SUPPLEMENTARY MATERIALS AND METHODS:

Patients, derivation of primary keratinocytes, and induction of keratinocyte differentiation: Kidney transplant recipients (KTRs) gave informed consent and were enrolled in the study approved by Northwestern University's Biomedical Institutional Review Board (IRB project number STU00069552). Full thickness abdominal skin biopsies (4 mm x 2) were taken at the time of a post-transplant kidney biopsy. KTRs with a history of skin cancer were excluded from the study. One biopsy was placed in formalin for histological processing and the other in Hank's Balanced Salt Solutions buffer (HBSS containing no $CaCl_2$ and no phenol red, GE Healthcare Life Sciences, Logan, UT) plus 10% calf serum plus gentamycin/amphotericin B (Life Technologies, Grand Island, NY, USA) for transport to the Northwestern University skin disease research center (SDRC). Patient privacy rights were observed by assigning each biopsy a unique study number prior to transport to the SDRC. The skin utilized for the immunocompetent patient (ICP) group was predominantly discarded tissue removed during abdominoplasty with 2 exceptions (buttock and breast skin, **Supplementary Table S1**). ICP-14 was consented since tissue was sent to pathology for processing as standard of care. Both consent and waiver of consent in the case of discarded tissue was approved (IRB project number STU00009443 through the SDRC). Skin cancer history in the ICPs was unknown. Primary keratinocytes were isolated by the SDRC as described (Halbert *et al.*, 1992) or purchased from Lifeline Cell Technology (Frederick, MD) or ATCC (Manassas, VA) (**Supplementary Table S2** reports cell line sources). Upon receipt in the SDRC, cells were propagated initially as co-cultures with J2-3T3 feeder cells in FAD medium (DMEM:Ham's F-12 with L-glutamine [3:1; Sigma-Aldrich, St, Louis, MO] supplemented with 10% fetal bovine serum, 1X E medium cocktail [180 μM

adenine, 5 μg/ml insulin, 5 μg/ml transferrin, 5 μg/ml T_3], 0.4 ng/ml hydrocortisone, 10 μg/ml cholera toxin, 10 ng/ml EGF, and 1000× gentamycin/amphotericin B solution) for 2-3 passages then switched to M154 medium supplemented with human keratinocyte growth supplement (HKGS, Life Technologies), 1000× gentamycin/amphotericin B solution, and 0.07 mM $CaCl_2$ (low calcium) for 1 passage prior to plating for the experiment. Confluent keratinocyte monolayers in low calcium M154 were induced to differentiate by addition of 1.2 mM $CaCl_2$ (final concentration high calcium) in M154 in the absence of HKGS for 48 or 72 hours. While the goal was to harvest each sample before the culture was 35 days old or ≤passage 5, this was not always possible due to the high number of cells needed to obtain confluent cultures and to freeze for repeat experiments.

Histology and indirect immunofluorescence microscopy: Histological sections from paraffin blocks from one ICP an all KTR biopsies were cut to a thickness of 4μm and hematoxylin and eosin (H&E) staining was performed by the SDRC pathology and tissue phenotyping core. Tissue microarrays (TMAs) containing normal human skin and staged SCC tissues cut to a thickness of 5μm (http://www.biomax.us/tissue-arrays/Skin/SK801b and http://www.biomax.us/tissue-arrays/Skin/SK802a) were purchased from US Biomax, Inc (Rockville, MD). These TMAs have recently been utilized in other publications (Siegle *et al.*, 2014; Yang *et al.*, 2015). All slides for indirect immunofluorescence microscopy were baked at 60°C overnight, de-paraffinized by xylenes, dehydrated with ethanol, rehydrated in PBS, and permeabilized by 0.5% Triton X-100 in PBS. Antigen retrieval was performed by incubation in 0.01 M citrate buffer (pH 6.0) at 95°C for 15 minutes. Sections were blocked in 1% BSA/0.05% Tween/PBS for 30 minutes at 37°C. Primary antibody incubation was carried out overnight at

