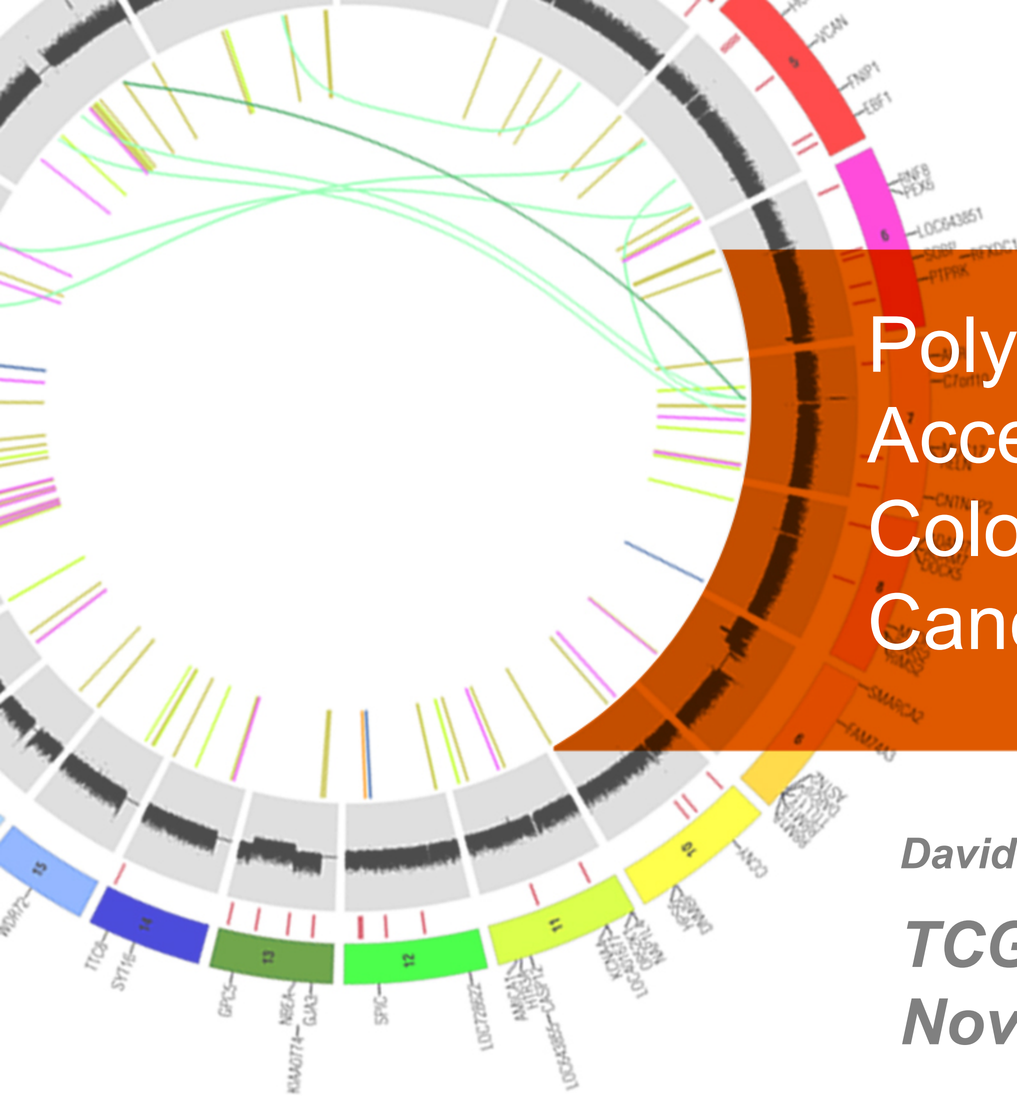


The Cancer Genome Atlas

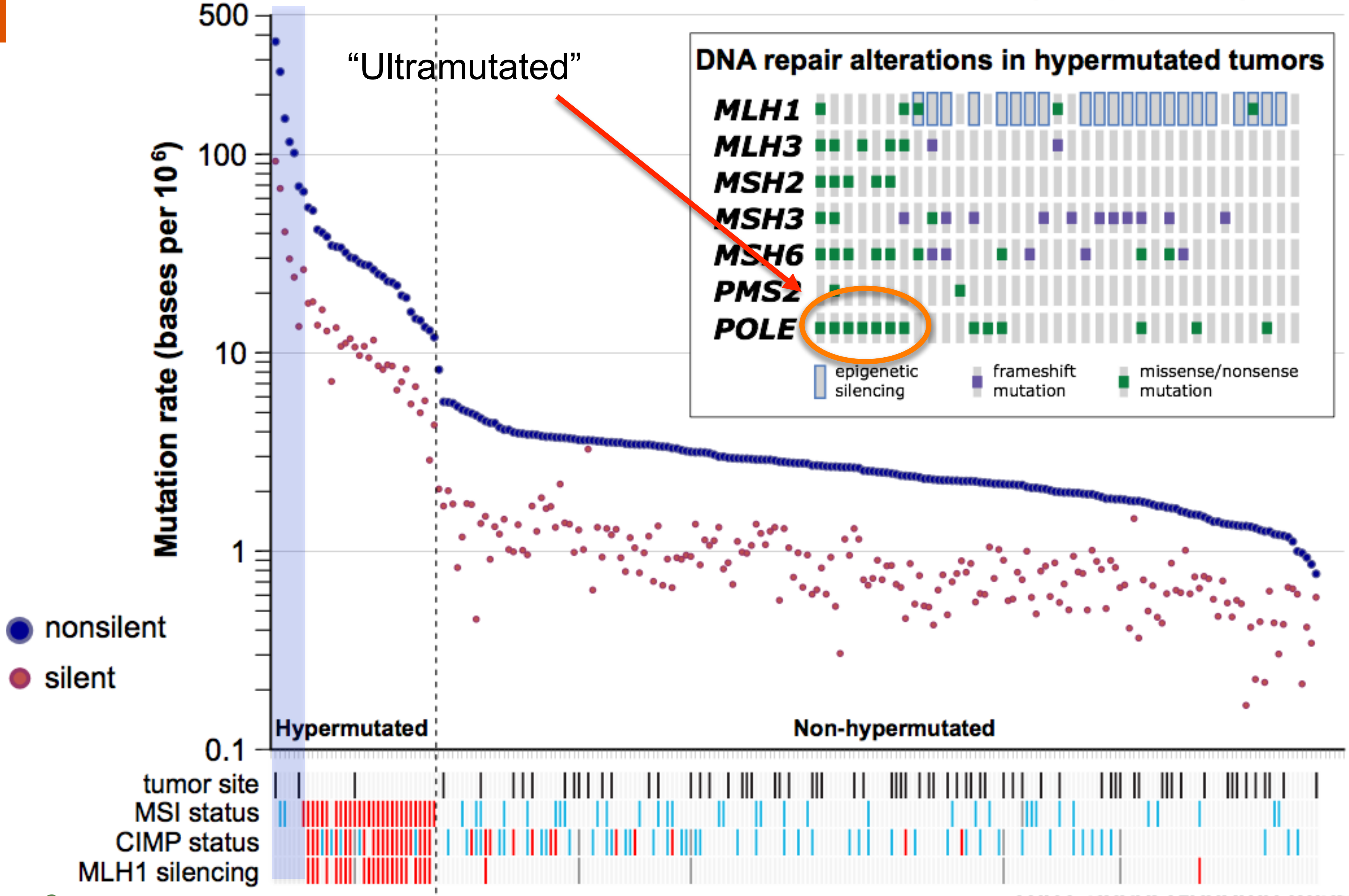
Polymerase ε Mutations Accelerate Mutation Rates in Colorectal and Endometrial Cancer

David A. Wheeler & TCGA Network

TCGA 2nd Annual Symposium
November 28, 2012

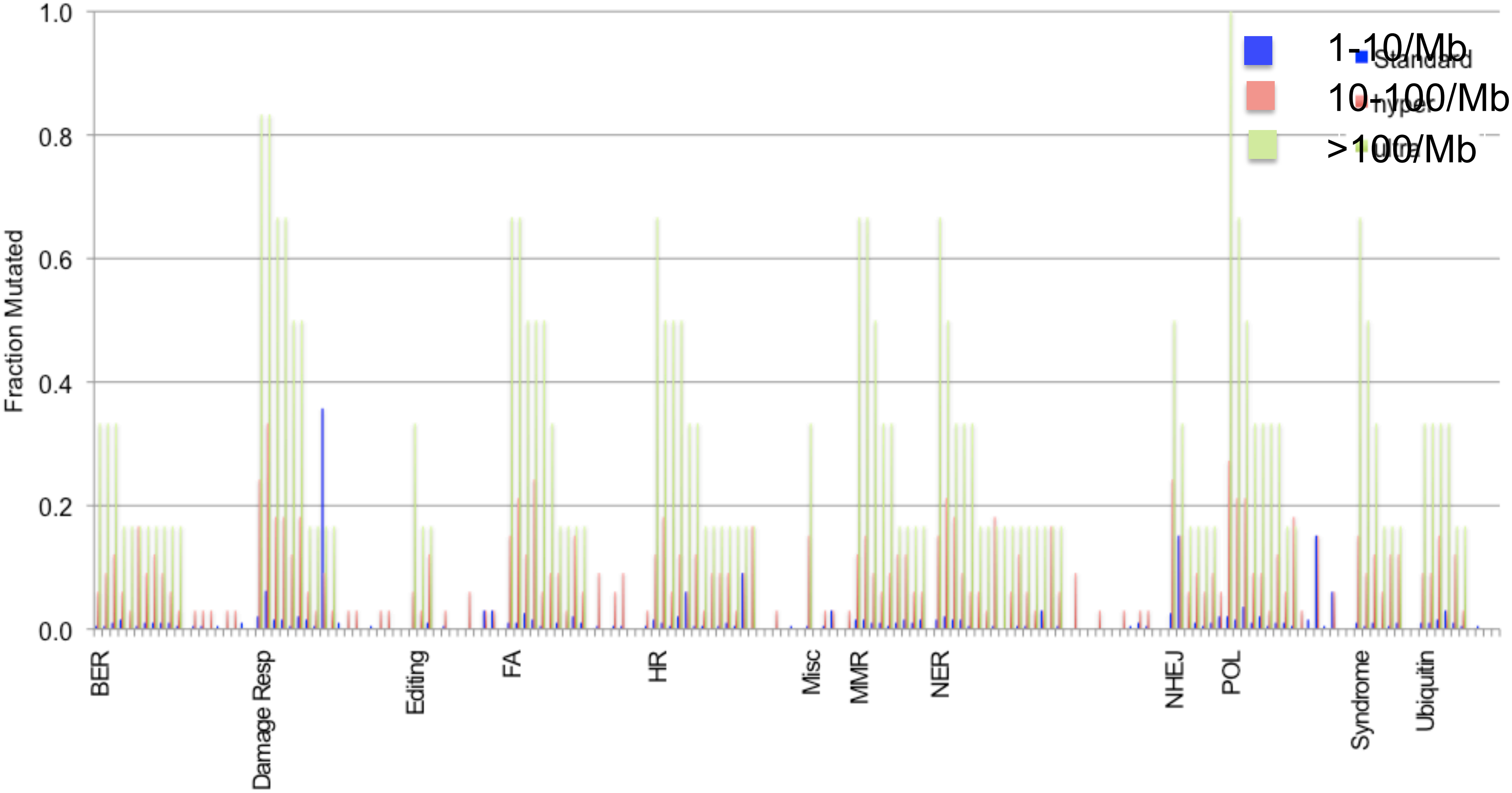
HumanGenomeSequencingCenter
BCM
Baylor College of Medicine

Mutation rates classify CRC patients



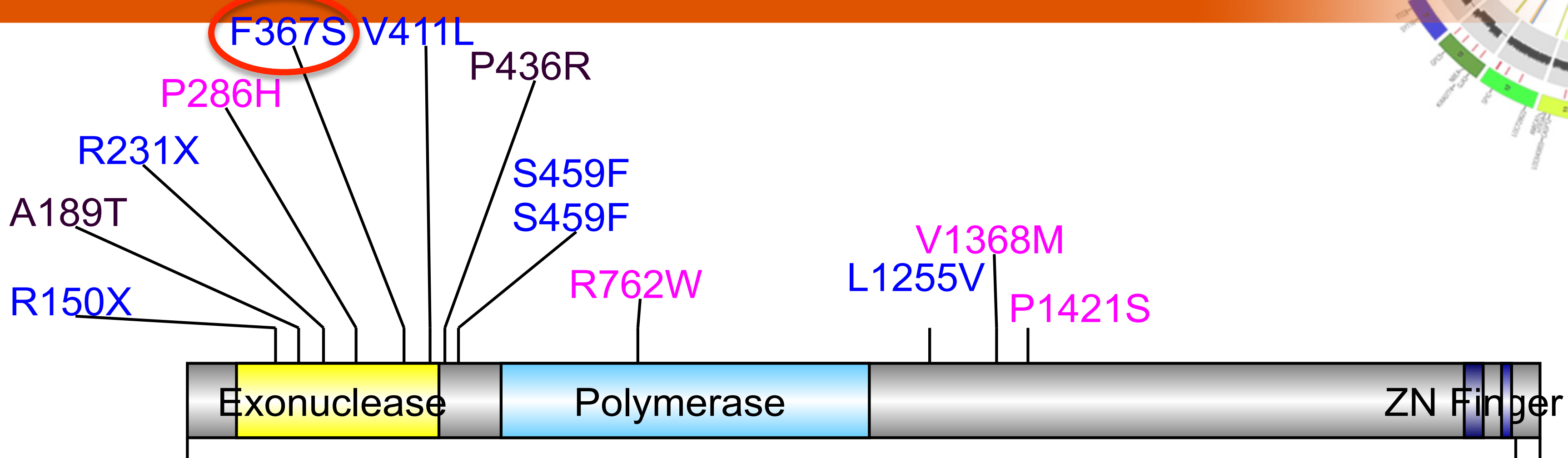
The Cancer Genome Atlas

Mutation frequencies in DNA repair genes in colorectal cancers



Categories according to RD Wood and colleagues:
http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html

POLE Mutations in Colorectal Cancer



Concurrent genetic alterations in DNA polymerase proofreading and mismatch repair in human colorectal cancer

Rintaro Yoshida[1], Kaname Miyashita[2], Mayuko Inoue[3], Akiyoshi Shimamoto[3], Zhao Yan[4], Akinori Egashira[1], Eiji Oki[1], Yoshihiro Kakeji[1], Shinya Oda*[,3] and Yoshihiko Maehara[1]

