Supplementary figures

Characterization of missense mutations in signal peptide and propeptide of FIX in hemophilia B by a cell-based assay

Running head: Various mechanisms lead to the FIX deficiency

Wenwen Gao[1*], Yaqi Xu[1*], Hongli Liu[1*], Meng Gao[1], Qing Cao[1], Yiyi Wang[1], Longteng Cui[1], Rong Huang[1], Yan Shen[1], Sanqiang Li[1], Haiping Yang[1,2], Yixiang Chen[1], Chaokun Li[3], Haichuan Yu[4], Weikai Li[5†], Guomin Shen[1†]

1. Institute of Hemostasis and Thrombosis, College of Medicine, Henan University of Science and Technology, Luoyang, Henan 471023, P. R. China

2. First Affiliated Hospital, Henan University of Science and Technology, Luoyang, Henan 471023, P. R. China

3. School of Basic Medical Sciences, Xinxiang Medical University, Xinxiang, Henan 453003, P. R. China

4. School of Medical Laboratory, Xinxiang Medical University, Xinxiang, Henan 453003, P. R. China

5. Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

*: This author contributed equally to this work

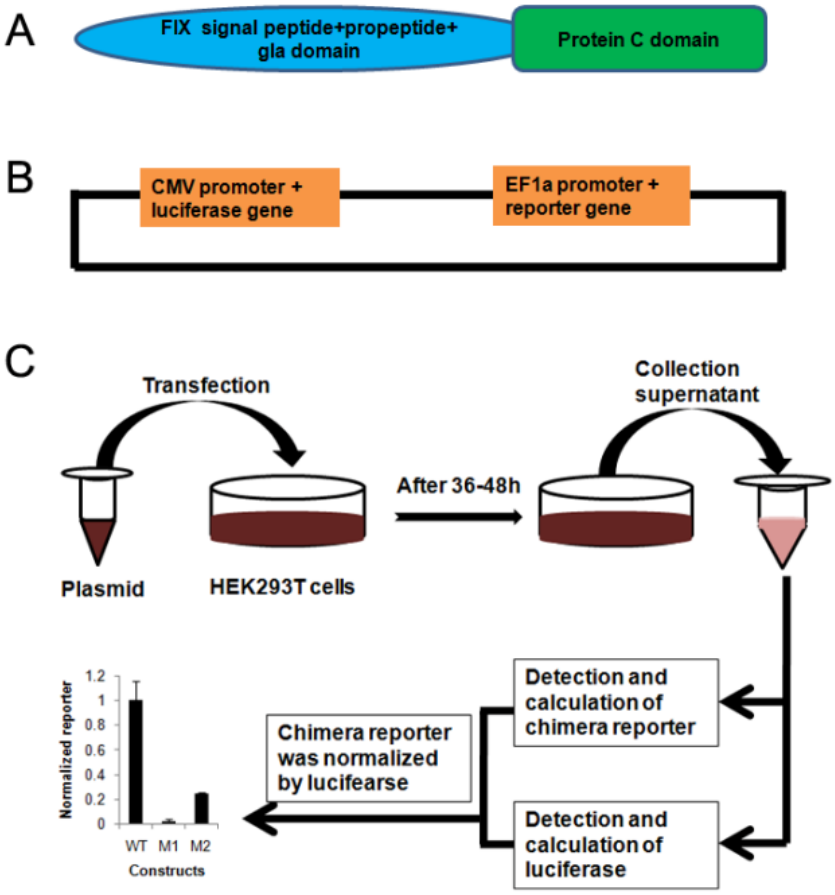
†Co-corresponding

Correspondence should be sent to:

