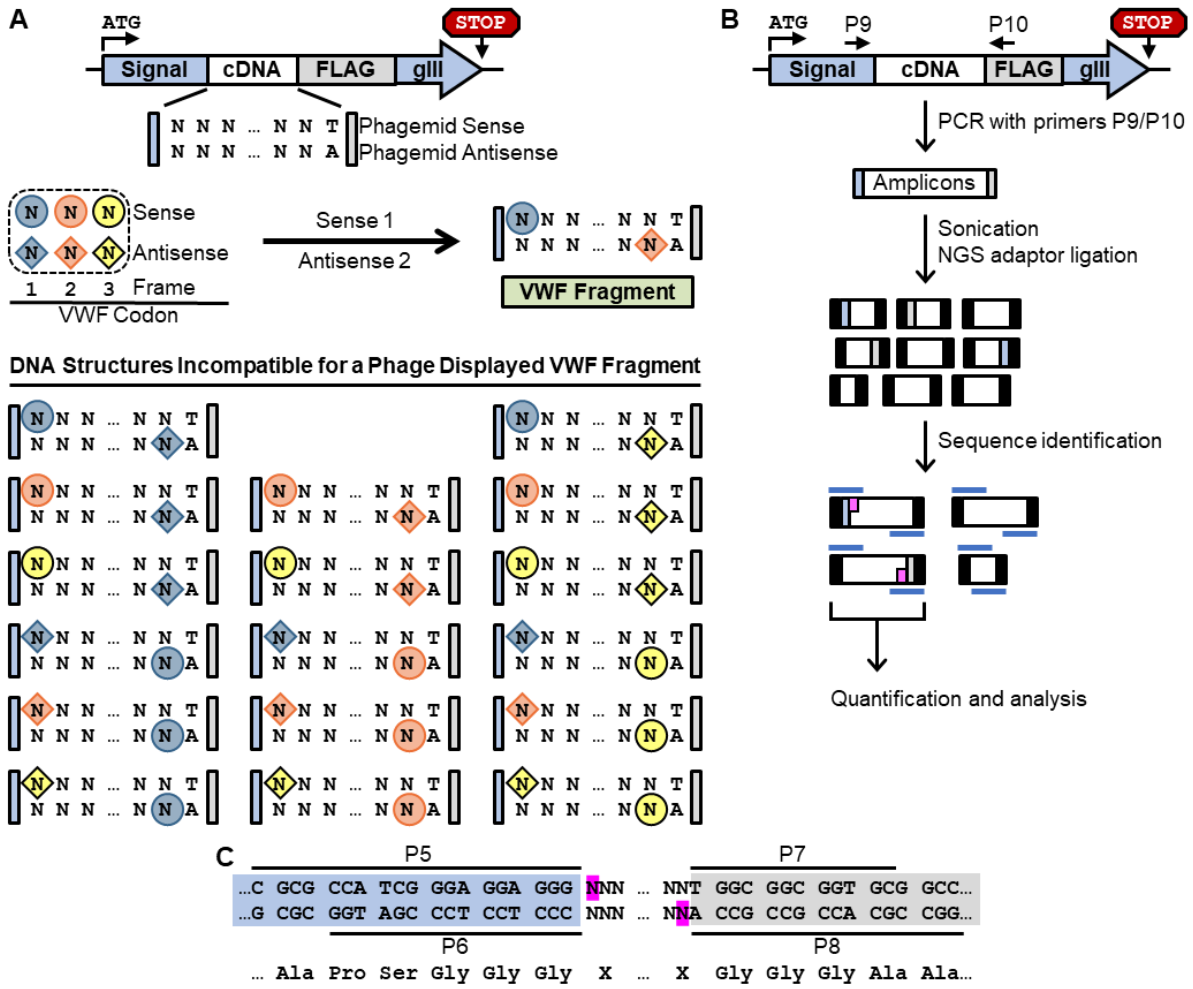

Figure S1. Schematic for expressing a VWF fragment fused to pIII and generating NGS-suitable DNA from the phage display library. A) Random fragments of the VWF plasmid (cDNA) were ligated between phagemid adaptors (P5/P6 and P7/P8) and cloned into the phagemid. Adaptor-adaptor ligation products (no intervening VWF cDNA) were mostly removed by gel electrophoresis and would introduce a frameshift between Signal and FLAG if cloned into the phagemid to limit the fraction of the library that would not contain or express VWF. All possible combinations of orientation and frame are shown, but only the Sense 1/Antisense 2 combination can generate a displayed VWF fragment. This combination has an orientation in which the sense strand of the cloned VWF cDNA is in line with the sense strand of the phagemid and a frame that is bounded by the 1st and 2nd nucleotide of a VWF codon on the 5' and 3' ends, respectively. B) For NGS, PCR amplicons (~500bp-~1,200bp) generated with primers P9 and P10 risked inefficient and biased clustering onto the sequencing platform and were thus sonicated to ~100bp – ~600bp prior to ligation of Illumina-compatible, barcoded adaptors (NGS adaptor, black boxes). Paired end sequencing (blue bars, 75 or 100 bases) provided information on all adaptor-amplicon junctions. Only VWF fragment sequence (magenta) immediately adjacent to the vector sequence were statistically analyzed. C) Magnification of quantifiable and analyzable sequences as depicted in (B). With the phagemid adaptors used for cloning (P5/P6 and P7/P8), display of a VWF fragment requires frames 1 and 2 of a VWF codon for the N- and C-terminal residues, respectively. Our NGS analysis identifies and quantifies the nucleotides highlighted in magenta. Thus, Sense, Frame 1 and Antisense, Frame 2 VWF nucleotides generate the appropriate architecture to express and display a VWF peptide. For reference, translation of this region in the phagemid relative to the initiating Met is shown below.

