Evaluating glioblastoma tumor sphere growth and migration in interaction with astrocytes using 3D collagen-hyaluronic acid hydrogels

Yixiao Cui[1], Paul Lee[2], Jesse J. Reardon[3,4], Anna Wang[1], Skylar Lynch[1], Jose J. Otero[3,5] , Gina Sizemore[3,4], Jessica O. Winter[1,2,3]

[1]Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA

[2]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH, USA

[3]Ohio State University Comprehensive Cancer Center - James, The Ohio State University, Columbus, OH, USA
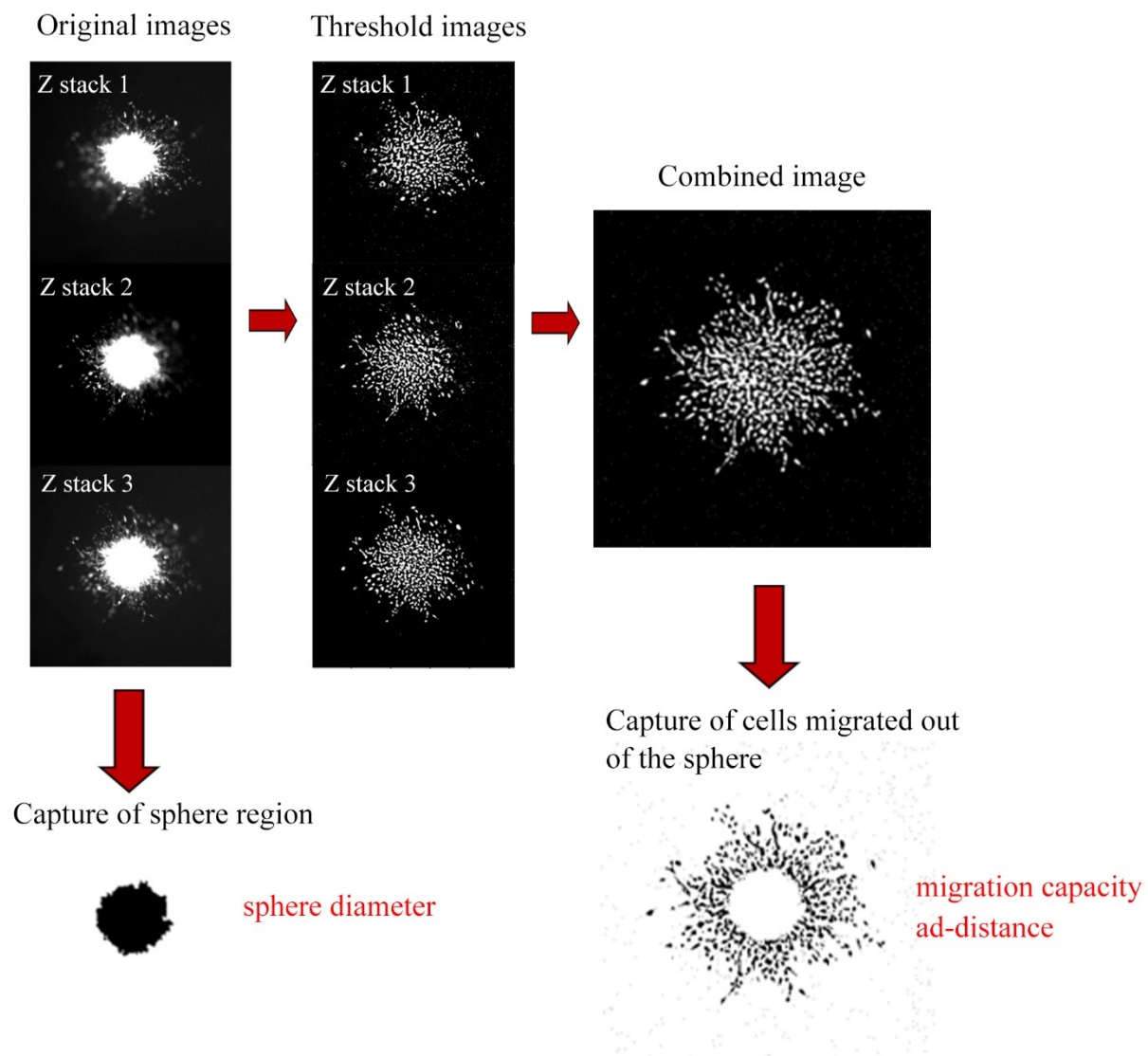
[4]Department of Radiation Oncology, The Ohio State University, Columbus, OH, USA

