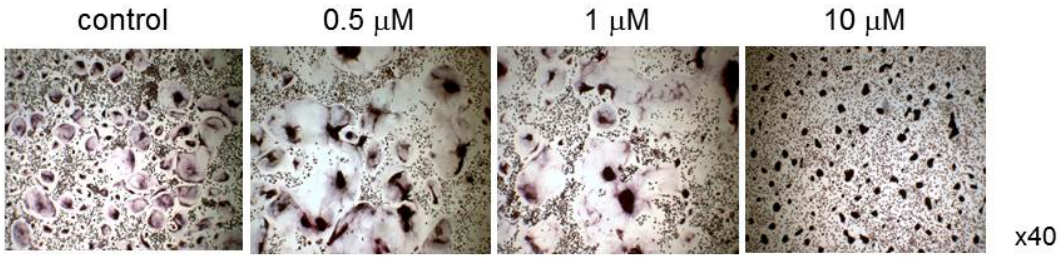


Figure S1

Figure S1. PCI-32765 inhibits osteoclastogenesis. TRAP staining was performed following 2-week OC culture from an additional human donor to identify mature OC, in the presence of serial dilutions of PCI-32765. Original magnification, x40.

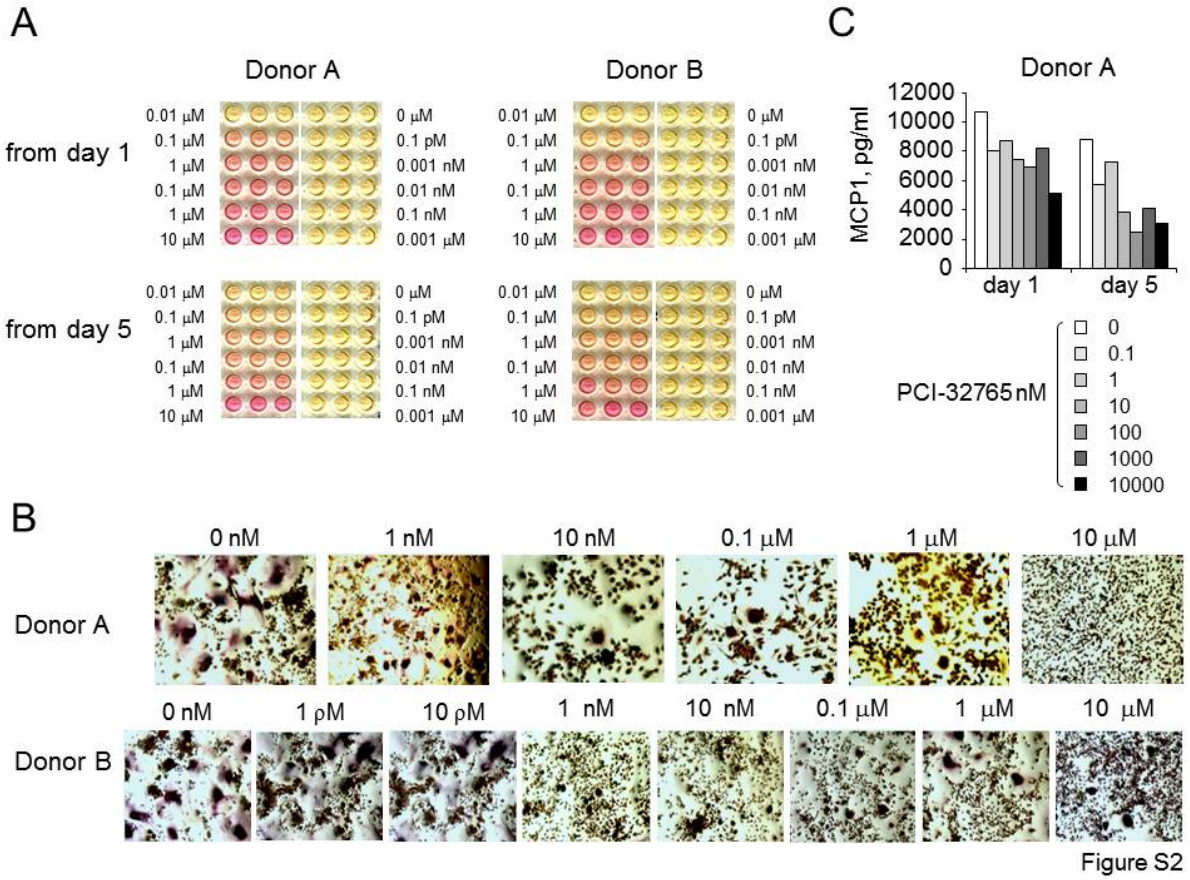


Figure S2. OC cell culture supernatants were affected by PCI-32765. (A) Supernatants were collected following 2-week cultures with M-CSF/RANKL to generate OC, in the presence or absence of PCI-32765. (B) MCP-1 was measured by ELISA. (C) TRAP staining was done to confirm effects of PCI-32765 on OC differentiation.

