

Supplementary Information for
PRMT1 reverts the immune escape of necroptotic colon cancer through RIP3
methylation

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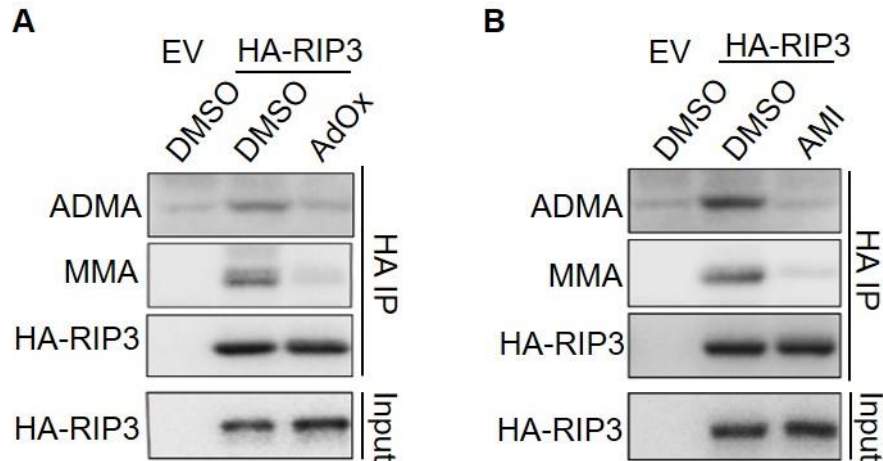


Figure S1 RIP3 undergoes arginine methylation. (A) The methylation of overexpressed RIP3 in HEK293T cells treated with or without AdOx was analyzed by immunoprecipitation and immunoblot. (B) The methylation of overexpressed RIP3 in HEK293T cells treated with or without AMI-1 was analyzed by immunoprecipitation and immunoblot.

HA-RIP3 IP Mass Spectrum

sp P07437 TUBB5_HUMAN	Tubulin beta chain OS=Homo sapiens OX=9606 GN=TUBB PE=1 SV=2	
sp Q9Y572 RIPK3_HUMAN	Receptor-interacting serine/threonine-protein kinase 3 OS=Homo sapiens OX=9606 GN=RIPK3 PE=1 SV=2	RIP3
sp Q71U36 TBA1A_HUMAN	Tubulin alpha-1A chain OS=Homo sapiens OX=9606 GN=TUBA1A PE=1 SV=1	
sp P63261 ACTG_HUMAN	Actin, cytoplasmic 2 OS=Homo sapiens OX=9606 GN=ACTG1 PE=1 SV=1	
sp Q96S66 CLCC1_HUMAN	Chloride channel CLIC-like protein 1 OS=Homo sapiens OX=9606 GN=CLCC1 PE=1 SV=1	
sp P09211 GSTP1_HUMAN	Glutathione S-transferase P OS=Homo sapiens OX=9606 GN=GSTP1 PE=1 SV=2	
sp P08670 VIME_HUMAN	Vimentin OS=Homo sapiens OX=9606 GN=VIM PE=1 SV=4	
sp Q99873 ANM1_HUMAN	Protein arginine N-methyltransferase 1 OS=Homo sapiens OX=9606 GN=PRMT1 PE=1 SV=3	PRMT1
sp Q9BY77 PDIP3_HUMAN	Polymerase delta-interacting protein 3 OS=Homo sapiens OX=9606 GN=POLDIP3 PE=1 SV=2	
sp P61247 RS3A_HUMAN	40S ribosomal protein S3a OS=Homo sapiens OX=9606 GN=RPS3A PE=1 SV=2	
sp P31943 HNRH1_HUMAN	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens OX=9606 GN=HNRNPH1 PE=1 SV=4	
sp Q53Q00 DHB12_HUMAN	Very-long-chain 3-oxoacyl-CoA reductase OS=Homo sapiens OX=9606 GN=HSD17B12 PE=1 SV=2	

Figure S2 PRMT1 interacts with RIP3. Mass spectrum identified PRMT1 as a RIP3 interaction protein.

