MDM4/HIPK2/p53 cytoplasmic assembly uncovers coordinated repression of molecules with anti-apoptotic activity during early DNA damage response

Mancini et al.,

Supplementary Information includes 7 Figures, 6 Tables.

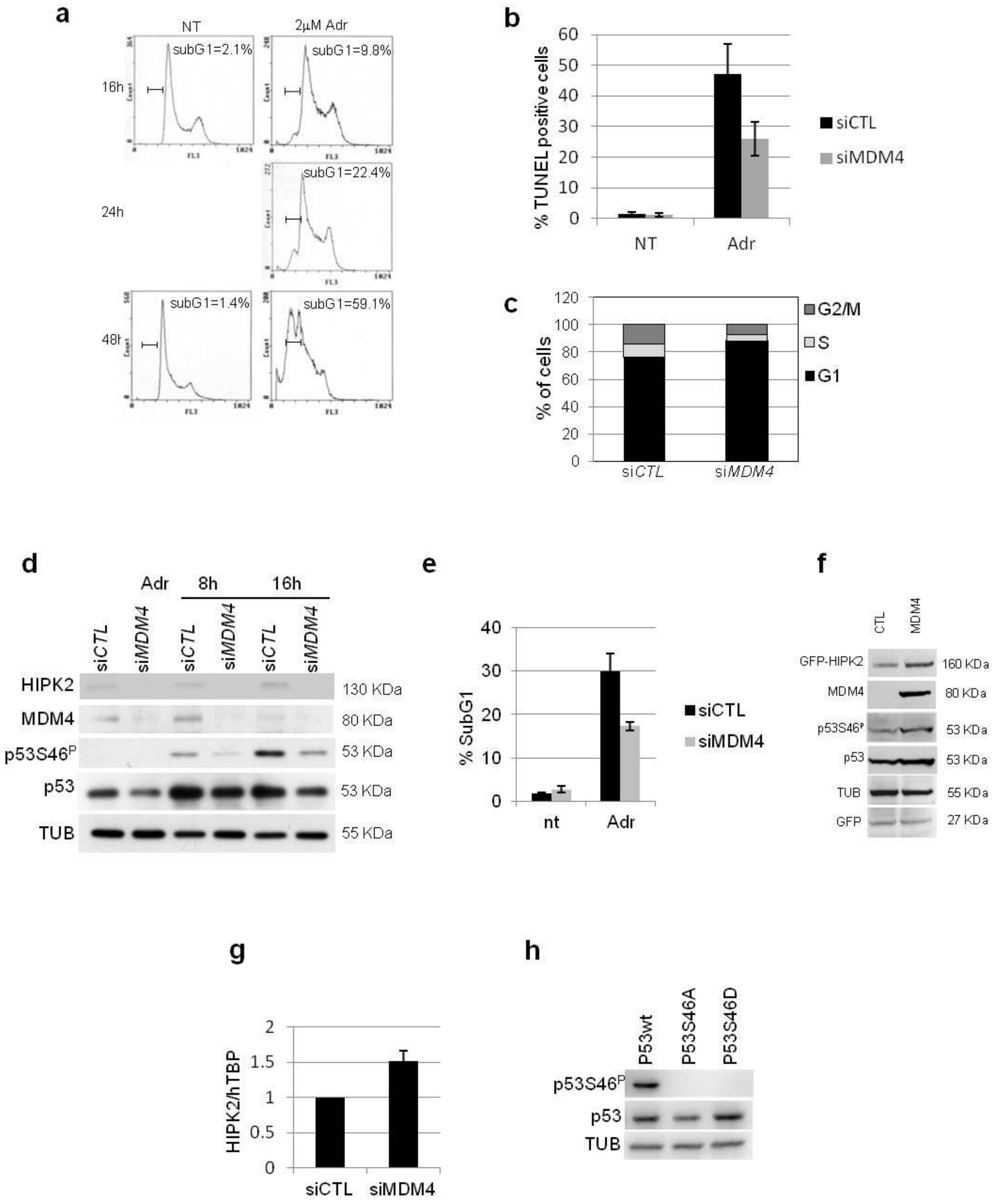

a
NT
2μM Adr
16h
subG1=2.1%
subG1=9.8%
24h
subG1=22.4%
48h
subG1=1.4%
subG1=59.1%
Count
FL3
1024
b
% TUNEL positive cells
60
50
40
30
20
10
0
NT
Adr
siCTL
siMDM4
c
% of cells
120
100
80
60
40
20
0
siCTL
siMDM4
G2/M
S
G1
d
Adr
8h
16h
siCTL
siMDM4
siCTL
siMDM4
siCTL
siMDM4
HIPK2
MDM4
p53S46P
p53
TUB
130 KDa
80 KDa
53 KDa
53 KDa
55 KDa
e
% SubG1
40
30
20
10
0
nt
Adr
siCTL
siMDM4
f
CTL
MDM4
GFP-HIPK2
MDM4
p53S46P
p53
TUB
GFP
160 KDa
80 KDa
53 KDa
53 KDa
55 KDa
27 KDa
g
HIPK2/hTBP
2
1.5
1
0.5
0
siCTL
siMDM4
h
P53wt
P53S46A
P53S46D
p53S46P
p53
TUB

Figure S1. Analysis of MDM4 activity. (**a**) FACS analysis of MCF10A cells untreated (NT) or treated with 2µM Adr at the indicated time points. Line indicates the subG1 area used to quantify % of cell death. (**b**) Analysis of TUNEL positivity of MCF10A cells transfected with stealth MDM4-specific (siMDM4) or stealth control (siCTL) RNA and untreated (NT) or treated with 2µM Adr for 16 hours. Mean±SD of two independent experiments are shown. (**c**) Distribution of untreated siCTL-MCF10A and siMDM4-MCF10A cells in G1, S and G2/M phases by PI flow cytometry (**d**) Western blot of indicated proteins in HMEC cells transfected with stealth MDM4-specific (siMDM4) or stealth control (siCTL) RNA and treated with 3.5µM Adr for indicated time points. (**e**) FACS analysis of HMEC cells transfected as in (a) and treated with 3.5µM Adr for 48 hours. Percentage of cells in subG1 is reported. Mean±SD of two independent experiments are shown. (**f**) WB of the indicated proteins in H1299 transfected with GFP-HIPK2, p53 and CTL or MDM4 expressing vectors. (**g**) RT-qPCR of HIPK2 mRNA, normalized to hTBP, in the MCF10A transfected as indicated. Mean±SD of four independent experiments are shown. (**h**) WB analysis of indicated proteins in Mdm4-/-p53-/-MEFs transfected with indicated plasmids.

