SUPPORTING APPENDIX

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Generation of *ΔNp63fl/fl* conditional and *ΔNp63-/-* knock out mice.

A) The *ΔNp63* targeting vector was generated by inserting loxP sites (triangles) flanking exon 3' and a neomycin cassette (neo) flanked by frt sites (squares). Arrows indicate location of primers used for genotyping. A dashed yellow line depicts the targeted region of the allele. Flox neo (fn) mice were crossed to the FLPeR mice expressing the flp recombinase to delete the neo cassette in vivo and to generate the flox (fl) allele. The knock out (KO) allele is shown after cre recombination. **B)** Southern analysis of genomic DNA from: $\Delta Np63^{fn/+}$, $\Delta Np63^{fn/fn}$, $\Delta Np63^{+/+}$ and $\Delta Np63^{-/-}$ mice. **C)** PCR analysis of genomic DNA from: $\Delta Np63^{fl/+}$, $\Delta Np63^{fl/fl}$, $\Delta Np63^{+/+}$ and $\Delta Np63^{-/-}$ mice. **D)** Quantitative (q) RT PCR analysis of *ΔNp63* mRNA from E9.5 and E18.5 wild-type and Δ$Np63^{-/-}$ embryos. Asterisks indicate statistical significance, ($p<0.001$). **E)** Western blot analysis for ΔNp63 using epidermal cell lines derived from wild-type and Δ$Np63^{-/-}$ embryos (E18.5) (left panel) and *p53-/-;p63-/-* MEFs expressing ΔNp63α and ΔNp63β cDNAs. Actin was used as a loading control. **F)** qRT-PCR analysis of *TAp63* mRNA from E9.5 and E18.5 wild-type and Δ$Np63^{-/-}$ embryos. qRT-PCR values are normalized to GAPDH. **G-J)** Embryos at day E18.5 of the following genotypes: wild-type **(G)**, $\Delta Np63^{+/-}$ **(H)**, $\Delta Np63^{-/-}$ **(I)**, and $p63^{-/-}$ **(J)**. Arrows in panel **(H)** indicate extra folds of skin and in panel **(I)** indicate non-adherent skin. **K-N)** Hematoxylin and eosin (H&E) stained cross sections of the skin of E18.5 embryos of the indicated genotypes.

Supplemental Figure 2. *ΔNp63* mutant mice exhibit epidermal abnormalities and defects in terminal differentiation.

A-B') Immunofluorescence (IF) or immunohistochemistry (IHC) of skin from day E18.5 embryos of the indicated genotypes. Antibodies used are as follows: **(A-D)** keratin 5 (K5), **(E-H)** keratin 10 (K10), **(I-L)** keratin 1 (K1), **(M-P)** filaggrin (Fila), **(Q-T)** keratin 14 (K14) (green) and K10 (red), **(U-X)** keratin 8 (K8), and **(Y-B')** keratin 18 (K18). DAPI was used as a counterstain for IF and hematoxylin was used for IHC. Magnification 400X. Black arrowheads indicate examples of K8 positive cells in the basal layer **(U)**. White arrowheads indicate K18

positive cells in the basal layer **(Y)**. Yellow arrows indicate K8 or K18 positive cells in the spinous layer **(V, W, Z, & A'). C'-H')** Double IF using skin from E18.5 day embryos of the indicated genotypes. Antibodies used are indicated. Magnification 400X. **C'-H')** White arrowheads indicate BrdU positive cells in the basal layer. **F'&H')** Yellow arrows indicate BrdU positive cells in the K10 expressing spinous layer **I')** Percentage of K5 positive cells expressing BrdU. **J')** Percentage of K10 positive cells expressing BrdU. Asterisks indicate statistical significance**,** ($p<0.001$). The dashed lines denote the dermal/epidermal interface.

Supplemental Figure 3. *DGCR8* expression is low in Δ*Np63+/-* and Δ*Np63-/-* epidermal cells.

A&B) qRT-PCR for *ΔNp63* **(A)** and *DGCR8* **(B)** using total RNA from wild-type (WT)*,* $\Delta Np63^{+/-}$ (+/-), and $\Delta Np63^{-/-}$ (-/-) epidermal cells. Values are normalized to GAPDH**.** Asterisks indicate statistical significance (p-value <0.001).

Supplemental Figure 4. miRNAs and mRNA signatures of *ΔNp63-/-* epidermal cell lines.

Heat map for microRNA-Seq (**A**) and RNA-Seq (**B**) performed using cells of the following genotypes: mouse *ΔNp63-/-* epidermal cell lines, (3 independent cell lines - *Δ*Np63-/-1, 2, 3), mouse iPS cells generated from fibroblasts using the Yamanaka factors ($miPS^{Yam}$), and mouse embryonic stem cells (mES). Low miRNA expression is indicated in green and high expression in red.

Supplemental Figure 5. Analysis of chimeric mouse embryos from *ΔNp63-/-* epidermal cell lines. Fluorescent (GFP) images of chimeric embryos at E13.5 (**A-D**) or E18.5 (**E&F**) generated from *ΔNp63*-/- epidermal cell lines expressing GFP**. A-F)** Double immunofluorescence for GFP (green) and the indicated tissue specific markers (red). DAPI (blue) was used as a counterstain. Magnification 200X.

Supplemental Figure 6. Analysis of NHEK-shΔNp63 and NHEK-shDGCR8 iPS cell lines.

A&B) Western blot analysis using the indicated antibodies of lysates from *ΔNp63-/-* epidermal cell lines, NHEKs (un) and NHEKs transduced with lentiviruses expressing the indicated shRNAs (shΔNp63 or shDGCR8) or a scrambled shRNA (scr). Actin was used as a loading

control. **C-F)** Micrographs of: normal human epidermal keratinocytes (NHEKs) cultured in keratinocyte media **(C),** human iPS cells generated using the Yamanaka factors (hiPS^{Yam}) **(D)**, and NHEKs transduced with lentiviruses expressing shΔNp63 **(E)** or shDGCR8 **(F)** cultured in ES media. **G&H)** Micrographs of embryoid bodies generated from NHEK cell lines expressing shΔNp63 **(G)** or shDGCR8 **(H)**. Arrows indicate embryoid bodies (200X magnification). Embryoid bodies of each genotype are further magnified in the inset in the lower right corner (400X magnification). **I)** Graphical representation of the number of days (D) required to reprogram NHEKs with shΔNp63 or shDGCR8 (6 days – D6) or the Yamanaka factors (OSKM) (Oct4, Sox2, Klf4, and c-myc) (7-10 days, D7-10). **J&K)** Western blot analysis of lysates from eight cell lines (#1-#8) of NHEKs transduced with lentiviruses expressing shΔNp63 (**J)** or shDGCR8 **(K)** at passage 10 using the indicated antibodies. Lysates from uninfected NHEKs were used as controls and actin was used as a loading control. **L-Q)** Q-RT PCR for *Oct4* **(L&O)**, *Sox2* **(M&P)**, and *Nanog* **(N&Q)** using total RNA from eight cell lines (#1-#8) of NHEKs transduced with lentiviruses expressing shΔNp63 **(L-N)** or shDGCR8 **(O-Q)** at passage 10.

