



Article

# The Transcription Coregulator RIP140 Inhibits Cancer Cell Proliferation by Targeting the Pentose Phosphate Pathway

Valentin Jacquier <sup>1</sup>, Delphine Gitenay <sup>2</sup>, Vincent Cavaillès <sup>1,†</sup> and Catherine Teyssier <sup>1,\*,†</sup>

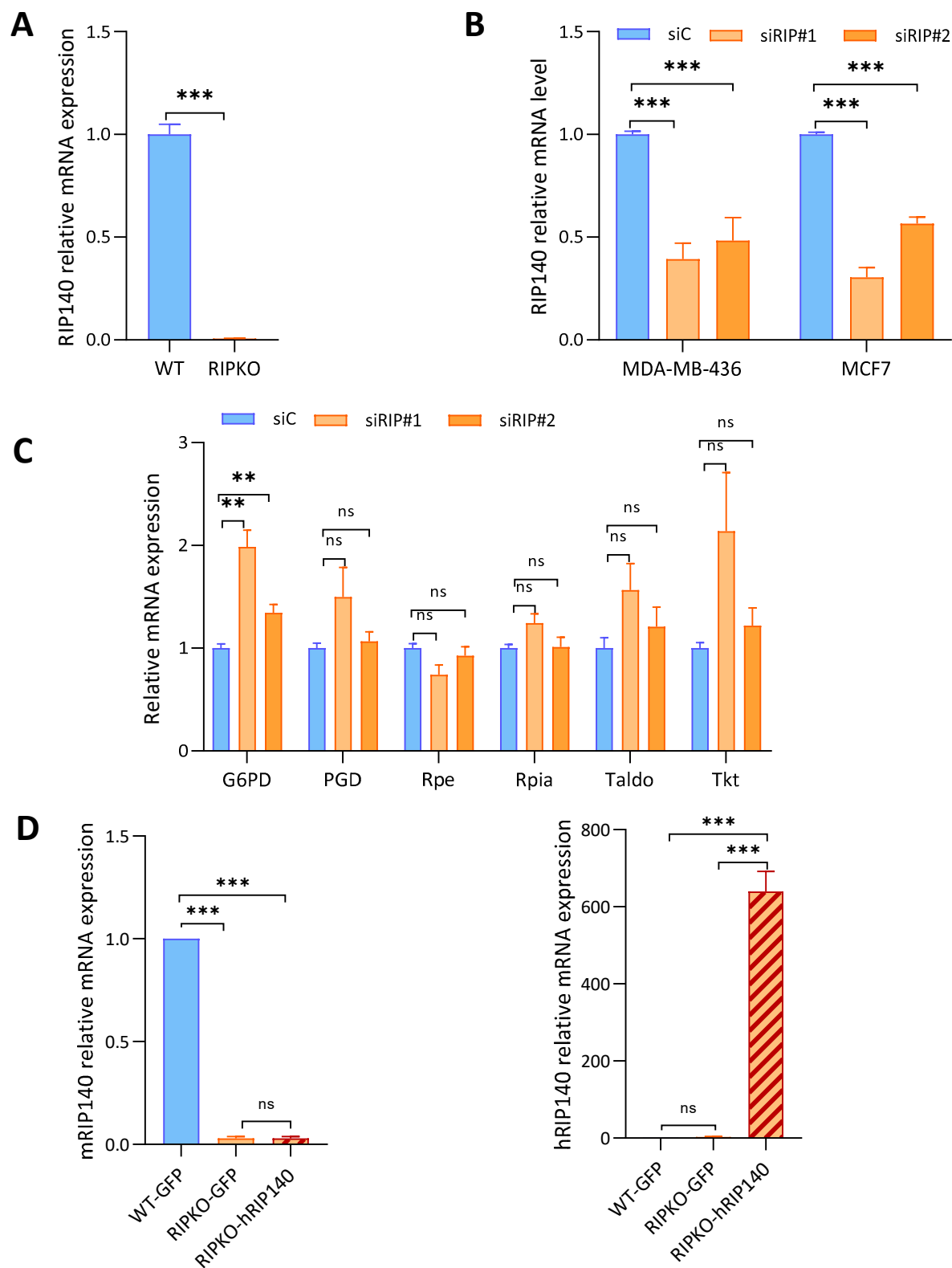
<sup>1</sup> IRCM, Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, University of Montpellier, 34298 Montpellier, France; valentinjacquier1993@gmail.com (V.J.); vincent.cavaillès@inserm.fr (V.C.)

