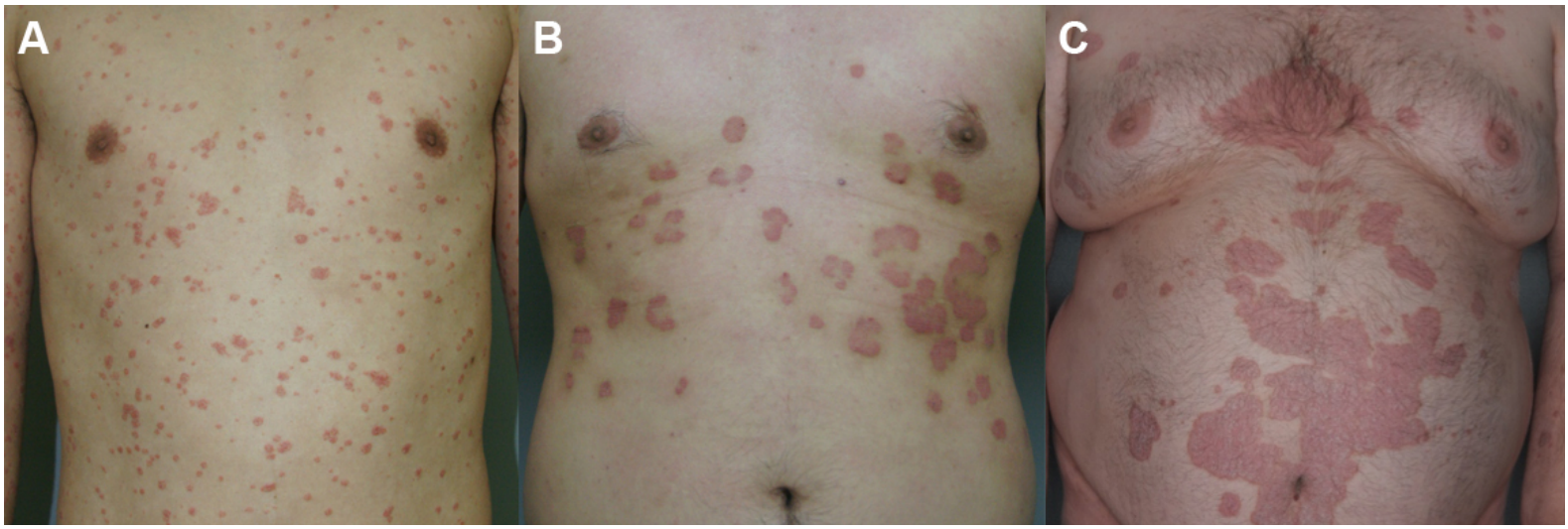
Molecular Phenotyping Small (Asian) versus Large (Western) Plaque Psoriasis Shows Common Activation of IL-17 Pathway Genes, but Different Regulatory Gene Sets

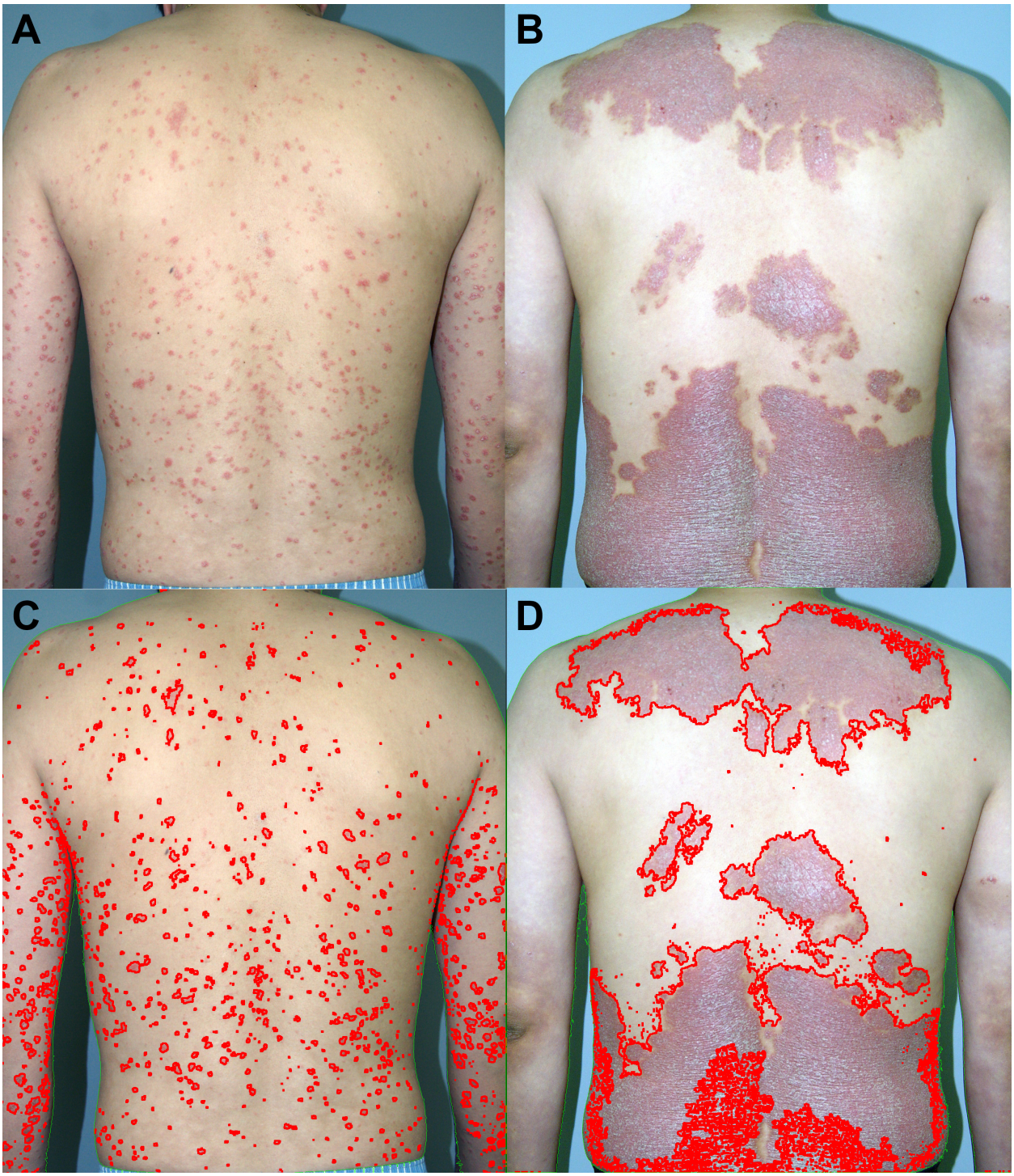
Jaehwan Kim, MD, PhD[1], **Chil-Hwan Oh**, MD, PhD[2], **Jiehyun Jeon**, MD, PhD[2], **Yoosang Baek**, MD[2], **Jaewoo Ahn**, MD[2], **Dong Joo Kim**, BS[1,3], **Hyun-Soo Lee**, MD[1], **Joel Correa da Rosa**, PhD[4], **Mayte Suárez-Fariñas**, PhD[4,5], **Michelle A. Lowes**, MD, PhD[1,6], **James G. Krueger**, MD, PhD[1*]

