HOKKAIDO UNIVERSITY

Title	The DNA methylation inhibitor 5-aza-2’-deoxycytidine retards cell growth and alters gene expression in canine mammary gland tumor cells
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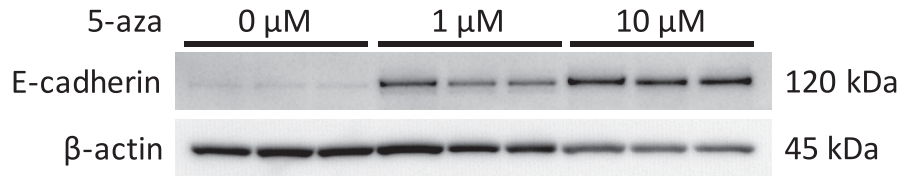

Supplemental Fig. 1. Western blot analysis of E-cadherin protein expression after 5-aza treatment. β-actin was the loading control.

Supplementary material and method

Cells were lysed in RIPA buffer (Nacalai Tesque, Kyoto, Japan) and resolved by 12.5% SDS-PAGE using approximately 10 μg of sample per lane. After electrophoresis, separated proteins were transferred to polyvinylidene difluoride membranes (ATTO, Tokyo, Japan). The membrane was then incubated for 1 h at room temperature with EzBlock BSA (ATTO, Tokyo, Japan) for blocking and rinsed with Tris-buffered saline (TBST; 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 0.05% Tween-20). We used 1 : 250-diluted anti-human E-cadherin mouse antibody (BD Transduction Laboratories, San Diego, CA, USA) or 1 : 1000-diluted anti-human β-actin rabbit antibody (Cell Signaling Technology, Danvers, MA, USA) as the primary antibodies. The membrane was incubated with the primary antibody for 16 hours at 4°C, rinsed with TBST and subsequently incubated with horseradish peroxidase-conjugated antibodies against mouse Ig or rabbit Ig (GE Healthcare, Piscataway, NJ, USA) for 1 h at room temperature with vigorous agitation. Signals were visualized with Western BLoT Ultra Sensitive HRP Substrate (TaKaRa Bio Inc.).