Supplementary Table S1. The sequences of primers for RT-PCR.

Target mRNA	Primer	Sequence
Human PML	Forward	GGATGAAGTGCTACGCCTCG
	Reverse	TCCCCTGGGTGATGCAAGA
Murine Pml	Forward	TGAACCAGTTTCCGTGCAGAA
	Reverse	TGAAATTCCTCCTGTATGGCTTG
Human TRAIL	Forward	TGCGTGCTGATCGTGATCTTC
	Reverse	GCTCGTTGGTAAAGTACACGTA
Murine Trail	Forward	ATGGTGATTTGCATAGTGCTCC
	Reverse	GCAAGC AGGGTCTGTTCAAGA
Human OAS1	Forward	AGTTGACTGGCGGCTATAAAC
	Reverse	GTGCTTGACTAGGCGGATGAG
Murine Oas1	Forward	GGGCCTCTAAAGGGGTCAAG
	Reverse	TCAAACTTCACTCCACAACGTC
Human RIG-I	Forward	CTGGACCCTACCTACATCCTG
	Reverse	GGCATCCAAAAAGCCACGG
Murine RIG-I	Forward	CAGATCCGAGACACTAAAGGGA
	Reverse	TCCTCATCAGCCTTGCTTTCA
Human OCT3/4	Forward	CTTGAATCCCGAATGGAAAGGG
	Reverse	GTGTATATCCCAGGGTGATCCTC
Murine Oct3/4	Forward	CACCATCTGTCGCTTCGAGG
	Reverse	AGGGTCTCCGATTTGCATATCT
Human NANOG	Forward	AAGGTCCCGGTCAAGAAACAG
	Reverse	CTTCTGCGTCACACCATTGC
Murine Nanog	Forward	AGGACAGGTTTCAGAAGCAGA
	Reverse	CCATTGCTAGTCTTCAACCACTG
Human SOX-2	Forward	TGGACAGTTACGCGCACAT
	Reverse	CGAGTAGGACATGCTGTAGGT
Murine Sox-2	Forward	GCGGAGTGGAAACTTTTGTCC

	Reverse	CGGGAAGCGTGTACTTATCCTT
Human STAT3	Forward	CAGCAGCTTGACACACGGTA
	Reverse	AAACACCAAAGTGGCATGTGA
Human β-actin	Forward	CATGTACGTTGCTATCCAGGC
	Reverse	CTCCTTAAT GTCACGCACGAT
Murine β-actin,	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT

Fig S1

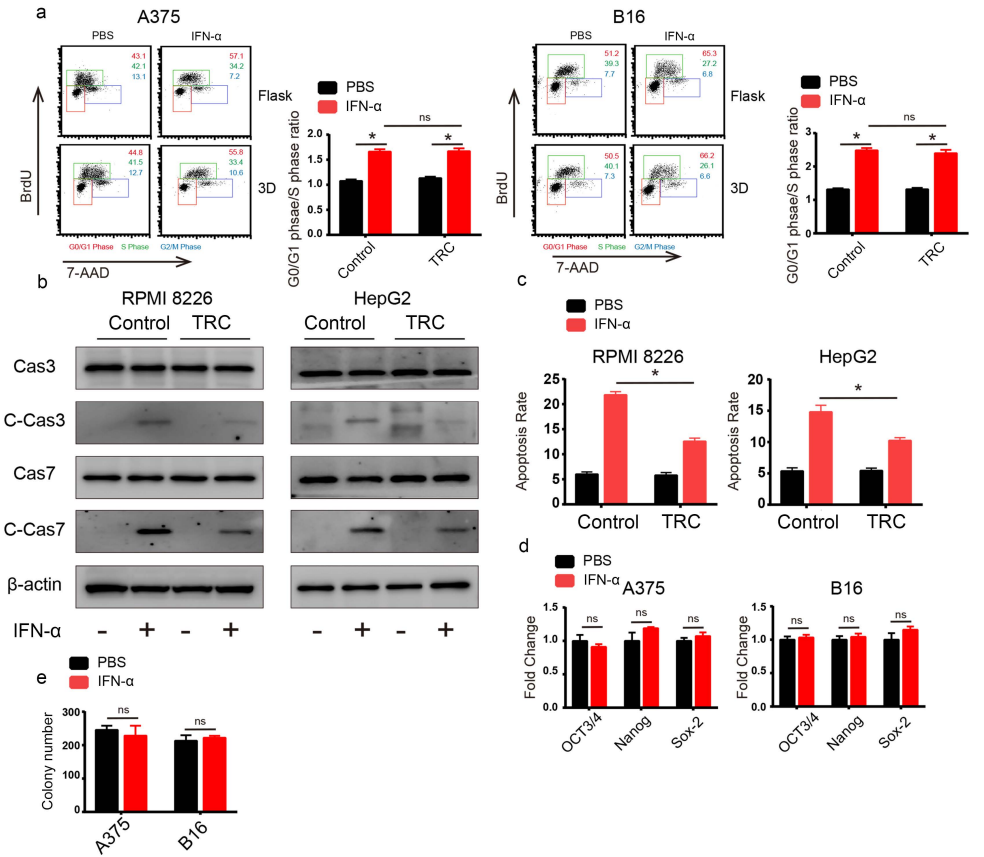

a
A375
PBS
IFN-α
Flask
3D
BrdU
7-AAD
G0/G1 Phase
S Phase
G2/M Phase
G0/G1 phsae/S phase ratio
Control
TRC
ns
B16
b
RPMI 8226
HepG2
Cas3
C-Cas3
Cas7
C-Cas7
β-actin
c
Apoptosis Rate
d
OCT3/4
Nanog
Sox-2
Fold Change
e
Colony number

