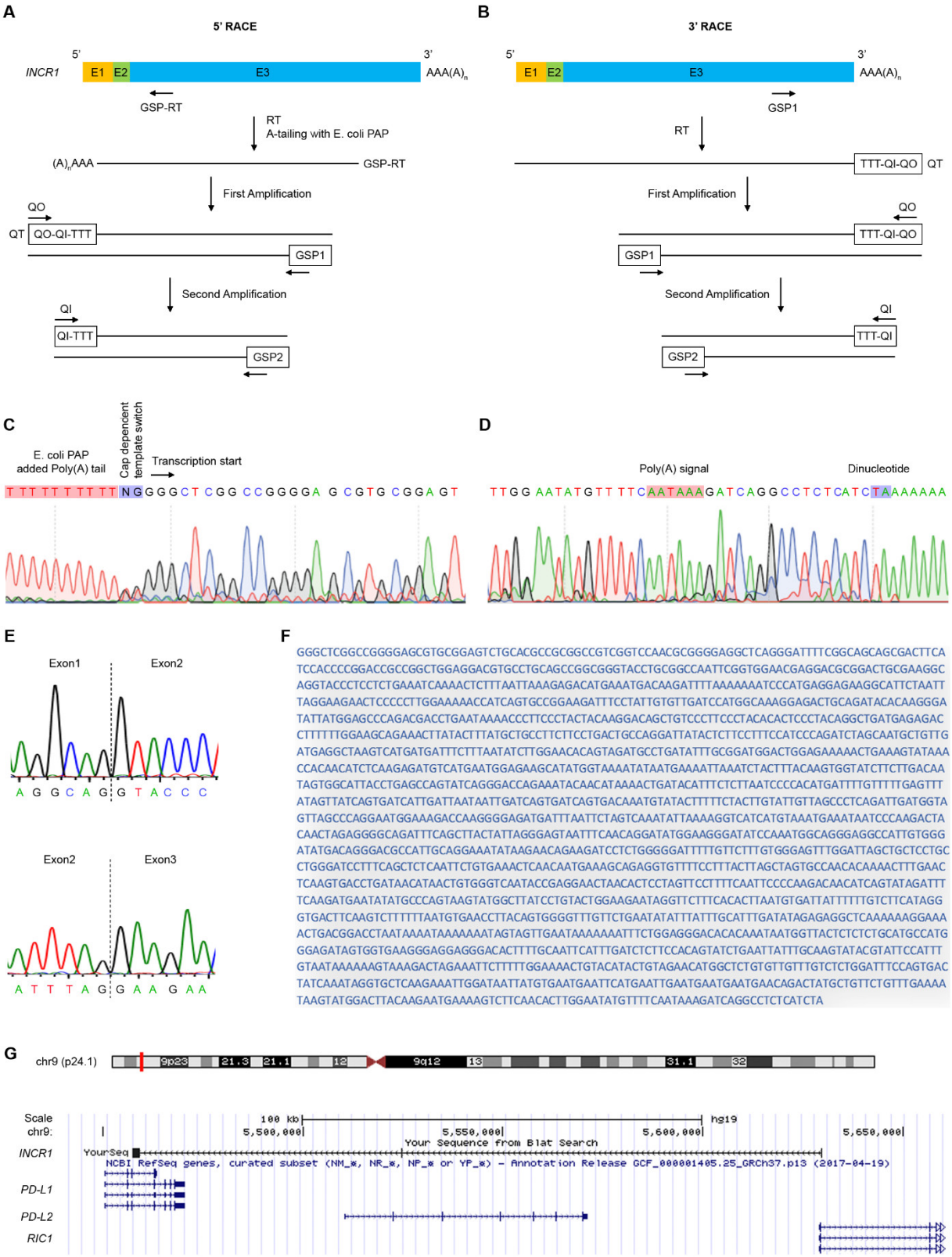
A
5' RACE
5'
3'
INCR1
E1
E2
E3
AAA(A)n
GSP-RT
RT
A-tailing with E. coli PAP
(A)nAAA
GSP-RT
First Amplification
QO
QT
QO-QI-TTT
GSP1
Second Amplification
QI
QI-TTT
GSP2
B
3' RACE
5'
3'
E1
E2
E3
AAA(A)n
GSP1
RT
TTT-QI-QO
QT
First Amplification
QO
TTT-QI-QO
GSP1
Second Amplification
QI
TTT-QI
GSP2
C
E. coli PAP
added Poly(A) tail
Cap depencent
template switch
Transcription start
T T T T T T T T T N G G G G C T C G G C C G G G G A G C G T G C G G A G T
D
Poly(A) signal
Dinucleotide
T T G G A A T A T G T T T C A A T A A A G A T C A G G C C T C T C A T C T A A A A A A A
E
Exon1
Exon2
A G G C A G G T A C C C
Exon2
Exon3
A T T T A G G A A A G A A
F
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G
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9p23
21.3
21.1
12
9q12
13
31.1
32
Scale
chr9:
100 kb
hg19
5,500,000
5,550,000
5,600,000
5,650,000
Your Sequence from Blat Search
INCR1
YourSeq
NCBI RefSeq genes, curated subset (NM_*, NR_*, NP_* or YP_*) - Annotation Release GCF_000001405.25_GRCh37.p13 (2017-04-19)
PD-L1
PD-L2
RIC1

