

INTRODUCTION

ARCI, a heterogeneous group of rare cornification diseases, is most commonly caused by mutations in Transglutaminase 1 (TGM1), an enzyme responsible for the formation of the cornified cell envelope. Loss of TGM1 function results in an impaired epidermal barrier with dramatically increased trans-epidermal water loss often resulting in clinical dehydration; TGM1 deficiency also leads to an increased risk of skin malignancies. Current therapeutic options for treating ARCI provide limited symptomatic relief without addressing the underlying genetic defect, necessitating novel targeted therapeutics. We have developed KB105, a replication-defective HSV-1 gene therapy vector encoding human TGM1 for molecular correction of ARCI.

MATERIALS/METHODS

Test Article

KB105: Krystal Biotech, Inc.'s proprietary replication-defective HSV-1 vector encoding optimized human TGM1.

Table 1. Critical Reagents

Antibody Description	Source	Cat. No.
Mouse anti-human TGM1	Zedira	A042
Rabbit anti-mouse Loricrin	Abcam	Ab85679
Rat anti-mouse CD49f (GoH3)	BD Biosciences	555734
Alexa Fluor® 488-conjugated anti-mouse	ThermoFisher	A-11029
Alexa Fluor® 594-conjugated anti-rabbit	Abcam	Ab150080
Anti-rat IgG (H+L) cross-adsorbed, Cyanine5	ThermoFisher	A10525

RESULTS

Dose-Response to KB105 via Multiple Routes of Topical Delivery to BALB/c Mice

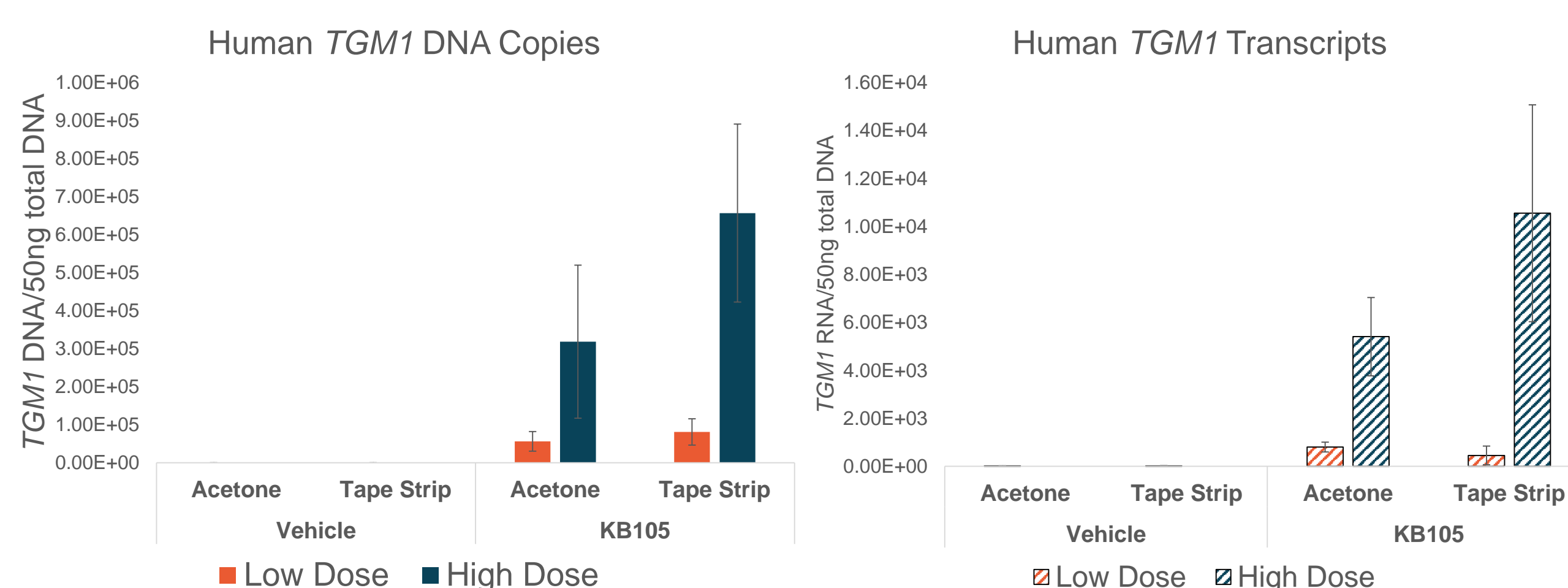


Figure 1. Robust TGM1 expression after topical KB105 administration to permeabilized and non-permeabilized skin

