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Supplemental Information

Epidermal E-Cadherin Dependent β-Catenin Pathway Is Phytochemical Inducible and Accelerates Anagen Hair Cycling

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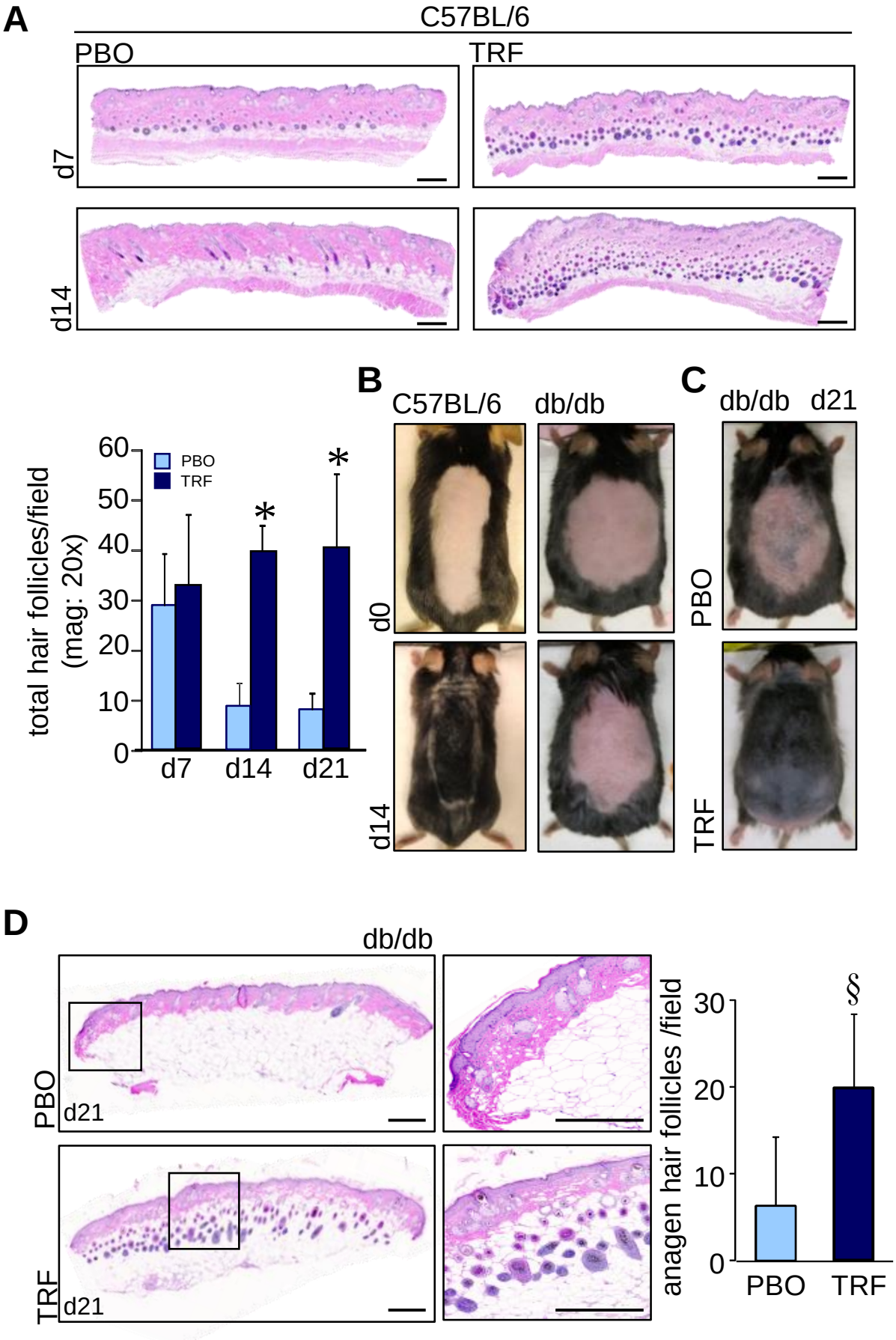
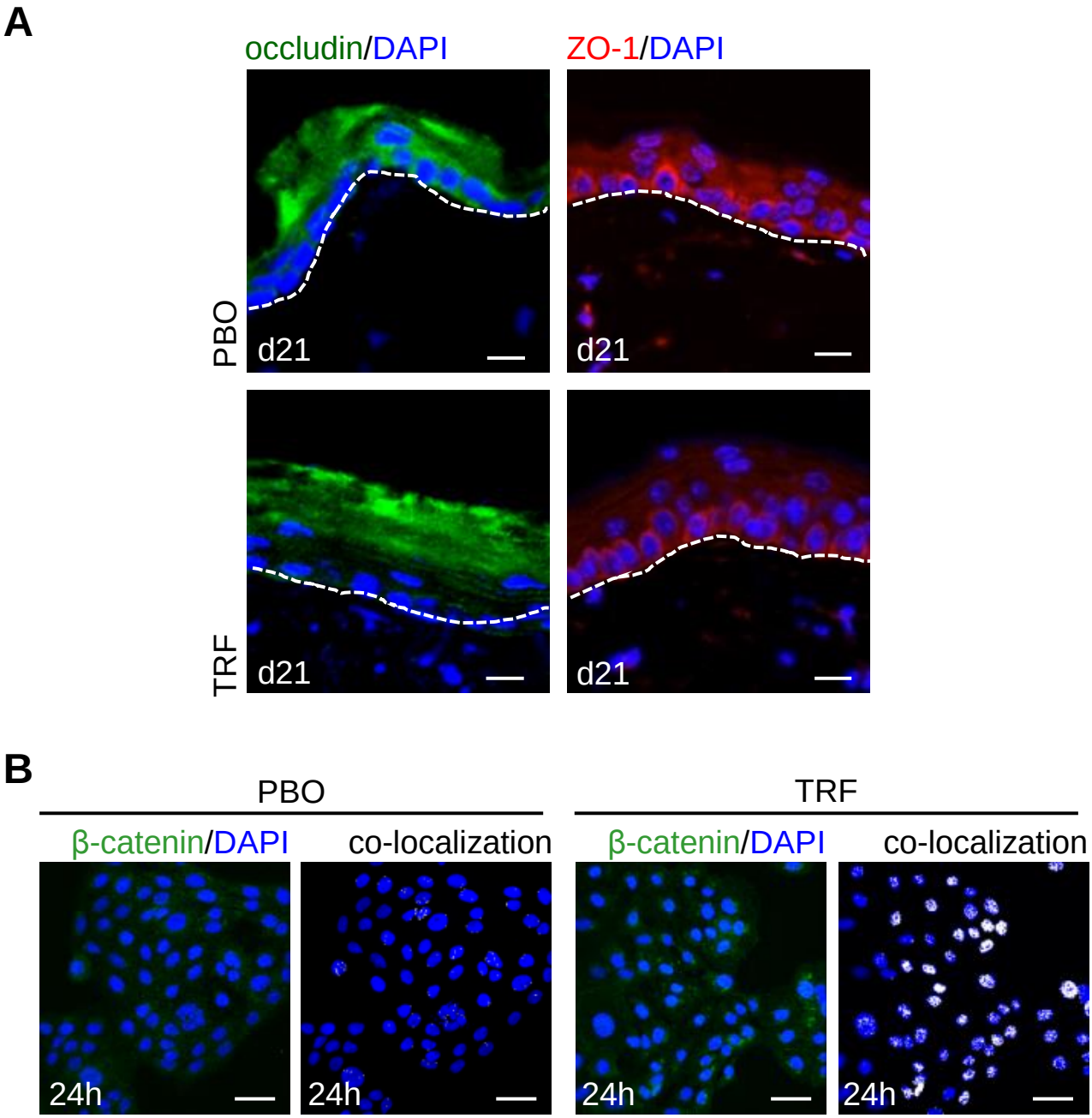


