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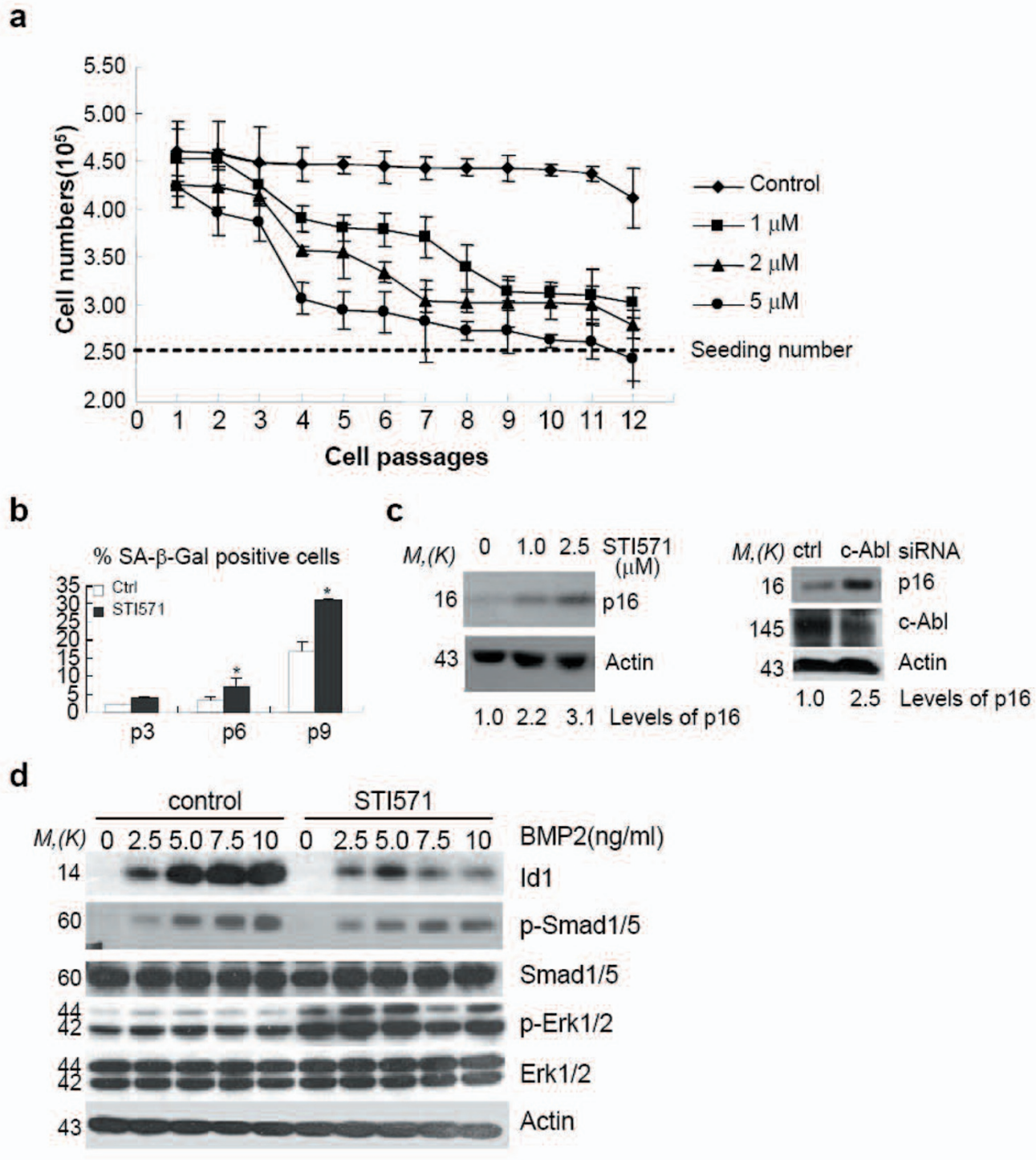

Figure S1 Inhibition of c-Abl mimics c-Abl deficiency in osteoblast proliferation, expression of Id1 and $p16^{INK4a}$, and activation of Smad1 and Erk1/2. (**a**) The effects of different doses of STI571 on osteoblast proliferation. Calvarial osteoblasts were isolated from newborn pups, pooled, and used for further experiments at p2. (**b**) STI571 treatment led to an increase in the number of SA-β-Gal positive cells. (**c**) The effects of different doses of STI571 (left panel) or c-Abl knockdown (right panel) on $p16^{INK4a}$ expression. Cells were treated with STI571 or c-Abl siRNA (Dharmacon On-Target Plus SMARTpool) for 2 days and the $p16^{INK4a}$ levels were determined by WB. (**d**) Inhibition of c-Abl with STI571 (1μM, a concentration shown to be effective) impeded Smad1 activation and Id1 expression, and enhanced Erk1/2 activation in response to BMP2 treatment. Data are means±s.e.m. (n=3 for a,b). * $p<0.05$, compared to untreated cells.

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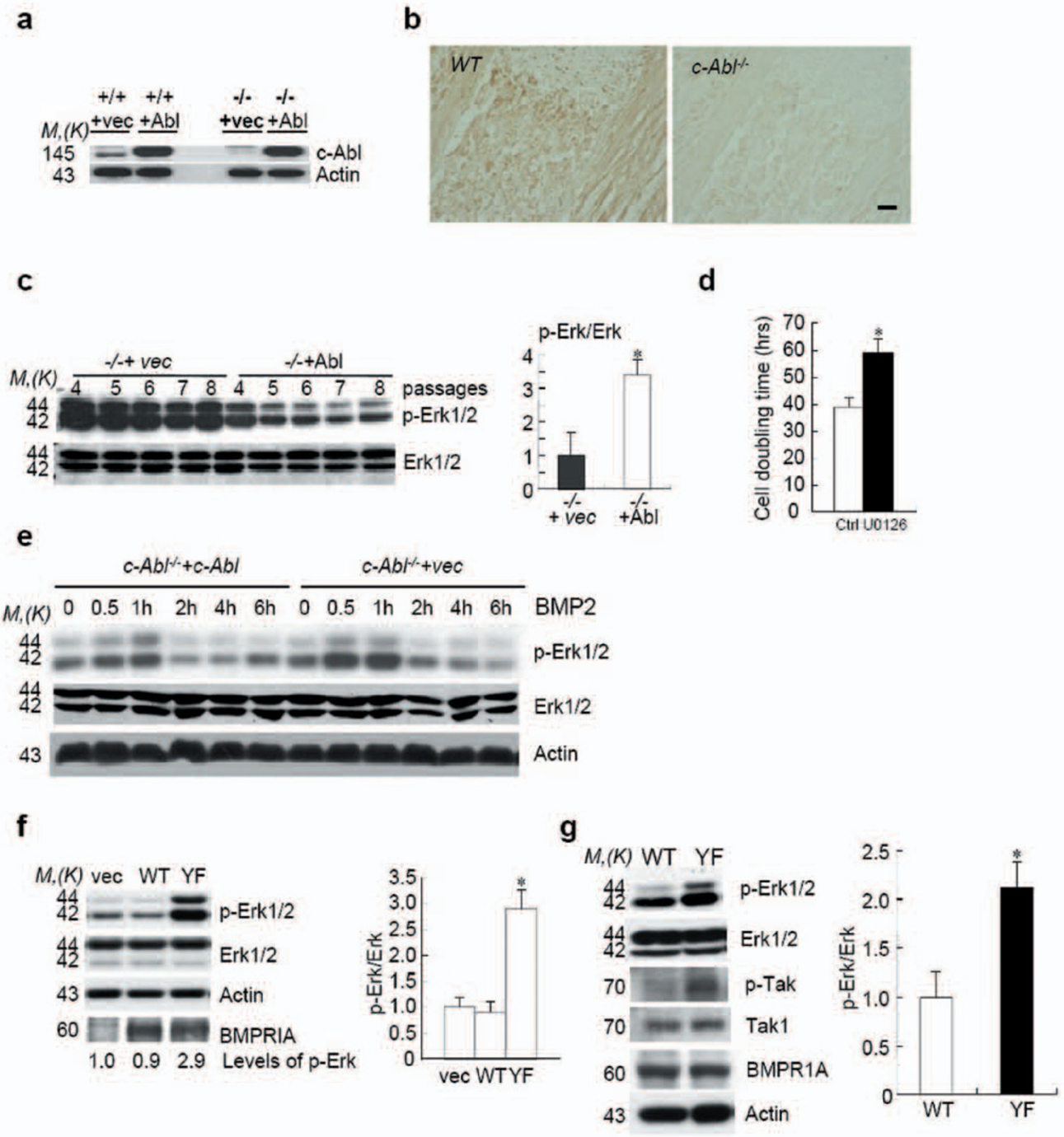

