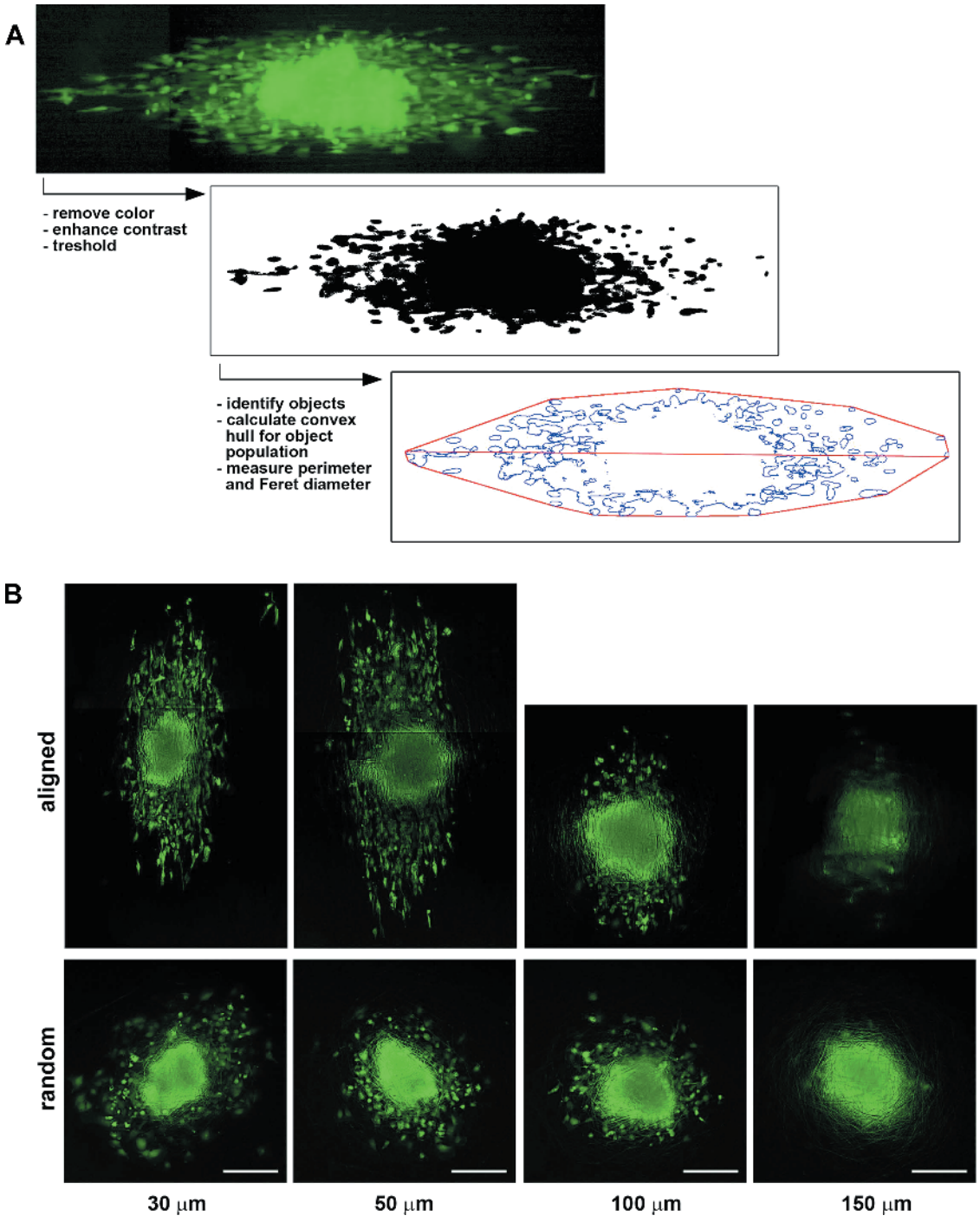

Figure W1. Analysis of cell migration on nanofiber scaffolds. (A) Images captured by fluorescence microscopy were analyzed using ImageJ software to quantify cell dispersion from the original spheroid. The maximum dispersion distance is the Feret diameter of the whole cell population at each time point. (B) Representative image of U251 glioma cells dispersing out of aggregates cultured on nanofiber scaffolds of different thickness (30-150 μm). Notice the elongated profile of cell dispersion on highly aligned nanofibers. Bars, 200 μm.

Table W1. Gene Up-regulation in Glioma Cells Migrating on Aligned Nanofibers.

