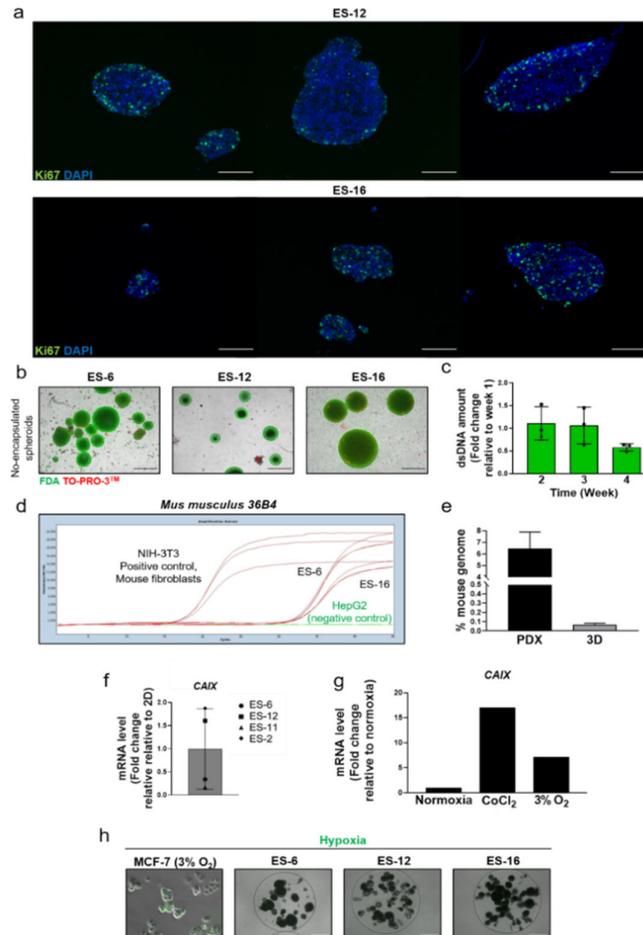
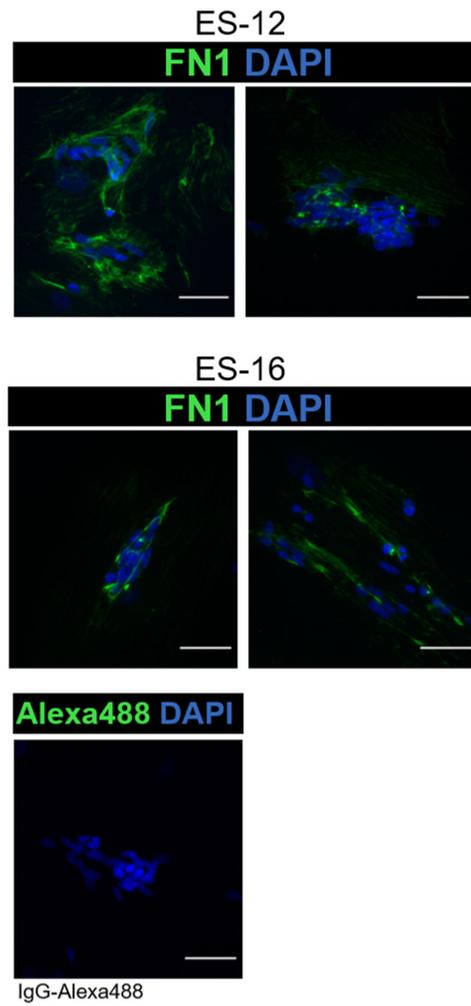


**Supplementary Materials: “PDX-Derived Ewing’s Sarcoma Cells Retain High Viability and Disease Phenotype in Alginate-Encapsulated Spheroid Cultures”**

