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Supplemental Information

NIX initiates mitochondrial fragmentation via DRP1 to drive epidermal differentiation

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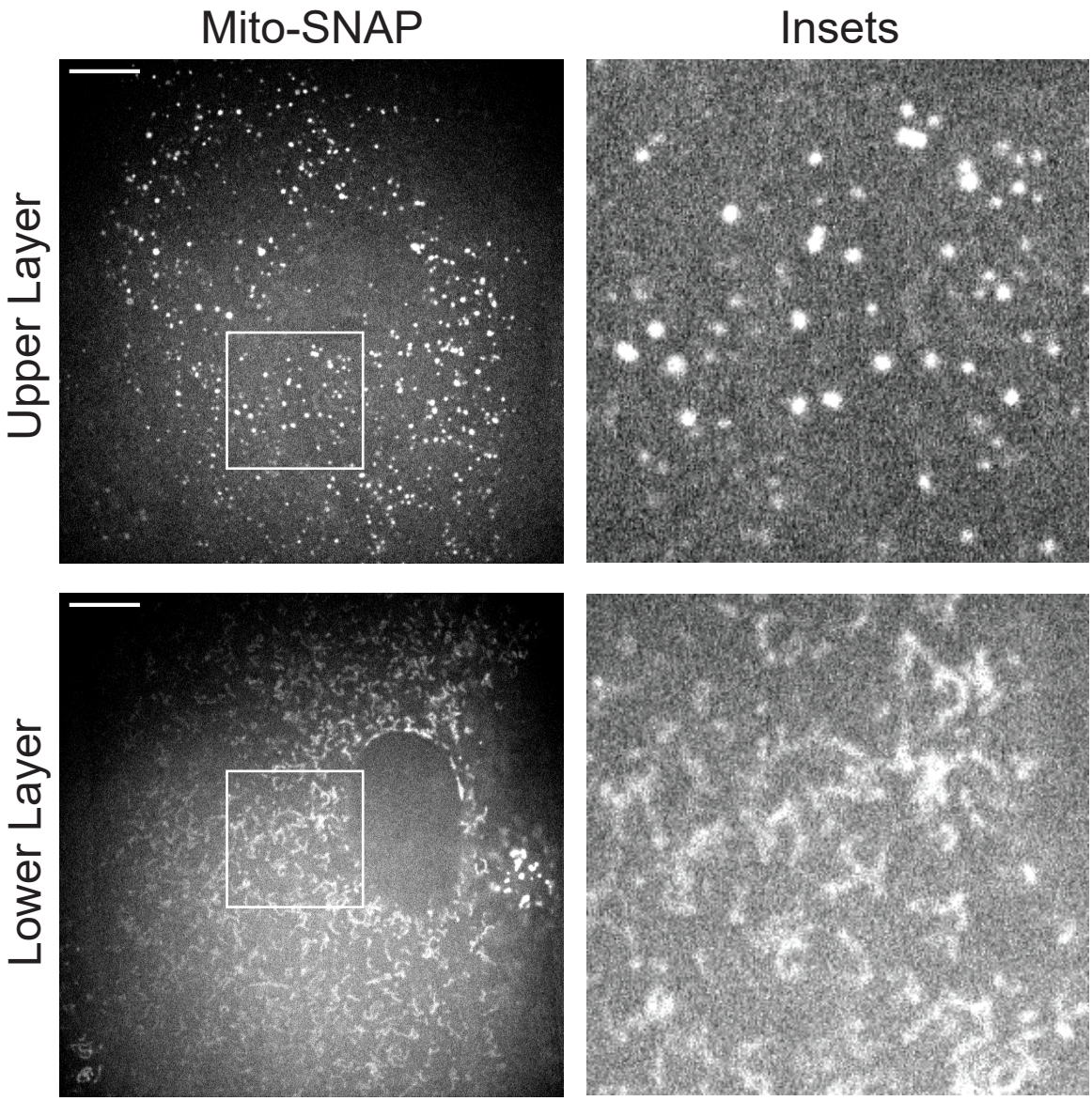

Figure S1: Keratinocyte mitochondria undergo fragmentation in the upper layers of organotypic epidermis, Related to Figure 1. SDC images of organotypic epidermis transduced with Mito-SNAP and treated with 647 nm-labeled cell-permeable SNAP tag. While branched and tubular mitochondria were present in lower layers (lower panels), differentiating cells within the upper layers (upper panels) exhibited spherical mitochondrial fragments. White bar=10 μm.