Figure S1. Characterization of the *INCR1* sequence. Related to Figure 1

(A-B) Schematic representation of 5' (A) and 3' (B) RACE experiments.

(C-D) PCR products from 5' and 3' RACE were purified and sequenced. Shown are DNA sequencing traces for 5' (C) and 3' (D) RACE.

(E) Full length *INCR1* was PCR amplified and purified product was sequenced. Shown are DNA sequencing traces for exon junctions of the *INCR1* expressed in patient derived GBM cell lines.

(F) The full-length sequence of *INCR1*.

(G) Genome Browser alignment of the *INCR1* sequence.

Data are representative of three independent experiments.

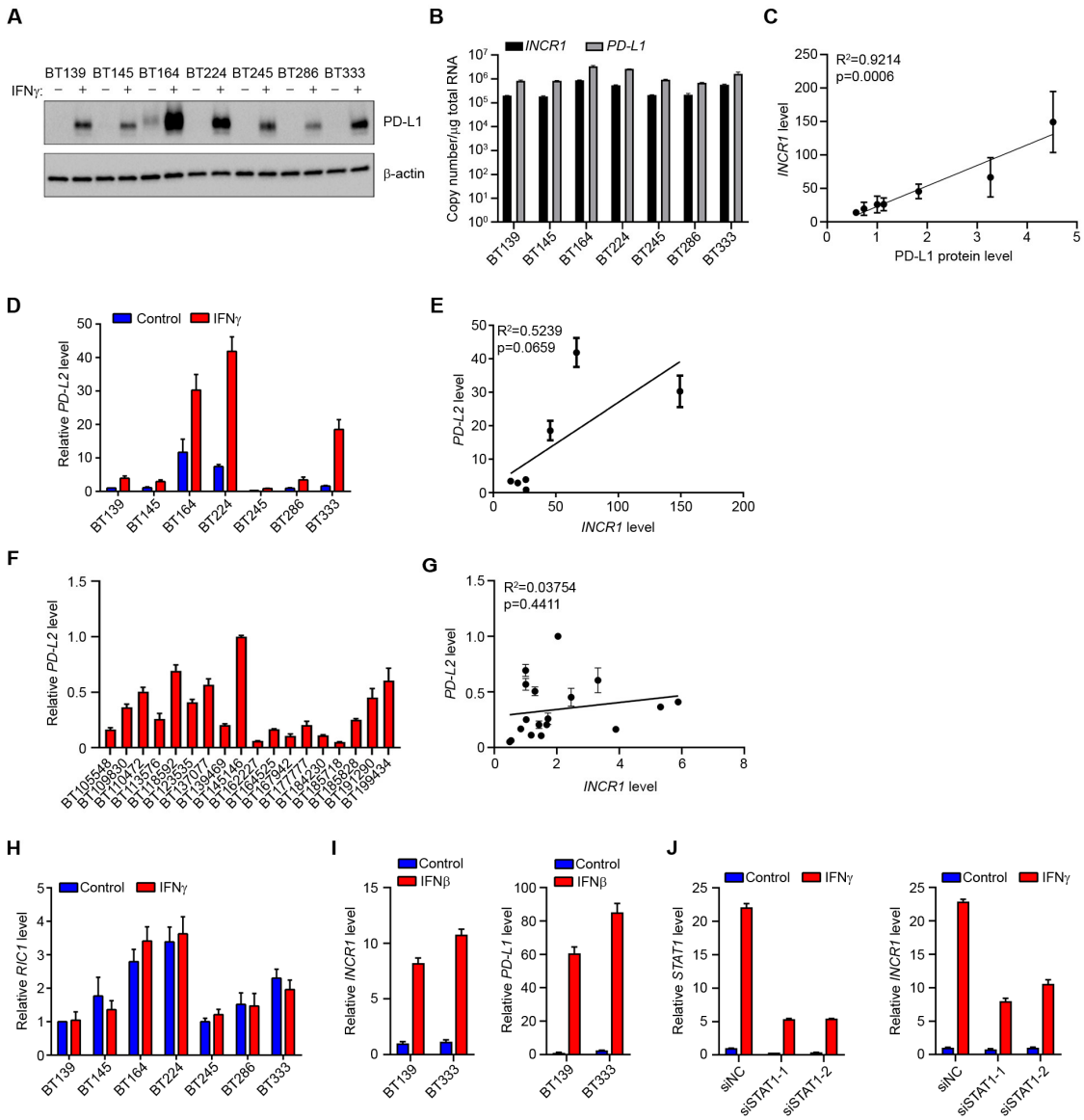

Figure S2. Interferon induced *INCR1* expression is STAT1 dependent and does not correlate with *PD-L2* or *RIC1* expression. Related to Figure 2

(A) Western blot analysis of PD-L1 expression in PDGCLs unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(B) qRT-PCR analysis of *INCR1* and *PD-L1* copy number in 7 IFNγ-stimulated (100 U/ml for 24 h) PDGCLs.

