Nociceptive transient receptor potential canonical 7 (TRPC7) mediates aging-associated tumorigenesis induced by ultraviolet B

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Aging Cell 2019

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Introduction

- Association between TRP channels and age-related diseases (e.g. cancer, Alzheimers disease, cardio-vascular disease)
  - TRPV1 knockout mice exhibit pain insensitivity and increased longevity
  - Naked mole-rats lack TRP pain receptors - primarily youthful and healthy, cancer resistance, low ROS production
- Specific roles roles of TRP channels in aging and development of diseases unclear

TRPC7

- All TRPC channels are nociceptive mechanoreceptors, expressed on keratinocytes
  - Involved in calcium homeostasis, which is a major regulator of epidermal keratinocyte turnover, influencing their differentiation and proliferation.
  - Operated by Phosphatidylinositol second messenger system activated by Receptor Tyrosine Kinases or G-Protein coupled receptors.
  - Activated by DAG / intracellular calcium store depletion

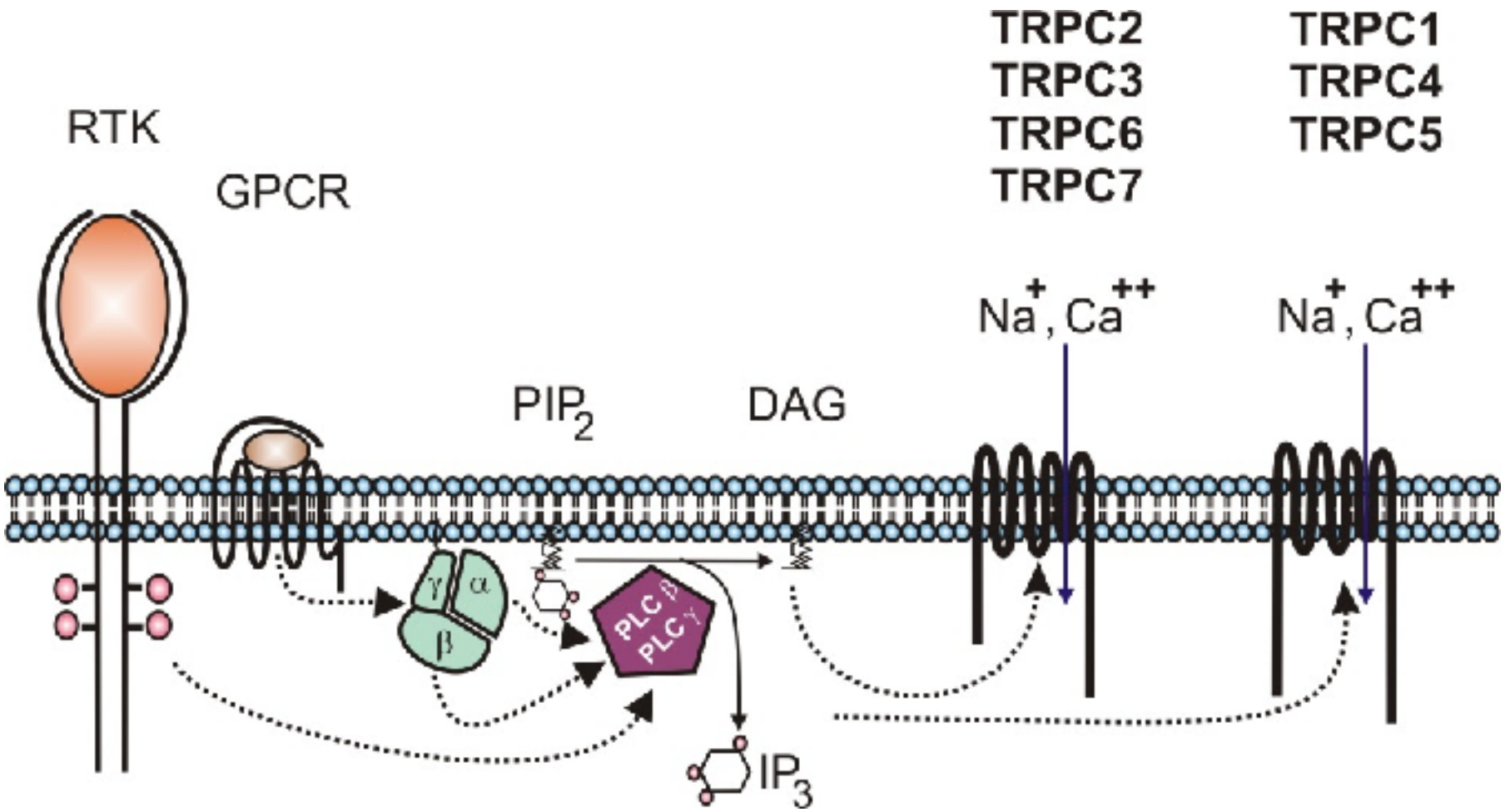
TRPC7

- Recent studies show that TRPC7 is involved in UVB-induced skin aging
  - This skin aging could be prevented by reducing the UVB-induced elevation of intracellular Ca2+ concentration.

- TRPC7 has been implicated in several (neurological, psychiatric) pathologic processes, unknown specificity of function or underlying mechanisms

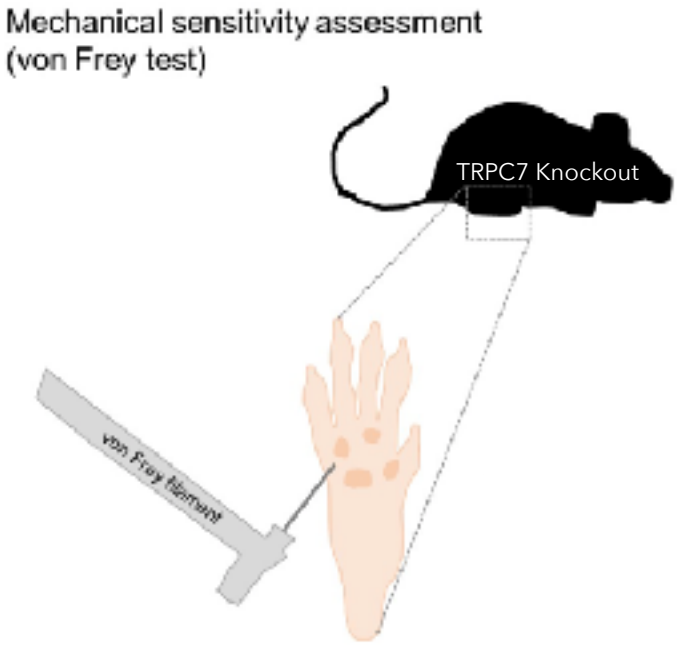
- **TRPC7 function in skin?**

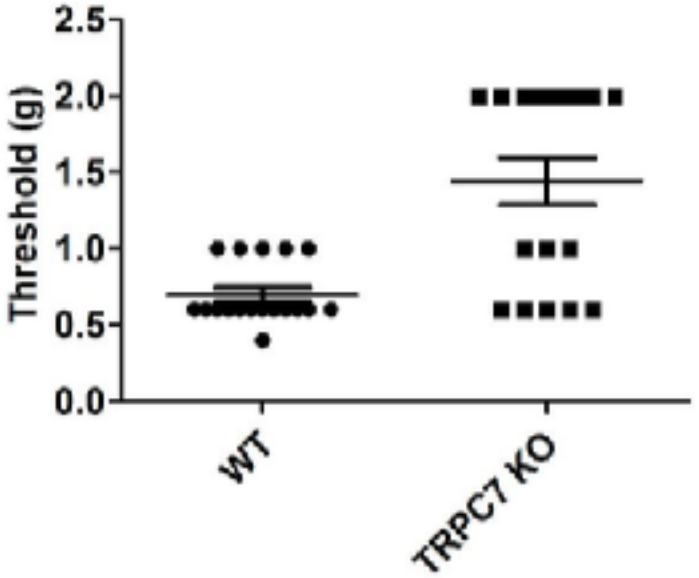
TRPC7

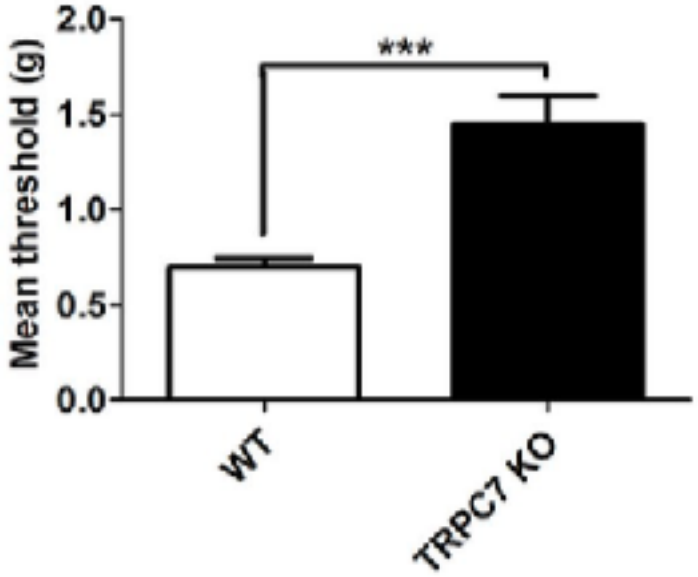

TRPC2
TRPC3
TRPC6
TRPC7
TRPC1
TRPC4
TRPC5
RTK
GPCR
Na^+, Ca^{++}
Na^+, Ca^{++}
PIP_2
DAG
γ
α
β
PLC β
PLC γ
IP_3

UVB-induced Ca2+ elevation is due to nociceptive mechanoreceptor TRPC7 in keratinocytes.

Physiologic role of TRPC7 in skin

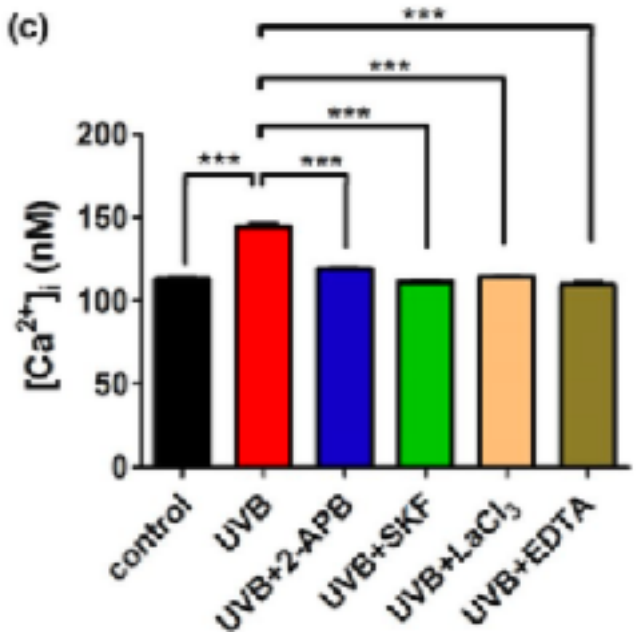






- TRPC7 knockout mice
- Von Frey filaments to assess mechanical hyperalgesie

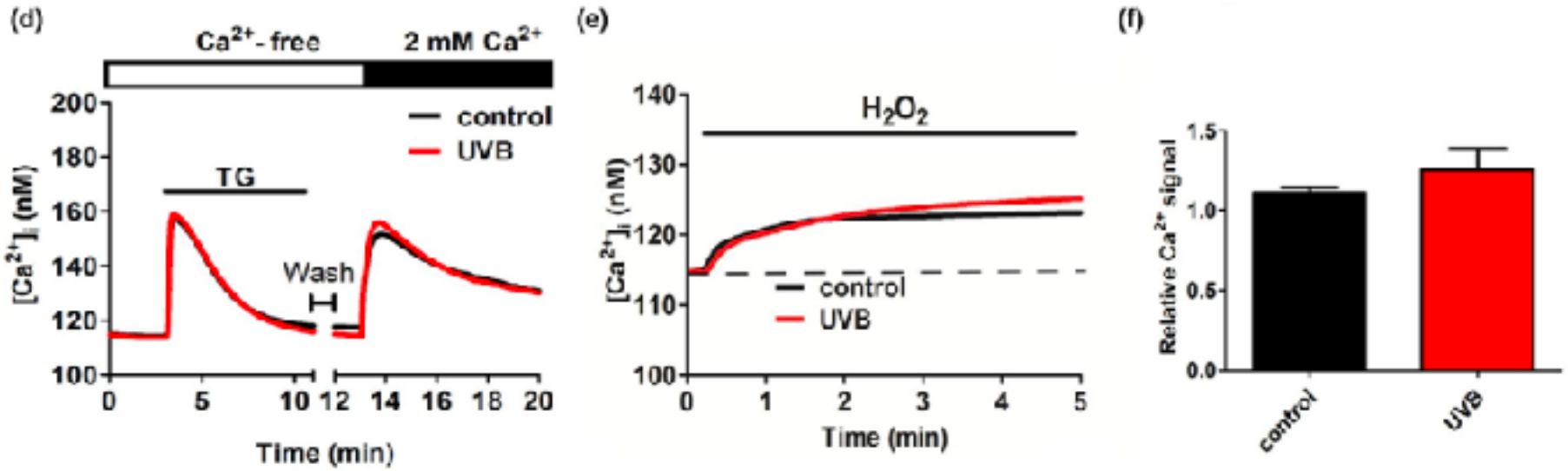
UVB induced Ca2+ elevation in keratinocytes



TRPC Inhibitors
2 Aminoethoxydiphenyl borate
SKF96365
LaCl3

- TRPC is the specific TRP channel involved in UVB-induced Ca2+ elevation
- Cultured human keratonocytes - UVB induced Ca2+ elevation attenuated pretreatment with TRPC inhibitors

