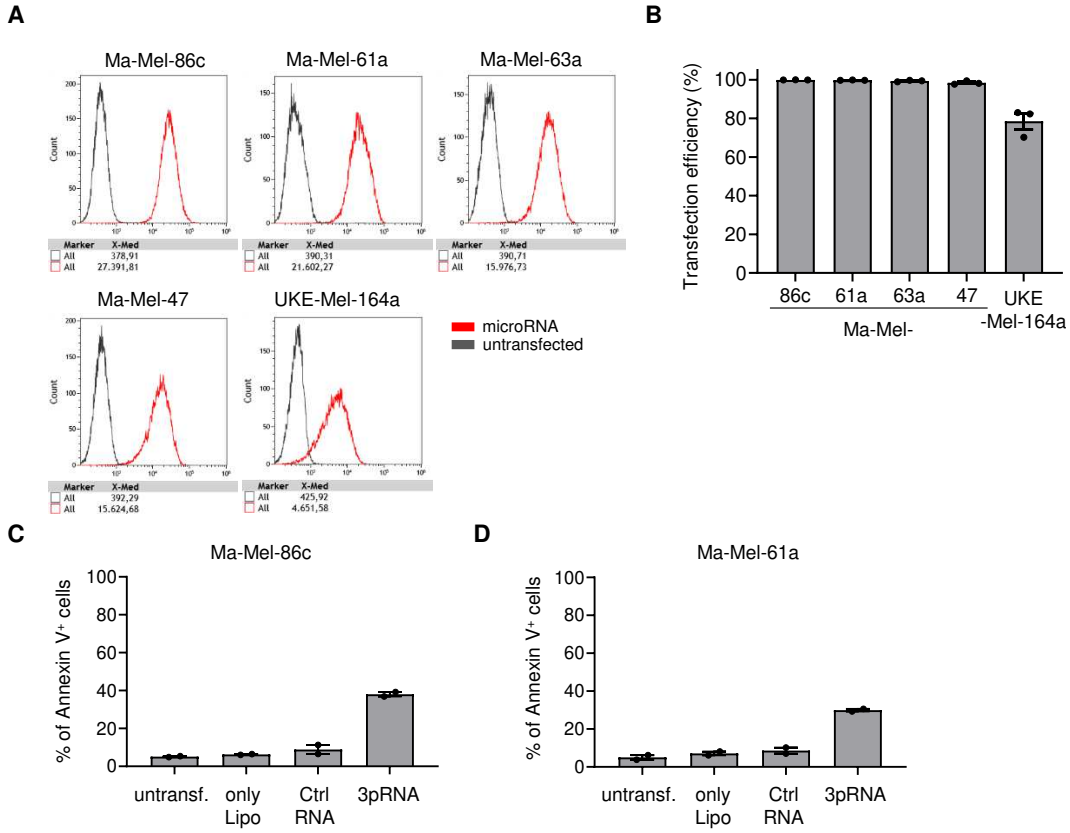
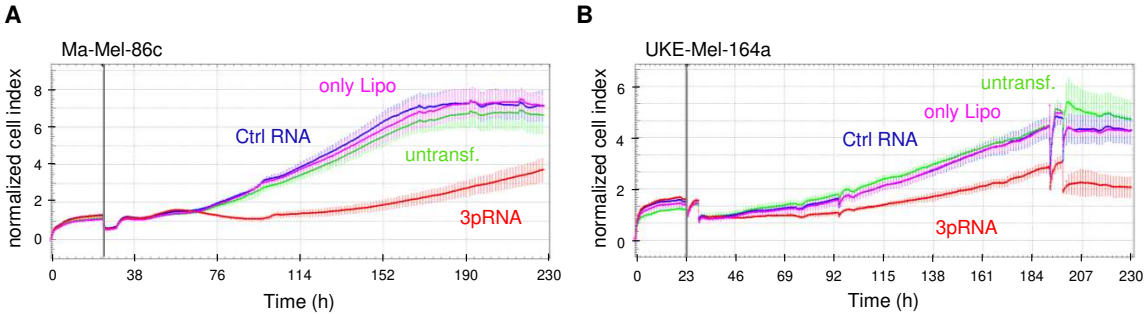
Thier et al., Supplementary file 1 – supplementary figures

