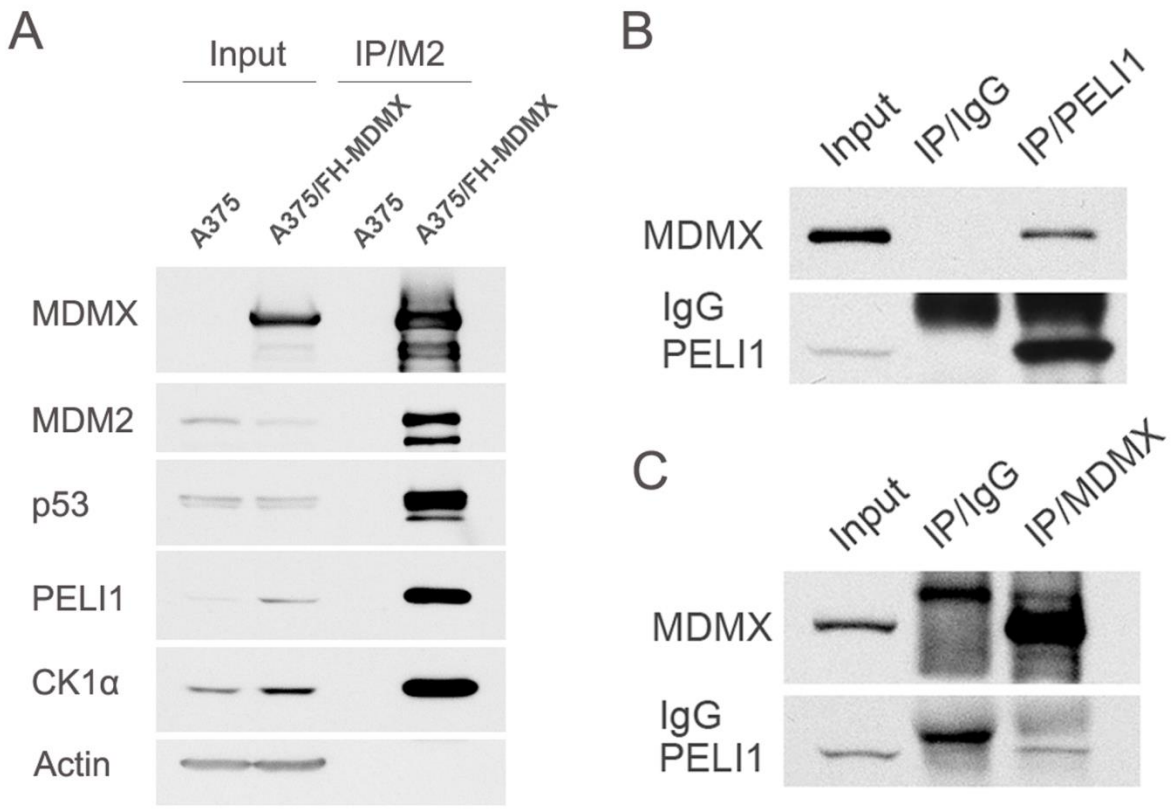
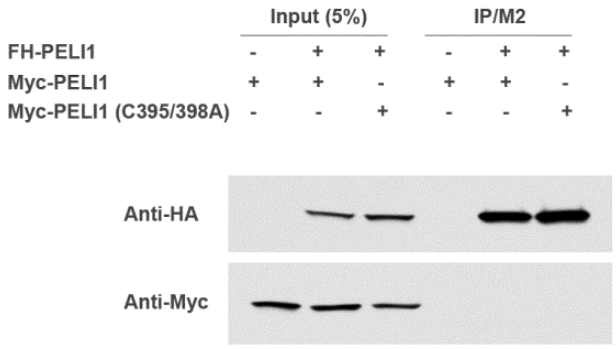


Supplementary Figure 1

(A) Whole cell extracts and immunoprecipitates with anti-FLAG antibody conjugated M2 beads (FLAG/M2) from parental A375 and FH-Mdmx A375 stable cells were subjected to western blot with antibody against Mdmx, Mdm2, p53, Peli1, CK1α and β-actin.

