PN	0,0626920,0055460,5643920,0872510,4517440,452948	2,559271	3,005958	29,78835	16,23366	1,21094	0,56174	3,021844	1,654655	1,041388					
s023	mix	0,0447340,0027060,7387940,0355330,159227	1,0262140,832442	3,023443	1,94376	2,3705060,920665	2,031218	2,373847	1,42014	1,023346						
s024	CL	0,1480140,144261	0,98368	0,134876	0,2081	1,108735	1,9222130,9637110,248148	5,380735	2,909518	8,918588	2,123012	3,469878	1,980662			
s025	CL	0,3686010,0210310,6646640,0759730,238224	2,012019	0,45911	0,1867210,055642	0,47459	1,262785	1,9553350,389367	1,65283	3,540112						
s026	mix	0,1461190,0067020,5062810,330752	2,471195	0,76193	0,094952	NA	0,0002410,0000010,0467270,0338860,0001060,050205	0,40693								
s027	MES	3,5486094,020323	5,152646	4,803758	2,5145860,3285640,1733160,0014880,0137090,037275	0,1615270,5512010,0072620,3296160,780085										
s028	MES	2,808029	NA	1,843538	3,911333	4,544921	1,3096280,1268060,0017990,060601	0,09824	0,067794	NA	0,0002230,1451820,855701					
s029	mix	3,284406	1,737785	1,89136	3,419077	1,2305990,4290040,390276	NA	0,0140470,0473770,428341	0,02842	0,0005530,432634	1,02534					
s030	MES	0,8753050,787815	1,233981	10,17887	1,906662	1,1186110,9448180,024995	NA	0,1455130,988446	0,18414	0,0090330,397877	1,840235					
s031	mix	0,0000530,0002880,1286330,0001060,0047050,0377030,5573280,016812	3,5672810,8872090,3000950,271744	3,148641	0,952609	1,218073										
s032	mix	NA	0,0022570,1348760,0005050,0287990,0234570,915631	0,11737	3,7842930,3124520,0121440,3950120,827448	0,6305090,332666										
s033	mix	0,0008570,0007010,1548580,0020030,1605560,0876850,5266430,002081	2,4457740,4099920,1851620,181809	1,1670680,3788270,760749												
s034	mix	0,0008410,001048	2,0753620,0151420,1306680,0253530,1168450,0053210,0000160,0019190,053126	1,947658	1,5825360,229594	0,59781										
s035	CL	0,0692130,086003	2,9629440,058371	0,61556	0,4570940,2827990,8232460,1030530,0418780,095625	1,697724	1,266418	1,401271	5,189586							
s036	mix	0,1032840,0001640,4057010,001475	0,07905	0,0142560,6695060,1438950,0600160,360519	0,39504	1,21738	0,825365	1,3249220,741346								
s037	mix	0,0174630,0010180,3011040,0109980,0968980,625959	1,006514	3,498168	3,899864	4,359523	2,18127	1,724127	1,648217	1,58282	0,72771					
s038	CL	0,2188690,024723	1,5169120,3742830,823548	2,0851	0,8225	0,566622	2,0951910,832297	1,5333870,645374	0,27235	1,318332	1,35806					
s039	CL	0,1291950,363283	2,40977	0,159954	3,862967	1,2645440,725185	3,820558	1,2863290,345714	2,26798	0,23963	0,35801	0,812023	1,724255			
s040	CL	0,1361	0,153832	1,6182580,108231	1,589296	2,166598	1,657394	NA	2,364336	1,4372680,7508890,313309	2,766898	2,3886430,951594				
s041	MES	10,207548,061938	1,5770880,7092024,9320830,9350340,5417060,0103870,001263	NA	0,005287	2,81084	NA	0,4470960,904167								
s042	mix	0,79876	2,247731	2,359017	1,8288870,5410630,7642740,4385180,029358	0,00888	0,112456	1,180543	2,7863720,845132	1,0840140,953197						
s043	mix	0,4911720,3422630,7890670,1991070,5348450,4177540,634038	1,013619	0,3305	0,2515650,713472	1,199158	3,609955	0,7230670,689825								
s044	mix	0,0964460,170519	0,35207	0,120508	0,14781	0,0833810,4620040,551022	1,540362	1,0514730,834152	1,0212050,705231	0,3846850,301943						
s045	PN	0,1772150,3868610,4502850,222484	0,45718	0,1297170,883595	1,50923	4,345073	3,9234730,5000910,949333	7,825623	6,2913760,666993							
s046	mix	1,440269	1,7534060,7295380,2670220,1532270,2640990,3912980,3510190,0264140,7010120,950706	1,161139	1,832965	1,6778180,793034										
s047	mix	0,0226280,041685	0,75995	0,1518660,395881	0,33706	0,69415	0,288272	2,27368	0,5006170,1067290,151358	1,465795	1,233821	1,702291				
s048	mix	0,5058270,398706	1,1607990,0383050,4485060,4275170,6851120,184248	NA	0,168631	3,0698180,618881	2,502464	1,247276	1,162164							
s049	mix	0,0874670,575067	0,272	0,0834280,0811680,2071710,2333690,0517010,0059950,1068550,295024	1,145973	1,318919	2,4098820,679848									
s050	mix	0,612476	0,58487	1,143947	2,336123	1,5509480,3999270,8622970,3202090,5717660,2717830,078308	0,08372	0,3811780,212951	1,313305							
s051	CL	3,169283	1,151673	1,048953	3,978698	1,217193	1,4196390,5606780,3206130,0276580,082101	0,37633	0,1817670,1705770,799669	3,090328						
s052	PN	0,111348	0,12249	0,2725170,1295910,1255370,107342	2,005383	0,87984	9,389925	3,700291	0,22763	0,460295	8,984523	1,797205	0,68204			
s053	MES	16,2825	2,273361	1,738287	4,221793	1,5470180,7240090,9530690,2510270,2330990,2612860,8808920,544448	1,172891	1,905386	1,159295							
s054	MES	9,936406	6,195340	8,15623	1,32067	0,4505170,8971990,4285530,0984840,059288	NA	0,7118290,0278960,230391	0,318308	0,650022						
s055	mix	0,2174520,1202480,2110680,1392290,1767350,1344910,0890680,0023350,0079960,0061370,0702310,1142010,0219530,1434180,760049														