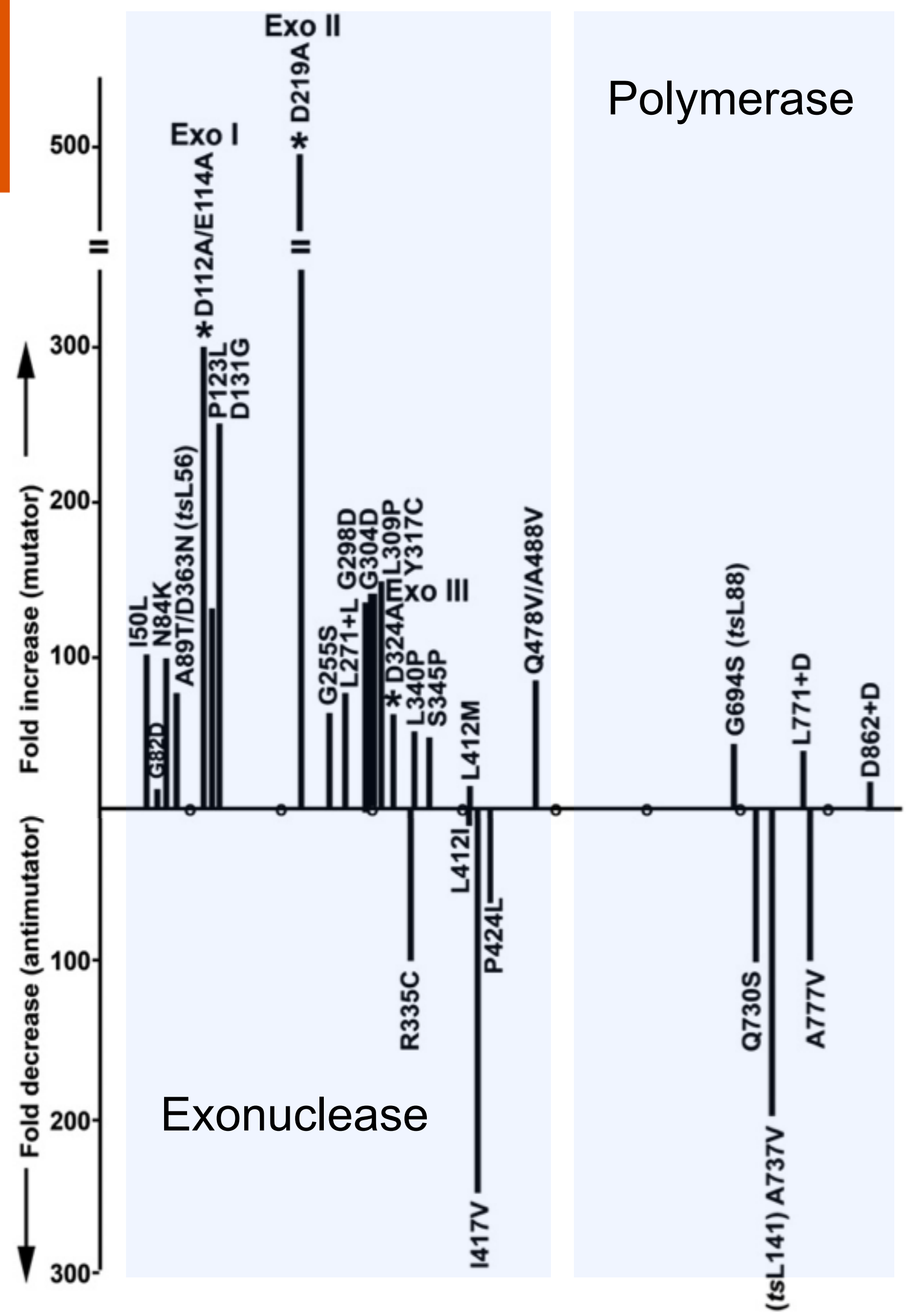
Genomic sequences encoding the 3′ exonuclease (proofreading) domains of both replicative DNA polymerases, pol delta and pol epsilon, were explored simultaneously in human colorectal carcinomas including six established cell lines. Three unequivocal sequence alterations, including one previously reported, were found, and all these were considered as dysfunctional mutations in light of the local amino-acid sequences. In particular, the F367S mutation found in the *POLE* gene encoding the pol epsilon catalytic subunit, which includes the proofreading domain, is the first found in human diseases. Surprisingly, the tumours carrying these proofreading domain mutations were all defective in DNA mismatch repair (MMR). In addition to the two cell lines with acknowledged MMR gene mutations, the third tumour was also demonstrated to harbour a distinct mutation in *MLH1*, and indeed exhibited a microsatellite-unstable phenotype. These findings suggest that, in concert with MMR deficiency, defective polymerase proofreading may also contribute to the mutator phenotype observed in human colorectal cancer. Our observations may suggest previously unrecognised complexities in the molecular abnormalities underlying the mutator phenotype in human neoplasms.

European Journal of Human Genetics (2011) **19**, 320–325; doi:10.1038/ejhg.2010.216; published online 15 December 2010

T4 exonuclease mutagenesis

- *rII*⁻ reversion rates
  - Sensitive to $1/10^{9-10}$
- Mutator phenotype also studied in bacteria, yeast, mice

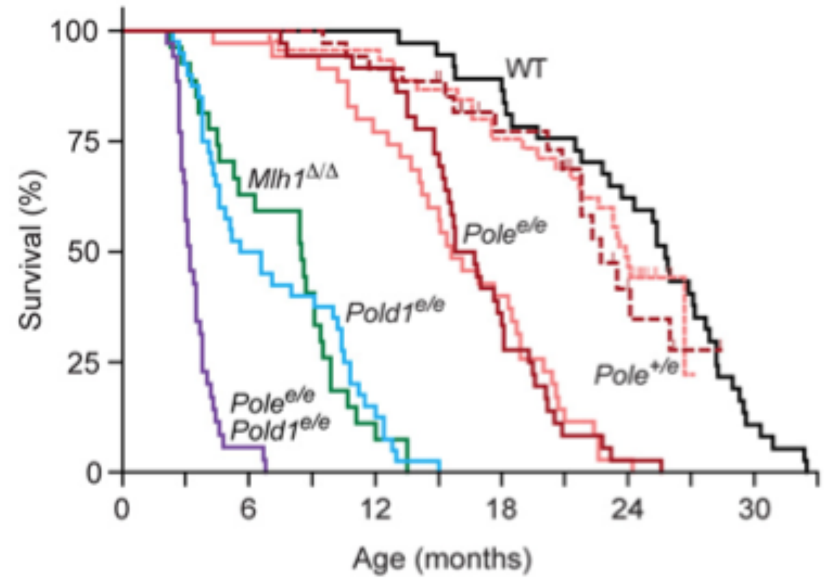
LJ Reha-Krantz, BBA 1804: 1049 (2010)



Pole, *Pold1* exonuclease KO in mice

- *Pole* and *Pold1* exonuclease KO exhibit mutator phenotype
- Homozygous mutants rapidly die of cancer
  - $Pole^{e/e}$ intestinal and lymphoma
  - $Pold1^{e/e}$ lymphoma and lung

A



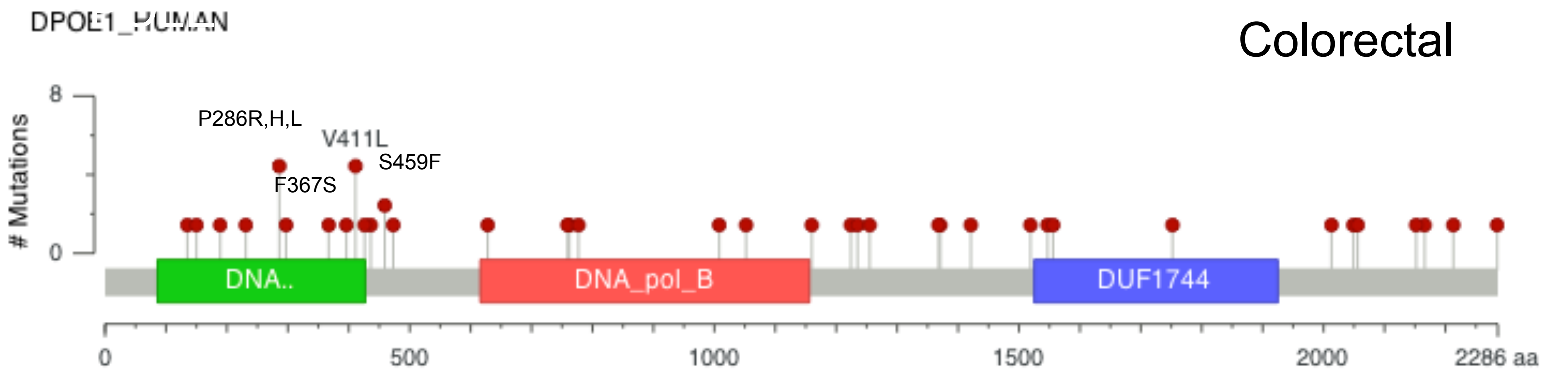
B

Tumor†	Incidence (%)* WT	$Pole^{e/e}$	$Pold1^{e/e}$	$Mlh1^{\Delta/\Delta}$	$Pole^{e/e}$ $Pold1^{e/e}$
Lymphoma					
Thymic	0	0	42	38	82
Nodal	6	24	14	4	12
Follicular	38	15	8	31	3
Squamous Papilloma/Carcinoma					
Tail Skin	0	0	25	0	6
Adenoma/Adenocarcinoma					
Intestine	6	45	14	42	3
Lung	9	12	28	4	3
Histiocytic Sarcoma	22	36	3	4	0

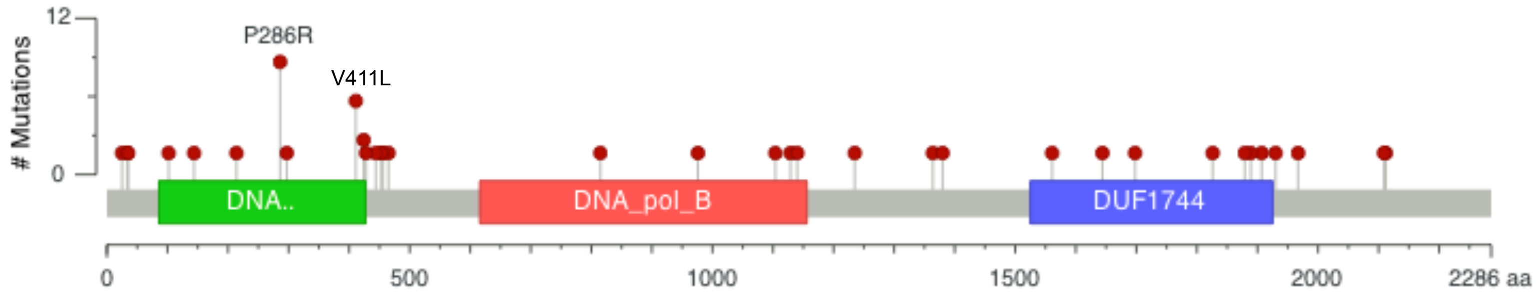
TA Albertson et al. PNAS 106: 17101 (2009)

