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Supplemental Information

Zika Virus Targets Glioblastoma Stem Cells through a SOX2-Integrin $\alpha_v\beta_5$ Axis

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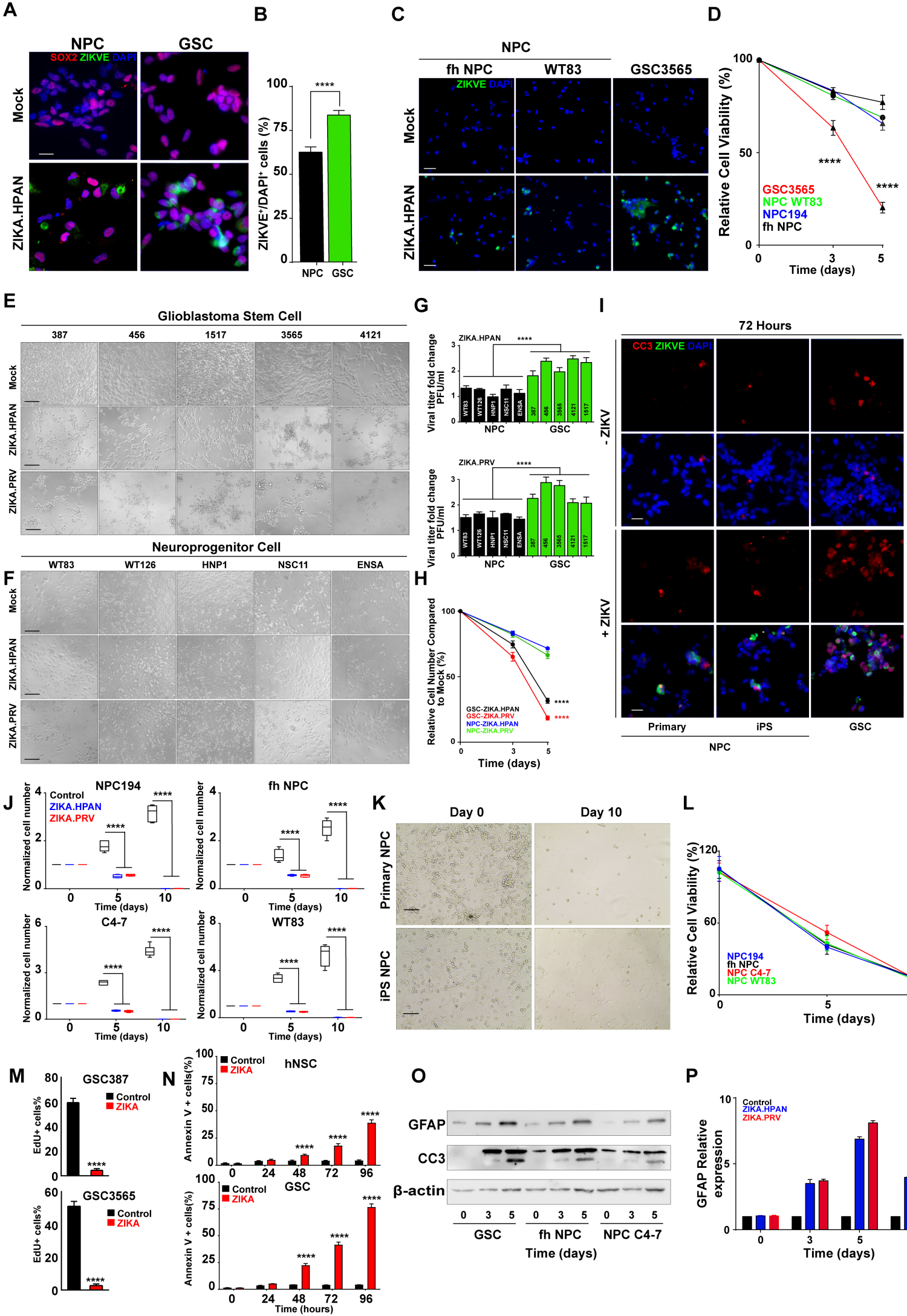

A
NPC
GSC
SOX2 ZIKVE DAPI
Mock
ZIKA.HPAN
B
ZIKVE+/DAPI+ cells (%)
****
NPC GSC
C
NPC
fh NPC
WT83
GSC3565
ZIKVE DAPI
Mock
ZIKA.HPAN
D
Relative Cell Viability (%)
GSC3565
NPC WT83
NPC194
fh NPC
****
Time (days)
E
Glioblastoma Stem Cell
387
456
1517
3565
4121
Mock
ZIKA.HPAN
ZIKA.PRV
Neuroprogenitor Cell
WT83
WT126
HNP1
NSC11
ENSA
F
G
ZIKA.HPAN
ZIKA.PRV
Viral titer fold change PFU/ml
NPC
GSC
H
Relative Cell Number Compared to Mock (%)
GSC-ZIKA.HPAN
GSC-ZIKA.PRV
NPC-ZIKA.HPAN
NPC-ZIKA.PRV
Time (days)
I
72 Hours
CC3 ZIKVE DAPI
- ZIKV
+ ZIKV
Primary
iPS
GSC
NPC
J
NPC194
fh NPC
C4-7
WT83
Control
ZIKA.HPAN
ZIKA.PRV
Normalized cell number
Time (days)
K
Day 0
Day 10
Primary NPC
iPS NPC
L
Relative Cell Viability (%)
NPC194
fh NPC
NPC C4-7
NPC WT83
Time (days)
M
GSC387
GSC3565
Control
ZIKA
EdU+ cells%
N
hNSC
GSC
Control
ZIKA
Annexin V + cells(%)
Time (hours)
O
GFAP
CC3
β-actin
GSC
fh NPC
NPC C4-7
Time (days)
P
Control
ZIKA.HPAN
ZIKA.PRV
GFAP Relative expression
Time (days)

SUPPLEMENTAL FIGURES & LEGENDS

Supplemental Figure 1. refers to Figure 1. Comparative infection efficiency of ZIKV on GBM stem cells (GSCs) and neural precursor cells (NPCs).

A. Primary NPCs and patient-derived GSCs underwent analysis for expression of the SOX2 neural progenitor marker (red) and the ZIKV envelope protein (ZIKV-E, green) under mock conditions or after ZIKV infection. Cell nuclei were imaged with DAPI. Scale bars, 20 μm.

B. The results of experiments in (A) were quantified. ****, $p < 0.0001$ two tailed Student's t-test.

C. 48 hours post ZIKV infection, patient-derived GSCs (GSC3565) and NPCs (primary and iPSC-derived) underwent immunostaining for ZIKV (green) with counterstaining by DAPI. GSCs displayed a greater fraction of $ZIKV^+$ cells than NPCs, with similar infection efficiency of primary and iPSC-derived NPCs. Scale bars, 40 μm.

D. The effects of ZIKV infection on cell viability was measured in GSCs and NPCs using the Cell Titer-Glo assay, revealing a greater loss of cell viability upon ZIKV infection in GSCs than either primary or iPSC-derived NPCs.

E. Representative brightfield images of GSCs (387, 456, 1517, 3565, and 4121) observed 5 days after infection with ZIKA.HPAN (MOI: 5 FFU/cell). Scale bars, 100 μm.

F. Representative brightfield images of NPCs (WT83, WT126, HNP1, NSC11, ENSA) observed 5 days after infection with ZIKA.HPAN (MOI: 5 FFU/cell). Scale bars, 100 μm.

G. Plaque assays were used to determine viral titers of NPCs and GSCs four days post infection with either ZIKA.HPAN (top) or ZIKA.PRV (bottom) at MOI of 0.1 FFU/cell. Values represent mean ± SEM and were normalized to day 0 levels as fold change. ****, $p < 0.0001$ by one-way ANOVA.

