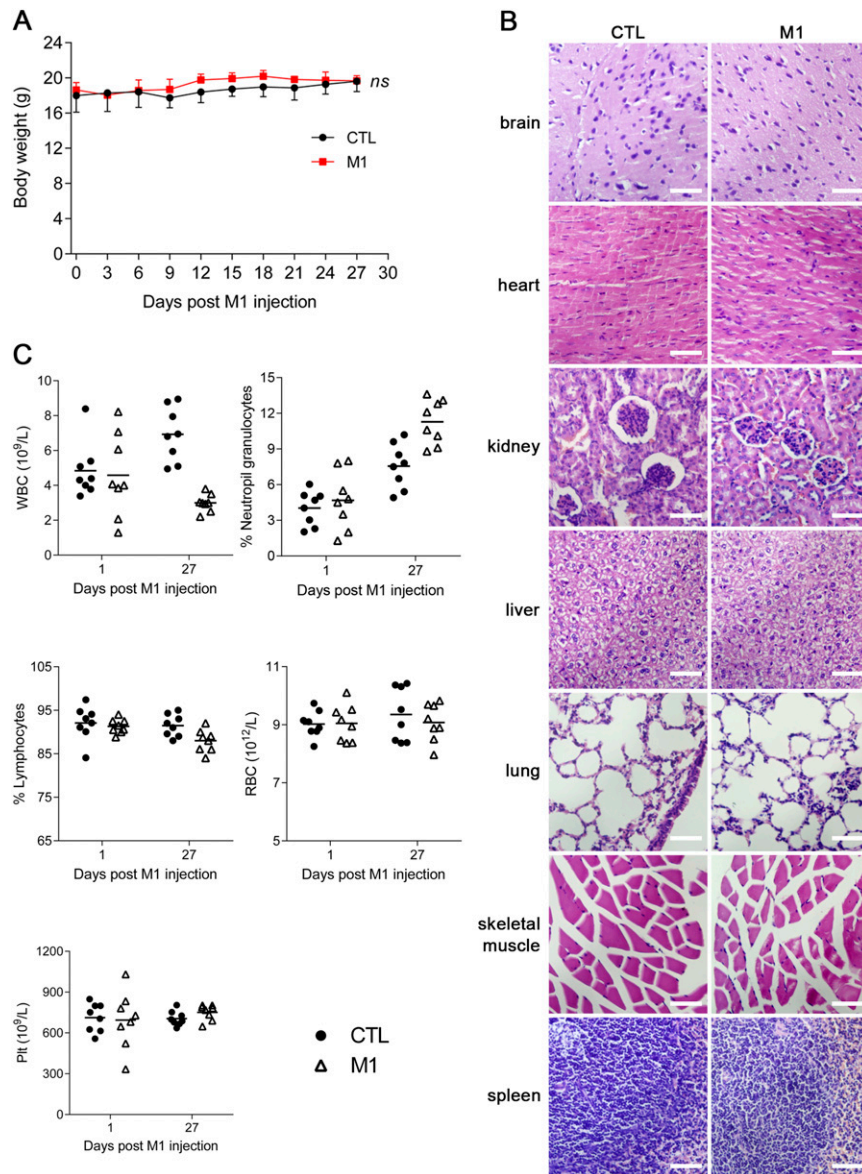
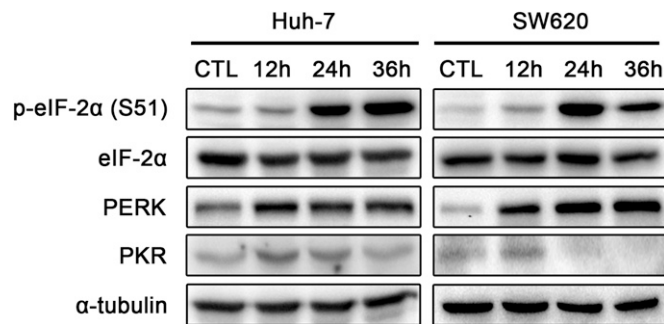


