Supplemental Experimental Procedures (Related to Experimental Procedures)

Cell Line Treatments

Colorectal cell lines HCT116 and HT29 were obtained from the American Type Tissue Collection and were cultured under recommended conditions. Ovarian cancer cell lines were obtained from the laboratory of Dr. Dennis Slamon and included A2780 Hey, Kuramochi, and TykNu. These cell lines were cultured according to ATCC recommended conditions.

Cell lines were treated with 500 nM of Aza, 100 nM of Dac, or 500 nM- 3 μM of carboplatin (Sigma; St. Louis, Missouri) for 72 hours while in log-growth phase, changing the media and drug every 24 hours for drug treatment. To select an appropriate chemotherapy control, the carboplatin dose that had a similar growth inhibitory effect to 500 nM Aza after 10 days was used to treat the cells (IC50s: A2780 (Aza = 848 nM, Carb = 457 nM), Hey (Aza = 4.1 μM, Carb = 12.2 μM), TykNu (Aza = 491 nM, Carb = 986.2 nM). 2 μM ruxolitinib (Invivogen #tlrl-rux), 0.625-5 U/mL of anti-IFNAR2 antibody (PBL Interferon Source #21385-1), 0.625-2.5 U/mL of anti-IFNB antibody (PBL Interferon Source #31400-1), or 1.25-5 U/mL of anti-IL10RB antibody (Abcam # ab89884) were added during DNMTi treatment. Cells were harvested at 1, 3, or 7 days following initial application of drug. DNA and RNA were obtained using standard protocols (Tsai et al., 2012) RNA from cell lines was sent for the Agilent 44K Expression Array (Li et al., 2014).

Bisulfite conversion

DNA bisulfite conversion was performed using a commercially available kit (EZ DNA Methylation Gold™ Kit, Zymo Research, Orange, CA).

DNA Methylation Analysis

Methylation-Specific PCR was performed as previously described (Herman et al., 1996) for the *IRF7* and *Fc2* genes. Primers are listed in Table S4. COBRA (Combined Bisulfite Restriction Analysis) was performed as previously described (Xiong and Laird, 1997) at the *Fc2* ERV located on chromosome 11. Primers are listed in Table S4. Bands and restriction fragments were resolved on 2% agarose gels, stained with ethidium bromide, and photoimaged with a UV camera.

RNAseq Expression Analysis of Tumors from Anti-CTLA-4- Treated Patients

Patients were described previously (Snyder et al., 2014) and samples were obtained with written informed consent per approved institutional review board (IRB) protocols. Expression data were obtained using RNASeq. RNA Sequencing was performed at New York Genome Center. Raw reads in the fastq format were aligned to Human Genome HG19 using RNA-seq STAR aligner (Dobin et al., 2013). Mapped reads for each sample were counted for each gene in annotation files in GTF format (gencode.v19.annotation.gtf available for download from GENECODE website (http://www.gencodegenes.org/releases/19.html)) using the FeatureCounts read summarization program(Liao et al., 2014). Individual count files were merged to the Matrix by an in-house R script. All expression data is deposited at GEO (accession number pending).

Statistical Analysis

qRT-PCR results are expressed as mean +/- SEM, with p values $\leq$ 0.05 considered statistically significant using the program IBM SPSS version 21. Different groups were compared by Mann-Whitney U test or Student *t*-test as appropriate, and 2-tailed p values are reported. Normalized, level 3 Agilent gene expression array data for high-grade serous ovarian cancer was downloaded from The Cancer Genome Atlas (TCGA) data portal (https://tcga-

data.nci.nih.gov/tcga/). A total of 489 ovarian tumors, which, in previous TCGA (Verhaak et al., 2013) have been classified into 4 subtypes (immunoreactive (IMR), proliferative (PRO), mesenchymal (MES), and differentiated (DIF)) by gene expression, were used for the analysis with our defined Viral Defense gene panel. All analyses were performed with the R statistical software suite and programing environment (www.**r**-project.org), using existing packages and customized routines. Consensus hierarchical clustering, as implemented in the ConsensusClusterPlus R-package (Wilkerson and Hayes, 2010) was used to identify stable clusters. Settings included Ward's method for innerLinkage and finalLinkage, Euclidean distance and 80% resampling. Solutions having anywhere from k=2 to 6 clusters were considered, and evaluated using consensus matrices to assess cluster stability, and cumulative distribution plots to measure the incremental value associated with adding additional clusters. Similar approaches were used to evaluate the gene expression pattern in other solid tumor types (Figure S7) and the anti-CTLA-4 treated metastatic melanoma dataset. Fisher exact *p* value test was used to test for association between clusters defined in this way using our Viral Defense panel and subtypes (IMR, PRO, MES, DIF) defined previously by TCGA ($p<0.05$ *; $p<0.01$ **; $p<0.001$ ***). Tumor surface measurements were imported in Graphpad Prism 6 and a two way ANNOVA was used to compare tumor growth between each of the group with anti-ctla4 monotherapy. Reported p values were adjusted using after Dunnett multiple comparison test (df=512).

