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Supporting Information

One-step immunoassay based on switching peptides for diagnosis of porcine epidemic diarrhea virus (PEDV) using screened F_V-antibodies

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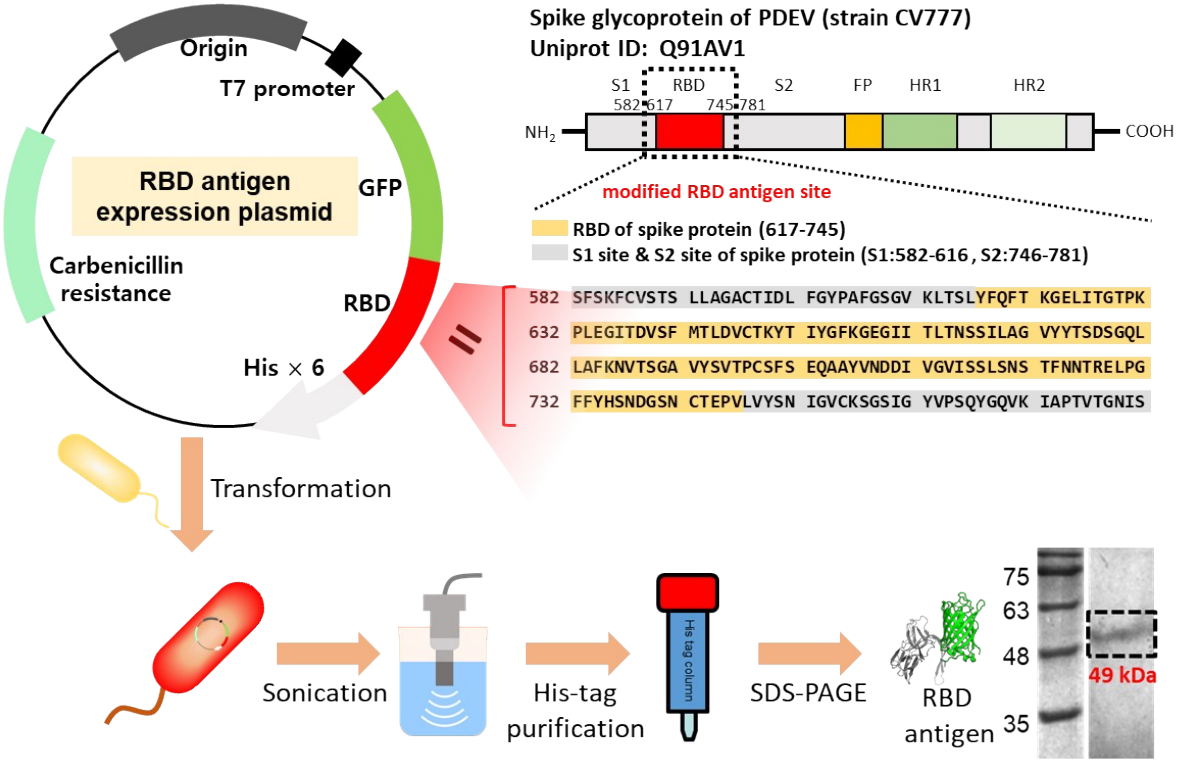

Fig. S1 Expression of RBD antigen from PEDV (strain CV777) S protein for the screening of Fv-antibody library. The RBD consisted of 129 amino acid residues (21.2 kDa), which included a part of the S protein (amino acid number from 582 to 781). SDS-PAGE showed the GFP (26.9 kDa) and His-tag were included in the structure of RBD antigen (49 kDa).

