<u>Supporting Information:</u>

Dose-related Mutagenic and Clastogenic Effects of Benzo[b]fluoranthene in Mouse Somatic Tissues Detected by Duplex Sequencing and the Micronucleus Assay

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Table S1. Peripheral blood micronucleus assay conducted 2 days after 28 days of oral exposure to BbF.

BbF Dose (mg/kg/day)	n	MN-RET[1]/1000 ± SEM	Adj. *p* value[2]	MN-RBC[3]/1000 ± SEM	Adj. *p* value[2]	%RET ± SEM	Adj. *p* value[2]
0	8	2.34 ± 0.1	-	1.63 ± 0.1	-	1.60 ± 0.1	
6.25	8	4.16 ± 0.4	0.0036	2.56 ± 0.2	0.0014	1.81 ± 0.1	0.5763
12.5	8	5.31 ± 0.3	<0.0001	3.00 ± 0.1	<0.0001	1.87 ± 0.1	0.2245
25	8	5.26 ± 0.4	<0.0001	3.00 ± 0.2	<0.0001	2.02 ± 0.1	0.0146
50	8	6.52 ± 0.3	<0.0001	3.90 ± 0.1	<0.0001	2.24 ± 0.1	<0.0001
100	8	8.11 ± 0.4	<0.0001	4.84 ± 0.2	<0.0001	2.17 ± 0.1	0.0005

[1] Micronucleated reticulocytes.

[2] Pairwise comparison to 0 mg/kg/day BbF using the Bonferroni multiple comparison test, significant if $p < 0.05$.

[3] Micronucleated red blood cells.

Table S2. Technical performance metrics of the libraries built on DNA from BM and liver from animals after exposure to BbF.

BbF Dose (mg/kg/day)	Animal Number	Raw Reads Passing Filter	Median Insert Size	Mean Duplex Depth	Peak Tag Family Size	Informative Duplex Bases	Raw Reads Passing Filter	Median Insert Size	Mean Duplex Depth	Peak Tag Family Size	Informative Duplex Bases
		Bone Marrow					Liver				
0	1	3.12^{+08}	299	15,550	14	1.17^{+09}	2.46^{+08}	252	16,764	13	1.04^{+09}
0	2	3.01^{+08}	269	17,271	11	1.34^{+09}	6.81^{+08}	267	17,433	40	1.07^{+09}
0	3	3.25^{+08}	285	15,948	14	1.22^{+09}	2.53^{+08}	276	19,600	11	1.18^{+09}
0	4	3.73^{+08}	249	18,109	13	1.47^{+09}	2.73^{+08}	259	19,867	12	1.23^{+09}
6.25	5	3.42^{+08}	292	13,438	19	1.00^{+09}	5.19^{+08}	268	25,014	20	1.53^{+09}
6.25	6	7.06^{+08}	425	10,718	63	7.06^{+08}	2.10^{+08}	248	16,886	9	1.05^{+09}
6.25	7	3.02^{+08}	311	12,297	19	9.08^{+08}	2.78^{+08}	276	16,524	18	9.60^{+08}
6.25	8	2.40^{+08}	278	14,593	9	1.11^{+09}	2.43^{+08}	261	18,443	10	1.13^{+09}
12.5	9	3.20^{+08}	271	19,131	10	1.47^{+09}	4.99^{+08}	261	26,398	19	1.63^{+09}
12.5	10	5.42^{+08}	308	14,064	29	1.07^{+09}	3.70^{+08}	248	23,489	13	1.49^{+09}
12.5	11	3.48^{+08}	276	18,558	12	1.43^{+09}	4.72^{+08}	261	23,011	20	1.44^{+09}
12.5	12	2.30^{+08}	284	13,785	10	1.04^{+09}	3.55^{+08}	272	21,224	16	1.28^{+09}
25	13	3.56^{+08}	276	19,780	11	1.52^{+09}	3.14^{+08}	268	21,397	13	1.30^{+09}
25	14	2.21^{+08}	279	14,154	9	1.06^{+09}	4.34^{+08}	286	22,006	20	1.33^{+09}
25	15	3.34^{+08}	319	11,071	22	8.31^{+08}	3.34^{+08}	267	17,970	18	1.10^{+09}
25	16	4.57^{+08}	220	19,177	15	1.62^{+09}	2.65^{+08}	202	15,613	9	1.10^{+09}
50	17	2.40^{+08}	273	14,280	11	1.10^{+09}	2.64^{+08}	268	21,146	10	1.28^{+09}
50	18	3.71^{+08}	251	19,331	12	1.55^{+09}	3.12^{+08}	255	22,785	12	1.39^{+09}
50	19	4.03^{+08}	294	13,726	21	1.04^{+09}	3.60^{+08}	254	21,148	16	1.31^{+09}
50	20	3.02^{+08}	268	16,705	12	1.27^{+09}	4.06^{+08}	275	19,002	21	1.17^{+09}
100	21	3.38^{+08}	230	16,505	9	1.39^{+09}	3.40^{+08}	286	19,003	17	1.15^{+09}
100	22	2.38^{+08}	266	13,861	11	1.06^{+09}	3.80^{+08}	253	24,706	14	1.54^{+09}
100	23	4.56^{+08}	282	21,661	14	1.67^{+09}	2.76^{+08}	236	19,811	11	1.26^{+09}
100	24	2.85^{+08}	271	16,844	10	1.30^{+09}	4.81^{+08}	249	21,772	21	1.39^{+09}