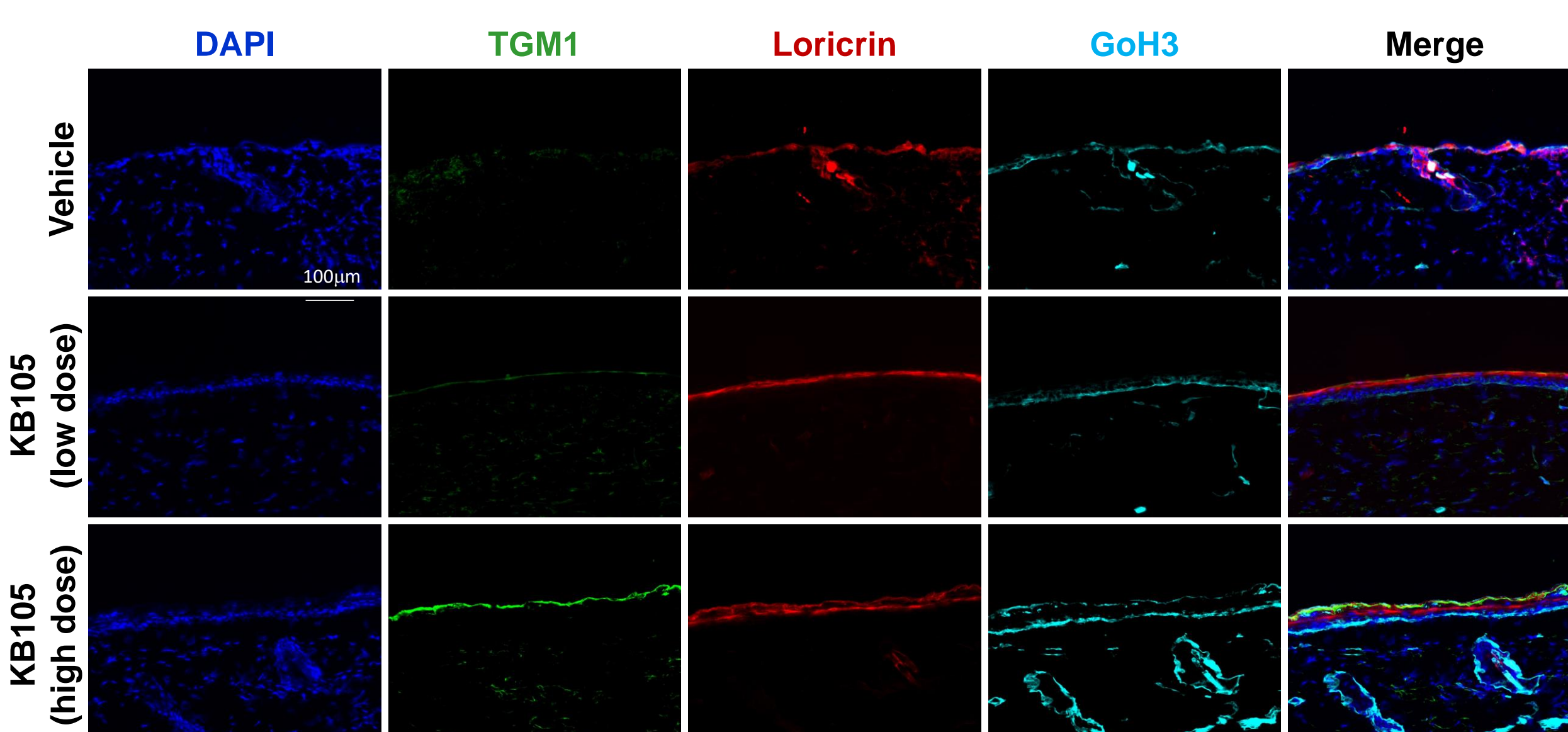


Figure 2. Proper localization of human TGM1 after topical KB105 administration in mice. Loricrin is a marker of the stratum granulosum

