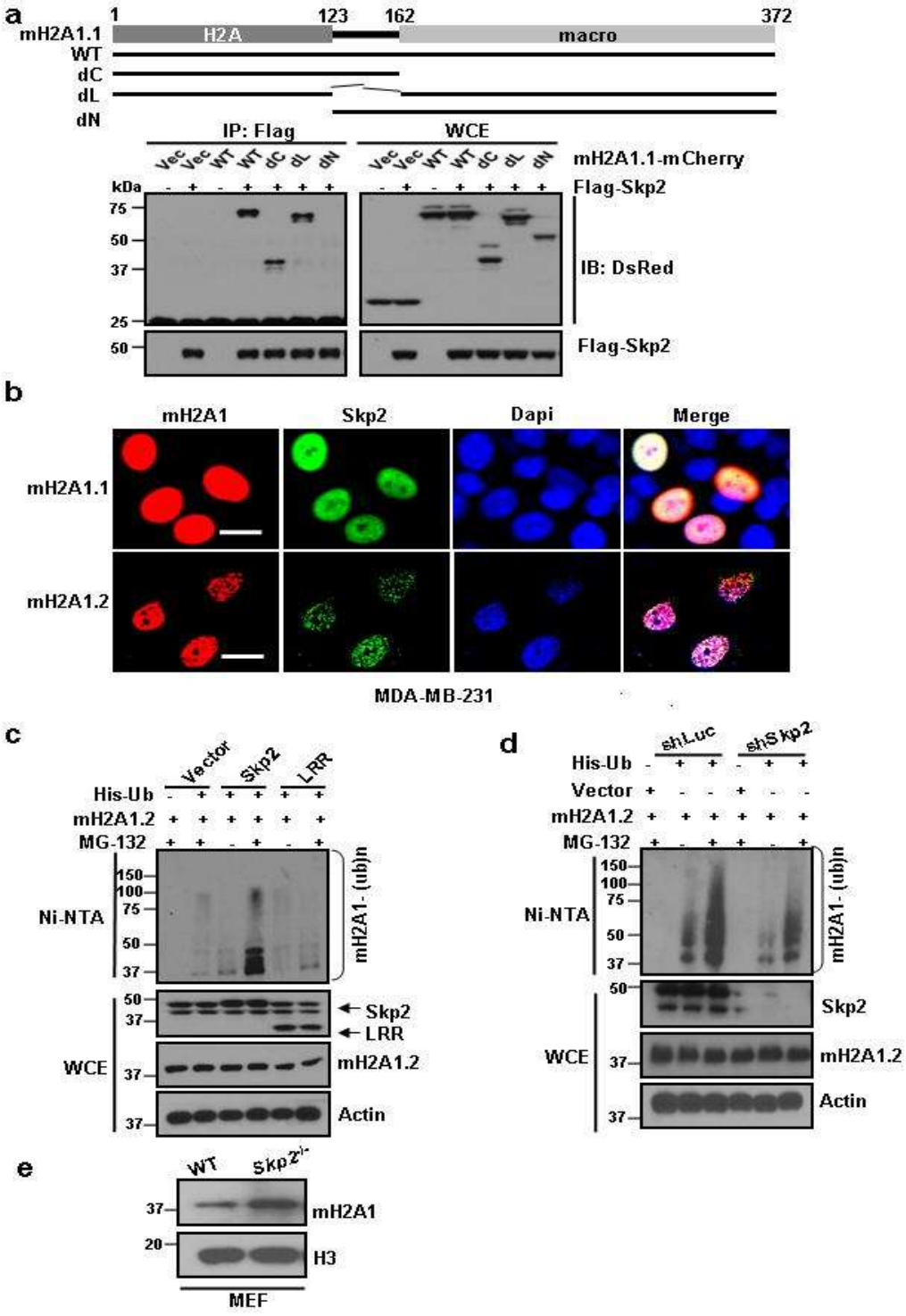
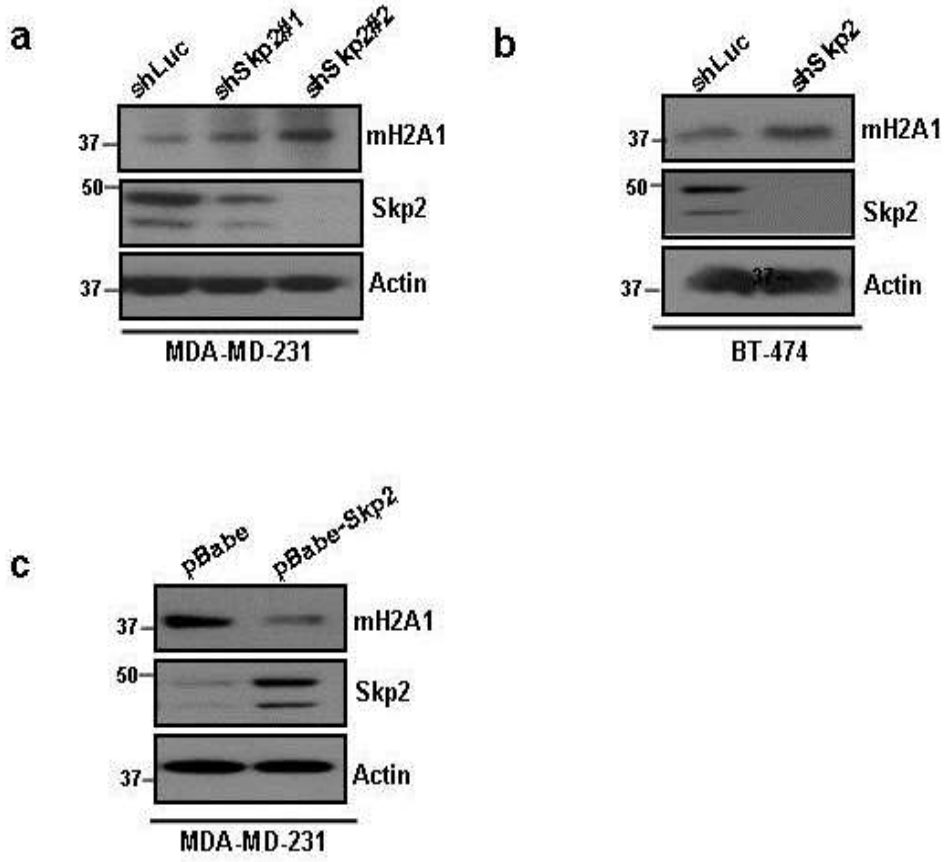
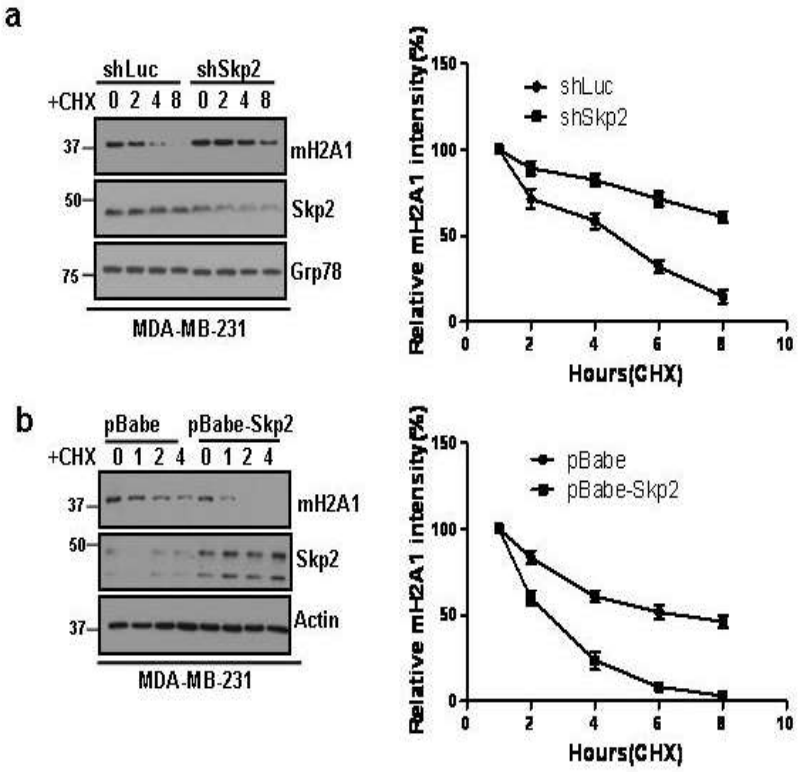

a
mH2A1.1
1
123
162
372
H2A
macro
WT
dC
dL
dN
IP: Flag
WCE
Vec Vec WT WT dC dL dN
mH2A1.1-mCherry
Flag-Skp2
kDa
75
50
37
25
IB: DsRed
Flag-Skp2
b
mH2A1
Skp2
Dapi
Merge
mH2A1.1
mH2A1.2
MDA-MB-231
c
Vector
Skp2
LRR
His-Ub
mH2A1.2
MG-132
Ni-NTA
mH2A1-(ub)n
WCE
Skp2
LRR
mH2A1.2
Actin
d
shLuc
shSkp2
His-Ub
Vector
mH2A1.2
MG-132
Ni-NTA
mH2A1-(ub)n
WCE
Skp2
mH2A1.2
Actin
e
WT
Skp2-/-
mH2A1
H3
MEF