(B) Co-immunoprecipitation of endogenous Mdmx with Peli1 from U2OS cells. Western blot analysis of indicated whole-cell extract (lane 1) and immunoprecipitates with a control IgG (lane 2) or a Peli1-specific antibody (lane 3) by anti-Mdmx polyclonal antibody (top) or anti-Peli1 monoclonal antibody (lower).

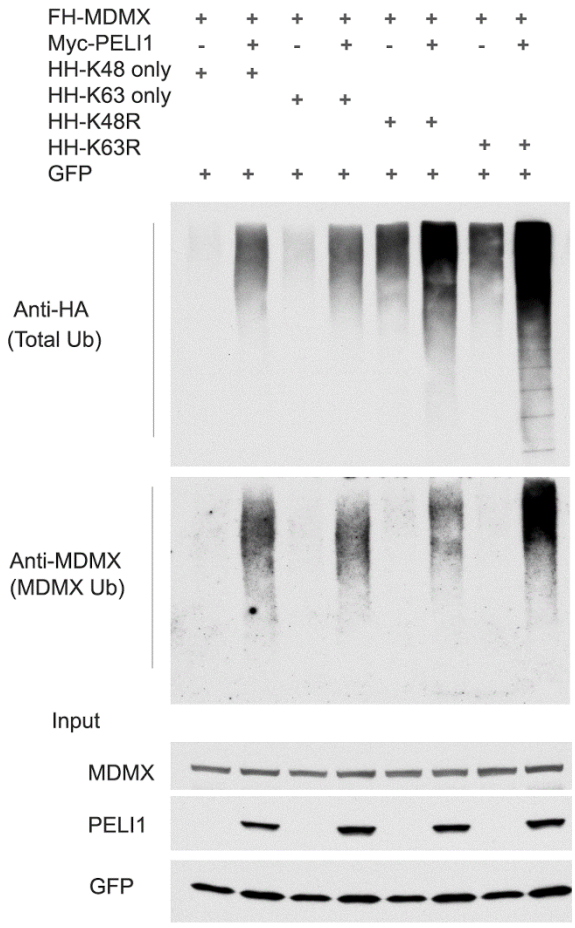
(C) Co-immunoprecipitation of endogenous Peli1 with Mdmx from U2OS cells. Western blot analysis of whole-cell extract (lane 1) or immunoprecipitates with a control IgG (lane 2) or an anti-Mdmx polyclonal antibody (lane 3) by an anti-Mdmx polyclonal antibody (top) or anti-Peli1 monoclonal antibody (lower).



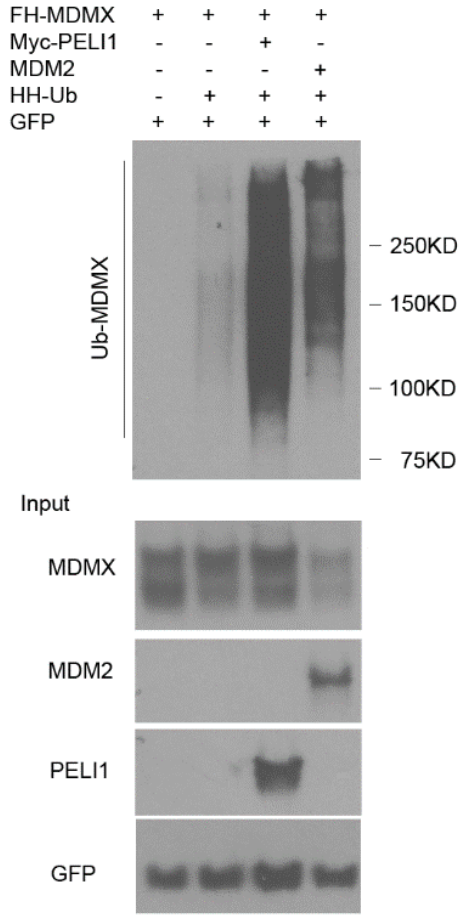
Supplementary Figure 2

Myc-Peli1 and Myc-Peli1 (C395/398A) mutant were individually transfected with FLAG-HA-Peli1 (FH-Peli1) in H1299 cells. Whole cell lysates and immunoprecipitates with FLAG/M2 were subjected to western blot with anti-HA (top) and anti-Myc (lower) monoclonal antibodies.

