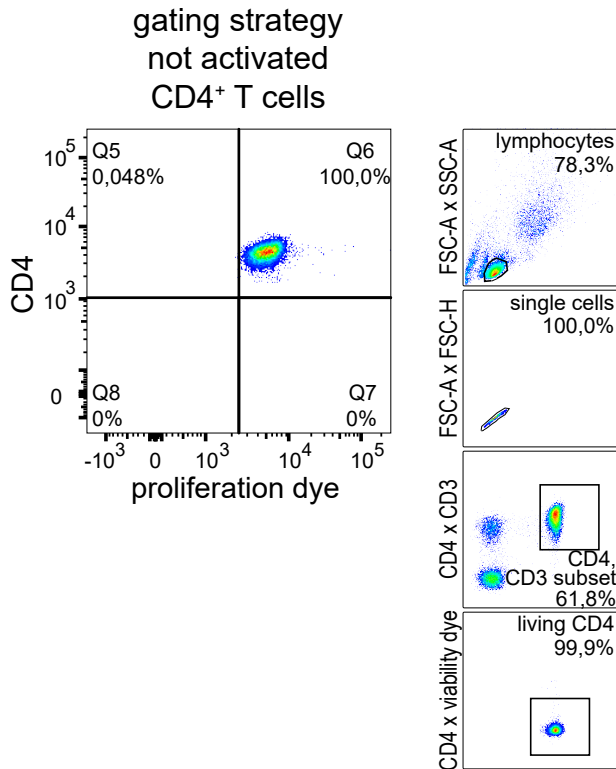


Figure S1

A



B

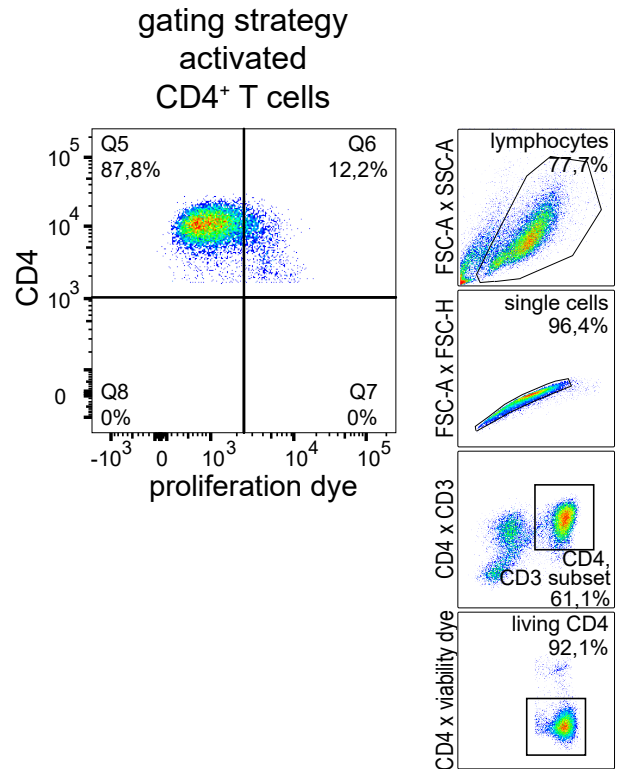


Figure S1: Flow cytometry gating strategy. (A) Gating for the proliferation of CD4⁺ T cells in unstimulated PBMCs and (B) in α CD3, α CD28 activated PBMCs. (A, B) First the lymphocyte population was gated (FSC-A x SSC-A). Then doublet exclusion was performed (FSC-A x FSC-H). Next CD3 and CD4 double positive T cells were gated (CD4 x CD3). After that the living cells were gated from the CD3, CD4 double positive T cells (CD4 x viability dye). Final evaluation of the proliferation of the living CD3, CD4 double positive T cells (proliferation dye x CD4). CD8⁺ T cells were gated accordingly.