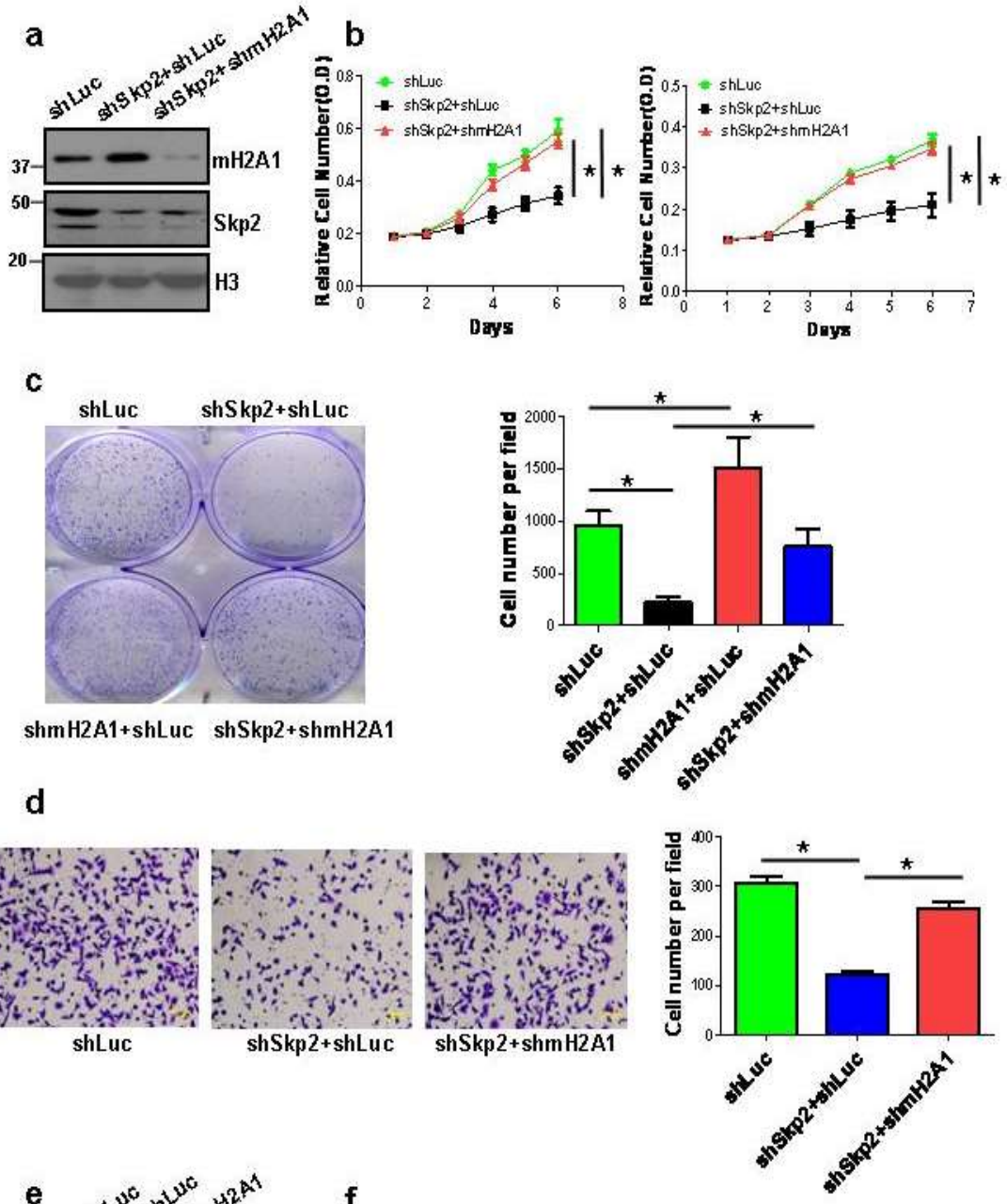
Supplementary Figure 1. Skp2 SCF is a direct E3 ligase for mH2A1. (a) 293T cells were transfected with indicated plasmids and harvested for immunoprecipitation assay. (b) MDA-MB-231 cells were transfected with indicated plasmids and harvested for immunofluorescence assay. Scale bar, 20μm. (c) In vivo ubiquitination assay of 293T cells transfected with various plasmids. Skp2-LRR: E3 ligase-dead mutant. (d) 293T cells with Luc or Skp2 knockdown were transfected with various plasmids and harvested for in vivo ubiquitination assay. (e) Lysates from primary *WT* and *Skp2*$^{-/-}$ MEFs were subjected to immunoblotting analysis.



Supplementary Figure 2. Skp2 negatively regulates mH2A1 protein expression. (a, b) MDA-MB-231 (a) or BT-474 cells (b) were infected with Luc or Skp2 shRNAs, selected and harvested for immunoblotting. (c) MDA-MB-231 cells stably expressing pBabe or pBabe-Skp2 were harvested for the immunoblotting assay.



Supplementary Figure 3. Skp2 regulates mH2A1 protein stability. (a) MDA-MB-231 cells with Luc or Skp2 knockdown were treated with cycloheximide (CHX) for the indicated times and harvested for the immunoblotting assay. (b) MDA-MB-231 cells stably expressing pBabe or pBabe-Skp2 were treated with cycloheximide (CHX) for the indicated times and harvested for the immunoblotting assay.


a
shLuc
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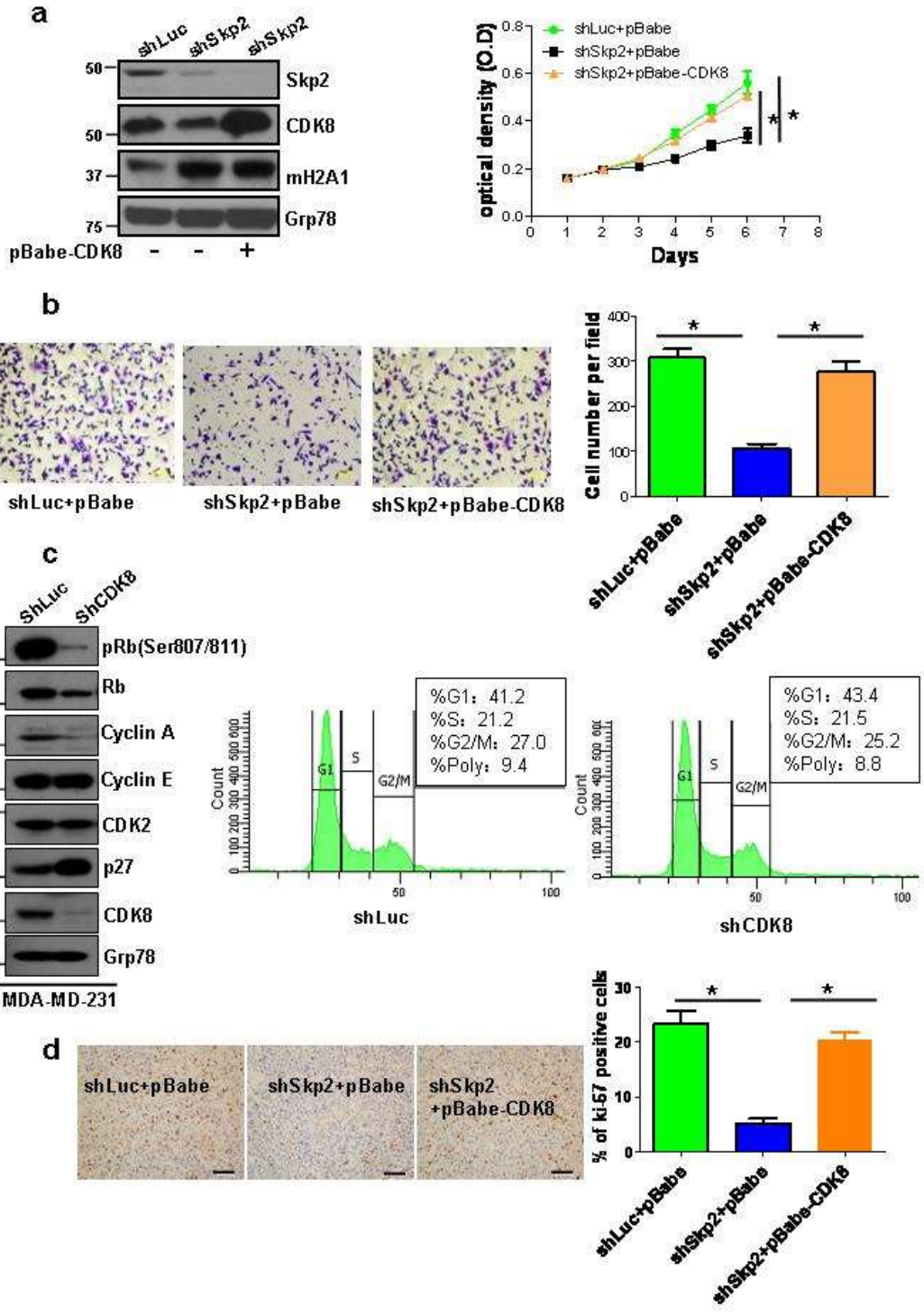
shSkp2+shmH2A1
37
50
20
mH2A1
Skp2
H3
b
shLuc
shSkp2+shLuc
shSkp2+shmH2A1
Relative Cell Number(O.D)
Days
c
shLuc
shSkp2+shLuc
shmH2A1+shLuc
shSkp2+shmH2A1
Cell number per field
d
shLuc
shSkp2+shLuc
shSkp2+shmH2A1
Cell number per field
e
shLuc
shSkp2+shLuc
shmH2A1+shLuc
shSkp2+shmH2A1
50
37
50
Skp2
mH2A1
Tubulin
f
shLuc
shSkp2+shLuc
shmH2A1+shLuc
shSkp2+ shmH2A1
caspase-3: –
caspase-3:+
caspase-3:–
caspase-3:+

