Supplementary figure 1 (Fig. S1) Methylation levels in relation to HPV status. Boxplots of DNA methylation levels relative to a reference gene ACTB (log2-transformed ΔΔCq ratios; y-axis) in relation to HPV status in the different histological categories of anal tissue samples of HIV-negative women and men combined (x-axis) for six markers: ASCL1, LHX8, SST, WDR17, ZIC1 and ZNF582. HPV status: positive for ≥1 HPV type in the WTS belonging to hierarchical (from low to high) risk classes (HPV-negative, hrHPV-positive, lrHPV other (non-HPV16 hrHPV-positive), HPV16-positive) [29, 30]. The box of the boxplots bounds the interquartile range (IQR) divided by the median and Tukey-style whiskers extend to a maximum of 1.5 IQR beyond the box. Differences between HPV status groups per histological categories are only visualised upon a significant Kruskal-Wallis omnibus test result and followed by post hoc testing using the Mann-Whitney U-test and Bonferroni multiple testing correction. * P < 0.05, ns: nonsignificant, # P < 0.05 on Kruskal-Wallis test but individual comparison using pairwise Mann-Whitney U-tests with Bonferroni correction were nonsignificant. Normal, normal control samples; AIN, anal intraepithelial neoplasia (grades 1-3); HPV, human papillomavirus; HPV-neg, HPV-negative; hrHPV, high-risk HPV; lrHPV, low-risk HPV; SCC, squamous cell carcinoma; transf., transformed; WTS, whole tissue section.

| Marker | Normal | AIN1 | AIN2 | AIN3 | SCC |
|--------|--------|------|------|------|-----|
| ASCL1  | 17     | 13   | 0    | 0    | 0   |
| LHX8   | 3      | 48   | 3    | 3    | 3   |
| SST    | 1      | 4    | 4    | 12   | 4   |
| WDR17  | 0      | 6    | 3    | 19   | 3   |
| ZIC1   | 7      | 2    | 3    | 28   | 3   |
| ZNF582 | 1      | 4    | 1    | 4    | 12  |