

SUPPLEMENTAL MATERIAL

A synthetic protein as efficient multitarget regulator against complement over-activation

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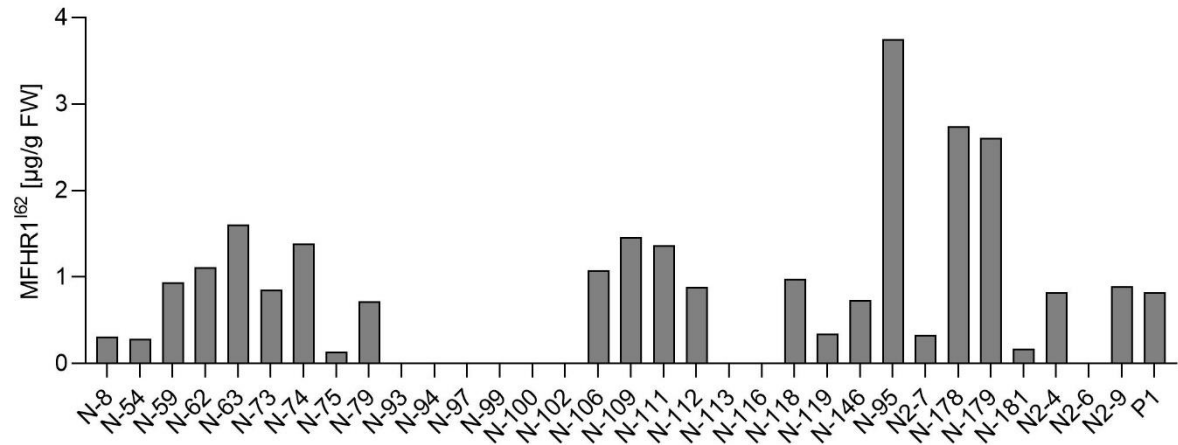
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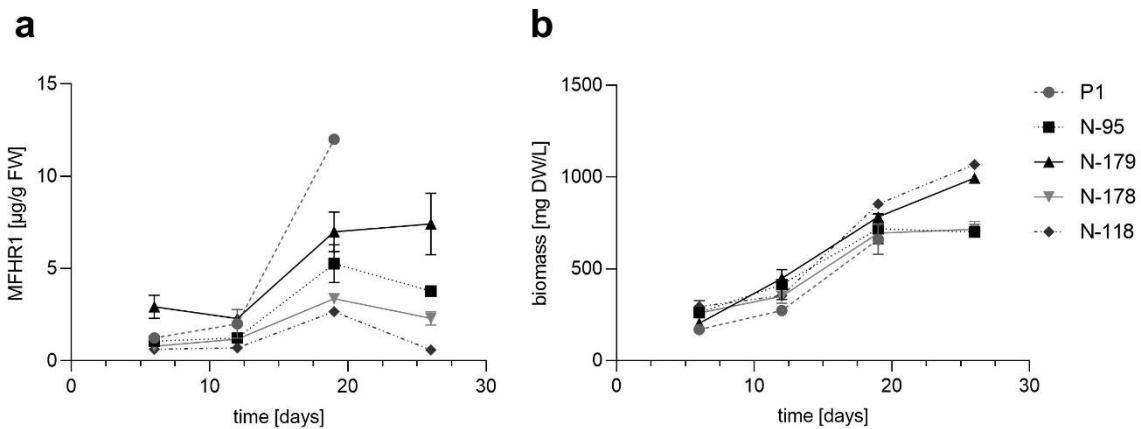
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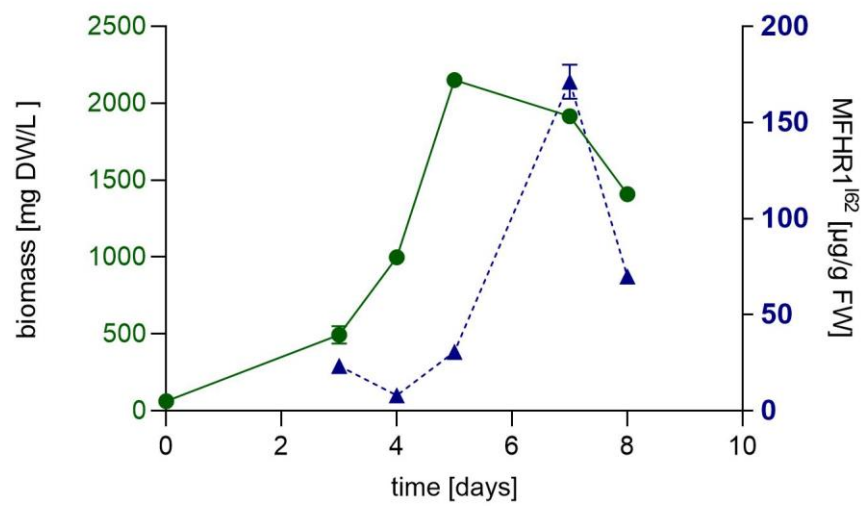