Figure S2 c-Abl and c-Abl-mediated BMPRIA phosphorylation regulate activation of Erk1/2 at the basal levels and in response to BMP2. (a) Reconstitution of c-Abl in *c-Abl*$^{-/-}$ osteoblasts. WT or *c-Abl*$^{-/-}$osteoblasts were infected with empty vector or c-Abl expressing retrovirus, selected, and the protein levels of c-Abl were determined by Western blot. (b) Immunostaining of c-Abl on bone sections. Bone sections of newborn WT and *c-Abl*$^{-/-}$pups were detected with anti-c-Abl antibodies. Scale bars, 200 μm. (c) Reconstitution of c-Abl restored Erk1/2 activation. *c-Abl*$^{-/-}$ osteoblasts infected with empty vector or c-Abl expressing retrovirus were passaged and Erk1/2 activation was determined by Western blot. Since p-Erk levels are similar in different passages, an average was shown on the right panel. The levels of p-Erk1/2 in WT cells were set at 1.0. Data are means±s.e.m. (n=3). * p<0.05, compared to *c-Abl*$^{-/-}$ cells. (d) Inhibition of Mek1/2 with U0126 (20 μM) reduced osteoblast proliferation rates. Data are means±s.e.m. (n=3). * p<0.05, compared to untreated cells. (e) *c-Abl*$^{-/-}$osteoblasts infected with empty vector or c-Abl expressing retrovirus were stimulated with BMP2 for different periods of time and Erk1/2 activation was determined by Western blot. Erk1/2 were detected on a 2nd gel. (f,g) Effects of YF BMPR1A overexpression in osteoblasts and C2C12 cells. Overexpression of YF BMPRIA, but not the WT BMPRIA, resulted in enhanced activation of Erks and Tak1 in osteoblasts (f) and C2C12 cells (g). Right panels: quantitation data. Data are means±s.e.m. (n=3). * p<0.05, compared to WT BMPRIA.

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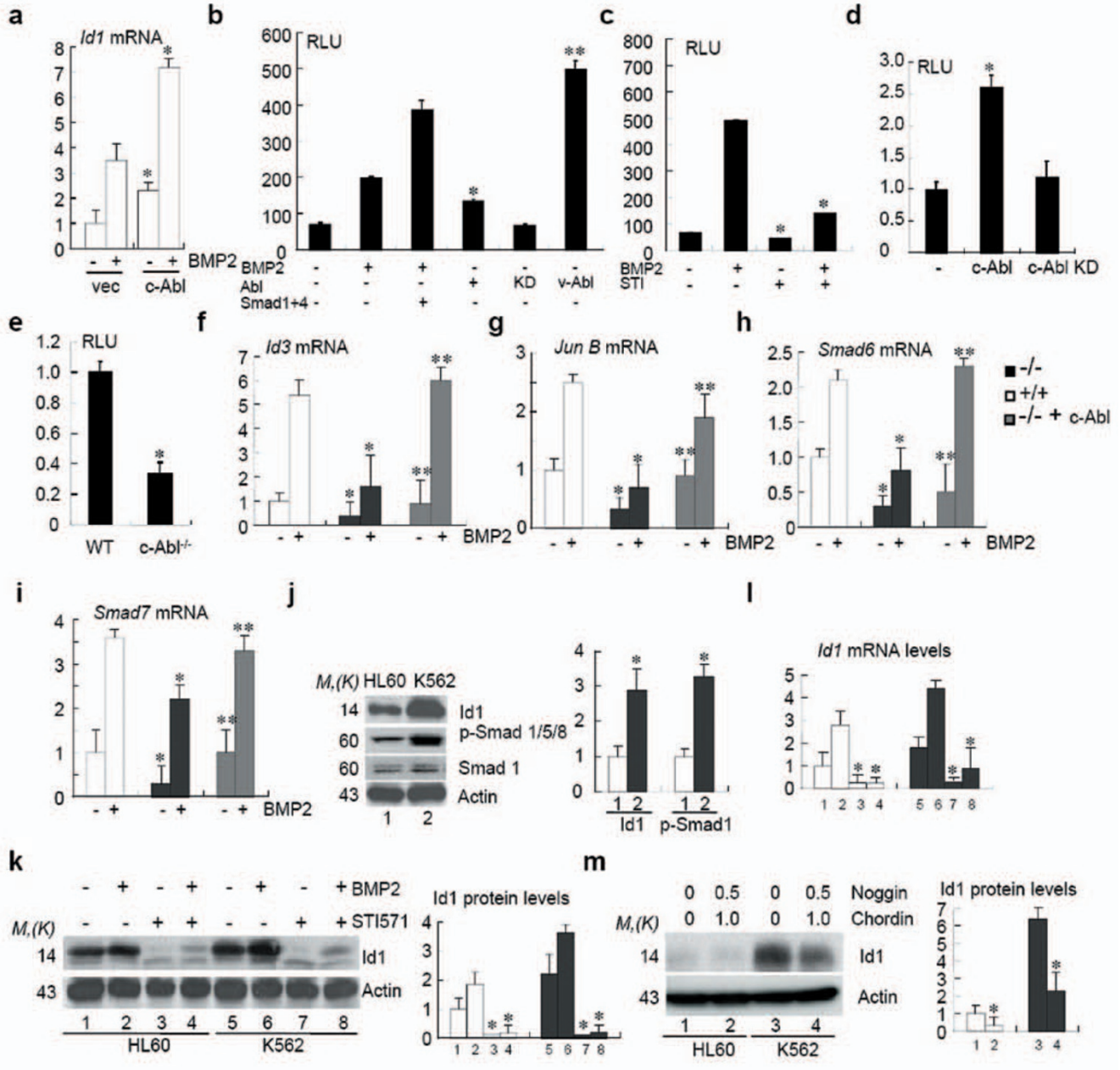

