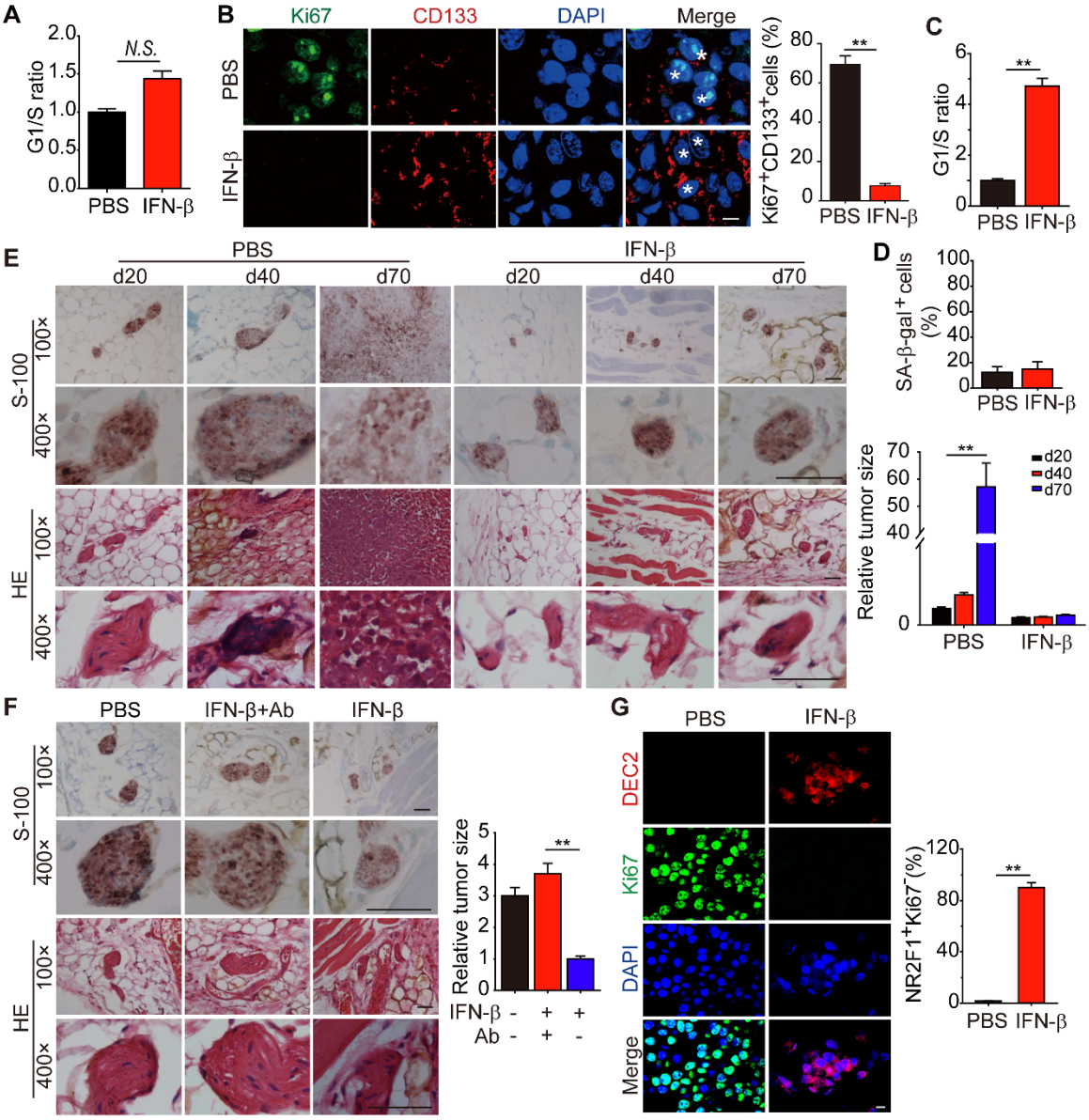
Supplemental figures and figure legends

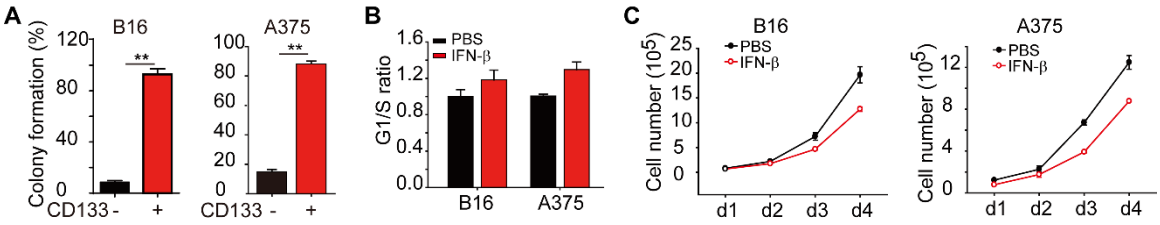
Figure S1



Supplemental Figure 1 IFN-β induces TRC dormancy in vivo. (**A**) Mice ($n = 5$) bearing B16 melanoma were intratumorally treated with IFN-β once per day for 3 days. Tumor cells isolated were stained with 7AAD, PE-conjugated anti-CD133 and APC-conjugated anti-CD45. The $CD45^-CD133^-$ tumor cells were gated to for cell cycle analysis ($n = 5$). (**B**)

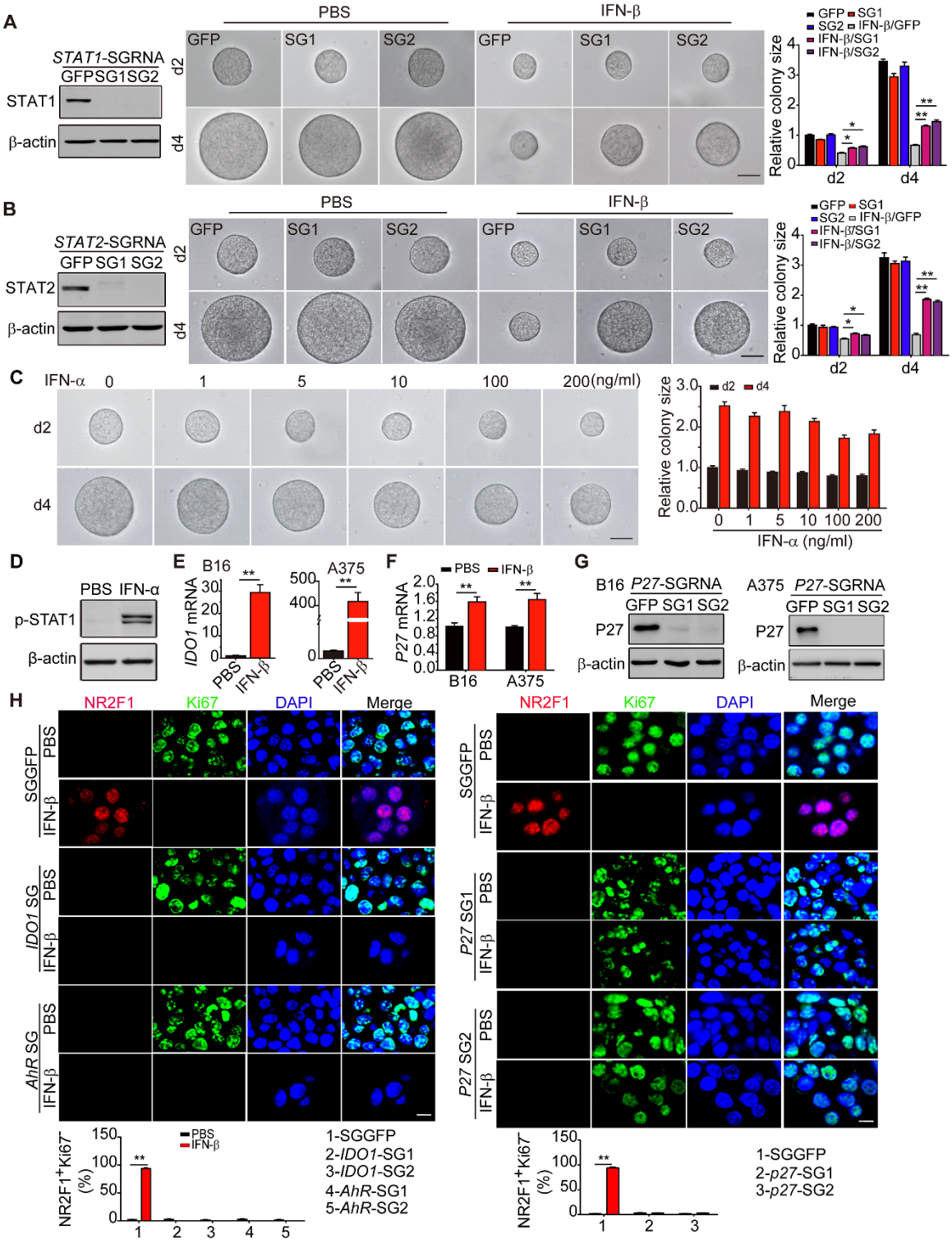
Mice with B16 melanoma were intratumorally treated with IFN-β once per day for 3 days. Tumor were immunostained with anti-Ki67 (green), CD133 (red) and DAPI (blue). $Ki67^+$ cell numbers were counted among 500 $CD133^+$ cells using 10 non-consecutive sections and the percentage of $Ki67^+CD133^+$ cells was determined (n = 5). *, $CD133^+$ tumor cells. Bar, 10 μm. (**C, D**) NOD-SCID mice bearing A375 melanoma were intratumorally treated with IFN-β once per day for 3 days. $CD133^{high}$ tumor cells were performed cell cycle analysis (**C**, n = 5) or stained with SA-β gal (**D**, n = 5). (**E**) A375 TRCs were injected to NOD-SCID mice. On day 3, IFN-β was injected into tumor site once every two days. On day 20, 40 and 70, tumor cell-injected tissues were immunostained against S100β or H&E staining. Tumor size was presented photographically (left) and graphically (right) (n = 6). (**F**) The same as (**E**), but mice were treated with IFN-β for 10 days, and further treated with IFN-β or IFN-β + anti-IFN-β antibody once every two days for 5 days. Injected tissues were immunostained against S100β or H&E staining (n = 6). Bar, 50 μm. (**G**) The same as (**E**), but cell-injected tissues with 20 days IFN-β treatment were immunostained against DEC2 and Ki67 (n = 5). Bar, 10 μm. Data represent mean ± SEM. **P<0.01 and *N.S.*, no significant difference, by 2-tailed Student's t test (**A-D** and **G**) and 1-way ANOVA (**E** and **F**).

