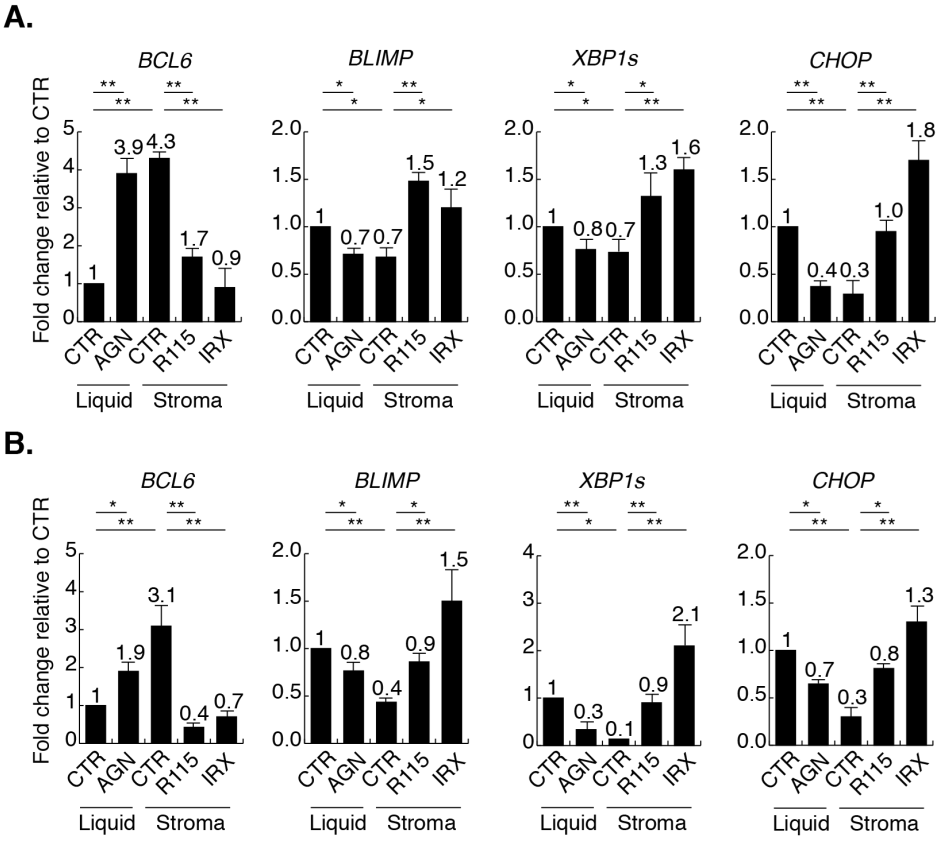
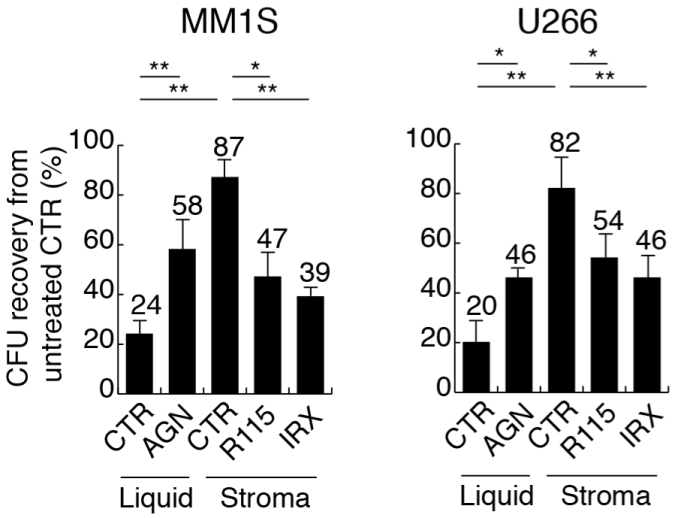
Supplemental Material: Microenvironment-mediated bortezomib resistance in multiple myeloma: role of crosstalk between Hedgehog and retinoid signaling

