

Figure S1. Specificity control of anti-glutamine staining of PDAC specimens. Cryostat sections (6 μm) of snap-frozen specimens from PDAC patients (all classified T3N1M0) were submitted to immunohistochemistry. Following an adapted crosslinking fixation protocol (see materials & methods), sections were probed with a rabbit Glutamine antibody (Abcam, ab9445) or a rabbit control antibody (Abcam, ab172730) at 1:100 dilution in *StainPerfect* antibody diluent and then further developed (see materials & methods). The result with two different PDAC specimens is shown.

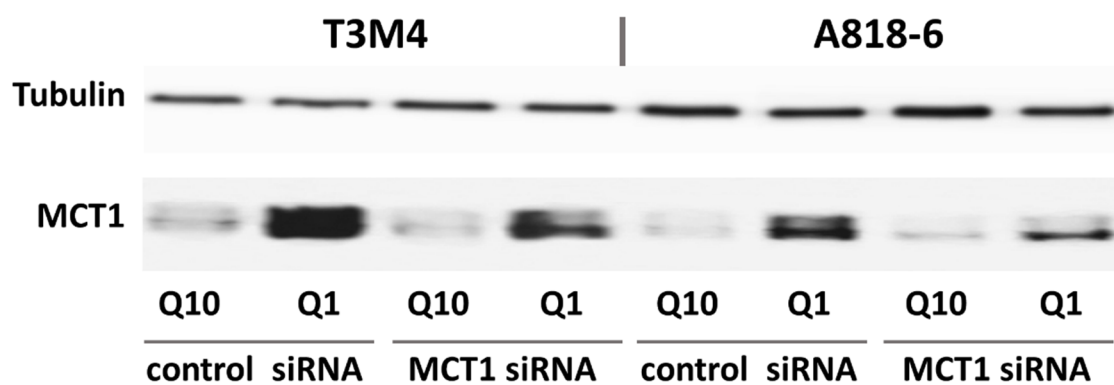


Figure S2. MCT1 knock-down in T3M4 and A818-6 cells. T3M4 and A818-6 cells were transfected with either control siRNA or MCT1 siRNA for 24h and then further cultured under normal glutamine (Q10) or low glutamine supply (Q1) for 48h. A western blot figure from a representative experiment is shown.

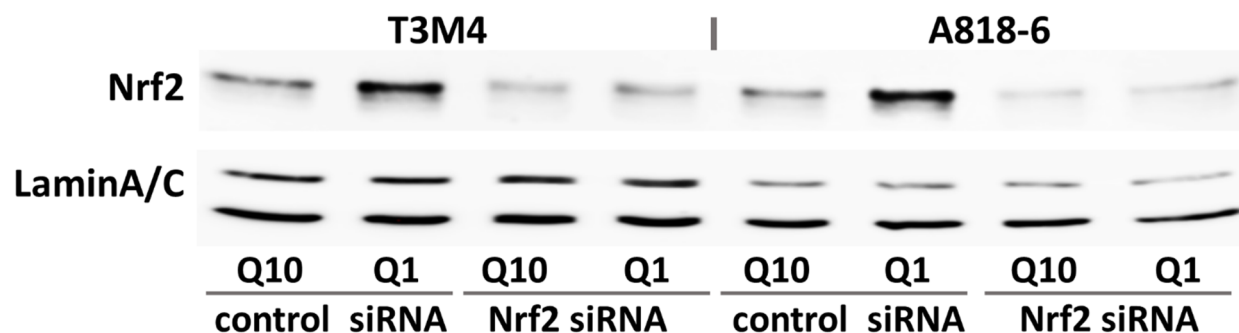


Figure S3. NRF2 knockdown in T3M4 and A818-6 cells. T3M4 and A818-6 cells were transfected with either control siRNA or Nrf2 siRNA for 24h and then further cultured under normal glutamine (Q10) or low glutamine (Q1) supply for 48h. A western blot with nuclear extracts from a representative experiment is shown detecting Nrf2 (Abcam 62352, 1:500 in 5% BSA/TBST) using Lamin A/C (Santa Cruz, 1:1000 in 5% skimmed milk/TBST) as loading control.

