

Supplementary information for

“GLIOBLASTOMA CELL MOTILITY AND INVASION IS REGULATED BY MEMBRANE-ASSOCIATED HEAT SHOCK PROTEIN HSP70”

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Table S1. Characteristics of patients with High-Grade Gliomas.

Patient code	Age (years)	Sex	KPS before surgery	Dexamethasone before surgery (mg)	Tumor site (lobe)	Hemi-sphere	Histology	MIB-1 index (%)	FGS
Adult patients									
ANI	57	male	70	12	frontal lobe	left	GB IDH-wt	20	no
GSN	44	male	60	12	frontal lobe	right	GB IDH-wt	35	no
Pediatric patients									
IBD	11	male	70	1	thalamus	left	pHGG	20	no
TMS	3	female	70	1	temporal	left	pHGG	70	yes

Notes: KPS – Karnofsky Performance Scale; GB – glioblastoma; wt – wildtype; pHGG – Diffuse paediatric-type high-grade glioma; FGS – Fluorescence-Guided Surgery with 5-aminolevulinic acid.

Table S2. The quantitative content (%) of mHsp70^{High} and mHsp70^{Low} subpopulations of primary glioblastoma cells based on gating by FACS.

Primary cells	mHsp70 ^{High} , %	mHsp70 ^{Low} , %
ANI	8.0 ± 4.6	18.3 ± 12.9
GSN	16.5 ± 2.1	17.0 ± 2.8
IBD	16.3 ± 4.9	18.8 ± 13.3
TMS	19.7 ± 1.5	21.0 ± 3.5

Table S3. Median ± 95% confidence interval of invaded ANI, GSN, IBD, TMS cell (%) in control group and treated with 1 µM PES or 50 nM JG-98.

Cell culture	Group	N _{images}	Median ± [95% CI]
ANI	Control	60	90.00 [75.00; 105.0]
	+PES	60	60.00 [45.00; 75.0]
	+JG-98	60	45.00 [30.00; 60.0]
GSN	Control	60	83.17 [71.29; 106.9]
	+PES	59	35.64 [35.64; 47.53]
	+JG-98	60	59.41 [47.53; 71.29]
IBD	Control	59	73.68 [50.00; 136.8]
	+PES	60	50.00 [36.84; 71.05]
	+JG-98	60	47.37 [31.58; 78.95]
TMS	Control	60	97.25 [68.07; 107.0]
	+PES	60	48.62 [38.90; 68.07]
	+JG-98	60	38.90 [29.17; 48.62]

Table S4. Tests for normal distribution for invaded ANI, GSN, IBD, TMS cell (%) in control group and treated with 1 µM PES or 50 nM JG-98. P significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ****p < 0.0001; ns, not significant.

Cell culture	Group	Shapiro-Wilk test		Kolmogorov-Smirnov test	
		W	P value	KS distance	P value
ANI	Control	0.8692	< 0.0001****	0.1325	0.0105*
	+PES	0.9460	0.0101*	0.1263	0.0185*
	+JG-98	0.8807	< 0.0001****	0.1630	0.0004***
GSN	Control	0.9190	0.0007***	0.1514	0.0016**
	+PES	0.8452	< 0.0001****	0.1970	< 0.0001****
	+JG-98	0.9261	0.0014**	0.1450	0.0031**
IBD	Control	0.9057	0.0002***	0.1574	0.0009***
	+PES	0.9349	0.0032**	0.1472	0.0024**
	+JG-98	0.9101	0.0003***	0.1787	< 0.0001****
TMS	Control	0.8972	0.0001***	0.1345	0.0087**
	+PES	0.8844	< 0.0001****	0.1906	< 0.0001****
	+JG-98	0.8621	< 0.0001****	0.2166	< 0.0001****

Table S5. Wilcoxon rank sum test for multiple pairwise comparison of invaded ANI, GSN, IBD, TMS cell (%) in control group and treated with 1 μ M PES or 50 nM JG-98. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

