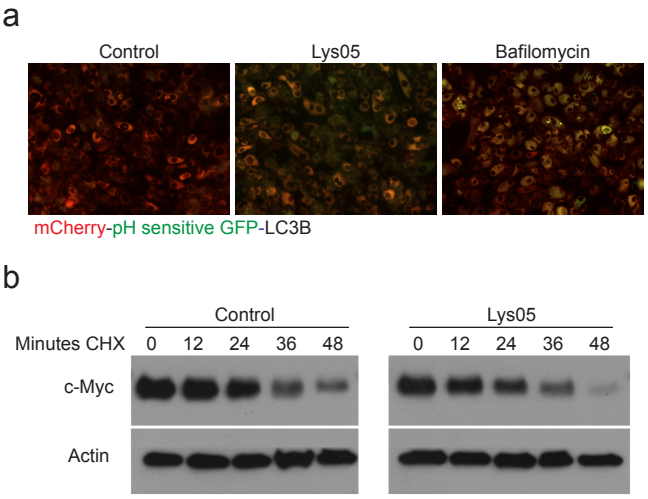


Figure S1. Lys05 and bafilomycin-A1 block lysosome-mediated degradation. (a) Keratinocytes were transduced with a double-tagged fluorescent LC3B (mCherry-GFP-LC3B), where the GFP tag is acid-sensitive and the mCherry tag is acid-insensitive (Hansen and Johansen, 2011). Keratinocytes treated with Lys05 and bafilomycin-A1 accumulated more GFP signal than control, indicating that autophagic cargo was not degraded. (b) Lys05 does not interfere with proteasome-mediated degradation. Cycloheximide (10µg/mL) was used to block translation in keratinocytes pre-treated with Lys05 for 48 hours versus control. Cells from monolayer cultures were lysed at time points indicated. Proteasome-mediated degradation was evaluated as a function of the expression of rapidly degraded c-Myc.

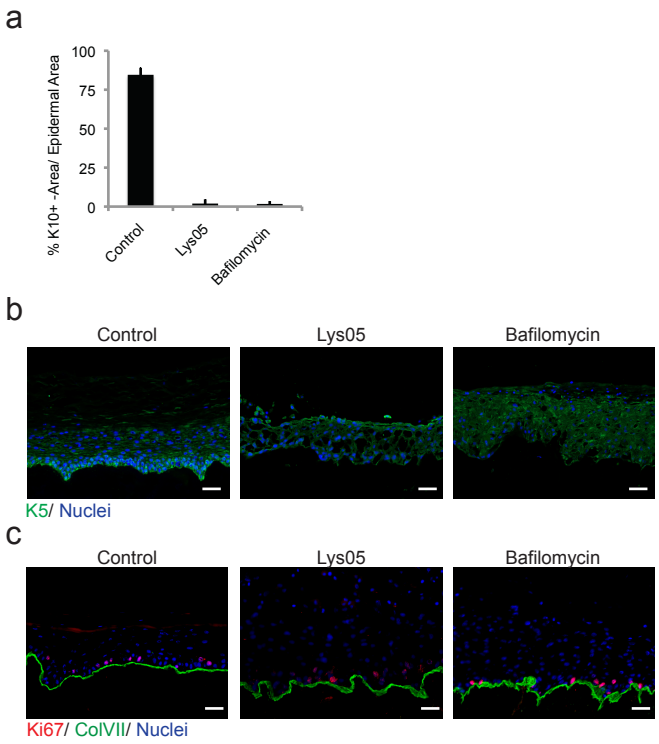


Figure S2. Lysosome inhibition in OTC does not affect expression of basal keratin-5 or basal cell proliferation. (a) Control, Lys05, and bafilomycin-A1 organotypic cultures were evaluated for differentiation by quantifying the area of keratin-10 positive epidermis over area of total epidermis. Lysosomal inhibition results in undifferentiated tissue. (b) Control and drug-treated tissues maintained keratin-5 (green) expression. (c) Proliferative basal keratinocytes are highlighted by Ki67 (red), with ColVII (green), and nuclei (blue). All scale bars = 100μM.

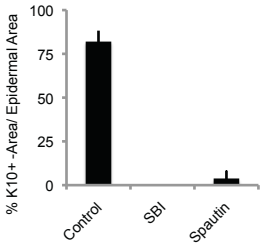


Figure S3. Blocking autophagy results in undifferentiated tissue. SBI and Spautin inhibit epidermal differentiation in organotypic cultures.