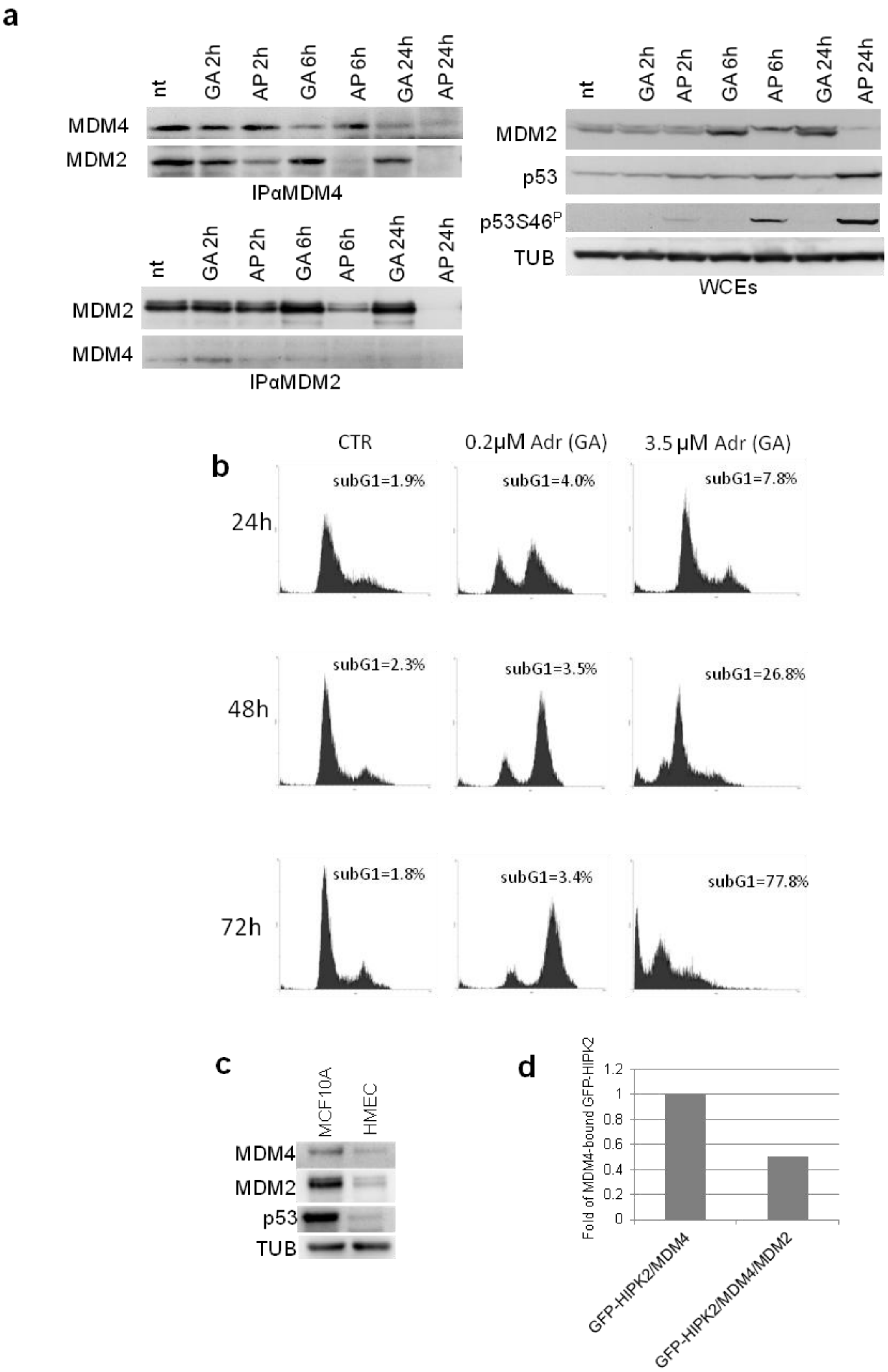

a
nt
GA 2h
AP 2h
GA 6h
AP 6h
GA 24h
AP 24h
MDM4
MDM2
IPαMDM4
MDM2
MDM4
IPαMDM2
MDM2
p53
p53S46P
TUB
WCEs
b
CTR
0.2μM Adr (GA)
3.5 μM Adr (GA)
24h
subG1=1.9%
subG1=4.0%
subG1=7.8%
48h
subG1=2.3%
subG1=3.5%
subG1=26.8%
72h
subG1=1.8%
subG1=3.4%
subG1=77.8%
c
MCF10A
HMEC
MDM4
MDM2
p53
TUB
d
Fold of MDM4-bound GFP-HIPK2
1.2
1
0.8
0.6
0.4
0.2
0
GFP-HIPK2/MDM4
GFP-HIPK2/MDM4/MDM2

Figure S2. Analysis of MDM4/MDM2 interaction in HMEC cells. (**a**) Western blot of the indicated proteins in HMEC cells untreated (nt) or treated with Adr 0.2µM (GA) and 3.5µM (AP) at the indicated time points. (**b**) FACS analysis of HMEC cells treated as in (a) at the indicated time points. Percentage of cells in subG1 area is shown. (**c**) Western blot of the indicated proteins in MCF10A and HMEC cells. (**d**) Densitometric analysis of GFP-HIPK2 levels in complex with MDM4 as resulting from Figure 4c. The levels of GFP-HIPK2 in the complex with MDM4 were normalized to the levels of MDM4 in the immunoprecipitation and to the levels of GFP-HIPK2 in the WCEs.

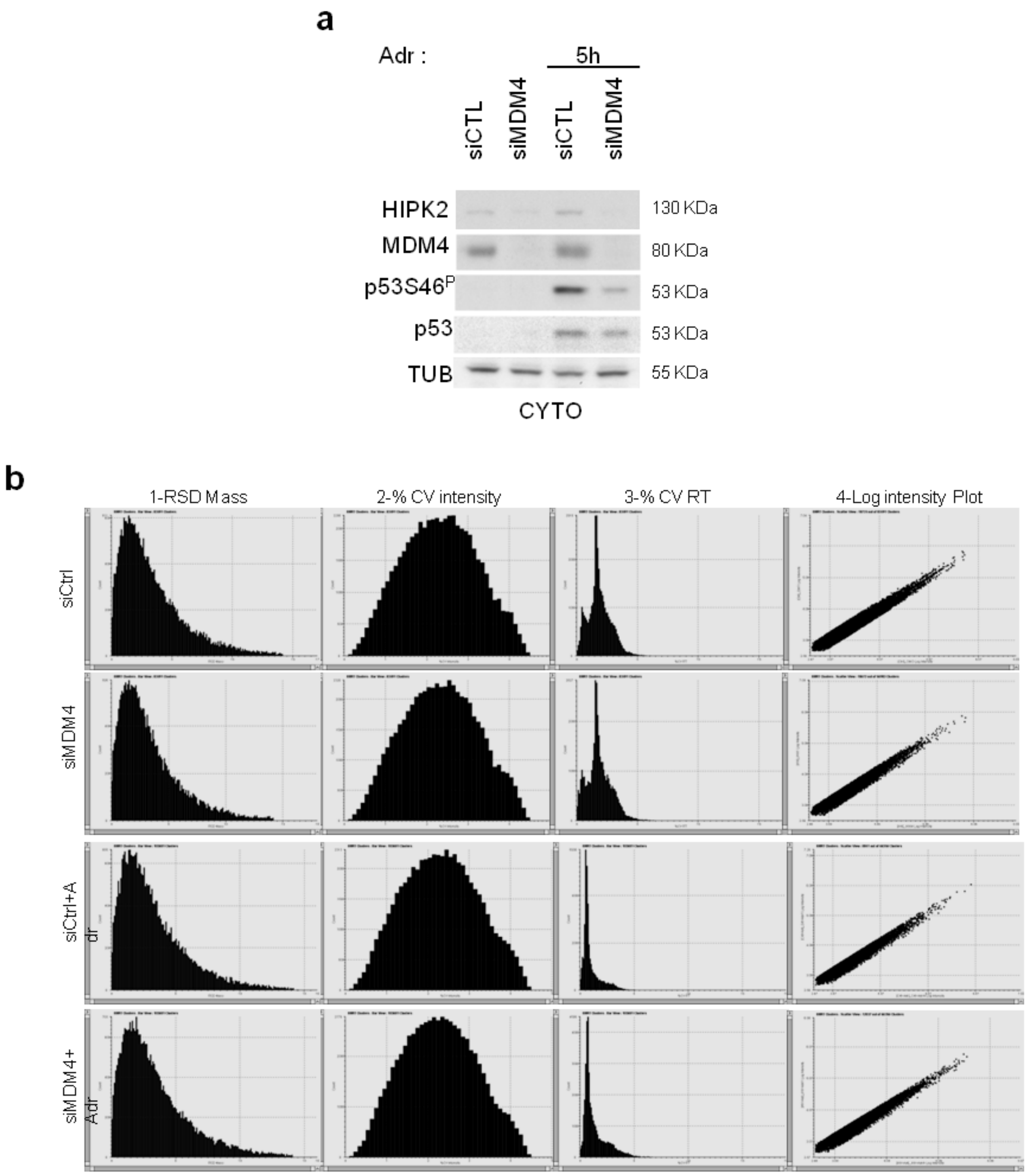


Figure S3. Proteomic analysis. (**a**) Western blot of a fraction of cytoplasmic extracts (CYTO) used for proteomic analysis. siCTL-MCF10A and siMDM4-MCF10A cells were untreated or treated with 2μM Adr for 5 hrs. (**b**) Analytical reproducibility of replicates from LC-MSE measurements in the four groups: siCTL, siMDM4, siCTL+Adr, siMDM4+Adr. 1: Relative standard deviation of EMRT components within 5 ppm of the mean mass measurements. 2:

Average coefficient of variation of the signal intensity of the clusters. 3: Average retention time coefficient of variation. 4: Binary comparison of the log intensity measurements obtained from the matched EMRT cluster for two replicate injections.