4°C in blocking buffer followed by washing in PBS. Secondary antibody incubation was carried out at 37°C for 45 minutes followed by washing in PBS. Sections were stained with 4′,6-Diamidino-2-phenylindole (DAPI - Sigma-Aldrich) at a final concentration of 5 ng/μl at room temperature for 2 minutes followed by washing in PBS and water. Cover slips were mounted using Gelvatol. Images were obtained with a 63x PL Fluotar, NA 1.0 objective on a Leica DMR microscope using a charge-coupled device camera (Orca 100, model C4742-95, Hamamatsu, Bridgewater, NJ) and MetaMorph 6.1 software (MDS Analytical Technologies, Union City, CA) for fluorescence or a Leica DFC320 digital camera and Photoshop software (Adobe Systems, Mountain View, CA) for H&E images. Automated imaging was achieved using a Zeiss upright AXIO scope (TissueGnostics GmbH, Vienna, Austria), with TissueFAXS version 3.5.5.0129 for image acquisition and TissueQuest version 4.01.0137 for image analysis. Settings for image acquisition were identical for each sample. Only cells with detectable nuclei (DAPI) were analyzed and identical masks and settings were applied to all samples for automated image analysis. For the KTR and ICP biopsies, only the epidermis was selected for automated Dsg1 staining intensity analysis, and the stratum corneum was excluded. For the TMAs, Dsg1 staining intensity was analyzed only within cells that co-stained for Pg (658 nm). Statistical comparisons were made between each patient group and the normal human skin samples using a 2-tailed Student's t test. The p values were not adjusted for multiple testing. A p value <0.05 was considered significant.

Antibodies: Mouse monoclonal antibodies used: P124 (Dsg1 extracellular domain utilized for histology; Progen, Heidelberg, Germany), 27B2 (Dsg1 cytodomain utilized for immunoblot; Invitrogen, Grand Island, NY, USA), and HECD1 E-cad (E-cadherin; Takara, Kyoto, Japan).

Rabbit polyclonal antibodies used: K10 (gift from J. Segre, National Human Genome Research Institute, Bethesda, MD), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase; Sigma-Aldrich). Chicken polyclonal antibody used: 1407 Pg (plakoglobin; Aves Laboratories, Tigard, OR) and Loricrin (gift from J. Segre, National Human Genome Research Institute, Bethesda, MD). Secondary antibodies for immunoblotting were goat anti–mouse, –rabbit, and –chicken peroxidase (Rockland; KPL, Gaithersburg, MD). Secondary antibodies for indirect immunofluorescence microscopy were goat anti–mouse, –rabbit, and –chicken linked to fluorophores of 488 nm, 568 nm, and 658 nm (Alexa Fluor; Invitrogen).

Immunoblot analysis of proteins: Whole cell lysates were collected in Urea-SDS buffer (8M Urea/ 1% Sodium dodecyl sulfate/ 60 mM Tris pH 6.8/ 5% β-mercaptoethanol/ 10% glycerol) and sonicated. Samples separated by SDS-PAGE were transferred to nitrocellulose, blocked for 1 hour at room temperature in 5% milk/PBS and probed with primary antibodies. Primary antibodies were incubated for 1 hour at room temperature in 5% milk/PBS. Secondary, HRP-conjugated antibodies diluted 1:5000 in milk were added to blots for 20 minutes at room temperature after washing with PBS. Protein bands were visualized using enhanced chemiluminescence and exposure to X-ray film (HyBlot CL Autoradiography film, Denville Scientific, Inc, Holliston, MA).

Immunoblot densitometry and statistical analysis: Immunoblot images were digitized using an HP Officejet 5610 All-in-One office scanner at 300 dpi and saved as grayscale mode TIFF documents. Adobe Photoshop (Creative Cloud Suite) was utilized for quantification of the bands by densitometry. The image was inverted (black bands made white) and a rectangular border of

uniform size was drawn around each protein band. Using the histogram of pixels, the mean (average intensity value) and the mean background value (film) were recorded in Excel, and the background subtracted from the average intensity for each band. To mitigate protein loading variability, all lanes were normalized to GAPDH protein loading controls. All values were normalized to a single sample (ICP-13 harvested at P4D22) loaded on each gel to allow blot-to-blot comparison and to mitigate film exposure variability. Averaged or individual densitometry values after double normalization were statistically analyzed to compare protein expression in keratinocytes from ICPs and KTRs using a 2-tailed Student's t test. A p value <0.05 was considered significant.

SUPPLEMENTARY FIGURE LEGENDS:

Supplementary Figure S1: Abdominal skin histology hematoxylin and eosin (H&E) staining of 1 immunocompetent control patient (ICP-2) and the 15 immunosuppressed kidney transplant recipients (KTR-1-15) who donated biopsies for this study. Bar = 50 μm. H&E staining from the tissue microarrays (TMAs) utilized in this study can be viewed at http://www.biomax.us/tissue-arrays/Skin/SK801b and http://www.biomax.us/tissue-arrays/Skin/SK802a.

