Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Divergent roles of HDAC1 and HDAC2 in the regulation of epidermal development and tumorigenesis

Winter M. *et al.*, The EMBO Journal (2013) 32, 3176–3191

Tanja Berger

Histone modifications

Histone acetylation by histone acetyltransferases (HATs)

→ Opening of local chromatin structures

→ Transcriptinal activation

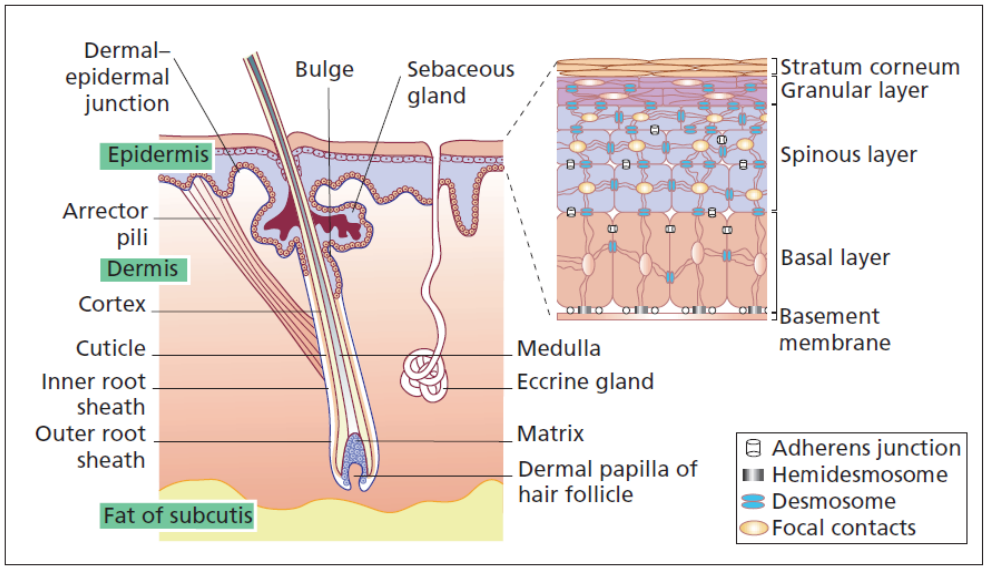
Histon deacetylation by histone deacetyltransferases (HDACs)

→ Repression

→ HDAC1 and HDAC2: components of the Sin3, NuRD, CoREST and NODE co-repressor complexes

➔ Potential regulatory functions of HDAC1 and HDAC2 in epidermal development?

Skin



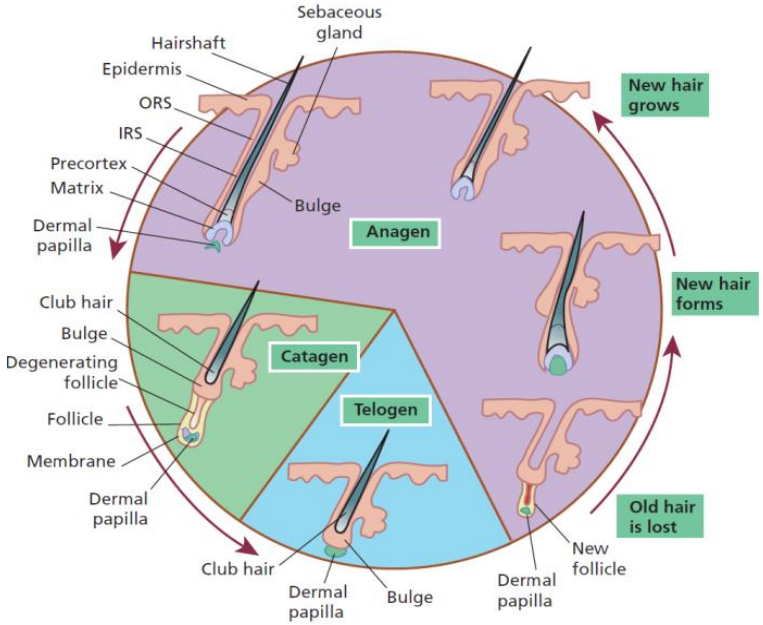
Burns *et al.*, 2004

→ physical barrier against the environment

→ differentiation of multipotent stem cells (SCs) into

- Interfollicular epidermis (IFE) lineage
- Sebaceous gland (SG) lineage
- Hair follicle (HF) lineage

Hair cycle



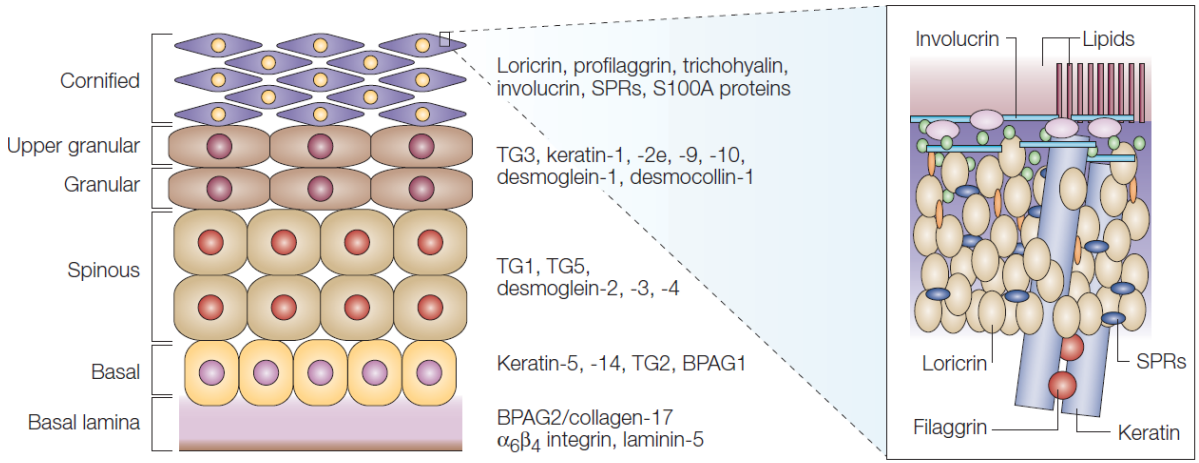
(Burns *et al.*, 2004)

→ **Anagen**: hair growth

→ **Catagen**: hair regression

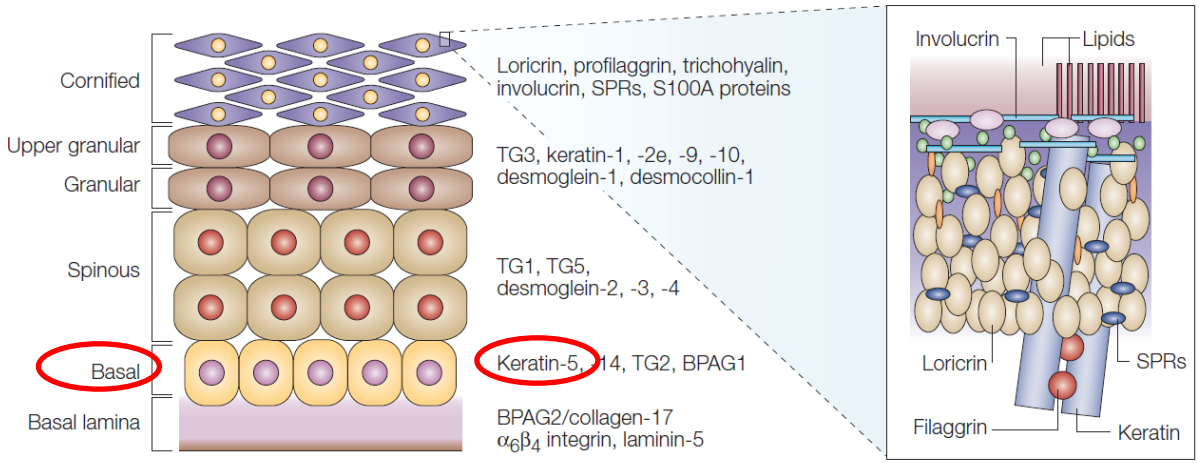
→ **Telogen**: resting phase

Keratinocyte differentiation



Candi *et al.*, 2005

Keratinocyte differentiation

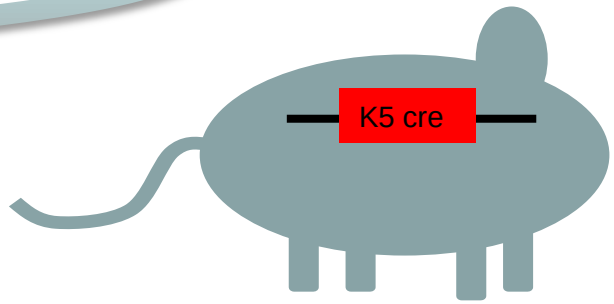


Candi *et al.*, 2005

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

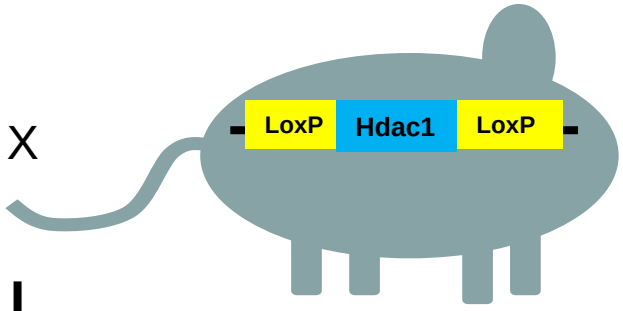
MEDIZINISCHE UNIVERSITÄT WIEN

Tissue specific knock out (epidermis)

