Supp. Table S1. List of mutations in 70 unilateral RB tumors detected by Sanger sequencing

Results of standard Sanger sequencing					Bar code used for deep sequencing
	ID	Exon/ Intron	Mutation	Change in amino acid/Splice site	
1	RBU1	1	g.2137_2139delGCC	in frame deletion	**1**
2	RBU2	2	g.5513-5514dupTA	p.Thr77X	**1**
3	RBU3	3	g.39486-39487delGT	p.Cys102TyrfsX6	**1**
4	RBU4	4	g.41954G>T	p.Glu137X	**1**
5	RBU5	5	g.44675G>T	p.Glu170X	**1**
6	RBU6	8	g.59727delA	p.Lys265AsnfsX2	**1**
7	RBU7	9	g.61752dupA	p.Asn295LysfsX14	**1**
8	RBU8	10	g.64348C>T	p.Arg320X	**1**
9	RBU9	11	g.65374delCA	p.Gln354GlufsX5	**1**
10	RBU10	12	g.70261dupC	p.Gln383ProfsX11	**1**
11	RBU10	(+1) Intron 12	g.70330G>A	splice site	**1**
12	RBU11	(-1) Intron 12	g.73752G>A	splice site	**1**
13	RBU12	14	g.76430C>T	p.Arg445X	**1**
14	RBU13	15	g.76898C>T	p.Arg467X	**1**
15	RBU14	16	g.77035-77036delTT	p.Leu486IlefsX5	**1**
16	RBU15	17	g.78238C>T	p.Arg552X	**1**
17	RBU3	19	g.153302C>T	p.Gln637X	**1**
18	RBU9	20	g.156705C>A	p.Ala658Asp	**1**
19	RBU16	21	g.160820C>T	p.His733Tyr	**1**
20	RBU2	22	g.162012-162015dupTTGA	p.Lys745AspfsX6	**1**
21	RBU1	23	g.162331delG	p.Gly818ValfsX7	**1**
22	RBU17	(-1) Intron 23	g.170371G>A	splice site	**1**
23	RBU18	(+2) Intron 25	g.173851T>C	splice site	**1**
24	RBU19	1	g.2104-2112del	p.delAla16-18	**2**
25	RBU7	2	g.5503delA	p.Arg73GlufsX3	**2**
26	RBU20	3	g.39486-39487delGT	p.Asp109X	**2**
27	RBU21	4	g.41983delTGCT	p.Asn145LysfsX5	**2**
28	RBU21	8	g.59695C>T	p.Arg255X	**2**
29	RBU22	9	g.61805G>T	p.Glu313X	**2**
30	RBU23	10	g.64348C>T; g.64414delA	p.Arg320X; p.Thr342LeufsX6	**2**
31	RBU24	11	g.65386C>T	p.Arg358X	**2**
32	RBU25	12	g.70280T>A	p.Leu389X	**2**