# Supporting Information

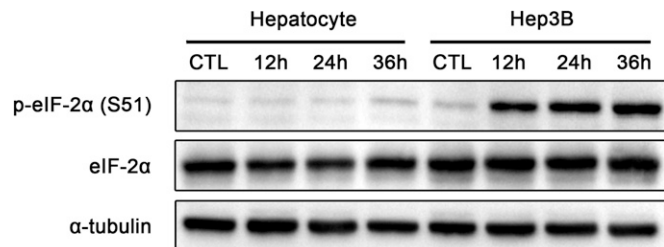
Lin et al. 10.1073/pnas.1408759111



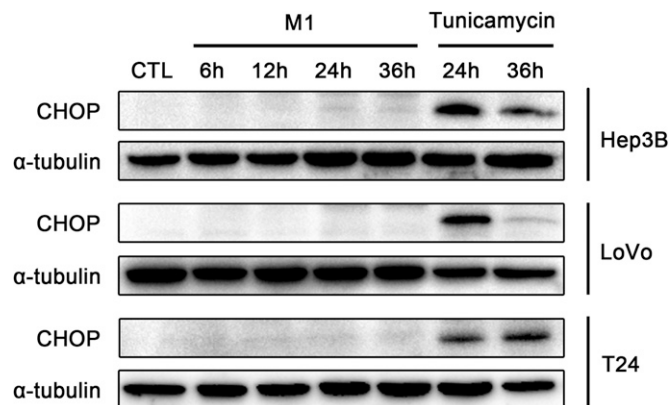
**Fig. S1.** Safety and potential toxicity evaluation of M1 in immunocompetent mice. Immunocompetent BALB/c mice received two doses of i.v. infusion of either M1 ( $3 \times 10^7$  pfu per dose) or vehicle. (A) Body weight was measured every 3 d. Means  $\pm$  SDs are shown.  $n = 8$  per group. (B) H&E staining of vital tissues. (Scale bars: 50  $\mu$ m.) (C) CBC analysis.  $n = 8$  per group. CTL, control; ns, not significant; Plt, platelet.



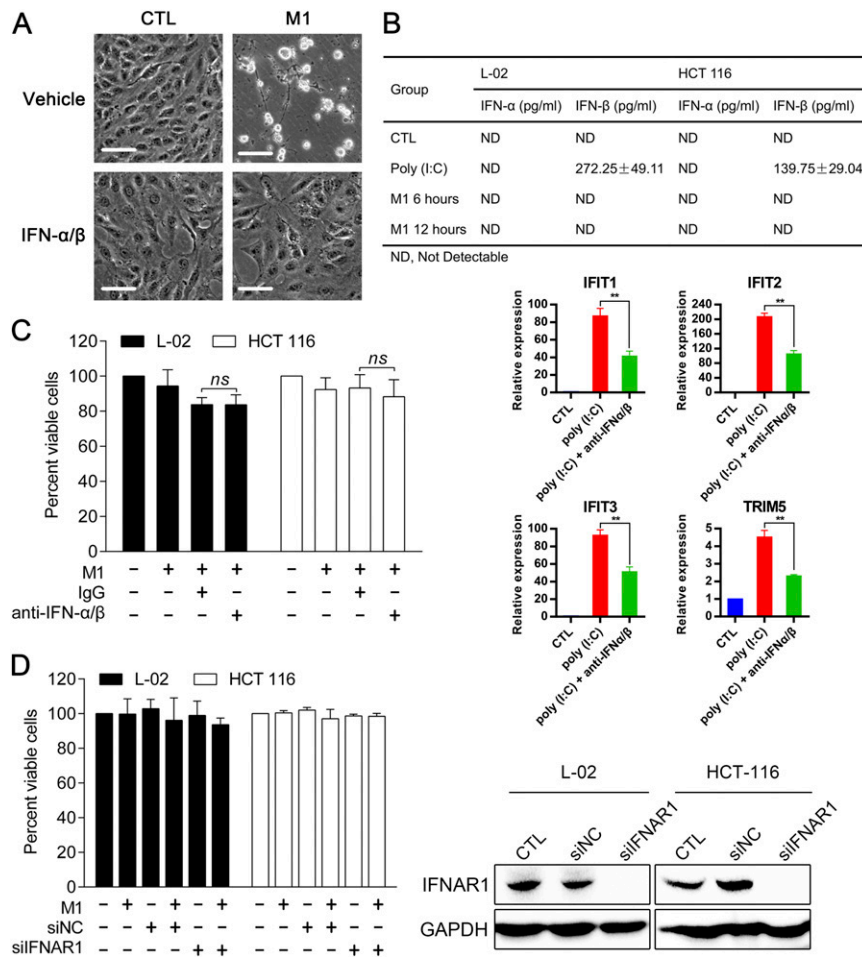
**Fig. S2.** Protein kinase R-like ER kinase (PERK), but not protein kinase R (PKR), is significantly induced by M1 infection. Sensitive cancer cells were treated with M1 (multiplicity of infection = 1) for 12, 24, or 36 h. Protein levels of eIF-2 $\alpha$  (eukaryotic translation initiation factor 2, subunit alpha) phosphorylation, PERK, and PKR were determined by Western blot, and  $\alpha$ -tubulin served as a loading control. CTL, control.