Involvement of other TRP channels

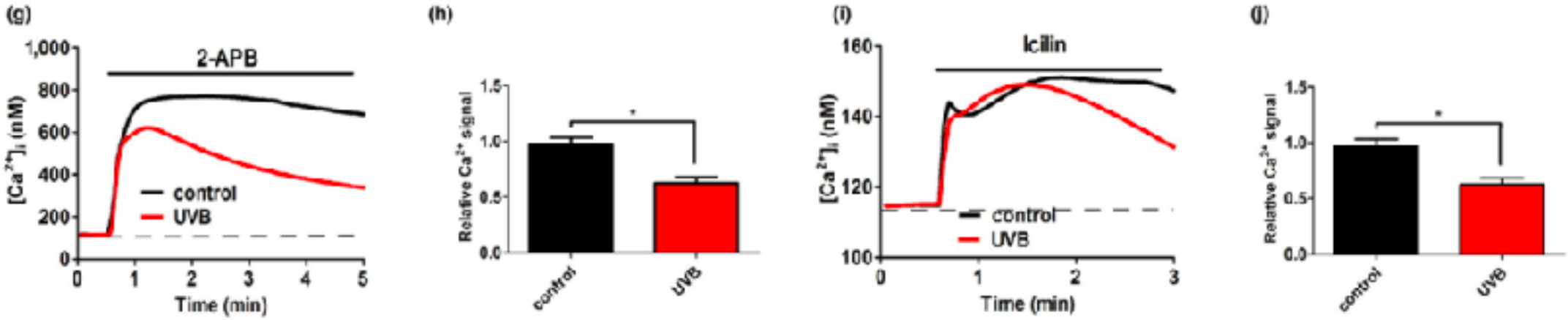


- Thapsigargin raises cytosolic (intracellular) calcium concentration (Store-depletion)

TRP Agonists	
TRPV6	Ca^{2+}, PIP2
TRPM2	Ca2+, H2O2…

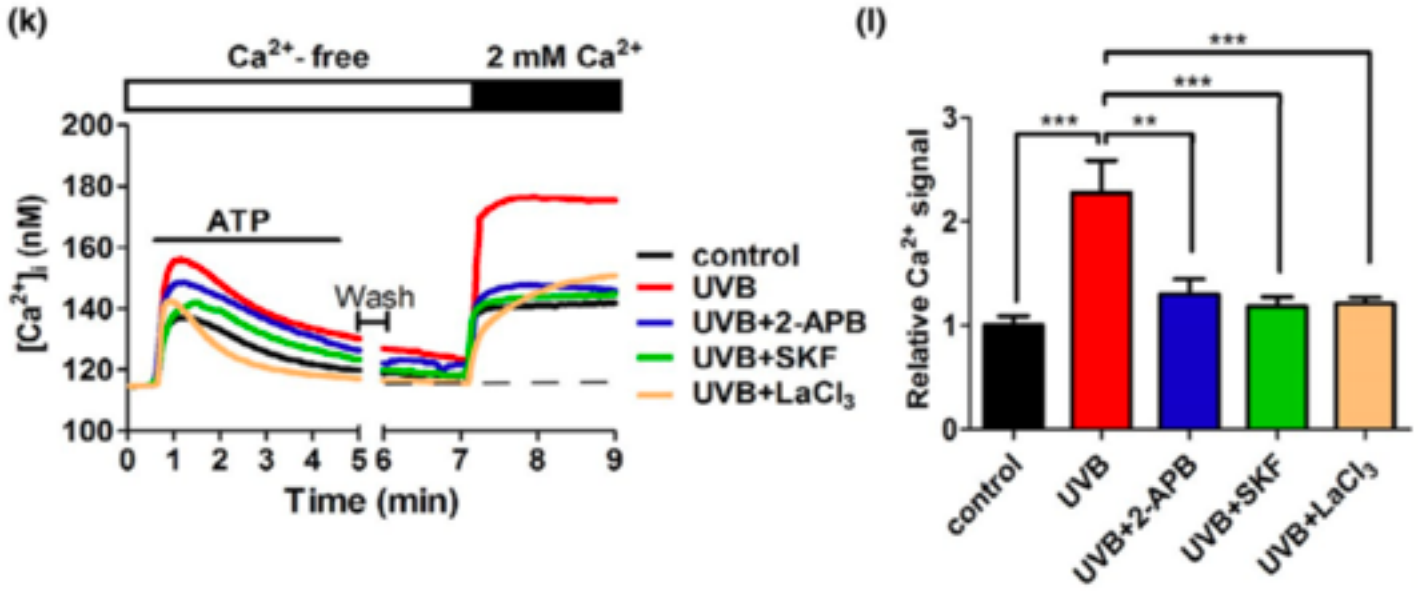
- TRPV6 & TRPM2 are not involved in UVB induced Ca2+ elevation

Involvement of other TRP channels



TRP Agonists	
TRPV1	2-APB
TRPV3	
TRPM8	Icillin
TRPA1	

Involvement of other TRP channels

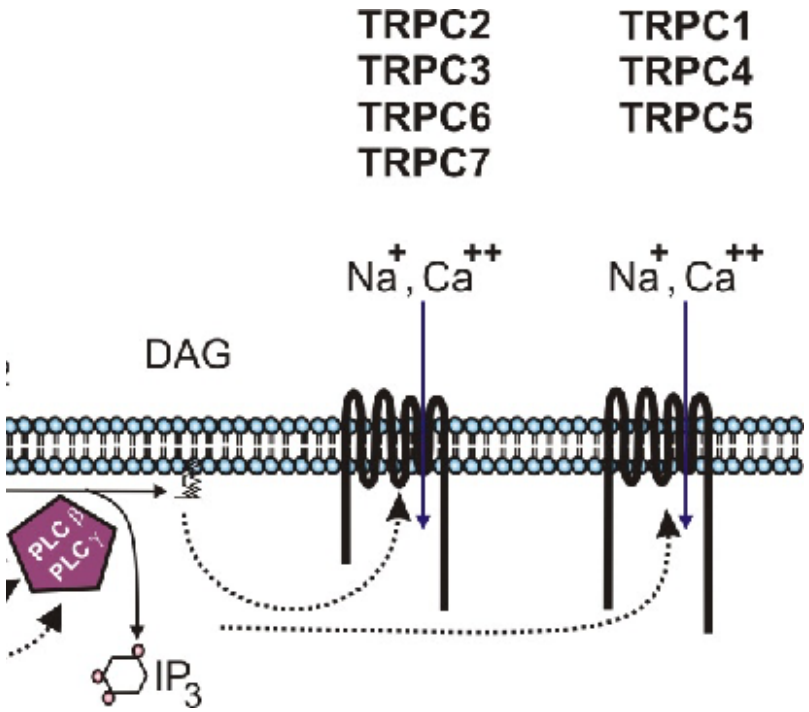


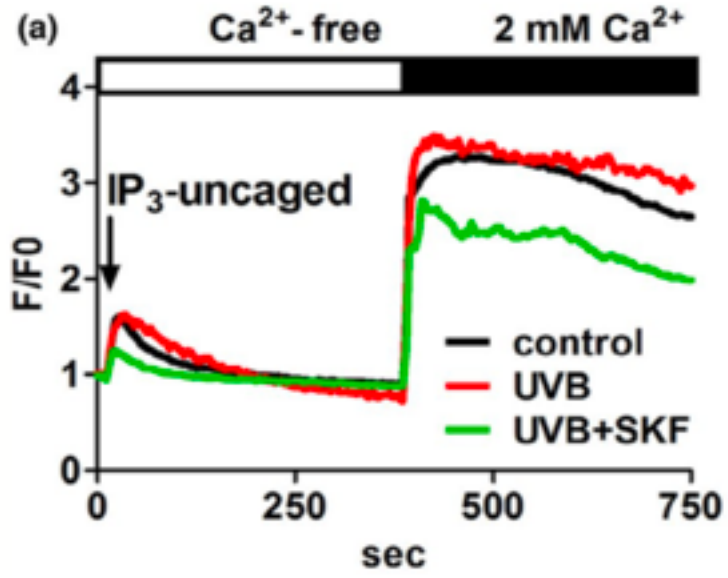
- ATP-induced Ca2+ mobilization via Phospholipase C pathway resulted in greatest extracellular influx of Ca2+ after UVB irradiation
- **TRPC specifically important in the initial stages of UVB induced Ca2+ elevation.**

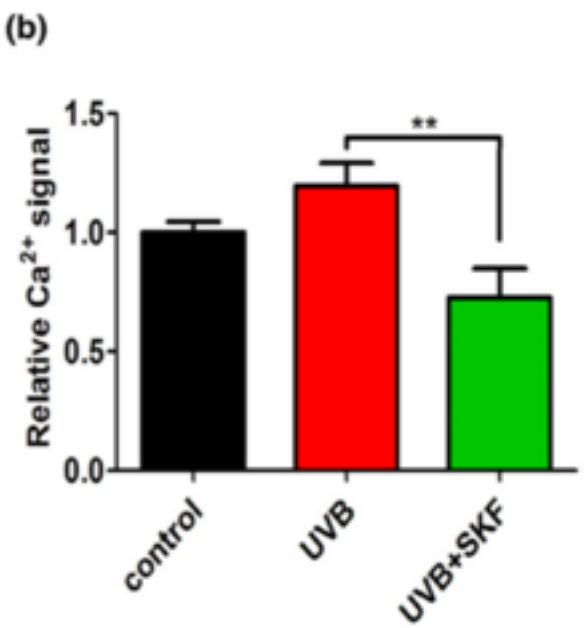
TRPC Agonists/Antagonists	
ATP (Phospholipase C Pathway)	Agonist
2-APB, SKF, LaCl3	Antagonist

UVB-induced ROS production specifically results from TRPC7-mediated Ca2+ influx

TRPC-mediated Ca2+ influx

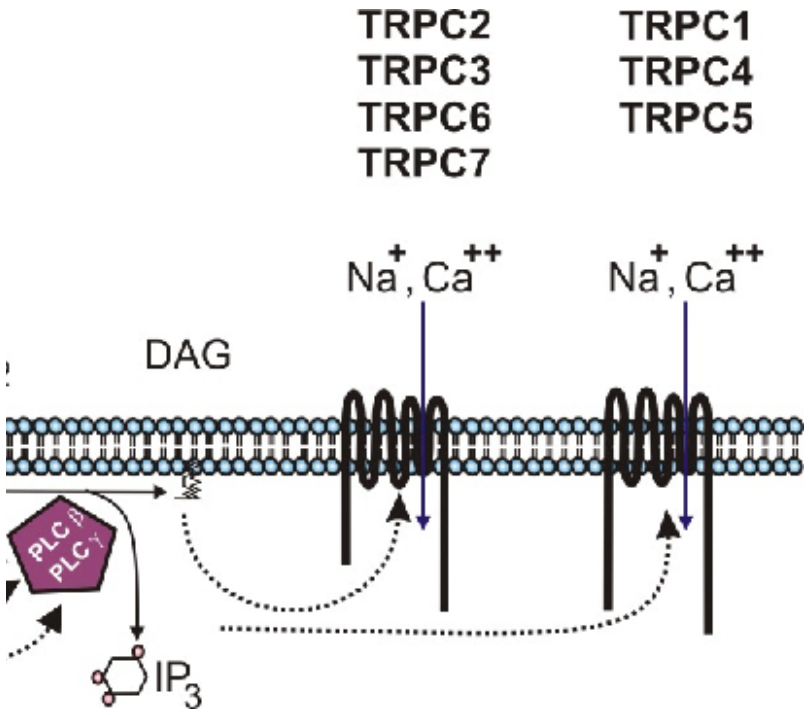


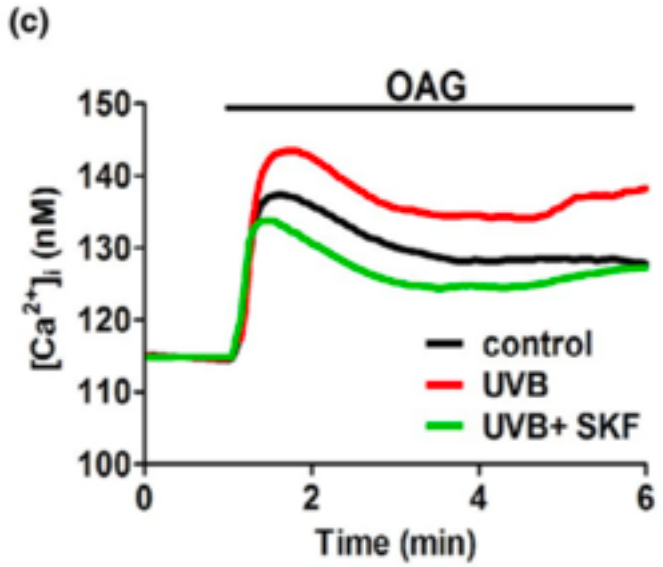


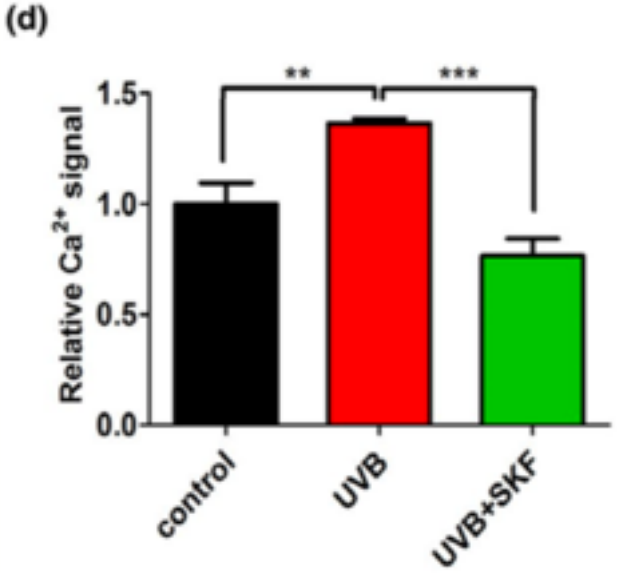


- No difference was observed in uncaged IP3 mediated activation of TRPC1,4,5 and subsequent Ca2+ influx in keratinocytes in the presence or absence of UVB pre exposure
- SKF positive-control: decreased UVB induced Ca2+ elevation

