Co-regulation of Sirt1 and Pin1 Contributes to UV A/B-Induced Skin Photo Ageing

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BACK GROUND

- Skin ageing can be caused by many external and internal factors; UVRs are one of the main causes
- UV light promotes ageing by increasing ROS levels which interfere with critical pathways and cause DNA damage
- Resultant collagen breakdown and elevated levels of matrix metalloproteases leads to appearance of ageing marks like wrinkles and lack of elasticity
- Sirtuins; NAD+ dependent deacetylases are critical to many processes from inflammation to longevity.
- Sirt1 being most studied sirtuin for diversity of roles it plays in mammals was considered for plausible role in skin ageing and regeneration
- Combination of Sirt1 and Pin1 may be critical for regulation of oncogenesis because Pin1 is a ubiquitously expressed cis/trans-isomerase, and it is renowned for playing critical role in various cell death related diseases
- Pin1 is also known to regulate cell cycle by phosphorylation of cyclin D1 proteins
- Our study focused on Roles of Pin1 and Sirt1 along with their inhibitors and activators in ageing and rejuvenation of skin
- Moreover, mechanism of co-regulation of Pin1 and Sirt1 was also aimed in this study (*Ref. Yaar M, Gilchrest BA, 2007. Langton et.al., 2010. Rache et.al., 2010. Finkel et.al., 2009*. Wulf et. al., 2001)

METHODOLOGY

In Vivo

Wound Healing Mice Models: Mice were divided in four groups; normal, vehicle, KSY-PH1 0.1% and KSY-PH1 1% each with n=3.

Wound Creation: Wound was created in the posterior dorsal region of mice using a 6mm biopsy punch.

Compound Treatment: 0.1% and 1% solutions of KSY-PH1 in a mixture of Ethanol, Propandiol and distilled water were applied topically on the wounded skin everyday for 10 days. Vehicle group was treated with solvent only.

Wound size and Physiological Factors:

Wound diameter was measured in centimeters everyday using a scale and average was taken for plotting. Physiological factors of skin like erythema and melanin content were measured using combo system (DermaLab® Combo, CORTEX TECHNOLOGY, Denmark).

In Vitro

Cells: NHDF and HaCaT cells were purchased from Korean Cell line Bank (Seoul National University, Korea) whereas other cells were already being grown in the lab.

UV Treatment: Almost 80% confluent NHDF and HaCaT cells were treated with UVB light at a dose of 144 mJ/cm^2

Compound Treatment: 1, 5 and 10 µM solutions of KSY-PH1 diluted in serum free media from 1mM stock were used for treatment.

Flow Cytometry: FACS Calibur® was used to measure ROS production after 24h of UVB and KSY-PH1 treatment.

Wound Healing Assay: Rate of cell migration after treatment of KSY-PH1 was checked by IncuCyte ZOOM (ESSEN BIOSCIENCE) in scratched cells every four hours of treatment.

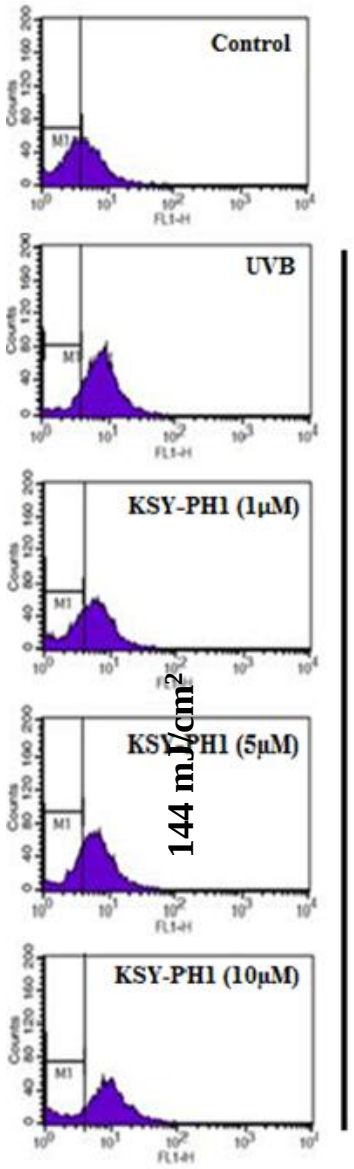
SDS-PAGE & Western Blot: Expression of Sirt1 and Pin1 was checked by running total cell lysate on SDS-PAGE and western blotting.

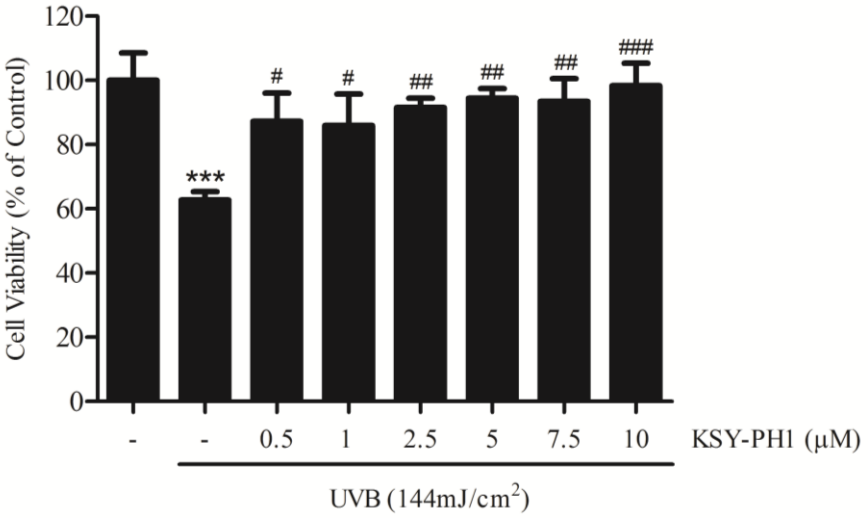
MTT Assay: Cell viability after compound treatment and UVB exposure was checked by MTT assay.

siRNA Knockdown: Pin1 and Sirt1 specific siRNAs (BIONEER) were transfected to 30-40% confluent cell plates using Lipofactamine (Invitrogen). Cells were harvested for western blot analysis after 24-48 hours.

