Supporting Information

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SI Text

Analysis of Microarrays. The following parameters were used for initial data analysis: global background correction, normalization to GAPDH, average density of pixels and nonclover-mode setting. Refined analysis excluded bleeding spots (if the average density of the spot was greater than the average value of all spots) and absent spots (if the average density of the spot was less than the mean value of the local backgrounds of the lower 75 percentile of all nonbleeding spots).

Conditions for QPCR Reactions. Samples with either no reverse transcriptase or water instead of RNA were used as negative controls. For 96-well QPCR plates, we used reaction mixture containing 12.5 μL of RT^2 qPCR master mix with SYBR Green (SABiosciences), 1 μL appropriate primers, 1 μL cDNA from RT reactions, and 10.5 μL nuclease-free water. For analysis we used a Stratagene Mx3000P instrument programmed as follows: 95 °C, 10 min, and 40 cycles of 95 °C for 15 sec, and 60 °C for 60 sec.

Conditions for Western Blots. Protein was isolated using RadioImmunoPrecipitation Assay (RIPA) buffer (Pierce Biotechnology) containing β mercaptoethanol (βME) and Proteinase Inhibitor Mixture Set III (1:1000; Calbiochem). When isolating activated NFκB-p65, we also added Phosphatase Inhibitor Mixtures I and II (Sigma Chemical) (1). Protein samples were mixed with equal volumes of SDS reducing buffer with βME), boiled for 10 min, and then placed on ice immediately. Twenty micrograms of protein were resolved on Tris-Hepes-SDS gels (Pierce Biotechnology) along with 15 μL of molecular weight marker (SeeBlue Plus 2 from Invitrogen) and then transferred onto nitrocellulose membranes as per manufacturer's protocol (MiniProtean 3; BioRad). Chemiluminecence was detected using Super Signal West Pico Chemiluminescent Substrate (Pierce Biotechnology) and quantified using GeneTools software (Syngene).

1. Royds JA, Dower SK, Qwarnstrom EE, Lewis CE (1998) Response of tumour cells to hypoxia: Role of p53 and NFkB. *Mol Pathol* 51(2):55–61.

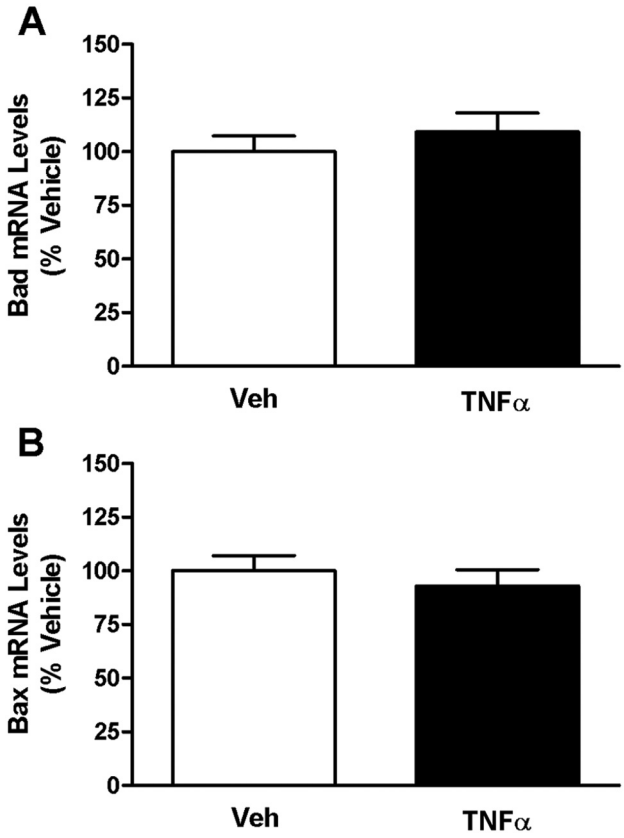

Fig. S1. Effects of TNFα on Bad (*A*) and Bax (*B*) mRNA levels in N42 hypothalamic cells. Cells were treated with 100 U of TNFα or vehicle (BSA) for 30 min and then medium was replaced. After 12 h, cells were harvested and Bad and Bax mRNA levels were measured using QPCR. Each bar represents mean $\pm$ SEM of 6 samples assayed in duplicate.

