SUPPORTING INFORMATION

Amorfrutins are Natural PPARγ Agonists with Potent Anti-inflammatory Properties

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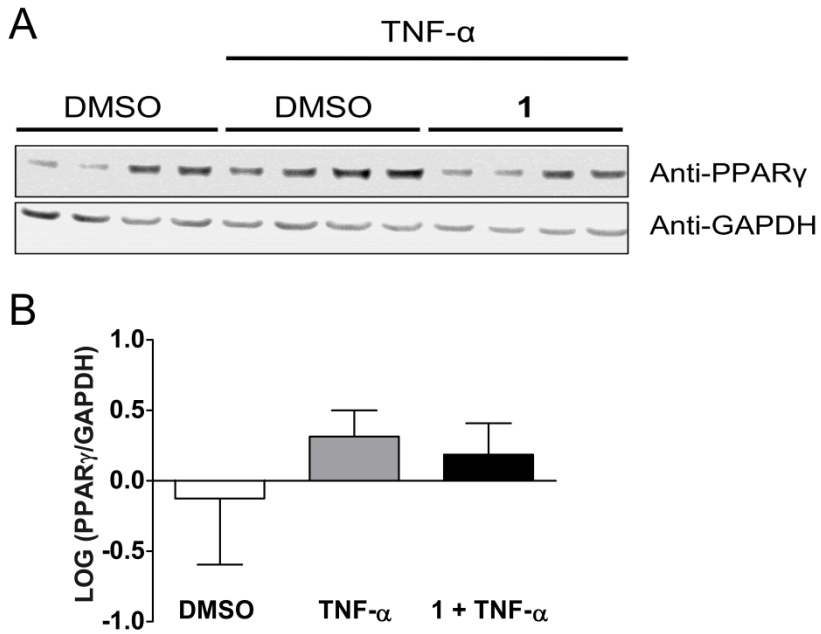
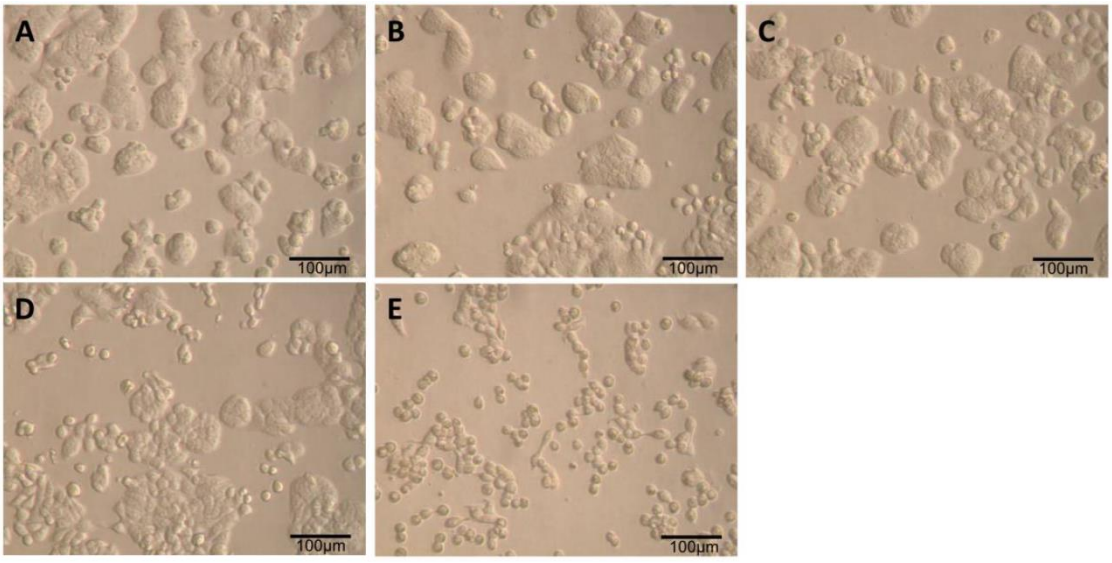
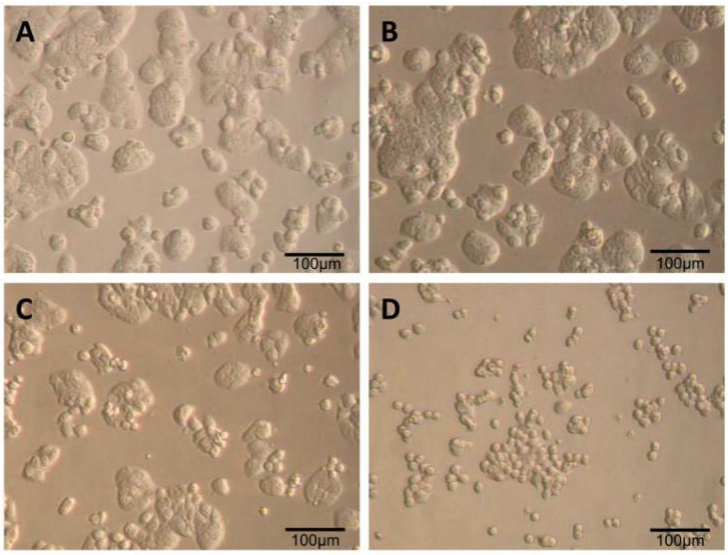