Figure S2

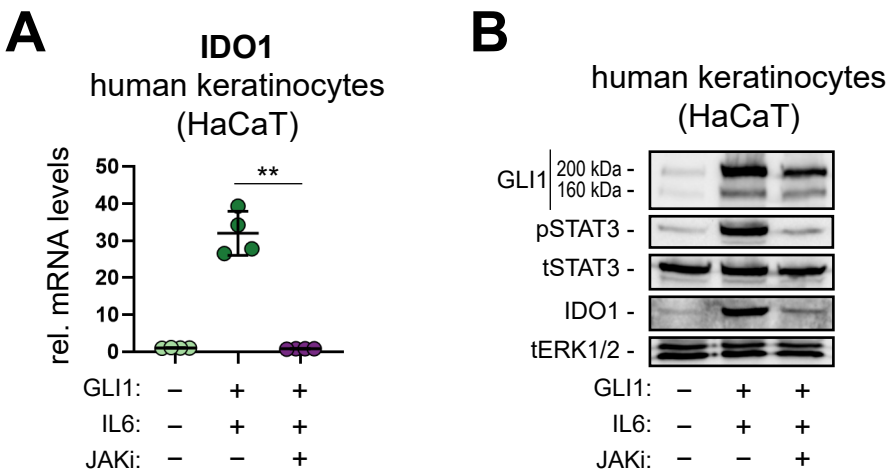


Figure S2: Pan JAK inhibition reverts the synergistic induction of IDO1 through combined IL6/STAT3 and GLI1 signaling. (A) qPCR analysis of relative IDO1 mRNA levels in dox-inducible GLI1 human HaCaT keratinocytes treated with dox [50 ng/mL] (48 h), JAKi [1 μ M] (for the last 24 h) and IL6 [75 ng/mL] (for the last 22 h) as described for Fig. 1 ($n = 4$). **(B)** Representative Western blot analysis of IDO1 expression in dox-inducible GLI1 human HaCaT keratinocytes treated with dox, JAKi and IL6 as described in (A). Active STAT3 signaling was assessed by measuring phospho-STAT3 (pSTAT3). Total ERK1/2 (tERK1/2) protein expression was used as loading control. Two protein bands for GLI1 are visible representing tagged and untagged GLI1 protein. One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis (** $p < 0.01$). (dox: doxycycline; JAKi: panJAK inhibitor 1; p: phospho; t: total).

