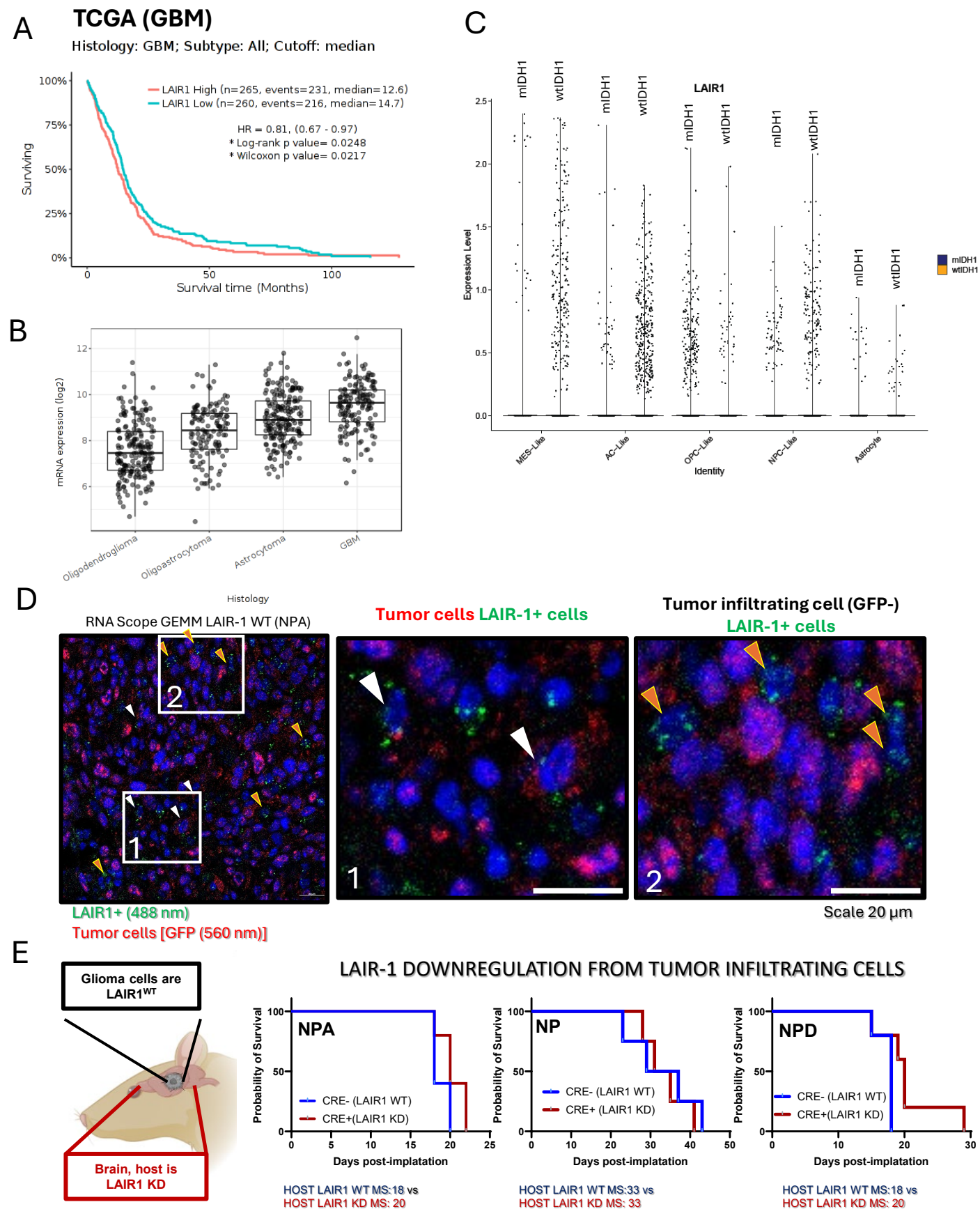


Supplementary Figures

Supp Fig 1



Supplemental Figure 1.

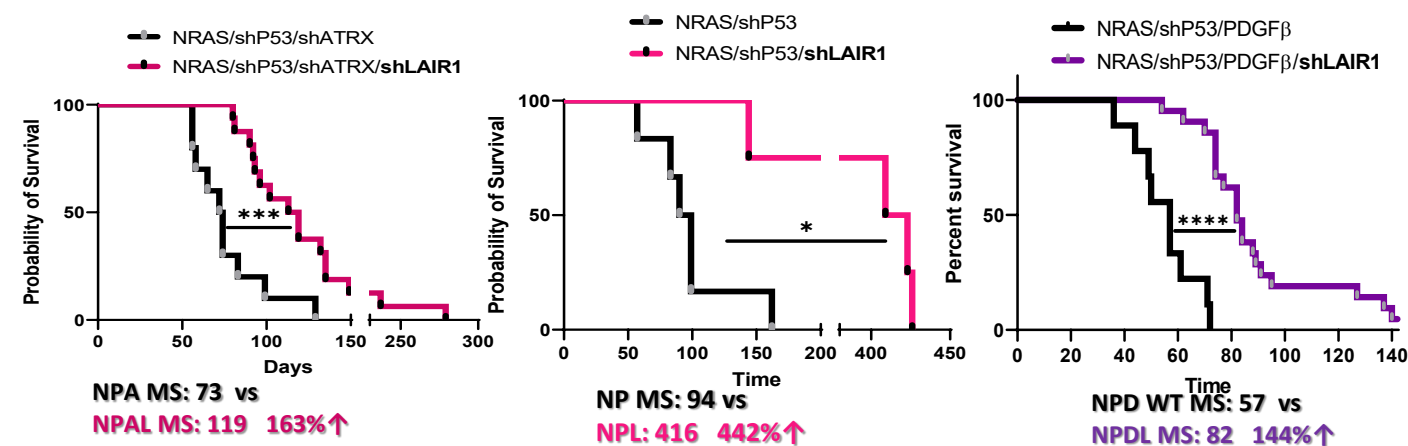
A. Survival curve of patients within the GBM database of TCGA stratified by LAIR-1 high (-) /low (-) expression. Low LAIR-1 expression has improved median survival. **B.** LAIR-1 expression stratified by histological glioma types. GBM, the most maligning type, has the highest expression of LAIR-1. **C.** LAIR-1 scRNAseq expression in glioma cells stratified by glioma cell state and dived by IDH1 Wild type (wtIDH1) and IDH1 mutant (mIDH1). All glioma cell states express LAIR-1, being MES-like the highest. **D.** RNA scope on brain sections of LAIR-1 WT mice stained for tumor cells (GFP (red)) and LAIR-1 (green). White arrows show LAIR-1⁺ GFP⁺ cells. Orange arrows show LAIR-1⁺ GFP⁻ cells. Scale bar is 20 μ m. **E.** Schematic of genetically engineered mouse model with LAIR-1 knockdown in host cells and implanted with LAIR-1 WT tumor cells. **F.** Survival curves of LAIR-1 Knockdown (KD) (●) mice bearing tumors with LAIR-1 WT (●) from multiple glioma models. NRAS/shP53/shATRX (NPA); NRAS/shP53 (NP); NRAS/shP53/PDGF β (NPD). There were no significant changes in survival, across three models.

