Supplementary Information：

Genetic load and potential mutational meltdown in cancer cell populations

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Supplementary material includes:

Supplementary Figures S1-S5

Supplementary Tables S1-S5

Supplementary Figures：

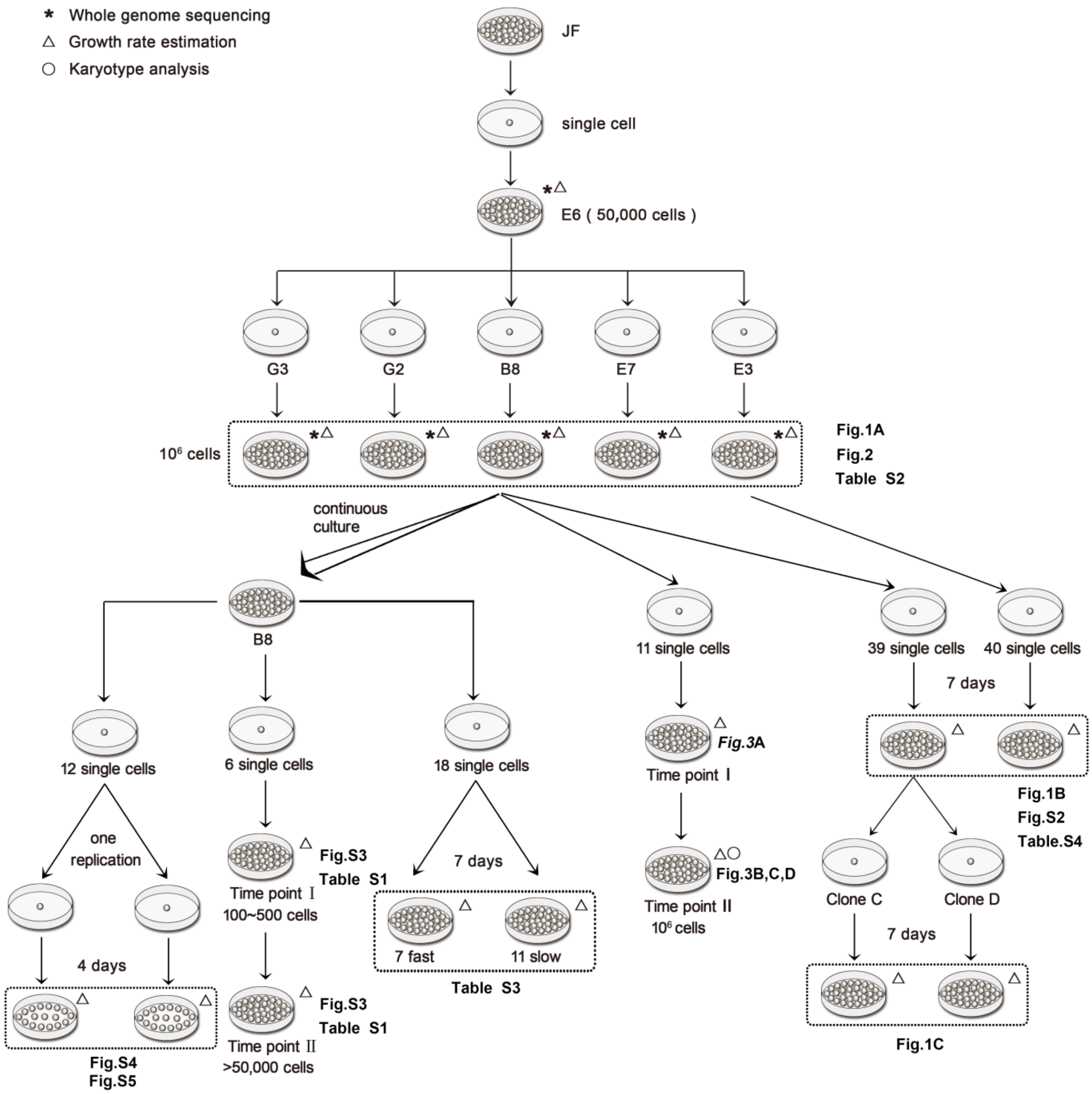

Fig. S1. Cell culture and experimental scheme. A single cell (E6) was randomly drew from a HeLa cell line (JF) and defined as the ancestral clone. Five clones (B8, G3, E3, E7, G2) were random chosen from E6 when the population size of E6 reached up to > 50,000 cells. The clones B8 and E3 kept cultured for further experiments which are shown in the schematic diagram and described in the *Results* and *Materials and Methods*.

