



Supplementary Material

Supplementary Figure. (A) HPV-positive UPCISCC90 and HPV-negative UMSCC23 cell lines were harvested and whole cells extracts were subjected to anti-UBC9 immunoprecipitation (IP). Immunoblotting with the indicated antibodies was then performed and whole cell extracts were used as input controls. (B) HPV-positive 93VU147T cell line was harvested and whole cells extracts were subjected to anti-SUMO1 or SUMO2 immunoprecipitation (IP). Immunoblotting with the indicated antibodies was then performed and whole cell extracts were used as input controls. (C) HPV-positive 93VU147T and HPV-negative UMSCC19 were transfected for 72h with specific siRNA against SUMO1 or Luciferase as control. After transfection total RNAs were isolated for RT-qPCR and SUMO1 expression was normalized to RpP0 and shown as means \pm SD. (D, E) UMSCC10A cell line was transduced with empty or HPV16E6/E7 recombinant retroviral vectors. After selection with G418 cells were transfected with specific siRNA against E-cadherin or Luciferase as control. 72h after transfection total RNAs were isolated for RT-qPCR and reported as means ±SD of fold changes. (F) Human Keratinocytes (HK) were transduced with empty or HPV16E6/E7 recombinant retroviral vectors. After selection with G418 cells were seeded on coverslips and after 24h fixed and stained with the indicated antibodies for Immunofluorescence. Coverslips were mounted and visualized using a Leica SP8 AOBS confocal microscopy. E-cadherin (green) and β-catenin (red) colocalization was evaluated in 4 different images and measured by Mander's coefficient (M1) as shown in the graph. *, P < 0.05 (unpaired t test).

| CELL LINE | HPV STATUS | SUBSITE | SEX | ORIGIN |
|------------|------------|-------------|--------|---|
| UMSCC4 | Negative | Oropharynx | Female | (Brenner et al., 2010) (Zhao et al., 2010) |
| UMSCC6 | Negative | Oropharynx | Male | (Brenner et al., 2010) (Zhao et al., 2010) |
| UMSCC10A | Negative | Larynx | Male | (Brenner et al., 2010) (Zhao et al., 2010) |
| UMSCC17A | Negative | Larynx | Female | (Carey <i>et al.</i> , 1989) |
| UMSCC17B | Negative | Larynx | Female | (Carey <i>et al.</i> , 1989) |
| UMSCC19 | Negative | Oropharynx | Male | (Brenner et al., 2010), (Zhao et al., 2010) |
| UMSCC23 | Negative | Larynx | Female | (Brenner <i>et al.</i> , 2010) |
| UDSCC2 | Positive | Hypopharynx | Male | (Balló <i>et al.</i> , 1999) |
| UMSCC47 | Positive | Oropharynx | Male | (Brenner et al., 2010), (Zhao et al., 2010) |
| UPCISCC90 | Positive | Oropharynx | Male | (Ragin, Reshmi and Gollin, 2004) |
| UMSCC104 | Positive | Oral cavity | Male | (Tang <i>et al.</i> , 2011) |
| UPCISCC152 | Positive | Hypopharynx | Male | (Kalu et al., 2017), (White et al., 2007) |
| UPCISCC154 | Positive | Oral cavity | Male | (White <i>et al.</i> , 2007) |
| 93VU147T | Positive | Oral cavity | Male | (Steenbergen et al., 1995) |

Supplementary Table1. List of used HNC cell lines.

| antibody | Company | Clone |
|-----------------|---------------------------|-----------------------|
| anti-UBC9 | Novus Biologicals | Catalog n: NBP1-86887 |
| anti-E-cadherin | Cell Signaling Technology | Catalog n: 3195 |
| anti-GAPDH | Abcam | 6C5 |
| anti-SUMO1 | SIGMA | Catalog n: S8070 |
| anti-vinculin | SIGMA | hVIN1 |
| anti-tubulin | SIGMA | DM 1A |
| anti-actin | Novus Biologicals | AC-15 |
| anti-E7(HPV16) | Santa Cruz Biotechnology | NM2 |
| anti-ubiquitin | Santa Cruz Biotechnology | P4D1 |
| anti-p53 | Santa Cruz Biotechnology | DO-1 |
| anti-β-catenin | BD Bioscences | 14/Beta-Catenin |

Supplementary Table2. List of used antibodies.

References

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