Supplementary Figure legends

Supplementary Figure 1. (a-b) Effect of PML constitutive silencing (sh4, sh5) on PML protein expression (**a**, representative of at least 3 experiments) and on the morphology (**b**, representative images, scale bar 50μm). (**c**) Schematic representation of one representative experiment of the defined populations represented in main Fig.1c upon inducible PML silencing on MDA-MB-231 cells. **(d-e)** Effect on the number of senescent cells upon constitutive PML silencing (**d**; n=13) and representative images of SA-β-Galactosidase assay in MDA-MB-231 cells, scale bar 50μm (**e**). **(f-g)** Impact on cell number upon either constitutive (**f**, n=12) or inducible (**g**, sh1 and sh5, n=4, sh4, n=7) PML silencing in MDA-MB-231 cells. **(h)** Effect of PML inducible silencing (sh4) on apoptosis using staurosporine (Stp) as a positive control. **(i)** Effect on the number of senescent cells after 150 nM arsenic trioxide treatment during 6 days in MDA-MB-231 cells. **(j-l)** Proteomics analysis of the secretome of MDA-MB-231 cells upon PML inducible silencing: **(j)** PML and tubulin levels in both cells extracts and secretome samples (dash lines indicate samples used in the analysis), **(k)** unsupervised exploratory data analysis by means of principal component analysis and **(l)** heat maps representing the proteins that were significantly over- and under-secreted upon PML silencing in MDA-MB-231 cells. Data analysis was based on spectral count data after exporting it from Scaffold software into R. The GLM model based on the Poisson distribution was used to test significance. Only the proteins with spectral counts of 2, Log2FC of 0.8 and adjusted p-value of 0.05 are present in the heatmap. Columns represent samples; rows are proteins. Red represents proteins that are over-secreted and green represents proteins that are under-secreted. The data rows are centred and scaled to 1 standard deviation prior to produce the heat map. **(m)** Immunofluorescence of macroH2A1.1 and DAPI upon inducible silencing of PML in MDA-MB-231 cells. **(n-o)** Levels of Lamin B1 protein upon PML inducible silencing in MDA-MB-231 cells (**n**, representative of 5 experiments) and **(o)** protein quantification (n=5). **(p)** Effect on ROS production (n=4) after inducible PML silencing in MDA-MB-231 cells. **(q-r)** Impact of inducible PML silencing

on tumour growth **(q)** and tumour weight **(r)** of established MDA-MB-231 xenografts (sh4 no dox, n=10; sh4 dox, n=12). Error bars represent s.e.m. p, p-value (*p< 0.05, **p< 0.01, ***p< 0.001) for figure f and g (* sh4 vs shC or dox; $ sh5 vs shC; # sh1 vs shC). One-tailed Student´s t-test (d, i) and one-tailed one sample t-test (f-g, o-p) were used for cell line data analysis, and one-tailed Mann-Whitney U-test for xenografts (q-r). shC: Scramble shRNA, sh1, sh4 and sh5: shRNA against *PML*, Dox: doxycycline, SA-β-gal: Senescence-associated beta-galactosidase, VC: vehicle control, ATO: arsenic trioxide, ROS: reactive oxygen species. Molecular weight markers (kDa) are shown to the right.

Supplementary Figure 2. (a-b) Protein levels of p27 and PML after either inducible (sh1) (**a**) or constitutive (**b**) PML silencing in MDA-MB-231 cells (representative of 3 experiments). **(c)** Quantification of p27 and PML protein levels along 6 days of doxycycline-inducible PML silencing on MDA-MB-231 cells (n=3) with sh1. **(d-f)** Representative western blots of inducible PML silencing with the 3 different short hairpins during 6 days of induction graphed on main Fig. 2b-c and Suppl. Fig. 2c. **(g-h)** Immunofluorescence quantification of nuclear p27 positive cells (**g**) and correlation of p27 positive cells and PML levels **(h)** (upon PML inducible silencing on MDA-MB-231 cells with sh1). **(i)** Schematic representation of the p27-Rb protein interaction and regulation. Error bars represent s.e.m. p, p-value (*p< 0.05, **p< 0.01, ***p< 0.001). One-tailed Student´s t-test (g-h) and one-sample t-test (c) were used for cell line data analysis. shC: Scramble shRNA, sh1, sh4 and sh5: shRNA against *PML*, Dox: doxycycline, D: day, RB: retinoblastoma protein. CDK: cyclin dependent kinases. Molecular weight markers (kDa) are shown to the right.

Supplementary Figure 3. (a) p27 and PML protein levels after constitutive silencing of either p27 or PML or both in MDA-MB-231 cells (representative of 4 experiments). **(b-c)** Effect on the number of senescent cells (n=4) **(b)** and cell growth (n=3) **(c)** after constitutive p27 and/or PML silencing. Error bars represent s.e.m. p, p-value (*p< 0.05, **p< 0.01, ***p< 0.001 compared with shC or as indicated, ns: not significant). One-tailed Student´s t-test (b) and one-tailed one-sample t-test (c)

were used for cell line data analysis. shC: Scramble shRNA, SA-β-gal: Senescence-associated beta-galactosidase. Molecular weight markers (kDa) are shown to the right.

Supplementary Figure 4. (a) Correlation analysis between PML and MYC (top panels) and between PML and PIM1 (bottom panels) mRNA levels in all breast cancer subtypes of tumor specimens of the indicated breast cancer datasets. Sample sizes: Ivshina (n=249), Lu (n=131), TCGA (n=522) and Wang (n=286). **(b-d)** MYC, p27 and PML protein levels (representative of 3 experiments) **(b)**, quantification of the protein (n=3) **(c)** and MYC gene levels **(d)** after inducible silencing of PML (sh4) in MDA-MB-231 cells (n=3). **(e)** PML, p27 and MYC mRNA levels upon doxycycline-inducible PML silencing (sh4) of established MDA-MB-231 xenografts. (**f-h)** MYC, p27 and PML protein levels (representative of 3 experiments) **(f)**, quantification of the protein (n=3) **(g)** and MYC gene levels **(h)** after inducible silencing of PML (sh4) in MDA-MB-468 cells (n=3). **(i-j)** MYC, p27 and PML protein levels (representative of 5 experiments) **(i)** and quantification of the protein (n=5) **(j)** after 150 nM arsenic trioxide treatment during 6 days in MDA-MB-231 cells. **(k-l)** Protein quantification from Fig. 4e **(k)** and impact in cell number (n=3) **(l)** of inducible MYC silencing (sh42) in MDA-MB-231 cells. **(m-n)** PIM1 gene levels after PML inducible silencing in MDA-MB-231 (n=3) **(m)** and MDA-MB-468 (n=3) **(n)** cells. **(o-q)** Protein quantification from Fig. 4i **(o)**, gene expression levels of PIM1, MYC and p27 **(p)** and impact in cell number (n=3) **(q)** of inducible PIM1 silencing (sh18) in MDA-MB-231 cells. Error bars represent s.e.m. p, p-value (*p< 0.05, **p< 0.01, ***p< 0.001, ns: not significant). One-tailed one sample t-test (c-d, g-h, j-q) was used for cell line data analysis and one-tailed Mann-Whitney U-test for xenografts (e). sh4: shRNA against *PML*, sh42: shRNA against *MYC*, sh18: shRNA against *PIM1*. Dox: doxycycline. VC: vehicle control. ATO: arsenic trioxide. Molecular weight markers (kDa) are shown to the right.

