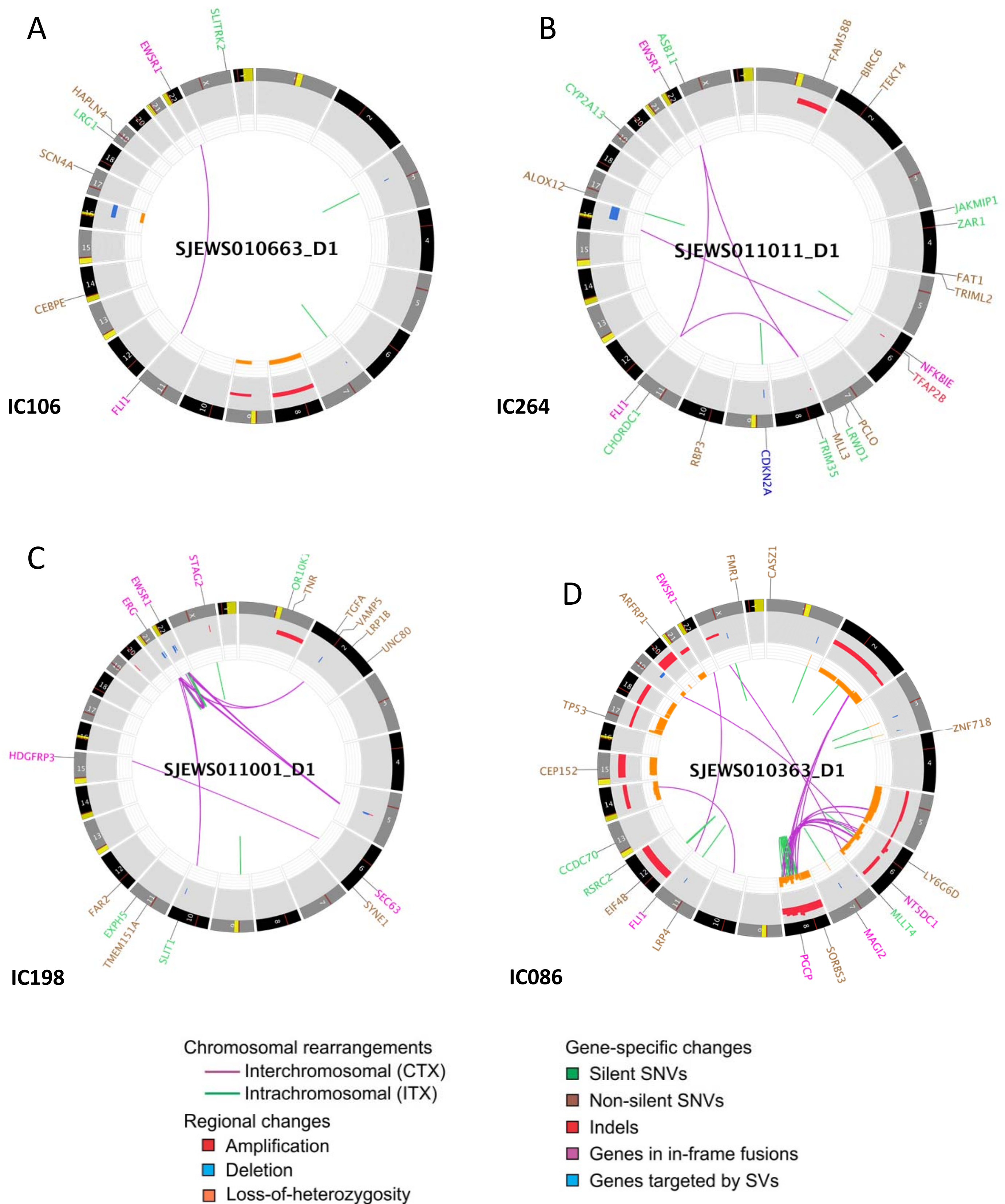
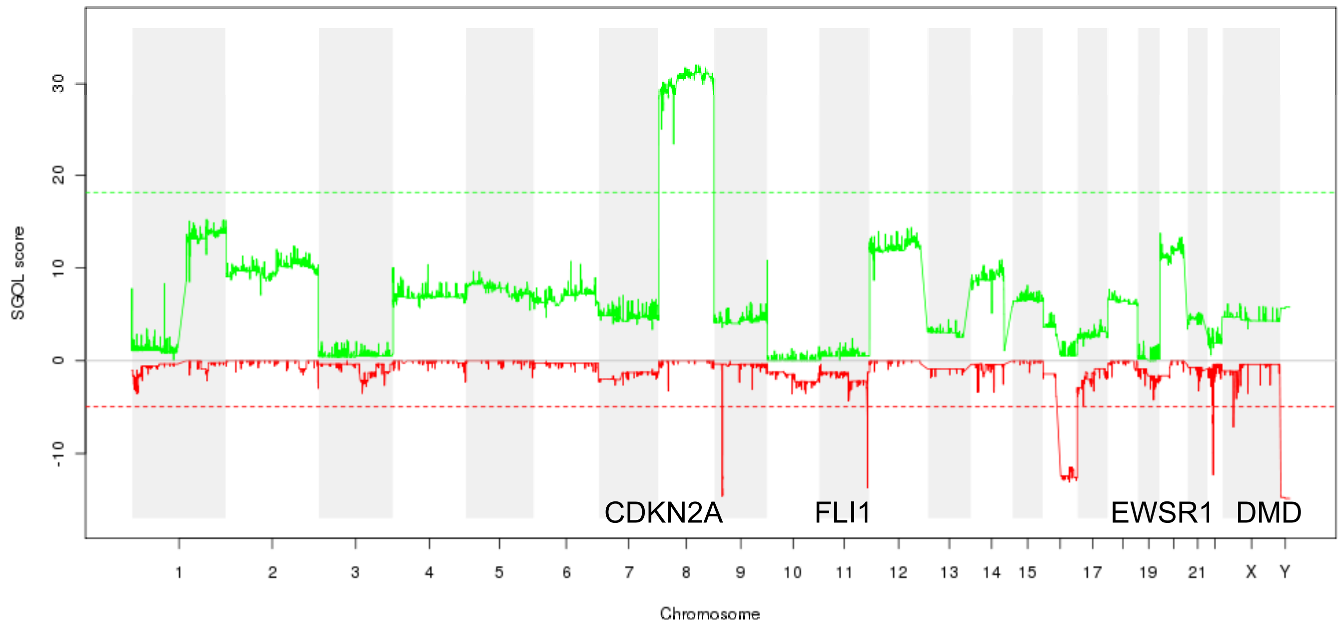


