Supporting Information

Oncogenic exon 2 mutations in Mediator subunit MED12 disrupt allosteric activation of Cyclin C-CDK8/19

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Running Title**:** *Oncogenic MED12 mutations disrupt CDK8/19 activity*

List of Materials:

Figure S1

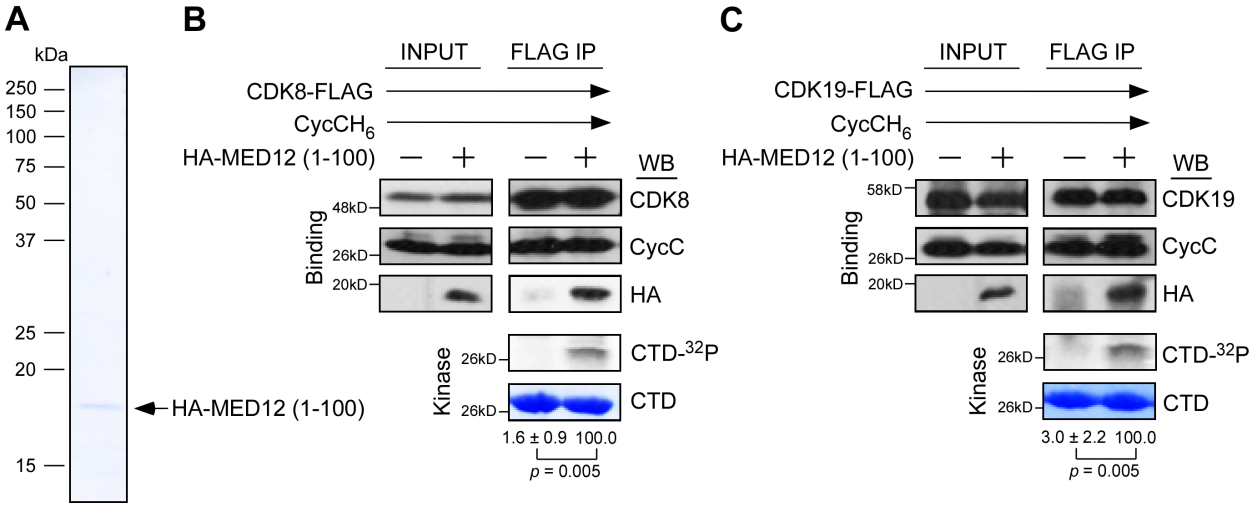

Figure S1. The MED12 N-terminus (amino acids 1-100) is sufficient to bind and activate CycC-CDK8/19. (**A**) Purified HA-MED12 (1-100) bearing tandem six-histidine and HA-epitope tags was expressed in *E. coli* and purified on Ni-NTA prior to resolution by SDS-15% PAGE and visualization by Coomassie blue staining. Molecular weight marker positions (kDa) are indicated. (**B** and **C**) Baculovirus-expressed CDK8-FLAG/CycCH$_6$ (**B**) or CDK19-FLAG/CycCH6 (**C**) were immunoprecipitated from infected insect cell lysates using FLAG epitope-specific antibodies in the absence (-) or presence (+) of HA-MED12 (1-100). FLAG-specific immunoprecipitates were processed by WB using the indicated antibodies (*top panels*) or incubated with [γ-^{32}P]-ATP and purified GST-CTD (*bottom panels*). INPUT corresponds to 5% of protein used in IP reactions. Horizontal arrows indicate the presence of specified Mediator subunits in parallel reactions. ^{32}P-GST CTD levels were quantified and expressed relative to the level in the presence of MED12 (1-100), which was assigned a value of 100%. Data represent the average +/- SEM of 3 independent experiments. *p* value calculated by Student's *t* test.