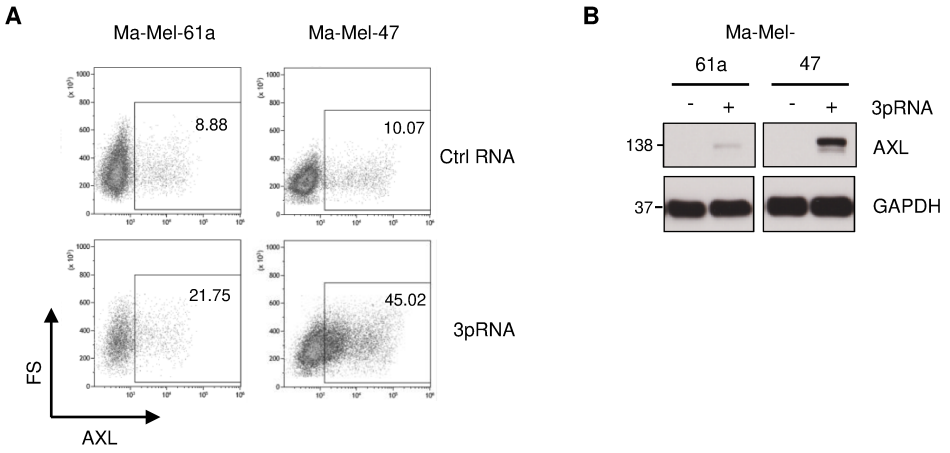
Innate immune receptor signaling induces transient melanoma dedifferentiation while preserving immunogenicity



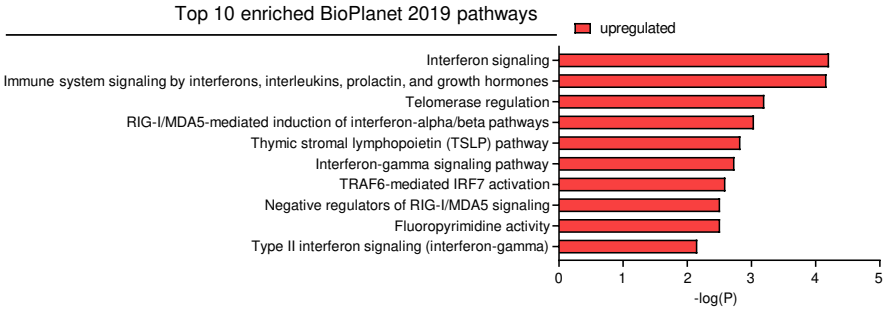
Supplementary figure S1: Control of transfection. (A,B) Melanoma cell lines were transfected with miRNA-Cy3 (red) or left untransfected (grey). After 24 h, transfection efficiency was determined by flow cytometry. (A) Representative histograms and (B) percentage of Cy3-positive cells given as mean±SEM from three independent experiments. (C,D) Transfection procedure does not alter apoptosis induction. Ma-Mel-86c (C) and Ma-Mel-61a (D) were transfected with 3pRNA or Ctrl RNA. As additional controls, cells were left untransfected (untransf.) or only treated with the transfection reagent Lipofectamine (only Lipo). Apoptosis was measured by flow cytometry on day 3 post-transfection. Percentage of Annexin V+ cells depicted as mean±SEM of two independent experiments.