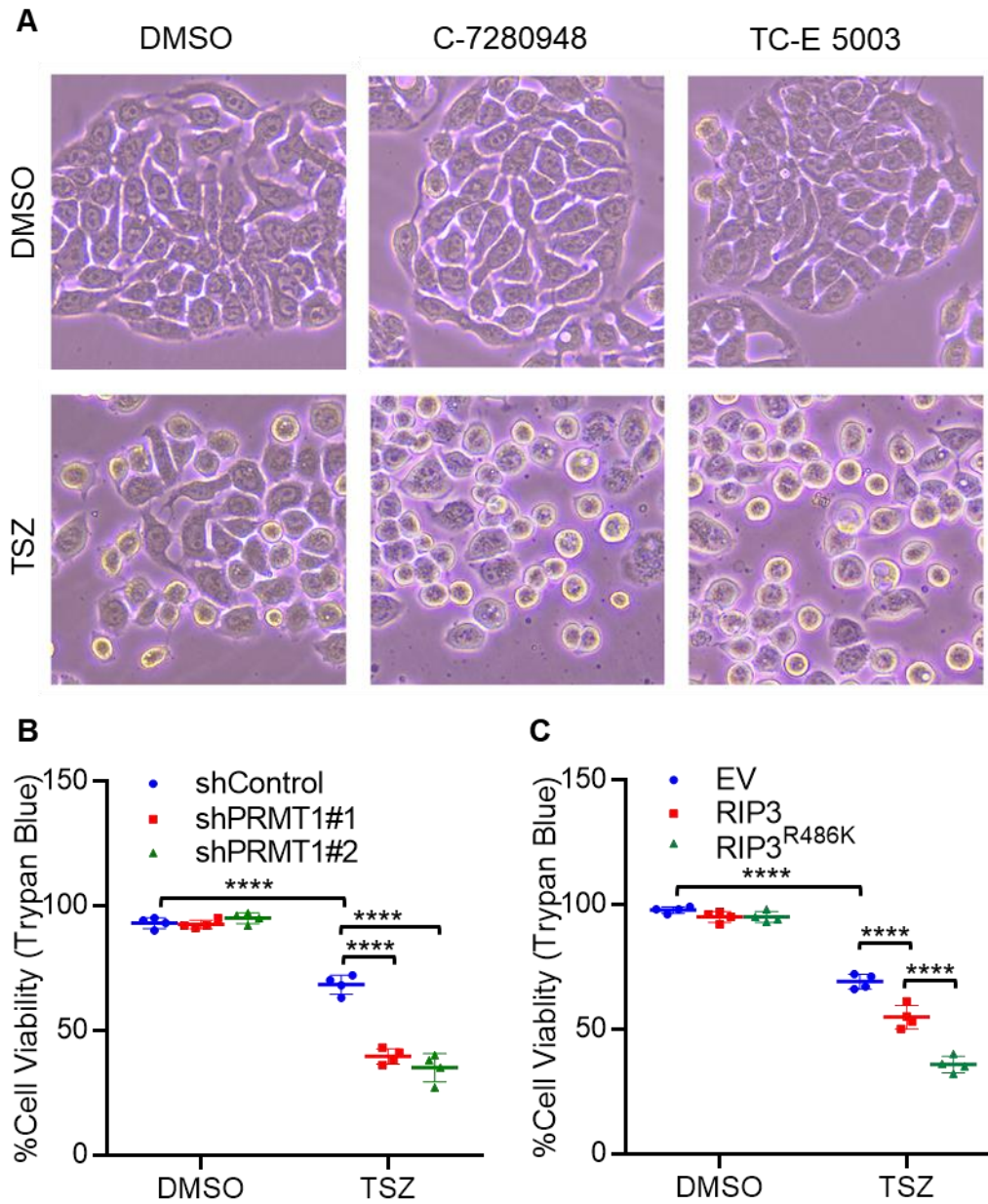


Figure S3 PRMT1 inhibits necroptosis. (A) The morphology of HT29 cells treated with or without TSZ and PRMT1 inhibitors C-7280948 (100nM), TC-E 5003 (25nM) for 8 hours. (B) The control and PRMT1 stable knockdown HT29 cells were treated with or without TSZ for 16 hours. Cell viability was calculated by Trypan Blue staining. n=4. **** $P < 0.0001$. (C) The control (EV), RIP3 and RIP3^{R486K} stable overexpression HT29 cells were treated with or without TSZ for 16 hours. Cell viability was calculated by Trypan Blue staining. n=4. **** $P < 0.0001$.

