

Figure S1. The workflow diagram of wet-laboratory steps (samples preparation and sequencing reaction).

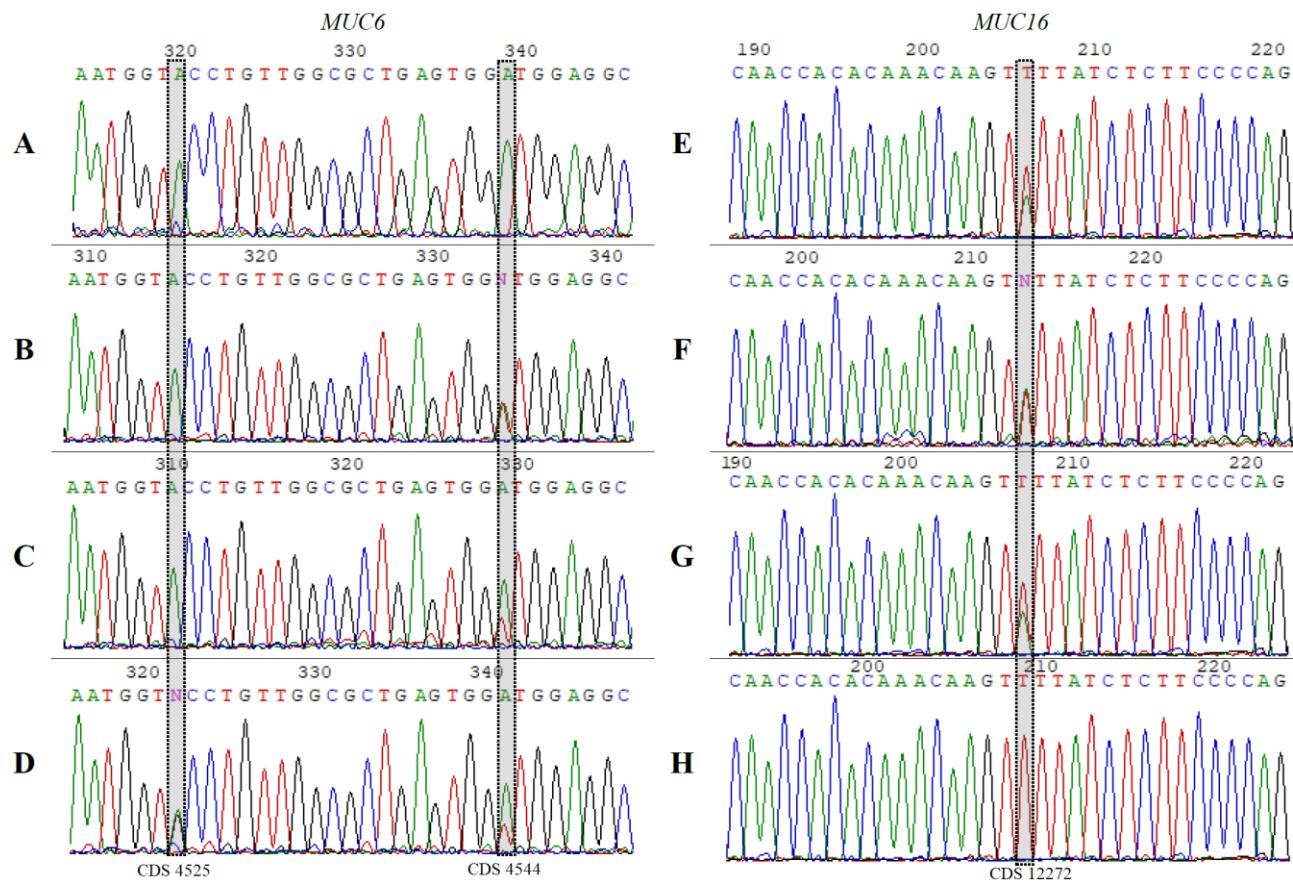


Figure S2. Electropherograms of Sanger sequencing traces for *MUC6* ENST00000421673.7 (A, B, C, D) and *MUC16* ENST00000397910.8 (E, F, G, H). (A) The absence of mutation A>G in CDS at position 4525 and the presence of a mutation C>A in CDS at position 4544 were found in the *MUC6* fragment from tumour sample ID 39. (B) The absence of mutation A>G in CDS at position 4525 and the presence of a mutation C>A in CDS at position 4544 were found in the *MUC6* fragment from tumour sample ID 47. (C) The absence of mutation A>G in CDS at position 4525 and the presence of a mutation C>A in CDS at position 4544 were found in the *MUC6* fragment from margin tissue sample ID 48. (D) The presence of mutation A>G in CDS at position 4525 and presence of mutation C>A in CDS at position 4544 were found in the *MUC6* fragment from margin tissue sample ID 90. (E) The presence of mutation T>A in CDS at position 12272 was found in the *MUC16* fragment from tumour sample ID 37. (F) The presence of mutation T>A in CDS at position 12272 was found in the *MUC16* fragment from tumour sample ID 47. (G) The presence of mutation T>A in CDS at position 12272 was found in the *MUC16* fragment from margin tissue sample ID 48. (H) The presence of mutation T>A in CDS at