**Supplementary material for**

**Phenotypic mapping of pathological crosstalk between glioblastoma and innate immune cells by synthetic genetic tracing**

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**Supplementary Figures with Legends**

**Supplementary Figure S1.** Extended characterization of selected reporters and reporter lines.

**Supplementary Figure S2.** Extended analysis of *in vivo* mesenchymal trans-differentiation.

**Supplementary Figure S3.** Extended analysis of mesenchymal sLCR activation by external signaling.

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**Supplementary Figure S6.** Extended characterization of microglia-driven mesenchymal transition in glioma-initiating cells.

**Supplementary Figure S7.** hMG cells induce mesenchymal glioblastoma with similar features as patients’ derived signatures.

**Supplementary Tables**

**Supplementary Table S1.** GBM-sLCR features

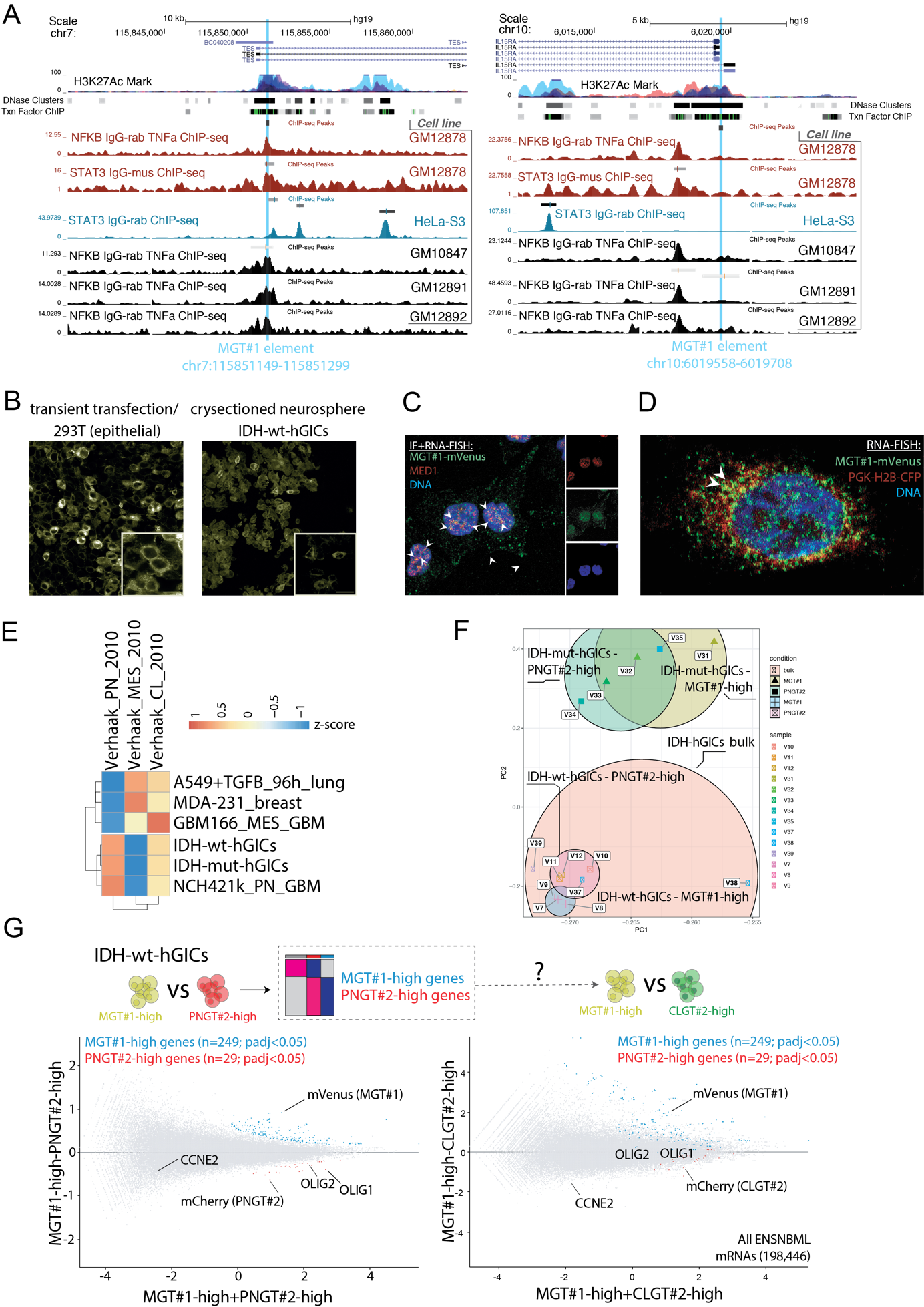
**Supplementary Table S2.** Phenotypic CRISPR-screen data analysis

