Expanded View Figures

Figure EV1. Quantitative analysis of phosphoproteome upon epidermal differentiation (related to Fig 1).

A Mass spectrometry quantification indicates the efficiency of SILAC labeling in cultured epidermal basal cells.

B Cell proliferation of control (Ctrl) and SILAC labeled cells (light or heavy isotope). Fold increase of cell numbers is quantified for all cell types (left panel). In the right panel, induction of cell differentiation after calcium shift is quantified for control and SILAC labeled cells. Error bars represent SD, $n = 3$.

C Cell lysates were prepared from undifferentiated or differentiated keratinocytes (12 or 24 h post-calcium shift) and subjected to immunoblots with different antibodies as indicated. Hr: hour. Numbers on left side indicate molecular weight markers. kD: kilodalton.

D Percentage of distribution of different phosphorylated residues in phosphoproteome of epidermal stem cells.

E Cell lysates were prepared from undifferentiated or differentiated keratinocytes (12 h post-calcium shift) and subjected to immunoblots with different antibodies as indicated.

F Annotated spectra of the three Pkp1 phosphorylation sites at the N-terminal head domain.

G Other potential phosphosites in Pkp1 head domain that were identified in our proteomics analysis are listed in the table.

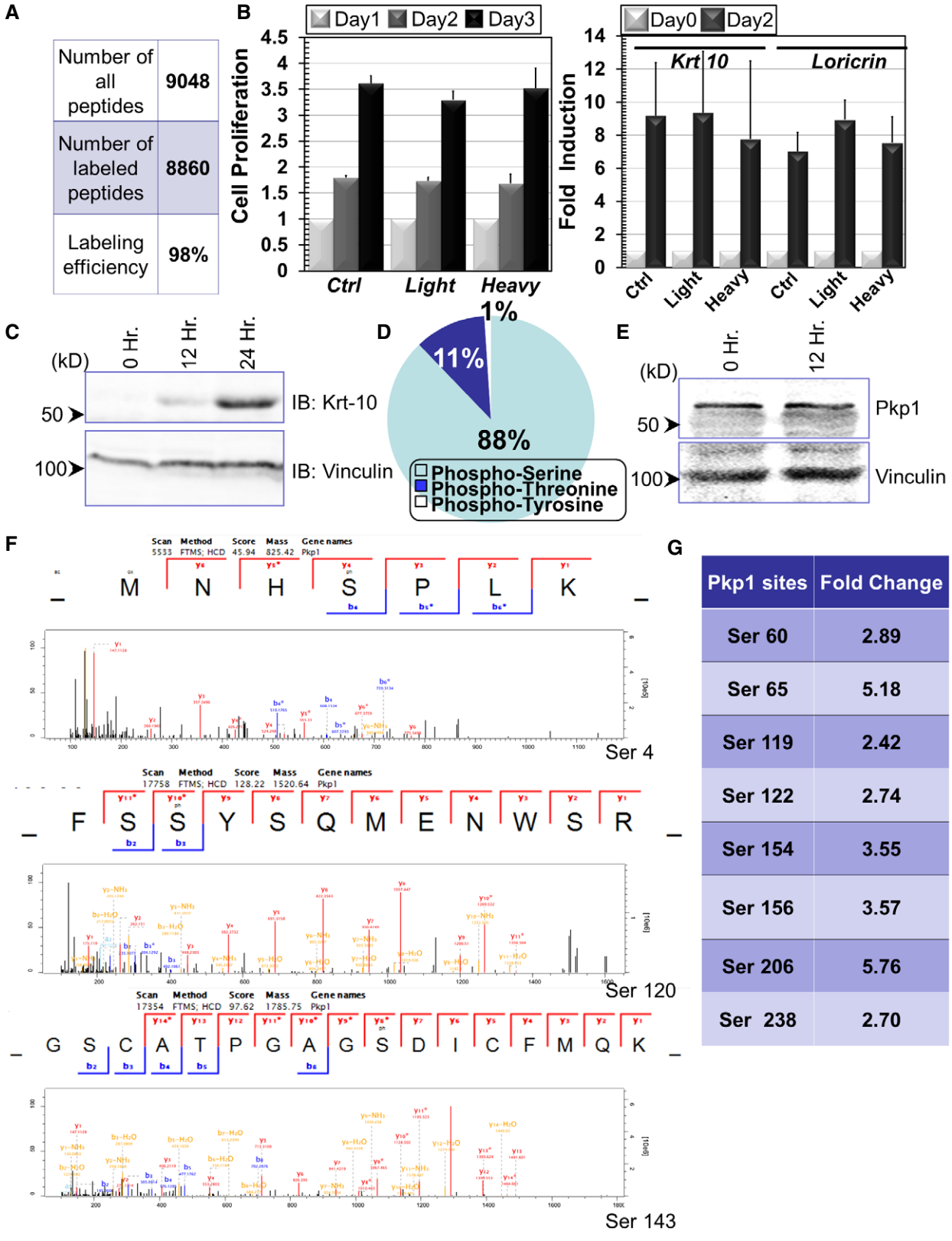

Figure EV1.

Figure EV2. Loss of *Pkp1* inhibits epidermal differentiation and promotes skin tumorigenesis (related to Figure 2).

A Cell proliferation of WT and *Pkp1* KO cells. Fold increase of cell numbers is quantified for both cell types. Error bars represent SD, $n = 3$.

B Western blot analysis of early (Krt10) and late (loricrin) differentiation marker expression in WT and KO keratinocytes upon calcium shift. Band intensity was determined by densitometry and quantified (Fig 2C).