TRPC-mediated Ca2+ influx

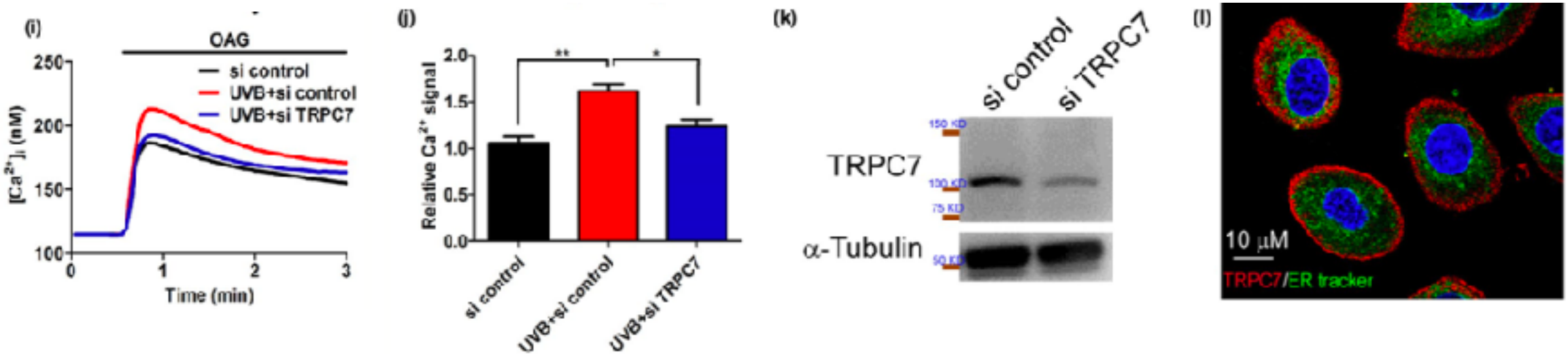






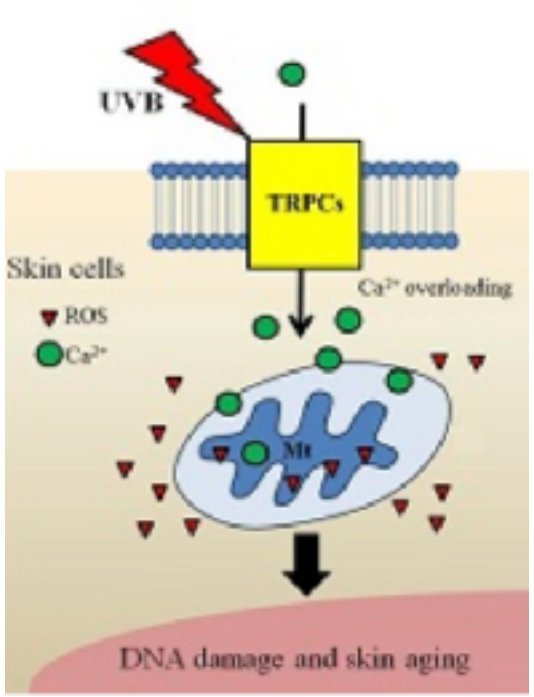
- UVB pre-exposure induced an increase in Ca2+ influx in the presence of the DAG analogue, OAG
- Effect was inhibited by SKF
- —> Channels most likely involved in UVB induced elevation are TRPC6 and 7.

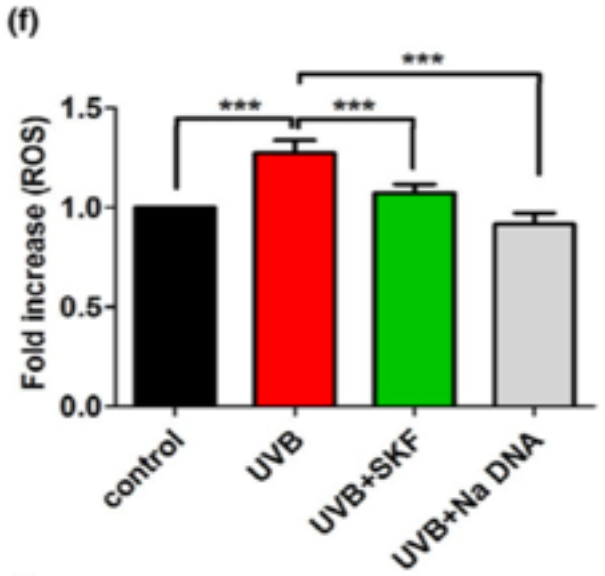
UVB induced Ca2+ elevation triggers ROS generation



- The knockdown of TRPC7 significantly decreased Ca2+ influx induced by the DAG analogue OAG after UVB irradiation
- TRPC7 was localized to the plasma membrane in keratinocytes
- **UVB induced Ca elevation results from the influx of extracellular Ca2+ and not the mobilization of Ca2+ from intracellular stores and that this specifically occurs through TRPC7.**

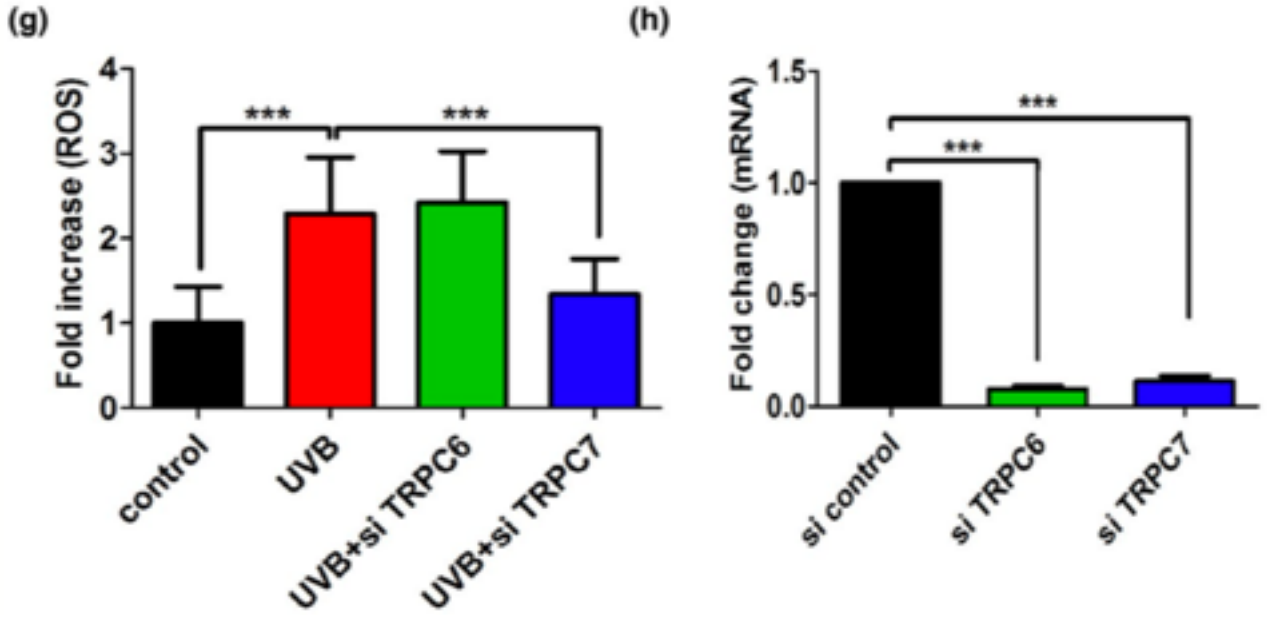
UVB induced Ca2+ elevation triggers ROS generation





- UVB-induced Ca2+ elevation via TRPC7 channels initiated intracellular reactive oxygen species (ROS) production in keratinocytes
- ROS production began within 30 minutes after UVB irradiation in keratinocytes

UVB induced Ca2+ elevation triggers ROS generation

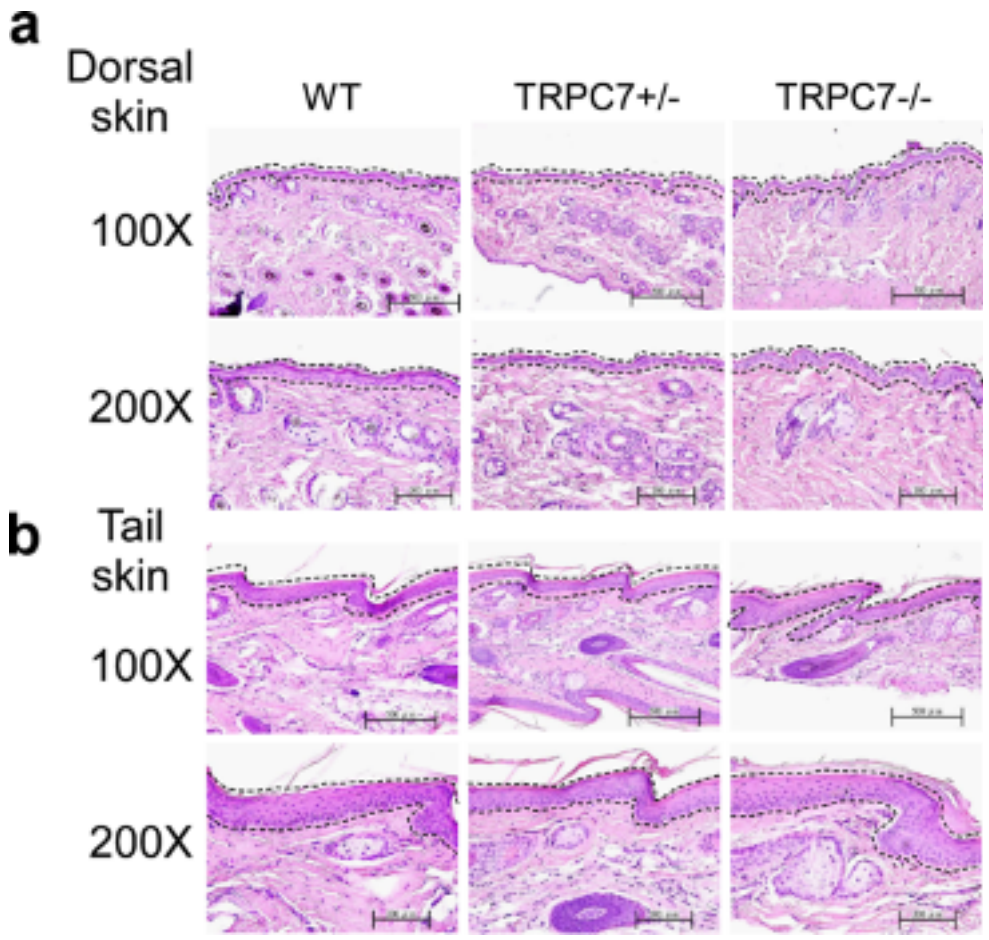


- Knockdown of TRPC7 but not TRPC6 with siRNA significantly inhibits UVB induced ROS production in keratinocytes

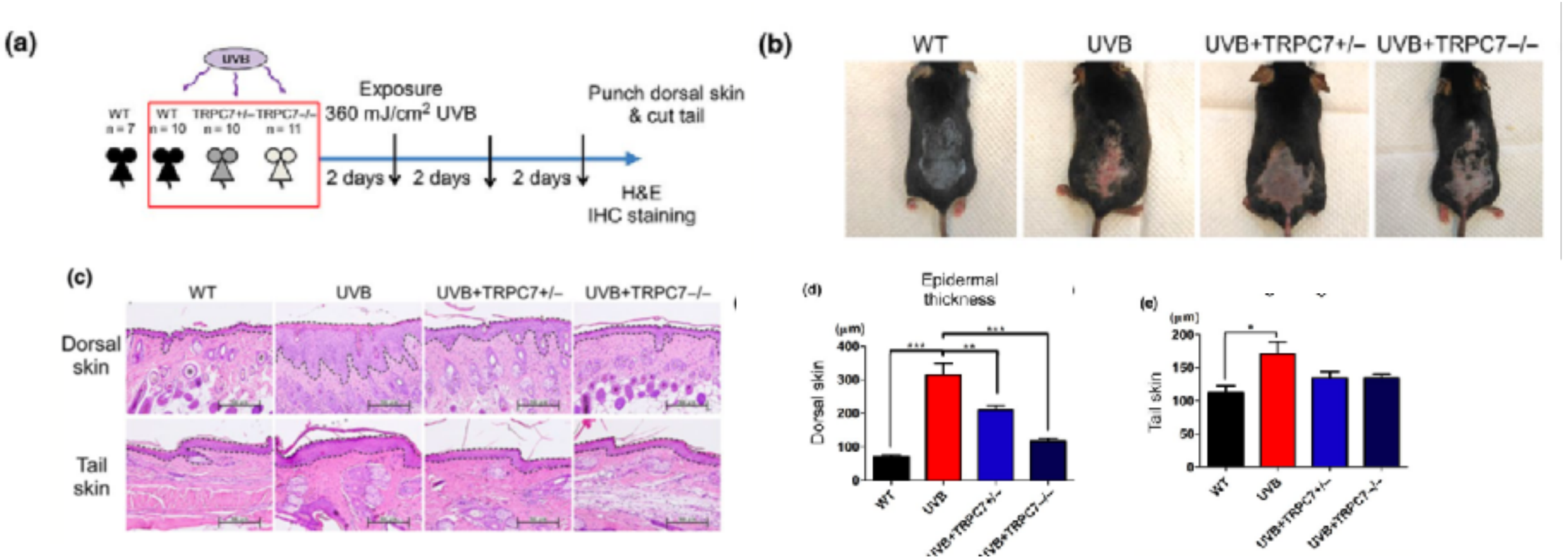
TRPC7 mediates UVB-induced epidermal pathology in mice

Ca2+ elevation via TRPC7 initiates cell senescence

- TRPC7+/– and TRPC7–/– knockout mice
- Does UVB induced Ca2+ elevation via TRPC7 initiates cell senescence through oxidative stress and activation of the DNA damage response (DDR) leading to abnormal differentiation and epidermal aging?
- After hair removal for 8 days, (a) dorsal skin and (b) tail skin of WT, and KO mice were punched and sectioned for staining
- In absence of UVB, skin phenotype was similar between wild type and knockout mice



