

Supplementary Information for

**Physical confinement alters sarcoma cell cycle progression and division**

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**Supplementary Table S1: Cell divisions decrease in confinement.** The mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for the fraction of cells that divide across varying degrees of confinement. Groups that do not share a letter are significantly different.

Fraction that Divide			
Degree of Confinement	Mean	Cell Count	ANOVA
2D	0.7	22	A
50 $\mu\text{m}$	0.23	131	B
20 $\mu\text{m}$	0.147	227	BC
10 $\mu\text{m}$	0.155	99	C
6 $\mu\text{m}$	0.096	202	CD
3 $\mu\text{m}$	0.05	160	D

**Supplementary Table S2: Cell and nuclear morphologies are altered in confinement.** The mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for cell area (Fig. 2D), cell aspect ratio (Fig. 2G), nuclear area (Fig. 2E), and nuclear aspect ratio (Fig. 2H) across all degrees of confinement and 2D controls. Groups in a column that do not share a letter are significantly different.

		Cell Area			Cell Aspect Ratio			Nuclear Area			Nuclear Aspect Ratio		
Cell Cycle Stage	Degree of Confinement	Mean	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value
G1	2D	958.27	249	A	3.33	249	GH	171.91	208	E	1.92	208	G
	50 $\mu$ m	468.91	144	EFG	3.17	144	GH	175.58	203	E	1.97	203	FG
	20 $\mu$ m	440.73	113	EFG	4.73	101	FG	196.89	138	DE	2.28	138	FG
	10 $\mu$ m	412.21	78	EFG	6.74	78	F	183.38	67	E	2.85	67	F
	6 $\mu$ m	328.99	99	G	15.88	99	D	169.92	135	EF	7.1	135	D
	3 $\mu$ m	576.61	87	CDE	27.65	87	B	172.72	94	EF	11.11	94	B
S/G2/M	2D	1460.17	246	B	2.56	246	H	284.43	194	B	1.83	194	G
	50 $\mu$ m	759.23	101	C	3.29	101	GH	305.81	93	B	1.93	93	FG
	20 $\mu$ m	683.2	63	CD	4.98	63	FG	356.93	72	A	2.19	72	FG
	10 $\mu$ m	541.04	67	DEF	9.09	67	E	279.84	72	BC	4.1	72	E
	6 $\mu$ m	465.94	60	DEFG	19.47	60	C	239.15	59	CD	10.05	59	C
	3 $\mu$ m	345.36	87	FG	32.83	87	A	135.09	80	F	15.96	80	A

**Supplementary Table S3: Nuclear morphology is altered in confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for the major (Fig. 2J) and minor (Fig. 2K) axis of the fitted nuclear ellipse across varying degrees of confinement and cell cycle stages. Groups in a column that do not share a letter are significantly different.

Nuclear Axis Length	Degree of Confinement	G1			S/G2/M		
		Mean	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value
Major	2D	11.13	150	E	13.8	138	C
	50 $\mu\text{m}$	13.52	66	D	20.05	46	B
	20 $\mu\text{m}$	15.84	95	D	22.72	61	B
	10 $\mu\text{m}$	20.26	38	C	28.03	61	A
	6 $\mu\text{m}$	28.91	120	B	22.49	56	B
	3 $\mu\text{m}$	35.36	94	A	28.03	80	A
Minor	2D	5.86	150	FG	7.31	138	E
	50 $\mu\text{m}$	6.66	66	F	10.42	46	D
	20 $\mu\text{m}$	7.26	95	F	10.51	61	D
	10 $\mu\text{m}$	7.3	38	F	6.74	61	E
	6 $\mu\text{m}$	4.11	120	GH	2.36	56	F
	3 $\mu\text{m}$	3.37	94	H	1.83	80	F

**Supplementary Table S4: Cell migration is altered in confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for cell speed (Fig. 3B) and chemotactic index (Fig. 3C) of both cell cycle stages across varying degrees of confinement. Groups in a column that do not share a letter are significantly different.

Cell Cycle Stage	Degree of Confinement	Cell Speed			Chemotactic Index		
		Mean	Count	ANOVA Value	Mean	Count	ANOVA Value
G1	2D	38.84	178	G	0.504	178	DE
	50 $\mu\text{m}$	115.22	162	B	0.51	163	DE
	20 $\mu\text{m}$	131.4	136	A	0.579	136	CDE
	10 $\mu\text{m}$	104.55	52	BCD	0.767	52	AB
	6 $\mu\text{m}$	100.61	51	BCD	0.634	51	ABCD
	3 $\mu\text{m}$	61.87	86	EF	0.729	86	AB
S/G2/M	2D	40.01	154	G	0.474	154	E
	50 $\mu\text{m}$	97.87	118	CD	0.531	118	DE
	20 $\mu\text{m}$	107.33	60	BC	0.611	60	BCDE
	10 $\mu\text{m}$	82.7	66	DE	0.718	66	ABC
	6 $\mu\text{m}$	55.2	64	FG	0.789	64	A
	3 $\mu\text{m}$	49.92	69	FG	0.739	69	AB

**Supplementary Table S5: S/G2/M stage is lengthened in confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) of time cell spends in the channel (Fig. 4A) across both cell cycle stages and varying degrees of confinement. Groups in a column that do not share a letter are significantly different.