	Group 1	Group 2	P value	P signif.
Invaded cells (%)	ANI Control	ANI +PES	< 0.0001	****
	ANI Control	ANI +JG-98	< 0.0001	****
	GSN Control	GSN +PES	< 0.0001	****
	GSN Control	GSN +JG-98	0.0001	***
	IBD Control	IBD +PES	0.0140	*
	IBD Control	IBD +JG-98	0.0186	*
	TMS Control	TMS +PES	< 0.0001	****
	TMS Control	TMS +JG-98	< 0.0001	****

Table S6. Median $\pm$ 95% confidence interval of cell track parameters of ANI cells + 1 μ M PES or 50 nM JG-98 inhibitors.

Parameter	mHsp70 ^{Wt}			mHsp70 ^{Low}			mHsp70 ^{High}		
	Control (N=399 8, Median $\pm$ [95% CI])	+PES (N=397 1, Median $\pm$ [95% CI])	+JG-98 (N=400 0, Median $\pm$ [95% CI])	Control (N=156 4, Median $\pm$ [95% CI])	+PES (N=154 9, Median $\pm$ [95% CI])	+JG-98 (N=151 3, Median $\pm$ [95% CI])	Control (N=105 3, Median $\pm$ [95% CI])	+PES (N=965, Median $\pm$ [95% CI])	+JG-98 (N=101 9, Median $\pm$ [95% CI])
Mean speed, μ m/h	29.51 [29.03; 30.06]	27.17 [26.65; 27.80]	21.08 [20.58; 21.57]	34.92 [32.71; 35.13]	33.84 [32.71; 35.13]	33.81 [32.86; 34.79]	41.85 [40.72; 43.17]	42.96 [41.40; 44.93]	44.79 [43.30; 46.37]
Straightness	0.3695 [0.3614; 0.3769]	0.3504 [0.3419; 0.3585]	0.3221 [0.3141; 0.3289]	0.2290 [0.2198; 0.2374]	0.2050 [0.1939; 0.2149]	0.2282 [0.2157; 0.2372]	0.2671 [0.2503; 0.2925]	0.2843 [0.2690; 0.3062]	0.2847 [0.2686; 0.3002]

Table S7. Tests for normal distribution for tracks mean speed and straightness in different experimental groups of ANI cells + 1 μ M PES or 50 nM JG-98 inhibitors experiment data. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

		Shapiro-Wilk test		Kolmogorov-Smirnov test	
		W	P value	KS distance	P value
Mean speed, μ m/h	mHsp70 ^{Wt} Control	0.9821	< 0.0001****	0.03169	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9746	< 0.0001****	0.03813	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9662	< 0.0001****	0.05631	< 0.0001****
	mHsp70 ^{Low} Control	0.9762	< 0.0001****	0.05437	< 0.0001****
	mHsp70 ^{Low} +PES	0.9740	< 0.0001****	0.05865	< 0.0001****

	mHsp70 ^{Low} +JG-98	0.9764	< 0.0001****	0.07038	< 0.0001****
	mHsp70 ^{High} Control	0.9787	< 0.0001****	0.05605	< 0.0001****
	mHsp70 ^{High} +PES	0.9672	< 0.0001****	0.06163	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9634	< 0.0001****	0.07002	< 0.0001****
Straightness	mHsp70 ^{Wt} Control	0.9771	< 0.0001****	0.08730	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9853	< 0.0001****	0.08638	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9728	< 0.0001****	0.09655	< 0.0001****
	mHsp70 ^{Low} Control	0.9505	< 0.0001****	0.07030	< 0.0001****
	mHsp70 ^{Low} +PES	0.9386	< 0.0001****	0.09258	< 0.0001****
	mHsp70 ^{Low} +JG-98	0.9354	< 0.0001****	0.08770	< 0.0001****
	mHsp70 ^{High} Control	0.9404	< 0.0001****	0.1019	< 0.0001****
	mHsp70 ^{High} +PES	0.9369	< 0.0001****	0.09364	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9484	< 0.0001****	0.07925	< 0.0001****

