Supporting Information

Omori et al. 10.1073/pnas.1116188109

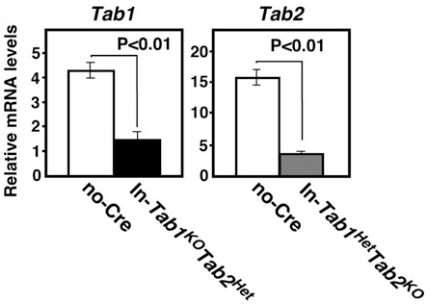

Fig. S1. *Tab1* and *Tab2* mRNAs were reduced in the $Tab1^{KO}Tab2^{Het}$ and $Tab1^{Het}Tab2^{KO}$ intestine. Real-time PCR analysis quantified mRNA levels in control (open bars) and intestinal epithelial-specific *Tab1* or *Tab2* knockout (solid and shaded bars) small intestines at P0. Relative mRNA levels of *Tab1* and *Tab2* were calculated using Gapdh mRNA. Data show the means ± SE of no-Cre, $n = 12$; In-$Tab1^{KO}Tab2^{Het}$, $n = 4$; and In-$Tab1^{Het}Tab2^{KO}$, $n = 7$.

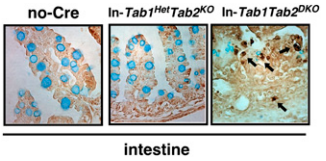

Fig. S2. Intestinal epithelial cells including goblet cells underwent apoptosis in *Tab1* and *Tab2* double-deficient intestinal epithelium. Sections of the small intestine from no-Cre, In-$Tab1^{Het}Tab2^{KO}$, and In-$Tab1Tab2^{DKO}$ at P0 were double stained with TUNEL (brown) and Alcian Blue (blue). Arrows indicate examples of TUNEL-positive cells.

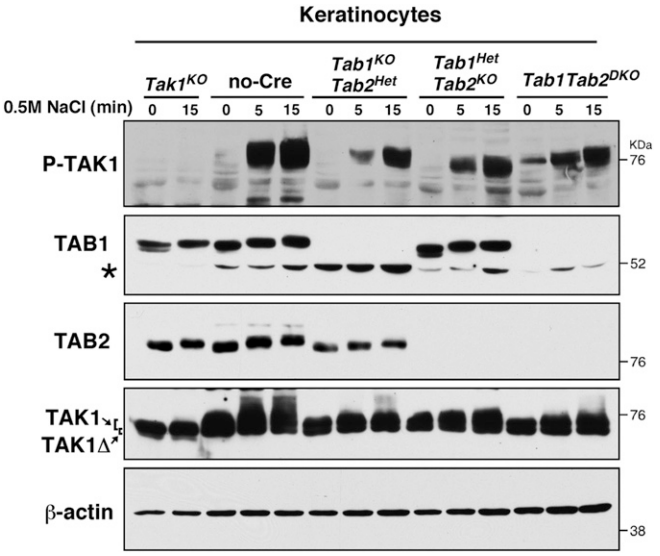

Fig. S3. TAB1 is not solely responsible for osmotic stress-induced TAK1 activation in keratinocytes. $Tak1^{KO}$, no-Cre, $Tab1^{KO}Tab2^{Het}$, $Tab1^{Het}Tab2^{KO}$, and $Tab1Tab2^{DKO}$ keratinocytes were stimulated with 0.5 M NaCl for 5 or 15 min. Immunoblotting was performed using the indicated antibodies. Asterisk indicates a nonspecific band.

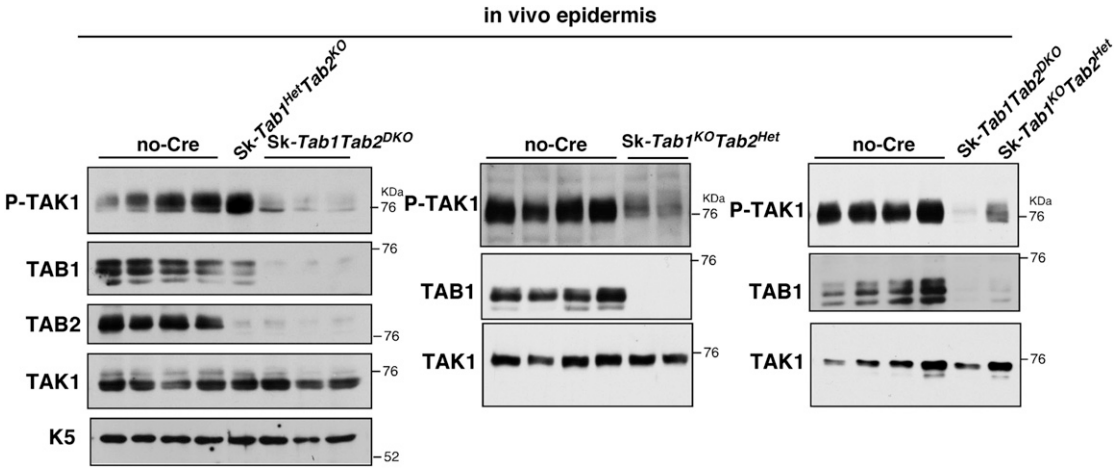

Fig. S4. Basal activity of TAK1 in in vivo epidermis. Shown is immunoblotting of phosphorylated TAK1, total TAK1, TAB1, and TAB2 in epidermal extracts of no-Cre control, *Sk-Tab1*^{Het}*Tab2*^{KO}, *Sk-Tab1*^{KO}*Tab2*^{Het}, and Sk-*Tab1Tab2*^{DKO} mice from three litters. Each set of panels represents one litter and each lane represents one animal.