Supplementary Figure S2: Protein expression levels of K10 (a), Ecad (b), and Pg (c) were compared in the same cultured keratinocytes derived from ICPs or KTRs and analyzed for Dsg1 expression in Figure 2. Keratinocytes were grown to confluence in low calcium-containing (0.07 mM) medium, switched to high calcium-containing medium (1.2 mM) to induce differentiation, and harvested after 48 hours. K10, Ecad, and Pg levels were not significantly different between the 2 groups ($p=0.136$, $p=0.246$, and $p=0.212$, respectively).

Supplementary Figure S3: Immunoblots from all samples that were quantified for this study (differentiated keratinocyte lysates derived from both kidney transplant recipients [K-1-15] and immunocompetent patients [I-1-16] harvested 48 hours [Dsg1, K10, Pg, Ecad, GAPDH] or 72 hours [loricrin, GAPDH denoted by black borders] after induction of differentiation by increasing calcium concentration). The numbers in parentheses indicate the number of gel lanes for each patient sample, representing individual experiments (replicates). GAPDH serves as a protein loading control for lane-to-lane normalization. The (*) above the second lane of the I-13 sample indicates that this was the reference sample included on each gel for purposes of blot-to-blot normalization.

SUPPLEMENTARY TABLES:

Supplementary Table S1: Characteristics of skin biopsy donors

ID	Age (y)	Sex	Ethnicity	Body site	Immunosup-pression (months)	CNI (μg/mL)	MMF (μg/mL)
ICP-1	35	F	W	Abdomen	NA	-	-
ICP-2	57	F	W	Abdomen	NA	-	-
ICP-3	46	F	W	Abdomen	NA	-	-
ICP-4	47	F	W	Abdomen	NA	-	-
ICP-5	40	F	W	Abdomen	NA	-	-
ICP-6	45	F	H	Abdomen	NA	-	-
ICP-7	50	F	H	Abdomen	NA	-	-
ICP-8	18	M	H	Buttocks	NA	-	-
ICP-9	39	F	H	Abdomen	NA	-	-
ICP-10	42	F	AA	Abdomen	NA	-	-
ICP-11	31	M	AA	Abdomen	NA	-	-
ICP-12	32	F	AA	Abdomen	NA	-	-
ICP-13	33	F	AA	Abdomen	NA	-	-
ICP-14	43	F	AA	Breast	NA	-	-
ICP-15	45	F	AA	Abdomen	NA	-	-
ICP-16	29	M	AA	Abdomen	NA	-	-
KTR-1	67	F	W	Abdomen	8	4.4	+
KTR-2	61	F	W	Abdomen	9	9.5	+

KTR-3	57	M	W	Abdomen	10	8.5	+
KTR-4	22	M	W	Abdomen	12	5.4	+
KTR-5	42	M	W	Abdomen	12	5.1	+
KTR-6	72	F	W	Abdomen	14	7.2	+
KTR-7	57	F	W	Abdomen	22	5.1	1.4
KTR-8	34	M	H	Abdomen	8	3.4	+
KTR-9	34	M	H	Abdomen	12	3.5	4
KTR-10	55	M	H	Abdomen	17	3.4	+
KTR-11	34	M	AA	Abdomen	10	Cyc 327	+
KTR-12	52	M	AA	Abdomen	12	4.9	+
KTR-13	63	F	AA	Abdomen	14	5.5	+
KTR-14	35	M	AA	Abdomen	24	9.3	+
KTR-15	66	F	AA	Abdomen	24	6.5	4.5

ID = patient study identifier, Y = years, F = female, M = male, W = white, H = Hispanic, AA = African American. NA indicates patient was included in the immunocompetent control group. A "-" indicates that a patient was not taking an agent, A "+" indicates the patient was taking an agent but blood levels were not measured at the time of skin biopsy. CNI = calcineurin inhibitor (tacrolimus blood level except patient KTR-11 who was taking cyclosporine [Cyc]), MMF = mycophenolate mofitil blood level.

Supplementary Table S2: Cell line characteristics at time of use in experiments and corresponding relative desmoglein 1 levels

