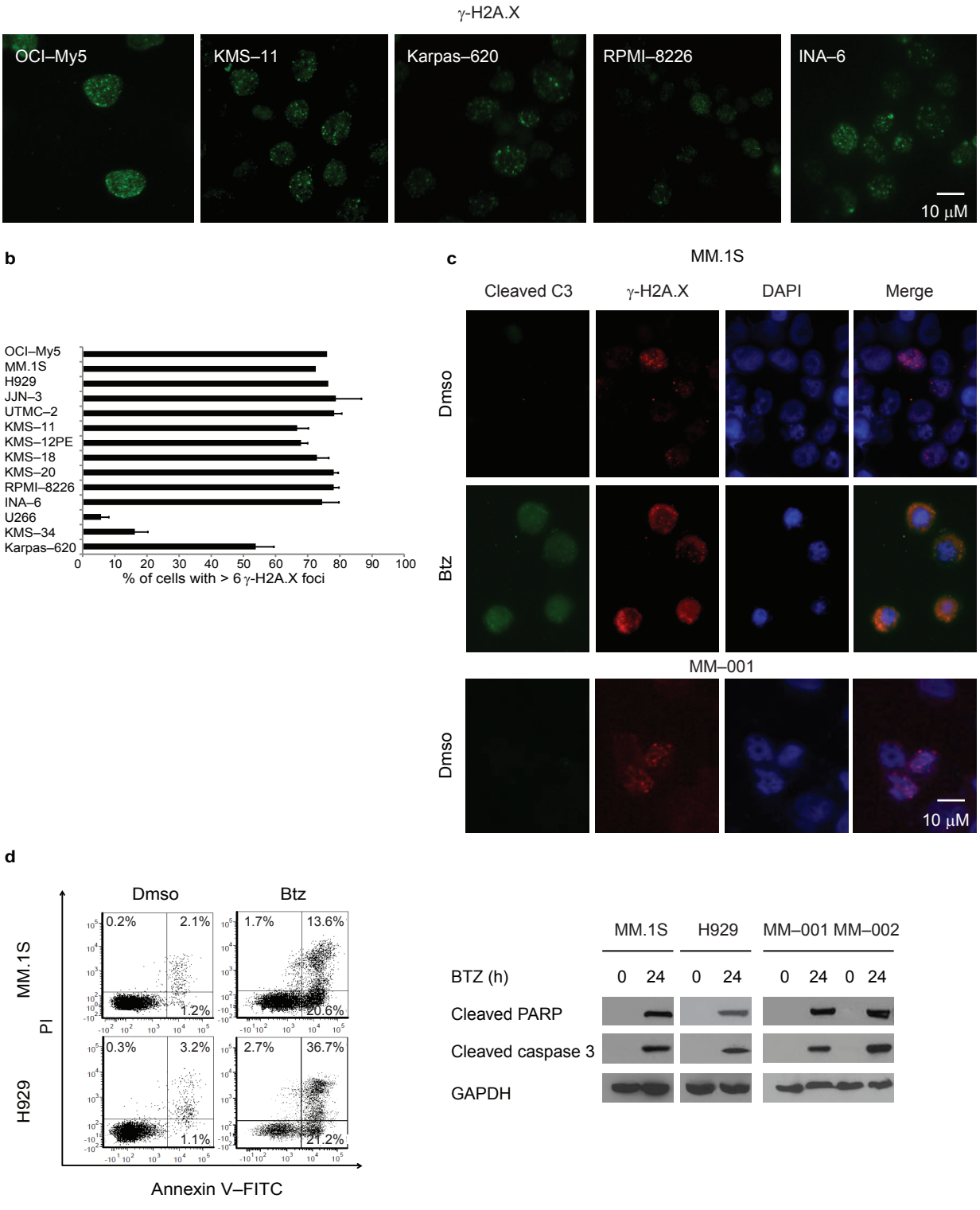
Supplementary Figure S1_Cottini



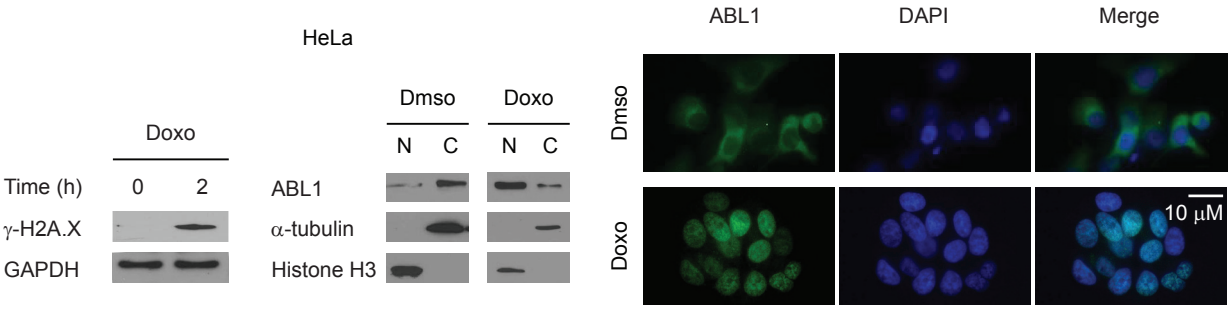
Supplementary Fig. 1

MM cells do not undergo apoptosis despite pervasive ongoing DNA damage

(**a**) γ-H2A.X immunofluorescence staining of OCI–My5, KMS–11, RMI–8226, and INA–6 MM cell lines.
(**b**) Quantitative evaluation of γ-H2A.X foci number. Mean values ± SD derived from triplicate experiments are shown.
(**c**) Immunofluorescence staining for cleaved caspase 3–Alexa 488, γ-H2A.X–Alexa 568, and DAPI in MM.1S cells and one representative patient (MM–001). As positive control, MM.1S were treated with 5 nM Bortezomib (Btz) for 24 h.
One representative experiment of three is shown.
(**d**) Annexin V−FITC/PI staining (left panel) and western blot analysis of cleaved caspase 3 (C3)/cleaved PARP in MM.1S and H929 MM cell lines and in two MM patient cells (right panel), before and after treatment with 5 nM Bortezomib (Btz) for 24 h.
One representative experiment of three is shown.