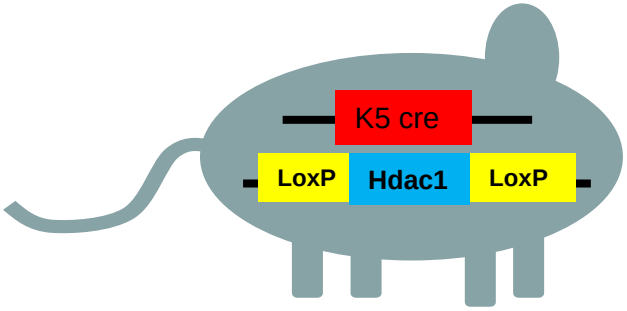


K5-cre mouse

X



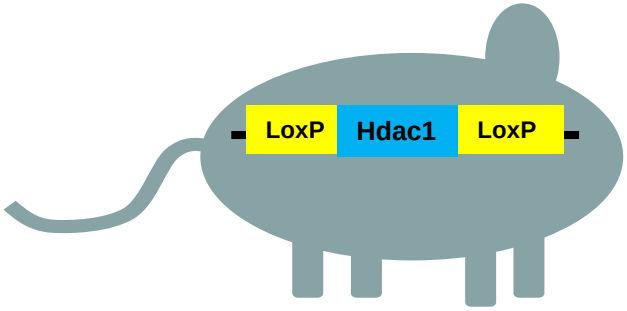
LoxP (floxed) mouse



active *K5 -Cre* → gene deletion of Hdac1

= ***Hdac1$^{f/f}$ K5-Cre* mouse**

= *Hdac1$^{\Delta/\Delta ep}$*



no active *K5 -Cre*

→ original gene function of Hdac1

= *Hdac1$^{f/f}$*

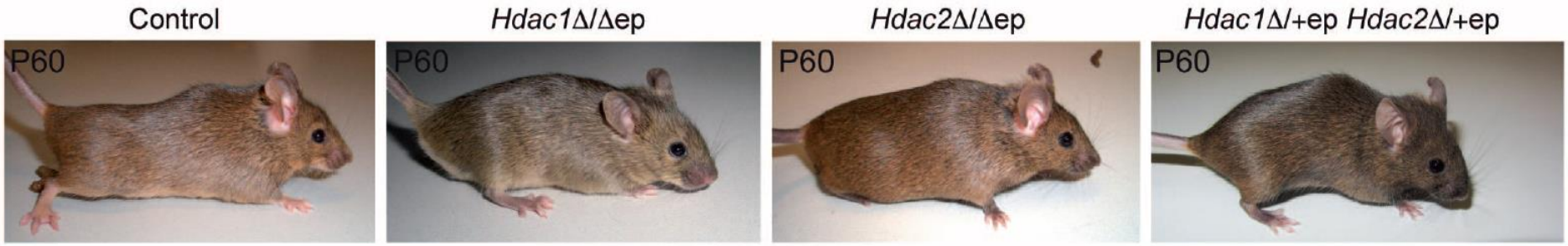
Results

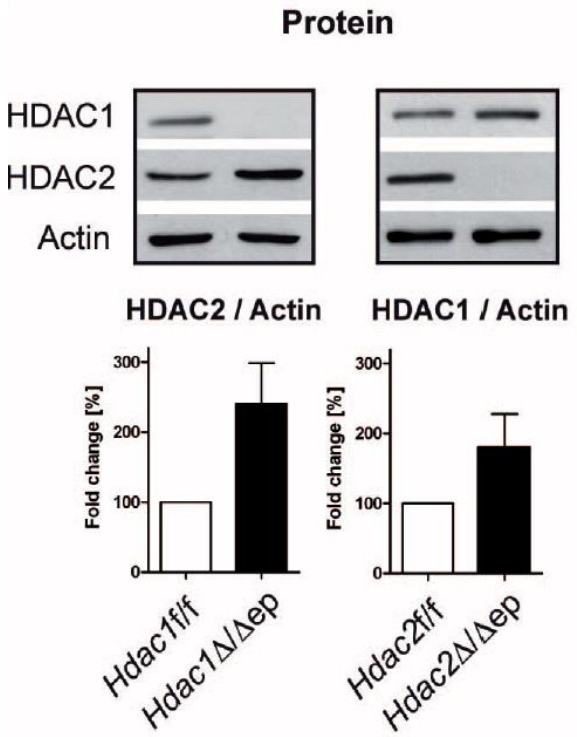
Epidermal development

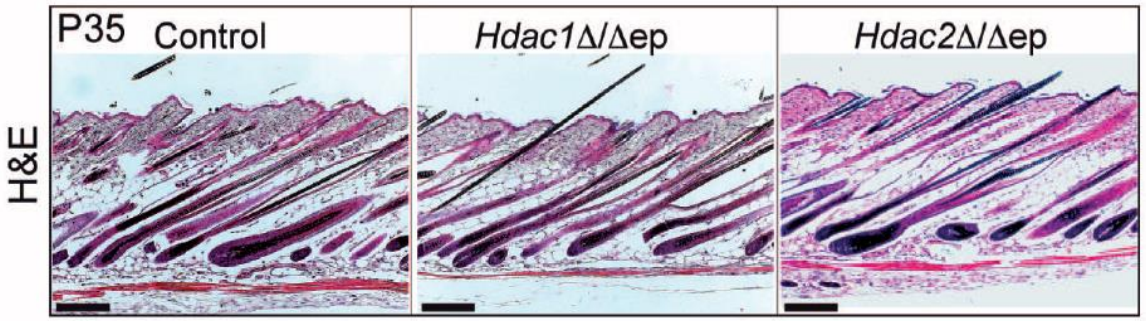
Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Single knock out of HDAC1 and HDAC2