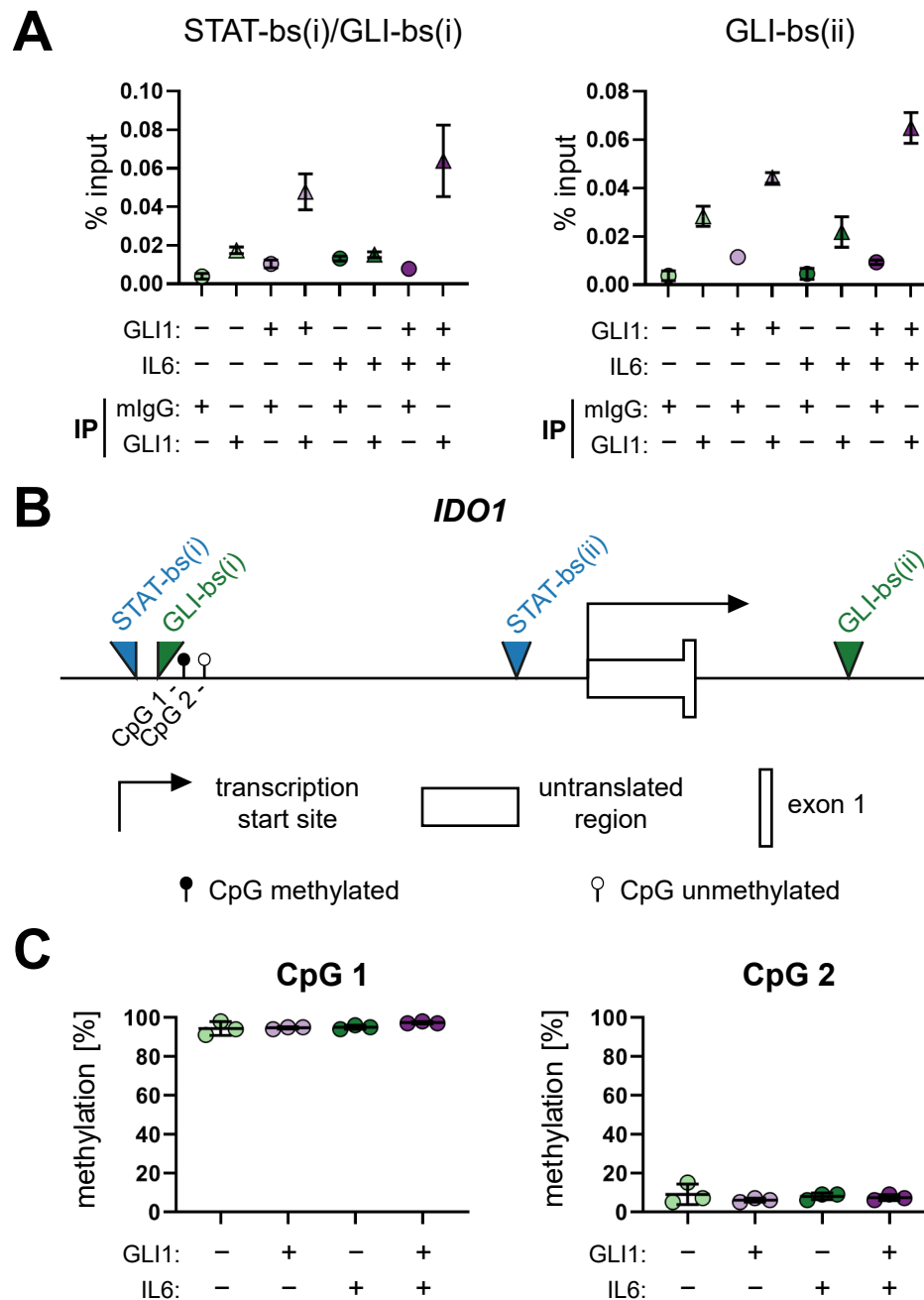
Figure S3

Figure S3: Analysis of GLI1 binding and CpG methylation in the *cis*-regulatory region of *IDO1*. (A) Targeted ChIP analysis showing GLI1 transcription factor binding to STAT-bs(i)/GLI-bs(i) as well as GLI-bs(ii) in the *IDO1* *cis*-regulatory region. Data shown as mean with range. (B) Illustration of the *IDO1* *cis*-regulatory region with *in silico* predicted binding sites for GLI (green) and STAT (blue) (not drawn to scale). CpG 1 and CpG 2 next to GLI-bs(i) are indicated. Position of CpG 1 is 2815 bp and CpG 2 2781 bp relative to the transcription start site. (C) Bisulfite pyrosequencing analysis of the methylation status of CpG 1 and CpG 2 in dox-inducible human keratinocytes (HaCaT). To induce GLI1 expression, cells were pre-treated with dox for 24 h [50 ng/mL] and then treated with IL6 [75 ng/mL] for another 24 h ($n = 3$). One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis. (dox: doxycycline, mlgG: mouse IgG).

Figure S4

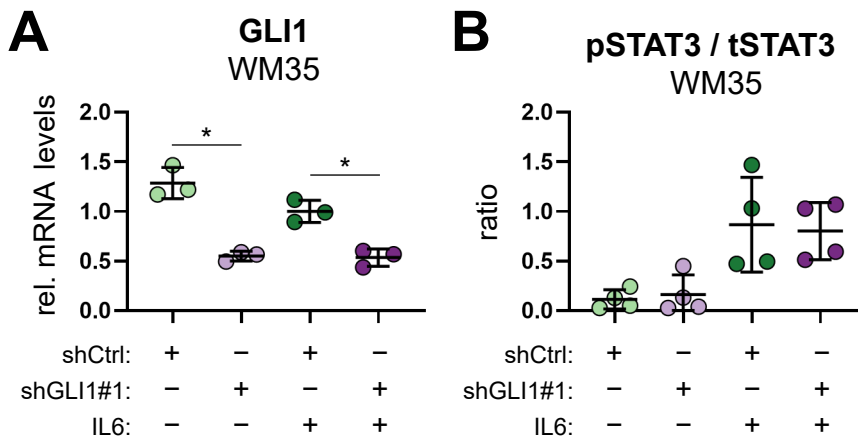


Figure S4: Validated GLI1 RNAi does not affect pSTAT3/tSTAT3 protein levels. (A) qPCR analysis of relative GLI1 mRNA levels ($n = 3$). **(B)** Ratio of pSTAT3 protein levels normalized to tSTAT3 ($n = 4$). Relative quantification of Western blot bands via densitometric image analysis was conducted using Image Lab 4.0 software (Bio-Rad, Vienna, Austria) ($n = 4$). (A, B) WM35 melanoma cells were treated with or without IL6 [75 ng/mL] for 24 h, and lentivirally transduced with shGLI1 (shGLI1#1) or control shRNA (shCtrl). One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis ($*p < 0.05$). (p: phospho; RNAi: RNA interference; t: total).