[5]Department of Neuroscience, The Ohio State University, Columbus, OH, USA
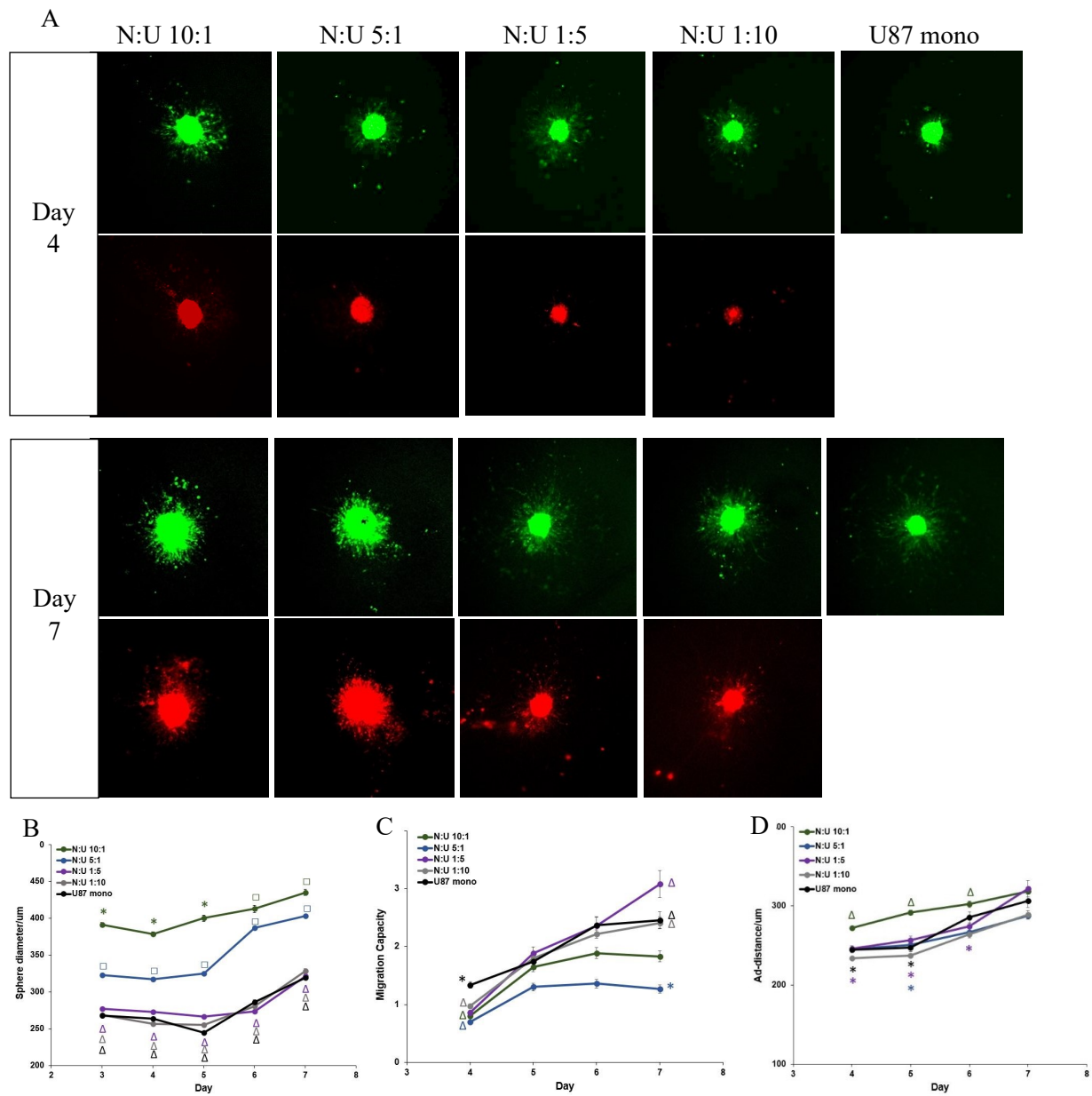
Supplementary Figures
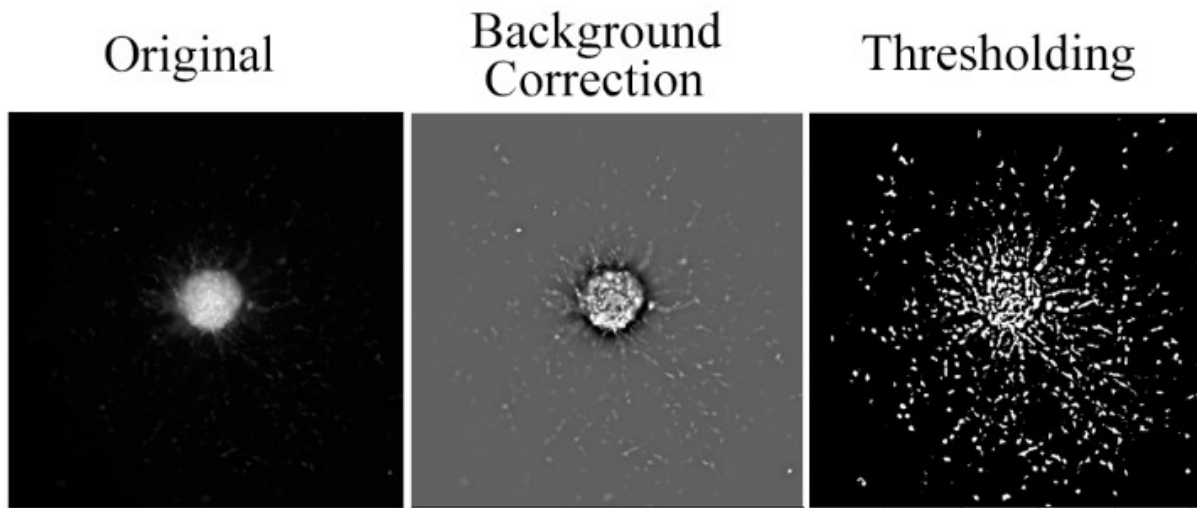
Supplementary Figure 1. Example of image processing methods for correcting uneven background and thresholding the images in R studio.