Supplementary Figure 4. mH2A1 deficiency rescues the defects in cell growth and migration upon Skp2 loss. (a) MDA-MB-231 cells were infected with various shRNAs, selected and harvested for Immunoblotting (Left panel). MTT assay of MDA-MB-231 cells infected with various shRNAs (Right panel). (b) MTT assay in BT474 cells with Luc knockdown, Skp2 knockdown or Skp2 plus mH2A1 knockdown. The quantified results are presented as means ± s.d. (n = 3). $P<0.05$. (c) Colony forming assay of BT474 cells with Luc knockdown, Skp2 knockdown or Skp2 plus mH2A1 knockdown. (d) MDA-MB-231 cells infected with various shRNAs were plated for cell transwell cell migration assay. The data are presented as means ± s.d. (*n* = 3). Scale bars, 100 μm. (e) MDA-MB-231 cells infected with various shRNAs were subcutaneously injected into nude mice, and breast tumours were harvested from nude mice at week 5 for Immunoblotting. (original magnification 20X). (f) MDA-MB-231 cells infected with various shRNAs were subcutaneously injected into nude mice, and breast tumours were harvested from nude mice at week 5 for cleaved caspase-3 staining by IHC Scale bars, 50μm. See the method for detailed scoring.


a
shLuc
shSkp2
shSkp2
50
Skp2
50
CDK8
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mH2A1
75
Grp78
pBabe-CDK8 - - +
shLuc+pBabe
shSkp2+pBabe
shSkp2+pBabe-CDK8
optical density (O.D)
Days
b
shLuc+pBabe
shSkp2+pBabe
shSkp2+pBabe-CDK8
Cell number per field
c
ShLuc
ShCDK8
100
pRb(Ser807/811)
100
Rb
50
Cyclin A
50
Cyclin E
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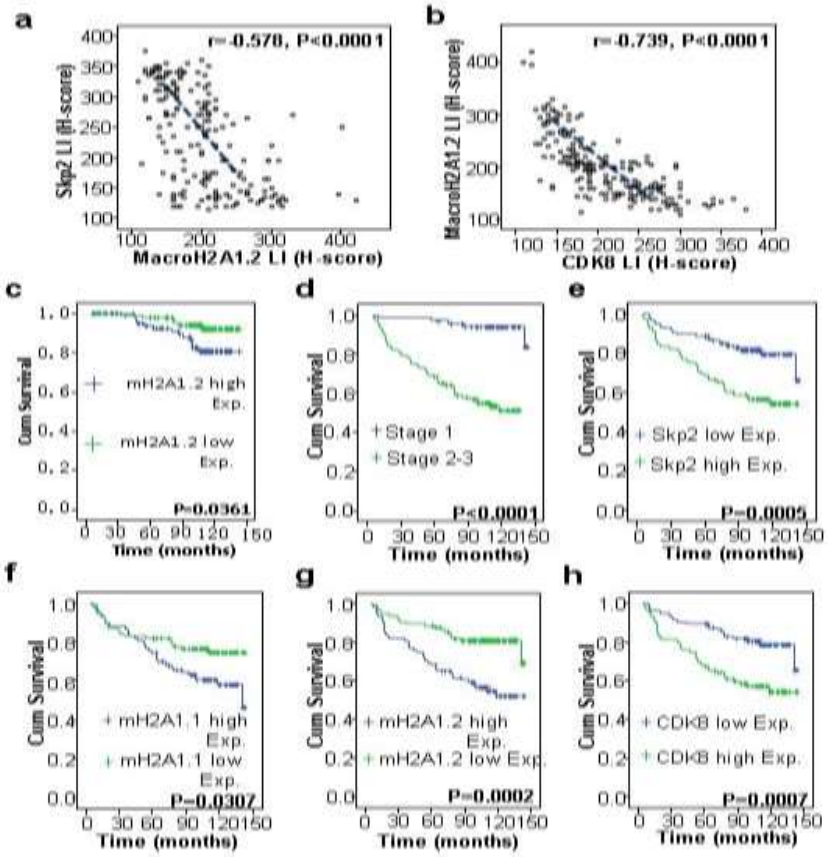
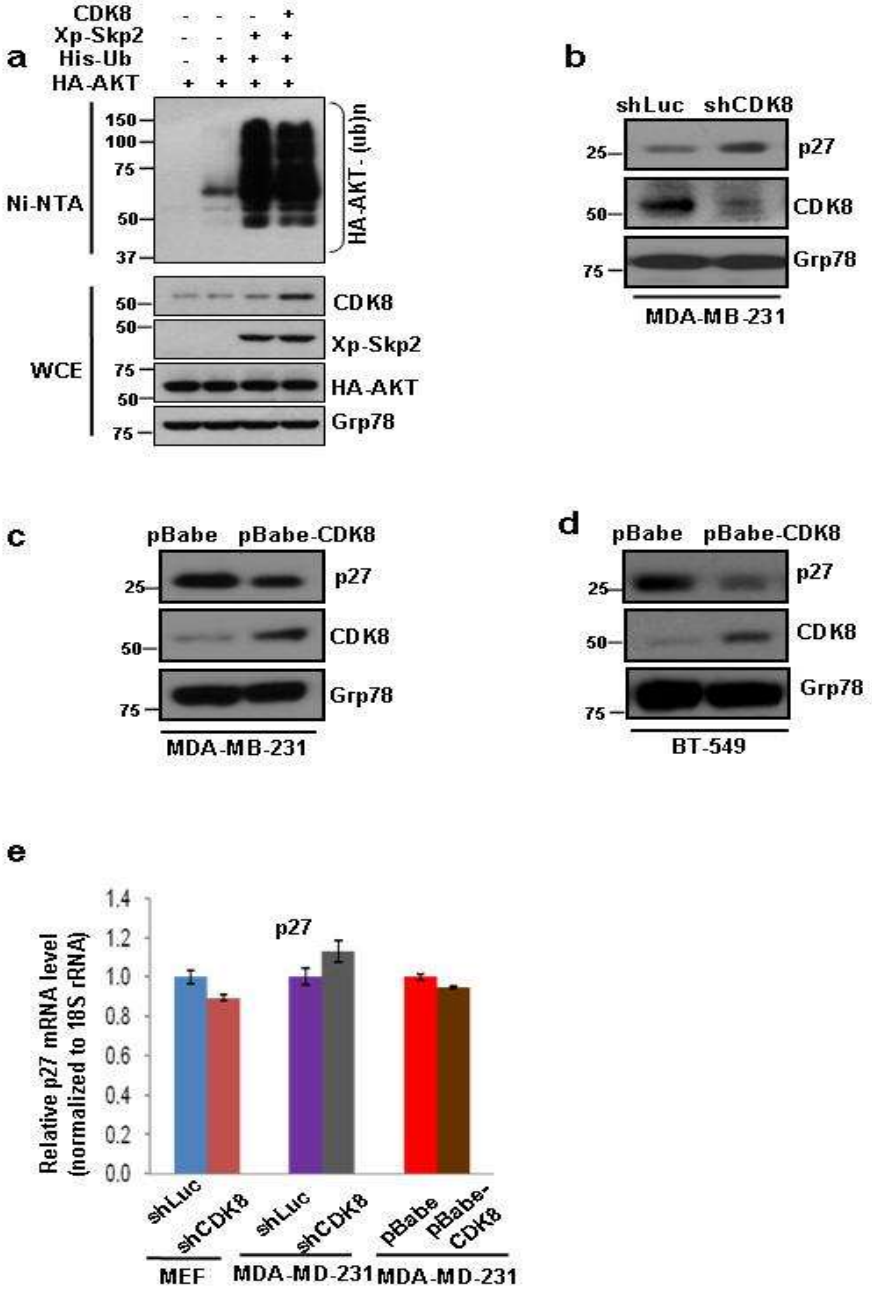
CDK2
25
p27
50
CDK8
75
Grp78
MDA-MD-231
%G1：41.2
%S：21.2
%G2/M：27.0
%Poly：9.4
shLuc
%G1：43.4
%S：21.5
%G2/M：25.2
%Poly：8.8
shCDK8
d
shLuc+pBabe
shSkp2+pBabe
shSkp2
+pBabe-CDK8
% of ki-67 positive cells