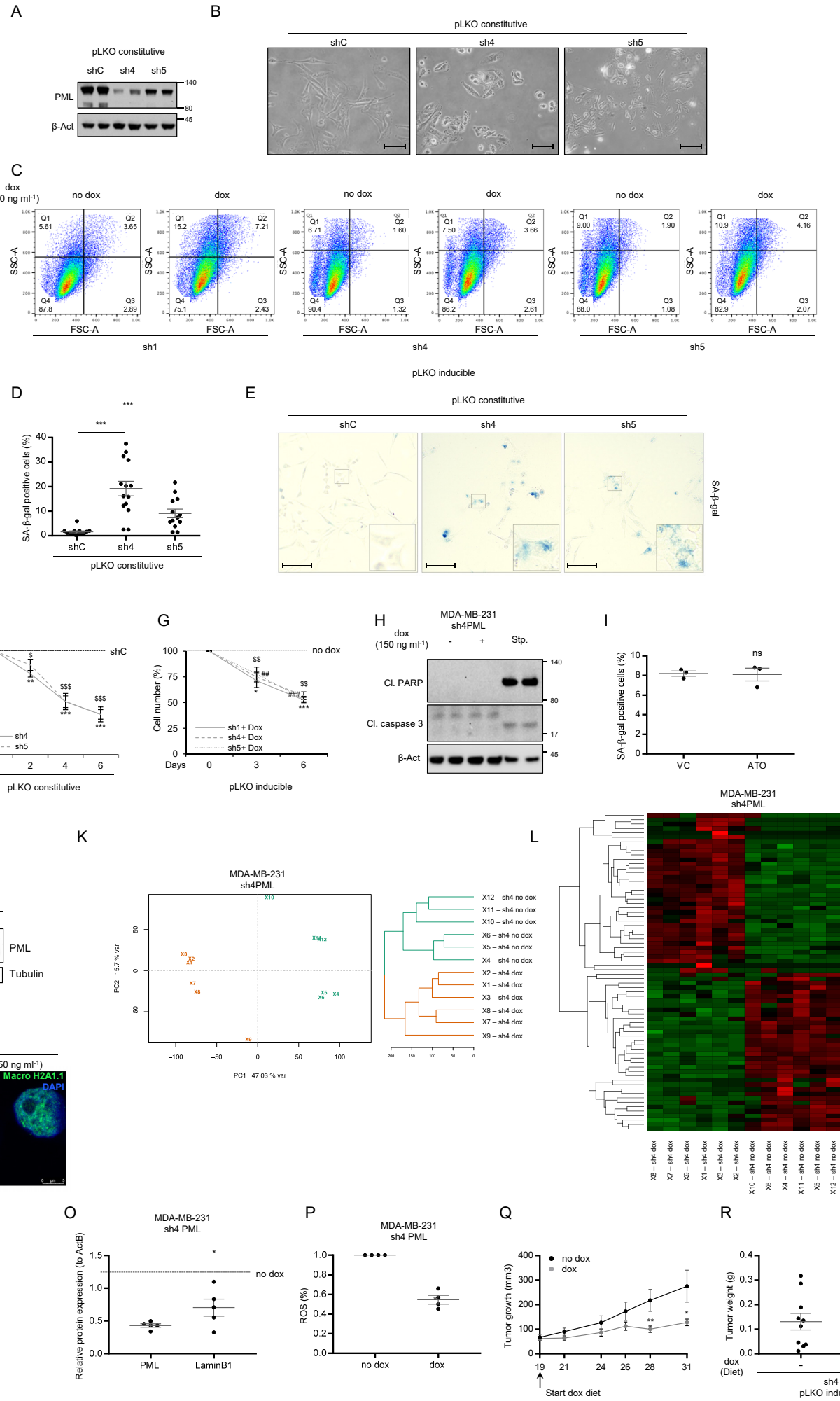
Supplementary Figure 5. (a-c) Impact of PML inducible silencing (sh4) on cell number in MDA-MB-468 **(a)**, MCF-7 **(b)** and Cama-1 **(c)** cell lines. **(d-e)** p27, MYC and PML protein levels **(d)** and

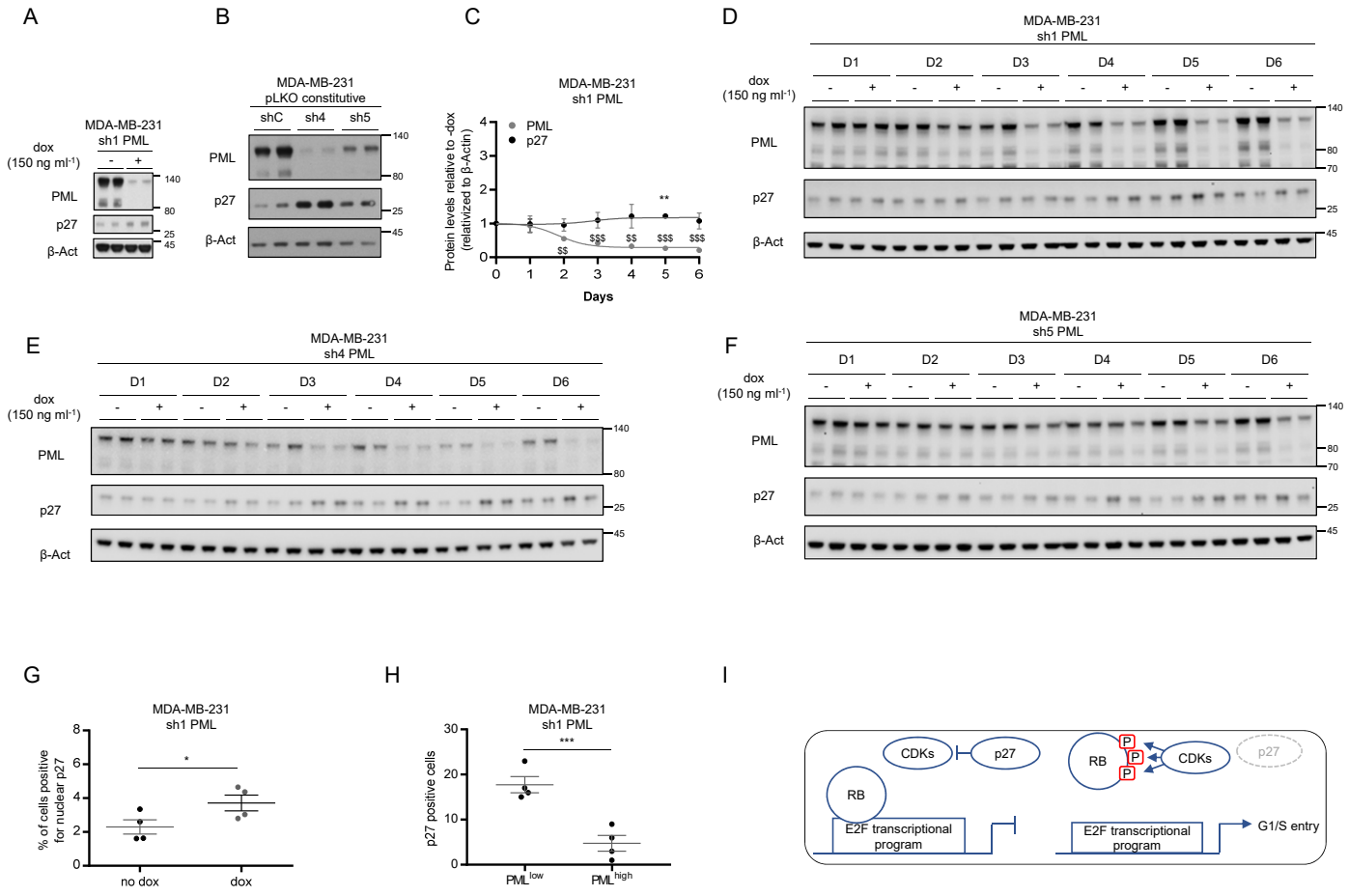
protein quantification **(e)** of PML inducible silencing in MCF-7 cells. **(f-g)** p27, MYC and PML protein levels **(f)** and protein quantification **(g)** of PML inducible silencing in Cama-1 cells. **(h)** Impact of PML inducible silencing (sh4) on cell morphology in MDA-MB-468, MCF-7 and Cama-1 cell lines, scale bar 50μm. **(i-k)** Proteomics analysis of the secretome of Cama-1 cells upon PML inducible silencing: **(i)** PML and tubulin levels in both cells extracts and secretome samples (dash lines indicate samples used in the analysis), **(j)** unsupervised exploratory data analysis by means of principal component analysis and **(k)** heat maps representing the proteins that were significantly over- and under-secreted, upon PML silencing in Cama-1 cells. Data analysis was based on spectral count data after exporting it from Scaffold software into R. The GLM model based on the Poisson distribution was used to test significance. Only the proteins with spectral counts of 2, Log_2FC of 0.8 and adjusted p-value of 0.05 are present in the heat map. Columns represent samples; rows are proteins. Red represents proteins that are over-secreted and green represents proteins that are under-secreted. The data rows are centered and scaled to 1 standard deviation prior to produce the heatmap. Error bars represent s.e.m. p, p-value (*p< 0.05, **p< 0.01, ***p< 0.001). One-tailed one sample t-test was used for cell line data analysis (a-c, e, g). sh4: shRNA against *PML*. Dox: doxycycline. Molecular weight markers (kDa) are shown to the right.

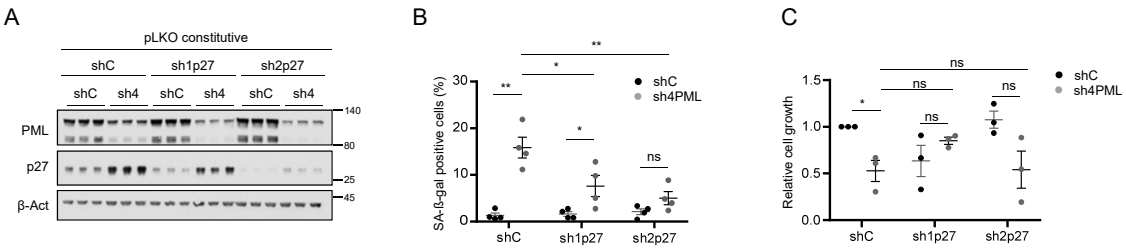
Supplementary Figure 6. Uncropped scans.

