

1 **Supplementary figure legends**

2 **Figure S1.** Work-flow of the study design and experimental approaches
3 adopted in this study

4 **Figure S2.** Details of patient enrolment, sampling and exclusion in this
5 study

6 **Figure S3.** a). number of mutations attributed to COSMIC SBS in each
7 patient, SBS was labelled in different colors; b). SBS with the highest
8 numbers of mutations from all samples, y-axis denotes the average
9 numbers of mutations attributed to the SBS among all samples. c).
10 hematoxylin and eosin (H&E) staining was used for pathological
11 evaluation ($\times 200$), and immunohistochemistry (IHC) assay was applied
12 to determine the expression of mutant p53 in *TP53^{mut}* TATs. Moderate
13 expression of mutant p53 was detected in nucleus (black arrow, $\times 400$).

14 **Figure S4.** Evolutionary relationship between TATs and primary tumors
15 in relapsed patients and patients with premalignant lesions in patients
16 with mutations identified in their TATs. Arrows with different colors
17 indicate the mutations that are detected in liquid biopsies: orange,
18 pre-operative plasma; green, pre-operative saliva; purple, post-operative
19 plasma; blue, post-operative saliva. TAT, tumor adjacent tissue; the
20 number of mutations identified were labeled near each branch or trunk;
21 Patients with premalignant lesion diagnosed pathologically in TATs are
22 labeled with star icons while relapsed patients are labeled with hexagon
23 icons

24 **Figure S5.** Clonal structures of TATs vs. Primary tumors in patients with
25 mutations identified in their TATs. VAF, variant allelic frequency;
26 mutations are labeled with different colors according to their clonal type
27 in TATs and primary tumors, indicated at the bottom right. Mutations
28 with VAF ratio greater than 0.8 are defined as clonal while other
29 mutations are defined as subclonal. Arrows with different colors indicate
30 the clones that are detected in post-operative plasma and saliva: purple,
31 post-operative plasma; blue, post-operative saliva. Patients with
32 premalignant lesion diagnosed pathologically in TATs are labeled with
33 star icons while relapsed patients are labeled with hexagon icons.

34 **Figure S6.** Mean VAF-ratio of mutations in all primary tumors (top,
35 n=27), TATs with premalignant lesions (bottom left, n=22) and
36 pathologically normal TATs (bottom right, n=4). Grey dots represent the
37 VAF-ratio of each mutation, while black dots denote the mean VAF-ratio
38 of corresponding mutation among all samples.

39 **Figure S7.** Number of patients with tumor-private, TAT-private or
40 tumor-TAT shared identified in each type of liquid biopsy; mutation
41 types are labeled at the top right, number of relapsed patients are labeled
42 with black squares; Types of liquid biopsies are labeled in the middle
43 panel; number of mutations detected are illustrated at the bottom.

44 **Figure S8.** a). Mutation tracking detected ctDNA shortly after surgery

1 in patient P19. By contrast, MRI scan revealed cervical lymph node
2 metastasis (white arrows) at 9.2 months follow-up. b). Tumor DNA
3 detected in saliva shortly after surgery in patient P03. Comparatively,
4 PET-CT scan revealed left buccal carcinoma relapse (white arrows) at
5 5.4 months follow-up.

Supplementary Table 1. Gene list

Part 1: Genes with all coding regions									
<i>ABL1</i>	<i>ABL2</i>	<i>AKT1</i>	<i>AKT2</i>	<i>AKT3</i>	<i>ALK</i>	<i>APC</i>	<i>AR</i>	<i>ARAF</i>	<i>ATM</i>
<i>ATR</i>	<i>AURKA</i>	<i>AURKB</i>	<i>AXL</i>	<i>BAPI</i>	<i>BCL2</i>	<i>BRAF</i>	<i>BRCA1</i>	<i>BRCA2</i>	<i>BRD2</i>
<i>BRD3</i>	<i>BRD4</i>	<i>BTK</i>	<i>C11orf30</i>	<i>C1Q1A</i>	<i>C1S</i>	<i>CBL</i>	<i>CCND1</i>	<i>CCND2</i>	<i>CCND3</i>
<i>CCNE1</i>	<i>CD274</i>	<i>CDH1</i>	<i>CDK13</i>	<i>CDK4</i>	<i>CDK6</i>	<i>CDK8</i>	<i>CDKN1A</i>	<i>CDKN1B</i>	<i>CDKN2A</i>
<i>CDKN2B</i>	<i>CHEK1</i>	<i>CHEK2</i>	<i>CRKL</i>	<i>CSF1R</i>	<i>CTNNB1</i>	<i>DDR1</i>	<i>DDR2</i>	<i>DNMT3A</i>	<i>EGFR</i>
<i>EPHA2</i>	<i>EPHA3</i>	<i>EPHA5</i>	<i>ERBB2</i>	<i>ERBB3</i>	<i>ERBB4</i>	<i>ERCC1</i>	<i>ERG</i>	<i>ESR1</i>	<i>EZH2</i>
<i>FAT1</i>	<i>FBXW7</i>	<i>FCGR2A</i>	<i>FCGR2B</i>	<i>FCGR3A</i>	<i>FGFR1</i>	<i>FGFR2</i>	<i>FGFR3</i>	<i>FGFR4</i>	<i>FLCN</i>
<i>FLT1</i>	<i>FLT3</i>	<i>FLT4</i>	<i>FOXA1</i>	<i>FOXL2</i>	<i>GAB2</i>	<i>GATA3</i>	<i>GNA11</i>	<i>GNAQ</i>	<i>GNAS</i>
<i>HDAC1</i>	<i>HDAC4</i>	<i>HGF</i>	<i>HRAS</i>	<i>IDH1</i>	<i>IDH2</i>	<i>IGF1R</i>	<i>IL7R</i>	<i>INPP4B</i>	<i>IRS2</i>
<i>JAK1</i>	<i>JAK2</i>	<i>JAK3</i>	<i>KDR</i>	<i>KIT</i>	<i>KRAS</i>	<i>MAP2K1</i>	<i>MAP2K2</i>	<i>MAPK1</i>	<i>MAPK3</i>
<i>MCL1</i>	<i>MDM2</i>	<i>MDM4</i>	<i>MED12</i>	<i>MET</i>	<i>MITF</i>	<i>MLH1</i>	<i>MLH3</i>	<i>MPL</i>	<i>MS4A1</i>
<i>MSH2</i>	<i>MSH3</i>	<i>MSH6</i>	<i>MTOR</i>	<i>MYC</i>	<i>MYD88</i>	<i>NFI</i>	<i>NF2</i>	<i>NOTCH1</i>	<i>NOTCH2</i>
<i>NOTCH3</i>	<i>NOTCH4</i>	<i>NRAS</i>	<i>NTRK1</i>	<i>NTRK3</i>	<i>PALB2</i>	<i>PDGFRA</i>	<i>PDGFRB</i>	<i>PDK1</i>	<i>PIK3CA</i>
<i>PIK3CB</i>	<i>PIK3R1</i>	<i>PIK3R2</i>	<i>PMS1</i>	<i>PMS2</i>	<i>PRKAA1</i>	<i>PSMB1</i>	<i>PSMB5</i>	<i>PTCH1</i>	<i>PTCH2</i>
<i>PTEN</i>	<i>PTPN11</i>	<i>RAFI</i>	<i>RARA</i>	<i>RB1</i>	<i>RET</i>	<i>RHEB</i>	<i>RHOA</i>	<i>RICTOR</i>	<i>RNF43</i>
<i>ROCK1</i>	<i>ROS1</i>	<i>RPS6KB1</i>	<i>SMARCA4</i>	<i>SMARCB1</i>	<i>SMO</i>	<i>SRC</i>	<i>STAT1</i>	<i>STAT3</i>	<i>STK11</i>
<i>SYK</i>	<i>TMPRSS2</i>	<i>TOPI</i>	<i>TP53</i>	<i>TSC1</i>	<i>TSC2</i>	<i>VEGFA</i>	<i>VHL</i>	<i>XPO1</i>	<i>XRCC1</i>
Part 2: Genes with promoter or selected introns									
<i>ABL1</i>	<i>ALK</i>	<i>BCL2L11</i>	<i>BCR</i>	<i>BRAF</i>	<i>BRD3</i>	<i>BRD4</i>	<i>EGFR</i>	<i>ERG</i>	<i>FGFR1</i>
<i>FGFR2</i>	<i>FGFR3</i>	<i>KIF5B</i>	<i>MET</i>	<i>NTRK1</i>	<i>NTRK3</i>	<i>PDGFRA</i>	<i>PDGFRB</i>	<i>RAFI</i>	<i>RARA</i>
Part 3: Genes with selected regions									
<i>ABCA10</i>	<i>ABCA8</i>	<i>ABCB7</i>	<i>ABCC8</i>	<i>ABCF2</i>	<i>ACE</i>	<i>ACER2</i>	<i>ACOT11</i>	<i>ACPP</i>	<i>ACSL1</i>
<i>ACSM5</i>	<i>ACSS3</i>	<i>ACTL6B</i>	<i>ADAM23</i>	<i>ADAM33</i>	<i>ADAMTS12</i>	<i>ADAMTS16</i>	<i>ADAMTS19</i>	<i>ADAMTS20</i>	<i>ADAMTS5</i>
<i>ADAMTS1</i>	<i>ADD2</i>	<i>AGMAT</i>	<i>AGTPBP1</i>	<i>AHCTFI</i>	<i>AK5</i>	<i>AKR1B10</i>	<i>AKR1C1</i>	<i>ALDH1A3</i>	<i>ALDH2</i>
<i>ALG5</i>	<i>ALX4</i>	<i>AMOT</i>	<i>ANK2</i>	<i>ANKRD13D</i>	<i>ANKRD20A4</i>	<i>ANKRD27</i>	<i>ANKRD28</i>	<i>ANKRD30A</i>	<i>ANKRD30B</i>
<i>ANKRD36B</i>	<i>ANO2</i>	<i>APIB1</i>	<i>APIG2</i>	<i>AP3B1</i>	<i>APAF1</i>	<i>APLP2</i>	<i>APMAP</i>	<i>APPL2</i>	<i>AQP12A</i>
<i>ARFGAPI</i>	<i>ARFRP1</i>	<i>ARHGAP35</i>	<i>ARHGAP40</i>	<i>ARHGEF1</i>	<i>ARHGEF7</i>	<i>ARNTL</i>	<i>ARPC4-TTLLASH2L</i>	<i>ASTN1</i>	
<i>ASXL2</i>	<i>ATAD2B</i>	<i>ATG9B</i>	<i>ATP10B</i>	<i>ATP10D</i>	<i>ATP12A</i>	<i>ATP2C1</i>	<i>ATP6V0A2</i>	<i>ATP8B2</i>	<i>ATXN2</i>
<i>ATXN7L2</i>	<i>BAX</i>	<i>BBS9</i>	<i>BCAS1</i>	<i>BCAS2</i>	<i>BLOC1S1</i>	<i>BMPR1B</i>	<i>BRF1</i>	<i>BRSK2</i>	<i>BRWD3</i>
<i>BSG</i>	<i>BTNL3</i>	<i>BTRC</i>	<i>C12orf5</i>	<i>C19orf38</i>	<i>Clof112</i>	<i>Clof35</i>	<i>C20orf112</i>	<i>C2orf47</i>	<i>C2orf62</i>
<i>C7orf53</i>	<i>C9orf114</i>	<i>C9orf43</i>	<i>CACNA1A</i>	<i>CACNA1D</i>	<i>CACNA1E</i>	<i>CADM2</i>	<i>CAMKK1</i>	<i>CAPRIN1</i>	<i>CARS</i>
<i>CARS2</i>	<i>CASC4</i>	<i>CASP8</i>	<i>CASP8AP2</i>	<i>CASQ2</i>	<i>CATSPER2</i>	<i>CBFB</i>	<i>CBX4</i>	<i>CCDC155</i>	<i>CCDC159</i>
<i>CCDC17</i>	<i>CCT3</i>	<i>CCT6B</i>	<i>CD1E</i>	<i>CD300LF</i>	<i>CD5L</i>	<i>CD9</i>	<i>CD97</i>	<i>CDH18</i>	
<i>CDH24</i>	<i>CDH26</i>	<i>CDK11A</i>	<i>CDK12</i>	<i>CDK14</i>	<i>CDK18</i>	<i>CDK19</i>	<i>CDS1</i>	<i>CEACAM20</i>	<i>CERC2</i>
<i>CELA2B</i>	<i>CGN</i>	<i>CHD3</i>	<i>CHD4</i>	<i>CHD6</i>	<i>CHI3L1</i>	<i>CISD3</i>	<i>CLCN7</i>	<i>CLEC16A</i>	<i>CLINT1</i>
<i>CNGB3</i>	<i>CNKS2</i>	<i>CNOT3</i>	<i>CNOT4</i>	<i>CNTN1</i>	<i>CNTN4</i>	<i>CNTN5</i>	<i>CNTNAP3B</i>	<i>CNTNAP5</i>	<i>COASY</i>
<i>COL14A1</i>	<i>COL16A1</i>	<i>COL19A1</i>	<i>COL1A1</i>	<i>COL25A1</i>	<i>COL4A5</i>	<i>COL4A6</i>	<i>COL5A1</i>	<i>COL5A2</i>	<i>COL5A3</i>
<i>COL6A5</i>	<i>COL6A6</i>	<i>COL9A1</i>	<i>COPA</i>	<i>COPG1</i>	<i>CPA1</i>	<i>CPSF3</i>	<i>CPSF6</i>	<i>CRTAM</i>	<i>CRTAP</i>
<i>CRYBG3</i>	<i>CSMD1</i>	<i>CSMD3</i>	<i>CSN3</i>	<i>CSNK1E</i>	<i>CSPP1</i>	<i>CTCF</i>	<i>CTIF</i>	<i>CTNNA2</i>	<i>CTSF</i>
<i>CYP2A13</i>	<i>CYP3A4</i>	<i>CYP4A11</i>	<i>CYTH4</i>	<i>DCLK2</i>	<i>DCST1</i>	<i>DDB1</i>	<i>DDX24</i>	<i>DDX3X</i>	<i>DEPDC4</i>
<i>DGKK</i>	<i>DHCR24</i>	<i>DHDDS</i>	<i>DHX9</i>	<i>DIAPH1</i>	<i>DKC1</i>	<i>DLST</i>	<i>DMD</i>	<i>DMXL1</i>	<i>DMXL2</i>
<i>DNAH10</i>	<i>DNAH5</i>	<i>DNAH9</i>	<i>DNAJC11</i>	<i>DNAJC9</i>	<i>DNTTIP1</i>	<i>DOCK11</i>	<i>DOCK3</i>	<i>DOTIL</i>	<i>DPP10</i>
<i>DPP4</i>	<i>DRGX</i>	<i>DUOX1</i>	<i>DYSF</i>	<i>DZANK1</i>	<i>ECHDC1</i>	<i>EDN1</i>	<i>EEF1A1</i>	<i>EFCAB5</i>	<i>EFCAB6</i>
<i>EFCAB7</i>	<i>EFHA2</i>	<i>EFNA5</i>	<i>EFLIA1X</i>	<i>EIF2B5</i>	<i>EIF2C2</i>	<i>EIF3E</i>	<i>EIF3I</i>	<i>EIF4ENIF1</i>	<i>EIF4H</i>
<i>ELAVL3</i>	<i>ELL3</i>	<i>EMID2</i>	<i>ENPP2</i>	<i>ENTPD6</i>	<i>EPB41L2</i>	<i>EPB41L4B</i>	<i>EPHB1</i>	<i>EPS8L3</i>	<i>ESD</i>
<i>ETNK2</i>	<i>ETV6</i>	<i>EXOC4</i>	<i>EXOC5</i>	<i>EXOC6</i>	<i>EXOC7</i>	<i>EXTL3</i>	<i>EYA4</i>	<i>F8</i>	<i>F9</i>
<i>FAH</i>	<i>FAM114A2</i>	<i>FAM131B</i>	<i>FAM135B</i>	<i>FAM13C</i>	<i>FAM157B</i>	<i>FAM177B</i>	<i>FAM17A</i>	<i>FAM3A</i>	<i>FAM49A</i>
<i>FAM49B</i>	<i>FAM5C</i>	<i>FAM86B1</i>	<i>FAN1</i>	<i>FANCC</i>	<i>FASTK</i>	<i>FATE1</i>	<i>FBN2</i>	<i>FDCSP</i>	<i>FLNC</i>
<i>FLOT2</i>	<i>FLT3LG</i>	<i>FMN2</i>	<i>FMNL3</i>	<i>FNDC4</i>	<i>FNIP2</i>	<i>FOLH1</i>	<i>FOXJ2</i>	<i>FRG1</i>	<i>FRG2B</i>
<i>FRMD4A</i>	<i>FRMPD2</i>	<i>FRMPD4</i>	<i>FSD2</i>	<i>FSHR</i>	<i>FUBP1</i>	<i>FUND1</i>	<i>GAB3</i>	<i>GABRD</i>	<i>GAD2</i>
<i>GALNT13</i>	<i>GALNT14</i>	<i>GFRAL</i>	<i>GIGYFY1</i>	<i>GINS4</i>	<i>GIPR</i>	<i>GKN2</i>	<i>GLB1L3</i>	<i>GLYR1</i>	<i>GMDS</i>
<i>GNPTAB</i>	<i>GOLGA4</i>	<i>GPAT2</i>	<i>GPATCH2</i>	<i>GPR114</i>	<i>GPR125</i>	<i>GPR133</i>	<i>GPR144</i>	<i>GPS2</i>	<i>GRIA3</i>
<i>GRIK2</i>	<i>GUCY1A3</i>	<i>GUCY2C</i>	<i>GYLTL1B</i>	<i>HAAO</i>	<i>HAPI</i>	<i>HAUS5</i>	<i>HAUS6</i>	<i>HCN1</i>	<i>HDAC6</i>
<i>HEATR7B2</i>	<i>HECTD4</i>	<i>HECW1</i>	<i>HECW2</i>	<i>HID1</i>	<i>HIST1H3B</i>	<i>HLA-DRB1</i>	<i>HLA-DRB5</i>	<i>HMCN1</i>	<i>HMAH1</i>
<i>HNF4A</i>	<i>HOMER2</i>	<i>HPS3</i>	<i>HPS4</i>	<i>HSPA12B</i>	<i>HSPD1</i>	<i>HYDIN</i>	<i>IBSP</i>	<i>IFT172</i>	<i>IGSF9</i>
<i>IKBKAP</i>	<i>IKBKE</i>	<i>IL11RA</i>	<i>IL13RA2</i>	<i>IL1RAP1</i>	<i>IL27RA</i>	<i>IMPG1</i>	<i>INHBA</i>	<i>INPP5J</i>	<i>IQCA1</i>
<i>ITFG2</i>	<i>ITGA8</i>	<i>ITGA9</i>	<i>ITIH1</i>	<i>ITLN2</i>	<i>ITM2A</i>	<i>ITPKB</i>	<i>ITPR1</i>	<i>KCNAB2</i>	<i>KCNH6</i>
<i>KCNQ2</i>	<i>KDM4A</i>	<i>KEAP1</i>	<i>KIAA0195</i>	<i>KIAA0226</i>	<i>KIAA0319</i>	<i>KIAA0922</i>	<i>KIAA1191</i>		<i>KIAA1199</i>
<i>KIAA1211L</i>	<i>KIF13A</i>	<i>KIF16B</i>	<i>KIF26B</i>	<i>KIFAP3</i>	<i>KIFC1</i>	<i>KIR2DL3</i>	<i>KIR3DL3</i>	<i>KLHL1</i>	<i>KLHL14</i>
<i>KLK1</i>	<i>KMT2B</i>	<i>KMT2C</i>	<i>KRT2</i>	<i>KRT9</i>	<i>KRTAP5-5</i>	<i>KTN1</i>	<i>L3MBTL1</i>	<i>LARPI</i>	<i>LCN10</i>
<i>LCT</i>	<i>LCTL</i>	<i>LETM1</i>	<i>LGALS13</i>	<i>LILRB3</i>	<i>LILRB4</i>	<i>LIPN</i>	<i>LMAN1L</i>	<i>LMBR1L</i>	<i>LPCAT4</i>
<i>LPHN3</i>	<i>LRBA</i>	<i>LRP1B</i>	<i>LRP2</i>	<i>LRP4</i>	<i>LRRC16B</i>	<i>LRRC2</i>	<i>LRRC7</i>	<i>LRRC72</i>	<i>LRRD1</i>
<i>LRRFP12</i>	<i>LRSAM1</i>	<i>LTBP1</i>	<i>LUZP4</i>	<i>MAEL</i>	<i>MAGII</i>	<i>MAN2A1</i>	<i>MAP2</i>	<i>MAP2K4</i>	
<i>MAP3K1</i>	<i>MAP4K1</i>	<i>MAPKAPK3</i>	<i>MAPRE3</i>	<i>MAST1</i>	<i>MBIP</i>	<i>MBTPS2</i>	<i>MCF2L2</i>	<i>MCOLN2</i>	<i>MDGA2</i>
<i>MDN1</i>	<i>MED23</i>	<i>MEFV</i>	<i>METTL14</i>	<i>METTL5</i>	<i>MGAM</i>	<i>MICALLI</i>	<i>MID1</i>	<i>MIER2</i>	<i>MLL3</i>
<i>MLPH</i>	<i>MORC1</i>	<i>MORN1</i>	<i>MRPL1</i>	<i>MRPL24</i>	<i>MRPS18B</i>	<i>MSII</i>	<i>MTA2</i>	<i>MTM1</i>	<i>MTR</i>
<i>MTTP</i>	<i>MUC5B</i>	<i>MUS81</i>	<i>MYB</i>	<i>MYBPC2</i>	<i>MYC1B</i>	<i>MYH15</i>	<i>MYH2</i>	<i>MYH4</i>	<i>MYH8</i>
<i>MYH9</i>	<i>MYL5</i>	<i>MYL6</i>	<i>MYLK2</i>	<i>MYO3A</i>	<i>MYOM1</i>	<i>NACAD</i>	<i>NARF</i>	<i>NAT10</i>	<i>NAV3</i>
<i>NBPF1</i>	<i>NBPF10</i>	<i>NCF2</i>	<i>NCKAP1</i>	<i>NCOR1</i>	<i>NCOR2</i>	<i>NEK5</i>	<i>NELL1</i>	<i>NFE2L2</i>	<i>NIPBL</i>
<i>NLGN3</i>	<i>NLRC3</i>	<i>NLRP4</i>	<i>NMI</i>	<i>NOP2</i>	<i>NOS1</i>	<i>NOS2</i>	<i>NRXN1</i>	<i>NRXN2</i>	<i>NT5C3L</i>
<i>NTM</i>	<i>NUCDC2</i>	<i>NUP205</i>	<i>NUP210</i>	<i>NUTM1</i>	<i>NWDL1</i>	<i>NXF1</i>	<i>NXF5</i>	<i>OBP2A</i>	<i>OTOG</i>
<i>OCA2</i>	<i>ODZ3</i>	<i>OR2T4</i>	<i>OR4A15</i>	<i>OR4C6</i>	<i>OR5L2</i>	<i>OR6F1</i>	<i>OSBPL10</i>	<i>OTOA</i>	<i>OTOG</i>
<i>OVCH1</i>	<i>P4HB</i>	<i>PABPC4</i>	<i>PAcs2</i>	<i>PAEP</i>	<i>PAGE1</i>	<i>PARK2</i>	<i>PARP4</i>	<i>PCK2</i>	<i>PCLO</i>
<i>PCNT</i>	<i>PCNXL2</i>	<i>PCSK5</i>	<i>PCYT1A</i>	<i>PDCD6</i>	<i>PDE1C</i>	<i>PDE2A</i>	<i>PDE4DIP</i>	<i>PDI15</i>	<i>PDLT</i>
<i>PDRG1</i>	<i>PEX6</i>	<i>PGAP1</i>	<i>PHACTR3</i>	<i>PHF20L1</i>	<i>PHYH</i>	<i>PI4KB</i>	<i>PIP4K2C</i>	<i>PIP5K1C</i>	<i>PIWIL1</i>
<i>PKD1L2</i>	<i>PKHD1</i>	<i>PKLR</i>	<i>PLAC8</i>	<i>PLCB4</i>	<i>PLCZ1</i>	<i>PLEC</i>	<i>PLK2</i>	<i>PLOD3</i>	<i>PLXNA1</i>
<i>POLDIP2</i>	<i>POLE</i>	<i>POLR2J</i>	<i>POLR3B</i>	<i>POLR3GL</i>	<i>POLRMT</i>	<i>POM121L12</i>	<i>POTE</i>	<i>PPA1</i>	<i>PPDPF</i>
<i>PPEF1</i>	<i>PPF1BP2</i>	<i>PPIL2</i>	<i>PPP1R17</i>	<i>PPP4R4</i>	<i>PQBPI</i>	<i>PREB</i>	<i>PREX2</i>	<i>PRKACA</i>	<i>PRKAG3</i>
<i>PRKCD</i>	<i>PRKDC</i>	<i>PRKX</i>	<i>PRRX1</i>	<i>PRSS1</i>	<i>PRUNE</i>	<i>PSG2</i>	<i>PSG5</i>	<i>PSIP1</i>	<i>PSMC4</i>

