SUPPLEMENTARY MATERIALS AND METHODS

Reagents

The selective PI3Kδ inhibitor, seletalisib (Allen et al., 2017) was synthesised at UCB Pharma. All titrations of seletalisib contained DMSO (Sigma) to a final concentration of 0.1%.

Human Subjects

15 subjects (13 men, 2 women) with psoriasis were recruited for this study (mean age 49.5 ±16.6 years), with a mean psoriasis area severity score (PASI) of 9.26 ±7.0 and a mean affected body surface area (BSA) of 12.7 ±15.5%. Individuals were excluded if they had used topical therapy within the previous 2 weeks, used systemic therapy or phototherapy within the previous 4 weeks, or had used biological therapies within the preceding 12 weeks. This study was approved by the local research ethics committee (REC reference 05/Q1701/117), and written informed consent was obtained from all participants.

T cell isolation and culture

Human PBMCs were isolated from venous blood by centrifugation on a Lymphoprep (Axis-Shield) gradient. From the lesional skin of psoriatic subjects, 6-mm punch biopsies were taken, the subcutaneous fat removed, and epidermis separated from dermis using 2 U/ml dispase (Gibco). The dermis was cultured in a 6-well plate at 37ºC, 5% CO_2, and migratory T cells collected after 72 h. All human T cells were maintained in RPMI 1640 enriched with L-glutamine (Gibco) supplemented with 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin.

Western blotting

$CD4^+$ T cells from human PBMCs were isolated by negative selection using MACs kits ($CD4^+$ T-cell isolation kit–human; Miltenyi Biotec). Cells were incubated alone or with 10 μg/ml anti-CD3 (UCHT1), with and without 10 μg/ml anti-CD28 (CD28.2; e-Bioscience), at 37ºC. After 2 min these antibodies were cross-linked for 5 min with 10 μg/mL goat anti–mouse IgG, F(ab')2 (Jackson

ImmunoResearch). Cells were pelleted by centrifugation, and lysed by incubation on ice in 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 1.0% NP-40, containing Phosphatase Inhibitor Cocktail I and II (Sigma) and Complete Protease Inhibitor Cocktail (Roche) for 30 min. Following centrifugation (13000 rpm, 10 min, 4^{o}C), lysates were mixed with sample loading buffer, run on a gel, blotted and probed with rabbit monoclonal antibodies against pS6$^{Ser235/236}$ (D57.2.2E) and total ribosomal protein S6 (5G10; both Cell Signaling Technology).

Human T cell activation and phospho-specific flow cytometry

Fresh T cells were incubated with seletalisib for 45 min, then stimulated with 1 μg/ml biotinylated anti-CD3 (OKT3) and 5 μg/ml biotinylated anti-CD28 (CD28.2; both eBioscience). After 2 min, cells were cross-linked with 50 μg/ml avidin (Invitrogen) and incubated for a further 10 min at 37^{o}C. Cells were fixed with Cytofix (BD Biosciences) for 10 min at 37^{o}C, washed, then permeabilised in Perm Buffer III (BD Biosciences) at -20^{o}C for 10 min. Prior to staining, cells were incubated with human Fc receptor binding inhibitor (eBioscience). Staining for CD markers was performed with the following directly conjugated antibodies: CD3 PerCP-Cy5.5 (UCHT1), CD8 Brilliant Violet (RPA-T8), CD4 PE-Cy7 (SK3) (all BioLegend) or CD4 FITC (SK3; eBioscience), and CD45RO PE (UCHL1; BD Biosciences); phosphorylation of ribosomal protein S6 was detected with the Alexa Fluor 647 rabbit monoclonal antibody against pS6$^{Ser235/236}$ (D57.2.2E, Cell Signaling Technology) and phosphorylation of Akt with the Alexa Flour 488 rabbit monoclonal antibody against pAktSer473 (D9E; Cell Signaling Technology). Isotype controls including the rabbit mAb IgG XP Alexa Fluor 647 (DA1E) were used to enable gating. Flow cytometry was performed on a FACSAria (BD Biosciences), and data were analysed using Cytobank (17), with median fluorescence intensity (MFI) results shown as the arcsinh ratio of medians normalised to unstimulated cells.