Supplementary Figure S2_Cottini

a



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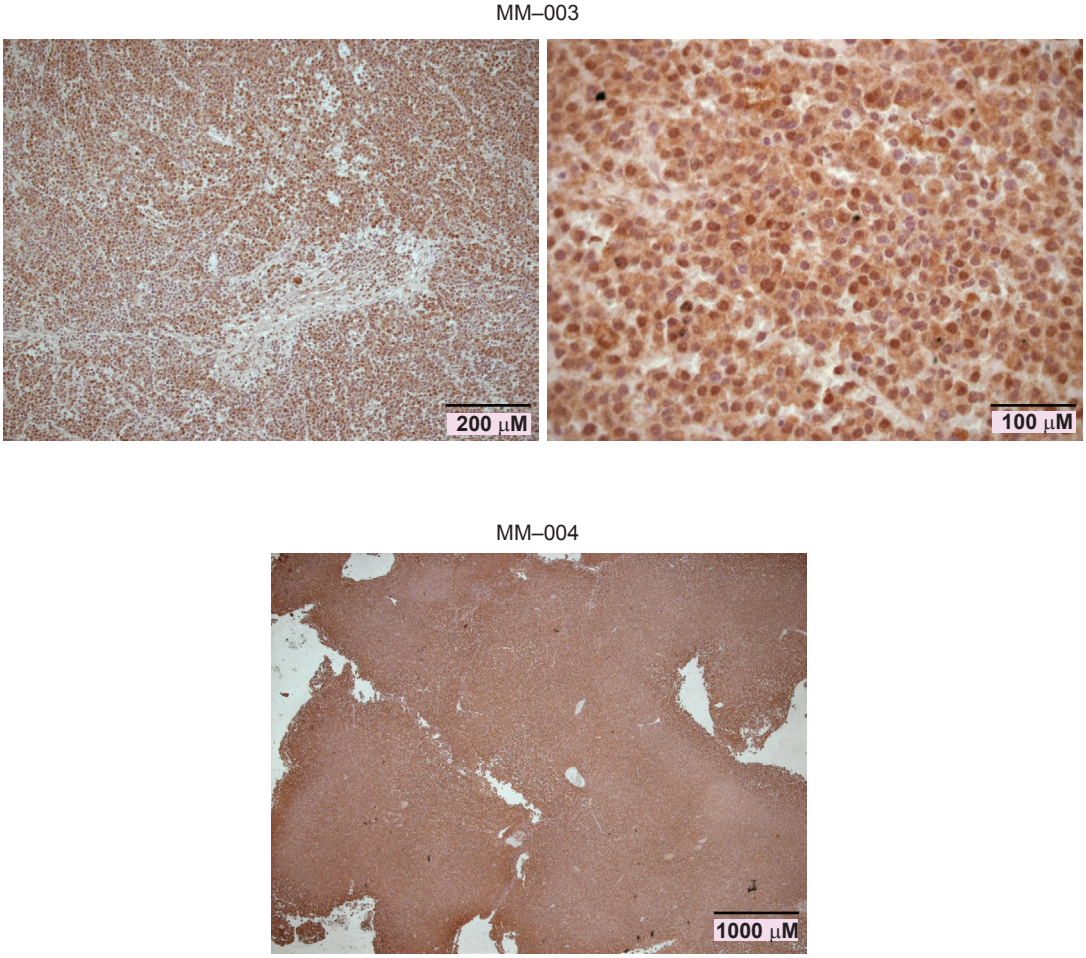
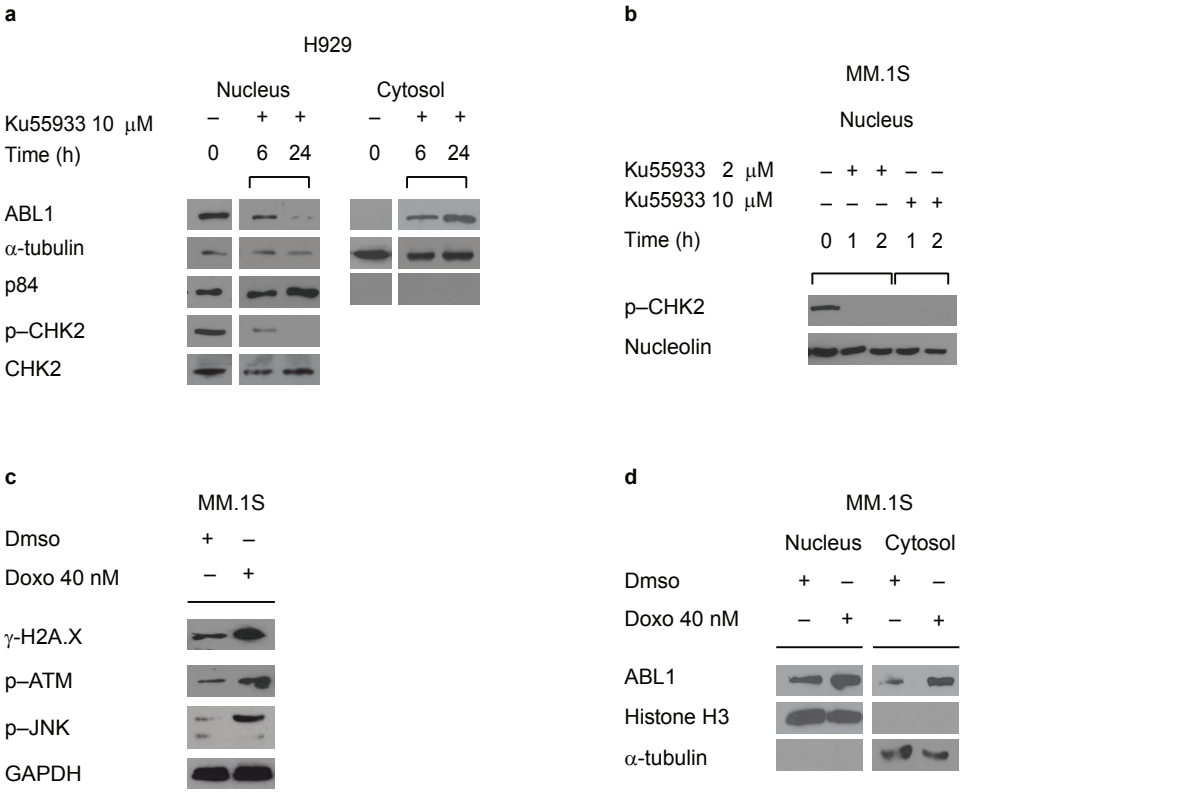


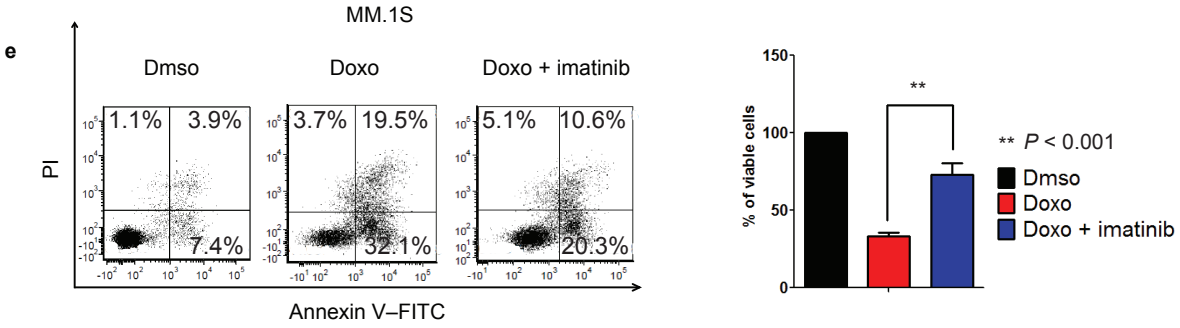
Supplementary Fig. 2

ABL1 localization in the nucleus of MM cells, but not of HeLa cells

(**a**) ABL1 cellular localization in HeLa cell line. HeLa cells were incubated for 2 h with Dmso or 1 μM doxorubicin (Doxo). Left panel: Western blot with total lysates for γ-H2A.X and with nuclear (N) and cytosolic (C) fractions for ABL1, α-tubulin, and Histone H3 are shown. Right panel: Immunofluorescence for ABL1 and DAPI after 2 h incubation with Dmso or 1 μM Doxo. One representative experiment of two is shown.
(**b**) Immuno–histochemical ABL1 staining in MM patient samples.
Upper panel, patient 3 (MM–003), 100X and 400X magnification. Lower panel, patient 4 (MM-004), 25x magnification.

