# Myeloma cell growth suppression by osteoblast-derived extracellular vesicles: the creation of a non-permissive niche for myeloma cells by bone-forming osteoblasts

## Authors

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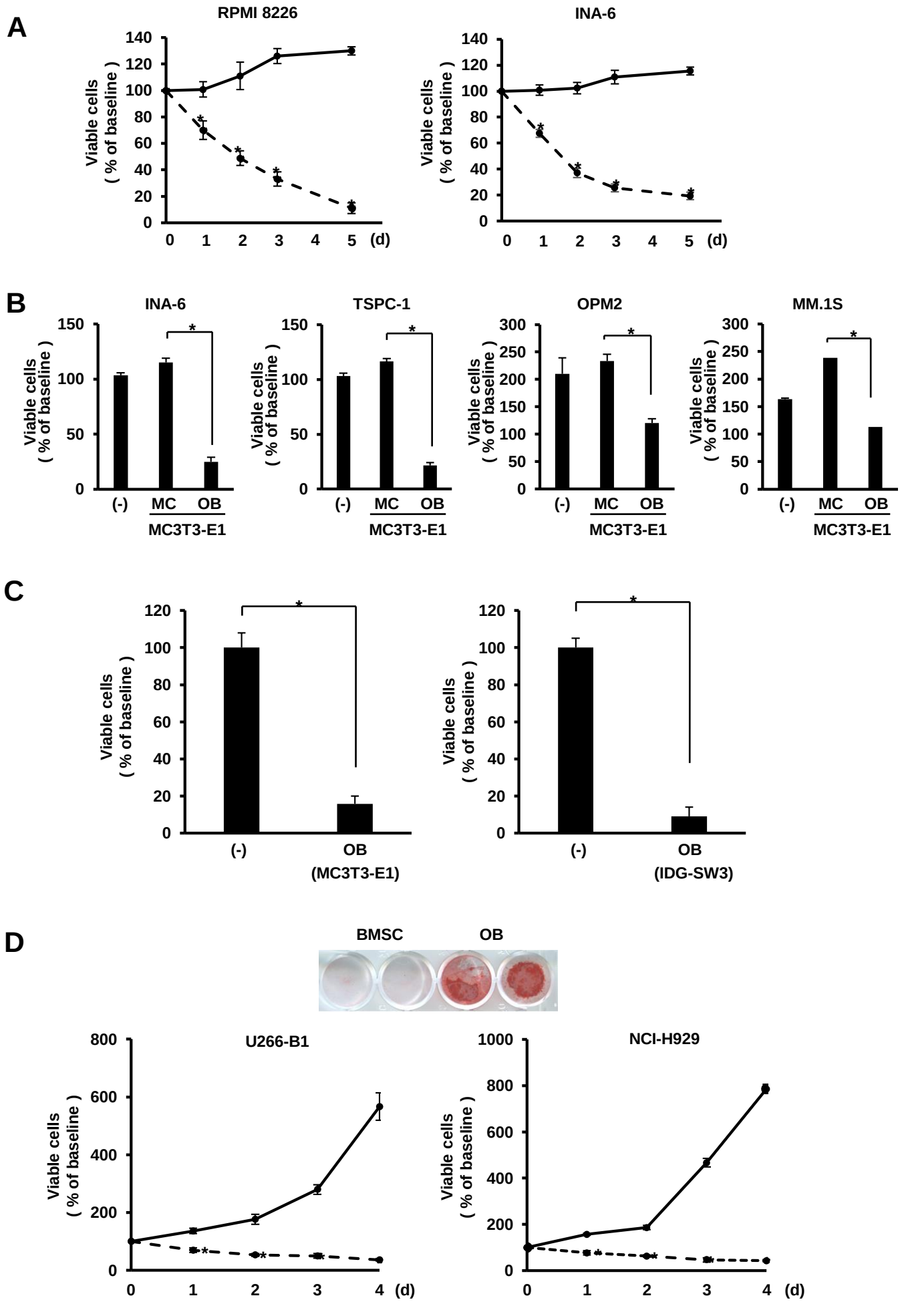
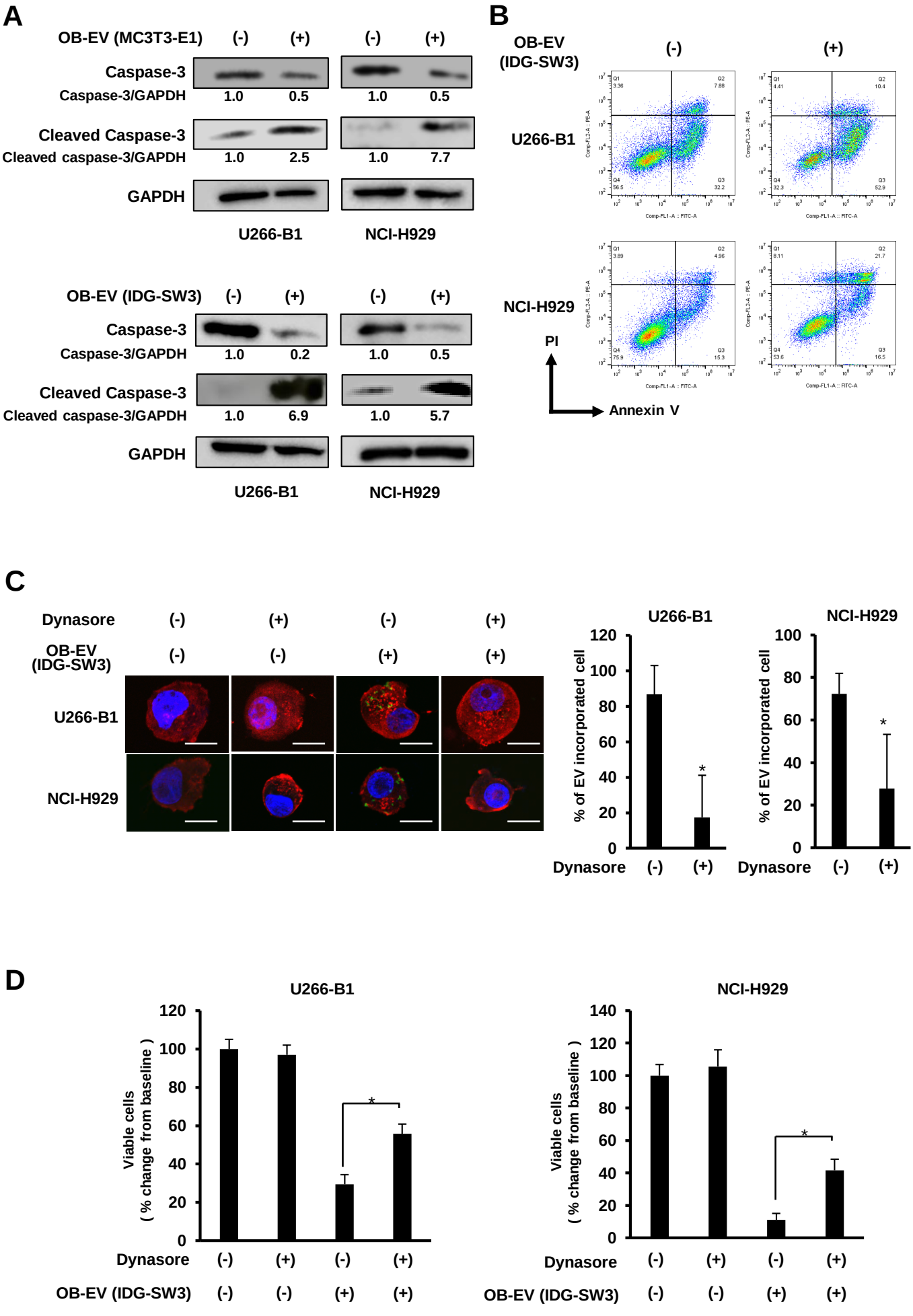
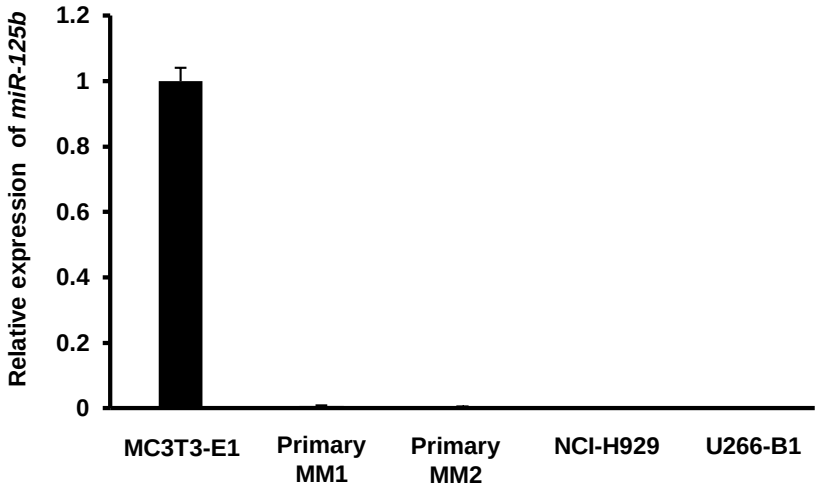