Fig S2

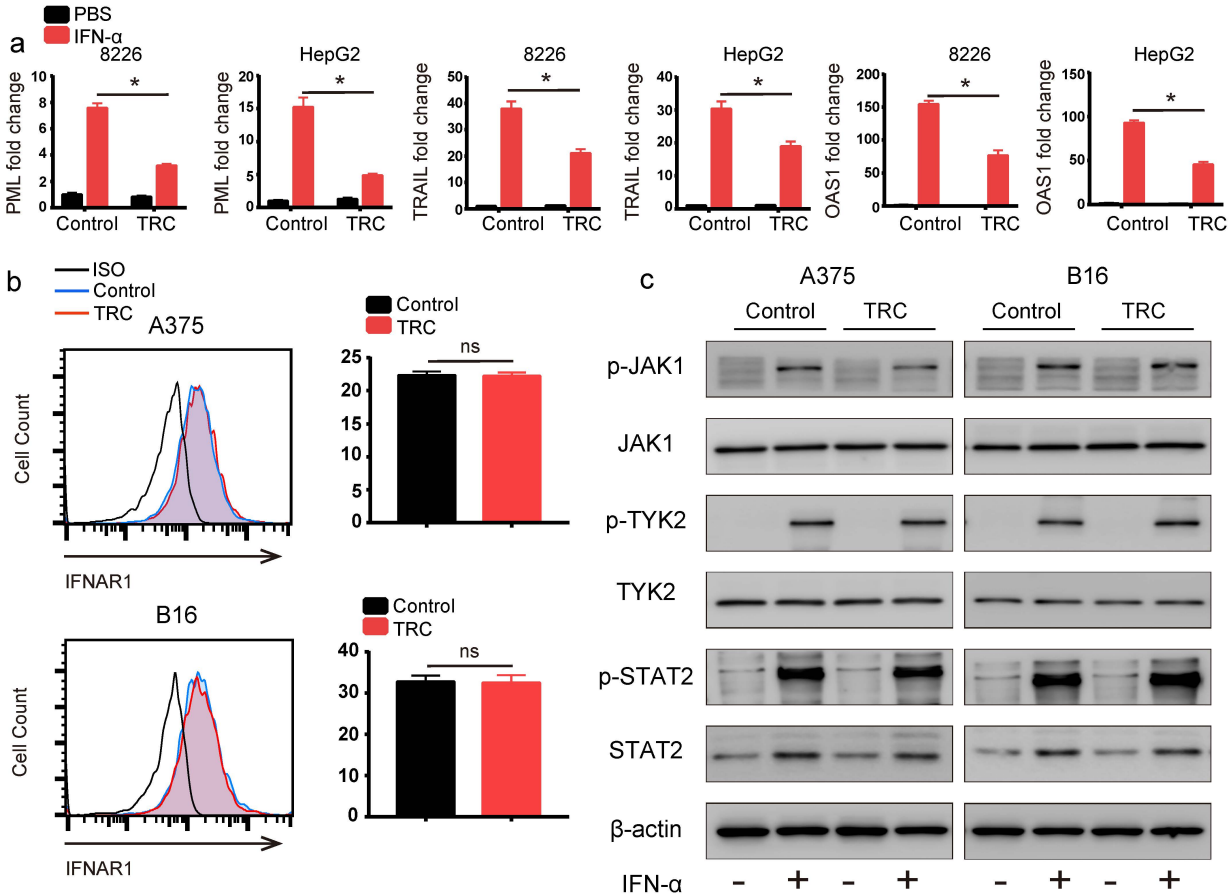

a
PBS
IFN-α
8226
HepG2
PML fold change
TRAIL fold change
OAS1 fold change
Control
TRC
*
b
ISO
Control
TRC
A375
B16
Cell Count
IFNAR1
ns
c
A375
B16
Control
TRC
p-JAK1
JAK1
p-TYK2
TYK2
p-STAT2
STAT2
β-actin
IFN-α

