Quiescence status of glioblastoma stem-like cells involves remodelling of Ca²⁺ signalling and mitochondrial shape

AUTHORS

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Supplementary tables

| Experiment # | Cell line | Culture conditions | cellular state |
|--------------|-----------|---|----------------------------------|
| 1 | TG1 | NS34, pH 7.4 | |
| 2 | TG1 | NS34, pH 7.4 | |
| 3 | TG1 | NS34, pH 7.4 | Proliferative (self- renewal) |
| 4 | TG1_C1 | NS34, pH 7.4 | |
| 5 | TG1_C1 | NS34, pH 7.4 | |
| 6 | TG1 | No replacement of NS34 medium during 9 days | |
| 7 | TG1_C1 | No replacement of NS34 medium during 9 days | |
| 8 | TG1_C1 | NS34 pH 6.2 during 5 days | Quiescence |
| 9 | TG1 | NS34 pH 6.2 during 5 days | |
| 10 | TG1 | NS34 pH 6.5 during 5 days | |
| 11 | TG1 | SKF-96365 (10μM) in NS34, pH 7.4 | |

Table S1. Culture conditions for TG1 and TG1-C1 cells

Table S2. List of Ca²⁺ toolbox genes expressed in GSLCs.

Among the 250 genes belonging to the Ca²⁺ toolkit, 107 genes are expressed in GSLCs either in the proliferative or quiescent conditions (see Table S1). A fold change < 1 corresponds to down-regulation in quiescent GSLCs (highlighted in blue). Fold change is computer from normalized log2 transformed data as described in the experimental procedures. For a given gene, fold change is 2^(mean value quiescent cells – mean value proliferative cells). CaBP, Calcium binding protein; TF, Transcription factor; ER/SR, Endoplasmic Reticulum/Sarcoplasmic Reticulum; ND, subcellular location not determined.

| Gene Symbol | Gene ID | Fold change | Function | Subcellular Location |
|-------------|---------|-------------|--------------------------|----------------------|
| VSNL1 | 7447 | 0.606635332 | CaBP | Cytosol / Membrane |
| S100A3 | 6274 | 0.623029709 | CaBP | Cytosol / Nucleus |
| SLC25A13 | 10165 | 0.643405378 | Ca ²⁺ outflow | Mitochondria |
| S100B | 6285 | 0.670835435 | CaBP | Cytosol / Nucleus |
| SLC24A3 | 57419 | 0.741691947 | Ca ²⁺ outflow | Plasma membrane |
| EFCAB13 | 124989 | 0.771550298 | CaBP | ND |
| RASGRP1 | 10125 | 0.776597738 | CaBP | ND |
| EFHD2 | 79180 | 0.777477086 | CaBP | ND |
| MCU | 90550 | 0.779598892 | Ca ²⁺ outflow | Mitochondria |
| MICU2 | 221154 | 0.779701352 | Ca ²⁺ outflow | Mitochondria |
| ITPR2 | 3709 | 0.791205823 | Ca entry | ER/SR |
| S100A16 | 140576 | 0.799640954 | CaBP | Cytosol |
| MYL6 | 4637 | 0.803082585 | CaBP | ND |
| CETN3 | 1070 | 0.806671381 | CaBP | Centrosome |
| PKD2 | 5311 | 0.824052572 | Ca entry | Plasma membrane |
| CACNA2D1 | 781 | 0.825506866 | Ca entry | Plasma membrane |
| S100A10 | 6281 | 0.829648793 | CaBP | Cytosol / Nucleus |
| CACNA1C | 775 | 0.830254197 | Ca entry | Plasma membrane |
| NUCB2 | 4925 | 0.838697314 | CaBP / DNA binding | Cytosol |
| ATP2B2 | 491 | 0.844160676 | Ca ²⁺ outflow | Plasma membrane |
| CALM1 | 801 | 0.85573566 | CaBP | Cytosol |
| RASEF | 158158 | 0.862091482 | CaBP | Cytosol |
| RCN2 | 5955 | 0.863518476 | CaBP | ER |
| CETN2 | 1069 | 0.864145756 | CaBP | Centrosome |
| CALM2 | 805 | 0.864191592 | CaBP | Cytosol |
| LETM1 | 3954 | 0.87683773 | Ca ²⁺ outflow | Mitochondria |
| EFHC1 | 114327 | 0.877512217 | CaBP | Cytosol |
| S100A4 | 6275 | 0.880372941 | CaBP | Cytosol / Nucleus |
| ORAI2 | 80228 | 0.882773757 | Ca entry | Plasma membrane |
| CALM3 | 808 | 0.889790297 | CaBP | Cytosol |
| ITPR3 | 3710 | 0.89111346 | Ca entry | ER/SR |
| STIM2 | 57620 | 0.891906142 | Ca entry | ER/SR |
| S100A1 | 6271 | 0.894057453 | CaBP | Cytosol / Nucleus |
| VDAC1 | 7416 | 0.89487958 | channel | Mitochondria |
| MYL12B | 103910 | 0.89888984 | CaBP | cytosol |

