Supplementary Methods and Figure Legends

Supplementary Methods

qRT-PCR analyses

Primers for qRT-PCR analyses were as follows:

Gene	Forward (5'-3')	Reverse (5'-3')
IFNb1	GCTTGGATTCCTACAAAGAAGCA	ATAGATGGTCAATGCGGCGTC
SP110	CCTATGCCATACACAAGCCATT	CCTCTCCAGTTGGGTGAGAAT
DCR2	CCGGGGGATGGTGGCAGAGT	CGCTCGAGCAGGGCGCTATC
DEC1	CGATGAGCCGGTGCGGCAAT	CCGGGACTGGAGCACGGAGA
P16	GGTTGTGGCGGGGGCAGTT	GGGGGCACCAGAGGCAGT
H3A	AAGCAGACTGCCCGCAAT	GGCCTGTAACGATGAGGTTTC
IFIT2	CAGCTGAGAATTGCACTGCAAC	GTAGGCTGCTCTCCAAGGAA
ISG58	TTGCAGGAAACACCCACTTCT	GCAAAGCCCTATCTGGTGATG
OAS1	AGCTTCGTACTGAGTTCGCTC	CCAGTCAACTGACCCAGGG
PKR	GCCGCTAAACTTGCATATCTTCA	TCACACGTAGTAGCAAAAGAACC
B2MG	TGTCACAGCCCAAGATAG	CCAGCAAGCAGAATTTGGAA
IFNAR1	ATTCCCGACAGACTCATCGC	TCCCAGATGATGGTCGTCCT
IFNAR2	GGCCATTTCCTAACCTGCCA	CTCGGACTCCGGGTCTATCA
PKCε	TGACGTGGACTGCACAATGA	CCATGCTGGTGGAGGAACAT
p21	CCGAAGTCAGTTCCTTGTGGA	AGTCGAAGTTCCATCGCTCA
p27	AGTGTCTAACGGGAGCCCTA	AGTAGAACTCGGGCAAGCTG
P53	TGCTCAAGACTGGCGCTAAA	TTTCAGGAAGTAGTTTCCATAGGT

RNA from cells treated as indicated were reverse transcribed to cDNA using High-Capacity Reverse Transcriptase kits, and qRT-PCR analyses were performed using Syber Green RT-PCR kits (Invitrogen, USA) on a Bio-Rad CFX Connect Real-Time system. The pRT-PCR cycles were as follows: 95^{o}C for 10 min; 50 cycles of 95^{o}C for 15 sec, 55^{o}C for 60 sec, 72^{o}C for 30 sec; 95^{o}C for 15 sec, 55^{o}C for 5 sec, and 95^{o}C for 30 sec. Expression levels normalized to H3A controls.

For qRT-PCR on the FFPE sections: FFPE sections (10 μm x 5 sections) for each sample were deparaffinized with xylene and washed with 100% ethanol. Total RNA was extracted with Tri-Reagent (Molecular Research Center, Cincinnati, OH). RNA was measured with the Quanti-iT RiboGreen RNA assay kit (Invitrogen, life technologies, Carlsbad CA). Subsequently, 4 μg of RNA was reverse-transcribed into cDNA as previously described [31]. The synthesized cDNA was then added to the PCR reaction mixture to make the final reaction volume of 12.5 μL. PCR amplification was performed with 2X PerfeCTa SYBR Green SuperMix for iQ (Quanta BioSciences, Inc. Gaithersburg, MD), 20 μM of each primer, and 2.5 μL of cDNA templates. The following thermal cycler condition was applied: Initial heating at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 15 sec and annealing at 55°C for 30 sec; lastly, extension at 72°C for 1 min. Each PCR reaction was performed in duplicates to verify the mRNA expression levels of IFNb1 marker. Furthermore, Beta-2-Microglobulin (B2MG) was used as an internal control in the assay.