**Supplementary Table S3.** List of reagents, software and deposited data

**Supplementary References**

**Figures with Figure Legends**

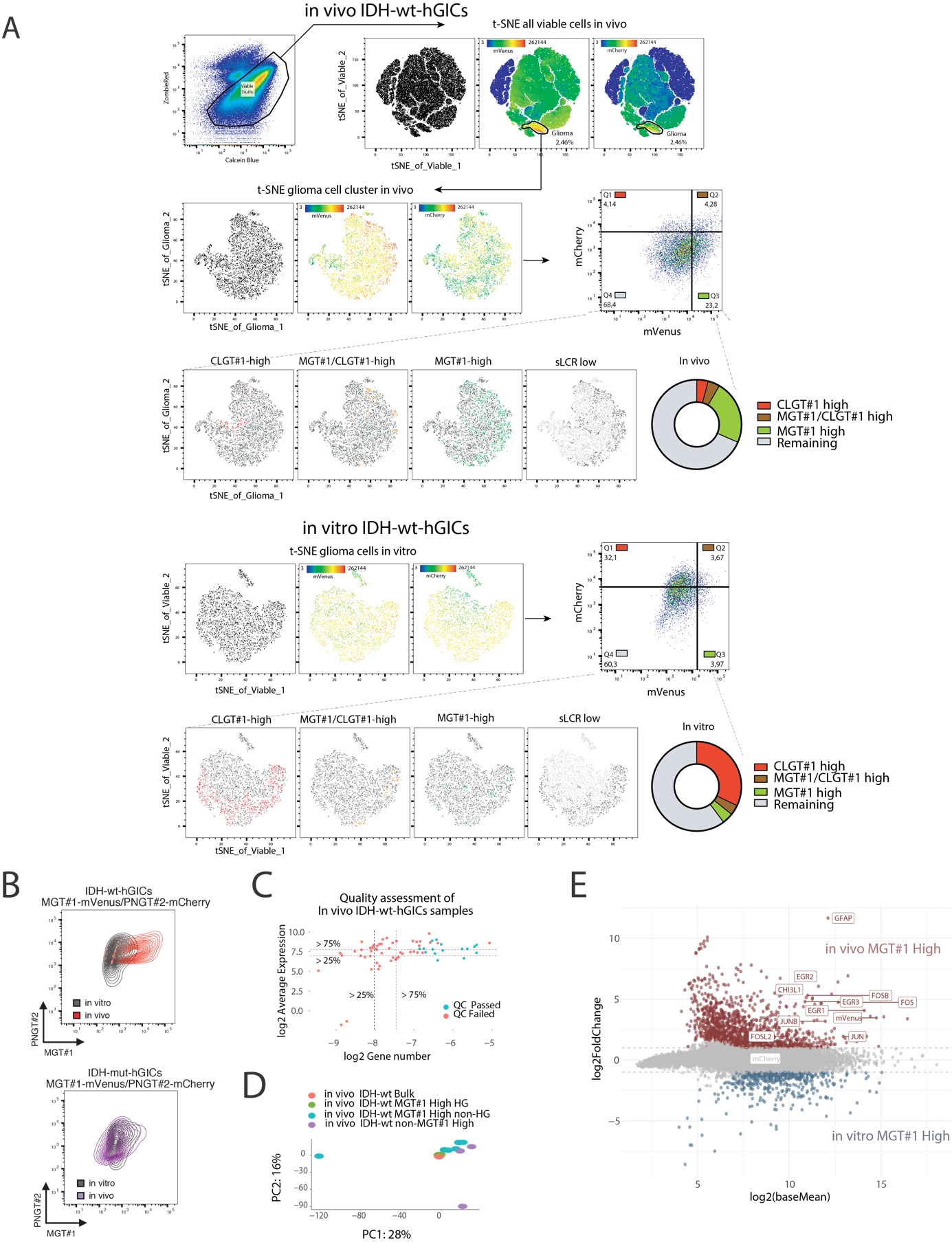
**Supplementary Figure S1**



**Supplementary Figure S1. Extended characterization of selected reporters and reporter lines.**

**(A)** UCSC Genome Browser view of ENCODE ChIP-seq for NFKB and STAT3 binding at two independent regions used as cis-regulators elements in MGT#1 (light blue column). **(B)** Left; confocal imaging of transiently MGT#1-transfected 293T; right, cryosectioned IDH-wt-hGICs tumorspheres upon lentiviral transduction. Scale=10μm. **(C)** Dual IF and smRNA-FISH. Images of the merged (left) and separate channels (right) are shown. Overlapping signal in yellow and arrowheads denote co-localization between MED1 and MGT#1-driven mVenus in TNF-stimulated A549 cells. **(D)** single-molecule RNA FISH quantification of MGT#1- and PGK-driven gene expression in TNF-stimulated A549 cells. Arrowheads/yellow denote cytoplasmic colocalization. **(E)** GSEA normalized scores for TCGA subtypes (3) of indicated cell lines from Fig.1D. **(F)** Principal component analysis of the indicated RNA-seq profiles. Bulk RNA-seq are generated by FACS sorting live and single hGICs. Note that bulk cells are more variable than reporter-expressing cells. **(G)** MA plot for the indicated FACS sorted RNA-seq profiles. Blue dots represent significant MGT#1 specific markers in the comparison MGT#1 vs PNGT#2 (padj<0.05). Reporter and selected genes are highlighted. Note that MGT#1 selected markers remain enriched in MGT#1 fraction, whereas PNGT#2 selected markers change when CLGT#2 is used in the comparison (schematic above).