Distribution of mutations in POLE

Colorectal

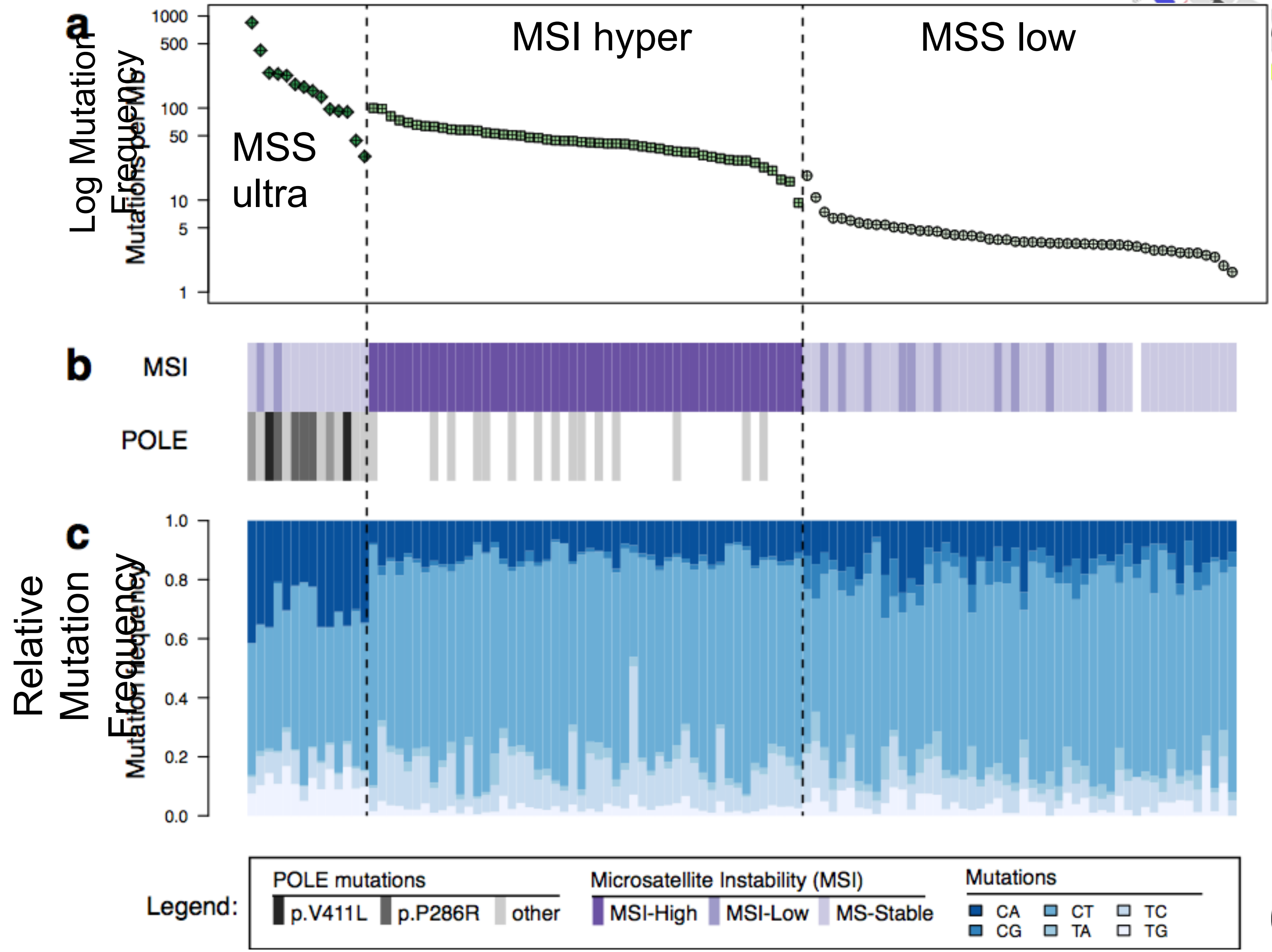


Endometrial



Eve Shinbrot, HGSC; cBIO Portal

Mutation properties of colorectal tumors



POLE mutations in CRC and Endometrial Cancer

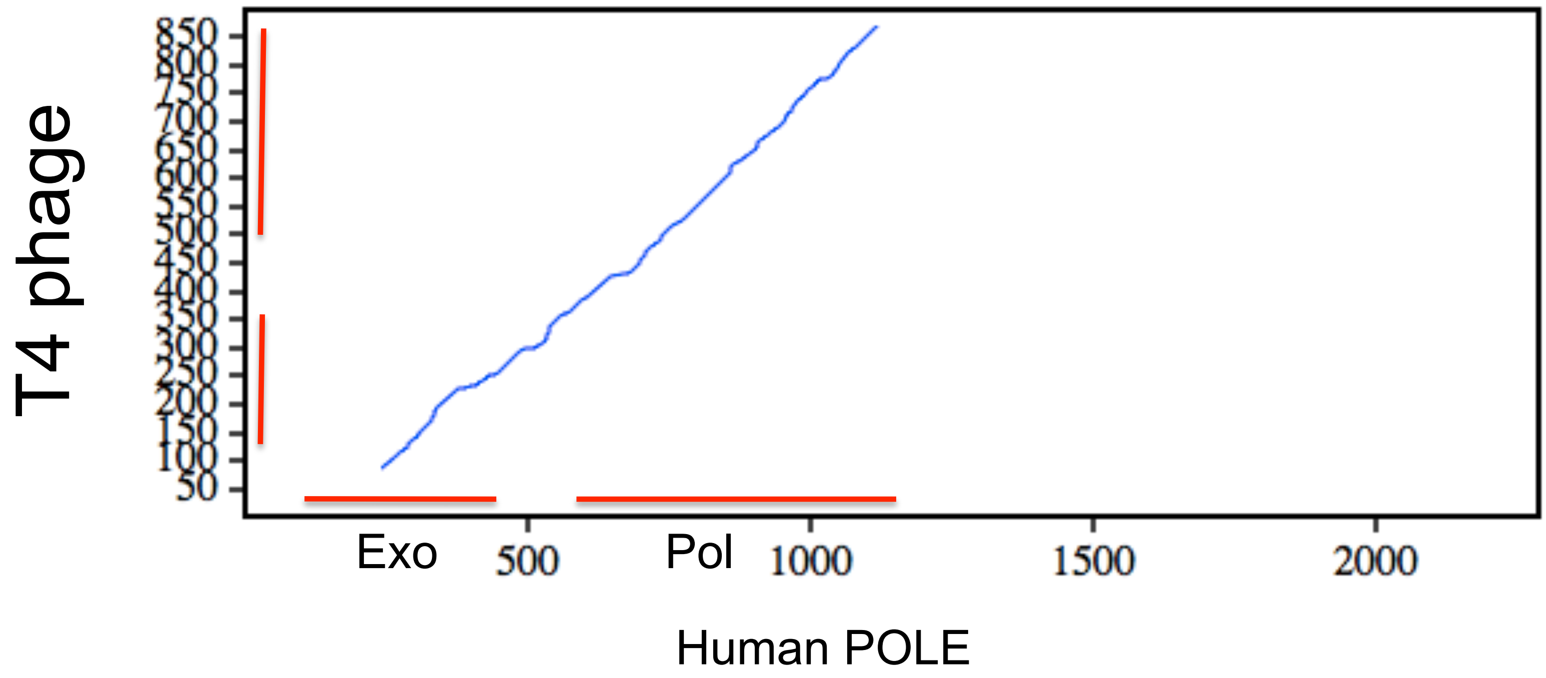
Occurance of POLE mutations in CRC

Subtype	Patients	All Sites (1-2268)	Exonuclease Domain (223-471)	Recurrent Sites (P286, F367, V411, S459)
MSS Low	412	4 (1%)	0	0
MSI Hypermutated	70	19 (27%)	3 (4%)	0
MSS Ultramutated	14	23 (164%)	14 (100%)	11 (79%)

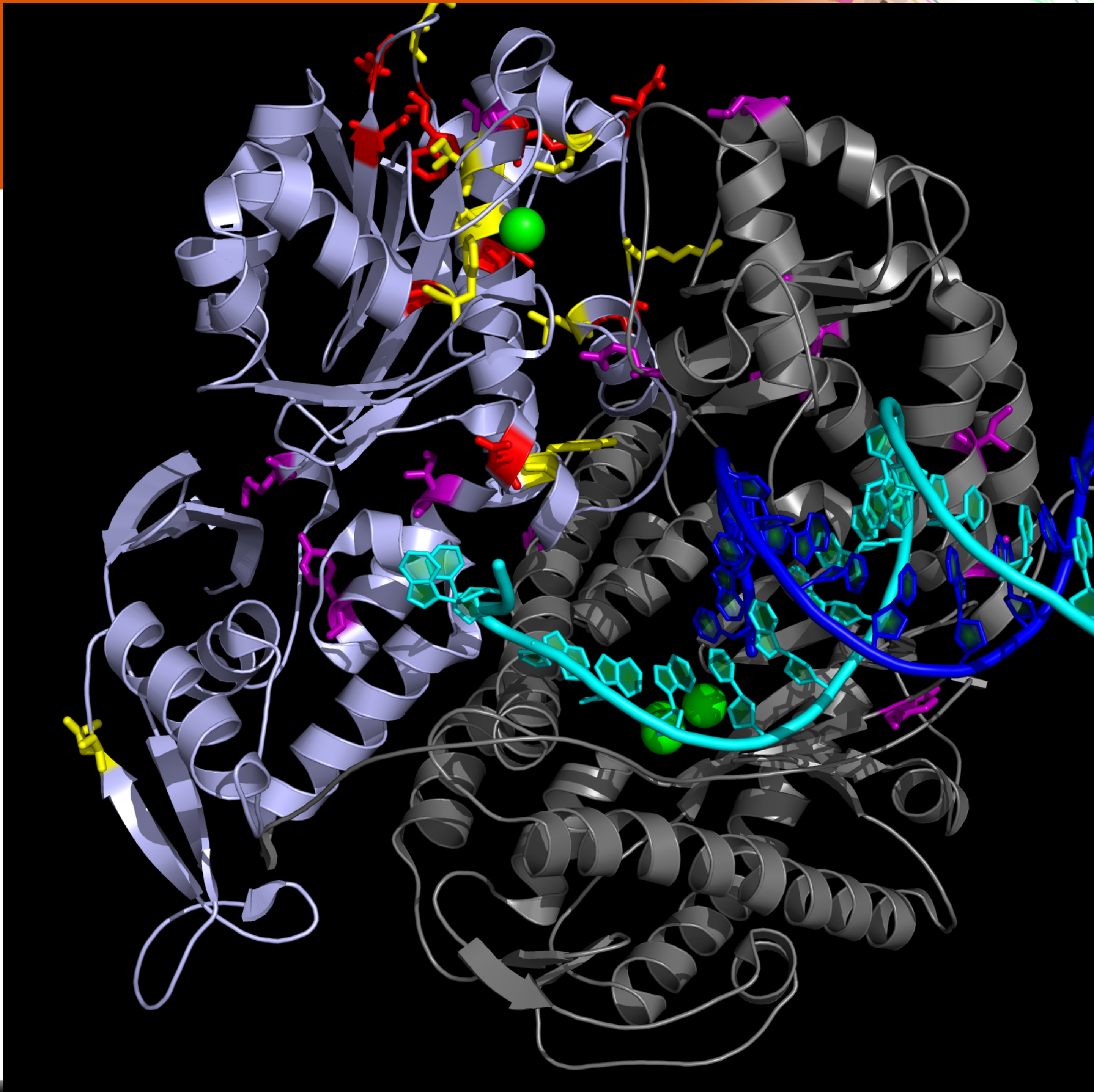
Occurance of POLE mutations in EEC

Subtype	Patients	All Sites (1-2286)	Exonuclease Domain (223-471)	Recurrent Sites (P286,V411)
MSS Low	166	6 (4%)	2 (1%)	0
MSI Hypermutated	65	9 (14%)	1 (2%)	0
MSS Ultramutated	17	45 (265%)	17 (100%)	13 (76%)

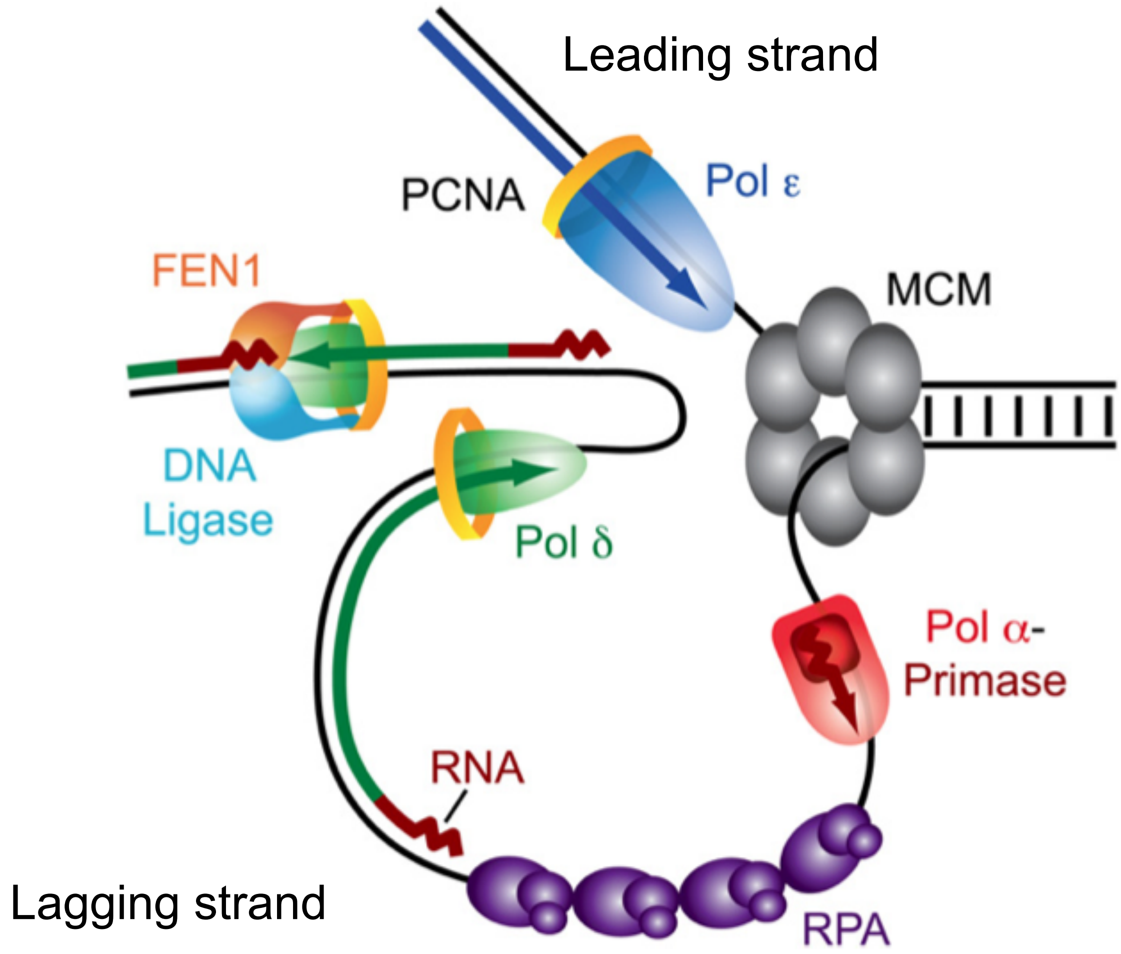
“Polymerase B” domain family sequence similarity over “a billion” years of evolution



POLE mutation



Asymmetric roles of major replicative DNA polymerases



Mutation profile is skewed at sites enriched for origins of replication*

**C.-L. Chen et al. Mol. Biol Evolution 28: 2327 (2011)*

Relative to ORI	CA	GT
UPstream	1049	721
DNstream	738	1041

- 60:40 bias of CA pattern on leading strand
- Caveat:
  - Whole exome sequence data is limited in resolution
  - Need to replicate in whole genome

Nils Weinhold, Niki Schultz, MSKCC

Pole, *Pold1* exonuclease KO in mice

- *Pole* and *Pold1* exonuclease KO exhibit mutator phenotype
- Homozygous mutants rapidly die of cancer
  - *Pole*$^{e/e}$ intestinal and lymphoma
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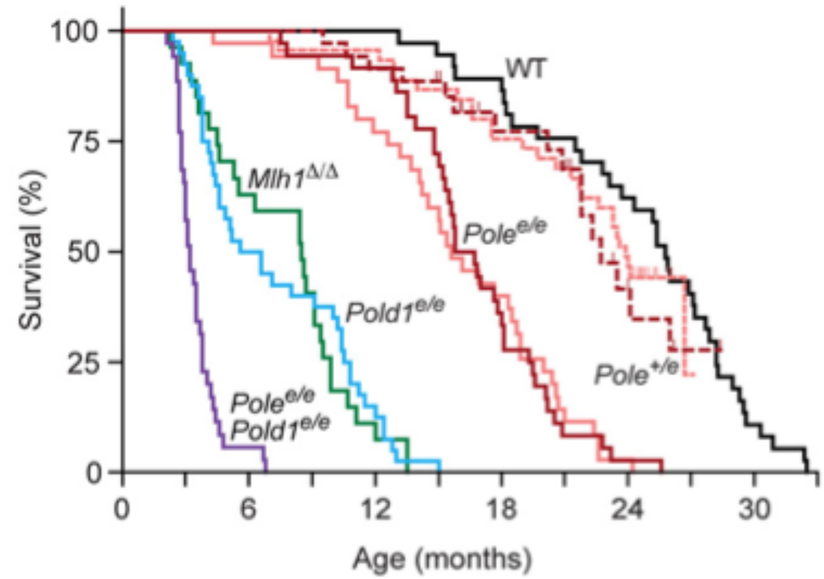
A