Supplementary Figure 5. CDK8 restoration rescues the defect in cell growth and migration upon *Skp2* loss. (a) Immunoblotting assay (Left panel) and MTT assay (Right panel) of MDA-MB-231 cells with Luc, Skp2 knockdown or Skp2 knockdown plus CDK8 overexpression. (b) Migration assay of MDA-MB-231 cells with GFP, Skp2 knockdown or Skp2 plus CDK8 overexpression. The quantified results are presented as means ± s.d. (n = 3). *$p<0.05$. (c) Immunoblotting assay (Left panel) and Flow cytometry analysis (Right panel) of MDA-MB-231 cells with shLuc or shCDK8 expression. (d) MDA-MB-231 cells infected with various shRNAs and/or CDK8 retroviral vector were subcutaneously injected into nude mice, and breast tumours were harvested from nude mice at week 5 for Ki-67 staining by IHC and quantitated (Scale bars, 50μm.*$p<0.05$ using Student's *t*-test)

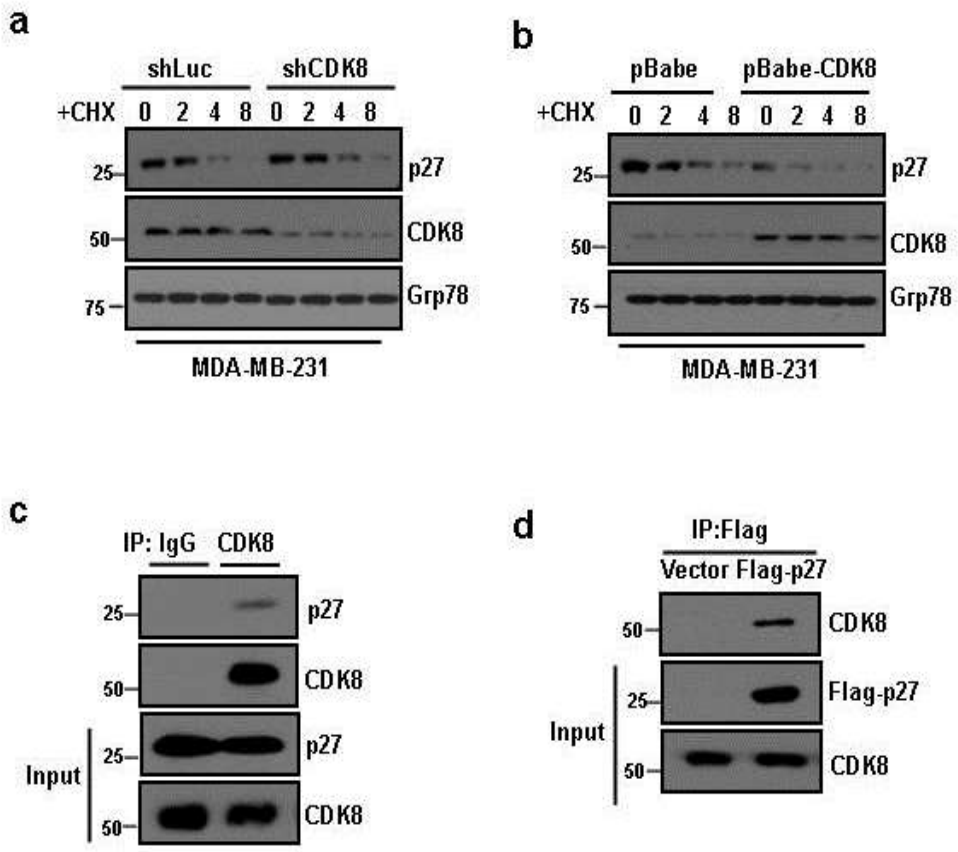


Supplementary Figure 6. Skp2, mH2A1.1, mH2A1.2 or CDK8 serves as an important biomarker for the survival outcome of breast cancer patients. (a) Skp2 expression was negatively associated with mH2A1.2 ($r=-0.578$, $p<0.001$) expression. (b) mH2A1.2 negatively correlated with CDK8 expression ($r=-0.638$, $p<0.001$ and $r=-0.739$, $p<0.001$, respectively). (c) Kaplan-Meier plot analysis of overall survival of 189 cases of breast cancer patients with low or high expression of mH2A1.2. (d-h), Kaplan-Meier plot analysis of metastasis-free of survival of 189 cases of breast cancer patients with different stages (d), with low or high expression of Skp2 (e), mH2A1.1 (f) mH2A1.2 (g) or CDK8 (h). p-value in all cases is < 0.01 by using Mann-Whitney U test.