Original images

Threshold images

Z stack 1

Z stack 1

Z stack 2

Z stack 2

Z stack 3

Z stack 3

Combined image

Capture of sphere region

sphere diameter

Capture of cells migrated out of the sphere

migration capacity
ad-distance

Supplementary Figure 2. Schematic and examples of image process methods to measure of sphere diameter, migration capacity, and adjusted migration distance (ad-distance).
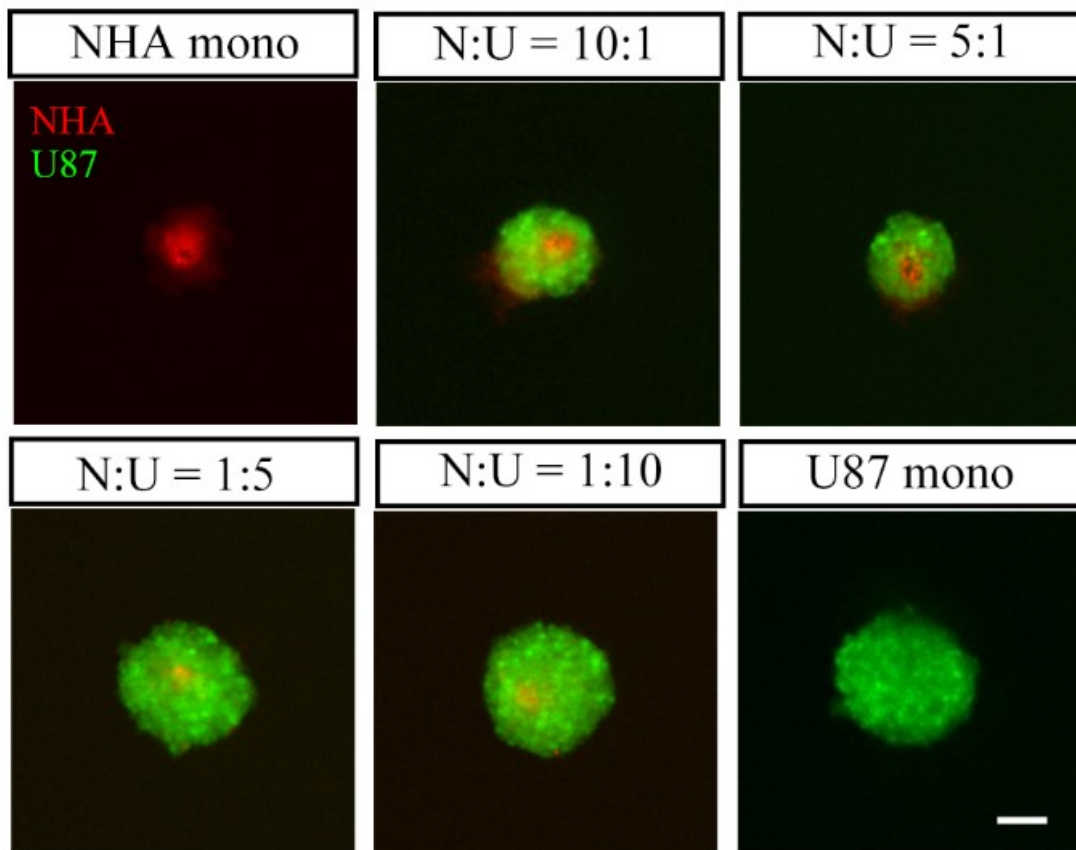
Supplementary Figure 3. Examples of normal human astrocyte (NHA) mono-sphere, U87-NHA multi-spheres at NHA:U87 (N:U) ratios of 10:1, 5:1, 1:5, and 1:10, and U87 mono-sphere in liquid culture at day 3. Scale bar = 100 μm. U87 glioblastoma (GB) cells are shown in green; NHAs are shown in red.

Supplementary Figure 4. U87 mono- and U87-NHA multi-spheres encapsulated in collagen-HA gels with an initial seeding density of 1000 U87 cells at different N:U ratios. GB A) Representative images; B) sphere diameter, C) migration capacity, and D) adjusted migration distance (ad-distance) are presented as average ± standard error. N = 9 or 10. *, □, and Δ are color coded to the respective condition. Different symbol groups indicated conditions that are statistically different on that day. P < 0.05.
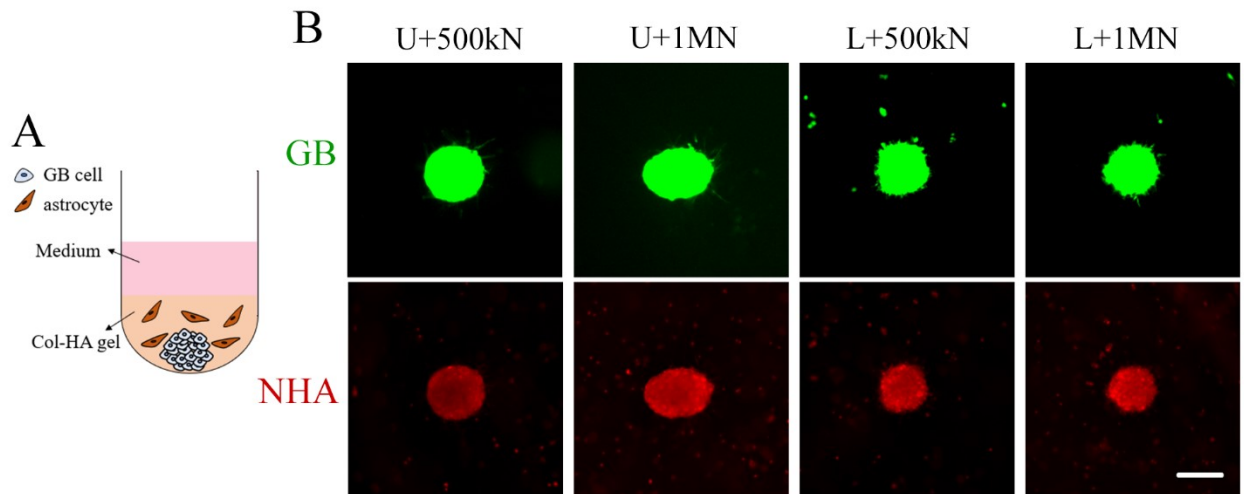
Supplementary Figure 5. U87 (U) and LN229 (L) mono-spheres encapsulated in collagen-HA gels loaded with NHAs at concentrations of 500k (500kN) or 1M (1MN) cells/ml: A) Model configuration. B) Representative images of NHA (red) co-localization with glioblastoma cells (green) after encapsulation at day 3. Scale bar = 200 μm.