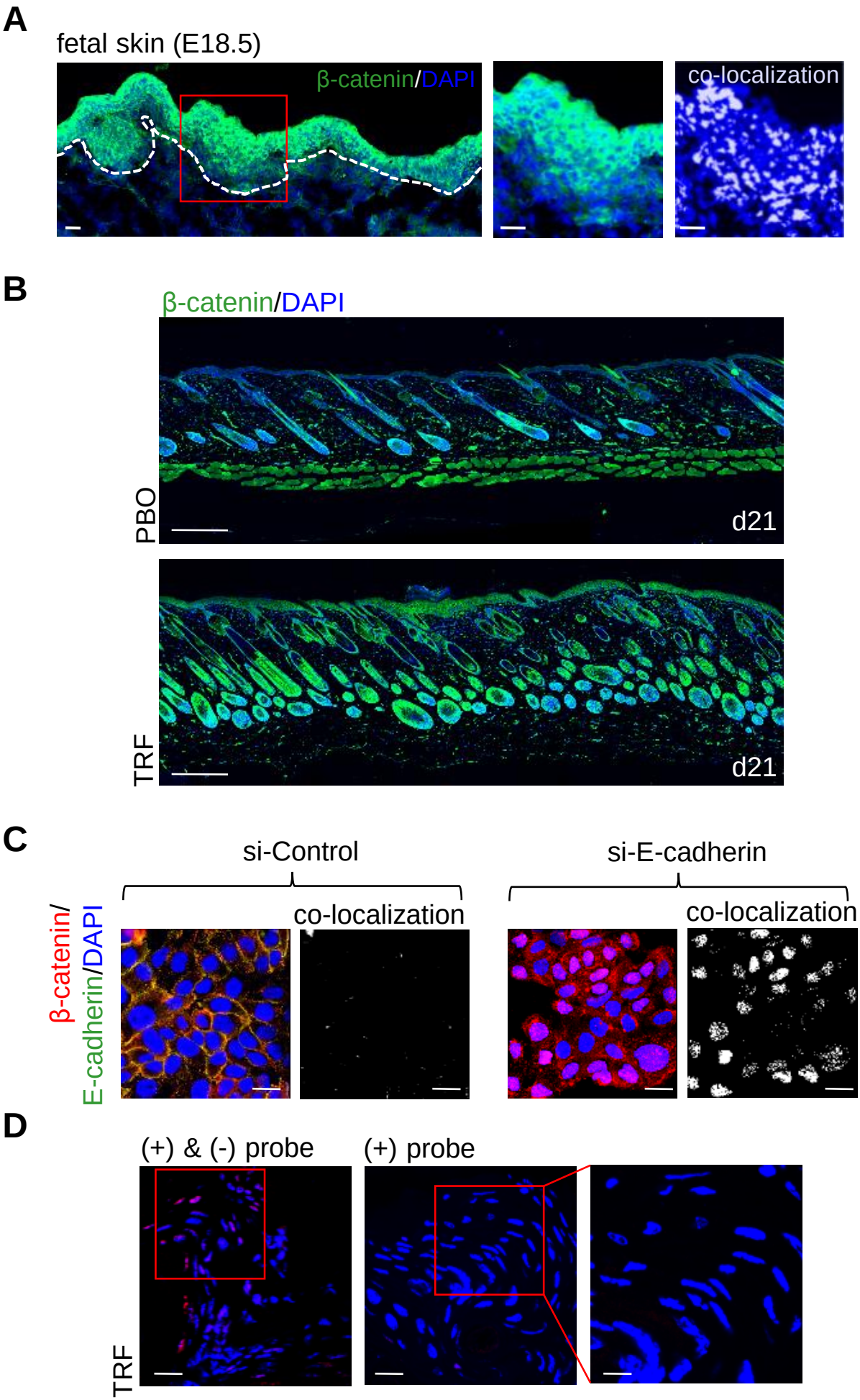
Supplemental Figure 1

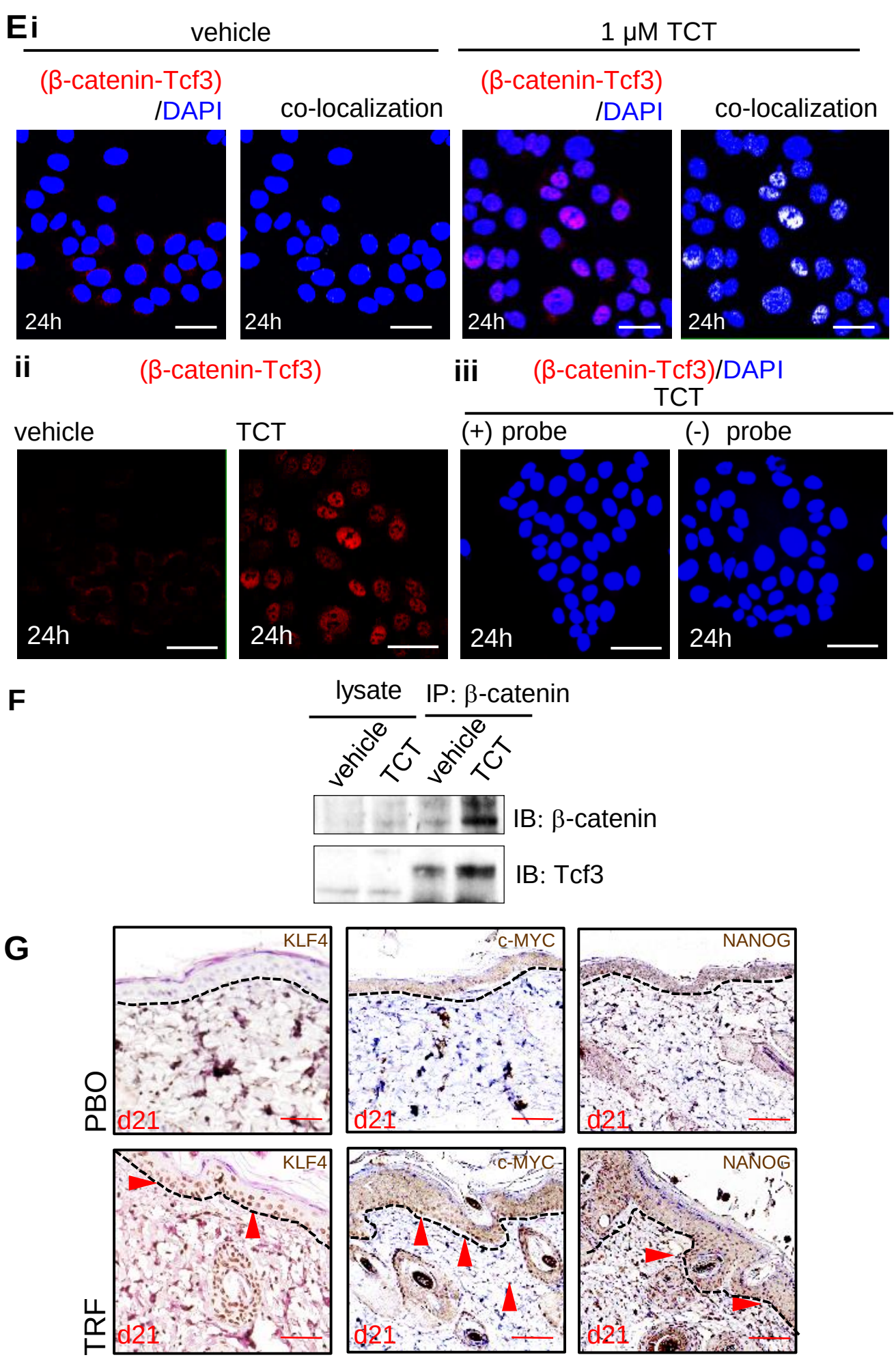
Figure S1: (**A**) Representative photomicrograph of formalin fixed paraffin embedded H&E stained sections showing larger number of anagen hair follicle in TRF treated (days 7 and 14) sections. Scale bar = 500μm. Total number of hair follicles were quantified from the H&E stained sections from each week and plotted graphically. Data are mean ± SD. (n=6) * $p < 0.001$. (**B**) Digital photomicrographs of C57BL/6 (wt) and db/db mice. db/db mice showing less hair growth in naired skin on day 14. (**C**) Application of TRF induced (day 21) hair growth in dorsal skin of diabetic mouse. (**D**) Representative photomicrograph of formalin fixed paraffin embedded H&E stained sections showing large number of anagen hair follicle in TRF-treated (day 21) db/db skin. Scale bar = 500μm. The number of anagen hair follicles on day 21 were quantified from H&E stained sections and plotted graphically. Data are mean ± SD. (n=6) § $p < 0.05$.