Supplemental Figure 7. Bioinformatic analysis of mouse to human iPS cell comparison.
A-C) Bioinformatic analysis of a human to mouse comparison of microRNA-Seq data of the following genotypes: mouse embryonic stem cells (mES), mouse iPS cells generated from fibroblasts using the Yamanaka factors (miPS^{Yam}), wild-type mouse keratinocytes ((m)Keratinocytes 1, 2), normal human epidermal keratinocyte cell lines transduced with shΔNp63 or shDGCR8 ((h)NHEKshΔNp63 or (h)NHEKshDGCR8), human iPS cells generated from fibroblasts using the Yamanaka factors (hiPS^{Yam}), and (h)NHEK cells. Low miRNA expression is indicated in green and high expression in red. Analyses shown are Pearson's correlation analysis **(A)**, Principal component analysis **(B)**, and supervised hierarchical clustering **(C)**. **(D & E)** Bioinformatic analysis of a human to mouse comparison of RNA-Seq data of the cells shown in (A-C). Analyses shown are Pearson's correlation analysis **(D)** and Principal component analysis (**E**).

Supplemental Figure 8. Gene expression pattern in mouse and human epidermal cell lines deficient for *ΔNp63* or *DGCR8* compared to ES and iPS cells.

Heat map for RNA–Seq performed using RNA from the following cell lines: mouse induced pluripotent cells (miPS^{Yam}), mouse embryonic stem cells (mES), mouse *ΔNp63-/-* epidermal cell lines [(m) *Δ*Np63-/- 1, 2], wild-type mouse epidermal cells [(m) keratinocytes 1, 2], human induced pluripotent stem cells (hiPS^{Yam}), and normal human epidermal keratinocytes (NHEK) transduced with lentiviral shRNA for ΔNp63 (NHEK-shΔNp63) or DGCR8 (NHEK-shDGCR8).

SUPPLEMENTAL METHODS

Genotyping

Genomic DNA from tail biopsies was genotyped by Southern blot analysis by digesting genomic DNA with BglI or by PCR using the following primers and annealing temperatures: 1) for wild-type: wt-F, 5'- ACAGTCCTCTGCTTTCAGC-3' and wt-R (fl-R), 5'- CACACAGCA CTGGCCTTGC -3', annealing temp: 62°C, 2) for ΔNp63flox: fl-F, 5' – TTAGGTGGA TCCCTAGGAAGAG - 3' and fl-R (wt-R), 5' – CACACAGCACTGGCCTTGC - 3', annealing temp: 62°C, and 3) for ΔNp63KO: ko-F, 5'- TACAGCTCCTGGAGGATC CCATGC-3' and wt-R, annealing temp: 62°C. Primers used to genotype for the CRE gene are as follows: Cre-F, 5' – TGGGCGGCATGGTGCAAGTT - 3' and Cre-R, 5' – CGGTGCTAACCAGCGTTTTC - 3', annealing temp: 60° C. The PCR products were electrophoresed on a 1% agarose gel.

Generation of ΔNp63 Conditional Knockout Mice

The cre-loxP strategy was used to generate the *ΔNp63* conditional knockout allele (*ΔNp63fl*). Genomic *p63* DNA from intron 3 to intron 3' was amplified from BAC clone DNA (BAC RP23-186N8, Children's Hospital Oakland Research Institute). LoxP sites flanking exon 3' of *p63* and neomycin (*neo*) gene flanked by frt sites inserted in intron 3' were cloned into pL253 (1). Mouse embryonic stem cells (ESCs) were analyzed by Southern blot analysis for proper targeting of the *ΔNp63* allele. Chimeras resulting from ESC clones injected into C57BL/6 blastocysts were mated with C57BL/6 albino females and genotyped as described below. Mice with germ line transmission of the targeted allele (conditional, flox neo allele, fn) were crossed to the FLPeR mice to delete the neo cassette (2). Resulting progeny were intercrossed with Zp3-Cre

(C57BL/6) (3) transgenic mice. *ΔNp63fl/+; Zp3-Cre* females were mated with C57BL/6 males to generate *ΔNp63+/-* mice. The *ΔNp63+/-* mice were intercrossed to generate *ΔNp63-/-* mice. All procedures were approved by the IACUC at U.T. M.D. Anderson Cancer Center.

Histology and immunostaining of E18.5 embryos

Pregnant females at day 18.5 of gestation were injected three times at 1 hour intervals with BrdU (100 mg / gram of total body weight). Embryos were extracted 1 hour later and fixed in 10% formalin at room temperature for 18 hours. Fixed embryos were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic analysis. For immunofluorescence (IF), paraffin-embedded sections were dewaxed in xylene and rehydrated using decreasing concentrations of ethanol. Sections were subjected to antigen unmasking in citrate buffer unmasking solution (Vector Laboratory) followed by incubation with blocking solution, and 18 hour incubation at 4^{o}C with the following primary antibodies: K5 (1:1000) (Abcam), K14 (1:500) (LifeSpan BioSciences), K10 (1:1000) (Covance), K1 (1:500) (gift from Dennis Roop), K18 (1:200) (Sigma), and filaggrin (1:1000) (Abcam). Visualization was performed using an anti-rabbit secondary antibody conjugated to Texas-red (1:5000) (Jackson ImmunoResearch Laboratories), an anti-guinea pig secondary antibody conjugated to FITC (1:1000, Jackson ImmunoResearch Laboratories), or an anti-chicken secondary antibody conjugated to Alexa 488 (1:1000) (Molecular Probes) followed by counterstaining with DAPI (Vector Laboratory). Incorporation of BrdU was analyzed using the BrdU detection kit II (Roche). For BrdU analysis by IF, an anti-rabbit secondary antibody conjugated to Texas Red was used with the BrdU detection kit II (Roche). For immunohistochemistry (IHC), paraffin-embedded sections were prepared as described above and incubated with K8 primary antibody (1:80) (University of Iowa) or DGCR8 primary antibody (1:100)(Abcam). Visualization was performed using the Vectastain Elite ABC and DAB Peroxidase Substrate Kits (Vector Laboratory).

Keratinocyte cells – proliferation assay

1 X 10^{3} *ΔNp63fl/fl* and *ΔNp63Δ/Δ* epidermal cell lines were plated on mitomycin c (Roche)-treated J2 3T3 feeder cells in F media as described (4-7). Colonies cultured for 7 days were trypsinized, counted, and plated at limiting dilution 10 cells, 100 cells, and 1000 cells per 6 cm

dish. At each passage, dishes were fixed in 10% formalin for 30 min and stained with 2% rhodamine B (Sigma). Some dishes were treated with 10 mM BrdU, fixed in 70% ethanol for 30 minutes, denatured with 0.01N NaOH, and double immunostained with anti-cytokeratin 5 (Abcam) (1:1000) and BrdU-FITC conjugated antibody (GeneTex, Inc.). To detect and visualize cytokeratin 5 staining, Texas Red-conjugated goat anti-rabbit secondary antibodies were used (Jackson ImmunoResearch) (1:500). BrdU positive cells were counted in 100 colonies per sample.

Normal Human Epidermal Keratinocytes (NHEK) culture and infection

NHEKs (Lonza) were cultured in serum free media containing supplements according to the manufacture's protocol (Lonza). NHEKs were infected with pGIPZ lentiviral vectors with shRNAs for human *p63* and *DGCR8* (Open Biosystems). Cells were selected for 48 hours with puromycin (2 μg/ml) to generate cell lines. Selected cell lines were subsequently used for the generation of embryoid bodies.