Supplementary Figure S3_Cottini



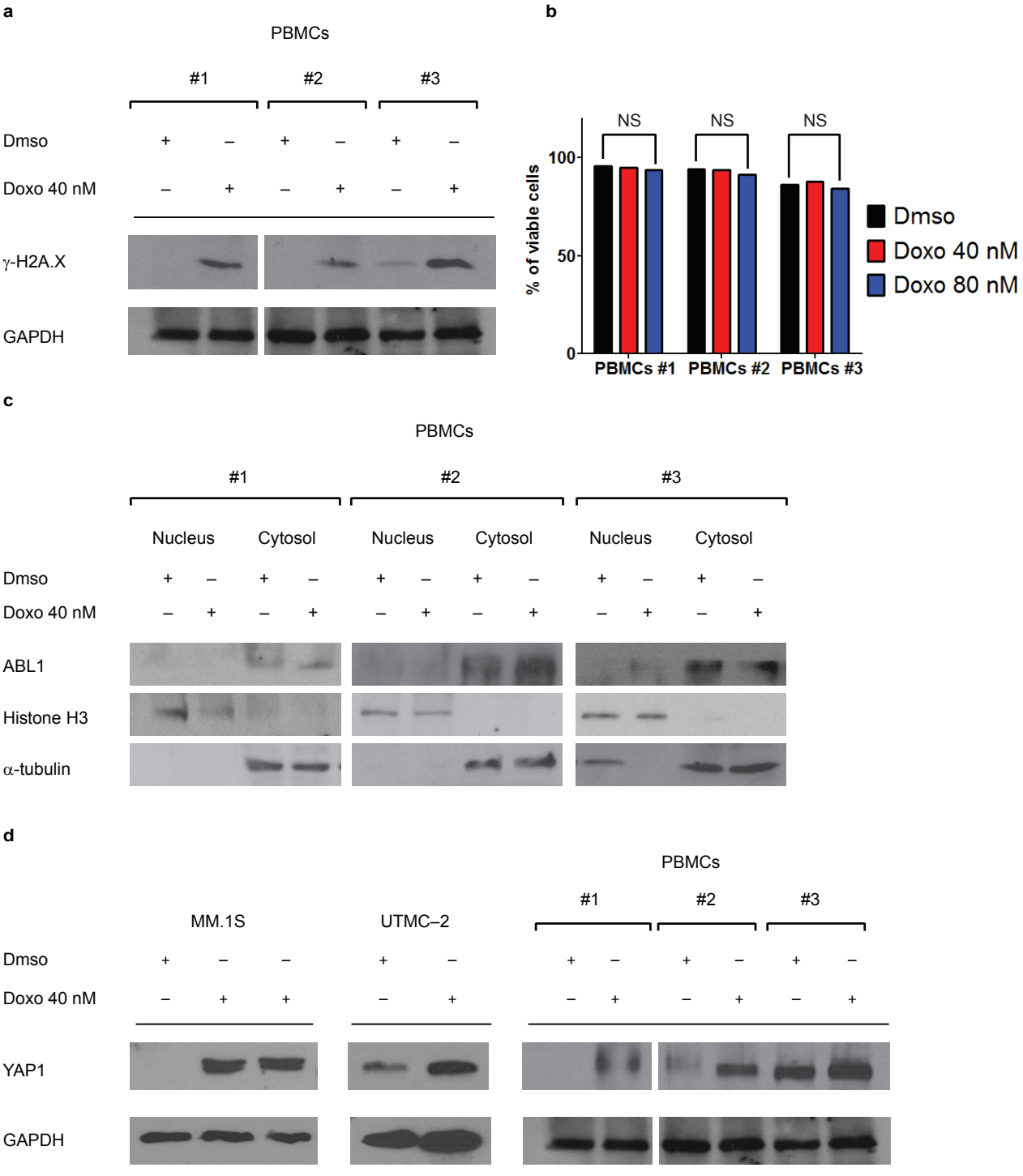


Supplementary Fig. 3

ABL1 nuclear localization is driven by ATM and JNK phosphorylation in MM cells

(**a**) H929 MM cells were treated with Dmso or 10 μM ATM kinase inhibitor Ku55933 for 6–24 h.
Cellular fractionation was performed, and cell lysates were blotted with ABL1, α-tubulin, and p84 (as loading control)
and p–CHK2 (Thr68) and total CHK2 (as control of Ku55933 activity). One representative experiment of two is shown.
(**b**) Western blot for p–CHK2 (Thr68) and nucleolin in MM.1S cells treated with Dmso or 2–10 μM Ku55933 for 1 or 2 h.
One representative experiment of two is shown.
(**c**) Western blot of γ-H2A.X, p–ATM(Ser1981), and p–JNK (Thr183/Tyr185) in MM.1S untreated cells or after incubation
with 40 nM Doxo for 72 h. One representative experiment of two is shown.
(**d**) Western blot for ABL1, Histone H3, and α-tubulin in MM.1S cells, before and after doxorubicin (Doxo; 40 nM) treatment.
One representative experiment of two is shown.
(**e**) Annexin V–FITC/PI staining and MTT absorbance assay in MM.1S cells after 48 h treatment.
Data are mean values ± SD of triplicates of two different experiments.

Supplementary Figure S4_Cottini

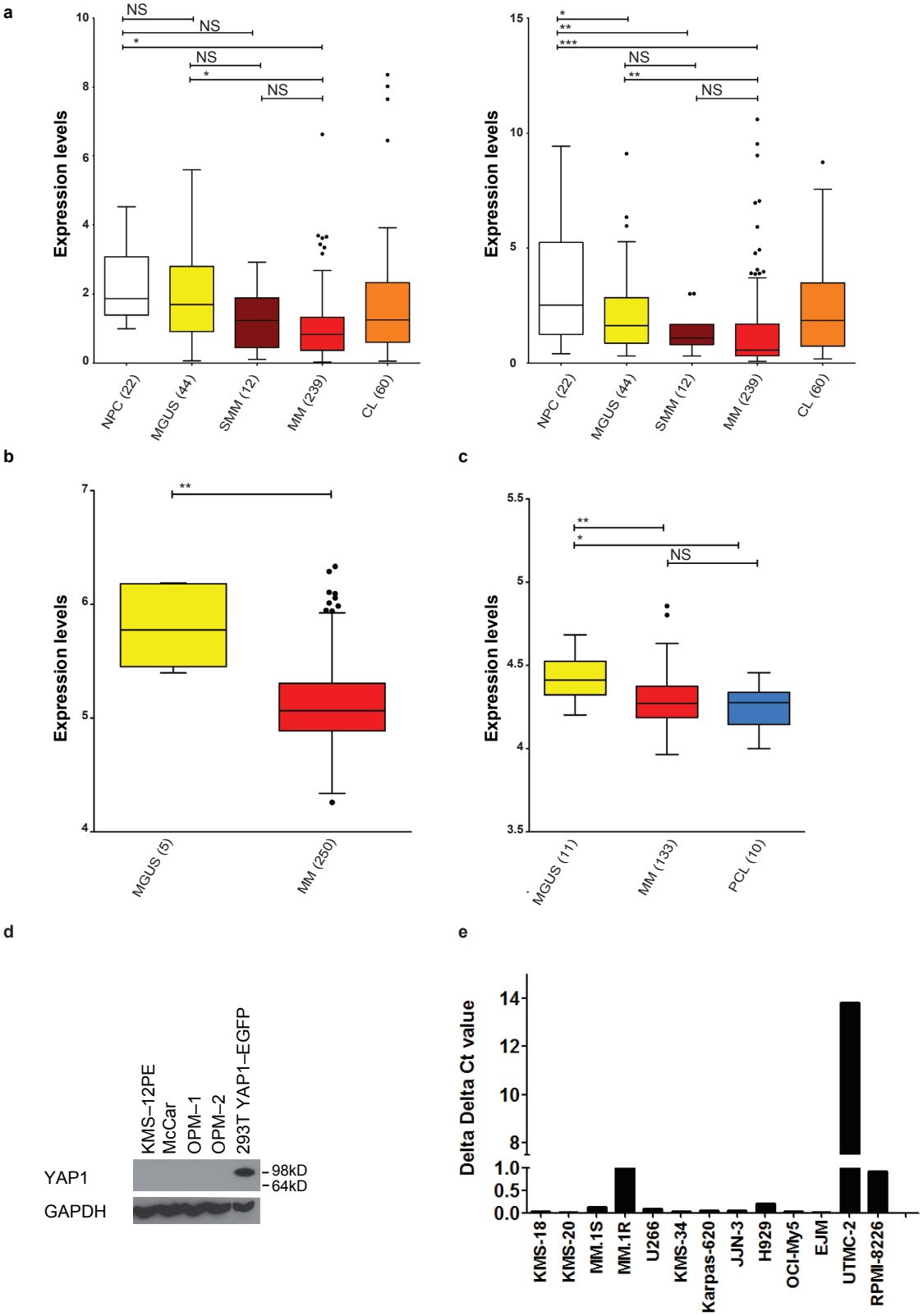


Supplementary Fig. 4

Peripheral blood mononuclear cells (PBMCs) response to induced DNA damage

(**a**) Western blot analysis for γ-H2A.X in PBMCs isolated from three healthy donors (#1, #2, #3), untreated or after incubation with 40 nM Doxo for 24 h.
(**b**) Annexin V–FITC/PI in PBMCs, untreated or after incubation with 40 nM and 80 nM Doxo for 48 h.
(**c**) Western blot for ABL1, Histone H3, and α-tubulin in PBMCs, untreated or after doxorubicin (Doxo; 40 nM) treatment for 2 h.
(**d**) Western blot for YAP1 in MM.1S, UTMC–2, and PBMCs, untreated or after incubation with 40 nM Doxo for 24–72 h.