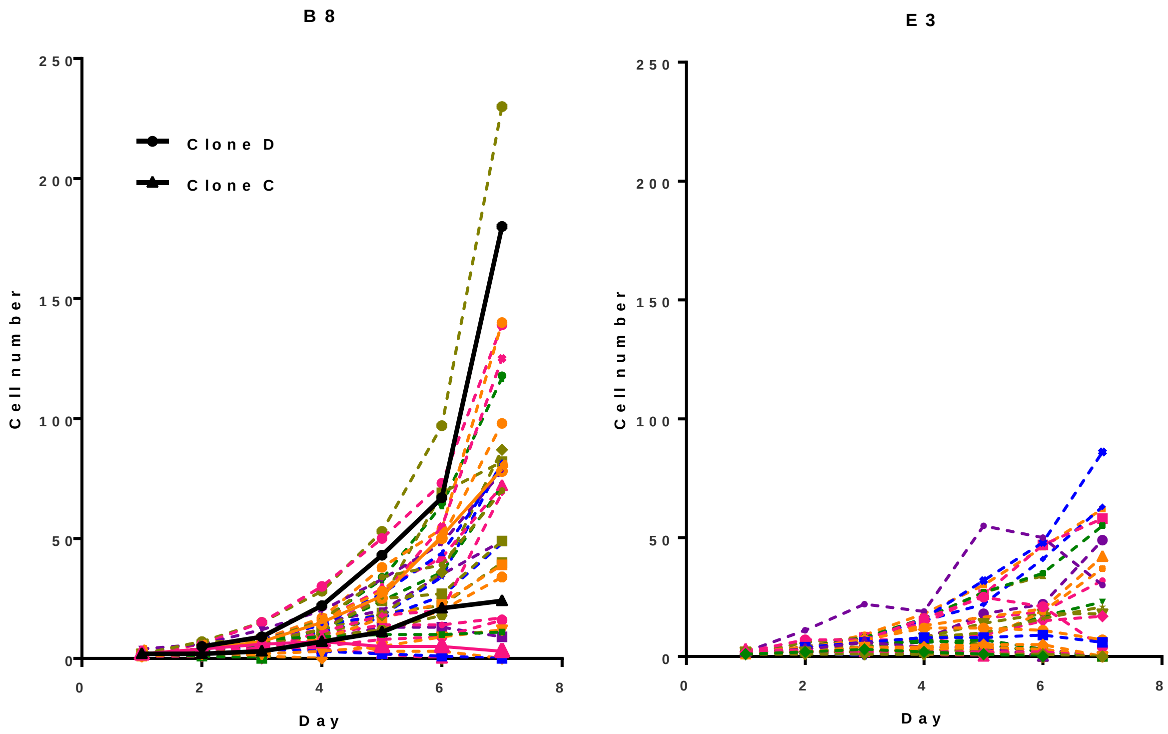

Fig. S2. Growth curves of cell clones from B8 (left) and E3 (right). Each curve represents a single cell clone from day 1 to day 7.

