Comparison of the Dosimetry and Cell Survival Effect of ^{177}Lu and ^{161}Tb Somatostatin Analog Radiopharmaceuticals in Cancer Cell Clusters and Micrometastases

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Supplementary data

Cell Culture

The AR42J (CRL1492) rat pancreatic tumor cell line (ATCC, Manassas, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM) high glucose (Euroclone, Milan, Italy) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco-Thermo Scientific, MA, USA), 1% antibiotics (penicillin/streptomycin) (Lonza-Euroclone, Milan, Italy), 1% Hepes buffer (Lonza-Euroclone, Milan, Italy), and 1% U-glutamine (Lonza-Euroclone, Milan, Italy). This solution will be referred to as a complete medium. The cells were grown in 75 cm^2 flasks, passed twice a week, and maintained in the incubator at 37 °C in a humidified atmosphere containing 5% CO_2.

Verification of the accuracy and homogeneity of the dose delivered by gamma irradiators

Cell irradiation for survival studies was performed using two gamma irradiators: 1) The Gammacell 220 irradiator (Atomic Energy of Canada, Ottawa, Canada) containing a cobalt-60 source with a dose rate of 7.5 Gy/h; 2) IBL 430 C biological sample irradiator (Schering/Cys bio-International Milan, Italy) containing a cesium-137 source at a dose rate of 120 Gy/h.

To ensure the accuracy and consistency of the dose delivered by the Gammacell 220 irradiator, direct ionization measurements were conducted using a secondary standard ionization chamber NE2611 (NPL, Middlesex, UK) certified by BIPM. Measurements were taken every 60 seconds at the central point of the field under controlled environmental conditions, with air density correction applied, until a dose of 10 Gy was reached. The results showed that the dose delivered by the Gammacell 220 irradiator had a total uncertainty of only 0.75% in the dose range studied.

The accuracy and uniformity of the dose delivered by the IBL 430 C irradiator during cell irradiation were evaluated using the Gafchromic EBT3 film. Prior to the commencement of the experiment, the EBT3 film batch underwent dose calibration. Calibration was performed according to the method proposed by Devic et al. (Devic S., et al. Medical Physics, 2005. **32**(7): 2245). An Epson Expression 10000 XL scanner (Epson, Japan) was used to read all films 48 hours after irradiation. Digitalized

images were acquired in RGB positive mode with 24-bit depth per color channel and a spatial resolution of 75 dots per inch (dpi). The red channel was used for dose measurements, and analysis was performed using DoseLab Pro software (Varian Medical Systems). The delivered dose was compared to the expected value. The relative difference between the expected and measured values was calculated. Orthogonal profiles were acquired on each irradiated film to verify the homogeneity of the irradiation. The maximum difference between the net optical density OD and the mean of the profile points was calculated to verify the uniformity of the irradiation. The resulting calibration curve was used to relate the film OD to the absorbed dose. Next, pieces of film, 10x10 cm^2, were irradiated at three different dose values in the region of interest, precisely 2.5, 5, and 10 Gy. The measurements were conducted in two different positions, at the base of the irradiation support and at a distance of 1 cm from it. Then calibration curve was employed to establish a relationship between the film OD and the absorbed dose. The percentage differences between the expected and measured dose values after evaluation of the Gafchromic film dosimetry for the IBL 430 C irradiator were found to be less than 5%. Uniformity indices were less than 5% and independent of the position of the measurement.

Table S1. Total number of cells and number of labeled cells for different percentage of labeling in 2D circular colonies or 3D spherical cell clusters models of different sizes (radius), used for the dose calculation with the MIRDcell software.

Size (µm)	2D colony				3D cluster			
	Total number of cells	Number of labeled cells			Total number of cells	Number of labeled cells		
		10%	40%	70%		10%	40%	70%
25	9	1	4	6	19	2	7	13
50	29	3	11	20	123	13	49	86
100	121	12	48	84	1021	102	408	714
200	489	49	195	342	8217	822	3286	5751
300	1101	110	440	770	27609	2761	11043	19326
500	3069	307	1227	2148	127675	12768	51070	89372
750	6909	691	2763	4836	431545	43155	172618	302081
1000	12281	1228	4912	8596	1023349	102335	409339	716344

Table S2. Relative enhancement of the mean dose to labeled cells (MDLC) and unlabeled cells (MDUC) resulting from an increase in cluster size from 25 to 1000 µm, calculated using a mean number of disintegrations per cell of 18,900 and 10%, 40%, or 70% of cells labeled with ^{177}Lu-RPs or ^{161}Tb-RPs.

Radiopharmaceutical	Cluster size (µm)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
^{177}Lu-DOTATOC	25	4.2	37.5	14.2	9.2
	1000	82.5	119	91.7	87.8
	Relative DI	1864	217	546	854
^{177}Lu-NLS	25	4.3	41.1	14.9	10.6
	1000	82.5	124	93	88.5
	Relative DI	1819	202	524	735
^{177}Lu-LM3	25	4.5	27.6	11.2	8.5
	1000	82.7	111	89.7	86.7
	Relative DI	1738	302	701	920
^{161}Tb-DOTATOC	25	11.7	131	43.2	29.4
	1000	101	232	134	119
	Relative DI	763	77	210	305
^{161}Tb-NLS	25	10.3	149	51.1	31.7
	1000	100	257	140	123
	Relative DI	871	72	174	288
^{161}Tb-LM3	25	10.5	102	37.1	25.8
	1000	101	202	126	116
	Relative DI	862	98	240	350

Table S3. Calculated MDC, MDUC and MDLC for 2D colonies and 3D cell clusters with radius of 25, 50, 100, 500 or 1000 μm considering 10, 40 or 70% of labeled cells and a mean number of disintegrations per cell of 18,900. MDC and MDUC are reported as mean values and standard deviations of calculations performed with different percentage of labeled cells (10, 40 and 70%).

