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Supplementary Materials for

MED12 methylation by CARM1 sensitizes human breast cancer cells to chemotherapy drugs

Lu Wang, Hao Zeng, Qiang Wang, Zibo Zhao, Thomas G. Boyer, Xiuwu Bian, Wei Xu

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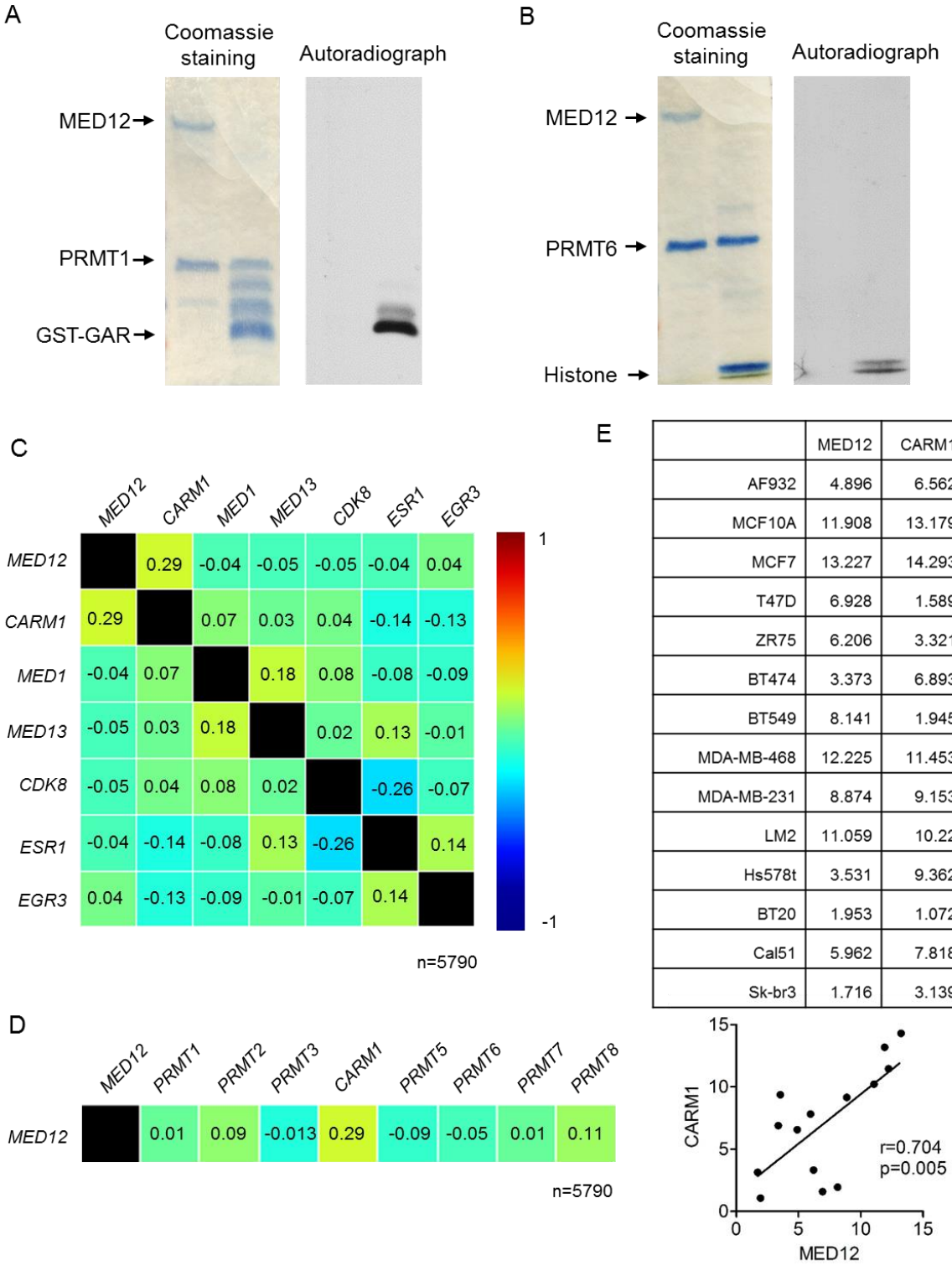
This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/1/9/e1500463/DC1)

