Materials and Methods

Cell lines, media, and reagents. Human A549 cells (ATCC® CCL-185™), validated by STR analysis at the University of Pennsylvania, were cultured in RPMI 1640 medium (Gibco, ThermoFisher Scientific), supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. HME cells, purchased from Clonetech and authenticated by STR analysis at Genetica DNA Labs (compared to ATCC CRL-4010) were cultured with mammary epithelial cell growth medium (MEGM, Lonza CC-3150). Human recombinant IFN-β (200–400 X 10^6 international units (IU)/mg; InterPharmLaboratories, was a gift from Ares-Serono, Rockland, MA).

Chemical Inhibitors. Valoneic acid dilactone (VAL), an RNase L inhibitor was provided by M. Talukar and F. Sicheri, Toronto. SP600125 and ZVAD-FMK were purchased from Santa Cruz Biotechnology.

Construction of gene knockout cells. The A549 RNase L KO, MAVS KO, ADAR1-MAVS DKO, ADAR1-RNase L DKO, and ADAR1-RNase L-MAVS TKO cells were described previously (1). The PDE12 KO, AKAP7 KO, PDE12-AKAP7 DKO A549 cells were generated by using the CRISPR Cas9 system. The sgRNA sequences were chosen from a published database (2). The guide RNA sequences were synthesized as DNA oligonucleotides by Integrated DNA Technologies. Primers were annealed, phosphorylated and ligated into the vector LentiCRISPR v2 (Addgene 52961, a gift from Feng Zhang) (3) that was prepared by digestion with BsmBI. The resulting plasmids were transformed into Stbl3 chemically competent E. *coli* (Invitrogen) and grown on a bacterial culture plate at 37°C. Colonies were screened by PCR using the U6 primer and gene specific reverse primer to generate an approximately 300 bp product. Positive clones were cultured and plasmid DNA was prepared and sequence verified using the U6 primer. The AKAP7 sgRNA sequences are: sgAKAP7_2 FW CACCG TGAGC GACTG GCCAA AGCAA and sgAKAP7_2 REV AAACT TGCTT TGGCC AGTCG CTCA C. The PDE12 sgRNA sequences are: sgPDE12_10 FW CACCG GGATG CCTGG CAAGA CGGCG and sgPDE12_10 REV AAACC GCCGT CTTGC CAGGC ATCCC. Cell cloning was done by limited dilution. To obtain PDE12 and AKAP7 DKO cells, a PDE12 knockout A549 cell line was infected with pseudo lentivirus expressing sgRNA for AKAP7, followed by single cell cloning. Resulting clones were screened for knockout of expression by the Western method. Single OAS1,2,3 KO HME cell lines were reported by us previously (1), and RNase L KO HME cells and HME DKO and TKO cell lines were made by CRISPR Cas9 gene editing as described (1).

Immunoblotting. Prior to lysis, cells were washed twice in cold phosphate-buffered saline (PBS). Cell extracts were prepared with RIPA lysis buffer, supplemented with phosphatase/protease inhibitors, followed by incubation on ice for 20 min. Lysates were subjected to centrifugation at 12,000 x *g* for 10 min, the supernatant solutions were collected, and the protein was quantified by the

Bradford assay (Bio-Rad Laboratories). Cell lysates (30-50 µg) were separated on 4-15% SDS PAGE gels (Bio-Rad Laboratories) and proteins were transferred to polyvinylidene difluoride membranes (0.45 µm) (Bio-Rad) and probed with antibodies according to the manufacturer's recommendations. Antibodies were against MAVS(Cat#3993S) (1:1000 dilution) and ADAR1(Cat#14175) 1:2000 dilution) (from Cell Signaling Technology), cleaved PARP1(Cat#9541S) (1:1000 dilution) (Cell Signaling Technology), Flag M2(1:5000 dilution) and ß-actin(1:50,000 dilution) (Sigma-Aldrich). A monoclonal antibody against human RNase L was previously described by us (4). OAS1 (Cat# sc-374656), OAS2 (Cat#sc-99097) and OAS3 (Cat# sc-49870) were from Santa Cruz Biotechnology. Anti-PDE12 (Abcam ab87738)(1:1000 dilution) and anti-AKAP7 (Proteintech: Cat# 12591-1-AP)(1:5000 dilution) were used to screen for knockouts of the respective genes.