(C) Correlation of *INCR1* expression with PD-L1 protein levels in IFNγ-stimulated PDGCLs. R^2=0.9214 calculated using linear regression analysis.

(D-E) qRT-PCR analysis of *PD-L2* (D) expression in 7 unstimulated or IFNγ-stimulated (100 U/ml for 24 h) PDGCLs, and correlation of *INCR1* expression with *PD-L2* expression in IFNγ-stimulated PDGCLs (E). R^2=0.5239 calculated using linear regression analysis.
(F-G) qRT-PCR analysis of *PD-L2* (F) expression in 18 GBM patient tumor specimens, and correlation of *INCR1* expression with *PD-L2* expression (G). R^2=0.03754 calculated using linear regression analysis.
(H) qRT-PCR analysis of *RIC1* expression in 7 unstimulated or IFNγ-stimulated (100 U/ml for 24 h) PDGCLs.
(I) qRT-PCR analysis of *INCR1* (left) and *PD-L1* (right) expression in 2 PDGCLs (BT139 and BT333) unstimulated or stimulated with 1000 U/mL of IFNβ for 24 h.
(J) qRT-PCR analysis of *STAT1* (left) and *INCR1* (right) expression in unstimulated or IFNγ-stimulated (100 U/ml for 24 h) patient derived BT139 cells transfected with siRNA control or two different siRNAs targeting *STAT1*.
Data shown as mean ± SD of three replicates.

