SUPPLEMENTAL MATERIAL

Kallikrein augments the anticoagulant function of the protein C system in thrombin generation.

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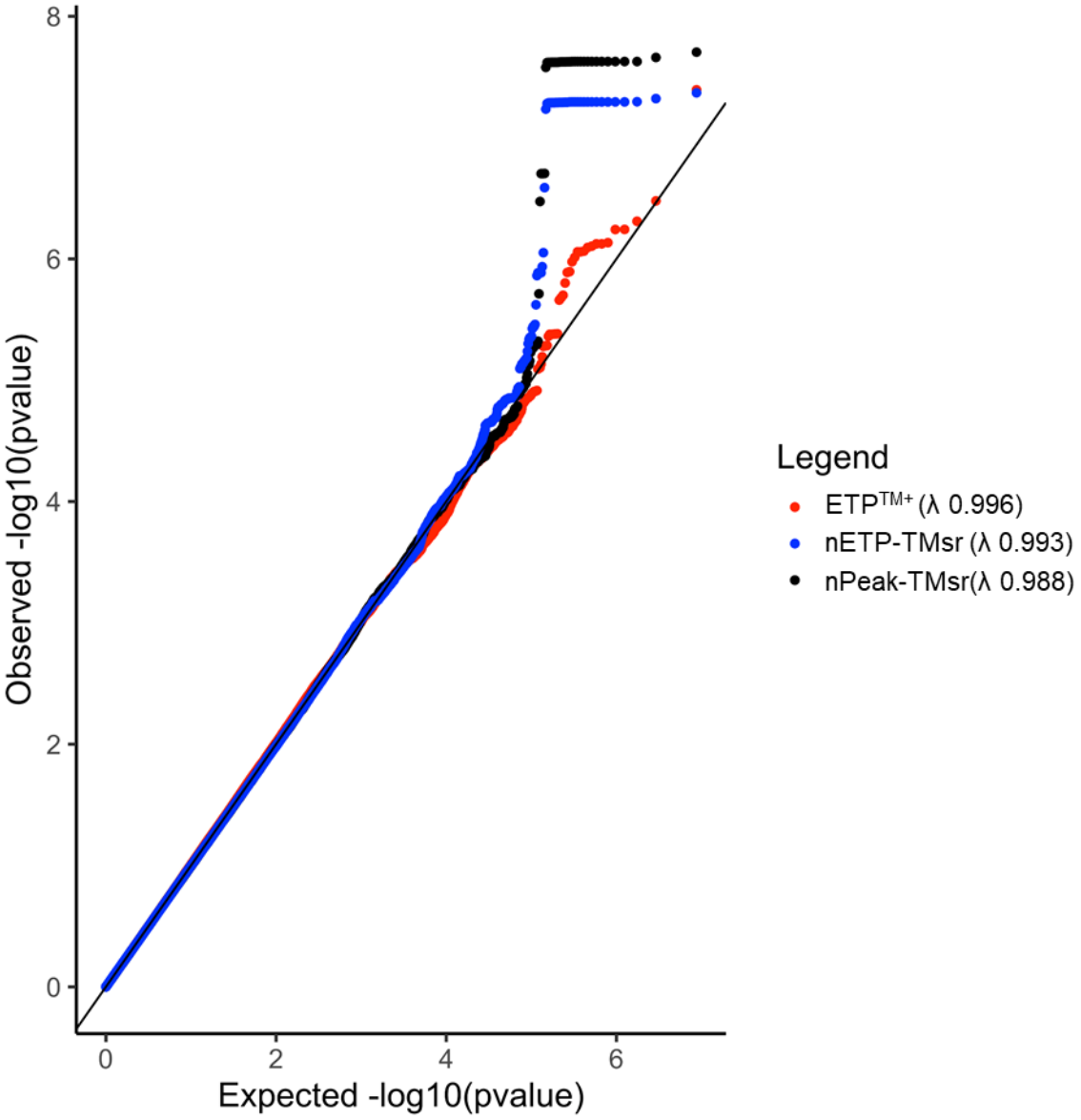
Supplemental Table I. Suggestive significant loci ($5 \times 10^{-6} > p\text{-value} > 5 \times 10^{-8}$) for thrombin generation traits.

