Supplementary files

Table S1. GBM (grade IV) patient characteristics

Patients characteristics	
N° sample	**66**
Sex	
Men	47% (n = 31)
Women	53% (n = 35)
Age, years	
< 45	55% (n =36)
>45	45% (n = 30)
GBM MGMT methylation	
Non detected	32% (n = 21)
Methylated	36% (n = 24)
Non methylated	32% (n = 21)
GBM Recidivated	
Not detected	9% (n = 6)
Not recidivated	14% (n = 9)
Recidivated	77% (n = 51)
Adjuvant therapy	
No	13% (n= 9)
STUPP	56% (n= 37)
Fotemustine	8% (n= 5)
RT	11% (n= 7)
Other	12% (n=8)
Temozolomide	*38% (3/8)*
Fotemustine + Bevacizumab	*38% (3/8)*
Fotemustine + RT	*12% (1/8)*
Fotemustine + RT + Bevacizumab	*12% (1/8)*

Table S2. TRPML1 and TRPML2 gene expression in GBM patients evaluated by qRT-PCR.

Pz. N°	TRPML1	TRPML2	Pz. N°	TRPML1	TRPML2	Pz. N°	TRPML1	TRPML2
	mRNA expression[a]			mRNA expression[a]			mRNA expression[a]	
#1	Low	High	#23	Neg	Neg	#45	High	Low
#2	High	Low	#24	Neg	Neg	#46	Low	High
#3	High	High	#25	Neg	High	#47	High	High
#4	High	Low	#26	Low	Neg	#48	Low	High
#5	Neg	Neg	#27	High	Low	#49	Low	Neg
#6	High	Low	#28	Neg	High	#50	Low	Neg
#7	Neg	High	#29	Neg	Neg	#51	Neg	High
#8	High	High	#30	Neg	High	#52	Neg	High
#9	High	High	#31	Neg	High	#53	Low	High
#10	Neg	Neg	#32	Low	High	#54	Low	Neg
#11	Low	Neg	#33	Low	High	#55	Neg	High
#12	Neg	High	#34	Neg	High	#56	Neg	High
#13	Low	Neg	#35	Neg	Neg	#57	Low	High
#14	High	Low	#36	High	Low	#58	Neg	Neg
#15	Neg	High	#37	Neg	High	#59	High	High
#16	Neg	High	#38	Neg	High	#60	High	High
#17	High	Low	#39	High	High	#61	Neg	High
#18	Neg	High	#40	Low	High	#62	High	High
#19	Neg	High	#41	High	High	#63	Neg	High
#20	Low	High	#42	Neg	High	#64	High	High
#21	Neg	High	#43	Neg	Neg	#65	High	High
#22	Neg	Neg	#44	High	Low	#66	High	High

[a]Relative mRNA expression; Neg: Negative; Low TRPML1 ≤ 1.0, Low TRPML2 ≤ 1.8; High TRPML1 > 1.0, High TRPML2 > 1.8 evaluated by ROC analysis.

