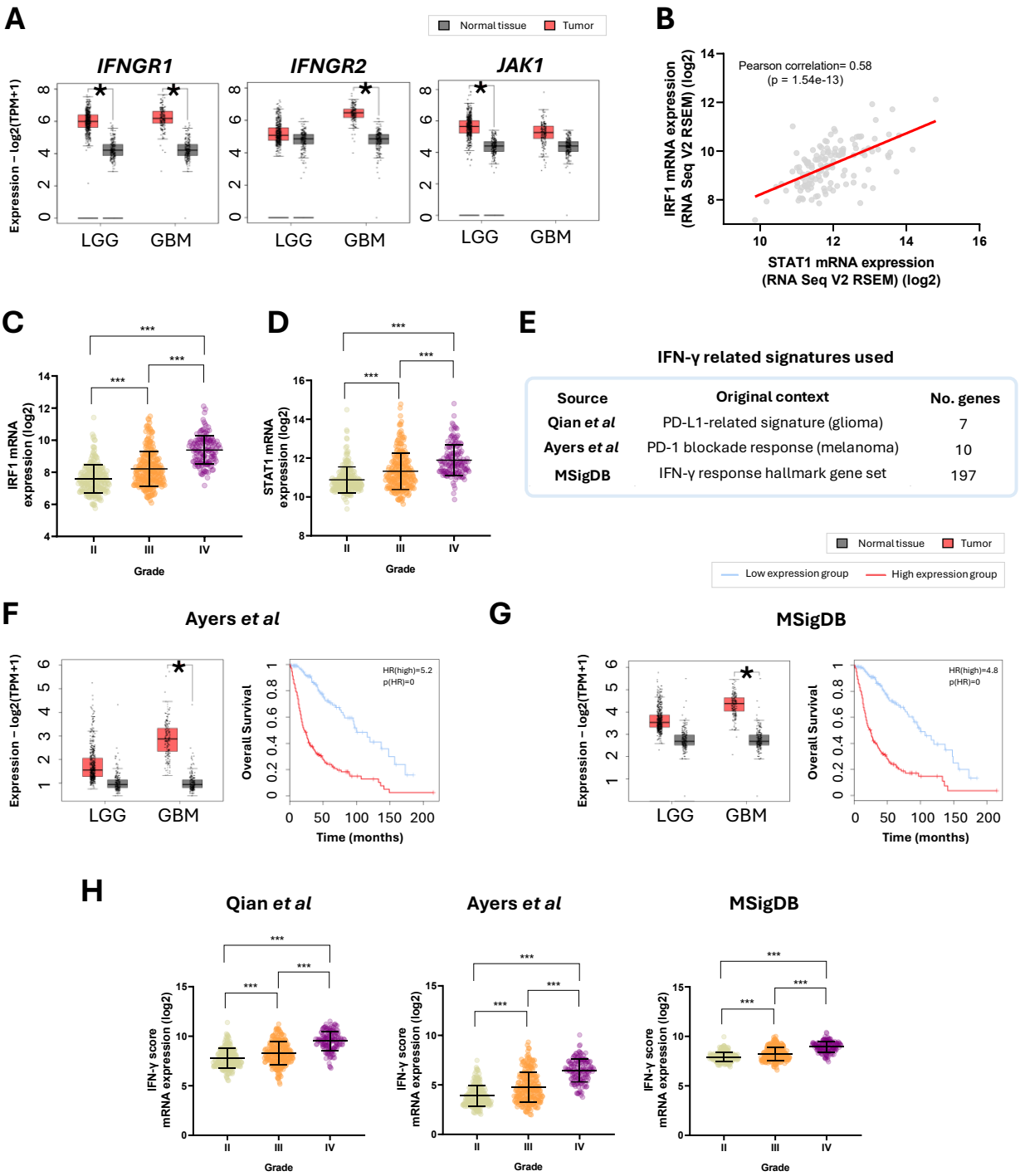
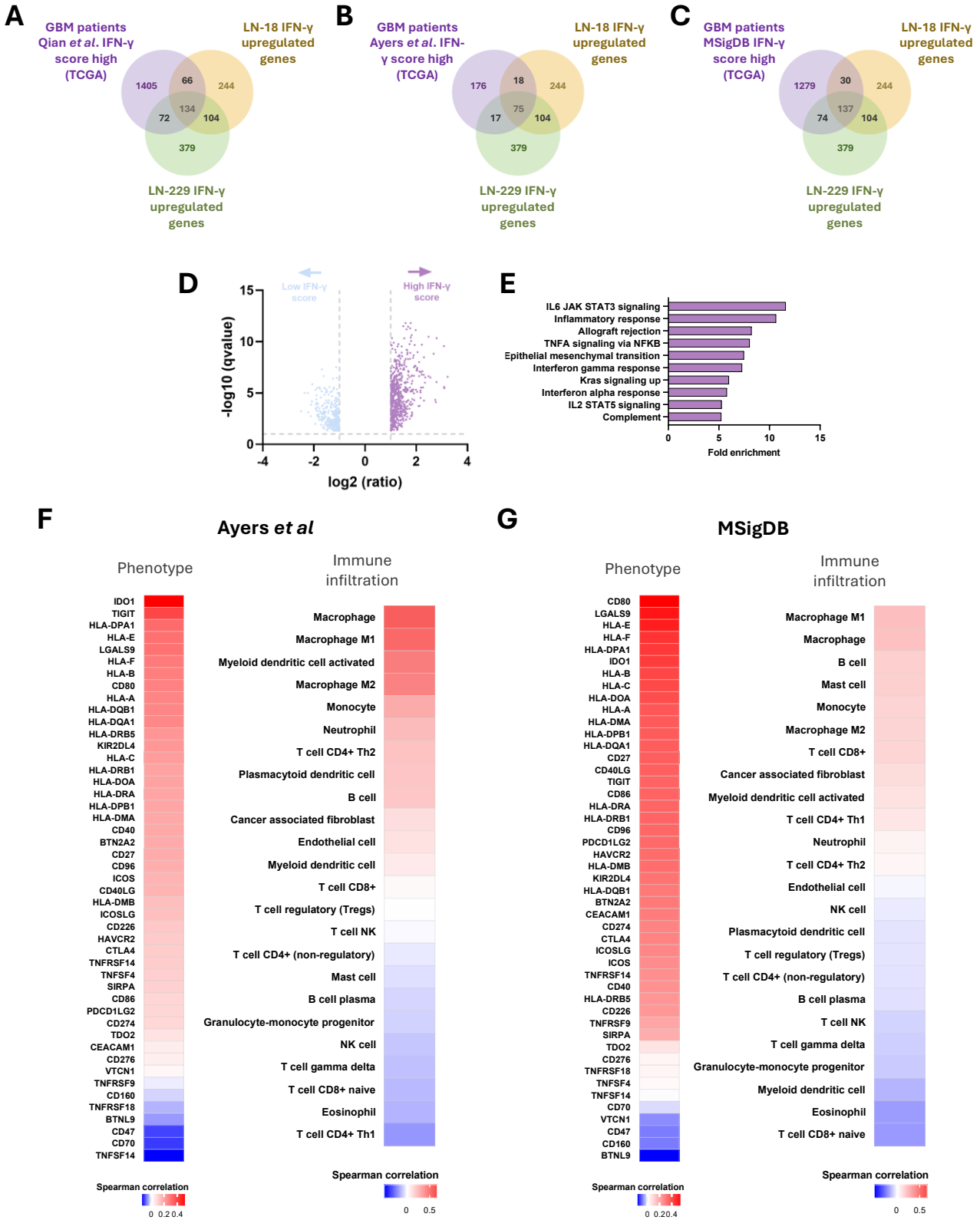
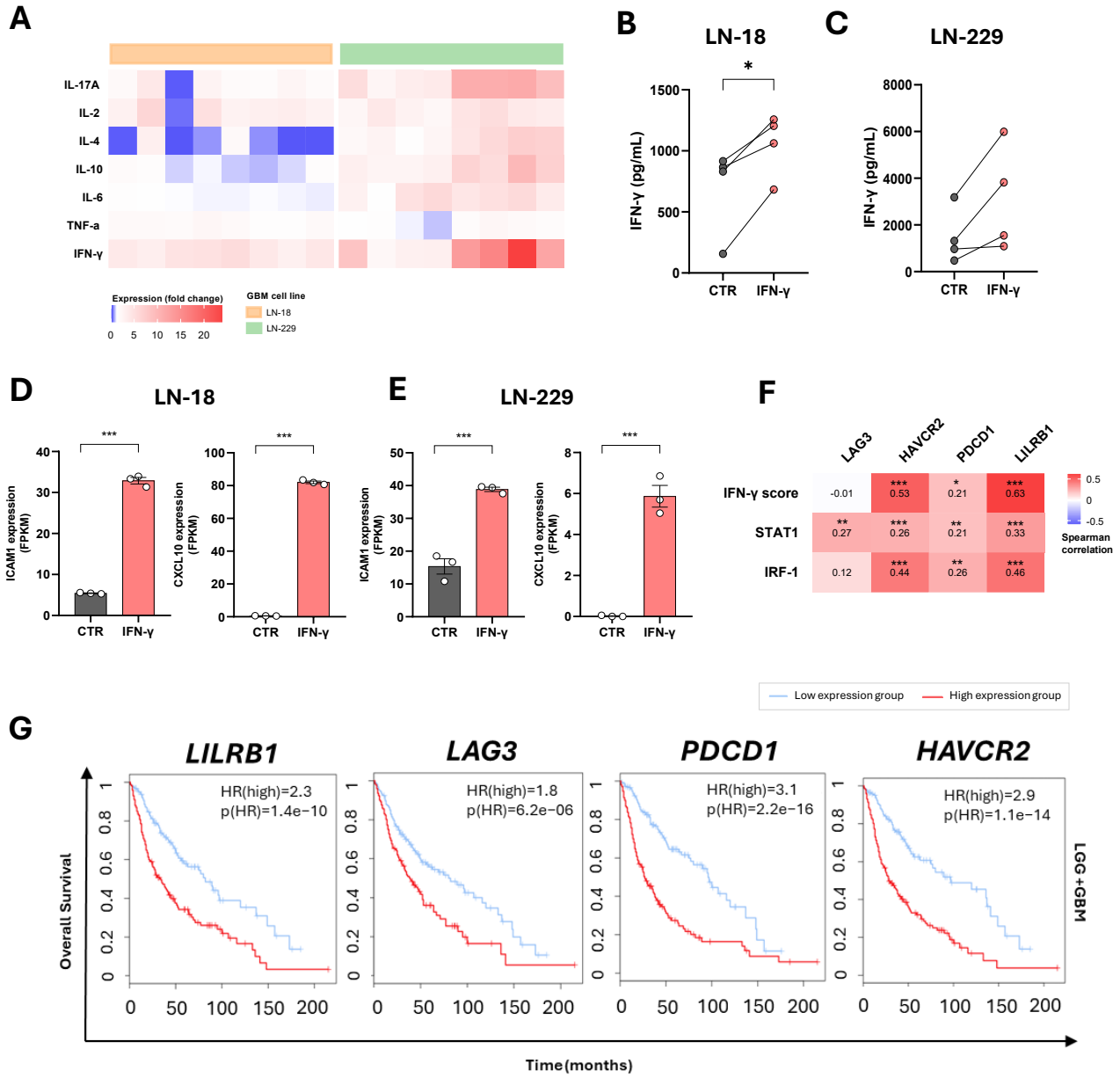


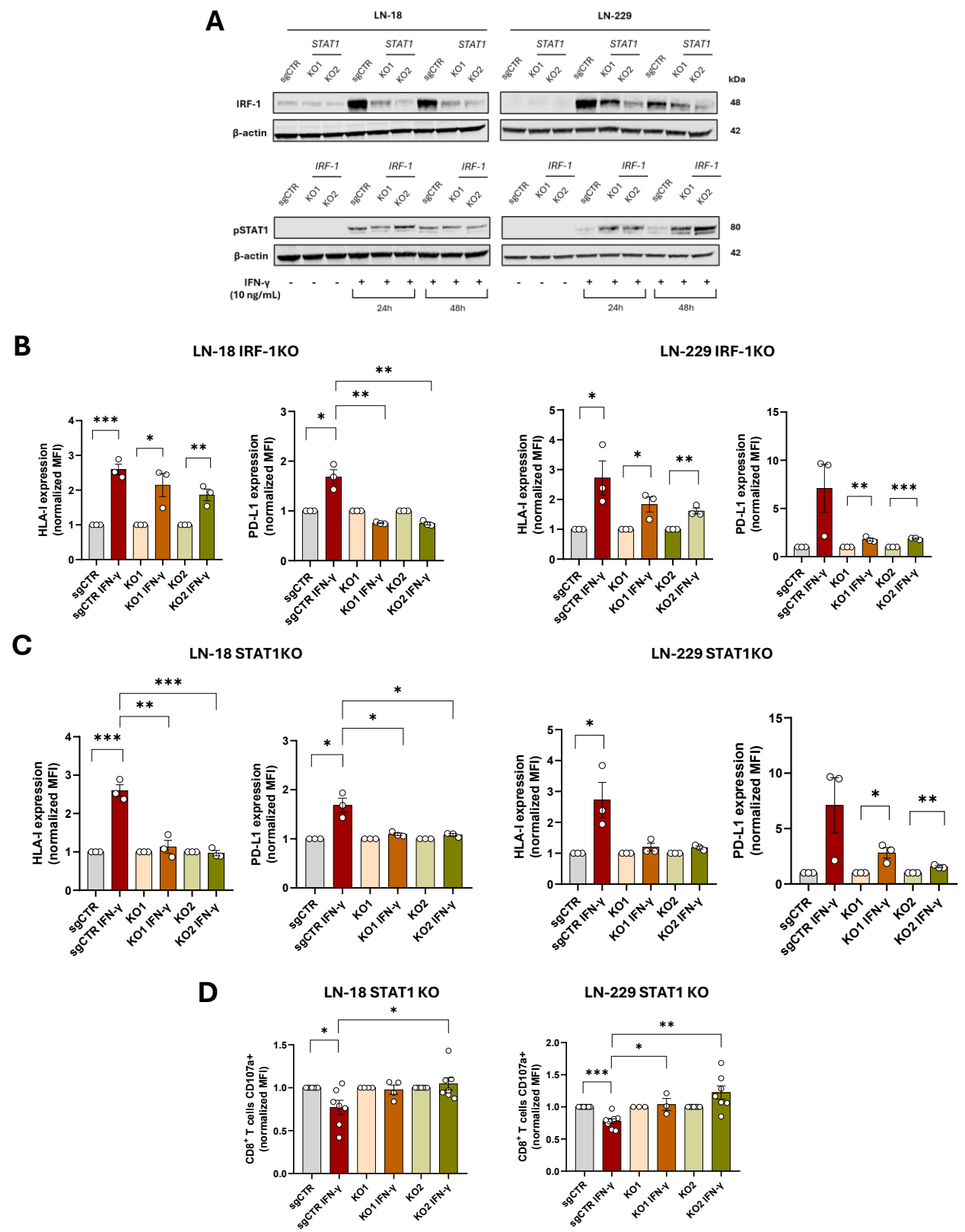
Supplementary Figure S1. Progressive activation of IFN-γ-related signaling pathways across glioma grades. A) mRNA expression levels of IFN-γ-related genes in low-grade glioma (LGG, n= 518) and glioblastoma (GBM, n= 163) samples (red bars) compared with normal brain tissue samples (control, n= 207; grey bars), analyzed using TCGA and GTEx datasets through the GEPIA2 platform. **B)** Spearman correlation analysis between *IRF-1* and *STAT1* mRNA expression in GBM samples from the TCGA Firehose Legacy dataset (n = 136). **C, D)** mRNA expression levels of *IRF-1* (C) and *STAT1* (D) across glioma grades II (n = 226), III (n = 244), and IV (n = 150), analyzed using the TCGA dataset through the GlioVis platform. **E)** Summary table describing the IFN-γ-related gene signatures used in this study. **F, G)** Expression levels and survival analyses associated with the Ayers *et al*. IFN-γ signature (F) and the MSigDB Hallmark IFN-γ Response signature (G) using the same TCGA and GTEx datasets analyzed in panel A. **H)** Expression levels of the three IFN-γ-related signature scores across glioma grades II–IV using the same TCGA datasets analyzed in panels C-D. ***p < 0.001.



