Supporting materials

Appendix S1

Immunohistochemistry (IHC)

Paraffin sections (5 μm) were deparafinized in xylene, rehydrated through graded alcohols, and placed in 0.1% hydrogen peroxide to quench any endogenous peroxidase activity. Sections were subjected to four rounds of an antigen retrieval technique (5 min in a 750 W microwave pretreatment in citrate buffer, pH 6.0), followed by treatment with 10% normal goat serum for 30 min. to block nonspecific antibody binding. The slides were then incubated with rat CTIP2 monoclonal antibody, 1/300 dilution (Abcam product number 18465; clone 25B6) in a humid chamber at 4°C overnight. Skin sections from CTIP2-null mice (since the CTIP2 antibody reacts with mouse CTIP2) (18), and sections incubated with IgG alone (isotype control) or without primary-antibody, were used as controls. The secondary staining was carried out using a biotin-labeled, rabbit anti-rat antibody at 1/500 dilution (Jackson Immuno Research Laboratories, Inc., catalogue number 112-065-143) for 2 hrs. at 37°C, followed by incubation with a streptavidin-conjugated horseradish peroxidase (Vector Laboratories, catalog numbe: SA-5704). We observed the reaction products by immersing the slides in freshly prepared diaminobenzidine solution for 10 min. and counterstaining with hematoxylin before dehydration and mounting. Images were captured at 40X magnification using Leica microscope and Hamamatsu C4742-95 digital camera, and processed using OpenLab software and Adobe Photoshop 7.0. We counted

the number of CTIP2-positive cells as a percentage of H&E positive cells (total cells) within a field of fixed size.

For immunofluorescence studies, paraffin sections were processed as described above. Slides were then incubated overnight in a humidified chamber with anti-CTIP2 and -Ki-67 (Abcam product number ab15580; 1:200 dilution) antibodies. Skin sections from CTIP2-null mice, and sections incubated without primary-antibody, or IgG alone (isotype cpntrols) were used as controls. The primary antibody incubation was followed by three washes with PBST and incubation with fluorescently-labeled (Cy2-donkey anti-rat [1:250] for CTIP2 and Cy3-goat anti-rabbit for Ki67 [1:500]; Jackson Immuno-Research] secondary antibodies for 2 h. Nuclei were counterstained with DAPI. Finally, sections were rinsed with PBST, dehydrated through sequential washes in 50%, 70%, 95%, and 100% ethanol and then cleared in xylene. Slides were mounted with DPX mounting media and allowed to dry overnight. Images were captured at 40X magnification using Leica DMRA fluorescent microscope and Hamamatsu C4742-95 digital camera and processed as above. $CTIP2^{+}Ki67^{+}$ double positive cells within a field of fixed size were quantified and expressed as a percentage of DAPI+ cells (total cells) in that same field. The expression analyses and cell counts were independently performed by two investigators.

Statistical analysis

Cell counts were performed on ten different fields at a fixed magnification for each sample (5 normal, 12 AD, and 4 ACD samples). Data are presented as

mean ± SEM. Data were analyzed by unpaired, 2-tailed *t* test and p values of < 0.5 were considered significant.