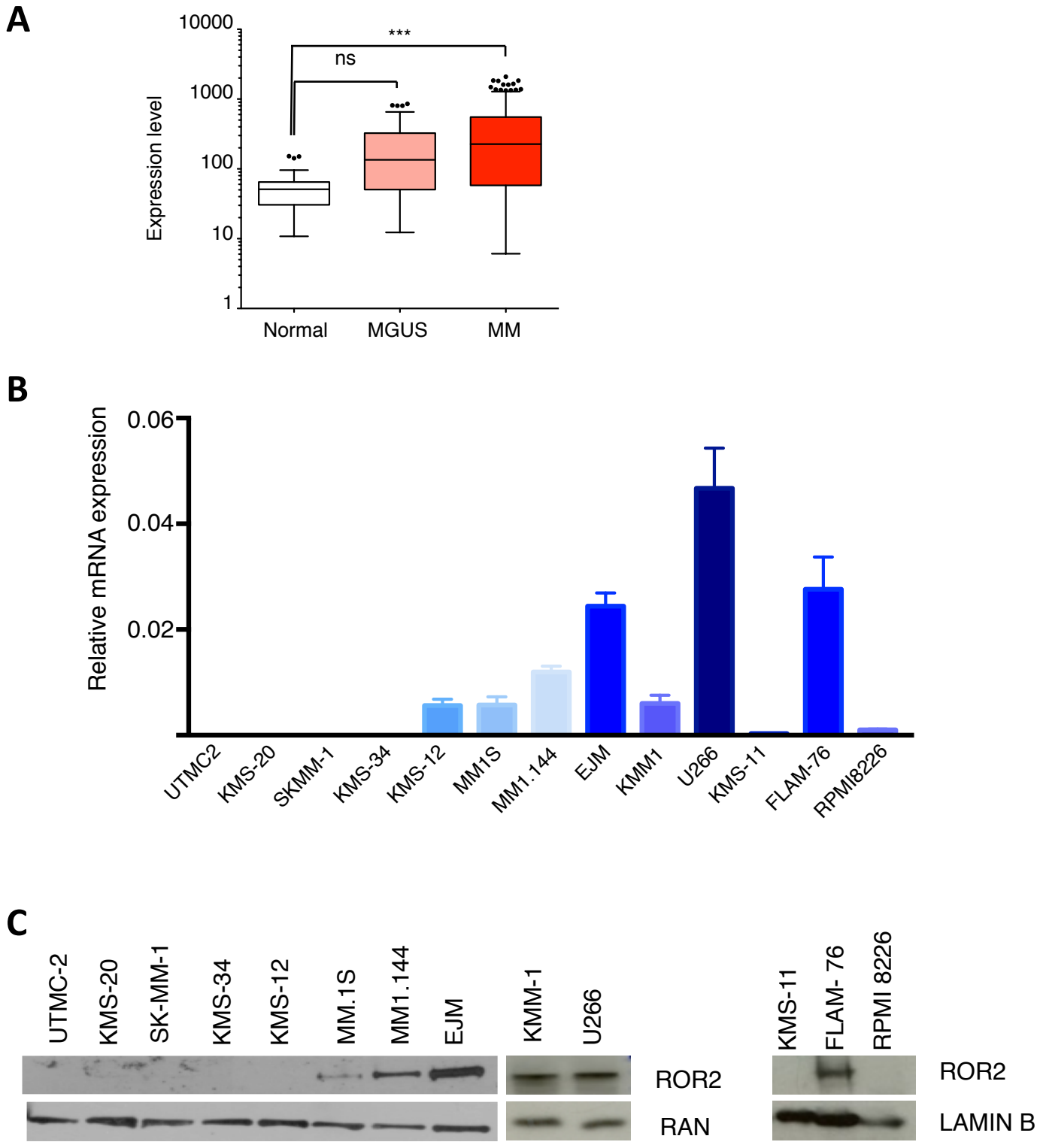
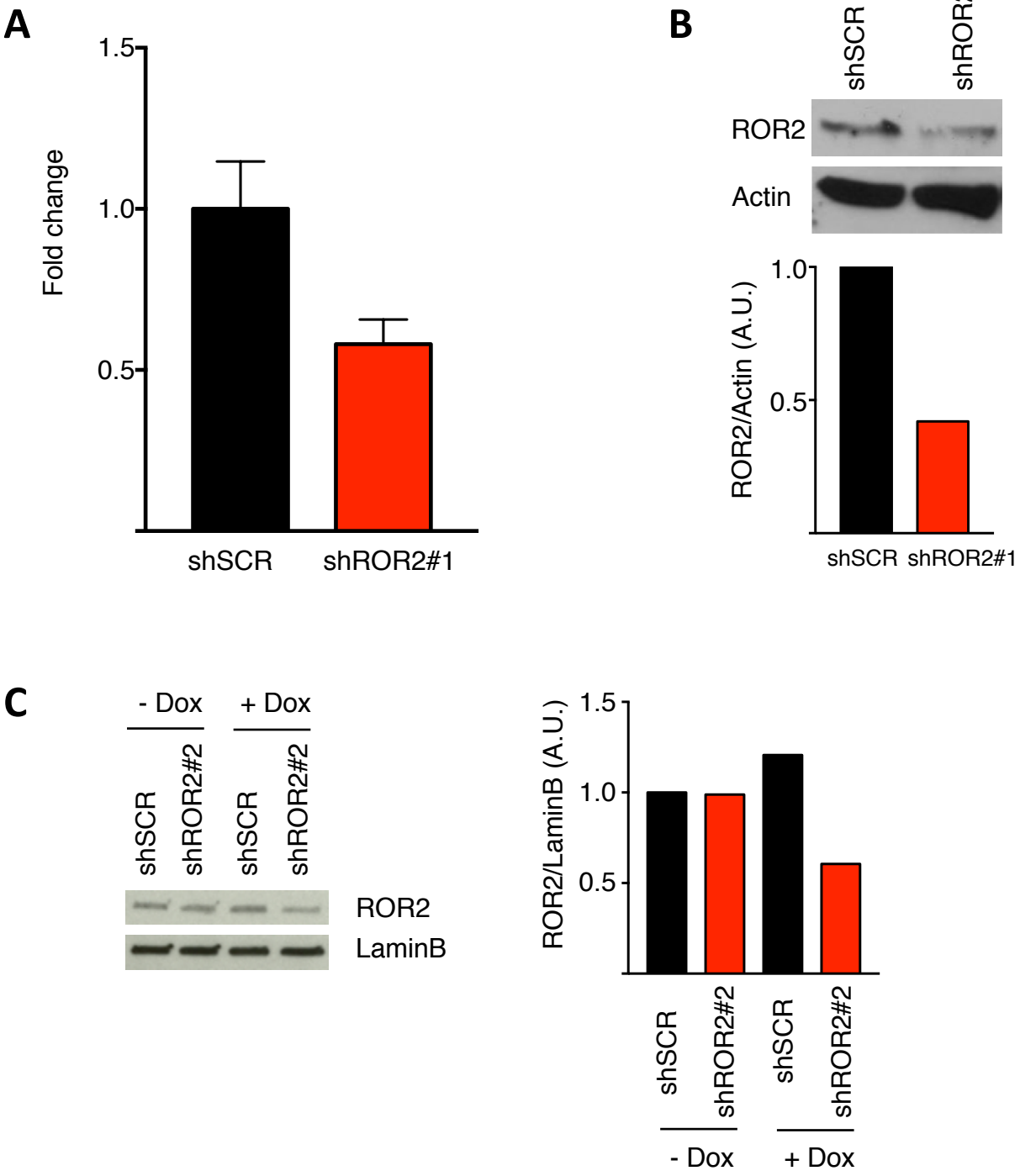