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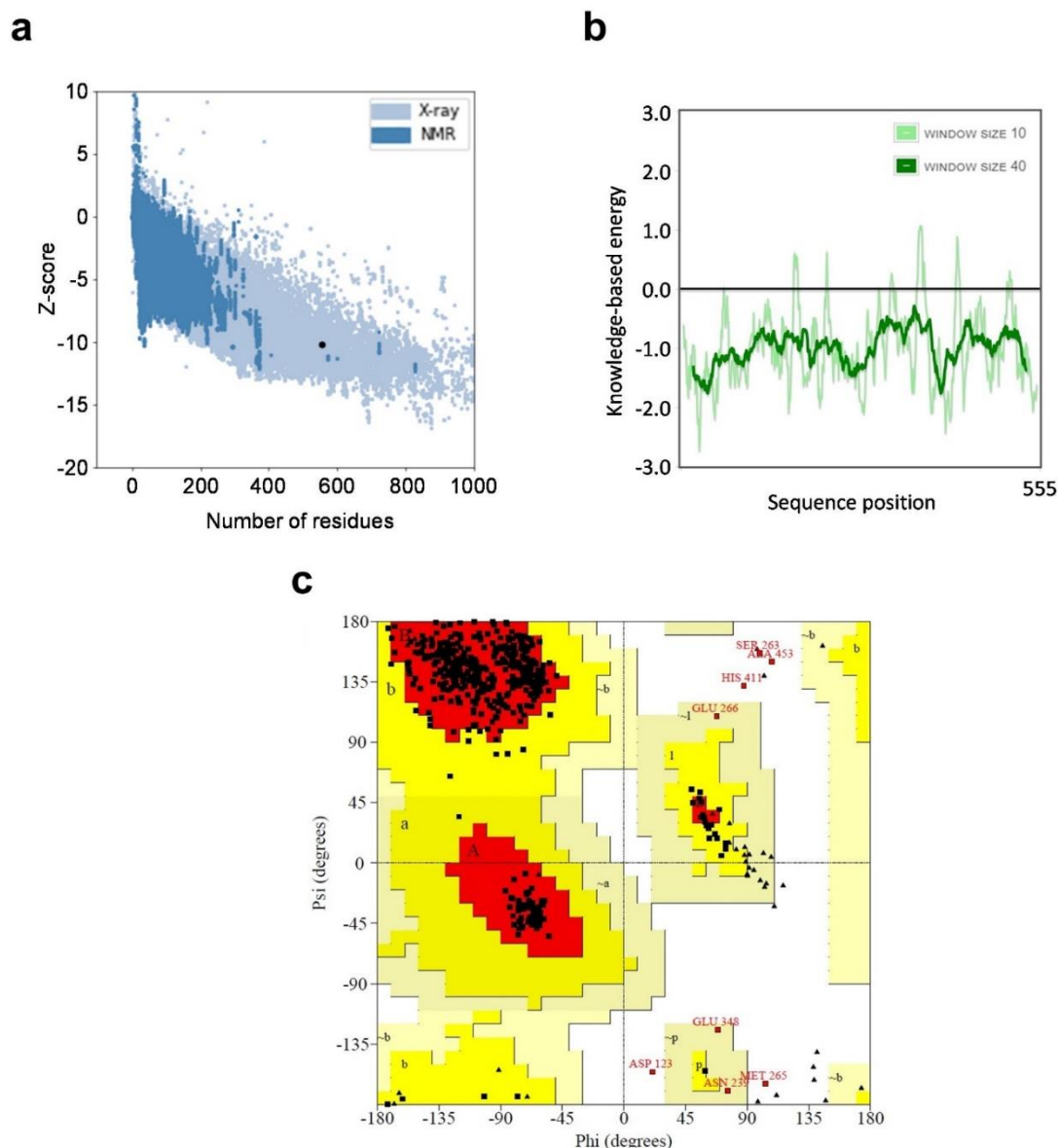
Supplementary Fig. 1: MFHR1^{I62} productivity. Plants surviving the selection were screened for productivity in suspension cultures in agitated flasks via ELISA. P1 is the MFHR1^{V62} moss producer line, which was included as an internal positive control