33	RBU26	(+1) Intron 23	g73870G>T	splice site	**2**
34	RBU18	14	g.76443G>T	p.Gly449Val	**2**
35	RBU27	15	g.76898C>T	p.Arg467X	**2**
36	RBU26	16	g.77051T>C	p.Leu491Pro	**2**
37	RBU28	17	g.78238C>T	p.Arg552X	**2**
38	RBU29	18	g.150037C>T	p.Arg579X	**2**
39	RBU20	19	g.153302C>T	p.Gln637X	**2**
40	RBU11	20	g.156713C>T	p.Arg661Trp	**2**
41	RBU14	21	g.160787A>T	p.Lys722X	**2**
42	RBU30	(-13) Intron 21	g.161984T>A	splice site	**2**
43	RBU31	23	g.162248C>G	p.Tyr790X	**2**
44	RBU32	(+1) Intron 24	g.170403G>A	splice site	**2**
45	RBU16	25	g.173711dupC	p.Glu843X	**2**
46	RBU33	1	g.2090G>T	p.Ala11Ser	**3**
47	RBU34	3	g.39485-39486delTG	p.Cys102TyrfsX6	**3**
48	RBU35	4	g.42008dupT	p.Tyr155LeufsX1	**3**
49	RBU36	8	g.59695C>T	p.Arg255X	**3**
50	RBU37	10	g.64411A>T	p.Lys341X	**3**
51	RBU38	11	g.65421T>C	p.Ile369Ile	**3**
52	RBU29	(+1) Intron 12	g.70330G>A	splice site	**3**
53	RBU34	(+1) Intron 13	g.73870G>T	splice site	**3**
54	RBU39	14	g.76430C>T	p.Arg445X	**3**
55	RBU40	15	g.76898C>T	p.Arg467X	**3**
56	RBU41	16	g.77051T>C	p.Leu491Pro	**3**
57	RBU42	17	g.78238C>T	p.Arg552X	**3**
58	RBU42	(+1) Intron 17	g.78280G>A	splice site	**3**
59	RBU39	18	g.150040G>T	p.Glu580X	**3**
60	RBU28	19	g.153352dupA	p.Val654SerfsX13	**3**
61	RBU13	20	g.156833G>A	p.Asp701Asn	**3**
62	RBU43	(-1) Intron 20	g.160729G>C	splice site	**3**
63	RBU44	22	g.162032T>A	p.Tyr749X	**3**
64	RBU45	23	g.162237C>T	p.Arg787X	**3**
65	RBU46	25	g.173711dupC	p.Glu843X	**3**
66	RBU47	1	g.2172_2174delGCCInsCT	p.Gly38AlafsX26	**4**
67	RBU48	2	g.5505-5506delAG	p.Arg73SerfsX35	**4**
68	RBU49	8	g.59695C>T	p.Arg255X	**4**
69	RBU50	(+1) Intron 10	g.64440G>A	splice site	**4**
70	RBU51	11	g.65386C>T	p.Arg358X	**4**
71	RBU52	(+1) Intron 12	g.70330G>A	splice site	**4**
72	RBU53	(+1) Intron 13	g.73870G>T	splice site	**4**

73	RBU5	14	g.76460C>T	p.Arg455X	**4**
74	RBU54	15	g.76898C>T	p.Arg467X	**4**
75	RBU55	17	g.78272G>A	p.Trp563X	**4**
76	RBU40	18	g.150025C>T	p.Gln575X	**4**
77	RBU55	19	g.153318T>A	p.Leu642X	**4**
78	RBU55	19	g.153320A>G	p.Lys643Glu	**4**
79	RBU56	(-2) Intron 19	g.156691A>T	splice site	**4**
80	RBU57	21	g.160832G>T	p.Glu737X	**4**
81	RBU58	23	g.162237C>T	p.Arg787X	**4**
82	RBU59	25	g.173711dupC	p.Glu843X	**4**
83	RBU60	1	g.2162C>T	p.Gln35X	**5**
84	RBU61	4	g.41991C>G	p.Ser149X	**5**
85	RBU62	8	g.59695C>T	p.Arg255X	**5**
86	RBU63	10	g.64348C>T	p.Arg320X	**5**
87	RBU64	11	g.65386C>T	p.Arg358X	**5**
88	RBU65	(+1) Intron 12	g.70330G>A	splice site	**5**
89	RBU66	(+1) Intron 13	g.73870G>A	splice site	**5**
90	RBU6	14	g.76430C>T	p.Arg445X	**5**
91	RBU67	15	g.76898C>T	p.Arg467X	**5**
92	RBU68	17	g.78250C>T	p.Arg556X	**5**
93	RBU15	18	g.150025C>T	p.Gln575X	**5**
94	RBU69	(+5) Intron 19	g.153358G>A	splice site	**5**
95	RBU70	23	g.162237C>T	p.Arg787X	**5**

Supp. Table S2. Summary of Ion PGM run of 20 barcoded bilateral RB libraries run on a single 318 chip

