Figure S1. Related to Figure 1

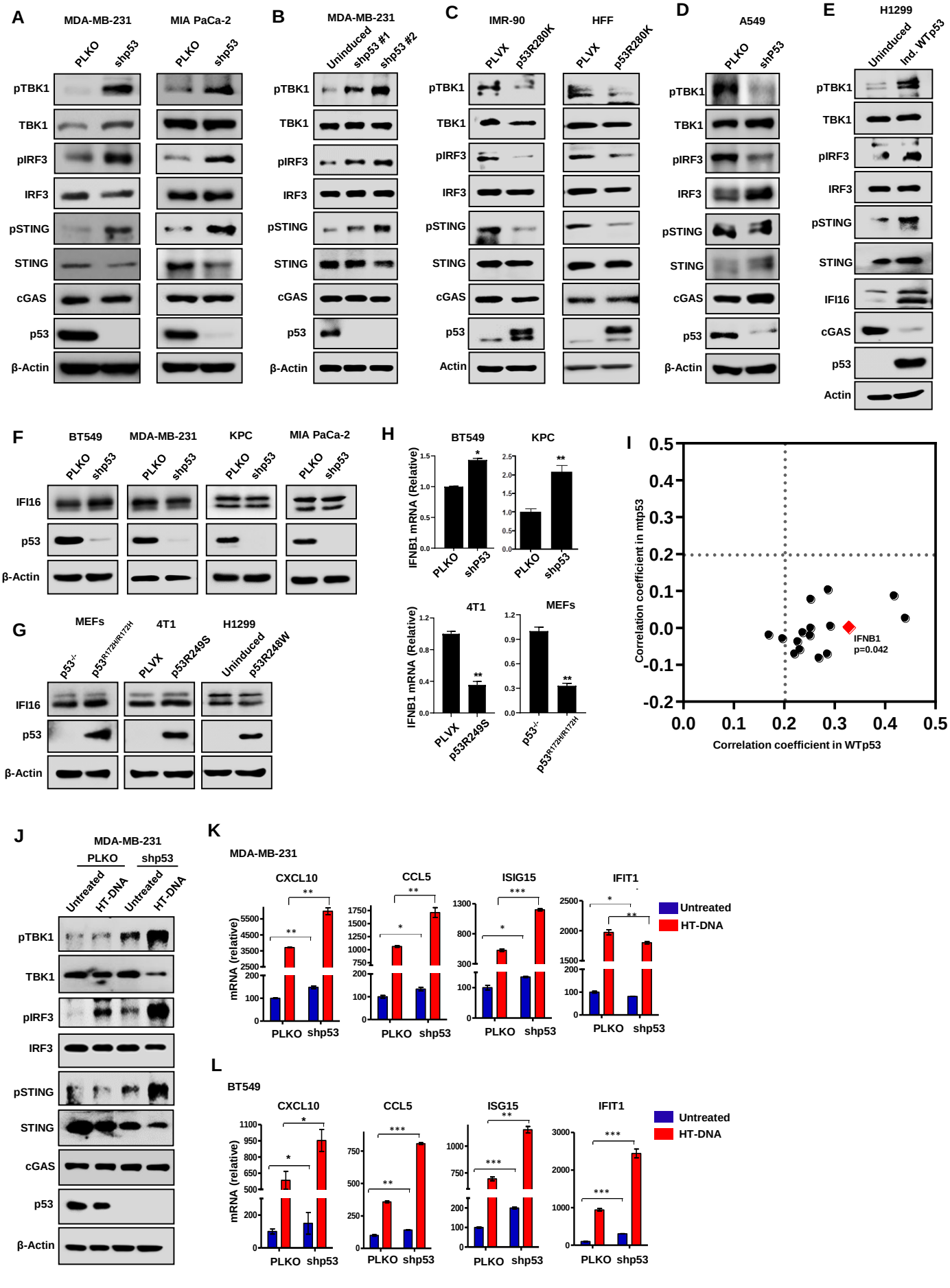


Figure S1. Related to Figure 1, Continue

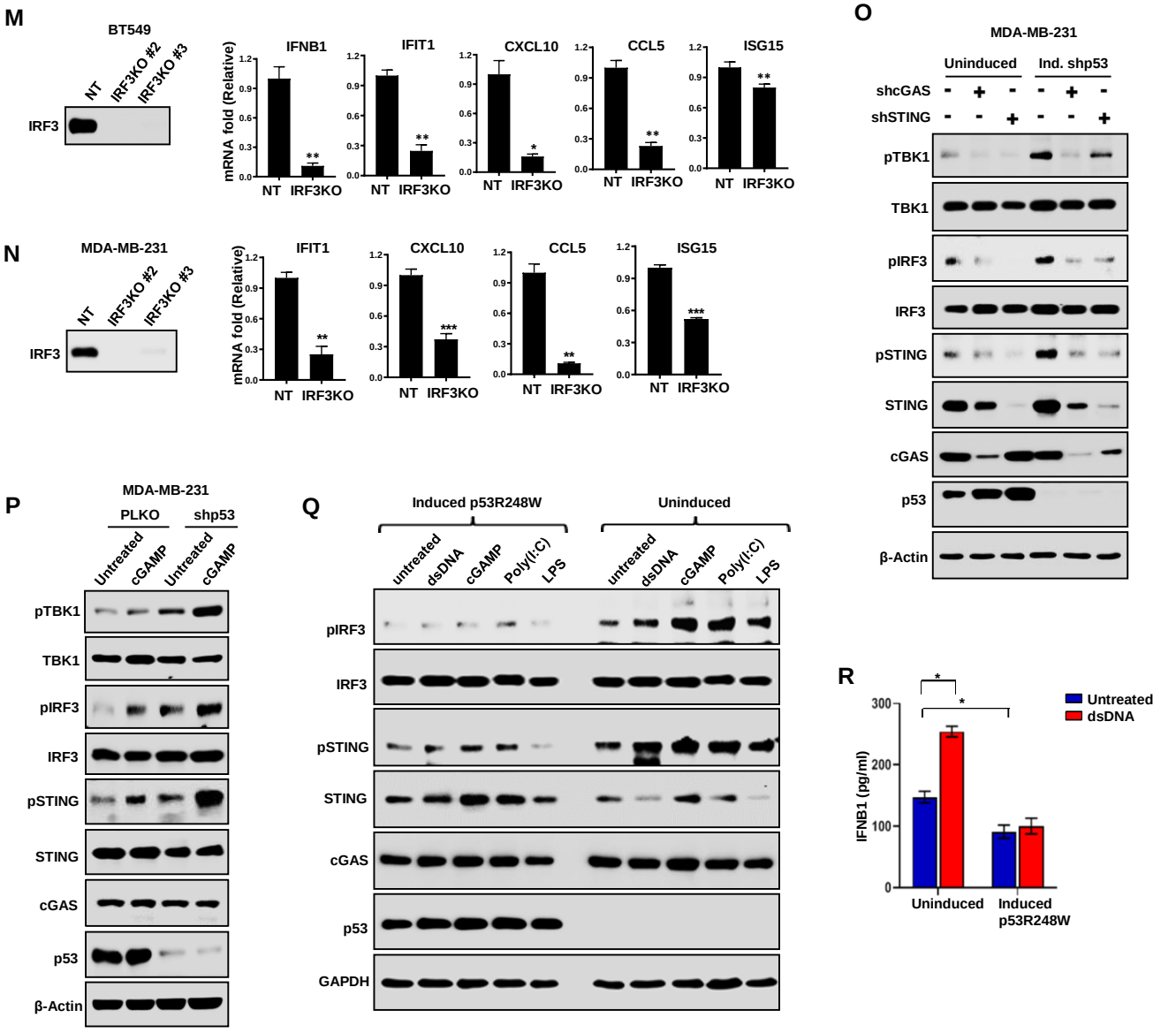


Figure S1. Mutant p53 suppresses innate immune signaling, Related to Figure 1

(A) Indicated cells were infected with virus containing control (PLKO) or p53 targeting shRNA (shp53) and subjected to Immunoblot analysis. (B) MDA MB-231 cells were induced to express two different p53 shRNA and subjected to western blot analysis. (C) Normal human fibroblasts IMR-90 and HFF cells were engineered to express p53R280K and subjected to western blotting. (D) A549 cells were infected with virus containing PLKO or shp53 and subjected to western blot. (E) P53 null H1299 cells were induced with doxycycline to express WTp53 and subjected to western blot. (F) Immunoblot analysis of shRNA targeting mutant p53 in BT549, MDA-MB-231, KPC and MIA PaCa-2. (G) Immunoblot analysis of $p53^{-/-}$ and $p53^{R172H/R172H}$ MEFs, p53 null 4T1 cells expressing p53R249S and p53 null H1299 cells expressing p53R248W. (H) Representative RT-PCR data showing mutant p53 attenuates IFNB1 mRNA in the indicated cells. (I) Pearson Correlation coefficient of Different chemokines and cytokines expression (Red marked IFNB1) with WTp53 (n=39) and mutant p53 (n=74) as determined from TCGA dataset. (J) MDA-MB-231 PLKO or shp53 cells were treated with 2 µg/ml of HT-DNA for 3 hrs, cells were harvested and subjected to western blot analysis. (K-L) p53 knock down MDA-MB-231 (K) or BT549 (L) cells were treated with HT-DNA for 18 hrs, cells were harvested, RNA was isolated from the cells for RT-PCR. (M-N) IRF3 knock out cells were prepared using CRISPR-Cas9 technique and cells were subjected to western blot and RT-PCR analysis. Graphs show mRNA expression of IFNB1, IFIT1, CXCL10, CCL5, ISG15 in IRF3KO #2 (M) BT549 and (N) MDA-MB-231 cells. (O) Immunoblots