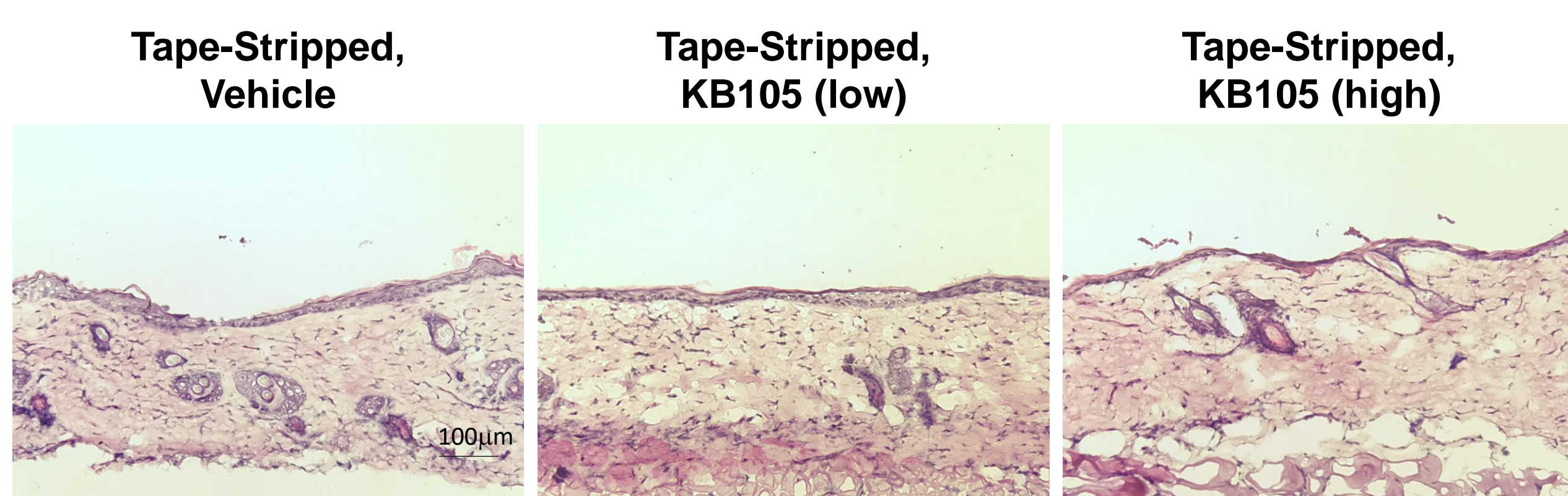


Figure 3. KB105-treated skin appears morphologically normal (comparable to vehicle-treated skin)

RESULTS (CONTINUED)

Short-Term Pharmacokinetics of KB105 Expression After Topical Delivery to BALB/c Mice

