

**Supplementary Table S3.** Description and clinical significance of detected alterations in ctDNA. *In silico* predictors (Sift, PolyPhen and FATHMM-MKL) for missense variants were used in those cases without ClinVar information or with inconclusive result GRCh38

| Gene Name | Transcript      | CDS Change         | AA Change      | Consequence        | Analysis       | COSMIC_ID    | ClinVar_ID | ClinVar_Significance                         | Sift        | PolyPhen          | FATHMM-MKL |
|-----------|-----------------|--------------------|----------------|--------------------|----------------|--------------|------------|--|-------------|-------------------|------------|
| PIK3CA    | ENST00000263967 | c.1035T>A          | p.N345K        | Missense variant   | SafeSeq        | COSV55873276 | 376050     | Pathogenic                                   | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1258T>C          | p.C420R        | Missense variant   | SafeSeq        | COSV55874020 | 31945      | Pathogenic                                   | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1346_1366del     | p.P449_1455del | Inframe deletion   | SafeSeq        | COSV55874439 | NA         | -  | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1624G>C          | p.E542Q        | Missense variant   | SafeSeq        | COSV55894248 | 376244     | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1624G>A          | p.E542K        | Missense variant   | dPCR + SafeSeq | COSV55873227 | 31944      | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1633G>A          | p.E545K        | Missense variant   | dPCR + SafeSeq | COSV55873239 | 13655      | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.1636C>A          | p.Q546K        | Missense variant   | SafeSeq        | COSV55873527 | 13657      | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.2176G>A          | p.E726K        | Missense variant   | SafeSeq        | COSV55875460 | 376476     | Pathogenic                                   | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.3129G>T          | p.M1043I       | Missense variant   | SafeSeq        | COSV55878974 | 217292     | Pathogenic                                   | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.3140A>T          | p.H1047L       | Missense variant   | SafeSeq        | COSV55873401 | 13653      | Pathogenic                                   | -           | -                 | -          |
| PIK3CA    | ENST00000263967 | c.3140A>G          | p.H1047R       | Missense variant   | dPCR + SafeSeq | COSV55873195 | 13652      | Pathogenic                                   | -           | -                 | -          |
| ESR1      | ENST00000440973 | c.1138G>C          | p.E380Q        | Missense variant   | SafeSeq        | COSV52782264 | 376206     | Likely_pathogenic                            | -           | -                 | -          |
| ESR1      | ENST00000440973 | c.1610A>C          | p.Y537S        | Missense variant   | dPCR + SafeSeq | COSV52783938 | NA         | -  | deleterious | probably damaging | pathogenic |
| ESR1      | ENST00000440973 | c.1609T>A          | p.Y537N        | Missense variant   | SafeSeq        | COSV52784978 | NA         | -  | deleterious | probably damaging | pathogenic |
| ESR1      | ENST00000440973 | c.1610A>G          | p.Y537C        | Missense variant   | SafeSeq        | COSV52782924 | NA         | -  | deleterious | probably damaging | pathogenic |
| ESR1      | ENST00000440973 | c.1613A>G          | p.D538G        | Missense variant   | dPCR + SafeSeq | COSV52781024 | NA         | -  | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.182insT          | p.G2E>* fs     | Frameshift variant | SafeSeq        | NA           | NA         | -  | -           | -                 | -          |
| TP53      | ENST00000269305 | c.215C>G           | p.P72R         | Missense variant   | SafeSeq        | COSV52666208 | 12351      | benign                                       | -           | -                 | -          |
| TP53      | ENST00000269305 | c.329G>C           | p.R110P        | Missense variant   | SafeSeq        | COSV52668419 | 233627     | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.331_333delinsTC  | p.L1115fs*12   | Frameshift variant | SafeSeq        | COSV53606624 | NA         | -  | -           | -                 | -          |
| TP53      | ENST00000269305 | c.337T>A           | p.F113I        | Missense variant   | SafeSeq        | COSV53351693 | NA         | -  | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.354A>T           | p.T118=        | Synonymous variant | SafeSeq        | COSV53220114 | NA         | -  | -           | -                 | -          |
| TP53      | ENST00000269305 | c.394A>G           | p.K132E        | Missense variant   | SafeSeq        | COSV52689323 | 376626     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.404G>T           | p.C135F        | Missense variant   | SafeSeq        | COSV52680475 | 376559     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.466_469delinsTCC | p.R156Sfs*14   | Frameshift variant | SafeSeq        | COSV52990383 | NA         | -  | -           | -                 | -          |