**Fig. S3.** Phosphorylation of eIF-2 $\alpha$  is slightly induced by M1 infection in primary human hepatocytes. Primary human hepatocytes and Hep3B cells were infected with M1 (multiplicity of infection = 10), and phosphorylation of eIF-2 $\alpha$  was determined by Western blot. Hep3B cells were used as a positive control of M1-induced phosphorylation of eIF-2 $\alpha$ . CTL, control.



**Fig. S4.** CCAAT-enhancer-binding protein homologous protein (CHOP) is not induced by M1 infection. Sensitive cancer cells were treated with M1 (multiplicity of infection = 1) or tunicamycin (0.5  $\mu$ g/mL; positive control) for 6–36 h. Protein level of CHOP was determined by Western blot, and  $\alpha$ -tubulin served as a loading control. CTL, control.



**Fig. S5.** M1 infection does not induce type I IFNs, and inhibition of type I IFN signal does not lead to M1 susceptibility in resistant cells. (A) T24 cells were pretreated with or without IFN- $\alpha/\beta$  (combination of 10,000 U/mL IFN- $\alpha$  and 30 ng/mL IFN- $\beta$ ) for 6 h before M1 infection [multiplicity of infection (MOI) = 1, 48 h]. Phase-contrast microscopy images were captured after infection. (Scale bars: 100  $\mu$ m.) (B) L-02 and HCT 116 cells were stimulated with poly(I:C) (5 mg/mL; positive control) or infected with M1 (MOI = 10). Supernatants were collected, and secreted IFN- $\alpha$  or IFN- $\beta$  was detected by ELISA. ND, not detectable. (C, Left) Cell viability in resistant cells infected with M1 (MOI = 10, 48 h) after 2 h of pretreatment with IFN- $\alpha$  and IFN- $\beta$  antibodies (800 ng/mL each). (C, Right) mRNA expression of IFN-stimulated genes.  $**P < 0.01$ . (D, Left) Resistant cells were transfected with a nontargeting siRNA or an IFNAR1-specific siRNA for 48 h before M1 infection. After 48 h of infection (MOI = 10), cell viability assays were performed. (D, Right) Detection of silencing efficiency. (C and D) Means  $\pm$  SDs from three independent experiments are shown. CTL, control; IFIT, interferon-induced protein with tetratricopeptide repeats; ns, not significant; siIFNAR1, IFN- $\alpha$  receptor subunit 1 siRNA; siNC, negative control siRNA; TRIM, tripartite motif-containing protein.