Supplemental Figure 1: ROR2 expression in Multiple Myeloma patients and cell lines.

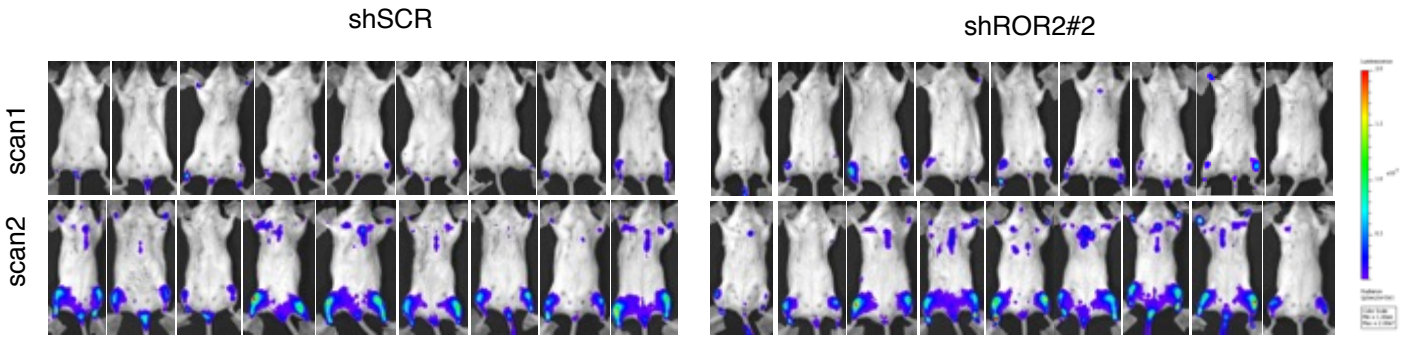
A) ROR2 mRNA expression in CD138+ plasma cells from healthy individuals (n=22), MGUS (n=44) and MM patients (n=559). ***p<0.001 (Tukey's multiple comparison test).
B) ROR2 mRNA expression in different MM cell lines. Data are expressed as relative to GAPDH expression (2^{-DCt}).
C) ROR2 protein expression in different MM cell lines. RAN and LaminB are used as loading controls.



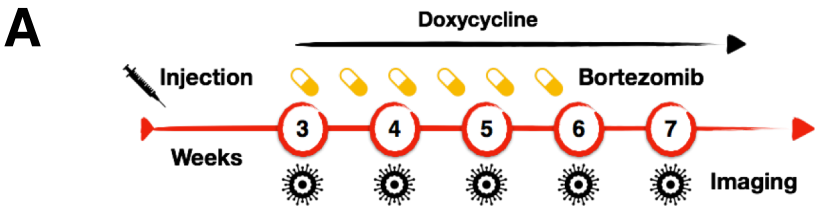
Supplemental Figure 2.