Table S3. Minimum mutation frequencies by duplex sequencing using the mouse mutagenesis panel in bone marrow and liver samples after exposure to increasing doses of BbF.

BbF Dose (mg/kg/day)	n	MF_{min}[1] ± SEM (×10^{-8})	Adj. *p* value[2]	MF_{min} Fold-change[3]
	Bone Marrow			
0	4	4.13 ± 0.45	-	-
6.25	4	5.65 ± 0.59	0.6271	1.4
12.5	4	7.54 ± 0.58	0.0052	1.8
25	4	7.95 ± 0.60	0.0025	1.9
50	4	11.66 ± 0.72	<0.0001	2.8
100	4	14.78 ± 0.76	<0.0001	3.6
	Liver			
0	4	5.33 ± 1.01	-	-
6.25	4	5.88 ± 1.05	0.9807	1.1
12.5	4	8.06 ± 1.10	0.3567	1.5
25	4	11.12 ± 1.43	0.0378	2.1
50	4	22.23 ± 1.94	<0.0001	4.2
100	4	36.27 ± 2.49	<0.0001	6.8

[1] Minimum mutation frequency considering unique mutations.

[2] Pairwise comparison to 0 mg/kg/day BbF by GLM, significant if $p < 0.05$.

[3] Fold-change of minimum mutation frequency versus 0 mg/kg/day BbF.

Table S4. Minimum and maximum mutation frequencies in bone marrow and liver samples from individual animals after exposure to increasing doses of BbF.

BbF Dose (mg/kg/day)	Animal	MF$_{min}$[1] ($\times10^{-8}$)	MF$_{max}$[2] ($\times10^{-8}$)	Fold-change[3]	MF$_{min}$[1] ($\times10^{-8}$)	MF$_{max}$[2] ($\times10^{-8}$)	Fold-change[3]
		Bone marrow			Liver		
0	1	4.51	5.24	1.2	6.32	6.75	1.1
0	2	3.87	4.10	1.1	6.61	12.0	1.8
0	3	4.04	4.16	1.0	5.01	5.75	1.1
0	4	4.09	4.51	1.1	3.39	3.39	1.0
6.25	5	4.51	5.36	1.2	6.72	7.87	1.2
6.25	6	9.29	33.3	3.6	5.70	8.26	1.4
6.25	7	4.15	4.30	1.0	4.60	4.75	1.0
6.25	8	4.63	5.69	1.2	6.51	6.51	1.0
12.5	9	6.72	7.53	1.1	8.38	8.65	1.0
12.5	10	9.49	16.8	1.8	8.74	10.9	1.2
12.5	11	7.38	8.20	1.1	7.11	7.53	1.1
12.5	12	6.58	7.14	1.1	8.02	8.92	1.1
25	13	6.38	7.06	1.1	9.97	10.5	1.1
25	14	8.20	9.98	1.2	11.1	12.9	1.2
25	15	8.34	8.85	1.1	11.4	11.9	1.0
25	16	8.88	9.77	1.1	12.0	12.0	1.0
50	17	9.63	11.2	1.2	22.1	22.3	1.0
50	18	12.0	13.3	1.1	29.0	32.0	1.1
50	19	14.4	16.6	1.2	15.4	15.5	1.0
50	20	10.6	11.7	1.1	22.4	23.1	1.0
100	21	16.6	20.5	1.2	31.5	33.9	1.1
100	22	15.9	19.1	1.2	48.5	58.0	1.2
100	23	13.1	15.4	1.2	32.1	33.2	1.0
100	24	13.5	15.5	1.2	33.0	33.9	1.0

[1] Minimum mutation frequency considering unique mutations.

[2] Maximum mutation frequency considering all detected mutations.

[3] Fold-change between MF$_{min}$ and MF$_{max}$

Table S5. Minimum mutation frequencies per loci detected in bone marrow samples after exposure to increasing doses of BbF.