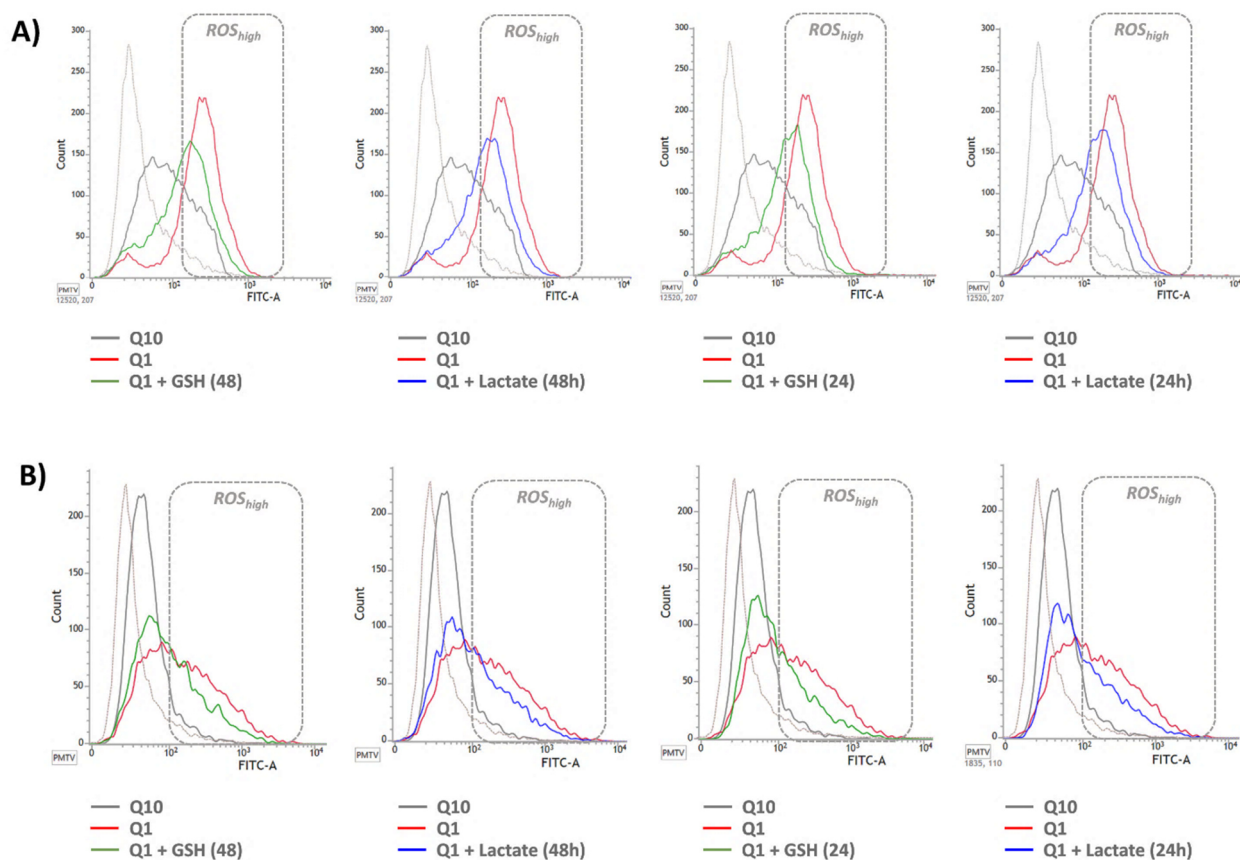


Figure S4. Lactate protects T3M4 and A818-6 cells from glutamine depletion induced ROS stress
A) T3M4 and **B)** A818-6 cells were cultured under normal glutamine (Q10) or low glutamine (Q1) supply for 48h, either in the absence or presence of 1.5 mM glutathione (GSH) or 20 mM lactate given at the same period or 24h later. Then, cells were DCFDA-stained and analyzed by flow cytometry. Those fluorescence signals >10-fold greater than the background signal with unstained cells (dotted line) were quantified as percentage of highly ROS-stressed (*ROS_{high}*) cells (see figure 3).

