Supplemental Data

Quantitative proteomics reveals β2 integrin-mediated cytoskeletal rearrangement in VEGF-induced retinal vascular hyperpermeability

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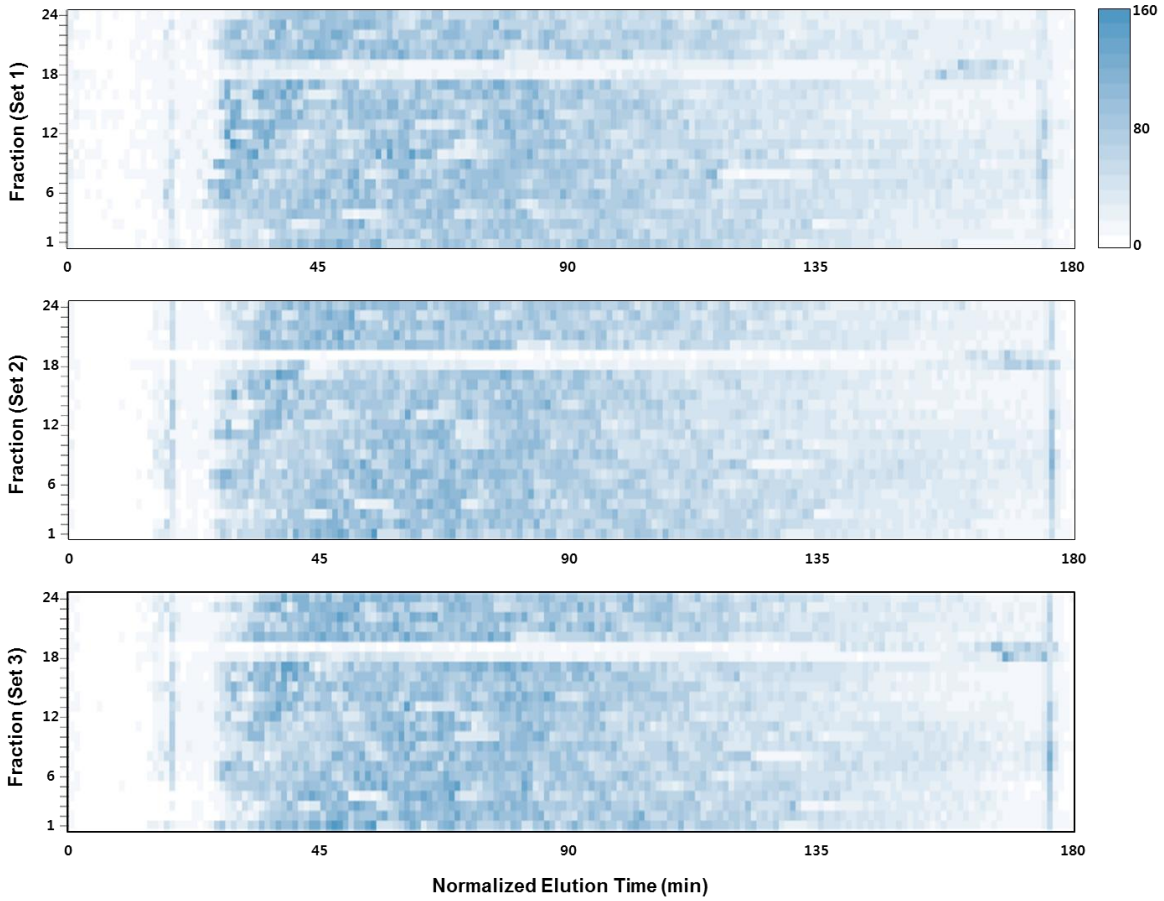

Fig. S1. Numbers of identified peptides over normalized elution time across 24 fractions for retinal proteome (experimental set #1, #2 and #3). Color bar, gradients of the numbers of identified peptides.