	0 mg/kg/day	6.25 mg/kg/day			12.5 mg/kg/day			25 mg/kg/day			50 mg/kg/day			100 mg/kg/day		
Location of locus	MF$_{min}$^{1} ± SEM (x 10^{-8})	MF$_{min}$^{1} ± SEM (x 10^{-8})	Fold-change^{2}	Adj. *p* value^{3}	MF$_{min}$^{1} ± SEM (x 10^{-8})	Fold-change^{2}	Adj. *p* value^{3}	MF$_{min}$^{1} ± SEM (x 10^{-8})	Fold-change^{2}	Adj. *p* value^{3}	MF$_{min}$^{1} ± SEM (x 10^{-8})	Fold-change^{2}	Adj. *p* value^{3}	MF$_{min}$^{1} ± SEM (x 10^{-8})	Fold-change^{2}	Adj. *p* value^{3}
Chr1	5.91 ± 1.69	15.51 ± 7.44	2.6	0.9986	9.07 ± 4.70	1.5	0.9994	5.14 ± 2.01	0.9	0.7675	7.18 ± 1.94	1.2	0.3633	9.18 ± 1.77	1.6	0.4251
Chr1.2	2.94 ± 1.12	2.31 ± 0.78	0.8	0.1287	2.46 ± 1.19	0.8	0.9299	5.02 ± 1.21	1.7	1.0000	7.46 ± 1.98	2.5	0.9920	6.84 ± 0.83	2.3	0.6902
Chr2	3.77 ± 1.63	5.72 ± 1.86	1.5	0.9564	2.35 ± 1.58	0.6	0.9809	4.16 ± 1.48	1.1	0.9983	3.64 ± 1.50	1.0	1.0000	11.54 ± 1.40	3.1	0.0742
Chr3	2.29 ± 0.98	3.17 ± 1.41	1.4	0.9980	5.80 ± 2.70	2.5	0.4562	7.27 ± 3.49	3.2	0.4201	6.00 ± 1.56	2.6	0.4289	3.80 ± 0.72	1.7	0.9053
Chr4	2.24 ± 0.87	7.07 ± 1.16	3.2	0.3101	4.97 ± 0.45	2.2	0.7340	5.85 ± 1.50	2.6	0.4198	13.75 ± 4.23	6.1	0.0069	13.28 ± 3.32	5.9	0.0090
Chr5	4.53 ± 1.93	4.45 ± 1.85	1.0	1.0000	13.26 ± 4.01	2.9	0.0863	7.37 ± 2.46	1.6	0.7349	13.73 ± 1.97	3.0	0.0421	13.21 ± 2.11	2.9	0.0500
Chr6	1.60 ± 0.98	3.77 ± 1.55	2.4	0.7973	2.85 ± 1.22	1.8	0.9495	4.31 ± 1.68	2.7	0.5109	8.06 ± 3.37	5.0	0.0654	8.29 ± 2.04	5.2	0.0436
Chr7	3.70 ± 1.29	3.22 ± 1.24	0.9	1.0000	1.51 ± 0.90	0.4	0.7279	5.09 ± 0.86	1.4	0.9386	7.80 ± 1.21	2.1	0.3573	8.60 ± 2.30	2.3	0.2542
Chr8	5.91 ± 1.29	10.13 ± 1.20	1.7	0.7182	12.41 ± 2.23	2.1	0.3124	14.86 ± 4.91	2.5	0.1643	28.10 ± 4.90	4.8	0.0002	34.73 ± 10.20	5.9	<0.0001
Chr9	3.92 ± 1.01	5.33 ± 1.84	1.4	0.9921	9.76 ± 4.34	2.5	0.1480	10.51 ± 4.60	2.7	0.2436	16.73 ± 3.36	4.3	0.0041	15.30 ± 0.98	3.9	0.0063
Chr10	1.09 ± 0.63	4.89 ± 1.66	4.5	0.2420	5.63 ± 1.40	5.2	0.1632	3.93 ± 1.46	3.6	0.4414	5.74 ± 1.33	5.3	0.1565	5.81 ± 1.68	5.3	0.1482
Chr11	8.94 ± 2.77	7.46 ± 1.25	0.8	0.9979	16.05 ± 1.77	1.8	0.2784	25.68 ± 5.33	2.9	0.0052	31.80 ± 2.76	3.6	0.0001	35.18 ± 6.74	3.9	<0.0001
Chr12	3.22 ± 1.01	2.12 ± 1.24	0.7	0.9872	4.78 ± 2.07	1.5	0.9858	5.15 ± 1.51	1.6	0.8300	9.50 ± 2.74	3.0	0.0796	7.48 ± 0.89	2.3	0.3355
Chr13	4.31 ± 1.94	5.45 ± 5.45	1.3	1.0000	5.90 ± 1.55	1.4	0.9614	4.61 ± 0.94	1.1	1.0000	4.18 ± 1.72	1.0	1.0000	4.41 ± 1.92	1.0	1.0000
Chr14	4.61 ± 1.34	4.55 ± 0.78	1.0	1.0000	14.40 ± 0.89	3.1	0.0308	17.88 ± 2.20	3.9	0.0047	20.73 ± 4.02	4.5	0.0009	37.50 ± 3.58	8.1	<0.0001
Chr15	1.57 ± 0.53	1.44 ± 0.85	0.9	1.0000	2.88 ± 0.80	1.8	0.9575	2.02 ± 0.76	1.3	0.9957	3.99 ± 1.24	2.5	0.6720	5.12 ± 1.88	3.3	0.3162
Chr16	10.00 ± 1.88	7.31 ± 1.70	0.7	0.9444	14.49 ± 3.40	1.4	0.7512	18.50 ± 1.54	1.9	0.2331	20.18 ± 2.70	2.0	0.1190	30.38 ± 6.63	3.0	0.0020
Chr17	6.23 ± 2.19	12.11 ± 2.72	1.9	0.4845	15.40 ± 2.14	2.5	0.0863	6.47 ± 1.40	1.0	1.0000	14.82 ± 3.87	2.4	0.1516	34.70 ± 3.78	5.6	<0.0001
Chr18	3.87 ± 1.68	5.34 ± 0.99	1.4	0.9899	7.78 ± 1.73	2.0	0.6284	6.34 ± 2.01	1.6	0.8538	5.70 ± 0.70	1.5	0.9361	14.53 ± 1.48	3.8	0.0108
Chr19	3.42 ± 0.70	3.92 ± 1.65	1.1	0.9999	3.16 ± 1.25	0.9	1.0000	3.64 ± 1.31	1.1	1.0000	9.96 ± 4.14	2.9	0.1563	9.08 ± 2.99	2.7	0.2403