Figure S1



	MED12	CARM1
AF932	4.896	6.562
MCF10A	11.908	13.179
MCF7	13.227	14.293
T47D	6.928	1.589
ZR75	6.206	3.321
BT474	3.373	6.893
BT549	8.141	1.945
MDA-MB-468	12.225	11.453
MDA-MB-231	8.874	9.153
LM2	11.059	10.22
Hs578t	3.531	9.362
BT20	1.953	1.072
Cal51	5.962	7.818
Sk-br3	1.716	3.139

Figure S1. In vitro methylation assays of MED12 and PRMT1/PRMT6 and correlation analyses of indicated genes in breast cancer specimens and cell lines. (A) In vitro methylation assay with MED12 and PRMT1. Purified GST-GAR was used as a positive substrate control for PRMT1. (B) In vitro methylation assay with MED12 and PRMT6. Purified histone from HEK293T CARM1^{KO} cells was used as a positive substrate control for PRMT6. (C)Correlation plot of the mRNA levels of *MED12*, *CARM1*, *MED1*, *MED13*, *CDK8*, *ESR1* and *EGR3* in 5790 human breast tumors generated using bc-GenExMiner v3.0 software. (D) Correlation plot of the mRNA levels of *MED12* and eight members of protein arginine methyltransferases in 5790 human breast tumors generated using bc-GenExMiner v3.0 software. (E) Quantitation of Western blots in Fig. 1D and Pearson correlation analysis of CARM1 and MED12.