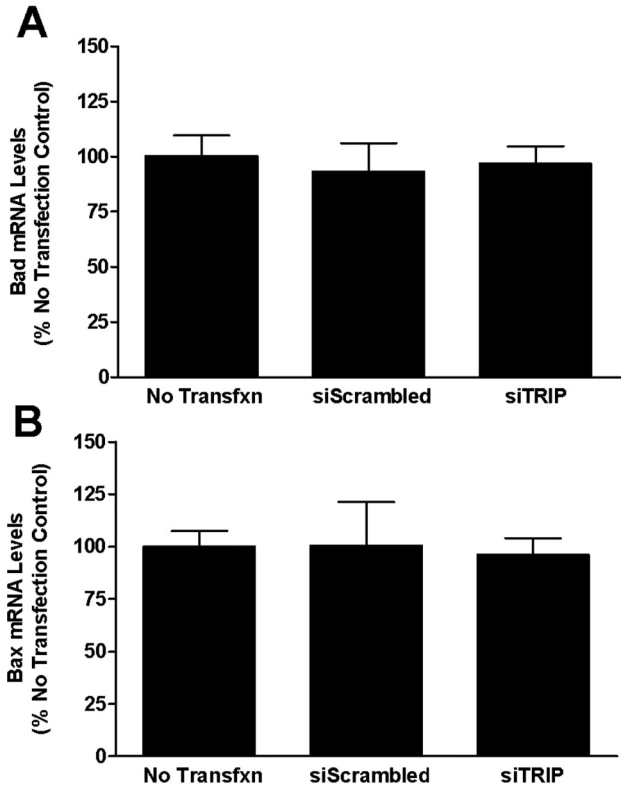

Fig. S2. Effects of TRIP knockdown on Bad (*A*) and Bax (*B*) mRNA levels in TNFα-treated N42 hypothalamic cells. Cells were treated with 100 U of TNFα for 30 min, and then 12 h later they were either left untransfected (No Transfxn) or transfected with either control vectors containing AllStars Negative Control siRNA (siScrambled) or vectors containing a combination of 2 constructs, S101454649 and S101454635 (siTRIP; to silence TRIP expression). RNA was isolated 24 h after transfection. Each bar represents mean $\pm$ SEM of at least 4 samples assayed in duplicate.

Table S1. Genes identified on rat apoptosis GEArray DNA microarrays (SABiosciences) as differing between sexes on postnatal day 2

Gene	Symbol	Accession number	Fold change (male:female)
Linked to TNF signaling			
Similar to TRAF interacting protein	TRIP	XM_345981	20.0
BH3 interacting domain	Bid 3	NM_057130	9.1
Myeloid cell leukemia sequence 1	Mcl 1	NM_021846	5.9
TNF ligand superfamily, 10	TNFSf10	NM_145681	5.0
DAP3-H; ESTs		XM_215627	3.0
Similar to ubiquitin protein ligase	Ube1x	XM_234520	2.0
B-cell CLL/lymphoma 10	Bcl 10	NM_031328	2.0
Ubiquitin-conjugating enzyme E2D3	Ube2d3	NM_031237	2.0
Ubiquitin-activating enzyme E1C	Ube1c	NM_057205	2.0
Ubiquitin-conjugating enzyme E21	Ube2i	NM_013050	2.0
Apoptotic peptidase activating factor	Apaf1	NM_023979	2.0
Coiled-coil, myosin-like Bcl2- interacting protein	Becn 1	NM_053739	2.0
B-cell leukemia/lymphoma 2	Bcl-2	NM_016993	−1.4
Traf family member-associated Nfκ B activator	TANK	NM_145788	−3.3
Not linked to TNF signaling			
Tumor protein p53	Tp53	NM_030989	10.0
Sodium channel associated protein	Sap 2	XM_342209	10.0
Brain-specific angiogenesis inhibitor 1-associated protein 2	Baiap2	NM_057196	3.0
Bcl2-associated X protein	Bax	NM_017059	2.0
Bcl2-associated death promoter	Bad	NM_022698	2.0
Bcl2/adenovirus E1B 19 Kda-interacting protein like	Bnip3l	NM_080888	2.0
Bcl2/adenovirus E1B 19 Kda-interacting protein 3	Bnip 3	NM_053420	2.0
Similar to Rad 1p mRNA	Rad 1	XM_215497	−3.3
Similar to UV excision repair protein RAD 23 mRNA	Rad23a	XM_341660	−3.3

Only genes identified on all 4 pairs of microarrays (male vs. female) are listed.

Table S2. PCR primers used to prepare cDNA transcriptional templates for cRNA probes used in situ hybridization studies

Gene	Accession number	Forward primer	Reverse primer
trip	XM_345981.2	5′-GCAATGCCTAATCCAGTGGT-3′	5′-GCTCAATTTGCTCCATGGTT-3′
tnfr2	AF498039.2	5-′AACCCTCTCCCAACACACTG-3′	5′-GGCAAAATAGCTGGCTTCAG-3′
traf2	XM_ 231032.3	5′-CTGAGCTCGTTCCTGGAGTC-3′	5′-AGCACACAGCAGTCCCTCTT-3′