Proteomic analysis of primary human airway epithelial cells exposed to the respiratory toxicant diacetyl

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Supplemental Excel File

Table S1. Run order and raw filename associations for proteome analysis; **Table S2.** Peptide expression table (proteome); **Table S3.** Proteins expression table (proteome); **Table S4.** Run order and raw filename associations for phosphoproteome analysis; **Table S5.** Phosphopeptide expression table (all quantified peptides) from TiO_2-enriched proteome; **Table S6.** Phosphopeptide expression table (quantified phosphopeptides only) from TiO_2-enriched; **Table S7.** Keratin isoform-specific peptide expression; **Table S8.** Recalculated keratin protein expression.

Supplemental Figures

Figure S1. Visualization of proteomic data by volcano plots. **Figure S2.** Extacted ion chromatograms the total and phosphorylated forms of a representative RSPH4A peptide. **Figure S3.** Western blotting of RSPH4A. **Figure S4**. Immunofluorescence staining of RSPH4A in donors 2-4. **Figure S5.** Immunofluorescence staining of TGM1 in donors 2 and 3. **Figure S6**. Immunofluorescence staining of repetin in donors 2-4. **Figure S7.** Summary of identified and quantified phosphorylation sites in K6 and K14. **Figure S8.** Overlay of quantified transitions for pSer31 and pSer35 peptides of K6.

Supplemental Electronic Data

The raw data for the label-free quantitative proteomic analyses was deposited at Chorus Project (www.chorusproject.org) under the project "EpiAir Diacetyl Proteomics" and experiment "EpiAir Diacetyl Proteome". Skyline files containing results of PRM analysis have been made public as part of the Panorama Targeted Proteomics data repository (panoramaweb.org).[65] Data can be viewed at https://goo.gl/e5gJRk and downloaded at https://goo.gl/3Z905f.