A
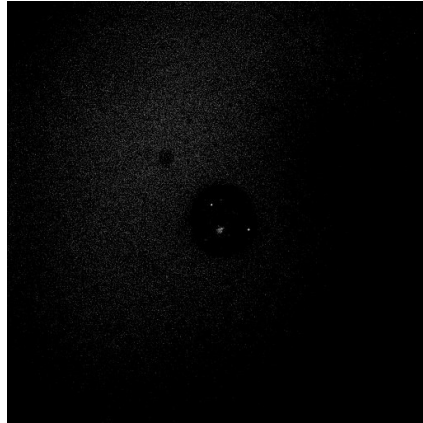
B
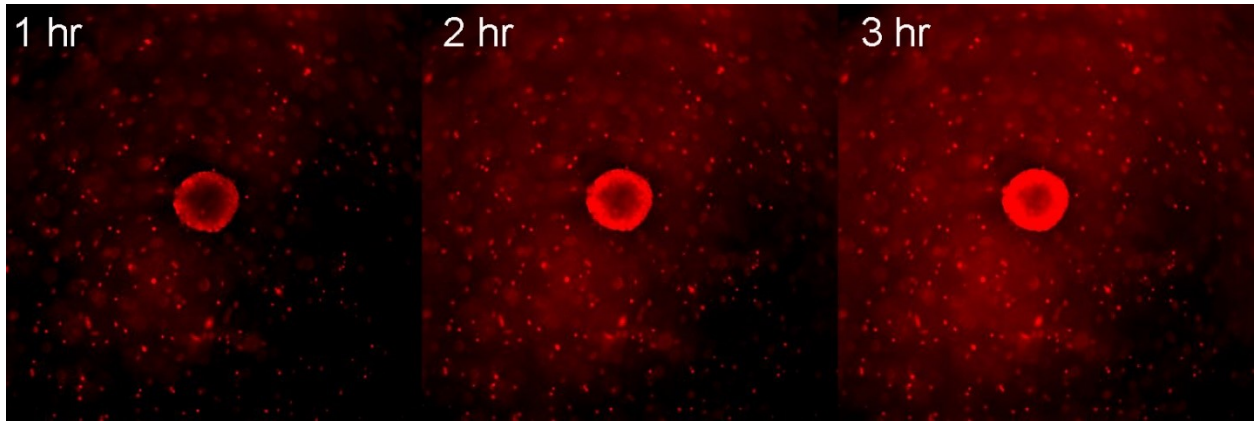
Supplementary Figure 6. A) Red (astrocyte) channel image of control sample containing only GB mono-sphere, indicating lack of significant bleed-through. B) Time lapse images of astrocyte-GB co-localization in GB mono-sphere plus dispersed astrocyte cultures on day 3 showing gradual co-localization.

Supplementary Figure 7. U87 (U) and LN229 (L) mono-spheres encapsulated in collagen-HA gels loaded with live (2MN-li) or fixed NHAs (2MN-fi) at 2M cells/ml: A) Model configuration B) Representative images of NHA (red) co-localization with glioblastoma cells (green) after encapsulation at day 3. Scale bar = 200 um.
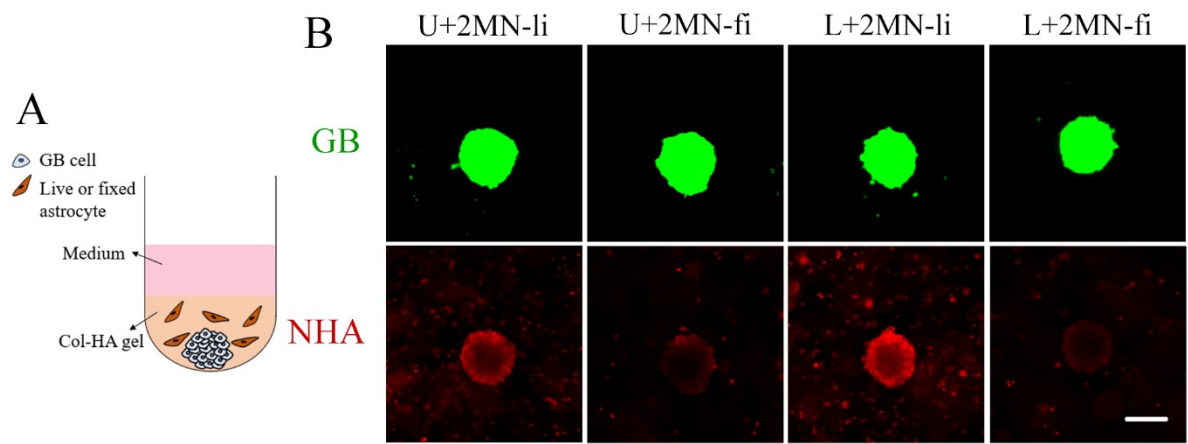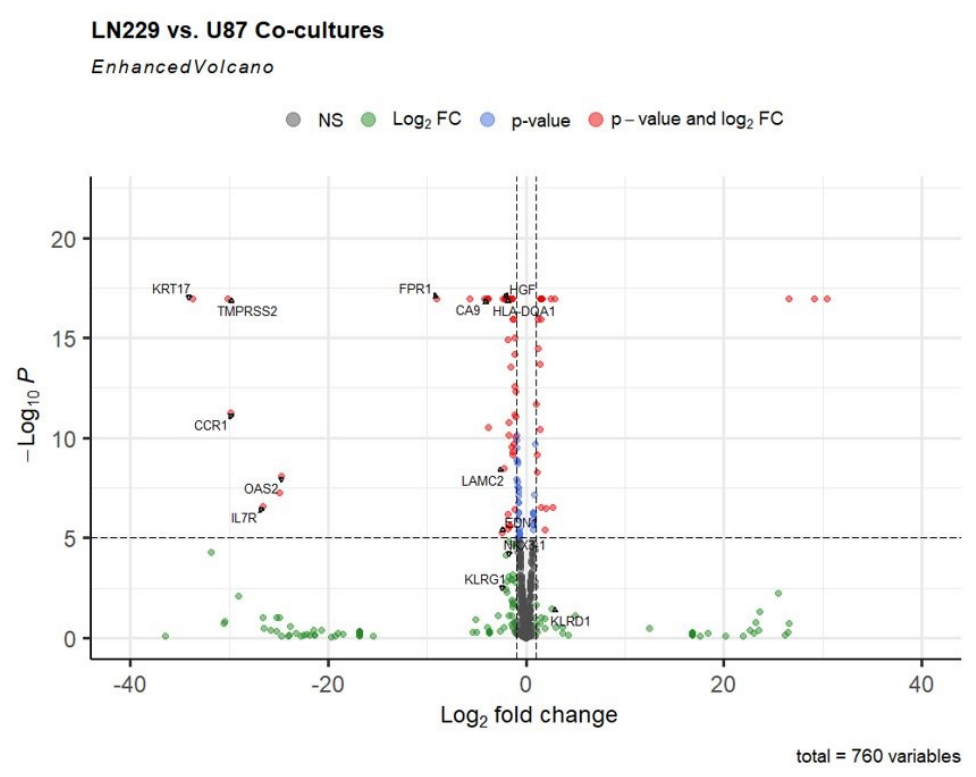