C H/E (hematoxylin and eosin) staining of skin organoid culture *in vitro* (WT cells). Scale bar = 100 μm.

D H/E staining of grafted skin (WT or *Pkp1* KO) after skin transplantation. Scale bar = 100 μm.

E WT and *Pkp1* KO cells were grafted to mice, and grafted tissue was collected and subjected to immunofluorescence staining with different antibodies as indicated. Lor: loricrin; β4: CD104, β4-integrin; DAPI: nuclear stain. The dashed line denotes the basement membrane that separates dermis and epidermis (Epi). Scale bar = 50 μm.

F Quantification of cell proliferation *in vivo*. Percentage of phospho-histone H3-positive cells in WT or *Pkp1* KO skin grafts was quantified and shown as bar graph. Error bars represent SD, $n = 8$.

G Grafted skins were stained with different antibodies as indicated. Boxed areas are magnified as insets that show only DSG1 or ZO1 staining. DSG1: desmoglein 1. Scale bar = 50 μm.

H Immunoblot of WT and *Pkp1* KO cells infected with virus encoding *Ha-Ras* mutant. Note increased phospho (Pi)-Erk level upon expression of *Ras* mutant. Loss of *Pkp1* leads to a further increase in the phospho-Erk level. The numbers below indicate relative Pi-Erk level in both WT and KO cells upon expression of *Ha-Ras* mutant, as quantified by densitometry.

I H/E staining of skin tumors generated from WT and *Pkp1* KO cells. Scale bar = 500 μm.

J, K Quantification of proliferative (phospho-histone) and apoptotic (active caspase-3 staining) cells in WT and *Pkp1* KO tumors (infected with virus encoding *Ha-Ras* mutant). Error bars represent SD. Apoptosis is significantly increased ($P < 0.05$, Student's *t*-test) and proliferation is mildly but significantly reduced ($P < 0.05$, Student's *t*-test) in the KO skin, $n > 10$.