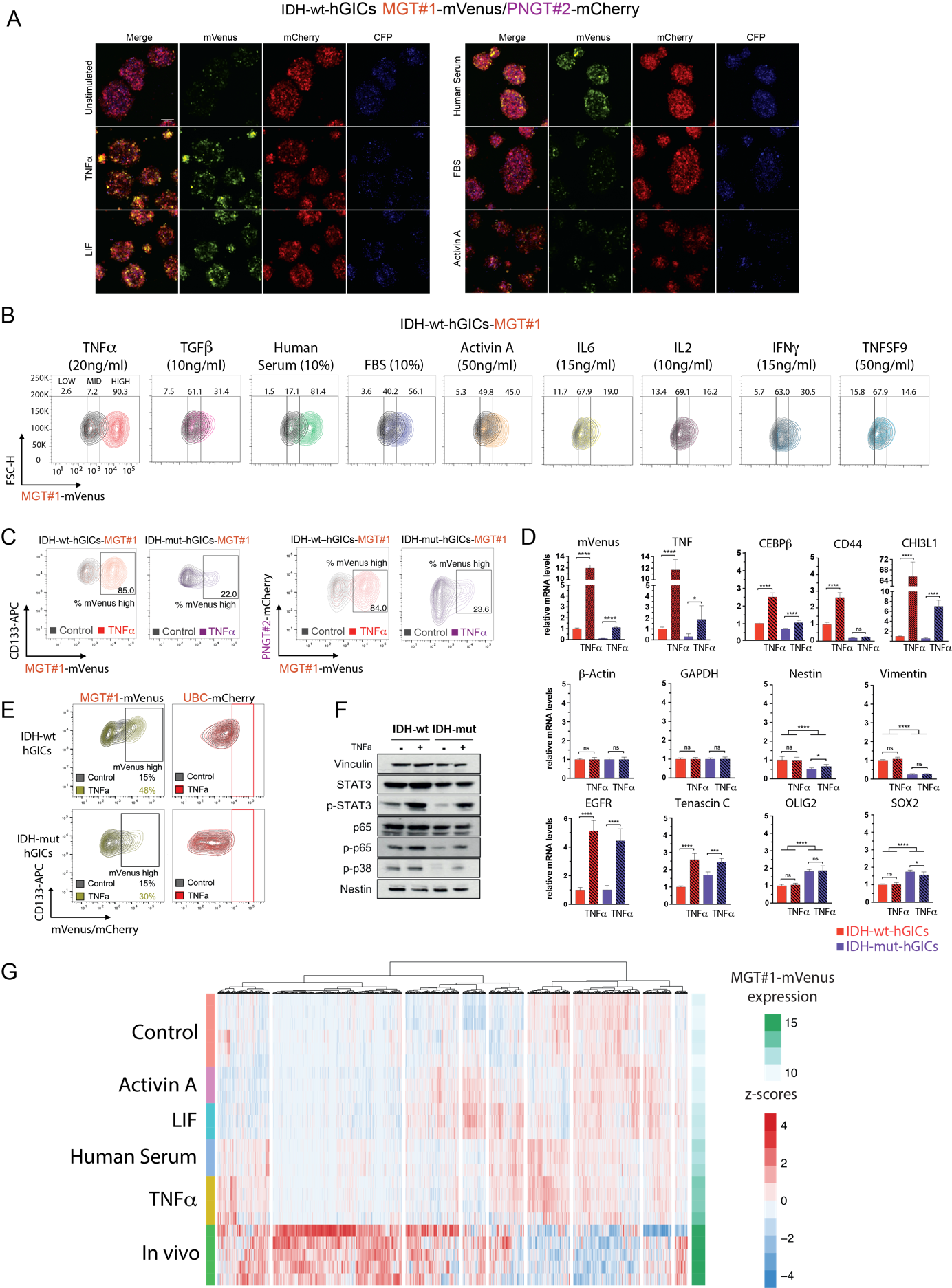
**Supplementary Figure S2**

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**Supplementary Figure S2. Extended analysis of *in vivo* mesenchymal trans-differentiation.**

**(A)** Example of gating strategy for FACS analysis of viable- and single-IDH-wt-hGICs-MGT#1-CLGT#1 *in vitro* and *in vivo*. (see **Methods**). **(B)** *In vivo/in vitro* FACS profile comparison of representative MGT#1/PNGT#2 dual-sLCR IDH-wt-hGICs and IDH-mut-hGICs. **(C)** Representation of the average expression (Y-Axis) and the number of expressed genes (X-axis) per *in vivo* expression profile profiles. Selected files (QC Passed) are shown in blue (see **Methods)**. **(D)** PCA of retained *in vivo* transcriptome profiles. Conditions for each sample are indicated in the legend. **(E)** MA plot of the comparison between *in vitro* MGT#1-high and *in vivo* MGT#1-high. Selected genes are highlighted.