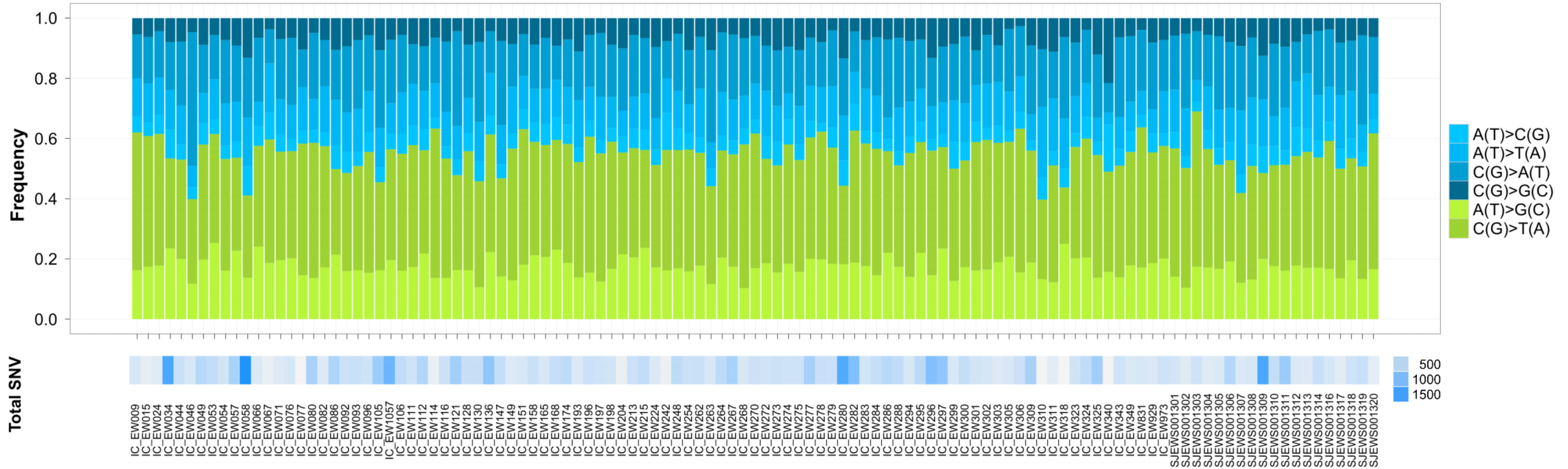
<u>Supplementary Figure 1</u>: CIRCOS Plots of representative Ewing cases

