

Article

Acriflavine, a potent inhibitor of HIF1 α , disturbs glucose metabolism and suppresses ATF4-protective pathways in melanoma under non-hypoxic conditions

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Supplementary Material

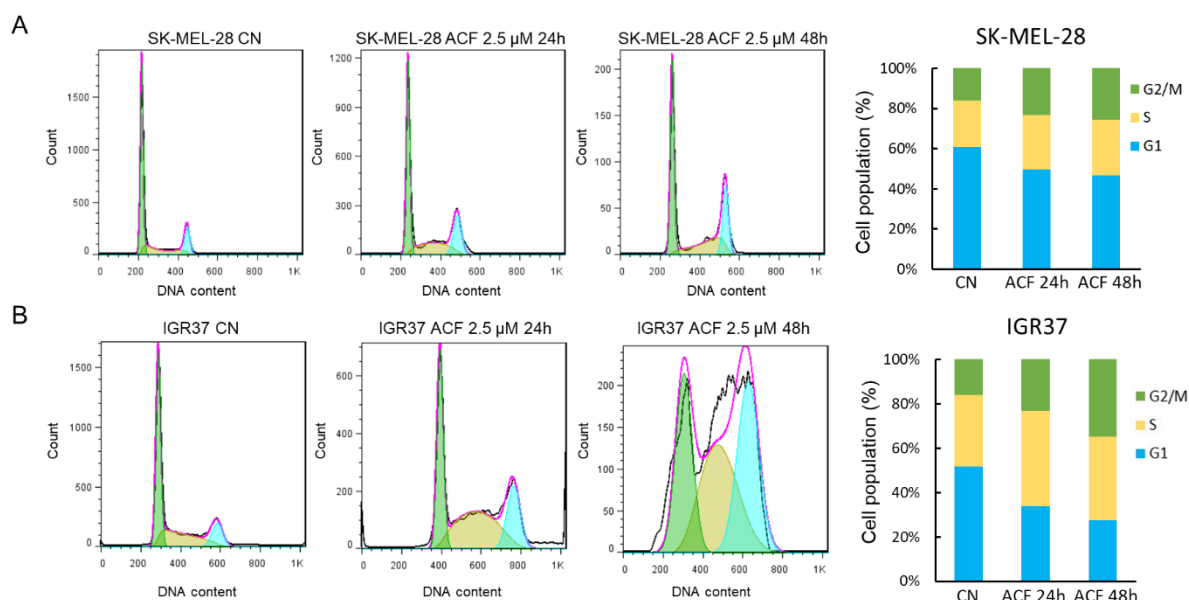


Figure S1. Cell cycle assays of SK-MEL-28 (A) and IGR37 (B) cells following ACF indicated treatments. Assays were performed as indicated in Figure 1B.