For statistical analyses of primary ovarian tumors the fold ratio of ISGs of the low ERV tumor group and high ERV tumor (T) cohort was determined. Using the results from real time PCR for ERVs (molecules/ng RNA) and ISGs (2^{-DDcT}) a ratio in AU was calculated as: ISG 2^{-DDcT} $T_{(1\text{-}19)}$ x *ERV* molecules $T_{(1\text{-}19)}$ / mean *ERV* molecules of controls (n=9). 2^{-DDcT} $T_{(1\text{-}19)}$ equaled R, which was the relative expression ratio of each ISG gene normalized to the two reference genes TBP and GAPDH and the ISG expression of the control ovarian tissues (n=9). Subsequently, all

ISG/ERV ratios were transformed into a color code (blue to red) for each tumor and ISG for display and number code (0 to >10.1) for statistical calculations. Using the number code a hierarchical cluster analysis using the Ward-Method cluster method and Interval Euclidian distance was performed with a maximum of 6 clusters (k=6). Using the different cluster results from the low ERV and high ERV expression cohorts a Fisher's exact test was executed.

IFN response gene validations (qRT-PCR)

After total cellular RNA was extracted using the Trizol method (Life Technologies, Carlsbad, California), RNA concentration was determined using the Nanodrop machine and software (Thermo Fisher Scientific, Rockville, Maryland). 1 µg total RNA was used to generate cDNA with the QuantiTect Reverse Transcription Kit (Qiagen, Venlo, The Netherlands). Quantitative reverse transcription PCR (q-RT-PCR) of *DDX41, DDX58, IFI16, IFI27, IFI44, IFI44L, IFI6, IFNB1, IFNL1, IFNL2, IFNL3, IFNLR1, IL-1B, IL-10RB, IL-15, IRF7, MAGEB2, MAVS, MB21D1, MX1, OASL, STAT1*, and *TMEM173* mRNA was performed using TaqMan assays or Custom Taqman Gene Expression Array Cards (Life Technologies, Carlsbad, California) and the Applied Biosystems 7500 Fast real-time PCR system and software. *TBP* and *GAPDH* were used as reference genes. The ΔΔCT method was used to calculate relative expression levels. All qRT-PCR assays were carried out in triplicate and then repeated with new cDNA synthesis. Reverse transcriptase negative cDNA synthesis reactions were performed for at least one sample per plate.

Immunoblotting

Protein was isolated from cell lines and measured by BCA (Pierce Biotechnology. Protein extracts were subjected to polyacrylamide gel electrophoresis using the 4%–12% NuPAGE gel system (Invitrogen), transferred to PVDF (Millipore) membranes, and

immunoblotted using antibodies that specifically recognize ERV-3, MDA5, cleaved PARP, RIG-I, STING (TMEM173), Syncytin-1, and TLR3. β-actin (Sigma #5441, 1:5000) was used as a loading control. Antibodies used were as follows: polyclonal goat anti-ERV-3 (1:1000, Everest), mouse anti-β-Actin (1:5000, Sigma), goat anti-mouse IgG-HRP (sc-2005, 1:5,000; Santa Cruz Biotechnology, Inc.), rabbit anti-MDA5 (1:1000, Cell Signaling #5321), rabbit anti-PARP (#9542, 1:1000; Cell Signaling Technology, Inc.), rabbit anti-RIG-I (1:1000, Cell Signaling #4200), rabbit anti-STING (TMEM173) (ab92605, 1:1000, Abcam), Syncytin-1 monoclonal mouse antibody 14A5 against the SU (1:350) (kind gift from Dr. Herve Perron, Geneuro, Geneva Switzerland), rabbit anti-TLR3 (#6961, 1:1000, Cell Signaling Technology, Inc.), sheep anti-mouse IgG-HRP (1:5000, NA931V, GE Healthcare Life Sciences, Inc.), and donkey anti-rabbit IgG-HRP (1:1000, NA934V, GE Healthcare Life Sciences, Inc.). Band intensities of Tiff images were quantified using the program ImageJ (http://imagej.nih.gov).

