TBK1 at the crossroads of inflammation and energy homeostasis in adipose tissue

Peng Zhao[1,2], Kai in Wong[1], Xiaoli Sun[1], Shannon M. Reilly[1,2], Maeran Uhm[2], Zhongji Liao[1], Yuliya Skorobogatko[1,2], Alan R. Saltiel[1,2,*]

Supplementary figure legends

Figure S1. Adipocyte-specific TBK1 deficiency does not affect energy homeostasis in ND-fed mice or body temperature in 16 weeks HFD-fed mice. Related to Figure 1. (A) Body weight of ND-fed Flox and ATKO mice. N=11-15. (B) DEXA imaging of ND-fed Flox and ATKO mice. (C, D) Fat mass (C) or percentage of fat mass (D) of ND-fed Flox and ATKO mice. N=6. (E) Tissues weight of ND-fed Flox and ATKO mice. N=11-15. (F, G) Respiratory exchange rate (RER) of ND (F) or HFD (G)-fed Flox and ATKO mice. N=5-6. (H, I) Activity of ND (H) or HFD (I)-fed Flox and ATKO mice. N=5-6. (J) Daily food intake of ND and HFD-fed Flox and ATKO mice. N=5-6. (K) Rectal temperature of HFD-fed Flox and ATKO mice. N=9. Data are represented as mean±s.e.m. *, $p<0.05$.

Figure S2. TBK1 deficiency increases expression of creatine cycle and lipase genes, without affecting catecholamine sensitivity or AMP/ATP in 16 weeks HFD-fed mice. Related to Figure 2. (A) Genes expression in BAT of HFD-fed Flox and ATKO mice. N=9. (B, C) Expression of creatine cycle genes in eWAT (B) and iWAT (C) of HFD-fed Flox and ATKO mice. N=9. (D, E) Expression of lipase genes in eWAT (D) and iWAT (E) of HFD-fed Flox and ATKO mice. N=9. (F) cAMP level in eWAT and iWAT of HFD-fed Flox and ATKO mice. N=4. (G, H) Glycerol released from explanted eWAT (G) and iWAT (H) of HFD-fed mice in the absence or presence of CL-316,243 (10μM). N=9. (I, J) Serum triglyceride (TG) (I) and NEFA (J) levels in HFD-fed Flox and ATKO mice. N=11-12. (K) IB analysis of BAT in HFD-fed Flox and ATKO mice. (L) *Tbk1* expression in BAT of ND or HFD-fed mice. N=4. (M, N) IB analysis of eWAT (M) and iWAT (N) in ND-fed Flox and ATKO mice. (O, P) Measurement of AMP/ATP (O) and ADP/ATP (P) ratio in eWAT and iWAT of HFD-fed Flox and ATKO mice. N=5. Data are represented as mean±s.e.m. *, $p<0.05$. (Q) IB analysis of WT and TKO (TBK1 KO) MEFs treated with Vehicle or TNFα (10nM). Quantification of pAMPK/AMPK ratio (bottom). Data are represented as mean±s.d. *, $p<0.05$.

Figure S3. Identification of TBK1-induced phosphorylation on AMPKα1 (PRKAA1) by LC-MS/MS. Related to Figure 3. (A) Coverage of AMPKα1 sequence in LC-MS/MS analysis. (B, C) MS chromatogram depicting the phosphopeptide showing AMPKα1 Ser459 (B) and Ser476 (C) phosphorylation (S+80).

Figure S4. Activation of AMPK induces TBK1 activity. Related to Figure 4. (A, B) IB analysis of HEK293T cells overexpressing HA-AMPKα1 (A) or HA-AMPKα2 (B) with Flag-AMPKβ1 and Myc-AMPKγ1. (C) Expression of *Tbk1* in eWAT and iWAT of C57BL6/J mice fed with ND or fasted for 48hrs. N=4. Data are represented as mean±s.e.m. *, $p<0.05$. (D) IB analysis of WT and ULK1 KO MEFs starved for 2hrs or not. Result is a representative experiment that was repeated three times.

