

## **Supplementary data.**

### **Contains:**

Figs S1 and S2

Tables S1-S4

### **Supplementary Figure legends**

Figure S1. Chromatograms showing *POLE* and *POLD1* EDMs reported in this study. The upper reads in each panel are the reference sequence generated by the Mutation Surveyor program.

Figure S2. Sequence alignment of human *POLE* exonuclease domain (residues 268-471) and orthologues with exo motifs highlighted and invariant residues indicated by asterisks. Mutated residues detected in this study and the TCGA dataset are indicated.

Figure S1.

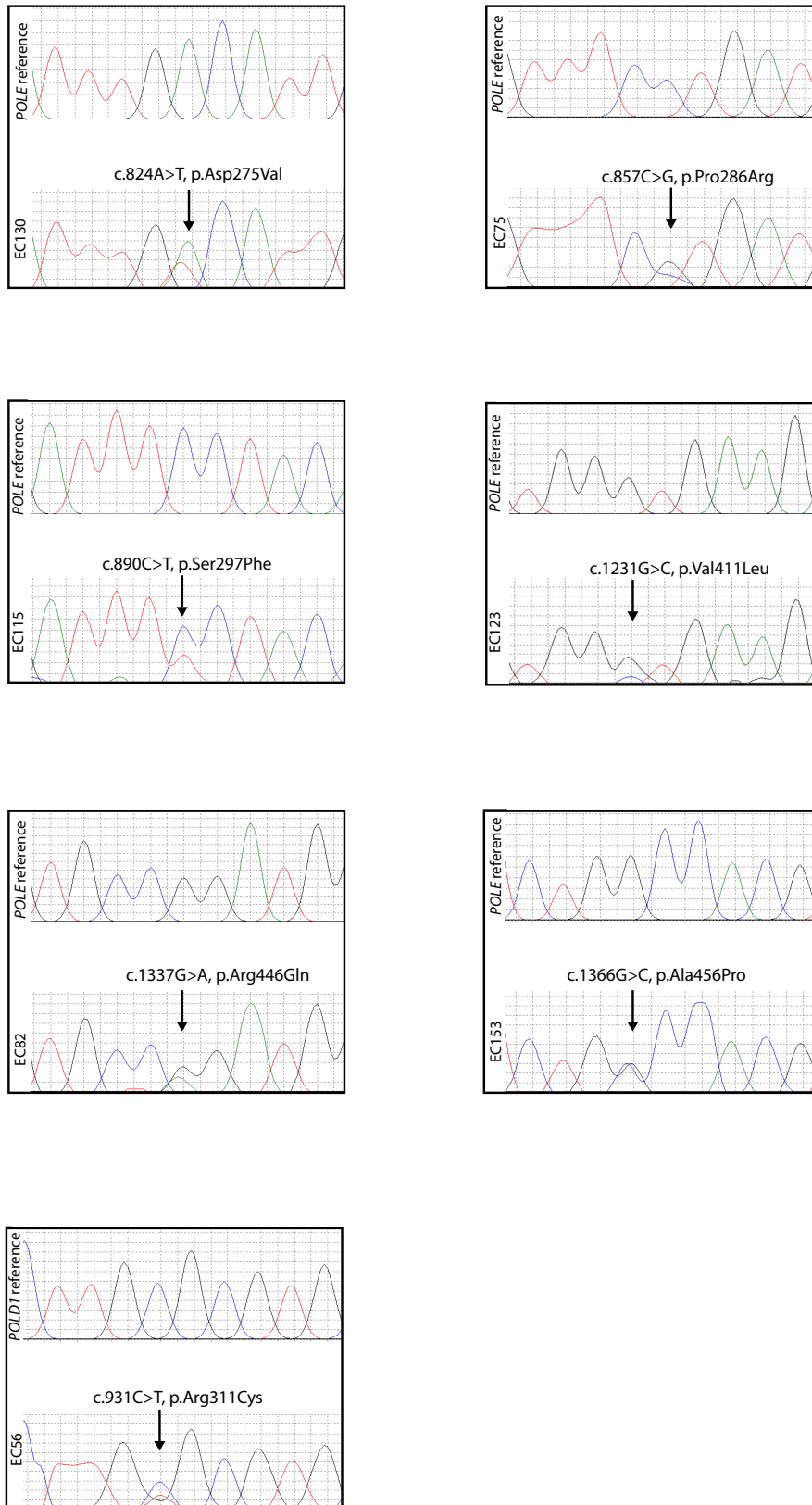




Table S1. Mutations in EC driver genes in non-EDM and *POLE*-EDM cancers

<b>Tumour ID</b>	<b>MSI</b>	<b>EDM status</b>	<b>Somatic mutation</b>	<b>Protein alteration</b>
EC8	MSS	Non-mutant	<i>PTEN</i> c.518G>A	PTEN p.Arg173His
EC25	MSS	Non-mutant	<i>PIK3CA</i> c.3127A>G <i>PIK3CA</i> c.263G>A	PK3CA p.Met1043Val PK3CA p.Arg88Gln
EC34	MSS	Non-mutant	<i>FBXW7</i> c.1385C>T¶	FBXW7 p.Ser462Phe¶
EC145	MSS	Non-mutant	<i>PIK3CA</i> c.278G>A <i>PTEN</i> c.389G>A	PK3CA p.Arg93Gln PTEN p.Arg130Gln
EC71	MSS	<i>POLE</i> Pro286Arg	<i>FBXW7</i> c.751G>T <i>PTEN</i> c.766G>T	FBXW7 p.Glu251* PTEN p.E256*