Gene Symbol	Entrez Gene Name	Location	Type(s)	Fold Change	*P*
IL8	**Interleukin 8**	**Extracellular space**	**Cytokine**	**3.450**	**2.38*e* – 03**
KLF2	Kruppel-like factor 2 (lung)	Nucleus	Transcription regulator	3.080	2.98*e* – 03
CTH	Cystathionase (cystathionine gamma-lyase)	Cytoplasm	Enzyme	2.780	8.53*e* – 03
BTBD11	BTB (POZ) domain containing 11	Nucleus	Transcription regulator	2.610	4.59*e* – 04
TSLP	**Thymic stromal lymphopoietin**	**Extracellular space**	**Cytokine**	**2.490**	**9.96*e* – 03**
CBS	Cystathionine-β-synthase	Cytoplasm	Enzyme	2.450	6.64*e* – 03
TMEM158	Transmembrane protein 158 (gene/pseudogene)	Plasma membrane	Other	2.410	1.49*e* – 03
SPHK1	**Sphingosine kinase 1**	**Cytoplasm**	**Kinase**	**2.370**	**4.99*e* – 03**
MGLL	Monoglyceride lipase	Plasma membrane	Enzyme	2.340	4.76*e* – 03
NT5E	5′-Nucleotidase, ecto (CD73)	Plasma membrane	Phosphatase	2.330	1.03*e* – 03
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	Cytoplasm	Enzyme	2.300	5.85*e* – 03
CXCL3	Chemokine (C-X-C motif) ligand 3	Extracellular space	Cytokine	2.250	4.44*e* – 04
PPP1R15A	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	Cytoplasm	Other	2.210	7.16*e* – 03
CD55	CD55 molecule, decay accelerating factor for complement	Plasma membrane	Other	2.140	7.77*e* – 03
TRIB3	Tribbles homolog 3 (*Drosophila*)	Nucleus	Kinase	2.030	7.96*e* – 03
ALDH1A3	Aldehyde dehydrogenase 1 family, member A3	Cytoplasm	Enzyme	2.020	2.27*e* – 03
NAV3	Neuron navigator 3	Unknown	Other	1.990	8.22*e* – 04
CAMK2N1	Calcium/calmodulin–dependent protein kinase II inhibitor 1	Plasma membrane	Kinase	1.970	1.77*e* – 03
PSAT1	Phosphoserine aminotransferase 1	Cytoplasm	Enzyme	1.940	4.10*e* – 03
DUSP5	**Dual-specificity phosphatase 5**	**Nucleus**	**Phosphatase**	**1.930**	**2.05*e* – 03**
HBEGF	**Heparin-binding EGF-like growth factor**	**Extracellular space**	**growth factor**	**1.920**	**2.89*e* – 03**
RGMB	RGM domain family, member B	Plasma membrane	Other	1.900	2.42*e* – 04
CXCL2	**Chemokine (C-X-C motif) ligand 2**	**Extracellular space**	**Cytokine**	**1.890**	**1.29*e* – 03**
ADAMTS6	ADAM metallopeptidase with thrombospondine type 1 motif, 6	Extracellular space	Peptidase	1.840	5.77*e* – 03
HMGA2	High-mobility group AT-hook 2	Nucleus	Other	1.840	1.64*e* – 03
EDG1	Sphingosine-1-phosphate receptor 1	Plasma membrane	G protein–coupled receptor	1.820	3.25*e* – 04
MICAL2	Microtubule-associated monoxygenase, calponin and LIM domain containing 2	Cytoplasm	Other	1.800	1.18*e* – 03
CCND1	**Cyclin D1**	**Nucleus**	**Other**	**1.780**	**6.51*e* – 03**
EPHB2	EPH receptor B2	Plasma membrane	Kinase	1.780	1.01*e* – 03
SERPINE1	**Serpine peptidase inhibitor, clade E, member 1**	**Extracellular space**	**Other**	**1.770**	**8.82*e* – 03**
DDR2	Discoidin domain receptor tyrosine kinase 2	Plasma membrane	Kinase	1.720	3.38*e* – 03