Fig S3

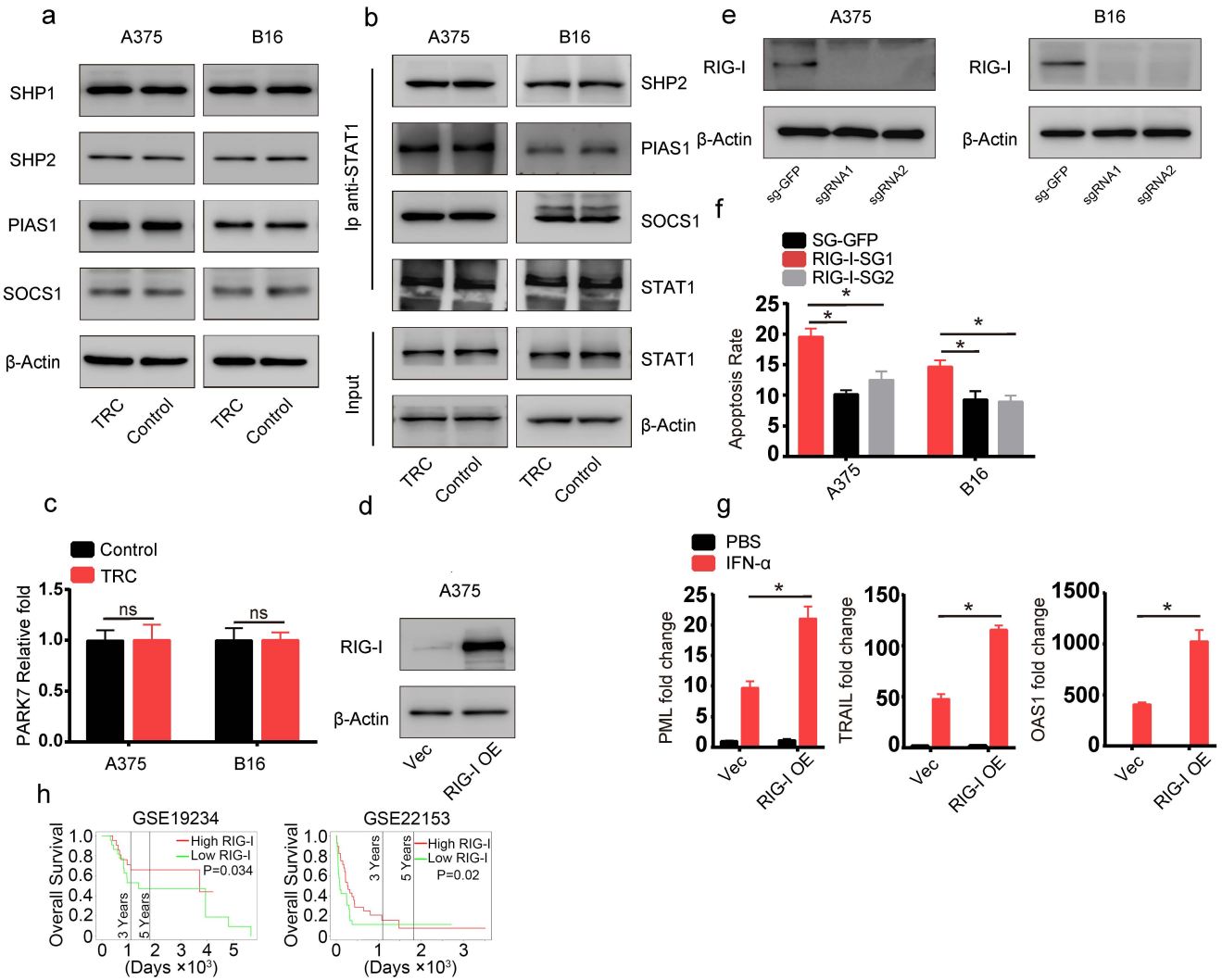

a
A375
B16
SHP1
SHP2
PIAS1
SOCS1
β-Actin
TRC
Control
b
Ip anti-STAT1
Input
SHP2
PIAS1
SOCS1
STAT1
STAT1
β-Actin
e
RIG-I
β-Actin
sg-GFP
sgRNA1
sgRNA2
f
SG-GFP
RIG-I-SG1
RIG-I-SG2
Apoptosis Rate
c
Control
TRC
PARK7 Relative fold
ns
d
RIG-I
β-Actin
Vec
RIG-I OE
g
PBS
IFN-α
PML fold change
TRAIL fold change
OAS1 fold change
h
GSE19234
GSE22153
High RIG-I
Low RIG-I
P=0.034
P=0.02
3 Years
5 Years
Overall Survival
(Days ×10³)