H. GSCs (GSC3565) and NPCs (NPC C4-7) were treated with mock conditions or infected with ZIKA.PRV or ZIKA.HPAN (MOI: 5 FFU/cell) with viability assayed over 5 days by CellTiter-Glo. Values represent mean ± SEM. ****, $p < 0.0001$ by one-way ANOVA.

I. Cell death of GSCs and NPCs upon ZIKV infection was measured by Cleaved Caspase-3 (CC3) staining, showing more apoptotic cells in GSCs than NPCs. Primary and iPSC-derived NPCs had similar rates of cell death upon ZIKV infection. Scale bars, 40 μm.

J. The effects of ZIKV on primary and iPSC-derived NPCs was measured over a time course. None of the NPCs displayed augmentation of proliferation, which would be expected with oncogenic transformation. ****, $p < 0.0001$ by one-way ANOVA.

K. Visualization of ZIKV infection on primary and iPSC-derived NPCs was assessed over a time course using brightfield images. No morphologic changes consistent with oncogenic transformation were detected over a time course. Scale bars, 40 μm.

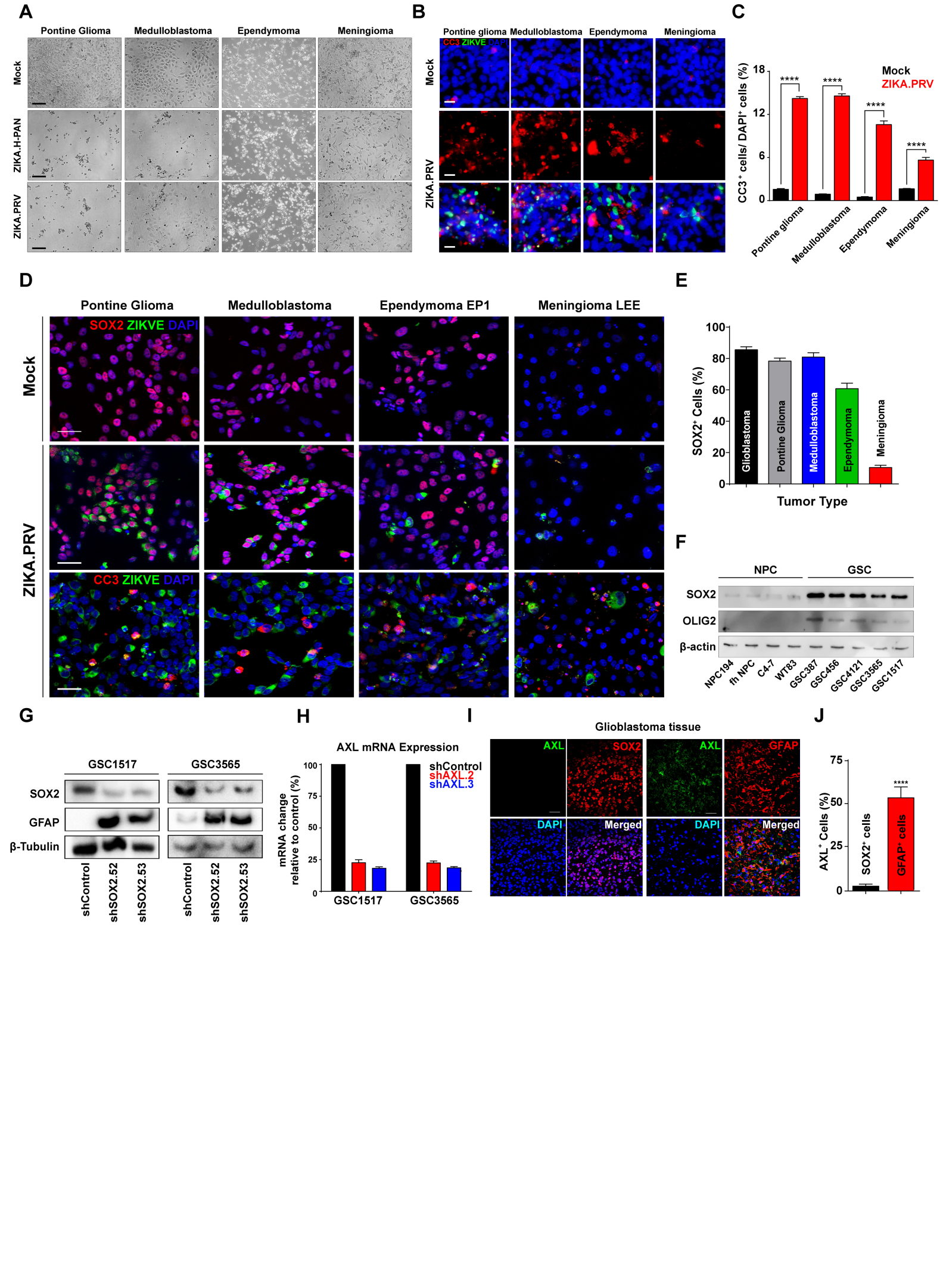
L. Results in (K) were quantified.

M. GSC387 and GSC3565 were treated with Zika virus at MOI of 5 FFU/cell, 72 hours after infection, EdU labeling assay was used to label proliferating cells. Mock treated GSCs were used as control. **** $p < 0.0001$ by two tailed Student's t-test.

N. hNSCs and GSCs were infected with Zika at MOI of 5 FFU/cell, Annexin V labeling were used over a time course from 0 hour to 96 hours and quantified. Uninfected (control) and ZIKV-infected (Zika) cells were compared at each timepoint. Values represent mean ± SEM. ****, $p < 0.0001$ by one-way ANOVA.

O. Effects of ZIKV on cellular differentiation and apoptosis were measured by immunoblotting for GFAP and Cleaved Caspase-3 (CC3), respectively, in GSCs, primary NPCs and iPSC-derived NPCs over a time course.

P. The effects of ZIKV infection on cellular differentiation was measured by qPCR of GFAP over one week time course.


A
Pontine Glioma
Medulloblastoma
Ependymoma
Meningioma
Mock
ZIKA.H-PAN
ZIKA.PRV
B
Pontine glioma
Medulloblastoma
Ependymoma
Meningioma
CC3 ZIKVE DAPI
Mock
ZIKA.PRV
C
Mock
ZIKA.PRV
CC3+ cells/ DAPI+ cells (%)
18
12
6
0
****
****
****
****
Pontine glioma
Medulloblastoma
Ependymoma
Meningioma
D
Pontine Glioma
Medulloblastoma
Ependymoma EP1
Meningioma LEE
Mock
ZIKA.PRV
SOX2 ZIKVE DAPI
CC3 ZIKVE DAPI
E
SOX2+ Cells (%)
100
80
60
40
20
0
Glioblastoma
Pontine Glioma
Medulloblastoma
Ependymoma
Meningioma
Tumor Type
F
NPC
GSC
SOX2
OLIG2
β-actin
NPC194
fh NPC
C4-7
WT83
GSC387
GSC456
GSC4121
GSC3565
GSC1517
G
GSC1517
GSC3565
SOX2
GFAP
β-Tubulin
shControl
shSOX2.52
shSOX2.53
shControl
shSOX2.52
shSOX2.53
H
AXL mRNA Expression
mRNA change relative to control (%)
100
75
50
25
0
shControl
shAXL.2
shAXL.3
GSC1517
GSC3565
I
Glioblastoma tissue
AXL
SOX2
AXL
GFAP
DAPI
Merged
DAPI
Merged
J
AXL+ Cells (%)
75
50
25
0
SOX2+ cells
GFAP+ cells
****

Supplemental Figure S2, refers to Figure 2. SOX2 expression in NPCs, GSCs and different brain tumors, which mediates infection of GSCs through integrin $\alpha_v\beta_5$.

A. Representative brightfield images of patient-derived cultures from pontine glioma, medulloblastoma, ependymoma and meningioma 5 days after mock infection or infection with one of two strains of Zika virus. MOI: 5 FFU/cell. Scale bars, 100 μm.