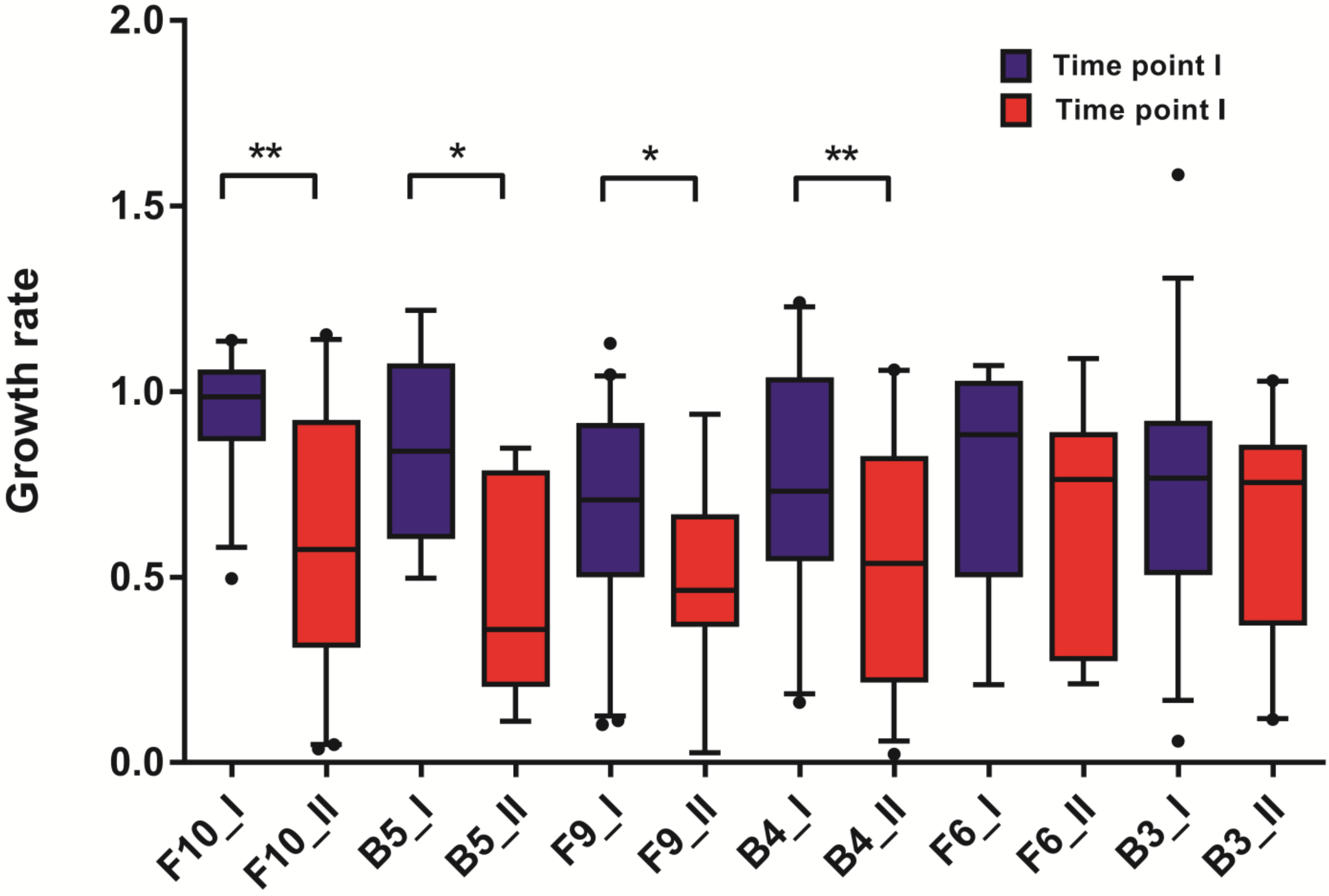

Fig. S3. The growth rates of six clones (F10 to B3, labeled on the x-axis) from B8 at the time point I and II. When cell numbers of these six single cell clones reached to 100-500 (after approximately 7-10 cell divisions; Table S1), 20~30% of cells from each clone were randomly drawn and separated into single cells. The growth of these cells was monitored for 8 days (time point I, blue boxplots). When cell numbers reached to at least 5000 cells (time point II, red boxplots), a number of single cell clones (Table S1) were generated, and the cell growth for those clones was monitored again. *: P-value < 0.05; **: P-value < 0.01, *t*-test.

