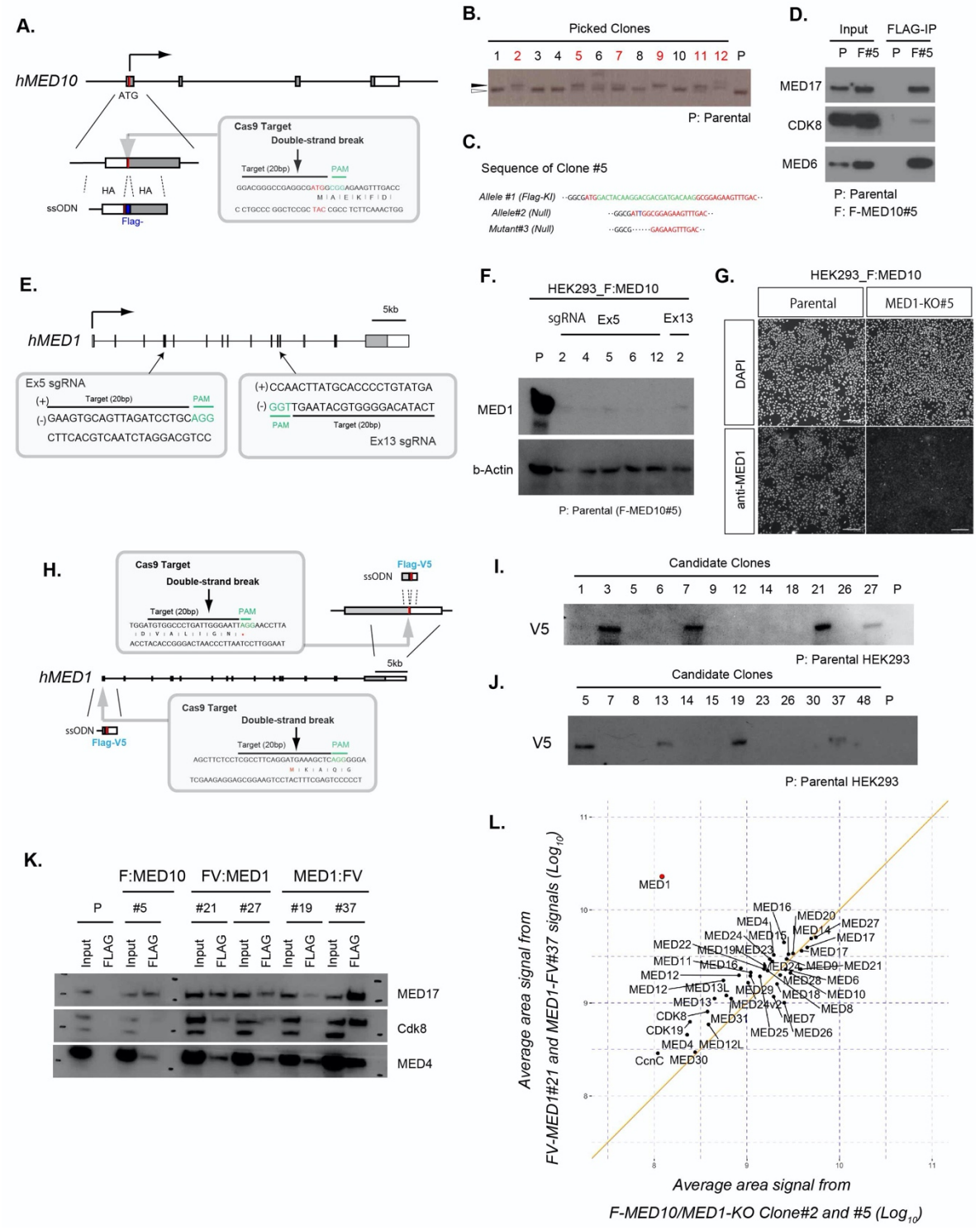
A.
hMED10
ATG
Cas9 Target
Double-strand break
Target (20bp)
PAM
HA
HA
ssODN
Flag-
B.
Picked Clones
1 2 3 4 5 6 7 8 9 10 11 12 P
P: Parental
C.