[1] Minimum mutation frequency considering unique mutations within a respective locus.

[2] Fold-change of minimum mutation frequency versus 0 mg/kg/day BbF.

[3] Pairwise comparison to 0 mg/kg/day BbF of the respective locus by GLMM, significant if $p < 0.05$.

Table S6. Minimum mutation frequencies per loci detected in liver samples after exposure to increasing doses of BbF.

	0 mg/kg/day	6.25 mg/kg/day			12.5 mg/kg/day			25 mg/kg/day			50 mg/kg/day			100 mg/kg/day		
Location of locus	MF$_{min}$[1] ± SEM (x 10^{-8})	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]
Chr1	2.77 ± 1.20	3.96 ± 1.63	1.4	0.9412	8.72 ± 2.22	3.1	0.0679	12.09 ± 2.21	4.4	0.0147	27.15 ± 7.81	9.8	< 0.0001	41.48 ± 4.59	15.0	<0.0001
Chr1.2	9.80 ± 5.52	8.92 ± 1.54	0.9	1.0000	12.93 ± 3.08	1.3	0.7896	23.64 ± 5.74	2.4	0.0097	28.18 ± 5.86	2.9	0.0010	45.28 ± 6.47	4.6	<0.0001
Chr2	2.63 ± 1.03	2.42 ± 0.84	0.9	0.9996	6.87 ± 1.66	2.6	0.3570	4.78 ± 1.63	1.8	0.6473	17.62 ± 4.60	6.7	0.0010	30.70 ± 8.09	11.7	<0.0001
Chr3	3.97 ± 1.79	6.18 ± 1.28	1.6	0.9429	7.04 ± 1.25	1.8	0.9090	8.98 ± 1.89	2.3	0.5024	15.88 ± 2.79	4.0	0.0113	35.23 ± 8.91	8.9	<0.0001
Chr4	5.59 ± 2.76	4.74 ± 2.01	0.8	0.9993	7.01 ± 1.90	1.3	0.9089	15.13 ± 1.96	2.7	0.0282	26.93 ± 2.22	4.8	0.0001	39.43 ± 7.87	7.1	<0.0001
Chr5	6.49 ± 1.59	5.46 ± 1.86	0.8	0.9850	4.82 ± 1.66	0.7	0.9964	9.39 ± 0.42	1.4	0.7252	17.43 ± 3.19	2.7	0.0153	42.63 ± 5.68	6.6	<0.0001
Chr6	5.21 ± 2.90	2.00 ± 1.30	0.4	0.7553	4.84 ± 1.06	0.9	1.0000	7.86 ± 0.52	1.5	0.8318	15.32 ± 4.15	2.9	0.0269	32.38 ± 8.14	6.2	<0.0001
Chr7	2.26 ± 0.90	3.15 ± 1.19	1.4	0.9871	9.21 ± 1.12	4.1	0.0454	11.43 ± 2.15	5.1	0.0125	14.56 ± 4.26	6.4	0.0016	29.85 ± 7.51	13.2	<0.0001
Chr8	4.52 ± 1.51	4.30 ± 2.59	1.0	1.0000	7.09 ± 1.11	1.6	0.9638	7.72 ± 1.45	1.7	0.8537	27.38 ± 3.01	6.1	0.0001	39.25 ± 6.63	8.7	<0.0001