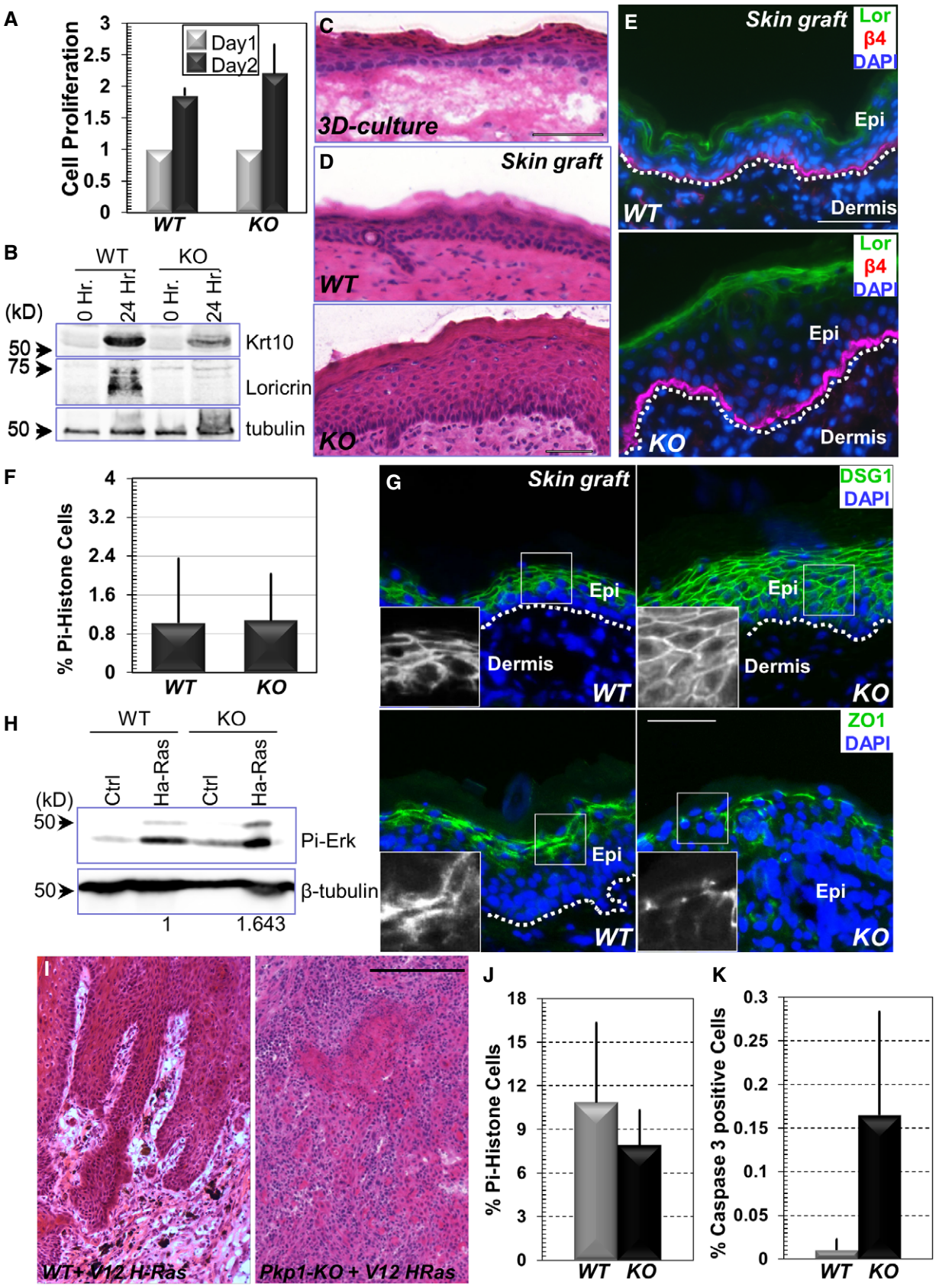

Figure EV2.