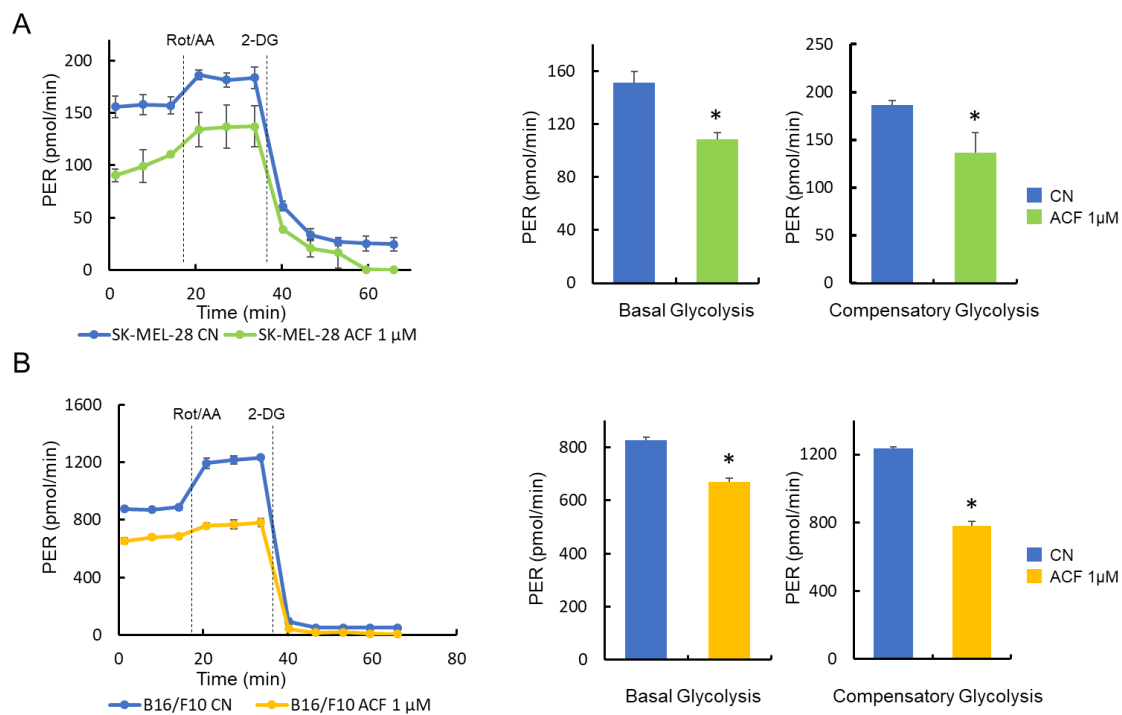
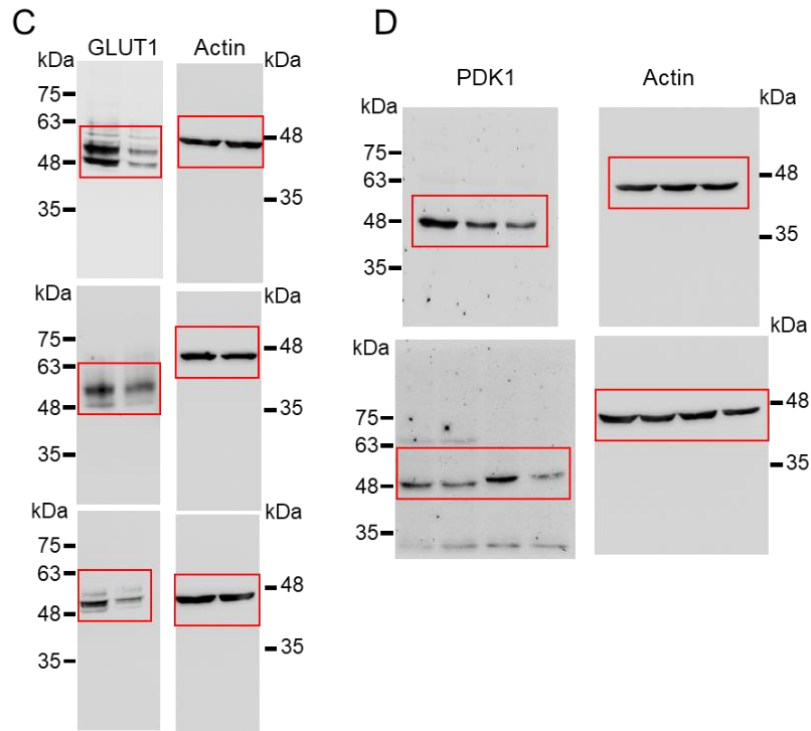