Supplemental Figure 2

Figure S2: (**A**) Representative photograph of formalin fixed paraffin embedded adult skin sections showing no change (day 21) in occludin and ZO-1 expression in the epidermis treated with TRF. Counterstained with DAPI. The dermal and epidermal junction is indicated by a dashed line. Scale bar = 20μm. (**B**) HaCaT keratinocytes treated with TRF (equivalent to 1μM tocotrienol) showed increased nuclear translocation of β-catenin (green) compared to PBO at 24h. Co-localization is shown in white. Scale = 50μm.


A
fetal skin (E18.5)
β-catenin/DAPI
co-localization
B
β-catenin/DAPI
PBO
d21
TRF
d21
C
si-Control
si-E-cadherin
co-localization
co-localization
β-catenin/
E-cadherin/DAPI
D
(+) & (-) probe
(+) probe
TRF


Ei
vehicle
1 μM TCT
(β-catenin-Tcf3)
/DAPI
co-localization
(β-catenin-Tcf3)
/DAPI
co-localization
24h
24h
24h
24h
ii
(β-catenin-Tcf3)
iii
(β-catenin-Tcf3)/DAPI
TCT
vehicle
TCT
(+) probe
(-) probe
24h
24h
24h
24h
F
lysate
IP: β-catenin
vehicle
TCT
vehicle
TCT
IB: β-catenin
IB: Tcf3
G
KLF4
c-MYC
NANOG
PBO
TRF
d21
d21
d21
d21
d21
d21