Supplementary Materials

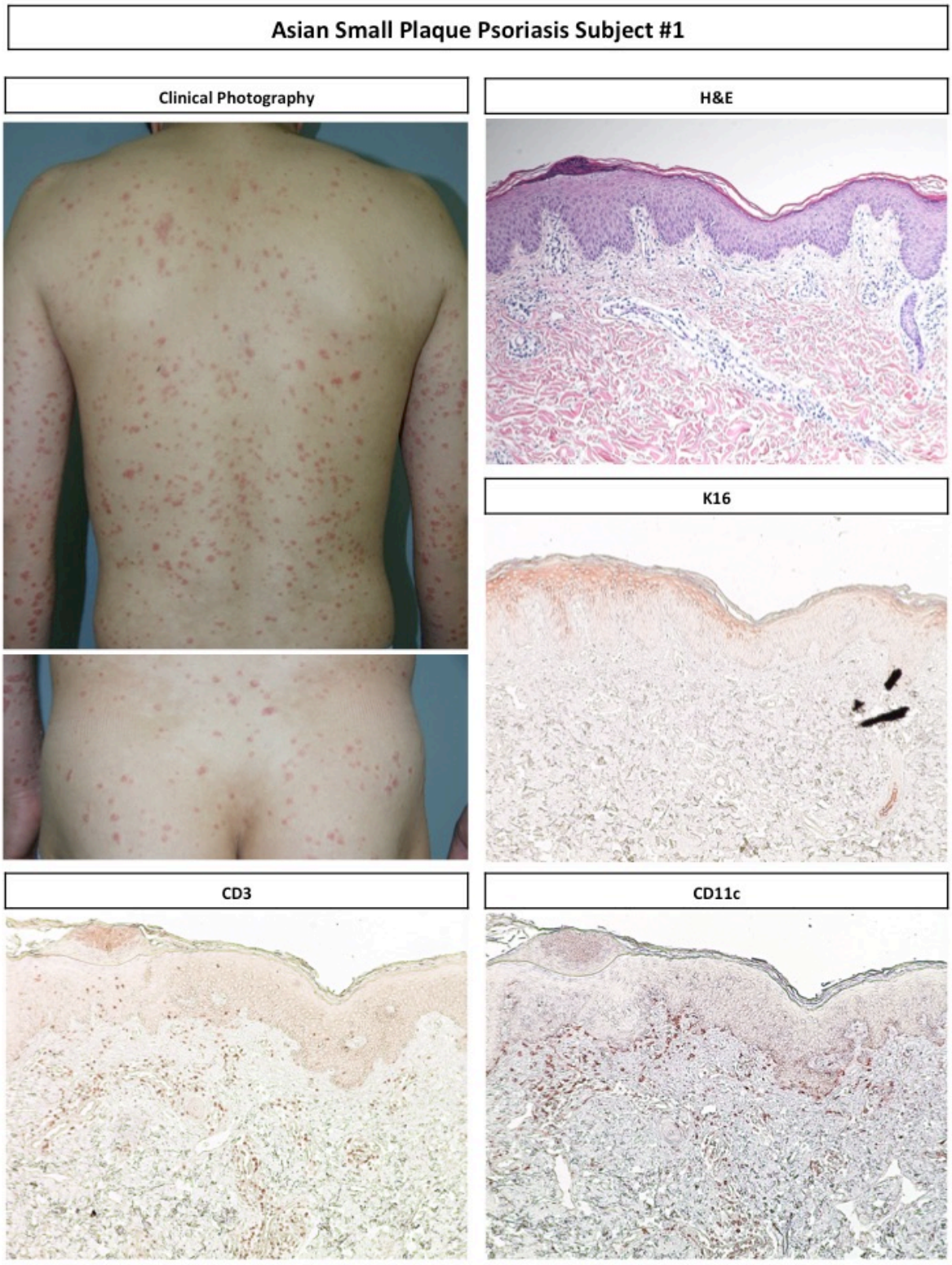
SUPPLEMENTARY FIGURES



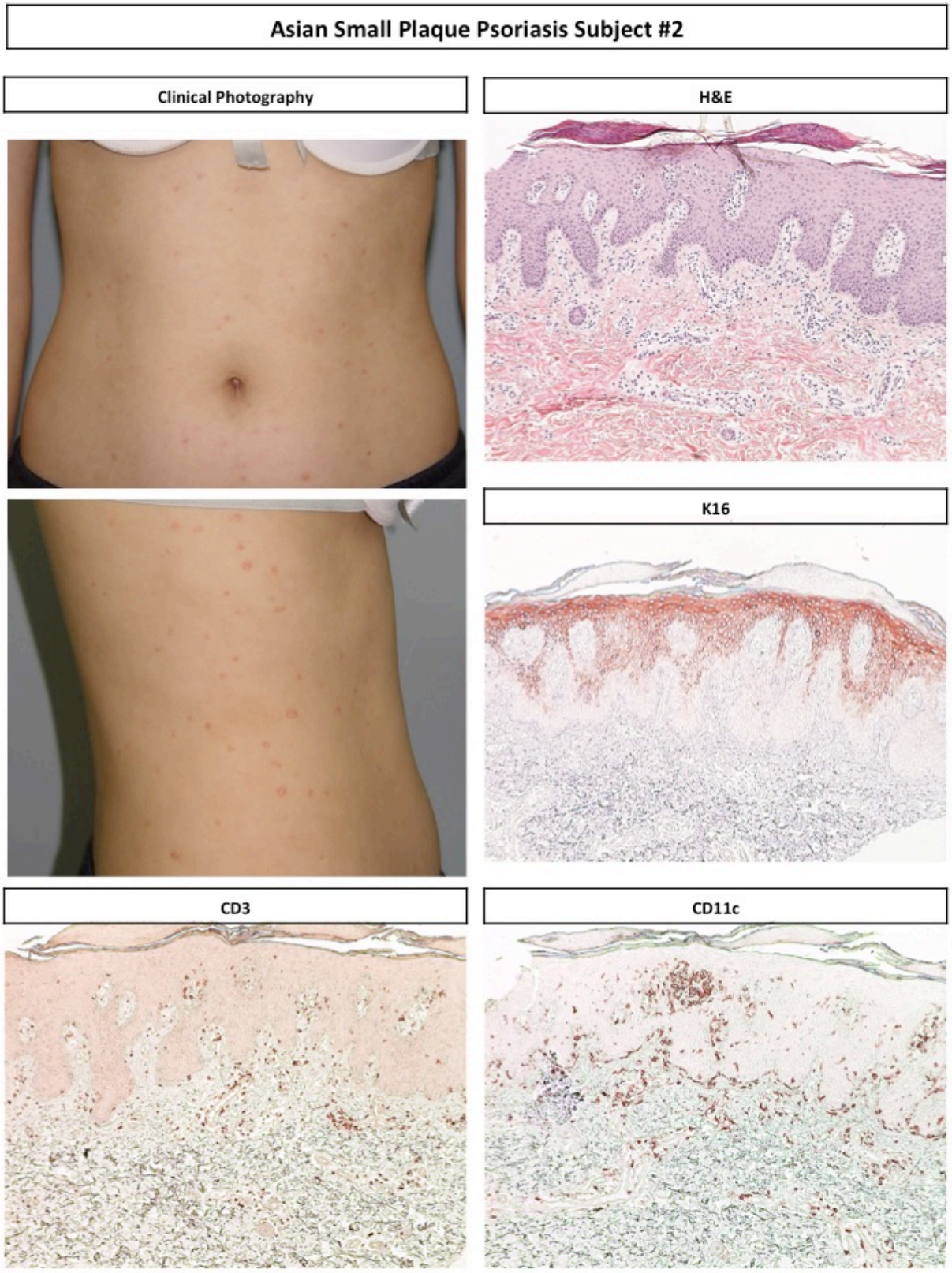
Supplementary Figure S1. Clinical stratification of Asian small and intermediate psoriasis in comparison to Western large psoriasis. (**A**) Asian small psoriasis (**B**) Asian intermediate psoriasis (**C**) Western large psoriasis



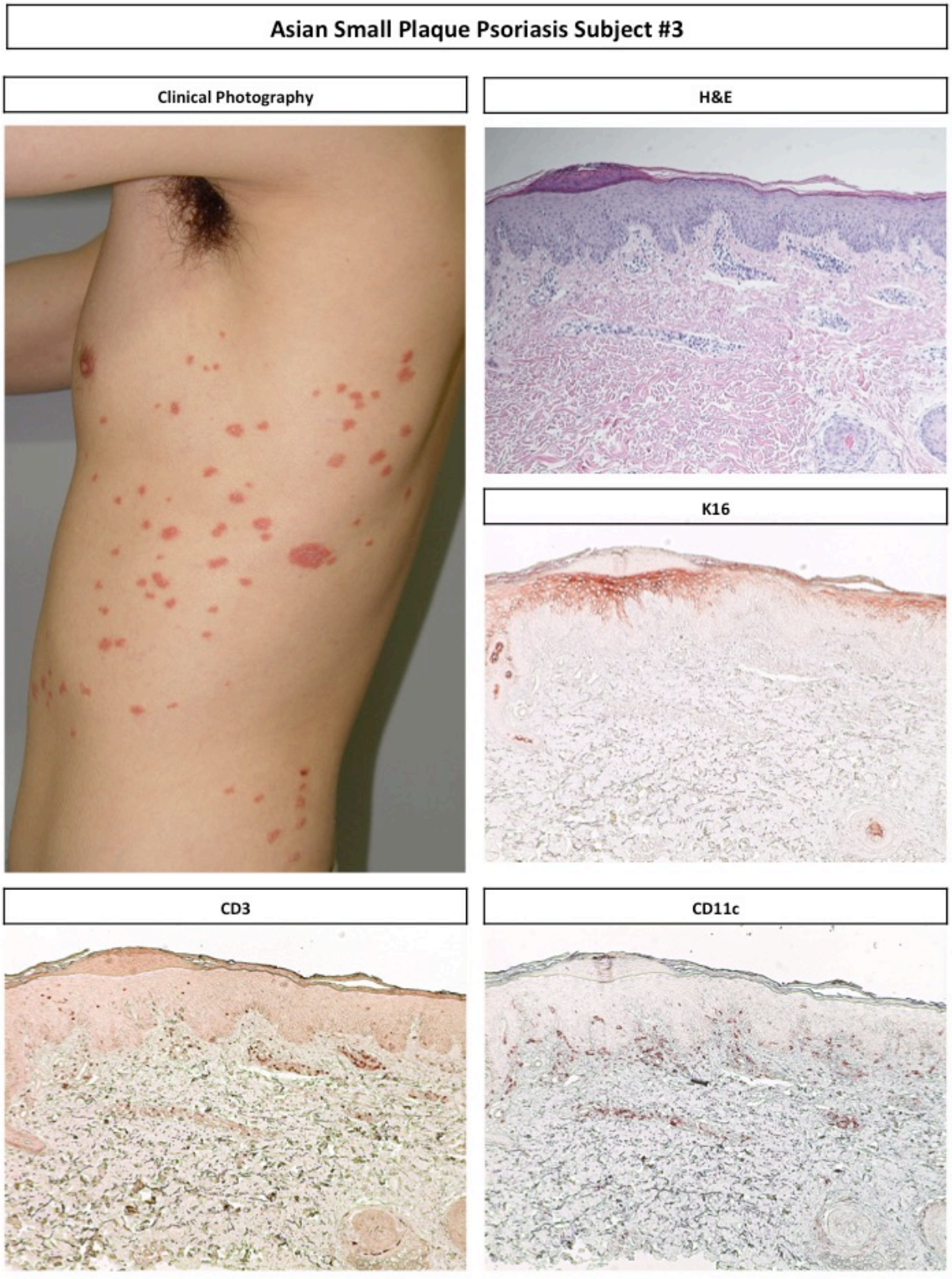
Supplementary Figure S2. Comparison of proportional area of involvement between small and large psoriasis. (**A, C**) Small psoriasis, (**B, D**) Large psoriasis. A computer vision algorithm was created to objectively measure the proportional area of involvement of psoriatic lesions (red outline). Both small and large psoriasis were widely dispersed on the back and arms, but small psoriasis involved only 5% of the identified skin, while large psoriasis occupied 36%.