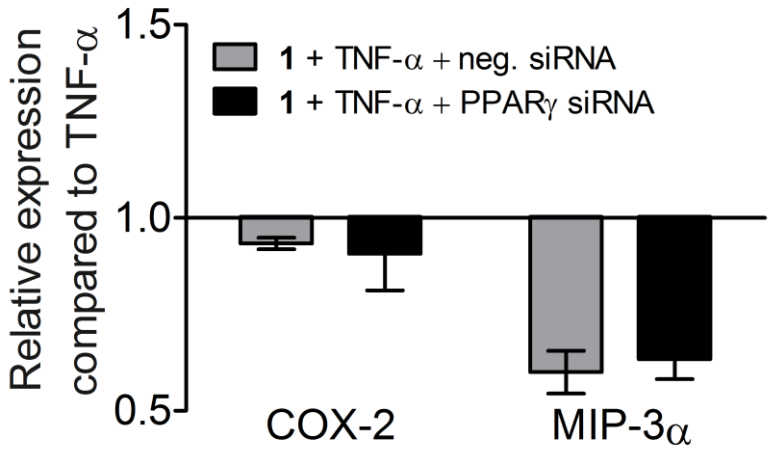
Supplementary Figure 1. PPARγ protein levels (isoform 1) in HT-29 cells. Cells were incubated with 0.1 % DMSO (vehicle control) or 10 µM **1** for 48 h and subsequently treated with 1 ng/mL TNF-α for 6 h. No significant changes in protein levels were observed after different treatment conditions. PPARγ protein levels were determined by western blotting (A) using a well-established antibody (see for example references 2-4) followed by densitometric analysis (B). Data are expressed as mean ± SD.



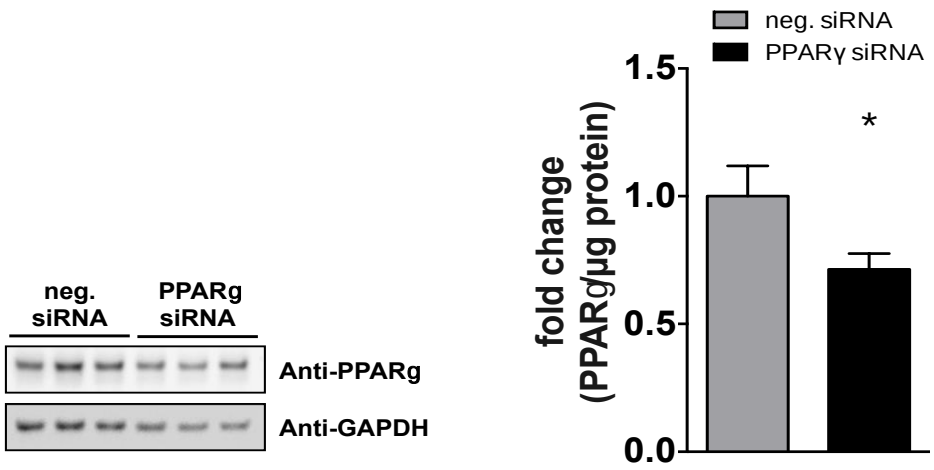
Supplementary Figure 2. HT-29 cells treated for 24 h with different concentrations of amorfrutin A (**1**).
A. 0.1 % DMSO. B. 1 µM of **1**. C. 10 µM of **1**. D. 20 µM of **1**. E. 50 µM of **1**.