**Table S1. Human cell line screening for M1 anticancer efficacy**

Cell line	Disease	Viability (%)	Cell line	Disease	Viability (%)
Hep3B	Hepatocellular carcinoma	3.8	Ca Ski	Cervix epidermoid carcinoma	87.0
Huh-7	Hepatocellular carcinoma	52.2	SiHa	Cervix squamous cell carcinoma	99.6
Huh-6	Hepatoblastoma	59.0	ME-180	Cervix epidermoid carcinoma	103.2
SK-HEP-1	Liver adenocarcinoma	71.2	22Rv1	Prostate carcinoma	39.1
Li-7	Hepatoma	72.1	PC-3	Prostate adenocarcinoma	72.7
PLC	Hepatoma	80.5	DU 145	Prostate carcinoma	91.4
HepG2	Hepatocellular carcinoma	88.6	A-375	Skin malignant melanoma	47.3
Bel-7402	Hepatocellular carcinoma	99.6	M14	Melanoma	92.1
L-02	Normal liver	100.3	MV3	Melanoma	114.0
LoVo	Colorectal adenocarcinoma	6.9	U-87 MG	Glioblastoma	32.4
HCT-8	Colorectal adenocarcinoma	35.2	U-251	Glioblastoma	34.7
SW620	Colorectal adenocarcinoma	43.7	T98G	Glioblastoma multiforme	38.2
SW480	Colorectal adenocarcinoma	53.8	U-138 MG	Glioblastoma	40.1
HCT 116	Colorectal carcinoma	81.3	MGR2	Glioma	63.2
SCaBER	Bladder squamous cell carcinoma	11.5	DBTRG-05MG	Glioblastoma	83.3
T24	Bladder transitional cell carcinoma	21.1	LN-229	Glioblastoma	98.6
UM-UC-3	Bladder transitional cell carcinoma	39.8	SF-767	Glioblastoma	98.6
5637	Bladder carcinoma	50.2	A-172	Glioblastoma	98.7
SW780	Bladder transitional cell carcinoma	88.0	HEB	Glia	98.8
EJ	Bladder transitional cell carcinoma	95.3	Capan-1	Pancreas adenocarcinoma	40.4
J82	Bladder transitional cell carcinoma	96.8	PANC-1	Pancreas epithelioid carcinoma	49.3
SV-HUC-1	Immortalized uroepithelial	97.2	SW1990	Pancreas adenocarcinoma	45.6
A549	Lung carcinoma	68.2	MIA PaCa-2	Pancreas carcinoma	49.1
NCI-H358	Nonsmall cell lung cancer	87.8	BxPC-3	Pancreas adenocarcinoma	98.8
H1299	Nonsmall cell lung cancer	96.3	NCI-N87	Gastric carcinoma	76.4
NCI-H460	Large cell lung cancer	99.5	HGC-27	Gastric carcinoma	79.2
HCC827	Lung adenocarcinoma	103.0	AGS	Gastric adenocarcinoma	92.3
MRC-5	Lung fibroblast	76.1	CNE-2	Nasopharyngeal carcinoma	24.5
MDA-MB-468	Breast adenocarcinoma	43.7	CNE-1	Nasopharyngeal carcinoma	48.2
MDA-MB-231	Breast adenocarcinoma	58.9	HNE-1	Nasopharyngeal carcinoma	76.5
T-47D	Mammary ductal carcinoma	86.5	HL-60	Acute promyelocytic leukemia	85.2
SK-BR-3	Breast adenocarcinoma	89.6	K-562	Chronic myelogenous leukemia	95.1
MCF7	Breast adenocarcinoma	95.2	CCRF-CEM	Acute lymphoblastic leukemia	110.3
C-33 A	Cervix carcinoma	14.8	HEL	Erythroleukemia	112.0
HeLa	Cervix adenocarcinoma	66.0	Reh	Acute lymphocytic leukemia	113.1

Different cells were treated with M1 (multiplicity of infection = 10) for 48 h, and cell viability was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

**Table S2. Cancer specificity of M1 in primary human cells**

Name	Tissue	Source	Time (hpi)	Viability (%)				
				0.01	0.1	1	10	100
HAEC	Human aortic endothelial cell	ScienCell*	96	101.4	99.9	95.3	98.5	96.7
HSkMC	Human skeletal muscle cell	ScienCell	96	103.4	101.2	99.3	95.7	95.5
HCM	Human cardiac myocyte	ScienCell	96	104.9	95.7	94.0	89.6	88.5
HBdSMC	Human bladder smooth muscle cell	ScienCell	96	102.7	116.4	115.4	112.1	113.6
HRGEC	Human renal glomerular endothelial cell	ScienCell	96	101.8	101.4	110.2	104.8	108.1
HH	Human hepatocyte	ScienCell	96	103.5	107.6	104.3	91.7	103.7
HCEpiC	Human colorectal epithelial cell	Isolated	96	101.9	109.6	109.0	104.6	104.1
HCRC1	Human colorectal carcinoma cell	Isolated	72	93.3	106.6	100.6	87.2	67.0
HCC1	Human hepatocellular carcinoma cell	Isolated	72	77.8	44.2	34.7	27.5	23.1
HCC2	Human hepatocellular carcinoma cell	Isolated	72	101.2	85.8	89.6	73.6	66.4
HCC3	Human hepatocellular carcinoma cell	Isolated	72	99.8	100.6	87.9	79.5	68.6

Primary human cells were treated with M1 (MOI = 0.01~100), and cell viability was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. hpi, hours postinfection.