Supplementary Figure S5_Cottini

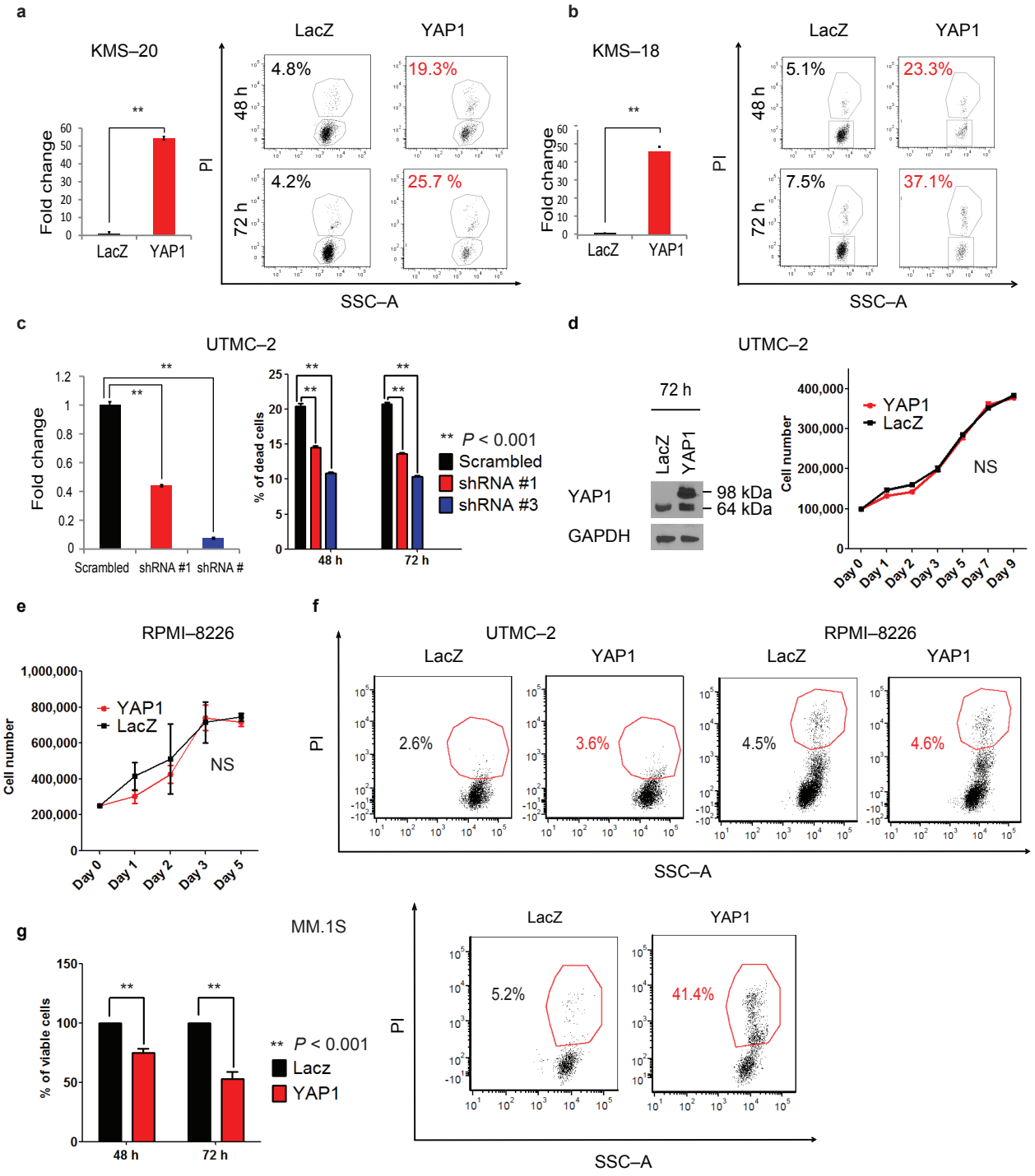


Supplementary Fig. 5

YAP1 is consistently down-regulated in MM cells

(**a**) YAP1 expression in dataset GSE2658 and GSE5900, as in Figure 3, but using probe sets 228494_at (left panel) and 213342_at (right panel).
(**b**) YAP1 expression in subjects with MGUS and MM obtained from E–MTAB–372, probe set 224895_at.
(**c**) YAP1 expression in subjects with MGUS, MM, and Plasma Cell Leukemia (PCL) obtained from the GSE13591 dataset.
*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
(**d**) Western blot analysis of YAP1 in MM cell lines.
(**e**) mRNA levels measured by qPCR analysis and calculated as delta delta Ct values on a panel of MM cell lines.

Supplementary Figure S6_Cottini

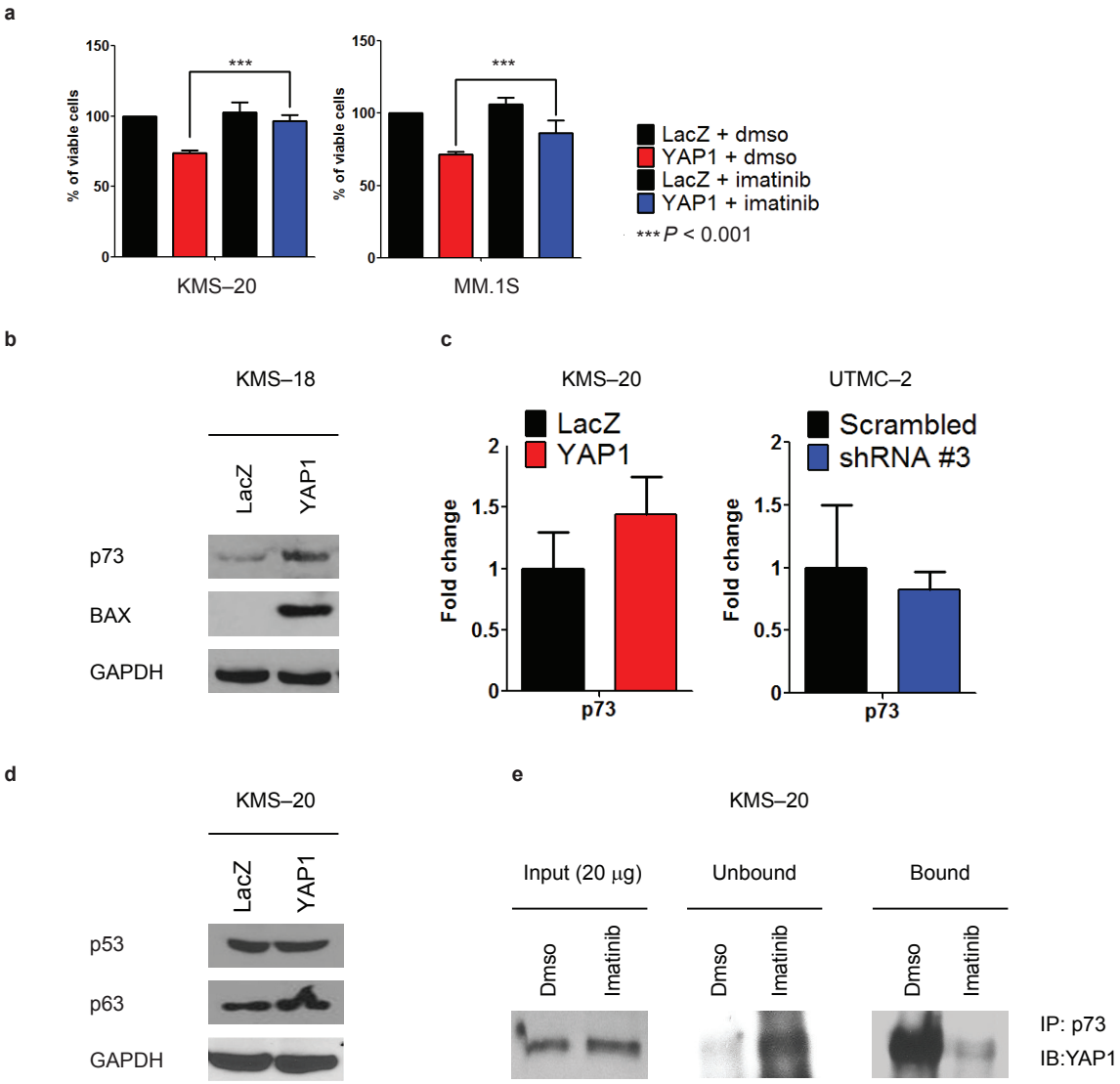


Supplementary Fig. 6

Effects on cell number and apoptosis after modulation of YAP1 expression in MM cells

(**a**) YAP1 re–expression in KMS–20 and (**b**) KMS–18 MM cells using AMAXA electroporation. qPCR (delta delta Ct) analysis and PI staining to detect dead cells after gating on GFP–positive cells are shown. Data shown are representative of three independent experiments.
(**c**) YAP1 silencing in UTMC–2 MM cell line using a lentiviral delivery system. Left panel: YAP1 mRNA levels assessed with qPCR (delta delta Ct); right panel: Cell death is measured with Annexin V–FITC/PI staining. The percentage of dead cells is the sum of Annexin V^+PI^+, Annexin V^+, PI^- and Annexin V^-, PI^+ cells. Mean values ± SD of triplicates of two different experiments.
(**d**) YAP1 re–expression in UTMC–2 MM cell line transfected using AMAXA electroporation (data shown are representative of two independent experiments). YAP1 levels were evaluated by western blot analysis. The two bands present in the western blot correspond to wild type YAP1 (64 kDa) and YAP1–EGFP (98 kDa). Right panel: cell number evaluated by cell counting with trypan blue exclusion in UTMC–2.
(**e**) Cell number evaluated by cell counting with trypan blue exclusion in RPMI–8226 MM cell line after YAP1 re–expression using AMAXA electroporation.
(**f**) PI staining in UTMC–2 and RPMI–8226 MM cells after transfection as in panel (**d**) and (**e**) at 72 h after transfection.
(**g**) YAP1 re–expression in MM.1S cells using AMAXA electroporation. Left panel: Cell viability is evaluated by MTT absorbance assay. Mean values ± SD of triplicates of two experiments are shown. Right panel: PI staining was used to detect dead cells after gating on GFP–positive cells at 72 h.