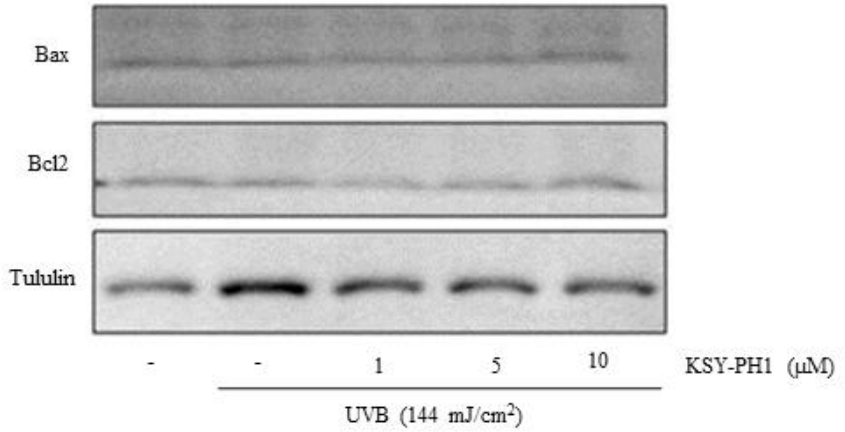
RESULTS

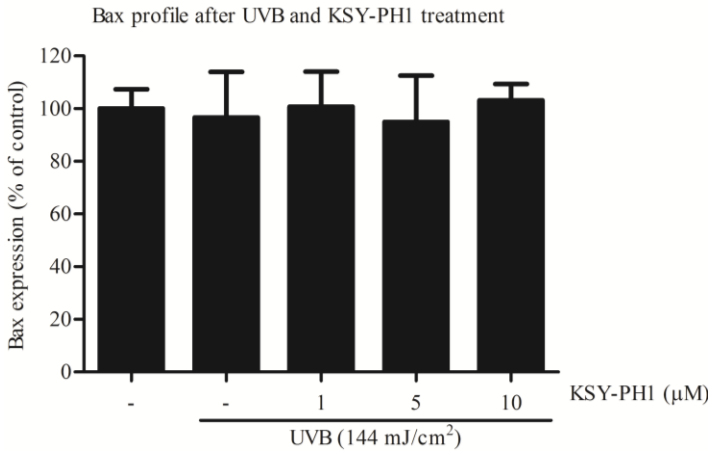
1. KSY-PH1 Decreases UVB Light-Induced ROS Production And Increases Cell Viability in NHDF Cells