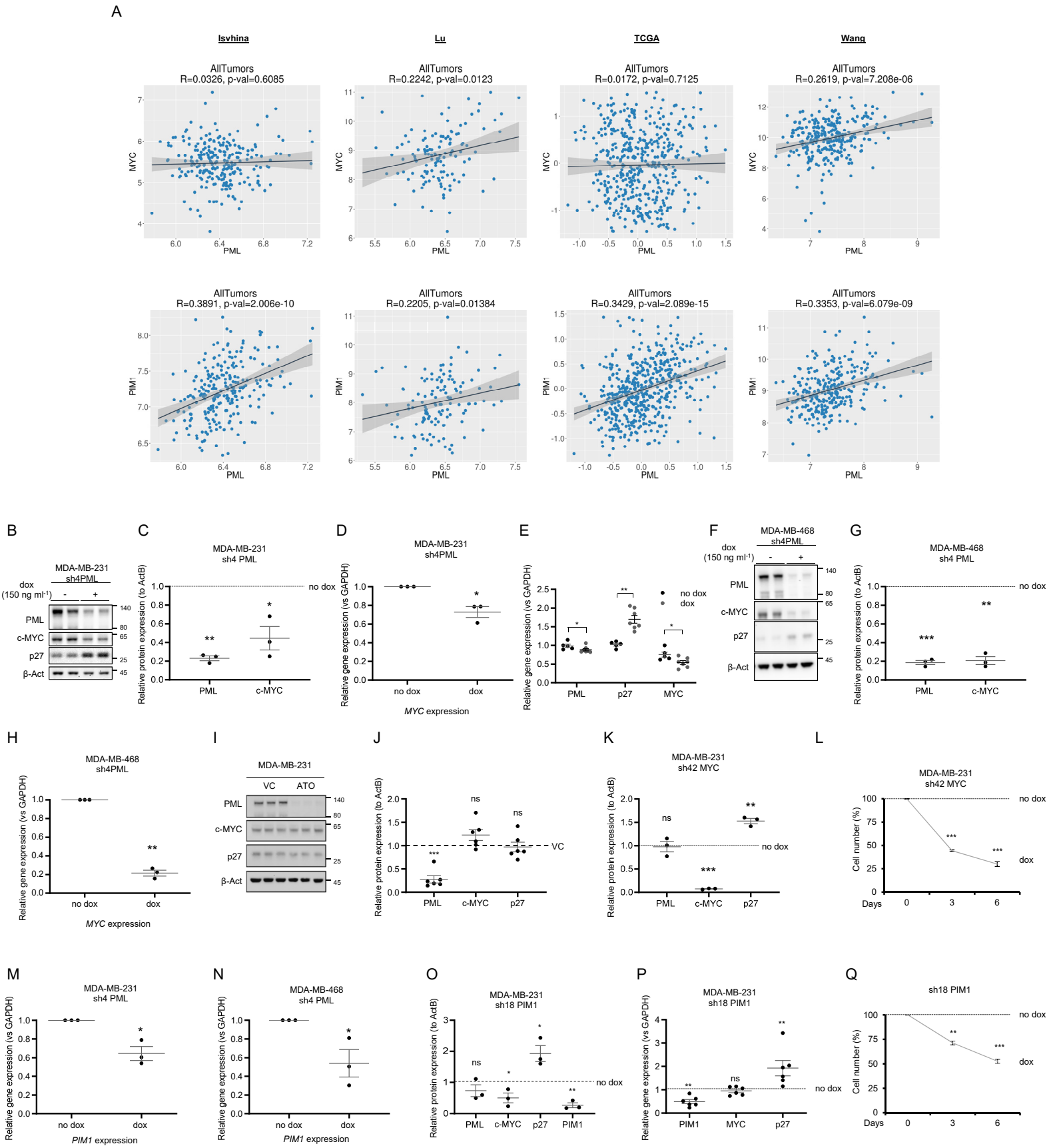
Supplemental Table 1. List of differentially secreted proteins upon silencing the PML protein in MDA-MB-231 cells.

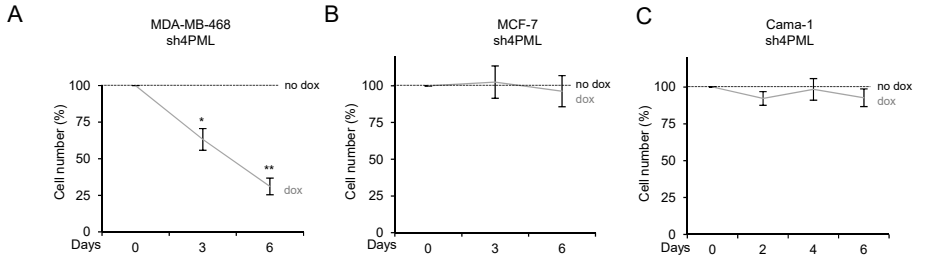
Supplemental Table 2. List of differentially secreted proteins upon silencing the PML protein in Cama-1 cells.

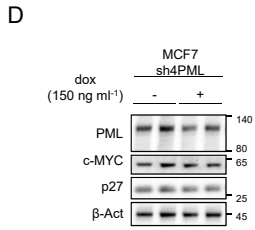

A
pLKO constitutive
shC
sh4
sh5
PML
β-Act
140
80
45
B
pLKO constitutive
shC
sh4
sh5
C
dox (150 ng ml-1)
no dox
dox
SSC-A
FSC-A
Q1 5.61
Q2 3.65
Q4 87.8
Q3 2.89
Q1 15.2
Q2 7.21
Q4 75.1
Q3 2.43
Q1 6.71
Q2 1.60
Q4 90.4
Q3 1.32
Q1 7.50
Q2 3.66
Q4 86.2
Q3 2.61
Q1 9.00
Q2 1.90
Q4 88.0
Q3 1.08
Q1 10.9
Q2 4.16
Q4 82.9
Q3 2.07
sh1
sh4
sh5
pLKO inducible
D
SA-β-gal positive cells (%)
shC
sh4
sh5
pLKO constitutive
E
pLKO constitutive
shC
sh4
sh5
SA-β-gal
F
Cell number (%)
shC
sh4
sh5
Days
pLKO constitutive
G
Cell number (%)
no dox
sh1+ Dox
sh4+ Dox
sh5+ Dox
Days
pLKO inducible
H
MDA-MB-231 sh4PML
dox (150 ng ml-1)
Stp.
Cl. PARP
Cl. caspase 3
β-Act
140
80
17
45
I
SA-β-gal positive cells (%)
ns
VC
ATO
J
MDA-MB-231 sh4PML
dox (150 ng ml-1)
cells
secretome
PML
Tubulin
K
MDA-MB-231 sh4PML
PC2 15.7 % var
PC1 47.03 % var
X12 – sh4 no dox
X11 – sh4 no dox
X10 – sh4 no dox
X6 – sh4 no dox
X5 – sh4 no dox
X4 – sh4 no dox
X2 – sh4 dox
X1 – sh4 dox
X3 – sh4 dox
X8 – sh4 dox
X7 – sh4 dox
X9 – sh4 dox
L
MDA-MB-231 sh4PML
M
MDA-MB-231 sh4PML
no dox
dox (150 ng ml-1)
Macro H2A1.1
DAPI
N
MDA-MB-231 sh4PML
dox (150 ng ml-1)
PML
LaminB1
β-Act
140
80
55
45
O
MDA-MB-231 sh4 PML
Relative protein expression (to ActB)
no dox
PML
LaminB1
P
MDA-MB-231 sh4 PML
ROS (%)
no dox
dox
Q
Tumor growth (mm3)
no dox
dox
Start dox diet
R
Tumor weight (g)
p=0.0901
dox (Diet)
sh4
pLKO inducible

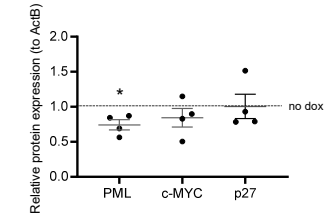

A
MDA-MB-231
sh1 PML
dox
(150 ng ml-1)
PML
p27
β-Act
B
MDA-MB-231
pLKO constitutive
shC
sh4
sh5
C
MDA-MB-231
sh1 PML
PML
p27
Protein levels relative to -dox
(relativized to β-Actin)
Days
D
MDA-MB-231
sh1 PML
D1
D2
D3
D4
D5
D6
E
MDA-MB-231
sh4 PML
F
MDA-MB-231
sh5 PML
G
MDA-MB-231
sh1 PML
% of cells positive
for nuclear p27
no dox
dox
H
MDA-MB-231
sh1 PML
p27 positive cells
PML low
PML high
I
CDKs
p27
RB
E2F transcriptional
program
G1/S entry

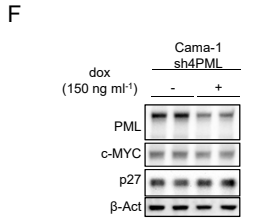

A
pLKO constitutive
shC
sh1p27
sh2p27
shC
sh4
shC
sh4
shC
sh4
PML
p27
β-Act
140
80
25
45
B
SA-ß-gal positive cells (%)
shC
sh4PML
shC
sh1p27
sh2p27
ns
C
Relative cell growth
shC
sh4PML
ns
ns
ns
shC
sh1p27
sh2p27

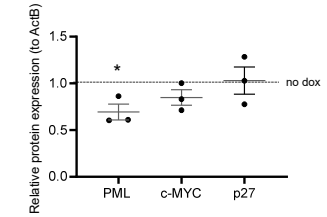

A
Isvhina
Lu
TCGA
Wang
AllTumors
R=0.0326, p-val=0.6085
R=0.2242, p-val=0.0123
R=0.0172, p-val=0.7125
R=0.2619, p-val=7.208e-06
R=0.3891, p-val=2.006e-10
R=0.2205, p-val=0.01384
R=0.3429, p-val=2.089e-15
R=0.3353, p-val=6.079e-09
MYC
PIM1
PML
B
MDA-MB-231
sh4PML
dox
(150 ng ml-1)
PML
c-MYC
p27
β-Act
C
MDA-MB-231
sh4 PML
Relative protein expression (to ActB)
no dox
PML
c-MYC
D
MDA-MB-231
sh4PML
Relative gene expression (vs GAPDH)
no dox
dox
MYC expression
E
Relative gene expression (vs GAPDH)
PML
p27
MYC
F
MDA-MB-468
sh4PML
G
MDA-MB-468
sh4 PML
H
MDA-MB-468
sh4PML
I
MDA-MB-231
VC
ATO
J
Relative protein expression (to ActB)
ns
VC
K
MDA-MB-231
sh42 MYC
L
MDA-MB-231
sh42 MYC
Cell number (%)
Days
M
MDA-MB-231
sh4 PML
PIM1 expression
N
MDA-MB-468
sh4 PML
O
MDA-MB-231
sh18 PIM1
P
MDA-MB-231
sh18 PIM1
Q
sh18 PIM1

