Biomechanical Regulation of Drug Sensitivity in an Engineered Model of Human Tumor

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Supplementary material

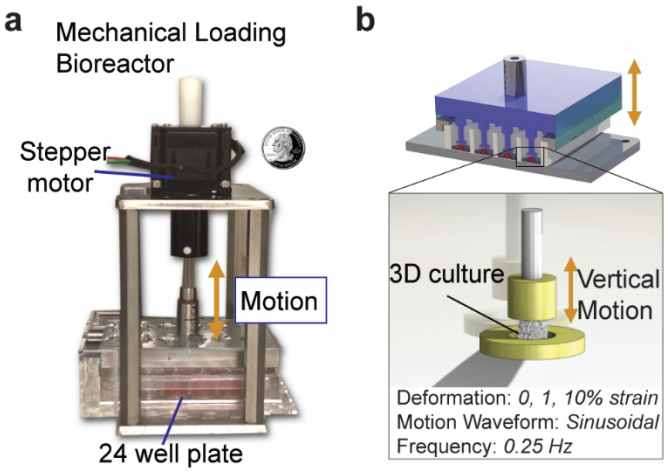

Fig.S1. Mechanical loading bioreactor overview. ***(a)*** Loading bioreactor designed to accommodate a standard tissue culture plate housing 24 samples. The motion is generated by a stepper motor and a linear actuator. ***(b)*** Experimental set-up and stimulation parameters.

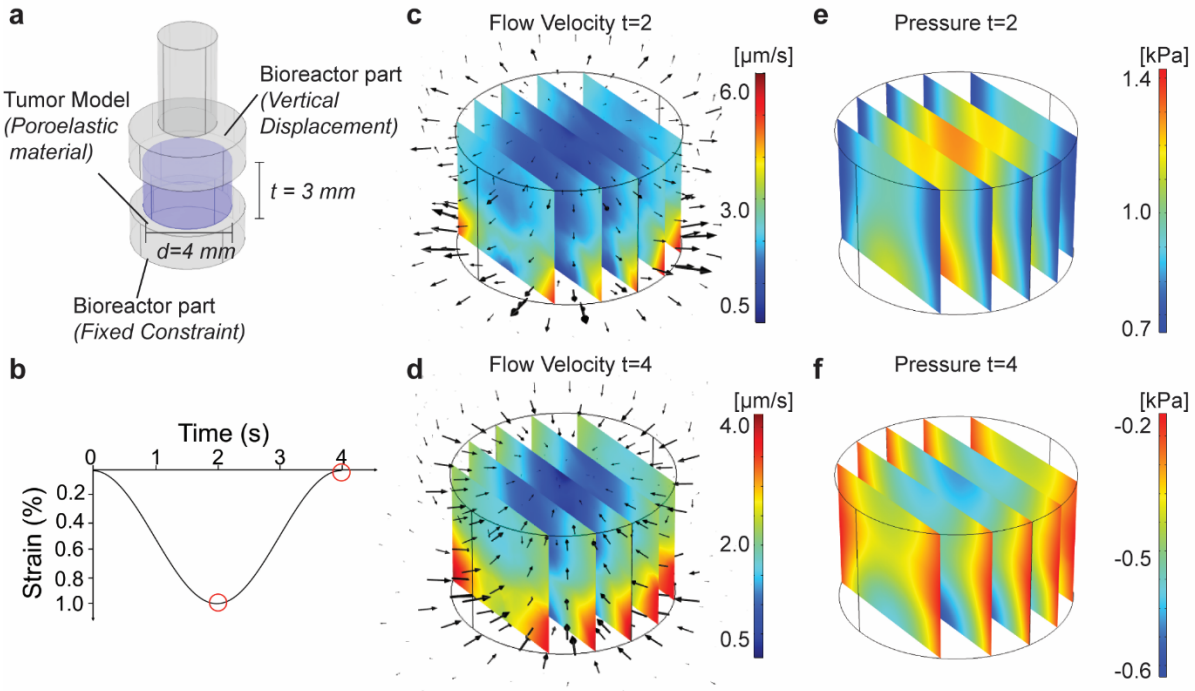

Fig.S2 Computational estimation of load-generated flow velocities and pressure. ***(a)*** Experimental setup, geometry and boundary conditions of the computational study. ***(b)*** Displacement waveform to generate 1% strain in the 3D model. ***(c,d)*** Flow velocities during loading (t=2s) and unloading (t=4s). ***(e,f)*** Pressure during loading (t=2s) and unloading (t=4s).