Figure S1

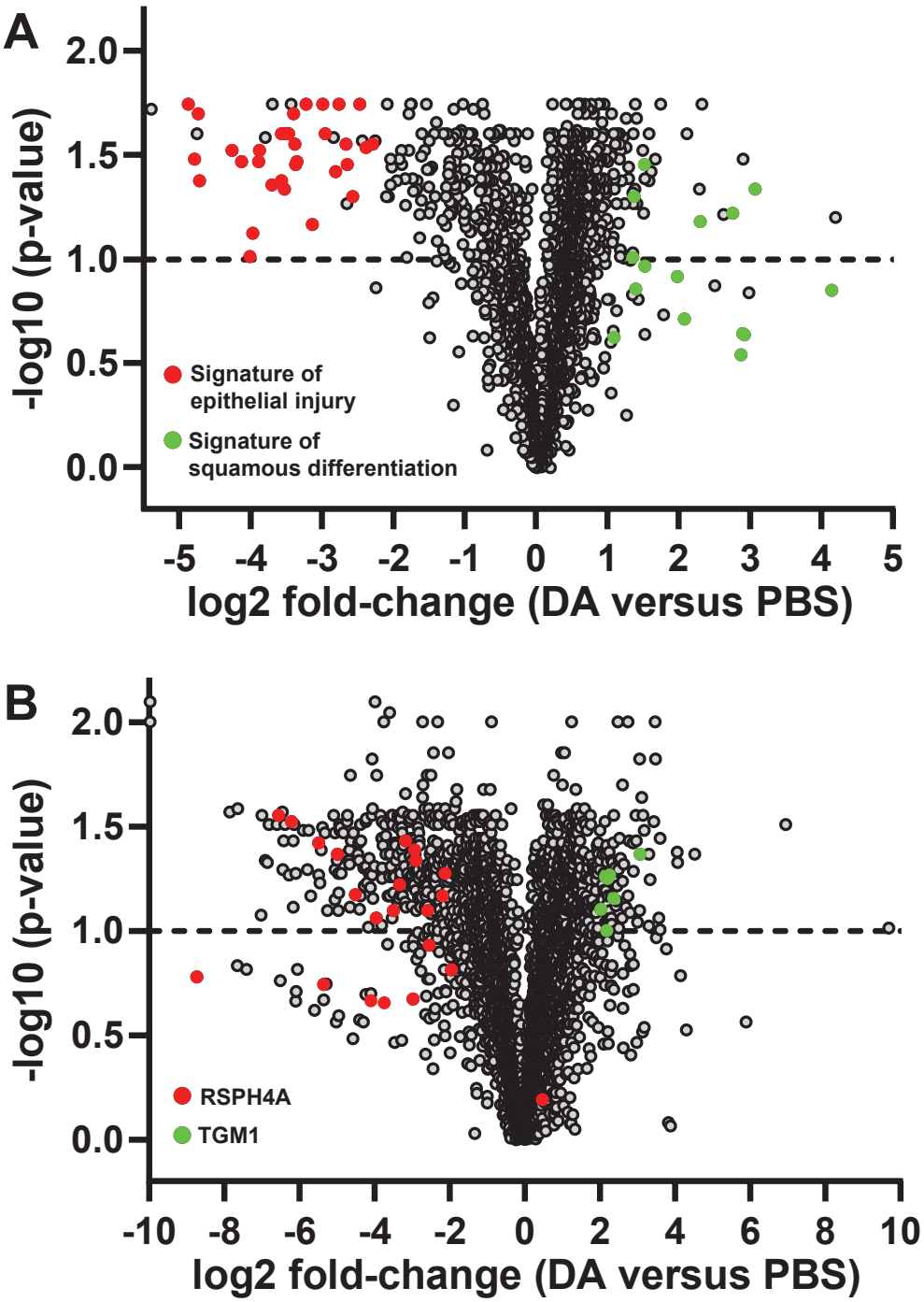

Figure S1. Visualization of proteomic data by volcano plots. A) Robustly quantified proteins (2 more more peptides and CV <30%) were visualized by a volcano plot: log2(mean fold change, DA versus PBS) versus -log(FDR-corrected p-value); each circle is individual protein; proteins above dashed line are p<0.1; proteins from Tables 1 and 3 are highlighted in red and green, respectively. **B)** Robustly quantified phosphopeptides were filtered to contain to contain peptides with a (CV <30%) and visualized by a volcano plot as in **(A)**. Phosphopeptides from RSPH4A and TGM1 are highlighted in red and green, respectively. Note that a maximum average fold change was set to ±1000 for purposes of visualization.

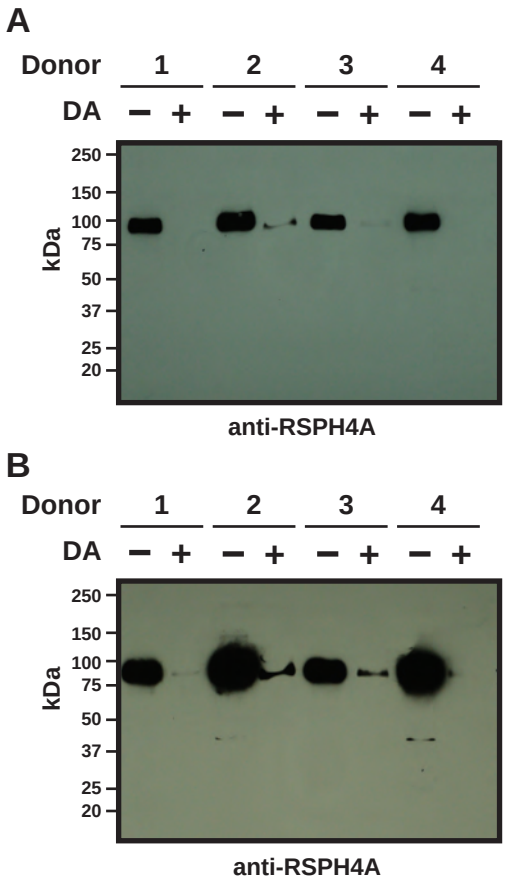

Figure S2. Western blotting of RSPH4A. An uncropped blot was used to perform western blotting of RSPH4A as in Fig. 3A and developed for 5 min using **(A)** standard ECL reagent or **(B)** Thermo SuperSignal West Femto Chemiluminescent Substrate ECL reagent.

