SUPPLEMENTAL INFORMATION

Germline mutations in shelterin complex genes are associated with familial chronic lymphocytic leukemia

Helen E. Speedy[1], Ben Kinnersley[1], Daniel Chubb[1], Peter Broderick[1], Philip J. Law[1], Kevin Litchfield[1], Sandrine Jayne[2], Martin J. S. Dyer[2], Claire Dearden[3], George A. Follows[4], Daniel Catovsky[5] and Richard S. Houlston[1,5,*]

[1]Division of Genetics and Epidemiology, The Institute of Cancer Research, London, UK; [2]Ernest and Helen Scott Haematological Research Institute, University of Leicester, Leicester, UK; [3]Department of Haemato-Oncology, Royal Marsden Hospital, Sutton, UK; [4]Department of Haematology, Cambridge University Hospitals NHS Foundation Trust, Addenbrooke's Hospital, Cambridge, UK; and [5]Division of Molecular Pathology, The Institute of Cancer Research, London, UK

Supplemental Table 1. Summary information for exome sequenced individuals

		Number of cases in family						
Round	Family ID	CLL	Other LPD	Sequenced[a]	Sample ID	Relationship to proband	Sex	Age at diagnosis
1	5	5	0	3	5201	Proband	Female	54
1	5	5	0	3	5202	Brother	Male	47
1	5	5	0	3	5203	Brother	Male	46
1	36	3	0	2	36202	Sister[b]	Female	76
1	36	3	0	2	36203	Brother	Male	63
1	37	4	0	4	37201	Proband	Male	84
1	37	4	0	4	37202	Sister	Female	85
1	37	4	0	4	37203	Sister	Female	78
1	37	4	0	4	37204	Brother	Male	59
1	42	2	2	2 (1)	42201	Proband	Male	58
1	42	2	2	2 (1)	42202	Brother	Male	52
1	42	2	2	2 (1)	42301	First cousin	Female	42
1	45	3	0	3	45201	Proband	Male	60
1	45	3	0	3	45202	Sister	Female	51
1	45	3	0	3	45203	Brother	Male	na
1	78	3	0	2	78104	Uncle	Male	53
1	78	3	0	2	78201	Proband	Male	31
1	166	3	0	3	166201	Proband	Female	68
1	166	3	0	3	166202	Brother	Male	59
1	166	3	0	3	166203	Brother	Male	59
1	208	3	0	4	208201	Proband	Male	78
1	208	3	0	4	208203	Brother	Male	79
1	208	3	0	4	208302	Daughter	Female	44
1	231	2	0	2	231201	Proband	Female	58
1	231	2	0	2	231202	First cousin	Male	62
1	254	3	0	2	254201	Proband	Male	59
1	254	3	0	2	254205	First cousin	Male	56
1	387	5	0	2	387201	Proband	Male	69
1	387	5	0	2	387304	Niece	Female	26
1	390	2	0	2	390201	Proband	Female	55
1	390	2	0	2	390306	Nephew	Male	35
1	392	3	0	2	392202	Sister[b]	Female	51
1	392	3	0	2	392301	Son	Male	47
1	4014	3	1	3	4014101	First cousin	Female	69
1	4014	3	1	3	4014201	Proband	Female	63
1	4014	3	1	3	4014202	Brother	Male	66
1	4027	2	0	2	4027201	Proband	Male	57
1	4027	2	0	2	4027202	First cousin	Female	60
1	4085	3	0	2	4085201	Proband	Female	49
1	4085	3	0	2	4085202	First cousin	Male	64
1	5034	2	0	2	5034103	Proband	Male	49