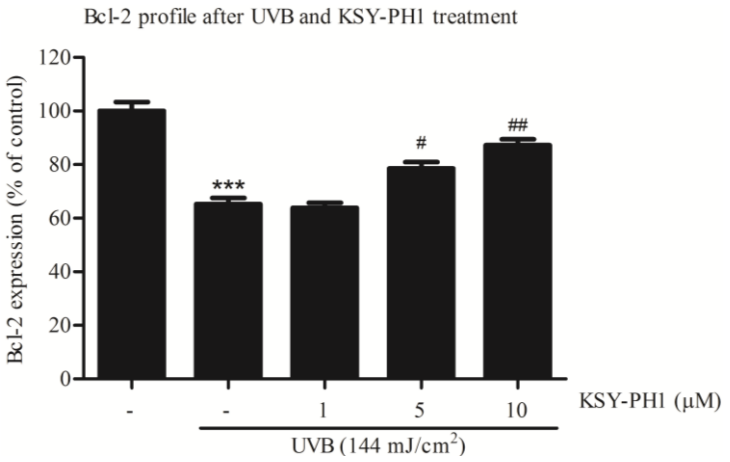




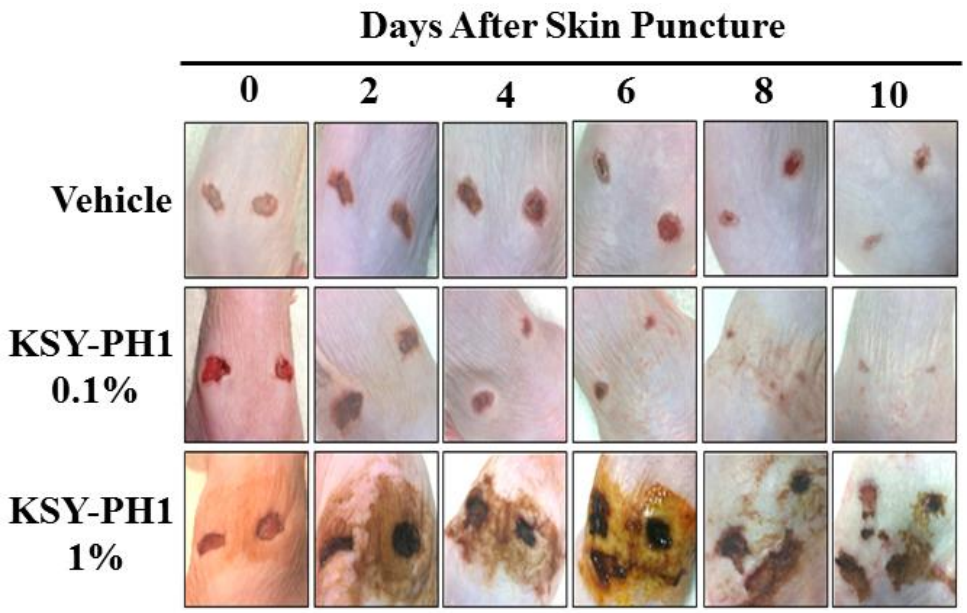
2. KSY-PH1 Reduces Cell Apoptosis in UVB Irradiated NHDF Cells

