

**Pre-existing immunity drives the response to neoadjuvant chemotherapy
in esophageal adenocarcinoma**

Supplementary Tables

Supplementary Table 1

| Pt N | Tumor Location | Age | Gender | Neoadjuvant protocol | Mandard | Response |
|------|--------------------------|-----|--------|----------------------|---------|----------|
| 12 | MiddleThird | 57 | Male | CROSS | 1 | CR |
| 13 | Distal third | 60 | Male | CROSS | 2 | CR |
| 26 | Esophagogastric junction | 60 | Male | CROSS | 1 | CR |
| 28 | Esophagogastric junction | 68 | Male | CROSS | 2 | CR |
| 30 | Middle-distal third | 77 | Male | CROSS | 1 | CR |
| 31 | Distal third | 66 | Male | CROSS | 2 | CR |
| 45 | Distal third | 53 | Male | CROSS | 2 | CR |
| 25 | Esophagogastric junction | 60 | Male | FLOT | 2 | CR |
| 34 | Esophagogastric junction | 62 | Male | FLOT | 1 | CR |
| 43 | Distal third | 57 | Male | FLOT | 2 | CR |
| 50 | Esophagogastric junction | 60 | Male | FLOT | 2 | CR |
| 54 | Distal third | 63 | Male | FLOT | 2 | CR |
| 5 | Esophagogastric junction | 27 | Male | CROSS | 4 | NR |
| 11 | Esophagogastric junction | 76 | Male | CROSS | 4 | NR |
| 17 | Distal third | 48 | Male | CROSS | 4 | NR |
| 33 | Distal third | 69 | Male | CROSS | 4 | NR |
| 35 | Esophagogastric junction | 75 | Male | CROSS | 4 | NR |
| 36 | Distal third | 54 | Male | CROSS | 4 | NR |
| 40 | Esophagogastric junction | 56 | Male | CROSS | 5 | NR |
| 1 | Esophagogastric junction | 56 | Male | Al-Sarraf | 4 | NR |
| 8 | Esophagogastric junction | 70 | Male | FLOT | 4 | NR |
| 39 | Distal third | 68 | Male | CAPOX | 5 | NR |
| 41 | Distal third | 45 | Male | FLOT | 4 | NR |
| 48 | MiddleThird | 62 | Female | CDDP+5-FU | 5 | NR |
| 55 | Esophagogastric junction | 38 | Male | FLOT | 4 | NR |
| 57 | Esophagogastric junction | 58 | Male | FLOT | 4 | NR |
| 10 | Esophagogastric junction | 48 | Male | CROSS | 3 | PR |
| 18 | Distal third | 56 | Male | CROSS | 3 | PR |
| 20 | Distal third | 59 | Male | CROSS | 3 | PR |
| 24 | Distal third | 56 | Male | CROSS | 3 | PR |
| 27 | MiddleThird | 69 | Female | CROSS | 3 | PR |
| 29 | MiddleThird | 61 | Male | CROSS | 3 | PR |
| 37 | Esophagogastric junction | 65 | Male | CROSS | 3 | PR |
| 15 | Distal third | 40 | Male | FLOT | 3 | PR |

Supplementary Table 1. Clinical characteristics of the EAC patients from the prospective cohort (protocol ESO-CA001)

Table reporting the patient codes, the tumor location (middle third, distal third, esophagogastric junction), the age at diagnosis, the gender, the neoadjuvant protocol acronym, the Mandard score (from 1 to 5), the pathological response category (complete responder=CR, partial responder=PR, non-responder= NR), for the patients enrolled in the protocol ESOCA-001

CROSS: combination of radiotherapy (41.4 Gy in 23 fractions) with carboplatin and paclitaxel; FLOT: combination of 5-fluorouracil, leucovorin, oxaliplatin and docetaxel; Al-Sarraf: combination of cisplatin and 5-fluorouracil; CAPOX: combination of capecitabine and oxaliplatin; FOLFOX: combination of folinic acid, 5-fluorouracil and oxaliplatin; CDDP+5-FU: cisplatin combined with 5-fluorouracil.