Differentiation Culture: keratinocytes and neurons

Twenty-four hours after adenoviral infection, *ΔNp63fl/fl* and *ΔNp63Δ/Δ* keratinocytes were trypsinized and plated at a density of 1 X 10^6 in a 10cm dish on J2-3T3 feeder cells in F media supplemented with high calcium at a final concentration of (1.6 mM) for 3 days. Keratinocyte differentiation was assessed using western blot analysis for K5, K10 and filaggrin as described above. *ΔNp63fl/fl* and *ΔNp63Δ/Δ* cell lines were also cultured in neuroectodermal media (8). Neuronal differentiation was assessed by qRT-PCR for *Nestin* and *NeuN* as described above.

Chromatin Immunoprecipitation

Wild type keratinocytes and *ΔNp63-/-* epidermal cell lines were grown to near confluence on J2-3T3m feeder cells in F media as described previously (5, 6). Feeder cells were removed with 0.02% EDTA 24 hours prior to collecting keratinocytes for chromatin extraction. Cellular proteins were crosslinked to DNA using 1% formaldehyde and chromatin was prepared as described earlier (5, 6, 9). p63 ChIP analysis was performed using a pan-p63 antibody (4A4, Abcam) as described previously. Each ChIP was performed in triplicate using keratinocytes from three embryos of each genotype. Q-RT–PCR was performed by using primers specific for the

indicated regions of the DGCR8 promoter: Site1 (-3393) - forward 5'-AGTCACCTTGGTGCC TCTCATAG-3' and (-3348) -reverse 5'-AAACAGGTGGCAAGGCTTCTT-3', Site2 (-1459) - forward 5'-CATTTTTTTCTGTGGATCTTTTGGT-3' and (-1397) - reverse 5'-CACAGGGCAGGC AGATCAG-3', and nonspecific site (-3893) - forward 5'-CAAATCAAAATCTGCATCCATAGG -3' and (-3833) - reverse 5'-GCCCTCCTGCCTGTAAACCT-3'.

Western blot analysis

Total cell lysates were generated from mouse ES, mouse iPS^{Yam}, keratinocytes or skin from E18.5 day embryos. 50 μg of protein were electrophoresed on a 10% SDS PAGE and transferred to PVDF membrane as described previously (5, 6). Blots were probed with anti-ΔNp63 (1:1000) (BioLegend), K5 (1:1000) (Abcam), K10 (1:1000) (Covance), filaggrin (1:1000) (Abcam), DGCR8 (1:200) (Abcam), Oct4 (1:1000) (Santa Cruz), Sox2 (1:500) (Santa Cruz), Nanog (1:1000)(Abcam), or p53 (1:1000)(Vector Labs) at 4^{o}C for 18 hours followed by incubation for one hour at room temperature with the appropriate secondary antibodies conjugated to horseradish peroxidase (1:5000)(Jackson Lab). Actin (Sigma 1:5000) was used as a loading control. Detection was performed using the ECL Plus Kit (Amersham) following the manufacturer's protocol and x-ray autoradiography.

RNA extraction and quantitative real time PCR

Total RNA was prepared using the RNeasy plus kit (Qiagen). cDNA was synthesized from 2 μg of total RNA using the SuperScript® III First-Strand Synthesis Kit (Invitrogen) according to the manufacturer's protocol followed by q-RT PCR using the Power SYBR Green PCR Master mix (AB Applied Biosystems). Primers for qRT-PCR were used as described previously: *DGCR8* (10), *Nestin* (11), *NeuN* (12), and Δ*Np63, TAp63* and *GAPDH* (5, 6).

Cloning of Dgcr8-luciferase reporter genes

To generate the dgcr8 S luciferase construct, DNA was amplified from wild type mouse genomic DNA (C57BL/6) using primers containing the p63 binding site shown by ChIP and 5' XhoI and 3' BglII cloning restriction enzyme sites: 1. forward - DGCR8- Luc F: XhoI 5'-CCGCTCGAGGCTTCTAGTTGTCTATTCC-3' and 2. reverse - DGCR8- Luc R: BglII 5'-

GGA AGATCTGCTCACCAGATAGCTTGGA -3'. PCR amplicons were digested with XhoI and BglII and ligated into pGL3 basic luciferase reporter vector (Promega). The QuickChange® Multi Site-Directed Mutagenesis kit was used to generate dgcr8 Sm using the dgcr8 S luciferase construct as a template and the following primers: 1. 5'-ATGCCTGTCTAAAGTCACTTTTGTGCCTCTCATAGGCCTG-3', 2. 5'-GCATGTATCTCCTAAGAAGCTTTTCCACCTGTTTACAACACCAG-3', and 3. 5'-TGGTGCCTCTCATAGGCCTGTTTTTATCTCCTAAGAAGCCTTGC-3'.

Dual-Luciferase Reporter Assay

Luciferase reporter assays were performed as described previously (13, 14). Briefly, *p53-/-;p63-/-* MEFs (9) were plated on 6-well plates (3.5 x 10^5 cells per well). Twelve hours after plating, the MEFs were transiently transfected using Fugene HD (Roche) with 1 μg of Dgcr8 S or Dgcr8 Sm, 0.5 μg of Renilla luciferase plasmid (transfection control), and 0.5 μg plasmids encoding the p63 isoforms [TAp63α, TAp63β, TAp63γ, ΔNp63α, ΔNp63β, or ΔNp63γ or 0.5 μg or empty vector (pcDNA3) (V)]. After 24 hr, cells were harvested and luciferase activity was measured using the Dual-Luciferase Reporter Assay system (Promega) and a Veritas microplate luminometer (Turner BioSystems). Each experiment was performed in triplicate and values were normalized to cells infected with the luciferase plasmid and pcDNA3 (V) vector alone.

Adeno-cre infection

Passage-0 *ΔNp63fl/fl* keratinocytes were plated at a density of 1 X 10^6 in 10cm dish on J2-3T3 feeder cells. When cell reached 80% confluency, cells were infected with adenovirus-Cre-GFP (5000 particles per cell) to generate *ΔNp63Δ/Δ* cell lines. Adenovirus-GFP (Vector Development Lab, Baylor College of Medicine) was used as a control. The efficiency of infection was calculated to be 100% by GFP expression. Western analysis was performed for ΔNp63 to score for efficient deletion of ΔNp63 after infection.

Cell Culture

Wild type, $\Delta Np63^{-/-}$, $\Delta Np63^{fl/fl}$ keratinocytes were isolated from E18.5 day embryos as described previously (5, 6). Cells were plated on J2-3T3m feeder cells and cultured in F media (Sigma) supplemented with 0.4 mg/ml hydrocortisone, 24 ng/ml adenine, 8.4 ng/ml cholera toxin, 5

mg/ml insulin, 13 ng/ml 3,3,5-triiodo-L-thyronine, and 10 ng/ml EGF as described earlier (4-6). Mouse ES cells or iPS^{Yam} (S3) cells (gift from Dr. Austin Cooney, Baylor College of Medicine) (15) were cultured on mitomycin treated feeder cells in the presence of ES cell media containing Knockout™ D-MEM(Invitrogen), 20% ESC screened FBS (Hyclone), 200mM L-glutamine (Gibco), 100ug/ml penicillin/streptomycin (Gibco), 0.1mM β-mercaptoethanol(Sigma) and 10ng/ml recombinant mouse Leukemia inhibitory Factor (LIF, Gibco). Human iPS^{Yam} cells (a gift from Dr. Brian Davis, MD Anderson Cancer Center) were maintained on hESC qualified Matrigel (BD Biosciences) following manufacturer's protocol in mTeSR1(Stemcell Technologies) media.