^{177}Lu-DOTATOC	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	6.12±0.20	2.49±0.37	33.10	11.40	7.60
	50	7.72±0.37	3.98±0.40	39.30	14.50	8.89
	100	8.98±0.04	5.45±0.13	39.90	14.40	10.60
	500	11.50±0.00	7.86±0.00	44.40	16.90	13.00
	1000	12.00±0.00	8.32±0.02	45.20	17.50	13.60
3D	25	7.65±0.33	4.19±0.40	37.50	14.20	9.18
	50	13.90±0.20	9.97±0.44	47.30	18.90	15.80
	100	24.33±0.15	20.70±0.20	57.70	29.90	25.80
	500	66.33±0.06	62.67±0.06	99.30	72.00	67.90
	1000	86.23±0.06	82.53±0.06	119.00	91.70	87.80
3D/2D	25	1.25±0.07	1.68±0.30	1.13	1.25	1.87
	50	1.80±0.09	2.50±0.27	1.20	1.30	2.13
	100	2.71±0.02	3.80±0.10	1.45	2.08	2.43
	500	5.77±0.01	7.97±0.01	2.24	4.26	5.22
	1000	7.19±0.01	9.92±0.03	2.63	5.24	6.46
^{177}Lu-NLS	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	6.52±0.17	2.90±0.09	37.40	10.80	8.24
	50	7.66±0.27	3.49±0.29	42.10	15.40	9.48
	100	9.45±0.07	5.38±0.12	46.90	15.60	11.30
	500	11.967±0.06	7.78±0.05	49.60	18.30	13.70
	1000	12.500±0.00	8.33±0.01	49.70	18.70	14.30
3D	25	8.41±0.20	4.34±0.28	41.10	14.90	10.60
	50	13.90±0.44	9.89±0.27	45.90	20.50	15.90
	100	24.333±0.47	20.37±0.49	60.00	30.50	26.40
	500	66.80±0.00	62.63±0.06	104.0	73.00	68.60
	1000	86.70±0.00	82.50±0.00	124.0	93.00	88.50
3D/2D	25	1.29±0.04	1.50±0.11	1.10	1.38	1.81
	50	1.82±0.09	2.83±0.25	1.10	1.33	2.16
	100	2.57±0.05	3.78±0.12	1.28	1.96	2.34
	500	5.58±0.03	8.05±0.05	2.10	3.99	5.01
	1000	6.94±0.00	9.91±0.01	2.49	4.97	6.19
^{177}Lu-LM3	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	5.11±0.25	2.90±0.11	25.20	7.51	6.08
	50	6.35±0.41	3.06±0.06	30.10	12.00	8.35
	100	8.30±0.15	5.69±0.16	32.40	12.10	9.31
	500	10.70±0.00	7.89±0.04	35.30	14.90	11.90
	1000	11.20±0.00	8.41±0.00	36.10	15.40	12.40
3D	25	7.08±0.18	4.49±0.29	27.60	11.20	8.470
	50	12.80±0.10	10.37±0.15	35.60	16.50	13.60
	100	23.27±0.25	20.53±0.29	47.80	27.40	24.70
	500	65.57±0.06	62.77±0.06	90.60	69.70	66.90
	1000	85.47±0.06	82.67±0.06	111.00	89.70	86.70
3D/2D	25	1.38±0.08	1.55±0.11	1.10	1.49	1.84
	50	2.01±0.13	3.39±0.09	1.18	1.37	1.98
	100	2.80±0.06	3.61±0.11	1.48	2.26	2.65
	500	6.13±0.01	7.95±0.04	2.57	4.68	5.62
	1000	7.63±0.01	9.83±0.01	3.07	5.82	6.99

^{161}Tb-DOTATOC	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	18.23±0.61	5.22±0.34	119.00	33.80	25.90
	50	20.70±1.15	7.72±0.84	129.00	43.70	26.80
	100	23.87±0.23	10.77±0.25	140.00	44.400	29.700
	500	27.03±0.06	13.83±0.12	147.00	47.000	32.700
	1000	27.67±0.06	14.43±0.06	146.00	47.400	33.300
3D	25	24.03±1.57	11.67±1.42	131.00	43.200	29.400
	50	32.83±1.10	19.57±0.55	139.00	54.800	38.600
	100	45.70±0.26	32.67±0.12	161.00	65.80	51.10
	500	91.00±0.00	77.83±0.06	209.00	111.00	96.60
	1000	114.0±0.00	101.00±0.00	232.00	134.00	119.00
3D/2D	25	1.32±0.10	2.23±0.31	1.10	1.28	1.67
	50	1.59±0.10	2.54±0.29	1.08	1.25	2.04
	100	1.91±0.02	3.03±0.07	1.15	1.48	1.72
	500	3.37±0.01	5.63±0.05	1.42	2.36	2.95
	1000	4.12±0.01	6.70±0.03	1.59	2.83	3.57
^{161}Tb-NLS	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	21.33±0.49	7.43±0.38	141.00	38.00	27.70
	50	23.10±0.61	7.67±0.77	154.00	50.30	30.10
	100	26.23±0.47	10.77±0.35	167.00	49.10	33.30
	500	29.40±0.00	13.73±0.12	172.00	52.90	36.10
	1000	30.07±0.06	14.47±0.06	171.00	53.50	36.70
3D	25	24.83±0.72	10.25±1.08	149.00	51.10	31.70
	50	35.53±0.49	20.00±0.17	165.00	60.20	41.90
	100	48.17±0.31	32.70±0.00	191.00	70.60	54.80
	500	93.30±0.00	77.70±0.00	234.00	117.00	100.00
	1000	116.00±0.00	100.00±0.00	257.00	140.00	123.00
3D/2D	25	1.16±0.04	1.38±0.16	1.06	1.34	1.84
	50	1.54±0.05	2.61±0.26	1.07	1.20	2.00
	100	1.84±0.04	3.04±0.10	1.14	1.44	1.65
	500	3.17±0.00	5.66±0.05	1.36	2.21	2.77
	1000	3.86±0.01	6.91±0.03	1.50	2.62	3.35
^{161}Tb-LM3	Radius (μm)	MDC (Gy)	MDUC (Gy)	MDLC 10% (Gy)	MDLC 40% (Gy)	MDLC 70% (Gy)
2D	25	16.10±0.46	7.07±0.50	90.60	26.00	20.80
	50	18.70±0.62	8.38±1.68	102.00	34.20	24.30
	100	21.33±0.23	11.17±0. 60	110.00	37.20	25.60
	500	24.27±0.06	14.23±0.06	115.00	39.40	28.60
	1000	24.93±0.06	14.83±0.06	116.00	40.10	29.20
3D	25	21.60±3.18	10.46±1.47	102.00	37.10	25.80
	50	30.80±1.35	21.10±1.48	113.00	43.10	35.30
	100	43.07±0.31	32.97±0.31	134.00	58.40	47.70
	500	88.47±0.06	78.43±0.12	179.00	104.00	92.80
	1000	111.00±0.00	101.00±0.00	202.00	126.00	116.00
3D/2D	25	1.34±0.20	1.48±0.23	1.13	1.43	1.78
	50	1.65±0.09	2.52±0.53	1.11	1.26	1.77
	100	2.02±0.03	2.95±0.16	1.22	1.57	1.86
	500	3.65±0.01	5.51±0.02	1.56	2.64	3.24
	1000	4.45±0.01	6.81±0.03	1.74	3.14	3.97