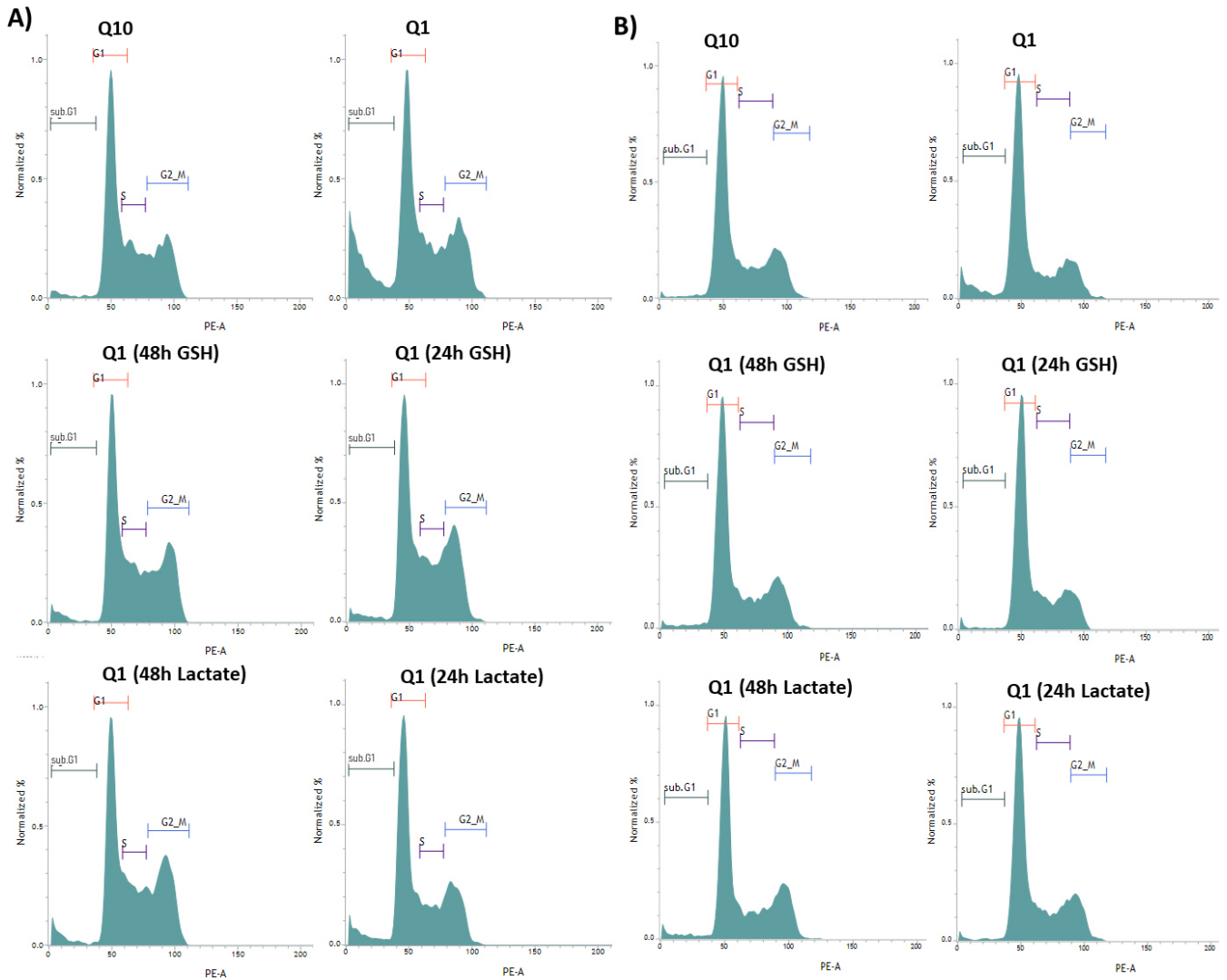


Figure S5. Lactate protects PDAC cells from cell death and cell cycle arrest under glutamine shortage. A) T3M4 and B) A818-6 cells cultured with Q10 or Q1 for 48h in the absence or presence of 1.5 mM GSH or 20 mM lactate, added in parallel (48h) or 24h later, were collected and stained with PI and then analyzed by flow cytometry. The subG1 fraction containing apoptotic cells as well as G1, S- and G2/M-cell cycle phase fractions were gated as indicated for quantification (see figure 6).