Supplementary Table 2

| Pt N | Tumor Location | Age | Gender | Neoadjuvant protocol | Mandard | Response |
|----------|--------------------------|-----|--------|----------------------|---------|----------|
| 51425244 | middle third | 64 | Male | CROSS | 1 | CR |
| 51621040 | esophagogastric junction | 53 | Male | CROSS | 2 | CR |
| 51626341 | esophagogastric junction | 62 | Male | CROSS | 2 | CR |
| 51628573 | distal third | 70 | Male | CROSS | 2 | CR |
| 51625062 | esophagogastric junction | 49 | Male | DCF | 1 | CR |
| 51432036 | distal third | 79 | Male | CROSS | 4 | NR |
| 51508168 | distal third | 75 | Male | CROSS | 4 | NR |
| 51511318 | esophagogastric junction | 64 | Male | CROSS | 4 | NR |
| 51513847 | esophagogastric junction | 39 | Male | CROSS | 5 | NR |
| 51517636 | distal third | 76 | Male | CROSS | 4 | NR |
| 51522996 | distal third | 61 | Male | CROSS | 4 | NR |
| 51609437 | distal third | 75 | Male | CROSS | 4 | NR |
| 51415418 | distal third | 67 | Male | ECF | 4 | NR |
| 51425917 | esophagogastric junction | 42 | Male | ECF | 4 | NR |
| 51428339 | esophagogastric junction | 26 | Male | ECF | 5 | NR |
| 51501054 | esophagogastric junction | 71 | Male | CT | 5 | NR |
| 51505797 | middle third | 48 | Male | TCX | 4 | NR |
| 51521219 | middle third | 55 | Male | TCF | 4 | NR |
| 51527513 | middle third | 59 | Female | CF | 4 | NR |
| 51603569 | distal third | 54 | Male | CF | 4 | NR |
| 51608842 | esophagogastric junction | 65 | Male | TCX | 5 | NR |
| 51629986 | esophagogastric junction | 72 | Male | CF | 4 | NR |
| 51700681 | esophagogastric junction | 62 | Female | CF | 4 | NR |
| 51704026 | esophagogastric junction | 53 | Male | ECF | 4 | NR |
| 51729958 | distal third | 67 | Male | FOLFOX | 5 | NR |
| 51418813 | distal third | 52 | Male | CROSS | 3 | PR |
| 51419455 | esophagogastric junction | 58 | Male | CROSS | 3 | PR |
| 51426513 | esophagogastric junction | 53 | Male | CROSS | 3 | PR |
| 51514406 | distal third | 52 | Male | CROSS | 3 | PR |
| 51518981 | esophagogastric junction | 57 | Male | CROSS | 3 | PR |
| 51607541 | distal third | 56 | Male | CROSS | 3 | PR |
| 51716931 | esophagogastric junction | 60 | Male | CROSS | 3 | PR |
| 51734213 | esophagogastric junction | 69 | Male | CROSS | 3 | PR |
| 51801483 | distal third | 70 | Male | CROSS | 3 | PR |

Supplementary Table 2. Clinical characteristics of the EAC patients from the additional retrospective cohort selected for immunohistochemistry and digital spatial profiling

Table reporting the patient histological code, the tumor location (middle third, distal third, esophagogastric junction), the age at diagnosis, the gender, the neoadjuvant protocol acronym, the Mandard score (from 1 to 5), and the pathological response category (complete responder=CR, partial responder=PR, non-responder= NR).

CROSS: combination of radiotherapy (41.4 Gy in 23 fractions) with carboplatin and paclitaxel; FLOT: combination of 5-fluorouracil, leucovorin, oxaliplatin and docetaxel; FOLFOX: combination of folinic acid, 5-fluorouracil and oxaliplatin; ECF: combination of epirubicin, cisplatin and 5-Fluorouracil; DCF: combination of epirubicin, cisplatin and 5-fluorouracil; TCF: combination of docetaxel, cisplatin and 5-fluorouracil; TCX: combination of docetaxel, cisplatin and capecitabine; CT: combination of cisplatin and docetaxel; CF: combination of cisplatin and 5-fluorouracil.