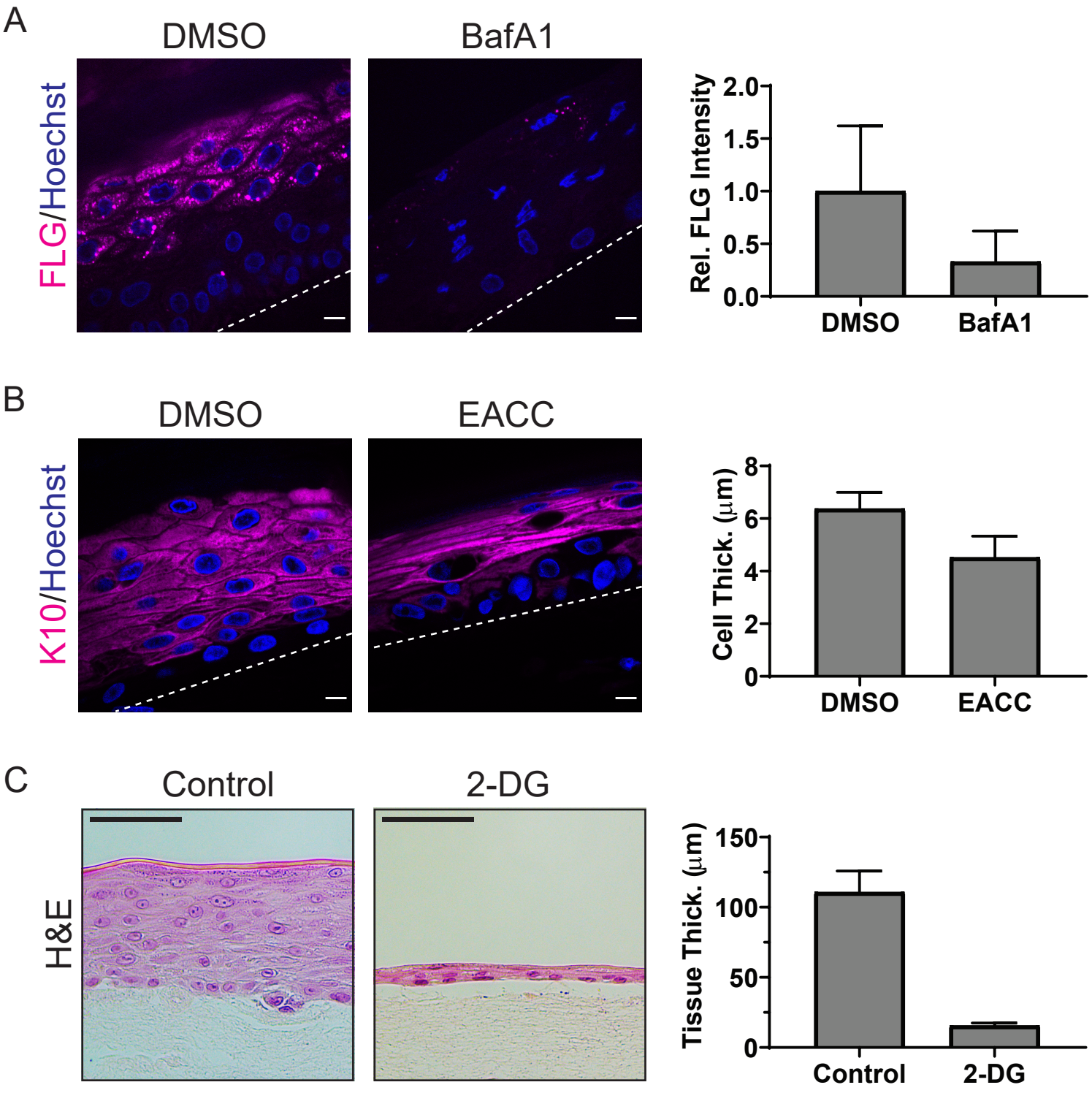

Figure S2: Lysosomal acidification, autophagosome-lysosome fusion, and glycolysis are critical for epidermal differentiation, Related to Figure 2. (A) ImF of FLG in DMSO- vs. BafA1-treated organotypic epidermis; (right) quantification of relative intensity of FLG ImF (mean+/-SD; n=8 fields). (B) K10 ImF in DMSO- vs. EACC-treated organotypic epidermis highlights morphology of suprabasal keratinocyte layers; (right) quantification of thickness of K10-positive cells (mean+/-SD; n=20 fields). (C) H&E staining of control vs. 2-DG-treated organotypic epidermis; (right) quantification of tissue thickness (mean+/-SD; n=8 fields). Dashed line marks bottom of epidermis; white bar=10 μm; black bar=50 μm.

