

## Supplementary Information

# **Distinct immune evasion in APOBEC-enriched, HPV-negative HNSCC**

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**Fig. S2:** The HPV-negative, APOBEC-enriched group shows higher predicted contributions of COSMIC SBS signatures 2 and 13 compared to the rest of the HPV-negative samples. Further, comparing all signatures within a sample, signatures 2 and 13 rank higher in the HPV-negative, APOBEC-enriched group.

**Fig. S3:** Survival analysis (OSS) of HPV-negative APOBEC-enriched (red) vs. APOBEC-negative (blue) cases did not show a significant difference between the groups ( $p = 0.63$ ).

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**Fig. S5:** An increase in other established immune population signatures (metagenes) including effector-memory CD8+ T-cells ( $p = 0.004$ ), MDSC ( $p = 0.0014$ ) and type 17 T-helper cells ( $p = 0.0014$ ) was seen in HPV-negative, APOBEC-enriched samples compared to HPV-negative APOBEC-negative cases.

**Fig. S6:** IFNG signature score in lung adenocarcinoma (LUAD) and bladder urothelial carcinoma (BLCA) for each group of APOBEC3 activity timing. Inflammation scores were not different in BLCA but significantly lower in LUAD cases with putative early APOBEC3 activation. Group sizes in the figure title correspond to x-axis labels, left to right.

**Fig. S7:** Gene expression (RSEM estimates) of APOBEC genes revealed increased expression of APOBEC3A among HPV-negative, APOBEC-enriched samples. P-values were adjusted for multiple testing between groups per gene (Benjamini-Hochberg), but not between genes.

**Fig S8:** Mutational profile of the HPV-negative case (patient 5) with enrichment for APOBEC activity in DTK Master cohort.

Table S1: Mapping of gene symbols to ENSEMBL ids for the IFNG signature (via ENSEMBL biomaRt).

Gene stable ID	Gene symbol
ENSG00000111537	IFNG
ENSG00000115415	STAT1
ENSG00000131203	IDO1
ENSG00000138755	CXCL9
ENSG00000169245	CXCL10
ENSG00000204287	HLA-DRA
ENSG00000206308	HLA-DRA
ENSG00000226260	HLA-DRA
ENSG00000227993	HLA-DRA
ENSG00000228987	HLA-DRA
ENSG00000230726	HLA-DRA
ENSG00000234794	HLA-DRA
ENSG00000277263	HLA-DRA

Table S2: Among HPV-negative samples, APOBEC-negative samples showed significant enrichment in the Inflamed/Mesenchymal gene expression subgroup as defined by Keck et al. ( $p = 0.018$ ).

	Basal	Classical	Inflamed/ Mesenchymal
HPV-positive	1	13	22
HPV-negative, APOBEC-enriched	14	6	19
HPV-negative, APOBEC-negative	90	59	52

Table S3: Top hits in genes from analysis in Table 1. Mutations in HLA-A showed the highest enrichment in APOBEC+ cases and remained significant after correcting for multiple testing (Benjamini-Hochberg).

Gene	p.adj	p	gene_mut_in_APOBEC-enriched	gene_mut_in_APOBEC-negative
HLA-A	0,01153211	5.56E-06	20	16
CASP8	0,23094184	2.23E-04	22	28
HLA-B	0,24745962	1.11E-03	11	10
ODF2	0,24745962	1.31E-03	8	5
SIK3	0,24745962	8.95E-04	7	3
KDM4B	0,24745962	4.20E-04	9	5

Table S4: Smokers were significantly underrepresented in the HPV-negative, APOBEC-enriched group ( $p = 0.01964$ ).

tobacco	HPV-negative/APOBEC-enriched	HPV-negative/APOBEC-negative
no	45	137
yes	39	211

Table S5: Tumor sites show a different distribution in HPV-negative, APOBEC-enriched samples ( $p = 9.999e-05$ ).

	HPV-negative/APOBEC-enriched	HPV-negative/APOBEC-negative
Alveolar Ridge	9	5
Base of tongue	2	8
Buccal Mucosa	6	16
Floor of mouth	11	48
Hard Palate	4	2
Hypopharynx	4	4
Larynx	10	98
Lip	0	3
Oral Cavity	22	44
Oral Tongue	16	105
Oropharynx	0	8
Tonsil	0	7

Table S6: Number of C>T mutations inside and outside the TCW motif with respective APOBEC enrichment p-value, IFNG signature score and expression pseudocounts of the genes in Supplementay table S1 for the DKTK Master cohort.

patient	inTCW	outTCW	APOBEC _enriched _p_adj	IFNG_ sig	ENSG0 0000111 537	ENSG0 0000115 415	ENSG0 0000131 203	ENSG0 0000138 755	ENSG0 0000169 245	ENSG0 0000204 287	ENSG0 0000206 308	ENSG0 0000226 260	ENSG0 0000227 993	ENSG00 0002289 87
patient1	54	380	1	3.24	0.21	19.66	0.39	1.36	3.92	30.89	130.49	29.62	65.9	0.69
patient2	72	437	1	3.91	0.05	95.18	5.05	12.7	15.12	0.2	109.96	350.82	0.2	76.41
patient3	40	211	1	4.44	3.27	141.13	153.06	167.08	151	0.52	1620.1	0	0.19	2.4
patient4	46	154	1	3.39	0.15	186.36	8.38	4.94	62.04	144.01	0	0.12	131.88	0
patient5	247	550	1.2E-10	5.92	1.81	357.49	149.66	138.26	222.02	146.14	0.09	1003.57	524.11	0.62
patient6	84	436	1	4.85	0	35.54	19.94	1.76	69.97	908.29	55.62	28.69	942.75	0.85
patient7	87	521	1	5.68	2.28	204.48	52.27	115.76	101.72	0.8	1163.08	66.05	1119.15	0.87
patient8	14	136	1	2.85	0.02	347.23	388.4	1.22	0.98	24.85	23.44	0	0	0
patient9	56	502	1	3.79	2.52	160.44	10.8	161.83	135.94	666.75	0	0.53	0.62	0.07
patient10	21	182	1	1.45	0.15	6.4	0.24	0.31	4.3	0.3	200.86	0	0.11	0.11