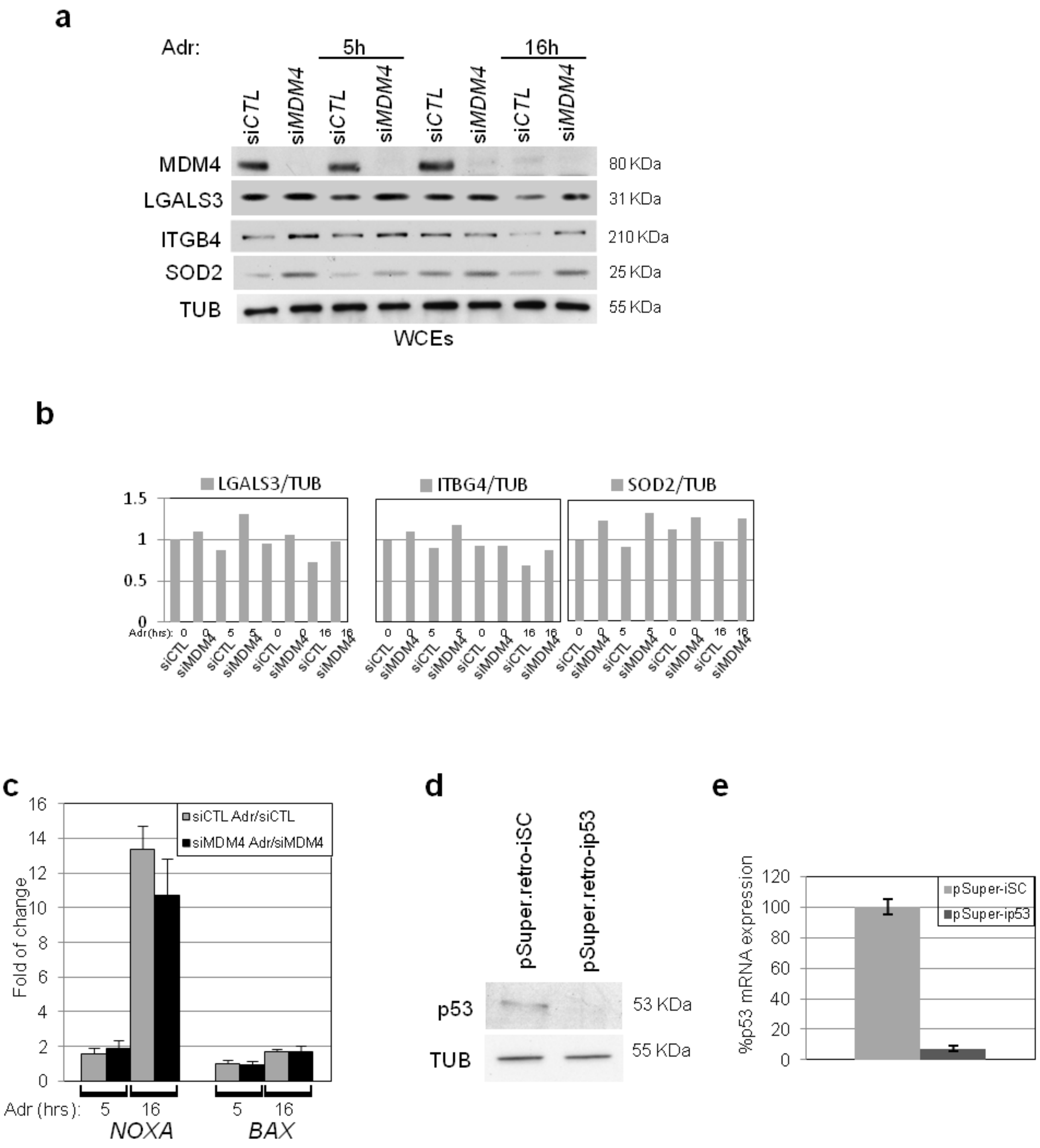
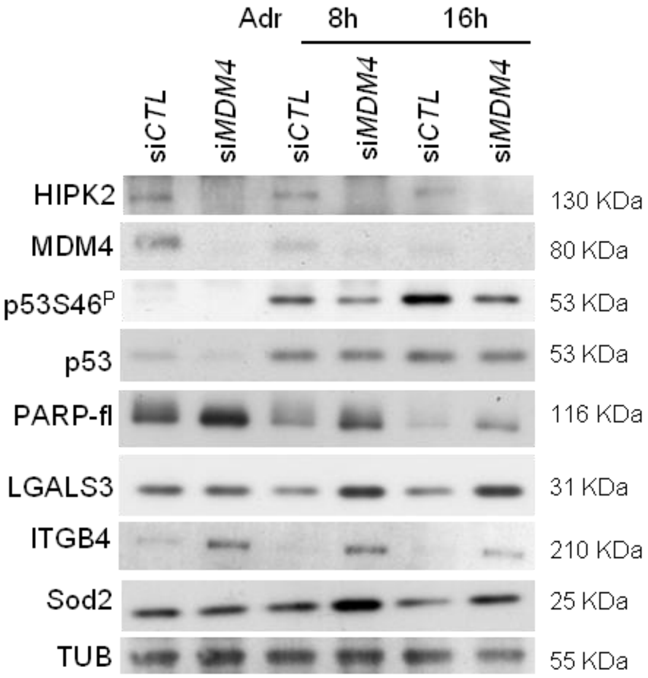

a
Adr:
5h
16h
siCTL
siMDM4
siCTL
siMDM4
siCTL
siMDM4
siCTL
siMDM4
MDM4
LGALS3
ITGB4
SOD2
TUB
80 KDa
31 KDa
210 KDa
25 KDa
55 KDa
WCEs
b
LGALS3/TUB
ITBG4/TUB
SOD2/TUB
1.5
1
0.5
0
Adr(hrs): 0 0 5 5 0 0 16 16
siCTL siMDM4 siCTL siMDM4 siCTL siMDM4 siCTL siMDM4
c
Fold of change
16
14
12
10
8
6
4
2
0
siCTL Adr/siCTL
siMDM4 Adr/siMDM4
Adr (hrs): 5 16 5 16
NOXA
BAX
d
pSuper.retro-iSC
pSuper.retro-ip53
p53
TUB
53 KDa
55 KDa
e
%p53 mRNA expression
120
100
80
60
40
20
0
pSuper-iSC
pSuper-ip53

Figure S4. Validation of proteomic analysis. (**a**) WB of whole cell extracts and (**b**) densitometric analysis of indicated proteins shown in (a). (**c**) RT-qPCR of indicated mRNAs from MCF10A treated as in (a). Mean±SEM of two experiments performed in triplicates are shown (**d**) WB and (**e**) RT-qPCR analysis of p53 in pSuper.retro-iSCMCF10A and pSuper.retro-ip53MCF10A cells. p53 mRNA expression was normalized to hTBP. The pSuper.retro-iSCMCF10A p53 value was arbitrarily set to 100%.

a


Adr
8h
16h
siCTL
siMDM4
siCTL
siMDM4
siCTL
siMDM4
HIPK2
MDM4
p53S46P
p53
PARP-fl
LGALS3
ITGB4
Sod2
TUB
130 KDa
80 KDa
53 KDa
53 KDa
116 KDa
31 KDa
210 KDa
25 KDa
55 KDa

b

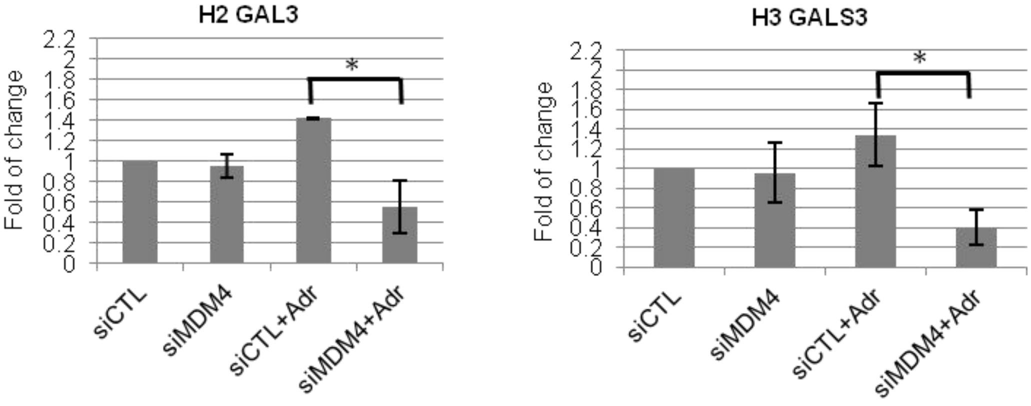

H2 GAL3
Fold of change
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
siCTL
siMDM4
siCTL+Adr
siMDM4+Adr
H3 GALS3
Fold of change
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
siCTL
siMDM4
siCTL+Adr
siMDM4+Adr

Figure S5. Analysis of MDM4 interference in HCT116. (**a**) WB of the indicated proteins in HCT116 cells transfected with stealth MDM4-specific (si*MDM4*) or stealth control (si*CTL*) and treated with 2µM Adr for indicated time points. (**b**) ChIP analysis of p53 on *LGALS3* promoter in HCT116 cells transfected and treated as in (a). Specific p53 binding on *LGALS3* promoter was performed using two different set of primers (H2Gal3 and H3Gal3). (N=3; * = $p<0.05$ Student's t-test).