EC74	MSS	<i>POLE</i> Pro286Arg	<i>PIK3CA</i> c.3141T>G <i>PIK3CA</i> c.1070G>A	PK3CA p.His1047Gln PK3CA p.Arg357Gln
EC167	MSS	<i>POLE</i> Pro286Arg	<i>FBXW7</i> c.1514G>T¶	FBXW7 p.Arg505Leu¶
EC173	MSS	<i>POLE</i> Ser297Phe	<i>PIK3CA</i> c.263G>A <i>PIK3CA</i> c.3062A>G <i>PTEN</i> c.388C>G	PK3CA p.Arg88Gln PK3CA p.Tyr1021Cys PTEN p.Arg130Gly

¶ Indicates variant validated by Sanger sequencing

Table S2. Site and predicted consequence of *POLE* and *POLD1* exonuclease domain mutations not detected in our panel but present in the TCGA EC set

Gene	Nucleotide change	Amino acid change	Number of tumours	Site	SIFT score	PolyPhen-2 score	PhyloCons Score	MutationTaster score
<i>POLE</i>	c.1270C>G	p.Leu424Val	2	Exo IV motif	0.01	0.993	1.00	0.87
	c.1282G>A	p.Ala428Thr	1	Exo IV motif	0.79	0.041 (Benign)	1.282	1.58
	c.1331T>A	p.Met444Lys	1	Between Exo III and V motifs	0.00	1.000	1.00	2.59
	c.1358A>G	p.Gln453Arg	1	Exo III motif	0.06	0.025 (benign)	1.00	1.17
	c.1394C>T	p.Ala465Val	1	Exo III motif	0.00	1.000	1.00	1.75
<i>POLD1</i>	c.1174G>A	p.Val392Met	1	Flanking Exo I motif	0.000	0.946	0.999	Polymorphism 0.57

Table S3. Frequency and type of base substitutions according to tumour POLE/POLD mutation status in TCGA dataset