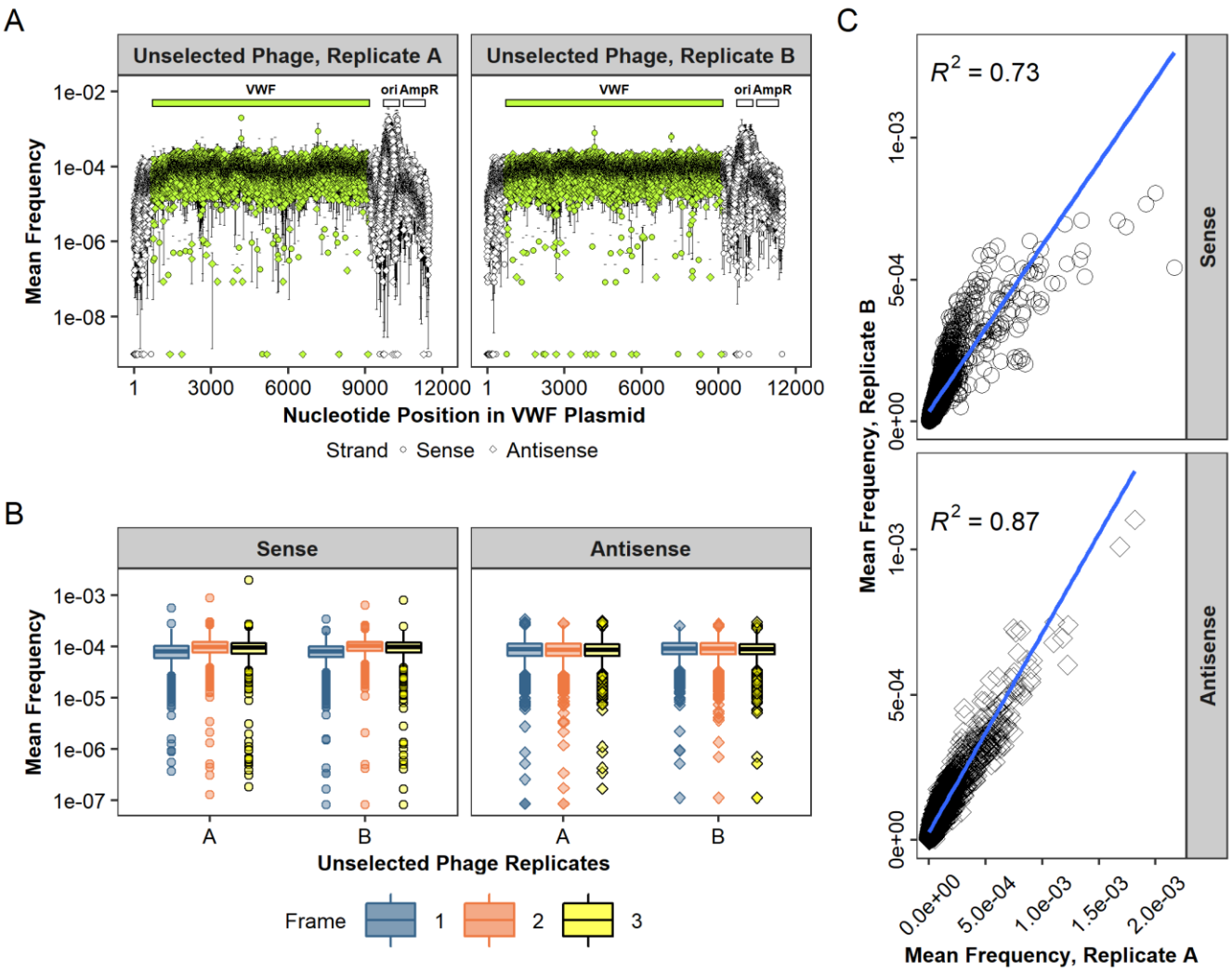

Figure S2. Packaging of VWF cDNA into phage virions is reproducible. A) The frequency (mean ± 1 standard deviation) of the nucleotides immediately adjacent to the phagemid adaptors (see Methods and Figure S1) was determined for the unselected phage (n = 3 NGS preparations for replicate A, n = 4 NGS preparations for replicate B) and mapped according to their position within the parental VWF plasmid. Each replicate of unselected phage was prepared from an aliquot of the expanded library stored at -80°C. Nucleotides with no reads are shown as a frequency of $1x10^{-9}$. Error bars represent 1 standard deviation. B) Boxplots of VWF nucleotides separated according to their strand (sense or antisense) and position within a VWF codon (Frame) show wide and differing distributions of frequencies but comparable medians. Nucleotides with no reads are not shown. The median and interquartile ranges (IQR) for the nucleotide frequencies are provided in Table S1. C) The frequencies of each fragment terminus were well correlated between the unselected phage of the different selection experiments (Sense, Pearson R = 0.86; Antisense, Pearson R = 0.93).

