

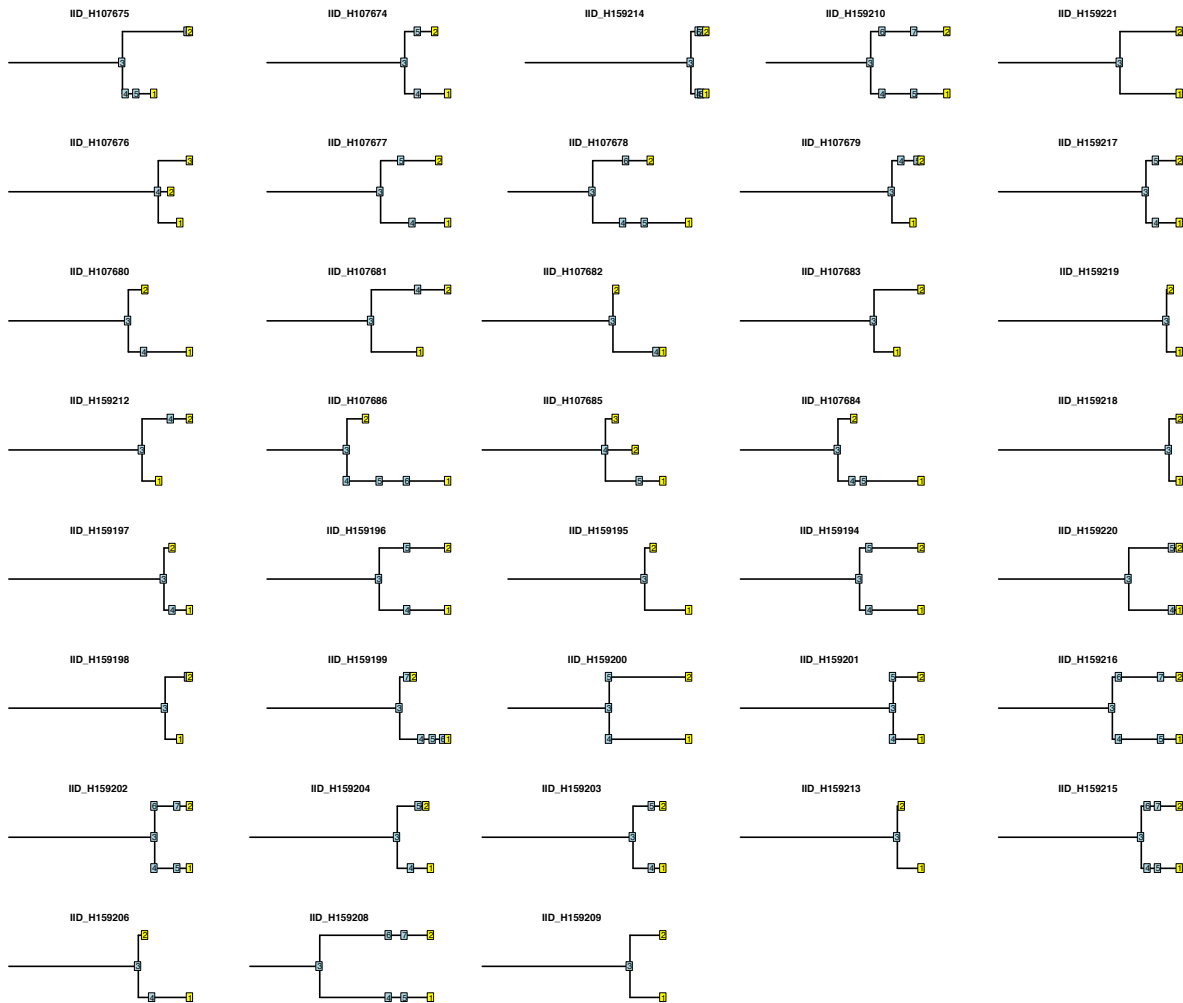
SUPPLEMENTARY INFORMATION

Accelerated single cell seeding in relapsed multiple myeloma

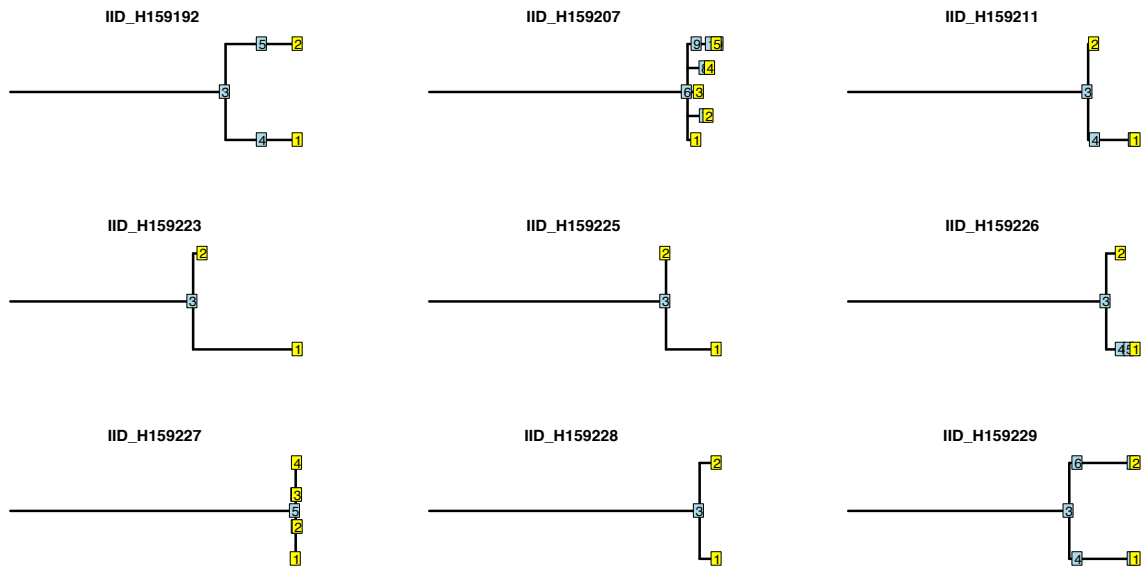
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Supplementary Figures

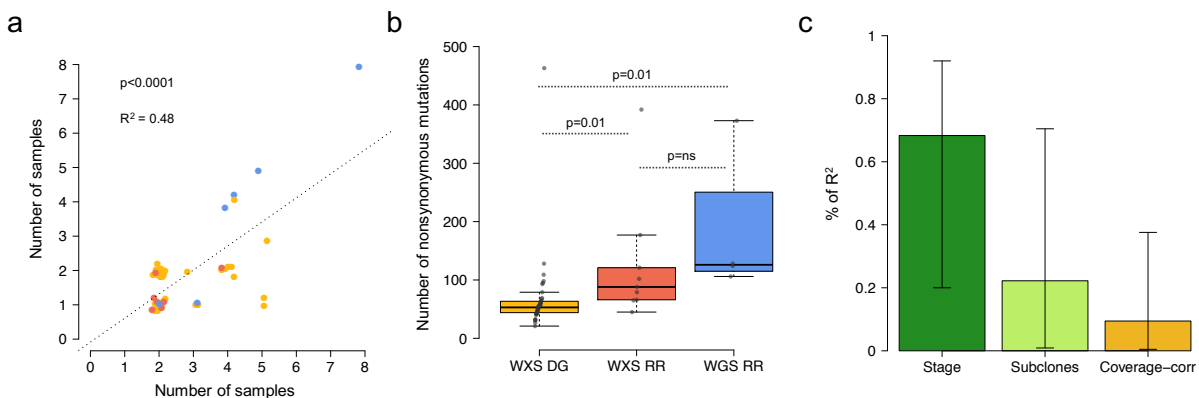
Supplementary Figure 1. Phylogenetic tree reconstruction of each newly diagnosed patient included in