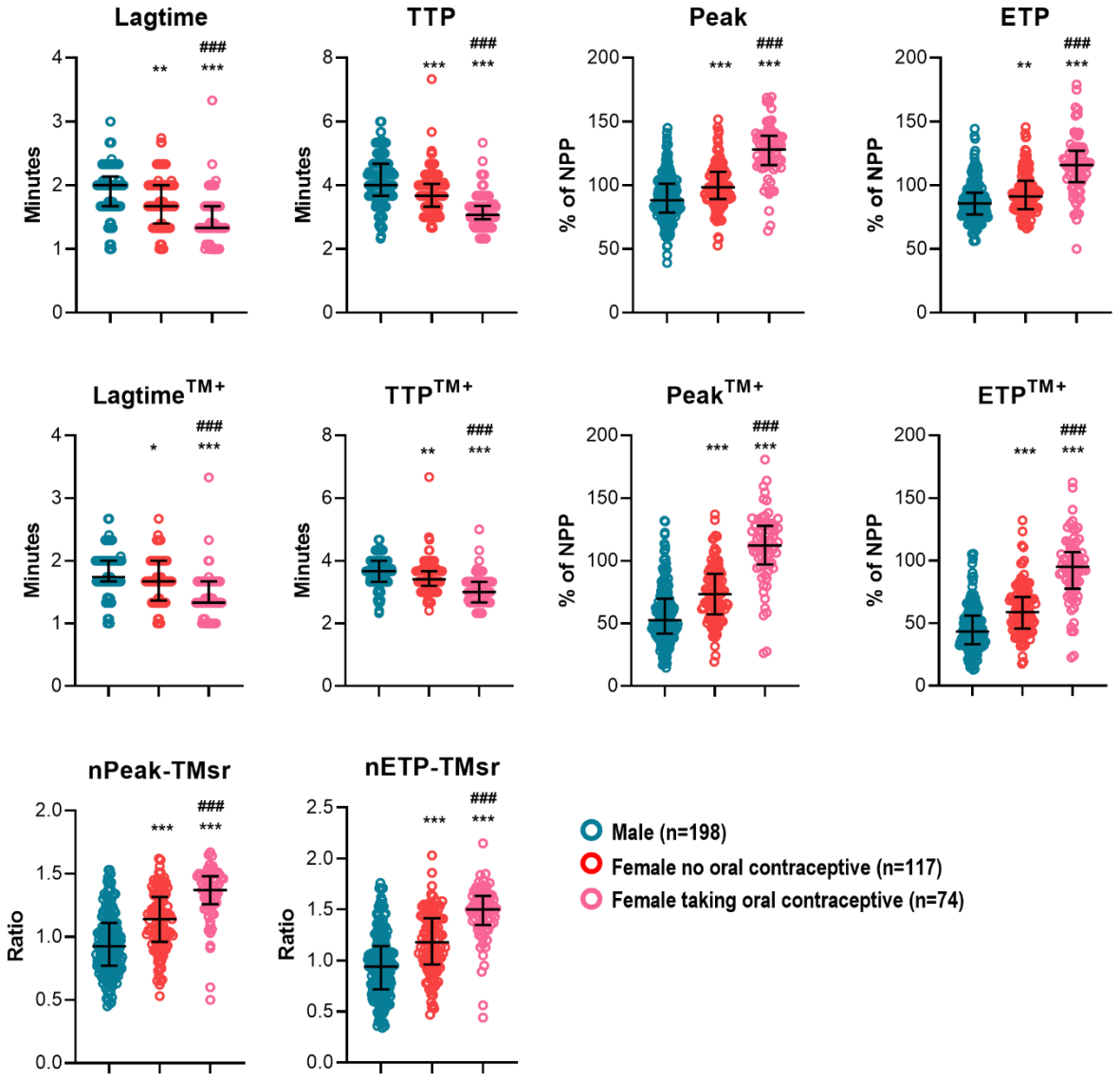
SNPs	Trait	Chr	Position (bp)	Effect /Alternative allele	MAF	P-value	Beta	Gene(s)
rs11616264	Lagtime	13	20898330	T/A	0.174	2.59×10^{-7}	0.55	*CRYL1*[*], *GJB6*[†]
rs1833710	Peak	5	147967746	A/G	0.11	1.88×10^{-7}	0.635	*HTR4*[*], *FBXO38*[‡]
rs10199793	TTP	2	84233916	G/T	0.292	3.80×10^{-7}	-0.374	*RP11-315I14.2*[*]
rs1767776	ETP	6	10923852	C/G	0.187	5.70×10^{-7}	0.458	*SNORD112*[*]
rs1985749	ETP	17	56417002	A/G	0.323	3.32×10^{-7}	0.399	*RP5-1171I10.4*[*], *RAD51C*[‡], *TRIM37*[‡], *SUPT4H1*[‡], *BZRAP1*[‡], *MSX2P1*[‡], *PRR11*[‡], *MTMR4*[‡], *SKA2*[‡], *CTD-2510F5.4*[‡], *SMG8*[‡], *hsa-mir-142*[‡], *MKS1*[‡], *AC099850.1*[‡]
rs11616264	Lagtime^{TM+}	13	20898330	T/A	0.174	9.59×10^{-7}	0.53	*CRYL1*[*]
rs610551	ETP $^{TM+}$	9	135968225	A/C	0.135	5.63×10^{-7}	-0.486	*snoU13*[*], *RALGDS*[†,‡], *GBGT1*[‡]
rs7045626	nETP-TMsr	9	2735203	A/G	0.357	8.88×10^{-7}	0.341	*KIAA0020*[*]