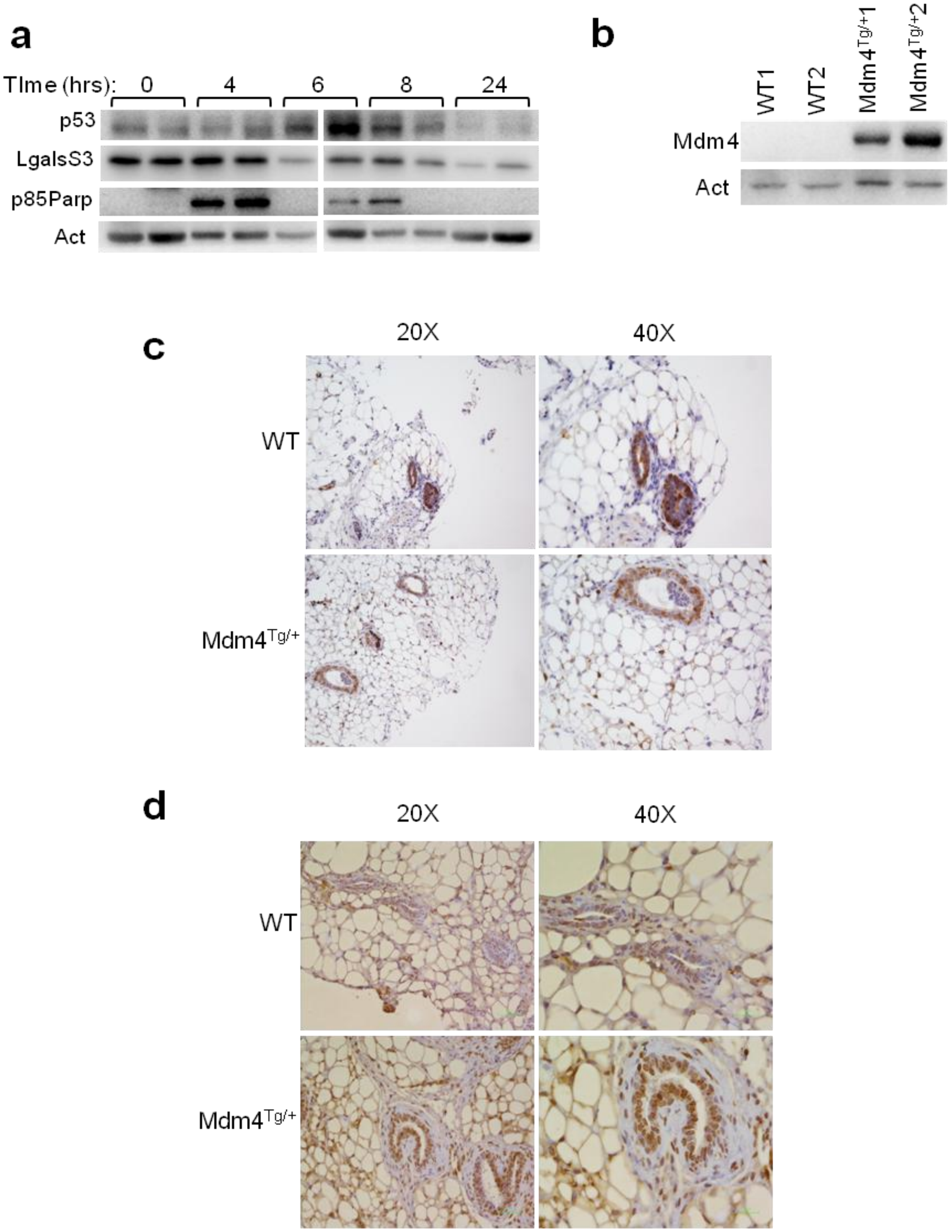
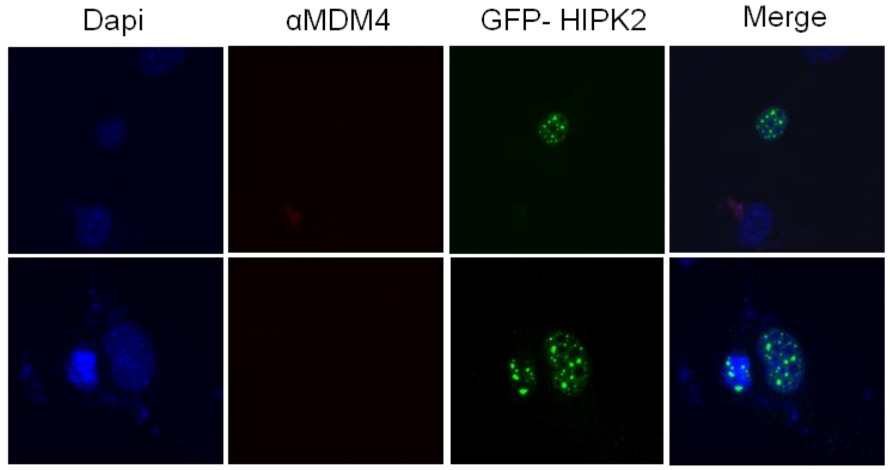
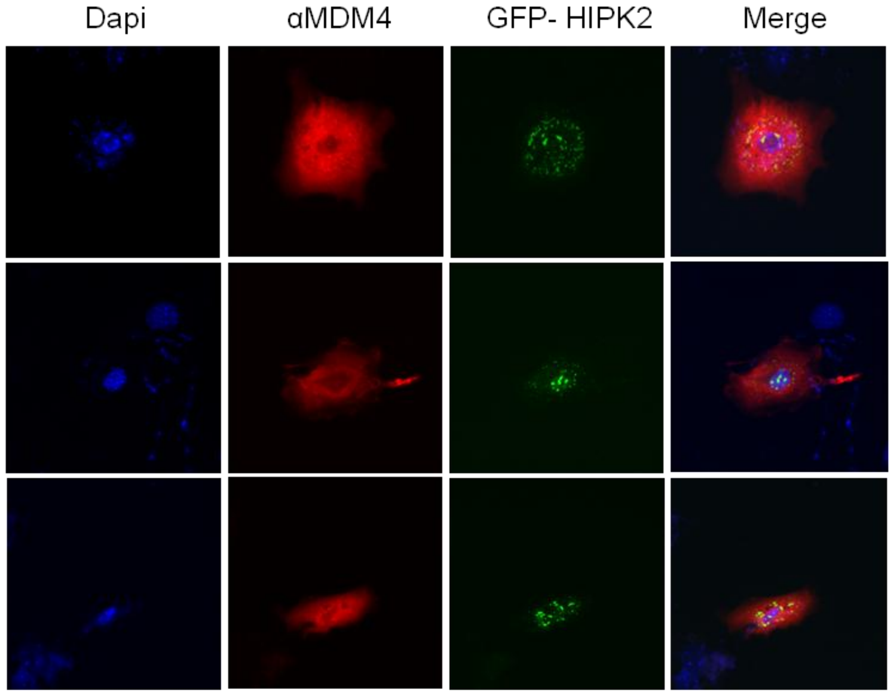

a
TIme (hrs):
0
4
6
8
24
p53
LgalsS3
p85Parp
Act
b
WT1
WT2
Mdm4Tg/+1
Mdm4Tg/+2
Mdm4
Act
c
20X
40X
WT
Mdm4Tg/+
d
20X
40X
WT
Mdm4Tg/+

Figure S6. Analysis of mouse mammary fat pad (MFP) samples. (**a**) WB of indicated proteins in MFP samples of C57Bl6 mice undergone to γ–irradiation and analysed at the indicated time points. Two animals were sacrificed at each indicated time point (**b**) WB of indicated proteins in MFP samples of 2 age-matched control (WT) and 2 *Mdm4* overexpressing transgenic female mice ($\mathrm{Mdm4}^{\mathrm{Tg/+}}$) (**c-d**) Analysis of murine Lgals3 (c) and HIPK2 (d) in Formalin-Fixed, Paraffin-Embedded (FFPE) mammary fat pad samples of WT and $\mathrm{Mdm4}^{\mathrm{Tg/+}}$ mice sacrificed 6 hrs after 6 Gy γ-irradiation. A representative image for each animal is shown at two different magnifications as indicated (20X and 40X).

a


Dapi
αMDM4
GFP- HIPK2
Merge

b


Dapi
αMDM4
GFP- HIPK2
Merge

c

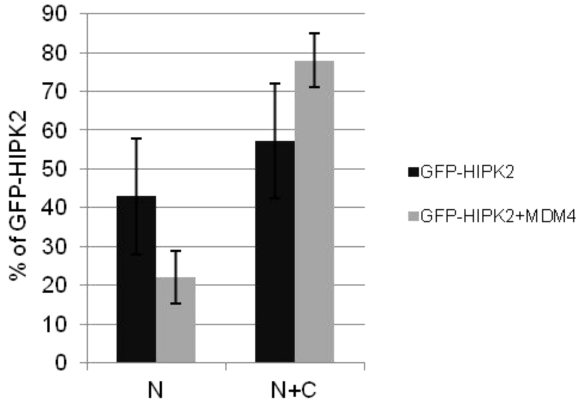

90
80
70
60
50
40
30
20
10
0
% of GFP-HIPK2
N
N+C
■GFP-HIPK2
■GFP-HIPK2+MDM4

Figure S7. Localization of HIPK2 in the presence or absence of MDM4. (a-b) Immunofluorescence of MEFs cells transfected with GFP-HIPK2 (a) or GFP-HIPK2 and pcDNA3.1MDM4 (b). GFP-HIPK2 is shown in green, MDM4 is stained in red. DNA (blue) is stained with DAPI. (**c**) Percentage of cells showing GFP-HIPK2 localization in the nuclei (N) and in the nuclei+cytoplasm (C+N) Mean±SD of three independent experiments are shown.

Supplementary Table 1. Summary of Shotgun proteomic analyses

Comparison	***Downregulated proteins***	***Upregulated proteins***
siMDM4 vs siCTL MCF10A cells	22	20
siMDM4+Adr vs siCTL+Adr MCF10A cells	20	52
siCTL+Adr vs siCTL MCF10A cells	50	21

Supplementary Table 2. siMDM4 vs siCTL MCF10A cells

Accession *	Unique protein sequence identifier according to UniProtKB/Swiss-Prot Protein Knowledgebase, release 2011_06 of 31-May-11.
PLGS Score †	ProteinLynx Global Server score.
Highly represented ‡	Protein appeared only in the indicated group and found in all three technical replicates.
siMDM4/siCTL §	Ratio of expression between the two experimental groups. An arbitrary ratio value of 0.1 or 10 is attributed to a protein found "highly represented" in the denominator or in the numerator group of the fraction, respectively.
Colours Code°	Green indicates a protein downregulated and Red a protein upregulated. That means green proteins are directly associated to the presence of MDM4, red proteins are inversely associated to MDM4.
Symbol, Location, Type #	According to IPA annotation, Build Version 124019/Content version: 11631407 ((Release Date: 2011-09-17).