the WXS cohort. Early and late cluster numbers are reported with blue and yellow rectangle, respectively.



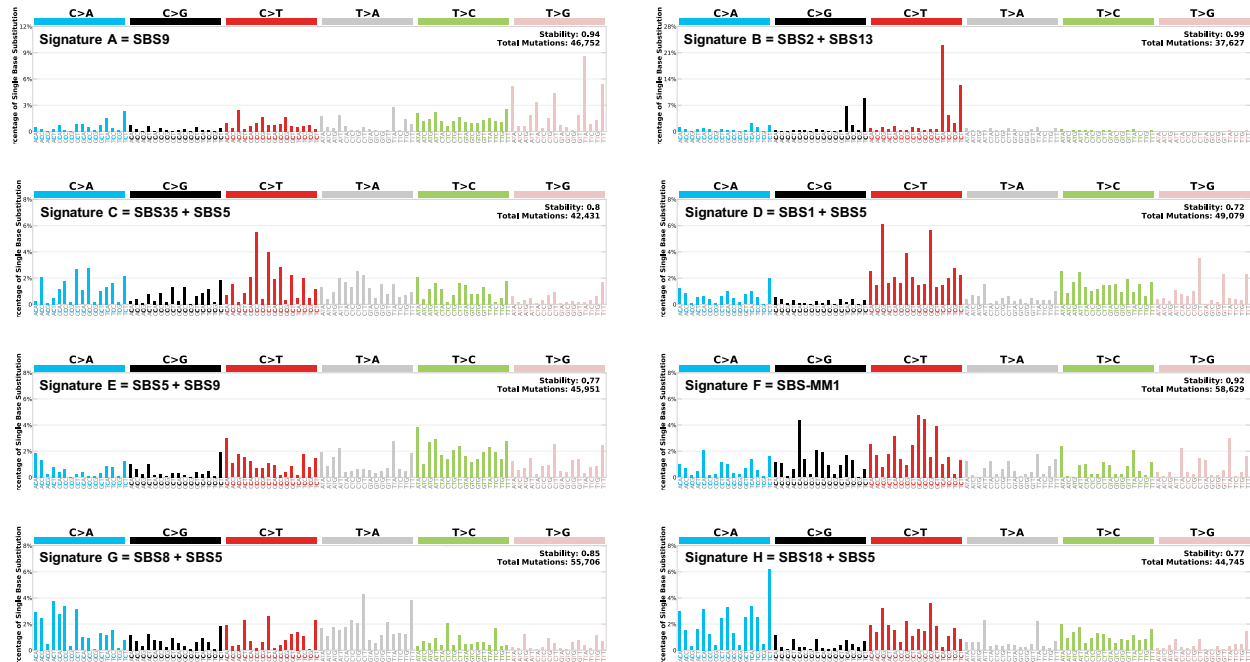
Supplementary Figure 2. Phylogenetic tree reconstruction of all patients with at least one sample collected at relapse included in the WXS cohort. Early and late cluster numbers are reported with blue and yellow rectangle, respectively.



