Supplementary Figures

Figure S1.

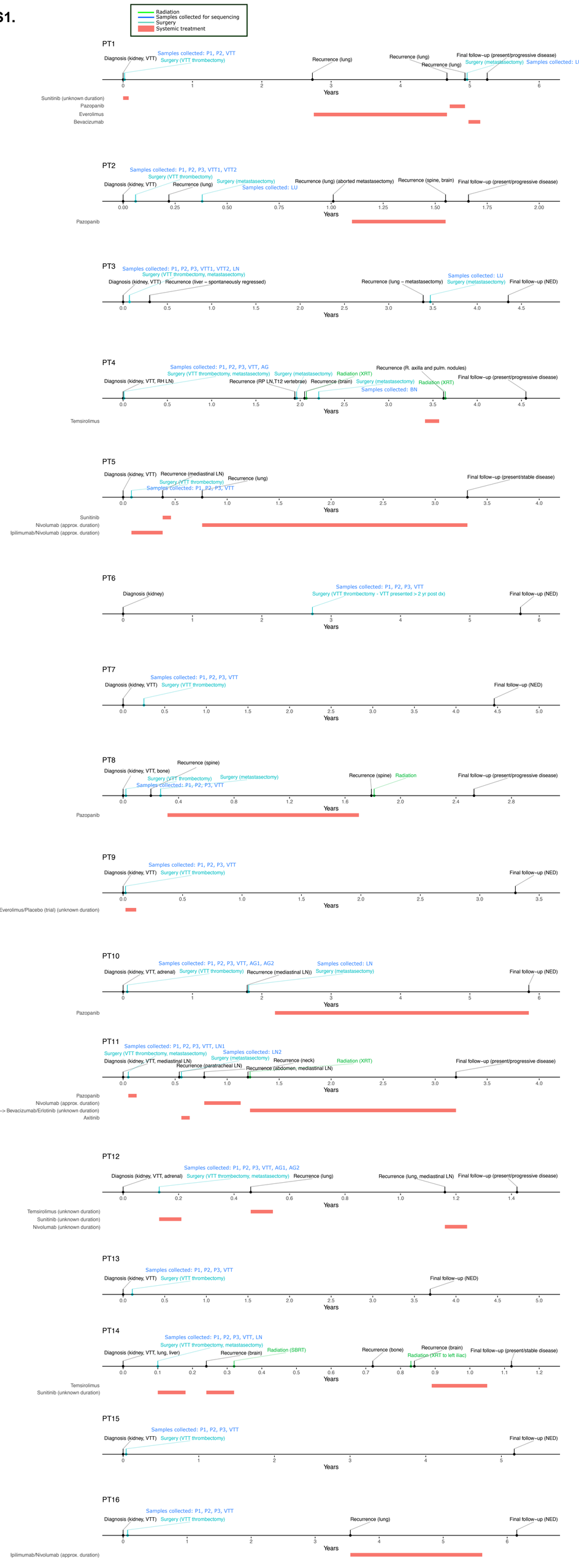
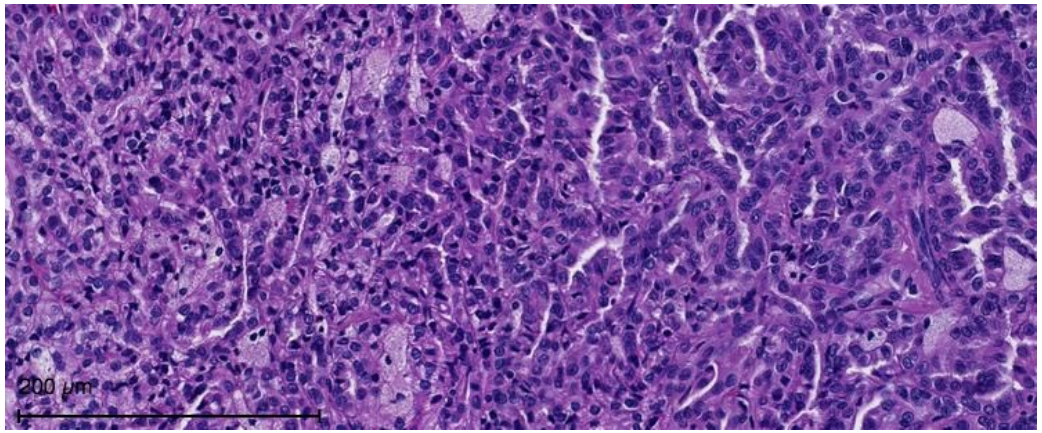

Radiation
Samples collected for sequencing
Surgery
Systemic treatment
PT1
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Samples collected: P1, P2, VTT
Recurrence (lung)
Recurrence (lung)
Recurrence (lung)
Surgery (metastasectomy)
Final follow-up (present/progressive disease)
Samples collected: LU
Years
Sunitinib (unknown duration)
Pazopanib
Everolimus
Bevacizumab
PT2
Samples collected: P1, P2, P3, VTT1, VTT2
Surgery (VTT thrombectomy)
Diagnosis (kidney, VTT)
Recurrence (lung)
Surgery (metastasectomy)
Samples collected: LU
Recurrence (lung) (aborted metastasectomy)
Recurrence (spine, brain)
Final follow-up (present/progressive disease)
Years
Pazopanib
PT3
Samples collected: P1, P2, P3, VTT1, VTT2, LN
Surgery (VTT thrombectomy, metastasectomy)
Diagnosis (kidney, VTT)
Recurrence (liver – spontaneously regressed)
Recurrence (lung – metastasectomy)
Samples collected: LU
Surgery (metastasectomy)
Final follow-up (NED)
Years
PT4
Samples collected: P1, P2, P3, VTT, AG
Surgery (VTT thrombectomy, metastasectomy)
Surgery (metastasectomy)
Radiation (XRT)
Recurrence (R. axilla and pulm. nodules)
Diagnosis (kidney, VTT, RH LN)
Recurrence (RP LN,T12 vertebrae)
Recurrence (brain)
Surgery (metastasectomy)
Radiation (XRT)
Final follow-up (present/progressive disease)
Samples collected: BN
Years
Temsirolimus
PT5
Diagnosis (kidney, VTT)
Recurrence (mediastinal LN)
Surgery (VTT thrombectomy)
Recurrence (lung)
Samples collected: P1, P2, P3, VTT
Final follow-up (present/stable disease)
Years
Sunitinib
Nivolumab (approx. duration)
Ipilimumab/Nivolumab (approx. duration)
PT6
Diagnosis (kidney)
Samples collected: P1, P2, P3, VTT
Surgery (VTT thrombectomy - VTT presented > 2 yr post dx)
Final follow-up (NED)
Years
PT7
Samples collected: P1, P2, P3, VTT
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Final follow-up (NED)
Years
PT8
Recurrence (spine)
Diagnosis (kidney, VTT, bone)
Surgery (VTT thrombectomy)
Surgery (metastasectomy)
Samples collected: P1, P2, P3, VTT
Recurrence (spine)
Radiation
Final follow-up (present/progressive disease)
Years
Pazopanib
PT9
Samples collected: P1, P2, P3, VTT
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Final follow-up (NED)
Years
Everolimus/Placebo (trial) (unknown duration)
PT10
Samples collected: P1, P2, P3, VTT, AG1, AG2
Diagnosis (kidney, VTT, adrenal)
Surgery (VTT thrombectomy)
Recurrence (mediastinal LN)
Samples collected: LN
Surgery (metastasectomy)
Final follow-up (NED)
Years
Pazopanib
PT11
Samples collected: P1, P2, P3, VTT, LN1
Surgery (VTT thrombectomy, metastasectomy)
Samples collected: LN2
Surgery (metastasectomy)
Diagnosis (kidney, VTT, mediastinal LN)
Recurrence (paratracheal LN)
Recurrence (neck)
Recurrence (abdomen, mediastinal LN)
Radiation (XRT)
Final follow-up (present/progressive disease)
Years
Pazopanib
Nivolumab (approx. duration)
Cabozantinib –> Bevacizumab/Erlotinib (unknown duration)
Axitinib
PT12
Samples collected: P1, P2, P3, VTT, AG1, AG2
Diagnosis (kidney, VTT, adrenal)
Surgery (VTT thrombectomy, metastasectomy)
Recurrence (lung)
Recurrence (lung, mediastinal LN)
Final follow-up (present/progressive disease)
Years
Temsirolimus (unknown duration)
Sunitinib (unknown duration)
Nivolumab (unknown duration)
PT13
Samples collected: P1, P2, P3, VTT
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Final follow-up (NED)
Years
PT14
Samples collected: P1, P2, P3, VTT, LN
Surgery (VTT thrombectomy, metastasectomy)
Diagnosis (kidney, VTT, lung, liver)
Recurrence (brain)
Radiation (SBRT)
Recurrence (bone)
Recurrence (brain)
Radiation (XRT to left iliac)
Final follow-up (present/stable disease)
Years
Temsirolimus
Sunitinib (unknown duration)
PT15
Samples collected: P1, P2, P3, VTT
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Final follow-up (NED)
Years
PT16
Samples collected: P1, P2, P3, VTT
Diagnosis (kidney, VTT)
Surgery (VTT thrombectomy)
Recurrence (lung)
Final follow-up (NED)
Years
Ipilimumab/Nivolumab (approx. duration)

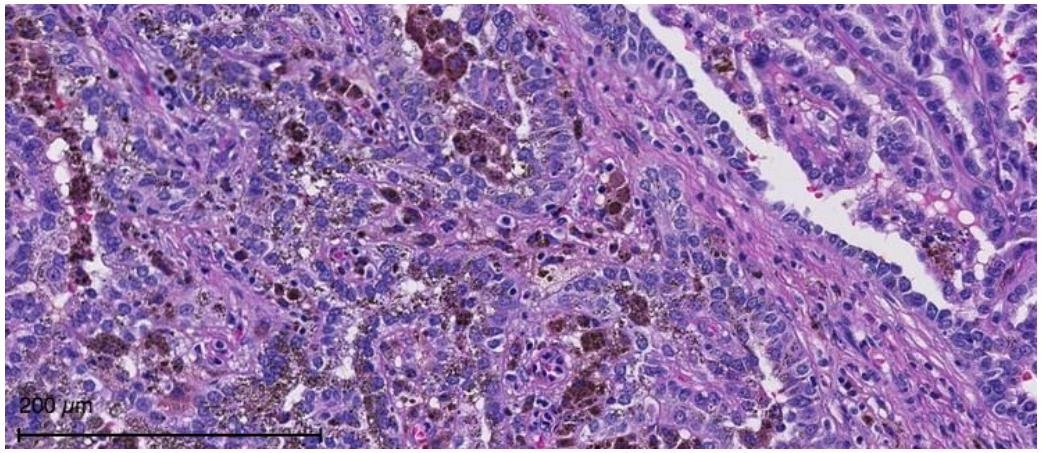
Figure S1. Timelines of patient diagnosis, surgeries, and other treatment.

Figure S2.

Low



Moderate



High

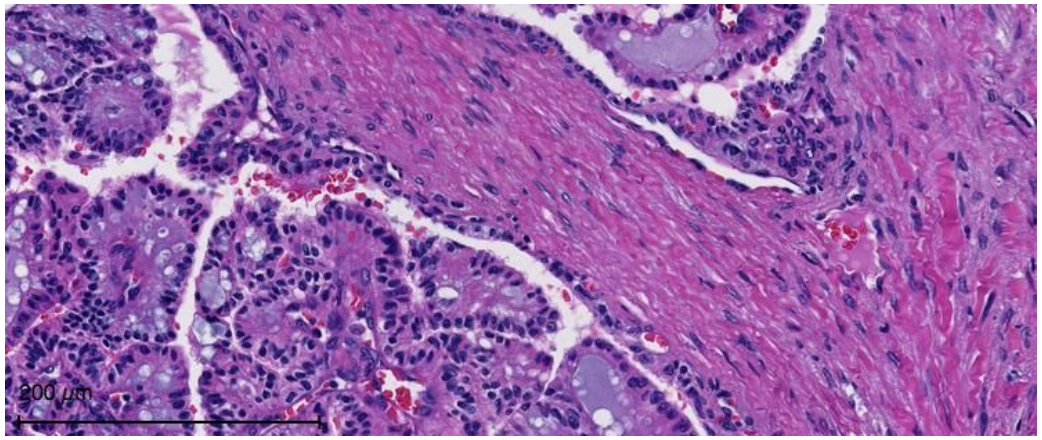
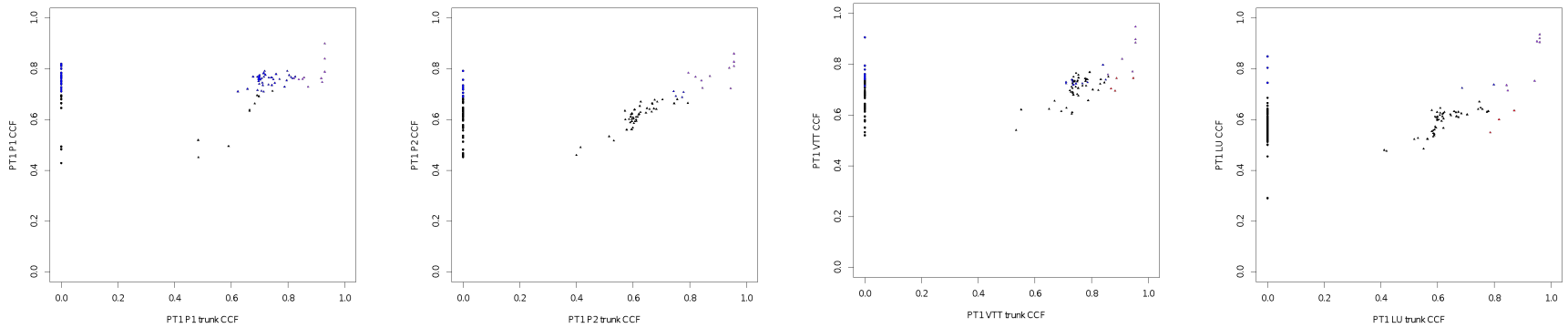
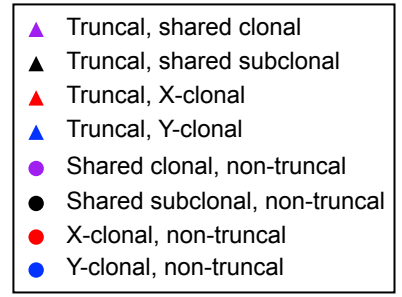


Figure S2: Semi-quantitative analysis of stromal abundance captured 3 levels (low, moderate or high) of stromal presence within tumors with little inflammatory response.

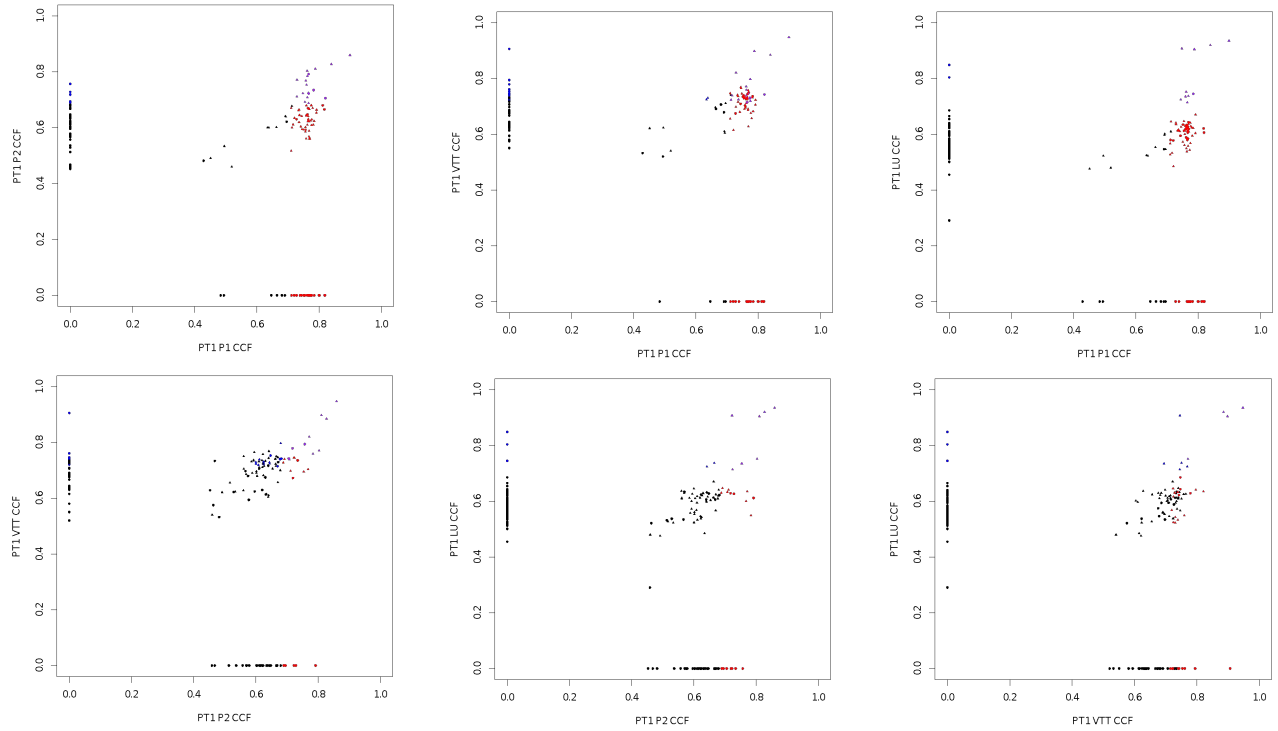
Figure S3.

A





B



C

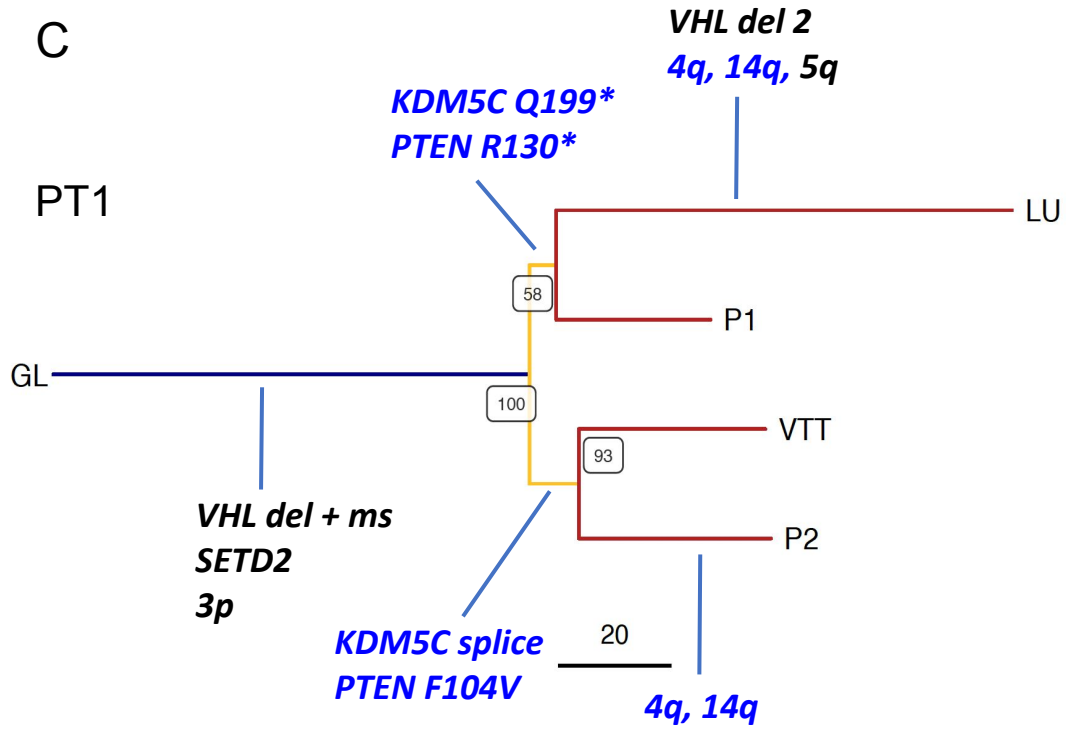
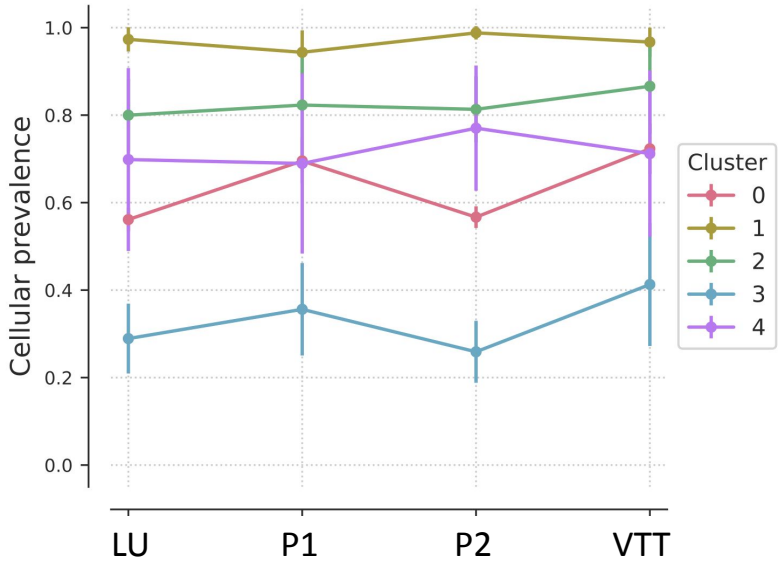


Figure S3.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
LU
P1
P2
VTT
Cluster
0
1
2
3
4

E

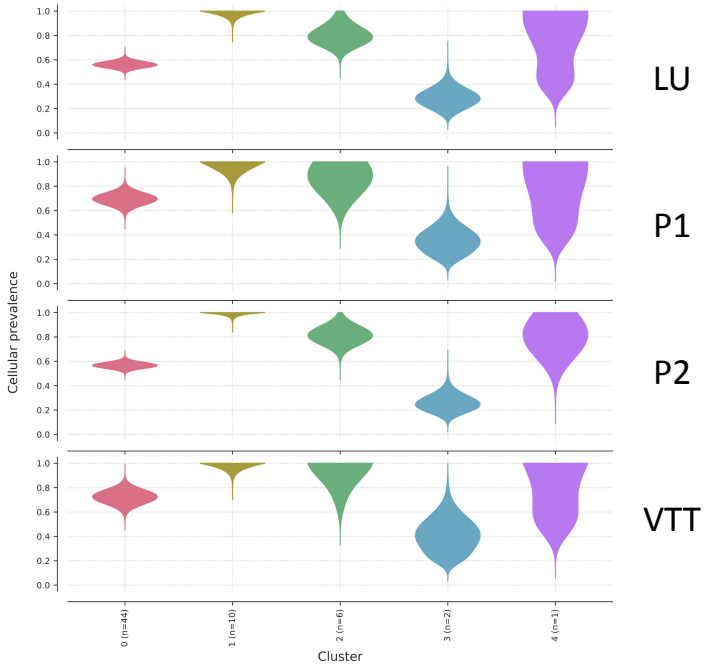
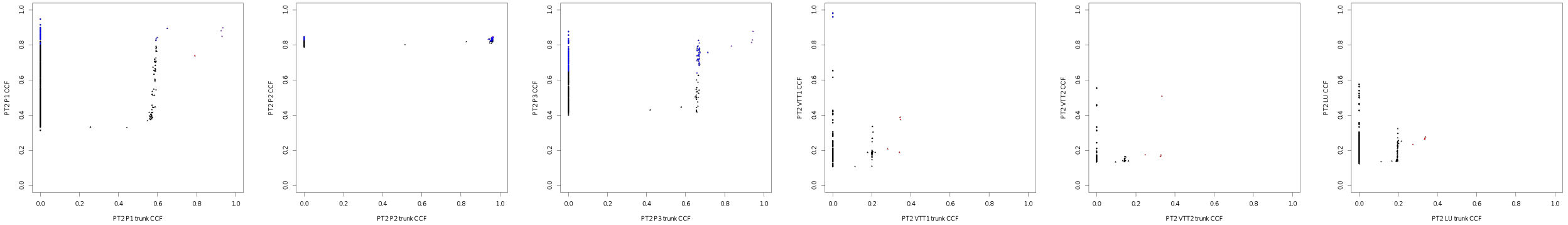
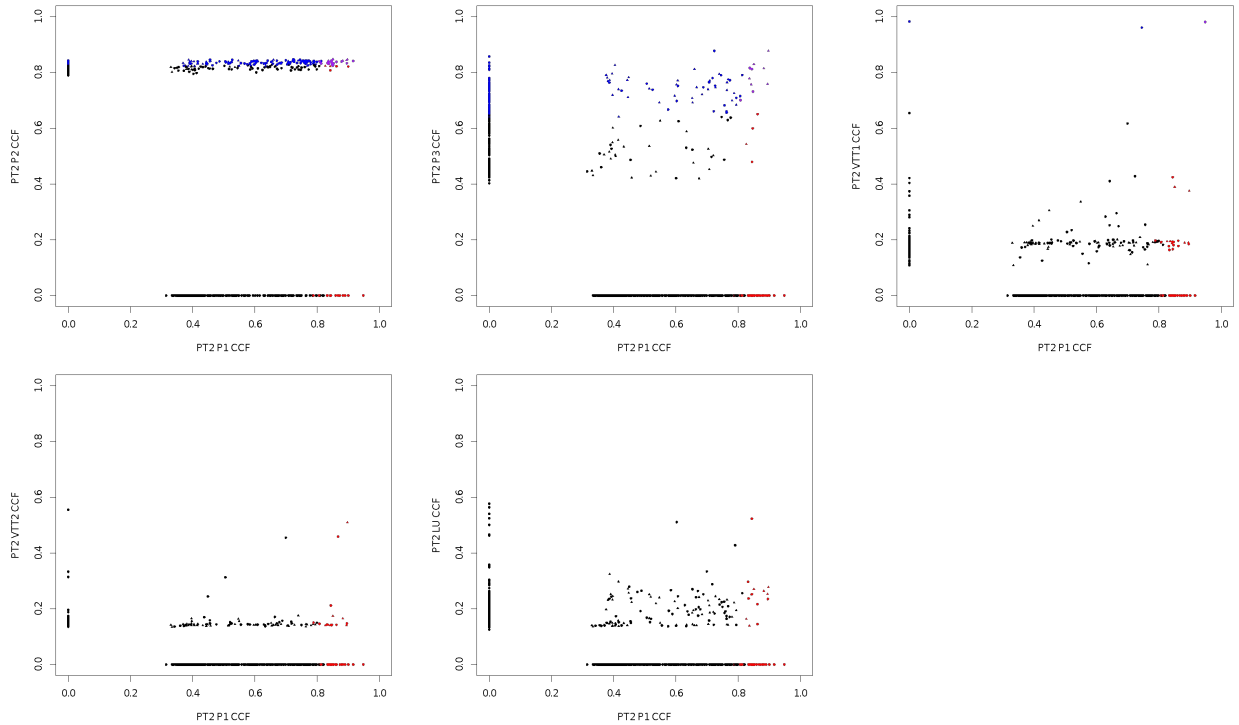

Cellular prevalence
LU
P1
P2
VTT
0 (n=44)
1 (n=10)
2 (n=6)
3 (n=2)
4 (n=1)
Cluster

Figure S4.

A


PT2 P1 CCF
PT2 P1 trunk CCF
PT2 P2 CCF
PT2 P2 trunk CCF
PT2 P3 CCF
PT2 P3 trunk CCF
PT2 VTT1 CCF
PT2 VTT1 trunk CCF
PT2 VTT2 CCF
PT2 VTT2 trunk CCF
PT2 LU CCF
PT2 LU trunk CCF

B


PT2 P2 CCF
PT2 P3 CCF
PT2 VTT1 CCF
PT2 VTT2 CCF
PT2 LU CCF
PT2 P1 CCF

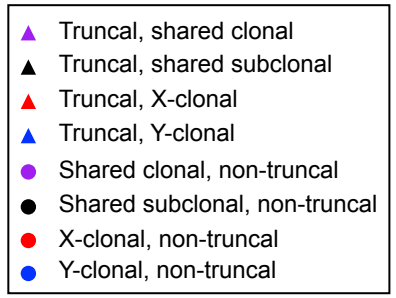
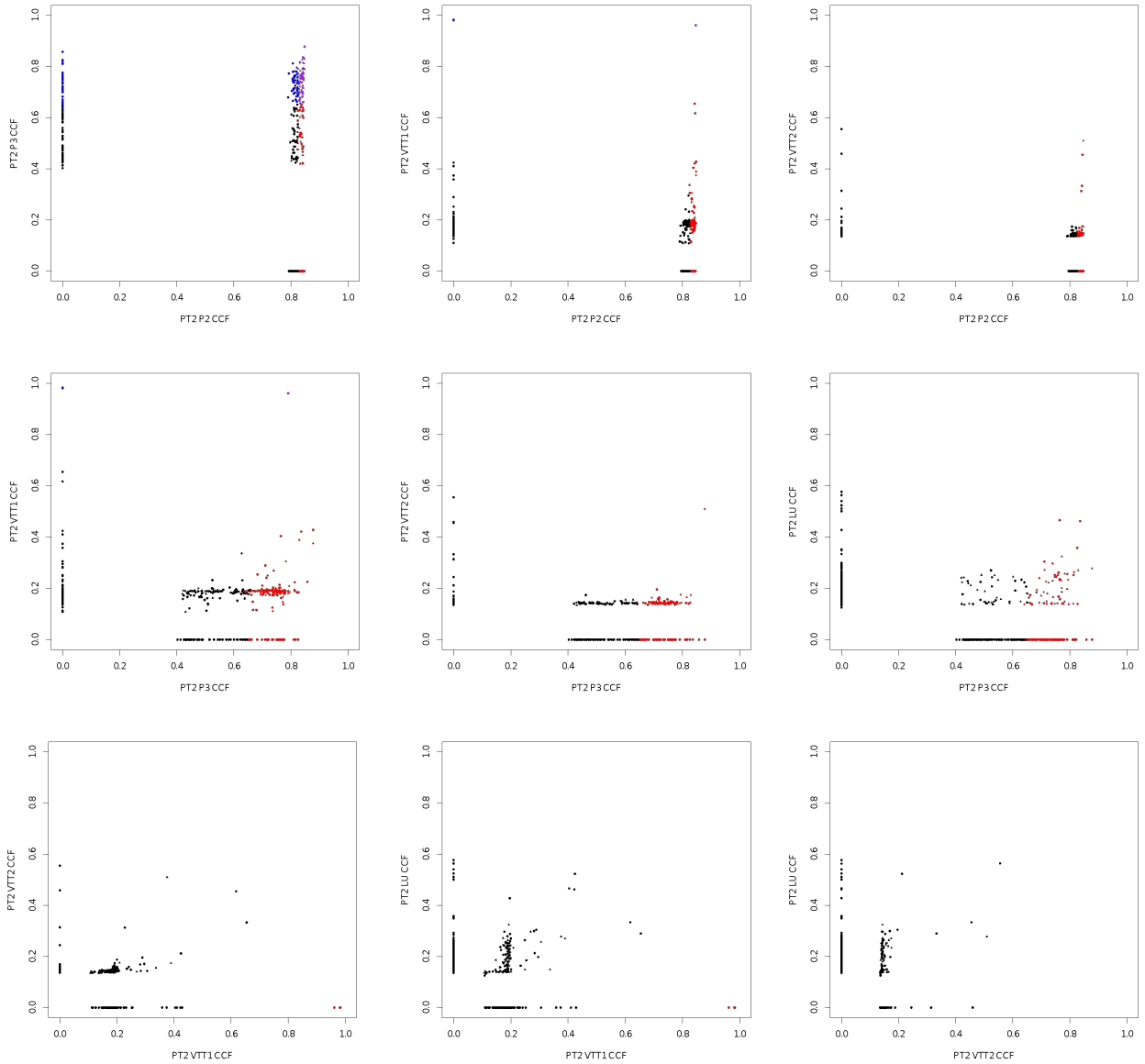

Truncal, shared clonal
Truncal, shared subclonal
Truncal, X-clonal
Truncal, Y-clonal
Shared clonal, non-truncal
Shared subclonal, non-truncal
X-clonal, non-truncal
Y-clonal, non-truncal

Figure S4.



C

PT2

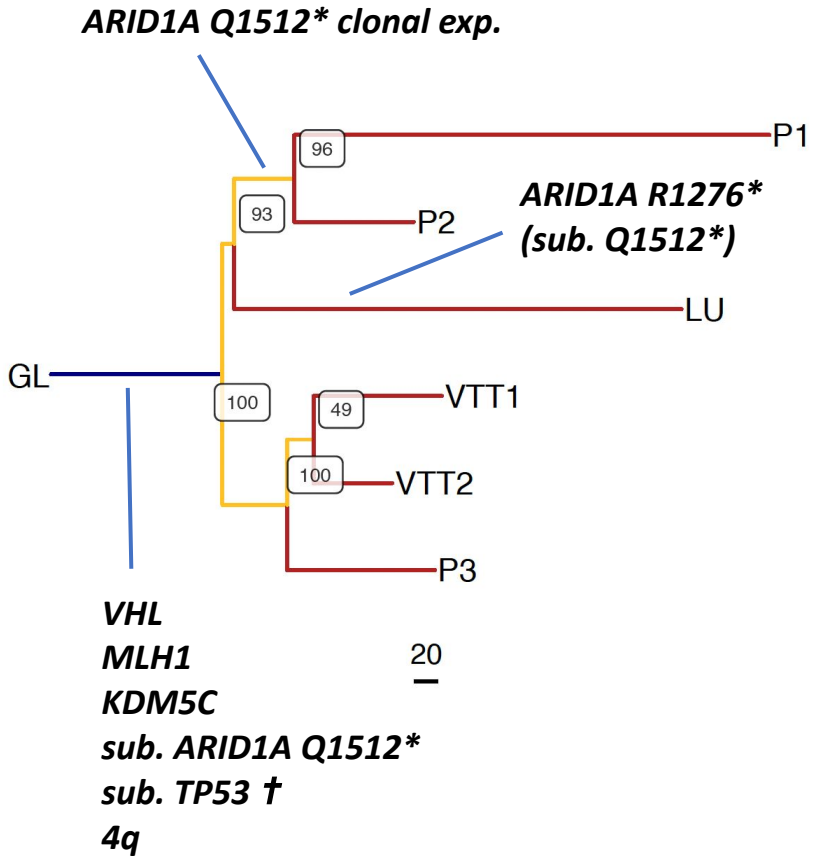
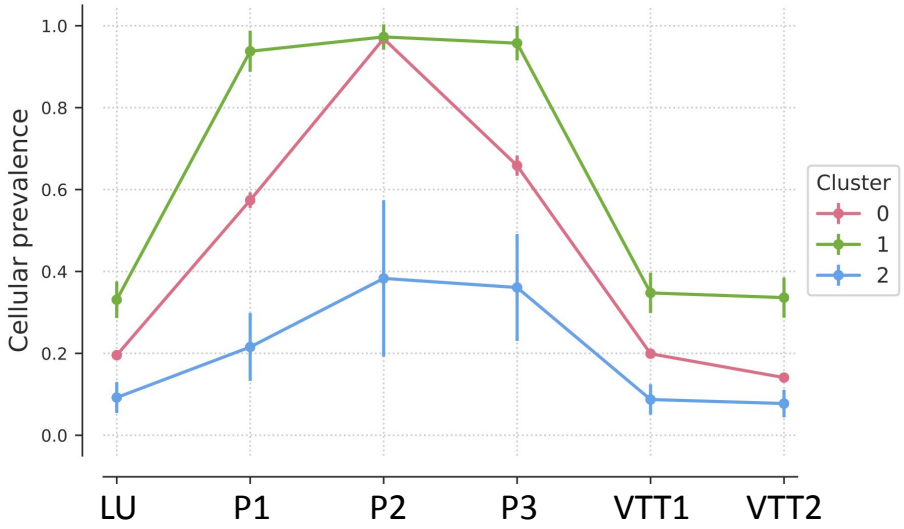


Figure S4.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
LU
P1
P2
P3
VTT1
VTT2
Cluster
0
1
2

E

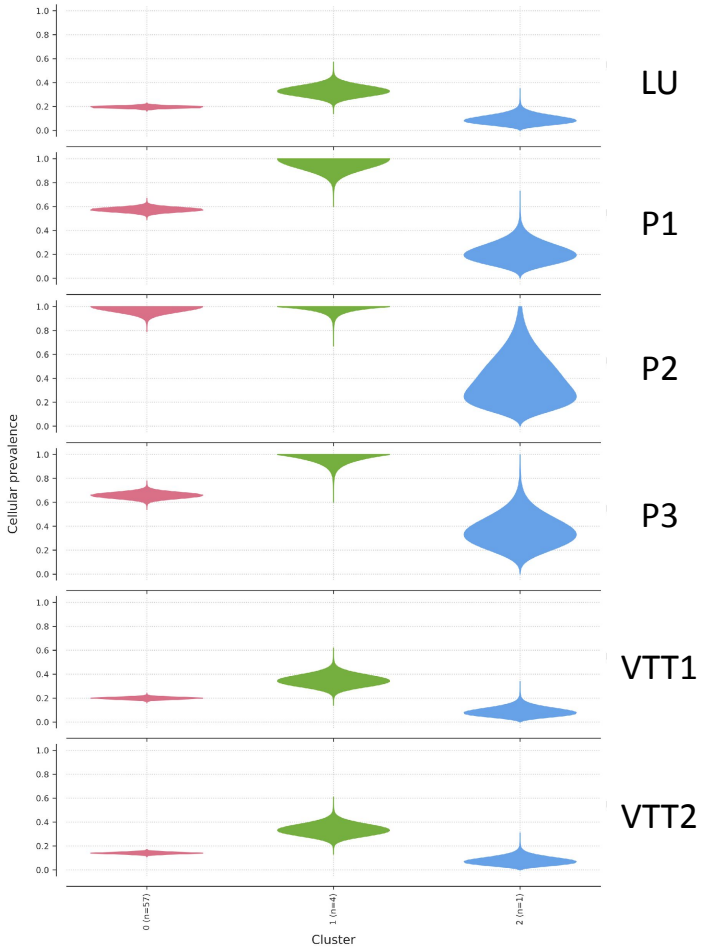
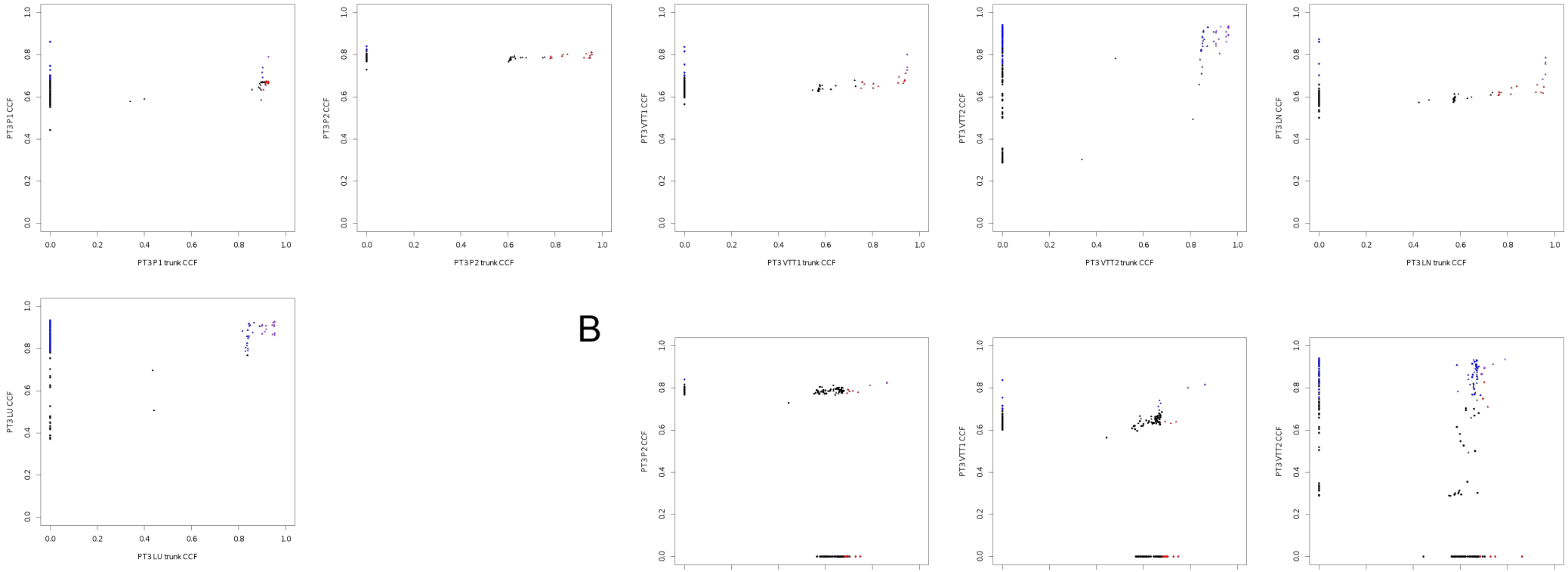
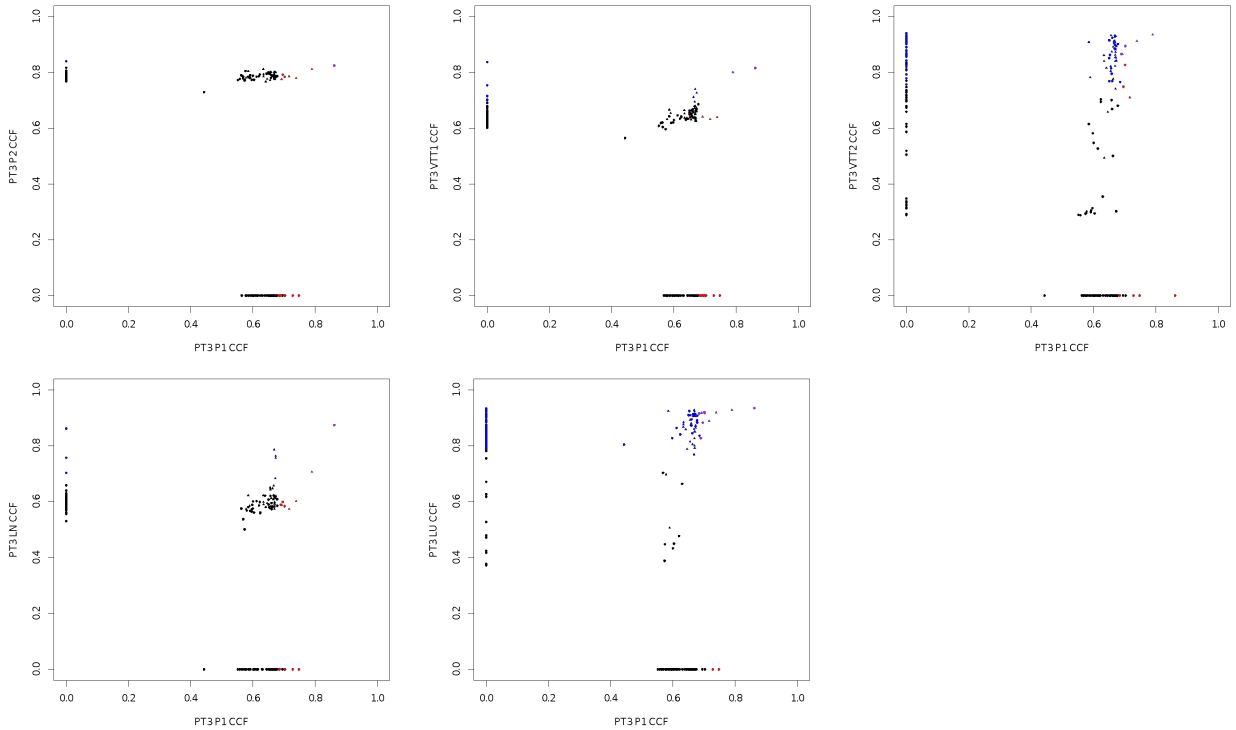

Cellular prevalence
LU
P1
P2
P3
VTT1
VTT2
0 (n=57)
1 (n=4)
2 (n=1)
Cluster

Figure S5.