PTHLH	Parathyroid hormone-like hormone	Extracellular space	Other	1.710	1.39*e* – 03
EMP1	Epithelial membrane protein 1	Plasma membrane	Other	1.690	4.14*e* – 03
FAM132B	Family with sequence similarity 132, member B	Unknown	Other	1.680	2.48*e* – 03
FRMD6	FERM domain containing 6	Cytoplasm	Other	1.680	4.08*e* – 03
SRGAP1	SLIT-ROBO Rho GTPase activating protein 1	Unknown	Other	1.680	6.70*e* – 04
KCNMA1	Potassium large conductance calcium-activated channel, subfamily M, alpha member 1	Plasma membrane	ion channel	1.670	9.85*e* – 04
PTPN22	Protein tyrosine phosphatase, nonreceptor type 22 (lymphoid)	Cytoplasm	Phosphatase	1.670	1.35*e* – 03
RND3	**Rho family GTPase 3**	**Cytoplasm**	**Enzyme**	**1.660**	**1.73*e* – 03**
BCAR3	Breast cancer antiestrogen resistance 3	Cytoplasm	Other	1.650	3.80*e* – 03
FLI32255	Hypothetical protein Loc643977	Unknown	Other	1.650	3.90*e* – 03
ANKRD55	Ankyrin repeat domain 55	Nucleus	Transcription regulator	1.620	2.20*e* – 03
GADD45A	Growth arrest and DNA damage–inducible, alpha	Nucleus	Other	1.620	2.25*e* – 03
PDGFA	Platelet-derived growth factor alpha polypeptide	Extracellular space	Growth factor	1.620	9.14*e* – 04
CD274	CD274 molecule	Plasma membrane	Other	1.610	4.15*e* – 04
PDCD1LG2	Programmed cell death 1 ligand 2	Plasma membrane	Other	1.610	3.80*e* – 03
F3	Coagulation factor III (thromboplastin, tissue factor)	Plasma membrane	Transmembrane receptor	1.600	1.74*e* – 03
MTHFD2	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2	Cytoplasm	Enzyme	1.600	7.73*e* – 03
DNER	Delta/notch–like EGF repeat containing	Plasma membrane	Transmembrane receptor	1.590	1.49*e* – 03
CEBPG	CCAAT/enhancer binding protein (C/EBP), gamma	Nucleus	Transcription regulator	1.580	6.97*e* – 03
FAM40B	Family with sequence similarity 40, member B	Unknown	Other	1.580	9.61*e* – 03
PIK3CD	**Phosphoinositide-3-kinase, catalytic, delta polypeptide**	**Cytoplasm**	**Kinase**	**1.570**	**2.64*e* – 03**
ARHGEF2	Rho/Rac guanine nucleotide exchange factor (GEF) 2	Cytoplasm	Other	1.560	5.52*e* – 03
STX1A	Syntaxin 1A (brain)	Cytoplasm	Transporter	1.560	3.76*e* – 03
TRIB1	Tribbles homolog 1 (*Drosophila*)	Cytoplasm	Kinase	1.560	2.19*e* – 03
ENOX1	Ecto-NOX disulfide-thiol exchanger 1	Unknown	Other	1.550	1.25*e* – 03
TMCC3	Transmembrane and coiled-coil domain family 3	Unknown	Other	1.550	1.42*e* – 03
GARS	Glycyl-tRNA synthetase	Cytoplasm	Enzyme	1.540	5.75*e* – 03
PIK3AP1	Phosphoinositide-3-kinase adaptor protein 1	Cytoplasm	Other	1.540	3.76*e* – 03
USP53	Ubiquitin-specific peptidase 53	Unknown	Enzyme	1.540	5.53*e* – 04
SLC38A1	Solute carrier family 38, member 1	Plasma membrane	Transporter	1.530	3.05*e* – 03
DUSP4	Dual-specificity phosphatase 4	Nucleus	Phosphatase	1.520	1.73*e* – 03
FOXQ1	Forkhead box Q1	Nucleus	Transcription regulator	1.520	1.51*e* – 03
IL11	**Interleukin 11**	**Extracellular space**	**Cytokine**	**1.520**	**8.34*e* – 04**