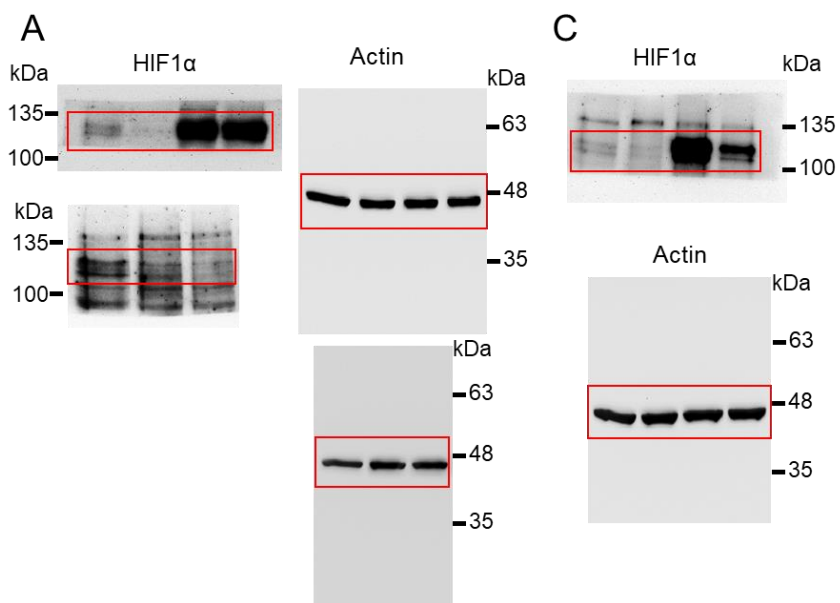
Figure S2. Glycolytic proton efflux rate (glycoPER) comparing untreated and ACF-treated melanoma cells. (A) SK-MEL-28 (B) B16/F10. Assays were performed as indicated in Figure 1F. P values for significant differences (Student's *t*-test) are summarized by one asterisk ($P < 0.05$) and groups are compared to ACF-untreated samples.