<sup>2</sup> IRMB, University of Montpellier, INSERM, 34295 Montpellier, France; delphine.gitenay@inserm.fr (D.G.)

\* Correspondence: catherine.teyssier@inserm.fr

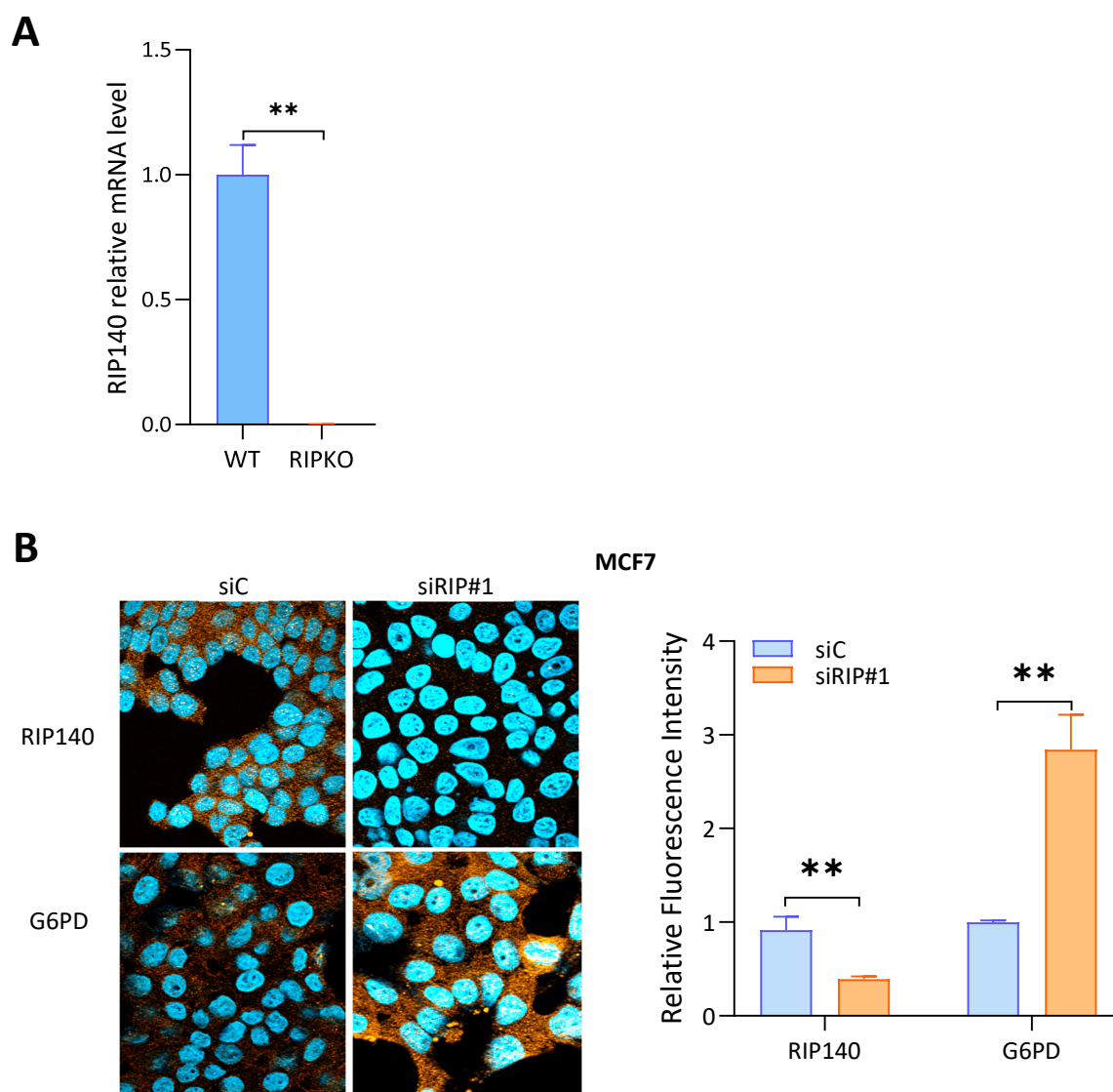
† These authors contributed equally to this work.

