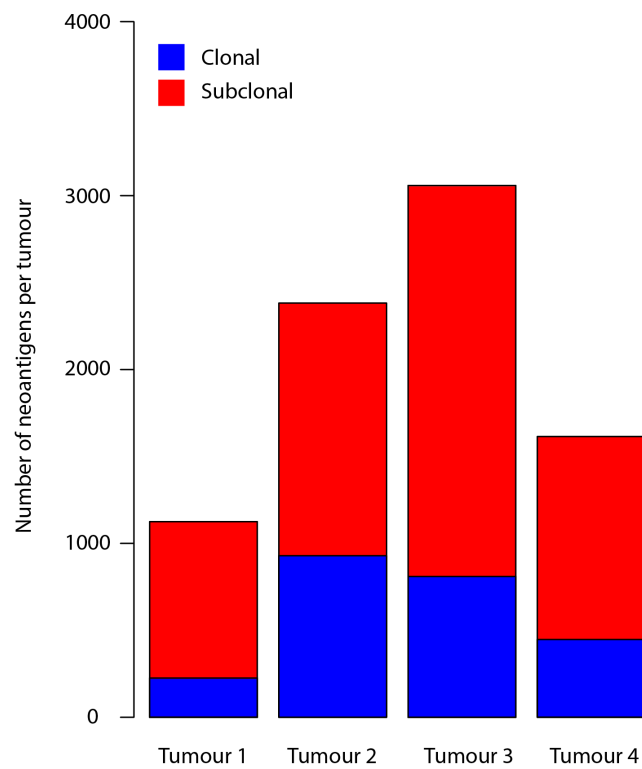


Extreme intratumour heterogeneity and driver evolution in mismatch repair deficient gastro-oesophageal cancer

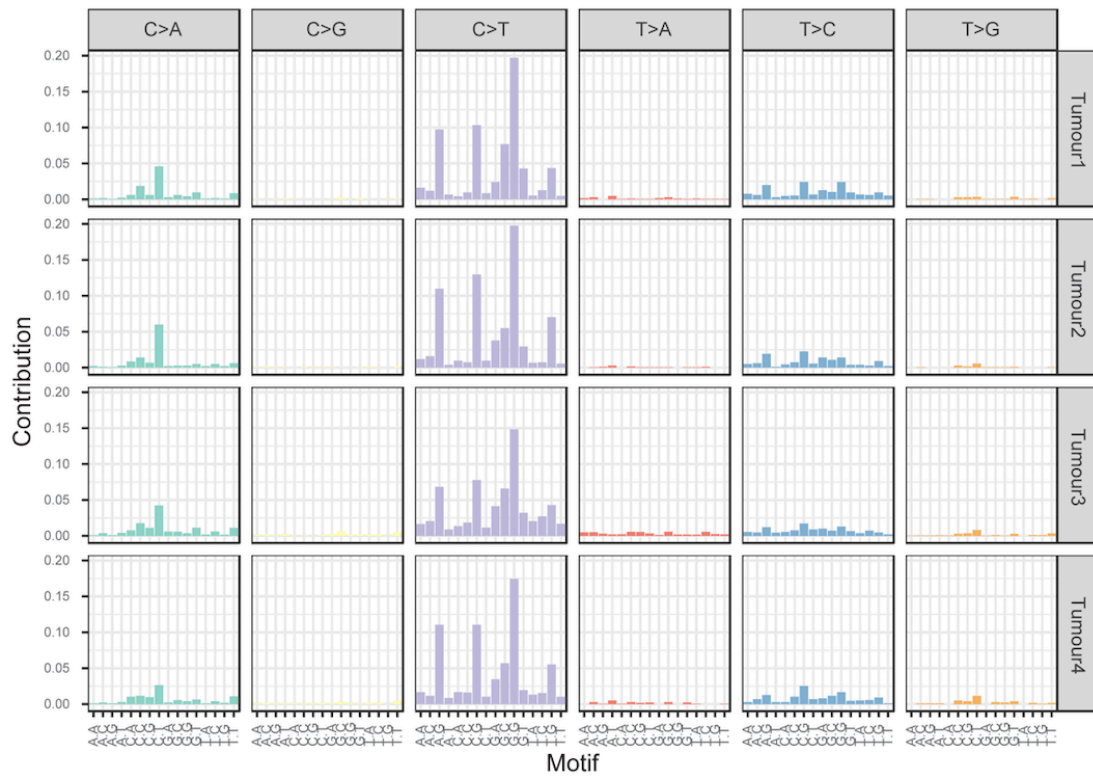
Supplementary Information

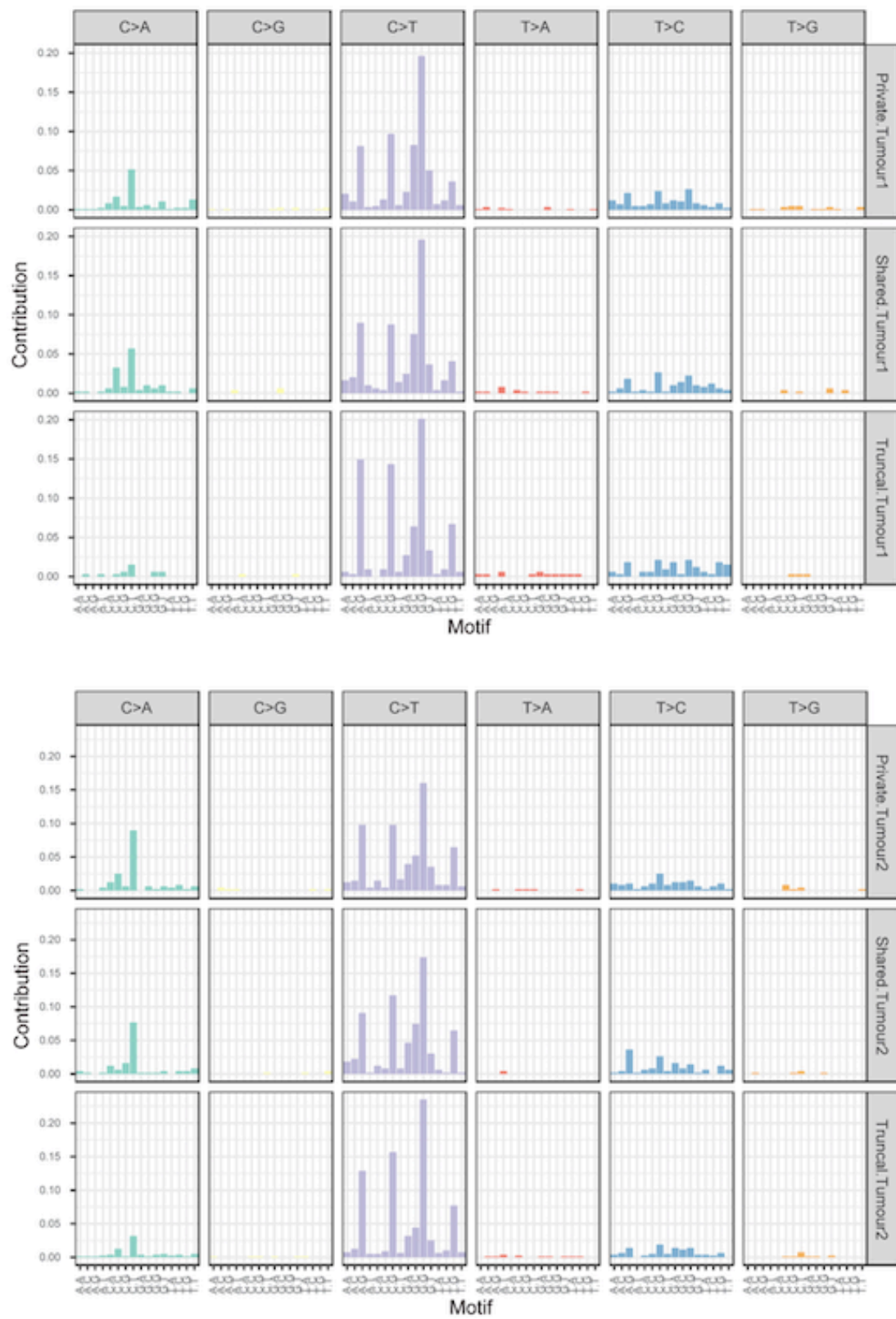
Supplementary Figure 1. Clonal and subclonal neoantigen burden.