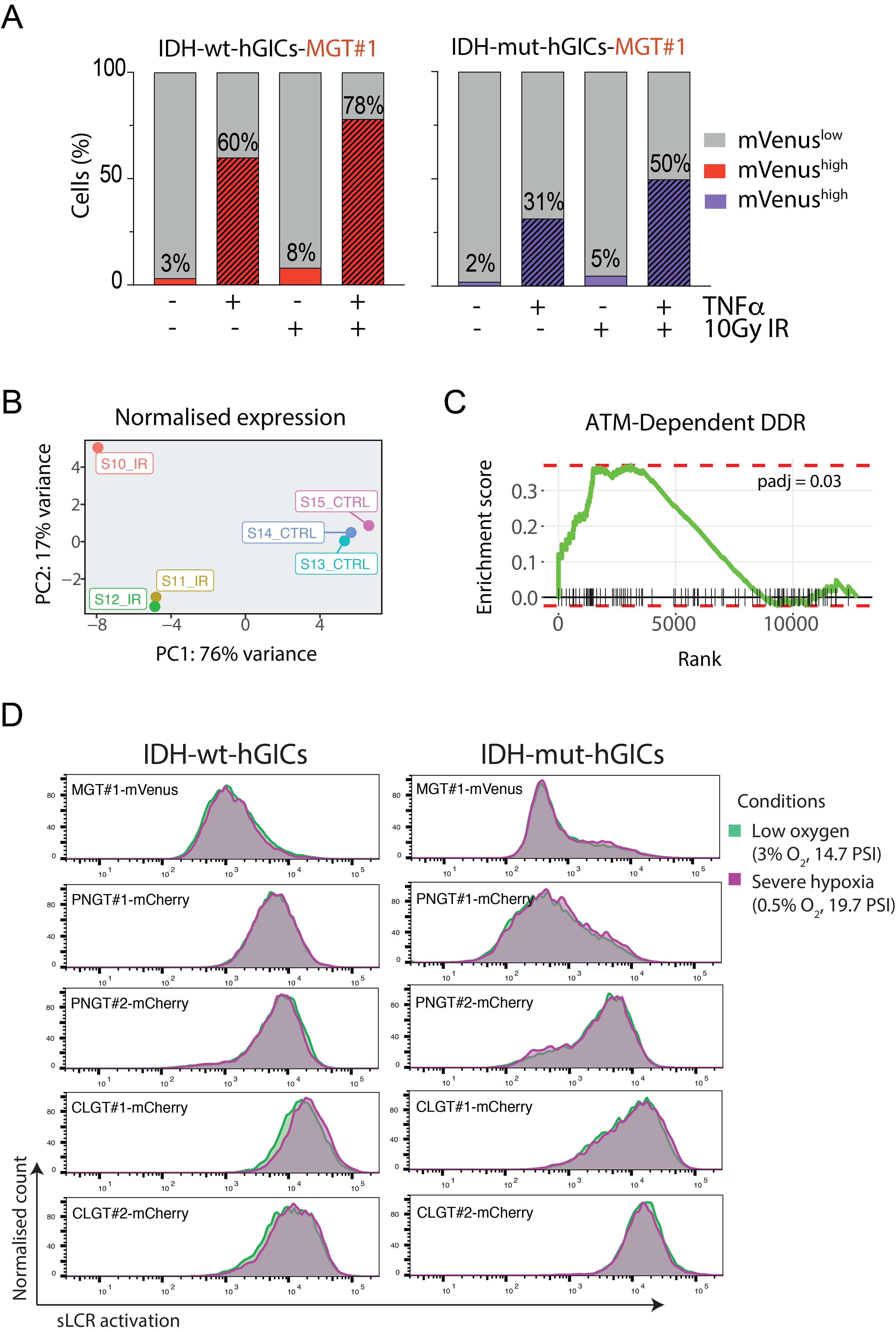
**Supplementary Figure S3**

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**Supplementary Figure S3. Extended analysis of mesenchymal sLCR activation by external signaling.**

**(A)** Representative confocal live cell imaging of MGT#1/PNGT#2 dual-sLCR IDH-wt-hGICs exposed to indicated stimuli after 48h as in (B). **(B)** Representative MGT#1 activation in IDH-wt-hGICs by FACS upon the indicated stimuli. **(C)** Representative FACS profile of selected markers expression in the indicated conditions. **(D)** RT-qPCR validation of the indicated genes in response to Tumor Necrosis Factor alpha (TNF) treatment in the indicated GICs. n=3 biologically independent samples, ANOVA followed by Dunnet’s post hoc test; \*\*\*\*=P<0.0001; mRNAs are normalized to b-Actin and IDH-wt-hGICs control cells. **(E)** Representative FACS profile of selected markers expression in the indicated conditions. UBC-mCherry is a control reporter generated by using a broadly expressed promoter (UBC). **(F)** Immunoblotting of the indicated conditions and antibodies. **(G)** Heatmap of combined up-regulated gene expression normalizes values for the indicated comparisons. MGT#1-mVenus expression is individually indicated for all the samples grouped by condition (green).