Figure S5

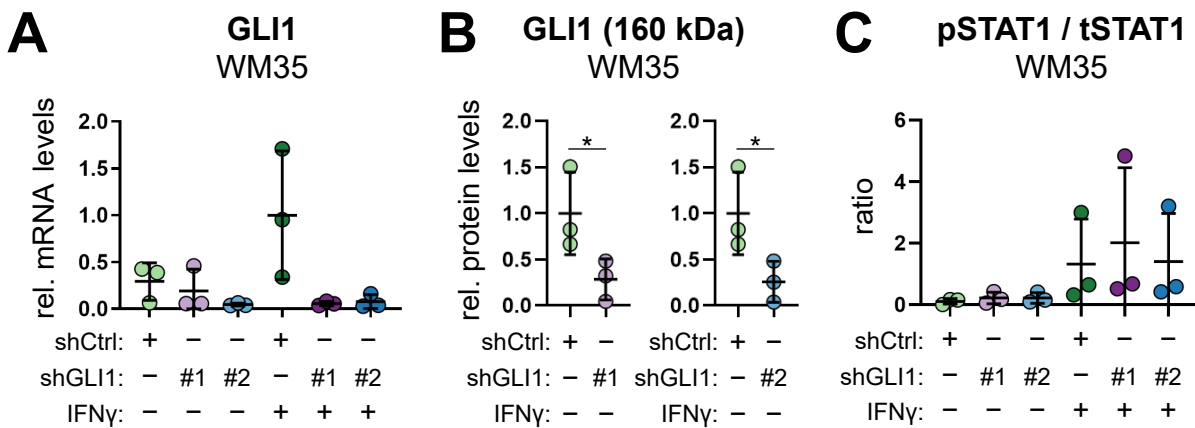


Figure S5: Validated GLI1 RNAi does not affect pSTAT1/tSTAT1 protein levels. (A) qPCR analysis of relative GLI1 mRNA levels in WM35 melanoma cells ($n = 3$). **(B)** Relative quantification of 160 kDa GLI1 Western blot bands of three independent experiments. Total ERK1/2 (tERK1/2) protein expression served as loading control. **(C)** Ratio of pSTAT1 protein levels normalized to tSTAT1 in WM35 melanoma cells ($n = 3$). (B, C) Relative quantification of Western blot bands via densitometric image analysis was conducted using Image Lab 4.0 software (Bio-Rad, Vienna, Austria). (A-C) WM35 melanoma cells were treated with or without IFN γ [10 ng/mL] for 18 h, and lentivirally transduced with shGLI1 (#1 or #2) or control shRNA (shCtrl). (A, C) One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis. (B) Student's t-test was used for statistical analysis ($*p < 0.05$). (p: phospho; RNAi: RNA interference; t: total).