Fig S4

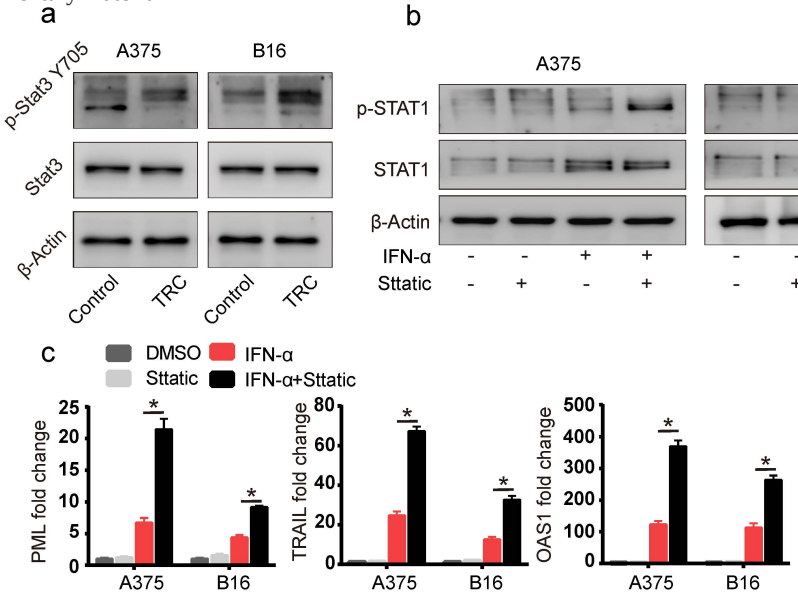
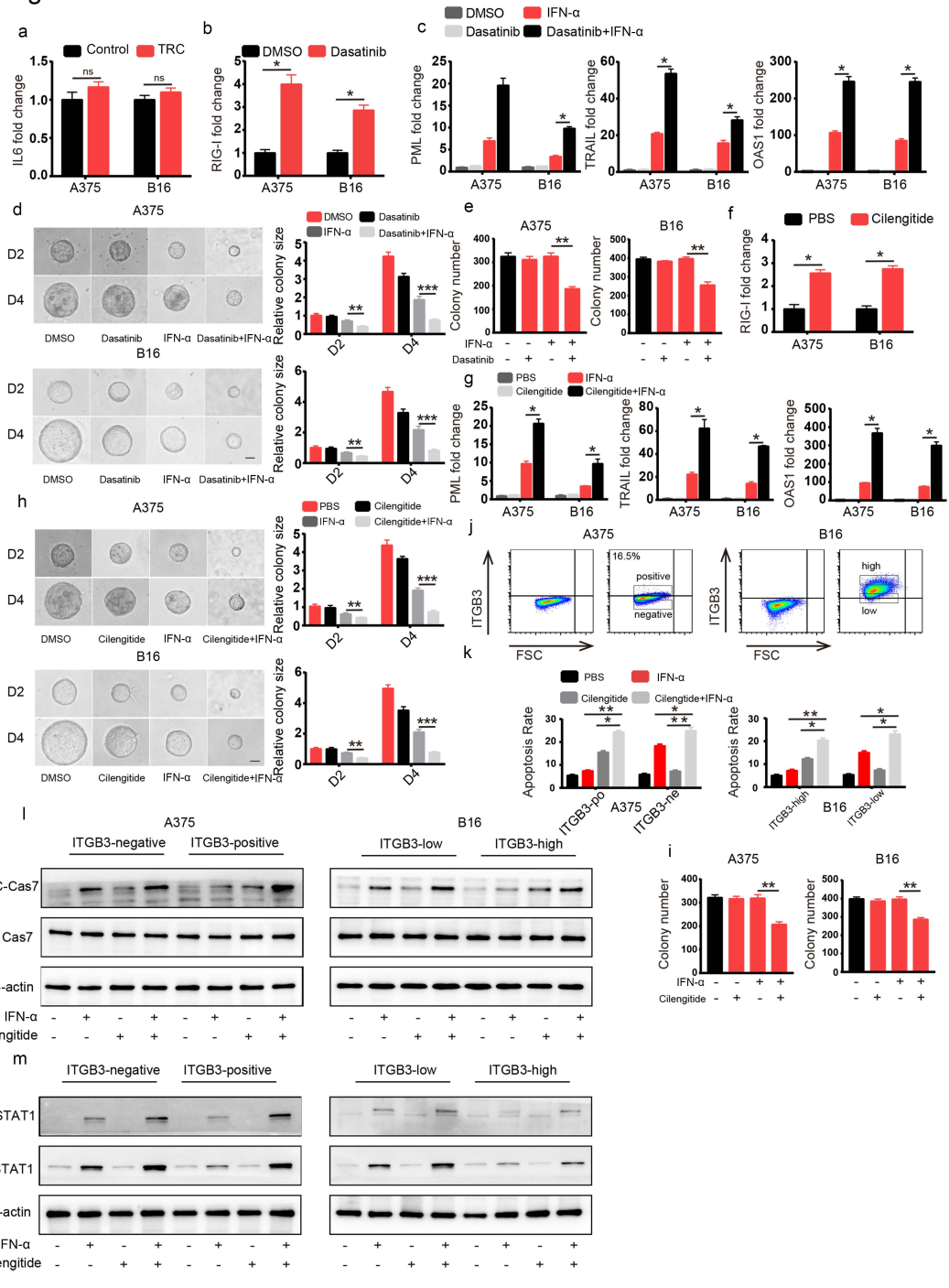

a
A375
B16
p-Stat3 Y705
Stat3
β-Actin
Control
TRC
Control
TRC
b
A375
B16
p-STAT1
STAT1
β-Actin
IFN-α
Sttatic
c
DMSO
IFN-α
Sttatic
IFN-α+Sttatic
PML fold change
TRAIL fold change
OAS1 fold change
A375
B16