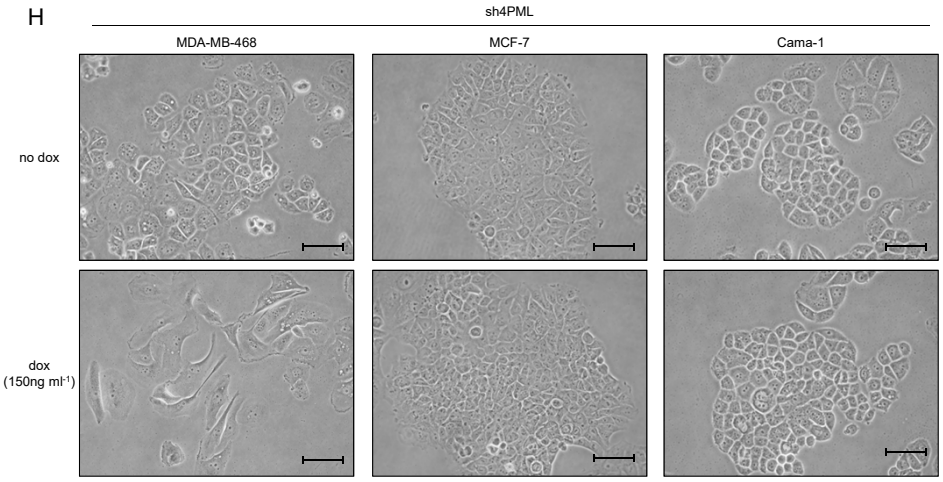

A
MDA-MB-468
sh4PML
Cell number (%)
no dox
dox
Days
B
MCF-7
sh4PML
C
Cama-1
sh4PML

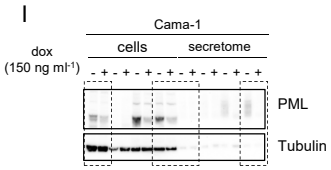

D
MCF7
sh4PML
dox
(150 ng ml-1)
PML
c-MYC
p27
β-Act

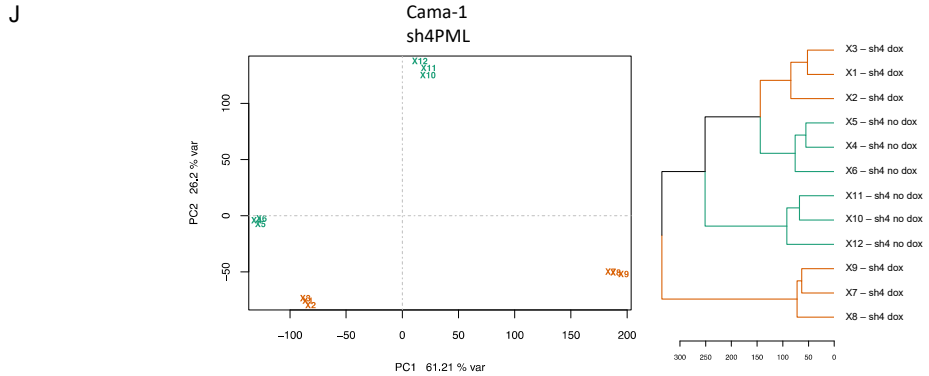

E
Relative protein expression (to ActB)
no dox
PML
c-MYC
p27


F
Cama-1
sh4PML
dox
(150 ng ml-1)
PML
c-MYC
p27
β-Act


G
Relative protein expression (to ActB)
no dox
PML
c-MYC
p27


H
sh4PML
MDA-MB-468
MCF-7
Cama-1
no dox
dox
(150ng ml-1)


I
Cama-1
dox
(150 ng ml-1)
cells
secretome
PML
Tubulin


J
Cama-1
sh4PML
PC2 26.2 % var
PC1 61.21 % var
X3 – sh4 dox
X1 – sh4 dox
X2 – sh4 dox
X5 – sh4 no dox
X4 – sh4 no dox
X6 – sh4 no dox
X11 – sh4 no dox
X10 – sh4 no dox
X12 – sh4 no dox
X9 – sh4 dox
X7 – sh4 dox
X8 – sh4 dox

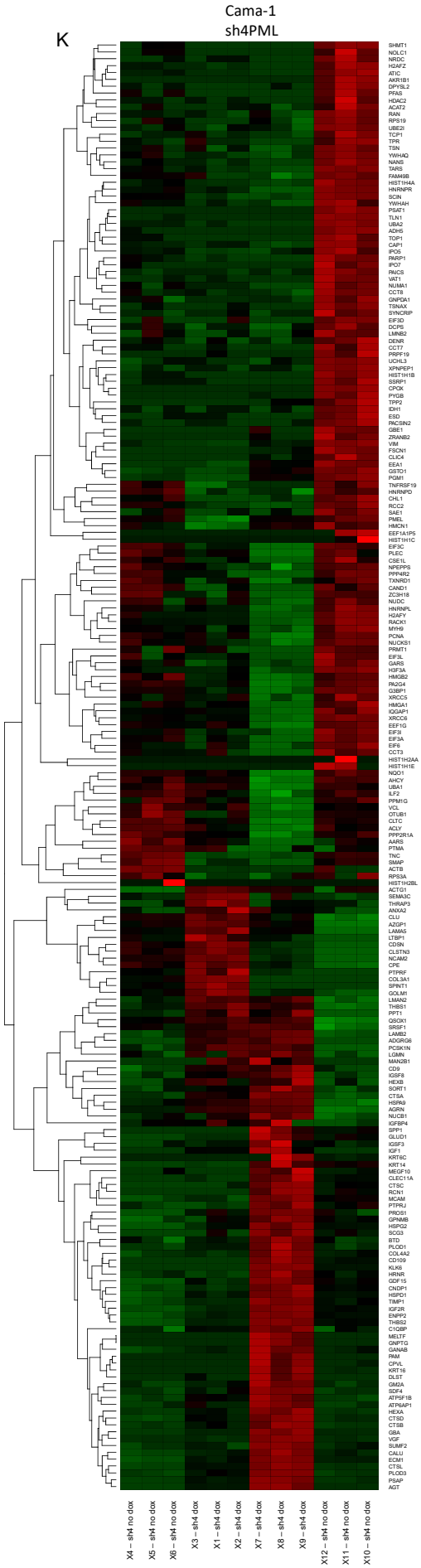
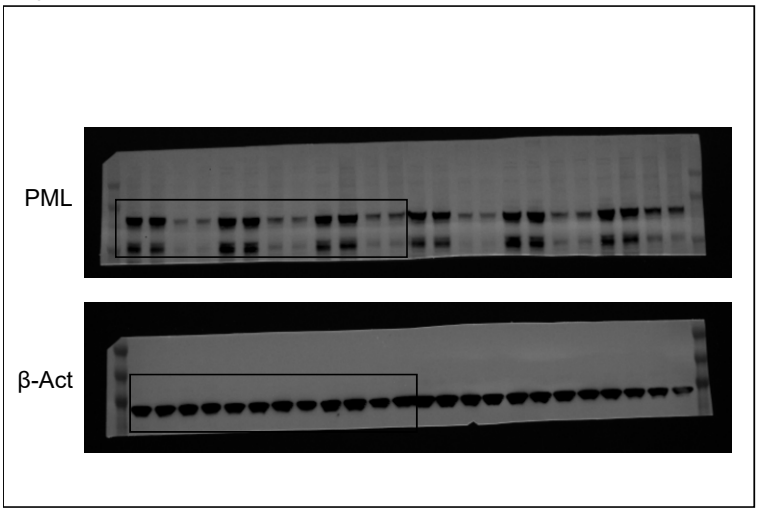

K
Cama-1
sh4PML
X4 – sh4 no dox
X5 – sh4 no dox
X6 – sh4 no dox
X3 – sh4 dox
X1 – sh4 dox
X2 – sh4 dox
X7 – sh4 dox
X8 – sh4 dox
X9 – sh4 dox
X12 – sh4 no dox
X11 – sh4 no dox
X10 – sh4 no dox

Figure 1A



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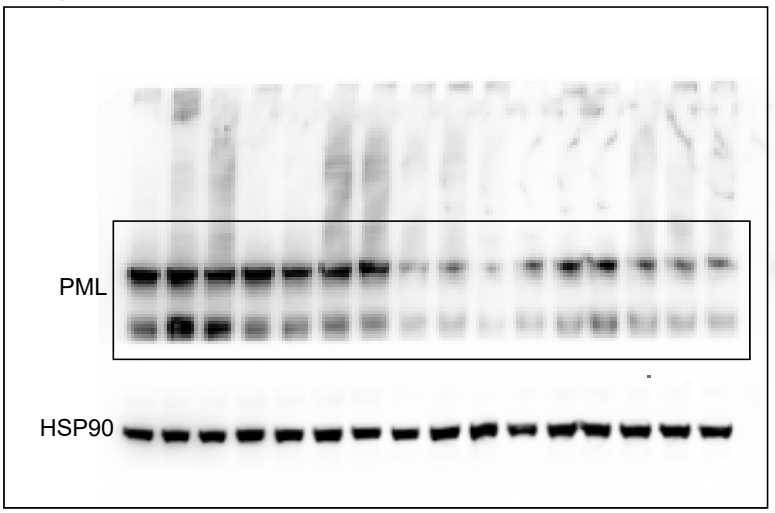


