Supplemental data

Materials and Methods

Specimens

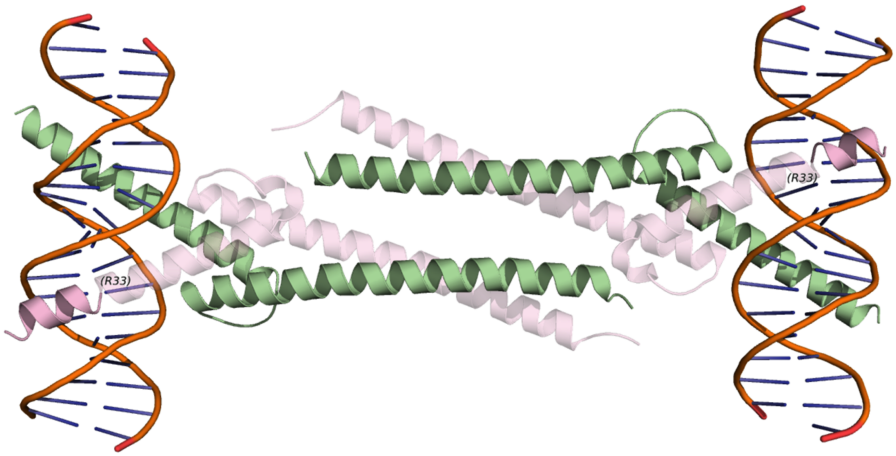
Ten tissues specimens of both adrenal glands of the patient were investigated. Three specimens were fresh frozen (FF) tissue from normal left and right adrenal gland and left nodular AMH. Seven specimens were formalin-fixed and paraffin-embedded (FFPE) tissues histologically determined as normal tissue, adrenal medullary hyperplasia or adrenal nodules.
From each specimen, lesional and normal DNA was isolated by manual or lazer microdissection of the tissue and extracted with a standard DNA extraction procedure, from the QIAamp DNA Mini kit (Qiagen, Courtaboeuf, France) following the manufacturer's instruction.

Pyrosequencing assay design

The amplification and sequencing primers for pyrosequencing were designed using Primer3 software. A biotin modification was added on the 5' extremity of the reverse PCR primer. A unique sequencing primer was created juxtaposed against the mutation point.

Pyrosequencing

The PCR reaction was performed in a final volume of 25 µL using the HotStarTaq Master Mix kit (Qiagen) following manufacturer's instruction. BSA was added in the reaction to counterbalance the effects of PCR inhibitors presents in tumor tissues. The PCR includes 50 PCR cycles (95°C for 15 minutes followed by 50 cycles at 95°C for 30 sec, 50°C for 30 sec and 72° for 30 sec, and a final extension at 72°C for 10 minutes).
The pyrosequencing assays were performed on the PyroMark MD96 with the PyroMark Gold Q96 Reagents. The DNA samples are analyzed in quadruplicate. Data were analyzed using PyroMark™ MD software.



Supplemental Figure 1: **Schematic cartoon representation of the 3D structure of Myc-Max complex bound to their DNA target.** The crystal structure of the ternary complex has been previously solved at 1.9A resolution (PDB code 1NKP). Myc and Max proteins are shown in pale green and light pink, respectively. The Myc-Max heterodimer dimerizes to form a bivalent heterotetramer (formed by 2 Myc and 2 Max proteins) that is essential for up-regulation of Myc-dependent genes. Mutation of Max residue R33 to a stop codon induces the loss (due to termination of translation) of all Max residues from R33 up to the C-terminus (shown as transparent) leading to the complete disruption of the interaction with Myc. The bivalent heterotetramer essential to Myc function is therefore no longer present.