SUPPLEMENTAL MATERIALS AND METHODS

Subcellular fractionation and mitochondrial purification

Subcellular fractionation experimental procedure was adapted and modified from a previously described method (Vander Heiden et al. 1997). Primary keratinocytes were washed with PBS, trypsinized and pelleted by centrifugation. Cells pellet were washed with NKM buffer (1 mM Tris-HCl, pH 7.4, 0.13 M NaCl, 5 mM KCl, 7.5 mM MgCl2) and centrifuged. Cells were resuspended in mitochondrial isolation buffer A (250 mM sucrose, 20 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, protease inhibitor cocktail, phosphatase inhibitor cocktail) and homogenized in an ice-cold dounce cell homogenizer. Unlysed cells and nuclei were pelleted twice via a 10 min, 750 × g spin. The supernatant was spun at 10,000 × g for 30 min. This pellet was resuspended in buffer A and represents the mitochondrial fraction. The supernatant was spun at 100,000 × g for 1 hr and the remaining supernatant represents the S100 cytoplasmic fraction. Mitochondrial pellets were washed 5 times with buffer A to avoid cytoplasmic contamination. Mitochondrial pellets were lysed with RIPA buffer.

Antibodies

Antibodies used from Cell Signaling Technology include, mouse anti Stat3, rabbit anti Stat3, rabbit anti COX IV, rabbit anti-phospo-Stat3 (S727), rabbit anti-phospho-Stat3 (Y705), rabbit anti GAPDH and rabbit anti Histone H3. Rabbit PKCδ, rabbit anti PKCε and mouse anti TFAM (mTFA) were purchased from Santa Cruz Biotechnology. Mouse monocolonal anti β-Actin was purchased from Sigma Aldrich.

Immunoflourescence staining and confocal microscopy

Cell lines used for confocal microscopy were plated on glass cover slips. Mitochondrial labeling was conducted in live cells by addition of 100 nM MitoTracker CMXRos to the

culture medium for 15 mins prior to fixation (Life Technologies). Mitochondrial DNA labeling was conducted by inhibiting nuclear DNA synthesis by 1hr by pretreatment with 20 μM aphidicolin, followed by incubation with 15 mM BrdU for 3 hrs (Davis and Clayton 1996). Cells in culture were fixed with ice cold methanol for 15 min, permeabilized with 0.25% Triton X-100 and incubated with the indicated antibodies. Paraffin embedded tissues were permeabilized by boiling in sodium citrate buffer for 10 min. Tissues sections were blocked with 10% normal goat serum, followed by overnight incubation with primary antibodies at 4°C. Primary antibody incubation was followed by detection with Alexa fluor secondary antibodies in fixed cells and paraffin embedded tissue sections (Life Technologies). Visualization was conducted using Leica SP5 X (White Light Laser) confocal microscope.

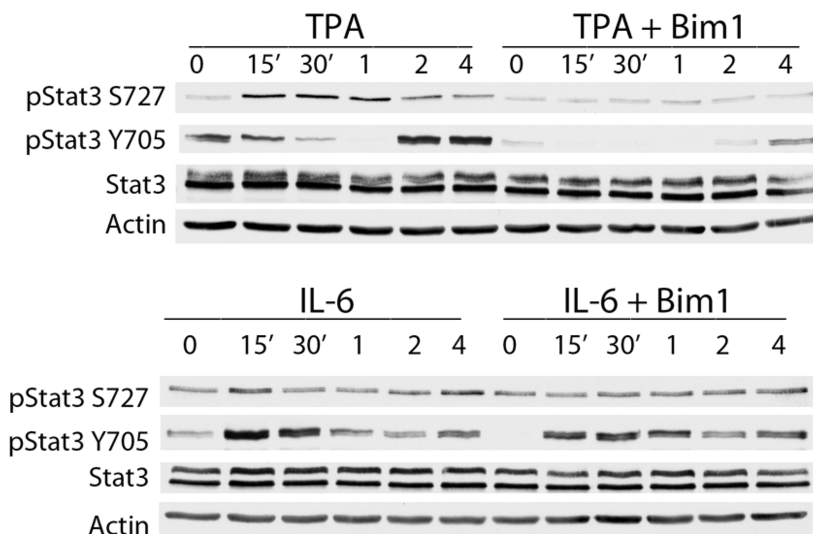
Real Time PCR

Total RNA was isolated from primary kerationocytes using RNEasy kit (Qiagen). On column Dnase treatment was conducted to avoid mitochondrial genomic DNA contamination. Reverse transcription of mRNA was carried according to manufacturers suggestions with 1 ug of total RNA using SuperScript III First-Strand cDNA synthesis kit (Life Technologies). Real time PCR was carried out on an Applied Biosystems ViiA 7 Real-Time PCR system using Sybr green dye and analyzed using comparative CT method. For each individual experiment triplicate samples were take, and the average of three individual experiments were taken and analyzed.

Supplemental Figures and Tables

Supplemental Figure S1

A.



B.

Supplemental Figure S1. Stat3 binds to PKCs in JB6 epidermal cells.