analysis of shRNA targeting cGAS and STING in presence and absence of mutant p53 in MDA MB-231 cells. (P) Control or p53 knockdown MDA MB-231 cells were treated with 2 µg/ml cGAMP for 3 hrs, cells were harvested and subjected to western blot analysis. (Q) H1299 cells were induced to express p53R248W or left uninduced and treated with dsDNA, cGAMP, poly(I:C) or LPS for 3 hrs and subjected to western blot analysis. (R) Representative graph shows ELISA data of IFNB1 secreted from H1299 cells uninduced and induced to express p53R248W and treated with 2 µg/ml dsDNA for 18 hrs.

Quantification graphs: In all panels, error bars represent Mean +/- SD. p values are based on Student's t test. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

Figure S2. Related to Figure 2

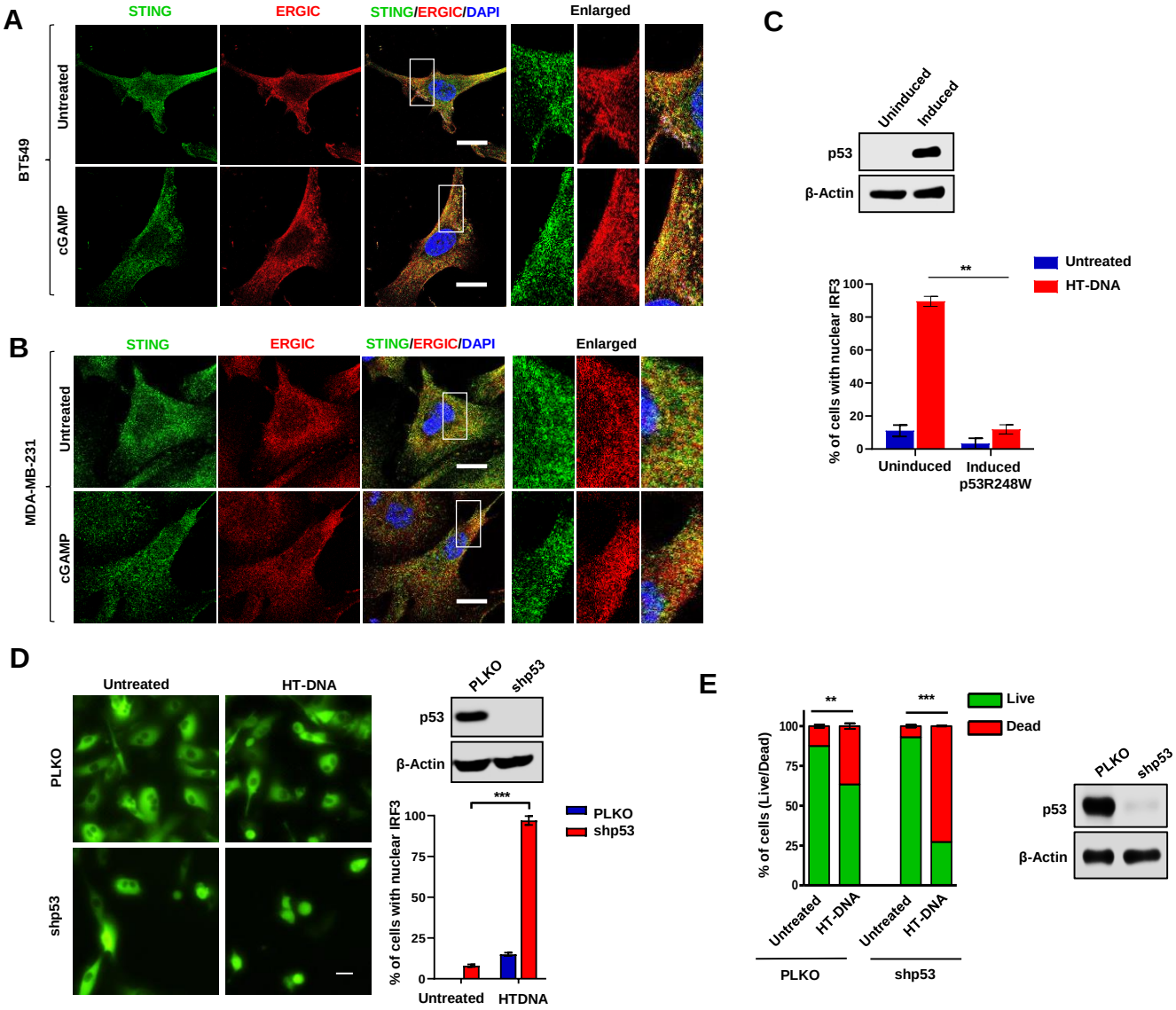


Figure S2. Mutant p53 blocks IRF3 nuclear translocation and IRF3-induced apoptosis, Related to Figure 2

(A-B) Representative confocal microscopy images of BT549 (A) and MDA-MB-231 (B) cells treated with cGAMP for 3 hrs and stained for STING, ERGIC and nucleus was stained with DAPI. Scale Bar 10 μm (C) Immunoblots showing p53R248W expression**.** Quantification analysis of cells with nuclear IRF3 localization post HT-DNA treatment in uninduced and p53R248W induced H1299 cells. (D) Representative fluorescent images depicting nuclear translocation of GFP-IRF3 in PLKO or mutant p53 knock down MDA-MB-231 cells. Cells were treated with 2 μg/ml HT-DNA for 3 hrs and IRF3 localization was visualized. Scale bar 20μm. Immunoblots showing p53 knock down efficiency and representative graph shows quantification of percent of cells with nuclear IRF3. (E) Graphical representation of apoptosis quantification by flow cytometry of PLKO or p53KD MDA-MB-231 cells that were treated with 2ug/ml of HT-DNA for 24 hrs. Immunoblots showing p53 knock down efficiency.