B. The baseline apoptotic index of cells derived from several brain tumor types was measured under mock conditions for apoptotic index (Cleaved Caspase-3, CC3, red). Total cell numbers were quantified through stained with DAPI. Scale bars, 20 μm.

C. Quantification of apoptotic cells upon ZIKV infection compared to mock in a spectrum of cells derived from different brain tumors. GBM and NPCs data were previously included. ****, $p < 0.0001$ by two tailed Student's t-test.

D. Representative confocal images of brain tumor cultures from (A) were immunostained 48 hours post ZIKA.PRV infection (MOI: 5 FFU/cell) for ZIKV envelope protein (ZIKVE, green), SOX2 or Cleaved Caspase-3 (red), and DAPI (blue). Scale bars, 100 μm.

E. Quantification of $SOX2^+$ cells in different brain tumor models used in (A) with two independent repeats.

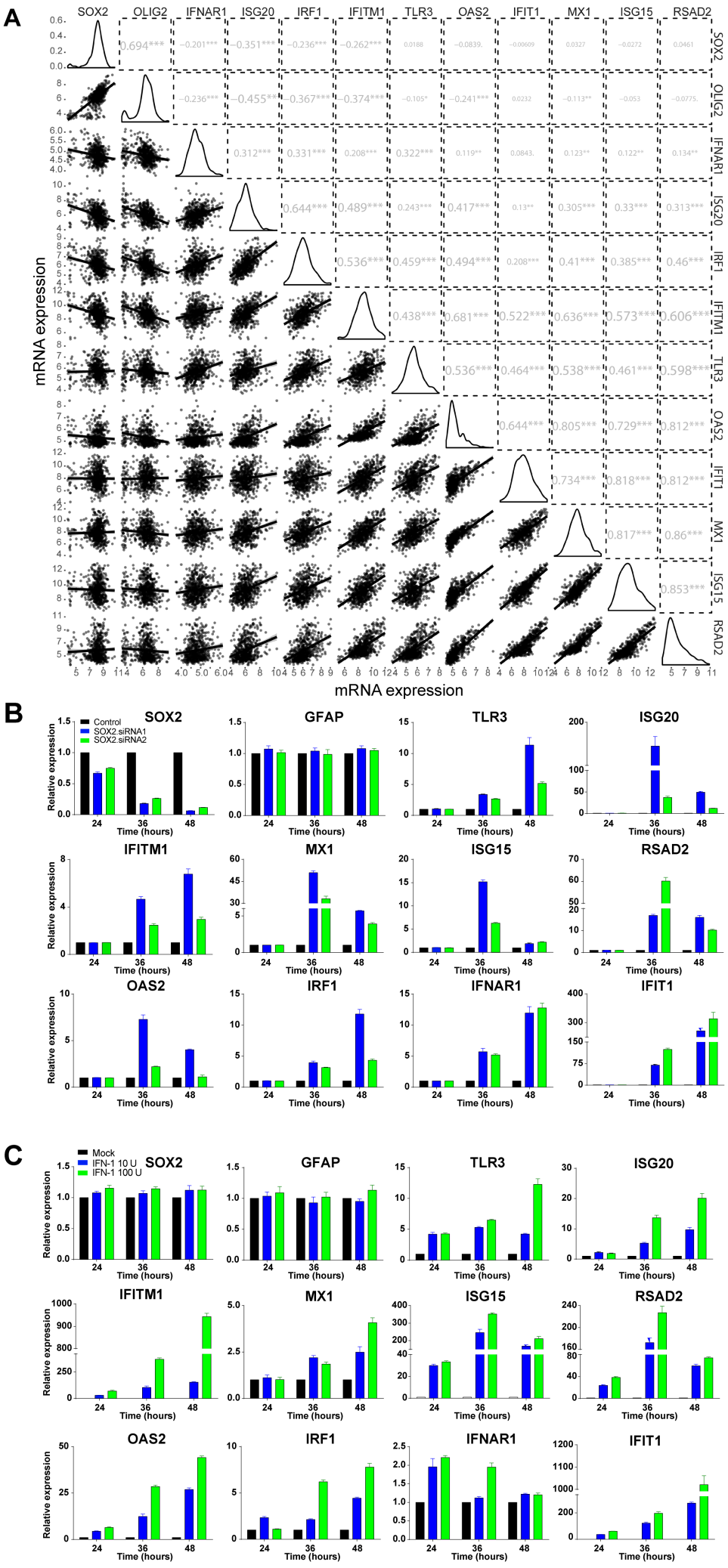
F. Basal levels of precursor markers, SOX2 and OLIG2, were measured in primary NPCs, iPSC-derived NPCs, and patient-derived GSCs by immunoblotting. β-actin served as a loading control. GSCs expressed higher levels of SOX2 and OLIG2, whereas primary and iPSC-derived NPCs expressed similar levels.

G. Two patient-derived GSCs (GSC3565 and GSC1517) were transduced with either a control shRNA (shCONT) or one of two shRNAs targeting SOX2 (shSOX2.52 or shSOX2.53). 72 hours later, whole cell lysates were generated and resolved by SDS-PAGE with SOX2 and GFAP protein levels measured by immunoblotting. Tubulin was used as a loading control.

H. Two patient-derived GSCs (GSC3565 and GSC1517) were transduced with either a control shRNA (shCONT) or one of two shRNAs targeting AXL (shAXL.2, or shAXL.3). *AXL* levels were measured by qPCR and normalized to the values from shCONT.

I. Representative images of primary human GBM surgical biopsy specimens stained for AXL (green), SOX2 or GFAP (red) and DAPI (blue). Scale bars, 100 μm. (Right)

79 **J.** Quantification of AXL^+ cells in $SOX2^+$ or $GFAP^+$ cells in primary human GBM surgical biopsy
80 specimens. Values represent mean ± SEM. ****, $p < 0.0001$ by two tailed Student's t-test.