Figure S3

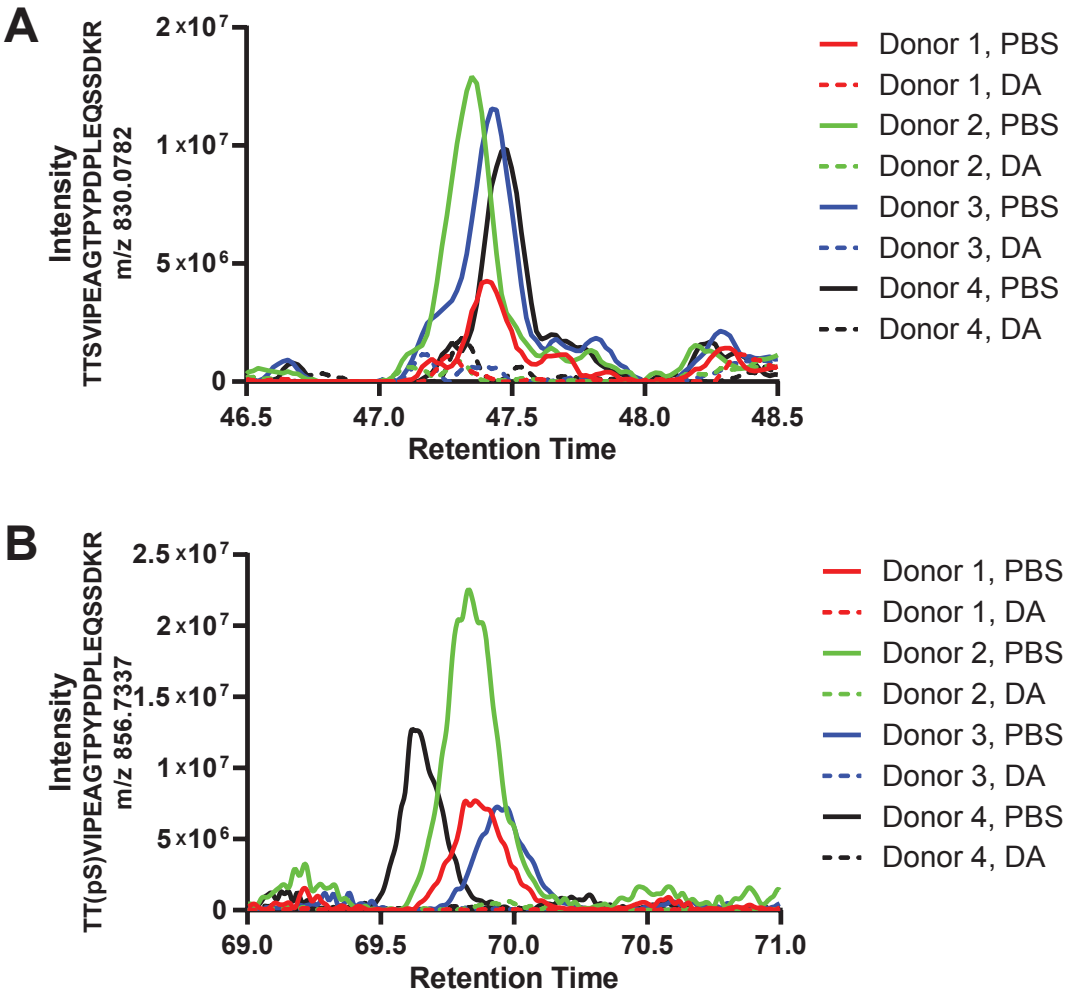

Figure S3. Extracted ion chromatograms (XICs) of a RSPH4A peptide and its corresponding phosphopeptide. XICs (sum of M, M+1, M+2 peaks) corresponding to the RSPH4A peptides **A**) TTSVIPEAGTPYPDPLEQSSDKR and **B**) TT(pS)VIPEAGTPYPDPLEQSSDKR were generated from unaligned, non-normalized raw data using Skyline.