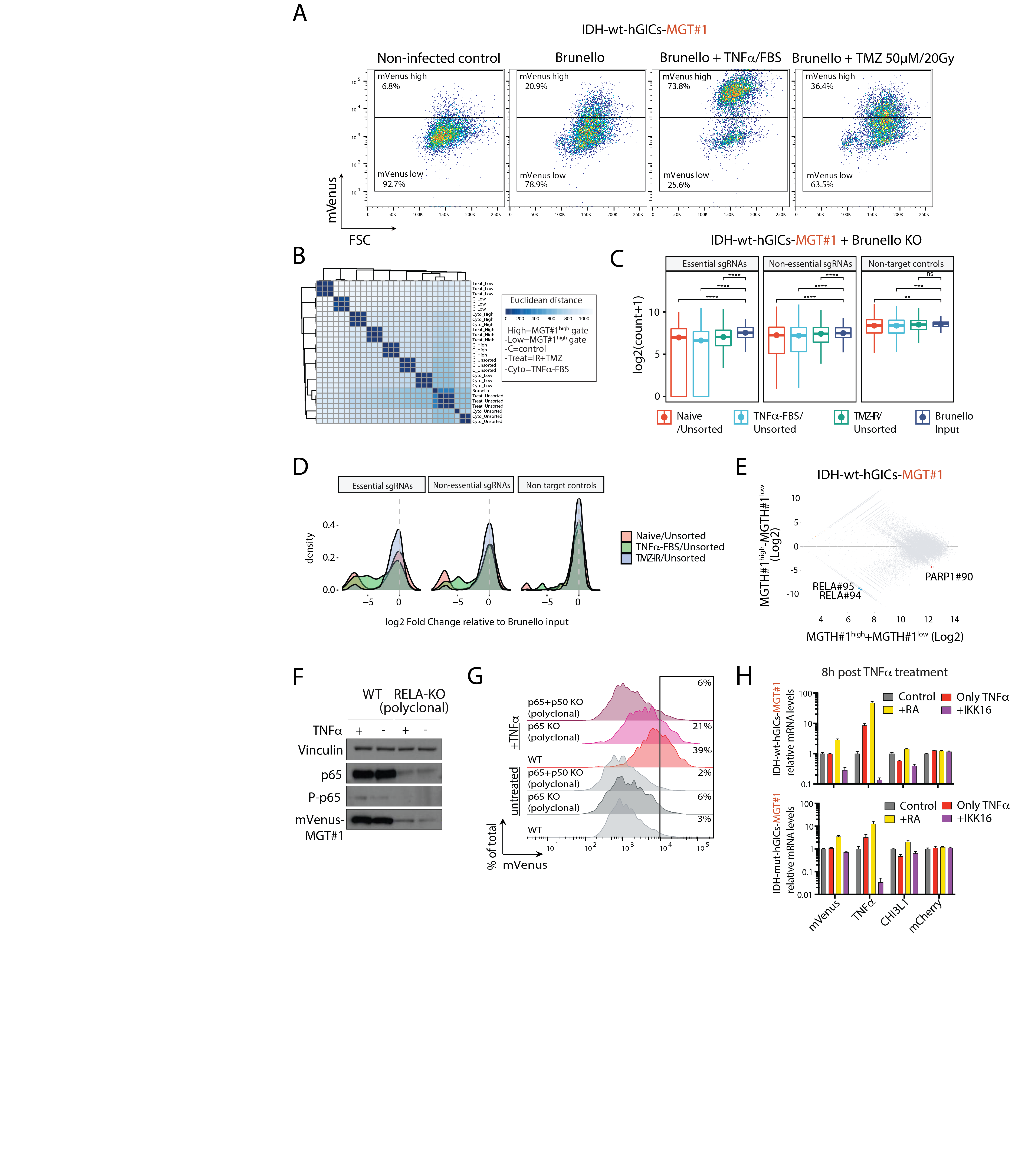
**Supplementary Figure S4**



**Supplementary Figure S4. Extended analysis of mesenchymal sLCR activation by environmental triggers.**

**(A)** Representative FACS quantification of MGT#1 activation at 48h in response to the indicated stimuli. **(B)** PCA of normalized transcriptome profiles of the irradiated and control samples. **(C)** Differential GSEA of the irradiated (n=2) versus control (n=3) samples for the indicated gene set. **(D)** Representative FACS profiles of sLCR expression in hGICs after 3d under severe hypoxia (magenta) compared to low oxygen (green).