Figure S1



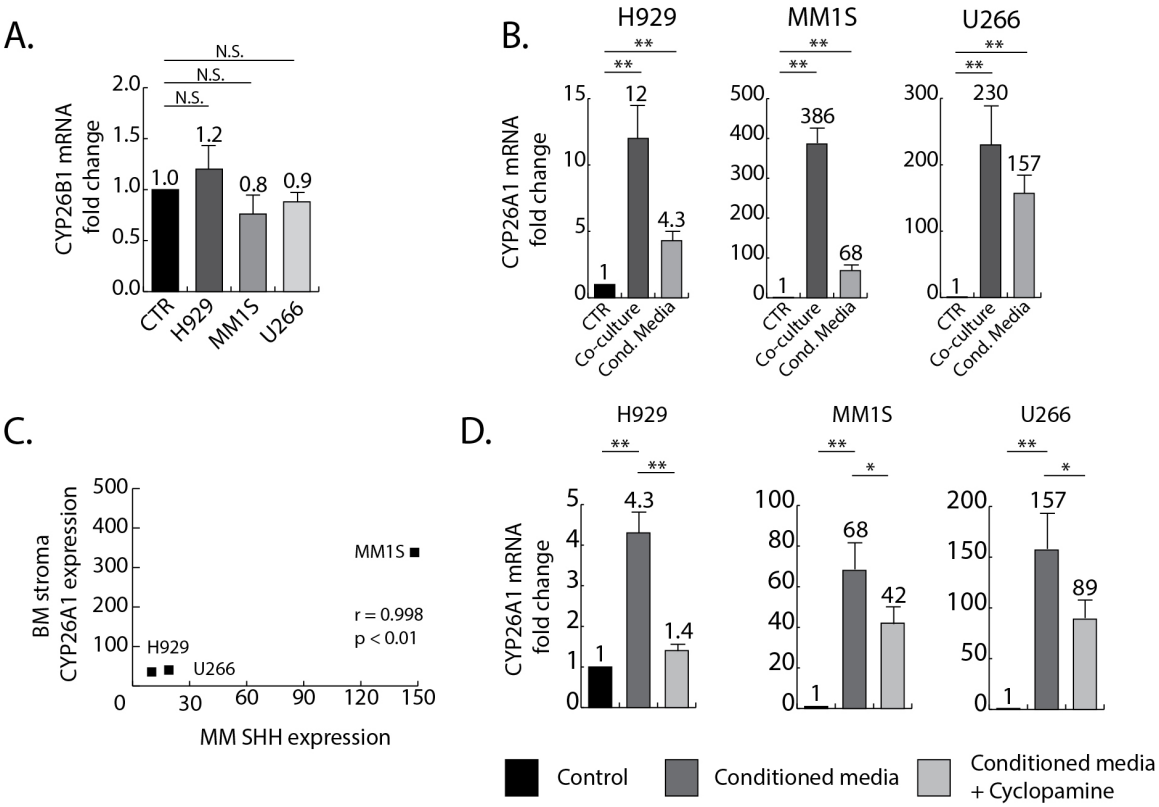
Expression of B and plasma cell markers in response to retinoid low or high conditions.(**A** and **B**) Relative quantification of *BCL6* (B cell marker), *BLIMP, XBP1s* and *CHOP* (plasma cell markers) in MM1S cells (A) or U266 cells (B) incubated for 5 days either in the absence of stroma (Liquid) ± AGN (1 μM) or in the presence of BM mesenchymal cells (Stroma) ± R115 (1 μM) or IRX (1 μM). Expression in untreated liquid conditions was set as 1. Data represents mean ± SEM of 3 independent experiments. Statistical significance between treatment groups was evaluated using RM one-way ANOVA, and p value was corrected for multiple comparisons using Dunnett's test. (*$P \leq 0.05$, **$P \leq 0.01$.).

Figure S2



BTZ sensitivity of MM cell line MM1S and U266 in various retinoid low and high conditions. Clonogenic recovery of MM MM1S or U266 cells upon treatment with BTZ (2.5 nM). MM cells were treated with BTZ for 48 hours after being incubated either in the absence of stroma (Liquid) ± AGN (1 μM) or in the presence of BM mesenchymal cells (Stroma) ± R115 (1 μM) or IRX (1 μM) for 5 days. Data represents mean ± SEM of CFU recovery from untreated CTR of 3 independent experiments. Statistical significance was evaluated using 2-tailed unpaired student *t* test. (*P ≤ 0.05, **P ≤ 0.01.)