<i>PSMC6</i>	<i>PSTPIP1</i>	<i>PTBP3</i>	<i>PTCD3</i>	<i>PTGES3L-AA</i>	<i>PTGS2</i>	<i>PTPLAD1</i>	<i>PTPN13</i>	<i>PTPRA</i>	<i>PTPRD</i>
<i>PTPRM</i>	<i>PYHIN1</i>	<i>QRICH2</i>	<i>RAB1B</i>	<i>RAB3GAP2</i>	<i>RAB6A</i>	<i>RAC2</i>	<i>RALBP1</i>	<i>RAPGEF2</i>	<i>RARB</i>
<i>RASEF</i>	<i>RBM6</i>	<i>RBMX</i>	<i>RCC1</i>	<i>REC8</i>	<i>REG1B</i>	<i>RELN</i>	<i>RERE</i>	<i>RFWD2</i>	<i>RFX3</i>
<i>RNF215</i>	<i>RNF219</i>	<i>RPL22</i>	<i>RPL36A</i>	<i>RPS5</i>	<i>RPS6KA1</i>	<i>RPTOR</i>	<i>RPUSD4</i>	<i>RREB1</i>	<i>RRP7A</i>
<i>RUND3A</i>	<i>RUNX1</i>	<i>RYR2</i>	<i>RYR3</i>	<i>SAFB2</i>	<i>SAG</i>	<i>SAGE1</i>	<i>SAMD8</i>	<i>SCN10A</i>	<i>SCN3A</i>
<i>SCN7A</i>	<i>SCN9A</i>	<i>SDK2</i>	<i>SEC14L4</i>	<i>SEC24B</i>	<i>SEH1L</i>	<i>SELP</i>	<i>SEMA6A</i>	<i>SEPT12</i>	<i>SERPIN A7</i>
<i>SETD1B</i>	<i>SETD2</i>	<i>SFI</i>	<i>SF3B1</i>	<i>SF3B14</i>	<i>SF3B3</i>	<i>SGCZ</i>	<i>SGIP1</i>	<i>SGK1</i>	<i>SGPL1</i>
<i>SH2D3A</i>	<i>SH3BGR</i>	<i>SH3PXD2A</i>	<i>SHISA4</i>	<i>SI</i>	<i>SIDT2</i>	<i>SIK3</i>	<i>SIM1</i>	<i>SIM2</i>	<i>SLC13A3</i>
<i>SLC17A6</i>	<i>SLC17A8</i>	<i>SLC25A1</i>	<i>SLC25A30</i>	<i>SLC26A3</i>	<i>SLC2A2</i>	<i>SLC30A5</i>	<i>SLC35B2</i>	<i>SLC35B4</i>	<i>SLC38A4</i>
<i>SLC38A5</i>	<i>SLC43A1</i>	<i>SLC45A1</i>	<i>SLC4A10</i>	<i>SLC4A4</i>	<i>SLC5A1</i>	<i>SLC6A5</i>	<i>SLC8A1</i>	<i>SLCO1B7</i>	<i>SLCO5A1</i>
<i>SMTN</i>	<i>SNTG1</i>	<i>SORCS3</i>	<i>SPAG16</i>	<i>SPATA13</i>	<i>SPG20</i>	<i>SPINT1</i>	<i>SPPL2A</i>	<i>SPPL3</i>	<i>SPRED1</i>
<i>SPTA1</i>	<i>SRRT</i>	<i>SSBP3</i>	<i>SSH2</i>	<i>SSPO</i>	<i>ST18</i>	<i>ST6GALNAC1</i>	<i>STAG2</i>	<i>STAT4</i>	<i>STAT6</i>
<i>STK11IP</i>	<i>STK31</i>	<i>STX3</i>	<i>SULT1A4</i>	<i>SUPT5H</i>	<i>SUPT6H</i>	<i>SYCP2L</i>	<i>SYNE1</i>	<i>SYNE2</i>	<i>SYNJ2</i>
<i>TAF1B</i>	<i>TAF6</i>	<i>TARBP1</i>	<i>TBC1D1</i>	<i>TBC1D21</i>	<i>TBC1D3</i>	<i>TBC1D5</i>	<i>TBLIX</i>	<i>TBP</i>	<i>TBX15</i>
<i>TBX22</i>	<i>TBX3</i>	<i>TCF20</i>	<i>TCF4</i>	<i>TCP10</i>	<i>TCP11</i>	<i>TEK</i>	<i>TESC</i>	<i>TEX35</i>	<i>TFDP1</i>
<i>TGDS</i>	<i>TGM2</i>	<i>TGM5</i>	<i>THBS2</i>	<i>THEM5</i>	<i>THOC1</i>	<i>THSD7A</i>	<i>THSD7B</i>	<i>TIMD4</i>	<i>TIMM44</i>
<i>TIMP3</i>	<i>TJP3</i>	<i>TLE1</i>	<i>TLL1</i>	<i>TMC2</i>	<i>TMED8</i>	<i>TMEM104</i>	<i>TMEM120B</i>	<i>TMEM132D</i>	<i>TMEM145</i>
<i>TMEM247</i>	<i>TMEM80</i>	<i>TMEM87A</i>	<i>TMTc4</i>	<i>TMX3</i>	<i>TNFAIP6</i>	<i>TNFSF4</i>	<i>TNN</i>	<i>TNNT1</i>	<i>TNR</i>
<i>TNS3</i>	<i>TP53BP1</i>	<i>TPCN1</i>	<i>TPH2</i>	<i>TPMT</i>	<i>TPTE</i>	<i>TRIM33</i>	<i>TRIM51</i>	<i>TRIM58</i>	<i>TRIML1</i>
<i>TRIO</i>	<i>TRIP11</i>	<i>TRMT112</i>	<i>TRPC5</i>	<i>TRUB1</i>	<i>TSGA10</i>	<i>TSKS</i>	<i>TSPAN12</i>	<i>TSR2</i>	<i>TTF2</i>
<i>TTN</i>	<i>TUBA3C</i>	<i>TUBGCP4</i>	<i>TUBGCP5</i>	<i>TYK2</i>	<i>TYRPI</i>	<i>U2AF1</i>	<i>U2AF2</i>	<i>UBASH3A</i>	<i>UBE2Q1</i>
<i>UBE4B</i>	<i>UCHL3</i>	<i>UCK2</i>	<i>UGT8</i>	<i>ULK3</i>	<i>UMOD</i>	<i>UNC13A</i>	<i>UNC13D</i>	<i>UNC5D</i>	<i>USP12</i>
<i>USP34</i>	<i>USP39</i>	<i>USP45</i>	<i>USP48</i>	<i>VAV1</i>	<i>VEZF1</i>	<i>VILL</i>	<i>VIT</i>	<i>VPS13A</i>	<i>VPS33B</i>
<i>VSIG4</i>	<i>WAS</i>	<i>WASL</i>	<i>WDR44</i>	<i>WDR52</i>	<i>WDR62</i>	<i>WDR66</i>	<i>WDR72</i>	<i>WDTCI</i>	<i>WLS</i>
<i>WSCD2</i>	<i>WWP2</i>	<i>XBPI</i>	<i>XPO4</i>	<i>XPO5</i>	<i>ZAP70</i>	<i>ZBTB8OS</i>	<i>ZC3H13</i>	<i>ZC3H7B</i>	<i>ZDHHC11</i>
<i>ZFC3H1</i>	<i>ZFR</i>	<i>ZMYM4</i>	<i>ZNF143</i>	<i>ZNF350</i>	<i>ZNF385A</i>	<i>ZNF414</i>	<i>ZNF512B</i>	<i>ZNF541</i>	<i>ZNF563</i>
<i>ZNF614</i>	<i>ZNF687</i>	<i>ZNF705B</i>	<i>ZNF705G</i>	<i>ZNF711</i>	<i>ZNF804B</i>	<i>ZSWIM8</i>			

