Stem Cell Reports, Volume *19*

Supplemental Information

An *in vitro* model of acute horizontal basal cell activation reveals gene regulatory networks underlying the nascent activation phase

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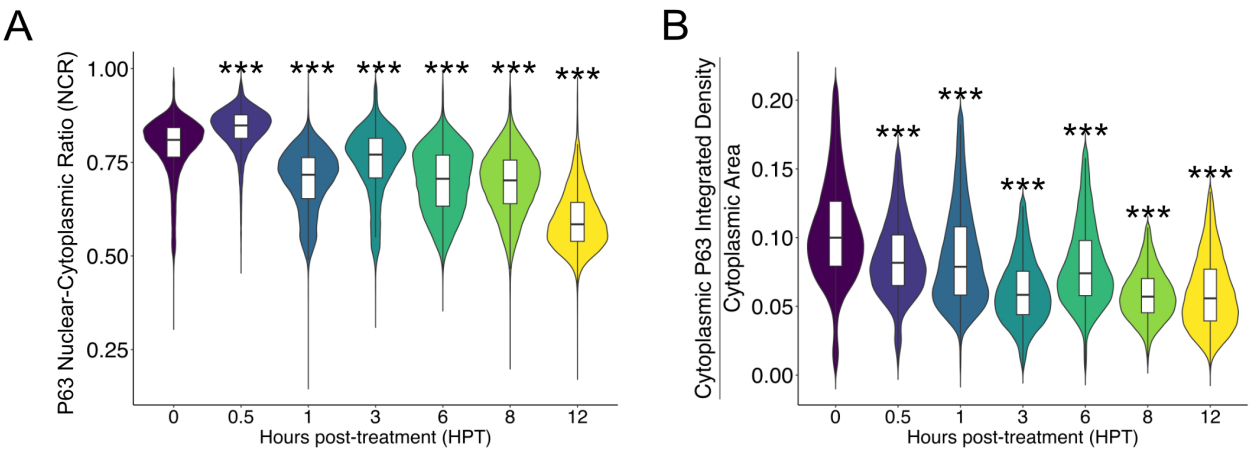
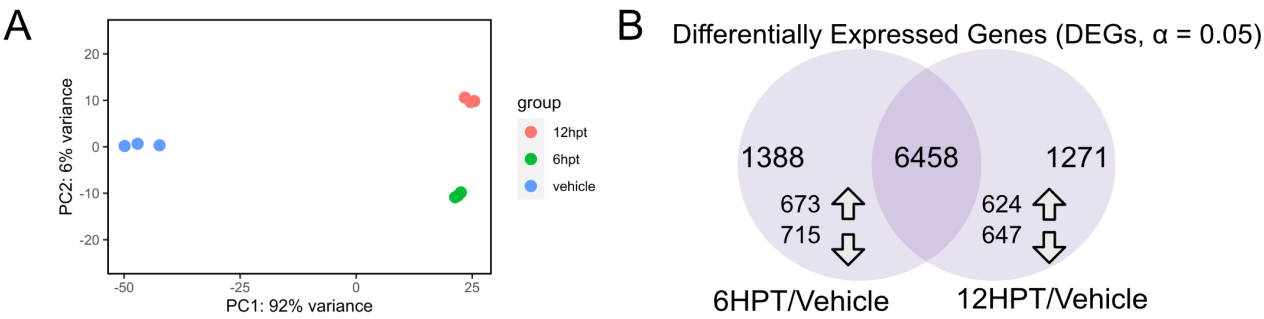


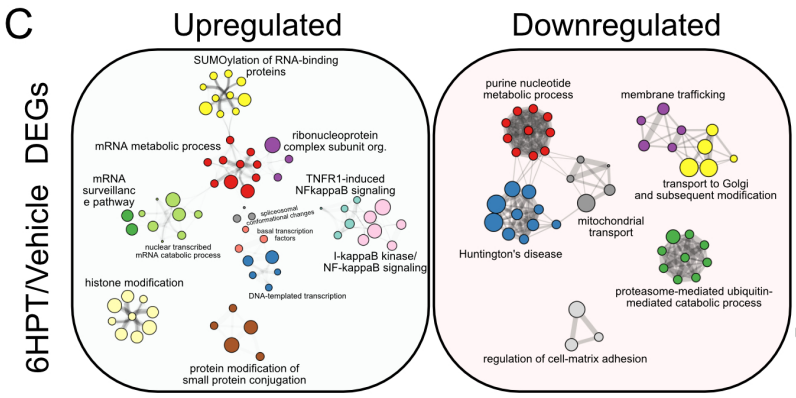
Supplemental Figure 1. PMA treatment does not lead to cytoplasmic translocation and leads do TP63 losses in the cytoplasm.