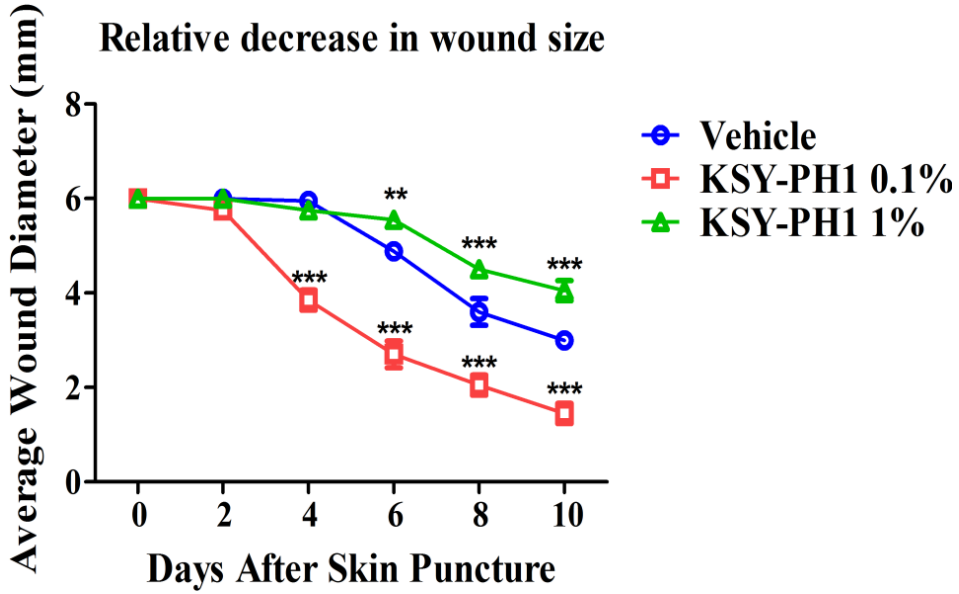




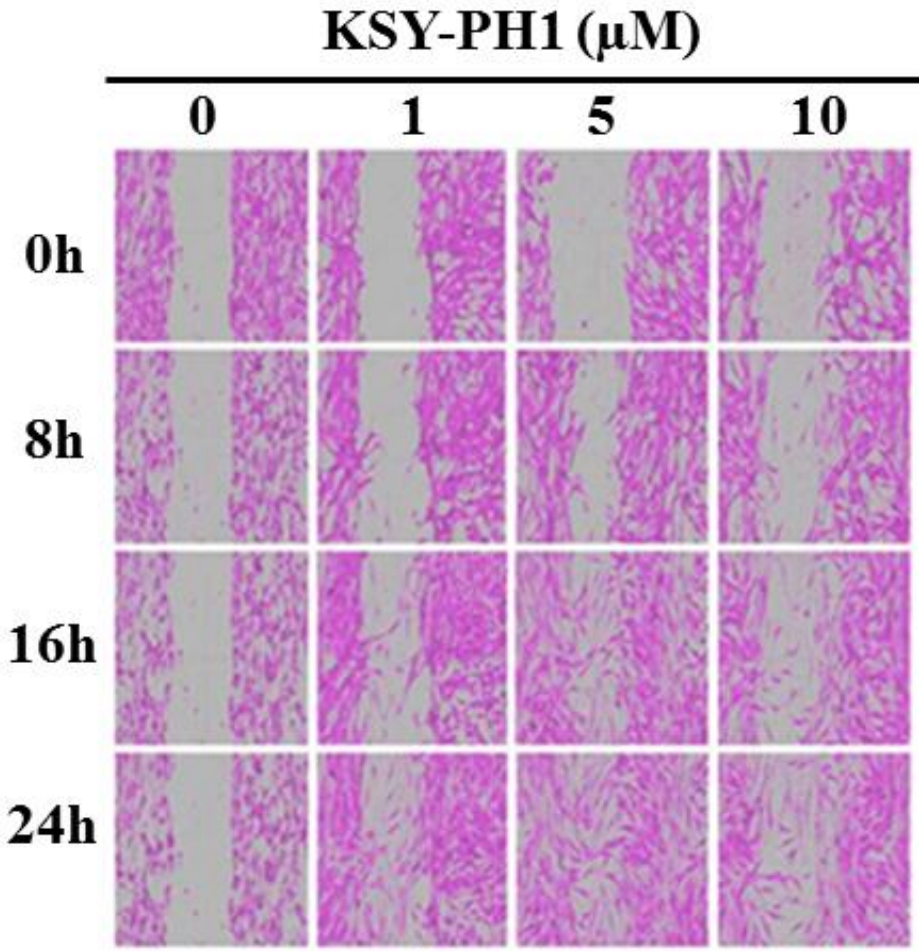


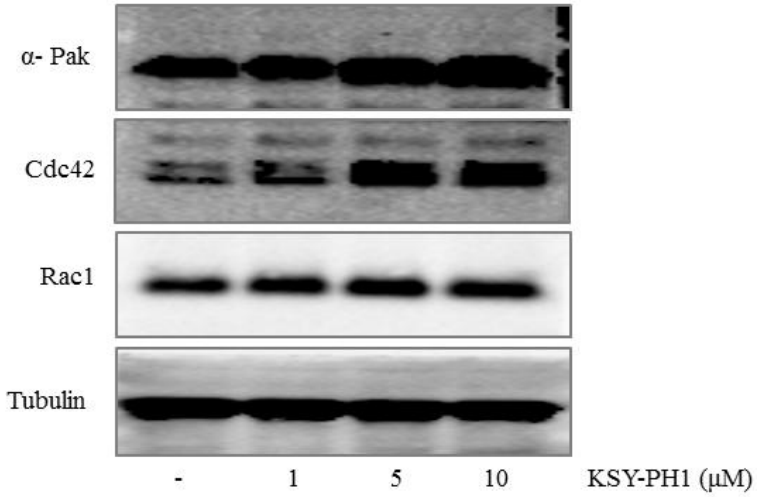
3. KSY-PH1 Enhances Wound Healing *In Vivo*

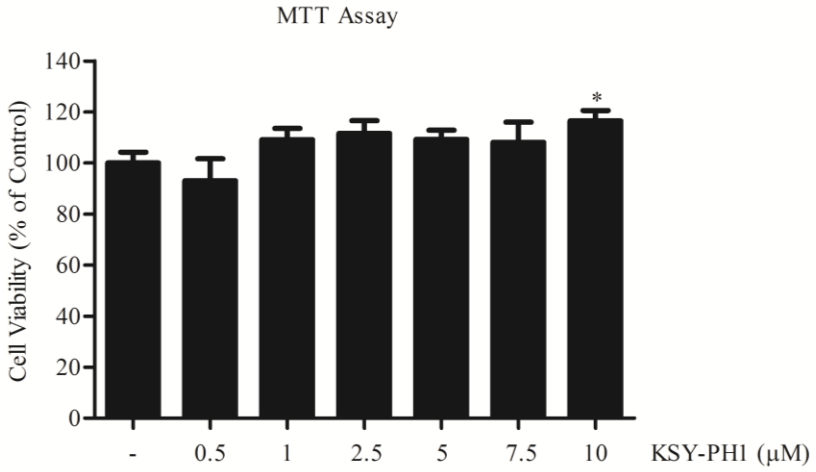




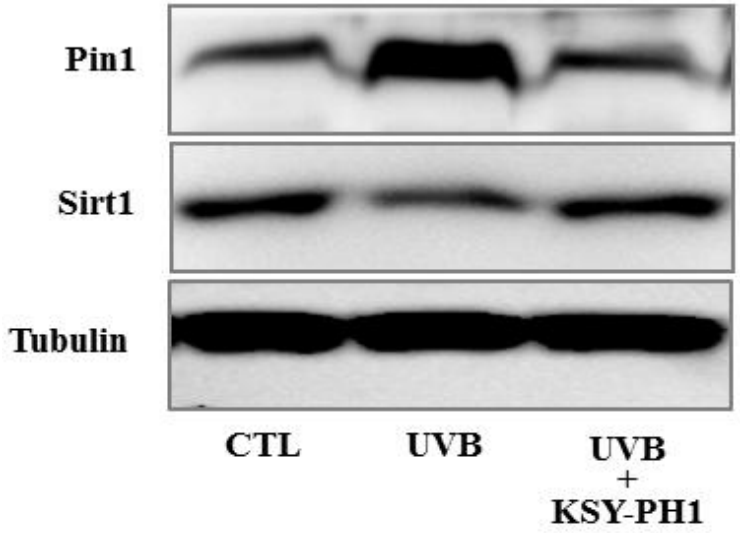
4. KSY-PH1 Enhances Wound Healing *In Vitro*