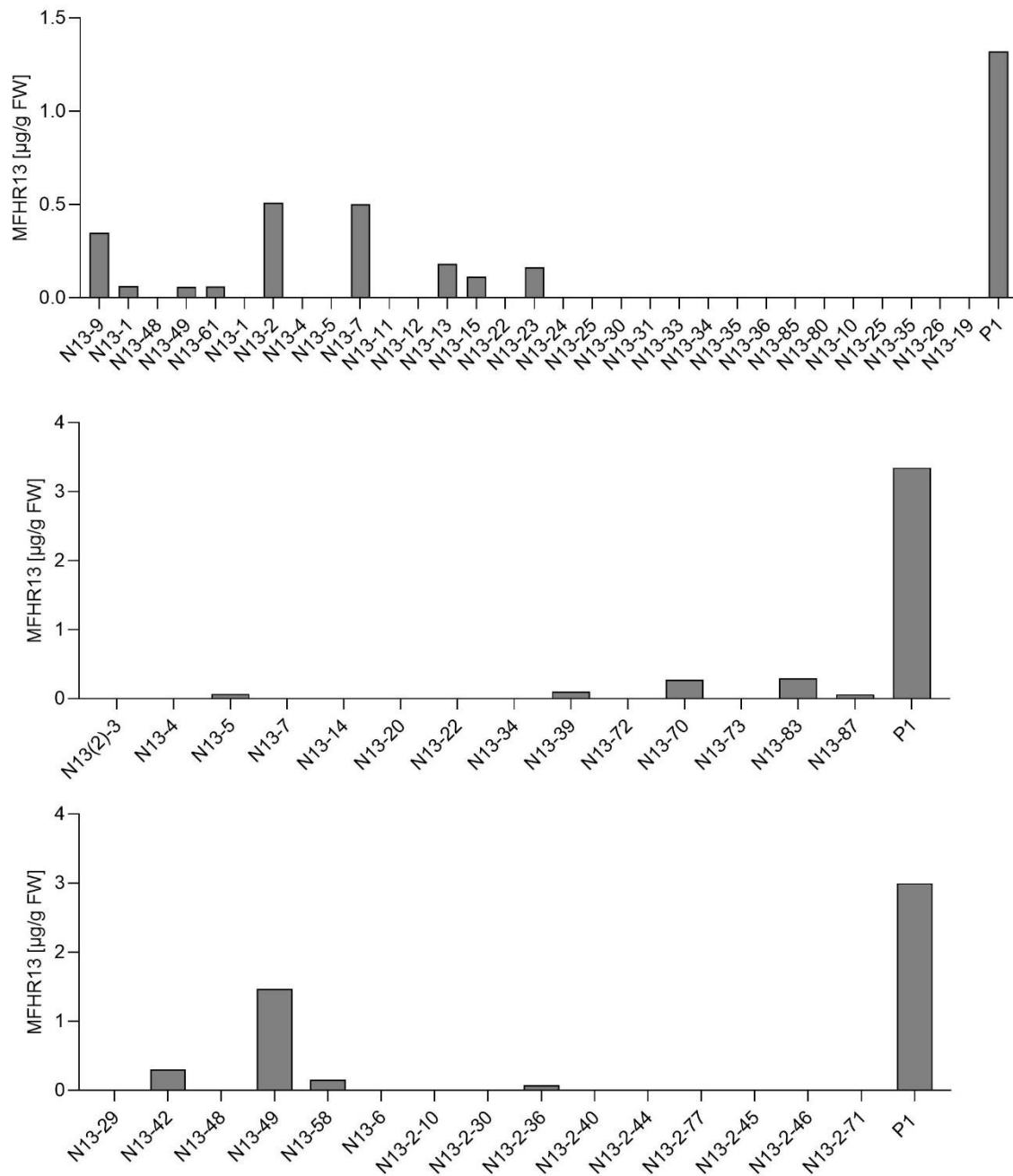
Supplementary Fig. 2: Kinetics of a) MFHR1^{I62} specific productivity and b) biomass accumulation of 4 of the best lines tested in agitated flasks.