Barcode ID	Mapped Reads	Mean Depth	Target Coverage at 100X
IonXpress_001	310,343	5,170.92	100.00%
IonXpress_002	291,126	4,812.42	100.00%
IonXpress_003	314,302	5,098.98	100.00%
IonXpress_004	386,915	5,617.44	100.00%
IonXpress_005	303,317	4,899.23	100.00%
IonXpress_006	263,944	4,558.00	100.00%
IonXpress_007	305,014	5,231.25	100.00%
IonXpress_008	320,411	5,105.73	100.00%
IonXpress_009	322,237	5,275.89	100.00%
IonXpress_010	316,455	5,237.92	100.00%
IonXpress_011	302,034	4,948.54	100.00%
IonXpress_012	266,918	4,625.56	100.00%
IonXpress_013	378,357	5,783.79	100.00%
IonXpress_014	438,254	5,889.83	100.00%
IonXpress_015	322,245	5,234.50	100.00%
IonXpress_016	470,035	6,039.94	100.00%
IonXpress_017	804,405	6,796.68	100.00%
IonXpress_018	811,778	6,728.76	100.00%
IonXpress_019	741,799	6,842.42	100.00%
IonXpress_020	842,794	7,022.60	100.00%
Average	**425,634**	**5,546.02**	**100.00%**

Supp. Table S3. List of known SNPs and false positive variant calls detected in bilateral RB cases

	Position	Intron/Exon	Type	Ref	Variant	Note
1	2261	1	INS	G	GC	Flanked by Homopolymer
2	2281	1	SNP	C	T	rs2854351
3	2302	1	SNP	G	A	rs2252544
4	39598	3	SNP	A	G	rs118072861
5	39606	3	SNP	T	C	rs3092882
6	42068	4	SNP	G	T	rs3092902
7	42091	4	SNP	A	G	rs3092901
8	44630	4	SNP	G	A	rs3092884
9	44748	5	SNP	G	A	Flanked by Homopolymer
10	56989	7	SNP	T	C	rs3092868
11	61715	8	SNP	C	A	Unreported SNP by LOVD
12	64497	10	SNP	G	A	rs4151502
13	65515	11	SNP	C	T	rs3092888
14	73724	12	SNP	A	G	rs3092887
15	76412	13	SNP	C	T	Flanked by Homopolymer
16	76721	14	SNP	C	G	rs3092894
17	76875	14	SNP	A	T	rs9535023
18	76936	15	SNP	T	A	Flanked by Homopolymer
19	76937	15	SNP	T	A	Flanked by Homopolymer
20	78086	17	INS	G	GT	Flanked by Homopolymer
21	156616	19	SNP	A	G	rs198580
22	161981	21	SNP	T	A	Flanked by Homopolymer
23	170327	23	SNP	A	G	rs4151610
24	173882	25	SNP	T	C	rs3020646
25	174351	25	SNP	T	A	rs3092904
26	174438	26	SNP	C	T	rs3020647

