

Supplemental Material

AMPK enhances transcription of selected Nrf2 target genes via negative regulation of Bach1

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Supplemental Table 1: Gene expression data from wt and Nrf2^{-/} or AMPK^{-/} MEFs after treatment (4h) with DMSO or Sfn (5 μ M) as obtained from an Affymetrix ClariomTM S Assay and subsequent statistical analysis

Supplemental Table 2: List of Nrf2-regulated genes and Nrf2-and AMPK-regulated genes in DMSO- and Sfn-treated MEF

Supplemental Materials and Methods

Expression of AMPK in AMPK α 1^{-/} MEFs

AMPK^{-/}-MEFs were seeded into 12-well plates and transfected with an expression plasmid for eGFP (Clontech) or GFP-AMPK α 1 (pEGFP-C1-PRKAA1 #30305 from Addgene) using Lipofectamine LTX and Plus reagent according to the manufacturers' instructions. After 42 hours, cells were lysed and probed for Bach1, GFP or actin.

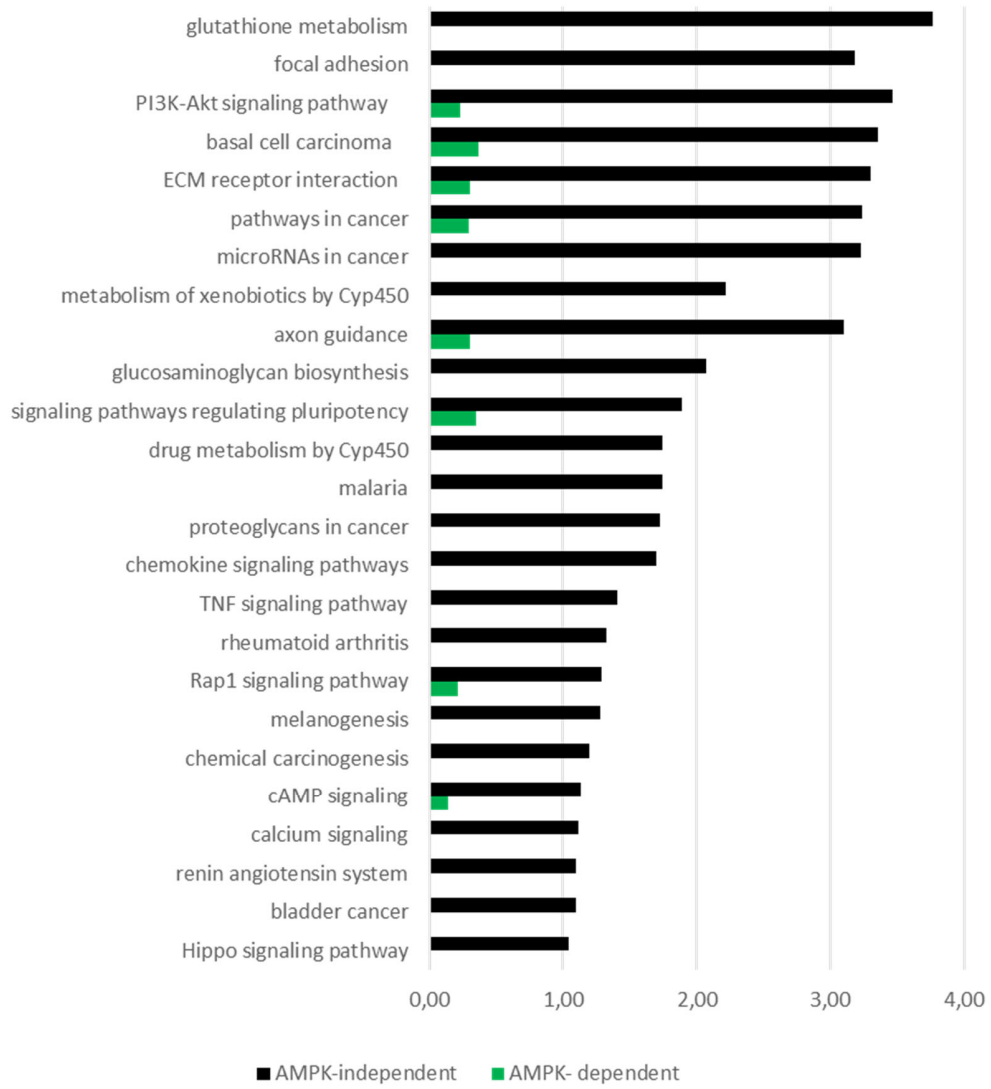
Supplemental Figures

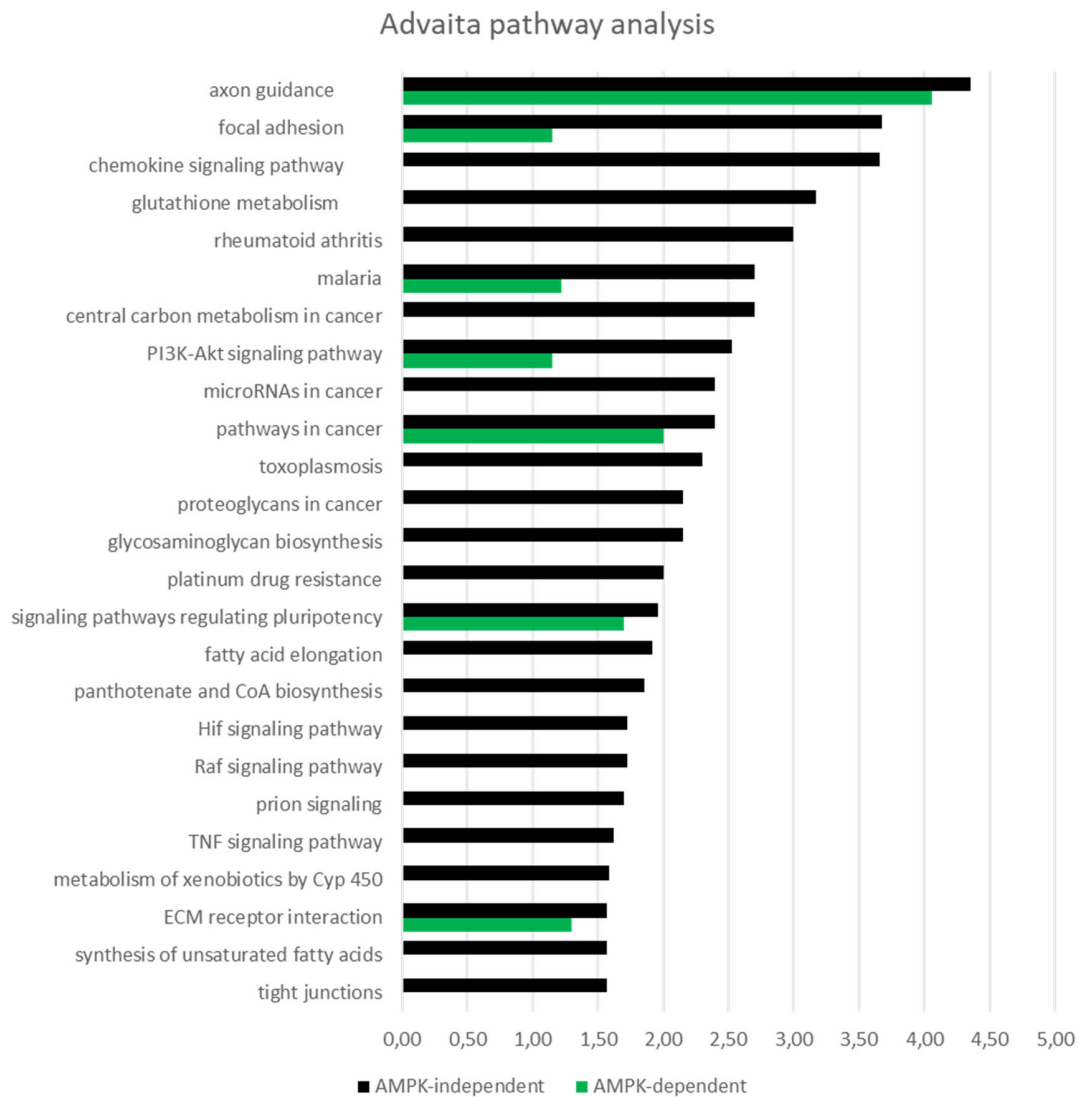
(A)