Supplementary Table 3

| Marker | Clone | Catalogue | Manufacturer | RRID |
|--------|---------|-----------|--------------|-----------------|
| CD3 | LN10 | PA0122 | BOND-LEICA | RRID:AB_2928983 |
| CD4 | SP35 | 790-4423 | VENTANA | RRID:AB_2335982 |
| CD8 | SP57 | 790-4460 | VENTANA | RRID:AB_2335985 |
| CD45RO | UCHL-1 | 790-2930 | VENTANA | RRID:AB_2336014 |
| CD68 | KP-1 | 790-2931 | VENTANA | RRID:AB_2335972 |
| CD163 | MRQ-26 | 760-4437 | VENTANA | RRID:AB_2335969 |
| Foxp3 | 236A/E7 | AB20034 | ABCAM | RRID:AB_445284 |

Supplementary Table 3. Monoclonal antibodies used for immunohistochemistry

Table reporting the antibody target protein, clone, catalogue number, manufacturer and RRID (key Research Resource IDentifiers).

Supplementary Table 4

| Panel N | Marker | Clone | Fluorochrome | Catalogue | Manufacturer | RRID |
|---------|--------|------------|--------------|------------|----------------|------------------|
| 1 | CD3 | UCHT1 | BV510 | 300448 | Biolegend | RRID:AB_2563468 |
| 1 | CD127 | HIL-7R-M21 | PECY7 | 560822 | BD Pharmigen | RRID:AB_2033938 |
| 1 | CD8 | HIT8a | APC-Cy7 | 300926 | Biolegend | RRID:AB_10613636 |
| 1 | CD4 | RPA-T4 | FITC | 300538 | Biolegend | RRID:AB_2562052 |
| 1 | CD25 | BC96 | PE | 302606 | Biolegend | RRID:AB_314276 |
| 1 | CD39 | A1 | PerCP-Cy5.5 | 328217 | Biolegend | RRID:AB_2562896 |
| 1 | CD103 | Ber-ACT8 | APC | 350215 | Biolegend | RRID:AB_2563906 |
| 2 | CD95 | DX2 | BB515 | 564596 | BD Biosciences | RRID:AB_2744470 |
| 2 | CXCR5 | T47-530 | BB660 | 624295 | BD Biosciences | Custom antibody |
| 2 | CD39 | TU66 | BB700 | 745904 | BD Biosciences | RRID:AB_2743331 |
| 2 | TIM-3 | 7D3 | PE | 563422 | BD Biosciences | RRID:AB_2716866 |
| 2 | CD127 | HIL-7R-M21 | PE-CF594 | 562397 | BD Biosciences | RRID:AB_11154212 |
| 2 | PD1 | 2A3 | PECY7 | 561272 | BD Biosciences | RRID:AB_10611585 |
| 2 | KLRG1 | MAFA | APC | 138412 | Biolegend | RRID:AB_10641560 |
| 2 | CD8 | SK1 | APC R700 | 565192 | BD Biosciences | RRID:AB_2739104 |
| 2 | CCR7 | 3D12 | BV480 | 566170 | BD Biosciences | RRID:AB_2739567 |
| 2 | CD103 | Ber-ACT8 | BV605 | 743653 | BD Biosciences | RRID:AB_2741655 |
| 2 | TIGIT | 741182 | BV650 | 747840 | BD Biosciences | RRID:AB_2872303 |
| 2 | CD28 | CD28.2 | BV711 | 563131 | BD Biosciences | RRID:AB_2738020 |
| 2 | CD69 | FN50 | BV786 | 563834 | BD Biosciences | RRID:AB_2738441 |
| 2 | CD137 | 4B4-1 | BUV395 | 745737 | BD Biosciences | RRID:AB_2743209 |
| 2 | CD3 | SK7 | BUV563 | 741448 | BD Biosciences | RRID:AB_2870922 |
| 2 | CD4 | SK3 | BUV661 | 566003 | BD Biosciences | RRID:AB_2739452 |
| 2 | LAG3 | RF8B2 | BUV737 | 624286 | BD Biosciences | Custom antibody |
| 2 | CD27 | M-T271 | BUV805 | 742012 | BD Biosciences | RRID:AB_2871310 |
| 2 | CD45RO | UCHL1 | APC-H7 | 561137 | BD Biosciences | RRID:AB_10562194 |