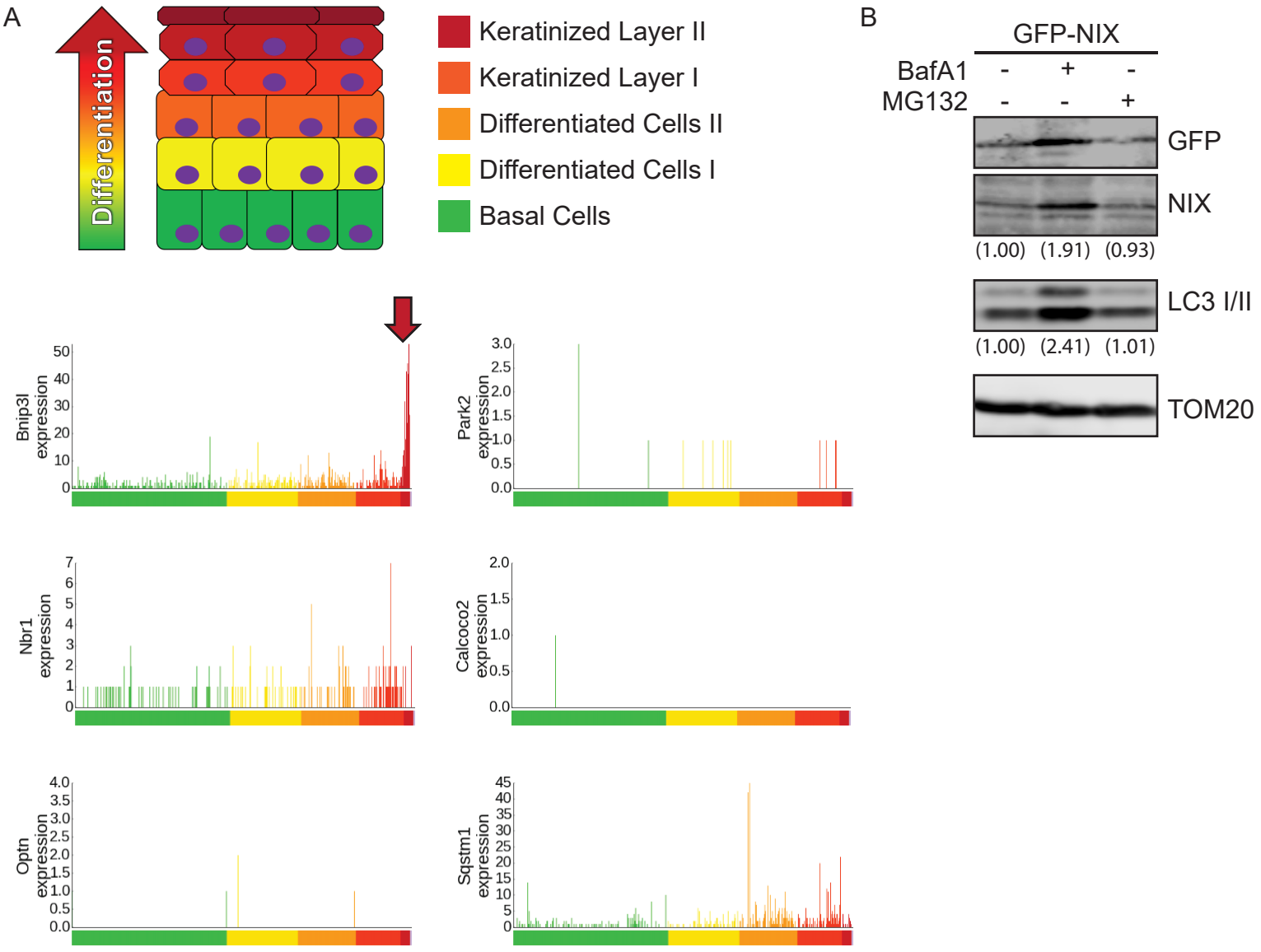

Figure S3: NIX expression is up-regulated in the upper epidermal layers and its protein levels are controlled by lysosomal degradation, Related to Figure 3. (A) Diagram of the epidermis depicting keratinocytes that progressively differentiate as they move upward in the epithelium with layers coded by color; (below) graphs generated from the public single-cell RNA sequencing database from murine epidermal keratinocytes (http://linnarssonlab.org/epidermis/) (Joost et al. 2016) depicting color-coded RNA expression levels in the different epidermal layers for the following genes: NIX (Bnip3l), Parkin (Park2), Nbr1, NDP52 (Calcoco2), optineurin (Optn), and p62 (Sqstm1); red arrow denotes a marked increase in NIX expression in the uppermost keratinocyte layers. (B) WB of lysates from NHEKs transduced with GFP-NIX and treated with DMSO vs. BafA1 to inhibit lysosomal degradation or MG132 to inhibit proteasomal degradation (below, relative band intensity normalized to TOM20).