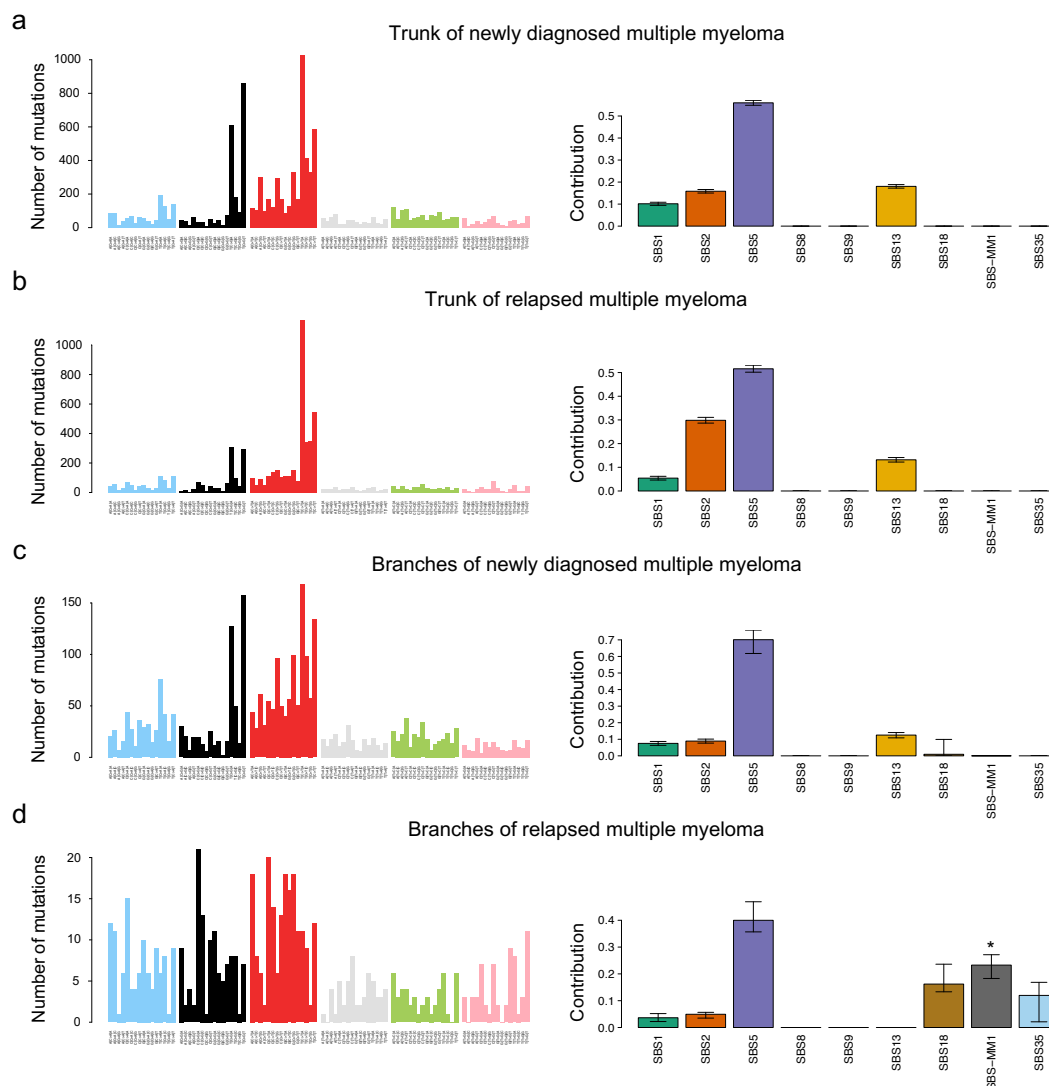
Supplementary Figure 3. Increased burden of nonsynonymous SNVs in relapsed multiple myeloma. a) Linear regression between the number of samples and evolutionary trajectories (number of clones); Blue, red and yellow dots represent relapsed WGS, relapsed WXS and newly diagnosed WXS samples, respectively. *lm* R package was used to estimate the p value ($p < 0.0001$) and R square ($R^2 = 0.48$). b) Boxplot showing increased burden of nonsynonymous SNVs in relapsed multiple myeloma. p-values were estimated using Wilcoxon (*pairwise.wilcox.test* R function). WGS RR = whole genome sequencing at relapsed, WXS DG = whole exome sequencing at diagnosis, WXS RR = whole exome sequencing at relapsed. Boxplots show the median and interquartile range; observations outside this interval are shown as dots. c) Relative contribution to the R square in a multivariate linear regression model shown in (a). Estimates and 95%CI were generated using the *relaimpo* R package.