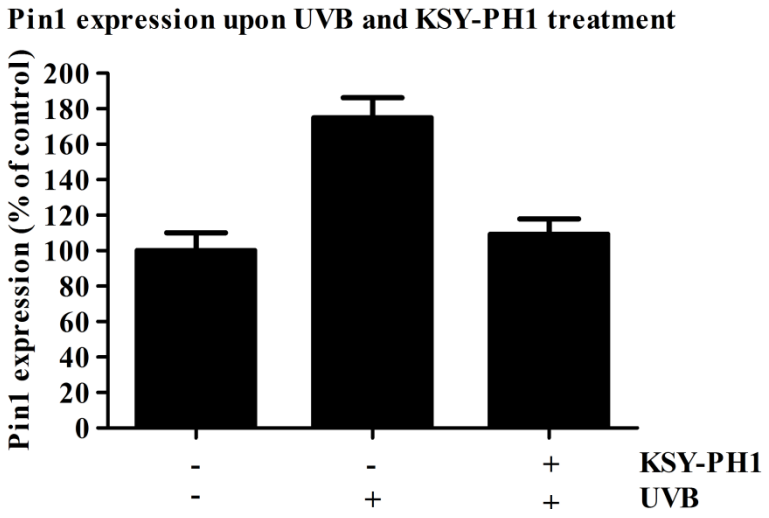


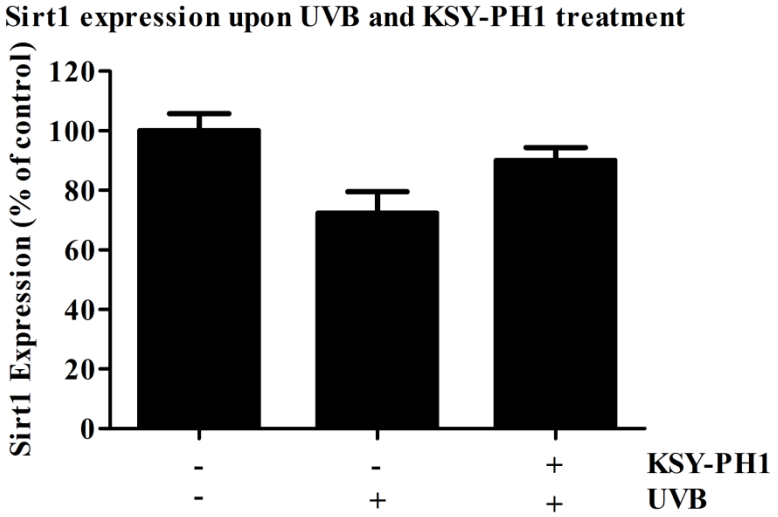




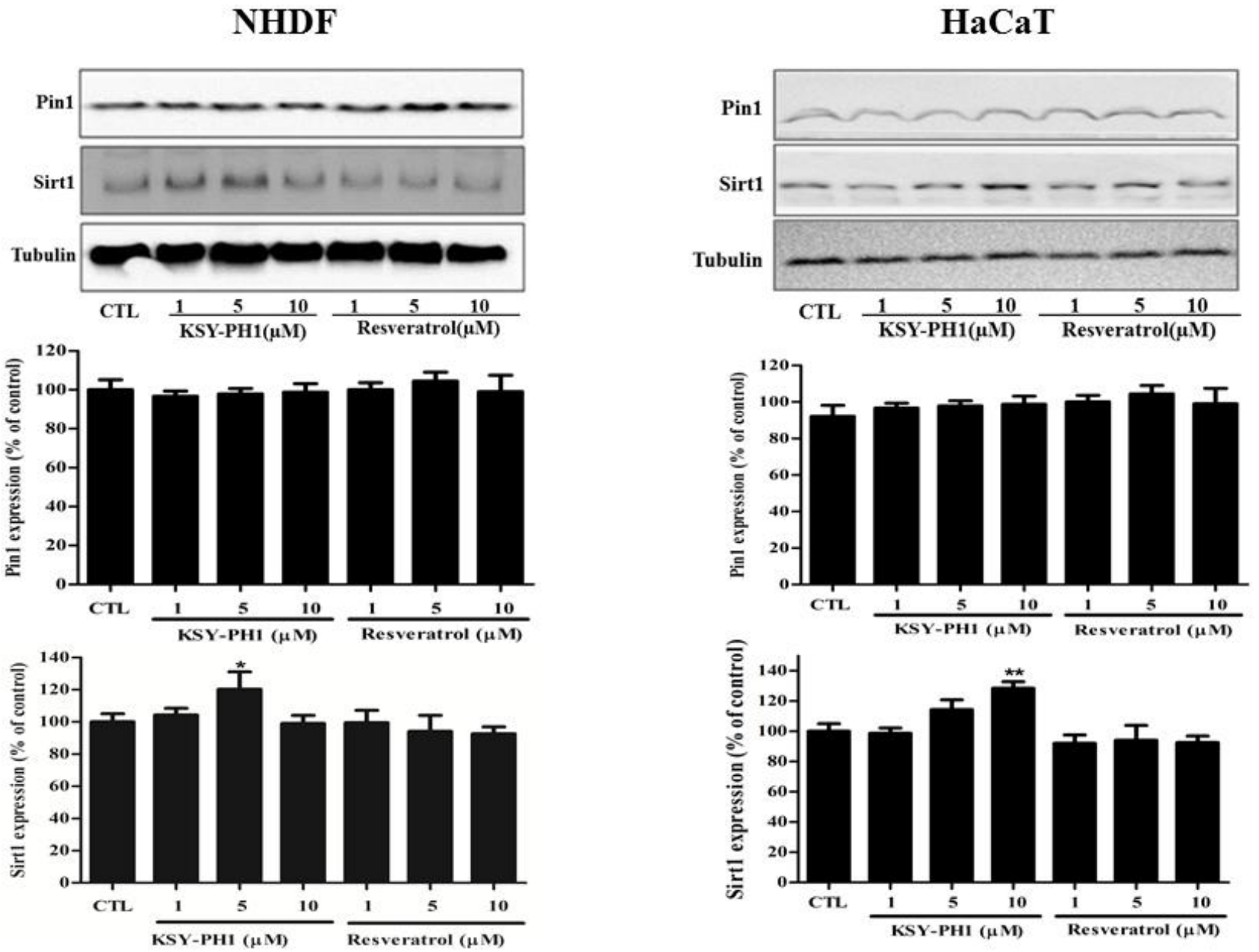
5. KSY-PH1 Restores Pin1 and Sirt1 Expression in UVB Treated Skin Cells