## Supplemental informations

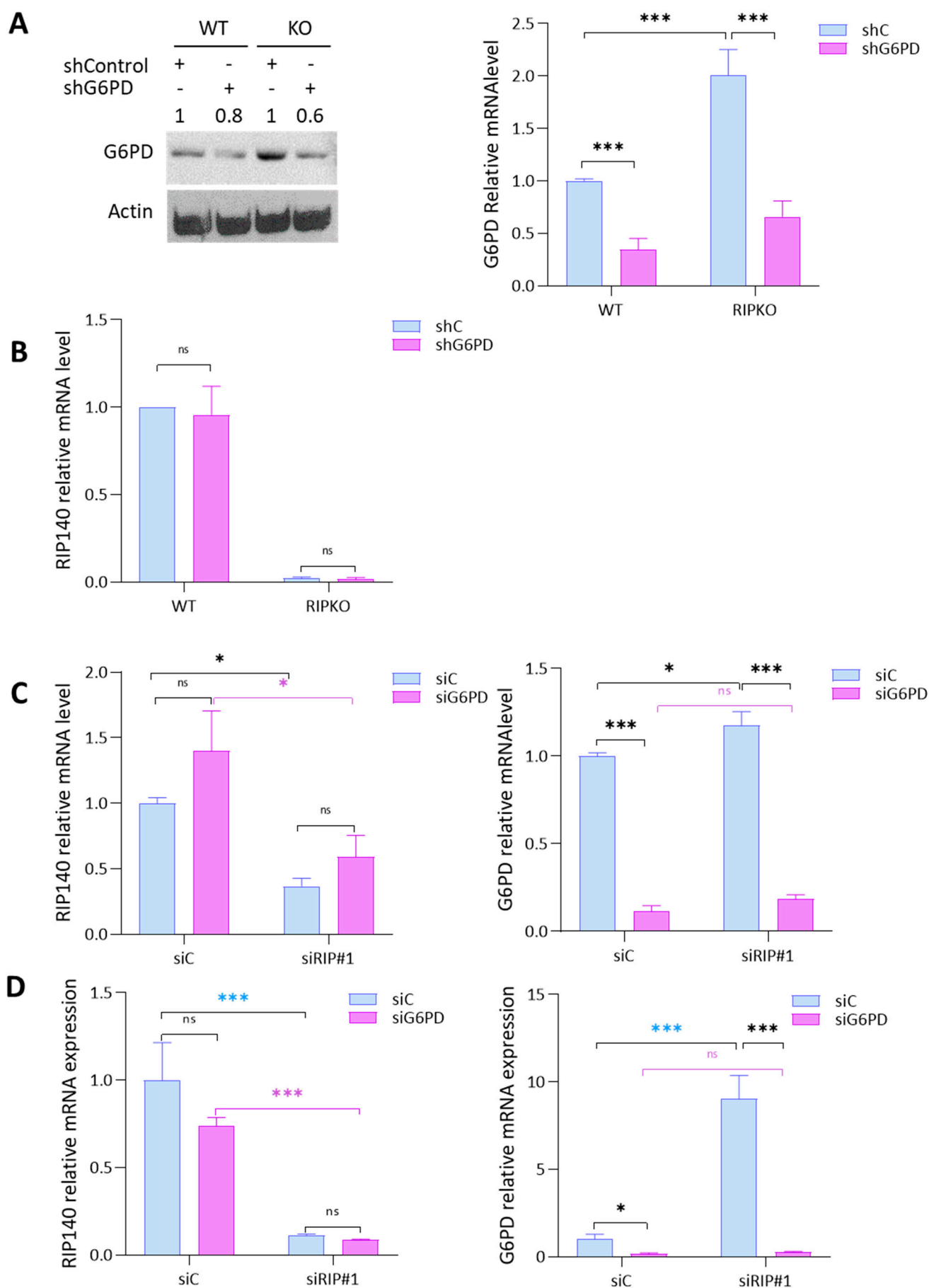


**Figure S1. RIP140 inhibits G6PD gene expression at the transcriptional level.** (A) RIP140 mRNA expression was assessed by RT-qPCR in MEF WT or RIP140 knock-out (RIPKO). The values are normalized to WT samples. (B) RIP140 mRNA expression was assessed by RT-qPCR in MCF7 and MDA-MB-436 cells transfected with control