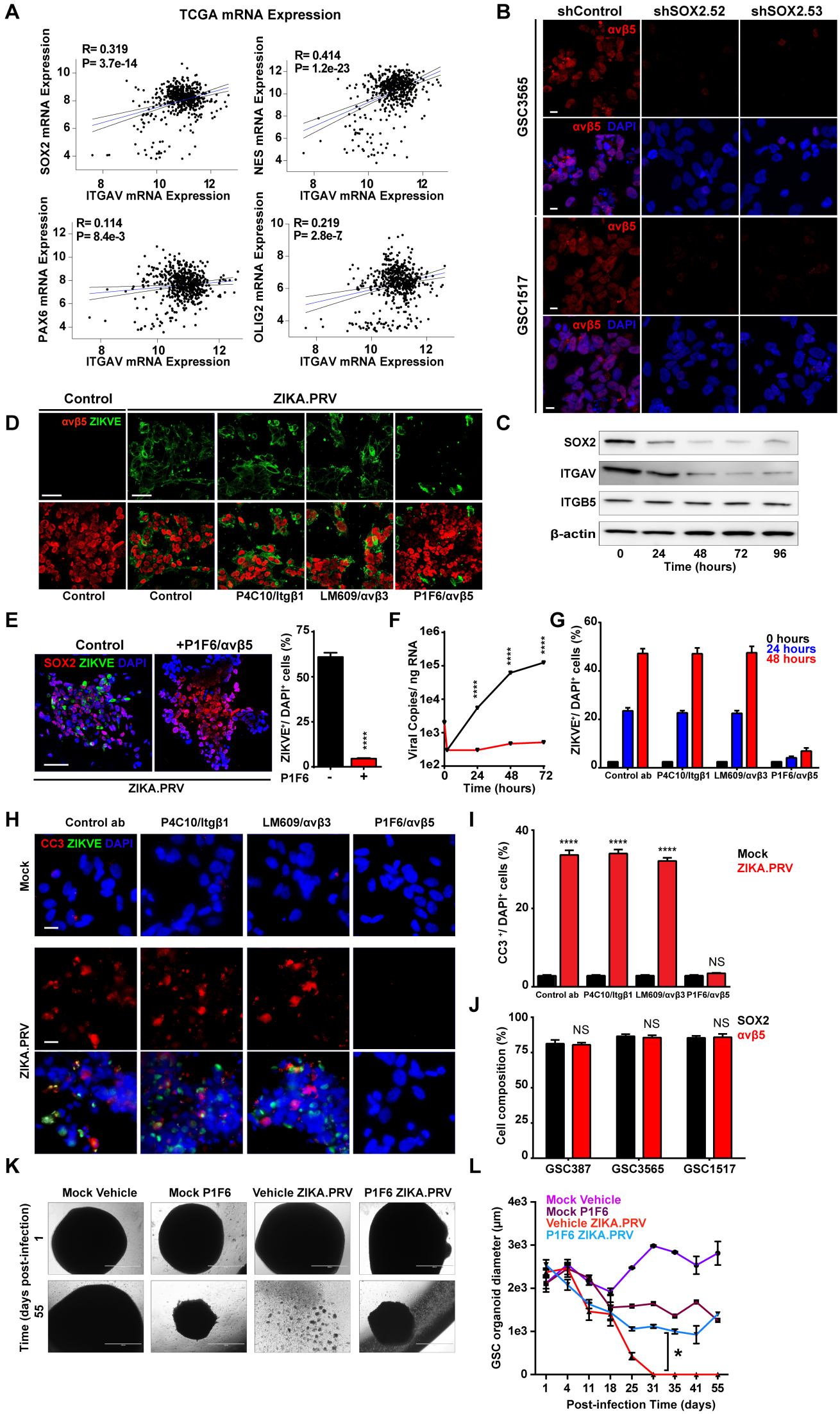

A
SOX2
OLIG2
IFNAR1
ISG20
IRF1
IFITM1
TLR3
OAS2
IFIT1
MX1
ISG15
RSAD2
mRNA expression
mRNA expression
B
Control
SOX2.siRNA1
SOX2.siRNA2
SOX2
GFAP
TLR3
ISG20
IFITM1
MX1
ISG15
RSAD2
OAS2
IRF1
IFNAR1
IFIT1
Relative expression
Time (hours)
C
Mock
IFN-1 10 U
IFN-1 100 U
SOX2
GFAP
TLR3
ISG20
IFITM1
MX1
ISG15
RSAD2
OAS2
IRF1
IFNAR1
IFIT1
Relative expression
Time (hours)

Supplemental Figure 3, refers to Figure 3. Targeting SOX2 induces ISG expression in GSCs.

A. SOX2 expression inversely correlates with ISG expression in GBM. The GBM TCGA database was interrogated for correlation between mRNA expression levels of SOX2 and OLIG2 with ISGs. R-values are presented for each gene-gene correlation.

B. Gene expression time course in GSCs upon SOX2 targeting. GSCs without ZIKV infection were transduced with a non-targeting control siRNA or one of two, non-overlapping siRNAs. mRNA expression of SOX2, GFAP and several ISGs following knockdown of SOX2 were monitored over 24-48 hours. All experiments were performed in the absence of ZIKV infection.

C. mRNA expression level of SOX2, GFAP, and several ISGs in GSC3565 following treatment with interferon at two concentrations over a time course. Each experiment was performed in 3 technical replicates and two biological repeats.


A
TCGA mRNA Expression
SOX2 mRNA Expression
R= 0.319
P= 3.7e-14
ITGAV mRNA Expression
NES mRNA Expression
R= 0.414
P= 1.2e-23
PAX6 mRNA Expression
R= 0.114
P= 8.4e-3
OLIG2 mRNA Expression
R= 0.219
P= 2.8e-7
B
shControl
shSOX2.52
shSOX2.53
GSC3565
GSC1517
αvβ5
αvβ5 DAPI
C
SOX2
ITGAV
ITGB5
β-actin
0 24 48 72 96
Time (hours)
D
Control
ZIKA.PRV
αvβ5 ZIKVE
Control
Control
P4C10/Itgβ1
LM609/αvβ3
P1F6/αvβ5
E
Control
+P1F6/αvβ5
SOX2 ZIKVE DAPI
ZIKA.PRV
ZIKVE+/ DAPI+ cells (%)
P1F6 - +
F
Viral Copies/ ng RNA
Time (hours)
G
ZIKVE+/ DAPI+ cells (%)
0 hours
24 hours
48 hours
Control ab
P4C10/Itgβ1
LM609/αvβ3
P1F6/αvβ5
H
Control ab
P4C10/Itgβ1
LM609/αvβ3
P1F6/αvβ5
Mock
ZIKA.PRV
CC3 ZIKVE DAPI
I
CC3 +/ DAPI+ cells (%)
Mock
ZIKA.PRV
NS
J
Cell composition (%)
SOX2
αvβ5
NS
GSC387
GSC3565
GSC1517
K
Mock Vehicle
Mock P1F6
Vehicle ZIKA.PRV
P1F6 ZIKA.PRV
Time (days post-infection)
1
55
L
Mock Vehicle
Mock P1F6
Vehicle ZIKA.PRV
P1F6 ZIKA.PRV
GSC organoid diameter (μm)
Post-infection Time (days)

Supplemental Figure 4, refers to Figure 4. Pharmacological Blocking of integrin $\alpha_v\beta_5$ attenuates ZIKV infection.

A. Plots showing correlation of mRNA levels between *ITGAV* with *SOX2*, *NES*, *PAX6* and *OLIG2* in GBMs derived from the HG-U133A microarray dataset in The Cancer Genome Atlas (TCGA) GBM dataset.

B. Patient-derived GSCs were transduced with a non-targeting control shRNA (shControl) or one of two non-overlapping shRNAs targeting SOX2 (labeled shSOX2.52 and shSOX2.53). Transduced GSCs underwent immunofluorescent staining 72 hours for the integrin $\alpha_v\beta_5$ heterodimer (red) and DAPI (blue). Scale bars, 10 μm.

C. Immunoblots were performed for SOX2, integrin α_v, and integrin β_5 over a 96 hours time course. β-actin was used as a loading control.

D. GSC3565 were cultured either under mock condition alone or with one of three blocking antibodies against integrins (Itgβ_1, P4C10; $\alpha_v\beta_3$, LM609; $\alpha_v\beta_5$, P1F6 at 50 μg/ml for 2 hours at 4^{o}C) then infected with ZIKA.PRV (MOI: 5 FFU/cell). Representative confocal immunofluorescent images for integrin $\alpha_v\beta_5$ (red) or ZIKV envelope protein (ZIKVE, green) were shown. Scale bars, 100 μm.

E. GSC3565 were cultured either under mock condition or with a neutralizing antibody against $\alpha_v\beta_5$ (P1F6 at 50 μg/ml for 2 hours at 4^{o}C) then infected with ZIKA.PRV (MOI: 5 FFU/cell). (Left) Representative confocal immunofluorescent images after 48 hours infection for ZIKV envelope protein (ZIKVE, green), SOX2 (red) and DAPI (blue) were shown. (Right) ZIKV$^+$ cells in GSCs subjected to mock conditions or with neutralizing antibodies against integrin $\alpha_v\beta_5$ were shown. Values represent mean ± SEM. ****, $p < 0.0001$ by two tailed Student's t-test. Scale bars, 100 μm.

F. GSC3565 were cultured either under mock condition or with a neutralizing antibody against integrin $\alpha_v\beta_5$ (P1F6 at 50 μg/ml for 2 hours at 4^{o}C) then infected with ZIKA.PRV (MOI: 0.1 FFU/cell). Kinetics of viral RNA copy number was measured by qRT-PCR over a 72 hours time course. Values represent mean ± SEM. ****, $p < 0.0001$ by one-way ANOVA.