Table S2 Clinicopathological characteristics of patients enrolled

Sample ID	Gender	Age at Diagnosis	Site	TAT Pathology	Smoking	Drinking Status	Stage	Adjuvant Treatment	Relapse	Follow-up Time (month)
P001	Male	55	Buccal	Negative	Yes	Yes	II	No	No	27.2
P002	Male	51	Buccal	Negative	Yes	No	III	Radiotherapy	No	26.8
P003	Male	49	Base of Tongue	Negative	Yes	No	II	No	Yes	5.4
P004	Male	53	Tongue	Negative	Yes	Yes	II	No	No	26.3
P005	Male	47	Gingiva	Negative	No	No	II	Radiotherapy	No	25.4
P006	Male	68	Larynx	Negative	No	No	IV	Radiotherapy	Yes	6.6
P007	Male	66	Hypopharynx	Negative	Yes	Yes	III	Radiotherapy	No	25.8
P008	Male	52	Hypopharynx	Dysplasia	Yes	Yes	IV	Chemoradiotherapy	No	24.3
P009	Male	57	Tonsil	Negative	Yes	Yes	I	No	Yes	7.7
P010	Female	57	Hypopharynx	Negative	No	No	II	No	No	24
P011	Male	64	Larynx	Negative	Yes	No	IV	Radiotherapy	No	22.7
P012	Male	69	Larynx	Negative	Yes	Yes	II	No	No	23.8
P013	Male	75	Larynx	Negative	Yes	No	IV	Radiotherapy	No	22.4
P014	Male	58	Larynx	Negative	Yes	Yes	II	No	No	22.6
P015	Male	59	Larynx	Negative	Yes	Yes	IV	Radiotherapy	No	21.2
P016	Male	67	Hypopharynx	Dysplasia	Yes	Yes	IV	Radiotherapy	No	21.1
P017	Male	61	Hypopharynx	Negative	Yes	Yes	IV	Radiotherapy	No	20.7
P018	Male	62	Larynx	Negative	Yes	Yes	IV	Radiotherapy	Yes	5.6
P019	Male	51	Hypopharynx	Dysplasia	Yes	Yes	IV	Chemoradiotherapy	Yes	9.2
P020	Male	51	Larynx	Negative	Yes	No	III	No	No	18.6
P021	Male	63	Larynx	Negative	Yes	Yes	IV	Radiotherapy	No	18.9
P022	Male	65	Hypopharynx	Negative	Yes	Yes	IV	Radiotherapy	No	19.5
P023	Male	65	Hypopharynx	Negative	No	No	IV	Chemoradiotherapy	No	20.3

			arynx					therapy		
P024	Male	52	Larynx	Negative	Yes	No	IV	Radiotherap y	No	18.6
P025	Male	46	Larynx	NA	Yes	Yes	II	No	Yes	4.6
P026	Male	61	Hypoph arynx	Dysplasia	Yes	Yes	IV	No	Yes	1.3
P027	Male	46	Hypoph arynx	Dysplasia	Yes	Yes	IV	Radiotherap y	Yes	2.2

Table S3. Association between mutational features with pathological diagnosis, disease recurrence and patients' clinicopathological characteristics

a.

		Premalignant Lesion	Negative	P value (Fisher's Exact test)
TAT <i>TP53 status</i>	Mutated	5	7	0.012 (*)
	Wild Type	0	14	

b.

Mutant p53 expression	negative	mild	moderate	strong	P value (Fisher's Exact test)
<i>TP53^{mut}</i> TATs (n=12)	0	3	7	2	
<i>TP53^{wt}</i> TATs (n=14)	8	5	1	0	<0.0001

c.

		Premalignant Lesion	Negative	P value (Fisher's Exact test)
Relapse	+	3	5	0.28
	-	2	16	

Patient ID	Tissue Type	Hugo Symbol	Chromosome	Start bp	End bp	Reference Allele	Mutated Allele	chGVS	phGVS	Variant Classification	ExIn ID
P001	Primary Tumor	TP53	17	7577083	7577084	T	A	c. 854A>T	p. E285V	missense	EX8
P002	Primary Tumor	TP53	17	7578525	7578526	C	A	c. 404G>T	p. C135F	missense	EX5
P003	Primary Tumor	TP53	17	7577538	7577539	G	A	c. 742C>T	p. R248W	missense	EX7
P003	TAT	TP53	17	7577537	7577538	A	G	c. 403T>C	p. C135R	missense	EX5
P003	TAT	TP53	17	7577537	7577538	C	T	c. 743G>A	p. R248Q	missense	EX7
P004	Primary Tumor	TP53	17	7577021	7577022	G	A	c. 916G>T	p. R306*	nonsense	EX8
P004	TAT	TP53	17	7577504	7577505	T	.	c. 776de1A	p. D259Afs*86	frameshift	EX7
P004	TAT	TP53	17	7579373	7579374	C	A	c. 313G>T	p. G105C	missense	EX4
P004	TAT	TP53	17	7577021	7577022	G	A	c. 916G>T	p. R306*	nonsense	EX8
P005	Primary Tumor	TP53	17	7577538	7577539	G	A	c. 742C>T	p. R248W	missense	EX7
P005	TAT	TP53	17	7578412	7578413	C	A	c. 517G>T	p. V173L	missense	EX5
P005	TAT	TP53	17	7577093	7577094	G	A	c. 844G>T	p. R282W	missense	EX8
P005	TAT	TP53	17	7578262	7578263	G	A	c. 586G>T	p. R196*	nonsense	EX6
P005	TAT	TP53	17	7578211	7578212	G	A	c. 637G>T	p. R213*	nonsense	EX6
P006	Primary Tumor	TP53	17	7579527	7579528	C	T	c. 159G>A	p. W53*	nonsense	EX4
P006	TAT	TP53	17	7579527	7579528	C	T	c. 159G>A	p. W53*	nonsense	EX4
P007	Primary Tumor	TP53	17	7577021	7577022	G	A	c. 916G>T	p. R306*	nonsense	EX8
P007	TAT	TP53	17	7578211	7578212	G	A	c. 637G>T	p. R213*	nonsense	EX6
P007	TAT	TP53	17	7577021	7577022	G	A	c. 916G>T	p. R306*	nonsense	EX8
P008	Primary Tumor	TP53	17	7578431	7578447	TGACTCTCTTGTAGATG	.	c. 485_500del1CTACAAGCAGTCACA	p. 1162Sfs*3	frameshift	EX5
P008	TAT	TP53	17	7579396	7579397	A	.	c. 290de1T	p. V97Afs*26	frameshift	EX4
P008	TAT	TP53	17	7577141	7577142	C	A	c. 796G>T	p. G266*	nonsense	EX8
P008	TAT	TP53	17	7577120	7577121	G	A	c. 817G>T	p. R273C	missense	EX8
P009	Primary Tumor	TP53	17	7579409	7579410	G	.	c. 274C[4>3] (std: c. 274C[3] alt: c. 277del1C)	p. L93Cfs*30 p. Leu93Cysfs*30	frameshift	EX3
P011	Primary Tumor	TP53	17	7578463	7578464	G	.	c. 464C[3>2] (std: c. 464C[2] alt: c. 466del1C)	p. R156Afs*14 p. Arg156Alafs*14	frameshift	EX4
P011	Primary Tumor	TP53	17	7579470	7579470	.	G	c. 211C[6>7] (std: c. 216dupC alt: c. 216_217insC)	p. V73Rfs*76 p. Val173Argfs*76	frameshift	EX3
P012	Primary Tumor	TP53	17	7576851	7576852	C	A	c. 993+1G>T	.	splice-5	IVS8
P013	Primary Tumor	TP53	17	7578405	7578406	C	T	c. 524G>A	p. R175H p. Arg175His	missense	EX4
P013	TAT	TP53	17	7577558	7577559	G	T	c. 722G>A	p. S241Y p. Ser241Tyr	missense	EX6
P014	Primary Tumor	TP53	17	7578473	7578473	.	A	c. 456_457insT	p. P1153Sfs*28 p. Pro153Serfs*28	frameshift	EX4
P015	Primary Tumor	TP53	17	7578238	7578239	C	A	c. 610G>T	p. E204* p. Glu204*	nonsense	EX5
P015	TAT	TP53	17	7578238	7578239	C	A	c. 610G>T	p. E204* p. Glu204*	nonsense	EX5
P016	Primary Tumor	TP53	17	7578454	7578455	C	G	c. 475G>C	p. A115P p. Ala115Pro	missense	EX4
P016	Primary Tumor	TP53	17	7577572	7577573	G	T	c. 708G>A	p. Y236* p. Tyr236E	missense	EX6
P016	TAT	TP53	17	7578223	7578224	T	A	c. 625A>T	p. R209* p. Arg209E	missense	EX5
P017	Primary Tumor	TP53	17	7578498	7578499	T	G	c. 431A>C	p. Q144P p. Gln144Pro	missense	EX4
P018	Primary Tumor	TP53	17	7578405	7578406	C	T	c. 524G>A	p. R175H p. Arg175His	missense	EX4
P019	Primary Tumor	TP53	17	7577532	7577533	G	A	c. 748G>T	p. P250S p. Pro250Ser	missense	EX6
P019	Primary Tumor	TP53	17	7577533	7577534	C	A	c. 747G>T	p. R249S p. Arg249Ser	missense	EX6
P019	Primary Tumor	TP53	17	7578207	7578208	T	C	c. 641A>G	p. H214R p. His214Arg	missense	EX5
P019	TAT	TP53	17	7577533	7577539	G	A	c. 742G>T	p. R248W p. Arg248Trp	missense	EX6
P019	TAT	TP53	17	7578189	7578190	T	C	c. 659A>G	p. Y220C p. Tyr220Cys	missense	EX5
P019	TAT	TP53	17	7578234	7578235	T	C	c. 614A>G	p. Y205C p. Tyr205Cys	missense	EX5
P021	Primary Tumor	TP53	17	7578418	7578419	C	A	c. 511G>T	p. E171* p. Glu171*	nonsense	EX4
P022	Primary Tumor	TP53	17	7577517	7577518	T	.	c. 763de1A	p. D255Sfs*90 p. Ile255Serfs*90	frameshift	EX6
P023	Primary Tumor	TP53	17	7577063	7577064	T	A	c. 874G>T	p. K292* p. Lys292*	nonsense	EX7
P025	Primary Tumor	TP53	17	7577547	7577548	C	T	c. 733G>A	p. G245S p. Gly245Ser	missense	EX6
P026	Primary Tumor	TP53	17	7577506	7577507	T	A	c. 774A>T	p. E258D p. Glu258Asp	missense	EX6
P026	TAT	TP53	17	7577021	7577022	G	A	c. 916G>T	p. R306* p. Arg306E	nonsense	EX7
P026	TAT	TP53	17	7577137	7577138	C	A	c. 800G>T	p. R267L p. Arg267Leu	missense	EX7
P026	TAT	TP53	17	7578234	7578235	T	C	c. 614A>G	p. Y205* p. Tyr205Cys	missense	EX5
P026	TAT	TP53	17	7578395	7578396	G	T	c. 534G>A	p. H178Q p. His178Gln	missense	EX4
P027	Primary Tumor	TP53	17	7577514	7577517	TGA	.	c. 761TCA[2>1] (std: c. 761TCA[1] alt: c. 764_766del1TCA)	p. I1254[2>1] (std: p. I1254[1] alt: p. I1255del1) p. I11e254[2>1] (std: p. I11e254[1] alt: p. I11e255del1)	cds-del	EX6
P027	TAT	TP53	17	7577514	7577517	TGA	.	c. 761TCA[2>1] (std: c. 761TCA[1] alt: c. 764_766del1TCA)	p. I1254[2>1] (std: p. I1254[1] alt: p. I1255del1) p. I11e254[2>1] (std: p. I11e254[1] alt: p. I11e255del1)	cds-del	EX6
P027	TAT	TP53	17	7578189	7578190	T	C	c. 659A>G	p. Y220C p. Tyr220Cys	missense	EX5
P027	TAT	TP53	17	7577120	7577121	G	T	c. 817C>A	p. R273S p. Arg273Ser	missense	EX7

a.Cox-proportional multivariate analysis of TP53 mutations in TATs together with other clinicopathological characteristics

	p value	HR	95% CI
TP53 mutations in TAT	0.010	6.764	1.595-28.687
site (oral cavity & oropharynx, larynx, hypopharynx)	0.923		
stage (early, advanced)	0.885		
age (<60, ≥60)	0.551		
smoking	0.330		
adjuvant treatment	0.062		

b.Sensitivity and specificity of tumor-specific mutations in post- operative plasma or saliva, and TP53 mutations in TATs in predicting HNSCC relapse

	State	Relapsing (n)	Non-relapsing(n)	Sensitivity	Specificity
Post-operative ctDNA	Positive (n)	5	1	62.5%	94.7%
	Negative (n)	3	18		
Post-operative SctDNA	Positive (n)	5	2	62.5%	89.5%
	Negative (n)	3	17		
TP53 mutation in TAT	Positive (n)	5	7	62.5%	63.2%
	Negative (n)	2	12		

c. Cox-proportional multivariate analysis of mutations detected in post-operative plasma together with other clinicopathological characteristics

	p value	HR	95% CI
post-operative ctDNA	0.005	8.030	1.889-34.131
site (oral cavity & oropharynx, larynx, hypopharynx)	0.958		
stage (early, advanced)	0.644		
age (<60, ≥60)	0.740		
smoking	0.764		
adjuvant treatment	0.219		

d. Cox-proportional multivariate analysis of mutations detected in post-operative saliva together with other clinicopathological characteristics

	<i>p</i> value	HR	95% CI
post-operative SctDNA	0.005	7.851	1.842-33.472
site (oral cavity & oropharynx, larynx, hypopharynx)	0.228		
stage (early, advanced)	0.732		
age (<60, ≥60)	0.709		
smoking	0.241		
adjuvant treatment	0.254		

e. Tumor- and/or TAT-specific mutations in post-operative plasma or saliva in predicting HNSCC relapse

	State	Relapsing (n)	Non-relapsing(n)	Sensitivity	Specificity
Post-operative ctDNA	Positive (n)	6	1	75.0%	94.7%
	Negative (n)	2	18		
Post-operative SctDNA	Positive (n)	7	4	87.5%	78.9%
	Negative (n)	1	15		

Table S6. Association between mutational features with patients' smoking history and TNM stages.

a.

