Additional file 1

Laminin 221 fragment is suitable for the differentiation of human induced pluripotent stem cells into brain microvascular endothelial-like cells with robust barrier integrity

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Components
Four Supplemental Figures

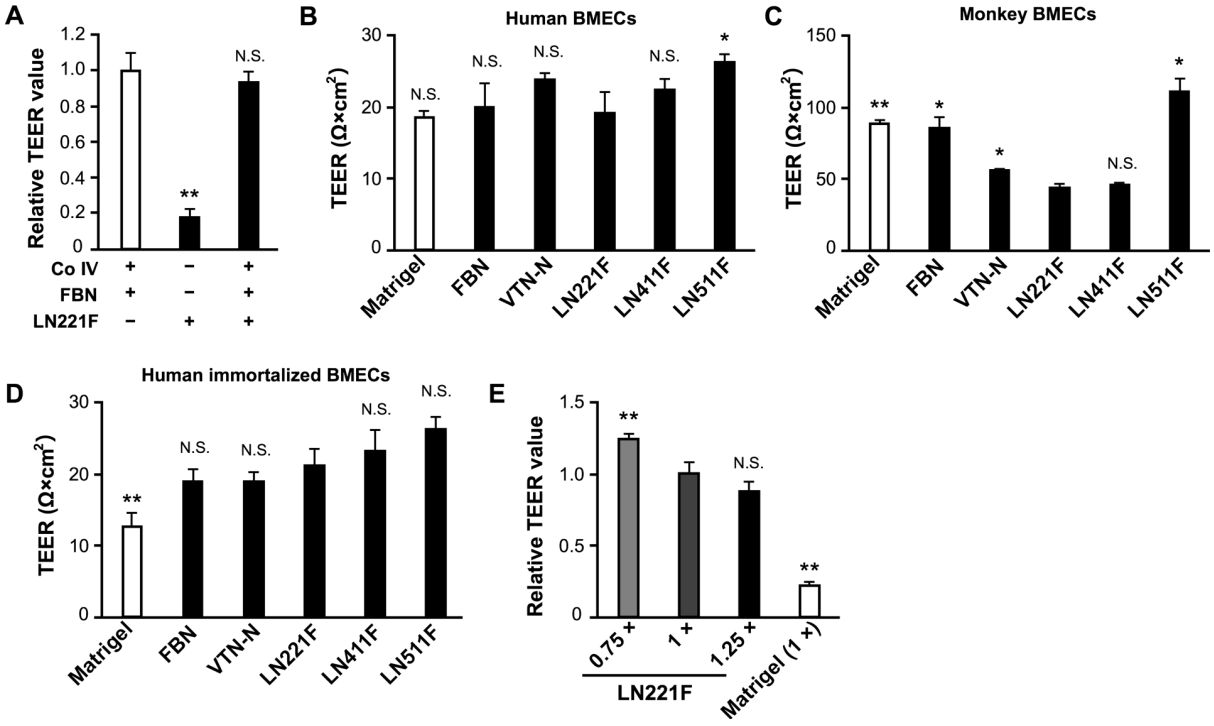

Fig. S1. Analyses of the effect of LN221F under various conditions

(A) Measurement of TEER values of 610B1-derived Matrigel-iBMELCs seeded on inserts coated with LN221F alone, a mixture of collagen type IV and FBN, or a mixture of collagen type IV, FBN, and LN221F (days 8–10). The relative TEER value of 610B1-derived Matrigel-iBMELCs seeded on inserts coated with a mixture of collagen type IV and FBN was defined as 1. Data are presented as mean ± SD ($n = 3$; N.S. = not significant, $^{**}p < 0.01$; Tukey's Honest Significant Difference test, collagen type IV, and fibronectin-coated insert group vs. others).

(B) Measurement of TEER values of BMECs derived from human (day 4) on Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F. Data are presented as mean ± SD ($n = 3$; N.S. = not significant, $^{*}p < 0.05$; Tukey's Honest Significant Difference test, LN221F group vs. others).