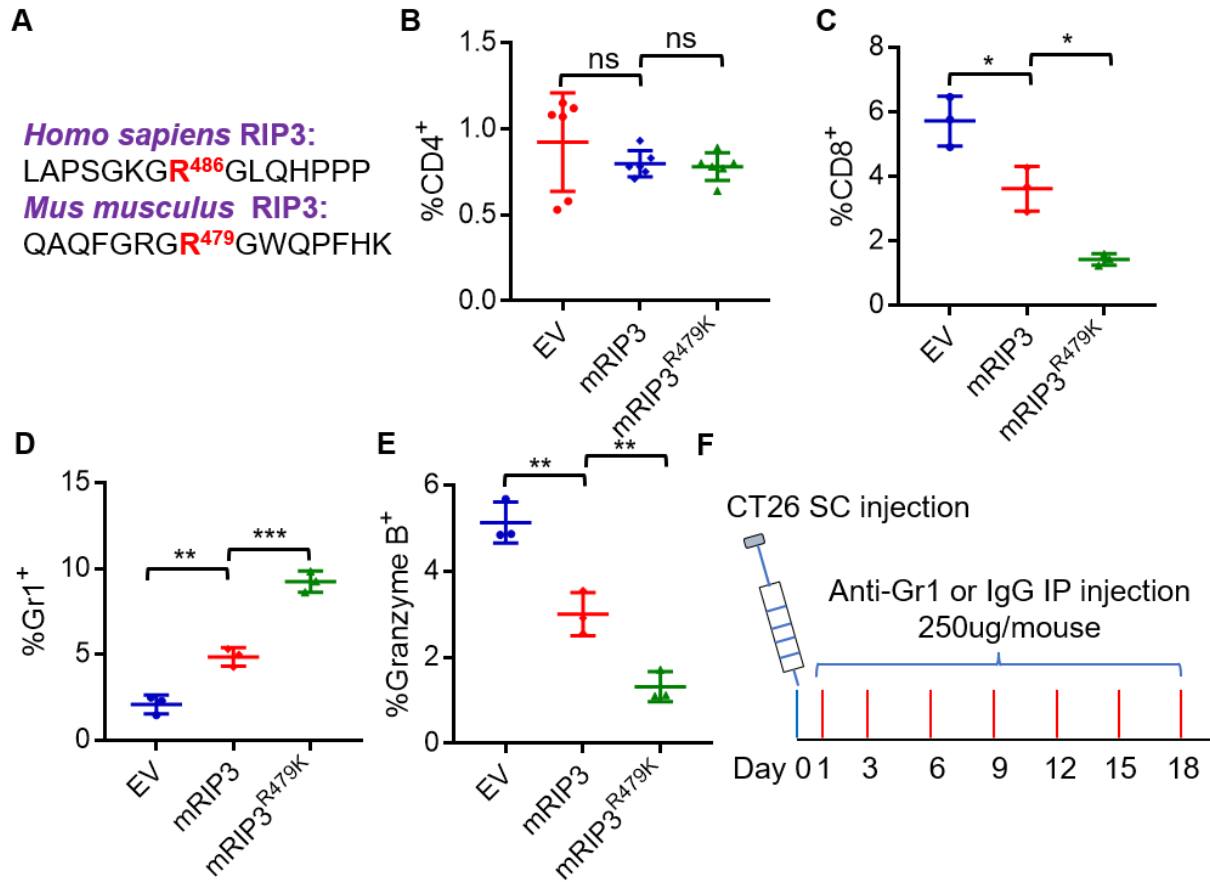


Figure S4 RIP3 methylation promotes anti-tumor immunity. (A) Sequence alignment identified murine RIP3 R479 as the homologous amino acid of human RIP3 R486. (B) Flowcytometry statistical analysis of CD4⁺ T cell percentage in the control (EV), mRIP3, mRIP3^{R479K} stable overexpression CT26 tumors. n=6. ns, not significant. (C) Immunohistochemistry statistical analysis of CD8⁺ T cell percentage in the control (EV), mRIP3, mRIP3^{R479K} stable overexpression CT26 tumors. n=3. *P<0.05, *P<0.05. (D) Immunohistochemistry statistical analysis of Gr⁺ MDSC percentage in the control (EV), mRIP3, mRIP3^{R479K} stable overexpression CT26 tumors. n=3. **P<0.01, ***P<0.001. (E) Immunohistochemistry statistical analysis of Granzyme B⁺ cytotoxic cell percentage in the control (EV), mRIP3, mRIP3^{R479K} stable overexpression CT26 tumors. n=3. **P<0.01, ** P <0.01. (F) *In vivo* treatment of anti-Gr1 antibody was performed by intraperitoneal (IP) injection with the dose of 250ug/mouse at day 1,3, 6, 9, 12, 15, 18 after subcutaneously (SC) tumor inoculation.