Chr9	6.71 ± 0.73	7.92 ± 3.11	1.2	0.9993	12.33 ± 2.56	1.8	0.2567	11.25 ± 2.00	1.7	0.4410	29.98 ± 4.61	4.5	< 0.0001	32.43 ± 4.93	4.8	<0.0001
Chr10	7.10 ± 1.18	5.30 ± 2.78	0.7	0.9913	3.28 ± 0.70	0.5	0.4860	11.61 ± 3.75	1.6	0.6971	11.09 ± 2.05	1.6	0.7661	24.75 ± 3.71	3.5	0.0014
Chr11	10.46 ± 4.19	8.65 ± 2.48	0.8	1.0000	6.57 ± 0.99	0.6	0.8447	18.65 ± 1.62	1.8	0.1140	29.75 ± 3.68	2.8	0.0005	37.95 ± 7.50	3.6	<0.0001
Chr12	2.12 ± 0.72	6.63 ± 1.52	3.1	0.5879	7.94 ± 1.25	3.7	0.2260	9.33 ± 2.56	4.4	0.0684	23.53 ± 5.03	11.1	0.0001	38.20 ± 4.77	18.0	<0.0001
Chr13	3.12 ± 1.49	3.07 ± 1.84	1.0	0.9999	3.81 ± 1.67	1.2	0.9844	8.84 ± 3.01	2.8	0.1332	9.67 ± 1.18	3.1	0.0789	17.00 ± 1.72	5.4	0.0013
Chr14	10.09 ± 2.96	9.40 ± 3.54	0.9	0.9879	9.59 ± 2.01	1.0	1.0000	14.90 ± 3.13	1.5	0.5698	29.23 ± 4.65	2.9	0.0011	63.35 ± 12.50	6.3	<0.0001
Chr15	3.16 ± 2.46	3.51 ± 2.13	1.1	0.9964	3.77 ± 1.00	1.2	0.9938	7.67 ± 2.81	2.4	0.3084	16.18 ± 1.36	5.1	0.0019	30.10 ± 4.28	9.5	<0.0001
Chr16	2.02 ± 0.68	6.87 ± 3.22	3.4	0.4963	12.29 ± 2.10	6.1	0.0225	12.86 ± 6.65	6.4	0.0496	24.50 ± 4.35	12.1	0.0001	38.85 ± 3.96	19.2	<0.0001
Chr17	4.43 ± 1.85	12.65 ± 2.01	2.9	0.1781	12.27 ± 3.76	2.8	0.1835	5.03 ± 1.85	1.1	0.9972	29.05 ± 4.35	6.6	< 0.0001	42.50 ± 5.09	9.6	<0.0001
Chr18	7.94 ± 3.88	8.18 ± 1.92	1.0	1.0000	11.42 ± 2.33	1.4	0.7965	12.58 ± 1.50	1.6	0.5288	33.00 ± 7.80	4.2	< 0.0001	44.88 ± 4.12	5.7	<0.0001
Chr19	6.78 ± 0.90	5.33 ± 1.17	0.8	0.9893	9.62 ± 2.38	1.4	0.7218	8.28 ± 2.36	1.2	0.9669	18.98 ± 3.27	2.8	0.0123	19.98 ± 1.78	2.9	0.0063

[1] Minimum mutation frequency considering unique mutations within a respective locus.

[2] Fold-change of minimum mutation frequency versus 0 mg/kg/day BbF.

[3] Pairwise comparison to 0 mg/kg/day BbF of the respective locus by GLMM, significant if $p < 0.05$.

Table S7. Minimum mutation frequencies of single base substitutions detected in bone marrow and liver samples after exposure to increasing doses of BbF.