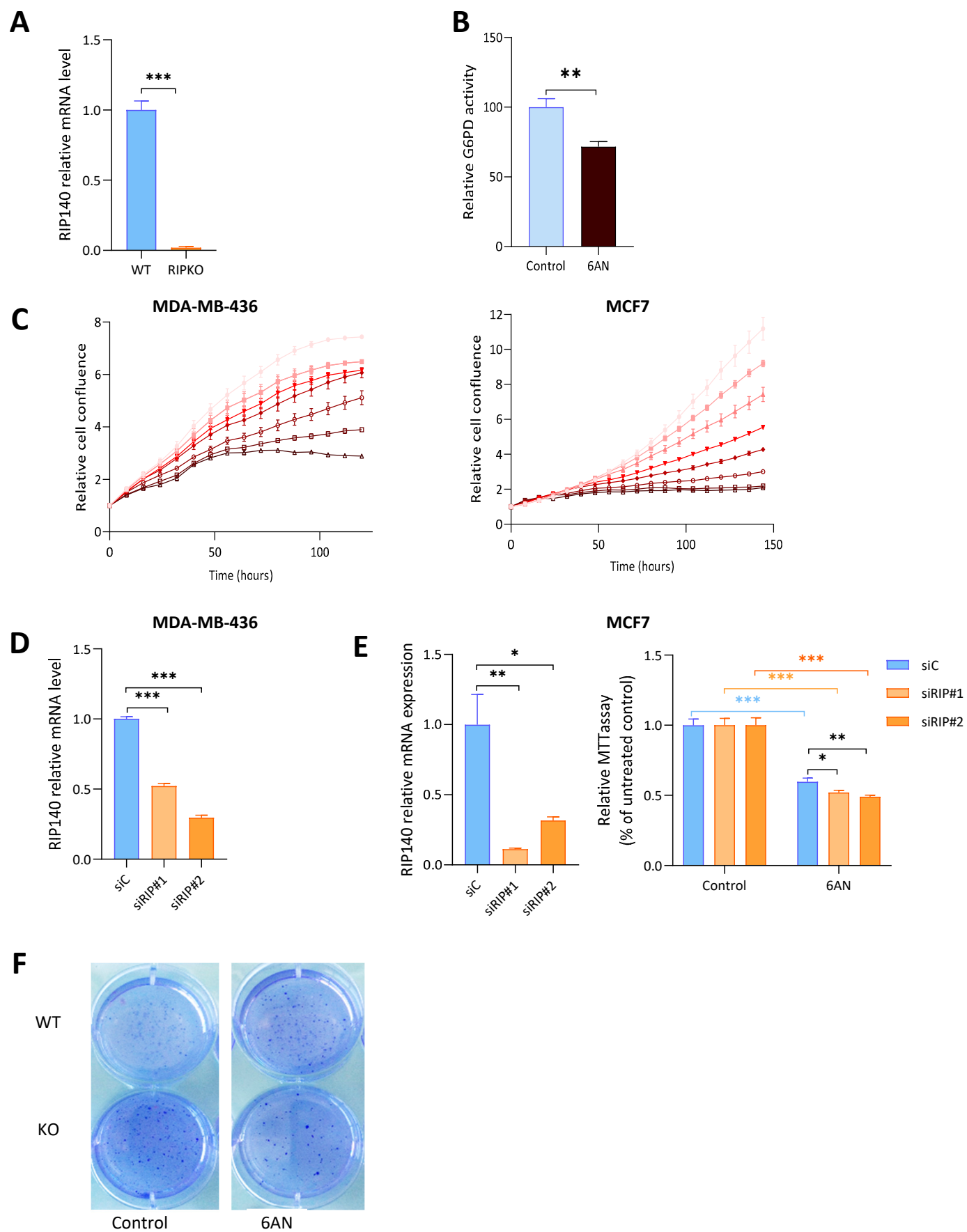
siRNA (siC) or RIP140 siRNAs (siRIP#1; siRIP#2). Values are normalized to control siRNA samples. (C) mRNA expression of the indicated genes was assessed by RT-qPCR in MCF7 cells transfected with control siRNA (siC) or RIP140 siRNAs (siRIP#1; siRIP#2). Values are normalized to control siRNA samples. (D) Murin (left panel) and human (right panel) RIP140 mRNA expression were assessed by RT-qPCR in MEF WT or RIP140 knock-out (RIPKO) overexpressing GFP or human RIP140 (hRIP140). The values are normalized to WT-GFP samples. All experiments were done at least three times. Values are means  $\pm$  SEM; \*\* $p$  < 0.01, \*\*\* $p$  < 0.001, ns = not significant.