\*ScienCell Research Laboratories.

**Table S3. Inclusion criteria for IFN-related genes**

Database or reference	Inclusion criteria
Interferome ( <a href="http://interferome.its.monash.edu.au/interferome/">http://interferome.its.monash.edu.au/interferome/</a> ) ISG Database ( <a href="http://lerner.ccf.org/labs/williams/">http://lerner.ccf.org/labs/williams/</a> )	ISGs reported in two or more of these three sets of ISGs were included in our study (type I IFN only)
1	
2	Genes reported in TLR-dependent and -independent pathways of type I IFN induction were included

ISG, IFN-stimulated gene; TLR, toll-like receptor.

- Schoggins JW, et al. (2011) A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472(7344):481–485.
- Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN (2007) TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. *Nat Med* 13(5):543–551.

**Table S4. Expression of IFN-related genes in L-02 and Hep3B cells**

Gene symbol	Fold change in L-02/Hep3B cells
GBP3	71.39
CHMP5	53.12
SCARB2	49.01
IFI16	48.52
ODC1	45.88
SEPHS2	45.06
EPAS1	42.49
GNAI1	42.34
ZC3HAV1	40.40
PNPT1	39.24
MAFF	39.17
EIF2AK2	37.96
ADAR	37.94
SLC1A1	37.87
CYP1B1	37.72
GCH1	35.75
FAM46A	35.11
HIST1H2AC	34.63
CRY1	33.73
STAT3	33.71
TBK1	29.20
TICAM2	28.77
RECQL	26.16
MICB	25.66
PMAIP1	25.03
OPTN	23.80
IMPA2	21.74
CLEC2B	21.50
UCP2	20.80
ADM	20.42
DYNLT1	19.94
IFIT3	19.87
LIPA	19.55
PDGFA	19.41
STAT1	18.69
NMI	17.72
UBE2N	16.98
TRIM5	16.76
NAMPT	16.52
SAMHD1	16.49
DDIT4	16.48
NET1	15.26
VEGFC	15.04
BST2	14.73
HLA-A	14.06
IFITM2	13.97





**Table S4. Cont.**

Gene symbol	Fold change in L-02/Hep3B cells
IRF9	1.07
UBE2L6	1.05
TRIM21	1.00
MT1X	1.00
CCL2	0.98
PLAUR	0.98
OAS3	0.97
PSMB10	0.96
MAX	0.95
TRAF6	0.94
TYK2	0.94
BTG1	0.92
IRF2	0.91
IRF3	0.90
PLSCR1	0.88
IFI44	0.87
FUT4	0.87
BAK1	0.85
ARNTL	0.83
DDX3X	0.83
PRIC285	0.83
RNF213	0.83
SAMD4A	0.82
IL6	0.81
MX2	0.80
LMO2	0.78
IFITM1	0.78
UBE2V1	0.78
SECTM1	0.78
JAK2	0.76
GEM	0.76
TLK2	0.75
RNF19B	0.75
OGFR	0.75
CD47	0.74
MX1	0.74
CXCL11	0.74
TLR7	0.71
TREX1	0.71
MT1M	0.70
TNFSF10	0.69
TRADD	0.68
IRF1	0.68
PPM1K	0.66
IL1B	0.65
HESX1	0.64
IKBKB	0.64
PDGFRL	0.63
TICAM1	0.63
MAPK1	0.63
CD58	0.63
TYMP	0.62
PIM1	0.61
CCNA1	0.60
STARD5	0.60
RNASEL	0.60
MAP3K14	0.60
IRF7	0.57
LY6E	0.56
TRIM14	0.56
MT1H	0.56
N4BP1	0.55