| 2 | CD25 | EH12.1 | BV421 | 564033 | BD Biosciences | RRID:AB_2738555 |
| 3 | CD45RO | UCHL1 | APC-H7 | 561137 | BD Biosciences | RRID:AB_10562194 |
| 3 | CD25 | EH12.1 | BV421 | 564033 | BD Biosciences | RRID:AB_2738555 |
| 3 | GATA3 | L50-823 | AF488 | 560077 | BD Biosciences | RRID:AB_1645303 |
| 3 | RORgt | Q21-559 | BB630 | 644294 | BD Biosciences | Custom antibody |
| 3 | CD4 | RPA-T4 | PerCP-Cy5.5 | 300530 | Biolegend | RRID:AB_893322 |
| 3 | CD127 | HIL-7R-M21 | PE | 557938 | BD Biosciences | RRID:AB_2296056 |
| 3 | EOMES | WD1928 | PE-CF594 | 61-4877-42 | e-Bioscience | RRID:AB_2574616 |
| 3 | Tbet | 4B10 | PECY7 | 25-5825-82 | e-Bioscience | RRID:AB_11042699 |
| 3 | Foxp3 | 259D/C7 | AF647 | 560045 | BD Biosciences | RRID:AB_1645411 |
| 3 | CD3 | UCHT1 | AF700 | 300424 | Biolegend | RRID:AB_493741 |
| 4 | CD45 | HI30 | FITC | 304006 | Biolegend | RRID:AB_314394 |
| 4 | CD15 | HI98 | PerCP-Cy5.5 | 560828 | BD Biosciences | RRID:AB_10563612 |
| 4 | CD1c | L161 | PE | sc-18886 | Santa cruz | RRID:AB_626959 |
| 4 | CD64 | 10.1 | PE-CF594 | 565389 | BD Biosciences | RRID:AB_2739213 |
| 4 | CD19 | HIB19 | PE-Cy5 | 560993 | BD Biosciences | RRID:AB_10562389 |
| 4 | CCR7 | 3D12 | PECY7 | 25-1979-42 | e-Bioscience | RRID:AB_2573422 |
| 4 | CD123 | 7G3 | APC | 560087 | BD Biosciences | RRID:AB_1645454 |
| 4 | CD11c | 3.9 | APC R700 | 566610 | BD Biosciences | RRID:AB_2869792 |
| 4 | HLA_DR | G46-6 | APC-H7 | 561358 | BD Biosciences | RRID:AB_10611876 |
| 4 | CD16 | 3G8 | BV421 | 562878 | BD Biosciences | RRID:AB_2737861 |
| 4 | CD33 | WM53 | BV510 | 563257 | BD Biosciences | RRID:AB_2738102 |
| 4 | CD303 | V24-785 | BV605 | 748004 | BD Biosciences | RRID:AB_2872465 |
| 4 | PDL1 | B7-H1 | BV650 | 563740 | BD Biosciences | RRID:AB_2738398 |
| 4 | CD141 | 1A4 | BV711 | 563155 | BD Biosciences | RRID:AB_2738033 |
| 4 | CD86 | 2331 | BV750 | 747383 | BD Biosciences | RRID:AB_2872076 |
| 4 | CD56 | NCAM16.2 | BV786 | 564058 | BD Biosciences | RRID:AB_2738569 |
| 4 | CD14 | MFP9 | BUV395 | 563562 | BD Biosciences | RRID:AB_2744288 |
| 4 | CD3 | SK7 | BUV496 | 612940 | BD Biosciences | RRID:AB_2870222 |
| 4 | CD54 | HA58 | BUV563 | 741374 | BD Biosciences | RRID:AB_2870873 |
| 4 | CD38 | HIT2 | BUV661 | 612970 | BD Biosciences | RRID:AB_2916888 |
| 4 | CD163 | GHI/61 | BUV737 | 741863 | BD Biosciences | RRID:AB_2871193 |
| 4 | CD11b | ICRF44 | BUV805 | 742004 | BD Biosciences | RRID:AB_2871303 |

Supplementary Table 4. Monoclonal antibodies used for flow cytometry staining

Table reporting the panel number (indicated in the main text), the antibody target protein, clone, fluorochrome conjugate, catalogue number, manufacturer and RRID (key Research Resource Identifiers) or alternatively the indication as Custom antibody.