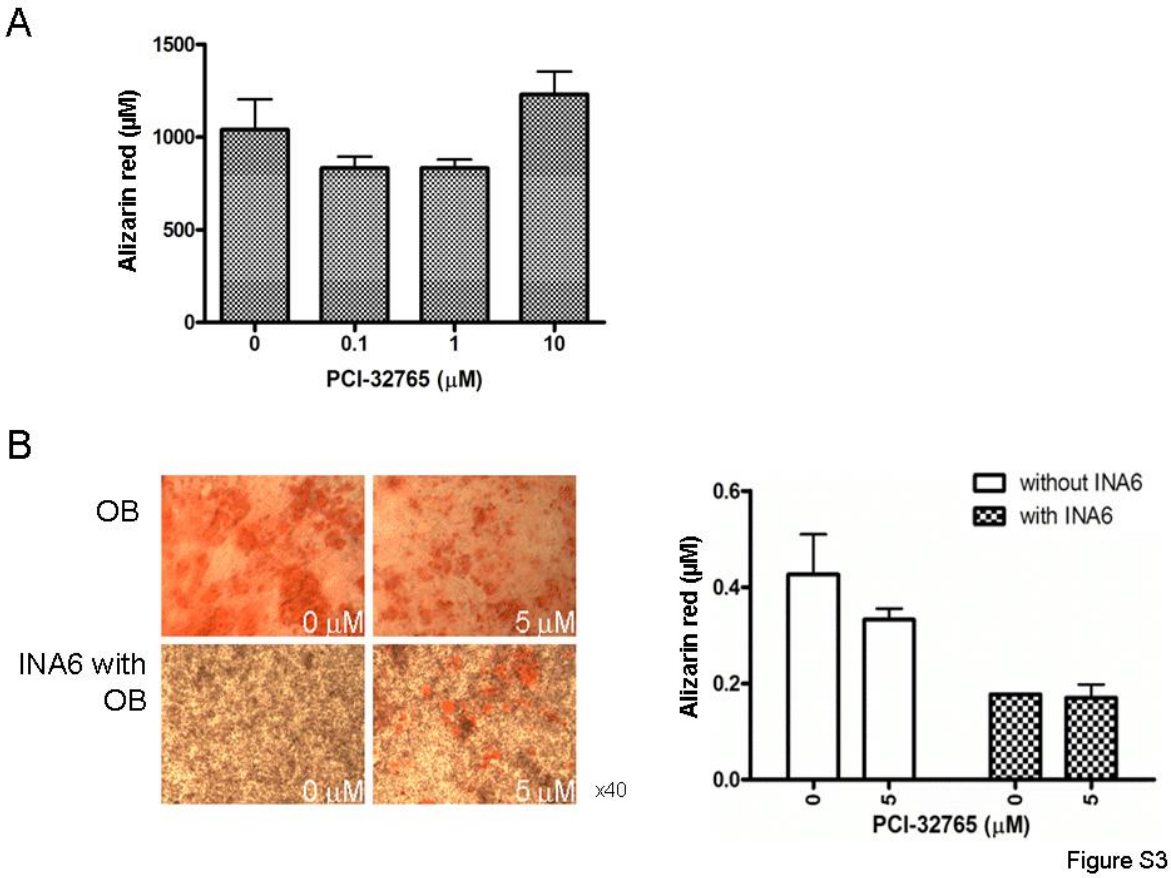


Figure S3. PCI-32765 has minimal effects on OB, alone or with MM cells. Human osteoprogenitor cells (pre-OB), obtained at 3 days from BM adherent cells from a MM patient, were stimulated with 2.16 mg/mL β-glycerolphosphate, 0.05 mg/mL ascorbic acid, and 10 nM dexamethasone (Sigma-Aldrich) for 3 weeks in the presence or absence of PCI-32765 (A), and with or without INA6 cells (B). At the end of the culture period, cells were fixed in 10% formaldehyde and stained with Alizarin Red for 30 minutes to assess the mineralizing activity. The amount of calcium deposition was quantified by ELISA, extracting the alizarin red with 10% acetic acid according to the manufacturer's instructions (Millipore).