**Supplementary Figure 1**




**Supplementary Figure 2**

**A**

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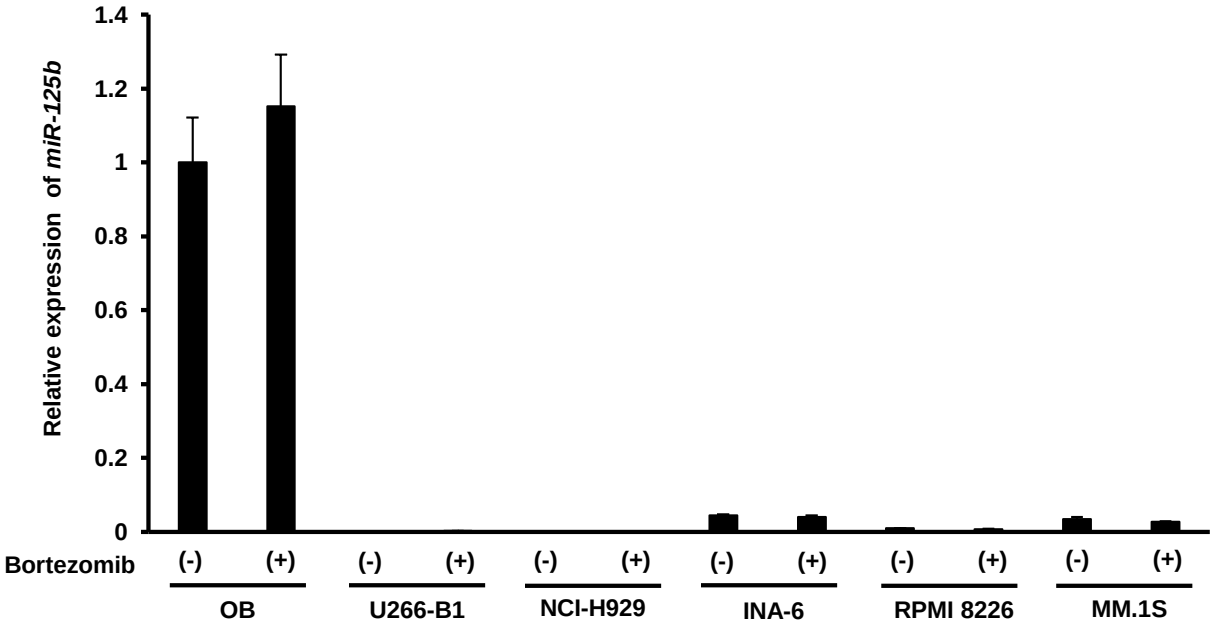