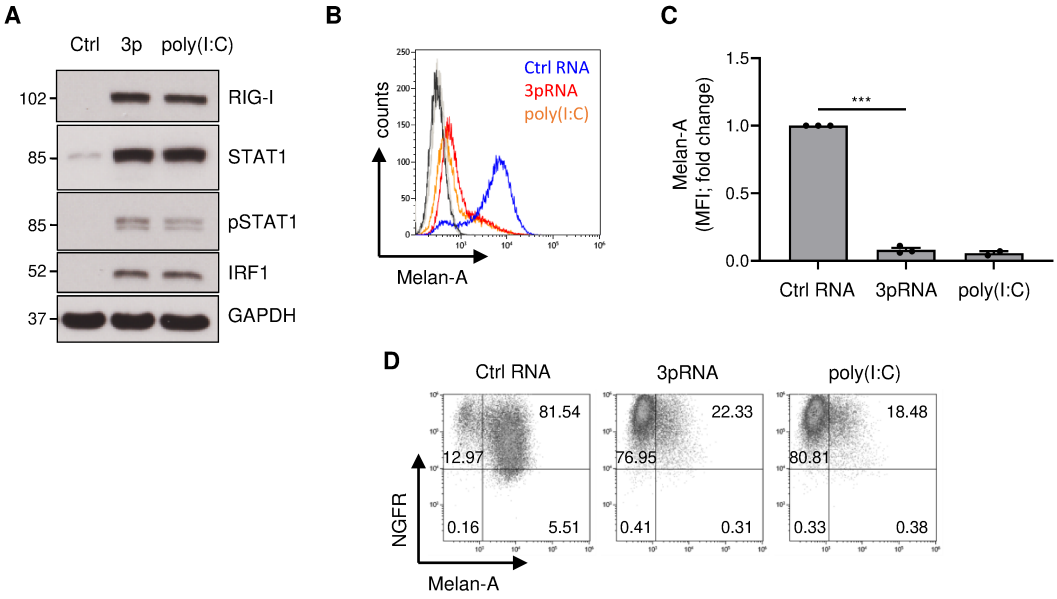
Supplementary figure S2: Transfection does not alter cell proliferation. (A,B) Real-time proliferation of Ctrl RNA (blue)- and 3pRNA (red)-transfected Ma-Mel-86c (A) and UKE-Mel-164a (B) cells, and of controls left untransfected (green) or only treated with Lipofectamine (purple). Vertical grey lines indicate time point of transfection. Representative data from three independent experiments.



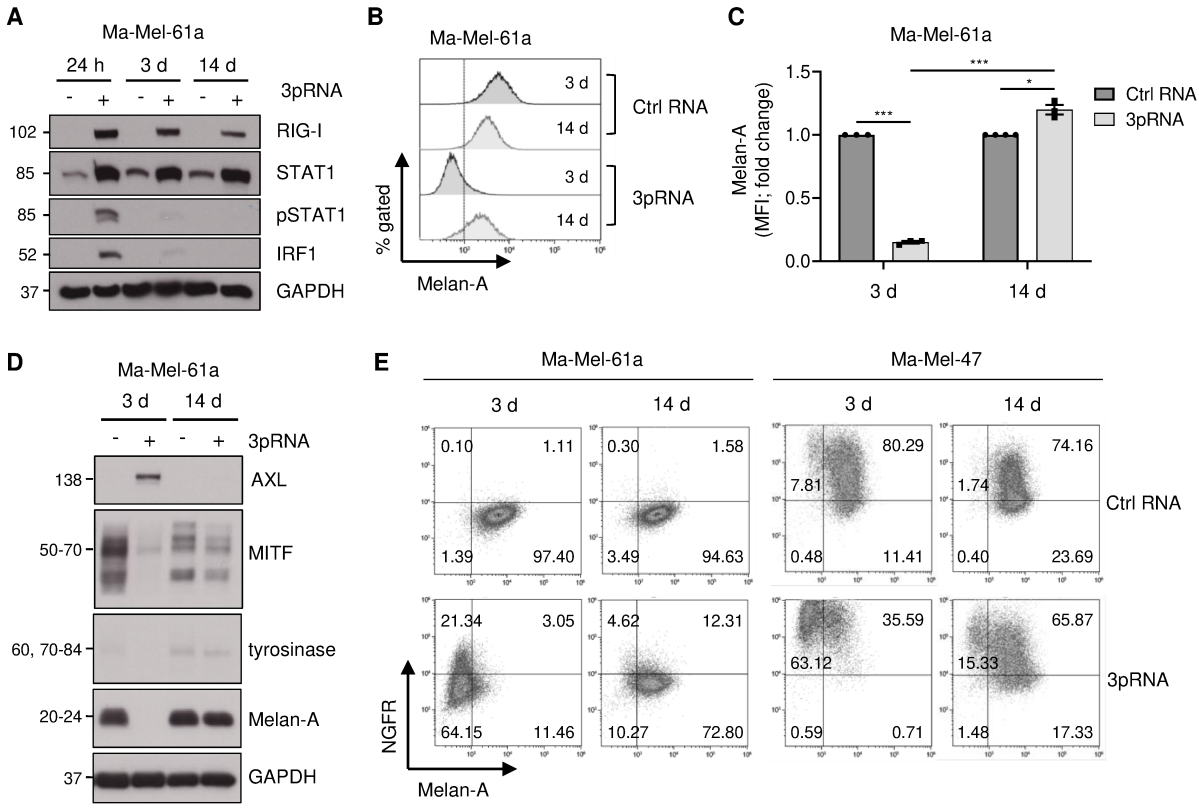
Supplementary figure S3: AXL expression upon RIG-I activation in Ma-Mel-61a and Ma-Mel-47. (A,B) Ma-Mel-61a and Ma-Mel-47 were transfected with 3pRNA or Ctrl RNA. AXL expression analyzed at single cell and bulk levels by flow cytometry (A) and Western Blot (B), respectively, on day 3 post-transfection. GAPDH, loading control. Representative data from one of three independent experiments.