Figure S3 c-Abl and BCR-ABL promote Id1 transcription and other BMP target genes. Overexpression of c-Abl promoted BMP2-induced Id1 expression. MC3T3 cells were transfected for 24 hrs, and then treated with 100 ng/ml of BMP2 for 6 hrs. The levels of Id1 mRNA were determined by realtime-PCR. (**b**) Abl acted directly on the Id1 promoter. Id1 promoter-luciferase reporter was co-transfected with Smad1+4, c-Abl, c-Abl KD (kinase dead), or v-Abl into C2C12 cells. These cells were treated with 100 ng/ml BMP2 for 16 hrs and the luciferase activity was measured using renilla as an internal control. (**c**) STI571 inhibited the Id1 promoter activity. The cells were pre-treated with STI571 (1 uM) for 2 hrs and then with BMP2 (100 ng/ml) for 16 hrs. (**d**) c-Abl, but not kinase-dead c-Abl, activated the BMP-responsive SBE-OC-Luc reporter in MC3T3 cells. The promoter activity at the basal level was set at 1.0. (**e**) The BMP-responsive SBE-OC-Luc reporter was inhibited in *c-Abl*$^{-/-}$osteoblasts. The promoter activity in WT cells was set at 1.0. (**f-i**) Realtime-PCR assays revealed reduced induction of Id3 and other target genes by BMP2 at the mRNA level in *c-Abl*$^{-/-}$osteoblasts. WT, *c-Abl*$^{-/-}$and c-Abl reconstituted osteoblasts were stimulated with 50 ng/ml BMP2 for different periods of time and the mRNA levels of Id3, JunB, Smad6 and Smad7 were assessed by realtime-PCR. The basal levels of individual mRNA were set at 1.0. (**j**) BCR-ABL positive K562 cells show Id1 upregulation and enhanced Smad1/5/8 activation compared to HL60 cells. Right panel: quantitation data. (**k**) Western blot analysis showed that STI571 (1 uM) treatment inhibited BMP2-induced Id1 expression at the protein levels in K562 cells. Right panel: quantitation data. (**l**) Realtime-PCR showed that BCR-ABL augmented BMP2 (100 ng/ml) induced Id1 expression at mRNA levels, which could be blocked by STI571. (**m**) Blockade of BMP activity with noggin/chordin down-regulates the protein levels of Id1. Right panel: quantitation data. Data are means±s.e.m. (n=3). * p<0.05, compared to empty vectors, WT cells, or untreated cells.

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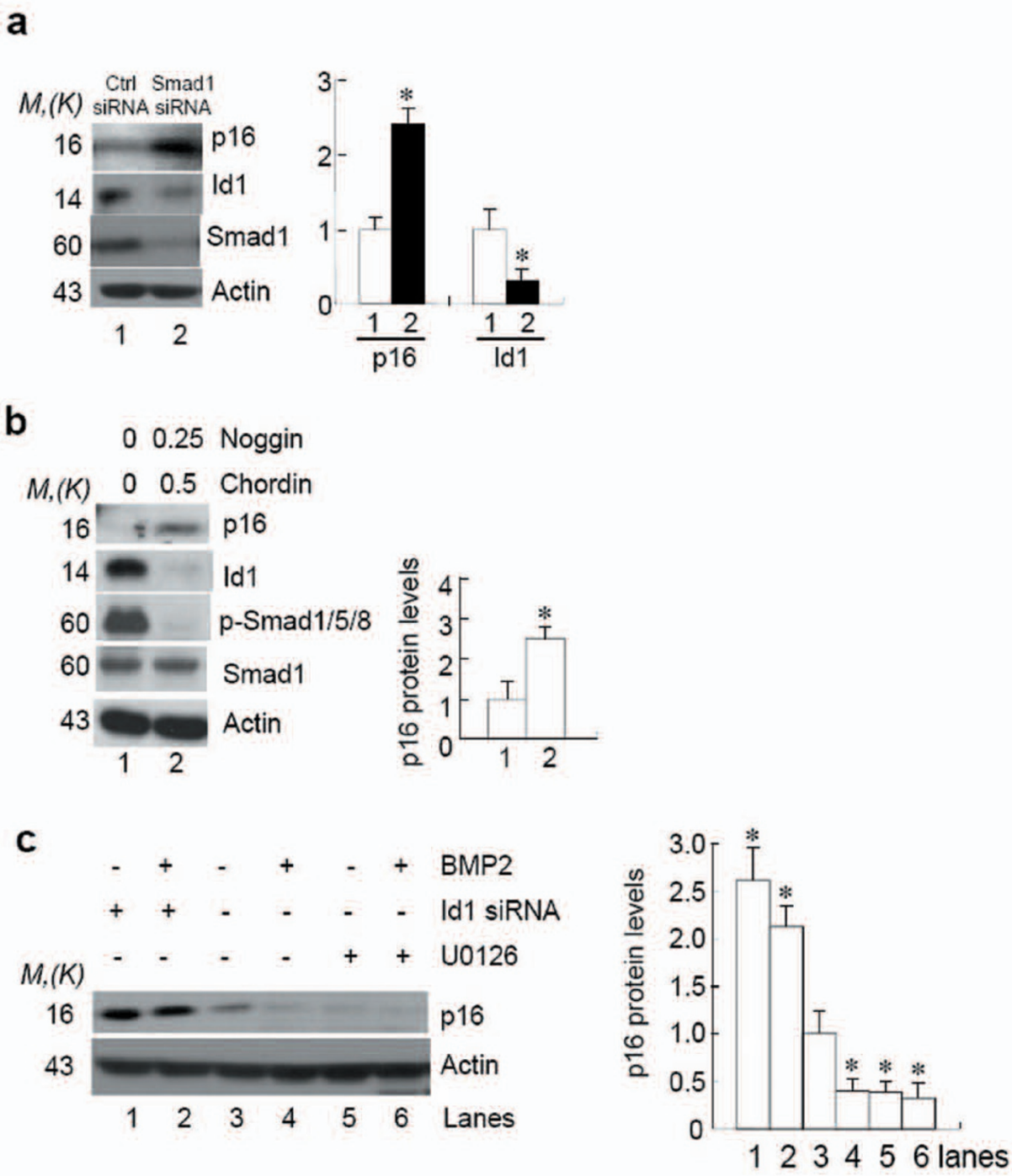

Figure S4 The roles of BMPs in $p16^{INK4a}$ expression in osteoblasts. (**a**) Knock-down of Smad1 in osteoblasts led to an up-regulation of $p16^{INK4a}$. Pooled siRNA was used to transfect primary osteoblasts for 2 days and the levels of $p16^{INK4a}$ and Id1 were analyzed by Western blot. (**b**) Blocking BMP signaling by noggin and chordin in osteoblasts down-regulated Id1 and up-regulated $p16^{INK4a}$. Wild type osteoblasts were cultured in the presence of noggin and chordin for 3 days and the levels of $p16^{INK4a}$, Id1, p-Smad1/5/8 were determined by Western blot analysis. Right panel: quantitation data of $p16^{INK4a}$. (**c**) The effects of U0126 and Id1 knockdown on $p16^{INK4a}$ expression in the presence of absence of BMP2 in osteoblast cultures. Normal osteoblasts were seeded at 1 x10^5 cells/60 mm plates, with Id1 knocked down by siRNA (Dharmacon On-Target Plus SMARTpool) or treated with U0126 for 24 hrs, followed by BMP2 treatment for 36 hrs. The levels of $p16^{INK4a}$ were determined by Western blot analysis. Right panel: quantitation data. The basal level of $p16^{INK4a}$ for wild type was set at 1.0. Data are means±s.e.m. (n=3 for all panels). * $p<0.05$, compared to empty vectors or untreated cells.