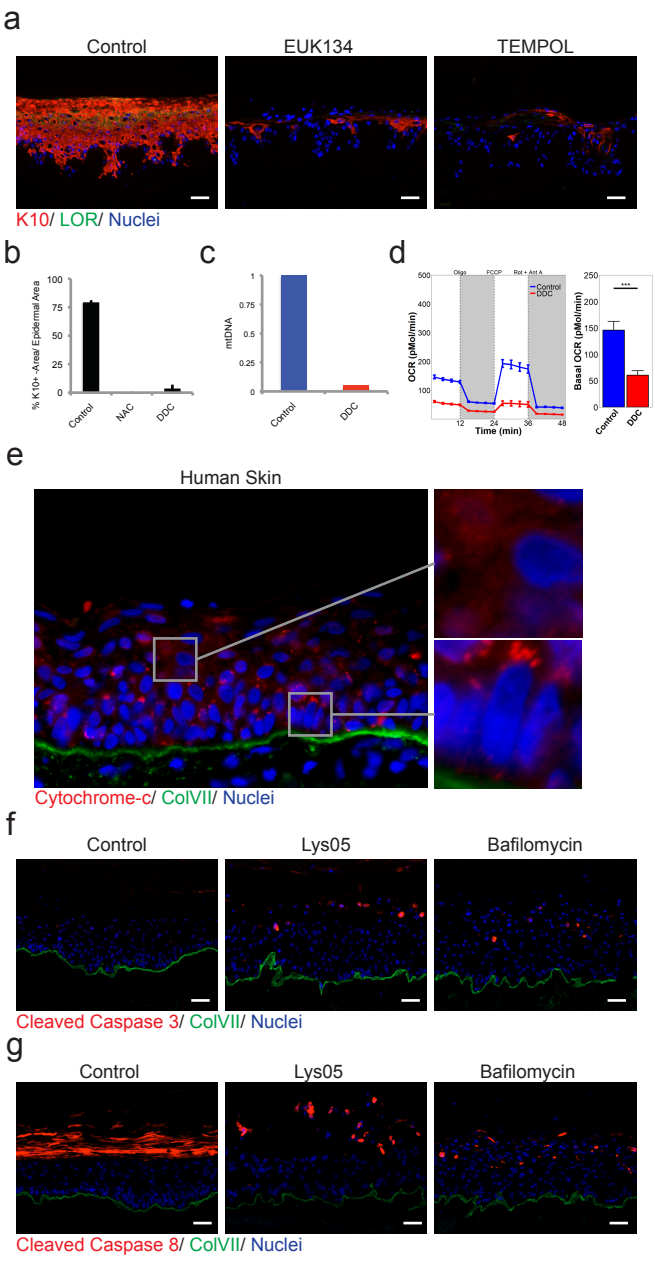


Figure S4. Epidermal differentiation is dependent on mtROS. (a) ROS-scavengers, EUK134 and TEMPOL, suppressed epidermal differentiation indicated by loss of keratin-10 (red) and loricrin (green). (b) NAC and DDC inhibit epidermal differentiation. (c-d) DDC interferes with mtDNA replication in keratinocytes resulting in (c) reduced levels of mtDNA and (d) diminished oxygen consumption. (e) Diffuse localization of cytochrome-c (red) indicates mitochondrial depolarization in human skin tissue. (f) Differentiating keratinocytes in control OTCs do not contain active caspase-3 (red), whereas some cells in upper layers of Lys05 and bafilomycin-A1 OTCs are cleaved caspase-3 positive. (g) Cleaved caspase-8 (red), which is upstream of caspase-3 in apoptosis, and signals independently of caspase-3 in cornification, is expressed in control OTC in a pattern consistent with terminal differentiation. Caspase-8 activation was reduced in Lys05 and bafilomycin-A1 OTCs and its mottled expression in this setting, similar to the pattern of cleaved caspase-3, suggests apoptosis rather than differentiation. ColVII (green) and nuclear signal (blue) were used as counterstains. All scale bars = 100μM.