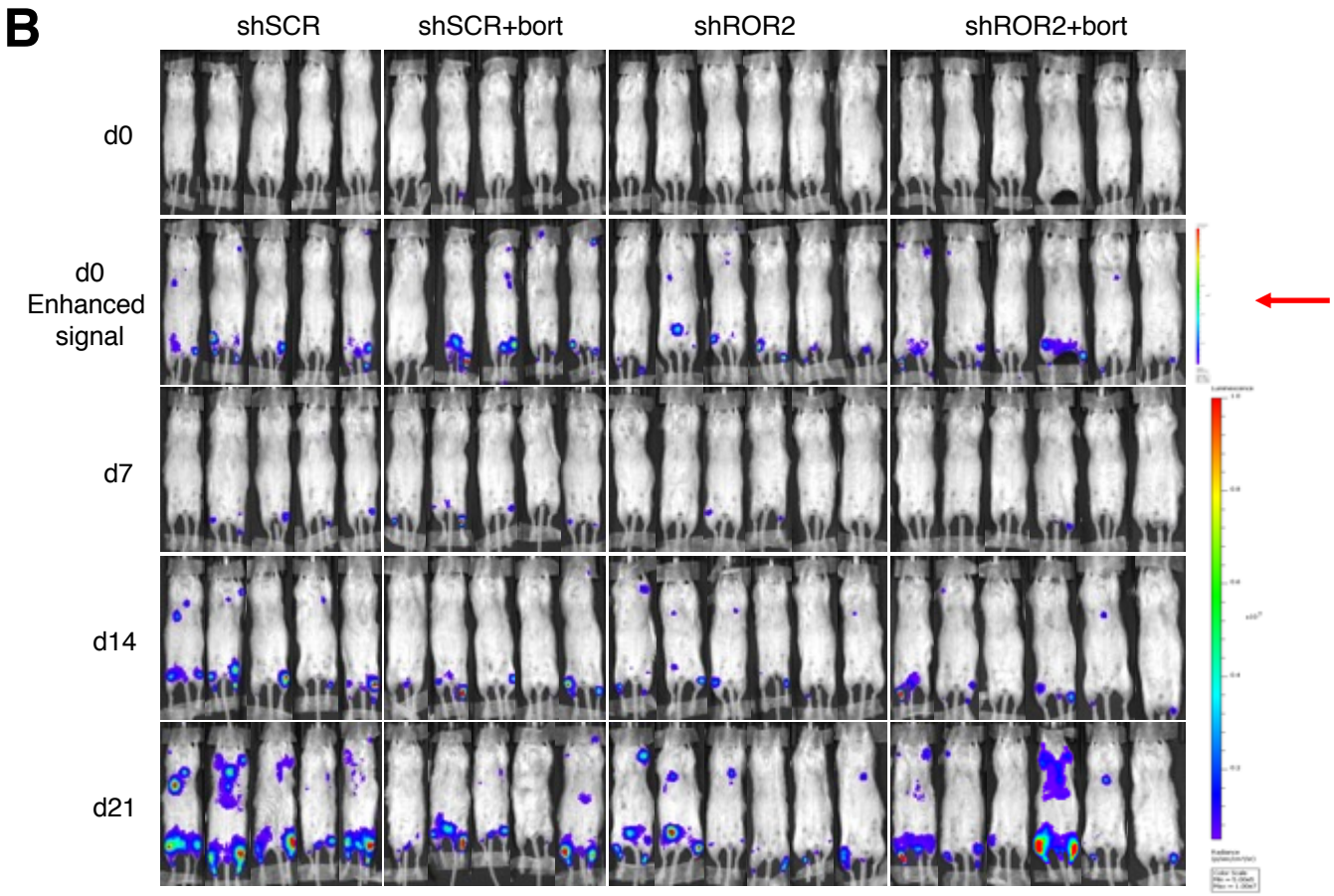
Downregulation levels of ROR2, analyzed by real time PCR (A) and western blot (B). Quantification of western Blot signals was performed using the ImageJ software and represented as the ratio between ROR2 and the loading control actin, using the shSCR sample as the baseline (Arbitrary Units: A.U.)

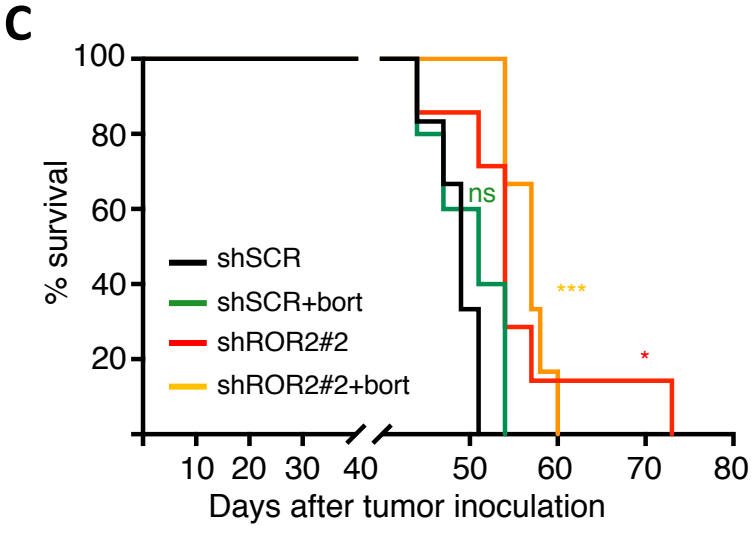
C) Efficiency of downregulation upon treatment with Doxycycline analyzed by western blot. Quantification of western Blot signals was performed using the ImageJ software and represented as the ratio between ROR2 and the loading control Lamin B, using the shSCR sample without doxycycline as baseline (Arbitrary Units: A.U.).



Supplemental Figure 3: ROR2 targeting delays tumor progression.
Mice injected with MM1.144Luc cells transduced with inducible shSCR or shROR2#2 were analyzed by in vivo imaging three weeks after injection (Scan1), then they started to be fed with doxycycline and imaged again after one week (Scan2). Images of each mouse from the first and second scans are shown.





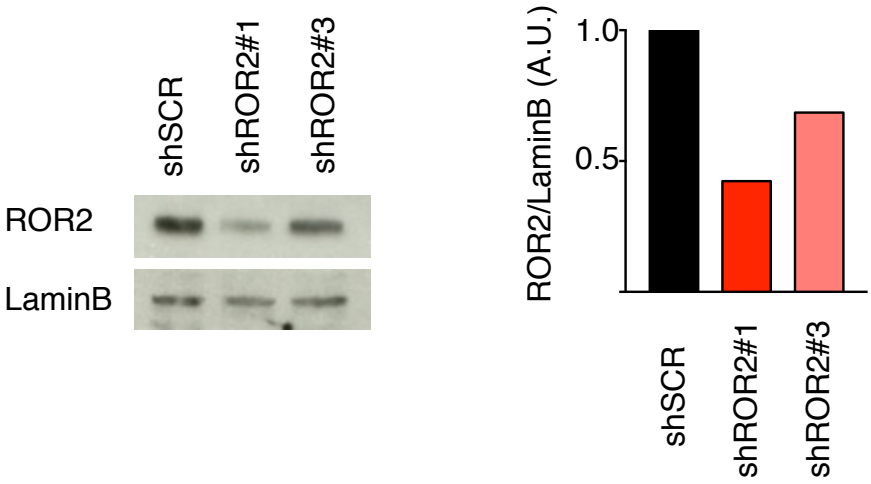


Supplemental Figure 4: ROR2 targeting delays tumor progression.

