Supplementary Material

Urolithin A gains in anti-proliferative capacity by reducing the glycolytic potential via the p53/TIGAR axis in colon cancer cells

Short Title: Growth inhibition by urolithin A profits from p53-dampened glycolysis

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Supplementary Material

Geraniin [1] and ellagic acid were kindly provided by Dr. Boakye, Kwame Nkrumah University of Science and Technology, Pharmaceutics Department, Ghana, and Dr. Krenn, Department of Pharmacognosy, University of Vienna., Austria, respectively.

The breast cancer cell lines MCF-7 and MDA-MB231 as well as the liver cancer cell line HepG2 were obtained from ATCC and maintained in DMEM medium supplemented with 10% serum. The liver cancer cell line HCC1.2 was kindly provided by M. Eisenbauer (Institute of Cancer Research, Medical University Vienna) and grown in RPMI medium supplemented with 10% serum. The stated p53 status of the cell lines is based on the information given in [2,3,4].

Supplementary Table 1: **Overview over IC_{50} values of test compounds in HCT116 cells**

HCT116 WT cells were seeded in 96-well plates and incubated with different concentrations of test compounds for 48 or 72 h prior to determination of (A) metabolic activity based on resazurin conversion or (B) residual biomass via crystal violet staining. IC_{50} values were determined as described in the method section and are means ± SD of at least three independent biological experiments.

Supplementary Table 2: **Overview over IC_{50} values of urolithin A in different cell lines –focus on the p53/TIGAR axis**

Cells were seeded in 96-well plates and incubated with different concentrations of urolithin A for 72 h prior to determination of residual biomass via crystal violet staining. IC_{50} values were determined as described in the method section and are means ± SD of at least three independent biological experiments.

Supplementary Figure 1

HCT116 WT or HepG2 cells were transfected with scrambled siRNA (scr) or siRNAs (mix of three3 different sequences) targeting TIGAR. An aliquot of those cells was treated with DMSO or urolithin A (30 µM) for 24 h before total cell lysates were subjected to immunoblot analysis for TIGAR and actin or tubulin as loading control. Representative blots of three independent experiments are shown. The residual cells were seeded in 96 well plates and subjected to biomass staining after urolithin (HCT116 and HepG2) or urolithin/oxaliplatin treatment (HCT116). Those results are depicted in figure 5 B and C as well as in supplementary table 2.

Supplementary Figure 2

Exemplary uncropped blot images for pivotal expression data

Supplementary References

[1] Boakye, Y. D. et al (2016) Anti-inflammatory activity of aqueous leaf extract of Phyllanthus muellerianus (Kuntze) Exell. and its major constituent, geraniin. J Ethnopharmacol, 187, 17-27.

[2] Waldherr,M. et al. (2018) Use of HuH6 and other human-derived hepatoma lines for the detection of genotoxins: a new hope for laboratory animals? Arch Toxicol., 92, 921-934

[3] Leroy, B. et al. (2014) Analysis of TP53 mutation status in human cancer cell lines: a reassessment. Hum Mutat, 35, 756-65.

[4] http://p53.iarc.fr/CellLines.aspx

A

	IC_{50} (48 h)	IC_{50} (72 h)
Enterolacton	>60 µM	>60 µM
S-Equol	>60 µM	>60 µM
Urolithin A	32.7 ± 2.3 µM	17.6 ± 2.9 µM
Oxaliplatin	3.6 ± 2.2 µM	1.9 ± 0.9 µM

