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Supplemental information

Inherited mutations affecting the SRCAP complex are central in moderate-penetrance predisposition to uterine leiomyomas

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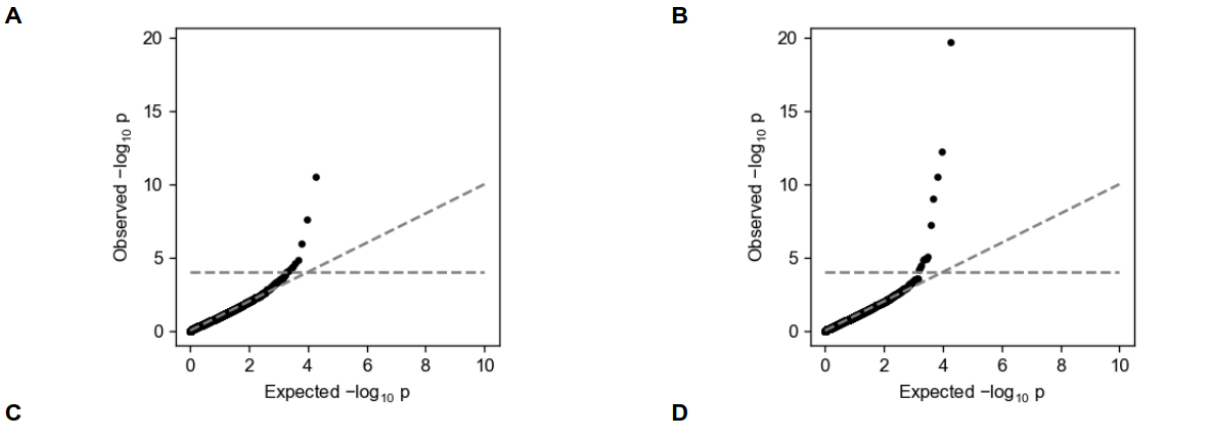
SUPPLEMENTAL FIGURES



Gene	Number of variants [a]	Ultra-rare variants [b]	MAC [c]	MAC [d] (controls)	Rank	OR	p
MSH6	4	101	140	127	1	3.3	1.9e-20
YEATS4 [e]	2	13	55	33	2	5.0	5.7e-13
ZNHIT1 [e]	3	9	54	61	3	2.6	3.0e-11
BRCA1	4	94	120	188	4	1.9	9.5e-10
CHEK2	9	40	452	990	5	1.4	6.4e-8
MSH2	0	31	30	29	6	3.1	9.2e-6
NAE1	0	33	35	40	7	2.6	1.2e-5
DMAP1 [e]	4	22	68	115	8	1.8	1.2e-5
FH	1	12	24	20	9	3.6	1.5e-5
MLH1	2	22	37	62	10	1.8	3.4e-5
GREB1	2	64	54	78	11	2.1	5.0e-5
ACAT2	1	22	18	55	12	1.0	7.0e-5
…							
ACTL6A [e]	0	8	9	4	21	6.7	4.8e-4
ACTR6	1	26	16	54	14,414	0.9	0.8
SRCAP	1	13	7	20	14,892	1.0	0.8
RUVBL2	0	6	3	9	17,113	1.0	1.0
VPS72	1	19	21	58	17,903	1.1	1.0
RUVBL1	0	2	0	2	n/a	n/a	n/a

SRCAP complex LoF					
At risk	399	398	325	190	12
Censored	0	0	0	31	201
Events	0	1	74	178	186
Other WES individuals					
At risk	232698	232621	218106	147082	9933
Censored	0	0	0	34589	164770
Events	0	77	14592	51027	57995

Figure S1: **Germline loss-of-function variant associations to individuals with uterine leiomyoma or hysterectomy operation.** A-B, quantile-quantile plots of SKAT-O test p values across all 18,899 genes: A, uterine leiomyoma (UL) phenotype, and B, the combined phenotype of UL or hysterectomy. The diagonal dashed line shows where the observed and expected null distributions match, and the horizontal dashed line shows p=1e-4. C-E, summary statistics for