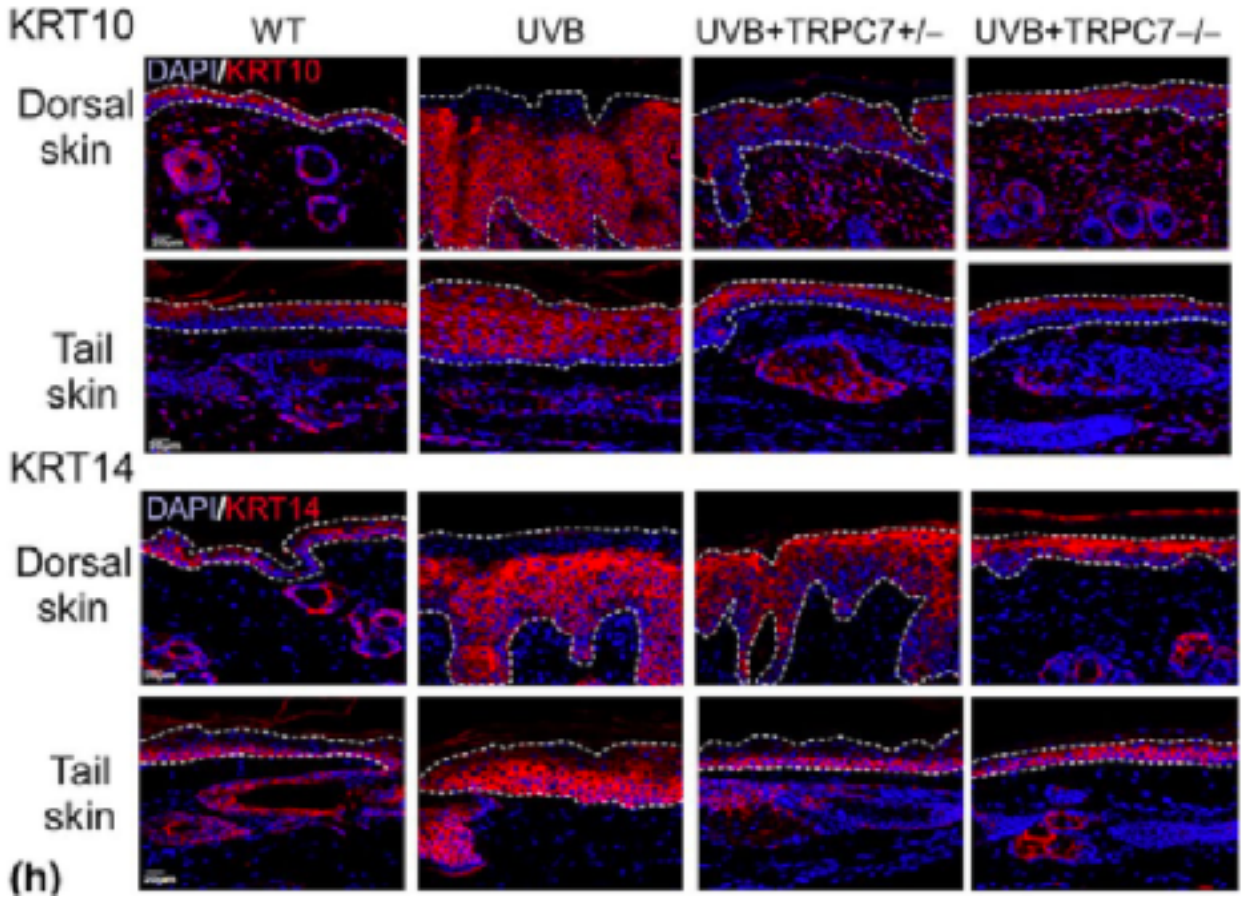
Ca2+ elevation via TRPC7 initiates cell senescence



- In WT mice, UVB exposure induced severe desquamation and erythema of the skin, however, in knockout mice UVB induced slight or no damage and less epidermal thickening than wild type mice

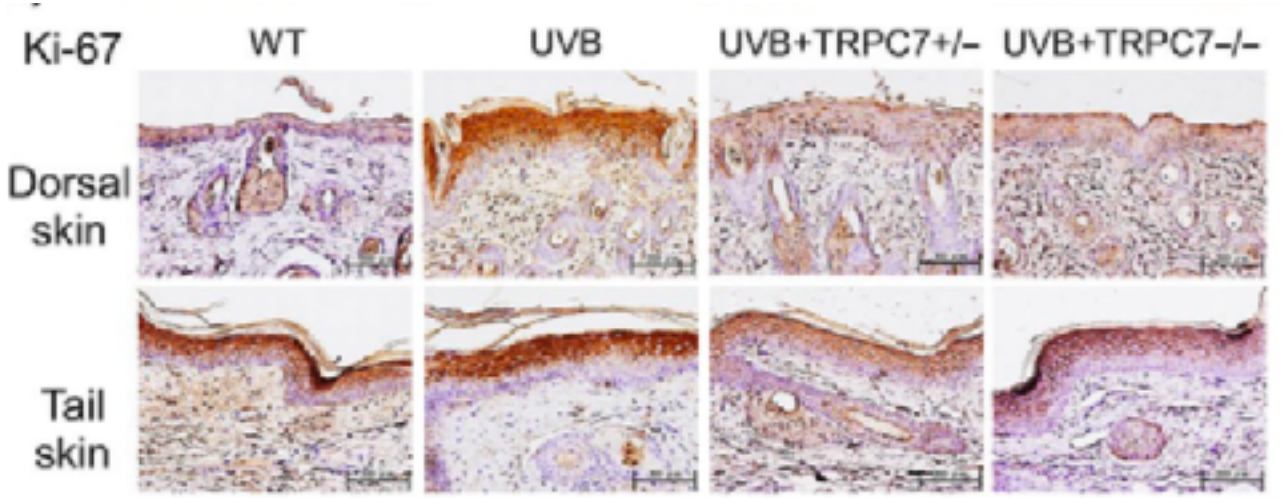
Ca2+ elevation via TRPC7 initiates cell senescence

- UVB exposure increased the expression of epidermal differentiation markers KRT10 and KRT14, indicating the abnormal differentiation of keratinocytes
- Ca2+ is a known regulator of keratinocyte differentiation, these results indicate that the abnormal differentiation induced by UVB was initiated by TRPC7 mediated Ca2+ elevation



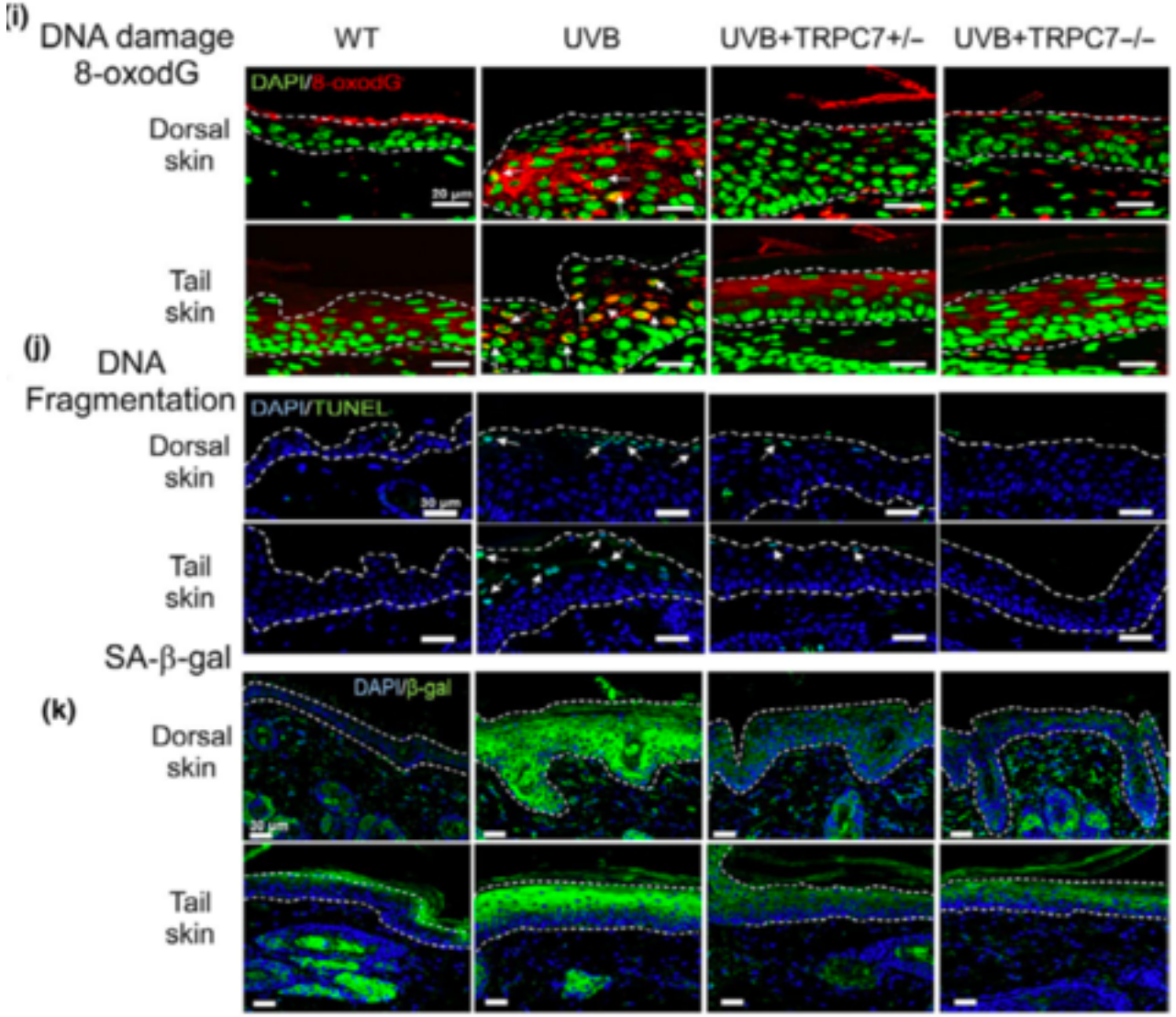
Ca2+ elevation via TRPC7 initiates cell senescence

- After UVB exposure, strong Ki-67 (proliferation marker) staining in the cytoplasm but not in the nuclei of differentiating keratinocytes
  - —> UVB may induce cell proliferation, followed by abnormal differentiation.
- The shift from proliferation to differentiation may depends on the accumulation of oxidative DNA damage.
- The repeated oncogenic stress from UVB irradiation breaks the balance between genomic instability and the DNA repair system, and increased Ca2+ signals initiate tumorigenesis through genomic instability.



Ca2+ elevation via TRPC7 initiates cell senescence

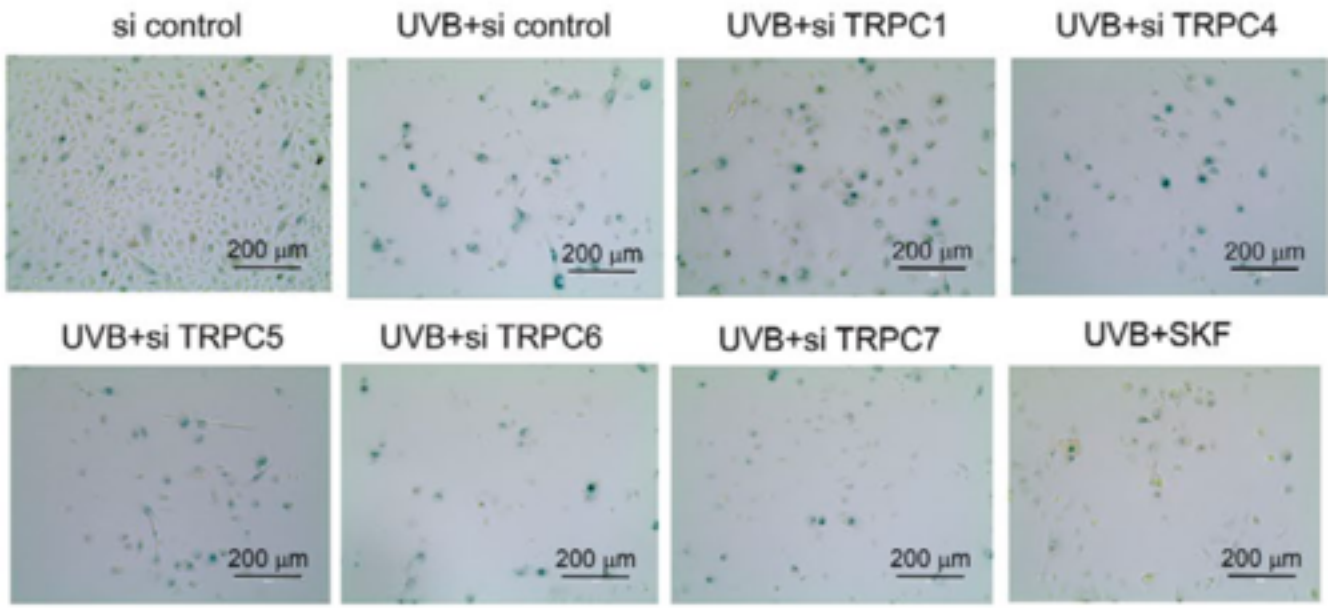
- 8-oxodG: Major marker of DNA oxidisation
- TUNEL staining: apoptotic DNA fragmentation
- After UVB exposure, TRPC7 knockout mice showed less 8-oxodG and Tunel staining in the epidermis than wild type mice, indicating reduced oxidative DNA damage and fragmentation in these mice.
- SA-β-gal staining, used to analyze cell senescence, was lower in epidermis of knockout mice than in WT mice after UVB exposure.