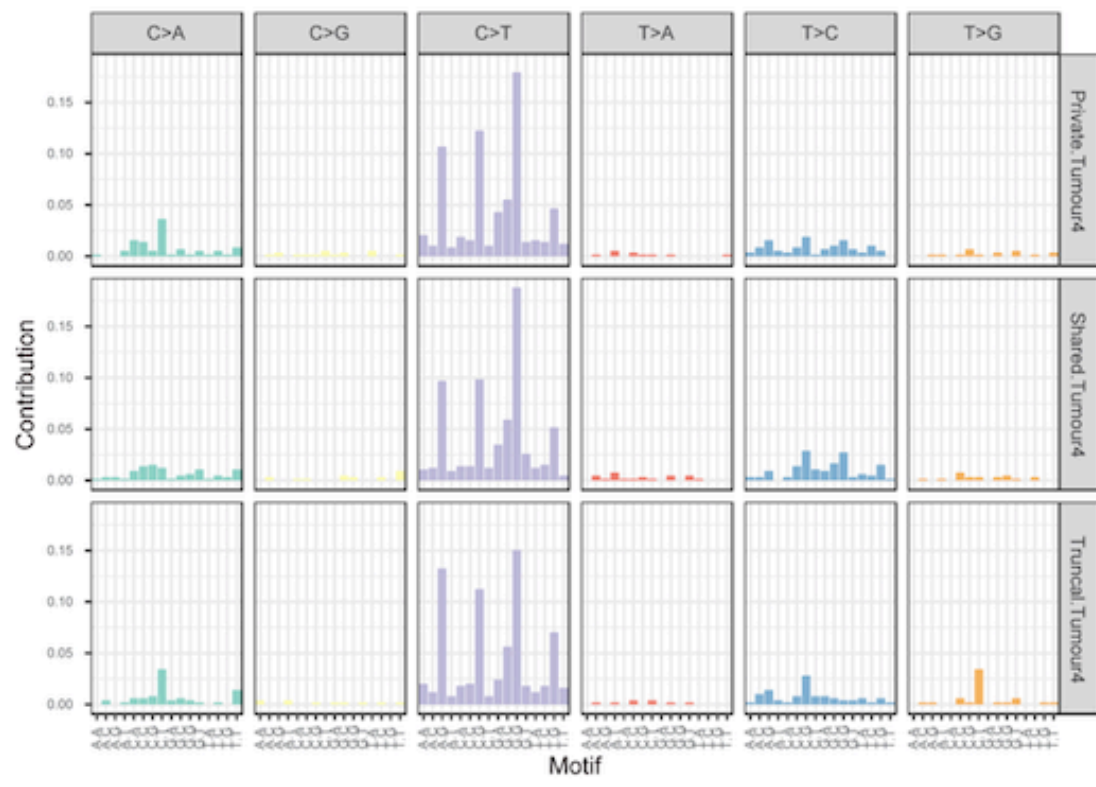
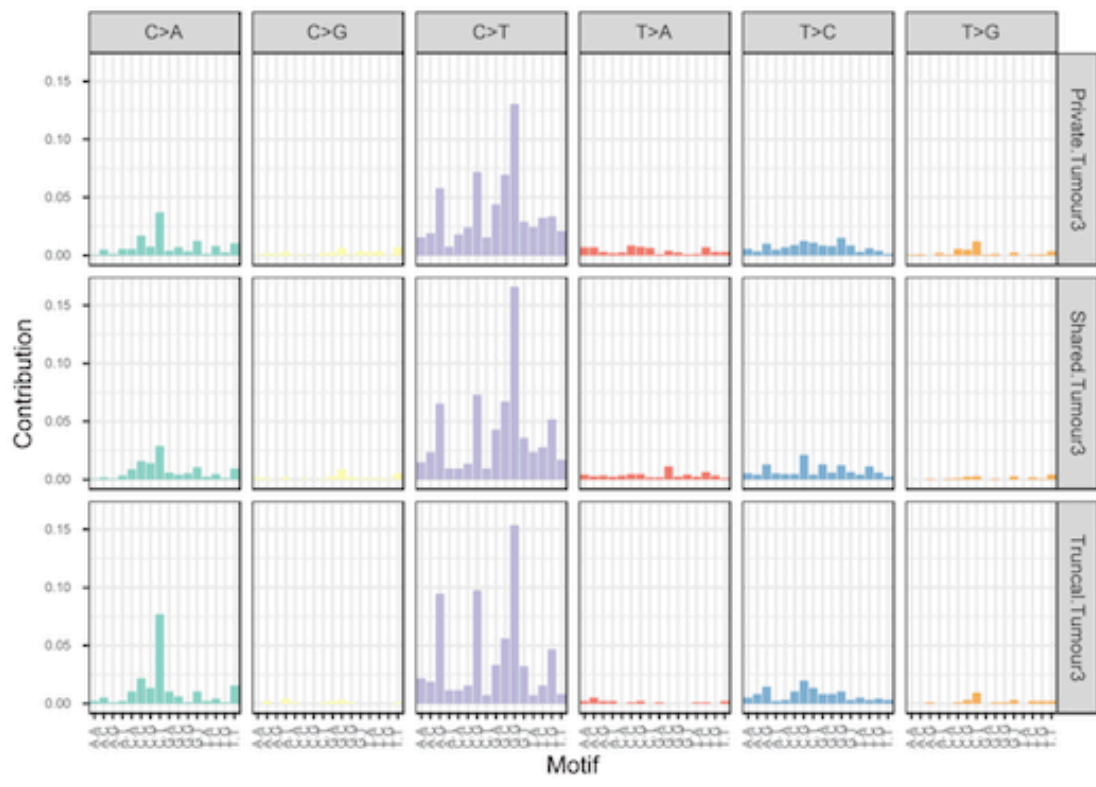


Supplementary Figure 2. Plots of somatic mutation counts split by the 96 mutation types.