| CALU | 813 | 0.899016738 | CaBP | ER | |
|----------|--------|-------------|--------------------------|---------------------|--|
| MYL12A | 10627 | 0.904072046 | CaBP | Cytosol | |
| S100A11 | 6282 | 0.906369627 | CaBP | Cytosol / Nucleus | |
| CALR | 811 | 0.907804906 | CaBP | ER | |
| CAB39 | 51719 | 0.908084214 | CaBP | Cytosol | |
| MICU1 | 10367 | 0.912965178 | Ca ²⁺ outflow | Mitochondria | |
| MYL6B | 140465 | 0.913026094 | CaBP | Cytosol | |
| TRPM8 | 79054 | 0.916101217 | Ca entry | Plasma membrane | |
| ATP2B1 | 490 | 0.916224957 | Ca ²⁺ outflow | Plasma membrane | |
| NCALD | 83988 | 0.91999191 | CaBP | Cytosol | |
| S100A2 | 6273 | 0.921293378 | CaBP | Cytosol / Nucleus | |
| MCTP1 | 79772 | 0.92139107 | CaBP | Membrane | |
| TPCN2 | 219931 | 0.922363162 | Ca entry | Membrane / Lysosome | |
| SLC25A12 | 8604 | 0.928004563 | Ca ²⁺ outflow | Mitochondria | |
| SRI | 6717 | 0.93116945 | CaBP | Cytosol / SR | |
| VDAC3 | 7419 | 0.931555867 | channel | Mitochondria | |
| CARHSP1 | 23589 | 0.940751493 | CaBP | Cytosol | |
| ATP2C1 | 27032 | 0.949672997 | Ca ²⁺ outflow | Plasma membrane | |
| S100A13 | 6284 | 0.951274991 | CaBP | Cytosol / Nucleus | |
| S100A6 | 6277 | 0.953499198 | CaBP | Cytosol / Nucleus | |
| TRPM7 | 54822 | 0.956226528 | Ca entry | Plasma membrane | |
| EFCAB11 | 90141 | 0.958138525 | CaBP | ND | |
| ATP2B4 | 493 | 0.961005092 | Ca ²⁺ outflow | Plasma membrane | |
| STC1 | 6781 | 0.964208543 | CaBP | Secreted | |
| ORAI1 | 84876 | 0.964939058 | Ca entry | Plasma membrane | |
| HPCAL1 | 3241 | 0.967616558 | CaBP | Membrane | |
| CAB39L | 61617 | 0.971555114 | CaBP | ND | |
| MYL9 | 10398 | 0.972350121 | CaBP | Cytosol | |
| NCS1 | 23413 | 0.975012004 | CaBP | Cytosol | |
| MICU3 | 286097 | 0.978178382 | Ca ²⁺ outflow | Mitochondria | |
| TRPC1 | 7220 | 0.988282204 | Ca entry | Plasma membrane | |
| VDAC2 | 7417 | 0.996251523 | channel | Mitochondria | |
| PSEN1 | 5663 | 0.999276936 | CaBP | Membrane | |
| GCA | 25801 | 0.999806046 | CaBP | Cytosol | |
| CAPS2 | 84698 | 1.00509083 | CaBP | ND | |
| CACNG4 | 27092 | 1.00808823 | Ca entry | Plasma membrane | |
| ATP2A2 | 488 | 1.01196682 | Ca ²⁺ outflow | ER/SR | |
| CACNB4 | 785 | 1.01560533 | Ca entry | Plasma membrane | |
| EFCAB14 | 9813 | 1.01783824 | CaBP | ND | |
| RCN1 | 5954 | 1.02335477 | CaBP | ER | |
| CHP1 | 11261 | 1.03763425 | CaBP | Cytosol / Nucleus | |
| NUCB1 | 4924 | 1.0463016 | TF | Golgi | |
| PSEN2 | 5664 | 1.05628753 | CaBP | Membrane | |
| CACFD1 | 11094 | 1.06460106 | Ca entry | Plasma membrane | |
| SLC24A1 | 9187 | 1.0790931 | Ca ²⁺ outflow | Plasma membrane | |
| CLGN | 1047 | 1.09286618 | CaBP | ER | |