58,709 individuals with UL or hysterectomy and 174,905 female controls. Genes that passed exome-wide significance at p<1e-4 are shown, including a summary of all nine SRCAP complex genes. The p values are from gene-based SKAT-O tests. Rank: rank among 18,899 genes; OR: odds ratio; MAC: minor allele count for loss-of-function (LoF) variants; [a] Number of LoF variants with minor allele frequency≤1%, excluding ultra-rare variants; [b] Number of ultra-rare LoF variants with MAC≤10; [c] MAC among individuals with UL/hysterectomy; [d] MAC among female controls; [e] SRCAP complex genes that passed p<0.05/9; n/a: no statistics were computed for MAC<3 genes. D-E**,** *YEATS4*, *ZNHIT1*, *DMAP1* and *ACTL6A* LoF variants contribute to younger age at UL diagnosis or hysterectomy (p=6.9e-8; two-sided Welch's t test). D, age at UL diagnosis or hysterectomy, stratified by the gene with a LoF variant. Box plots show the median and the first and third quartiles of the data; whiskers extend up to 1.5 interquartile range (IQR) and dots show the individual observations. *FH* is shown as a reference. No LoF: individuals without LoF variants among the genes highlighted. E, Kaplan-Meier estimates (p=3.8e-31; two-sided log-rank test) and at risk, censored and event counts for the five time points on the X-axis. Individuals with *FH* LoF variants were excluded from the Kaplan-Meier estimates.

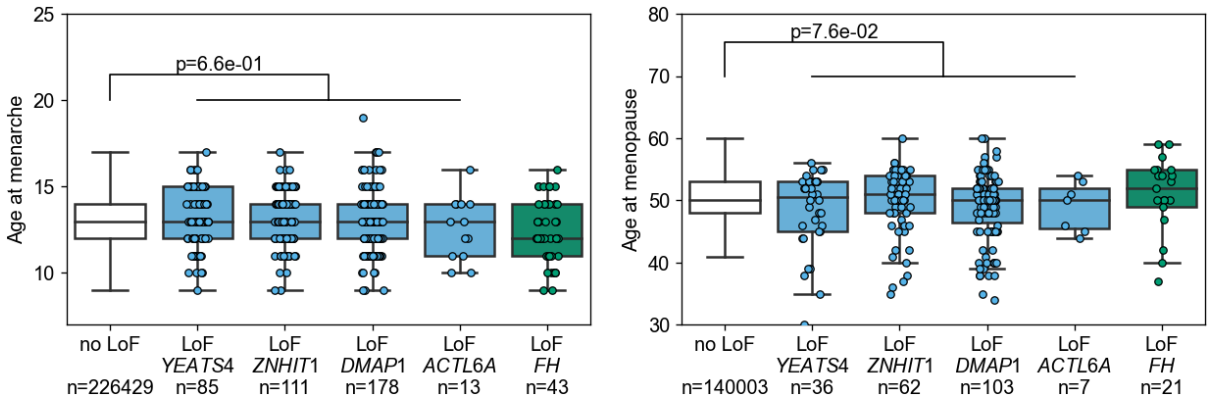


Figure S2. No differences in age at menarche or menopause were observed for women with loss-of-function of SRCAP complex genes. *YEATS4*, *ZNHIT1*, *DMAP1* and *ACTL6A* were examined for loss-of-function (LoF) variant association to age at menarche and menopause. Box plots stratified by the gene with a LoF variant, showing the median and the first and third quartiles of the data; whiskers extend up to 1.5 IQR. Coloured dots show the individual observations with LoF variants. *FH* is shown as a reference. On the left, age at menarche stratified by LoF status. No difference was observed (p=0.66; two-sided Welch's t test). On the right, age at menopause stratified by the gene with a LoF variant. No difference was observed (p=0.076; two-sided Welch's t test). Altogether 226,859 and 140,232 women had age at menarche and menopause information available, respectively. No LoF: women with no LoF variants among the genes highlighted.

