SUPPLEMENTARY INFORMATION

Figure S1. NAm inhibits both secreted and cell associated forms of TNF-α. Splenic cells were stimulated by CpG in complete media or in media supplemented with the protein trafficking inhibitor Brefeldin A. TNF-α protein content was evaluated both in culture supernatants and total cell extracts (RIPA) by ELISA. As expected, Brefeldin A inhibited TNF-α release in the supernatant and led to an accumulation of TNF-α proteins in cell extracts (compare black and grey columns in the control group). NAm inhibited of TNF-α protein accumulation in both compartments.

Figure S2. Metabolic pathways of NAD biosynthesis in mammals.
Three biosynthetic routes have been shown to contribute to NAD biosynthesis in mammals including the de novo pathway using tryptophan (trp) as a precursor, the Press-Handler pathway allowing use of nicotinic acid (NA) as a precursor and the salvage pathway leading to the recycling of nicotinamide (NAm) and nicotinamide riboside (NR) to NAD. The site of action of APO866 is also shown in the Figure. Finally, note in contrast to yeasts, vertebrates do not express a nicotinamidase, and cannot therefore convert NAm into NA. These two NAD precursors contribute therefore to the intracellular NAD pool through distinct, non overlapping metabolic pathways.

Figure S3. Preferential inhibition of TNF-α protein synthesis by sirtuin and NAmPRT inhibitors. (A) RAW 264.7 cells were pretreated overnight with APO866 (10 nM) and subsequently stimulated for 2 hours with LPS (100 ng/ml). (B) and (C) RAW 264.7 cells pre-treated with the indicated sirtuin inhibitors for 1 h after which cells were stimulated for 2 hours with LPS (100 ng/ml) and in the presence of inhibitors. Relative expression of TNF-α protein and mRNA (relative to the RPL-32 housekeeping gene) were determined respectively by ELISA and quantitative RT-PCR.

Figure S4. Values of a single representative experiment used to calculate the translational efficiency of TNF-α. Pools of 293T cells were transiently transfected with three constructs encoding respectively for human TNF-α, mouse IL-2 and selected murine sirtuins as indicated. Control cells (Ctrl) were transfected with hTNF and mIL-2 encoding vectors alone

(Ctrl). 48h after transfection cells were washed, and either used for mRNA evaluation by quantitative RT-PCR or cultured an additional 6 hours for cytokine secretion. (A). Cytokine concentration in the 6h supernatant. (B) mRNA content, expressed as % of control values. (C) relative values of TNF-α vs IL-2 protein and mRNA. (D) Translational efficiency: ratio of TNF-α protein / TNF-α mRNA values, relative to IL-2 protein / IL-2 mRNA determinations.

Figure S5. The NAmPRT inhibitor APO866 displays anti-inflammatory properties *in vivo* and leads to accumulation of acetylated forms of p53 *in vitro*. (A). Naïve C57BL/6 mice (4 mice per group) were injected i.p. with solvent or APO866 (10 mg/kg). 4 hours later, mice were challenged with LPS and serum TNF-α levels determined as described in the legend of Figure 1. This experiment is representative of three independent experiments (n=12) and illustrates the ability of APO866 to significantly inhibit an *in vivo* inflammatory response. (B). The human 293T cell line was incubated in the presence of APO866 (10 nM), TSA (100 nM) or a combination of both. Cell extracts were analyzed by western blots for the acetylation status of p53, a known substrate of both the class I/II HDAC and SIRT1 deacetylases [46] using an antibody specific for the acetylated form of human p53 (anti-acetylated p53 rabbit polyclonal antibody (Abcam, Cambridge, USA)). This experiments suggests that by lowering intracellular NAD levels, APO866 inhibits the enzymatic activity of sirtuins.

Supplementary methods

Expression plasmids. The final hTNF-α expression plasmids, pCMV-SPORT6-hTNF and the low-level expression vector hTNF-pSEAP, were obtained using a standard PCR-based cloning strategy. Source plasmids (pATHTNF) were obtained from BCCM/LMBP-Plasmid Collection, Ghent, Belgium (http://bccm.belspo.be/db/lmbp_search_form.php). For hTNF-pSEAP, the SEAP coding sequence from pSEAP2-Control (clontech) was replaced with the hTNF-α cDNA.