Supp Fig 2

A

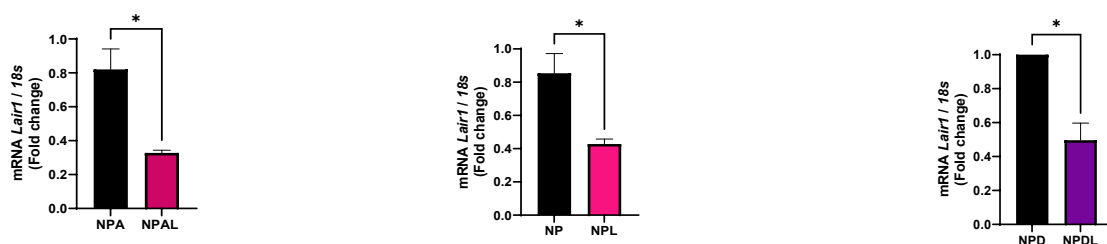
MOUSE GLIOMA MODEL WITH LAIR-1 DOWNREGULATION

SURVIVAL



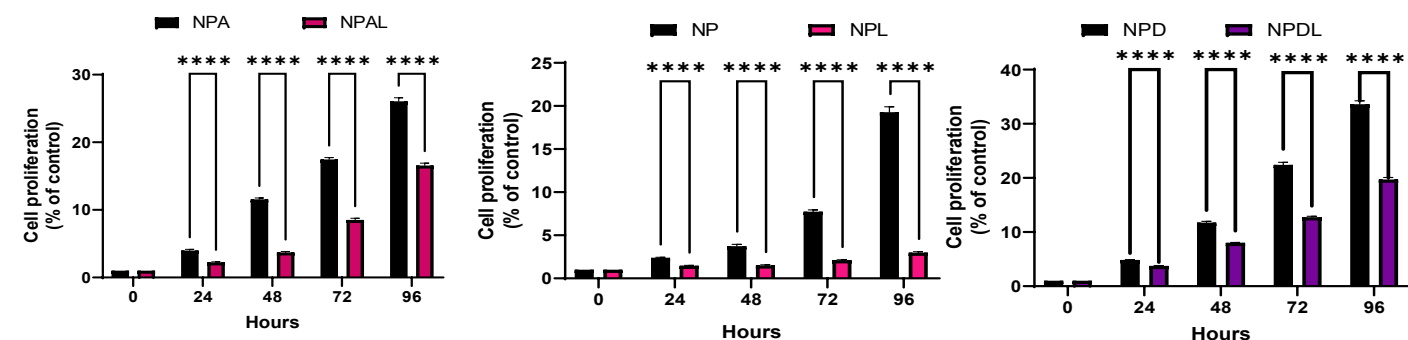
B

LAIR-1 EXPRESSION



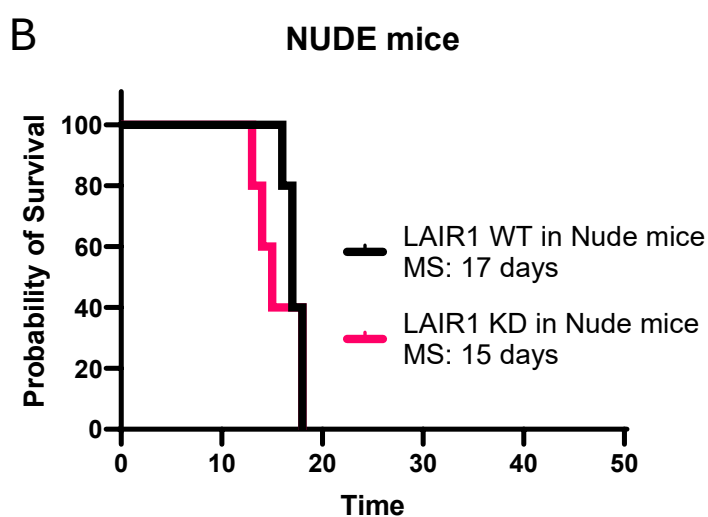
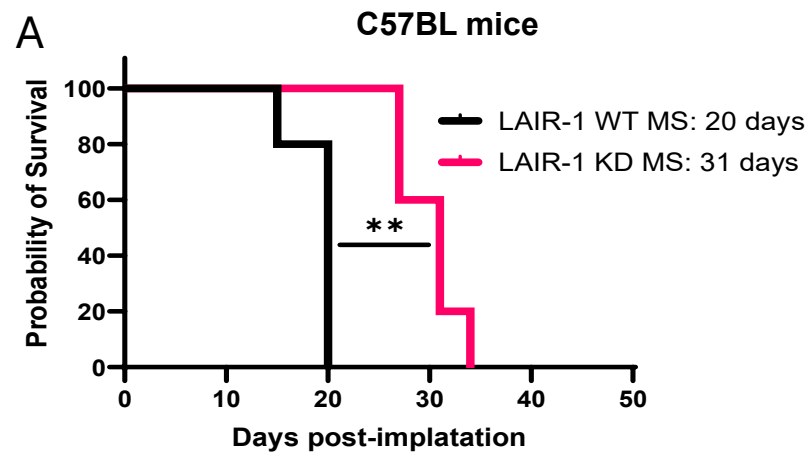
C

IN VITRO PROLIFERATION



Supplemental Figure 2. A. Survival curves from wild type (C57BL) mice bearing Sleeping Beauty induced GEMMs with LAIR-1 KD. (i) NRAS/shP53/shATRX/shLAIR1 (NPAL) ($\bullet$) group median survival (MS) is 113 days, significantly more than the respective LAIR-1 WT(NPA) ($\bullet$) (MS: 73 days). (ii)NRAS/shP53/shLAIR1 (NPL) ($\bullet$) group median survival (MS) is 416 days, significantly more than LAIR-1 WT(NP) ($\bullet$) (MS: 94 days). (iii)NRAS/shP53/PDGF β /shLAIR1 (NPDL) ($\bullet$)group median survival (MS) is 82 days, significantly more than LAIR-1 WT group (NPD) ($\bullet$) (MS: 57 days). **B.** Fold change of *LAIR-1* *in vitro* gene expression of glioma cells derived from each different glioma models, measured by qPCR, relative to 18s expression. **C.** *In vitro* cell proliferation assay of LAIR-1 WT and LAIR-1 KD cells from glioma models. Bar graphs show mean and SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supp Fig 3

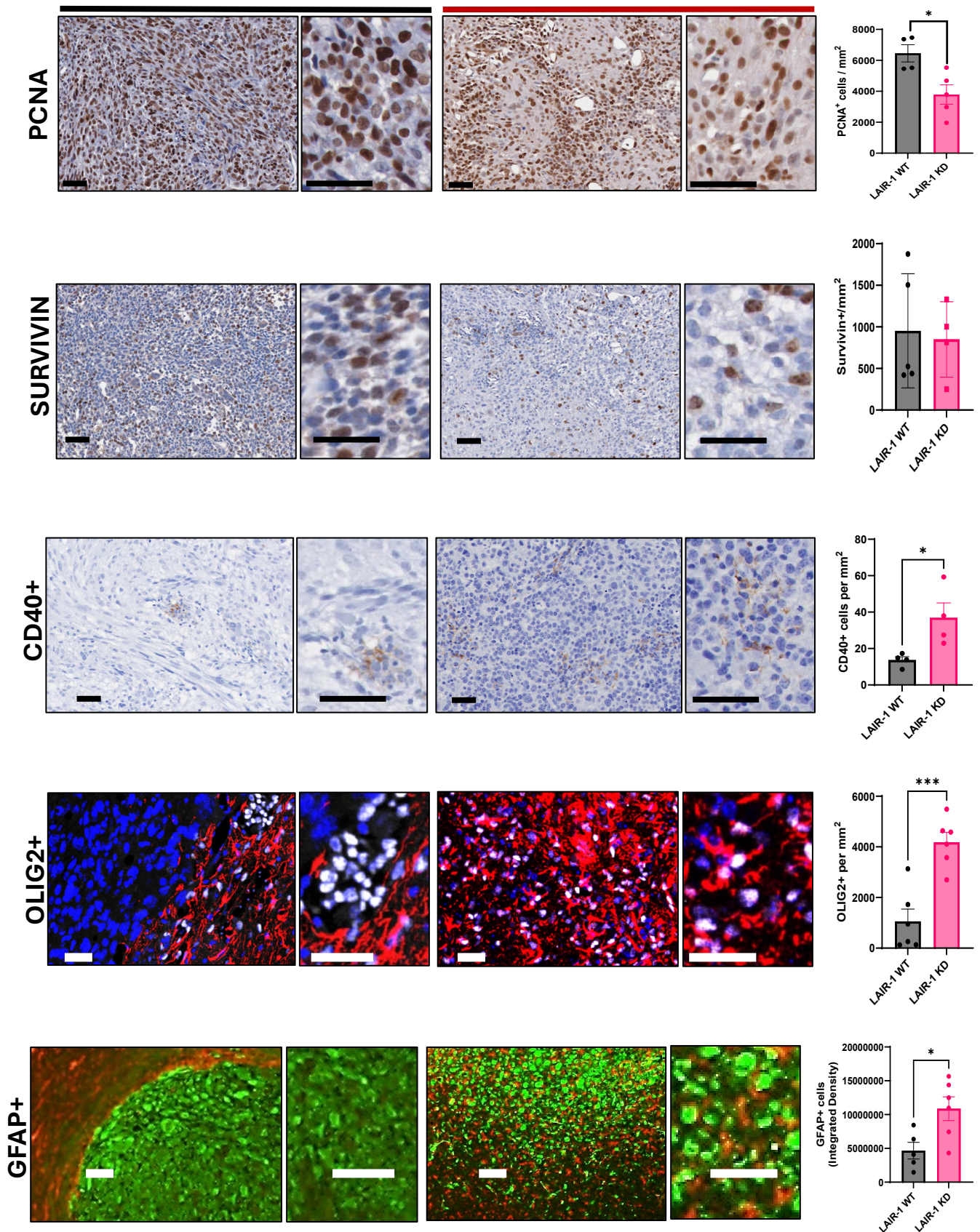


Supplemental Figure 3. A. Survival curve of wild type(C57BL) mice implanted with LAIR-1 KD (NPAL) glioma cells (●) (MS: 31 days) and LAIR-1 WT glioma cells (NPA) (●) (MS: 20 days). LAIR-1 KD bearing-mice survived longer than LAIR-1 WT. $**p=0.0035$. **B.** Survival curve of immunocompromised NUDE mice implanted with LAIR-1 KD (NPAL) glioma cells (●) (MS: 15 days) and LAIR-1 WT glioma cells (NPA) (●) (MS: 17 days). No statistical difference.