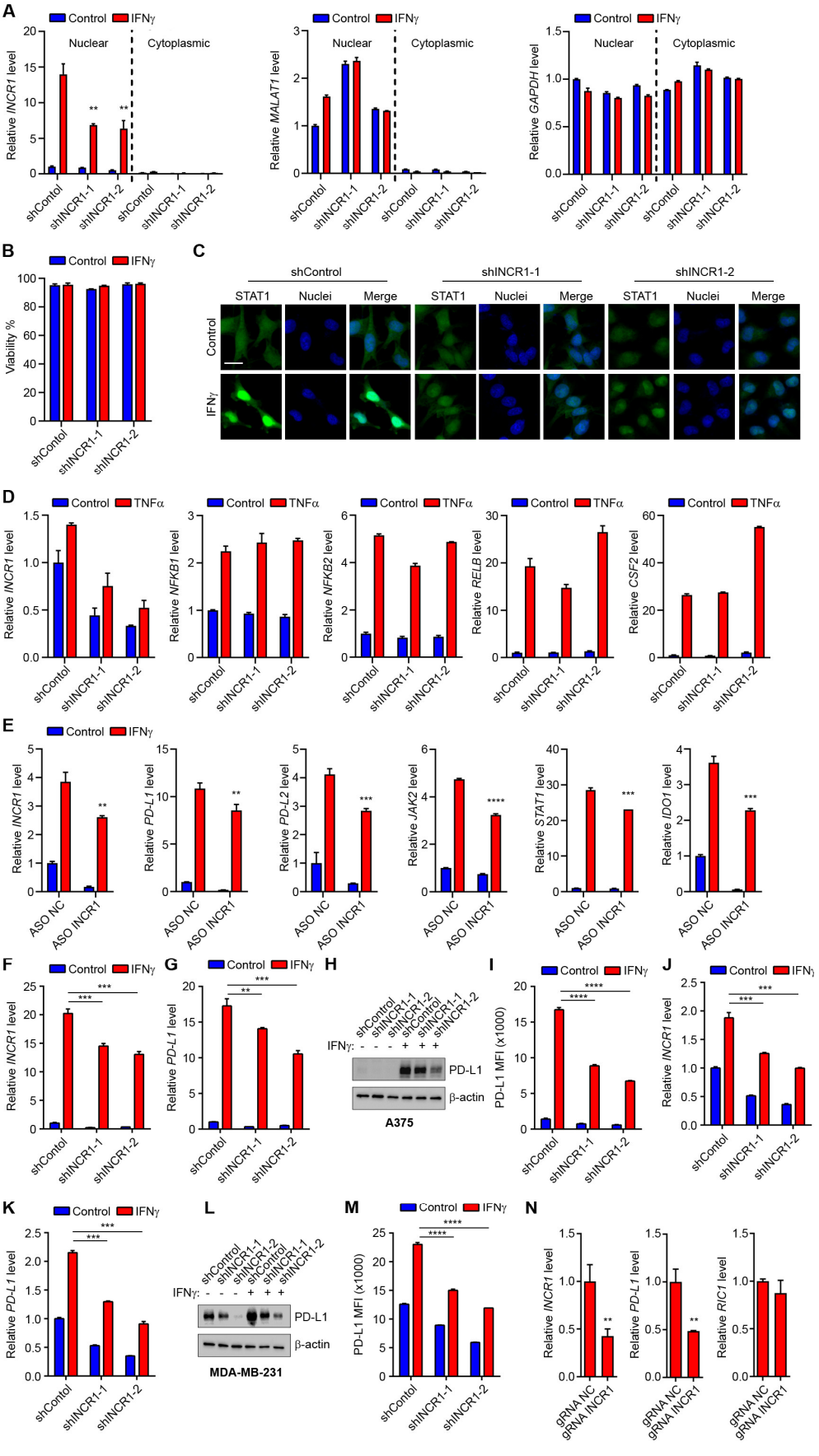
A
Control
IFNγ
Nuclear
Cytoplasmic
Relative INCR1 level
Relative MALAT1 level
Relative GAPDH level
shContol
shINCR1-1
shINCR1-2
B
Viability %
C
shControl
shINCR1-1
shINCR1-2
STAT1
Nuclei
Merge
Control
IFNγ
D
TNFα
Relative INCR1 level
Relative NFKB1 level
Relative NFKB2 level
Relative RELB level
Relative CSF2 level
E
Relative INCR1 level
Relative PD-L1 level
Relative PD-L2 level
Relative JAK2 level
Relative STAT1 level
Relative IDO1 level
ASO NC
ASO INCR1
F
G
H
IFNγ:
PD-L1
β-actin
A375
I
PD-L1 MFI (x1000)
J
K
L
MDA-MB-231
M
N
gRNA NC
gRNA INCR1
Relative RIC1 level