Table S8. Wilcoxon rank sum test for multiple pairwise comparison of migration parameters of ANI cells + 1 μ M PES or 50 nM JG-98 inhibitors experimental data groups. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

	Group 1	Group 2	P value	P signif.
Mean speed, μm/h	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	0.0539	ns
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	0.1869	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.2026	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.0233	*
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	< 0.0001	****
Straightness	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	0.7203	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.4113	ns

	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.5875	ns
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	< 0.0001	****

Table S9. Median $\pm$ 95% confidence interval of cell track parameters of GSN cells + 1 μ M PES or 50 nM JG-98 inhibitors.

Parameter	mHsp70 ^{Wt}			mHsp70 ^{Low}			mHsp70 ^{High}		
	Control (N=332 7, Median $\pm$ [95% CI])	+PES (N=267 0, Median $\pm$ [95% CI])	+JG-98 (N=357 1, Median $\pm$ [95% CI])	Control (N=745 9, Median $\pm$ [95% CI])	+PES (N=493 4, Median $\pm$ [95% CI])	+JG-98 (N=594 8, Median $\pm$ [95% CI])	Control (N=315 4, Median $\pm$ [95% CI])	+PES (N=258 4, Median $\pm$ [95% CI])	+JG-98 (N=327 4, Median $\pm$ [95% CI])
Mean speed, μ m/h	31.93 [31.32; 32.36]	29.97 [29.43; 30.50]	29.32 [28.74; 29.80]	18.69 [18.23; 19.18]	19.33 [18.97; 19.77]	15.18 [14.84; 15.48]	34.27 [33.67; 34.93]	33.53 [32.71; 34.51]	30.16 [29.55; 30.89]
Straightness	0.1818 [0.1753; 0.1872]	0.1700 [0.1637; 0.1761]	0.1593 [0.1551; 0.1634]	0.1754 [0.1646; 0.1839]	0.1402 [0.1312; 0.1498]	0.1268 [0.1199; 0.1243]	0.1928 [0.1867; 0.1981]	0.1759 [0.1700; 0.1816]	0.1795 [0.1751; 0.1856]

Table S10. Tests for normal distribution for tracks mean speed and straightness in different experimental groups of GSN cells + 1 μ M PES or 50 nM JG-98 inhibitors experiment data. P significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001; ns, not significant.

		Shapiro-Wilk test		Kolmogorov-Smirnov test	
		W	P value	KS distance	P value
Mean speed, μ m/h	mHsp70 ^{Wt} Control	0.9872	< 0.0001****	0.03537	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9843	< 0.0001****	0.04033	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9861	< 0.0001****	0.03314	< 0.0001****
	mHsp70 ^{Low} Control	0.9526	< 0.0001****	0.07749	< 0.0001****
	mHsp70 ^{Low} +PES	0.9522	< 0.0001****	0.07034	< 0.0001****
	mHsp70 ^{Low} +JG-98	N too large	-	0.08696	< 0.0001****
	mHsp70 ^{High} Control	0.9807	< 0.0001****	0.03999	< 0.0001****
	mHsp70 ^{High} +PES	0.9857	< 0.0001****	0.04310	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9750	< 0.0001****	0.04225	< 0.0001****
Straightness	mHsp70 ^{Wt} Control	0.9249	< 0.0001****	0.08835	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9157	< 0.0001****	0.08784	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9143	< 0.0001****	0.09304	< 0.0001****
	mHsp70 ^{Low} Control	0.9094	< 0.0001****	0.1031	< 0.0001****
	mHsp70 ^{Low}	0.9131	< 0.0001****	0.09069	< 0.0001****

	+PES				
	mHsp70 ^{Low} +JG-98	N too large	-	0.1098	< 0.0001****
	mHsp70 ^{High} Control	0.9261	< 0.0001****	0.08468	< 0.0001****
	mHsp70 ^{High} +PES	0.9170	< 0.0001****	0.09392	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9221	< 0.0001****	0.08750	< 0.0001****

Table S11. Wilcoxon rank sum test for multiple pairwise comparison of migration parameters of GSN cells + 1 μ M PES or 50 nM JG-98 inhibitors experimental data groups. P significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ****p < 0.0001; ns, not significant.