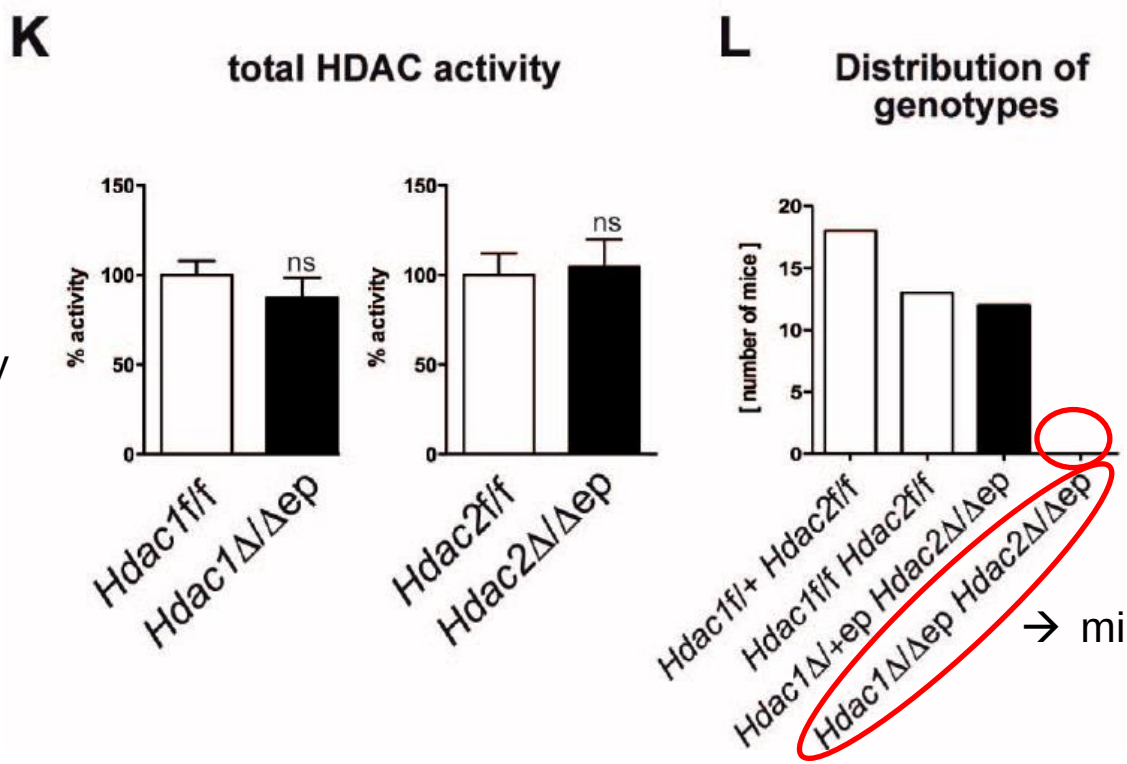




→ Normal epidermal development in the absence of either HDAC1 or HDAC2

Double knock out of HDAC1 and HDAC2

→ No altered HDAC activity



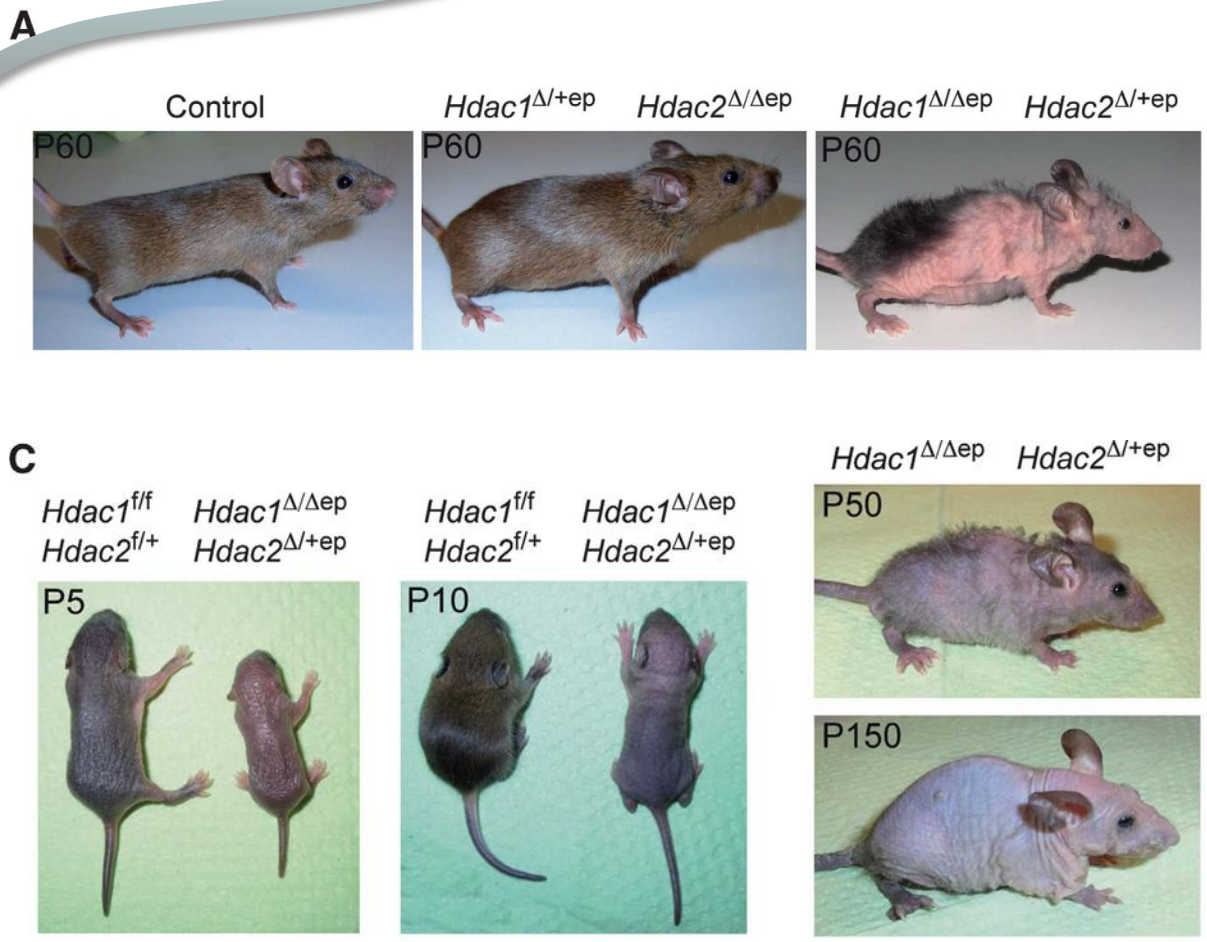
→ mice were not viable

➔ Indispensable role of HDAC1 and HDAC2 for epidermal development

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Developmental abnormalities in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$



→ Progressive alopecia

→ Smaller after birth

→ Reduced body weight

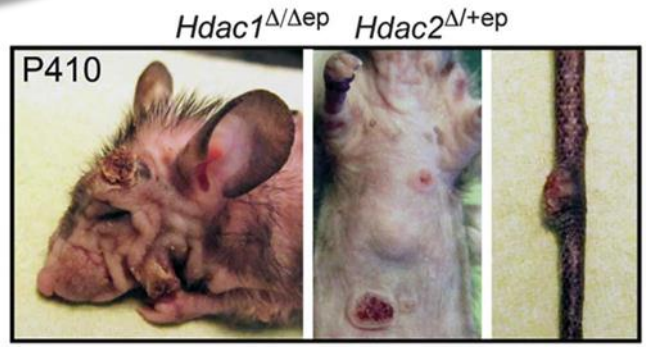
→ Scaly tail regions

Ankersmit Laboratory

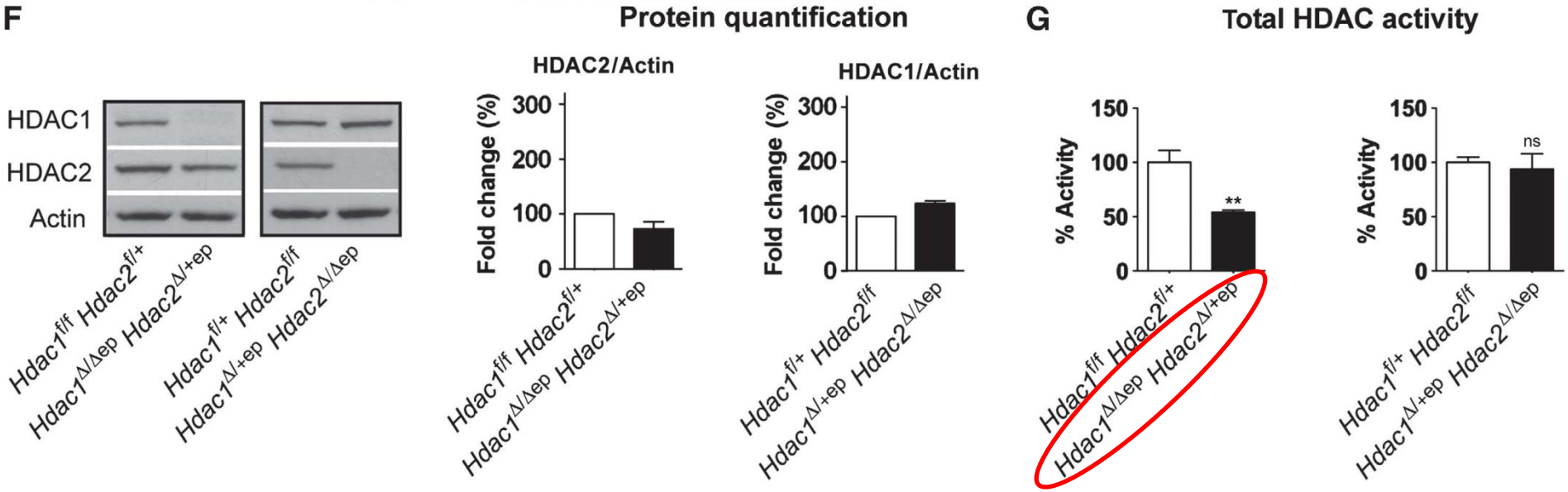
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Severe phenotype of *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$



→ Papilloma like lesions



➔ Single *Hdac1* allele can compensate for HDAC2 deficiency

but not the other way around

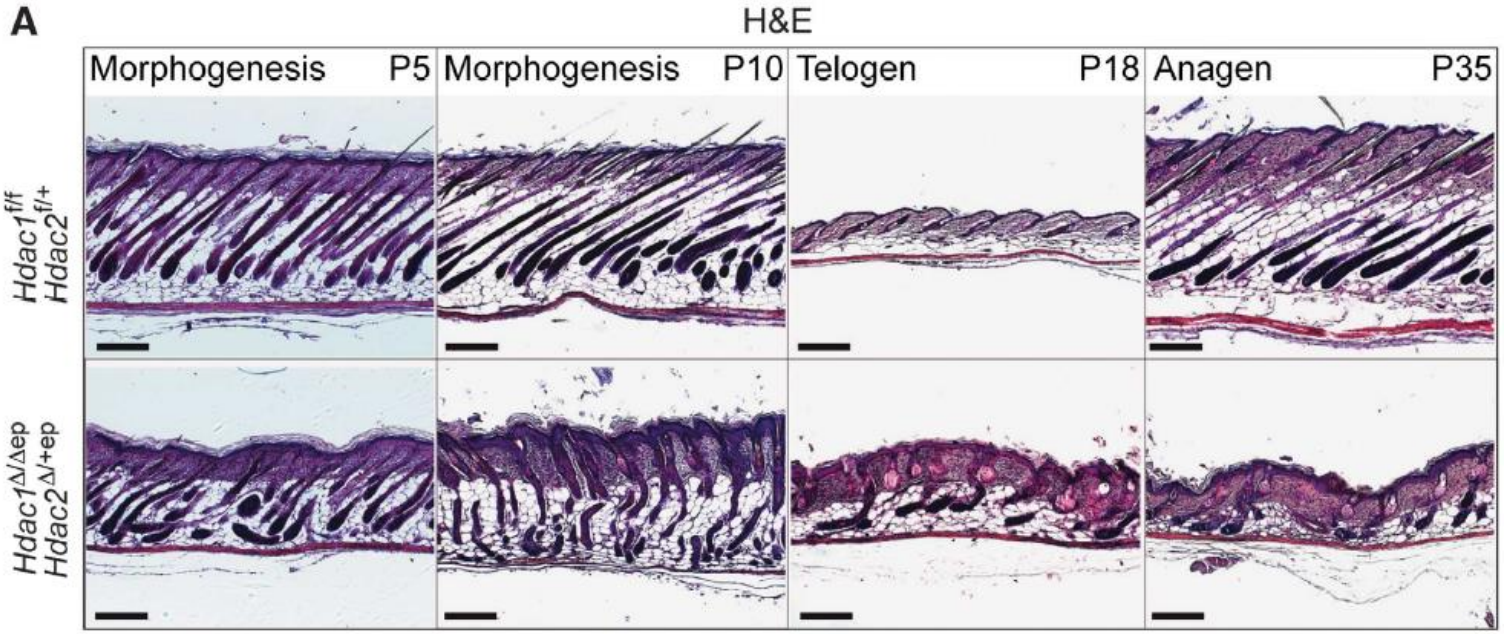
Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Disturbed hair follicle development

Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$

back skin



Mutant hair follicles: → mostly shorter and disordered

→ failed to enter the telogen phase in a synchronized manner

→ became atrophied

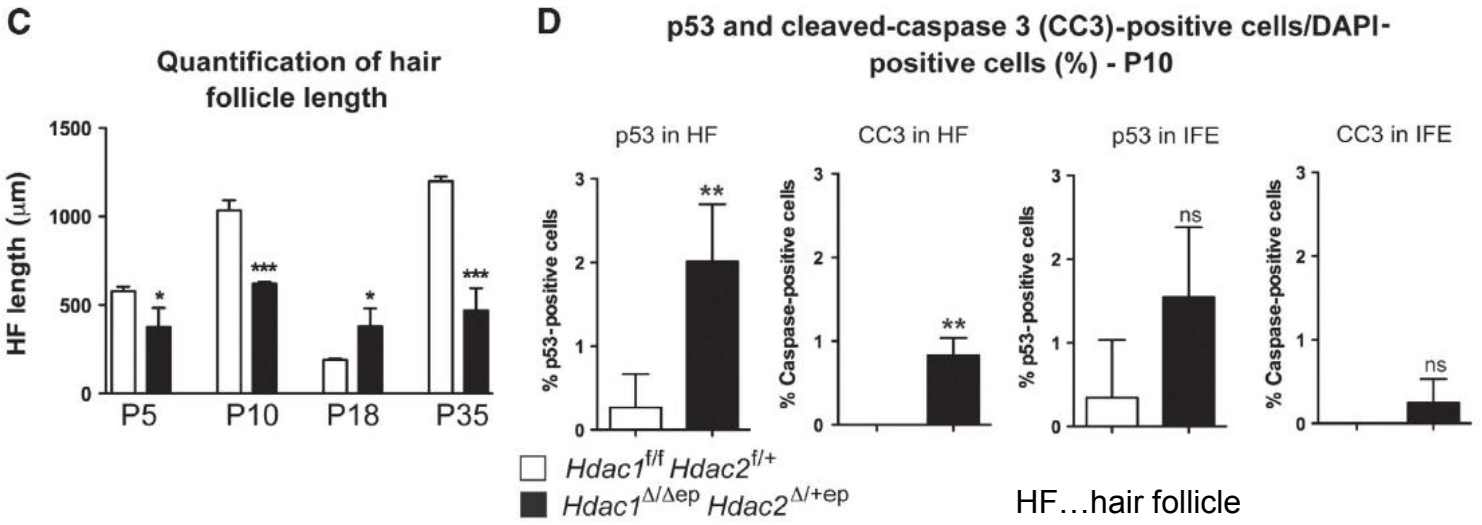
→ failed to enter the anagen phase

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Disturbed hair follicle development

Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$



HF…hair follicle

IFE…interfollicular epidermis

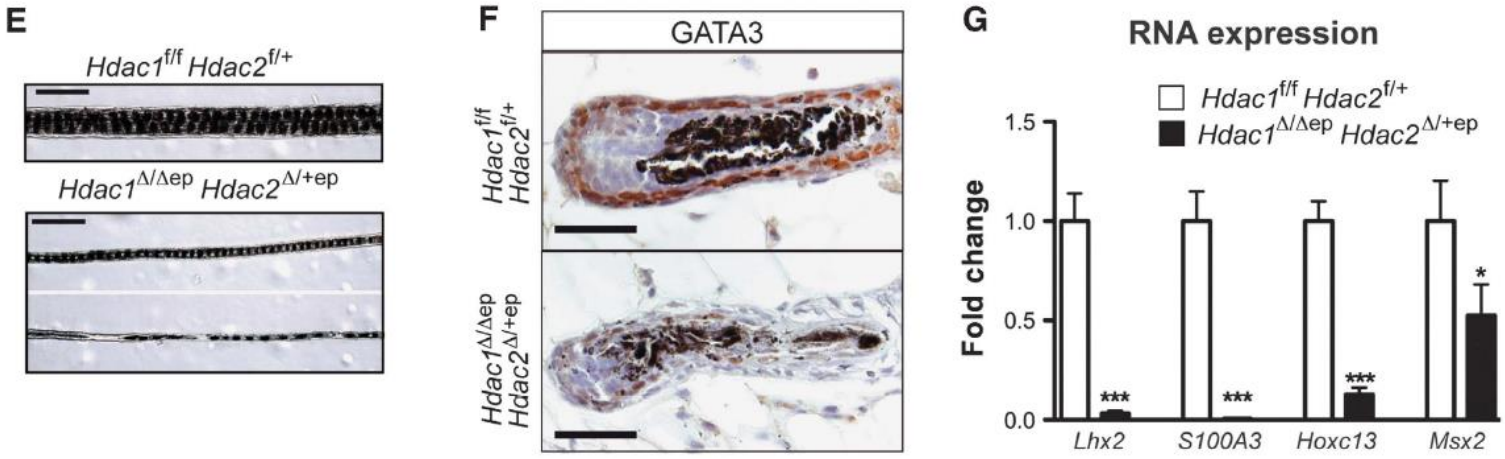
Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ HF

→ Increased p53 expression and apoptosis (cleaved caspase-3)

Disturbed hair follicle development

Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$

TF GATA3: epidermal lineage determination and differentiation of the inner root sheath



Lhx2, S100A3, Hoxc13, Msx2 genes important for hair development

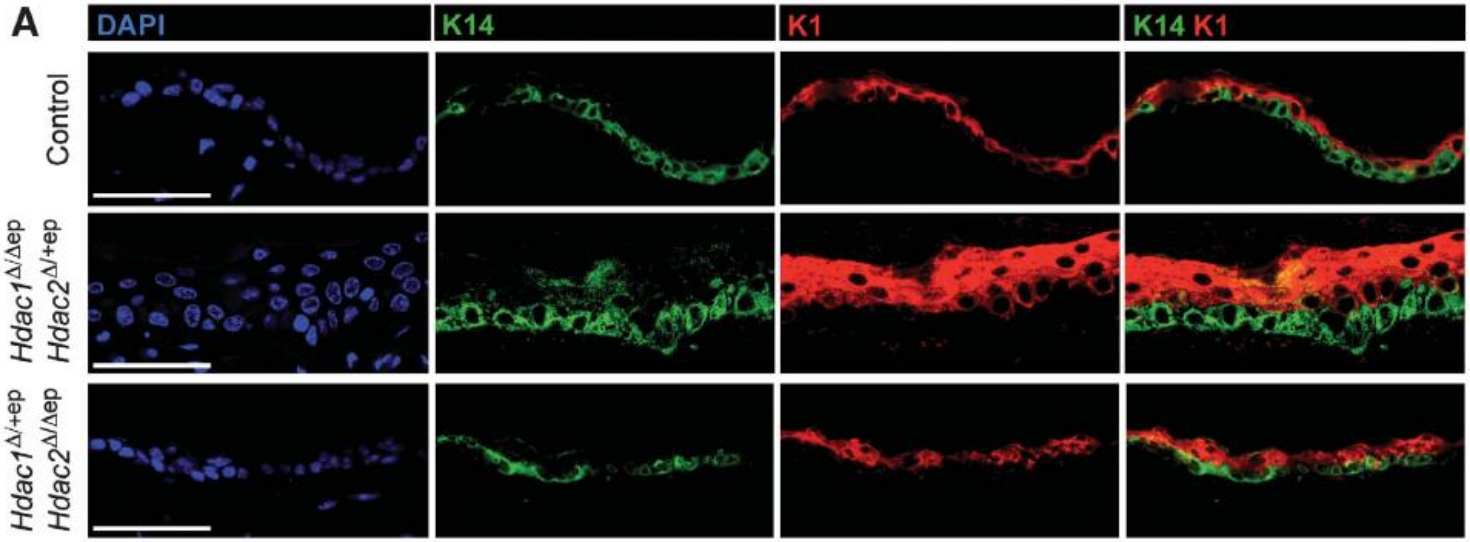
→Reduced in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$

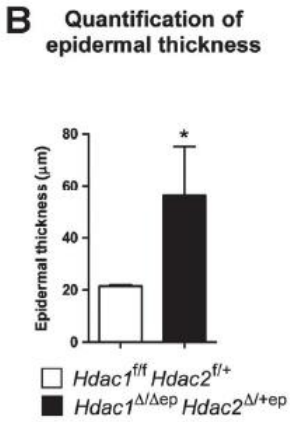
Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