A

Gene	Variant category	Number of QV (individuals with UL)	Number of QV (controls)	OR	p
YEATS4 [a]	ptv5pcnt/ptv	23	58	4.96	1.4e-08
DMAP1 [a]	ptv5pcnt/ptv	22	74	3.72	1.6e-06
YEATS4 [a]	ptvraredmg/flexdmg	23	94	3.06	1.5e-05
TMED7	UR	11	23	5.98	2.1e-05
ZNHIT1 [a]	ptv5pcnt/ptv	21	84	3.13	2.6e-05
SRPK2	flexdmg	44	264	2.09	2.8e-05
ZNF697	rec	7	8	10.94	4.6e-05
MMP11	raredmg	38	218	2.18	5.9e-05
MMP11	ptvraredmg	42	259	2.03	9.1e-05
DNASE2	rec	9	18	6.25	9.2e-05
ZNHIT1 [a]	ptvraredmg/flexdmg	32	178	2.25	9.9e-05

B

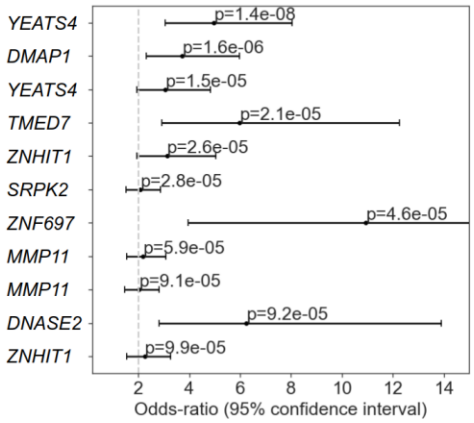


Figure S3. Summary of moderate-penetrance associations to uterine leiomyoma. A uterine leiomyoma endpoint comprising 15,780 individuals with ULs and 197,159 female controls was inspected for gene-level associations across ten different variant categories and 19,000 genes. Altogether eleven associations displayed a moderate effect size (gene-based collapsing test, odds-ratio [OR]>2.0 and p value<1e-4). A, details for numbers qualifying variants (QV), odds-ratios (OR) and p values (Fisher's exact test). ptv5pcnt/ptv: Protein-truncating variants (MAF≤5% had the same variants as MAF≤0.1%). ptvraredmg, flexdmg, raredmg: rare non-synonymous, predicted damaging variants (MAF≤0.025%, MAF≤0.1%, MAF≤0.025%, respectively). UR: ultra-rare damaging variants (MAF≤0.005%). rec: recessive variant model (MAF≤1%). [a] SRCAP complex member genes. B, odds ratios, p values and 95% confidence intervals. The dashed-line shows the OR>2.0 cutoff.