IFNβ1 chromatin immunoprecipitation (ChIP) assay

501Mel cells treated as indicated in the main text were washed in room temperature PBS and fixed in 1% formaldehyde in PBS at RT for 10 min. The cells were pelleted and resuspended in 10 ml of neutralization buffer (0.125 M glycine/1X PBS) and incubated

for 5 min at RT. The cells were washed twice with 10 ml of cold PBS, resuspended well in 1 ml of lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.0), and subjected to sonication on ice to shear the DNA to ~500-bp fragments. The sonicated chromatin was cleared of debris by centrifugation at 16,000 g at 4°C for 5 min. The sonicated chromatin was then diluted 1:10 with dilution buffer (1% Triton X-100, 0.01% SDS, 150 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0) and precleared by incubation with protein A/G beads (Santa Cruz Biotech, USA) with rotation at 4^{o}C for 2 h. Equal concentrations of sonicated chromatin were incubated with 2 µg of control IgG (IgG) or ATF2 (C-19X, Santa Cruz Biotechnologies, USA) antibody (αATF2) and allowed to incubate overnight at 4^{o}C. Next, 25 µl of protein A/G beads were incubated with the samples for 4 hours with rotation at 4^{o}C. After 3 washes in wash buffer 1 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl pH8.0), followed by 1 wash in wash buffer 2 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl, 20 mM Tris-HCl pH 8.0). Next, the ATF2-bound chromatin was eluted from the beads in 300 µl of elution buffer (1% SDS, 100 mM $NaHCO_3$) for 15 min with constant agitation. Next, 6 µl of protease K (10 mg/ml) and 3 µl of RNase A (20 mg/ml) were added to each sample and incubated at 37 °C for 30 min with agitation. The supernatants were transferred from the mixture with beads following centrifugation at 16,000g, and 12 µl of 5 M NaCl was added and incubated further at 65 °C overnight to reverse the formaldehyde cross-link. The chromatin was then purified using a QiaQuick PCR purification kit (Qiagen, USA). The 2µl of eluted chromatin was next used in a PCR reaction to detect ATF2 binding to the IFNb1 promoter as follows: 95^{o}C for 10 min; 50 cycles of 95^{o}C for 15 sec, 55^{o}C for 60 sec, 72^{o}C for 30 sec. The primers used to detect the IFNb1 promoter were as follows (5'-3'): Forward (AACATTAGAAAACCTCACAGTTTGT) and reverse (ATTTCCCACTTTCACTTCTCCCTT).

Immunocytochemical analysis for Mre11 foci

After indicated treatments, poly-L-lysine-coated coverslip-grown cells were hypotonically extracted in 0% CSK buffer (10 mM PIPES, pH 7.0/100 mM NaCl/300 mM sucrose/3 mM $MgCl_2$/1 mM EGTA) for 2 min followed by extraction in 0.5% CSF buffer (0.5%TX-100/10 mM PIPES, pH 7.0/100 mM NaCl/300 mM sucrose/3 mM $MgCl_2$/1 mM EGTA) for an additional 2 min. The cells were then fixed in fixation buffer (4% paraformaldehyde/2% Sucrose/PBS) for 20 min at room temperature. Coverslips were then rinsed twice in phosphate buffer solution and permeabilized in permeabilization buffer for 20 min. Primary antibodies were applied at 1:250 dilution in staining buffer overnight at 4^{o}C in a humid chamber. Coverslips were subsequently subject to 5 standing 5-minute washes in wash buffer. Secondary antibodies (AlexaFluor secondary 350, 488 or 568, Invitrogen) were applied at 1:250 dilution in staining buffer for 2-3 hours at room temperature in a humid chamber in the dark. Prior to mounting with Vectorshield with DAPI (Vector Laboratories, CA), coverslips were washed twice more in wash buffer. Immunofluorescent analysis was conducted on an Olympus TH4-100 fluorescent microscope. $\geq$ 3 Z-planes per field were captured and analyzed using Slidebook V.4.1, and Z-projection images of average intensity were constructed using ImageJ V.1.48.