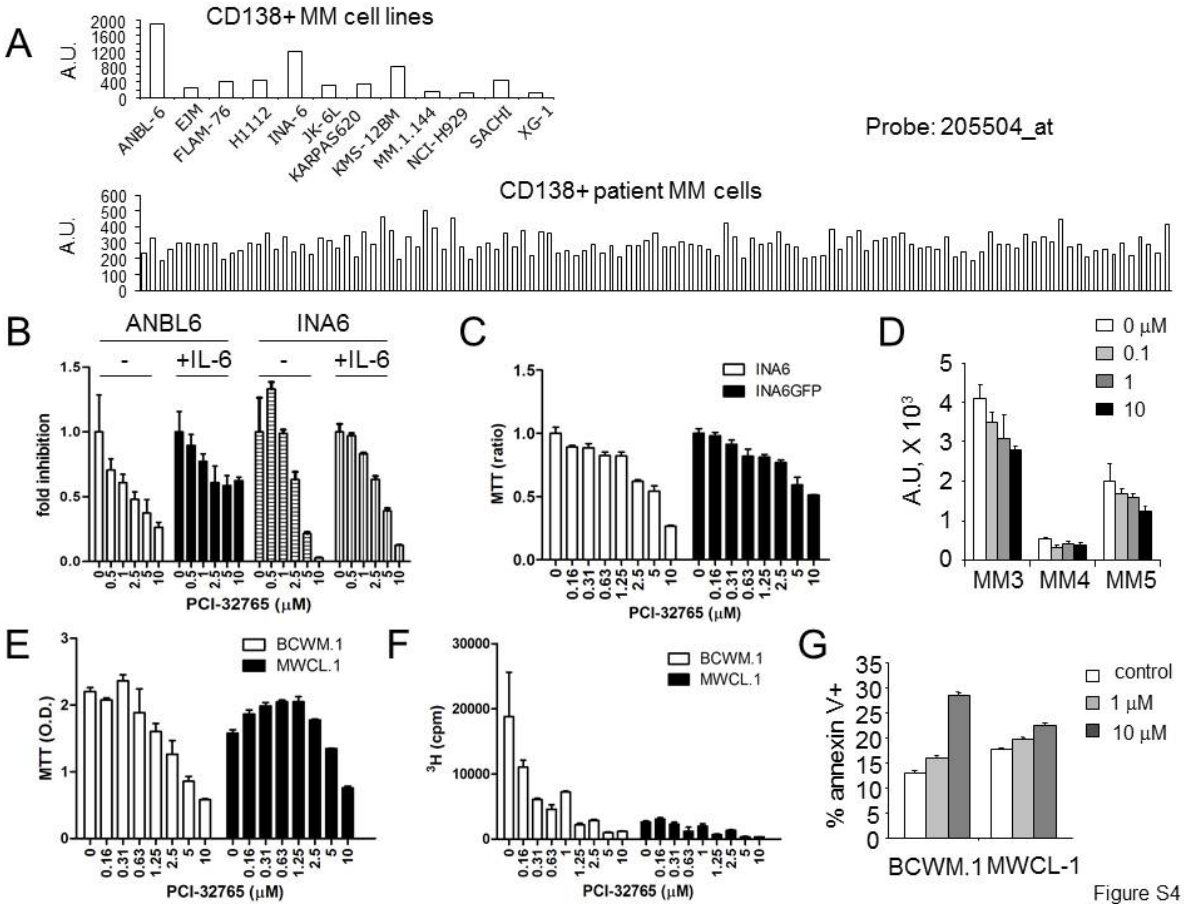


Figure S4. Btk is expressed in MM cells and PCI-32765 induces direct cytotoxicity against MM and WM cells. (A, upper panel) Btk mRNA expression was analyzed with probes 205504_at in MM cell lines and CD138+ MM patient cells (n= 117) using Affymetrix U133A and U133Aplus2 array data, respectively. (B) IL-6-dependent INA6 and ANBL6 MM cells, with or without IL-6, were cultured with PCI-32765 for 3 days followed by [^{3}H] thymidine incorporation. Viability of INA6 and INA6GFP MM cells (C) as well as 2 WM cell lines (E) was decreased, evidenced by MTT assay. PCI-32765 also inhibited viability in purified CD138+cells from 3 MM patients, evidenced by luminescence assay (D). DNA synthesis assay and Annexin V-staining followed by flow cytometry were also examined in 2 WM cell lines (F & G).