Figure S2



Supplemental Figure 2 The effect of IFN-β on differentiated melanoma cells. (**A**) C57BL/6 or NOD-SCID mice with 5×5 mm B16 or A375 melanoma were intratumorally treated with 250 ng IFN-β once per day for 3 days. $CD133^{high}$ or $CD133^{-}$ tumor cells were sorted by flow cytometry and seeded in the soft 3D fibrin gels for 4 days. The colony number was counted (n = 5). (**B**) B16 or A375 cells growing in the rigid flask were treated with IFN-β (5 ng/ml) for 72h. The cell cycle analysis was performed (n = 3). (**C**) Same as (**B**), but cells were treated for indicated time and the cell number was counted (n = 3). The data represent mean ± SEM. $^{**}P<0.01$, by 2-tailed Student's t test (**A** and **B**)

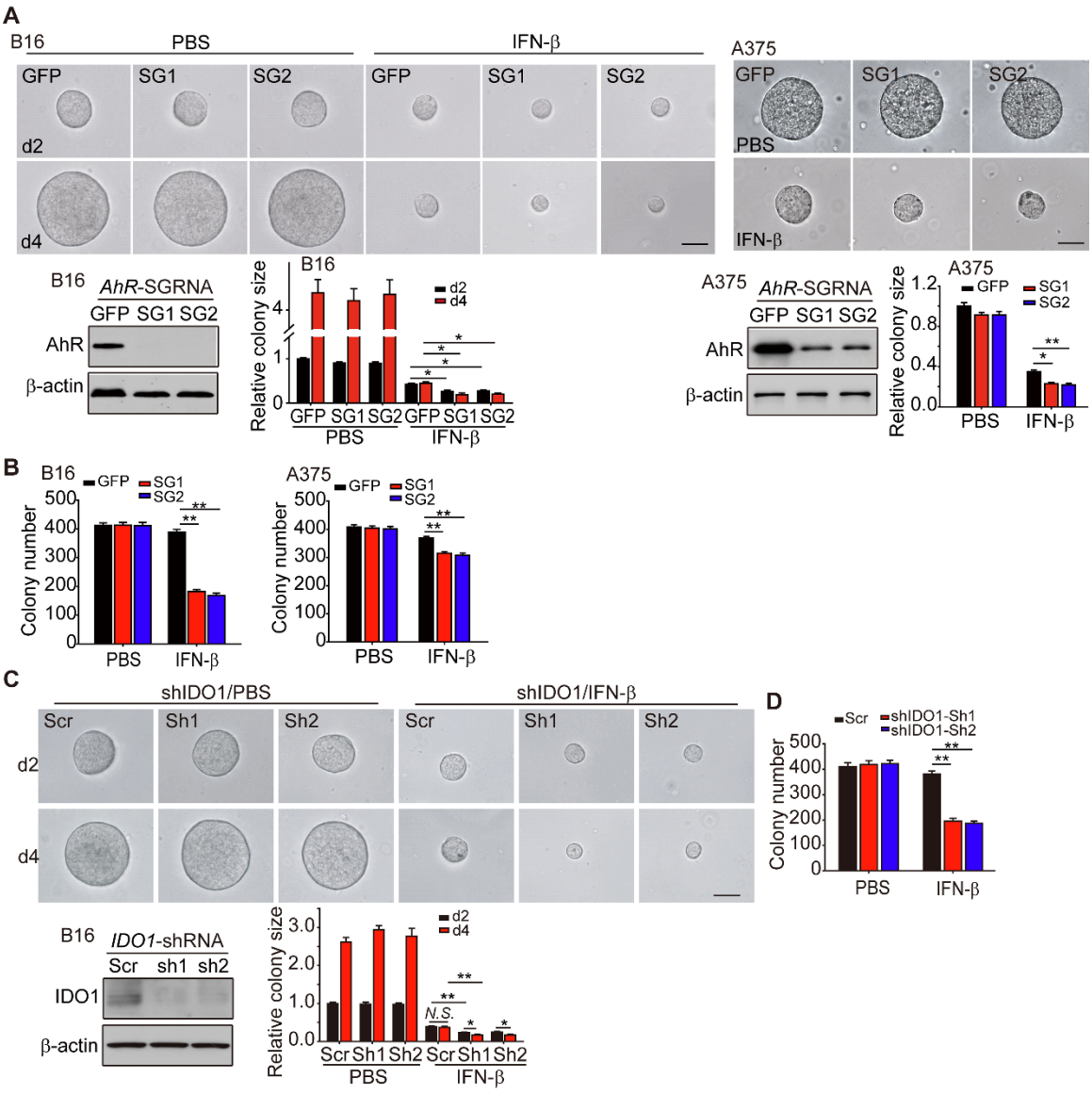
Figure S3



Supplemental Figure 3 STATs-IDO1signaling pathway is involved in IFN-β induced TRC dormancy. (**A**) B16-STAT1-SGGFP and B16-STAT1-SGs TRCs were treated with