A



B

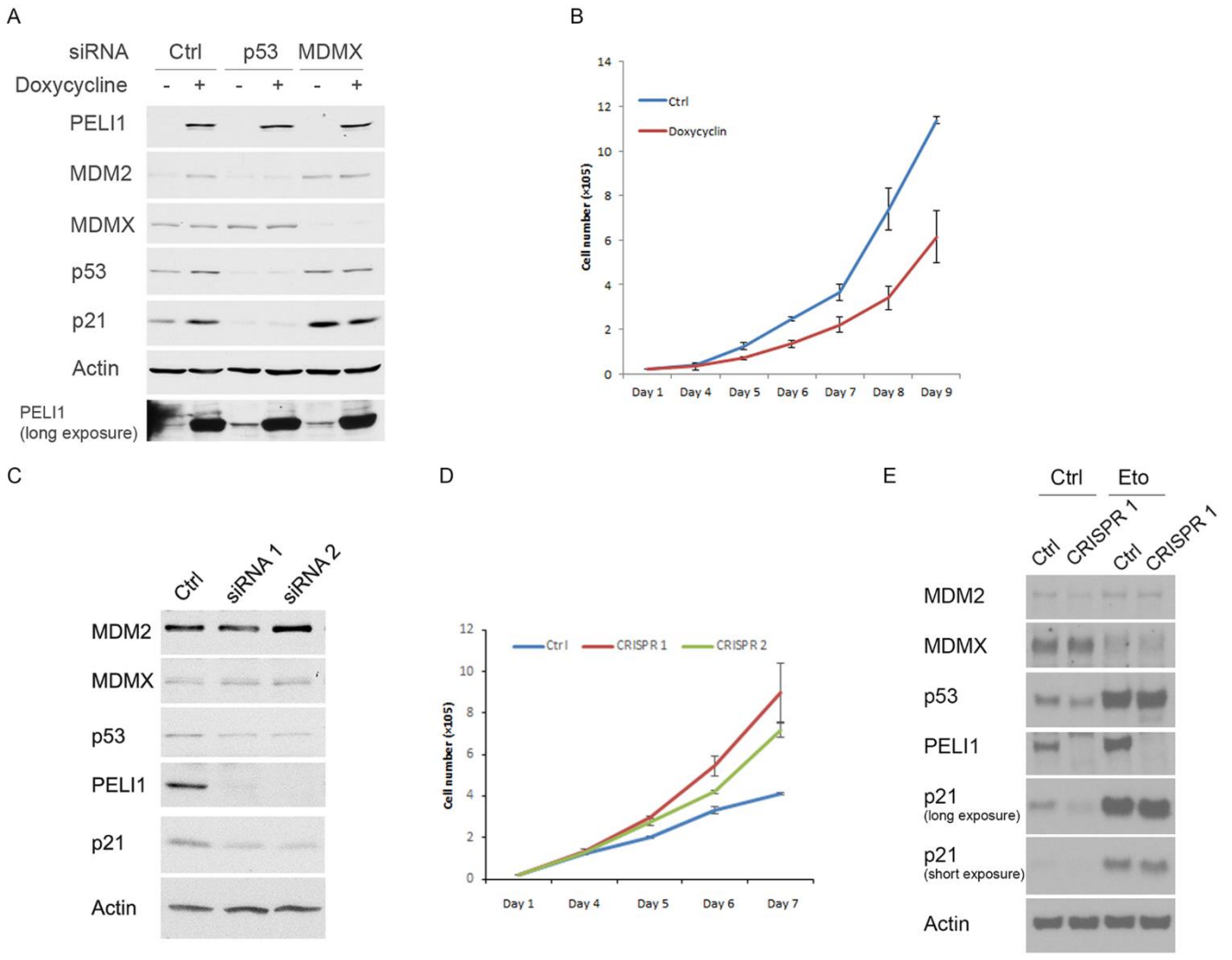


Supplementary Figure 3

(A) Peli1 promotes Mdmx K48 and K63 poly-ubiquitination. FH-Mdmx was transfected with HA-His-Ubiquitin K48 only mutant (lysine to arginine mutations on all lysine but on K48; HH-Ub-K48, lane 1), or with Myc-Peli1 and HH-Ub-K48 only mutant (lane 2); or with HA-His-Ubiquitin K63 only mutant (lysine to arginine mutations on all lysine but on K63; HH-Ub-K63, lane 3), or with Myc-Peli1 and HH-Ub-K63 only mutant (lane 4); or with HA-His-Ubiquitin K48R mutant (lysine to arginine mutation only at K48; HH-Ub-K48R, lane 5), or with Myc-Peli1 and HH-Ub-K48R (lane 6); or with HA-His-Ubiquitin K63R mutant (lysine to arginine mutation only at K63; HH-Ub-K63R, lane 7), or with Myc-Peli1 and HH-Ub-K48R (lane 8) in H1299 cells. The cell lysates were immunoprecipitated with Ni-NTA agarose followed by western blot with Anti-HA

monoclonal antibody (top panel) and anti-MDMX polyclonal antibodies (middle panel). The crude cell extracts were also detected with anti-HA (lower panel, top) and