	Group 1	Group 2	P value	P signif.
Mean speed, μm/h	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	0.0489	*
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	< 0.0001	****
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.3841	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	< 0.0001	****
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	< 0.0001	****
Straightness	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	0.0002	***
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	< 0.0001	****
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	< 0.0001	****
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.0001	***
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	0.0001	***

Table S12. Median $\pm$ 95% confidence interval of cell track parameters of IBD cells + 1 μ M PES or 50 nM JG-98 inhibitors.

Parameter	mHsp70 ^{Wt}			mHsp70 ^{Low}			mHsp70 ^{High}		
	Control (N=195 7, Median $\pm$ [95% CI])	+PES (N=147 1, Median $\pm$ [95% CI])	+JG-98 (N=179 7, Median $\pm$ [95% CI])	Control (N=110 5, Median $\pm$ [95% CI])	+PES (N=139 5, Median $\pm$ [95% CI])	+JG-98 (N=103 7, Median $\pm$ [95% CI])	Control (N=189 7, Median $\pm$ [95% CI])	+PES (N=182 9, Median $\pm$ [95% CI])	+JG-98 (N=198 3, Median $\pm$ [95% CI])

Mean speed, $\mu\text{m/h}$	9.847 [9.308; 10.65]	7.814 [7.202; 8.475]	7.125 [6.930; 7.335]	7.550 [7.333; 7.787]	7.474 [7.235; 7.731]	7.298 [6.890; 7.661]	10.89 [10.13; 11.68]	10.74 [9.986; 11.50]	11.56 [10.97; 12.21]
Straightness	0.1873 [0.1783; 0.1971]	0.2032 [0.1965; 0.2139]	0.1342 [0.1266; 0.1398]	0.2282 [0.2120; 0.2420]	0.1370 [0.1308; 0.1456]	0.1727 [0.668; 0.1816]	0.2426 [0.2331; 0.2499]	0.2412 [0.2289; 0.2489]	0.2388 [0.2289; 0.2489]

Table S13. Tests for normal distribution for tracks mean speed and straightness in different experimental groups of IBD cells + 1 μM PES or 50 nM JG-98 inhibitors experiment data. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

		Shapiro-Wilk test		Kolmogorov-Smirnov test	
		W	P value	KS distance	P value
Mean speed, $\mu\text{m/h}$	mHsp70 ^{Wt} Control	0.8937	< 0.0001****	0.1462	< 0.0001****
	mHsp70 ^{Wt} +PES	0.8800	< 0.0001****	0.1569	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9030	< 0.0001****	0.1403	< 0.0001****
	mHsp70 ^{Low} Control	0.9139	< 0.0001****	0.1241	< 0.0001****
	mHsp70 ^{Low} +PES	0.9175	< 0.0001****	0.1201	< 0.0001****
	mHsp70 ^{Low} +JG-98	0.9004	< 0.0001****	0.1252	< 0.0001****
	mHsp70 ^{High} Control	0.8993	< 0.0001****	0.1285	< 0.0001****
	mHsp70 ^{High} +PES	0.9169	< 0.0001****	0.1079	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9087	< 0.0001****	0.1119	< 0.0001****
Straightness	mHsp70 ^{Wt} Control	0.9401	< 0.0001****	0.08361	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9450	< 0.0001****	0.07270	< 0.0001****
	mHsp70 ^{Wt} +JG-98	0.9251	< 0.0001****	0.09735	< 0.0001****
	mHsp70 ^{Low} Control	0.9309	< 0.0001****	0.08624	< 0.0001****
	mHsp70 ^{Low} +PES	0.9213	< 0.0001****	0.09448	< 0.0001****
	mHsp70 ^{Low} +JG-98	0.9360	< 0.0001****	0.08959	< 0.0001****
	mHsp70 ^{High} Control	0.07962	< 0.0001****	0.07962	< 0.0001****
	mHsp70 ^{High} +PES	0.9431	< 0.0001****	0.08800	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9457	< 0.0001****	0.07918	< 0.0001****