Mutation Subtype	0 mg/kg/day	6.25 mg/kg/day			12.5 mg/kg/day			25 mg/kg/day			50 mg/kg/day			100 mg/kg/day		
	MF$_{min}$[1] ± SEM (x 10^{-8})	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]	MF$_{min}$[1] ± SEM (x 10^{-8})	Fold-change[2]	Adj. *p* value[3]
								Bone marrow								
C:G>A:T	2.08 ± 0.40	3.28 ± 0.89	1.6	0.7761	5.48 ± 0.76	2.6	0.0184	4.17 ± 0.32	2.0	0.0912	8.84 ± 1.32	4.3	0.0002	12.78 ± 1.41	6.1	<0.0001
C:G>G:C	0.39 ± 0.23	0.86 ± 0.45	2.2	0.0340	1.45 ± 0.16	3.7	0.0340	1.48 ± 0.16	3.8	0.0523	1.61 ± 0.17	4.1	0.0189	2.50 ± 0.29	6.4	0.0013
C:G>T:A	2.34 ± 0.24	2.79 ± 0.67	1.2	0.9935	2.59 ± 0.64	1.1	0.9998	3.90 ± 0.30	1.7	0.2643	5.63 ± 0.91	2.4	0.0037	7.84 ± 0.81	3.4	0.0001
T:A>A:T	0.26 ± 0.13	0.45 ± 0.10	1.7	0.8744	1.17 ± 0.16	4.5	0.0489	1.76 ± 0.42	6.8	0.0103	2.04 ± 0.16	7.8	0.0044	2.28 ± 0.56	8.8	0.0027
T:A>C:G	0.95 ± 0.15	1.44 ± 0.35	1.5	0.9263	1.24 ± 0.33	1.3	0.8778	1.65 ± 0.24	1.7	0.6433	2.15 ± 0.57	2.3	0.0439	1.75 ± 0.43	1.8	0.2853
T:A>G:C	0.35 ± 0.05	0.61 ± 0.15	1.7	0.5553	0.98 ± 0.11	2.8	0.0052	0.62 ± 0.09	1.8	0.2107	0.81 ± 0.17	2.3	0.0169	0.81 ± 0.11	2.3	0.0168
Insertion	0.12 ± 0.08	0.10 ± 0.06	0.8	1.0000	0.17 ± 0.07	1.4	0.9570	0.24 ± 0.09	2.0	0.9409	0.32 ± 0.08	2.7	0.3450	0.21 ± 0.07	1.8	0.7503
Deletion	0.95 ± 0.17	0.70 ± 0.17	0.7	0.8069	0.99 ± 0.05	1.0	0.9996	1.05 ± 0.08	1.1	0.9949	1.28 ± 0.22	1.3	0.7746	1.03 ± 0.26	1.1	0.9999
MNV[4]	0.06 ± 0.03	0.28 ± 0.14	4.6	0.5715	0.26 ± 0.18	4.3	0.6452	0.19 ± 0.08	3.2	0.5672	0.20 ± 0.05	3.3	0.6186	0.41 ± 0.04	6.8	0.1553
								Liver								
C:G>A:T	1.21 ± 0.29	2.53 ± 0.46	2.1	0.8117	3.85 ± 0.15	3.2	0.4030	9.03 ± 0.76	7.5	0.0319	21.38 ± 4.07	17.7	0.0009	35.48 ± 5.62	29.3	0.0001
C:G>G:C	0.38 ± 0.20	0.44 ± 0.20	1.2	1.0000	1.26 ± 0.33	3.3	0.4026	2.31 ± 0.69	6.1	0.0541	4.56 ± 0.79	12.0	0.0055	7.77 ± 0.94	20.4	0.0007
C:G>T:A	4.75 ± 0.57	4.88 ± 1.29	1.0	0.9910	5.53 ± 0.75	1.2	0.9015	5.34 ± 0.57	1.1	0.9964	10.41 ± 0.85	2.2	0.0043	16.53 ± 1.37	3.5	<0.0001
T:A>A:T	0.87 ± 0.18	0.75 ± 0.33	0.9	0.9617	1.36 ± 0.34	1.6	0.8657	2.47 ± 0.21	2.8	0.1372	4.42 ± 0.55	5.1	0.0034	7.52 ± 1.04	8.6	0.0034
T:A>C:G	1.05 ± 0.30	1.00 ± 0.38	1.0	0.9935	1.54 ± 0.11	1.5	0.6879	1.32 ± 0.09	1.3	0.9461	2.22 ± 0.33	2.1	0.0909	3.47 ± 0.51	3.3	0.0016
T:A>G:C	0.40 ± 0.17	0.65 ± 0.14	1.6	0.8476	0.62 ± 0.08	1.6	0.8725	0.80 ± 0.30	2.0	0.6951	0.96 ± 0.1	2.4	0.3025	1.66 ± 0.32	4.2	0.0137
Insertion	0.24 ± 0.07	0.12 ± 0.05	0.5	0.9145	0.36 ± 0.16	1.5	0.8505	0.23 ± 0.04	1.0	1.0000	0.25 ± 0.1	1.0	0.9986	0.45 ± 0.03	1.9	0.5266
Deletion	0.81 ± 0.27	0.67 ± 0.16	0.8	1.0000	0.76 ± 0.07	0.9	1.0000	0.67 ± 0.23	0.8	0.9999	1.00 ± 0.13	1.2	0.9244	1.65 ± 0.33	2.0	0.0609
MNV[4]	0.14 ± 0.14	0.15 ± 0.01	1.1	0.9975	0.20 ± 0.04	1.4	0.9325	0.39 ± 0.08	2.8	0.2172	0.68 ± 0.09	4.9	0.0298	1.06 ± 0.09	7.6	0.0039

[1] Minimum mutation frequency considering unique mutations.
[2] Fold-change of minimum mutation frequency versus 0 mg/kg/day BbF.
[3] Pairwise comparison to 0 mg/kg/day BbF by GLMM, significant if $p < 0.05$.
[4] Multi-nucleotide variants.