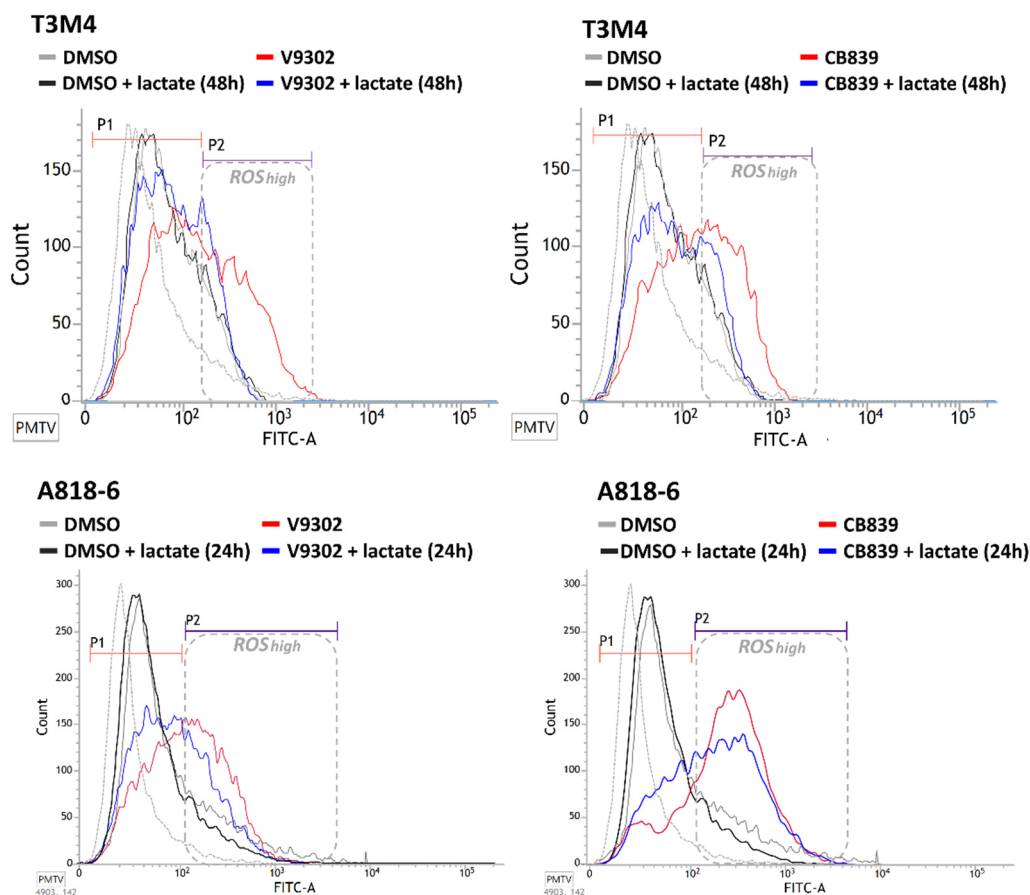


Figure S6. T3M4 and A818-6 cells were treated with V9302 (2 μ M), CB839 (10 M) or DMSO for 48h either in the absence or presence of 20 mM lactate as indicated. Then, cells were DCFDA-stained and analyzed by flow cytometry. Those fluorescence signals >10-fold greater than the background signal with unstained cells (dotted line) were quantified as percentage of highly ROS-stressed (ROS_{high}) cells (see figure 8).