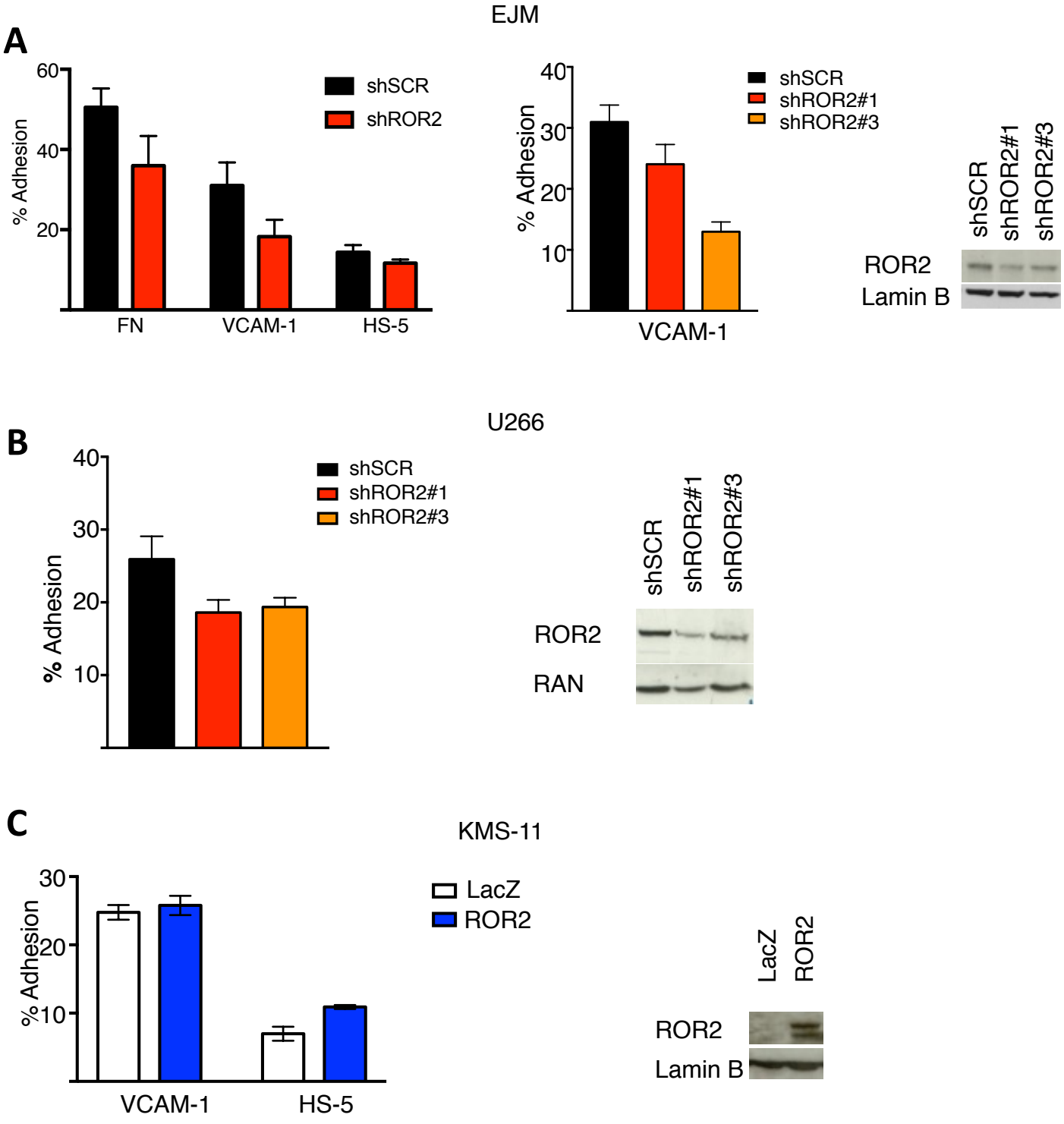
A) Experimental outline. Rag2–/–γc–/– mice were injected with MM1.144Luc cells transduced with inducible shRNAs, either control non-targeting shSCR or ROR2 specific shROR2#2. Twenty-one days after MM cell injection, mice were assessed by in vivo bioluminescent imaging and started to be fed with doxycycline-containing food. Mice were randomized according to the initial BLI signal and treated with saline or bortezomib (0,5 mg/Kg) twice a week for three weeks and monitored by in vivo imaging until terminally ill.

B) Images of each mouse from each scan performed. The first scan (d0) is shown also as enhanced signal (scale marked by red arrow) to evidence the presence of disease.