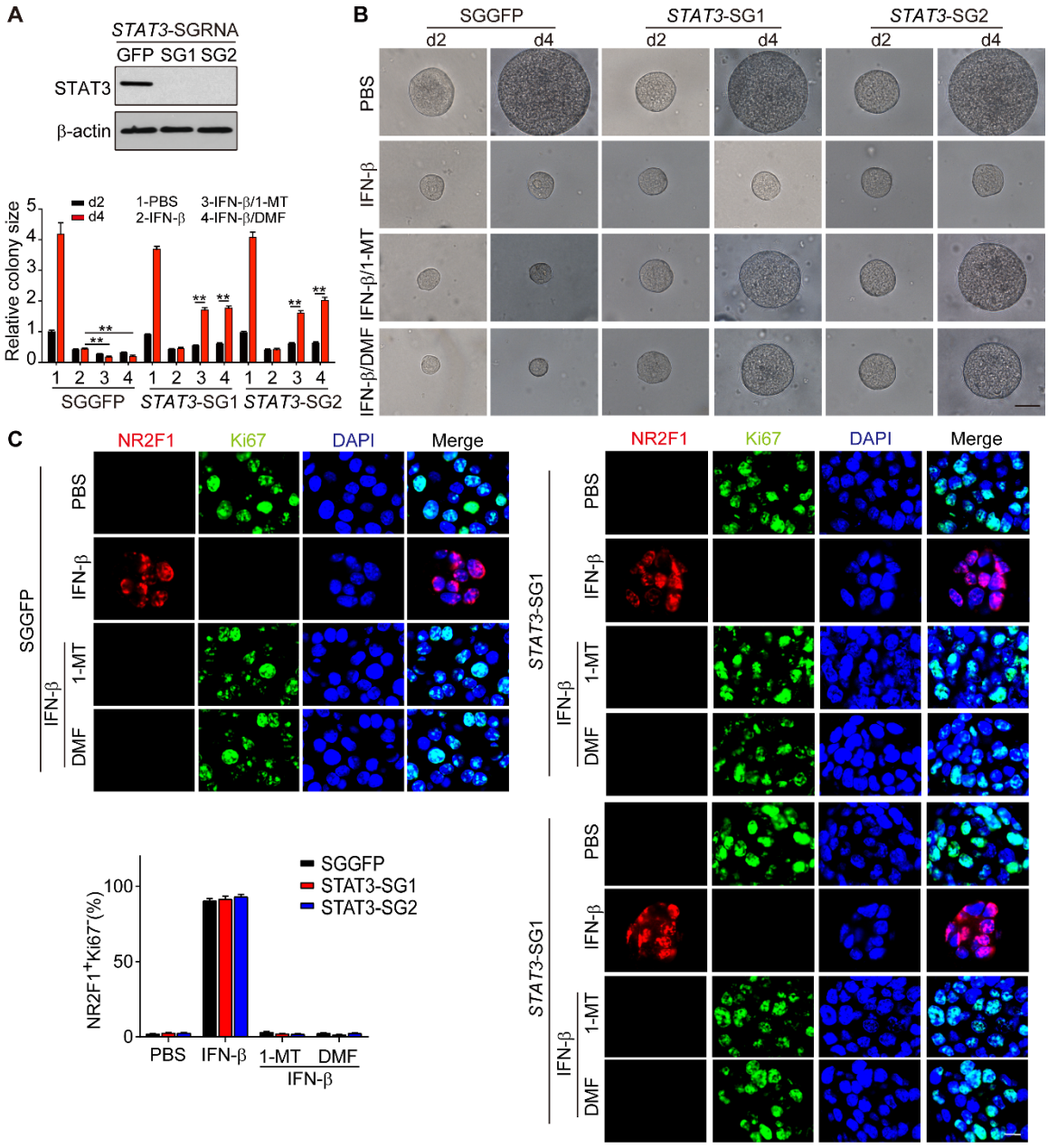
IFN-β (5 ng/ml) for 2 days (d2) or 4 days (d4). The efficiency of *STAT1* knockdown was determined by western blot (left). The colony size was represented (middle) and quantified (right). Bar, 50 μm. (**B**) B16-*STAT2*-SGGFP and B16-*STAT2*-SGs TRCs were treated with IFN-β for 2 or 4 days. The efficiency of *STAT2* knockdown was determined by western blot (left). The colony size was represented (middle) and quantified (right). Bar, 50 μm. (**C**) B16 TRCs in 90 Pa 3D fibrin gel were treated with different dose of IFN-α. The colony size was represented (left) and quantified (right). Bar, 50 μm. (**D**) B16 TRCs were treated with IFN-α (5 ng/ml) for 24h. The cell lysates were extracted to perform western blot analysis. (**E, F**) The mRNA expression of *IDO1* (**E**) or *p27* (**F**) in B16 or A375 TRCs treated with IFN-β (5 ng/ml) for 48h. (**G**) The efficiency of *p27* knockdown was determined by western blot. (**H**) SG-GFP, *IDO1*-SG, *AhR*-SG or *p27*-SGs B16 TRCs were treated with IFN-β (5 ng/ml) for 72h. The cells were then fixed and immunostained with anti-NR2F1 and Ki-67 antibodies. Bar, 10 μm. Graphs represent mean ± SEM of 3 independent experiments. **P*<0.05 and ***P*<0.01, by 1-way ANOVA (**A** and **B**) and 2-tailed Student's t test (**E, F** and **H**).

Figure S4



Supplemental Figure 4 Blocking IDO-AhR pathway abrogates dormant melanoma TRCs. (**A** and **B**) *AhR*-SGGFP and AhR-SGs-B16 or A375 TRCs were seeded in 3D fibrin gels for 2 days. Some cells were lysed to detect the efficiency of *AhR* knocking out (**A**, left). Some cells were then treated with IFN-β (5 ng/ml) for another 2 days (d2) or 4 days (d4). The colony size was represented and quantified (**A**, right) and the colony number was counted (**B**). Bar, 50 μm. (**C** and **D**) The same as (**A** and **B**), but scramble and B16-IDO1-shRNAs TRCs were used. The relative colony size was calculated by comparing the colony size in groups with that in GFP (d2) group, which was set to 1. Bar, 50 μm. Graphs represent mean ± SEM of 3 independent experiments. **P*<0.05, ***P*<0.01 and *N.S.*, no significant difference, by 1-way ANOVA (**A-D**).

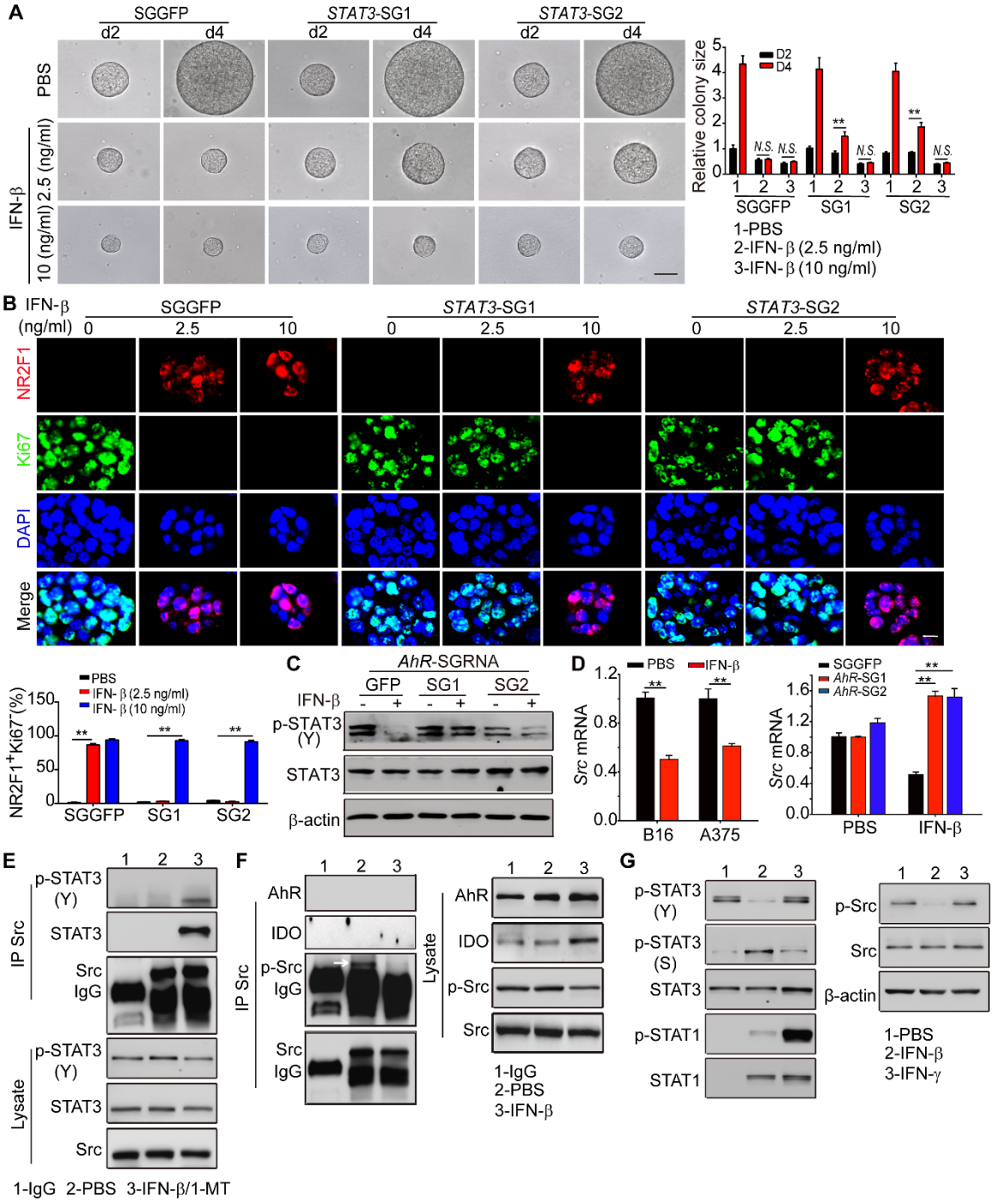
Figure S5



Supplemental Figure 5 STAT3 knockout rescues the death of dormant TRC by IFN-β/1-MT or DMF. (**A**) *STAT3*-SGGFP and *STAT3*-SGs-B16 TRCs were seeded in 3D fibrin gels for 2 days. STAT3 knockout efficiency was determined. (**B**) *STAT3*-SGGFP and *STAT3*-SGs-B16 TRCs were treated with IFN-β (10 ng/ml) for 2 days (d2) or 4 days (d4). Colony size was represented and quantified. Bar, 50 μm. (**C**) *STAT3*-SGGFP and *STAT3*-