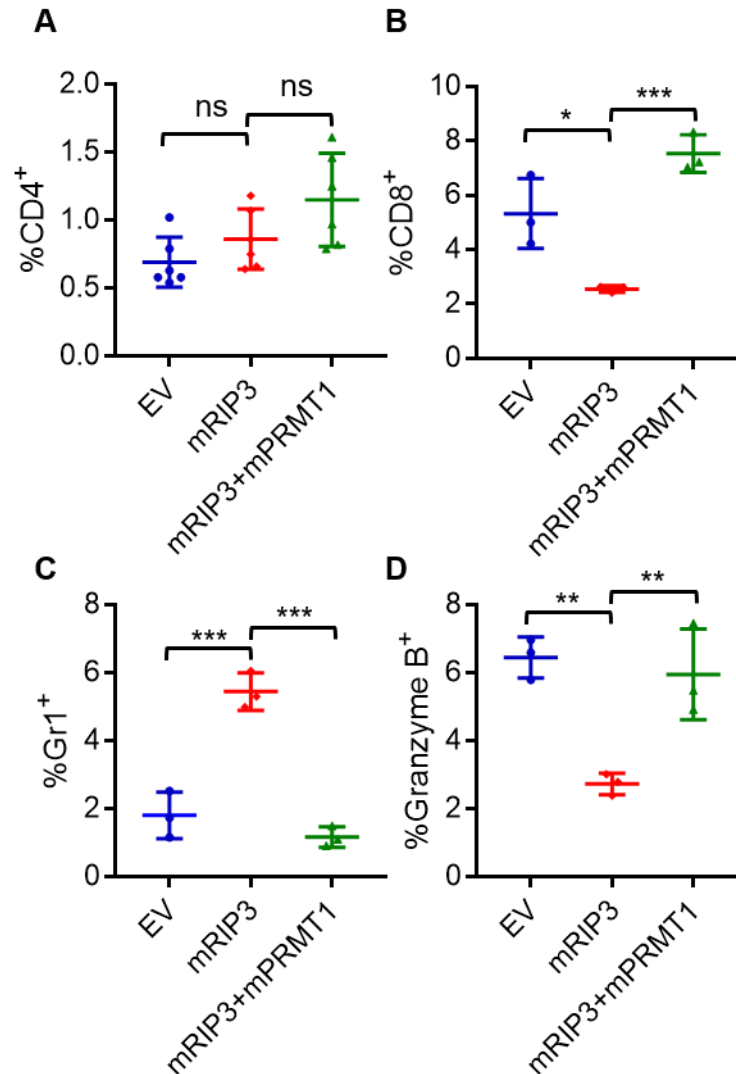


Figure S5 PRMT1 promotes anti-tumor immunity. (A) Flowcytometry statistical analysis of CD4⁺ T cell percentage in the control (EV), mRIP3, mRIP3+mPRMT1 stable overexpression CT26 tumors. n=6. ns, not significant. (B) Immunohistochemistry statistical analysis of CD8⁺ T cell percentage in the control (EV), mRIP3, mRIP3+mPRMT1 stable overexpression CT26 tumors. n=3. **P*<0.05, ****P*<0.001. (C) Immunohistochemistry statistical analysis of Gr⁺ MDSC percentage in the control (EV), mRIP3, mRIP3+mPRMT1 stable overexpression CT26 tumors. n=3. ****P*<0.001, ****P*<0.001. (D) Immunohistochemistry statistical analysis of Granzyme B⁺ cytotoxic cell percentage in the control (EV), mRIP3, mRIP3/mPRMT1 stable overexpression CT26 tumors. n=3. ***P*<0.01, ***P*<0.01.