Figure S1A

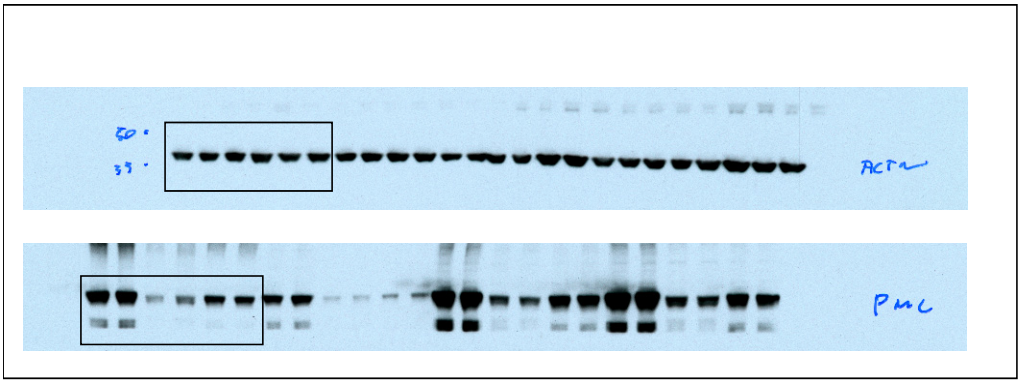
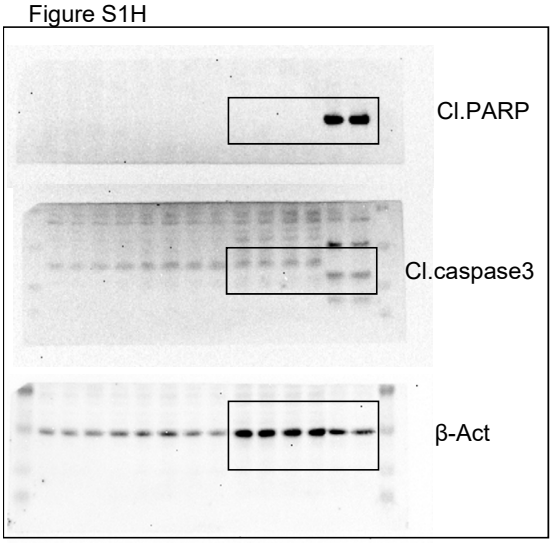


Figure S1H



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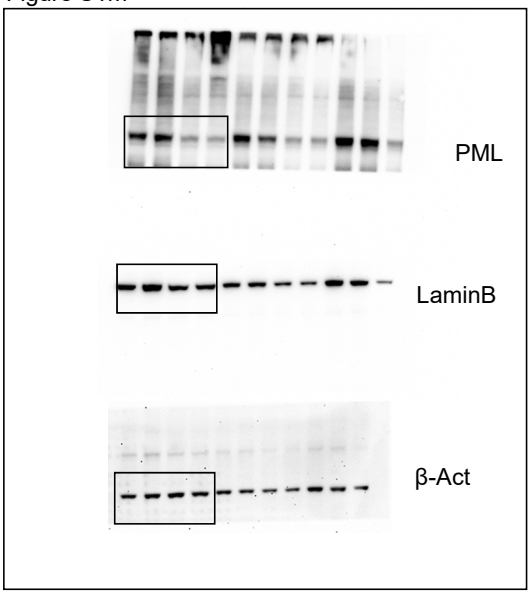

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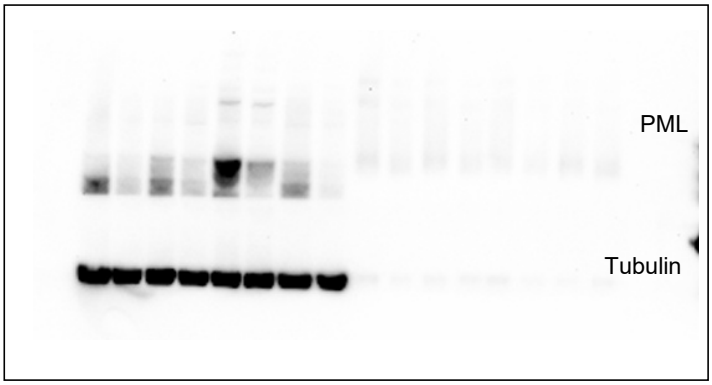
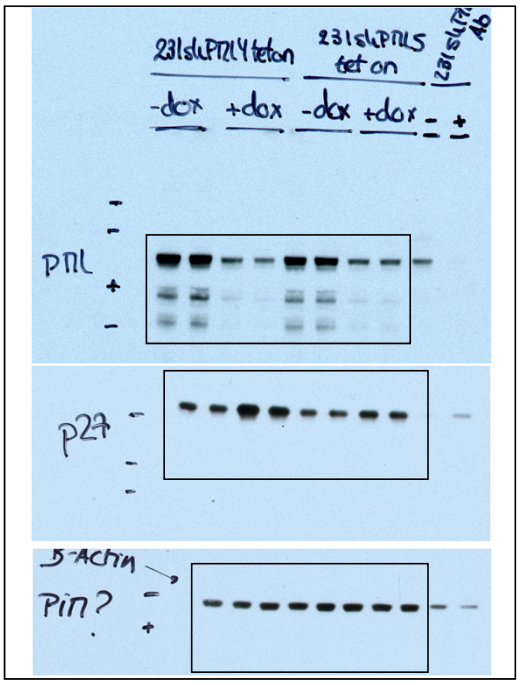


Supp. Figure 6 (cont)

Figure 2A



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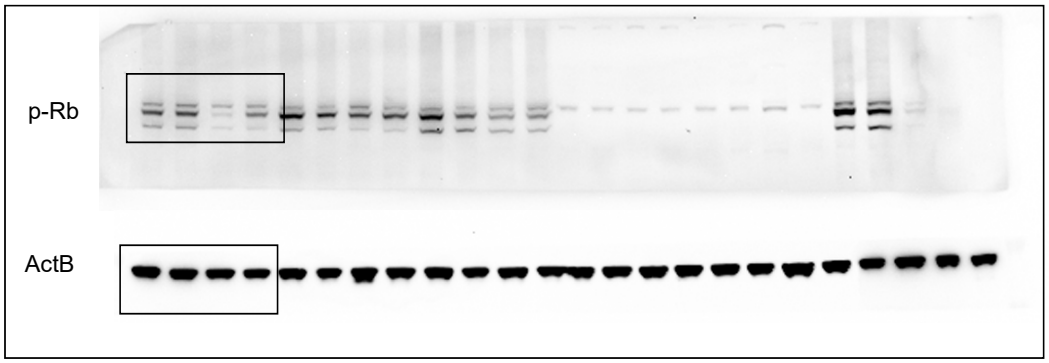
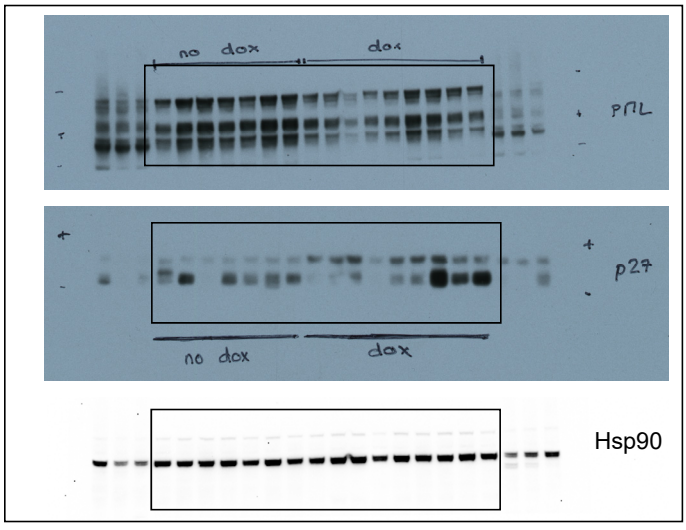


Figure 2J



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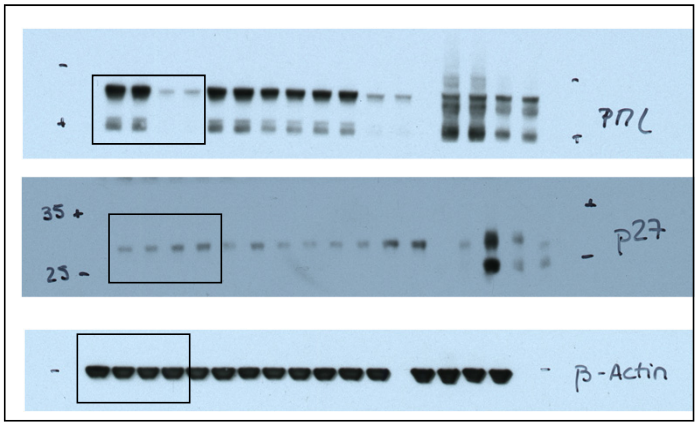