Figure S2

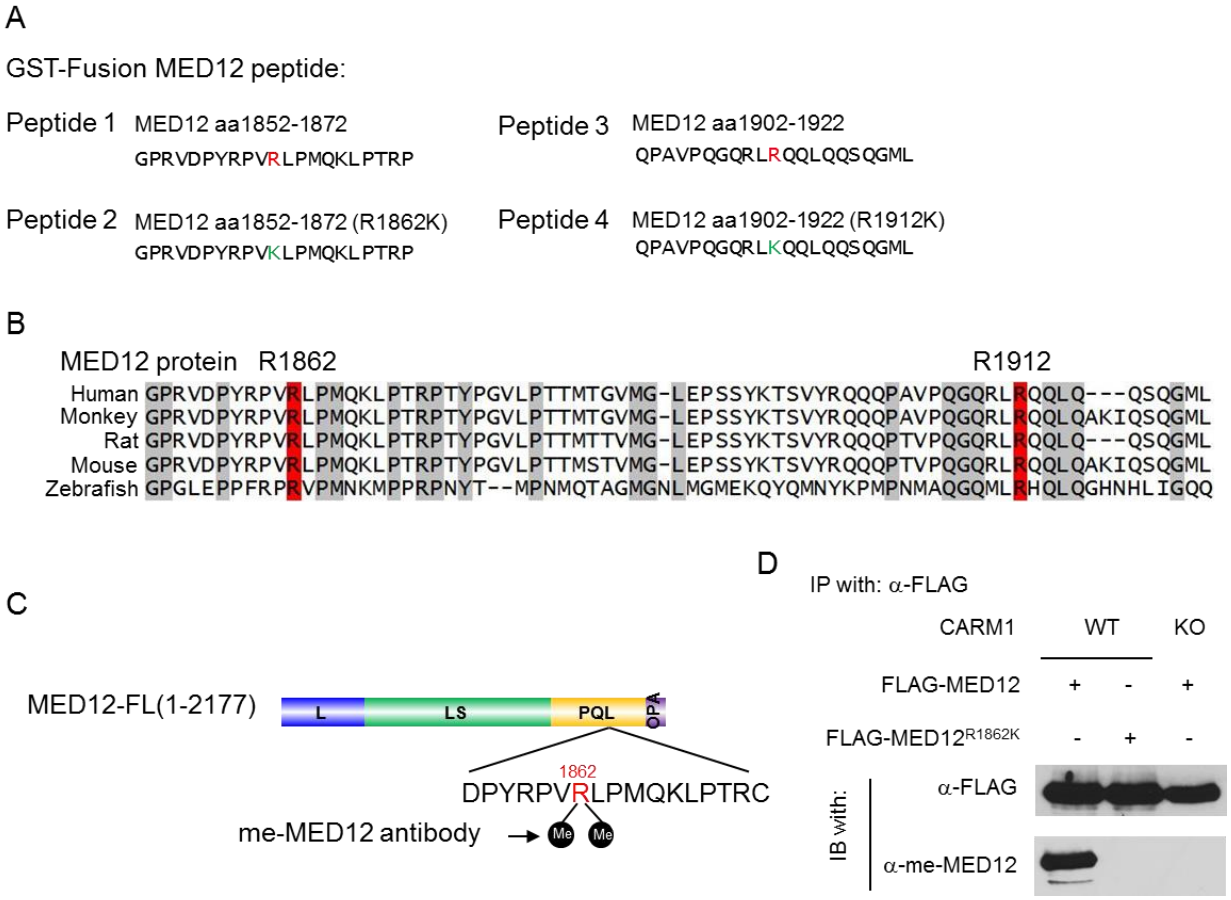


Figure S2. Western blotting analysis of endogenous dimethylated MED12^{R1862} using a methyl-specific MED12 rabbit polyclonal antibody.

(A) Wild type or mutant MED12 peptide sequences used for generation of GST-peptide fusions in a bacterially expressed vector. (B) CLUSTALW alignment of vertebral MED12 sequences flanking the methylation sites R1862 and R1912 (in red) on human MED12. (C) A polyclonal peptide antibody was generated using the annotated peptide encompassing asymmetrically dimethylated-R1862 as antigen. (D) FLAG-tagged MED12^{WT} or MED12^{R1862K} proteins were expressed and immunoprecipitated from

HEK293 CARM1^{WT} or CARM1^{KO} cells. Western blotting was performed utilizing the rabbit polyclonal antibody in (C).

