

Supplementary Materials: IQGAP1 in Podosomes/Invadosomes Is Involved in the Progression of Glioblastoma Multiforme Depending on the Tumor Status

Deborah Rotoli, Natalia Dolores Pérez-Rodríguez, Manuel Morales, María del Carmen Maeso, Julio Ávila, Ali Mobasher and Pablo Martín-Vasallo

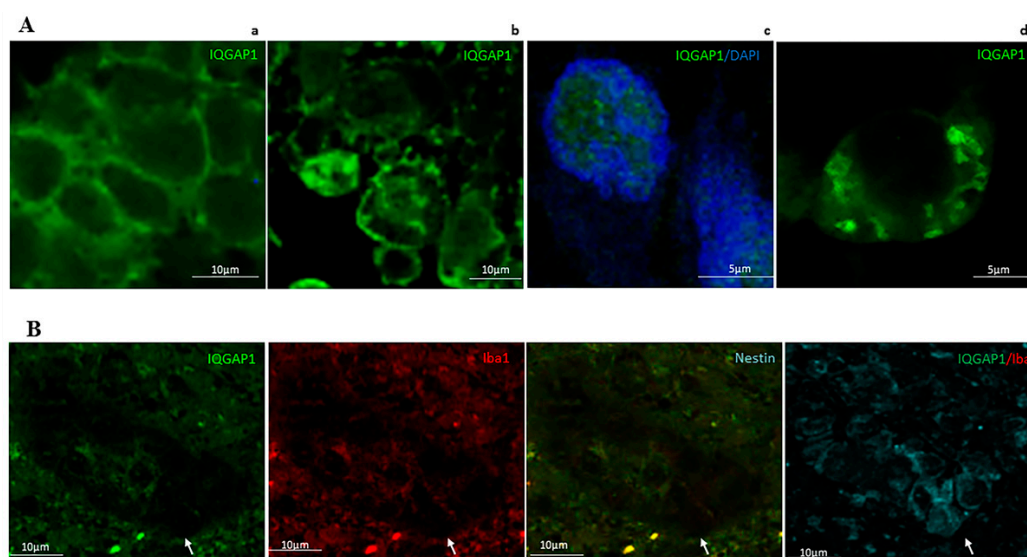


Figure S1. (A) Higher magnification of Figure 1A–D. IQGAP1 protein in GBM detected in: (a) plasma membrane; (b) cell protrusions, plasma membrane and cytosol; (c) nucleus; and (d) cell with podosome-like structures highly positive for IQGAP1; (B) Higher magnification of Figure 2K–O. Triple immunolocalization of IQGAP1 protein (green), Iba1 (red) and nestin (cyan). Arrow points to a nestin⁺/IQGAP1⁻/Iba1⁻ cell flanked by nestin⁺/IQGAP1⁺/Iba1⁺ cells.

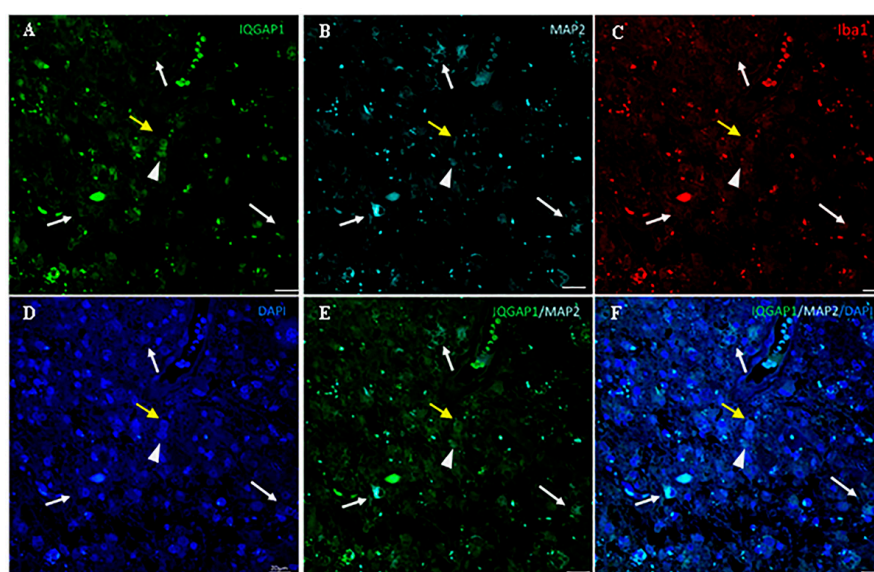


Figure S2. Triple immunolocalization of IQGAP1 protein (green), the microglia/macrophage marker Iba1 (red) and the Microtubule-associated protein 2 (MAP2, cyan) in GBM tissue sections. (A) IQGAP1; (B) MAP2; (C) Iba1; (D) DAPI; (E) IQGAP1/MAP2 merge; (F) IQGAP1/MAP2/DAPI merge. White arrows point to Map2⁺ neurons IQGAP1⁻/Iba1⁻. Yellow arrows points to a cell IQGAP1⁺/Iba1⁺. Arrowhead points a cell IQGAP1⁺/nestin⁺. Bar 20 μm.