Note: *, Genes in close proximity to thrombin generation associated SNPs; †, Genes in close proximity to high LD variants ($r^2 \geq 0.8$); ‡, eQTL effect of thrombin generation associated SNPs based on publicly available databases[1, 2]. Abbreviations: SNP, single-nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency; nETP-TMsr, normalized sensitivity ratio of endogenous thrombin potential to thrombomodulin; ETP TM+, endogenous thrombin potential in presence of thrombomodulin



Supplemental Figure I. Quantile-quantile (Q-Q) plot of the p values for ETP^{TM+}, nETP-TMsr and nPeak-TMsr. Inflation factors are shown in the graph and are all around 1.

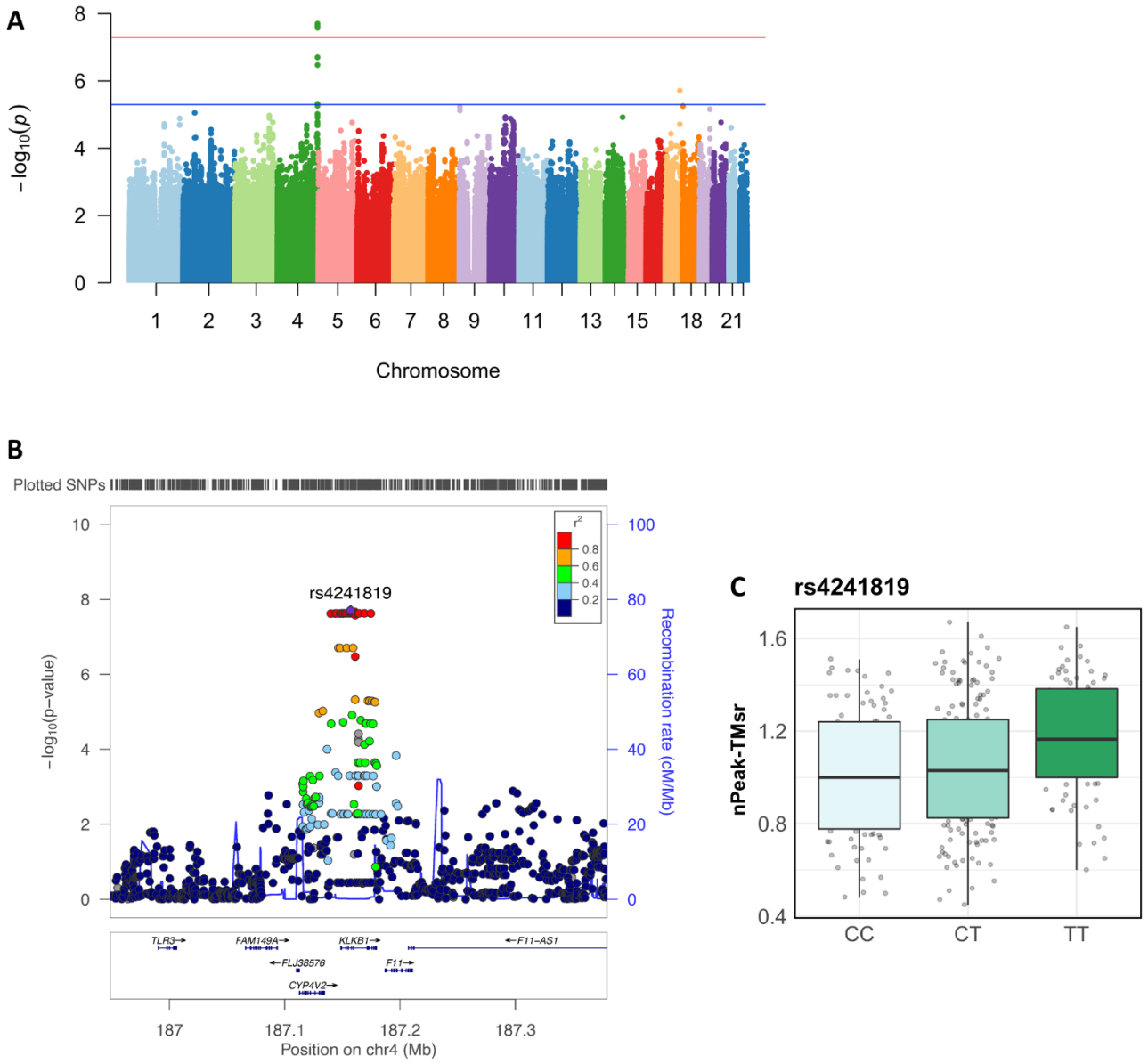
Abbreviations: ETP^{TM+}, endogenous thrombin potential in presence of thrombomodulin; nETP-TMsr, normalized sensitivity ratio of ETP to thrombomodulin; nPeak-TMsr, normalized sensitivity ratio of peak to thrombomodulin.