| SLC25A23 | 79085 | 1.09671128 | Ca ²⁺ outflow | Mitochondria |
|-----------|--------|------------|--------------------------|----------------------|
| CACNA1H | 8912 | 1.10686553 | Ca entry | Plasma membrane |
| CACNB3 | 784 | 1.11599135 | Ca entry | Plasma membrane |
| ATP2A3 | 489 | 1.13170516 | Ca ²⁺ outflow | ER/SR |
| ATP2A1 | 487 | 1.13189495 | Ca ²⁺ outflow | ER/SR |
| PKD1L2 | 114780 | 1.13193941 | Ca entry | Membrane |
| NECAB3 | 63941 | 1.13303959 | CaBP | Golgi |
| STIM1 | 6786 | 1.13753235 | Ca entry | ER/SR |
| CALCOCO2 | 10241 | 1.14259505 | CaBP | Cytosol |
| ITPR1 | 3708 | 1.15209162 | Ca entry | ER/SR |
| TRPM4 | 54795 | 1.19094431 | Ca entry | Plasma membrane |
| RAB11FIP3 | 9727 | 1.19934535 | CaBP | Cytosol / Centrosome |
| PKD1 | 5310 | 1.19998717 | Ca entry | Plasma membrane |
| GUCA1B | 2979 | 1.25257576 | CaBP | Membrane |
| ZZEF1 | 23140 | 1.29527771 | TF | ND |
| RCN3 | 57333 | 1.35777569 | CaBP | ER |
| SLC8B1 | 80024 | 1.40965879 | Ca entry | Mitochondria |
| CALCOCO1 | 57658 | 1.41236198 | CaBP | Cytosol |
| ORAI3 | 93129 | 1.43146002 | Ca entry | Plasma membrane |
| MYL5 | 4636 | 1.44176078 | CaBP | Cytosol |
| CARF | 79800 | 1.45379829 | TF | Nucleus |
| TPCN1 | 53373 | 1.50876069 | Ca entry | Membrane / Lysosome |
| CAPS | 828 | 1.87185323 | CaBP | Cytosol |
| EFHD1 | 80303 | 1.93805242 | CaBP | Mitochondria |
| CACNB1 | 782 | 2.0890739 | Ca entry | Plasma membrane |

Table S3. List of antibodies used.

| Name | Type, Company | Dilution |
|------------|--|----------|
| Anti-Sox2 | Rabbit Polyclonal, Abcam | 1/100 |
| Anti-Nanog | Goat Polyclonal, R&D Systems | 1/100 |
| Anti-Olig2 | Rabbit Polyclonal, Millipore Bioscience | 1/500 |
| Anti-TOM20 | Rabbit Polyclonal, Santa Cruz Bioechnology | 1/100 |
| Anti-Ki67 | Mouse, Dako | 1/100 |
| Anti-GFP | Mouse, Roche | 1/1000 |