position 12272 was found in the *MUC16* fragment from margin tissue sample ID 90. Electropherograms of Sanger sequencing traces were prepared in GeneStudio™ Pro 2.2.0.0 software (GeneStudio Inc., Suwanee, GA, USA).

Table S1. Accepted and rejected sequencing results.

| Patient | Tumour samples | | | Margin samples | | |
|---------|----------------|---------------------------|----------------------------|----------------|---------------------------|----------------------------|
| | ID | <i>MUC6</i> sequencing | <i>MUC16</i> sequencing | ID | <i>MUC6</i> sequencing | <i>MUC16</i> sequencing |
| 1 | 11 | Yes | Yes | 12 | No | No |
| 2 | 15 | No | Yes | 16 | Yes | Yes |
| 3 | 25 | No | No | 26 | Yes | No |
| 4 | 29 | Yes | No | 30 | No | Yes |
| 5 | 33 | No | Yes | 34 | Yes | No |
| 6 | 37 | Yes | Yes | 38 | No | No |
| 7 | 39 | Yes | No | 40 | No | No |
| 8 | 41 | No | Yes | 42 | Yes | Yes |
| 9 | 45 | Yes | No | 46 | No | No |
| 10 | 47 | Yes | Yes | 48 | Yes | Yes |
| 11 | 49 | Yes | Yes | 50 | Yes | Yes |
| 12 | 53 | Yes | No | 54 | Yes | Yes |
| 13 | 55 | Yes | No | 56 | Yes | Yes |
| 14 | 57 | Yes | Yes | 58 | Yes | Yes |
| 15 | 61 | Yes | No | 62 | Yes | Yes |
| 16 | 63 | No | Yes | 64 | Yes | Yes |
| 17 | 79 | Yes | Yes | 80 | Yes | Yes |
| 18 | 89 | No | No | 90 | Yes | Yes |

ID - sample identifier

Table S2. Detailed information of investigated MUC6 (ENSG00000184956) mutations.