Figure S3. *INCR1* regulates PD-L1 expression in different tumor types. Related to Figure 3

(A) qRT-PCR analysis of RNAs extracted from cytoplasmic and nuclear compartments of unstimulated and IFNγ-stimulated (100 U/ml for 24 h) U251 control and two independent *INCR1*-knockdown cells. *MALAT1* and *GAPDH* were used to assess fractionation efficiency.

(B) Cell viability analysis of control and two independent *INCR1*-knockdown U251 cells. Cell viability was determined by Muse Cell Analyzer.

(C) Immunofluorescence analysis of STAT1 (green) in control and two independent *INCR1*-knockdown U251 cells unstimulated (Control) or stimulated with 100 U/ml IFNγ for 3 h. Nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm.

(D) qRT-PCR analysis of *INCR1*, *NFKB1*, *NFKB2*, *RELB* and *CSF2* expression in U251 control and two independent *INCR1*-knockdown cells unstimulated or stimulated with 20 ng/ml TNFα.

(E) qRT-PCR analysis of *INCR1*, *PD-L1*, *PD-L2*, *JAK2*, *STAT1* and *IDO1* expression in patient derived BT333 cells transfected with LNA gapmer antisense oligo negative control (ASO NC) or ASO targeting *INCR1*, unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(F-G) qRT-PCR analysis of *INCR1* (F) and *PD-L1* (G) expression in control or two independent *INCR1*-knockdown A375 melanoma cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(H) Western blot analysis of PD-L1 expression in control or two independent *INCR1*-knockdown A375 melanoma cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(I) Flow cytometry analysis of cell surface levels of PD-L1 in control or two independent *INCR1*-knockdown A375 melanoma cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(J-K) qRT-PCR analysis of *INCR1* (J) and *PD-L1* (K) expression in control or two independent *INCR1*-knockdown MDA-MB-231 breast cancer cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(L) Western blot analysis of PD-L1 expression in control or two independent *INCR1*-knockdown MDA-MB-231 breast cancer cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(M) Flow cytometry analysis of cell surface levels of PD-L1 in control or two independent *INCR1*-knockdown MDA-MB-231 breast cancer cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

(N) qRT-PCR analysis of *INCR1* (left), *PD-L1* (center) and *RIC1* (right) expression in dCAS9-KRAB expressing patient-derived BT333 cells transfected with sgRNA control (gRNA NC) or sgRNA targeting the *INCR1* promoter (gRNA INCR1).

Data shown as mean ± SD of three replicates. Data were analyzed by unpaired t-test: $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