Figure S2B

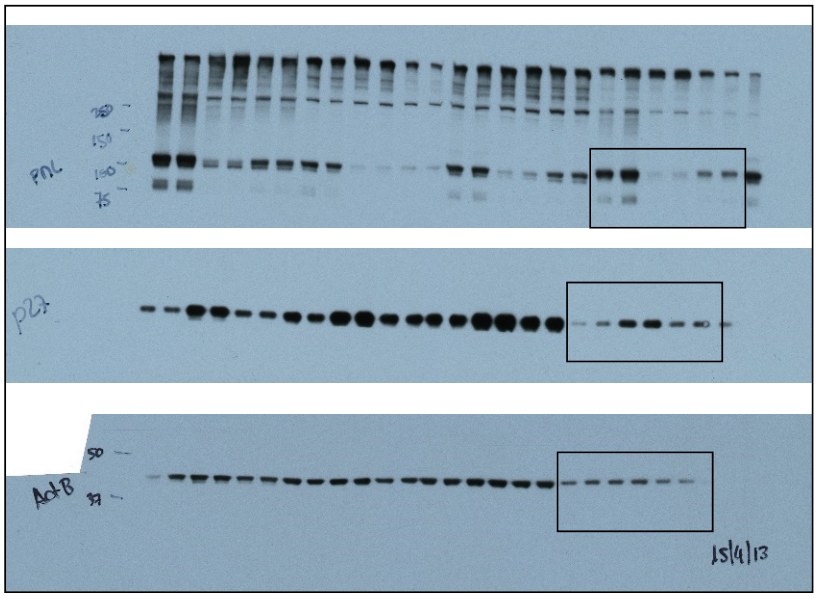

Figure S2D

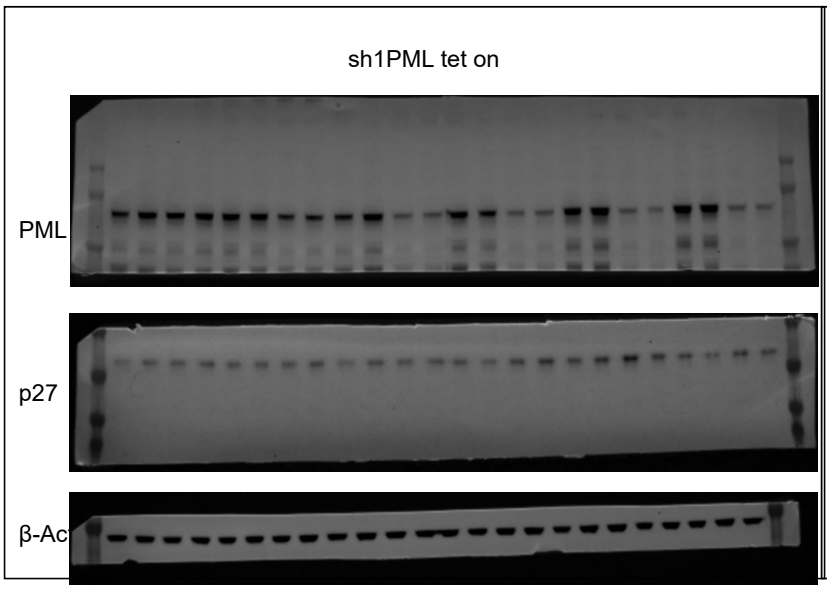

Figure S2E

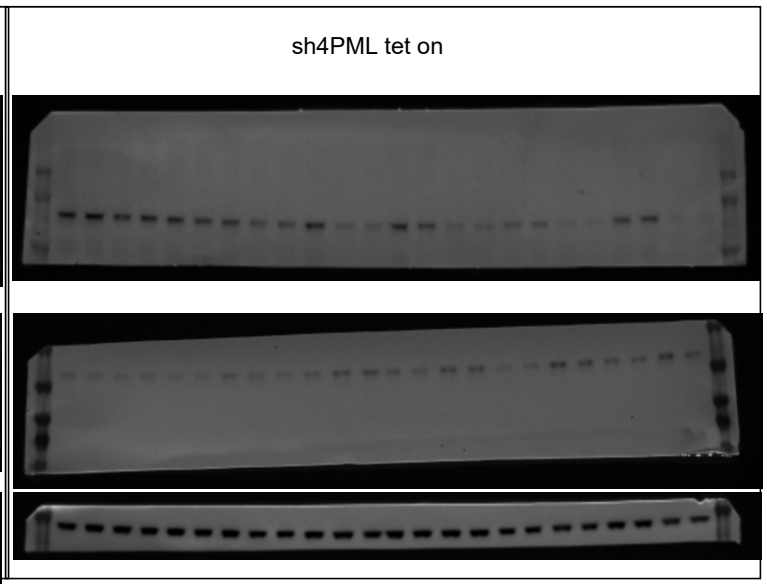

Figure S2F

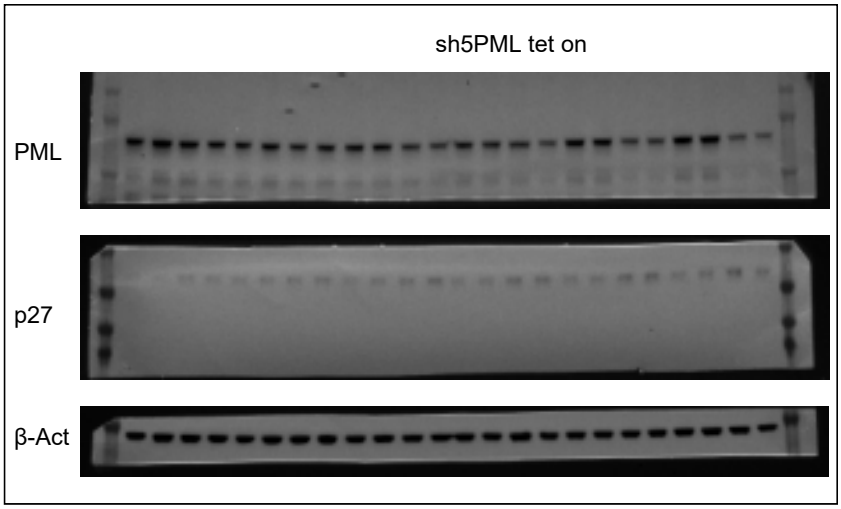

Figure 3B

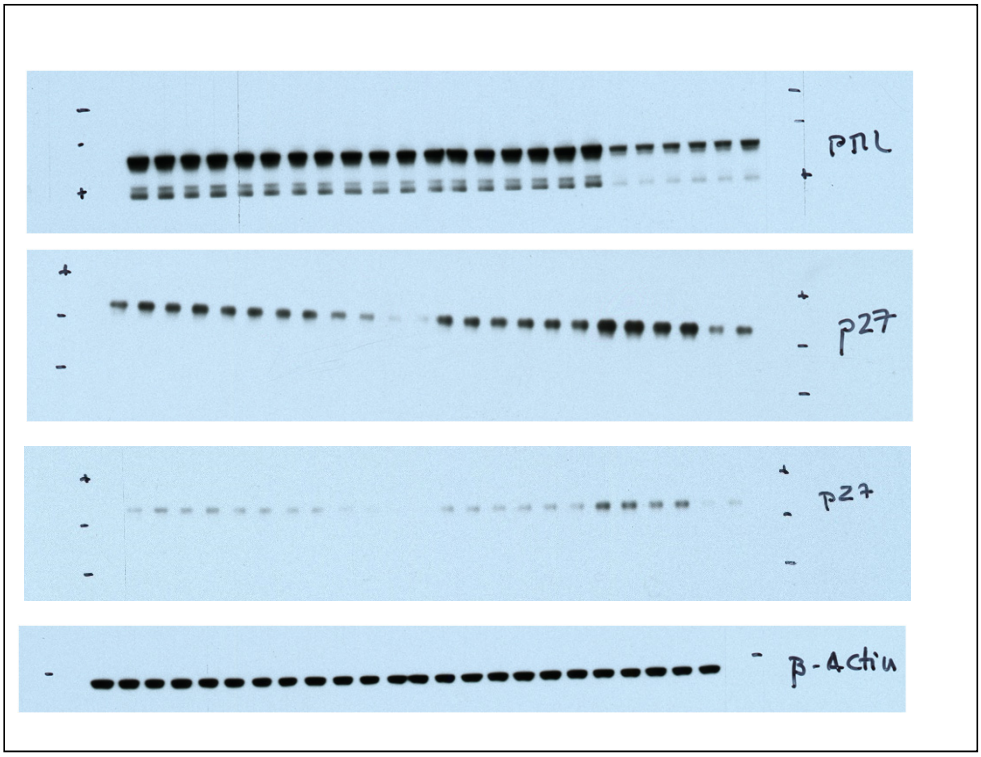

