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Article

Salinomycin Treatment Specifically Inhibits Cell Proliferation of Cancer Stem Cells Revealed by Longitudinal Single Cell Tracking in Combination with Fluorescence Microscopy

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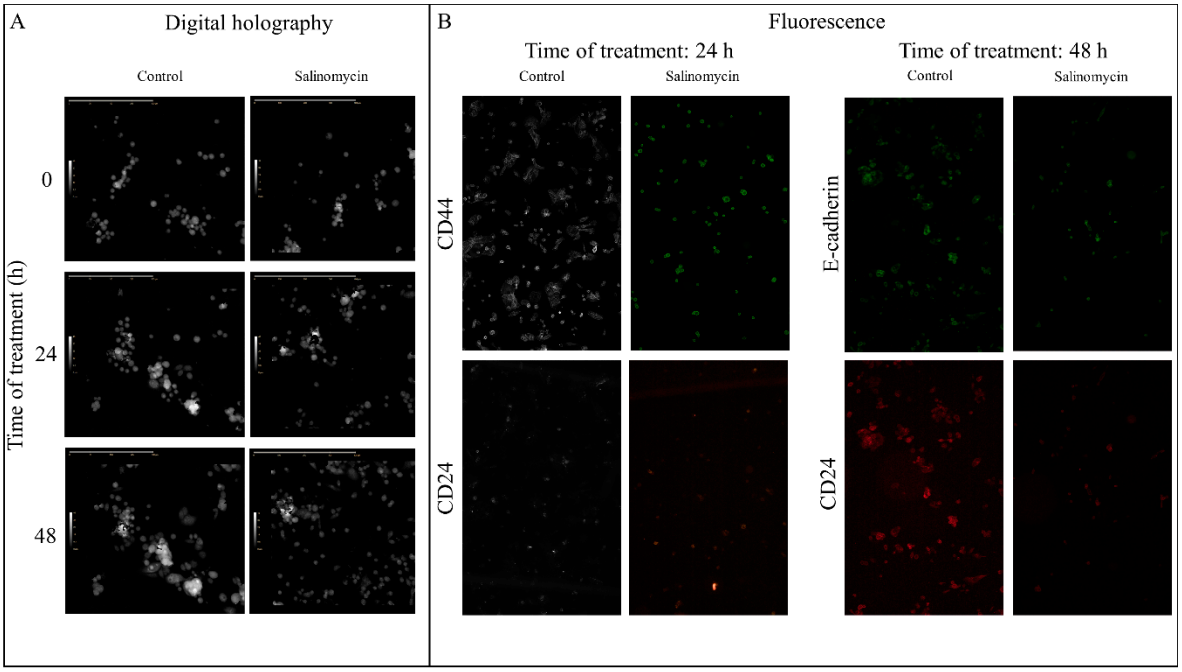

Figure S1. Representative images from DHM time-lapses and fluorescence. **(**A) Images from time-point 0, 24 and 48 h of a representative DHM time-lapse of JIMT-1 cells in the absence (control) or presence of 0.5 μM salinomycin. The salinomycin was added immediately before the start of the time-lapse. The scale bar on top represents 300 μm for control and 400 μm in salinomysin-treated samples, and the scalebar to the left represents the optical thickness 0-46 μm in control and 0-48 μm in salinomycin-treated samples. (B) Fluorescence images of samples fixed after DHM imaging. The cells were labelled with anti-CD24-PE and anti-CD44-FITC or anti-CD24-PE and anti-E-cadherin-Alexa Flour 488 after 24 or 48 h of treatment.