Cell death assay. Cells at 2 x 10^4 per well were seeded in 24-well plates (about 30-40% confluent) or 8 x 10^3 cells per well in 96-well plates (about 10% confluent). The cells were incubated with 250 nM Sytox-Green dye (Thermo Fisher), a nucleic acid stain that is an indicator of dead cells and that does not penetrate live cells, and 250 nM of cell-permeable dye SytoTM 60-Red (ThermoFisher), which allows quantification of the total number of cells present in each field, using an IncuCyte Live-Cell Imaging System and software (Essen Instruments 2015A) for up to 100 h. Cell death was measured by counting the number of green objects per well (dead cells) which was then normalized to the total number of cells per well (red objects) at each time point, using IncuCyte software (version 2016B).

Caspase-3/7 assays. Cells were seeded in 96-well plates and incubated without or with AZA. The cells were incubated with IncuCyte™ Kinetic Caspase-3/7 Apoptosis Assay Reagent (green) according to the manufacturer's protocol for 24 h. The apoptotic index was determined by determining the total count [green Integrated Intensity (GCU x µm²/Image)] and then normalizing by count confluence (percent) using IncuCyte software. To inhibit apoptosis, cells were treated with ZVAD-FMK (50 µM).

IR. IR was generated with a J.L. Shepherd & Associates Mark 1 gamma irradiator containing Cesium-137.

Immunofluorescence. WT A549 cells were treated with 50 µM AZA for 48 h. Cells were fixed with 4% formaldehyde diluted in PBS for 15 min, washed three times with PBS and incubated in blocking buffer according to the manufacturer's protocol. J2 antibody against dsRNA (Cat#10010200) was purchased from Scicons. Cells were incubated with primary antibody solution (J2-antibody) overnight, washed three times with PBS, and incubated with 1:500 anti-mouse-Alexa 488-conjugated antibody for 2 h in the dark. After washing with PBS, coverslips containing cells were mounted on glass slides with Vectashield

(Vector Labs) mounting medium containing DAPI and observed with a confocal microscope.

Statistics. All p values are calculated by two-way ANOVA using Graphpad Prism 7 software.