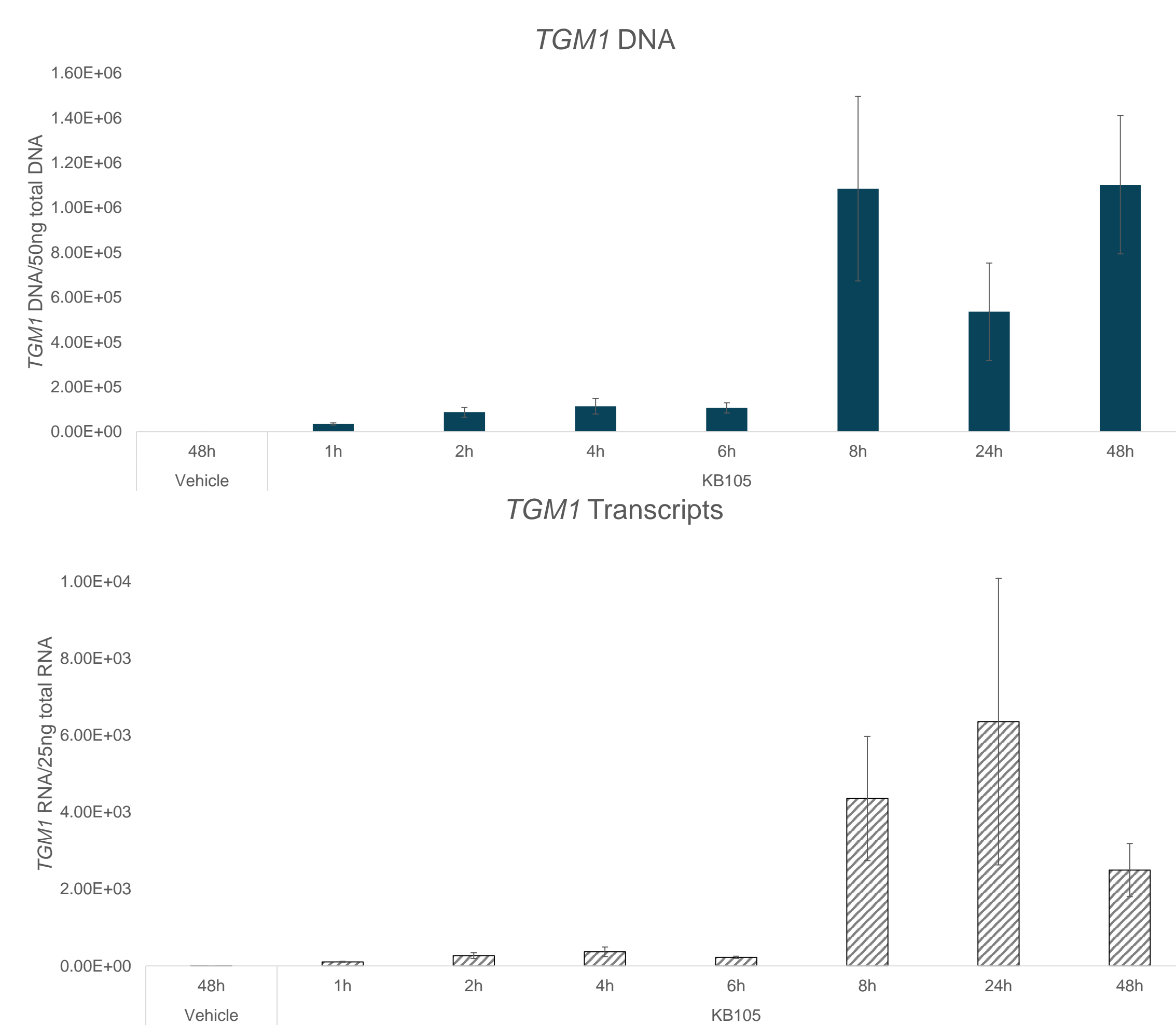


Figure 4. TGM1 transcript expression peaks 24 hours after topical KB105 administration