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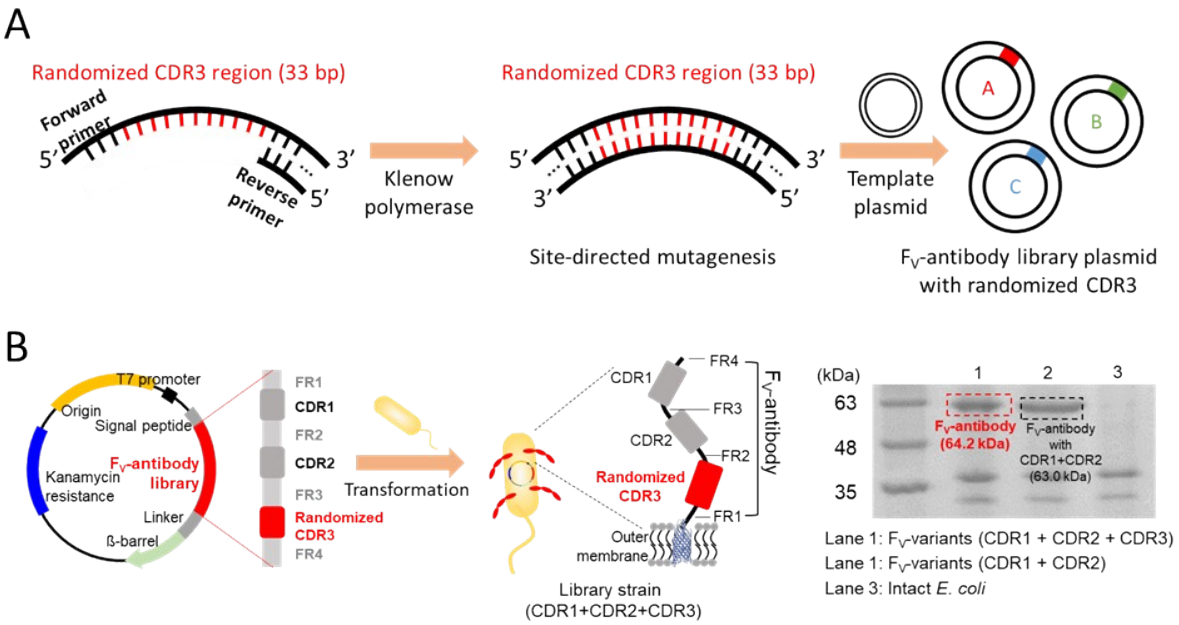

Fig. S2 An autodisplayed F_V-library with a randomized CDR3 region was used to screen for F_V-antibodies against PEDV S protein. (A) Preparation of the F_V-library with a randomized CDR3 region in the V_H using site-directed mutagenesis. (B) Expression of the F_V-library on the outer membrane of *E. coli* using an autodisplay vector (SDS-PAGE of the autodisplayed F_V-variant, control strain with CDR1 and CDR2, and intact *E. coli*).

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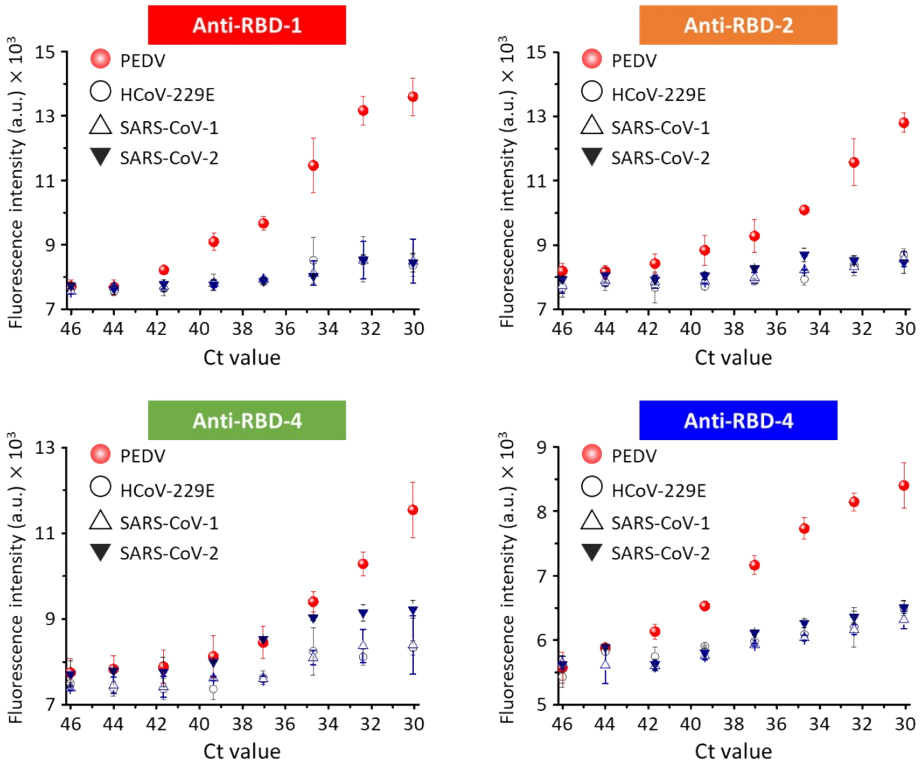

Fig. S3 Specificity test of Fv-antibodies (Anti-RBD-1, Anti-RBD-2, Anti-RBD-3, and Anti-RBD-4) using the positive sample (PEDV) and negative control samples (HCoV-229E, SARS-CoV-1, and SARS-CoV-2) to Fv-antibodies.