s056	PN	0,8622981,1928131,0291281,1398291,1681310,3396352,739298										NA	7,9938638,4902830,566048										1,26396	3,58491	1,1725351,033517								
s057	mix	0,1810430,1908970,6869663,1690780,5245750,4015921,1609810,3630362,0575891,0415210,1280550,056239										3,77515	0,88432										0,710062										
s058	mix	0,2624770,6969141,1010540,3870381,3104280,5584710,5768510,2668110,5935520,5247270,7467542,1667971,5751171,1353410,855542																															
s059	mix	0,0823980,2379250,6642620,0465850,2993830,366682										0,33153	0,0665440,0000090,1234221,2556113,0472291,6258211,8645910,889711																				
s060	mix	1,0745742,631891										0,80808	2,7342280,7195070,4607081,2376930,4204690,0157280,7470950,7650161,4583192,0843832,0679021,748391																				
s061	mix	0,208731										NA	1,0125831,0629961,0716550,5357992,1906921,1513234,6352344,1208880,6681730,1834482,5922211,1107530,913997																				
s062	mix	0,385735										NA	0,426794										4,00968	0,6026370,2485510,316615	0,06627	0,0545720,124204	0,62503	0,2531690,2831680,3553410,570901					
s063	mix	0,036391										NA	0,0784350,1723570,1344010,0459530,0872330,274462										NA	0,0028750,0037260,0042550,0034530,0281040,614451									
s064	mix	0,0841880,0624390,3545350,0629080,1592560,1354860,526629										0,56925	0,930005										0,48103	0,6116750,2978041,3234760,6414020,506385									
s065	mix	1,1843161,8664291,075497										0,57469	0,6025350,2851970,2191730,0106850,0142710,0579140,1878670,2096850,0195960,2292710,680522																				
s066	mix	0,0508280,2144990,7333150,1739041,1904590,1287591,7554580,545233										1,05903	0,7327070,8085370,1621752,5923591,2594270,573841																				
s067	mix	0,3438741,0665141,0983541,8509361,9882190,5720030,5955680,0556020,2479260,176846										0,25176	0,0755450,1166370,2817020,641783																				
s068	mix	0,2308620,6809950,8411270,0815990,5129260,4349641,6520730,1259410,0198680,0884350,7505030,0836950,4185190,341346										0,96208																					
s069	mix	0,066743										NA	0,0972140,1799120,1499570,0331241,2162940,3127250,2592741,0546110,164098										0,03663	0,3060470,6229130,299773									
s070	mix	0,0713980,3131730,1927930,1487570,1869740,0404770,3392360,2081870,1223380,3843241,012747										0,92193	0,7765560,2919940,565369																				
s071	mix	1,3306										NA	0,8508723,0882791,2895360,6884041,9440810,4251860,030598										3,25883	0,7217031,2156013,594798	1,68744	1,030808							
s072	mix	0,0695470,2433430,6030770,2837190,3907080,4287970,6851050,3474340,3430340,6694470,5908910,6641330,7747170,4487130,519476																															
s073	mix	0,1321690,3630410,1484620,4217120,369017										0,09637	1,7423370,8448655,0760476,2732820,0870820,1151067,0389621,0815010,579174																				
s074	mix	0,0427210,0939670,1522660,2250730,1205980,0592340,5755410,3742522,7487572,5608170,1026630,1384272,0892981,019025										0,44415																					
s075	mix	0,0993210,1272210,7774680,0461020,1074860,1646550,5285060,2417650,0648080,1287633,3145694,2830572,8259230,8299320,707406																															