Sequence of Clone #5
Allele #1 (Flag-KI)
Allele#2 (Null)
Mutant#3 (Null)
D.
Input
FLAG-IP
P F#5 P F#5
MED17
CDK8
MED6
P: Parental
F: F-MED10#5
E.
hMED1
5kb
Ex5 sgRNA
Target (20bp)
PAM
CTTCACGTCAATCTAGGACGTCC
Ex13 sgRNA
F.
HEK293_F:MED10
sgRNA Ex5 Ex13
P 2 4 5 6 12 2
MED1
b-Actin
P: Parental (F-MED10#5)
G.
HEK293_F:MED10
Parental
MED1-KO#5
DAPI
anti-MED1
H.
Cas9 Target
Double-strand break
Flag-V5
ssODN
5kb
hMED1
I.
Candidate Clones
1 3 5 6 7 9 12 14 18 21 26 27 P
V5
P: Parental HEK293
J.
Candidate Clones
5 7 8 13 14 15 19 23 26 30 37 48 P
V5
P: Parental HEK293
K.
F:MED10
FV:MED1
MED1:FV
P
#5
#21
#27
#19
#37
Input
FLAG
MED17
Cdk8
MED4
L.
MED1
MED16
MED4
MED20
MED14
MED27
MED24
MED15
MED17
MED22
MED19
MED23
MED11
MED16
MED21
MED12
MED28
MED6
MED12
MED13L
MED29
MED18
MED10
MED13
MED24v2
MED8
CDK8
MED31
MED7
CDK19
MED25
MED26
MED4
MED12L
CcnC
MED30
Average area signal from FV-MED1#21 and MED1-FV#37 signals (Log10)
Average area signal from F-MED10/MED1-KO Clone#2 and #5 (Log10)

Figure S1: Assessment of Mediator subunits in the MED1-KO Mediator

A) Design of CRISPR/Cas9 mediated Flag-tag knock-in strategy at the *MED10* locus in HEK293 cells. **B**) Genomic PCR of a region flanking the first ATG of *MED10*. Bands with slower migration (marked with black arrowhead) correspond to FLAG-tag inserted alleles. **C**) Sequence of the amplified product of clone #5 shown in (B). Note that this clone carried three copies of MED10, and alleles that were untagged resulted in a non-functional allele. **D**) Immunoprecipitation followed by immunoblotting of Mediator subunits (MED17, CDK8, MED6). Parental HEK293 cells were used as a control. Flag-tagged MED10 efficiently precipitates other Mediator subunits. **E**) Design of MED1-knockout by CRISPR/Cas9. sgRNAs were designed to sequences present in either exon 5 or exon 13. **F**) Immunoblot of lysates showing the absence of MED1 in established *MED1* knockout clones. Parental cells (HEK293_F:MED10#5) were used as a control. **G**) Representative image of immunocytochemical analysis of MED1 in parental cells and in one of the derived MED1-KO clones. Note the absence of a nuclear MED1 signal in the knockout clone. White bars indicate 100 µm. **H**) Design of Flag-V5 tandem tag *MED1* knock-ins at the N- and C-termini of MED1. (**I and J**) Immunoblot of lysates showing the successful V5-tag knock-in either at the N-terminus (I) or at the C-terminus (J) of MED1. **K**) Immunoprecipitation followed by immunoblotting of Mediator subunits (MED17, CDK8, MED4). A parental HEK293 and F-MED10#5 clone was used as a control. FlagV5-tagged MED1 efficiently precipitates other Mediator subunits. **L**) Comparison of Mediator subunit abundance by mass spectrometry. Two independent Mediator preparations from MED1 knockout clones (KO#2 and KO#5) and two independent MED1-containing Mediator preparations (FV-MED1#21 and MED1-FV#37) were compared. The abundance of each subunit was calculated based on the three most abundantly detected peptides for each subunit. Signals between samples were normalized to the abundance of MED14 (a structural backbone subunit) and averaged between replicates, and all Mediator subunits were plotted. Despite the loss of MED1, other subunits were detected at comparable levels.