A

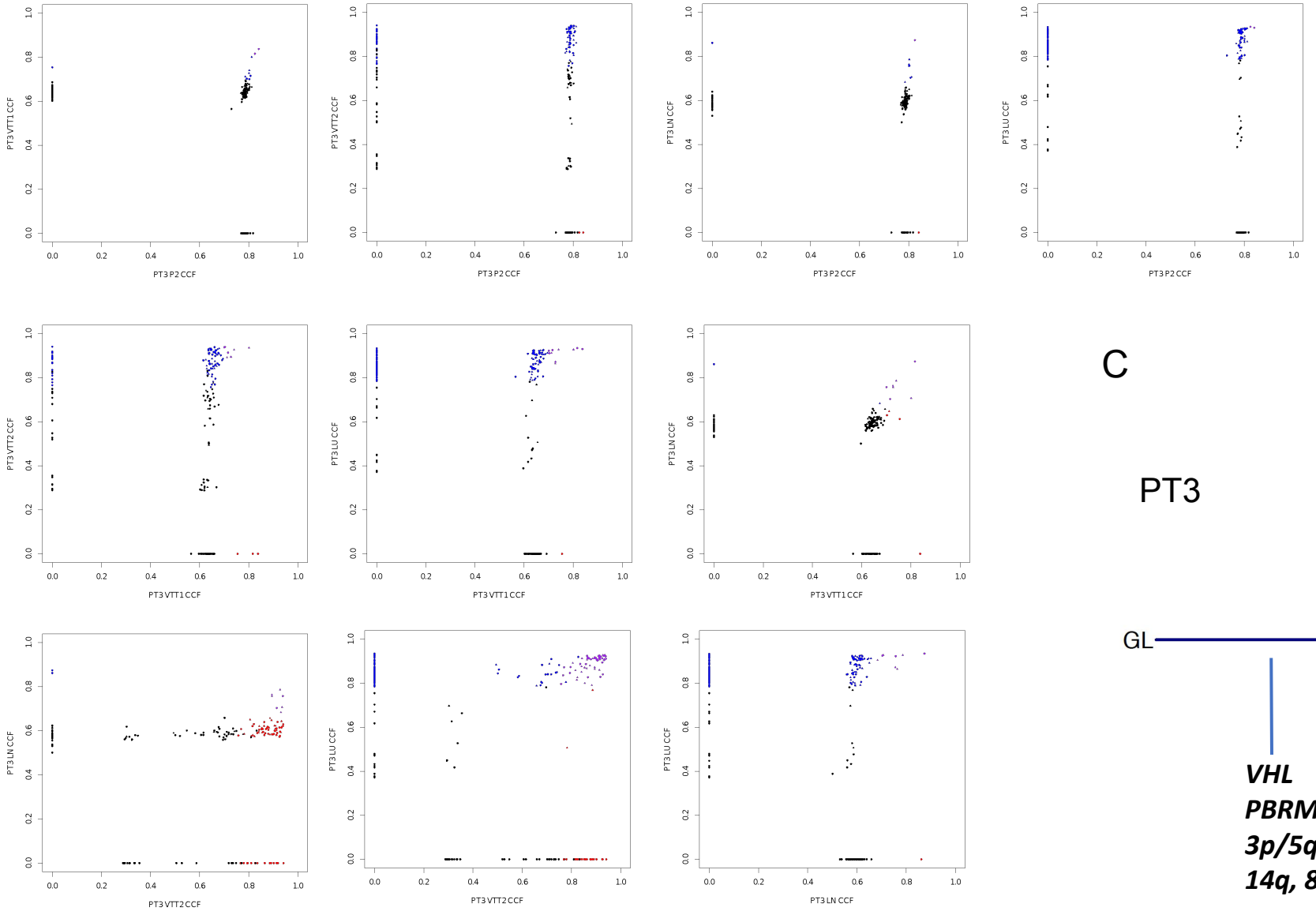


B



- Truncal, shared clonal
- Truncal, shared subclonal
- Truncal, X-clonal
- Truncal, Y-clonal
- Shared clonal, non-truncal
- Shared subclonal, non-truncal
- X-clonal, non-truncal
- Y-clonal, non-truncal

Figure S5.


PT3 VTT1 CCF
PT3 P2 CCF
PT3 VTT2 CCF
PT3 LN CCF
PT3 LU CCF

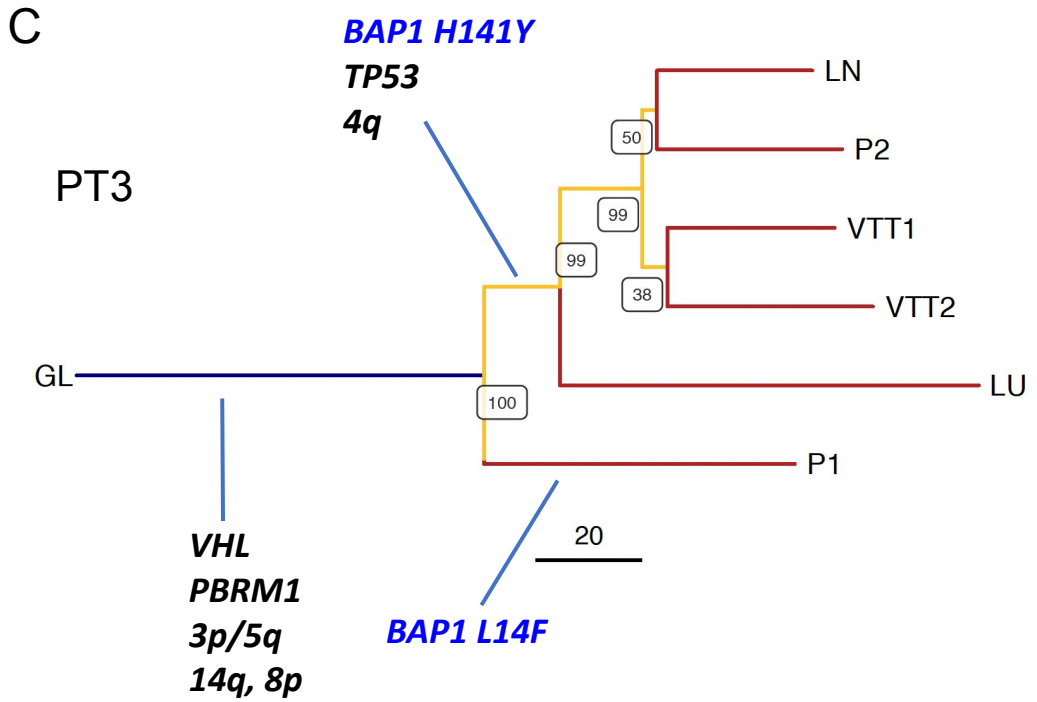
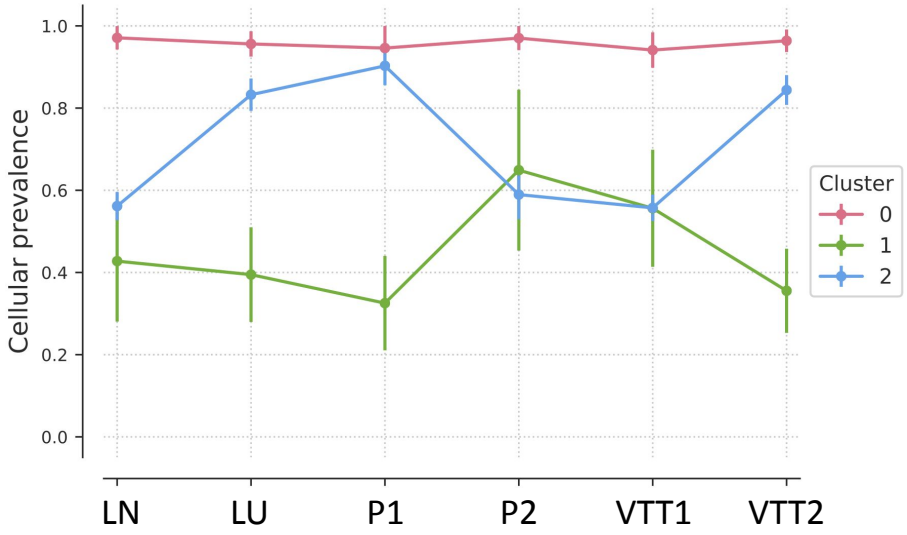

C
BAP1 H141Y
TP53
4q
PT3
LN
P2
VTT1
VTT2
LU
P1
GL
50
99
99
38
100
20
VHL
PBRM1
3p/5q
14q, 8p
BAP1 L14F

Figure S5.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
LN
LU
P1
P2
VTT1
VTT2
Cluster
0
1
2

E

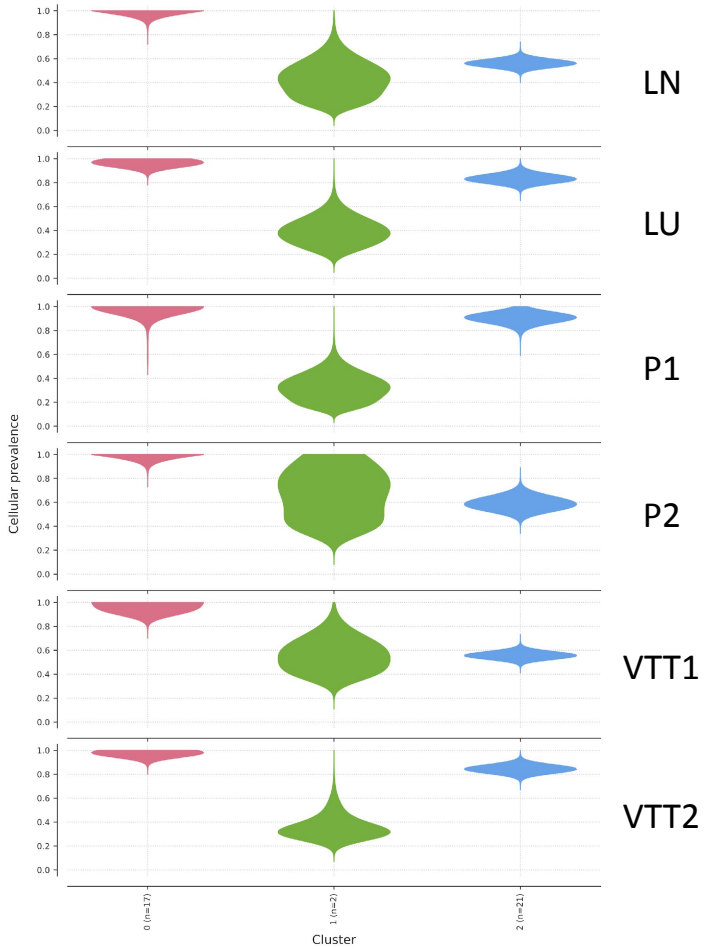
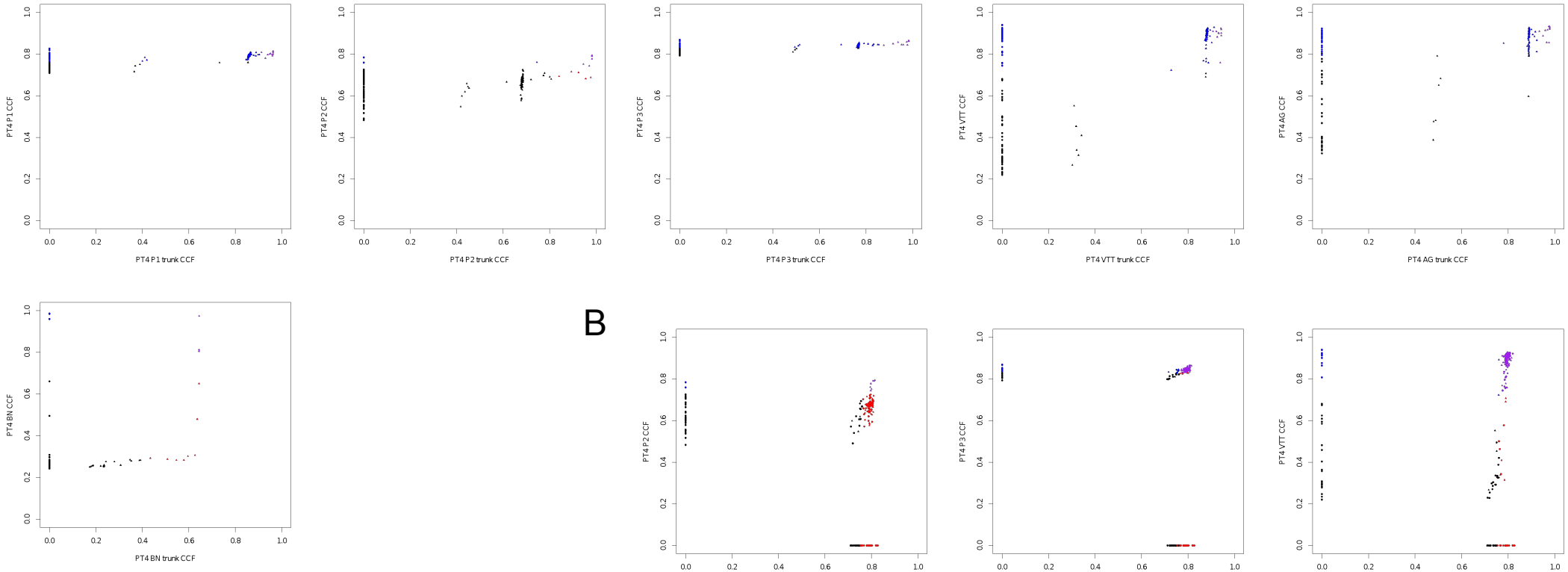
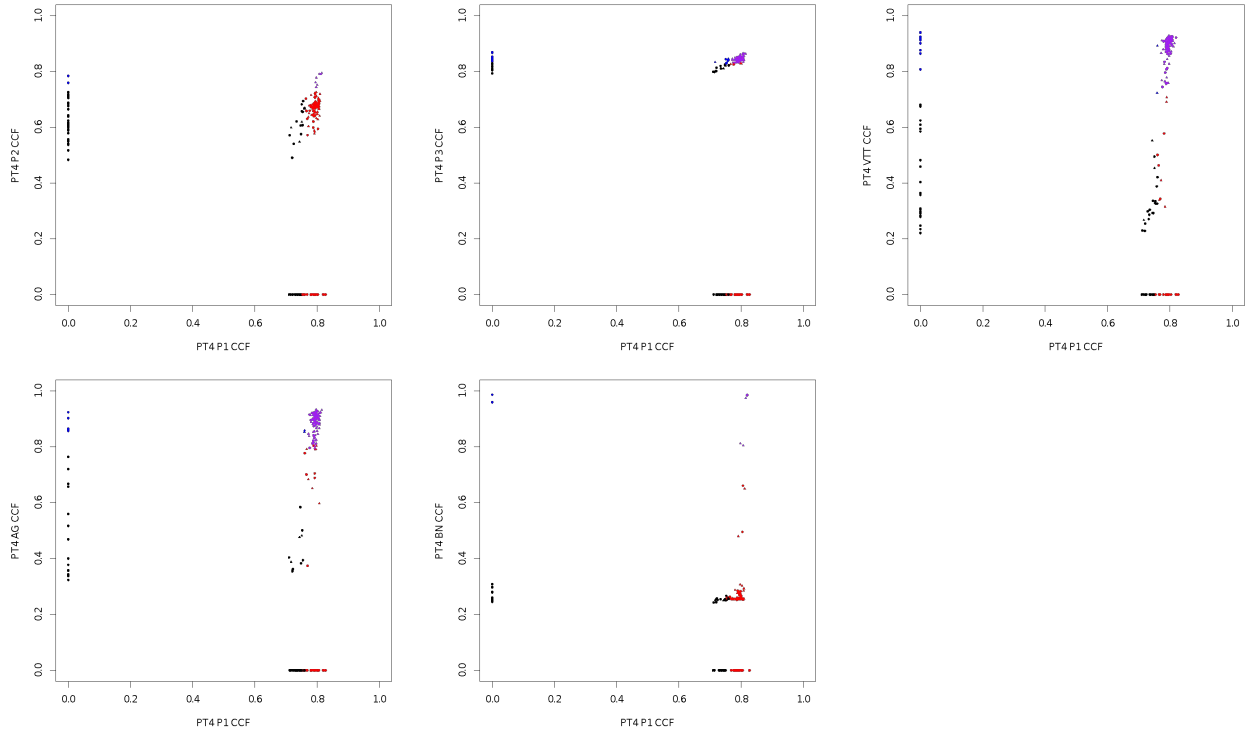

Cellular prevalence
LN
LU
P1
P2
VTT1
VTT2
Cluster

Figure S6.

A

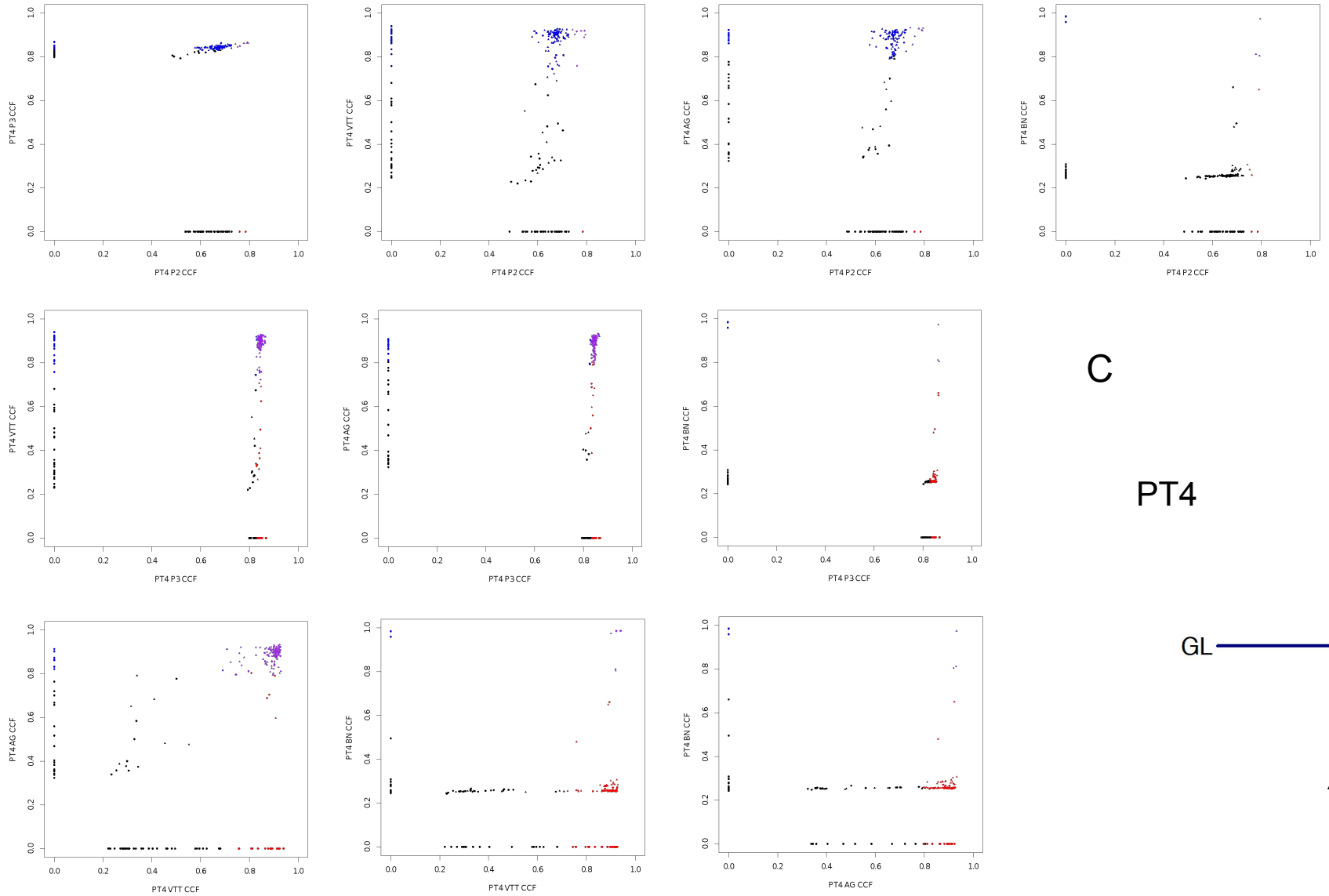


B



- Truncal, shared clonal
- Truncal, shared subclonal
- Truncal, X-clonal
- Truncal, Y-clonal
- Shared clonal, non-truncal
- Shared subclonal, non-truncal
- X-clonal, non-truncal
- Y-clonal, non-truncal

Figure S6.


PT4 P3 CCF
PT4 P2 CCF
PT4 VTT CCF
PT4 AG CCF
PT4 BN CCF

C

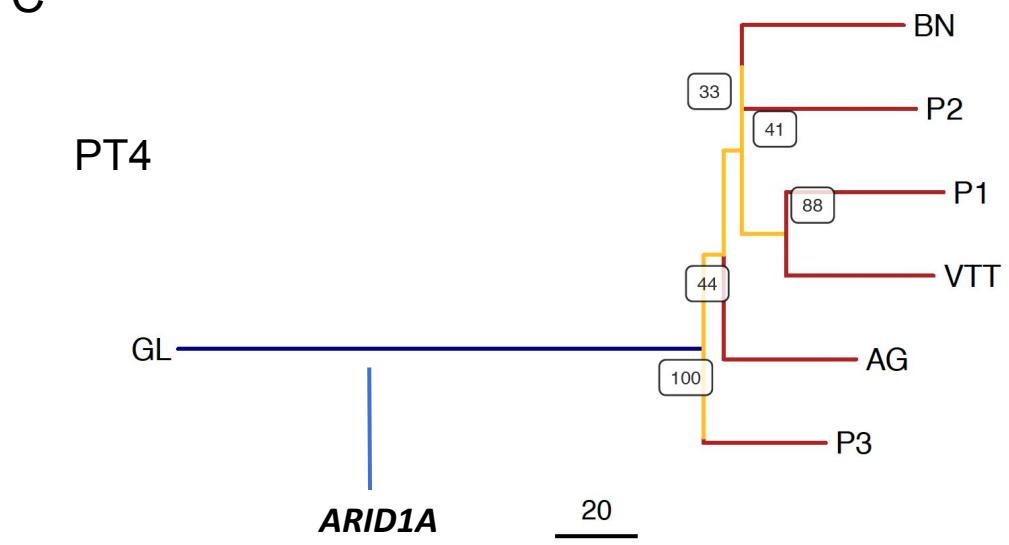
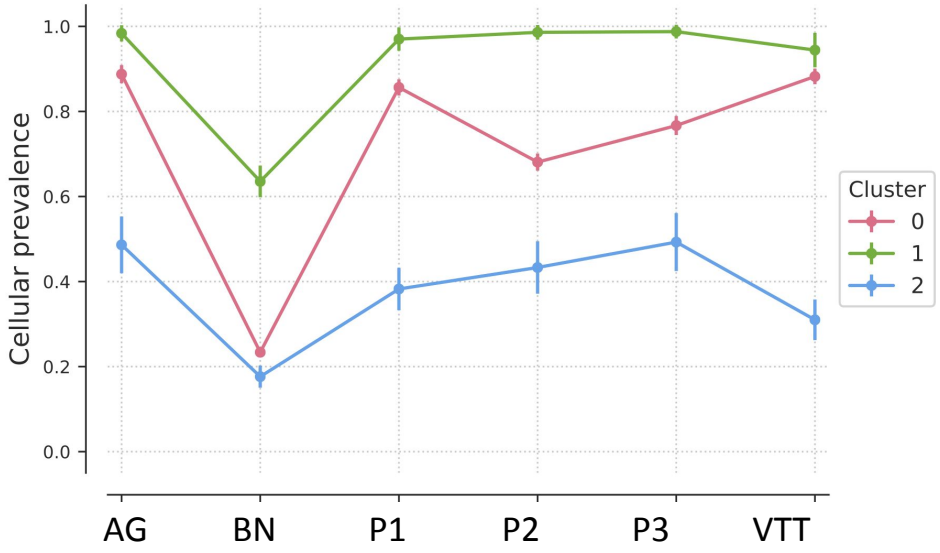

PT4
BN
P2
P1
VTT
AG
P3
GL
33
41
88
44
100
ARID1A
20

Figure S6.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
AG
BN
P1
P2
P3
VTT
Cluster
0
1
2

E

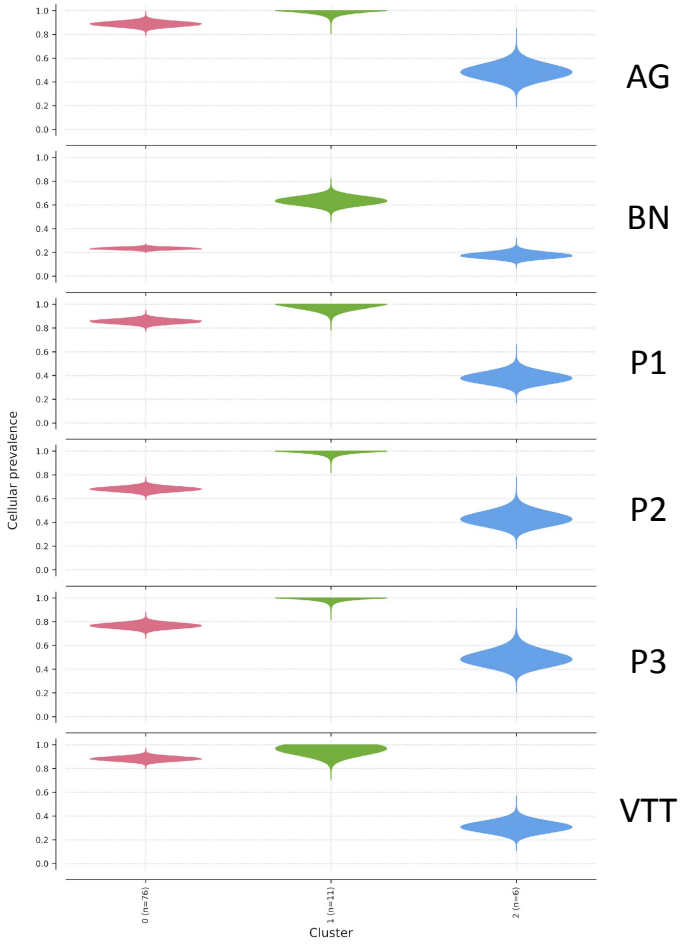
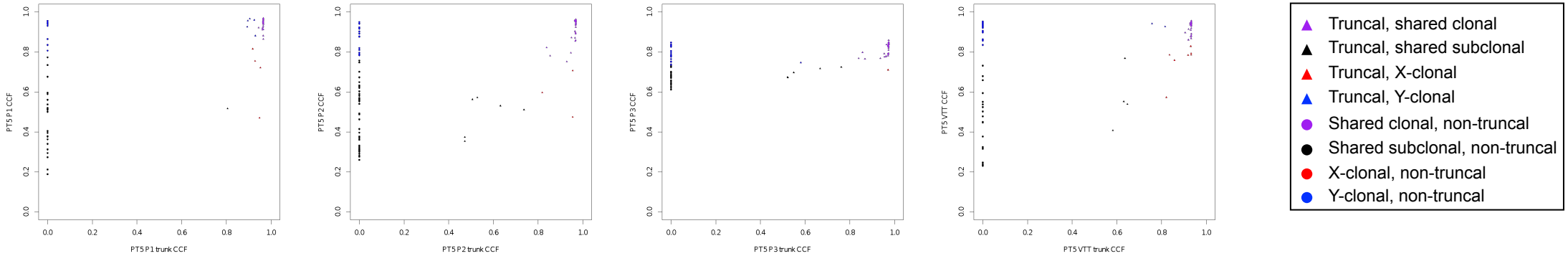

Cellular prevalence
AG
BN
P1
P2
P3
VTT
Cluster

Figure S7.

A



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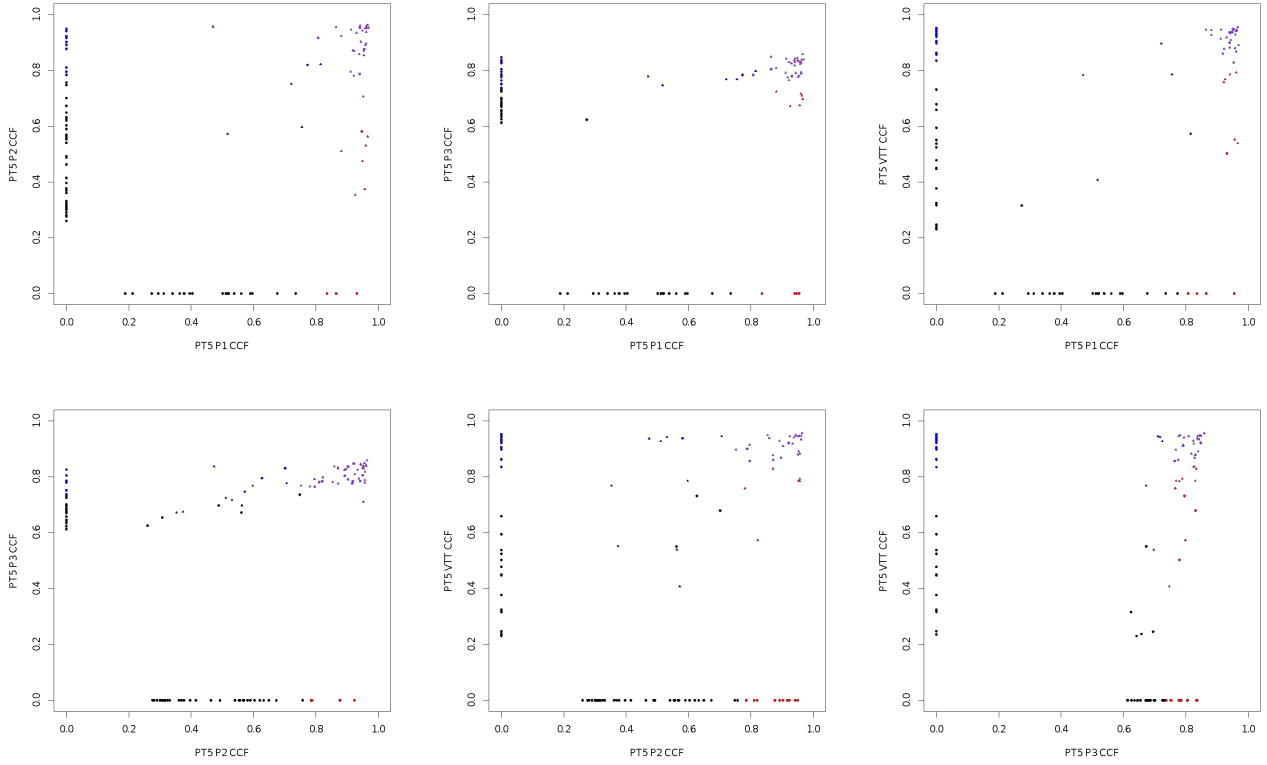

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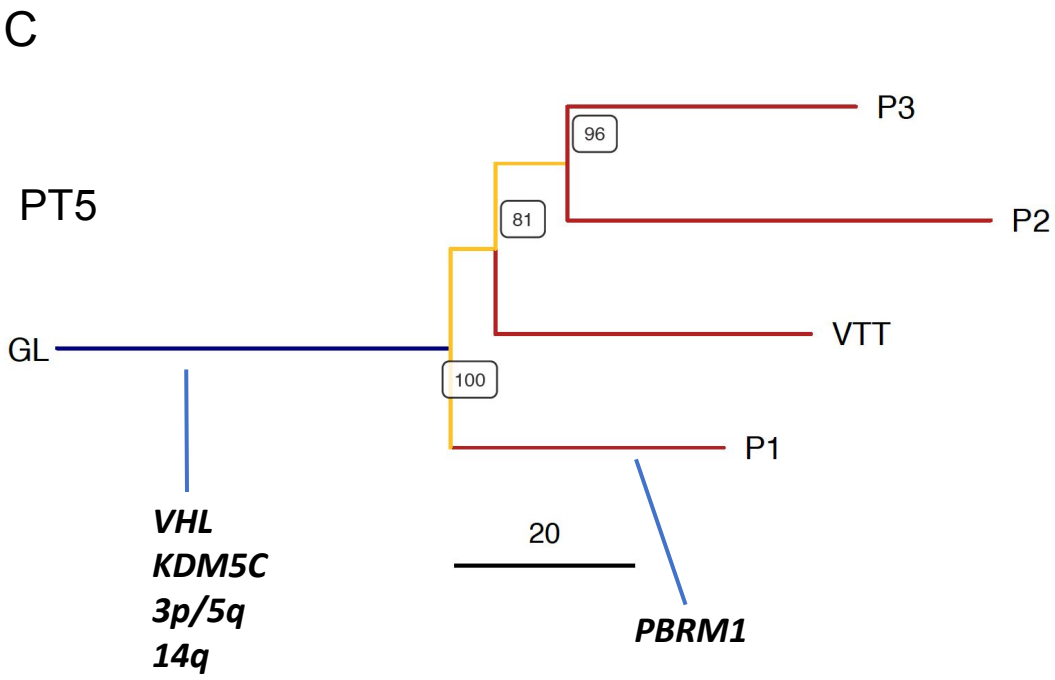
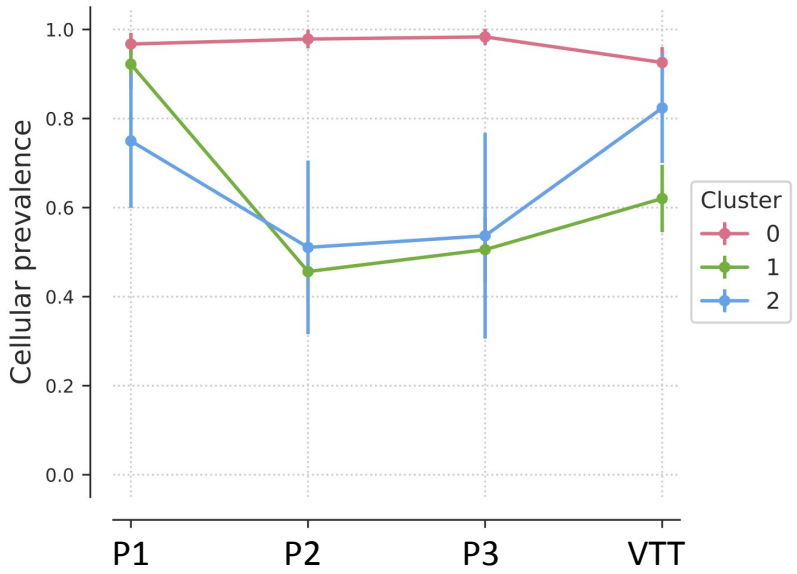


Figure S7.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
P1
P2
P3
VTT
Cluster
0
1
2

E

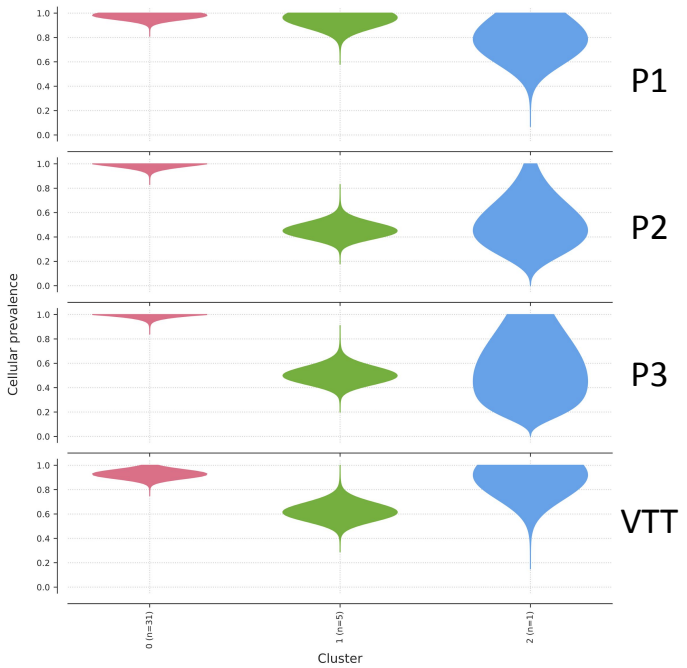
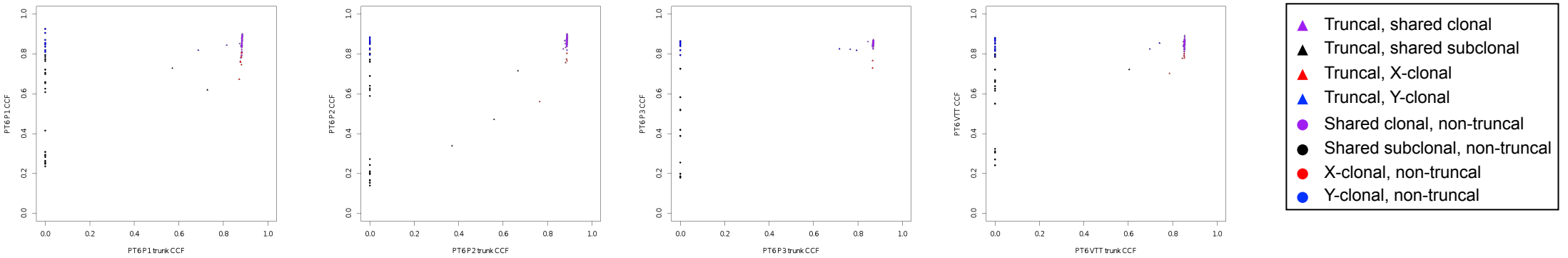
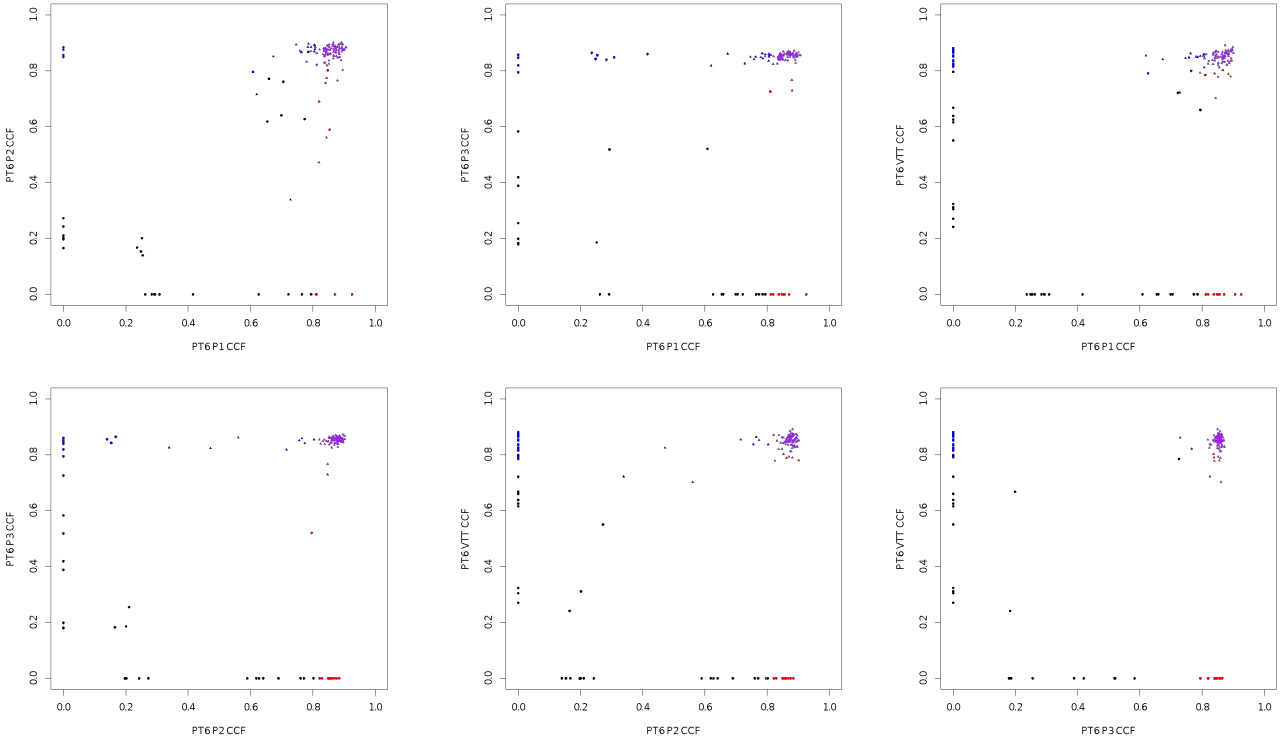

P1
P2
P3
VTT
Cellular prevalence
Cluster

Figure S8.