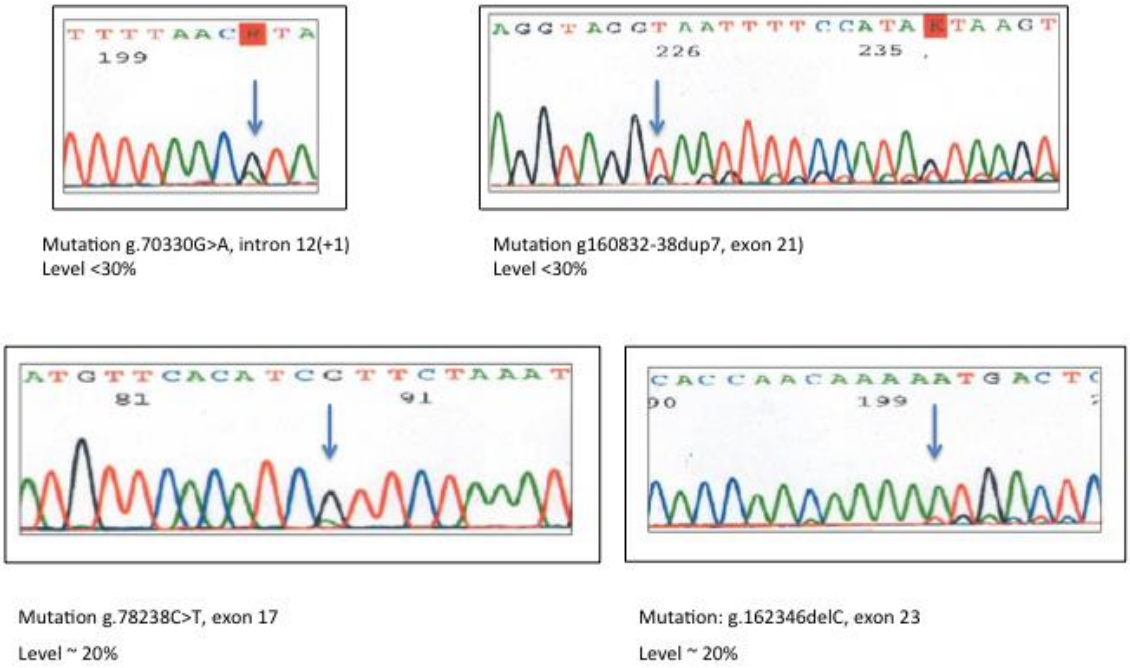
Note 1. LOVD stands for RB1 LSDB (Leiden Open source Variation Database) V2.0 Build 31
Note 2: Positions of variants defined by the numbering system of the *RB1* reference sequence (GenBank Accession L11910.1)

Supp. Table S4. Summary of Ion PGM run of 5 barcoded unilateral RB libraries on a single 316 chip

Barcode ID	Mapped Reads	Mean Depth	Target Coverage at 100X
IonXpress_001	308,794	4,650.82	100.00%
IonXpress_002	604,514	6,341.70	100.00%
IonXpress_003	591,884	5,826.33	100.00%
IonXpress_004	530,232	5,313.68	100.00%
IonXpress_005	453,975	4,019.11	100.00%
Average	497,880	5,230.33	100.00%

Supp. Table S5. Primers and probes of Taqman genotyping assays for seven recurrent nonsense mutations

Assay ID	Forward primer	Reverse primer	C allele Reporter (5'-VIC)	T allele Reporter (5'-FAM)
R251X	AGAAACAGCTGTTATACCCATTAATGGT	CCGTGCACTCCTGTTCTGA	CTGGGTGTTCGAGGTGA	TGGGTGTTCAAGGTGA
R320X	GTCAGTGACTTTTTTCTTTCAAGGTTGA	AGAGTTTTATCATGATCCAAAAATAATCTTGCATC	AAATTTCTTCGTATCGTTTAGAA	AAATTTCTTCGTATCATTTAGAA
R455X	GCGATACAAACTTGGAGTTCGC	GCCCGGCTGAAATTTTTTTATATTGTTTTTAA	ATTCCATTACTCGGTAATAC	TTCCATTACTCAGTAATAC
R552X	CAGAAGGCAACTTGACAAGAGAAAT	AGCCATGCAAGGGATTCCAT	ATGTTCACATCGTTCTAAA	ATGTTCACATCATTCTAAA
R556X	AGAAGGCAACTTGACAAGAGAAATGAT	GCTACTTACTGAGAGCCATGCAA	TTCCATGATTCGATGTTC	CCATGATTCAATGTTC
R579X	TCATCATGTTTCATATAGGATTCACCTTTATTTGA	CAAGCAGATTCAAGGTGATCAGTTG	CCTTCTCGGTCCTTTG	TCCTTCTCAGTCCTTTG
R787X	CCCCTACCTTGTCACCAATACCT	CGTAAGGGTGAACTAGGAAACTTGT	AAGGGCTTCGAGGAAT	AAGGGCTTCAAGGAAT