Table S14. Wilcoxon rank sum test for multiple pairwise comparison of migration parameters of IBD cells + 1 μM PES or 50 nM JG-98 inhibitors experimental data groups. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

	Group 1	Group 2	P value	P signif.
Mean speed, $\mu\text{m/h}$	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****

	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	0.8211	ns
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	0.4302	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.0089	**
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.2558	ns
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	< 0.0001	****
Straightness	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	0.0013	**
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	< 0.0001	****
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.5340	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.7898	ns
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	0.0029	**

Table S15. Median $\pm$ 95% confidence interval of cell track parameters of TMS cells + 1 μ M PES or 50 nM JG-98 inhibitors.

Parameter	mHsp70 ^{Wt}			mHsp70 ^{Low}			mHsp70 ^{High}		
	Control (N=575 4, Median $\pm$ [95% CI])	+PES (N=291 8, Median $\pm$ [95% CI])	+JG-98 (N=550 8, Median $\pm$ [95% CI])	Control (N=569 9, Median $\pm$ [95% CI])	+PES (N=939, Median $\pm$ [95% CI])	+JG-98 (N=112 8, Median $\pm$ [95% CI])	Control (N=566 9, Median $\pm$ [95% CI])	+PES (N=734, Median $\pm$ [95% CI])	+JG-98 (N=862, Median $\pm$ [95% CI])
Mean speed, μm/h	27.44 [26.98; 27.89]	23.06 [22.50; 23.68]	24.07 [23.60; 24.49]	17.71 [17.37; 18.09]	15.13 [14.22; 15.89]	17.58 [16.74; 18.47]	24.05 [23.66; 24.42]	25.51 [24.45; 26.33]	24.68 [23.90; 25.56]
Straightness	0.1929 [0.1858; 0.2002]	0.2488 [0.2378; 0.2586]	0.2458 [0.2401; 0.2540]	0.2002 [0.1921; 0.2073]	0.2049 [0.1914; 0.2212]	0.2420 [0.2263; 0.2609]	0.2509 [0.2435; 0.2576]	0.2152 [0.2036; 0.2311]	0.1830 [0.1705; 0.1920]

Table S16. Tests for normal distribution for tracks mean speed and straightness in different experimental groups of TMS cells + 1 μ M PES or 50 nM JG-98 inhibitors experiment data. P significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001; ns, not significant.

		Shapiro-Wilk test		Kolmogorov-Smirnov test	
		W	P value	KS distance	P value
Mean speed, μm/h	mHsp70 ^{Wt} Control	N too large	-	0.04895	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9743	< 0.0001****	0.05472	< 0.0001****
	mHsp70 ^{Wt}	N too large	-	0.06031	< 0.0001****