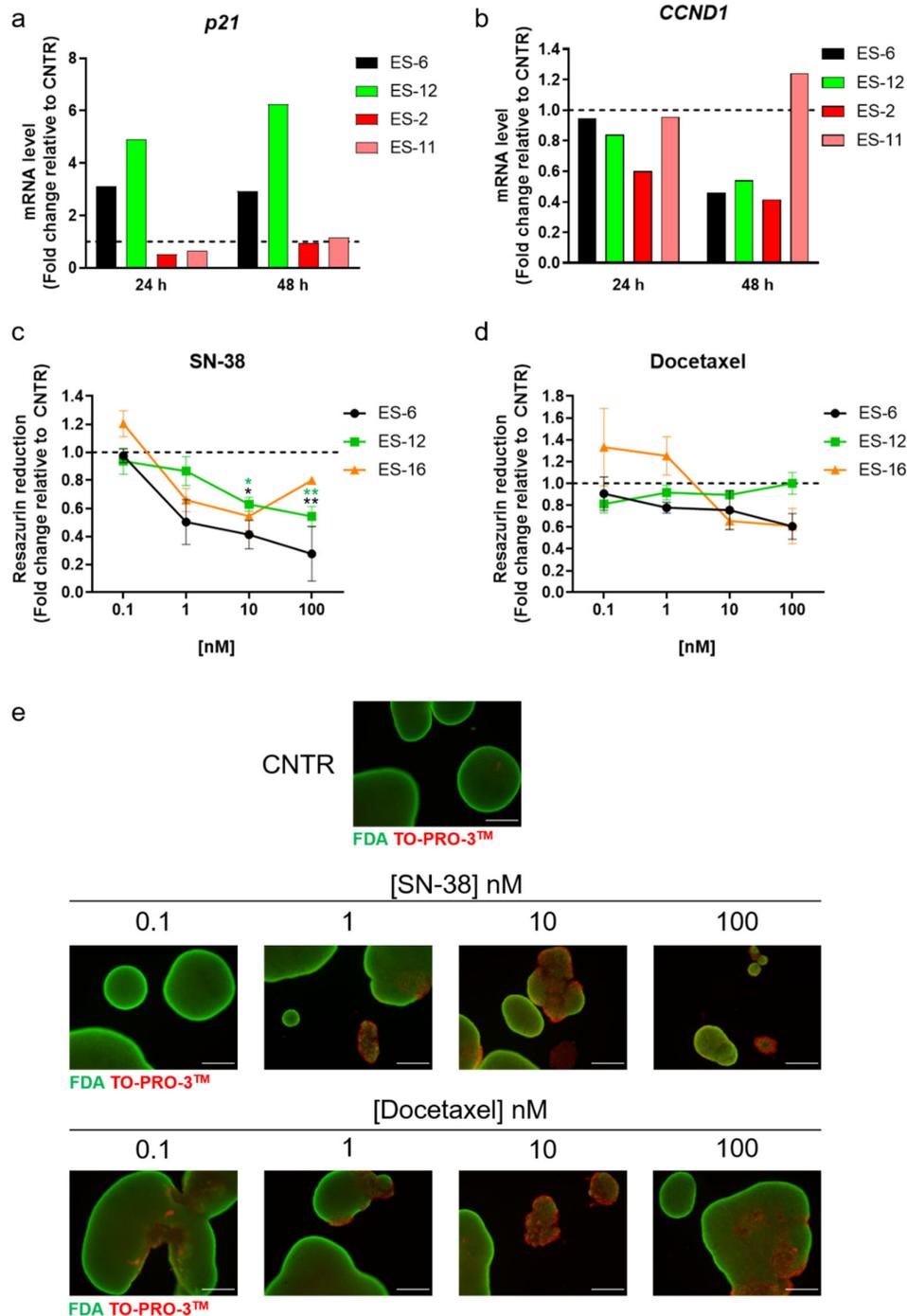
Giacomo Domenici, Rodrigo Eduardo, Helena Castillo-Ecija, Gorka Orive, Ángel Montero Carcaboso and Catarina Brito