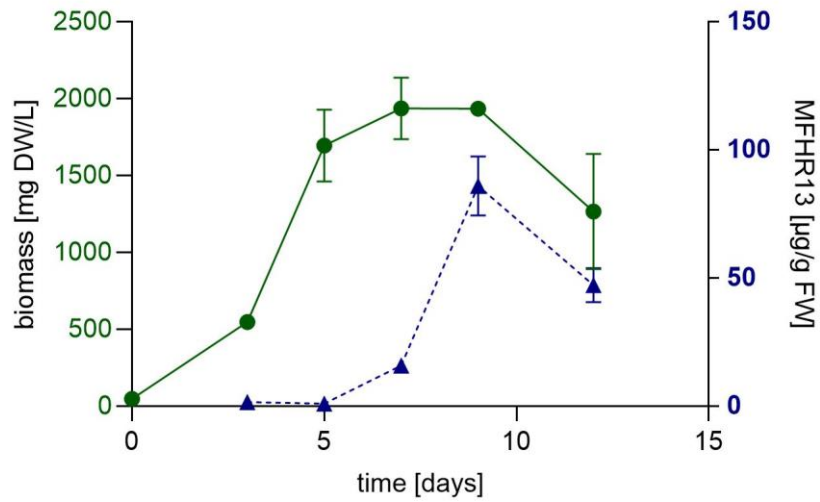
Supplementary Fig. 3: Kinetics of biomass accumulation and MFHR1¹⁶² levels of plant N-179 in a 5 L stirred bioreactor.