Immunofluorescence – Cell culture

Mouse iPS^{Yam}, mouse ES, and *ΔNp63-/-* epidermal cell lines were cultured in 24 well dishes on feeder cells. These cells were fixed in 4% paraformaldehyde for 30 minutes and incubated with the following antibodies: SSEA-1(1:200 Developmental Studies Hybridoma Bank), Oct4 (1:100 Santa Cruz), Nanog (1:100 Abcam) for 18 hours at 4°C. Visualization was performed using an anti-mouse secondary antibody conjugated to Texas-red (1:5000) (Jackson ImmunoResearch Laboratories), or an anti-rabbit secondary antibody conjugated to FITC (1:1000, Jackson ImmunoResearch Laboratories) for one hour, followed by counterstaining with DAPI (Vector Laboratory). Human iPS cells were fixed in 4% paraformaldehyde for 30 minutes at room temperature and incubated with SEEA-4 (1:200 Developmental Studies Hybridoma Bank), Tra-1-60 (1:200 Millipore), Oct4 (1:100 Santa Cruz), Sox2 (1:100 Santa Cruz), Nanog (1:100 Abcam) for 18 hours at 4°C. Secondary antibodies were used as mentioned above and DAPI was used for visualization. Cells were rinsed in PBS and photomicrographs were taken with a Zeiss AxioObserver A1 inverted fluorescence microscope.

Embryoid body generation

Embryoid bodies were cultured using the hanging drop method. Each drop of 30 μL of ES cell media without LIF contained 800 cells. Hanging drops were incubated for 48 hours at 37°C. Hanging drops were aggregated (20 drops per well) in a six well low attachment plate and maintained for 7 days at 37°C.

miR-Seq

Two micrograms of total RNA was isolated from cells and purified using the miRvana microRNA isolation kit (Ambion) in preparation for miR-Seq.

Small RNA Sequencing and Analysis

For small RNA library construction, RNA samples were prepared using the DGE-Small RNA Sample Prep Kit (Illumina, San Diego, CA) as described previously (16, 17). A total of two Solexa-ready small RNA templates were analyzed on an Illumina GA-IIx Genome Analyzer at University of Houston. Cluster generation was performed and clusters were sequenced. Initial sequence process and analysis was followed as described previously(16, 17). Small RNA-Seq sequencing data was uploaded and processed using the Genboree Small RNA Toolset (http://genboree.org). The Illumina adapter was trimmed, and reads with length between 11 and 30, a copy number of at least 4, and finishing in monomers with length less than 10, were selected for further processing, similar to the processing described previously (16, 17). The reads were mapped to the mouse genome and build UCSC mm9 (NCBI 37) using Pash 3.0(18). Reads mapping up to 100 locations were selected for further analysis. The miRNA definitions from miRBase (19-22) were used to construct a known miRNA profile for each sample; the abundance of the known miRNAs were normalized as a fraction of the usable reads. For each species, a combined profile of all samples was computed; miRNA abundance was mean-centered and z-score transformed for each miRNA individually. Principal component analysis was performed using the implementation within the R statistical analysis system. Hierarchical clustering of samples was performed by first computing the symmetrical sample distance matrix using the Pearson correlation between microRNA profiles as a metric. Supervised sample analysis was performed using the t-test statistics, and heatmaps were generated using the heatmap.2 package in R.

RNA Sequencing and Analysis

Approximately 5 μg of polyA+ RNA was used to construct RNA-Seq libraries using the standard Illumina protocol. Mouse and human mRNA sequencing yielded 30-40million read pairs for each sample. The mouse mRNA-Seq reads were mapped using TopHat (23) onto the mouse

genome and build UCSC mm9 (NCBI 37) and the RefSeq mouse genes. The human mRNA-Seq reads were mapped using TopHat onto the human genome and build UCSC hg19 (NCBI 37) and the RefSeq human genes. Gene expression and gene expression differences were computed using Cufflinks (23). For each species, a combined profile of all samples was computed; miRNA abundance was mean-centered and z-score transformed for each miRNA individually. Principal component analysis was executed using the implementation within the R statistical analysis system. Hierarchical clustering of samples was executed by first computing the symmetrical sample distance matrix using the Pearson correlation between mRNA profiles as a metric, supervised sample analysis was performed using the t-test statistics, and heatmaps were generated using the heatmap.2 package in R. For gene signatures, we further explored gene enrichment using DAVID (15) and the GSEA implementation at the Molecular Signature Database (MSigD) (24).

Multi-species analysis of smRNA-Seq and RNA-Seq data.

To integrate the smRNA-seq datasets between mouse and human, first we selected the conserved microRNAs between mouse and human; next, we combined the z-score transformed miRNA abundance obtained from the human and the mouse cell lines. For integrated mRNA-seq analysis, the z-score transformed mRNA abundance datasets for mouse and human were combined by selecting only the conserved miRNAs. For each resulting dataset, principal component analysis and hierarchical clustering using the Pearson correlation coefficient metric were carried out using the implementations available within the R statistical analysis system

Generation of an inducible DGCR8 lentiviral vector

A tetracycline inducible *DGCR8* lentiviral vector was generated by cloning a PCR amplified product from pFLAG/HA-DGCR8 (25). Primers used to amplify the DGCR8 cDNA were as follows:

FOR -GGATCCCATGGAGACAGATGAGAGC

REV -GAATTCGGTGCACAGGGGCTCAC

The resulting PCR product was cloned into the EcoRI sites in the pLVX-Tight-Puro vector (Clontech).

Southern Blot Analysis of mouse ΔNp63-/- epidermal cell lines and iPS^{Yam} cells

Genomic DNA was extracted from mouse iPS cells generated using the Yamanaka factors (miPS^{Yam}) and Δ*Np63-/-* epidermal cell lines, digested with EcoRI, and separated on a 0.7% agarose gel. Hybridization was performed as described previously (6) using a probe for Klf4 amplified from miPS^{Yam} DNA using forward primer 5'-GCAGCCACCTGGCGAGTCTGA-3' and reverse primer 5'-GAGCCCTCCACCTGTGTTGCT-3'.

Calculation of Timing and Efficiency of Reprogramming

1 x 10^5 normal human epidermal keratinocytes (NHEK) were plated on 6 cm dishes coated with 0.1% gelatin and infected for 48 hours with the following lentiviruses: shTRP63-pGIPz (Open BioSystems), shDGCR8-pGIPz (Open BioSystems), or OKSIM (Addgene) (15). On day 3, ES cell media was added to the cells. The cells were fixed with 4% paraformaldehyde on day 6, 8, and 14 to determine the timing of reprogramming by immunofluorescence staining with Oct-4 (Santa Cruz; 1:100), Tra-1-60 (Millipore; 1:200) or Nanog (1:100; Abcam) antibodies. DAPI (Vector Laboratory) was used as counterstain. To determine percentage of reprogramming efficiency the cells were fixed at days 8 and 14 and visualized by immunofluorescence using the antibodies listed above. Photomicrographs were taken with a Zeiss AxioObserver A1 inverted fluorescence microscope at 10x magnification. Percent efficiency was calculated by counting Tra-1-60 positive colonies, dividing by 1 x 10^5 cells, and multiplying by 100.