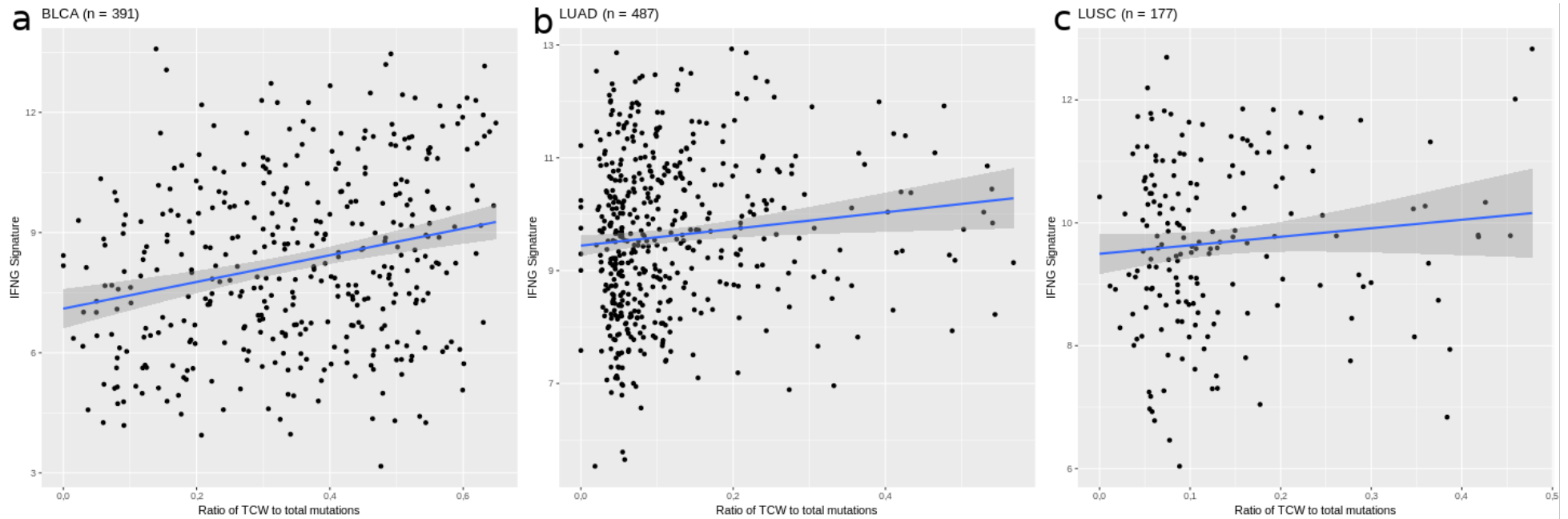


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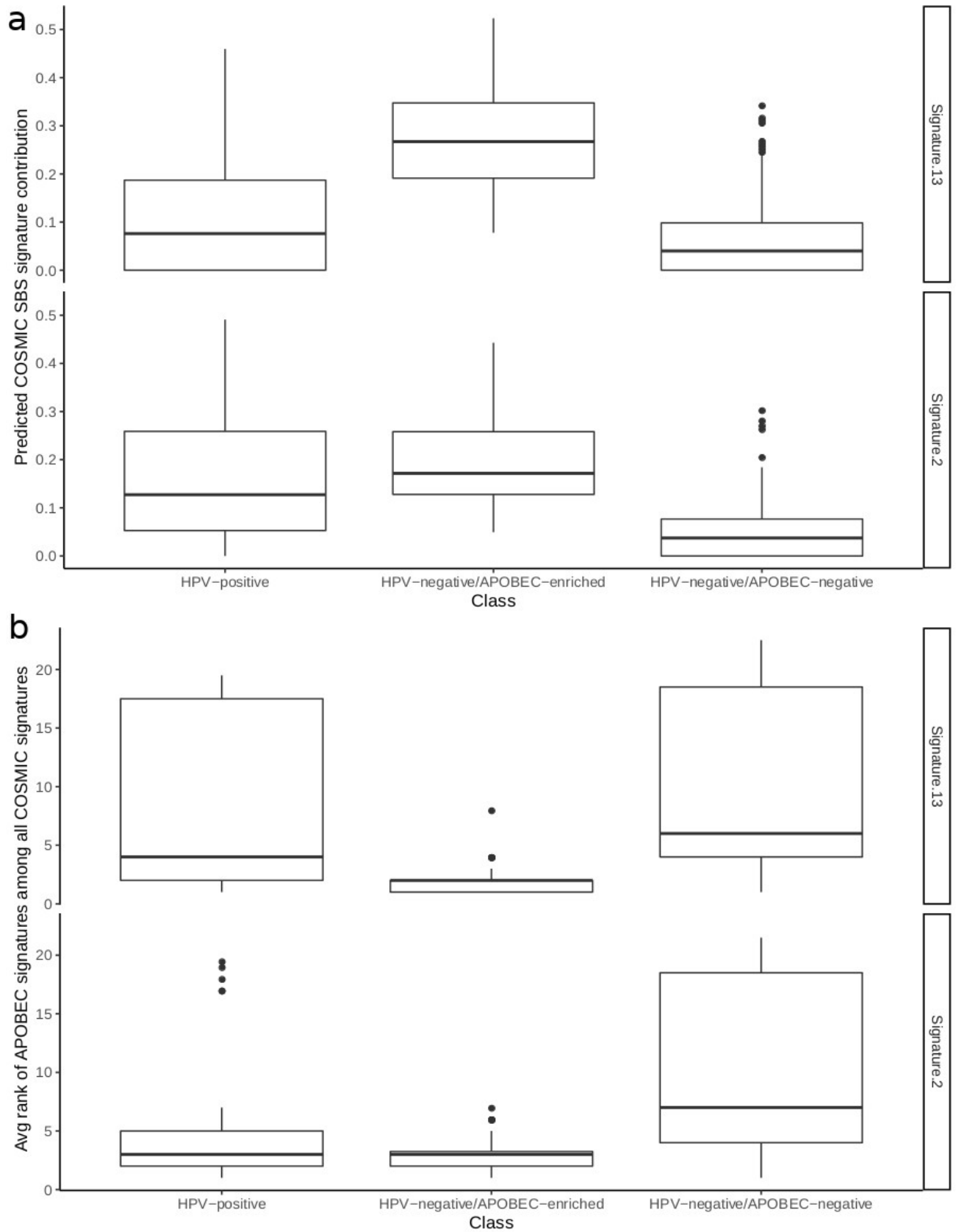