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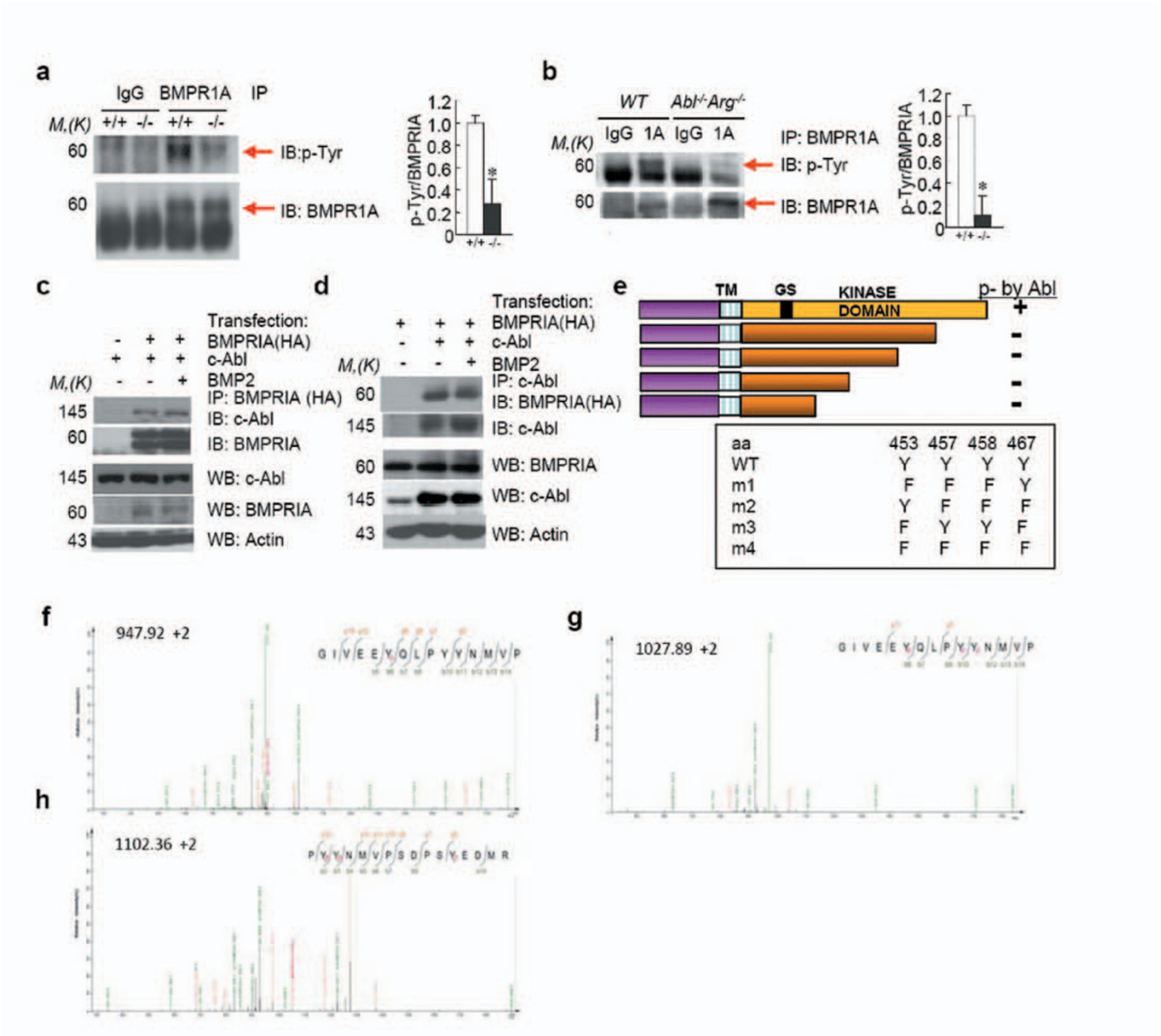

Figure S5 c-Abl interacts with BMPRIA and phosphorylates BMPRIA at four Tyr residues. (**a,b**) Reduced tyrosine phosphorylation of BMPRIA in *c-Abl*$^{-/-}$ (**a**) and *c-Abl*$^{-/-}$*Arg*$^{-/-}$ (**b**) MEFs. Endogenous BMPRIA was immunoprecipitated from the cell lysate of the mutant and control cells and its tyrosine phosphorylation was determined by Western blot. Right panel: quantitation data. (**c**) BMPRIA and c-Abl were expressed in COS7 cells, which were treated with 100 ng/ml BMP2 for 1 hr. BMPRIA and associated proteins were precipitated with anti-HA antibodies, and c-Abl was detected with anti-Abl antibodies. Cells transfected with DNA expressing c-Abl alone were used as control. (**d**) BMPRIA and c-Abl were expressed in COS7 cells, which were treated with 100 ng/ml BMP2 for 1 hr. c-Abl and associated proteins were precipitated with anti-Abl antibodies, and BMPRIA was detected with anti-HA antibodies. Cells transfected with DNA expressing BMPRIA alone were used as control. (**e**) Four different C' terminal truncated BMPRIA mutants were co-expressed with c-Abl and their tyrosine phosphorylation was determined by Western blot. Bottom panel: Tyr mutations of Fig. 5f. (**f-g**) Phosphorylation sites identification of BMPRIA peptide 1 (the sequence was shown) by mass spectrometric analysis. Single phosphorylation (f) or triple phosphorylation (g) could be detected in the in vitro kinase reaction using purified c-Abl. (**h**) Phosphorylation sites identification of BMPRIA peptide 2 (the sequence was shown) by mass spectrometric analysis. Triple phosphorylation could be detected in the in vitro kinase reaction using purified c-Abl. Data are means±s.e.m. (n=3 for a,b). * p<0.05, compared to WT cells.