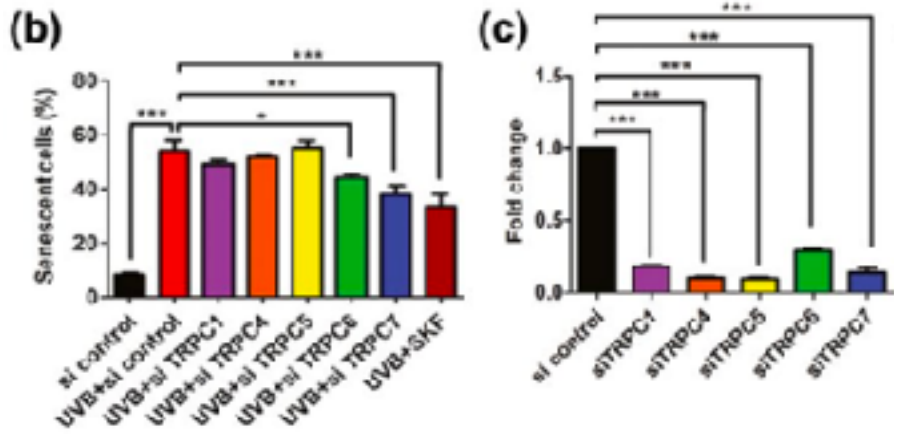


TRPC7 mediates UVB induced epidermal aging in mice

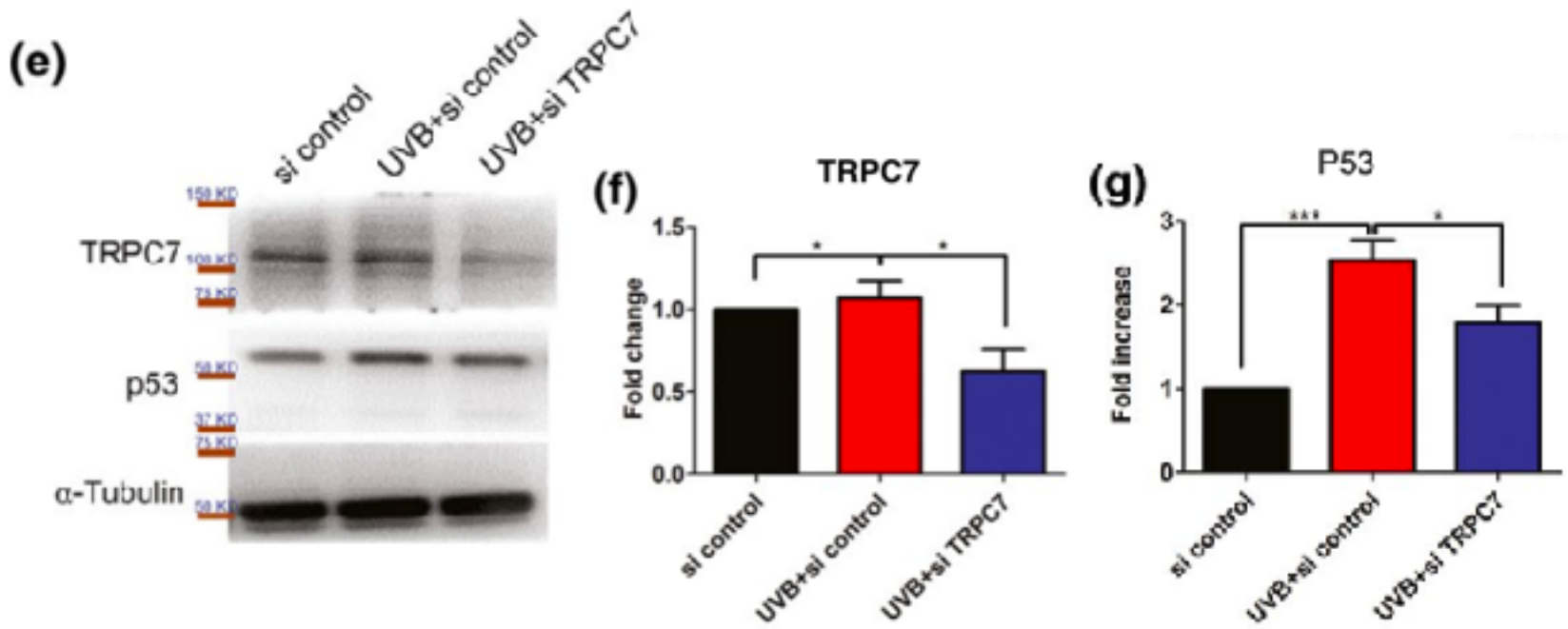
TRPC7 mediates UVB induced epidermal aging in mice

- After UVB irradiation, knockdown of TRPC7 in keratinocytes significantly decreased the percentage of senescent cells as determined by SA-β-gal staining.
- Knockdown of TRPC7 increased the keratinocyte survival ratio after exposure to UVB



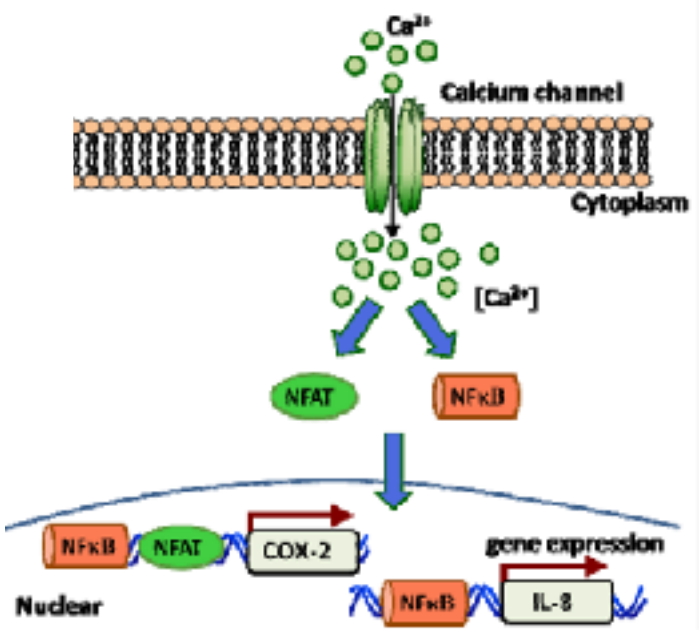


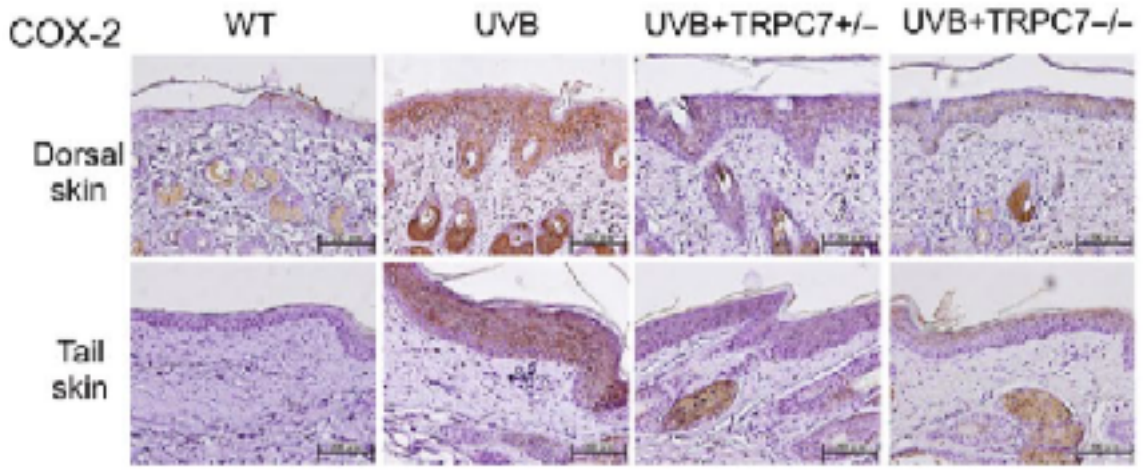
TRPC7 mediates UVB induced epidermal aging in mice



- Knockdown of TRPC7 inhibites p53 expression in keratinocytes

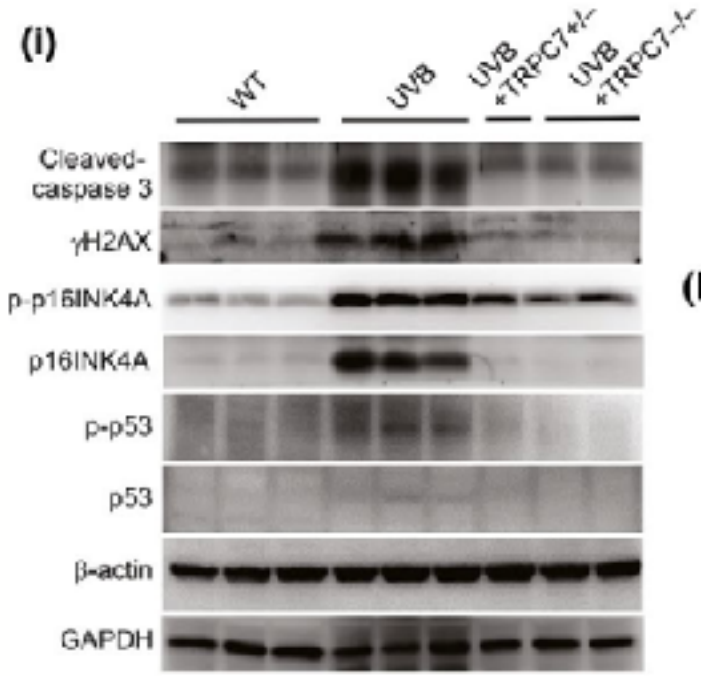
TRPC7 mediates UVB induced epidermal aging in mice

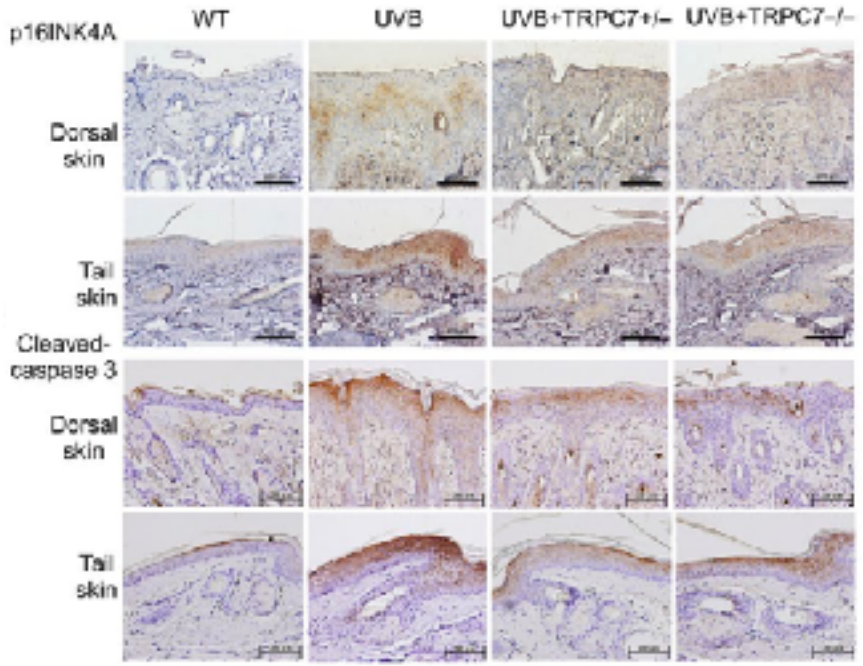




- Extracellular Ca2+ influx is known to induce COX 2 expression which is known to increase p53 pathway activation and promotes aging process.
- After UVB exposure, the high levels of COX 2 protein expression observed in the epidermis of WT mice were decreased to minimal levels in TRPC7 knockout mice.

TRPC7 mediates UVB induced epidermal aging in mice

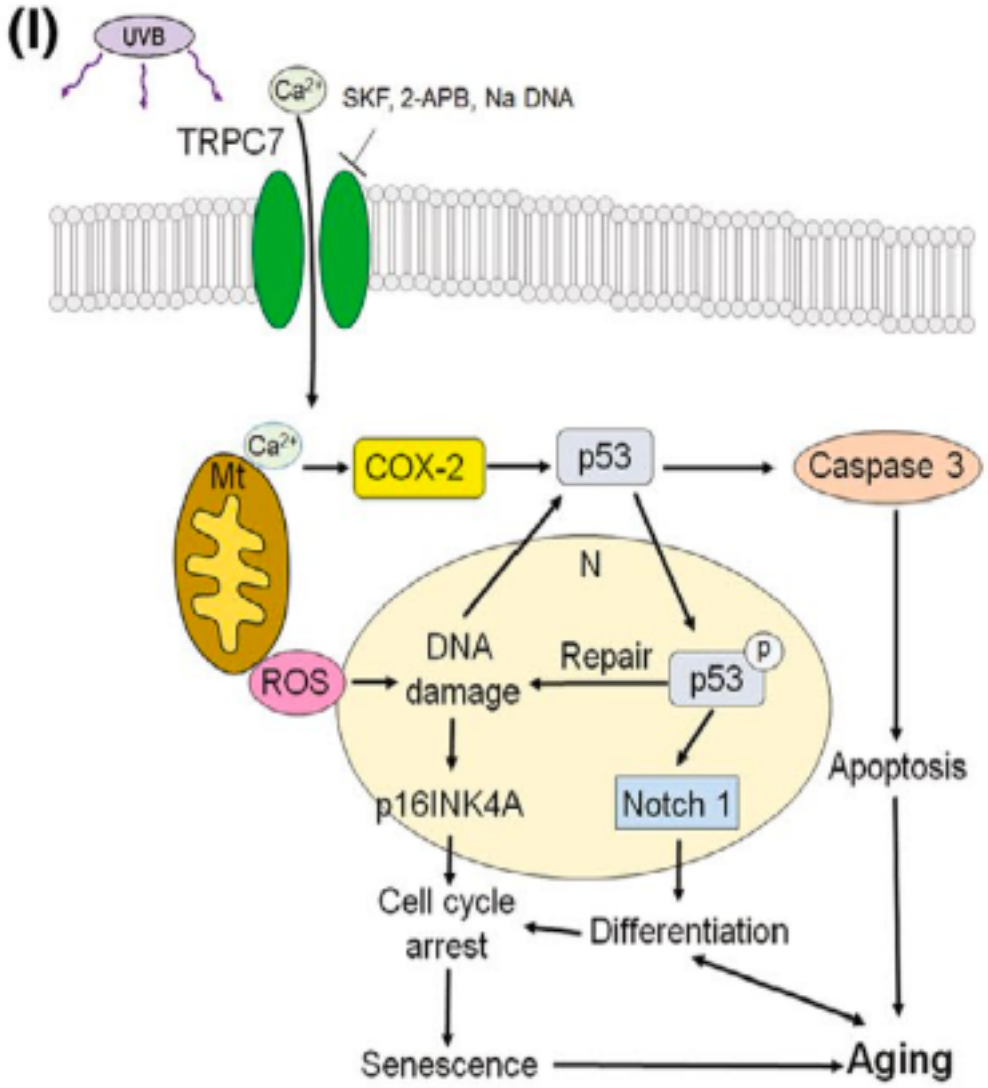




- UVB exposure increases the levels of γH2AX, p16INK4A, and cleaved Caspase-3 in the epidermis of WT mice, indicating increased senescence and apoptosis.
- Imbalance of proliferation and apotposis together with the accumulation of senescent and apoptotic cells results in cell aging.