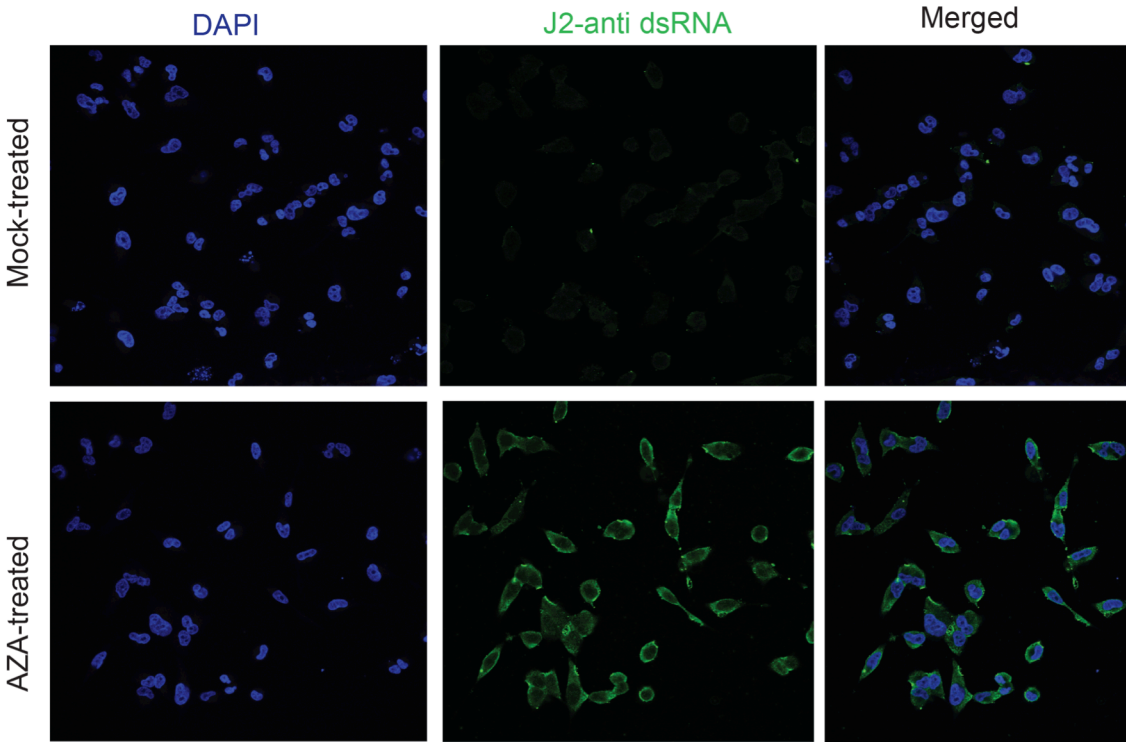
References

1. Li Y, et al. (2016) Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. Proc Natl Acad Sci U S A 113(8):2241-2246.
2. Wang T, Wei JJ, Sabatini DM, & Lander ES (2014) Genetic screens in human cells using the CRISPR-Cas9 system. Science 343(6166):80-84.
3. Sanjana NE, Shalem O, & Zhang F (2014) Improved vectors and genome-wide libraries for CRISPR screening. Nat Methods 11(8):783-784.
4. Dong B & Silverman RH (1995) 2-5A-dependent RNase molecules dimerize during activation by 2-5A. J Biol Chem 270(8):4133-4137.