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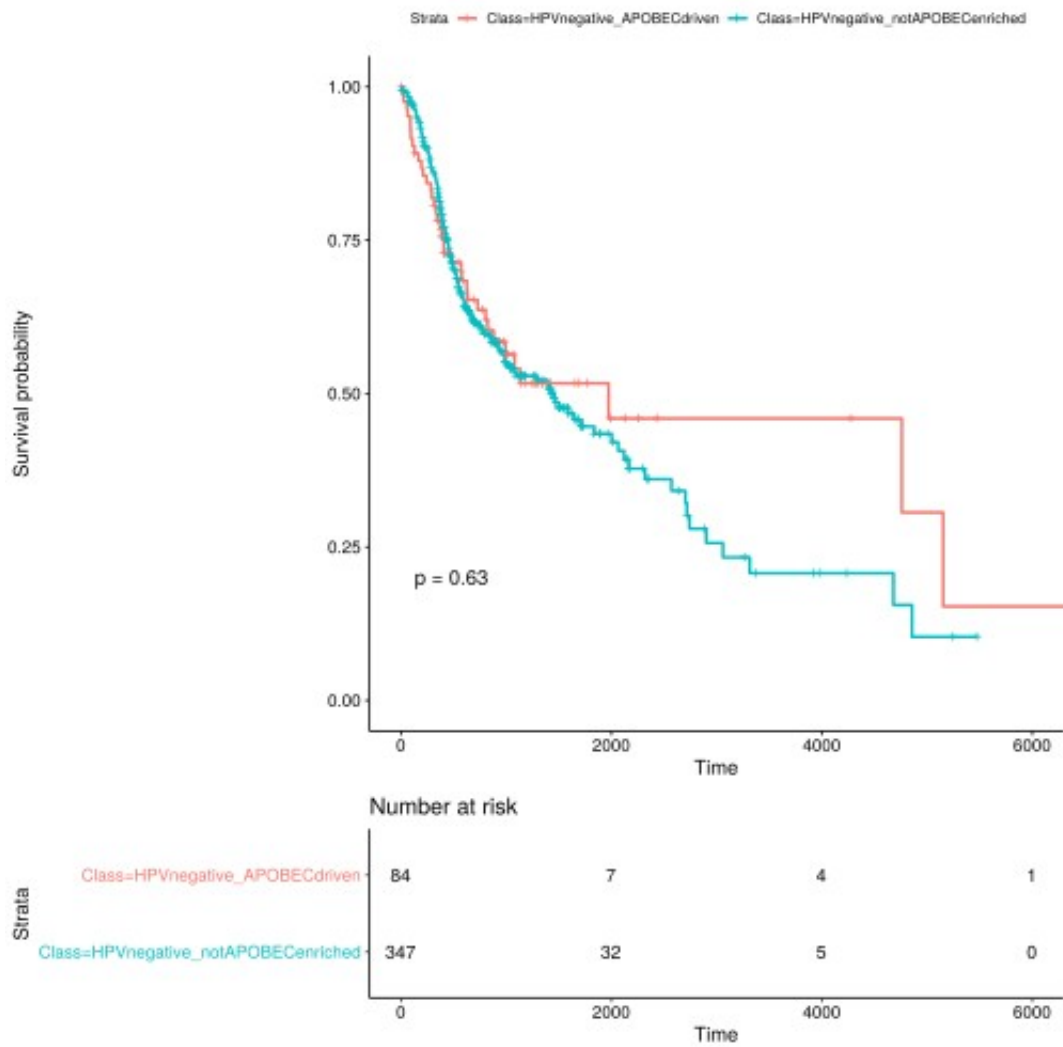


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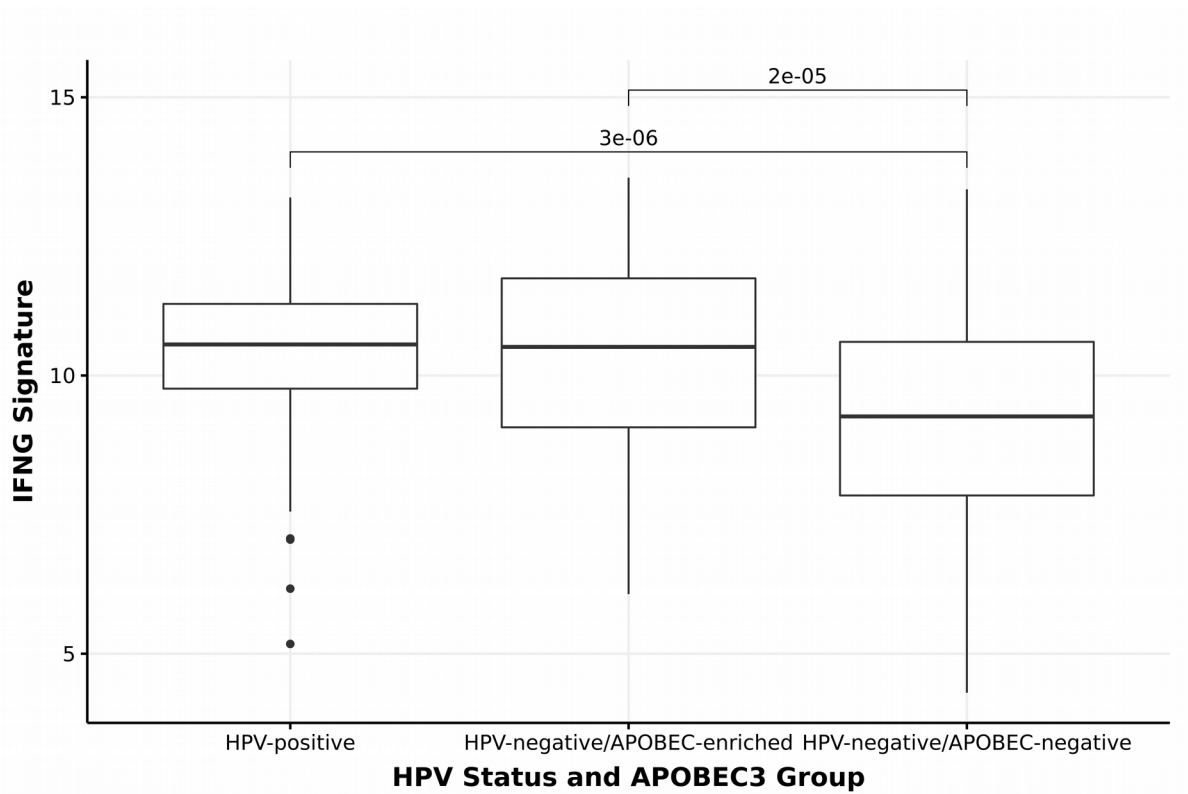