Figure S3. Uncropped Western blots for indicated Figures

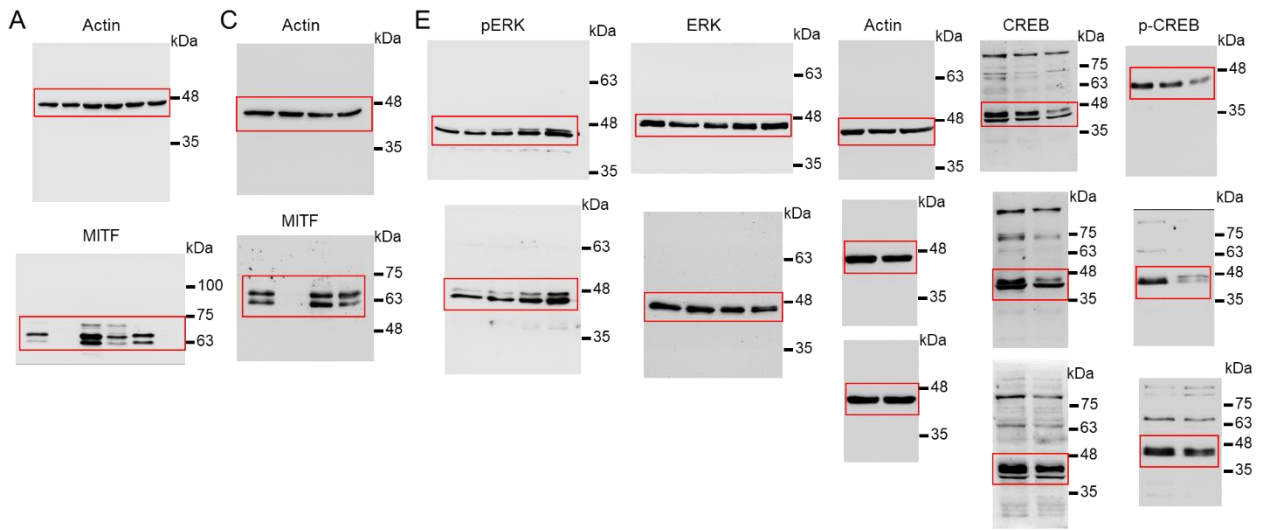
Blots from Figure 1



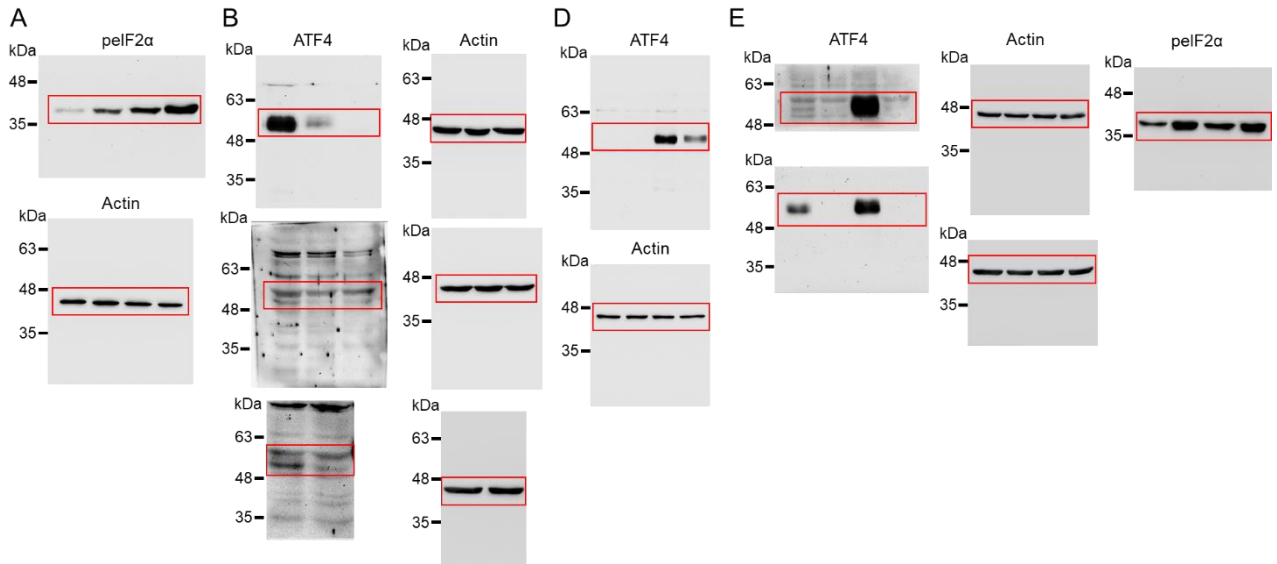
Blots from Figure 2



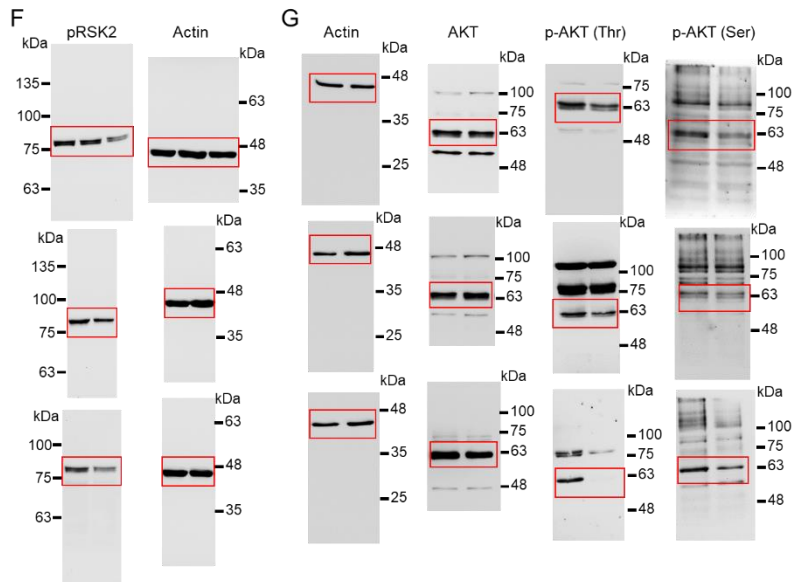
Blots from Figure 3



Blots from Figure 4 (A-E)



Blots from Figure 4 (F-G)