DAPI Calreticulin TRPML1 Merge

DAPI LAMP1 TRPML1 Merge

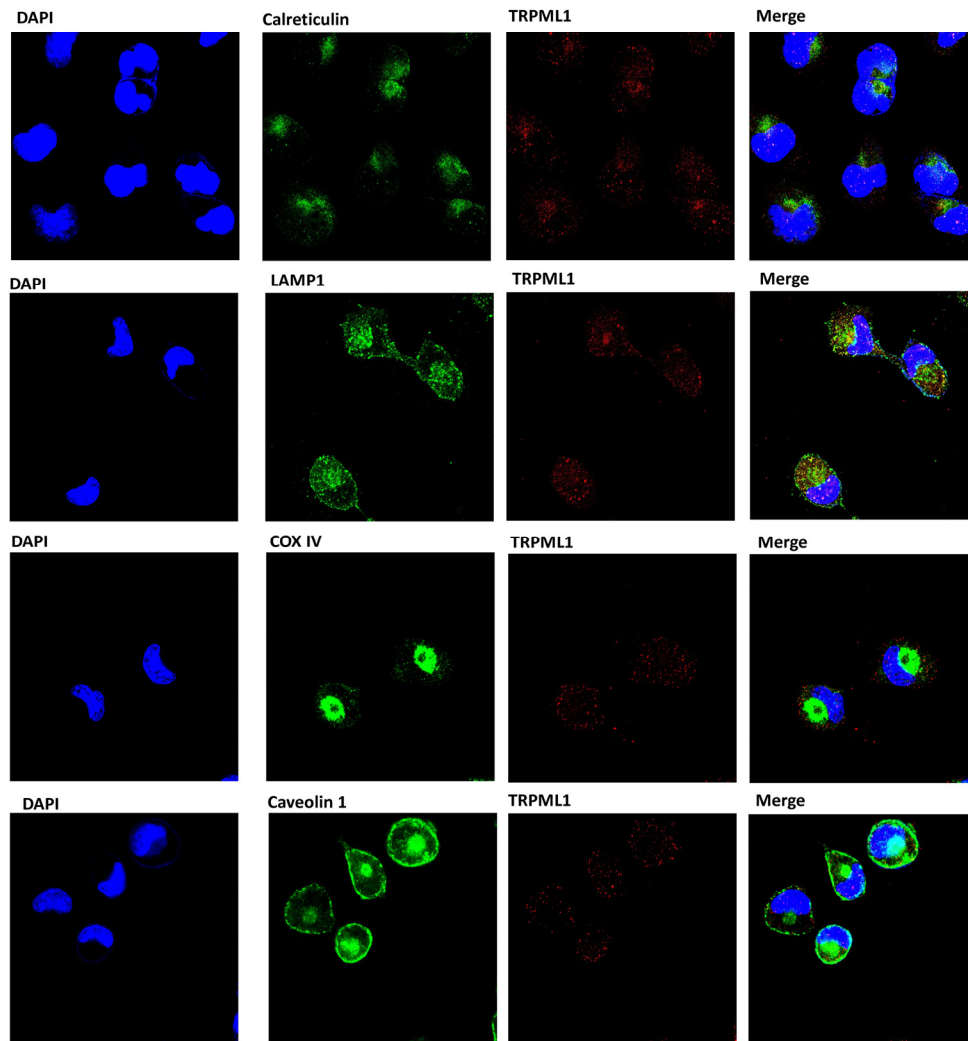DAPI COX IV TRPML1 Merge

DAPI Caveolin 1 TRPML1 Merge

Figure S1. Confocal microscopy analysis to evaluate colocalization of TRPML1 different cellular organelles in T98 cells using specific Abs. Endoplasmic reticulum (ER): anti-calreticulin Ab; plasma membrane: anti-caveolin-1 Ab; lysosome: anti-LAMP1; mitochondria: anti-COX IV. 40,6-diamidino-2-phenylindole (DAPI) was used to counterstain nuclei. Magnification 60x.
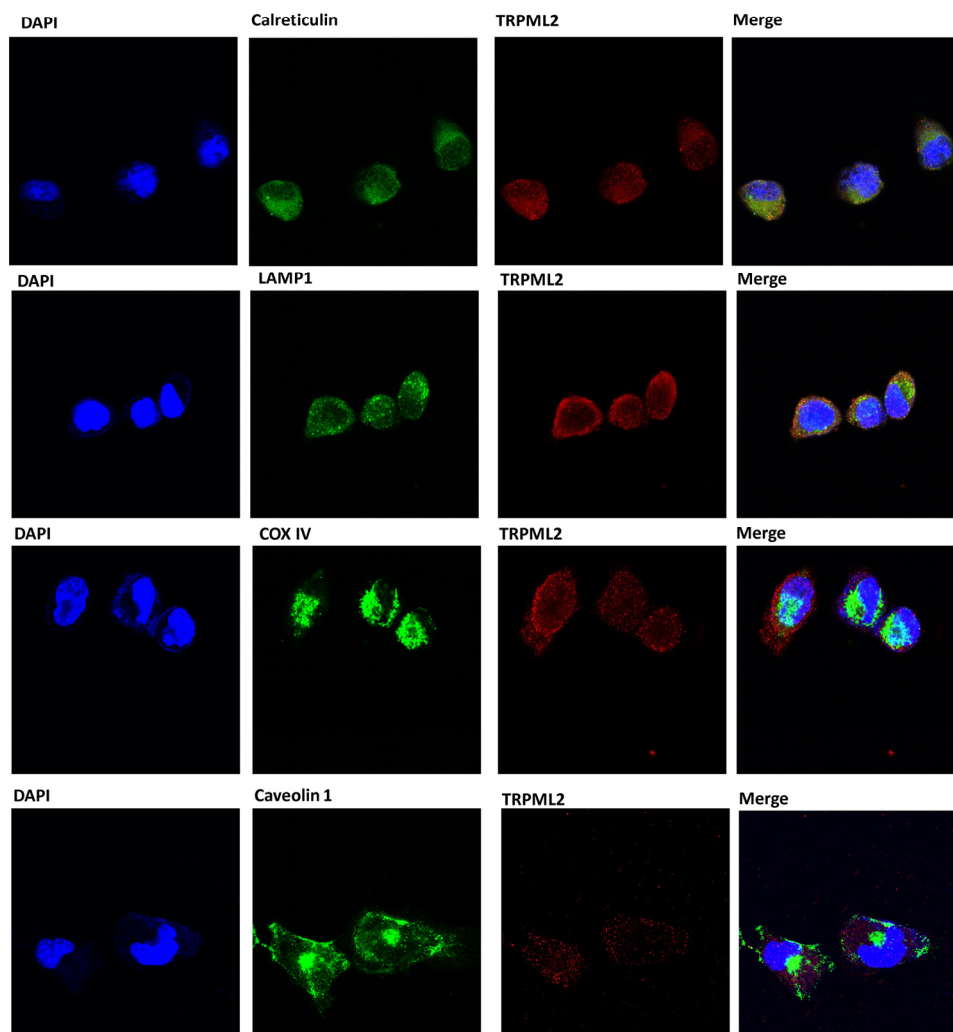