Fig S5


a
Control
TRC
IL6 fold change
ns
A375
B16
b
DMSO
Dasatinib
RIG-I fold change
c
DMSO
IFN-α
Dasatinib
Dasatinib+IFN-α
PML fold change
TRAIL fold change
OAS1 fold change
d
A375
D2
D4
DMSO
Dasatinib
IFN-α
Dasatinib+IFN-α
Relative colony size
B16
e
Colony number
f
PBS
Cilengitide
g
h
Cilengitide
Cilengitide+IFN-α
i
j
ITGB3
FSC
16.5%
positive
negative
high
low
k
Apoptosis Rate
ITGB3-positive
ITGB3-negative
ITGB3-high
ITGB3-low
l
C-Cas7
Cas7
β-actin
m
p-STAT1
STAT1

Supplementary Figure legends

Figure S1.(a) B16 TRCs and A375 TRCs were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 48 h. Cells were collected, and analysed cell cycle with BD cell-cycle analysing kit. Flask-cultured tumor cells were used as control (b) RPMI 8226 TRCs and HepG2 TRCs were treated with 1000 U/mL human recombinant IFN-α for 48 h. Cell lysates were collected, and caspase 3, caspase 7 and their cleaved forms were analysed by immunoblotting. Flask-cultured tumor cells were used as control. (c) RPMI 8226 TRCs and HepG2 TRCs were treated with 1000 U/mL human recombinant IFN-α for 48 h. The cells were isolated, stained with annexin V and 7-AAD and analysed by flow cytometry. Columns indicate three independent replicate experiments. Flask-cultured tumor cells were used as control. (d) A375 and B16 TRCs were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 48 h. Total RNA was extracted and reverse transcribed, and gene expression was analysed by RT-qPCR.(e) A375 and B16 TRCs were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 48 h. Cells were collected and seeded on corning ultra-low adhesion plate with 1000 cells/ml. Cultured with serum free DMEM-F12 containing B27,20ng/ml EGF, 0.4% bovine serum albumin, and 4 mg/ml insulin. After culturing for ten days, the spheres were counted. The data shown are representative of three independent experiments and represent the mean± S.E.M. (indicated by errors bars). **P* < 0.05.

Figure S2. (a) RPMI 8226 TRCs and HepG2 TRCs were treated with 1000 U/mL human recombinant IFN-α for 24 h. Total RNA was extracted and reverse transcribed, and gene expression was analysed by RT-PCR with specific primers for PML, TRAIL and OAS1. Flask-cultured tumor cells were used as control. (b) A375 TRCs and B16 TRCs were isolated, stained with anti-IFNAR1 and analysed by flow cytometry. Flask-cultured tumor cells were used as control. Columns indicate three independent replicate experiments. (c) A375 TRCs and B16 TRCs were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-a for 6 h, and cell lysates

were collected and analysed by immunoblotting. The data shown are representative of three independent experiments and represent the mean± S.E.M. (indicated by error bars). ns: not significant, *P < 0.05.

Figure S3. (a) The lysates of flask-cultured control cells and TRCs of A375 or B16 cells were analysed by immunoblotting using the indicated antibodies. (b) A375 TRCs and B16 TRCs were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 6 h. Cell lysates were collected for immunoprecipitation with anti-STAT1 antibody, and protein interactions were analysed by immunoblotting with the indicated antibodies. Flask-cultured tumor cells were used as control. (c) Total RNA from TRCs of A375 or B16 and flask-cultured control tumor cells was extracted, reverse transcribed and analysed with specific primers for PARK7. (d) RIG-I overexpression in A375 cells was verified via immunoblotting with anti-RIG-I antibody. (e) RIG-I knockout in A375 and B16 cells by CRISPR/Cas9 was verified via immunoblotting with anti-RIG-I antibody. (f) Flask-cultured RIG-I knockout A375 and B16 cells generated by CRISPR targeting were treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 48 h. Cells were isolated and stained with annexin V and 7-AAD and analysed by flow cytometry. Columns indicate three independent replicate experiments. (g) TRCs of A375 RIG-I-overexpressing cells and control cells were treated with human recombinant IFN-A for 24 h. The total RNA was extracted and gene expression was analysed by RT-qPCR. (h) Two melanoma datasets obtained from Gene Expression Omnibus (GEO) profiles (PROGgeneV2) and clinical data were analysed via Kaplan-Meier survival analysis The data shown are representative of three independent experiments and represent the mean± S.E.M. (indicated by errors bars). ns: not significant.