Supplementary Figure S7_Cottini

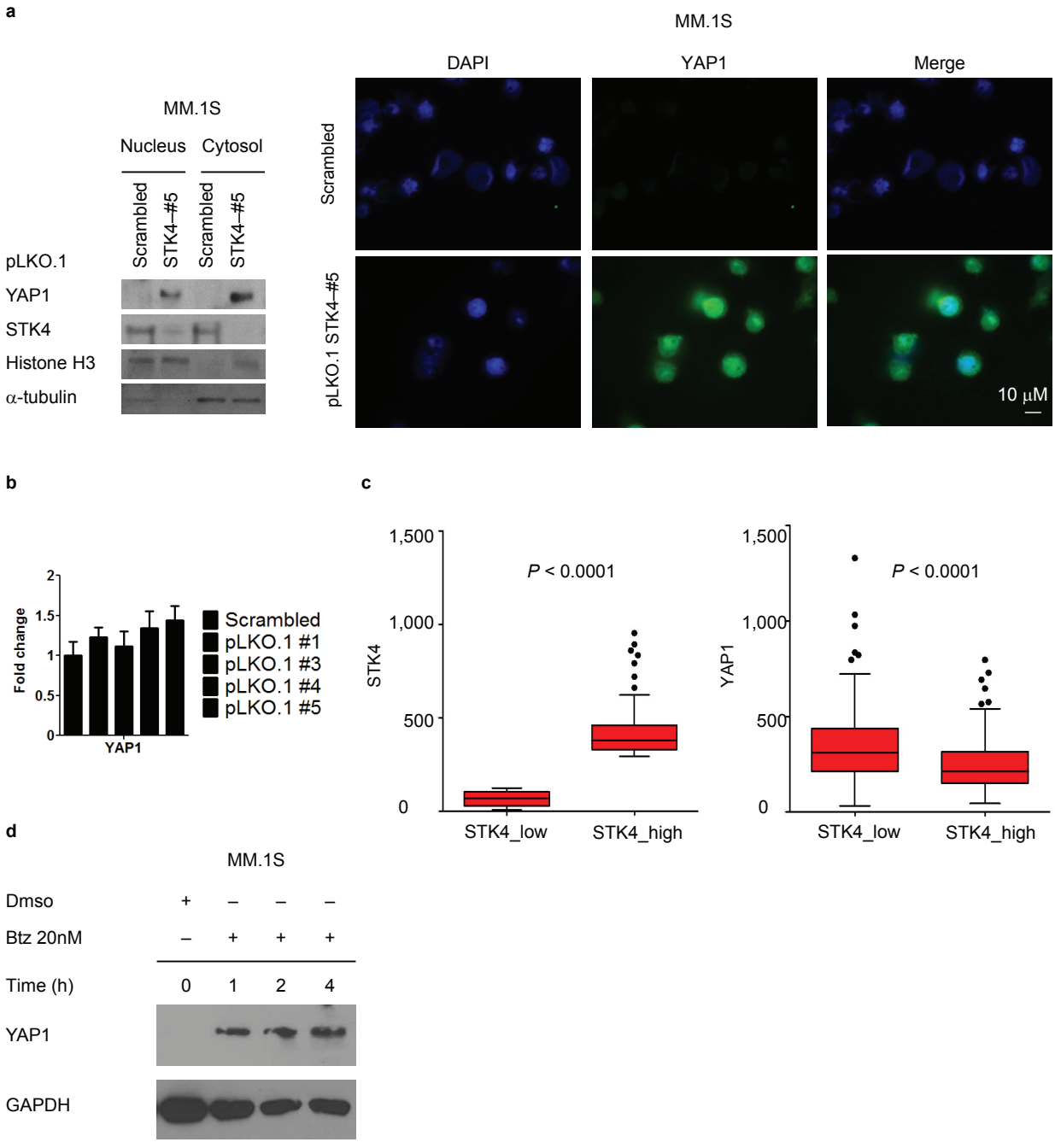


Supplementary Fig. 7

Role of ABL1 and engagement of p73 in YAP1–mediated apoptosis in MM cells

(**a**) Cell viability measured by MTT absorbance assay in KMS–20 and MM.1S transfected cells, as described in **Fig. 4a** and **4d** respectively after treatment with 10 μM Imatinib initiated at day 0 of transfection. Percentage of viable cells was calculated normalizing to absorbance values of LacZ + dmso. Mean values ± SD of triplicates of two experiments are shown.
(**b**) Western blot analysis for p73 and BAX in KMS–18 MM cells transfected with pLENTI4–YAP1–EGFP or LacZ.
(**c**) p73 mRNA levels in KMS–20 MM cells transfected with YAP1–EGFP or LacZ (left panel), and UTMC–2 cells infected with a YAP1–specific shRNA or with scrambled shRNA (right panel). 72 h data after transfection and infection, respectively. Mean values ± SD of triplicates of two experiments are shown.
(**d**) Western blot analysis for p53 and p63 in KMS–20 MM cells transfected with pLENTI4–YAP1–EGFP or LacZ, 48 h after transfection.
(**e**) Co–immunoprecipitation between p73 and YAP1 in KMS–20–transfected cells after 48h treatment with Dmso or 10 μM Imatinib.