Table W1. (*continued*)

Gene Symbol	Entrez Gene Name	Location	Type(s)	Fold Change	*P*
MYADM	Myeloid-associated differentiation marker	Nucleus	Other	1.520	4.26*e* – 03
MB2	Metastasis-related protein mb2	Unknown	Other	1.520	5.95*e* – 03
AKAP12	**A kinase (PRKA) anchor protein 12**	**Cytoplasm**	**Transporter**	**1.510**	**6.02*e* – 03**
PHACTR2	Phosphatase and actin regulator 2	Unknown	Other	1.510	5.86*e* – 03
RASA3	RAS p21 protein activator 3	Plasma membrane	Other	1.510	1.01*e* – 04
SARS	Seryl-tRNA synthetase	Cytoplasm	Enzyme	1.510	8.21*e* – 03
FOSL1	**FOS-like antigen 1**	**Nucleus**	**Transcription regulator**	**1.510**	**1.60*e* – 03**
KLF6	Kruppel-like factor 6	Nucleus	Transcription regulator	1.510	6.73*e* – 04
RGS7	Regulator of G protein signaling 7	Cytoplasm	Enzyme	1.500	8.44*e* – 04
GEM	GTP binding protein overexpressed in skeletal muscle	Plasma membrane	Enzyme	1.500	7.04*e* – 04
IRAK2	Interleukin-1 receptor–associated kinase 2	Plasma membrane	Kinase	1.500	1.93*e* – 03
SH2D5	SH2 domain containing 5	Unknown	Other	1.500	1.76*e* – 03
WIPF3	WAS/WASL interacting protein family, member 3	Plasma membrane	Other	1.500	4.22*e* – 03

Summary of microarray data analysis (Gene Expression Omnibus data set GSE28167) showing messenger RNA up-regulation (>1.5 fold) in U251 glioma cells migrating on aligned *versus* randomly oriented nanofibers. Genes in bold face emphases have been described in the literature as regulators or targets of JAK/STAT signaling [24–26]. Results were analyzed by BRB-Array Tools software using a modified *t* test with random variance ($P < .05$ indicates statistically significant differences).

Table W2. Gene Ontology Analysis Identifies a Cell Motility Signature Upregulated in Glioma Cells Migrating on Aligned Nanofibers.

(A) Ingenuity Pathway Analysis (Ingenuity Systems)

Top Functional Networks

ID	Score	Top Functions	Upregulated Genes Associated with This Network
1	52	**Cellular movement**, hematological system development and function, hematopoiesis	*ALDH1A3*, *ARHGEF2*, *ASNS*, *CD55*, *CD274*, *CEBPG*, *CTH*, ***CXCL2***, *CXCL3*, ***DUSP5***, *F3*, *GARS*, ***HMGA2***, ***IL11***, ***IL8***, *IRAK2*, *KLF2*, *MTHFD2*, *PDGFA*, *PSAT1*, ***RND3***, ***SERPINE1***, ***SPHK1***, *TRIB3*, ***TSLP***
2	40	Tissue morphology, **cellular movement**, skeletal and muscular system development and function	***AKAP12***, *CAMK2N1*, ***CCND1***, *DDR2*, *DNER*, *DUSP4*, *EMP1*, *EPHB2*, ***FOSL1***, *GEM*, ***IL11***, *KLF6*, *PDGFA*, ***PIK3CD***, *PTHLH*, *RGS7*, *TMEM158*, *TRIB1*
3	23	Cellular development, cellular growth and proliferation, cellular function and maintenance	*BTBD11*, *DNER*, *FAM40B*, *FOXQ1*, *FRMD6*, *MGLL*, *PHACTR2*, *PTPN22*, *RASA3*, *SARS*, *SH2D5*, *USP53*
4	20	Immunologic disease, inflammatory disease, renal nephritis	*BCAR3*, *CBS*, *GADD454*, ***HBEGF***, ***IL11***, *KCNMA1*, *MICAL2*, *NT5E*, *PDCD1LG2*, *PIK3AP1*, *PPP1R15A*, *PTPN22*, *STX1A*
5	9	Cell death, cellular development, cellular growth and proliferation	*ENOX1*, *NAV3*, *RGS7*, ***SERPINE1***, *SLC38A1*, *SRGAP1*, *WIPF3*

(B) DAVID Bioinformatics Resources (NIH/NIAID):

Top Annotation Clusters

Annotation Cluster 1: Enrichment Score: 2.4295

GO Category	Term	Upregulated Genes Associated with This Cluster	*P*	Fold Enrichment
GOTERM_BP_FAT	GO:0040017~**positive regulation of locomotion**	***IL8***, *PDGFA*, *F3*, ***SPHK1***, ***HBEGF***	.00081	11.6984
GOTERM_BP_FAT	GO:0042060~wound healing	*KLF6*, *PDGFA*, *F3*, ***SERPINE1***, ***HBEGF***, ***IL11***	.00133	7.2028
GOTERM_BP_FAT	GO:0032101~regulation of response to external stimulus	***IL8***, *PDGFA*, *F3*, ***SERPINE1***, *NT5E*	.00476	7.2103
GOTERM_BP_FAT	GO:0030193~regulation of blood coagulation	*PDGFA*, *F3*, ***SERPINE1***	.01036	19.1073
GOTERM_BP_FAT	GO:0050818~regulation of coagulation	*PDGFA*, *F3*, ***SERPINE1***	.01330	16.7772