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Supplemental Table 1: Quantification of BrdU positive keratinocyte

	PASSAGE 1		PASSAGE 3		PASSAGE 5	
	ΔNp63fl/fl	ΔNp63Δ/Δ	ΔNp63fl/fl	ΔNp63Δ/Δ	ΔNp63fl/fl	ΔNp63Δ/Δ
1	30%	30%	25%	90%	0%	100%
2	25%	100%	60%	90%	5%	90%
3	65%	50%	60%	95%	5%	50%
4	50%	25%	50%	100%	50%	95%
5	10%	35%	15%	45%	0%	95%
6	50%	85%	50%	95%	0%	100%
7	5%	90%	15%	90%	0%	100%
8	30%	90%	10%	90%	0%	100%
9	45%	35%	10%	35%	0%	40%
10	40%	50%	10%	50%	0%	45%
11	45%	50%	10%	50%	0%	45%
12	50%	90%	50%	90%	5%	90%
13	40%	90%	10%	90%	50%	90%
14	85%	35%	70%	35%	0%	95%
15	35%	50%	5%	100%	0%	95%
16	45%	50%	10%	100%	0%	50%
17	50%	85%	15%	85%	0%	100%
18	40%	50%	20%	90%	50%	50%
19	25%	50%	20%	50%	0%	50%
20	20%	50%	20%	50%	5%	50%
21	15%	40%	20%	95%	5%	100%
22	10%	95%	20%	90%	10%	90%
23	30%	95%	25%	90%	55%	95%
24	30%	50%	15%	50%	10%	50%
25	35%	45%	20%	45%	10%	40%
26	50%	45%	60%	45%	5%	40%
27	40%	100%	15%	100%	10%	100%
28	50%	100%	60%	80%	0%	95%
29	40%	100%	20%	80%	60%	90%
30	45%	45%	20%	40%	0%	95%
31	40%	45%	20%	35%	0%	95%
32	35%	50%	20%	50%	0%	50%
33	40%	45%	20%	45%	0%	40%
34	50%	50%	50%	80%	5%	40%
35	50%	50%	50%	85%	5%	50%
36	40%	50%	20%	90%	50%	45%
37	35%	50%	15%	90%	25%	50%
38	35%	50%	10%	100%	20%	50%
39	40%	40%	10%	100%	50%	100%
40	35%	95%	20%	95%	50%	100%
41	35%	95%	20%	90%	0%	90%
42	40%	35%	25%	40%	0%	40%
43	45%	50%	25%	50%	0%	50%
44	50%	50%	60%	50%	50%	100%
45	40%	15%	25%	40%	0%	100%

46	40%	50%	25%	50%	0%	50%
47	40%	95%	30%	95%	0%	90%
48	50%	50%	55%	50%	10%	50%
49	45%	50%	35%	100%	10%	100%
50	45%	45%	40%	90%	50%	45%
AVERAGE	**39%**	**59%**	**28%**	**73%**	**13%**	**73%**

percent of colonies matching the following criteria:

- 0-50% BrdU positive
- 50% BrdU positive
- >50% BrdU positive

Supplemental Table 2: *p63/p53* response elements

Element	Location	Sequence			MM / spacer
DGCR8-1	-3394 to -3366	ctgCATGtat	ctcctaaga	agcCTTGcca	5 / 9
DGCR8-2	-1459 to -1434	ttcCATGtgg	tctcct	cccCTAGacc	8 / 6

Sequences of elements assayed by ChIP analysis. MM indicates the number of mismatches to p63/p53 consensus binding site and spacer indicates the number of nucleotides within the spacer region.

Supplemental Table 3: ***Embryos generated from iPS cells***

Cell Lines	# of blastocysts	# of embryos @ E13.5	# of GFP +ve
ΔNp63-/- 1	17	4	2
ΔNp63-/- 2	12	3	1
ΔNp63-/- 3	14	0	N/A
ΔNp63-/- 4	15	1	0
ΔNp63-/- 5	15	4	2
ΔNp63-/- 6	20	0	N/A
ΔNp63-/- 7	21	0	N/A
iPS^{Yam} -1	14	0	N/A
iPS^{Yam} -2	12	2	1
iPS^{Yam} -3	16	9	4
iPS^{Yam} -4	16	9	3
iPS^{Yam} -5	21	6	2
iPS^{Yam} -6	21	7	3

ΔNp63-/-**:** epidermal cell lines derived from 7 individual ΔNp63-/- E18.5 day embryos
iPS^{Yam}**:** mouse fibroblasts reprogrammed with Yamanaka factors
E13.5: embryonic day 13.5
N/A: not applicable

Supplemental Table 4: ***Mice generated from iPS cells***

Cell Lines	# of blastocysts	# of pups @ P1	# of pups @ P7	coat color
ΔNp63-/- 1	17	2	0	N/A
ΔNp63-/- 2	13	3	0	N/A
ΔNp63-/- 3	14	0	0	N/A
ΔNp63-/- 4	16	3	3	white
ΔNp63-/- 5	17	2	0	N/A
ΔNp63-/- 6	21	0	0	N/A

ΔNp63-/-**:** epidermal cell lines derived from 7 individual ΔNp63-/- E18.5 day embryos
P1: postnatal day 1
P7: postnatal day 7

Supplemental Table 5: ***Efficiency of Reprogramming of NHEK cells***

NHEK	**OKSM**	**shDGCR8**	**shΔNp63**
% Efficiency	0.03	0.10	0.07
# of days to reprogramming	10	6	6

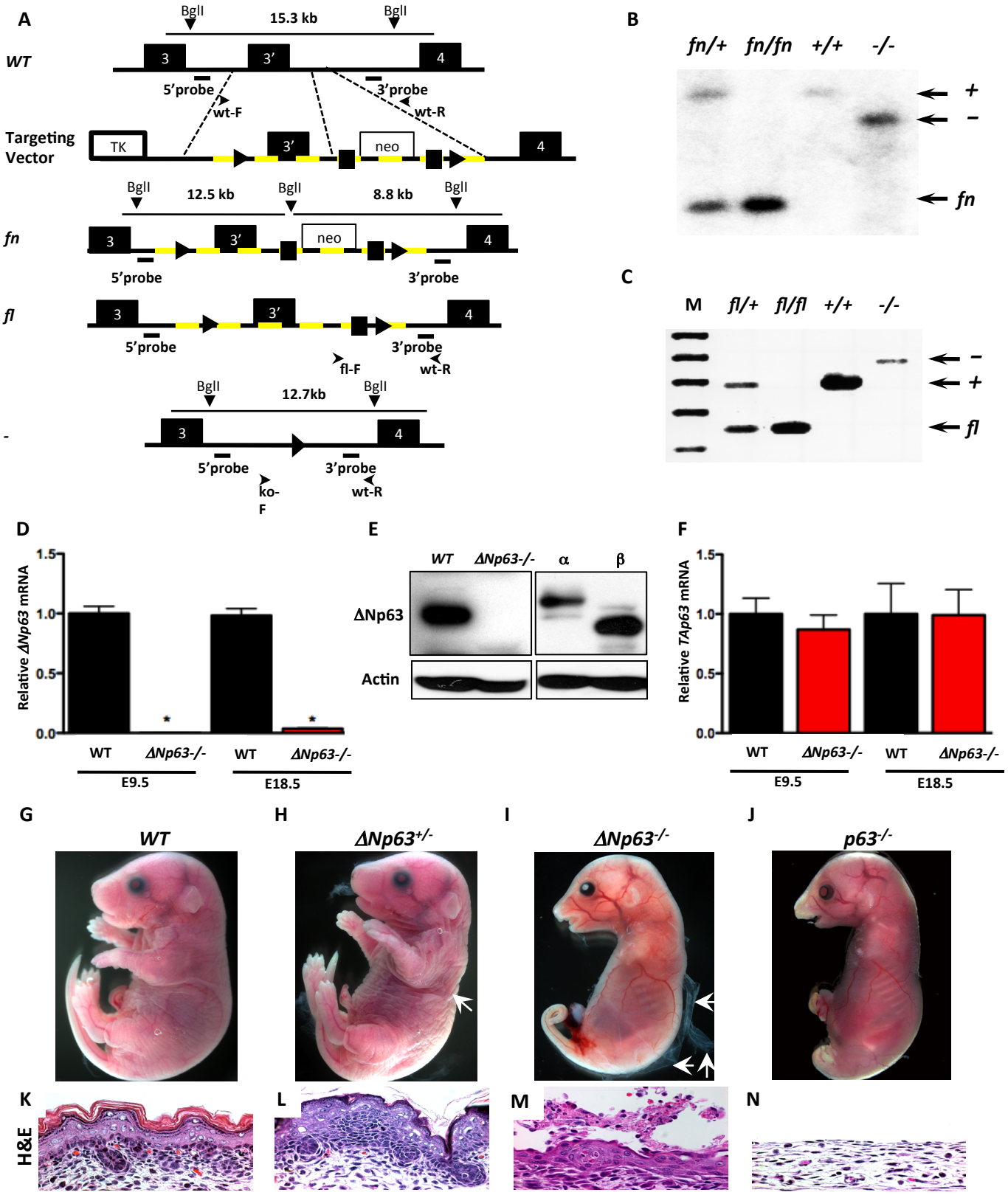
% efficiency is represented by number of Tra-1-60 positive colonies divided by $1X10^5$ multiplied by 100.