B

Tumor†	Incidence (%)* WT	*Pole*$^{e/e}$	*Pold1*$^{e/e}$	*Mlh1*$^{\Delta/\Delta}$	*Pole*$^{e/e}$ *Pold1*$^{e/e}$
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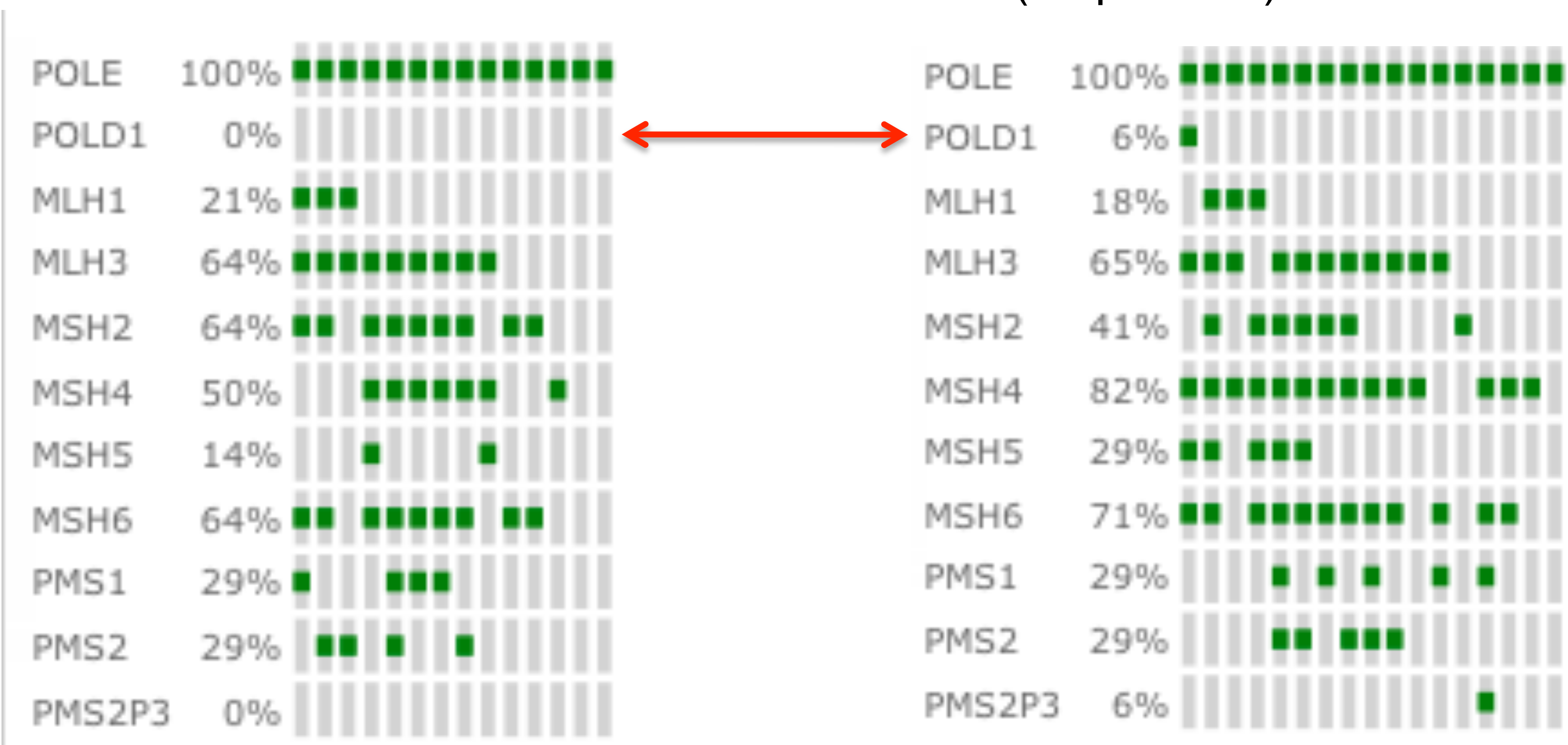
Lung	9	12	28	4	3
Histiocytic Sarcoma	22	36	3	4	0

TA Albertson et al. PNAS 106: 17101 (2009)

POLD1: no exonuclease domain mutations in ultramutated patients

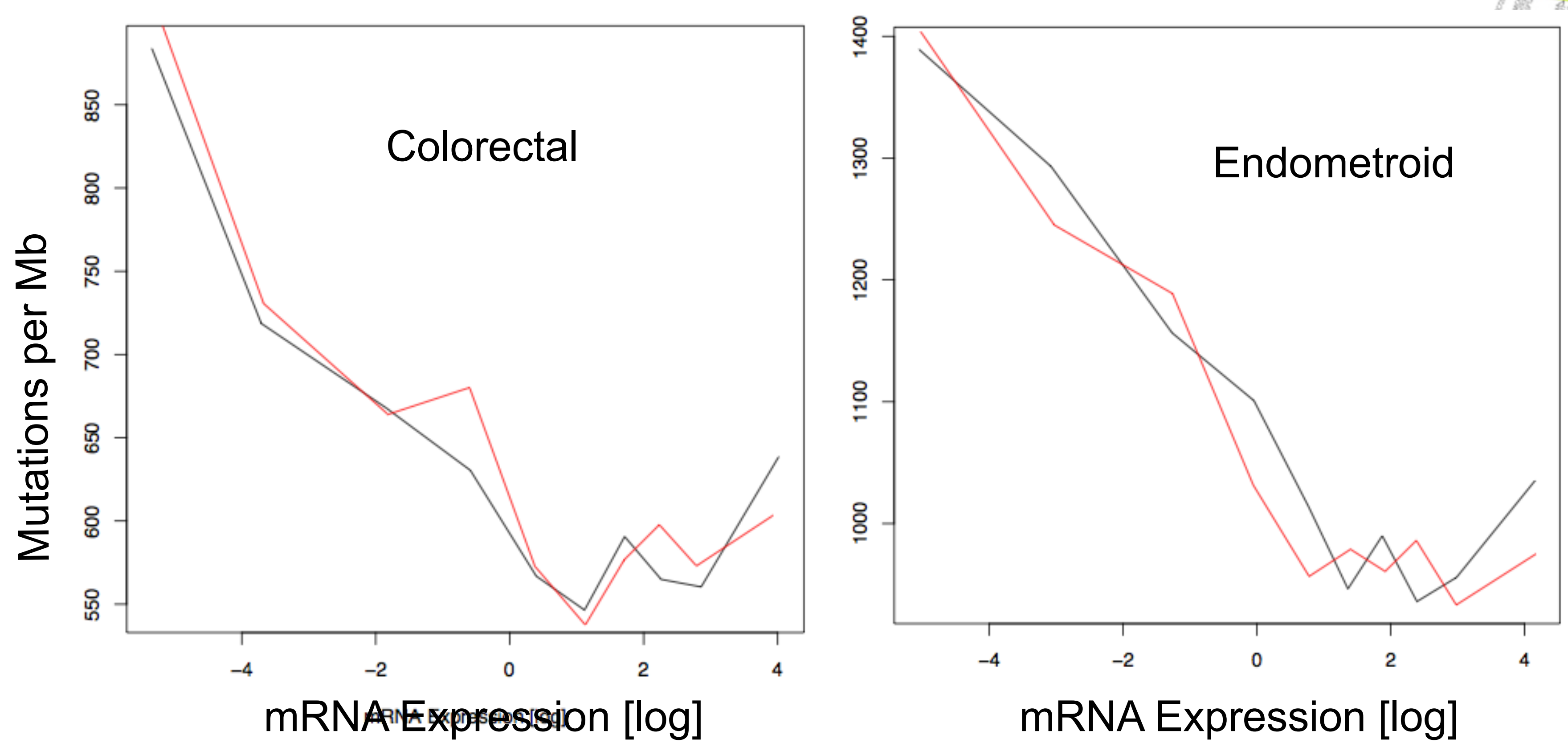
Colorectal
(14 patients)

Endometriod
(17 patients)



Eve Shinbrot, HGSC; cBIO Portal

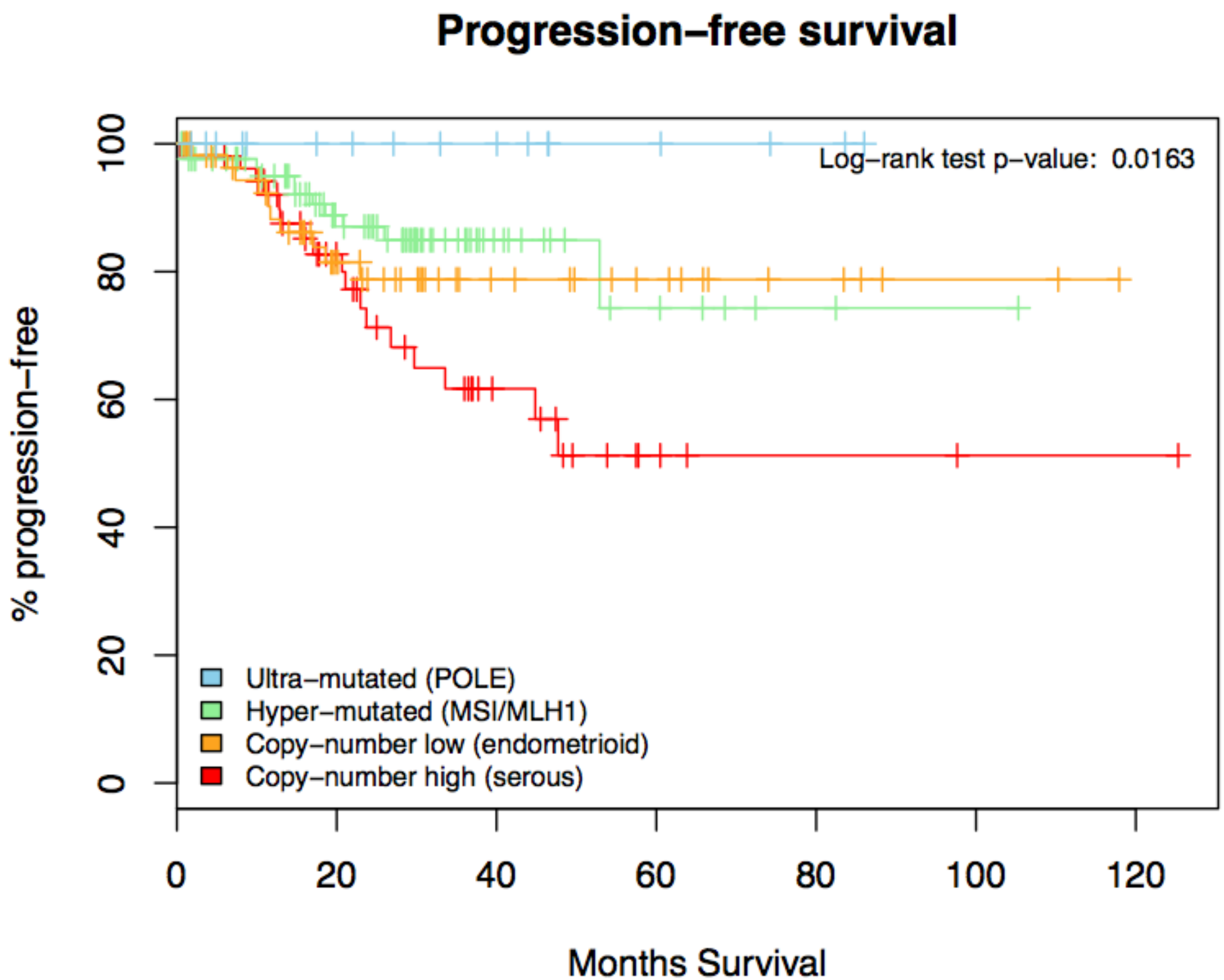
Mutation frequencies are anticorrelated with expression level in ultramutated patients



- ~2X reduction in mutation rate on highly transcribed genes

Nils Weinhold, Niki Schultz, MSKCC

UCEC Progression free survival favors ultramutated patients



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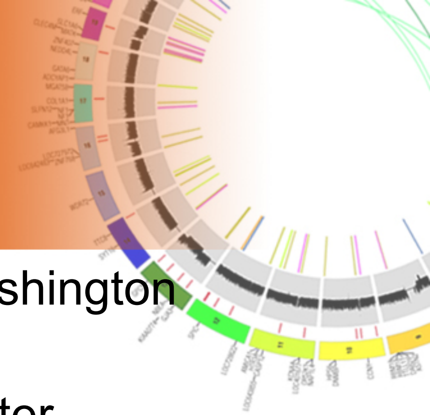
Conclusions and future directions

- Rare exonuclease-mutation in POLE leads to an ultramutator phenotype in colorectal and endometrioid cancers.
- The ultramutator phenotype defines a new subtype of these tumors that may have unique prognostic features and interesting biological properties.
  - Need further mutation profiling in colorectal and endometriod cohorts with clinical outcomes.
- Ultramutator patients exhibit a signature of transcription coupled repair.
- Absence POLD1 ultramutators suggests it may perform an essential function in this new subtype of colorectal and endometrioid cancers (role in TCR?).
- Strand-specific mutation pattern associated with putative origins of replication in humans is first suggestive evidence for confirmation of yeast model of replication in a higher eukaryote.
- Whole genome sequencing should help to separate the effects of transcriptional repair and strand-specific mutation effects.

Acknowledgements



- Human Genome Sequencing Center, Baylor College of Med
  - Richard Gibbs
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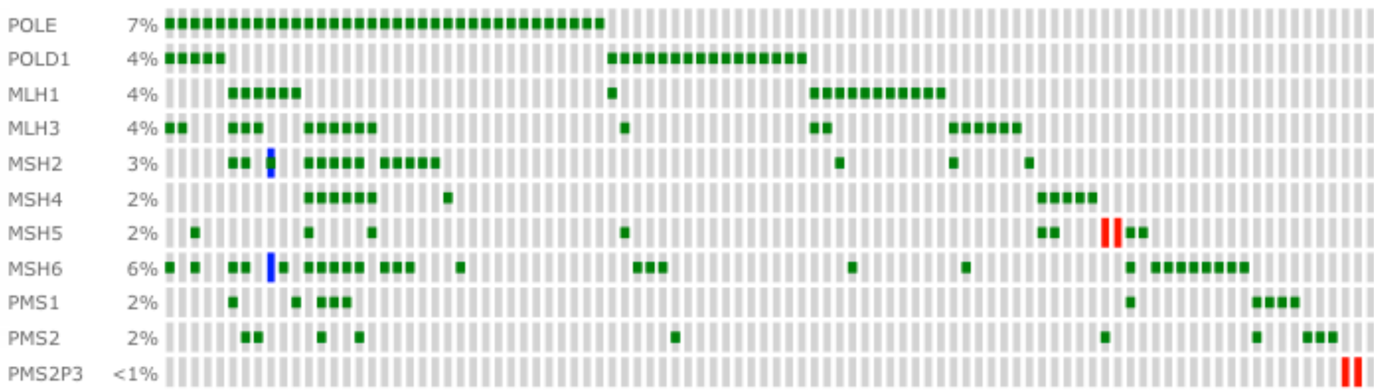
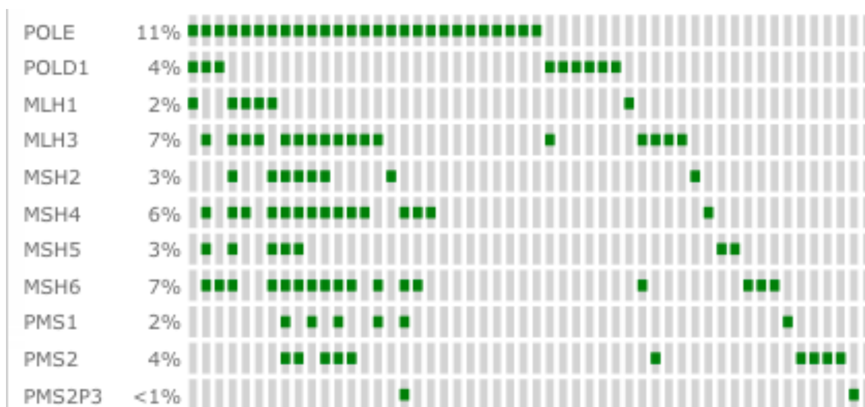
- Broad Institute of MIT and Harvard
  - Mike Lawrence
  - Gaddy Getz
- National Human Genome Research Institute
- National Cancer Institute
- The TCGA Network