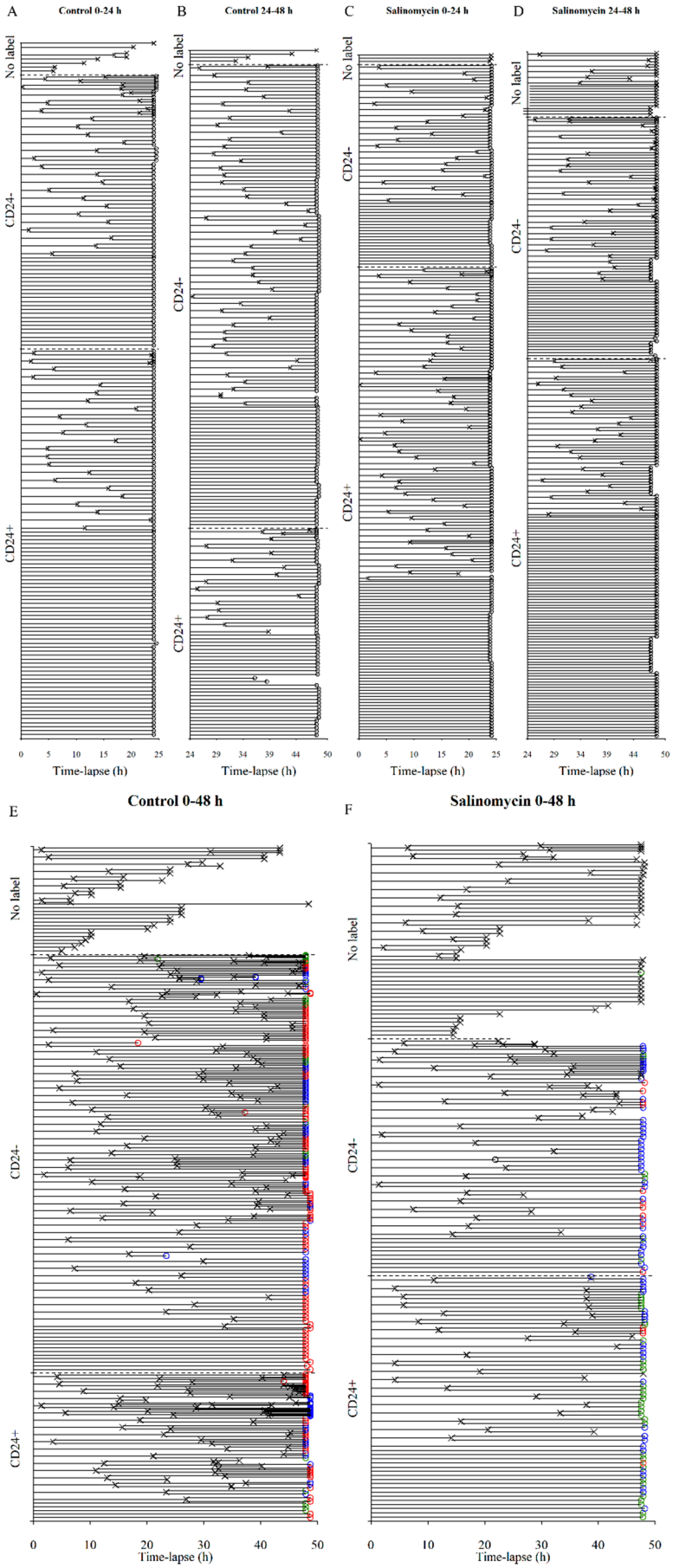
A
Control 0-24 h
B
Control 24-48 h
C
Salinomycin 0-24 h
D
Salinomycin 24-48 h
No label
CD24-
CD24+
Time-lapse (h)
E
Control 0-48 h
F
Salinomycin 0-48 h

Figure S2. Cell family trees of JIMT-1 cells tracked through time-lapses of digital holographic images. Images was acquired every 15 min for 24 (A-D) or 48 (E, F) hours. Salinomycin (0.5 μM) was added immediately before the start of the time-lapse (C, F) or 24 h before the start of the time-lapse (D). Immediately after the image acquisition the cells were fixed and labelled with anti-CD24-PE and anti-CD44-FITC (A-D) or anti-CD24-PE and anti-E-cadherin-Alexa Flour 488 (E, F). The tracked cells were matched to their expression of the fluorescent markers after tracking. The CD24-expression was characterized as positive or negative. The cell trees are grouped according to their CD24-expression, divided by a dashed line. The expression of E-cadherin is characterized as low (red), mid (blue) or high (green). No label means tracked cells that escaped the frame before the fixation and therefor don't have any knows expression. X denotes an un-known expression, either due to escape out of frame or due to division during the time-lapse.

Table S1. Interpretation of number of cell divisions seen in Figure S2 in relation to labelling.