Number of positive colonies was counted from two duplicate plates for each method of reprogramming at day 14.

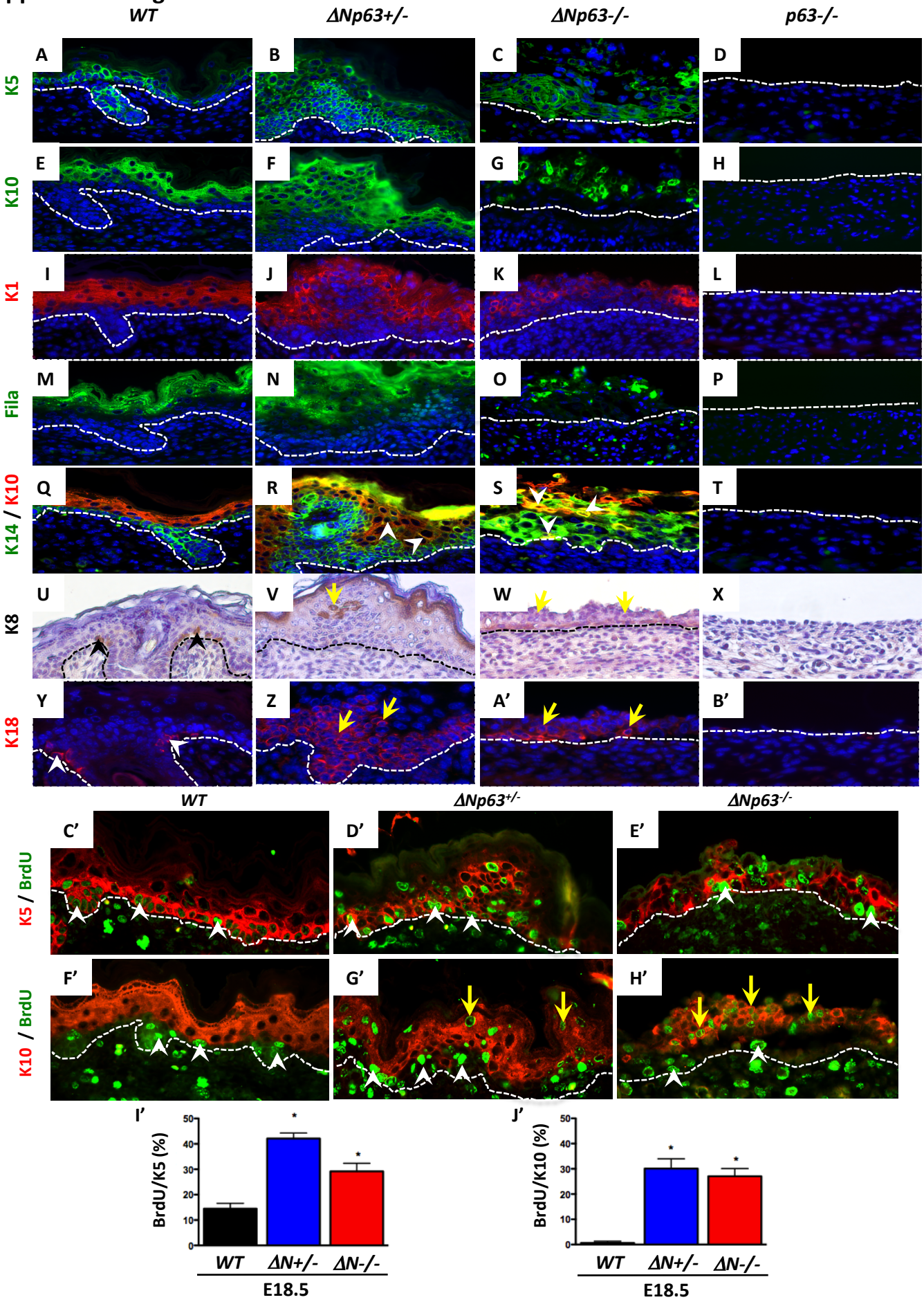
NHEK: normal human epidermal keratinocytes