(A) PKC mediated serine phosphorylation of Stat3. Primary mouse keratinocytes were pretreated for 1 hr with Bim-1, followed by treatment with TPA (680 nM) or IL-6 (10 ng/ml) and harvested at the indicated time points. Lysates were immunoblotted as indicated.

(B) Whole cell lysates from JB6 cells were immunoprecipitated with an anti-Stat3 antibody and normal rabbit IgG (negative control). Immunoprecipitates were electrophoresed and western blots were carried out for PKCδ and PKCε to assess association with Stat3.

Supplemental Figure S2

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15421 tcttgcgtacataaatttacatagtacaatagtacatttatgtatatcgtacattaaact
15481 attatccccaagcatataagcaagtacatttaatcaatgatataggccataaaacaatta
15541 tcaacataaactgatacaaaccatgaatattatactaatacatcaaattaatgctttaaa
15601 gacatatctgtgttatctgacatacaccatacagtcataaactcttctcttccatatgac
15661 tatccccttccccatttggtctattaatctaccatcctccgtgaaaccaacaacccgccc
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15901 gtcatgcatttggtatttttttattttggcctactttcatcaacatagccgtcaaggcat
15961 gaaaggacagcacacagtctagacgcacctacggtgaagaatcattagtccgcaaaaccc
16021 aatcacctaaggctaattattcatgcttgttagacataaatgctactcaataccaaattt
16081 taactctccaaacccccccaacccccctcctcttaatgccaaccccaaaaacactaagaac
16141 ttgaaagacatatattattaactatcaaaccctatgtcctgatcgattctagtagttccc
16201 aaaatatgactcatattttagtacttgtaaaaattttacaaaatcatgctccgtgaacca
16261 aaactctaatcacactctattacgcaataaatattaacaa
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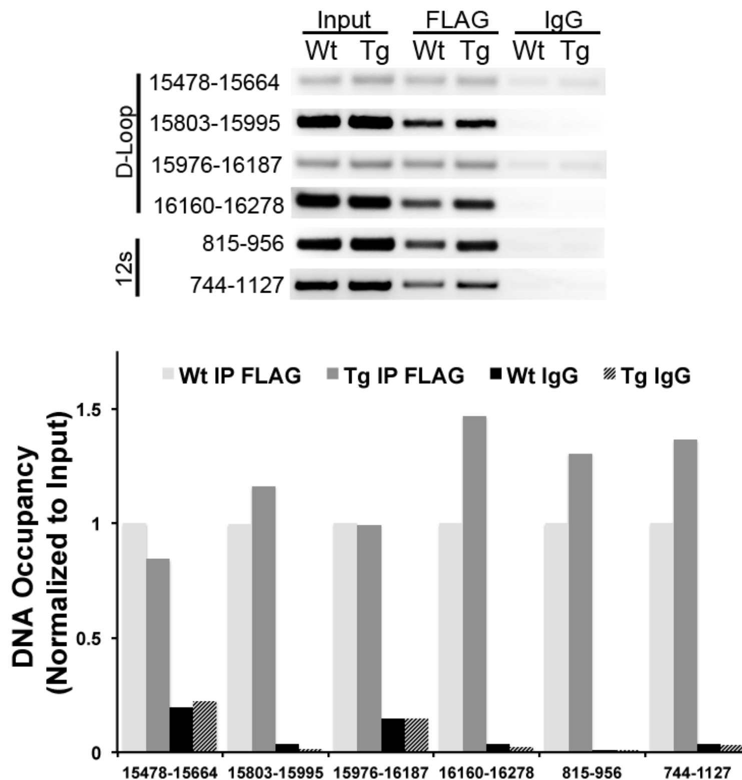
Supplemental Figure S2. Stat3 binding sites in mouse mitochondrial genome d-loop region. Sequence analyses of Stat3 consensus binding sites (TT$N_{4\text{-}6}$AA) in d-loop region of *Mus musculus musculus* mitochondrion complete genome NCBI Reference Sequence: NC_010339.1 (d-loop nucleotide position 15426-16300).