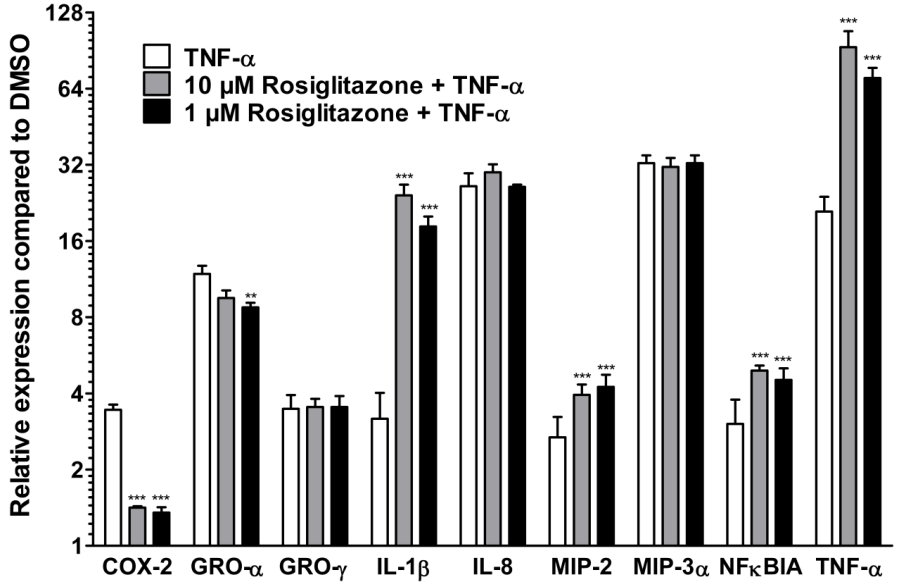
Supplementary Figure 3. HT-29 cells treated for 24 h with different concentrations of amorfrutin B (**2**).
A. 0.1 % DMSO. B. 1 µM of **2**. C. 10 µM of **2**. D. 20 µM of **2**.



Supplementary Figure 4. Effects of amorfrutin A (**1)** on COX-2 and MIP-3α gene expression were not altered after PPARγ knockdown. Gene expression analysis of COX-2 and MIP-3α in HT-29 cells after siRNA-mediated PPARγ knockdown. Gene expression analysis was performed using qPCR. Data are shown relative to TNF-α stimulated cells. Data are expressed as mean ± SD (n=3).



Supplementary Figure 5. siRNA mediated knockdown of PPARγ in HT-29 cells. Cells were transfected with PPARγ silencer select validated siRNA or silencer select negative control #1 siRNA (vehicle control) using HT-29 transfection reagent. PPARγ protein levels were determined via western blotting (left) followed by densitometric analysis (right). Expression of PPARγ was normalized by overall protein content, as the expression of GADPH and in particular other house-keeping proteins such as tubulin or actin seemed to be at least slightly reduced, indicating major cellular effects and technical limitations of knockdown of PPARγ in HT-29 cells. Data are expressed as mean ± SD. *$p \leq 0.05$ vs. neg. siRNA. The exact knockdown efficiency was 28.6% ($p = 0.02$). (Using alternatively GADPH for normalization resulted in knockdown efficiency of 19.79% ($p = 0.04$). Data analysis figure is not shown.)



Supplementary Figure 6. Gene expression analysis of pro-inflammatory genes in HT-29 cells treated with rosiglitazone for 48 hours and subsequently stimulated with TNF-α for 6 hours. Cells were treated with 0.1 % DMSO, 10 µM rosiglitazone or 1 µM rosiglitazone for 48 hours and subsequently treated with 1 ng/mL TNF-α for 6 h. Gene expression analysis was performed using qPCR. Data are shown relative to DMSO-treated cells. Data are expressed as mean ± SD (n=3). **$p \leq 0.01$, ***$p \leq 0.001$ vs. TNFα.

Supplementary Table1. Primers

Gene	Forward primer	Reverse primer
COX-2	CAGCACTTCACGCATCAGTT	CGCAGTTTACGCTGTCTAGC
GAPDH	CTCCTCCTGTTCGACAGTCA	CGACCAAATCCGTTGACTCC
GRO-α/ CXCL1	GCGGAAAGCTTGCCTCAATC	GGTCAGTTGGATTTGTCACTGT
GRO-γ/ CXCL3	GAAAAGATACTGAACAAGGGGAGC	GCAGGAAGTGTCAATGATACGC
IL-1β	GGACAGGATATGGAGCAACAAG	AACACGCAGGACAGGTACAG
IL-8	CTGATTTCTGCAGCTCTGTG	GGGTGGAAAGGTTTGGAGTATG
MIP-2/ CXCL2	ACAGTGTGTGGTCAACATTTCTC	TCGAAACCTCTCTGCTCTAACAC
MIP-3α / CCL20	CTGGCTGCTTTGATGTCAGTG	AGTCAAAGTTGCTTGCTGCTTC
NFκBIA	CTTCGAGTGACTGACCCCAG	TCACCCCACATCACTGAACG
TNF-α	AGGGACCTCTCTCTAATCAGC	CTCAGCTTGAGGGTTTGCTAC