Supplementary Figure 8. Volcano plot of differential gene expression for LN229 vs. U87 mono-spheres in co-culture with dispersed astrocytes. NS = not significant, $\log_2$ FC = $\log_2$ fold change > 2, p-value = p-value < 1E-5. (Note that software cannot detect p < 1E-12). p-value and $\log_2$ FC = p value < 0.05 and $\log_2$ FC > 2. The differential expression of these genes is considered statistically significant.
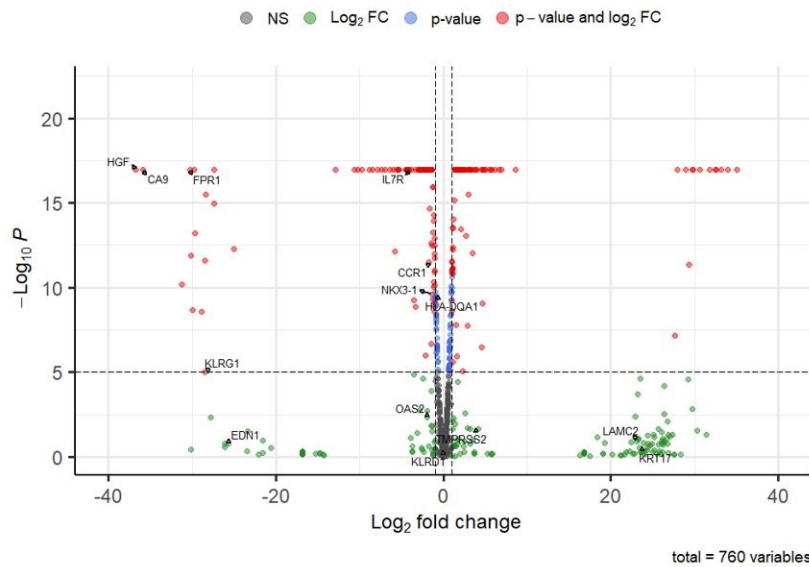
Supplementary Figure 9. Volcano plot of differential gene expression for LN229 vs. U87 mono-spheres cultured without astrocytes. NS = not significant, Log_2 FC = log_2 fold change > 2, p-value = p-value value < 1E-5. (Note that software cannot detect p < 1E-12). p-value and log_2 FC = p value < 0.05 and log_2 FC > 2. The differential expression of these genes is considered statistically significant.
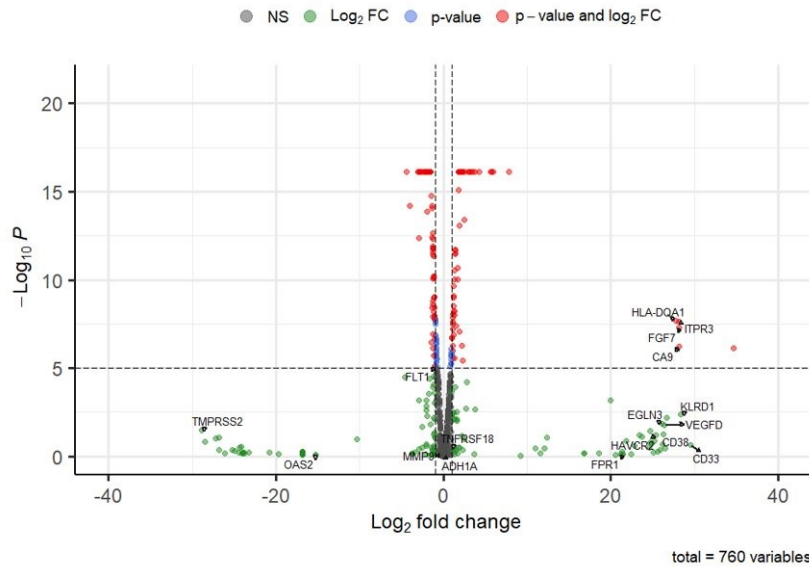
Supplementary Figure 10. Volcano plot of differential gene expression for LN229 spheroid co-cultures with dispersed normal human astrocytes and U87 spheroid and astrocyte separate cultures. NS = not significant, Log_2 FC = log_2 fold change > 2, p-value = p-value value < 1E-5. (Note that software cannot detect p < 1E-12). p-value and log_2 FC = p value < 0.05 and log_2 FC > 2. The differential expression of these genes is considered statistically significant. *MAGEC2 (log FC = -43.8, p value =0.62) and KLKR9 (log FC = 83.2, p value = 0.74) could not fit on plot. Neither is statistically significant.