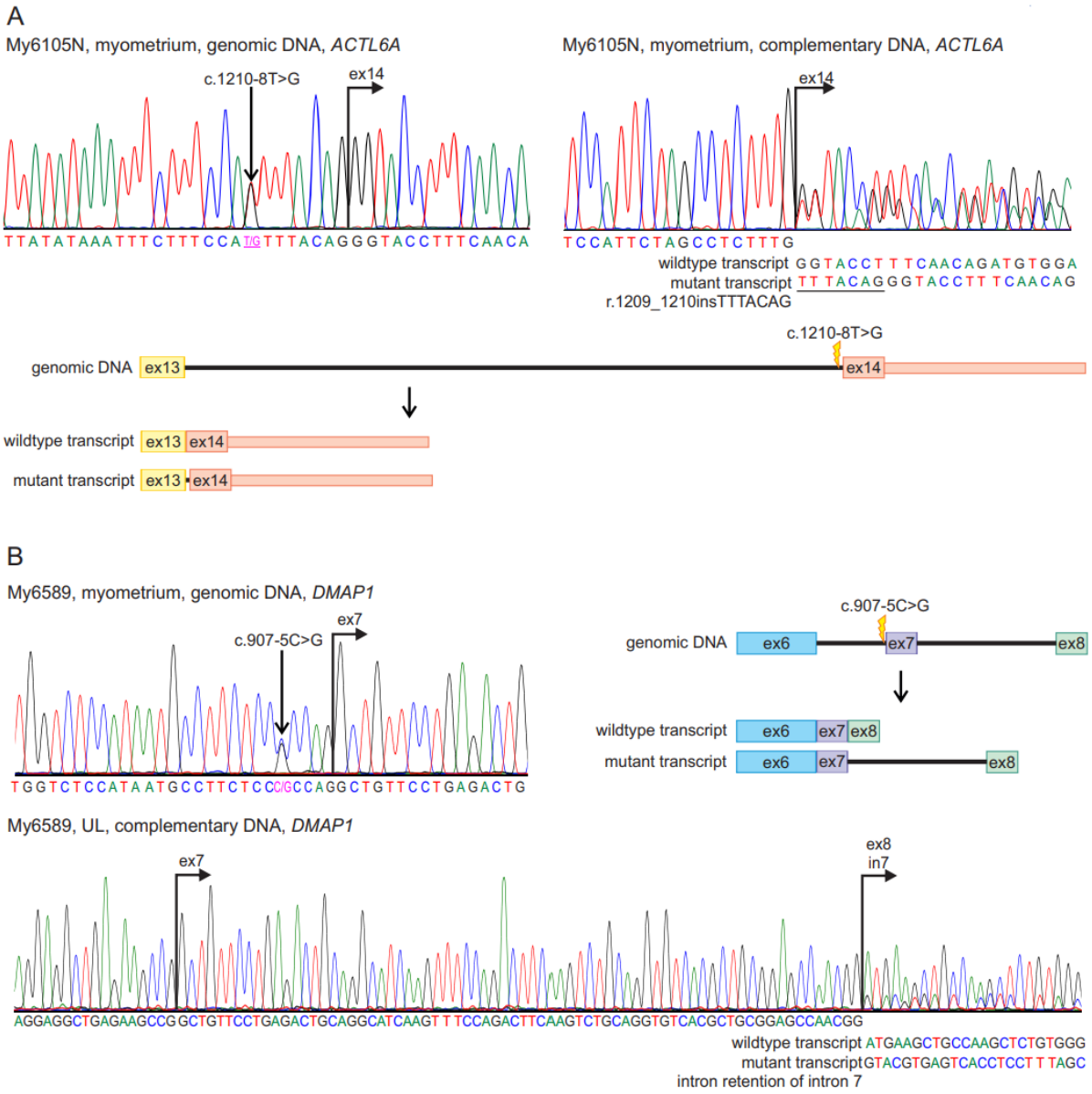


Figure S4. Splice-site variants of SRCAP complex members in two individuals with ULs.

A) Sanger sequencing of genomic DNA from normal myometrium confirmed that individual My6105 has an intronic germline variant c.1210-8T>G in *ACTL6A*. Sequencing of complementary DNA (cDNA) revealed that this mutation creates a new splice site, resulting in a 7bp insertion c.1209_1210insTTTACAG at the transcript level, breaking the reading frame. B) Individual My6589 harbored a germline mutation *DMAP1* c.907-5C>G. At the transcript level, cDNA sequencing revealed intron retention of intron 7.

SUPPLEMENTAL TABLES

Table S1. Primers used for the mutation screening.

Gene and exon(s)	Primer sequences 5'-3'	Amplicon length
ACTL6A exon 1	GGCTATCGCTCCTCGAGAC CGGTTACAAACCCCACACG	172 bp
ACTL6A exons 2-3	TATTTGGAAGACTGAGGCGG TGTCTGGAAATCAGTTTGTGG	680bp
ACTL6A exon 4	TTACATTTGGGACAGGTCTGG CCATTAGGGTGTTTTGTTGTTC	560bp
ACTL6A exon 5	TGAGATATTATGAAAGATGATTTCCC GGAACAAGCAATAATTGGCG	345bp
ACTL6A exon 6	GAGCCTGCCCTTTACAATAAAC TCAACACATAAATAAAGCCATGC	355bp
ACTL6A exons 7-8	TCACATAAATACACCCACAGAGG CCATATTTTACTCAAGGATTTACAGC	477bp
ACTL6A exons 9-10	AAACATGGTCCAATTTCATTCAG CAAAACATTTTAGCCACAAAAGC	550bp
ACTL6A exon 11	GTGGCTAAAATGTTTTGGATATG ATGATTTGGGGACATTTCAGTC	221bp
ACTL6A exon 12	CCAGGATGGAGAATTGATGG ACCAGGCACACACAAAATATC	382bp
ACTL6A exon 13	GCGAGGAAGACCCTGTCTC AGGTCTTCCACTCATTTGACAG	318bp
ACTL6A exon 14	GGTTGTAAGCTACAATCACGCC AAAGATGGTCATTCTTTTCCTG	302bp
DMAP1 exon 1	TGAGCCTGGTCCTTCTTCAG AGTGTAGCACCCATCCCTTG	261bp
DMAP1 exon 2	GCTTTGTTCAGGGATTGAGC AACTAGGAGTTAGGGGCATGG	247bp
DMAP1 exon 3	AGGGTAGGGTAGATCAGCTCC AGCTCGCACAAATGGCTC	350bp
DMAP1 exons 4-5	GTCACTGGGGCCTGGAG AGTGGGAGTTGAGGGGATG	602bp
DMAP1 exons 6-7	TCACCTCCGTGTCTACCCTC CTGCTCCATCCCCACCTG	582bp
DMAP1 exon 8	TTCCACCATCTTTCCCTCTG CCTGCCCAACCTATATCCAC	226bp
DMAP1 exon 9	GAGGGGAGTTCACATTGCTG CACTAGAGGGAGAGGAGCCC	451bp
DMAP1 exon 10	TGAAGCACATGCACTAAGCC GAAAGGAAAAGGAAGCAGCC	210bp
YEATS4 exon 1	CCAAGTAACTCGCCCTCCTT GAGAAAAGGCGCGAAAGGAA	291bp