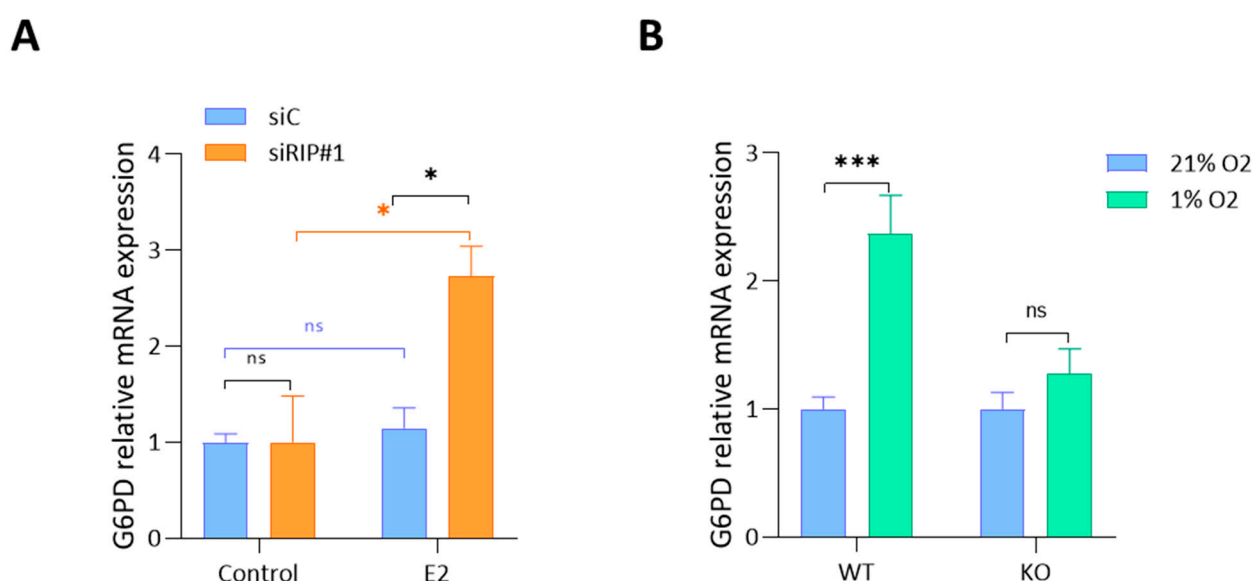
**Figure S2. RIP140-deficiency increases G6PD activity.** (A) RIP140 mRNA expression was assessed by RT-qPCR in MEF WT or RIP140 knock-out (RIPKO). The values are normalized to WT samples. Values are means  $\pm$  SEM of at least three independent experiments; \*\* $p$  < 0.01. (B) RIP140 and G6PD protein level was assessed by immunofluorescence using anti-RIP140 and anti-G6PD antibodies in MCF7 cells transfected with control siRNA (siC) or RIP140 siRNAs (siRIP#1). Hoechst 33342 was used for nuclei detection (left panel). Fluorescence intensity was quantified using Image J software in 6 different fields randomly chosen for each condition. Values are normalized to that of control siRNA. Values are means  $\pm$  SEM; \*\* $p$  < 0.01 (right panel).