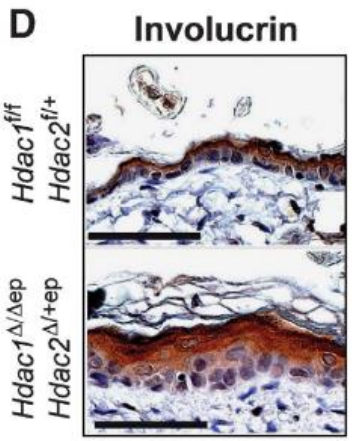
MEDIZINISCHE UNIVERSITÄT WIEN

Hyperkeratosis in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ mice

back skin







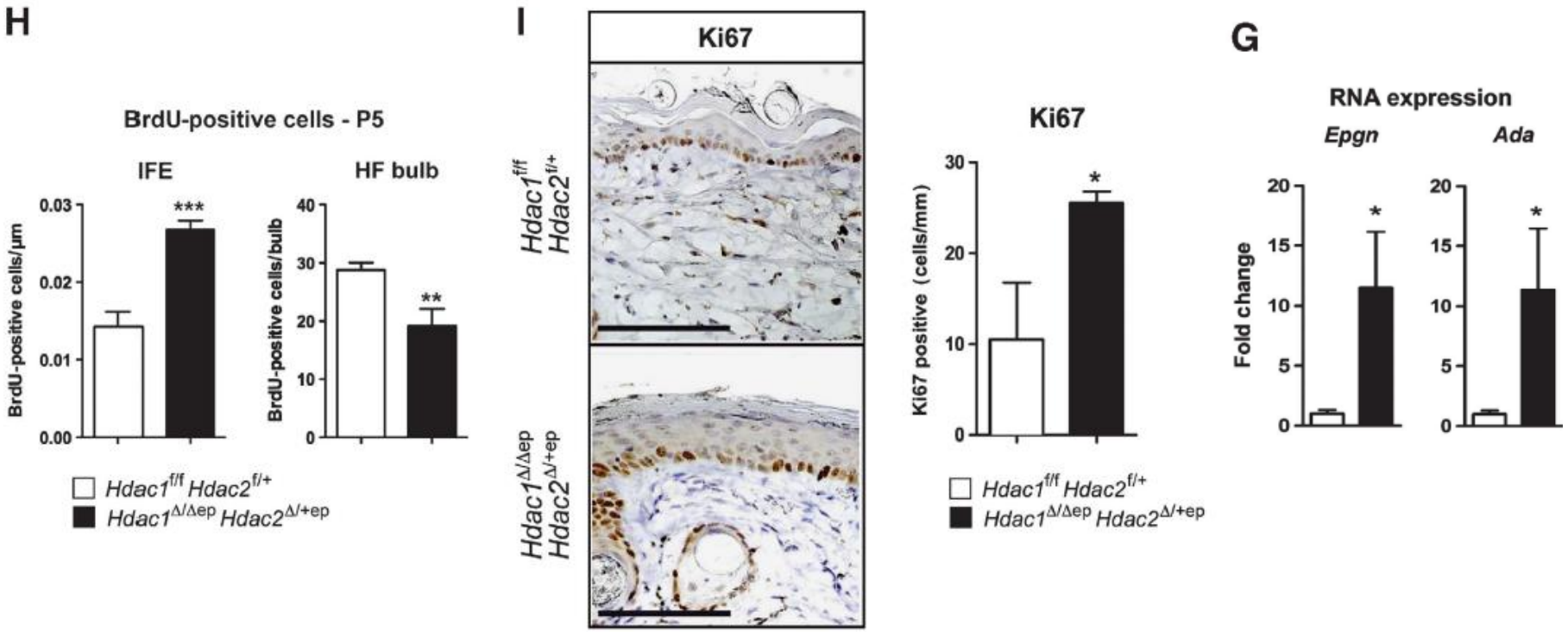
→ thickening of the epidermis

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Hyperkeratosis in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ mice

Quantification of proliferating cells



Epgn and Ada… genes crucial for epithelial morphogenesis and proliferation

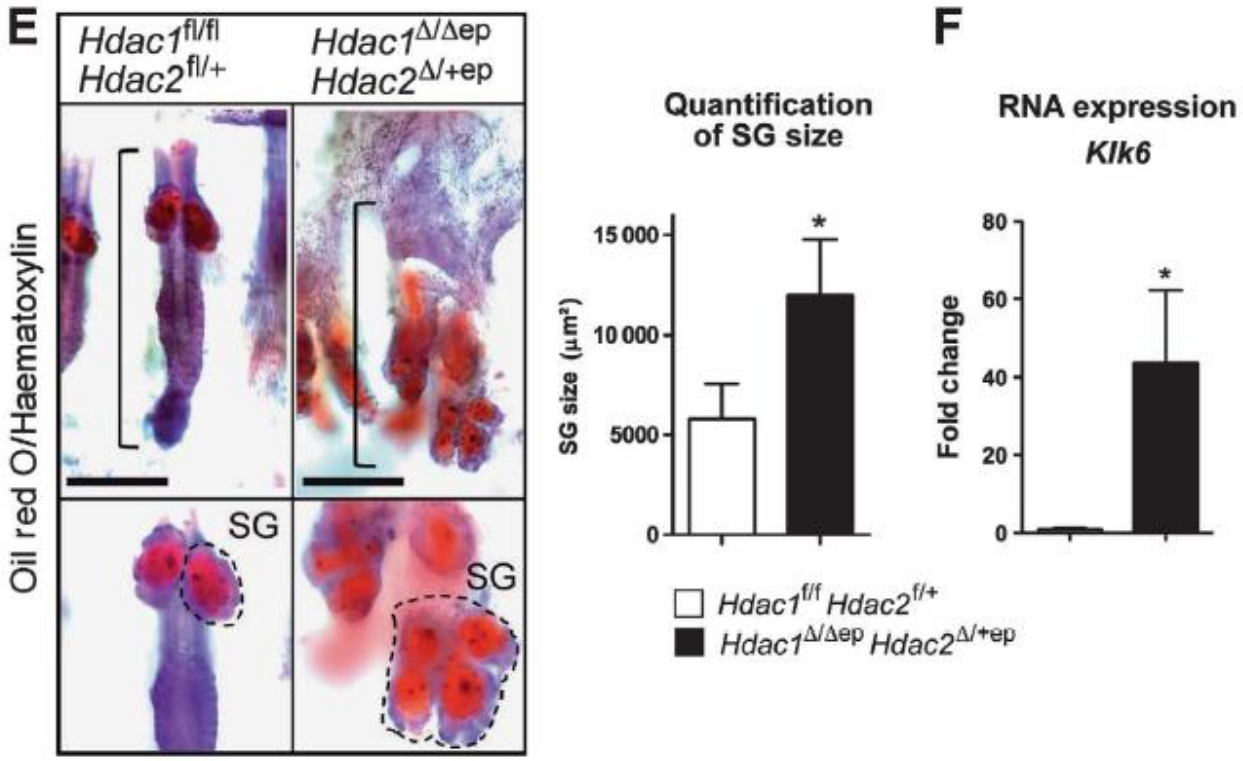
➔ Hyperpoliferation of the IFE in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ mice

Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Enlarged sebaceous gland in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ mice



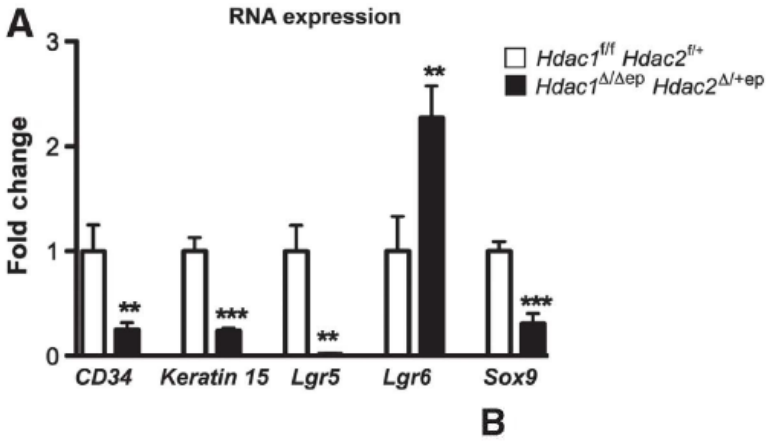
Klk6…serine protease predominatly expressed in SGs

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Changes in lineage determination

Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ epidermis



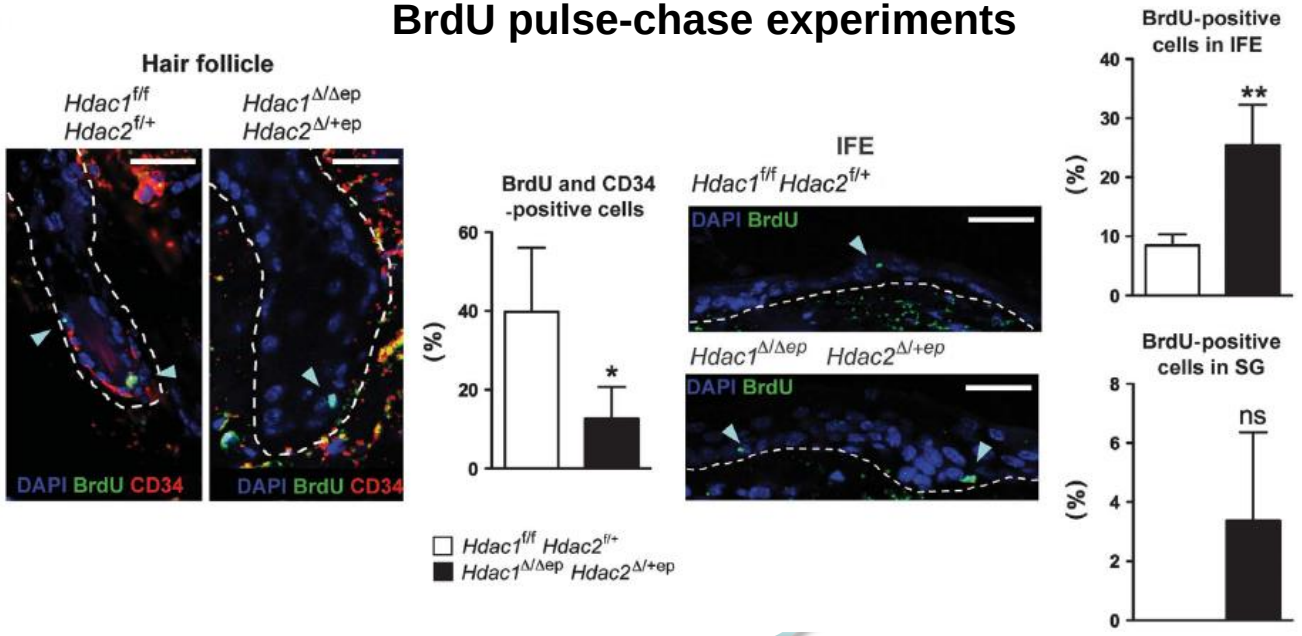
→ Downregulation of SC markers in the HF bulge (*CD34, Keratin 15*) and hair sheath (*Lgr5 and Sox9*)

→ *Upregulation Lgr6* (SC marker for SG and IFE growth)