G. Quantification of ZIKV$^+$ cells in GSC3565 using different integrin antibody treatments according to different time points.

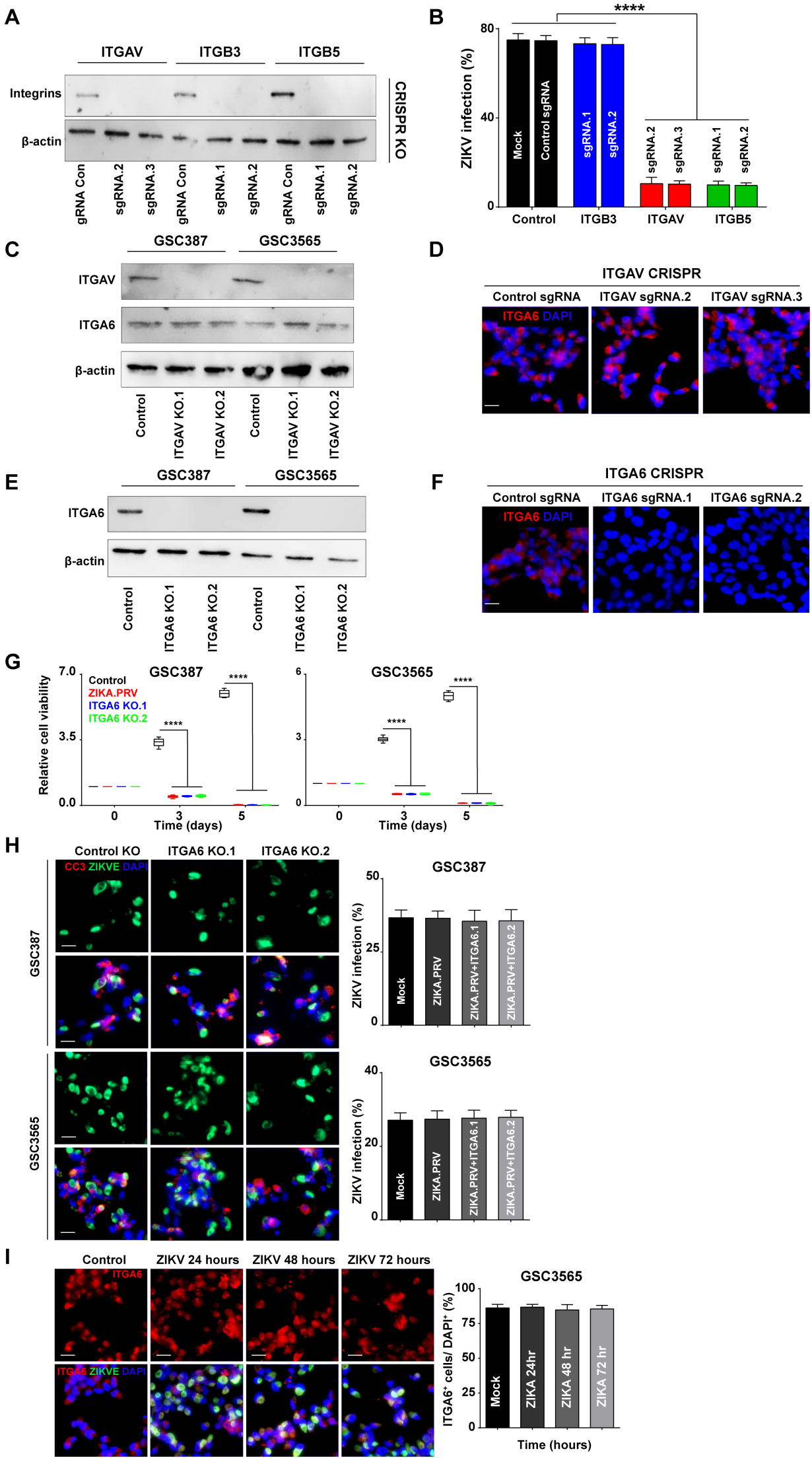
H. Patient-derived GSCs were cultured under mock (upper panel) or ZIKV (lower panel) conditions with IgG control or blocking antibodies against several integrins (pan-β_1, $\alpha_v\beta_3$, or $\alpha_v\beta_5$). GSCs were immunostained for ZIKVE (green), CC3 (red) and DAPI (blue). Scale bars, 20 μm.

I. Quantification of $CC3^+$ cells upon ZIKV infection compared to mock control, after incubation with antibodies targeting several integrins. ****, $p < 0.0001$, by two tailed Student's t-test.

J. Quantification of $\alpha_V\beta_5$ and SOX2 cells in total DAPI cells by GSC387, GSC3565 and GSC1517 models. The percentages were similar between $\alpha_V\beta_5^+$ and $SOX2^+$ cells (NS, no significance).

K. Organoids were derived from GSC3565 and subjected to treatment with mock condition, a blocking antibody against integrin $\alpha_v\beta_5$ (P1F6 at 50 μg/ml twice a week), ZIKA.PRV infection (MOI: 5 FFU/cell), or the combined antibody and ZIKV treatment. Brightfield images were taken over a time course until day 55. N = 5. Scale bars, 1 mm.

L. GSC-derived organoids from (H) were assayed for their diameter over a time course. Values represent mean ± SEM. *, $p < 0.05$ by two-way ANOVA.


A
ITGAV
ITGB3
ITGB5
Integrins
β-actin
CRISPR KO
gRNA Con
sgRNA.2
sgRNA.3
gRNA Con
sgRNA.1
sgRNA.2
gRNA Con
sgRNA.1
sgRNA.2
B
****
ZIKV infection (%)
80
40
0
Mock
Control sgRNA
sgRNA.1
sgRNA.2
sgRNA.2
sgRNA.3
sgRNA.1
sgRNA.2
Control
ITGB3
ITGAV
ITGB5
C
GSC387
GSC3565
ITGAV
ITGA6
β-actin
Control
ITGAV KO.1
ITGAV KO.2
Control
ITGAV KO.1
ITGAV KO.2
D
ITGAV CRISPR
Control sgRNA
ITGAV sgRNA.2
ITGAV sgRNA.3
ITGA6 DAPI
E
GSC387
GSC3565
ITGA6
β-actin
Control
ITGA6 KO.1
ITGA6 KO.2
Control
ITGA6 KO.1
ITGA6 KO.2
F
ITGA6 CRISPR
Control sgRNA
ITGA6 sgRNA.1
ITGA6 sgRNA.2
ITGA6 DAPI
G
GSC387
GSC3565
Control
ZIKA.PRV
ITGA6 KO.1
ITGA6 KO.2
Relative cell viability
7.0
3.5
0.0
6
3
0
****
****
****
****
0
3
5
0
3
5
Time (days)
Time (days)
H
Control KO
ITGA6 KO.1
ITGA6 KO.2
CC3 ZIKVE DAPI
GSC387
GSC3565
GSC387
ZIKV infection (%)
50
25
0
Mock
ZIKA.PRV
ZIKA.PRV+ITGA6.1
ZIKA.PRV+ITGA6.2
GSC3565
ZIKV infection (%)
40
20
0
Mock
ZIKA.PRV
ZIKA.PRV+ITGA6.1
ZIKA.PRV+ITGA6.2
I
Control
ZIKV 24 hours
ZIKV 48 hours
ZIKV 72 hours
ITGA6
ITGA6 ZIKVE DAPI
GSC3565
ITGA6+ cells/ DAPI+ (%)
100
75
50
25
0
Mock
ZIKA 24hr
ZIKA 48 hr
ZIKA 72 hr
Time (hours)

Supplemental Figure 5, refers to Figure 5. CRISPR-mediated targeting of ITGAV and ITGB5 integrins, but not ITGB3 attenuates ZIKV infection of GSCs

A. Patient-derived GSCs were transduced with sgRNAs for a non-targeting control sequence (Control sgRNA) or one of two sgRNAs targeting selected integrins. Whole cell lysates from transduced GSCs were collected and resolved by SDS-PAGE. Immunoblotting confirmed successful knockout of integrins at the protein level.