ID	Purchase source	Passage/ day 1 (Dsg1 level)	Passage/ day 2 (Dsg1 level)	Passage/ day 3 (Dsg1 level)	Passage/ day 4 (Dsg1 level)	Passage/ day 5 (Dsg1 level)	Avg. Dsg1 level after normalizing to selected control (ICP-13)	SD
ICP-1	SDRC	NA[2]	NA[2]	P5D23 (70.4)	P5D23 (73.8)	P6D25 (106)	83.4	16.1
ICP-2	SDRC	NA[2]	NA[2]	P5D27 (70.5)	P5D28 (148.2)	P6D31 (174.5)	131.1	44.1
ICP-3	SDRC	NA[2]	NA[2]	P5D30 (19.6)	P5D35 (15.4)	P6D39 (33)	22.6	7.5
ICP-4	SDRC	NA[2]	NA[2]	P5D25 (155)	P5D28 (143.8)	P6D32 (246)	181.6	45.7
ICP-5	SDRC	NA[2]	NA[2]	P4D21 (51.7)	P5D26 (52.8)	P5D24 (86.5)	63.7	16.2
ICP-6	Lifeline	NA[2]	NA[2]	P4 (71.1)	P4 (108.5)	P6 (8.3)	62.6	41.3
ICP-7	Lifeline	NA[2]	NA[2]	P5 (15.1)	NA[3]	NA[3]	15.1	-
ICP-8	Lifeline	NA[2]	NA[2]	P5	NA[3]	NA[3]	48.6	-

				(48.6)				
ICP-9	SDRC	NA[2]	NA[2]	P4D17 (78.7)	P5D22 (76.9)	P5D22 (65.9)	73.8	5.6
ICP-10	Lifeline	NA[2]	NA[2]	P5 (48.8)	P6 (26.2)	P7 (25.4)	33.5	10.8
ICP-11	Lifeline	NA[2]	NA[2]	P5 (1.1)	NA[3]	NA[3]	1.1	-
ICP-12	SDRC	NA[2]	NA[2]	P3D14 (70.3)	P5D20 (59.4)	P5D20 (56.9)	62.2	5.8
ICP-13	SDRC	NA[2]	NA[2]	P3D18 (54.9)	P4D22 (21.2)	P5D38 (0.8)	25.6	22.3
ICP-14	SDRC	NA[2]	NA[2]	P3D19 (25)	P4D26 (15.5)	NA[3]	20.3	4.8
ICP-15	ATCC	NA[2]	NA[2]	P5 (10.8)	P4 (23)	P5 (1.9)	11.9	8.7
ICP-16	ATCC	NA[2]	NA[2]	P5 (25.2)	P6 (0.3)	P7 (1.1)	8.9	11.6
KTR-1	SDRC	D42 (0.14)	P4D40 (1)	NA[3]	NA[3]	P3D47 (59.8)	20.3	28
KTR-2	SDRC	D40 (1.97)	P4D27 (9.2)	P5D40 (29)	P5D39 (42.9)	P5D31 (61.1)	28.8	21.7
KTR-3	SDRC	NA[1]	P4D24 (14.1)	P5D33 (19.5)	P5D31 (36.4)	P5D25 (33.1)	25.8	9.2

KTR-4	SDRC	NA[1]	P5D27 (27.8)	P6D29 (27.5)	P6D30 (34.3)	P6D31 (35.5)	31.3	3.6
KTR-5	SDRC	NA[1]	P5D31 (15)	P6D37 (28.1)	P6D36 (37.4)	P6D37 (34.1)	28.7	8.6
KTR-6	SDRC	D31 (19.2)	P3D27 (20.7)	P4D37 (24.8)	P3D32 (0.6)	P4D37 (20.1)	17.1	8.5
KTR-7	SDRC	D32 (2.14)	P5D40 (1.3)	P5D41 (55.1)	P4D26 (47.2)	P4D26 (27.6)	26.7	22.3
KTR-8	SDRC	D33 (27.4)	P4D31 (4.1)	NA[3]	P4D33 (30)	P4D26 (22.6)	21	10.1
KTR-9	SDRC	D19 (27.2)	P3D20 (26.2)	NA[3]	P4D32 (68.2)	P4D27 (49.7)	42.8	17.4
KTR-10	SDRC	D27 (7.27)	P4D32 (5.5)	NA[3]	P4D25 (28)	P4D27 (17.7)	14.6	9
KTR-11	SDRC	D45 (3.67)	NA[3]	NA[3]	P4D25 (2.7)	P4D22 (4.5)	3.6	0.8
KTR-12	SDRC	P6D39 (30.9)	P4D24 (27.2)	P5D28 (18.8)	P5D29 (90.6)	P4D19 (35.6)	40.6	25.6
KTR-13	SDRC	D53 (23.3)	NA[3]	NA[3]	P5D29 (35.4)	P4D23 (36.5)	31.7	5.9
KTR-14	SDRC	D41 (12.6)	P5D27 (8.6)	P6D31 (7.9)	P6D33 (20.4)	P6D32 (19.2)	13.7	5.2
KTR-15	SDRC	D25	P5D31	P5D31	P4D22	P4D18	49	11

		(39.6)	(32.3)	(60.2)	(55.2)	(57.7)		

ID = patient study identifier, SDRC = Northwestern University Skin Disease Research Center, ATCC = American Type Culture Collection, Lifeline = Lifeline Cell Technology. NA indicates that the experiment was not performed on the cell line at that time[1], the cell line was not available to us at the time of that particular experiment[2], or the cell line was not reproducibly able to be grown to confluence after being frozen down[3]. If a passage number (P) or day after biopsy (D) is not given, it indicates that the information was unknown. Desmoglein 1 (Dsg1) levels are reported based on densitometry of images taken following immunoblot (**Supplementary Figure S3**) followed by normalization to a reference sample as discussed in Materials and Methods. SD = standard deviation.