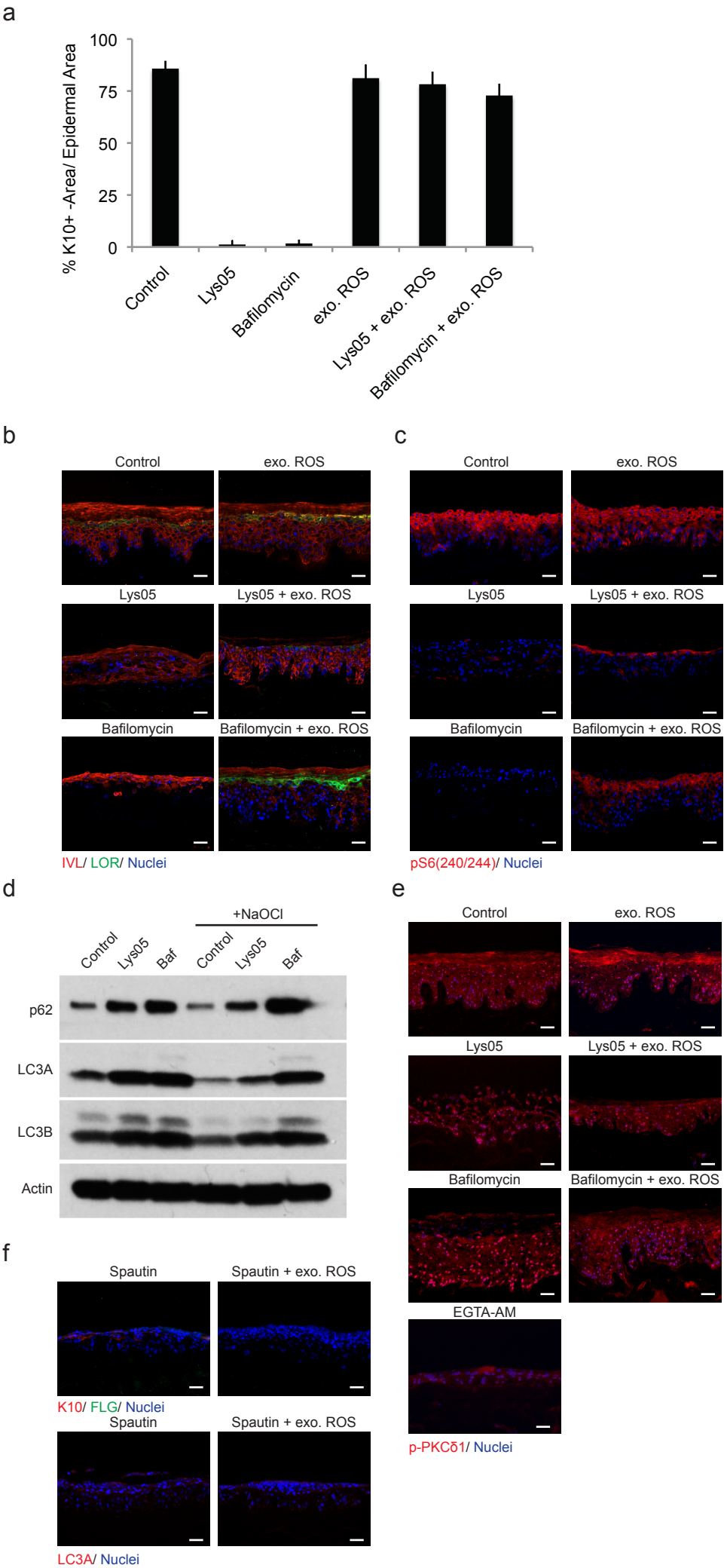


Figure S5. Exogenous ROS restores differentiation in epidermis with inhibited lysosomes. (a) While Lys05 and bafilomycin-A1 block differentiation in organotypic cultures, this effect was rescued by exogenous ROS. (b) While exogenous ROS largely restores epidermal architecture, ROS did not fully normalize the assembly and crosslinking of involucrin (red) and loricrin (green). (c) Immunostaining for active S6 (red) and nuclei (blue) demonstrated that exogenous ROS did not completely restore mTOR-pS6K-pS6 signaling. (d) In western analysis from monolayer cultures, NaOCl did not interfere with the capacity of Lys05 or bafilomycin-A1 to inhibit lysosome-mediated degradation. Both compounds lead to accumulation of p62, and lipidated forms of LC3A and LC3B, independent of ROS. (e) Lys05, bafilomycin-A1, and exogenous ROS (NaOCl) do not affect activation or localization of the calcium signaling molecule, p-PKC∂1 (red). As a negative control, calcium chelation via EGTA-AM prevented phosphorylation of PKC∂1 (red). (f) Exogenous ROS did not rescue differentiation in Spautin-1 OTCs as seen by immunofluorescent microscopy for Keratin 10 (red) and filaggrin (green) with nuclei (blue). NaOCl also did not restore the distribution of LC3A in Spautin-1 OTCs (red), counterstained with nuclei (blue). All scale bars = 100μM.