		Smoker	Non-smoker	P value (Fisher's Exact test)
Pre-ctDNA	Positive	17	2	0.56
	Negative	6	2	

b.

		Smoker	Non-smoker	P value (Fisher's Exact test)
Pre-SctDNA	Positive	13	4	0.26
	Negative	10	0	

c.

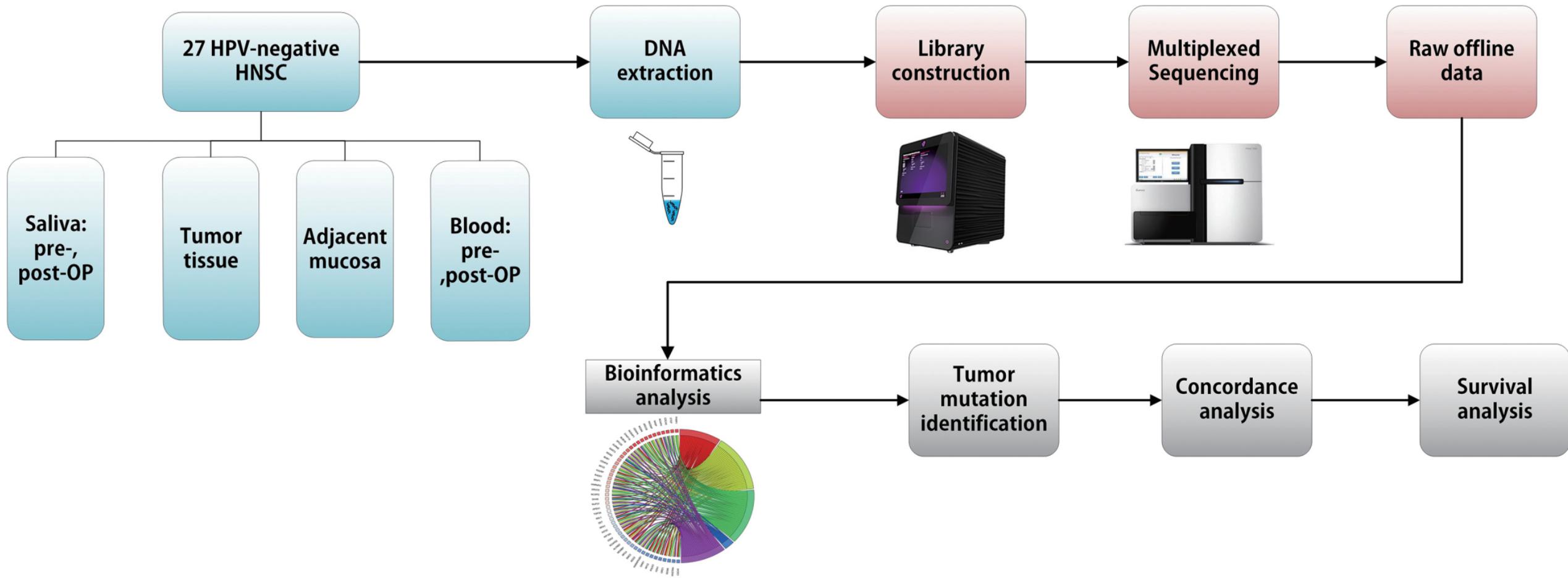
		Smoker	Non-smoker	P value (Fisher's Exact test)
Post-ctDNA	Positive	6	1	1.00
	Negative	17	3	

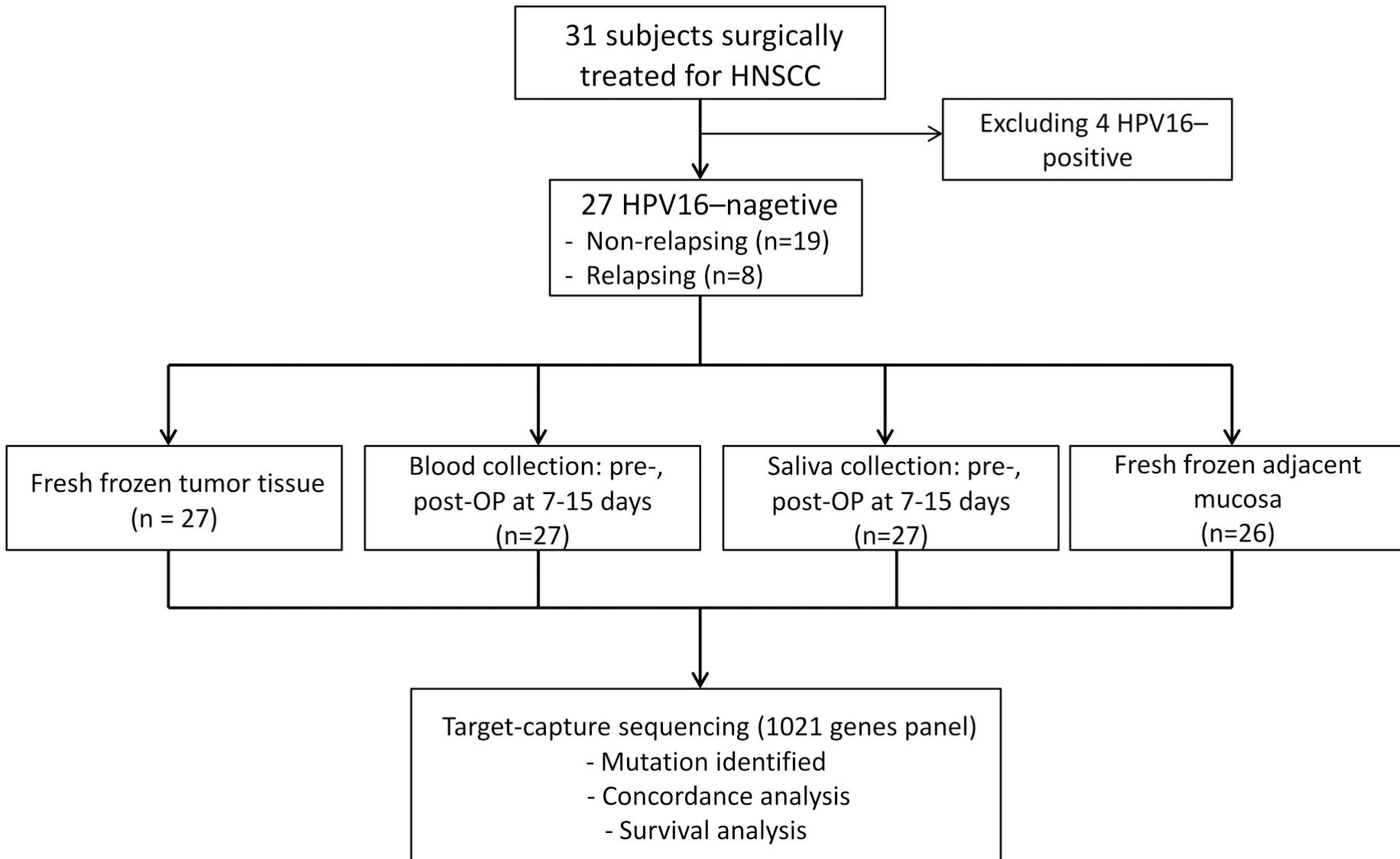
d.

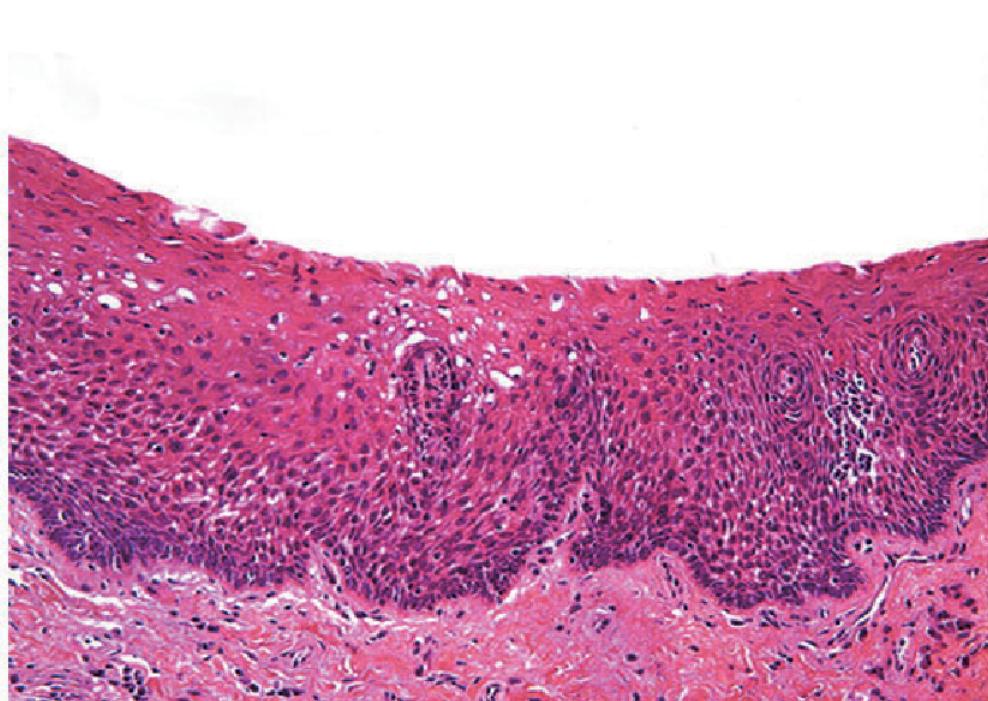
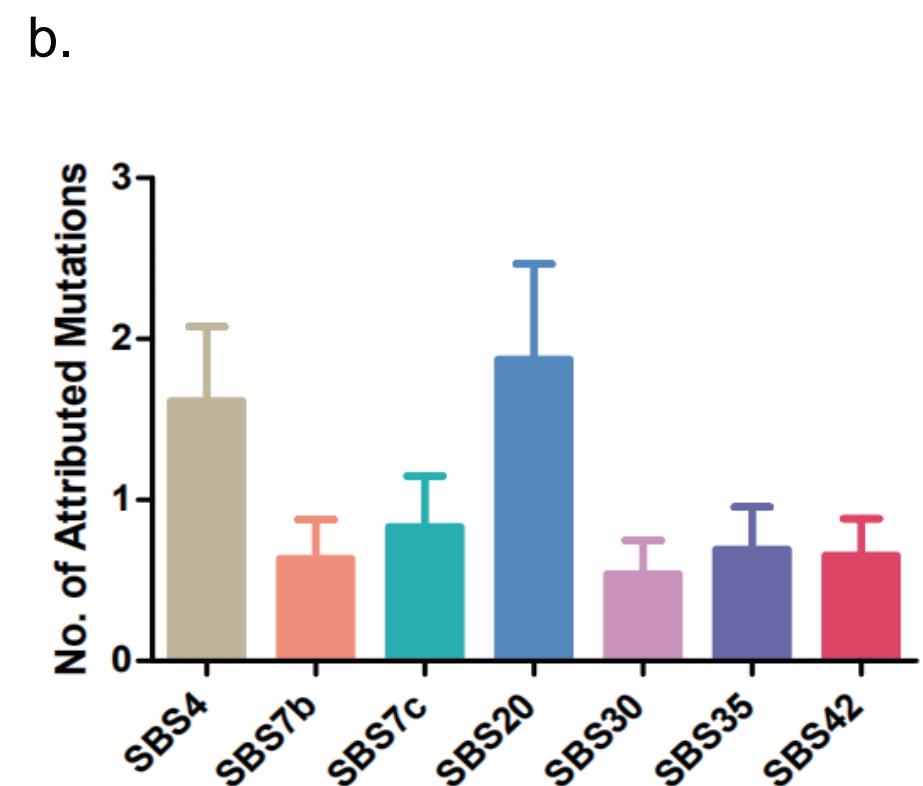
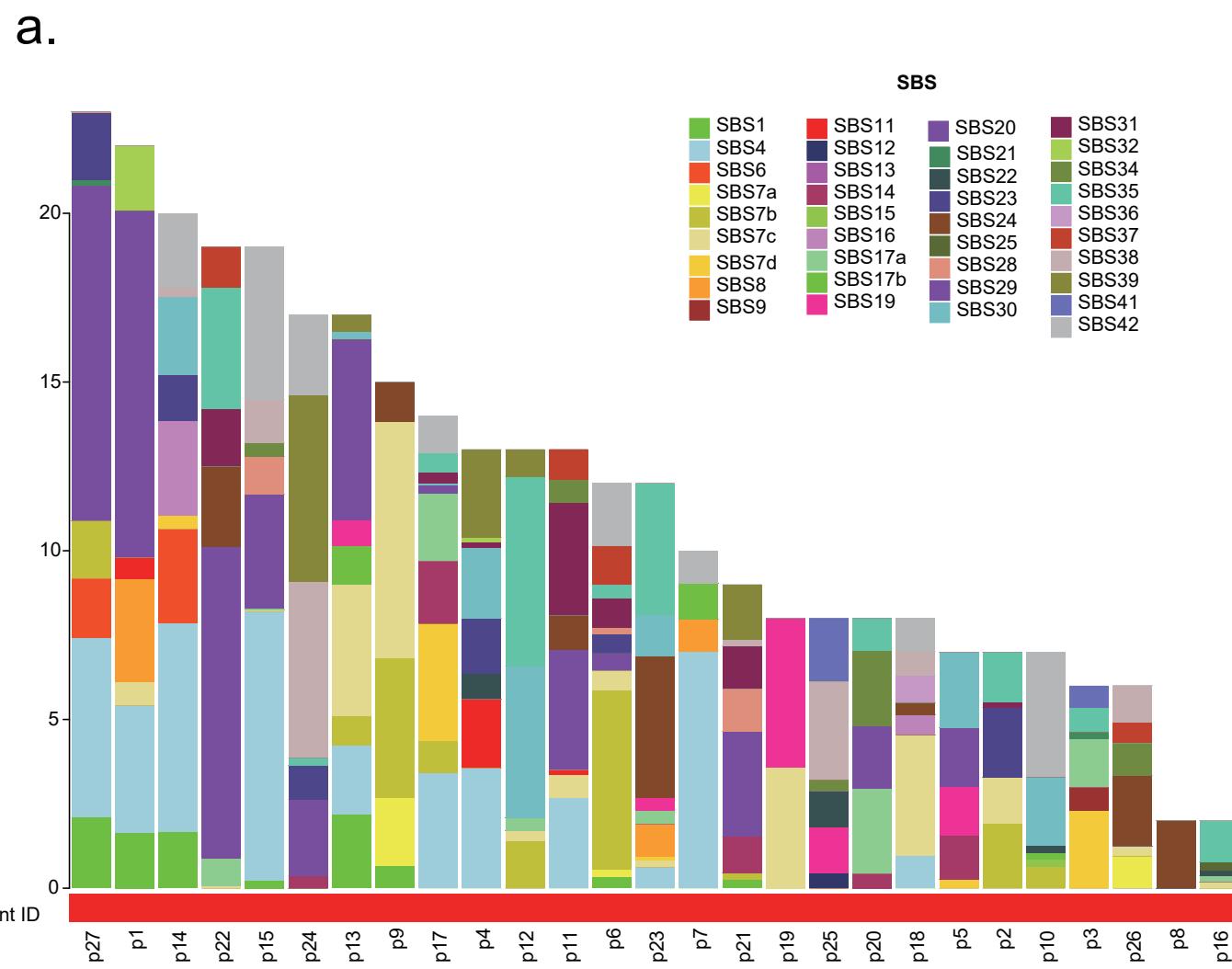
		Smoker	Non-smoker	P value (Fisher's Exact test)
Post-SctDNA	Positive	8	3	0.27
	Negative	15	1	