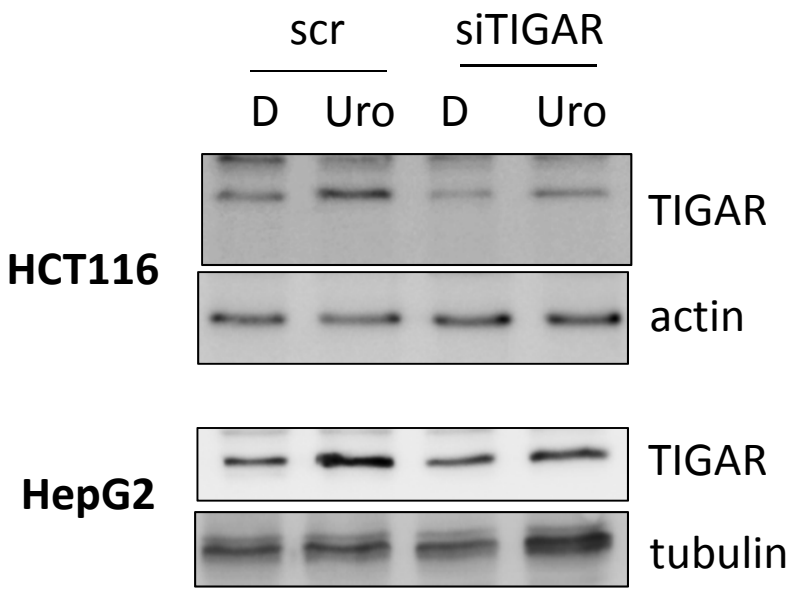
B

	IC_{50} (72 h)
Geraniin	44.2 ± 5.2 µM
Ellagic acid	83.0 ± 7.5 µM

Supplementary Table 1

	IC_{50} (72 h) of Uro A
HCT116 (WTp53)	19.6 ± 2.8 µM
HCT116 (p53 -/-)	38.4 ± 1.6 µM
MCF-7 (WT p53)	9.6 ± 0.7 µM
MDA-MB231 (mutant p53)	24.9 ± 3.3 µM
HepG2 (WT p53)	14.4 ± 3.6 µM
HCC1.2 (mutant p53)	24.9 ± 2.2 µM
HCT116-si-scr	25.9 ± 4.1 µM
HCT116-si-TIGAR	45.3 ± 1.7 µM
HepG2-si-scr	21.6 ± 3.6 µM
HepG2-si-TIGAR	38.2 ± 2.5 µM
HCT116 (p53-/-)-si-scr	47.1 ± 4.3 µM
HCT116 (p53 -/-)-si-TIGAR	50.2 ± 5.2 µM