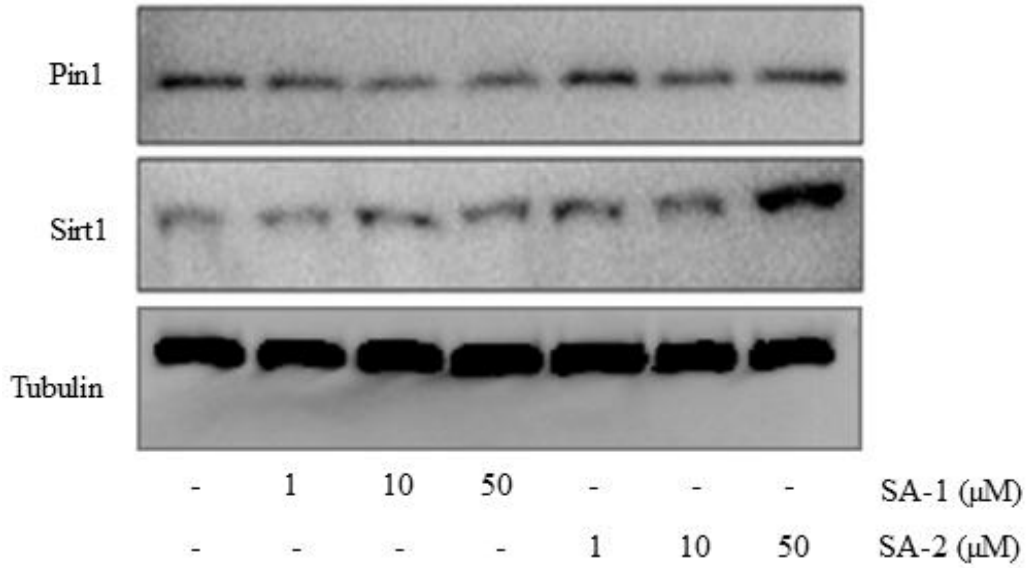


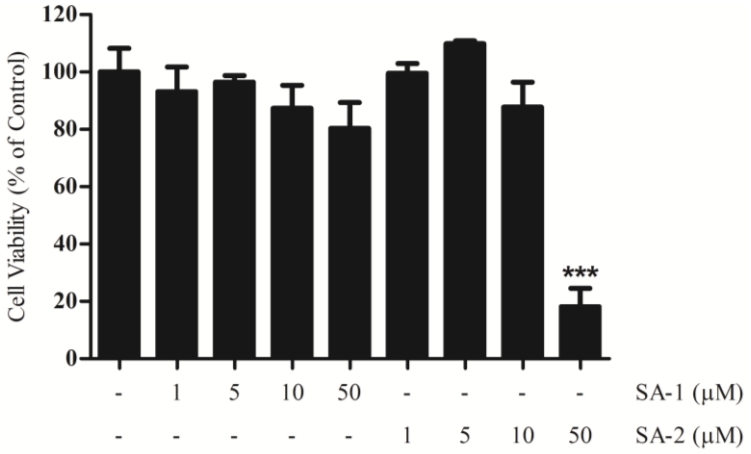


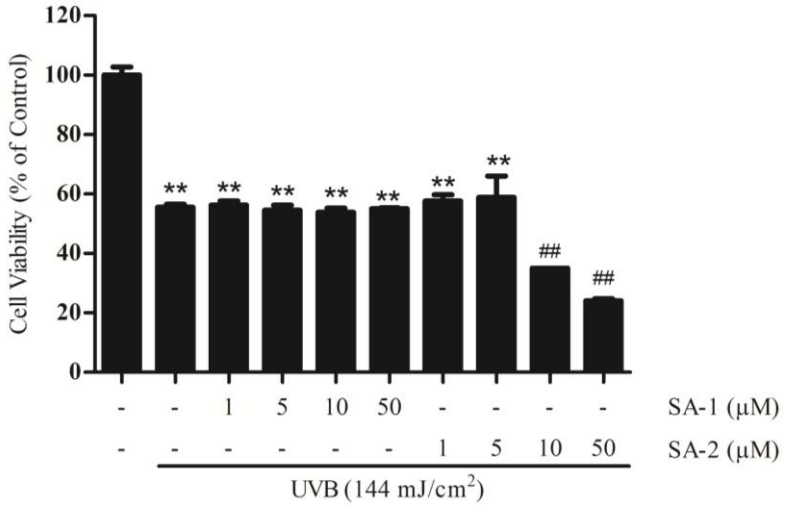
6. KSY-PH1 Up-regulates Sirt1 in Skin Cells