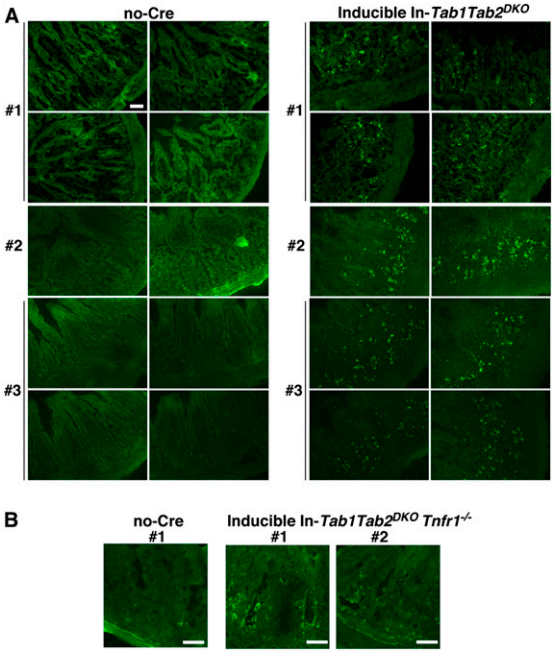

Fig. S5. Double deficiency of *Tab1* and *Tab2* causes ROS accumulation. (*A*) ROS were detected in unfixed fresh frozen intestine sections by CM-H2DCFDA. Images of two different positions of the small intestine from three no-Cre control and three inducible double-knockout mice (nos. 1, 2, and 3) are shown. (Scale bars, 50 μm.) (*B*) Control no-Cre and two (nos. 1 and 2) inducible In-*Tab1Tab2*^{DKO} *Tnfr1*$^{-/-}$ mice were treated with tamoxifen for 3 consecutive days, and intestinal sections were prepared 2 wk after the tamoxifen treatment. ROS were detected in unfixed fresh frozen intestine sections by CM-H2DCFDA. (Scale bars, 40 μm.)

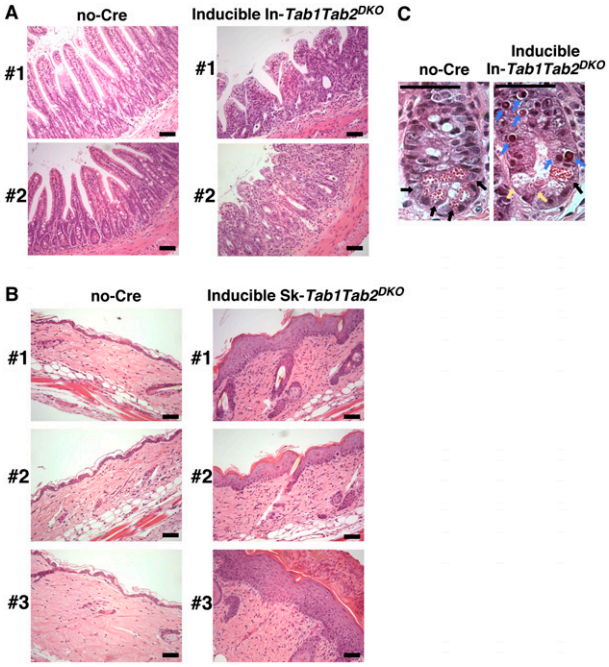

Fig. S6. Double deficiency of *Tab1* and *Tab2* causes tissue damage. (*A*) Control no-Cre and inducible In-*Tab1Tab2*^{DKO} mice or Sk-*Tab1Tab2*^{DKO} mice were treated with tamoxifen for 2 or 5 consecutive days, respectively. Intestinal sections were prepared 2 d after the second tamoxifen treatment and skin sections were prepared 9 d after the fifth tamoxifen treatment. Images of the small intestine and the epidermis from two or three no-Cre control and two or three inducible double-knockout mice (nos. 1 and 2 or nos. 1, 2, and 3) are shown. (Scale bars, 50 μm.) (*B*) The crypts of control no-Cre and inducible In-*Tab1Tab2*^{DKO} are shown. Black arrows indicate intact paneth cells, yellow arrows indicate damaged paneth cells, and blue arrows indicate morphologically apoptotic epithelial cells. (Scale bars, 40 μm.)