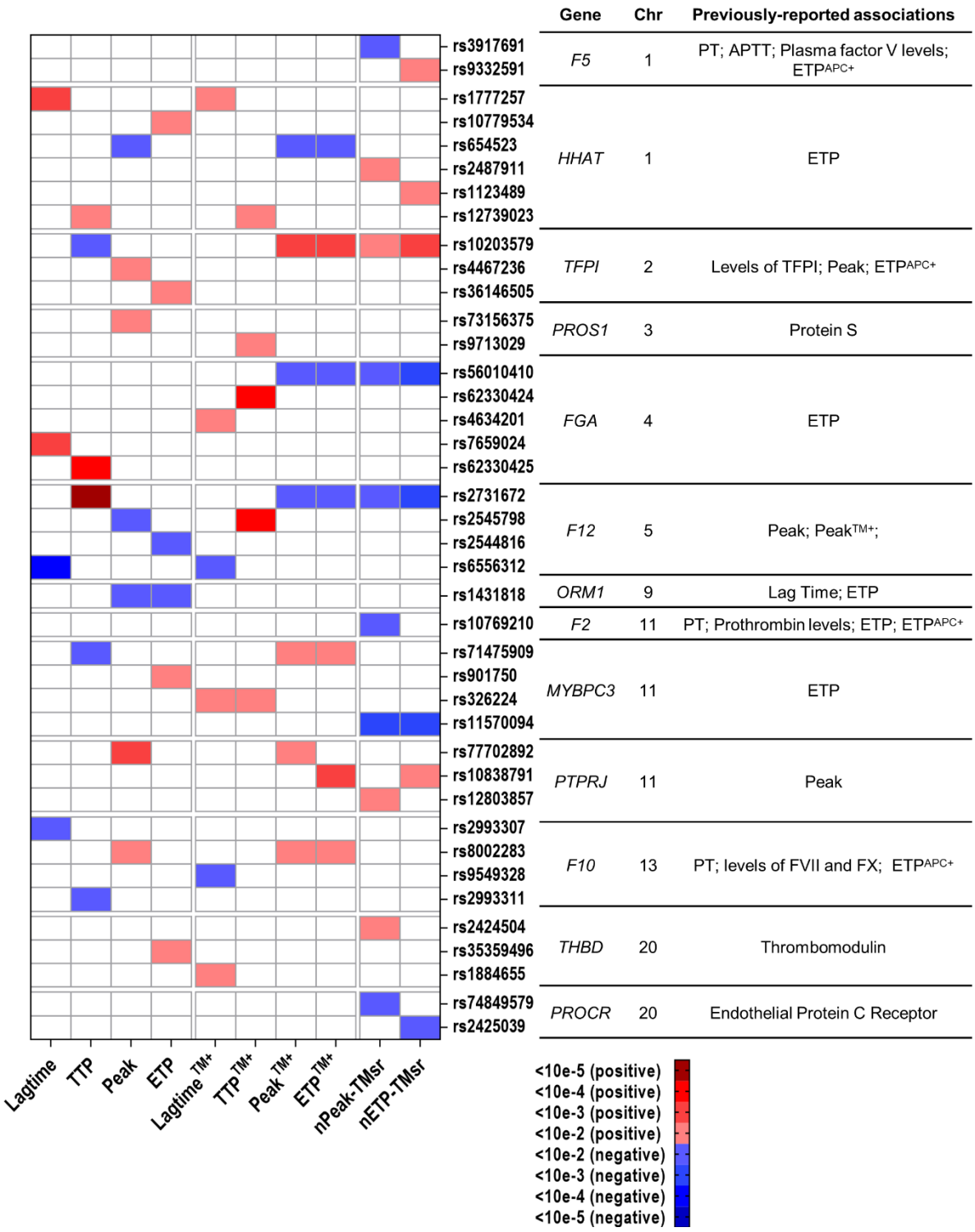
Supplemental Figure II. Thrombin generation (TG) parameters in males (n=198), females not on oral contraceptives (n=117) and females taking oral contraceptives (n=74).

(A) Comparison of the TG parameters without thrombomodulin (TM) between groups. (B) Comparison of the TG parameters with thrombomodulin (TM). (C) Comparison of the TM sensitivity ratios. Comparisons between groups were done with one way ANOVA and the turkey HSD multiple comparison test. * p<0.05, ** p<0.01 and *** P<0.001 compared with males. ### p<0.001 compared with females not on oral contraceptives.