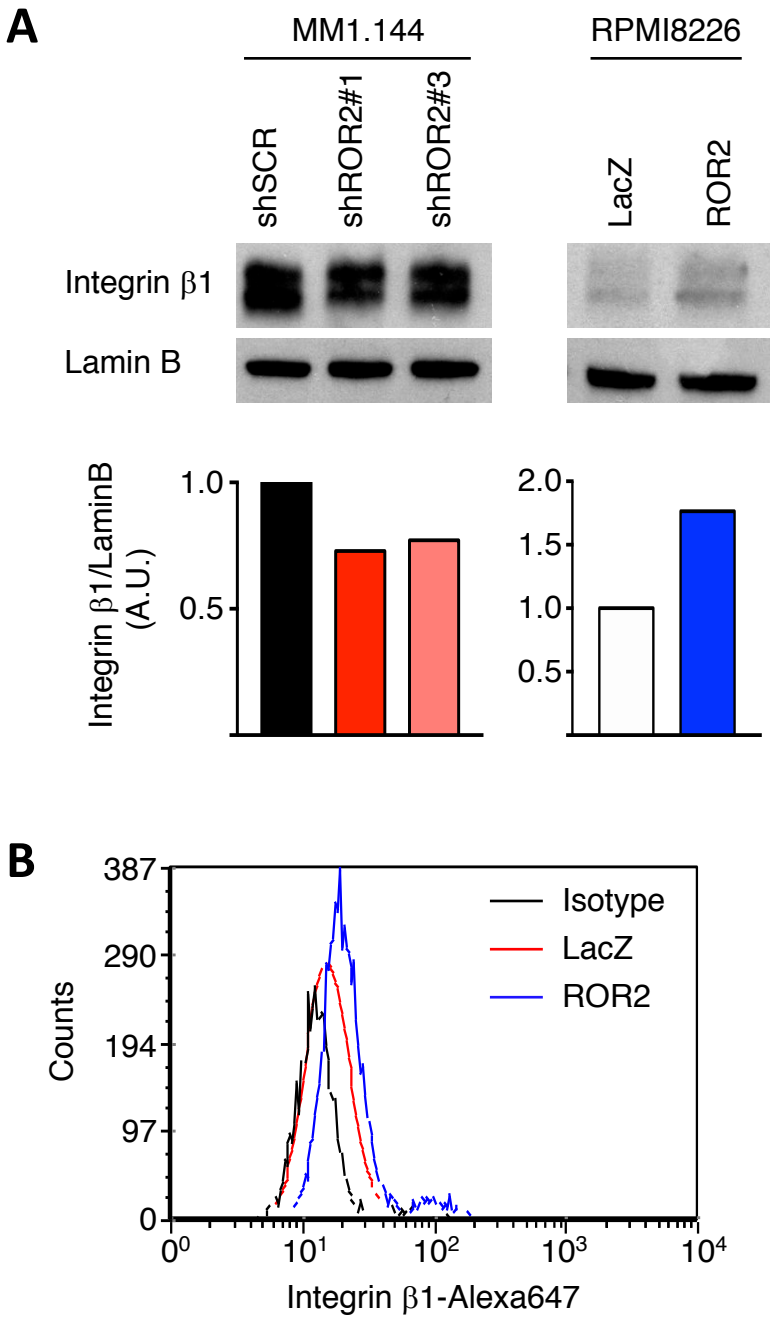
C) Survival curves of mice as in A). Statistical analysis was performed using the log-rank Mantel-Cox test comparing each group to the shSCR group. * $p<0.05$, *** $p<0.001$.



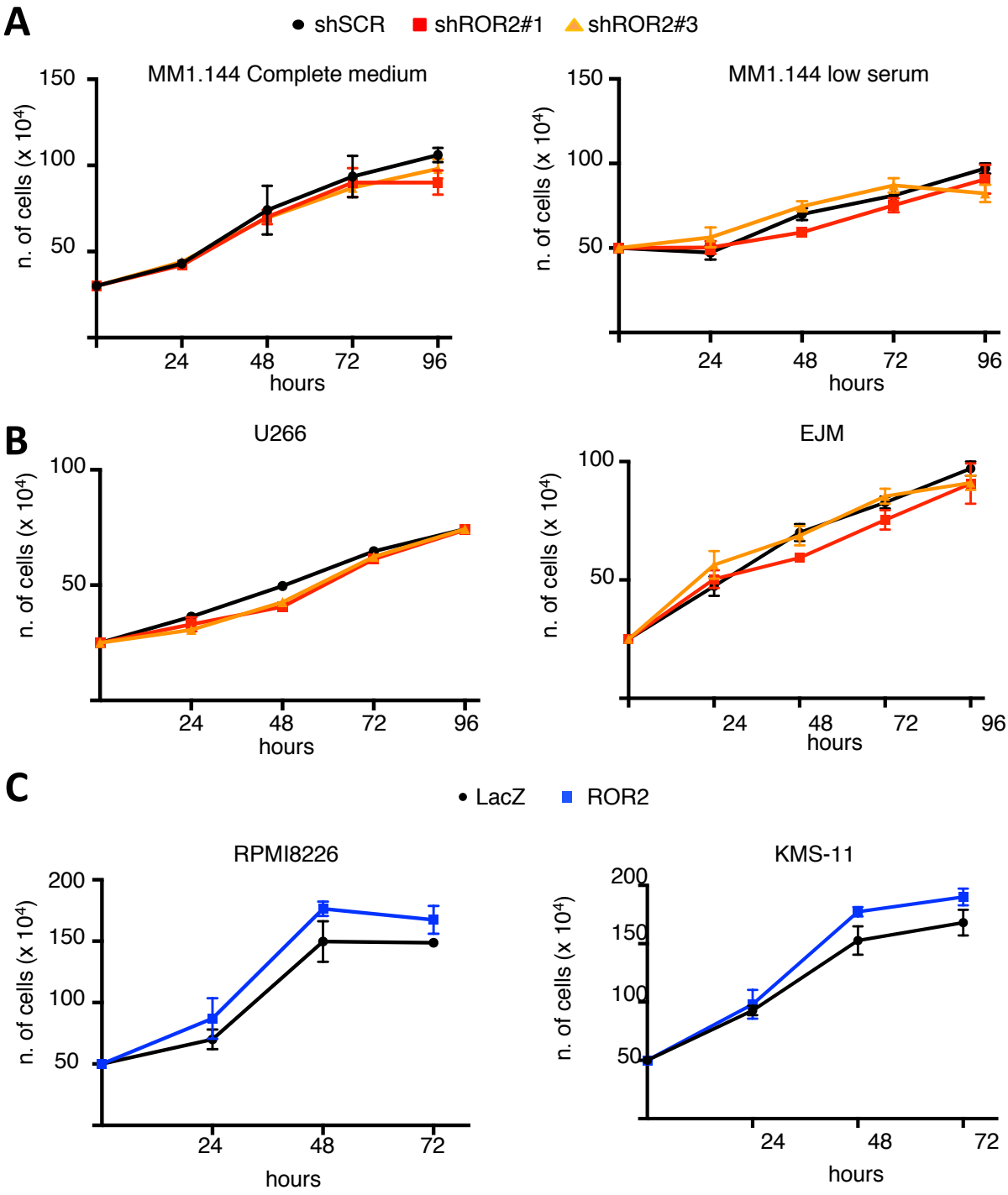
Supplemental Figure 5: ROR2 knockdown efficiency.
Total cell lysates from MM1.44 cells transduced with a a control non-targeting shRNA (shSCR) or two ROR2-specific shRNAs (shROR2#1 and shROR2#3) were resolved on SDS-PAGE and membranes were probed with ROR2, and LaminB.