| SAMPLE ID | SAMPLE TYPE | CHROM | POS | REF | ALT | HGVSc | HGVSp | Consequence | IMPACT | Amino acids | Existing variation | VARIANT CLASS | SIFT | PolyPhen |
|-----------|-------------|-------|---------|-----|-----|---------------------------------|------------------------------------|--------------------|----------|-------------|--------------------|---------------|-----------------------------------|---------------------------|
| 11 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 11 | G | chr11 | 1018258 | C | T | ENST00000421673.7:c. 4543T>A | ENSP00000406861.2:p. Ala1515Thr | missense variant | MODERATE | A/T | | SNV | deleterious low confidence (0.04) | benign (0.026) |
| 16 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 29 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 34 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 34 | M | chr11 | 1018465 | T | C | ENST00000421673.7:c. 4336C>G | ENSP00000406861.2:p. Asn1446Asp | missense variant | MODERATE | N/D | COSV70139063 | SNV | deleterious (0.02) | benign (0.019) |
| 39 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 39 | G | chr11 | 1018322 | A | G | ENST00000421673.7:c. 4479A>C | ENSP00000406861.2:p. Thr1493%3D | synonymous variant | LOW | T | | SNV | | |
| 39 | G | chr11 | 1018506 | C | T,A | ENST00000421673.7:c. 4295C>A | ENSP00000406861.2:p. Ser1432Asn | missense variant | MODERATE | S/N | | SNV | tolerated low confidence (0.14) | benign (0.212) |
| 42 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 42 | M | chr11 | 1018543 | A | G | ENST00000421673.7:c. 4258G>C | ENSP00000406861.2:p. Cys1420Arg | missense variant | MODERATE | C/R | | SNV | tolerated (0.06) | probably damaging (0.921) |
| 45 | G | chr11 | 1018111 | C | T,A | ENST00000421673.7:c. 4690G>A | ENSP00000406861.2:p. Ala1564Thr | missense variant | MODERATE | A/T | | SNV | tolerated (0.2) | benign (0.007) |
| 45 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 47 | G | chr11 | 1018243 | C | T | | ENSP00000406861.2:p. Glu1520Lys | missense variant | MODERATE | E/K | | SNV | | |
| 47 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 48 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 50 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 53 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 54 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |

| SAMPLE ID | SAMPLE TYPE | CHROM | POS | REF | ALT | HGVSc | HGVSp | Consequence | IMPACT | Amino acids | Existing variation | VARIANT CLASS | SIFT | PolyPhen |
|-----------|-------------|-------|---------|-----|---------|---------------------------------|------------------------------------|--------------------|----------|-------------|--------------------|---------------|-----------------------------------|----------------|
| 55 | G | chr11 | 1018180 | C | T | ENST00000421673.7:c. 4621T>A | ENSP00000406861.2:p. Ala1541Thr | missense variant | MODERATE | A/T | | SNV | tolerated (0.86) | benign (0.021) |
| 55 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 56 | M | chr11 | 1018381 | C | T | ENST00000421673.7:c. 4420G>A | ENSP00000406861.2:p. Ala1474Thr | missense variant | MODERATE | A/T | | SNV | tolerated low confidence (0.6) | benign (0) |
| 56 | M | chr11 | 1018513 | C | A,T | ENST00000421673.7:c. 4288A>T | ENSP00000406861.2:p. Ala1430Ser | missense variant | MODERATE | A/S | | SNV | tolerated low confidence (0.21) | benign (0.045) |
| 57 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 58 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 58 | M | chr11 | 1018297 | G | A | ENST00000421673.7:c. 4504A>T | ENSP00000406861.2:p. Leu1502%3D | synonymous variant | LOW | L | COSV70138702 | SNV | | |
| 61 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 62 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | MODERATE | L/* | | SNV | | |
| 64 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 79 | G | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 90 | M | chr11 | 1018257 | A | T | ENST00000421673.7:c. 4544C>A | ENSP00000406861.2:p. Leu1515Ter | stop gained | HIGH | L/* | | SNV | | |
| 90 | M | chr11 | 1018276 | T | C, A | ENST00000421673.7:c. 4525A>G | ENSP00000406861.2:p. Thr1509Ala | missense variant | HIGH | T/A | | SNV | deleterious low confidence (0.02) | benign (0.037) |