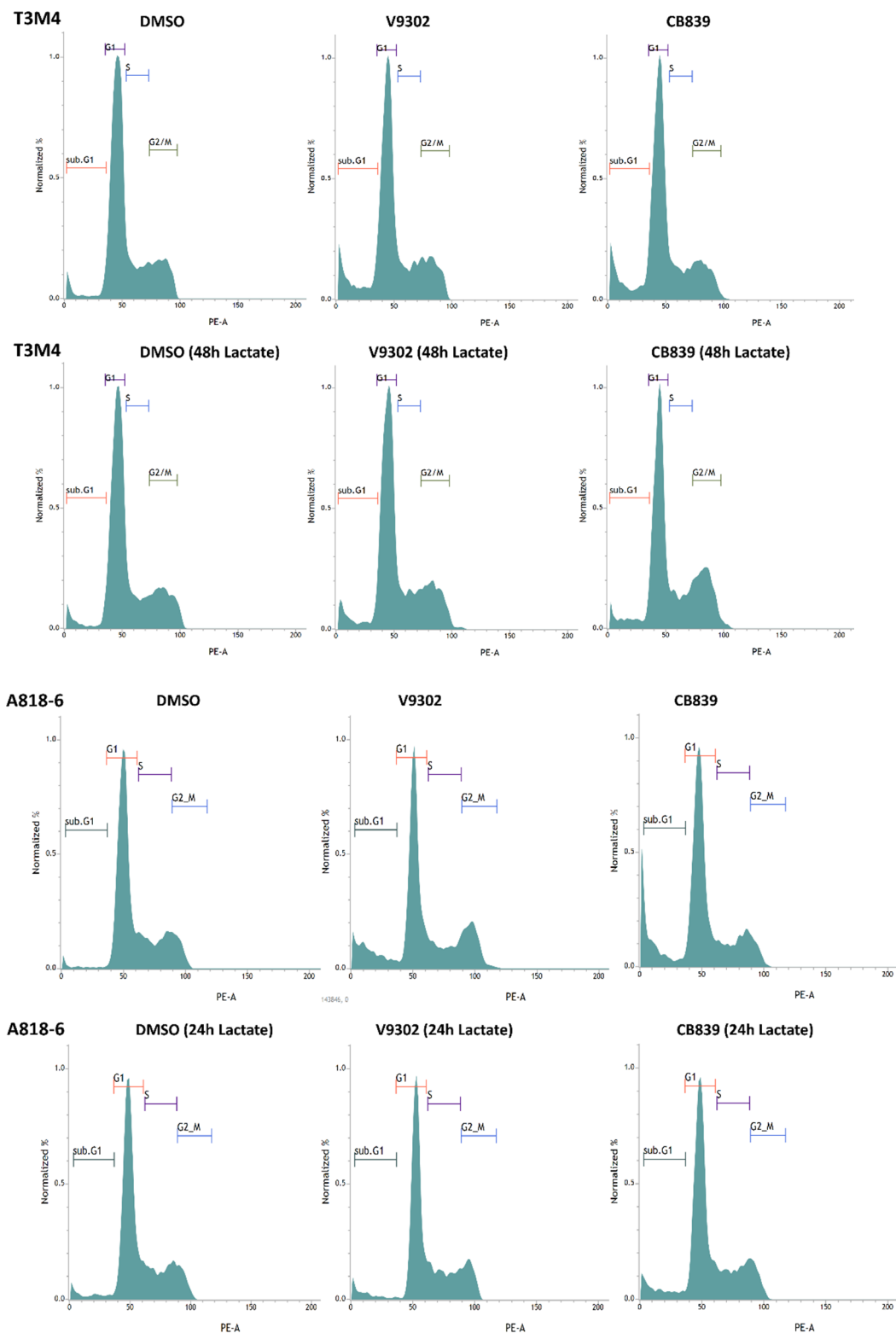


Figure S7. Lactate protects PDAC cells from V9302 or CB839 induced cell death and cell cycle arrest. T3M4 and A818-6 cells were treated with V9302 (2 μ M), CB839 (10 μ M) or DMSO for 48h either in the absence or presence of 20 mM lactate as indicated. Then, cells were collected, stained with PI and analysed by flow cytometry. The subG1 fraction containing apoptotic cells as well as G1, S- and G2/M-cell cycle phase fractions were gated as indicated for quantification (see figure 8).

T3M4	24h treatment	G1	S	G2/M
	Q10	43,26 ± 4,15	34,21 ± 6,20	22,53 ± 6,91
	Q1	53,22 ± 1,98*	28,35 ± 4,12*	18,43 ± 5,12
	Q1+24h GSH	45,81 ± 2,85	33,27 ± 5,62	20,92 ± 5,88
	Q1+24h Lactate	49,97 ± 2,69*	31,18 ± 5,27	18,86 ± 5,27
T3M4	48h treatment	G1	S	G2/M
	Q10	57,28 ± 5,17	21,17 ± 3,69	21,55 ± 4,12
	Q1	68,15 ± 5,58*	16,23 ± 3,25*	15,62 ± 3,49*
	Q1+48h GSH	57,66 ± 3,04*	21,01 ± 3,44 [#]	21,33 ± 3,88*
	Q1+48h Lactate	61,55 ± 5,22*	20,10 ± 2,78 [#]	18,35 ± 3,62
	Q1+24h GSH	60,64 ± 4,75*	19,82 ± 2,65 [#]	19,54 ± 4,21
	Q1+24h Lactate	63,18 ± 5,18	19,14 ± 4,42	17,67 ± 4,24
T3M4	72h treatment	G1	S	G2/M
	Q10	63,19 ± 7,07	18,80 ± 5,14	18,01 ± 2,84
	Q1	68,85 ± 6,21*	15,59 ± 3,25*	15,56 ± 3,49
	Q1+72h GSH	65,62 ± 3,94*	16,74 ± 3,16	17,64 ± 3,47
	Q1+72h Lactate	63,57 ± 5,25*	19,34 ± 3,51 [#]	17,09 ± 2,69
	Q1+48h GSH	65,04 ± 4,16*	17,43 ± 3,08	17,53 ± 2,97
	Q1+48h Lactate	65,25 ± 5,29*	18,42 ± 4,34 [#]	16,33 ± 3,26
	Q1+24h GSH	66,43 ± 5,86	17,94 ± 3,58	15,63 ± 2,55
	Q1+24h Lactate	66,02 ± 4,99	17,91 ± 3,68	16,07 ± 3,53
A818-6	24h treatment	G1	S	G2/M
	Q10	54,51 ± 4,67	30,14 ± 4,58	15,35 ± 1,77
	Q1	59,84 ± 3,84*	27,99 ± 3,65*	12,17 ± 3,23
	Q1+24h GSH	55,20 ± 4,02*	31,18 ± 5,84	13,62 ± 2,63
	Q1+24h Lactate	58,37 ± 5,79	28,78 ± 5,27	12,85 ± 2,72
A818-6	48h treatment	G1	S	G2/M
	Q10	50,07 ± 5,03	33,77 ± 4,01	16,16 ± 3,44
	Q1	57,78 ± 2,93*	27,29 ± 3,07*	14,93 ± 3,49
	Q1+48h GSH	52,29 ± 3,42*	32,62 ± 4,07 [#]	15,09 ± 3,88
	Q1+48h Lactate	53,08 ± 3,39	31,74 ± 5,14	15,18 ± 3,62
	Q1+24h GSH	51,91 ± 3,22*	31,95 ± 2,65 [#]	16,14 ± 3,08
	Q1+24h Lactate	51,35 ± 3,32*	32,82 ± 2,82 [#]	15,83 ± 3,19
A818-6	72h treatment	G1	S	G2/M
	Q10	55,81 ± 3,75	27,74 ± 4,25	16,45 ± 3,61
	Q1	67,59 ± 6,33*	20,22 ± 4,17*	12,19 ± 3,17*
	Q1+72h GSH	57,24 ± 3,66*	26,74 ± 4,31 [#]	16,02 ± 3,82
	Q1+72h Lactate	58,78 ± 3,83*	25,54 ± 2,97 [#]	15,68 ± 3,00
	Q1+48h GSH	59,14 ± 3,27*	25,34 ± 4,81 [#]	15,52 ± 3,24
	Q1+48h Lactate	58,25 ± 3,92*	26,78 ± 3,99 [#]	14,98 ± 2,84
	Q1+24h GSH	62,06 ± 5,56	23,92 ± 4,11	14,02 ± 2,45
	Q1+24h Lactate	57,99 ± 3,52*	27,57 ± 4,05 [#]	14,44 ± 2,78