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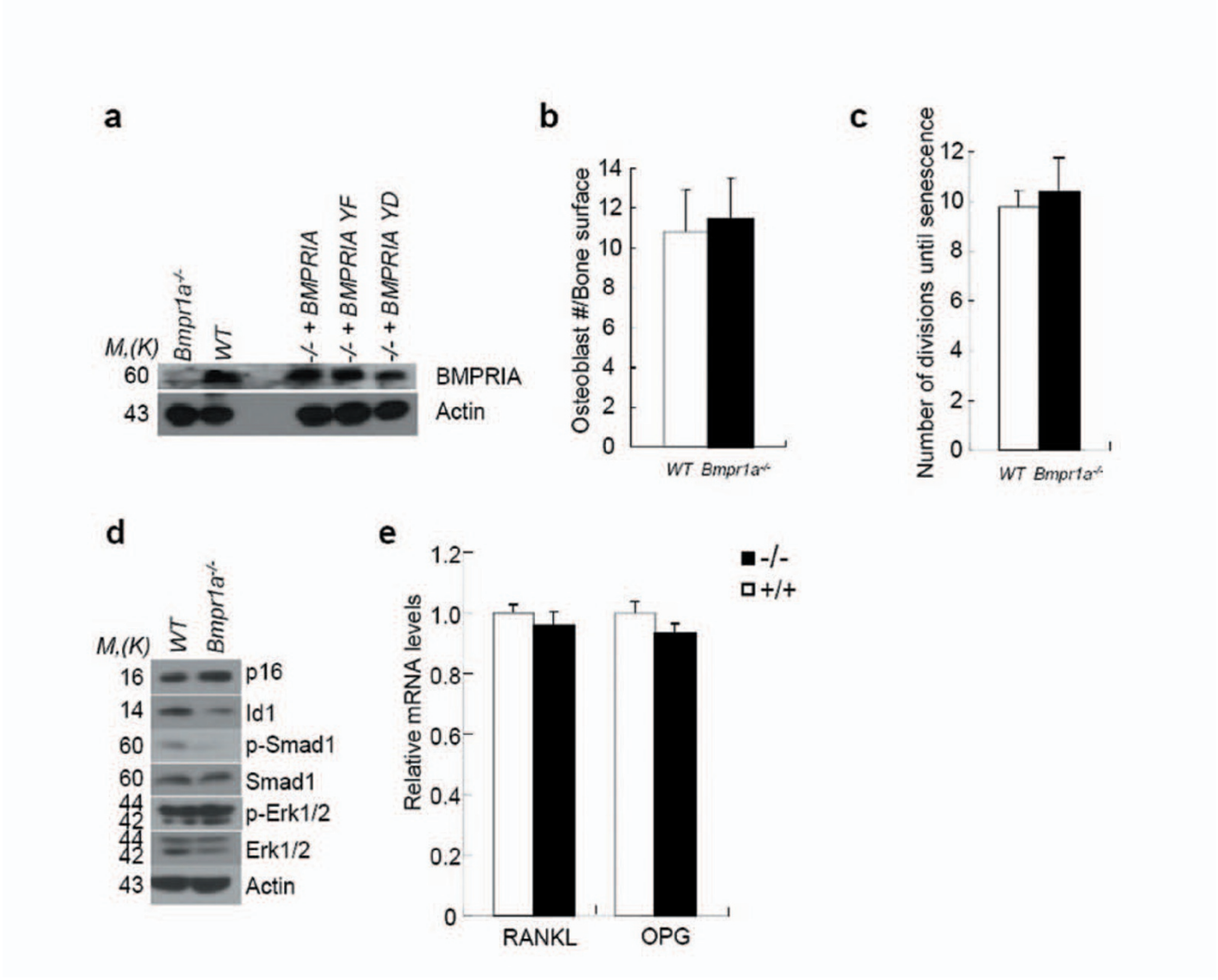

Figure S6 *Bmpr1a*$^{-/-}$ calvarial osteoblasts showed no significant alteration in expansion and senescence. (**a**) Western blot shows the protein levels of BMPRIA in WT, Cre-expressing cells, and cells reconstituted with WT, YF, or YD BMPRIA. (**b**) Osteoblast-specific deletion of BMPRIA did not alter the number of osteoblasts. Femur bones of 2 month-old control or *Bmpr1a*$^{-/-}$ mice were used for histomorphometric analysis. (n=6). (**c**) Osteoblast-specific deletion of BMPRIA did not alter the lifespan of osteoblasts. Bmpr1a f/f osteoblasts were infected with retrovirus vector or retroviruses expressing Cre, selected for 5 days. The doubling times (until senescence) were counted after passage 3. For expression of BMPRIA, see Fig. S6a. (**d**) The effects of BMPRIA knockout on activation of Smad1 and Erk1/2, and the expression of Id1 and p16^{INK4a}. A modest increase in p16^{INK4a} was observed, but Erk activation was not altered. (**e**) *c-Abl*$^{-/-}$osteoblasts did not show difference from WT cells in the expression of RANKL or OPG. WT and *c-Abl*$^{-/-}$osteoblasts were cultured in differentiation medium for 4 days, which were harvested. Total RNA was isolated and the RANKL and OPG levels were determined by realtime PCR using the primers (sequences) from Roche. Data are means±s.e.m. (n=3 for b,c,e).

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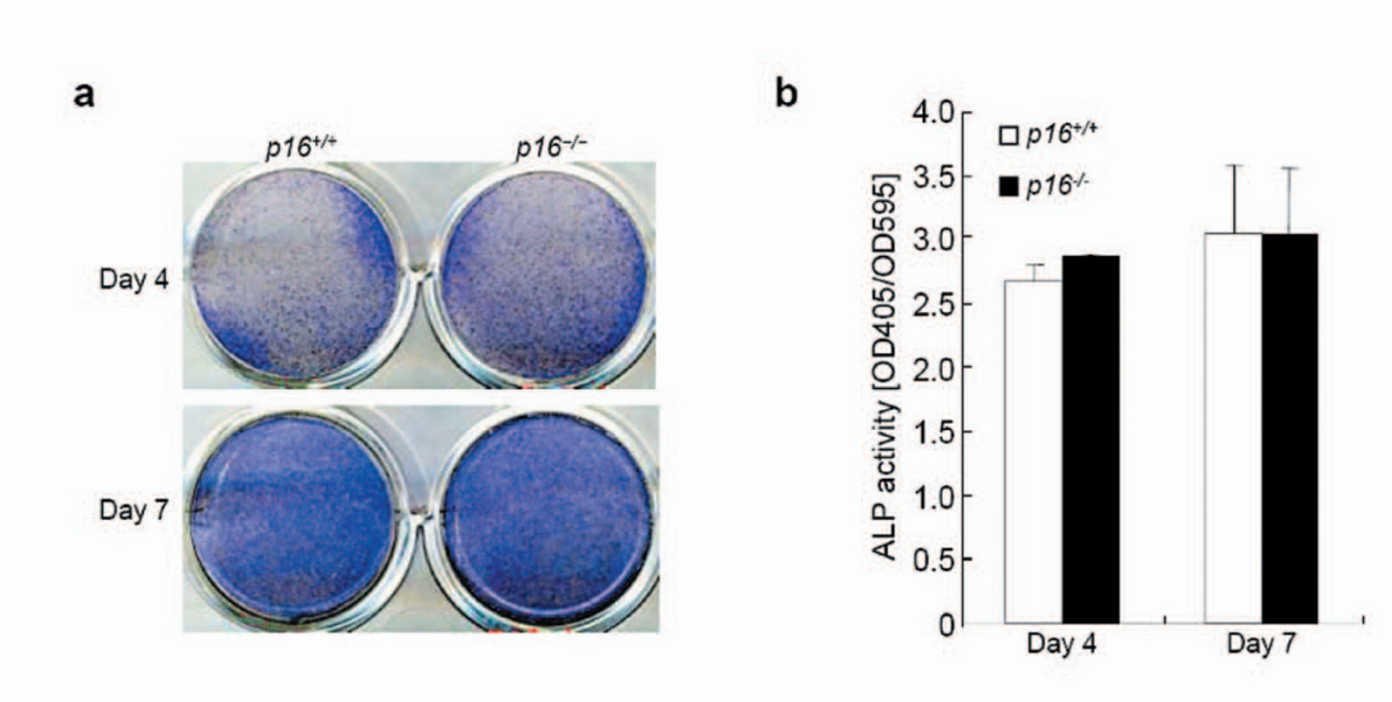