Supplementary Table 5

| Pt N | Hugo Symbol | Variant Class | HGVSc | HGVSp | IMPACT | COSMIC ID |
|------|-------------|-------------------|----------------|--------------------|----------|---------------|
| 8 | ANAPC1 | Missense Mutation | c.4922C>G | p.Thr1641Ser | MODERATE | n.a. |
| 17 | AREL1 | 5'UTR | c.-505G>A | n.a. | MODIFIER | n.a. |
| 27 | ASB7 | Missense Mutation | c.11A>C | p.His4Pro | MODERATE | n.a. |
| 20 | BTBD1 | Missense Mutation | c.364C>T | p.Pro122Ser | MODERATE | n.a. |
| 48 | CANX | Missense Mutation | c.913G>A | p.Asp305Asn | MODERATE | n.a. |
| 11 | CBLB | 3'UTR | c.-505A>G | n.a. | MODIFIER | n.a. |
| 35 | CBL2 | Missense Mutation | c.526C>A | p.His176Asn | MODERATE | n.a. |
| 48 | CDC23 | Missense Mutation | c.1087C>T | p.Arg363Trp | MODERATE | COSV105334329 |
| 40 | CDC27 | 3'UTR | c.*102C>A | n.a. | MODIFIER | n.a. |
| 41 | CUL1 | 5'UTR | c.1674+24C>T | n.a. | MODIFIER | n.a. |
| 17 | DYNC1H1 | Nonsense Mutation | c.11182C>T | p.Arg3728Ter | HIGH | n.a. |
| 26 | FBXW10 | 3'UTR | c.*21A>G | n.a. | MODIFIER | COSV57059706 |
| 11 | HECTD1 | 3'UTR | c.203T>C | n.a. | MODIFIER | n.a. |
| 8 | HECTD2 | 3'UTR | c.*105A>G | n.a. | MODIFIER | n.a. |
| 29 | HERC1 | Missense Mutation | c.2330C>A | p.Pro777His | MODERATE | n.a. |
| 40 | HERC1 | Missense Mutation | c.7675A>C | p.Met2559Leu | MODERATE | n.a. |
| 41 | HERC1 | Missense Mutation | c.9034C>T | p.Arg3012Cys | MODERATE | COSV71247403 |
| 33 | HERC6 | Frame Shift Del | c.1277_1278del | p.Gly426AspfsTer8 | HIGH | n.a. |
| 41 | HSPA5 | Missense Mutation | c.1322C>T | p.Thr441Ile | MODERATE | n.a. |
| 57 | HSPA5 | Missense Mutation | c.1219G>A | p.Gly407Ser | MODERATE | n.a. |
| 34 | HUWE1 | Missense Mutation | c.11008C>G | p.Gln3670Glu | MODERATE | n.a. |
| 48 | HUWE1 | Missense Mutation | c.5491C>A | p.Pro1831Thr | MODERATE | n.a. |
| 33 | KLHL11 | 3'UTR | c.105_109del | n.a. | MODIFIER | n.a. |
| 8 | KLHL41 | 3'UTR | c.*148del | n.a. | MODIFIER | n.a. |
| 33 | LY96 | Frame Shift Del | c.438del | p.Cys148AlafsTer17 | HIGH | n.a. |
| 48 | MRC1 | Missense Mutation | c.343T>A | p.Leu115Ile | MODERATE | n.a. |