Supplementary Table S3: Average K10, Ecad, and Pg levels across experiments

ID	Avg. K10 level after normalizing	SD	Avg. Ecad level after normalizing	SD	Avg. Pg level after normalizing	SD
ICP-1	34.1	0.6	38.4	3.0	47.3	3.7
ICP-2	39.7	10.2	43.6	7.1	54.1	12.2
ICP-3	27.3	7.9	29.6	3.1	34.0	1.7
ICP-4	79.9	9.7	45.2	4.0	46.0	7.6
ICP-5	42.6	13.7	41.7	7.2	51.0	6.7
ICP-6	70.8	38.6	49.6	7.9	56.6	7.9
ICP-7	7.9	-	13.9	-	29.7	-
ICP-8	24.7	-	26.2	-	38.9	-
ICP-9	180.6	4.1	37.0	6.3	67.1	12.1
ICP-10	82.4	44.2	24.3	4.7	28.9	4.7
ICP-11	7.8	-	19.2	-	7.4	-
ICP-12	71.9	3.0	49.3	2.7	49.2	4.9
ICP-13	34.7	18.5	27.9	12.0	35.5	5.2
ICP-14	36.2	8.5	47.6	2.9	56.5	2.8
ICP-15	12.5	5.8	7.2	2.7	31.7	4.9
ICP-16	15	19.7	12.6	9.8	35.4	8.3
KTR-1	15.2	18.2	17.6	3.8	47.1	9.0
KTR-2	30.4	21.2	30.7	6.6	49.2	8.2
KTR-3	25.2	9.2	30.3	2.7	38.5	4.9

KTR-4	35.4	7.8	25.2	3.7	28.2	1.2
KTR-5	22.6	6.6	26.8	4.8	36.9	3.7
KTR-6	15.5	6.1	22.1	4.4	32.7	6.2
KTR-7	35.7	26.3	29.6	7.2	33.9	14.6
KTR-8	36.0	20.2	29.1	3.7	36.7	4.9
KTR-9	32.2	8.5	29.4	3.0	31.8	13.3
KTR-10	41.3	25.7	23.6	4.0	29.9	6.3
KTR-11	12.1	1.4	17.3	6.0	36.6	4.6
KTR-12	32.1	17.8	35.3	11.6	44.4	16.0
KTR-13	53.3	16.0	29.5	36.6	31.9	1.7
KTR-14	16.1	4.6	31.4	3.5	23.4	3.0
KTR-15	51.6	7.3	35.6	3.7	46.9	8.2

ID = patient study identifier, SD = standard deviation. Average keratin 10 (K10), E-cadherin (Ecad), and plakoglobin (Pg) levels are reported based on densitometry of images taken following immunoblot followed by normalization to a reference sample (ICP-13) as discussed in Materials and Methods.