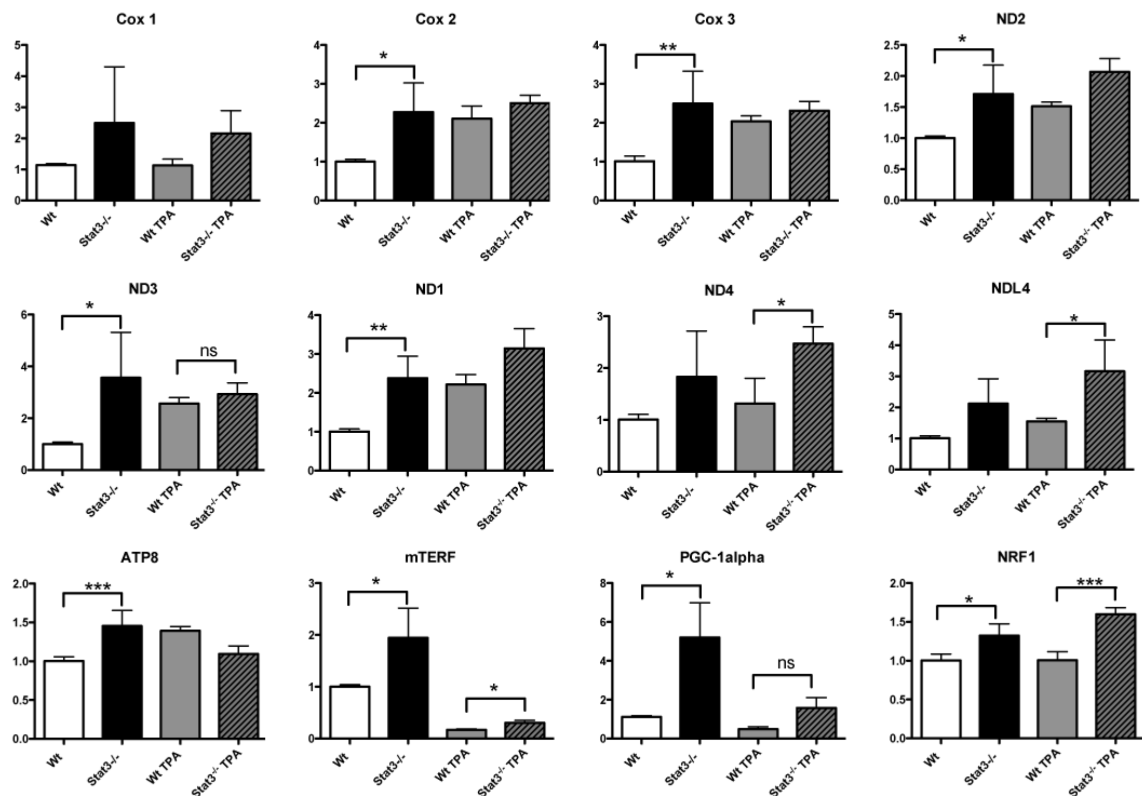
Supplemental Figure S3



Supplemental Figure S3. Stat3C binds to mtDNA. Mitochondrial ChIP assays were carried out from wild type (Wt) and BK5.Stat3C transgenic (Tg) epidermal keratinocyte

cross-linked mitochondrial DNA-protein lysates. Pull downs were conducted for FLAG tagged Stat3C with a mouse anti-FLAG and IgG (negative control). PCR was carried out with primers spanning putative STAT binding sites priming at the indicated nucleotide positions with DNA from input and isolated DNA fragments. DNA occupancy at indicated nucleotide positions was quantified and graphed by normalizing bound DNA to input and taken relative to DNA bound in Wt.

Supplemental Figure S4



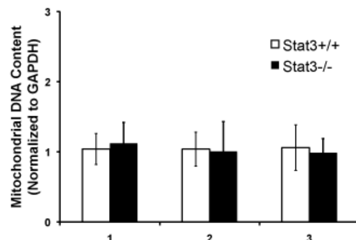


Figure S4. (A) Quantitative analysis of mitochondrial and nuclear encoded genes. Adult primary keratinocytes from Stat3$^{fl/fl}$ (Wt) and HK5.Cre;Stat3$^{fl/fl}$ (Stat3$^{-/-}$) were treated with DMSO (control) or 680 nM TPA for 1hr. Real time quantitative PCR was carried out using Sybr green on an ABI Viia 7 RT-PCR system using comparative Ct method.

(B) mtDNA content normalized to nuclear DNA (GAPDH) content was conducted using qPCR as above from epidermal keratinocytes. Primer set 1, 2, and 3 (x axis) correspond to amplicons on mtDNA nucleotide positions, 744-959, 744-1127, and 15976-90, respectively. Values represent mean ± SD (n=3). No significant difference was found between Wt and Stat3$^{-/-}$ mtDNA by Mann-Whitney *U* test.

Supplemental Table S1.

Primer Set	Nucleotide Position	Primer Set	Nucleotide Position	Primer Set	Nucleotide Position	Primer Set	Nucleotide Position
	66-493	11	4279-4679	21	8440-8824	31	12540-12892
2	467-787	12	4735-5173	22	8850-9273	32	12964-13360
3	763-1222	13	5183-5583	23	9343-9781	33	13330-13634
4	1194-1644	14	5636-6026	24	9874-10230	34	13603-14032
5	1597-2035	15	6021-6417	25	10281-10607	35	14011-14381
6	2056-2430	16	6477-6832	26	10625-11069	36	14424-14759
7	2441-2885	17	6834-7261	27	11076-11430	37	14776-15178
8	2968-3300	18	7277-7600	28	11443-11803	38	15192-15401
9	3376-3707	19	7621-8056	29	11833-12147	39	15721-16034
10	3814-4251	20	8047-8429	30	12170-12494	40	16006-90

Supplemental Table S1. Amplicons from Stat3-mitochondrial genome wide ChIP assay. Nucleotide positions of primer sets 1-40 spanning 300-400 bases throughout the entire mouse mitochondrial genome are listed (NCBI Reference Sequence: NC_010339.1).