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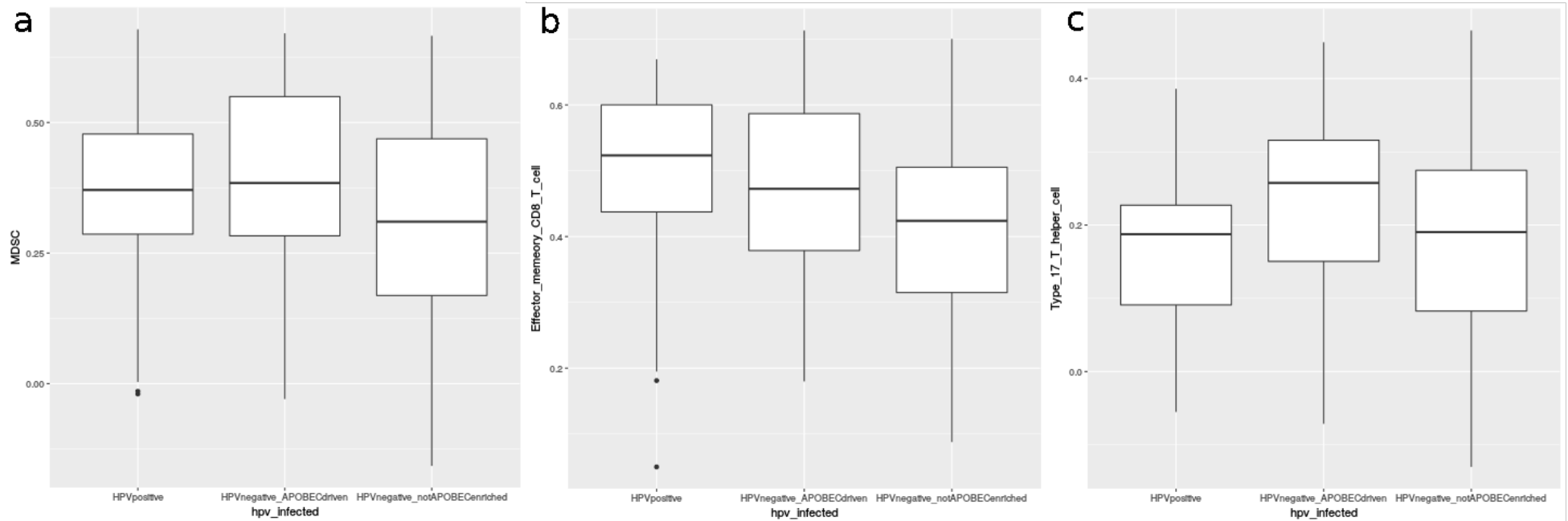