A



B



C

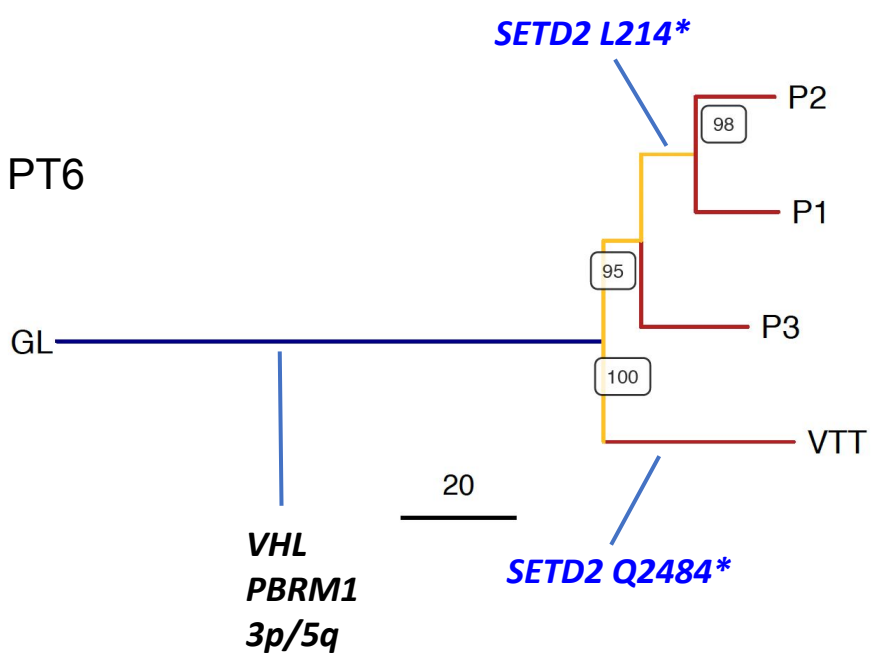
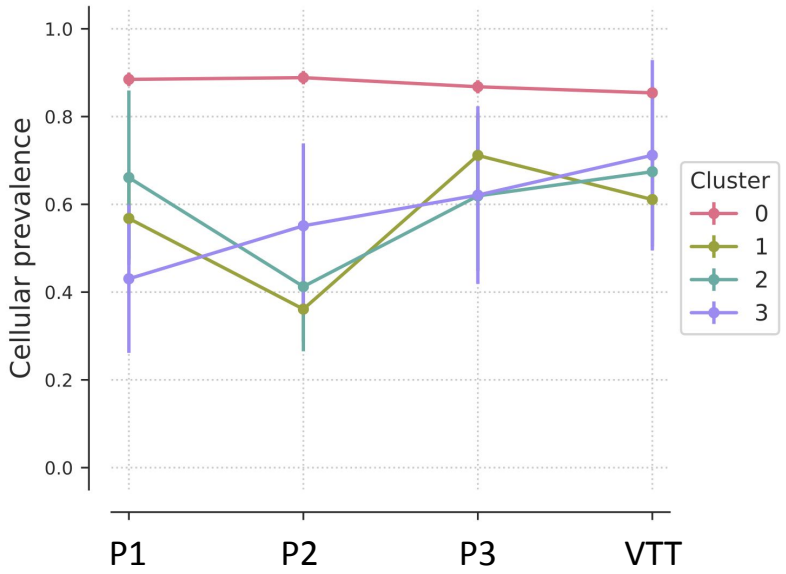


Figure S8.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
P1
P2
P3
VTT
Cluster
0
1
2
3

E

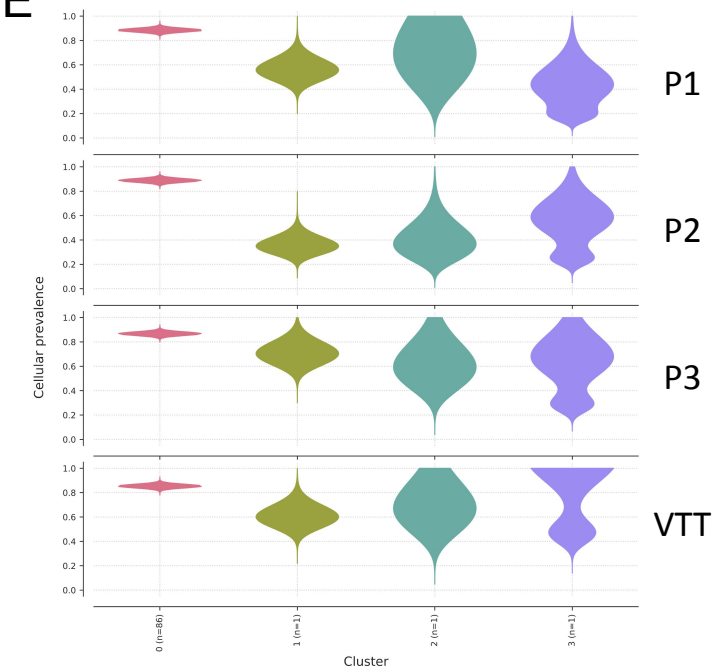
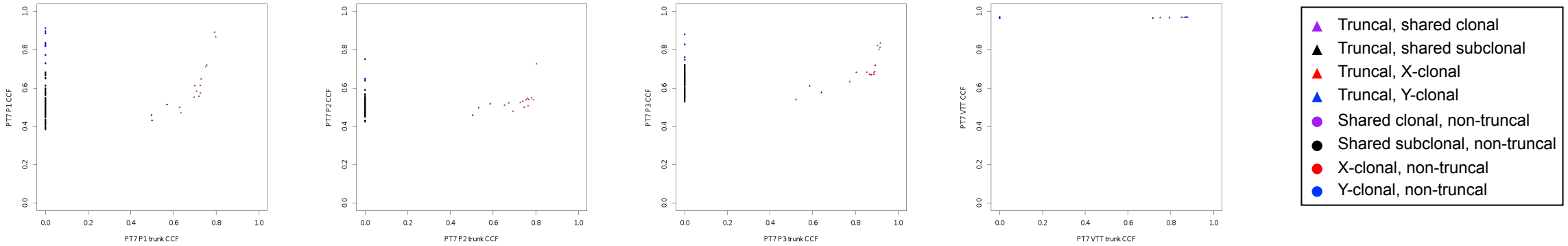
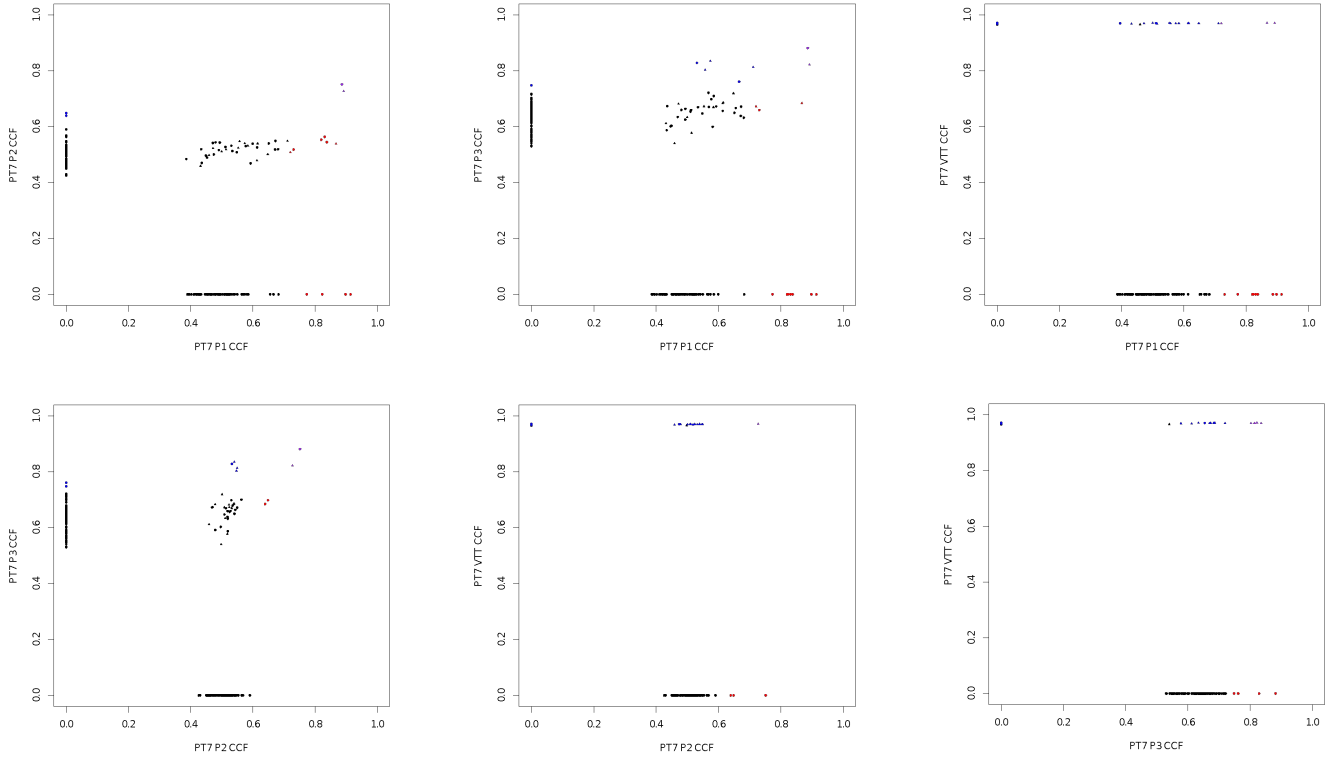

Cellular prevalence
P1
P2
P3
VTT
Cluster

Figure S9.

A



B



C

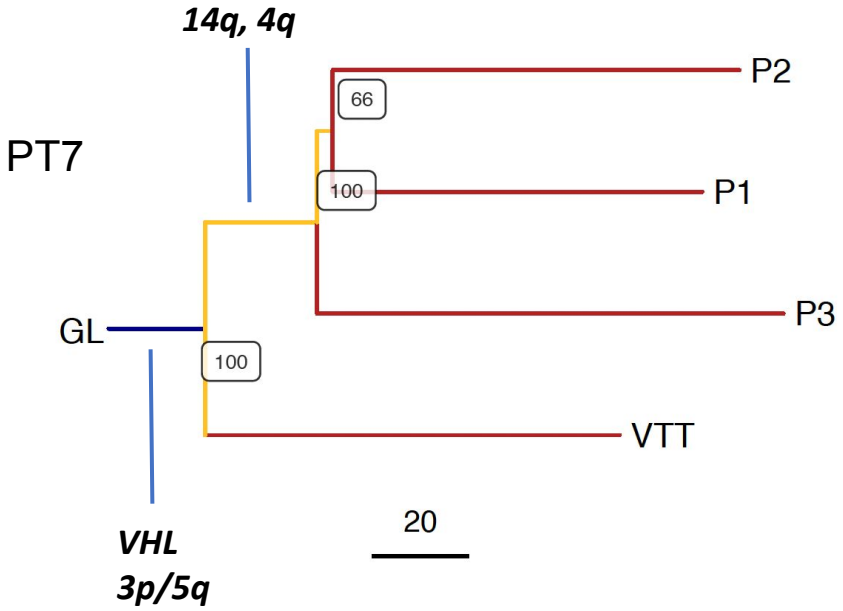
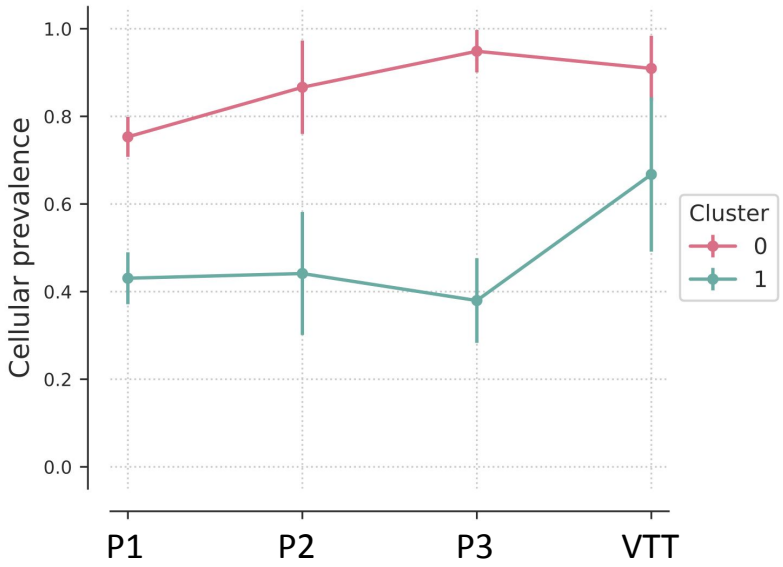


Figure S9.

D


Cellular prevalence
1.0
0.8
0.6
0.4
0.2
0.0
P1
P2
P3
VTT
Cluster
0
1

E

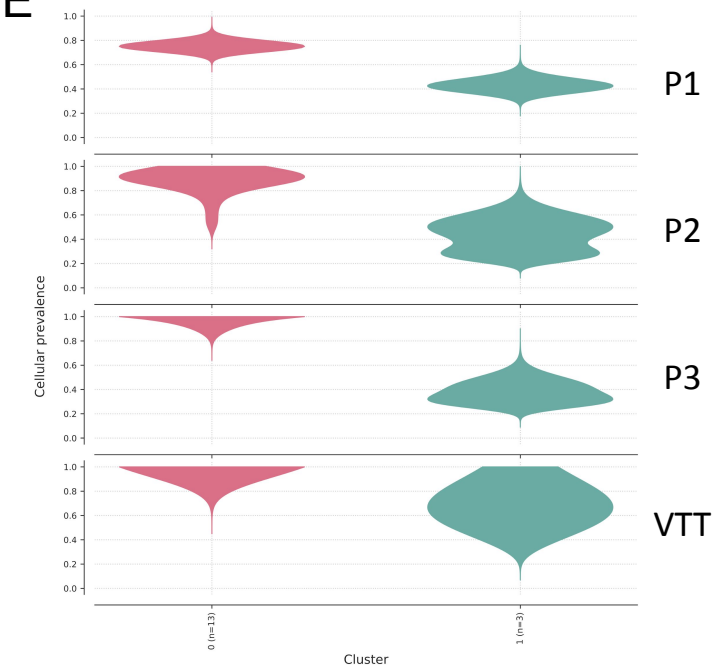
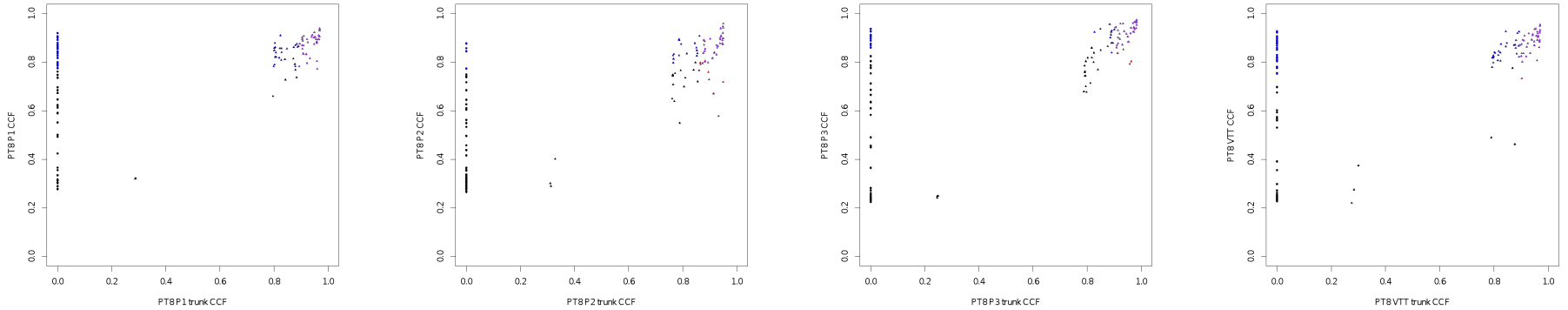
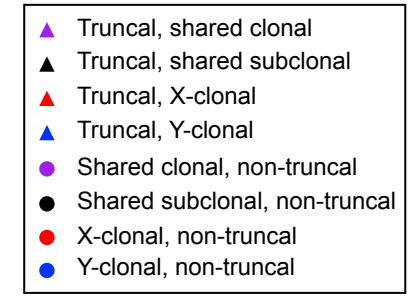

Cellular prevalence
P1
P2
P3
VTT
0 (n=13)
1 (n=3)
Cluster

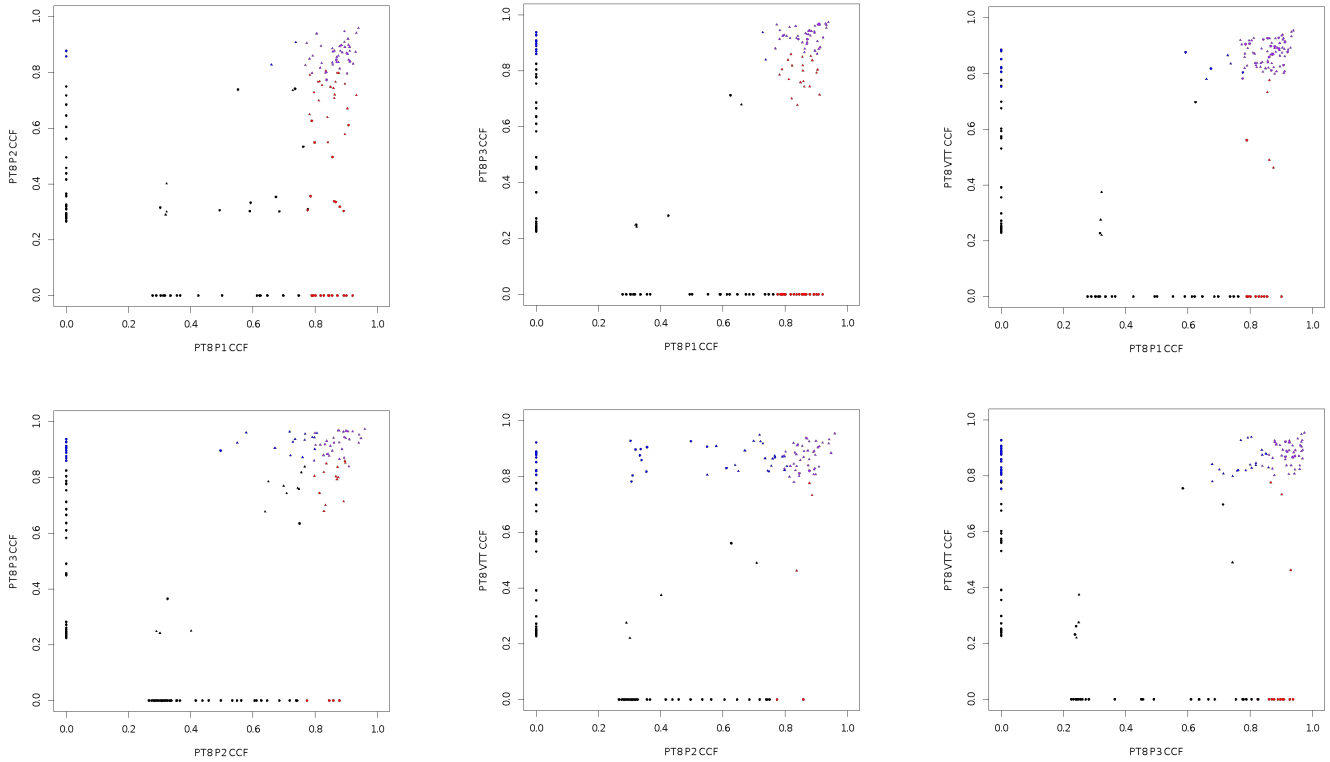
Figure S10.

A





B



C

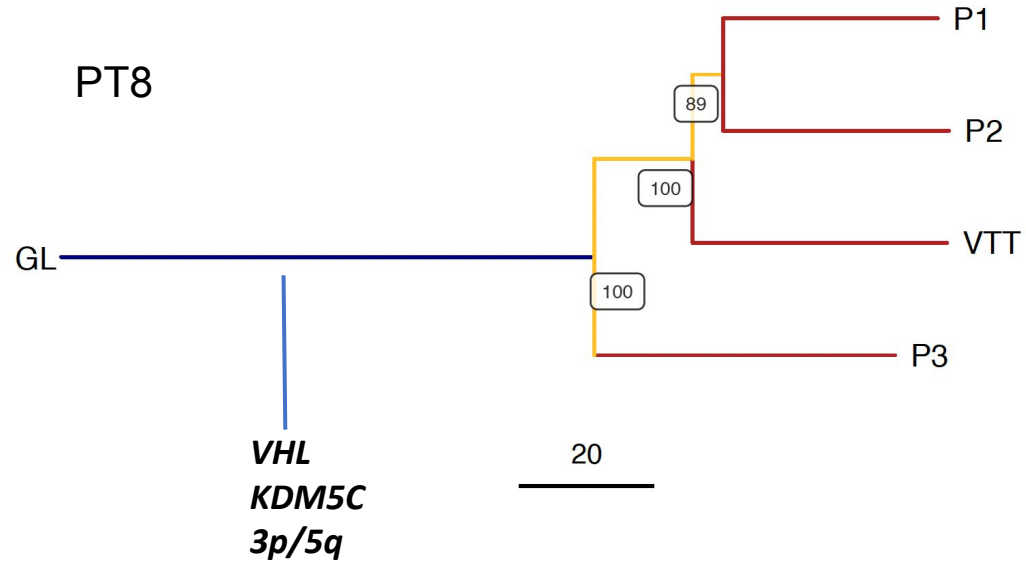
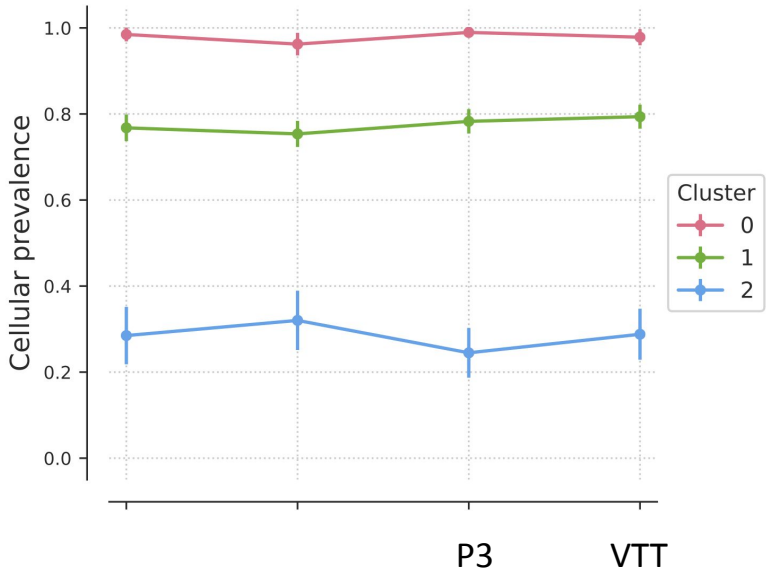


Figure S10.

D



E

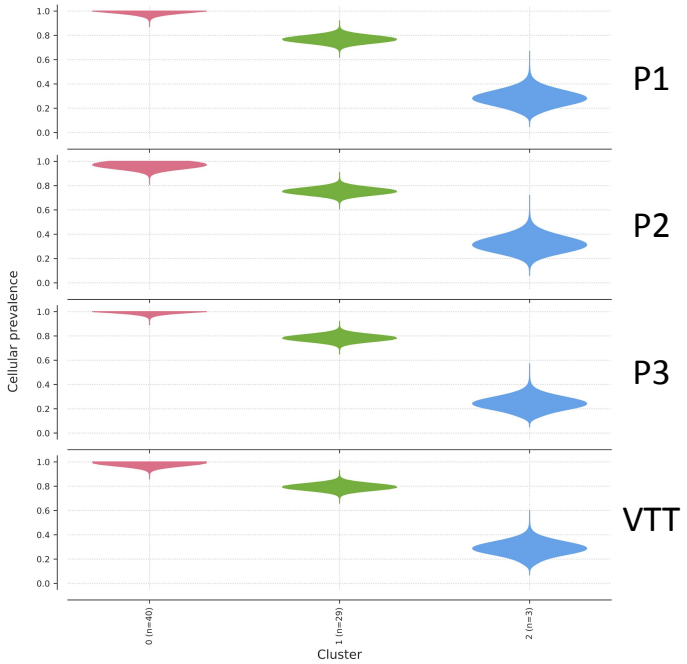
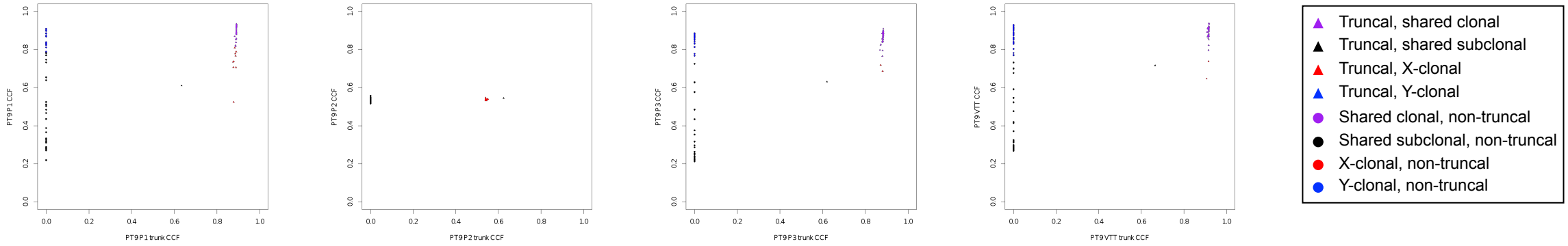
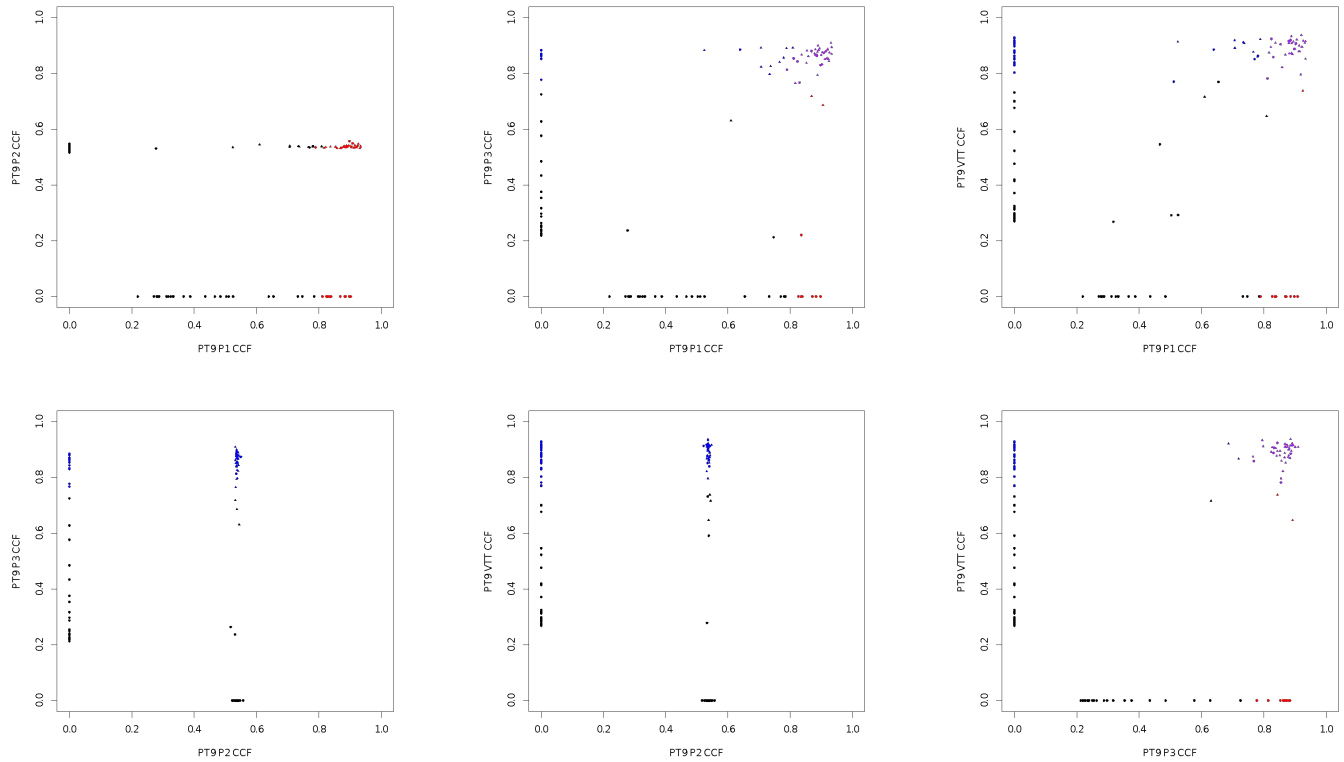


Figure S11.

A



B



C

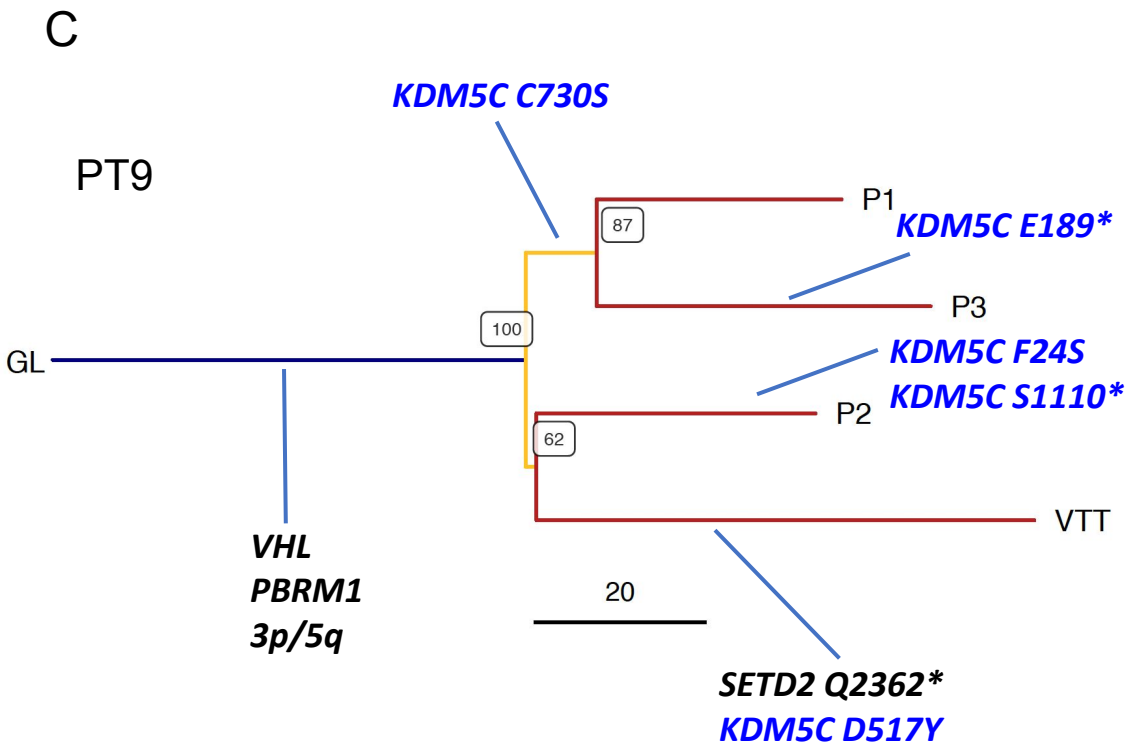
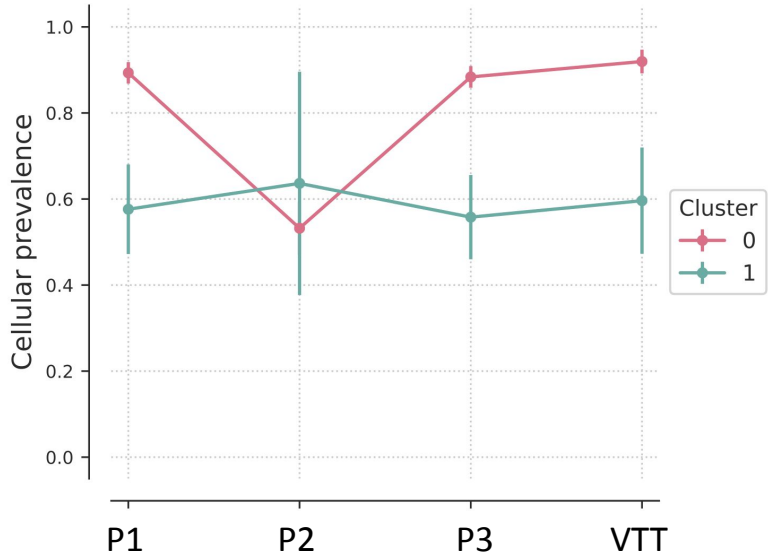


Figure S11.

D



E

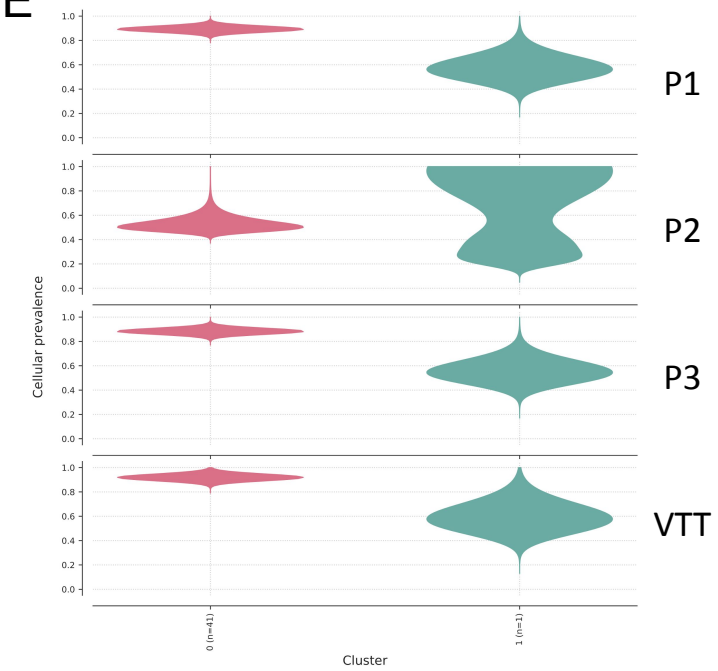
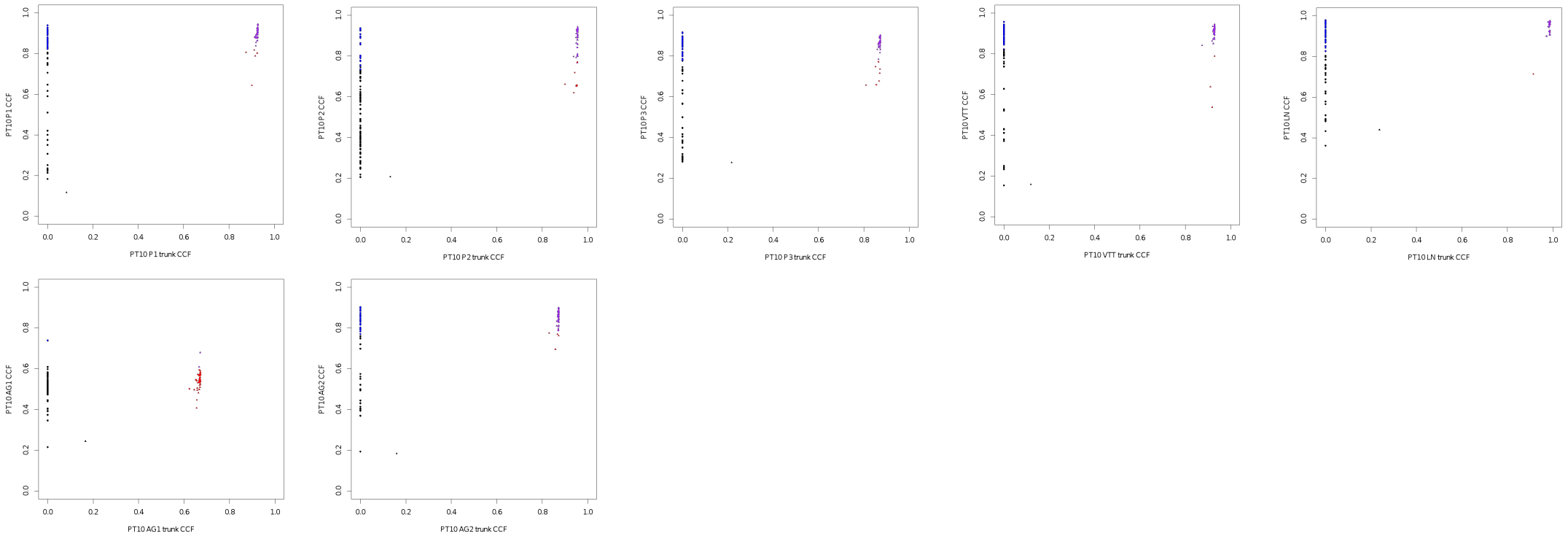


Figure S12.

A



- Truncal, shared clonal
- Truncal, shared subclonal
- Truncal, X-clonal
- Truncal, Y-clonal
- Shared clonal, non-truncal
- Shared subclonal, non-truncal
- X-clonal, non-truncal
- Y-clonal, non-truncal

Figure S12.

B

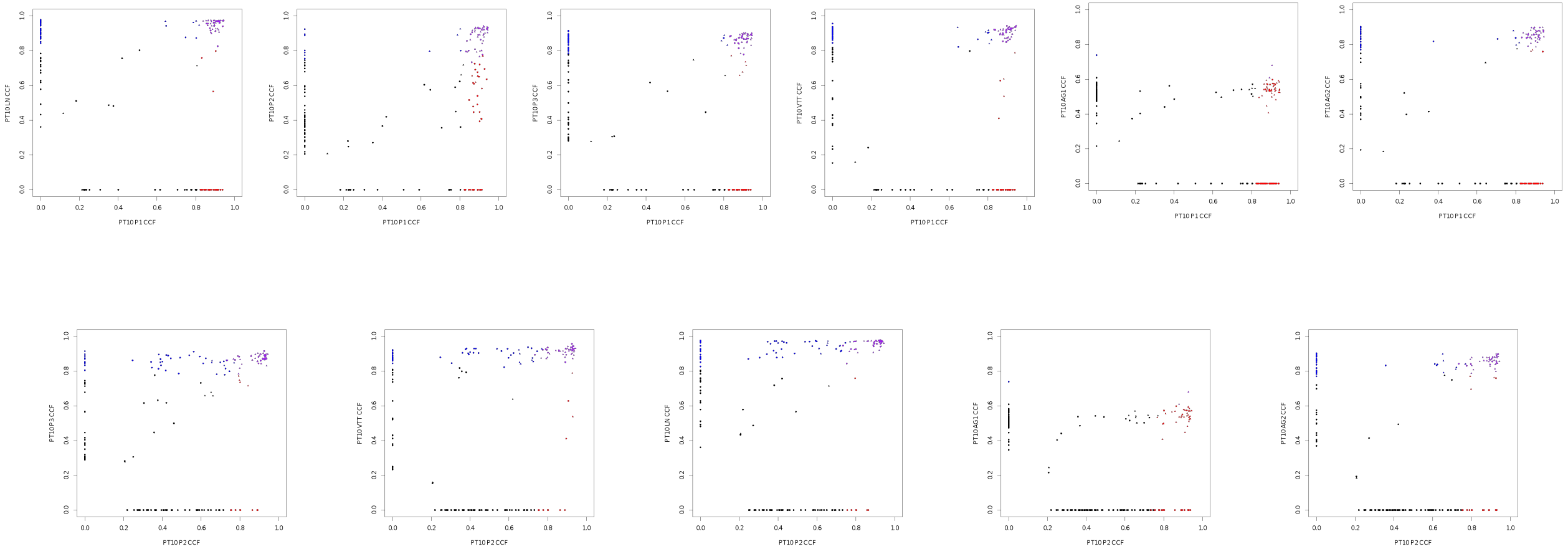
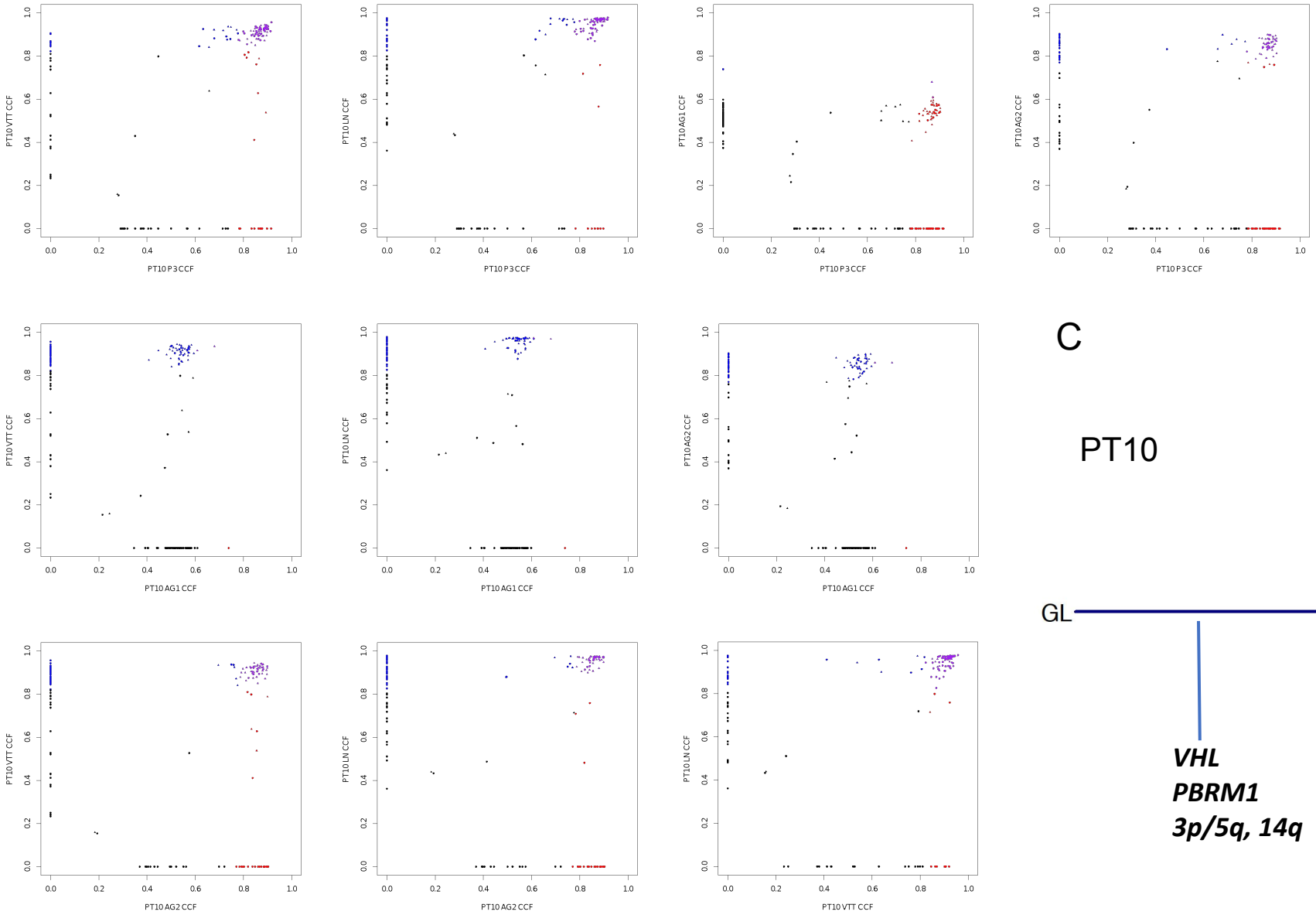

PT10LN CCF
PT10P2 CCF
PT10P3 CCF
PT10VTT CCF
PT10AG1 CCF
PT10AG2 CCF
PT10P3 CCF
PT10VTT CCF
PT10LN CCF
PT10AG1 CCF
PT10AG2 CCF
PT10P1 CCF
PT10P2 CCF

Figure S12.