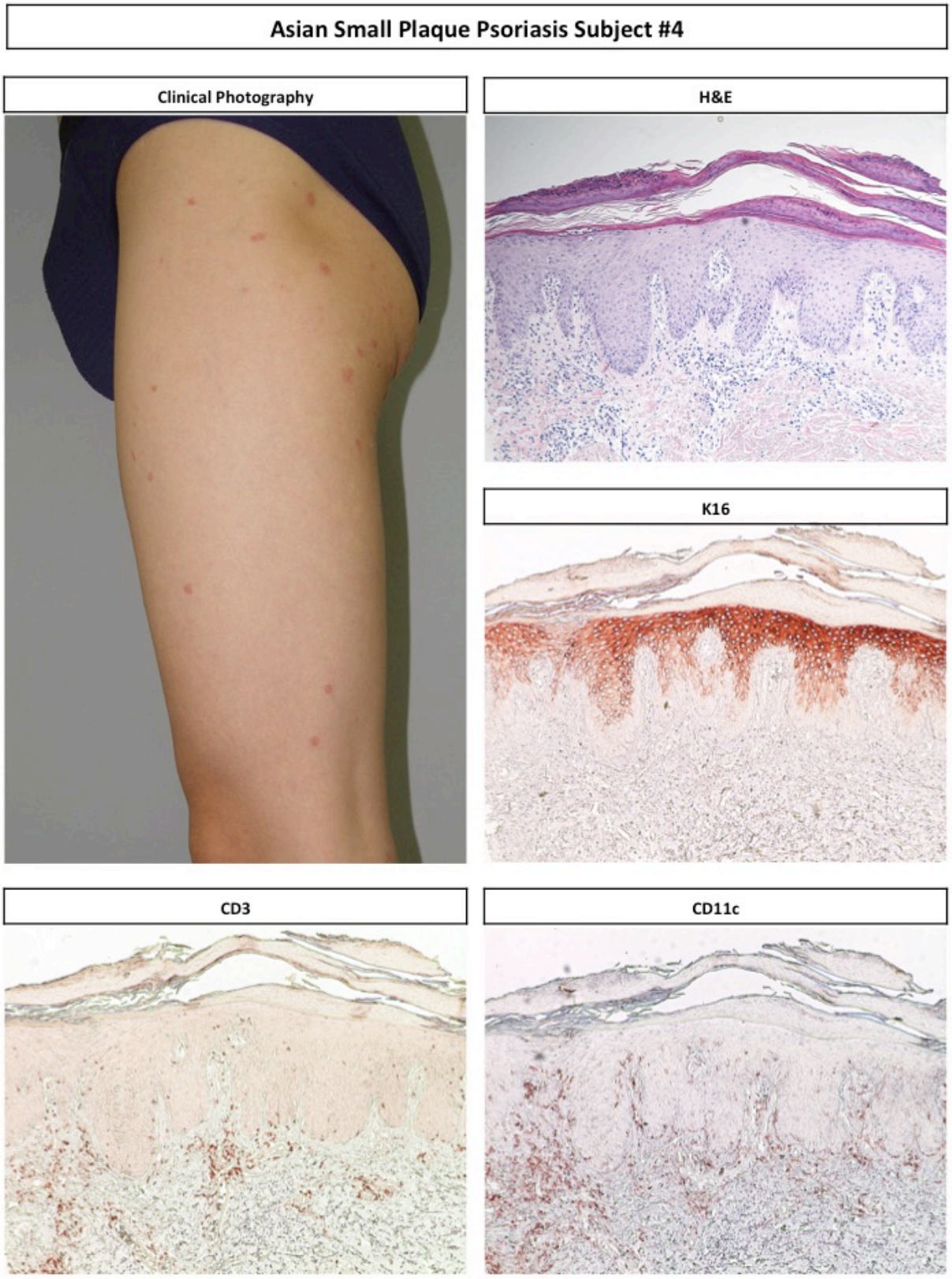
Supplementary Figure S3. Clinical photos and immunohistochemical images of 10 representative Asian psoriasis patients (5 small and 5 intermediate psoriasis patients, K16 for Keratin16, CD3 for T cells, CD11c for dendritic cells, original magnification ×10).



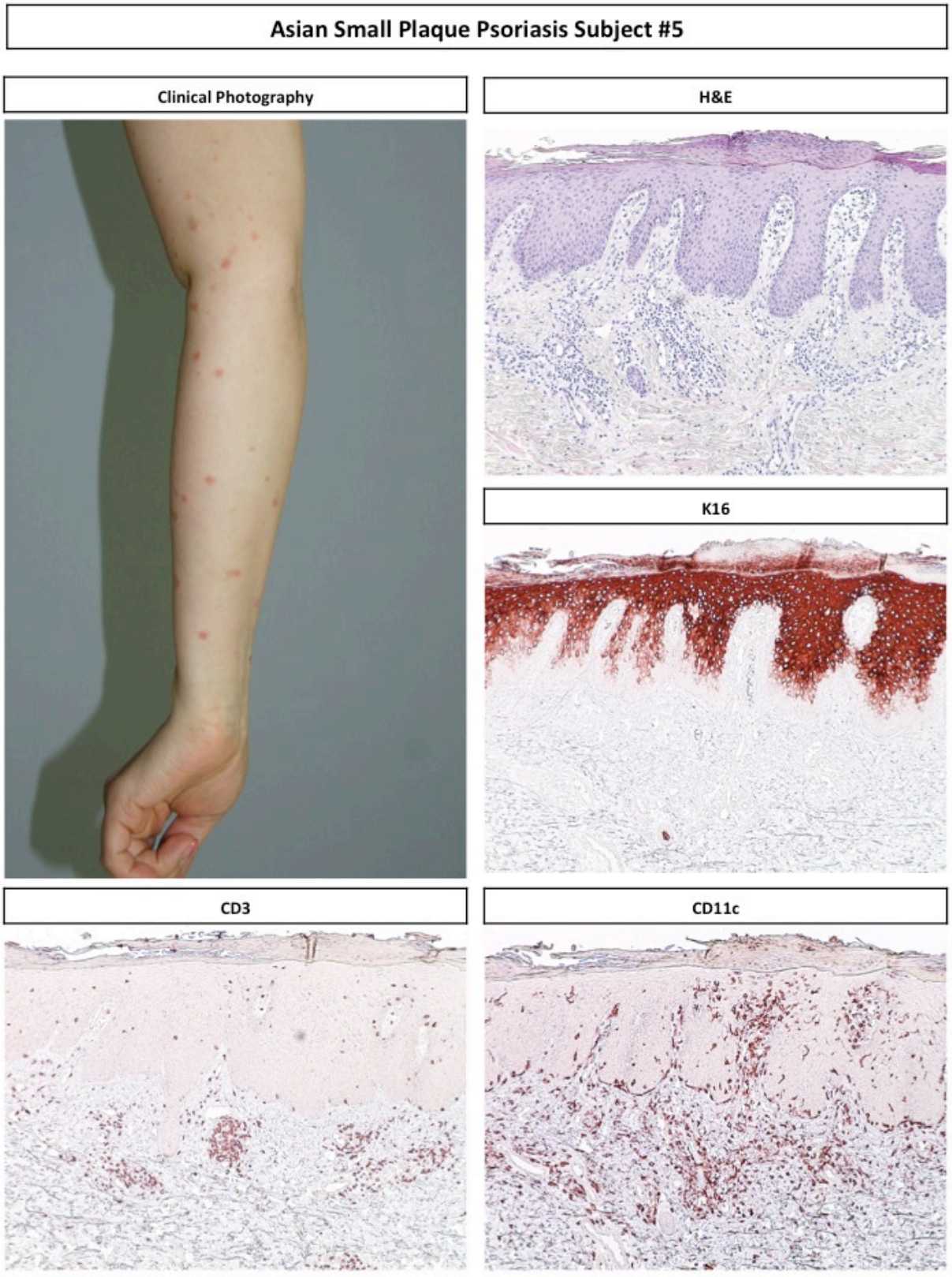
Supplementary Figure S3. Continued.



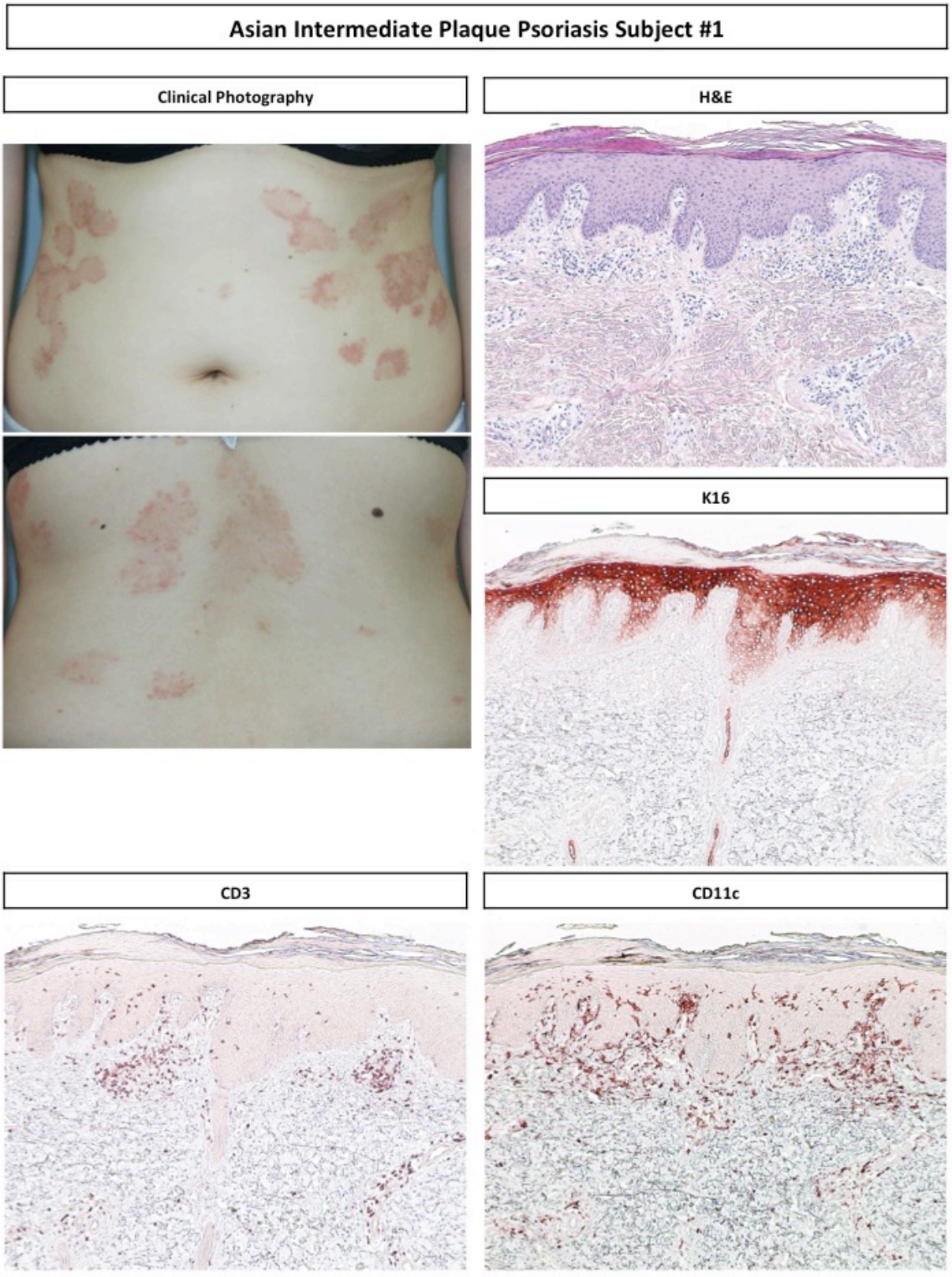
Supplementary Figure S3. Continued.