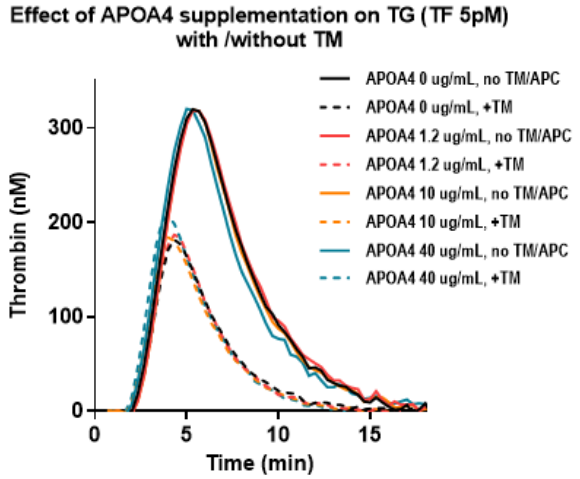
Abbreviations: ETP, endogenous thrombin potential; NPP, normal pool plasma; nPeak-TMsr, normalized sensitivity ratio of peak to TM.

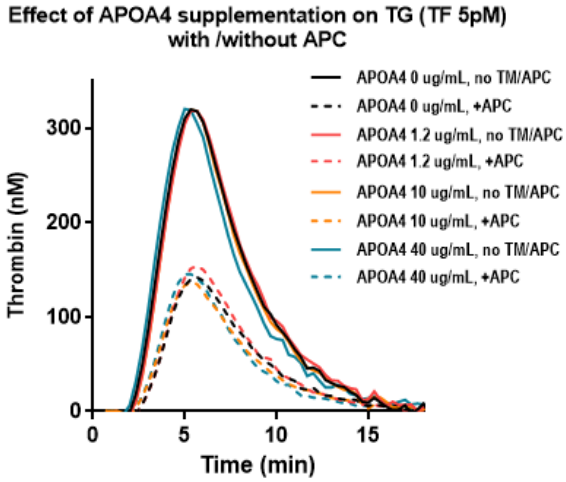


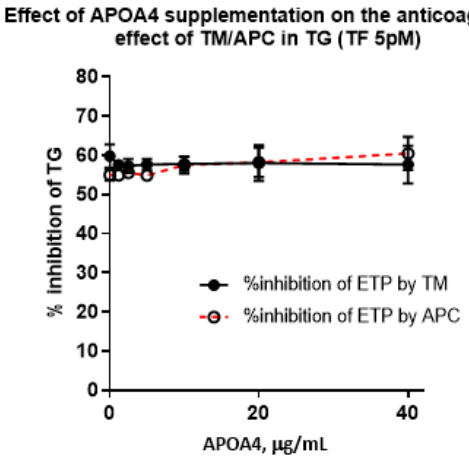
Supplemental Figure III. Genome-wide significant loci associated with the normalized sensitivity ratio of peak to thrombomodulin (nPeak-TMsr). (A) Manhattan plot of SNPs associated with nPeak-TMsr. The red line indicates the threshold for genome-wide significance (5×10^{-8}). (B) Regional association plot of genome-wide significant SNPs at chromosome 4. (C) Genotype stratified nPeak-TMsr.



Supplemental Figure IV. Associations between single-nucleotide polymorphisms (SNPs) located in previously reported TG associated-candidate gene regions and TG parameters in our cohort. The colour legend indicates the range of p-values. Only the SNPs associated with each TG trait with a p-values <0.01 are shown. p-values were obtained from a linear regression model of TG parameters on genotype data. Abbreviations: TTP, time to peak; TM, thrombomodulin; ETP, endogenous thrombin potential; nPeak-TMsr, normalised sensitivity ratio of peak to thrombomodulin; nETP-TMsr, normalised sensitivity ratio of peak to thrombomodulin; PT, prothrombin time; APTT, activated partial thromboplastin time; APC, activated protein C; TFPI, tissue factor pathway inhibitor.