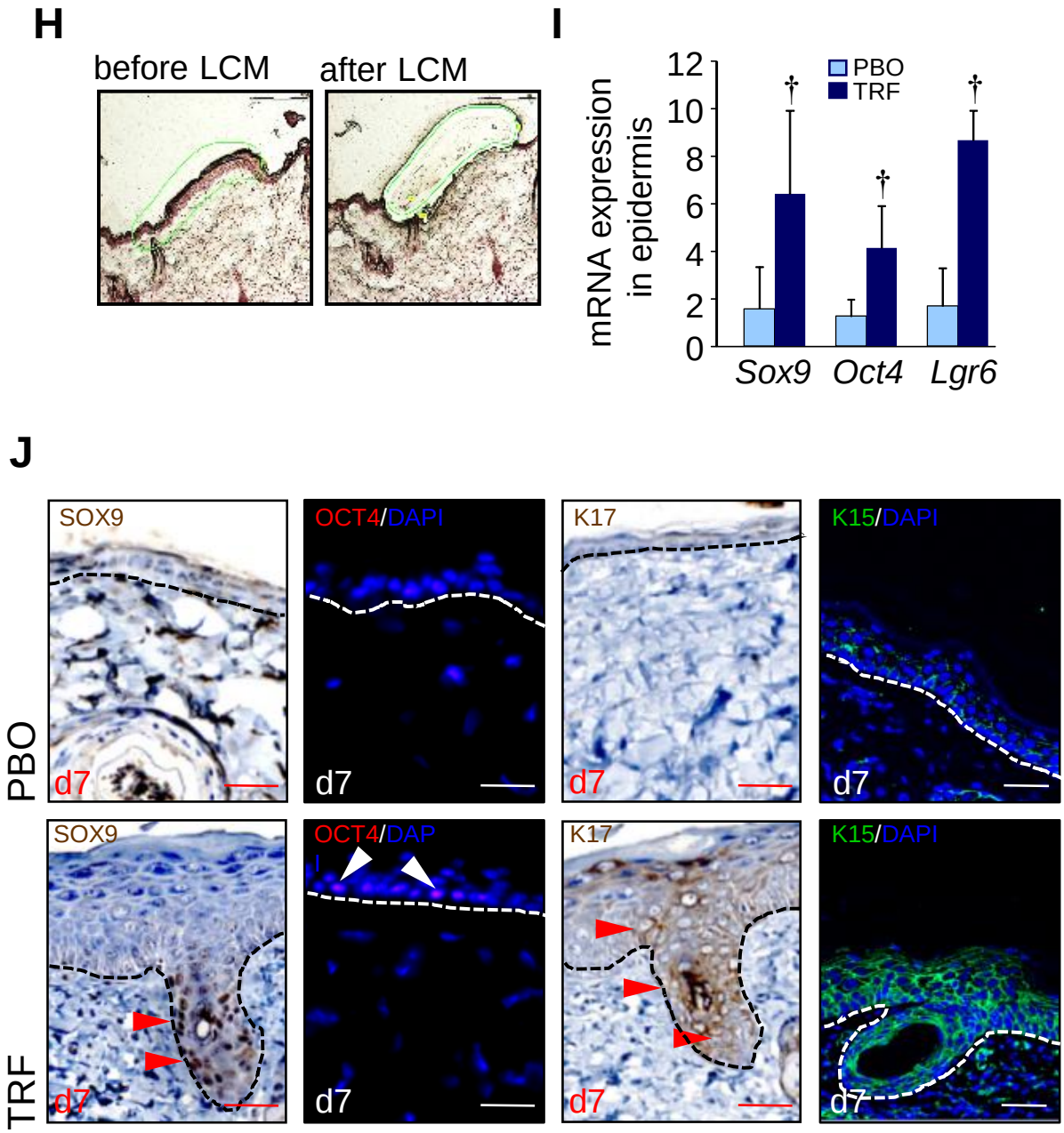

H
before LCM
after LCM
I
mRNA expression in epidermis
12
10
8
6
4
2
0
PBO
TRF
Sox9
Oct4
Lgr6
J
PBO
TRF
SOX9
OCT4/DAPI
K17
K15/DAPI
d7

Figure S3: (**A**) β-catenin is abundantly expressed in fetal skin (E18.5). Confocal microscopic image showing translocation of β-catenin (green) into the nucleus. Counterstained with DAPI. Dermal and epidermal junction is indicated by dashed line in the left panel. Scale bar = 200μm. Co-localization is shown in white in the right panel. Scale bar = 20μm. (**B**) β-catenin expression in the adult skin epidermis counterstained with DAPI at day 21 post-treatment with TRF or PBO. Scale bar = 200μm. (**C**) E-cadherin knockdown in HaCaT keratinocytes induced nuclear translocation of β-catenin. White dots marks co-localization. Scale bar=20μm. (**D**) Proximity ligation assay (PLA) with both + and – probe and + probe alone validating the assay for which data is shown in **Fig. 5C**. (**E**) (*i*) Proximity ligation assay (PLA) showing the interaction of β-catenin and Tcf3 in the nucleus 24h after treatment with 1μM pure tocotrienol. (ii) PLA showing the β-catenin and Tcf3 co-localization as red dots (related to fig S3Ei. (iii) PLA with (+) probe and (–) probe alone validating the assay. Scale bar = 50μm (**F**) The nuclear lysates from HaCaT keratinocytes after 24h treatment with either vehicle or 1μM pure tocotrienol were subjected to immunoprecipitation with β-catenin antibody. The immunoprecipitates (*IP*) were subjected to SDS-PAGE and subjected to immunoblotting (*IB*) for the detection of Tcf3.