Annotation Cluster 2: Enrichment Score: 2.26307

GO Category	Term	Genes	*P*	Fold Enrichment
GOTERM_BP_FAT	GO:0040017~**positive regulation of locomotion**	***IL8***, *PDGFA*, *F3*, ***SPHK1***, ***HBEGF***	.00081	11.69834
GOTERM_BP_FAT	GO:0042060~wound healing	*KLF6*, *PDGFA*, *F3*, ***SERPINE1***, ***HBEGF***, ***IL11***	.00133	7.2028
GOTERM_BP_FAT	GO:0040012~**regulation of locomotion**	***IL8***, *PDGFA*, *F3*, ***SPHK1***, ***HBEGF***, *TRIB1*	.00136	7.1652
GOTERM_BP_FAT	GO:0030334~**regulation of cell migration**	*PDGFA*, *F3*, ***SPHK1***, ***HBEGF***, *TRIB1*	.00590	6.7836
GOTERM_BP_FAT	GO:0030335~**positive regulation of cell migration**	*PDGFA*, *F3*, ***SPHK1***, ***HBEGF***	.00654	10.3051
GOTERM_BP_FAT	GO:0051272~**positive regulation of cell motion**	*PDGFA*, *F3*, ***SPHK1***, ***HBEGF***	.00852	9.3587
GOTERM_BP_FAT	GO:0051270~**regulation of cell motion**	*PDGFA*, *F3*, ***SPHK1***, ***HBEGF***, *TRIB1*	.00935	5.9401
GOTERM_CC_FAT	GO:0009986~cell surface	*KCNMA1*, *IRAK2*, *PDGFA*, *F3*, *CD274*, ***HBEGF***	.01544	4.0069
PANTHER_PATHWAY	P00005:Angiogenesis	*PDGFA*, *DNER*, *F3*, ***PIK3CD***, ***SPHK1***, *EPHB2*	.06108	2.6626

Genes upregulated in U251 glioma cells migrating on aligned *versus* randomly oriented nanofibers (Table W1) were analyzed using Ingenuity Pathway Analysis software (A) and DAVID bioinformatics resources (B). Results from both analyses revealed a cluster of genes associated with increased cell locomotion. Genes in bold face emphases have been described in the literature as targets and/or regulators of JAK/STAT signaling.

Table W3. List of Primers Used for Quantitative RT-PCR.

Gene	Forward Sequence 5′ → 3′	Reverse Sequence 5′ → 3′
CAMK2N1	TATTGAAGATGATAGGATTG	AATTTTGACAAATAGCTGCA
CAPS	AGCTGCATTTGCCAAGCTGG	GCTGTAGTAGTCCTGGAATT
CCND1	GCGGRAGTAGGACAGGAAGTT	GAACAAACAGATCATCCGCAAAC
CXCL2	ACTGAACTGCGCTGCCAGTG	TTCTGCCCATTCTTGAGTGT
CXCL3	TCAAGAACATCCAAAGTGTG	TTGTTCAGTATCTTTTCGAT
EDG1	TATGCCAGTATGCTCTGGCT	CGATGAGTGATCCAGGCTTT
EDNRB	ATGTGTAAGCTGGTGCCTTT	TTTGGAACCCCAATTCCTTT
EMP1	ATGGAGAAGGGAAACCGGTT	GATACTGCGTTCCATCACGA
IL-8	AGACATACTCCAAACCTTTC	TCTGCACCCAGTTTTCCTTG
IL-11	AAATTCCCAGCTGACGGGGA	AAATAAATAAGATCTGGCTTTG
KLF2	GACTTAGGGTGGTAAAGGC	CATGGACAGGATGAACTCCA
MMP2	CCATCGAGACCATGCGGAAG	CCTGTATGTGATCTGGTTCTTG
MMP9	TCATCCAGTTTGGTGTCGCG	GACCACAACTCGTCGTCGTC
MMP13	GAGGTGACTGGCAAACTTGA	ATATCAGGGGTGTAATTCAC
PARP9	AAATGTCCTGTGCCTCCAAC	ACTCTGCATACCACATTGCA
PDGFA	AGTGAGGATTCTTTGGACCAC	TTGACACTGCTCGTGTTGCA
PDGFRA	TACCAGGGAGGTCAAAGAAA	TTCCTGAATCTTTTCCACAT
PI15	GATTATGCTTTTCCATATCCC	ATCCTATCCGATTGGAAGTG
RND3	AATAGAGTTGAGCCTGTGGG	ATCAGACTTGCAGCCGACCA
SERPINE1	TTTCAGAGGTGGAGAGAGCC	AAGGGAGTCTTCCACTGGCC
SESN3	GTTCATGTCAATCTACTTTT	TCATGATTTATGATCAGTAT
SOX4	TAGTTCTTGCACGCTCTTTA	TTCCCTGAAGCAGTTGATTC
SOX13	AAGGAGCTCCAGCTTCTGGT	AGGAGGTTGATCTTATGCTG
SOX21	AAGATGCACAACTCGGAGAT	CGACGAGATCTCTGCCATTT
SPHK1	CTGGTGGTCATGTCTGGAGA	CAGCAATAGCGTGCAGTTGG
STC1	TTGCATGCCTGGAAAACTCC	CCGTTGGCGATGCATTTTAA
STX1A	TCAAGTACCAGAGCAAGGC	GCAGATGATGATCATGATTT
VANGL2	AGCGTCGCTGGATTTTCTCT	ATCTCGACTCTTAGAGCGGT