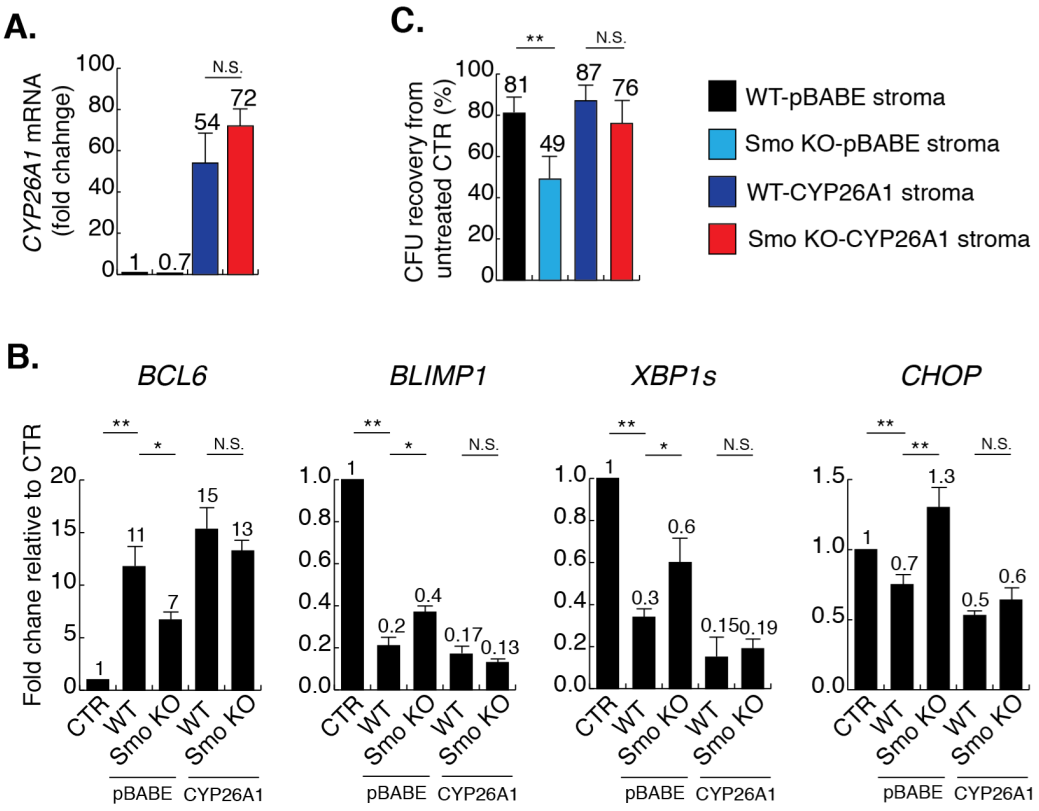
Figure S3



Effects of MM cells on the expression of *CYP26* isoenzymes in BM mesenchymal cells. (**A**) Relative quantification of *CYP26B1* mRNA on human primary BM mesenchymal cells after being co-cultured with MM cell lines (H929, MM1S, U266) for 24 hours. (B) Relative quantification of *CYP26A1* mRNA in human BM mesenchymal cells incubated for 24 hours either in the absence (CTR) or presence (Co-culture) of MM cells (H929, MM1s, U266) or their conditioned media. Expression in untreated BM stroma (CTR) was arbitrarily set as 1. (**C**) Correlation between *SHH* mRNA levels in MM cells and up-regulation of *CYP26A1* mRNA in BM stroma cells after being co-cultured with MM cells (H929, MM1S, U266). R and P values were calculated using Pearson's correlation coefficient. (**D**) Relative quantification of *CYP26A1* mRNA on human primary BM stroma cells treated with conditioned media derived from MM cells (H929, MM1S, U266) ± cyclopamine (SMO antagonist, 1 μM). Expression of *CYP26A1* in untreated BM stroma cells (Control) was arbitrarily set as 1. Data depicted in panels A

and C represent mean ± SEM of 3 independent experiments. Statistical significance was evaluated using 2-tailed unpaired student *t* test. (*P ≤ 0.05, **P ≤ 0.01.)

Figure S4



Effects of paracrine SHH signaling on plasma cell differentiation and BTZ sensitivity of MM cells. (**A**) Relative quantification of *CYP26A1* mRNA (mean ± SEM of 3 independent experiments) in mouse WT and SMO KO stroma cells transduced with an empty vector (pBABE) or a lentivirus encoding CYP26A1 (pBABE-*CYP26A1*). Expression in WT-pBABE stroma cells was arbitrarily set as 1. (**B**) Relative quantification of *BCL6* (B cell marker), *BLIMP*, *XBP1s* and *CHOP* (plasma cell markers) in H929 cells co-cultured for 5 days with WT or *Smo* KO stroma cells transduced with either pBABE or pBABE-CYP26A1. Expression in untreated liquid conditions (CTR) was arbitrarily set as 1. Data represents mean ± SEM of 3 independent experiments. (**C**) BTZ sensitivity of H929 cells co-cultured for 5 days with WT or *Smo* KO stroma cells transduced with either an empty vector (pBABE) or a lentivirus encoding *CYP26A1*

(pBABE-*CYP26A1*). H929 cells were treated with BTZ (2.5 nM) for 48 hours after being incubated with respective stroma for 5 days. Data represents mean ± SEM of CFU recovery from untreated CTR of 3 independent experiments. Statistical significance between treatment groups was evaluated using RM one-way ANOVA, and p value was corrected for multiple comparisons using Dunnett's test. (*$P \leq 0.05$, **$P \leq 0.01$.).