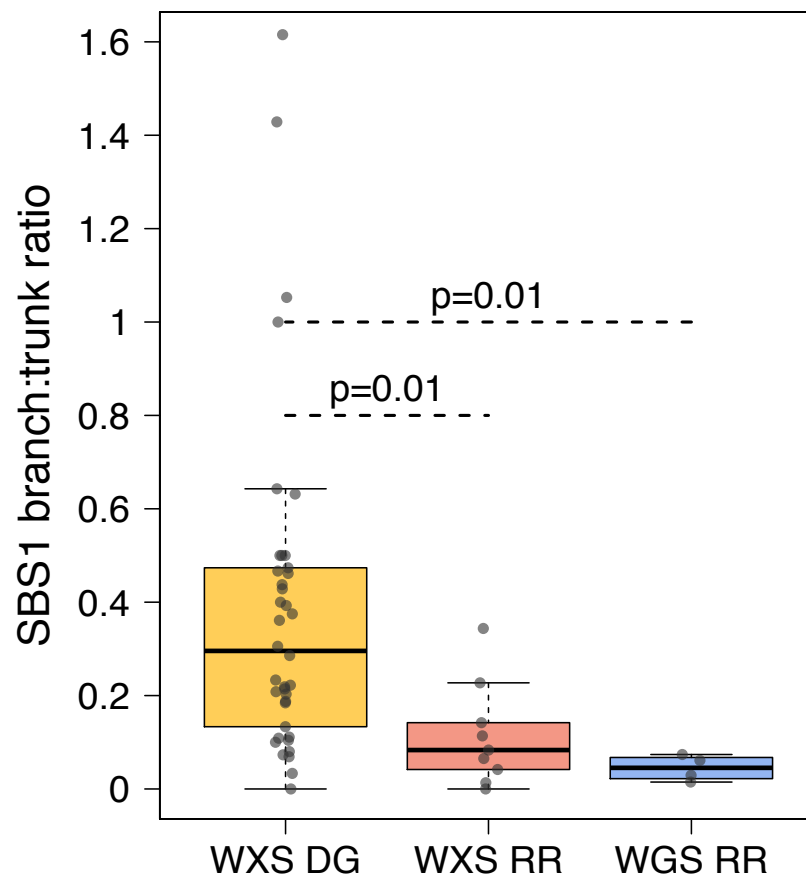
Supplementary Figure 4. The 8 mutational signatures extracted by SigProfiler. On the top left of each 96-mutational profile is annotated the signature(s) that contribute to the profile.



Supplementary Figure 5. The 96-mutational profile and mutational contribution of the trunks and the branches of all newly diagnosed and relapsed multiple myeloma included in the WXS cohort. The asterisk in d reflect the presence of transcriptional strand bias for SBS-MM1. Confidence interval of each mutational signature was generated by drawing 1000 mutational profiles from the multinomial distribution, each time repeating the signature fitting procedure, and finally taking the 2.5th and 97.5th percentile for each signature.



Supplementary Figure 6. Difference in SBS1 branches:trunk ratio between WXS at diagnosis, whole genome (WGS) and exome (WXS) relapsed multiple myeloma. p-value were estimated using Wilcoxon test ($p=0.01$). DG= diagnosis; RR= relapse. Boxplots show the median and interquartile range; observations outside this interval are shown as dots.



Supplementary Tables

Supplementary Table 1. WGS cohort demographic data.

Patients	Gender	Isotype	Age
I-H-106917	Male	IgG Kappa	53
I-H-130718	Male	IgG Kappa	60
I-H-130719	Male	IgG Lambda	54
I-H-130720	Male	IgA Kappa	64

Supplementary Table 2. Coverage, purity and ploidy of the WGS cohort.