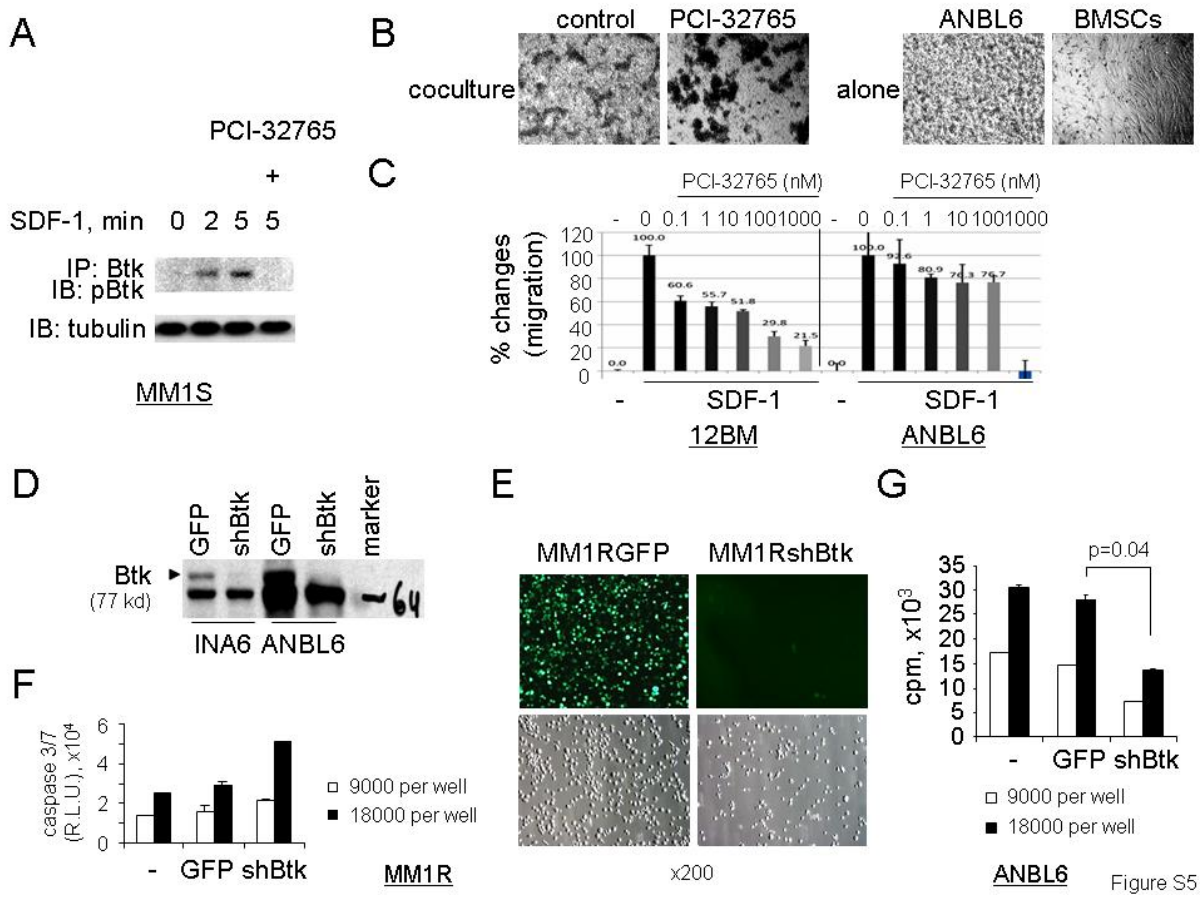


Figure S5. SDF-1-induced MM cell adhesion and migration was blocked by PCI-32765 and Btk directly regulates MM cell survival. (A) MM1S cells expressing low Btk (Figure 4A) preincubated with PCI-32765 (100 nM, +) or control media were stimulated with SDF-1 for 2 and 5 min. Lysates were immunoprecipitated (IP) with anti-Btk, and the IPs probed with anti-pBtk. (B) ANBL6 cells were cultured, alone or with BMSCs, in the presence or absence of PCI-32765 (0.2 μM) (C) SDF-1-induced MM transmigration assay was performed in the presence of PCI-32765. (D) INA6 and ANBL6 MM cells, transduced with GFP or shBtk lentiviruses for 3 days, were harvested, and lysates were subjected to immunoblotting for Btk levels. (E) Four days following lentiviral infections of MM1R cells with GFP lentiviruses, cell images demonstrated high gene transduction efficiency by the majority of GFP positive cells (>85%) (x200). Reduced cell number and adherent phenotype was observed in MM1RshBtk vs MM1RGFP cells. (F) Caspase 3/7 activation was examined in MM1R cells without any virus infection (-) or after infection with either lentiviral control GFP or shBtk particles. (G) Cell proliferation was assessed in ANBL6 cells without virus infection (-) or after infection with either lentiviral control GFP or shBtk particles.

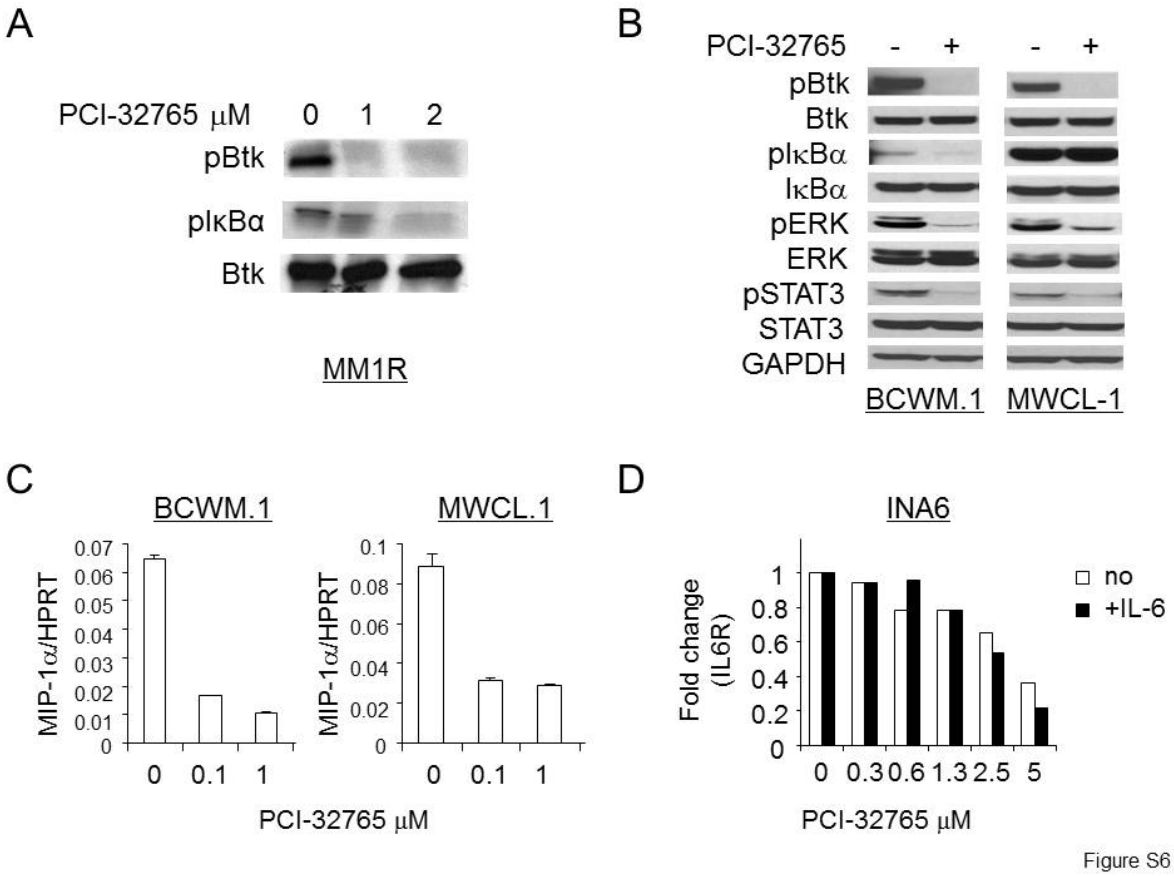
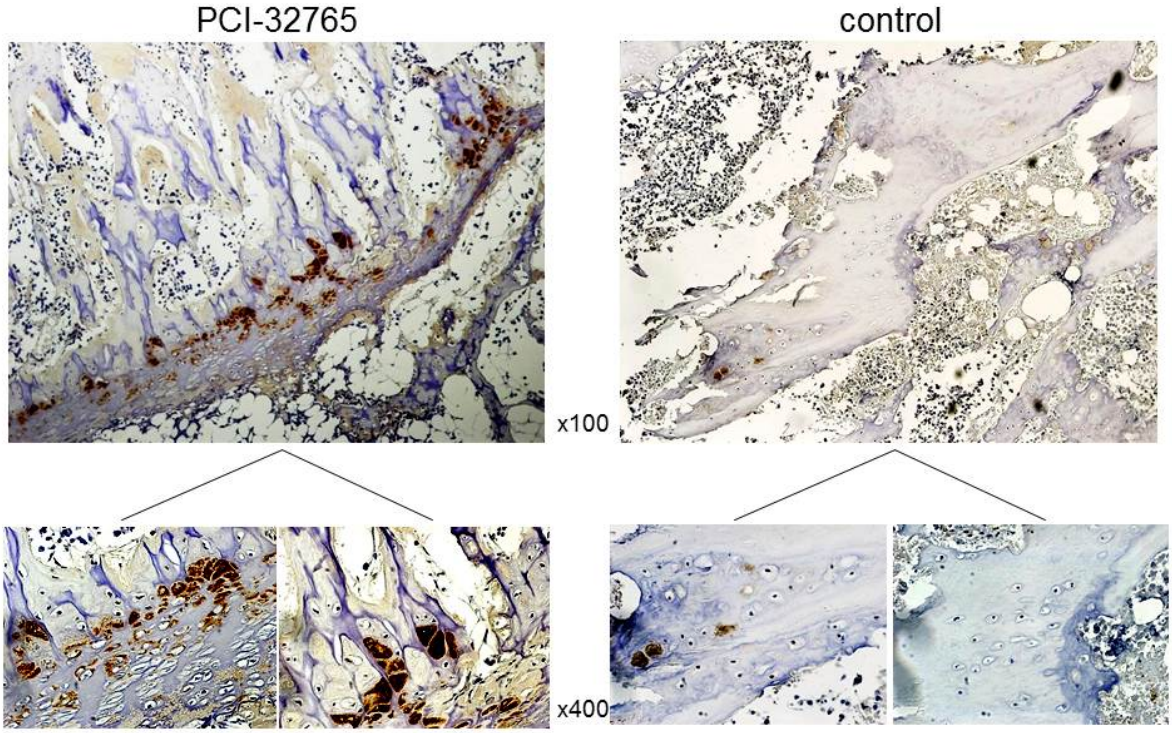