Supplementary Figure S8_Cottini

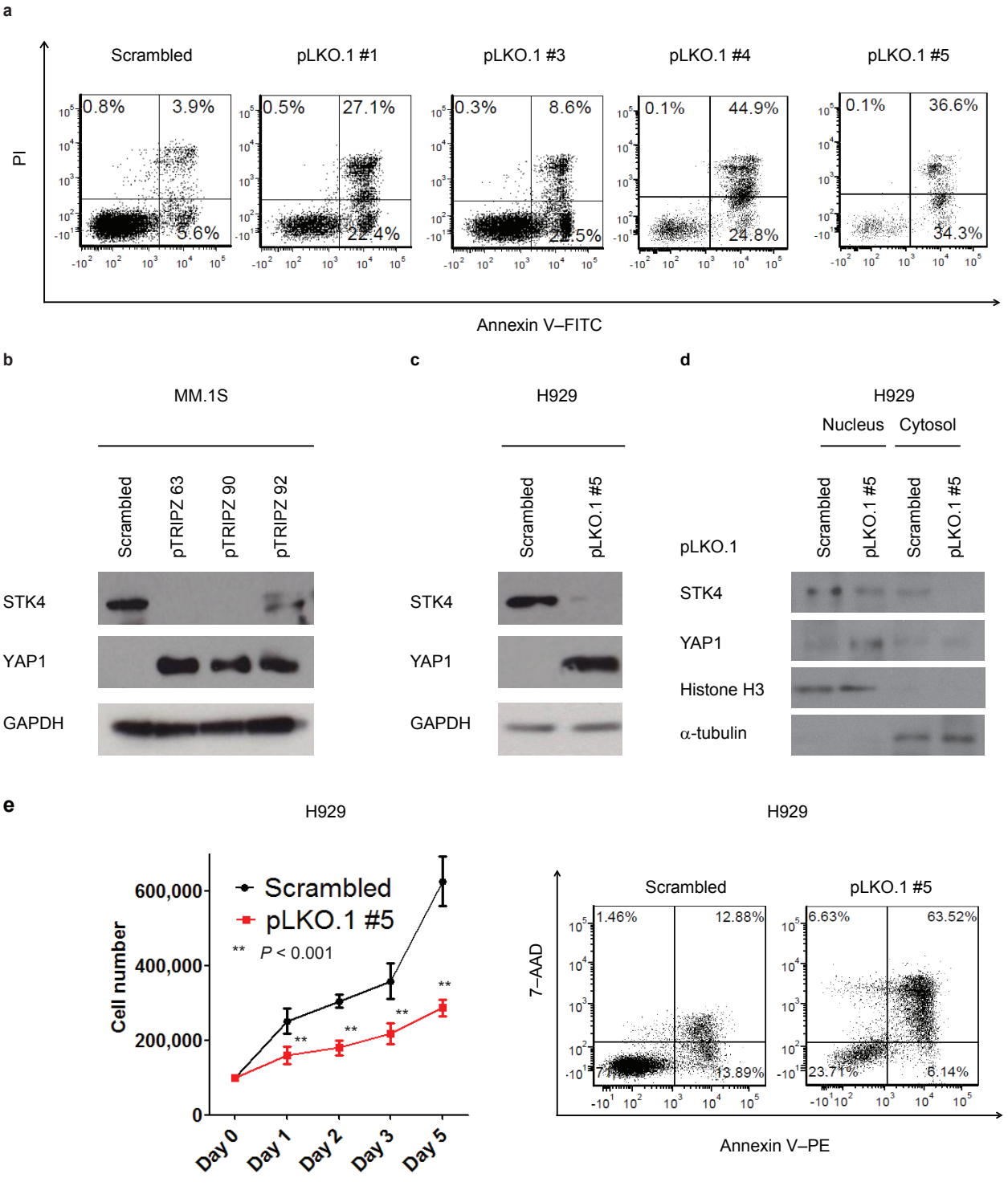


Supplementary Fig. 8

YAP1 and STK4 regulation in MM cells

(**a**) YAP1 cellular localization in MM.1S, after STK4 silencing. Left panel: Western blot with nuclear (N) and cytosolic (C) fractions for STK4, YAP1, α-tubulin, and Histone H3. Right panel: Immunofluorescence for YAP1 after STK4 silencing in MM.1S cells.
(**b**) qPCR for YAP1 mRNA levels in MM.1S after STK4 silencing. One representative experiment of two is shown.
(**c**) Left panel: GSE2658 gene expression dataset was sorted according to STK4 expression (probe set 211085_s).
Top and bottom quartiles were selected (140 individuals affected by MM for each group) and STK4 expression was compared using box–and–whisker plots. Right panel: Levels of expression for YAP1 (probe set 224895_at) in the same groups.
(**d**) Western blot for YAP1 and GAPDH in MM.1S after incubation with 20 nM Bortezomib (Btz) for 1, 2, and 4 h.
One representative experiment of two is shown.

Supplementary Figure S9_Cottini

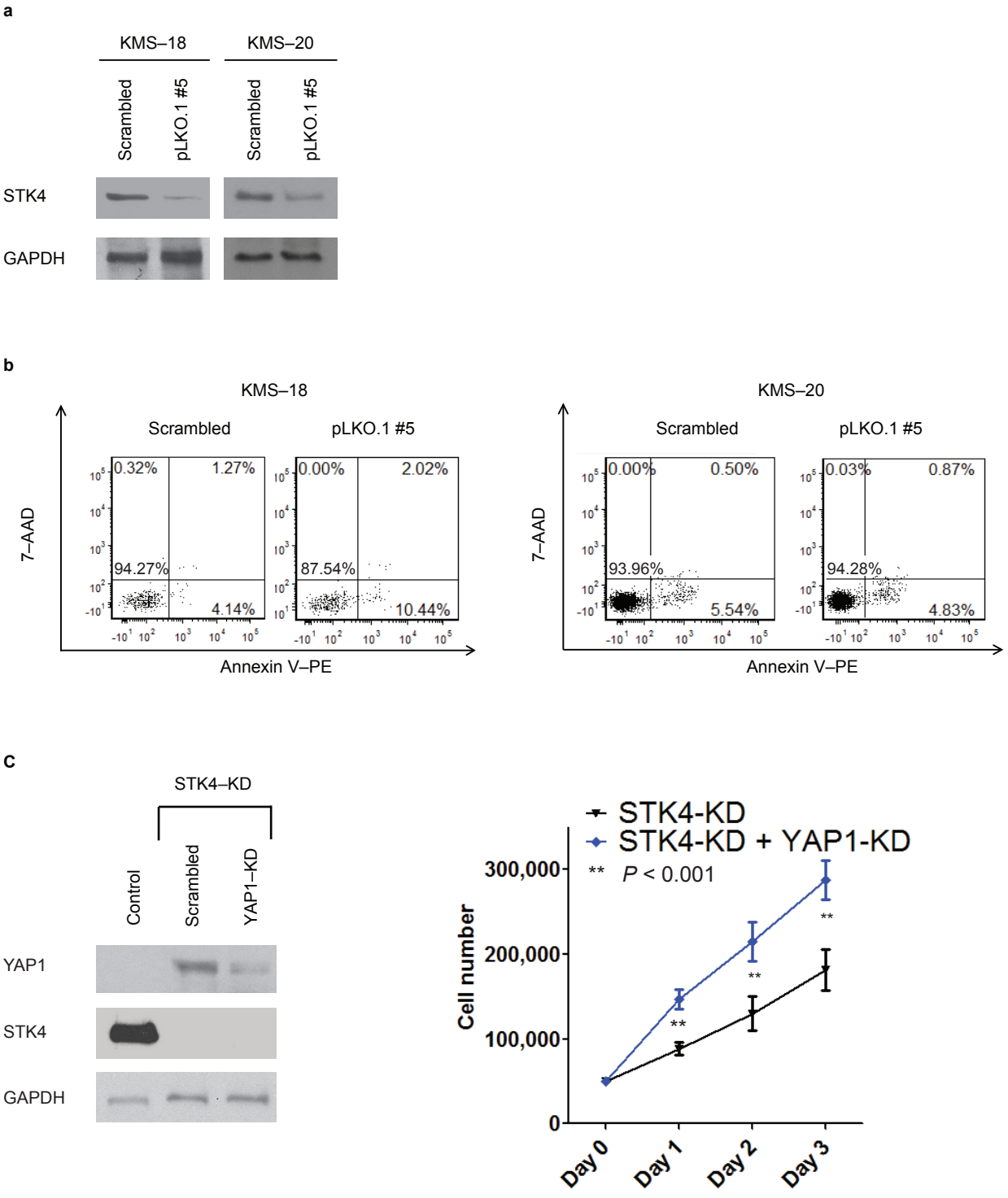


Supplementary Fig. 9

Effects of STK4 silencing in MM.1S and H929 cells

(**a**) Annexin V–FITC/PI staining in scrambled and STK4–silenced MM.1S cells. Data shown are representative of two independent experiments.
(**b**) Western blot for STK4, YAP1, and GAPDH in MM.1S cells infected with inducible lentiviral vectors, and selected with 2 μg mL^{-1} puromycin for 48 h. Cells were then exposed for 72 h to 2 μg mL^{-1} doxycycline. One representative experiment of three is shown.
(**c**) Western blot for STK4, YAP1, and GAPDH in H929 cells transfected with pLKO.1 containing a scrambled sequence or shRNA #5 targeting STK4. One representative experiment of two is shown.
(**d**) Western blot with nuclear (N) and cytosolic (C) fractions for STK4, YAP1, α-tubulin, and Histone H3 in H929 MM cell lines. One representative experiment of two is shown.
(**e**) Left panel: Cell number evaluated by cell counting in triplicates with trypan blue exclusion. Mean values ± SD of triplicates of two different experiments. Right panel: Annexin V–PE/7–AAD staining after gating on GFP–positive cells in STK4–silenced H929 cells. Data shown are representative of two independent experiments.

