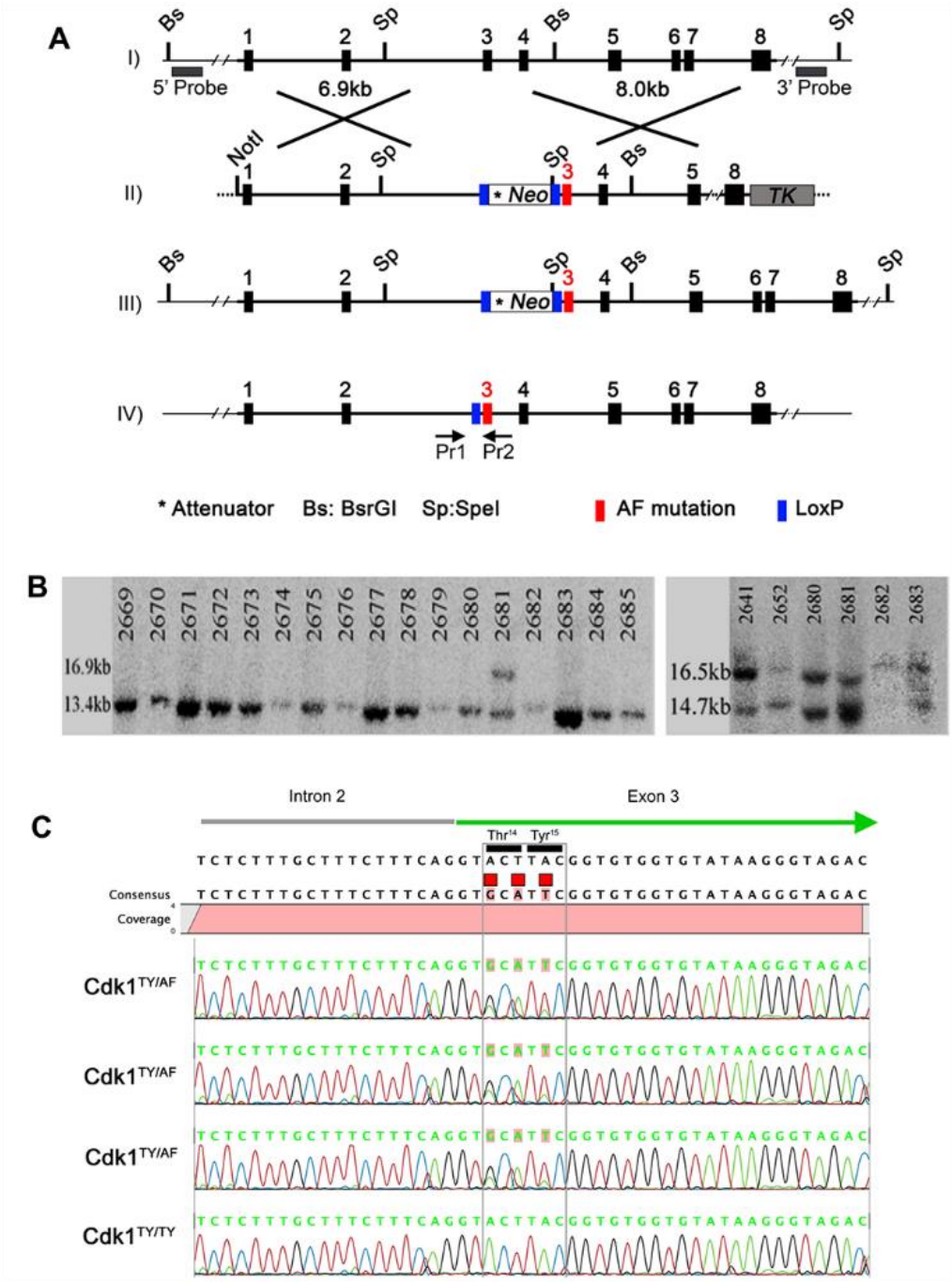

Figure S1. Generation of *Cdk1*$^{+/SAF}$ knockin mice. (A) The mouse *Cdk1* genomic locus (I) was modified in ES cells by homologous recombination with the targeting vector (II). A splice acceptor-STOP/neomycin-selection cassette, flanked by *loxP* recombination sites (blue rectangles), was placed on the 5' side of exon 3 (red rectangle) bearing the *Cdk1*^{AF} knockin mutation, and this generated the *Cdk1*$^{\underline{Stop}\underline{AF}}$ (*Cdk1*^{SAF}) locus (III). Upon expression of Cre recombinase, the splice acceptor-STOP/neomycin cassette was removed, and only a single *loxP* site next to the mutated exon 3 remained in the locus (IV). After initial verification of the presence of the *Cdk1*^{SAF} knock-in allele by sequencing (see below), the presence of the *Cdk1*^{SAF} allele was routinely tested by PCR using neomycin and the Cdk1 locus-specific primers (PKO2226 and PKO2237). Recombination-induced conversion to the *Cdk1*^{AF} allele was verified using genotyping

primers indicated as Pr1 and Pr2 (PKO2226 and PKO174) (Supplementary information, Table S1). (B) Genomic DNA isolated from double-selected ES cell colonies was digested with BsrGI and analyzed by Southern blot using a 5' probe (PKO380/PKO381). Homologous recombination at the 5' site yields a 16.9 kb recombinant fragment vs. a 13.4 kb wild-type fragment (left panel). Genomic DNA was analyzed as above using a 3' probe (PKO382/PKO383). Homologous recombination at the 3' site yields a 14.7 kb recombinant fragment vs. a 16.5 kb wild-type fragment (right panel). One of the ES cell clones (#2681) that had undergone homologous recombination at both the 5' and 3' sites of exon 3 was selected for the generation of chimera. (C) Due to the nature of the homologous recombination strategy we had to use for modifying exon 3, screening strategies by Southern blot or PCR do not confirm the presence of the AF mutation. For this reason, a DNA fragment encompassing these mutations was amplified by PCR (PKO2212 and PKO174) and sequenced using an internal sequencing primer (PKO2222). Sequencing trace chromatograms from three different *Cdk1*$^{StopAF/WT}$ mice (TY/AF) displayed double peaks in the modified locations. Below, a sequencing trace chromatogram from a wild-type mouse (TY/TY) is shown. T, threonine; Y, tyrosine; A, alanine; and F, phenylalanine.