Supplementary Figure 11. Volcano plot of differential gene expression for U87 spheroid co-cultures with dispersed normal human astrocytes and U87 spheroid and astrocyte separate cultures. NS = not significant, Log$_2$ FC = log$_2$ fold change > 2, p-value = p-value value < 1E-5. (Note that software cannot detect p < 1E-12). p-value and log$_2$ FC = p value < 0.05 and log$_2$ FC > 2. The differential expression of these genes is considered statistically significant.
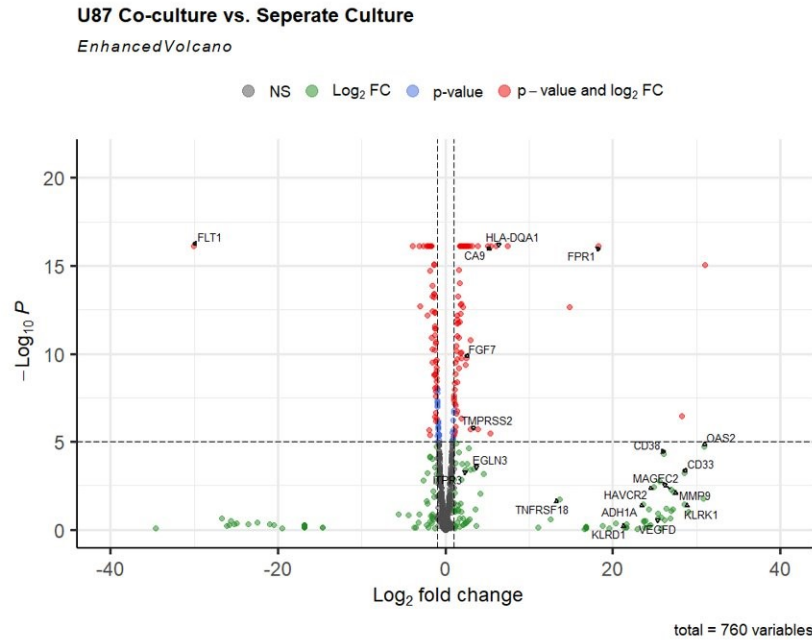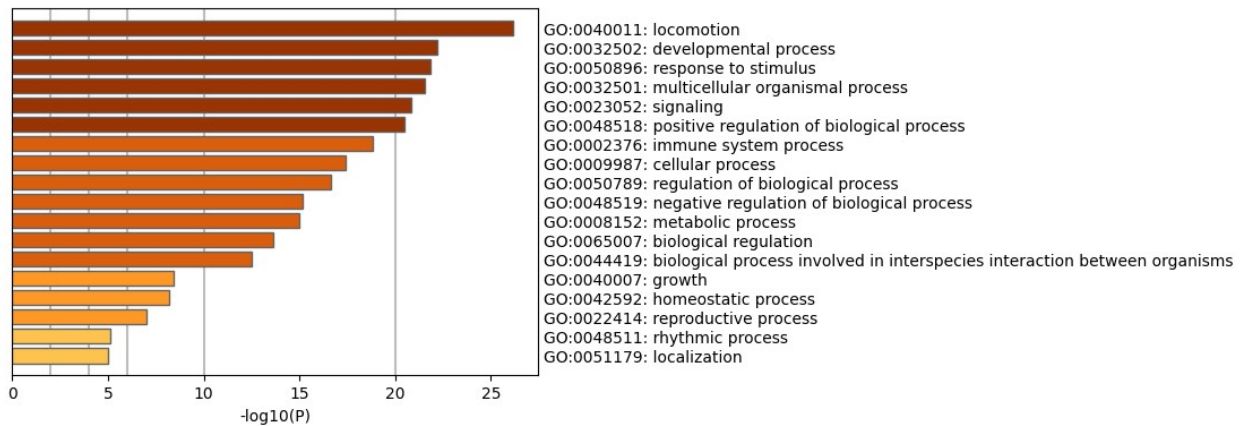

Supplementary Figure 12. Metascape Gene Ontology (GO) biological processes enrichment analysis of differentially expressed genes in LN229 vs. U87 mono-sphere cultures without astrocytes.
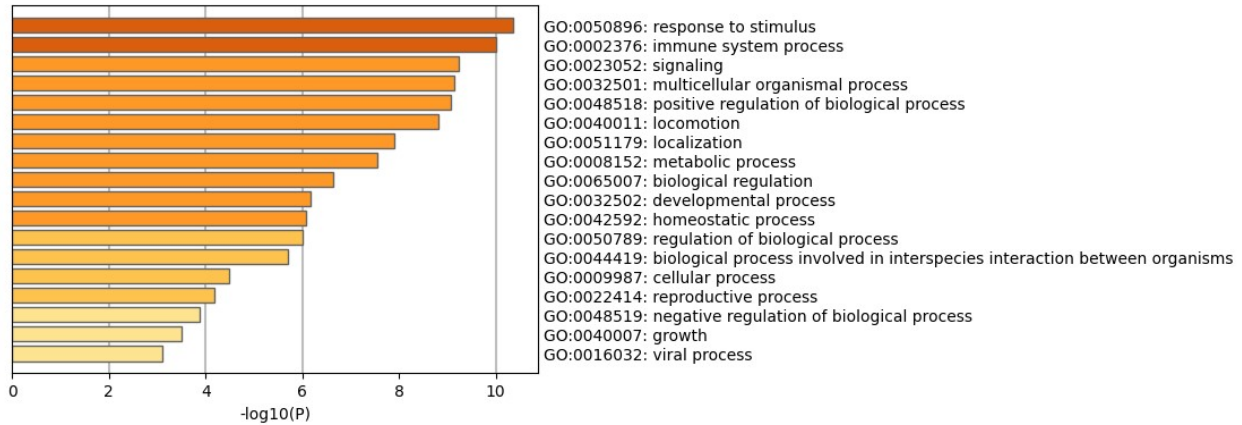
Supplementary Figure 13. Metascape Gene Ontology (GO) biological processes enrichment analysis of differentially expressed genes in U87 co-cultures vs. combined RNA from separate U87 and astrocyte cultures.
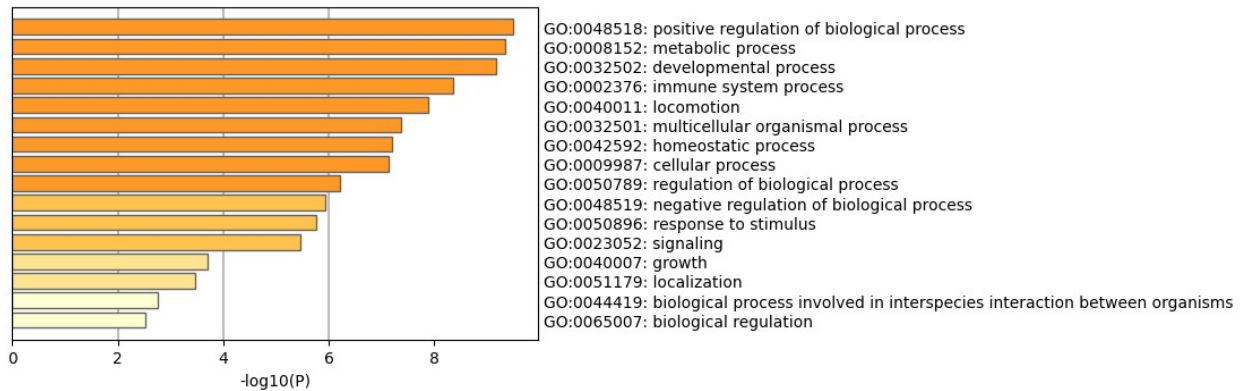
Supplementary Figure 14. Metascape Gene Ontology (GO) biological processes enrichment analysis of differentially expressed genes in LN229 co-cultures vs. combined RNA from separate U87 and astrocyte cultures.