(**G**)KLF4, c-MYC, and NANOG expression in adult skin epidermis on day 21 post-treatment with PBO and TRF. Dermal and epidermal junction is indicated by black dashed line. Scale bar = 20μm. (**H**) Laser captured epidermis was subjected to (**I**) quantitative PCR analysis of *Sox9* , *Oct4* and *Lgr6* (day 21 after PBO and TRF treatment). Data are mean ± SD, † $p<0.01$ compared to PBO. (**J**) SOX9, OCT4, K15 and K17 expression in adult skin epidermis on day 7 post-treatment with TRF. Dermal and epidermal junction is indicated by a dashed line. Scale bar = 20μm.

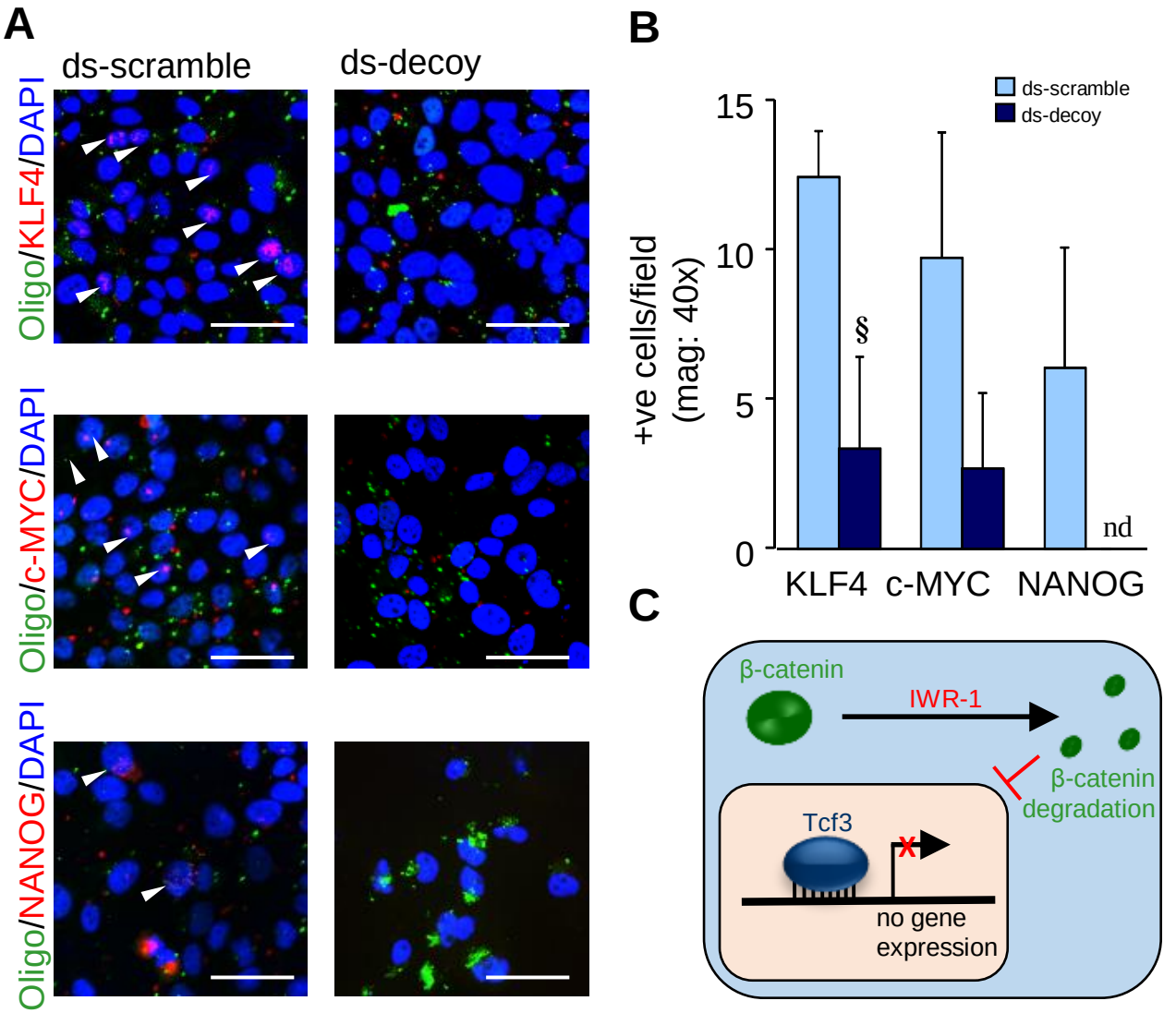


Figure S4: (**A**) Expression of KLF4, c-MYC, and NANOG in Tcf3 scramble and decoy transfected cells after pure tocotrienol treatment (1μM, 24h). Scale bar = 50μm. (**B**) The number of positive cells per field were quantified and plotted graphically. Data are mean ± SD. (n=3) § $p<0.05$. nd, not detected. (**C**) Schematic representation of how IWR-1 is inhibiting β-catenin nuclear translocation and subsequent activation of target gene.