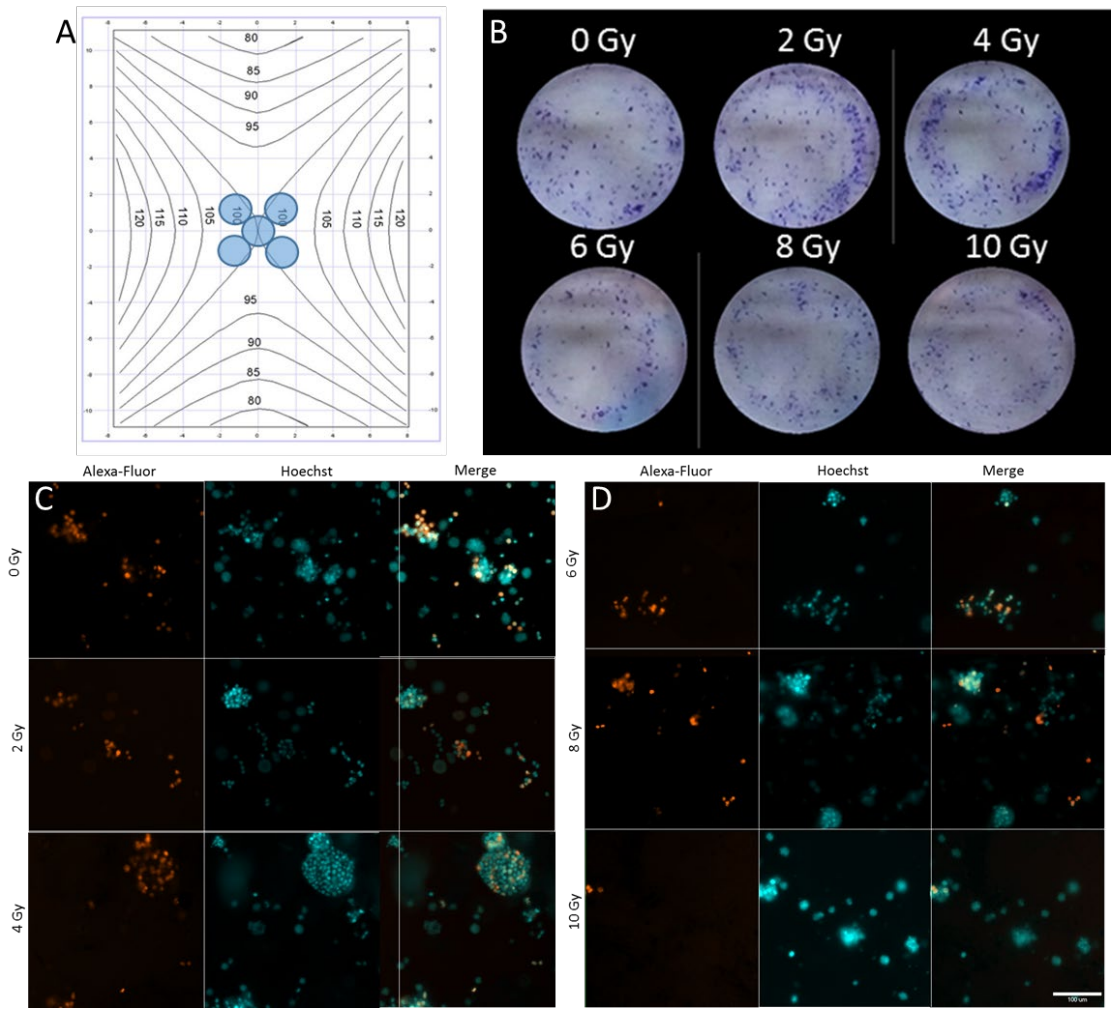

Figure S1. Representative images of the cell proliferation result. A) Scheme of the experimental setup used for the irradiation of the well plates containing the cells for the clonogenic assay, B) representative image of the colonies formed by the AR42J cells that were exposed to different ADs in the clonogenic assay; C) and D) representative fluorescence image of EdU thymidine analog incorporation by cells in EdU proliferation assay (first column in red), counter-stained with Hoechst (second column, blue) to determine the total number of cells and the combined image of both fluorophores (third column, merge).