Figure S3A

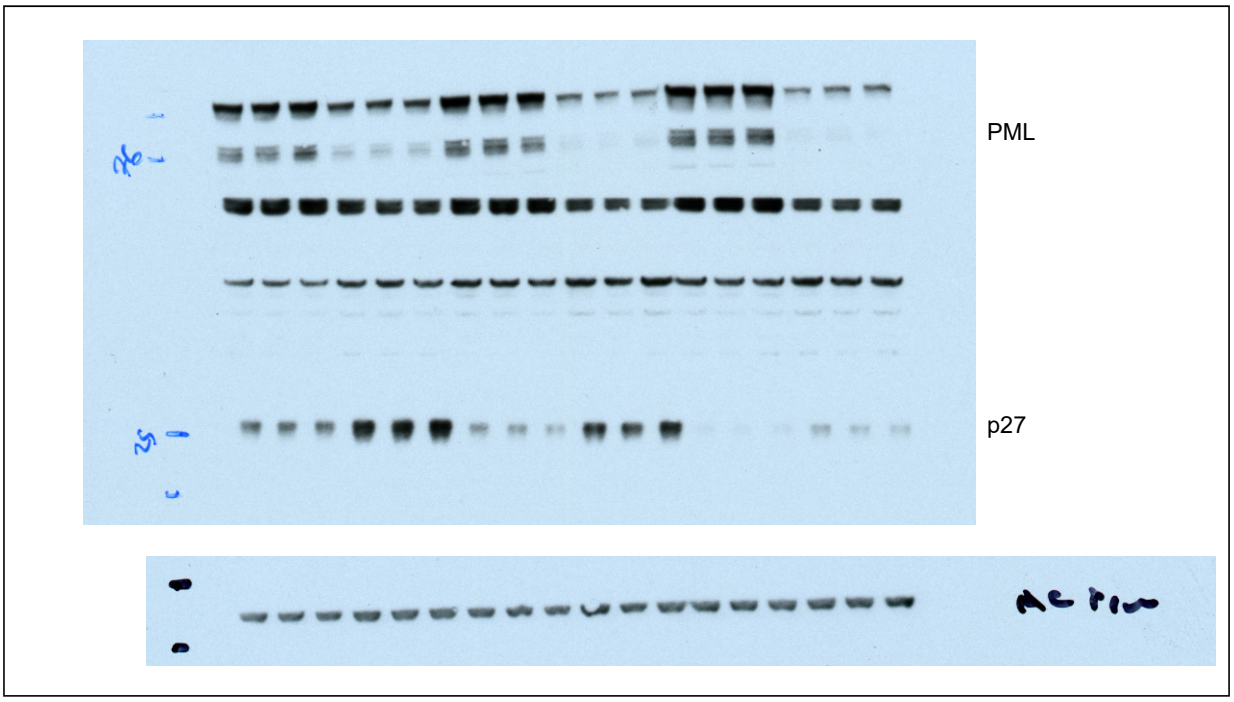

Figure 4B

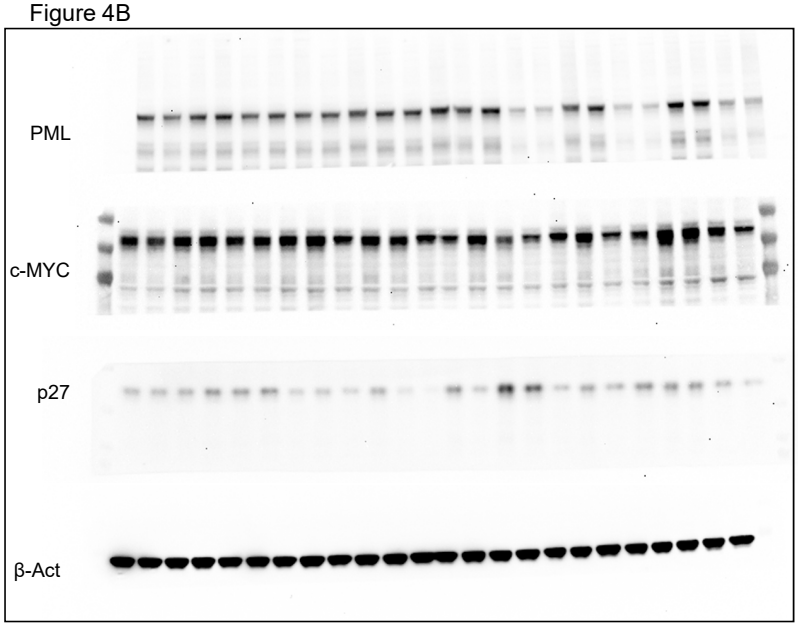

Figure 4E

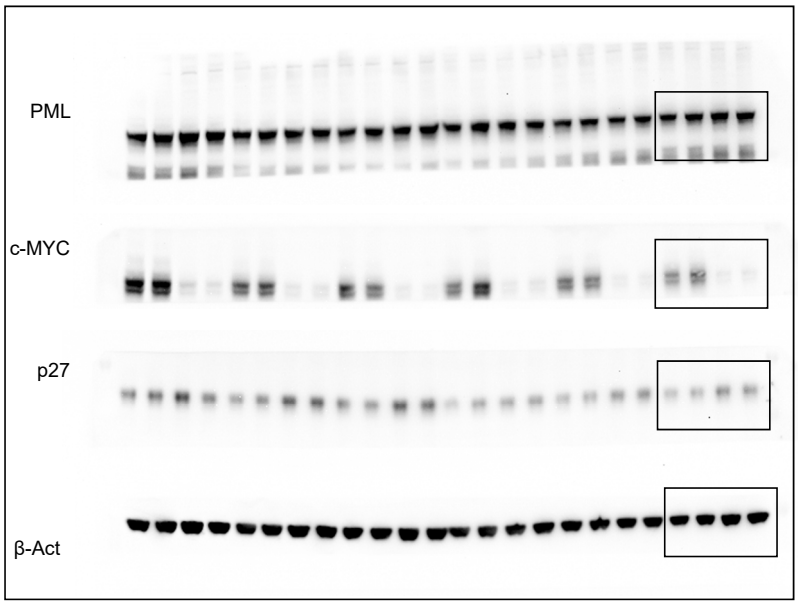

Figure 4I

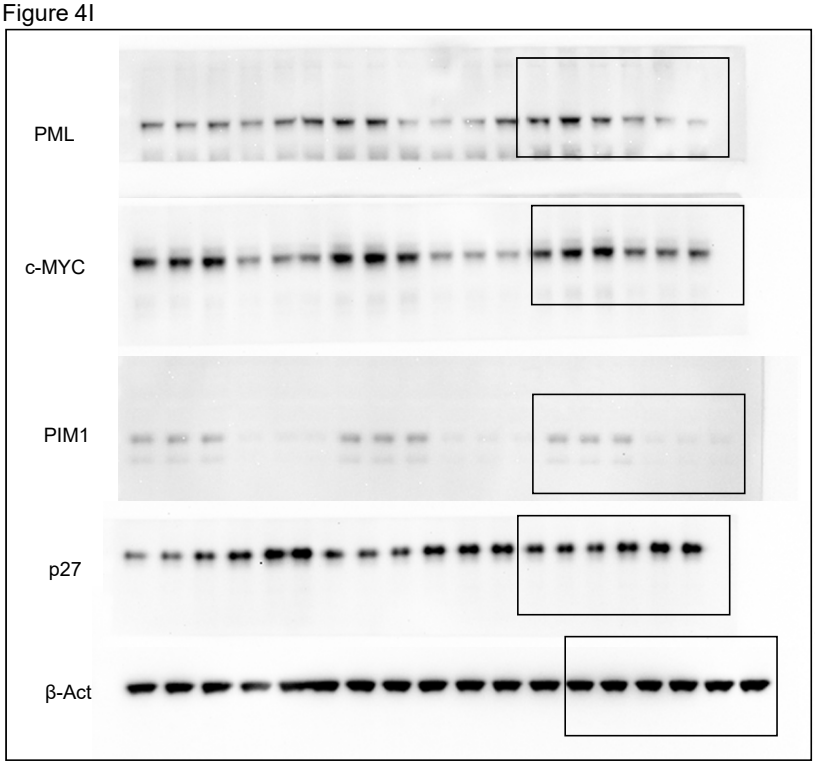

Figure S4B