DAVID	<i>p</i> -value	Advaita	<i>p</i> -value
glutathione metabolism	1.7 e-4	axon guidance	4.4 e-5 8.7 e-5
focal adhesion	6.5 e-4	focal adhesion	2.1 e-4 0.07
PI3K-Akt signaling pathway	3.4 e-4 5.9 e-1	chemokine signaling pathway	2.2 e-4
basal cell carcinoma	4.4 e-4 4.3 e-1	glutathione metabolism	6.8 e-4
ECM receptor interaction	5.0 e-4 5.0 e-1	rheumatoid arthritis	0.001
pathways in cancer	5.8 e-4 5.1 e-1	malaria	0.002 0.06
microRNAs in cancer	5.9 e-4	central carbon metabolism in cancer	0.002
metabolism of xenobiotics by Cyp450	6.1 e-3	PI3K-Akt signaling pathway	0.003 0.07
axon guidance	8.0 e-4 5.0 e-1	microRNAs in cancer	0.004
glucosaminoglycan biosynthesis	8.5 e-3	pathways in cancer	0.004 0.01
signaling pathways regulating pluripotency	1.3 e-2 4.5 e-1	toxoplasmosis	0.005
drug metabolism by Cyp450	1.8 e-2	proteoglycans in cancer	0.007
malaria	1.8 e-2	glycosaminoglycan biosynthesis	0.007
proteoglycans in cancer	1.9 e-2	platinum drug resistance	0.010
chemokine signaling pathways	2.0 e-2	signaling pathways regulating pluripotency	0.011 0.02
TNF signaling pathway	3.9 e-2	fatty acid elongation	0.012
rheumatoid arthritis	4.7 e-2	panthotenate and CoA biosynthesis	0.014
Rap1 signaling pathway	5.1 e-2 6.1 e-1	Hif signaling pathway	0.019
melanogenesis	5.3 e-2	Raf signaling pathway	0.019
chemical carcinogenesis	6.3 e-2	prion signaling	0.020
cAMP signaling	7.3 e-2 7.2 e-1	TNF signaling pathway	0.024
calcium signaling	7.7 e-2	metabolism of xenobiotics by Cyp 450	0.026
renin angiotensin system	8.0 e-2	ECM receptor interaction	0.027 0.05
bladder cancer	8.0 e-2	synthesis of unsaturated fatty acids	0.027
Hippo signaling pathway	9.0 e-2	tight junctions	0.027

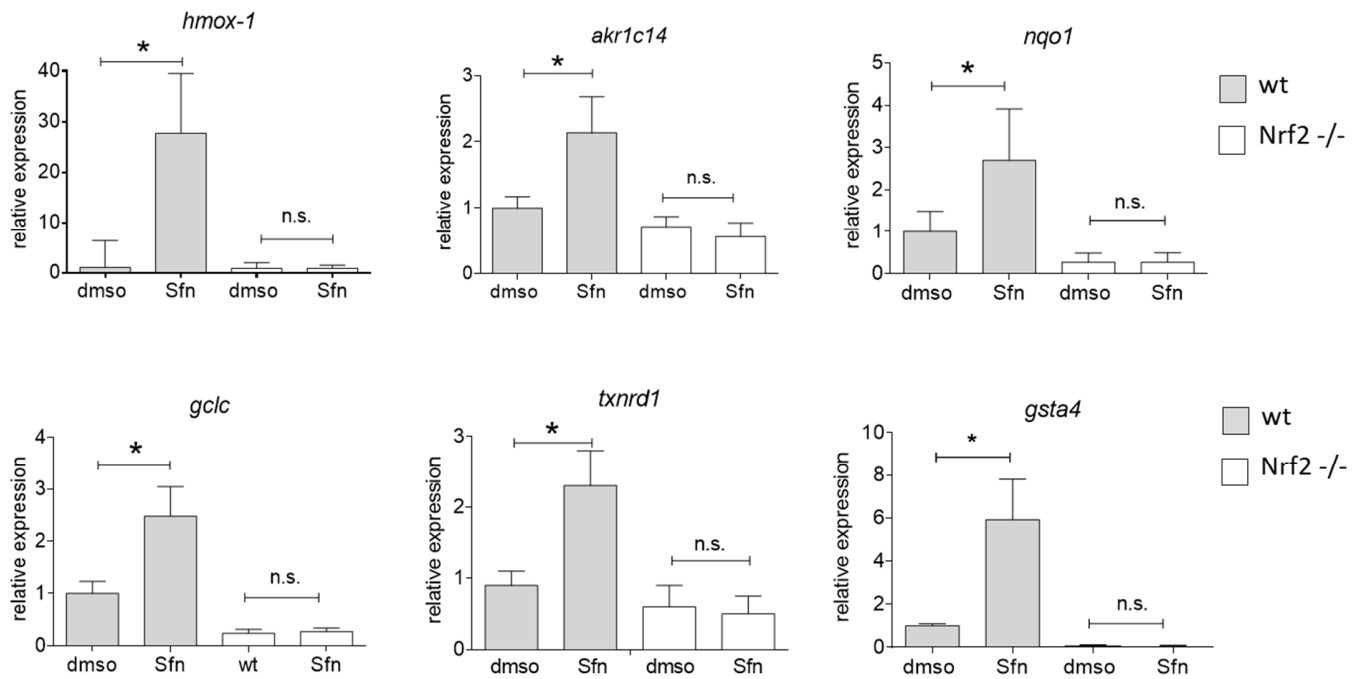
(B)