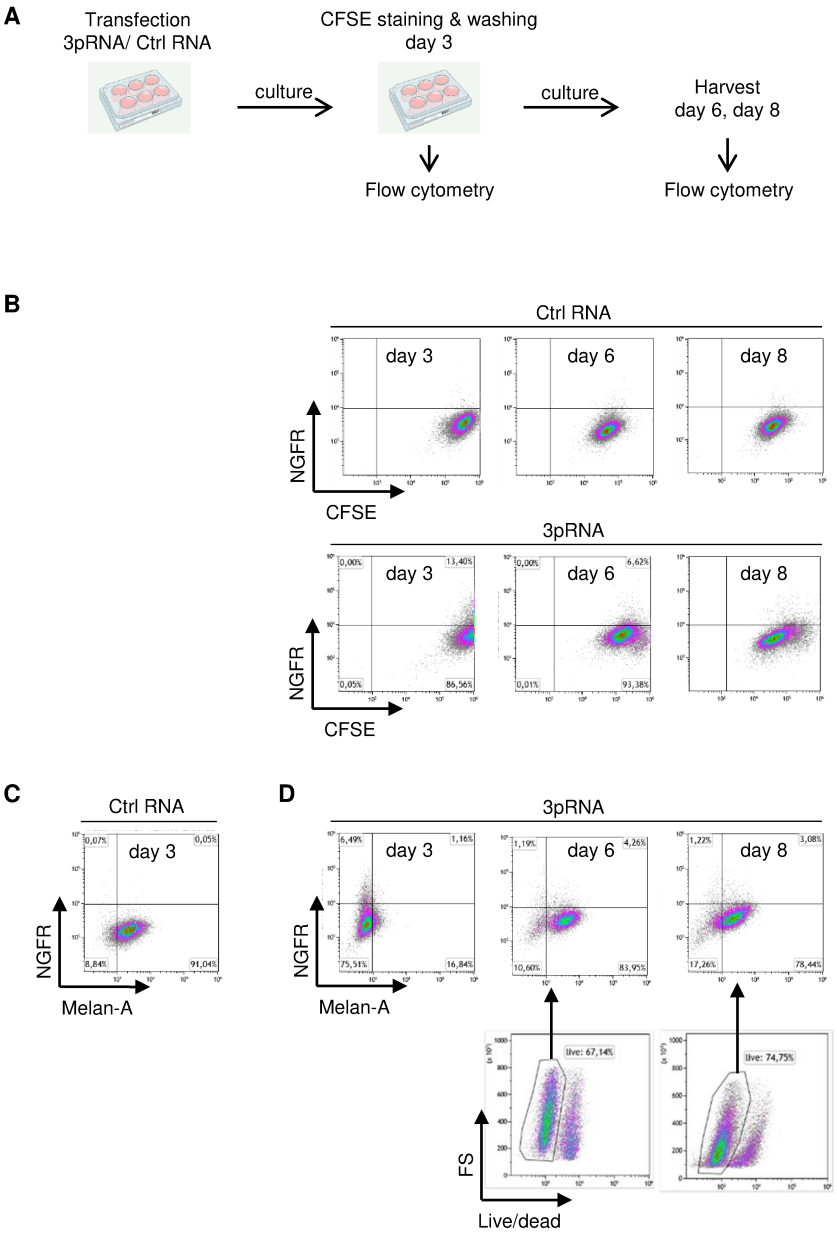
Supplementary figure S4: Significantly upregulated pathways in *RIG-I/DDX58*^{high} melanoma cell lines. BioPlanet database pathways enriched for genes co-regulated with *DDX58* mRNA in the Tsoi transcriptomic data set [27].