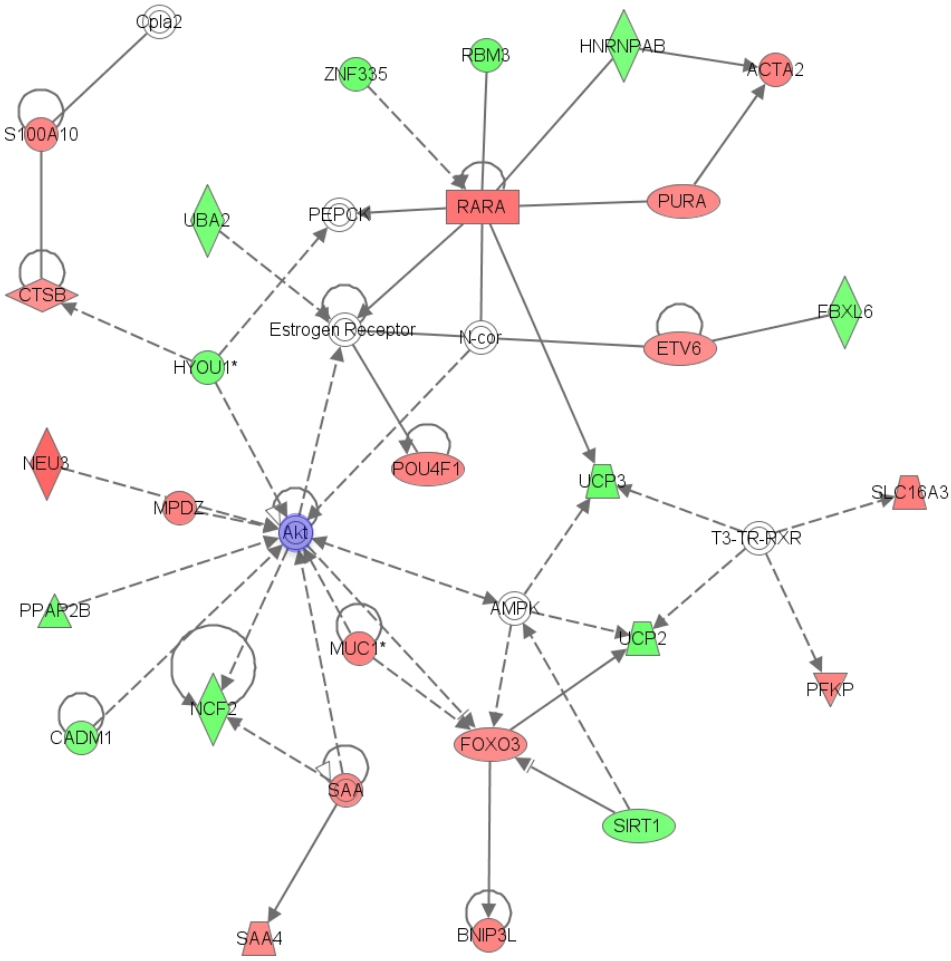
Supplemental Figure 6: ROR2 expression modifies adhesion properties of myeloma cells.
A) Adhesion of EJM cells shSCR and shROR2#1 to recombinant VCAM-1, to Fibronectin (FN) and to the stromal cell line HS-5 (left). Adhesion to VCAM-1 was also assessed for an additional ROR2-specific shRNA (shROR2#3) (right). Results are shown as percentages of adhering cells with respect to the total plated cells. One representative experiment of three is shown (technical replicates are shown). Efficiency of knockdown was evaluated by western blot.
B) Adhesion of U266 cells shSCR, shROR2#1 and shROR2#3 to VCAM-1. Results are shown as percentages of adhering cells in respect to the total cells plated. One representative experiment of three is shown (technical replicates are shown). Efficiency of knockdown was evaluated by western blot.
C) Adhesion of KMS-11 cells expressing LacZ or ROR2 to recombinant VCAM-1 (left) and to HS5 cells (right). Two experiments were performed. Overexpression was assessed by western blot.



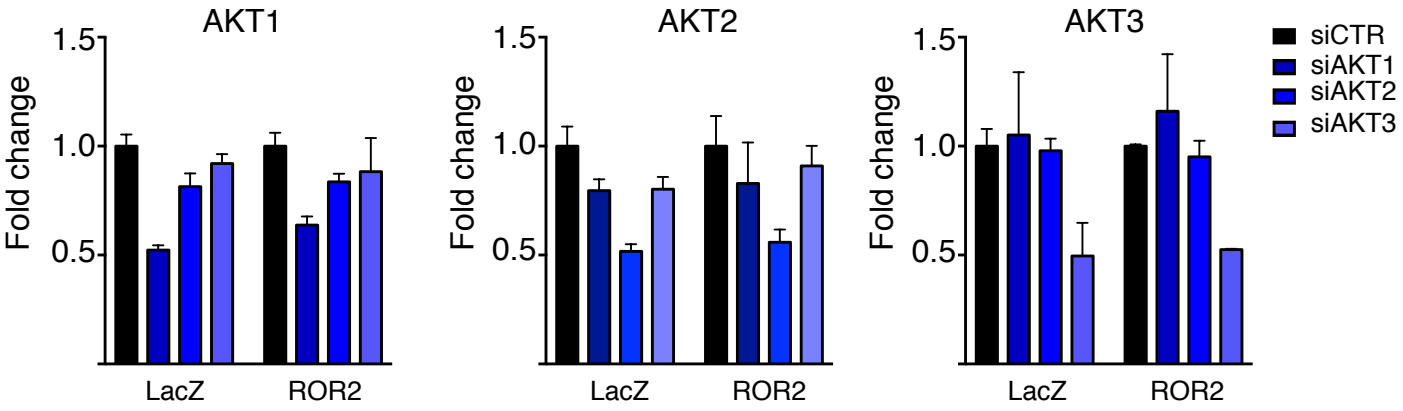
Supplemental Figure 7: ROR2 affects Integrin β1 expression. A)Total cell lysates of MM1.144 cells transduced with a a control non-targeting shRNA (shSCR) or two ROR2-specific shRNAs (shROR2#1 and shROR2#3) and RPMI8226 cells expressing LacZ or ROR2 were resolved on SDS-PAGE and membranes were probed with Integrin β1. LaminB was used as loading control. Quantification of western Blot signals was performed using the ImageJ software and represented as the ratio between Integrin β1 and the loading control LaminB, using the shSCR sample(left) or LacZ(right) as the baseline (Arbitrary Units: A.U.).
B) RPMI8226 cells cells expressing LacZ or ROR2 were stained with Integrinβ1 or a Isotype control IgG, followed by AlexaFluor647-conjugated secondary antibody and analyzed by flow-cytometry.