Figure S7 *p16*$^{INK4a-/-}$ calvarial osteoblasts showed no significant alteration in differentiation. (**a**) ALP staining at Day 4 and 7. Calvarial cells were seeded at a density of 1.5 x 105 cells per well in a 12-well plate and maintained in differentiation medium. ALP staining were done on Day 4 and 7. (**b**) ALP biochemical assay at Day 4 and 7. Experiments were carried out like (**a**) but cells were harvested and ALP activities were assayed on Day 4 and 7. Data are means±s.e.m. (n=3 for b).

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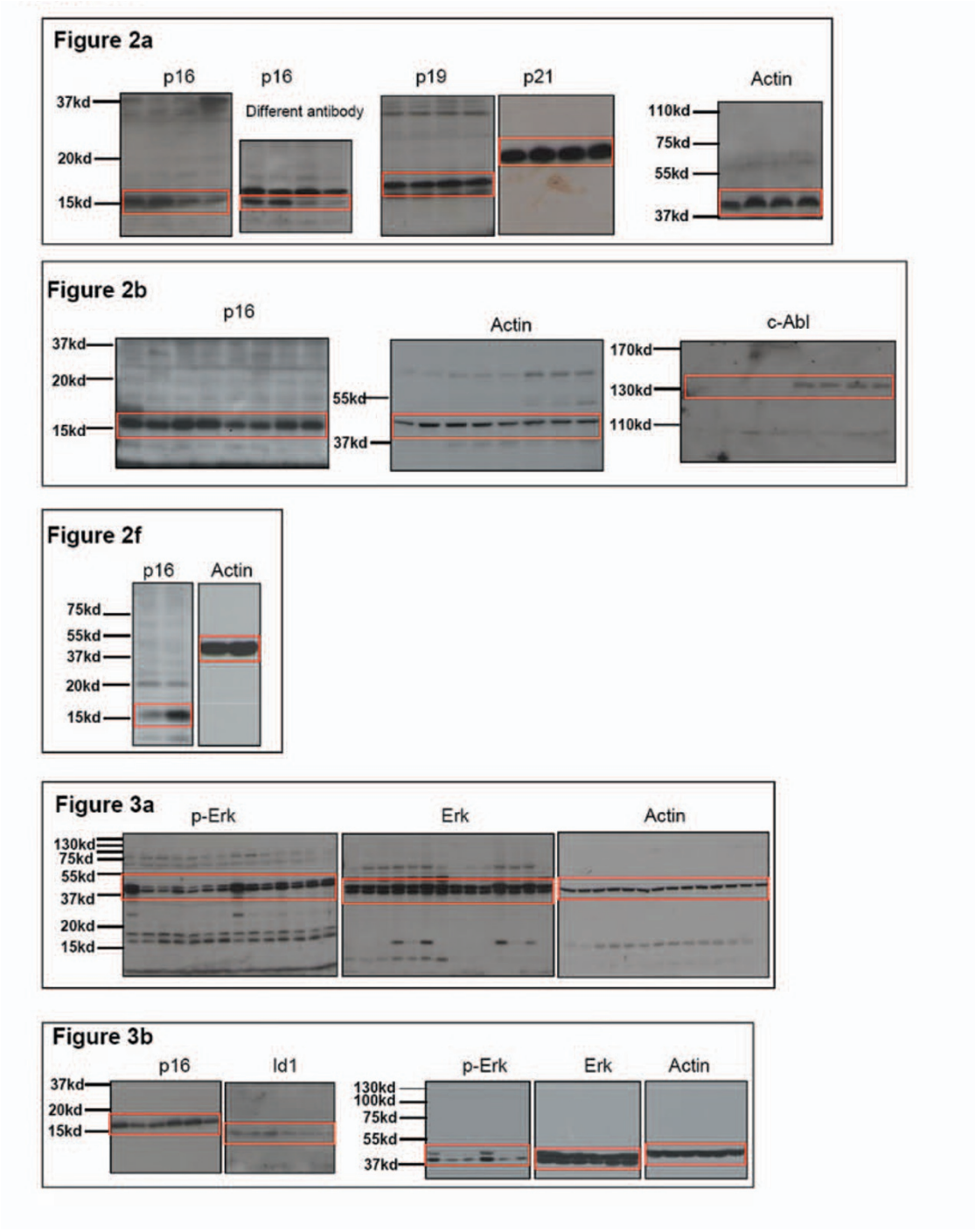

Figure S8 Full scans of immunoblot data. In most of the experiments, protein samples were quantified, normalized, aliquoted, frozen or directly analyzed on 7.5% and/or 12% SDS-PAGE gels. The membranes were stripped and reprobed with various antibodies.