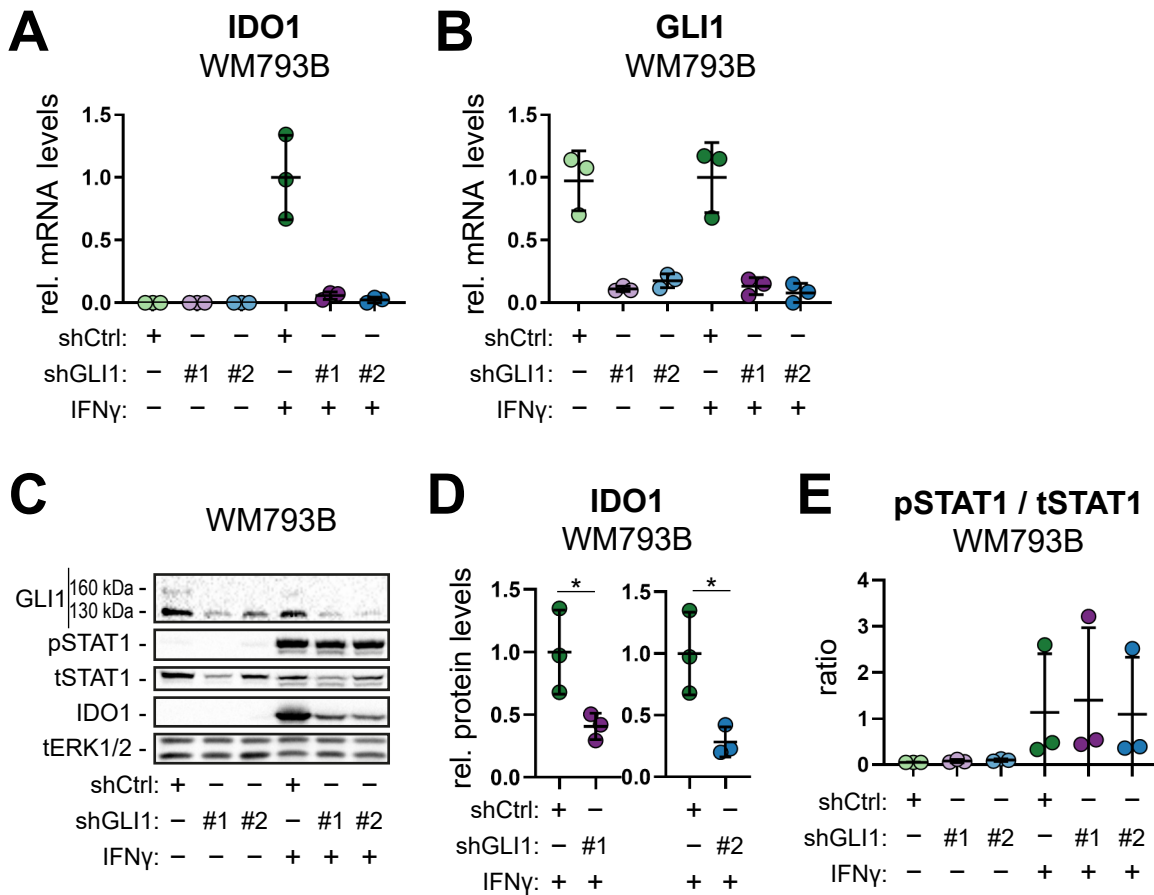
Figure S6

Figure S6: Validated GLI1 RNAi does not affect pSTAT1/tSTAT1 protein levels. (A, B) qPCR analysis of (A) relative IDO1 and (B) relative GLI1 mRNA levels in WM793B melanoma cells ($n = 3$). (C) Representative Western blot analysis of GLI1, total- and phospho-STAT1 (tSTAT1 and pSTAT1) and IDO1 expression in WM793B melanoma cells. Of note, WM793B melanoma cells displayed full-length GLI1 at 160 kDa and a splice variant at around 130 kDa on the blot. (D) Relative quantification of IDO1 Western blot bands of three independent experiments. Total ERK1/2 (tERK1/2) protein expression served as loading control. (E) Ratio of pSTAT1 protein levels normalized to tSTAT1 in WM793B melanoma cells ($n = 3$). Relative quantification of Western blot bands via densitometric image analysis was conducted using Image Lab 4.0 software (Bio-Rad, Vienna, Austria). (A-E) WM793B melanoma cells were treated with or without IFN γ [10 ng/mL] for 18 h, and lentivirally transduced with shGLI1 (#1 or #2) or control shRNA (shCtrl). (A, B, E) One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis. (D) Student's t-test was used for statistical analysis ($*p < 0.05$). (p: phospho; RNAi: RNA interference; t: total).

Figure S7

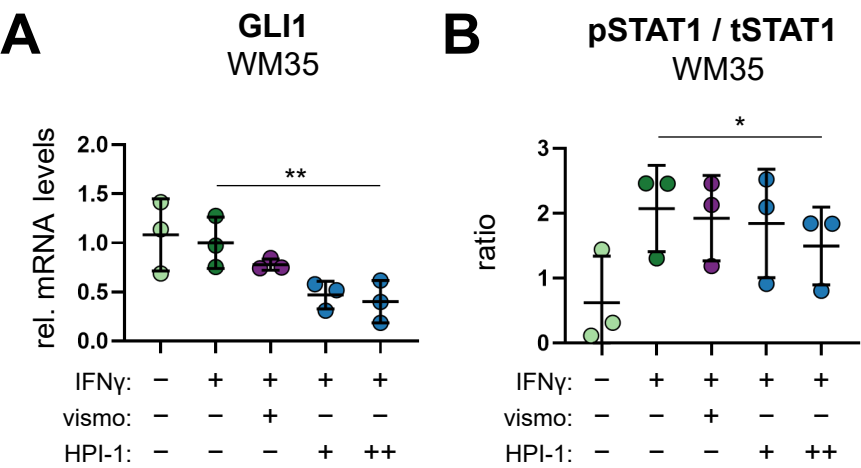


Figure S7: Validation of the effects of the pharmacological inhibitors vismodegib and HPI-1 on relative GLI1 mRNA levels and pSTAT1/tSTAT1 protein levels. (A) qPCR analysis of relative GLI1 mRNA levels in WM35 melanoma cells ($n = 3$). **(B)** Ratio of pSTAT1 protein levels normalized to tSTAT1 in WM35 melanoma cells ($n = 3$). Relative quantification of Western blot bands via densitometric image analysis was conducted using Image Lab 4.0 software (Bio-Rad, Vienna, Austria). (A, B) WM35 melanoma cells were treated for 24 h with solvent, vismo [0.5 μ M], HPI-1 [5 μ M] (+), [10 μ M] (++) and/or IFN γ [10 ng/mL]. IFN γ was added following inhibitor pretreatment for 6 h. One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis (* $p < 0.05$; ** $p < 0.01$). (HPI-1: hedgehog pathway inhibitor 1; p: phospho; t: total; vismo: vismodegib).