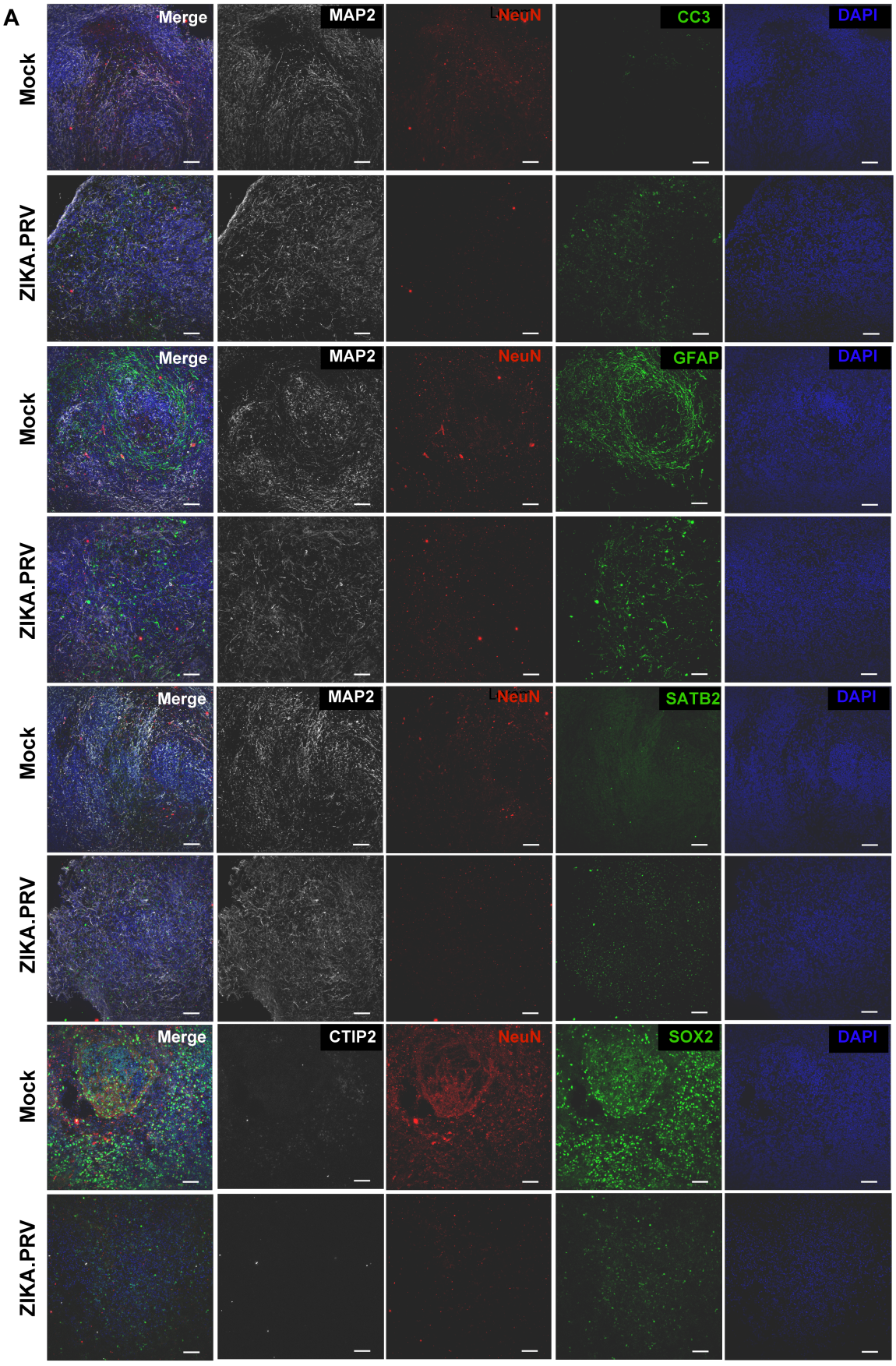
B. GSC3565 under mock conditions or transduced with indicated sgRNAs were examined for ZIKV infection efficiency.

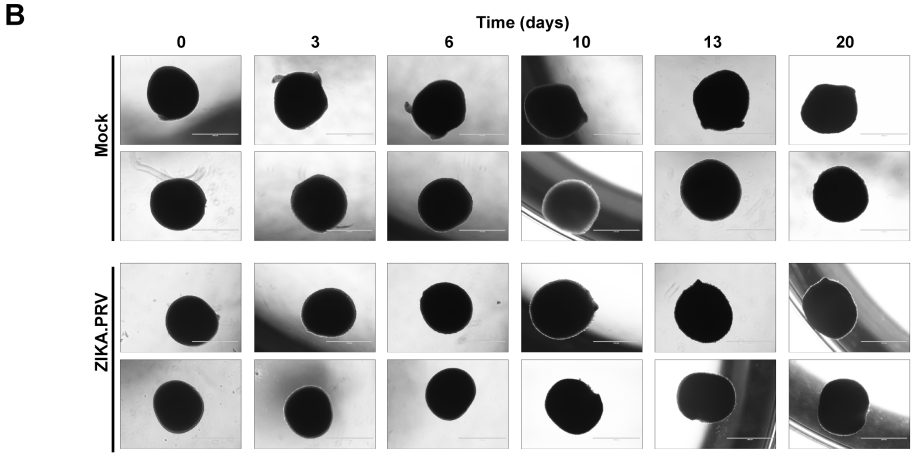
C-F. Patient-derived GSCs (GSC387 and GSC3565) were transduced with a non-targeting control sgRNA or one of two sgRNAs targeting either (E,F) ITGA6 or (C, D) ITGAV. A and B. ITGA6 and/or ITGAV expression was assessed by immunoblotting. β-actin was used as a loading control. D and F. ITGA6 expression was analyzed by immunofluorescence after targeting (F) ITGA6 or (D) ITGAV. DAPI (blue) was used as counterstain. Scale bars, 20 μm.

G. Patient-derived GSCs were either infected with ZIKV or transduced with sgRNAs targeting ITGA6. The CellTiter-Glo assay was performed over a time course.

H. Patient-derived GSCs were transduced with either a non-targeting control sgRNA or one of two, non-overlapping sgRNAs targeting ITGA6. Left, immunostaining for ZIKVE (green), CC3 (red) with DAPI (blue). Right, quantification of $ZIKVE^+$ cells in $DAPI^+$ cells.

I. Left, immunostaining for ZIKV (green), ITGA6 (red) and DAPI (blue). Right, quantification of $ITGA6^+$ cells in $DAPI^+$ cells. Scale bars, 20 μm. Values represent mean ± SEM. ****, $p < 0.0001$ by one-way ANOVA.