Figure S2

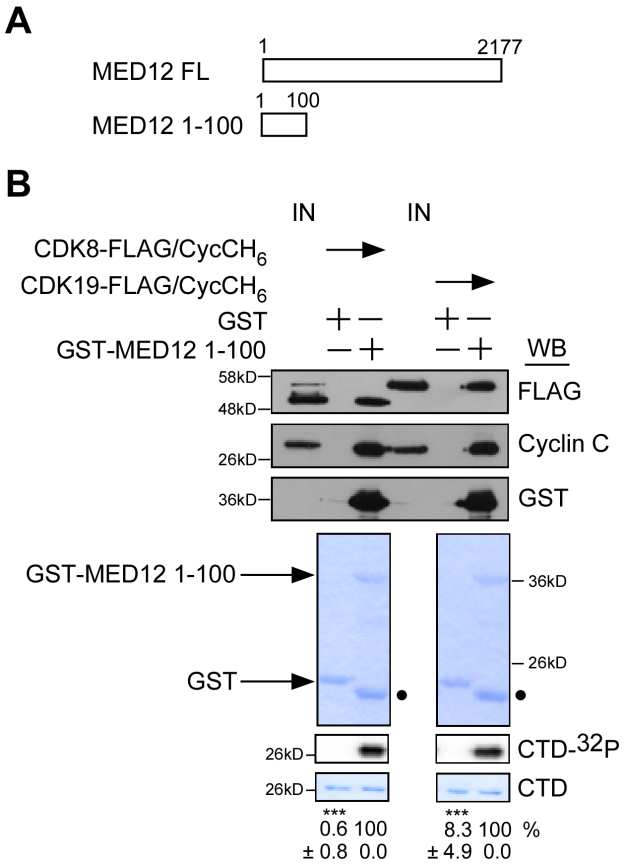

Figure S2. MED12 amino acids 1-100 bind and activate CycC-CDK8/19. (**A**) Schematic diagram of full-length (FL) MED12 and MED12 1-100 that was fused to GST to generate GST-MED12 1-100 for use in binding and kinase assays. (**B**) Glutathione-sepharose-immobilized GST or GST-MED12 1-100 as indicated were incubated with lysates from insect cells co-expressing baculovirus-produced $CycCH_6$/CDK8-FLAG or $CycCH_6$/CDK19-FLAG. Bound proteins were eluted with with Laemmli sample buffer and processed by WB using the indicated antibodies (top panels) or subjected to in vitro kinase assay (described in the legend to Fig. 1) prior to resolution by SDS-PAGE and phosphorimager analyses (bottom panels). Input (IN) corresponds to 10% of insect cell lysate used in binding reactions. Horizontal arrows indicate the presence of specified Mediator subunits in parallel reactions. ^{32}P-GST-CTD levels were quantified and expressed relative to the level in the CDK8 (or CDK19)/CycC/MED12 1-100 reaction. Bullets denote degradation products derived from GST-MED12 1-100. Data represent the average +/- SEM of 3 independent experiments. Asterisks denote statistically significant differences (Student's *t* test, *** $p < 0.001$).

Figure S3

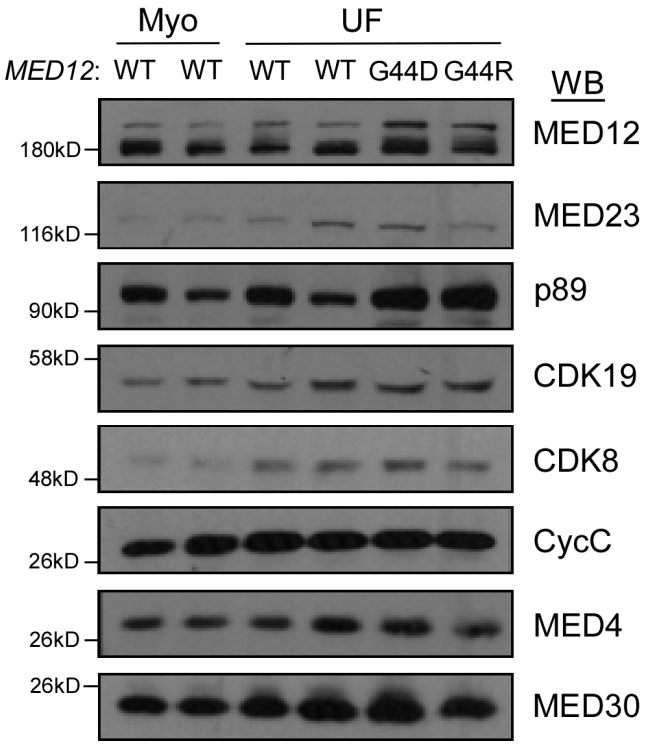

Figure S3. CDK19 is expressed in myometrium and uterine fibroid tissues. Whole tissue lysates from patient-matched myometrium (Myo) and four different uterine fibroid (UF) tumors (two MED12 WT, one MED12 mutant G44D, one MED12 mutant G44R) were resolved by SDS-10% PAGE and processed by WB analysis using the indicated Mediator subunit-specific antibodies. Note that CDK19, like CDK8 and other Mediator subunits is expressed in both myometrium and uterine fibroid tumors. The p89 subunit of the general transcription factor TFIIH served as a loading control. *MED12* mutation status in Myo and UF tumors is indicated.

Figure S4 (Related to Figure 4)