**Supplementary Figure 3**

**Supplementary Figure legends**

**Supplementary Figure 1**

(A) MM cell lines RPMI8226 and INA-6 were cultured in triplicate at $5\times10^4$/mL alone (solid line) or co-cultured with mature OBs differentiated from MC3T3-E1(dashed line). (B) MM cell lines, INA-6, TSPC-1, OPM-2 and MM1.S, were cultured in triplicate for 2 days at $1\times10^5$/mL alone (-), or co-cultured either with untreated MC3T3-E1 cells (MC) or with mature OBs derived from MC3T3-E1 cells (OB). (C) MM cells were isolated from patients with MM after receiving informed consent. Primary MM cells were cultured in triplicate for 4 days at $5\times10^4$/mL alone or co-cultured with mature OBs from MC3T3-E1 or IDG-SW3. Viable MM cell numbers were counted. (D) U266-B1 and NCI-H929 cells were cultured in triplicate at $5\times10^4$/mL alone (solid line) or co-cultured with mature OBs generated from mouse primary BMSCs (dashed line). Viable MM cell numbers were counted at the indicated time points. Percent changes from the baseline were compared with those in MM cells cultured alone. The values are mean ± SD (*$P < 0.05$).

**Supplementary Figure 2**

U266-B1 and NCI-H929 cells were cultured for 24 hours in the presence or absence of EVs isolated from mature OBs derived from MC3T3-E1 (OB-EV (MC3T3-E1)) and IDG-SW3 cells (OB-EV (IDG-SW3)). Cell lysates were then collected, and caspase 3 cleavage was analyzed using western blotting analysis (A). GAPDH was used as a loading control. The cells were further analyzed to detect apoptosis by flow cytometry using annexinV and propidium iodide (PI) dual staining (B). (C) MM cells were cultured in the presence or absence of 50 μg/mL Dynasore. After 30 min, RNA-labeled EVs isolated from mature OBs derived from IDG-SW3 cells were added and further cultured for 24 hours. The incorporation of the EVs into MM cells was detected using confocal microscopy. Actin filaments and nuclei were stained with Phalloidin (red) and DAPI (blue), respectively. Representative cell images are shown (left). Scale bars represent 10 μm. The proportion of cells with incorporated EVs in the presence or absence of 50 μg/mL Dynasore was counted under a confocal microscope. The efficiency of the incorporation is shown (right). (D) The indicated MM cell lines were cultured in triplicate in the presence or absence of 50 μg/mL Dynasore. After 30 min, EVs isolated from mature OBs derived from IDG-SW3 cells were added, and MM cells were further cultured for 2 days. Viable cell numbers were counted, and percent changes from the baseline are shown. The values are mean ± SD (*$P < 0.05$).

**Supplementary Figure 3**

(A) miR-125b levels were analyzed by TaqMan MicroRNA Assays (Thermo Fisher Scientific) in MC3T3-E1, primary MM cells from patients and MM cell lines. (B) mature OBs derived from MC3T3-E1 (OB) and the indicated MM cell lines were cultured with or without pulsatile treatment of bortezomib at 200 nM for 1 hour, which simulates serum pharmacokinetics of bortezomib in patients as previously reported[1]. The cells were then washed to remove bortezomib and further cultured without bortezomib for 24 hours. RNA was collected and miR-125b levels were analyzed by TaqMan MicroRNA Assays. *U6* served as an endogenous control to normalize the data.

**Reference**

1. Nakaue E, Teramachi J, Tenshin H, et al. Mechanisms of preferential bone formation in myeloma bone lesions by proteasome inhibitors. Int J Hematol. 2023;118(1):88-98.