OKSM: Yamanaka factors

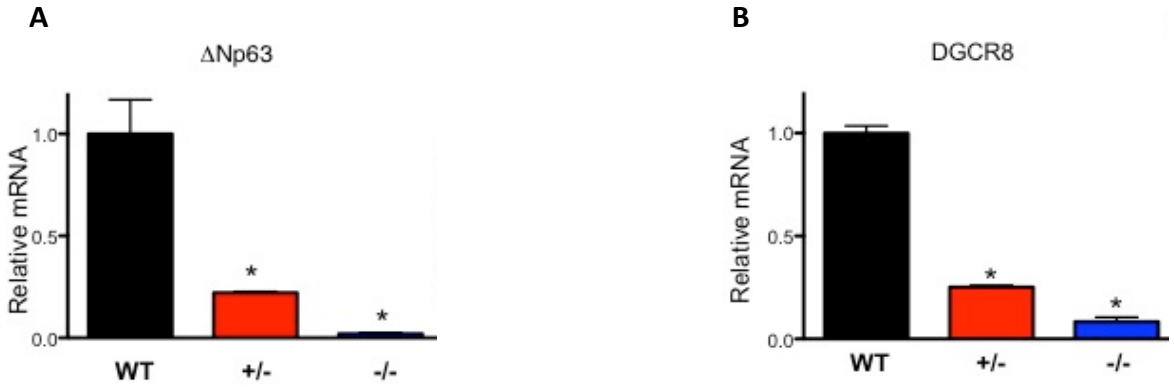
Supplemental Figure 1



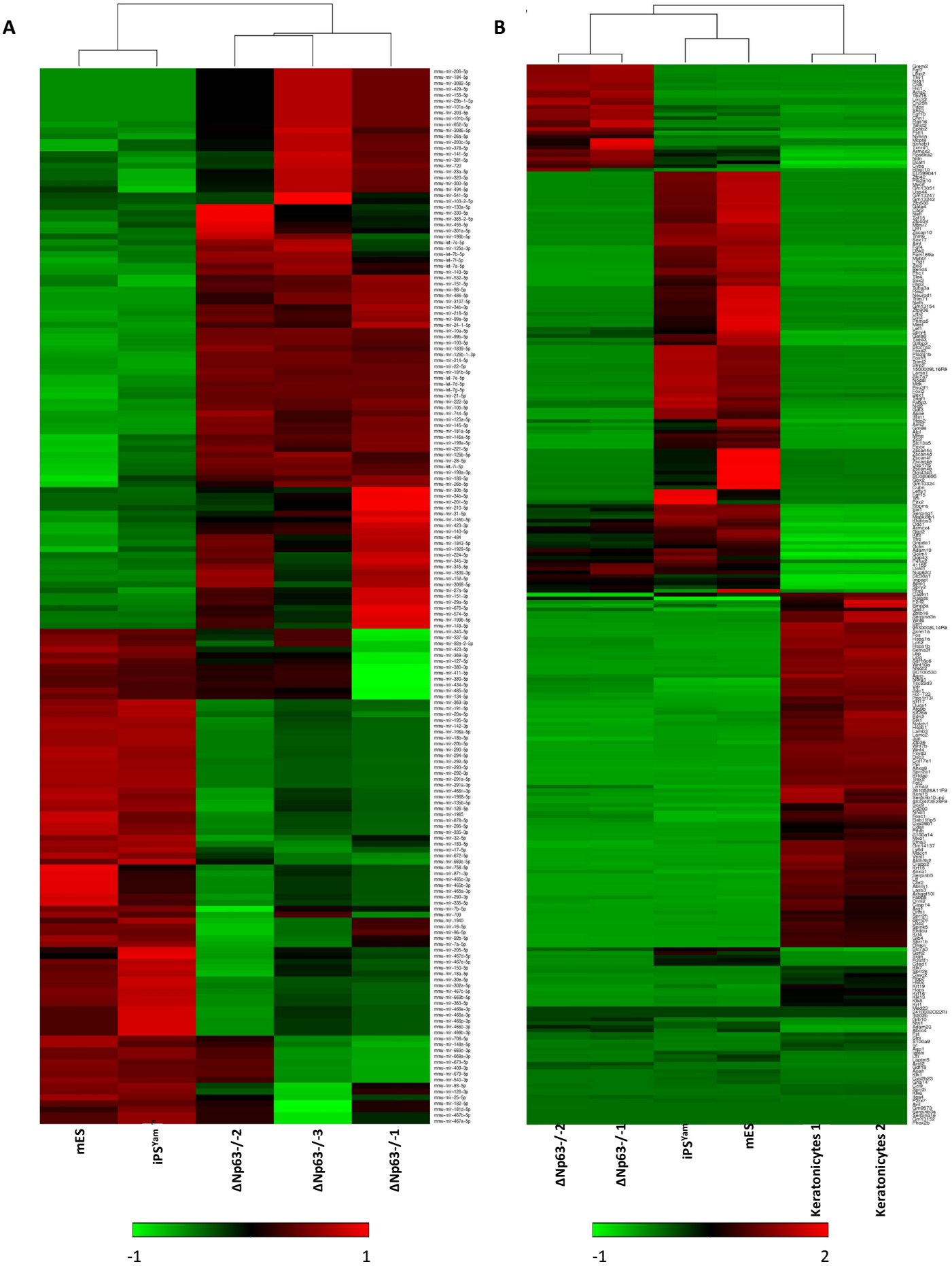
Supplemental Figure 2



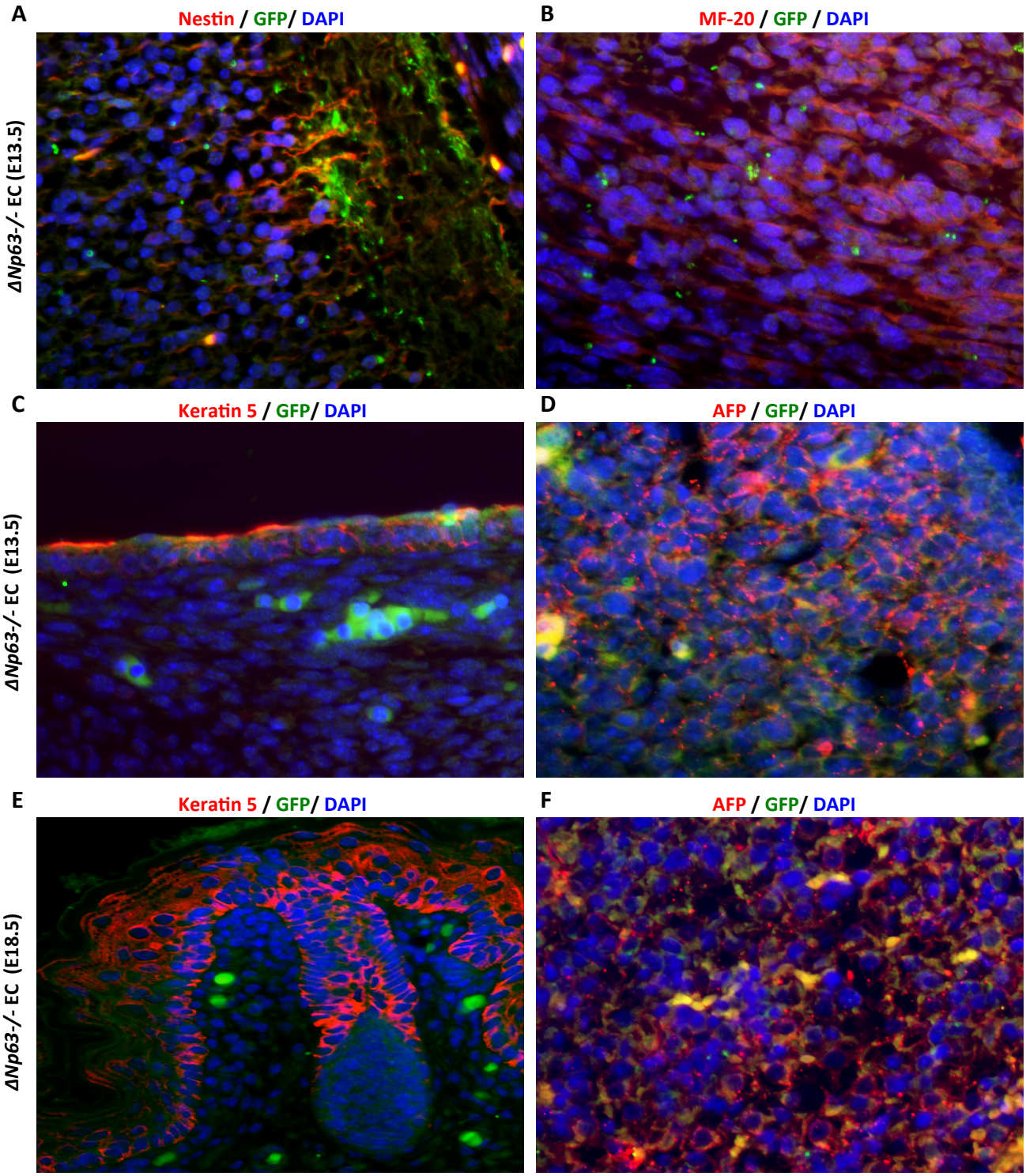