Figure S2. Confocal microscopy analysis to evaluate colocalization of TRPML2 different cellular organelles in T98 cells using specific Abs. Endoplasmic reticulum (ER): anti-calreticulin Ab; plasma membrane: anti-caveolin-1 Ab; lysosome: anti-LAMP1; mitochondria: anti-COX IV. 40,6-diamidino-2-phenylindole (DAPI) was used to counterstain nuclei. Magnification 60x.
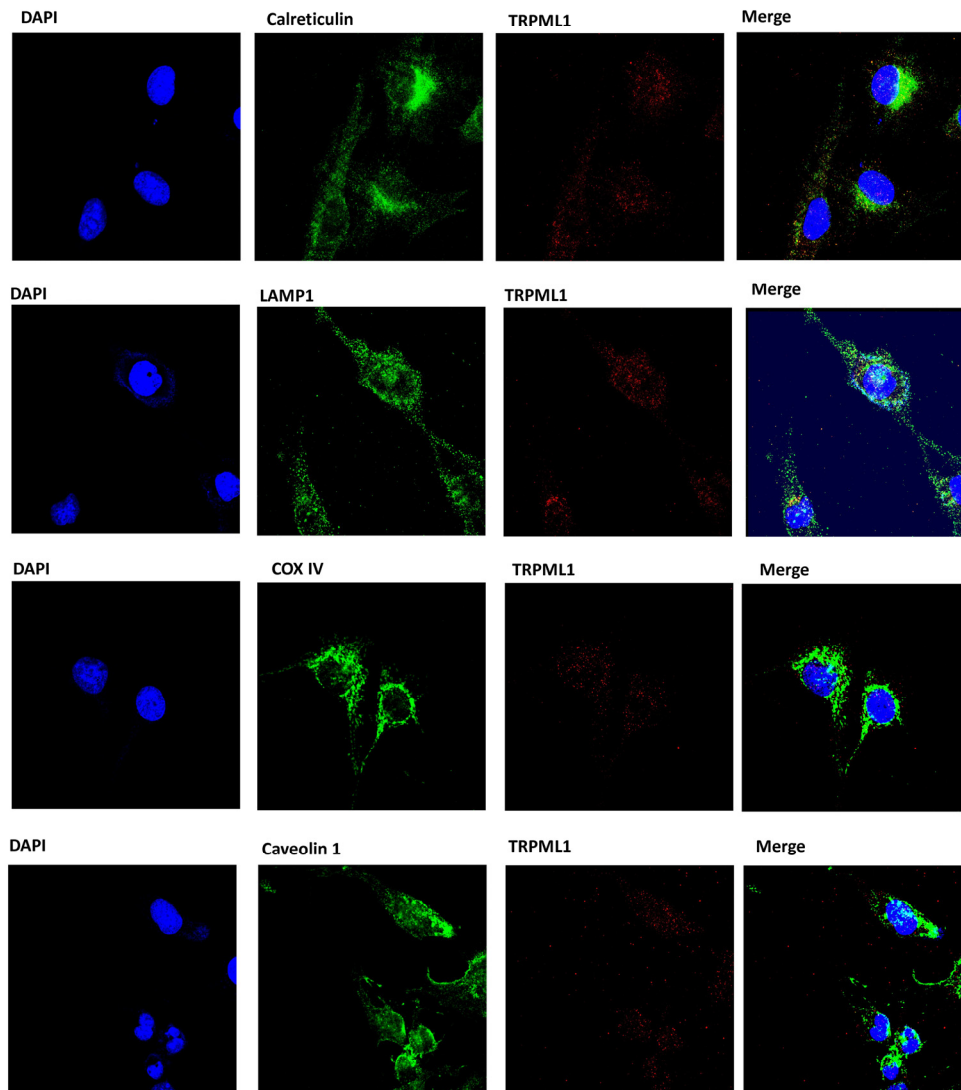
Figure S3. Confocal microscopy analysis to evaluate colocalization of TRPML1 different cellular organelles in U251 cells using specific Abs. Endoplasmic reticulum (ER): anti-calreticulin Ab; plasma