Figure S6. PCI-32765 altered gene transcription in WM and INA6 MM cells. (A) PCI-32765-inhibited Btk signaling cascade was seen in MM1R cells (A) and 2 WM cells (B). WM cells (C) and INA6 MM cells (D), with or without IL-6, were treated with PCI-32765 overnight, and then subjected to MIP-1α and IL6R qRT-PCR, with HPRT as an internal control.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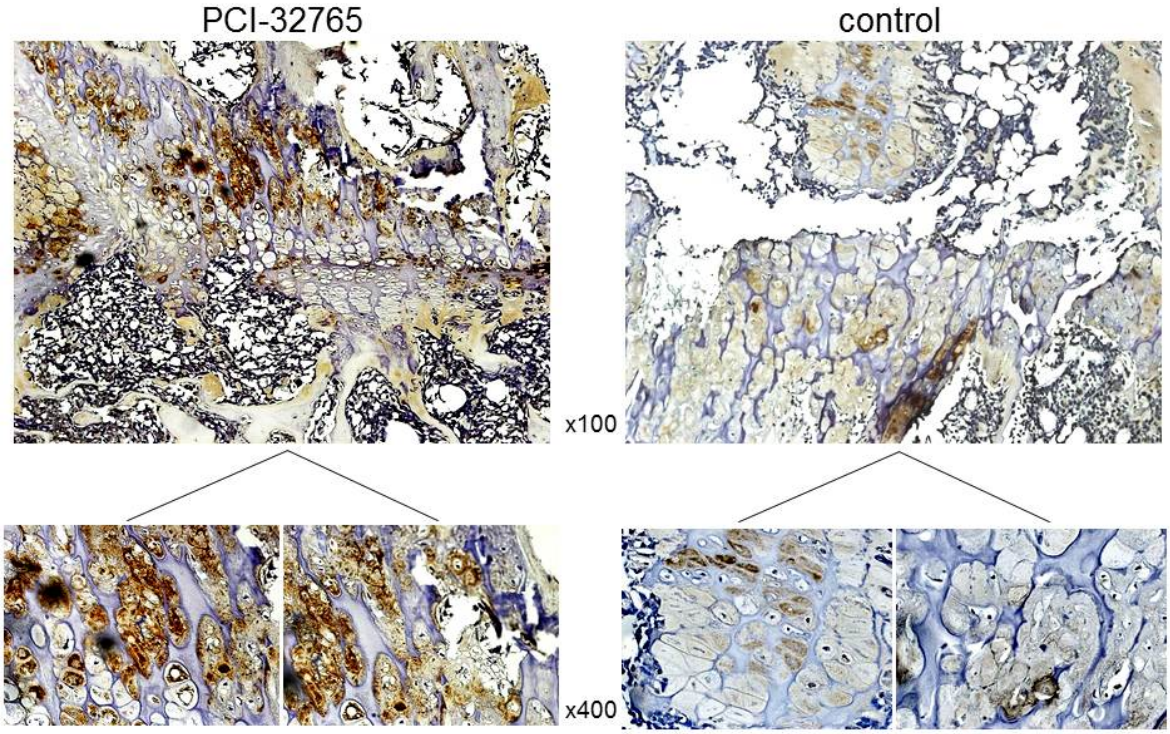
control
x100
x400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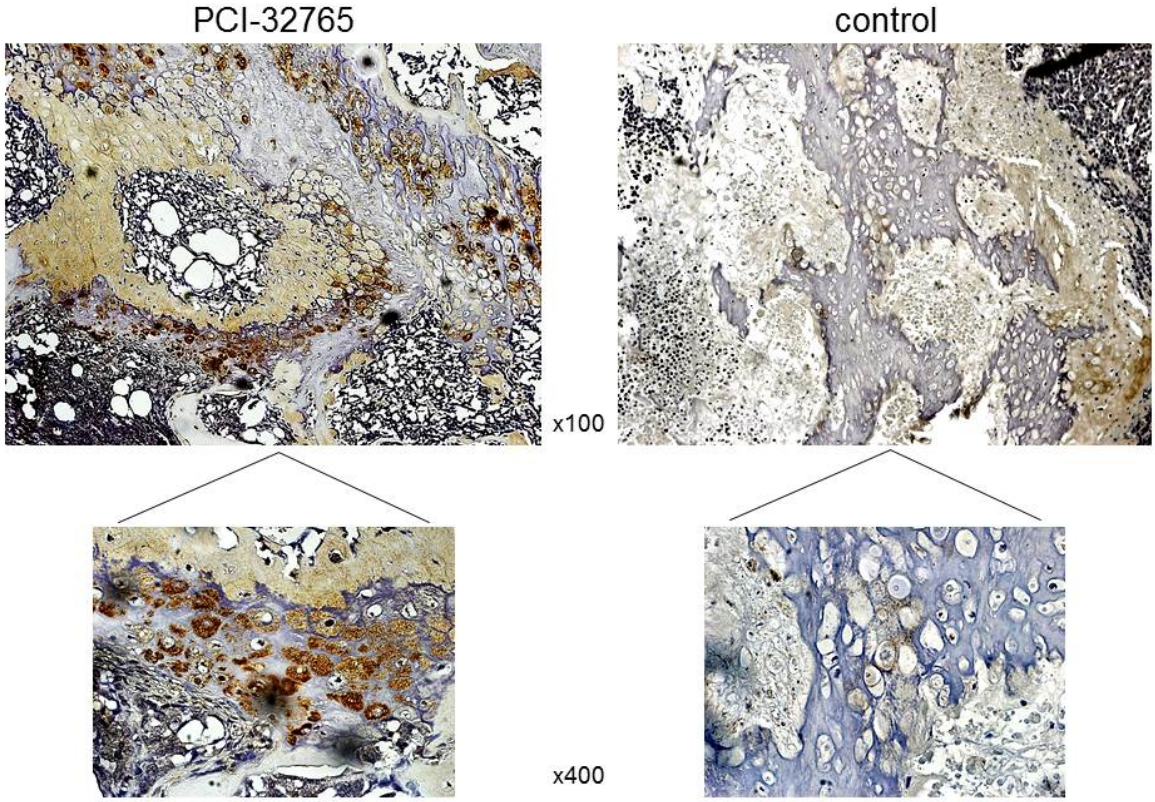
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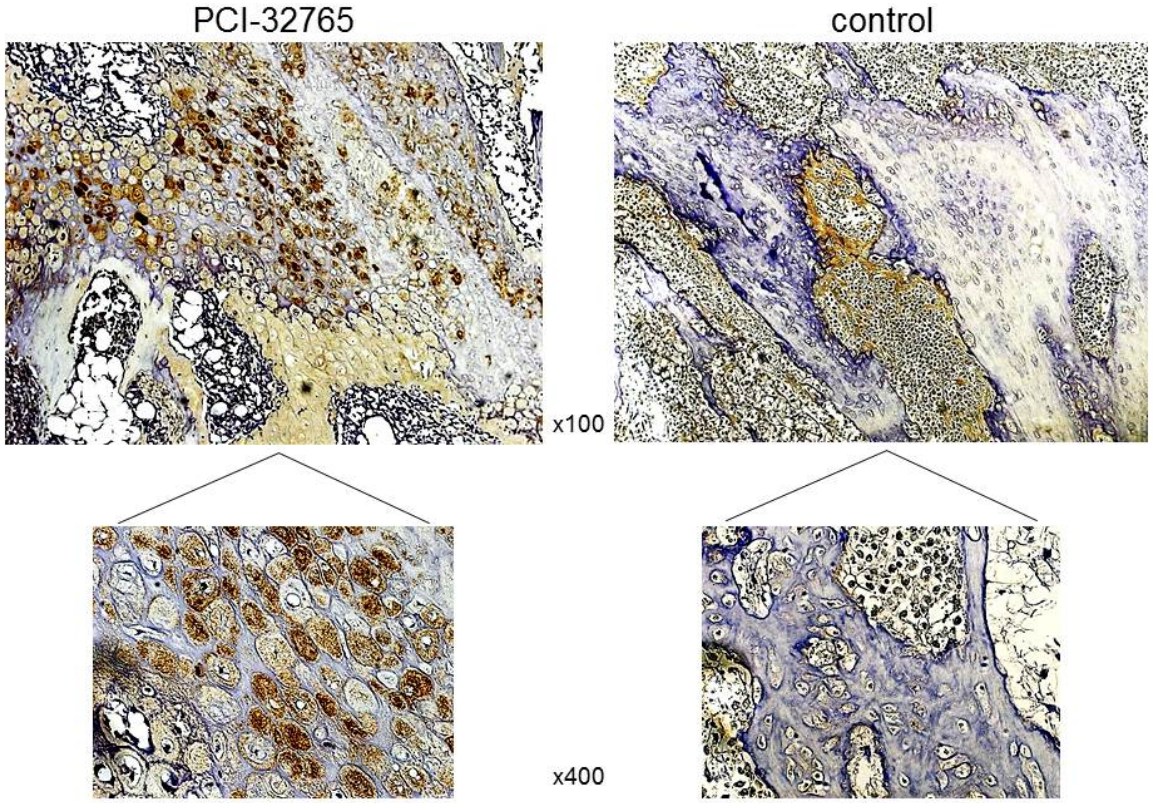
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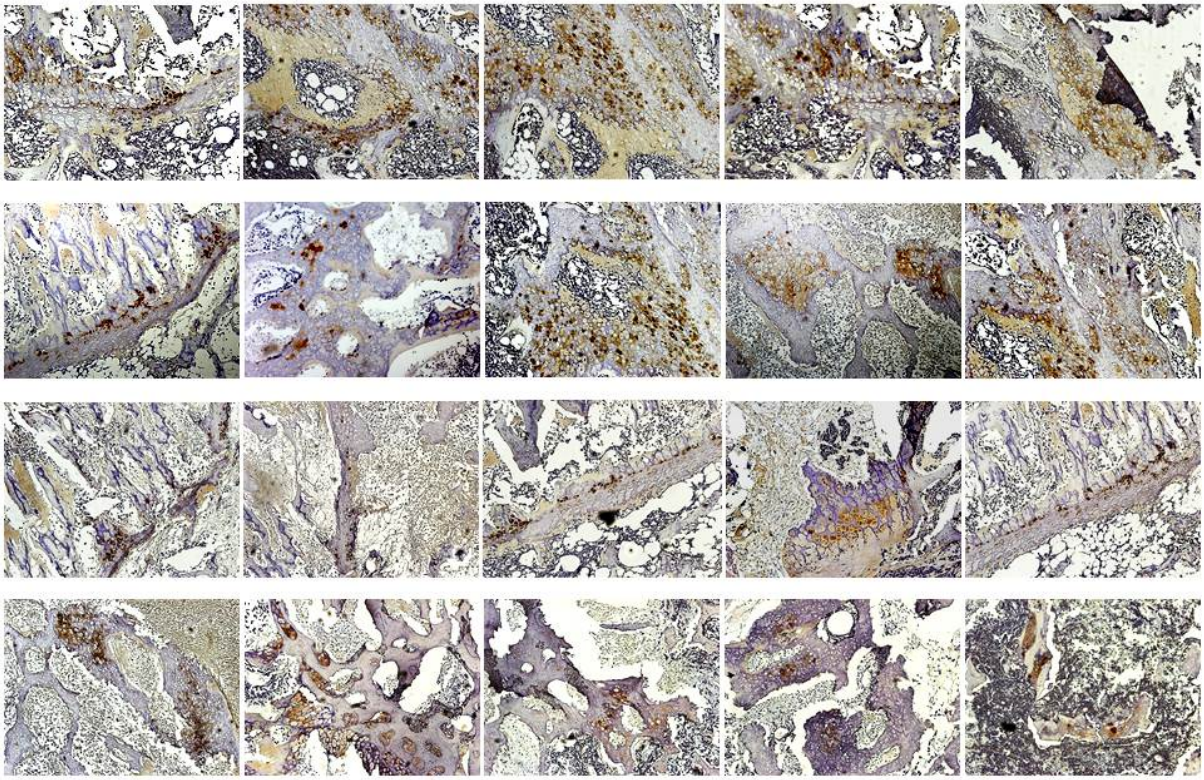