A

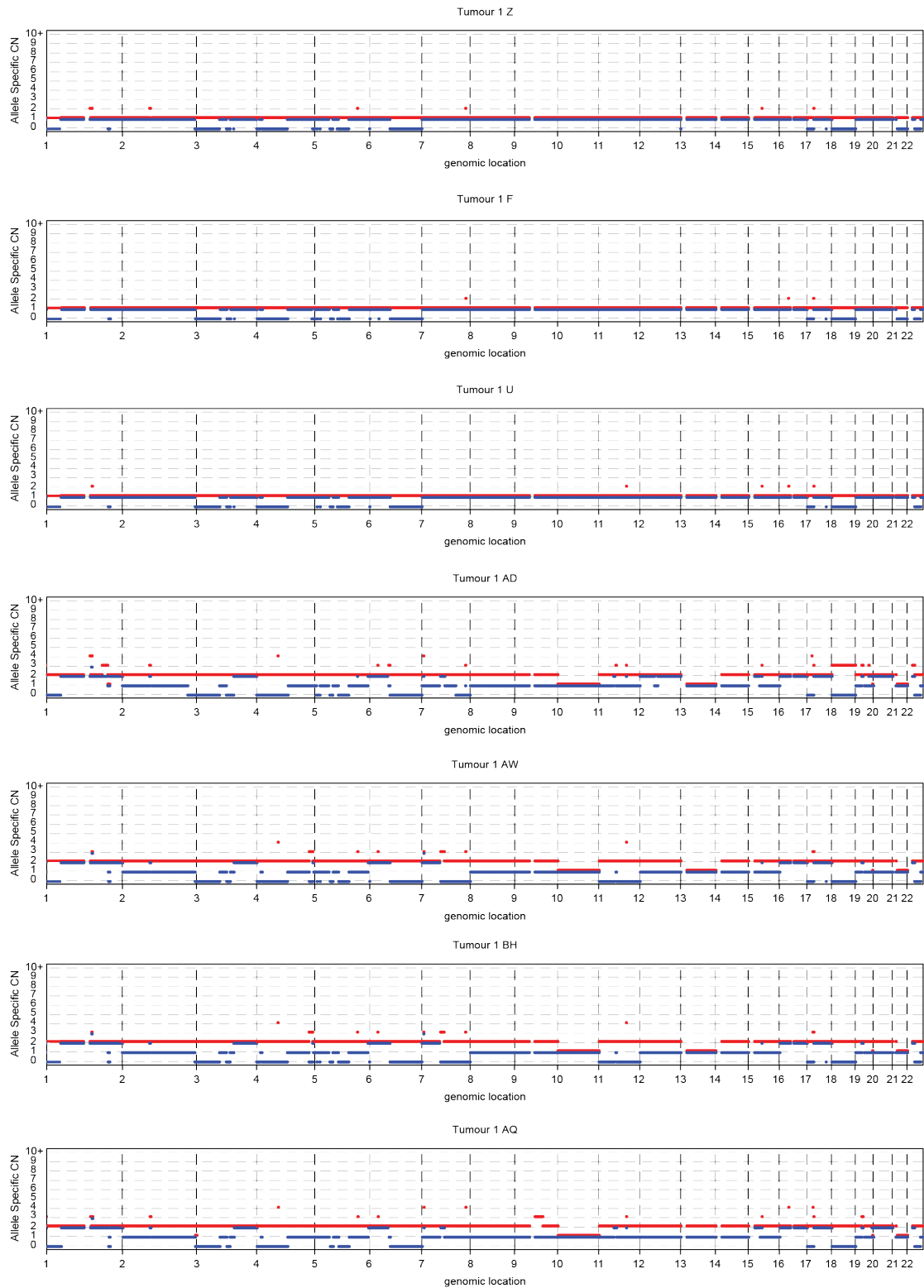


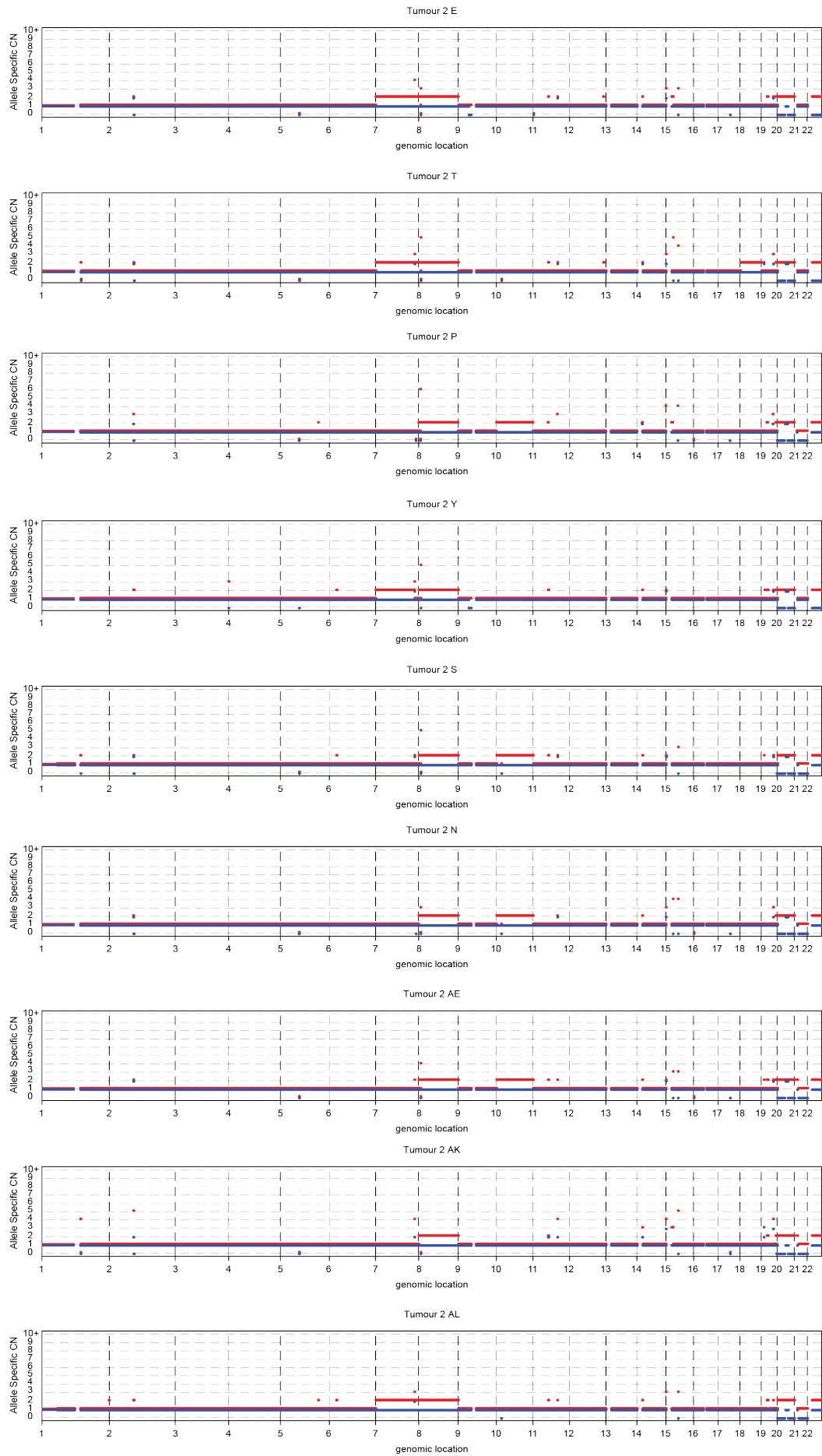
B

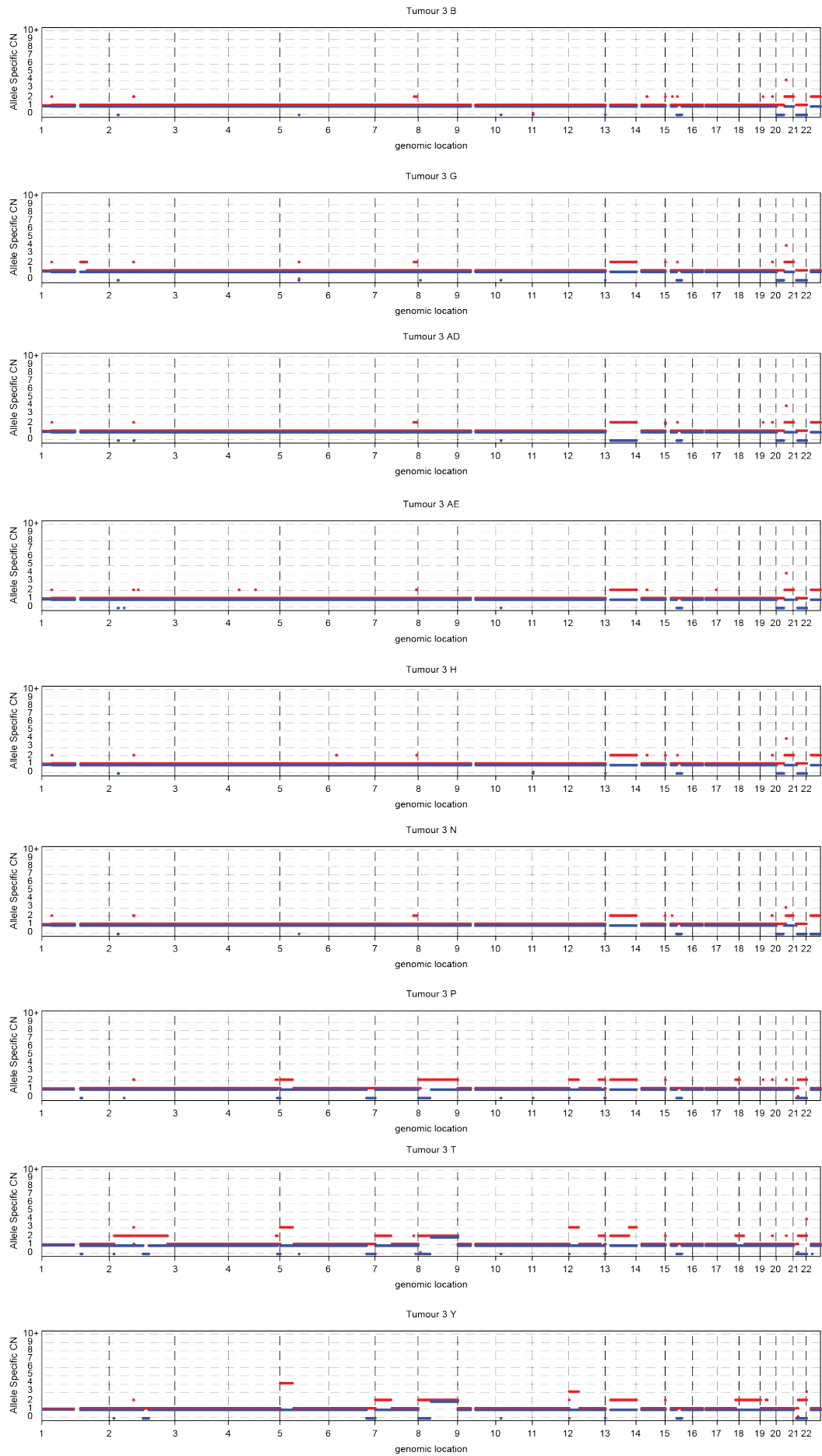


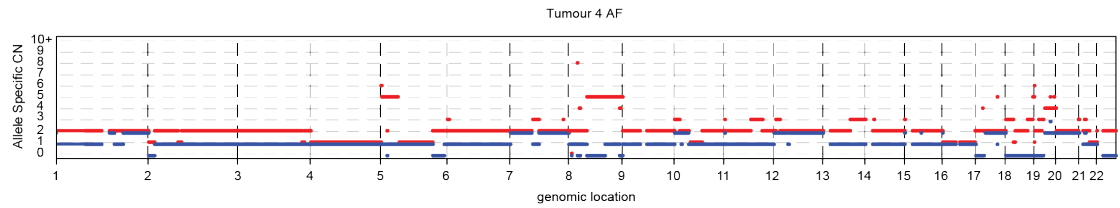
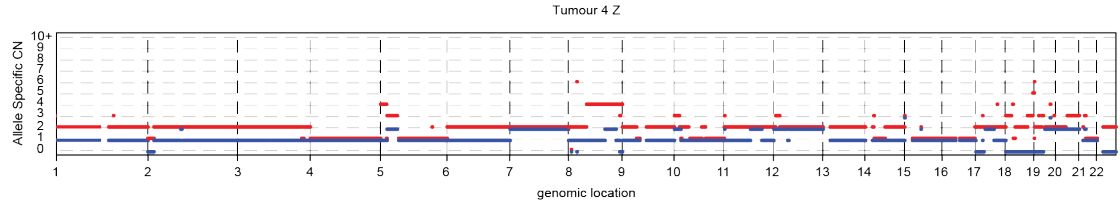
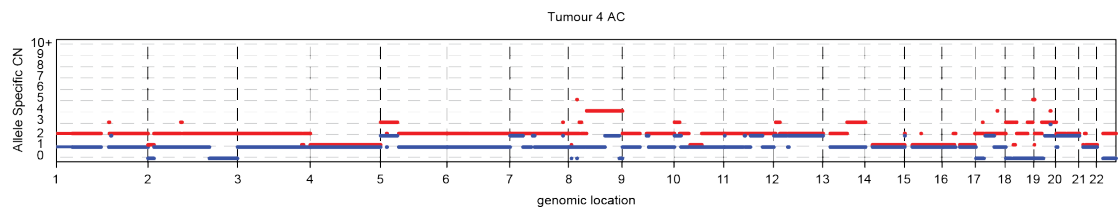
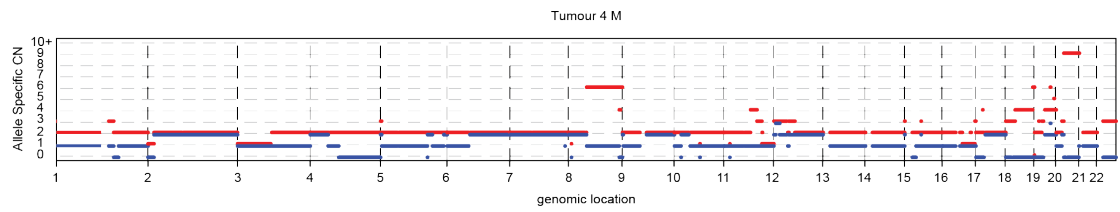
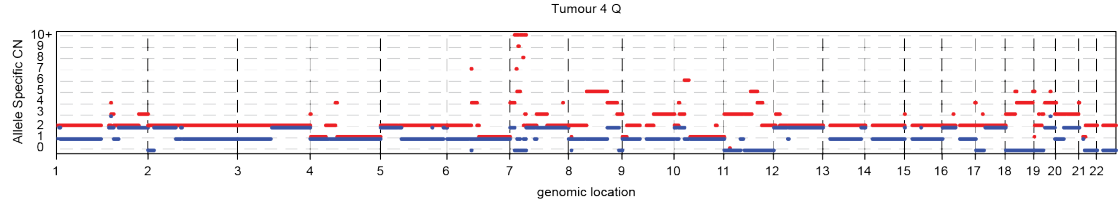
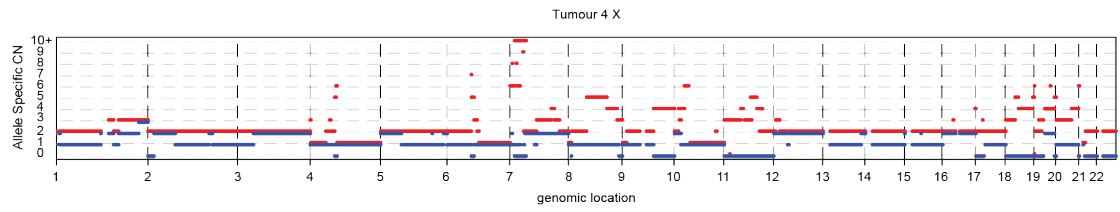
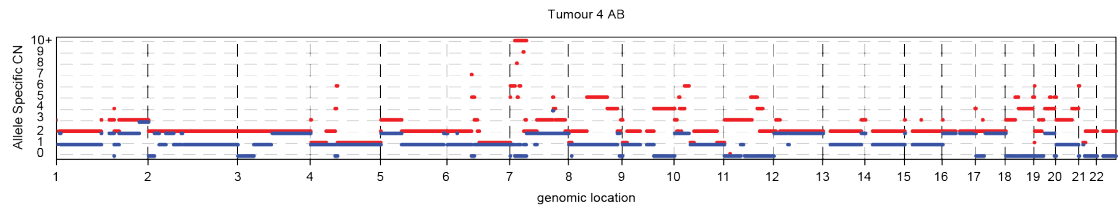
A. Normalised mutation spectrums for Tumours 1-4. Plots are based on the following SNV burdens: Tumour 1: 1653, Tumour 2: 1766, Tumour 3: 4662 and Tumour 4: 1738. **B.** Normalised mutation profiles split by private, shared and truncal mutations for each tumour. Plots are based on the following SNV burdens: Tumour 1: 835 private, 490 shared, 328 truncal, Tumour 2: 480 private, 495 shared, 791 truncal, Tumour 3: 2002 private, 1698 shared, 962 truncal, Tumour 4: 580 private, 660 shared, 498 truncal.