Intracellular IFNβ1 FACS analysis

Intracellular FACS analysis for IFNβ1 was performed as previously described by Seeds and Miller (Current Protocols in Immunology 2011). Briefly, the cells were treated as indicated overnight. The cells were further incubated in the presence of 10 μg/ml brefeldin A for 4 hours, harvested and fixed in fixation buffer (1% paraformaldehyde/PBS). After a single wash in PBS, the cells were blocked in blocking buffer (5% BSA/PBS) for 30 min on ice and then washed once in wash buffer (0.2% saponin/0.5% BSA/5 mM EDTA). The cells were then stained with anti-IFNb1 antibody

(2.5 µg/ml antibody in block buffer overnight at 4^{o}C). After 3 washes in wash buffer, the cells were resuspended in wash buffer containing the goat-anti-rabbit AlexaFluor 488 (1:250 dilution) and incubated at room temperature for 1 h. After 3 further washes in wash buffer, the cells were immediately subjected to FACS analysis. Flow cytometric data were analyzed using FlowJo software (TreeStar, OR).

Secreted luciferase assays

Secreted promoter luciferase constructs were generated as detailed in Figure 2A. Each of the IFNb1 full promoter or promoter fragments were cloned into the pMCS Gaussia-Luc vector (ThermoScientific) using EcoRI and BamHI restriction enzyme sites.

Primers used for the generation of the promoter luciferase constructs were designated as follows (for constructs 1, 2, 3, 4, self-annealing primers were used; the primers used to generate the ΔE1 construct were mutagenic primers that were used to mutagenize the FL construct using the Stratagene Lightning Mutagenesis kit according to manufacturer's protocols):

Construct	Forward Primer (5'-3')	Reverse Primer (5'-3')
FL	CGCGGAATTCAACATTAGAAAACCTCACAGTTTGT	CGCGGGATCCGGAGGAGACACTTGTTGGTCA
1	AATTCAACATTAGAAAACCTCACAGTTTGTAAAG	GTTGTAATCTTTTGGAGTGTCAAACATTTCCTAG
2	AATTCGTAAATGACATAGGAAAAG	GCATTTACTGTATCCTTTTCCTAG

3	AATTCGCCTTTGCTCTGGCACAACA GGTAGTAG	GCGGAAACGAGACCGTGTTGTCCAT CATCCTAG
4	AATTCGCCTTTGCTCTGGCACAACA GGTAGTAGGCGACACTGTTCGTGTT GTCAACGATGACCAAG	GCGGAAACGAGACCGTGTTGTCCAT CATCCGCTGTGACAAGCACAACAGT TGCTACTGGTTCCTAG
123	CGCGGAATCAACATTAGAAAACCTC ACAGTTTGT	CGCGGGATCCCGGAAACGAGACGT GTTGTCCATCAT
234	CGCGGAATCAAAATGTAAATGACAT AGGAAAACTGAA	CGCGGGATCCGGAGGAGACACTTG TTGGTCA
ΔE 1	CATTAGAAAACCCACCAGTTTGTAAA TC	TTTTTTAAAGTTTCAGTGAATGAC

A pCMV-Cypridina Luc (a constitutively CMV promoter-driven secreted Cypridina luciferase) construct was used as a normalization control.

After the indicated treatments, media samples were collected and measured for Gaussia and Cypridina luciferase activity using a standard luminometer. Gaussia luciferase activity values were normalized to Cypridina luciferase activity values and were plotted relative to the values indicated in the figure legends. The data are representative of 3 independent experiments.