SGs-B16 TRCs were treated with IFN-β for 72h. Cells were immunostained with anti-NR2F1 and Ki-67 antibodies. The percentage of $NR2F1^{+}Ki67^{-}$ cells were calculated. Bar, 10 μm. Graphs represent mean ± SEM of 3 independent experiments. $^{**}P<0.01$, by 1-way ANOVA (**B**)

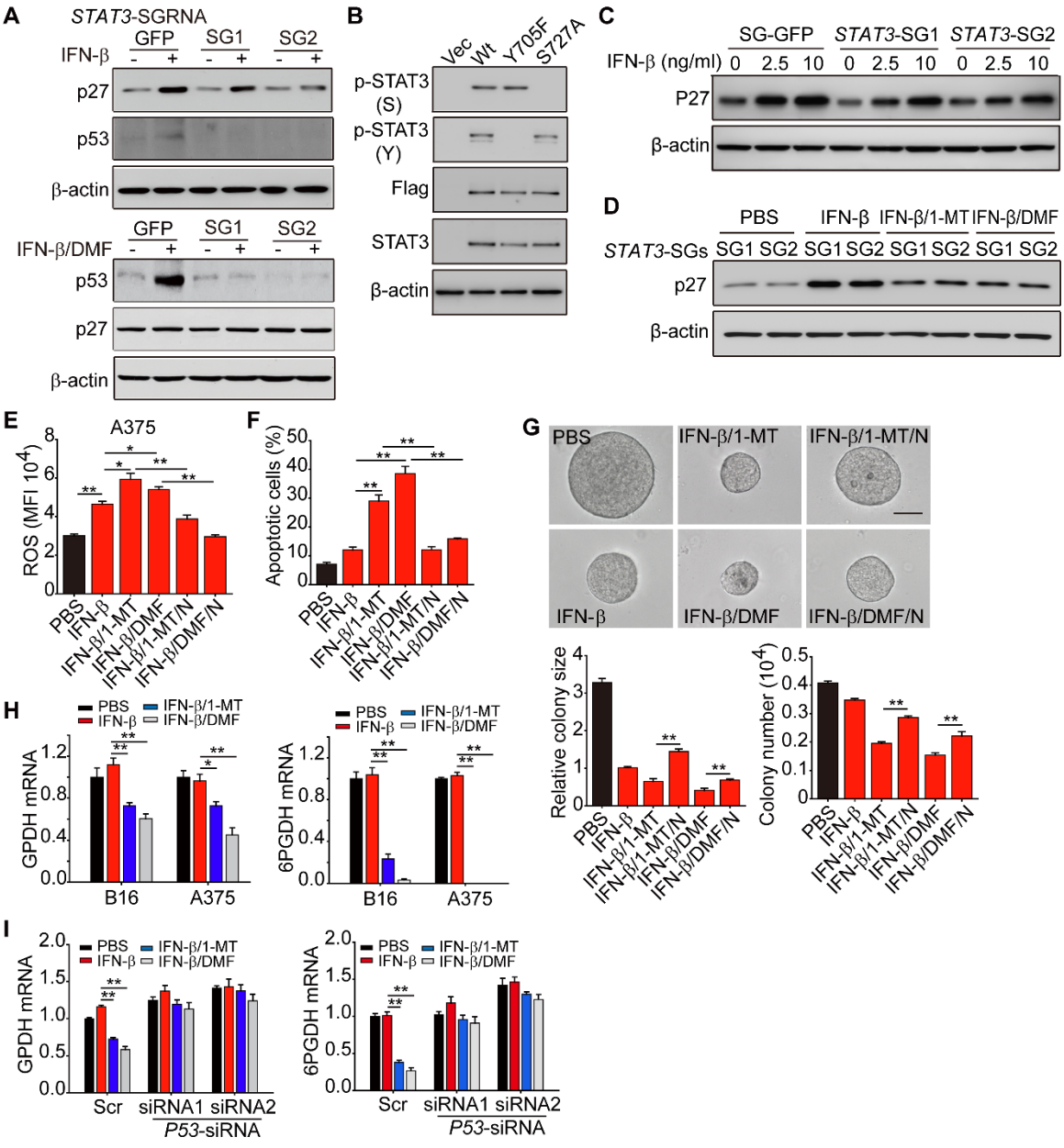
Figure S6



Supplemental Figure 6 Src-STAT3 signaling regulates dormant TRC apoptosis by IFN-β/1-MT or DMF. (**A**) SGGFP or *STAT3*-SGs B16 TRCs were treated with IFN-β (2.5 or 10 ng/ml) for 48h. Colony size was present (up) and quantified (down). Bar, 50 μm. (**B**)

STAT3-SGGFP and *STAT3*-SGs-B16 TRCs were treated with IFN-β (5 or 10 ng/ml) for 72h. Cells were immunostained with anti-NR2F1 and Ki-67 antibodies. Bar, 10 μm. (**C**) Western blot analysis of p-STAT3 (Y) and STAT3 in B16-*AhR*-SGGFP and B16-*AhR*-SGs TRCs treated with IFN-β (5 ng/ml) for 48h. (**D**) The *Src* mRNA expression from B16 TRCs, A375 TRCs (left), B16-*AhR*-SGGFP and B16-*AhR*-SGs TRCs (right) treated with or without IFN-β for 48h (right). (**E**) B16 TRCs were treated with IFN-β/1-MT for 48h. Cell lysates were immunoprecipitated with anti-Src antibody and blotted with anti-p-STAT3 (Y), STAT3 or Src antibody. (**F**) B16 TRCs were treated with IFN-β for 48h. Cell lysates were immunoprecipitated with anti-Src antibody and blotted with anti-AhR, IDO1, p-Src or Src antibody. (**G**) B16 TRCs were treated with IFN-β or IFN-γ for 48h. Cell lysates were extracted for western blot analysis of STAT1, STAT3, Src and their phosphorylated forms. Graphs represent mean ± SEM of 3 independent experiments. $^{**}P<0.01$ and *N.S.*, no significant difference, by 1-way ANOVA (**B** and **D**, right) and 2-tailed Student's t test (**A** and **D**, left).