s076	mix	1,0738561,1065691,0440490,7154080,3949430,7738330,6204670,2283150,3046540,2575420,973971										0,63288	0,4769060,8409080,776479																				
s077	CL	0,0873880,041475										1,15721	0,0061680,2382190,1536791,4106680,8406410,2099830,2444111,7933843,1234040,0531034,3612581,489326																				
s078	mix	0,1629630,4705151,0089450,6841581,4360050,7794551,3569621,9359892,9868932,104729										1,00126	0,1151110,8537150,8611460,865285																				
s079	mix	0,0447680,0964330,5228920,0841850,2895270,1089590,2504010,3183560,0117430,0887941,1348812,4874530,8380221,0409660,642422																															
s080	mix	0,0044810,0036280,2215740,0115420,0589260,0160690,8457471,2871072,194599										1,73867	0,61123										0,4662980,7519170,1735310,252088										
s081	mix	0,2656820,9840222,4454763,4790370,9045490,713911										NA	0,0293890,0154610,0229290,3847551,7287240,1370220,2992330,640945																				
s082	mix	0,2487080,1499750,4014890,0322010,1242140,0514410,3759181,107428										0,78606	0,76698										1,4633180,5964350,3272140,6244010,235083										
s153	mix	1,8137030,233101										1,28324	0,3360450,5537270,1853770,9887890,7728573,6699290,8208311,1466120,2357052,4694131,5147540,703353																				
s154	mix	0,1838290,0456751,0917570,0774920,2279110,1148591,2548260,4695081,1181440,7437471,1572490,3472080,9848010,4250860,390933																															
s156	CL	0,3407220,876858										2,52071	0,1511441,4067242,9431651,0233880,4596780,1297540,4641131,0234912,3557471,4681883,1784591,177528																				
s157	mix	0,1150330,2562990,9591450,7876530,4150180,219543										0,54452	0,5999250,2853460,9360320,3625520,6782121,5299260,9999260,457483																				
s158	mix	0,7195990,7421030,9116271,8918730,7539080,3903170,435278										0,58244	0,2564910,2109210,2048940,2559190,3356541,1691410,823789																				
s159	mix	1,3886170,5184420,9062610,0909190,3645210,2219140,7119780,598255										0,17056	0,6859241,0606471,5598441,5834842,1469480,755996																				
s160	mix	0,0839550,4234221,6638940,7386150,622259										0,31139	0,3850450,0814280,0834640,4129620,0831640,2828490,5039910,3171860,740798																				
s161	PN	0,083971										NA	2,3428120,1974551,2828310,1918044,5601778,29931710,959466,0128981,5164550,072288										3,82944	0,4062820,754017									
s162	mix	0,4094770,8073390,8442710,9182761,0081170,206438										0,82124	0,4944842,3872280,7084970,2298470,1279071,2993690,4291360,893916																				
s163	mix	0,0795050,2663160,6530070,0865170,1911370,1851980,527909										1,67599	0,4443640,2707790,2391241,3383451,3400010,4918210,266051																				
s164	PN	0,0814270,3853981,772612										0,0485	0,3410120,0654163,6532934,9335026,6212325,9727831,1740620,153662										2,4633	0,3253190,742077									
s178	MES	14,1921626,212121,5633111,0121461,0021561,4463520,3576250,0813740,0490580,0499930,0259450,784206										0,00723	0,3464510,678689																				

