

LEGENDS FOR SUPPLEMENTAL FIGURES

Supplementary Table S1. Summary of statistical significance

Datasets shown in Figure 4A

E/T ratio	VBL vs Vehicle	VBL vs CDDP	CDDP vs Vehicle
3	7.9×10^{-3}	7.8×10^{-3}	> 0.1
10	2.1×10^{-2}	2.1×10^{-2}	> 0.1
30	1.3×10^{-2}	7.9×10^{-3}	> 0.1
100	7.9×10^{-3}	7.9×10^{-3}	> 0.1

Datasets shown in Figure 5C

E/T ratio	VBL vs Vehicle	VBL vs CDDP	CDDP vs Vehicle
3	1.6×10^{-2}	9.1×10^{-5}	> 0.1
10	9.5×10^{-5}	1.1×10^{-5}	> 0.1
30	3.3×10^{-4}	1.8×10^{-5}	> 0.1
100	2.7×10^{-4}	3.1×10^{-5}	> 0.1

Datasets shown in Figure 6A

Tumor size	VBL vs Vehicle	CDDP vs Vehicle	VBL vs CDDP
30 mm^2	2.0×10^{-4}	4.7×10^{-2}	2.1×10^{-4}
50 mm^2	3.0×10^{-4}	6.9×10^{-3}	4.9×10^{-5}
70 mm^2	2.0×10^{-4}	1.5×10^{-3}	9.0×10^{-5}

Datasets shown in Figure 6B

Tumor size	VBL vs Vehicle	CDDP vs Vehicle	VBL vs CDDP	Vehicle vs VBL*
30 mm^2	9.1×10^{-4}	9.1×10^{-4}	1.7×10^{-5}	> 0.1
50 mm^2	6.0×10^{-4}	6.0×10^{-4}	2.0×10^{-5}	1.6×10^{-2}
70 mm^2	1.8×10^{-4}	1.8×10^{-4}	2.1×10^{-5}	> 0.1

VBL*: VBL injection to normal skin of tumor-bearing mice

Datasets shown in Figure 6C

Tumor size	VBL vs Vehicle	CDDP vs Vehicle	VBL vs CDDP
30 mm^2	2.1×10^{-2}	2.1×10^{-2}	> 0.1
50 mm^2	2.0×10^{-3}	2.0×10^{-3}	> 0.1
70 mm^2	1.0×10^{-3}	3.4×10^{-3}	5.0×10^{-2}

Supplemental Figure S1. Effects of VBL on CCR7 expression by DCs. (A) BM-DCs propagated from BALB/c mice were incubated for 24 h with 0.3 μ M VBL or vehicle alone and then stained with anti-CCR7 mAb (filled histograms) or isotype-matched control IgG (open histograms). (B) BM-DCs were examined for CCR7 expression in triplicates after 24 h incubation with 1 μ M VBL or vehicle alone. Data shown are the means \pm SD ($n = 3$) of the median fluorescence intensity within the CD11c $^{+}$ populations. (C) After 24 h incubation with 0.3 μ M VBL or vehicle alone, BM-DCs were examined for their migratory capacity toward CCL19 or PBS alone. Data shown are the means \pm SD ($n = 3$) of % DC migration. In all panels, statistically significant difference compared to the vehicle-treated control is indicated with asterisks (** $P < 0.01$).

Supplemental Figure S2. *In vitro* impacts of VBL on B16 melanoma cells. B16 melanoma cells were incubated with VBL at the indicated concentrations and then examined for growth, apoptosis, and HMGB1 release. Data shown are the means \pm SD ($n = 3$) of ^{3}H -thymidine uptake harvested on day 2 (A), percentages of PI-negative/Annexin V-positive cells on day 1 (B), and amounts of HMGB1 detected in supernatants on day 1 (C). Statistically significant differences compared with the vehicle-treated control are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). B16 melanoma cells were incubated with VBL for 24 h and then stained with anti-calreticulin antibodies (filled histograms or closed circles) or control IgG (open histograms or open circles) (D and E). (F) B16 melanoma cells were pre-incubated for 24 h with 1 μ M VBL, washed extensively, and then added to BM-DC cultures in the presence or absence of freshly added VBL (1 μ M). IL-12 was then tested in 24 h culture supernatants ($n = 3$, ** $P < 0.01$, $^{\#}$ statistically significant synergy between the two stimuli).

Supplemental Movie S1. Time-lapse images of dynamic LC behaviors exacerbated by local

VBL injection. I-A β -EGFP knock-in mice received s.c. injection of VBL (3.6 μ g/animal or 40 μ l of 90 μ g/ml solution) or vehicle alone (40 μ l) into the right ear and anesthetized 24 h later to visualize dynamic behaviors of EGFP $^+$ LCs in the injection sites under confocal microscopy.

Three-dimensional images of EGFP $^+$ LCs recorded every 2 minutes were compiled to illustrate VBL-induced augmentation of LC behaviors. Quantitative measurements of the dendrite motion and the cell body movement are shown in Figure 2. Each movie is representative of three independent imaging experiments.