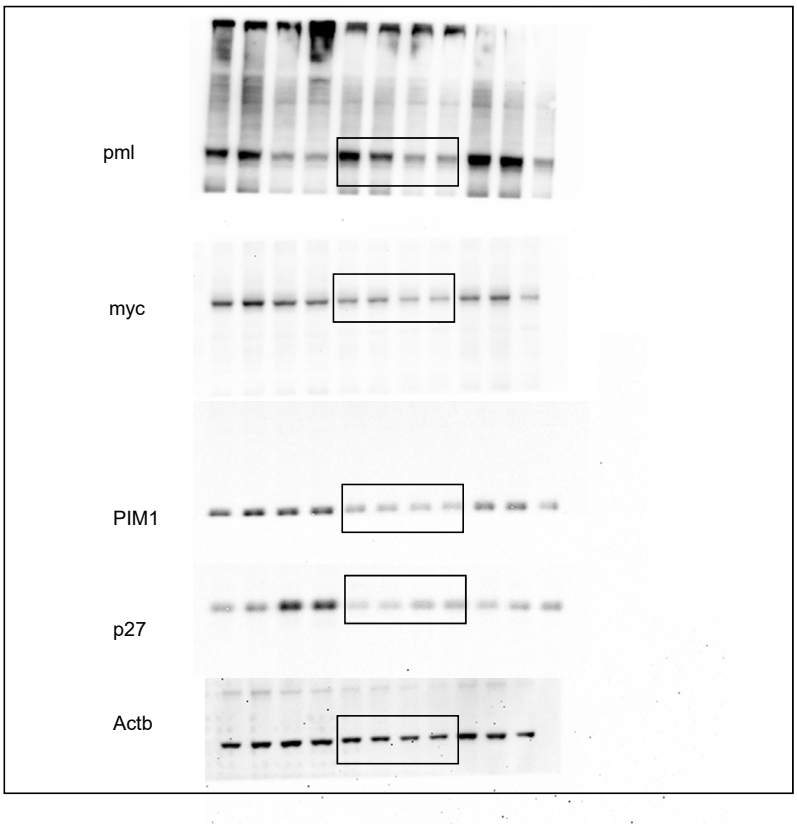

Figure S4F

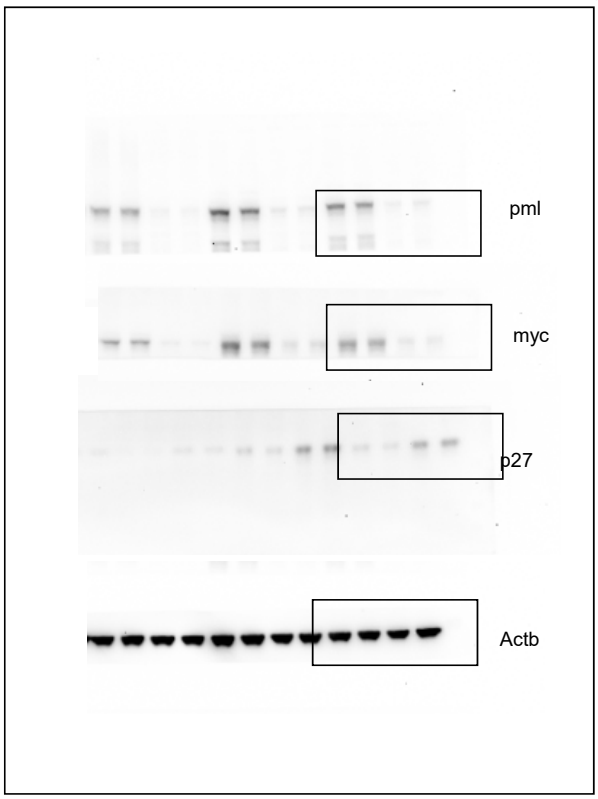

Figure S4I

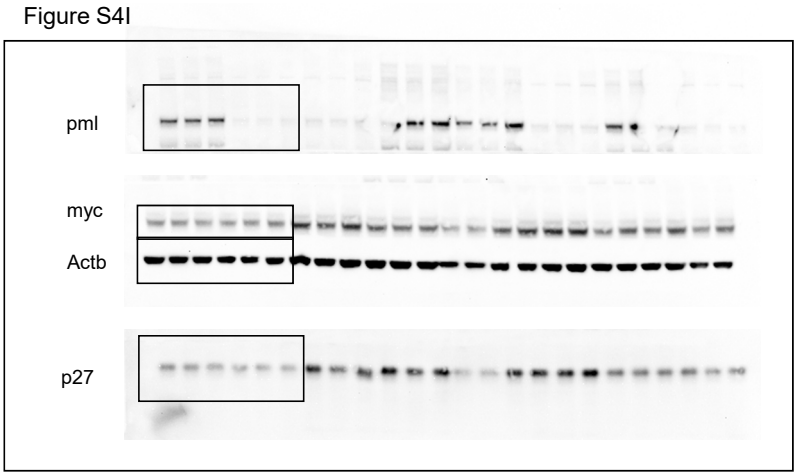

Figure 5A

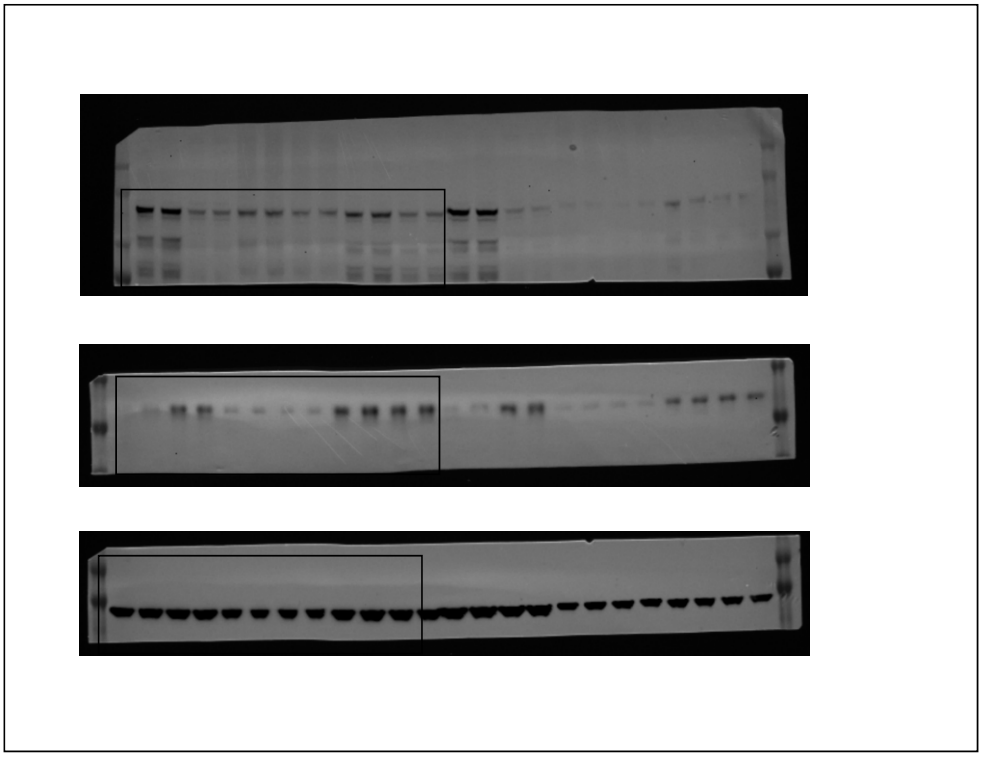

Figure S5D and S5F

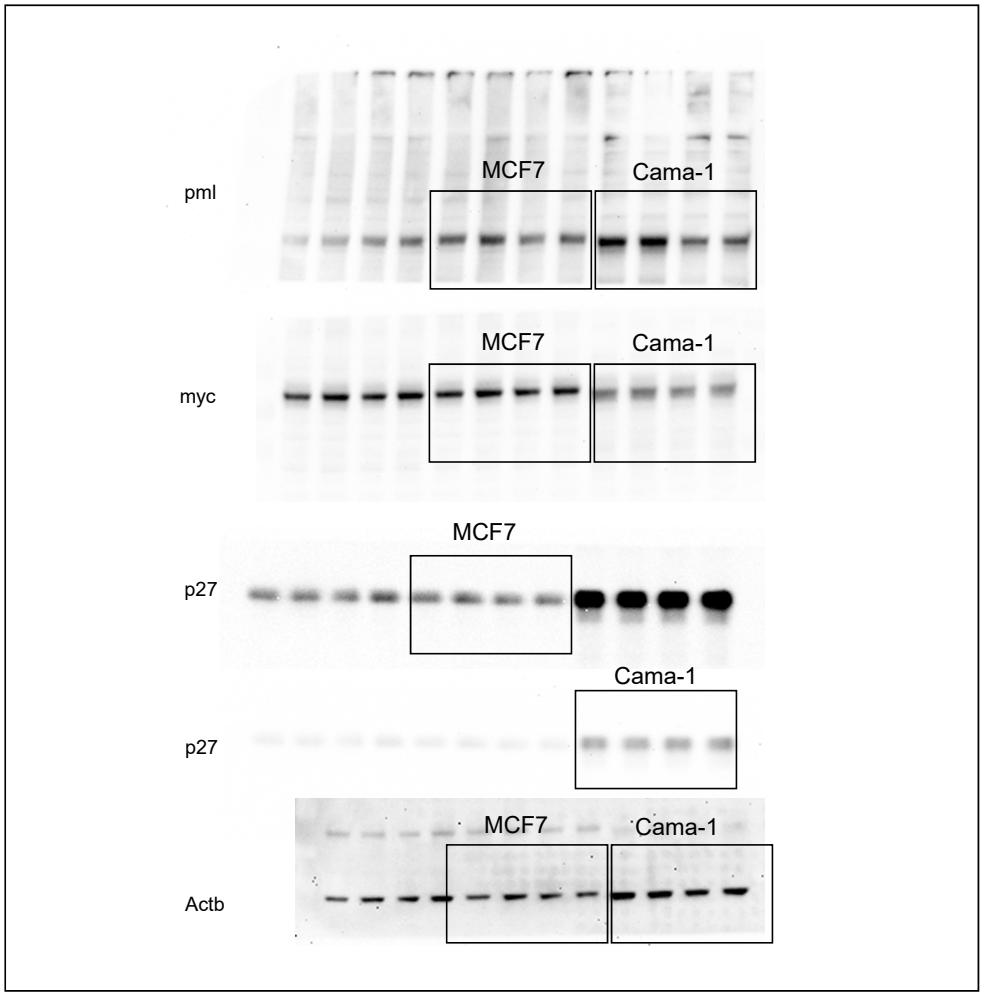

Figure S5I