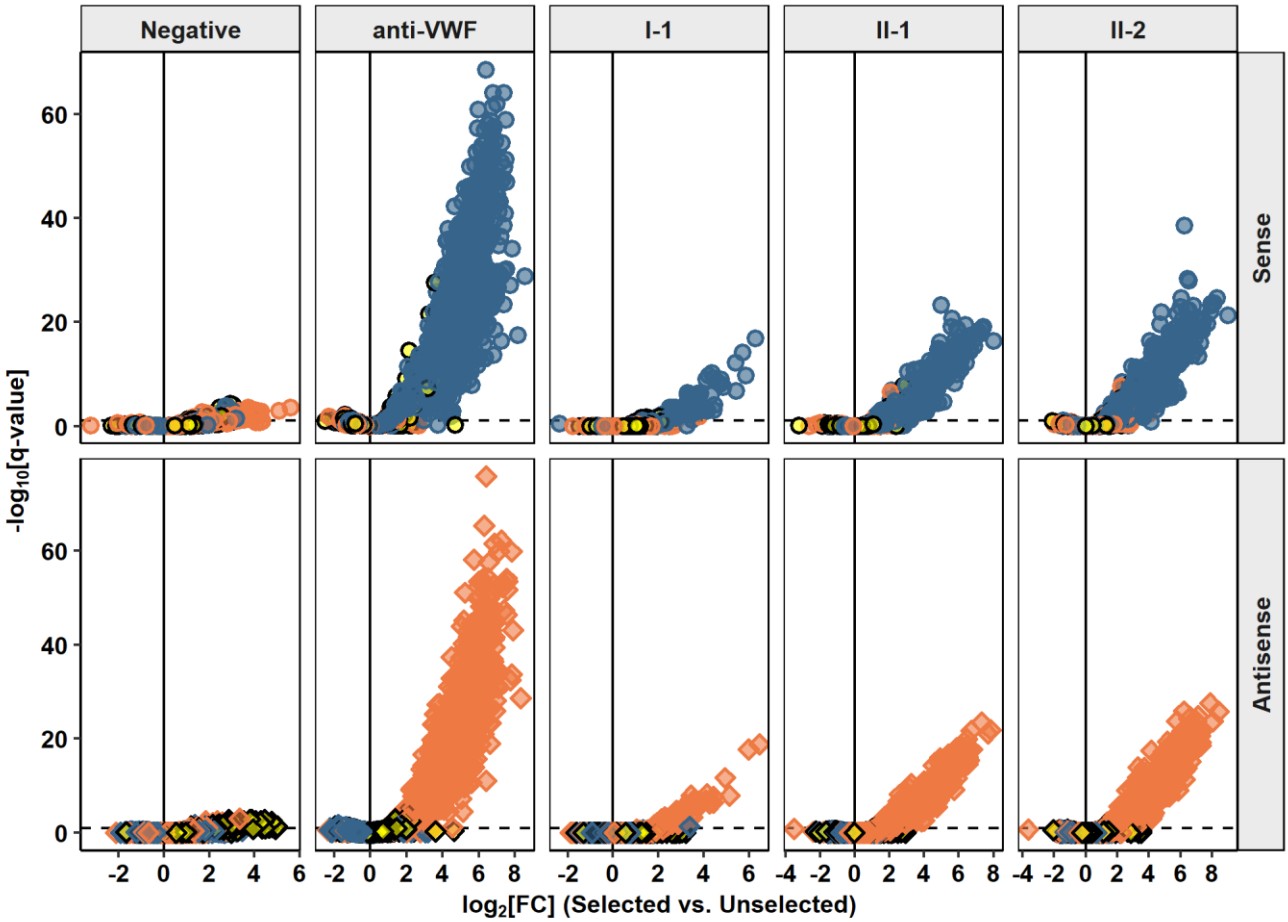

Figure S3. Anti-VWF antibodies significantly enriched phage clones with VWF nucleotides in the orientation and frame needed to express and display a VWF protein fragment, demonstrating that our NGS analysis is appropriate for our cloning strategy. Sequences contiguous with the phagemid were identified and quantified for unselected phage and for phage selected in the absence (Negative) or presence of the commercial antibody (anti-VWF) or patient alloantibodies (I-1, II-1, and II-2). Orientation (sense, circle and antisense, diamond) and frame (1, blue; 2, orange; or 3, yellow) are referenced to the position within the VWF coding sequence as described in Figure S1. Each marker indicates the VWF nucleotide adjoining the phagemid adaptor sequence as described in Figure S1. Phage displayed VWF peptides are represented by Sense, Frame 1 (blue circles) and Antisense, Frame 2 (orange diamonds) nucleotides (see Figure S1). Log transformed (base 2) fold-changes (FC, selected vs. unselected) and log transformed (base 10) q-values (p-values adjusted for multiple comparison) were calculated in DESeq2[1]. The dashed line marks the significance threshold (FDR < 0.1). We note a slight enrichment of non-VWF coding nucleotides in the absence of antibodies (Negative: Sense, Frame 2 and Antisense, Frame 3) may reflect background binding of a non-VWF peptide to protein G beads.