Supplementary Table 2

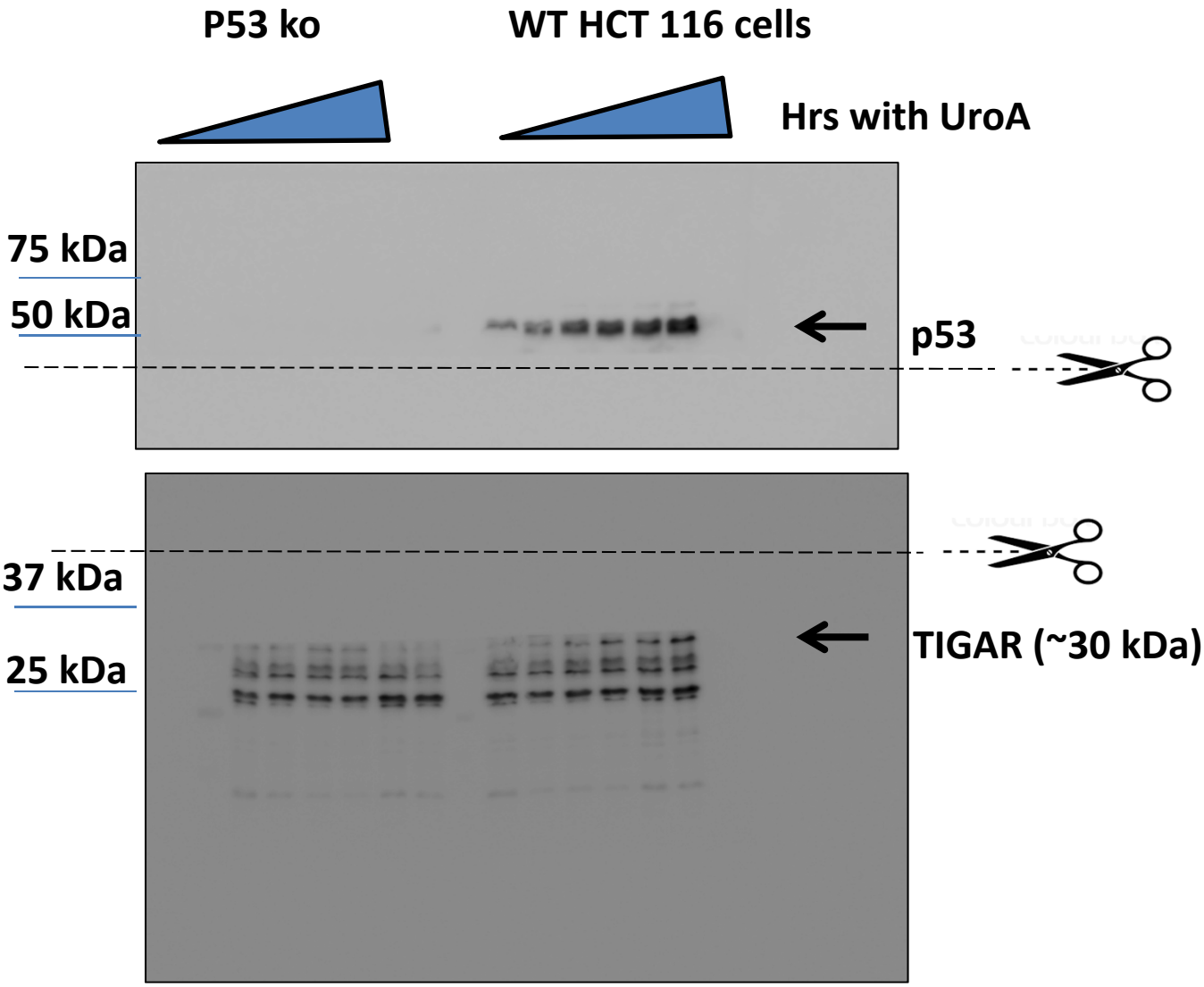


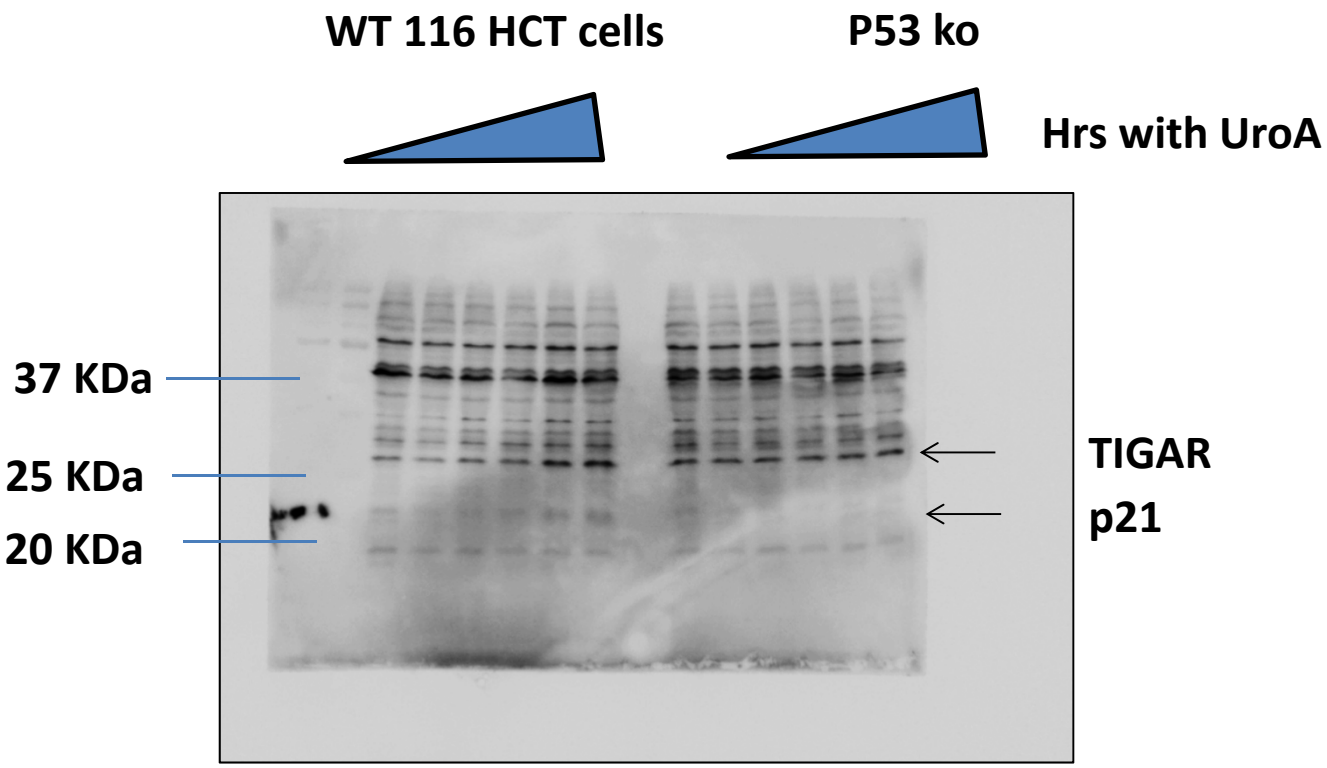
Supplementary Figure 1

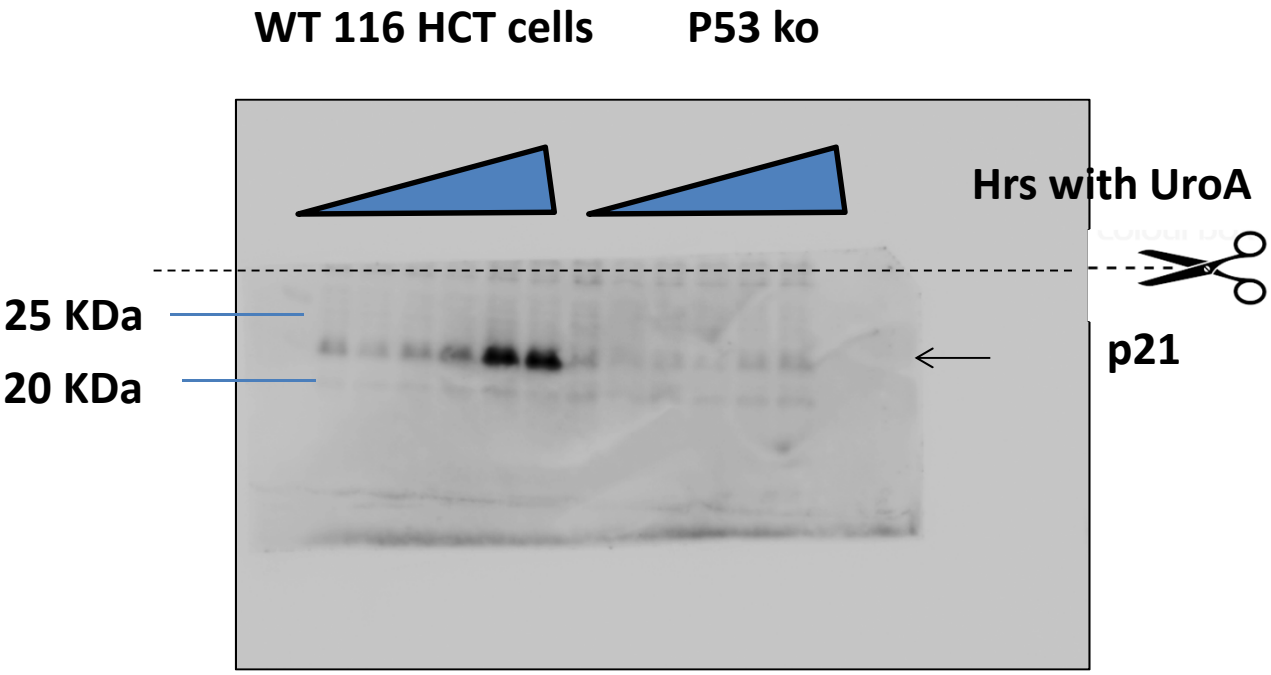
Supplementary Figure 2

UNCROPPRED EXEMPLARY IMMUNOBLOTS

- *Time dependent induction of p53 target genes by urolithin A*




WT 116 HCT cells
P53 ko
Hrs with UroA
37 KDa
25 KDa
20 KDa
TIGAR
p21


WT 116 HCT cells
P53 ko
Hrs with UroA
25 KDa
20 KDa
p21

- *siRNA mediated knockdown of TIGAR*