Supplementary Figure S2. Integration of IFN-γ-driven transcriptional programs in GBM and their association with immune-related features. A-C) Overlap between genes upregulated in GBM patients with high IFN-γ signaling activity (defined using the Qian *et al*. (A), Ayers *et al*. (B), or MSigDB Hallmark IFN-γ Response signatures (C)) and IFN-γ-stimulated LN-18 and LN-229 cells. **D)** Differential gene expression analysis of GBM patients stratified by the Qian *et al*. IFN-γ score. **E)** Hallmark pathway enrichment analysis of genes upregulated in high IFN-γ score patients in D. **F, G)** Correlations between IFN-γ signaling activity (Ayers *et al*. (F) or Hallmark IFN-γ Response signatures (G) and immune-related gene expression or immune cell infiltration estimated by xCell 2.0. Analyses were performed using the TCGA Firehose Legacy GBM cohort.



Supplementary Figure S3. Functional and transcriptomic evidence of IFN-γ-driven immunosuppression in GBM. A) Heatmap showing cytokine secretion profiles from PBMCs cocultured for 48 h with GBM cell lines (LN-18 and LN-229) pretreated with IFN-γ (10 ng/mL) versus untreated controls. **B, C)** Dot plot quantification of IFN-γ concentration in coculture supernatants by ELISA. **D, E)** Using the same RNA-seq dataset as in Figure 3, expression levels (FPKM, mean ± SEM) of IFN-γ-induced adhesion-related genes, including *ICAM1* and *CXCL10*, are shown for LN-18 and LN-229 cells. **F)** Heatmap of Spearman correlation coefficients between IFN-γ signaling markers (IFN-γ score from Qian *et al.*, STAT1, IRF-1) and immunosuppressive checkpoint genes (*LAG3*, *HAVCR2*, *PDCD1*, *LILRB1*) in GBM patient samples (TCGA, Firehose Legacy). **G)** Kaplan-Meier survival analysis of LGG and GBM patients stratified by high versus low average expression of the genes in panel F (n = 338 each group). HR, hazard ratio. ***p< 0.001, **p<0.01,*p< 0.05.



Supplementary Figure S4. STAT1 mediates IFN-γ-induced signaling and immune checkpoints expression in GBM cells. A) Western blot analysis of IRF-1 expression in *STAT1* knockout (STAT1KO) LN-18 (left) and LN-229 (right) cells, and of total and phosphorylated STAT1 levels in *IRF-1* knockout (IRF-1KO) LN-18 and LN-229 cells. Cells were left untreated or stimulated with IFN-γ (10 ng/mL) for 24 or 48 h. β-Actin was used as a loading control. **B, C)** Flow cytometric analysis of classical HLA-I molecules and PD-L1 expression in IRF-1KO (B) and STAT1KO (C) cells compared with sgCTR controls. Cells were analyzed under basal conditions or after 48 h of IFN-γ stimulation. Graphs show relative mean fluorescence intensity (MFI) normalized to untreated controls (set to 1). **D)** Flow cytometric assessment of CD107a expression in $CD8^+$ T cells from PBMCs cocultured for 4 h with control or IFN-γ-pretreated GBM cells. Graphs represent normalized CD107a expression relative to the corresponding untreated condition. Data are presented as mean ± SEM from at least three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001.