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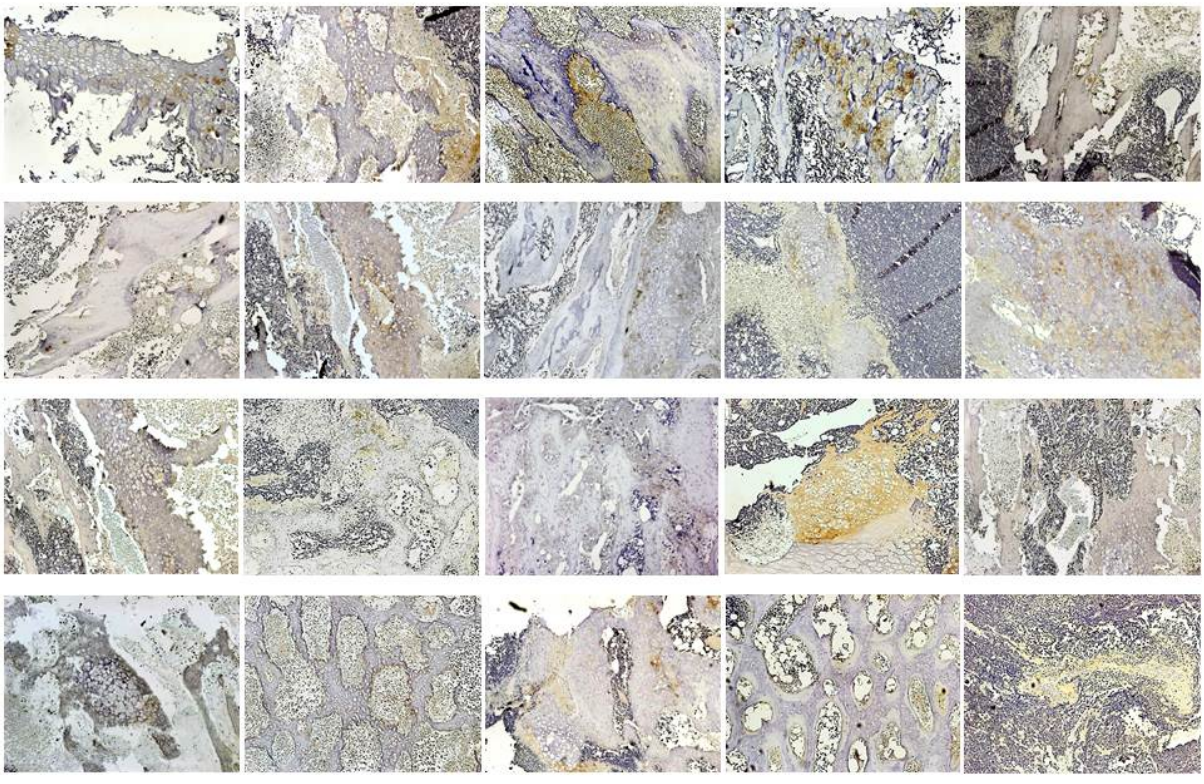