Supplemental Figure 3



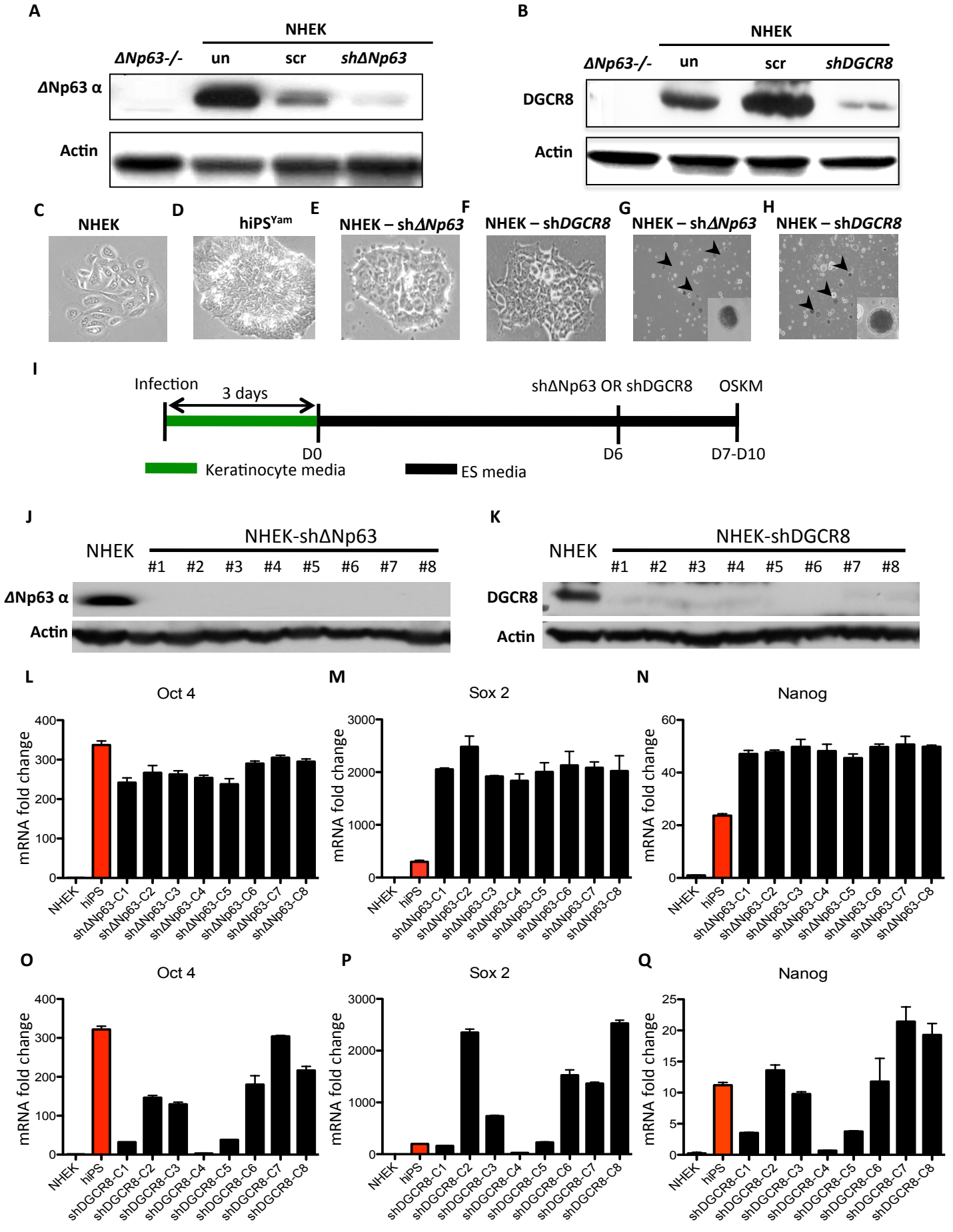
Supplemental Figure 4



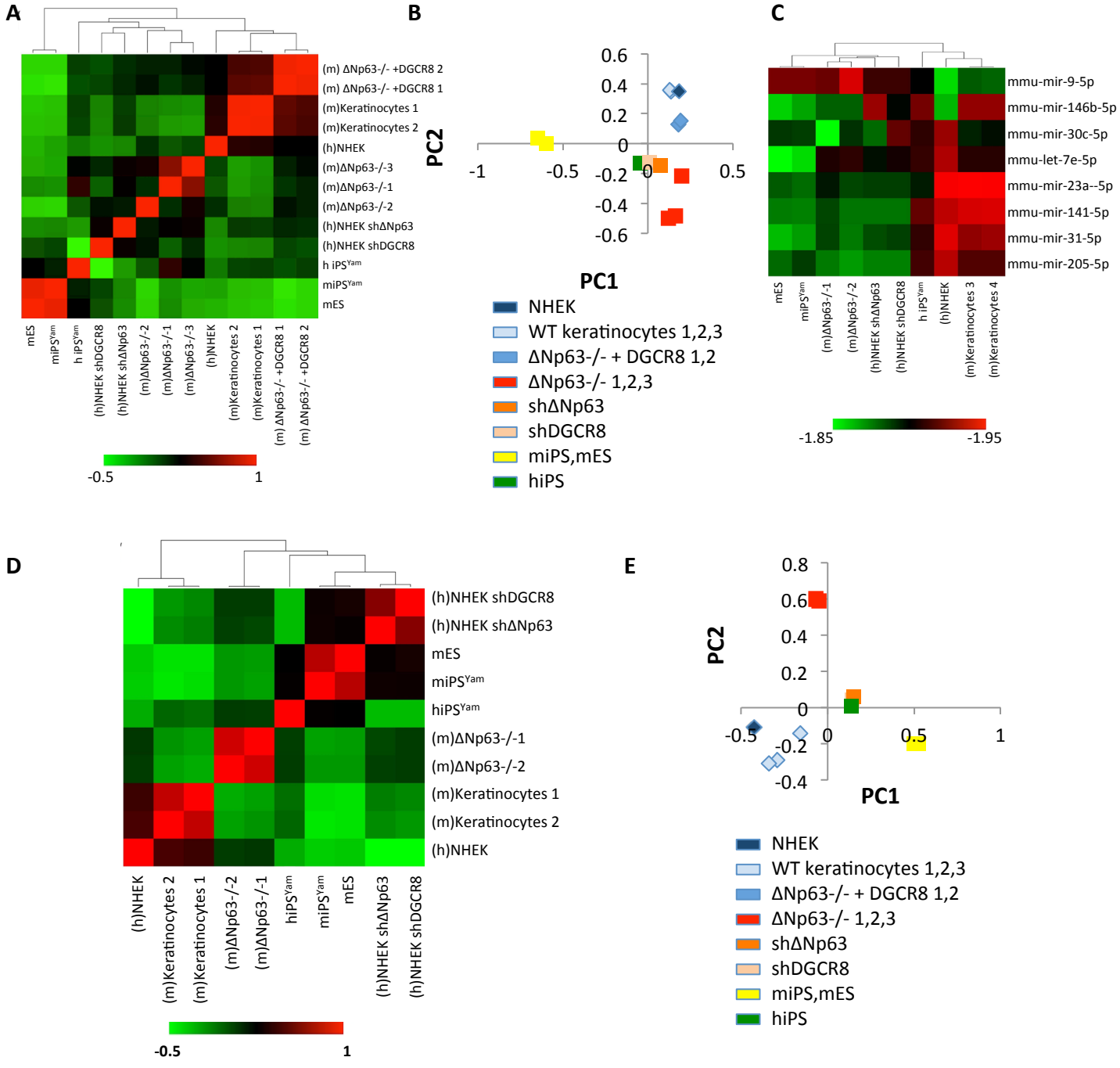
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8