		Control 0-24 h	
Number of divisions	**No labelling**	**CD24$^-$**	**CD24$^+$**
0	6	23	52
1	1	18	21
2	0	5	2

		Control 24-48 h	
Number of divisions	**No labelling**	**CD24$^-$**	**CD24$^+$**
0	4	34	28
1	0	47	14
2	0	1	1

		Salinomycin 0-24 h	
Number of divisions	**No labelling**	**CD24$^-$**	**CD24$^+$**
0	3	21	52
1	0	23	50
2	0	0	2

		Salinomycin 24-48 h	
Number of divisions	**No labelling**	**CD24$^-$**	**CD24$^+$**
0	11	27	78
1	3	27	27
2	3	1	1

		Control 0-48 h					
	No labelling	**CD24$^-$**			**CD24$^+$**		
Number of divisions		**E-cad low**	**E-cad mid**	**E-cad high**	**E-cad low**	**E-cad mid**	**E-cad high**
0	14	12	0	0	2	0	3
1	6	9	6	0	1	1	0
2	2	18	10	3	6	4	0
3	0	3	2	2	3	3	0

		Salinomycin 0-48 h					
	No labelling	**CD24$^-$**			**CD24$^+$**		
Number of divisions		**E-cad low**	**E-cad mid**	**E-cad high**	**E-cad low**	**E-cad mid**	**E-cad high**
0	20	1	6	2	1	7	10
1	12	4	11	1	0	5	8
2	2	3	4	1	1	3	3
3	0	0	0	0	0	0	0

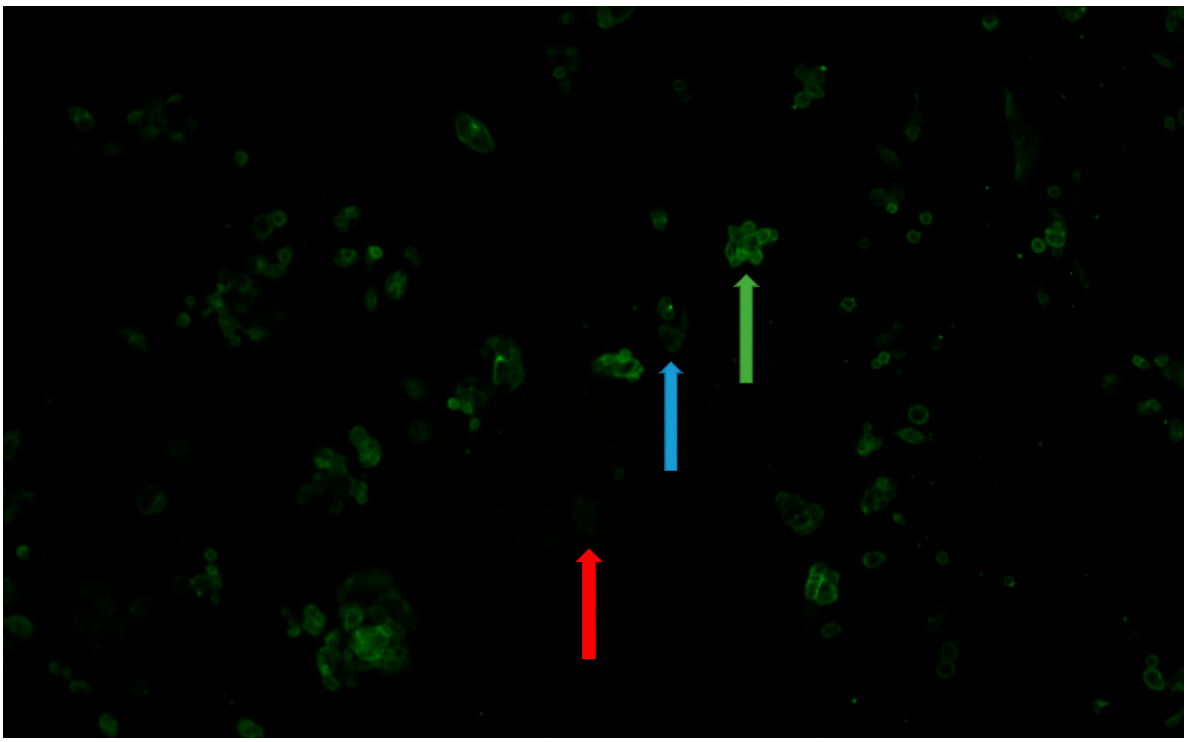

Figure S3. Fluorescence image displaying the visual evaluation of E-cadherin -labeled cells as low (red arrow), mid (blue arrow) or high (green arrow). The cell with the red arrow is barely visible on a computer screen.