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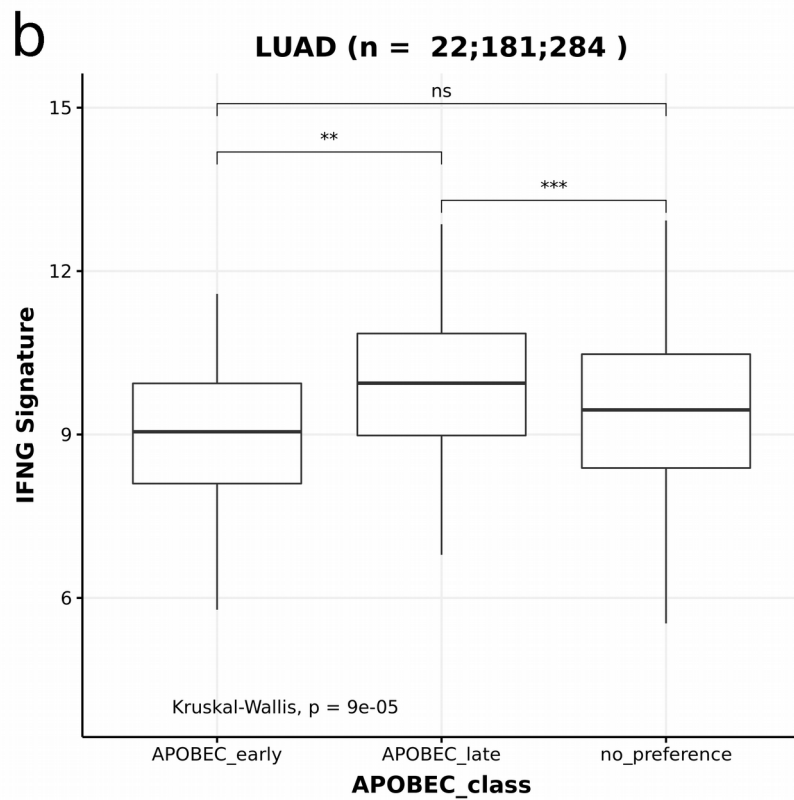
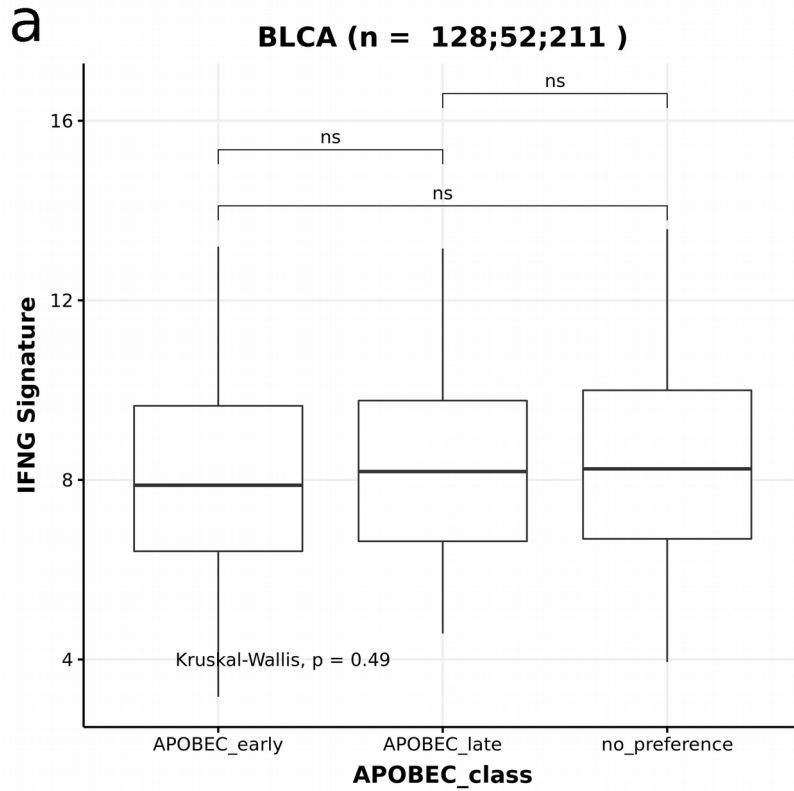