Immunofluorescence staining

Skin biopsies, from subjects in this study (and, for supplementary figure S2, taken at day 0 from subjects reported in Helmer et al. (2017)), were snap-frozen in OCT embedding medium in an

isopentane bath. Frozen skin sections were cut to a section thickness of 5 μm using a cryostat. Sections were fixed in 4% formaldehyde for 10 min at room temperature, then permeabilised with 100% methanol for 10 min at -20°C. A goat blocking buffer (0.3% Triton X-100 / 5% goat serum (Sigma) / 1% BSA) was applied for 1 h at room temperature, and then the sections were incubated with the primary antibodies, anti-CD3 (1:300; Dako) and $pS6^{Ser235/236}$ (1:100; Cell Signaling Technology), overnight at 4°C. The secondary antibodies, goat anti-rabbit IgG Alexa Fluor 488 (1:200; Invitrogen) and goat anti-mouse IgG1 Alexa Fluor 555 (1:200; Invitrogen), were then added for 1 h at room temperature. Slides were counterstained with DAPI and mounted using Mowiol. Images were captured on a confocal microscope (Leica SP5) or on a Hamamatsu NanoZoomer XR slide scanner and analysed with Definiens image analysis software.

Cell proliferation

Cryopreserved PBMCs were thawed and rested for 2 h at 37°C, 5% CO_2. The cells were stained in 5 μM CFSE (BioLegend) and added to a 96-well plate containing plate-bound 5 μg/ml anti-CD3 (OKT3; eBioscience) and titrated seletalisib. Cells were incubated for 72 h, and then stained with a LIVE/DEAD Fixable Aqua Dead cell stain (Invitrogen), CD3 PerCP-Cy5.5, CD4 PE-Cy7 and CD8 Brilliant Violet 421. Flow cytometry was performed on a FACSAria (BD Biosciences), and data were analysed with FlowJo.