DAVID pathway analysis

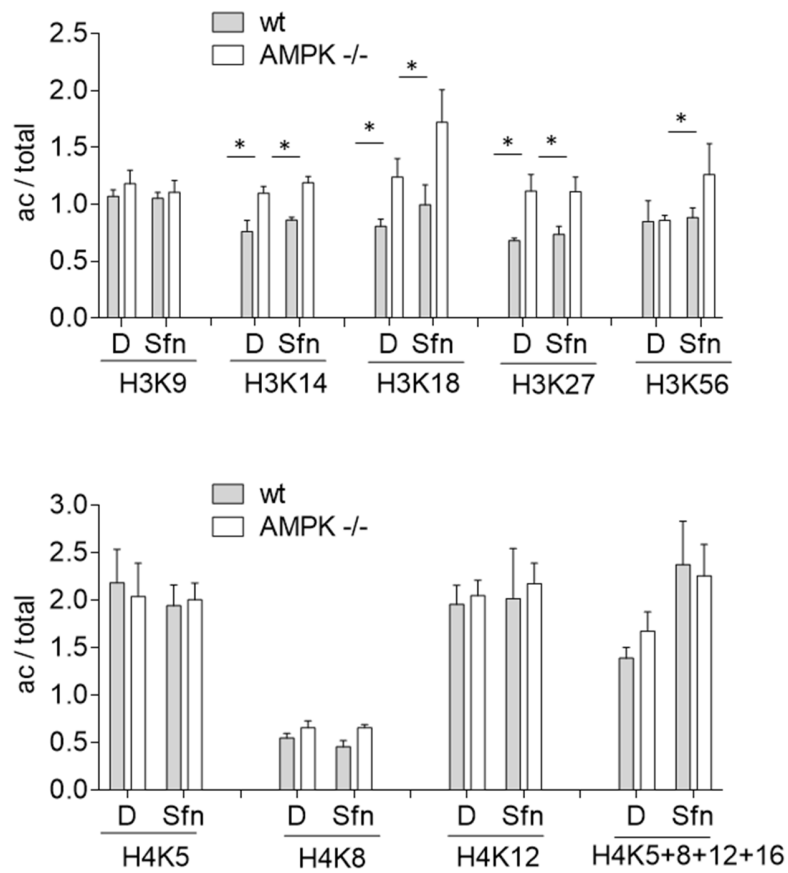




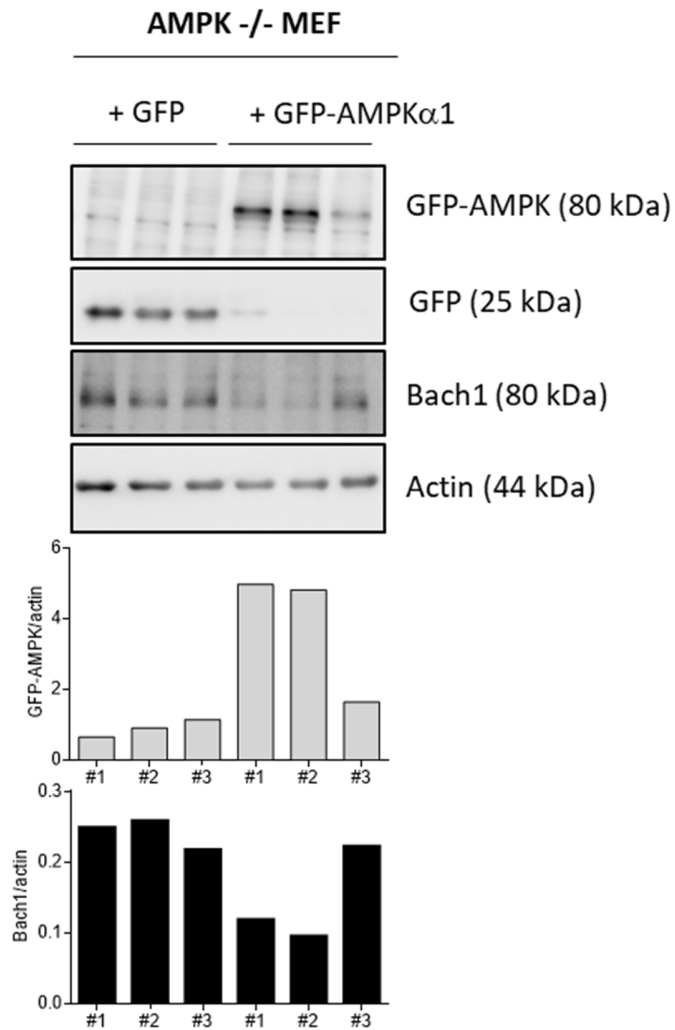
Supplemental Figure 1: Pathway analysis of only Nrf2-regulated and of AMPK and Nrf2-regulated genes using DAVID and Advaita analysis tools. (A) Pathways in bold were consistently found to be enriched by the two analysis tools, pathways in black were only susceptible to regulation by Nrf2, pathways in green are suggested to be under the control of both Nrf2 and AMPK. (B) Bar graph presentation.