Supp Figure 4

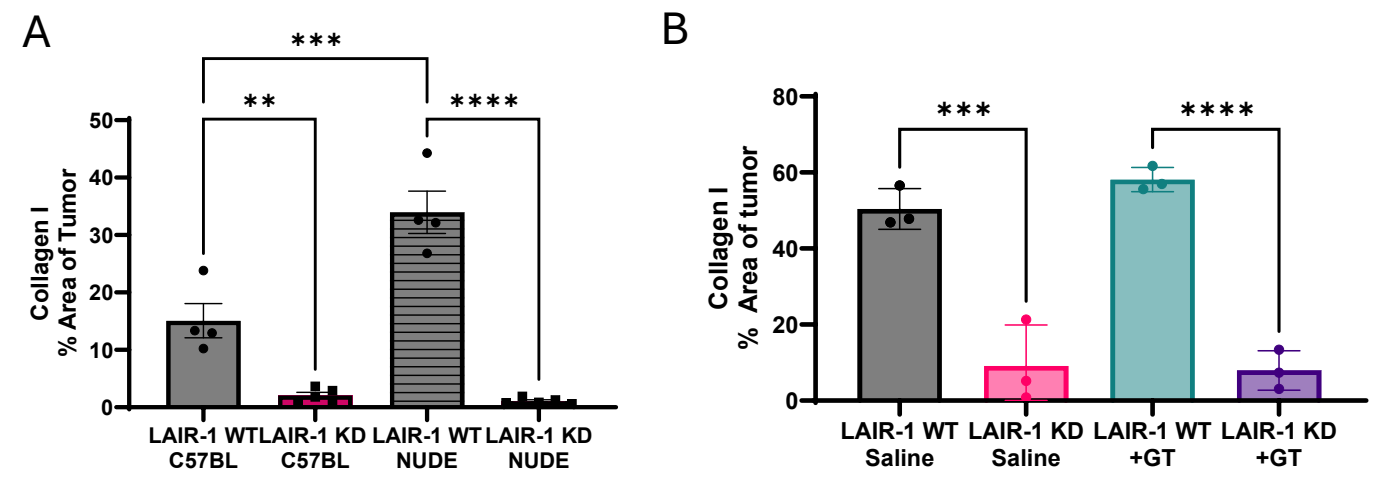
SB-LAIR-1 WT

SB-LAIR-1 KD



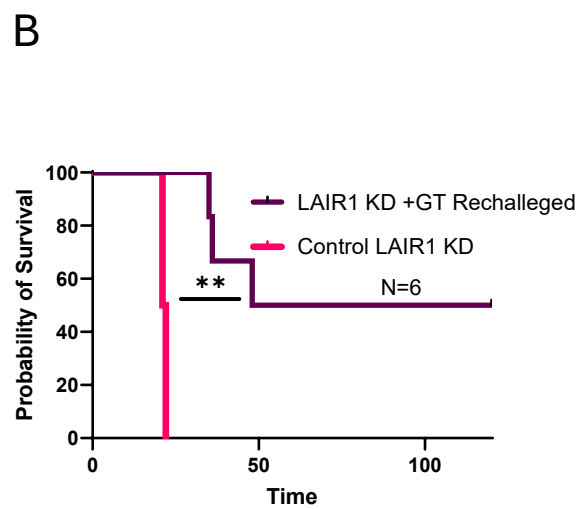
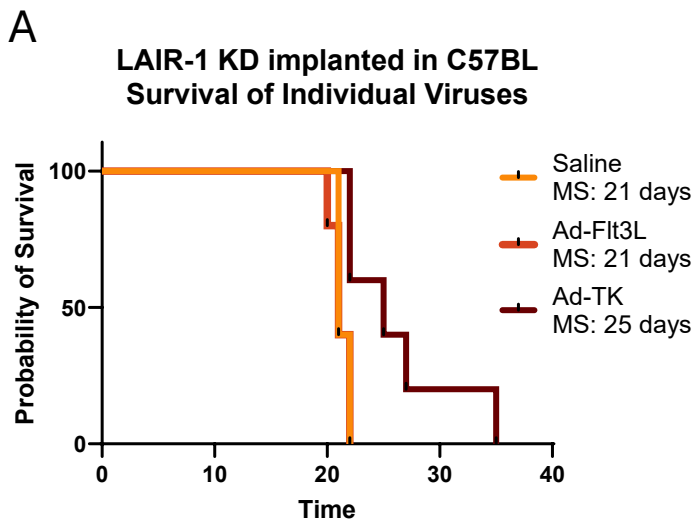
Supplemental Figure 4. Immunohistochemistry analyses of brain sections of Sleeping Beauty induced GEMMs with LAIR-1 KD. **PCNA** (proliferating cells), **SURVIVIN** (apoptosis resistant cells), **CD40+** cells, and **OLIG2** (OPCs and oligodendrocytes) and **GFAP** (astrocytes). Quantifications show positive cells per mm² within tumor area. Bar graphs show mean and SEM. *p<0.05, **p<0.01, ***p<0.001.