Blots from Figure 5

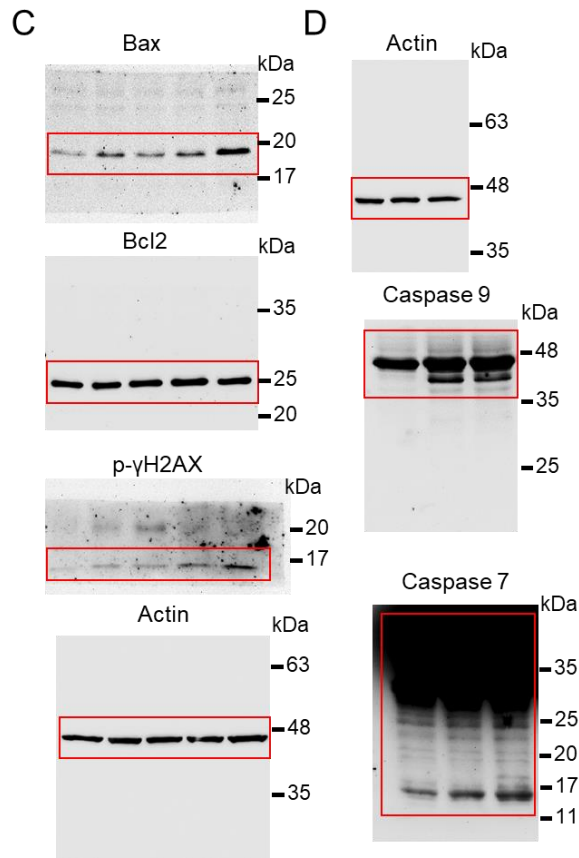


Table S1. Used primary antibodies.

| Antibody | Source | Catalogue number |
|---|-----------------|------------------|
| Mouse monoclonal anti- β -Actin (AC-15) | Merck/Sigma | Cat# A5441 |
| Rabbit polyclonal anti-pCREB (Ser133) | Merck/Millipore | Cat# 06-519 |
| Mouse monoclonal anti-pERK1/2 | Merck/Sigma | Cat# M9692 |
| Rabbit monoclonal anti-ATF4 (D4B8) | Cell Signaling | Cat# 11815 |
| Mouse monoclonal p- γ H2AX (Ser139) | Merck/Millipore | Cat# 05-636 |
| Mouse monoclonal anti-MITF | Merck/Millipore | Cat# MAB3747 |
| Mouse monoclonal anti-BCL2 | Merck/Sigma | Cat# B 3170 |
| Rabbit polyclonal anti-BAX | Santa Cruz | Cat# sc-493 |
| Mouse monoclonal anti-HIF1 α | Novus | Cat# NB100-105 |
| Rabbit monoclonal anti-peIF2 α (Ser51) | Cell Signaling | Cat# 3597 |
| Rabbit polyclonal anti-ERK2 | Santa Cruz | Cat# sc-154 |
| Rabbit polyclonal anti-CREB | Merck/Millipore | Cat# 06-863 |
| Mouse monoclonal anti-Caspase-9 | Santa Cruz | Cat# sc-17784 |
| Rat monoclonal anti-Caspase-7 | Merck/Sigma | Cat# C 1104 |
| Mouse monoclonal anti-GLUT1 | Abcam | Cat# ab40084 |
| Rabbit monoclonal anti-p-RSK2 | Abcam | Cat# ab75820 |
| Mouse monoclonal anti-p-AKT (Thr308) | Santa Cruz | Cat# sc-271966 |
| Mouse monoclonal anti-p-AKT (Ser473) | Merck/Millipore | Cat# 05-1003 |
| Rabbit monoclonal anti-AKT | Abcam | Cat# ab200195 |
| Mouse monoclonal anti-PDK1 | Thermo Fisher | Cat# MA5-15797 |

Table S2. The following primers for human genes were used.

| Genes | Source |
|------------------------------------|---------------|
| MITF RT qPCR primers | Thermo-Fisher |
| F 5'-3': GCGCAAAAGAAGCTTGAAAAC | |
| R 5'-3': CGTGGATGGAATAAGGGAAA | |
| ATF4 RT qPCR primers | Thermo-Fisher |
| F 5'-3': TCAAACCTCATGGGTTCTCC | |
| R 5'-3': GTGTCATCCAACGTGGTCAG | |
| PDK1 RT qPCR primers | Thermo-Fisher |
| F 5'-3': CGGATCAGAAACCGACACA | |
| R 5'-3': ACTGAACATTCTGGCTGGTGA | |
| HIF1 α RT qPCR primers | Thermo-Fisher |
| F 5'-3': GAGATGTTAGCTCCCTATATCCCA | |
| R 5'-3': TAGGTTCTTGTATTTGAGTCTGCTG | |
| VEGFA RT qPCR primers | Thermo-Fisher |
| F 5'-3': TACTGCCATCCAATCGAGAC | |
| R 5'-3': GCATGGTGATGTTGGACT | |
| β -Actin RT qPCR primers | Thermo-Fisher |
| F 5'-3': AGAAAATCTGGCACCACACC | |
| R 5'-3': GGGGTGTTGAAGGTCTCAA | |