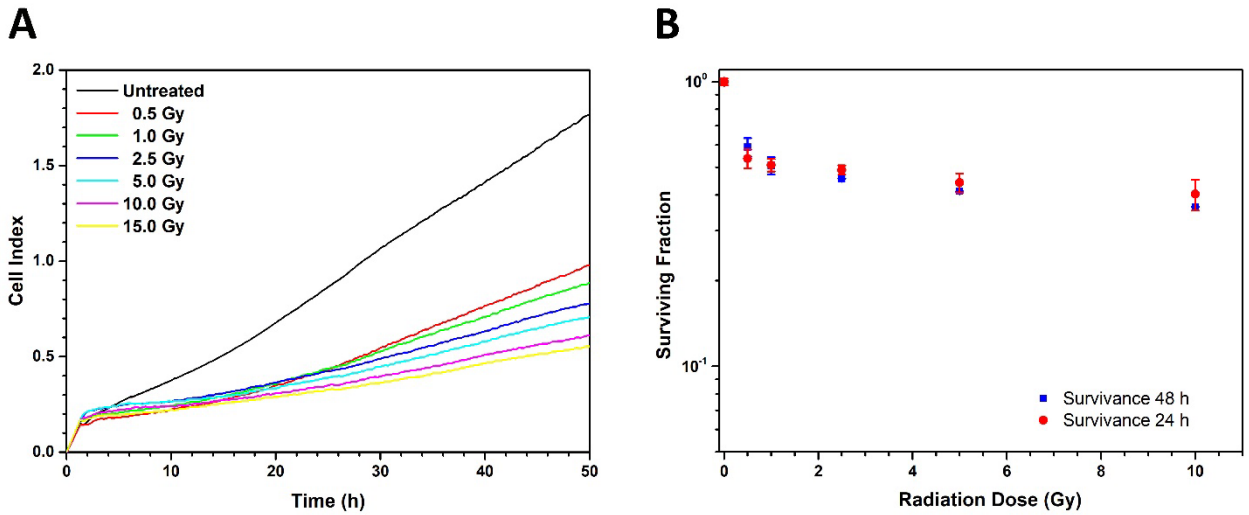

Figure S2. A) Average of cell index values of 3 experiments monitored by impedance-based real-time cell analysis from 15 min to 50 h. B) Survival curves obtained at 24 and 48 h after irradiation. Points represent the mean values of six experiments. Bars are the standard errors.