Accession*	Description	PLGS Score †	Highly Represented‡	siMDM4/ siCTL§°	Symbol#°	Location #	Type(s)#
P34897	Serine hydroxymethyltransferase, mitochondrial (SHMT2)	246.58	siCTL	0.10	SHMT2	Cytoplasm	enzyme
Q16629	Serine/arginine-rich splicing factor 7 (SRSF7)	142.01	siCTL	0.10	SRSF7	Nucleus	other
P49368	T-complex protein 1 subunit gamma(CCT3)	365.84	siCTL	0.10	CCT3	Cytoplasm	other
Q5XKE5	Keratin, type II cytoskeletal 79 (KRT79)	263.79	siCTL	0.10	KRT79	Unknown	other
P08779	Keratin, type I cytoskeletal 16 (KRT16)	352.26	siCTL	0.10	KRT16	Cytoplasm	other
P51572	B-cell receptor-associated protein 31 (BCAP31)	206.19	siCTL	0.10	BCAP31	Cytoplasm	transporter
P19012	Keratin, type I cytoskeletal 15 (KRT15)	311.39	siCTL	0.10	KRT15	Cytoplasm	other
P52597	Heterogeneous nuclear ribonucleoprotein F (HNRNPF)	158.79	siCTL	0.10	HNRNPF	Nucleus	other
P08727	Keratin, type I cytoskeletal 19 (KRT19)	273.42	siCTL	0.10	KRT19	Cytoplasm	other
P62158	Calmodulin(CALM1)	120.12	siCTL	0.10	CALM1	Cytoplasm	
O15533	Tapasin (TAPBP)	171.41	siCTL	0.10	TAPBP	Cytoplasm	
P39656	Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit(DDOST)	119.56	siCTL	0.10	DDOST	Cytoplasm	enzyme
O95678	Keratin, type II cytoskeletal 75 (KRT75)	351.29	siCTL	0.10	KRT75	Cytoplasm	other
P62873	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1(GNB1)	216.52	siCTL	0.10	GNB1	Plasma Membrane	enzyme
Q04695	Keratin, type I cytoskeletal 17 (KRT17)	524.13	siCTL	0.10	KRT17	Cytoplasm	other
P84103	Serine/arginine-rich splicing factor 3 (SRSF3)	155.36	siCTL	0.10	SRSF3	Nucleus	other

Q96A08	Histone H2B type 1-A (HIST1H2BA)	119.08	siCTL	**0.10**	**HIST1H2BA**	Nucleus	other
Q01546	Keratin, type II cytoskeletal 2 oral (KRT76)	300.82		**0.60**	**KRT76**	Cytoplasm	other
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1(HNRNPA2B1)	237.57		**0.63**	**HNRNPA2B1**	Nucleus	other
P08670	Vimentin (VIM)	444.93		**0.66**	**VIM**	Cytoplasm	other
P04259	Keratin, type II cytoskeletal 6B (KRT6B)	443.53		**0.69**	**KRT6B**	Cytoplasm	other
P08195	4F2 cell-surface antigen heavy chain (SLC3A2)	791.90		**0.69**	**SLC3A2**	Plasma Membrane	transporter
P31949	Protein S100-A11 (S100A11)	516.82		**1.35**	**S100A11**	Cytoplasm	other
A5A3E0	POTE ankyrin domain family member F (POTEF)	750.92		**1.36**	**POTEE/POTEF**	unknown	other
Q6S8J3	POTE ankyrin domain family member E (POTEE)	778.22		**1.38**	**POTEE/POTEF**	unknown	other
P07437	Tubulin beta chain (TUBB)	360.55		**1.40**	**TUBB**	Cytoplasm	other
P50454	Serpin H1 (SERPINH1)	257.23		**1.49**	**SERPINH1**	Extracellular Space	other
P04179	Superoxide dismutase [Mn], mitochondrial (SOD2)	341.11		**1.93**	**SOD2**	Cytoplasm	enzyme
P08648	Integrin alpha-5 (ITGA5)	302.16		**2.44**	**ITGA5**	Plasma Membrane	other
P08865	40S ribosomal protein SA (RPSA)	194.41	siMDM4	**10.00**	**RPSA**	Cytoplasm	translation regulator
P35221	Catenin alpha-1 (CTNNA1)	491.79	siMDM4	**10.00**	**CTNNA1**	Plasma Membrane	other
P55084	Trifunctional enzyme subunit beta, mitochondrial (HADHB)	457.95	siMDM4	**10.00**	**HADHB**	Cytoplasm	enzyme
Q9HAV7	GrpE protein homolog 1, mitochondrial (GRPEL1)	198.76	siMDM4	**10.00**	**GRPEL1**	Cytoplasm	other
Q13751	Laminin subunit beta-3 (LAMB3)	475.82	siMDM4	**10.00**	**LAMB3**	Extracellular Space	transporter
P04083	Annexin A1 (ANXA1)	187.23	siMDM4	**10.00**	**ANXA1**	Plasma Membrane	other
P25398	40S ribosomal protein S12 (RPS12)	151.99	siMDM4	**10.00**	**RPS12**	Cytoplasm	other
O43707	Alpha-actinin-4 (ACTN4)	649.75	siMDM4	**10.00**	**ACTN4**	Cytoplasm	other
P68104	Elongation factor 1-alpha 1 (EEF1A1)	227.90	siMDM4	**10.00**	**EEF1A1**	Cytoplasm	translation regulator
Q5VTE0	Putative elongation factor 1-alpha-like 3 (EEF1A1P5)	232.41	siMDM4	**10.00**	**EEF1AL3**	unknown	other
P30049	ATP synthase subunit delta, mitochondrial (ATP5D)	128.84	siMDM4	**10.00**	**ATP5D**	Cytoplasm	transporter
Q16352	Alpha-internexin (INA)	220.02	siMDM4	**10.00**	**INA**	Cytoplasm	other
P04264	Keratin, type II cytoskeletal 1 (KRT1)	370.80	siMDM4	**10.00**	**KRT1**	Cytoplasm	other

Supplementary Table 3. siMDM4+Adr vs siCTL+Adr MCF10A cells

Accession *	Unique protein sequence identifier according to UniProtKB/Swiss-Prot Protein Knowledgebase, release 2011_06 of 31-May-11.
PLGS Score †	ProteinLynx Global Server score.
Highly represented ‡	Protein appeared only in the indicated group and found in all three technical replicates.
siMDM4/siCTL §	Ratio of expression between the two experimental groups. An arbitrary ratio value of 0.1 or 10 is attributed to a protein found "highly represented" in the denominator or in the numerator group of the fraction, respectively.
Colours Code°	Green indicates a protein downregulated and Red a protein upregulated. That means green proteins are directly associated to the presence of MDM4, red proteins are inversely associated to MDM4.
Symbol, Location, Type #	According to IPA annotation, Build Version 124019/Content version: 11631407 ((Release Date: 2011-09-17).

Accession*	Description	PLGS Score †	Highly Represented‡	siMDM4 +Adr/ siCTL+ Adr§°	Symbol#°	Location#	Type(s)#
P35232	Prohibitin (PHB)	254.63	siCTL+ Adr	0.10	PHB	Nucleus	transcription regulator
P06733	Alpha-enolase (ENO1)	357.78	siCTL+ Adr	0.10	ENO1	Cytoplasm	transcription regulator
Q99623	Prohibitin-2 (PHB2)	260.54	siCTL+ Adr	0.10	PHB2	Cytoplasm	transcription regulator
Q13838	Spliceosome RNA helicase DDX39B (DDX39B)	194.65	siCTL+ Adr	0.10	DDX39B	Nucleus	enzyme
Q9H0C2	ADP/ATP translocase 4 (SLC25A31)	174.63	siCTL+ Adr	0.10	SLC25A31	Cytoplasm	transporter
P11279	Lysosome-associated membrane glycoprotein 1(LAMP1)	174.49	siCTL+ Adr	0.10	LAMP1	Plasma Membrane	other
P62158	Calmodulin (CALM1)	203.74	siCTL+ Adr	0.10	CALM1	Cytoplasm	
P54709	Sodium/potassium-transporting ATPase subunit beta-3(ATP1B3)	97.40	siCTL+ Adr	0.10	ATP1B3	Plasma Membrane	transporter
Q14697	Neutral alpha-glucosidase AB (GANAB)	392.52	siCTL+ Adr	0.10	GANAB	Cytoplasm	enzyme
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 (GNB2)	231.89	siCTL+ Adr	0.10	GNB2	Plasma Membrane	enzyme
P30048	Thioredoxin-dependent peroxide reductase, mitochondrial (PRDX3)	222.11	siCTL+ Adr	0.10	PRDX3	Cytoplasm	enzyme
P61026	Ras-related protein Rab-10 (RAB10)	167.35	siCTL+ Adr	0.10	RAB10	Cytoplasm	enzyme
Q00839	Heterogeneous nuclear ribonucleoprotein U (HNRNPU)	312.99	siCTL+ Adr	0.10	HNRNPU	Nucleus	transporter
O00148	ATP-dependent RNA helicase DDX39A (DDX39A)	178.69	siCTL+ Adr	0.10	DDX39A	Nucleus	enzyme
P27824	Calnexin (CANX)	266.82	siCTL+ Adr	0.10	CANX	Cytoplasm	other