x100

Figure S7. Enhanced bone formation in PCI-32765-treated human bone implants containing MM in SCID-hu mice. Human bone chips were retrieved from SCID-hu mice, decalcified, and sectioned. Tissue slides were immunohistochemically analyzed for ALP for osteogenic activity. Shown are representative examples of bone structure from PCI-32765 (n = 6) and control (n = 5) treated mice. Multiple photos were taken of these chips. Significantly augmented bone formation activity was observed in PCI-32765-treated bone tissue slides, indicating that PCI-32765-blocked in vivo anti-MM activity and osteolytic activity. Original magnification: x100 and x400.

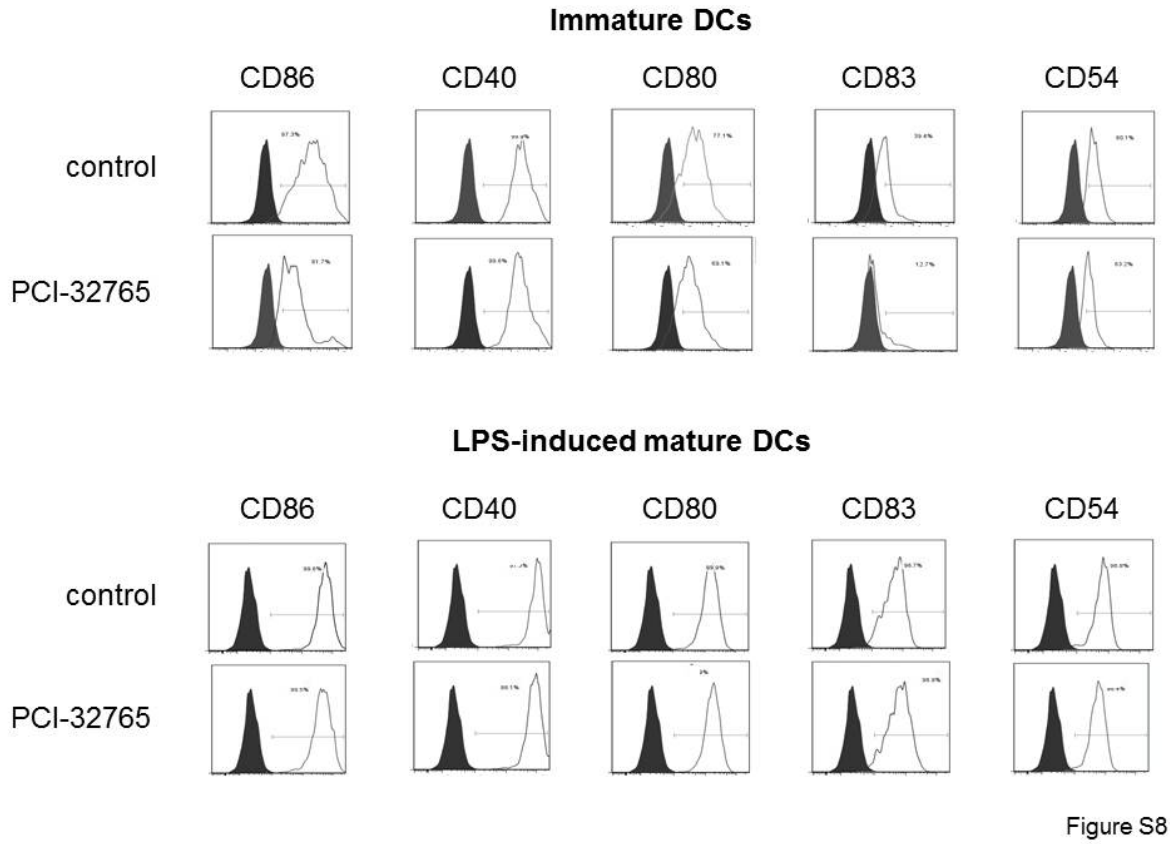

Figure S8. No adverse effect of PCI-32765 was observed on dendritic cells. Immature or mature dendritic cells were generated by in vitro differentiation of CD14+ monocytes using IL-4 and GM-CSF containing medium in the presence or absence of PCI-32765 (100 nM), followed by flow cytometric analysis for listed antigens. LPS was used to induce mature dendritic cells.