	+JG-98				
	mHsp70 ^{Low} Control	N too large	-	0.06501	< 0.0001****
	mHsp70 ^{Low} +PES	0.9606	< 0.0001****	0.06272	< 0.0001****
	mHsp70 ^{Low} +JG-98	0.9609	< 0.0001****	0.05543	< 0.0001****
	mHsp70 ^{High} Control	N too large	-	0.05754	< 0.0001****
	mHsp70 ^{High} +PES	0.9679	< 0.0001****	0.06625	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9780	< 0.0001****	0.06584	< 0.0001****
Straightness	mHsp70 ^{Wt} Control	N too large	-	0.06842	< 0.0001****
	mHsp70 ^{Wt} +PES	0.9473	< 0.0001****	0.08094	< 0.0001****
	mHsp70 ^{Wt} +JG-98	N too large	-	0.08137	< 0.0001****
	mHsp70 ^{Low} Control	N too large	-	0.1150	< 0.0001****
	mHsp70 ^{Low} +PES	0.9350	< 0.0001****	0.09691	< 0.0001****
	mHsp70 ^{Low} +JG-98	0.9357	< 0.0001****	0.08733	< 0.0001****
	mHsp70 ^{High} Control	N too large	-	0.1122	< 0.0001****
	mHsp70 ^{High} +PES	0.9436	< 0.0001****	0.08750	< 0.0001****
	mHsp70 ^{High} +JG-98	0.9449	< 0.0001****	0.08522	< 0.0001****

Table S17. Wilcoxon rank sum test for multiple pairwise comparison of migration parameters of TMS cells + 1 μ M PES or 50 nM JG-98 inhibitors experimental data groups. P significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

	Group 1	Group 2	P value	P signif.
Mean speed, μm/h	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +PES	< 0.0001	****
	mHsp70 ^{Low} Control	mHsp70 ^{Low} +JG-98	0.4544	ns
	mHsp70 ^{High} Control	mHsp70 ^{High} +PES	0.0004	***
	mHsp70 ^{High} Control	mHsp70 ^{High} +JG-98	0.0121	*
	mHsp70 ^{High} Control	mHsp70 ^{Low} Control	< 0.0001	****
Straightness	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +PES	< 0.0001	****
	mHsp70 ^{Wt} Control	mHsp70 ^{Wt} +JG-98	< 0.0001	****
	mHsp70 ^{Low}	mHsp70 ^{Low}	0.8656	ns

	Control	+PES		
	mHsp70 ^{Low}	mHsp70 ^{Low}	< 0.0001	****
	Control	+JG-98		
	mHsp70 ^{High}	mHsp70 ^{High}	< 0.0001	****
	Control	+PES		
	mHsp70 ^{High}	mHsp70 ^{High}	< 0.0001	****
	Control	+JG-98		
	mHsp70 ^{High}	mHsp70 ^{Low}	< 0.0001	****
	Control	Control		