YEATS4 exon 2	AGCCTGCCATTCTTTAAAGCA ACAGGTTGTCTTTAAGCAAAACA	290bp
YEATS4 exons 3-4	ACTTCCCAGGTGTAGTTCATGT AGGATTTTGAAAGGCGCTACA	581bp
YEATS4 exons 5-6	TGGGTGATTTGCTGCAATAAGT GGCACGAATCATACAACCCT	496bp
YEATS4 exons 7	CATTGTCGTCAGGAAATGCC CCATTTCTCCAGTGAAGCCC	328bp
ZNHIT1 exon 1	ACGCGCAGAAGTACAAGCTA GCGAAAGAGCGAGACCAAAA	220bp
ZNHIT1 exon 2	TGGGGATGAGATCAGAGAGC GTCCTCCCACAGCCTGAGT	373bp
ZNHIT1 exon 3	TGAACCAGAGAAAGCTGCTG GCCGGCTCTAGAACTCCTC	246bp
ZNHIT1 exon 4	GTTGGAGGAGCAGGTGAGAG AGGATGAGGGGAGAGAGGTC	373bp
ZNHIT1 exon 5	CTGTCTGTGGCTTCCCATCC CAGAATCTCTCGCGATCAGGG	585bp
Primers to validate splice effect of the *ACTL6A* c.1210-8T>G germline mutation		
Forward primer aligning to exon 12	GCAGTGTAATAGTGGCAGGAG	wildtype 336bp mutated 343bp
Reverse primer aligning to exon 14	AGATGGTCATTCTTTTCCTGAGT	
Primers to validate splice effect of the *DMAP1* c.907-5C>G germline mutation		
Forward primer aligning to exon 5	GCTTGAGCGTCTCTACAACC	wildtype 342bp mutated 670bp
Reverse primer aligning to exon 8	TGTTCCAGGGCCTTGATCTT	

Table S2. *MSH6* and *BRCA1* hysterectomy associations arose with endometrial and ovarian cancer. Summary numbers of individuals with loss-of-function (LoF) variants stratified by hysterectomy and cancer endpoints. Among individuals with *MSH6* and *BRCA1* LoF germline mutations, hysterectomy operations were explained to a large degree by endometrial and ovarian cancer, respectively. Breast cancer and *CHEK2* are shown for reference. Odds ratios (OR) and p values are from a two-sided Fisher's exact test.

		No hysterectomy		Hysterectomy operation			
Gene	**Phenotype**	**No cancer**	**Individuals with cancer**	**No cancer**	**Individuals with cancer**	**OR**	**p**
MSH6	Breast cancer	114	13	126	13	0.9	8.4E-01
MSH6	Endometrial cancer	127	0	89	50	n/a [a]	6.0E-17
MSH6	Ovarian cancer	127	0	125	14	n/a [a]	1.0E-04
MSH6	Malignant neoplasms of female genital organs	123	4	71	68	29.5	3.7E-19
BRCA1	Breast cancer	133	55	69	51	1.8	2.0E-02
BRCA1	Endometrial cancer	185	3	113	7	3.8	5.1E-02
BRCA1	Ovarian cancer	184	4	91	29	14.7	1.2E-09
BRCA1	Malignant neoplasms of female genital organs	178	10	79	41	9.2	6.9E-11
CHEK2	Breast cancer	826	160	356	93	1.3	4.4E-02
CHEK2	Endometrial cancer	985	1	427	22	50.7	9.0E-11
CHEK2	Ovarian cancer	982	4	430	19	10.8	4.5E-07
CHEK2	Malignant neoplasms of female genital organs	948	38	395	54	3.4	2.1E-08

[a] n/a: no odds ratio estimates available.

