



ALDH1A1 overexpression in melanoma cells promotes tumor angiogenesis by activating the IL-8/Notch signaling cascade

This is the peer reviewed version of the following article:

Original:

Ciccone, V., Terzuoli, E., Ristori, E., Filippelli, A., Ziche, M., Morbidelli, L., et al. (2022). ALDH1A1 overexpression in melanoma cells promotes tumor angiogenesis by activating the IL-8/Notch signaling cascade. INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE, 50(1) [10.3892/ijmm.2022.5155].

Availability:

This version is available <http://hdl.handle.net/11365/1212434> since 2022-07-06T15:34:08Z

Published:

DOI: <http://doi.org/10.3892/ijmm.2022.5155>

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Figure S1. Apoptosis genes-related expression in HUVEC 2D vs. HUVEC 3D sorted from multicellular skin 3D spheres. Data are reported as fold change relative to HUVEC 2D, which were assigned to 1. HUVECs, human umbilical vein endothelial cells.

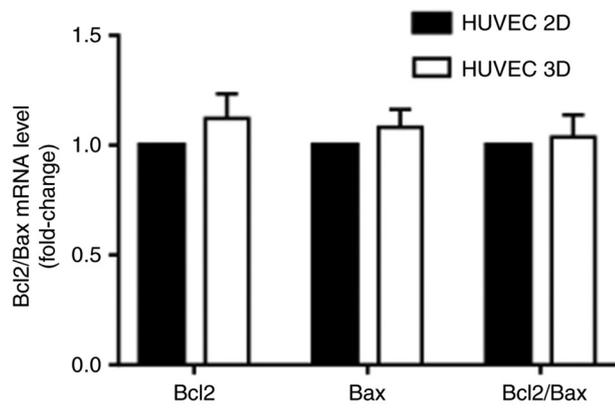


Figure S2. Pharmacological characterization of CM037. ALDH1A1 activity measured by the formation of NADH in melanoma cells. (A) A375 and (B) WM-266-4 lysates were pretreated with CM037 (50 μ M, 10 min) and absorbance at 340 nm (corresponding to NADH production) was measured. *** P <0.001 vs. untreated cells. Cell viability in (C) A375 and (D) WM-266-4 exposed to increasing concentrations of CM037 in complete medium with 10% FBS for 48 h. Cell survival, reported as absorbance at 540 nm, was measured by MTT assay. $n=3$. *** P <0.001 vs. Ctr untreated.