**Figure S1.** Cell viability and proliferation in ES spheroid cultures (a) Immunofluorescence detection of Ki67 (green) in encapsulated ES spheroids; nuclei were counterstained with DAPI (blue). Scale bar: 100  $\mu$ m. (b) Representative images of non-encapsulated spheroid cultures derived from ES-6, ES-12 and ES-16. Live cells (FDA, green) and dead cells (TO-PRO-3<sup>TM</sup>). Scale bar: 500  $\mu$ m. (c) dsDNA amount along non-encapsulated spheroid cultures derived from 3 distinct ES-PDX, shown as fold change at each culture time relative to week 1 (set as 1, dashed line). Statistical analysis was performed by the non-parametric Kruskal Wallis test. (d) Representative RT-qPCR amplification curves of 36B4 mRNA in mouse NIH-3T3 cells, encapsulated spheroid cultures (derived from ES-6 and ES-16) and human hepatocarcinoma cell line HepG2. (e) Percentage of mouse genome in encapsulated spheroid cultures derived from ES6 and ES12 after one-month culture relative to the original PDX cells ( $n = 2$ ). (f) Fold change in mRNA expression levels of CAIX in in 2D monolayer and encapsulated spheroid cultures derived from 4 different ES-PDX ( $n = 4$ ). (g) MCF-7 cells were exposed to 800  $\mu$ m CoCl<sub>2</sub> (in normoxia) or to 3% O<sub>2</sub>, for 24 h. Data are presented as fold change in mRNA level in each condition relative to normoxia control condition. (h) Environmental hypoxia analysis by Image-iT<sup>TM</sup> Green Hypoxia Reagent in encapsulated ES spheroid cultures and control MCF-7 2D monolayers exposed to 3% O<sub>2</sub>. MCF-7 cells: scale bar: 50  $\mu$ m; encapsulated ES spheroids: scale bar: 500  $\mu$ m.



**Figure S2.** Detection of fibronectin (FN1) in ES12 and ES16-derived 2D cultures. Nuclei were counterstained with DAPI. Secondary antibody control (IgG-Alexa488) is shown as well. Scale bar: 50  $\mu\text{m}$ .



**Figure S3.** *CCND1* and *p21* expression in encapsulated ES spheroids and drug response in non-encapsulated spheroids (a) *CCND1* mRNA expression in encapsulated ES spheroid cultures exposed to 10 nM SN-38, for 24 or 48 h. Data are shown as fold change in *CCND1* mRNA level relative to vehicle control (DMSO; set as 1, dashed line). (b) *p21* mRNA expression in encapsulated ES spheroid cultures exposed to 10 nM SN-38, for 24 or 48 h. Data are shown as fold change in *p21* mRNA level relative to vehicle control (DMSO; set as 1, dashed line). (c) Resazurin reduction in non-encapsulated ES spheroid cultures, exposed to SN-38 for 7 days (ES-6, -12:  $n = 3$ ; ES-16:  $n = 2$ ). (d) Resazurin reduction in non-encapsulated ES spheroid cultures exposed to Docetaxel (ES-6, -12:  $n = 3$ ; ES-16:  $n = 2$ ). Data are shown as fold change in resazurin reduction relative to vehicle control (DMSO; set as 1, dashed line)  $\pm$  SEM. For ES-12 and ES-16 derived cultures, one-way ANOVA followed by Dunnett's test was performed, comparing each drug concentration with CNTR, (control, vehicle-challenged

cultures \* =  $p < 0.05$ , \*\* =  $p \leq 0.01$ ). (e) Representative FDA (live cells, green) and TOPRO-3™ (dead cells, red) staining of encapsulated spheroid cultures derived from ES-6, exposed to increasing concentration of SN-38 and Docetaxel. Scale bar: 500  $\mu\text{m}$ .