Fractionation Experiments

Preparation of nuclear and cytoplasmic fractions of cultured cells was performed as previously described (O'Hagan et al., 2011). Cytoplasmic RNA was depleted of ribosomal RNA using the Ribominus kit (Invitrogen), according to the manufacturer's instructions. PolyA+ and PolyA- RNA were isolated using the Oligotex Direct mRNA Mini Kit (Invitrogen), according to the manufacturer's instructions. Nucleic acids were treated with 1 U/μg of RNase III (Ambion), 10 U/μg of RNaseH (Invitrogen) or 3 U/μg calf intestine alkaline phosphatase (New England Biolabs) according to the manufacturer's instructions. Subsequent to treatment, RNA concentration was measured with a Nanodrop, and 400 ng of each nucleic acid or 1 μg/mL PolyI:C (Invivogen) was transfected into HT29 recipient cells.

ELISA

IFNB ELISA was performed with the *Verikine-HS*™ Human Interferon Beta Serum ELISA kit (PBL Interferon Source) and IFNL ELISA was performed with the DuoSet ELISA for Human IL-29/IL28-B (IFNL 1/3) kit (R & D Systems).

Lentiviral transduction to create stable knockdowns

TLR3, MAVS, STING and GFP knockdowns were created in A2780, Hey, and TykNu cell lines. Virus production and infections were carried out according to established methods (Stewart et al., 2003).

The short hairpin sequences used were:

shTLR3A 5′-CCTTACACATACTCAACCT-3′

shTLR3D 5′-CCTCTTCGTAACTTGACCA-3′

shGFP 5′- GCAAGCTGACCCTGAAGTTCAT3-3′

shMAVS 5’ -GCATCTCTTCAATACCCTT-3’

shSTINGC 5’- GCATGGTCATATTACATCG-3’

shSTINGD 5’-GTCCAGGACTTGACATCTTAA-3’

ERV absolute quantitative real time PCR (qPCR).

PCR cDNA amplification was performed and fragments cloned into TopoTA vectors (Invitrogen) for all new ERV genes not previously analyzed as stated above (Strissel et al., 2012) (Table S2). qPCR analyses of all cloned *env* and other ERV genes with a known copy number was used as an external standard to generate a standard curve with a cycle threshold (CT) value against the log of amount of standard. Importantly, a similar PCR efficiency (over 97 %) between all *env* genes was needed for comparison. Similar standard curves of all *env* genes

were obtained for the SYBR-Green based qPCR with the following slopes and calculations (Strissel et al., 2012) and Table S2).

ERV *env* genes were amplified by qPCR from 40 ng of cell line cDNA with SYBR-green technology and analyzed with an ABI7300 (ABI, Darmstadt, Germany) according to Strissel et al. (Strissel et al., 2012). Expression values were calculated as molecules (mol) per ng total RNA using a standard curve of each cloned *env* gene determined by real time PCR and calculated as mean +/- standard deviation of the mean (S.E.M.) according to (Ruebner et al., 2013) (Ruebner et al., 2012). qPCR analyses of all cloned *env* and other ERV genes with a known copy number was used as an external standard to generate a standard curve with a cycle threshold (CT) value against the log of amount of standard. Importantly, a similar PCR efficiency (over 97 %) between all *env* genes was needed for comparison. Similar standard curves of all env genes were obtained for the SYBR-green based qPCR with the following slopes and calculations (Strissel et al., 2012) and Table S2).

Transfection and Overexpression of ERV-env vectors

For transfections (per 35mm dish and 2 ml media) 3μg of each vector in 100 μl 150 mM NaCl in a ratio with 6μl JetPEI in 100 μl 150 mM NaCl was used according to manufacturer's instructions (JetPEI, PeqLab). Syncytin-1 on 7q21.2 (NM_014590.3) was amplified from human control placenta (1617 bp) and cloned via Eco R1 into pcDNA3.1/myc-HisB (Invitrogen) with an N-terminal CMV-promoter and C-terminal myc and 6x-His epitopes. The ERV-W1 URE/ 5'LTR without the U5 region (URE-U3-R) (-613 to +133 nt) on chromosome 7q21.2 was amplified with PCR from human control placenta and cloned via Kpn1 and Hind III into pGL3-basic (Promega) (Ruebner et al. 2013). The new pGL3-LTR vector was incubated with Nco I and Xba 1 deleting the luciferase gene and Syncytin-1 (1617 bp) was cloned into the Nco I and Xba 1 sites of pGL3-basic without luciferase and sequenced (5' LTR-Syn-1-pGL3). The vector of ERV-W2 *env*