Table S3. Phenome-wide associations of loss-of-function variants in SRCAP complex genes. The nine SRCAP complex genes were tested for loss-of-function (LoF) variant associations against 15,500 binary phenotypes. A putative significance threshold was chosen at $p<0.0005$ to account for multiple testing over the nine SRCAP complex genes and 15,500 binary phenotypes. The table gives summary statistics for a gene-based collapsing test, including the gene symbol, phenotype, variant model (details in Material and methods), category of the phenotype, p value (two-sided Fisher's exact test; details in Material and methods) and numbers of individuals with the phenotype and numbers of controls. Numbers of qualifying variant (QV) are also given. OR: odds ratio; LCI and UCI: lower and upper 95% confidence intervals, respectively.

Table S3 is provided as a separate Excel file.

Table S4. Results of H2A.Z prescreening IHC. Uterine leiomyoma (UL) sections were stained together with the *YEATS4* and *MED12* mutated ULs used as negative and positive controls, respectively. Samples were classified based on the immunoreaction intensity into three groups: 0 = negative or weak, 1 = moderate, 2= strong.

Individual ID	Tumor	H2A.Z score
My6451	My6451m1	2
My6458	My6458m1	1
My6463	My6463m1	1
	My6463m2	2
My6499	My6499m1	2
My6512	My6512m1	2
My6517	My6517m1	2
My6519	My6519m1	2
My6527	My6527m1	2
My6533	My6533m1	2
My6542	My6542m1	2
My6544	My6544m1	2
My6547	My6547m1	2
My6564	My6564m1	1
My6569	My6569m1	2
My6571	My6571m1	2
My6576	My6576m1	2
My6589	My6589m1	1
My6593	My6593m1	2
My6601	My6601m1	2
My6604	My6604m1	2
My6605	My6605m1	2
	My6605m2	0
	My6605m3	2
My6615	My6615m1	2
My6621	My6621m1	1
My6623	My6623m1	2
My6624	My6624m1	2

My6626	My6626m1	2
My6634	My6634m3	2
My6635	My6635m5	2
My6636	My6636m1	2
My6638	My6638m1	1
	My6638m2	1
	My6638m3	0
My6640	My6640m1	2
My6644	My6644m1	2
My6645	My6645m2	2
	My6645m6	2
My6652	My6652m2	2
My6655	My6655m1	2
My6660	My6660m1	1
My6661	My6661m1	1
My6665	My6665m1	1
My6667	My6667m3	2
My6668	My6668m1	2
	My6668m4	2
My6673	My6673m3	2
My6674	My6674m1	2
My6675	My6675m4	2
	My6675m7	0
My6676	My6676m4	2
My6677	My6677m1	0
My6679	My6679m4	2
My6680	My6680m1	2
My6687	My6687m1	2
My6692	My6692m1	2
My6696	My6696m1	1
My6701	My6701m1	0

Table S5. Reduction of H2A.Z levels in germline mutated *ACTL6A*, *YEATS4*, and *DMAP1* ULs. Individual My6105 had a germline *ACTL6A* mutation, and individuals My6564 and My6606 shared a germline mutation in *YEATS4*. Four individuals (My6638, My6589, My6621, My6660) harbored *DMAP1* germline mutations. Samples are classified based on the immunoreaction intensity into three groups: 0 = negative/weak, 1 = moderate, 2= strong.

Individual ID	Tumor	H2A.Z score
My6105	My6105m1	0
	My6105m4	0
	My6105m5	0
My6564	My6564m1	1
My6606 [a]	MH19-A	1
	MH19-B	1
	MH19-C	0
	MH19-D	0
	MH19-E	0
	MH19-F	0
	MH19-H	0
	MH19-I	1
	MH19-K	0
	MH19-L	0
	MH19-M	0
My6589	My6589m1	1
My6621	My6621m1	1
My6638	My6638m1	1
	My6638m2	1
	My6638m3	0
My6660	My6660m1	1

[a] formalin fixed paraffin embedded blocks of this individual from diagnostic laboratory