Figure S4

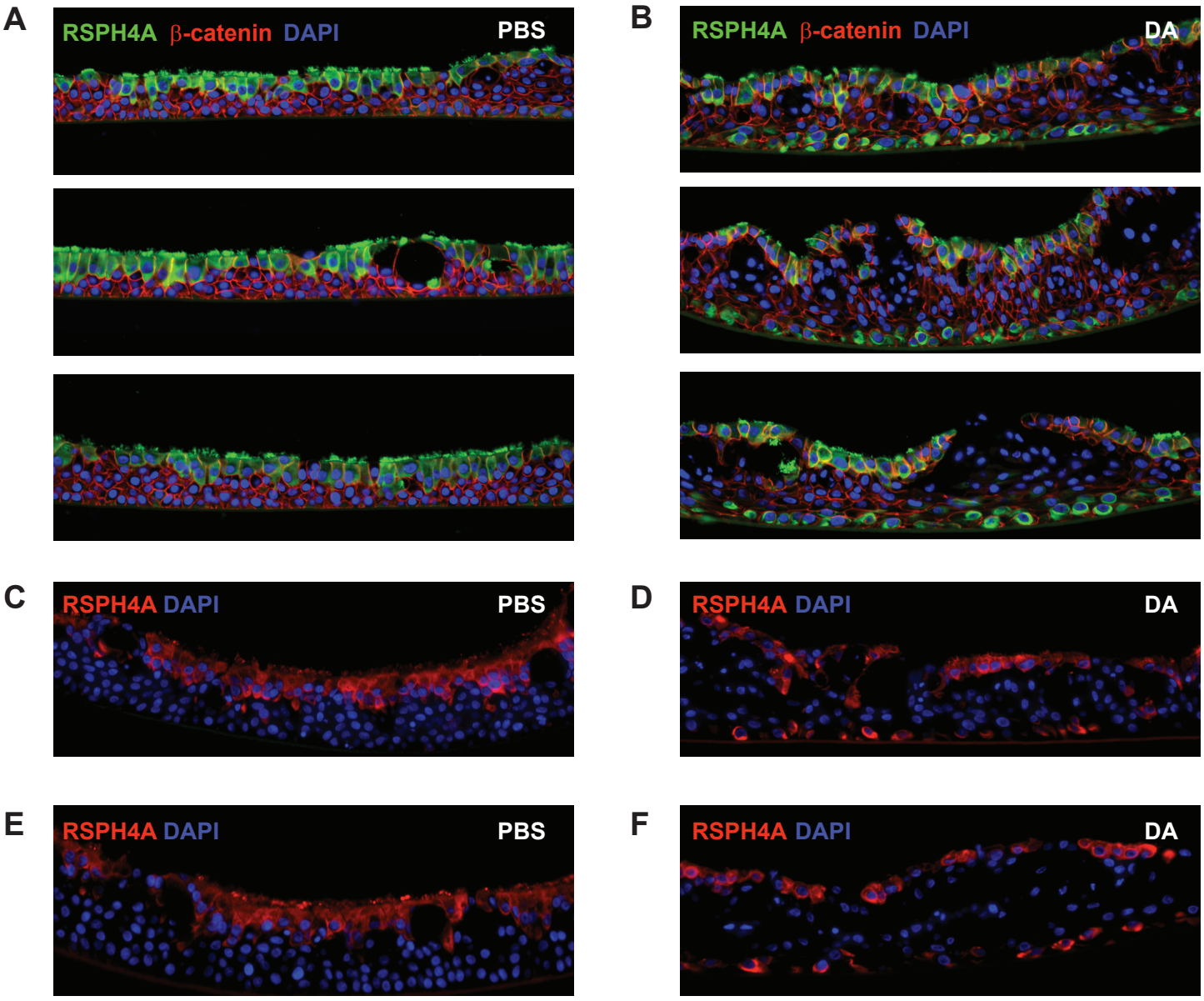

Figure S4. Immunofluorescence staining of RSPH4A in donors 2-4. (A-B) IF images are shown for n=3 replicate sections from cells from donor 3 (including an uncropped image from Fig. 3) exposed to PBS and DA, respectively. **(C-D)** IF images are shown for cells from donor 2 exposed to PBS and DA, respectively. **(E-F)** IF images are shown for cells from donors 4 exposed to PBS and DA, respectively.