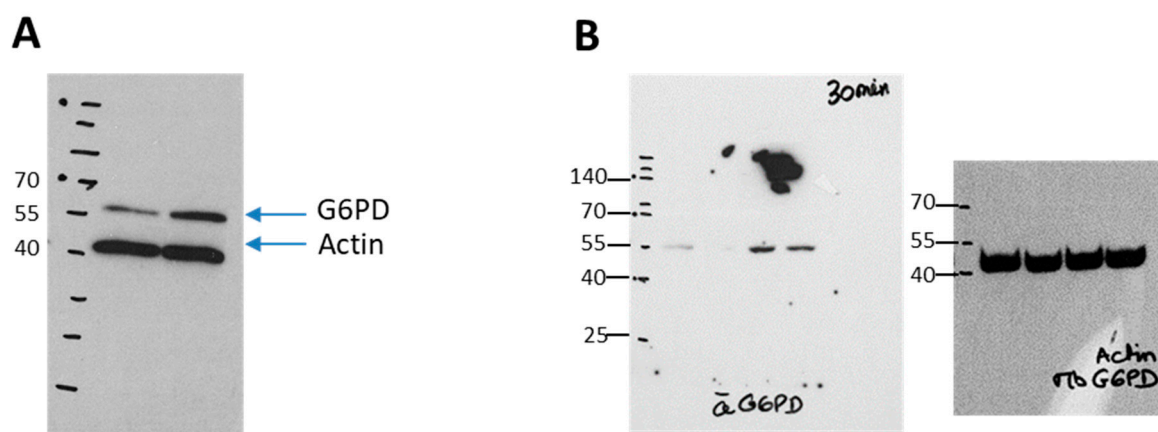
**Figure S3. G6PD is required for the proliferative advantage of RIP140-deficient cells.** (A) G6PD expression level was assessed by Western blot (left panel) quantified by Image J software and by RT-qPCR (right panel) in MEF transfected with shRNA control or shG6PD expressing lentivirus. (B) RIP140 expression level quantified by RT-qPCR in MEF used in Figure 3. (C–D) siRNA efficiency was verified by RT-qPCR for RIP140 (left panel) and G6DP (right panel) 48h after transfection in MDA-MD-436 (C) and in MCF7 (D) cells used in Figure 3C. Data are presented as the mean  $\pm$  SEM of at least three independent experiments. The statistical significance of differences between groups was evaluated via one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test, using the GraphPad Prism software. \* $p < 0.05$ , \*\*\* $p < 0.001$ , ns = not significant.



**Figure S4. RIP140-deficiency sensitizes cells to G6PD-inhibition.** (A) RIP140 mRNA expression was assessed by RT-qPCR in MEF WT or RIP140 knock-out (RIPKO). The values are normalized to WT samples. (B) The effect of 6-aminonicotinamide (6-AN, 10 $\mu$ M) on G6PD enzymatic activity was evaluated in MEF WT samples. Values are normalized to protein content and to that of untreated samples. (C) To fix the 6-AN concentration to be used in breast cancer cells, cell proliferation of MDA-MD-436 (left panel) and MCF7 (right panel) cells was assessed using the IncuCyte® Live Cell Analysis Imaging System in a 96-well plate with 2000 cells per well for the indicated times. Cells were treated at day 1 with increasing doses of 6-AN (0, 1, 2, 5, 10, 20, 50, 100 $\mu$ M). Values are normalized to that of untreated samples at day 1. 20 $\mu$ M of 6-AN inhibited cell proliferation about 70% in both breast cancer cell lines and was used in the subsequent experiments. (D) RIP140 mRNA expression in MDA-MB-436 cells transfected with control siRNA (siC) or RIP140 siRNAs (siRIP#1; siRIP#2). Values are normalized to control siRNA samples. (E) Right panel: RIP140 mRNA expression in MCF7 cells transfected with control siRNA (siC) or RIP140 siRNAs (siRIP#1; siRIP#2). Values are normalized to control siRNA samples. Left panel: MTT assay in MCF7 cells transfected with control siRNA (siC) or RIP140 siRNA (siRIP#1, siRIP#2) and treated for seven days with 6AN (20 $\mu$ M). Values are normalized to that of untreated control siRNA (Right panel). (E) Crystal violet staining of soft agar colonies quantified in Figure 4E. All experiments were done at least three times. Values are means  $\pm$  SEM; \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001, ns = not significant.



**Figure S5. Regulation of G6PD expression by RIP140 under estrogenic and hypoxia conditions.** (A) G6PD mRNA expression was assessed by RT-qPCR in MCF7 cells transfected with control siRNA (siC) or RIP140 siRNA (siRIP#1) and treated for 24h with Estradiol (E2 10E-8M). The values are normalized to untreated siC samples. (B) G6PD mRNA expression was assessed by RT-qPCR in MEF WT or RIP140 knock-out (RIPKO) exposed to hypoxia (1% O<sub>2</sub>) for 24h. The values are normalized to that of samples under normoxia (21% O<sub>2</sub>). All experiments were done at least three times. Values are means  $\pm$  SEM; \* $p$  < 0.05, \*\*\* $p$  < 0.001, ns = not significant.



**Figure S6. Unprocessed original scans of blots.** Unprocessed images of Western blots used in Figure 2 (A) and in Figure 3 (B). Molecular size markers in kDa.