Supplementary Figure 3. Allele specific copy number profiles of individual tumour regions.



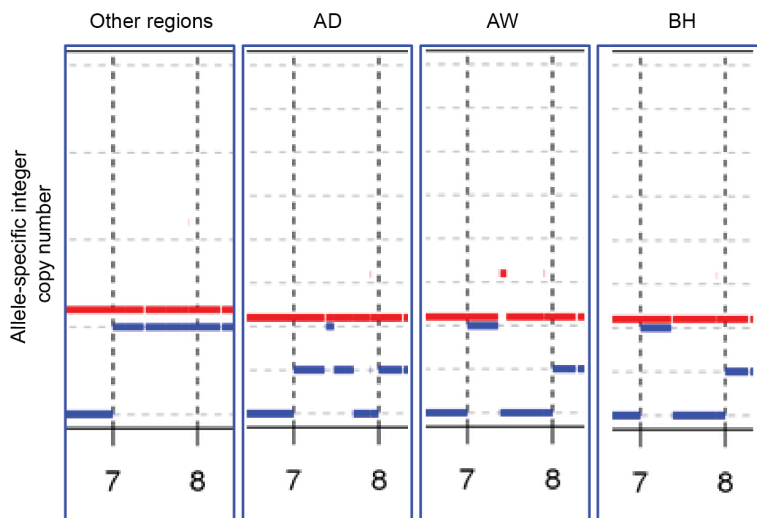






Supplementary Figure 4. An Illustration of Phylogenetic conflict in Tumour 1.

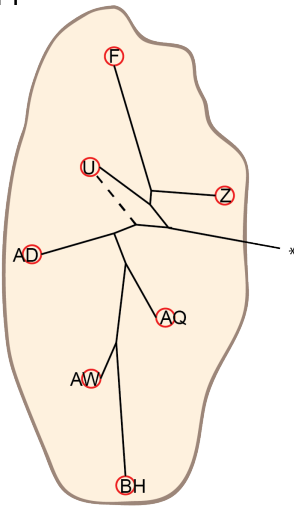
Tumour 1



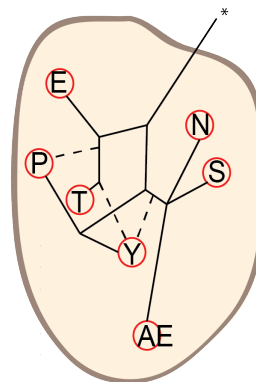
Chr7q LOH in AW and BH and shorter segment LOH in AD which explain one phylogenetic conflict in Tumour 1

Supplementary Figure 5. Mapping of phylogenetic trees onto tumour maps.

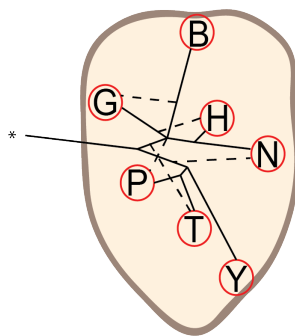
Tumour 1



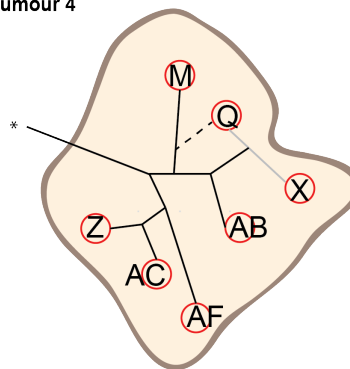
Tumour 2



Tumour 3



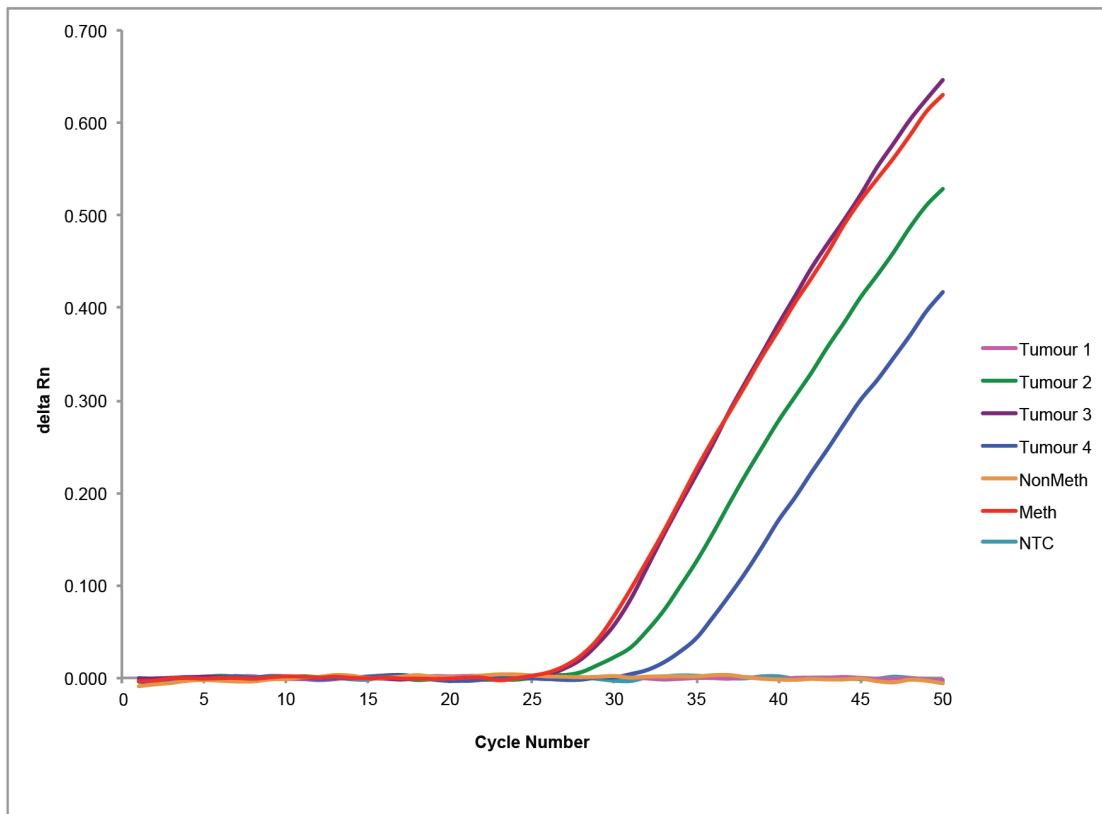
Tumour 4



- Phylogenetic tree trunk or branches indicating ancestral relationships of major clones
 - - - Phylogenetic tree branches indicating ancestral relationships of minority subclones
 - Connecting line indicating subclones which appeared identical in the phylogenetic deconvolution
- Trunk and branch lengths are scale free

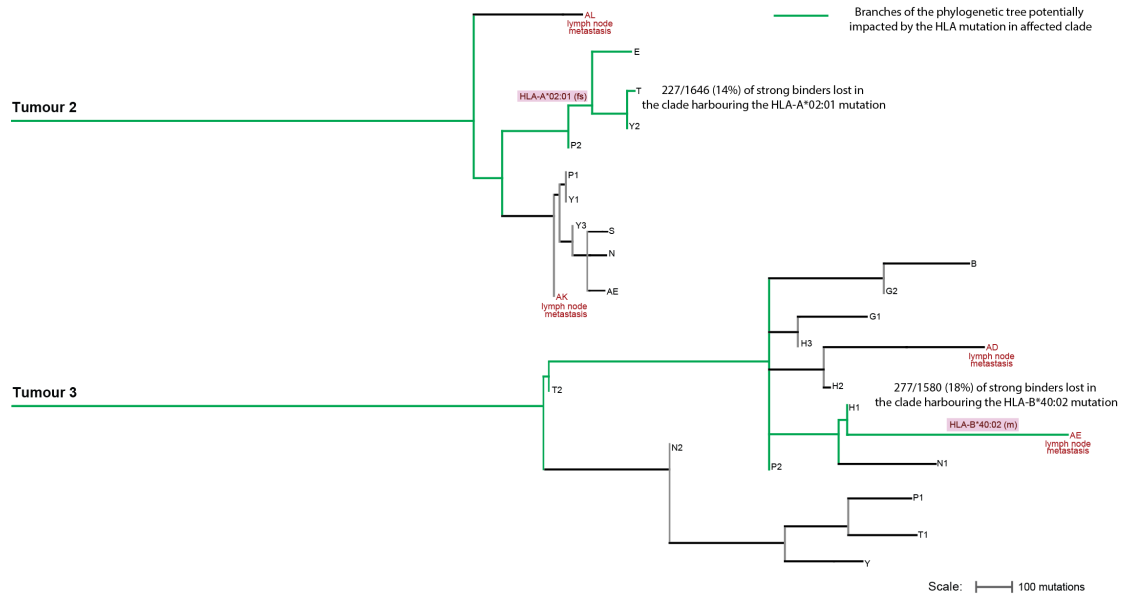
*Origin of the phylogenetic tree, mapped outside of the tumour area by default

Supplementary Figure 6. *MLH1* promoter methylation.