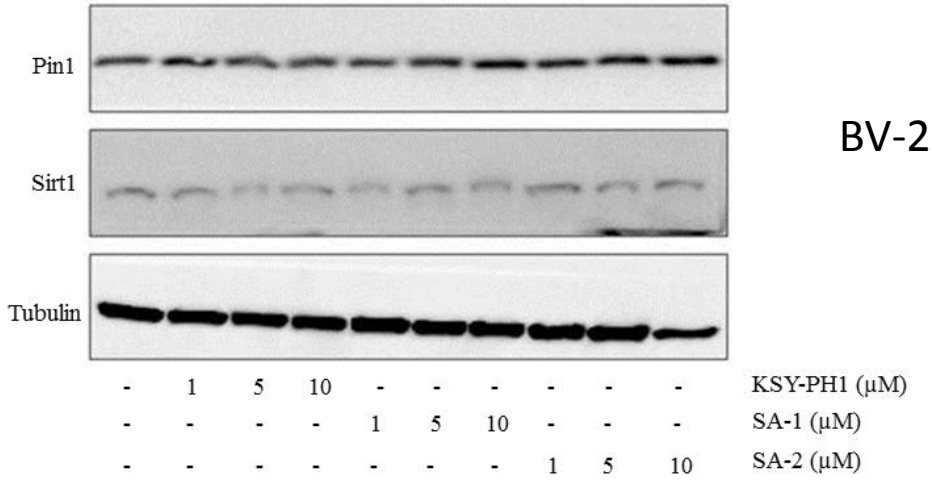
7. KSY-PH1 is More Effective Than Its Structural Analogues