Supplemental Figure 8: the modulation of ROR2 expression levels does not affect MM cell proliferation.
A) MM1.144 cells transduced with a control non-targeting shRNA (shSCR) or two ROR2-specific shRNAs (shROR2#1 and shROR2#3) were plated in complete RPMI medium (left) or in low serum RPMI (right) and counted every 24 hours for four consecutive days. Each time point was counted in triplicate.
B) U266 (left) and EJM (right) cells transduced with a control non-targeting shRNA (shSCR) or two ROR2-specific shRNAs (shROR2#1 and shROR2#3) were plated in complete RPMI medium and counted every 24 hours for four consecutive days. Each time point was counted in triplicate.
C) RPMI8266 (left) and KMS-11 (Right) cells expressing LacZ or ROR2 wereplated in complete RPMI medium and counted every 24 hours for three consecutive days. Each time point was counted in triplicate.

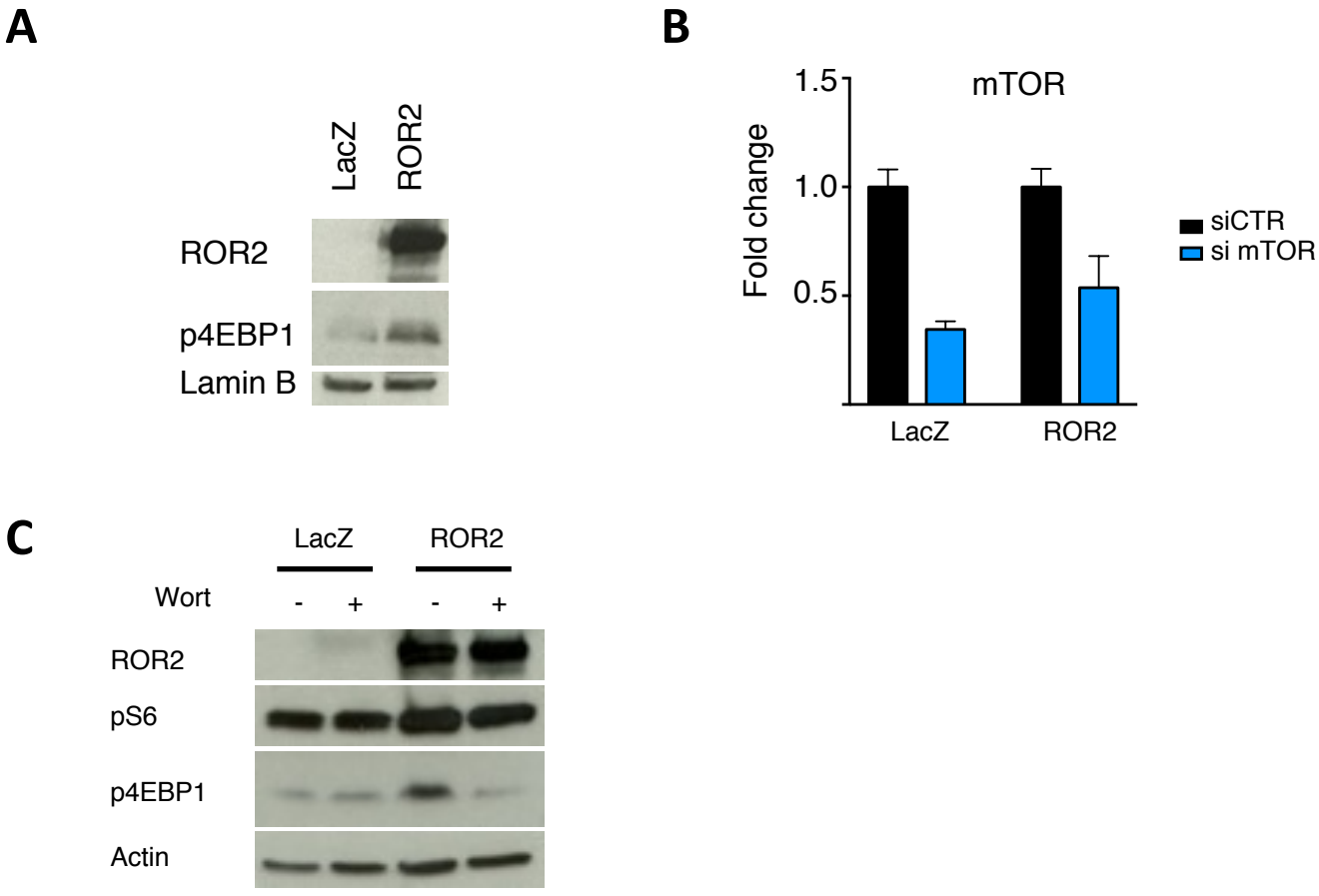


Supplemental Figure 9: ROR2 downregulation affects AKT pathway
Ingenuity Pathway Analysis of gene expression profiling data of MM1.144 cells infected with shSCR or shROR2#1. In green, downregulated, and red, upregulated genes between shROR2#1 and shSCR cells.



Supplemental Figure 10: AKT downregulation

RPMI8226 cells expressing LacZ or ROR2 were transfected with control siRNA (siCTR) or AKT isoform specific siRNAs and 72h post transfection knock-down of the different genes was assessed. One representative experiment is shown. Levels of mRNA expression for AKT isoforms are shown as fold change relative to control, as measured by quantitative RT-PCR.