Figure S3

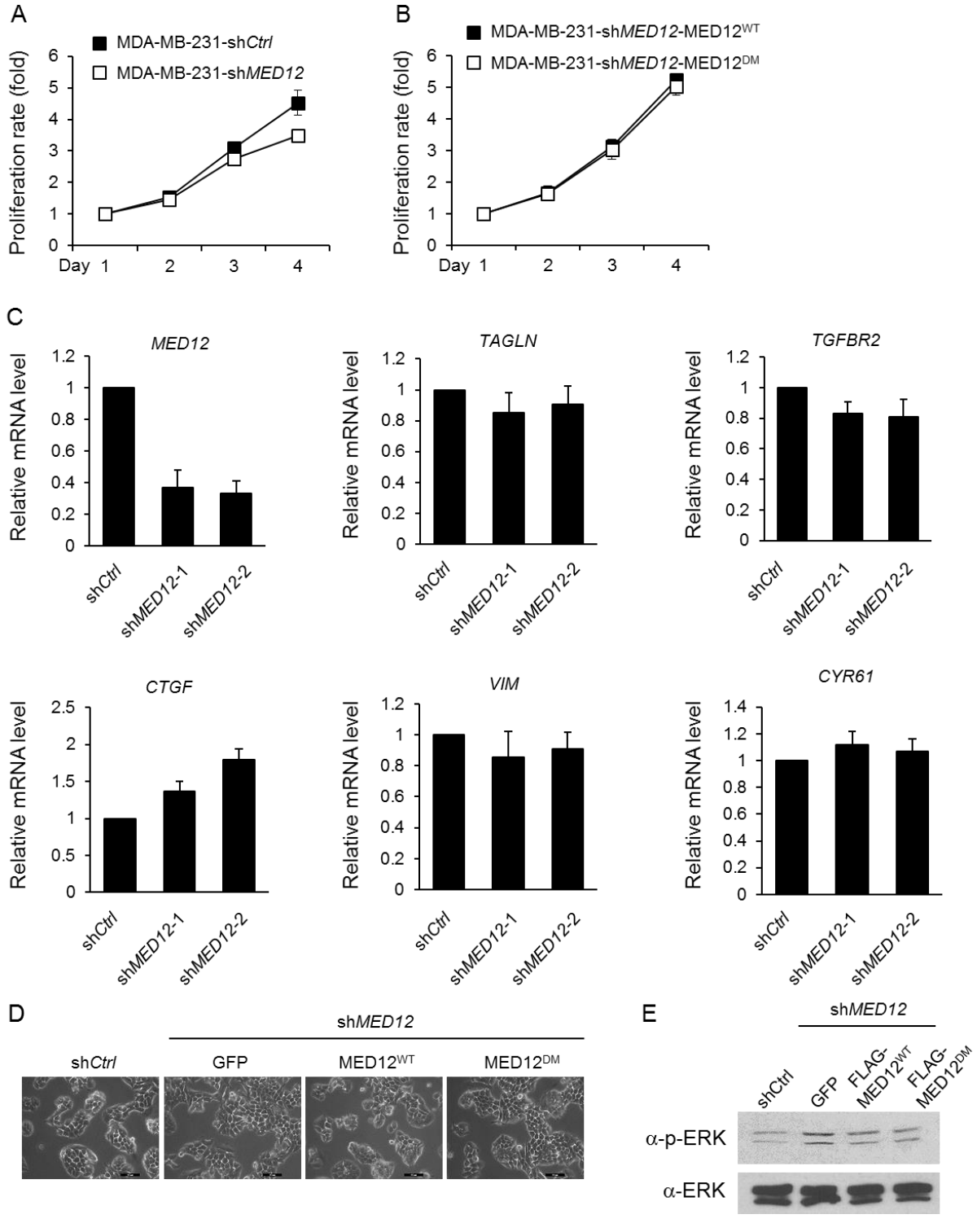

A
MDA-MB-231-sh*Ctrl*
MDA-MB-231-sh*MED12*
Proliferation rate (fold)
Day 1 2 3 4
B
MDA-MB-231-sh*MED12*-MED12^{WT}
MDA-MB-231-sh*MED12*-MED12^{DM}
Proliferation rate (fold)
Day 1 2 3 4
C
MED12
TAGLN
TGFBR2
CTGF
VIM
CYR61
Relative mRNA level
shCtrl
shMED12-1
shMED12-2
D
shMED12
shCtrl
GFP
MED12^{WT}
MED12^{DM}
E
shMED12
shCtrl
GFP
FLAG-MED12^{WT}
FLAG-MED12^{DM}
α-p-ERK
α-ERK

Figure S3. Mutation of MED12 methylation sites does not affect cell growth or EMT-associated gene expression.

(A) Normalized cell growth rate measured by MTT assay in control shRNA (sh*Ctrl*) or sh*MED12* expressing MDA-MB-231 cells. (B) Normalized cell growth rate measured by MTT assay in sh*MED12* expressing MDA-MB-231 cells restored with $MED12^{WT}$ or $MED12^{DM}$. (C) Real-time qPCR analyses of *MED12* and five TGF-β target genes and EMT marker genes in control shRNA (sh*Ctrl*) or sh*MED12* expressing MCF7 cells. (D) Representative images of cell morphology (scale bars=50 μm) of MCF7-sh*Ctrl*-GFP, MCF7-sh*MED12*-GFP, MCF7-sh*MED12*-$MED12^{WT}$, MCF7-sh*MED12*-$MED12^{DM}$ cells. (E) Western blotting analysis of total ERK and phosphorylated ERK in MCF7-sh*Ctrl*-GFP, MCF7-sh*MED12*-GFP, MCF7-sh*MED12*-$MED12^{WT}$, MCF7-sh*MED12*-$MED12^{DM}$ cells.