Figure S5

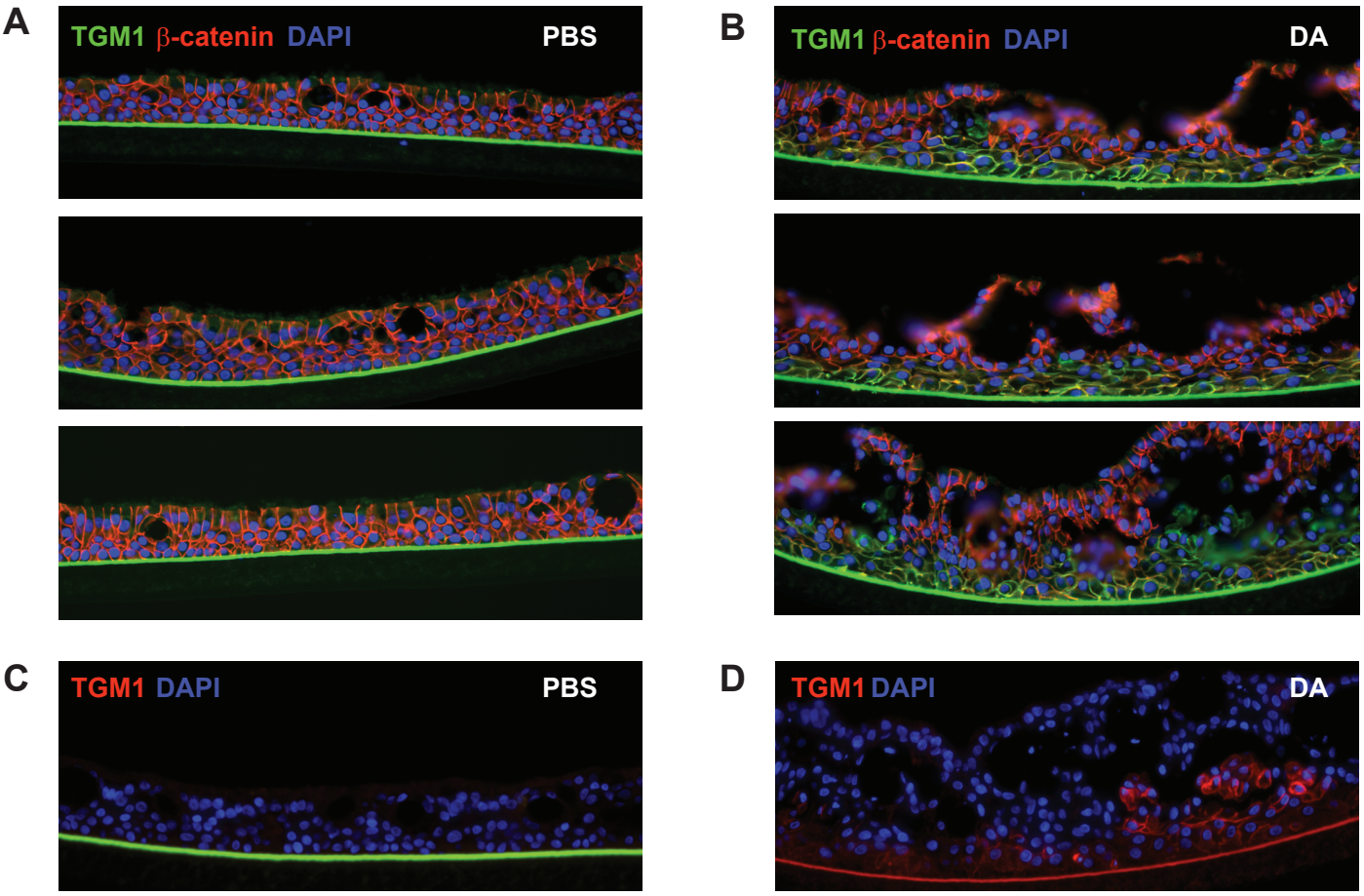

Figure S5. Immunofluorescence staining of TGM1 in donors 2 and 3. (A-B) IF images are shown for n=3 replicate sections from cells from donor 3 (including an uncropped image from Fig. 4) exposed to PBS and DA, respectively. **(C-D)** IF images are shown for cells from donor 2 exposed to PBS and DA, respectively. Note: there is non-specific staining of the transwell membrane.