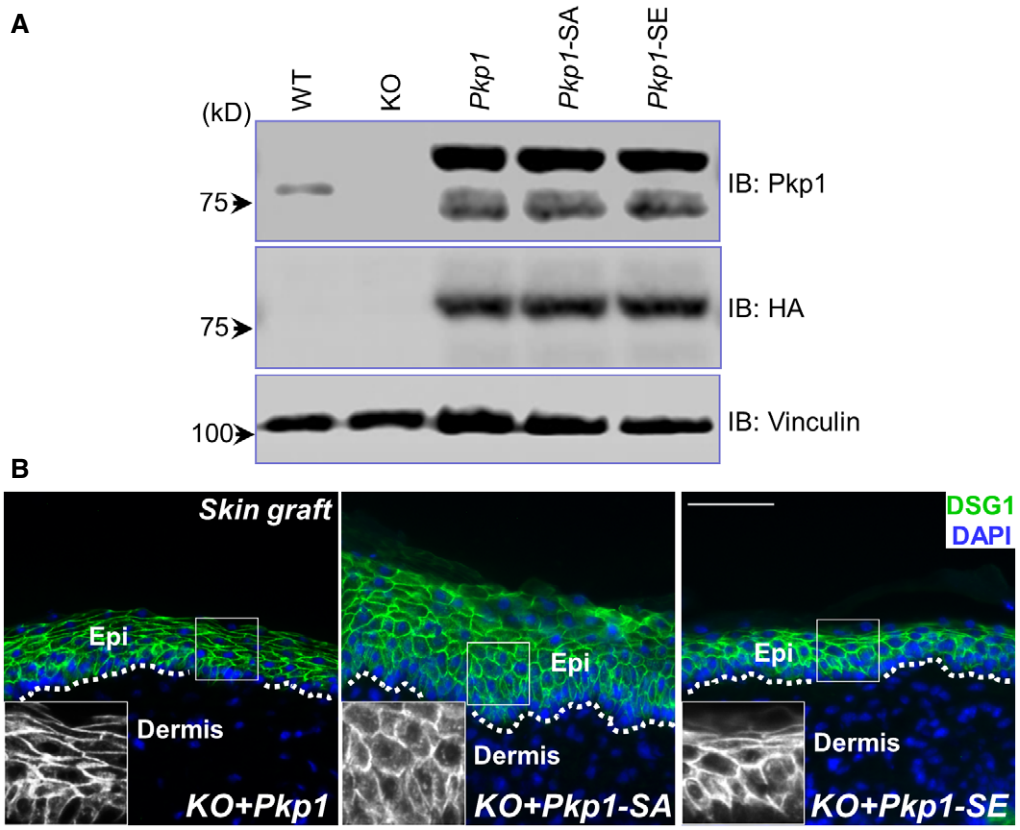

Figure EV3. Exogenous expression of *Pkp1* or its mutants in *Pkp1* KO cells (related to Fig 3).

A Cell lysates were collected from KO and rescued cells, and examined by immunoblots with different antibodies as indicated.

B Skin sections from different grafted skin were immunostained with antibodies as indicated. Boxed areas are magnified as insets that show only DSG1 staining. Scale bar = 50 μm.