Sample	Purity	Ploidy	Median Coverage
I-H-106917-T2-1-D1-2	0.6678	2.53	88.3745
I-H-106917-T2-2-D1-2	0.76726185	2.89423837	129.051
I-H-106917-T2-3-D1-2	0.78763	2.6048	101.449
I-H-106917-T2-4-D1-2	0.77841	2.4564	145.224
I-H-130718-T1-1-D1-2	0.94771604	2.15905083	89.9814
I-H-130718-T1-10-D1-2	0.9108	2.15	88.266
I-H-130718-T1-11-D1-1	0.74708	2.1824	121.605
I-H-130718-T1-12-D1-1	0.7	2.25	132.268
I-H-130718-T1-2-D1-2	0.92814	2.15	98.4156
I-H-130718-T1-4-D1-2	0.92	2.15	87.7814
I-H-130718-T1-6-D1-2	0.96419548	2.24056118	89.4443
I-H-130718-T1-9-D1-2	0.82908	2.2	89.8941
I-H-130719-T1-2-D1-2	0.8159	2.35	109.137
I-H-130719-T1-4-D1-2	0.2755	2.4	92.357
I-H-130719-T1-5-D1-2	0.69156	2.35	89.085
I-H-130719-T1-6-D1-2	0.68136	2.4	99.8962
I-H-130720-T1-2-D1-2	0.97924447	2.04353711	91.9502
I-H-130720-T1-3-D1-2	0.9598966	2.01730807	93.0744
I-H-130720-T1-4-D1-2	0.92907	2.05	88.1103
I-H-130720-T1-5-D1-2	0.97575174	2.05223504	88.2675
I-H-130720-T1-8-D1-2	0.95006146	2.02236114	88.3816
I-H-130720-T1-9-D1-1	0.26769043	2.8866776	107.828

Supplementary Table 3. Clinical and spatial characteristic of the whole exome cohort.

Study ID	Sample	Stage	Platinum-based	Melphalan-based	Posterior iliac crest	Biopsy
1	IID_H107686	Diagnosis	-	-	left	L4
2	IID_H107681	Diagnosis	-	-	left	Ilium
3	IID_H107677	Diagnosis	-	-	right	Symphysis
4	IID_H107675	Diagnosis	-	-	right	Ilium
5	IID_H107685	Diagnosis	-	-	right	Ilium
6	IID_H107683	Diagnosis	-	-	left	Sacrum
7	IID_H107679	Diagnosis	-	-	right	T8, Ilium
8	IID_H107682	Diagnosis	-	-	left	Rib, Pelvis, L1
9	IID_H107678	Diagnosis	-	-	left	Ilium
10	IID_H107684	Diagnosis	-	-	right	L1
11	IID_H107674	Diagnosis	-	-	left	L4
12	IID_H107676	Diagnosis	-	-	left	T5, Sacrum, Ilium x 2
13	IID_H107680	Diagnosis	-	-	right	T8
14	IID_H159193	Diagnosis	-	-	right	Iliac
15	IID_H159194	Diagnosis	-	-	right	Sacrum, Sacrum
16	IID_H159195	Diagnosis	-	-	right	T5
17	IID_H159196	Diagnosis	-	-	left	Acetabulum
18	IID_H159197	Diagnosis	-	-	left	Ilium
19	IID_H159198	Diagnosis	-	-	right	Clavicle
20	IID_H159199	Diagnosis	-	-	right	T12, T8, Acetabulum
21	IID_H159200	Diagnosis	-	-	left	L3
22	IID_H159201	Diagnosis	-	-	left	L5
23	IID_H159202	Diagnosis	-	-	left	Sacrum
24	IID_H159203	Diagnosis	-	-	right	T7
25	IID_H159204	Diagnosis	-	-	left	T8, L2, L3
26	IID_H159205	Diagnosis	-	-	left	Sacrum
27	IID_H159206	Diagnosis	-	-	left	L1
28	IID_H159207	Diagnosis	-	-	left	Ischium, Ilium
28	IID_H159207	Diagnosis	no	no	right	Pelvis
29	IID_H159208	Diagnosis	-	-	left	Sacrum
30	IID_H159209	Diagnosis	-	-	left	Anterior
31	IID_H159210	Diagnosis	-	-	left	Ilium, T10, L1
48	IID_H159227	Diagnosis	no	no	left	Ischium, Ilium, T8
32	IID_H159211	Diagnosis	-	-	right	Sacrum
33	IID_H159212	Diagnosis	-	-	right	Sacrum
34	IID_H159213	Diagnosis	-	-	right	Pleural
35	IID_H159214	Diagnosis	-	-	left	Posterior
38	IID_H159217	Diagnosis	-	-	right	Sacrum
39	IID_H159218	Diagnosis	-	-	right	Sacrum
40	IID_H159219	Diagnosis	-	-	right	Ilium
41	IID_H159220	Diagnosis	-	-	left	T8
42	IID_H159221	Diagnosis	-	-	left	Posterior
45	IID_H159224	Relapse	yes	no	-	T3 x2
32	IID_H159211	Relapse	yes	yes	right	Sacrum
43	IID_H159222	Relapse	yes	yes	right	Sacrum, T9
44	IID_H159223	Relapse	yes	yes	right	Sacrum

46	IID_H159225	Relapse	yes	yes	left	L4
47	IID_H159226	Relapse	yes	yes	left	Sacrum
49	IID_H159228	Relapse	yes	yes	left	Right
50	IID_H159229	Relapse	yes	yes	right	L2, Ilium
51	IID_H159192	Relapse	yes	yes	right	T12

Supplementary Table 4. Coverage and purity characteristics of the whole exome cohort.