Supp Fig 5

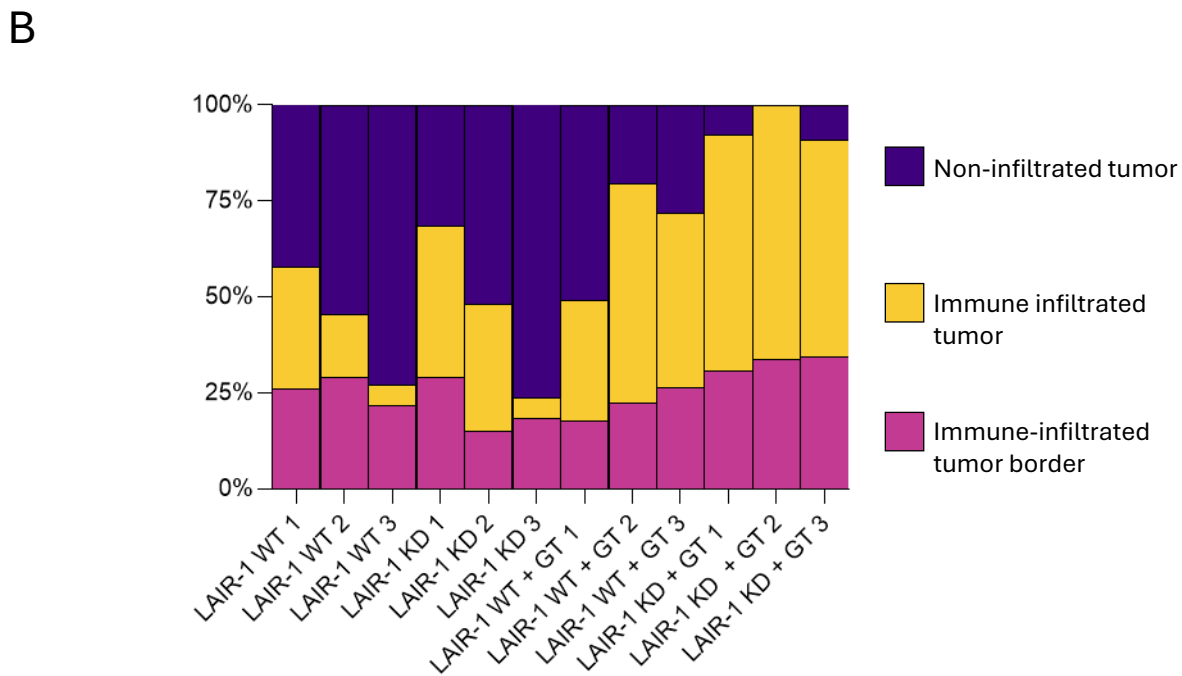
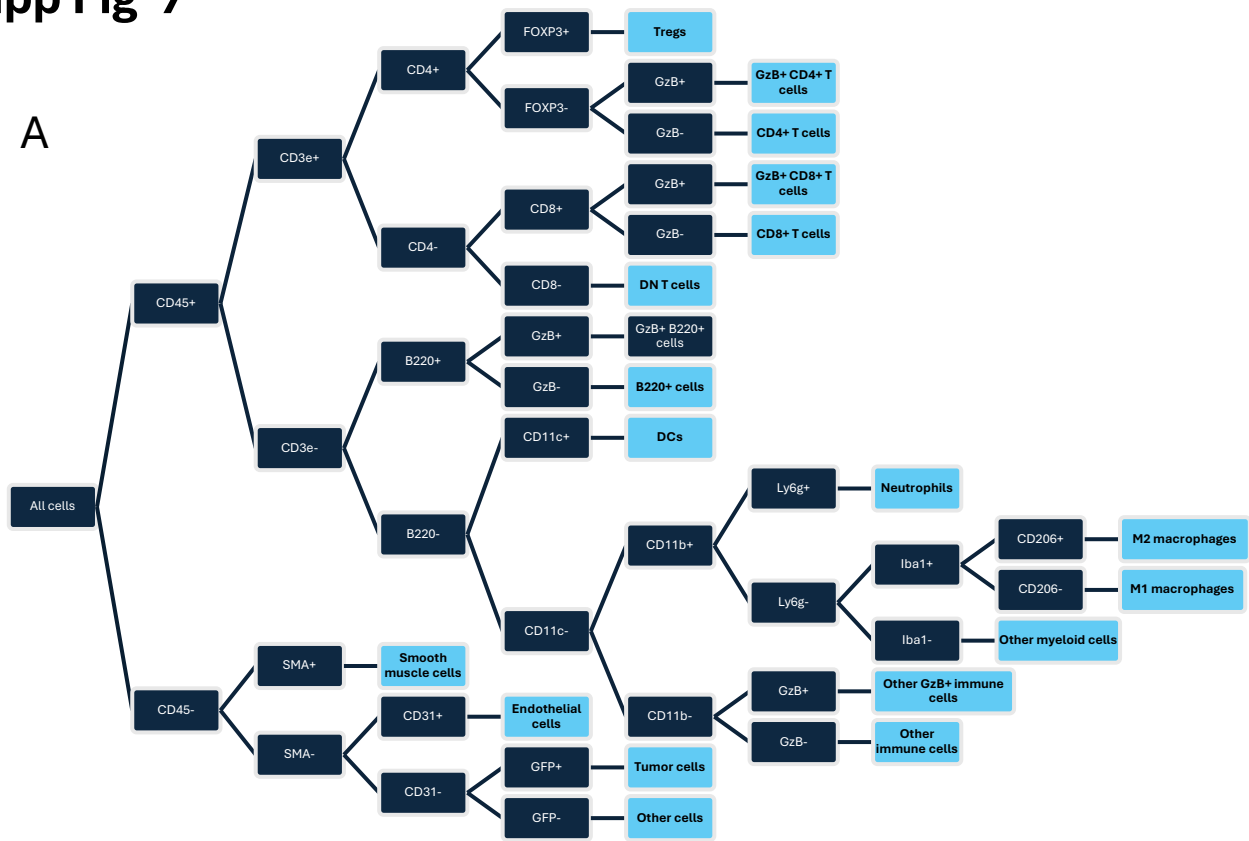


Supplemental Figure 5. A-B. Quantification of Collagen I area within tumor area detected by immunostaining. **A.** Quantification of Collagen 1 content of LAIR-1 WT and KD tumor implanted into immune competent (wild type C57BL) or immune compromised NUDE mice. **B.** Quantification of Collagen 1 content of LAIR-1 WT and KD tumor implanted treated with GT or saline control. Bar graphs show mean and SEM. ** $p < 0.01$, *** $p < 0.001$.

Supp Fig 6

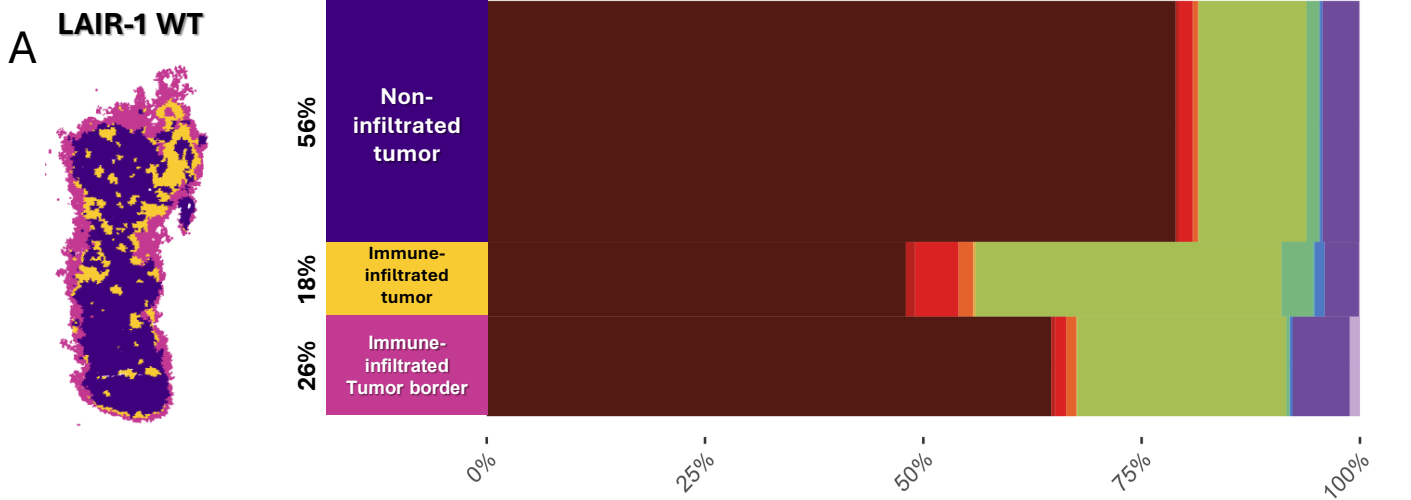


Supplemental Figure 6.A. Survival curve of wild type (C57BL) mice implanted with LAIR-1 KD (NPAL) glioma cells treated with individual virus vectors (Ad-TK (●) or Ad-Flt3L (●)) or saline (●) . There were no significant changes in MS among groups. **B.** Survival curve of long-term survivors (●), or treatment naïve mice (●), re-implanted with LAIR-1 KD glioma cells with no further treatment. N=6 **p=0.0018.



Supplemental Figure 7. A. Multiplex proteomics hierarchy. Cell markers used to define cell types. **B.** Average of neighborhood prevalence (%) within samples. Samples were divided into 3 neighborhood: Non-infiltrated tumor, Immune-infiltrated tumor and immune-infiltrated tumor border. There is a significant variation of neighborhood proportions between groups. Global PERMANOVA $p=0.0100$; $R^2=0.6616$.

Supp Fig 8



B LAIR WT Cell Percentage composition per Neighborhood

	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.30	0.80	0.20
CD4+ T cells	1.30	3.50	0.50
CD8+ T cells	0.50	1.10	0.50
Dendritic cells	0.10	0.20	0.00
Double negative T cells	1.60	3.50	0.80
Endothelial cells	3.40	2.80	2.80
GzB+ B220+ cells	0.00	0.00	0.00
GzB+ CD4+ T cells	0.00	0.10	0.00
GzB+ CD8+ T cells	0.00	0.00	0.00
GzB+ immune cells	0.00	0.10	0.10
M1 macrophages	9.90	24.60	10.20
M2 macrophages	1.20	2.50	0.20
Neutrophils	0.10	0.10	0.00
Other cells	11.60	11.00	52.30
Other immune cells	2.50	9.20	2.40
Other myeloid cells	3.60	5.90	1.80
Smooth muscle cells	0.10	0.10	0.50
Tregs	0.20	0.80	0.10
Tumor cells	63.70	33.70	27.50
	100	100	100