**Table S4. Cont.**

Gene symbol	Fold change in L-02/Hep3B cells
ISG20	0.54
TIRAP	0.54
DUSP5	0.54
TNFSF13B	0.54
THBS1	0.53
FAM46C	0.53
CDK18	0.53
APOL6	0.52
GBP4	0.51
CCL8	0.51
IRAK1	0.51
SERPINE1	0.51
CD69	0.50
TMEM140	0.49
VEGFA	0.48
HLA-F	0.48
BRF2	0.48
PML	0.48
IKBKE	0.47
CCR1	0.47
GMPR	0.45
CD163	0.44
OAS2	0.44
IFI27	0.44
SERPINB3	0.43
AIM2	0.42
SERPING1	0.42
IL15RA	0.42
C4BPA	0.42
FUT2	0.41
HSH2D	0.41
ATF3	0.40
RELA	0.39
NAPA	0.39
AXL	0.39
TLR8	0.38
APOL3	0.37
SHMT2	0.37
CD274	0.37
HBEGF	0.36
DHX58	0.36
C10orf10	0.36
PLEKHA4	0.36
SPATS2L	0.36
HLA-G	0.35
RTP4	0.35
FZD5	0.34
MAZ	0.34
BCL2L14	0.34
EGR1	0.34
APOL1	0.34
EHD4	0.33
CD38	0.33
CX3CL1	0.32
MAPK3	0.31
EPST11	0.31
SLC15A3	0.31
ADAM11	0.31
CXCL10	0.31
CDKN1A	0.30
LGALS9	0.29
SERPINB9	0.29

**Table S4. Cont.**

Gene symbol	Fold change in L-02/Hep3B cells
MYC	0.29
IRF5	0.29
RARRES3	0.28
UBA7	0.28
XAF1	0.28
CCL5	0.27
IL1RN	0.27
RSAD2	0.27
CD74	0.27
ETV7	0.26
BATF2	0.25
P2RY6	0.25
TAB1	0.25
CXCL9	0.24
TNF	0.24
SAT1	0.23
IDO1	0.22
JUN	0.21
INHBA	0.20
TAGAP	0.19
SOCS1	0.19
VAMP5	0.18
FST	0.16

**Table S5. RNAi screening of IFN-related genes**

Gene	(siX + M1)/siX	z Score	Gene	(siX + M1)/siX	z score
ZC3HAV1	0.71	-0.40	DDIT4	1.20	0.01
IFIT2	0.87	-0.26	IFIH1	1.21	0.02
IFI6	0.90	-0.24	IFI16	1.22	0.03
RNF114	0.91	-0.23	TLR3	1.23	0.04
GBP3	0.94	-0.20	NC	1.23	0.04
IL8	0.94	-0.20	IFIT3	1.24	0.04
UBE2N	0.97	-0.18	RBCK1	1.25	0.05
ADAR	0.98	-0.17	ISG15	1.26	0.07
IFIT1	1.01	-0.15	WARS	1.26	0.07
MAVS	1.01	-0.15	NT	1.26	0.07
MYD88	1.01	-0.14	USP4	1.27	0.07
PSME1	1.01	-0.14	NMI	1.28	0.08
IFIT5	1.02	-0.14	TRAF3	1.31	0.11
STAT1	1.02	-0.14	TRIM25	1.34	0.13
JAK1	1.03	-0.13	IFI30	1.34	0.13
STAT2	1.03	-0.13	TAP1	1.34	0.13
EIF2AK2	1.05	-0.11	BST2	1.35	0.14
PNPT1	1.06	-0.11	OAS1	1.37	0.16
TAB2	1.06	-0.10	TBK1	1.39	0.17
IER3	1.07	-0.10	TRIM5	1.40	0.18
PSMB8	1.08	-0.09	TRIM22	1.40	0.19
PMAIP1	1.09	-0.08	NAMPT	1.43	0.21
PRKRA	1.09	-0.08	SAMHD1	1.43	0.21
PARP12	1.14	-0.04	GBP1	1.49	0.26
IFITM3	1.15	-0.03	TICAM2	1.50	0.26
TAP2	1.15	-0.03	IFITM2	1.55	0.31
ATF2	1.15	-0.03	BTN3A3	1.94	0.64
DDX60	1.17	-0.01			

Data are ranked by relative cell count (siX + M1/siX). z Scores are included to show distribution. NC, scramble siRNA; NT, nontransfected.