Figure S8

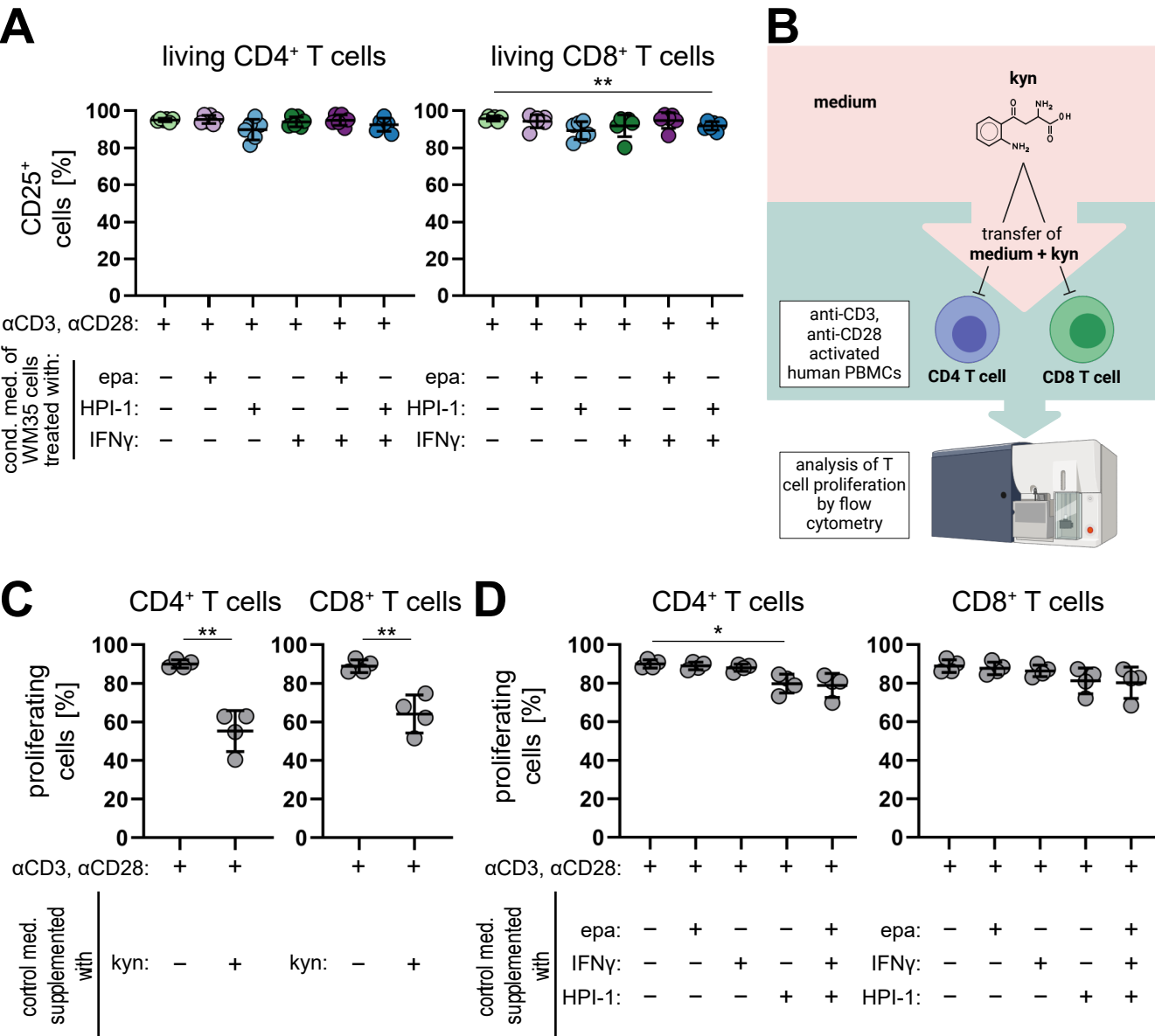


Figure S8: Analysis of CD25 and treatment of PBMCs with control media. (A) Percentage of CD25⁺ cells of living CD4⁺ and CD8⁺ T cells from anti-CD3/-CD28 stimulated PBMC cultures from six different human donors after transfer of conditioned medium as described in (Fig. 5A) (*n* = 6). (B) Illustration of the preparation of kyn supplemented control medium, transfer to αCD3 and αCD28 activated PBMCs and treatment for 72 h with a final treatment concentration of 100 μM kyn, followed by flow cytometric analysis of the proliferation of (C) CD4⁺ and CD8⁺ T cells (*n* = 4). (D) PBMCs were also activated with αCD3 and αCD28 and treated for 72 h with control media supplemented with epa, IFNγ or HPI-1, followed by flow cytometric analysis of the proliferation of CD4⁺ and CD8⁺ T cells (*n* = 4). The final treatment concentrations were: epa [0.75 μM], IFNγ [5 ng/mL], HPI-1 [5 μM]. (B) Student's t-test was used for statistical analysis. (A, C) One-way ANOVA with Tukey's multiple-comparison test was used for statistical analysis (**p* < 0.05; ***p* < 0.01). (epa: epacadostat; HPI-1: hedgehog pathway inhibitor 1; kyn: kynurenine).