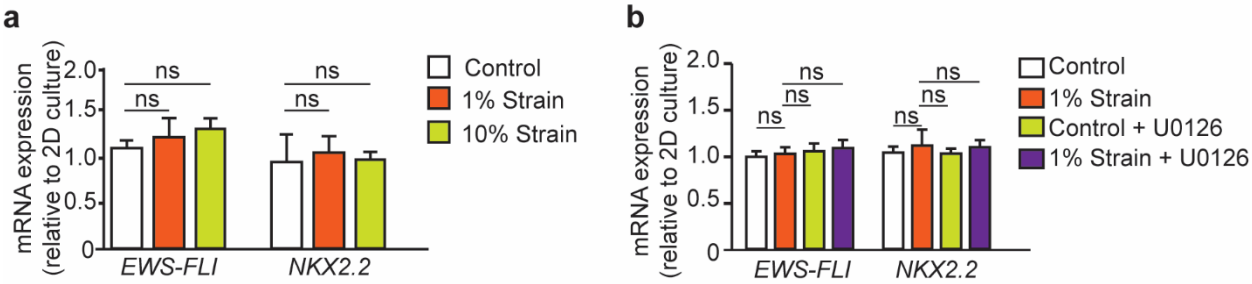

Fig.S3. Ewing sarcoma oncogene expression following mechanical stimulation. ***(a)*** qRT-PCR analysis of the ES oncogene *EWS-FLI* and its target *NKX2.2* mRNA levels, when cells were exposed to increased amplitudes of strain (1or 10%). ***(b)*** qRT-PCR analysis of the ES oncogene *EWS-FLI* and its target *NKX2.2* mRNA levels, when cells were exposed to 1% strain and cultured in the presence of the MEK1/2 inhibitor U0126 (10 μM).

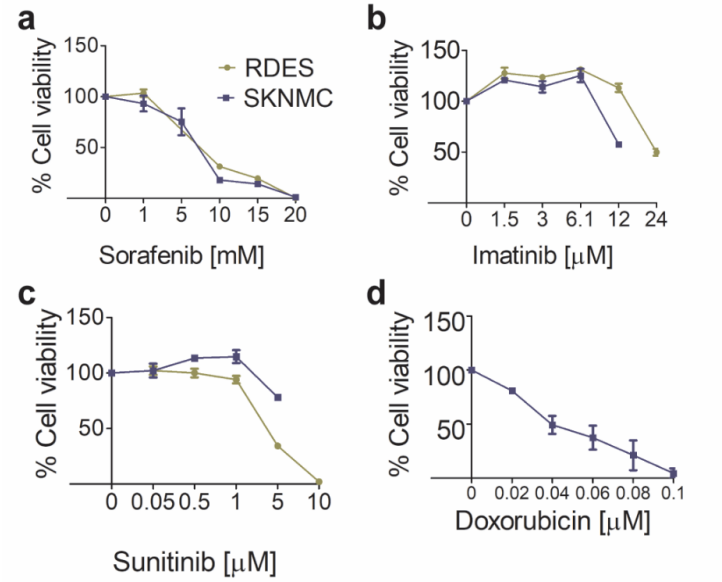

Fig.S4 ES cells lines drug sensitivity in 2D culture. ES cell lines SK-N-MC and RDES were exposed to increasing concentrations of sorafenib, sunitinib, imatinib and doxorubicin for 48 hours. Cell viability (MTA assay) is expressed as percentage of non-treated cells.

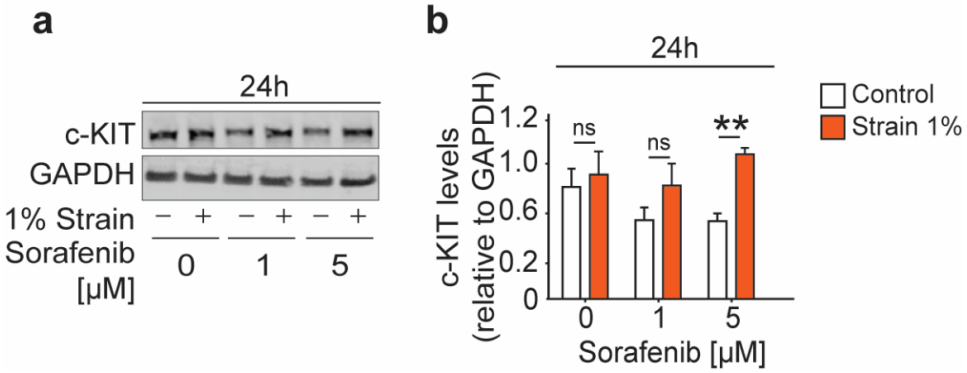

Fig.S5 Mechanical stimulation modulates c-KIT expression. ***(a)*** Western blot analysis showing c-KIT protein levels in ES cells exposed to mechanical stimulation (+) or in the controls (-), treated with sorafenib for 24 hours. ***(b)*** Quantified c-KIT protein levels represented as relative changes in band density normalized to GAPDH. Data is represented as average ± SD (n= 3; * $p<0.05$; ** $p<0.01$ line indicates statistical comparison between groups). P values are determined by Student's t-test (two-tailed).