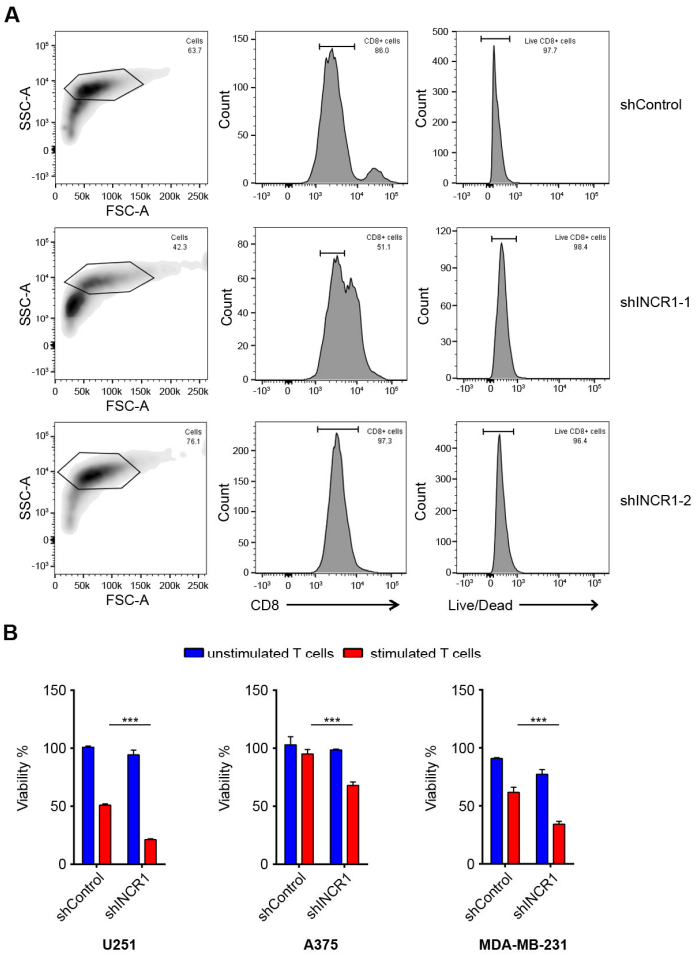

Figure S4. *INCR1* promotes tumor cell escape from T cell-mediated killing in different tumor types. Related to Figure 4

(A) Flow cytometry analysis of cell viability in $CD8^+$ T cells co-cultured for 96 h with control or two independent *INCR1*-knockdown U251 cells.

(B) Flow cytometry analysis of cell viability in control and *INCR1*-knockdown (shINCR1-2) U251 (left), A375 (middle) and MDA-MB-231 (right) cells co-cultured with activated $CD8^+$ T cells for 96 h.

Data shown as mean ± SD of at least three replicates. Data were analyzed by unpaired t-test: ***$p < 0.001$.