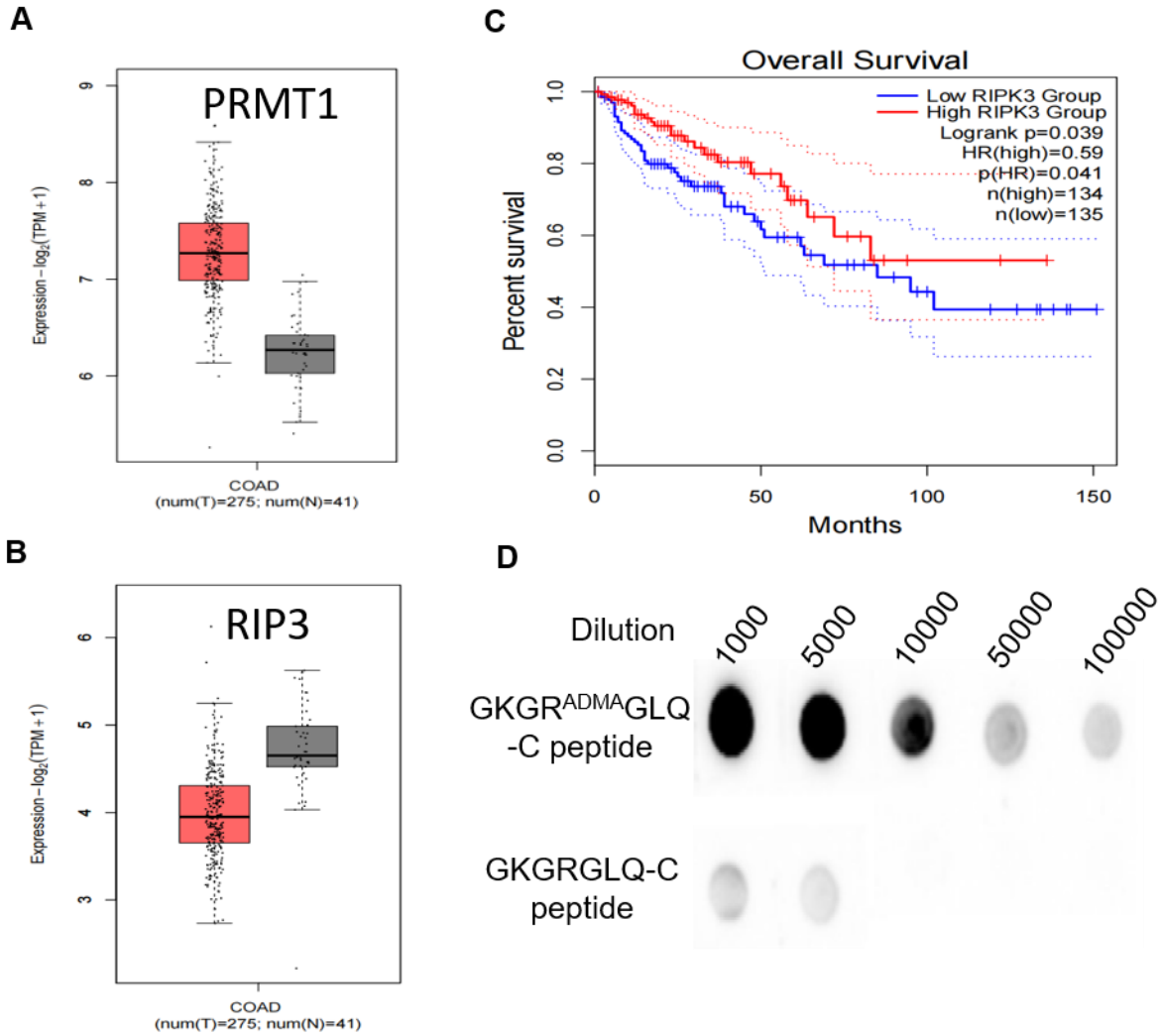


Figure S6 Clinical analysis of PRMT1 and RIP3 methylation in colon cancer patient samples. (A) GEPIA analysis of PRMT1 mRNA expression in colon carcinoma tissues and adjacent normal tissues in TCGA database. T, tumor tissues; N, adjacent normal tissues. (B) GEPIA analysis of RIP3 mRNA expression in colon carcinoma tissues and adjacent tissues in TCGA database. T, tumor tissues; N, adjacent normal tissues. (C) GEPIA overall survival analysis of colon carcinoma patients with low and high RIP3 mRNA expression in TCGA database. (D) Dot blot verification of RIP3 R486 methylation specific antibody RIP3^{ADMA}.