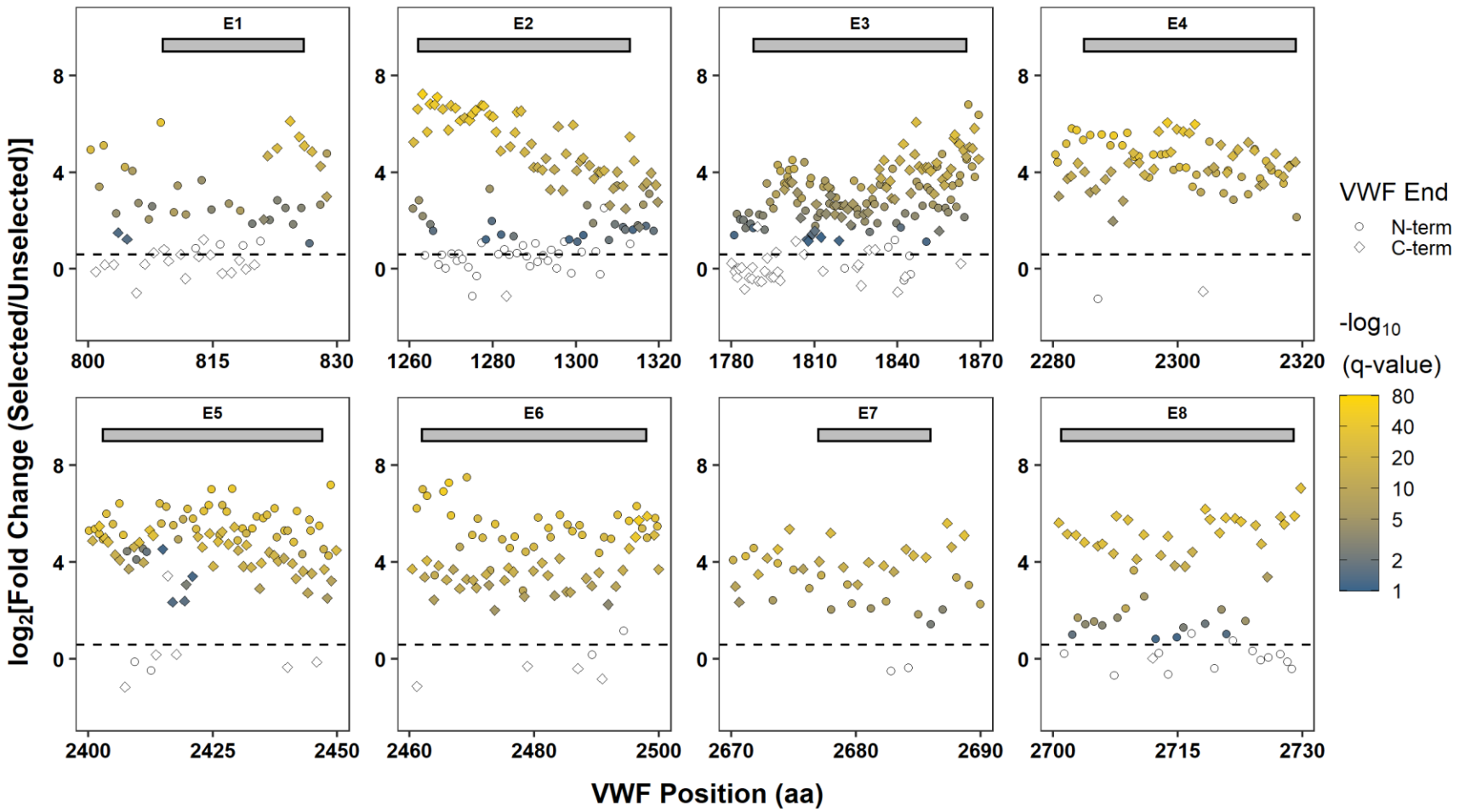

Figure S4. Magnification of anti-VWF in Figure 1 for the eight, previously reported immunoreactive regions, E1-E8[2]. The black dashed line denotes a fold change of 1.5. Terminal residues of phage displayed VWF fragments are marked (VWF End) according to their location and enrichment. Only VWF fragment termini with a significant fold change (FDR adjusted p-value < 0.1) are indicated in shading from blue to yellow, which scales with the significance of fold change ($-\log_{10}$(q-value)).

