Supplementary Information:

Article Title: Impact of Phospholipase C β1 in Glioblastoma: a study on the main mechanisms of tumor aggressiveness

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Supplementary Tables:

| MGMT | IDH1 | EGFR | IDH2 | H3F3A | TERT | Date of Birth | Age at diagnosis | Gender |
|------|------|------|------|-------|------|---------------|-----------------|--------|
| Patient 1 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:29%) | 01/01/23 | 68 F |
| Patient 2 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 01/01/23 | 68 M |
| Patient 3 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:31%) | 25/01/23 | 66 M |
| Patient 4 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:28%) | 25/01/23 | 69 M |
| Patient 5 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 25/01/23 | 66 M |
| Patient 6 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 25/01/23 | 66 M |
| Patient 7 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 25/01/23 | 66 M |
| Patient 8 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:49%) | 25/01/23 | 66 M |
| Patient 9 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:49%) | 25/01/23 | 66 M |
| Patient 10 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 11 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 12 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 13 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 14 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 15 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 16 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 17 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 18 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 19 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 20 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 21 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 22 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 23 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 24 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 25 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 26 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 27 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 28 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 29 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 30 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 31 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 32 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 33 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 34 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 35 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 36 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 37 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 38 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 39 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 40 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 41 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 42 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 43 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 44 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 45 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 46 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 47 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 48 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 49 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |
| Patient 50 | UMT | WT | WT | WT | WT | g.1,295,113 G>A (VAF:35%) | 07/01/23 | 72 M |

Supplementary Table 1: Data and Molecular characterization of samples from 50 Glioblastoma patients

Among the 50 glioblastoma samples, only six were detected mutant for isocitrate dehydrogenase 1 (IDH1), p.R132H and none for isocitrate dehydrogenase 2 (IDH2) and histone H3-3A. These IDH1-mutated samples will be classified as Adult-type diffuse astrocytoma, IDH mutant, grade 4 considering the recent tumor classification update(1). The Telomerase Reverse Transcriptase (TERT) promoter was found to be mutated in 32 cases (29 for g.1,295,113 G>A and 3 for g.1,295,135 G>A). O-6-Methylguanine-DNA Methyltransferase (MGMT) was detected hypermethylated in 23 cases.
Supplementary Figures:

**Supplementary Fig. 1: Quantitative analysis of PLCβ1 protein expression**

Panels **a**, **b** and **c**: Quantitative analysis of PLCβ1 protein expression in U87-MG (**a**), U-251 MG (**b**) and HA (**c**). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) cells. WT cells were used as reference samples. Columns show the mean ± SD of three independent experiments with *p < 0.05, **p < 0.01 and ***p < 0.001.
Supplementary Fig. 2: Quantitative analysis of Mesenchymal markers and MMPs protein expression

Panels a, b and c: Quantitative analysis of Slug and N-Cadherin protein expression in U87-MG (a), U-251 MG (b) and HA (c). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) cells. WT cells were used as reference samples. Columns show the mean ± SD of three independent experiments with *p < 0.05, **p < 0.01 and ***p < 0.001.

Panels d and e: Quantitative analysis of MMP-2 and MMP-9 protein expression in U87-MG (d) and HA (e). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) cells. WT cells were used as reference samples. Columns show the mean ± SD of three independent experiments with *p < 0.05, **p < 0.01 and ***p < 0.001.
Supplementary Fig. 3: Quantitative analysis of the protein expression of the molecules belonging to the main survival pathways

Panels a, b and c: Quantitative analysis of the protein expression of the molecules belonging to the main survival pathways in U87-MG (a), U-251 MG (b) and HA (c). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) cells. WT cells were used as reference samples. Columns show the mean ± SD of three independent experiments with *p < 0.05, **p < 0.01 and ***p < 0.001.
Supplementary Fig. 4: Consequences of PLCβ1 modulation on PLCγ1 expression

Panels a, c and e: Western blot analysis of PLCγ1 expression after PLCβ1 silencing on U87-MG (a), U-251 MG (c) and HA primary astrocytes (e). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) samples. Densitometric analysis was performed with total protein normalization through the iBright analysis software. Panels b, d and f: PLCγ1 mRNA expression in U87-MG (b), U-251 MG (d) and HA primary astrocytes (f). PLCβ1-silenced cells (shPLCβ1) were compared to wild type (WT) and mock-transduced (shCTRL) samples. GAPDH was used as housekeeping gene and all the analysis derived from three independent experiments.