CIRCOS plots of genetic alterations in representative ES cases, depicting structural genetic variants, including DNA copy number alterations, intra- and inter-chromosomal translocations, and sequence alterations. A) A case with EWSR1-FLI1 fusion as the sole intrachromosomal rearrangement. B) A case with EWSR1-FLI1 fusion and interstitial deletion of CDKN2A. C) A case with EWSR1-ERG fusion caused by chromothripsis between chromosome 21 and chromosome 22. The case also harbors an exon duplication in STAG2.D) A case with EWSR1-FLI1 fusion and chromothripsis on chr 6 and 8.



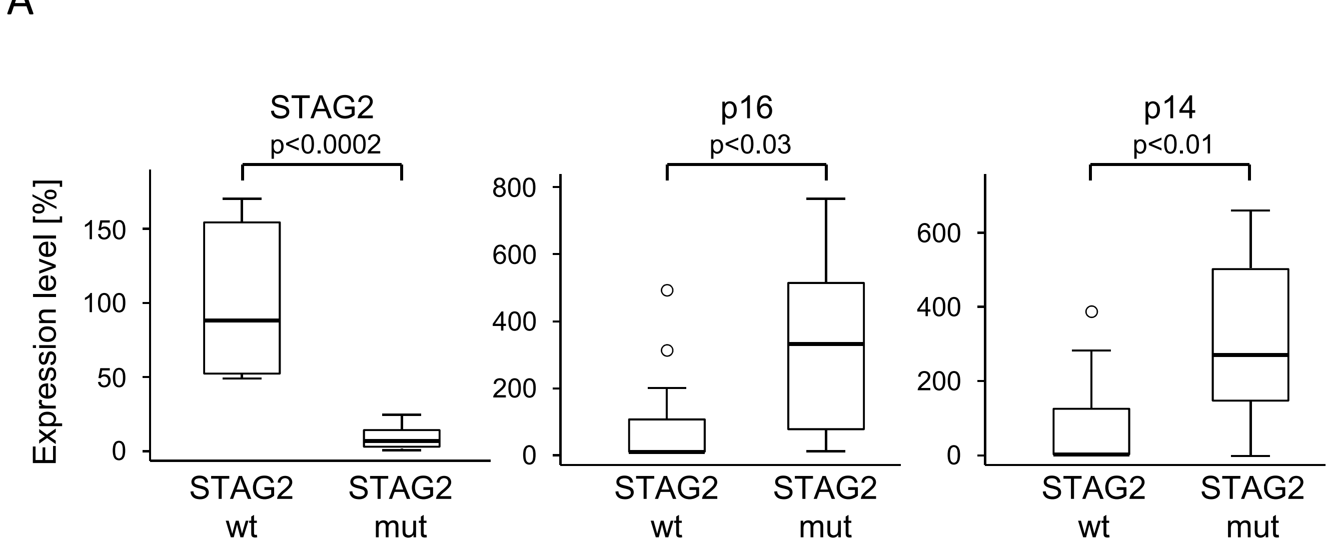
Supplementary Figure 2: Chromosomal regions with recurrent copy number loss or gain.

Chromosomal regions with recurrent copy number loss or gain. The x-axis shows the genomic location while the y-axis represents the score of segment gain or loss (SGOL score). Copy number gain is shown in green with positive score while copy number loss is shown in red with negative score. The dotted horizon green and red lines represent SGOL score threshold for regions with recurrent gain or loss, respectively. Gain of chromosome 8, loss of *CDKN2A*, *FLI1*, *EWSR1*, *DMD* and 16q passed the threshold. Loss of *FLI1* and *EWSR1* were caused by *EWSR1-FLI1* translocation.

