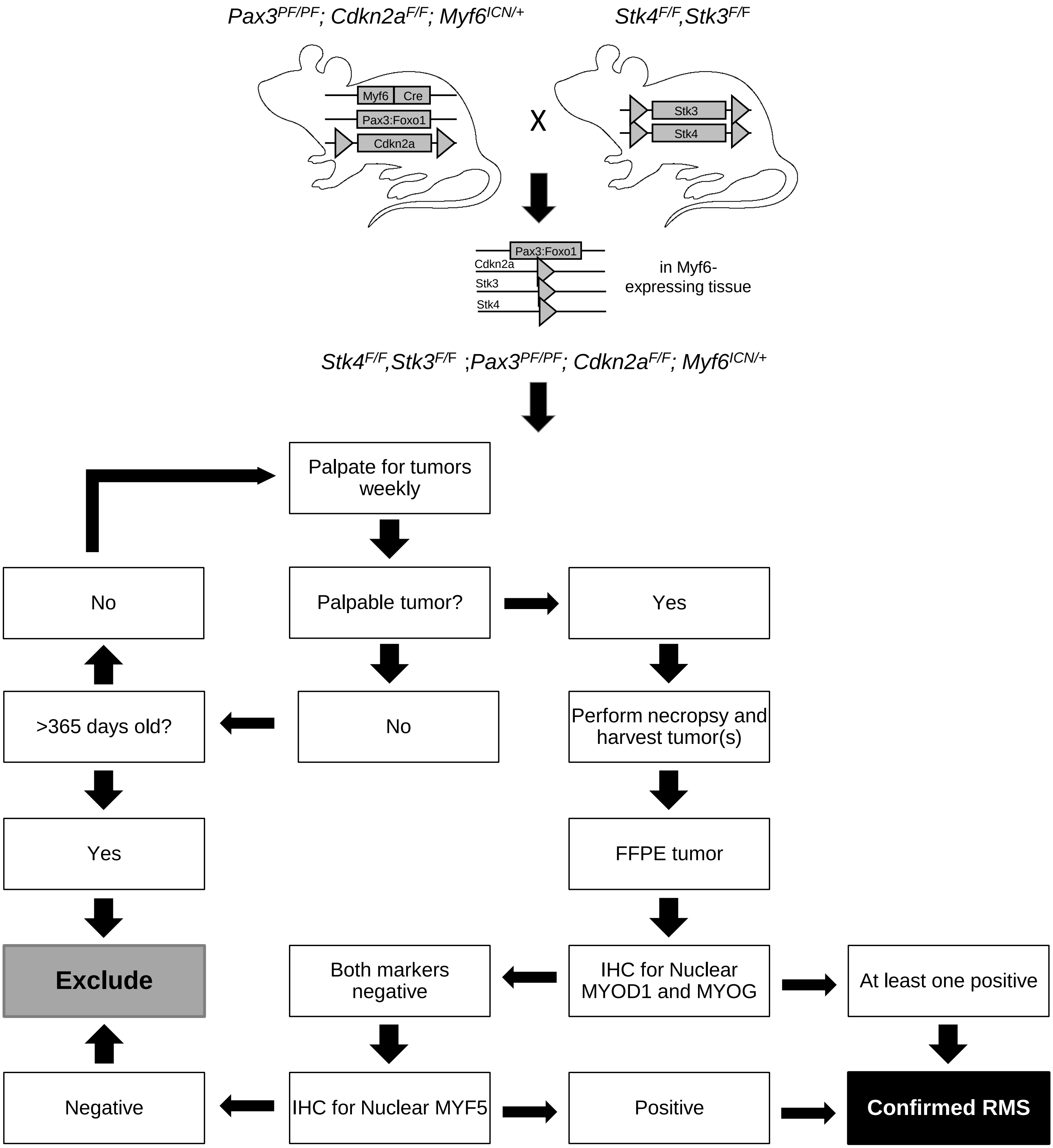

Supplementary Figure 1. Use of the Cre-Lox system to create and diagnose a genetically engineered mouse model (GEMM) of Pax3:Foxo1-positive aRMS with conditional deletion of the MST1 (*Stk4*) and MST2 (*Stk3*) alleles. The *Pax3*$^{PF/PF}$; *Cdkn2a*$^{F/F}$; *Myf6*$^{ICN/+}$ aRMS GEMM was re-derived from founder mice according to the original description of this model (Keller et al, 2004). The re-derived GEMM was then crossed with mice harboring floxed *Stk4*$^{F/F}$,*Stk3*$^{F/F}$ alleles. Expression of *Cre* results in expression of *Pax3:Foxo1* but loss of *Cdkn2a*, *Stk3*, and *Stk4* in cells and tissues expressing *Myf6*. Mice were obtained from sources described in Materials and Methods. Triangles indicate LoxP sites. A series of parameters were established for inclusion and analysis of tumors arising from the MST^{WT} and MST^{Null} aRMS model cohorts for further analysis based on palpability on physical examination, age of animal, cellular morphology, and histologic markers.