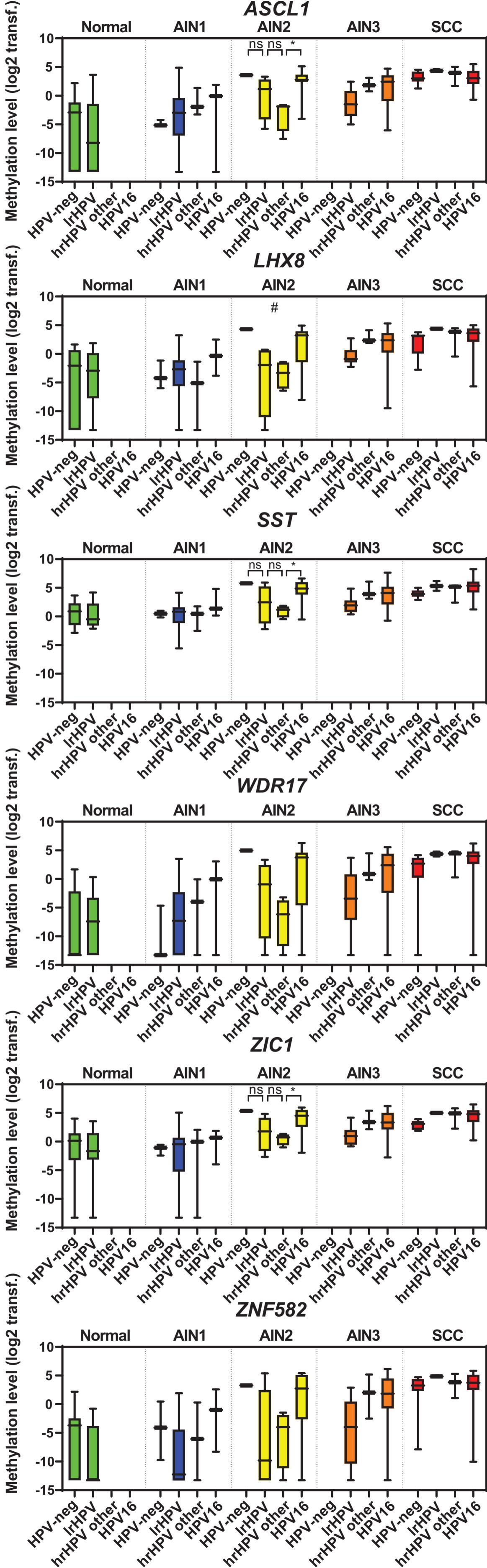


**Supplementary figure 1 (Fig. S1)**  
**Methylation levels in relation to HPV status.** Boxplots of DNA methylation levels relative to a reference gene *ACTB* (log<sub>2</sub>-transformed  $\Delta\Delta Cq$  ratios; y-axis) in relation to HPV status in the different histological categories of anal tissue samples of HIV-negative women and men combined (x-axis) for six markers: *ASCL1*, *LHX8*, *SST*, *WDR17*, *ZIC1* and *ZNF582*. HPV status: positive for  $\geq 1$  HPV type in the WTS belonging to hierarchical (from low to high) risk classes (HPV-negative, IrHPV-positive, hrHPV other (non-HR HPV-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; IrHPV, low-risk HPV; SCC, squamous cell carcinoma; transf., transformed; WTS, whole tissue section.



	HPV-	hrHPV	HPV	
n	neg	IrHPV	other	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