(C) Measurement of TEER values of BMECs derived from *Macaca irus* (day 4) on Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F. Data are presented as mean ± SD ($n = 3$; N.S. = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$; Games–Howell test, LN221F group vs. others).

(D) Measurement of TEER values of hCMEC/D3 cells (day 4) on Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F. Data are presented as mean ± SD ($n = 3$; N.S. = not significant, $^{**}p < 0.01$; Tukey's Honest Significant Difference test, LN221F group vs. others).

(E) The TEER values of 610B1-derived LN221F-iBMELCs on day 10 depending on seeding cell density before the start of differentiation. The seeding cell number were set to 6×10^5 cells/well (0.75 × group), 8×10^5 cells/well (1 × group), or 10×10^5 cells/well (1.25 × group). The relative TEER value of 1 × group was defined as 1. Data are presented as mean ± SD ($n = 3$; N.S. = not significant, $^{**}p < 0.01$; Tukey's Honest Significant Difference test, 1 × group vs. others).

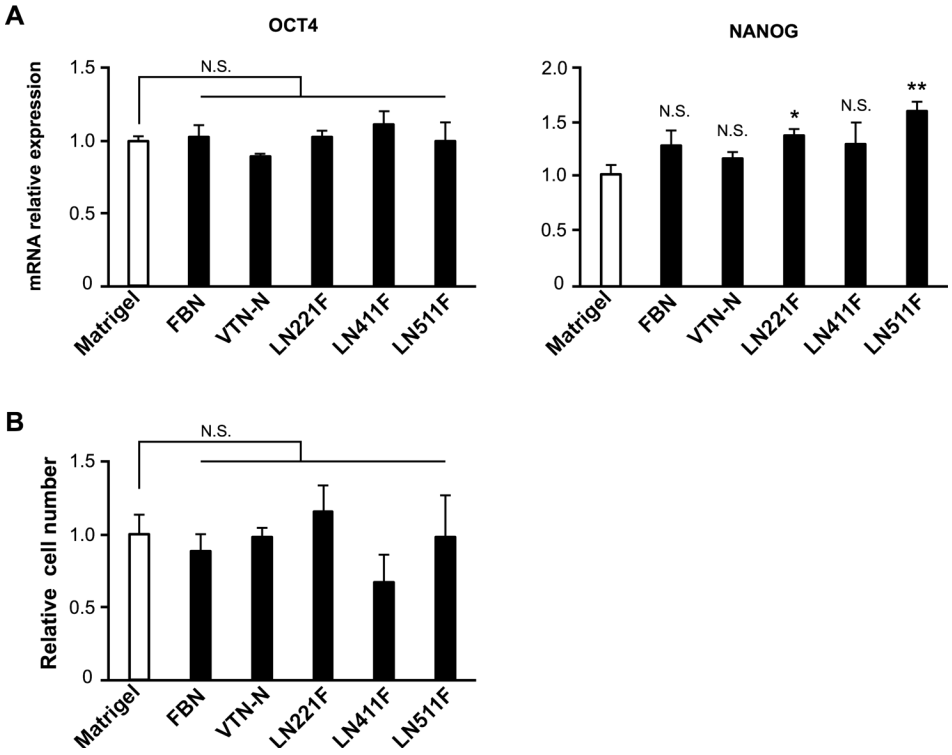

Fig. S2. Analyses of the effects of Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F on human iPS cells

(A) Relative mRNA expression levels of OCT-4 and NANOG in human iPS cells cultured on Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F from days −3 to 0. The values are normalized to those of HPRT1. The relative mRNA expression levels of 610B1 cultured on Matrigel were defined as 1. Data are presented as mean ± SD (n = 3; N.S. = not significant, *p < 0.05, **p < 0.01; Tukey's Honest Significant Difference test or Games–Howell test, Matrigel group vs. others).