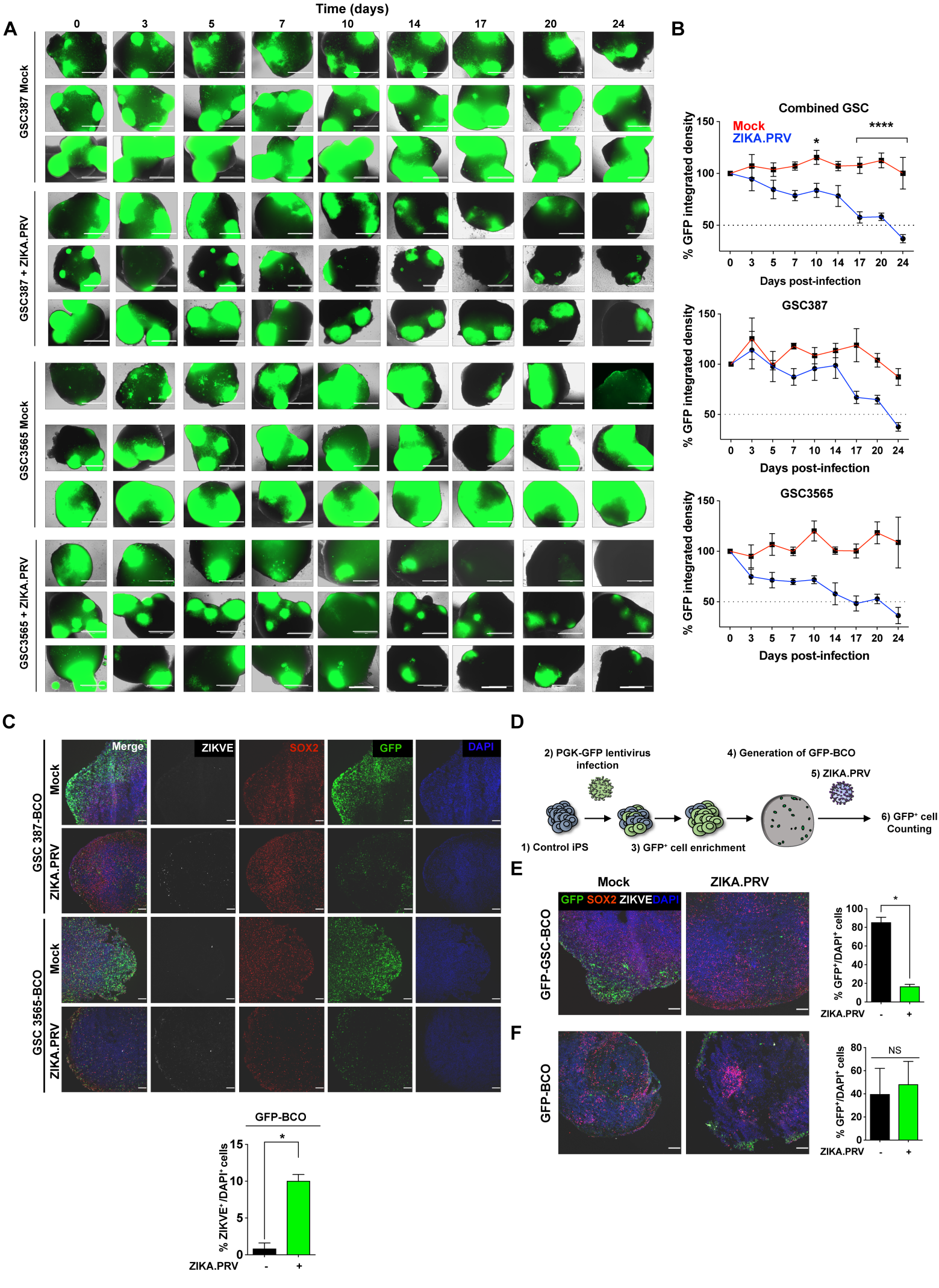

A
Mock
ZIKA.PRV
Merge
MAP2
NeuN
CC3
DAPI
GFAP
SATB2
CTIP2
SOX2


B
Time (days)
0
3
6
10
13
20
Mock
ZIKA.PRV

Supplemental Figure 6, refers to Figure 6. Effects of ZIKV on normal brain cortical organoid cellular types and size.

A. Representative images of mock or ZIKV-infected brain cortical organoids immunostained with MAP2, NeuN, CC3, GFAP, CTIP2, SATB2, SOX2 and DAPI. Scale bar = 100μm.

B. Representative brightfield images of brain cortical organoids derived from induced pluripotent stem cells that were subjected to mock conditions or infected with ZIKA.PRV (MOI: 5 FFU/cell) over a time course until 20 days post infection. Scale bar, 1 mm.


A
Time (days)
0
3
5
7
10
14
17
20
24
GSC387 Mock
GSC387 + ZIKA.PRV
GSC3565 Mock
GSC3565 + ZIKA.PRV
B
Combined GSC
Mock
ZIKA.PRV
*
****
% GFP integrated density
Days post-infection
GSC387
GSC3565
C
Merge
ZIKVE
SOX2
GFP
DAPI
GSC 387-BCO
GSC 3565-BCO
Mock
ZIKA.PRV
GFP-BCO
% ZIKVE+ /DAPI+ cells
ZIKA.PRV
D
2) PGK-GFP lentivirus infection
4) Generation of GFP-BCO
5) ZIKA.PRV
1) Control iPS
3) GFP+ cell enrichment
6) GFP+ cell Counting
E
Mock
ZIKA.PRV
GFP-GSC-BCO
GFP SOX2 ZIKVE DAPI
% GFP+/DAPI+ cells
F
GFP-BCO
NS

Supplemental Figure 7, refers to Figure 7. ZIKV infection preferentially targets GBM in GSC-brain cortical organoids models

A. Brain cortical organoids were derived from induced pluripotent stem cells then confronted with GFP-labeled GSCs (GSC387 or GSC3565), permitting integration. Brightfield images showing GSC-brain cortical organoids after being subjected to mock conditions or infection with ZIKA.PRV (MOI: 5 FFU/cell) were taken over a time course until day 24. Scale bars, 1 mm.

B. GFP integrated density shown in A was measured using ImageJ software. Mock infection vs. ZIKA.PRV infection. Day 10: $p = 0.03$; *, $p < 0.05$; days 17, 20 and 24: ****, $p < 0.0001$ by two-way ANOVA.

C. Representative images of GSC-brain cortical organoids with or without ZIKV immunostained with NS1, staining for ZIKV, SOX2, GFP and DAPI (GSC387 top panel, GSC3565 bottom panel). Scale bars, 50 μm. The quantification of $ZIKV^+$ (quantified by NS1 immunostaining) within all cells and $ZIKV^+$ cells within the GFP^+ cells in GSC-brain cortical organoid (BCO) mock vs GSC-BCO ZIKV respectively. Significance was assessed by two tailed Student's t-test, experiments were performed in two batches with 12 organoids per group, per batch (*$p < 0.05$).

D. Schematic of GFP-brain cortical organoid (BCO) generation. Control iPS were firstly infected with a lentivirus containing the PGK-GFP vector, then the GFP^+ cells were enriched using FACs sorting to generate brain cortical organoids by sorted GFP-iPS. These organoids were then infected with ZIKA.PRV (MOI: 5 FFU/cell) and the GFP^+ cells were counted.

E. GFP-BCO and GFP-GSC-BCO were immunostained with SOX2, ZIKVE and DAPI antibodies with or without ZIKV. Quantification of GFP^+ cells in GFP-brain cortical organoid (BCO) with or without ZIKV infection respectively. Scale bars, 100 μm. Significance was assessed by two tailed Student's t-test, experiments were performed in two batches with 12 organoids per group for each GSCs cell line (GSC387 and GSC3565), per batch (*$p < 0.05$; NS, no significance). Experiments were performed in two batches with 12 organoids per group, per batch.