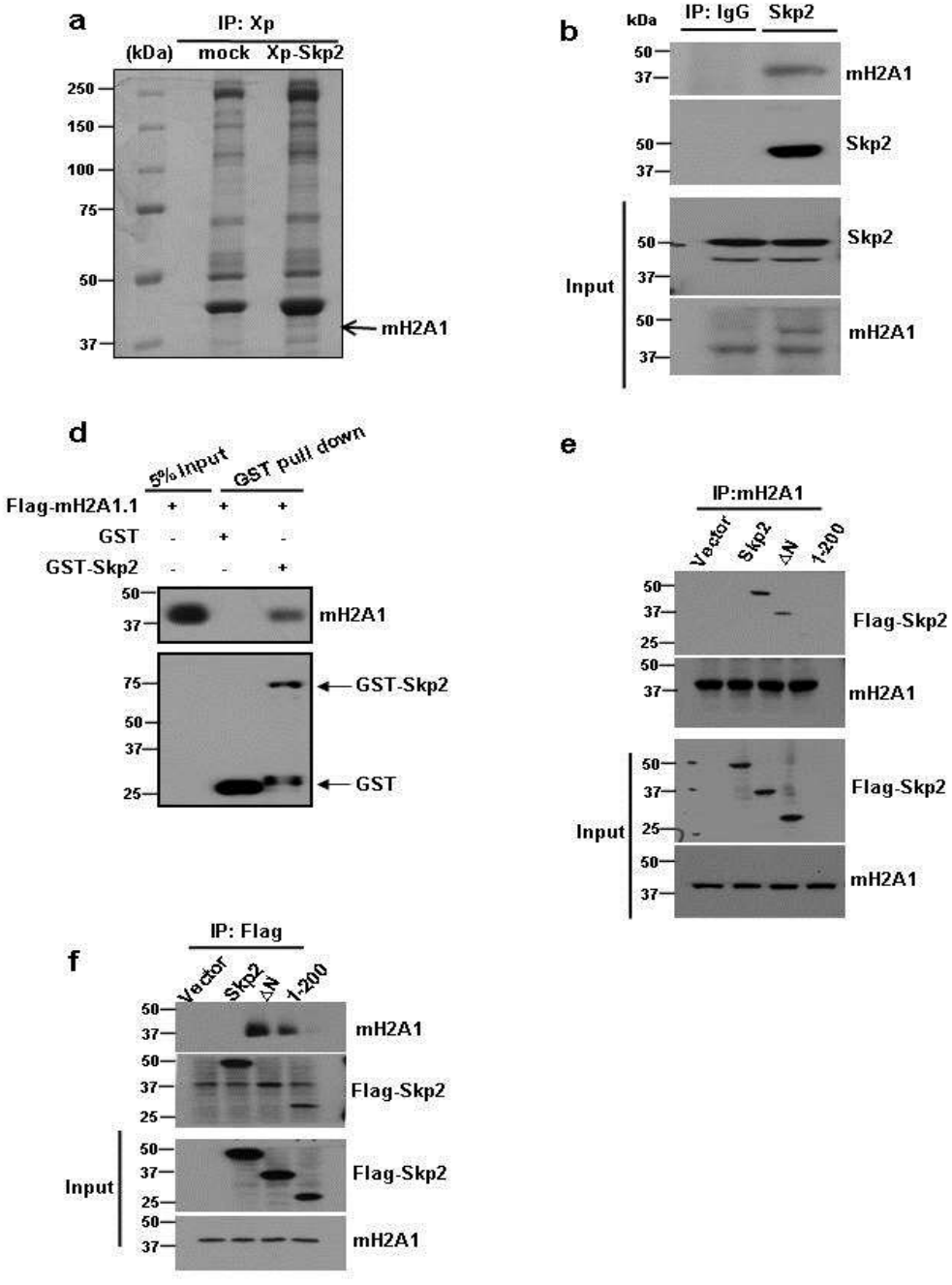
Supplementary Figure 7. CDK8 reduces p27 protein expression and stability, but not p27 mRNA level. (a) 293T cells were transfected with the indicated plasmids and harvested for in vivo ubiquitination assay. (b) MDA-MB-231 cells with Luc or Skp2

knockdown were harvested for the immunoblotting assay. (c) MDA-MB-231 cells with stably expressing pBabe or pBabe-Skp2 were harvested for the immunoblotting assay. (d) BT-549 cells with stably expressing pBabe or pBabe-Skp2 were harvested for the immunoblotting assay. (e) The mRNA levels of p27 were measured by real-time PCR in MEF and MDA-MB-231 cells with Luc or Skp2 knockdown or stably expressing pBabe or pBabe-Skp2 (n=3).



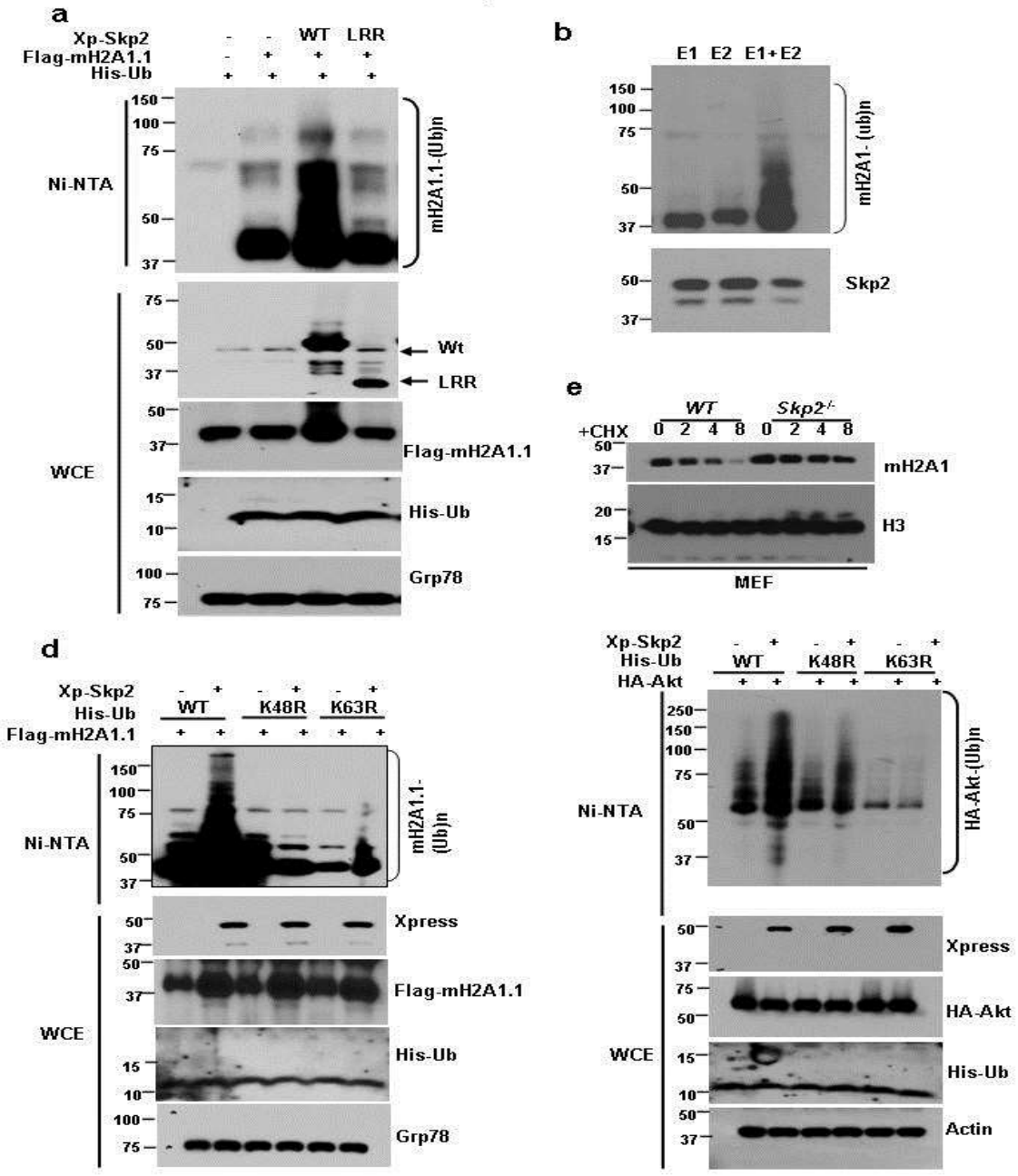
Supplementary Figure 8. CDK8 interacts with p27 and reduces 27_stability. (a) MDA-MB-231 cells with Luc or CDK8 knockdown were treated with cycloheximide (CHX) for the indicated times and harvested for the immunoblotting assay. (b) MDA-MB-231 cells stably expressing pBabe or pBabe-CDK8 were treated with cycloheximide (CHX) for the indicated times and harvested for the immunoblotting assay. (c-d) 293T cells were transfected with the indicated plasmids and harvested for immunoprecipitation, followed by the immunoblotting assay.