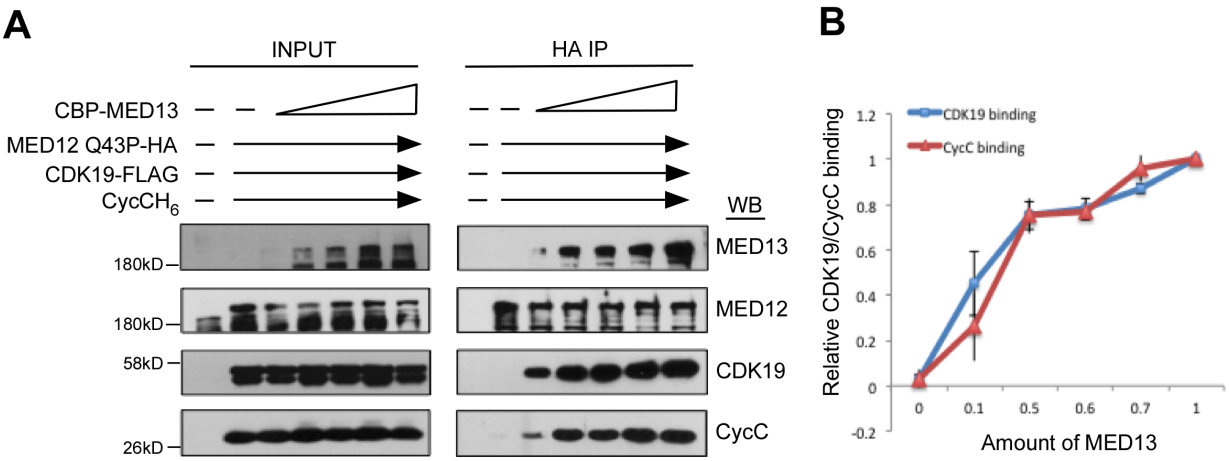

Figure S4. MED13 promotes the binding of mutant MED12 to CycC-CDK19. (A) Lysates from insect cells co-expressing baculovirus-produced $CycCH_6$, CDK19-FLAG, MED12-HA (mutant Q43P) and increasing amounts of CBP-MED13 as indicated were subjected to IP with HA epitope-specific antibodies. HA-specific IPs were processed by WB using the indicated antibodies. Input corresponds to 10% of cell lysate used in IP reactions. Horizontal arrows indicate the presence of specified Mediator subunits in parallel reactions. (**B**) WBs were quantified and levels of CycC and CDK19 in each IP were normalized to those of MED12 and expressed relative to their corresponding normalized levels in the CDK19/CycC/MED12/MED13 IP from lysates expressing the highest levels of CBP-MED13. Note that IP/kinase reactions in **Fig. 4** were performed using insect cell lysates that express CBP-MED13 at levels at least 2-fold above the highest level shown here, and thus saturating for CycC-CDK19 association with MED12.

Figure S5

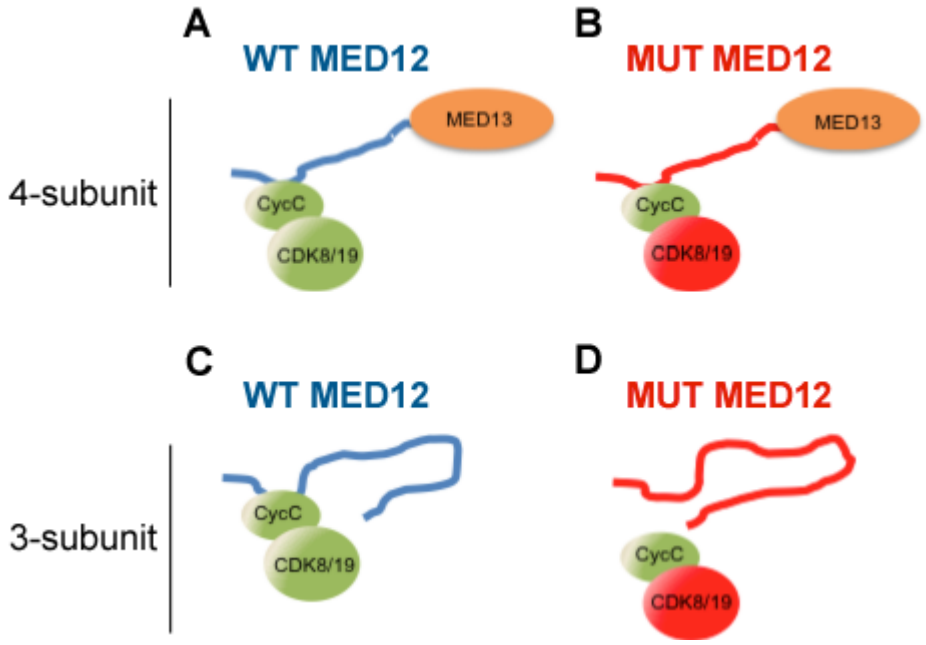

Figure S5. Schematic model for subunit interactions within WT and mutant MED12-containing Mediator kinase modules. Predicted protein interactions within 4-subunit (**A** and **B**) or 3-subunit (**C** and **D**) Mediator kinase modules reconstituted from recombinant proteins, including MED12 WT (**A**, **C**) or MED12 exon 2 mutant (**B, D**) derivatives. Blue and red lines represent WT and mutant MED12 proteins, respectively. (**A** and **C**) MED12 binds to CycC and activates CDK8/19 in the presence (**A)** or absence (**C**) of MED13. Active CDK8/19 indicated by green coloring. (**B** and **D**) MED13 bound to the MED12 C-terminus suppresses an exon 2 mutation-induced conformational change in MED12 (shown in **D**) that otherwise disrupts its association with CycC-CDK8/19. In the presence of MED13 (**B**), mutant MED12 can bind to CycC, but not activate CDK8/19. Inactive CDK8/19 indicated by red coloring.