

R2FL (n=12)

VH		DH		JH	Del.	Add.	PNuc.	<u>μHom</u>	CDR3
tgtgcaagag	aga	gatggttactac	ga	ttgactactgg	12	5	1	0	38
tgtgcaagaga	ta	gggctac		tgg	24	2	1	4	23
tgtgcaagac	gggca	actggga	acc	actactgg	14	8	0	0	33
tgtgcaaga	c	atga	cccagttttt	actactttgactactgg	15	11	0	0	41
tgtgcaaga	cgggg	tatggtaact		gactactgg	15	5	0	1	33
tgtgcaaga		ctatggtaactac	g	cctggtttgcttactgg	6	1	1	0	40
tgtgcaaga		gcta	aagggtgc	tgcttactgg	21/28	7	0	3	30
tgtgcaaga	c	atg		ctgg	29	1	0	0	17
tgtgcaa	at	tagctac	gtt	ctactttgactactgg	21	5	2	0	35
tgtgcaaga	g	cagc	ccccagttga	actactgg	23/24	11	0	0	32
tgtgcaaga	catacc	gggac	gtg	gg					
tgtgcaaga	c	atacc	gggacgtg	gg	19/26	4/14	0/2	0	25
tgtgcaaga	c	agac	ccccag	tttgactactgg	17	7	0	0	32
Average:					17.2	5.5	0.5	0.6	31

R2FL-N474S (n=12)

VH		DH		JH	Del.	Add.	PNuc.	<u>μHom</u>	CDR3
tgtgcaaga	cccccc			actactttgactactgg	2	6	0	0	32
tgtgcaagg	ccccgcgcgctc	actacggtagtagctac	cctccagg	cctggtttgcttactgg	7	20	5	0	64
tgtgcaaga	ca	agac	tggg	cctggtttgcttactgg	14	6	2	0	36
tgtgcaag	ctagc	tactaccgtagta	ataaatagggga	gactactgg	21	17	0	0	47
tgtgcaaga	cataaga	ctaactgggac		tactgg	13	7	0	3	33
tgtgcaa		tattactacggtagt		tttgactactgg	17	0	0	0	34
tgtgcaaga	catg	actgggac		tactgg	18	4	0	3	27
tgtgcaaga	cttt	ctatggtaac	gg	ctactgg	19	6	0	0	32
tgtgcaagac	g	aact	tt	actactttgactactgg	8/14	3	1	0	34
tgtgcaaga		taact		gactactgg	16/22	0	0	1	23
tgtgcaaga	c	atgg		actactgg	24	1	0	1	22
tgtgcaaga	caag	actggg		cctggtttgcttactgg	7	4	0	0	36
Average:					14.3	6.2	0.7	0.7	35

Supplementary Table 1. Comparative sequences analysis shows that RAG2-N474S mutant induces endogenous IgH rearrangements with wild-type features. PCR products from endogenous V_H7183(D)J rearrangements in pro-B cells transduced with FNT-tagged full-length wild-type RAG2 (R2FL) or R2FL-N474S mutant were cloned into pGEM-T easy vector (Promega) for sequencing. Length of nucleotide deletion or addition (indicated in bold on the sequence), as well as the P-nucleotides (in italic) and the micro-homologies (underlined) are indicated on right, and the resulting length of the CDR3.

R2FL (n = 12)

Vk	Jk	Del.	Add.	PNuc.	<u>μHom</u>	CDR3
tgatcatcaatacctctcct	tcacgttc	4	0	0	0	27
tgtaaacagtggagtagttccccaccca	gctcacgttc	0	0	0	0	38
tgtaacagcacagtagggagcttcctcc	cacgttc	3	0	0	0	33
tgtaacagcacagtagggagcttc	gctcacgttc	3	0	<i>1</i>	0	33
tgtaacagcacagtagtaagctt	acacgttc	8	0	0	1	29
tgcaagcaatcttataatatttc	gctcacgttc	1	1	0	0	33
tgatcatcaatacctctcctc	tcacgttc	3	0	0	1	28
tgccatcagcggagtagttaccca	gctcacgttc	0	2	0	0	36
tgtaaacatttttggagtactcctc	ttc	8	3	0	0	37
tgccagcagtaggagtagtaaccc	tcacgttc	7	0	0	0	31
tgtaaacagtaggagtagttacccactca	cggttc	5	0	0	4	33
tgtaacagcaatattatagctatcctcc	gctcacgttc	0	0	0	0	36
tgccaacagggtaatacgttc	gttc	9	0	0	1	27
tgtaacagcagttgggagattcc	attcacgttc	3	3	2	0	36
tgtaaacaggttatgacgttcctcc	attcacgttc	0	0	0	0	36
tgtaacaaaagtaataagctggccaac	ctcacgttc	1	0	0	0	35
tgtaaacagtaggagtagttacccactca	cacgttc	3	1	0	0	36
tgccagcagtagcagtggttacccatc	acgttc	6	0	0	2	32
tgatcatcaatacctctcctc	ttcacgttc	1	2	2	0	32
tgatcatcaatacctctcctc	ctcacgttc	1	0	0	1	30
Average:		3.3	0.6	0.25	0.5	32.9