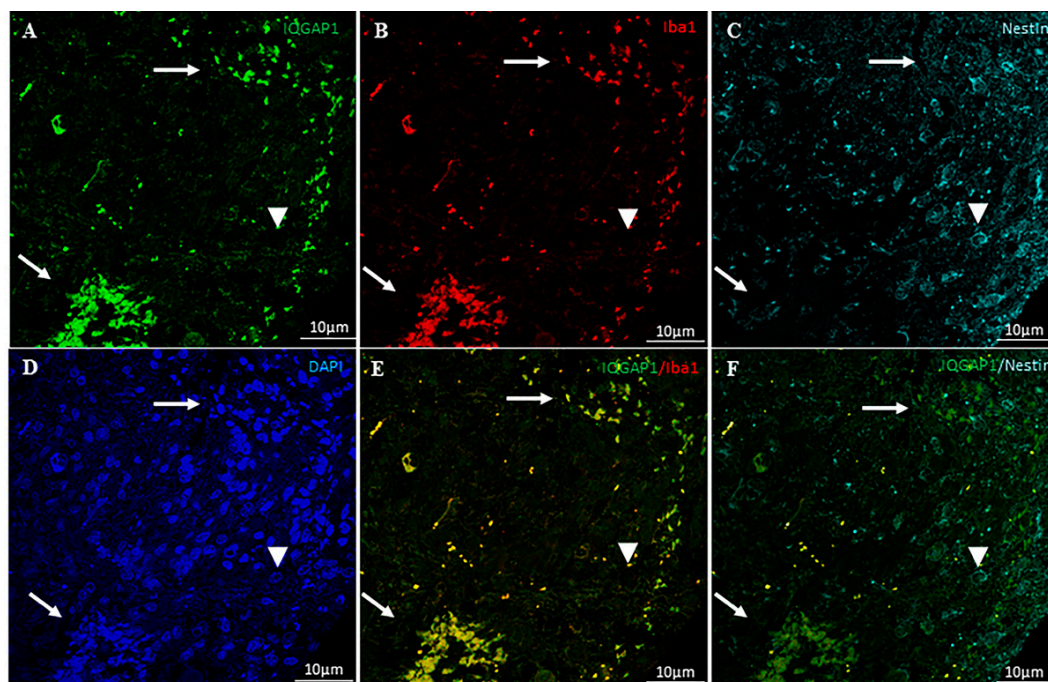


Figure S3. Triple immunolocalization of IQGAP1 protein (green), the microglia/macrophage marker Iba1 (red) and the CSC marker nestin (cyan). (A) IQGAP1; (B) Iba1; (C) Nestin; (D) DAPI; (E) IQGAP1/Iba1 merge; (F) IQGAP1/Nestin merge. In this highly vascularized area of a GBM tissue section, many nestin+ cells are observed (arrowhead). Note the massive presence of macrophages (Iba1+/IQGAP1+/nestin-) in the periphery (arrows). Scale bar: 10 μ m.

Table S1. IQGAP1/PCNA, IQGAP1/GFAP and IQGAP1/Iba1 colocalization. Mean and standard deviation of values obtained with the ImageJ plug-in “Mander’s coefficients”.

	<i>n</i>	<i>R</i>	Ch1/Ch2	M1	M2	Ch1 Thresh, Ch2 Thresh
Ch1 = GFAP Ch2 = IQGAP1	27	0.59 ± 0.05	0.995 ± 0.02	0.969 ± 0.02	0.971 ± 0.01	1;255
Ch1 = PCNA Ch2 = IQGAP1	20	0.677 ± 0.13	0.750 ± 0.29	0.851 ± 0.14	0.739 ± 0.20	1;255
Ch1 = Iba1 Ch2 = IQGAP1	14	0.84 ± 0.04	0.801 ± 0.15	0.954 ± 0.04	0.835 ± 0.09	1;255

n = samples analyzed. *R* = Mander’s Overlap coefficient. Value Range: 0–1, with 0 = low colocalization and 1 = high colocalization; Ch1/Ch2 = red/green pixel ratio. Ratio ≈ 1 allows the use of Mander’s Overlap coefficient (*R*); M1, M2 = Mander’s Colocalization coefficients for channel 1 (M1) and channel 2 (M2). Value range: 0–1, with 0 = no colocalization 1 = all pixels colocalize; Ch1 thresh, Ch2 thresh = ch1 and ch2 threshold.