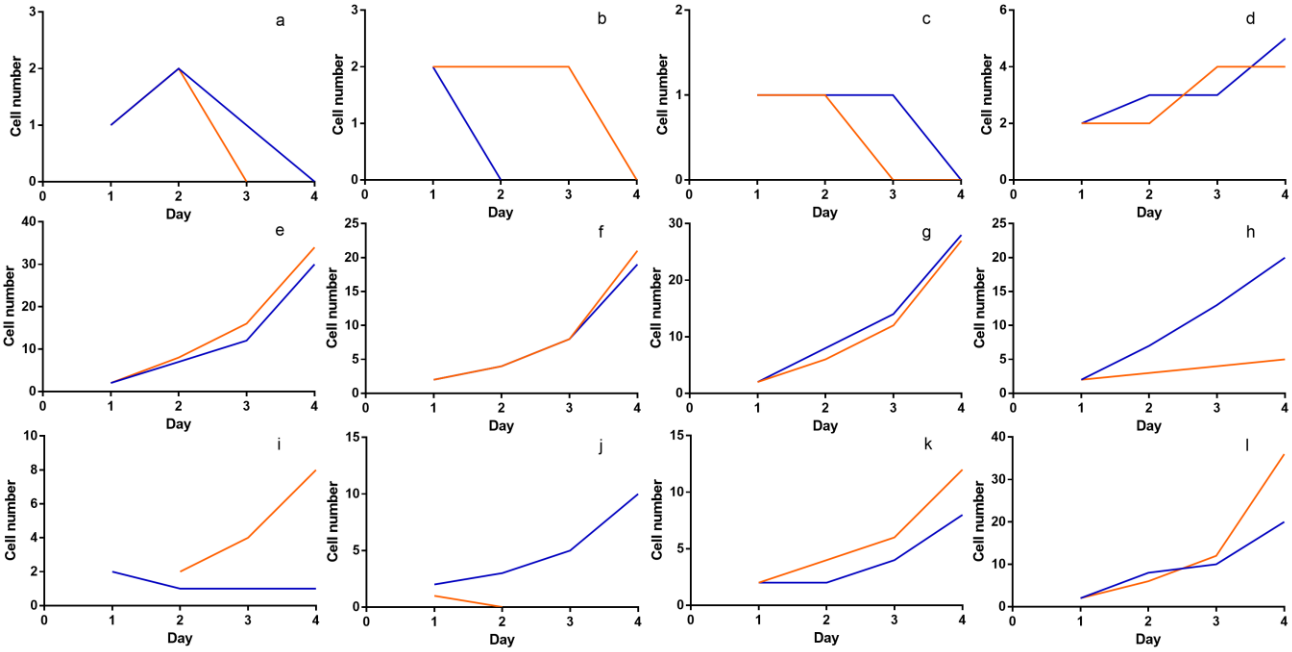

Fig. S4. Proliferation rate differences between pairs of daughter cells. Growth graph of two daughter cells (blue and orange line) derived from a single cell. The number (y-axis) of cells in 12 pairs (a to l) of daughter cells was counted every day for four days.