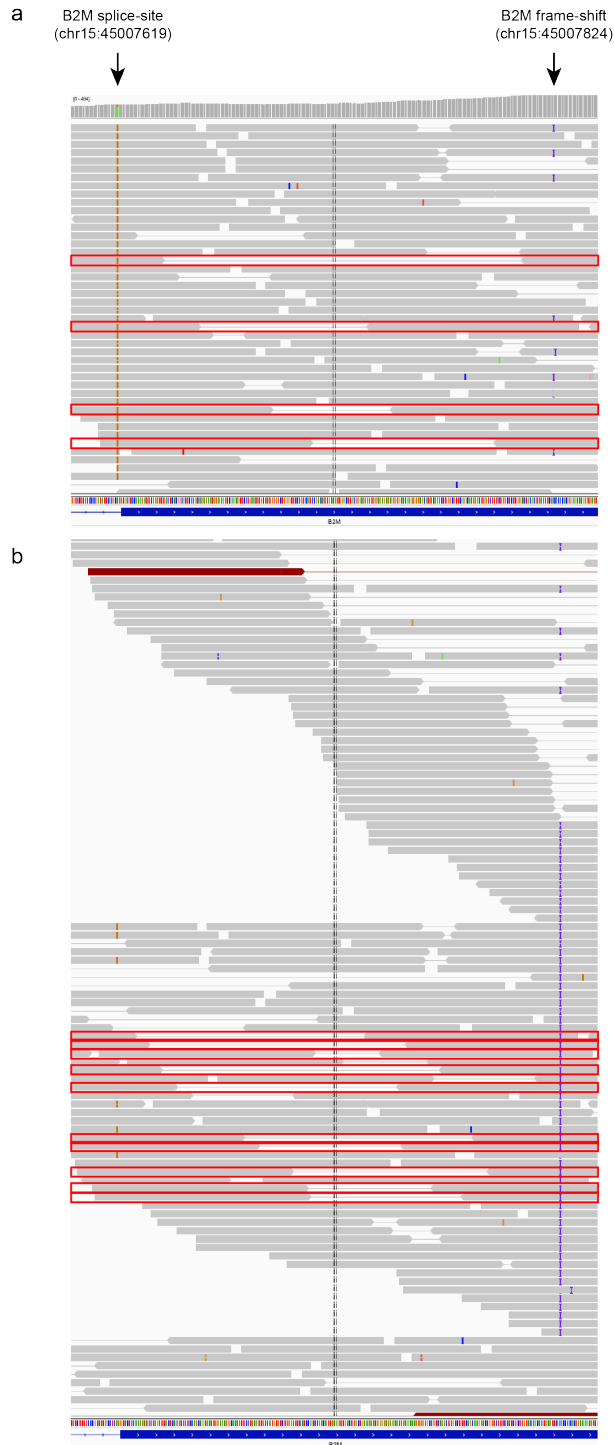


qPCR of bisulfite converted DNA confirms *MLH1* promoter methylation in Tumour 2-4 but not Tumour 1. NonMeth=Human Non-Methylated DNA Standard; Meth=Human Methylated DNA Standard; NTC=Non-template control.

Supplementary Figure 7. Possible neoantigen loss due to HLA mutations.

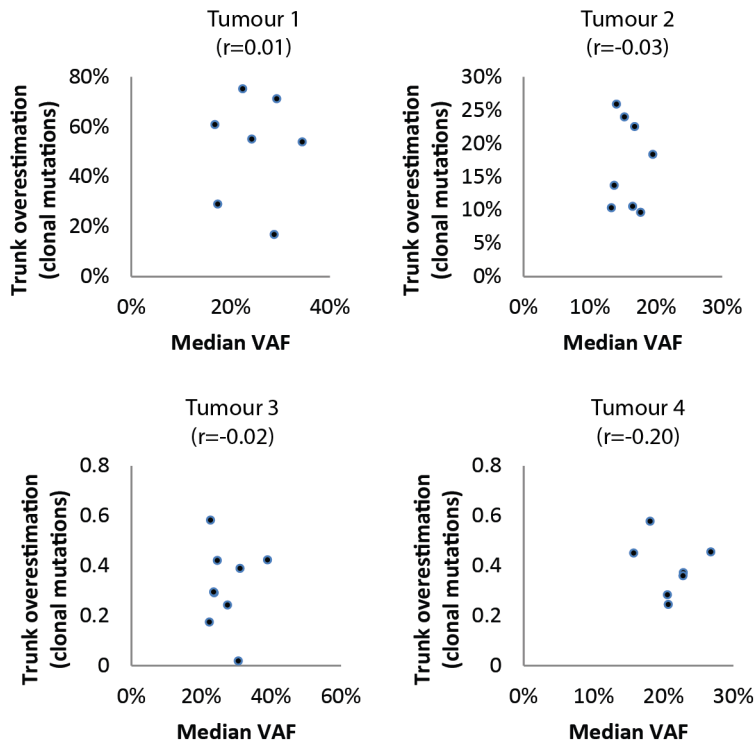


Supplementary Figure 8. Paired-end reads demonstrate biallelic inactivation of *B2M*.