SAMPLE ID - sample identifier; **CHROM** - chromosome number; **POS** - start coordinate of the variant; **REF** - reference allele; **ALT** - alternative allele; **Consequence** - probable effect of this variant; **IMPACT** - classification of the severity of this variant consequence; **Amino acids** - the change in amino acids in the protein coding sequence (reference/alteration); ; **Existing variation** - identifier available in database (dbSNP or COSMIC); **Variant class** Sequence Ontology variant class (single nucleotide variation – SNV, insertion, or deletion). **SIFT** - Scale Invariant Feature Transform prediction and score; **PolyPhen** - Polymorphism Phenotyping v2 prediction and score

Table S3. Detailed information of investigated *MUC16* (ENSG00000181143) mutations.

| SAMPLE ID | SAMPLE TYPE | CHROM | POS | REF | ALT | HGVSc | HGVSp | Consequence | IMPACT | Amino acids | Existing variation | VARIANT CLASS | SIFT | PolyPhen |
|-----------|-------------|-------|---------|-----|-----|------------------------------|--------------------------------|--------------------|----------|-------------|--------------------|---------------------------------|----------------|----------|
| 11 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 11 | G | chr19 | 8964755 | A | C,T | ENST00000397910.8:c.12015T>G | ENSP00000381008.2:p.Ala4005%3D | synonymous variant | LOW | A | SNV | | | |
| 15 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 15 | G | chr19 | 8964627 | A | T | ENST00000397910.8:c.12143G>A | ENSP00000381008.2:p.Ile4048Lys | missense variant | MODERATE | I/K | SNV | tolerated low confidence (0.09) | benign (0.013) | |
| 16 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 30 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 33 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 33 | G | chr19 | 8964627 | A | T | ENST00000397910.8:c.12143G>A | ENSP00000381008.2:p.Ile4048Lys | missense variant | MODERATE | I/K | SNV | tolerated low confidence (0.09) | benign (0.013) | |
| 37 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 37 | G | chr19 | 8964627 | A | T | ENST00000397910.8:c.12143G>A | ENSP00000381008.2:p.Ile4048Lys | missense variant | MODERATE | I/K | SNV | tolerated low confidence (0.09) | benign (0.013) | |
| 47 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 48 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 56 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 57 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 62 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 63 | G | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 63 | G | chr19 | 8964627 | A | T | ENST00000397910.8:c.12143G>A | ENSP00000381008.2:p.Ile4048Lys | missense variant | MODERATE | I/K | SNV | tolerated low confidence (0.09) | benign (0.013) | |
| 64 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 64 | M | chr19 | 8964627 | A | T | ENST00000397910.8:c.12143G>A | ENSP00000381008.2:p.Ile4048Lys | missense variant | MODERATE | I/K | SNV | tolerated low confidence (0.09) | benign (0.013) | |

| SAMPLE ID | SAMPLE TYPE | CHROM | POS | REF | ALT | HGVSc | HGVSp | Consequence | IMPACT | Amino acids | Existing variation | VARIANT CLASS | SIFT | PolyPhen |
|-----------|-------------|-------|---------|-----|-----|------------------------------|--------------------------------|------------------|----------|-------------|--------------------|--------------------------------|----------------|----------|
| 80 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |
| 90 | M | chr19 | 8964498 | A | T | ENST00000397910.8:c.12272T>A | ENSP00000381008.2:p.Met4091Lys | missense variant | MODERATE | M/K | SNV | deleterious low confidence (0) | benign (0.028) | |

SAMPLE ID - sample identifier; **CHROM** - chromosome number; **POS** - start coordinate of the variant; **REF** - reference allele; **ALT** - alternative allele; **Consequence** - probable effect of this variant; **IMPACT** - classification of the severity of this variant consequence; **Amino acids** - the change in amino acids in the protein coding sequence (reference/alteration); **Existing variation** - identifier available in database (dbSNP or COSMIC); **Variant class** Sequence Ontology variant class (single nucleotide variation – SNV, insertion, or deletion). **SIFT** - Scale Invariant Feature Transform prediction and score; **PolyPhen** - Polymorphism Phenotyping v2 prediction and score