e.

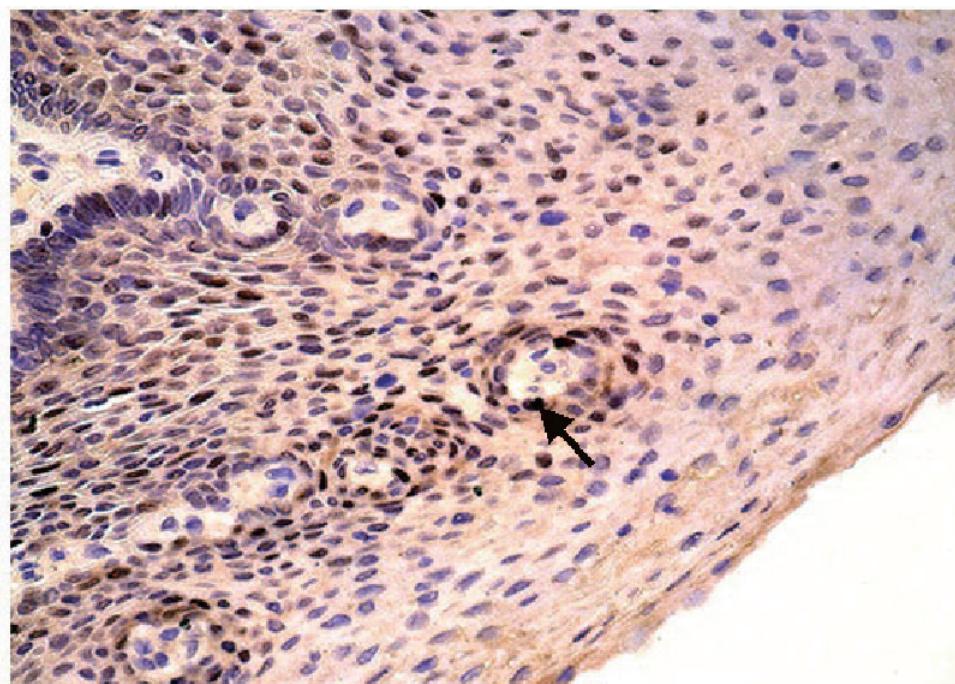
		TNM I-II	TNM III-IV	P value (Fisher's Exact test)
Alterations in TAT	Positive	6	13	1.00
	Negative	2	5	