(B) Relative cell viabilities (610B1) on day 0 cultured on Matrigel, FBN, VTN-N, LN221F, LN411F, or LN511F from days −3 to 0 were analyzed by CCK-8 assay. The relative cell number of 610B1 cultured on Matrigel was defined as 1. Data are presented as mean ± SD (n = 3; N.S. = not significant; Games–Howell test, Matrigel group vs. others).

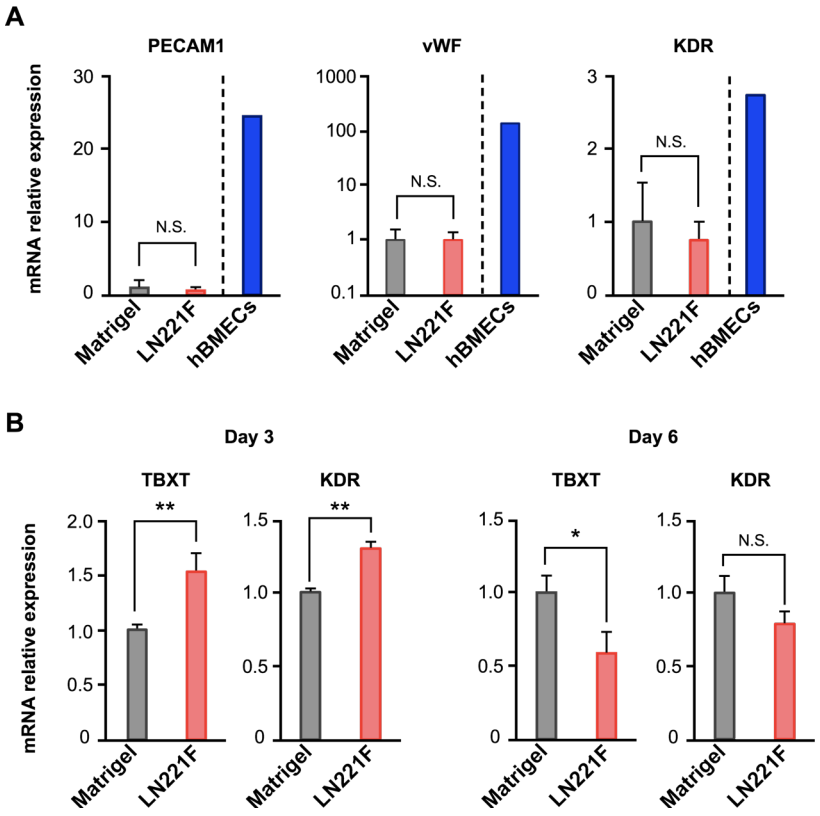

Fig. S3. Gene expression analyses of endothelial cell markers in iBMELCs and mesodermal markers in the intermediate state of iBMELCs

(A) Relative mRNA expression levels of PECAM1, vWF, and KDR in LN221F-iBMELCs and Matrigel-iBMELCs from 610B1 on day 10. The values are normalized to those of HPRT1. The relative mRNA expression levels of Matrigel-iBMELCs were defined as 1. Data are presented as mean ± SD (n = 3; N.S. = not significant; Student's t-test).

(B) Relative mRNA expression levels of TBXT and KDR in differentiated cells (on day 3 or 6) derived from 610B1. The values are normalized to those of HPRT1. The relative mRNA expression levels of Matrigel-iBMELCs were defined as 1. Data are presented as mean ± SD (n = 3; N.S. = not significant, *$p < 0.05$, **$p < 0.01$; Student's t-test).