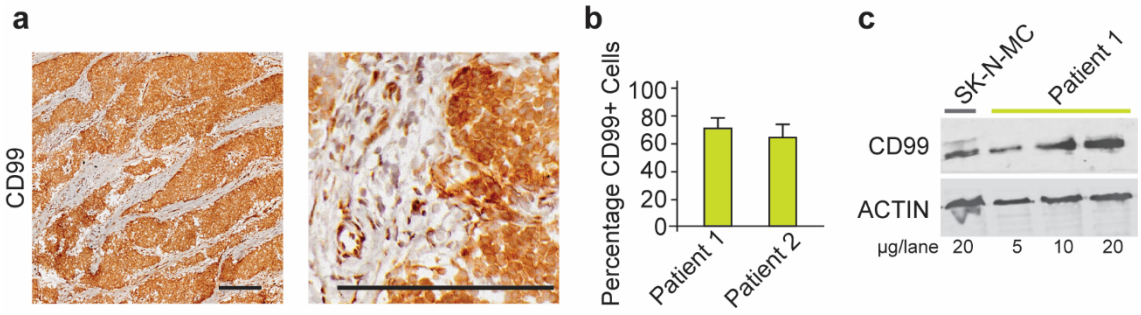

Fig. S6 Analysis of tumor versus stromal fraction in ES tumor samples.
(a) Histological analysis showing the expression of the diagnostic marker CD99 used to assess the presence of tumor cells with respect to stromal cells. Scale bars: 500 µm. ***(b)*** Quantification of CD99 positive fraction expressed as percentage area (n=3). **(c)** Western blot analysis showing CD99 protein levels in ES cell lines cultured in 2D compared to the levels in tumors. Protein extracted from ES tumors was loaded with increasing amounts (5, 10, 20 µg/lane), while ES cell lines protein was kept constant (20 µg/lane).

IC50 (µM)	Cell lines		PDX
	SK-N-MC	RD-ES	PS3
Sorafenib	6.5	7	22
Sunitinib	7	5	15
Imatinib	12	24	25
Doxorubicin	0.03	n/c	0.05

Table S1. IC50 values in 2D culture.

Sorafenib [µM]	Viable cells (%)		
	Strain	Control	2D culture
1	153.0 ± 9.0	107.9 ± 7.1	74.5 ± 2.5
5	116.8 ± 3.1	66.9 ± 3.8	54.6 ± 3.8
10	46.8 ± 2.0	37.2 ± 3.2	18.7 ± 4.5
15	2.6 ± 1.9	4.2 ± 3.0	9.8 ± 1.6
20	7.4 ± 2.0	1.8 ± 1.4	4.3 ± 0.3
IC50 [µM]	10	7.8	5

Table S2. ES cells drug sensitivity after 3D model dissociation

Gene	PrimerBank ID
EWS-FLI1 fusion isoform type 8 (EWS-FLI)	633772a1
NK2 homeobox 2 (NKX2-2)	32307133b1
Secreted phosphoprotein 1 (SPP1) ALIAS: OPN	38146097b1
Integrin-binding sialoprotein (IBSP) ALIAS: BSP	167466186b1
Matrix metallopeptidase 3 (MMP3)	73808272b2
Matrix metallopeptidase 9 (MMP9)	74272286b1
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	83641890b1
Parathyroid hormone-like hormone (PTHLH) ALIAS: PTHrP	39995087c1
Runt-related transcription factor 2 (RUNX2)	225690525c1

Table S3. List of primers

Description	Symbol	Value	reference
Scaffold thickness [mm]	t	3	This study
Scaffold diameter [mm]	h	4	This study
Porosity [%]	ε	0.85	(46)
Young's Modulus [kPa]	E	3	This study
Poisson's ratio	v	0.2	This study
Permeability [m^2]	k	0.6*10^-14	(48)
Biot-Willis coefficient	α_b	0.95	(49)
Density [kg/m^3]	ρ	157	(48)
Dynamic viscosity medium [Pa s]	μ	8.1*10^4	(50)
Density medium [kg/m^3]	ρ_f	1000	(50)
Displacement Function [m]	w	(15*10^-6)*cos(1.57*t)-(15*10^-6)	This study

Table S4. Computational parameters

Video S1. Bioreactor overview

Video S2. Computational estimation of pressure and flow velocity