Figure S4. (a) Cell lysates from A375 TRCs and B16 TRCs were extracted and analysed via immunoblotting with the indicated antibodies. Flask-cultured tumor cells were used as control. (b) A375 TRCs and B16 TRCs were pretreated with stattic (1 μM) for 12 h and then treated with human recombinant IFN-α (1000 U/mL) and murine recombinant IFN-α (200 ng/mL) for 6 h. Cell lysates were extracted and analysed via

immunoblotting with the indicated antibodies. (c) A375 TRCs and B16 TRCs were pretreated with stattic (1 μM) for 12 h and then treated with human recombinant IFN-α (1000 U/mL) and murine recombinant IFN-α (200 ng/mL) for 16 h. Total RNA from TRCs was extracted, reverse transcribed and analysed by RT-PCR with specific primers for PML, TRAIL and OAS1. The data shown are representative of three independent experiments and represent the mean± S.E.M. (indicated by error bars). ns: not significant, $*P < 0.05$.

Figure S5. (a) The expression of IL6 in A375 TRCs and B16 TRCs was analyzed by RT-qPCR. (b) A375 TRCs and B16 TRCs were treated with dasatinib (1 μM) for 12h. The expression of RIG-I was analyzed by RT-qPCR. (c) A375 TRCs and B16 TRCs were pretreated with dasatinib (1 μM) for 12h and then treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-a for 24 h. The expression of PML, TRAIL and OAS1 was analyzed by RT-qPCR. (d-e) A375 and B16 cells were seeded in fibrin, and after two days, the cells were treated with dasatinib (1 μM), IFN-α (1000 U/mL for A375 cells, 200 ng/mL for B16 cells) or both for 4 days (d). The colonies size was determined at day 2. Scale bar: 25 μm (e) The numbers of colonies were counted at day 4. (f) A375 TRCs and B16 TRCs were treated with cilengitide (1 μM) for 12 h. The expression of RIG-I was analyzed by RT-qPCR. (g) A375 TRCs and B16 TRCs were pretreated with cilengitide (1 μM) for 12 h and then treated with 1000 U/mL human recombinant IFN-α or 200 ng/mL murine recombinant IFN-α for 24 h. The expression of PML, TRAIL and OAS1 were analyzed by RT-qPCR. (h-i) A375 and B16 cells were seeded in fibrin, and after two days, the cells were treated with cilengitide (1 μM), IFN-α (1000 U/mL for A375 cells, 200 ng/mL for B16 cells) or both for 4 days (h). The colonies size was determined at day 2. Scale bar: 25 μm (i) The numbers of colonies were counted at day4. (j) Representative images of sorting strategy. (k-m) The sorted cells were seeded in 6-well plate after 12h, the cells were treated with cilengitide (1uM), IFN-α (human recombinant IFN-α (1000 U/mL) and murine recombinant IFN-α (200 ng/mL)) or cilengitide/IFN-α for 2 days. (k) Cells were collected and analysed apoptosis via flow cytometry. (l-m) Cell lysates were collected

and analysed via immunoblotting with the indicated antibodies. The data shown are representative of three independent experiments and represent the mean± S.E.M. (indicated by error bars). ns: not significant, **P* < 0.05, ***P*<0.01, ****P*<0.001.

Li Y, *et al. J Immunother Cancer* 2020; 8:e000111. doi: 10.1136/jitc-2019-000111