Category	Proportion of total base substitutions, % (95 CI)						Number of substitutions (range)
	C>T G>A	A>G T>C	C>G G>C	G>T C>A	A>C T>G	A>T T>A	
No <i>POLE/POLD1</i> mutation	59.1 (57.6-59.1)	11.8 (10.9-11.8)	8.7 (7.6-8.7)	13.5 (12.9-13.5)	3.5 (3.1-3.5)	3.4 (3-3.4)	173.9 (140-208)
<i>POLE</i> EDM							
p.Pro286Arg	42.9 (38.7-47)	7.6 (6.6-8.6)	0.3 (0.2-0.3)	37.4 (34.2-40.5)	11.1 (9.3-12.9)	0.8 (0.6-0.9)	7355.1 (4887-9824)
p.Ser297Phe	56.4	6.4	0.1	32.4	3.5	1.2	12699
p.Val411Leu	55.1 (49.3-61)	10.8 (9.3-12.3)	0.5 (0.4-0.7)	23.3 (21-25.6)	9.5 (6.1-12.9)	0.7 (0.6-0.8)	7743 (2474-13012)
p.Leu424Val	58.1	9.9	0.8	26.5	3.2	1.4	4749
p.Ala428Thr	59.3	3.7	11.1	11.1	11.1	3.7	27
p.Met444Lys	38.6	4.8	0.3	44.9	10.1	1.2	1523
p.Gln453Arg	65.1	20.0	0.9	10.0	1.5	2.5	976
p.Ala456Pro	60.1	8.3	0.3	25.1	5.0	1.3	8385
p.Ala465Val	85.0	5.3	0.4	8.3	0.5	0.6	5592
<i>POLE</i> non-ED mutated	58.49 (40.1-76.9)	22.47 (4.8-40.2)	2.4 (0.6-4.2)	9.8 (5.8-13.8)	3.0 (1.4-4.5)	3.9 (2.0-5.9)	636 (205.0-1067.0)
<i>POLD1</i> EDM							
p.Val392Met	81.3	9.8	1.3	5.7	0.9	1.1	1637
<i>POLD1</i> non-ED mutated	63.7 (59.0-68.4)	15.1 (8.6-21.6)	3.8 (0.3-7.2)	11.9 (8.7-15.2)	2.1 (0.7-3.4)	3.5 (2.3-4.7)	606 (72.5-1139.5)

Table S4. Pattern of mutation of EC driver alterations in EDM tumours in TCGA dataset