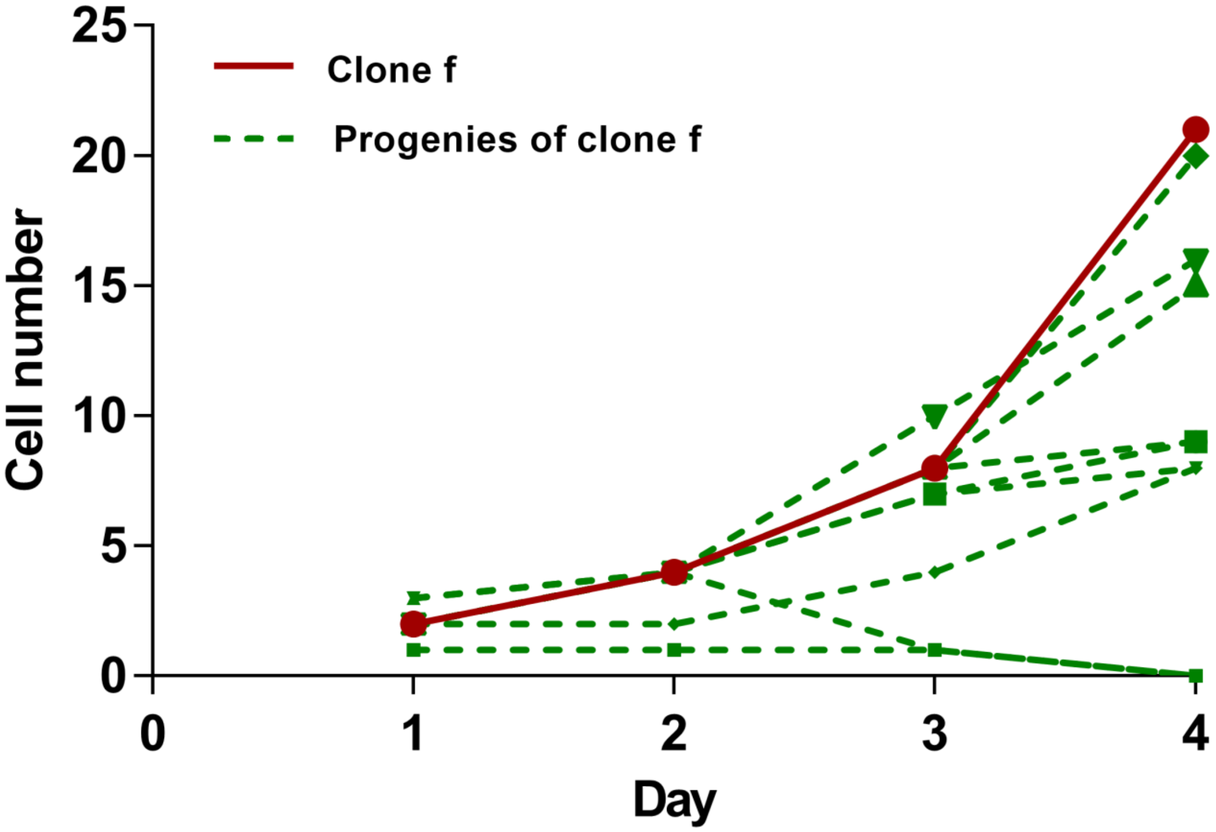

Fig. S5. Proliferation rate differences between clone f and its progenies. Growth graph of progeny cells from clone f. The progeny cells were isolated from clone f and monitored for four days. Maroon solid line: clone f; Green dashed line: progenies of clone f.

Supplementary Tables：

Table S1. Number of clones that were generated from six clones at time point I and II

Clones from B8[a]	Time point	Cell number[b]	Clone number[c]
F10	I	182	61
	II	5000	81
F6	I	133	27
	II	54000	18
E3	I	271	74
	II	27100	33
F9	I	430	62
	II	16500	23
B4	I	100	33
	II	10000	33
B5	I	100	18
	II	10000	12

[a] Single cell isolations and growth rate measurements were performed for the 6 cell clones (B4 ~ F10) at the two time points. See Fig. S3 and Results for the details of the growth rate measurement.

[b] The number of cells in the six clones at the time point I and II.

[c] The number of clones that were randomly isolated and generated from the 6 clones at the time point I and II.

Table S2. Copy number gain and loss of five descendant clones compared to their ancestor clone (E6).

Descendant clones from E6	Chromosome	Start	End	Length	Median ratio	Copy number gain/ loss
B8	17	0	18,000,000	18,000,000	1.30	gain
	18	24,300,000	70,850,000	46,550,000	1.36	gain
G3	5	0	47,000,000	47,000,000	0.73	loss
	18	30,900,001	78,077,248	47,177,247	1.30	gain
E3	2	0	43,000,000	43,000,000	0.71	loss
	3	185,650,000	197,800,000	12,150,000	0.80	loss
	4	0	16,700,000	16,700,000	0.78	loss
	4	170,350,000	190,850,000	20,500,000	1.45	gain
	5	15,600,000	38,600,000	23,000,000	0.77	loss
	10	35,650,000	53,660,000	18,010,000	0.79	loss
	10	105,250,000	123,850,000	18,600,000	0.79	loss
	15	90,450,000	102,531,392	12,081,392	1.30	gain
	X	0	33,950,000	33,950,000	0.55	loss
G2	1	30,550,000	119,600,000	89,050,000	0.79	loss
	4	0	178,750,000	178,750,000	0.74	loss
	5	0	46,400,000	46,400,000	0.64	loss
	6	0	25,350,000	25,350,000	1.38	gain
	10	0	17,200,000	17,200,000	0.80	loss
E7	4	0	16,700,000	16,700,000	0.80	loss
	8	2,500,000	19,150,000	16,650,000	0.75	loss
	11	117,950,000	135,006,516	17,056,516	0.72	loss
	14	0	40,860,000	40,860,000	0.73	loss
	14	52,550,000	107,349,540	54,799,540	0.71	loss
	20	15,000,000	29,800,000	14,800,000	0.51	loss
	20	29,800,000	60,700,000	30,900,000	0.79	loss
	X	0	24,000,000	24,000,000	0.80	loss