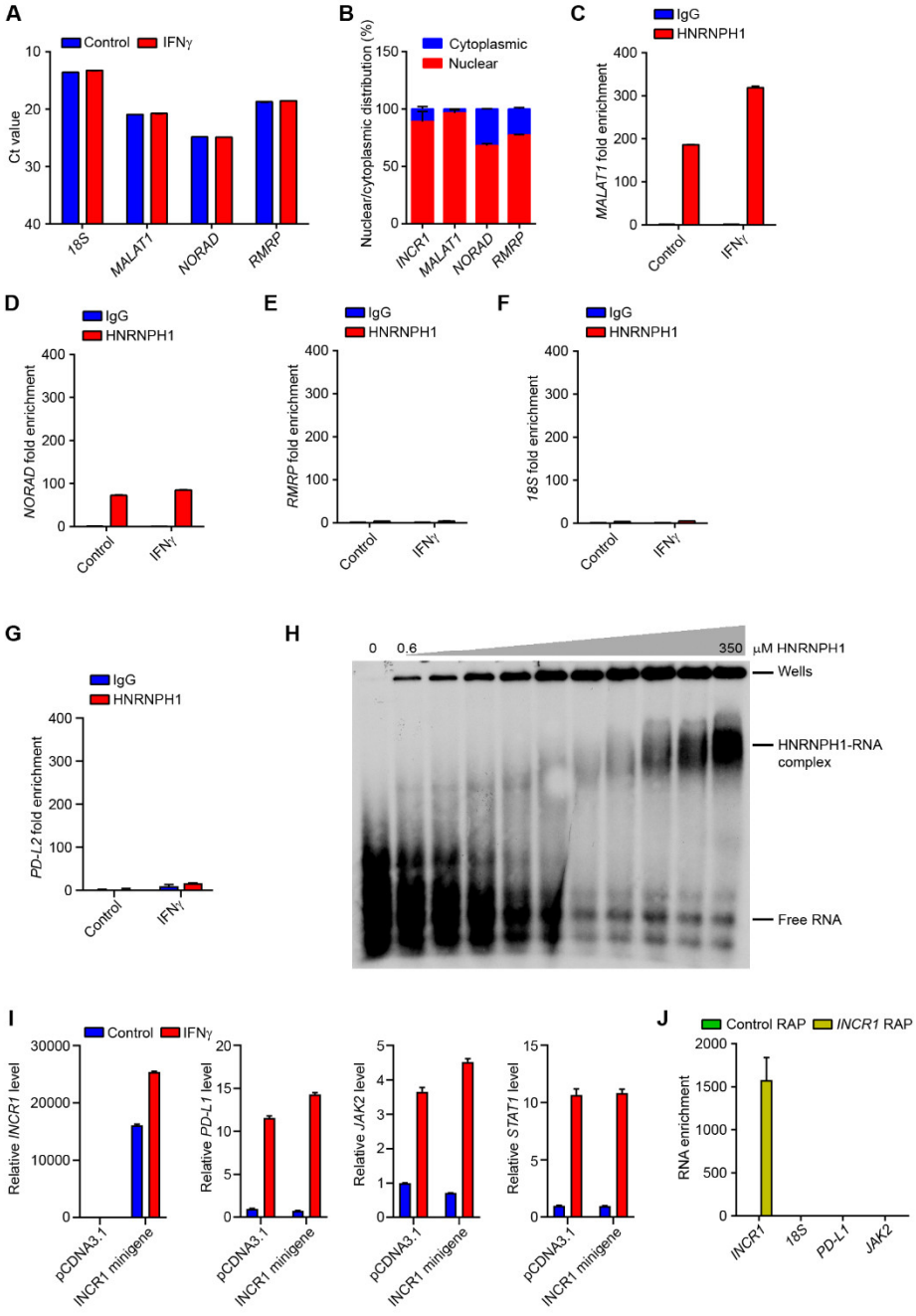

Figure S5. Validation of HNRNPH1 RNA-immunoprecipitation. Related to Figure 5

(A) qRT-PCR analysis of the expression of the lncRNAs *MALAT1*, *NORAD* and *RMRP* in PDGCLs stimulated with 100 U/ml IFNγ for 24 h.

(B) qRT-PCR analysis of RNAs extracted from cytoplasmic and nuclear compartments of IFNγ-stimulated (100 U/ml for 24 h) PDGCLs.

(C-F) HNRPNH1 RIP followed by qRT-PCR analysis of co-purified RNAs in UV-crosslinked cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h. *MALAT1* (C) and *NORAD* (D)

lncRNAs were used as positive control of HNRNPH1 binding. *RMRP* (E) and *18S* (F) lncRNAs were used as negative control.
(G) HNRPNH1 RIP followed by qRT-PCR analysis of co-purified *PD-L2* in PDGCLs stimulated with 100 U/ml IFNγ for 24 h.
(H) EMSA analysis of HNRNPH1 binding to radiolabeled oligonucleotide (50 bases) whose sequence represents the major eCLIP peak. The highest protein concentration used was 350 μM, and 2-fold serial dilutions were assayed. No protein was added to the lane marked "0".
(I) qRT-PCR analysis of *INCR1*, *PD-L1*, *JAK2* and *STAT1* expression in unstimulated or IFNγ-stimulated (100 U/ml for 24 h) U251 cells upon *INCR1* overexpression in *trans*. Cells were stimulated with IFNγ 48 h after transfection with pCDNA3.1 (empty vector) or pCDNA3.1-INCR1 minigene.
(J) MDA-MB-231 cells were stimulated with 100 U/ml IFNγ for 12 h and RAP-RNA was performed using biotinylated probes antisense to *INCR1* (yellow) or scramble. RNA co-purified with *INCR1* was analyzed by qRT-PCR.