(phCMV-Xq22.3 env FL) was a kind gift from Dr. K. Ruprecht, Charite, University Medicine, Berlin and represents the env-W2 cDNA but only has a partial codogenic env due to a stop codon (Table S1) (Roebke et al., 2010). The p3Xflag-CMV-14 vector contains the ERV-3 *env* full length cDNA and was contributed by Dr. Neal Rote, Case Western University. The pEGFP-N1 vector was purchased (CloneTech Laboratories, Inc.). A pCMV-Tag vector (Stratagene) contains the human wild type ERα cDNA and is called HEGO-CMV-ERα and was a kind gift from Dr. R.X. Song, University of Virginia, USA. Following transfection of HEGO-CMV-ERα into an ER minus carcinoma cell line an ERα full length 66 KD protein was observed (Strissel et al Oncotarget, 2012).

ERV siRNA Knockdown Experiments

A2780, Hey and TykNu ovarian cancer cell lines were treated with 500 nM of Aza (Sigma; St. Louis, Missouri) for 72 hours while in log-growth phase, changing the media and drug every 24 hours. The next day (Day 4) following Aza treatment, the cells were transfected using Hyperfect reagent (Qiagen) according to manufacturer's instructions with siAlexa scrambled (80nM) (Allstars Neg siRNA AF488, Qiagen) to achieve transfection efficiency above 85% and also to represent a negative mock control. Cells were also transfected with two 21bp siRNAs targeting Syncytin-1 (each 40nM) (Ambion, Life Technologies, IDs26922; IDs26920) or two siRNAs targeting ERV-3 (40nM) (Ambion, Life Technologies, IDs4817; IDs4818) or one siRNA combined together from each gene (each 40nM). RNA was isolated at 72 hours post transfection to ensure gene knockdown and at Day 10 from initial Aza treatment to address the expression of genes in the interferon response.

First strand cDNA synthesis and strand specific PCR for detection of sense and antisense ERV transcripts using TASA-TD methodology

Components from the SuperScript III First-Strand Synthesis System for RT-PCR (Life technologies, Germany) were adapted to perform reverse transcription with RNA from ovarian cancer lines A2780, Hey, TykNu the colon cancer cell line DKO and its parental cell line HCT116. For *first strand cDNA synthesis* 50ng RNA was used for *ß-actin* with all cell lines; *Syncytin-1* (400ng for A2780, Hey, TykNu, HCT116 and 200ng for DKO) and env-Fc2, [A2780 (200ng), Hey and TykNu (400ng), HCT116 (200ng), DKO (100ng)]. 1µM of a gene specific primer ligated to a TAG-sequence not specific for the human genome (GSP sense/antisense (RT) TAG) was implemented in the reaction. RNA and primers were preheated at 65°C for 5min. For the total reaction: the GSP-TAG, 0.5mM dNTP, 5mM $MgCl_2$, 10mM DTT, 40U RNaseOUT, 100U SuperScriptIII® RT (life technologies, Germany) and 240ng Actinomycin D (Sigma, Germany) were added with the RNA for a 20µl reaction. Synthesis was performed at 50°C for 50 min and terminated at 85°C for 5 min. RT with extremely low intrinsic RNase H activity (for cleavage of RNA from RNA/DNA duplexes) and Actinomycin D was added to prevent second strand cDNA RT resulting in antisense artifacts (PMID 17897965). After cDNA synthesis 2U recombinant RNase H (life technologies) was added to each reaction and incubated 20 min at 37°C. Finally the first strand cDNA mix was ethanol precipitated and resuspended in 10 µl sterile water. Afterwards *gene and strand specific PCR* was performed. PCR reactions were implemented with the Fast-Start Taq-Polymerase Kit (Roche) as described above (PCR analysis). To amplify sense cDNA and antisense cDNA a TAG-primer and GSP sense (PCR) and a TAG-primer and GSP antisense (PCR) were used, respectively. We performed sense and antisense specific PCR using both sense and antisense cDNA of *ß-actin* as an internal negative control that was previously demonstrated to have no antisense transcript (PMID 15356298). All cDNA products were electrophoresed on 1% agarose gels, visualized using ethidium bromide and then Tif-images quantified using ImageJ (http://imagej.nih.gov).