membrane: anti-caveolin-1 Ab; lysosome: anti-LAMP1; mitochondria: anti-COX IV. 40,6-diamidino-2-phenylindole (DAPI) was used to counterstain nuclei. Magnification 60x.
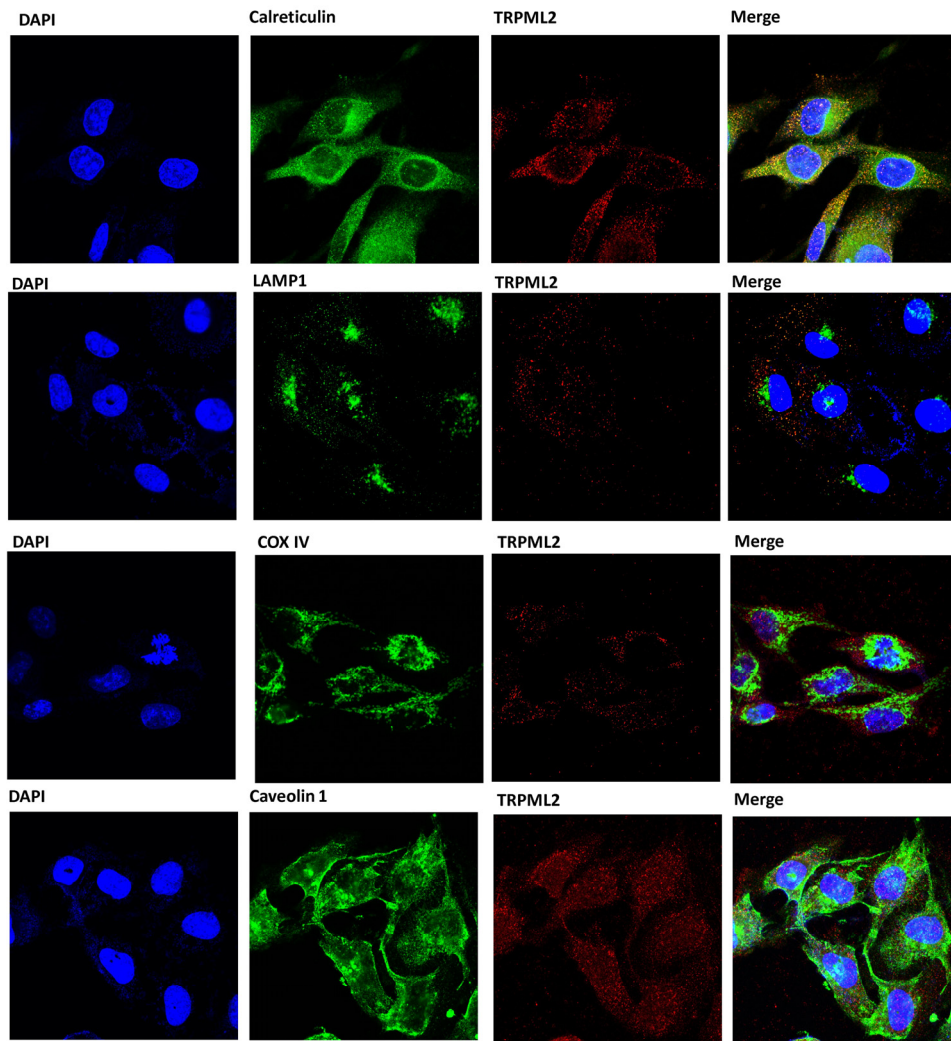
Figure S4. Confocal microscopy analysis to evaluate colocalization of TRPML2 different cellular organelles in U251 cells using specific Abs. Endoplasmic reticulum (ER): anti-calreticulin Ab; plasma membrane: anti-caveolin-1 Ab; lysosome: anti-LAMP1; mitochondria: anti-COX IV. 40,6-diamidino-2-phenylindole (DAPI) was used to counterstain nuclei. Magnification 60x.