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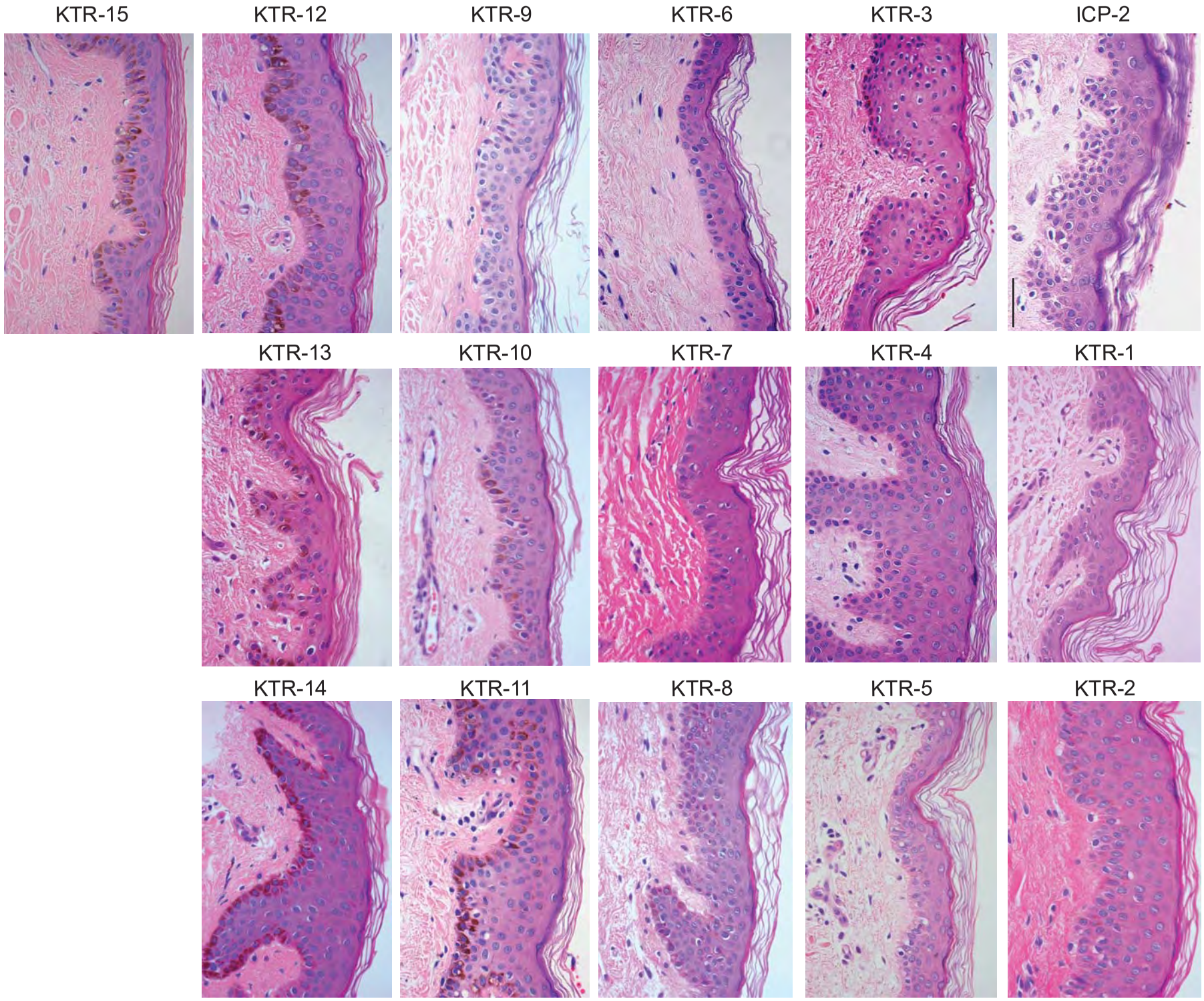
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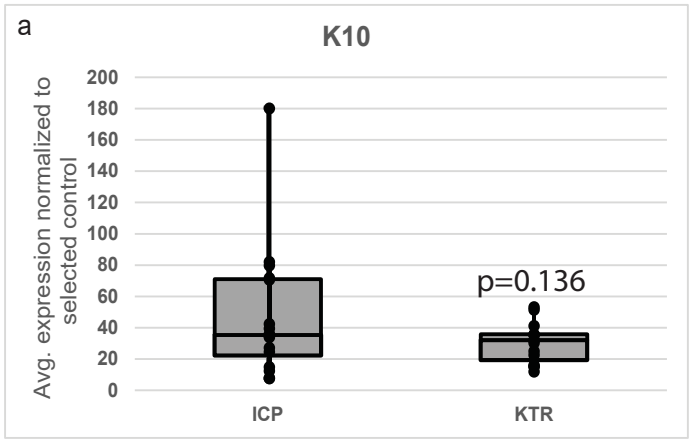
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