Sample	Patients	Study ID	Purity	Ploidy	Median Coverage
IID_H107674_T05_01_WE01	IID_H107674	11	0.8002272	2.2694711	128
IID_H107674_T06_01_WE01	IID_H107674	11	0.6091187	2.2481825	116
IID_H107675_T05_01_WE01	IID_H107675	4	0.60931558	1.86780442	159
IID_H107675_T06_01_WE01	IID_H107675	4	0.50668536	1.93331034	99
IID_H107676_T05_01_WE01	IID_H107676	12	0.97001951	2.09682618	88
IID_H107676_T06_01_WE01	IID_H107676	12	0.96428541	2.09482283	82
IID_H107676_T07_01_WE01	IID_H107676	12	0.94218106	2.13657702	70
IID_H107676_T08_01_WE01	IID_H107676	12	0.95948579	2.12620523	87
IID_H107676_T09_01_WE01	IID_H107676	12	0.50818037	2.20878185	82
IID_H107677_T05_01_WE01	IID_H107677	3	0.8665444	2.05411238	73
IID_H107677_T06_01_WE01	IID_H107677	3	0.74822104	2.01652858	124
IID_H107678_T05_01_WE01	IID_H107678	9	0.94894169	1.97271947	80
IID_H107678_T06_01_WE01	IID_H107678	9	0.89273631	1.97978789	82
IID_H107679_T05_01_WE01	IID_H107679	7	0.5838939	2.3652043	59
IID_H107679_T06_01_WE01	IID_H107679	7	0.8354561	2.3706632	62
IID_H107679_T07_01_WE01	IID_H107679	7	0.8047266	2.3729548	62
IID_H107680_T05_01_WE01	IID_H107680	13	0.8438806	2.1559941	86
IID_H107680_T06_01_WE01	IID_H107680	13	0.8333836	2.1744959	91
IID_H107681_T05_01_WE01	IID_H107681	2	0.9630114	2.3173836	98
IID_H107681_T06_01_WE01	IID_H107681	2	0.6284588	2.5577561	103
IID_H107682_T05_01_WE01	IID_H107682	8	0.8939267	2.177801	82
IID_H107682_T06_01_WE01	IID_H107682	8	0.921053	2.1734415	83
IID_H107682_T07_01_WE01	IID_H107682	8	0.8197613	2.176688	125
IID_H107682_T08_01_WE01	IID_H107682	8	0.9217819	2.2564858	127
IID_H107683_T04_01_WE01	IID_H107683	6	0.7353756	2.5173613	110
IID_H107683_T05_01_WE01	IID_H107683	6	0.7644068	2.5169572	136
IID_H107684_T05_01_WE01	IID_H107684	10	0.73348935	1.9112504	90
IID_H107684_T06_01_WE01	IID_H107684	10	0.94217719	1.99039462	65
IID_H107685_T05_01_WE01	IID_H107685	5	0.8603543	2.6715024	111
IID_H107685_T06_01_WE01	IID_H107685	5	0.560074	2.3485615	73
IID_H107686_T05_01_WE01	IID_H107686	1	0.94766558	1.93627571	110
IID_H107686_T06_01_WE01	IID_H107686	1	0.6659103	2.2234326	100
IID_H159192_T01_01_WE01	IID_H159192	51	0.83804261	2.07896957	95
IID_H159192_T02_01_WE01	IID_H159192	51	0.82906367	2.08565467	95
IID_H159193_T01_01_WE01	IID_H159193	14	0.2853044	1.6461001	77
IID_H159193_T02_01_WE01	IID_H159193	14	0.2793778	1.70384482	77
IID_H159194_T01_01_WE01	IID_H159194	15	0.949149	2.1787565	71
IID_H159194_T02_01_WE01	IID_H159194	15	0.97119946	2.13396304	91