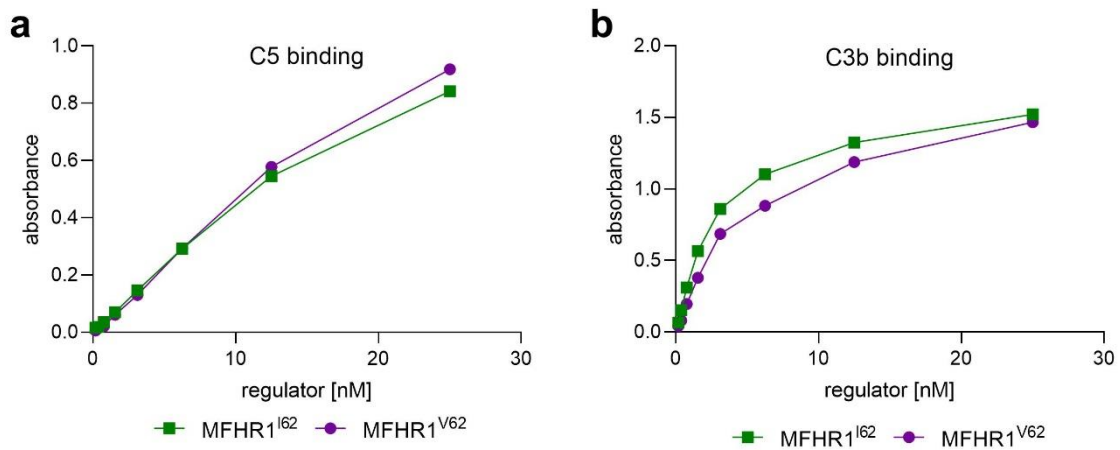
Supplementary Fig. 4: Assessment of the overall and local quality of the MFHR13 structure model. (a) Overall model quality of MFHR13 obtained by ProsaWEB. Z-score: -10.07. (b) Local model quality. Diagram of energy as a function of residues sequence position. Average energy over 40 or 10 amino acid fragments was calculated by ProsaWEB. (c) Ramachandran plot analysis by PROCHECK web-based tool of the model MFHR13. The most favored regions are colored red (labeled A, B, L), allowed regions are colored dark yellow (labeled a, b, l, p), and generously allowed regions are colored in shades of light yellow (labeled ~a, ~b, ~l, ~p), while amino acids in disallowed regions are indicated as red squares. The analysis revealed that 97.6, 1.3 and 1.1% of the residues are located in favored, allowed and outlier regions, respectively.



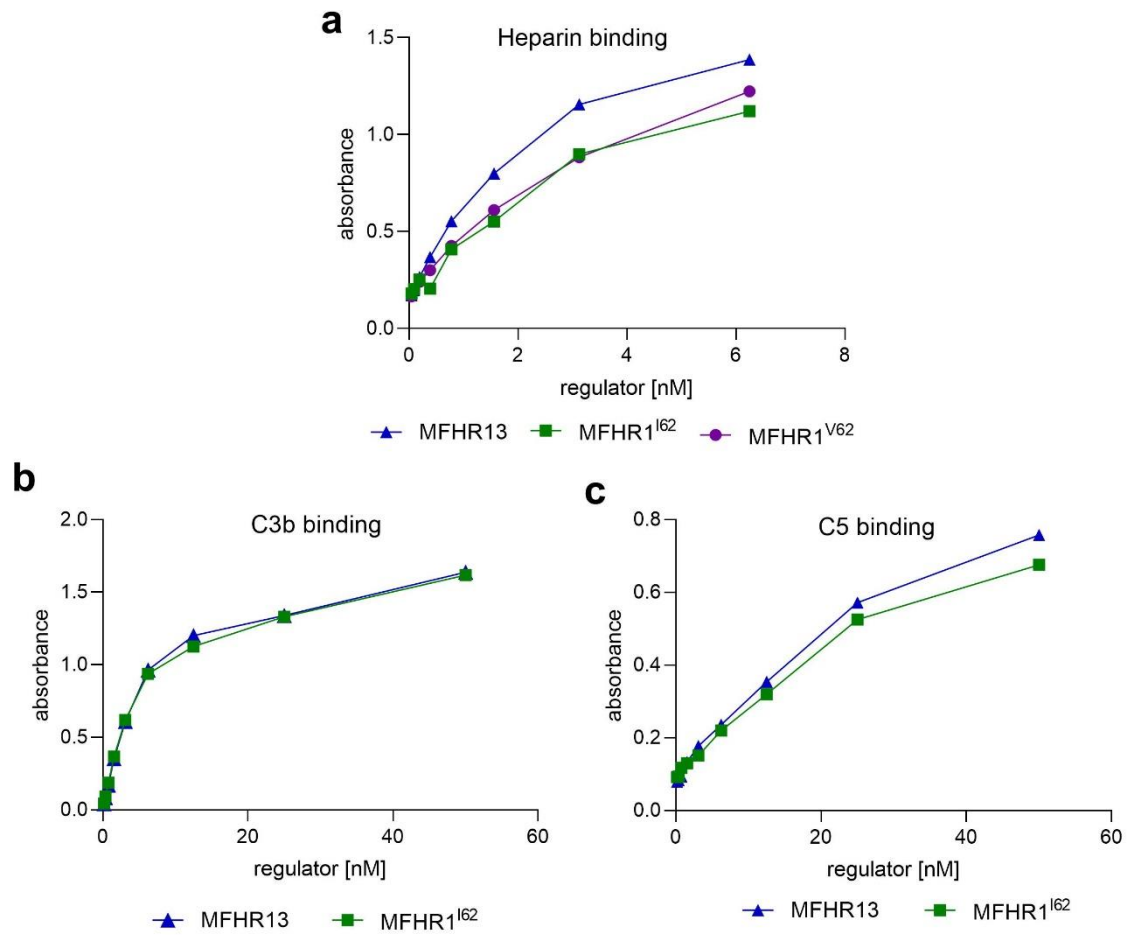
Supplementary Fig. 5: MFHR13 productivity. All plants surviving the selection were screened for productivity via ELISA in suspension cultures in agitated flasks. P1 is the MFHR1^{V62} moss producer line, which was included as an internal positive control.