Table S1: T3M4 and A818-6 cells cultured with Q10 or Q1 for 24h, 48h or 72h in the absence or presence of 1.5 mM GSH or 20 mM lactate added in parallel or later, as indicated, were collected and stained with PI and analyzed by flow cytometry (see Figure 6C). The G1, S and G2/M cell cycle-fractions were quantified. Data are the mean values ± SD from six independent experiments; *p<0.05 compared to Q10, [#]p<0.05 compared to Q1.

T3M4	siRNA	48h treatment	G1	S	G2/M
	Control	Q10	62.70 ± 4.98	19.12 ± 2.55	18.18 ± 4.12
	Control	Q1	72.90 ± 6.02*	16.45 ± 2.31*	10.65 ± 2.90*
	Control	Q1+48h Lactate	65.59 ± 4.86 [#]	19.15 ± 3.00 [#]	15.27 ± 3.25 [#]
	Control	Q1+24h Lactate	64.74 ± 4.09 [#]	18.76 ± 3.32	16.50 ± 2.77 [#]
	MCT1	Q10	63.09 ± 3.99	19.37 ± 3.09	18.16 ± 3.85
	MCT1	Q1	72.03 ± 5.74*	16.83 ± 2.81*	10.42 ± 3.01*
	MCT1	Q1+48h Lactate	69.68 ± 5.12	16.38 ± 1.95	13.31 ± 2.78
	MCT1	Q1+24h Lactate	69.87 ± 5.36	16.14 ± 2.23	13.99 ± 3.27
T3M4	siRNA	72h treatment	G1	S	G2/M
	Control	Q10	65.24 ± 3.55	19.74 ± 2.71	15.02 ± 3.80
	Control	Q1	69.93 ± 4.69*	17.28 ± 2.60*	12.79 ± 2.13*
	Control	Q1+72h Lactate	65.64 ± 3.79 [#]	19.53 ± 2.85	14.83 ± 2.42 [#]
	Control	Q1+48h Lactate	65.31 ± 3.60 [#]	20.03 ± 2.78 [#]	14.66 ± 2.61
	Control	Q1+24h Lactate	66.62 ± 4.25	18.63 ± 2.34	14.75 ± 2.33
	MCT1	Q10	67.33 ± 4.52	18.16 ± 2.77	14.51 ± 2.60
	MCT1	Q1	70.08 ± 4.87	17.01 ± 1.93	12.91 ± 2.24
	MCT1	Q1+72h Lactate	68.79 ± 4.81	17.93 ± 2.44	13.28 ± 2.09
	MCT1	Q1+48h Lactate	67.98 ± 4.57	17.49 ± 2.38	14.53 ± 1.92
	MCT1	Q1+24h Lactate	68.53 ± 4.66	16.70 ± 1.83	14.77 ± 2.42
A818-6	siRNA	48h treatment	G1	S	G2/M
	Control	Q10	49.47 ± 3.82	30.65 ± 3.01	19.88 ± 2.75
	Control	Q1	66.57 ± 4.68*	16.80 ± 1.67*	16.63 ± 2.33*
	Control	Q1+48h Lactate	56.33 ± 3.94 [#]	24.67 ± 2.56 [#]	19.00 ± 2.61 [#]
	Control	Q1+24h Lactate	54.01 ± 3.79 [#]	27.22 ± 2.65 [#]	18.77 ± 2.50 [#]
	MCT1	Q10	50.40 ± 4.17	29.24 ± 2.80	20.37 ± 3.00
	MCT1	Q1	66.73 ± 4.96*	18.56 ± 1.85*	15.37 ± 2.22*
	MCT1	Q1+48h Lactate	61.54 ± 5.03	20.26 ± 2.18	18.20 ± 3.02
	MCT1	Q1+24h Lactate	59.87 ± 4.60 [#]	23.76 ± 2.57 [#]	16.37 ± 2.80
A818-6	siRNA	72h treatment	G1	S	G2/M
	Control	Q10	56.55 ± 3.57	25.02 ± 4.01	18.43 ± 3.21
	Control	Q1	61.08 ± 4.15*	20.17 ± 3.27*	18.75 ± 3.64
	Control	Q1+72h Lactate	59.43 ± 3.66	22.44 ± 3.71	18.13 ± 3.10
	Control	Q1+48h Lactate	57.53 ± 3.72 [#]	23.21 ± 3.52 [#]	19.07 ± 4.05
	Control	Q1+24h Lactate	54.84 ± 2.99 [#]	23.39 ± 3.16 [#]	19.58 ± 3.23
	MCT1	Q10	60.30 ± 3.73	22.42 ± 3.47	17.28 ± 3.36
	MCT1	Q1	61.10 ± 3.47	21.19 ± 2.90	16.93 ± 3.44
	MCT1	Q1+72h Lactate	60.64 ± 4.13	22.23 ± 3.14	17.13 ± 3.51
	MCT1	Q1+48h Lactate	60.77 ± 4.00	22.27 ± 3.19	16.96 ± 3.70
	MCT1	Q1+24h Lactate	59.76 ± 3.54	21.46 ± 2.82	17.44 ± 3.53