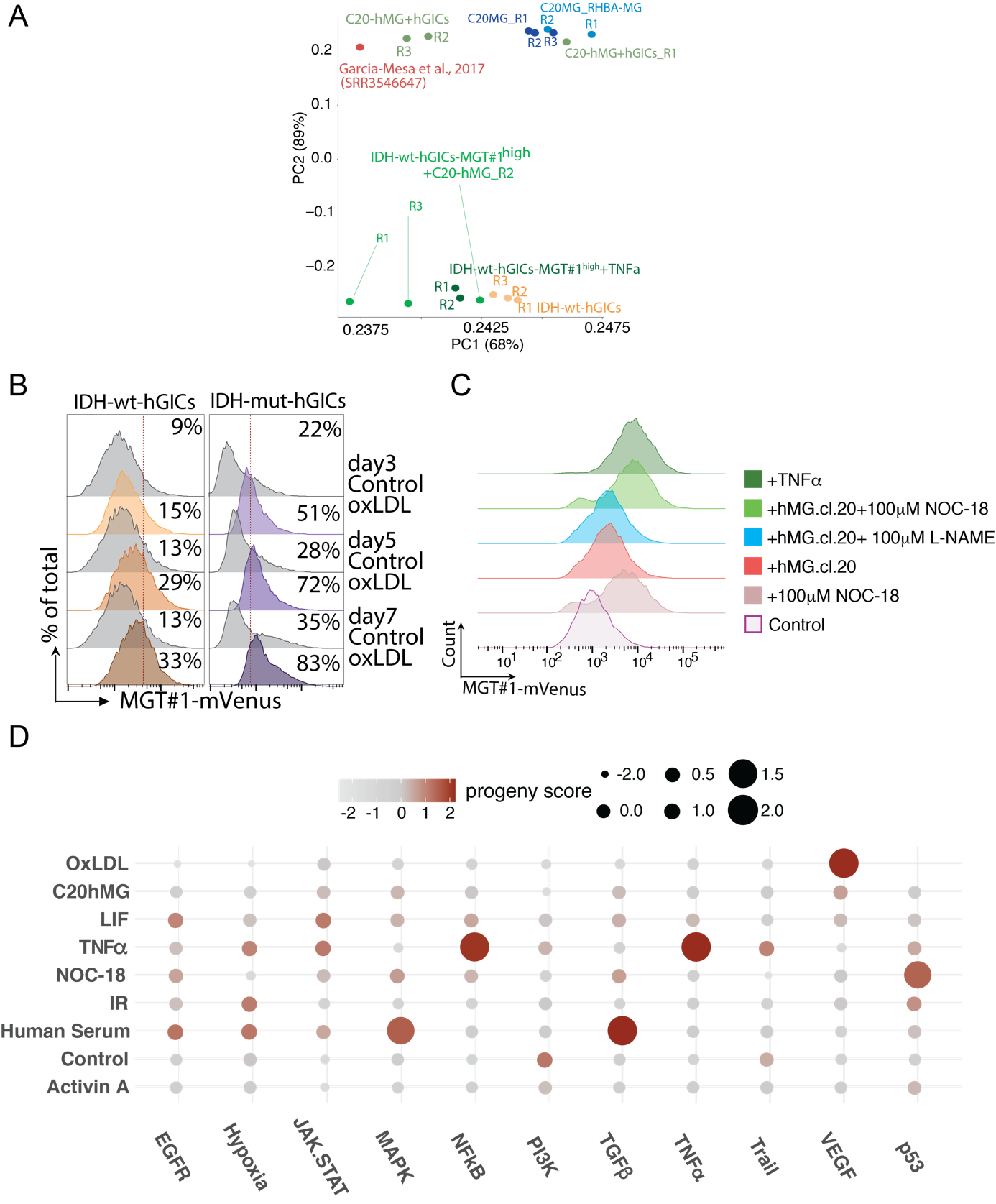
**Supplementary Figure** **S5**



**Supplementary Figure S5. Phenotypic CRISPR/Cas9 forward genetic screens using sLCRs.**

**(A)** FACS plots prior sorting of the MGT#1-high and MGT#1-low fractions and subsequent sgRNA amplification for indicated conditions. **(B)** Correlation heatmap showing Euclidian distance between the indicated samples post regularized log2 normalization of the sgRNAs abundance. **(C)** Box plot showing data quality assessment for the indicated conditions at the time-point chosen to perform the phenotypic screen by comparing the distribution of validated essential sgRNAs to all non-essential or non-targeting sgRNAs (P-value = Wilcoxon rank-sum test). **(D)** Distribution of log2-fold changes for sgRNAs targeting the indicated gene sets in the averaged unsorted IDH-wt-hGICs conditions (n=3) relative to the Brunello input. **(E)** MA plot of sgRNA abundance (x-axis) and fold-change (y-axis) for FACS sorted MGT#1-high and MGT#1-low fraction in naïve IDH-wt-hGICs carrying the Brunello library. The gRNAs were normalized to the largest dataset and log2-transformed. The highlighted sgRNAs are depleted compared to MGT#1-high fraction. **(F)** Immunoblotting of the indicated conditions and antibodies. **(G)** Representative FACS quantification of MGT#1 activation by the indicated treatments at 48h and 72h, respectively. **(H)** RT-qPCR of the indicated genes upon sequential treatment with the indicated treatments and challenging with TNF.