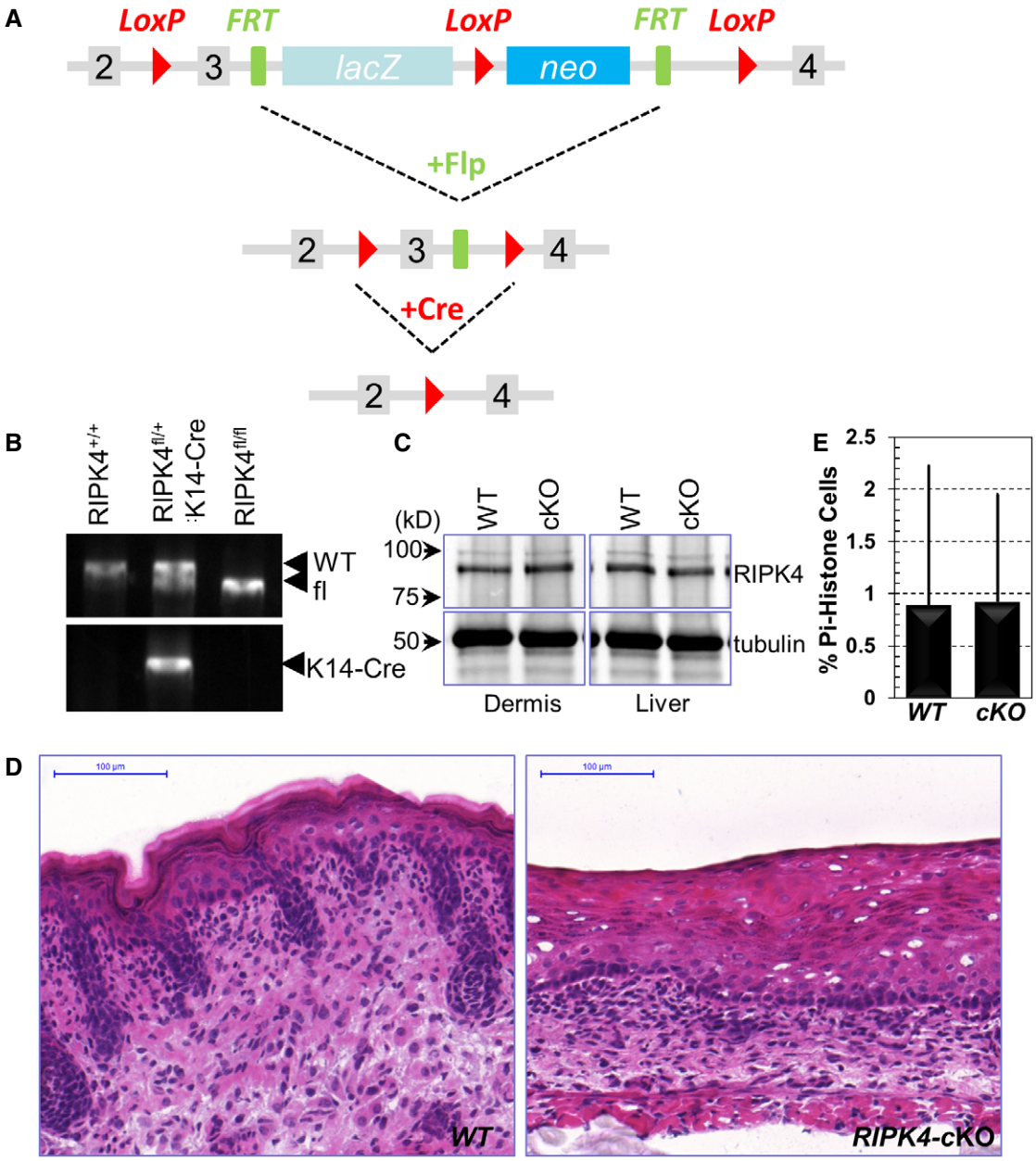

Figure EV4. Generation of *RIPK4* skin cKO strain (related to Fig 4).

A Diagram of the strategy for generation of *RIPK4* skin cKO strain.
B Typical genotyping results for *RIPK4* cKO.
C Lysates were prepared from skin dermis and liver from WT and *RIPK4* cKO animals. Lysates were immunoblotted with different antibodies as indicated.
D H/E staining of WT and *RIPK* cKO skin (E18.5). Scale bar = 100 µm.
E Quantification of proliferative (phospho-histone) cells in WT and *RIPK4* cKO skin. Error bars represent SD, $n > 10$.

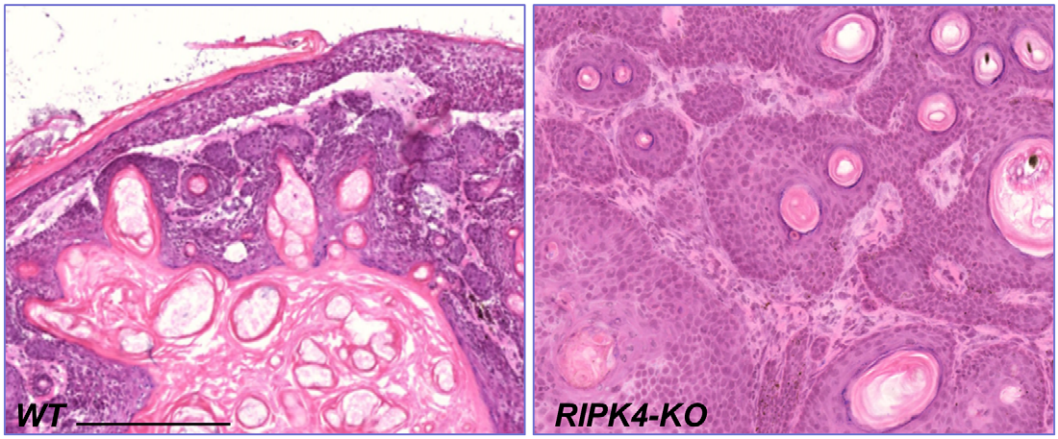

Figure EV5. Loss of *RIPK4* enhances skin tumorigenesis (related to Fig 5).

H/E staining of skin tumors generated from WT and *RIPK4* cKO animals upon DMBA/TPA treatment. Scale bar = 200 μm.