Table S3. The number of cells in single cell clones[a] from B8 and parameter estimation[b].

	Clone	Day							Parameter estimation			
		1	2	3	4	5	6	7	R_0	μ	s	$\mu \times s$
Fast growing cells	#1	2	4	8	15	30	45	101	2.22	0.26	0.17	0.04
	#2	2	4	8	20	52	99	182	2.48	0.29	0.18	0.05
	#3	2	5	10	23	51	84	150	2.41	0.30	0.19	0.06
	#4	2	4	8	20	38	57	107	2.29	0.30	0.18	0.05
	#5	2	4	14	32	61	112	190	2.52	0.29	0.20	0.06
	#6	2	4	12	20	41	100	187	2.44	0.26	0.17	0.04
	#7	2	4	8	20	33	55	104	2.26	0.30	0.17	0.05
Slow growing cells	#8	2	4	7	18	23	35	66	2.13	0.31	0.18	0.06
	#9	2	4	7	17	27	46	88	2.19	0.27	0.17	0.05
	#10	2	4	7	13	19	27	46	1.99	0.27	0.17	0.05
	#11	2	4	6	10	17	30	61	2.04	0.25	0.16	0.04
	#12	2	4	8	15	27	48	82	2.17	0.27	0.19	0.05
	#13	2	4	4	5	6	9	11	1.66	0.32	0.18	0.06
	#14	1	4	6	9	15	25	34	1.96	0.29	0.20	0.06
	#15	2	3	5	7	11	13	20	1.76	0.28	0.20	0.06
	#16	2	2	4	8	13	19	23	1.89	0.31	0.20	0.06
	#17	2	4	8	11	18	26	32	1.97	0.33	0.18	0.06
	#18	2	4	8	15	30	51	88	2.21	0.27	0.18	0.05

[a] Single cells were randomly drawn from B8, followed by cell culture for seven days. The cell numbers in the single cell clones were counted every day. According to their cell numbers on the 7th day, newly derived cell clones are grouped into fast (cell number > 100) and slow (cell number < 100) growing clones.

[b] The Approximate Bayesian Computation (ABC) method was performed to estimate R_0, u and s for each clone (see *Materials and Methods*).

Table S4. R_0 estimation with constant μ and s.