Quantification graphs: FoV= Field of View, (n=20) In all panels, error bars represent Mean +/- SD. p values are based on Student's t test. $^{***}p < 0.001$, $^{**}p < 0.01$.

Figure S3. Related to Figure 3

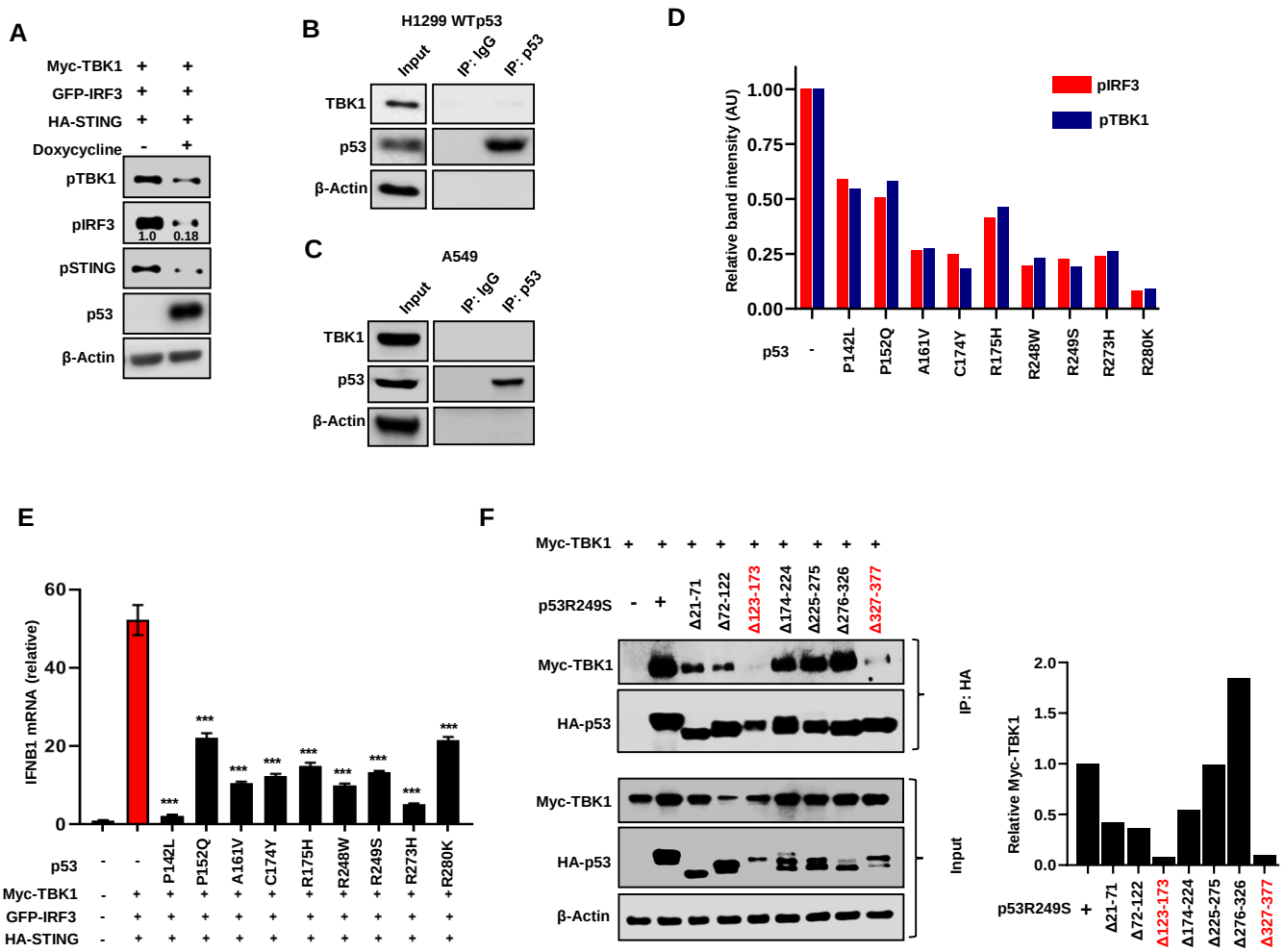


Figure S3. Mutant p53 prevents formation of the trimeric TBK1/STING/IRF3 complex, Related to Figure 3

(A) Myc-TBK1, GFP-IRF3 and HA-STING were co-transfected with or without p53R248W and subjected to western blot analysis. (B) p53 null H1299 cells expressing inducible WTp53 were induced with doxycycline treatment for 24 hrs and p53 was immunoprecipitated from the whole cell lysate. Lysates and immunoprecipitates (IP) were analyzed by western blotting.(C) Endogenous WTp53 in A549 was immunoprecipitated with p53 antibody. Cell Lysates and IP were analyzed by western blot. (D) Representative graph indicates relative pIRF3 and pTBK1 levels with each p53 mutants. (E) Quantification of IFNB1 mRNA expression in p53 null H1299 cells that were transfected with Myc-TBK1, GFP-IRF3 and HA-STING in absence or presence different p53 mutants. Cells were harvested after 24 hrs and RT-PCR for IFNB1 was performed. (F) H1299 cells were transfected with Myc-TBK1 and seven different deletion mutants of HA-p53R249S. Cells were lysed and mutant p53 was immunoprecipitated using HA antibody and analyzed by western blot. Representative graph indicated relative Myc-TBK1 IP'ed with different p53 mutants.

Quantification graphs: In all panels, error bars represent Mean +/- SD. p values are based on Student's t test. $^{***}p < 0.001$, $^{**}p < 0.01$.