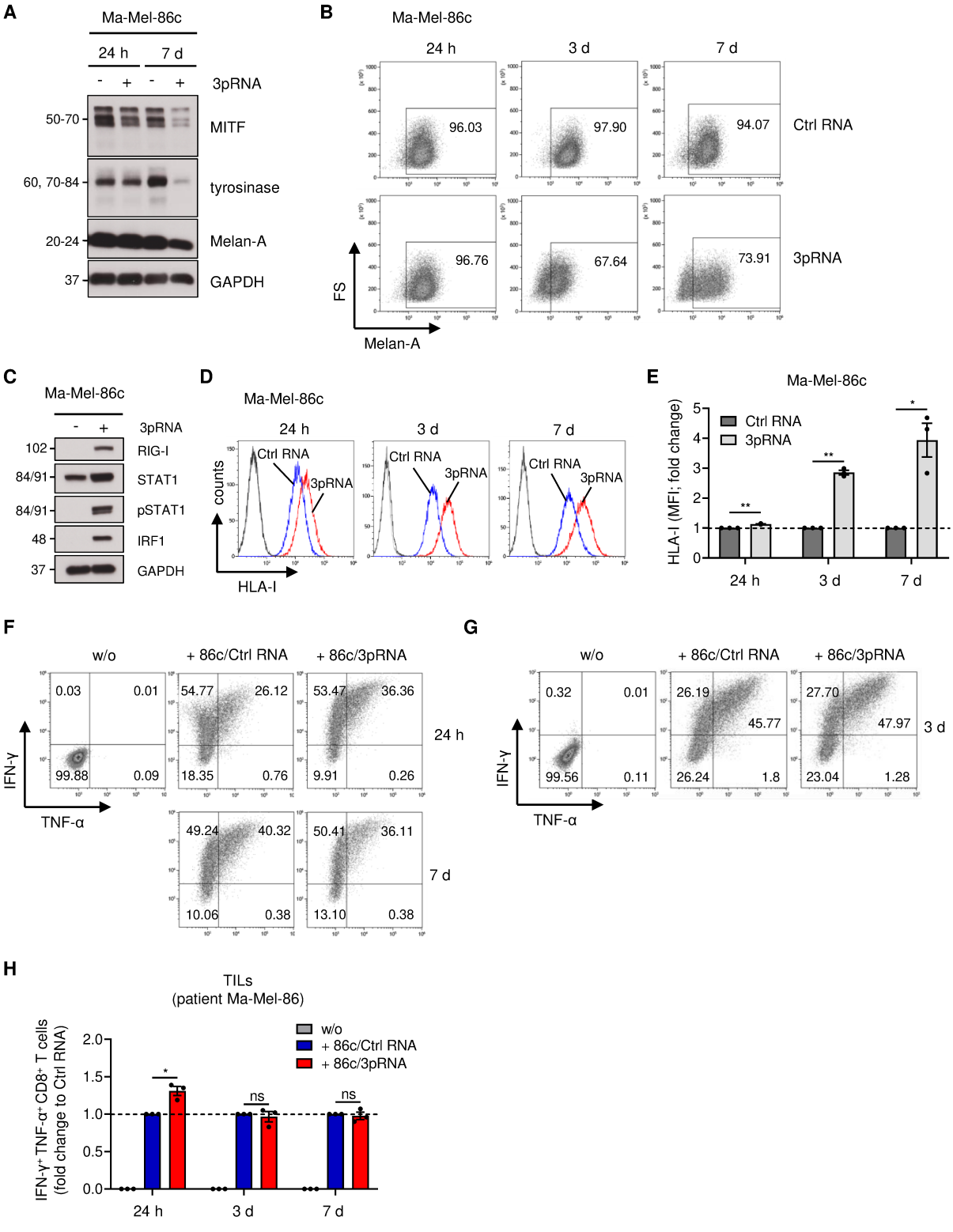
Supplementary figure S5: Dedifferentiation of Ma-Mel-47 cells upon poly(I:C) transfection. Ma-Mel-47 were transfected with 3pRNA, poly(I:C) or Ctrl RNA and subjected to further analyses on day 3 post-transfection. (A) RIG-I expression and signaling pathway activation analyzed by Western blot. GAPDH, loading control. Representative data of two independent experiments. (B,C) Melan-A expression determined by flow cytometry. (B) Representative histograms and (C) fold change of MFI given as mean±SEM of two or three independent experiments. (D) Melan-A and NGFR co-expression in Ma-Mel-47 cells analyzed by flow cytometry. Representative dot plots from two or three independent experiments, numbers indicate percentage of cells. Significantly different experimental groups: *** $p < 0.005$ by two-tailed paired t-test.