Primary ovarian serous carcinomas. Following dissection of primary serous ovarian carcinoma tissues (n=19) and healthy control ovarian tissues (n=9) (performed by a pathologist at the Institute of Pathology, Erlangen), all tissues were flash frozen in liquid nitrogen and then stored at -80°C. Please note that Tumor 12 was not represented. Clinical data for the primary serous ovarian carcinomas include mean age (55.1 years) and tumor stage and grading (IIB/G2 n=1; IIC/G2 n=1; IIIC G1 n=1; IIIC/G2 n=3; IIIC/G3 n=8; IV/G2 n=1; IV/G3 n=4). Clinical data for healthy control ovarian tissues include mean age (53.7 years) where healthy tissues were dissected from ovaries with ovarian cysts (n=4), uterine leiomyoma (n=1); dermoid (n=1); and patient matched n=1 and non-patient matched normal ovarian tissues from ovarian carcinomas.

For experiments, 20-50mg of tissue was demembranated and the RNA isolated and cDNA synthesized according to (Strissel et al., 2012). All cDNAs were quantified for 22 ERV env genes to determine molecules / ng RNA (identical env genes as in Figure 5 but not including gag-W5, env-MER34, ERV-FXA34 and ERV9-1).

B16-F10 Melanoma Mouse Model

All mouse procedures were performed in accordance with the institutional protocol guidelines at the Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY). C57BL/6J mice (6-8-week-old females) were obtained from The Jackson Laboratory. C57BL/6J mice were subcutaneously injected with $1x10^5$ B16-F10 tumor cells. On days 4, 8, 11, 14, 18, the mice were treated intraperitoneally with anti-ctla-4 (100 µg in 100 µL). For Aza treatment, mice received two cycles of intraperitoneal injection of 0.1 to 0.75 mg/kg Aza in PBS for 5 consecutive days followed by 7 days off treatment, starting at day 8. Control groups received a corresponding dose of non-specific isotype antibody control and PBS intraperitoneally. Mice with no palpable tumors on day 8 were eliminated from our study and tumor surface was measured with a caliper using the ellipse surface formula (Length*Width*π)/400. Tumor surface

measurements were imported in Graphpad Prism 6 and a two way ANNOVA was used to compare tumor growth between each of the group with anti-ctla4 monotherapy. Reported p values were adjusted using after Dunnett multiple comparison test (df=512).

Table S1. Analyzed codogenic and partially codogenic ERVs: 28 *env*, 1 *gag*, 2 *pol*s. Italics and parenthesis indicated ERV genes with only partial ORF. Related to Experimental Procedures.

	ERV	HUGO	gag/pol/env	chromosome	accession	coding/ ORF (aa)
1	E4-1		env-E	19q12	AB062274.1	428
2	F(c)2	ERVFC1-1	env-Fc2	7q36.2	AC016222	528
3	*F(c)2*		*(env)*	*2p11*	*AC104395.4*	*(229)*
4	*F(c)2*		*(env)*	*7q11*	*AC073349.11*	*(391)*
5	*F(c)2*		*(env)*	*11p15*	*AC019088*	*(252)*
6	*F(c)2*		*(env)*	*Yq11*	*AC007241*	*(125)*
7	F(c)1		env-Fc1	Xq21.33	AL354685	584
8	FRD	ERVFRD-1	Syncytin-2	6p24.1	AL136139	538
9	H1		env-H1 (p62)	2q24.3	AJ289709	585
10	H2		env-H2 (p60)	3q26	AJ289710	563
11	H3		env-H3 (p59)	2q24.1	AJ289711	555
12	K1 HERV-K74261	ERVK-21	env-K1	12q14.1	AC074261.3	698
13	K2 HML-2.HOM / K108	ERVK-6	env-K2	7p22.1	AC072054	699
14	K3 HERV-K17833 C19	ERVK-19	env-K3	19q12	Y17833	694
15	K4 HML-2 (K109)	ERVK-9	env-K4	6q14.1	AF164615	698
16	K5 HERV-K113	ERVK-22	env-K5	19p13.11	AY037928.1	699
17	K6 HERV-K115	ERVK-8	env-K6	8p23.1	AY037929.1	699
18	K7 HERV-K102	ERVK-7	env-K7	1q22	AF164610.1	588
19	MER34	ERVMER34-1	env-MER34	4q12	336594227	563
20	P(b)		Syncytin-3	14q32.12	ABB52637.1	665
21	R	ERV3-1	erv-3	7q11.21	AC073210	604
22	R(b)	ERVPABLB-1	env-Rb	3p24.3	AC093488	514
23	T	ERVS71-1	env-T	19p13.11	AC078899	626
24	V1	ERVV-1	env-V1	19q13.41	NM_152473	477
25	V2	ERVV-2	env-V2	19q13.41	NM_001191055	535
26	W	ERVW-1	Syncytin-1	7q21.2	AC000064	538
27	ERVW-5	ERVW-5	gag–W5 (p30)	3q26.32	AF156961	363
28	*Fb1/ C21orf105*	*ERVH48-1*	*(env-Fb1)*	*21q22.3*	*AP001629*	*(179)*
29	*ERVF(XA34)*		*(pol)*	*7p31.1*	*U29659.1*	*(125)*
30	*ERV9-1*	*ERV9-1*	*(pol)*	*11q13*	*X57147*	*(129)*
31	*W2*	*ERVW-2*	*(envW2)*	*Xq22.3*	*FN689795*	*(38)*