Figure S6

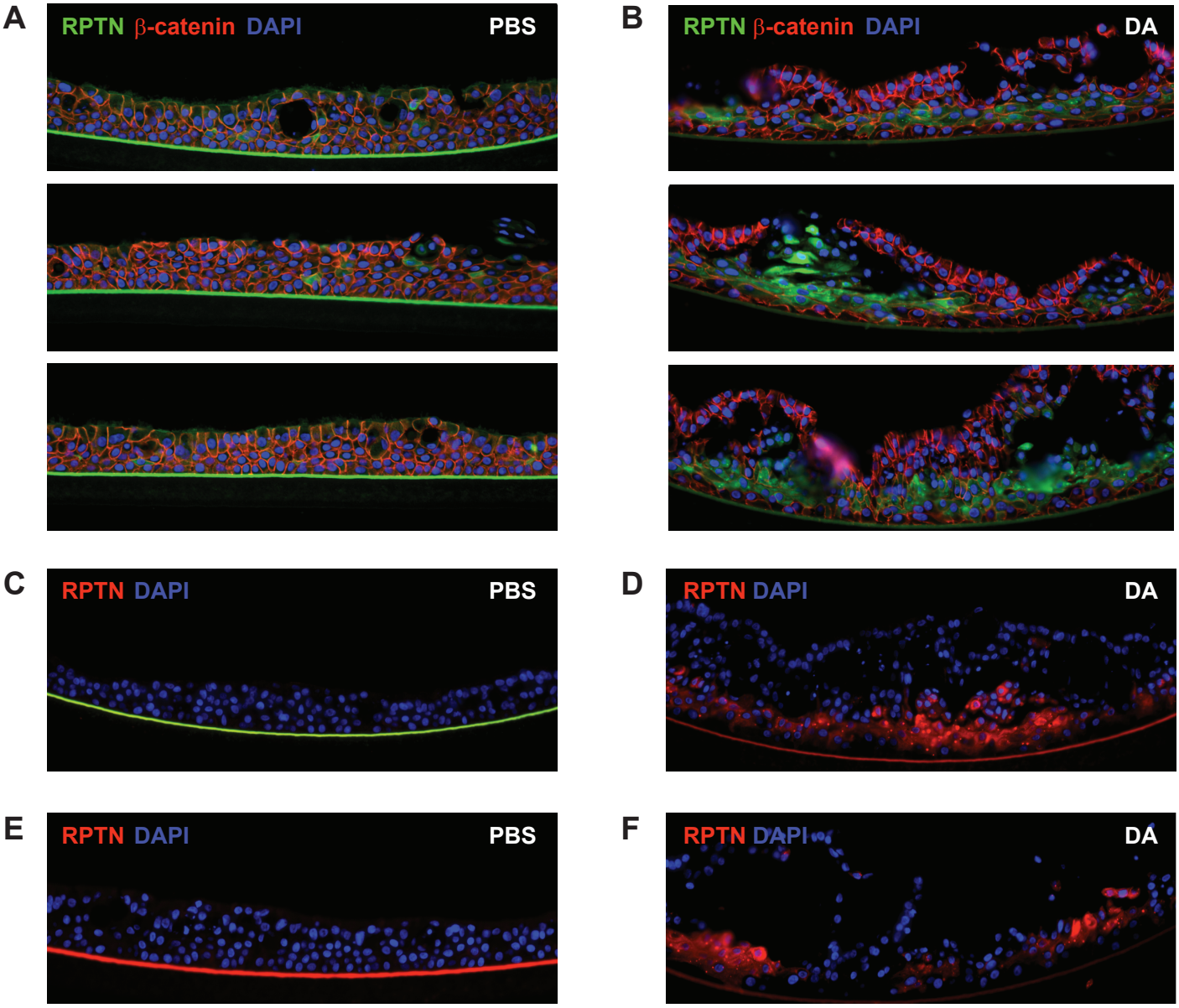

Figure S6. Immunofluorescence staining of repetin in donors 2-4. (A-B) IF images are shown for n=3 replicate sections from cells from donor 3 (including an uncropped image from Fig. 4) exposed to PBS and DA, respectively. **(C-D)** IF images are shown for cells from donor 2 exposed to PBS and DA, respectively. **(E-F)** IF images are shown for cells from donor 4 exposed to PBS and DA, respectively.