C LAIR WT Cell Percentage composition by Neighborhood per total tumor area

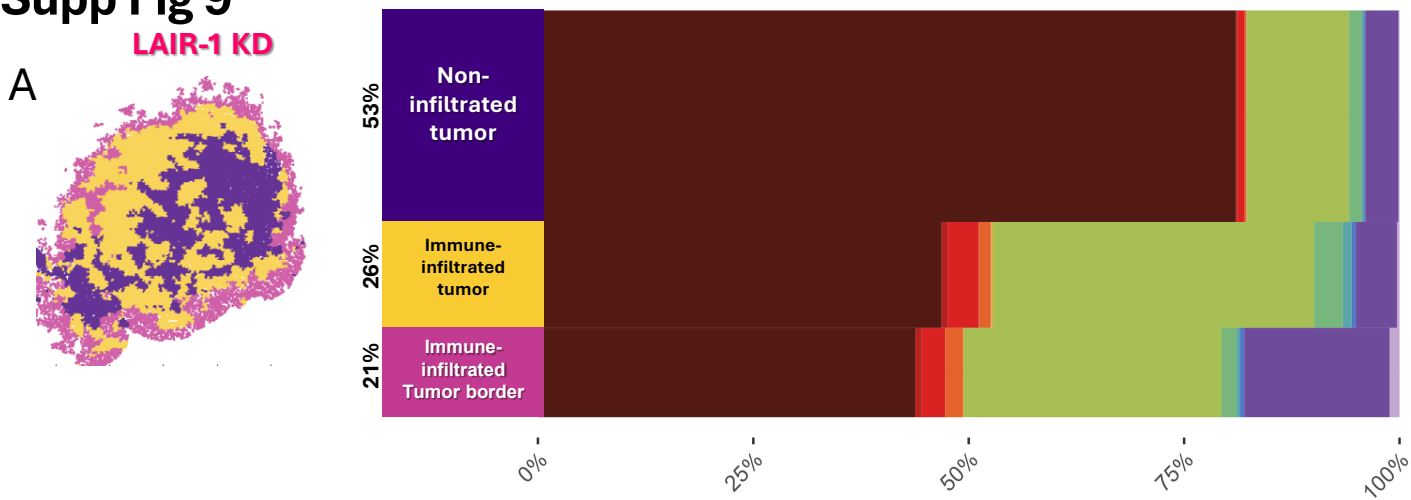
	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.16959	0.143093	0.051173
CD4+ T cells	0.73489	0.626033	0.127933
CD8+ T cells	0.28265	0.196753	0.127933
Dendritic cells	0.05653	0.035773	0
Double negative T cells	0.90448	0.626033	0.204693
Endothelial cells	1.92202	0.500827	0.716427
GzB+ B220+ cells	0	0	0
GzB+ CD4+ T cells	0	0.017887	0
GzB+ CD8+ T cells	0	0	0
GzB+ immune cells	0	0.017887	0.025587
M1 macrophages	5.59647	4.40012	2.60984
M2 macrophages	0.67836	0.447167	0.051173
Neutrophils	0.05653	0.017887	0
Other cells	6.55748	1.967533	13.38183
Other immune cells	1.41325	1.645573	0.61408
Other myeloid cells	2.03508	1.055313	0.46056
Smooth muscle cells	0.05653	0.017887	0.127933
Tregs	0.11306	0.143093	0.025587
Tumor cells	36.00961	6.027807	7.036333
	56.6	17.9	25.6

Supplemental Figure 8. A. Average neighborhood composition and prevalence (%) of LAIR-1 WT tumor. **B.** Specific cell types proportion (%) per neighborhood. **C.** Specific cell types proportion (%) per total tumor area.

Supp Fig 9

LAIR-1 KD

A



B LAIR KD Cell Percentage composition per Neighborhood

	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.20	0.50	0.30
CD4+ T cells	0.70	2.60	1.00
CD8+ T cells	0.20	0.90	0.80
Dendritic cells	0.00	0.10	0.00
Double negative T cells	0.60	3.00	1.80
Endothelial cells	3.20	3.50	6.40
GzB+ B220+ cells	0.00	0.00	0.00
GzB+ CD4+ T cells	0.00	0.10	0.00
GzB+ CD8+ T cells	0.00	0.00	0.00
GzB+ immune cells	0.00	0.10	0.00
M1 macrophages	9.80	26.70	11.30
M2 macrophages	1.20	2.40	0.70
Neutrophils	0.20	0.70	0.10
Other cells	5.80	7.60	48.40
Other immune cells	5.50	6.90	6.30
Other myeloid cells	5.10	10.50	5.70
Smooth muscle cells	0.10	0.20	0.40
Tregs	0.10	0.30	0.20
Tumor cells	67.20	33.60	16.60
	100	100	100

C LAIR KD Cell Percentage composition by Neighborhood per total tumor area

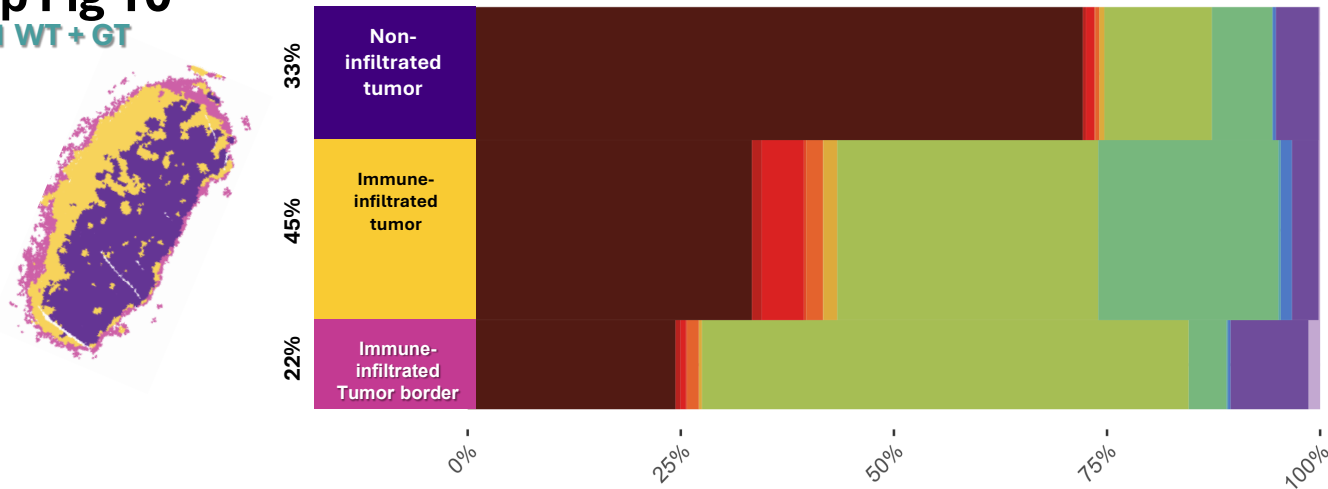
	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.1061667	0.130433	0.06249
CD4+ T cells	0.3715833	0.678253	0.2083
CD8+ T cells	0.1061667	0.23478	0.16664
Dendritic cells	0	0.026087	0
Double negative T cells	0.3185	0.7826	0.37494
Endothelial cells	1.6986667	0.913033	1.33312
GzB+ B220+ cells	0	0	0
GzB+ CD4+ T cells	0	0.026087	0
GzB+ CD8+ T cells	0	0	0
GzB+ immune cells	0	0.026087	0
M1 macrophages	5.2021667	6.96514	2.35379
M2 macrophages	0.637	0.62608	0.14581
Neutrophils	0.1061667	0.182607	0.02083
Other cells	3.0788333	1.982587	10.08172
Other immune cells	2.9195833	1.79998	1.31229
Other myeloid cells	2.70725	2.7391	1.18731
Smooth muscle cells	0.0530833	0.052173	0.08332
Tregs	0.0530833	0.07826	0.04166
Tumor cells	35.672	8.76512	3.45778
	53	26	21

Supplemental Figure 9. A. Average neighborhood composition and prevalence (%) of LAIR-1 KD tumor. B. Specific cell types proportion (%) per neighborhood. C. Specific cell types proportion (%) per total tumor area.