Supplemental Figure 2. The investigated genes are Nrf2-dependently induced by Sfn. Wt and Nrf2^{-/-} MEFs were treated with DMSO (0.1%) or sulforaphane (Sfn, 5 μ M) for 4 h before RNA was isolated, reversely transcribed and subjected to qPCR analysis for *hmx1*, *akr1c14*, *txnr1*, *gsta4*, *gclc* and *nqo1* as indicated (*hprt1* as reference gene). Bar graphs present the mean + 95% CI (* p<0.05, ANOVA, Tuckey)

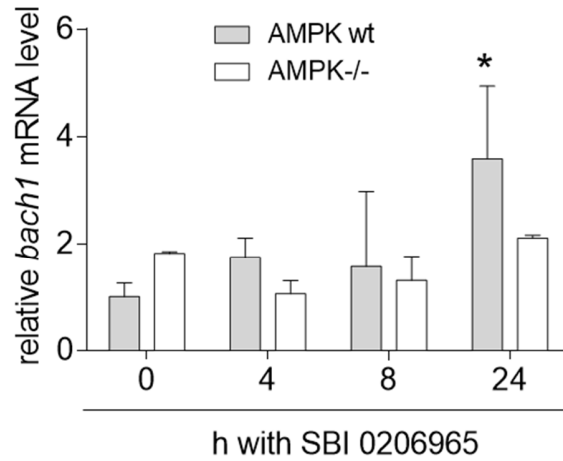


Supplemental Figure 3. AMPK^{-/-} cells show increased global histone H3 acetylation. Histones 3 and 4 from wt and AMPK^{-/-} MEFs were tested for acetylation at specific lysine residues by immunoblot. Compiled densitometric analyses are depicted (n=3, mean + SD; * p<0.05; ANOVA, Tuckey).

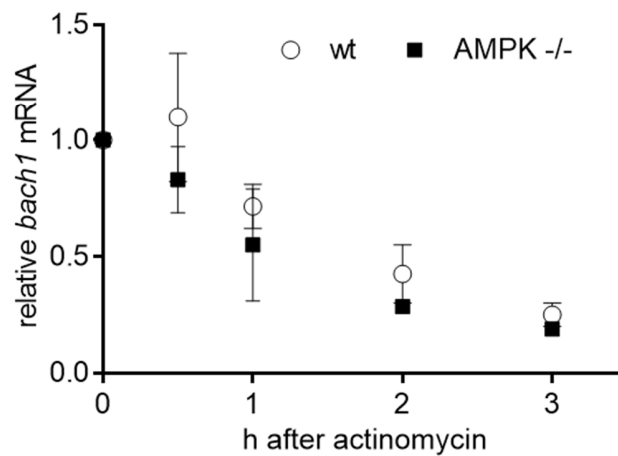


Supplemental Figure 4: Forced expression of AMPK α 1 in AMPK^{-/-} cells negatively correlates with Bach1 levels. AMPK^{-/-} cells were transiently transfected with eGFP- or GFP-AMPK α 1 expression plasmids (independent experiments #1-3). After 42 hours, cell lysates were probed for GFP, Bach1 and actin, respectively.

(A)



(B)



Supplemental Figure 5: *bach1* mRNA is AMPK-dependently suppressed and its half-life does not differ between wt and AMPK^{-/-} MEFs. (A) Wt and AMPK^{-/-} cells were treated with 0.1% DMSO or the AMPK inhibitor SBI0206965 (SBI, 30 μ M) for the indicated time periods. Bach1 mRNA expression was then analyzed by qPCR (*hprt1* as reference). (n=3, * $P \leq 0.05$ to t_0 (wt), ANOVA, Dunnett post-test). **(B)** Wt AMPK^{-/-} cells were treated with actinomycin D (5 μ M) for the indicated periods of time before mRNA was extracted, and *bach1* mRNA levels were determined by qPCR (*actinb* as reference gene, Qiagen) (n=2).