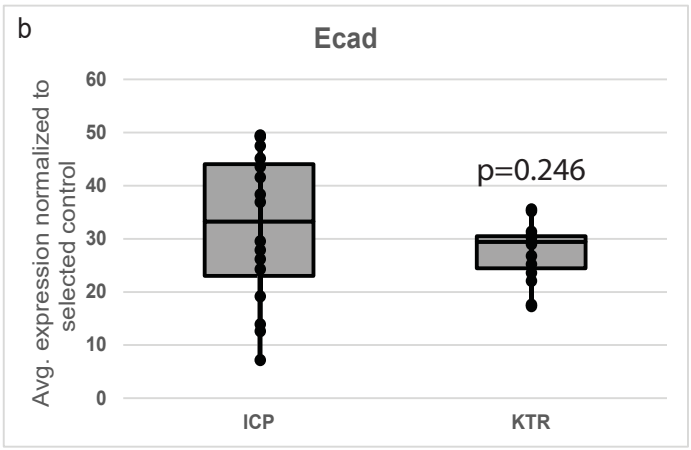
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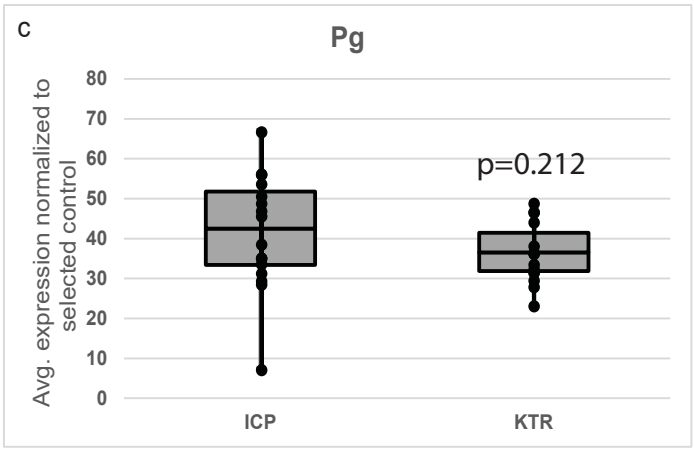
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Pg