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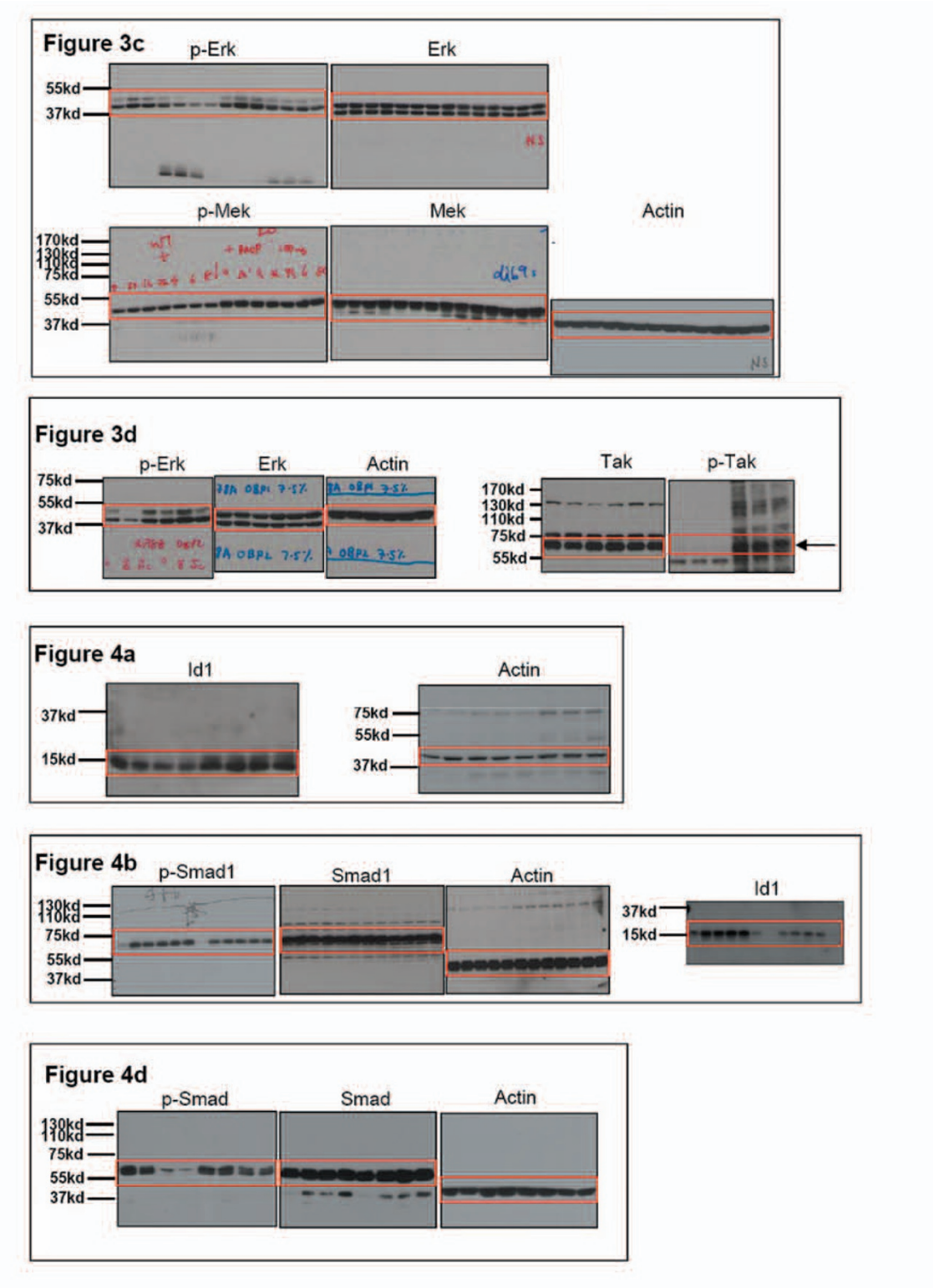

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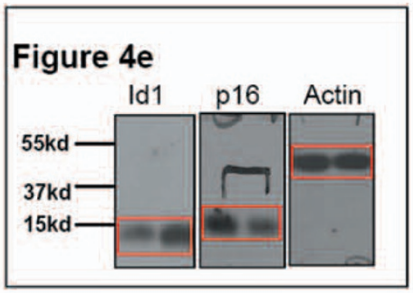

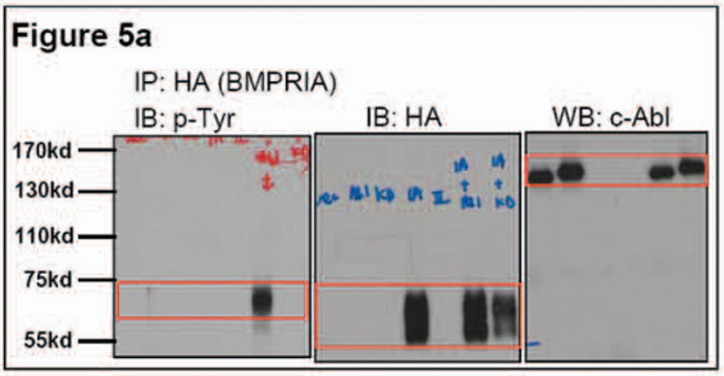

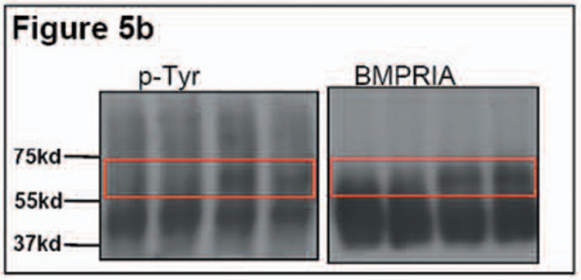

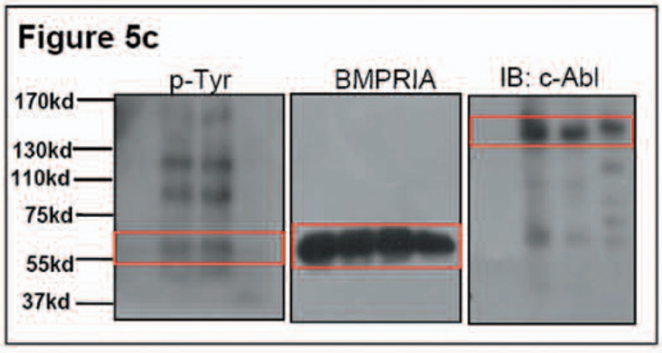

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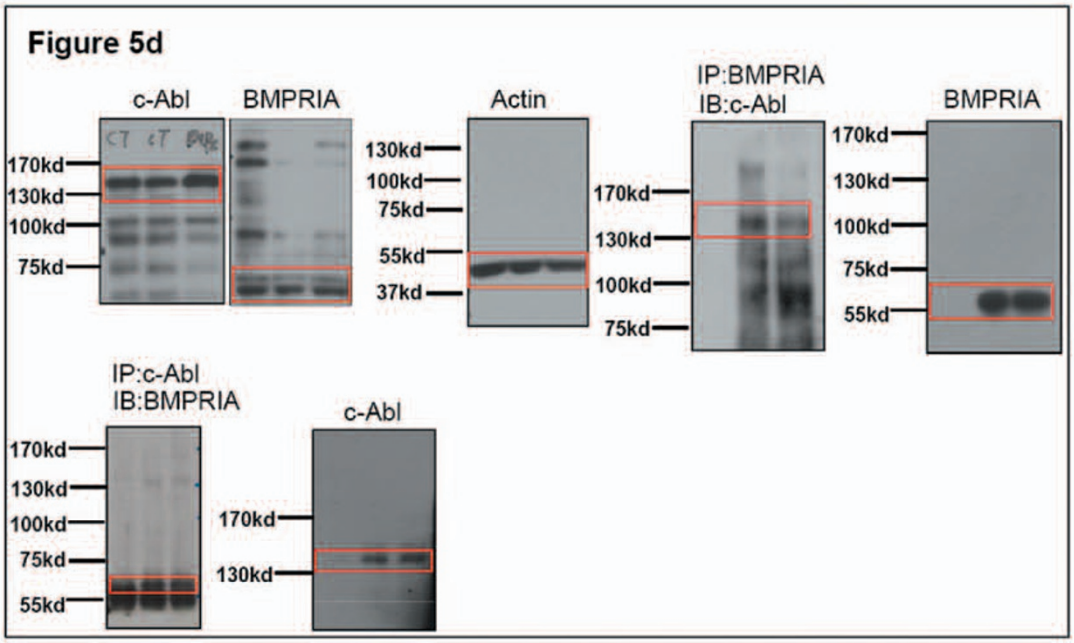