Supplementary figure S6: Transient dedifferentiation upon RIG-I activation. Ma-Mel-61a cells were transfected once with 3pRNA or Ctrl RNA and analyzed on day 3 (3 d) and 14 (14 d) post-transfection. (A) RIG-I expression and JAK-STAT pathway activation analyzed by Western Blot. GAPDH, loading control. Representative data of three independent experiments. (B,C) Melan-A expression determined by flow cytometry. (B) Representative histograms with dotted line representing unstained control and (C) fold change of MFI given as mean±SEM of three independent experiments. (D) Differentiation status of Ma-Mel-61a cells determined by expression of indicated proteins. GAPDH, loading control. Representative data of three independent experiments. (E) Melan-A and NGFR co-expression in Ma-Mel-61a and Ma-Mel-47 cells analyzed by flow cytometry. Representative dot plots of three independent experiments, numbers indicate percentage of cells. Significantly different experimental groups: * $p < 0.05$; *** $p < 0.005$ by two-tailed paired t-test.

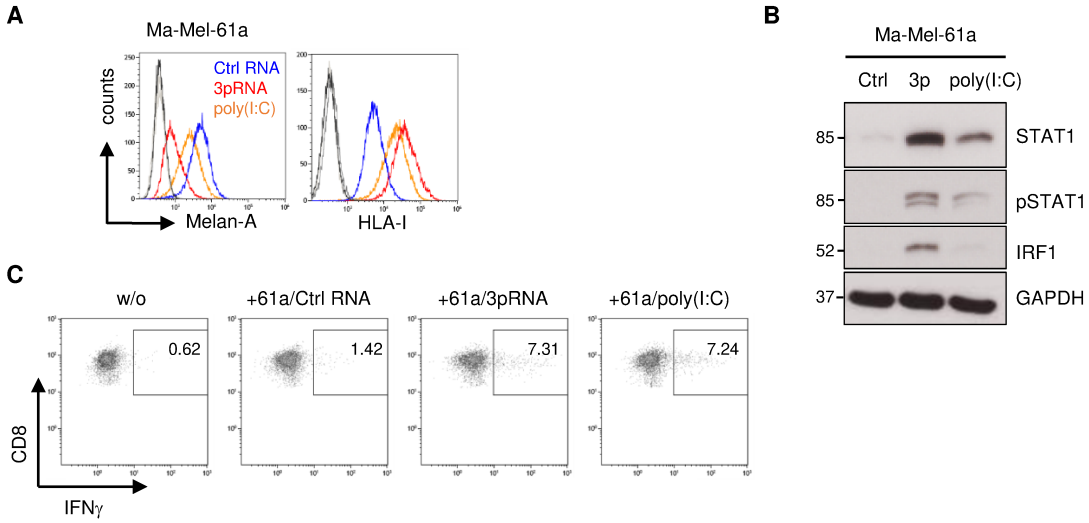


Supplementary figure S7: Monitoring reproliferation of 3pRNA-induced persisters. (A) Scheme of workflow. Ma-Mel-61a cells were transfected with Ctrl RNA or 3pRNA. On day 3 post-transfection, adherent cells were stained with CFSE, washed and directly analysed by flow cytometry or cultured until analysis at indicated time points. (B) Loss in CFSE signal intensity upon proliferation. CFSE-labeled cells co-stained for intracellular NGFR. Note, Ctrl RNA-treated cells: day 6/8 – high cell density, arrest in proliferation. (C, D) Differentiation status of viable Ctrl RNA- and 3pRNA-treated Ma-Mel-61a cells measured by co-staining for intracellular NGFR and Melan-A, in parallel to proliferation. (C) Differentiation status of control cells on day 3 post-transfection. (D) Differentiation status of viable persisters on day 3, day 6 and day 8 post-transfection. Cell death determined by live/dead staining.