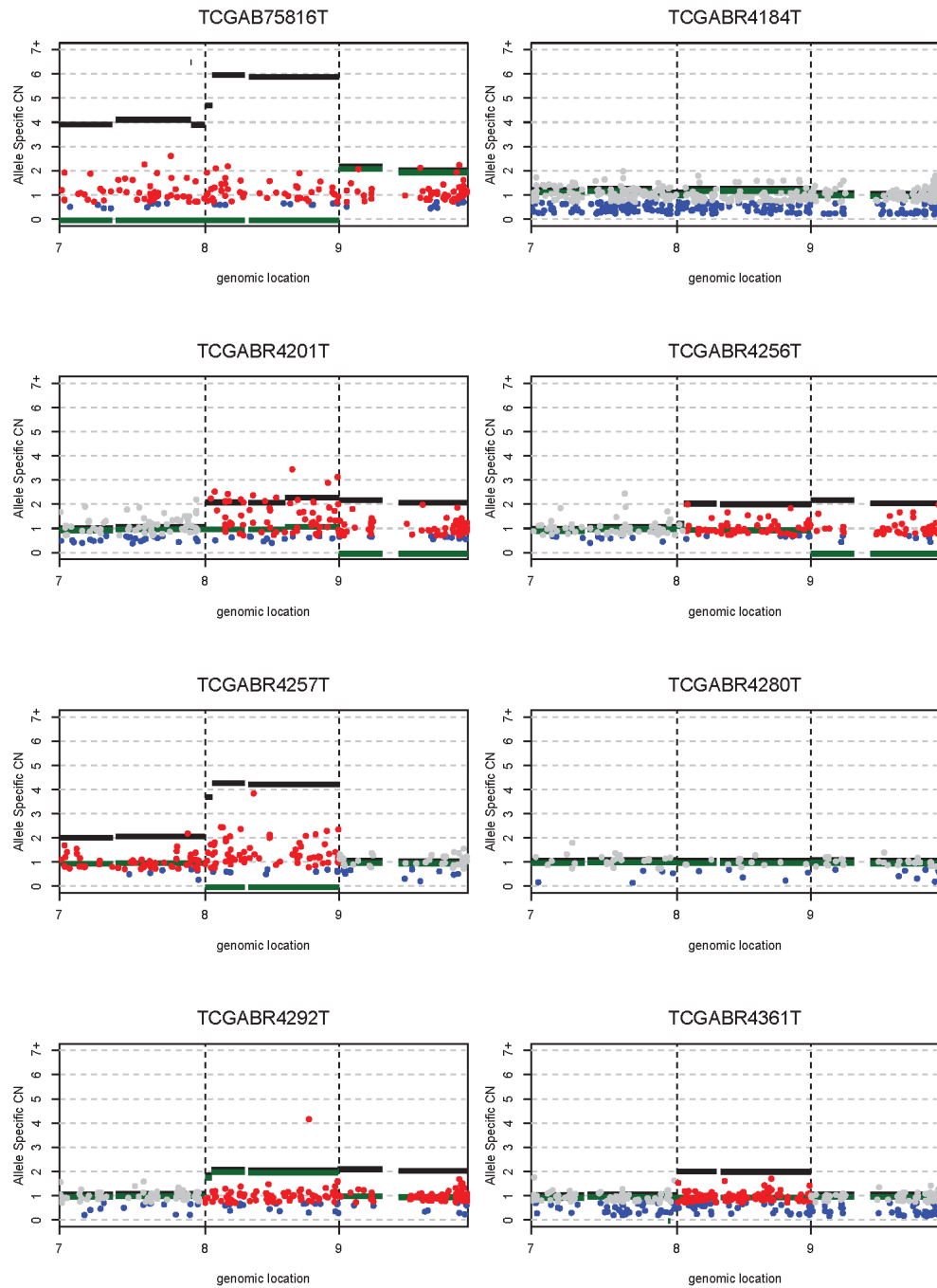


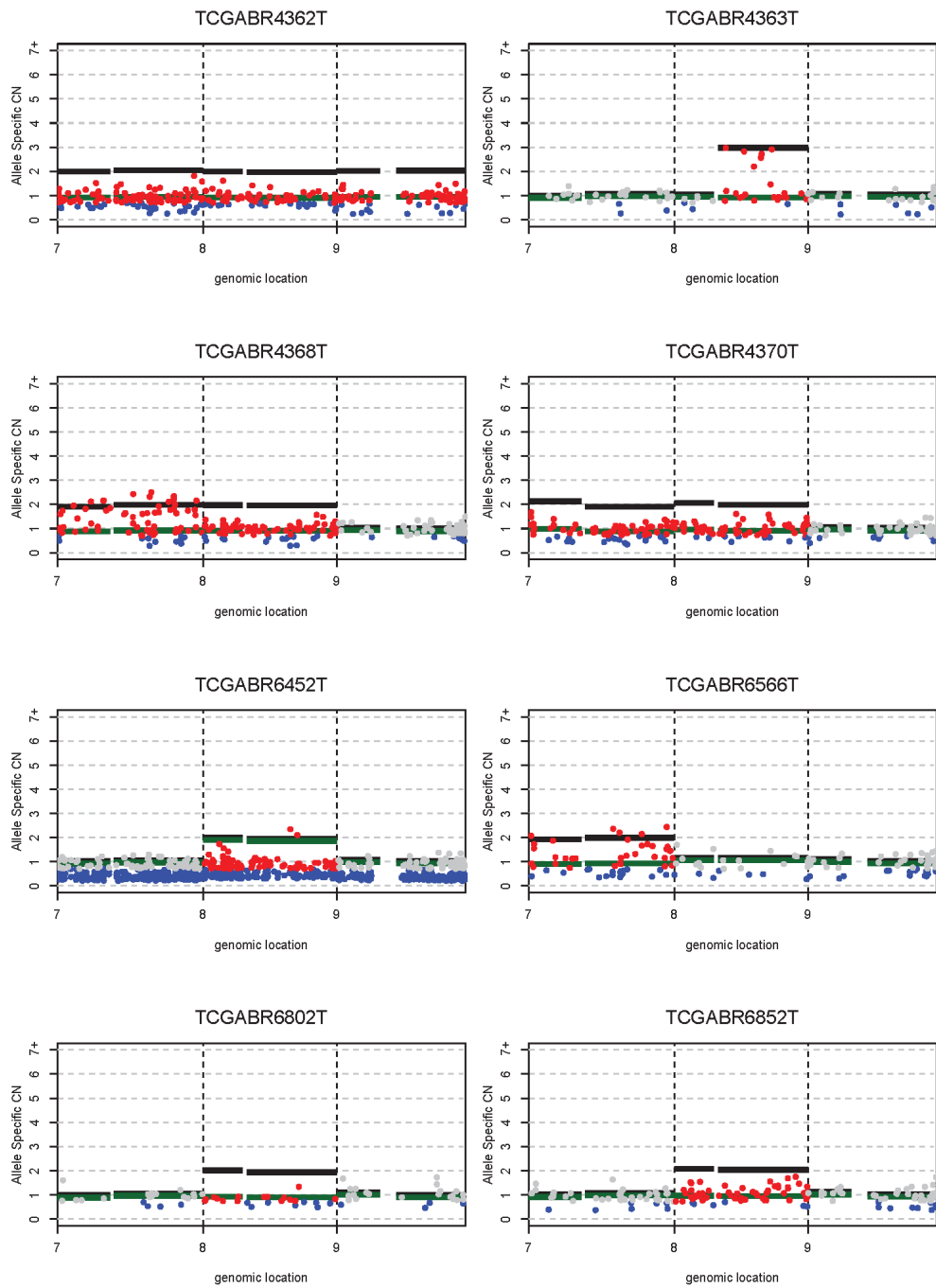
Screenshots from the Interactive Genome Viewer of Tumour 2 AE displaying the *B2M* splice-site and frameshift mutations. Paired reads that cover both mutation loci are highlighted with red boxes. **A.** A total of 4 read pairs showed the *B2M* splice-site mutation and covered the location of the *B2M* frameshift mutation but none showed the frameshift mutation. **B.** A total of ten read pairs showed the *B2M* frameshift mutation and covered the location of the *B2M* splice site mutations but none showed the *B2M* splice-site mutation.

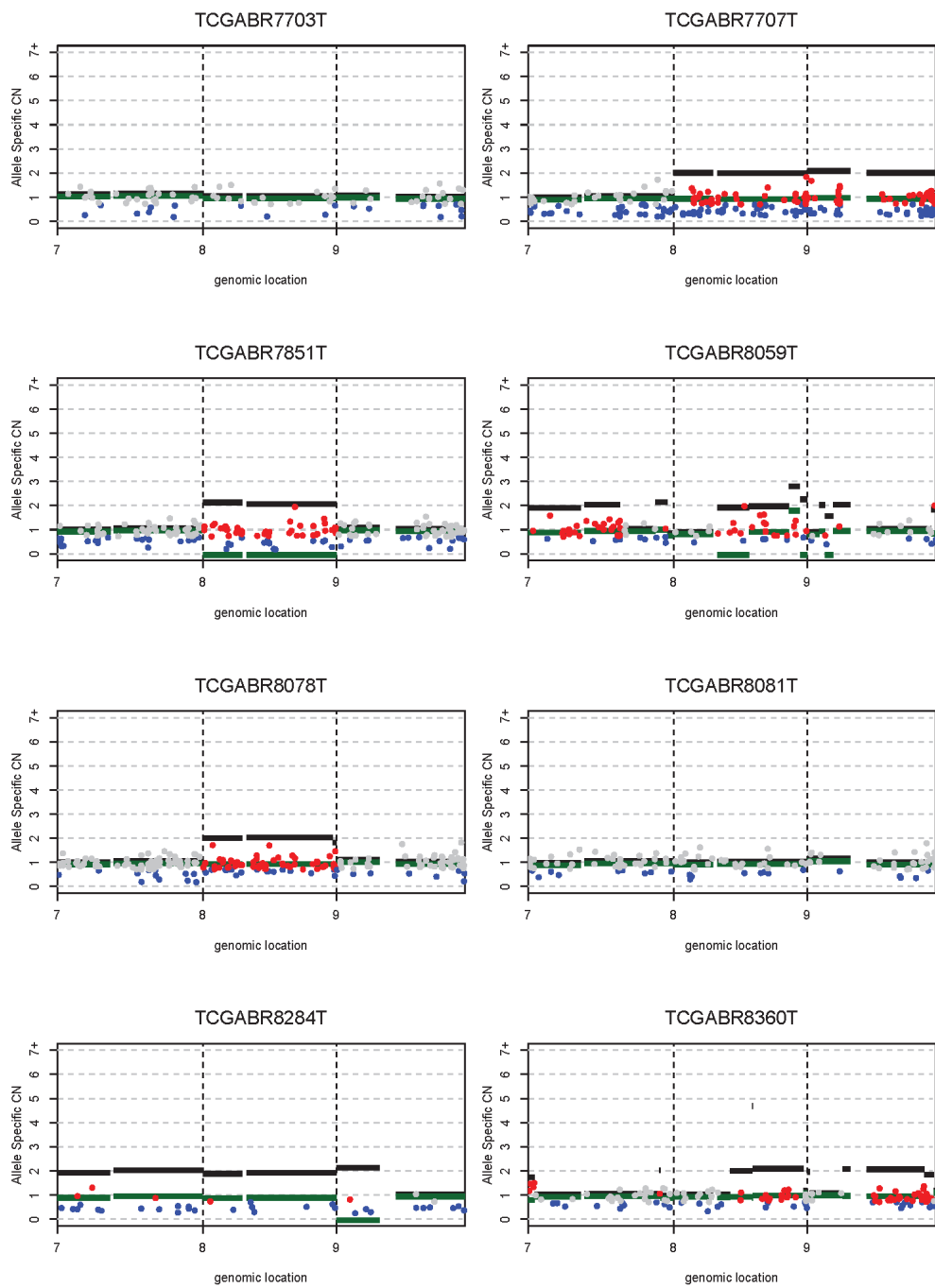
Supplementary Figure 9. Trunk overestimation by single region analysis.