Table S8. Benchmark doses of measured genotoxic endpoints in blood, bone marrow and liver.

Genotoxic endpoint	n	CES[1]	BMDL[2]	BMDU[3]	Log10 BMDL	Log10 BMDU	Bootstrap runs
MN RET	8	0.5	0.408	4.06	-0.3893	0.6085	200
MN RBC	8	0.5	2.23	9.34	0.3483	0.9703	200
MF BM	4	0.5	3.6	12.8	0.5563	1.1072	200
MF Liver	4	0.5	10.1	18.4	1.0043	1.2648	200

[1] Critical effect size used to perform benchmark dose modeling.

[2] Benchmark dose lower confidence limit.

[3] Benchmark dose upper confidence limit.

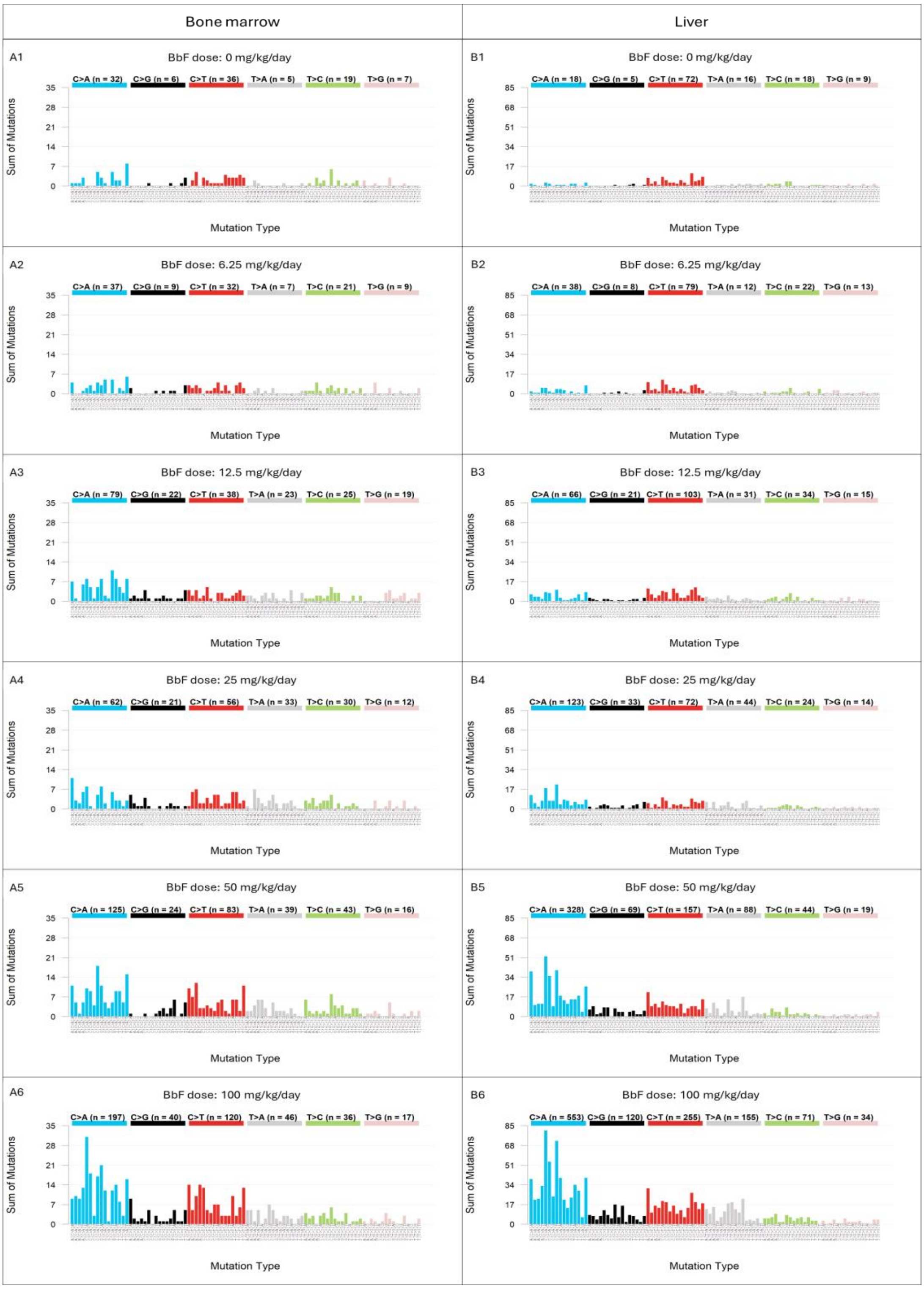

Figure S1. Trinucleotide mutation pattern of bone marrow (A) and liver (B) samples after 28-day exposure to the vehicle control (A1, B1) and increasing doses of BbF (A2 = 6.25, A3 = 12.5, A4 = 25, A5 = 50, A6 = 100, B2 = 6.25, B3 = 12.5, B4 = 25, B5 = 50 and B6 = 100 mg/kg/day). The panels represent the sum of mutations of each 96-mutational subtypes for n = 4 samples of each dose group. The sum of all mutation for each single base substitution type; C>A (blue), C>G (black), C>T (red), T>A (gray), T>C (green) and T>G (pink), are indicated.