Supplementary Figure S10_Cottini

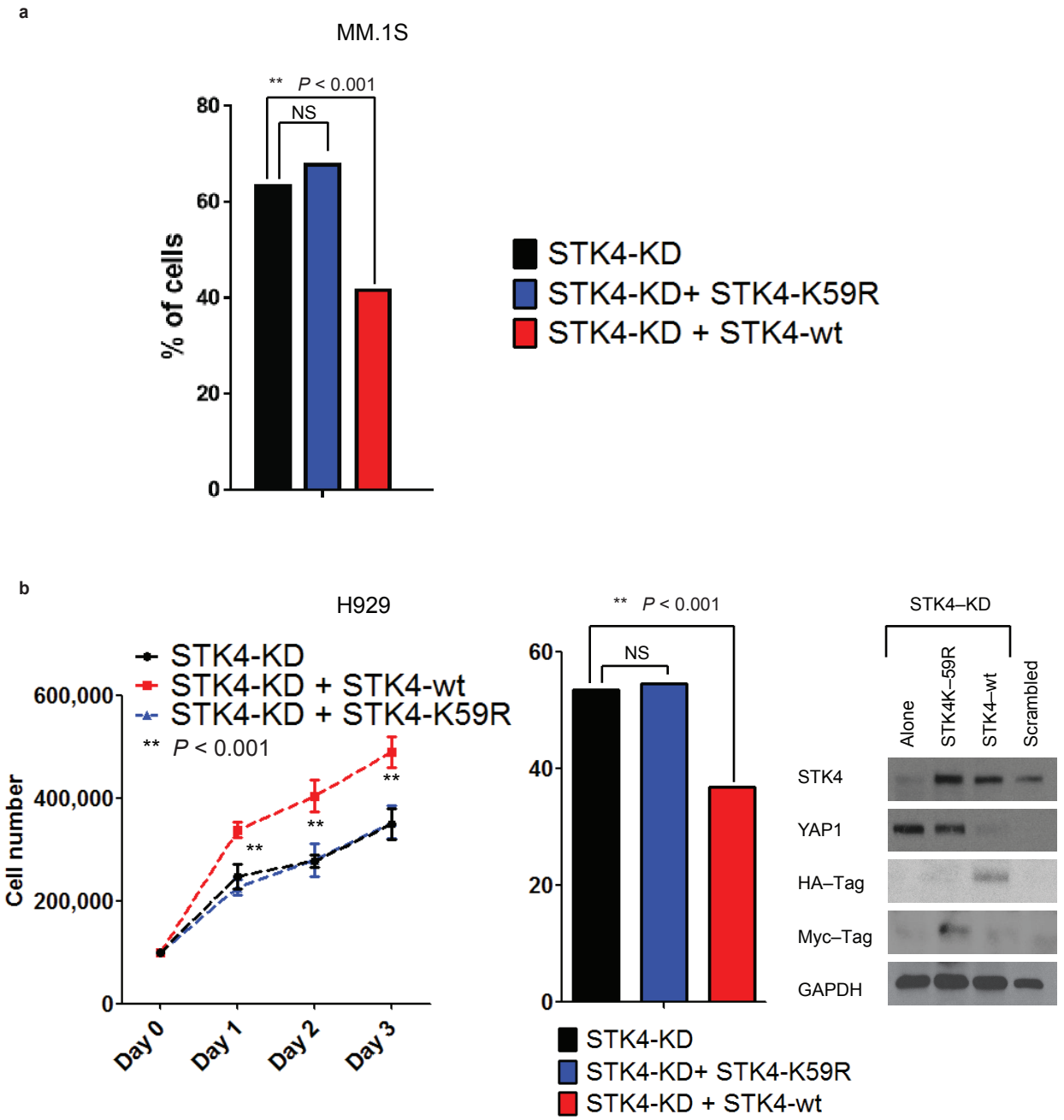


Supplementary Fig. 10

YAP1 is required for STK4–mediated growth inhibitory effects in MM cells

(**a**) Western blot for STK4 and GAPDH in KMS–18 and KMS–20 cells transfected with pLKO.1 containing a scrambled sequence or shRNA #5 targeting STK4.
(**b**) Annexin V–PE/7–AAD staining in KMS–18 and KMS–20 cells after gating on GFP–positive cells at 72 h.
(**c**) STK4 silencing using an inducible shRNA (pLKO.1–TET #4) by lentiviral infection, followed by YAP1 silencing (shRNA #5 against YAP1) in MM.1S cells. Left panel: Western blot for YAP1, STK4 and GAPDH. Right panel: Cell counting in triplicates.

Supplementary Figure S11_Cottini



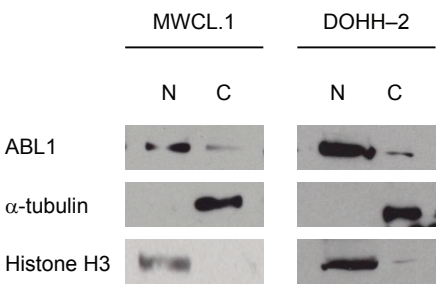
Supplementary Fig. 11

STK4 kinase activity is required for YAP1 inactivation

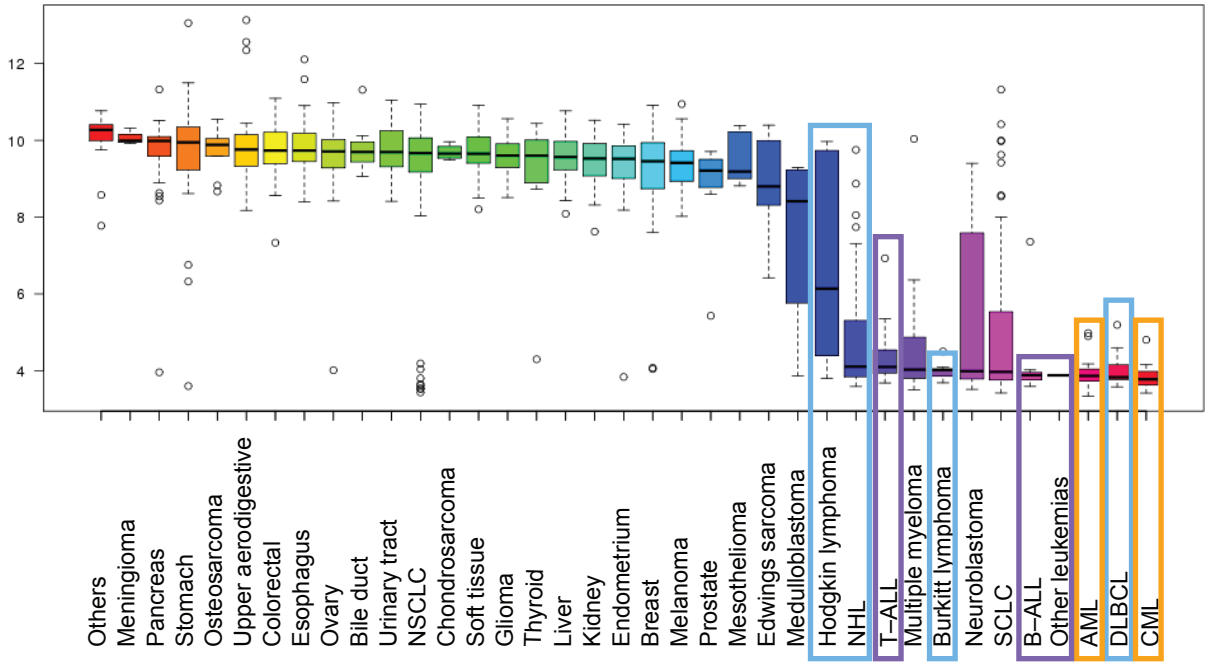
(**a**) Annexin V–FITC/PI staining in MM.1S silenced for STK4 and then transfected with a vector coding for pJ3H–STK4 wild type (STK4–wt) or pJ3H–STK4 K59R mutant (STK4–K59R mutant).
(**b**) H929 cells were co–transfected with pLKO.1 shRNA #5 against STK4 alone, or together with STK4–wt or STK4–K59R mutant. Cells transfected with scrambled shRNAs are used as internal control.
Left panel: Cell growth evaluated by cell counting with trypan blue exclusion. Middle panel: Annexin V–FITC/PI staining.
Right panel: Western blot for STK4, YAP1, HA–Tag, Myc–Tag, and GAPDH at 48 h after transfection.