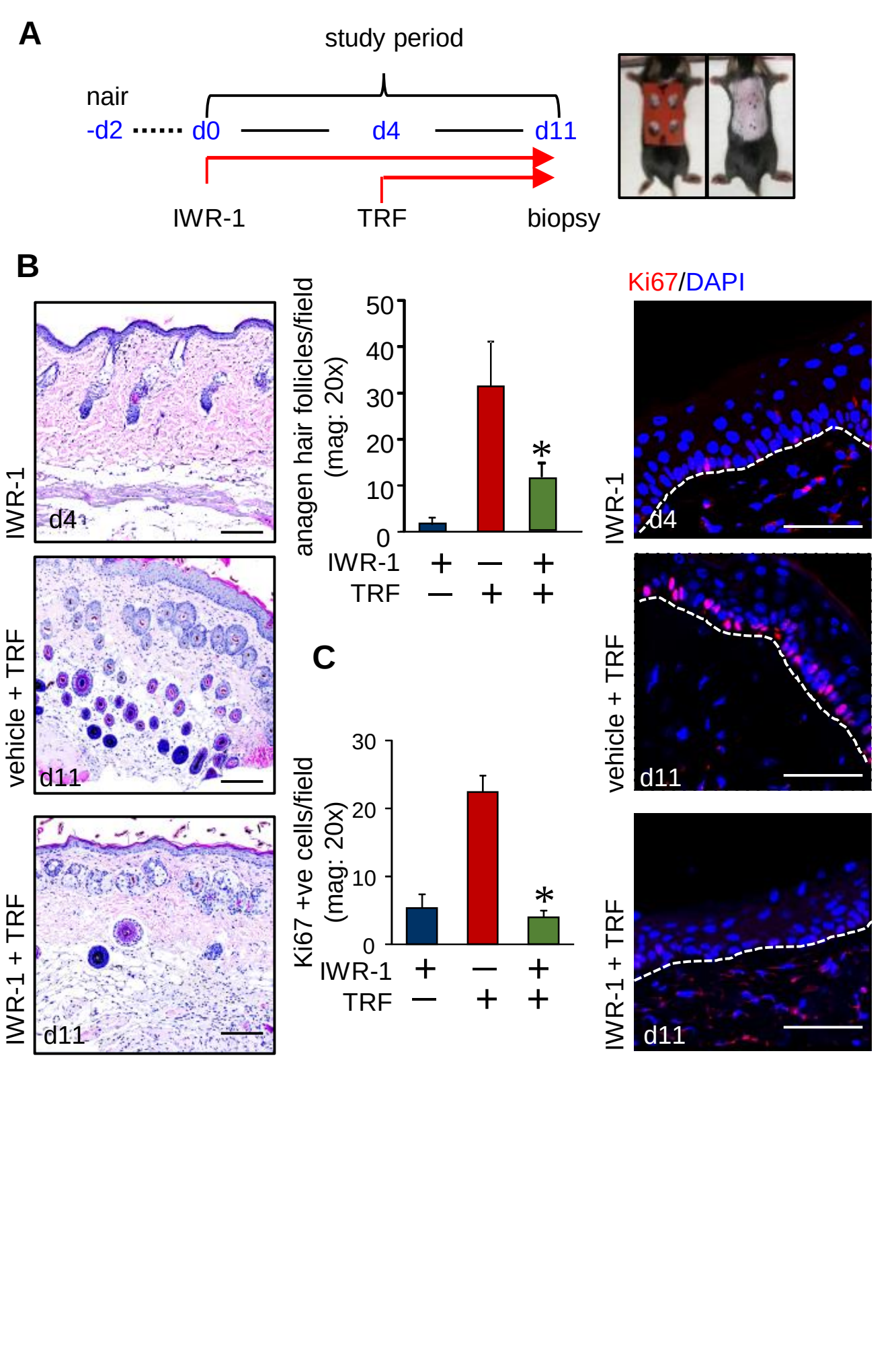

A
study period
nair
-d2
d0
d4
d11
IWR-1
TRF
biopsy
B
IWR-1
d4
vehicle + TRF
d11
IWR-1 + TRF
d11
anagen hair follicles/field
(mag: 20x)
50
40
30
20
10
0
*
IWR-1 + —
TRF — +
Ki67/DAPI
IWR-1
d4
vehicle + TRF
d11
IWR-1 + TRF
d11
C
Ki67 +ve cells/field
(mag: 20x)
30
20
10
0
*
IWR-1 + —
TRF — +