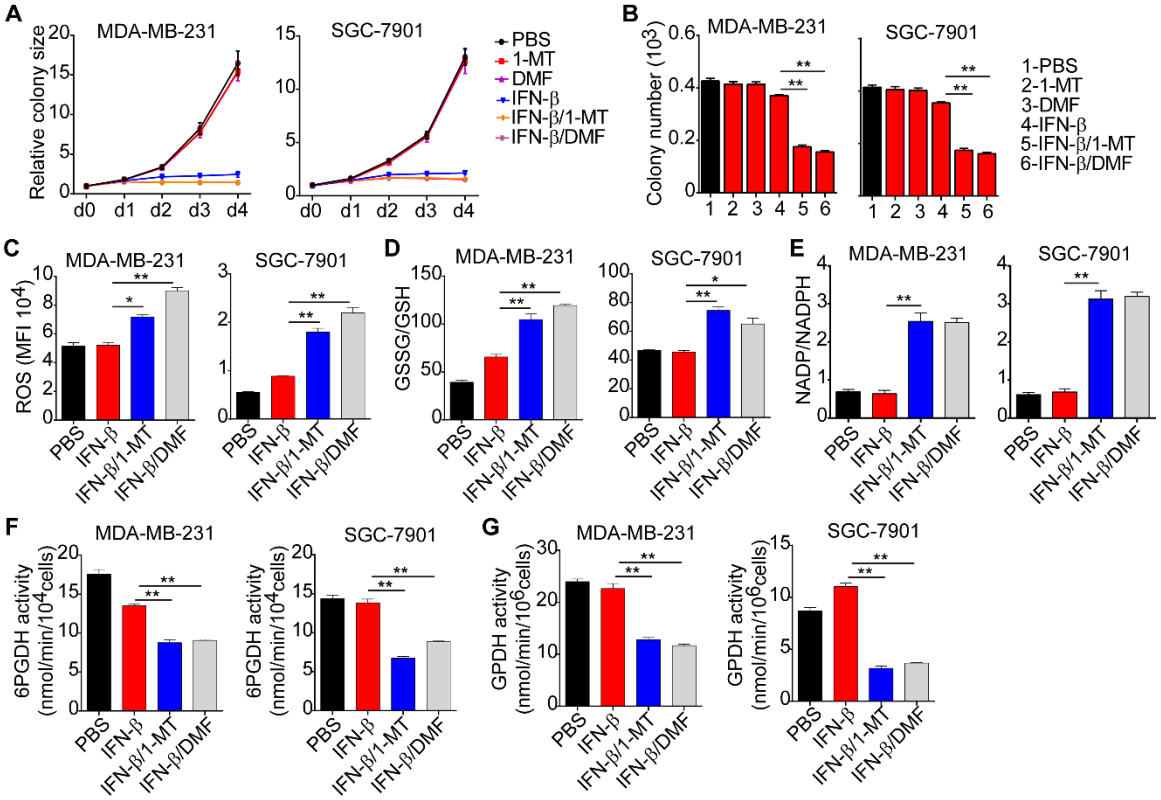
Figure S7



Supplemental Figure 7 p53-GPDH/6PGDH-ROS pathway participates in regulating TRC apoptosis. (**A**) Western blot analysis of p27 and p53 from STAT3-SGRNA-GFP or SGs-B16 TRCs treated with or without IFN-β or IFN-β/DMF for 48h. (**B**) *STAT3*-SGRNA-B16 cells stably expressing Flag-Wt-STAT3, Flag-Y705F-STAT3 or Flag-S727A-STAT3 were seeded in 3D fibrin gels and then treated with IFN-β (5 ng/ml) for 24h. The cells were

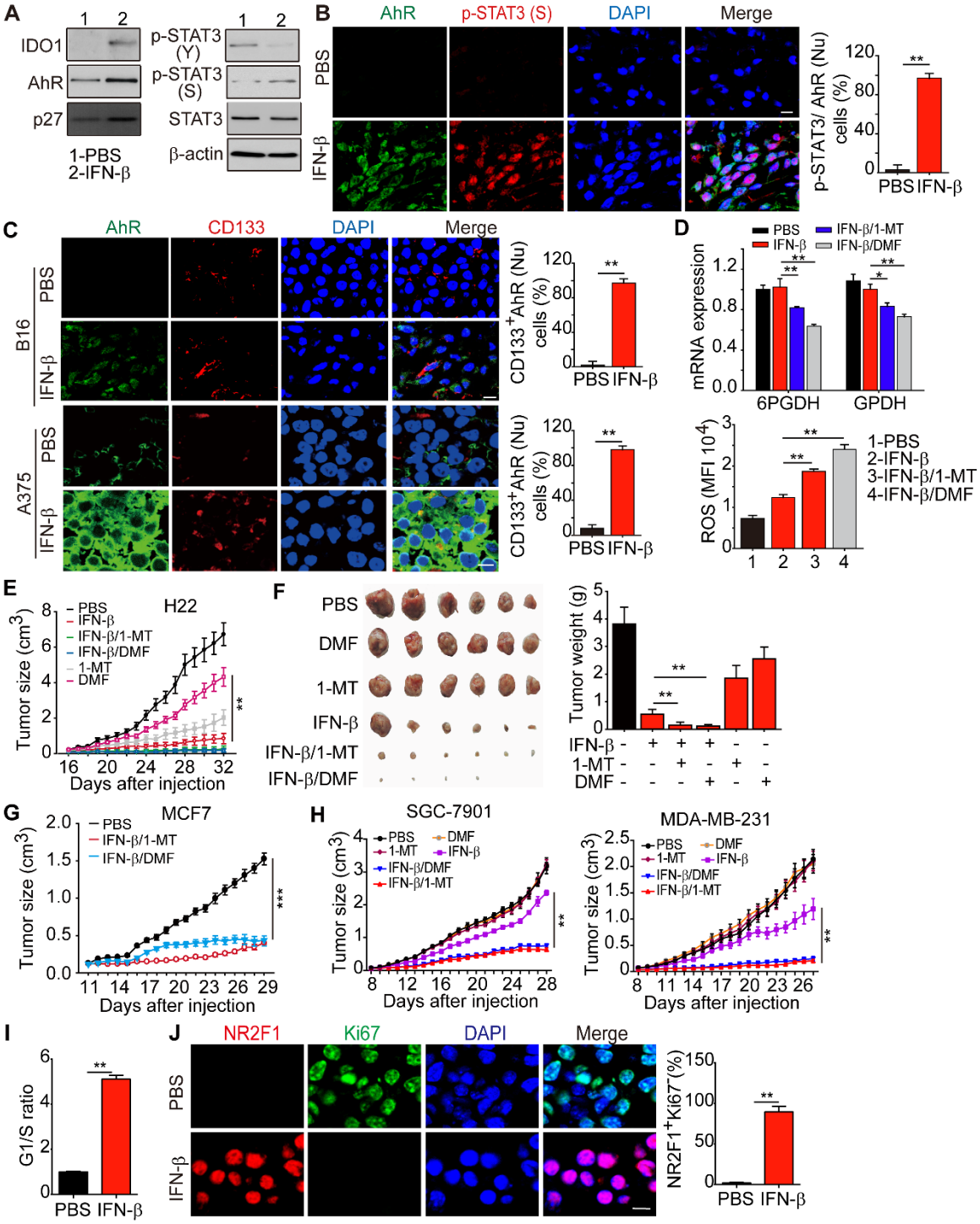
lysised and performed the western blot analysis. (**C**) SGGFP or *STAT3*-SGs B16 TRCs were treated with low or high concentration of IFN-β (2.5 or 10 ng/ml) for 48h. Cell lysates were extracted for western blot. (**D**) *STAT3*-knockout B16 TRCs were treated with PBS, IFN-β, IFN-β/1-MT or IFN-β/DMF for 48h. The protein expression of p27 was determined by western blot analysis. (**E**) A375 TRCs were treated with PBS, IFN-β, IFN-β/1-MT (200 μM), IFN-β/DMF (20 μM), IFN-β/1-MT/NAC (10 mM) or IFN-β/DMF/NAC for 48h. The ROS level was determined by flow cytometry. (**F**) B16 TRCs were treated with PBS, IFN-β, IFN-β/1-MT, IFN-β/DMF, IFN-β/1-MT/NAC or IFN-β/DMF/NAC for 48h. The percentage of cell apoptosis was measured by flow cytometry. (**G**) B16 TRCs were seeded in 3D fibrin gels for 2 days, and then treated with IFN-β, IFN-β/1-MT, IFN-β/DMF, IFN-β/1-MT/NAC or IFN-β/DMF/NAC for 72h. The colony size was presented and quantified (left) and the colony number was counted (right). Bar, 50 μm. (**H**) Real-time PCR analysis of *GPDH* (left) and *6PGDH* (right) mRNA expression in B16 or A375 TRCs treated with IFN-β, IFN-β/1-MT or IFN-β/DMF for 48h. (**I**) The mRNA expression of *GPDH* (left) and *6PGDH* (right) in Scramble-B16 TRCs or p53-siRNAs TRCs treated with IFN-β, IFN-β/1-MT or IFN-β/DMF for 48h. Graphs represent mean ± SEM of 3 independent experiments. **P*<0.05 and ***P*<0.01, by 1-way ANOVA (**E-I**).