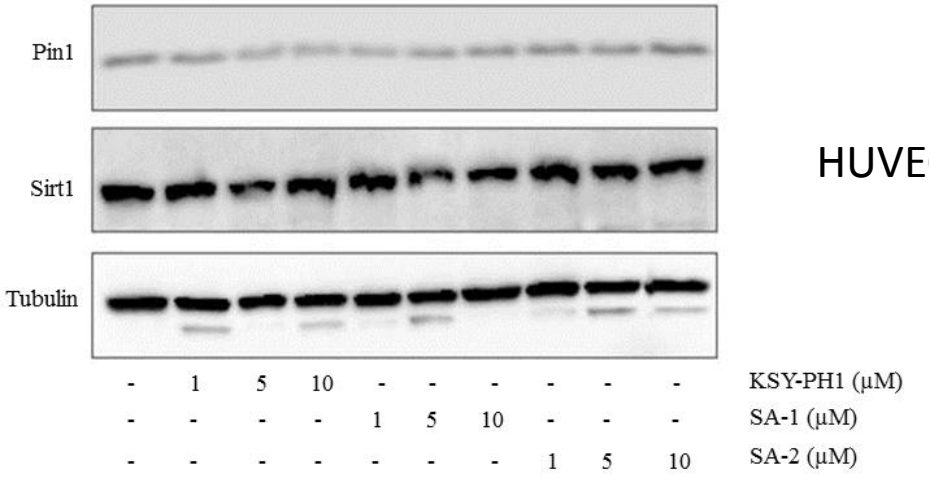




8. KSY-PH1 Acts As Sirt1 Activator Predominantly in Skin Cells

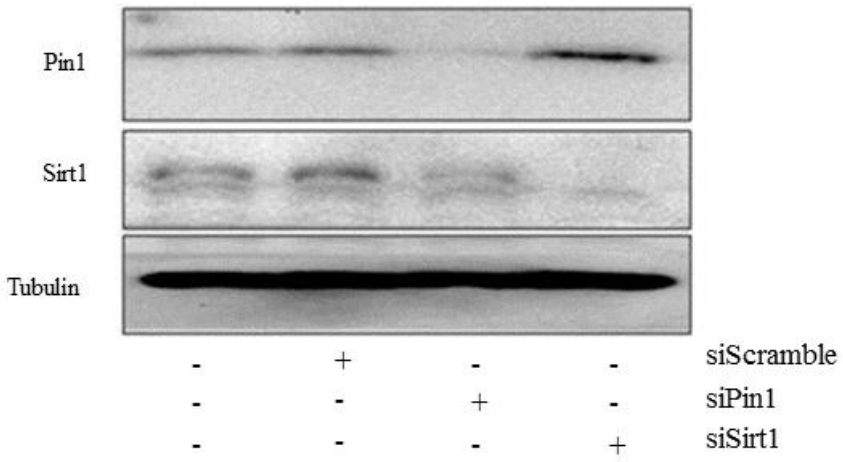


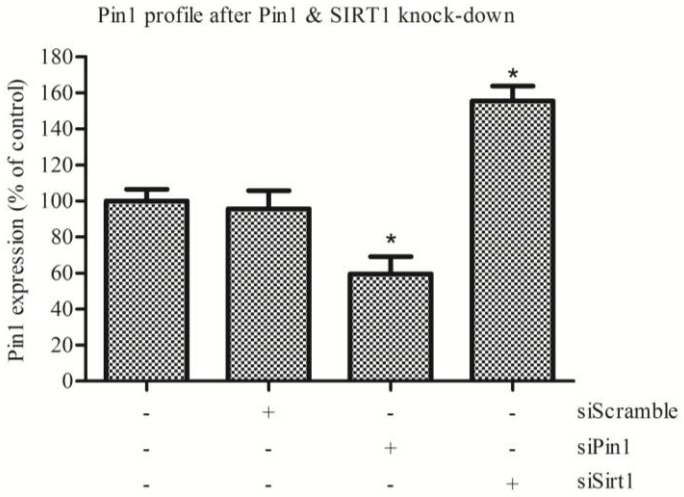
BV-2 Cells

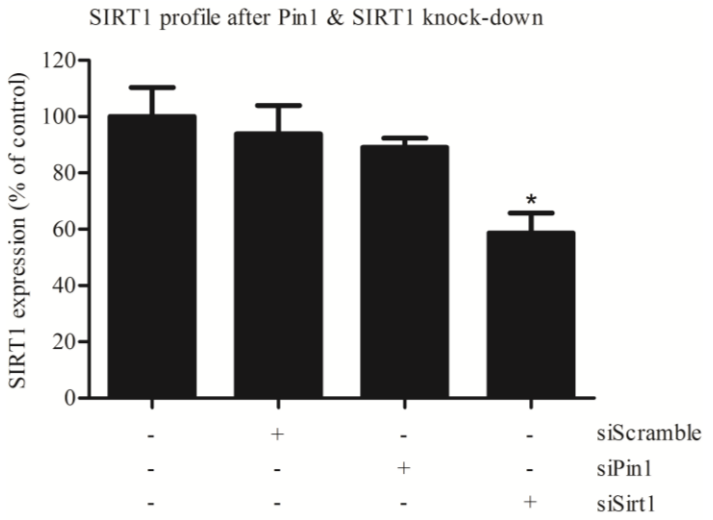


HUVEC Cells

9. Sirt1 Suppresses the Level of Pin1 in NHDFs







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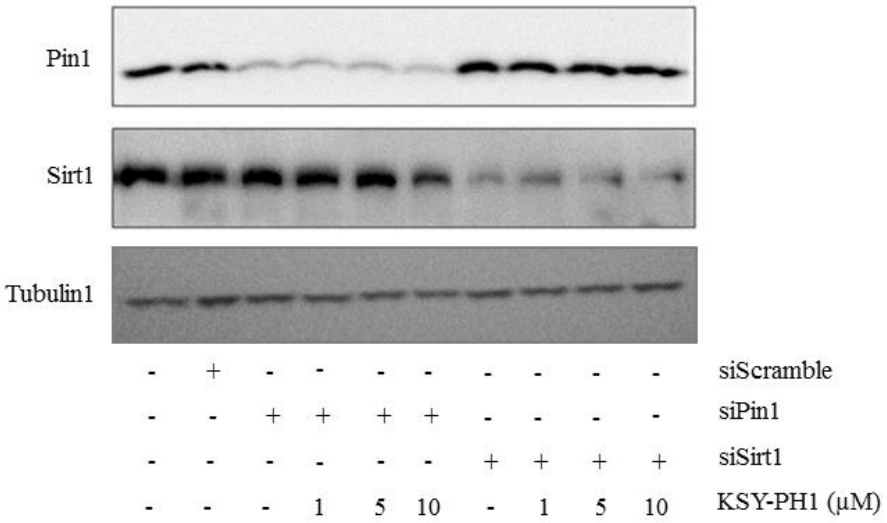

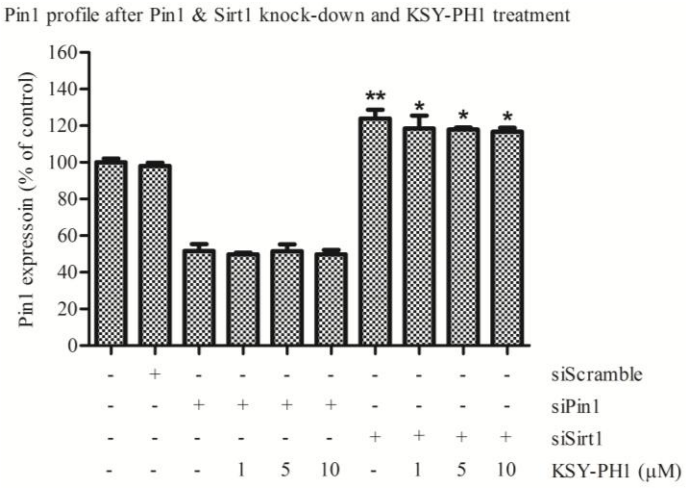

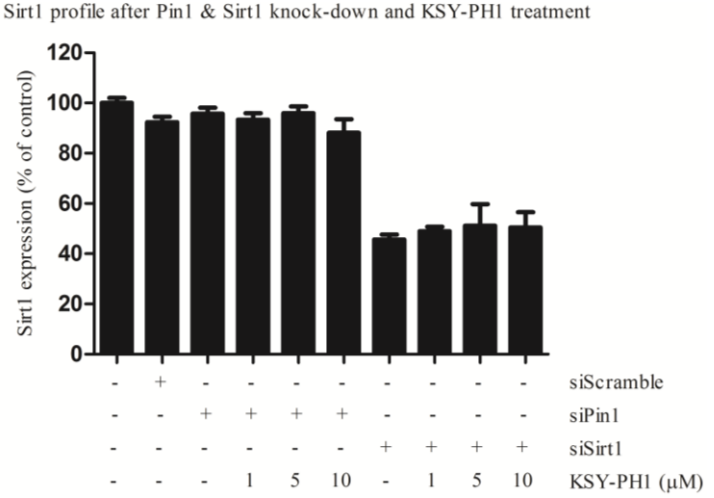

SUMMARY

- KSY-PH1 is a natural compound found in some plants which can act as a protective substance against ageing in skin cells
- KSY-PH1 promotes wound healing and rejuvenation *In Vitro* and *In Vivo*
- Being a novel activator of Sirt1, KSY-PH1 can have many important implications yet to be discovered
- Sirt1 can directly or indirectly suppress the expression of Pin1 which turns out to be its novel target

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