Figure S7

A

MASTSTTIRS HSSSRRGFSA NSARLPGVSR SGFSSVSVSR SRGSGGLGGA CGGAGFGSRS 60
LYGLGGSKRI SIGGGSCAIS GGYGSRAGGS YGFGGAGSGF GFGGGAGIGF GLGGGAGLAG 120
GFGGPGFPVC PPGGIQEVTV NQSLLTPLNL QIDPTIQRVR AEEREQIKTL NNKFASFIDK 180
VRFLEQQNKV LETKWTLLQE QGTKTVRQNL EPLFEQYINN LRRQLDSIVG ERGRLDSELR 240
GMQDLVEDFK NKYEDEINKR TAAENEFVTL KKDVDAAYMN KVELQAKADT LTDEINFLRA 300
LYDAELSQMQ THISDTSVVL SMDNNRNLDL DSIIAEVKAQ YEEIAQRSRA EAESWYQTKY 360
EELQVTAGRH GDDLRNTKQE IAEINRMIQR LRSEIDHVKK QCANLQAAIA DAEQRGEMAL 420
KDAKNKLEGL EDALQKAKQD LARLLKEYQE LMNVKLALDV EIATYRKLLE GEECRLNGEG 480
VGQVNISVVQ STVSSGYGGA SGVGSGLGLG GGSSYSYGSG LGVGGGFSSS SGRAIGGGLS 540
SVGGGSSTIK YTTTSSSSRK SYKH

B

MTTCSRQFTS SSSMKGSCGI GGGIGGGSSR ISSVLAGGSC RAPSTYGGGL SVSSSRFSSG 60
GACGLGGGYG GGFSSSSSSF GSGFGGGYGG GLGAGLGGGF GGGFAGGDGL LVGSEKVTMQ 120
NLNDRLASYL DKVRALEEAN ADLEVKIRDW YQRQRPAEIK DYSPYFKTIE DLRNKILTAT 180
VDNANVLLQI DNARLAADDF RTKYETELNL RMSVEADING LRRVLDELTL ARADLEMQIE 240
SLKEELAYLK KNHEEEMNAL RGQVGGDVNV EMDAAPGVDL SRILNEMRDQ YEKMAEKNRK 300
DAEEWFFTKT EELNREVATN SELVQSGKSE ISELRRTMQN LEIELQSQLS MKASLENSLE 360
ETKGRYCMQL AQIQEMIGSV EEQLAQLRCE MEQQNQEYKI LLDVKTRLEQ EIATYRRLLE 420
GEDAHLSSSQ FSSGSQSSRD VTSSSRQIRT KVMDVHDGKV VSTHEQVLRT KN

Figure S7. Summary of identified and quantified phosphorylation sites in **(A)** K6 and **(B)** K14. Sites of phosphorylation from Table S6 are highlighted. Peptides identified and quantified in the unenriched proteome (from Table S2) are underlined. The site of p38 MAPK phosphorylation in K6, which is unaccessible to trypsin, is highliighted in green.

Figure S8

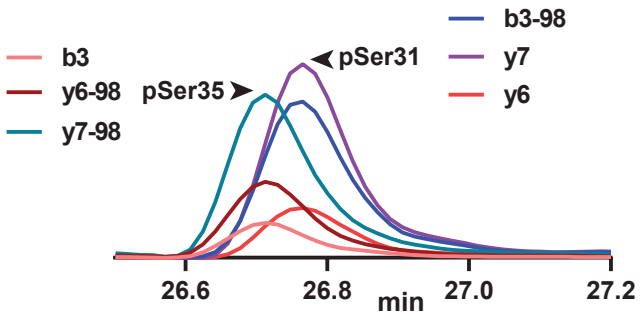

Figure S8. Overlapping chromtagrams of stable isotope-labeleld peptides used for site localization and quantitation of K6 phosphorylation. Parallel reaction monitoring was used to analyze a mixture of mono-phosphorylated, stable isotope-labeled peptide standards with sequence ^{31}SGFSSVSVSR^{40} (precursor m/z 551.7438). Overlay of quantified transitions for pSer31 and pSer35 peptides of K6, which are nearly overlapping but an be distinguished based on unique product ions and retention times. The position of the properly assigned peak is indicated by an arrow Data show SIL peptides only.