Table S4. Sequences of primers used

| Tata box BP NM_003194 GAGCTGTGATGTGAAGTTTCC 1059 117 bp (1) CDKN1A (p21) NM_000389 GACTCTCAGGTCGAAAGG 531 94 bp (2) GGATTAGGGCTTCCTCTTGG 623 0 0 0 0 CCNB1 NM_0131966 TCTTCCAGTGTGGCAT 1464 0 0 G052 NM_015714.3 AAGGGGAAGATGGTGAAGCTG 315 85 BP primer3 CTGCACACAGTCTCATCAGG 400 CCNA2 NM_015203 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCCTACTAGTGACCC 896 0 0 0 CCND3 NM_001760 AGGATCACTGGAAGT 909 102 bp Primer3 CCNE2 NM_057749 TTGGCTATGCTGAGAGGAGT 1193 103 bp Primer3 AGGGTCATGGAGGCATTATAC 618 89 bp (4) GGGGTAGGTCATGGAGAGCAT 100 100 bp (5) FCN2 NM_002524.3 ACCTCCGAGGGCCTAATAC 618 89 bp (4) | Gene name | Accession number | Primer sequences (5'-3') | Position | Amplicon size | References |
|--|--------------|---------------------|-----------------------------|----------|------------------|------------|
| TCTGGGTTTGATCATTCTGTAG 1176 CDKN1A (p21) NM_00388 GACTCTCAGGGTCGAAAAGG 531 94 bp (2) GGATTAGGGCTCCTCTGG 623 < | Tata box BP | NM_003194 | GAGCTGTGATGTGAAGTTTCC | 1059 | 117 bp | (1) |
| CDKN1A (p21) NM_000389 GACTCTCAGGGTCGAAAACG 531 94 bp (2) CCNB1 NM_031966 TCTTCCAGTTAGCAGCACCT 1381 103 bp primer3 AGCATGCTTCGATGTGGCAT 1464 G052 NM_015714.3 AAGGGAAGATGGTGAAGCTG 315 85 BP primer3 CCNA2 NM_001237 GGAAGTGAAAAGCCAGTGGACT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCCTACTACCGCCTCA 878 59 bp (3) CAGTCCGGGTCACACTGGAAGGC 971 CCMD2 Primer3 CCND2 NM_001760 AGGGATCACTGGCACACTGAAGGC 971 CCND2 NM_001760 AGGGATCACTGGCACACTGAAGGC 971 CCND2 NM_057749 TTTGCCTGCAGAGGACG 1193 103 bp Primer3 AGTGCTCTCGGGTGCACACTTGA 1276 HES1 NM_05524.3 ACGTGCCAGGGTCTACACTG 120 bp Primer3 GGGTAGGTCTCGGAGGCCTCAGTATGA | | | TCTGGGTTTGATCATTCTGTAG | 1176 | | |
| GGATTAGGGCTTCCTTTGG 623 CCNB1 NM_031966 TCTTCCAGTTATGCAGCACCT 1381 103 bp primer3 AGCATGCTTCGATGTGGCACCT 1381 103 bp primer3 G052 NM_015714.3 AAGGGGAAGATGGTGAAGCTG 315 85 BP primer3 CCRA2 NM_01237 GAAGATGAAAAGCCAGTGACTG 798 120 bp primer3 TGGCTGTTCTTCATGTAACCC 896 CCND1 NM_053056.2 CCTGCTCACACTGACGCTCA 878 59 bp (3) CAGTCCGGGTCACACTTGA 937 <t< td=""><td>CDKN1A (p21)</td><td>NM_000389</td><td>GACTCTCAGGGTCGAAAACG</td><td>531</td><td>94 bp</td><td>(2)</td></t<> | CDKN1A (p21) | NM_000389 | GACTCTCAGGGTCGAAAACG | 531 | 94 bp | (2) |
| CCNB1 NM_031966 TCTTCCAGTTATGCAGCACCT 1381 103 bp primer3 G052 NM_015714.3 AAGGGGAAGATGGAGAGCTG 315 85 BP primer3 CTGCACACAGTCTCCATCAGG 400 CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCTACTACCGCGCTCA 878 59 bp (3) CCND3 NM_001760 AGGGATCACTGGCACAGTGAGG 909 102 bp Primer3 CCND3 NM_057749 TTTGGCTATGCTGAGGAGAGT 1193 103 bp Primer3 AGTGCTCTTCGGTGGAGGAGGCGTTAATAC 618 89 bp (4) GGGGTAGGCCTGAACG 991 PCNA NM_002592 GCCACTCCACTCTCTCAACG 991 Primer3 ATCCTGGAGCAGAG 991 STIM1 NM_003156.3 TGTGGAGCAGGCGTTAATAC 618 89 bp (4) GGGGTAGGCCTCACACTCTCCAGTATG 1000 108 bp (5) CTTCAGCACAGTGCCCCAGTCCTCAGTATG STIM1 NM_003156.3 TGTGCAC | | | GGATTAGGGCTTCCTCTTGG | 623 | | |
| AGCATGCTTCGATGTGGCAT 1464 G052 NM_015714.3 AAGGGAAGATGGTGAAGCTG 315 85 BP primer3 CTGCACACAGTCTCCATCAGG 400 C CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAAGTGT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCCTCATCACGCCTCA 878 59 bp (3) CCND3 NM_001760 AGGATCACACTGCACACTGAAGT 999 102 bp Primer3 CCNE2 NM_057749 TTTGGCTATGCGGAGGAGAGT 1193 103 bp Primer3 AGGGGTAGGTCATGGCGGTGTGAGAGAGAGT 1193 103 bp Primer3 AGGGGTAGGTCATGGCATTGA 707 PCNA NM_005524.3 ACGTGCGAGGAGCATTGA 707 Primer3 AGGGGTAGGTCCTCCACTGTCA 1108 STIM1 NM_002592 GCCACTCCACTCTCATGGAGACG 991 120 bp Primer3 ATCCTGGACACGGAGGCC 991 120 bp Primer3 GGGGTAGGTCATGGCCA 991 STIM1 NM_002592 GCCACTCCACTCTCATGAG 1193 103 bp (5) CTTCAGCACAGTCCCCCGTGCA | CCNB1 | NM_031966 | TCTTCCAGTTATGCAGCACCT | 1381 | 103 bp | primer3 |