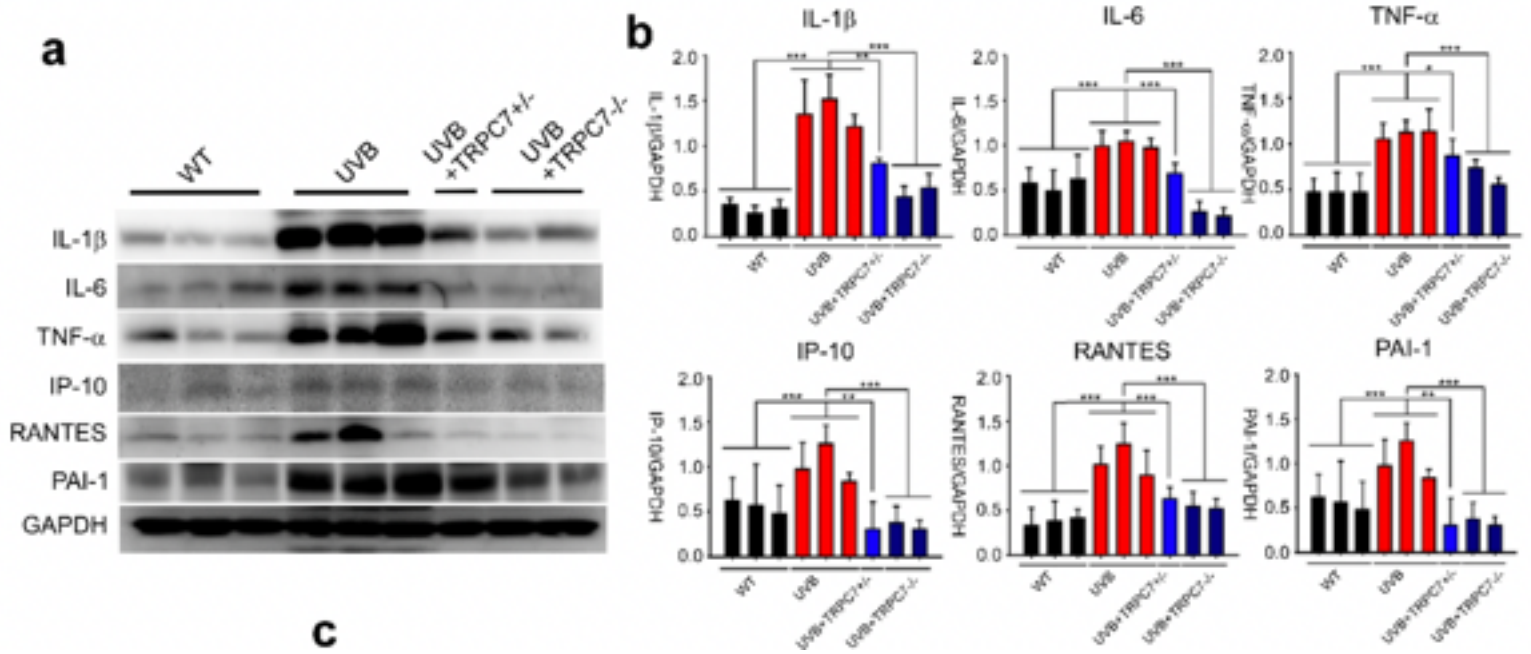
TRPC7 mediates UVB induced epidermal aging in mice

- **UVB induced epidermal aging is attentuated in TRPC7 KO mice, showing that TRPC7 is necessary for the initial increase in ca2+ and for activating the cascade of cellular processes that lead to skin aging.**



TRPC7 mediates UVB induced tumor initiation and growth

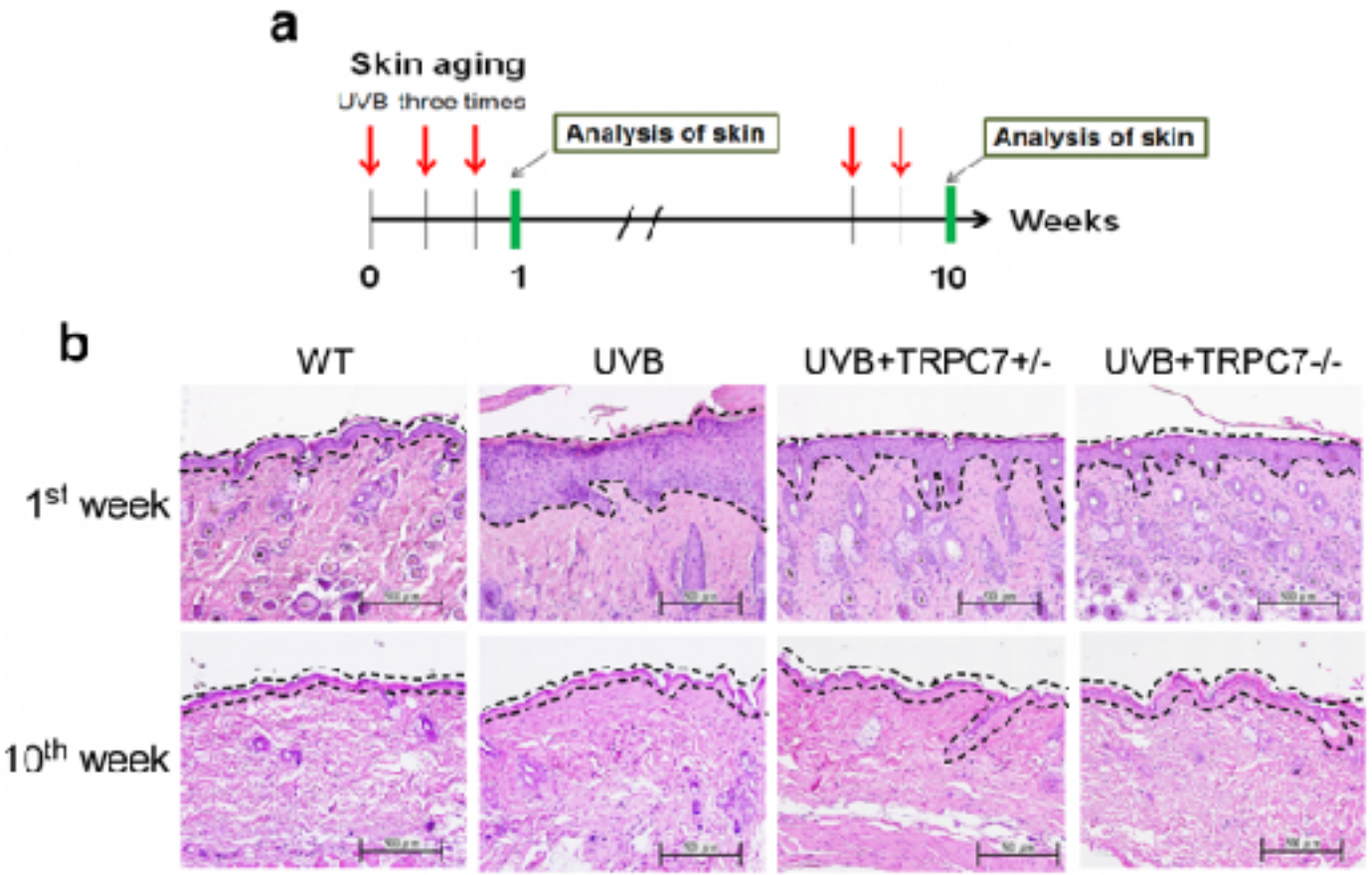
TRPC7 mediates UVB induced tumor initiation and growth



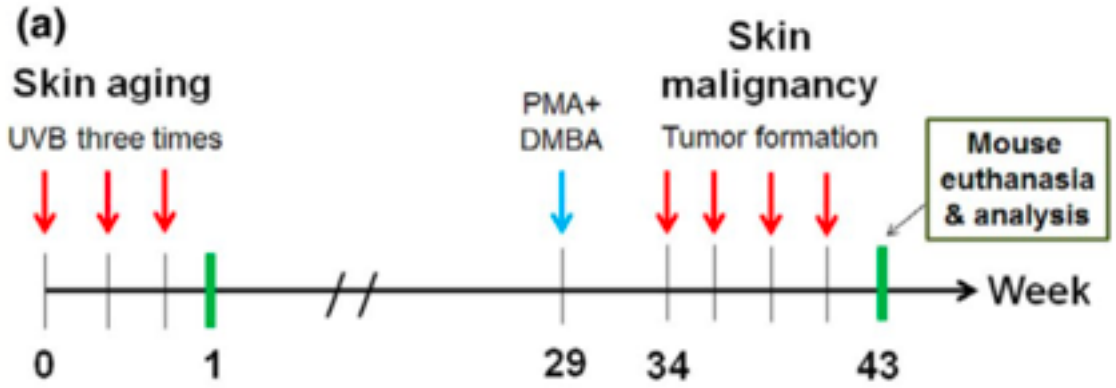
- Aging associated tumorigenesis is dependent on the activation of SASP (Senescence associated secretory phenotype) (proteins IL-1β, IL-6, TNF-α, IP-10, RANTES, and PAI-1)
- —> UVB induced SASP activation was reduced in the epidermis of TRPC7 knockout mice

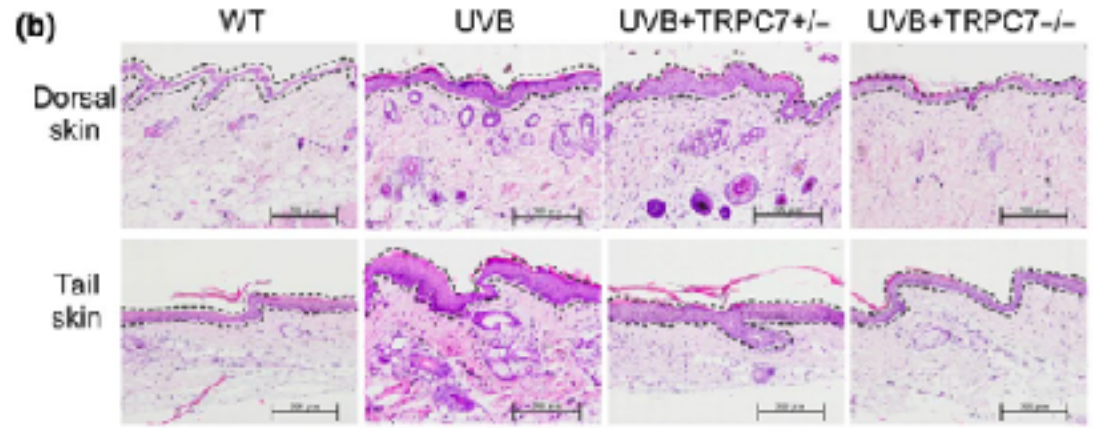
TRPC7 mediates UVB induced tumor initiation and growth

- In normal keratinocytes exposed to UVB, p53 family of proteins is activated and tissue repair is initiated
- Repeated UVB irradiation for 10 weeks and epidermal thickening at 1 week, the epidermis in all UVB exposed mice was repaired and restored to a single layer at the 10th week, resembling non irradiated skin.