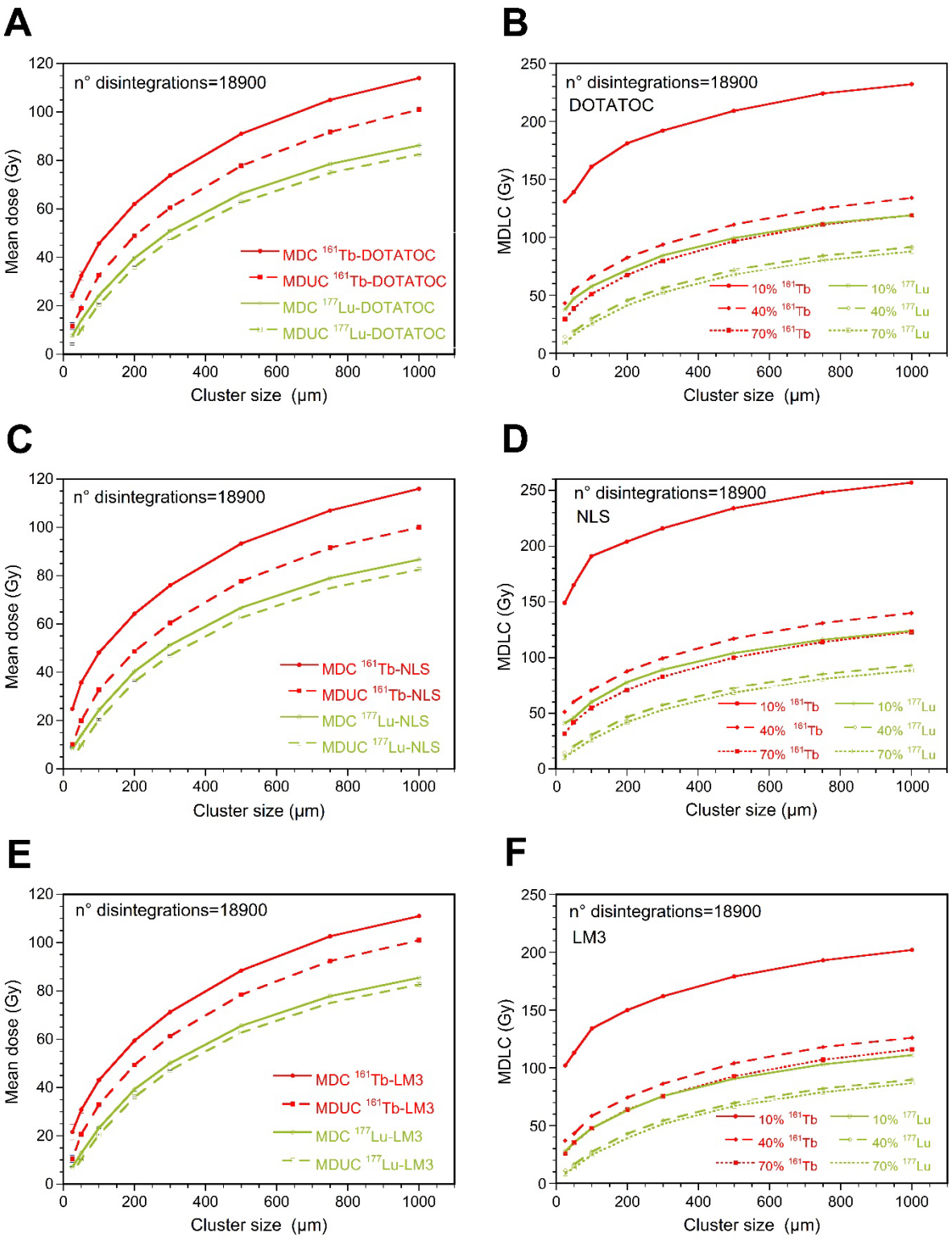

Figure S3. Comparison of MDC, MDUC and MDLC (for 10, 40 and 70% of labeled cells) of ^{177}Lu- and ^{161}Tb-RPs, obtained using the same mean number of disintegrations per cell (18,900), vs. the radius of the spherical cell cluster.

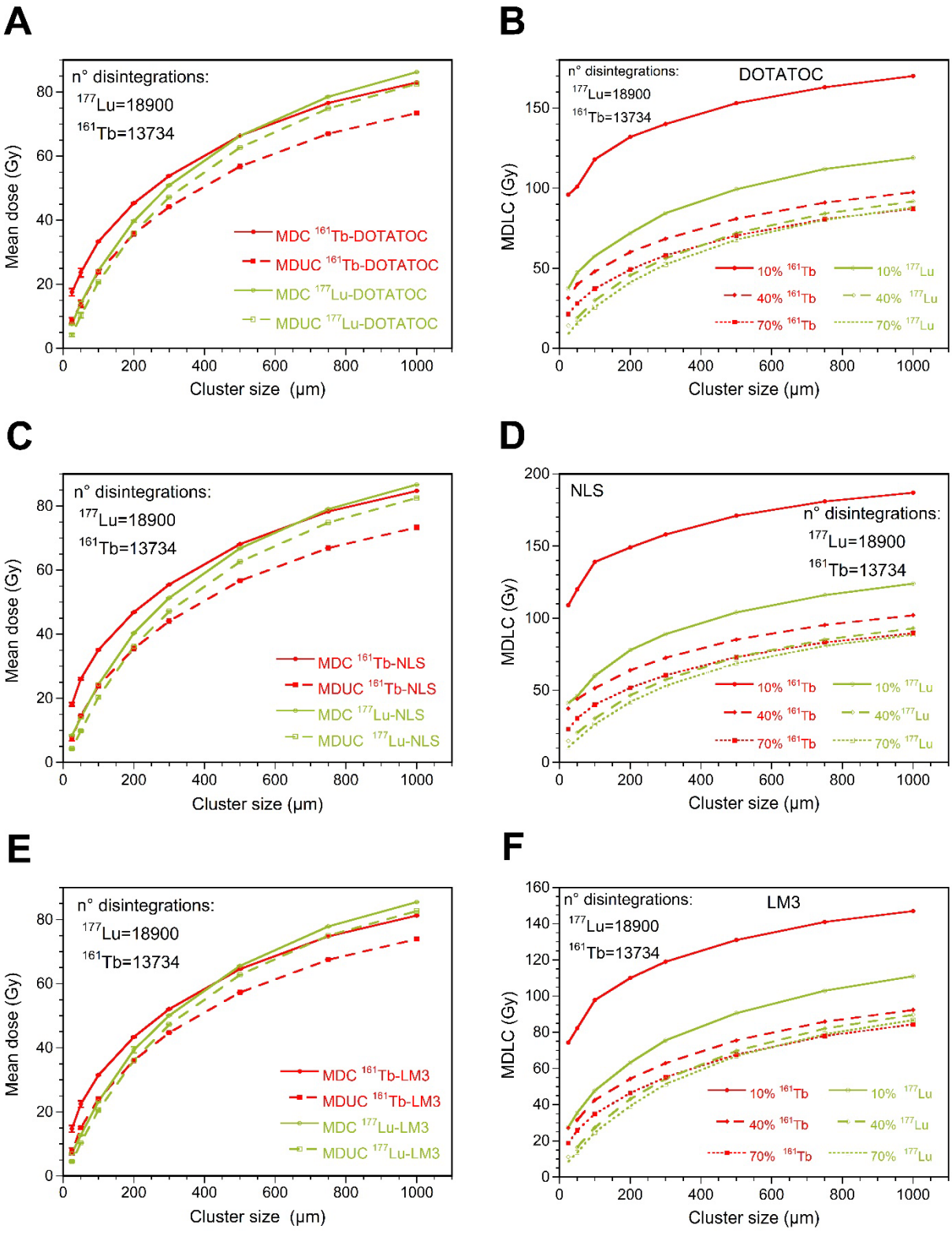

Figure S4. Comparison of ^{177}Lu- and ^{161}Tb-RPs MDC, MDUC and MDLC (for 10, 40 and 70% of labeled cells) obtained using a mean number of disintegrations per cell of 18,900 for ^{177}Lu-RPs and of 13,734 for ^{161}Tb-RPs vs. the radius of the spherical cell cluster.