Figure S4. Related to Figure 4

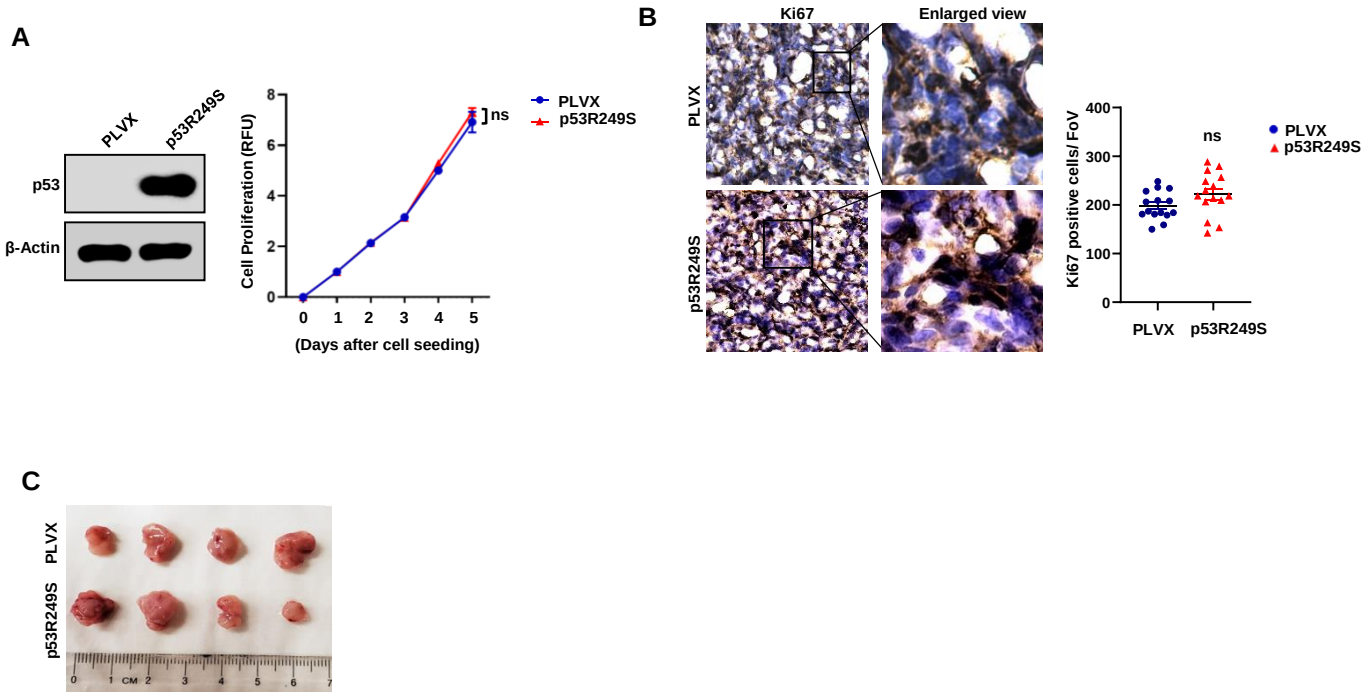


Figure S4. Mutant p53 tumors exhibit accelerated tumor growth in hosts with intact immune system, Related to Figure 4
(A) Immunoblots show p53 null 4T1 cells infected with PLVX or p53R249S expression vectors. Representative graphical quantification of *in vitro* cell proliferation between 4T1 PLVX and p53R249S cohorts. (B) Representative images and quantitation of tumor cryo-section stained with Ki67 in 4T1 PLVX and p53R249S tumors. (C) 5 x 10^4 4T1 cells expressing PLVX or p53R249S were injected into immunodeficient NOD/SCID mice and the image depicts similar tumor size between the two cohorts (n=4).
Quantification graphs: FoV= Field of View (n=15), Error bars represent Mean +/- SE. p values are based on Student's t test. ns=non-significant.

Figure S5. Related to Figure 5

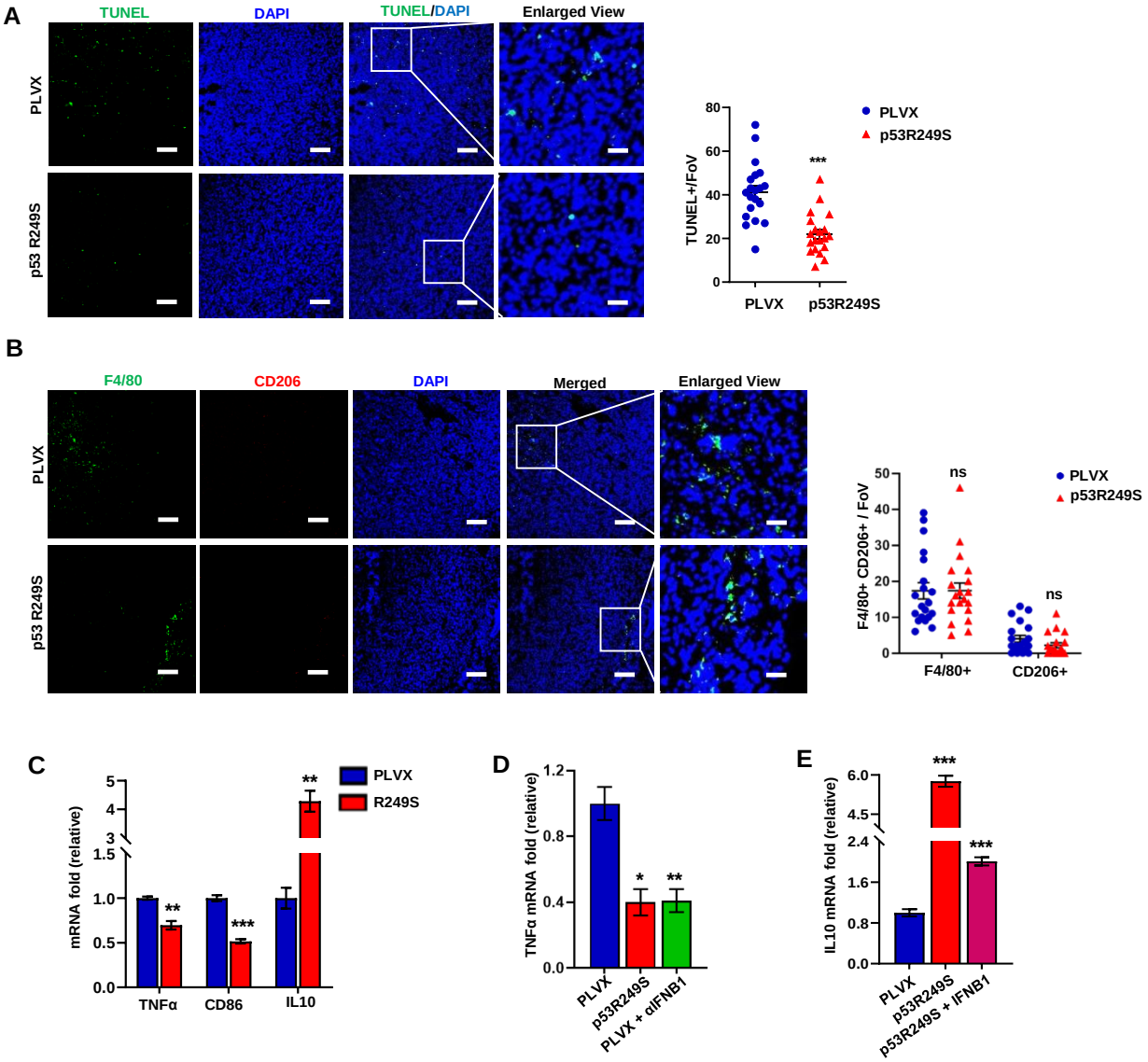


Figure S5. Mutant p53 suppresses immune surveillance to support tumor growth *in vivo*, Related to Figure 5

(A) Representative immunostaining for tumor cell death (TUNEL) in 4T1 PLVX and p53R249S tumor sections in Balb/C mice. (Right) Representative graph shows quantification of TUNEL positive cells (n=20). (B) Representative confocal images depicting the F4/80^{+}CD206^{+} M2 type of TAMs in PLVX and p53R249S expressing tumors isolated from NOD/SCID mice on Day 21. (Right) Representative graphs show quantitation of F480+, CD206+ M2 macrophage subsets in NOD/SCID mice (right) (n=20). (C) Quantification of the expression of the indicated mRNAs expressed in RAW264.7 cells when cultured either in PLVX or p53R249S expressing 4T1 cell conditioned media. (D) RT-PCR analysis of TNFα mRNA expression in RAW264.7 when cultured in PLVX conditioned media containing anti-αIFNB1 antibody. (E) RT-PCR analysis of IL-10 expression in RAW264.7 when cultured in p53R249S conditioned media supplemented with IFNB1.

Scale bar 25 μm except in enlarged panel which is 100 μm. Quantification graphs: Figure S5A and S5B error bars represent Mean +/- SE and in Figure S5C, S5D and S5E error bars represent Mean +/- SD. p values are based on Student's t test. $^{***}p < 0.001$, $^{*}p < 0.05$, ns=non-significant.