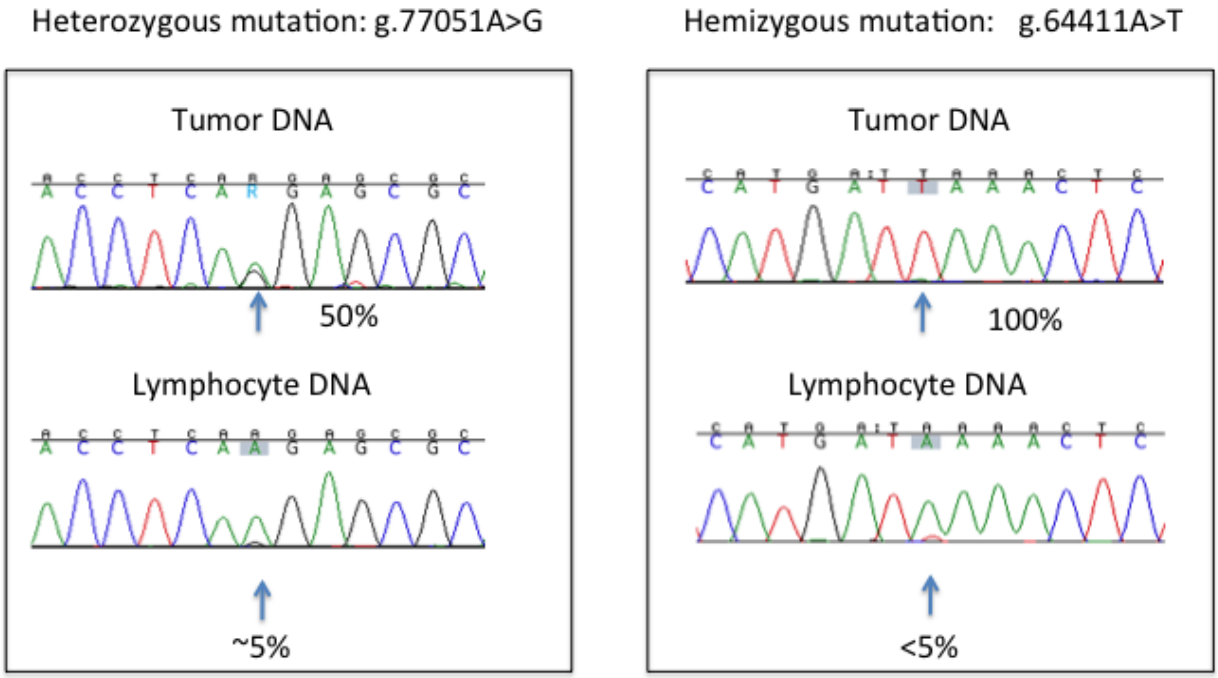
Mutation g.70330G>A, intron 12(+1)
Level <30%

Mutation g160832-38dup7, exon 21)
Level <30%

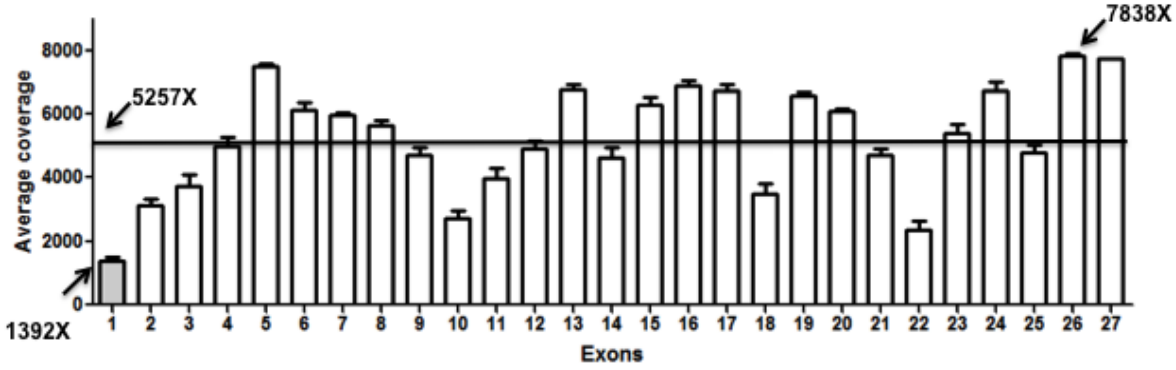
Mutation g.78238C>T, exon 17
Level ~ 20%

Mutation: g.162346delC, exon 23
Level ~ 20%

Supp. Figure S1. Examples of germline mosaicism detected by Sanger sequencing in bilateral RB cases. The sequence chromatograms obtained by analysis of lymphocyte DNA samples showing different mutations present at different levels are indicated.