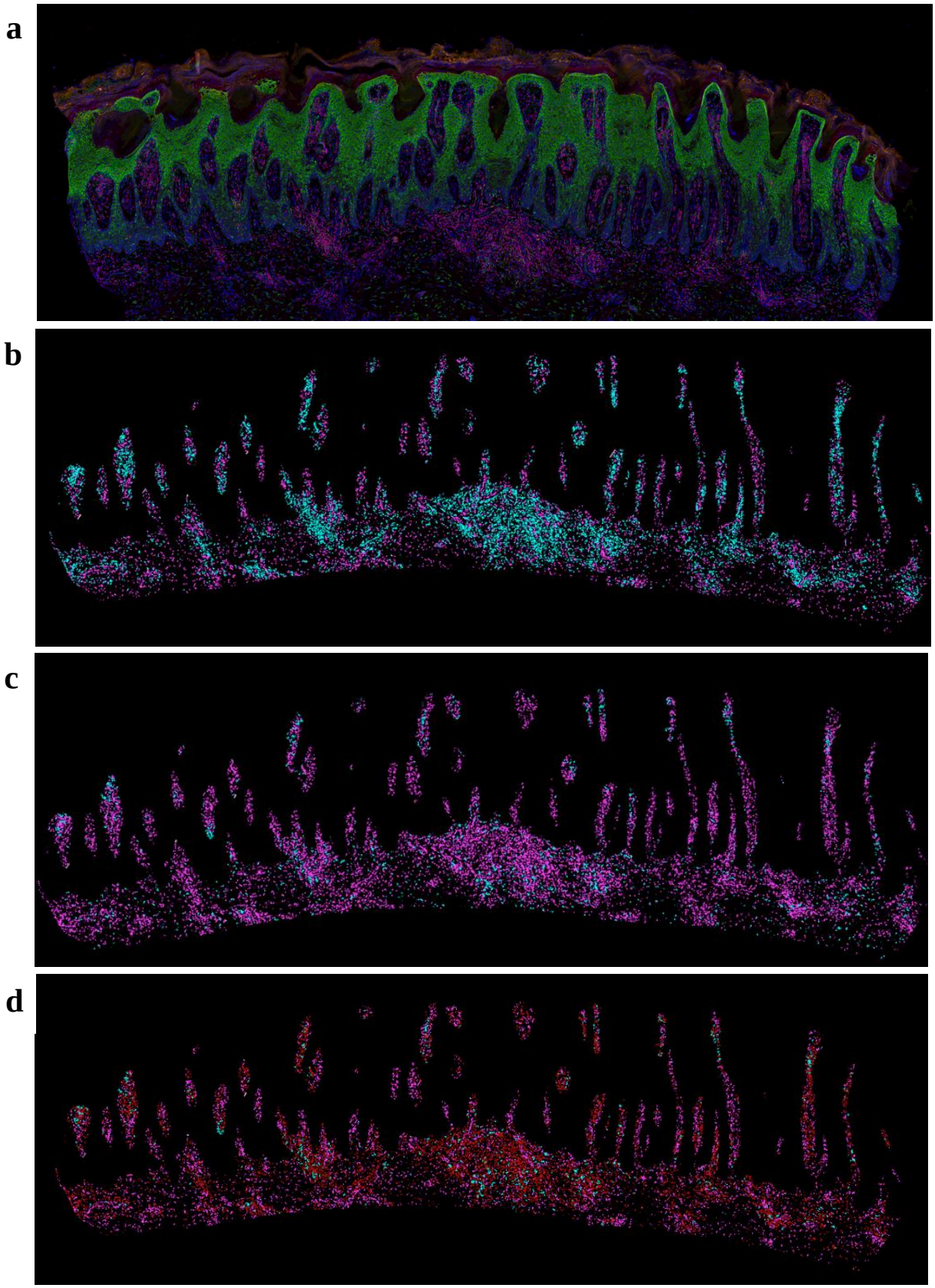
Cytokine detection

Cryopreserved PBMCs were thawed and rested for 2 h at 37°C, 5% CO_2. The cells were added to a 96-well plate containing plate-bound 5 μg/ml anti-CD3 and titrated seletalisib. Cells were incubated for 48 h, centrifuged and supernatant used for cytokine analysis, using human ELISA assays from R&D Systems.

Statistical analysis

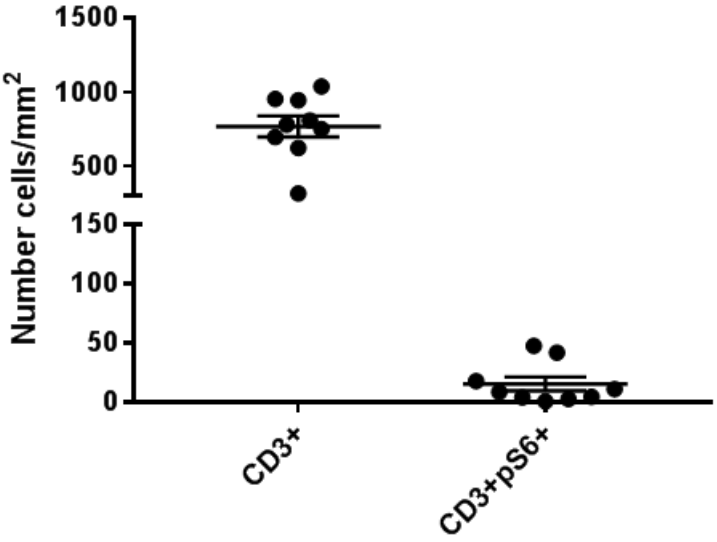
Data were analysed using GraphPad Prism software. One-way ANOVA or the non-parametric Kruskal-Wallis tests were used. A value of $p<0.05$ was considered statistically significant.

Supplementary Figure S1



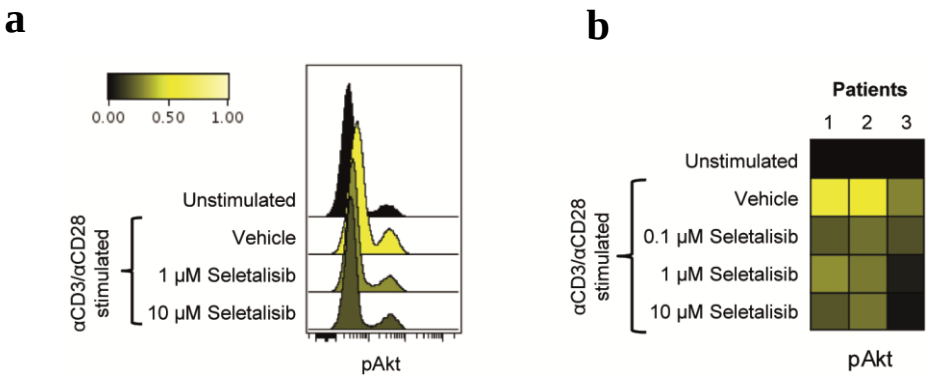
Supplementary Figure S1: Immunofluorescence microscopy showing $pS6^+$ T cells in psoriatic lesional skin. (a) Original Hamamatsu Nanozoomer slide scanner image; blue = DAPI, green = pS6, red = CD3. (b-d) Definiens analysed images. (b) cyan = $CD3^+$, pink = $CD3^-$. (c) cyan = $pS6^+$, pink = $pS6^-$. (d) cyan = $CD3^+pS6^+$, pink = $CD3^-pS6^-$, red = all other $CD3^+$cells. Note that as PI3Kδ is restricted to immune cells, it is unlikely that the expression of pS6 in the epidermis represents pI3Kδ activity.