Fig. 1

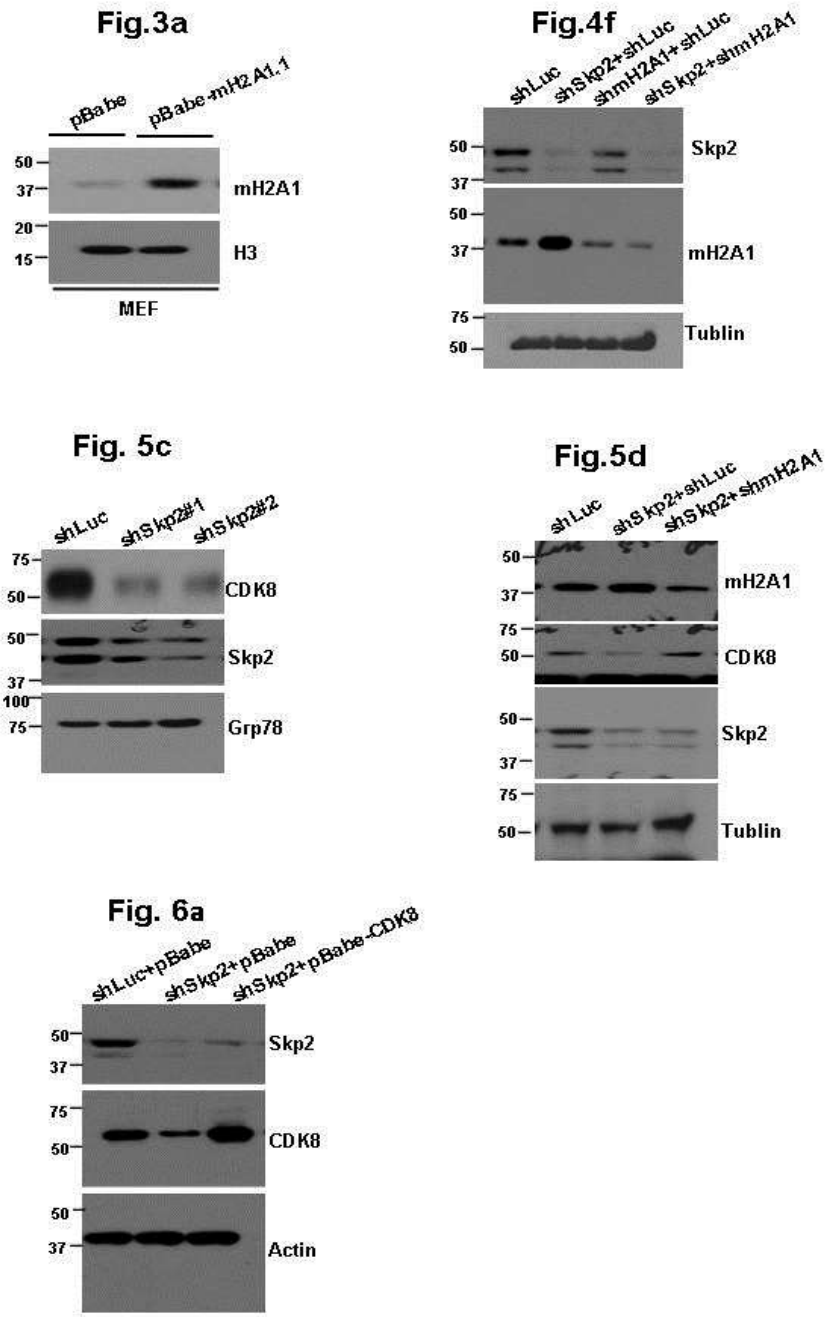


Supplementary Figure 9. Full scans of Western blots

Fig. 2

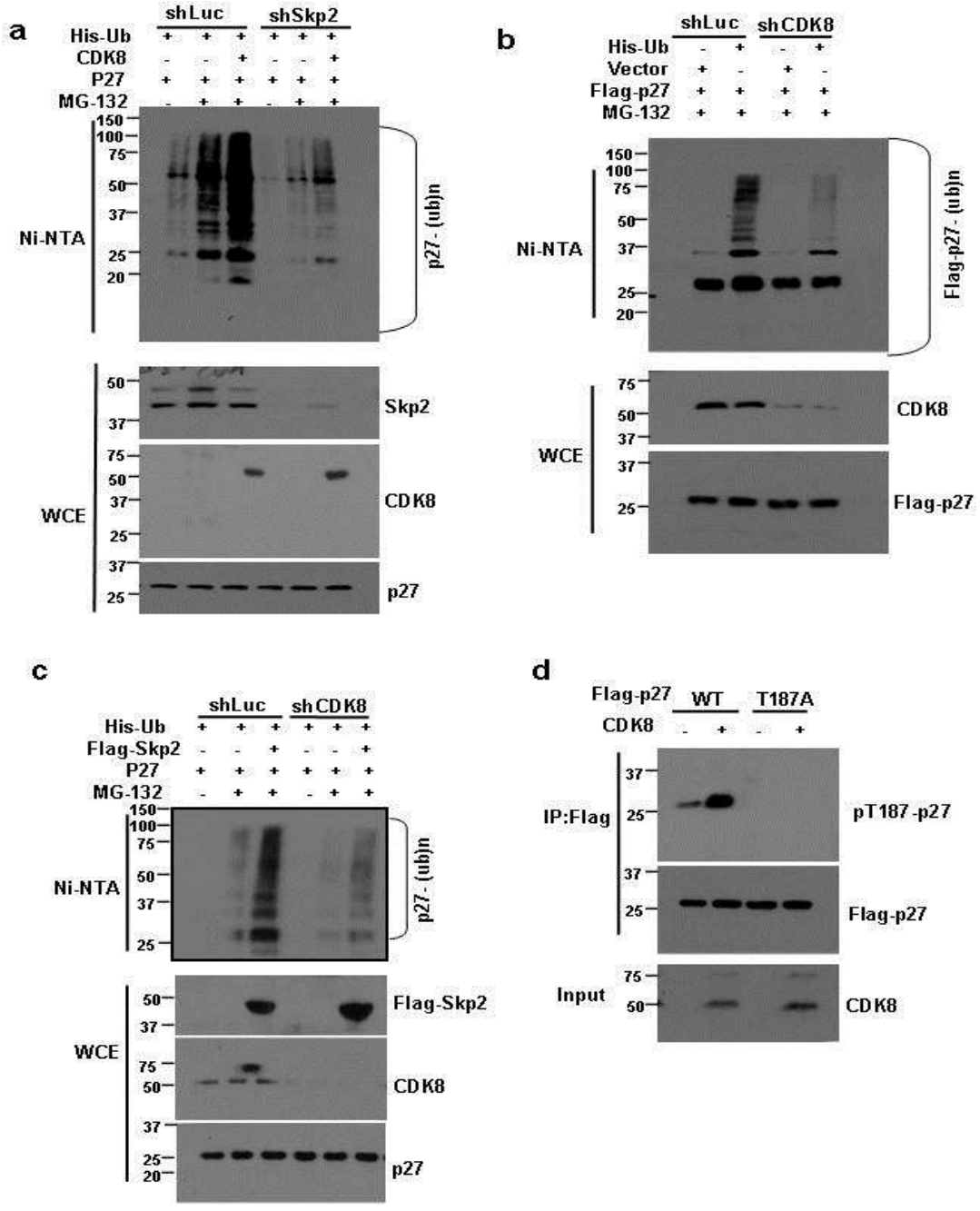


Supplementary Figure 10. Full scans of Western blots



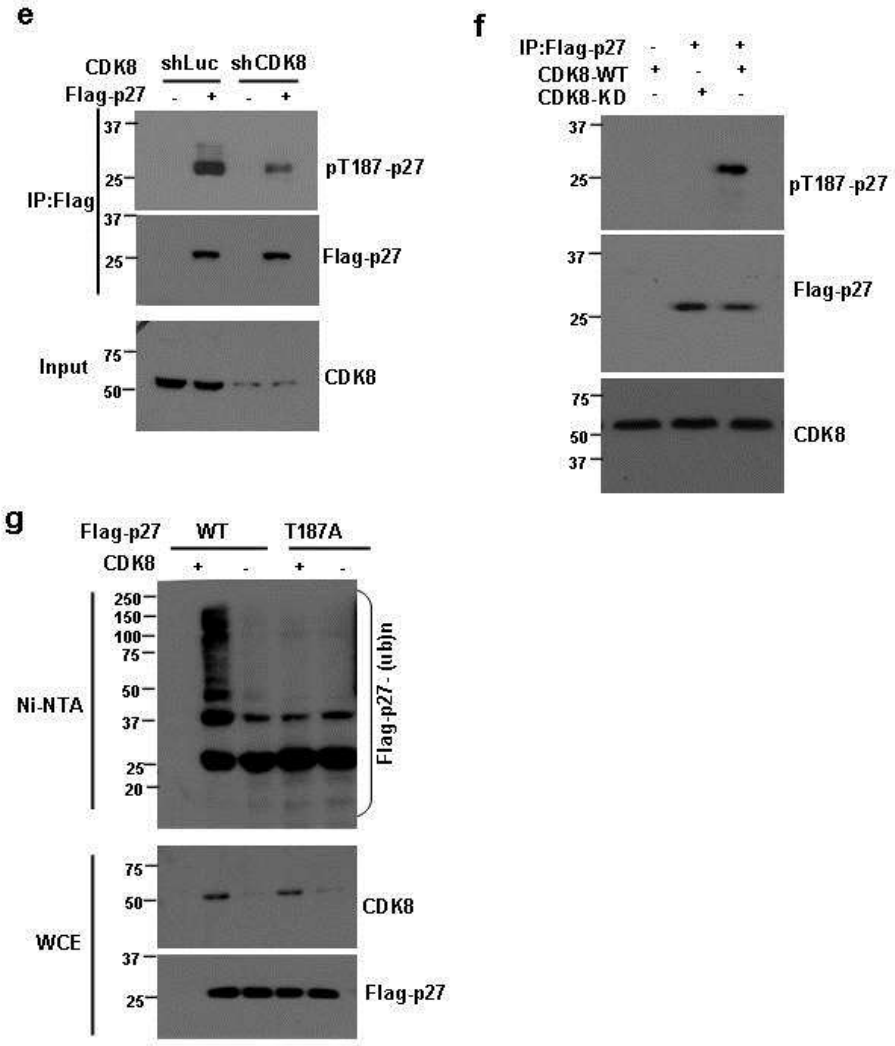
Supplementary Figure 11. Full scans of Western blots

