



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 $\Delta\Delta Cq$ 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, lrHPV-positive, hrHPV 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.

	HPV-neg	lrHPV	hrHPV other	HPV 16
Normal	17	13	0	0
AIN1	3	48	3	3
AIN2	1	4	4	12
AIN3	0	6	3	19
SCC	7	2	3	28