PT10 VTT CCF
PT10 LN CCF
PT10 AG1 CCF
PT10 AG2 CCF
PT10 P3 CCF
PT10 AG1 CCF
PT10 AG2 CCF
PT10 VTT CCF

C

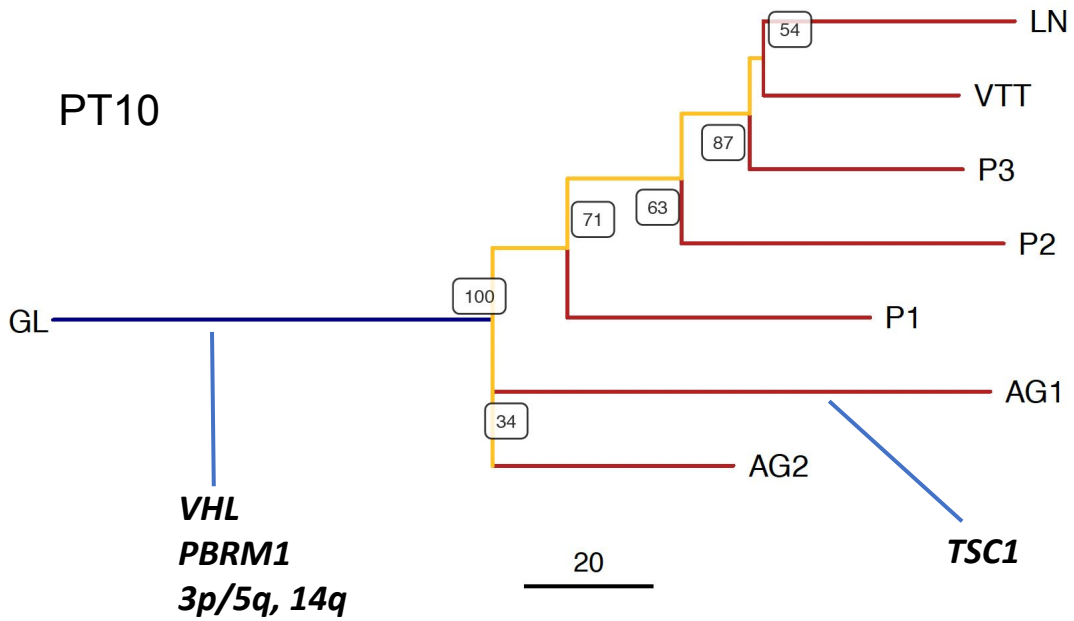
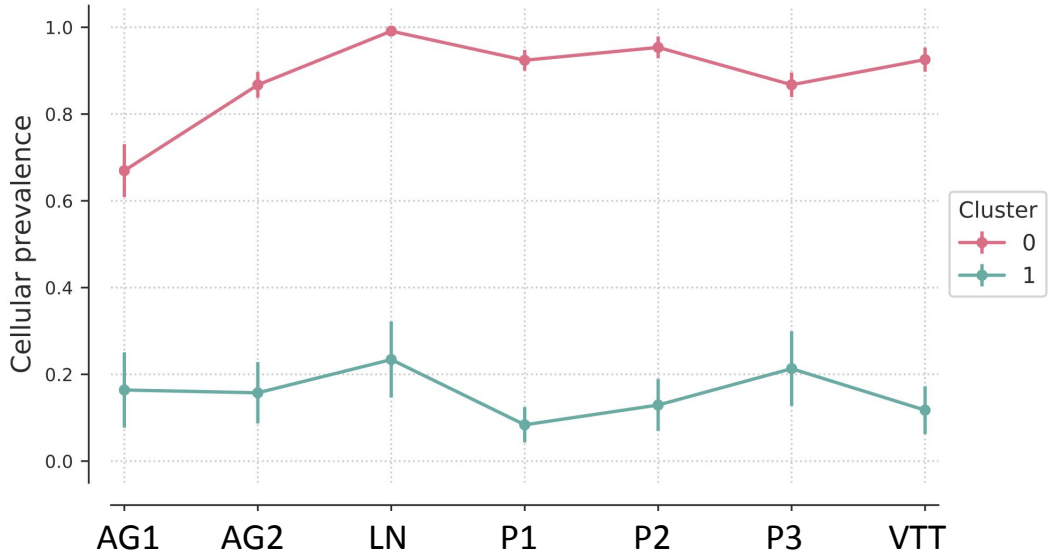

PT10
GL
100
71
63
87
54
LN
VTT
P3
P2
P1
34
AG1
AG2
20
VHL
PBRM1
3p/5q, 14q
TSC1

Figure S12.

D



E

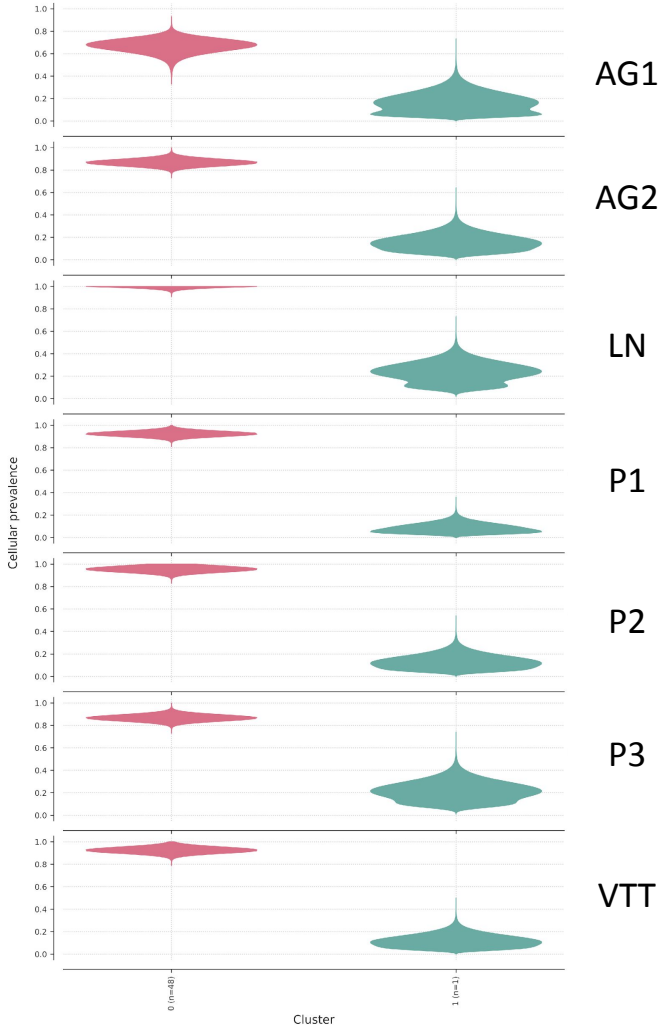
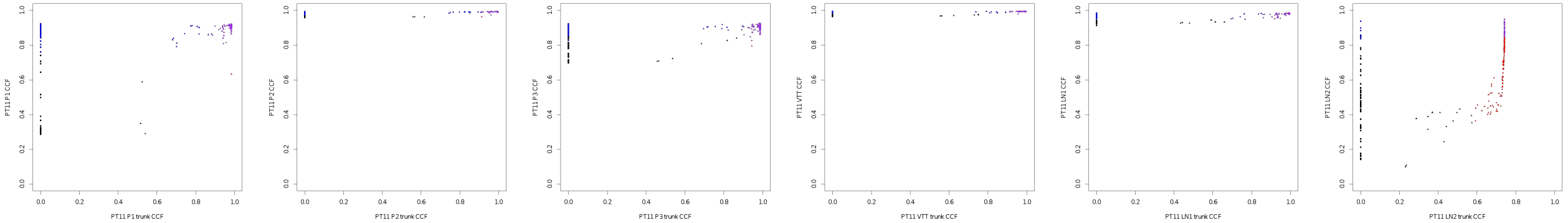
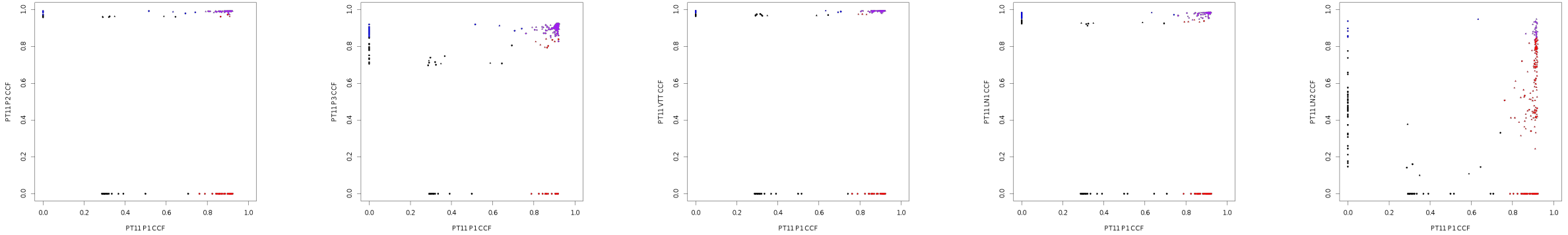


Figure S13.

A

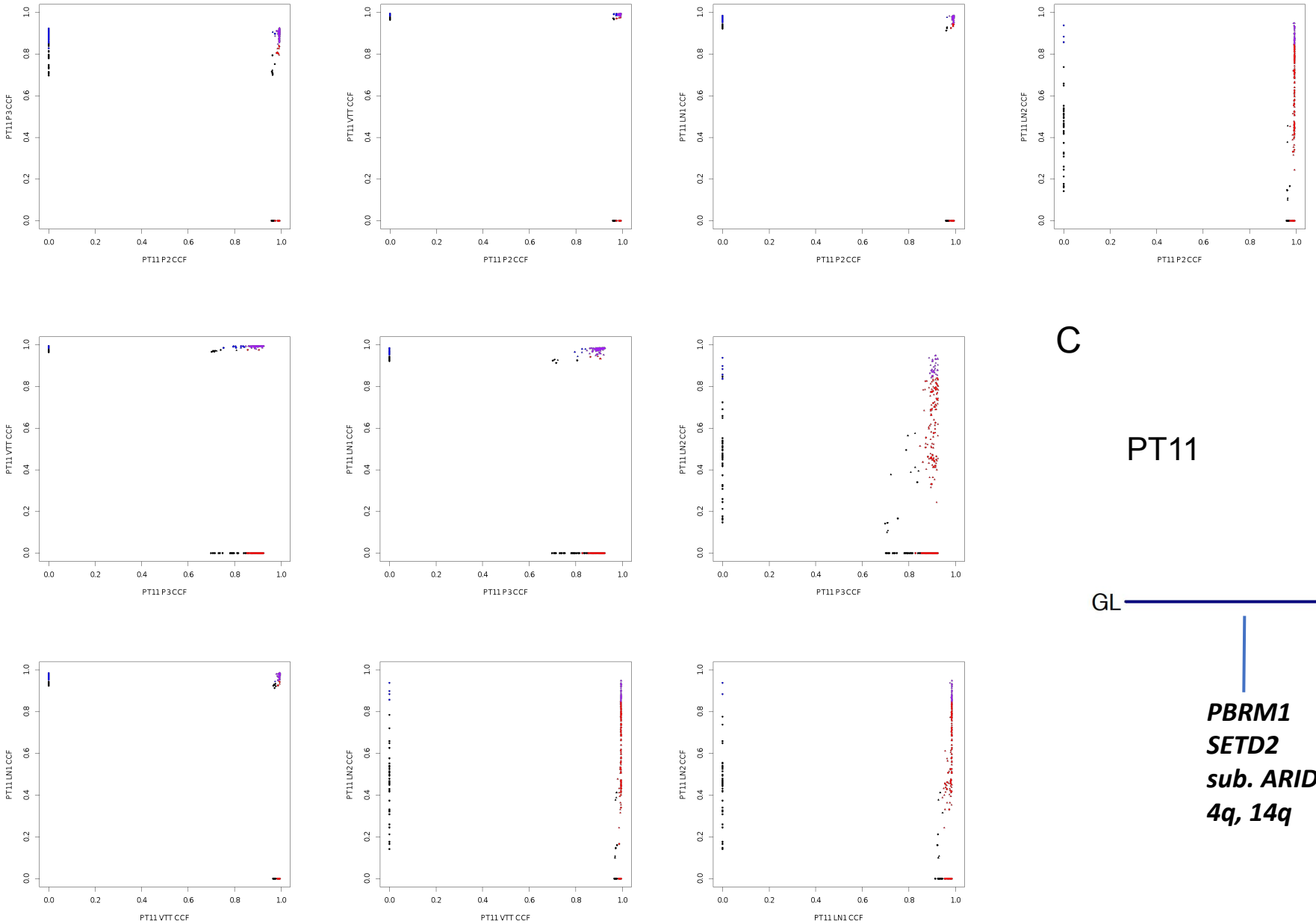


B



- Truncal, shared clonal
- Truncal, shared subclonal
- Truncal, X-clonal
- Truncal, Y-clonal
- Shared clonal, non-truncal
- Shared subclonal, non-truncal
- X-clonal, non-truncal
- Y-clonal, non-truncal

Figure S13.


PT11 P3 CCF
PT11 P2 CCF
PT11 VTT CCF
PT11 LN1 CCF
PT11 LN2 CCF
PT11 VTT CCF
PT11 LN1 CCF
PT11 LN2 CCF
PT11 P3 CCF
PT11 LN1 CCF
PT11 LN2 CCF
PT11 VTT CCF
PT11 LN1 CCF
PT11 LN2 CCF
PT11 VTT CCF
PT11 LN1 CCF
PT11 P2 CCF
PT11 P3 CCF
PT11 VTT CCF
PT11 LN1 CCF
PT11 LN2 CCF

C

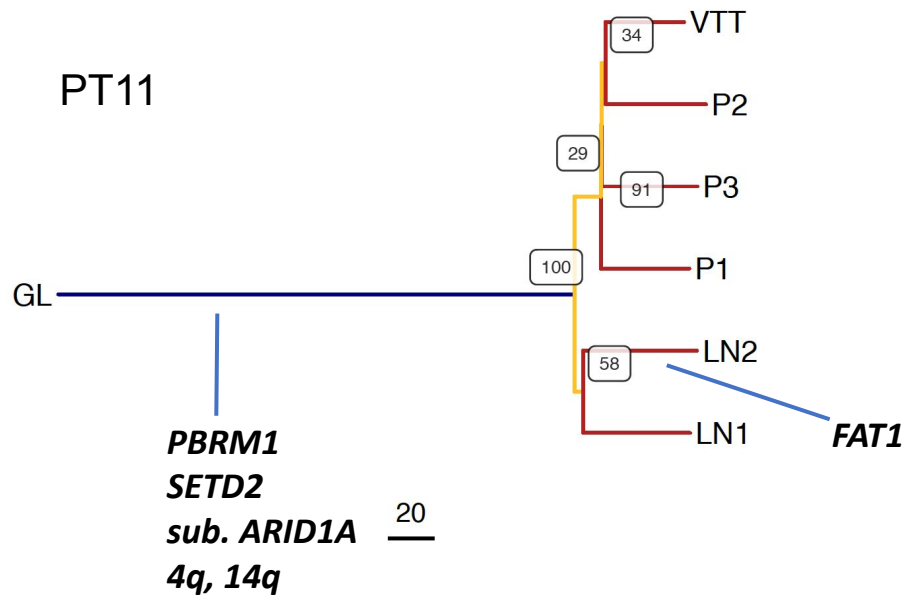
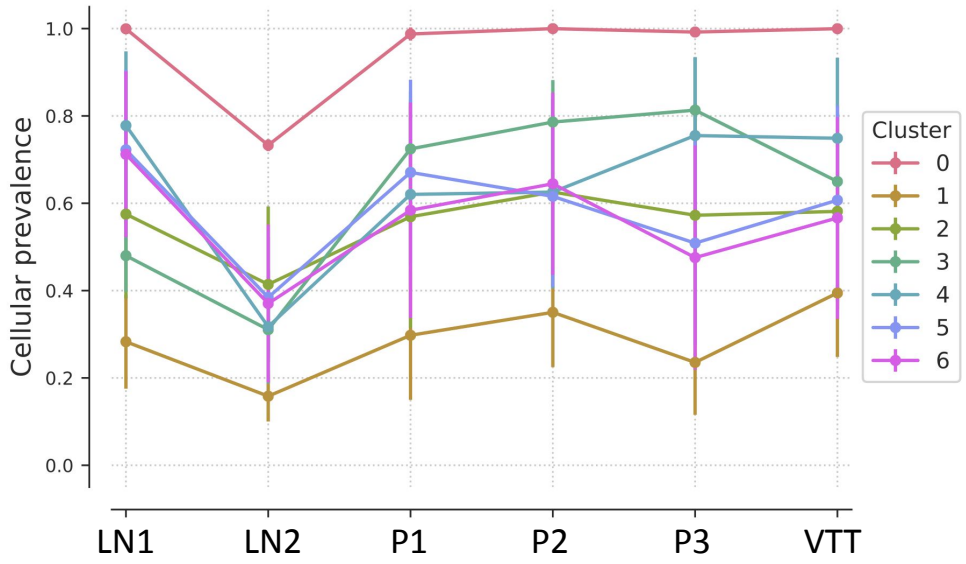

PT11
GL
34
VTT
P2
29
91
P3
P1
100
58
LN2
LN1
FAT1
PBRM1
SETD2
sub. ARID1A
4q, 14q
20

Figure S13.

D



E

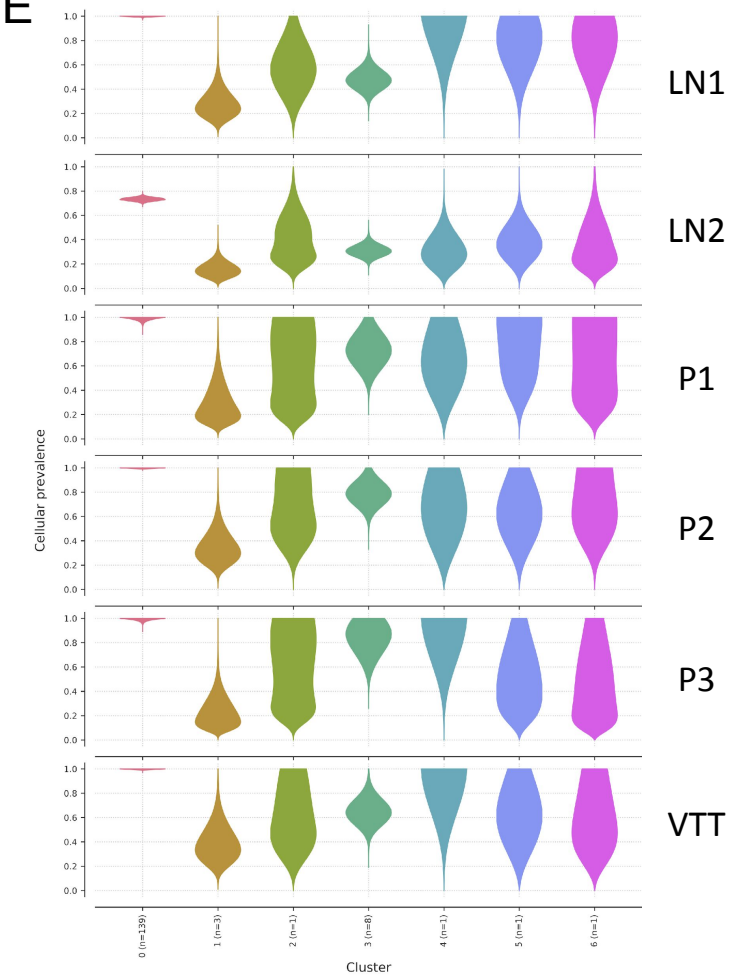
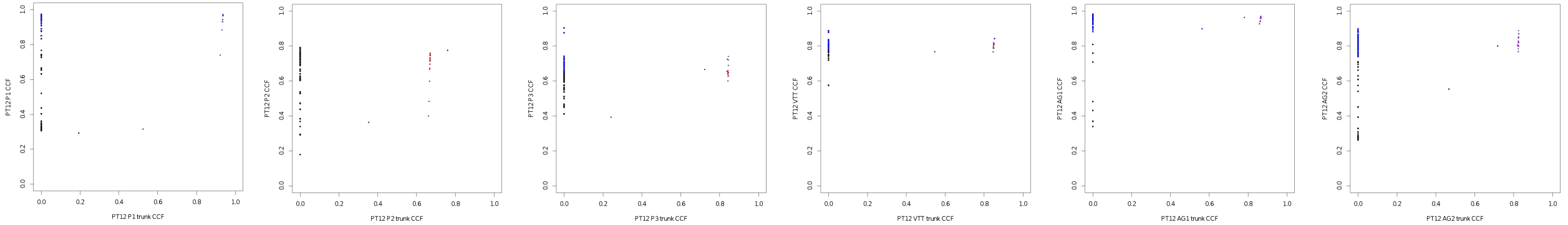
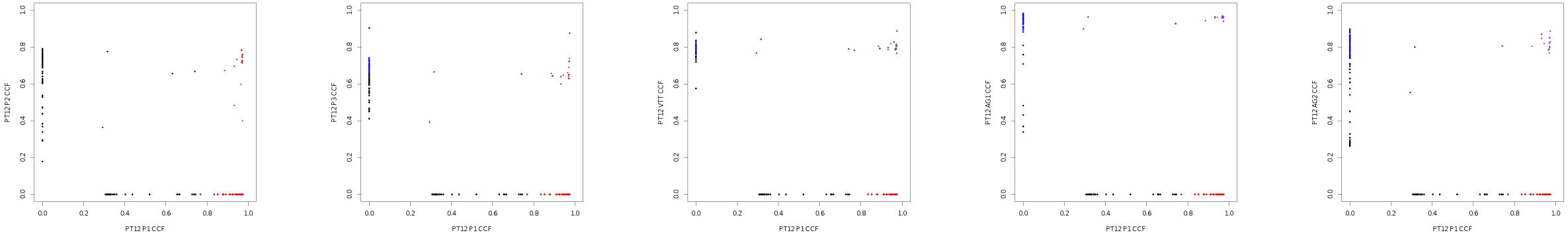


Figure S14.

A



B



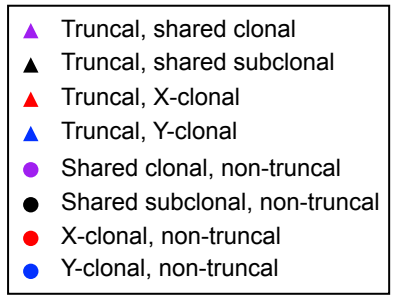
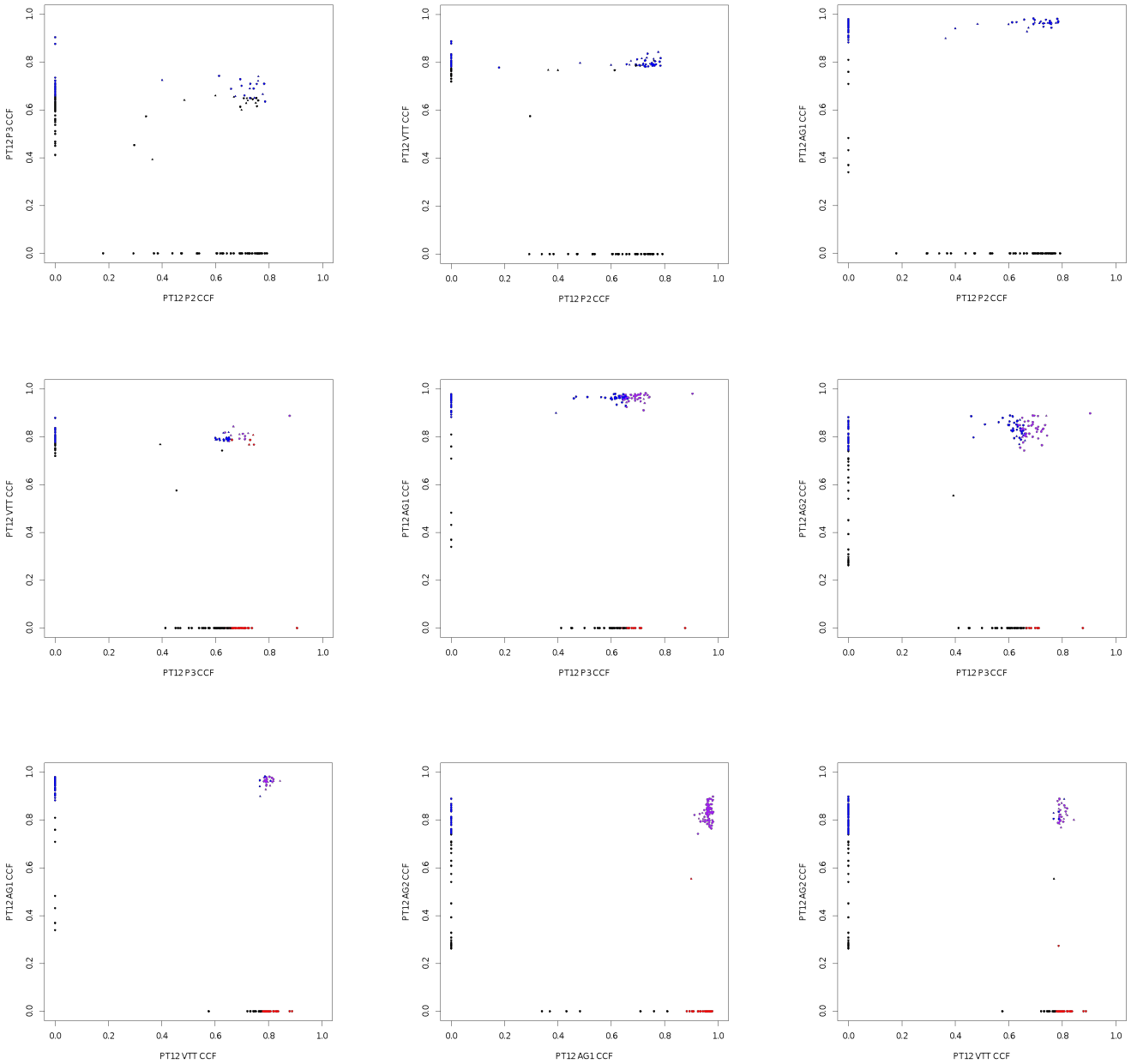


Figure S14.


PT12 P3 CCF
PT12 P2 CCF
PT12 VTT CCF
PT12 AG1 CCF
PT12 AG2 CCF

C

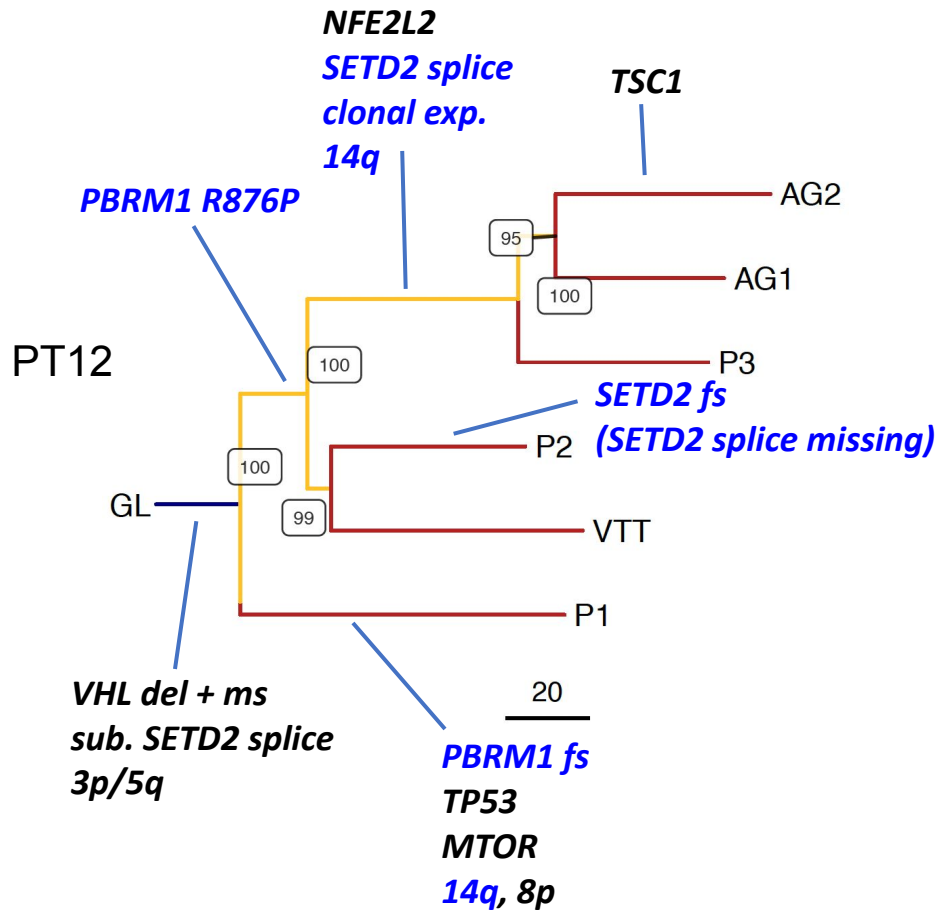
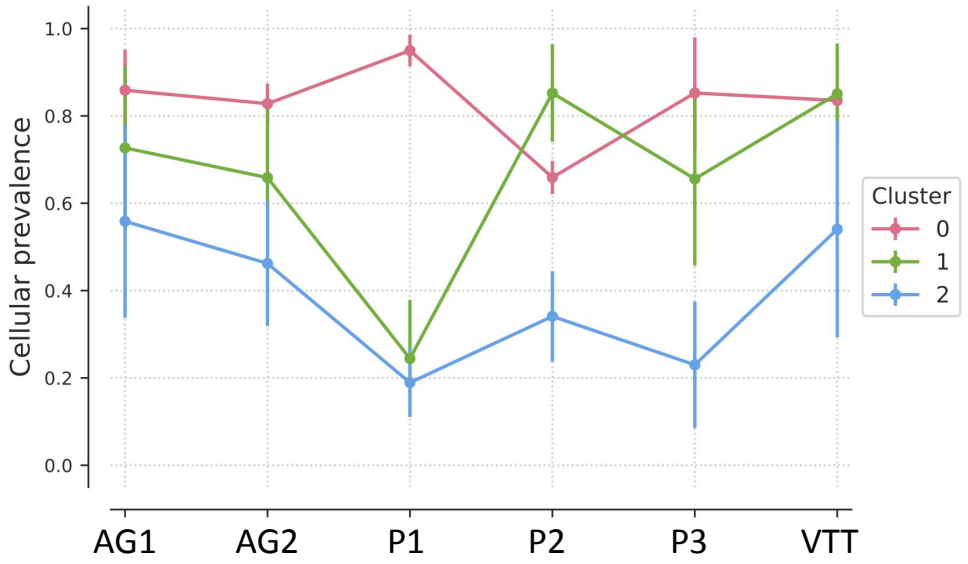

NFE2L2
SETD2 splice
clonal exp.
14q
TSC1
PBRM1 R876P
AG2
AG1
P3
P2
VTT
P1
GL
PT12
95
100
100
100
99
SETD2 fs
(SETD2 splice missing)
VHL del + ms
sub. SETD2 splice
3p/5q
20
PBRM1 fs
TP53
MTOR
14q, 8p

Figure S14.

D



E

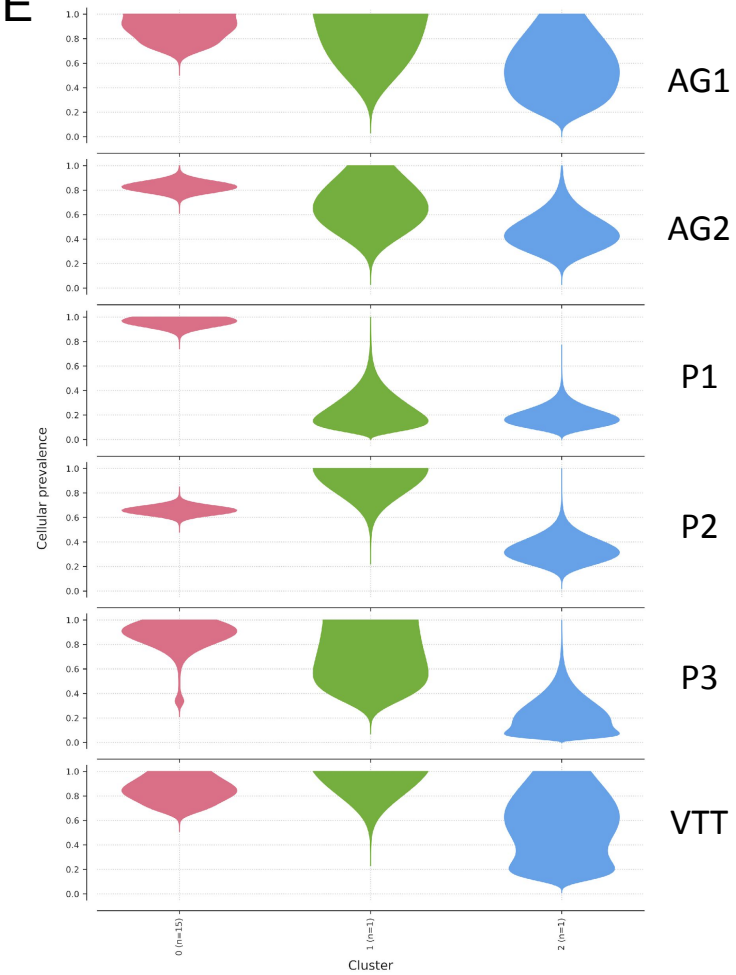
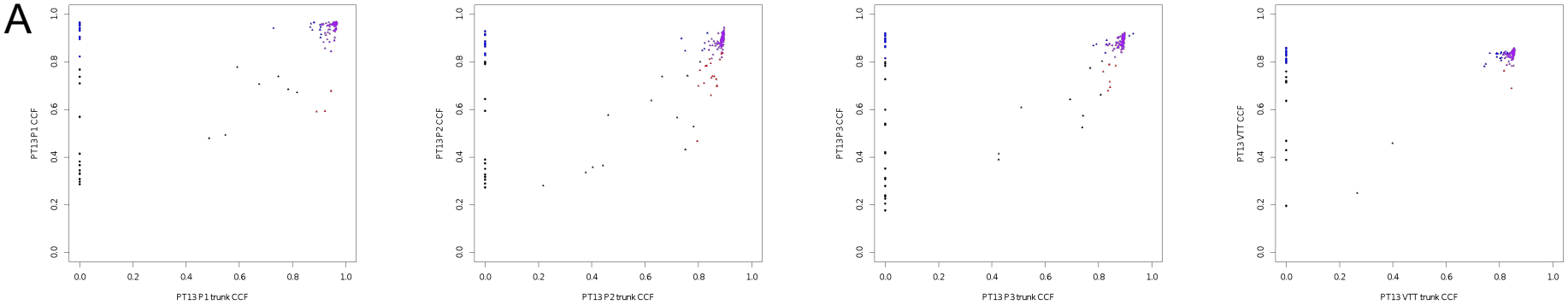
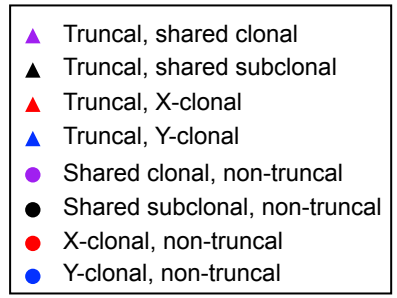


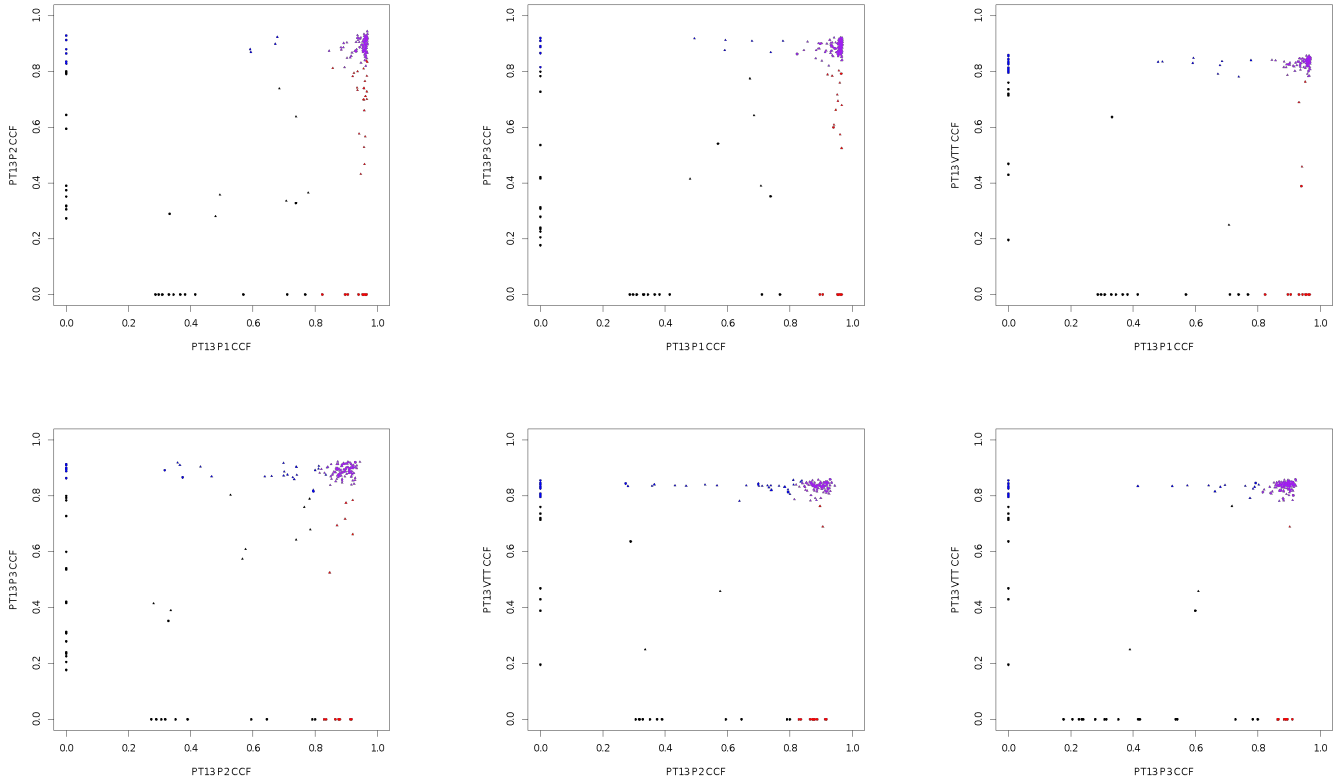
Figure S15.

A





B



C

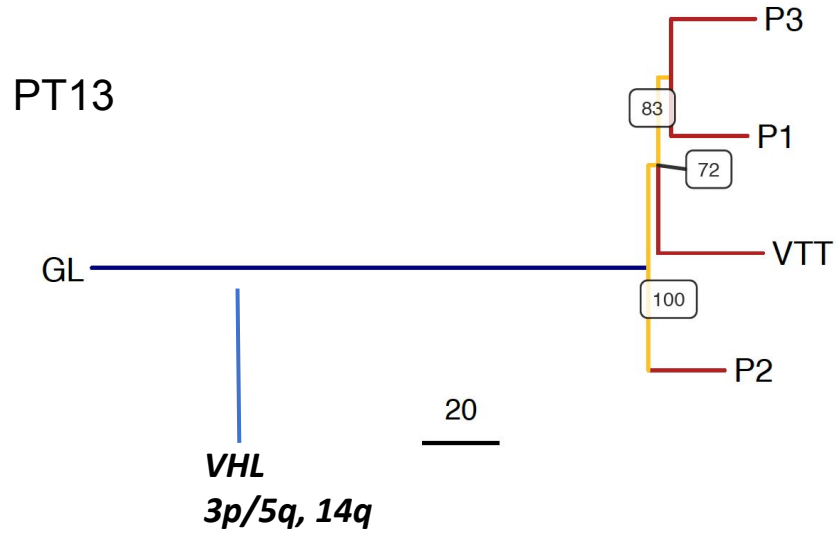
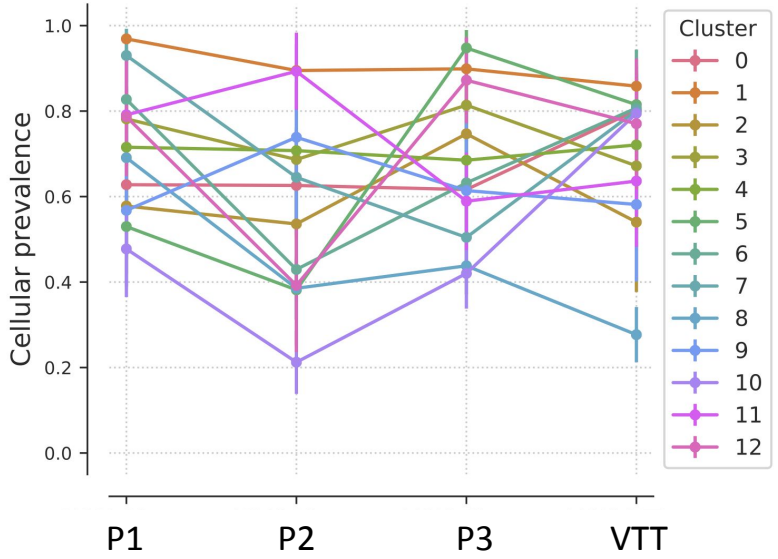


Figure S15.