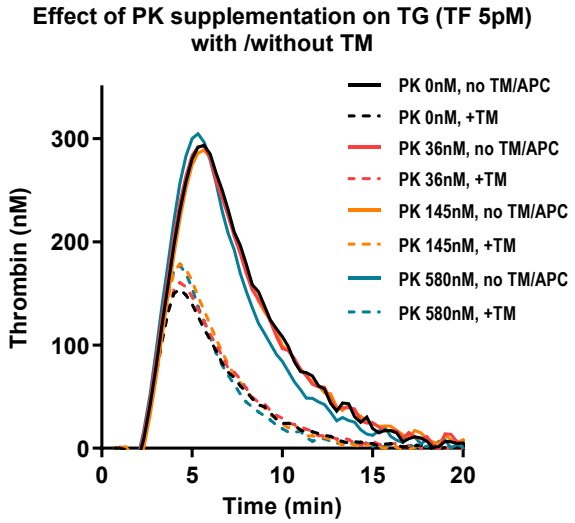


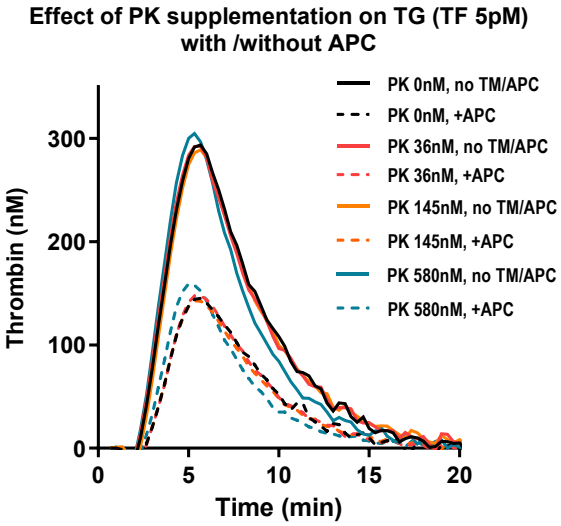


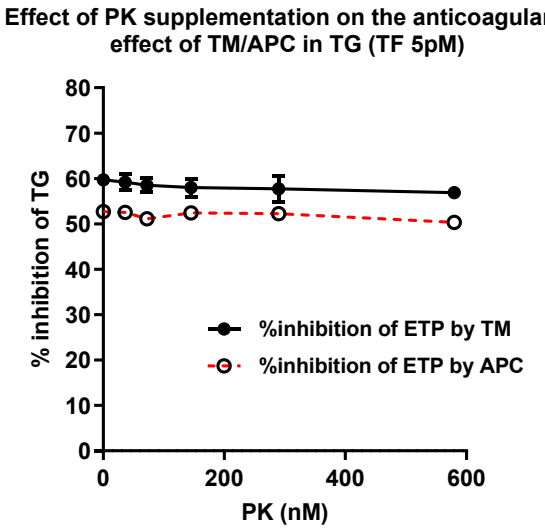


Supplemental Figure V. Effect of apolipoprotein A-IV (APOA4) supplementation on the anticoagulant effect of thrombomodulin (TM) and activated protein C (APC) in thrombin generation (TG).

TG was triggered with 5 pM tissue factor (TF) in the presence or absence of 7nM TM (or 3 nM APC) in normal pooled plasma supplemented with varying doses of APOA4 (1.2, 2.5, 5, 10, 20 or 40 µg/mL). Note that the normal concentration of APOA4 is approximately 150 µg/mL.

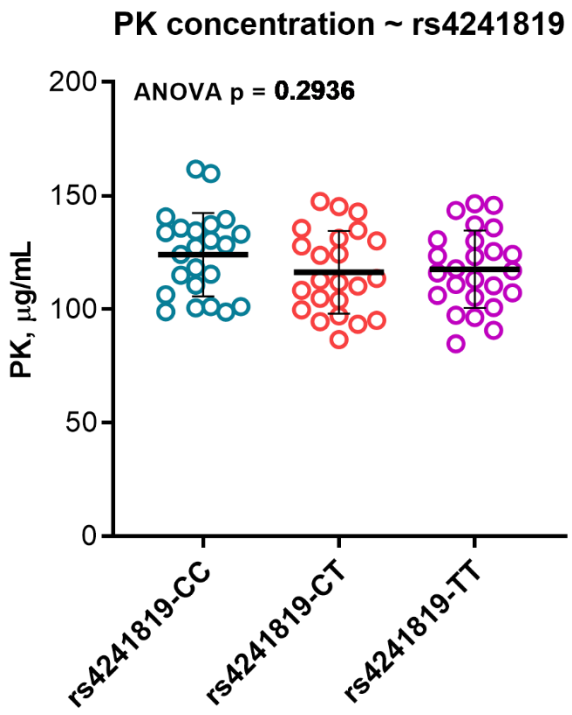




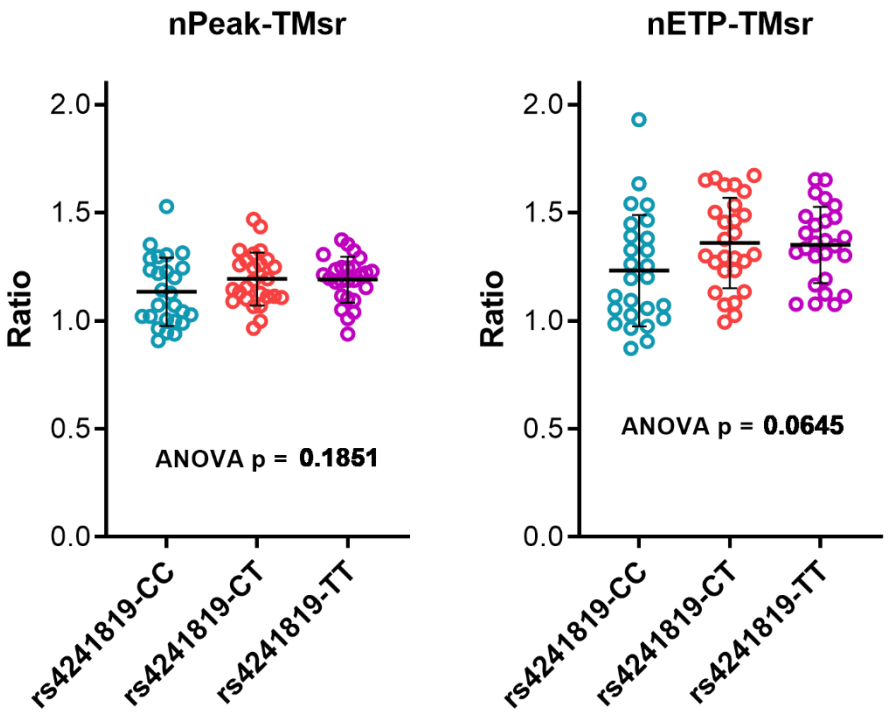


Supplemental Figure VI Effect of prekallikrein (PK) supplementation on the anticoagulant effect of thrombomodulin (TM) /activated protein C (APC) on TG.

TG was initiated with 5pM TF in the presence or absence of 7 nM TM (or 3 nM APC) in normal pooled plasma supplemented with varying doses of human PK (36, 72, 145, 290 or 580 nM). Note that the normal concentration of PK is approximately 580nM.



Supplemental figure VII. Plasma concentration of prekallikrein (PK) in the 500FG cohort in relation to the variations of the SNP rs4241819.
ELISA was used to determine the PK concentration in part of the cohort (n=70). Bars in the graph are mean ± standard deviation. One way ANOVA was used for statistical analyses.



Supplemental figure VIII. Normalized thrombomodulin sensitivity ratios (nTMsr) in presence of corn trypsin inhibitor (CTI).

In 79 samples of the 500FG cohort, TG both in absence and presence of TM was tested in presence of 1.45 µM of CTI to prevent contact activation. nTMsr of peak and ETP were calculated as described in the method section. Bars in the graph are mean ± standard deviation. Comparisons of nTMsr values were done with one way ANOVA.

Supplemental References

1. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* **2020,** *369* (6509), 1318-1330.
2. Westra, H.-J.; Peters, M. J.; Esko, T.*, et al.*, Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* **2013,** *45* (10), 1238-1243.