Table S2. Primers used to amplify ERVs and ERV absolute quantification. Related to Experimental Procedures.

gene	ERV	chromosome	Acc. number	Cloning primer (5'-3')	real time PCR primer (5'-3')
env	ERVMER34-1	4q12	336594227	TF: GACACAGACAATCCACCTCTGTATTGC BR: CACTGACAAGGAGCTGTGCTGATTGCTG	TF: GAATTCAGTGCCACTAAGCAGAC BR: TCGGTATATCCAAGACATGATCC
env	ERV-Fb1 (ERVH48-1) (C21orf105)	21q22.3	BC005107.1	TF: ATGGCCTGTATCTACCCAACCACTTTCTA BR: TTATAGTTTTTGTATAAAGGAATGGAAATG	TF: ATATCCCTCACCACGATCCTAATA BR: CCCTCTGTAGTGCAAAGACTGATA
pol	ERV9-1	11q13	38332	TF: ATGGATCCCTGGATACAGCGAGACAGC BR: TTACATCATAAGTAGTCTAGAAGTGAG	TF: TCTTGGAGTCCTCACTCAAACTC BR: ACTGCTGCAACTACCCTTAAACA
pol	ERV-F (XA34)	7q31.1	U29659.1	TF: TAGCCCTCGGTGTCTTAGGACAACAG BR: TCAGTGGAAGAATGAGTAGGAAGGGAAG	TF: CAGGAAACTAACTTTCAGCCAGA BR: TAAAGAGGGCATGGAGTAATTGA

	γ	t	R^2
ERVMER34-1	-3.2876	32.3023	0.9967
ERV-Fb1	-3.5287	34.418	0.9926
ERV9-1	-3.3507	33.579	0.9866
ERV-F (XA34)	-3.4066	34.2405	0.9957

Table S3. Primers used for TASA-TD method. Related to Experimental Procedures.

primer	5'-3'
Fc2-tag-TF	GCACACGACGACAGACGACGCACTAGATGGAGGACTATATGAGCACA
Fc2-tag-BR	GCACACGACGACAGACGACGCACGAGGCTAAGGAGATGCCTAGTACT
ERVFc2all-TF	TAGATGGAGGACTATATGAGCACA
ERVFc2all-BR	GAGGCTAAGGAGATGCCTAGTACT
Syn1-tag-TF	GCACACGACGACAGACGACGCACATGGAGCCCAAGATGCAGTCCAAGA
Syn1-tag-BR	GCACACGACGACAGACGACGCACACTGCTTCCTGCTGAATTGGGGCGTA
Syn1-TF	ATGGAGCCCAAGATGCAG
Syn1-full BR	CTAACTGCTTCCTGCTGAATTGGGGCGTAG
hbAct-tag-TF	GCACACGACGACAGACGACGCACGCTCGTCGTCGACAACGGCTCCGGCAT
hbAct-tag-BR	GCACACGACGACAGACGACGCACCAAACATGATCTGGGTCATCTTCTC
hbAct-TF	GCTCGTCGTCGACAACGGCTCCGGCA
hbAct-BR	CAAACATGATCTGGGTCATCTTCTC
TAG-TF	GCACACGACGACAGACGACGCAC

Table S4. Primers used for MSP and COBRA. Related to Experimental Procedures.

gene	Primers
IRF7	MF: CGTAGGTCGGATTTTGCG MR: CGAAATTCCGCGAAACTAAA UMF: AGATGGGAAAGGTGATGTTA UMR: CACAAAACTAAAAACCAAACAA
Fc2 MSP	MF: CGGGCGAGGTTTATTGGTTCG MR: CGCYTATTCCCWTTTATTCCCG UMF: TGGGTGAGGTTTATTGGTTTGTAGG UMR: CACYTATTCCCWCCATTTATTCCCA
Fc2 COBRA	F: GTATAATGGGGAAAAATGGAA R: ACTTCCCTAACCCTCCC

Table S5. Anti-CTLA-4 melanoma trial clinical data. Related to Figure 7.