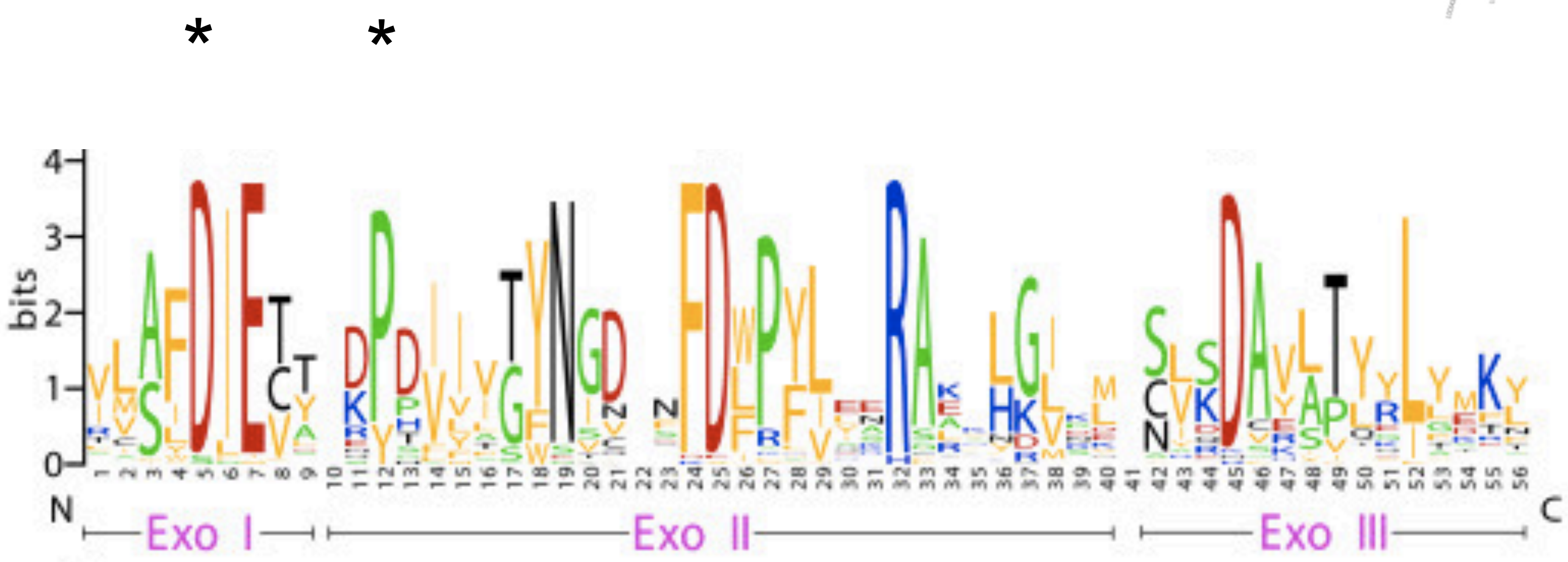
CRC-total sar
MMR



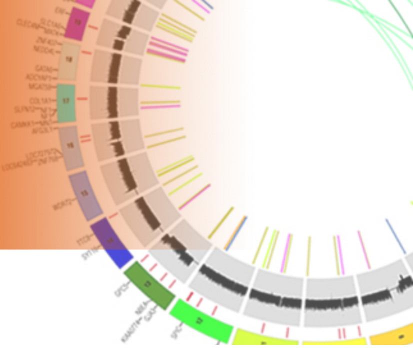
Endometrial-total samples
MMR



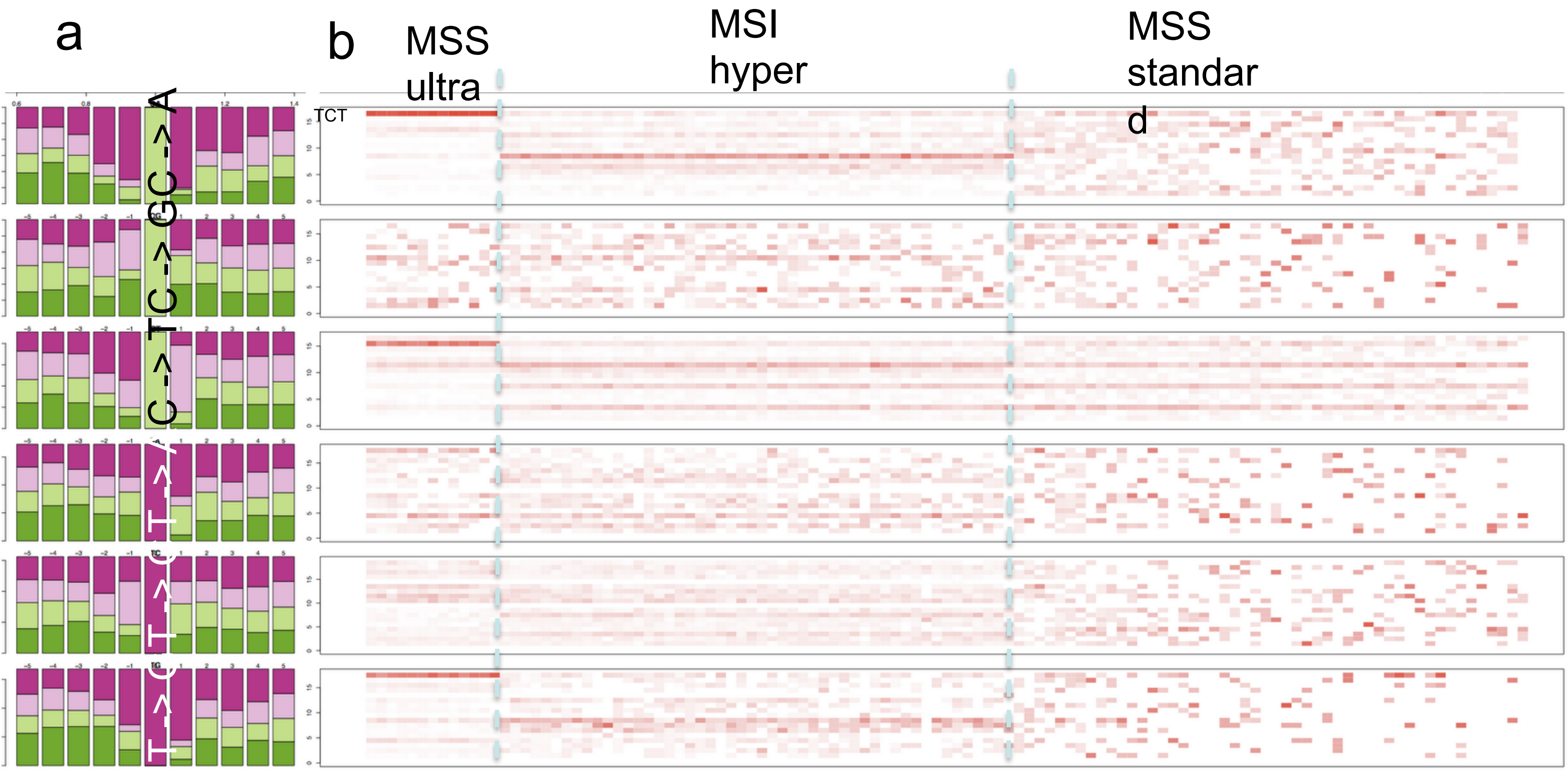
Exonuclease motifs of B family DNA polymerases



Nucleotide context of POLE mutations

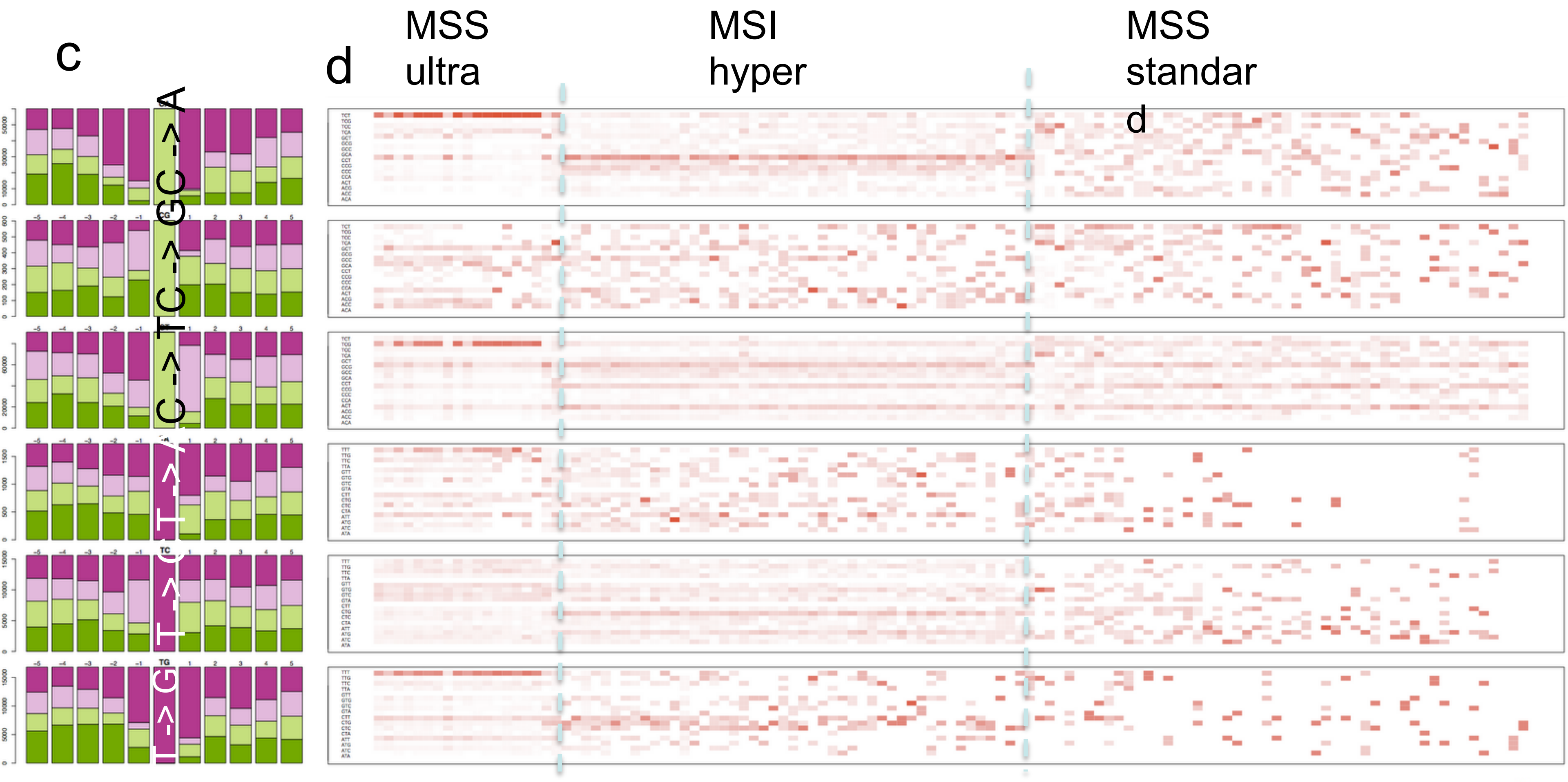


Colorectal cancer



Nucleotide context of POLE mutations

Endometrial cancer



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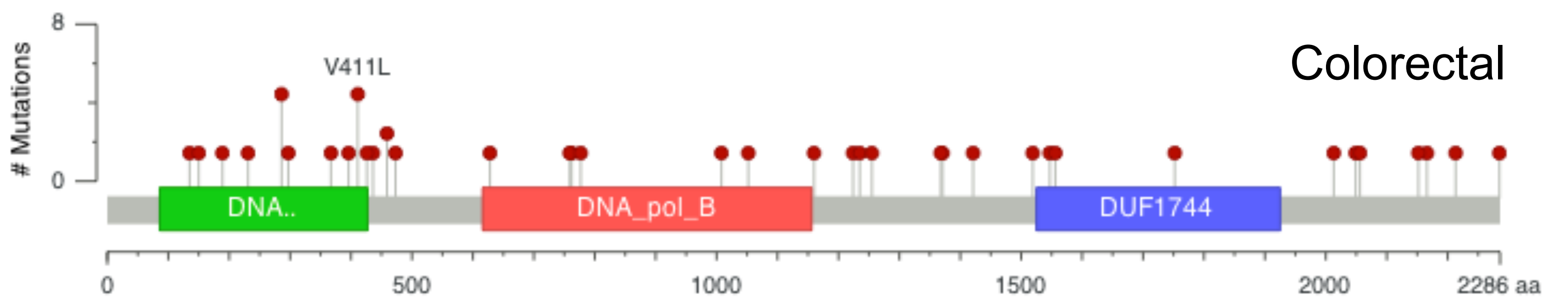
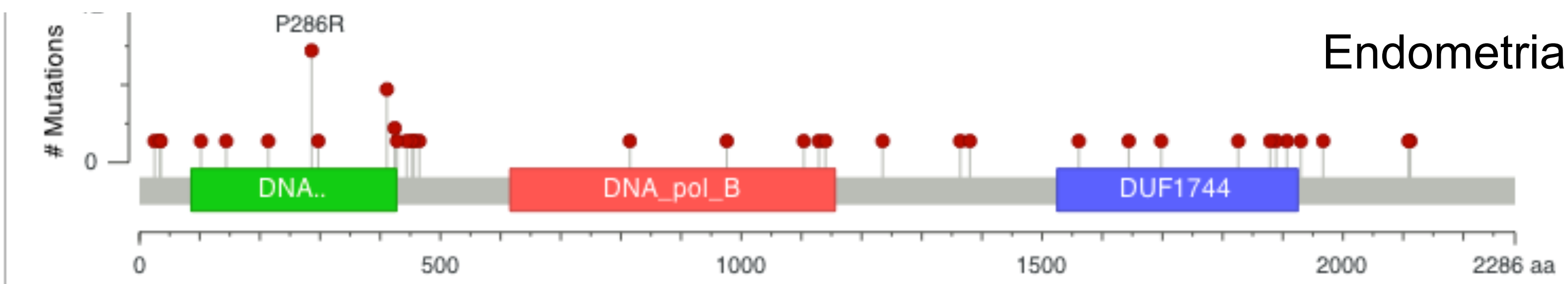
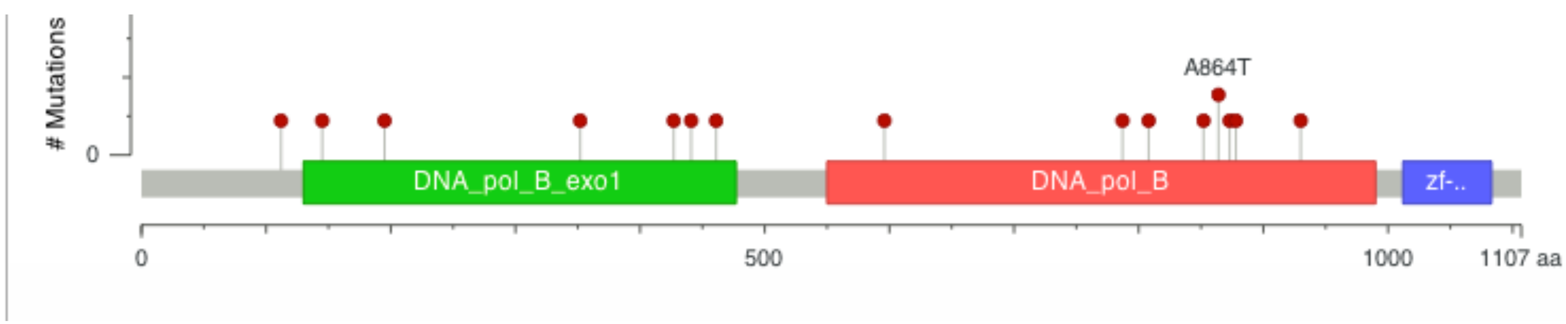
DPOE1_HUMAN