Figure S4

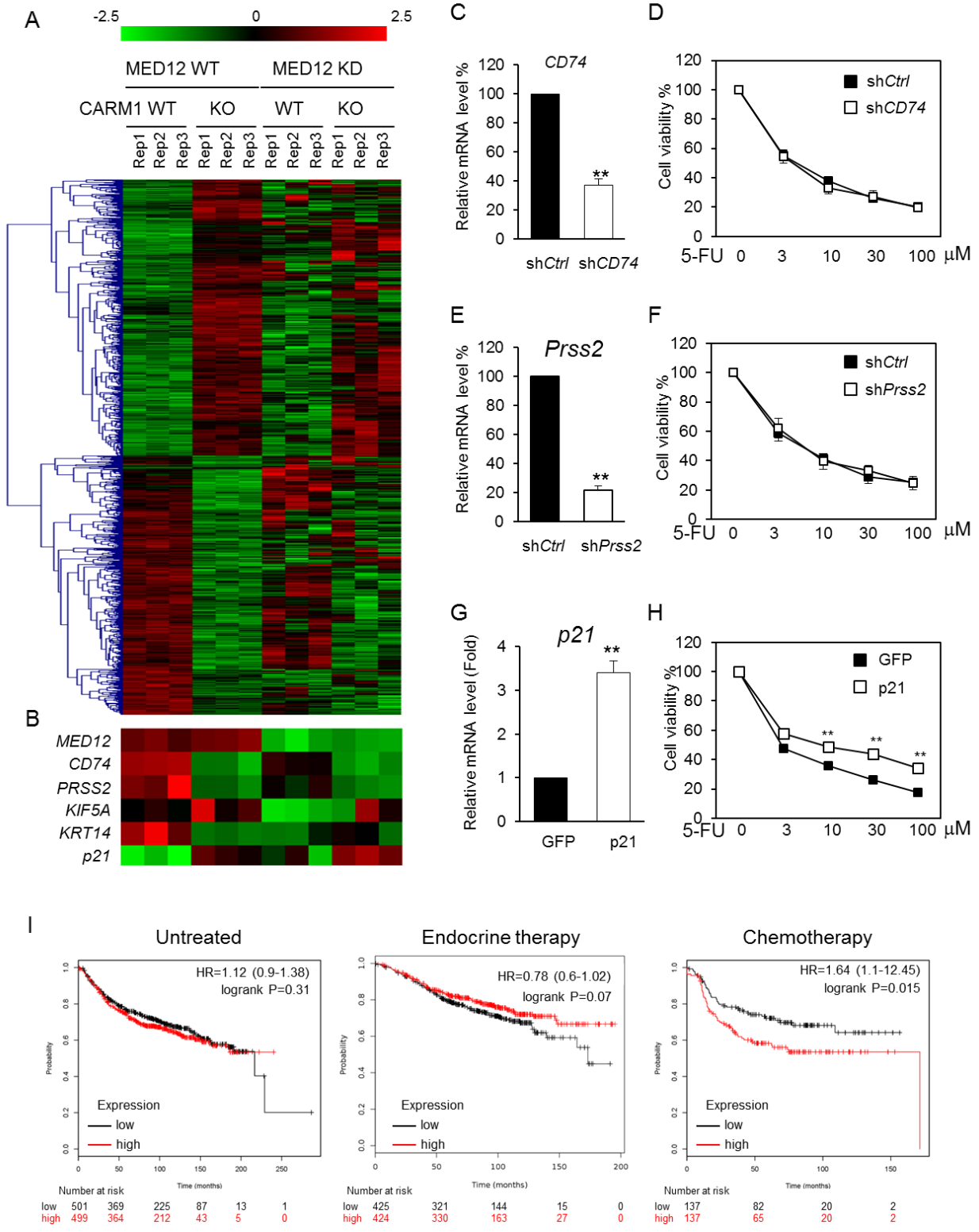

A
-2.5
0
2.5
MED12 WT
MED12 KD
CARM1 WT
KO
WT
KO
Rep1
Rep2
Rep3
B
MED12
CD74
PRSS2
KIF5A
KRT14
p21
C
CD74
Relative mRNA level %
shCtrl
shCD74
D
Cell viability %
shCtrl
shCD74
5-FU
μM
E
Prss2
shPrss2
F
G
p21
Relative mRNA level (Fold)
GFP
p21
H
I
Untreated
Endocrine therapy
Chemotherapy
HR=1.12 (0.9-1.38)
logrank P=0.31
HR=0.78 (0.6-1.02)
logrank P=0.07
HR=1.64 (1.1-12.45)
logrank P=0.015
Probability
Expression
low
high
Time (months)
Number at risk

Figure S4. The mRNA level of *CDKN1A/p21*, a MED12 and CARM1 co-regulated gene, correlates with 5-FU response in vitro and predicts the probability of recurrence-free survival in breast cancer patients.

(A) Affymetrix gene expression microarrays were performed in four cell lines: MDA-MB-231 CARM^{WT} and CARM1^{KO} cells and these cells expressing either sh*Ctrl* or sh*MED12*. Among the genes with 1.5-fold change ($p<0.05$) by loss of CARM1 and MED12, 444 genes were increased and 409 genes were decreased. (B) Heat map of