| TP53      | ENST00000269305 | c.475G>C           | p.A159P        | Missense variant   | SafeSeq        | COSV52661847 | 182929     | Uncertain significance                       | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.490A>G           | p.K164E        | Missense variant   | SafeSeq        | COSV52728094 | 246416     | Uncertain significance                       | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.532C>G           | p.H178D        | Missense variant   | SafeSeq        | COSV52693338 | 482223     | Likely_pathogenic                            | -           | -                 | -          |
| TP53      | ENST00000269305 | c.578A>T           | p.H193L        | Missense variant   | SafeSeq        | COSV52663304 | 185822     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.578A>G           | p.H193R        | Missense variant   | SafeSeq        | COSV52662414 | 184979     | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| TP53      | ENST00000269305 | c.583A>T           | p.I195F        | Missense variant   | SafeSeq        | COSV52677568 | 376617     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.639A>G           | p.R213=        | Synonymous variant | SafeSeq        | COSV52679610 | NA         | -  | -           | -                 | -          |
| TP53      | ENST00000269305 | c.658T>C           | p.Y220H        | Missense variant   | SafeSeq        | COSV52760651 | 376687     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.707A>G           | p.Y236C        | Missense variant   | SafeSeq        | COSV52662150 | 376693     | Conflicting interpretations of pathogenicity | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.711G>A           | p.M237I        | Missense variant   | SafeSeq        | COSV52661887 | 142714     | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.716A>G           | p.N239S        | Missense variant   | SafeSeq        | COSV52661127 | 376637     | likely_pathogenic                            | -           | -                 | -          |
| TP53      | ENST00000269305 | c.730G>A           | p.G244S        | Missense variant   | SafeSeq        | COSV52676997 | 376600     | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.743G>A           | p.R248Q        | Missense variant   | SafeSeq        | COSV52661580 | 12356      | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.757A>C           | p.T253P        | Missense variant   | SafeSeq        | COSV52873550 | NA         | -  | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.764T>A           | p.I255N        | Missense variant   | SafeSeq        | COSV52714133 | 232289     | Uncertain significance                       | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.799C>T           | p.R267W        | Missense variant   | SafeSeq        | COSV52678166 | 141764     | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| TP53      | ENST00000269305 | c.817C>T           | p.R273C        | Missense variant   | SafeSeq        | COSV52662066 | 43594      | Pathogenic;Likely_pathogenic                 | -           | -                 | -          |
| TP53      | ENST00000269305 | c.818G>T           | p.R273L        | Missense variant   | SafeSeq        | COSV52664805 | 376655     | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.839G>C           | p.R280T        | Missense variant   | SafeSeq        | COSV52677783 | 12368      | Uncertain significance                       | deleterious | probably damaging | pathogenic |
| TP53      | ENST00000269305 | c.991C>T           | p.Q331*        | Nonsense variant   | SafeSeq        | COSV52664188 | 922893     | Pathogenic                                   | -           | -                 | -          |
| TP53      | ENST00000269305 | c.1018A>T          | p.M340L        | Missense variant   | SafeSeq        | NA           | NA         | -  | tolerated   | benign            | pathogenic |
| AKT1      | ENST00000349310 | c.47-30G>A         | p.?            | Missense variant   | SafeSeq        | NA           | NA         | -  | -           | -                 | -          |
| AKT1      | ENST00000349310 | c.49G>A            | p.E17K         | Missense variant   | SafeSeq        | COSV62571334 | 15017      | Pathogenic                                   | -           | -                 | -          |
| ERBB2     | ENST00000269571 | c.937C>G           | p.L313V        | Missense variant   | SafeSeq        | COSV54067264 | NA         | -  | tolerated   | benign            | pathogenic |
| KRAS      | ENST00000311936 | c.34G>T            | p.G12C         | Missense variant   | SafeSeq        | COSV55497469 | 12578      | pathogenic                                   | -           | -                 | -          |
| KRAS      | ENST00000311936 | c.35G>T            | p.G12V         | Missense variant   | SafeSeq        | COSV55497419 | 12583      | pathogenic;likely_pathogenic                 | -           | -                 | -          |