Supp Fig 10

LAIR-1 WT + GT

A



B

LAIR WT + GT Cell Percentage composition per Neighborhood

	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.30	0.90	0.20
CD4+ T cells	0.80	3.70	0.30
CD8+ T cells	0.40	1.40	0.50
Dendritic cells	0.50	1.20	0.10
Double negative T cells	0.90	3.20	0.40
Endothelial cells	4.10	2.30	3.60
GzB+ B220+ cells	0.00	0.00	0.00
GzB+ CD4+ T cells	0.10	0.20	0.10
GzB+ CD8+ T cells	0.00	0.10	0.00
GzB+ immune cells	0.10	0.10	0.10
M1 macrophages	10.40	22.80	22.70
M2 macrophages	5.80	15.80	1.80
Neutrophils	0.10	0.20	0.10
Other cells	4.80	6.90	50.90
Other immune cells	5.70	9.30	6.90
Other myeloid cells	6.30	5.80	1.90
Smooth muscle cells	0.10	0.10	0.50
Tregs	0.30	1.00	0.10
Tumor cells	59.30	24.90	9.70
	100	100	100

C LAIR WT + GT Cell Percentage composition by Neighborhood per total tumor area

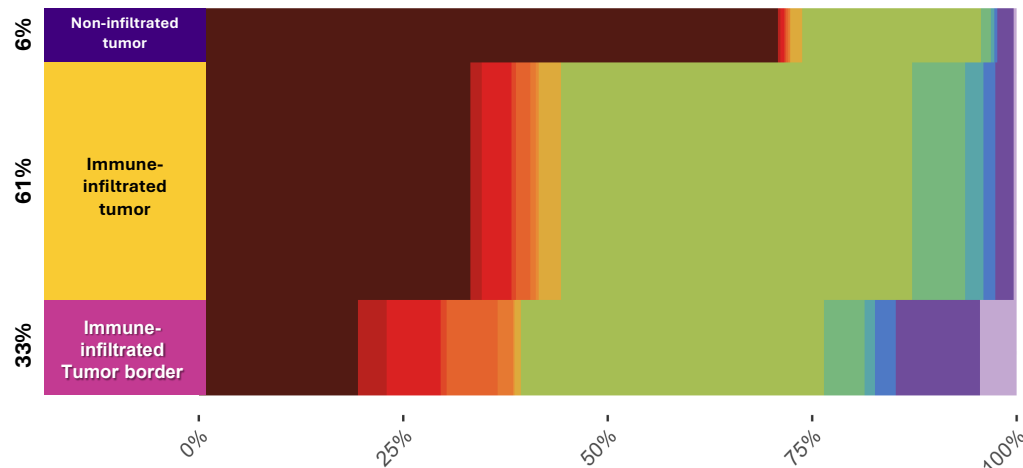
	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.099	0.405	0.044
CD4+ T cells	0.264	1.665	0.066
CD8+ T cells	0.132	0.63	0.11
Dendritic cells	0.165	0.54	0.022
Double negative T cells	0.297	1.44	0.088
Endothelial cells	1.353	1.035	0.792
GzB+ B220+ cells	0	0	0
GzB+ CD4+ T cells	0.033	0.09	0.022
GzB+ CD8+ T cells	0	0.045	0
GzB+ immune cells	0.033	0.045	0.022
M1 macrophages	3.432	10.26	4.994
M2 macrophages	1.914	7.11	0.396
Neutrophils	0.033	0.09	0.022
Other cells	1.584	3.105	11.198
Other immune cells	1.881	4.185	1.518
Other myeloid cells	2.079	2.61	0.418
Smooth muscle cells	0.033	0.045	0.11
Tregs	0.099	0.45	0.022
Tumor cells	19.569	11.205	2.134
	33	45	22

Supplemental Figure 10. A. Average neighborhood composition and prevalence (%) of LAIR-1 WT + GT tumor. B. Specific cell types proportion (%) per neighborhood. C. Specific cell types proportion (%) per total tumor area.

Supp Fig 11

LAIR-1 KD + GT

A



B

LAIR KD+ GT Cell Percentage composition per Neighborhood

	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.30	1.00	1.20
CD4+ T cells	0.40	2.60	2.20
CD8+ T cells	0.20	1.20	2.10
Dendritic cells	1.10	2.00	0.30
Double negative T cells	0.60	2.80	4.20
Endothelial cells	1.60	1.70	3.50
GzB+ B220+ cells	0.10	0.20	0.10
GzB+ CD4+ T cells	0.10	0.40	0.20
GzB+ CD8+ T cells	0.20	0.50	0.60
GzB+ immune cells	0.50	0.70	0.40
M1 macrophages	16.90	31.10	12.50
M2 macrophages	1.00	4.70	1.70
Neutrophils	0.30	1.60	0.40
Other cells	7.50	7.60	48.50
Other immune cells	8.50	9.40	4.80
Other myeloid cells	5.70	7.10	8.20
Smooth muscle cells	0.30	0.20	1.50
Tregs	0.30	1.00	0.90
Tumor cells	54.60	24.00	6.60
	100	100	100

C

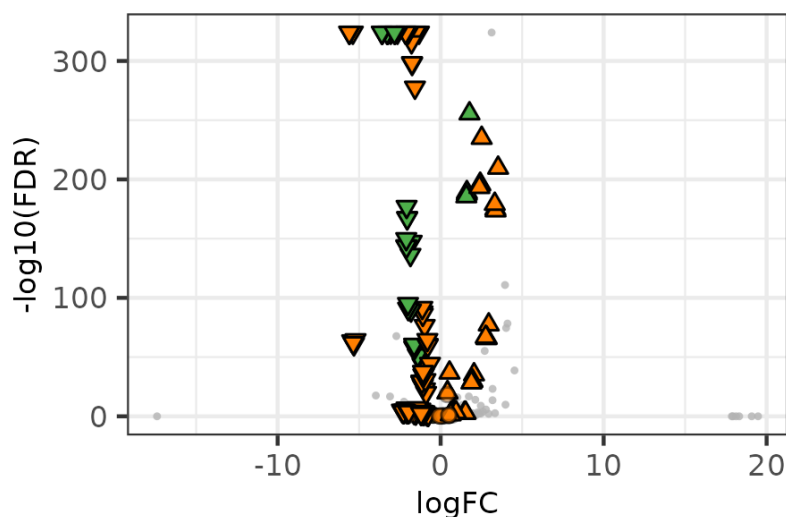
LAIR KD+ GT Cell Percentage composition by Neighborhood per total tumor area

	Non-infiltrated tumor	Immune-infiltrated tumor	Immune-infiltrated tumor border
B cells	0.018	0.61	0.396
CD4+ T cells	0.024	1.586	0.726
CD8+ T cells	0.012	0.732	0.693
Dendritic cells	0.066	1.22	0.099
Double negative T cells	0.036	1.708	1.386
Endothelial cells	0.096	1.037	1.155
GzB+ B220+ cells	0.006	0.122	0.033
GzB+ CD4+ T cells	0.006	0.244	0.066
GzB+ CD8+ T cells	0.012	0.305	0.198
GzB+ immune cells	0.03	0.427	0.132
M1 macrophages	1.014	18.971	4.125
M2 macrophages	0.06	2.867	0.561
Neutrophils	0.018	0.976	0.132
Other cells	0.45	4.636	16.005
Other immune cells	0.51	5.734	1.584
Other myeloid cells	0.342	4.331	2.706
Smooth muscle cells	0.018	0.122	0.495
Tregs	0.018	0.61	0.297
Tumor cells	3.276	14.64	2.178
	6	61	33

Supplemental Figure 11. A. Average neighborhood composition and prevalence (%) of LAIR-1 KD + GT tumor. **B.** Specific cell types proportion (%) per neighborhood. **C.** Specific cell types proportion (%) per total tumor area.