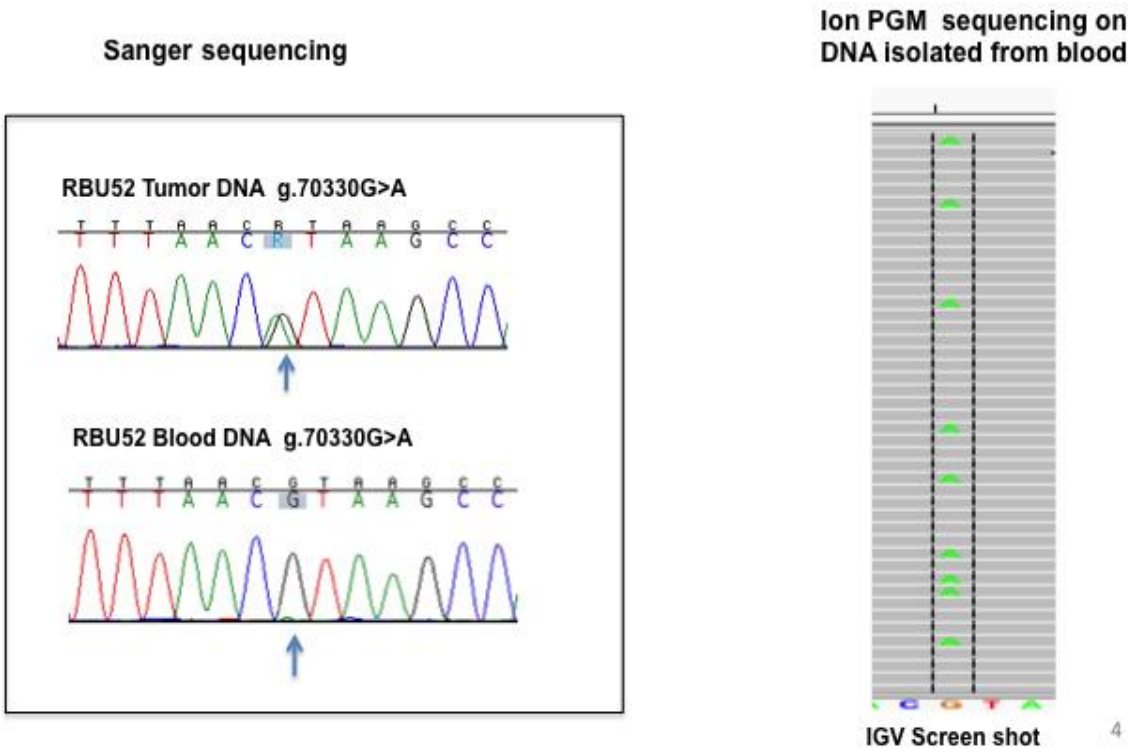


Supp. Figure S2. Examples of germline mosaicism detected by Sanger sequencing in unilateral RB cases. The sequence chromatograms obtained by analysis of tumor and lymphocyte DNA samples are included for comparison.



Supp. Figure S3. Average depth of coverage of individual *RB1* exons of 20 bilateral RB samples from two sequencing runs on the 318 chips. Each run with 10 samples per chip was performed in duplicate. The coverage data was obtained using the pre-installed Coverage Analysis plugin of Ion Torrent Suite.

ID	Mutation	Total coverage	Variant coverage	p Value	Mosaic %
RBU52	g.70330G>A	5591	873	1.00 E-10	13.5%



Supp. Figure S4. Sanger sequencing chromatograms of a mosaic mutation identified in tumor and blood DNA of one RB sample (RBU52) and a screen shot of alignment of Ion PGM runs on Integrative Genomics Viewer are displayed**.**