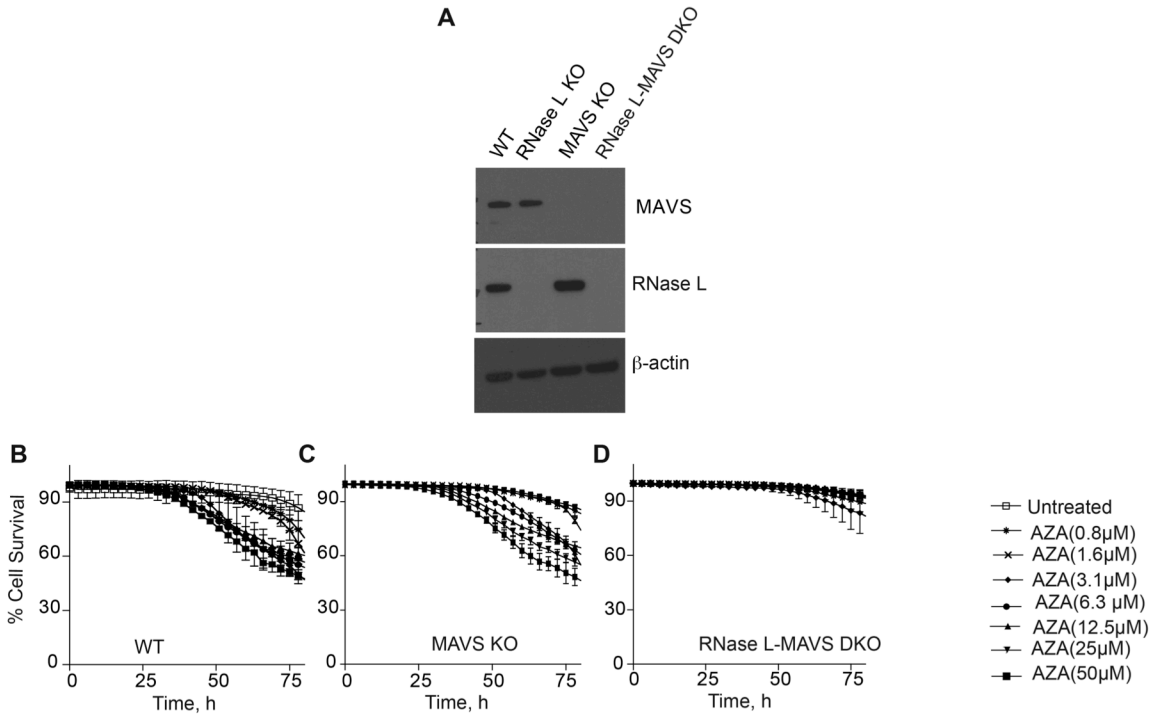
Supplementary Table and Figures

		Doubling Time in Hours	Concentration of 5-azacytidine in micromolar that produced 50% growth inhibition (GI50) 6 days after drug addition	Concentration of 5-azacytidine in micromolar that produced 50% growth inhibition (GI50) 2 days after drug addition	202869_at	205552_s_at	204972_at	206553_at	218400_at	205660_at	210797_s_at	221287_at
Cell Line name	Tissue of Origin	DoublingTime	AZAD6GI50	AZAD2GI50	OAS1	OAS1	OAS2	OAS2	OAS3	OASL	OASL	RNASEL
786-0	Renal	22.4	2.333	0.843	43.6758	19.1630	32.0406	55.3976	157.5713	76.4857	91.2794	49.0253
A498	Renal	66.8	0.767	0.495	93.8828	39.8232	55.6167	70.6082	192.7644	98.3476	138.5505	58.4399
A549-ATCC	Non-SmallCellLung	22.9	0.855	0.809	61.7392	32.9642	43.7841	65.2759	186.5021	92.5647	96.6684	61.5451
ACHN	Renal	27.5	0.432	0.330	80.8688	45.2552	35.1639	58.0664	179.3593	83.0399	83.5327	42.6075
BT-549	Breast	53.9	1.496	1.045	167.6166	68.7818	203.1080	67.6396	362.1468	170.9669	137.6839	53.0213
CAKI-1	Renal	39	0.328	0.160	79.8681	42.5268	38.4233	63.0776	138.7671	70.5838	83.0887	55.3320
CCRF-CEM	Leukemia	26.7	0.150	1.770	39.5388	16.9522	54.3774	53.4527	158.7543	79.6331	92.0419	52.5794
COLO205	Colon	23.8	0.480	0.984	101.8381	77.6481	43.4367	49.4525	146.1431	162.3093	144.4513	46.6940
DU-145	Prostate	32.3	0.269	0.439	46.1356	15.6871	54.9057	60.6120	130.3253	139.6674	148.7033	57.4615
EKVX	Non-SmallCellLung	43.6	5.534	12.882	49.2333	16.9929	38.6795	61.9920	89.1258	91.6338	97.0788	60.7116
HCC2998	Colon	31.5	0.166	0.794	155.7843	109.2238	59.6710	61.4221	278.6660	131.9111	154.6830	48.1707
HCT116	Colon	17.4	0.091	0.207	46.7242	15.3064	38.2690	69.4703	186.5327	79.4418	102.7016	48.3502
HCT15	Colon	20.6	0.769	0.895	51.0628	21.8358	35.5539	64.8979	226.3822	87.7371	93.0638	51.1069
HL60TB	Leukemia	28.6	0.143	0.977	43.3924	19.1049	51.3433	62.2425	109.3605	88.0255	80.1004	60.4428
HOP62	Non-SmallCellLung	39	0.597	0.778	38.2375	17.6884	29.6196	44.0413	128.9150	73.3838	100.2656	41.0922
HOP92	Non-SmallCellLung	79.5	1.663	2.188	61.0527	19.1575	41.9741	58.6128	85.5269	167.7624	171.9063	46.5118
HS578T	Breast	53.8	3.357	0.867	40.2068	13.3480	44.2591	47.8071	58.1390	71.8563	65.8764	41.0058
HT29	Colon	19.5	0.467	1.009	1130.4880	720.9533	1104.0295	157.7500	2236.2194	1874.2777	1459.3589	49.6788
IGR-OV1	Ovarian	31	4.121	1.086	52.5101	13.2038	44.6464	60.6720	130.3813	76.7994	72.6045	46.9203
K-562	Leukemia	19.6	1.318	0.735	33.5386	17.2045	27.9704	54.5065	59.8553	76.7118	64.8823	66.9518
KM12	Colon	23.7	0.231	0.682	63.8182	24.9029	39.6473	75.2488	112.3102	92.5879	90.1813	49.1554
LOXIMVI	Melanoma	20.5	0.298	0.332	80.9905	51.7858	93.3317	66.3598	498.2607	242.8088	305.9449	46.8337
M14	Melanoma	26.3	0.589	0.995	93.8427	41.1541	101.5400	71.3410	162.9447	72.6571	86.6093	58.8878
MALME-3M	Melanoma	46.2	1.469	1.535	58.8408	25.2440	45.8736	59.0767	144.1392	77.8014	88.5521	54.3057
MCF7	Breast	25.4	0.973	0.938	42.4842	14.8713	31.4686	51.7962	102.3772	70.6072	95.6264	72.7501
MDA-MB-231ATCC	Breast	41.9	1.346	0.740	39.2286	12.8553	50.0165	59.2021	90.2902	114.6388	98.4318	46.2983
MDA-MB-435	Melanoma	25.8	0.394	0.845	57.0606	24.3420	41.5946	45.6883	119.9891	72.1755	76.3419	52.2644
