Supplemental Fig. A:

In vivo occurrence of t(10;22) and 22q11 breakpoints as demonstrated by double-color interphase FISH. *a*: Schematic representation of the results obtained from double-color FISH experiments using *SMARCB1/INI1* flanking probes (RP11-61P17 and RP11-80O7) on touch preparations of frozen tissue-derived nuclei (case 1) or paraffin embedded tissue (case 2). In case 1, the nuclei displaying splitting of the two signals indicate the presence of a demonstrable subclone of tumor cells with a 22q11 breakpoint located between the two probes, as observed in the short-term cell colture. Additional nuclei that retain only 1 signal for one or both probes suggest the presence of subclones of tumor cells that lost a single or both derivative chromosomes. As control, touch preparation of frozen tissue-derived nuclei of case 8, that does not harbor any alteration of SMARCB1/INI1, were used. In case 2, the vast majority of the tumor cells harbor homozygous deletion of both clones and only a small fraction of the nuclei displayed splitting of the two signals (0.7%, ie 2/300 nuclei). *b*: Representative result of double-color FISH from case 1 using as probes two pools of BAC clones spanning chromosomal bands 10q26 (red signal) and 22q11 (green signal) and displaying evidence of fusion signals that are not detected in normal interphase nuclei. *c*: Representative result of double-color FISH from case 1 using RP11-61P17 (green signal) and RP11-80O7 (red signal) BAC probes flanking *SMARCB1/INI1* locus. Splitted signal is evident in case 1, demonstrating that the 22q11 breakpoint lies between the two BAC clones, as observed in the short-term cell colture (Fig. 1C of the manuscript). No splitted signals are present in control case 8. *d*: Representative result of double-color FISH from paraffin-embedded tissue of case 2 using BAC probes RP11-61P17 (green signal) and RP11-80O7 (red signal) flanking *SMARCB1/INI1* locus and demonstrating the splitted signals in case 2 but not in normal control tissue adjacent to the tumor.

Supplemental Fig. B:

FISH analysis of 22q11 homozygous deletion (HD) in cases 1, 2 and 5. In order to better define the extent of HD, we performed double-color interphase FISH experiments using BAC probe RP11-71G19 (containing *SMARCB1/INI1*) in combination with each of the flanking clones (RP11-61P17 and RP11-80O7) on touch preparations of frozen tissue-derived nuclei (case 1) or paraffin-embedded tissue (cases 2 and 5). *a*: Tabulated results of FISH experiments. Notably, in case 1 the region of HD is confined to clone RP11-71G19 and therefore spans less then 150 kb. As a control, the results obtained from case 8, which does not carry any alteration of *SMARCB1/INI1*, are reported. In case 2, results demonstrated homozygous deletion of all the three BAC clones. The telomeric boundary of the HD region in case 2 was delimited by array-CGH and FISH with clone RP11-91K24, that retained normal copy number (see Fig. 2*B* and 2*D*). The centromeric boundary of the HD region in case 2 was delimited by array-CGH to clone RP11-22M5, that displayed a normal dosage (see Fig. 2*B*).Therefore, the extent of the region of HD in case 2 is between 450 Kb and 5 Mb. Representative FISH results obtained from case 1 (*b*) and case 5 (*c*) are shown.