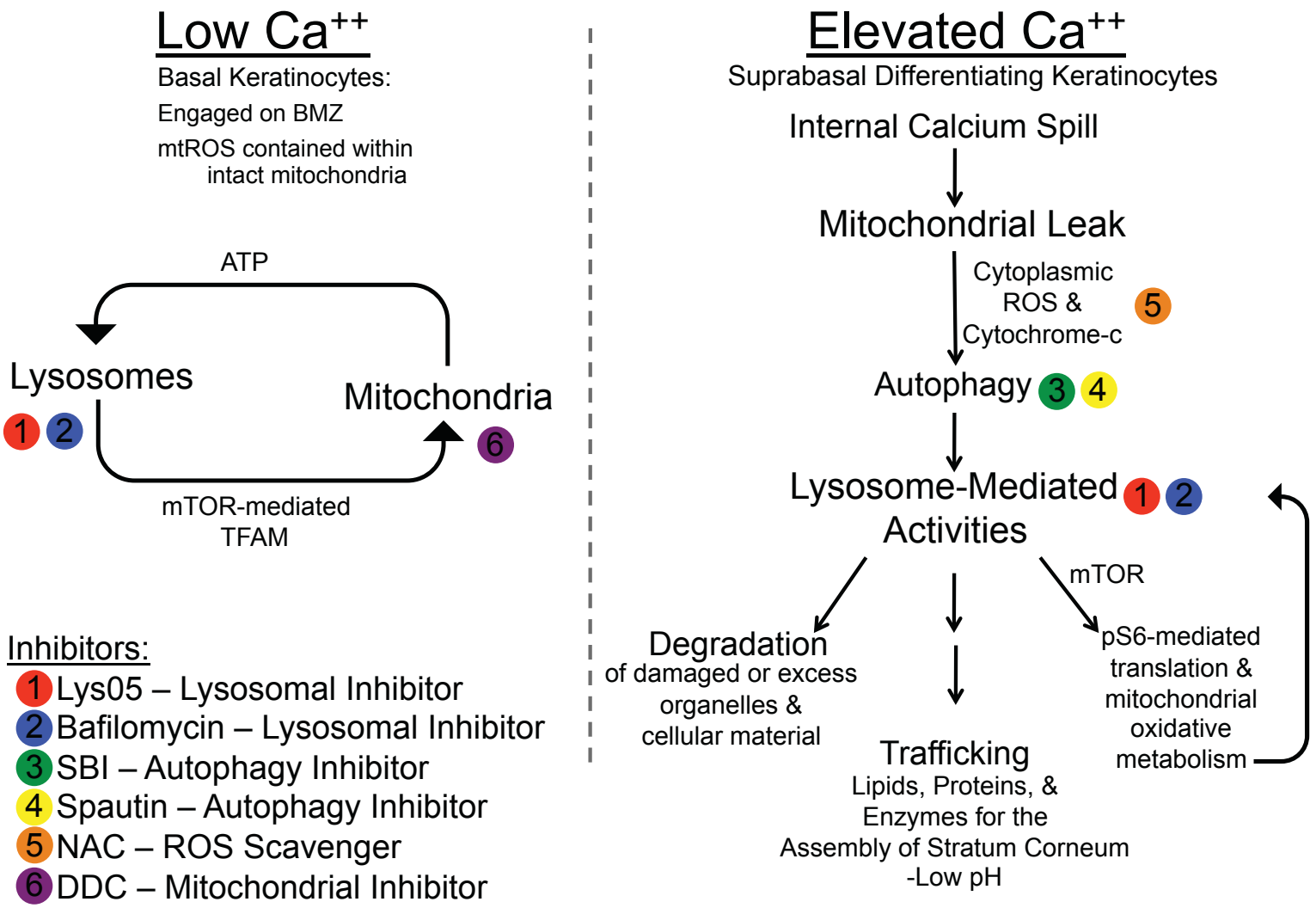


Figure S6. Schematic model detailing the relationships between lysosomes and mitochondria in normal epidermal homeostasis. In basal keratinocytes, lysosomes support mitochondrial metabolism, while mitochondria generate the ATP required for lysosomal function. Suprabasal keratinocytes undergo an internal calcium spill that induces mitochondrial leak of mtROS, and autophagy necessary for epidermal differentiation.