→ Reduction of BrdU+/ CD34+ in HF

→ Increase of BrdU+ cells in the IFE

BrdU pulse-chase experiments



Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

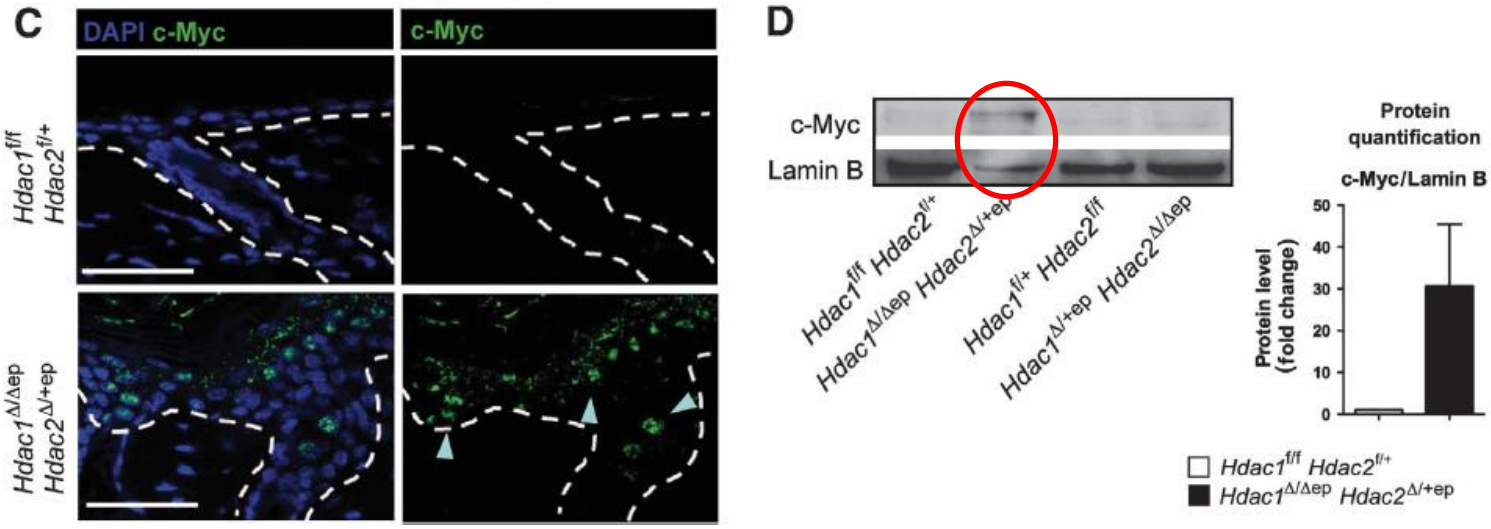
c-Myc activation in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ epidermis

c-Myc overexpressing mice:

Epidermal hyperprolifration along the SG and IFE lineages

at the expense of HF differentiation

back skin

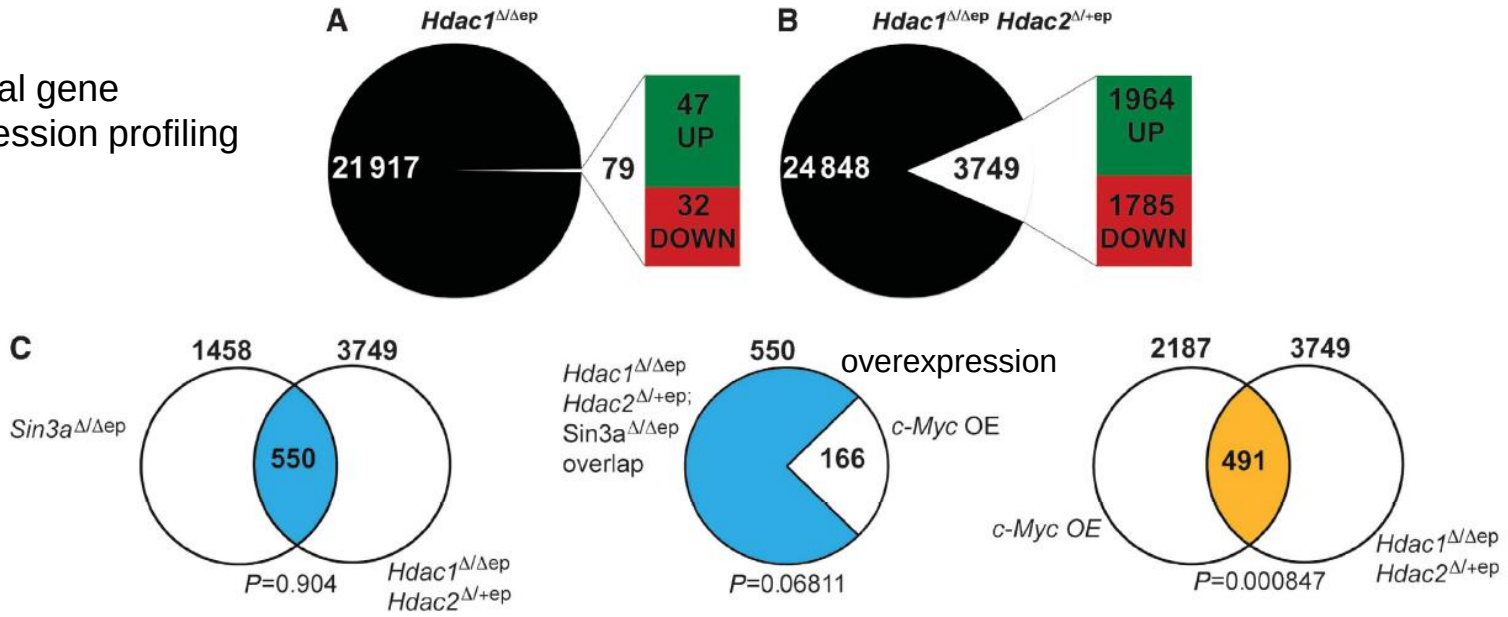


Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Gene expression changes in *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$ epidermis

Global gene expression profiling



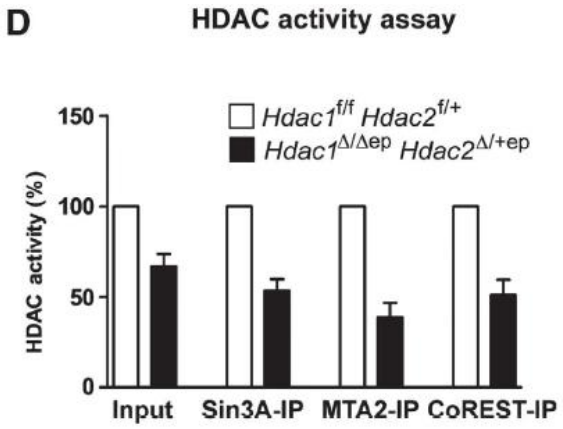
Sin3a$^{\Delta/\Delta ep}$ displayed c-Myc upregulation similar to the phenotype of *Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$

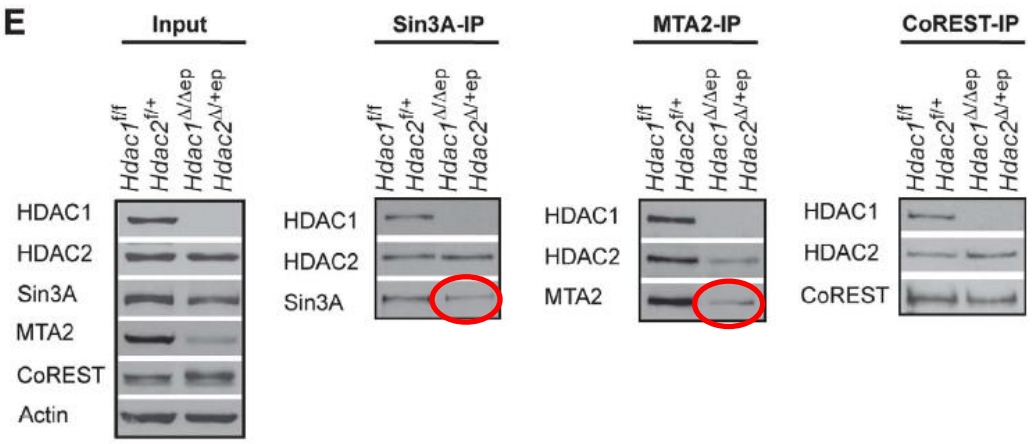
Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

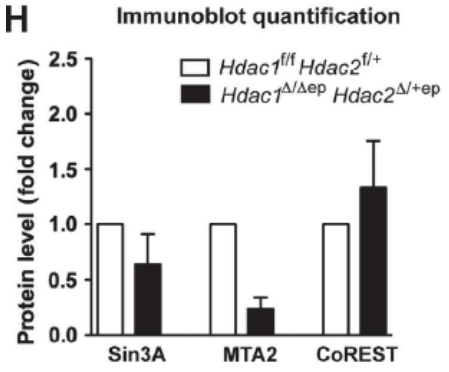
MEDIZINISCHE UNIVERSITÄT WIEN

Alteration in repressor complex function

Hdac1$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+ep}$







→ HDAC1 and HDAC2: components of the Sin3, MTA2 and CoREST co-repressor complexes