Q9BYX7	Putative beta-actin-like protein 3 (POTEKP)	332.53		**0.64**	**POTEKP**	unknown	other
P08195	4F2 cell-surface antigen heavy chain (SLC3A2)	971.90		**0.66**	**SLC3A2**	Plasma Membrane	transporter
P33778	Histone H2B type 1-B (HIST1H2BB)	218.56		**0.66**	**HIST1H2BB**	Nucleus	other
P27797	Calreticulin (CALR)	628.54		**0.72**	**CALR**	Cytoplasm	transcription regulator
P63261	Actin, cytoplasmic 2(ACTG1)	1640.98		**0.73**	**ACTG1**	Cytoplasm	other
P07237	Protein disulfide-isomerase (P4HB)	422.43		**1.38**	**P4HB**	Cytoplasm	enzyme
P14625	Endoplasmin (HSP90B1)	1056.58		**1.40**	**HSP90B1**	Cytoplasm	other
P09651	Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1)	245.25		**1.42**	**HNRNPA1**	Nucleus	other
P06748	Nucleophosmin (NPM1)	434.73		**1.46**	**NPM1**	Nucleus	transcription regulator
P19338	Nucleolin (NCL)	319.40		**1.46**	**NCL**	Nucleus	other
P01892	HLA class I histocompatibility antigen, A-2 alpha chain (HLA-A)	317.82		**1.51**	**HLA-A**	Plasma Membrane	transmembrane receptor
P01891	HLA class I histocompatibility antigen, A-68 alpha chain (HLA-A)	319.94		**1.51**	**HLA-A**	Plasma Membrane	transmembrane receptor
P10809	60 kDa heat shock protein, mitochondrial (HSPD1)	1219.55		**1.52**	**HSPD1**	Cytoplasm	enzyme
P40926	Malate dehydrogenase, mitochondrial (MDH2)	530.45		**1.52**	**MDH2**	Cytoplasm	enzyme
P35613	Basigin (BSG)	298.16		**1.55**	**BSG**	Plasma Membrane	transporter
Q08380	Galectin-3-binding protein(LGALS3BP)	296.64		**1.62**	**LGALS3BP**	Plasma Membrane	transmembrane receptor
P26006	Integrin alpha-3 (ITGA3)	533.38		**1.67**	**ITGA3**	Plasma Membrane	other
P04259	Keratin, type II cytoskeletal 6B (KRT6B)	421.37		**1.68**	**KRT6B**	Cytoplasm	other
P61604	10 kDa heat shock protein, mitochondrial (HSPE1)	378.58		**1.68**	**HSPE1**	Cytoplasm	enzyme
P48668	Keratin, type II cytoskeletal 6C (KRT6C)	383.20		**1.72**	**KRT6C**	unknown	other
P38646	Stress-70 protein, mitochondrial (HSPA9)	1197.09		**1.77**	**HSPA9**	Cytoplasm	other
P13987	CD59 glycoprotein (CD59)	194.47		**1.79**	**CD59**	Plasma Membrane	other
P02538	Keratin, type II cytoskeletal 6A(KRT6A)	389.16		**1.80**	**KRT6A**	Plasma Membrane	other
Q04837	Single-stranded DNA-binding protein, mitochondrial (SSBP1)	206.71		**1.80**	**SSBP1**	Cytoplasm	other
P05556	Integrin beta-1 (ITGB1)	482.32		**1.84**	**ITGB1**	Plasma Membrane	transmembrane receptor
P30101	Protein disulfide-isomerase A3 (PDIA3)	749.29		**1.84**	**PDIA3**	Cytoplasm	peptidase
Q9Y6N5	Sulfide:quinone oxidoreductase, mitochondrial (SQRDL)	396.06		**1.99**	**SQRDL**	Cytoplasm	enzyme
P61769	Beta-2-microglobulin (B2M)	282.06		**2.01**	**B2M**	Plasma Membrane	transmembrane receptor
Q8NBS9	Thioredoxin domain-containing protein 5 (TXNDC5)	255.48		**2.10**	**TXNDC5**	Cytoplasm	enzyme
P09382	Galectin-1 (LGALS1)	477.23		**2.10**	**LGALS1**	Extracellular Space	other

Q5VTE0	Putative elongation factor 1-alpha-like 3 (EEF1A1P5)	266.91		**2.23**	**EEF1AL3***	unknown	other
Q9HCY8	Protein S100-A14 (S100A14)	262.18		**2.39**	**S100A14**	Cytoplasm	other
A5A3E0	POTE ankyrin domain family member F O(POTEF)	676.94		**2.64**	**POTEE/POTEF**	unknown	other
Q6S8J3	POTE ankyrin domain family member E (POTEE)	700.81		**2.66**	**POTEE/POTEF**	unknown	other
P04179	Superoxide dismutase [Mn], mitochondrial (SOD2)	188.75		**3.74**	**SOD2**	Cytoplasm	enzyme
P31949	Protein S100-A11 (S100A11)	147.95		**3.86**	**S100A11**	Cytoplasm	other
P06753	Tropomyosin alpha-3 chain (TPM3)	252.92	siMDM4 +Adr	**10.00**	**TPM3**	Cytoplasm	other
Q96AE4	Far upstream element-binding protein 1 (FUBP1)	361.72	siMDM4 +Adr	**10.00**	**FUBP1**	Nucleus	transcription regulator
P60174	Triosephosphate isomerase (TPI1)	206.04	siMDM4 +Adr	**10.00**	**TPI1**	Cytoplasm	enzyme
Q9HAV7	GrpE protein homolog 1, mitochondrial (GRPEL1)	188.49	siMDM4 +Adr	**10.00**	**GRPEL1**	Cytoplasm	other
P36957	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial (DLST)	230.70	siMDM4 +Adr	**10.00**	**DLST**	Cytoplasm	enzyme
P15153	Ras-related C3 botulinum toxin substrate 2 (RAC2)	160.46	siMDM4 +Adr	**10.00**	**RAC2**	Cytoplasm	enzyme
Q13753	Laminin subunit gamma-2 (LAMC2)	639.87	siMDM4 +Adr	**10.00**	**LAMC2**	Extracellular Space	other
P04083	Annexin A1 (ANXA1)	293.03	siMDM4 +Adr	**10.00**	**ANXA1**	Plasma Membrane	other
Q9NSB2	Keratin, type II cuticular Hb4 (KRT84)	380.86	siMDM4 +Adr	**10.00**	**KRT84**	Cytoplasm	other
P16144	Integrin beta-4 (ITGB4)	780.89	siMDM4 +Adr	**10.00**	**ITGB4**	Plasma Membrane	transmembrane receptor
P08670	Vimentin (VIM)	319.24	siMDM4 +Adr	**10.00**	**VIM**	Cytoplasm	other
O95678	Keratin, type II cytoskeletal 75 (KRT75)	295.74	siMDM4 +Adr	**10.00**	**KRT75**	Cytoplasm	other
P47914	60S ribosomal protein L29 (RPL29)	135.97	siMDM4 +Adr	**10.00**	**RPL29**	Cytoplasm	other
P46782	40S ribosomal protein S5 (RPS5)	125.83	siMDM4 +Adr	**10.00**	**RPS5**	Cytoplasm	other
Q8N1N4	Keratin, type II cytoskeletal 78 (KRT78)	312.51	siMDM4 +Adr	**10.00**	**KRT78**	unknown	other
P17931	Galectin-3 (LGALS3)	165.35	siMDM4 +Adr	**10.00**	**LGALS3**	Extracellular Space	other
P26038	Moesin (MSN)	518.77	siMDM4 +Adr	**10.00**	**MSN**	Plasma Membrane	other
P05787	Keratin, type II cytoskeletal 8 (KRT8)	253.07	siMDM4 +Adr	**10.00**	**KRT8**	Cytoplasm	other
P13647	Keratin, type II cytoskeletal 5(KRT5)	274.68	siMDM4 +Adr	**10.00**	**KRT5**	Cytoplasm	other
P14314	Glucosidase 2 subunit beta (PRKCSH)	415.90	siMDM4 +Adr	**10.00**	**PRKCSH**	Cytoplasm	enzyme
P09972	Fructose-bisphosphate aldolase C (ALDOC)	274.36	siMDM4 +Adr	**10.00**	**ALDOC**	Cytoplasm	enzyme

Supplementary Table 4. siCTL+Adr vs siCTL MCF10A cells

Accession *	Unique protein sequence identifier according to UniProtKB/Swiss-Prot Protein Knowledgebase, release 2011_06 of 31-May-11.
PLGS Score †	ProteinLynx Global Server score.
Highly represented ‡	Protein appeared only in the indicated group and found in all three technical replicates.
siMDM4/siCTL §	Ratio of expression between the two experimental groups. An arbitrary ratio value of 0.1 or 10 is attributed to a protein found “highly represented” in the denominator or in the numerator group of the fraction, respectively.
Colours Code°	Green indicates a protein downregulated and Red a protein upregulated. That means green proteins are directly associated to the presence of MDM4, red proteins are inversely associated to MDM4.
Symbol, Location, Type #	According to IPA annotation, Build Version 124019/Content version: 11631407 ((Release Date: 2011-09-17).