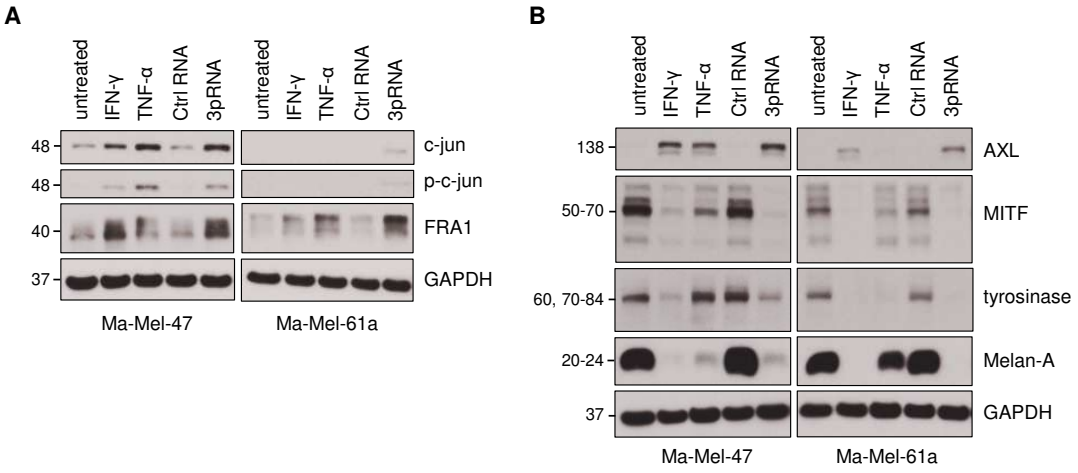


Supplementary figure S8

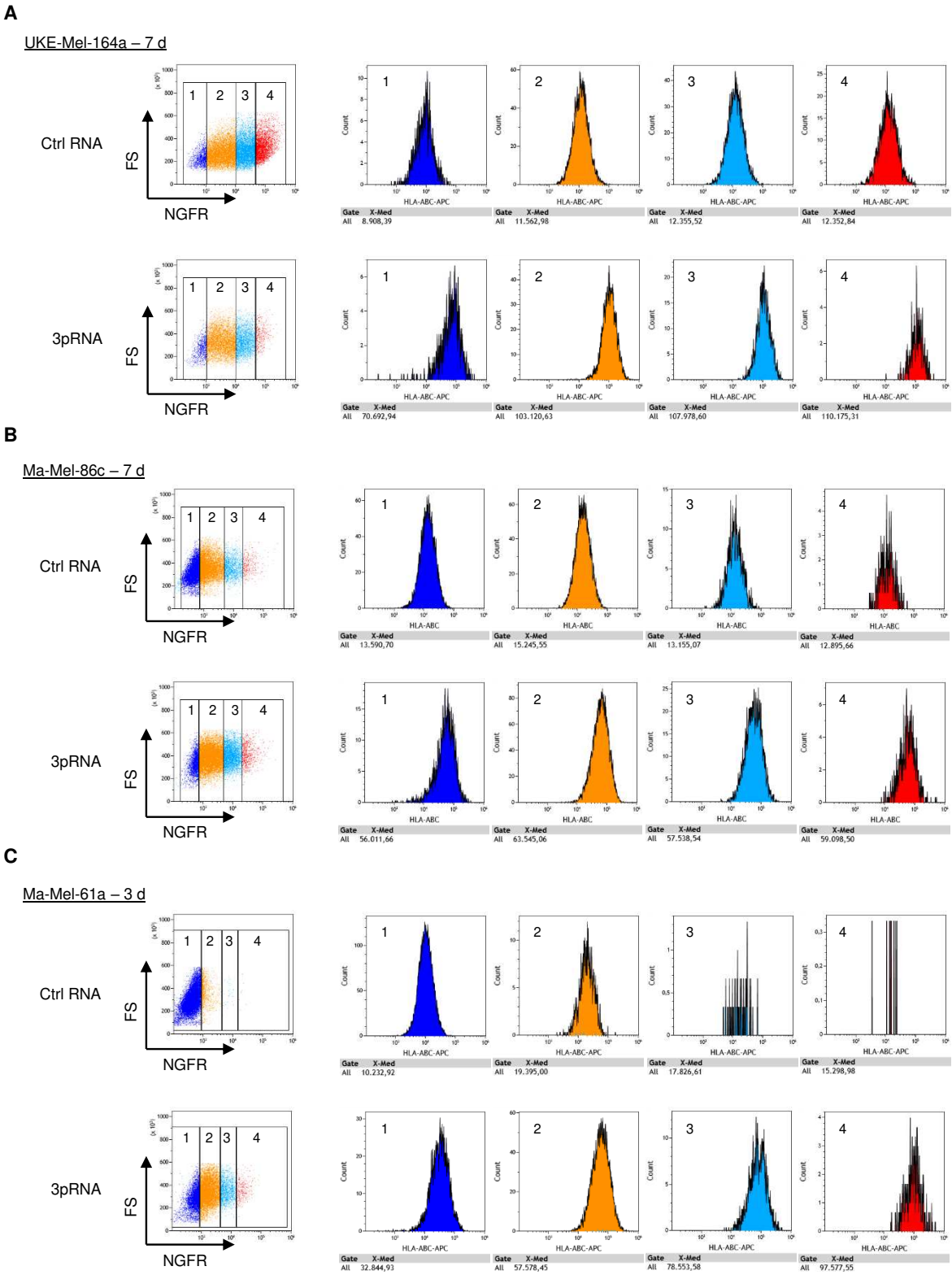
Supplementary figure S8: CD8 T-cell reactivity toward 3pRNA-induced dedifferentiated Ma-Mel-86c persisters. (A-F) Melanoma cells Ma-Mel-86c were transfected with 3pRNA (+) or Ctrl RNA (-) and subjected to further analyses. Cells analyzed at 24 h post-transfection or on day 3 post-transfection were transfected once (day 0), cells analyzed on day 7 post-transfection were transfected twice (day 0 and 6). (A) Differentiation status of Ma-Mel-86c cells determined by expression of indicated proteins. GAPDH, loading control. Representative data from three independent experiments. (B) Melan-A expression analyzed by flow cytometry. Representative data from three independent experiments. (C) RIG-I expression and JAK-STAT pathway activation analyzed 24 h post-transfection by Western Blot. GAPDH, loading control. Representative data from three independent experiments. (D,E) HLA-I cell surface expression measured by flow cytometry. (D) Representative histogram and (E) fold change of MFI given as mean±SEM from three independent experiments. (F-H) Activation of autologous TILs by melanoma cells transfected with 3pRNA or Ctrl RNA. TIL activation analyzed by intracellular cytokine staining via flow cytometry. (F,G) Representative dot plots, (F) 24 h and day 7, (G) day 3. (H) Quantification of IFN-γ^+ TNF-α^+ $CD8^+$ T cells. Fold change given as mean±SEM from three independent experiments. Significantly different experimental groups: * $p < 0.05$; ** $p < 0.01$ by two-tailed paired t-test.