#### Supplemental Materials and Methods

**Immunofluorescence.** Cells grown on glass coverslips were fixed with 3,7% Formaldehyde for 10 minutes, then washed with 0,05% Tween-20-PBS, permeabilized with 1% Triton-X100-PBS for 10 minutes followed by a wash with 0,05% Tween-20-PBS. The slides were then blocked with 1% bovine serum albumin for 3 hours, before applying primary antibodies overnight. The primary antibodies used: RIP140 antibody (1:100, Ab42126; Abcam Cambridge, UK), G6PD antibody (1:200, ab993; Abcam, Cambridge, UK). The secondary antibody used was AlexaFluor555-conjugated goat anti-rabbit (1:250, #A27039, Thermo Fisher Scientific, Waltham, USA). The cells were counterstained with Hoechst 33342 (Merck, Darmstadt, Germany) and analyzed with a AxioVision Microscope (Zeiss, Lena, Germany).

**IncuCyte® Live Cell Analysis.** Cell proliferation was directly determined and imaged using IncuCyte® Live Cell Analysis Imaging System[1] (Essen BioScience Co., Ltd., Ann Arbor, Michigan, USA). Briefly, 2000 cells were plated into a 96-well plate and put into the IncuCyte® 24 hours later. Scans were scheduled every 8 hours using Phase Image channel and 4x magnification for 7 days. Scans were retrieved using the Analysis Imaging System measuring classic confluence.



**Table D1. Murine primer sequences.**

m-G6pd2-FOR	GGC TTC TTG GTC ATC ATC GT
m-G6pd2-REV	TCC GAG ATA TAC CAG GCG AC
mPgD-FOR	CATCGATGATGATGTCACCC
mPgD-REV	GGGTCATCCTGCTTGTGAAG
mRpia-FOR	CACTTCCAATTCCCAGCACT
mRpia-REV	CCTCCACGATGTCCAAGG
mRpe-FOR	CACAGCCATTGGTTTTACCC
mRpe-REV	GGTCACCCTGTGGTTGAAAG
mTkt-FOR	ATGGAAAAACAGGACAGCCA
mTkt-REV	GCATCAGCTCCATCCAGG
mTaldo1-FOR	CTTGGGGCTTGTATTCATCG
mTaldo1-REV	GGTAAAGCGCCAGAGGATG
mRipP140-FOR	AGAACGCACATCAGGTGGCA
mRip140-REV	GATGGCCAGACACCCCTTTG
RS9-FOR	CGGCCCGGAGCTGTTGACG
RS9-REV	CTGCTTGCGGACCCTAATGTGACG

**Table S2. Human primer sequences.**

h-G6PDH-FOR	CAC CAG ATG GTG GGG TAG AT
h-G6PDH-REV	AGA GCT TTT CCA GGG CGA
hPGD-FOR	GCCTTGGAAGATGGTCTTGA
hPGD-REV	GTCAGTGGTGGAGAGGAAGG
hRPIA-FOR	CGATCCAGATCACTGAGGGT
hRPIA-REV	GCTGAAAGGGTGAAGCAAGA
hRPE-FOR	GGCTTTACCCACTGTTCTGG
hRPE-REV	GGATGCTAGACTCTGGGGC
hTKT-FOR	GCATGGTGTGAAAAAGAGG
hTKT-REV	CGCCTACGTATCAGCTCCA
hTALDO1-FOR	ATCCTGGGGCTTGTACTCGT
hTALDO1-REV	GAAGCGTCAGAGGATGGAGT
hRIP140-FOR	AATGTGCACTTGAGCCATGATG
hRIP140-REV	TCGGACACTGGTAAGGCAGG
28S-FOR	CGATCCATCATCCGCAATG
28S-REV	AGCCAAGCTCAGCGCAAC

**Table S3. siRNA sequences.**

Name	sequence
siControl	(UAAUGUAUUGGAACGCAUA)TT
siRIP140#1	(GAAGCGUGCUAACGAUAAA)TT
siRIP140#2	(AUACGAAUCUCCUGAUGU)TT

**Reference**

1. Quantitative Measurement of Cancer Cell Proliferation Using CellPlayer™ Kinetic Proliferation Assay Available online: <https://www.news-medical.net/whitepaper/20161003/Quantitative-Measurement-of-Cancer-Cell-Proliferation-Using-CellPlayer-Kinetic-Proliferation-Assay.aspx> (accessed on 24 June 2022).