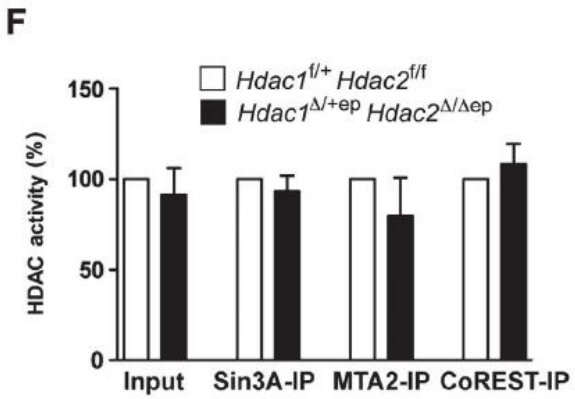
→ Reduced deacetylase activities

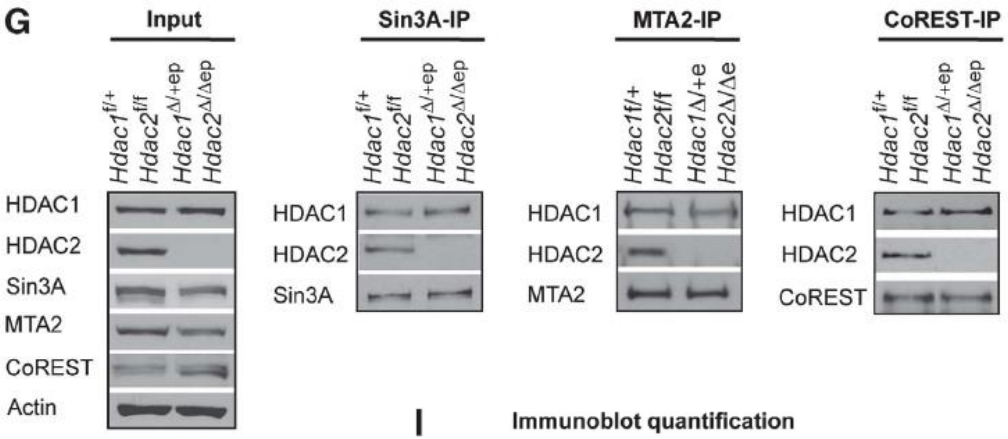
→ Reduced protein levels of Sin3A and MTA2

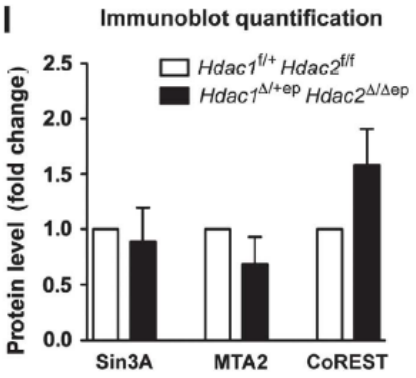
Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Repressor complex function in *Hdac1*$^{\Delta/+ep}$ *Hdac2*$^{\Delta/\Delta ep}$ mice







→ HDAC1 and HDAC2: components of the Sin3, MTA2 and CoREST co-repressor complexes

→ No changes in co-repressor associated deacetylase activities

Results

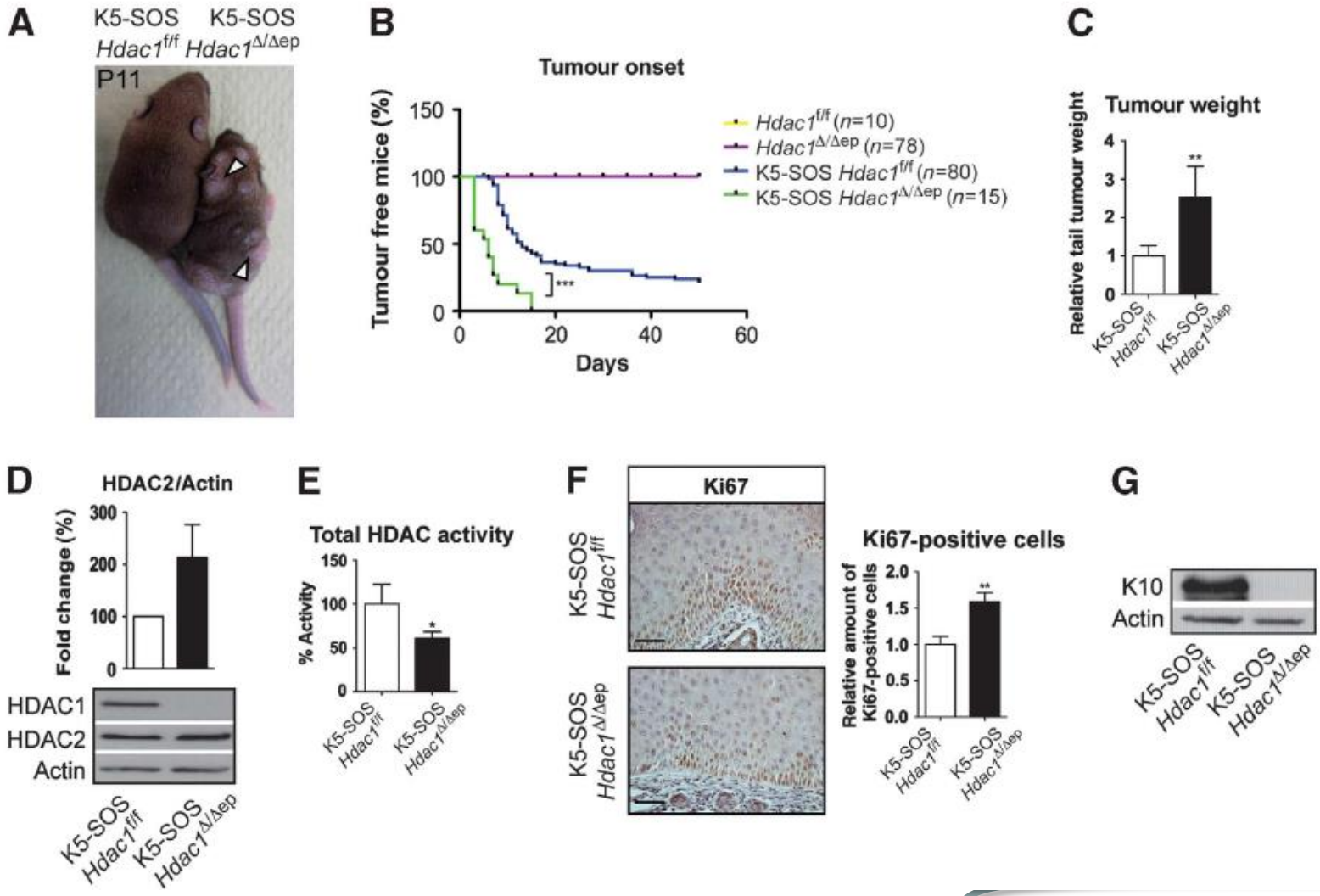
Tumour development

Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

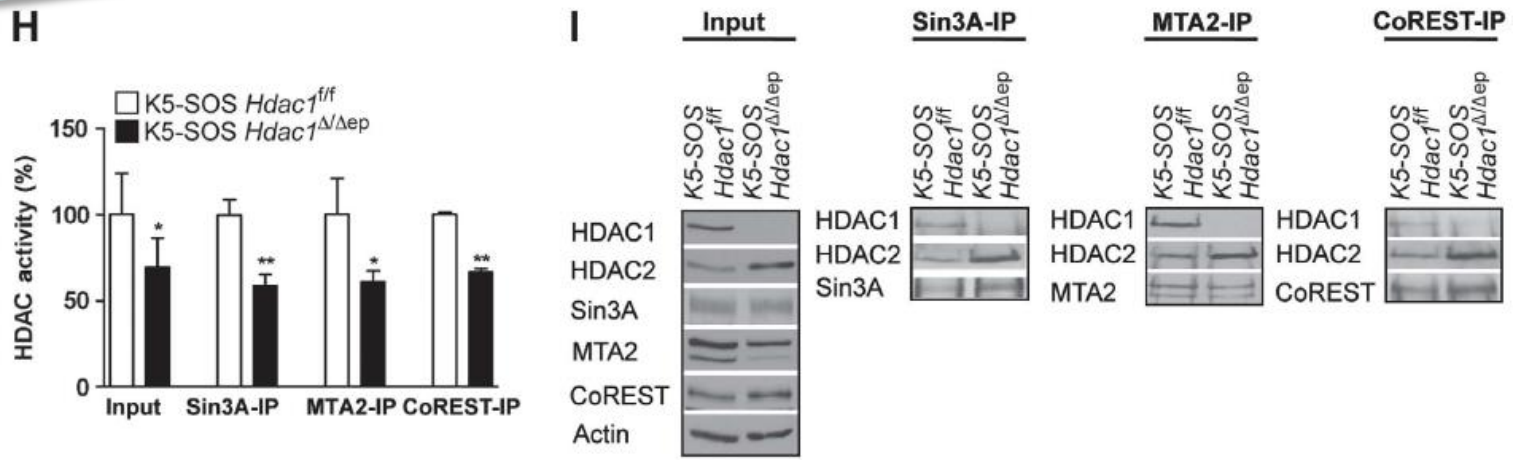
Tumor development in *K5-SOS Hdac1$^{\Delta/\Delta ep}$* mice

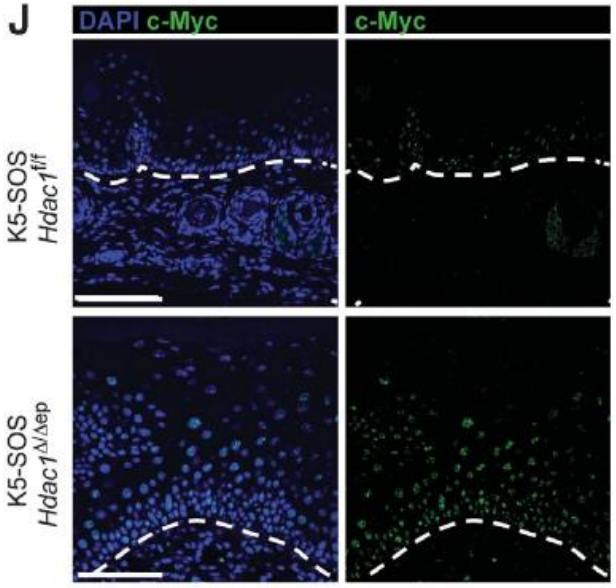


Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Tumor development in *K5-SOS Hdac1*$^{\Delta/\Delta ep}$ mice