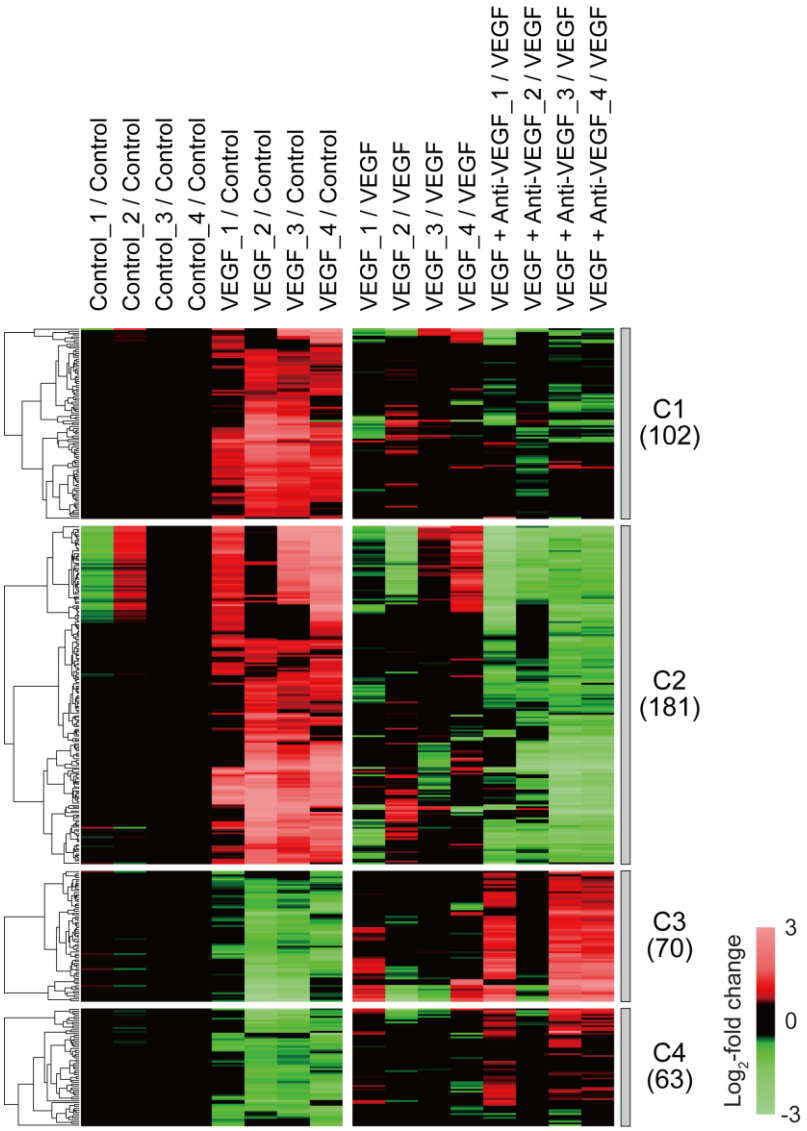

Fig. S2. Alterations of the DEPs in four major clusters in all the replicates. Log_2-fold-changes of the DEPs in each cluster were computed as the ratios of the median intensities of differentially expressed peptides for individual DEPs. For each DEP, the log_2-fold-changes were normalized by the median value of four replicates in Control for VEGF versus Control (VEGF/Control, left panels of the heat map including eight samples) or in VEGF for anti-VEGF versus VEGF (anti-VEGF/VEGF, right panels of the heat map including eight samples) in order to clearly show the difference between the two conditions being compared. The underscored number followed by the condition indicates the replicate sample in the condition (e.g., VEGF_2 indicates the 2^{nd} replicate sample under VEGF condition). The dendrogram shows how the DEPs in each cluster were clustered using a hierarchical clustering method (complete linkage and Euclidian distance). Color bar, gradient of the normalized log_2-fold-changes in the samples indicated in the top of the columns.