MDA-N	Breast	.	0.597	1.318	48.0801	22.2773	45.7313	44.9390	117.7364	67.4782	63.6743	52.7835
MOLT-4	Leukemia	27.9	0.628	1.282	45.0691	19.1164	69.8990	61.3662	163.5123	93.7216	95.5189	47.7984
NCI-ADR-RES	Ovarian	34	1.799	1.486	52.0259	14.0989	37.7534	62.7428	75.0604	96.0960	77.9482	56.1674
NCI-H226	Non-SmallCellLung	61	1.005	0.989	169.9985	116.5058	236.0440	80.1622	465.7077	165.0802	181.3203	49.0396
NCI-H23	Non-SmallCellLung	33.4	2.312	1.466	43.9772	15.8063	37.7292	50.6113	118.5568	73.6474	86.9957	44.8791
NCI-H322M	Non-SmallCellLung	35.3	1.014	0.721	34.6050	23.2847	37.6607	48.8560	238.9161	118.4318	106.6283	53.8479
NCI-H460	Non-SmallCellLung	17.8	0.145	0.119	370.3529	179.4326	34.9043	58.0099	193.6110	100.5879	123.0612	55.5602
NCI-H522	Non-SmallCellLung	38.2	0.528	0.735	41.9126	17.0849	34.0360	55.0026	70.5034	76.9140	94.5013	45.5438
OVCAR-3	Ovarian	34.7	0.713	1.552	43.2042	18.2200	43.6180	54.7115	90.5336	72.9210	81.3225	53.2258
OVCAR-4	Ovarian	41.4	0.374	1.225	53.6065	21.6913	39.9797	56.4850	79.1060	93.8003	103.1607	52.2159
OVCAR-5	Ovarian	48.8	1.581	3.097	79.5931	39.9161	37.4344	54.1149	116.9728	97.3222	108.3312	52.8407
OVCAR-8	Ovarian	26.1	0.655	0.652	49.8588	16.6586	42.8373	58.4513	124.6652	70.7955	88.6929	41.6358
PC-3	Prostate	27.1	1.202	1.274	75.0021	36.6865	54.1058	69.5544	141.2624	104.3382	107.5540	50.6757
RPMI-8226	Leukemia	33.5	0.412	0.293	576.4989	305.4177	556.5008	123.7441	149.6787	238.3692	299.7045	54.2761
RXF393	Renal	62.9	0.087	0.589	367.5921	199.6693	359.1374	73.9115	798.7205	128.6042	145.0199	51.1729
SF-268	CNS	33.1	2.443	1.596	33.7752	16.5541	37.2038	52.0369	63.3659	56.3093	72.7847	43.8847
SF-295	CNS	29.5	1.742	1.340	50.9867	25.1900	46.7346	53.0522	158.5851	105.4042	100.1552	42.5057
SF-539	CNS	35.4	0.845	0.863	42.5600	19.6393	52.8222	62.2370	186.3340	82.7320	96.0364	50.7660
SK-MEL-2	Melanoma	45.5	0.252	0.365	64.0637	30.4586	30.8890	58.2007	131.5448	114.1995	111.0072	58.3750
SK-MEL-28	Melanoma	35.1	1.117	1.750	75.7969	38.8835	63.4331	54.2337	146.3269	56.2565	71.0512	39.2151
SK-MEL-5	Melanoma	25.2	0.811	1.746	47.8044	17.8693	34.3153	65.6810	143.2204	79.5222	88.2697	53.4159
SK-OV-3	Ovarian	48.7	7.962	2.286	59.4772	19.3159	42.8259	75.5662	159.2933	99.6584	107.8684	61.7914
SN12C	Renal	29.5	1.205	1.076	54.7160	26.5410	42.6995	64.1910	157.7641	78.9070	84.7199	53.9929
SNB-19	CNS	34.6	1.791	3.251	41.2184	14.5585	40.8056	45.8864	103.6093	60.8089	88.1028	56.6488
SNB-75	CNS	62.8	2.018	0.603	48.9287	17.8724	46.3070	52.2597	199.9273	83.6961	95.6304	54.4263
SR	Leukemia	28.7	0.568	2.198	77.7801	45.7879	373.0916	95.1388	176.0238	165.1172	135.8657	56.4681
SW-620	Colon	20.4	0.659	1.247	62.5654	29.7102	66.6692	52.2913	142.0973	66.1477	83.0848	48.1659
T-47D	Breast	45.5	2.612	0.887	52.1371	17.1170	59.5715	61.8434	215.9101	78.5393	90.5066	57.0151
TK-10	Renal	51.3	1.675	0.753	62.0289	21.5804	43.9632	64.1952	91.0889	236.0648	258.3013	55.0055
U251	CNS	23.8	0.815	1.153	36.9377	15.2685	33.4074	51.8664	126.5839	98.3026	108.2353	51.1680
UACC-257	Melanoma	38.5	3.597	1.687	42.1751	31.7835	40.1491	57.5907	142.0158	87.1711	91.4526	49.0189
UACC-62	Melanoma	31.3	0.259	0.764	47.5814	30.3795	37.9355	58.6312	107.0415	78.0237	98.7107	50.8944
UO-31	Renal	41.7	6.067	0.671	59.5781	38.4206	54.7387	53.4154	176.2299	80.5702	74.2686	51.0996