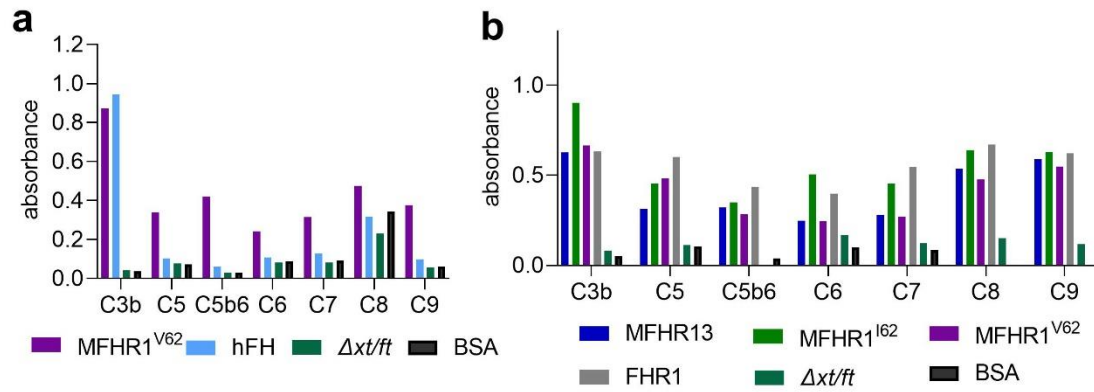
Supplementary Fig. 6: Kinetics of biomass accumulation and MFHR13 specific productivity of moss cell line N13-49 in a 5 L stirred bioreactor.



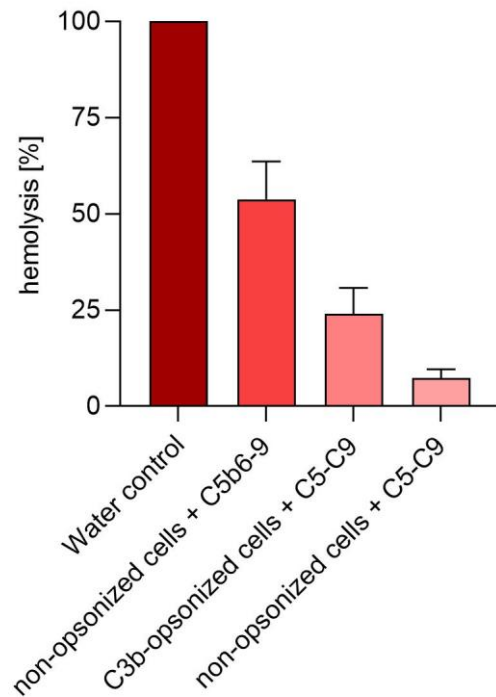
Supplementary Fig. 7: Evaluation of C5 and C3b binding to MFHR1^{I62} and MFHR1^{V62}. One representative experiment for the results presented in (a) Figure 2a and (b) 2b is shown with absolute magnitudes for the absorbance.