| G0S2 NM_015714.3 AAGGGGAAGATGGTGAAGCTG 315 85 BP primer3 CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCCTACTACCGCCTCA 878 59 bp (3) CCND3 NM_001760 AGGGATCACTGGCACTGAAGG 909 102 bp Primer3 CCND3 NM_001760 AGGGATCACTGGAGGAGGC 971 CCNE2 NM_057749 TTTGGCTATGCTGAGAGGCG 971 CCNE2 NM_005524.3 ACGTGCGAGGGCTTAATAC 618 89 bp (4) GGGGTAGGCCTCCTCTCTCTCAAGG 911 120 bp Primer3 ATCCTCGATCTTGGGAGCCA 991 STIM1 NM_002592 GCCACTCCACTCTCTCTCAAGG 911 120 bp STIM2 NM_020860 GACGTCAGTGCTCCAGTAG 1000 108 bp (5) GCAACTGCTGGCACAGTGCTCCAG | | | AGCATGCTTCGATGTGGCAT | 1464 | | |
| CTGCACACAGTCTCCATCAGG 400 CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 TGGCTGTTTCTTCATGTAACCC 896 (3) CAGTCCGGGTCACACTGAAGT 937 CCND1 NM_053056.2 CCTGTCCTACTACCGCCTCA 878 59 bp (3) CCND3 NM_001760 AGGGATCACTGGCACTGAAGT 909 102 bp Primer3 CCND3 NM_057749 TTTGGCTATGCTGGAGGAAGT 1193 103 bp Primer3 AGTGCTCTCGGTGGTGTCA 1276 PCNA NM_005524.3 ACGTGCGAGGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCACAGTGCA 191 120 bp Primer3 ATCCTCGATCTTCGGTGGCTCA 111 120 bp Primer3 ATCCTCGATCTGGAACAG 1108 p (5) CTTCAGCACAGTCCTCGTGCA 11000 108 bp (5) CTTCAGCACAGTCCTCGAGAGACAG 1478 87 bp (5) GACCAACTGCTTCTCAGTGGTGCA 1108 121 bp (5) CTTCAGCACAGTCCTGGAGAACAG 1478 87 bp | G0S2 | NM_015714.3 | AAGGGGAAGATGGTGAAGCTG | 315 | 85 BP | primer3 |
| CCNA2 NM_001237 GAAGATGAAAAGCCAGTGAGTGT 798 120 bp primer3 CCND1 NM_053056.2 CCTGTCTTCTTCATGTAACCC 896 (3) CCND3 NM_01760 AGGGATCACCGGGTCACACTTGA 937 (3) CCND3 NM_001760 AGGGATCACTGGCACTGAAGGT 909 102 bp Primer3 CCND2 NM_057749 TTTGGCTAGCGGGGGGTAATATC 618 89 bp (4) GGGGTAGGTCATGGCAGGGGGGTAATAC 618 89 bp (4) GGGGTAGGTCACTGGAGGCAC 911 120 bp Primer3 ACTCCGAGGCGCGTAATAC 618 89 bp (4) GGGGTAGGTCATGGAGCAC 911 120 bp Primer3 STIM1 NM_005524.3 ACGTGCAACCTCCTCTTCAACG 911 120 bp Primer3 ATCCTCGATCTGGAGGCCTAATAC 618 89 bp (4) GGGGTAGGTCTTCGACGACA 991 STIM1 NM_003156.3 TGTGGAACCTGCTCAGTATG 1000 108 bp (5) GACCAACTGCTTCCAGTTC 1108 STIM2 NM_020860 GACGTCAGTAGGAACAG 1478 87 bp | | | CTGCACACAGTCTCCATCAGG | 400 | | |
| TGGCTGTTTCTTCATGTAACCC 896 CCND1 NM_053056.2 CCTGTCCTACTACCGGCTCA 878 59 bp (3) CCND3 NM_001760 AGGGATCACTGGACACTTGA 937 909 102 bp Primer3 CCND3 NM_001760 AGGGATCACTGGACAGTGAAGT 909 102 bp Primer3 CCNE2 NM_057749 TTTGGCTGTGGAGGAGGAGT 1193 103 bp Primer3 AGTGCTCTTCGGTGGTGTCA 1276 1193 103 bp Primer3 AGTGCTCTTCGGAGGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGGA 707 7 PCNA NM_002592 GCCACTCCACTCTCTCAACG 911 120 bp Primer3 ATCCTCGATCTGGAGCCACAGTGCCTCAGTATG 1000 108 bp (5) 101 STIM1 NM_003156.3 TGTGGAGCTCAGTATGCAGAACAG 1478 87 bp (5) GACAACTGCTTCCAGTTC 1565 1000 108 bp (5) 104 STIM2 NM_023280 GACGACACTGGTGGGAG 494 121 bp (5) | CCNA2 | NM_001237 | GAAGATGAAAAGCCAGTGAGTGT | 798 | 120 bp | primer3 |
| CCND1 NM_053056.2 CCTGTCCTACTACCGCCTCA 878 59 bp (3) CCND3 NM_001760 AGGGATCACTGGCACTGAAGGT 909 102 bp Primer3 CCND3 NM_057749 TTTGGCTACCTGGAGAGAGT 917 103 bp Primer3 CCNE2 NM_057749 TTTGGCTATCCTGGAGGAGAGT 1193 103 bp Primer3 AGTGCTCTTCGGTGGGTGTCA 1276 102 bp Primer3 AGTGCTCTTCGGTGGCATTGA 707 PCNA NM_005592 GCCACTCCACTCTCTTCAGAGACG 911 120 bp Primer3 ATCCTCGATCTGGAAGCGCCCTGA 991 120 bp Primer3 ATCCTCGATCTGGCATTGGCATTG 910 108 bp (5) CTTCAGCACAGTCCTCGTCA 1108 511///1 NM_002156.3 TGTGGAGACTGCTCAGTAT 1000 108 bp (5) CTTCAGCACAGTCCTGGAACCG 1478 87 bp (5) GACCAACTGCTTCCAGTACT 155 ORA/11 NM_02290 ATGGTGGCAACAGG 1478 87 bp (5) GGGGTACTGGTACTGGAACAGG 1478 121 bp (5) GCGGGTACTG | | | TGGCTGTTTCTTCATGTAACCC | 896 | | |