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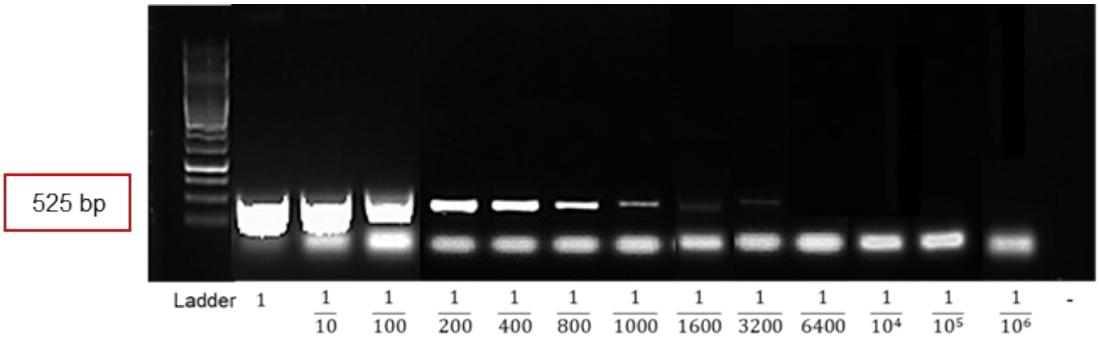

Fig. S4 Quantitative analysis of the RNA from PEDV in supernatant media of Vero cell was extracted using Patho Gene-spin™ DNA/RNA extraction kit from iNtRON Biotechnology (Gyeonggi-do, Korea). The amount of RNA from each diluted PEDV samples were analyzed using PEDV PCR reaction kit from iNtRON Biotechnology (Gyeonggi-do, Korea). The PCR reaction was performed according to the manufacturer's instructions. After PCR reaction. the band intensity was calculated using software (ImageJ).

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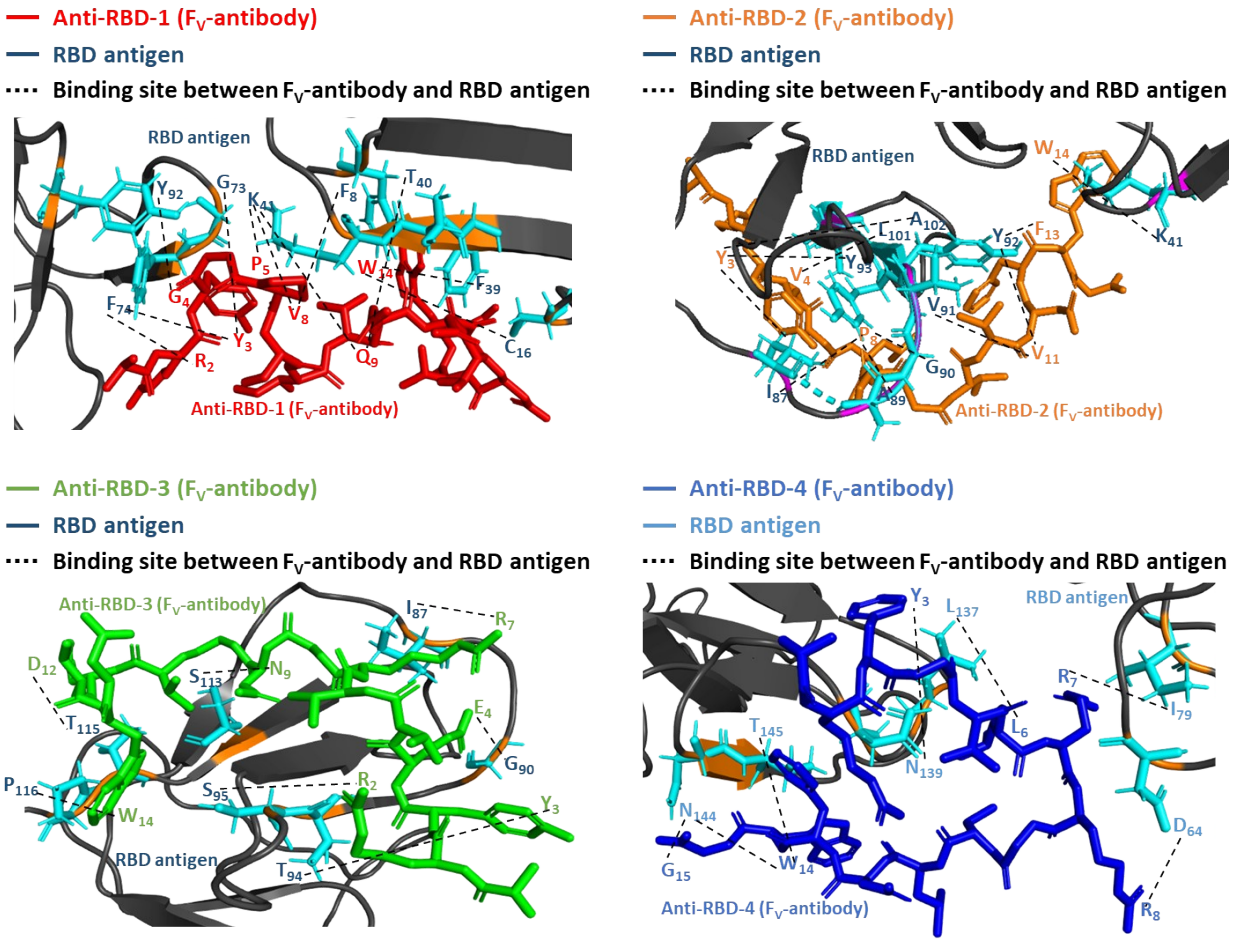