Supplementary Fig. 8: MFHR13 binds to heparin, C3b and C5. Binding was analyzed by ELISA and data from one representative experiment presented in (a) Figure 5a (b) Figure 6a (c) Figure 7a (c) is shown with absolute magnitudes for the absorbance.



Supplementary Fig. 9: MFHR13 binds to the proteins of the terminal complement pathway. Binding was analyzed by ELISA and complement components were immobilized. Data from one representative experiment presented in Figure 7b and 7c are shown with absolute magnitudes for the absorbance. (a) MFHR1 binds to the TCC proteins but hFH does not. (b) MFHR13, MFHR1 and FHR1 bind to the TCC proteins.



Supplementary Fig. 10: Comparison of hemolysis achieved by convertase-dependent and convertase-independent activation of C5. The extent of hemolysis achieved after activating C5 in the absence of convertases by using C3b-opsonized sheep erythrocytes exposed to C5-C9 mixture (equivalent to 20% normal human serum) is weaker than the C5b6-mediated hemolysis in non-opsonized erythrocytes. Additionally, a negative control of non-opsonized cells exposed to C5-C9 is included. Cells in water were set to 100%. Data represent mean values $\pm$ SD from three independent experiments.

Fig. 4a SDS-PAGE

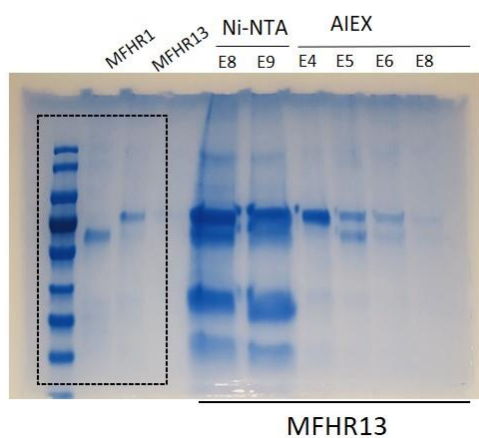
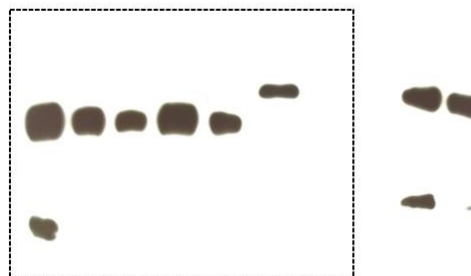


Fig. 4b Western blot



Supplementary Fig. 11: Uncropped scans shown in Fig. 4a, b.

Supplementary Table 1: Primers used to create the expression constructs pAct5-MFHR1^{I62} and pAct5-MFHR13, by directed mutagenesis and Gibson assembly respectively. Mismatch for codon exchange is shown in red and the overhangs (overlapping regions for Gibson assembly) in lowercase.