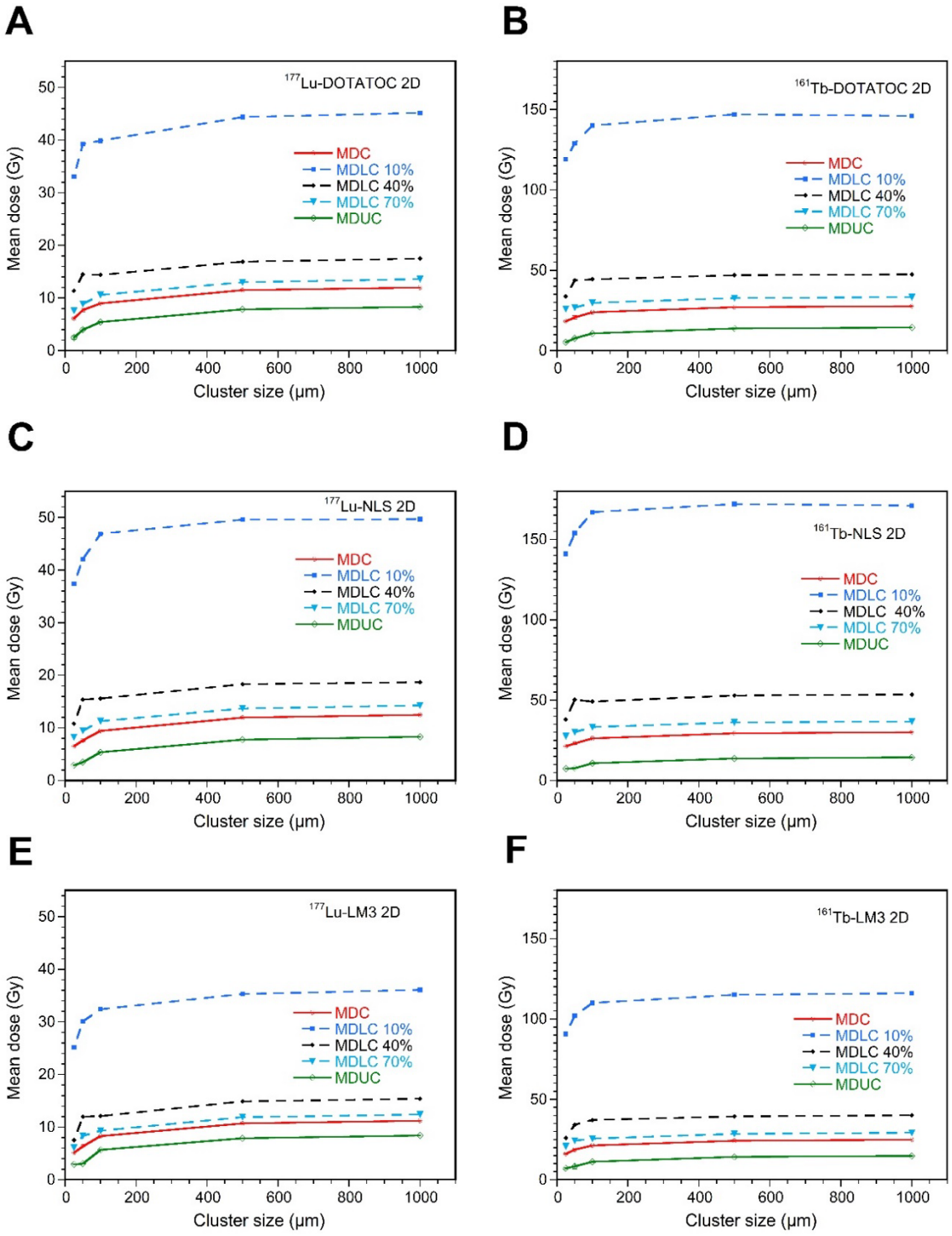

Figure S5. MDC, MDUC and MDLC considering 10, 40 or 70% of labeled cells and a mean number of disintegrations per cell of 18,900 for both ^{177}Lu-RPs and ^{161}Tb-RPs vs. the radius of the circular cell colony. For ^{177}Lu-DOTATOC (A), the increase in MDUC, MDLC-10%, MDLC-40% and MDLC-70% by moving from a 25 µm to a 1000 µm colony is 234, 37, 54 and 79%, respectively. Similar values were obtained for ^{177}Lu-NLS (187, 33, 73 and 62%) (C) and ^{177}Lu-LM3 (190, 43, 105 and 104%) (E). For ^{161}Tb-DOTATOC (B), the increase in MDUC, MDLC-10%, MDLC-40%, and MDLC-70%, by increasing the radius of the circular colony from 25 µm to 1000 µm, is 176, 23, 40 and 29%, respectively. Similar values were obtained for the other ^{161}Tb-RPs (95, 21, 41 and 32% for ^{161}Tb -NLS (D) and 110, 28, 54 and 40% for ^{161}Tb-LM3 (E), respectively). In all cases, the increment of values was much lower in 2D compared to 3D models (see Table S2).