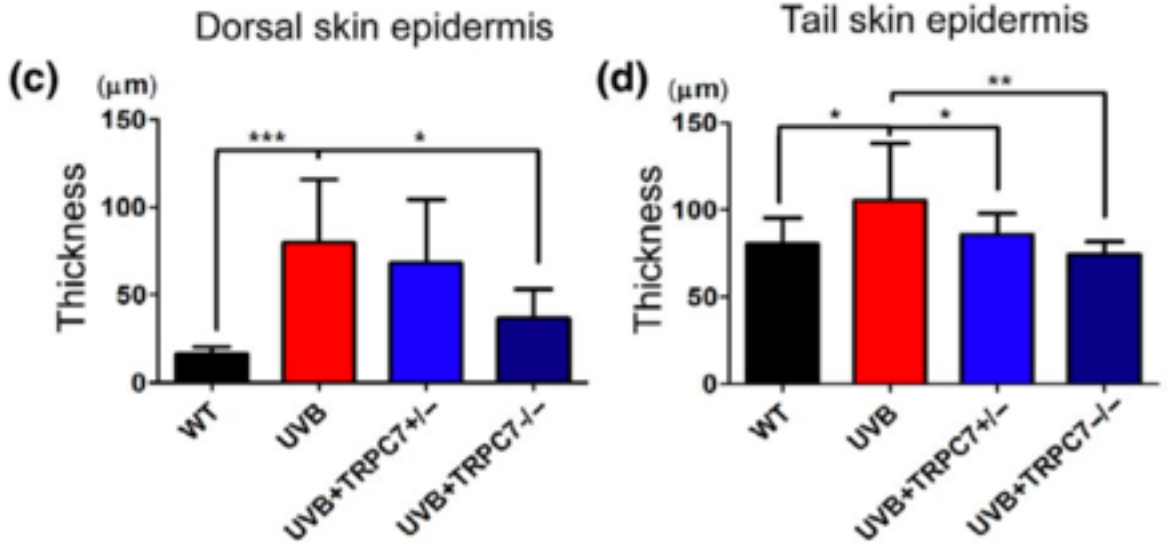


TRPC7 mediates UVB induced tumor initiation and growth

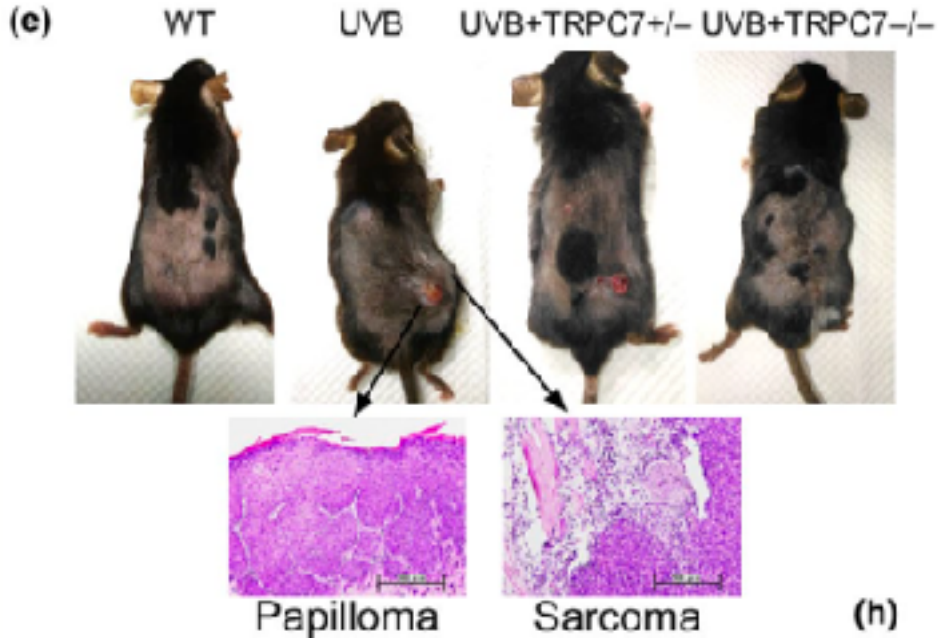


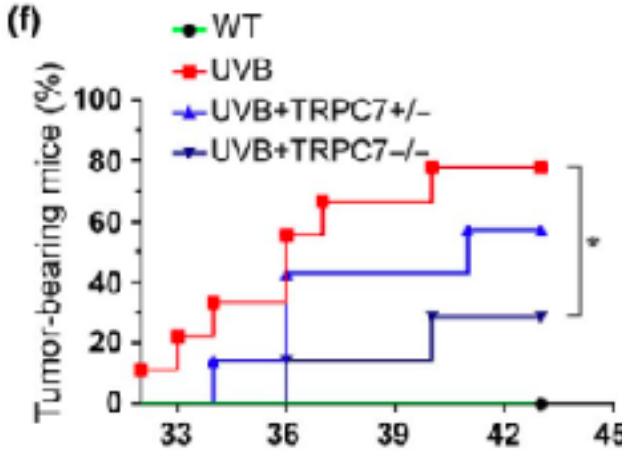


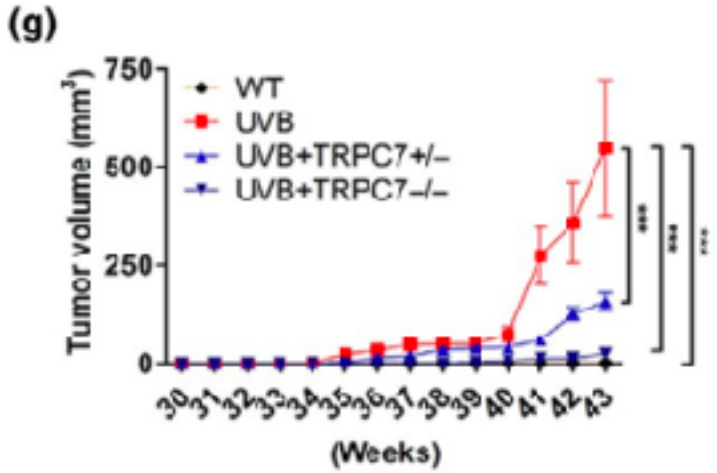
- Once the repair system no longer provides sufficient recovery from injury, the aging process begins to advance in the damaged tissue —> epidermis thickens.



TRPC7 mediates UVB induced tumor initiation and growth



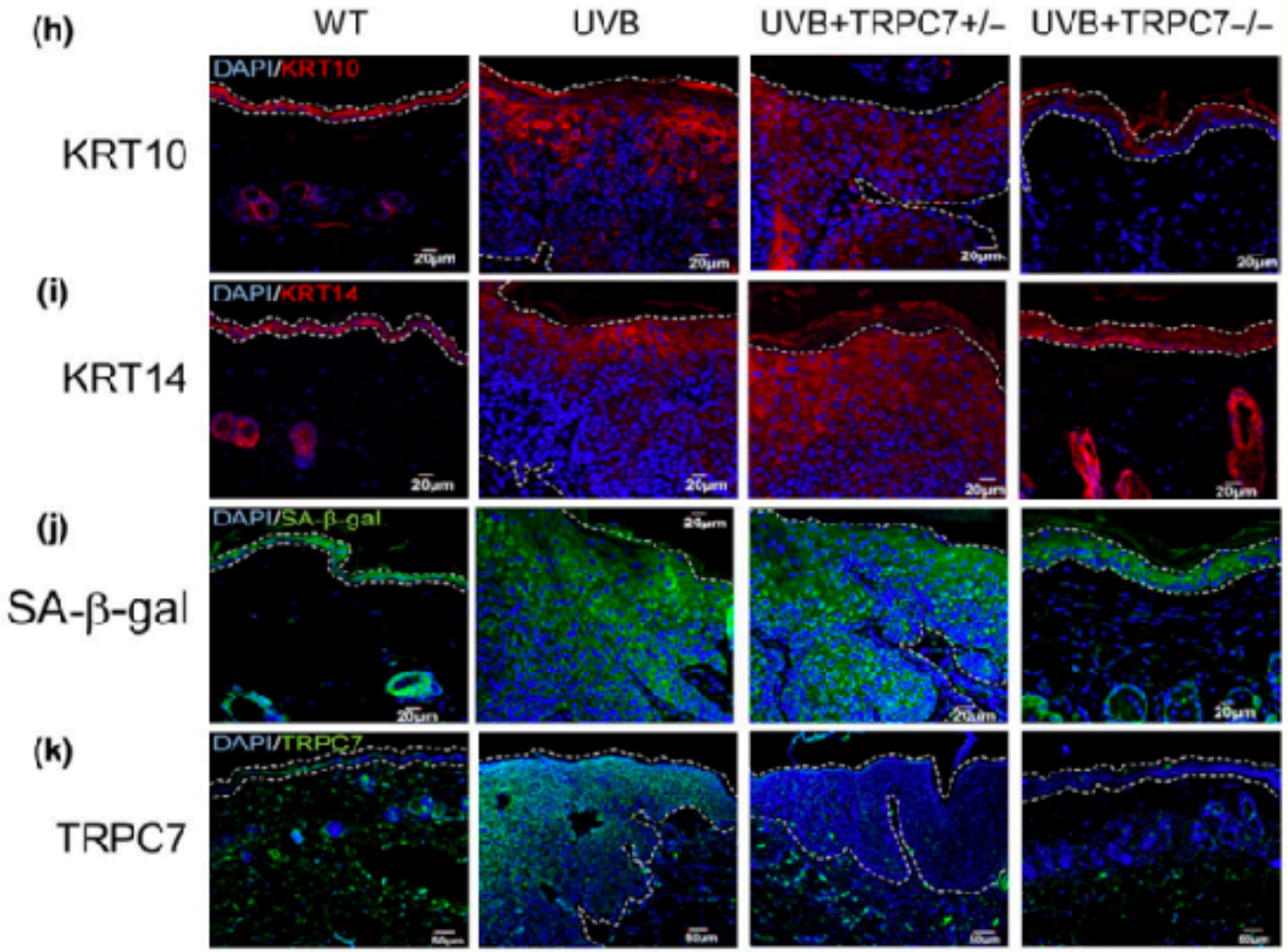




- Significantly fewer knockout mice developed tumors and the tumors that did develop were significantly smaller volume
- TRPC7 deficiency did not completely eliminate tumor formation.

TRPC7 mediates UVB induced tumor initiation and growth

- KRT10, KRT14 and SA-β-gal were up-regulated at the boundary of the tumor in UVB exposed WT type mice
- Cell senescene leads to SASP activation and carcinogen stimulated tumor formation
- Overexpression of TRPC7 in UVB exposed skin especially in papilloma
- **The overexpression of TRCP7 in tumors raises the possibility that TRPC7 promotes tumorigenesis.**



UVB induced p53 mutations are prevented in TRPC7 knockout mice

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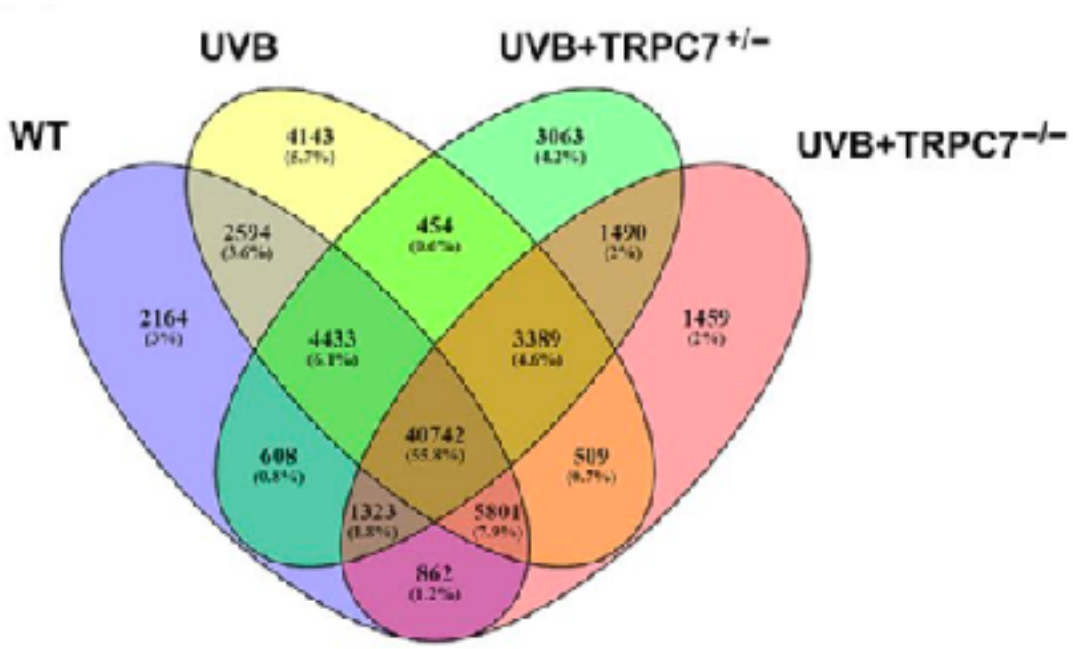
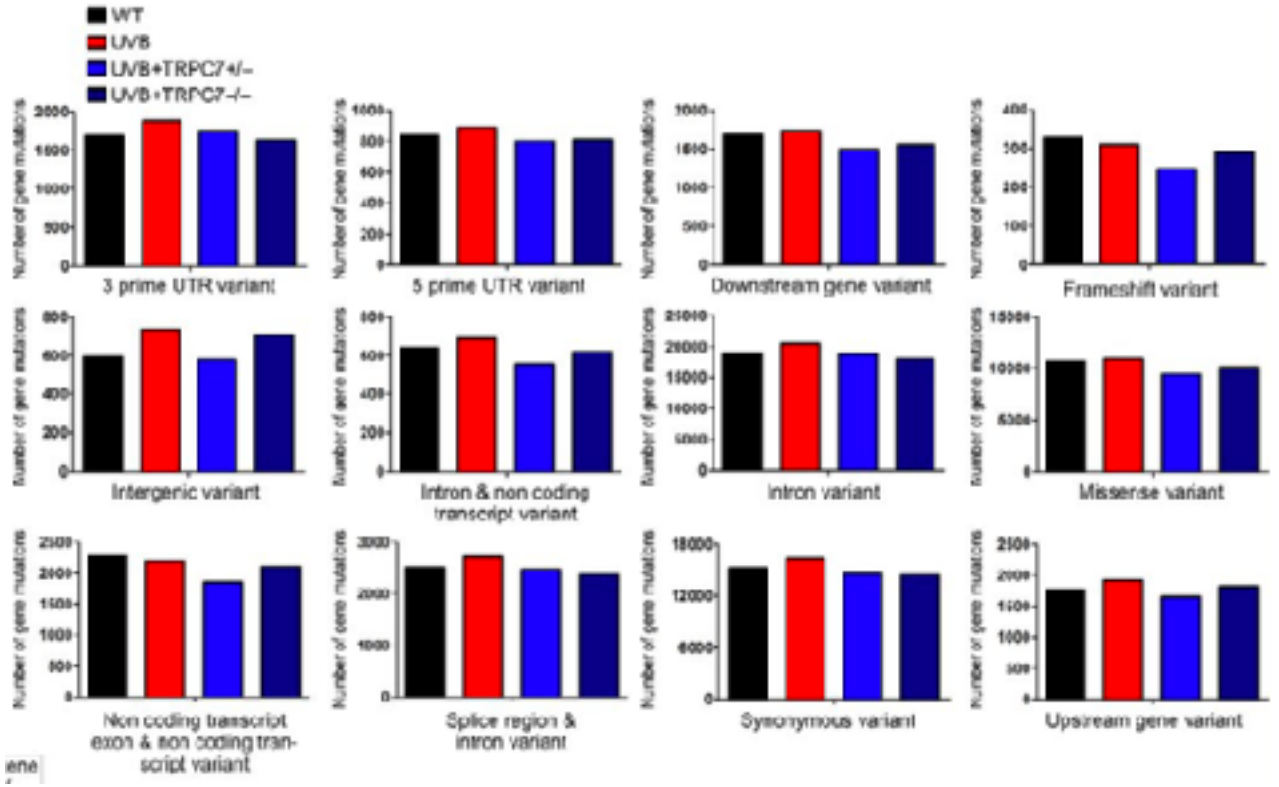
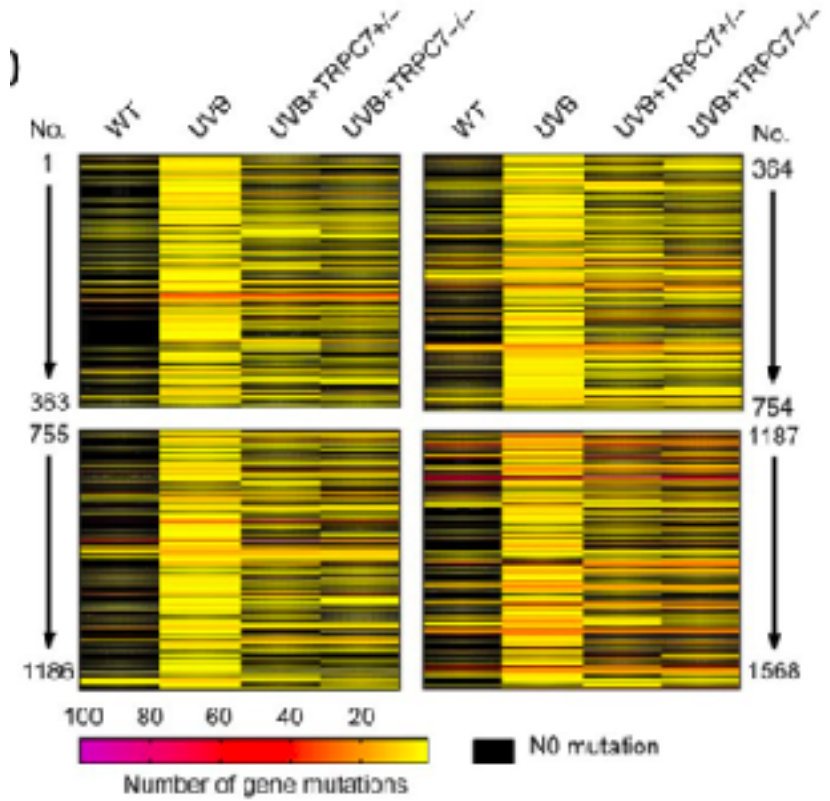
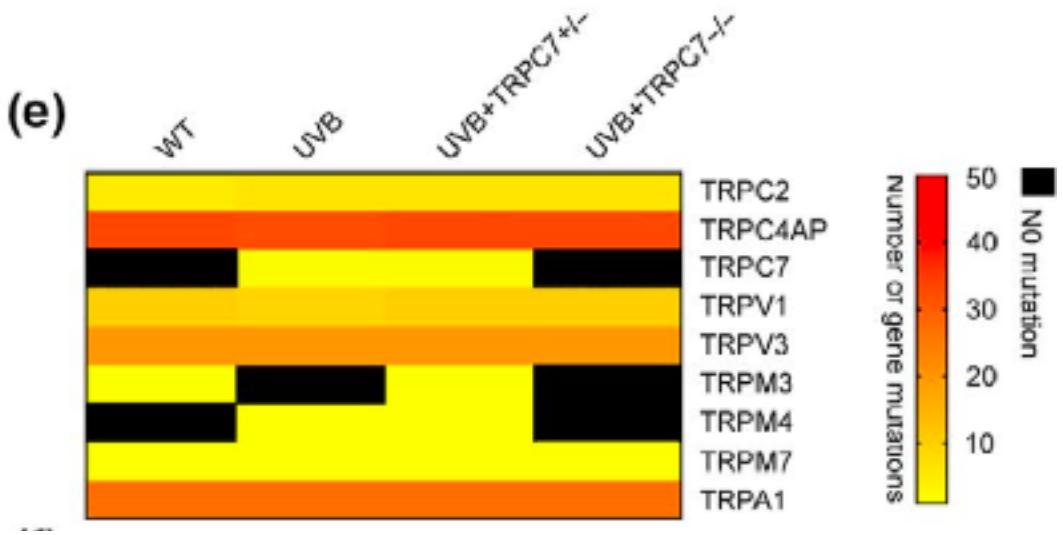