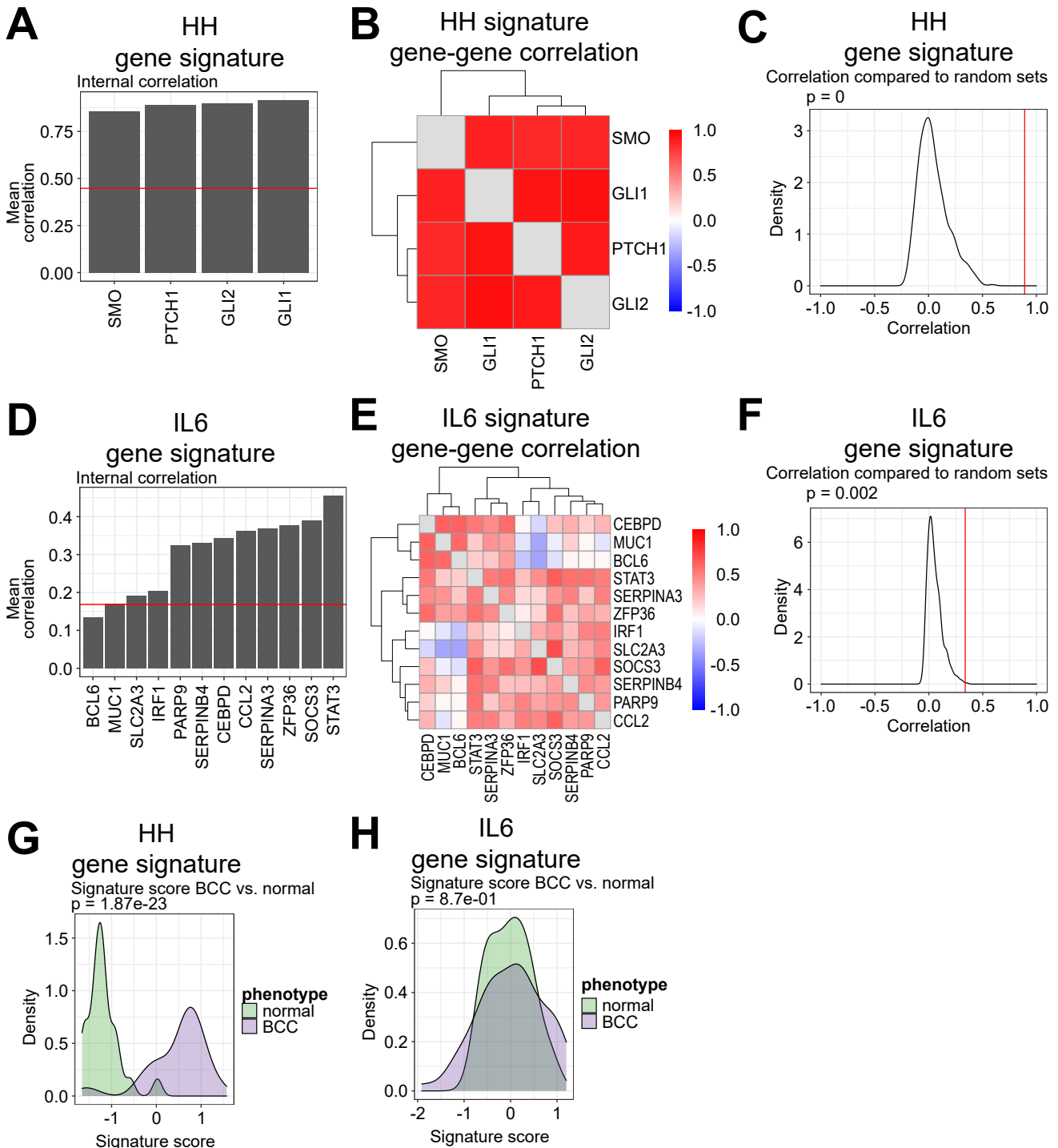
Figure S9


Figure S9: Information on HH and IL6 gene signatures. (A) Internal correlation of the HH signature genes. (B) Heatmap of the gene to gene correlation of the HH signature genes. (C) Density plot of the correlation of random gene sets with the same number of genes as the HH signature. The red line indicates the correlation of the HH gene signature. (D) Internal correlation of the IL6 signature genes. (E) Heatmap of the gene to gene correlation of the IL6 signature genes. (F) Density plot of the correlation of random gene sets with the same number of genes as the IL6 signature. The red line indicates the correlation of the IL6 gene signature. (G) Density plot of the HH signature score in normal (green) and BCC samples (violet). (H) Density plot of the IL6 signature score in normal (green) and BCC samples (violet).