| CAGTCCGGGTCACACTTGA 937 CCND3 NM_001760 AGGGATCACTGGCACTGAAGT 909 102 bp Primer3 CCND2 NM_057749 TTTGGCTATGCTGGAGGAGC 971 103 bp Primer3 AGTGCTCTCGGTGGTGTCA 1276 103 bp Primer3 HES1 NM_005524.3 ACGTGCCGAGGGCTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGGA 707 PCNA NM_002592 GCCACTCCACTCTCTTCAACG 911 120 bp Primer3 ATCCTCGATCTGGGAGGCCA 991 103 bp (5) CTTCAGCACAGTCCCTGATATG 1000 108 bp (5) STIM1 NM_0203156.3 TGTGGAGCTGCCTCAGTATG 1000 108 bp (5) GACCAACTGCTTCCCAGTATG 1000 108 bp (5) (5) (5) (5) GACCAACTGCTCCTCAGTATG 100 108 bp (7) (5) (6) (5) GCMAI1 NM_0202080 GACCAACTGCTCCAGTTC 349 175 bp (6) (6) (6) (7) (CGGATCATGGTACTCGGGACT 349 | CCND1 | NM_053056.2 | CCTGTCCTACTACCGCCTCA | 878 | 59 bp | (3) |
| CCND3 NM_001760 AGGGATCACTGGCACTGAAGT 909 102 bp Primer3 CCNE2 NM_057749 TTTGGCTATGCTGGAGGAGC 971 103 bp Primer3 AGTGCTCTTCGGTGGTGTCA 1276 1193 103 bp Primer3 HES1 NM_005524.3 ACGTGCGAGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGA 707 7 7 7 PCNA NM_002592 GCCACTCCACTCTCTTCAACG 911 120 bp Primer3 ATCCTCGATCTTGGGAGCCA 991 1000 108 bp (5) STIM1 NM_0020860 GACCTAACTGCTGAALAGG 1478 87 bp (5) GACCAACTGCTTCAGTCTC 1565 0 | | | CAGTCCGGGTCACACTTGA | 937 | | |
| CTGGAGCTGGTCTGAGAGGC 971 CCNE2 NM_057749 TTTGGCTATGCTGGAGGAAGT 1193 103 bp Primer3 AGTGCTCTTCGGTGGTGTCA 1276 1276 1276 1276 HES1 NM_005524.3 ACGTGCCGAGGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGGA 707 707 707 707 PCNA NM_002592 GCCACTCCACTCTTCAACG 911 120 bp Primer3 ATCCTCGATCTTGGGAGCCA 991 700 108 bp (5) STIM1 NM_003156.3 TGTGGAGCTGCCTCAGTATG 1108 707 STIM2 NM_020860 GACGTCAGTATGCAGAACAG 1478 87 bp (5) GACCAACTGCTTCTCAGTTC 1565 707 707 707 707 ORAI1 NM_032790 ATGGTGGCAATGGTGGAGA 494 121 bp (5) 70 CGGGTACTGGAACTGGTACTGGGTC 349 175 bp (6) 70 70 CGGGTAGGTCTCCAGGTACTGGGTGCT 524 70 70 70 70 | CCND3 | NM_001760 | AGGGATCACTGGCACTGAAGT | 909 | 102 bp | Primer3 |
| CCNE2 NM_057749 TTTGGCTATGCTGGAGGAAGT 1193 103 bp Primer3 HE51 NM_005524.3 ACGTGCCAGGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGA 707 Primer3 ATCCTCGATCATGGCATTGA 707 PCNA NM_002592 GCCACTCCACTCTTCTAACG 911 120 bp Primer3 STIM1 NM_003156.3 TGTGGAGCCCCAGGAGCCA 991 STIM2 NM_020860 GACGTCAGTGCCTGTCA 1108 STIM2 NM_020860 GACGCAACTGCTTCTCAGTTC 1565 STIM2 NM_020860 GACCAACTGCTTCCAGTTC 1565 STIM2 NM_032790 ATGGTGGCAATGGTGGAG 494 121 bp (5) CTGATCATGAGCGCCAAGG 615 STIM2 NM_032831 GCAGCTACTGGAACTGGTC 349 175 bp (6) CGGGTACTGGTACTCGTGGT 524 S24 | | | CTGGAGCTGGTCTGAGAGGC | 971 | | |
| AGTGCTCTTCGGTGGTGCA 1276 HES1 NM_005524.3 ACGTGCGAGGGCGTTAATAC 618 89 bp (4) GGGGTAGGTCATGGCATTGA 707 707 707 707 PCNA NM_002592 GCCACTCCACTCTCTTCAACG 911 120 bp Primer3 ATCCTCGATCTTGGGAGCCA 991 1000 108 bp (5) STIM1 NM_003156.3 TGTGGAGCTGCCTCAGTATG 1000 108 bp (5) STIM2 NM_020860 GACGTCAGTAGCAGAACAG 1478 87 bp (5) GACCAACTGCTTCCAGTAGCAGAACAG 1478 87 bp (5) (5) GACAACTGGCTACTGAGAGAG 494 121 bp (5) (5) CTGATCATGAGCCAAACAG 615 (6) (6) (7) CGGGTACTGGTACCTGGAACTGGTC 349 175 bp (6) (6) CGGGTACGTGGTACTGGGACTCCTGGGT 484 (7) (7) (7) (7) CCTGATGATATGGTGCTCAAAGCTCCTCAGTCT 338 104 bp (7) (7) (7) (7) (7) (7) </td <td>CCNE2</td> <td>NM_057749</td> <td>TTTGGCTATGCTGGAGGAAGT</td> <td>1193</td> <td>103 bp</td> <td>Primer3</td> | CCNE2 | NM_057749 | TTTGGCTATGCTGGAGGAAGT | 1193 | 103 bp | Primer3 |
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| | | — | AAGAGGAAGTCTCGTGGTGTC | 400 | · | . / |