Contact-dependent interactions

High in experimental → LAIR-1 KD

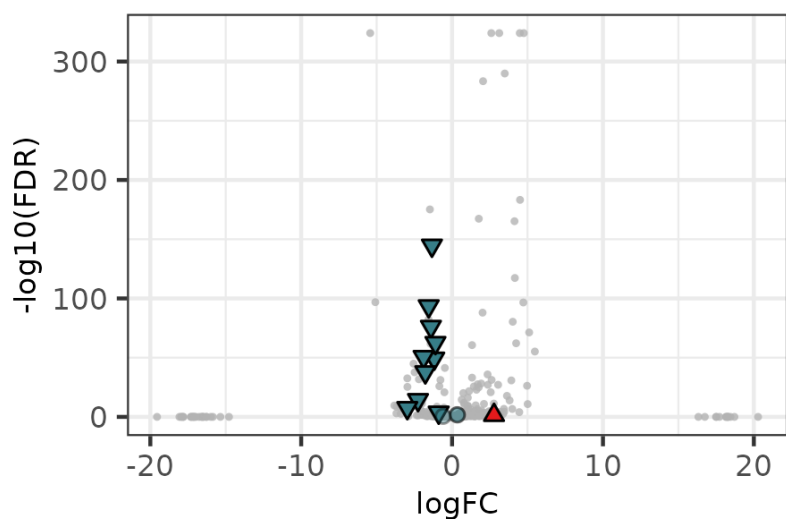


Significance

- △ Up
- ▽ Down
- n/s

Diffusion-dependent interactions

High in experimental → LAIR-1 KD



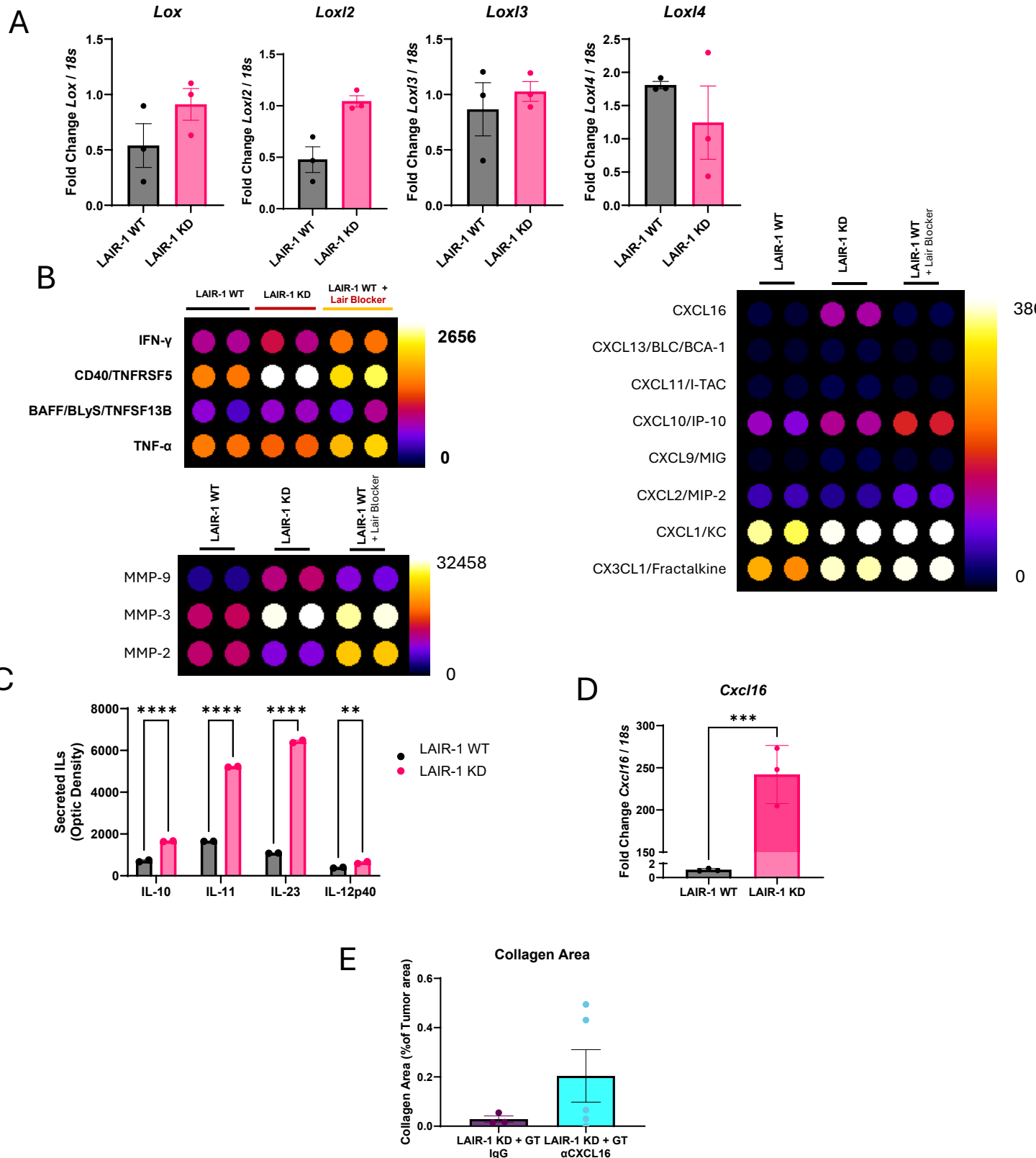
Category

- Fibrillar Collagen
- Non-Fibrillar Collagen
- TGF
- CXCL16-CXCR6

Supplemental Figure 12. Spatial transcriptomics analysis of CytoSignal Ligand-receptor interactions. The volcano plots show differential signaling test statistics between LAIR-1 WT and KD. Each marker represents a ligand-receptor (LR) pair. Positive log fold change indicates higher signaling in the LAIR-1 KD group. Significant LR pair were defined as $FDR < 0.05$ and fold-change > 1.5 . LR pairs of interest were highlighted with colors.

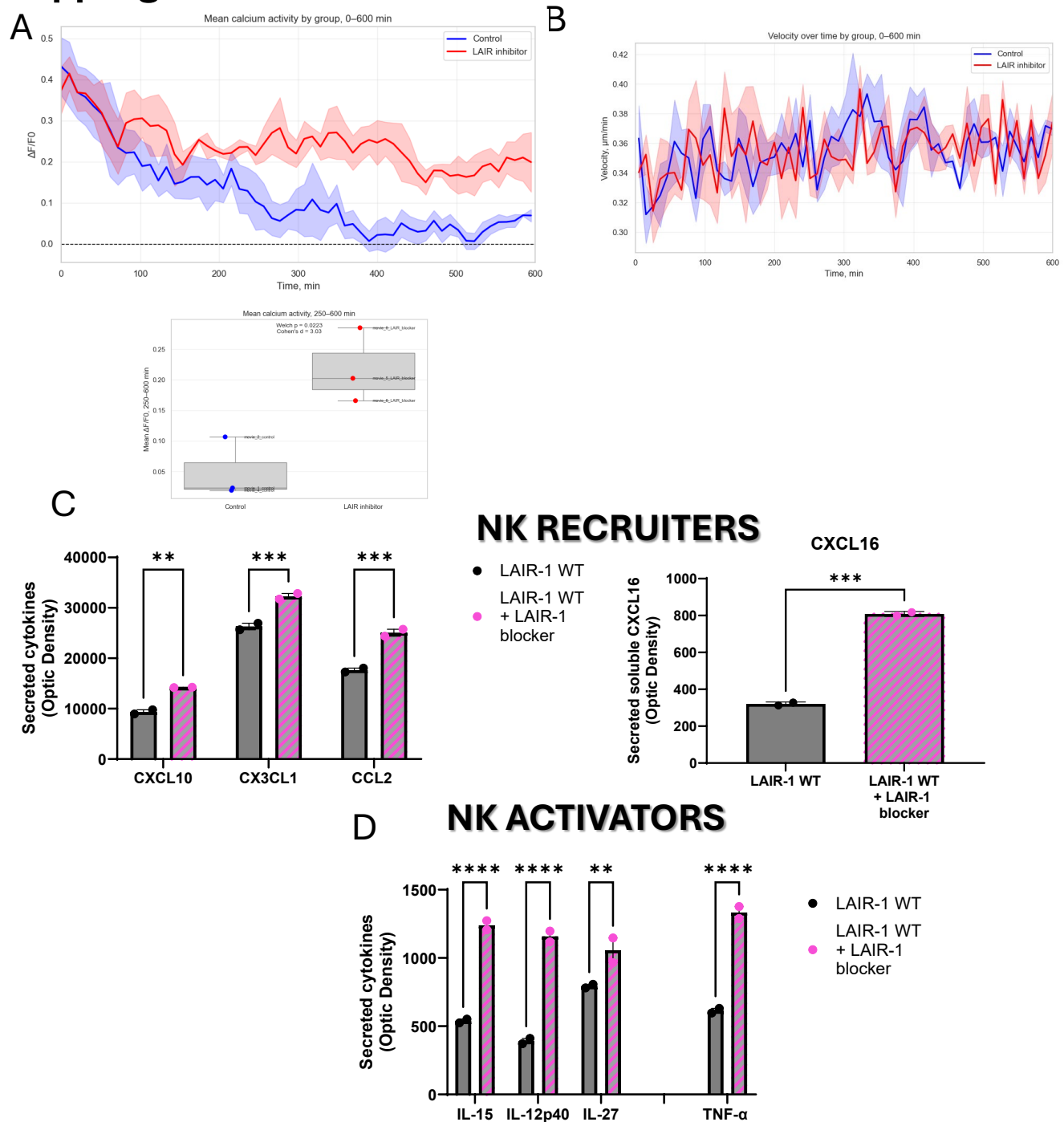
Supp Fig 13

OTHER LOX GENE EXPRESSION



Supplemental Figure 13. **A.** Fold change of LOX family genes *in vitro* in LAIR-1 WT and LAIR-1 KD measured by qPCR, relative 18s. **B.** Heatmap *in vitro* cytokine secretion of LAIR-1 WT, LAIR-1 KD and LAIR-1 WT +LAIR-1 blocker cell supernatants. **C.** *In vitro* ILs secretion of LAIR-1 WT and LAIR-1 KD cell supernatants. **D.** Fold change of *CXCL16* *in vitro* in LAIR-1 WT and LAIR-1 KD measured by qPCR, relative 18s. **E.** Quantification of Collagen 1 area within tumor area detected by immunostaining. **A.** Quantification of Collagen 1 content in LAIR-1 KD + GT treated with IgG or α CXCL16 brain tumor sections. Bar graphs show mean and SEM. ** $p < 0.01$, *** $p < 0.001$.