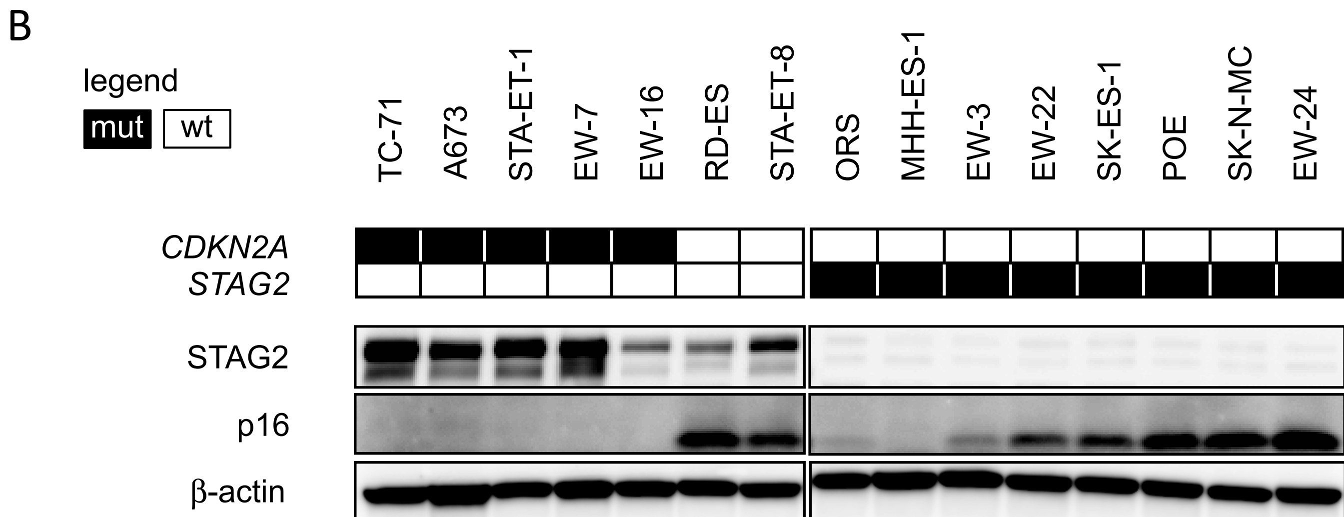


Supplementary Figure 3: Mutation spectrum of Ewing sarcomas
which plots the frequency of the six substitution patterns observed in the somatic SNVs detected in each case. Transitions C(G)>T(A) and A(T)>G(C) are shown in light and dark green, respectively while transversions are shown in various shades of blue. The total numbers of SNVs detected in each tumor genome are shown as a heatmap at the bottom.