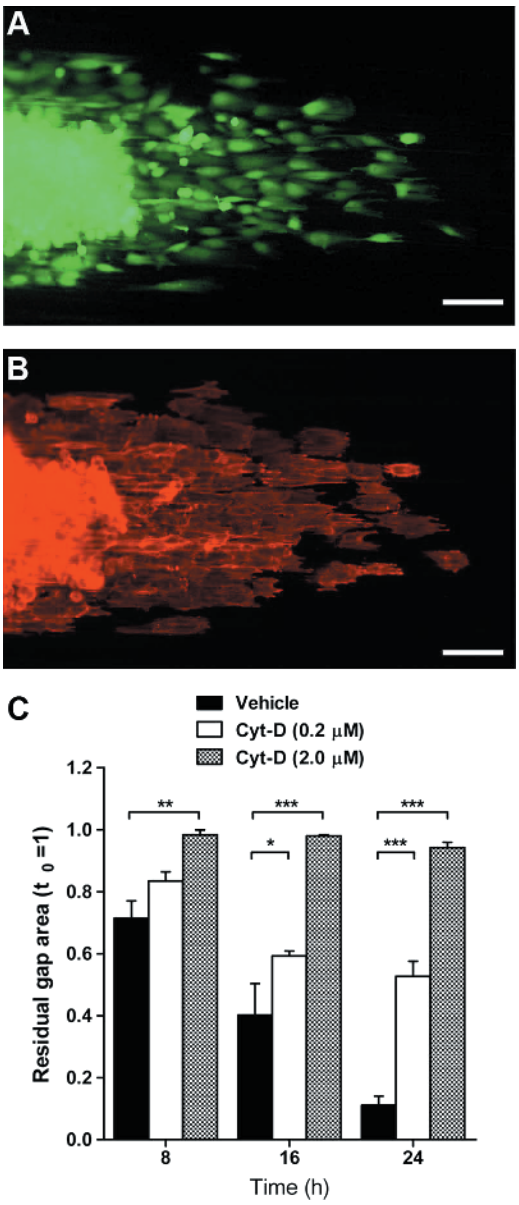

Figure W2. Inhibition of actin polymerization disrupts two-dimensional glioma cell migration. (A) Representative image of unfixed, CMFDA-labeled U251 glioma cells migrating out of a spheroid deposited on aligned nanofibers. (B) Same cells from A, imaged after fixation and staining with phalloidin–Alexa 594 to detect actin F. The staining was mostly cortical and diffuse. Bars, 100 μm. (C) Effect of cytochalasin D (Cyt-D) on the migration of U251 cells, measured using a wound healing assay. Cell migration was significantly inhibited at 2.0 μM Cyt-D and completely abolished at 2 μM due in part to cell detachment. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ by two-way ANOVA for repeated measures.