Supplementary Table 1. Contribution of variables to principal components (PC) from principal component analysis (PCA).

| | U87 | | LN229 | |
	PC1 (%)	PC2 (%)	PC1 (%)	PC2 (%)
sphere diameter	25.12	72.48	24.57	73.10
migration capacity	35.70	22.07	35.93	21.54
ad-distance	39.18	5.45	39.40	5.36

Supplementary Table 2: Differentially Expressed Genes Between LN229 versus 87 spheroids in co-culture with dispersed normal human astrocytes. Log FC = log$_2$ fold change. Genes highlighted in gray are also differentially expressed in mono-sphere cultures without astrocytes at similar levels, and thus indicate differences between cell lines not astrocyte-related behaviors. *p value = 0 indicates p < 1E-12.

Gene	Log FC	p value*
BCL2A1	2.5	0
BLK	-29.1	0.0081
C3AR1	-24.9	5.8E-08
CA9	-3.9	0
CBX2	2.7	3.1E-07
CCL5	-3.8	3.1E-11
CCR1	-29.8	5.6E-12
CD22	30.4	0
CSF2RA	25.5	0.0058
EDN1	-2.5	5.6E-06
ERBB3	29.1	0
FLT1	26.6	0
FPR1	-9.1	0
HGF	-2.3	0
HLA-DQA1	-2.2	0
IL11	-4.2	0
IL6	-5.6	0
IL7R	-26.6	2.7E-07
ITGA5	-2.1	0
KLRD1	2.6	0.034
KLRG1	-2.0	0.0038
KRT17	-33.7	0
LAMC2	-2.2	3.3E-09
LAT	-3.8	0
MAGEC2	-31.8	5.1E-05
NFATC2	2.9	0
NKX3-1	-2.1	7.4E-05
OAS2	-24.7	8.6E-09
PTGS2	-3.9	0
TMPRSS2	-30.2	0
VEGFA	-2.0	0

Supplementary Table 3: Differentially Expressed Genes Between LN229 versus U87 spheroids in mono-culture without astrocytes. Log FC = log$_2$ fold change. Genes highlighted in gray are also differentially expressed in spheroid co-cultures with astrocytes at similar levels, indicating cell lines differences. *p value = 0 indicates p < 1E-12.

Gene	Log FC	p value*
ABCC4	-2.6	0
ADM	-2.5	0
ADORA2A	-2.4	0
ALK	-27.8	0.0049
BCL2	2.6	9.0E-14
BCL2A1	4.6	0
BIRC3	-3.1	0.038
BLK	-29.8	0
C3AR1	-28.5	2.4E-12
CA9	-35.9	0
CBX2	6.6	0
CCL2	3.3	0
CCL5	-3.5	5.4E-10
CD22	33.2	0
CD274	-4.4	0
CD38	2.9	0.021
CD40	-7.4	0
CD53	-2.8	0.0017
CD6	-30.1	1.2E-12
CD68	-5.4	0
CD80	26.8	0.0085
CDCP1	-31.2	6.7E-11
CDH11	4.7	0
CEACAM1	29.4	4.5E-12
COL4A1	32.6	0
COL4A2	8.6	0
COL6A3	-9.8	0
CSF2RA	30.6	0
CTLA4	26.4	6.8E-05
CYBB	30.4	0.029
DCN	2.4	0
DOCK2	-4.4	0
EHHADH	2.9	0
ENTPD1	23.2	0.00020
EPCAM	-23.4	0.033
ERBB2	4.6	0
ERBB3	35.1	0
FCRL2	26.0	0.036
FGF7	-2.9	0
FGFR3	29.8	0.0015
FLT1	32.5	0
FOLH1	29.3	2.8E-05
FOS	2.3	9.2E-06
FOXP1	-7.9	0
FPR1	-30.2	0
FZD1	3.2	0
FZD4	26.4	0.0028
FZD7	5.4	0
FZD8	2.2	3.5E-14
GALNT3	3.5	9.2E-13
GPNMB	6.2	0
GRAP2	3.2	0.023
GREM1	-8.8	0
HGF	-36.8	0
HLA-B	-2.6	0
HSD11B1	-29.9	2.1E-09
IFNGR2	2.5	0
IGF1R	3.1	0
IGFBP3	3.1	0
IL10	31.4	0.049
IL11	-4.3	0
IL6	-10.6	0
IL7	-3.0	0
IL7R	-4.1	0
INHBA	5.7	0
ITGA11	3.1	0
ITGA5	-2.0	0
ITGAX	-4.1	0
ITGB8	5.1	0
ITK	-6.0	0
ITPR1	-3.3	0
JUN	-2.5	0
KDR	-28.9	2.7E-09
KIT	28.0	0
KLRG1	-28.5	9.5E-06
LAG3	2.6	0.0027
LAIR1	27.7	6.7E-08
LAMA2	-27.4	1.1E-15
LAMA4	5.7	0
LAMB1	2.5	0
LAMC1	2.2	0
LAT	-4.5	0
LCN2	4.0	0
LOX	-12.9	0
LRRC32	-5.8	7.7E-13
LTBP1	-3.8	0
LY96	-5.6	0
MAGEC2	-25.1	5.2E-13
MET	-2.6	0
MYC	-2.2	0
MYL9	-27.4	0
NFATC2	6.9	0
NOTCH3	-28.4	3.3E-16
NT5E	-2.6	0
OAS1	3.0	3.3E-16
PDCD1LG2	-4.4	0
PDGFB	25.7	0.022
PDGFRA	2.9	1.8E-08
PDGFRB	-2.8	0
PFKP	-2.2	0
PGF	3.0	0
PIK3CD	-5.2	0
PLAU	-10.3	0
PLEK2	-29.7	6.2E-14
PRKCA	-2.1	0
PTCH1	3.7	0
PTCH2	22.9	0.0040
PTGER2	-3.3	1.3E-09
PTGS2	-2.2	0
RAC3	2.3	0
SELL	4.6	3.3E-07
SERPINE1	-8.5	0
SIRPA	2.3	0
SLC7A5	2.2	0
SLIT2	2.0	0
SMO	34.0	0
SNAI1	4.9	0
SOCS1	-2.8	0
SOX2	28.9	0
SPINT1	29.7	0
STAT4	-2.4	2.3E-05
STING1	29.8	0
TEK	-3.6	1.4E-05
TERT	27.3	0.049
THBS1	3.9	0
THBS2	3.5	0
TLR3	2.0	0
TLR4	31.8	0
TMPRSS2	4.2	0.022
TNFRSF9	-6.4	0
TNFSF14	-2.2	1.0E-06
TOX3	23.5	2.3E-05
TP53	2.4	0
TP73	4.7	8.9E-10
TSPAN1	-4.3	0
VEGFA	-5.4	0
VEGFC	-7.0	0
WWTR1	2.6	0