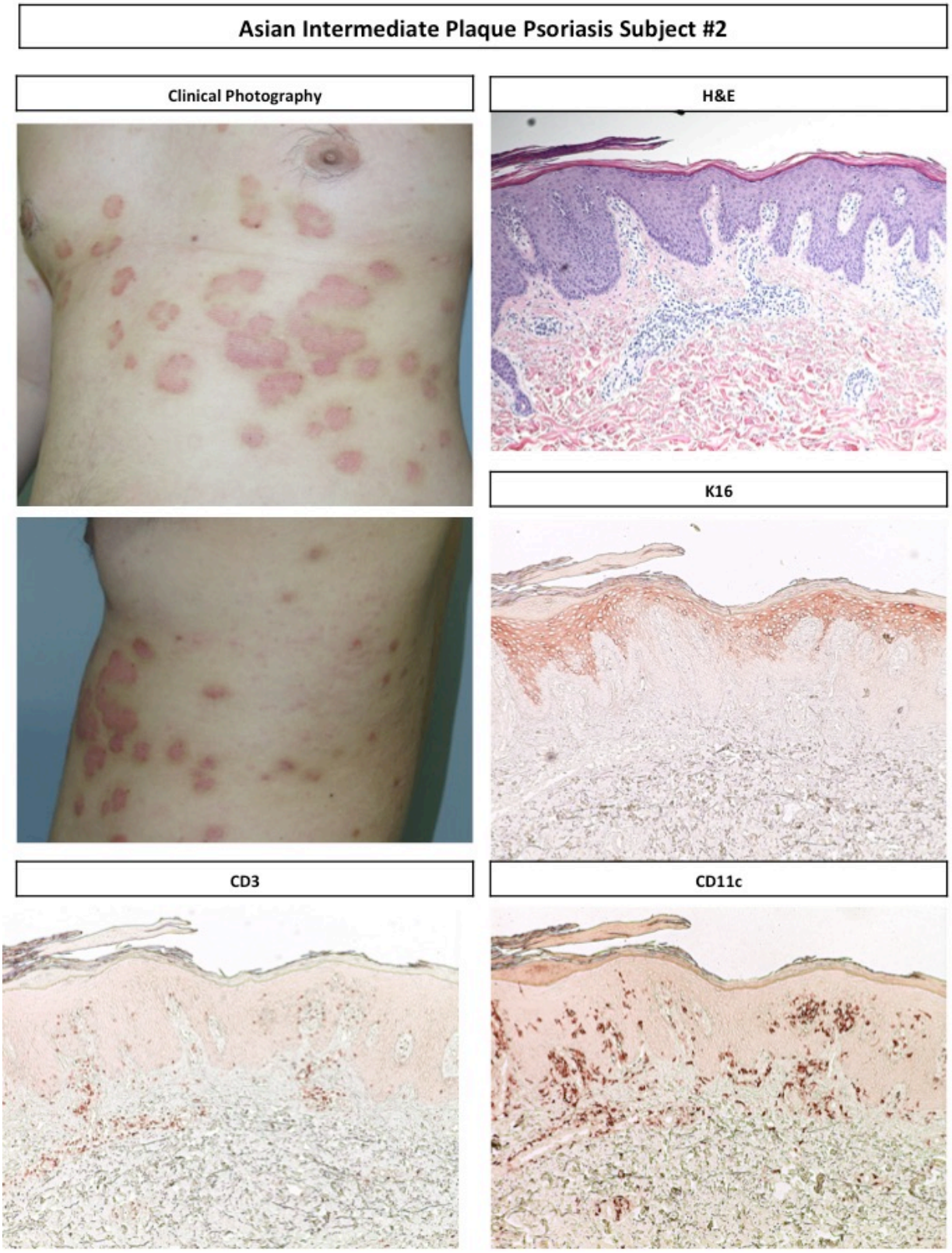
Supplementary Figure S3. Continued.



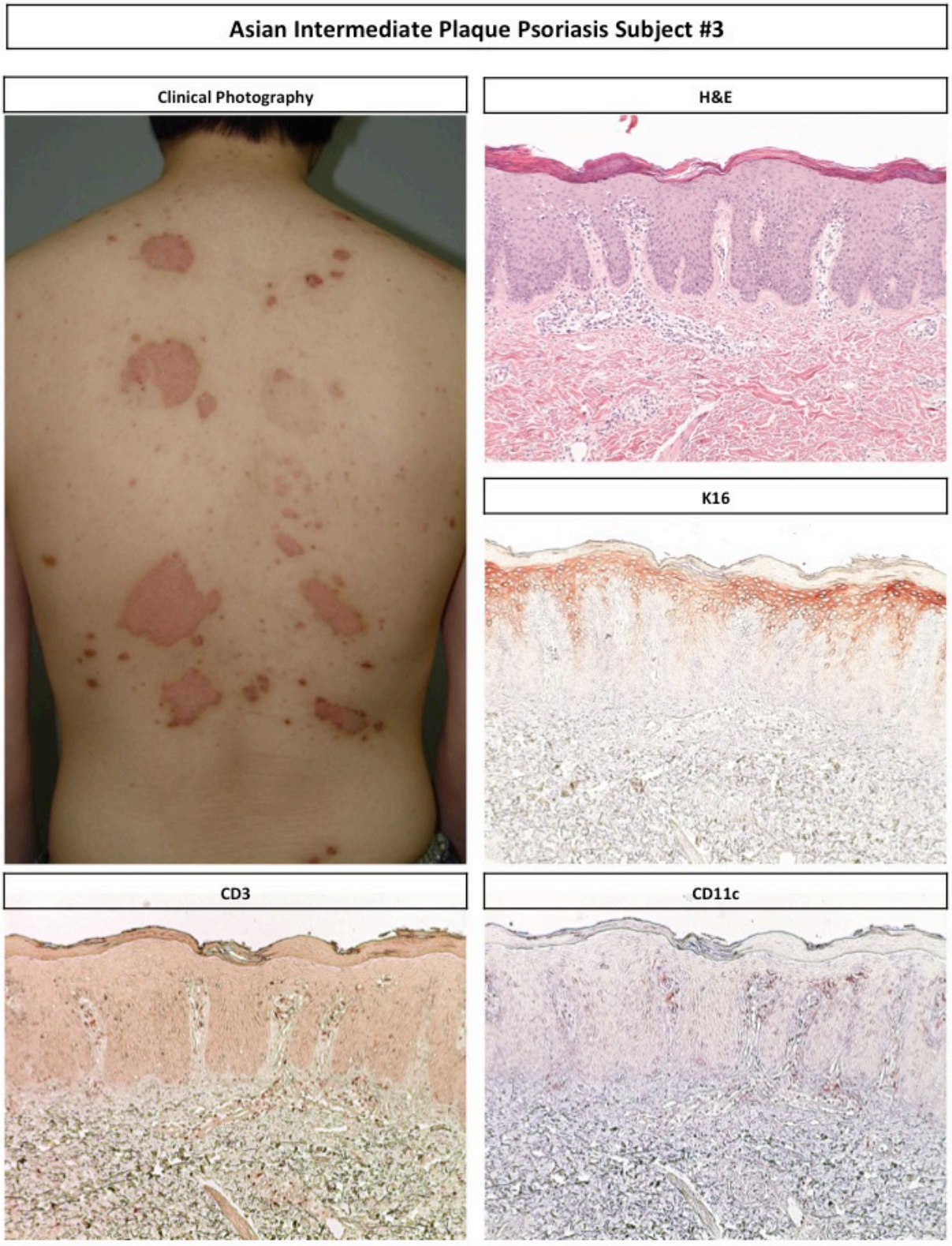
Supplementary Figure S3. Continued.



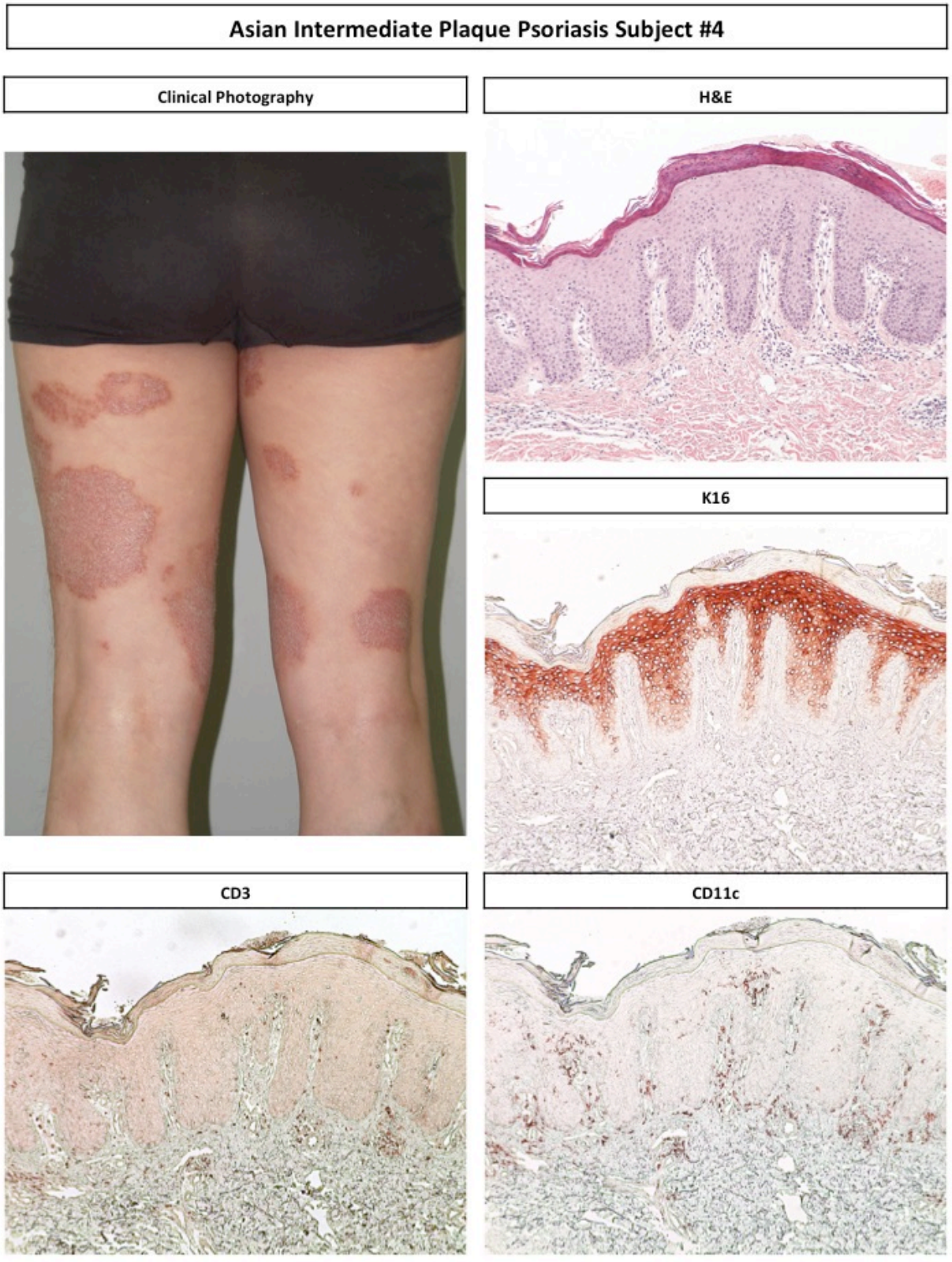
Supplementary Figure S3. Continued.