- Whole-exome sequencing in dorsal skin of mice to characterize genetic mutations.
- Knockout mice had the smallest percentage of mutations despite being exposed to UVB.
- KO mice consistently had fewer mutations of each variant type than did UVB exposed WT mice.

UVB induced p53 mutations are prevented in TRPC7 knockout mice

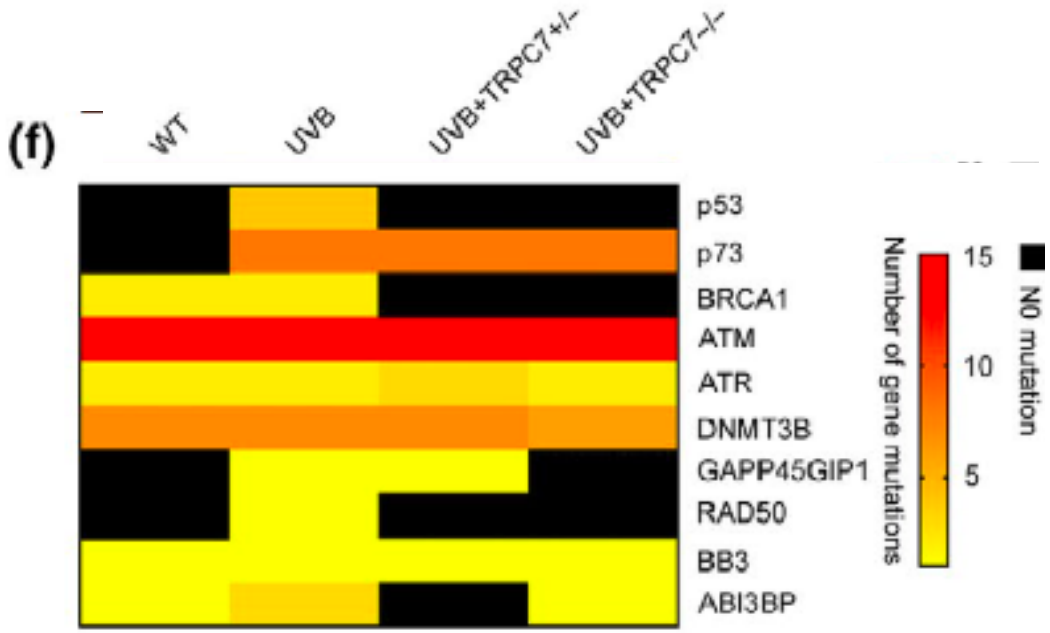




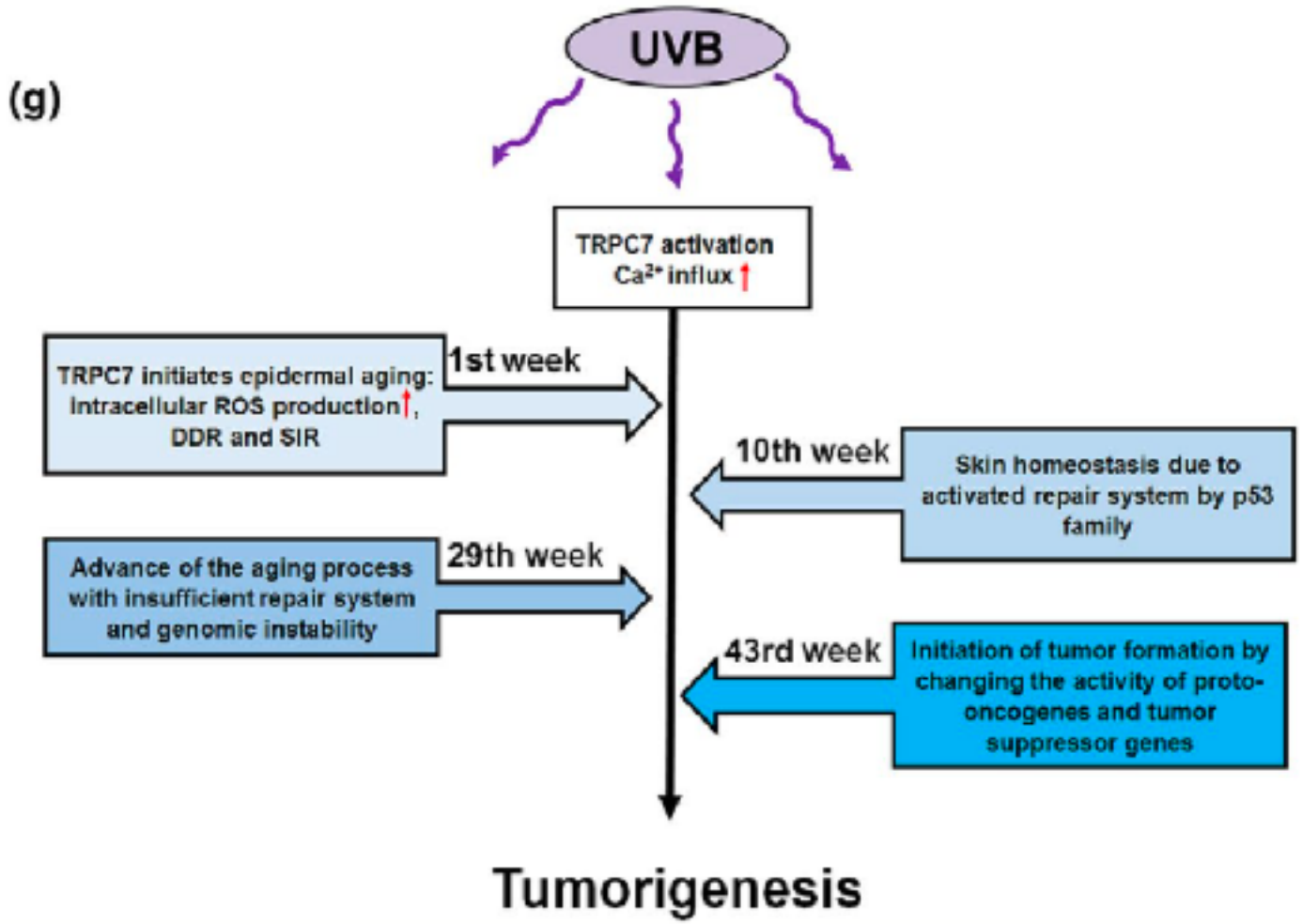
- Knockout mice had a lower number of mutations than did wild type mice
- Greater number of TRPC7 Gene mutations in wild type mice than in knockout mice

UVB induced p53 mutations are prevented in TRPC7 knockout mice

- p53 and p53 dependent DNA repair molecules were mutated less frequently after UVB exposure and thus maintained their protective functions against UVB induced tumorigenesis in TRPC7 knockout mice.
- **In response to UVB, TRPC7 is a primary initiator of epidermal aging and skin tumorigenesis, contributes to mutations in the p53 gene family and promotes the development of cancerous tumors**



UVB induced p53 mutations are prevented in TRPC7 knockout mice



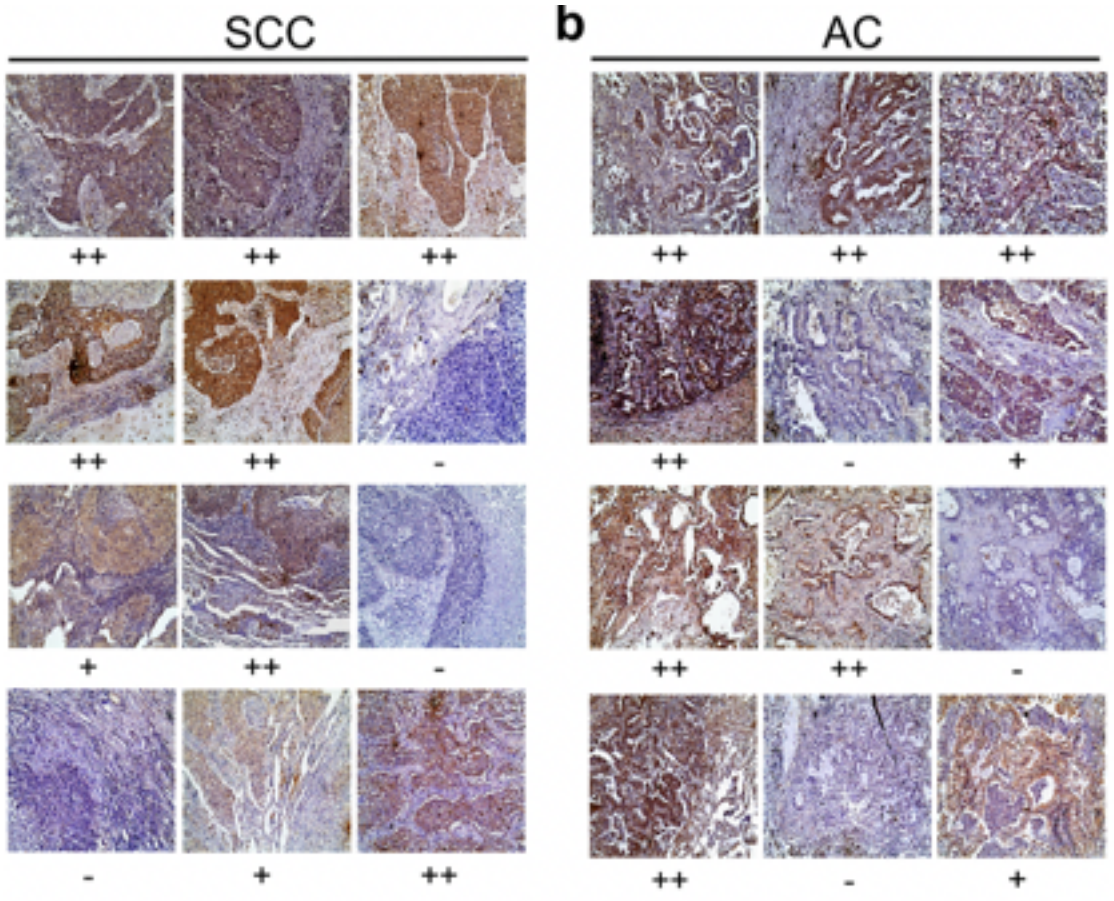
Summary

- TRP channels transduce stimulation into neuronal impulses for perception, also respond with Ca2+ influx to protect tissue against harm from extreme mechanical pressure, temperature, irradiation.
- TRPC7 activation through UVB initiates skin aging, results in mutations in the p53 gene family, promotes tumor development.

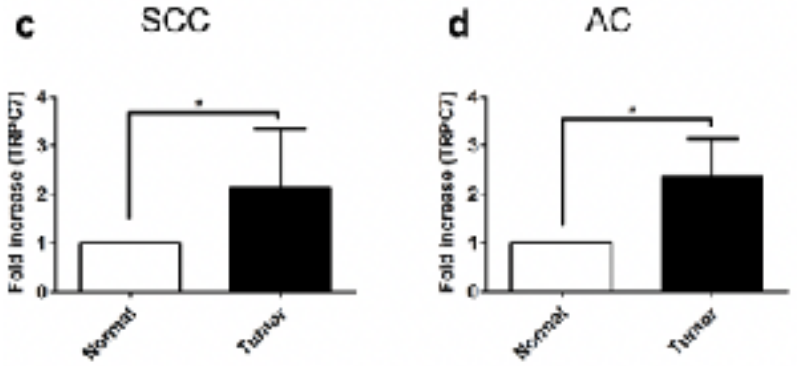
Final Thoughts

- TRPC7 blockage inhibits tumor initiation, but cannot completely prohibit tumor formation
- TRP channel expression has shown to increase with age related degeneration - Process of aging may be due to excess Ca2+ signaling from TRP upon continual environmental stimuli
- 55.8% of gene mutations occur from natural process of skin aging
    - Mutations naturally occur during skin aging, but external trigger such as UVB is required for aging associated diseases.

Final Thoughts



- TRPC7 was found to be overexpressed in tumor biopsies from patients with non NSCLC (Squamus Cell- / Adenocarcinoma)



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Aging Cell 2019

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