Figure S6. Related to Figure 6

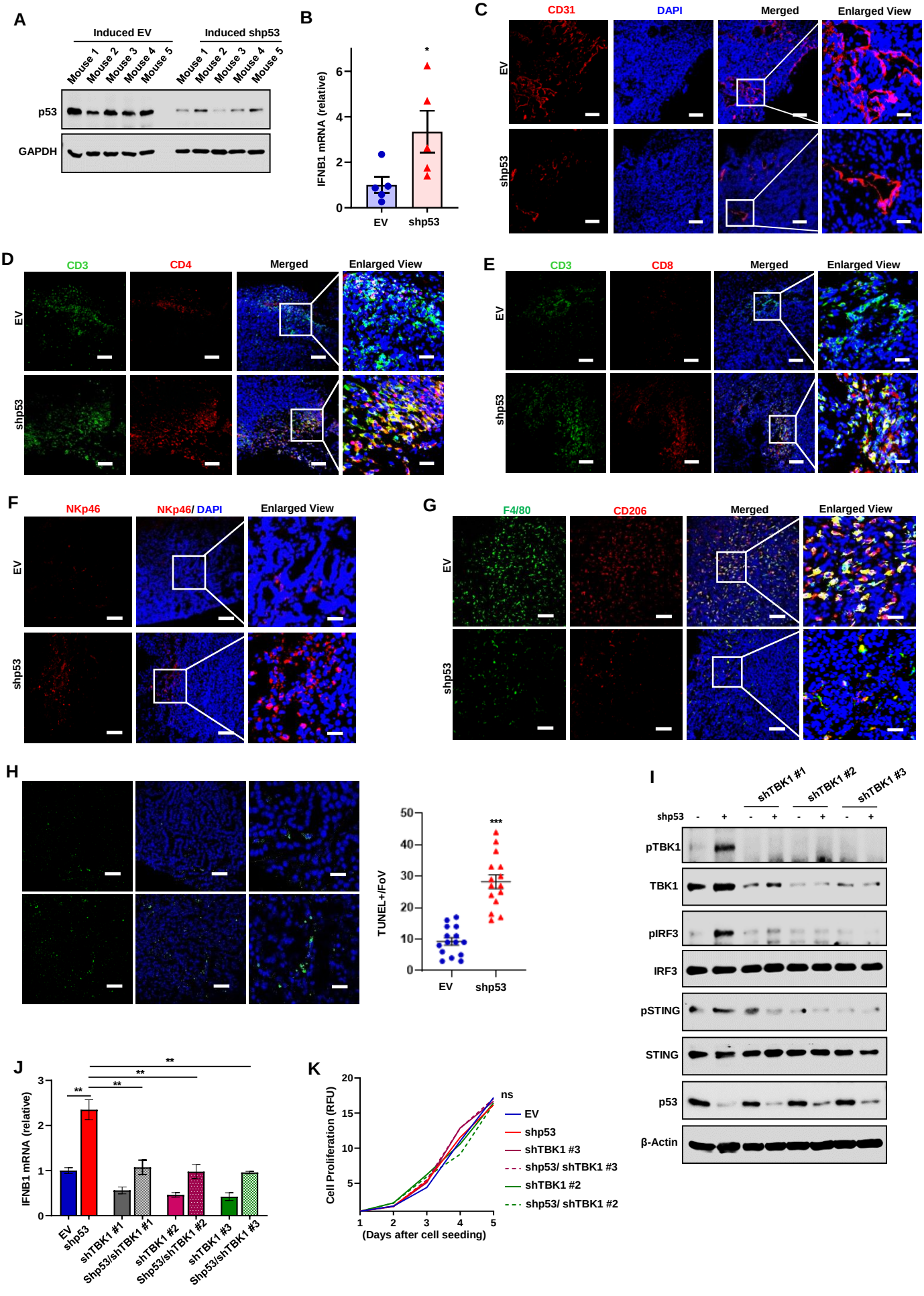
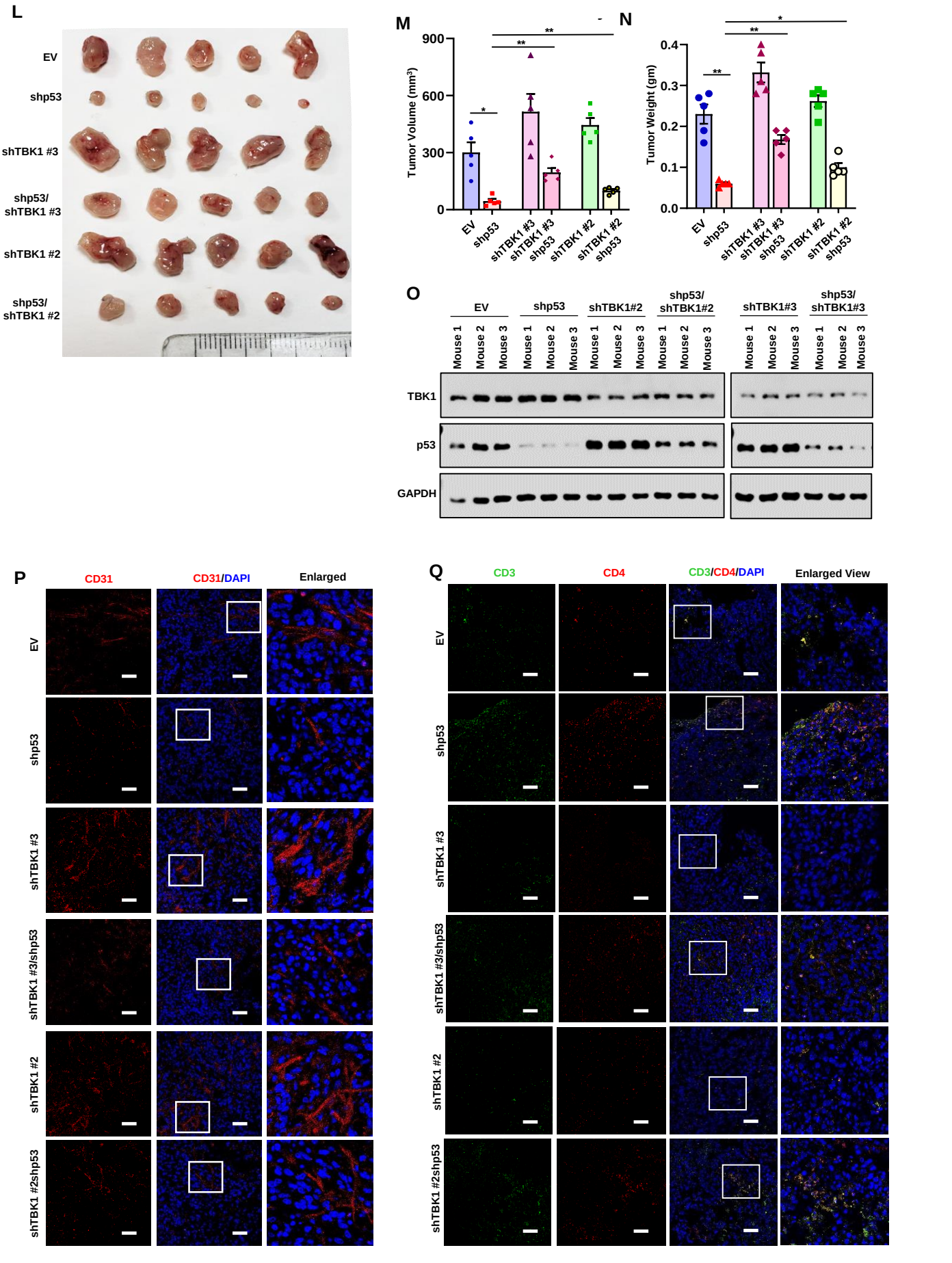