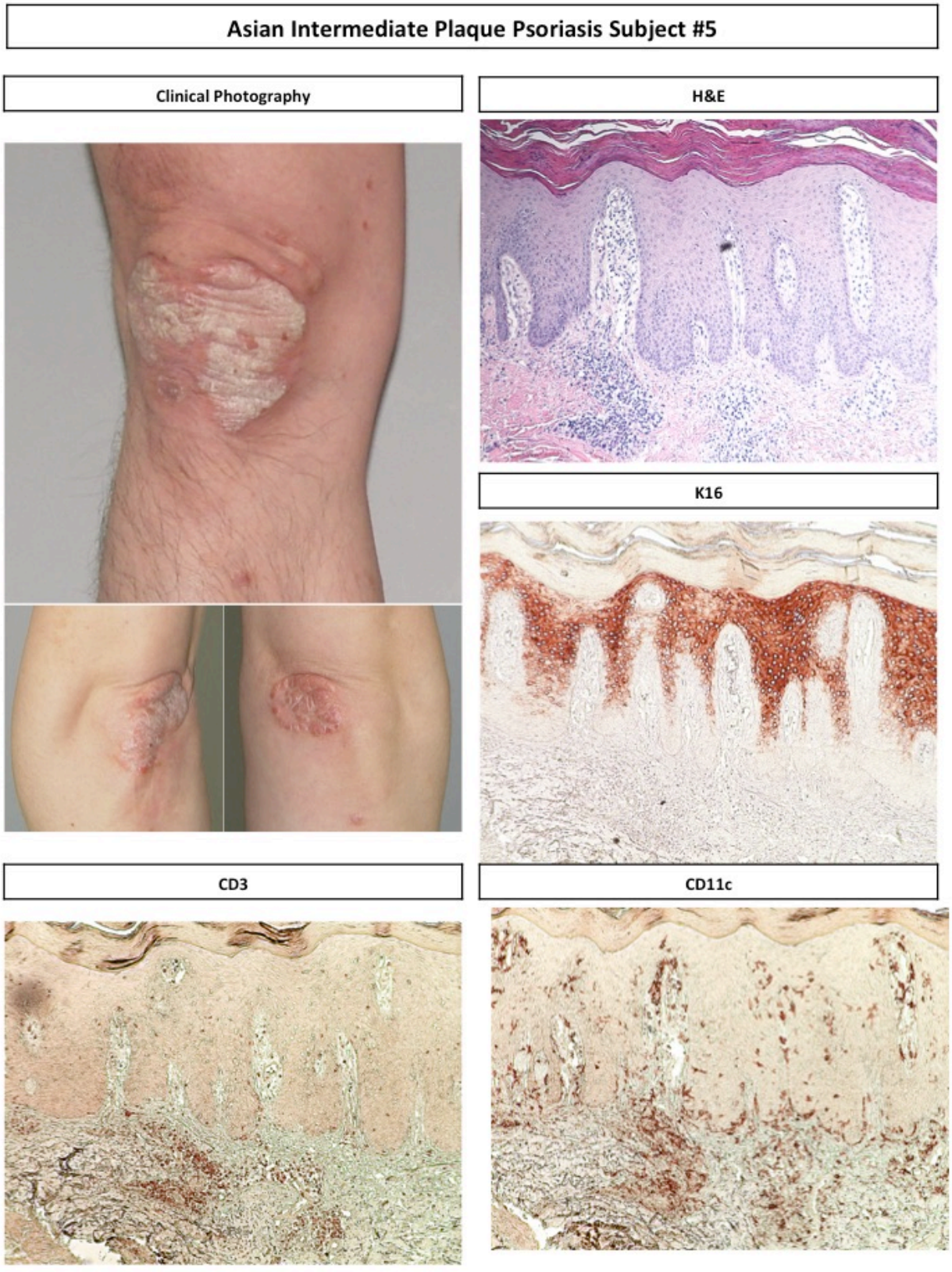
Supplementary Figure S3. Continued.



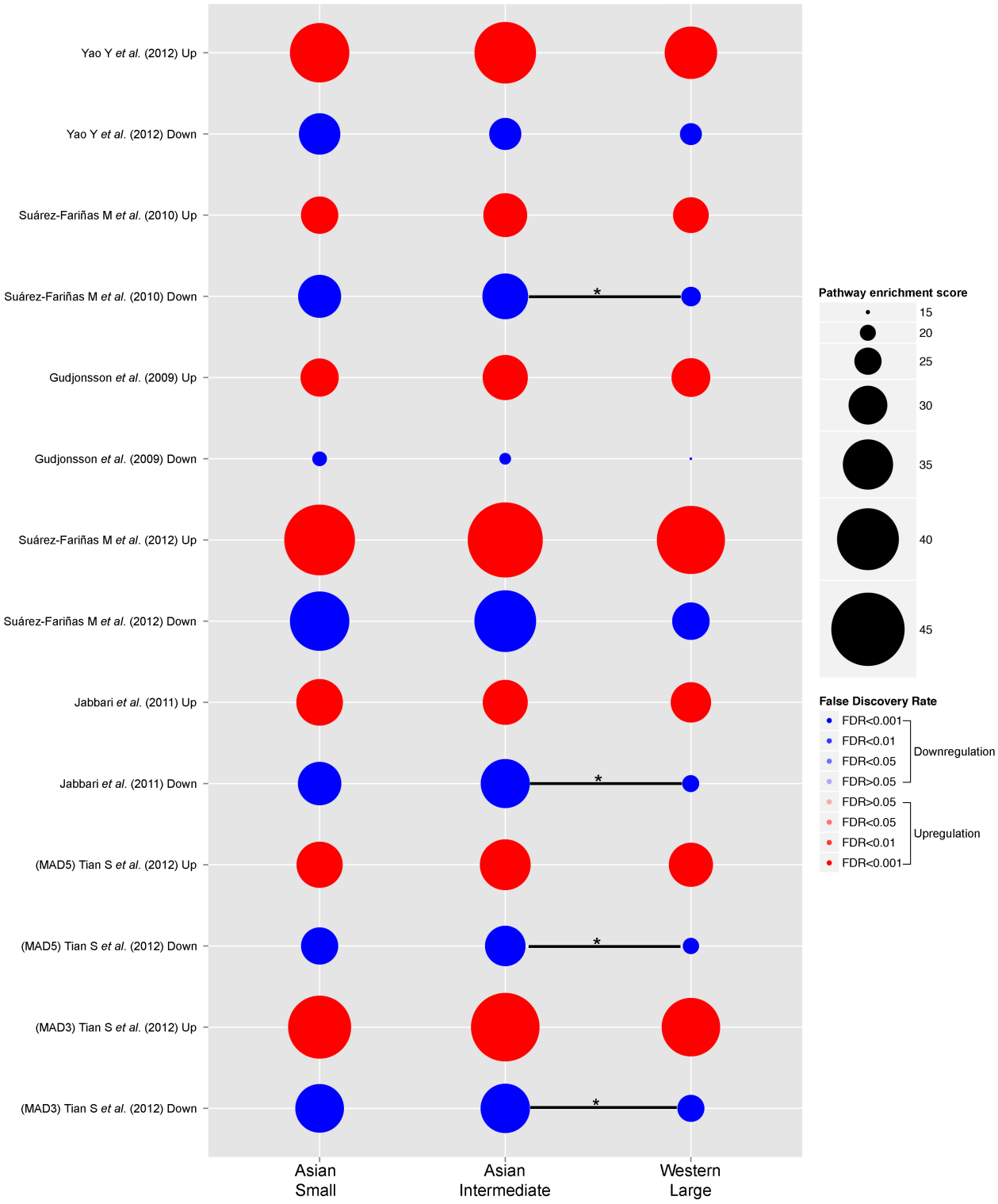
Supplementary Figure S3. Continued.