Senescence assay

Cells were seeded at 5 X 10^3 cells per well into clear-bottom 96-well tissue culture plates and treated the next day as indicated in the figure legend. Following treatment, the plates were briefly centrifuged at 500 g, and the media was aspirated from each well and replaced immediately with senescence assay buffer (per well: 100 µl of galactosidase reaction buffer (37 mM $C_6H_8O_7$/127 mM Na_2HPO_4/5 mM $K_3(Fe(CN)_6)$/5 mM $K_4(Fe(CN)_6)$/150 mM NaCl/ 2mM $MgCl_2$)) + 10µl substrate (2 mM FDG)). The plates were incubated at 37°C in atmospheric oxygen for ~2 h. Next, the top and bottom absorbances (excitation: 485 nm; emission: 535 nm) of the plates were measured using a fluorometer (ABI).

Direct melanoma:lymphocyte co-culture assay and assessment of lymphocyte activation

Stably GFP- and empty vector- or $ATF2^{T34E}$-expressing YUMM 1.3 cells were seeded at 2.5 X 10^4 cells per well in 48-well tissue culture plates in 250-µl volumes. The next day, the cells were subjected to 0, 5, or 10 Gy of ionizing radiation using a Gammacell 40 Exactor (Low Dose Irradiator, Canada). The spleens of wild-type C57B6 mice previously inoculated with 1 X 10^6 YUMM 1.3 cells (1 month before the experiment) were subjected to ACK lysis, and lymphocytes were washed and immediately plated at 7.5 X 10^5 cells in 200 µl on top of the YUMM cells in the presence or absence of the 10 µg/ml of anti-IFNb1 antibody. At the indicated time points, the well bottoms were imaged for residual GFP-positive YUMM cells. Using ImageJ, melanoma cell viability was measured as follows: the YUMM images were contrast-adjusted to visualize all of the YUMM cells, converted to binary, and quantitated for cell areas. The measurement of lymphocyte activation was performed as follows: the co-cultured lymphocytes were immediately harvested, pelleted, washed with staining buffer (2% FBS/PBS), stained for 20 min on ice with anti-Gr-1 PerCP Cy5.5, anti-CD3 APC-Cy7, anti-CD4 PE-Cy7, anti-CD8

eFluor450, anti-NKp46 APC, anti-CD69 FITC, and anti-CD25 PE (all antibodies for these experiments were purchased from eBioscience, except for anti-Gr-1 and anti-CD69, which were purchased from BD Biosciences), washed twice, and subjected to flow cytometric analysis. n = 50,000 cells per replicate for Fig. 5a,b and Supplementary Fig. 4; lymphocytes for each replicate were derived from 3 individual tumor-bearing mice for Fig. 5a,b and Supplementary Fig. 4. The FACS data were subsequently analyzed using FlowJo software (TreeStar, OR).

Supplementary Figure Legends

Supplementary Figure 1. Inverse correlation between high PKCε and phosphorylated (pT52)-ATF2 levels and reduced expression of IFNB1 and IFN-related genes in melanoma cells.

(**a**) Upper: Western blot analysis of the indicated proteins from melanoma cell lines, arranged from low to high PKCε and pT52-ATF2 levels (left to right). "ATF2" indicates total ATF2. Lower: Western blot analysis of the indicated proteins in WM793 cells that were transfected with empty vector (EV), caPKCε, or $ATF2^{T52E}$. (**b**) Left: Gene profiling of WM793 cells expressing ATF2-targeting shRNA and reconstituted with wild-type ATF2 ($ATF2^{WT}$), $ATF2^{T52A}$, or $ATF2^{T52E}$ for 48 h and then treated with DMSO or 10 μM ETO. Gene expression profiles were subjected to Ingenuity Pathway Analysis clustering and "Canonical Pathways" analysis. The 4 most significantly altered pathways affected upon expression of $ATF2^{T52E}$ and ETO treatment are shown. Right: Ingenuity Pathway Analysis network clustering of IFNB1, SP110, IRF9, IL9R, IFI44L, and ISGF3. (**c-e**) qRT-PCR analysis of the indicated gene transcripts in WM793 (**c**), LU1205 (**d**), or 501Mel (**e**) cells treated with DMSO or 10 μM etoposide (ETO). (**f**) qRT-PCR analysis of the type 1 IFN-1downstream targets IFIT2, ISG56, OAS2, and PKR in 501Mel (grey) and WM793 (green) cells. Light-to-dark shading indicates increasing times (0, 4, 8, and 20 h) after ETO treatment. (**g**) qRT-PCR analysis of IFNB1 expression levels in WM793 cells treated with the indicated concentrations of SBI-410 for 24 h. In (c-g), results represent the mean values ± SD of experiments performed in triplicate. (**h**) qPCR analysis for PKCε and IFNβ1 in LU1205 cells that were transfected with control siRNA (siCTL) or PKCε-targeted siRNA (siPKCε) that were incubated in the presence of DMSO or ETO.