→ Reduced co-repressor associated deacetylase activity

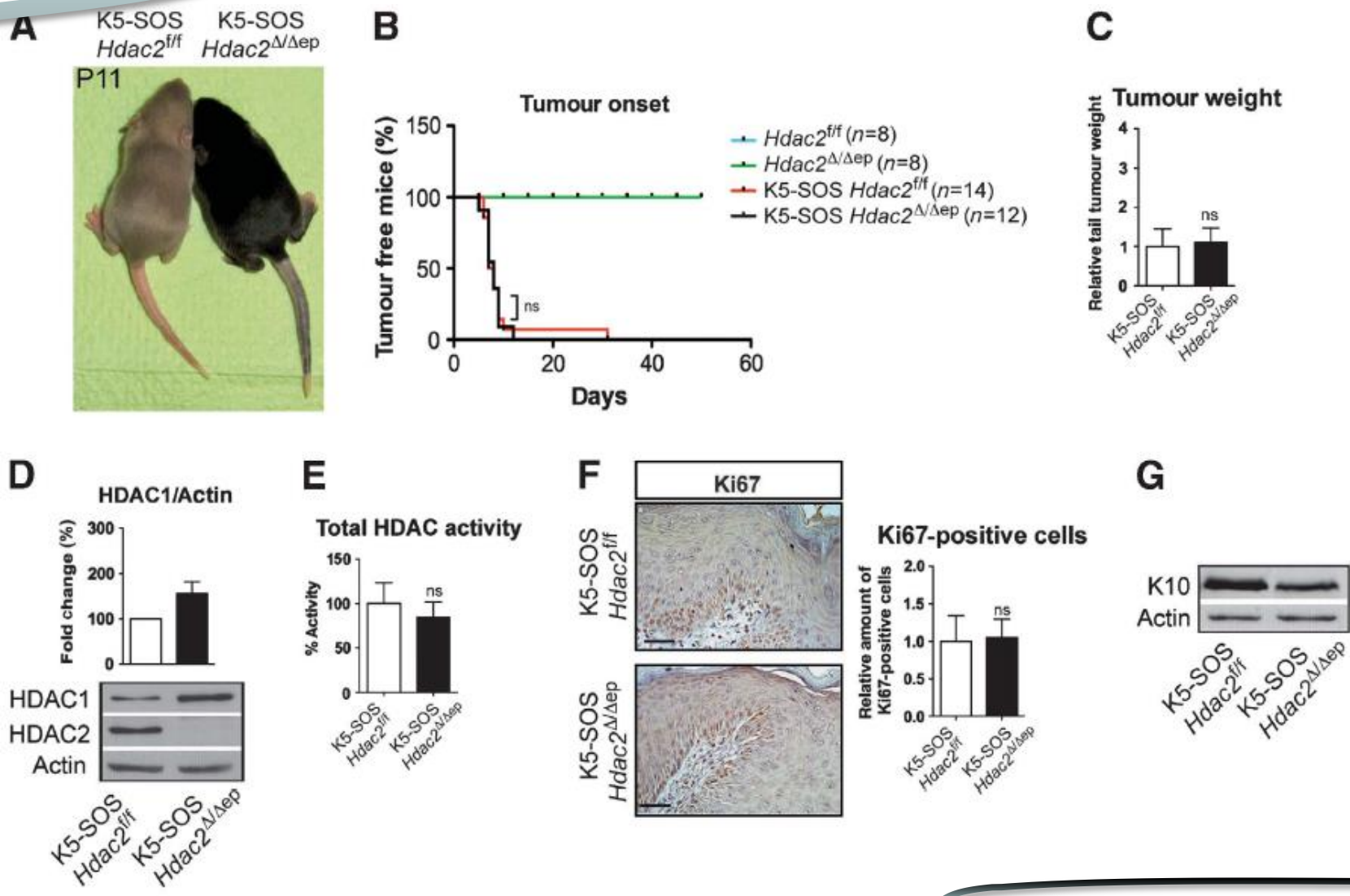
→ Incrased levels of c-Myc protein

Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

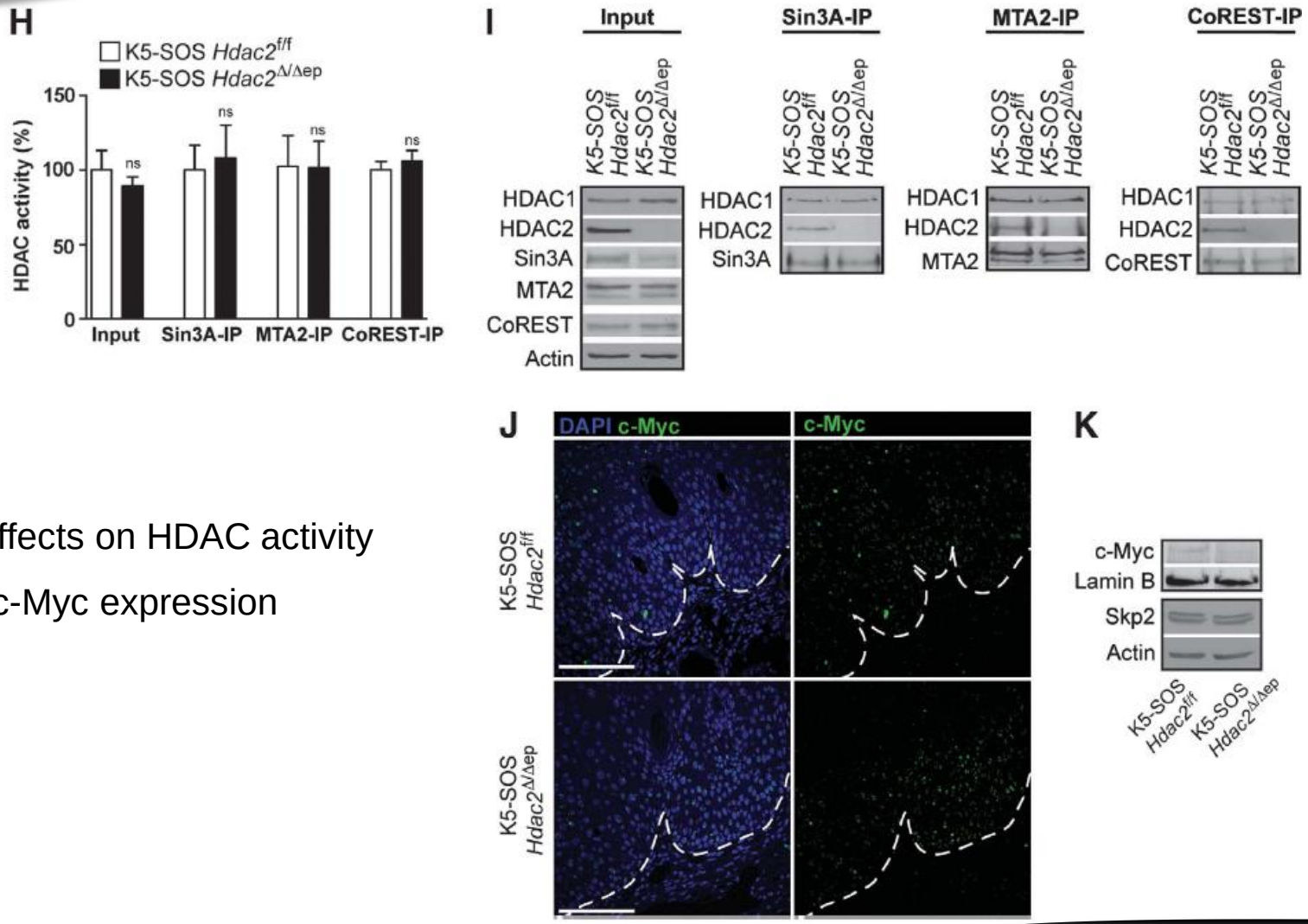
K5-SOS Hdac2$^{\Delta/\Delta ep}$ mice



Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

K5-SOS Hdac2$^{\Delta/\Delta ep}$ mice



→ No effects on HDAC activity and c-Myc expression

Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Discussion

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

Divergent roles of HDAC1 and HDAC2

- Single *Hdac1* allele sufficient to maintain proper epidermal development
- But a single *Hdac2* allele displayed a severe developmental phenotype in the epidermis (hyperkeratosis, hair loss and sebaceous gland enlargement)

- HDAC1 play a role in embryonic development (Lagger *et al.*, 2002), in B cells (Reichert *et al.*, 2012) and T cells (Grausenburger *et al.*, 2010; Dovey *et al.*, 2013; Heideman *et al.*, 2013)
- Contrary, single *Hdac2* allele sufficient for normal oocyte (Ma et al, 2012) and brain development (Hagelkruys, Lagger et al, manuscript inrevision)

→HDAC1 and HDAC2 specific functions in differentiation and development

Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN

HDAC1 acts as a tumour suppressor in the epidermis

- Under mechanical or oncogenic stress conditions HDAC2 cannot fully compensate for the loss of HDAC1 in the epidermis

- Sin3A and HDAC1 as negative regulators of the proto-oncogene c-Myc

- *Lck-Cre Hdac1*$^{\Delta/\Delta ep}$ *Hdac2*$^{\Delta/+}$ resulted in neoplastic transformation of immature T cells (Dovey *et al.*, 2013)

Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

MEDIZINISCHE UNIVERSITÄT WIEN