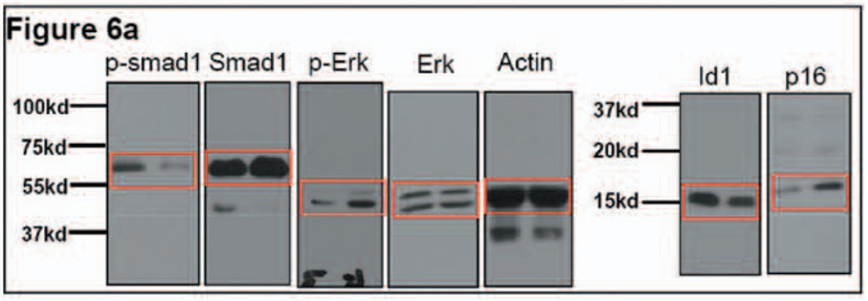

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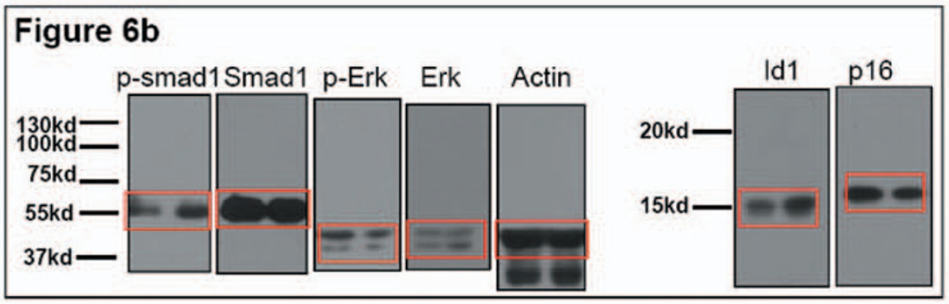

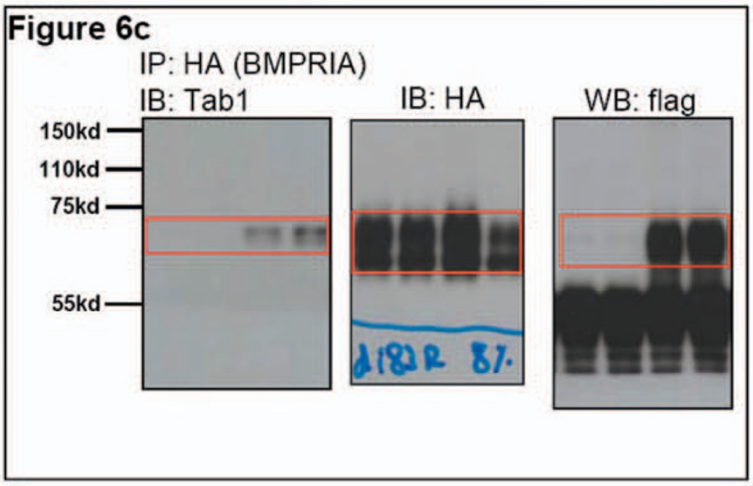

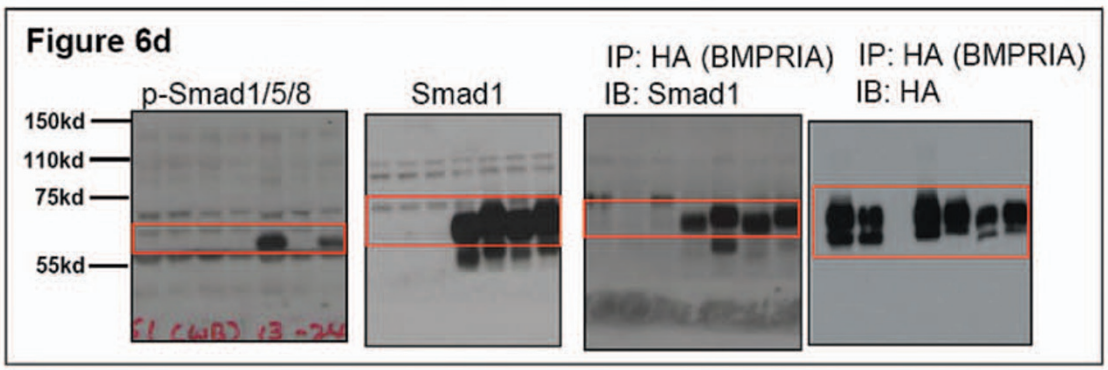

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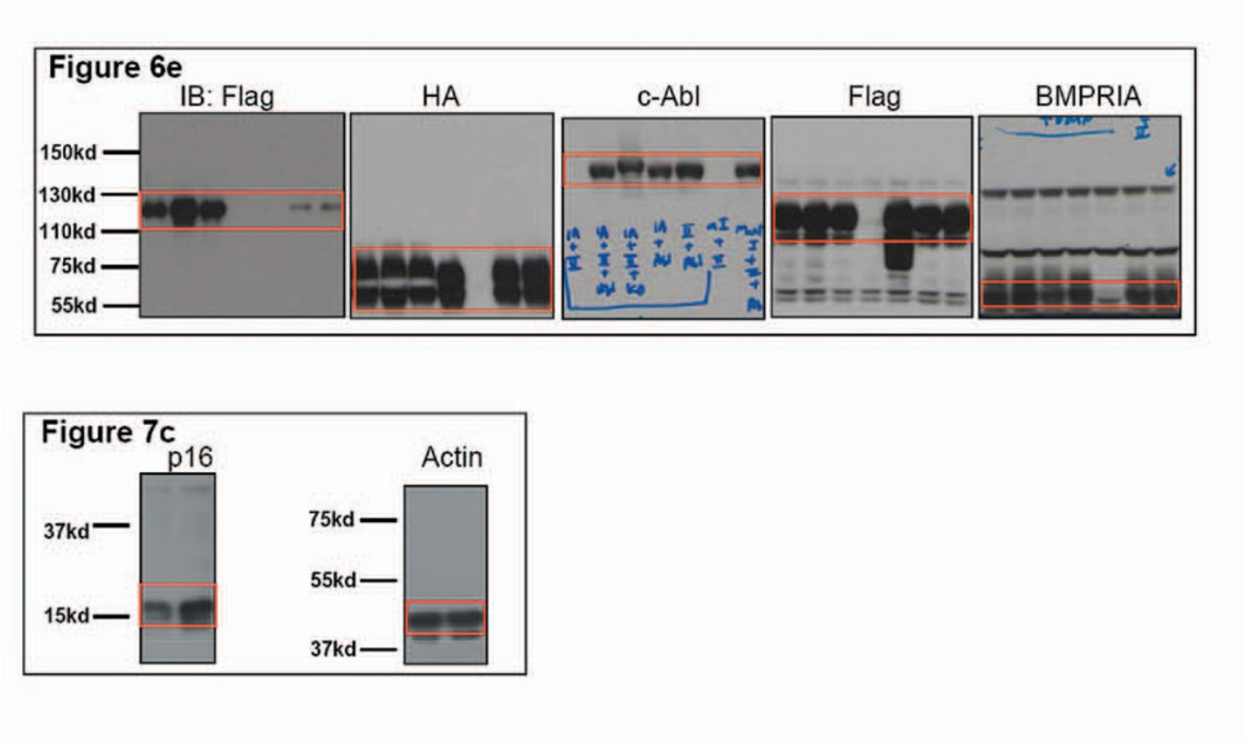

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