Supplementary Figure 2. shRNA-mediated knockdown of IFNAR1 and IFNAR2, and caPKCε- or ATF2^{T52E}-mediated rescue of cell viability during genotoxic stress.

(**a**) qRT-PCR analysis of IFNAR1 and IFNAR2 in WM793 cells infected with PLKO empty vector (shEV) or lentiviruses coding for IFNAR1 (shA3 and shH3)- or IFNAR2 (shF3)-targeted shRNA for 24 h. Cells were selected on puromycin (1 μg/ml) for 7 days before analysis. Results represent the mean values ± SD of experiments performed in triplicate and are expressed relative to values in shEV-infected cells. (**b**) Cell cycle phase distribution of WM793 cells transfected with EV, caPKCε, or ATF2^{T52E} and incubated with DMSO or 10 μM etoposide (ETO) for 48 h. Cell cycle analysis was performed by flow cytometry. Results represent the mean values ± SD of experiments performed in triplicate, n = 10,000 cells per sample. (**c**) Quantification of cell cycle phase distribution of WM793 cells treated with 600 IU/ml human IFNβ1 (6 h pretreatment) ± 10 μM ETO. (**d**) Cell cycle analysis of WM793 cells performed at 144 h after treatment with 600 IU/ml human IFNβ1 (6 h pretreatment) ± 5 μM PLX4720. #P < 0.005. For (c,d), results represent the mean values ± SD of triplicates, with 10,000 cells per sample.

Supplementary Figure 3. Altered melanoma viability during lymphocyte co-culture.

The viability of GFP-expressing YUMM1.3 cells that were transfected with empty vector (EV) or vector encoding ATF2^{T34E} and either untreated or exposed to 5 or 10 Gy ionizing radiation (IR) and cultured for 24 h with or without lymphocytes (melanoma to lymphocyte ratio: 1:30) from the spleens of B6 mice that were previously inoculated subcutaneously with YUMM1.3 tumors (for 1 month). Anti-IFNβ1 (aIFNb1) was added at 10 μg/ml as indicated. Cell viability was measured as described in the Supplementary Methods section. The results represent the mean values ± SD of triplicates. $^{\#}P \leq 0.005$; $^{*}P$ = 0.0009; n.s. = not significant.

Supplementary Figure 4. Influence of IR-induced IFNβ1 expression on lymphocyte activation.

(**a-d**) FACS analysis of lymphocytes co-cultured with YUMM1.3 cells as described in Figure 5c. Cells were stained for CD4 (**a**), CD8 (**b**), NKp46 (**c**), and Gr-1 (**d**), as well as activation markers CD25 and CD69, at 24 h of co-culture with YUMM1.3 cells ± IR ± neutralizing anti-IFNb1 (aIFN) antibody as indicated. The results represent the mean values ± SD of experiments performed using lymphocytes harvested from 3 C57B6 donor mice that had been previously inoculated with 1 X 10^6 GFP- and empty vector-expressing YUMM1.3 cells at 1 month prior to the experiment, with N = 50,000 cells per sample.

