Supporting online materials

Uncarboxylated Osteocalcin Induces Antitumor Immunity against Mouse Melanoma Cell Growth

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Supplementary methods

Depletion of NK cells from mouse splenocytes

To deplete NK cells from mouse splenocytes, an NK cell isolation kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) was used according to the manufacturer's instructions. Briefly, a cell pellet of splenocytes was resuspended in 90 µl of buffer (phosphate-buffered saline containing 0.5% bovine serum albumin and 2 mM EDTA) and 10 µl CD49b (DX5) MicroBeads (130-052-501, Miltenyi Biotec) per 1×10^7 cells was added, followed by incubation for 15 min at 4°C. The beads were washed, resuspended in the buffer, and passed through a 30 µm nylon mesh (130-041-407, Miltenyi Biotec). Then, the cell suspension was applied to a column placed in the magnetic field of a MACS Separator (Miltenyi Biotec). Unlabeled cells that passed through the column were collected. After checking the expression level of CD49b by immunoblot analysis (data not shown), the collected cells were used as NK cell-depleted splenocytes.

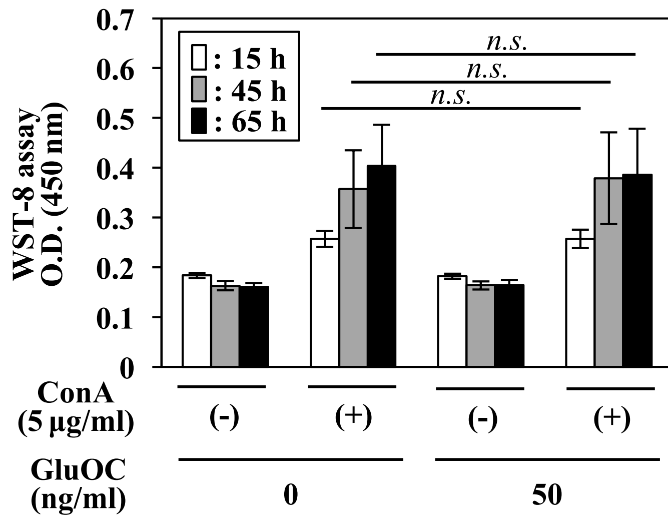
Supplementary figure legends

Supplementary Figure 1. Effects of NK cell depletion on the ConA-stimulated responses of mouse splenocytes *in vitro*. Splenocytes without NK cells collected from C57Bl/6N female mice were stimulated by ConA in the presence or absence of GluOC (50 ng/ml) for 15 h (white columns), 45 h (gray columns), and 65 h (black columns). A lymphocyte blast transformation assay (A) and ELISA of IFN-γ (B) in culture supernatants were performed at 15, 45, and 65 h after stimulation. Each experiment was repeated three times. Data represent the mean ± SEM (n=3–6).

Supplementary Figure 2. Effects of GlaOC on ConA-stimulated splenocytes of C57BL/6 mice *in vitro*. Splenocytes collected from C57Bl/6N female mice were stimulated by ConA in the presence or absence of GlaOC (50 ng/ml) for 15 h (white columns), 45 h (gray columns), and 65 h (black columns). A lymphocyte blast transformation assay (A) and ELISA of IFN-γ (B) in culture supernatants were performed at 15, 45, and 65 h after stimulation. Each experiment was

repeated three times. Data are the mean ± SEM (n=3–6).

A



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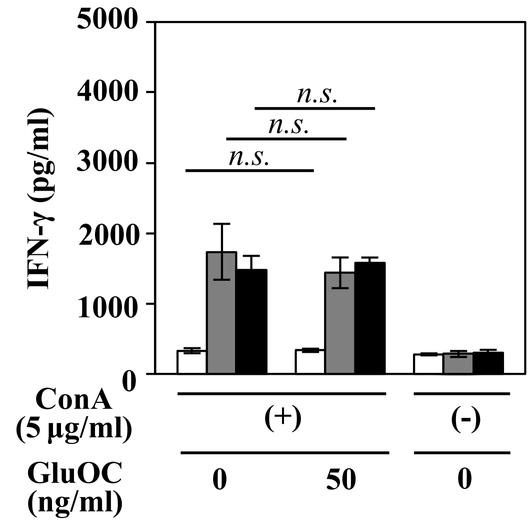
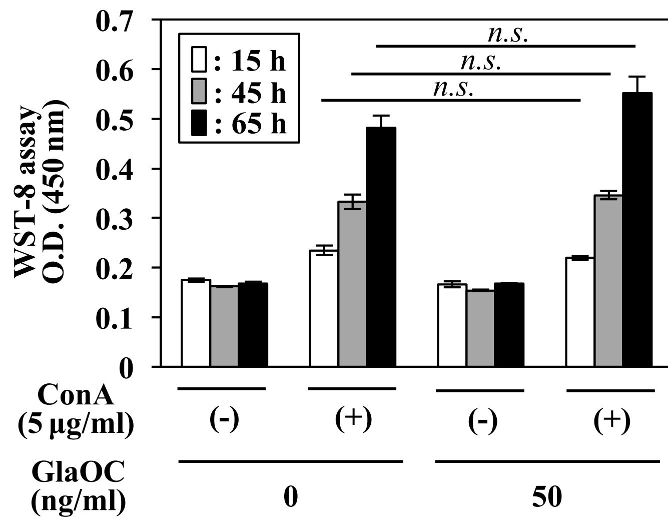


Fig. S1 Hayashi et al.

A



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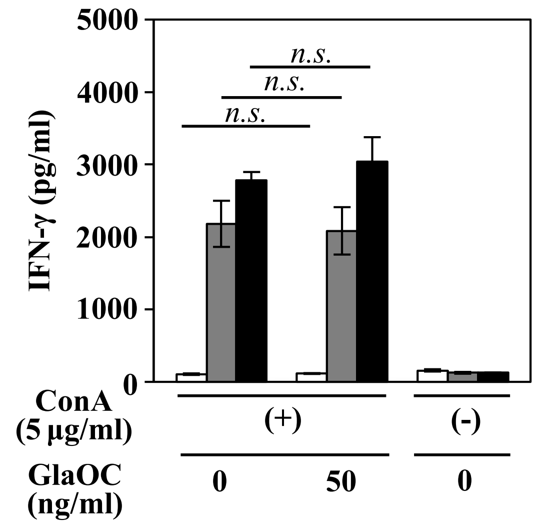

Fig. S2 Hayashi et al.