D



E

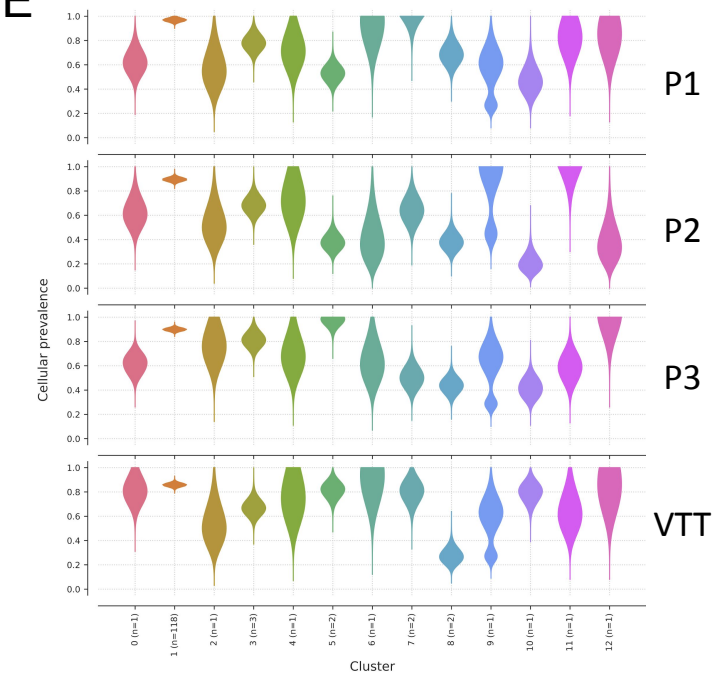
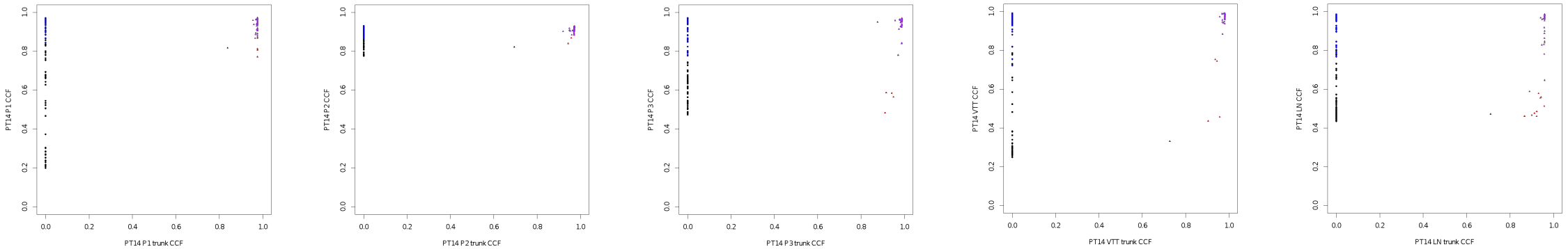


Figure S16.

A



B

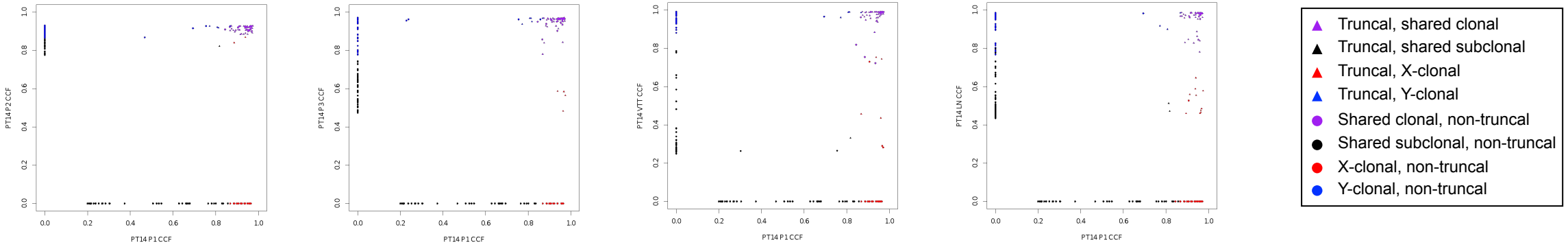
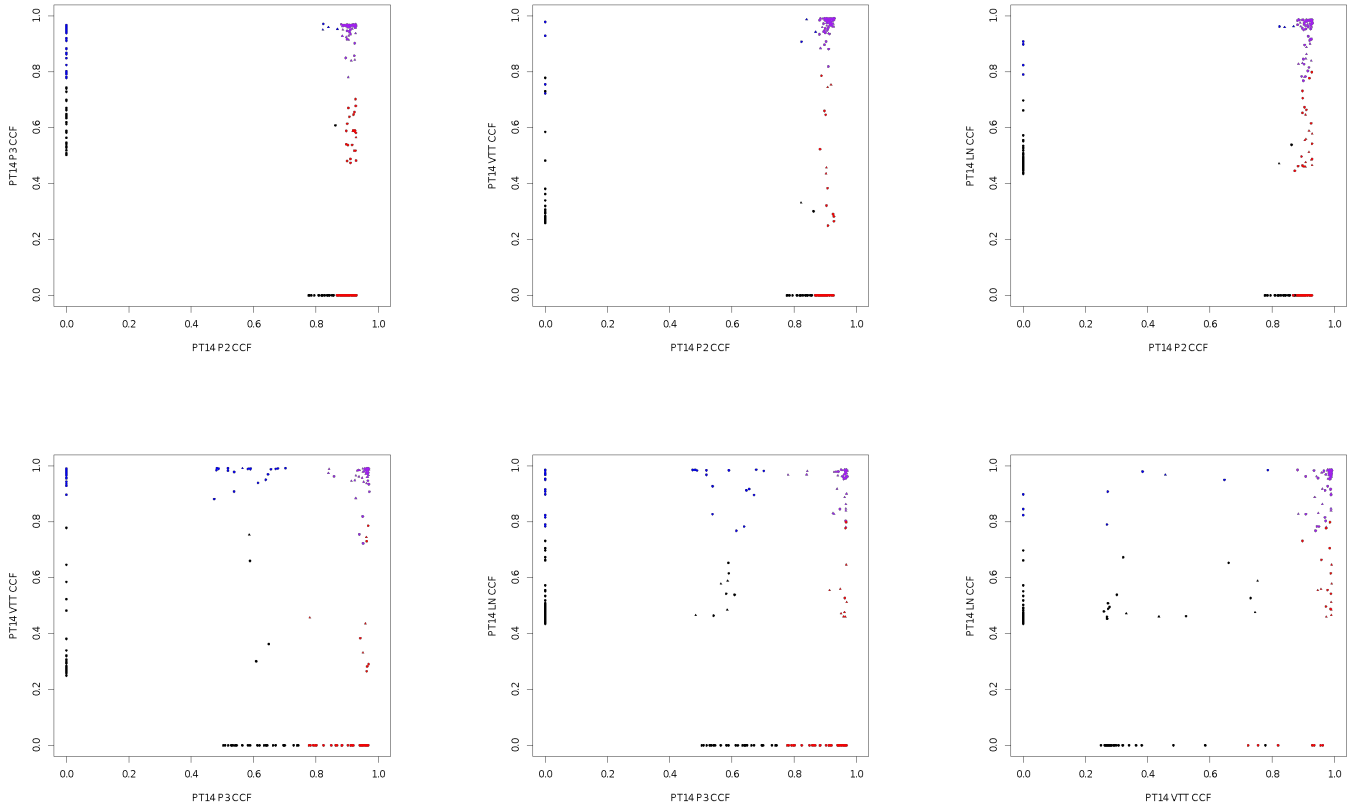


Figure S16.


PT14 P3 CCF
PT14 P2 CCF
PT14 VTT CCF
PT14 LN CCF
PT14 P3 CCF
PT14 VTT CCF

C

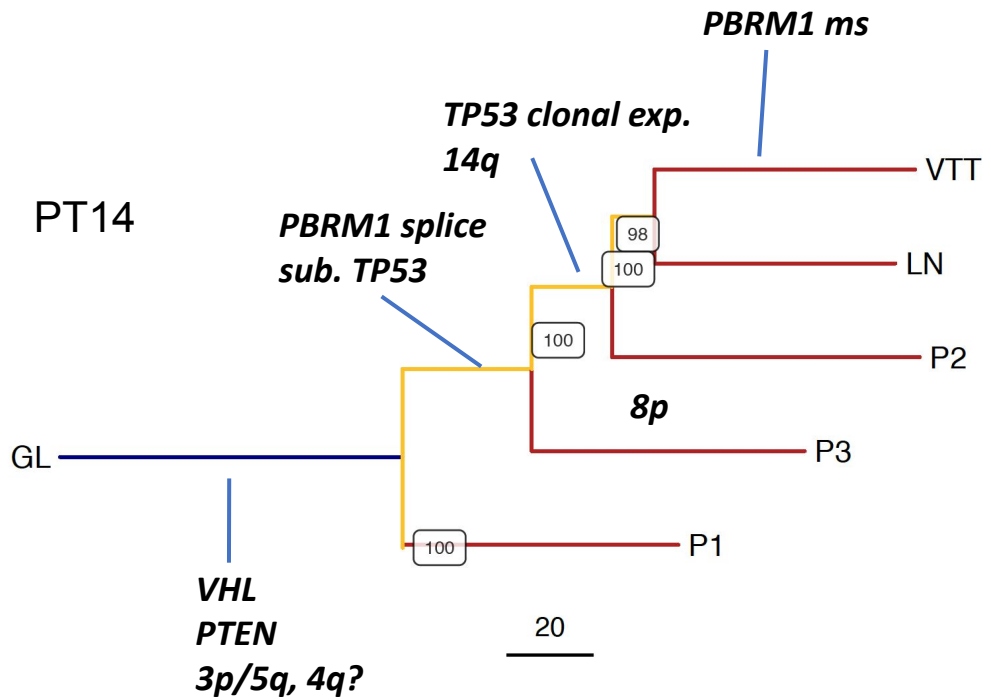
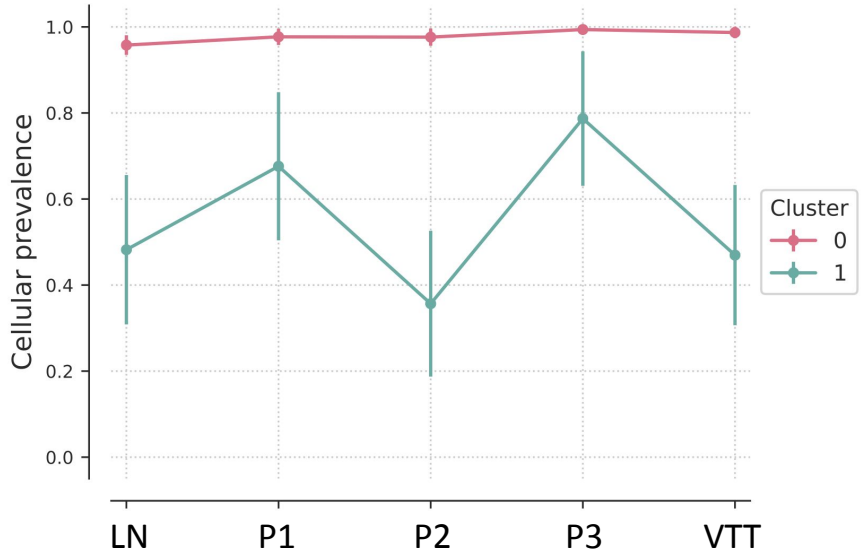

PBRM1 ms
TP53 clonal exp.
14q
PT14
PBRM1 splice
sub. TP53
VTT
LN
P2
P3
P1
GL
8p
VHL
PTEN
3p/5q, 4q?
98
100
100
100
20

Figure S16.

D



E

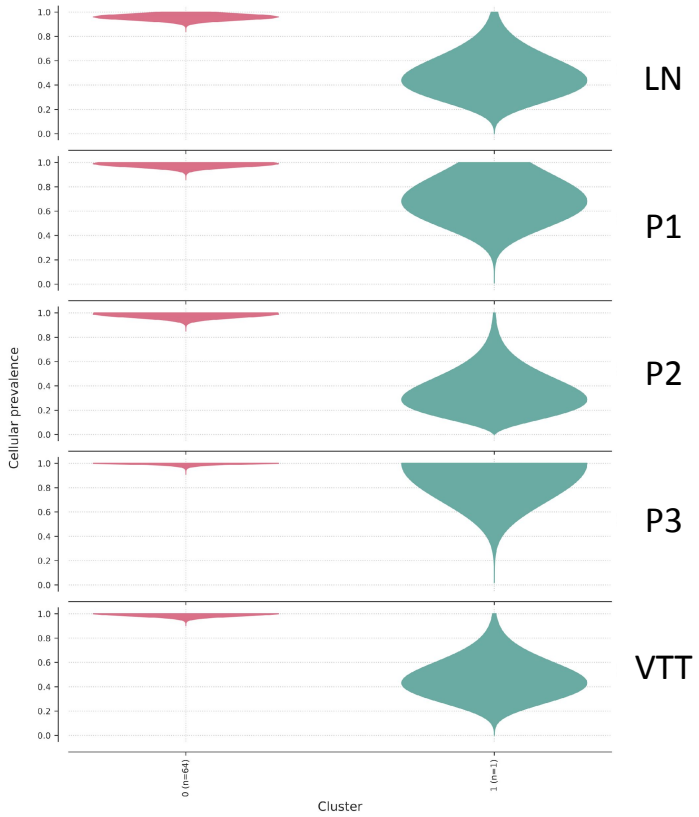
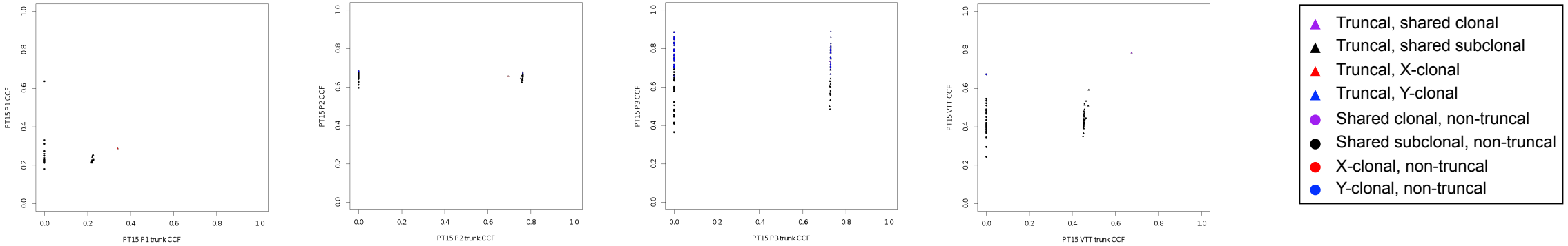
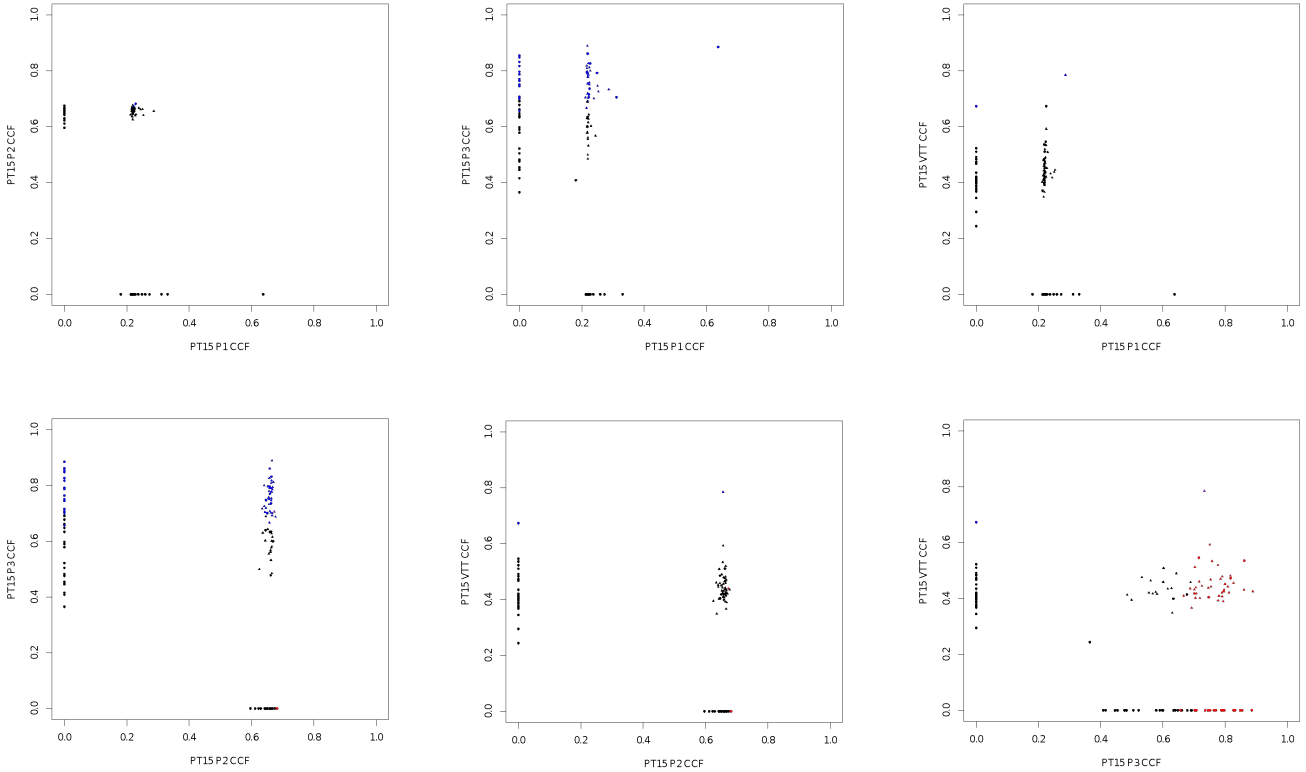


Figure S17.

A



B



C

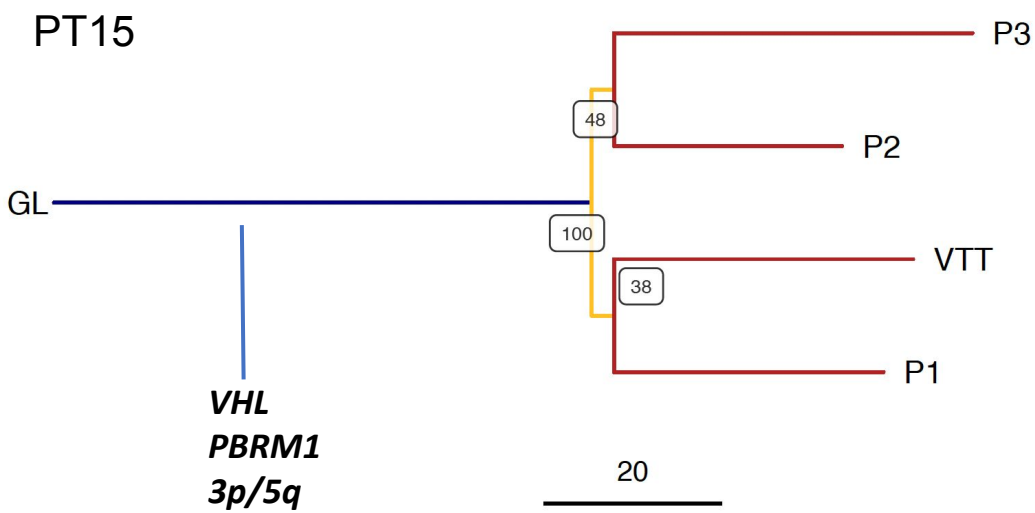
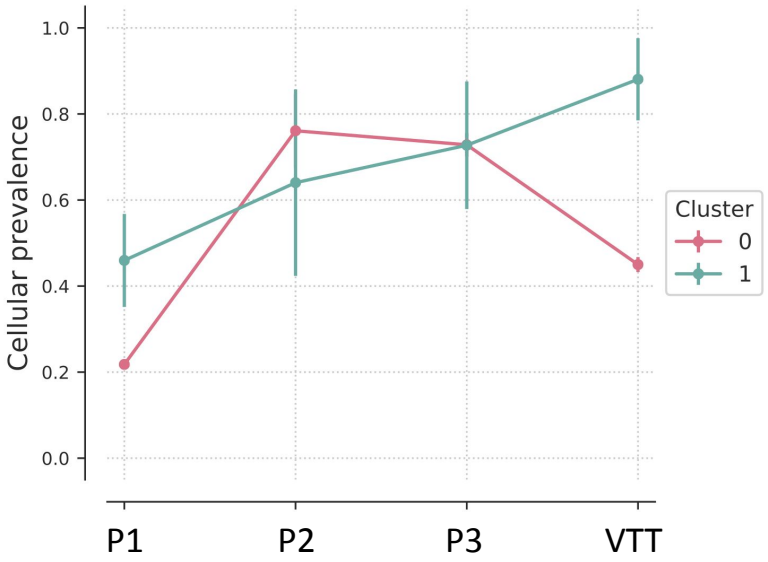


Figure S17.

D



E

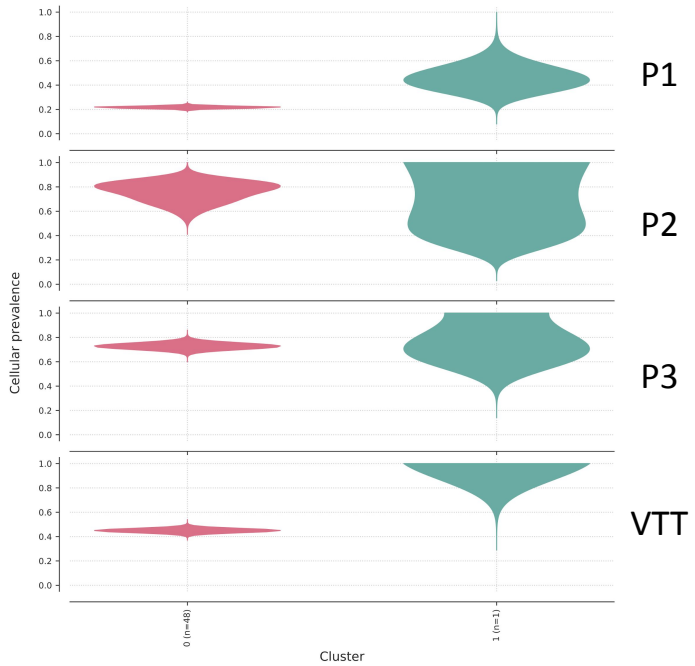
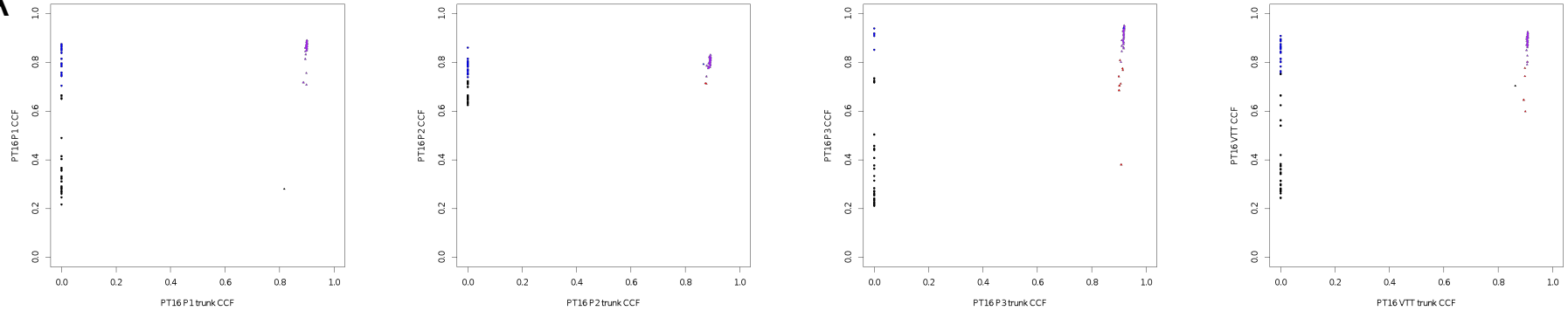
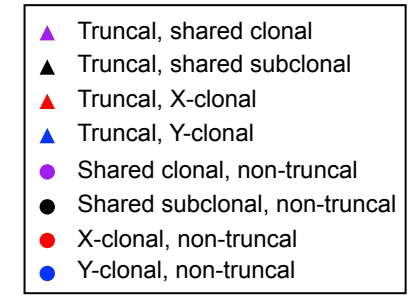


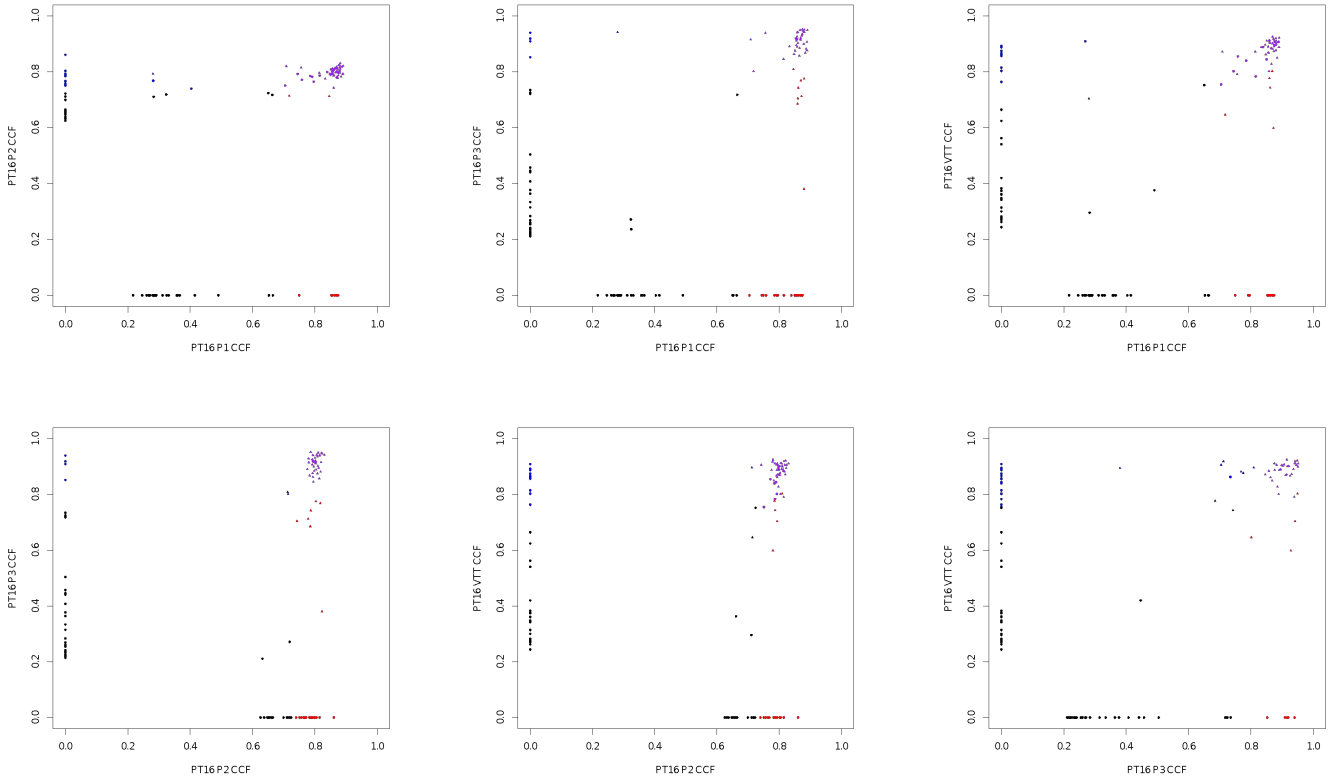
Figure S18.

A





B



C

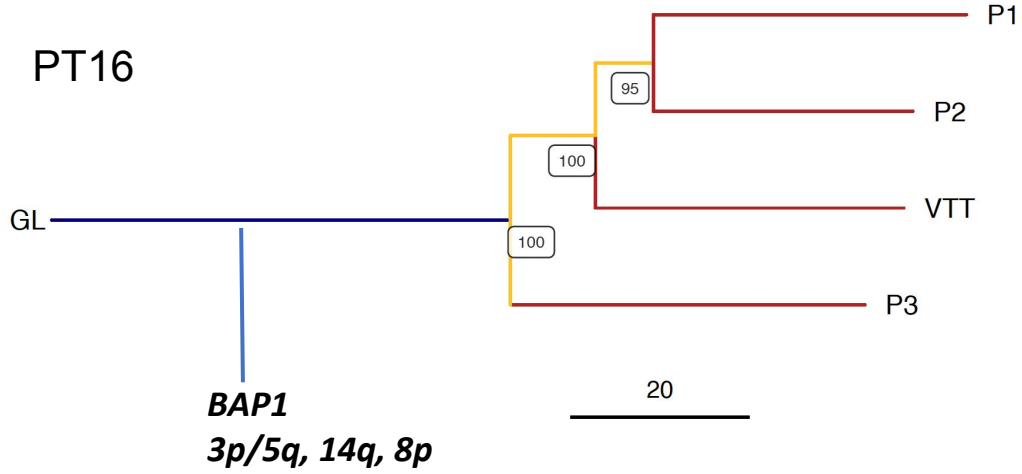
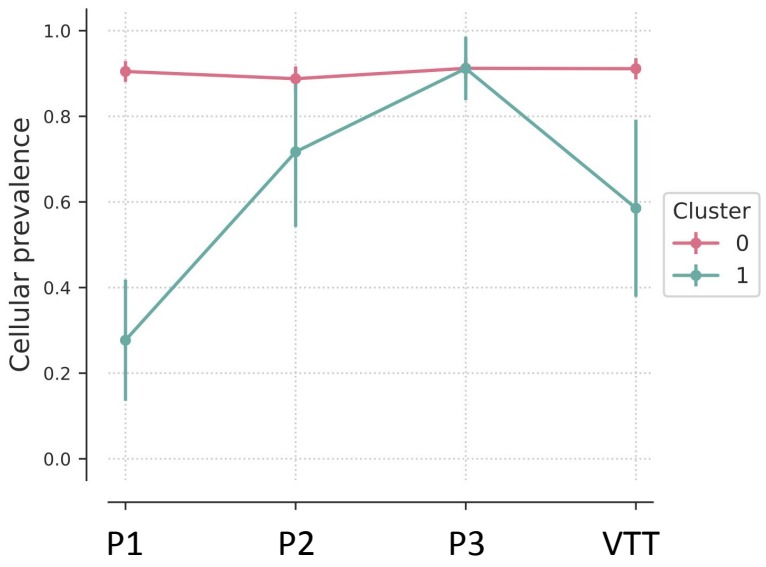
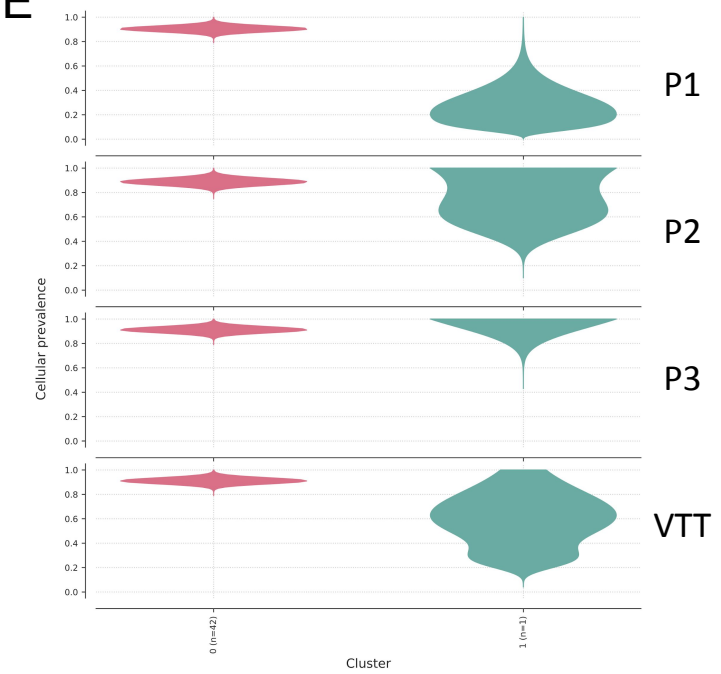


Figure S18.

D



E



Figures S3-S18. Integrative evolutionary analysis of each tumor, overlaying mutation clonality, phylogenies, and driver alterations. Two-way cancer cell fraction (CCF) plots show (A) all mutations from each tumor region versus truncal mutations, and (B) all possible pairs of tumor regions. (C) Tumor phylogenies annotated with driver alterations. Alterations in blue indicate parallel mutations. "Sub.", subclonal; "clonal exp.", clonal expansion; †, TP53 clone in PT2 persists only in P3, VTT1, VTT2, P2 (minor subclone in P2). (D) Average CCF value with standard error for each mutation cluster (clone) across tumor regions, as determined by PyClone. Only mutations present in all regions are included. (E) CCF density distributions for each mutation cluster (clone) across tumor regions, as determined by PyClone. Numbers of mutations per cluster are indicated in parentheses at bottom.

Figure S19.

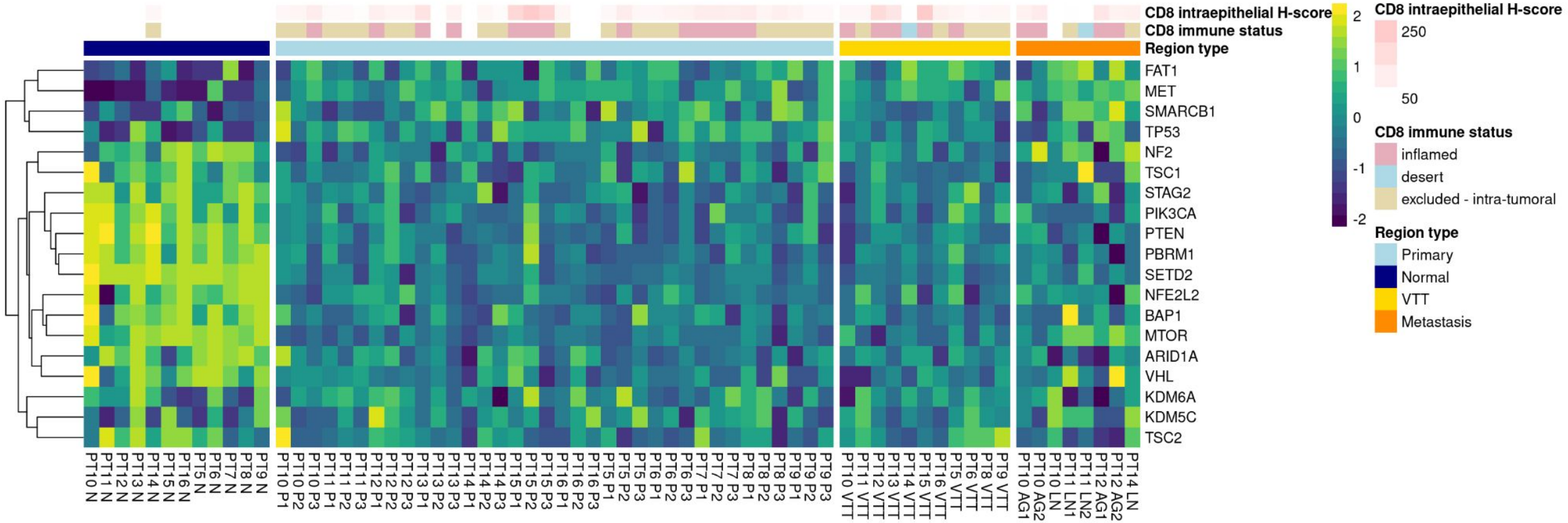

CD8 intraepithelial H-score
CD8 immune status
Region type
FAT1
MET
SMARCB1
TP53
NF2
TSC1
STAG2
PIK3CA
PTEN
PBRM1
SETD2
NFE2L2
BAP1
MTOR
ARID1A
VHL
KDM6A
KDM5C
TSC2
2
1
0
-1
-2
CD8 intraepithelial H-score
250
50
CD8 immune status
inflamed
desert
excluded - intra-tumoral
Region type
Primary
Normal
VTT
Metastasis
PT10 N
PT11 N
PT12 N
PT13 N
PT14 N
PT15 N
PT16 N
PT5 N
PT6 N
PT7 N
PT8 N
PT9 N
PT10 P1
PT10 P2
PT10 P3
PT11 P1
PT11 P2
PT11 P3
PT12 P1
PT12 P2
PT12 P3
PT13 P1
PT13 P2
PT13 P3
PT14 P1
PT14 P2
PT14 P3
PT15 P1
PT15 P2
PT15 P3
PT16 P1
PT16 P2
PT16 P3
PT5 P1
PT5 P2
PT5 P3
PT6 P1
PT6 P2
PT6 P3
PT7 P1
PT7 P2
PT7 P3
PT8 P1
PT8 P2
PT8 P3
PT9 P1
PT9 P2
PT9 P3
PT10 VTT
PT11 VTT
PT12 VTT
PT13 VTT
PT14 VTT
PT15 VTT
PT16 VTT
PT5 VTT
PT6 VTT
PT8 VTT
PT9 VTT
PT10 AG1
PT10 AG2
PT10 LN
PT11 LN1
PT11 LN2
PT12 AG1
PT12 AG2
PT14 LN

Figure S19. Expression status of putative driver genes across all regions of all patients, excepting CSMD3, which had no uniquely mapped counts in any region. Expression values were normalized by variance-stabilizing transformation, then turned into z-scores normalized within each gene and patient prior to visualization.

Figure S20.