Figure S6. Related to Figure 6, Continue



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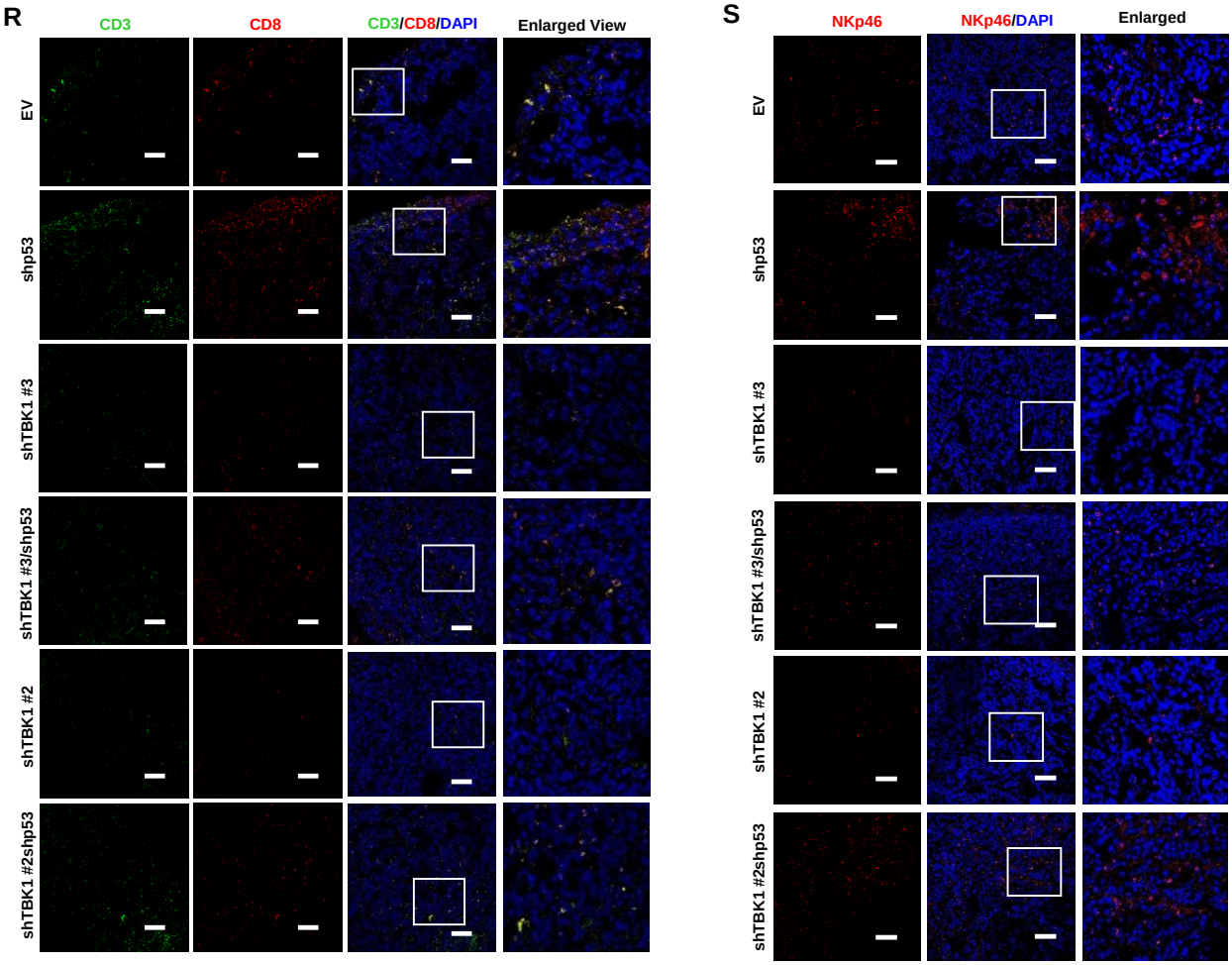

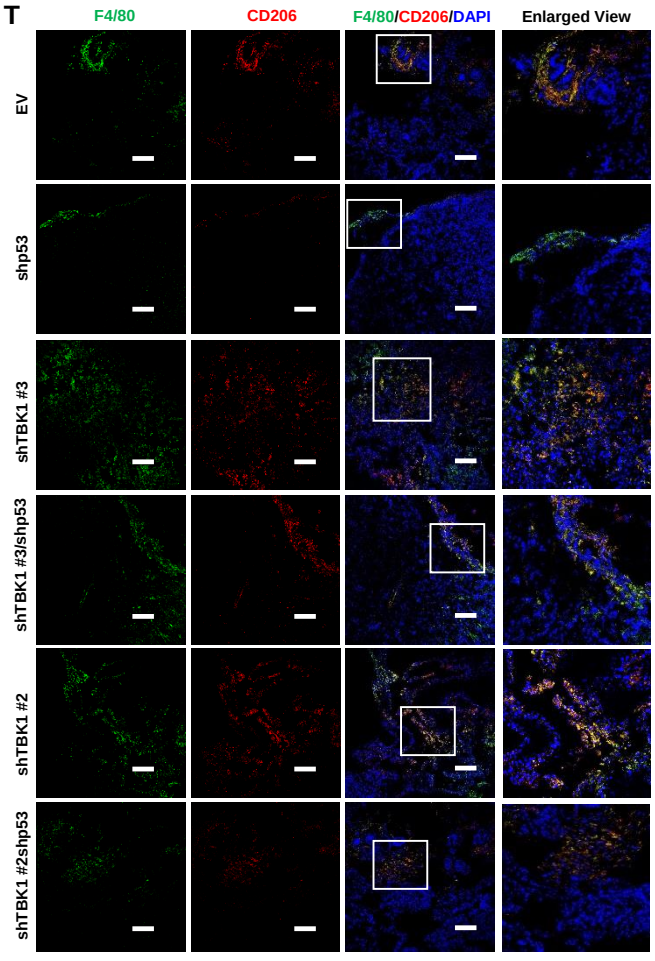

Figure S6. Related to Figure 6, Continue

Figure S6. Loss of mutant p53 triggers immune surveillance in a TBK1-dependent manner, Related to Figure 6
(A) Representative immunoblots showing p53 knockdown efficiency in EV and shp53 KPC tumor cell lysates. (B) Representative graph showing IFNB1 mRNA in EV and shp53 KPC tumor. (C) Representative confocal micrographs of EV and shp53 KPC tumor sections stained with angiogenic marker CD31. Representative confocal micrographs of (D) $CD3^+CD4^+$ T-helper and (E) $CD3^+CD8^+$ cytotoxic T-lymphocyte infiltration in KPC induced EV or shp53 tumor sections. (F) Representative confocal micrographs depicting the expression of NKp46 positive NK cells in EV or shp53 induced KPC tumors. (G) Representative confocal images depicting the $F4/80^+CD206^+$ M2 type of TAMs in EV and shp53 tumors isolated from C57BL/6 mice. (H) Representative immunostaining for TUNEL in KPC tumor sections. (Right) Representative graph showed quantification of TUNEL positive cells. (I-K) KPC shTBK1 cells were induced for 24 hrs to express EV or shp53. Immunoblots representing TBK1 knockdown status in presence and absence of shp53 (I). Quantitative RT-PCR analysis of IFNB1 mRNA expression in the indicated cells (J). Representative graphical quantification of *in vitro* cell proliferation between indicated KPC cells (K). (L) Representative image showed tumor volume difference between TBK1 knockdown EV and shp53 KPC tumors. (M-N) Representative graphs indicate tumor volume (M) and tumor weight (N) in TBK1 knockdown EV and shp53 KPC tumors. (O) Representative immunoblots showing TBK1 knockdown efficiency in presence and absence of mutant p53 *in vivo* in indicated KPC tumors. (P) Representative confocal micrographs of TBK1 knockdown EV and shp53 KPC tumor sections stained with angiogenic marker CD31. (Q-R) Representative confocal micrographs depicts recruitment of cytotoxic $CD3^+CD4^+$ T-helper cells (Q) and Cytotoxic $CD3^+CD8^+$ T-cells (R) infiltration in the tumor microenvironment of the indicated tumor sections. (S-T) Representative confocal micrographs depicts recruitment of $NKp46^+$ NK cells (S) and $F480^+CD206^+$ M2 macrophage (T) infiltration in indicated tumor sections.
Scale bar 25 μm except in enlarged panel which is 100 μm. Quantification graphs: In all panels, error bars represent mean +/- SE except in Figure S6J, where data presented as Mean +/- SD. In scatter dot plots, each dots represent one mice, p values are based on Student's t test. $^{***}p < 0.001$, $^{*}p < 0.05$, ns=non-significant.