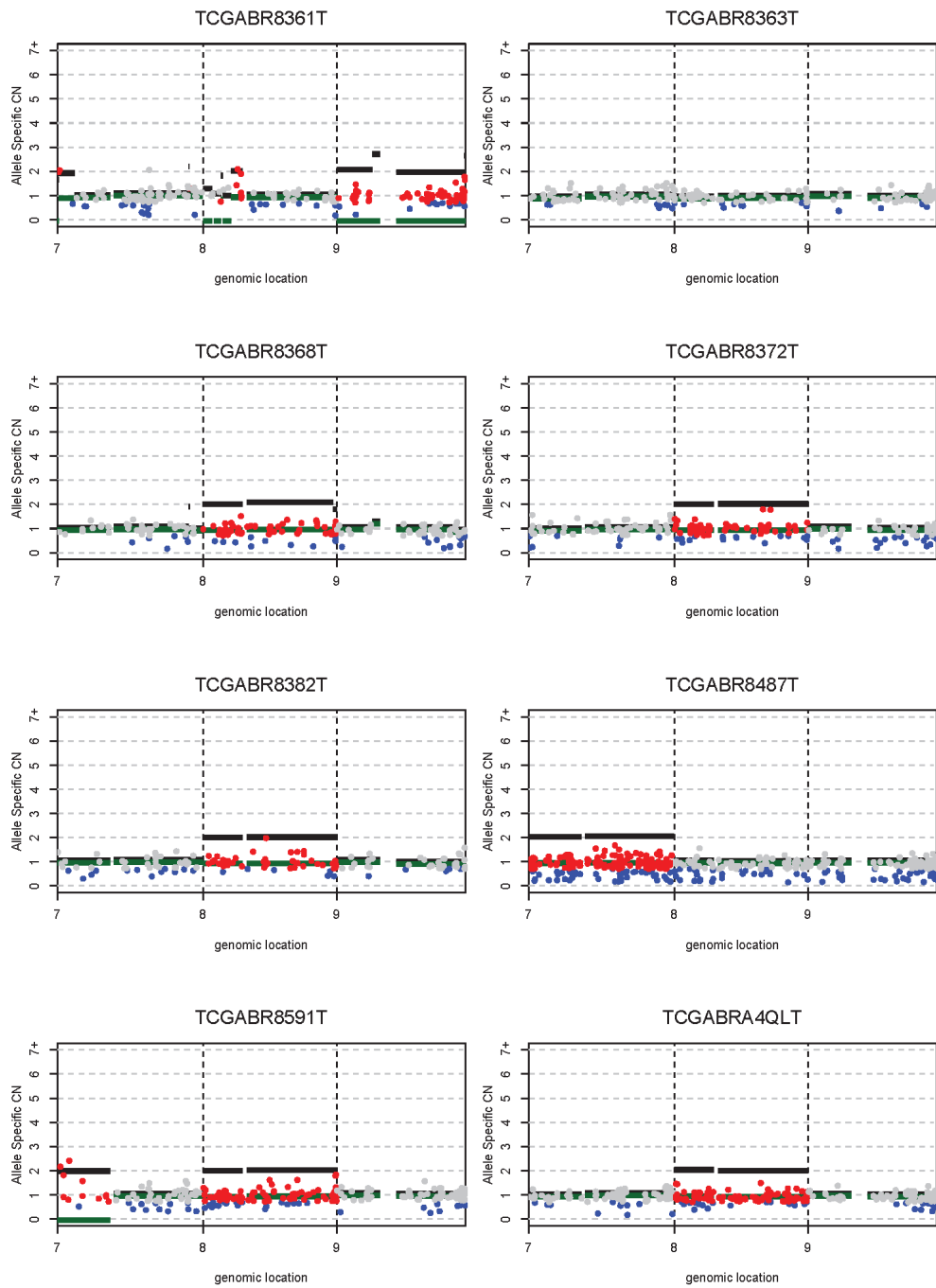


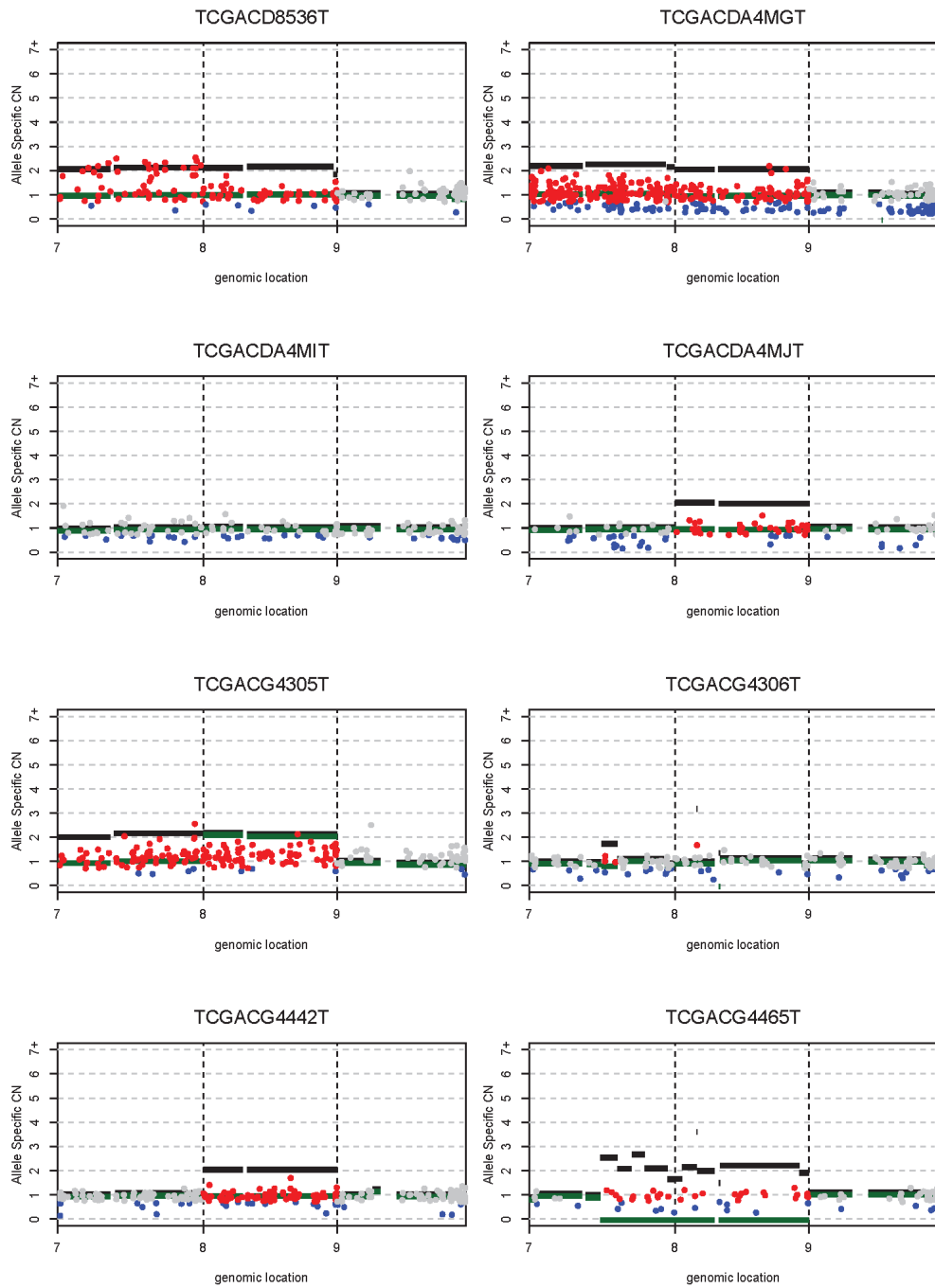
Supplementary Figure 10. Mutation copy number of chromosome 8 in the STAD TCGA series.