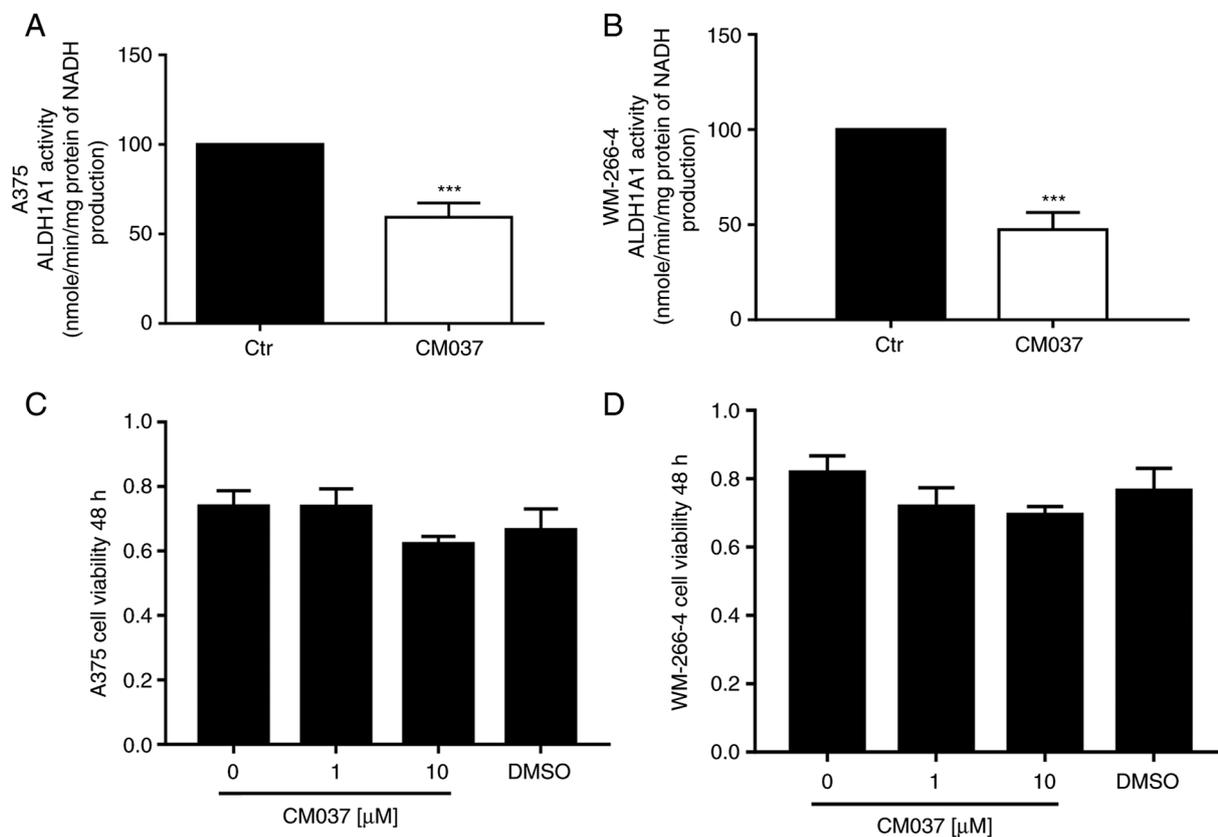


Figure S3. In A375 ALDH1A1 regulates IL-8 and NF- κ B expression through retinoic acid signaling. (A) RT-qPCR analysis of IL-8 in A375 ALDH1A1SC, A375 ALDH1A1KD and A375ALDH1A1⁺ cultured in medium with 2% FBS for 48 h. Data are reported as Δ Ct (Ct gene of interest-Ct Housekeeping gene). #P<0.05 vs. ALDH1A1KD. (B) IL-8 gene expression evaluated by RT-qPCR in A375 ALDH1A1SC and A375 ALDH1A1⁺ treated with retinoic acid receptor antagonist (AGN 193109; 1 μ M) for 48 h. *P<0.05 vs. untreated cells. (C) IL-8 gene expression evaluated by RT-qPCR in A375 ALDH1A1KD treated with exogenous retinoic acid (1 μ M) for 24 h. *P<0.05 vs. untreated cells. (D) Immunofluorescent images for NF- κ B in A375 cells were obtained by confocal microscope (Nikon Eclipse TE 300). A375 ALDH1A1KD were starved for 4 h and then treated with retinoic acid (1 μ M, 1 h). Scale bars=50 μ m. (E) NF- κ B protein expression in A375 treated with retinoic acid (1 μ M, 24 h) or 2% FBS. β -actin was used as loading control. Gel shown is representative of three experiments with similar results. RT-qPCR, reverse transcription-quantitative PCR.