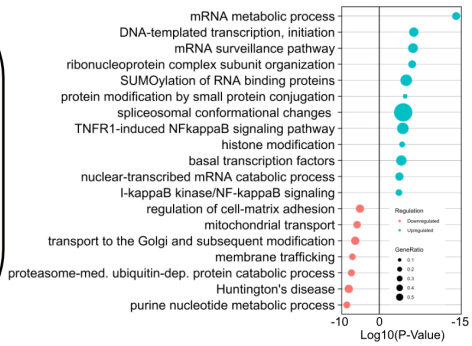
A) An integrated violin and boxplot representing the P63 Nuclear CytoplasmicRatio (NCR) quantification results at each time point (n=3).

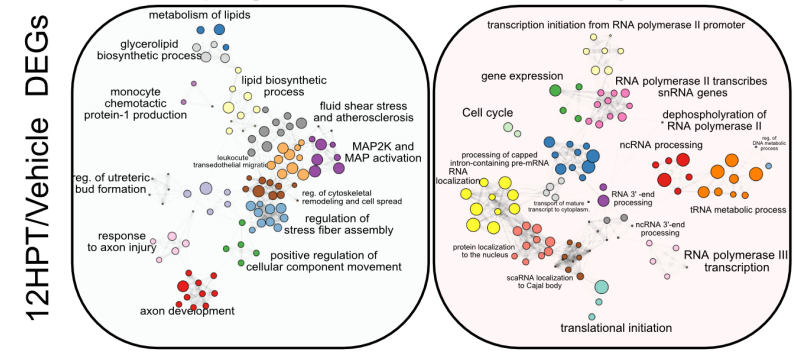
B) An integrated violin and boxplot representing the Cytoplasmic P63 Integrated Density/ Nuclear Area quantification results each time point (n=3).

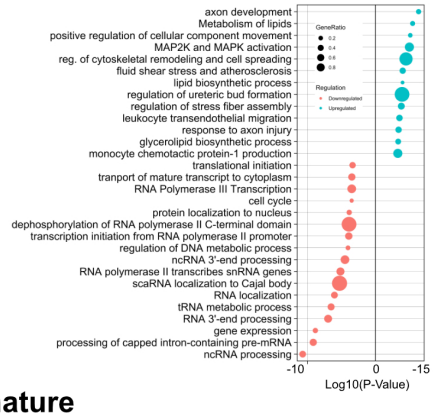
For A) and B) Extreme outliers were removed utilizing the Tukey method (1.5 x IQR) (A, B). For (A, B) statistical significance was determined by one-way ANOVA followed by Dunnett's test. For (A, B): *p< 0.05,**p< 0.001,***p< 0.0001.



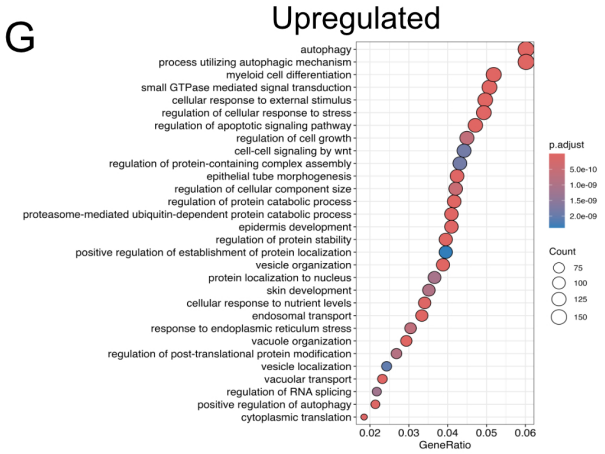


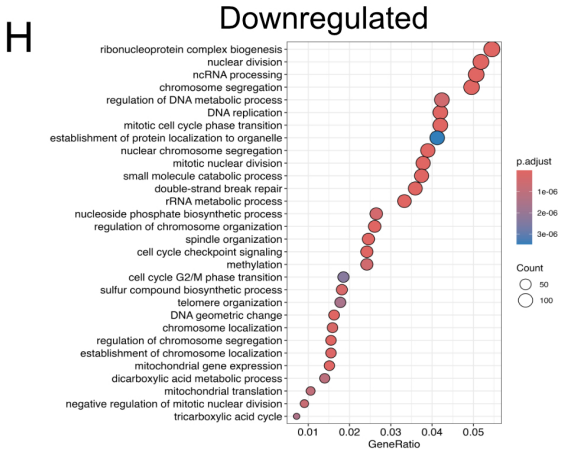






PMA-induced ontology signature
(96% concordant enrichment between 6HPT/Vehicle and 12HPT/Vehicle; n=6175/6458)