Table S2: T3M4 and A818-6 cells pretreated with control or MCT1 siRNA were cultured under Q10 or Q1 for 48h or 72h in the absence or presence of 20 mM lactate added in parallel or later, as indicated. PI-stained cells were quantified for the G1-, S-, and G2/M-cell cycle-fractions (see Figure 6D). Data are the mean values ± SD from four independent experiments; *p<0.05 compared to Q10, [#]p<0.05 compared to Q1.

T3M4	<u>48h treatment</u>	<u>G1</u>	<u>S</u>	<u>G2/M</u>
	<i>DMSO</i>	60.09 ± 2.06	21.56 ± 1.44	18.35 ± 1.75
	<i>DMSO+48h Lactate</i>	60.54 ± 2.40	21.46 ± 1.61	18.00 ± 1.78
	<i>DMSO+24h Lactate</i>	60.38 ± 1.97	20.46 ± 1.67	19.16 ± 2.06
	<i>V9302</i>	79.28 ± 6.23*	12.04 ± 3.14*	8.68 ± 1.86*
	<i>V9302+48h lactate</i>	68.79 ± 4.05 [#]	17.33 ± 2.25 [#]	12.88 ± 1.21 [#]
	<i>V9302+24h lactate</i>	69.22 ± 4.72 [#]	16.95 ± 2.47 [#]	12.83 ± 1.55 [#]
	<i>CB839</i>	68.54 ± 4.23*	16.44 ± 2.66*	15.01 ± 1.93*
	<i>CB839+48h lactate</i>	61.34 ± 3.37 [#]	20.76 ± 3.40 [#]	17.90 ± 1.69 [#]
	<i>CB839+24h lactate</i>	60.93 ± 3.72 [#]	21.03 ± 3.25 [#]	18.04 ± 1.65 [#]

A818-6	<u>48h treatment</u>	<u>G1</u>	<u>S</u>	<u>G2/M</u>
	<i>DMSO</i>	61.62 ± 3.73	29.17 ± 1.95	9.21 ± 1.08
	<i>DMSO+48h Lactate</i>	62.00 ± 3.18	28.35 ± 2.01	9.65 ± 1.34
	<i>DMSO+24h Lactate</i>	61.83 ± 3.31	28.93 ± 1.82	9.24 ± 1.15
	<i>V9302</i>	67.74 ± 3.79*	26.35 ± 2.12*	5.91 ± 0.98*
	<i>V9302+48h lactate</i>	64.28 ± 2.89	27.14 ± 1.82	8.58 ± 1.09 [#]
	<i>V9302+24h lactate</i>	63.05 ± 3.35 [#]	27.63 ± 1.73	9.31 ± 1.35 [#]
	<i>CB839</i>	70.44 ± 3.63*	22.41 ± 1.57*	7.15 ± 1.49*
	<i>CB839+48h lactate</i>	64.46 ± 2.55 [#]	25.88 ± 2.74 [#]	9.66 ± 1.25 [#]
	<i>CB839+24h lactate</i>	63.56 ± 2.91 [#]	26.73 ± 2.16 [#]	9.71 ± 1.02 [#]

Table S3: T3M4 and A818-6 cells were treated with V9302 (2 μM), CB839 (10 μM) or DMSO for 48h either in the absence or presence of 20 mM lactate for 48h and 24h, respectively. Then, cells were collected, stained with PI and analyzed by flow cytometry (see Figure 8C). Data are the mean values ± SD from four independent experiments; *p<0.05 compared to DMSO, #p<0.05 compared to V9302 and CB839, respectively, without lactate.