Data shown as mean ± SD of three replicates.

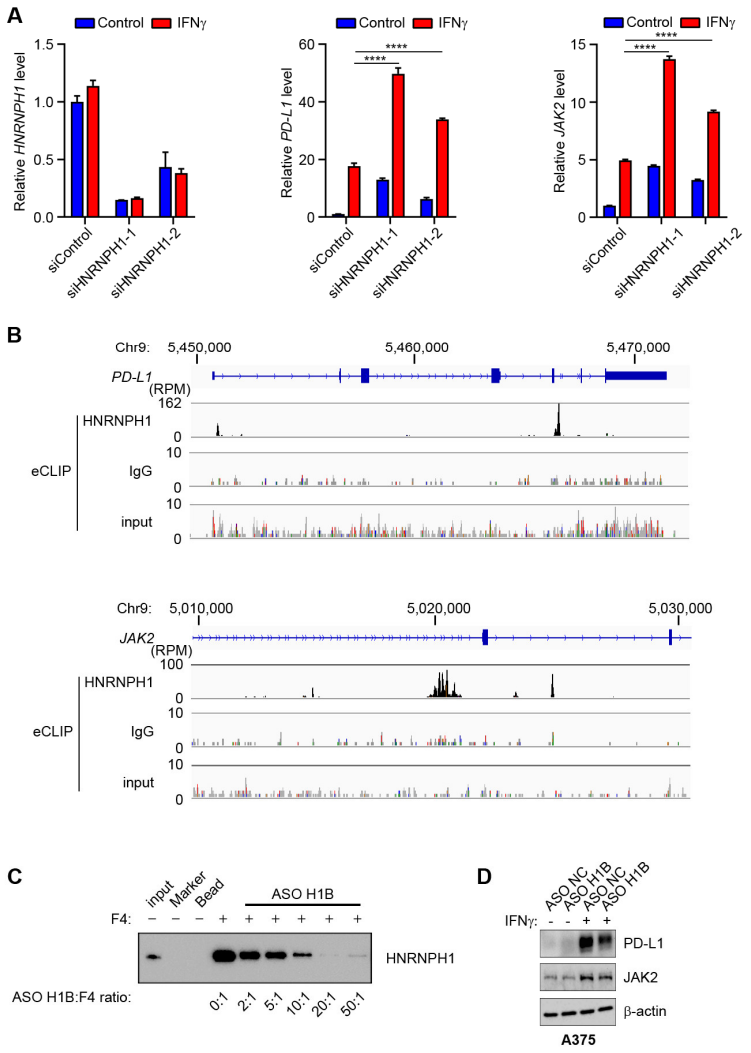

Figure S6. HNRNPH1 regulates PD-L1 and JAK2 expression in an *INCR1*-dependent manner. Related to Figure 6

(A) qRT-PCR analysis of *HNRNPH1* (left), *PD-L1* (center) and *JAK2* (right) expression in unstimulated or IFNγ-stimulated (100 U/ml for 24 h) patient derived BT139 cells transfected with siRNA control or two different siRNAs targeting *HNRNPH1*.

(B) Identification of HNRNPH1 binding sites in *PD-L1* (top) and *JAK2* (bottom) by eCLIP in A375 cells stimulated with 100 U/ml IFNγ for 6 h. Read density in reads per million (RPM) are shown for HNRNPH1, IgG and input.

(C) RNA pull-down analysis of biotinylated fragment 4 (F4) in the presence of increasing concentrations of antisense oligonucleotide targeting HNRNPH1 binding site (ASO H1B). No RNA fragment was added in the lanes marked "-".

(D) Western blot analysis of PD-L1 and JAK2 expression in ASO NC or ASO H1B transfected A375 melanoma cells unstimulated or stimulated with 100 U/ml IFNγ for 24 h.

Data shown as mean ± SD of three replicates. Data were analyzed by unpaired t-test: ****$p < 0.0001$.