representative genes in Fig. S4A were shown. (C, E, G) Real-time qPCR analyses of mRNA levels of *CD74*, *Prss2*, and *p21* in control MDA-MB-231 cell lines and those expressing sh*CD74*, sh*Prss2* or *p21*, respectively. (D, F, H) Cell viability determined by MTT assay after treatment with different doses of 5-FU in cell lines shown in (C, E, G). (I) Kaplan Meier curves stratified by *p21* levels depicting the probability of relapse-free survival in untreated, endocrine therapy treated, or chemotherapy treated breast cancer patients. Patient samples were divided into *p21*^{high} and *p21*^{low} groups based on the median of the expression level of *p21*. Affymetrix gene ID 202284_s_at was used to plot the survival curves of *p21* using datasets from GEO (Affymetrix HGU133A and HGU133+2 microarrays, Santa Clara, CA, USA), EGA and TCGA.

Figure S5

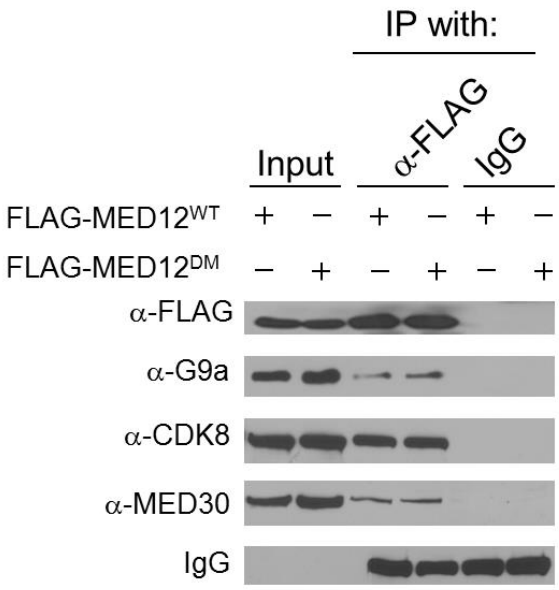


Figure S5. Mutation of MED12 methylation sites does not affect the interaction of MED12 with other known interacting proteins.