A



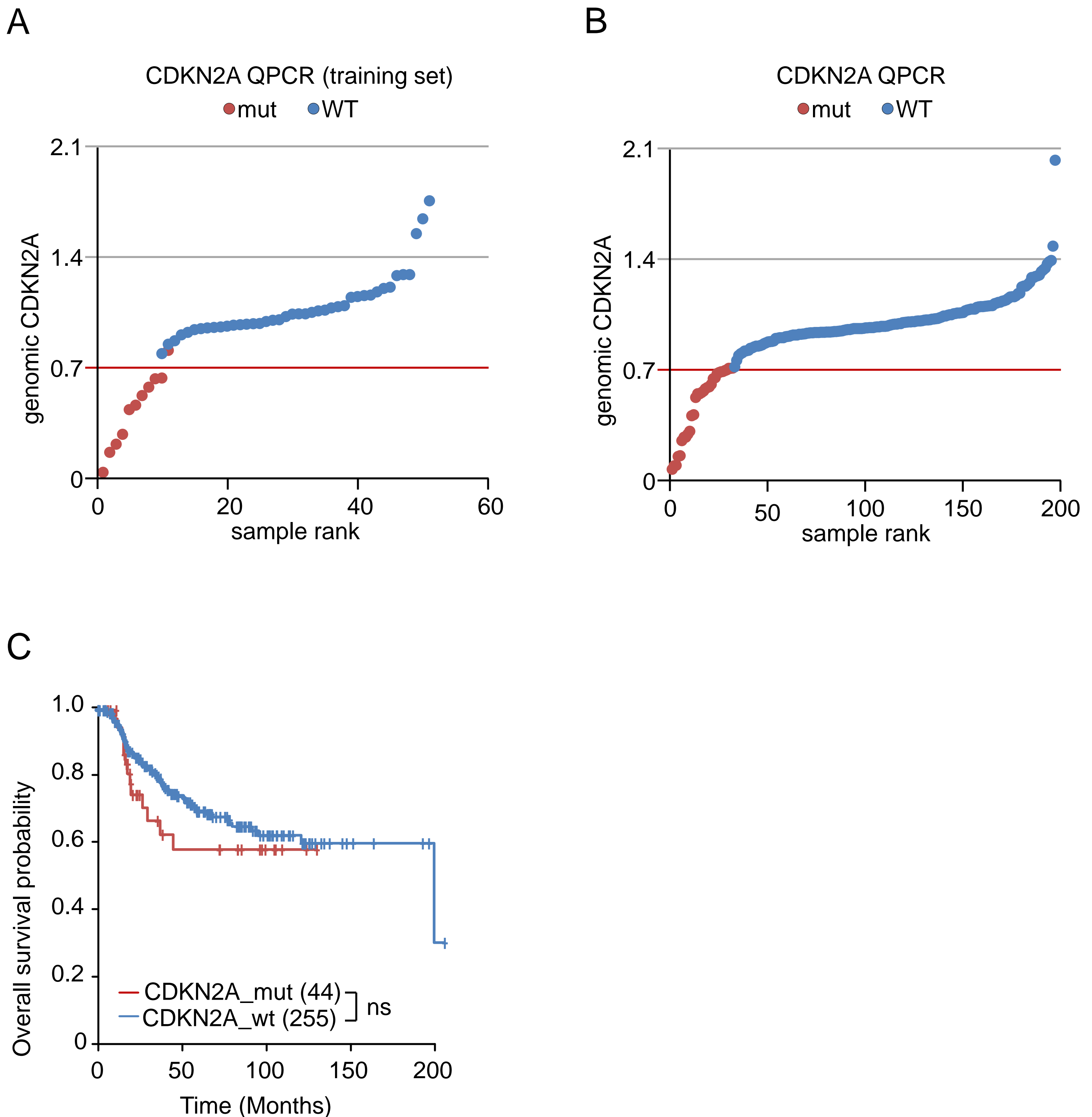
B



Supplementary Figure 4: RT-QPCR and western blot for STAG2 and CDKN2A in Ewing cell lines.

A. RT-QPCR for *STAG2*, p16 and p14 transcripts were assessed in 22 Ewing cell lines using Taqman gene expression assays (Life technologies) as described in (Louis-Brennetot et al. Genes Chromosomes Cancer. 2011;50(11):896-907) except for STAG2 where primer (F: AACAGAATGGAGTGGAAAACATG R: AAGTGCTATATCTCGGTCATGC) were designed to perform a sybrgreen assay. The relative expression level (%) for each gene are displayed on the y axis, *RPLP0* was used as house keeping gene. Group comparisons were done using Welch Two Sample t-test.

B. Expression of STAG2 (clone J-12, sc-81852, Santa Cruz biotechnology) and p16 (clone C-20, sc-468, Santa Cruz Biotechnology) were determined by western blot in 15 Ewing cell lines. Beta-actin was used as loading control (clone AC-74, A- 5316, Sigma). The genomic status of *STAG2* and *CDKN2A* in these cell lines is indicated in the table above the western blot.



Supplementary Figure 5 : *CDKN2A* genomic level in Ewing tumor samples

A: the genomic level of *CDKN2A* (average value of Exon1A and Exon2) was determined by QPCR and normalized to the genomic level of *TGFBR*2 (located on chromosome 3, the most stable chromosome across the WGS cohort) in Ewing tumor samples. 11 Ewing tumor samples with known *CDKN2A* deletion (mut) and 42 Ewing tumor samples with wild type (WT) *CDKN2A* status determined by WGS were used as training set. The thresold was set to 0.7.
B: Normalized genomic level of *CDKN2A* was determined in the remaining 199 Ewing tumor samples. C.) Overall survival among 299 patients according to *CDKN2A* status. The number of patients in the different groups is indicated in brackets.