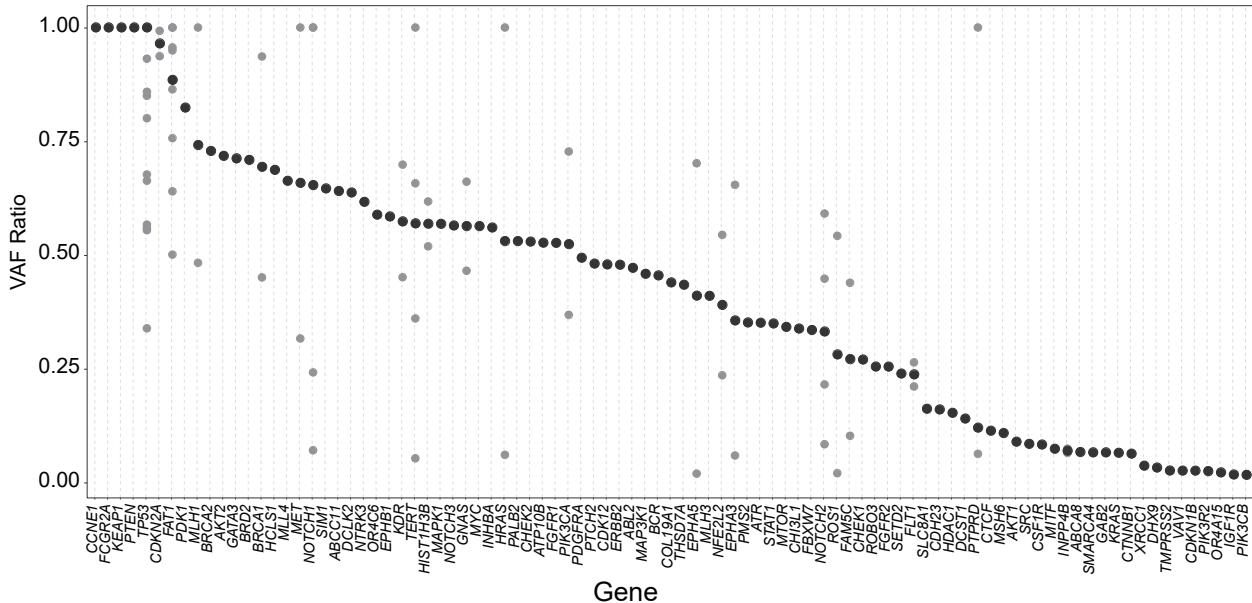


H&E staining



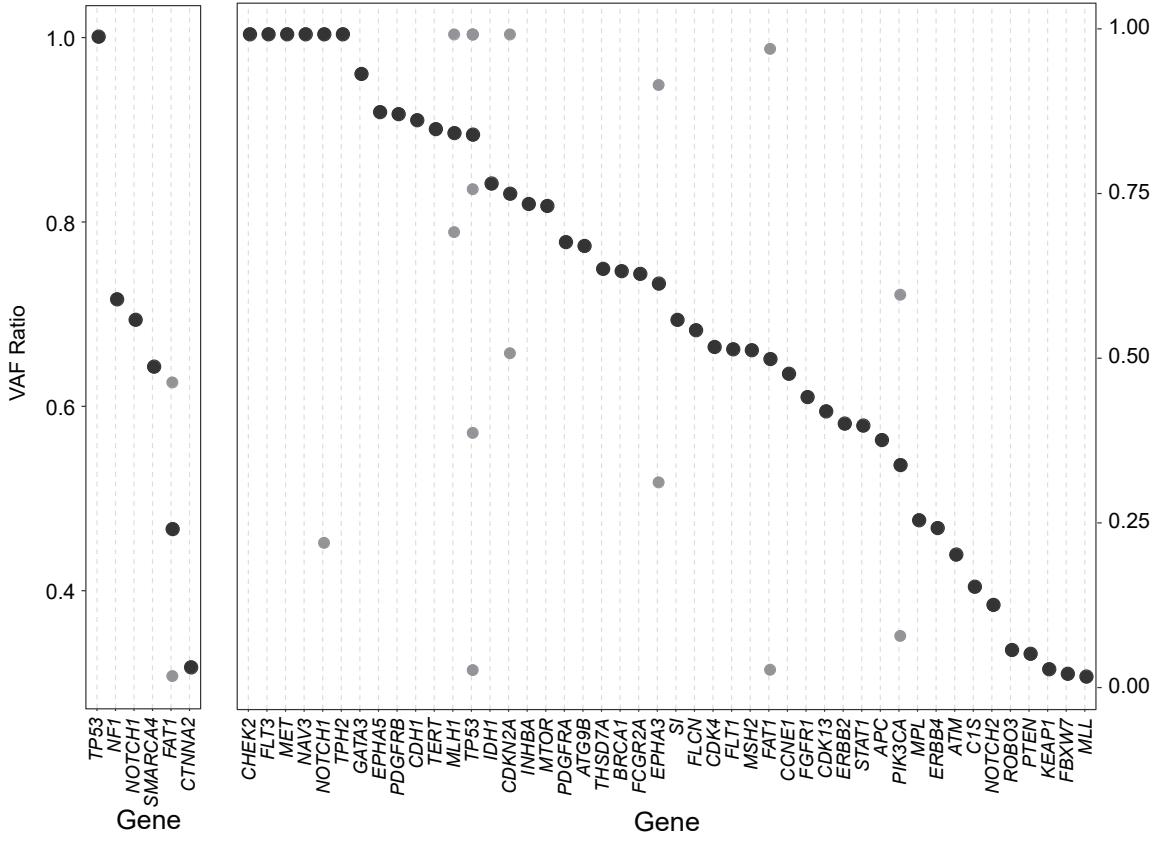
mutant p53

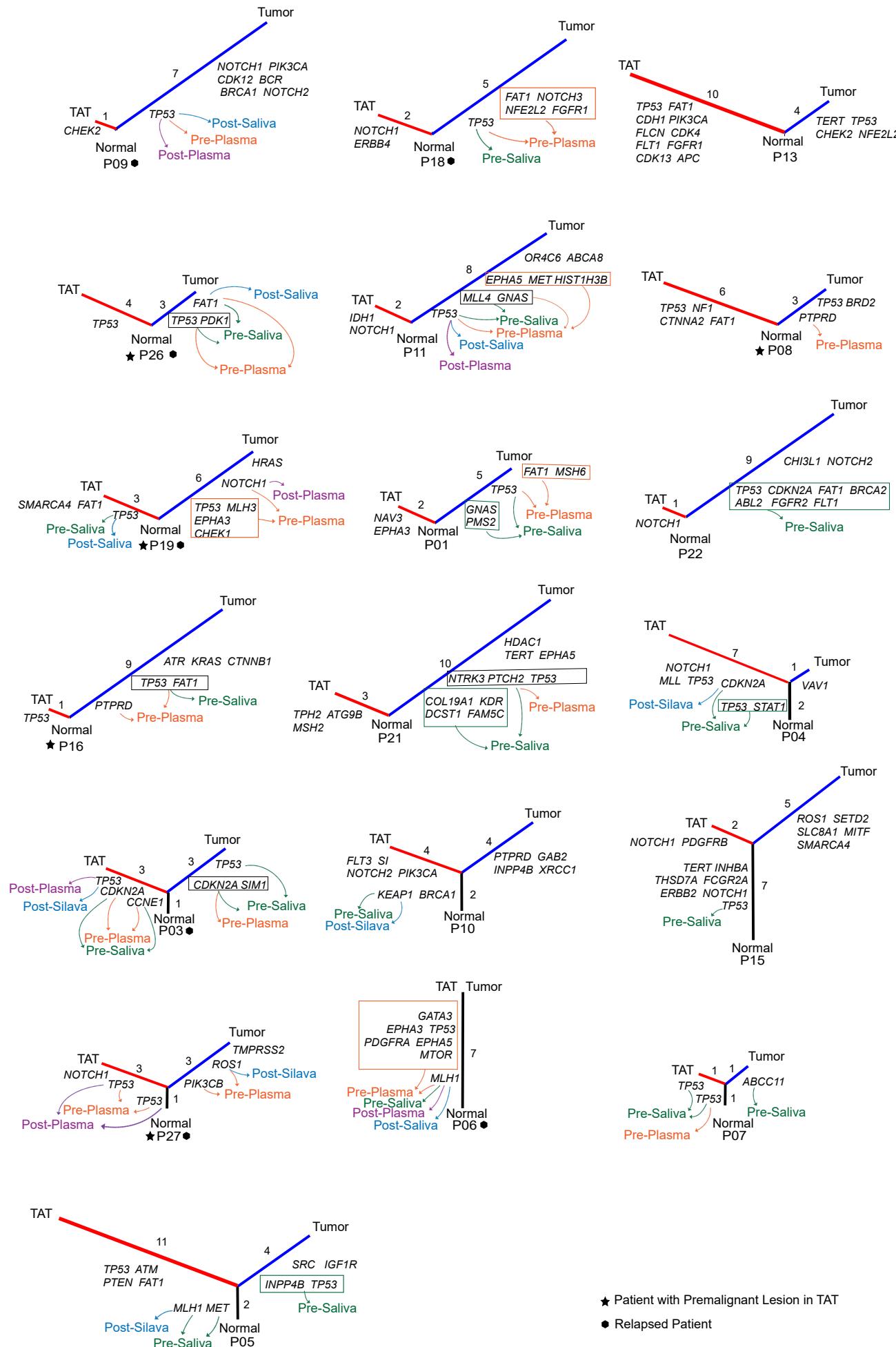
Primary Tumor

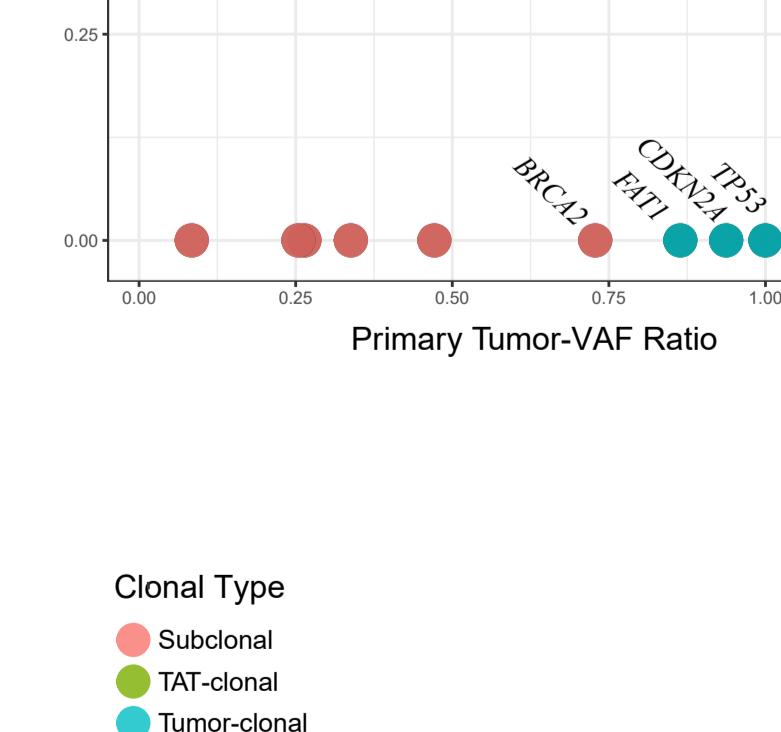
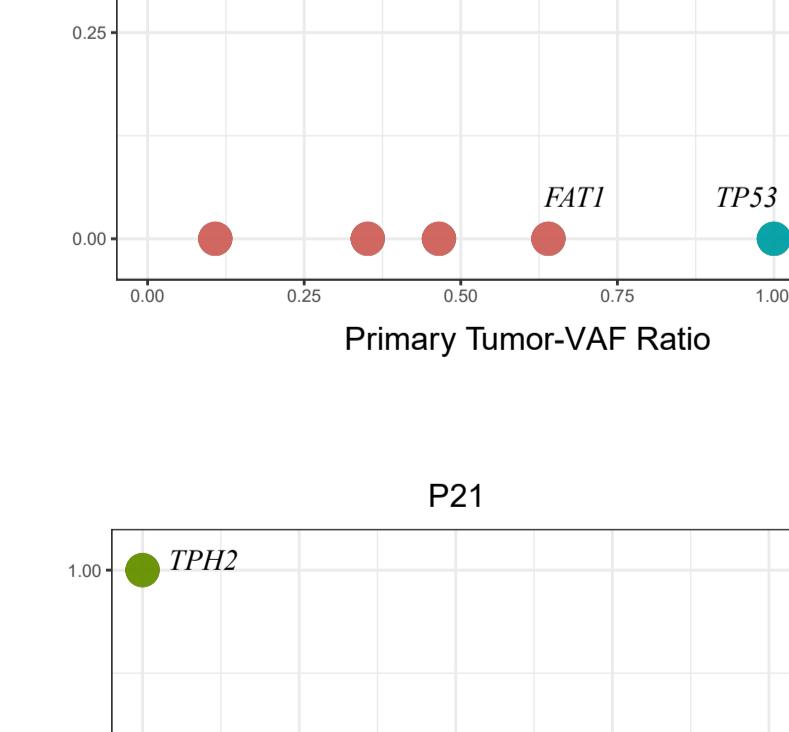
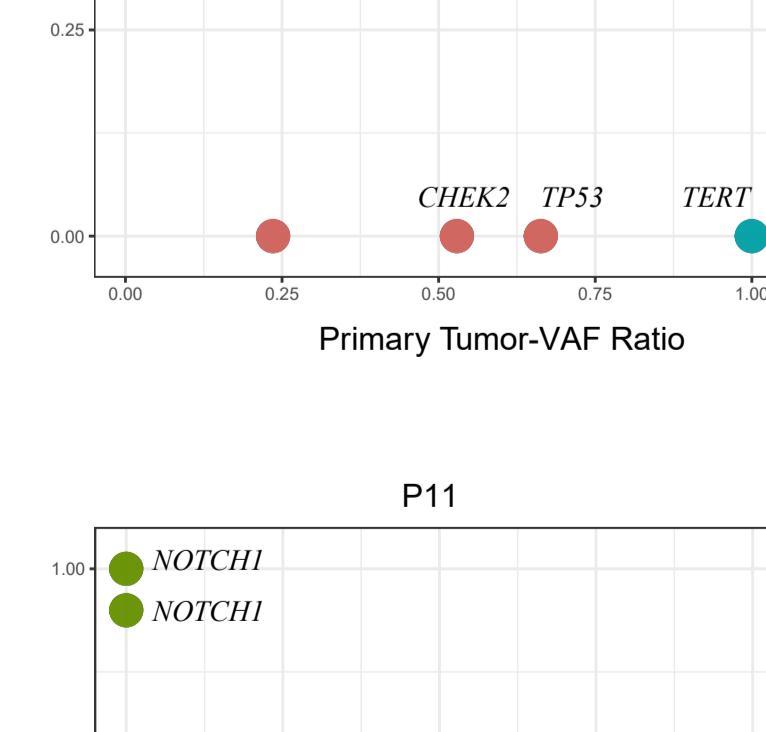
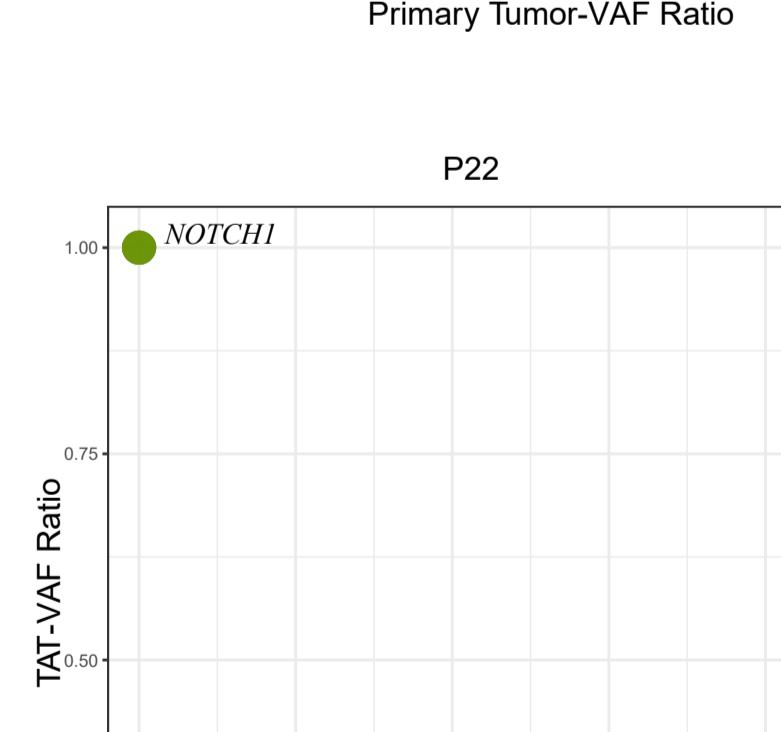
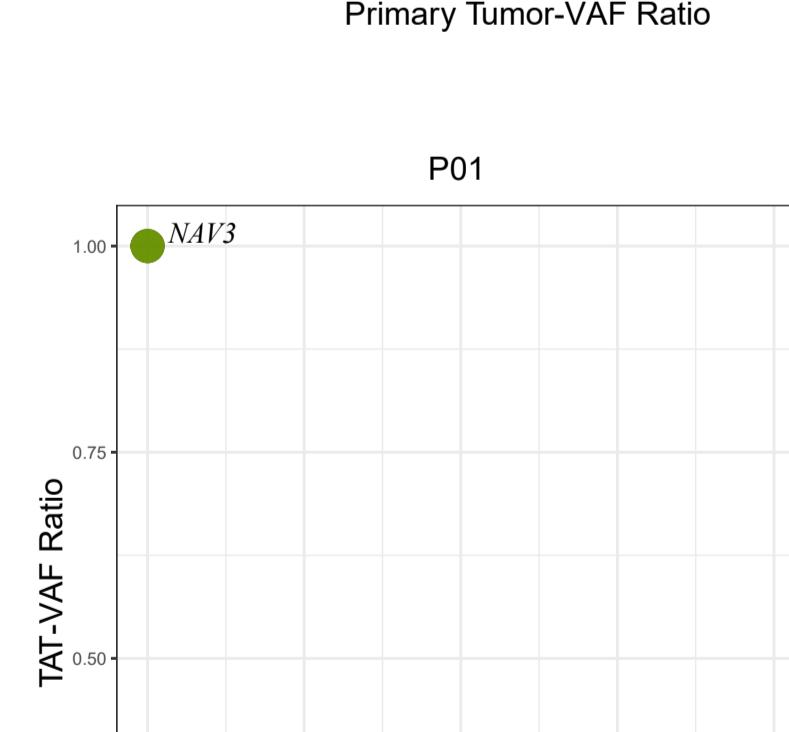
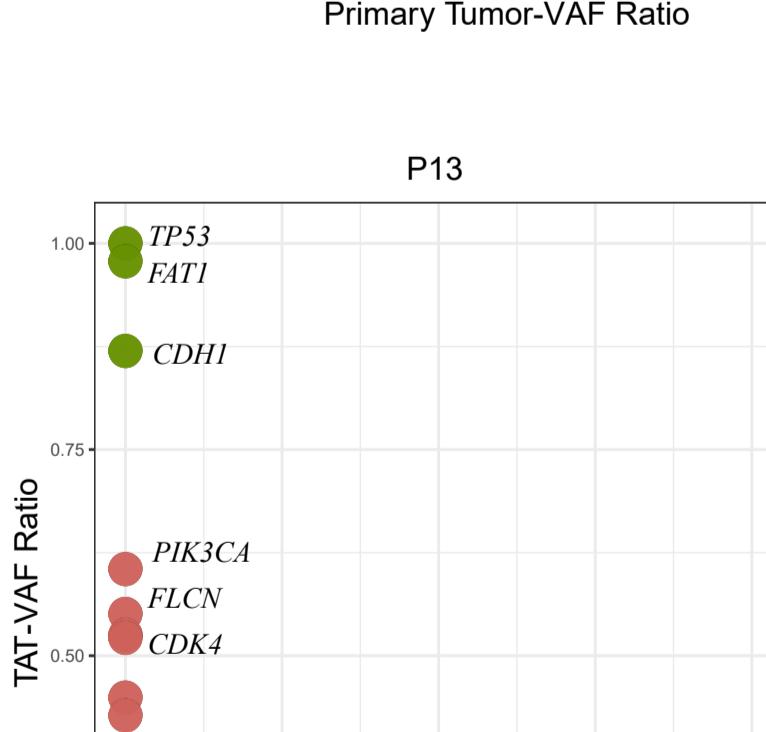