Fig. S5 Analysis of interaction between screened F_V-antibodies (Anti-RBD-1, Anti-RBD-2, Anti-RBD-3, Anti-RBD-4) and RBD antigen from PEDV S protein (Uniprot ID: Q91AV1) using molecular docking software.

Table S1. Primer sequences of randomized CDR3 region of the F_V-antibody library. Randomized forward primer (75 bases) and corresponding reverse primer (22 bases) and composition of nucleotides at each position were listed in the table.

Primer type	Oligonucleotide sequence
Randomized forward primer (75 bases)	5'-GTCTATTATTGCGCTCGT **^{1}KRYVNN ^{7}VNNVNN ^{13}VNNVNN ^{19}VNNVNN ^{25}VNNGAT ^{31}KWY**TGGGGTCAAGGTACTACGGTTACG-3'
Corresponding reverse primer (22 bases)	3'-CCCAGTTCCATGATGCCAATGC-5'
Composition of nucleotides at each position	**N** = A, C, G, T / **R** = A, G / **K** = G, T / **Y** = C, T / **W** = A, T / **V** = A, C, G

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Table S2. Amino acid and corresponding oligonucleotide sequences of F_V-antibody. The CDR3 sequence was randomized for the preparation of F_V-antibody library from the template using site-directed mutagenesis.

Region	Amino acid (N → C-term)	Oligonucleotide sequence (5' → 3')
Frame-1	EVQLVESAAEVRRPGASVKITCKASGYSFS	GAA GTG CAG CTC GTG GAA AGC GCT GCC GAA GTT CGG CGT CCT GGG GCT AGC GTG AAG ATC ACC TGC AAA GCG TCC GGC TAT TCA TTC AGC
CDR1	TYGIQ	ACC TAT GGG ATT CAG
Frame-2	WMRQAPGQRPEWLG	TGG ATG CGC CAA GCG CCA GGC CAG CGT CCG GAA TGG CTT GGG
CDR2	WIHAGTGGTKYSRKFQG	TGG ATA CAT GCA GGC ACA GGT GGG ACT AAG TAC TCG CGC AAA TTT CAG GGT
Frame-3	RITITRDTSANTVYLDLNSLTSEDTAVYYCAR	CGC ATT ACT ATC ACC CGT GAT ACC AGC GCG AAT ACC GTC TAT CTG GAT CTG AAC TCT CTG ACA TCG GAG GAT ACG GCC GTC TAT TAT TGC GCT CGT
CDR3 (Template)	DKVTVWACQDN	GAC AAA GTT ACA GTC TGG GCT TGT CAG GAT AAT
Frame-4	WGQGTTVTVSS	TGG GGT CAA GGT ACT ACG GTT ACG GTC AGC AGT

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Table S3. Analytic parameters of L1-switching peptide. The peptide was synthesized from Peptron Co. (Daejeon, Korea) using solid-phase Fmoc chemistry under consecutive flow conditions. The synthesized peptides were purified using reverse-phase HPLC (High-Performance Liquid Chromatography) and lyophilized.

Switching peptide	Amino acid sequence	Molecular weight (g/mol)	Purity
L1-peptide	TYLEW^{5} YPQKP^{10} GQSPK^{15} LLIYK^{20}	2,452	90 %