SIRT1-Flag and SIRT1H355Y-Flag fragments were amplified by PCR using pHan-SIRT1 kindly provided by V. Sartorelli as a template and cloned into pCMV-SPORT6. To generate SIRT2/3/5/6/7-FLAG the corresponding coding region were amplified using Image clone as

template and inserted into pCMV-SPORT6 vector. The SIRT4 coding region was amplified from C57/B6 testis cDNA by RT-PCR and cloned into pCMV-SPORT6.

Oligonucleotide used for sirtuins construct

SIRT1-Forward : aat tgg tac cgt aat ggc gga cga ggt ggc gct cgc
SIRT1-Reverse : a att tct aga tta ctt atc gtc gtc atc ctt gta atc tga ttt gtc tga tgg ata gtt tac

SIRT2 Image clone 5039278 (NM_022432)
SIRT2-Forward :Aat tgg tac cgc cat ggc cga gcc gga ccc ctc
SIRT2-Reverse : aat tgc ggc cgc tta ctt atc gtc gtc atc ctt gta atc ctg ctg ttc ctc ttt ctc ttt ggt cc

SIRT3 Image clone 5253854 (NM_022433)
SIRT3-Forward : a att ggt acc gta atg gtg ggg gcc ggc atc agc
SIRT3-Reverse : a att tct aga tta ctt atc gtc gtc atc ctt gta atc tct gtc ctg tcc atc cag ctt gcc acg

SIRT4-Forward : aat tgg tac cgt aat gagc gga ttg act ttc agg cc
SIRT4-Reverse : a att tct aga tta ctt atc gtc gtc atc ctt gta atc ggg atc ttg agc agc gga act cag agg

SIRT5 Image clone 4014119 (NM_178848)
SIRT5-Forward : a att ggt acc gta atg cga cct ctc ctg att gct c
SIRT5-Reverse : a att tct aga tta ctt atc gtc gtc atc ctt gta atc aga agt cct ttc agt ttc atg agg agc

SIRT6 Image clone 6513375 (NM_181586)
SIRT6-Forward : aat tga tat cgg atc cac gat gtc ggt gaa tta tgc agc agg g
SIRT6-Reverse : a att tct aga tca ctt atc gtc gtc atc ctt gta atc gct ggg ggc agc ctc ggt ctt cac

SIRT7 Image clone 4987746 (NM_153056)
SIRT7-Forward : aat tgg tac cgt aat ggc agc cgg tgg cgg tct g
SIRT7-Reverse : aa ttt cta gac tac tta tcg tcg tca tcc ttg taa tct gcc act ttc ttc ctt ttt gca cgc ttg gc

Oligonucleotides for site-directed mutagenesis (mutated nucleotides are underlined)

SIRT6 G52A

Forward : cca gcg tgg ttt tcc aca ccg cgg ccg gca tca gca ccg cc

Reverse : gg cgg tgc tga tgc cgg ccg cgg tgt gga aaa cca cgc tgg

SIRT6 H133Y

Forward : tcg ggc ttc ccc agg gac aag ctt gca gag ctg tac gga aac atg ttt gta gag gaa tgt c

Reverse : g aca ttc ctc tac aaa cat gtt tcc gta cag ctc tgc aag ctt gtc cct ggg gaa gcc cga

Sirt7 H188Y

Forward : ccg cca tct cag agc tct atg gga ata tgt ata ttg aag tct gc

Reverse : gc aga ctt caa tat aca tat tcc cat aga gct ctg aga tgg cgg

Quantitative PCR primer list.

Gene Name Forward Primer Sequence 5'-3' Reverse Primer Sequence 5'-3'

mTNF: gcctccctctcatcagttcta - gctacgacgtgggctacag

hTNF: gcccgactatctcgactttg - tttgggaaggttggatgttc

mIL2: aaagggctctgacaacacattt - cctcagaaagtccaccacagt

RPL-32: ggcaccagtcagaccgatat - caggatctggcccttgaac

GaPDH: caactcactcaagattgtcagcaa - ggcatggactgtggtcatga

All primers were designed with Primer3 online design tool.