Table S3. Kaplan-Meier p-values.

P value	TRPML2 low TRPML1 high	TRPML2 low TRPML1 neg	TRPML2 high TRPML1 high	TRPML2 high TRPML1 low	TRPML2 high TRPML1 neg	TRPML2 neg TRPML1 neg
TRPML2 low TRPML1 high		0,0219 *	<0,0001 *	<0,0001 *	<0,0001 *	<0,0001 *
TRPML2 low TRPML1 neg	0,0219 *		0,2334	0,576	0,0088 *	0,0004 *
TRPML2 high TRPML1 high	<0,0001 *	0,2334		0,0259 *	0,0942	<0,0001 *
TRPML2 high TRPML1 low	<0,0001 *	0,576	0,0259 *		0,0019 *	<0,0001 *
TRPML2 high TRPML1 neg	<0,0001 *	0,0088 *	0,0942	0,0019 *		<0,0001 *
TRPML2 neg TRPML1 neg	<0,0001 *	0,0004 *	<0,0001 *	<0,0001 *	<0,0001 *	

Table S4 Pearson's Correlation Coefficient

Markers	PCC T98	PCC U251
TRPML2 Calreticulin	0.86	0.82
TRPML2 Caveolin 1	0.23	0.26
TRPML2 COX IV	0.27	0.29
TRPML2 LAMP1	0.81	0.86
TRPML1 Calreticulin	0.66	0.64
TRPML1 Caveolin 1	0.25	0.19
TRPML1 COX IV	0.18	0.20
TRPML1 LAMP1	0.67	0.65

0<PCC<0.3= weak positive correlation; 0.3<PCC<0.5= moderate positive correlation; >0.05 strong positive correlation. PCC= Pearson's Correlation Coefficient