Tumour ID	Gene	Protein alteration	Hypermutated	MLH1 methylation	Somatic mutation	Protein alteration
A05Z	<i>POLE</i>	p.Pro286Arg	+	-	<i>P TEN</i> c.389G>A, <i>P TEN</i> c.601G>T	PTEN p.Arg130Gln PTEN p.Glu201*
A0J0	<i>POLE</i>	p.Pro286Arg	+	-	<i>CTNNB1</i> c.110C>G <i>P TEN</i> c.389G>A	CTNB1 p.Ser37Cys PTEN p.Arg130Gln
A0JY	<i>POLE</i>	p.Pro286Arg	+	-	<i>CTNNB1</i> c.94G>T <i>P TEN</i> c.19G>T, c.140delG:	CTNB1 p.Asp32Tyr PTEN p.Glu7*, p.Arg47fs
A11N	<i>POLE</i>	p.Pro286Arg	+	-	<i>FBXW7</i> c.1394G>A <i>KRAS</i> c.182A>T <i>P TEN</i> c.19G>T:	FBXW7 p.Arg465His KRAS p.Gln61Leu PTEN p.Glu7*
A0UF	<i>POLE</i>	p.Pro286Arg	+	-	<i>P TEN</i> c.389G>A <i>TP53</i> c.637C>T:	PTEN p.Arg130Gln TP53 p.Arg213*
A0UV	<i>POLE</i>	p.Pro286Arg	+	-	<i>PIK3CA</i> c.263G>A <i>P TEN</i> c.389G>A, c.720C>G <i>TP53</i> c.637C>T	PK3CA p.Arg88Gln PTEN p.Arg130Gln, p.Tyr240* TP53 p.Arg213*
A16X	<i>POLE</i>	p.Pro286Arg	+	-	<i>P TEN</i> c.389G>A, c.601G>T	PTEN p.Arg130Gln, p.Glu201*
A17Q	<i>POLE</i>	p.Pro286Arg	+	-	<i>FBXW7</i> c.711G>A <i>PIK3CA</i> c.353G>A <i>P TEN</i> c.1008C>A	FBXW7 p.Trp237* PK3CA p.Gly118Asp PTEN p.Tyr336*
A059	<i>POLE</i>	p.Ser297Phe	+	-	<i>TP53</i> c.718A>G	TP53 p.Ser240Gly
A0GP	<i>POLE</i>	p.Val411Leu	+	-	<i>FBXW7</i> c.1393C>T <i>KRAS</i> c.35G>T <i>PIK3CA</i> c.263G>A, c.353G>A <i>P TEN</i> c.G389A, c.697C>T	FBXW7 p.Arg465Cys KRAS p.Gly12Val PK3CA p.Arg88Gln, p.Gly118Asp PTEN p.Arg130Gln, p.Arg233*
A056	<i>POLE</i>	p.Val411Leu	+	-	<i>FBXW7</i> c.1105G>T <i>KRAS</i> c.38G>T <i>PIK3CA</i> c.263G>A, c.3062A>G <i>P TEN</i> c.389G>A	FBXW7 p.Glu369* KRAS p.Gly13Val PK3CA p.Arg88Gln, p.Tyr1021Cys PTEN p.Arg130Gln
A0LM	<i>POLE</i>	p.Val411Leu	+	-	<i>FBXW7</i> c.1513C>G	FBXW7 p.Arg505Gly
A11E	<i>POLE</i>	p.Val411Leu	+	-	<i>KRAS</i> c.35G>T <i>P TEN</i> c.895G>T <i>TP53</i> c.1024C>T	KRAS p.Gly12Val PTEN p.Glu299* TP53 p.Arg342*
A16Y	<i>POLE</i>	p.Val411Leu	+	-	<i>FBXW7</i> c.1972C>T <i>P TEN</i> c.259C>T, c.389G>A <i>TP53</i> c.638G>A	FBXW7 p.Arg658* PTEN p.Gln87*, p.Arg130Gln TP53 p.Arg213Gln
A051	<i>POLE</i>	p.Leu424Val	+	+	Nil found	Nil found
A0VX	<i>POLE</i>	p.Leu424Val	-	-	<i>CTNNB1</i> c.94G>T <i>PIK3CA</i> c.1637A>G, c.263G>A	CTNB1p.Asp32Tyr PK3CA:p.Gln546Arg, p.Arg88Gln
A1DQ	<i>POLE</i>	p.Ala428Lys	-	-	<i>PIK3CA</i> c.317G>T <i>TP53</i> c.524G>A	PK3CA p.Gly106Val TP53 p.Arg175His
A0TC	<i>POLE</i>	p.Met444Lys	+	-	<i>KRAS</i> c.35G>T <i>PIK3CA</i> c.323G>A <i>P TEN</i> c.388C>G, c.437T>A	KRAS p.Gly12Val PK3CA p.Arg108His PTEN p.Arg130Gly, p.Leu146*
A11H	<i>POLE</i>	p.Gln453Arg	+/-	-	<i>P TEN</i> c.526_528del, c.606_607del	PTEN p.176_176del, p.202_203del
A103	<i>POLE</i>	p.Ala456Pro	+	+	<i>FBXW7</i> c.1972C>T, c.2065C>T <i>P TEN</i> c.19G>T	FBXW7 p.Arg658*X, p.Arg689Trp PTEN p.Glu7*
A0J1	<i>POLE</i>	p.Ala465Val	+	+	<i>P TEN</i> c.389G>A	PTEN p.Arg130Gln
A0VP	<i>POLD1</i>	p.Val392Met	+	-	<i>FBXW7</i> c.40C>T <i>PIK3CA</i> c.G263G>A, c.1625A>C <i>P TEN</i> c.518G>A, c.697C>T	FBXW7 p.Arg14* PK3CA p.Arg88Gln, p.Glu542Ala PTEN p.Arg173His, p.Arg233*