Supplementary Figure S2



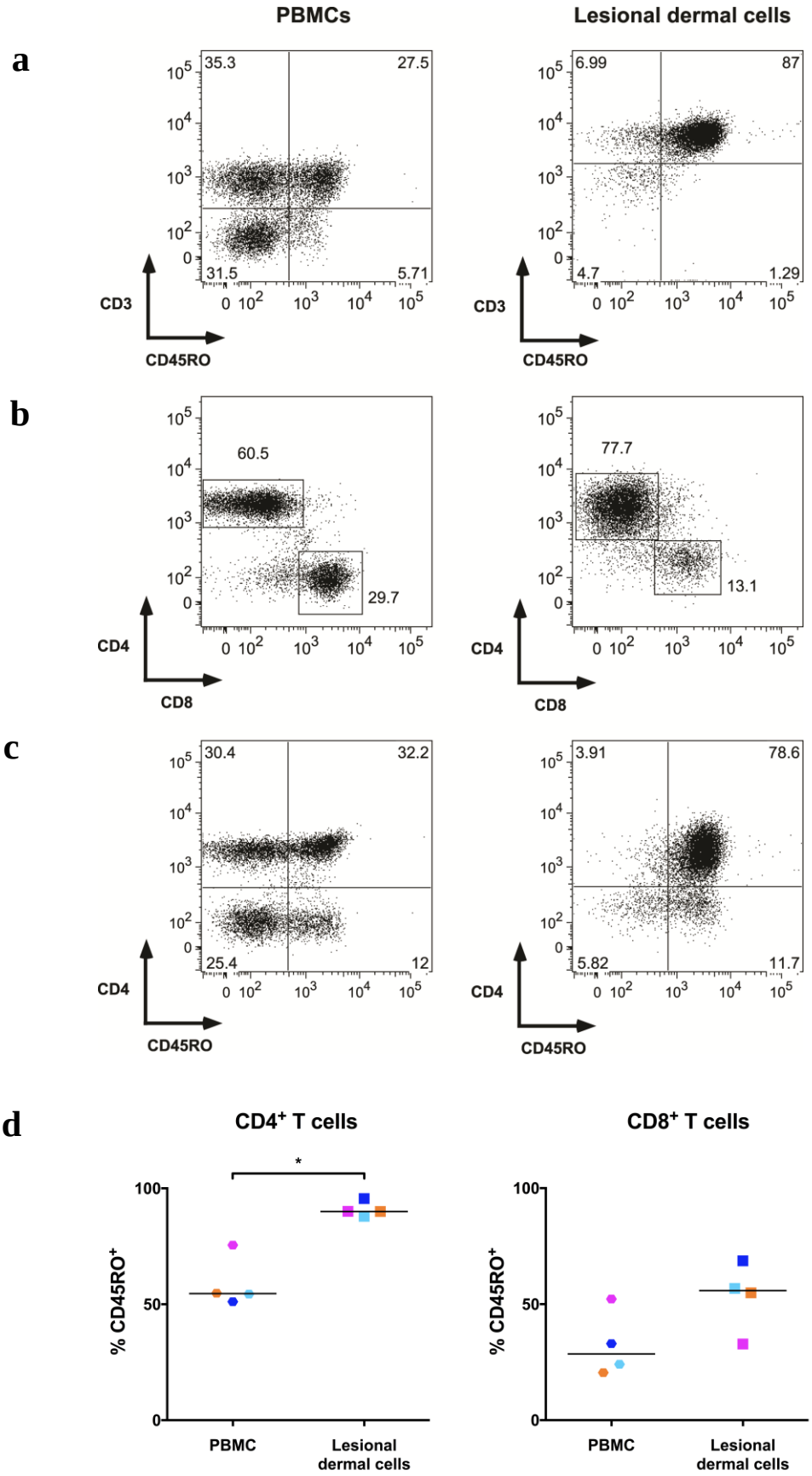
Supplementary Figure S2: Number of $CD3^+$ and $CD3^+pS6^+$ cells in lesional psoriatic skin (n=9 subjects). Lesional skin biopsies were taken from subjects with psoriasis and fixed, sectioned and stained for CD3 and pS6 using immunofluorescence, then stained samples were scanned and analysed using a Hamamatsu NanoZoomer XR and Definiens image analysis software respectively.

Supplementary Figure S3



Supplementary Figure S3: (a) Representative histograms of Akt phosphorylation (pAktSer473), which is further upstream in the PI3K pathway than pS6, during a phosflow assay, gated on $CD3^+$ cells in a lymphocyte population determined by FSC and SSC. (b) pAkt data from three psoriatic subject as shown as a heat map based on MFI of $CD3^+$ cells. The colour (based on the scale bars in (b) indicates the relative change in phospho-signal compared to unstimulated cells (black, 0.00).

Supplementary Figure S4



Supplementary Figure S4: Representative plots of naïve ($CD45RO^-$) and effector/memory ($CD45RO^+$) T cells in psoriatic PBMCs and lesional dermis. (a – c) Flow cytometry plots for (a) CD3 and CD45RO, (b) CD4 and CD8, (c) CD4 and CD45RO in psoriatic PBMCs (on left) and lesional dermis (on right). (d) Percentage of memory $CD4^+$ cells (on left) and $CD8^+$ cells (on right).

In d, each colour represents a single psoriatic subject, with the colours indicating the same 4 subjects which are depicted in Figure 1f and g.