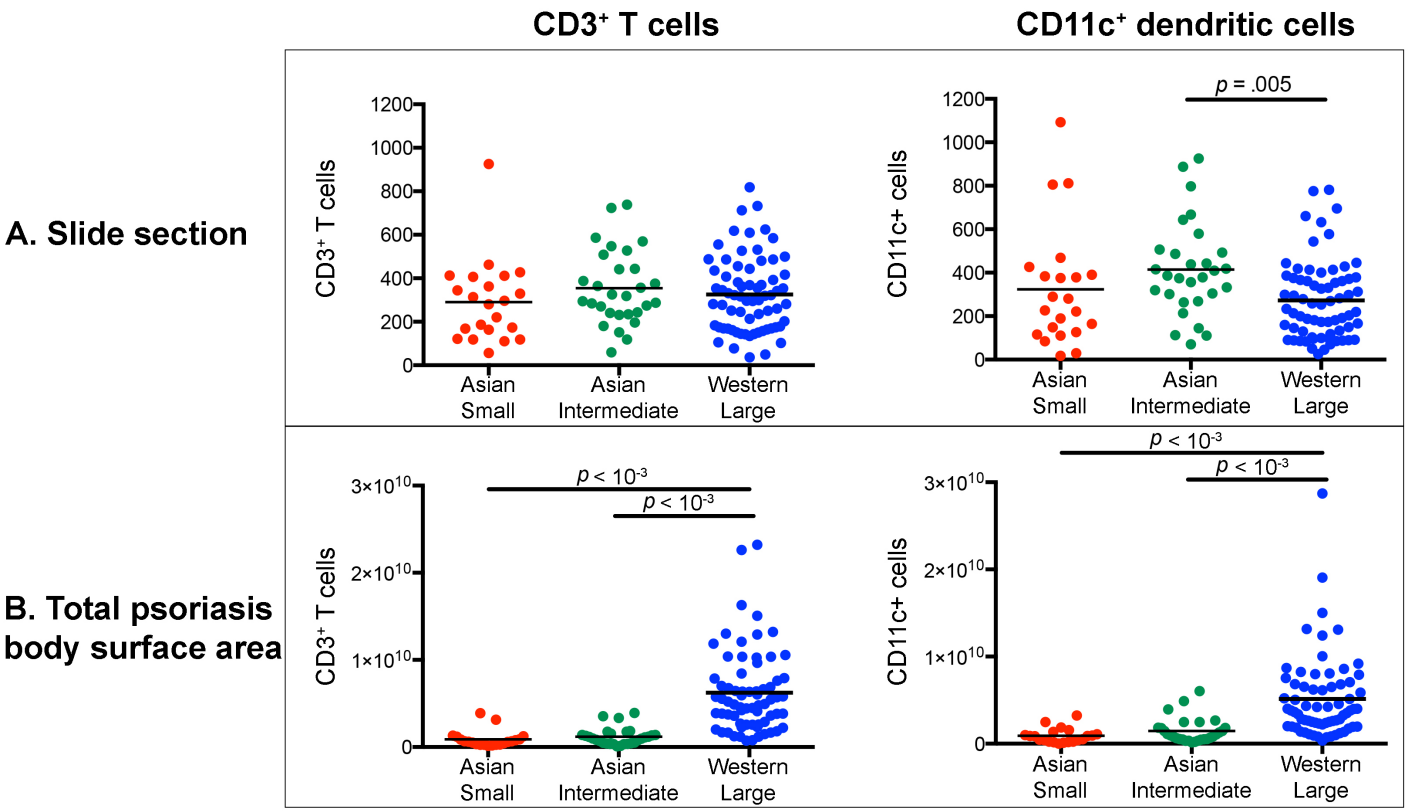
Supplementary Figure S3. Continued.



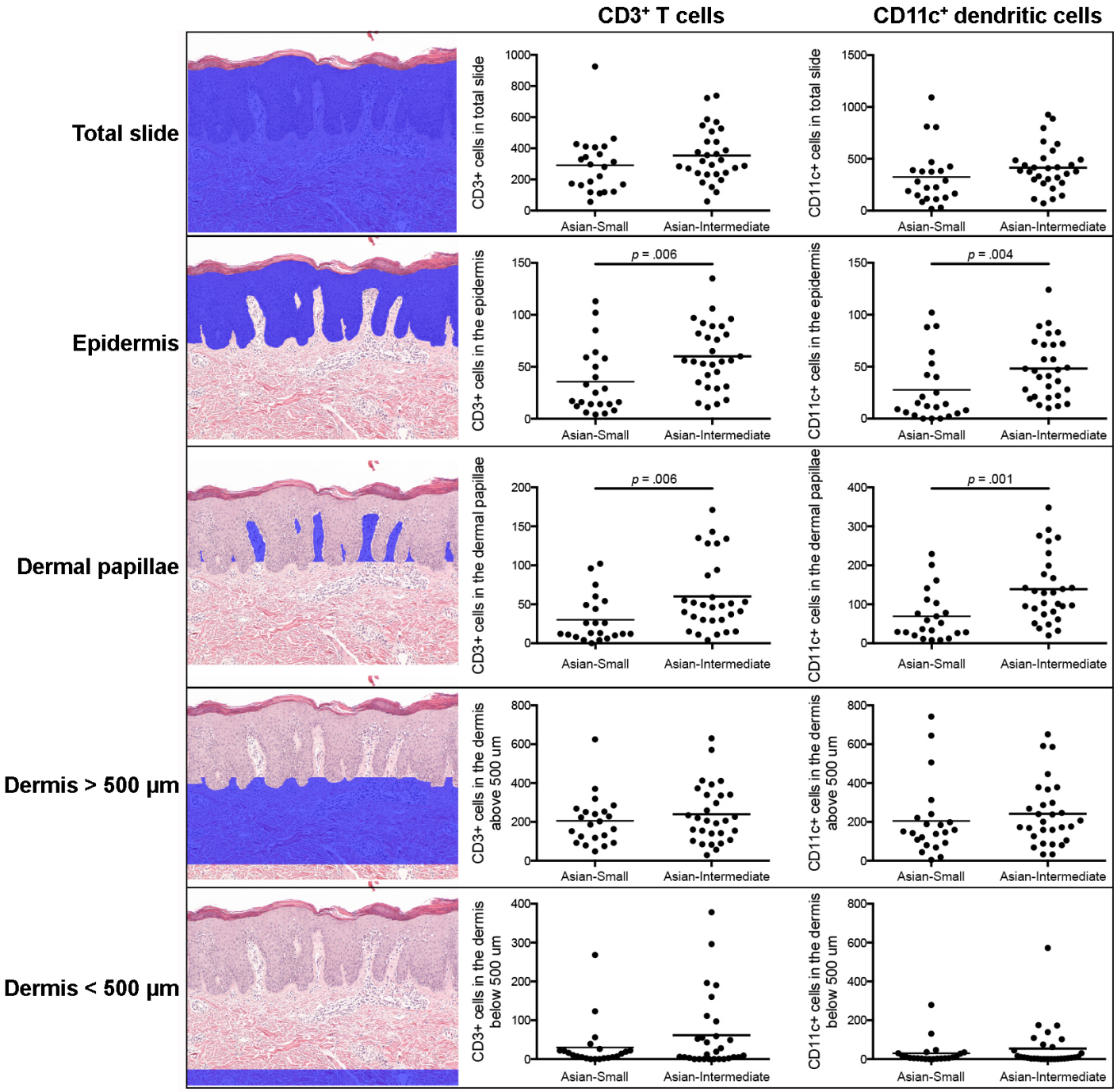
Supplementary Figure S3. Continued



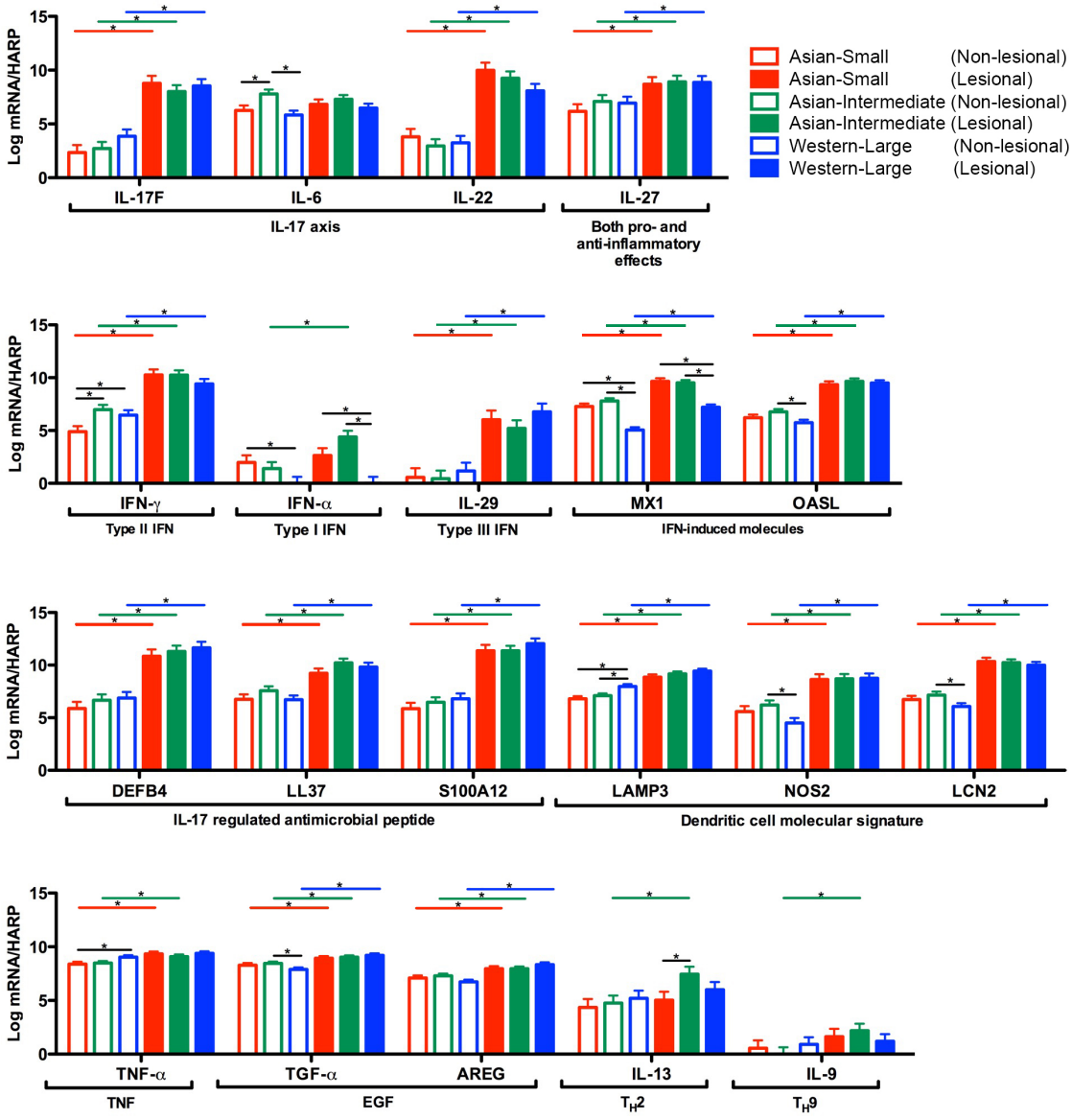
Supplementary Figure S4. **Psoriasis transcriptome enrichment scores.** The scores of Asian small and intermediate psoriasis were not different from, or were even higher than the scores of Western large psoriasis. The scores were generated by GSVA with the combined z-score method (*$p < 0.01$ and FDR < 0.01).