	Time in Channel			
Cell Cycle Stage	Degree of Confinement	Mean	Cell Count	ANOVA
G1	2D	282	10	EF
	50 $\mu\text{m}$	316.92	13	F
	20 $\mu\text{m}$	338.57	7	F
	10 $\mu\text{m}$	290	10	F
	6 $\mu\text{m}$	298	10	F
	3 $\mu\text{m}$	317.78	9	F
S/G2/M	2D	480	7	DE
	50 $\mu\text{m}$	714.29	7	C
	20 $\mu\text{m}$	810.19	3	BC
	10 $\mu\text{m}$	650	2	CD
	6 $\mu\text{m}$	972.5	4	B
	3 $\mu\text{m}$	1206	5	A

**Supplementary Table S6: Cells continue to divide less frequently after exiting confinement.**

Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for the fraction of cells that divide during and after confinement (Fig. 5B) across varying channel widths. Groups that do not share a letter are significantly different.

	Fraction that Divide			
	Degree of Confinement	Mean	Cell Count	ANOVA
During Confinement	2D	0.7	22	A
	50 $\mu\text{m}$	0.23	131	BC
	20 $\mu\text{m}$	0.15	227	CDE
	10 $\mu\text{m}$	0.15	99	BCDE
	6 $\mu\text{m}$	0.1	202	DF
	3 $\mu\text{m}$	0.05	160	EF
Post-Confinement	50 $\mu\text{m}$	0.45	7	AB
	20 $\mu\text{m}$	0.24	10	BCDEF
	10 $\mu\text{m}$	0.22	13	BCDEF
	6 $\mu\text{m}$	0.2	10	BCDEF
	3 $\mu\text{m}$	0.25	29	BCD

**Supplementary Table S7: Cell cycle stage time returns to normal after cells leave confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for the time cells spend in each stage of the cell cycle, during and post confinement, across varying degrees of confinement (Fig. 5C). Groups that do not share a letter are significantly different.

	Cell Cycle Stage	Time in Channel			
		Degree of Confinement	Mean	Cell Count	ANOVA
During Confinement	G1	2D	282	10	F
	S/G2/M	2D	480	7	DE
	G1	50 $\mu\text{m}$	316.923	13	F
		20 $\mu\text{m}$	338.571	7	EF
		10 $\mu\text{m}$	290	10	F
		6 $\mu\text{m}$	298	10	F
		3 $\mu\text{m}$	317.778	9	EF
	S/G2/M	50 $\mu\text{m}$	714.286	7	C
		20 $\mu\text{m}$	810.187	3	BC
		10 $\mu\text{m}$	650	2	CD
		6 $\mu\text{m}$	972.5	4	B
		3 $\mu\text{m}$	1206	5	A
Post- Confinement	G1	50 $\mu\text{m}$	259.091	11	F
		20 $\mu\text{m}$	266.667	9	F
		10 $\mu\text{m}$	370	11	EF
		6 $\mu\text{m}$	318.889	9	EF
		3 $\mu\text{m}$	327.5	8	EF
	S/G2/M	50 $\mu\text{m}$	598.333	6	CD
		20 $\mu\text{m}$	728.333	6	B
		10 $\mu\text{m}$	755	6	BC
		6 $\mu\text{m}$	716.667	6	C
		3 $\mu\text{m}$	680	2	BCD



**Supplementary Table S8: Cell and nuclear area, but not shape are altered post-confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for cell area (Fig. 5D), cell aspect ratio (Fig. 5F), nuclear area (Fig. 5E), and nuclear aspect ratio (Fig. 5G) in both stages of the cell cycle and across varying degrees of confinement. Groups in a column that do not share a letter are significantly different.