anti-Myc (lower panel, middle) monoclonal antibodies. GFP was used as loading control. Total ubiquitination proteins were also detected by an anti-ubiquitin antibody and similar results were acquired (data not shown).

(B) MDMX ubiquitination mediated by Peli1 and MDM2. Peli1 significantly promotes low to middle molecular weight MDMX ubiquitination, whereas MDM2 only induces middle to high molecular weight MDMX ubiquitination. FH-Mdmx was co-transfected with or without HA-His-Ubiquitin (HH-Ub) and Myc-Peli1 or MDM2 in H1299 cells. The cell lysates were immunoprecipitated with Ni-NTA agarose followed by western blot with Anti-MDMX polyclonal antibody (top panel). The crude cell extracts were also detected with anti-HA (lower panel, top) and anti-MDM2 (lower panel, middle) monoclonal antibodies. GFP was used as loading control.



Supplementary Figure 4

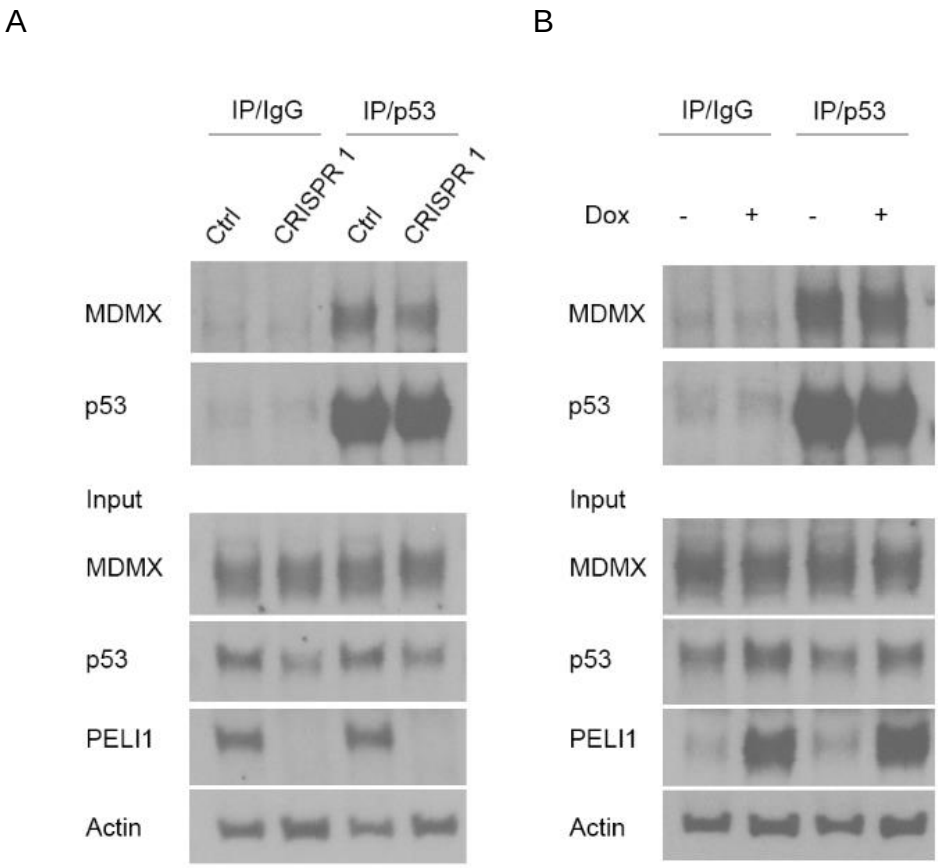
(A) Related to Figure 4A, Western blot analysis of cell extracts with antibody against Mdm2, Mdmx, p53, Peli1, p21 and β-actin. Peli1 long exposure was shown.