Figure S8



Supplemental Figure 8 P53 mutants does not affect its function in regulating the PPP pathway. (**A** and **B**) MDA-MB-231 or SGC-7901 TRCs were treated with PBS, IFN-β, 1-MT, DMF, IFN-β/1-MT or IFN-β/DMF for different time point. The colony size was calculated (**A**) and the colony number was counted (**B**). (**C-E**) MDA-MB-231 or SGC-7901 TRCs were treated with PBS, IFN-β, IFN-β/1-MT (200 μM) or IFN-β/DMF (20 μM) for 48h. The ROS level was determined by flow cytometry (**C**). The level of GSH, GSSG, NADP or NADPH was measured and the ratio of GSSG to GSH or NADP to NADPH was calculated (**D** and **E**). (**F** and **G**) The activity of GPDH and 6PGDH in MDA-MB-231 or SGC-7901 TRCs treated with PBS, IFN-β, IFN-β/1-MT (200 μM) or IFN-β/DMF (20 μM) for 48h. Graphs represent mean ± SEM of 3 independent experiments. **P*<0.05 and ***P*<0.01, by 1-way ANOVA (**B-G**).

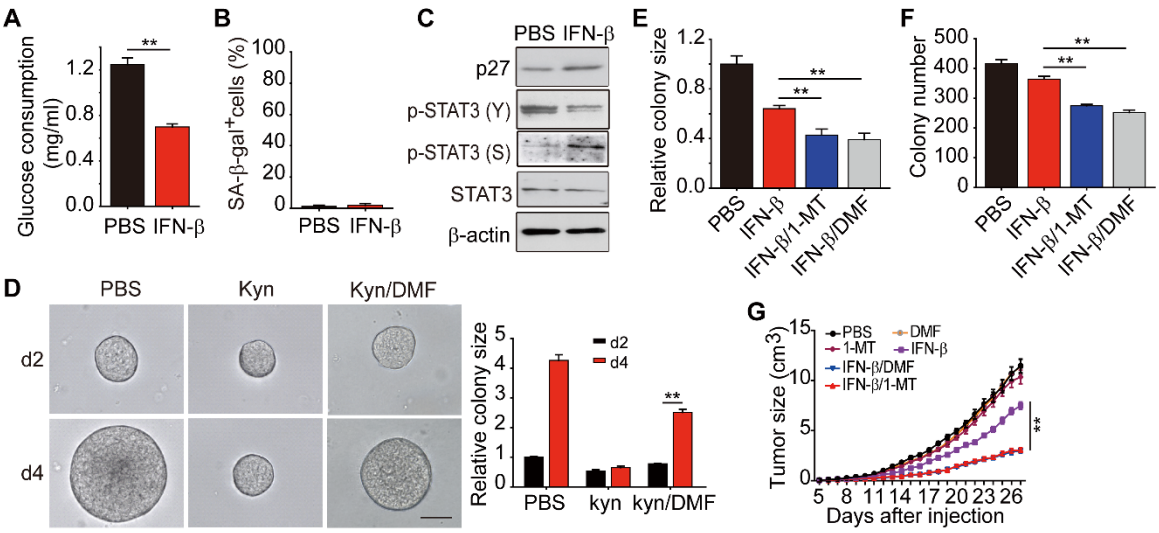
Figure S9



Supplemental Figure 9 IFN-β/1-MT or DMF disrupts dormant melanoma TRCs in vivo. (**A**) NOD-SCID mice bearing A375 melanoma were treated with IFN-β once daily

for 3 days. Tumor cells isolated were analyzed by western blot (n = 5). (**B**) Same as (**A**), but tumor was immunostained with anti-AhR, S100, p-STAT3 (S), and DAPI (n = 4). Bar, 10 μm. (**C**) C57BL/6 or NOD-SCID mice with 5×5 mm B16 or A375 melanoma were intratumorally treated with IFN-β once daily for 3 days. Tumor was stained with anti-AhR (green), CD133 (red) and DAPI (blue) for microscopy. Cell number with AhR nuclear localization was counted among 500 $CD133^+$ cells using 10 non-consecutive sections and the percentage of $CD133^+$ cells with AhR nuclear localization was determined (n = 5). Bar, 10 μm. (**D**) The same as (**C**), but $CD133^{high}$ cells were sorted. G6PDH and 6PGDH expression and ROS levels were measured in $CD133^{high}$ tumor cells (n = 5). (**E** and **F**) BALB/c mice with H22 hepatocarcinoma were treated with IFN-β, 1-MT, IFN-β/1-MT, DMF or IFN-β/DMF for 10 days. Tumor growth (**E**) and weight (**F**) were measured (n = 6). (**G**) NOD-SCID mice (n = 8) with MCF-7 tumor were treated with IFN-β/1-MT or IFN-β/DMF for 10 days. Tumor growth was measured. (**H**) NOD-SCID mice (n = 8) with MDA-MB-231 or SGC-7901 tumor were treated with IFN-β, 1-MT, IFN-β/1-MT, DMF or IFN-β/DMF for 10 days. Tumor growth was measured. (**I** and **J**) NOD-SCID mice with B16 melanoma were intratumorally treated with IFN-β once per day for 3 days. Isolated primary tumor cells were assayed for cell cycle analysis (**I**, n = 5) and immunostained against NR2F1 and Ki-67 (**J**, n = 5). Data represent mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001, by 2-tailed Student's t test (**B**, **C**, **I** and **J**) and 1-way ANOVA (**D-H**).

Figure S10



Supplemental Figure 10 IFN-β induces primary human melanoma TRCs into dormancy. (**A** and **B**) Primary human melanoma TRCs treated with IFN-β for 3 days were subjected to glucose consumption assay (**A**) (n = 3) or SA-β gal staining (**B**) (n = 3). (**C**) The same as (**A**), but these cells were subjected to western blot against p27, p-STAT3(Y), p-STAT3(S) or STAT3 (n = 3). (**D**) Primary human melanoma TRCs were treated with Kyn (200 μM) in the presence or absence of DMF (20 μM) for indicated time. The colony size was calculated (n = 3). Bar, 50 μm. (**E** and **F**) Primary human melanoma TRCs were treated with IFN-β (10 ng/ml) in the presence or absence of 1-MT (200 μM) or DMF (20 μM) for 3 days. The colony size (n = 3) was quantified (**E**) and colony number (n = 3) was counted (**F**). (**G**) NOD-SCID mice with 7×7 mm primary human melanoma by s.c. injection with 1×10^5 bulk B16 cells were treated with PBS, IFN-β, 1-MT, IFN-β/1-MT, DMF or IFN-β/DMF for 10 days. The tumor growth was measured (n = 8). The data represent mean ± SEM. $^{**}P<0.01$, by 2-tailed Student's t test (**A** and **D**) and 1-way ANOVA (**E-G**).

Supplemental Experimental Procedures

Determining Kyn production

Kyn production was measured in cell lysates by ELISA (MYBioSource, Cat. Cat#MBS495082) according to the supplier's instructions.

Transient transfection of siRNA and stable transfection of shRNAs

RNAimax reagent (Invitrogen, Cat. 13778150) was used to transiently transfect B16 TRCs following the manufacturer's instructions. B16 cells grown in conventional rigid plate were transfected with p53-siRNA for 12h. After trypsinization, these cells were seeded into soft 3D fibrin gels for another 12h culture. Then, these cells were used for the following experiments. Stable IDO1-shRNA cell lines were generated by lentiviral transduction and isolated by FACS. Briefly, Pspax2 (Addgene, Cat.12260), pMD2.G (Addgene, Cat. 12259) and pGFP-C-shLenti-shIDO1 or scrambled control (Origene, Cat. TL511273) were transfected into HEK 293T cells using FuGENE HD transfection reagent (Promega Cat. E2311). Polybrene was mixed with the virus containing supernatants to a final concentration of 8 μg/ml before addition to B16 cells. Cells with stable knockdown of IDO1 were FACS-sorted and cutured in 0.5 μg/ml puromycin with 10% FBS. The following shRNAs from Origene were used: scrambled shRNA: GCACTACCAG AGCTAACTCAGATAGTACT (sense) and AGTACTATCTGAGTTAGCTCTGGTA GTGC (antisense); *IDO1* shRNA1, TCCTAAGGAGTGTGAAAGATACAACCGAG (sense) and CTCGGTTGTATCTTT CACACTCCTTAGGA (antisense); *IDO1* shRNA2, AAACACGAGGCTGGCAAAGAATCTCCTGC (sense) and GCAGGA GATTCTTTGC CAGCCTCGTGTTT (antisense).

Stable overexpression of STAT3 and mutants

Pspax2, pMD2.G and pLenti-Flag-STAT3-Wt, pLenti-Flag-STAT3-S727A, pLenti-Flag-STAT3-Y705A or vector control were transfected into HEK 293T cells using FuGENE HD transfection reagent. Polybrene (Sigma-Aldrich, Cat.107689) was mixed with the virus containing supernatants to a final concentration of 8 μg/ml before addition to STAT3-SGRNA-B16 cells. Cells with stable overexpression of Flag-STAT3 and mutants were sorted by FACS and cutured in 0.5 μg/ml puromycin with 10% FBS.