Supplementary Table 4: Differentially Expressed Genes between U87 spheroid co-cultures with dispersed normal human astrocytes versus U87 spheroid and astrocyte separate cultures. Log FC = log$_2$ fold change. Genes highlighted in gray are also differentially expressed in LN229 co-cultures vs. separate cultures at similar levels, indicating behaviors that are the same for both cell lines. *p value = 0 indicates p < 1E-12.

Gene	Log FC	p value*
ACTG2	-3.9	0
ADH1A	23.6	0.037
ADM	2.7	0
AMOTL2	-2.2	0.023
BCL2A1	4.6	0.00070
CA9	5.4	0
CCL5	5.4	3.2E-06
CCR1	3.0	0.00041
CD33	28.5	0.00061
CD38	26.1	5.1E-05
EDN1	-2.1	6.9E-13
EGF	2.6	0.00018
EGLN3	3.4	0.00035
EOMES	-2.6	0.0015
EPAS1	2.1	0
FGF7	2.5	1.8E-10
FLT1	-30.1	0
FPR1	18.3	0
GPNMB	2.1	0
HAVCR2	24.9	0.0036
HGF	2.4	0
HLA-B	2.4	0
HLA-DQA1	6.0	0
HLA-DRB1	2.6	0
HSD11B1	31.1	8.9E-16
ICAM1	7.5	0
IDO1	25.6	0.0017
IL3RA	-2.1	0.033

Gene	Log FC	p value*
IL7	2.8	2.3E-05
INHBA	-2.6	0
ITGA8	3.2	0
ITPR3	2.4	0.00038
JAG1	-3.1	0
KIT	-3.1	1.9E-13
KLRG1	4.1	0.0090
KLRK1	28.5	0.036
KRT17	5.0	0
LAT	2.4	4.9E-10
LCN2	14.9	2.2E-13
LY96	2.5	0
MAGEC2	27.0	0.0053
MMP9	27.3	0.0072
OAS1	3.9	2.0E-06
OAS2	31.0	1.9E-05
PIK3R1	2.1	0
PLAU	-2.9	0
PTGS2	2.9	0
SDC1	-2.3	0
SHH	30.8	0.017
STAT4	2.0	0.00018
TLR3	3.0	1.6E-11
TMPRSS2	3.0	2.0E-06
TNFRSF18	13.6	0.019
TNFSF11	3.9	0
TNFSF13B	28.2	3.4E-07
TNS1	-2.2	0.00057
TPD52	2.1	2.24E-13
VEGFA	2.0	0

Supplementary Table 5: Differentially Expressed Genes between LN229 spheroid co-cultures with dispersed normal human astrocytes versus LN229 spheroid and astrocyte separate cultures. Log FC = log$_2$ fold change. Genes highlighted in gray are also differentially expressed in U87 co-cultures vs. separate cultures at similar levels, indicating behaviors that are the same for both cell lines. *p value = 0 indicates p < 1E-12.

Gene	Log FC	p value*
ACKR3	-4.0	6.2E-15
ACTG2	-2.8	0
ADM	3.5	0
ALDH1A3	2.3	0
AMOTL2	-2.9	0.00069
BCL2	2.8	6.3E-05
BCL2A1	2.5	4.0E-14
CA9	28.2	6.2E-07
CD22	5.7	0
DCN	2.2	0
DOCK2	-2.0	0.00064
E2F2	-2.6	0.020
EDN1	-4.4	0
EGLN3	26.1	0.014
ERBB3	3.3	0
ESR2	2.0	0
FAS	2.2	5.5E-07
FGF7	28.2	4.9E-08
GPNMB	3.1	0
GREM1	-2.2	0
HGF	5.7	0
HLA-DQA1	27.7	2.0E-08
HLA-DRB1	2.6	0
HSD11B1	34.7	7.0E-07
ICAM1	4.2	0
IDO1	26.7	0.0062
IL11	-2.1	0
IL6	-3.0	0
INHBA	-2.7	0
ITGA5	-2.0	0
ITGA8	7.8	0
ITGB8	2.3	0
ITPR3	28.1	2.4E-08
JAG1	-2.8	0
KAT2B	3.0	0
KLRD1	28.4	0.0044
LCN2	5.9	0
LYN	2.6	0.0020
MDM2	2.2	0
NOTCH3	-2.9	4.4E-13
OAS1	3.8	0.0021
PIK3R1	2.1	0
PLAU	-2.2	0
PLEK2	-4.6	3.3E-05
PLK1	-2.1	0
PTCH1	2.3	3.6E-06
SERPINE1	-2.1	0
SHH	24.7	0.038
SNAI2	-3.1	0
SOCS1	-2.5	0
TCF7	-2.1	0.0024

Gene	Log FC	p value*
TMPRSS2	-28.9	0.034
TNFSF11	3.8	0
TNFSF13B	19.9	0.00070
VEGFD	26.4	0.017

Supplementary Video 1. Time lapse video of U87 mono-sphere after gel encapsulation. Scale Bar = 100 μm.