(B) Growth curve of Peli1 inducible U2OS cell line. 2×10^4 cells were plated in 60 mm dish at day 1. After 24 hours, cells were without treatment (Ctrl) or treated with 0.1 μg/ml of doxycycline. 3 plates were counted for each day from day 4-9. The medium with or without doxycycline was changed every 2-3 days. The experiment was repeated two times and a representative curve was shown (p<0.05 or p<0.01 from day 5-9).

(C) Western blot analysis of cell extracts from U2OS cells transfected with either control siRNA or siRNA against Peli1 (siRNA1 and siRNA2) with antibody against Mdm2, Mdmx, p53, Peli1, p21 and β-actin.

(D) Growth curve of Peli1 CRISPR-Cas9 knock-out U2OS cell lines. 2×10^4 cells from control parental and two Peli1 knock-out cell lines were plated in 60mm dish. 3 plates were counted for each day from day 4-7. The medium was replaced every 2-3 days. The experiment was repeated two times and a representative curve was shown ($p<0.01$ or $p<0.05$ from day 5-7).

(E) U2OS and Peli1 knock-out cells were plated on 60 mm dishes. After overnight culture, the cells at 60% confluence were without treatment (Ctrl) or treated with 20 μM etoposide (Eto) 18 hours. Western blot analysis of cell extracts with antibody against Mdm2, Mdmx, p53, Peli1, p21 and β-actin.



Supplementary Figure 5

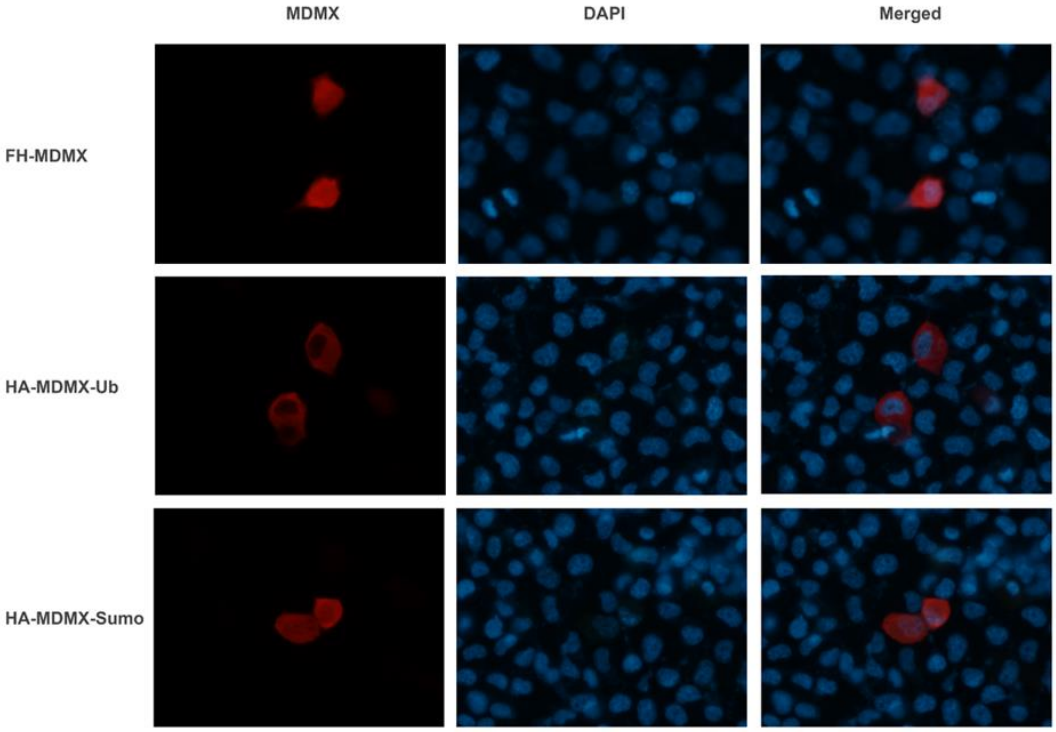
(A) Co-immunoprecipitation of endogenous p53 with Mdmx from the control parental and Peli1 CRISPR-Cas9 knock-out cells. Western blot analysis of the immunoprecipitates with a control mouse IgG (lane 1 and 2) or a p53-specific antibody (DO-1, lane 3 and 4) by anti-Mdmx polyclonal antibody (upper panel, top band) or anti-p53 rabbit polyclonal antibody (FL393, Santa Cruz; upper panel, lower band). The whole-cell extract (lower panel) was analyzed by antibody against Mdmx, p53, Peli1 and Actin.