Supplementary Figure 5. Representative tumor sections from a biochemotherapy responder and non-responder immunostained for IFNβ1 and ATF2 in the JWCI cohort

Immunofluorescence staining for ATF2 (red) and IFNβ1 (green) in representative melanoma tumor sections from a responder (**a**) and non-responder (**b**) patient from the JWCI cohort, prior to (Pre-Tx; upper) and after (Post-Tx; lower) biochemotherapy treatment as indicated in the text, is shown. Scale bars represent 100μm.

Supplementary Figure 6. Blinded scoring of melanoma patient tumor samples from JWCI and UPCC cohorts

(**a,b**) Immunofluorescent staining of ATF2 and IFNβ1 in pre- and post-treatment melanoma tumor sections from the indicated patients. The tumor sections were imaged (3-4 fields per tumor) and scored by 3 blinded scorers. The subcellular localization of ATF2 (nuclear vs. cytosolic on a scale of low (+) to high (++++) signal intensity) within each patient section, and the induction of IFNβ1 was scored as a comparison of each

pair of pre- and post-treatment patient sections. The scores were compiled and the consensus scores are shown. Total intensity changes scores were based on a blinded comparison between each pre- and post-treatment section. The 2nd-to right-most column indicates the clinical outcome/responsiveness (classified as Non-Responsive" (NR) or "responsive" (R) in the JWCI cohort and as "Complete Response" (CR), "Partial Response" (PR) or "No response" (NR) in the UPCC cohort) was correctly and blindly predicted based on ATF2 and IFNB1 status. The right-most column indicates the status of CD8+ T-cells in after treatment sections compared to before treatment sections as follows: + = increased; - = reduced; and ø = no change compare to before treatment. Yellow highlight indicates samples with correlation between CD8+ T-cells, ATF2, and IFNb1 status. (**c**) qRT-PCR analysis of IFNB1 transcripts in tumor samples from 8 non-treated and 15 treated, non-responder patients from the JWCI cohort. Shown is the dCq plot for the 23 patients, which indicates a 9-fold reduced IFNB1 expression in samples from treated, non-responder patients. $P = 0.032$.

Supplementary Figure 7. Expression of caPKCε and ATF2^{T52E} reduces the effects of IFNβ1 and/or etoposide treatment on melanoma cells

Flow cytometric analysis of WM793 (black) and 501Mel (red) cells transfected with empty vector (EV), ATF2^{T52E}, or caPKCε for 48 h and then treated with the indicated combinations of DMSO, IFNβ1 (6 h pretreatment with 600 IU/ml human IFNβ1), and 10 μM etoposide for an additional 48 h. The results represent the mean values ± SD of experiments performed in triplicate. *$P < 0.0005$; n.s. = not significant.

Supplementary Figure 8. Representative tumor sections from IFN-α2a–based therapy immunostained for IFNβ1 and ATF2.

Immunofluorescence staining for ATF2 (red) and IFNβ1 (green) in representative melanoma tumor sections from 1 of 5 non-responder patients from the UZH cohort, prior to (Pre-Tx; upper) and after (Post-Tx; lower) IFN-α2a treatment as indicated in the text, is shown. Scale bars represent 100μm. Table shows quantification of IFNβ1 expression and ATF2 subcellular localization status before and after treatment for each patient.

Supplementary Table 1. Top 100 differentially expressed genes in etoposide-treated WM793 depleted of endogenous ATF2 and reconstituted with ATF2^{T52E} (compared to ATF2^{T52A}).

The top 100 genes exhibiting significantly altered expression ($P < 0.05$) in 10 μM etoposide-treated (24 h) WM793 cells expressing ATF2^{T52E} (compared to ATF2^{T52A}) are shown. The genes shown are listed in ascending log2-fold change order, with *P* values shown in the right-most column. Yellow highlight denotes IFN-related hits. The expression profiles were generated using Illumina HumanHT-12 v4 BeadChip and RNA samples derived from cells prepared in 3 biological replicates. The expression data were normalized by quantile approach and linear analysis [32] using Illumina GenomeStudio.