Supplementary Figure S12_Cottini

a



b

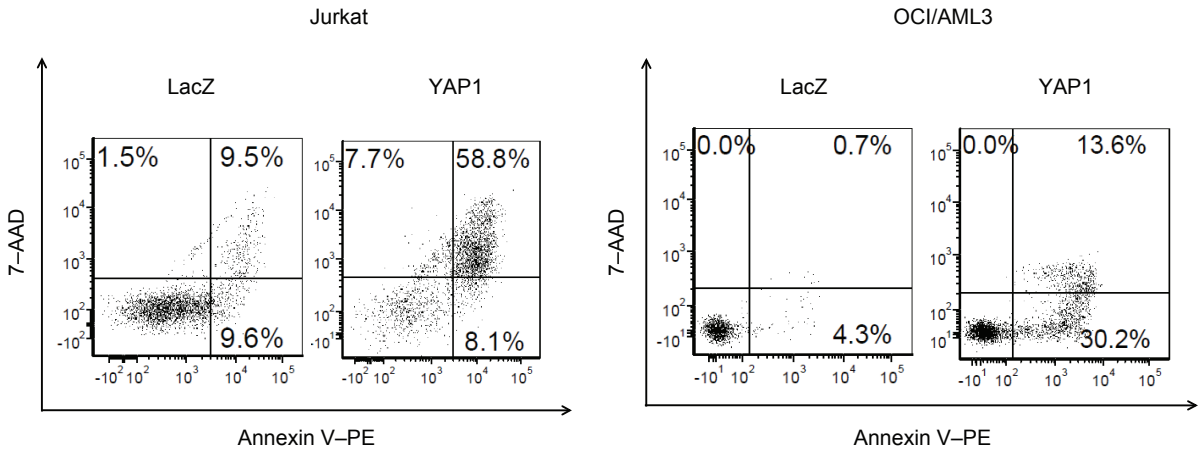


Supplementary Fig. 12

ABL1 cellular localization and YAP1 expression levels in hematological cancer cell lines

(**a**) Subcellular fractionation of MWCL.1 (Waldenström's macroglobulinemia) and DOHH–2 (lymphoma) hematological cancer cell lines. Cell lysates from cytoplasmic (C) and nuclear (N) fractions were analyzed by western blot for ABL1 expression. α-tubulin and Histone H3 were used as loading controls for C and N fractions, respectively.
(**b**) YAP1 mRNA expression in solid tumors and hematological cancer cell lines (Broad–Novartis Cancer Cell Line Encyclopedia, at http://www.broadinstitute.org/ccle/home). Abbreviations: NSCLC (Non–small–cell lung carcinoma), NHL (Non–Hodgkin lymphoma), T–ALL (T–cell acute lymphoblastic leukemia), SCLC (small–cell lung carcinoma), B–ALL (B–cell acute lymphoblastic leukemia), AML (acute myeloid leukemia), DLBCL (Diffuse large B–cell lymphoma), and CML (chronic myeloid leukemia).

Supplementary Figure S13_Cottini

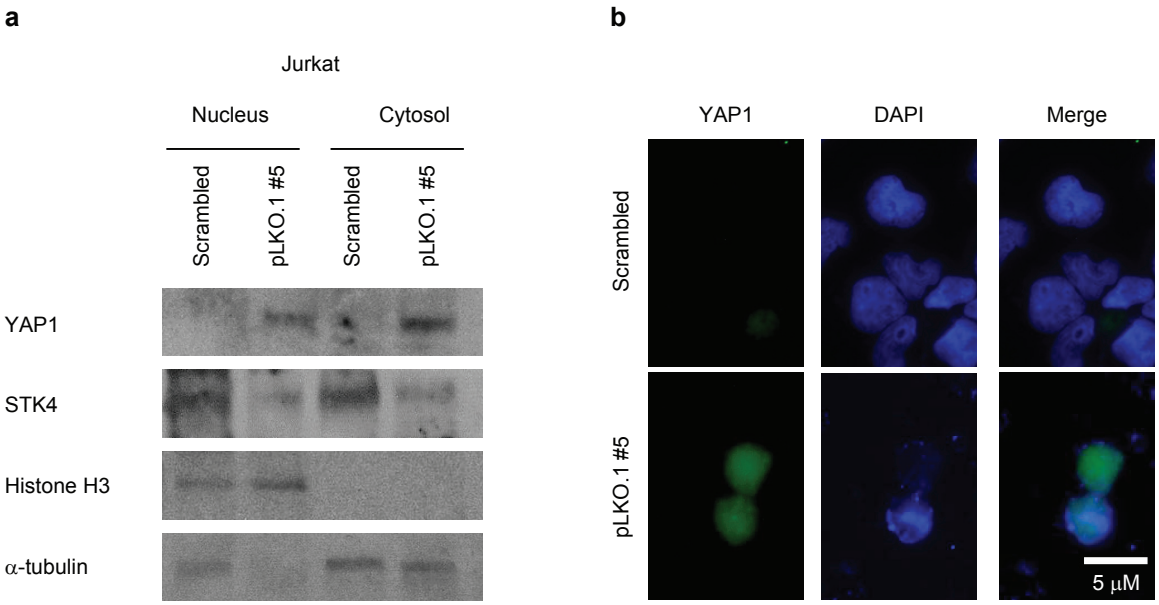


Supplementary Fig. 13

Effect on apoptosis following YAP1 re–expression in Jurkat and OCI/AML3 cells

Annexin V–PE/7–AAD staining to detect apoptotic cells after gating on GFP–positive in Jurkat and OCI/AML3 cells after YAP1 overexpression. Data shown are representative of two independent experiments.

Supplementary Figure S14_Cottini

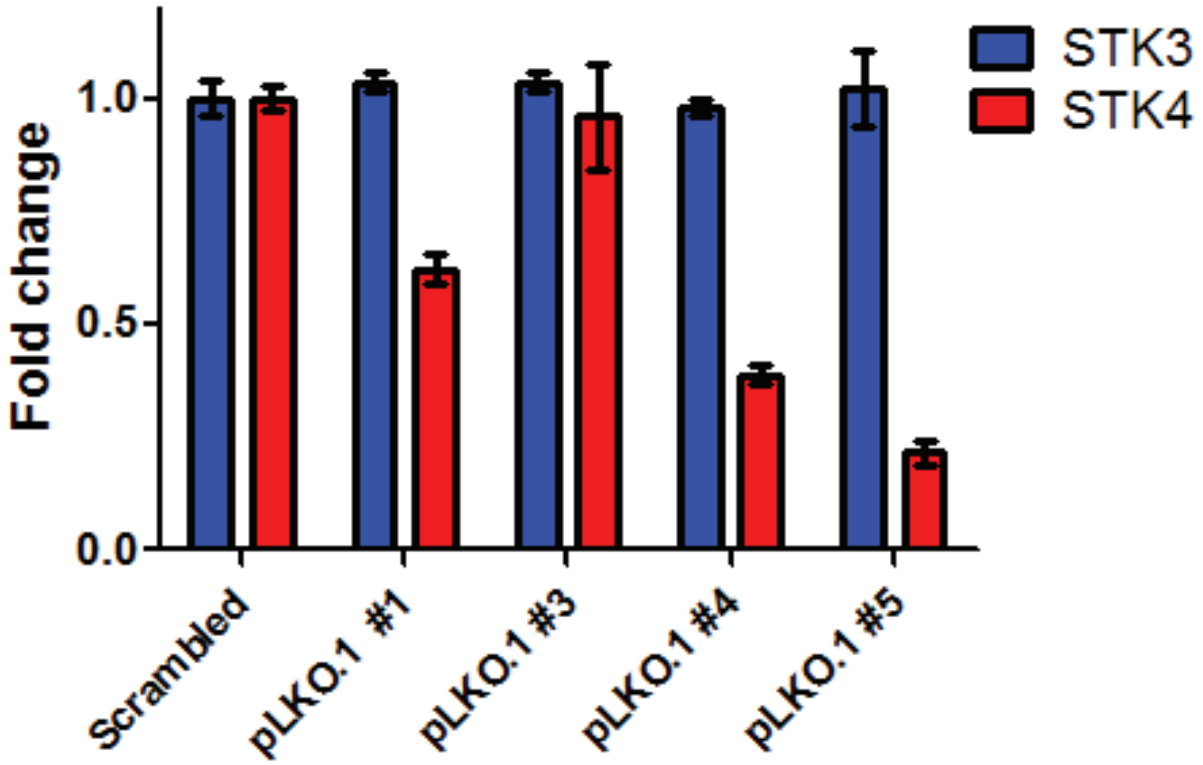


Supplementary Fig. 14

YAP1 cellular localization in Jurkat cells, after STK4 silencing.

(**a**) Western blot with nuclear (N) and cytosolic (C) fractions for STK4, YAP1, α-tubulin, and Histone H3 in Jurkat cells.
(**b**) Immunofluorescence for YAP1 after STK4 silencing in Jurkat cells.

Supplementary Figure S15_Cottini



Supplementary Fig. 15

Effects of STK4 knockdown with specific shRNAs on STK3 mRNA levels

qPCR analysis for STK4 and STK3 mRNA in scrambled and STK4–silenced MM.1S cells.