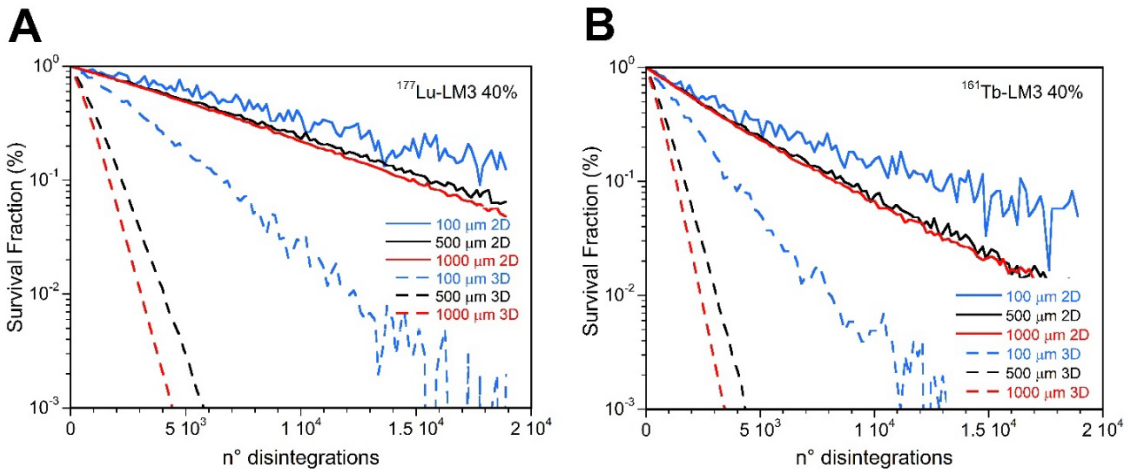

Figure S6. Survival fraction versus the mean number of disintegrations per cell for 100, 500 and 1000 µm colony and cluster radius in both the 2D and 3D geometry after A) ^{177}Lu-LM3 treatment and B) ^{161}Tb-LM3 treatment, considering 40% of labeled cells. No significative differences were found in the 2D case between the SF curves for 500 µm and 1000 µm colony, unlike the 3D case. Large differences in the SF curves are evident between the 2D and the 3D case.

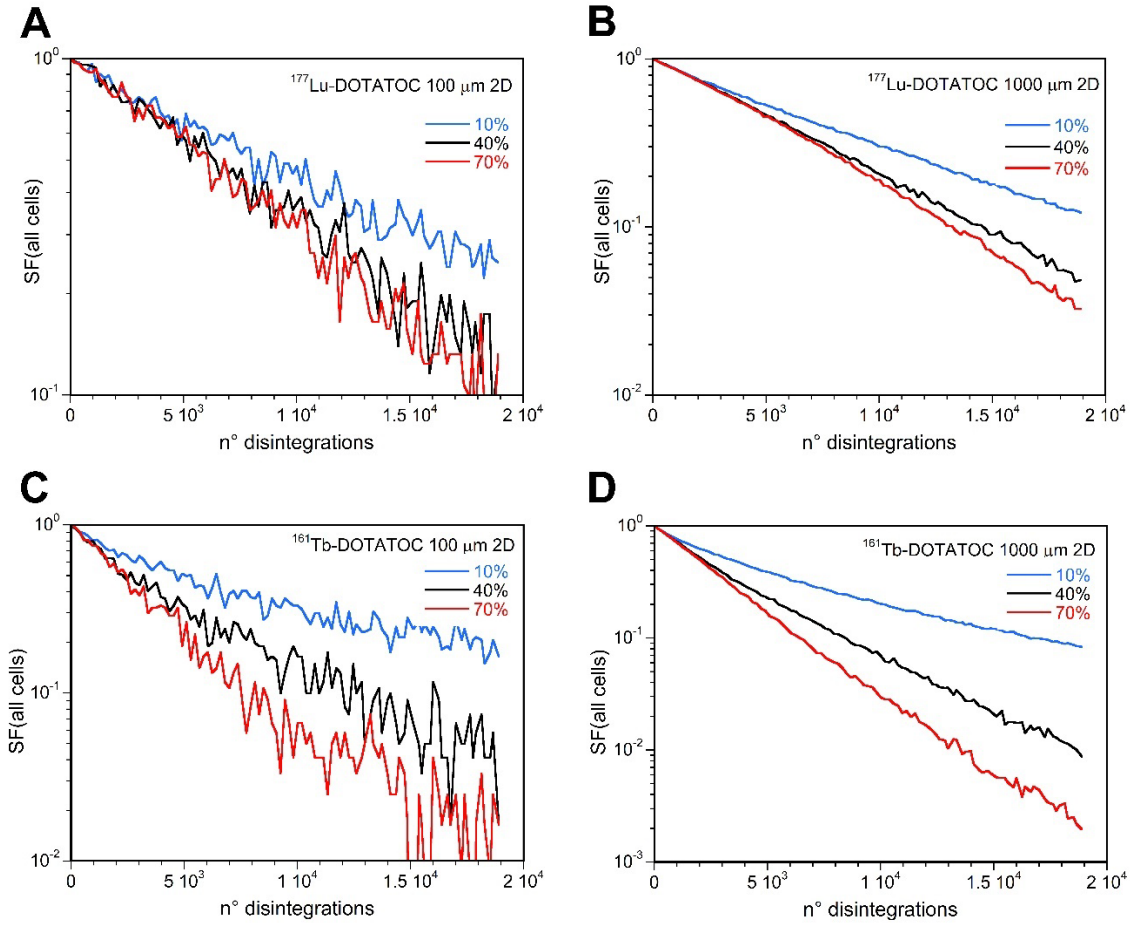

Figure S7. Survival fraction versus the mean number of disintegrations per cell for 100 and 1000 µm colony considering 10, 40 and 70% of labeled cells after ^{177}Lu-DOTATOC treatment (A and B) and ^{161}Tb-DOTATOC treatment (C and D).