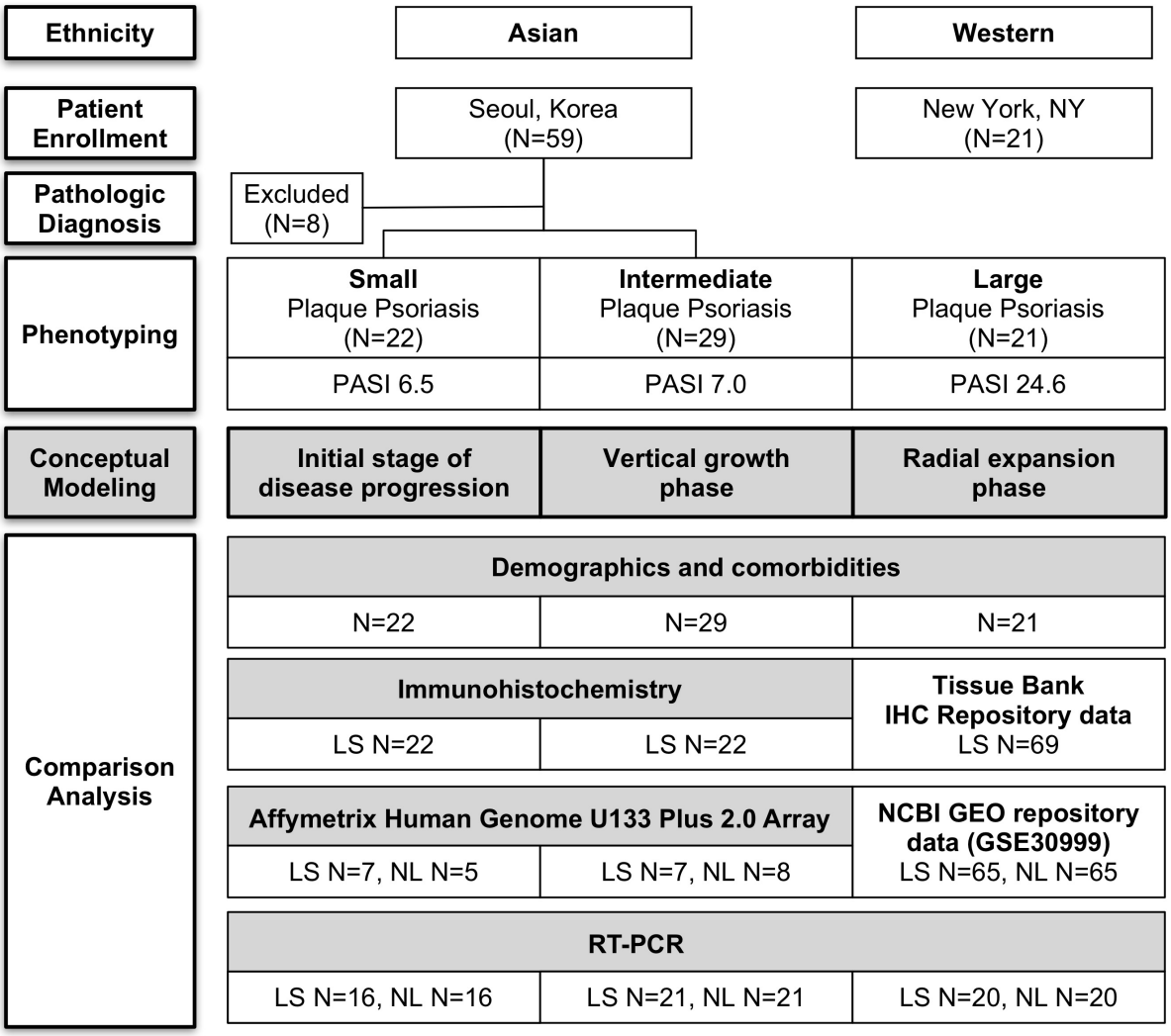
Supplementary Figure S5. Comparison of T cell and dendritic cell accumulation in slide section and total psoriasis body surface area. (A) When cell counts in slide section were compared, there was no significant difference in numbers of $CD3^+$ T cells between Asian small, Asian intermediate, and Western large psoriasis. Asian intermediate psoriasis had more $CD11c^+$ dendritic cells compared to Western large psoriasis. **(B)** When cell counts in total psoriasis body surface area were compared, Western large psoriasis had exponentially higher numbers of $CD3^+$ T cells and $CD11c^+$ dendritic cells compared to Asian small and intermediate psoriasis (Cell count in total psoriasis body surface area = cell count in the slide section × body surface area × proportion of psoriasis involvement).



Supplementary Figure S6. Comparison of T cell and dendritic cell infiltrates in different skin regions between Asian small and intermediate psoriasis. Compared to Asian small psoriasis, Asian intermediate psoriasis had more $CD3^+$ T cells and $CD11c^+$ dendritic cells in the epidermis and dermal papillary area (cell counting regions: purple, original magnification ×10).



Supplementary Figure S7. Quantitative comparison of gene expression in psoriatic lesional and non-lesional skin between Asian small (N=16), Asian intermediate (N=21), and Western large (N=20) psoriasis. Expression levels of genes involved in psoriasis disease progression were quantified by RT-PCR (Gene expression: Log_2 conversion of mRNA expression normalized to human acidic ribosomal protein (HARP), $*p < 0.05$).



Supplementary Figure S8. Asian psoriasis patients were enrolled in Seoul, Korea and Western psoriasis patients were enrolled in New York, NY, USA. Asian small, Asian intermediate, and Western large psoriasis were compared to explore models of initial stage of disease progression, vertical growth phase, and radial expansion phase. For immunohistochemistry and gene set variation analysis of Western large psoriasis, established repository data was utilized. (PASI: Psoriasis Area and Severity Index, LS: Lesional skin, NL: Non-lesional skin, Tissue bank: Rockefeller University Laboratory for Investigative Dermatology Tissue Bank, IHC: Immunohistochemistry, NCBI: National Center for Biotechnology Information, GEO: Gene Expression Omnibus).

TABLE LEGENDS

Supplementary Table S1. Clinical phenotyping definitions of small plaque psoriasis in comparison to psoriasis vulgaris and guttate psoriasis (Lew *et al.*, 2004)

	Psoriasis vulgaris	**Small plaque psoriasis**	Guttate psoriasis
Proportion in psoriasis	90%	**Common in Asians**	1.9%
Duration	**Chronic (> 24 weeks)**		Acute (< 20 weeks)
Age	**Adult**		Child and adolescent
Streptococcal infection	**No**		Yes
Size of psoriatic plaque	> 5 cm	**< 2 cm**	
Predominant location	Elbows, knees, scalp	**Upper trunk and proximal extremities**	

Supplementary Table S2. Correlation between disease progression and immune signatures.

Disease progression	Immune signatures	Location	Correlation				
			Linear		Monotonic		
			r	*p*	**ρ**	*p*	N
Epidermal thickness	$CD3^+$ T cell	Epidermis & dermal papillae	**0.558**	**<0.0001**	**0.641**	**<0.0001**	51
	$CD11c^+$ dendritic cell		**0.716**	**<0.0001**	**0.629**	**<0.0001**	51
PASI	$CD3^+$ T cell	Total psoriasis body surface area	**0.707**	**<0.0001**	**0.771**	**<0.0001**	98
	$CD11c^+$ dendritic cell		**0.644**	**<0.0001**	**0.762**	**<0.0001**	97
	CD69	Lesional skin	**-0.55**	**<0.0001**	**-0.600**	**<0.0001**	41
		Non-lesional skin	**-0.455**	**0.003**	**-0.481**	**0.001**	41
	FAS	Lesional skin	**-0.586**	**0.001**	**-0.511**	**0.001**	41
		Non-lesional skin	**-0.487**	**<0.0001**	**-0.591**	**<0.0001**	41
	CTLA4	Lesional skin	**-0.337**	**0.031**	**-0.380**	**0.012**	41
		Non-lesional skin	-0.089	0.579	-0.188	0.239	41
	PD-L1	Lesional skin	-0.092	0.568	-0.072	0.654	41
		Non-lesional skin	-0.162	0.312	-0.208	0.191	41
	FoxP3	Lesional skin	-0.158	0.325	-0.202	0.206	41
		Non-lesional skin	-0.245	0.122	**-0.350**	**0.025**	41
	TNF-α	Lesional skin	**0.454**	**0.047**	0.204	0.200	41
		Non-lesional skin	**0.312**	**0.003**	**0.407**	**0.008**	41

r = Pearson (parametric) correlation coefficient
ρ = Spearman (non-parametric) correlation coefficient
PASI: Psoriasis Area and Severity Index
Gene expression: Log_2 conversion of mRNA expression normalized to human acidic ribosomal protein (HARP)
Bolding indicates $p < 0.05$

Supplementary Table S3. Demographics and comorbidities of Asian small, Asian intermediate, and Western large psoriasis.