Study ID	Age	Gender	Primary Melanoma type	M stage	Priors	Therapy (n mg/kg x no. doses)[b]	Response duration (weeks)	LDH (normal <256)	BRAF/NRAS status	OS (yr)	Biopsy
SD7357	50	F	unknown	M1c	2	3 x4	21	234	BRAF P367L	2	post
NR4631	59	M	cutaneous	M1c	1	3x4	13	341	WT BRAF, NRAS	0.5	post
NR5784	58	F	cutaneous	M1c	0	10 x4	11	266	WT BRAF, NRAS	0.5	pre
SD1494[a]	70	M	cutaneous	M1c	0	3 x4	51	324	BRAF V600E	2	pre
SD5038	55	M	cutaneous	M1c	2	10 x9	24	133	NRAS Q61L	1.2	post
CR6126[a]	66	F	cutaneous	M1b	1	3x4	101	211	NRAS Q61K	1.9	pre
SD2051	61	F	acral	M1c	1	3 x5 +vemurafenib	16	439	BRAF V600E	0.8	pre
CR1509[a]	54	F	cutaneous	M1c	1	10 x14	72	181	BRAF V600E	4.5	post
NR4810	48	F	cutaneous	M1c	1	3 x4	12	330	BRAF V600E	0.4	post
SD0346	43	F	cutaneous	M1b	0	10 x3 +dacarbazinex6	68	210	NRAS Q61K	2.9	post
CR7623[a]	62	M	cutaneous	M1c	3	10 x17	47	357	WT BRAF, NRAS	4	post
NR9705	63	F	acral	M1c	0	3x4	29	167	BRAF V600E	1.2	post
SD5118	55	F	acral	M1c	2	10 x4 ('09), 3 x3 ('12)	24	183	WT BRAF, NRAS	2.7	post
NR3549	50	M	cutaneous	M1c	1	10 x3	16	309	NRAS Q61K	0.5	pre
NR9521	74	M	cutaneous	M1b	0	3x4	12	169	NRAS Q61L	2.7	post
SD2056[a]	39	F	cutaneous	M1b	1	10 x17	208	195	NRAS Q61K	3.9	pre
SD6336[a]	53	M	cutaneous	M1c	1	10 x34	361	N/A	BRAF V600E	6.9	post
CR9699[a]	70	M	cutaneous	M1c	0	10 x19	52	191	BRAF V600K	4.4	post
LSD0167[a]	33	M	cutaneous	M1c	0	3x3	140	156	BRAF V600E	2.7	pre
SD6494	63	F	unknown	M1c	2	3 x2	13	322	WT BRAF, NRAS	0.7	post
NR9449	64	F	cutaneous	M1c	0	3x4	10	202	NRAS Q61R	0.6	post

[a]Alive at time of censure.

[b]Ipilimumab unless otherwise noted.

N/A : Not available

Table S6. RNA-Seq data from patients on Anti-CTLA-4 melanoma trial. Related to Figure 7.