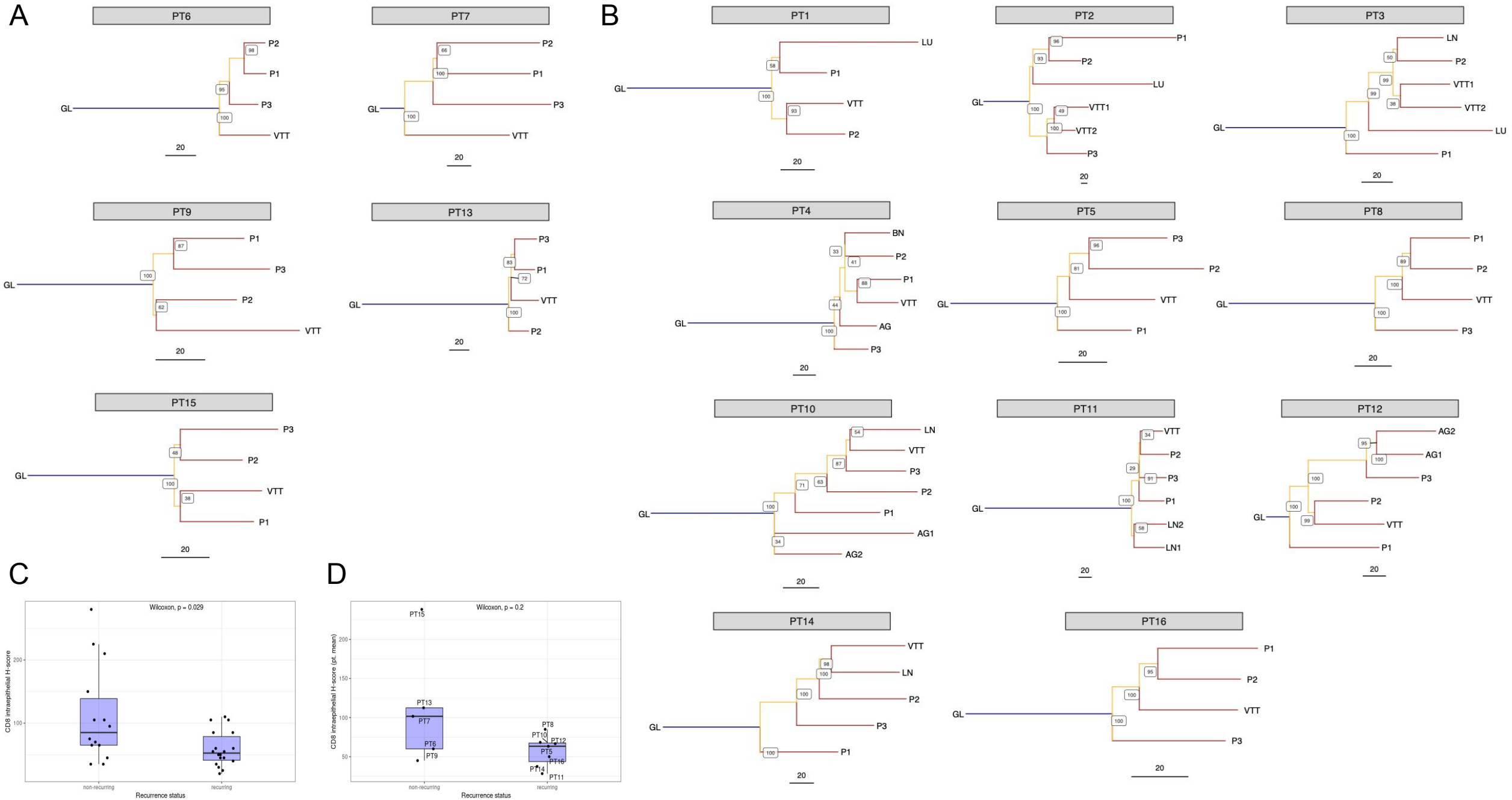
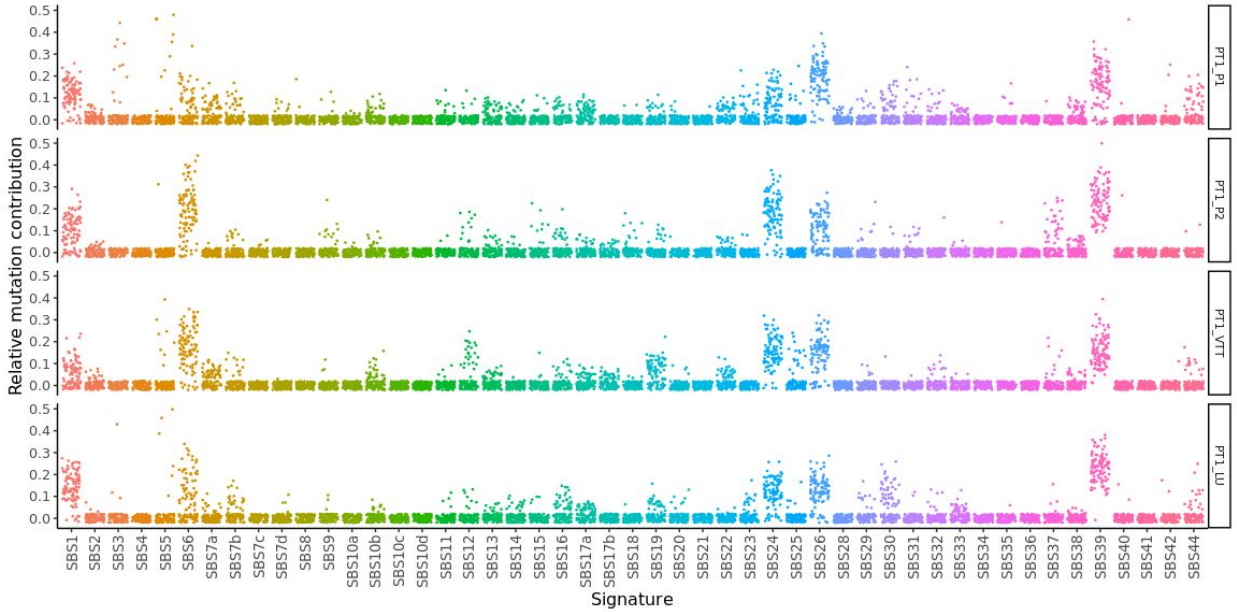
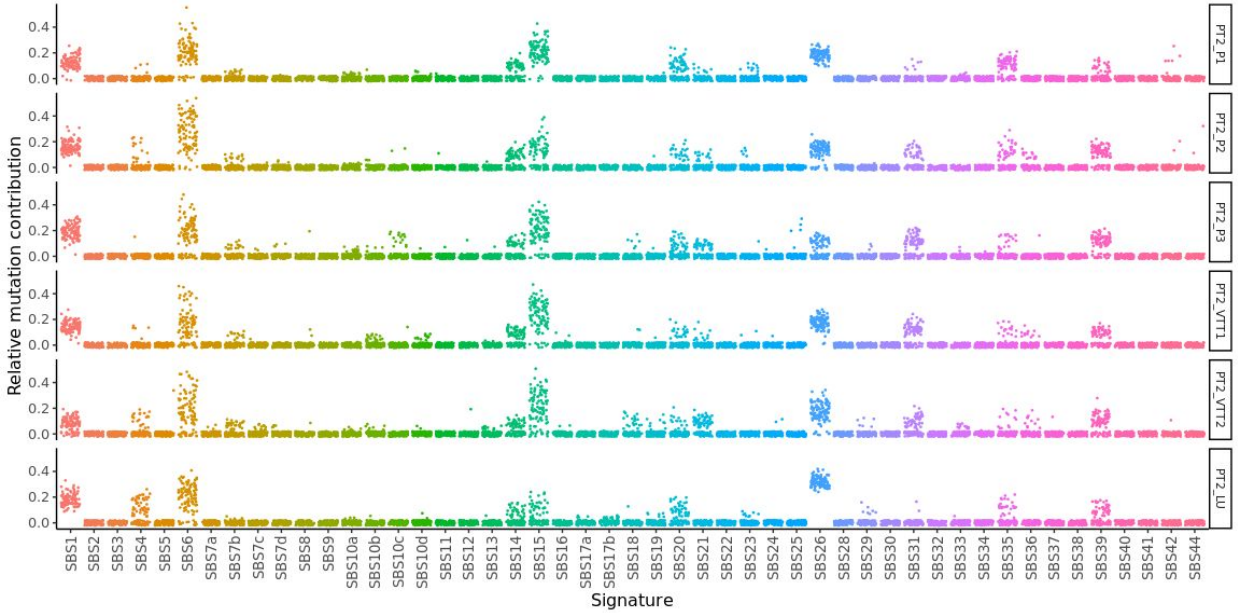
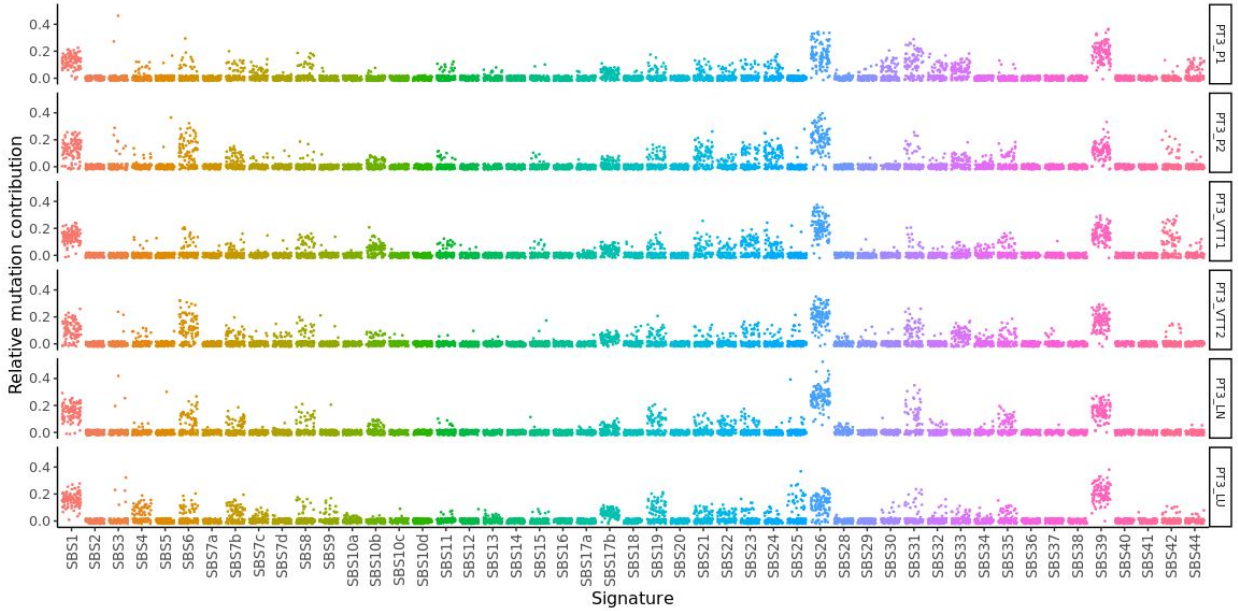

A
PT6
P2
P1
P3
VTT
GL
20
PT7
P2
P1
P3
VTT
GL
20
PT9
P1
P3
P2
VTT
GL
20
PT13
P3
P1
VTT
P2
GL
20
PT15
P3
P2
VTT
P1
GL
20
B
PT1
LU
P1
VTT
P2
GL
20
PT2
P1
P2
LU
VTT1
VTT2
P3
GL
20
PT3
LN
P2
VTT1
VTT2
LU
P1
GL
20
PT4
BN
P2
P1
VTT
AG
P3
GL
20
PT5
P3
P2
VTT
P1
GL
20
PT8
P1
P2
VTT
P3
GL
20
PT10
LN
VTT
P3
P2
P1
AG1
AG2
GL
20
PT11
VTT
P2
P3
P1
LN2
LN1
GL
20
PT12
AG2
AG1
P3
P2
VTT
P1
GL
20
PT14
VTT
LN
P2
P3
P1
GL
20
PT16
P1
P2
VTT
P3
GL
20
C
Wilcoxon, p = 0.029
CD8 intraepithelial H-score
non-recurring
recurring
Recurrence status
D
Wilcoxon, p = 0.2
CD8 intraepithelial H-score (pt. mean)
PT15
PT13
PT7
PT6
PT9
PT8
PT10
PT12
PT5
PT16
PT14
PT11
non-recurring
recurring
Recurrence status

Figure S20. Comparison of phylogenetic trees and CD8 IHC for non-recurring versus recurring tumors. (A) Phylogenetic trees of non-recurring tumors. (B) Phylogenetic trees of non-recurring tumors. (C) CD8 intraepithelial H-scores for recurring versus non-recurring tumors for all primary tumor regions included in panel A. (D) Patient averages (mean) of the CD8 intraepithelial H-scores for recurring versus non-recurring tumors.

Figure S21.


Relative mutation contribution
Signature
PT1_P1
PT1_P2
PT1_VTT
PT1_LU


Relative mutation contribution
Signature
PT2_P1
PT2_P2
PT2_P3
PT2_VTT1
PT2_VTT2
PT2_LU


Relative mutation contribution
Signature
PT3_P1
PT3_P2
PT3_VTT1
PT3_VTT2
PT3_LN
PT3_LU

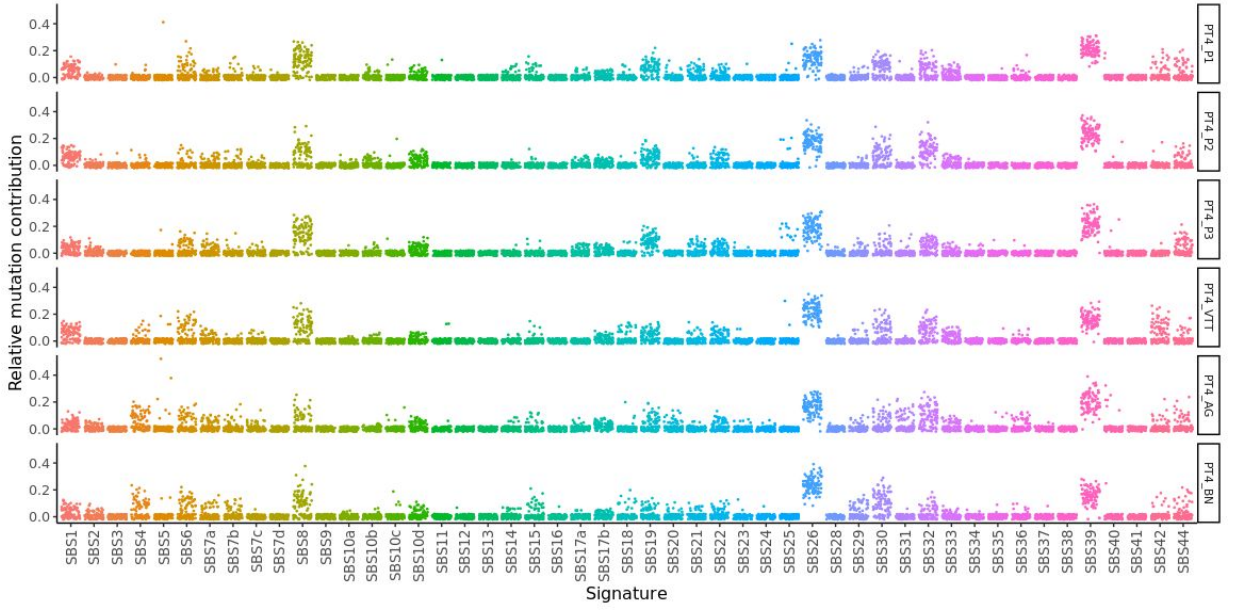
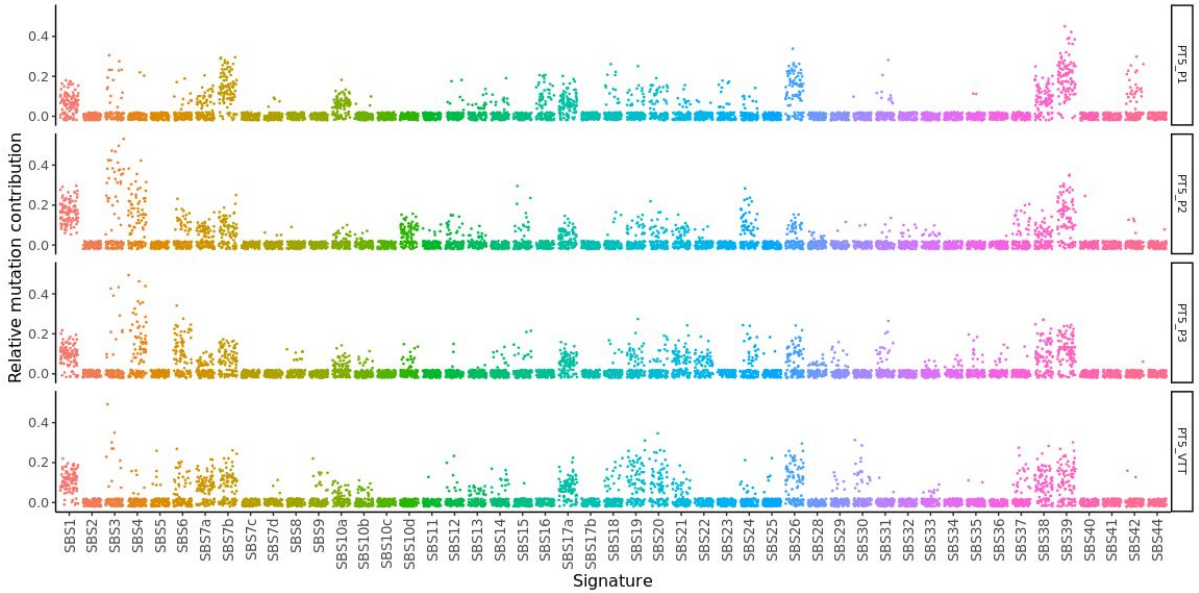
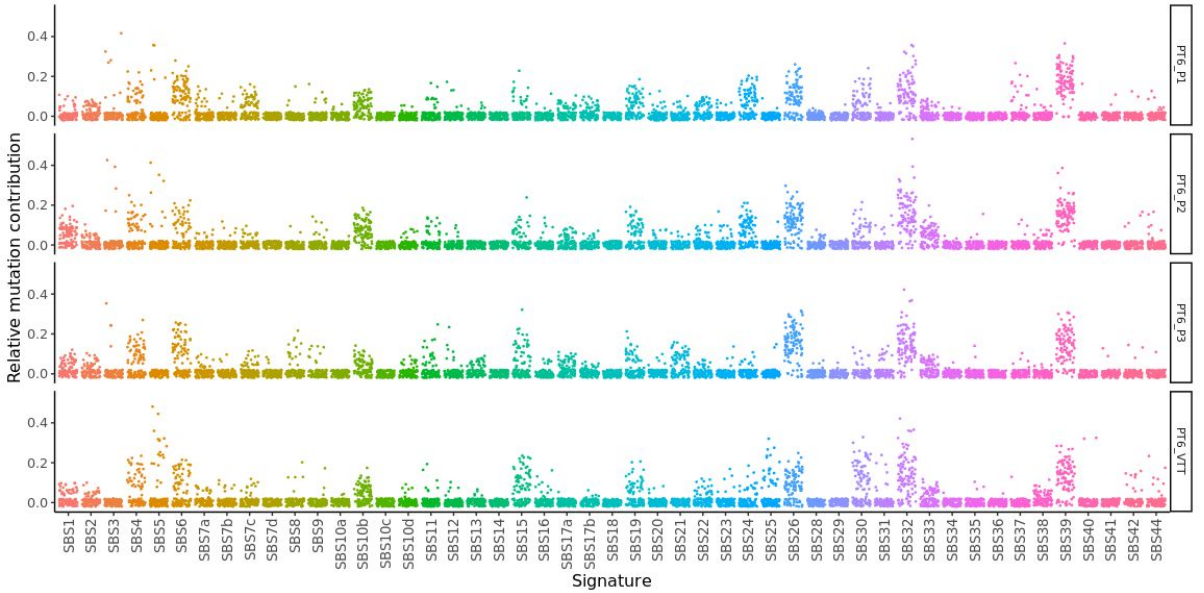
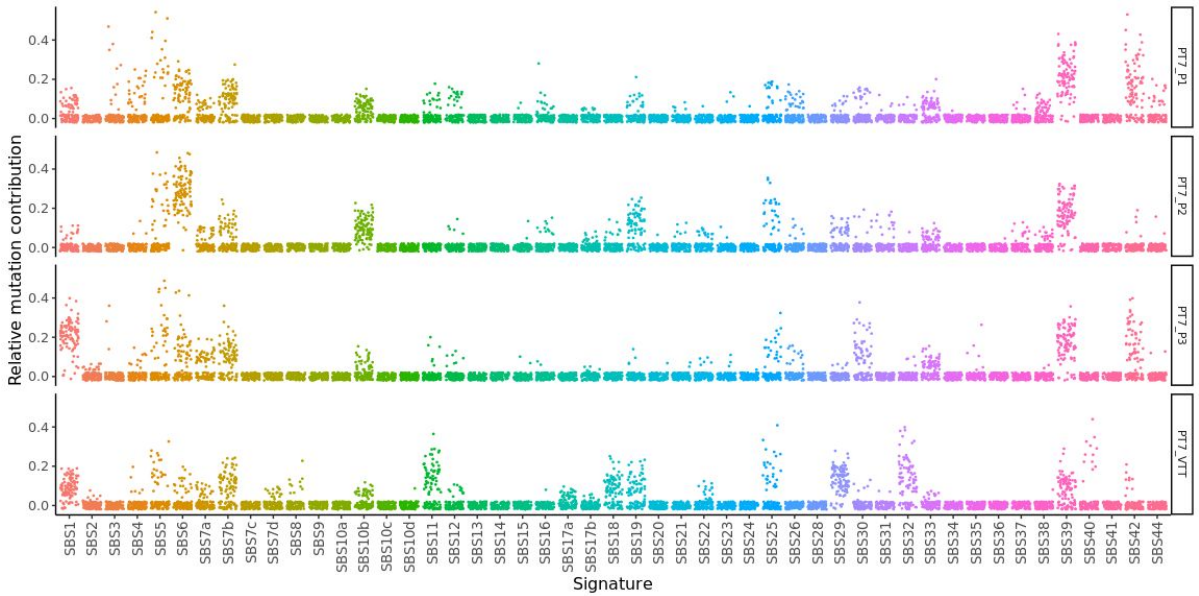

Relative mutation contribution
Signature
PT4_P1
PT4_P2
PT4_P3
PT4_VTT
PT4_AG
PT4_BN

Figure S21.


Relative mutation contribution
0.0
0.2
0.4
PT5_P1
PT5_P2
PT5_P3
PT5_VTT
SBS1 SBS2 SBS3 SBS4 SBS5 SBS6 SBS7a SBS7b SBS7c SBS7d SBS8 SBS9 SBS10a SBS10b SBS10c SBS10d SBS11 SBS12 SBS13 SBS14 SBS15 SBS16 SBS17a SBS17b SBS18 SBS19 SBS20 SBS21 SBS22 SBS23 SBS24 SBS25 SBS26 SBS28 SBS29 SBS30 SBS31 SBS32 SBS33 SBS34 SBS35 SBS36 SBS37 SBS38 SBS39 SBS40 SBS41 SBS42 SBS44
Signature


Relative mutation contribution
0.0
0.2
0.4
PT6_P1
PT6_P2
PT6_P3
PT6_VTT
SBS1 SBS2 SBS3 SBS4 SBS5 SBS6 SBS7a SBS7b SBS7c SBS7d SBS8 SBS9 SBS10a SBS10b SBS10c SBS10d SBS11 SBS12 SBS13 SBS14 SBS15 SBS16 SBS17a SBS17b SBS18 SBS19 SBS20 SBS21 SBS22 SBS23 SBS24 SBS25 SBS26 SBS28 SBS29 SBS30 SBS31 SBS32 SBS33 SBS34 SBS35 SBS36 SBS37 SBS38 SBS39 SBS40 SBS41 SBS42 SBS44
Signature


Relative mutation contribution
0.0
0.2
0.4
PT7_P1
PT7_P2
PT7_P3
PT7_VTT
SBS1 SBS2 SBS3 SBS4 SBS5 SBS6 SBS7a SBS7b SBS7c SBS7d SBS8 SBS9 SBS10a SBS10b SBS10c SBS10d SBS11 SBS12 SBS13 SBS14 SBS15 SBS16 SBS17a SBS17b SBS18 SBS19 SBS20 SBS21 SBS22 SBS23 SBS24 SBS25 SBS26 SBS28 SBS29 SBS30 SBS31 SBS32 SBS33 SBS34 SBS35 SBS36 SBS37 SBS38 SBS39 SBS40 SBS41 SBS42 SBS44
Signature

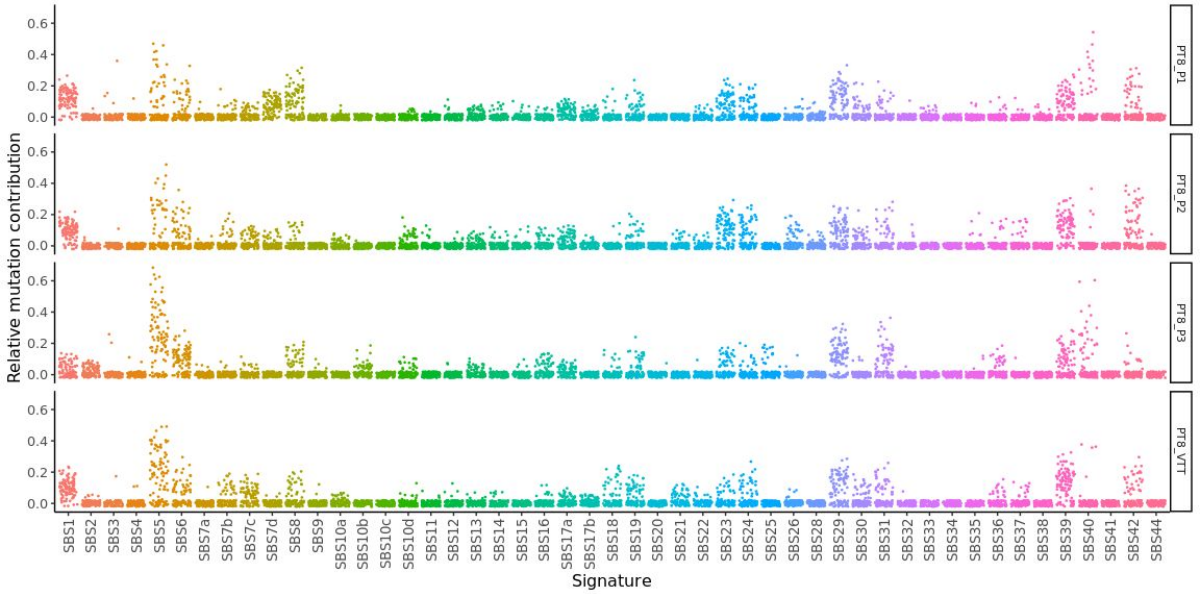
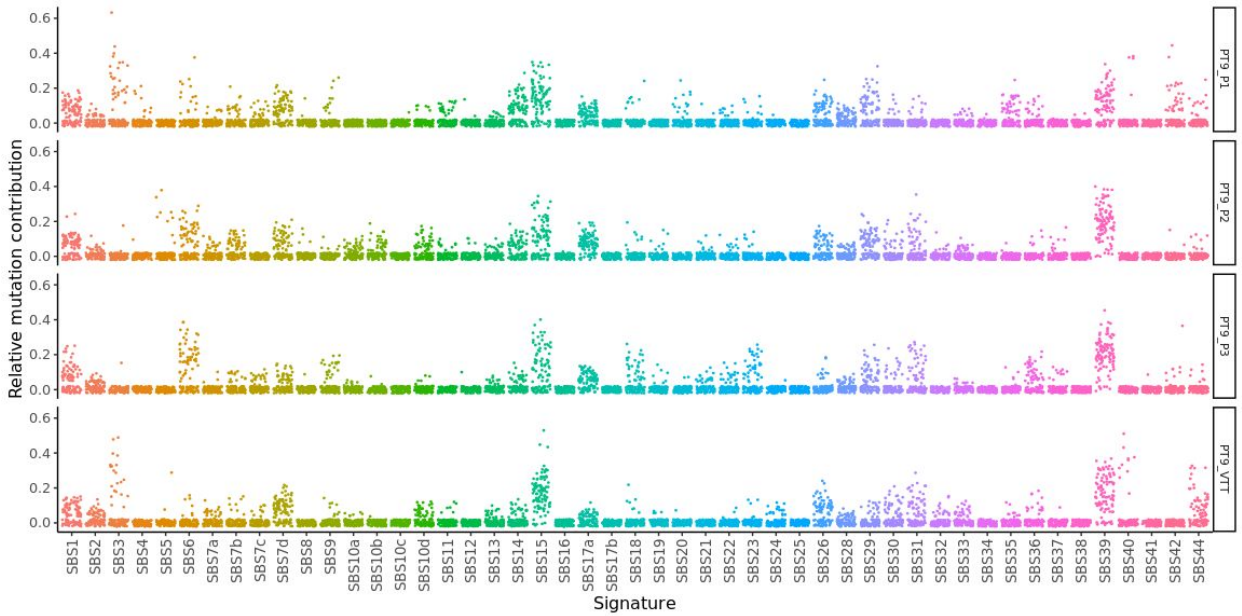
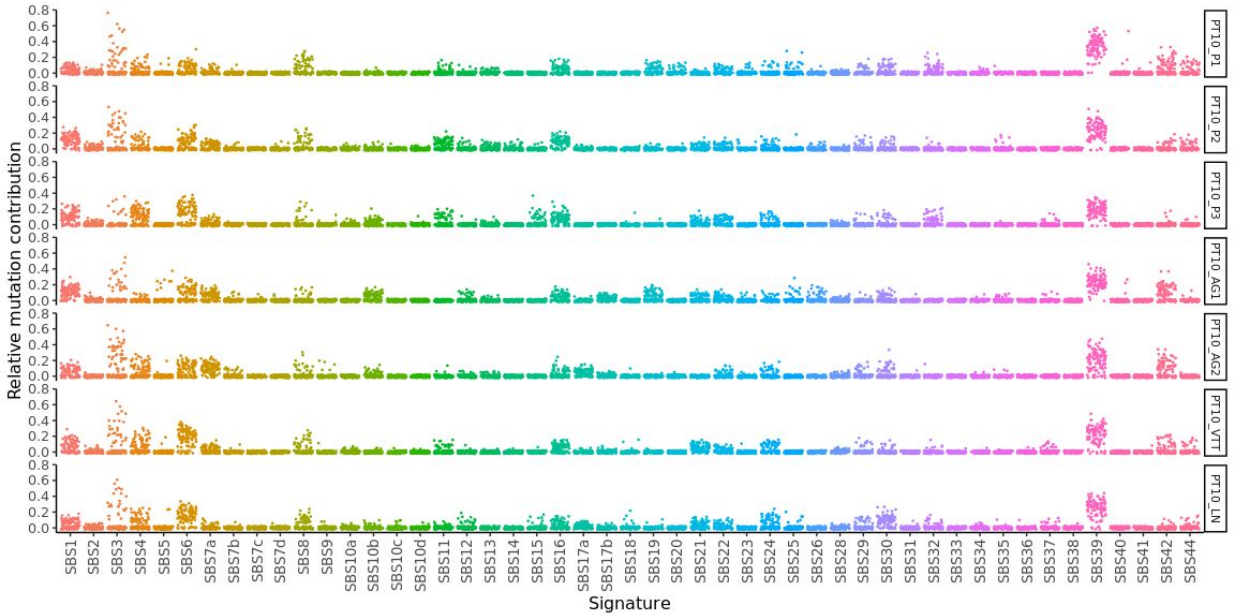
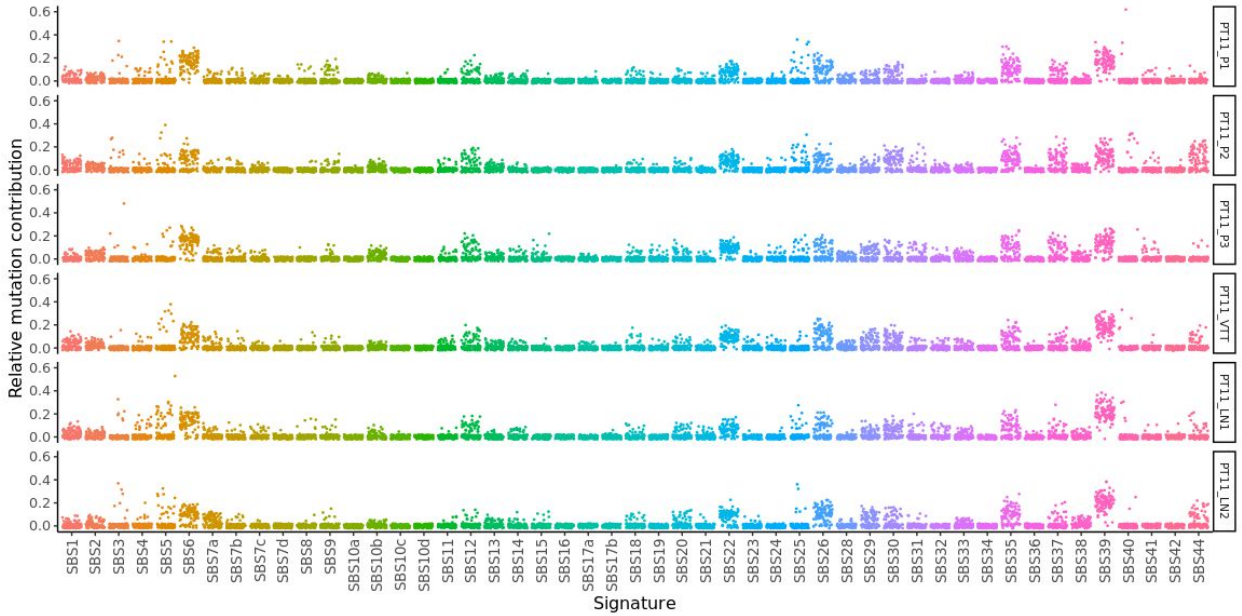

Relative mutation contribution
0.0
0.2
0.4
0.6
PT8_P1
PT8_P2
PT8_P3
PT8_VTT
SBS1 SBS2 SBS3 SBS4 SBS5 SBS6 SBS7a SBS7b SBS7c SBS7d SBS8 SBS9 SBS10a SBS10b SBS10c SBS10d SBS11 SBS12 SBS13 SBS14 SBS15 SBS16 SBS17a SBS17b SBS18 SBS19 SBS20 SBS21 SBS22 SBS23 SBS24 SBS25 SBS26 SBS28 SBS29 SBS30 SBS31 SBS32 SBS33 SBS34 SBS35 SBS36 SBS37 SBS38 SBS39 SBS40 SBS41 SBS42 SBS44
Signature

Figure S21.


Relative mutation contribution
Signature
PT9_P1
PT9_P2
PT9_P3
PT9_VTT


Relative mutation contribution
Signature
PT10_P1
PT10_P2
PT10_P3
PT10_AG1
PT10_AG2
PT10_VTT
PT10_LN


Relative mutation contribution
Signature
PT11_P1
PT11_P2
PT11_P3
PT11_VTT
PT11_LN1
PT11_LN2

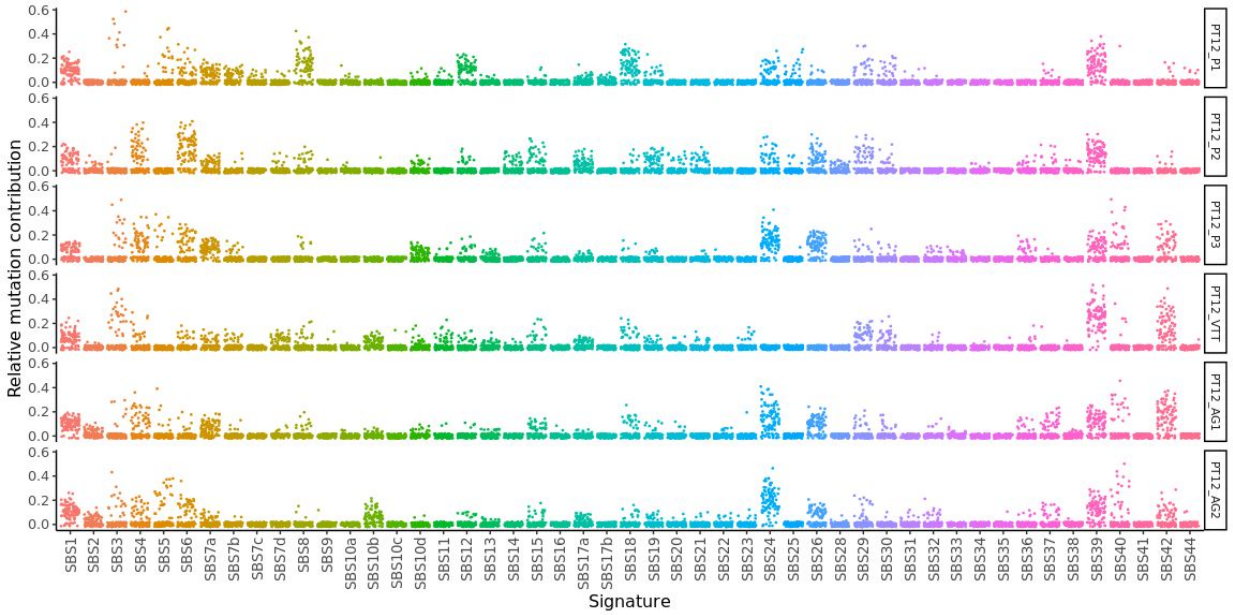
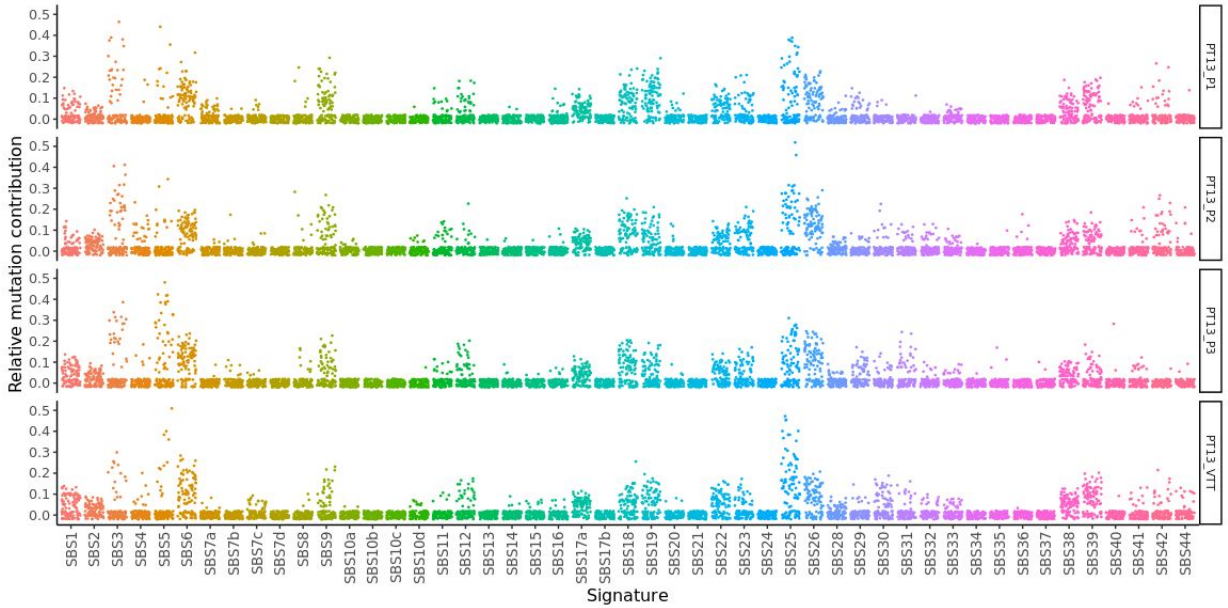
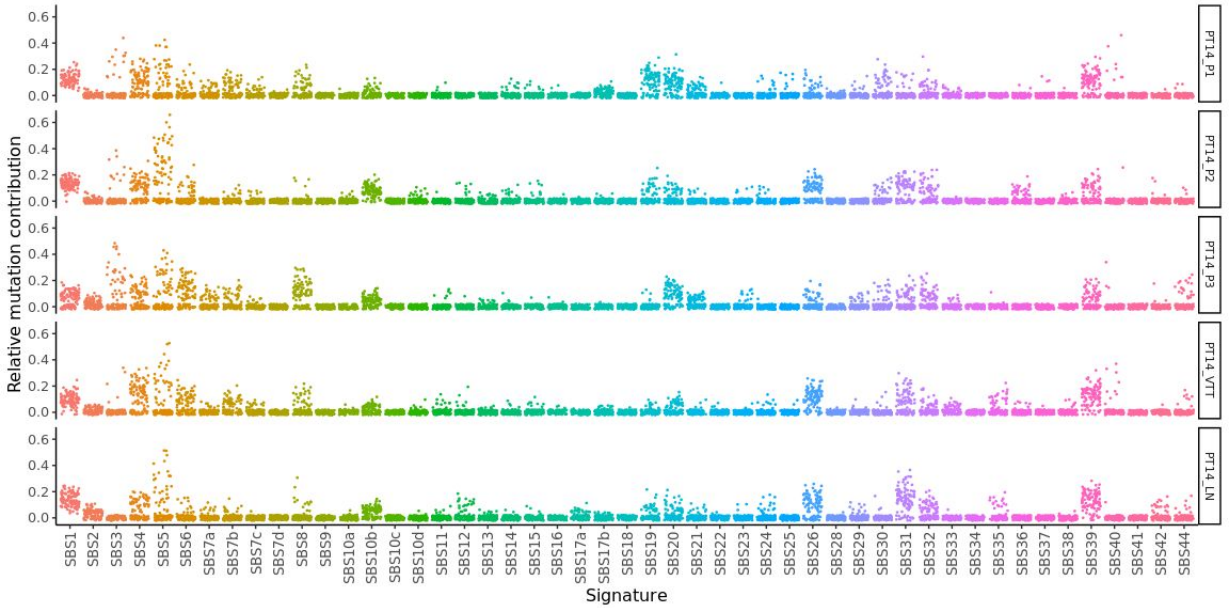
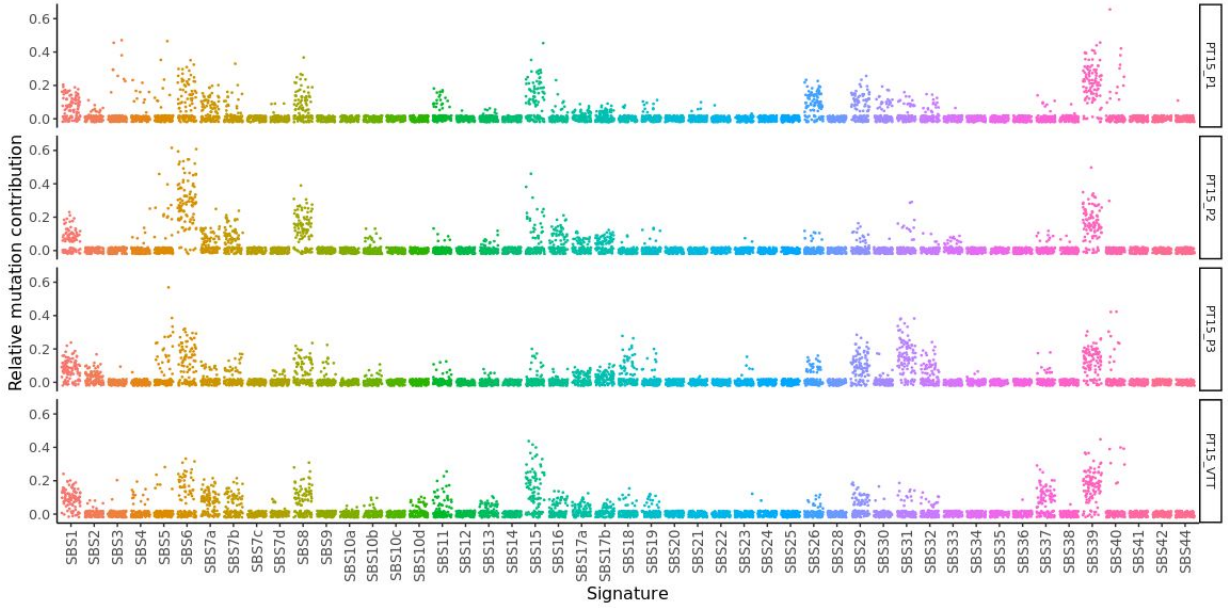

Relative mutation contribution
Signature
PT12_P1
PT12_P2
PT12_P3
PT12_VTT
PT12_AG1
PT12_AG2

Figure S21.