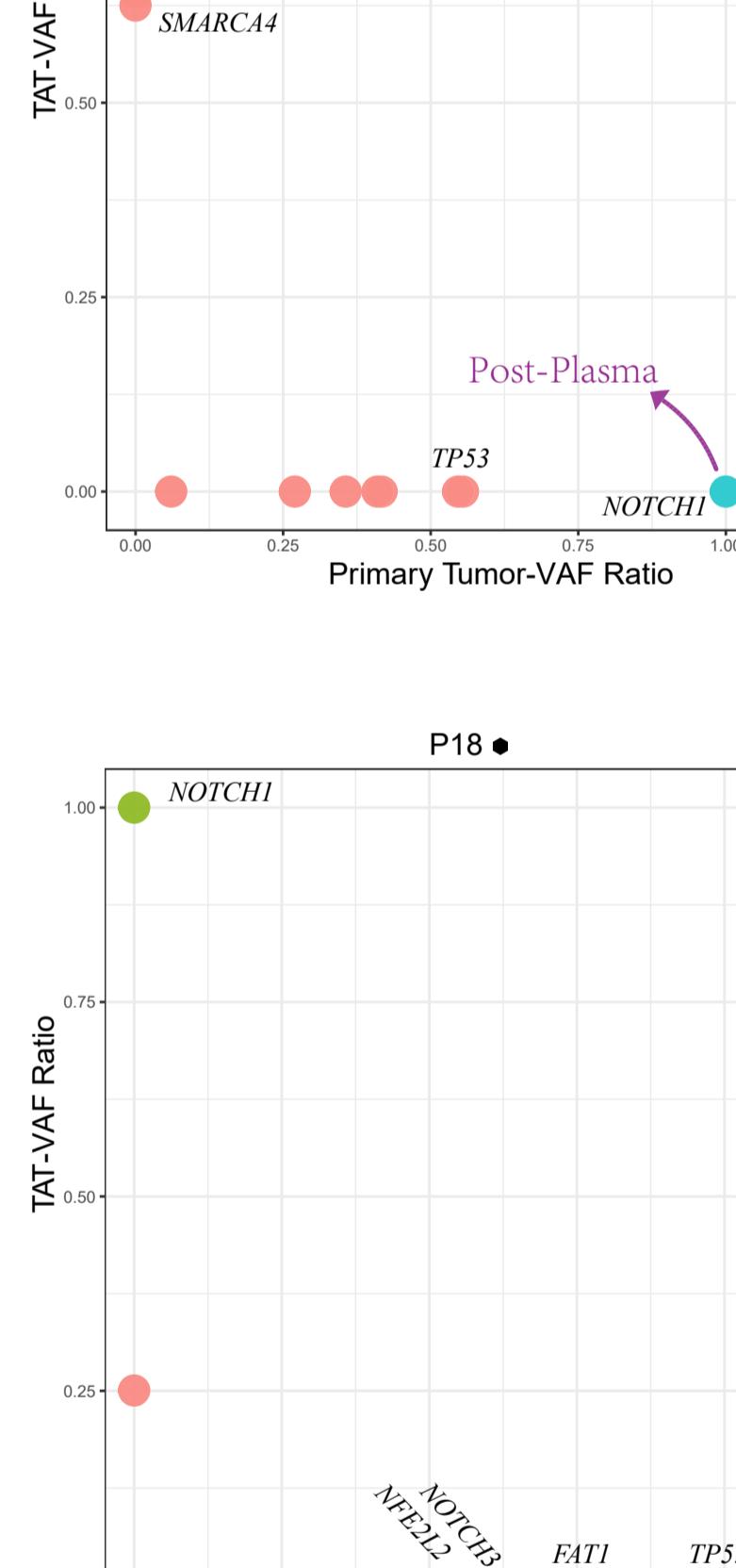
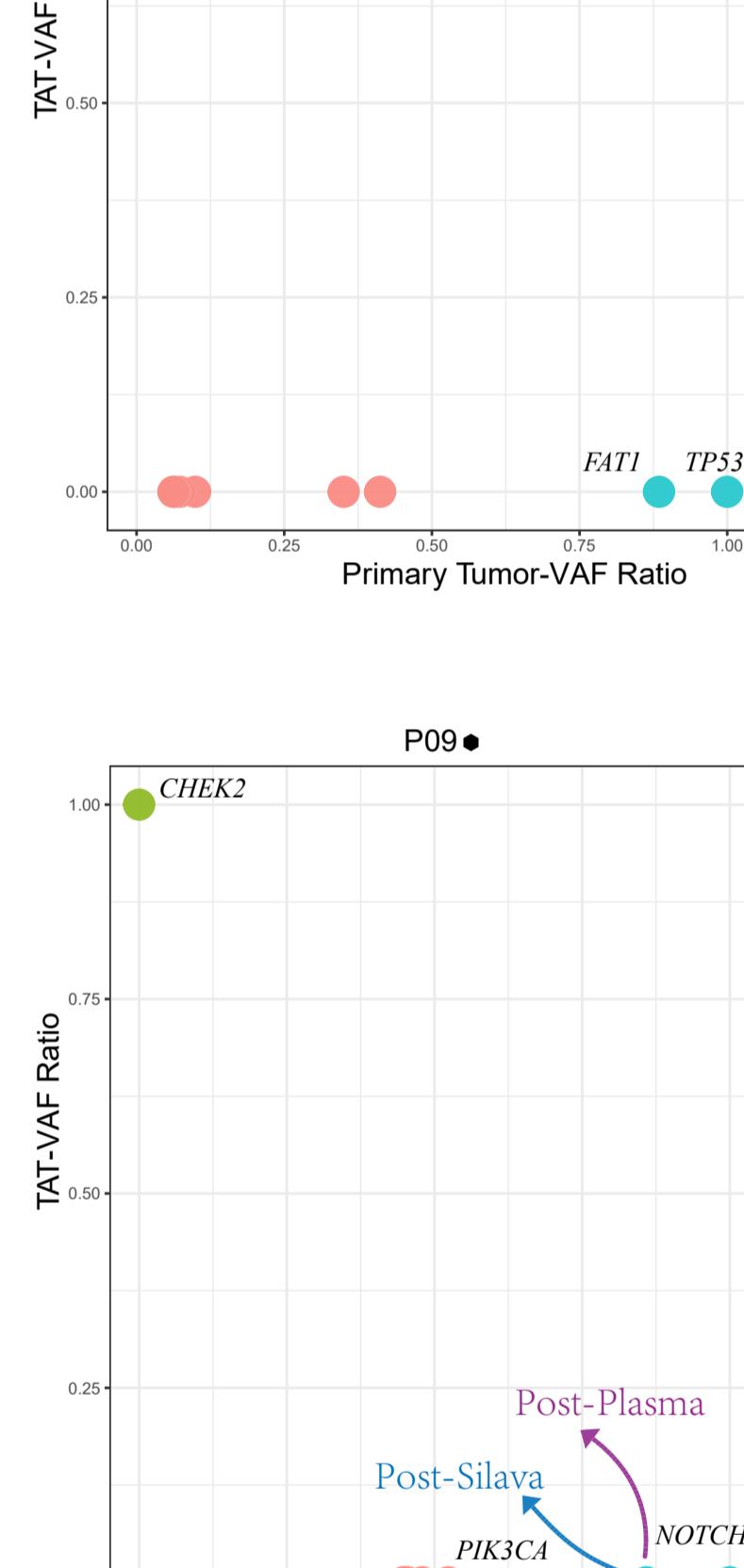
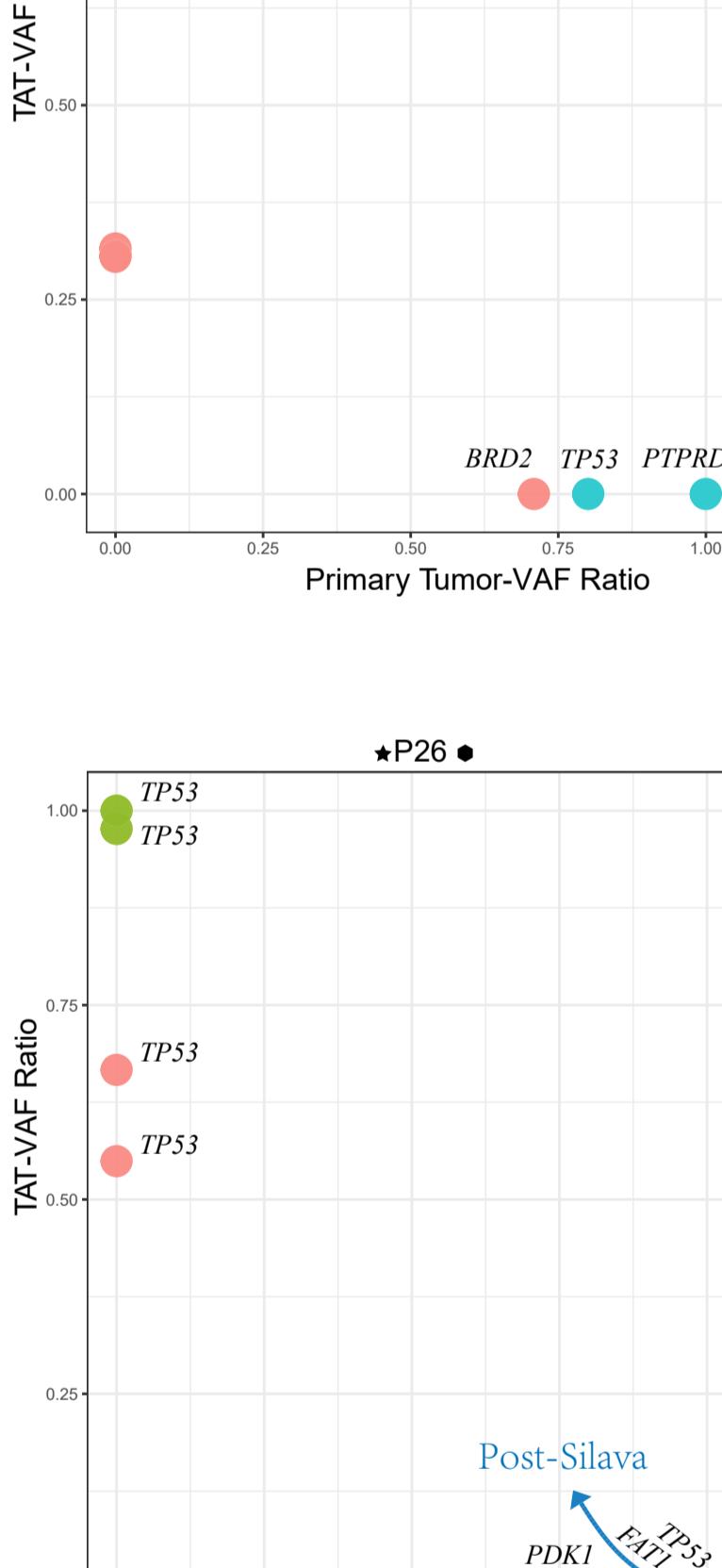
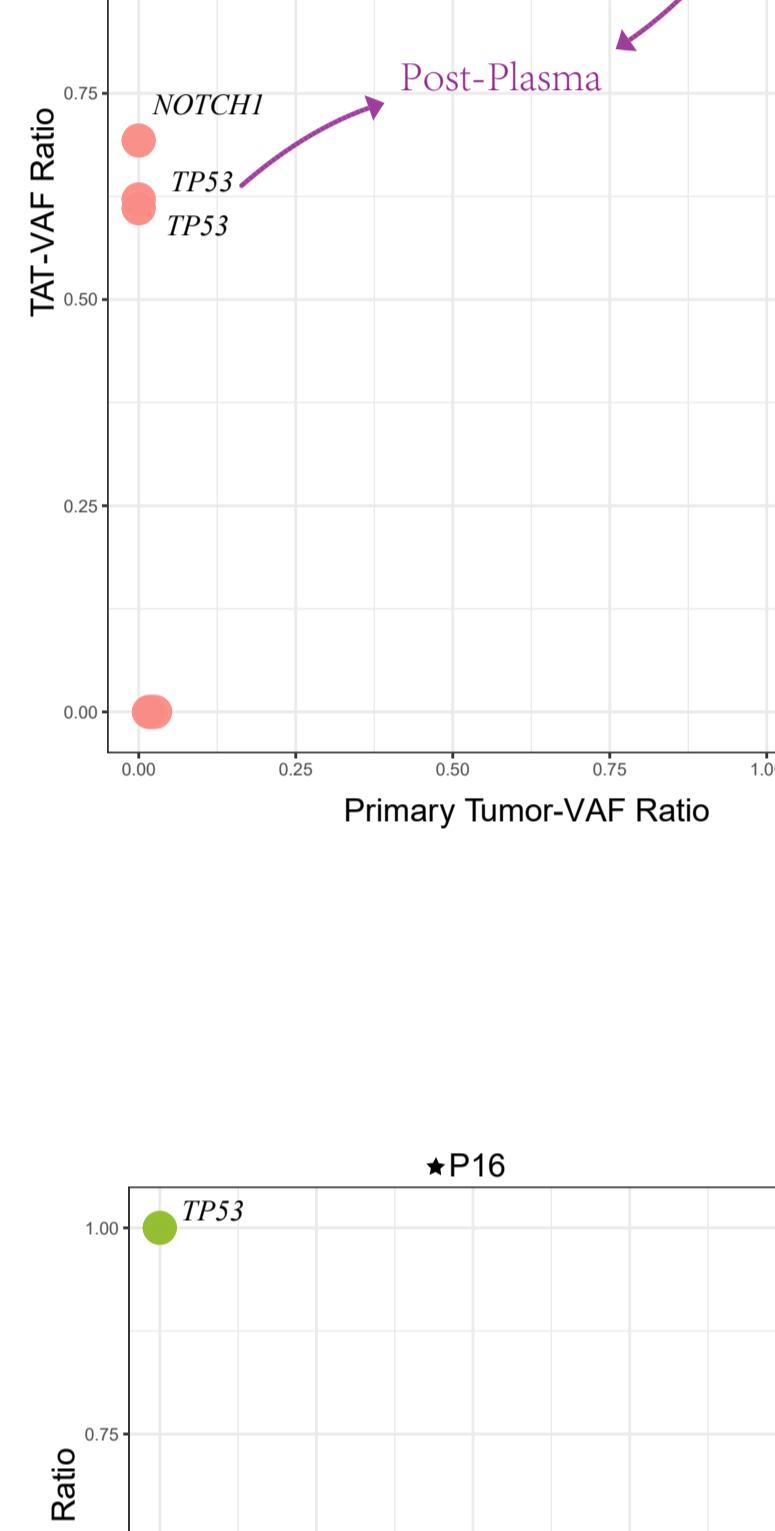
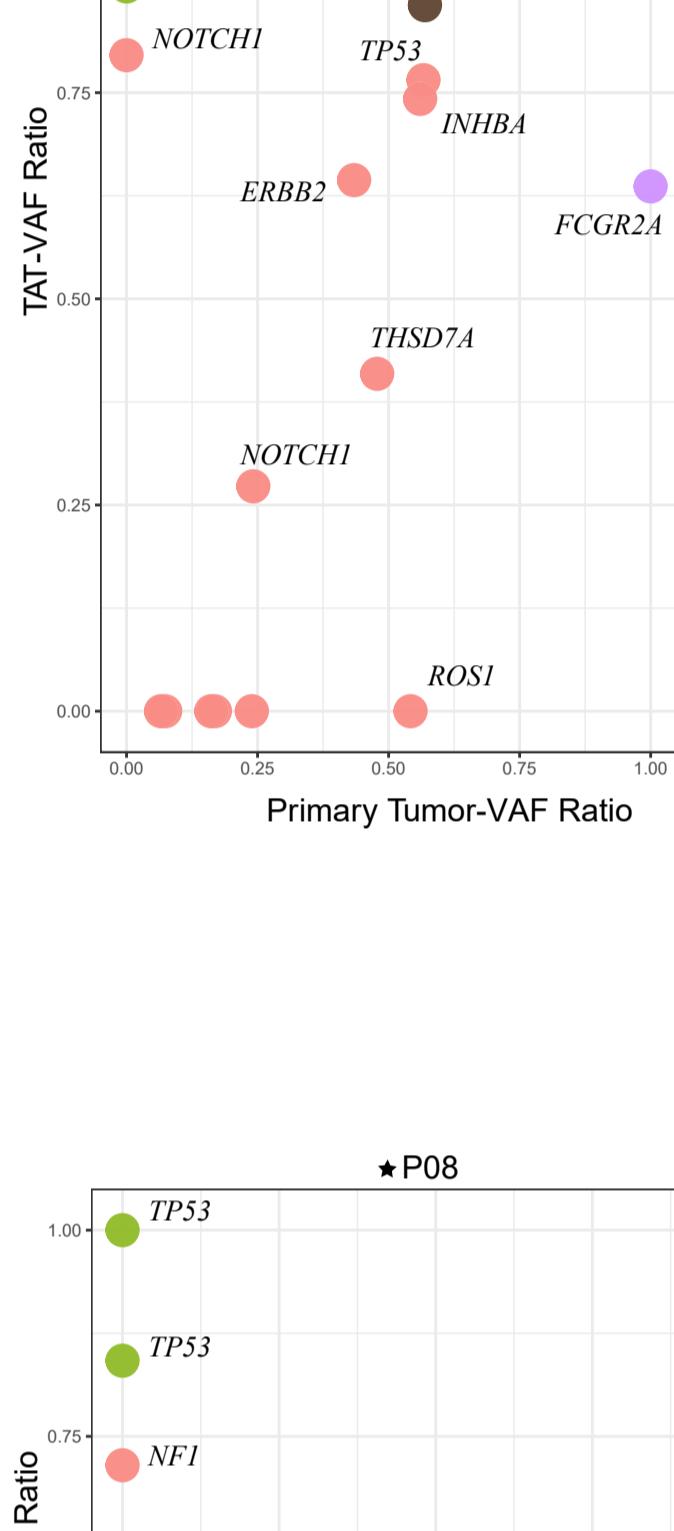
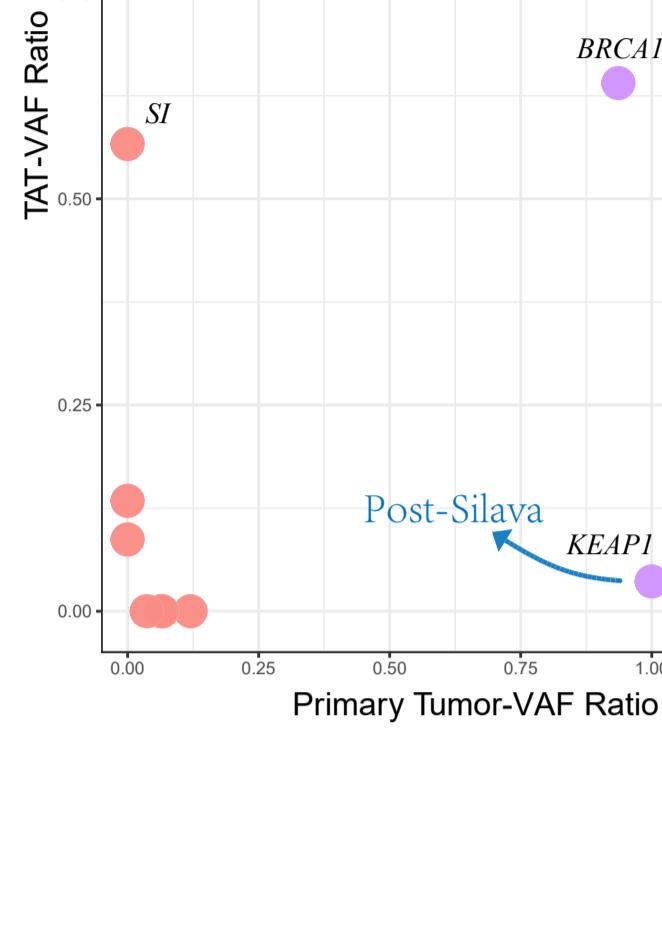
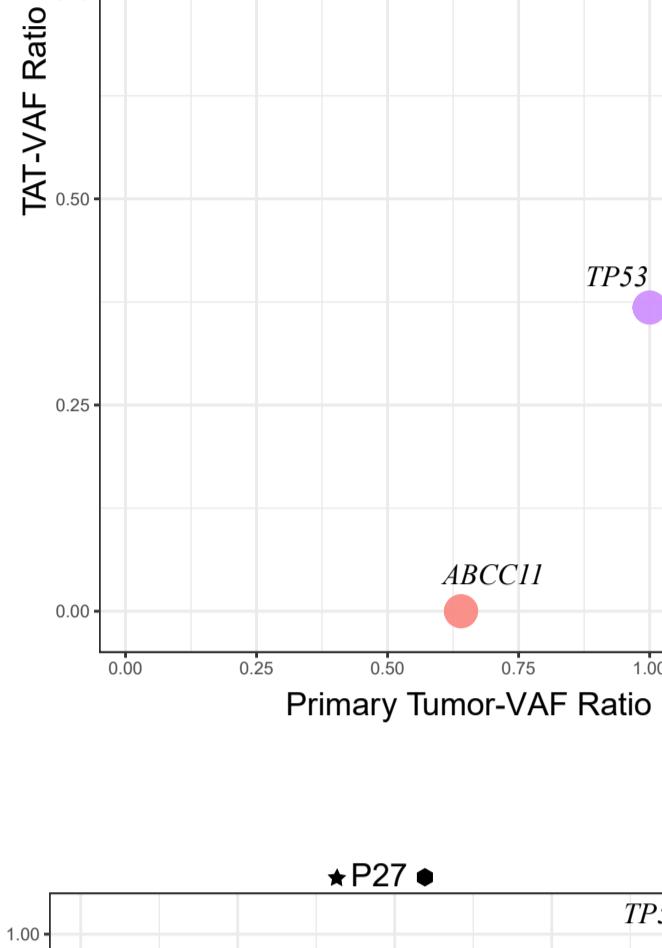
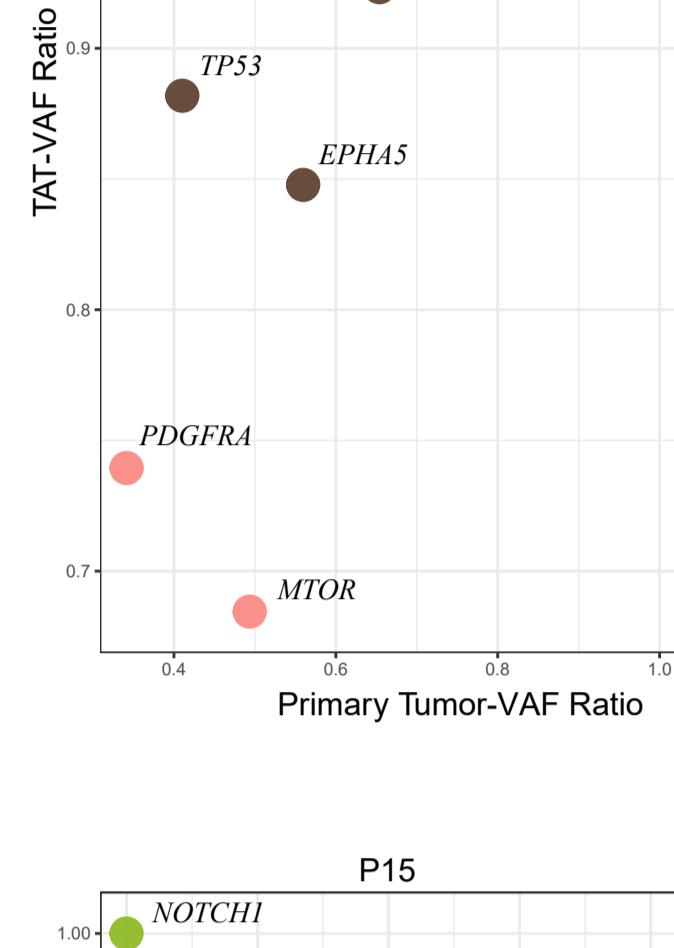
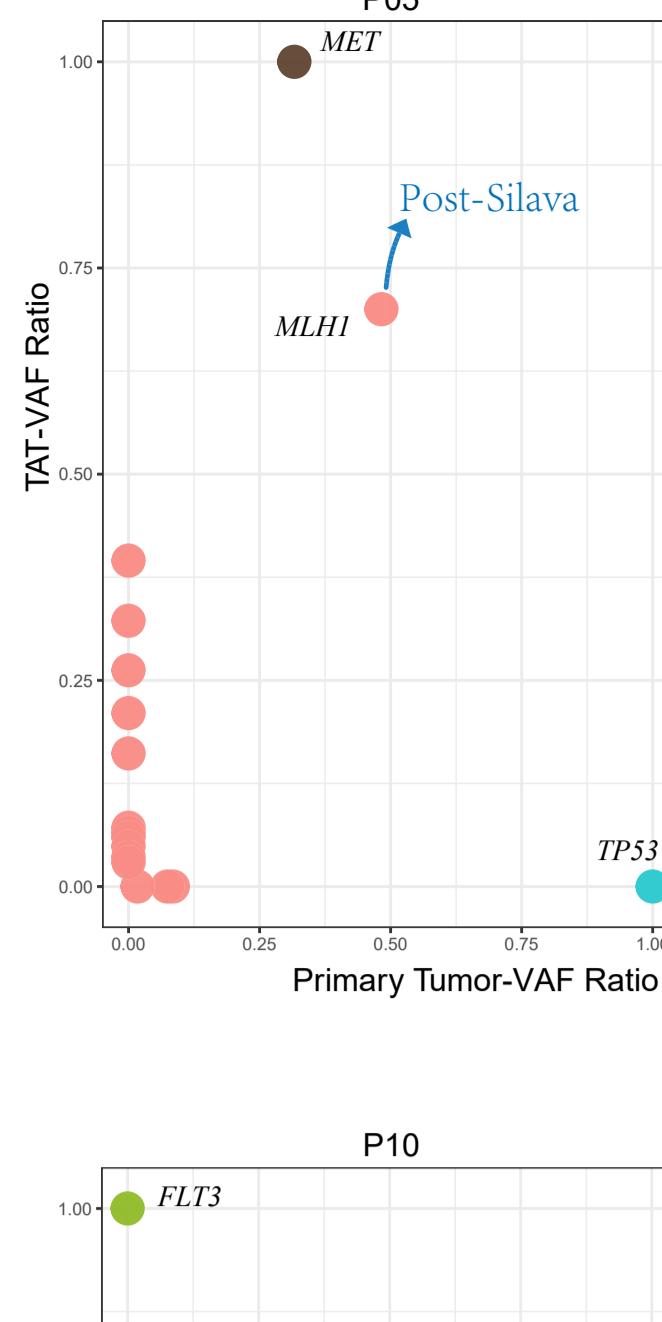
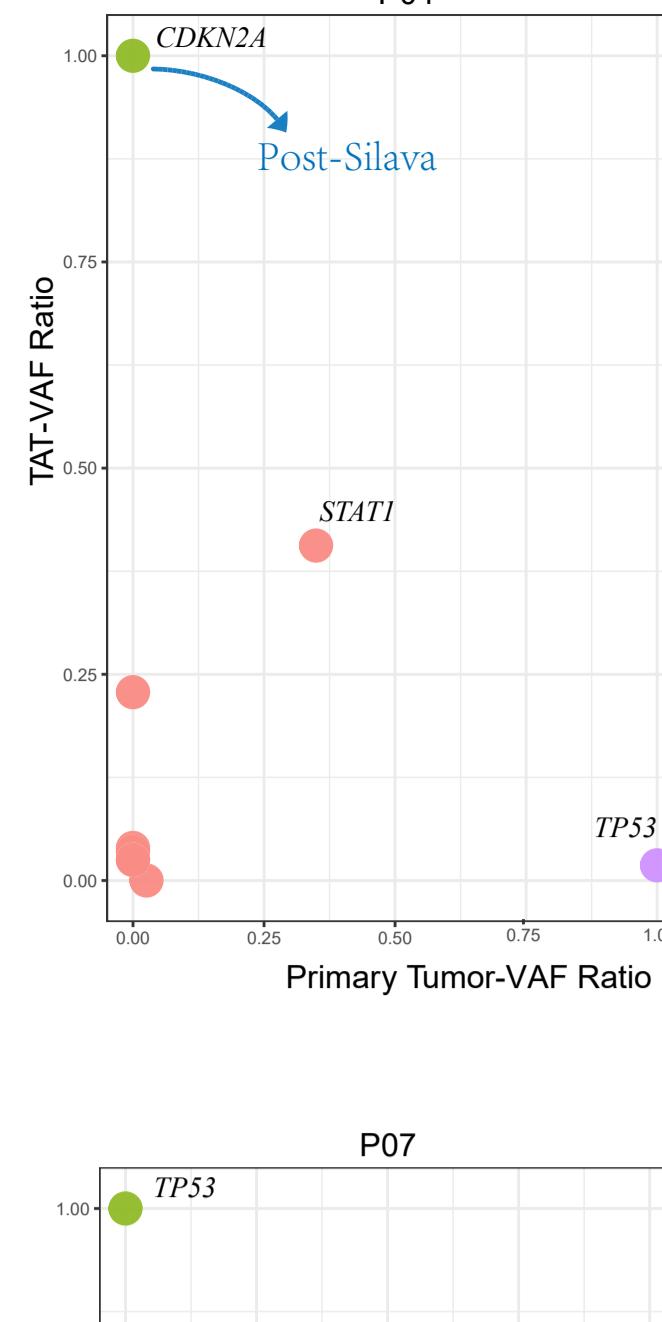
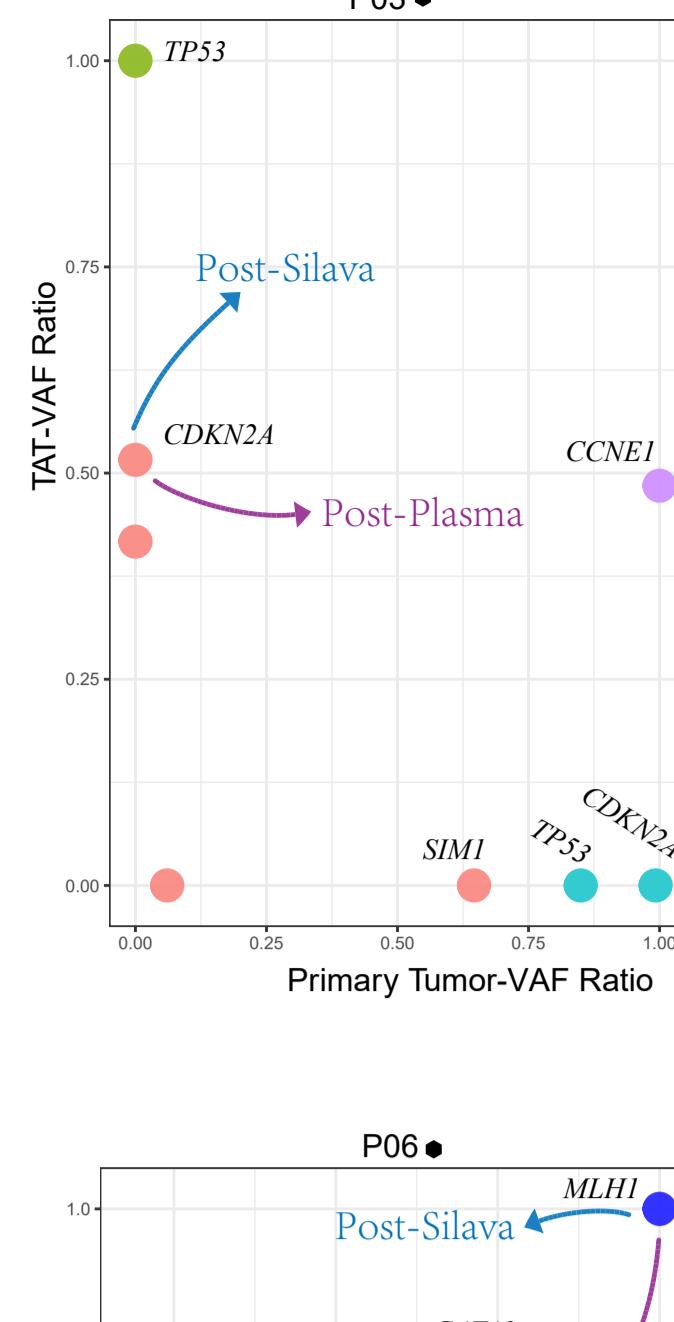