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Quiescence status of glioblastoma stem-like cells involves remodelling of Ca²⁺ signalling and mitochondrial shape

AUTHORS

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Supplementary Legends

Figure S1. In vitro induction of quiescence in TG1 cells.

(A) Trypan blue viability test. TG1 cells at pH7.4 (filled black bars) and pH 6.5 (striped black bars) and TG1_C1 cells at pH7.4 (filled grey bars) and at pH6.5 (striped grey bars). Each measure was done in triplicates and with 3 independent experiments. (B) Histogram plot of the percentage of EdU and Ki67 positive cells in NS34 at pH 7.4 (black bars) or pH 6.5 (white bars), determined by analysis of 6 confocal microscopy fields. Error bars are derived from 3 independent experiments. (C) TG1 cells induced to quiescence continue to express stemness markers. Immunofluorescence analysis of stemness markers (NANOG, OLIG2 and SOX2) after 5 days in culture at pH 7.4 (proliferating condition, left panel) and at pH 6.5 (quiescent condition, right panel). Nuclei were visualized by Draq5 staining. Pictures taken with a 63X 1.40 N.A. objective on a Leica SP8 upright confocal microscope. Scale bar = 20 μm.

Figure S2. The cell-cycle arrest induced by low extracellular pH is reversible.

(A) Proliferating TG1 cells were cultured for 5 days in NS34 medium at pH 6.5 to induce quiescence and then either transferred back to NS34 at pH 7.4 (black dots) or to NS34 at pH 6.5 (grey squares) for an additional 5 days and the number of viable cells counted. (B) Histogram plot of the percentage of EdU and Ki67 positive cells in NS34 at pH 7.4 (black bars), pH 6.5 (open bars) and reversible conditions (pH 6.5 to pH 7.4; grey bars), determined by analysis of 6 confocal microscopy fields. Error bars are derived from 3 independent experiments. (C) Expression of *CDKN1A*, *CCNB1* and *G0S2* was assessed by QRT-PCR in TG1 cells cultured for 5 days in proliferating (NS34 at pH7.4, black bars), quiescent (NS34 at pH 6.5, open bars) and reversible conditions (NS34 at pH 6.5 and then 5 days at pH 7.4, grey bars). Results are given relative to *TBP* (TATA-Box Binding Protein) expression level. Error bars are derived from 11 independent experiments (pH 7.4 and pH 6.5) and from 3 independent experiments (pH 7.4).

Figure S3. In vitro induction of quiescence in BTIC25 cells.

(A) BTIC25 cells were cultured for 5 days in NS34 medium at pH 7.5 (a) or pH 6.5 (b) to assess their ability to proliferate or to stop proliferation and go to quiescence. In NS34 medium, cells are floating and never form monolayer. (B) Cell proliferation measured by counting the number of viable cells over 5 days in NS34 at pH 7.4 in absence (black bars) or presence of SKF96365 (10 μ M) (striped bars) and in NS34 at pH 6.5 (open bars) for TG1 cells (left panel) and for BITC25 cells (right panel). Each measure were done in duplicate and with 3 independent experiments. (C) Expression of *CDKN1A*, *CCNB1* and *G0S2* was assessed by QRT-PCR in TG1 cells after 5 days in NS34 at pH7.4 in absence (black bars) or presence of SKF96365 (10 μ M) (striped bars) and in NS34 at pH 6.5 (open bars) for BITC25 cells. Results

are given relative to *TBP* (TATA-Box Binding Protein) expression level. Error bars are derived from 11 independent experiments. Error bars are derived from 3 independent experiments. Pictures were taken with a 20X 0.40 N.A. objective on Nikon eclipse TS100 microscope. Scale bars: 100 μ m in A.

Figure S4. Remodeling of SOCE activity between proliferative and quiescent GSLCs.