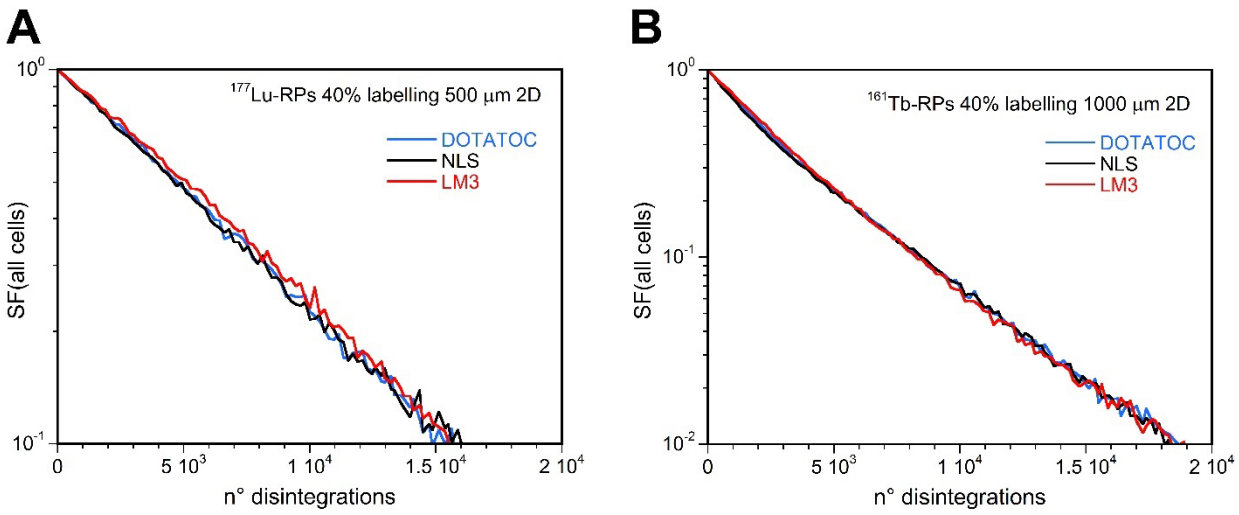

Figure S8. Comparison of the survival fraction versus the mean number of disintegrations per cell after treatment of a A) 500 µm radius colony with ^{177}Lu-RPs and B) 1000 µm radius colony with ^{161}Tb-RPs, considering 40% labeled cells in both cases.

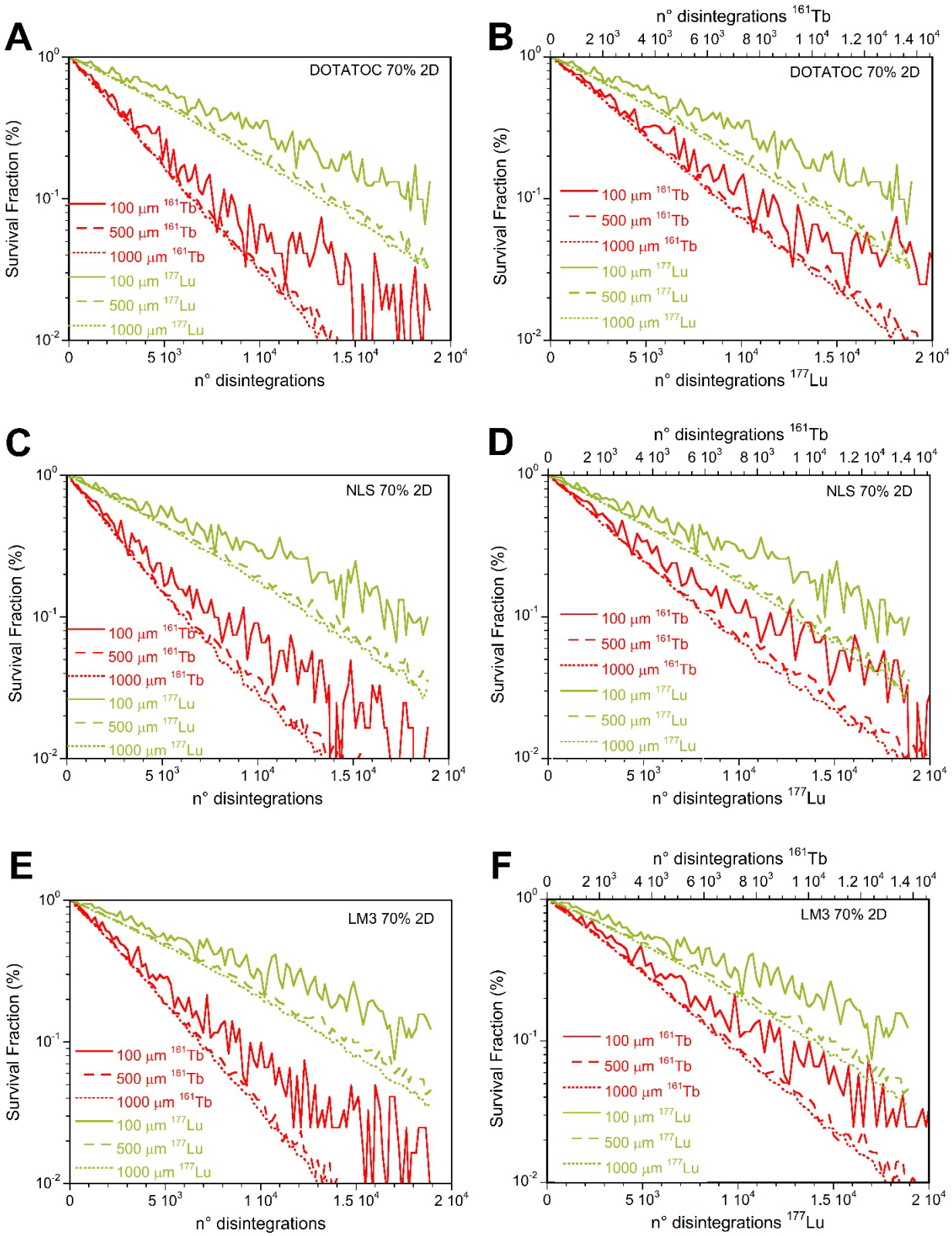

Figure S9. Comparison of the survival fraction in the 2D model after administration of ^{177}Lu-RPs and ^{161}Tb-RPs in cell colony of 100, 500 and 1000 µm considering different percentage of labeled cells and the same mean number of disintegrations per cell for the two radionuclides (A, C, E) or by scaling the mean number of disintegrations of ^{161}Tb by a factor of 1.37 (B, D,F).