Colorectal



Endometrial



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Human POLE vs T4 POL


```
human  YNVRYRGNAFPVEITRRDDLVERPDPVVLAFDIETTKLPLKFPDA-----ETDQIMMISY
T4     FKLAYISDTYGSEIVYDRKFVR-----VANCDIEVTGD--KFPDPMKAEYEIDAITHYDS

human  MIDGQGYL--ITNREIVSEDIEDFEFTPKPEYEG----PFCVFNE------PDEAHLIQR
T4     -IDDRFYVFDLLNSMYGSVSKWDAKLAAKLDCEGGDEVPQEILDRVIYMPFDNERDMLME

human  WFEHVQETKPTIMVTYNGDFFDWPFVEARAAVHGLSMQQEIGFQKDSQGEYKAPQCIHMD
T4     YINLWEQKRPAIFTGWNIEGFDVPYIMNRV-------------KMILGE---------

human  CLRWVKRDSYLPVGSHNLKAAAKAKLGYDPVELDPEDMCRMATEQPQTLATYSVSDAVAT
T4     --RSMKR--FSPIGR------VKSKL--------IQNM--YGSKEIYSIDGVSILDYLD-

human  YYLYMKYVHPFI--FALCTIIPMEPDEVLRKGSGTLCEALLMVQAFHANIIFPNKQEQEF
T4     --LYKKFAFTNLPSFSLESVAQHET----KKGK----------------LP--YDGPI

human  NKLTDDGH-------VLDSETY-----VGGHVEALESGVFRSDIPCRFRMNPAAFDFLLQ
T4     NKLRETNHQRYISYNIIDVESVQAIDKIRGFIDLVLSMSYYAKMPFSGVMSP------IK

human  RVEKTLRHALEEEEKVPVEQVTNFEEVCDEIKSKLASLKDVPSRIECPLIYHLDVGAMYP
T4     TWDAIIFNSLKGEHKVIPQQGSHVKQ------SFPGAFVFEPKPIARRYIMSFDLTSLYP

human  NIILTNRLQPSAMVDEATCAACDFNKPGANCQRKMAWQWRGEFMPASRSEYHRIQHQLES
T4     SIIRQVNISPETI-------------------------RGQF------KVHPIHEYIAG
```

P286R/H

F367S

V411L

S459F

```
###  QRIAPLRVLSFDIECAGRKGIFPEP   329
###  --RPDPVVLAFDIETTKLPLKFPDA   288
           **:****  :     **:
```

P286

```
441  TGRRDTKVVSMVGRVQMDMLQVLLREYKL--RSYTLNAVSFHFLGEQKEDVQHSIITDLQ  498
394  QGEYKAP-----QCIHMDCLRWVKRDSYLPVGSHNLKAAAKAKLGYDPVELDPEDMCRM-  447
      *.  .:          ::** *: : *:  *   *:.*:*.:   ** :  ::: . :  :
```

V411

```
499  NGNDQTRRRLAVYCLKDAYLP----LRLLERLMVLVNAVEMARVTGVPLSYLLSRGQQVK  554
448  --ATEQPQTLATYSVSDAVATYYLYMKYVHPFIFAL-----CTIIPMEPDEVLRKGSGTL  500
        :  :  **.*.:.**       :: :. ::. :     . :  :  . :* :*. .
```

S459

Waterman-Eggert score: 99; 33.1 bits; E(1) < 5.9e-06
26.7% identity (49.1% similar) in 116 aa overlap (5-110:15-126)

P326

```
          10        20        30        40                50        60          70
POLD1 QRIAPLRVLSFDIECAGRKGIFPEPERDPVIQICSLGLRWGE--------PEPFLRLALTLRPC--APILGAKVQSYEKE
      .:  :. ::.:::: .     ::. : : ...:  .    :          : .  . .: .:   .:.    : .   :
POLE  ERPDPV-VLAFDIETTKLPLKFPDAETDQIMMISYMIDGQGYLITNREIVSEDIEDFEFTPKPEYEGPFC---VFNEPDE
          20         30        40        50        60        70        80           90
```

P286

470 C312

```
         160       170         180       190       200       210
POLD1 VQMDMLQVLLREYKLR--SYTLNAVSFHFLGEQKEDVQHSIITDLQNGNDQTRRRLAVYCLKDA
      ..:: :. . :.  :   :..:.:..   :: .  ...   .  . . . ::   ::.: ..::
POLE  IHMDCLRWVKRDSYLPVGSHNLKAAAKAKLGYDPVELDPEDMCRMATEQPQT---LATYSVSDA
           160       170       180       190       200          210
```

V411 S459

Supplementary Table 2. POLE mutations in TCGA endometroid cancer patients

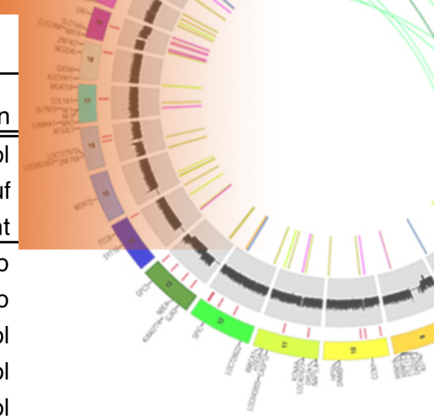
Tumor_Sample_Barcode	aa1	aa2	aa3	a4	a5	a6	Subtype	Position	Domain
TCGA-B5-A11Y-01A-21D-A10M-09	R34C	P102L					Standard	34	uracil
TCGA-BG-A0VX-01A-11D-A122-09	L424V						Standard	424	exo
TCGA-AP-A1DQ-01A-11D-A135-09	A428T						Standard	428	exo
TCGA-BG-A18A-01A-21D-A12J-09	R976S						Standard	976	pol
TCGA-B5-A11Y-01A-21D-A10M-09	P1779?						Standard	1779	duf
TCGA-B5-A11H-01A-11D-A122-09	Q453R						Hyper	453	exo
TCGA-AX-A0J1-01A-11W-A062-09	A465V						Hyper	465	exo
TCGA-D1-A16N-01A-11D-A12J-09	Y473?						Hyper	473	exo
TCGA-BS-A0UM-01A-11W-A10C-09	A566T						Hyper	566	pol
TCGA-A5-A0VP-01A-21D-A10B-09	A1140T						Hyper	1140	pol
TCGA-AX-A0J1-01A-11W-A062-09	G1256?						Hyper	1256	int
TCGA-BG-A0LX-01A-11W-A062-09	E1698D						Hyper	1698	duf
TCGA-D1-A167-01A-11D-A12J-09	Y1865?						Hyper	1865	duf
TCGA-AP-A054-01A-11W-A062-09	Y1889C						Hyper	1889	duf
TCGA-D1-A17F-01A-11D-A12J-09	E2272?						Hyper	2272	int
TCGA-AX-A05Z-01A-11W-A027-09	P286R	F1907L	S1930?				Ultra	286	exo
TCGA-AX-A0J0-01A-11D-A117-09	P286R						Ultra	286	exo
TCGA-B5-A0JY-01A-11D-A10B-09	P286R	F815L					Ultra	286	exo
TCGA-B5-A11N-01A-11D-A122-09	P286R						Ultra	286	exo
TCGA-BS-A0UF-01A-11D-A10B-09	P286R	C2109R					Ultra	286	exo
TCGA-BS-A0UV-01A-11D-A10B-09	P286R	S1380L	L1561P				Ultra	286	exo
TCGA-D1-A16X-01A-11D-A12J-09	P286R	D1129G	F1553?				Ultra	286	exo
TCGA-D1-A17Q-01A-11D-A12J-09	P286R	E714?					Ultra	286	exo
TCGA-AP-A059-01A-21D-A122-09	L35M	A25T	S297F	S554	L2112M		Ultra	297	exo
TCGA-AP-A0LM-01A-11D-A122-09	Q214R	V411L					Ultra	411	exo
TCGA-A5-A0GP-01A-11W-A062-09	V411L						Ultra	411	exo
TCGA-AP-A056-01A-11W-A027-09	V411L	L1235I	R1826W	I2199			Ultra	411	exo
TCGA-B5-A11E-01A-11D-A10M-09	V411L	G904	R1364C	V1555	R1879C	C2238	Ultra	411	exo
TCGA-D1-A16Y-01A-31D-A12J-09	V411L						Ultra	411	exo
TCGA-AP-A051-01A-21W-A027-09	L424I	G1425	L2083				Ultra	424	exo
TCGA-BS-A0TC-01A-11D-A10B-09	M444K						Ultra	444	exo
TCGA-D1-A103-01A-11D-A10M-09	H144Q	A456P	T1104M	S1644L	A1967V		Ultra	456	exo

e Atlas

Supplementary Table 1. POLE mutations in TCGA colorectal cancer patients

TCGA Sample ID	aa1	aa2[a]	aa3	Subgroup[b]	Position[c]	Domain
TCGA-CM-6678-01A-11D-1835-10	G628R			standard	628	pol
TCGA-AA-3678-01A-01W-0900-09	D1752N			standard	1752	duf
TCGA-AG-A01W-01A-21W-A096-10	D2013N			standard	2013	int
TCGA-A6-6141-01A-11D-1771-10	S297F			hyper	297	exo
TCGA-AD-6964-01A-11D-1924-10	Y473C			hyper	473	exo
TCGA-EI-6882-01A-11D-1924-10	R759C			hyper	759	pol
TCGA-AA-3525-01A-02W-0833-10	R762W	?2213?		hyper	762	pol
TCGA-AA-3525-01A-02W-0833-11	K1008?			hyper	1008	pol
TCGA-AZ-6598-01A-11D-1771-10	T1052M			hyper	1052	pol
TCGA-AA-3833-01A-01W-0900-09	R1160H			hyper	1160	pol
TCGA-D5-6540-01A-11D-1719-10	A1224T			hyper	1224	int
TCGA-AA-3518-01A-02W-0833-10	V1368M			hyper	1368	int
TCGA-AA-3710-01A-01W-0995-10	P1421S			hyper	1421	int
TCGA-AZ-6601-01A-11D-1771-10	R1519C			hyper	1519	duf
TCGA-A6-6781-01A-22D-1924-10	P1547S			hyper	1547	duf
TCGA-AA-A00J-01A-02W-A005-10	A2056T			hyper	2056	int
TCGA-CM-5861-01A-01D-1650-10	V2152M			hyper	2152	int
TCGA-AA-3864-01A-01W-0995-10	R231H			ultra	231	exo
TCGA-CA-6718-01A-11D-1835-10	P286R			ultra	286	exo
TCGA-F5-6814-01A-31D-1924-10	P286R			ultra	286	exo
TCGA-AA-3555-01A-01W-0831-10	P286H			ultra	286	exo
TCGA-CA-6717-01A-11D-1835-10	P286S	L1235I	R1371X	ultra	286	exo
TCGA-AA-3977-01A-01W-0995-10	F367S	K777N		ultra	367	exo
TCGA-EI-6917-01A-11D-1924-10	V411L	A426V		ultra	411	exo
TCGA-AA-3984-01A-02W-0995-10	V411L			ultra	411	exo
TCGA-AA-A00N-01A-02W-A00E-09	V411L	L1255V		ultra	411	exo
TCGA-AZ-4315-01A-01D-1408-10	V411L	R1556W		ultra	1556	exo
TCGA-AA-A010-01A-01W-A00E-09	A189T	P436R		ultra	436	exo
TCGA-AA-3510-01A-01D-1408-10	P135S	A456P		ultra	135	exo
TCGA-AG-A002-01A-01W-A005-10	R150X	S459F		ultra	459	exo
TCGA-AG-3892-01A-01W-1073-09	S459F			ultra	459	exo

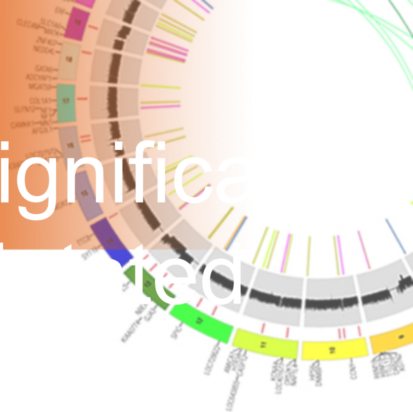
a, Six colorectal cancer patients harbor multiple POLE mutations, 5/6 are in the ultramutated. The phasing is not established; however we assume the R150X termination codon is in cis with the S459F.
b, Subgroups are defined by mutation rate, see main text
c, Position relative to POLE GenBank accession NP_006222



HUGO Symbol	Title	Mutations	Patients	q
APC	adenomatous polyposis coli	199	146	<3.15e-08
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	86	86	<3.15e-08
TP53	tumor protein p53	113	112	<3.15e-08
PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	29	25	<3.15e-08
FBXW7	F-box and WD repeat domain containing 7	23	21	3.15E-08
SMAD4	SMAD family member 4	18	18	3.15E-08
TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)	13	13	2.84E-07
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	16	16	0.000035
CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	10	9	0.0025
ACVR1B	activin A receptor, type IB	8	7	0.0026
SMAD2	SMAD family member 2	10	10	0.007
GRIK3	glutamate receptor, ionotropic, kainate 3	11	11	0.019
WBSCR17	Williams-Beuren syndrome chromosome region 17	9	9	0.02
AHNAK2	AHNAK nucleoprotein 2	15	12	0.038
TTN	titin	71	58	0.038
MLK4	mixed lineage kinase 4	8	8	0.04
FAM123B	family with sequence similarity 123B	13	13	0.04
GPC6	glypican 6	7	7	0.064
SOX9	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	9	8	0.064