IID_H159194_T03_01_WE01	IID_H159194	15	0.90459305	2.14247739	79
IID_H159195_T01_01_WE01	IID_H159195	16	0.95128944	2.01012549	66
IID_H159195_T02_01_WE01	IID_H159195	16	0.93960744	1.99212335	85
IID_H159196_T01_01_WE01	IID_H159196	17	0.8384426	2.3350139	105
IID_H159196_T02_01_WE01	IID_H159196	17	0.4478289	2.29280087	84
IID_H159197_T01_01_WE01	IID_H159197	18	0.9224657	2.5700855	98
IID_H159197_T02_01_WE01	IID_H159197	18	0.8507106	2.539911	114
IID_H159198_T01_01_WE01	IID_H159198	19	0.94679	2.3540743	90
IID_H159198_T02_01_WE01	IID_H159198	19	0.8608714	2.3666977	90
IID_H159199_T01_01_WE01	IID_H159199	20	0.7013097	2.400672	96
IID_H159199_T02_01_WE01	IID_H159199	20	0.8488204	2.3950974	97
IID_H159199_T03_01_WE01	IID_H159199	20	0.9053691	2.3947028	82
IID_H159199_T04_01_WE01	IID_H159199	20	0.8603973	2.3947498	94
IID_H159200_T01_01_WE01	IID_H159200	21	0.96572423	2.04134535	133
IID_H159200_T02_01_WE01	IID_H159200	21	0.86389517	2.04768843	131
IID_H159201_T01_01_WE01	IID_H159201	22	0.49243245	1.99047269	143
IID_H159201_T02_01_WE01	IID_H159201	22	0.76782059	1.97094179	153
IID_H159202_T01_01_WE01	IID_H159202	23	0.8718508	2.206232	89
IID_H159202_T02_01_WE01	IID_H159202	23	0.7162207	2.2211288	123
IID_H159203_T01_01_WE01	IID_H159203	24	0.7806844	2.4000219	91
IID_H159203_T02_01_WE01	IID_H159203	24	0.8748083	2.4049286	95
IID_H159204_T01_01_WE01	IID_H159204	25	0.662403	2.230165	105
IID_H159204_T02_01_WE01	IID_H159204	25	0.68983017	2.19421531	133
IID_H159204_T03_01_WE01	IID_H159204	25	0.7695526	2.196245	126
IID_H159204_T04_01_WE01	IID_H159204	25	0.6537956	2.223384	117
IID_H159205_T01_01_WE01	IID_H159205	26	0.1108276	2.6798439	75
IID_H159205_T02_01_WE01	IID_H159205	26	0.95525302	2.06748533	78
IID_H159206_T01_01_WE01	IID_H159206	27	0.6056648	2.3623408	65
IID_H159206_T02_01_WE01	IID_H159206	27	0.7959656	2.3905351	77
IID_H159207_T01_01_WE01	IID_H159207	28	0.7339352	4.2380534	114
IID_H159207_T01_01_WE01	IID_H159207	28	0.7339352	4.2380534	114
IID_H159207_T02_01_WE01	IID_H159207	28	0.8440228	4.1297769	119
IID_H159207_T02_01_WE01	IID_H159207	28	0.8440228	4.1297769	119
IID_H159207_T03_01_WE01	IID_H159207	28	0.7653815	2.4118943	141
IID_H159207_T03_01_WE01	IID_H159207	28	0.7653815	2.4118943	141
IID_H159207_T04_01_WE01	IID_H159207	28	0.5479146	4.457577	103
IID_H159207_T04_01_WE01	IID_H159207	28	0.5479146	4.457577	103
IID_H159207_T05_01_WE01	IID_H159207	28	0.4643885	2.5469944	118
IID_H159207_T05_01_WE01	IID_H159207	28	0.4643885	2.5469944	118
IID_H159208_T01_01_WE01	IID_H159208	29	0.7877964	2.00249734	80
IID_H159208_T02_01_WE01	IID_H159208	29	0.79442943	1.9885857	84
IID_H159209_T01_01_WE01	IID_H159209	30	0.9637269	2.1989709	114