Supplementary figure S9: CD8 T-cell reactivity toward poly(I:C)-induced Ma-Mel-61a persisters. Ma-Mel-61a melanoma cells were transfected with 3pRNA, poly(I:C) or Ctrl RNA and subjected to further analyses. (A) Melan-A and HLA-I expression determined by flow cytometry on day 3 post-transfection. Representative histograms from two independent experiments. (B) JAK/STAT signaling pathway activation analyzed 24 h post-transfection by Western Blot. GAPDH, loading control. Representative data from two independent experiments. (C) Activation of autologous CD8 T cells (PBMCs) by Ma-Mel-61a cells on day 3 post-transfection with 3pRNA, poly(I:C) or Ctrl RNA. T cell activation analyzed by intracellular cytokine staining via flow cytometry. w/o: spontaneous cytokine release in the absence of tumor cells. Representative dot plots from two independent experiments.



Supplementary figure S10: Signaling pathway activation and differentiation status of melanoma cells upon 3pRNA and cytokine treatment. Ma-Mel-47 and Ma-Mel-61a cells were treated with IFN-γ or TNF-α, or transfected with 3pRNA or Ctrl RNA and subjected to further analyses. (A) Signaling pathway activation analyzed 24 h post-treatment/post-transfection, and (B) differentiation status determined on day 3 post-treatment/post-transfection by Western Blot. Representative data from two independent experiments.



Supplementary figure S11

Supplementary figure S11: Correlation of HLA-I and NGFR expression. (A,B) UKE-Mel-164a (A) Ma-Mel-86c (B) were transfected twice (day 0 and day 6) with 3pRNA or Ctrl RNA and subjected to further analyses on day 7. (C) Ma-Mel-61a cells were transfected once with 3pRNA or Ctrl RNA and subjected to further analyses on day 3 post-transfection. (A-C) Surface expression of NGFR and HLA-I measured by flow cytometry. Left panel, melanoma cells were divided into subpopulations (1-4) according to NGFR expression intensity (1: lowest; highest: 4). Right panel, histograms of HLA-I surface expression in NGFR subpopulations with median MFI.