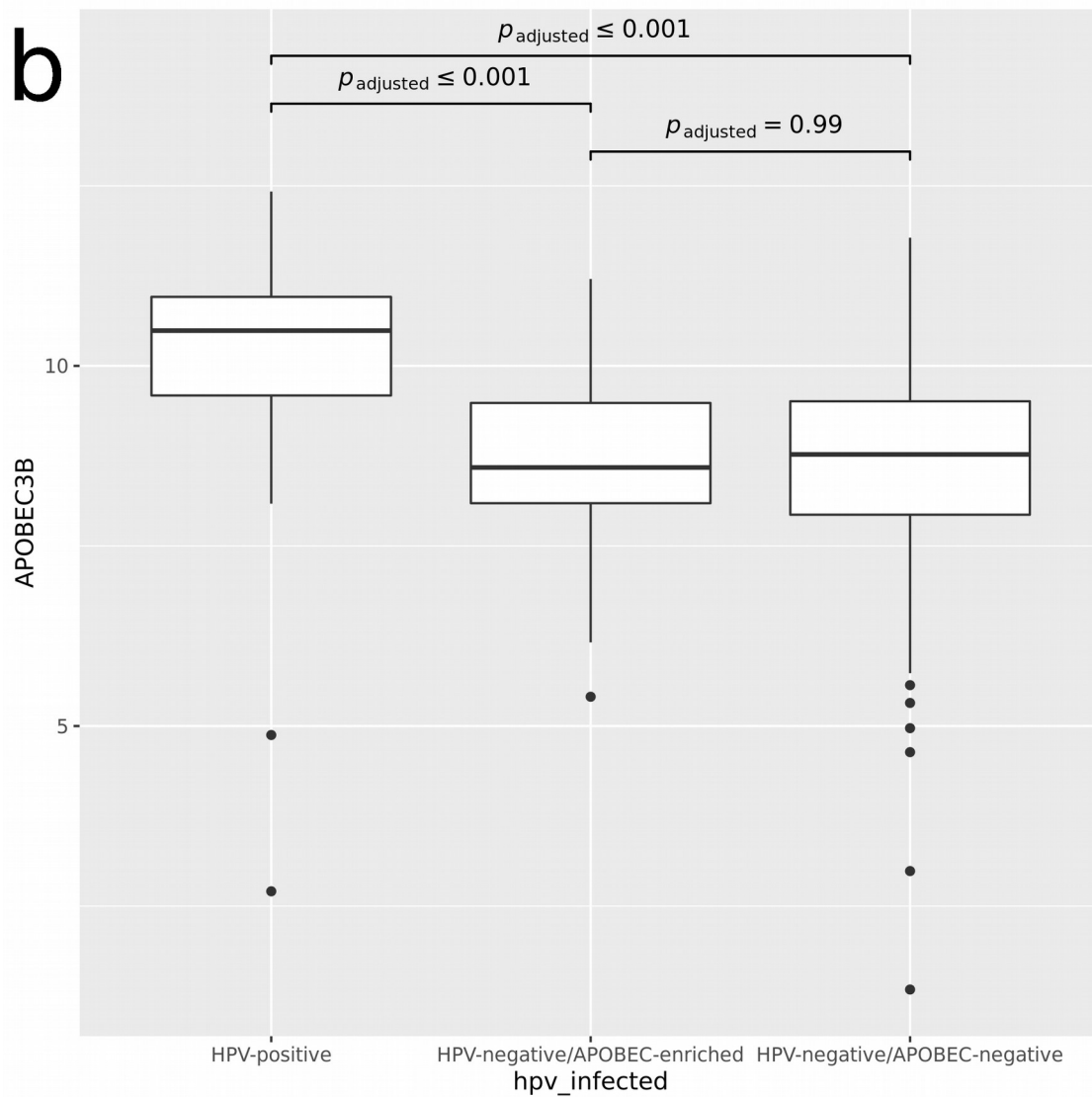
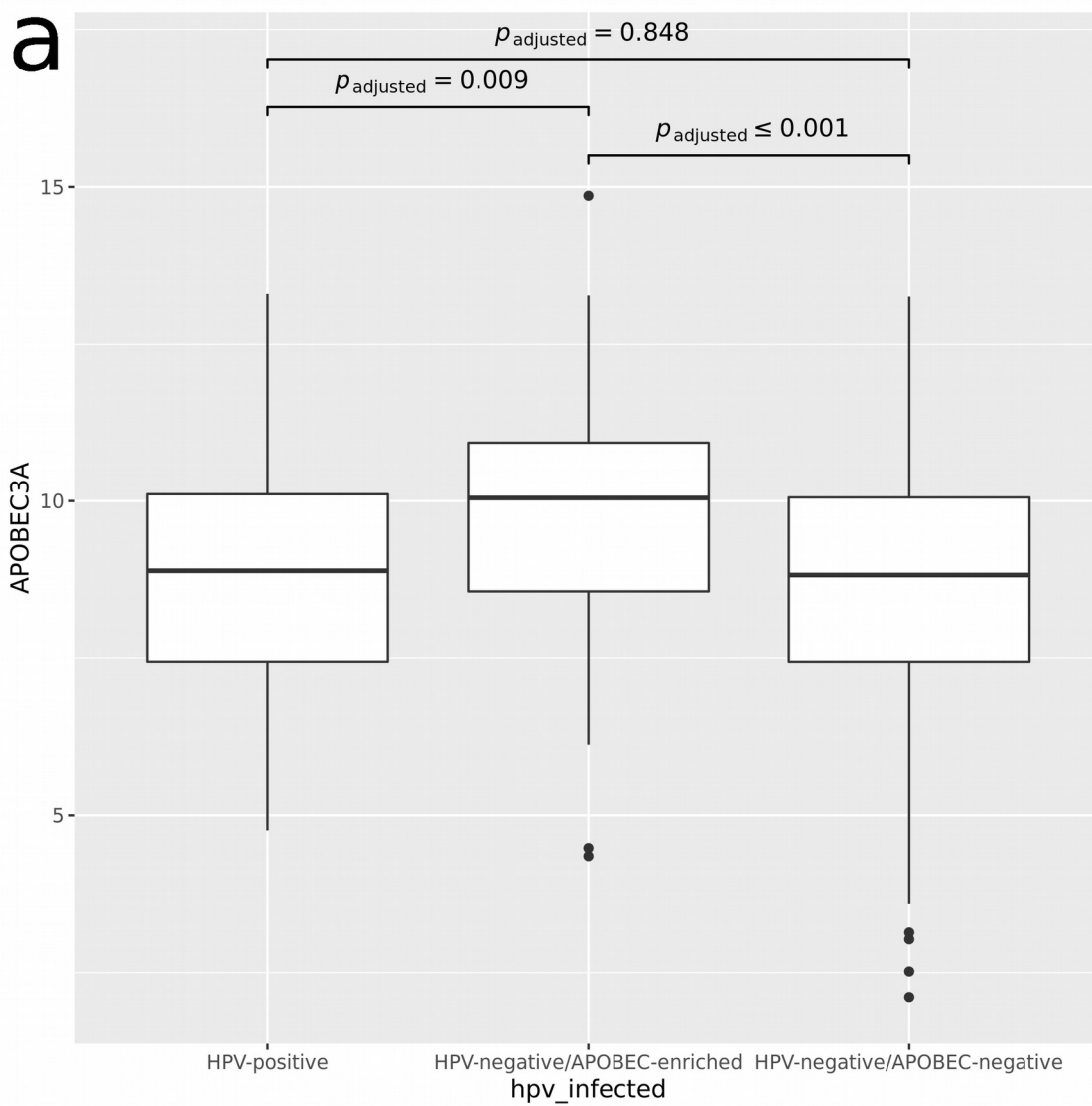
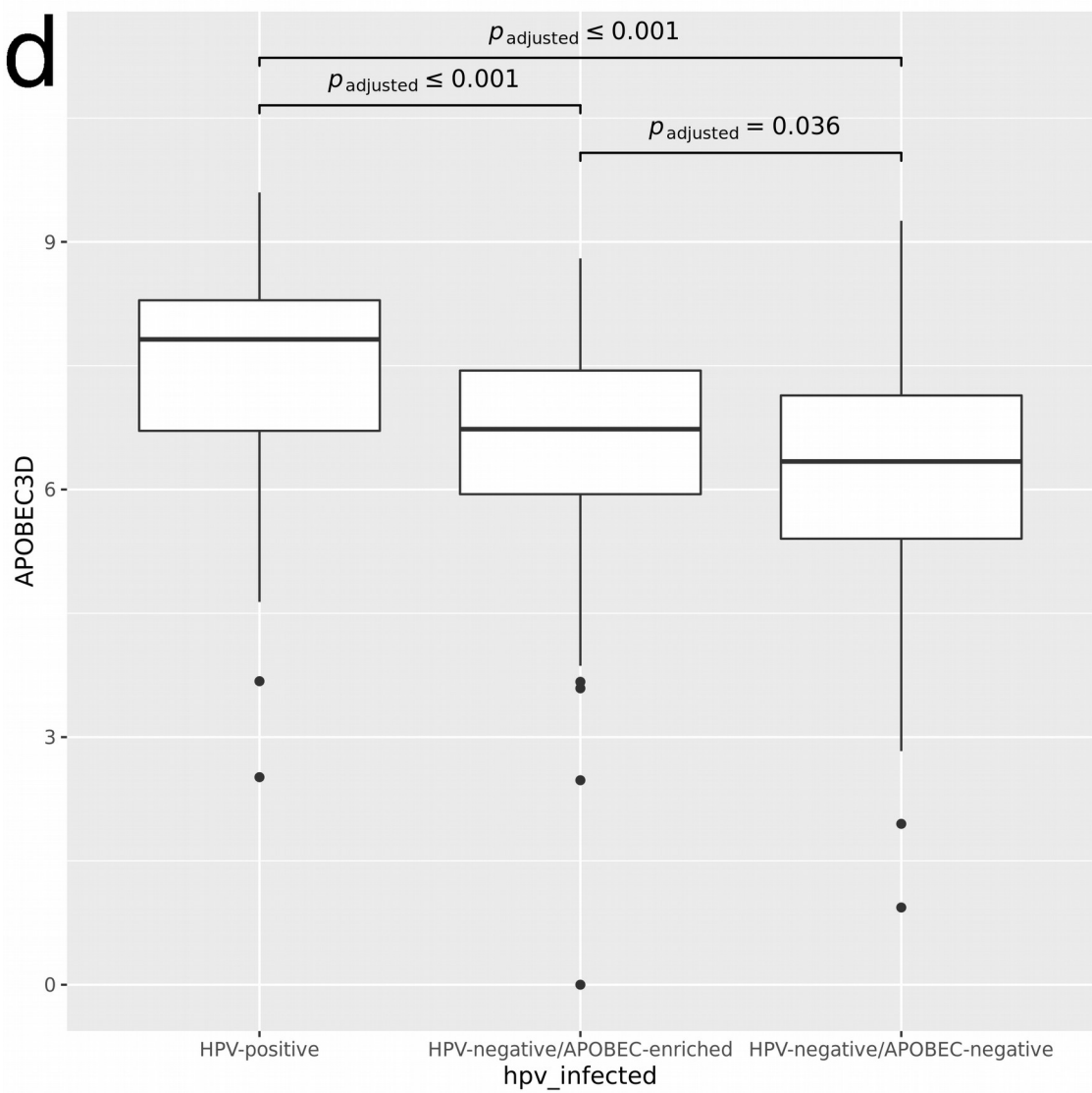