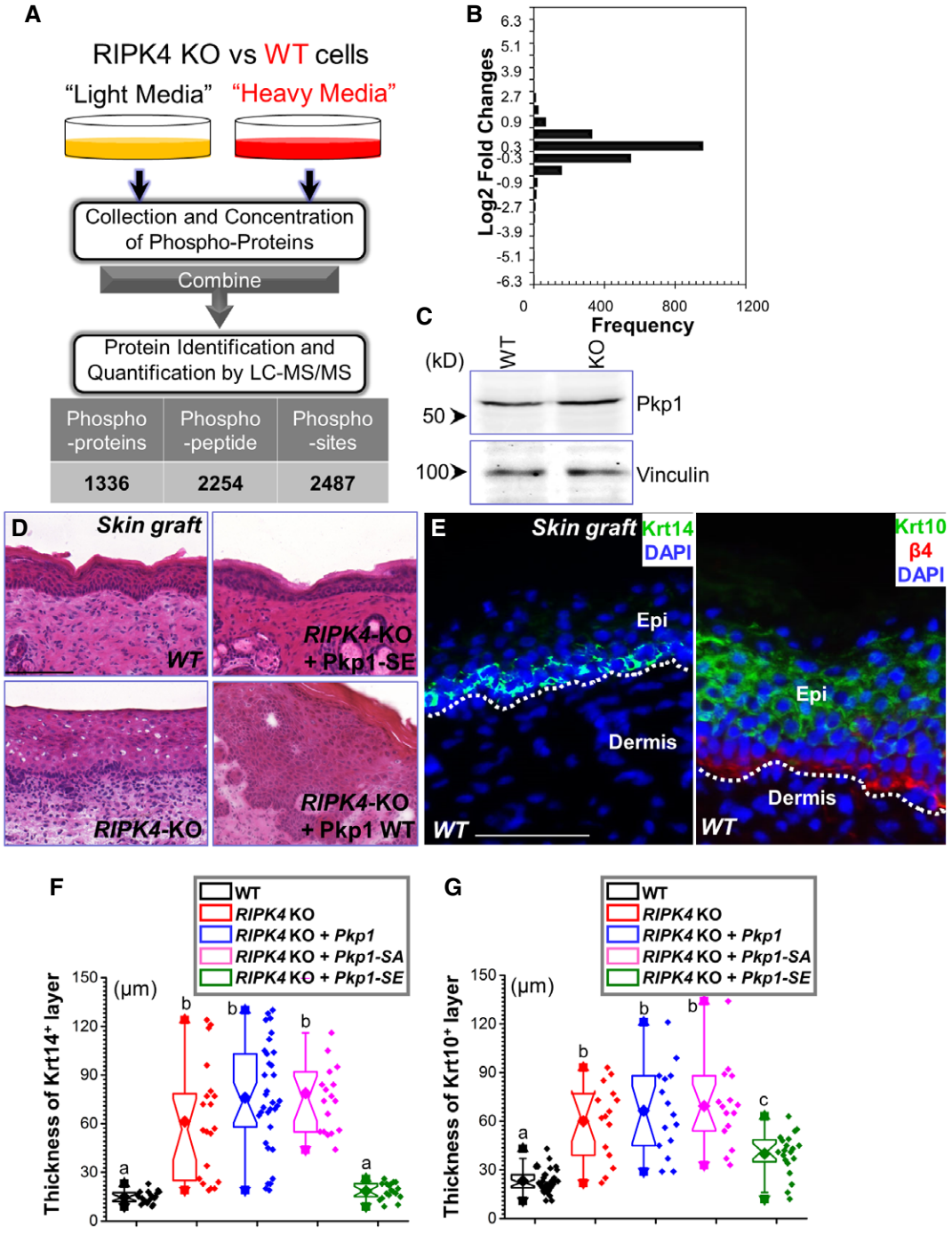

Figure EV6. Phosphoproteomic changes upon loss of *RIPK4* (related to Fig 6).

A Diagram of SILAC-MS workflow. Differentiated *RIPK4* KO cells (labeled with regular arginine and lysine) and differentiated WT cells (labeled with arginine and lysine containing heavy isotope) were mixed together and examined by LC-MS/MS. Number of identified phosphorylation (Pi) peptide, protein, and sites are listed in the table below.

B The distribution of normalized $\log_2$-transformed ratios of phosphopeptides enriched in *RIPK4* KO cells compared with WT epidermal stem cells.

C Cell lysates were prepared from WT or *RIPK4* KO keratinocytes and subjected to immunoblots with different antibodies as indicated.

D H/E staining of WT and *RIPK4* KO skin grafts, as well as skin grafts generated from rescued cells. Scale bar = 100 μm.

E Skin sections from WT skin grafts were immunostained with different antibodies as indicated. Scale bar = 50 μm.

F, G Thickness of Krt14 and Krt10 layers in different skin grafts were quantified and shown as box-and-whisker plots. The plots indicate the mean (solid diamond within the box), 25th percentile (bottom line of the box), median (middle line of the box), 75th percentile (top line of the box), 5th and 95th percentile (whiskers), 1st and 99th percentile (solid triangles) and minimum and maximum measurements (solid squares). Note significant rescue of *RIPK4* KO phenotype upon expression of *Pkp1* SE mutant transgene. One-way ANOVA test was used for statistical analysis (data with different superscript letters $P < 0.05$), $n > 15$.