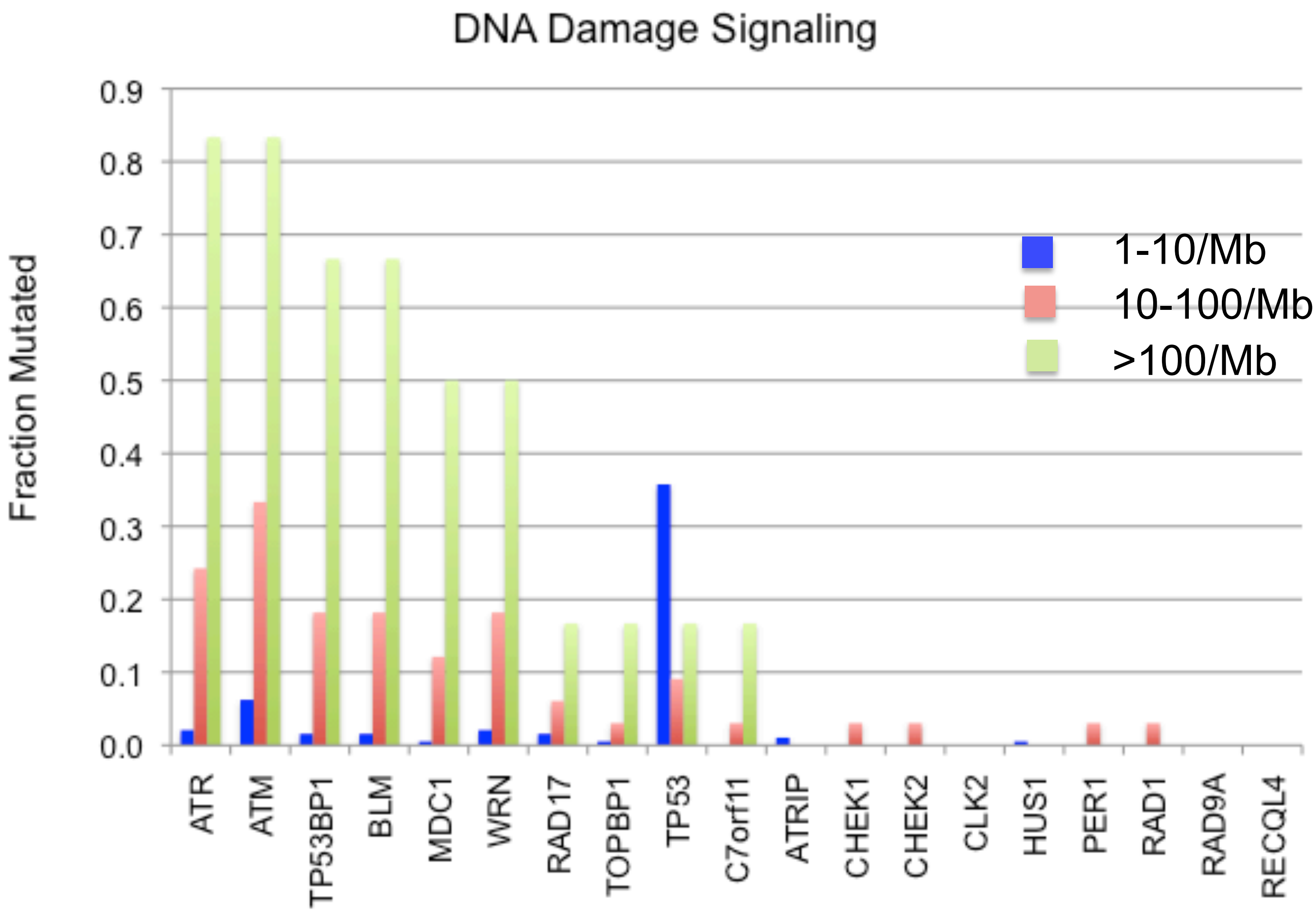
HUGO Symbol	Title	Mutations	Patients	q
APC	adenomatous polyposis coli	44	17	1.89E-07
BRAF	v-raf murine sarcoma viral oncogene homolog B1	18	17	2.37E-06
FBXW7	F-box and WD repeat domain containing 7	22	17	0.000055
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	11	11	0.0015
CASP8	caspase 8, apoptosis-related cysteine peptidase	11	10	0.0038
TMEM132D	transmembrane protein 132D	19	15	0.014
SMAD4	SMAD family member 4	11	7	0.025
SLC16A7	solute carrier family 16, member 7 (monocarboxylic acid transporter 2)	10	7	0.04
ZNF585A	zinc finger protein 585A	12	8	0.095

Significa
ted

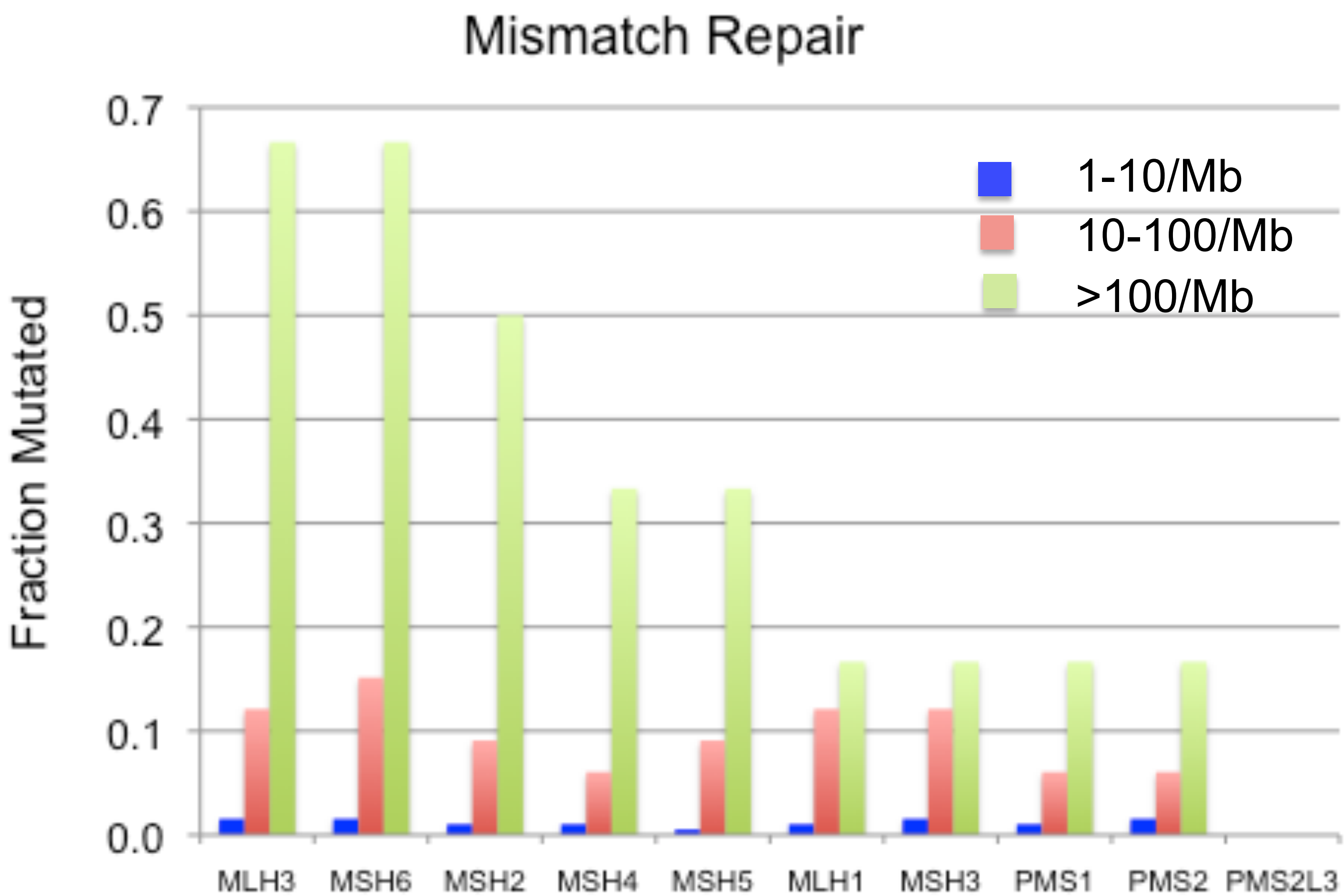


Mike Lawrence, Gaddy Getz, Broad

he Cancer Genome Atlas



The Cancer Genome Atlas

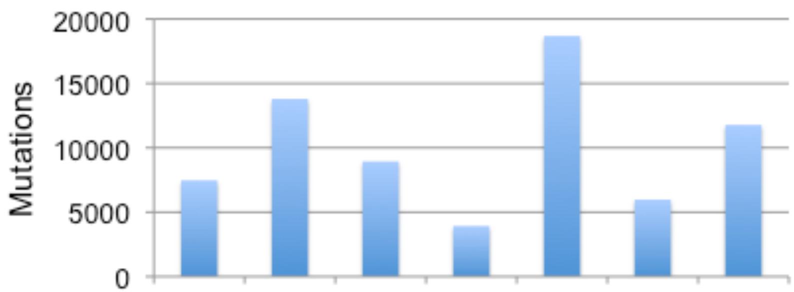


Mismatch Repair
Fraction Mutated
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1-10/Mb
10-100/Mb
>100/Mb
MLH3
MSH6
MSH2
MSH4
MSH5
MLH1
MSH3
PMS1
PMS2
PMS2L3

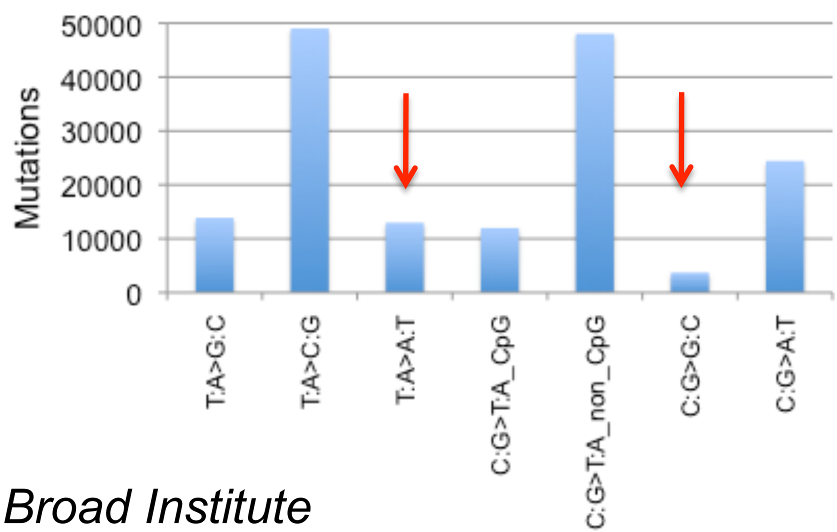
Mutation spectrum changes with increased MMR

- Transversions that reverse paired bases appear less frequently in hypermutated patients