(A) Structure of the chimeric gene encoding the CytGA probe. The open-reading frame (ORF) encoding the Green Fluorescent Protein (EGFP) is linked to the ORF encoding the apo-aequorin as described previously¹. Confocal microscopy observation of the GFP signal of electroporated TG1 cells showed good expression of the probe in the cytosol (green). Nucleus was labelled with Draq5 (blue). Scale bar: 5 μ m. (B) Structure of the chimeric gene encoding the MitGA probe. The open-reading frame (ORF) encoding the Green Fluorescent Protein (EGFP) is linked to the ORF encoding the apo-aequorin and to the mitochondrial targeting sequence Cox VIII. Confocal microscopy observation of the GFP signal of electroporated TG1 cells showed good expression of the probe in the mitochondria (merge). Nucleus was labeled with Draq5 (blue) and mitochondria with anti-TOM20 (red). (C) Representative PMT traces obtained from CytGA expressing TG1_C1 cells in proliferating (left panel, pH 7.4) or in quiescent (right panel, pH 6.5) conditions. Values are plotted as L/L_{TOTAL} and every trace is the mean of 3 independent experiments. Prior to recording, cells were washed with Ca²⁺-free medium. The arrows indicate the time at which medium containing 1 mM Ca²⁺ is perfused. Pictures in A and B were taken with a 63X 1.40 N.A. objective on a Leica SP8 upright confocal microscope.

Figure S5. Calcium signaling is involved in the induced-quiescent state.

(A) Cell proliferation measured by counting the number of viable cells over 5 days in NS34 medium at pH 7.4 in absence (black bars) or presence of EGTA (5 or 10 μM) (grey bars). Each measure was done in triplicates; 3 independent experiments were performed. (B). Expression of CDKN1A, CCNB1 and GOS2 was assessed by QRT-PCR after 5 days of culture in TG1 cells in NS34 at pH7.4 in absence (black bars) or presence of EGTA (grey bars) and compared to TG1 cells in NS34 at pH 6.5 (open bars). Results are given relative to TBP (TATA-Box Binding Protein) expression level. Error bars are derived from 11 independent experiments. (C) Expression of cyclins required during the different phases of the cell cycle; CCND1, D3 (G1), E2 (G1/S transition) and A2 (S/G2 transition), of PCNA (Proliferating Cell Nuclear Antigen) and of Hes1 which is known to be involved in the control of the reversibility of cellular quiescence. Expression of these cell cycle markers was assessed by QRT-PCR after 5 days of culture in TG1 cells in NS34 at pH7.4 in absence (black bars) or presence of EGTA (grey bars) and compared to TG1 cells in NS34 at pH 6.5 (open bars). Results are given relative to TBP (TATA-Box Binding Protein) expression level. Error bars are derived from 4 independent experiments. (D) Immunofluorescence for Ki67 of TG1 cells after 5 days in culture at pH 7.4 in presence of EGTA (10 μ M). Nuclei were visualized by Draq5. Scale bar = 20 μm. (E) EdU incorporation (upper panels) and Ki67 positive cells (lower panels) at pH 7.4 and pH 6.5 and at pH7.4 in presence of SKF96365 (10 μ M) for TG1 cells. Scale bar: 5 μ m. Pictures taken with a 63X 1.40 N.A. objective on a Leica SP8 upright confocal microscope.

Figure S6. Expression of the members of the Store-Operated Calcium Entry.

Expression of *STIM* and *ORAI* family members in TG1 cells was assessed by QRT-PCR cultured for 5 days either in NS34 at pH7.4 (black bars, proliferative condition) or in NS34 at pH 6.5 (open bars, quiescent condition). Results are given relative to *TBP* (TATA-Box Binding Protein) expression level. Error bars are derived from 3 independent experiments.

Figure S7. Mitochondrial morphology remodeling in BTIC25 cells. Confocal microscopy analysis of mitochondria shapes in proliferating (left panel, pH 7.4), in quiescent BTIC25 cells (middle panel, pH 6.5) and in SKF96365-treated BTIC25 cells (right panel, pH 7.4 + SKF96365 10 μ M). Mitochondria were labeled with TOM20 antibody (green) and nucleus with Draq5 (red). Tubular mitochondria are found in proliferating BTIC25 cells (left panel and Inset) and donut-shaped mitochondria in quiescent and SKF96365-treated BTIC25 cells (Middle and right panel). Pictures taken with a 63X 1.40 N.A. objective on a Leica SP8 upright confocal microscope. Scale bars: 10 μ m.



С

pH 7.4

pH 6.5

 Nanog
 Draq5
 Merge
 Nanog
 Draq5
 Merge

 Olig-2
 Draq5
 Merge
 Olig-2
 Draq5
 Merge

 SOX2
 Draq5
 Merge
 SoX2
 Draq5
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NS34, pH 7.4

NS34, pH 6.5

NS34, pH 7.4 + SKF96365