Fig.7

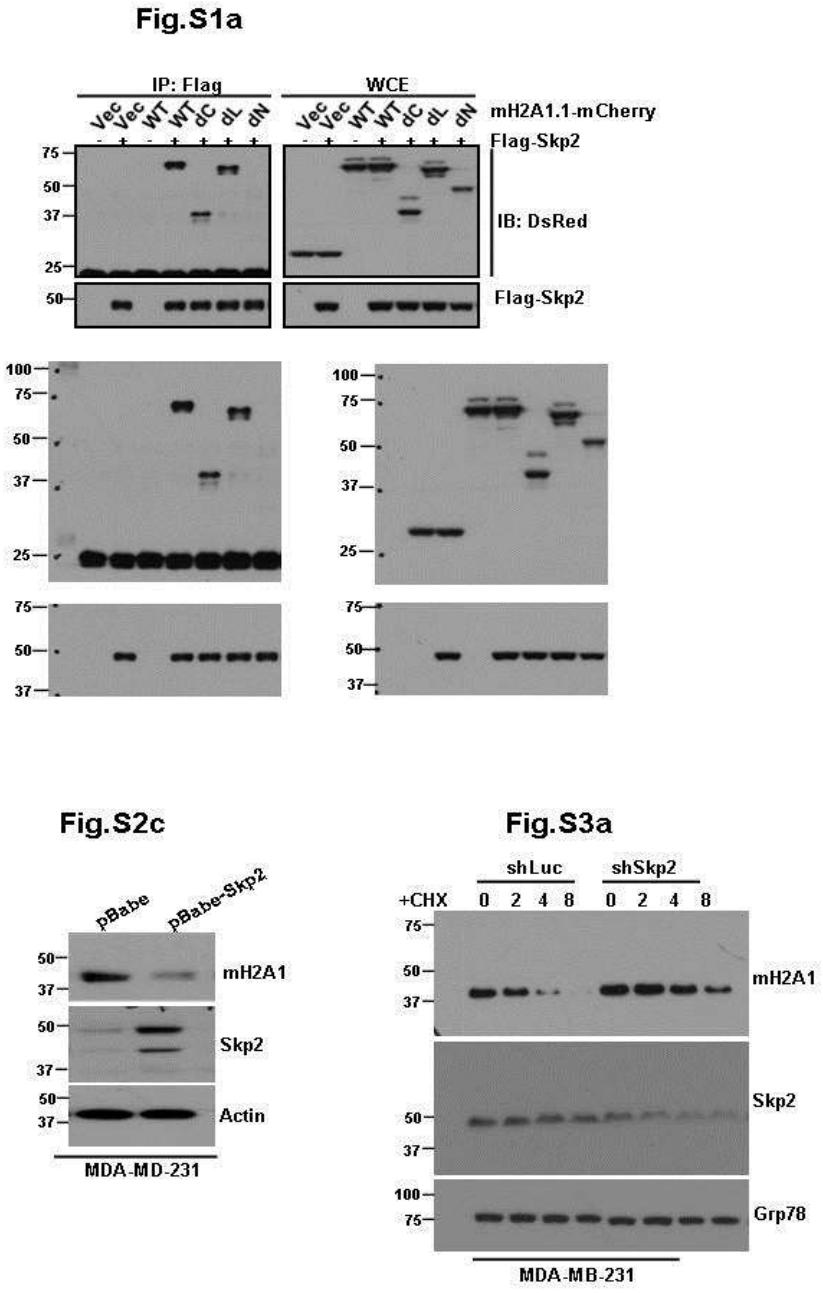


Supplementary Figure 12. Full scans of Western blots

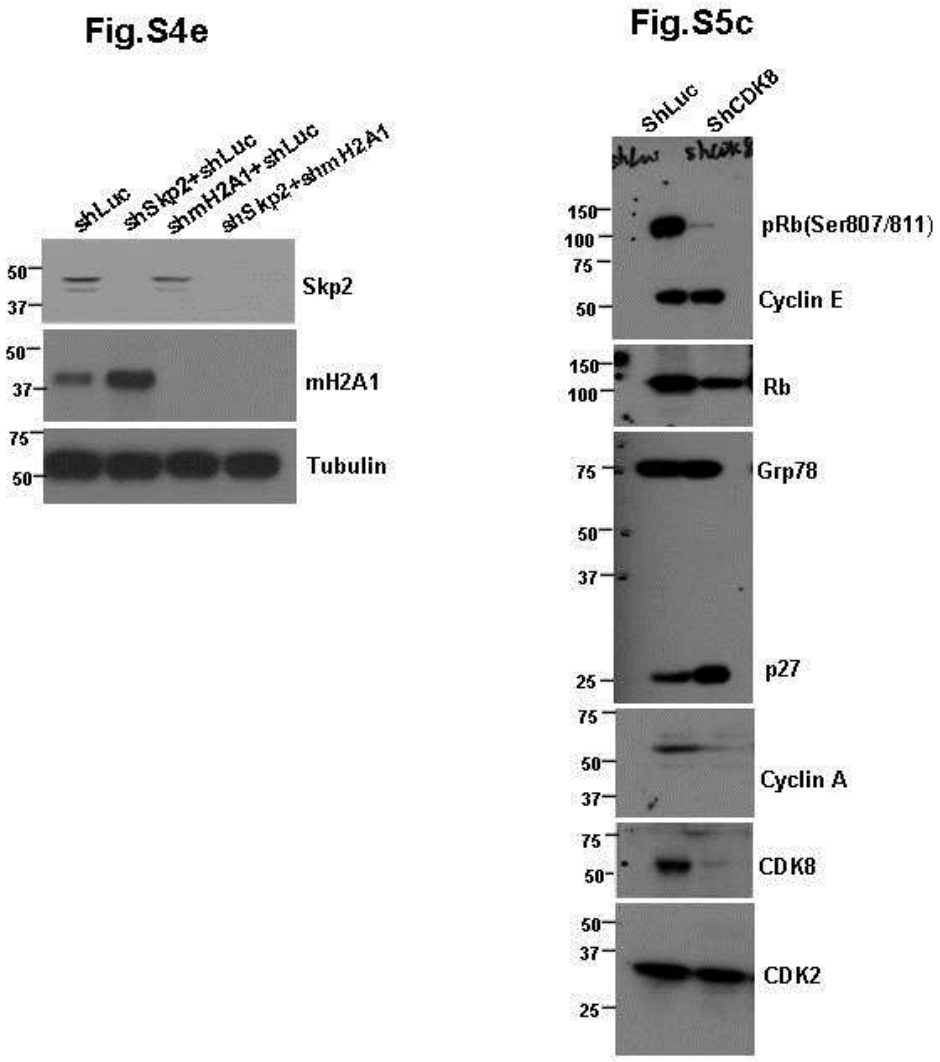
Fig.7



Supplementary Figure 13. Full scans of Western blots

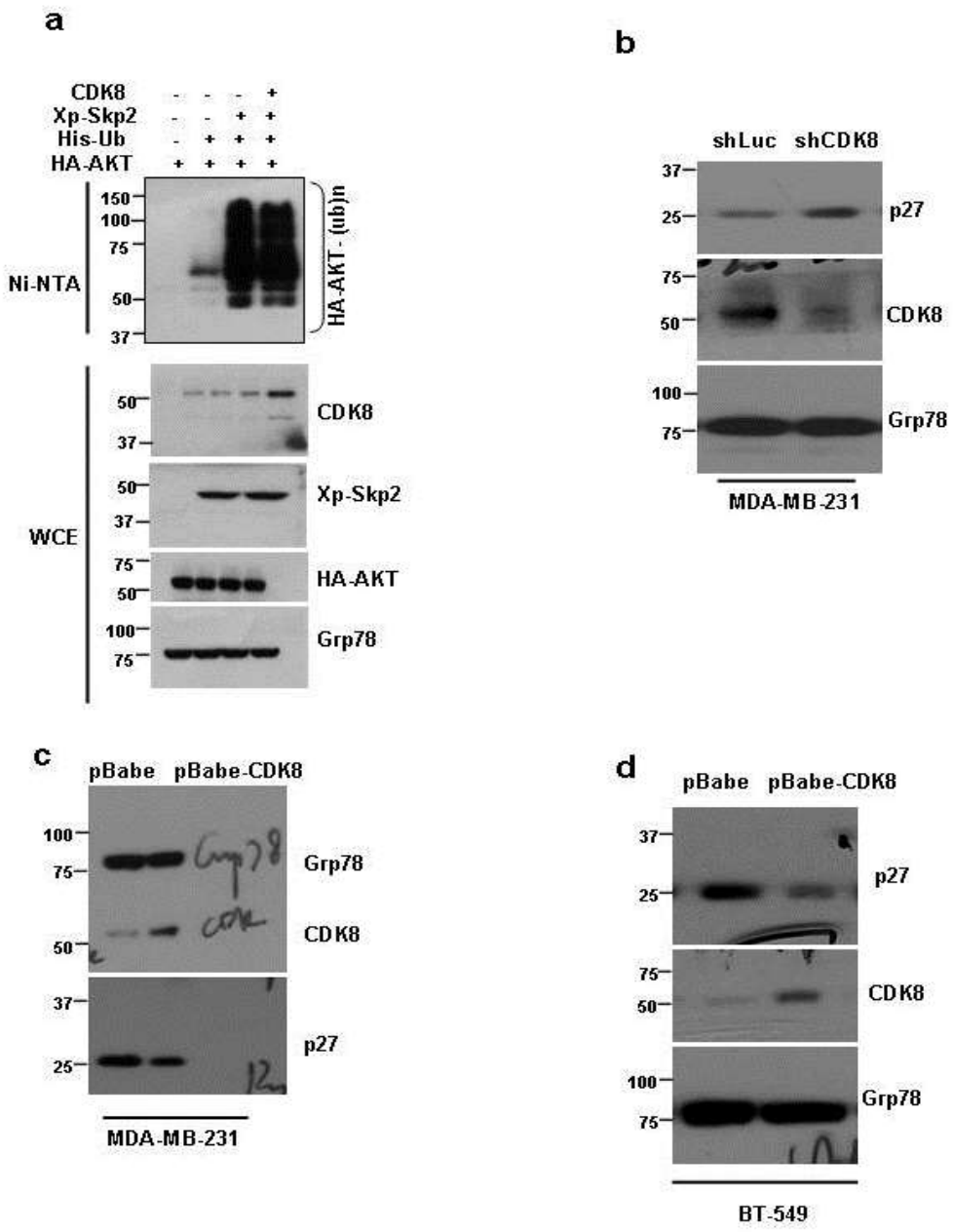


Supplementary Figure 14. Full scans of Western blots



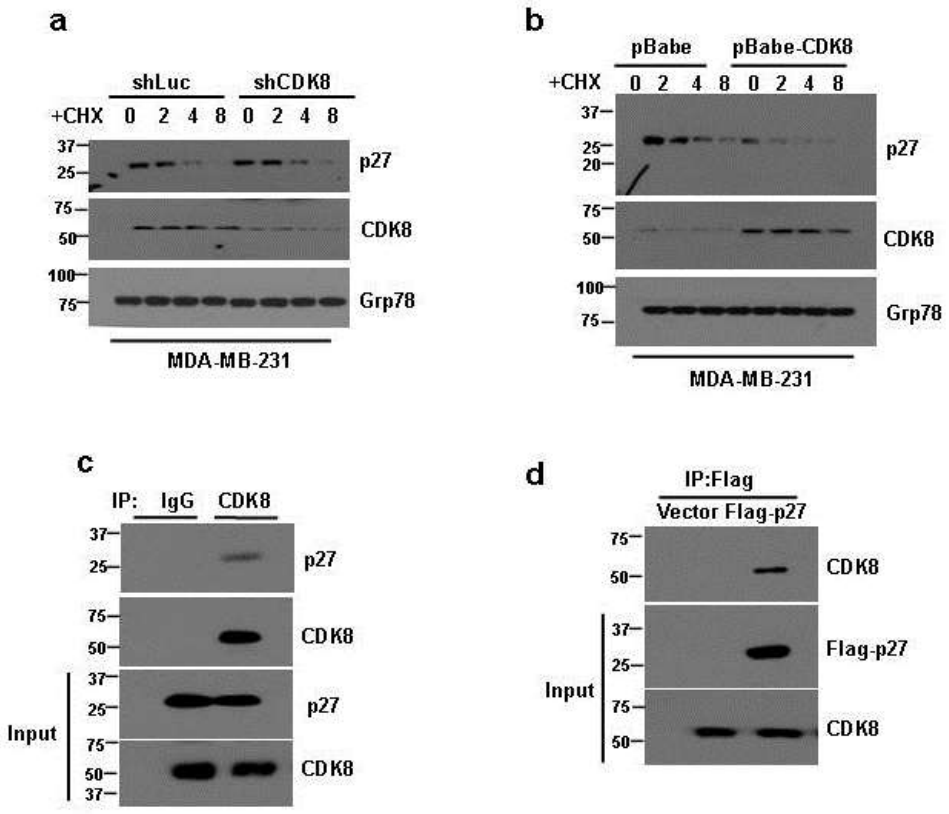
Supplementary Figure 15. Full scans of Western blots

Fig. S7



Supplementary Figure 16. Full scans of Western blots

Fig.S8



Supplementary Figure 17. Full scans of Western blots

Supplementary Table 1. The effect of Skp2 on gene expression profile of LT-HSCs

Total RNAs from *WT* and *Skp2*$^{-/-}$ LT-HSCs were subjected to microarray analysis. The result was shown as a ratio between *Skp2*$^{-/-}$ and *WT* HSCs (*Skp2*$^{-/-}$ verses *WT*). The data were deposited to Gene Expression Omnibus (GEO), the record number is GSE23064; Link:

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=hfinxomsugyialo&acc=GSE23064.

Gene Name	Description	Ratio
CDK5R1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	4.26
CDK5R2	cyclin-dependent kinase 5, regulatory subunit 2	2.69
CDK6	Mus musculus cyclin-dependent kinase 6 (Cdk6),	2.48
CDK10	Mus musculus cyclin-dependent kinase (CDC2-like) 10 (Cdk10)	2.13
CDK4	Mus musculus cyclin-dependent kinase 4 (Cdk4)	0.53
CDK7	Mus musculus cyclin-dependent kinase	0.09