	Clone	Observed data							R_0	Expected data							Chi-square test（P-value)
		1	2	3	4	5	6	7		1	2	3	4	5	6	7	
B8	#1	2	7	7	16	34	39	70	2.13	2.1	4.3	8.2	14.9	25.6	41.8	64.5	0.672
	#2	2	4	8	9	10	10	11	1.64	1.6	2.5	3.8	5.2	6.9	8.7	10.4	0.468
	#4	4	6	12	20	33	48	78	2.18	2.2	4.5	8.8	16.4	28.8	48.0	75.9	0.773
	#5	-	4	4	11	18	20	69	2.16	2.2	4.4	8.6	15.8	27.5	45.4	71.2	5.208e-08***
	#6	2	4	8	16	28	50	98	2.26	2.3	4.8	9.8	18.9	34.5	59.6	97.7	0.611
	#8	2	4	8	16	29	42	72	2.17	2.2	4.5	8.7	16.0	28.1	46.7	73.5	0.994
	#9	1	2	2	3	5	9	12	1.67	1.7	2.6	4.0	5.6	7.6	9.7	11.8	0.387
	#11	2	3	6	12	24	36	72	2.18	2.2	4.5	8.8	16.4	28.8	48.0	75.9	0.182
	#12	2	4	8	14	18	34	82	2.20	2.2	4.6	9.1	17.0	30.1	50.7	80.9	0.008**
	#13	2	4	8	15	20	35	49	2.04	2.0	3.9	7.2	12.6	20.7	32.3	47.7	0.994
	#14	-	4	8	12	26	55	125	2.33	2.3	5.1	10.8	21.4	40.2	71.6	120.9	6.323e-04***
	#16	-	4	7	12	24	69	82	2.23	2.2	4.7	9.4	17.9	32.3	55.0	89.0	0.071
	#17	2	6	15	30	50	73	139	2.36	2.4	5.3	11.2	22.5	42.8	77.3	132.3	0.594
	#18	1	4	3	8	17	23	39	1.97	2.0	3.7	6.5	10.9	17.4	26.2	37.4	0.352
	#19	2	4	7	9	12	18	-	1.82	1.8	3.1	5.1	8.0	11.7	16.3	21.5	0.961
	#20	2	4	7	15	26	51	79	2.19	2.2	4.5	8.9	16.7	29.5	59.4	78.4	0.852
	#21	2	4	8	16	33	64	117	2.31	2.3	5.1	10.5	20.6	38.5	68.0	113.9	0.717
	#23	2	2	3	7	11	21	24	1.89	1.9	3.4	5.7	9.3	14.1	20.4	27.9	0.458
	#24	2	3	6	10	14	14	17	1.76	1.8	2.9	4.6	7.0	9.9	13.3	17.0	0.865
	#26	2	7	15	28	53	97	230	2.54	2.5	6.1	14.0	30.2	61.8	120.2	221.3	0.252
	#27	-	4	5	5	8	9	16	1.68	1.7	2.7	4.0	5.8	7.8	10.1	12.3	0.883
	#28	1	3	6	8	12	20	34	1.92	1.9	4.5	6.0	9.9	15.3	22.4	31.2	0.752
	#29	2	4	8	12	19	22	40	1.94	1.9	3.6	6.2	10.3	16.1	23.9	33.5	0.884
	#32	2	4	6	10	18	36	87	2.21	2.2	4.6	9.2	17.3	30.8	52.1	83.5	6.214e-04***
	#33	2	4	7	12	25	27	49	1.99	2.0	3.8	6.7	11.4	18.3	27.8	40.0	0.741
	#35	-	5	9	22	43	67	180	2.45	2.5	5.7	12.5	26.1	51.6	96.8	171.9	3.548e-03**
	#36	2	4	8	14	27	44	80	2.19	2.2	4.5	8.9	16.7	29.5	59.4	78.4	0.385
	#37	2	4	8	17	38	54	140	2.37	2.4	5.3	11.3	22.9	43.7	79.3	136.2	0.011*
	#38	2	3	6	11	17	26	48	2.01	2.0	3.8	6.9	11.8	19.2	29.5	43.0	0.946
E3	#1	1	2	3	8	16	19	32	1.93	1.9	3.5	6.1	10.1	15.7	23.1	32.4	0.361
	#2	2	4	9	18	30	46	62	2.12	2.1	4.3	8.1	14.6	25.1	40.6	62.4	0.901
	#4	2	2	4	8	9	17	23	1.83	1.8	3.2	5.2	8.1	12.0	16.8	22.3	0.908
	#7	-	4	8	14	26	47	58	2.10	2.1	4.2	7.9	14.1	23.9	38.4	58.4	0.882
	#13	2	4	8	16	27	35	55	2.06	2.1	4.0	7.4	13.1	21.7	34.2	51.1	0.928
	#14	2	4	7	16	32	48	86	2.21	2.2	4.6	9.2	17.3	30.8	52.1	83.5	0.967
	#18	-	4	6	8	10	16	21	1.77	1.8	3.0	4.7	7.1	10.1	13.8	17.7	0.918
	#22	2	4	8	15	22	41	63	2.11	2.1	4.2	8.0	14.4	24.5	39.5	60.4	0.998
	#30	2	4	8	13	-	20	42	1.96	2.0	3.6	6.4	10.7	16.9	25.4	36.0	0.687
	#31	2	4	4	8	14	18	18	1.78	1.8	3.0	4.8	7.3	1.1	14.2	18.4	0.039*
	#34	2	4	7	7	9	15	17	1.74	1.7	2.9	4.5	6.6	9.3	12.4	15.7	0.935
	#35	1	2	2	4	7	19	37	1.97	2.0	3.7	6.5	10.9	17.4	26.2	37.4	1.364e-07***
	#38	2	2	4	7	18	22	49	2.04	2.0	3.9	7.2	12.6	20.7	32.3	47.7	0.028*
	#40	1	6	8	16	27	34	-	2.11	2.1	4.2	8.0	14.4	24.5	39.5	60.4	0.695