| 34 | MYD88 | Missense Mutation | c.170A>T | p.Glu57Val | MODERATE | n.a. |
| 15 | PJA1 | Nonsense Mutation | c.157G>T | p.Glu53Ter | HIGH | n.a. |
| 25 | PRKN | 3'UTR | c.*50T>C | n.a. | MODIFIER | n.a. |
| 24 | PSMB3 | Missense Mutation | c.111G>T | p.Gln37His | MODERATE | n.a. |
| 8 | PSMB9 | Missense Mutation | c.133G>T | p.Ala45Ser | MODERATE | n.a. |
| 55 | PSMC6 | 3'UTR | c.442-40C>T | n.a. | MODIFIER | n.a. |
| 35 | PSMD1 | Missense Mutation | c.2641C>A | p.Pro881Thr | MODERATE | n.a. |
| 33 | RBBP6 | Missense Mutation | c.5167A>G | p.Ser1723Gly | MODERATE | n.a. |
| 18 | RLIM | 3'UTR | c.*8383T>G | n.a. | MODIFIER | n.a. |
| 20 | RLIM | 3'UTR | c.*8406A>T | n.a. | MODIFIER | n.a. |
| 35 | RNF213 | Missense Mutation | c.15209C>T | p.Ala5070Val | MODERATE | n.a. |
| 35 | RNF217 | Missense Mutation | c.182C>A | p.Thr61Lys | MODERATE | n.a. |
| 55 | SEC23A | 3'UTR | c.1398+3A>C | n.a. | LOW | n.a. |
| 30 | SEC24C | Missense Mutation | c.1265C>G | p.Pro422Arg | MODERATE | n.a. |
| 29 | TAPBP | Missense Mutation | c.449C>T | p.Ala150Val | MODERATE | n.a. |
| 33 | TLR6 | Frame Shift Del | c.1036del | p.Met346CysfsTer13 | HIGH | n.a. |
| 8 | TRIM32 | Nonsense Mutation | c.1060C>T | p.Gln354Ter | HIGH | n.a. |
| 39 | TRIM37 | Nonsense Mutation | c.58G>T | p.Glu20Ter | HIGH | n.a. |
| 35 | TRIM69 | Missense Mutation | c.967T>C | p.Ser323Pro | MODERATE | n.a. |
| 39 | TRIM71 | Missense Mutation | c.583C>T | p.Arg195Cys | MODERATE | n.a. |
| 27 | TRIM9 | Missense Mutation | c.1576A>C | p.Lys526Gln | MODERATE | n.a. |
| 57 | TRIM9 | Missense Mutation | c.266C>A | p.Thr89Asn | MODERATE | n.a. |
| 17 | TRIP12 | Missense Mutation | c.4022G>A | p.Arg1341Lys | MODERATE | n.a. |
| 20 | UBE2D4 | 3'UTR | c.*65del | n.a. | MODIFIER | n.a. |
| 27 | UBE2J2 | Nonsense Mutation | c.158G>A | p.Trp53Ter | HIGH | n.a. |
| 33 | UBE2J2 | In Frame Del | c.109_111del | p.Cys37del | MODERATE | n.a. |
| 27 | UBE3B | Missense Mutation | c.2904G>C | p.Glu968Asp | MODERATE | n.a. |
| 8 | WWP1 | Missense Mutation | c.293C>T | p.Thr98Met | MODERATE | COSV55359464 |