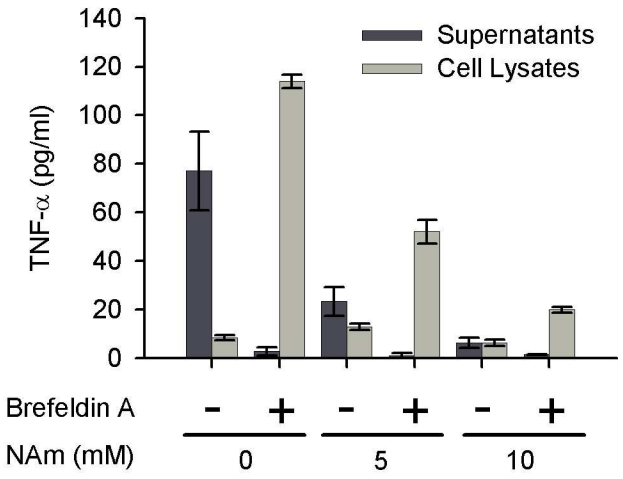

140
120
100
80
60
40
20
0
TNF-α (pg/ml)
Supernatants
Cell Lysates
Brefeldin A
-
+
-
+
-
+
NAm (mM)
0
5
10

Figure S1

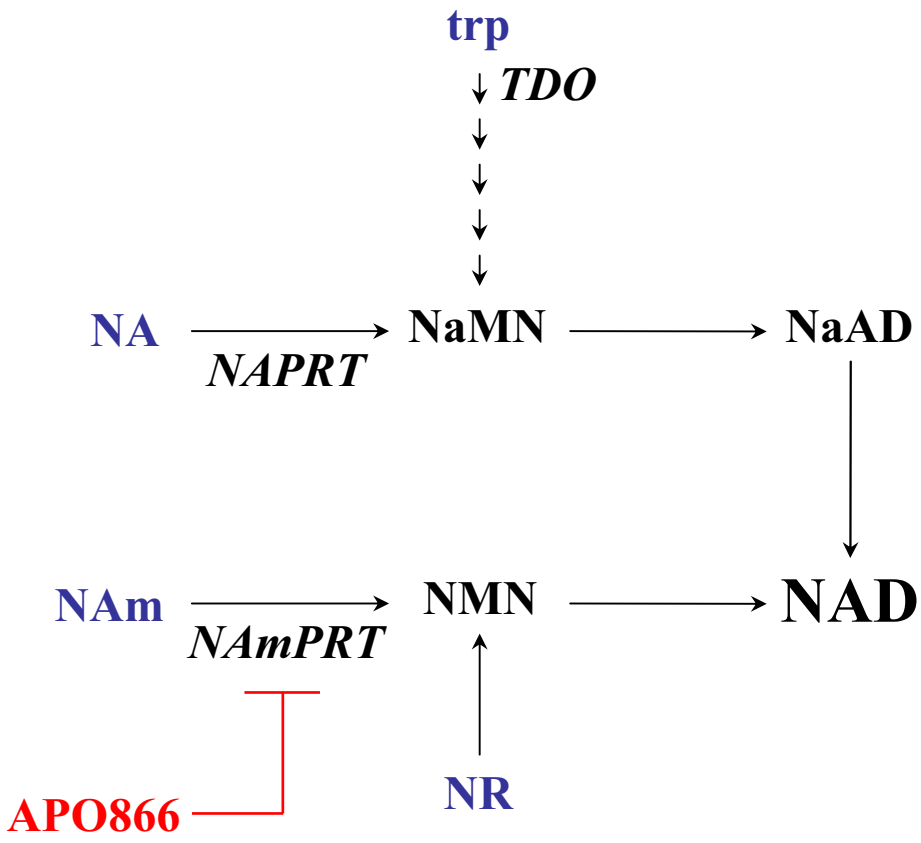


Figure S2

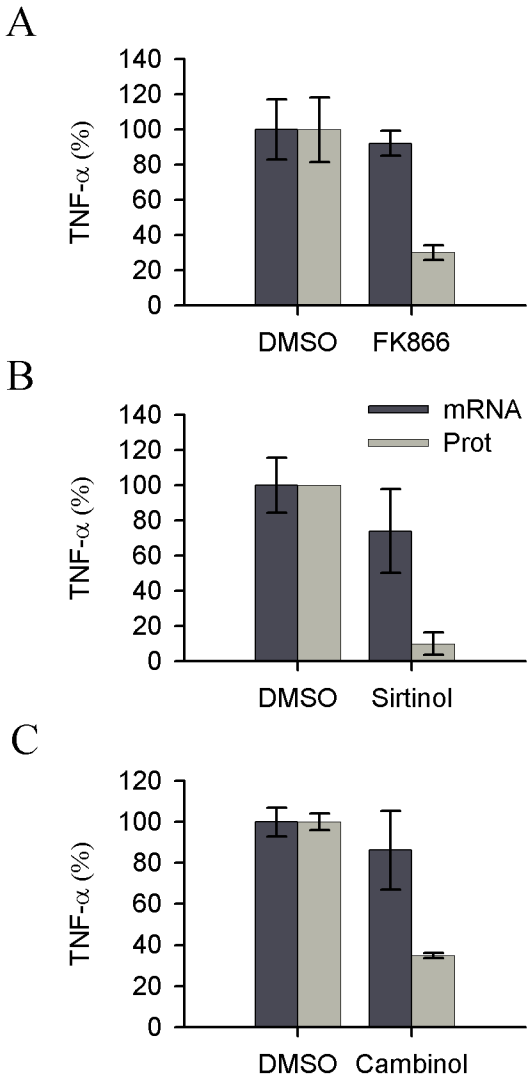