MFHR1_I62_fwd	TCTTGGAAATATAATAATGGTATGCAG
MFHR1_I62_rev	GATCTATATCCAGGGCGG
SCR13_overlapSCR4_fwd	gatggcgtccgtgacctcatgtGTGGCAATAGATAAACTTAAGAAG
SCR13_overlapSCR19_rev	tgtcaataggtggagggggccacaTAATTGTATTTGTGCCATTG
SCR4_overlapSCR13_rev	cttcttaagttatctattgccacACATGAAGGCAACGGACGC
SCR19_overlapSCR13_fwd	ctgctcaatggcacaataacaattaTGTGGGCCCCCTCCACC

Supplementary Table 2: Estimated IC₅₀ values for C3b and C5 binding of MFHR1^{I62} and MFHR1^{V62} by fitting the data shown in Figure 2a,b with 4PL nonlinear regression model. CI: Confidence Interval, R²: Goodness of Fit. Comparison of fits was carried out using the extra sum-of-squares F test C3b binding: P= 0.0437, F(DFn, Dfd)= 4,338 (1,40) C5 binding: P= 0.9328, F(DFn, Dfd)= 0.007207 (1,40). DF= degrees of freedom

	MFHR1^{I62}	MFHR1^{V62}
C3b binding		
IC ₅₀ (nM)	4.70	6.32
95% CI	3.82 - 5.80	5.88 - 6.80
R ²	0.99	0.99
C5 binding		
IC ₅₀ (nM)	26.83	26.83
95% CI	18.15 - 56.97	18.15 - 56.97
R ²	0.99	0.99

Supplementary Table 3: Estimated IC₅₀ values for heparin binding of MFHR13 and MFHR1 variants fitting the data shown in Figure 5a with 4PL nonlinear regression model. CI: Confidence Interval, R²: Goodness of Fit. Comparison of fits was carried out using the extra sum-of-squares F test. P< 0.0001, F(DFn, Dfd)= 29.76 (2, 94). MFHR13 vs MFHR1^{I62} P< 0.0001, F(DFn, Dfd) = 23.42 (1, 66). MFHR13 vs MFHR1^{V62} P< 0.0001, F(DFn, Dfd) = 42.53 (1, 67). DF= degrees of freedom,

Heparin binding	MFHR13	MFHR1 ^{I62}	MFHR1 ^{V62}
IC ₅₀ (nM)	0.24	0.50	0.76
95% CI	0.20 - 0.28	0.4 - 0.65	0.58 - 1.17
R ²	0.98	0.98	0.98

Supplementary Table 4: Estimated IC₅₀ values for C3b and C5 binding of MFHR13 and MFHR1^{I62} by fitting the data shown in Figure 6a and 7a with 4PL nonlinear regression model. CI: Confidence Interval, R²: Goodness of Fit. Comparison of fits was carried out using the extra sum-of-squares F test. C3b binding P= 0.8426 F(DFn, Dfd)= 0.0397 (1, 71). C5 binding P= 0.2565 F(DFn, Dfd)= 1.309 (1, 69). DF= degrees of freedom

C3b binding	MFHR13	MFHR1 ^{I62}
IC ₅₀ (nM)	16.05	16.05
95% CI	8.79 - 635.1	8.79 - 635.1
R ²	0.93	0.91
C5 binding		
IC ₅₀ (nM)	52.5	52.5
95% CI	Not calculated	Not calculated
R ²	0.86	0.96

Supplementary Table 5. IC₅₀ calculated by 4PL nonlinear regression model for decay acceleration activity (DAA), fitting the data shown in Figure 6d. CI: Confidence Interval, R²: Goodness of Fit. Comparison of fits was carried out using the extra sum-of-squares F test. MFHR13 vs MFHR^{I62} P<0.0001 DFn, DFd = 196.6 (1, 28). MFHR13 vs MFHR^{V62} P<0.0001 DFn, DFd = 131.5 (1, 31). MFHR13 vs hFH P=0.2022 F (DFn, DFd) 1.7 (1, 30). DF= degrees of freedom

	MFHR13	MFHR1 ^{I62}	MFHR1 ^{V62}	hFH
IC ₅₀ (nM)	2.70	7.8	9.45	3.84
95% CI	2.2 - 3.3	6.2 - 9.99	7.7 - 11.7	2.95 - 5.07
R ²	0.99	0.96	0.95	0.96