Accession*	Description	PLGS Score †	Highly Represented‡	siCTL+Adr/siCTL§	Symbol#°	Location#	Type(s)#
P34897	Serine hydroxymethyltransferase, mitochondrial (SHMT2)	246.58	siCTL	**0.1**	**SHMT2**	Cytoplasm	enzyme
P20674	Cytochrome c oxidase subunit 5A, mitochondrial (COX5A)	153.2	siCTL	**0.1**	**COX5A**	Cytoplasm	enzyme
Q16629	Serine/arginine-rich splicing factor 7 (SRSF7)	142.01	siCTL	**0.1**	**SRSF7**	Nucleus	other
P49368	T-complex protein 1 subunit gamma(CCT3)	365.84	siCTL	**0.1**	**CCT3**	Cytoplasm	other
Q5XKE5	Keratin, type II cytoskeletal 79 (KRT79)	263.79	siCTL	**0.1**	**KRT79**	unknown	other
P08779	Keratin, type I cytoskeletal 16 (KRT16)	352.26	siCTL	**0.1**	**KRT16**	Cytoplasm	other
P51572	B-cell receptor-associated protein 31 (BCAP31)	206.19	siCTL	**0.1**	**BCAP31**	Cytoplasm	transporter
P15153	Ras-related C3 botulinum toxin substrate 2 (RAC2)	162.9	siCTL	**0.1**	**RAC2**	Cytoplasm	enzyme
P19012	Keratin, type I cytoskeletal 15 (KRT15)	311.39	siCTL	**0.1**	**KRT15**	Cytoplasm	other
P61916	Epididymal secretory protein E1(NPC2)	157.64	siCTL	**0.1**	**NPC2**	Extracellular Space	other
P16144	Integrin beta-4 (ITGB4)	775.97	siCTL	**0.1**	**ITGB4**	Plasma Membrane	transmembrane receptor
P08670	Vimentin (VIM)	444.93	siCTL	**0.1**	**VIM**	Cytoplasm	other
P53634	Dipeptidyl peptidase 1(CTSC)	152.78	siCTL	**0.1**	**CTSC**	Cytoplasm	peptidase
O95678	Keratin, type II cytoskeletal 75 (KRT75)	351.29	siCTL	**0.1**	**KRT75**	Cytoplasm	other
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPA2B1)	237.57	siCTL	**0.1**	**HNRNPA2B1**	Nucleus	other
P08648	Integrin alpha-5 (ITGA5)	302.16	siCTL	**0.1**	**ITGA5**	Plasma Membrane	other
Q8N1N4	Keratin, type II cytoskeletal 78 (KRT78)	257.35	siCTL	**0.1**	**KRT78**	unknown	other
P62857	40S ribosomal protein S28 (RPS28)	161.87	siCTL	**0.1**	**RPS28**	Cytoplasm	other

P05787	Keratin, type II cytoskeletal 8 (KRT8)	311.84	siCTL	**0.1**	**KRT8**	Cytoplasm	other
P13647	Keratin, type II cytoskeletal 5 (KRT5)	441.45	siCTL	**0.1**	**KRT5**	Cytoplasm	other
Q07065	Cytoskeleton-associated protein 4 (CKAP4)	412.42	siCTL	**0.1**	**CKAP4**	Cytoplasm	other
P00505	Aspartate aminotransferase, mitochondrial (GOT2)	280.07	siCTL	**0.1**	**GOT2**	Cytoplasm	enzyme
P23229	Integrin alpha-6 (ITGA6)	529.79	siCTL	**0.1**	**ITGA6**	Plasma Membrane	other
P14314	Glucosidase 2 subunit beta (PRKCSH)	510.81	siCTL	**0.1**	**PRKCSH**	Cytoplasm	enzyme
P07919	Cytochrome b-c1 complex subunit 6, mitochondrial (UQCRH)	154.22	siCTL	**0.1**	**UQCRH**	Cytoplasm	enzyme
P31949	Protein S100-A11 (S100A11)	516.82		**0.37**	**S100A11**	Cytoplasm	other
P35908	Keratin, type II cytoskeletal 2 epidermal (KRT2)	316.85		**0.46**	**KRT2**	Cytoplasm	other
P61769	Beta-2-microglobulin (B2M)	556.9		**0.46**	**B2M**	Plasma Membrane	transmembrane receptor
P04179	Superoxide dismutase [Mn], mitochondrial (SOD2)	341.11		**0.49**	**SOD2**	Cytoplasm	enzyme
P61604	10 kDa heat shock protein, mitochondrial (HSPE1)	529.12		**0.49**	**HSPE1**	Cytoplasm	enzyme
P02538	Keratin, type II cytoskeletal 6A (KRT6A)	389.85		**0.52**	**KRT6A**	Plasma Membrane	other
Q01546	Keratin, type II cytoskeletal 2 oral (KRT76)	300.82		**0.52**	**KRT76**	Cytoplasm	other
Q8NBS9	Thioredoxin domain-containing protein 5 (TXNDC5)	275.85		**0.55**	**TXNDC5**	Cytoplasm	enzyme
P19338	Nucleolin (NCL)	499.84		**0.57**	**NCL**	Nucleus	other
Q9HCY8	Protein S100-A14 (S100A14)	318.36		**0.57**	**S100A14**	Cytoplasm	other
P30101	Protein disulfide-isomerase A3 (PDIA3)	811.84		**0.59**	**PDIA3**	Cytoplasm	peptidase
P01891	HLA class I histocompatibility antigen, A-68 alpha chain (HLA-A)	430.54		**0.61**	**HLA-A**	Plasma Membrane	transmembrane receptor
P08729	Keratin, type II cytoskeletal 7 ((KRT7)	537.74		**0.62**	**KRT7**	Cytoplasm	other
Q07000	HLA class I histocompatibility antigen, Cw-15 alpha chain (HLA-C)	466.26		**0.67**	**HLA-A***	Plasma Membrane	transmembrane receptor
Q08380	Galectin-3-binding protein (LGALS3BP)	278.86		**0.68**	**LGALS3BP**	Plasma Membrane	other
P13987	CD59 glycoprotein (CD59)	186.8		**0.68**	**CD59**	Cytoplasm	transcription regulator
P27797	Calreticulin (CALR)	749.28		**0.68**	**CALR**	Extracellular Space	other
P09382	Galectin-1 (LGALS1)	473.76		**0.69**	**LGALS1**	Plasma Membrane	transmembrane receptor
P05556	Integrin beta-1 (ITGB1)	625.91		**0.7**	**ITGB1**	Cytoplasm	enzyme
P07237	Protein disulfide-isomerase (P4HB)	688.06		**0.7**	**P4HB**	Plasma Membrane	transmembrane receptor
P16188	HLA class I histocompatibility antigen, A-30 alpha chain (HLA-A)	510.89		**0.71**	**HLA-A**	Cytoplasm	enzyme
P13667	Protein disulfide-isomerase A4 (PDIA4)	510.8		**0.72**	**PDIA4**	Cytoplasm	other
Q04837	Single-stranded DNA-binding protein, mitochondrial (SSBP1)	229.67		**0.73**	**SSBP1**	Cytoplasm	other
P38646	Stress-70 protein, mitochondrial (HSPA9)	1440.29		**0.73**	**HSPA9**	Cytoplasm	enzyme
P31930	Cytochrome b-c1 complex subunit 1, mitochondrial (UQCRC1)	406.49		**0.73**	**UQCRC1**	Cytoplasm	other