Study ID	SD7357	NR4631	NR5784	SD1494	SD5038	CR6126	SD2051	CR1509	NR4810	SD0346	CR7623	NR9705	SD5118	NR3549	NR9521	SD2056	CR6336	CR9699	LSD0167	SD6494	NR9449
IFI6	0.02	-0.80	-0.69	1.53	-0.57	-0.29	-0.71	0.90	-0.56	-0.77	-0.59	-0.82	3.09	1.28	-0.80	0.57	0.16	-0.16	0.25	-0.30	-0.73
ISG15	-0.60	-0.83	-0.52	1.47	-0.04	0.02	-0.60	0.98	-0.69	-0.79	-0.45	-0.83	2.57	0.50	-0.80	1.58	-0.60	-0.20	1.31	-0.77	-0.70
OASL	-0.23	-0.76	-0.44	0.79	-0.54	1.08	-0.67	1.37	-0.68	-0.76	-0.54	-0.76	3.05	-0.49	-0.61	0.58	-0.50	0.06	1.27	-0.64	-0.58
DDX58	-0.35	-0.46	-0.18	-0.04	0.20	0.40	-0.44	0.61	-0.34	-0.55	-0.38	-0.55	4.11	-0.36	-0.43	-0.15	-0.45	-0.20	0.39	-0.50	-0.33
IFIH1	0.20	-0.78	-0.42	0.88	-0.64	0.53	-0.49	1.59	-0.68	-0.73	-0.68	-0.74	3.30	-0.43	-0.57	0.27	-0.49	0.36	0.65	-0.65	-0.47
IFIT2	-0.03	-0.85	-0.44	0.79	-0.48	0.26	-0.48	1.13	-0.65	-0.82	-0.52	-0.87	3.45	-0.62	-0.59	0.69	-0.55	0.80	0.33	0.08	-0.63
IFIT1	-0.48	-0.66	-0.44	0.17	-0.10	1.70	-0.59	0.09	-0.57	-0.61	-0.33	-0.70	3.55	-0.12	-0.67	0.35	-0.62	0.42	0.57	-0.34	-0.62
OAS2	-0.15	-0.86	-0.27	1.05	-0.27	0.55	-0.63	1.74	-0.66	-0.82	-0.61	-0.85	2.97	-0.41	-0.77	0.84	-0.42	0.24	0.72	-0.75	-0.65
STAT1	0.62	-0.95	-0.80	1.81	-0.59	-0.01	-0.33	2.83	-0.60	-0.97	-0.40	-0.86	0.40	-0.51	-0.55	1.58	-0.06	0.50	0.03	-0.62	-0.52
IFI44	-0.37	-0.72	-0.44	0.28	1.47	0.70	-0.50	-0.28	-0.59	-0.69	-0.54	-0.68	3.32	-0.16	-0.54	0.06	-0.39	-0.24	1.50	-0.58	-0.60
OAS1	-0.14	-0.72	-0.33	0.71	-0.54	0.65	-0.60	0.88	-0.56	-0.68	-0.51	-0.71	3.54	-0.40	-0.66	0.62	-0.46	0.00	1.04	-0.54	-0.58
IRF7	-0.20	-0.81	0.16	1.58	-0.12	-0.26	-0.70	0.67	-0.49	-0.84	-0.60	-0.89	3.12	-0.34	-0.71	1.03	-0.63	0.29	1.00	-0.61	-0.64
IFITM1	-0.18	-0.96	-0.29	1.06	-0.09	-0.32	-0.57	2.04	-0.75	-0.92	-0.54	-0.89	1.85	-0.44	-0.90	1.07	0.65	0.10	1.81	-0.95	-0.78
IFITM3	0.02	-1.12	-0.35	2.31	-0.09	-0.39	-0.15	1.17	-0.15	-0.94	-0.41	-1.25	1.51	-0.21	-1.30	1.82	0.54	-0.22	0.53	-1.04	-0.30
IRF9	0.06	-1.05	1.14	1.94	-0.74	-1.26	-0.69	0.53	-1.13	-1.03	-0.60	-0.88	0.96	-0.36	-0.68	0.75	-0.51	0.70	2.05	0.64	0.15
MX2	-0.38	-0.57	-0.15	-0.05	-0.35	0.48	-0.45	1.07	-0.45	-0.55	0.37	-0.54	3.91	-0.34	-0.55	-0.22	-0.44	-0.32	0.55	-0.48	-0.53
ISG20	-0.11	-1.13	1.93	2.22	0.86	-0.80	-0.53	0.16	-0.98	-0.82	0.00	-1.19	0.55	0.03	-0.93	1.29	-0.67	0.57	0.89	-0.78	-0.56
IFI44L	-0.41	-0.71	-0.47	0.95	-0.08	0.27	-0.57	0.07	-0.64	-0.65	-0.49	-0.72	3.21	-0.18	-0.63	0.29	-0.55	0.28	2.10	-0.56	-0.51
MX1	-0.47	-0.70	-0.35	0.17	-0.49	0.34	-0.52	0.96	-0.48	-0.68	-0.54	-0.70	3.52	-0.08	-0.66	0.59	-0.50	0.05	1.49	-0.52	-0.44
IFI27	-0.34	-0.84	-0.64	1.63	-0.66	1.20	-0.60	1.47	-0.81	-0.77	-0.61	-0.83	0.98	-0.71	-0.80	2.03	0.15	-0.23	1.54	-0.76	-0.39

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