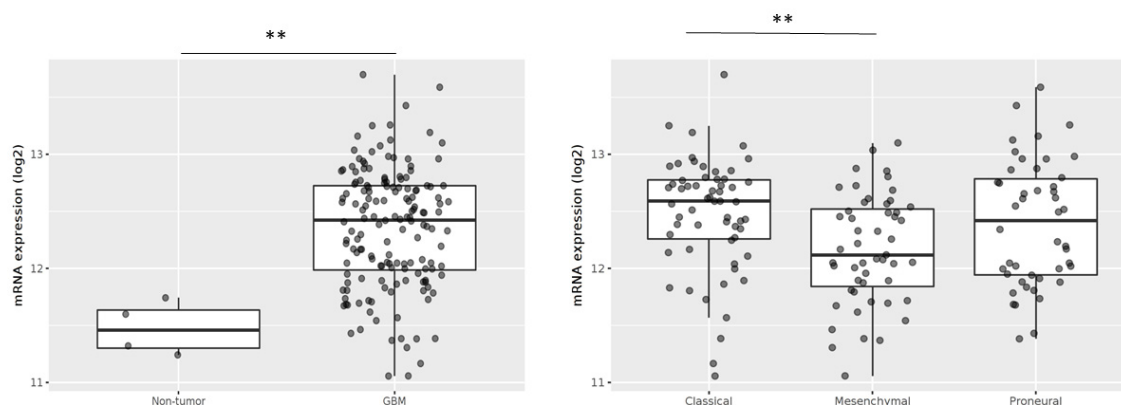


Figure S1. *TRIM28* gene expression in glioma tissues and GB tissue subtypes. *TRIM28* mRNA expression levels were determined in GB (n=156), non-cancerous brain tissues (N, n=4) and in GB subtypes CL (n=59), PN (n= 46) and MES (n=51). Expression *TRIM28* values were analysed and visualised in TCGA GlioVIS database. (<http://gliovis.bioinfo.cnio.es/>). Tukey's post hoc test was used for multiple comparison between groups with significance levels (** P < 0.01).

Histology: GBM; Subtype: All; Cutoff: median

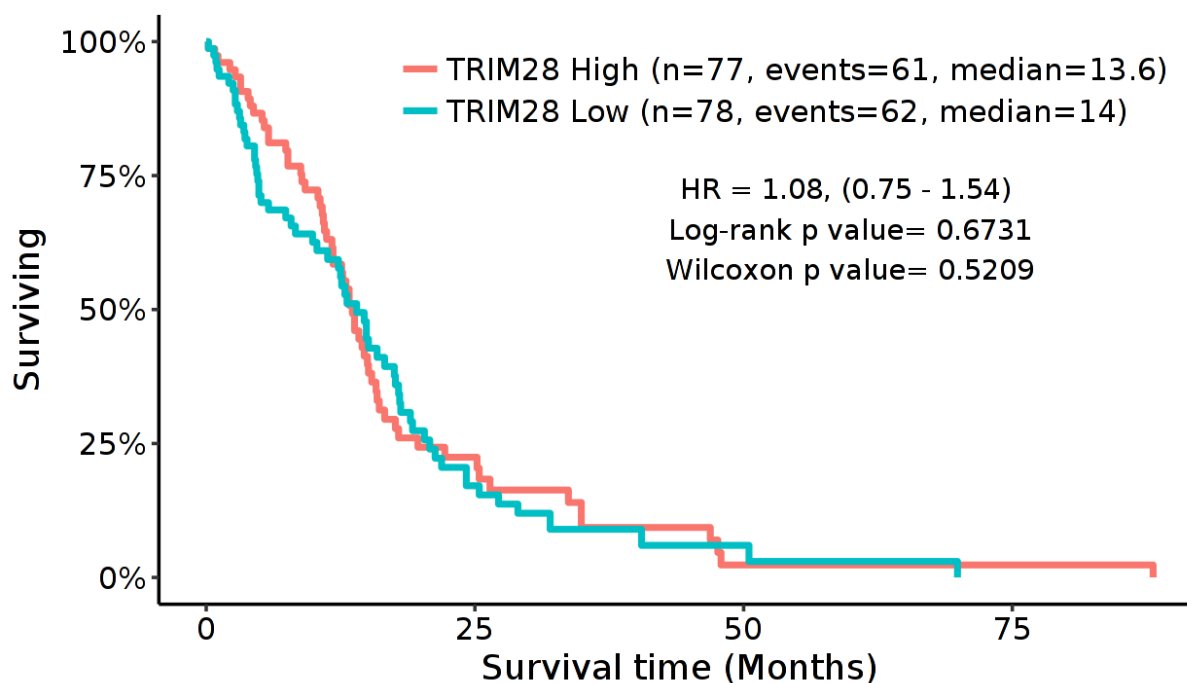


Figure S2. Kaplan-Meier survival curves for a cohort of 155 GB patients with high (red) and low (blue) expression of *TRIM28* based on median gene expression value analysed and visualised in GlioVIS database.

(<http://gliovis.bioinfo.cnio.es/>). *TRIM28* is not related to survival rate in this validation cohort of patients, similar to our experimental cohort (Fig.3).