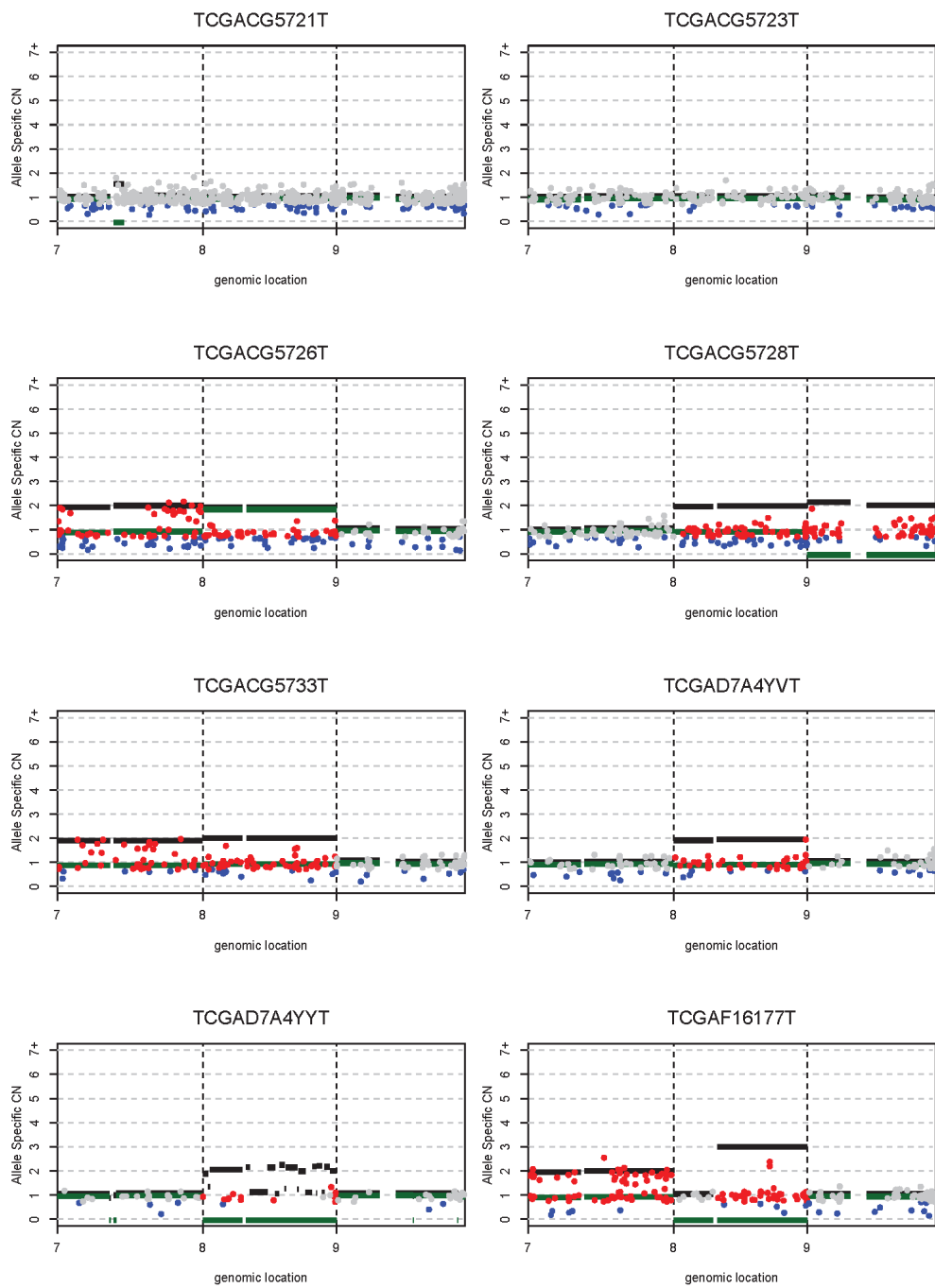


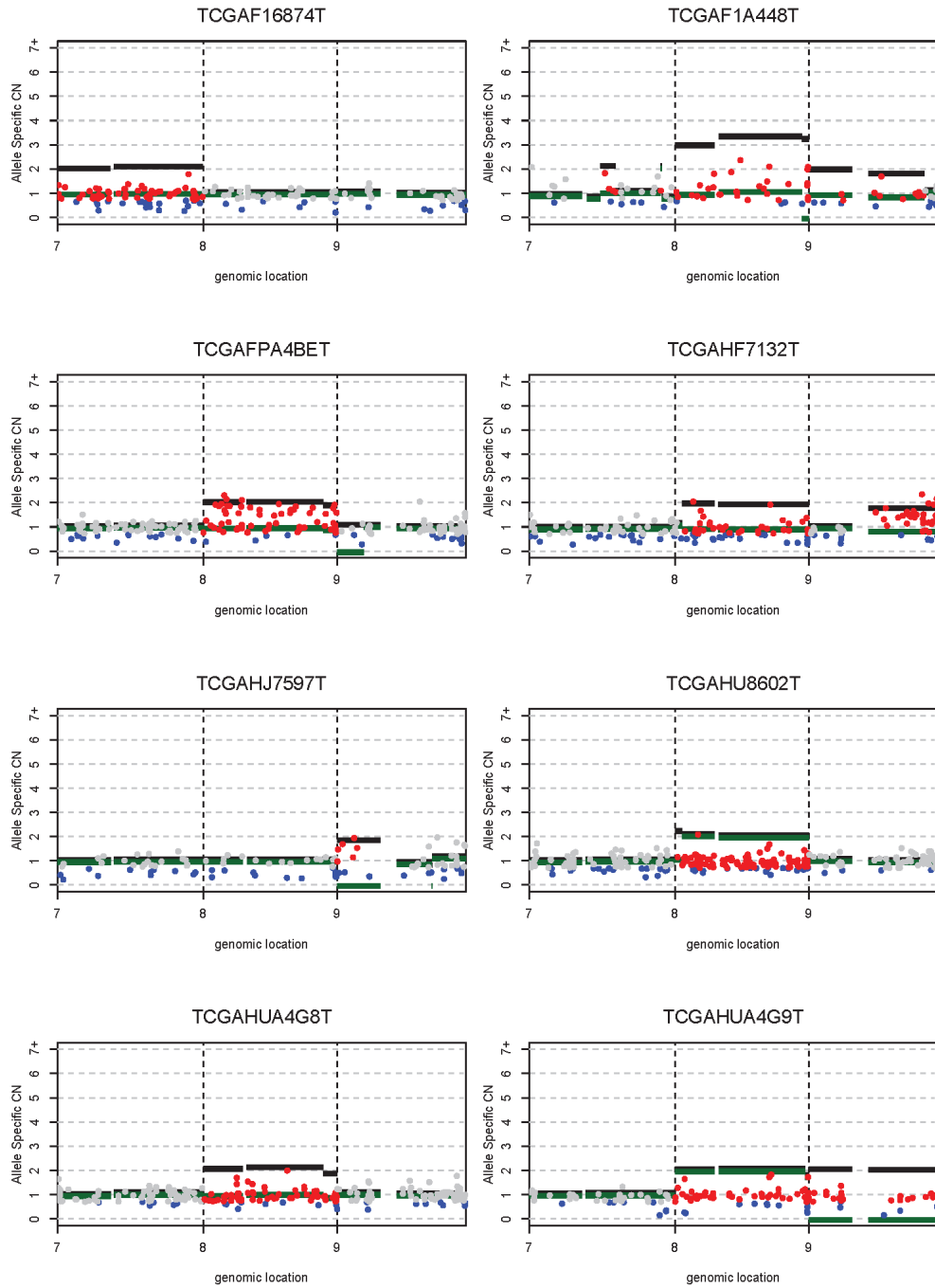


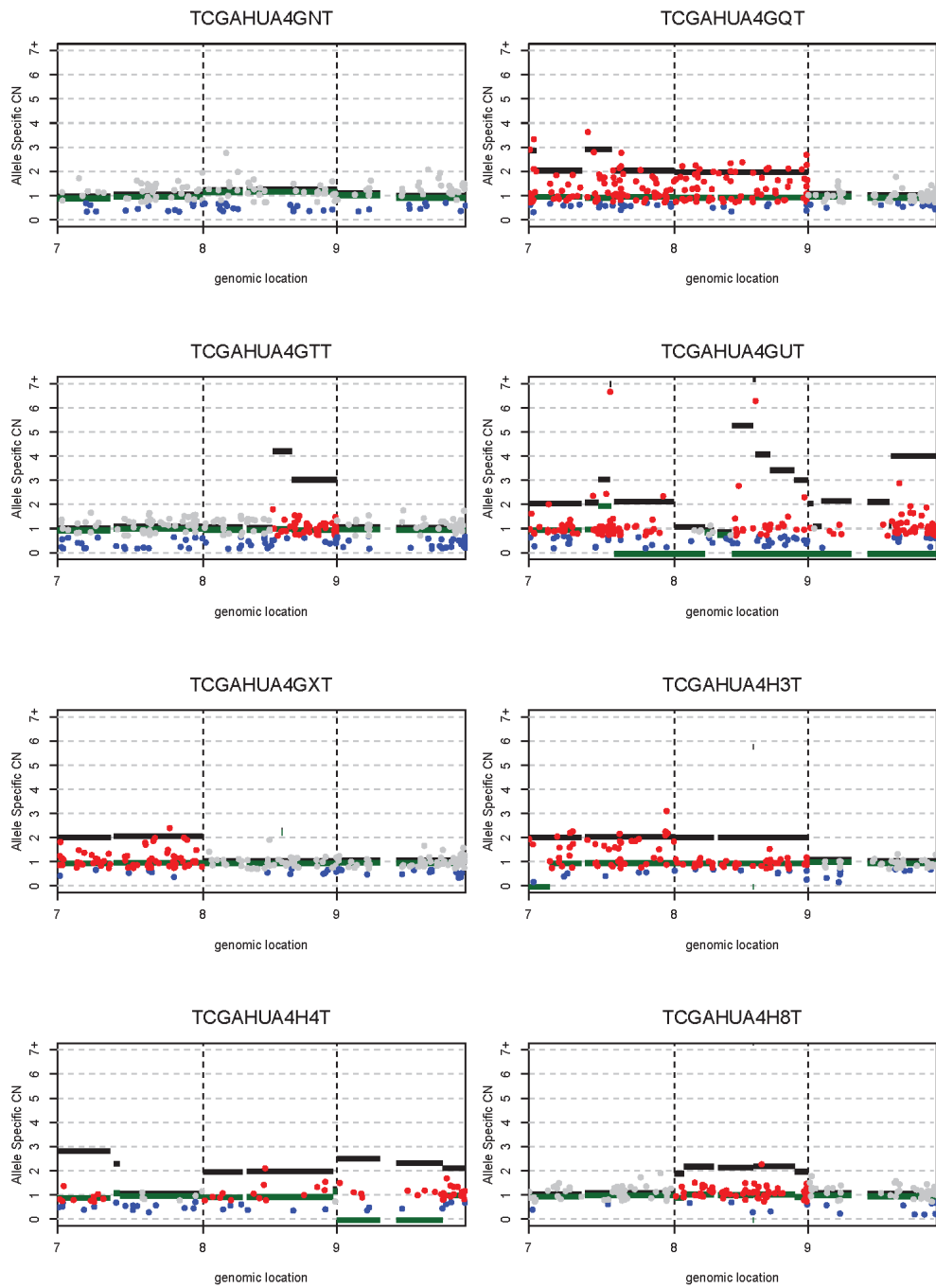












Supplementary Table 1. Cell purity and ploidy

Sample	Purity	Ploidy
Tumor 1 F	0.72	1.82
Tumor 1 U	0.6	1.8
Tumor 1 Z	0.61	1.9
Tumor 1 AD	0.23	3.19
Tumor 1 AQ	0.26	3.15
Tumor 1 AW	0.37	2.97
Tumor 1 BH	0.33	2.79
Tumor 2 Y	0.34	2.07
Tumor 2 AL	0.37	2.1
Tumor 2 S	0.32	2.1
Tumor 2 T	0.28	2.12
Tumor 2 AE	0.41	2.07
Tumor 2 AK	0.15	2.15
Tumor 2 E	0.32	2.14
Tumor 2 N	0.28	2.07
Tumor 2 P	0.26	2.1
Tumor 3 Y	0.5	2.22
Tumor 3 AE	0.82	2.04
Tumor 3 H	0.51	2.12
Tumor 3 N	0.54	2.03
Tumor 3 P	0.64	2.15
Tumor 3 T	0.62	2.12
Tumor 3 B	0.49	2.02
Tumor 3 G	0.5	2.05
Tumor 3 AD	0.43	2.02
Tumor 4 M	0.38	3.81
Tumor 4 Q	0.34	3.63
Tumor 4 X	0.37	3.69
Tumor 4 Z	0.25	3.43
Tumor 4 AB	0.37	3.76
Tumor 4 AC	0.32	3.27
Tumor 4 AF	0.33	3.45

Supplementary Table 2. COSMIC signatures

	COSMIC Signature						
	3	4	6	7	14	15	17
Lymph Node Metastases (subclonal only)	0.075143	0.0609702	0.4732131	0.0693594	0.0595752	0.1420577	0.0575242

Supplementary Table 3. dN/dS

	dN/dS	CI _{95%}
Tumour1.Truncal	1.06	0.83-1.35
Tumour1.Shared	1.07	0.87-1.31
Tumour1.Private	1.16	0.99-1.37
Tumour2.Truncal	1.04	0.89-1.21
Tumour2.Shared	1.05	0.86-1.28
Tumour2.Private	1.31	1.05-1.63
Tumour3.Truncal	1.01	0.87-1.17
Tumour3.Shared	1.01	0.9-1.12
Tumour3.Private	1.01	0.91-1.12
Tumour4.Truncal	0.95	0.77-1.16
Tumour4.Shared	0.83	0.7-0.98
Tumour4.Private	0.90	0.75-1.09