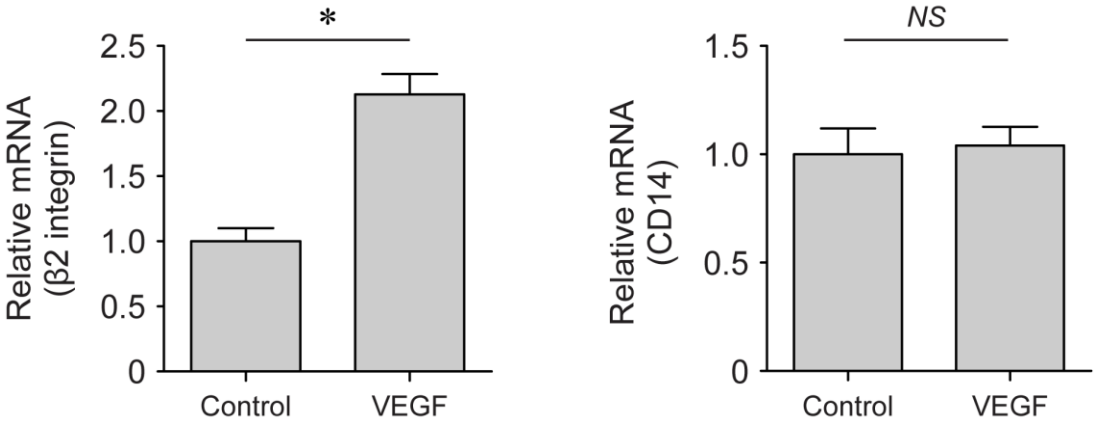

Fig. S3. mRNA expression levels of β2 integrin and CD14 in confluent HRMECs on F2 and FN1-coated dish, respectively (n = 4). The data are normalized to that of GAPDH and are shown as means ± SEM. *NS*, $P > 0.05$; *, $P < 0.05$ from Mann-Whitney U-test.

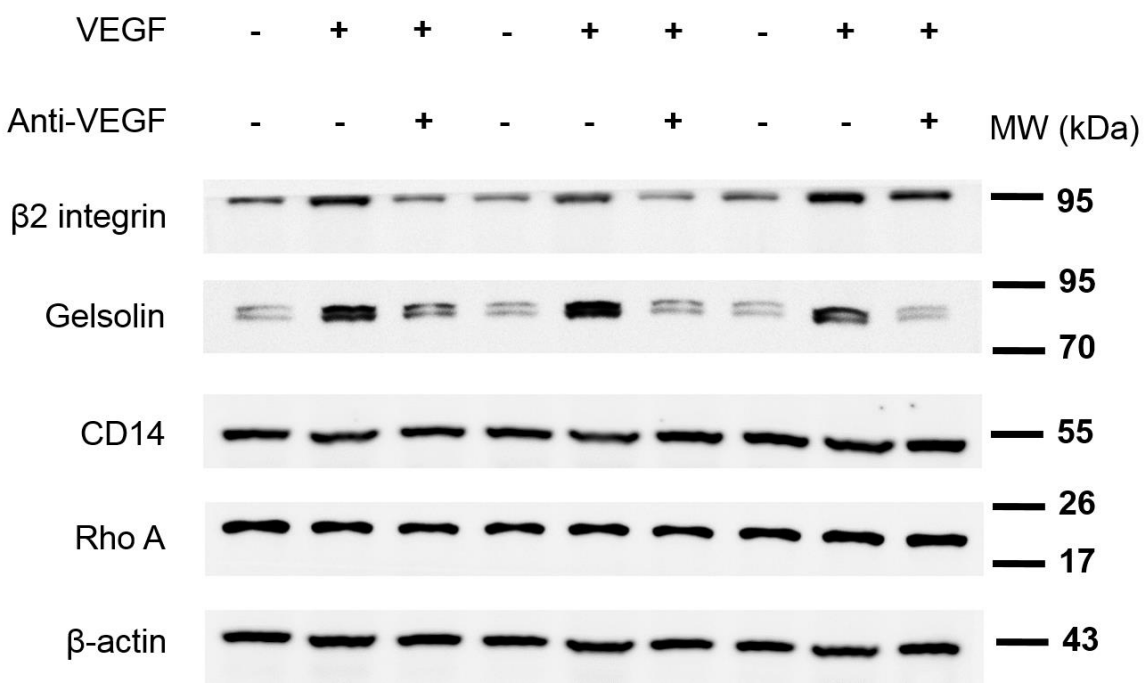

Fig. S4. Protein expression levels of β2 integrin, gelsolin (GSN), CD14, and RhoA in the retina measured by western blotting analysis (n = 3). The retinas were prepared at 24 hours after the injection of VEGF or VEGF plus anti-VEGF antibody (anti-VEGF). MW, molecular weight.