Supplementary Table 5. Baseline mutation in genes within the Reactome MHC class I antigen processing in EAC tumors from the prospective cohort

Table reporting the code for the patients enrolled in the protocol ESO-CA001, the gene Hugo Symbol, the mutation category as referred on main Figure 1D, the mutated nucleotides, the mutated amino acids, the predicted impact (high, moderate, modifier, low), and the ID for mutations present in the Catalogue of somatic mutations in cancer (COSMIC). n.a.: not available

Supplementary Table 6

| Pt N | Hugo Symbol | Variant Class | HGVSc | HGVSp | IMPACT | COSMIC ID |
|------|-------------|-------------------|----------------|---------------|----------|--------------|
| 29 | ABC1 | 3'UTR | c.*10G>A | n.a. | MODIFIER | n.a. |
| 18 | APC | Nonsense Mutation | c.4588G>T | p.E1530* | HIGH | COSV57335138 |
| 55 | APC | Missense Mutation | c.4139C>A | p.T1380N | MODERATE | n.a. |
| 57 | APC | Missense Mutation | c.414G>T | p.E138D | MODERATE | n.a. |
| 27 | ARID1A | Nonsense Mutation | c.199C>T | p.Q67* | HIGH | n.a. |
| 27 | ARID1A | Missense Mutation | c.6200T>G | p.L2067S | MODERATE | n.a. |
| 31 | ARID1A | Missense Mutation | c.6167T>C | p.L2056P | MODERATE | n.a. |
| 8 | ARID1A | Frame Shift Del | c.4693del | p.T1565Qfs*47 | HIGH | n.a. |
| 57 | ARID1B | Missense Mutation | c.5602G>A | p.D1868N | MODERATE | COSV99271200 |
| 8 | ARID1B | Frame Shift Del | c.6610del | p.D2204ifs*24 | HIGH | n.a. |
| 54 | ARID2 | Nonsense Mutation | c.3926C>G | p.S1309* | HIGH | COSV57594609 |
| 33 | CDH1 | Missense Mutation | c.2603G>A | p.R868H | MODERATE | COSV55741662 |
| 8 | CDH1 | In Frame Del | c.38_46del | p.L13_L15del | MODERATE | n.a. |
| 34 | CDKN2A | In Frame Del | c.220_222del | p.D74del | MODERATE | n.a. |
| 20 | CDKN2A | Nonsense Mutation | c.358G>T | p.E120* | HIGH | COSV58683444 |
| 24 | CDKN2A | Frame Shift Del | c.225_243del | p.A76Cfs*64 | HIGH | COSV58696968 |
| 27 | CDKN2A | Missense Mutation | c.188T>A | p.L63Q | MODERATE | COSV58694651 |
| 8 | CDKN2A | Missense Mutation | c.247C>T | p.H83Y | MODERATE | COSV58682852 |
| 35 | CHD4 | Missense Mutation | c.1801C>T | p.R601C | MODERATE | COSV58907163 |
| 30 | EPHA2 | Frame Shift Del | c.44del | p.G15Afs*40 | HIGH | n.a. |
| 54 | EPHA2 | Frame Shift Ins | c.729_730dup | p.R244Pfs*150 | HIGH | n.a. |
| 45 | EPHA3 | Missense Mutation | c.2716A>G | p.S906G | MODERATE | n.a. |
| 57 | EPHA3 | Missense Mutation | c.569T>C | p.V190A | MODERATE | n.a. |
| 30 | ERBB2 | Missense Mutation | c.929C>T | p.S310F | MODERATE | COSV54062198 |
| 48 | ERBB2 | Missense Mutation | c.2329G>T | p.V777L | MODERATE | COSV54062767 |
| 8 | FGFR1 | Missense Mutation | c.1802A>G | p.E601G | MODERATE | n.a. |
| 15 | KCNQ3 | Missense Mutation | c.1064T>A | p.L355Q | MODERATE | n.a. |
| 20 | LIN7A | Missense Mutation | c.573A>T | p.K191N | MODERATE | n.a. |
| 24 | LIN7A | Missense Mutation | c.115G>T | p.V39L | MODERATE | n.a. |
| 20 | MAP3K1 | Nonsense Mutation | c.4027G>T | p.E1343* | HIGH | COSV68127321 |
| 12 | MET | Missense Mutation | c.4002G>A | p.M1334I | MODERATE | n.a. |
| 54 | MUC6 | Frame Shift Del | c.6424_6427del | p.S2142Lfs*33 | HIGH | n.a. |
| 26 | NAV3 | Missense Mutation | c.980G>T | p.S327I | MODERATE | n.a. |
| 41 | NAV3 | Missense Mutation | c.2203A>G | p.S735G | MODERATE | COSV99892697 |
| 27 | NIPBL | Missense Mutation | c.2248A>G | p.K750E | MODERATE | n.a. |
| 18 | NOTCH1 | Nonsense Mutation | c.4497C>A | p.Y1499* | HIGH | COSV53097144 |