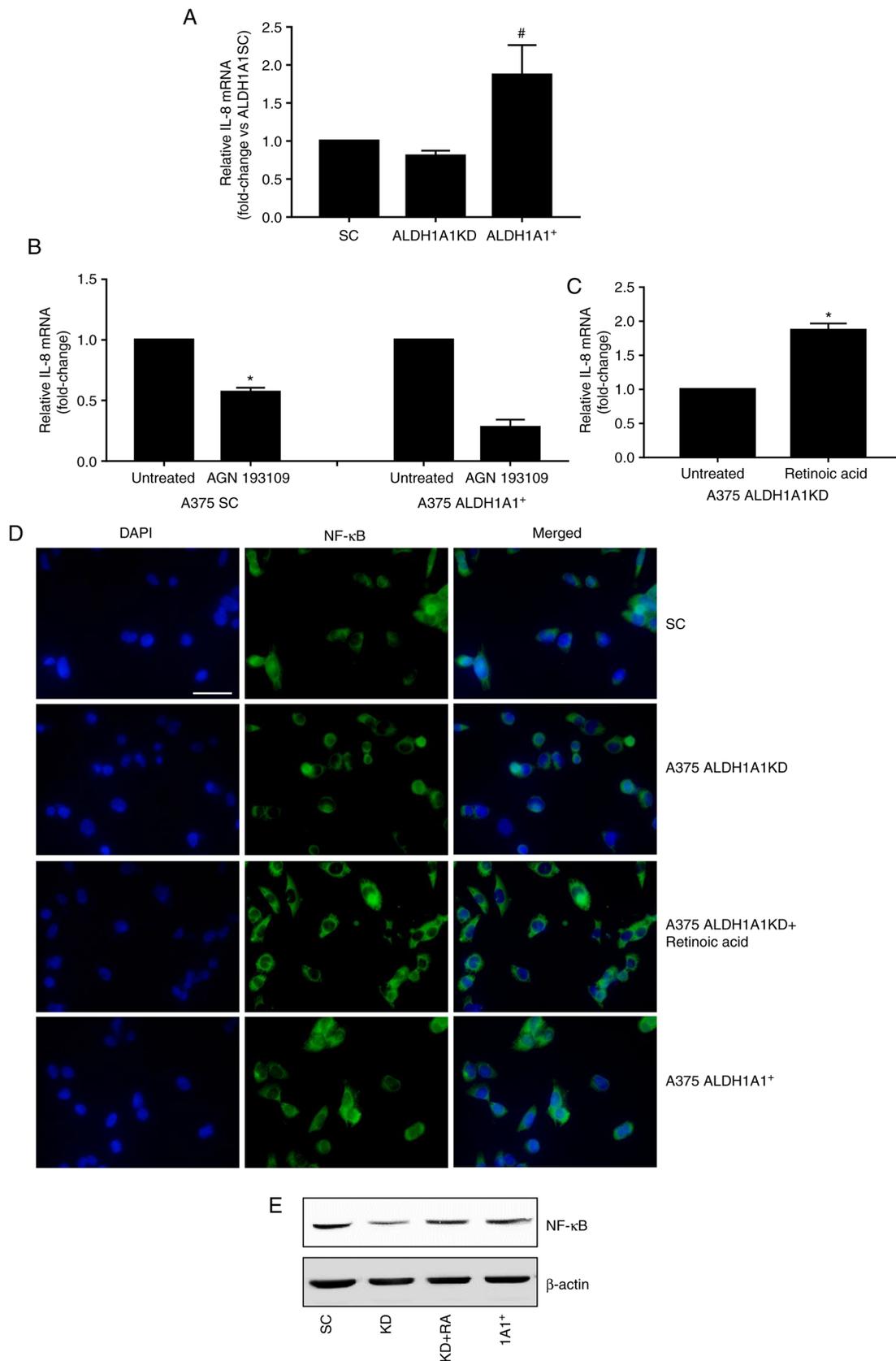


Figure S4. HUVEC proliferation, migration and tube formation in Matrigel when co-cultured with melanoma cells. (A) A375 and (B) WM-266-4 were pretreated with CM037 (10 μ M, 1 h) and then co-cultured with HUVEC for 48 h. HUVEC were fixed, stained, and counted (5 random fields for well). Data are reported as number of HUVEC counted/well (n=3). ***P<0.001 vs. untreated cells. (C) A375 and (D) WM-266-4 were pretreated with CM037 (10 μ M, 1 h) and the co-cultured with HUVEC for 18 h. Cell migration was evaluated by scratch assay. Images were captured after 18 h of incubation on fixed and stained cells. (E and F) Organization of network was evaluated in HUVEC co-cultured with melanoma cells (pretreated with CM037 for 1 h) on Matrigel after 18 h. Images are representative of n=3 experiments. (G) Endothelial cells were exposed to increasing concentrations of IL-8 in EBM with 1% FBS for 48 h. Cells were then fixed, stained, and randomly counted at x20 original magnification in 5 fields. The values are mean viable cell number \pm SEM (bars) of triplicate culture. This is representative of three experiments. *P<0.05 vs. Ctr untreated. HUVECs, human umbilical vein endothelial cells.