A
140
120
100
80
60
40
20
0
TNF-α (%)
DMSO
FK866
B
mRNA
Prot
140
120
100
80
60
40
20
0
TNF-α (%)
DMSO
Sirtinol
C
120
100
80
60
40
20
0
TNF-α (%)
DMSO
Cambinol

Figure S3

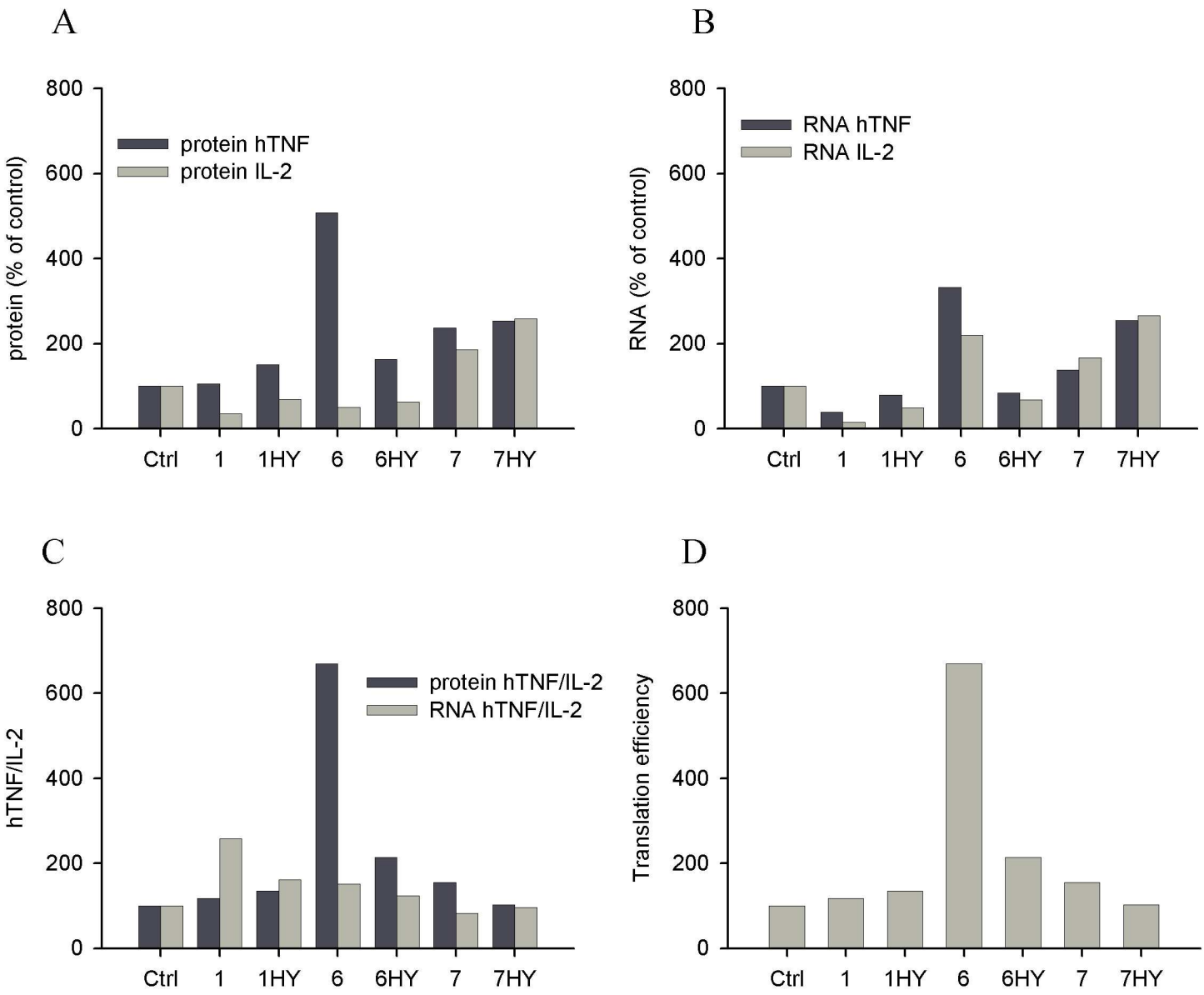
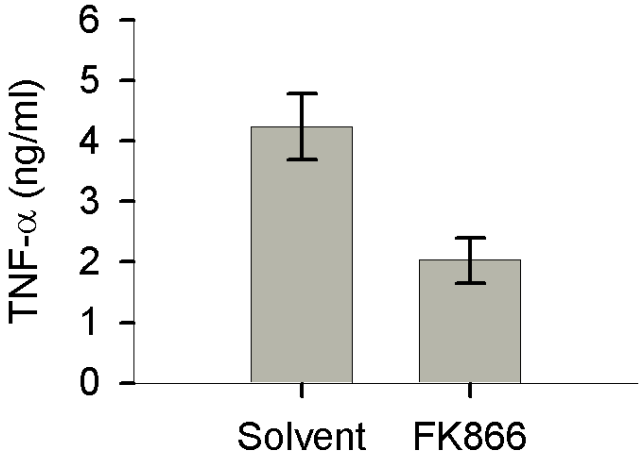