CDK8	**Mus musculus cyclin-dependent kinase 8 (Cdk8)**	**0.25**
CDC42	Mus musculus CDC42 effector protein (Rho GTPase binding) 5 (Cdc42ep5)	2.83
cdca3	Mus musculus cell division cycle associated 3 (Cdca3)	2.33
cdc8	Mus musculus cell division cycle associated 8 (Cdca8)	2.31
cdc34	Mus musculus cell division cycle 34 homolog (S. cerevisiae) (Cdc34)	2.22
cdc27	Mus musculus cell division cycle 27 homolog (S. cerevisiae)	2.14
cdc37	Mus musculus cell division cycle 37 homolog (S. cerevisiae) (Cdc37)	0.54
UBE2	Mus musculus ubiquitin-conjugating enzyme E2O (Ube2o)	10.14
Dub1	Mus musculus deubiquitinating enzyme 1 (Dub1)	4.3
USP36	PREDICTED: Mus musculus ubiquitin specific peptidase 36	3.84
USP14	Mus musculus ubiquitin specific peptidase 14 (Usp14	3.82
USP43	Mus musculus ubiquitin specific peptidase 43 (Usp43)	3.22
USP42	Mus musculus ubiquitin specific peptidase 42 (Usp42)	2.5
USP4	Mus musculus ubiquitin specific peptidase 43 (Usp43)	0.49
Smurf1	Mus musculus SMAD specific E3 ubiquitin protein ligase 1 (Smurf1)	0.33
ube3a	Mus musculus ubiquitin protein ligase E3A (Ube3a)	0.3
EDD1	Mus musculus E3 ubiquitin protein ligase, HECT domain containing, 1 (Edd1)	0.3
Smurf2	Mus musculus SMAD specific E3 ubiquitin protein ligase 2 (Smurf2)	0.21
EDD1	Mus musculus E3 ubiquitin protein ligase, HECT domain containing, 1 (Edd1)	0.1
Ubc	Mus musculus 17 days embryo kidney cDNA, RIKEN full-length enriched library	0.37