		Number of cases in family						
Round	Family ID	CLL	Other LPD	Sequenced[a]	Sample ID	Relationship to proband	Sex	Age at diagnosis
1	5034	2	0	2	5034201	Uncle	Male	71
1	5047	3	2	3	5047201	Proband	Female	53
1	5047	3	2	3	5047202	Sister	Female	61
1	5047	3	2	3	5047203	Brother	Male	61
2	13	2	0	2	13201	Proband	Female	70
2	13	2	0	2	13203	Brother	Male	53
2	32	2	0	2	32201	Proband	Male	50
2	32	2	0	2	32202	Brother	Male	54
2	96	2	0	2	96201	Proband	Male	63
2	96	2	0	2	96202	Brother	Male	60
2	110	2	1	2	110201	Proband	Male	60
2	110	2	1	2	110202	Brother	Male	66
2	121	4	0	2	121201	Proband	Male	52
2	121	4	0	2	121202	Sister	Female	55
2	134	3	0	2	134201	Proband	Male	71
2	134	3	0	2	134202	Sister	Female	70
2	148	2	1	2	148201	Proband	Female	63
2	148	2	1	2	148202	Brother	Male	57
2	156	2	0	2	156201	Proband	Male	42
2	156	2	0	2	156202	Brother	Male	42
2	162	2	0	2	162201	Proband	Male	54
2	162	2	0	2	162202	Brother	Male	60
2	190	3	0	2	190201	Proband	Female	59
2	190	3	0	2	190202	Sister	Female	75
2	191	2	1	2	191201	Proband	Male	51
2	191	2	1	2	191202	Brother	Male	64
2	198	2	0	2	198201	Proband	Male	68
2	198	2	0	2	198202	Brother	Male	68
2	209	3	0	2	209201	Proband	Female	43
2	209	3	0	2	209202	Sister	Female	69
2	233	2	0	2	233201	Proband	Female	46
2	233	2	0	2	233203	Brother	Male	53
2	239	2	0	2	239201	Proband	Male	na
2	239	2	0	2	239202	Sister	Female	51
2	243	2	0	2	243201	Proband	Female	46
2	243	2	0	2	243202	Brother	Male	75
2	255	2	1	2	255201	Proband	Female	76
2	255	2	1	2	255202	Sister	Female	84
2	267	2	0	2	267201	Proband	Male	49
2	267	2	0	2	267202	Brother	Male	59
2	276	2	0	2	276201	Proband	Female	49
2	276	2	0	2	276202	Brother	Male	46
2	301	2	0	2	301201	Proband	Male	55

		Number of cases in family						
Round	Family ID	CLL	Other LPD	Sequenced[a]	Sample ID	Relationship to proband	Sex	Age at diagnosis
2	301	2	0	2	301202	Sister	Female	49
2	305	2	0	2	305201	Proband	Female	65
2	305	2	0	2	305202	Brother	Male	59
2	332	2	0	2	332201	Proband	Female	54
2	332	2	0	2	332202	Sister	Female	65
2	333	3	0	2	333201	Proband	Male	57
2	333	3	0	2	333202	Sister	Female	58
2	372	3	0	2	372201	Proband	Male	55
2	372	3	0	2	372202	Brother	Male	65
2	374	2	0	2	374201	Proband	Female	61
2	374	2	0	2	374202	Brother	Male	63
2	379	2	0	2	379201	Proband	Male	61
2	379	2	0	2	379202	Brother	Male	60
2	395	2	0	2	395201	Proband	Female	73
2	395	2	0	2	395205	Sister	Female	62
2	399	2	0	2	399201	Proband	Male	66
2	399	2	0	2	399202	Sister	Female	82
2	1796	2	0	2	1796201	Proband	Male	73
2	1796	2	0	2	1796202	Brother	Male	63
2	4001	2	0	2	4001201	Proband	Male	75
2	4001	2	0	2	4001202	Brother	Male	70
2	4002	2	0	2	4002201	Proband	Male	49
2	4002	2	0	2	4002203	Sister	Female	53
2	4006	3	1	2	4006201	Proband	Female	72
2	4006	3	1	2	4006202	Brother	Male	68
2	4009	2	0	2	4009201	Proband	Male	60
2	4009	2	0	2	4009202	Sister	Female	67
2	4013	3	0	2	4013201	Proband	Male	68
2	4013	3	0	2	4013202	Sister	Female	65
2	4029	4	0	2	4029201	Proband	Male	61
2	4029	4	0	2	4029202	Brother	Male	58
2	4033	2	0	2	4033201	Proband	Male	63
2	4033	2	0	2	4033202	Brother	Male	62
2	4038	2	0	2	4038201	Proband	Male	64
2	4038	2	0	2	4038202	Brother	Male	62
2	4042	2	0	2	4042201	Proband	Female	75
2	4042	2	0	2	4042202	Sister	Female	56
2	4043	2	0	2	4043201	Proband	Female	58
2	4043	2	0	2	4043202	Brother	Male	58
2	4047	3	1	2	4047201	Proband	Female	58
2	4047	3	1	2	4047202	Brother	Male	65
2	4065	2	0	2	4065201	Proband	Female	71
2	4065	2	0	2	4065202	Sister	Female	76

		Number of cases in family						
Round	Family ID	CLL	Other LPD	Sequenced[a]	Sample ID	Relationship to proband	Sex	Age at diagnosis
2	4089	2	0	2	4089201	Proband	Female	71
2	4089	2	0	2	4089202	Sister	Female	56
2	4092	3	0	2	4092201	Proband	Female	54
2	4092	3	0	2	4092203	Brother	Male	64
2	5000	2	2	2	5000201	Proband	Male	31
2	5000	2	2	2	5000102	Mother	Female	41
2	5009	2	0	2	5009201	Proband	Male	74
2	5009	2	0	2	5009202	Sister	Female	78
2	5033	2	0	2	5033201	Proband	Male	66
2	5033	2	0	2	5033205	Brother	Male	64
2	5044	2	0	2	5044201	Proband	Female	65
2	5044	2	0	2	5044202	Brother	Male	70
2	5065	2	0	2	5065201	Proband	Male	62
2	5065	2	0	2	5065202	Sister	Female	62

LPD= lymphoproliferative disease; na = not accurately known

[a] CLL samples(other LPD samples)

[b] Proband sample failed quality control and was not exome sequenced

Supplemental Table 2. Primer sequences for mutation verification and RT-PCR

Purpose	Gene	Mutation Position	Forward	Reverse	Product size (bp)
Mutation verification	*POT1*	7:g.124481233C>T	AGACAATCAGCTTAGCATTGACA	CCAGGCATAGAAATCACTGGT	748
Mutation verification	*POT1*	7:g.124532337T>C	CTGTGTGCATTGCCTTATTTCAG	TGCATCAGTGTTGTTTGGCA	399
Mutation verification	*POT1*	7:g.124482952_124482953insA	GCTAGTGGGCCTTGGGTATA	TGCCTTTATCAGAGTGACTAAGC	568
Mutation verification	*POT1*	7:g.124482897T>C	CATCAGTATTTGGAGAGGACACC	AGATCTGAAAATAGCACCCACT	353
Mutation verification	*ACD*	16:g.67692984T>G	AGCATGGTTCCTGAGTCCTG	CCAAGCAAATCCCCAGACTG	396
Mutation verification	*TERF2IP*	16:g.75682090G>C	AGCTCCATGTCCTTCTACGT	CTTGAGCTTCTGGGAGGAGG	552
Mutation verification	*TERF2IP*	16:g.75682178G>A	AGGCGATGGATTTGGGCA	GGACTGCCACGAGTGCTG	530
RT-PCR	*POT1*	7:g.124481233C>T	GCATCCGAGCAAAATTGAGG	TCCAAAAGTTCCAGGTCTTCG	336/379[a]
Telomere length	*TEL*[b]	na	CGGTTTGTTTGGGTTTGGGTTTGGGTT TGGGTTTGGGTT	GGCTGGCCTTACCCTTACCCTTACC CTTACCCTTACCCT	na
Telomere length	*CON*[c]	na	GCTTCTGACACAACTGTGTTCACTAGC	CACCAACTTCATCCACGTTCACC	na

na, not applicable

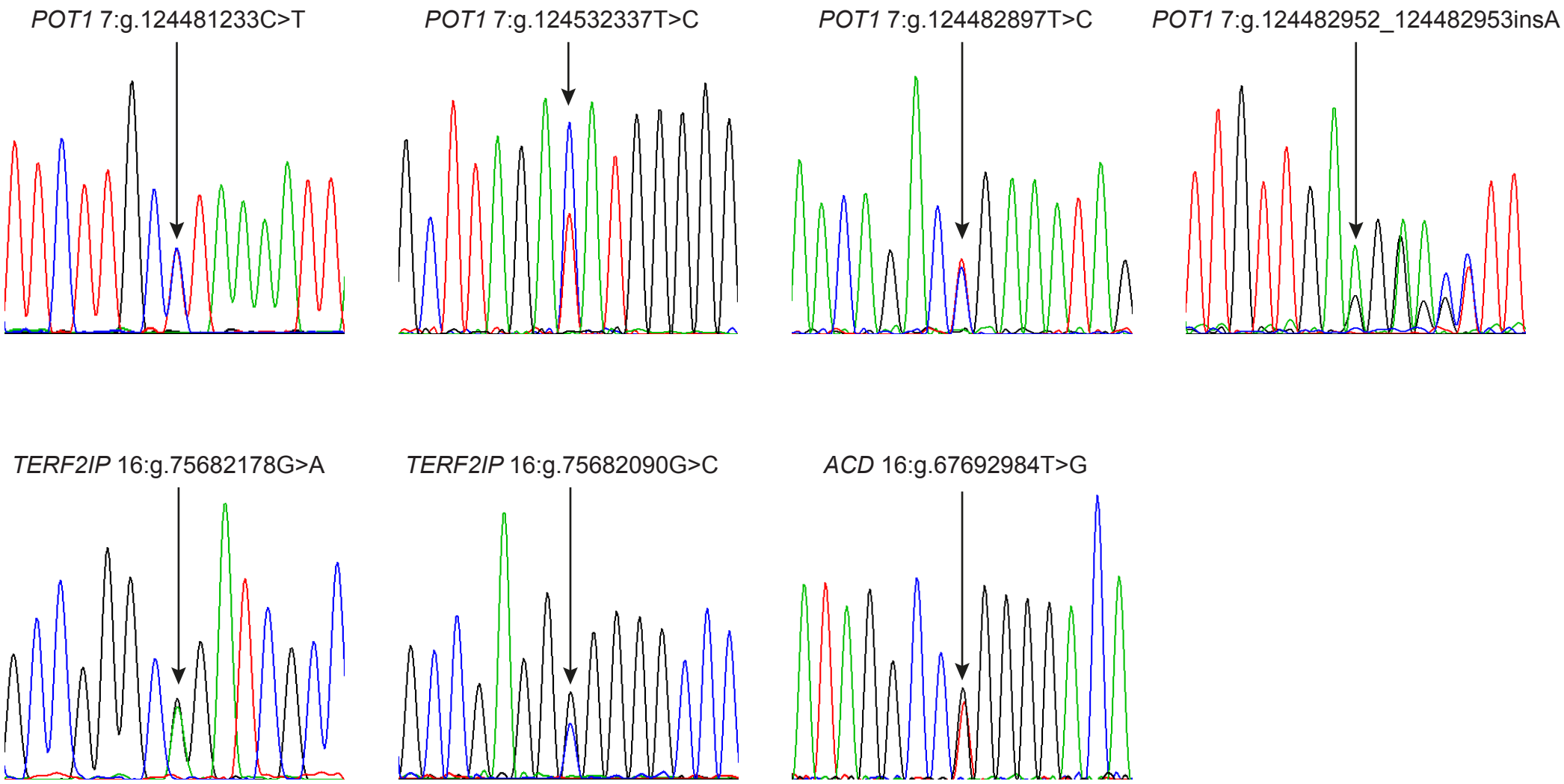
[a] RT-PCR product size without splice acceptor mutation/with mutation (predicted)

[b] TEL = telomeric repeat unit (fluorescence detected in reaction proportional to the number of telomeric repeats)

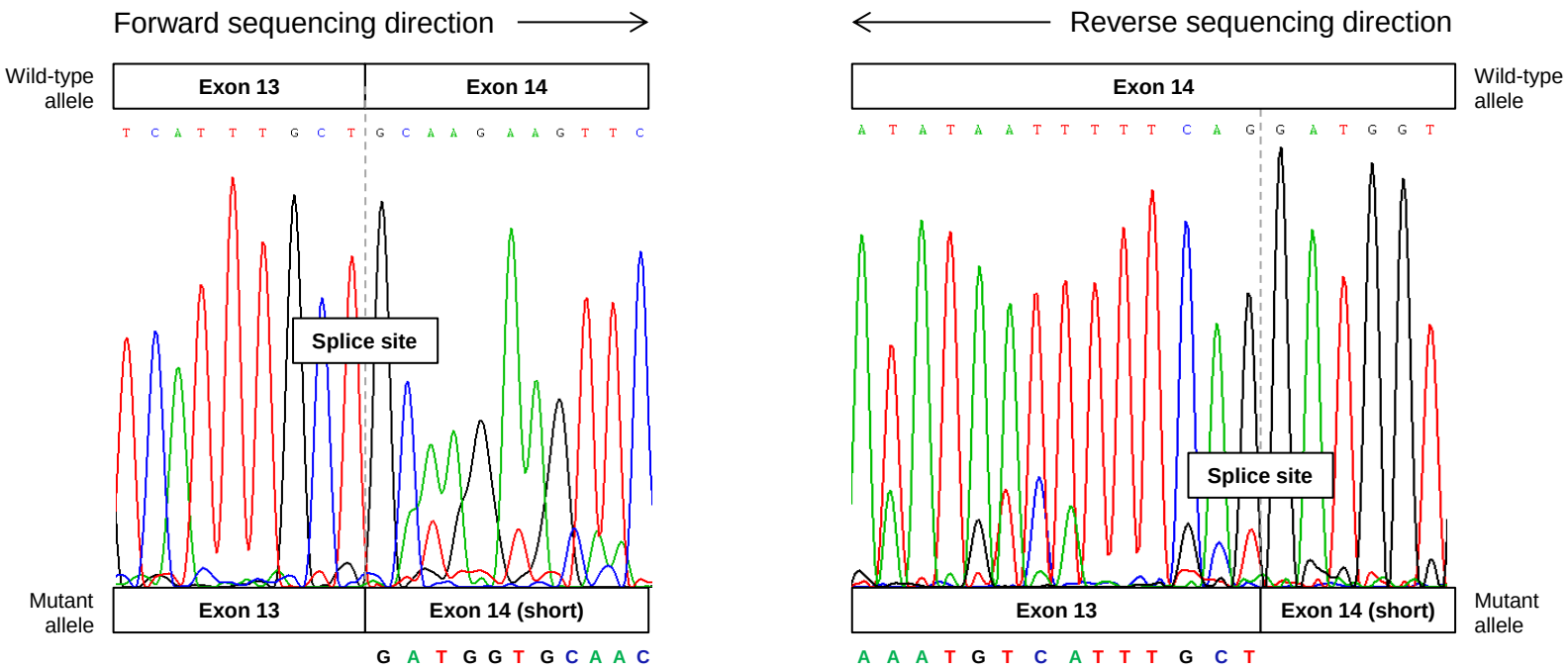
[c] CON= β-globin, single copy gene

Supplemental Table 3. Predicted impact of *POT1* and *TERF2IP* non-synonymous variants on protein stability using INPS

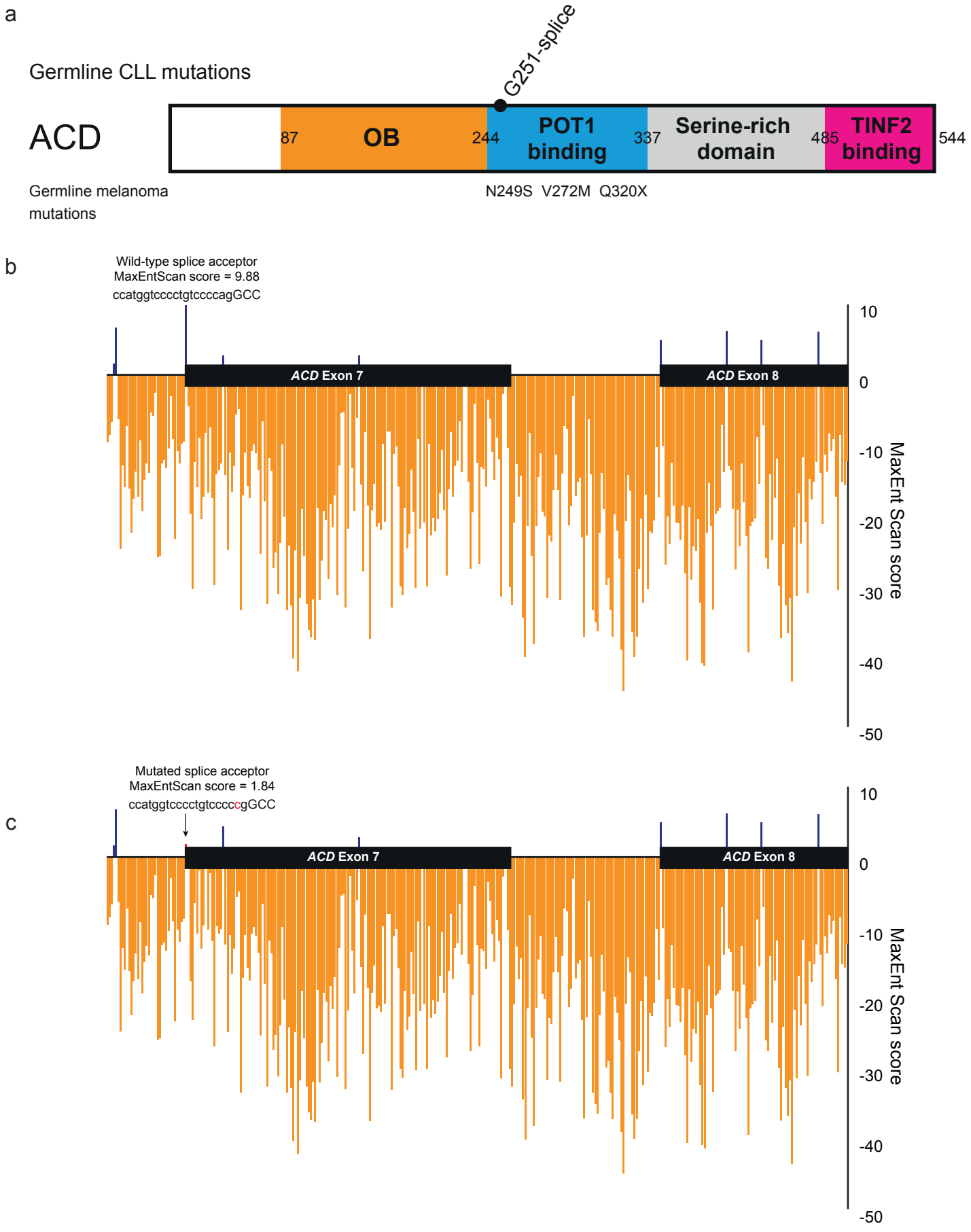
Protein	Mutation	Stability Change ($\Delta\Delta$G, kcal/mol)
POT1	Q376R	-0.05
TERF2IP	A104P	+0.23
TERF2IP	R133Q	-1.32



Supplemental Figure 1. Sanger sequencing confirmation of variants identified by whole exome sequencing.
Representative sequencing traces from one carrier of each mutation are shown. Reactions were performed using mouthwash-derived DNA



Supplemental Figure 2. Sanger sequencing to confirm utilization of predicted alternative *POT1* splice donor site
Sequencing of cDNA from proband of pedigree 5047, performed in both forward and reverse orientations, demonstrates the use of the alternative splice acceptor site predicted by MaxEntScan. Nucleotides derived from the wild-type allele are shown above sequence traces and mutant sequences are shown below.



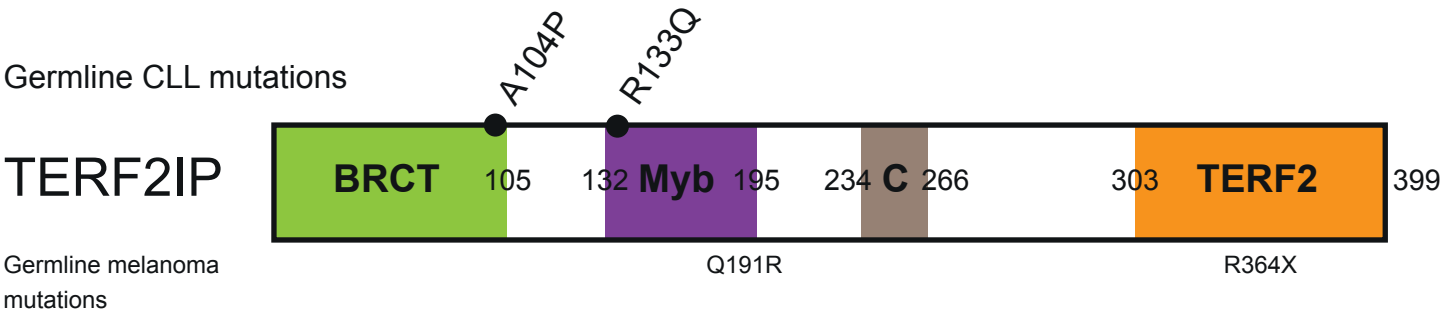
Supplemental Figure 3. Impact of rare familial mutation on ACD protein

(a) Schematic showing position of germline ACD mutation identified in family 233 relative to OB domains (orange) and POT1 binding region (blue). A serine-rich domain (grey) and the TINF2 protein binding domain (pink) are also shown. Germline mutations found in familial cutaneous melanoma are illustrated below the ACD protein.

(b) Splice acceptor site consensus scores predicted by MaxEntScan for each base from the wild-type ACD intron 6/exon 7 boundary. Positive scores are marked in blue, negative scores in orange. The sequence and predicted score for the wild-type splice site is labelled.

(c) MaxEntScan splice acceptor consensus scores for the same region based upon the sequence of c.752-2A>C mutation carriers. The mutated splice acceptor sequence is also labelled.

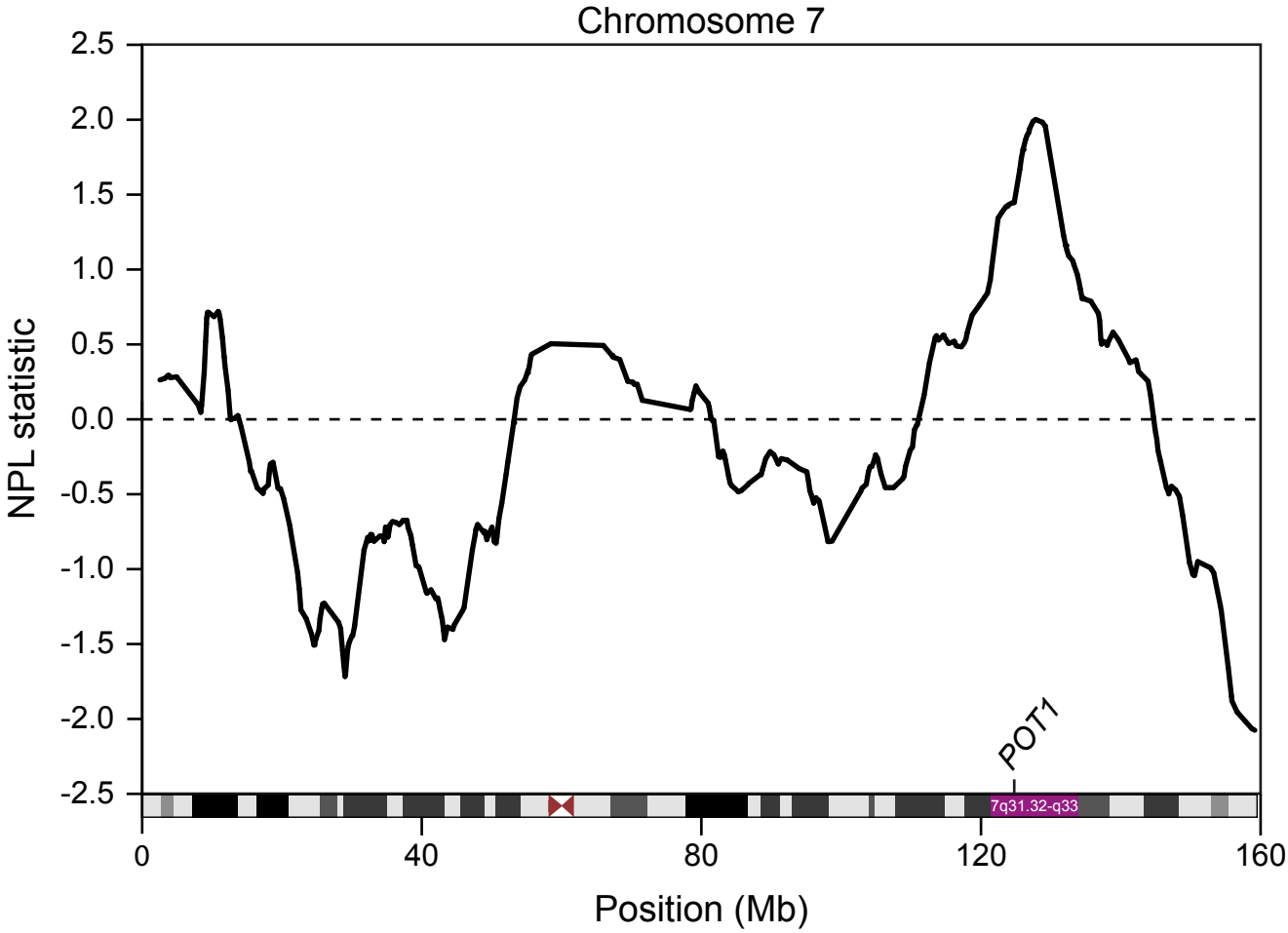
a

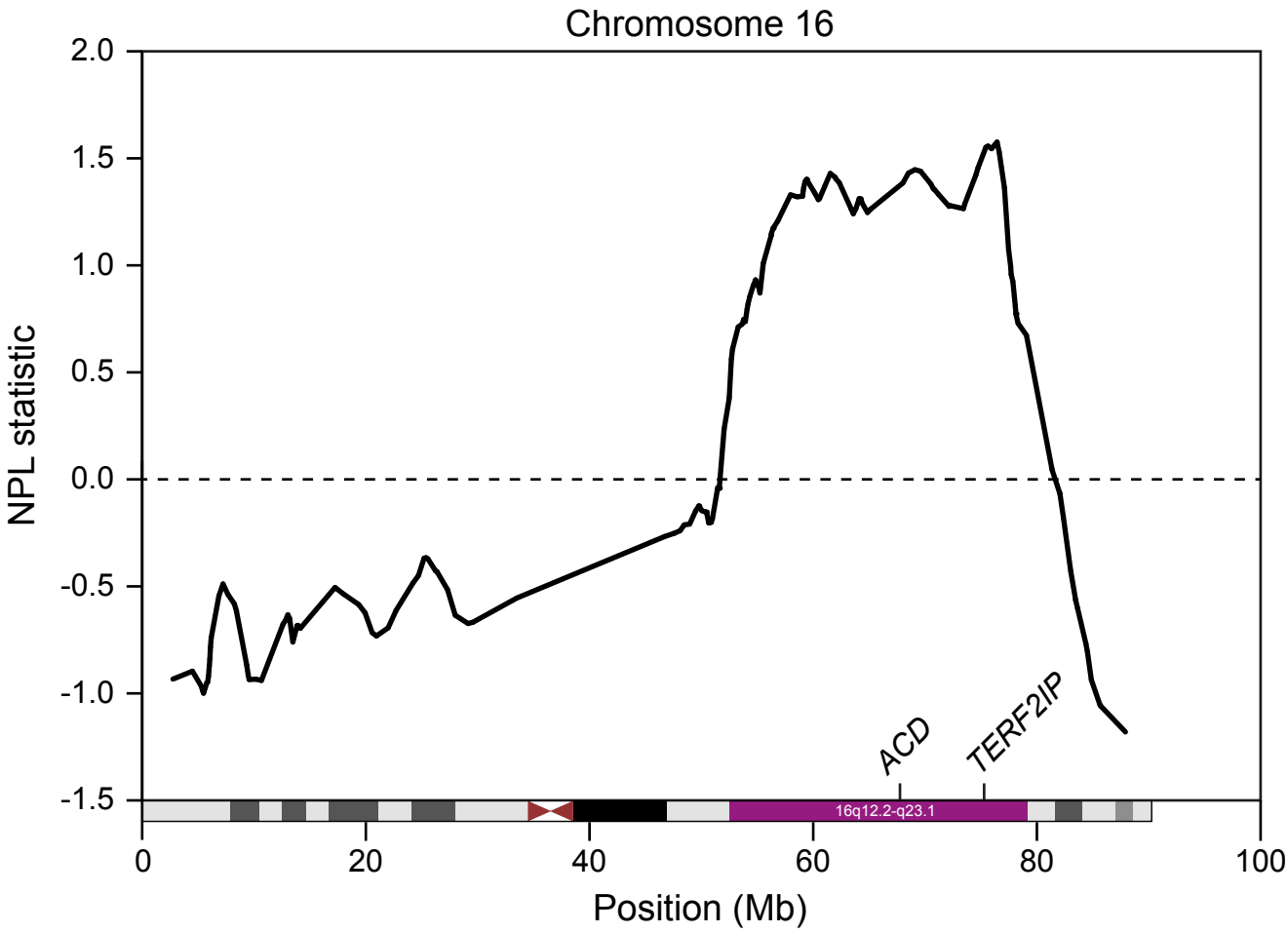


b

	p.Ala104	p.Arg133
Human	G - P A S A A	H A G R I A F
Chimpanzee	G - P A S A A	L A G R I A F
Orangutan	G - P A S A A	L A G R I A F
Marmoset	G - P A S A A	H T G R V A Y
Tree shrew	G - R V A A A	L T V R I A F
Mouse	G - L T E - -	L T G R I A Y
Rabbit	G - L A S G A	P A G R I S F
Cow	G - P A P A A	Q A G R M V F
Dog	G - S A A - -	L A G R I A F
Horse	R - P A P A A	Y T G R M G F
Bat	G - L A P A A	H T G R I A F
Elephant	G - S A - - A	L T G R T V F
Armadillo	G - P A S A A	P T G R M A F
Platypus	G - K A A A G	L S G R I P F
Chicken	S G P A P - -	P R G R L P F
Frog	D - G S H G R	Q T G R N P F

Supplemental Figure 4. Impact of rare familial mutations on TERF2IP protein
(a) Schematic showing position of germline TERF2IP mutations identified in families 4014 and 4092. The BRCT (BRCA1 C-terminus, green), Myb (purple), coil (brown) and TERF2 protein binding (orange) domains are also shown. Germline mutations found in familial cutaneous melanoma are illustrated below the TERF2IP protein.
(b) Cross-species conversion of TERF2IP amino acids subject to missense mutation in CLL families.





Supplemental Figure 5. Linkage analysis of familial CLL for chromosomes 7 and 16
Data from Sellick *et al*., (2007), based on 182 CLL families. 1-LOD support intervals are marked in purple. Positions of *POT1*, *ACD* and *TERF2IP* are labelled. NPL=non-parametric linkage