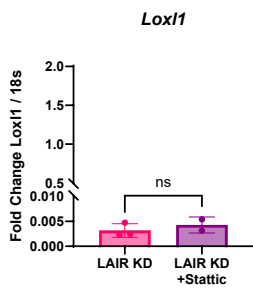
Supp Fig 14



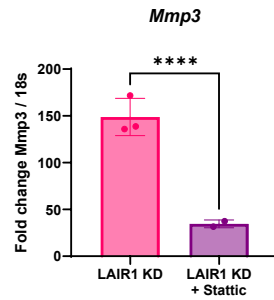
Supplemental Figure 14. A. Calcium-associated fluorescence quantified from time-lapse movies of WT glioma cells treated with LAIR-1 inhibitor, or saline, and expressed as $\Delta F/F_0$. Group traces show mean $\pm$ SEM from 0 to 600 min. For statistical analysis, mean $\Delta F/F_0$ was calculated for each movie during the 250–600 min interval, with each movie treated as one replicate. After QC-based exclusion of technically compromised movies, LAIR-1 inhibitor-treated samples showed significantly higher mean $\Delta F/F_0$ than controls by Welch's t-test: Control, 0.0498 ± 0.0285 SEM; LAIR inhibitor, 0.2177 ± 0.0352 SEM; $p = 0.0223$; Cohen's $d = 3.03$; $n = 3$ movies/group. **B.** Velocity over time quantifications from movies show no difference between LAIR WT and LAIR WT treated with LAIR-1 inhibitor. **C.** *In vitro* secretion of NK recruiting cytokines in LAIR-1 WT treated with LAIR-1 inhibitor, or saline, cell supernatants. **D.** *In vitro* secretion of NK activating cytokines in LAIR-1 WT treated with LAIR-1 inhibitor, or saline, cell supernatants. Bar graphs show mean and SEM. ** $p < 0.01$, *** $p < 0.001$.

Supp Fig 15

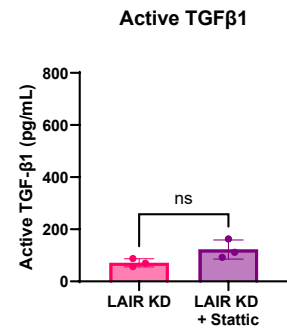
A



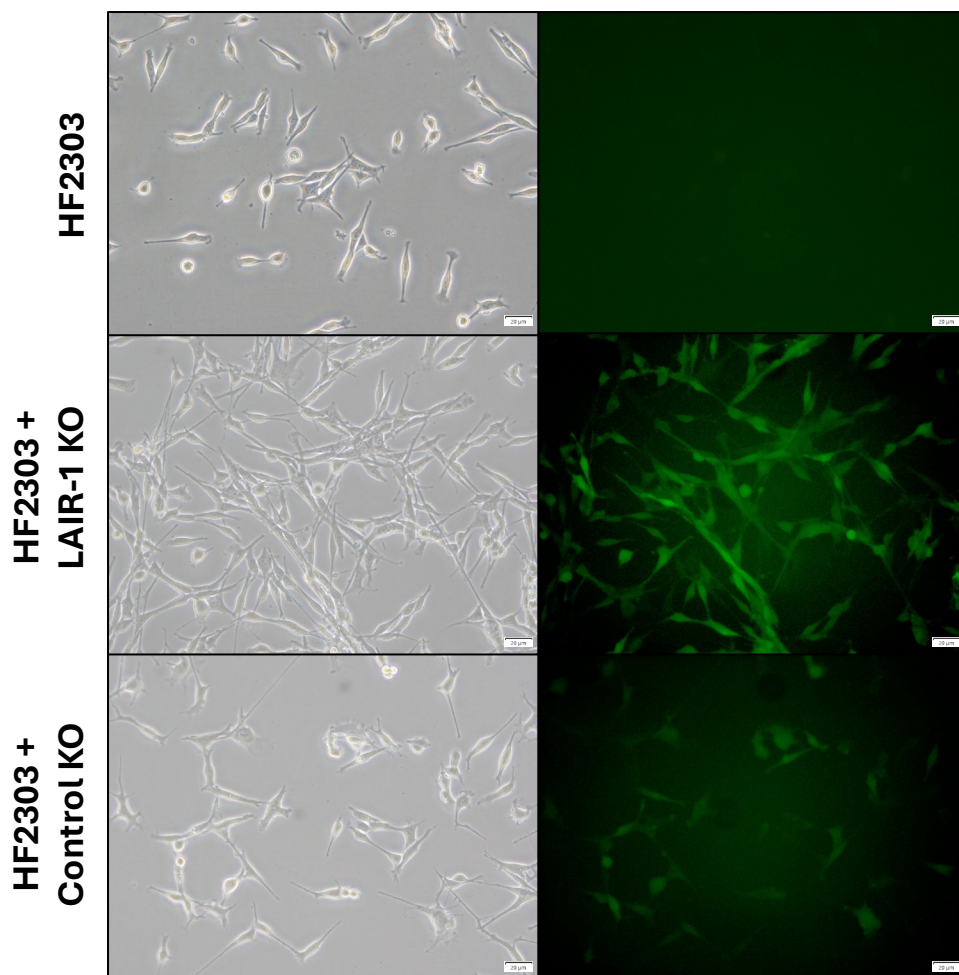
B



C



Supplemental Figure 15. A-B. Fold change of *Lox11* (A) and *Mmp3* (B) *in vitro* gene expression of LAIR-KD cells treated with STAT3 inhibitor (Stattic), or saline, by qPCR, relative to 18s expression. **C.** Active TGF-β1 secreted by LAIR-KD cells treated with STAT3 inhibitor (Stattic), or saline, *in vitro*.



Supplemental Figure 16. Brightfield and fluorescent microscopy images of human HF2303 cells, HF2303 LAIR1 KO cells, and HF2303 KO control cells. Successful plasmid recombination shown in the HF2303+LAIR-1 KO is corroborated by GFP expression. Scale bar is 50 µm.

Supp Table 1

Immunohistochemistry / Immunofluorescence Antibodies

Antibody	Catalog Number	Company	Dilution
ADAM10	Ab1997	Abcam	1:500
B220/CD45R	14045282	Invitrogen	1:100
CD20	70168	Cell Signaling	1:400
CD4	Ab183685	Abcam	1:300
CD68	Ab955	Abcam	1:500
CD8	HS-361OU3	Cedar Lane	1:250
Collagen 1	Ab279711	Abcam	1:1000
CXCL16	AF503	R&D Systems	1:250
GFAP	Ab5541	Abcam	1:1000
GFP (Chicken)	Ab13970	Abcam	1:500
GFP (goat)	600-101-215	Rockland	1:500
Granzyme B	46890S	Cell Signaling	1:200
LOXL1	Sc-166632	Santa Cruz	1:500
NK1.1 (CD161)	NB10077528SS	Novus	1:250
OLIG2	Ab109186	Abcam	1:400
PCNA	2586S	Cell Signaling	1:1000
PDL1	MAB90782	Novus	1:500
Survivin	2808S	Cell Signaling	1:500
CD11c	97585	Cell Signaling	1:300
CD206	87887SF	Cell Signaling	1:200
CD40	E2Z7J	Cell Signaling	1:400

Western Blot Antibodies

Antibody	Catalog Number	Company	Dilution
Actin	4970T	Cell Signaling	1:1000
ERK1/2	4695S	Cell Signaling	1:1000
p-ERK1/2	4370S	Cell Signaling	1:1000
p-SHP2	3751S	Cell Signaling	1:1000
p-STAT3	9145S	Cell Signaling	1:1000
SHP2	3397S	Cell Signaling	1:1000
STAT3	124H6	Cell Signaling	1:1000
p-SAPK/JNK	4668	Cell Signaling	1:1000
SAPK/JNK	9252	Cell Signaling	1:1000
p-C-JUN	3270	Cell Signaling	1:1000
C-JUN	9165	Cell Signaling	1:1000
p-AKT	9271	Cell Signaling	1:1000
AKT	4691	Cell Signaling	1:1000

RNA Scope Probes

Probe	Catalog Number	Company
LAIR1	509151	Advanced Cell Diagnostics
GFP	400281-C3	Advanced Cell Diagnostics
PTPRC	318651-C2	Advanced Cell Diagnostics