Standard Mutation

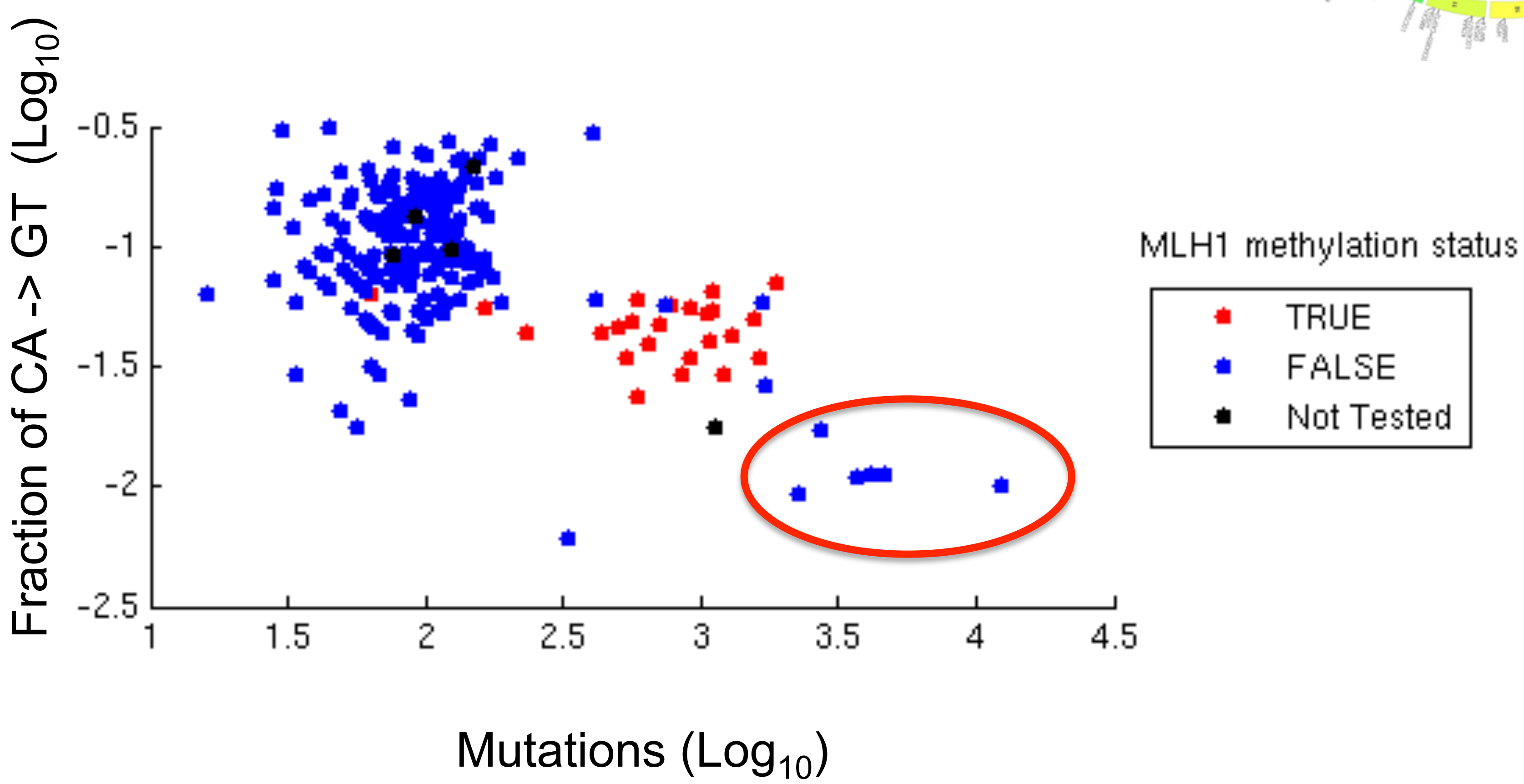


Hypermutated



Mike Lawrence, Broad Institute

Flip transversions in ultramutated



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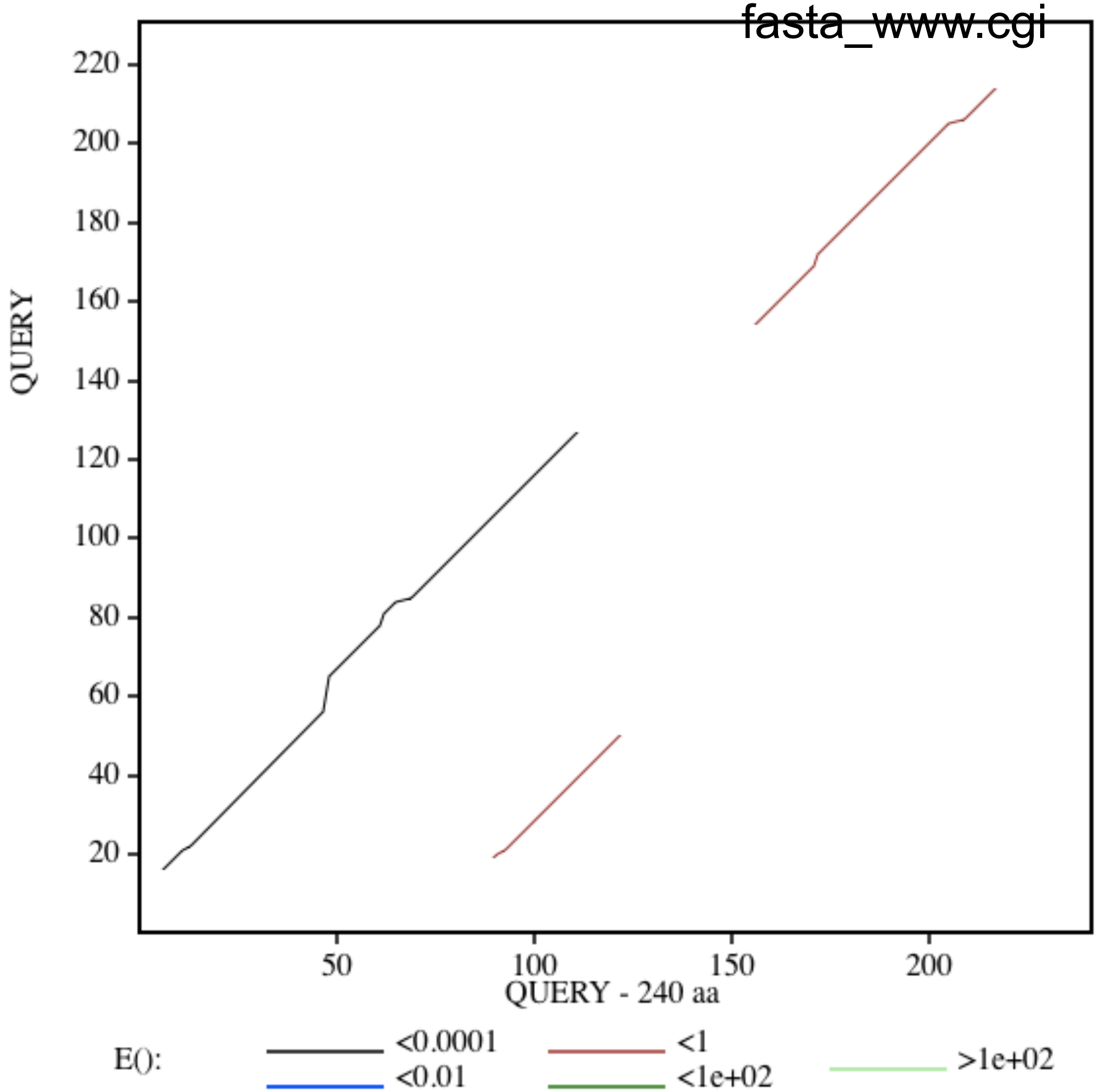
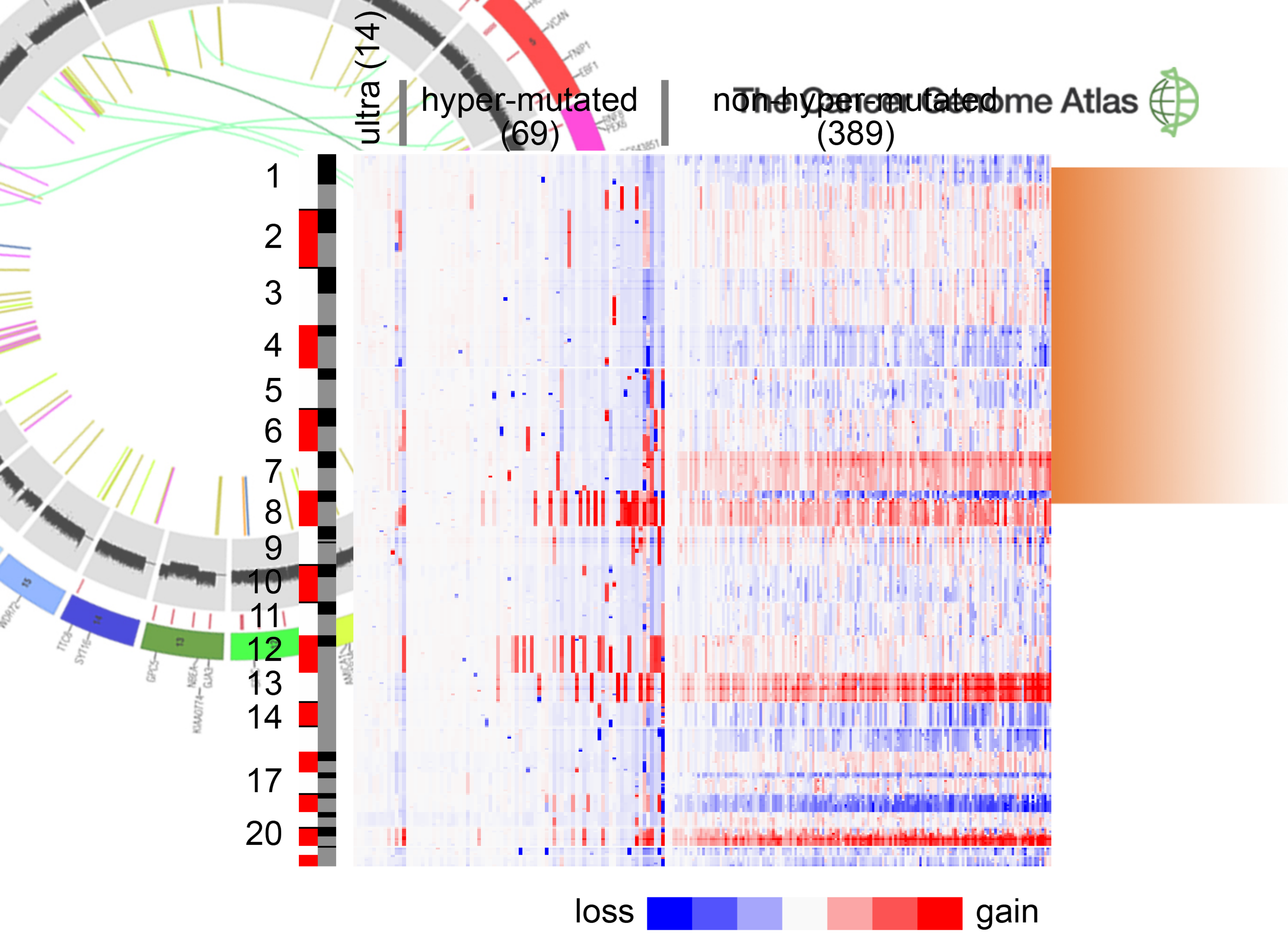
http://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi





ultra (14)
hyper-mutated
(69)
non-hyper-mutated
(389)
The Cancer Genome Atlas
1
2
3
4
5
6
7
8
9
10
11
12
13
14
17
20
loss
gain

P286R/H

```
3|Cilio|Paramecium_tetraurelia_strain_d4-2          222 YLAFDIETYK--QPLKF------------------------------------------------PDAK--NDQIM--
Apico|Cryptosporidium_parvum_Iowa_II                 278 SLAWDIETTK--DPLKF------------------------------------------------PNVE--KDQIM--
Mycet|Dictyostelium_discoideum_AX4                   269 VLAYDIETTK--LPLKF------------------------------------------------PDSS--IDSIM--
ungi|Saccharomyces_cerevisiae                        286 VMAFDIETTK--PPLKF------------------------------------------------PDSA--VDQIM--
Fungi|Schizosaccharomyces_pombe                      272 IMAFDIETTK--LPLKF------------------------------------------------PDSS--FDKIM--
Fungi|Cryptococcus_neoformans_var-_neoformans_JEC21  314 VMAYDIETTK--QPLKF------------------------------------------------PDQQ--TDQIM--
'|Fungi|Malassezia_globosa_CBS_7966                  287 VMAFDIETTK--QPLKF------------------------------------------------PDAE--IDAIM--
i|Choan|Monosiga_brevicollis_MX1                     230 VLAWDIETTK--LPLKF------------------------------------------------PNSE--IDQIM--
Metaz|Drosophila_melanogaster                        269 VLAFDIETTK--LPLKF------------------------------------------------PDAQ--TDQVM--
i|Metaz|Ciona_intestinalis                           257 VLAYDIETTK--LPLKF------------------------------------------------PDAE--TDNII--
3|Metaz|Trichoplax_adhaerens                         258 VLAFDIETTK--LPLRF------------------------------------------------PDSS--SDAIM--
Metaz|Homo_sapiens                                   271 VLAFDIETTK--LPLKF------------------------------------------------PDAE--TDQIM--
i|Metaz|Nematostella_vectensis                       237 VLAFDIETTK--LPLKF------------------------------------------------PDSA--TDSIM--
)|Chlor|Ostreococcus_tauri                           292 VCAFDIETTK--LPLKF------------------------------------------------PDAE--HDQVF--
i|Strep|Physcomitrella_patens_subsp-_patens          283 VCAFDIETTK--LPLKF------------------------------------------------PDAS--YDSVM--
'|Strep|Vitis_vinifera                               251 VCAFDIETTK--LPLKF------------------------------------------------PDAE--YDLVM--
Strep|Oryza_sativa_Japonica_Group                    274 VCAFDIETTK--LPLKF------------------------------------------------PDAE--YDTVM--
```

F367S

```
-------VLERFFSEIKREKPHIFVSYNGD-MFDWPFVESRAETHGLSMF--------------------HQIGFR-----
-------LLQRFFEHIRDVRPTVISTFNGD-FFDWPFIHNRSKIHGLDMF--------------------DEIGFAP----
-------LLHRFFKHIRSAKPSVIVTYNGD-FFDWPFVDARAAFHGLNLT--------------------EETGFFR----
-------VIRRWFEHIRDSKPTVIATYNGD-SFDFPFVDARAKIHGISMY--------------------EEIGFKP----
-------LLRRFFAHIQEARPTVIATYNGD-SFDFMFVDVRARIHGINMK--------------------HEIGFAK----
-------TIARFFEHIQSEKPHVMVTYNGD-SFDWPFLERRAEINDMRMF--------------------YEIGFRP----
-------LLQRFFDHIMEVRPHIIVTYNGD-FFDWPFVETRAAVYDLDMK--------------------QEIGFSK----
-------TINRFFDHVLEVKPHVFVTYNGD-FFDWPFVESRARILGLDML--------------------REIGFSK----
-------LLNRFFQHIIELKPTIYATYNGD-AFDWPFIEARAAFHDINMS--------------------EEIGFSK----
-------LIQRWFEHVQETKPTIMVTYNGD-FFDWPFVEARAAVHGLSMQ--------------------QEIGFQK----
-------LLRRFFEHILEVKPNIFVTYNGD-SFDWPFVEARASHHGINMM--------------------EEIGFSP----
-------LLRRWFDHMREAQPSIYVTYNGD-YFDFPFIETRAKKNGMDMY--------------------REIGFKC----
-------LLVSWFSHMREVKPSIYVTYNGD-FFDWPFIQARALHHGMRMH--------------------DEIGFQH----
-------LLRMWFAHMREVKPGIYVTYNGD-FFDWPFLESRAAHHGLKMS--------------------DEVGFQC----
-------LLKAWFSHMQEVKPGIYVTYNGD-FFDWPFLEKRAAHHGIKMN--------------------EEIGFQC----
```

Eukaryotic POLE alignments

- All mutator positions are invariant across eukaryotes

V411L

S459F

```
IDAFCWVKRDSYLPH-----------GSHG---LKAVTREKLRYDPL--------------------ELD-------PELMLKSAQED----------------------------PETLANY----------------------------SVSDAVATYYLY
IDCFRWVKRDSYLPQ-----------GSQG---LKAVTQSKLGYNPI--------------------ELD-------PELMTPYAFEK----------------------------PQHLSEY----------------------------SVSDAVATYYLY
IDAFRWVKRDSYLPQ-----------GSQG---LKAVTVSKLGYNPI--------------------ELD-------PELMTPYASEK----------------------------PQVLAQY----------------------------SVSDAVATYFLY
IDCFRWVKRDSYLPQ-----------GSQG---LKAVTTAKLGYNPI--------------------ELD-------PELMTPYAIEQ----------------------------PQILAQY----------------------------SVSDAVATYYLY
DCFRWVKRDSYLPQ-----------GSQG---LKAVTVAKLGYDPM--------------------ELD-------PELMTPYASEQ----------------------------PQTLAQY----------------------------SVSDAVATYYLY
DCFRWVKRDSYLPV-----------GSHG---LKAVTREKLRYDPL--------------------EIH-------YEEICRMAAEQ----------------------------PQELANY----------------------------SVSDAVATYYLY
IDCLCWVKRDSYLPV-----------GSQG---LKAVAKAKLRYDPV--------------------ELD-------PEDMCRMAVEQ----------------------------PQVLANY----------------------------SVSDAVATYYLY
IDAYKWVKRDSYLPV-----------GSQN---LKAAAKAKLRYDPV--------------------ELD-------PEEICRLASEE----------------------------PQTLANY----------------------------SVSDAVATYYLY
IDCYRWVKRDSYLPV-----------GSHN---LKAVTKAKLRYNPI--------------------ELD-------PEEMCRMASEQ----------------------------PQILANY----------------------------SVSDAVATYYLY
IDCLRWVKRDSYLPV-----------GSHN---LKAAAKAKLGYDPV--------------------ELD-------PEDMCRMATEQ----------------------------PQTLATY----------------------------SVSDAVATYYLY
IDAFRWVKRDSYLPV-----------GSQN---LKATTKAKLRYDPI--------------------ELD-------PEEMCRMASEQ----------------------------PQELSNY----------------------------SVSDAVATYYLY
DCFCWVKRDSYLPA-----------GSHG---LKAVTKAKLKFDPV--------------------EVD-------PEDMLPFAQSR----------------------------PQHMAQY----------------------------SVSDAVSTYYLY
DCFAWVKRDSYLPQ-----------GSQG---LKAVTKAKLGFDPL--------------------EVN-------PEDMVRFAIEQ----------------------------PQTMASY----------------------------SVSDAVSTYYLY
DCFAWVKRDSYLPQ-----------GSQG---LKAVTKAKLGYDPL--------------------EVN-------PEDMVRFAKEL----------------------------PQMMASY----------------------------SVSDAVSTYYLY
DCFAWVKRDSYLPQ-----------GSHG---LKAVTKAKLGYDPL--------------------EVN-------PEDMVRFAMEQ----------------------------PQTMASY----------------------------SVSDAVATYYLY
```

Mutation properties of endometroid tumors