Characteristics	Asian Small Psoriasis (n = 22)	Asian Intermediate Psoriasis (n = 29)	Western Large Psoriasis[a] (n = 21)	*P*
Demographics				
Gender, No (%)				
Female	10 (45.5)	6 (20.7)	8 (38.1)	.156
Male	12 (54.5)	23 (79.3)	13 (61.9)	
Age, y				
Mean	40	43	53	.016
Range	18-70	20-70	23-70	
PASI[1] (0~72)				
Mean	6.5	7.0	24.6	<.0001
Range	1.9-15.8	1.6-23.5	12.0-58.4	
Duration of psoriasis (yr)				
Mean	12.0	9.2	18.8	.009
Range	.04-40	.17-60.0	2.0-38.0	
BMI				
Mean	22.2	24.9	30.3	<.0001
Range	16.5-31.0	20.2-38.9	20.2-52.7	
Smoking	12/21 (57.1)	18/29 (62.1)	10/15 (66.7)	.897
Alcohol	16/21 (76.2)	21/29 (72.4)	10/16 (62.5)	.691
Comorbidities, No (%)				
Psoriatic arthritis	0/22 (0.0)	0/29 (0.0)	9/19 (47.4)	<.0001
Obesity	2/21 (9.5)	3/29 (10.3)	9/19 (47.4)	.004
Diabetes mellitus	0/22 (0.0)	6/29 (20.7)	3/21 (14.3)	.062
Hypertension	3/22 (13.6)	5/29 (17.2)	10/20 (50.0)	.016
Hypercholesterolemia	0/22 (0.0)	2/29 (6.9)	4/21 (19.0)	.066
Myocardial infarction	0/22 (0.0)	0/29 (0.0)	1/21 (4.8)	.292
Osteoporosis	0/22 (0.0)	0/29 (0.0)	2/21 (9.5)	.082
Depression	0/22 (0.0)	3/29 (10.3)	5/21 (23.8)	.036

a. In subsequent pairwise comparison with Bonferroni correction ($p < 0.017$), Western large psoriasis patients had more severe psoriasis (PASI, duration of psoriasis) with higher prevalence of comorbidities (obesity and psoriatic arthritis) than both Asian small and intermediate psoriasis ([1]*PASI: Psoriasis Area and Severity Index*).

SUPPLEMENTARY MATERIAL & METHODS

Human samples

Lesional skin biopsy tissue was obtained at a representative psoriatic plaque, and non-lesional skin biopsy tissue was obtained adjacent to the psoriatic plaque where the lesional skin biopsy was performed. Skin biopsy tissues and relevant clinical information collected at Korea University Guro Hospital were stored at Korea University Guro Hospital Biobank. After reviewing the study by Korea University Guro Biobank Review Board committee and quality control process, de-identified samples and information were transferred to the tissue bank in the Laboratory for Investigative Dermatology at the Rockefeller University under material transfer agreement.

Immunohistochemical analyses

For immunohistochemical analysis of psoriasis skin biopsy samples, tissues were fixed in 4% buffered formaldehyde, embedded in paraffin. For H&E staining, the sections were stained with hematoxylin (Fisher Scientific) and eosin (Shandon). For immunostaining, the sections were processed for antigen retrieval with Diva Decloaker, RTU (Biocare Medical) and stained with CD3 (DAKO, clone F7.2.38, dilution 1:50), CD11c (Novocastra, clone 5D11, dilution 1:100) and K16 (Bio-Rad, clone LL025, dilution 1:40). Biotin-labeled horse anti-mouse antibodies (Vector Laboratories) were used to detect the mouse monoclonal antibodies. The staining signal was amplified with avidin-biotin complex (Vector Laboratories) and developed using chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich). Epidermal thickness and immunostaining positive cell counts were measured by computer-assisted image analysis (NIH ImageJ 1.48).

Cell counts in total psoriasis body surface area were calculated under the assumption that the number of cells in the skin biopsy tissue slide section represents the number of cells in the skin area of 5 mm (average diameter of a skin biopsy) × 6 μm (average thickness of a slide section): Cell counts in total psoriasis body surface area = (Cell counts in the slide section per 5 mm × 6 μm area) × [(Body surface area calculated from height and weight using DuBois formula, m^2) × (proportion of psoriasis involvement, %)].

Isolation of total RNA from skin biopsies

Frozen tissue was mechanically disaggregated before RNA extraction. RNA was extracted with AllPrep DNA/RNA/Protein Mini Kit (Qiagen) according to the manufacturer's protocol. The RNA yield and quality were determined with NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies), respectively.

Real-time PCR

Real-time PCR was performed on QuantStudio™ 6 Flex Real-Time PCR System (Life Technologies) with TaqMan expression assays. Complementary DNA (cDNA) synthesis was performed with the High Capacity cDNA Reverse Transcription Kit (Life Technologies) according to the manufacturer's instructions. After pre-amplification with TaqMan® PreAmp Master Mix (Life Technologies), gene expression was determined with Taqman expression assays: K16 (Hs00955082_g1), CCL20 (Hs01011368_m1), DEFB103B (Hs04194486_g1), DEFB4 (Hs00823638_m1), LL37 (Hs00189038_m1), S100A12 (Hs00942835_g1), IL-17A (Hs00174383_m1), IL-17F (Hs00369400_m1), IL-23A (Hs00900828_g1), IL-1B (Hs01555410_m1), IL-6 (Hs00985639_m1), IL-8 (Hs00174103_m1), FAS (Hs00236330_m1),

FOXP3 (Hs01085834_m1), CTLA4 (Hs03044418_m1), PD-L1 (Hs01125301_m1), CD69 (Hs00934033_m1), IL-27 (Hs00377366_m1), IL-12 (Hs01011518_m1), IFN-γ (Hs00989291_m1), IFN-α (Hs00855471_g1), IL-29 (Hs00601677_g1), MX1 (Hs00895608_m1), OASL (Hs00984387_m1), IL-22 (Hs01574154_m1), IL-20 (Hs00218888_m1), IL-19 (Hs00604657_m1), LAMP3 (Hs00180880_m1), NOS2 (Hs01075529_m1), LCN2 (Hs01008571_m1), TNF-α (Hs01113624_g1), TGF-α (Hs00608187_m1), AREG (Hs00950669_m1). The results were quantified by standard curve method and normalized to Human acidic ribosomal protein (HARP) housekeeping gene.

RNA microarray experiments

RNA extracted from Asian small and intermediate psoriasis skin biopsy tissues was amplified and labeled (Ovation RNA Amplification System V2, NuGEN Techologies). A total of 100 ng of biotinylated cDNA was hybridized to GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). The expression values were obtained using GCRMA algorithm (Wu *et al.*, 2004), while normalization across samples was carried out using quantile normalization. As the first step of data filtering, only those probe sets with at least 1 sample with expression values larger than 4 and standard deviation/SD >0.33 were kept for further analyses.

The gene expression data of Western large psoriasis skin biopsy tissues was obtained from the NIH's GEO (Gene Expression Omnibus) repository (GSE30999). The skin biopsy samples were collected from histologically confirmed Western large psoriasis patients who were enrolled into an IRB-approved Phase 3, multicenter, randomized trial protocol (ACCEPT trial)(Griffiths *et al.*, 2010). The platform of expression profiling was identical Affymetrix Human Genome U133 Plus 2.0 Array. The raw Affymetrix data (CEL files) were downloaded from GEO repository and

expression values were obtained with the identical GCRMA algorithm (Wu *et al.*, 2004) and data filtering.

We next combined the expression data of Asian small and intermediate psoriasis with Western large psoriasis. The expression data of Asian small and intermediate psoriasis was normalized based upon the quantiles of the distribution of Western large psoriasis, using the function normalize.quantiles.target available in the Bioconductor R package 'preprocessCore'.

Raw data of Asian small and intermediate psoriasis have been deposited in NCBI's Gene Expression Omnibus together and are accessible through accession number GSE67853.

Statistics

Analysis of Clinical information and Immunohistochemistry: To compare clinical information and immunohistochemistry between Asian small, Asian intermediate, and Western large psoriasis, we used Kruskal-Wallis test and Fisher's exact test and performed subsequent pairwise comparisons with Bonferroni correction.

Correlation Analysis of Cutaneous and Systemic Progression: Correlation between the measurements of disease progression (epidermal thickness or PASI) and immune signatures ($CD3^+$ T cell and $CD11c^+$ dendritic cell counts in skin biopsy tissue of lesional skin, and $\log_2$ converted gene expression measured by RT-PCR in lesional/non-lesional skin) was explored by both Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (ρ). Association between epidermal thickness or PASI and gene expressions in lesional and non-lesional skin was also explored through a multivariable linear regression model that included epidermal thickness or PASI as dependent variable and gene expression as independent variable. The selection of a subset of genes that significantly correlated with epidermal thickness or PASI

was carried by stepwise linear regression as implemented in IBM SPSS statistics Version 22.

Analysis of Arrays, RT-PCR: For microarray and RT-PCR data, $\log_2$-transformed expression values were modeled using linear mixed-effects models with psoriasis phenotype groups (Asian small/Asian intermediate/Western large psoriasis) and tissue type (lesional/non-lesional) as fixed effects and a random effect term for each subject. After fitting the interaction model, comparisons of interest were assessed using linear contrasts via restricted log-likelihood maximization (REML). Analysis was conducted under the general framework of *limma* package. *P*-values from moderated (paired) t-test were adjusted for multiple hypotheses across genes using Benjamini-Hochberg procedure. FDR < 0.05 and fold change ratio > 2 were used as cut-offs to define differentially expressed genes between lesional and non-lesional skin biopsy samples.

Pathway Analysis: The activity of entire signaling pathways for each sample was quantified by using a per-patient GSEA (Gene Set Enrichment Analysis)-like method. GSVA (Gene Set Variation Analysis) is an unsupervised sample-wise enrichment method described by Hanzelmann *et al.* (Hänzelmann *et al.*, 2013). Enrichment scores were obtained by setting the parameter z-score in gsva function available in *R* package. The formulation for scores evaluation was described by Lee *et al.* (Lee *et al.*, 2008). The authors proposed the linear combination of normalized expressions with weights in the combination being defined as $1/\sqrt{k}$ for *k* being the number of genes in the pathway. This methodology was applied to obtain pathway enrichment scores in microarray analysis. These enrichment scores were used as inputs for the same linear mixed model framework, described previously for gene expressions, in order to evaluate significant differences between Asian small, Asian intermediate, and Western large psoriasis. *P* < 0.01 and FDR < 0.01 were used as cut-offs to define significantly increased pathway enrichment score.

Most of the statistical analysis was carried out in the R language version 2.12 (www.r-project.org), and packages were from the Bioconductor project (www.bioconductor.org). IBM SPSS Statistics Version 22 was used for stepwise linear regression modeling. Results are expressed as means ± SEM.