Table S1.**List of colon cancer patients.**

Patient No.	Live-0, Dead-1	Live months	Gender	Age	T	N	M	Stage
1	1	13	Female	47	T4b	N0	M0	2
2	1	12	Male	65	T4b	N2	M0	3
3	0	68	Male	71	T3	N0	M0	2
4	0	68	Male	58	T3	N0	M0	2
5	1	17	Male	49	T3	N0	M1	4
6	0	68	Male	72	T2	N0	M0	1
7	0	68	Male	57	T4a	N1	M0	3
8	1	16	Male	83	T3		M0	
9	1	27	Female	66	T3		M0	
10	1	37	Female	60	T3	N1	M0	3
11	1	26	Male	47	T4b	N1	M1	4
12	1	25	Male	57	T3	N0	M1	4
13	0	68	Female	68	T3	N0	M0	2
14	0	68	Male	59	T3	N0	M0	2
15	1	14	Female	58	T3	N2	M0	3
16	1	8	Male	81	T3	N1	M0	3
17	1	8	Female	81	T3	N0	M0	2
18	0	67	Male	60	T3	N0	M0	2
19	1	24	Male	22	T3	N1	M0	3
20	1	31	Female	80	T3	N2	M0	3
21	1	29	Male	84	T3	N0	M0	2
22	1	65	Male	78	T3	N0	M0	2
23	1	56	Male	82	T3	N0	M0	2
24	0	67	Male	53	T3	N0	M0	2
25	1	42	Male	83	T3	N0	M0	2
26	1	5	Male	57	T3	N1	M0	3
27	1	23	Female	64	T4b	N0	M0	2
28	0	67	Female	63	T4a	N0	M0	2
29	0	67	Male	53	T3	N1	M0	3
30	1	47	Female	81	T3	N1	M0	3
31	0	67	Male	74	T4a	N0	M0	2
32	0	66	Male	60	T3	N0	M0	2
33	0	66	Female	78	T3	N0	M0	2
34	1	18	Female	55	T3	N1	M0	3
35	0	66	Female	51	T3	N0	M0	2
36	1	16	Female	53	T4a	N0	M0	2
37	1	20	Male	56	T3	N2	M0	3
38	1	45	Male	68	T3	N1	M0	3
39	1	25	Female	62	T3	N2	M0	3

40	1	23	Male	77	T3	N0	M0	2
41	0	65	Male	45	T2	N0	M0	1
42	1	34	Male	79	T4a	N0	M0	2
43	0	65	Female	41	T3	N1	M0	3
44	0	65	Male	82	T3	N0	M0	2
45	1	1	Male	70	T3	N1	M0	3
46	0	65	Male	46			M0	
47	1	46	Female	78	T3	N1	M1	4
48	1	26	Male	59	T3	N1	M0	3
49	0	65	Male	52	T3	N0	M0	2
50	0	65	Male	69	T3	N0	M0	2
51	1	27	Female	75	T4b	N2	M0	3
52	0	65	Male	70	T3	N1	M0	3
53	1	25	Male	72	T3	N0	M0	2
54	1	42	Female	61	T3	N1	M0	3
55	0	65	Male	78	T3	N1	M0	3
56	0	65	Male	73	T2	N0	M0	1
57	1	33	Male	85	T2	N0	M0	1
58	1	14	Female	56	T3	N0	M0	2
59	0	64	Male	45	T3	N1	M0	3
60	1	30	Female	86	T3	N1	M0	3
61	0	64	Male	76	T3	N0	M0	2
62	1	15	Female	71	T4b	N1	M0	3
63	1	32	Male	80	T3	N1	M0	3
64	0	64	Female	70		N0	M0	
65	1	16	Male	68	T3	N1	M0	3
66	0	64	Male	50	T3	N0	M0	2
67	0	64	Female	70	T3	N0	M0	2
68	0	63	Male	56		N0	M0	
69	0	63	Male	85	T3	N0	M0	2
70	0	63	Female	71		N0	M0	
71	0	63	Female	77		N1	M0	3
72	0	63	Female	61	T4a	N0	M0	2
73	0	63	Female	60	T2		M0	
74	1	17	Male	80	T3	N0	M0	2
75	0	63	Female	84	T3	N0	M0	2
76	0	63	Female	63	T3	N0	M0	2
77	1	1	Female	81		N1	M0	3
78	1	62	Female	87	T3	N0	M0	2
79	0	63	Male	58	T3	N0	M0	2
80	1	2	Female	58		N1	M0	3
81	0	62	Female	53	T4b		M0	
82	1	15	Female	93	T4b	N0	M0	2
83	0	62	Female	22	T3	N1	M0	3
84	1	27	Female	87	T4a	N1	M0	3

85	1	21	Male	82	T3	N2	M0	3
86	1	48	Male	80		N0	M0	
87	1	48	Female	85	T3	N0	M0	2
88	1	5	Male	55	T3	N2	M0	3
89	1	7	Female	76	T4b	N2	M0	3
90	1	5	Male	64	T3	N0	M0	2
91	1	67	Female	64	T3	N0	M0	2
92	1	18	Female	67	T3	N0	M0	2
93	1	12	Male	80	T3	N1	M0	3