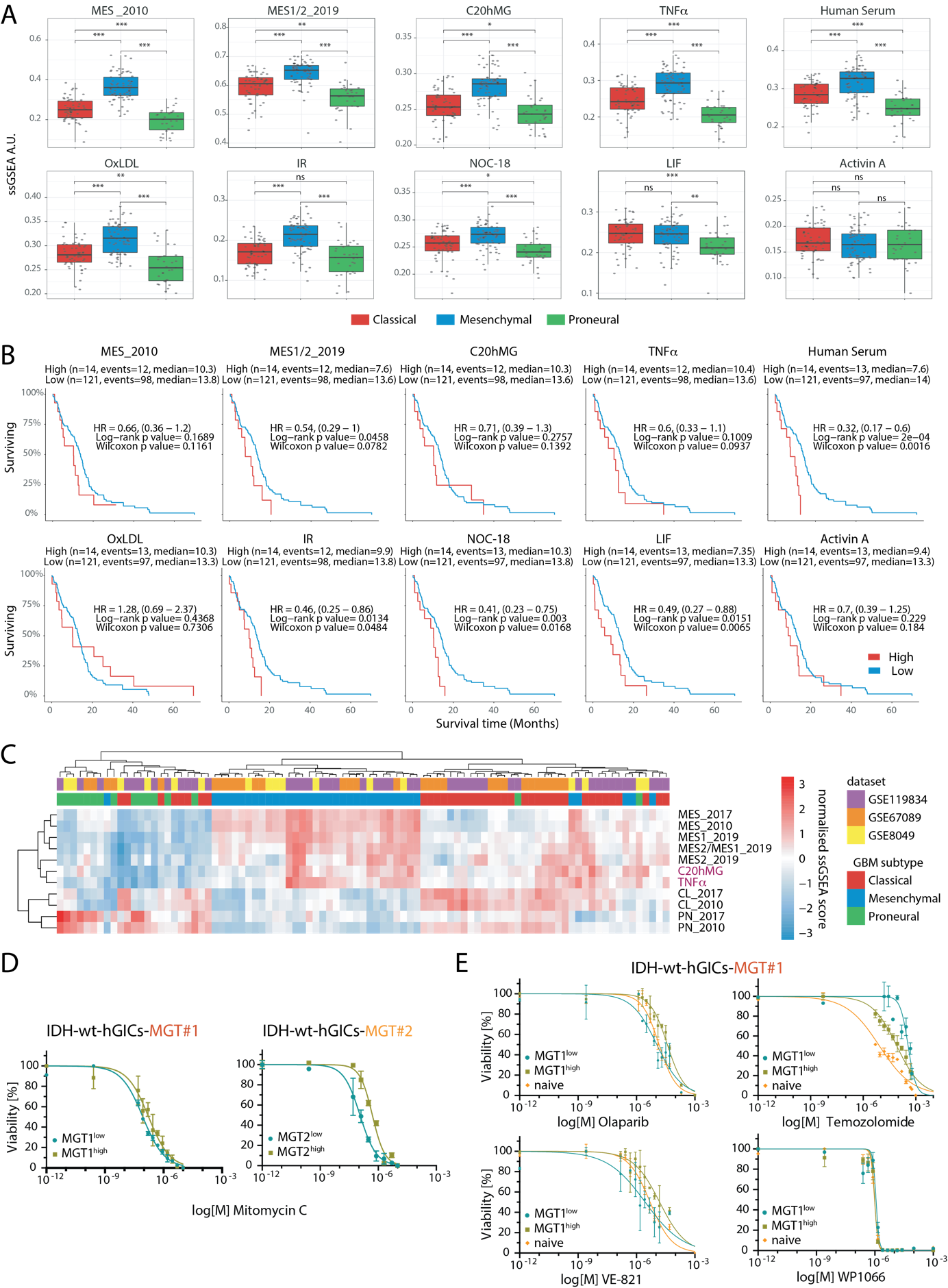
**Supplementary Figure S6**



**Supplementary Figure S6. Extended characterization of microglia-driven mesenchymal transition in glioma-initiating cells.**

**(A)** Principal component analysis of the indicated RNA-seq profiles. Distances were calculated based on the average expression level of selected human MG markers obtained from (60). **(B)** Representative FACS profiles of IDH-wt and IDH-mut-hGICs-MGT#1 treated with 15µg/ml oxLDL or control for the indicated time. **(C)** Representative FACS profiles of IDH-wt-hGICs-MGT#1 treated as indicated for 48h. In co-culture experiments, hMG cells were pre-treated for 24h with the indicated drug concentrations before IDH-wt-hGICs-MGT#1 were seeded. Drugs were refreshed at the time of seeding IDH-wt-hGICs for co-culture. **(D)** Pathway analysis of condition-specific genes for each MGT#1 activator using PROGENy.

**Supplementary Figure S7**



**Supplementary Figure S7. hMG cells induce mesenchymal glioblastoma with similar features as patients’ derived signatures.**

**(A)** Box-plot representation of single-sample GSEA normalised scores for the indicated gene sets for each TCGA GBM patients’ expression profile, grouped by TCGA-assigned GBM subtype. P-values are calculated by t-test followed by Benjamini-Hochberg post hoc test. (ns=not significant; \*\*\*=P<0.001; see **Methods)**. **(B)** Kaplan-Meier survival plots of TCGA-GBM patients grouped by high and low expression of indicated gene sets(see **Methods**). **(C)** Heatmap of the relative single-sample GSEA score for the indicated gene sets in patient-derived GSCs from the indicated profiles. The corresponding GBM subtypes are also indicated. **(D)** Dose-response curve of FACS-sorted IDH-wt-hGICs subjected to increasing concentrations of Mitomycin C for IDH-wt-hGICs fractions FACS sorted using either MGT#1- or MGT#2-high. **(E)** Dose-response curve of MGT#1-high, MGT#1-low and naïve IDH-wt-hGICs cells subjected to increasing concentrations of the indicated compounds.

**Supplementary References**

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