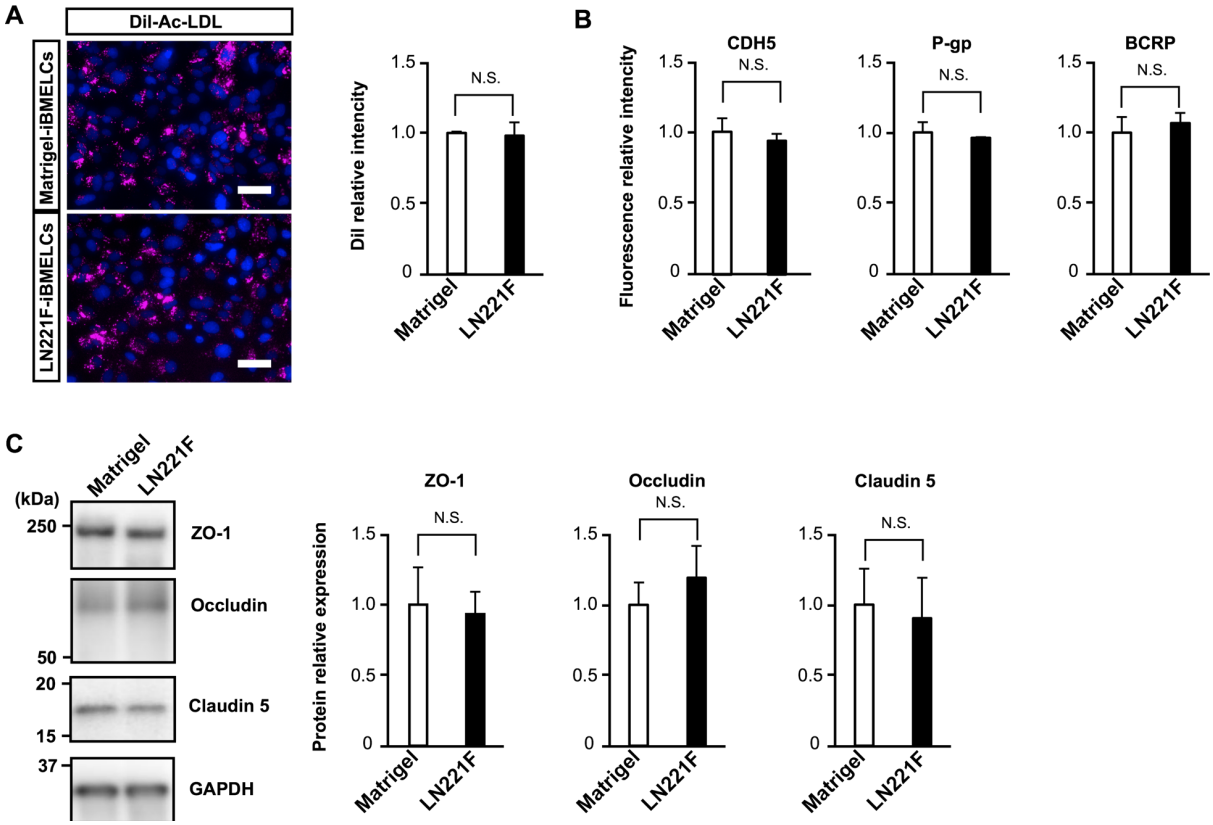

Fig. S4. Quantification of protein expression and uptake of Dil-Ac-LDL

(A) Uptake of Dil-Ac-LDL by iBMELCs on day 10 derived from 610B1. Dil-Ac-LDL = pink; Hoechst 33342 = blue. Scale bars = 50 μm. Mean fluorescence intensities of Dil-Ac-LDL on cellular cytosol. The mean fluorescence intensity of Matrigel-iBMELCs was defined as 1. Data are presented as mean ± SD (n = 3, 6 fields/well).

(B) Mean fluorescence intensities of CDH5, P-gp, and BCRP immunostaining of cellular cytosol. The mean fluorescence intensities of Matrigel-iBMELCs were defined as 1. Data are presented as mean ± SD (n = 3, 6 fields/well; N.S. = not significant; Student's t-test).

(C) The protein expression levels of ZO-1, occludin, and claudin 5 in Matrigel- and LN221F-iBMELCs on day 10 derived from 610B1 were subjected to western blot analysis. The protein expression levels were normalized to GAPDH levels. The protein expression levels of Matrigel-iBMELCs were defined as 1. Data are presented as mean ± SD. (n = 3; N.S. = not significant; Student's t-test).