Figure S5: (**A**) Design of topical IWR-1 studies. (**B**) H&E stained skin sections showing that IWR-1 attenuated TRF-induced increase in the number of hair follicles at day 11 (day 7 post TRF application). Scale bar = 50μm. Hair follicles were enumerated and expressed graphically as mean ± SD, * $p<0.001$ compared to vehicle TRF with vehicle. (**C**) Graphical representation of $Ki67^{+}$ (red) cells in formalin fixed paraffin embedded skin sections of mice treated with IWR-1 or vehicle DMSO followed by TRF treatment. IWR-1 treatment blunted TRF-induced cell proliferation in the epidermis. Scale bar = 50μm

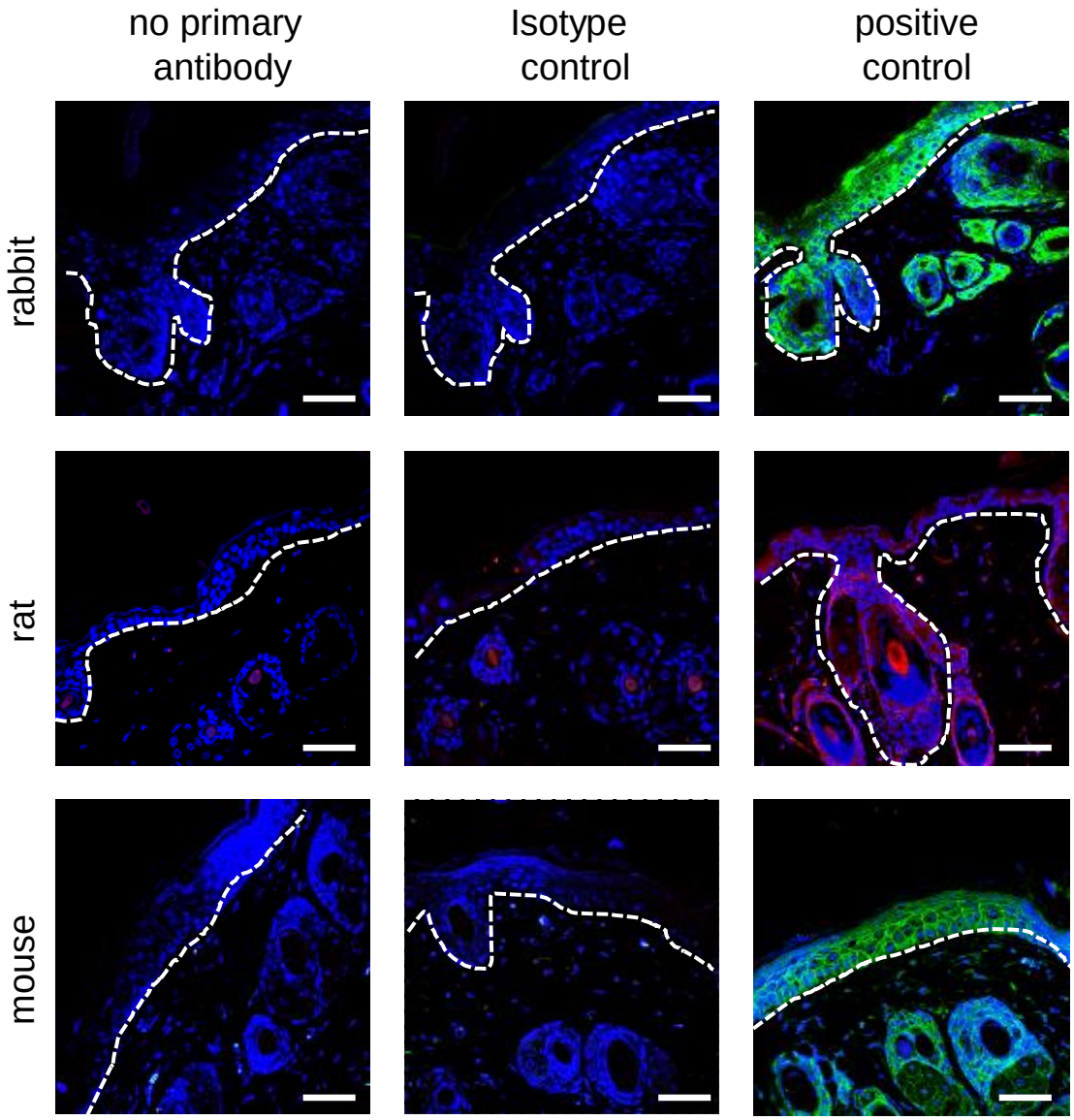


Figure S6: Specificity of the antibodies used in the study was validated using no antibody control and isotype controls of respective host species. The white dash line indicates the epidermal and dermal junctions. Scale bar = 50μm.