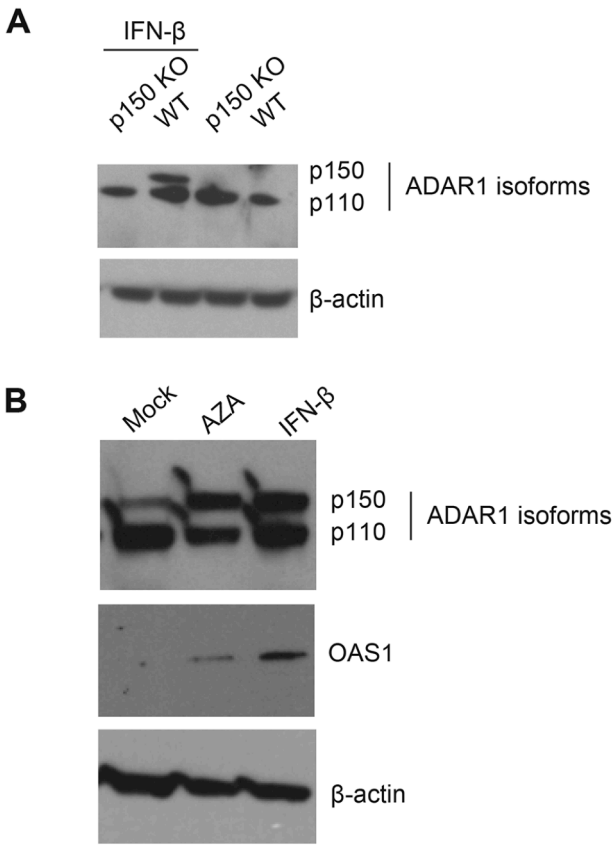
Supplementary Table 1. Correlation between sensitivity to 5-azacytidine and expression levels of OAS family members. Drug sensitivity to 5-azacytidine is represented as GI50, the drug concentration resulting in a 50% growth reduction quantified by measurement of total protein at day 6 (raw data downloaded from NCI DTP - http://dtp.nci.nih.gov)(higher GI50 = less sensitive to drug). GI50 was correlated with expression of OAS1 and OASL in the cell lines (gene expression values by microarray from Geo Database GSE5846).



Supplementary Fig. 1. Visualization of self dsRNA induced by AZA treatment. WT A549 cells were incubated without (mock) or with 50 μM AZA for 48 h. The intracellular dsRNA was detected by IFA with J2 monoclonal antibody against dsRNA (Green). The nuclei were stained with DAPI (blue). The experiment was reproduced in three biological replicates.



Supplementary Fig. 2. MAVS has a minimal effect on cell survival after AZA treatment. (***A***) Western blots of WT, RNase L KO, MAVS KO and RNase L-MAVS DKO A549 cells. (***B-D***) Percent cell survival after AZA treatment. The data are the averages ± SD of four identical replicates. Three biological replicates were performed, each with a minimum of three technical replicates.



Supplementary Fig. 3. Induction of ADAR1 p150 and OAS1 by IFN-β or AZA. (A) Western blots of ADAR1 from WT and p150 KO A549 cells that were either untreated or treated with IFN-β (200 IU/ml for 18 h). Upper panel shows p110 and p150 isoforms of ADAR1, lower panel shows β-actin as loading control. **(B)** Western analyses of WT A549 cells untreated (mock) or treated with 50 μM AZA (for 48 h) or 10 IU per ml of IFN-β (for 48 h). The blots were probed with antibodies against ADAR1, OAS1 and β-actin antibody.