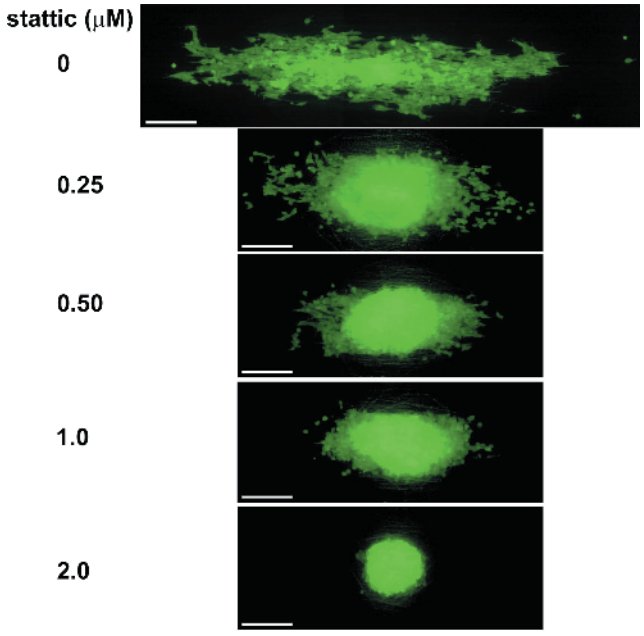

Figure W3. STAT3 inhibitors disrupt glioma cell migration. Representative images of G9 spheroids (glioblastoma-derived initiating cells) cultured on aligned nanofibers scaffolds in the presence of increasing concentrations of the STAT3 inhibitor stattic. Notice the reduction of cell dispersion along the major axis of the scaffold, without a noticeable decrease in fluorescence (ratio of integrated fluorescence/area remained approximately constant). Bars, 200 μm.

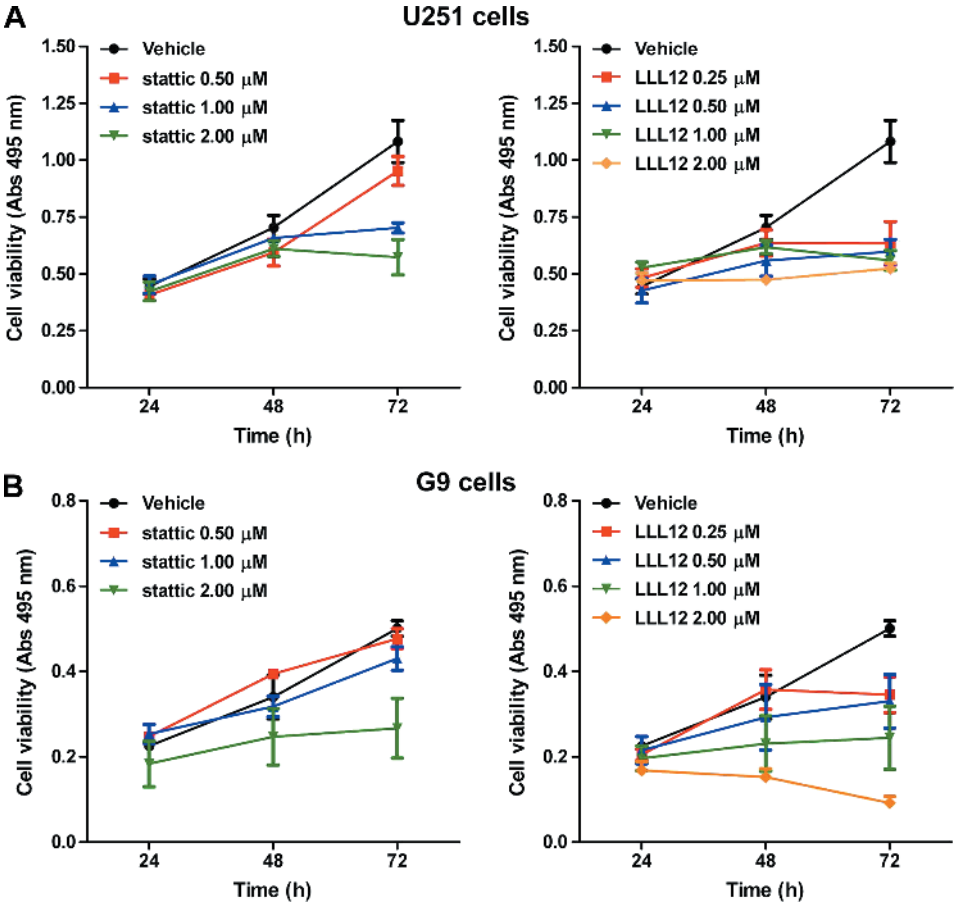

Figure W4. STAT3 inhibitors do not disrupt cell viability at early culture times. U251 (A) and G9 (B) glioma cell were cultured on aligned nanofiber for 24 to 72 hours in the presence of the STAT3 inhibitors stattic and LLL12. Toxicity of these compounds was measured using an assay for metabolic reduction of tetrazolium. Results showed that neither stattic nor LLL12 reduced cell viability in the conditions at which they inhibited cell migration (0.5-2 μM for up to 24 hours). Negative effects on cell viability were observed only at the highest concentrations tested and longer incubation times (>48 hours).