Figure S10

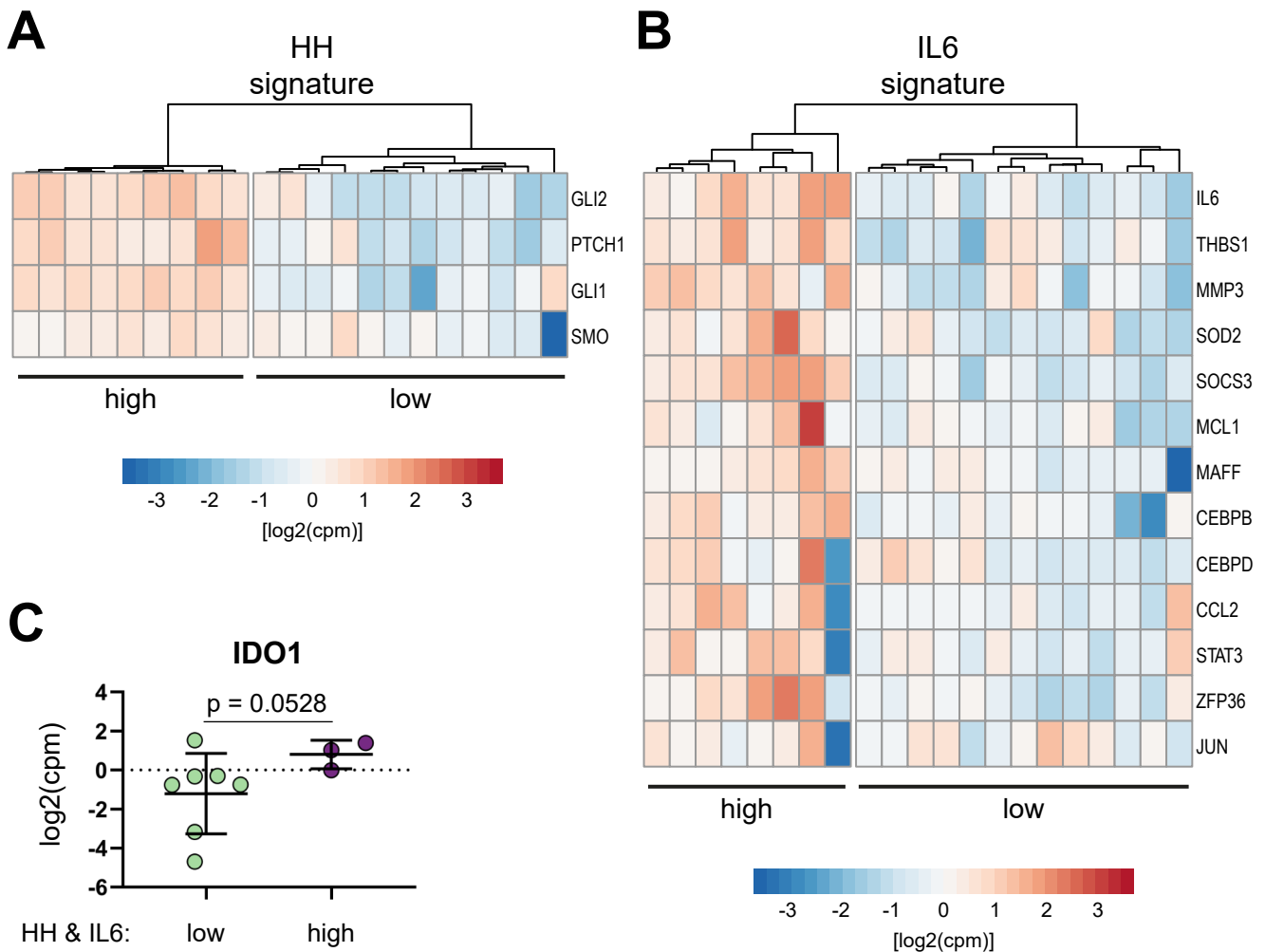


Figure S10: IDO1 is elevated in human BCC patient samples with active HH/GLI and IL6/STAT3 signaling. Clustering analysis of log2(cpm) mRNA expression values of an RNA-seq dataset of human BCC patient and normal skin samples ($n = 21$) from the GEO database (GEO accession: GSE58375) using **(A)** HH/GLI and **(B)** IL6/STAT3 pathway signature genes. **(C)** IDO1 log2(cpm) mRNA expression values of BCC patient and normal skin samples grouped into HH, IL6 low ($n = 7$) or HH, IL6 high ($n = 3$) signaling activity according to the results of the clustering analysis shown in (A) and (B) ($n = 21$). Unpaired Welch's t test was used for statistical analysis ($*p < 0.05$). (cpm: counts per million).