Knock-out by CRISPR/Cas9

For construction of the stable knockdown of *p27*-, *STAT1*-, *STAT2*-, *STAT3*- or *AhR*-B16 cell lines, B16 cells were transfected with Linear Selection Markers-puromycin resistance (Clontech, Cat. 631626) and pX330-U6-Chimeric_BB-CBh-hSpCas9-p27-SGRNAs, pX330-U6-Chimeric_BB-CBh-hSpCas9-STAT1-SGRNAs, pX330-U6-Chimeric_BB-CBh-hSpCas9-STAT2-SGRNAs, pX330-U6-Chimeric_BB-CBh-hSpCas9-STAT3-SGRNAs, or pX330-U6-Chimeric_BB-CBh-hSpCas9-AhR-SGRNAs (Addgene, Cat. 42230) for 24h. The following primers were used: SGGFP: CACCGGGGCGAGGAGCTG TTCACCG (sense) and AAACCGGTGAACAGCTCCTCGCCCC (antisense); *p27*-SGRNA1, CACCGGCGGATGGACGCCAGACAAG (sense) and AAACCTTGTCTG GCGTCCATCCGCC (antisense); *p27*-SGRNA2, CACCGTGGGTCTCAGGCAAACTC TG (sense) and AAACCAGAGTTTGCCTGAGACCCAC (antisense); *STAT1*-SGRNA1, CACCGGTACGATGACAGTTTCCCCA (sense) and AAACTGGGGAAACTGTCAT CGTACC (antisense); *STAT1*-SGRNA2, CACCGGGTACTGTCTGATTTCCATG (sense) and AAACCATGGAAATCAGACAGTACCC (antisense); *STAT2*-SGRNA1, CACCGATAACTTGCGAAAATTCAGC (sense) and AAACGCTGAATTTTCGCAAG

TTATC (antisense); *STAT2*-SGRNA2, CACCGGTT CCTGGACCAGTTACACC (sense) and AAACGGTGTAACTGGTCCAGGAACC (antisense); *STAT3*-SGRNA1, CACCG GTACAGCGACAGCTTCCCCA (sense) and AAACTGGGGAAGCTGTCGCTGTA CC (antisense); *STAT3*-SGRNA2, CACCGAGAGTCTCGCCTCCTCCAGA (sense) and AAACTCTGGAGGAGGCGAGACTCTC (antisense); *AhR*-SGRNA1 (human), CACCG TCAAGTCAAATCCTTCCAAG (sense) and AAACCTTGGAAGGATTTGACTTGAC (antisense); *AhR*-SGRNA2 (human), CACCGAAGGCAGCAGGCTAGCCAAA (sense) and AAACTTTGGCTAGCCTGCTGCCTTC (antisense); *AhR*-SGRNA1 (mouse), CAC CGGCTCAGCGTCAGCTACCTGA (sense) and AAACTCAGGTAGCTGACGCTGAG CC (antisense); *AhR*-SGRNA2 (mouse), CACCGGCTGGCGCTGGAATTCCGCC (sense) and AAACGGCGGAATTCCAGCGCCAGCC (antisense). Puromycin (1 μg/ml) (Invitrogen, Cat. A1113803) was used to select the cells for the first 2 weeks, afterwards the cells were maintained in puromycin (0.5 μg/ml). In order to construct stable overexpression cells, B16 cells were transfected with pLenti-C-Myc-DDK-IDO1 or empty vector by using FuGENE HD transfection reagent. Cellular populations expressing Myc-IDO1 or Myc-vector were sorted by FACS and cultured with 0.5 μg/ml puromycin.

RT-PCR

Trizol (Invitrogen, Cat. 15596018) was used to extract total RNA from cells which was then transcribed to cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Cat. 4368813). The primer sequences are shown as follows: *IDO1* (mouse), GAGGATGCGTGACTTTGTGG (sense) and ATCAAGACTCTGGAAGATGCTG (antisense); *IDO1* (human), CTCACAGACCACAAGTCACAG (sense) and TCCAGTTT GCCAAGACACAG (antisense); *p27* (mouse), AGGAGAGCCAGGATG TCAG (sense)

and AAGAAGAATCTTCTGCAGCAGG (antisense); *p27* (human), AACGTGCGAGTG TCTAACGG (sense) and CCCTCTAGGGGTTTGTGATTCT (antisense); *Src* (mouse), TGGCAAGATCACCAGACGG (sense) and GGCACCTTTCGTGGTCTCAC (antisense); *Src* (human), TTTGGCAAGATCACTAGACGGG (sense) and GAGGCAGTAGG CACCTTTTGT (antisense); *GAPDH* (mouse), AGGTCGGTGTGAACGGATTTG (sense) and TGTAGACCATGTAGTTGAGGTCA (antisense); *GPDH* (mouse), ATGAAGCACA CAGGCATTTGG (sense) and TCCAGGTATAGCTGAAACAGTCC (antisense); *GAPDH* (human), ACAGAACTCGGGACCTTTTTCC (sense) and CAGCATCCACGG TCTCTTTCA (antisense); *6PGDH* (mouse), TGAAGGGTCCTAAGGTGGTCC (sense) and CCGCCATAATTGAGGGTCCAG (antisense); *6PGDH* (human), GTGGCCCCAC ATCAAGACC (sense) and GTCCCCATACTCTATCCCGTT (antisense); *GAPDH* (human), ACAACTTTGGTATCGTGGAAGG (sense) and GCCATCACGCCACAGT TTC (antisense). Real-time PCR was performed using ABI stepone plus (Applied Biosystems, MA, USA).

Chromatin immunoprecipitation (ChIP) followed by qPCR (ChIP-qPCR)

ChIP-qPCR was performed by using MAGnity™ Chromatin Immunoprecipitation System (Invitrogen, Cat.492024) according to the supplier's instruction. Briefly, B16 TRCs were crosslinked and chromatin was extracted and sheared. IP was performed by using anti-AhR (Abcam, Cat. Ab2769), anti-p-STAT3 (Y) (Cell signaling technology, Cat. 9145)or anti-p-STAT3 (S) (Cell signaling technology, Cat. 9134). The primer sequences used for ChIP-qPCR are provided as followings: Src, GCAGTGGAGGA GGTAACAGGAA (sense) and CAGCTGTGTCACACTCCTGGTA (antisense); p27, AGTCGCAGAACTTCGAAGA GG (sense) and CACTCTCACGTTTGACATCTTCC (antisense); p53, AAGGAACGAC

TTTGCCTACAC (sense) and AAACTGTAGAAATTATGCGCCC (antisense). The results were from 3 independent experiments followed by normalization to input signals and showed as mean ± SEM.

Western blotting

Cells were harvested, lysed in M2 lysis buffer and sonicated and protein concentrations were determined by the BCA kit (Applygen Technologies Inc., Cat. P1511). Next, the proteins were run on an SDS-PAGE gel and transferred to nitrocellulose membranes. These were blocked with 5% bovine serum albumin (BSA) and incubated with the following antibodies overnight: anti-β-actin (Sigma-Aldrich, Cat. A2228), Flag-tag (Sigma-Aldrich, Cat. F1804) and TopBP1 (Sigma-Aldrich, Cat. PLA0030); anti-p-STAT3 (Y) (Cell signaling technology, Cat. 9145), p-STAT3 (S) (Cell signaling technology, Cat. 9134), STAT3 (Cell signaling technology, Cat. 9139), p-Src (Cell signaling technology, Cat. 6943), Src (Cell signaling technology, Cat. 2109), phospho-STAT1 (Y) (Cell signaling technology, Cat. 9167) , STAT1 (Cell signaling technology, Cat. 9172), p53 (Cell signaling technology, Cat. 2524) and p27 (Cell signaling technology, Cat. 3688); anti-β3 tubulin (Millipore, Cat. AB9354) and IDO1 (Millipore, Cat. 05-840); anti-AhR (R&D Systems, Cat. AF6697). After washing, secondary antibody: anti-rabbit IgG (Cell signaling technology, Cat. 7074), anti-mouse IgG (Cell signaling technology, Cat. 7076) and anti-sheep (G+L) (Invitrogen, Cat. 81-8620) conjugated to horseradish peroxidase was added followed by enhanced chemiluminescence (Thermo fisher, MA). Results were confirmed in at least three independent experiments.