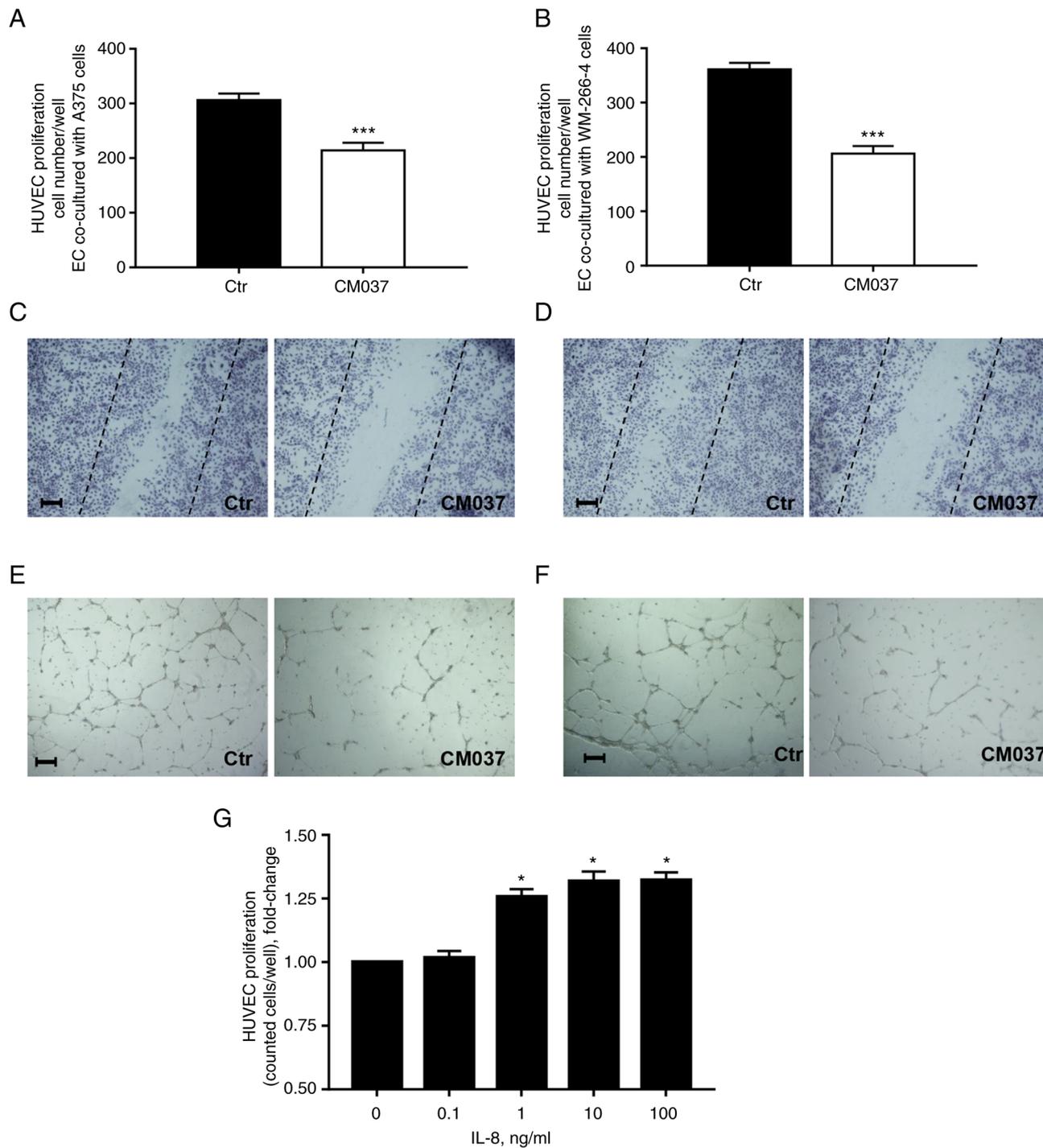


Figure S5. Human Notch signaling transcription clustergram. HUVECs were seeded on the bottom of 6 well multiplates (8×10^4 cells) and tumor cells on the top of transwell (5×10^4 cells). It is reported the profiling of 84 genes representative of Notch pathway in HUVEC co-cultured with A375 cells (A375 ALDH1A1SC, ALDH1A1KD and ALDH1A1⁺) for 6 days. HUVECs, human umbilical vein endothelial cells.