Supplemental Figure 2. Transcriptomic characterization of PMA-treated HBCs

A) Principal component analysis (PCA) plot of 12h-, 6h- and vehicle- PMA treated HBCs (n=3).
B) Venn diagram of differentially expressed genes (DEGs) of 12h- and 6h-PMA treated HBCs relative to vehicle. DEGs were identified with a false discovery rate (FDR) adjusted p-value of < 0.05.
C) Metascape enrichment of 6HPT/Vehicle DEGs genes
D) Accompanying ontology dotplot of significantly regulated ontological categories from (C)
E) Metascape enrichment of 6HPT/Vehicle DEGs genes
F) Accompanying ontology dotplot of significantly regulated ontological categories from (E)
G) ClusterProfiler dotplot of GO Biological Process ontology for shared, upregulated 6HPT and 12HPT genes, relative to vehicle.
H) ClusterProfiler dotplot of GO Biological Process ontology for shared, downregulated 6HPT and 12HPT genes, relative to vehicle.

For (C) and (F) Significance testing determined by Metascape. For (G) and (H) FDR adjusted p-value < 0.05 and q-value < 0.10. Ontology categories were simplified utilizing the Wang method, with a similarity cutoff of 0.7.

Supplemental Tables

Table S1. Primary antibodies, their source, and concentration

Target	Source and vendor	Concentration
Ms α-P63	ATCC	1:100
Ch α-KERATIN 5	Biolegend (905904)	1:500
Rb α-HOPX	Proteintech (11419-1-AP)	1:250
Rb α-RELA	CST (D14E12)	1:250
Rat α-SOX2	Invitrogen (14-9811-92)	1:200

Table S2. Excel file. Quantitative image analysis: sample sizes and immunofluorescent thresholds (if applicable). Related to Figure 1B; 1C; 1E; 1G; 1F; 1I; 1J; 3E; S1A; S1B. Each tab refers to the specific Figure and panel. Listed are the timepoints after PMA and/or MTZ injection.

Supplementary experimental procedures

Bulk RNA sequencing of PMA-treated HBCs

Cultured HBCs were washed 3x with PBS and incubated with Accutase at RT for 15 minutes. Suspended cells were spun and washed with 1X PBS before being flash frozen in liquid nitrogen and kept at -80°C overnight. Total RNA was harvested from frozen cell pellets utilizing the PureLink RNA Mini Kit per manufacturer's instructions. Residual DNA was removed utilizing the PureLink DNAse Set, according to manufacturer's instructions, before being subjected to NanoDrop analysis. RNA QC, cDNA synthesis, and library preparation was carried out by Novogene Corporation Inc. Samples underwent robust quality control (QC) assessment by Novogene Corporation, which included an assessment of RNA quality (RNA Integrity Number [RIN], all samples ≥ 9.7), cDNA library QC, which included library quantification and insert size assessment, as well as sequencing quality control. Samples were loaded onto an Illumina NovaSeq 6000 sequencer and samples were underwent paired end 150bp sequencing (PE150). Following demultiplexing, samples were delivered via FTP.

Transplantation of PMA-treated HBCs

The anterior neck of the host animals was shaved to permit access to the trachea. The skin was sanitized and disinfected with prep pads pre-soaked with iodine and then 70% isopropyl alcohol (Covidien, Catalogue #57520). A tracheotomy was performed, and the palate was elevated with a 3 cm piece of PE-100 tubing to appose the wall of the nasopharynx, preventing the infusion mixture from reaching the rest of the respiratory tree. Cells were gently resuspended and aspirated into a 1 ml plastic syringe (Fisher Scientific, Catalogue #14955456). Cells were then slowly infused into one naris until liquid could be seen starting to exit the other naris. The tracheotomy was then sutured, rats were injected with 2 ml of saline, and rats were placed onto a warm heat pad singly housed and allowed to recover overnight.

Single-cell RNA seq analysis – iterated subclustering

Principal components for dimensionality reduction were chosen at each stage of subclustering according to visual inspection of the Elbow Plot. Clustering was performed in Seurat using the Louvain algorithm; coarse-versus-fine-grained clustering was optimized at each stage of subclustering according to the concordance between cluster assignment and the expression of established cell type markers. *Kit*, *NeuroD1*, *Sox2*, and *Ascl1* were used to identify and remove GBC populations. *Cyp2g1* was used to identify and remove Sus cells. *Omp* and *Gap43* were used to identify and remove populations of olfactory sensory neurons. HBCs were identified based on *Krt5*, *Krt14*, and *Tp63* expression. Cycling HBCs were identified by expression of HBC markers plus expression of *Top2a*, *Mki67*, and *Ccnb1*. Activated HBCs were positively identified by expression of HBC markers plus *Hopx*, *Krt6a*, *Sprr1a*, *Sbsn*, and *Gpx2* expression. For pipeline to reproduce this data, please see our GitHub repository.