KM analysis

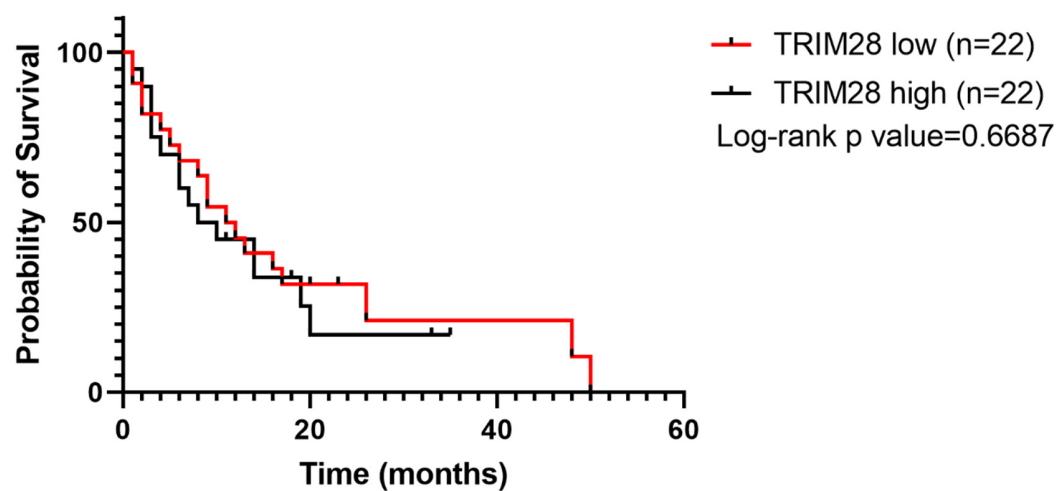


Figure S3. Kaplan–Meier survival curves for the two-quartile cut-off high/low *TRIM28* expression in the top/bottom 25% of the samples. Kaplan–Meier survival curves for a cohort of 44 GB samples with high (black line)/low (red line) *TRIM28* expression in the top/bottom 25% of the samples. *TRIM28* expression is not related to survival rate in our cohort of patients. Kaplan–Meier survival curves analyses were performed using GraphPad Prism software.

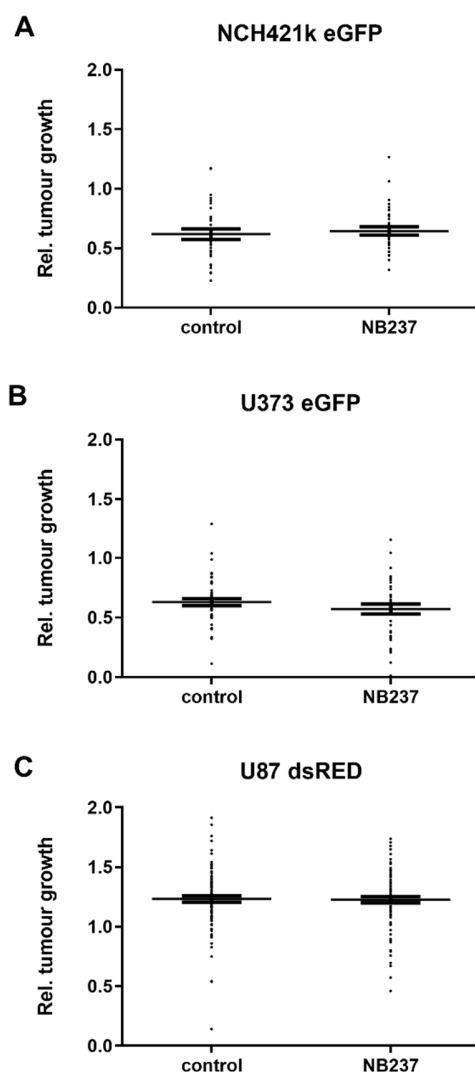


Figure S4. In vivo imaging of tumour growth in the brain of zebrafish embryos. Embryos 72h after the xenotransplantation of (A) NCH421k eGFP, (B) U373eGFP and (C) U87DsRed in the brain (visible as red and green fluorescence) without (control) or with the TRIM28 nanobody NB237. Data are shown as means \pm S.D., dots on graphs A, B and C represent each embryo. Fluorescence intensity of GB xenograft at 72h was normalised to fluorescence intensity of GB xenograft at 24h after xenotransplantation to determine relative tumour growth. Number of embryos in control and with NB237 in the experiment with U373 n=37-50, U87 n=94-108, NCH421k n=13-38. Statistical analyses were performed using GraphPad Prism software, using Mann–Whitney test.