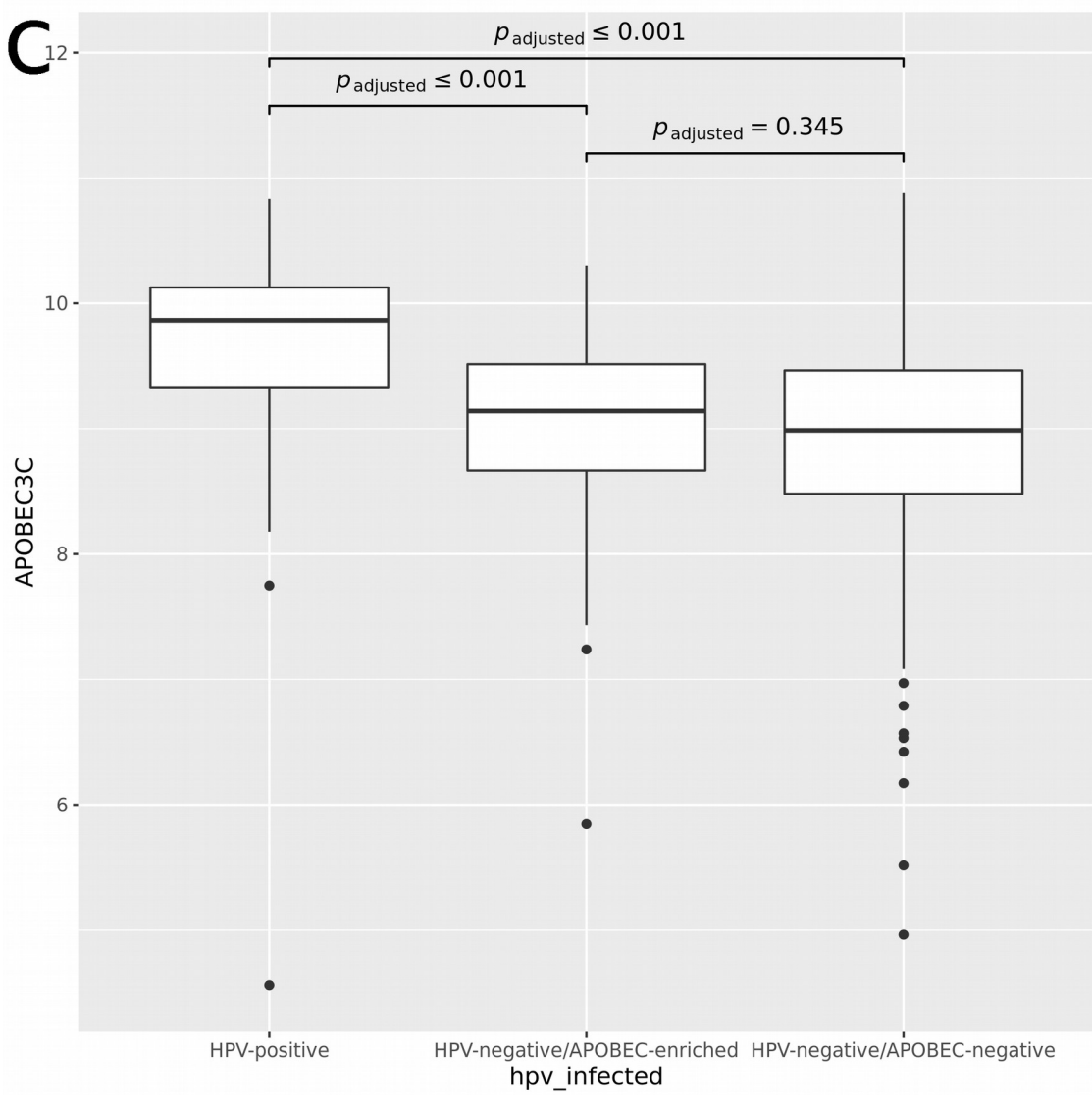
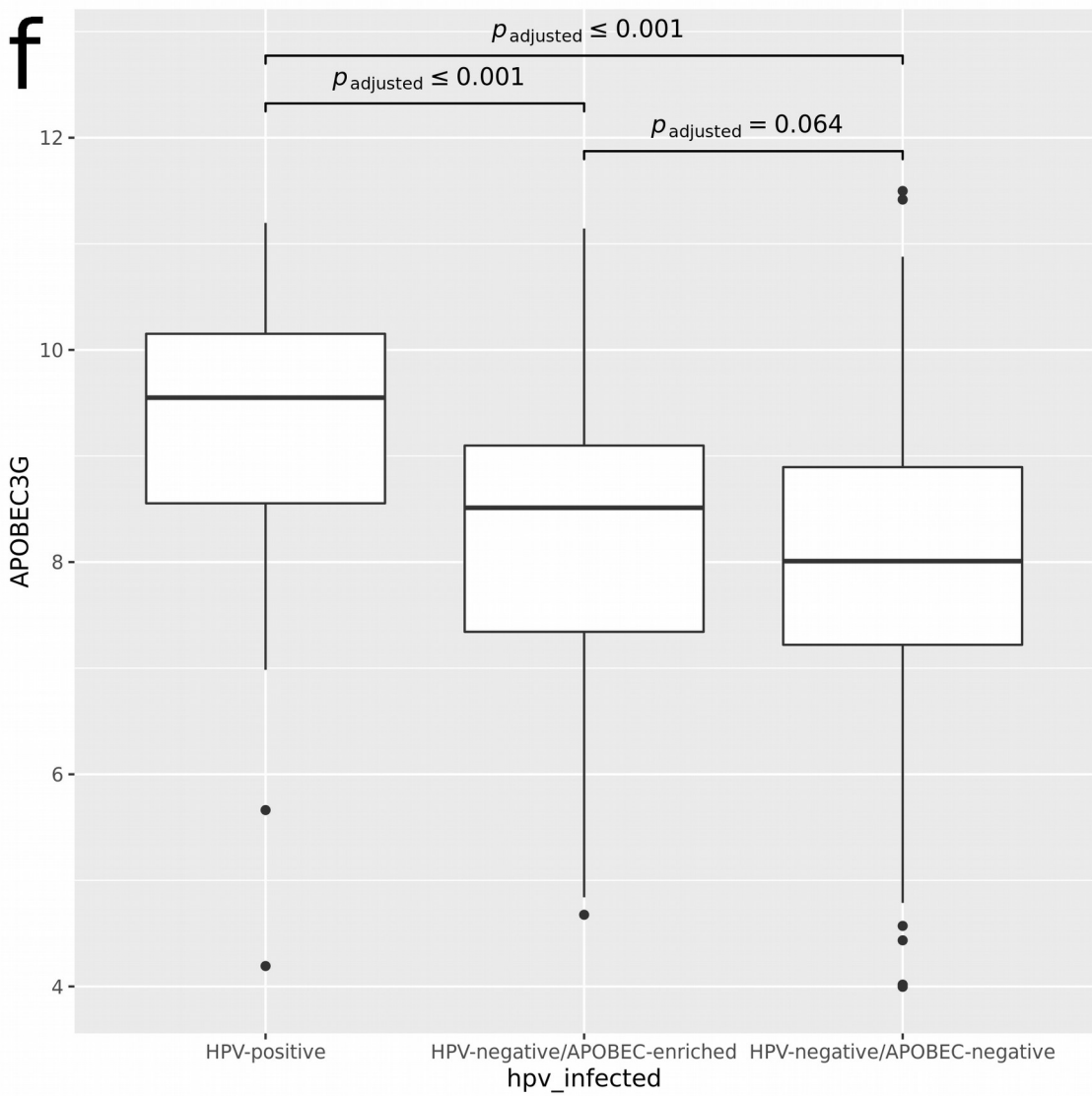
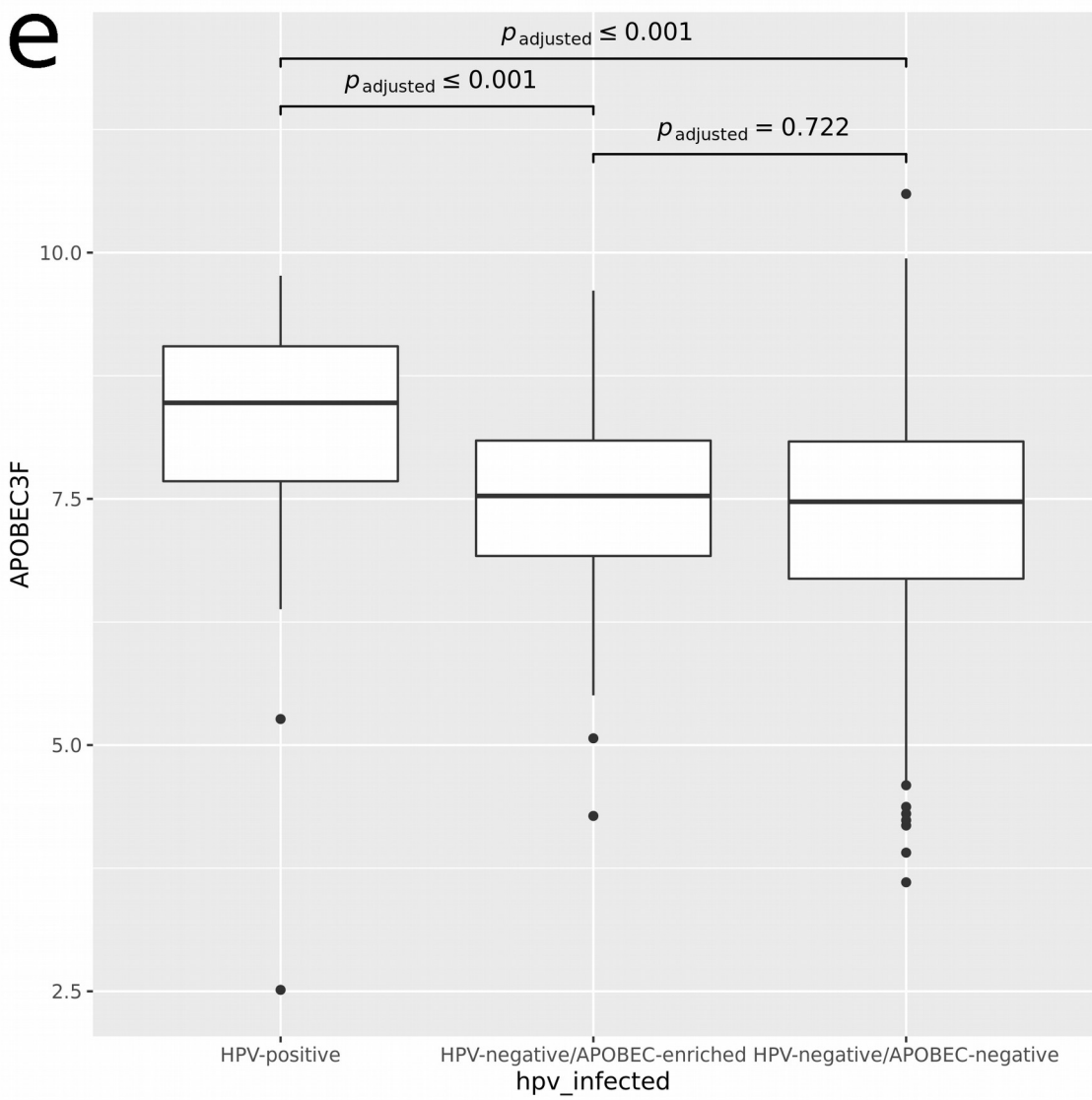