A
protein (% of control)
protein hTNF
protein IL-2
B
RNA (% of control)
RNA hTNF
RNA IL-2
C
hTNF/IL-2
protein hTNF/IL-2
RNA hTNF/IL-2
D
Translation efficiency
0
200
400
600
800
Ctrl
1
1HY
6
6HY
7
7HY

Figure S4

A


TNF-α (ng/ml)
6
5
4
3
2
1
0
Solvent
FK866

B

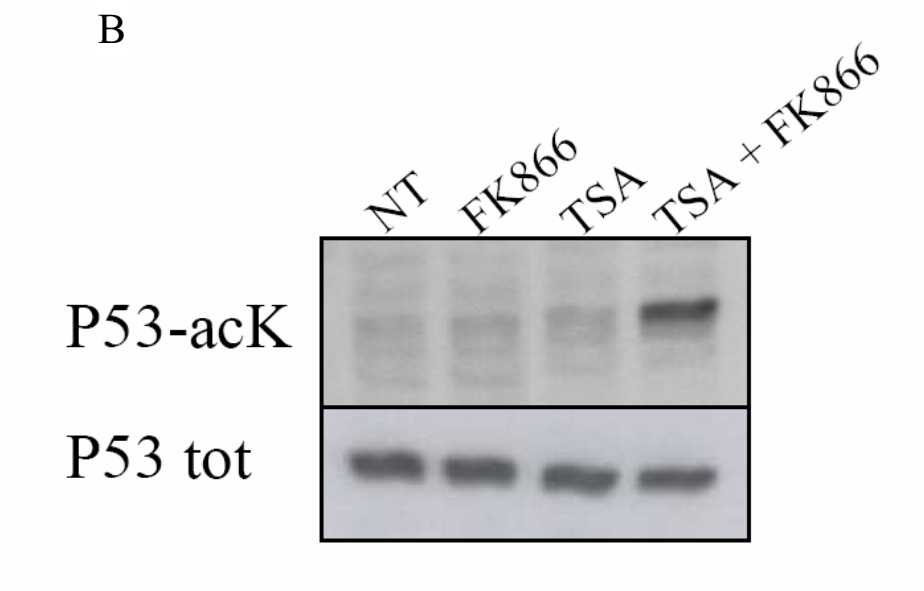

NT
FK866
TSA
TSA + FK866
P53-acK
P53 tot

Figure S5