R2FL-N474S (n = 12)

Vk	Jk	Del.	Add.	PNuc.	<u>μHom</u>	CDR3
tgtaacagcacagttgggagattcctcc	tcacgttc	2	0	0	1	36
tgccagcggtaggagtagttaccc	gctcacgttc	5	0	<i>1</i>	0	33
tgccagcagtagcagtggttac	ctcacgttc	8	0	0	0	30
tgatcatcaatacctctcctc	cacgttc	3	2	2	0	30
tgcttgacagtagcacattttcctc	attcacgttc	1	3	1	0	35
tgtaacagcacagttgggagattcc	gctcacgttc	3	3	1	0	36
tgtaacagcaggattatagctctcc	gctcacgttc	3	0	0	0	33
tgccagcagtaggagtagttacccaccc	ttcacgttc	2	0	0	0	36
tgtaacagcaagtaatagagtagctcctcc	tcacgttc	2	1	0	0	35
tgtaaacagagtaaacagctggcctca	ttcacgttc	1	0	0	1	35
tgccagcaagagtagtagttaccca	ttcacgttc	5	0	0	1	33
tgtaacagcaggattatagctctcctc	acgttc	5	0	0	3	31
tgtaacagcaaaataatgagtagctcctcc	gtacacgttc	1	0	0	0	36
tgtaacagcacagttgggagattcctc	acgttc	5	0	0	2	31
tgctttcaagggttacagcaccttc	cggttc	5	1	0	0	32
tgccaacagggtaatacgttcctcc	gctcacgttc	0	2	2	0	38
tgccagcagtaggagtagtaacccac	attcacgttc	3	0	0	0	35
tgtaacatcattatggtactcctc	acgttc	5	3	3	0	34
tgtaacagcaagtaaggaggttc	acgttc	8	0	0	3	28
tgctttcaagggttacagcacacc	ctcacgttc	4	0	0	0	32
Average:		3.45	0.75	0.5	0.5	31.65

Supplementary Table 2. Comparative sequences analysis shows that RAG2-N474S mutant induces endogenous Igκ rearrangements with wild-type features. PCR products from endogenous VJκ rearrangements in pro-B cells transduced with FNT-tagged full-length wild-type RAG2 (R2FL) or R2FL-N474S mutant were cloned into pGEM-T easy vector (Promega) for sequencing. Length of nucleotide deletion or addition (indicated in bold on the sequence), as well as the P-nucleotides (in italic) and the micro-homologies (underlined) are indicated on right, and the resulting length of the CDR3.

		Expression				Endogenous Recombination			
RAG2	Protein Degradation	WCE	CE	NE	Interaction with Histones	D-J _H	V-DJ _H	D-J _K	Patient Diagnosis
WT	+	++++	-	++++	+++	+++	+++	+++	Healthy
W416L	++	+++	+	++	-	+	-	-	OS ^{1,2} , T-B-SCID ²
C446W	+++	++	+	+/-	-	+/-	-	-	T-B-SCID ³
W453R	+	++++	+++	+	-	+	-	-	OS ^{4,5}
N474S	+	++++	-	++++	+++	+++	+++	++	SCID with MEF ^{6,7}
C478Y	+++	+	-	+/-	-	+/-	-	-	T-B-SCID ⁶ , SCID with MEF ⁶
H481P	++	+++	+/-	++	++/-	++	-	+/-	<i>leaky</i> T-B-SCID ⁵

¹Sobacchi et al., 2006; ²Haq et al., 2007; ³This study; ⁴Gomez et al., 2000; ⁵Noordzij et al., 2002; ⁶Villa et al., 2001; ⁷Erkin et al., 2005

Supplementary Table 3. Summary of protein expression, degradation, histone interaction and endogenous recombination activity observed in mouse proB cells (Figures 1, 2 and 3) for mouse Rag2 PHD mutants and wildtype protein. Whole cell extract (WCE), cytoplasmic extracts (CE) and nuclear extracts (NE).