IID_H159209_T02_01_WE01	IID_H159209	30	0.7879392	2.1929875	126
IID_H159210_T01_01_WE01	IID_H159210	31	0.92712086	2.00858273	83
IID_H159210_T02_01_WE01	IID_H159210	31	0.93806964	1.97343159	107
IID_H159210_T03_01_WE01	IID_H159210	31	0.91711508	1.99323632	98
IID_H159210_T04_01_WE01	IID_H159210	31	0.86551271	1.96390226	120
IID_H159211_T01_01_WE01	IID_H159211	32	0.8783528	2.03188819	137
IID_H159211_T01_01_WE01	IID_H159211	32	0.8783528	2.03188819	137
IID_H159211_T02_01_WE01	IID_H159211	32	0.63023737	2.04069739	131
IID_H159211_T02_01_WE01	IID_H159211	32	0.63023737	2.04069739	131
IID_H159211_T03_01_WE01	IID_H159211	32	0.56188275	2.01511523	176
IID_H159211_T03_01_WE01	IID_H159211	32	0.56188275	2.01511523	176
IID_H159211_T04_01_WE01	IID_H159211	32	0.81070812	2.01084752	104
IID_H159211_T04_01_WE01	IID_H159211	32	0.81070812	2.01084752	104
IID_H159212_T01_01_WE01	IID_H159212	33	0.8652078	2.2682235	114
IID_H159212_T02_01_WE01	IID_H159212	33	0.5051255	2.27083046	115
IID_H159213_T01_01_WE01	IID_H159213	34	0.96980921	1.96990208	102
IID_H159213_T02_01_WE01	IID_H159213	34	0.75708204	1.97584576	125
IID_H159214_T01_01_WE01	IID_H159214	35	0.7750537	2.4587212	75
IID_H159214_T02_01_WE01	IID_H159214	35	0.7905361	2.461937	73
IID_H159214_T03_01_WE01	IID_H159214	35	0.7797573	2.4679043	70
IID_H159215_T01_01_WE01	IID_H159215	36	0.92918503	2.01415484	98
IID_H159215_T02_01_WE01	IID_H159215	36	0.69855941	2.01105364	99
IID_H159216_T01_01_WE01	IID_H159216	37	0.8330706	2.1822886	129
IID_H159216_T02_01_WE01	IID_H159216	37	0.82611333	2.15428486	135
IID_H159217_T01_01_WE01	IID_H159217	38	0.716789	1.9613428	133
IID_H159217_T02_01_WE01	IID_H159217	38	0.6839017	2.0669228	107
IID_H159218_T01_01_WE01	IID_H159218	39	0.94526328	1.93725482	88
IID_H159218_T02_01_WE01	IID_H159218	39	0.93183277	1.93351012	84
IID_H159219_T01_01_WE01	IID_H159219	40	0.89509	1.8119822	77
IID_H159219_T02_01_WE01	IID_H159219	40	0.572952	1.82642918	113
IID_H159220_T01_01_WE01	IID_H159220	41	0.8937164	2.2900012	99
IID_H159220_T02_01_WE01	IID_H159220	41	0.8173879	2.2983591	95
IID_H159221_T01_01_WE01	IID_H159221	42	0.8370796	2.6851918	117
IID_H159221_T02_01_WE01	IID_H159221	42	0.9003912	2.7185433	110
IID_H159222_T01_01_WE01	IID_H159222	43	0.743228	3.047736	77
IID_H159222_T02_01_WE01	IID_H159222	43	0.7252859	3.1409718	79
IID_H159222_T03_01_WE01	IID_H159222	43	0.79253508	1.86817258	84
IID_H159223_T01_01_WE01	IID_H159223	44	0.96868429	2.11916229	95
IID_H159223_T02_01_WE01	IID_H159223	44	0.47157677	2.22985945	77
IID_H159224_T01_01_WE01	IID_H159224	45	0.8738939	2.609495	66
IID_H159224_T02_01_WE01	IID_H159224	45	0.7960685	4.6081039	95
IID_H159225_T01_01_WE01	IID_H159225	46	0.5272777	1.9398966	102

IID_H159225_T02_01_WE01	IID_H159225	46	0.47170094	1.93603211	120
IID_H159226_T01_01_WE01	IID_H159226	47	0.92537319	1.99920882	96
IID_H159226_T02_01_WE01	IID_H159226	47	0.53255266	1.91373547	123
IID_H159227_T01_01_WE01	IID_H159227	48	0.9107675	2.3705624	117
IID_H159227_T02_01_WE01	IID_H159227	48	0.9751333	2.3660556	115
IID_H159227_T03_01_WE01	IID_H159227	48	0.9383707	2.3171541	129
IID_H159227_T04_01_WE01	IID_H159227	48	0.7306523	2.3878758	115
IID_H159228_T01_01_WE01	IID_H159228	49	0.9660542	2.3084885	103
IID_H159228_T02_01_WE01	IID_H159228	49	0.8801174	2.2496426	96
IID_H159229_T01_01_WE01	IID_H159229	50	0.8335068	2.2233683	67
IID_H159229_T02_01_WE01	IID_H159229	50	0.9234834	2.2469325	66
IID_H159229_T03_01_WE01	IID_H159229	50	0.8911536	2.2505918	67

Supplementary Table 5. *SigProfiler* assignment of mutational signatures.

De novo extracted	Global NMF Signatures
Signature 96-A	Signature SBS9 (94.66%)
Signature 96-B	Signature SBS2 (64.42%) & Signature SBS13 (35.58%)
Signature 96-C	Signature SBS5 (57.54%) & Signature SBS35 (42.46%)
Signature 96-D	Signature SBS1 (17.80%) & Signature SBS5 (75.48%)
Signature 96-E	Signature SBS5 (63.62%) & Signature SBS9 (25.56%)
Signature 96-F	Signature SBS-MM1
Signature 96-G	Signature SBS5 (27.52%) & Signature SBS8 (72.48%)
Signature 96-H	Signature SBS5 (47.20%) & Signature SBS18 (40.36%)