Figure S7. Related to Figure 7

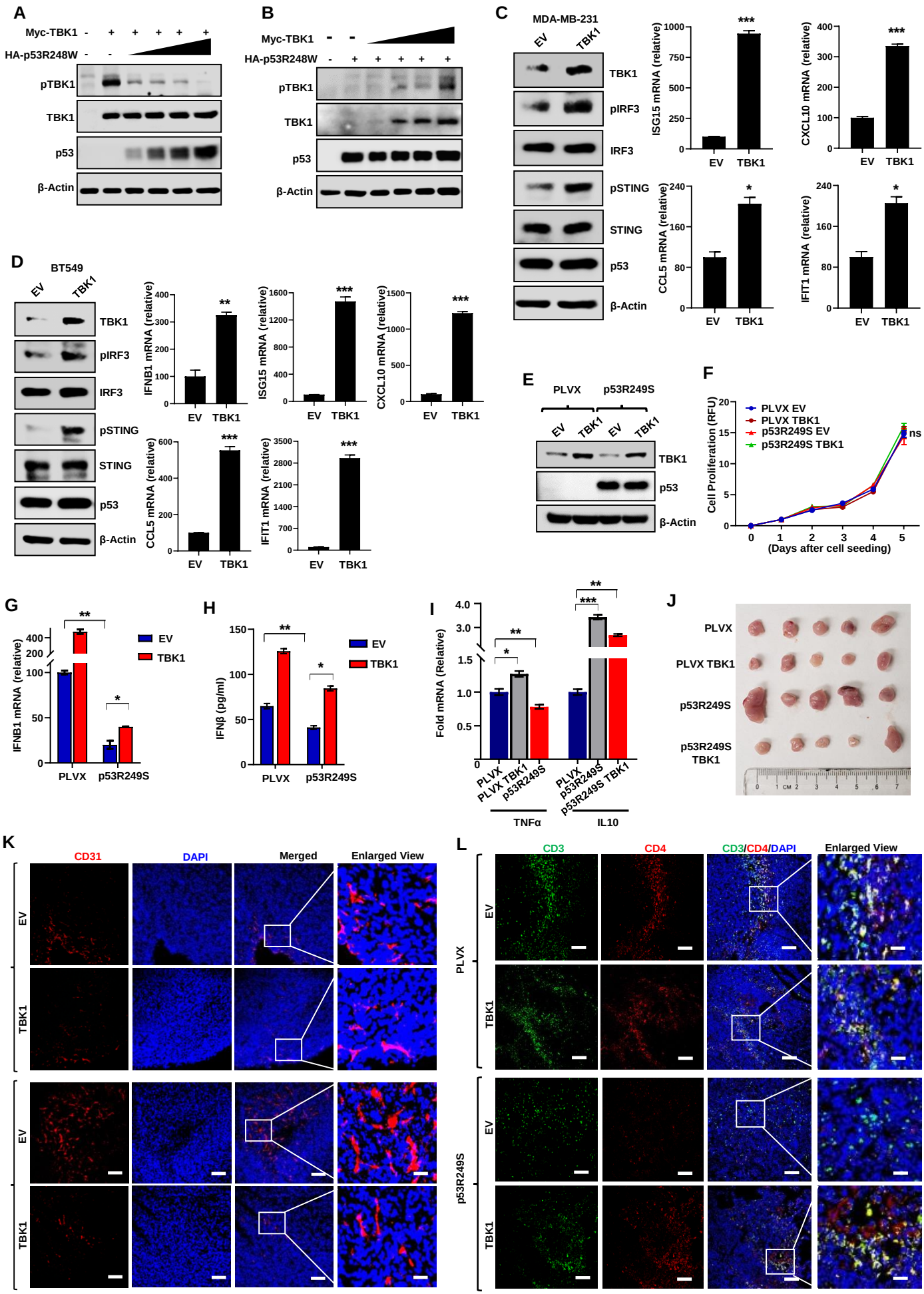

Figure S7. Related to Figure 7, Continue

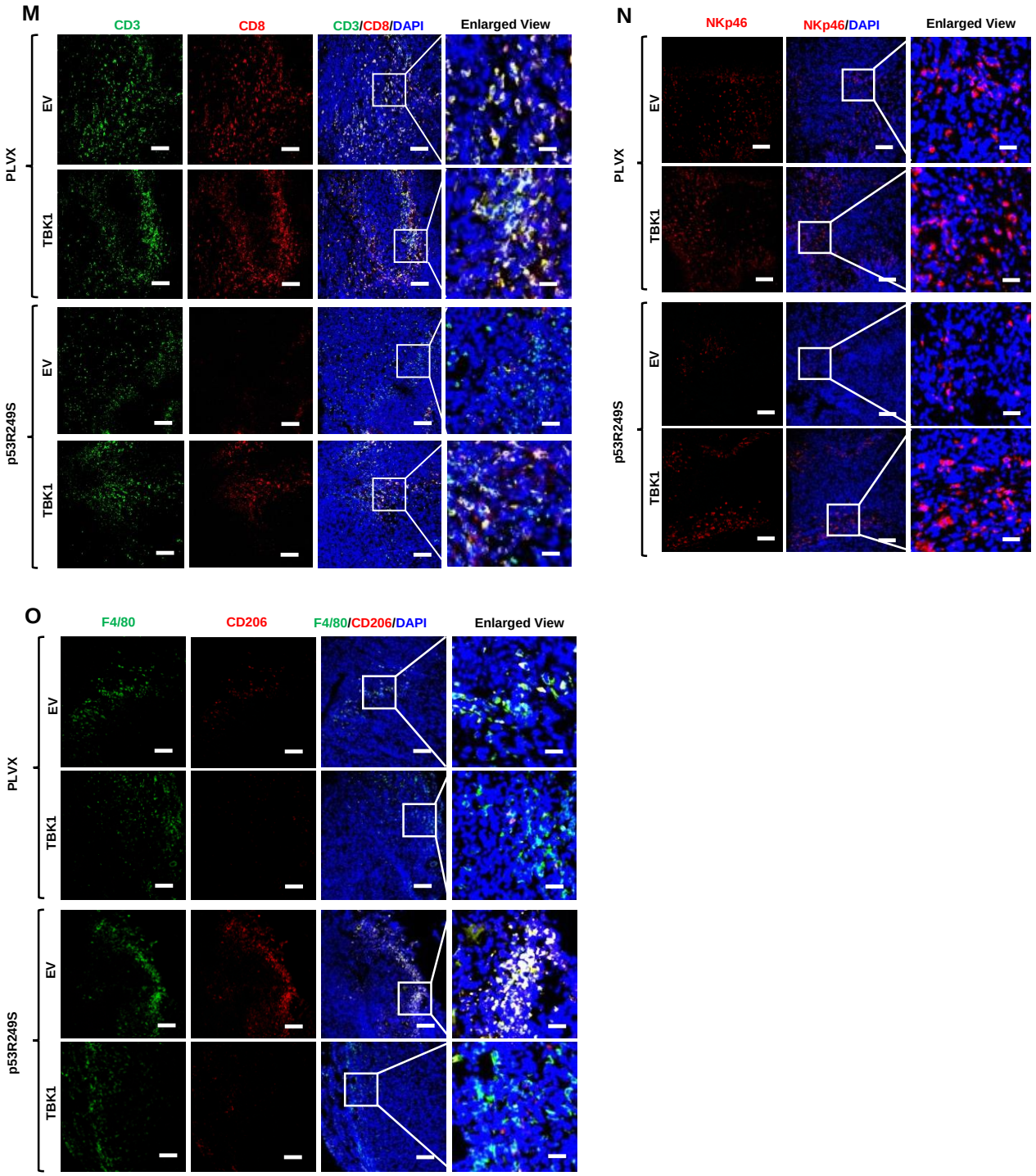

Figure S7. Ectopic TBK1 expression overrides mutant p53's effect, Related to Figure 7