(B) Co-immunoprecipitation of endogenous p53 with Mdmx from Peli1 inducible cells. Peli1 expression is induced by 0.1 µg/ml of doxycycline 48 hours. The cells are lysed and co-immunoprecipitation is performed with control mouse IgG (lane 1 and 2) or a p53-specific antibody (DO-1, lane 3 and 4). The immunoprecipitates (upper panel) and whole cell extract (lower panel) were detected as shown in (A).

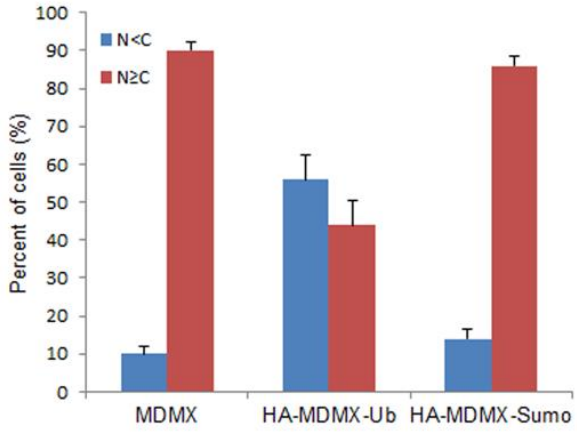
A


HA
MDMX
Ubiquitin
HA-MDMX-Ub
HA
MDMX
Sumo
HA-MDMX-Sumo

B


MDMX
DAPI
Merged
FH-MDMX
HA-MDMX-Ub
HA-MDMX-Sumo

C

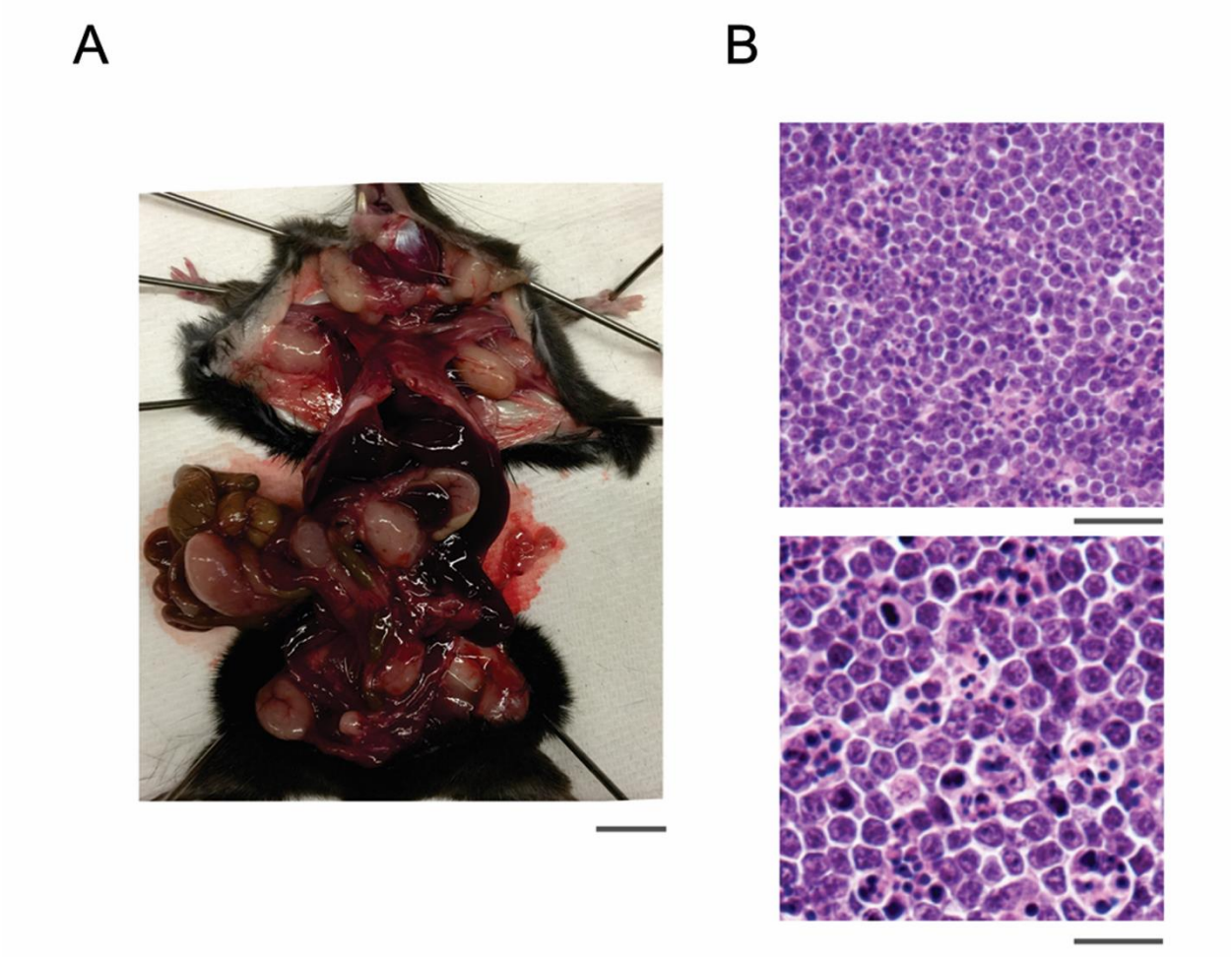

100
90
80
70
60
50
40
30
20
10
0
N<C
N≥C
Percent of cells (%)
MDMX
HA-MDMX-Ub
HA-MDMX-Sumo

Supplementary Figure 6

(A) Schematic representation of Mdmx derivatives (HA-Mdmx-Ubiquitin and HA-Mdmx-Sumo) that have one copy of the ubiquitin or sumo sequence fused to the C terminus.

(B) FH-Mdmx, HA-Mdmx-Ubiquitin and HA-Mdmx-Sumo were individually transfected in H1299 cells. Twenty-four hours after transfection, the cells were fixed, permeabilized and then incubated with anti-HA rat antibody followed by Alexa Fluor 568 (red) conjugated anti-rat secondary antibody. DAPI was used for nuclear staining. The representative images of Mdmx, DAPI and merged staining are shown.

(C) FH-Mdmx, HA-Mdmx-Ubiquitin and HA-Mdmx-Sumo staining were scored as cytoplasmic staining stronger than nuclear (N<C) and nuclear staining stronger or equal to cytoplasmic (N≥C). At least 100 cells were counted for each group. Shown is the average from 3 independent experiments. Error bars represent the SEM.



Supplementary Figure 7

(A) Early onset lymphoma in an 83 day-old Peli1 null and Eμ-Myc mouse: dissection reveals severe enlargement of the spleen, liver and most lymph nodes (notably, the mesenteric, cervical, axillary, mediastinal and inguinal nodes). Bar represents 1 cm.

(B) High grade B-cell lymphoma in Peli1 null and Eμ-Myc mice. The lymph node architecture is replaced with the solid sheets of the infiltrated middle size lymphocytes, the apoptotic lymphoma cells and the large multinucleated macrophages. The intermediate size lymphocytes have moderate to high anisocytosis, high mitotic index and abnormal mitosis (H&E, Bars represent 40 μm in upper panel and 20 μm in lower panel).