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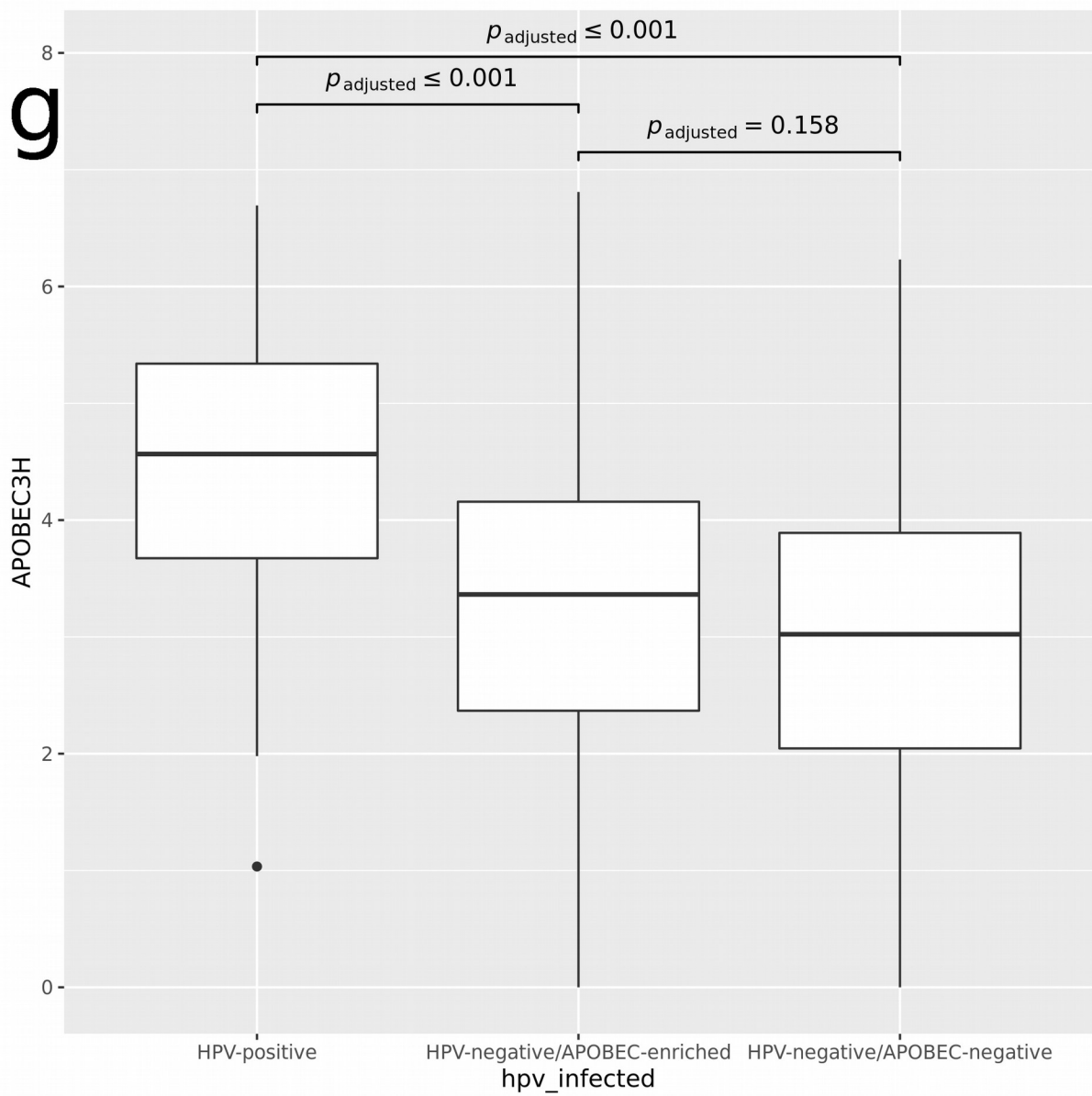


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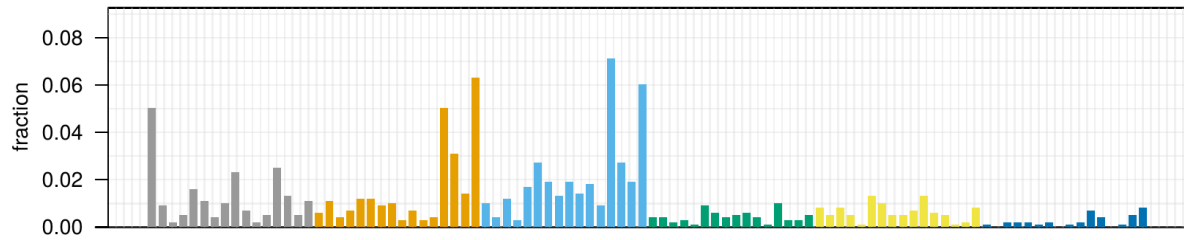


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