TAT-Premalignant
Lesion

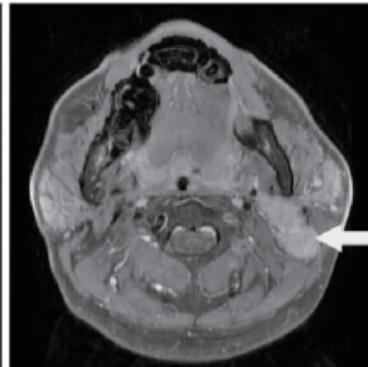
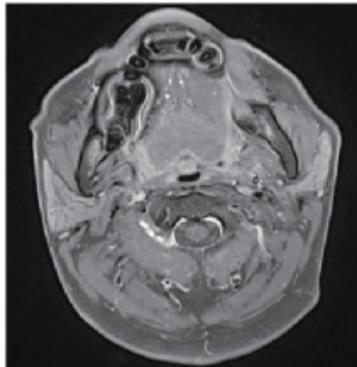
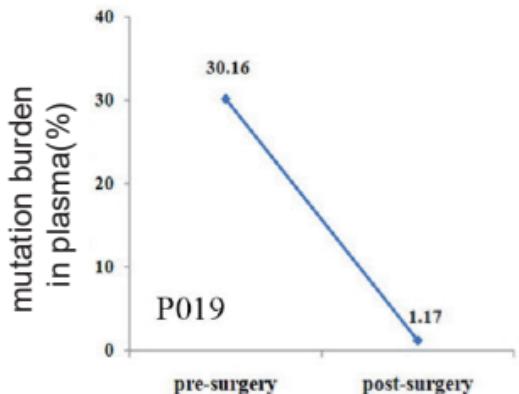
TAT-Pathologically Normal







a.



b.