| Pt N | Hugo Symbol | Variant Class | HGVSc | HGVSp | IMPACT | COSMIC ID |
|------|-------------|-------------------|--------------|--------------|----------|---------------|
| 17 | NOTCH1 | Missense Mutation | c.1070T>C | p.F357S | MODERATE | COSV530373359 |
| 35 | PBRM1 | Missense Mutation | c.638C>T | p.S213F | MODERATE | n.a. |
| 30 | PCDH17 | Missense Mutation | c.374C>G | p.T125S | MODERATE | n.a. |
| 8 | PCDH17 | Missense Mutation | c.2968G>A | p.V990M | MODERATE | n.a. |
| 27 | POLQ | Nonsense Mutation | c.4289T>A | p.L1430* | HIGH | COSV51750601 |
| 45 | PTEN | Missense Mutation | c.105G>T | p.M35I | MODERATE | COSV64295455 |
| 8 | RNF43 | Frame Shift Ins | c.1976dup | p.P660Sfs*87 | HIGH | COSV68456721 |
| 34 | SMARCA4 | Missense Mutation | c.4060G>T | p.V1354L | MODERATE | n.a. |
| 39 | STK11 | Frame Shift Del | c.545_567del | p.L182Pfs*76 | HIGH | n.a. |
| 17 | TGFBR2 | Missense Mutation | c.923T>C | p.L308P | MODERATE | COSV55454008 |
| 8 | TGFBR2 | Missense Mutation | c.1555G>A | p.E519K | MODERATE | COSV55442699 |
| 12 | TP53 | Missense Mutation | c.713G>A | p.C238Y | MODERATE | COSV52661646 |
| 25 | TP53 | Frame Shift Del | c.801del | p.N268Tfs*77 | HIGH | COSV52740807 |
| 30 | TP53 | Nonsense Mutation | c.592G>T | p.E198* | HIGH | COSV52678088 |
| 31 | TP53 | Missense Mutation | c.853G>A | p.E285K | MODERATE | COSV52661732 |
| 34 | TP53 | Missense Mutation | c.844C>T | p.R282W | MODERATE | COSV52662048 |
| 45 | TP53 | Missense Mutation | c.725G>A | p.C242Y | MODERATE | COSV52661189 |
| 54 | TP53 | Missense Mutation | c.578A>G | p.H193R | MODERATE | COSV52662414 |
| 15 | TP53 | Missense Mutation | c.731G>T | p.G244V | MODERATE | COSV52724858 |
| 18 | TP53 | Missense Mutation | c.818G>A | p.R273H | MODERATE | COSV52660980 |
| 20 | TP53 | Missense Mutation | c.742C>T | p.R248W | MODERATE | COSV52662035 |
| 24 | TP53 | Missense Mutation | c.332T>C | p.L111P | MODERATE | COSV52985195 |
| 27 | TP53 | Missense Mutation | c.536A>G | p.H179R | MODERATE | COSV52661712 |
| 29 | TP53 | Nonsense Mutation | c.687T>A | p.C229* | HIGH | COSV52903842 |
| 33 | TP53 | Nonsense Mutation | c.1024C>T | p.R342* | HIGH | COSV52665487 |
| 39 | TP53 | Missense Mutation | c.722C>T | p.S241F | MODERATE | COSV52661688 |
| 48 | TP53 | Missense Mutation | c.811G>A | p.E271K | MODERATE | COSV52700326 |
| 55 | TP53 | Missense Mutation | c.527G>T | p.C176F | MODERATE | COSV52661329 |
| 57 | TP53 | Missense Mutation | c.722C>T | p.S241F | MODERATE | COSV52661688 |
| 8 | TP53 | Nonsense Mutation | c.637C>T | p.R213* | HIGH | COSV52665560 |
| 37 | TRPA1 | Missense Mutation | c.2210T>C | p.V737A | MODERATE | n.a. |
| 12 | TSHZ3 | Missense Mutation | c.2332G>A | p.D778N | MODERATE | COSV53675488 |
| 24 | TSHZ3 | Missense Mutation | c.434A>C | p.K145T | MODERATE | n.a. |
| 25 | ZFHX3 | Missense Mutation | c.6314T>C | p.M2105T | MODERATE | n.a. |
| 18 | ZFHX3 | Missense Mutation | c.6311C>T | p.T2104M | MODERATE | n.a. |

Supplementary Table 6. Baseline mutation in genes within the EAC Driver Genes in EAC tumors from the prospective cohort

Table reporting the patient code for the patients enrolled in the protocol ESOCA-001, the gene Hugo Symbol, the mutation category as referred in Supplementary Figure 2G, the mutated nucleotides, the mutated amino acids, the predicted impact (high, moderate, modifier, low), and the ID for mutations present in the Catalogue of somatic mutations in cancer (COSMIC). n.a.: not available