P63261	Actin, cytoplasmic 2 (ACTG1)	1412.46		**1.36**	**ACTG1**	Cytoplasm	transcription regulator
P06733	Alpha-enolase (ENO1)	352.24		**1.39**	**ENO1**	Cytoplasm	other
P04350	Tubulin beta-4 chain (TUBB4)	253.7		**1.42**	**TUBB4**	Nucleus	other
Q96FQ6	Protein S100-A16 (S100A16)	220.28		**1.55**	**S100A16**	Cytoplasm	other
P07437	Tubulin beta chain (TUBB)	360.55		**1.63**	**TUBB**	Cytoplasm	transporter
P06576	ATP synthase subunit beta, mitochondrial (ATP5B)	1149.16		**1.7**	**ATP5B**	Cytoplasm	transporter
P62158	Calmodulin (CALM1)	120.12		**1.79**	**CALM1**	Cytoplasm	
P35232	Prohibitin (PHB)	254.63	siCTL+ Adr	**10**	**PHB**	Nucleus	transcription regulator
P08865	40S ribosomal protein SA (RPSA)	224.18	siCTL+ Adr	**10**	**RPSA**	Cytoplasm	translation regulator
Q99623	Prohibitin-2 (PHB2)	260.54	siCTL+ Adr	**10**	**PHB2**	Cytoplasm	transcription regulator
P12814	Alpha-actinin-1 (ACTN1)	772.81	siCTL+ Adr	**10**	**ACTN1**	Cytoplasm	other
Q9H4B7	Tubulin beta-1 chain (TUBB1)	195.91	siCTL+ Adr	**10**	**TUBB1**	Cytoplasm	other
P54709	Sodium/potassium-transporting ATPase subunit beta-3 (ATP1B3)	97.4	siCTL+ Adr	**10**	**ATP1B3**	Plasma Membrane	transporter
Q14697	Neutral alpha-glucosidase AB (GANAB)	392.52	siCTL+ Adr	**10**	**GANAB**	Cytoplasm	enzyme
O43707	Alpha-actinin-4 (ACTN4)	825.22	siCTL+ Adr	**10**	**ACTN4**	Cytoplasm	other
P62879	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 (GNB2)	231.89	siCTL+ Adr	**10**	**GNB2**	Plasma Membrane	enzyme
Q5VTE0	Putative elongation factor 1-alpha-like 3 (EEF1A1P5)	266.91	siCTL+ Adr	**10**	**EEF1AL3***	unknown	other
P30048	Thioredoxin-dependent peroxide reductase, mitochondrial (PRDX3)	222.11	siCTL+ Adr	**10**	**PRDX3**	Cytoplasm	enzyme
P61026	Ras-related protein Rab-10 (RAB10)	167.35	siCTL+ Adr	**10**	**RAB10**	Cytoplasm	enzyme
P17066	Heat shock 70 kDa protein 6 (HSPA6)	392.44	siCTL+ Adr	**10**	**HSPA6**	unknown	other
P04264	Keratin, type II cytoskeletal 1 (KRT1)	314.23	siCTL+ Adr	**10**	**KRT1**	Cytoplasm	other

Supplementary Table 5. IPA analysis

Comparison	Network ID	Molecules in Network	Score	Focus Molecules	Associated Network Functions
siMDM4 vs siCTL					
	1	BAD,CANX,CCNL2,E2F1,EIF2S1,GNB1,HADHB,IgG,ITGA5,KRT14,KRT15,KRT16,KRT18,LAMB3,Lh,MLL2,NXF1,PDIA3,PLAU,PPP2R2B,PRDM1,PRKD1,SLC3A2,SNAP23,SP1,SRSF3,SRSF7,TAP1,TAP2,TAPBP,TNF,**TP53**,TUBB,VIM,ZNF148	23	13	Cellular Development, Cellular Growth and Proliferation, Gene Expression
	2	ACTN4,AGTR1,APEX1,CCNC,CCT2,CCT3,CCT4,CCT5,CCT7,CCT8,CCT6A,CDK8,CDK19,CDKN1A,CTNNA1,CTNNB1,EEF1A1,GCN1L1,HGF,HNRNPA2B1,HNRNPF,INA,KRT18,KRT19,MED12,MED13,MYC,PLAU,PPIA,RPSA,S100A11,SERPINF1,SHMT2,TCP1,TNPO1	19	11	Cell Death, Cell Cycle, Cancer
siMDM4+Adr vs siCTL+Adr					
	1	ANXA1, ATP1B3, B2M, BSG, CALR, CANX, ERK, ERK1/2, HLA-A, HLA-B27, HSPD1, IgG, ITGA3, ITGB1, ITGB4, LAMC2, Laminin, LAMP1, LGALS1, LGALS3, LGALS3BP, MHC Class I (complex), NCL, NFkB (complex), NPM1, PDIA3, PI3K (complex), RNA	44	23	Cell-To-Cell Signaling and Interaction, Cell Death, Tissue Development
	2	ACTG1, BMI1, CTNNB1, CXCL12, DDX39A, E2F4, HNRNPA1, HNRNPC, HNRNPK, HSP90B1, ITGB4, KRT5, LGALS3, MYCN, NCL, P4HB, PABPC1, PABPN1, PHB2, PHB, PPP2CA, PRKCSH, RBL1, RBL2, SARNP, SATB1, SSBP1, TCF7L2, THOC2, THOC4, Thymidine Kinase, **TP53**, TPI1, YWHAG,	22	14	Cancer, Gene Expression, Cell Death
	3	26s Proteasome, Actin, BTRC, CD59, ENO1, EP300, ESR1, ESRRA, EZR, FOXP3, FUBP1, HNRNPU, HSPE1, Jnk, KRT8, KRT19, LAMC2, Lamin b, MDH2, MSN, MYB, PLEC, POSTN, PPP2CA, PPP2R1A, RAC2, SERPINA1, SNAI2, SRC, TGFA, TGFB1, TNF, TNFSF11, VIM, WISP2	16	11	Cellular Movement, Cancer, Dermatological Diseases and Conditions

The score associated to each network indicates the degree of relevance of a network to the molecules of the input dataset and takes into account the number of Network Eligible Genes and the size of the network. Only a score value >15 was considered. Fischer's exact test was used to evaluate the probability that each biological function assigned to that network is due to a random event.

Supplementary Table 6. IPA Protein Function Profiles

siMDM4 vs siCTL				
Category	Functions Annotation	p-Value	Molecules	# Molecules
Cellular Growth and Proliferation	growth of cells	1,12E-04	ACTN4, ANXA1, BCAP31, CCT3, HADHB, HNRNPF, ITGA5, RPSA, S100A11, SERPINH1, SLC3A2, SOD2	12
	growth of tumor cells	4,60E-03	ACTN4, ITGA5, SLC3A2	3
	proliferation of breast cancer cell lines	1,13E-02	EEF1A1, ITGA5, SOD2	3
	colony formation of tumor cell lines	1,41E-02	ANXA1, HNRNPA2B1, SOD2	3
	size of breast cancer cell lines	1,44E-02	ITGA5	1
	colony formation of embryonic cell lines	1,68E-02	SOD2	1
	colony formation of epithelial cell lines	2,15E-02	SOD2	1
	proliferation of carcinoma cell lines	3,18E-02	ANXA1, HNRNPA2B1	2
	growth of tumor cell lines	3,43E-02	ACTN4, ANXA1, ITGA5, SLC3A2, SOD2	5
	proliferation of tumor cell lines	4,58E-02	ANXA1, EEF1A1, HNRNPA2B1, ITGA5, SOD2	5
siMDM4+Adr vs siCTL+Adr				
Category	Functions Annotation	p-Value	Molecules	# Molecules
Cell Death	apoptosis of tumor cell lines	1.53E-07	B2M, CALR, CD59, FUBP1, HNRNPA1, HSPD1, ITGB1, ITGB4, LGALS1, LGALS3, LGALS3BP, MSN, NCL, NPM1, P4HB, PHB, S100A11, SOD2	18
	cell death of tumor cell lines	2.83E-07	B2M, CALR, CD59, FUBP1, HNRNPA1, HSPD1, ITGB1, ITGB4, LAMP1, LGALS1, LGALS3, LGALS3BP, MSN, NCL, NPM1, P4HB, PHB, S100A11, SOD2	19
	cell death of cancer cells	3.27E-07	B2M, CALR, CD59, ENO1, FUBP1, HNRNPA1, HSP90B1, HSPD1, ITGB1, ITGB4, LAMP1, LGALS1, LGALS3, LGALS3BP, MSN, NCL, NPM1, P4HB, PHB, S100A11, SOD2	21
	apoptosis of breast cancer cell lines	5.90E-05	B2M, HSPD1, ITGB1, ITGB4, LGALS3, PHB, SOD2	7
	activation-induced cell death	1.30E-04	CD59, ITGB1, RAC2	3
	cytolysis of tumor cell lines	1.72E-03	B2M, CD59	2
	killing of cells	1.97E-03	ITGB1, LGALS1, LGALS3	3
	apoptosis of carcinoma cells	2.22E-03	ENO1, ITGB4	2
	inhibition of apoptosis	3.73E-03	ANXA1, HSP90B1, HSPA9, TXNDC5	4
	apoptosis of breast cell lines	5.99E-03	ITGB1, NPM1	2
	cell death of epithelial cell lines	1.07E-02	DLST, HSPD1, ITGB1, PRDX3	4
	apoptosis of cancer cells	1.07E-02	ENO1, ITGB4, LGALS1	3
	cell death of organ	1.20E-02	DLST, ITGB1, LGALS1, PRDX3, SOD2	5
	apoptosis of organ	1.21E-02	ITGB1, LGALS1, SOD2	3
	toxicity of cells	1.24E-02	CD59	1
	anoikis	1.90E-02	ITGB1, LGALS3	2
	cell viability of tumor cell lines	2.40E-02	HSP90B1, P4HB, SOD2	3
	apoptosis of epithelial cell lines	2.53E-02	HSPD1, ITGB1, PRDX3	3
	anoikis of breast cancer cell lines	3.68E-02	LGALS3	1
	survival of breast cancer cell lines	3.69E-02	LGALS3, SOD2	2