Supplementary Figure S1 | Genetic Lesions associated with “Fusion Negative” Alveolar cases. Ten cases were found to be fusion negative alveolar by RT-PCR and transcriptome sequencing. a, clinical characteristics of the 10 cases. b, Genetic lesions, Purple, translocation, Black, missense mutations, Orange, indel mutation, red, copy gain; green, loss of heterozygozity. Median centered Z-scores of the FPKM from classifier genes of c, embryonal versus d, alveolar tumors (25).

Supplementary Figure S2 | Loss of heterozygosity across RMS tumor samples. 2.5 million segmented and normalized snp arrays visualized across the genome (chromosome position indicated on the x-axis) analyzed for loss of heterozygozity (purple) frequency across 134 rhabdomyosarcoma tumors.

Supplementary Figure S3 | Germline alteration of TP53 in patient RMS212. a, DNA sequencing reveals a heterozygous germline mutation of TP53 at the known COSMIC site chr17: 7577538. In the tumor DNA and RNA exclusive expression is seen of the variant allele. b, protein map produced by Protein Painter tool (Pediatric Cancer Genome Project) resulting in an arginine to glutamine change at codon 248. c, Circos plot of the tumor reveals loss of heterozygosity of Chromosome 17.

Supplementary Figure S4 | BCOR alterations. Locations of somatic mutations on linear protein domain model of BCOR produced using Protein Painter tool (Pediatric Cancer Genome Project). In total 10 tumors were found to have alterations of the BCOR gene including both frameshift insertions and deletions. One tumor (RMS 217) was found to have a stop gain in the ankyrin repeat region of the gene. Two tumors (RMS 2015 and RMS 2035) had areas of focal deletion on the X chromosome that included the BCOR gene.

Supplementary Figure S5 | Expressed mutations summary. 58% of all somatic alterations were found to have evidence of expression. a, A small percentage of changes were found to have a mutant allele bias (>80% variant) or reference allele bias (<20%). b, A range of correlation was found between the DNA and RNA variant allele frequency. Each color represents variants found in an individual tumor.
Supplementary Figure S6 | Recurrent Recurrence peaks of focal copy number change. Frequency analysis of copy number changes in rhabdomyosarcoma with amplification (red) and homozygous deletion (blue) across the genome (chromosome position indicated on the x-axis).

Supplementary Figure S7 | Loss of heterozygosity on Chromosome 11p15.5. Green-fusion negative tumors; red-fusion positive tumors

Supplementary Figure S8 | Recurrent amplicons. Recurrence peaks of focal amplification shown as heat maps plotting the row probe level Log Relative Ratios and identified the minimum common regions using the significant peaks in Nexus. An asterisk in front of the sample name denotes a PAX gene fusion is present in the tumor. a, amplicon of 15q26 including IGF1R. b, amplicon of chromosome 12q13.13. c, amplicon of chromosome 12q15. d, amplicon of chromosome 2p24.3. e, amplicon of PAX7 gene f, amplicon of chromosome 13q31.3-13q32.1.

Supplementary Figure S9 | Genome wide copy number profile. Amplifications (red) and deletions (blue) determined by segmentation analysis of normalized signal intensities from 2.5 million SNP arrays (See Methods) are displayed across the genome (chromosome position indicated on the y axis) for 134 rhabdomyosarcoma. Chromosome level gain of chromosome 8 is notable in the fusion-negative population.

Supplementary Figure S10 | Determination of a somatic score cutoff for WGS based on the verification by WES. Somatic single nucleotide variants (SNVs) reported from WGS and WES on 30 RMS patients are compared in the genomic region covered by WES. a, most reported WGS somatic SNVs are of low somatic score; b, WGS somatic SNVs verified by WES are of high somatic score.

Supplementary Figure S11 | Reverse Transcription PCR of PAX3-FOXO1 in cell line 7250_PF.

Supplementary Table S1 | Whole genome sequencing summary by sample

Supplementary Table S2 | Clinical characteristics for 147 RMS cases

Supplementary Table S3 | High confidence structural variations

Supplementary Table S4 | Genes affected by recurrent structural variations
Supplementary Table S5 | Small variants with somatic score ≥ 0.

Supplementary Table S6 | Expressed mutations

Supplementary Table 7 | Gene Ontology results for expressed mutations

Supplementary Table 8 | Altered genes in 44 WGS samples with Drug Annotation

Supplementary Table S9 | Altered genes in the 7250_PF cell line

Supplementary Table S10 | Common Genes in the Leading Edge Set of GSEA Analysis