Luciferase assays

B16 cells were transfected with 100 ng renilla luciferase plasmid pRL-SV40 (Promega, Cat. E2231), 1 μg firefly luciferase plasmid pGL3-p27 promoter-luc or pGL3-CYP1A1 promoter driven luciferase and 1 μg of either vector or pCMV6-AhR for 12h. Then, these cells were treated with or without IFN-β (R&D Systems, Cat.8234-MB) (5 ng/ml) for 24h. Cells lysates were analyzed using the Dual Luciferase Reporter Assay (Promega, Cat. E1910) on a GloMax Multi Plus (Promega). Firefly luciferase activity was normalized to Renilla luciferase.

SA-β-gal activity assay

SA-β-gal activity was analyzed in B16, A375 or primary human melanoma TRCs using the β-Galactosidase Staining Kit (Cell signaling technology, Cat. 9860) according to the provided protocol. SA-β-gal-positive and negative cells were then counted under the microscope.

Cell cycle analysis

Cells cycle analysis was performed using BD Pharmingen APC/FITC-BrdU Flow Kits in accordance with the manufacturer's protocol (BD Bioscience, Cat. 552598). The samples were analyzed by flow cytometry on a BD Accuri C6 Flow Cytometer (BD Bioscience). The cell cycle phases were determined as a percentage of the total population: sub-G0 (apoptotic cells), G0/G1 (2n, BrdU-negative), S (2n to 4n, BrdU-positive) and G2/M phase (4n, BrdU-negative). For in vivo cell cycle analysis, BrdU (1 mg, Sigma-Aldrich, Cat. B5002) was i.p. injected into mice 18h before mice were sacrificed. The primary tumor cells were isolated from tumor or ascites and analysed according to the BD Pharmingen kit.

Cell fractionation

Cells were collected and washed twice in cold PBS. Then, the cytoplamic and nuclear proteins were seperated by using the Cell Fractionation kit (BioVison, Cat. K266-100) according to the supplier's instruction. Equal cell equivalents were run on SDS-PAGE gels and immunoblotted.

Immunofluorescence

Cells were plated on fibronectin(Sigma-Aldrich, Cat. F2006) (1μg/ml)-coated coverslips, fixed in 4% paraformaldehyde and permeablized with 0.2% Triton X-100. Fixed cells were blocked in 5% BSA and incubated with monoclonal anti-AhR (Gene Tex, GTX113124), NR2F1 (Abcam, ab181137), Ki67 (Abcam, ab15580), S100 (Abcam, ab52642), p-STAT3 (Y) (Abcam, ab76315), p-STAT3 (S) (Abcam, ab32143), or anti-CD133 (Abcam, ab19898) antibody in 5% BSA, followed by Alexa Fluor 488-conjugated goat anti-mouse antibody (Invitrogen, Cat. A-11029) or Alexa Fluor 488-conjugated goat anti-rabbit antibody (Invitrogen, Cat. A-11008) or Alexa Fluor 594-conjugated goat anti-rabbit antibody (Invitrogen, Cat. A-21211). Cells were mounted with Vectashield mounting media containing DAPI (Vector Laboratories, Cat. H-1200). A Olympus laser scanning confocal microscope (FVI000MPE) was used to image the cells.

Measurement of the level of ROS and the activity of GSH, GSSG, NADP, NADPH, GPDH and 6PGDH

The cell lysates were extracted. According to the supplier's instruction, the level of ROS was detected by using CellROX^{TM} Green Reagent (Invitrogen, Cat. C10444) and the activity of GSSG/GSH, NADPH, GPDH and 6PGDH was measured by Glutathione Fluorometric Assay Kit (BioVision, Cat. 264), NADP/NADPH Quantitation Kit (Biovision,

Cat. K347), Glucose 6 Phosphate Dehydrogenase Assay Kit (Abcam, Cat. ab83426) and 6PGDH Test Kit (BioLab, Cat. SK019), respectively.

$CD133^+$ tumor cells sorting

Freshly isolated tumor tissue from mice was digested with collagenase and hyaluronidase for 1 h. After grinding with semi-frosted slides and lysis of RBC, the non-dissolved connective tissue was pelleted by centrifugation at 500 rpm for 1 min and the cell containing supernatant was incubated on a plastic surface for 1 h to get rid of adhesive cells. The suspension cells were stained with PE conjugated anti-CD133 antibody (Invitrogen Cat. 12-1331-80) at room temperature for 30 min. After washing, these cells were filtered through a 40 um cell strainer (JET Biofil, China) to obtain single cell suspension for sorting on a BD FACSARIA III (BD Bioscience, NJ, USA).

Animal experiments and treatment protocol

C57BL/6, BALB/c or NOD-SCID mice were injected s.c. with different amount of B16, A375, primary human melanoma, H22 or MCF7 TRCs into the right flank. Three days later after inoculation or after the tumor size reached 5×5 mm or 7×7 mm, mice were randomized into different groups (sample size was n=5-10) based on similar tumor size and body weight. And then, these mice were treated with or without IFN-β (intratumoral injection of 250 ng/day, once every two days), 1-MT (5 mg/ml in drinking water, 3-4 ml/mouse/day), IFN-β/1-MT, DMF (intragrastric injection of 10 mg/kg, once every two days), IFN-β /DMF or anti-IFN-β neutralizing antibody (Biolegend, Cat. 508104) (250 μg/mouse) for the indicated time. The mice in the control groups received an equal volume of saline as a mock treatment. The incidence of tumor growth in the mice and the survival of the mice was recorded daily. For some experiments, mice were sacrificed and tumors

were dissected and weighed or fixed in 37% formalin on the indicated day after inoculation. Tumor volume was calculated according to the formula: tumor volume = length×width^2/2. No blinding was performed.

Histological and immunohistochemical staining

The subcutaneous tissue at the inoculation site surgically excised from mice or the patients' tissues were fixed in 10% formalin, embedded in paraffin and sectioned for H&E staining. Immunohistochemistry was performed using the Vectastain Universal Elite ABC kit (Vector Laboratories, Cat. PK-6100) according to the manufacturer's instructions. Briefly, the sections of paraffin embedded tissues were incubated with anti-S100 (1:200, Abcam, Cat. Ab52642) or anti-IFN-β (Abcam, Cat. ab85803) at 4℃ overnight. After development, tissue sections were moderatly counterstained with methyl green. In some experiments, immunohistochemistry was implemented by using Tyramide Signal Amplification (TSA) Plus Cyanine 3/Fluorecein system (PerkinElmer, Cat. NEL753001KT) according to the supplier's instructions. In brief, the sections were dewaxed, rehydrated, the endogenous peroxidase quenched, blocked and incubated with anti-S100 (1:3000, Abcam, Cat. Ab52642), anti-AhR (1:3000, Abcam, Cat. Ab2769), anti-CD133 (Invitrogen, Cat. PA5-38014), anti-Ki67 (Abcam, Cat. ab15580), NR2F1 (Abcam, Cat. Ab2796), DEC2 (Abcam, Cat. Ab175544) and anti-p-STAT1 (1:3000, Cell signaling technology, Cat. 9167) at 4℃ overnight. Afterwards, slides were sequentially incubated with two HRP-conjugated secondary antibodies for one hour at room temprature. The staining was developed in TSA Plus Working Solution for 5min, before conterstaining with DAPI and finally mounting for confocal analysis.

Isolating primary human melanoma cells

Melanoma tissues were obtained from patients at the Peking Union Medical College. Ethical permission was granted by the Clinical Trial Ethics Committee of Peking Union Medical College. All patients provided written informed consent to participate in the study. The surgically removed tumor tissues were cut into small pieces of 1-3mm, minced and incubated for 1 hour at 37 ℃ under continuous rotation with RPMI 1640 medium supplemented with collagenase type Ⅳ (Sigma, 32 μg/ml, Cat. C5138), hyaluronidase (Sigma, 500 μg/ml, Cat. H1136) and DNAase I (Sigma, 5 μg/ml, Cat. 11284932001). After the digestion, the samples were filtered through Cell Strainer (BIOFIL) before being pelleted by centrifugation and then exposed to RBC lysis buffer. Finally, the tumor cells were resuspended in RPMI1640 medium with 10% FBS, penicillin and streptomycin. The identity of the primary melanoma cells was confirmed by immunostaining with anti-S100β antibody.