Relative mutation contribution
Signature
PT13_P1
PT13_P2
PT13_P3
PT13_VTT


Relative mutation contribution
Signature
PT14_P1
PT14_P2
PT14_P3
PT14_VTT
PT14_LN


Relative mutation contribution
Signature
PT15_P1
PT15_P2
PT15_P3
PT15_VTT

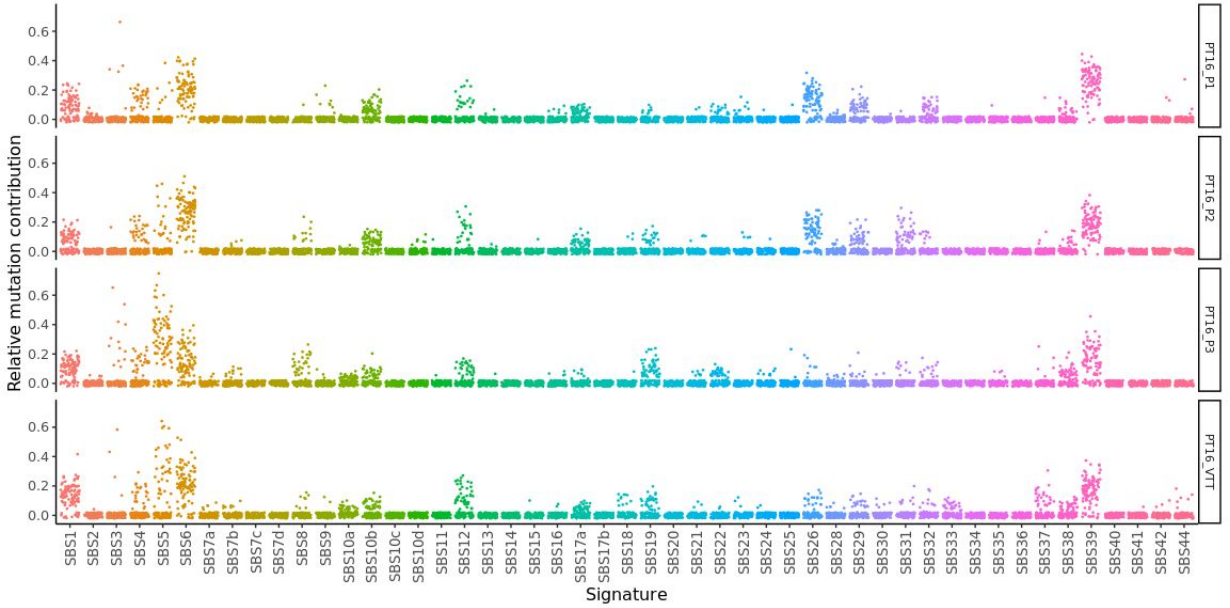
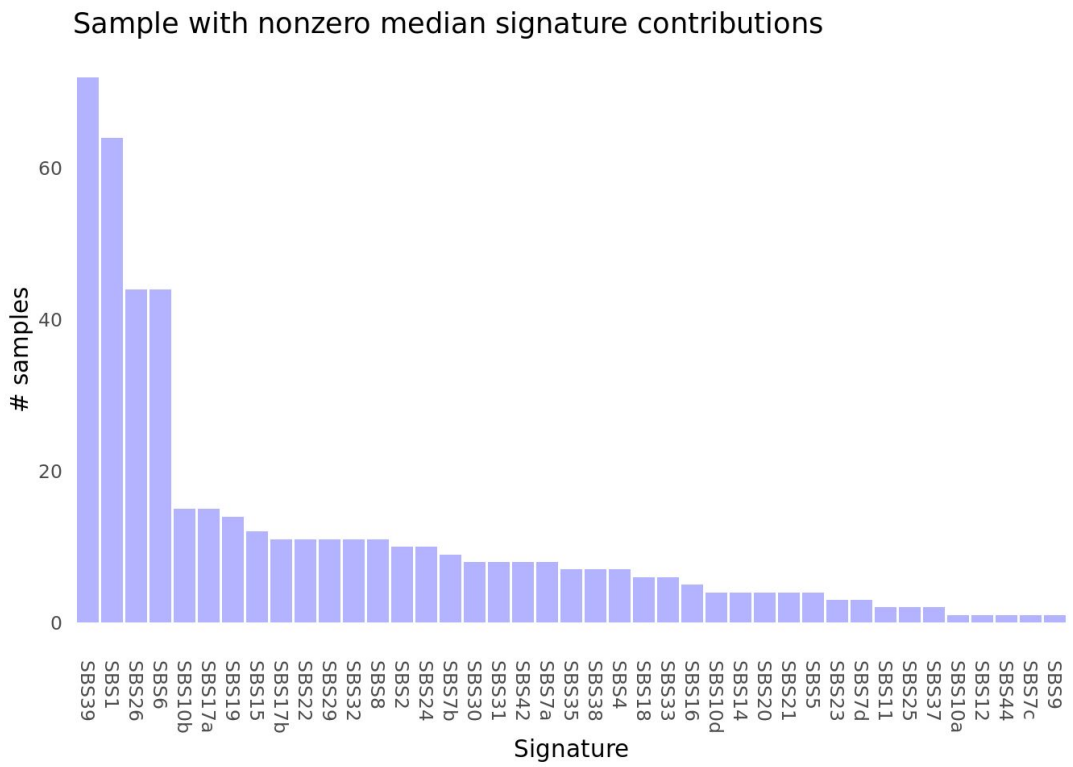

Relative mutation contribution
Signature
PT16_P1
PT16_P2
PT16_P3
PT16_VTT

Figure S21. Relative contribution of the 49 Alexandrov mutation signatures (37) to each sample, for all 100 bootstrap replicates.

Figure S22.

A



B

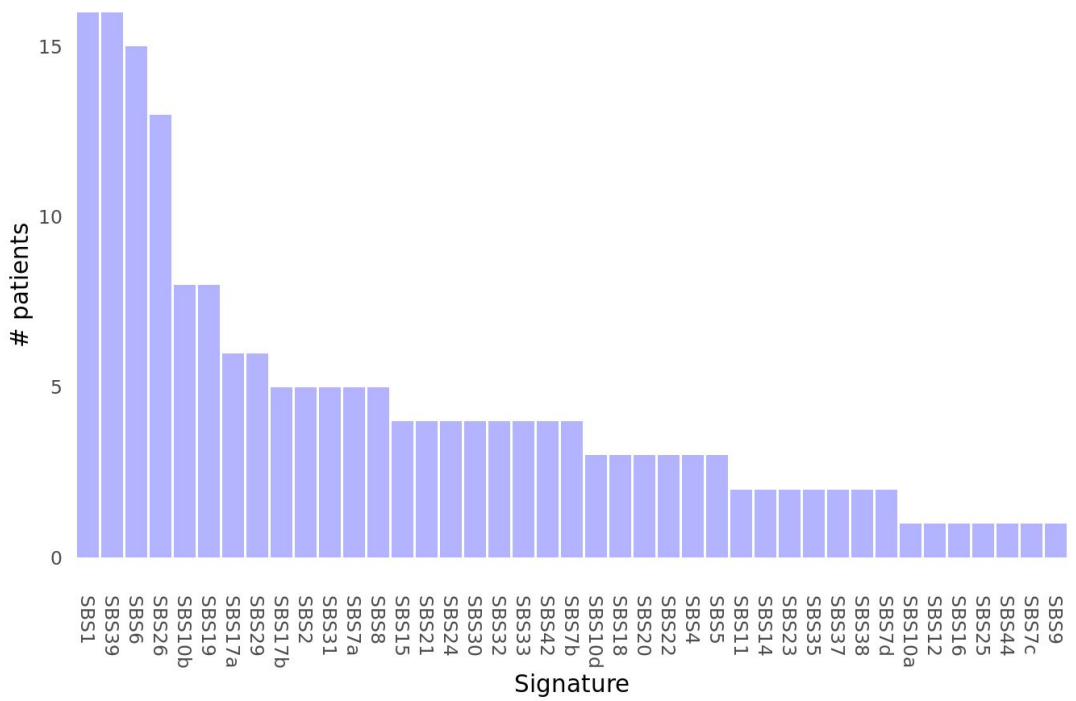
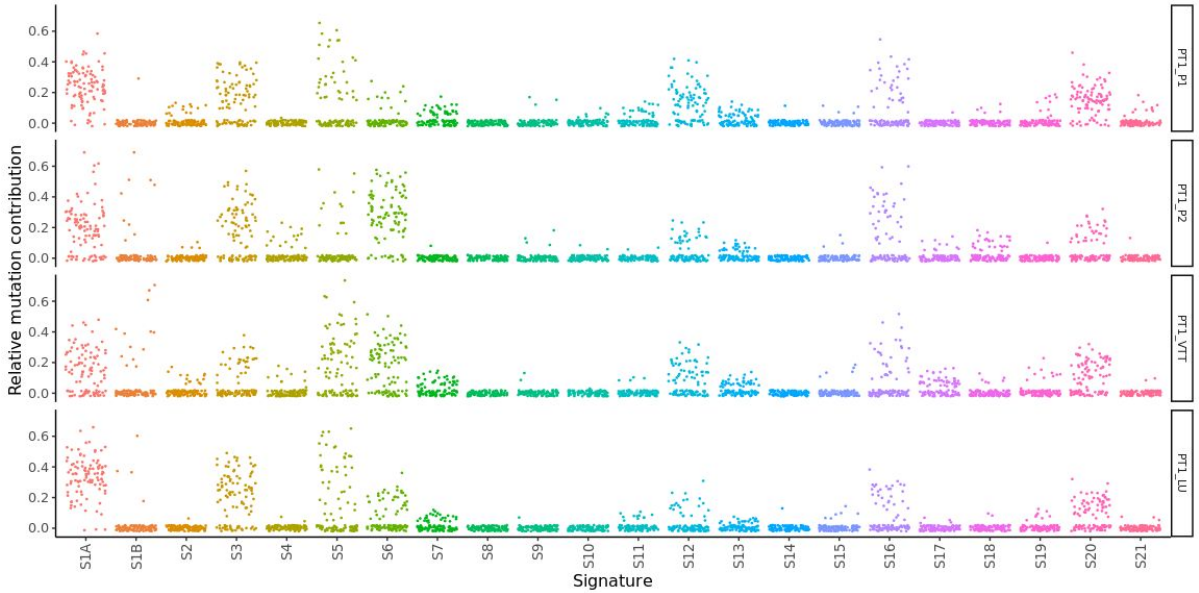
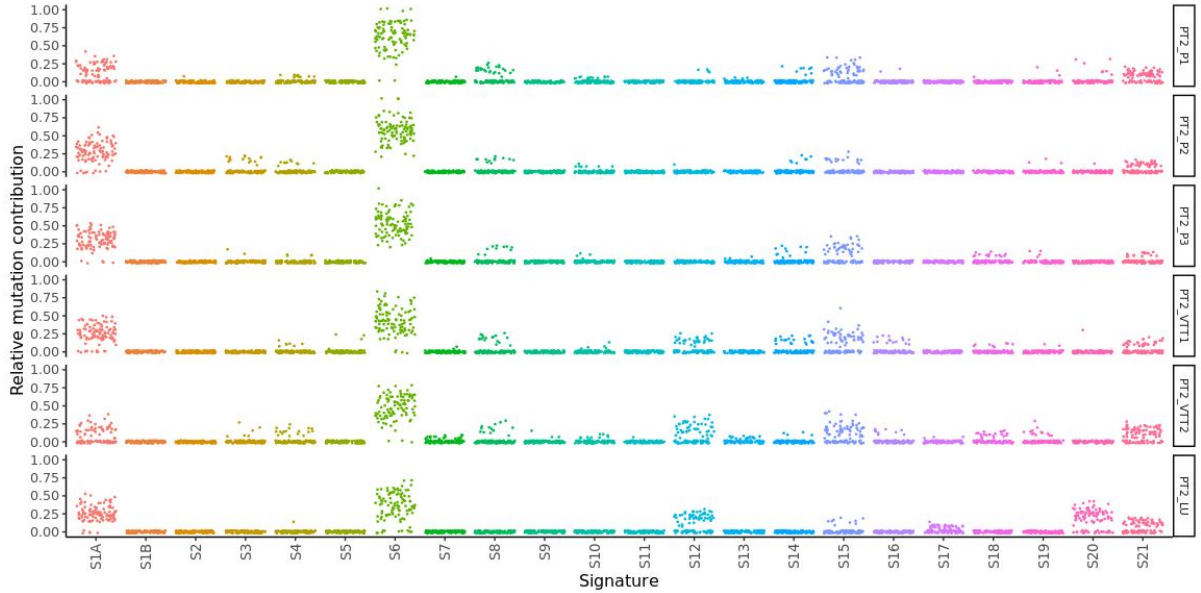
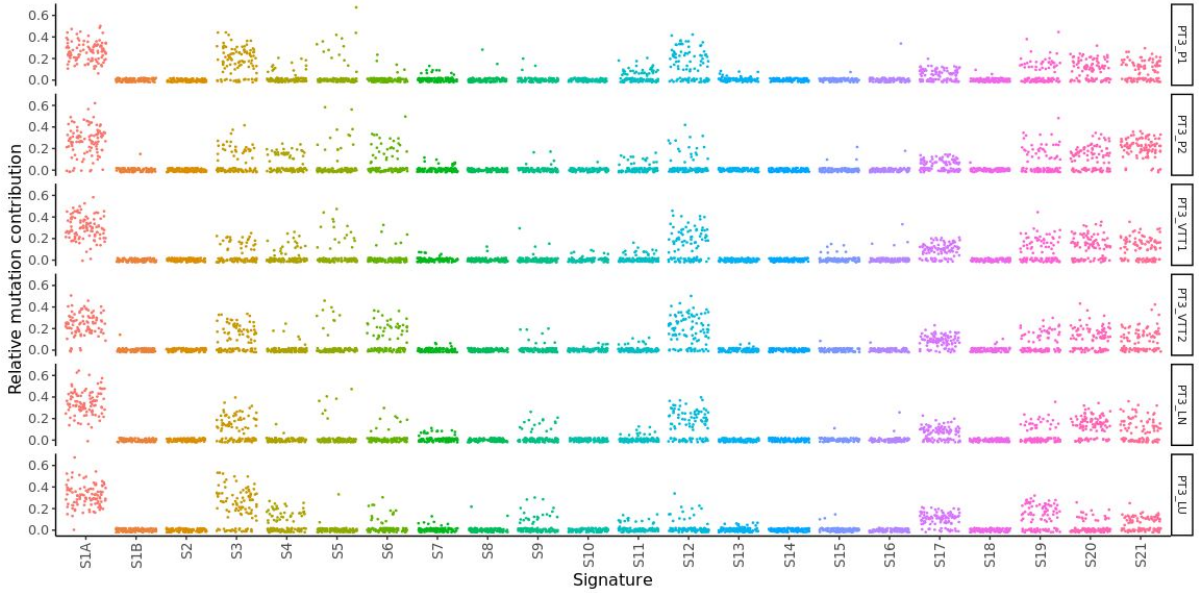


Figure S22. Number of a) samples and b) patients in whom a given mutation signature (of the 49 derived from reference (37)) has a non-zero median contribution over 100 bootstrap replicates.

Figure S23.


Relative mutation contribution
Signature
PT1_P1
PT1_P2
PT1_VTT
PT1_LU


Relative mutation contribution
Signature
PT2_P1
PT2_P2
PT2_P3
PT2_VTT1
PT2_VTT2
PT2_LU


Relative mutation contribution
Signature
PT3_P1
PT3_P2
PT3_VTT1
PT3_VTT2
PT3_LN
PT3_LU

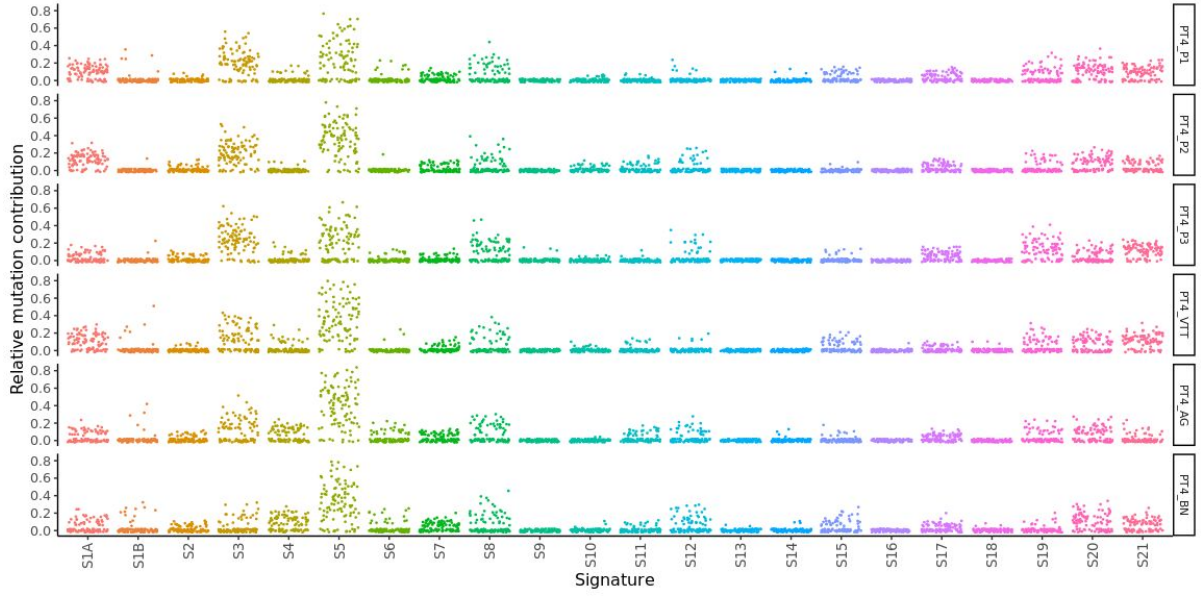
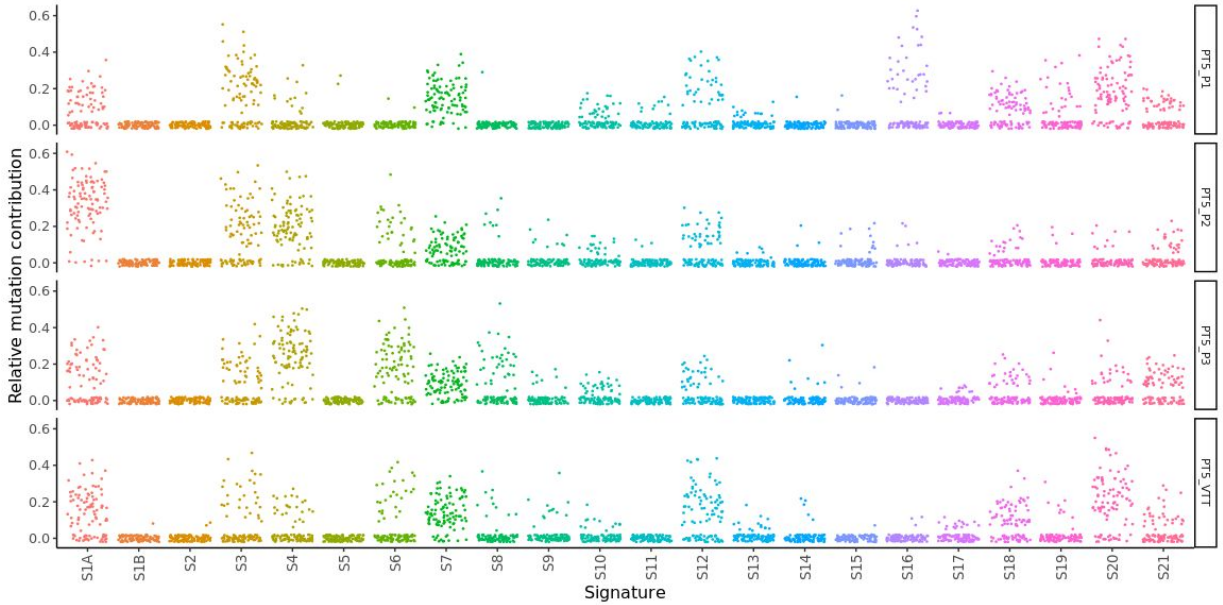
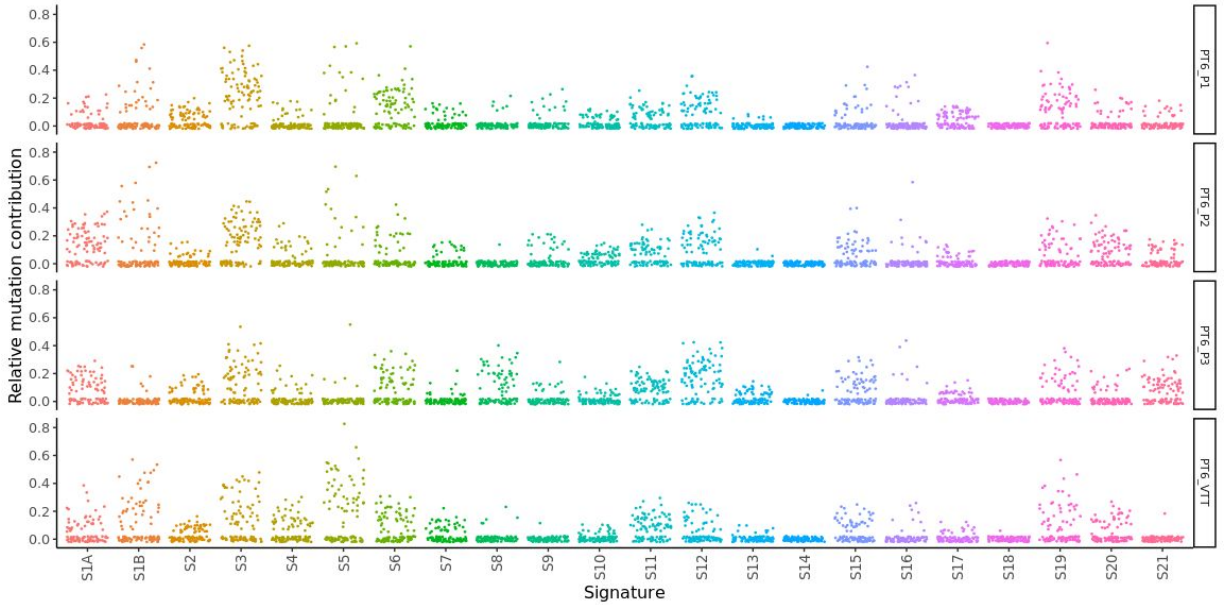
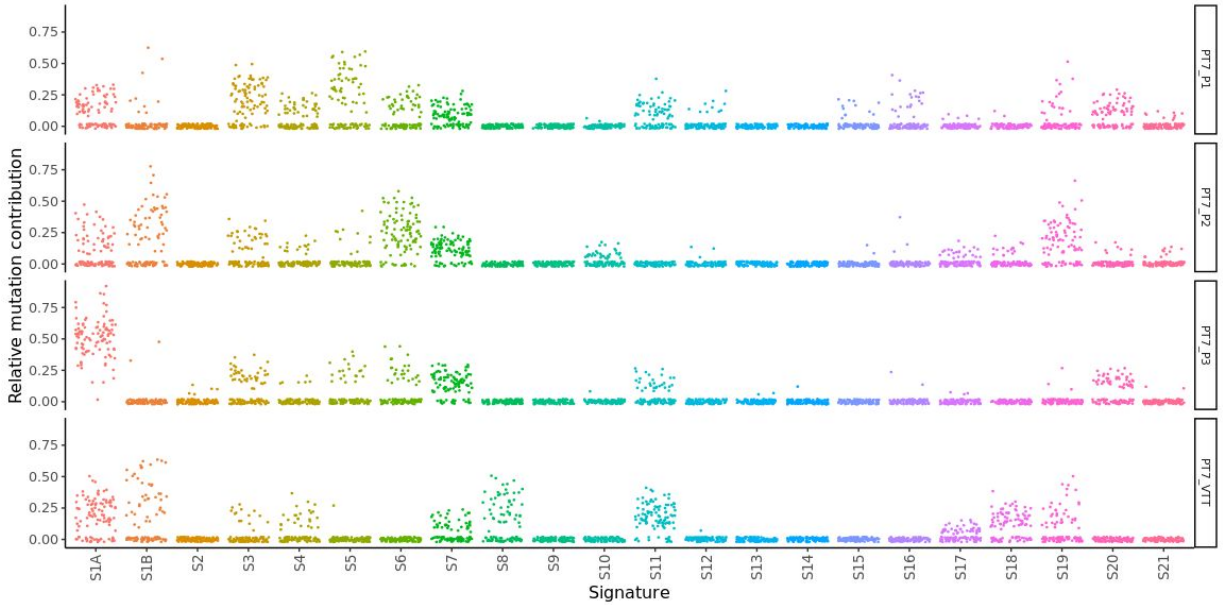

Relative mutation contribution
Signature
PT4_P1
PT4_P2
PT4_P3
PT4_VTT
PT4_AG
PT4_BN

Figure S23.


Relative mutation contribution
Signature
PT5_P1
PT5_P2
PT5_P3
PT5_VTT


Relative mutation contribution
Signature
PT6_P1
PT6_P2
PT6_P3
PT6_VTT


Relative mutation contribution
Signature
PT7_P1
PT7_P2
PT7_P3
PT7_VTT

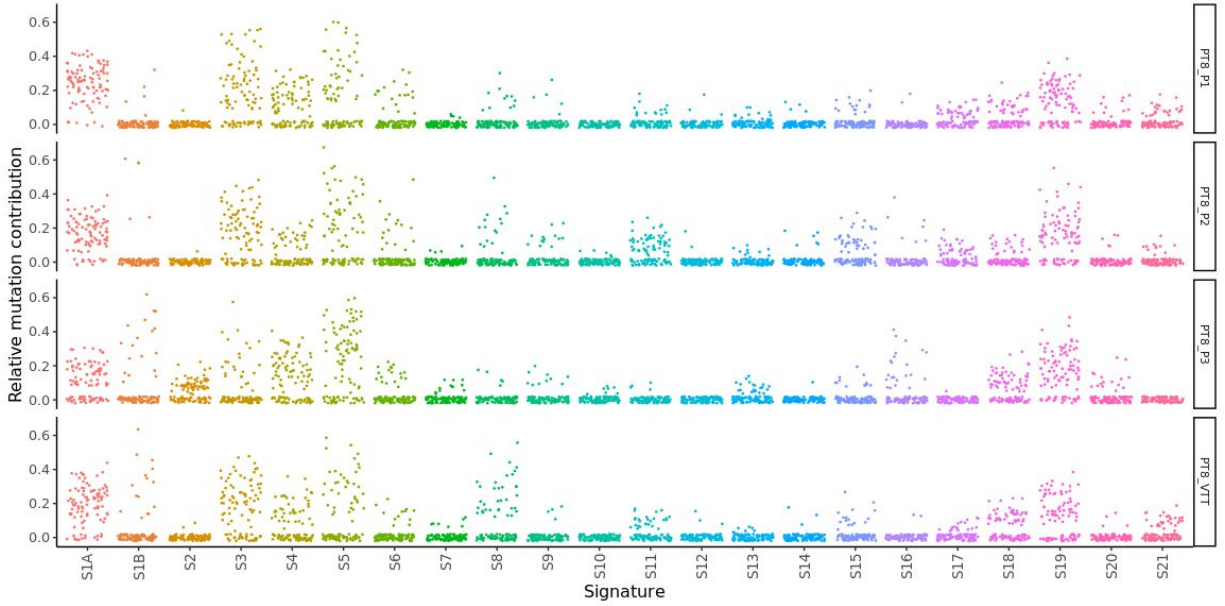
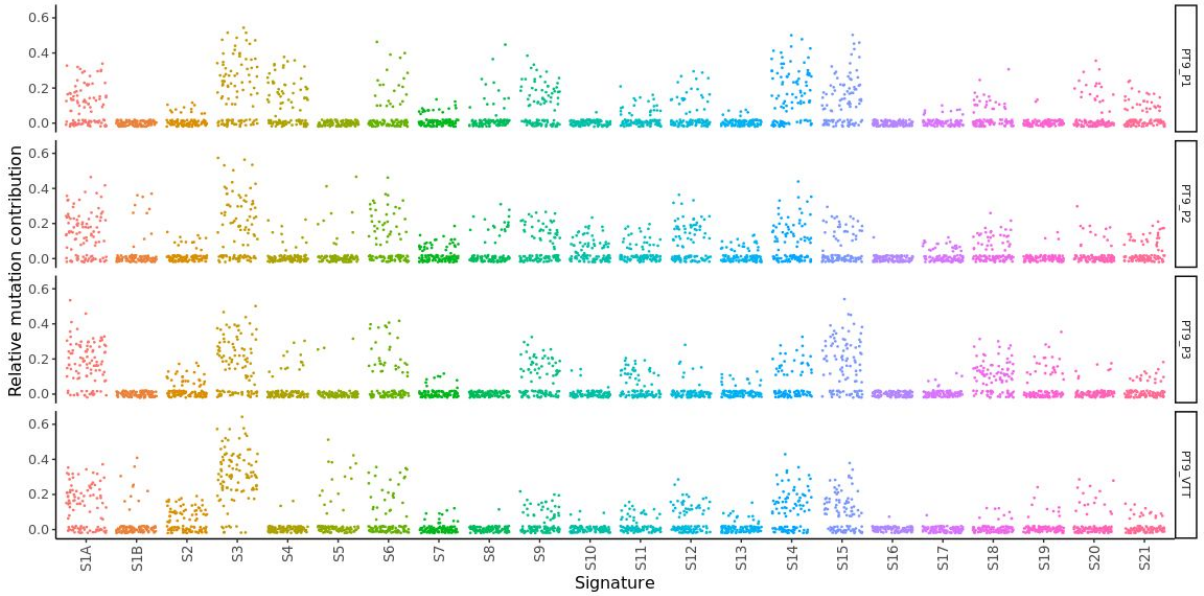
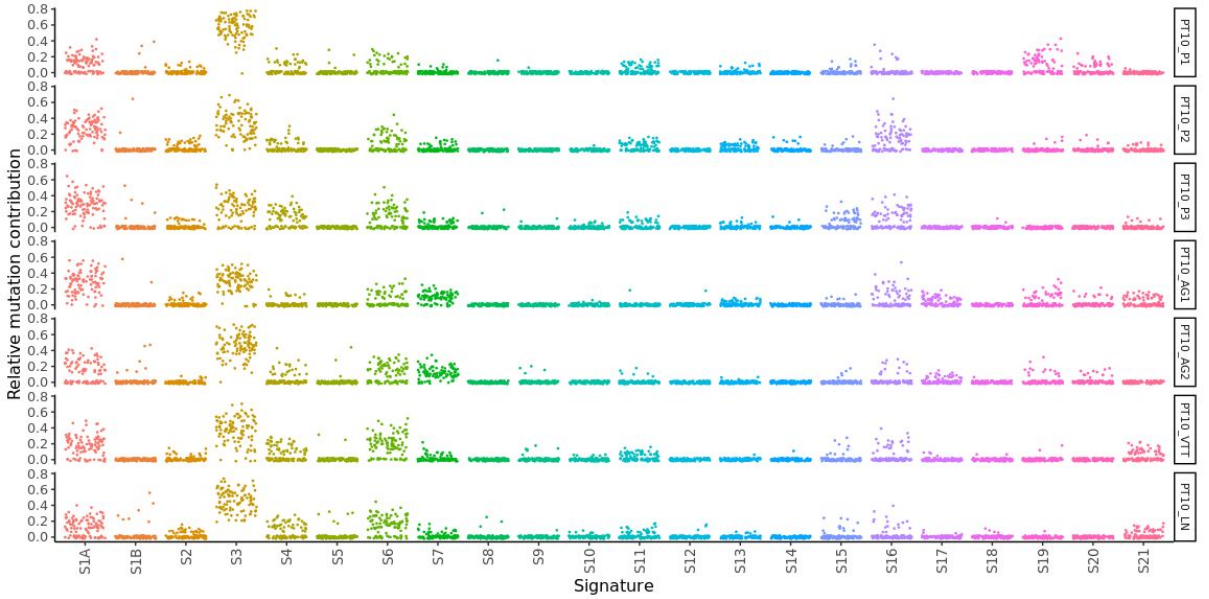
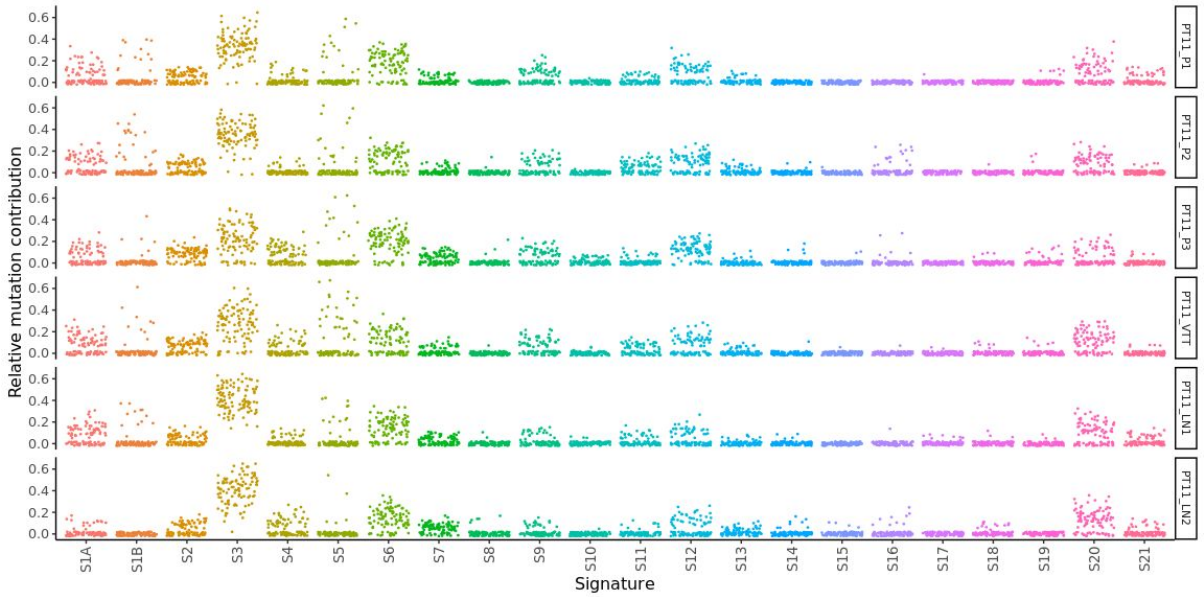

Relative mutation contribution
Signature
PT8_P1
PT8_P2
PT8_P3
PT8_VTT

Figure S23.


Relative mutation contribution
Signature
PT9_P1
PT9_P2
PT9_P3
PT9_VTT
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21


Relative mutation contribution
Signature
PT10_P1
PT10_P2
PT10_P3
PT10_AG1
PT10_AG2
PT10_VTT
PT10_LN
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21


Relative mutation contribution
Signature
PT11_P1
PT11_P2
PT11_P3
PT11_VTT
PT11_LN1
PT11_LN2
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21

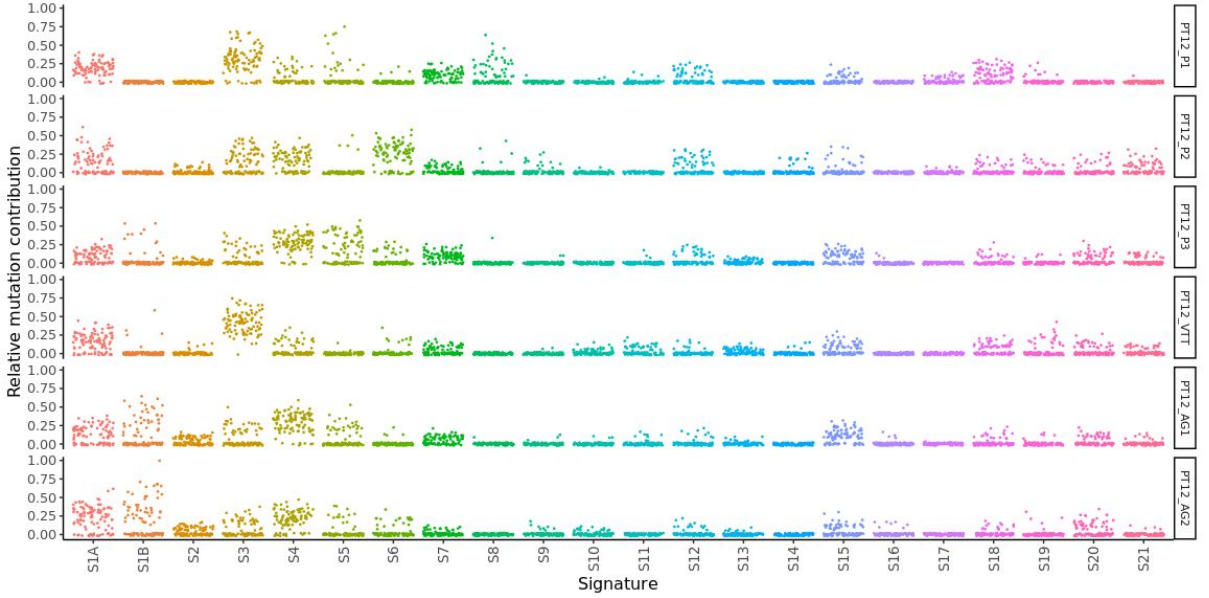
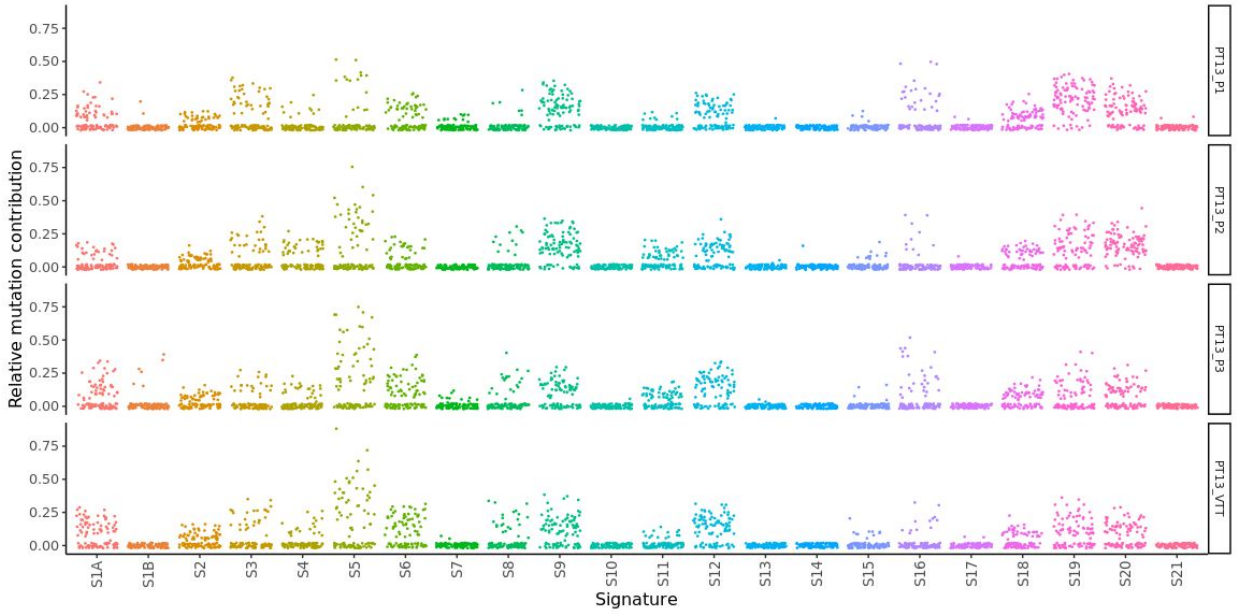
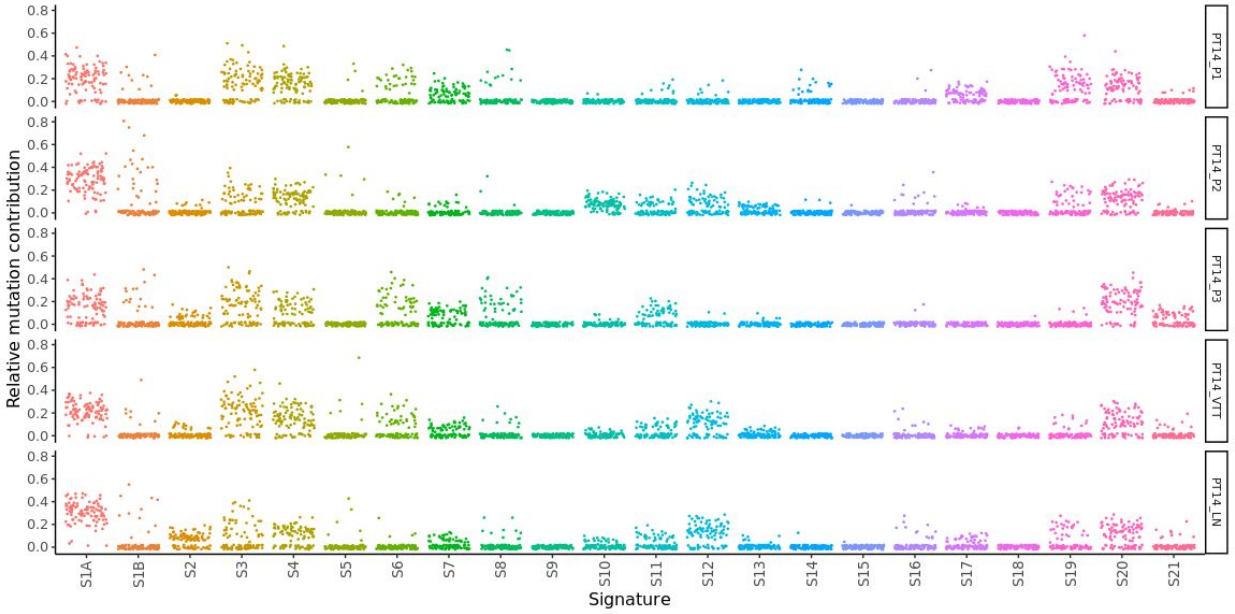
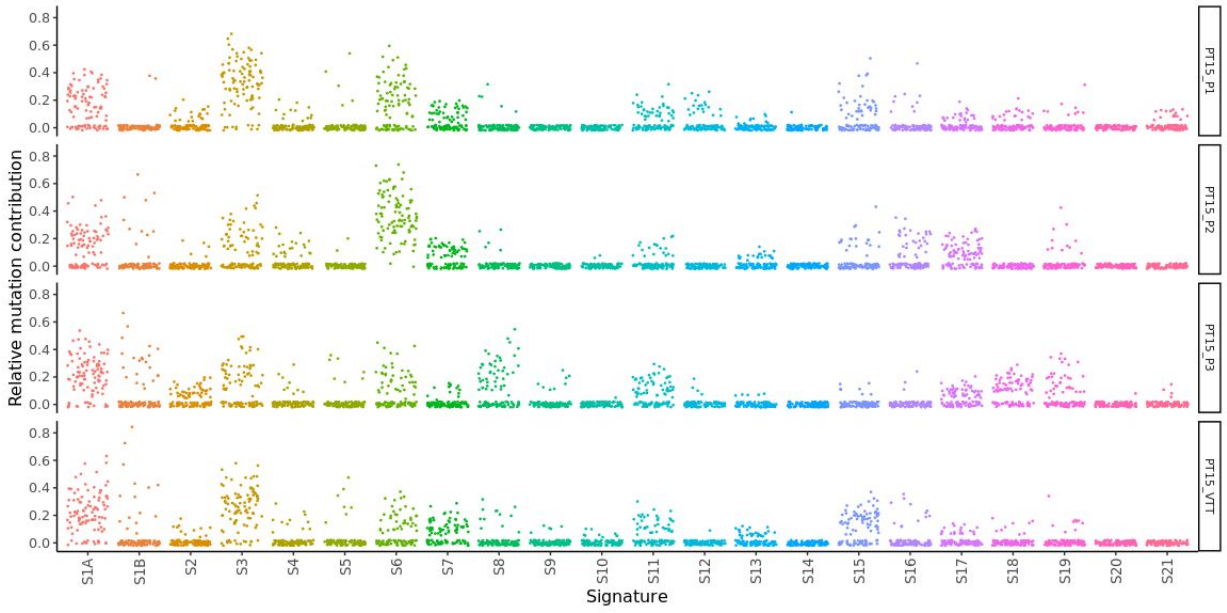

Relative mutation contribution
Signature
PT12_P1
PT12_P2
PT12_P3
PT12_VTT
PT12_AG1
PT12_AG2
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21

Figure S23.