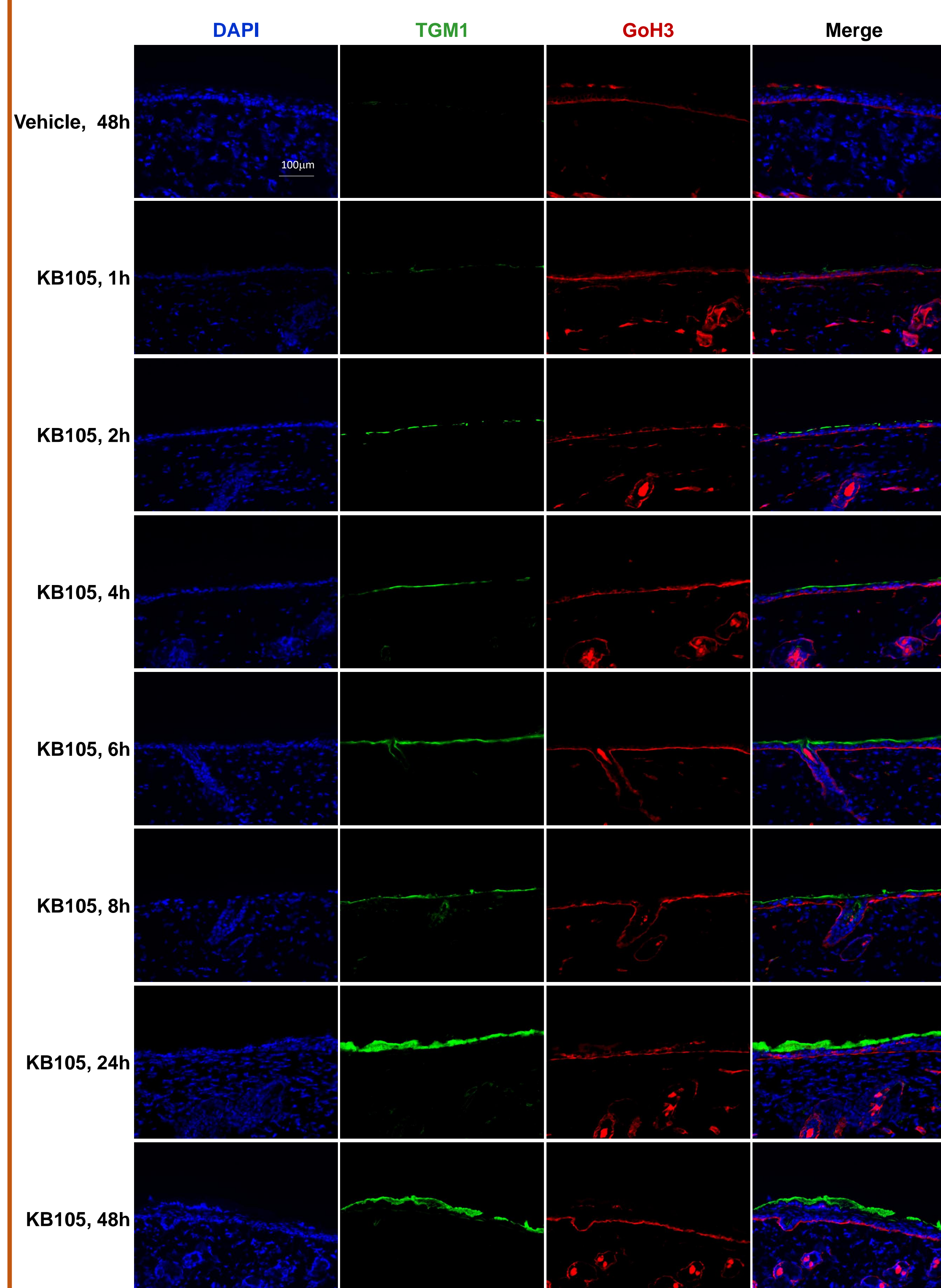


Figure 5. Proper localization of human TGM1 after topical KB105 administration in mice. GoH3 is a marker of the basal layer of the epidermis

RESULTS (CONTINUED)