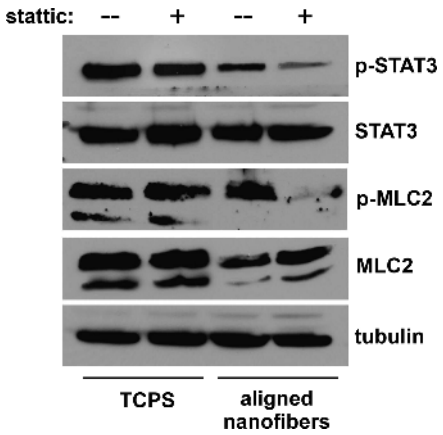

Figure W5. STAT3 inhibition reduces MLC2 phosphorylation of cells cultured on aligned nanofibers. U251 glioma cells were cultured on aligned nanofibers or TCPS for 24 hours in the presence of 1 μM stattic, collected, and processed for Western blot analysis. Results showed that a low concentration of the STAT3 inhibitor partially reduced STAT3 phosphorylation in cells of myosin II, MLC2. In contrast, neither STAT3 nor MLC2 phosphorylation was affected by the same treatment when cells were cultured on TCPS.

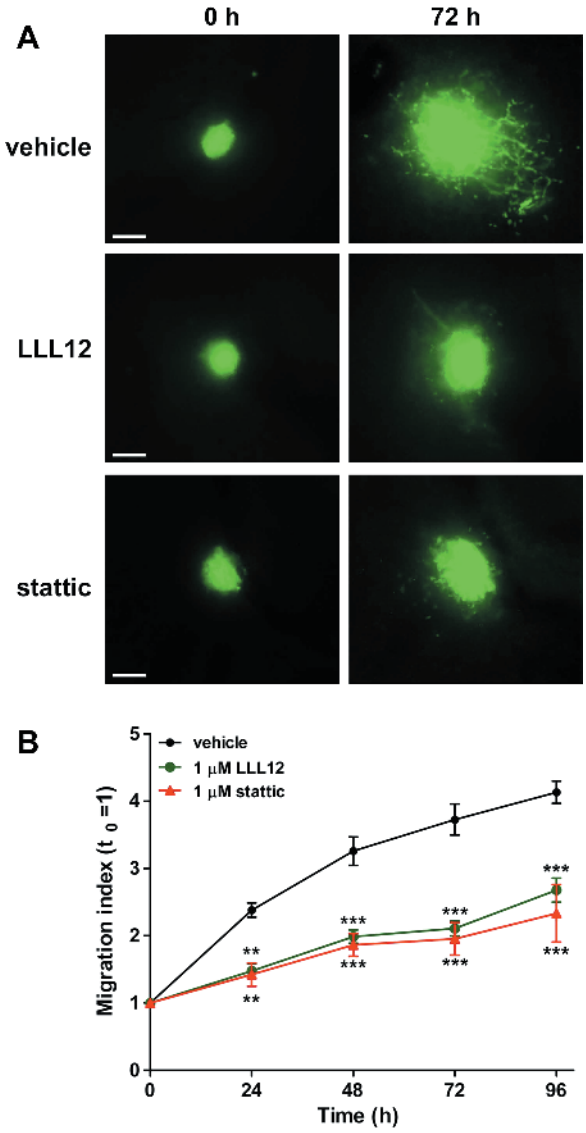

Figure W6. STAT3 inhibition reduces cell dispersion in cultured brain slices. G9 glioma cells were treated with 1 μM stattic of 1 μM LLL12 overnight and deposited on brain slices prepared as described in the Materials and Methods section. Dispersion of the cells in the tissue slice was followed by fluorescence microscopy for 96 hours. (A) Cell migration followed a pattern of dispersion with typical trails of cells dispersing out of tumorspheres, which was abolished by the pharmacological treatments. (B) Quantitative results indicated that cell dispersion had been significantly reduced by treatment with low concentrations of the STAT3 inhibitors, in agreement with the results observed using nanofiber scaffolds. $^{**}P < .01$, $^{***}P < .001$ by two-way ANOVA for repeated measures. Bars, 200 μm.