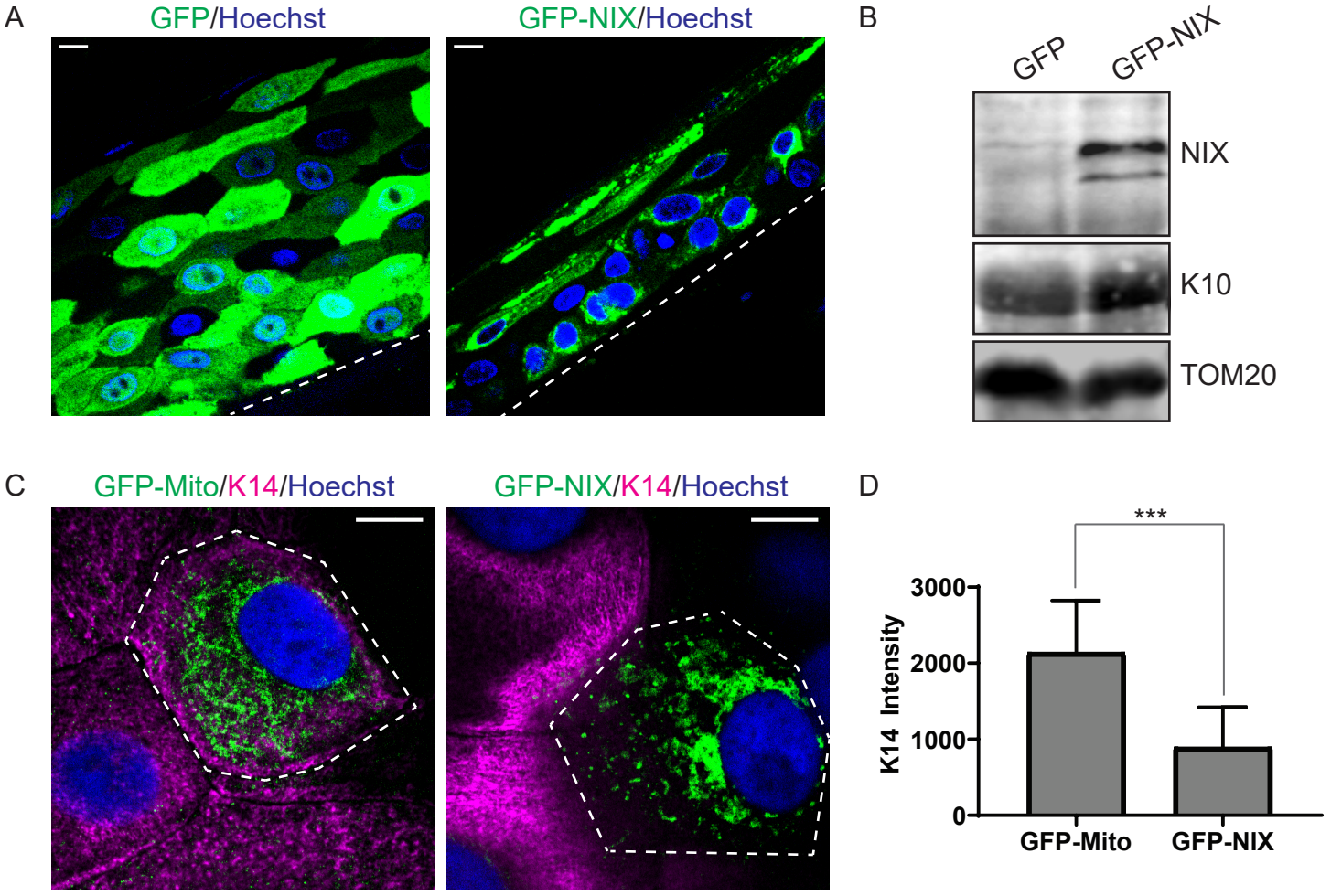

Figure S4: Premature NIX expression accelerates the epidermal differentiation program, Related to Figure 5. (A) NHEKs transduced with GFP or GFP-NIX were grown as organotypic cultures for 5 days, then were fixed and tissue sections were immunostained with anti-GFP to localize expression of the transgene and Hoechst. Dashed line marks bottom of epidermis; bar=10 μm. (B) WB of day 5 organotypic culture lysates, which demonstrated transgenic NIX expression in cultures transduced with GFP-NIX while keratin 10 (K10) was expressed in both GFP- and GFP-NIX-transduced cultures; TOM20 serves as a protein loading control for mitochondria. (C) NHEKs transduced with GFP-Mito or GFP-NIX were fixed in methanol and immunostained for K14 and Hoechst. Dashed line marks cell outline; bar=10 μm. (D) The average intensity of K14 immunostaining was quantified in cells expressing GFP-Mito compared to GFP-NIX (mean+/-SD; n=101 cells from 4 donors, ***p<0.0001).