Repeat Administration of KB105 by Topical Delivery to BALB/c Mice

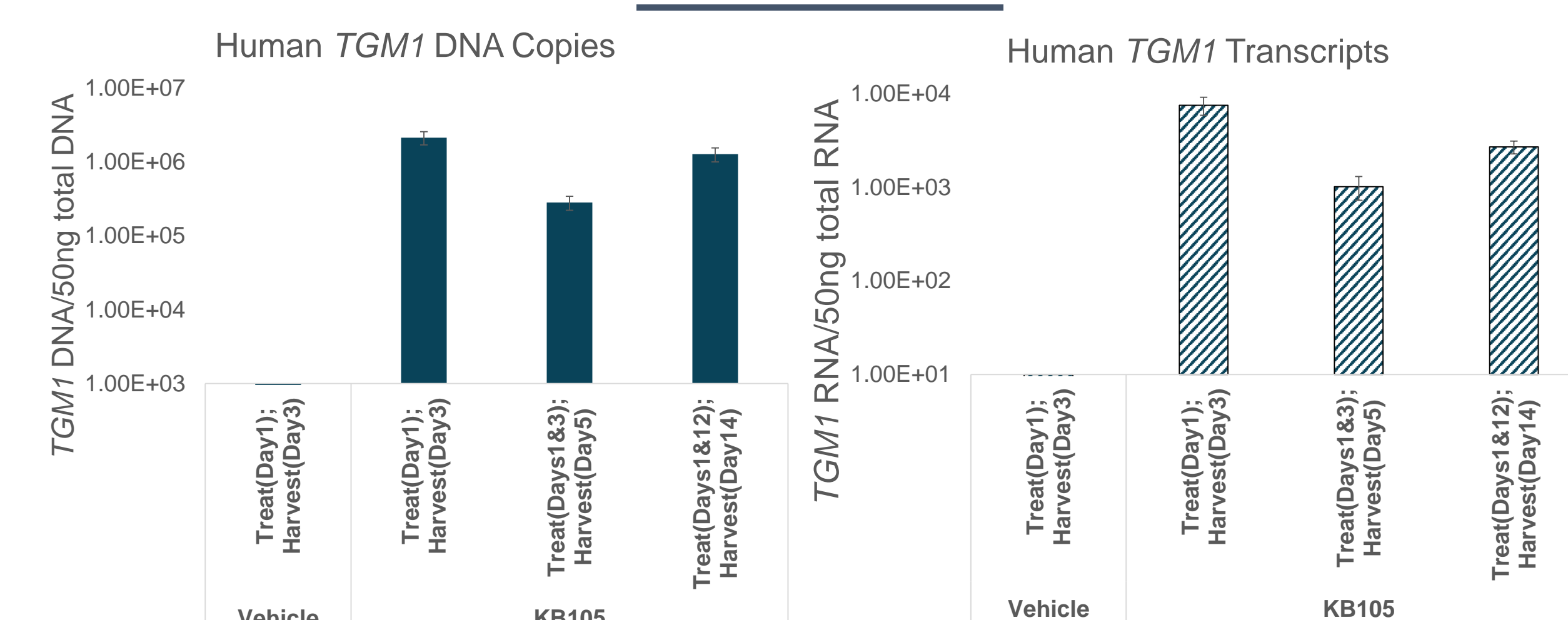


Figure 7. Efficient vector transduction and robust TGM1 expression after repeated topical KB105 administration

GLP Repeat-Dose Toxicity and Biodistribution

Table 2. Toxicity and Biodistribution Study Design

Group	Number of Animals*	Topical Dosing Regimen	Termination	Dose (PFU/day)
Control	6 Males	Days 1, 8, 15, 22, 29	Day 3 (Interim)	-
KB105	6 Females		Day 30 (Terminal) Day 63 (Recovery)	1.07x10 ⁹

*For each termination time point.

Toxicity parameters: mortality, clinical observations, body weights, food consumption, dermal observations, and clinical and anatomic pathology.

Histological assessment: application/dose site, sternum with bone, marrow, brain, epididymis, heart, kidneys, lesions, liver, lungs, axillary lymph node, inguinal lymph node, ovaries, oviducts, prostate, spleen, testes, thymus, uterus with cervix).

Biodistribution (qPCR): Dose site, blood, femur with bone marrow, brain, heart, kidney, liver, lung, lymph nodes, spleen, ovaries and testes.

Results

Five weekly administrations of 1.07x10⁹ pfu/day KB105 via topical application to the dorsal skin of male and female mice were well tolerated.

- NOAEL dose: 1.07x10⁹ pfu/day
- No KB105-related mortality, clinical observations, body weight or food consumption changes, macroscopic findings, or effects on organ weight parameters were noted.
- Minor increase in incidence of edema of the dose site in males between Days 16 and 30 of the dosing phase and persistence of erythema at the dose site during the recovery phase in females were not considered adverse based on severity.
- High copy levels of KB105 detected at the (and limited to) dose site of all KB105-treated animals
 - On Day 3, 3.6x10⁷copies/ug tissue DNA
 - On Day 30 1.3x10⁷copies/ug tissue DNA – **demonstrates successful repeat dosing.**

CONCLUSIONS

- Topical KB105 efficiently transduces permeabilized skin and expresses human TGM1 *in vivo*, in a dose-dependent manner. KB105 application on tape stripped skin samples results in better TGM1 expression compared to non-abraded or acetone treated skin.
- KB105-expressed TGM1 colocalizes with native TGM1 substrates, loricrin, indicating delivery to the appropriate epidermal layer.
- Histology indicates KB105 is well tolerated, even at high doses.
- Biodistribution and toxicity data indicate that KB105 can be safely and repeatedly administered to the skin at high doses without systemic vector exposure.
- Taken together, these data support KB105 as a novel topical gene therapy candidate for the treatment of TGM1-deficient ARCI.