

## Supplemental Figure 1: <u>Controls for mBD3 and CRAMP Immunostaining</u>:

Immunostaining was performed for mBD3 (A-C) and CRAMP (D-F) and in CRAMP k.o. mice (B, D). Panels C and F were immunostained with secondary antibody only. Note marked decrease in CRAMP staining in CRAMP k.o. mice (E); in contrast, the epidermis of some CRAMP k.o. mice demonstrates increased mBD3 protein (B). Mag bars = 50 \_m. G: The full-length mBD3 (3.5-4 kDa) is detectible on a 10% tricine gel between 110 and 140 kDa. The western immunoblotting shows the full-length peptide (left) and mBD3 protein in normal (non-stressed) mouse epidermis (right).



Supplemental Figure 2: Exogenous GC Down-Regulate CRAMP (but not mBD3)

**<u>mRNA Expression In Vitro</u>**: Second-passage, cultured human keratinocytes were treated with a single dose of dexamethasone (10 ng/ml ethanol) or same volume of vehicle alone (controls) for 18 hrs, followed by RNA extraction and quantitation by rt-PCR, as in Methods.



Supplementary Figure 3: Normal Permeability Barrier Recovery in

Adrenalectomized Mice: Adrenalectomized and sham-operated mice (n=5 each) were

tape-stripped until TEWL levels  $\geq$  5x normal, and recovery rates were compared 2 and 4 hours later. The differences in recovery rates in the two groups were not significant.



**Supplementary Figure 4:** <u>Exogenous Physiologic Lipids Restore Lamellar Bodies in</u> <u>Psychologically Stressed (PS) Epidermis</u>: A: PS epidermis under basal conditions (PS + Ba) displays few lamellar bodies (LB) in cytosol of outer stratum granulosum (SG) cells. Note proximity of secreted LB contents at SG-stratum corneum (SC) interface (open arrows). B: PS animals treated with vehicle alone (PS + Veh). C: PS animals treated with physiologic lipids (PS + L). Few LB are present in SG cytosol of vehicletreated, PS mice (arrowheads), and secreted LB contents at SG-SC interface remain diminished (B, open arrows). In contrast, PS and lipid-treated animals reveal numerous LB in SG cytosol (C, arrowheads), and abundant, newly-secreted LB contents at SG-SC interface (C, solid arrows). A, ruthenium textroxide post-fixation; B & C, osmium tetroxide post-fixation. Mag bars = 0.5 \_m.



Supplementary Figure 5: <u>Topical Physiologic Lipids Do Not Normalize</u> <u>Immunostaining for CRAMP after PS/GC</u>: Hairless mice (n= 4 or 5 in each cohort) received either equimolar mixture of ceramides, cholesterol and free fatty acids (1:1:1 molar ratio; 2% final concentration) in propylene glycol:ethanol (7:3 vols) vehicle (60 \_1 vols to  $3 \text{ cm}^2$ ), or vehicle alone, while being co-treated with either PS (E, F) or topical clobetasol (GC) (B, C), as above. Frozen sections were immunostained for CRAMP and mBD3, as above. Mag bars = 50 \_m.