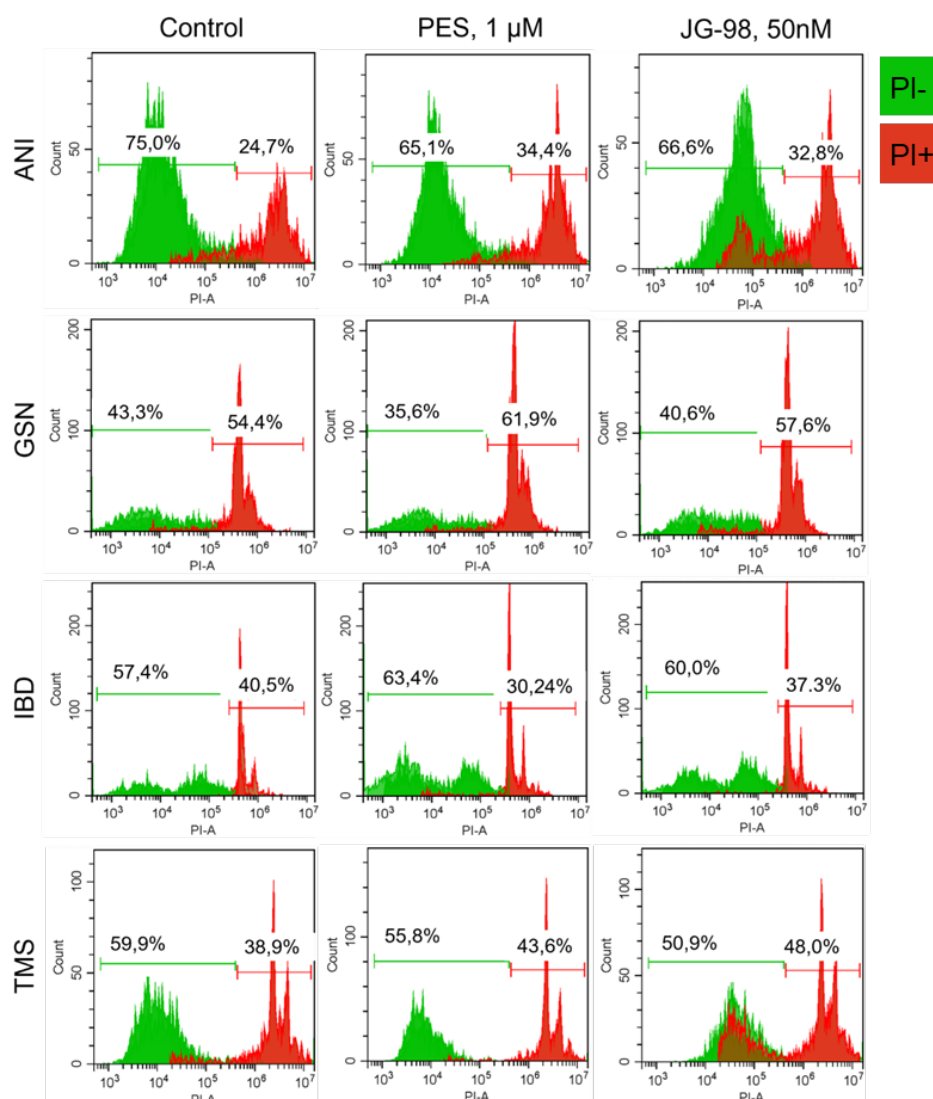


Figure S1. Hsp70 inhibitors PES and JG-98 at concentrations of 1 μM and 50 nM, respectively, do not affect the viability of primary glioblastoma cells. Flow cytometry analysis of cells stained with PI. Green color indicates living cells (PI-), red color indicates dead cells (PI+).

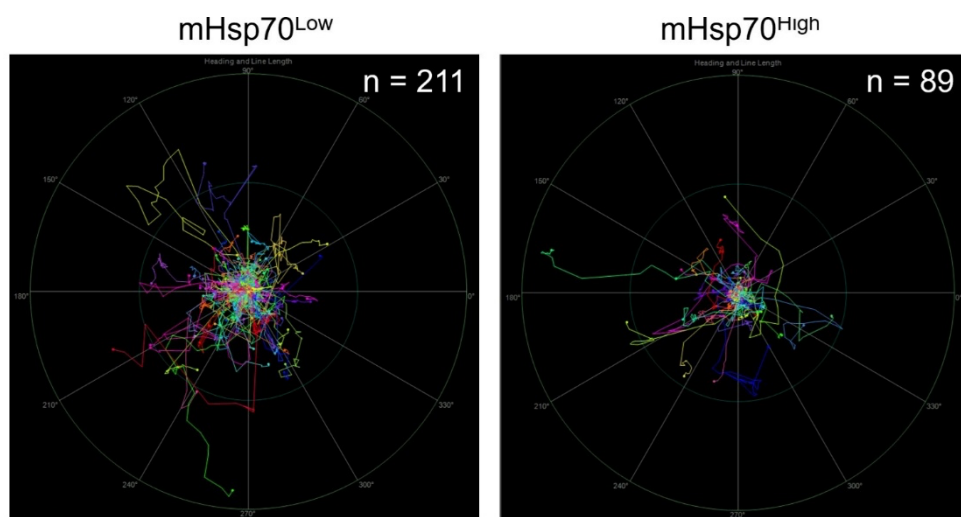


Figure S2. Example of normalized tracks (rose plots) of mHsp70^{Low} and mHsp70^{High} subpopulations of IBD cells.