T3M4	siRNA	48h treatment	G1	S	G2/M
	<i>Control</i>	<i>DMSO</i>	61.23 ± 5.17	20.09 ± 3.47	18.68 ± 3.53
	<i>Control</i>	<i>DMSO + Lactate (48h)</i>	61.46 ± 4.75	21.00 ± 3.33	17.54 ± 3.22
	<i>Control</i>	<i>V9302</i>	78.41 ± 6.76*	13.21 ± 2.19*	8.38 ± 1.84*
	<i>Control</i>	<i>V9303 + Lactate (48h)</i>	68.21 ± 5.63 [#]	18.29 ± 2.95 [#]	13.50 ± 2.43 [#]
	<i>Control</i>	<i>CB839</i>	67.92 ± 5.50*	16.55 ± 2.63*	15.52 ± 2.22*
	<i>Control</i>	<i>CB839 + Lactate (48h)</i>	62.38 ± 4.60 [#]	19.47 ± 3.52 [#]	18.15 ± 3.40 [#]
	<i>MCT1</i>	<i>DMSO</i>	60.67 ± 3.91	20.38 ± 3.76	18.51 ± 3.31
	<i>MCT1</i>	<i>DMSO + Lactate (48h)</i>	61.05 ± 4.26	20.17 ± 4.00	18.78 ± 3.64
	<i>MCT1</i>	<i>V9302</i>	76.52 ± 7.30	14.15 ± 2.52	9.33 ± 2.07
	<i>MCT1</i>	<i>V9303 + Lactate (48h)</i>	74.28 ± 6.51	15.19 ± 2.49	10.53 ± 2.18
	<i>MCT1</i>	<i>CB839</i>	69.07 ± 6.11	17.02 ± 2.68	13.91 ± 2.09
	<i>MCT1</i>	<i>CB839 + Lactate (48h)</i>	68.53 ± 6.26	18.45 ± 3.20	13.02 ± 2.33

A818-6	siRNA	48h treatment	G1	S	G2/M
	<i>Control</i>	<i>DMSO</i>	60.83 ± 4.62	29.09 ± 4.76	10.07 ± 2.89
	<i>Control</i>	<i>DMSO + Lactate (24h)</i>	61.36 ± 5.14	27.42 ± 4.20	11.22 ± 2.95
	<i>Control</i>	<i>V9302</i>	68.17 ± 6.25*	25.75 ± 4.74*	6.08 ± 1.52*
	<i>Control</i>	<i>V9303 + Lactate (24h)</i>	62.84 ± 5.20 [#]	27.29 ± 5.11 [#]	9.87 ± 2.84 [#]
	<i>Control</i>	<i>CB839</i>	69.59 ± 6.49*	22.12 ± 4.57*	8.29 ± 1.97*
	<i>Control</i>	<i>CB839 + Lactate (24h)</i>	63.12 ± 5.43 [#]	26.04 ± 4.08 [#]	10.84 ± 2.93 [#]
	<i>MCT1</i>	<i>DMSO</i>	61.29 ± 4.85	27.92 ± 4.98	10.79 ± 2.73
	<i>MCT1</i>	<i>DMSO + Lactate (24h)</i>	61.91 ± 5.06	27.15 ± 4.72	10.94 ± 2.58
	<i>MCT1</i>	<i>V9302</i>	69.27 ± 5.70	24.45 ± 3.54	6.28 ± 2.07
	<i>MCT1</i>	<i>V9303 + Lactate (24h)</i>	68.03 ± 5.28	24.98 ± 4.02	6.99 ± 2.27
	<i>MCT1</i>	<i>CB839</i>	70.67 ± 6.72	21.63 ± 3.16	7.70 ± 2.38
	<i>MCT1</i>	<i>CB839 + Lactate (24h)</i>	69.57 ± 5.88	22.28 ± 3.81	8.15 ± 2.33

Table S4: T3M4 and A818-6 cells subject control or MCT1 siRNA transfection for 24h were treated with V9302 (2 µM), CB839 (10 µM) or DMSO for 48h either in the absence (-) or presence of 20 mM lactate for 48h and 24h, respectively. Then, cells were collected, stained with PI and analyzed by flow cytometry (see Figure 8D). Data are the mean values ± SD from four independent experiments; *p<0.05 compared to DMSO, [#]p<0.05 compared to V9302 and CB839, respectively, without lactate.