(A) In H1299 cells, Myc-TBK1 was co-transfected with increasing amount of HA-p53R248W and subjected to western blot. (B) In H1299 cells, HA-p53R248W was co-transfected with increasing amount of Myc-TBK1 and subjected to western blot. (C-D) Immunoblots showing that overexpression of TBK1 in mutant p53 harboring MDA-MB-231 (C) and BT549 (D) cells leads to the phosphorylation of IRF3 and STING. Quantitative graphs showing mRNA expression of the indicated cytokines upon TBK1 overexpression. (E) 4T1 PLVX or p53R249S expressing 4T1 cells were induced with doxycycline for 24 hrs to induce TBK1. Immunoblots representing TBK1 expression status within the cells. (F) Representative graphical quantification of *in vitro* cell proliferation between indicated cohorts. (G) Quantitative RT-PCR analysis of IFNB1 mRNA expression in the indicated cells. (H) Graph represents quantitative IFNB1 secretion as analyzed using ELISA. (I) Quantification of the expression of mRNA of indicated genes in RAW264.7 cells when cultured in PLVX and p53R249S induced TBK1 expressing 4T1 cell conditioned media. (J) 5 x 10^4 4T1 PLVX and p53R249S cells were injected into BALB/c mice and 20 mg/kg of Doxycycline was administered orally to induce TBK1 (n=5). Image depicts tumor volume of the indicated cohorts. (K) Confocal micrographs depict CD31 expression in the respective tumor cryo-sections. (L-O) Representative confocal micrographs depicts recruitment of cytotoxic $CD3^+CD4^+$ T-helper cells (L) Cytotoxic $CD3^+CD8^+$ T-cells (M) NKp46+ NK cells (N) and F480+CD206+ M2 macrophage (O) infiltration to the tumor microenvironment in the indicated tumor sections.

Quantification graphs: In all panels error bars represent mean +/- SD. p values are based on Student's t test. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, ns=non-significant. Scale bar 25 μm except in enlarged panel which is 100 μm.

Table S2: List of primers, Related to STAR Methods

Gene name	Forward Primer	Reverse Primer	Comments
IRF3 #2	CACCGCCAGTGGTGCCTACACCCCG	AAACCGGGGTGTAGGCACCACTGGC	sgRNA targeting human IRF3
IRF3 #3	CACCGGCAACACTTCTTTCCGGTTC	AAACGAACCGGAAAGAAGTGTTGCC	sgRNA targeting human IRF3
p53 (h) #1	CTAGCGACTCCAGTGGTAATCTACTTACTAGTAGTAGATTACCACTGGAGTCttttttG	AATTCAAAAAGACTCCAGTGGTAATCTACTACTAGTAAGTAGATTACCACTGGAGTCG	Inducible shRNA targeting human p53
p53 (h) #2	CTAGCCAGCATCTTATCCGAGTGGAATACTAGTTTCCACTCGGATAAGATGCTGttttttG	AATTCAAAAACAGCATCTTATCCGAGTGGAAACTAGTATTCCACTCGGATAAGATGCTGG	Inducible shRNA targeting human p53
p53 (m)	CTAGCGTACATGTGTAATAGCTCCTACTAGTGGAGCTATTACACATGTACTTTTTG	AATTCAAAAAGTACATGTGTAATAGCTCCACTAGTAGGAGCTATTACACATGTACG	shRNA targeting mouse p53
PLVXTBK1	ATATCTCGAGATGCAGAGCACTTCTAATCATCTG	ATATGCGGCCGCCTAAAGACAGTCAACGTTGCGA	Sub cloned in PLVX
MYC-TBK1	ATATGTCGACCATGCAGAGCACTTCTAATCATCTG	ATATGCGGCCGCCTAAAGACAGTCAACGTTGCGA	Sub cloned in PCMV
HA-STING	ATATGCGGCCGCCTAAAGACAGTCAACGTTGCGA	ATATGCGGCCGCTCAAGAGAAATCCGTGCGG	Sub cloned in PCMV
HA-p53R249SΔ21-71	CCCGTGGCCCCTGCACCA	TGAAAATGTTTCCTGACTCAGAGGGGGCTC	Site directed mutagenesis
HA-p53R249SΔ72-122	ACTTGCACGTACTCCCCTG	GGGAGCAGCCTCTGGCAT	Site directed mutagenesis
HA-p53R249SΔ123-173	AGGCGCTGCCCCCACCAT	CACAGACTTGGCTGTCCCAGAATGC	Site directed mutagenesis
HA-p53R249SΔ174-224	GTTGGCTCTGACTGTACC	CACAACCTCCGTCATGTG	Site directed mutagenesis
HA-p53R249SΔ225-275	GCCTGTCCTGGGAGAGAC	CTCAGGCGGCTCATAGGG	Site directed mutagenesis
HA-p53R249SΔ276-326	TATTTCACCCTTCAGATCC	ACAAACACGCACCTCAAAG	Site directed mutagenesis
HA-p53R249SΔ327-377	TCCCGCCATAAAAAACTC	TTCTCCATCCAGTGGTTTC	Site directed mutagenesis
IFNβ (h)	GTCAGAGTGGAAATCCTAAG	TATGCAGTACATTAGCCATC	qPCR primers
IFIT1 (h)	TACAGCAACCATGAGTACAA	TCACATAGGCTAGTAGGTTG	qPCR primers
CCL5 (h)	AGCAGTCGTCTTTGTCAC	TAGCTCATCTCCAAAGAGTT	qPCR primers
CXCL10 (h)	TACCTGCATCAGCATTAGTA	TGTAGCAATGATCTCAACAC	qPCR primers
ISG15 (h)	GAACTCATCTTTGCCAGTA	ATCTTCTGGGTGATCTGC	qPCR primers

Table S2: List of primers, Related to STAR Methods, Continue

Gene name	Forward Primer	Reverse Primer	Comments
IL6 (h)	CCCCCAATAAATATAGGACT	GATAGAGCTTCTCTTTCGTT	qPCR primers
IFNβ (m)	AAGATCAACCTCACCTACAG	AAAGGCAGTGTAACTCTTCT	qPCR primers
CCL5 (m)	AGTGGGTTCAAGAATACATC	CTAGGACTAGAGCAAGCAAT	qPCR primers
CXCL10 (m)	AAGTTTACCTGAGCTCTTTT	AGTATCTTGATAACCCCTTG	qPCR primers
ISG15 (m)	ACAGTGATGCTAGTGGTACA	AAGACCTCATAGATGTTGCT	qPCR primers
IL6 (m)	GTCTTCTGGAGTACCATAGC	TATCTGTTAGGAGAGCATTG	qPCR primers
TNFα	ATCTTCTCAAAATTCGAGTG	ACCACTAGTTGGTTGTCTTT	qPCR primers
CD86	GAACAACCAGACTCCTGTAG	GTCACAAAGATAAGGATTGC	qPCR primers
IL10	TTACCTGGTAGAAGTGATGC	TGTAGACACCTTGGTCTTG	qPCR primers