Relative mutation contribution
0.75
0.50
0.25
0.00
PT13_P1
PT13_P2
PT13_P3
PT13_VTT
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21
Signature


Relative mutation contribution
0.8
0.6
0.4
0.2
0.0
PT14_P1
PT14_P2
PT14_P3
PT14_VTT
PT14_LN
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21
Signature


Relative mutation contribution
0.8
0.6
0.4
0.2
0.0
PT15_P1
PT15_P2
PT15_P3
PT15_VTT
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21
Signature

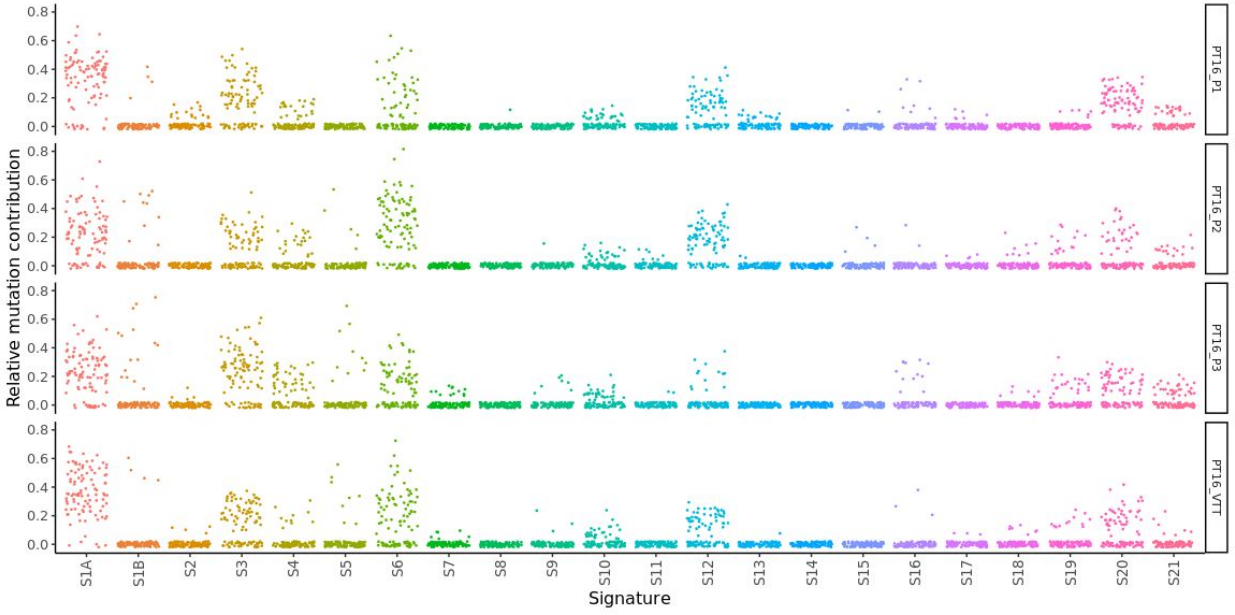

Relative mutation contribution
0.8
0.6
0.4
0.2
0.0
PT16_P1
PT16_P2
PT16_P3
PT16_VAT
S1A S1B S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14 S15 S16 S17 S18 S19 S20 S21
Signature

Figure S23. Relative contribution of the 22 original Alexandrov mutation signatures (36) to each sample, for all 100 bootstrap replicates.

Figure S24.

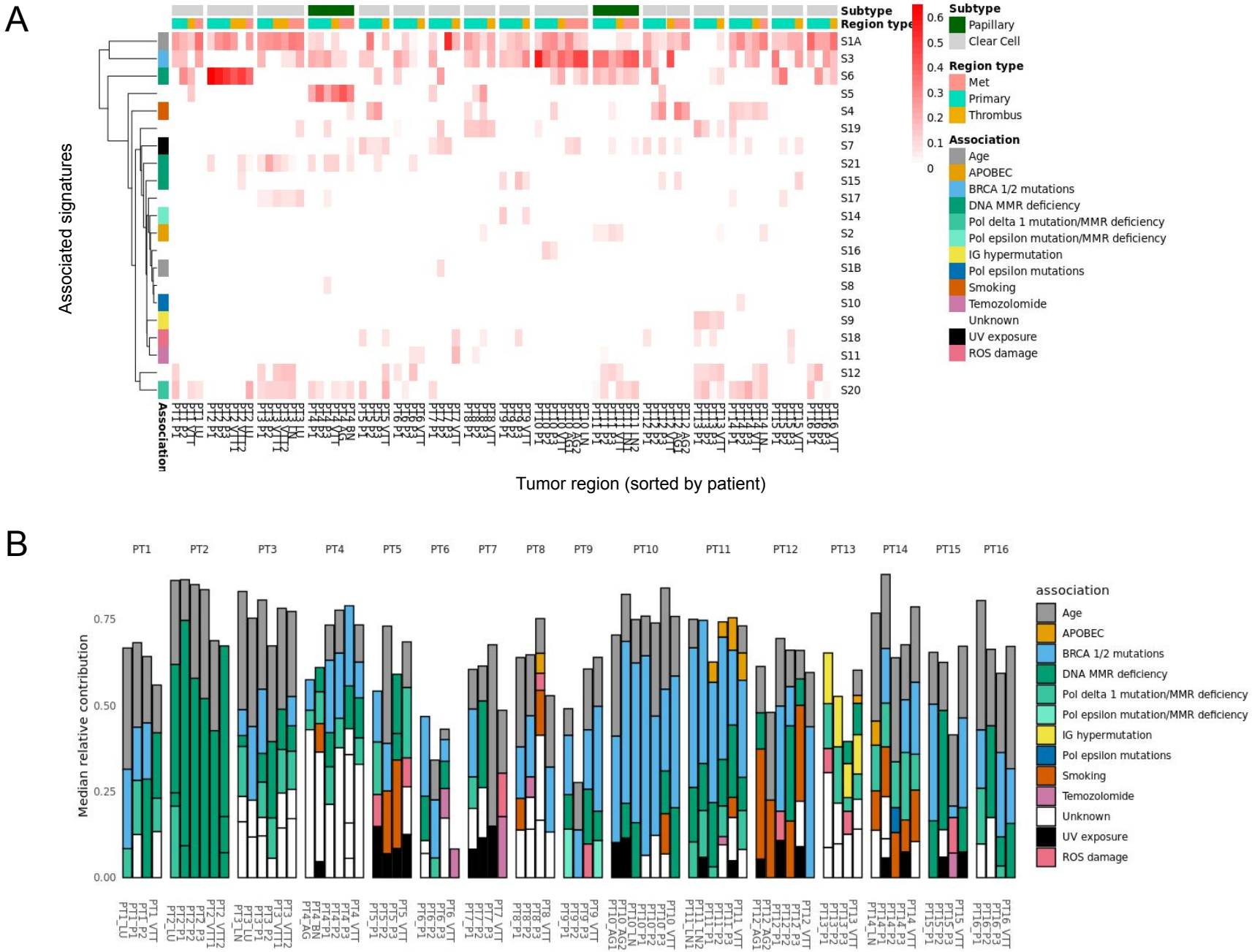
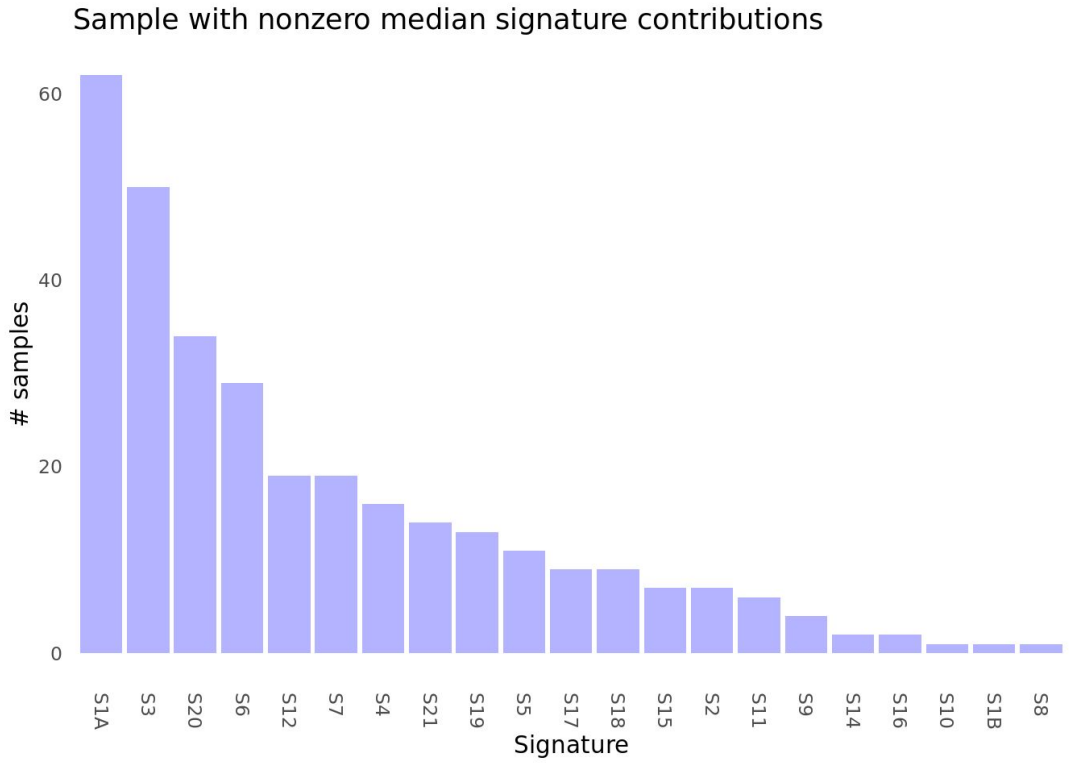

A
Associated signatures
Tumor region (sorted by patient)
Subtype
Region type
Association
S1A
S3
S6
S5
S4
S19
S7
S21
S15
S17
S14
S2
S16
S1B
S8
S10
S9
S18
S11
S12
S20
Subtype
Papillary
Clear Cell
Region type
Met
Primary
Thrombus
Association
Age
APOBEC
BRCA 1/2 mutations
DNA MMR deficiency
Pol delta 1 mutation/MMR deficiency
Pol epsilon mutation/MMR deficiency
IG hypermutation
Pol epsilon mutations
Smoking
Temozolomide
Unknown
UV exposure
ROS damage
B
Median relative contribution
association
PT1
PT2
PT3
PT4
PT5
PT6
PT7
PT8
PT9
PT10
PT11
PT12
PT13
PT14
PT15
PT16

Figure S24. Median mutation signature contributions of 22 mutation signatures (36) shown as a) a heatmap of median relative contributions for all signatures with a non-zero median contribution to at least one sample and b) a bar plot showing the median relative contributions for all signatures with a non-zero value for a given sample

Figure S25.

A



B

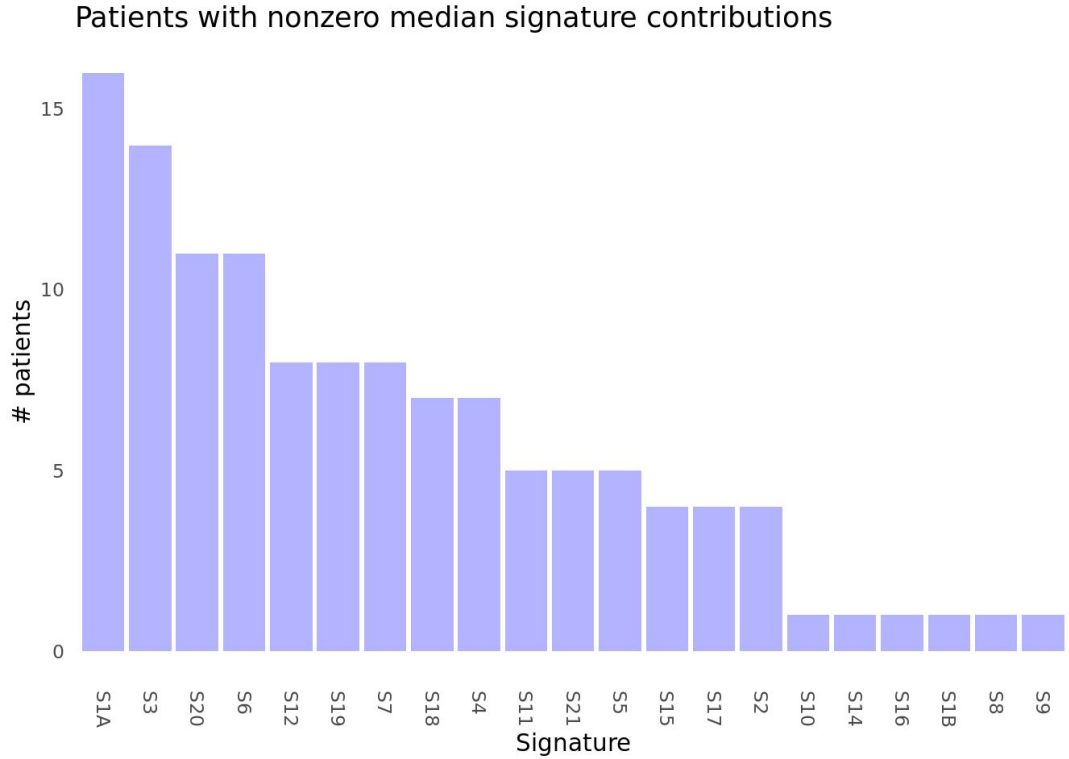


Figure S25. Number of a) samples and b) patients in whom a given mutation signature (36) has a non-zero median contribution over 100 bootstrap replicates.

Figure S26.

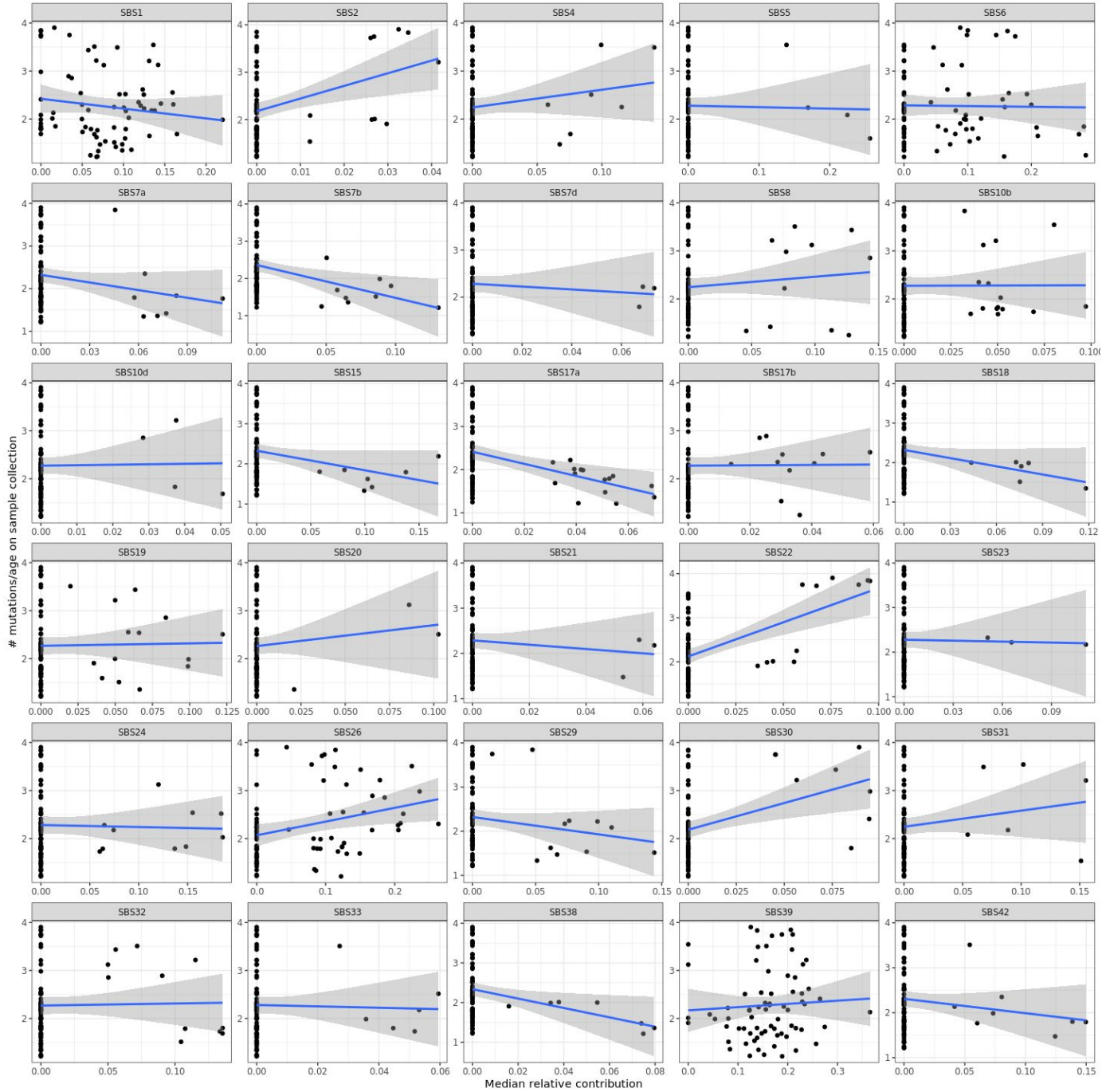

SBS1
SBS2
SBS4
SBS5
SBS6
SBS7a
SBS7b
SBS7d
SBS8
SBS10b
SBS10d
SBS15
SBS17a
SBS17b
SBS18
SBS19
SBS20
SBS21
SBS22
SBS23
SBS24
SBS26
SBS29
SBS30
SBS31
SBS32
SBS33
SBS38
SBS39
SBS42
mutations/age on sample collection
Median relative contribution

Figure S26. Relationship between the ratio of mutation burden and age and median relative contribution of different mutation signatures. 49 signatures (37) were assessed -- only those with a non-zero median relative contribution in > 1 patient and > 2 samples were plotted.

Figure S27.

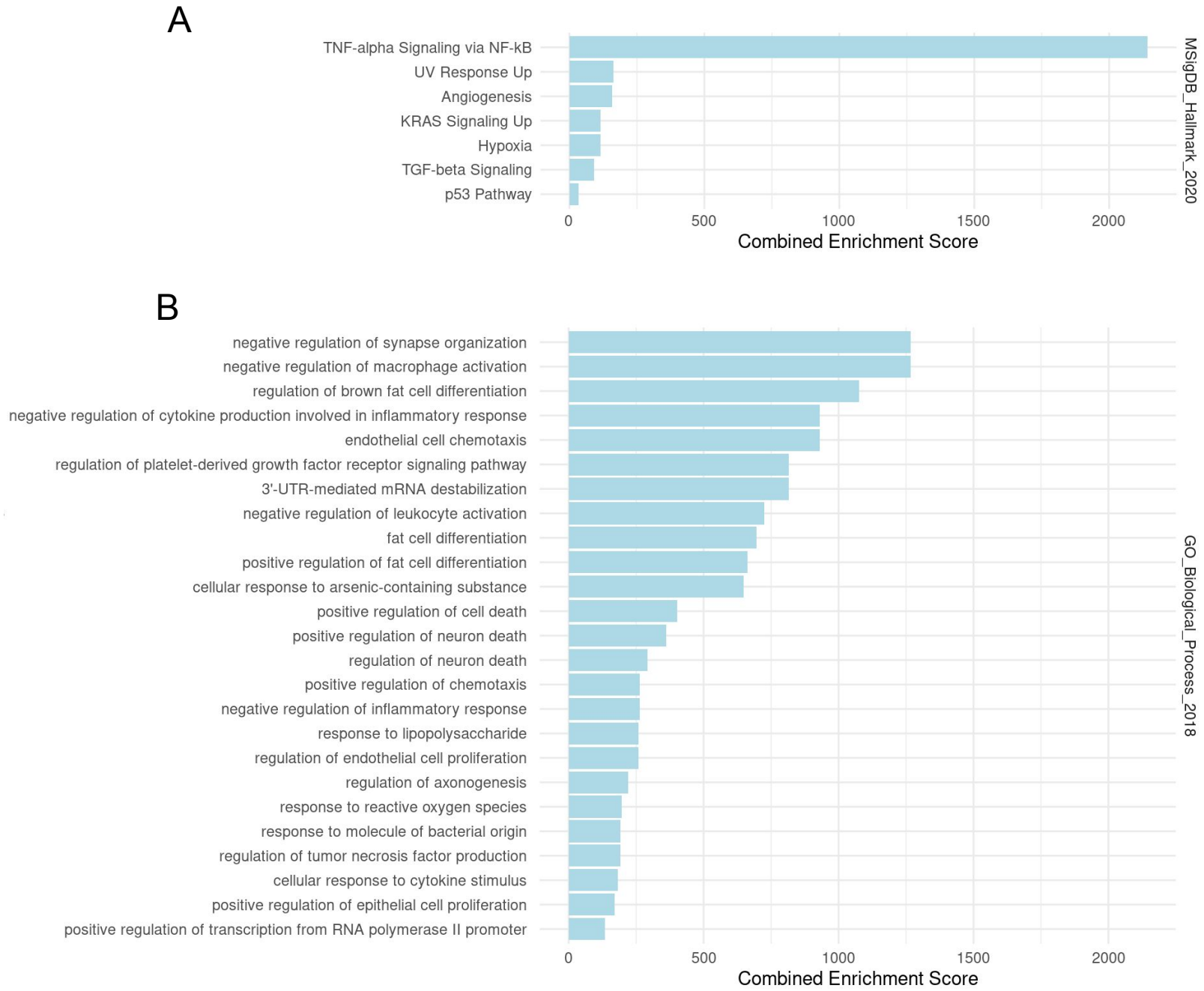

A
TNF-alpha Signaling via NF-kB
UV Response Up
Angiogenesis
KRAS Signaling Up
Hypoxia
TGF-beta Signaling
p53 Pathway
0
500
1000
1500
2000
Combined Enrichment Score
MSigDB_Hallmark_2020
B
negative regulation of synapse organization
negative regulation of macrophage activation
regulation of brown fat cell differentiation
negative regulation of cytokine production involved in inflammatory response
endothelial cell chemotaxis
regulation of platelet-derived growth factor receptor signaling pathway
3'-UTR-mediated mRNA destabilization
negative regulation of leukocyte activation
fat cell differentiation
positive regulation of fat cell differentiation
cellular response to arsenic-containing substance
positive regulation of cell death
positive regulation of neuron death
regulation of neuron death
positive regulation of chemotaxis
negative regulation of inflammatory response
response to lipopolysaccharide
regulation of endothelial cell proliferation
regulation of axonogenesis
response to reactive oxygen species
response to molecule of bacterial origin
regulation of tumor necrosis factor production
cellular response to cytokine stimulus
positive regulation of epithelial cell proliferation
positive regulation of transcription from RNA polymerase II promoter
0
500
1000
1500
2000
Combined Enrichment Score
GO_Biological_Process_2018

Figure S27. Enrichr set enrichment of differentially expressed genes between primary and VTT regions. (A) Significantly enriched terms from MSigDB Hallmark pathways. (B) Significantly enriched terms from GO Biological Processes. Significance is defined as adjusted P-value $\leq$ 0.05.

Figure S28.

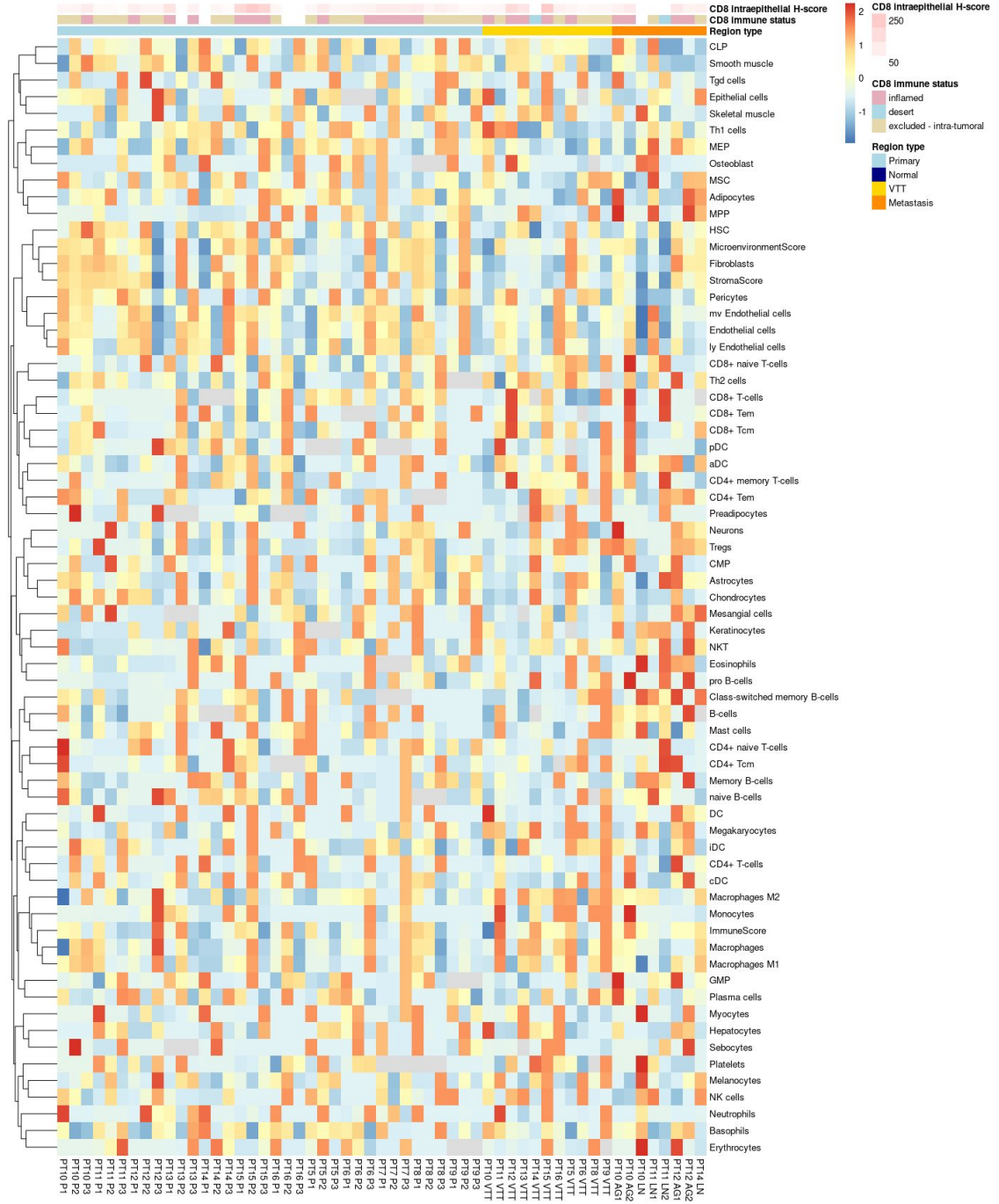
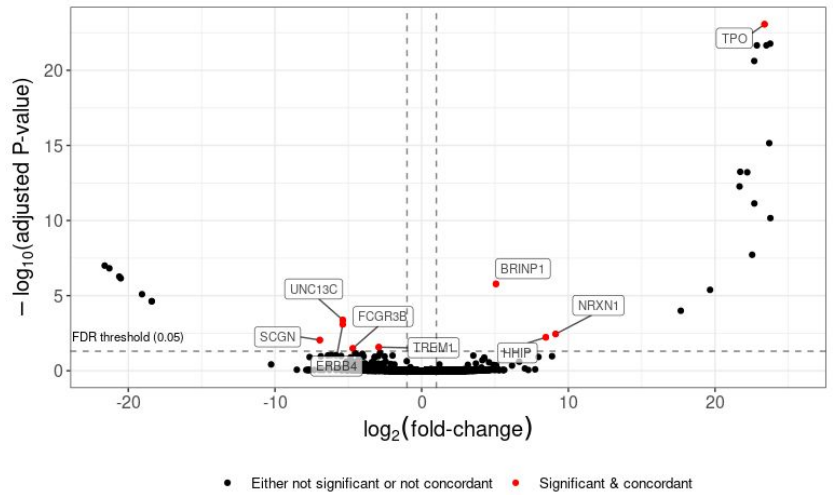

CD8 intraepithelial H-score
CD8 immune status
Region type
CLP
Smooth muscle
Tgd cells
Epithelial cells
Skeletal muscle
Th1 cells
MEP
Osteoblast
MSC
Adipocytes
MPP
HSC
MicroenvironmentScore
Fibroblasts
StromaScore
Pericytes
mv Endothelial cells
Endothelial cells
ly Endothelial cells
CD8+ naive T-cells
Th2 cells
CD8+ T-cells
CD8+ Tem
CD8+ Tcm
pDC
aDC
CD4+ memory T-cells
CD4+ Tem
Preadipocytes
Neurons
Tregs
CMP
Astrocytes
Chondrocytes
Mesangial cells
Keratinocytes
NKT
Eosinophils
pro B-cells
Class-switched memory B-cells
B-cells
Mast cells
CD4+ naive T-cells
CD4+ Tcm
Memory B-cells
naive B-cells
DC
Megakaryocytes
iDC
CD4+ T-cells
cDC
Macrophages M2
Monocytes
ImmuneScore
Macrophages
Macrophages M1
GMP
Plasma cells
Myocytes
Hepatocytes
Sebocytes
Platelets
Melanocytes
NK cells
Neutrophils
Basophils
Erythrocytes
PT10 P1
PT10 P2
PT10 P3
PT11 P1
PT11 P2
PT11 P3
PT12 P1
PT12 P2
PT12 P3
PT13 P1
PT13 P2
PT13 P3
PT14 P1
PT14 P2
PT14 P3
PT15 P1
PT15 P2
PT15 P3
PT16 P1
PT16 P2
PT16 P3
PT75 P1
PT75 P2
PT75 P3
PT76 P1
PT76 P2
PT76 P3
PT77 P1
PT77 P2
PT77 P3
PT78 P1
PT78 P2
PT78 P3
PT79 P1
PT79 P2
PT79 P3
PT10 VTT
PT11 VTT
PT12 VTT
PT13 VTT
PT14 VTT
PT15 VTT
PT16 VTT
PT75 VTT
PT76 VTT
PT78 VTT
PT79 VTT
PT10 AG1
PT10 AG2
PT10 LN
PT11 LN1
PT11 LN2
PT12 AG1
PT12 AG2
PT14 LN
2
1
0
-1
CD8 intraepithelial H-score
250
50
CD8 immune status
inflamed
desert
excluded - intra-tumoral
Region type
Primary
Normal
VTT
Metastasis

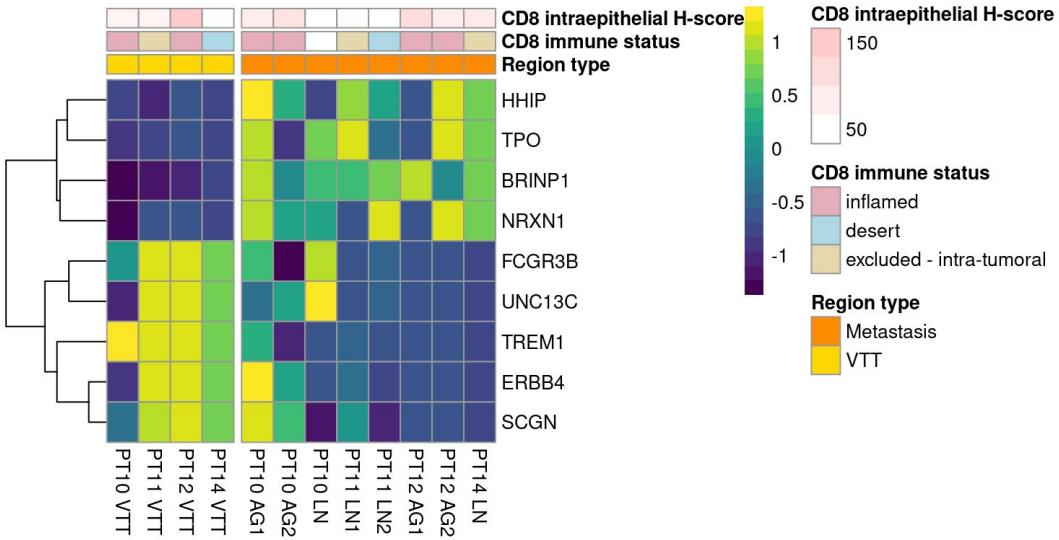
Figure S28. xCell enrichment scores for 67 cell types across all regions of all patients. Scores were normalized within each cell type and patient prior to visualization.

Figure S29.

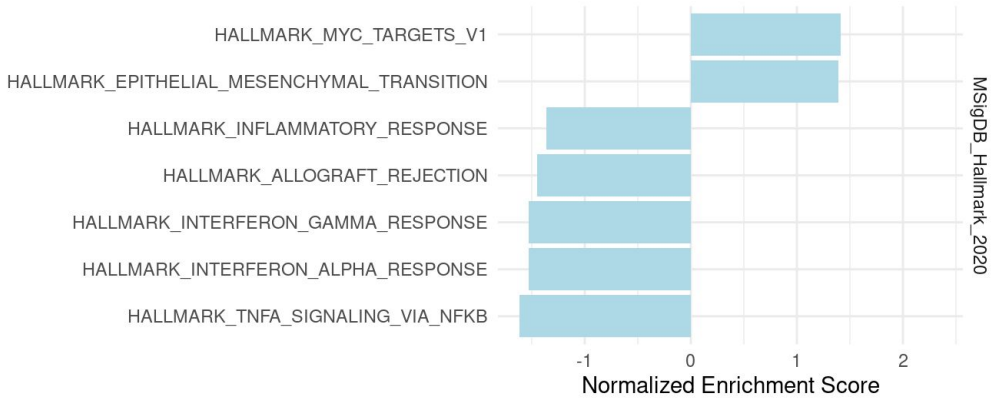
A



B



C



D

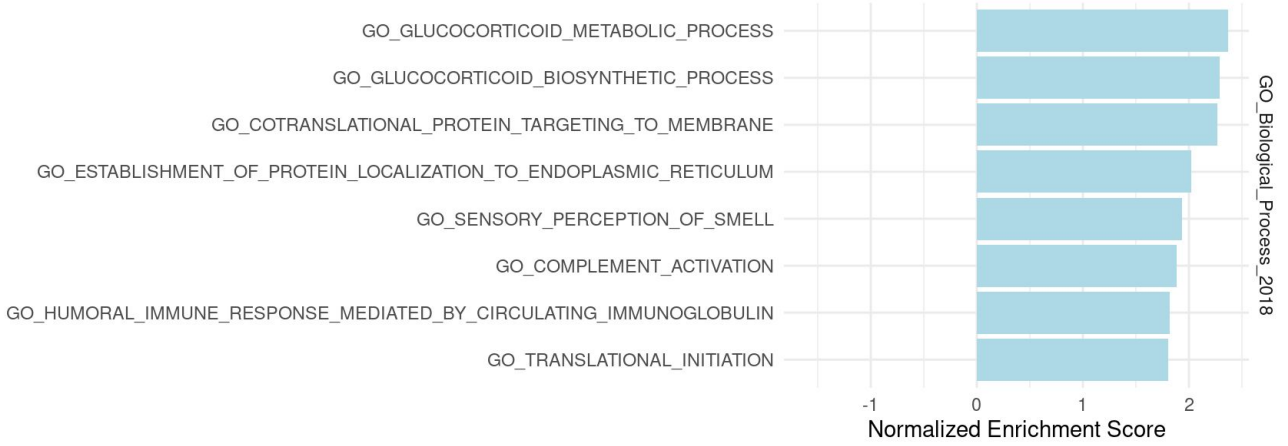
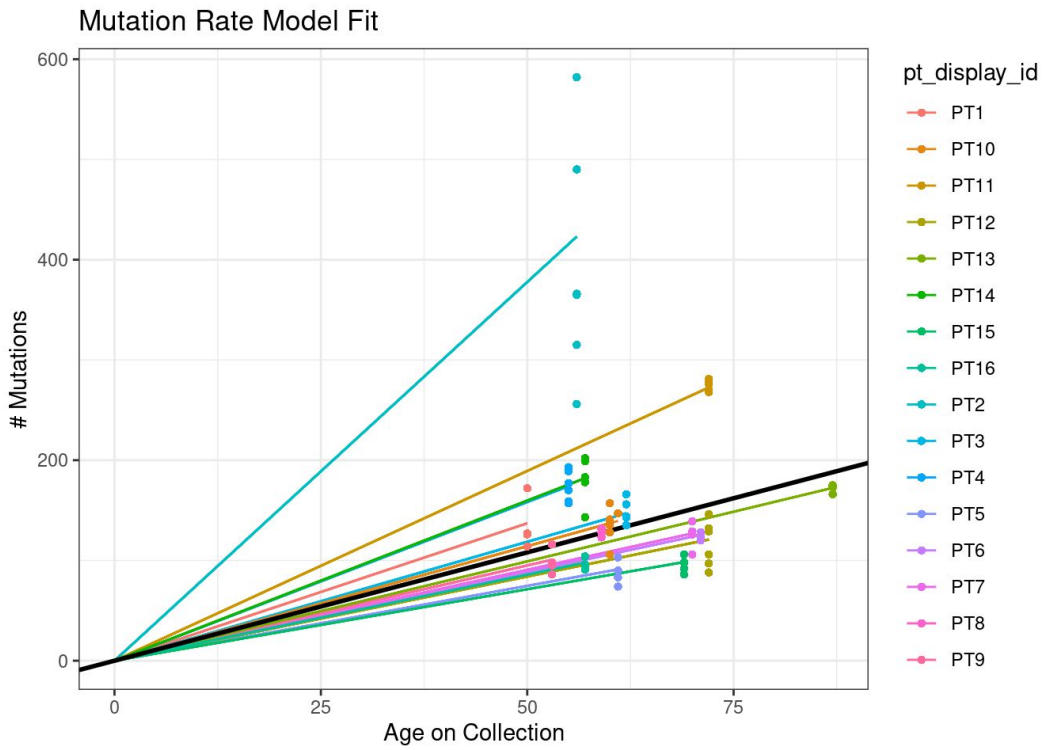


Figure S29. Differential expression between VTT and metastatic regions. (A) Volcano plot showing log-fold changes and adjusted P-values for differentially expressed genes. (B) Heatmap showing expression values of genes determined to be differential between VTT and metastatic regions. Expression values are normalized per gene and per patient. (C) Significantly enriched or depleted Hallmark pathways in metastases relative to VTT regions as determined by GSEA. (D) Significantly enriched GO Biological Process terms enriched or depleted in metastases relative to VTT regions as determined by GSEA. Significance is defined as adjusted p-value $\leq$ 0.05 (Benjamini-Hochberg).

Figure S30.

A.



B.

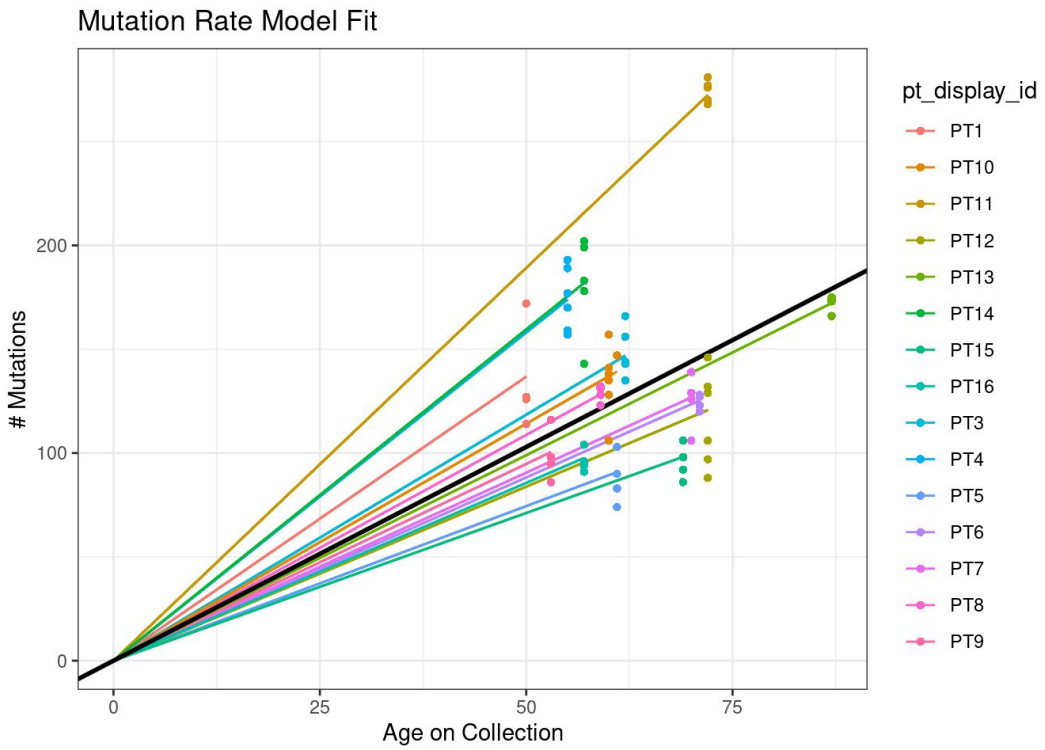


Figure S30. LME mutation rate model fits (A) with and (B) without PT2.