			Cell Area			Cell Aspect Ratio			Nuclear Area			Nuclear Aspect Ratio		
	Cell Cycle Stage	Degree of Confinement	Mean	Cell Count	ANOVA Value	Hide	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value	Mean	Cell Count	ANOVA Value
	G1	2D	958.27	249	BC	3.33	249	HI	171.91	208	F	1.92	208	GH
	S/G2/M	2D	1460.17	246	A	2.56	246	HI	284.43	194	B	1.83	194	GH
During Confinement	G1	50 $\mu$ m	468.91	144	JKL	3.17	144	HI	175.58	203	F	1.97	203	GH
		20 $\mu$ m	440.73	113	JKL	4.73	101	G	196.89	138	EF	2.28	138	FG
		10 $\mu$ m	412.21	78	JKL	6.74	78	F	183.38	67	EF	2.85	67	F
		6 $\mu$ m	328.99	99	L	15.88	99	D	169.92	135	FG	7.1	135	D
		3 $\mu$ m	576.61	87	KL	27.65	87	B	172.72	94	FG	11.11	94	B
	S/G2/M	50 $\mu$ m	759.23	101	DEFG	3.29	101	GHI	305.81	93	B	1.93	93	GH
		20 $\mu$ m	683.2	63	EFGHI	4.98	63	FG	356.93	72	A	2.19	72	FGH
		10 $\mu$ m	541.04	67	HIJK	9.09	67	E	279.84	72	BC	4.1	72	E
		6 $\mu$ m	465.94	60	IJKL	19.47	60	C	239.15	59	CD	10.05	59	C
		3 $\mu$ m	345.36	87	GHIJ	32.83	87	A	135.09	80	GH	15.96	80	A
Post-Confinement	G1	50 $\mu$ m	659.085	152	GH	3.73	152	GH	131.195	142	EF	1.89	142	GH
		20 $\mu$ m	617.658	161	GHI	2.65	161	HI	120.362	170	H	1.63	170	H
		10 $\mu$ m	693.582	217	GH	2.92	217	HI	127.228	224	H	1.73	224	H
		6 $\mu$ m	638.496	165	GHI	2.89	165	HI	115.362	191	H	1.88	191	GH
		3 $\mu$ m	692.144	90	FGH	2.2	90	I	127.827	109	H	1.72	109	GH
	S/G2/M	50 $\mu$ m	833.568	134	CDEF	2.77	134	HI	182.34	133	EF	1.7	133	GH
		20 $\mu$ m	920.007	141	BCD	2.13	141	I	209.458	105	DE	1.73	105	GH
		10 $\mu$ m	869.395	123	CDE	2.61	123	HI	186.341	125	EF	1.79	125	GH
		6 $\mu$ m	850.348	121	CDEF	2.75	121	HI	181.163	126	EF	1.93	126	GH
		3 $\mu$ m	1057.62	72	B	2.19	72	HI	189.287	71	EF	1.81	71	GH

**Supplementary Table S9: Cell migration reverts back to an unconfined state post-confinement.** Mean, cell count, and ANOVA letters (from post-hoc Tukey tests) for cell speed (Fig. 5H) and chemotactic index (Fig. 5I) across both cell cycle stages and varying degrees of confinement. Groups in a column that do not share a letter are significantly different.

			Cell Speed			Chemotactic Index		
	Cell Cycle Stage	Degree of Confinement	Mean	Count	ANOVA Value	Mean	Count	ANOVA Value
	G1	2D	38.84	178	GHI	0.504	178	DEF
	S/G2/M	2D	40.01	154	FGH	0.474	154	EF
During Confinement	G1	50 $\mu$ m	115.22	162	B	0.51	163	DEF
		20 $\mu$ m	131.4	136	A	0.579	136	CDE
		10 $\mu$ m	104.55	52	BC	0.767	52	AB
		6 $\mu$ m	100.61	51	BCD	0.634	51	ABCD
		3 $\mu$ m	61.87	86	E	0.729	86	AB
	S/G2/M	50 $\mu$ m	97.87	118	CD	0.531	118	DEF
		20 $\mu$ m	107.33	60	BC	0.611	60	BCDE
		10 $\mu$ m	82.7	66	D	0.718	66	ABC
		6 $\mu$ m	55.2	64	EF	0.789	64	A
		3 $\mu$ m	49.92	69	EFG	0.739	69	AB
Post-Confinement	G1	50 $\mu$ m	38.77073	150	GHI	0.559	150	DEF
		20 $\mu$ m	32.04606	199	HIJ	0.519	199	D
		10 $\mu$ m	29.16349	157	HIJ	0.544	157	DEF
		6 $\mu$ m	26.87913	162	J	0.57	162	DE
		3 $\mu$ m	28.47004	147	HIJ	0.525	147	DEF
	S/G2/M	50 $\mu$ m	35.25458	145	GHIJ	0.497	145	DEF
		20 $\mu$ m	28.38235	146	HIJ	0.519	146	DEF
		10 $\mu$ m	27.91323	149	IJ	0.509	149	DEF
		6 $\mu$ m	24.5743	131	J	0.506	131	DEF
		3 $\mu$ m	24.20096	132	J	0.449	132	F

### **Supplementary Movie Captions:**

**Supplementary Movie 1.** Example of abnormal division event in Fucci-expressing mouse sarcoma cell in 3  $\mu\text{m}$  wide fibronectin-coated channel. Cell divides into two daughter cells, and then fuses into one multinucleated cell. Total length of movie is 1000 minutes.

**Supplementary Movie 2.** Example of abnormal division event in Fucci-expressing mouse sarcoma cell in 6  $\mu\text{m}$  wide fibronectin-coated channel. Cell divides into two daughter cells, and then fuses into one multinucleated cell. Total length of movie is 830 minutes.

**Supplementary Movie 3.** Example of abnormal division event in Fucci-expressing mouse sarcoma cell in 20  $\mu\text{m}$  wide fibronectin-coated channel. Cell divides into three individual daughter cells. Total length of movie is 250 minutes.

**Supplementary Movie 4.** Example of normal division event in Fucci-expressing mouse sarcoma cell in 10  $\mu\text{m}$  wide fibronectin-coated channel. Total length of movie 380 minutes.