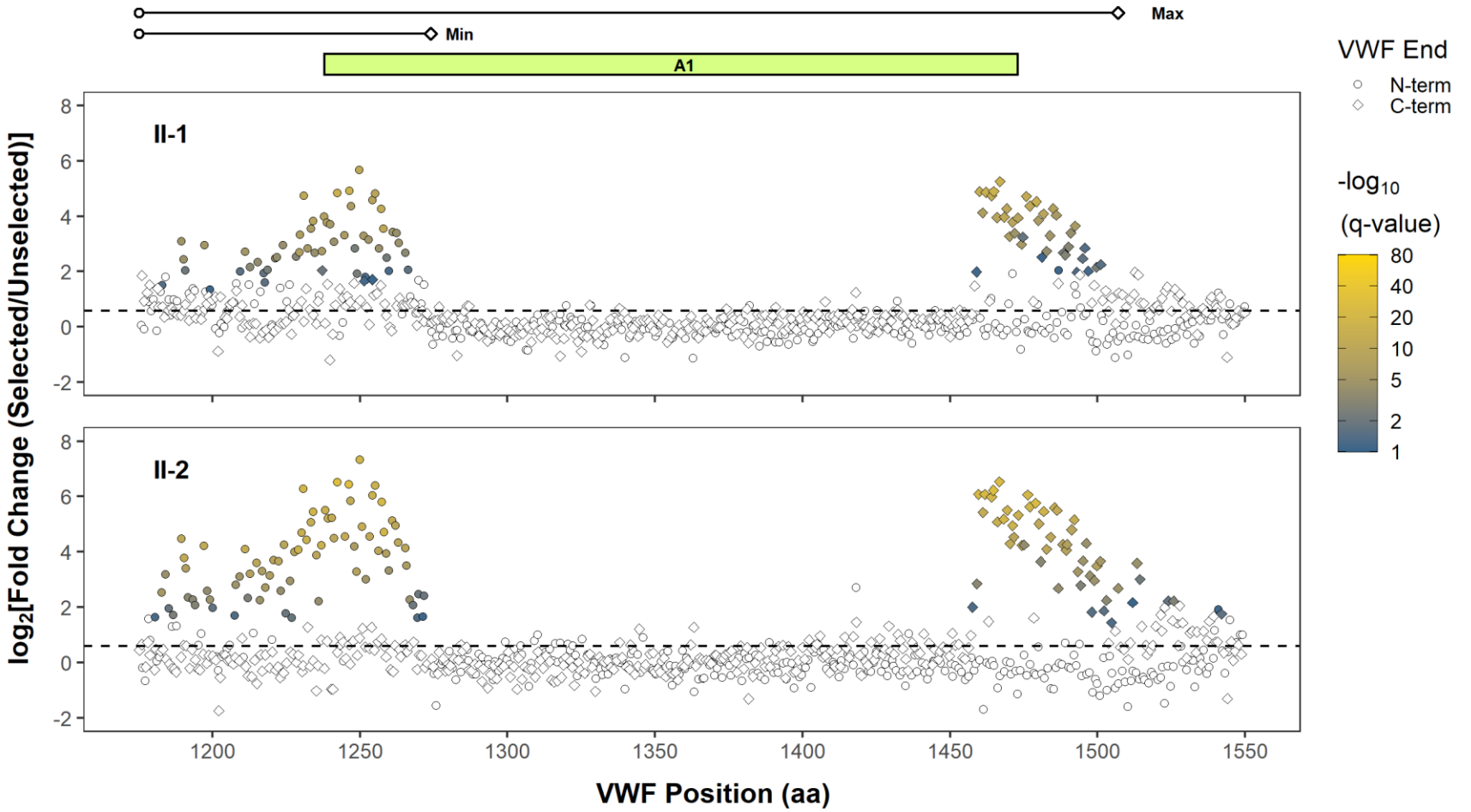

Figure S5. Magnification of Figure 1 around the VWF A1 domain for patients II-1 and II-2. As in Figure 1, the minimal (~100 amino acids, Min) and maximal (~333 amino acids, Max) are shown to scale of the x-axis. The boundaries of the VWF A1 domain are annotated in light green. The black dashed line denotes a fold change of 1.5. Terminal residues of phage displayed VWF fragments are marked (VWF End) according to their location and enrichment. Only VWF fragment termini with a significant fold change (FDR adjusted p-value < 0.1) are indicated in shading from blue to yellow, which scales with the significance of fold change ($-\log_{10}$(q-value)).

Table S1. Median and IQR values for boxplots in Figure S2B.

Strand	Replicate	Frame	Median Frequency (IQR)
Sense	A	1	$7.91x10^{-5}$ ($5.98x10^{-5}$ – $1.02x10^{-4}$)
Sense	A	2	$9.84x10^{-5}$ ($7.71x10^{-5}$ – $1.23x10^{-4}$)