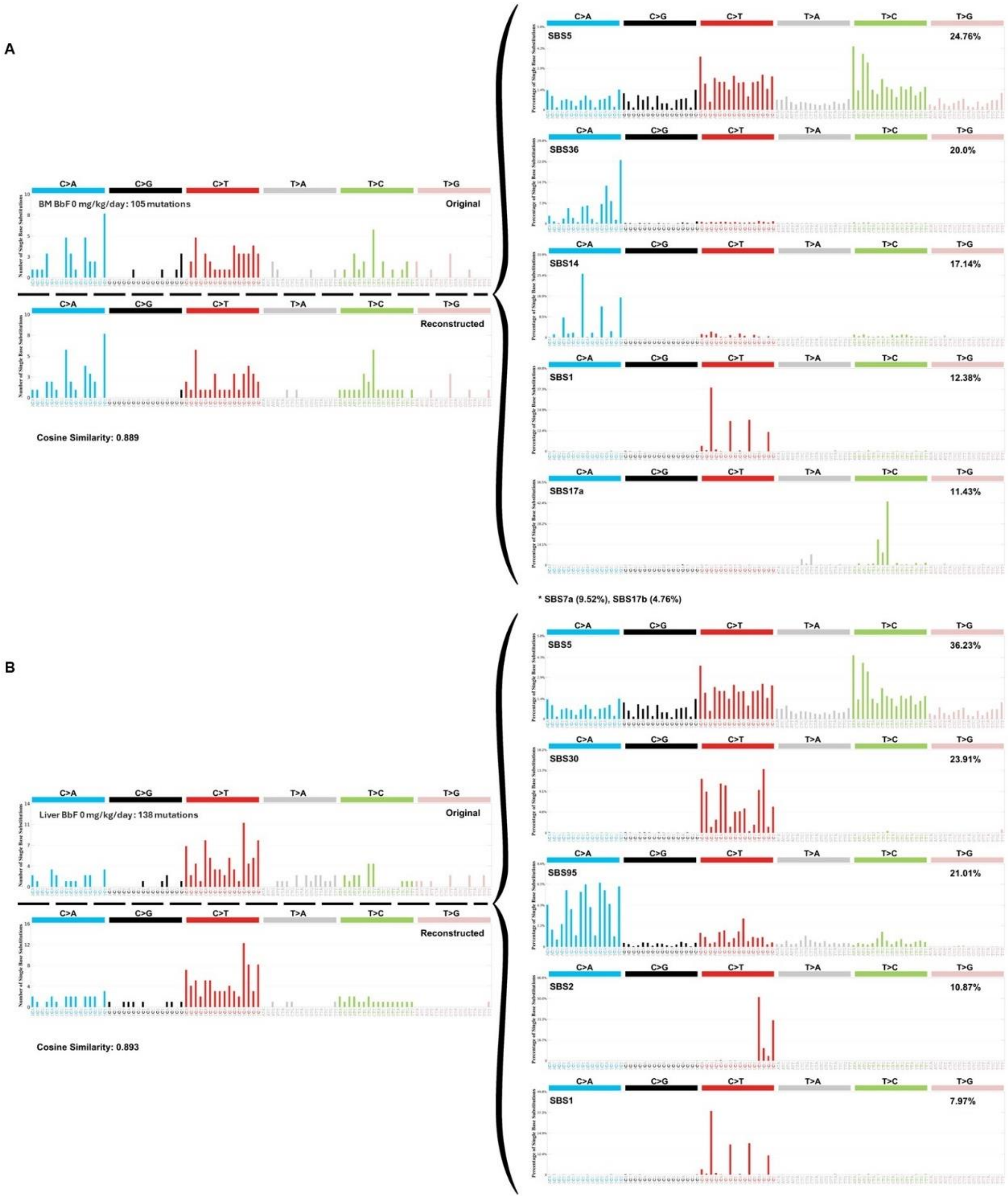

Figure S2. Mutation signature analyses for vehicle control in bone marrow (A) and liver (B). The original trinucleotide mutation profile is shown on the top left. SigProfilerAssignment used the single base substitution (SBS) signatures of the Catalogue of Somatic Mutations in Cancer (COSMIC) database to reconstruct the signatures. The SBS signatures and their relative contributions are shown on the right. The reconstructed mutation pattern, number of substitutions used for the assignment and cosine similarity between reconstructed and observed trinucleotide mutation profile are shown on left below the original profile. The total amount of mutations of the original signature are indicated on the left.