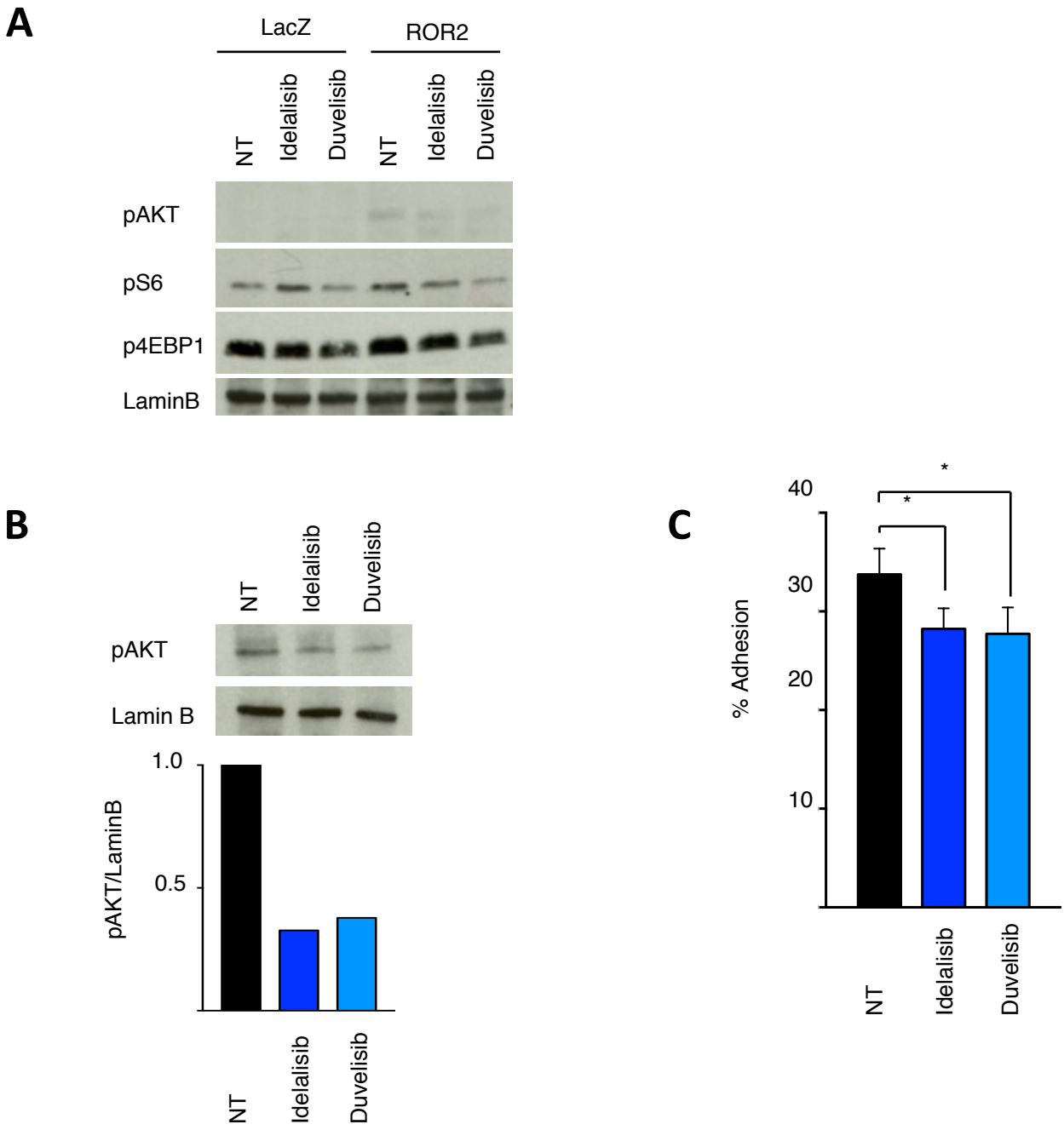


Supplemental Figure 11: ROR2 expression modulates mTOR activation.

A) Total cell lysates of RPMI8226 cells expressing lacZ or ROR2 were separated on SDS-PAGE and probed with ROR2 and phospho-4EBP1. LaminB was used as loading control.

B) RPMI8226 cells expressing LacZ or ROR2 were transfected with control siRNA (siCTR) or mTOR specific siRNAs and 72h post transfection mTOR knock- was assessed. One representative experiment is shown. Levels of mRNA expression for are shown as fold change relative to control, as measured by quantitative RT-PCR.

C) RPMI8226 cells expressing LacZ or ROR2 were treated or not with wortmannin (20nM) for two hours and then total cell lysates were collected and subjected to SDS-PAGE and membranes probed with phospo-S6 and phosphor-4EBP1. Actin is used as loading control.



Supplemental Figure 12: Pharmacological or genetic AKT inhibition affects adhesion properties.

A) RPMI8226 cells expressing LacZ or ROR2 incubated for two hours with the PI3K inhibitors Idelalisib (1uM) or Duvelisib (1uM) were washed with ice-cold PBS and then total cell lysates were separated on on a SDS-PAGE and membranes were probed with phospho- AKT, phosphor-S6, phosphor-4EBP1 and LaminB.

B) MM1.144 cells were either left untreated or treated with Idelalisib (1uM) or Duvelisib (1uM). After two hours cells were washed with PBS, lysed in Laemmli and then total cell lysates were separated on SDS-PAGE and probed with phospho-Akt. Lamin was used as loading control.

C) MM1.144 cells were plated onto a VCAM-1 coated multiwell plate in presence or not of the isoform specific PI3K inhibitors Idelalisib (1uM) and Duvelisib (1uM). After a 2h incubation, plates were gently washed with PBS and adhering cells were counted. Results are expressed as percentages of adhering cells with respect to the total cell plated. Graph shows the average from three independent experiments combined. Statistical analysis was performed with two-tailed Student's t test. ** $p<0.01$.