Western blotting analysis of MED12, G9a, CDK8 and MED30 in anti-FLAG immunoprecipitates of cell lysates derived from HEK293 cells transiently transfected with FLAG-tagged MED12^{WT} or MED12^{DM}.

Figure S6

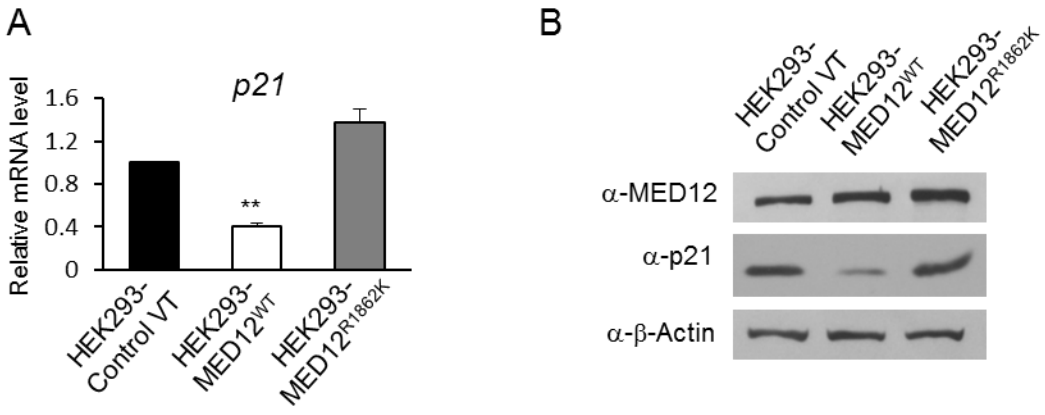


Figure S6. Suppression of p21 mRNA and protein levels is retained in MED12^{WT}- but not MED12^{DM}-expressing HEK293 cells.

(A) Real-time qPCR analysis of *p21* mRNA level in HEK293 cells transfected with control, MED12^{WT} or MED12^{R1862K} expressing mammalian vectors. (B) Western blotting analysis of p21 protein levels in HEK293-VT, HEK293-MED12^{WT} and HEK293-MED12^{R1862K} cells.

Table S1. Primary hits from the FDA-approved oncology drug screening.

Table S1

A FDA approved Oncology Drugs Set III

Well No.	Inhibition rate (sh*Ctrl* minus sh*MED12*)	NSC No.	Drug Name
H04	48.76494 %	105014	Cladribine
F02	24.6054 %	762	Mechlorethamine
G05	20.91573 %	3088	Chlorambucil
F03	13.0879 %	760766	**Floxuridine (FUdR)**
B02	16.34715 %	19893	**Fluorouracil (5FU)**
C05	15.08945 %	122758	Tretinoin(ATRA)
G02	14.62496 %	6396	Thiotepa
G11	14.3828 %	9706	Triethylenemelamine
G07	13.03225 %	609699	Topotecan hydrochloride
D09	10.17675 %	721517	Zoledronic acid
F07	10.14687 %	14229	Quinacrine hydrochloride

B

Well No.	Inhibition rate (WT minus DM)	NSC No.	Drug Name
B02	20.36798 %	19893	**Fluorouracil (5FU)**
A02	18.73072 %	1390	antineoplastic-41390
B03	17.35848 %	92859	Arsenic Trioxide
A03	16.25519 %	45388	Dacarbazine
H08	12.02172 %	701852	Vorinostat
F07	11.6451 %	14229	Quinacrine hydrochloride
B07	11.2092 %	71423	Megestrol acetate
F03	10.81928 %	27640	**Floxuridine (FUdR)**
G06	10.49247 %	122819	Teniposide