Sense	A	3	$9.45x10^{-5}$ ($7.19x10^{-5}$ – $1.17x10^{-4}$)
Sense	B	1	$8.03x10^{-5}$ ($6.22x10^{-5}$ – $9.93x10^{-5}$)
Sense	B	2	$1.03x10^{-4}$ ($8.24x10^{-5}$ – $1.23x10^{-4}$)
Sense	B	3	$9.82x10^{-5}$ ($7.64x10^{-5}$ – $1.19x10^{-4}$)
Antisense	A	1	$8.90x10^{-5}$ ($6.57x10^{-5}$ – $1.15x10^{-4}$)
Antisense	A	2	$8.73x10^{-5}$ ($6.54x10^{-5}$ – $1.15x10^{-4}$)
Antisense	A	3	$8.64x10^{-5}$ ($6.60x10^{-5}$ – $1.11x10^{-4}$)
Antisense	B	1	$9.14x10^{-5}$ ($7.13x10^{-5}$ – $1.17x10^{-4}$)
Antisense	B	2	$9.04x10^{-5}$ ($7.04x10^{-5}$ – $1.15x10^{-4}$)
Antisense	B	3	$8.91x10^{-5}$ ($6.98x10^{-5}$ – $1.11x10^{-4}$)

Supporting Information References

1 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014; **15**: 550. s13059-014-0550-8 [pii]
10.1186/s13059-014-0550-8.
2 Tan FL, Ginsburg D. What a polyclonal antibody sees in von Willebrand factor. *Thromb Res*. 2008; **121**: 519-26. S0049-3848(07)00229-0 [pii]
10.1016/j.thromres.2007.05.015.