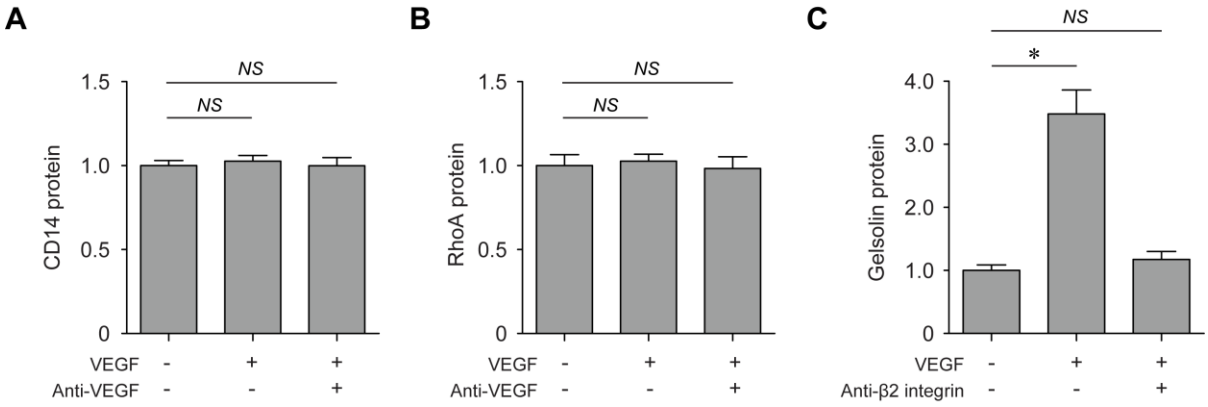

Fig. S5. Quantification of the protein expression levels of CD14, RhoA, and gelsolin (GSN) in the retina measured by western blotting analysis (n = 3). Anti-VEGF, VEGF plus anti-VEGF antibody. *NS*, $P > 0.05$; *, $P < 0.05$ from Kruskal-Wallis test with post-hoc Dunn's multiple comparison test.

Supplemental Tables

Table S1. The alignment table of the 205,730 peptides identified with PSM-level FDR < 1%. For each peptide, sequence, precursor m/z, charge state (CS), score [-log (SpecEValue)], and peptide intensities are shown. SpecEValue is the score obtained from MS-GF+ search engine, which represents the reliability of the PSM. In the alignment table, the percentages of missing data were found to be 25.8, 26.9, and 26.4% in Control, VEGF, and anti-VEGF conditions, respectively, and 24.1, 28.5, and 26.5% in three iTRAQ experiments.

See the attached excel file.

Table S2. The list of proteins identified from retinal tissues. For each protein, the SwissProt accession number, sequence coverage (%), the number of identified unique peptides, and whether the protein was detected ("O" symbol) or not (empty) are shown. The protein-level missing value rate were found to be 7.3, 7.8, and 7.3% in Control, VEGF, and anti-VEGF conditions, respectively, and 6.7, 8.7, and 6.9% in the three iTRAQ experiments. Also, all proteins were quantified in all the three conditions, and 7,393 proteins (85.1%) were quantified in all the 12 samples (4 biological replicate samples per condition).

See the attached excel file.

Table S3. 479 DEPs from the two comparisons (VEGF versus Control and Anti-VEGF versus VEGF). For each DEP, the cluster to which the protein belongs to and log2-fold-changes in the two comparisons are shown together with SwissProt ID, EntrezID, and gene symbols and descriptions. For each DEP, the $\log_2$-fold-changes in each comparison were computed as the ratios of the median intensities of differentially expressed peptides between the two conditions being compared in the comparison (see Experimental Procedures).

See the attached excel file.

Table S4. Organizing of the 479 DEPs into six clusters (Clusters 1-6) based on their differential expression in the two comparisons. The colors represent up-regulation (red) and down-regulation (green) in the corresponding comparison: For example, the red color in the comparison of VEGF/Control represents up-regulation in VEGF-treated samples, compared to control samples.

Cluster	VEGF/Control	Anti-VEGF/VEGF	Number of proteins
1			**102**
2			**181**
3			**70**
4			**63**
5			16
6			47
Total			**479**

Table S5. Gene ontology biological processes (GOBPs) enriched by the DEPs in the individual clusters. Enrichment P-values computed by DAVID are shown.

See the attached excel file.