Figure 2A Gel

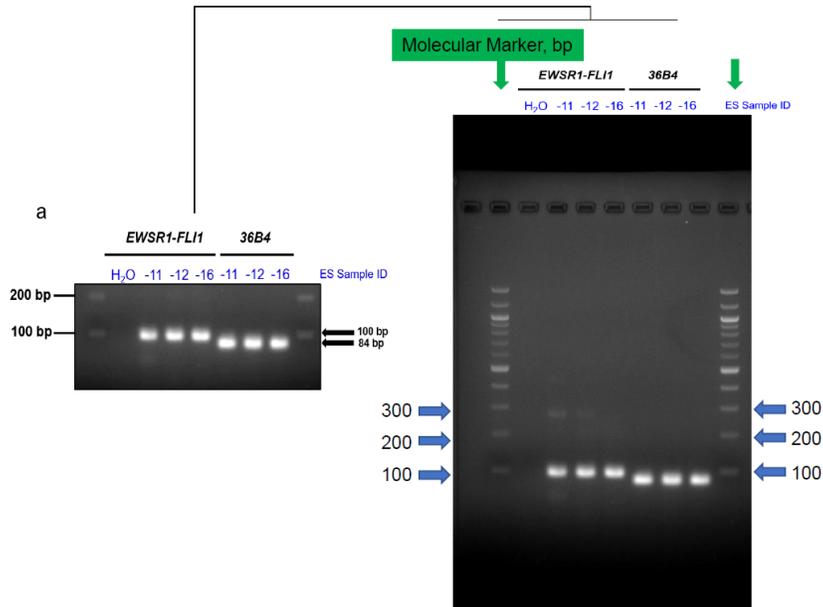


Figure S4. Uncropped and unadjusted agarose gel image relative to Figure 2A. bp: base pair.

Table S1. Characterization of PDX models.

Pdx Model	Patient	Disease Stage	Translocation	Time to Endpoint <sup>b</sup> (day)	Pdx Passage in Mice
HSJD-ES-002	1	Diagnosis <sup>a</sup>	<i>EWSR1-FLI1</i> , ex10-ex5	52	6
HSJD-ES-006	1	Relapse	<i>EWSR1-FLI1</i> , ex10-ex5	46	4
HSJD-ES-011	2	Relapse	<i>EWSR1-FLI1</i> , ex7-ex6	62	3
HSJD-ES-012	3	Relapse	<i>EWSR1-FLI1</i> , ex7-ex6	94	5
HSJD-ES-016	4	Relapse	<i>EWSR1-FLI1</i> , ex7-ex6	29	9

<sup>a</sup>No previous treatment at the time of the biopsy, <sup>b</sup>Defined as the time to achieve 1500 mm<sup>3</sup>.

Table S2. Primer List.

Gene	Forward 5'-3'	Reverse 5'-3'
<i>EWSR1</i>	CCAAGTCAATATAGCCAACAG	-
<i>FLI1</i>	-	GGCCAGAATTCATGTTATTGC
<i>36B4</i>	GTGTTTCGACAATGGCAGCAT	GACACCTCCAGGAAGCGA
<i>RPL22</i>	CACGAAGGAGGAGTGACTGG	TGTGGCACACCACTGACATT
Mouse <i>36B4</i>	TGCTCGACATCACAGAGCAG	ACGCGCTTGTACCCATTGAT
<i>CCND1</i>	AGACCTTCGTTGCCCTCTGT	TGTGAGGCGGTAGTAGGACA
<i>p21</i>	AGTCAGTTCCTTGTGGAGCC	GCATGGGTTCTGACGGACAT

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<i>CAIX</i>	TTTGCCAGAGTTGACGAGGC	TCTCCAAGCGAGACAGCAAC
<i>DAX1</i>	GGGGACCGTGCTCTTAACC	GCGTCATCCTGGTGTGTTCA
Mouse <i>PTGER2</i>	CCTGCTGCTTATCGTGGCTG	GCCAGGAGAATGAGGTGGTC
Human <i>PTGER2</i>	GCTGCTTCTCATTGTCTCGG	GCCAGGAGAATGAGGTGGTC

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