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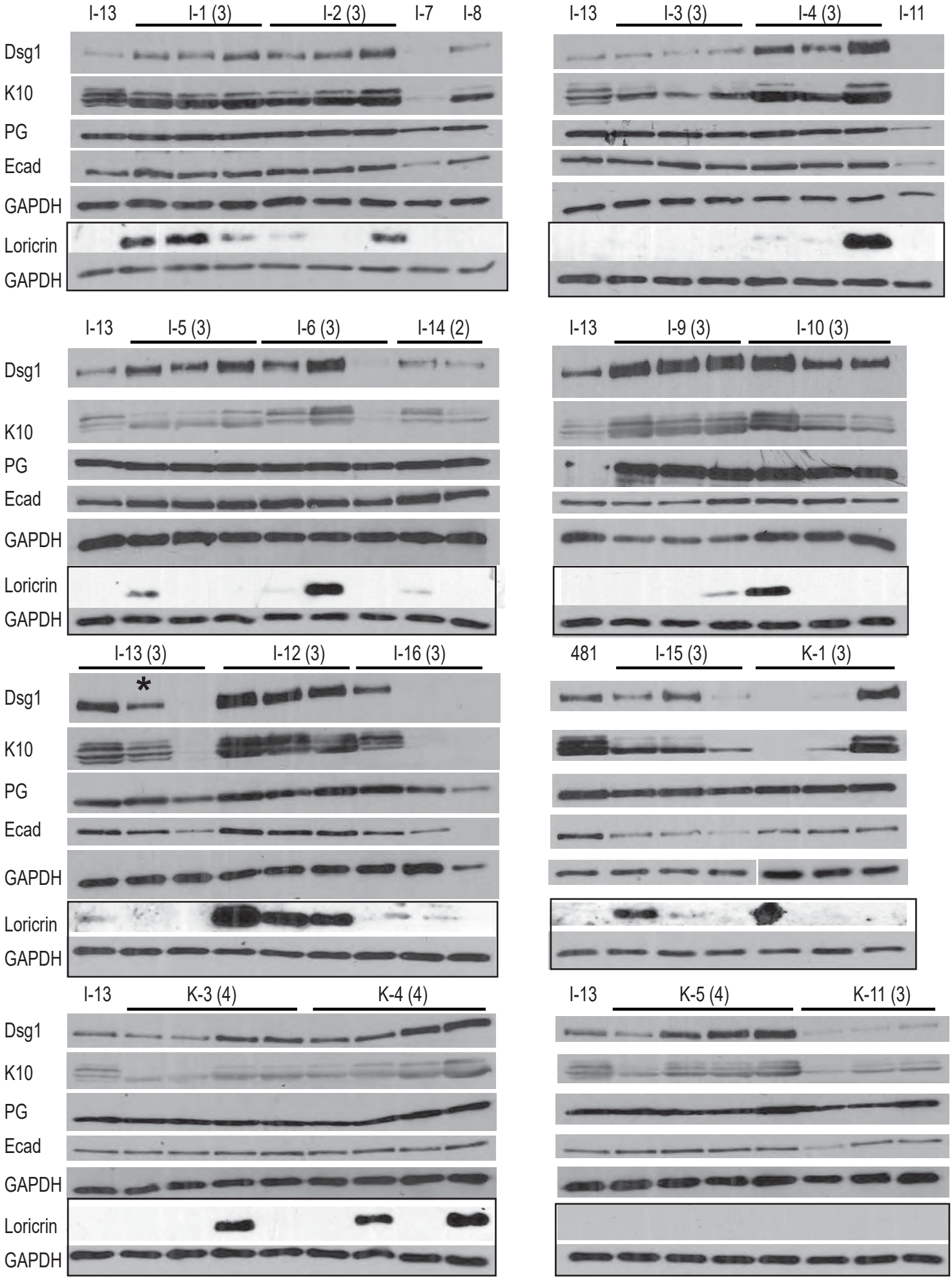
KTR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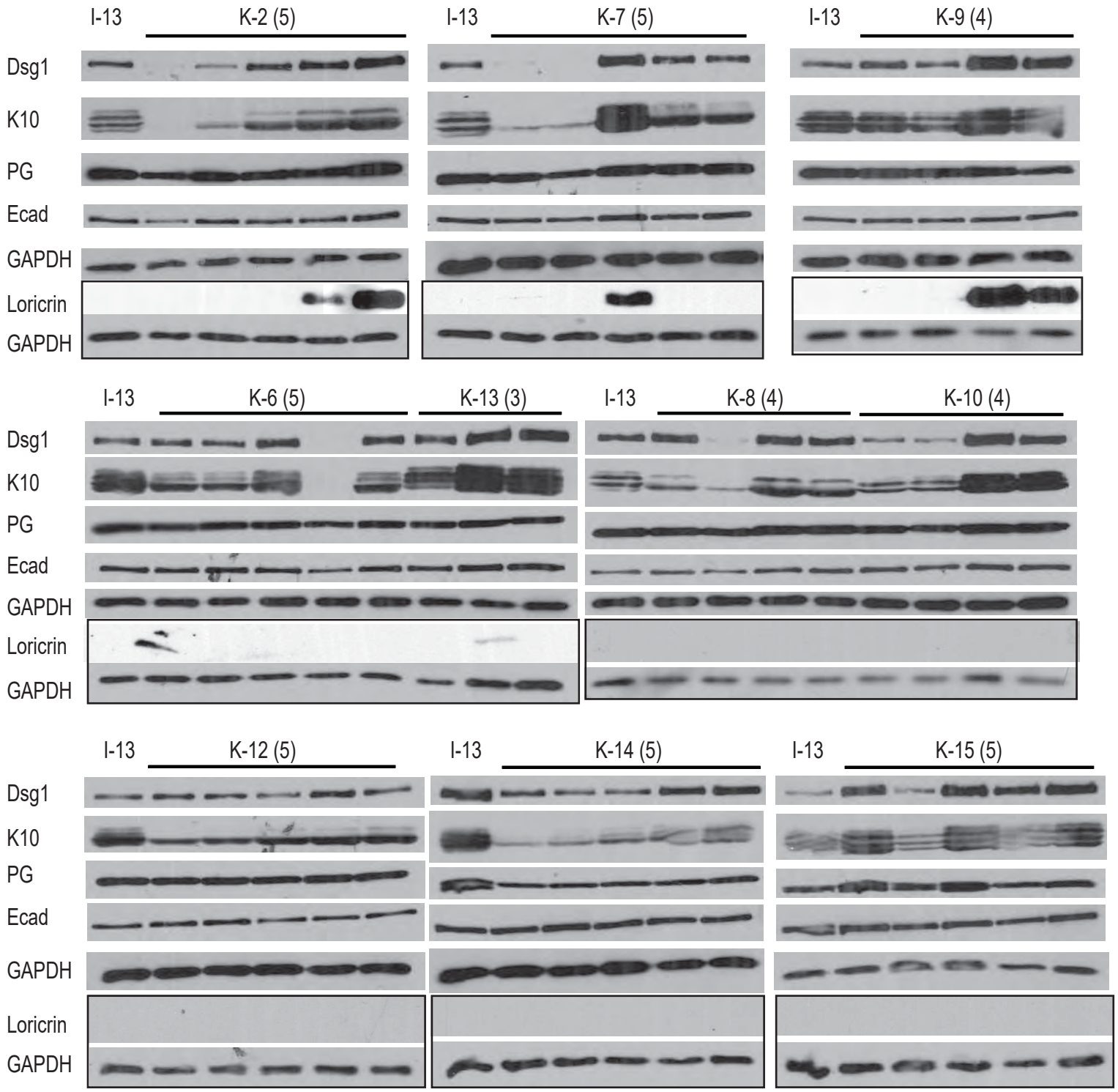
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