NOTE. — Using ABC method with the fixed μ (0.29) and s (0.18), R_0 was estimated from the cell clones derived from B8 and E3. The expected data were calculated from equation (1) using the estimated R_0. Chi-square test was used to test whether the expected data are fitted with the observed data. *, P-value < 0.05; **, P-value < 0.01; ***, P-value < 0.001.

Table S5. Cell numbers of single cell clones in different cancer cell lines.

Cell line	Clone	Day							Parameter estimation			
		1	2	3	4	5	6	7	R_0	μ	s	$\mu \times s$
PC3 (Prostate cancer)	#1	2	2	4	4	10	13	-	1.79	0.30	0.18	0.05
	#2	1	2	4	7	6	13	25	1.87	0.28	0.18	0.05
	#3	2	4	4	8	11	17	-	1.85	0.31	0.20	0.06
	#4	2	2	4	9	15	32	55	2.11	0.27	0.17	0.05
	#5	1	2	2	5	7	11	18	1.80	0.29	0.18	0.05
	#6	1	1	2	3	3	8	13	1.69	0.28	0.18	0.05
	#7	1	1	2	2	4	8	15	1.68	0.27	0.17	0.05
	#8	1	2	3	3	7	13	23	1.83	0.28	0.16	0.04
	#9	1	2	2	4	8	12	25	1.84	0.26	0.17	0.04
	#10	1	2	4	4	8	13	18	1.80	0.29	0.18	0.05
A204 (Rhabdosarcoma)	#1	3	4	7	8	13	21	26	1.89	0.33	0.21	0.07
	#2	2	2	2	3	6	13	26	1.87	0.28	0.18	0.05
	#3	2	2	3	9	12	15	16	1.83	0.33	0.22	0.07
	#4	2	3	6	7	14	23	30	1.92	0.30	0.20	0.06
	#5	2	4	6	7	13	21	26	1.89	0.31	0.20	0.06
	#6	2	4	9	15	18	28	40	2.07	0.32	0.21	0.07
	#7	1	2	4	4	8	11	20	1.81	0.28	0.18	0.05
A375 (Melanoma)	#1	2	3	6	16	24	29	36	2.14	0.34	0.23	0.08
	#2	2	4	14	28	59	97	117	2.54	0.34	0.23	0.08
	#3	2	5	10	23	54	91	172	2.42	0.28	0.17	0.05
	#4	2	3	6	9	17	46	115	2.28	0.27	0.17	0.05
	#5	1	4	6	13	32	67	129	2.33	0.30	0.16	0.05
	#6	2	2	8	14	27	50	86	2.20	0.29	0.18	0.05
	#7	2	4	6	13	25	52	101	2.20	0.27	0.17	0.05
	#8	1	4	6	20	37	74	117	2.41	0.33	0.20	0.07
	#9	2	3	4	11	14	25	38	1.95	0.29	0.18	0.05
	#10	2	4	8	17	47	114	201	2.48	0.28	0.17	0.05
	#11	2	5	13	22	37	56	103	2.31	0.30	0.20	0.06
	#12	2	4	10	22	52	137	262	2.51	0.26	0.18	0.05
	#13	2	5	14	32	62	166	298	2.64	0.29	0.18	0.05
	#14	2	4	7	16	39	75	116	2.32	0.30	0.18	0.05
	#15	1	5	13	18	29	66	124	2.35	0.30	0.18	0.05
	#16	1	5	9	19	38	94	184	2.47	0.28	0.18	0.05
	#17	2	4	10	22	42	89	193	2.49	0.29	0.18	0.05