Figure S5. Loss of TBK1 does not affect glucose metabolism in ND-fed mice or genes expression and H&E staining of liver in HFD-fed mice. Related to Figure 5. (A, B) Fasting blood glucose (A) and insulin (B) levels of 12 weeks ND or HFD-fed Flox and ATKO mice. N=7-11. (C) Glucose (2g/kg BW) tolerance test on 12 weeks ND-fed Flox and ATKO mice.

N=7. (D) Insulin (1U/kg BW) tolerance test on 12 weeks ND-fed Flox and ATKO mice. N=7-8. Data are represented as mean±s.e.m. *, $p<0.05$. (E) Blood insulin levels at 0 min or 30 min during GTT on HFD-fed Flox and ATKO mice. N=10-11. (F) H&E staining of livers in 16 weeks HFD-fed Flox and ATKO mice. (G) Genes expression in livers of 16 weeks HFD-fed Flox and ATKO mice. N=10-11. Data are represented as mean±s.e.m. *, $p<0.05$.

Figure S6. Loss of TBK1 increases TNFα-induced NFκB activation. Related to Figure 6. (A) IB analysis of BAT from HFD-fed Flox and ATKO mice. (B, C) IB analysis of eWAT (B) and iWAT (C) from ND-fed Flox and ATKO mice. (D) IB analysis of WT or TKO MEFs treated with TNFα (10nM) for indicated time. (E) *Ccl2* expression in WT or TKO MEFs treated with TNFα (5nM) for 16hrs. N=3. Data are represented as mean±s.d. *, $p<0.05$. (F) IB analysis of WT or TKO MEFs treated with TNFα (10nM) for indicated time. (G) IB analysis of HEK293T cells overexpressing HA-NIK with Flag-TBK1 WT or Flag-TBK1 KD. (H) IB analysis of product from immunoprecipitation with HA-antibody. (I) IB analysis of WT, NKO, TKO or DKO MEFs treated with proteasome inhibitor MG132 (20μM) for 6hrs.

Figure S7. TBK1 deficiency attenuates inhibition of NFκB signaling by AMPK. Related to Figure 7. (A) IB analysis of WT and TKO MEFs pretreated with Vehicle or AICAR (500μM) for 7hrs, and then treated with TNFα (10nM) for 45min. (B) *Ccl2* expression in WT and TKO MEFs pretreated with Vehicle or AICAR (500μM) for 7hrs, and then treated with TNFα (10nM) for 2hrs. Data are represented as mean±s.d. *, $p<0.05$. (C) Proposed model for roles of TBK1 in modulating inflammation and energy metabolism.

Supplementary table

Table S1. Identification of TBK1-induced phosphorylation sites on AMPKα1. Related to Figure 3.

Table S2. Q-PCR primer sequences

Gene	Forward Primer	Reverse Primer
Adgre1	CCCCAGTGTCCTTACAGAGTG	GTGCCCAGAGTGGATGTCT
Atp5d	AAGATGCCAAAGGCTCCAG	GATGTCCTTCACCTTTGCCT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Cox4i1	GCAGACAGCATCGTGACAT	GAGAGCCATTTCTACTTCGGT
Cox5b	GCGAAGTAACCTTGAAGCCA	CCGCCCATCTTGCTCAG
Cox8a	CTTCGAGTGGACCTGAGC	CATCTTGACTCCCTGACCTTG
Fasn	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
G6pc	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Gapdh	TGAAGCAGGCATCTGAGGG	CGAAGGTGGAAGAGTGGGAG
Itgax	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
Lipe	GGCTCACAGTTACCATCTCACC	GAGTACCTTGCTGTCCTGTCC
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC

Ndufs7	CTTCTGTTCACGCTTGATCTTC	GGCTACTACCACTACTCCTACT
Pck1	ACACACACACATGCTCACAC	ATCACCGCATAGTCTCTGAA
Pnpla2	CAACGCCACTCACATCTACGG	TCACCAGGTTGAAGGAGGGAT
Ppargc1a	CCACTTCAATCCACCCAGAAA	TATGGAGTGACATAGAGTGTGCT
Scd1	GCTGGAGTACGTCTGGAGGAA	TCCCGAAGAGGCAGGTGTAG
Srebf1	TGACCCGGCTATTCCGTGA	CTGGGCTGAGCAATACAGTTC
Tnfa	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
Tbk1	GACAGCATAGAGATCACCAGTT	CAGAGCACCTCCAACCATC
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT