Supplementary Information for Manuscript "Characterisation of a Portuguese origin founder missense variant in *MSH6*".

This PDF contains all supplementary information to accompany the manuscript as mentioned above. This includes images of IHC staining for the MMR proteins from two probands, a table detailing each family's cancer history, and a table detailing the interpretation of our variant.

Table S1. Clinicopathological features of *MSH6* c.2061T>G carrier families

Family	Country	Clinical Criteria Fulfilled	N	Relationship to Proband	Sex	*MSH6* Status	Diagnosis	Age	IHC			
									MLH1	MSH2	MSH6	PMS2
I	Canada	Bethesda	1	Proband	F	+/-	HL	30s*		-		
							EC	40s*	retained	retained	absent	retained
							BlC	50s*		-		
							RC	50s*	equivocal	weak	weak	absent [a]
							CRC	50s*		retained		
			2	Father	M	UNK	LC	60s		-		
			3	Mother	F	+/+	-			-		
			4	Daughter	F	+/-	-			-		
			5	Son	M	+/+	-					
			6	Sister	F	+/-	EC	50s*		retained [c]		
			7	Sister	F	+/-	-[b]			-		
			8	Niece	F	+/-	-			-		
			9	Nephew	M	+/-	-			-		
			10	Nephew	M	+/+	HL			-		
II	Canada	Bethesda	1	Proband	F	+/-	EC [d]	50s*	retained	retained	absent	retained
			2	Daughter	F	+/+	BCC	<20*		-		
							ThC	30s*		-		
			3	Brother	M	UNK	CRC	50s		-		
			4	Brother	M	UNK	BlC	70s		-		
			5	Brother	M	UNK	GC	60s		-		
			6	1st Cousin (Mat)	F	UNK	CRC	40s		-		
			7	1st Cousin (Mat)	M	UNK	GC	40s		-		
			8	1st Cousin (Mat)	F	UNK	CRC	50s		-		
			9	Uncle (Mat)	M	UNK	GC	50s		-		
			10	Aunt (Mat)	F	UNK	GlC	70s		-		
III	Portugal	Amsterdam II	1	Proband	F	+/-	EC [e]	40s*	retained	retained	absent	retained
			2	Mother	F	+/-	EC	50s		-		
			3	Uncle (Mat)	M	+/-	CRC	60s*	retained	retained	absent	retained
							BlC	70s		retained		
							SkC	70s		-		
			4	Aunt (Mat)	F	+/-	EC	50s*	retained	absent	absent	retained
IV	Portugal	Bethesda	1	Proband	F	+/-	CRC [g]	80s		-		
							EC	80s		retained		
			2	Mother	F	UNK	CRC	80s		-		
			3	Sister	F	UNK	CRC	50s		-		
							GynC	50s		-		
			4	1st Cousin (Mat)	F	UNK	BrC	30s*		-		
V	Portugal	None	1	Proband	F	+/-	SSL w/D x 1	40s*		retained		
							SSL x 1	40s*		-		
							TA(LGD) x 9	40s*		-		
							TvA(LGD) x 1	40s*		-		
							TA(HGD) x 1	60s*		-		
			2	Mother	F	UNK	GynC	80s		-		
			3	Father	M	UNK	CRC	60s		-		
			4	Aunt (Pat)	F	UNK	GynC	30s		-		
			5	Grandfather (Pat)	M	UNK	CRC	60s		-		
VI	Portugal	Amsterdam II	1	Proband	F	+/-	CRC	40s*	retained	retained	N/A	retained
							EC	50s*	retained	absent	retained	retained
			2	Father	M	UNK	DC	50s		-		
			3	Daughter	F	+/-	TA(LGD) x 1	20s		-		
			4	Son	M	+/+	TA(LGD) x 1	30s		-		
			5	Sister	F	+/+	-			-		
			6	Brother	M	UNK	CRC	50s		-		
			7	Nephew	M	UNK	GBL	<20		-		
			8	Uncle (Mat)	M	UNK	GC	70s		-		
			9	1st Cousin (Mat)	F	UNK	OvC	30s		-		
VII	Portugal	Amsterdam	1	Proband	M	+/-	CRC	50s		-		
			2	Father	M	UNK	CRC	60s		-		
			3	Mother	F	UNK	BlC	80s		-		
			4	Sister	F	UNK	ThC	50s		-		
			5	Sister	F	UNK	BrC	30s		-		
							CRC	40s		-		
			6	Uncle (Pat)	M	UNK	CRC	60s		-		
			7	Aunt (Pat)	F	UNK	CRC	80s		-		
			8	1st Cousin (Pat)	M	UNK	CRC	80s		-		
			9	1st Cousin (Pat)	F	UNK	OvC	70s		-		
			10	1st Cousin Once Removed (Pat)	M	UNK	CRC	30s		-		
			11	Aunt (Mat)	F	UNK	CRC	70s		-		
							GC			-		
			12	Aunt (Mat)	F	UNK	CRC			-		
			13	Uncle (Mat)	M	UNK	LC (smoker)	60s		-		
			14	1st Cousin (Mat)	M	UNK	CRC	60s		-		
			15	1st Cousin (Mat)	F	UNK	CRC			-		
VIII	Switzerland	None	1	Proband	M	-/VUS	MB	<10*	Loss of MSH6 in non-neoplastic cells [f]			
							GlC	20s				
							MG					
							HGG					
IX	USA	None	1	Proband	UNK	+/-	CRC	50s		-		
X	USA	Bethesda	1	Proband	F	+/-	CRC	30s		-		
							EC	40s		-		
XI	USA	Bethesda	1	Proband	M	+/-	CRC [g]	60s	retained	retained	absent	retained
			2	Father	M	UNK	CRC	60s		-		
XII	USA	Amsterdam II	1	Proband	F	+/-	DCIS [h]	30s		-		
							CRC	40s*	retained [c]	retained	partial loss	retained
			2	Sister	F	+/+	-			-		
			3	Father	M	+/+	-			-		
			4	Mother	F	UNK	BrC	50s		-		
							EC	50s		-		
			5	Aunt (Mat)	F	UNK	BrC	50s		-		
			6	Grandfather (Pat)	M	UNK	CRC	60s		-		
			7	Grandmother (Mat)	F	UNK	NHL	50s		-		
							EC	60s		-		
			8	Great aunt (Mat)	F	UNK	GynC					
			9	2nd Cousin (Mat)	M	UNK	CRC	40s				
			10	2nd Cousin (Mat)	F	+/+	BrC	50s		-		
XIII	Portugal	Amsterdam II	1	Proband	M	+/-	CRC	30s*		retained		
			2	Mother	F	UNK	RC	50s		-		
			3	Aunt (Pat)	F	UNK	BrC	50s		-		
			4	Grandmother (Mat)	F	UNK	CRC	60s		-		
			5	Great aunt (Mat)	F	UNK	CRC	60s		-		
			6	1st Cousin Once Removed (Mat)	M	UNK	CRC	60s				
XIV	Portugal	Bethesda	1	Proband	F	+/-	CRC	40s		-		
			2	Mother	F	UNK	BrC	60s		-		
							EC	60s		-		
			3	Aunt (Mat)	F	UNK	ThC	50s		-		

Legend
BCC - basal cell carcinoma
BlC - bladder cancer
BrC - breast cancer
CRC - colorectal cancer
DC - duodenal cancer
DCIS - ductal carcinoma in situ
EC - endometrial cancer
GBL - gastric Burkitt lymphoma
GC - gastric cancer
GIC - gastrointestinal cancer (origin unspecified)
GynC - gynecological cancer of unknown origin
HGG - high-grade glioma
HL - Hodgkin's lymphoma
LC - lung cancer
MB - medulloblastoma
MG - meningioma
NHL - non-Hodgkin's lymphoma
OvC - ovarian cancer
RC - renal cancer
SkC- skin cancer
SSL w/D - sessile serrated lesion with dysplasia
SSL - sessile serrated lesion
TA(LGD) - tubular adenoma with low grade dysplasia
ThC - thyroid cancer
TvA(LGD) - tubulovillous adenoma with low grade dysplasia
UC - urothelial cancer
UNK - unknown
[a] expression also absent in internal control
[b] total abdominal hysterectomy at 53, now 73
[c] tumour also microsatellite stable
[d] *MSH6* c.3261del, p.(Phe1088SerfsTer2) in tumour
[e] *MSH6* c.3261del, p.(Phe1088SerfsTer2) in tumour at 31% allele frequency
[f] clinical CMMRD, carrier of *MSH6* VUS c.1196C>T (p.Pro399Leu)
[g] two primary colorectal cancers
[h] multifocal DCIS
* confirmed with medical records

Table S2. Interpretation of *MSH6* c.2061T>G (p.Cys687Trp)

CanVIG-UK Guidelines

Rule Applied	Strength	Evidence	Points
Population data (PM2)	Moderate	Absent from gnomAD	2
Cosegregation (PP1)	Supporting	<1/8 LR	1
Phenotypic specificity/Case counting (PP4)	Strong	≥3 independent CRC/Endometrial MSI-H tumours in ≥2 families using a standard panel of 5-10 markers and/or loss of MMR protein expression consistent with the variant location (Families I, II, III, VIII, XI)	4
In silico predictions (PP3)	Supporting	Variant is predicted as pathogenic by REVEL (score = 0.825)	1
Enrichment/constraint (PM1)	Supporting	Variant located in established connector domain; changes at amino acid positions 686 and 688 are damaging	1
		Total:	**9**

ACMG Guidelines

Rule Applied	Strength	Evidence	Points
Phenotypic specificity (PP4)	Strong	≥3 independent CRC/Endometrial MSI-H tumours in ≥2 families using a standard panel of 5-10 markers and/or loss of MMR protein expression consistent with the variant location (Families I, II, III, VIII, XI)	4
Population data (PM2)	Supporting	Absent from gnomAD	1
Alternate Molecular Basis (BP5)	Supporting	MMR protein expression not consistent with variant location (Families I, III, IV, V, VI, XII, XIII)	-1
		Total:	**4**

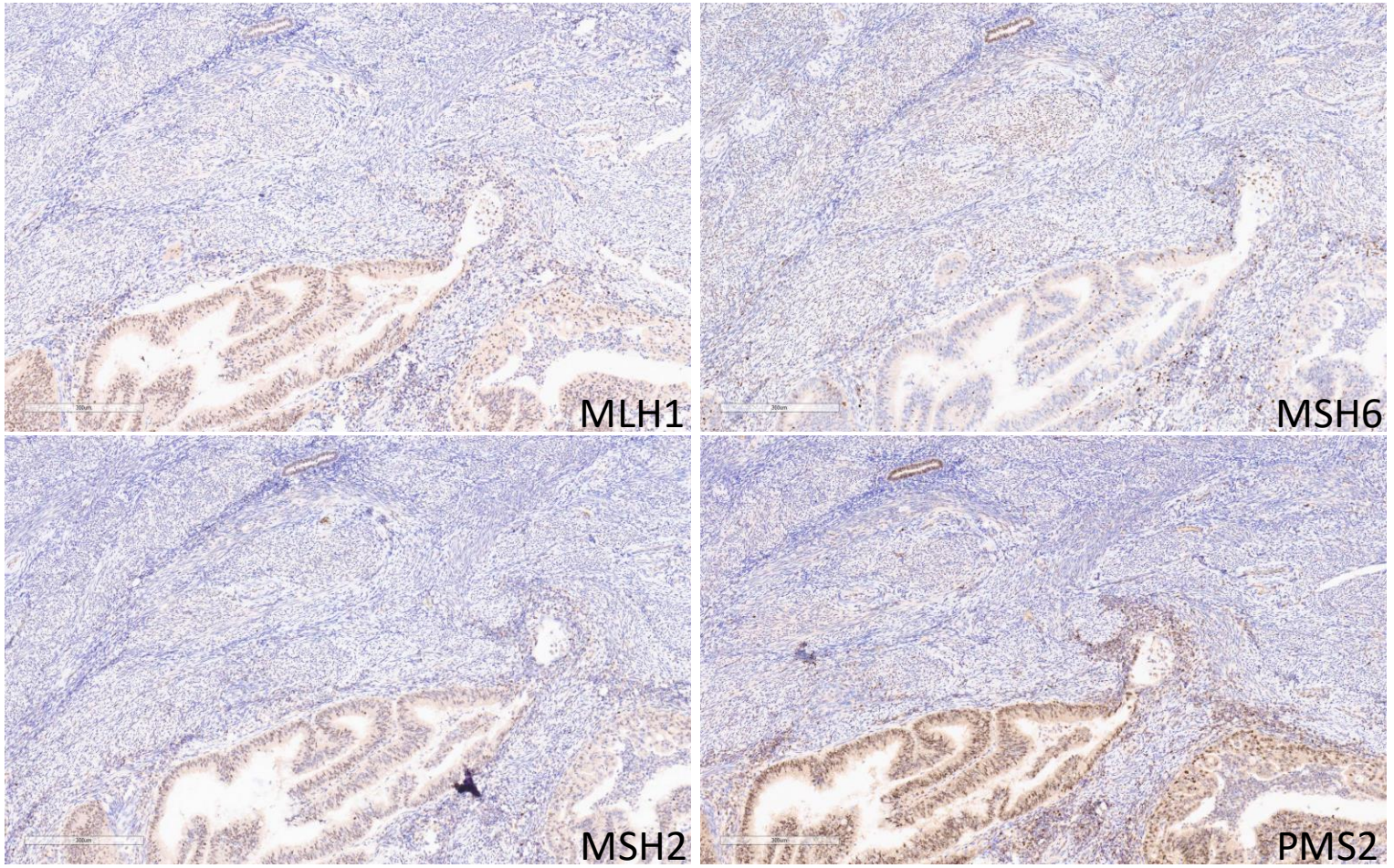

Figure S1. Immunohistochemistry of MMR proteins in endometrial cancer from proband of Family II

Female proband was diagnosed with MSH6-deficient endometrial cancer in her 50s. This individual is known to carry *MSH6* c.2061T>G in the germline. A second variant was identified in the tumor [*MSH6* c.3261del p.(Phe1088SerfsTer2)]. Magnification 100x. IHC performed using ready-to-use anti-bodies for Dako Omnis: anti-MLH1 (clone ES05) antibody, anti-MSH2 (clone FE11) antibody, anti-MSH6 (clone EP49) antibody, and anti-PMS2 (clone EP51) antibody.

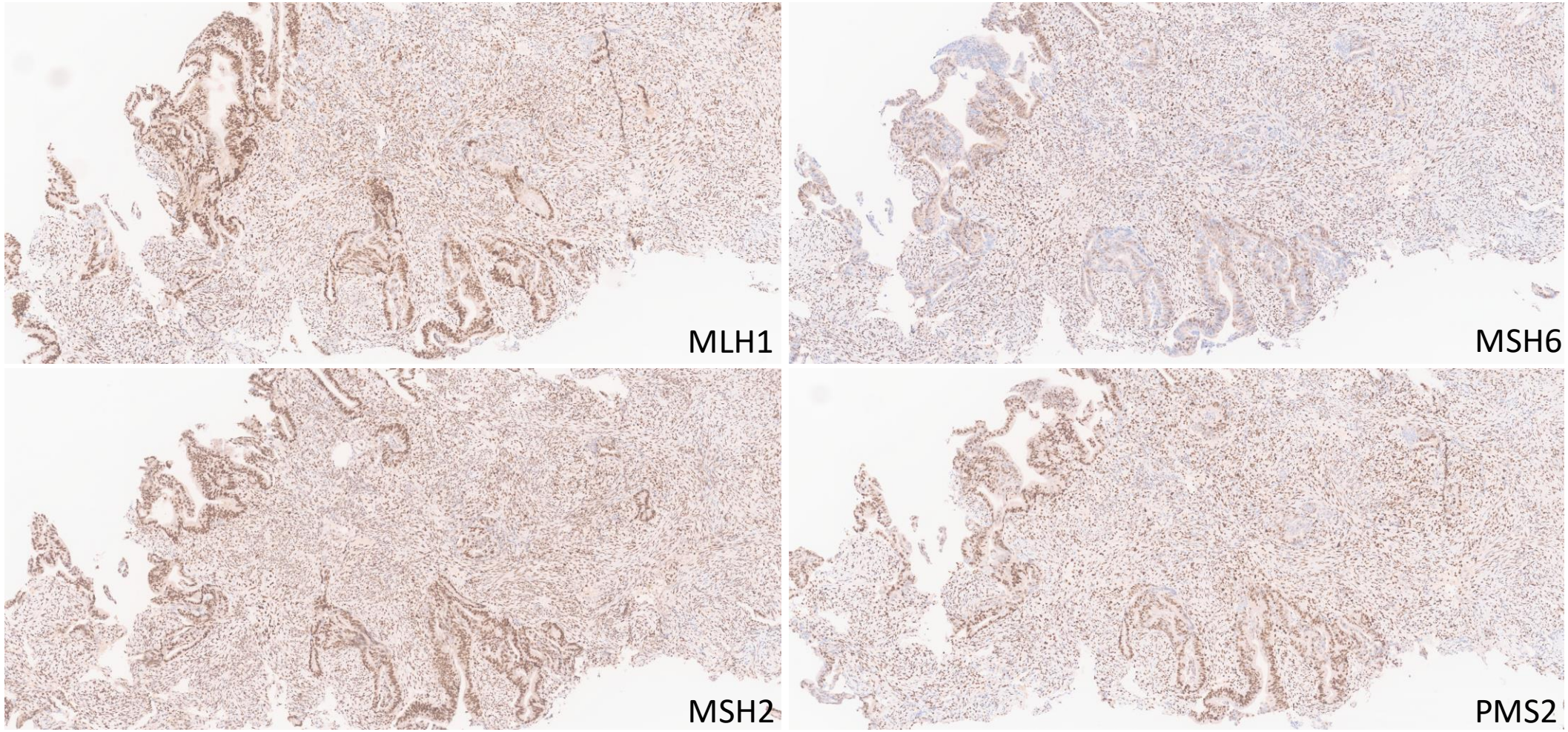

Figure S2. Immunohistochemistry of MMR proteins in colon cancer from proband of Family XIII
Male proband was diagnosed with colon cancer demonstrating partial loss of MSH6 in his 30s. This individual is known to carry *MSH6* c.2061T>G in the germline. Magnification 10x. IHC performed using Leica Biosystems anti-MLH1 (clone ES05) antibody, anti-MSH2 (clone C219-1129) antibody, anti-MSH6 (clone EP49) antibody, and anti-PMS2 (clone EP51) antibody.