196 **Table S1. All Cell lines used in the manuscript.**

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Glioblastoma Stem Cell Model or Tissue	Patient Age (Years)	Patient Sex	Tumor Grade
GSC387	76	Female	Glioblastoma (Grade IV)
GSC3565	32	Male	Glioblastoma (Grade IV)
GSC23	63	Male	Recurrent Glioblastoma (Grade IV)
MGG8	Restricted by Institutional Requirements	Female	Glioblastoma (Grade IV)
GSC1517	54	Female	Glioblastoma (Grade IV)
3752	5	Female	Pontine Glioma(Milde *et al.*, 2011)
007	6	unknown	Pontine Glioma
CH-157MN	41	Female	Meningioma
IOMM-Lee	61	Male	Meningioma
DAOY	4	Male	Medulloblastoma
D283	6	Male	Medulloblastoma
HDMB03	3	Male	Medulloblastoma
D341	3.5	Male	Medulloblastoma

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200 **Table S2. Primers and Oligos for PCR and shRNA assay.**

Gene Name	Forward Primer	Reverse Primer
SOX2	5’-GCCGAGTGGAAACTTTTGTCG-3’	5’-GGCAGCGTGTACTTATCCTTCT-3’
OLIG2	5’-CCAGAGCCCGATGACCTTTTT-3’	5’-CACTGCCTCCTAGCTTGTCC-3’
GFAP	5’-CTGCGGCTCGATCAACTCA-3’	5’-TCCAGCGACTCAATCTTCCTC-3’
ITGAV	5’-ATCTGTGAGGTCGAAACAGGA-3’	5’-TGGAGCATACTCAACAGTCTTTG-3’
ITGB1	5’-CCTACTTCTGCACGATGTGATG-3’	5’-CCTTTGCTACGGTTGGTTACATT-3’
ITGB3	5’-GTGACCTGAAGGAGAATCTGC-3’	5’-CCGGAGTGCAATCCTCTGG-3’
ITGB5	5’-TCTCGGTGTGATCTGAGGG-3’	5’-TGGCGAACCTGTAGCTGGA-3’
ITGB6	5’-TCCATCTGGAGTTGGCGAAAG-3’	5’-TCTGTCTGCCTACACTGAGAG-3’
ITGB8	5’-ACCAGGAGAAGTGTCTATCCAG-3’	5’-CCAAGACGAAAGTCACGGGA-3’
TLR3	5’-TTGCCTTGTATCTACTTTTGGGG-3’	5’-TCAACACTGTTATGTTTGTGGGT-3’
ISG20	5’-CTCGTTGCAGCCTCGTGAA-3’	5’-CGGGTTCTGTAATCGGTGATCTC-3’
IFITM1	5’-CCAAGGTCCACCGTGATTAAC-3’	5’-ACCAGTTCAAGAAGAGGGTGTT-3’
MX1	5’-GTTTCCGAAGTGGACATCGCA-3’	5’-CTGCACAGGTTGTTCTCAGC-3’
ISG15	5’-CGCAGATCACCCAGAAGATCG-3’	5’-TTCGTCGCATTTGTCCACCA-3’
RSAD2	5’-TGGGTGCTTACACCTGCTG-3’	5’-GAAGTGATAGTTGACGCTGGTT-3’
OAS2	5’-CTCAGAAGCTGGGTTGGTTTAT-3’	5’-ACCATCTCGTCGATCAGTGTC-3’
IRF1	5’-ATGCCCATCACTCGGATGC-3’	5’-CCCTGCTTTGTATCGGCCTG-3’
IFNAR1	5’-AACAGGAGCGATGAGTCTGTC-3’	5’-TGCGAAATGGTGTAAATG AGTCA-3’

IFIT1	5’-TTGATGACGATGAAATGCCTGA-3’	5’-CAGGTCACCAGACTCCTCAC-3’
ZIKV835	5’-TTGGTCATGATACTGCTGATTGC-3’	
ZIKV911c	5’-CCTTCCACAAAGTCCCTATTGC-3’	
ZIKV860FAM	5’-CGGCATACAGCATCAGGTGCATAG GAG-3’	
Gene Name and start site of shRNA targeting	**shRNA sequence**	
ShAXL-2	5’-CCGGCGTGGAGAACAGCGAGATTTACTCGAGTAAATCTCGCTGTTCTCCACGTTTTTTG-3’	
ShAXL-3	5’-CCGGCCTAAGCATCTAAGTTATAAGCTCGAGCTTATAACTTAGATGCTTAGGTTTTTTG-3’	
ShSOX2-52	5'-CCGGGAAGAAGGATAAGTACACGCTCTCGAGAGCGTGTACTTATCCTTCTTCTTTTT-3'	
ShSOX2-53	5'-CCGGCTGCCGAGAATCCATGTATATCTCGAGATATACATGGATTCTCGGCAGTTTTT-3'	
ShITGB1-1	5’-CCGGGCCTTGCATTACTGCTGATATCTCGAGATATCAGCAGTAATGCAAGGCTTTTTG-3’	
ShITGB1-2	5’-CCGGGCCCTCCAGATGACATAGAAACTCGAGTTTCTATGTCATCTGGAGGGCTTTTTG-3’	
ShITGB3-3	5’-CCGGGTCGTCAGATTCCAGTACTATCTCGAGATAGTACTGGAATCTGACGACTTTTT-3’	
ShITGB3-2	5’-CCGGCCACGTCTACCTTCACCAATACTCGAGTATTGGTGAAGGTAGACGTGGTTTTT-3’	
ShITGB5-1	5’-CCGGGGCTCGCAGGTCTCAACATATCTCGAGATATGTTGAGACCTGCGAGCCTTTTTG-3’	
ShITGB5-3	5’-CCGGGGATCAGCCTGAGGATCTTAACTCGAGTTAAGATCCTCAGGCTGATCCTTTTTG-3’	
ShITGB6-1	5’-CCGGGCCTCCAAACATTCCCATGATCTCGAGATCATGGGAATGTTTGGAGGCTTTTTG-3’	
ShITGB6-2	5’-CCGGCCATTGACAAATGATGCTGAACTCGAGTTCAGCATCATTTGTCAATGGTTTTTG-3’	
ShITGB8-1	5’-CCGGGCTCAGTTGATTCAATAGAATCTCGAGATTCTATTGAATCAACTGAGCTTTTTG-3’	
ShITGB8-2	5’-CCGGCGAGCAATGATGAAGTTCTTTCTCGAGAAAGAACTTCATCATTGCTCGTTTTTG-3’	

Table S3. ChIP-PCR primers, refers to Figure 3.

Target Name	Target Region (hg19)	Forward Primer	Reverse Primer
Negative Control #1	chr11:35,158,607-35,159,750	AGGGTGAGGGCTCTGAAGAT	GCCATCCCCCTATGCATTCA
SOX2 Enhancer Primer #1	chr2:187,477,736-187,478,211	AGGACCACTGGAATTGCTCA	GCAGAACCAGAGGAAAACAGG
SOX2 Enhancer Primer #2	chr2:187,477,736-187,478,211	GGCCTGTATTCTTTGTGCTGC	AGCTCATTTCACTGGGATTGGT

Table S4. sgRNA sequences and plasmids used in the manuscript

Target Name	Forward Sequence	CRISPR Type
Non-Targeting	CACCGCTCTGCTGCGGAAGGATTCG	LentiCrisprV2 (Addgene #52961)
ITGAV Gene 1	GGAATTGATAGCGTATCTGC	LentiCrisprV2 (Addgene #52961)

ITGAV Gene 2	AGGCAATAGAGATTATGCCA	LentiCrisprV2 (Addgene #52961)
ITGAV Gene 3	GCCTTAACAATCAATGTCAG	LentiCrisprV2 (Addgene #52961)
ITGB3 Gene 1	GCGAGGTGAGCCCAGAGGCA	LentiCrisprV2 (Addgene #52961)
ITGB3 Gene 2	TCACAGCGAGGTGAGCCCAG	LentiCrisprV2 (Addgene #52961)
ITGB3 Gene 3	TTCTCCTTCAGGTCACAGCG	LentiCrisprV2 (Addgene #52961)
ITGB5 Gene 1	TTTTTCCCTGCCTAGGACTT	LentiCrisprV2 (Addgene #52961)
ITGB5 Gene 2	ACCGAGAGGTGATGGACCGT	LentiCrisprV2 (Addgene #52961)
ITGB5 Gene 3	CACCGAGAGGTGATGGACCG	LentiCrisprV2 (Addgene #52961)
ITGA6 Gene 1	CGGATCGAGTTTGATAACGA	LentiCrisprV2 (Addgene #52961)
ITGA6 Gene 2	TTTCCAGTTATAAGTACCCG	LentiCrisprV2 (Addgene #52961)
ITGA6 Gene 3	GGAGCTCCGTATGATGACTT	LentiCrisprV2 (Addgene #52961)

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