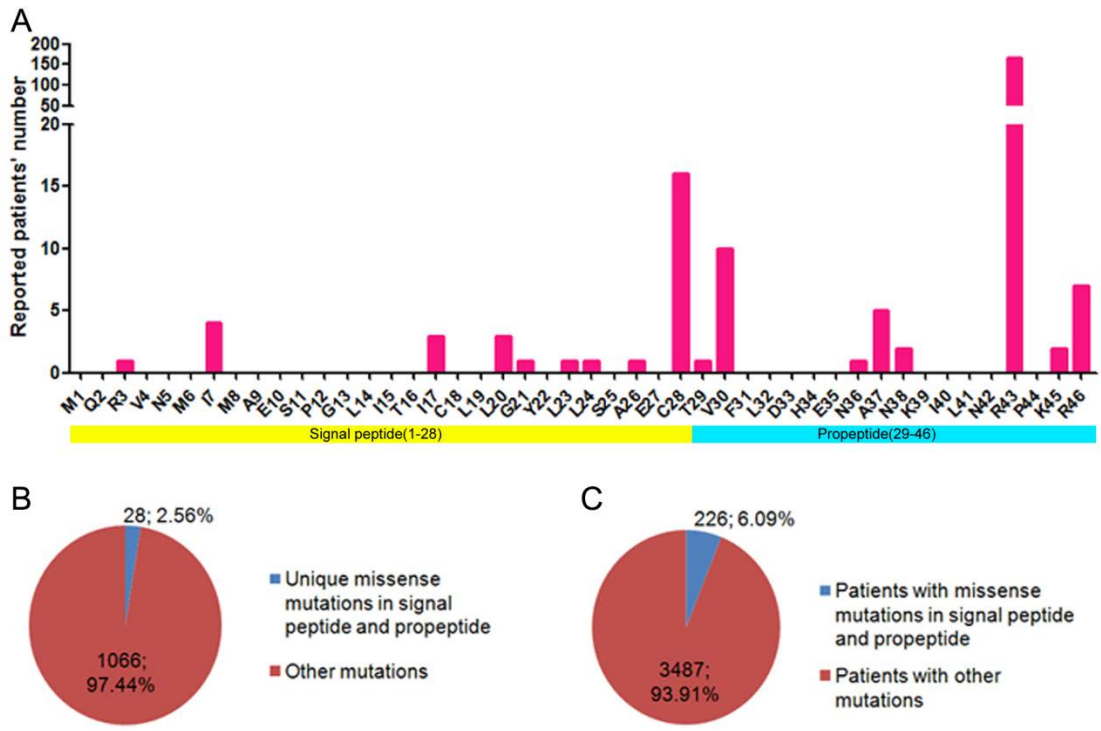
Guomin Shen, Institute of Hemostasis and Thrombosis, College of Medicine, Henan University of Science and Technology, NO. 263 Kaiyuan Avenue, Luoyang, Henan, 471003, P. R. China.Tel: +86 17737913688. Email: shenba433@163.com or shenguomin@haust.edu.cn

or

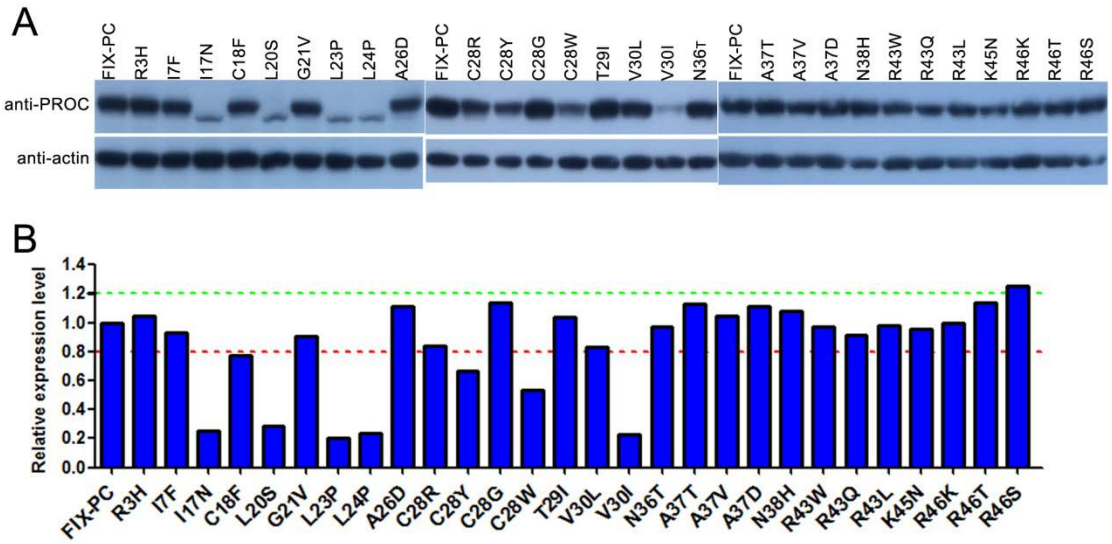
Weikai Li, Department of Biochemistry and Molecular Biophysics, Washington University in St. Louis School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA, Tel: +1 314-362-8687, E-mail: weikai@wustl.edu



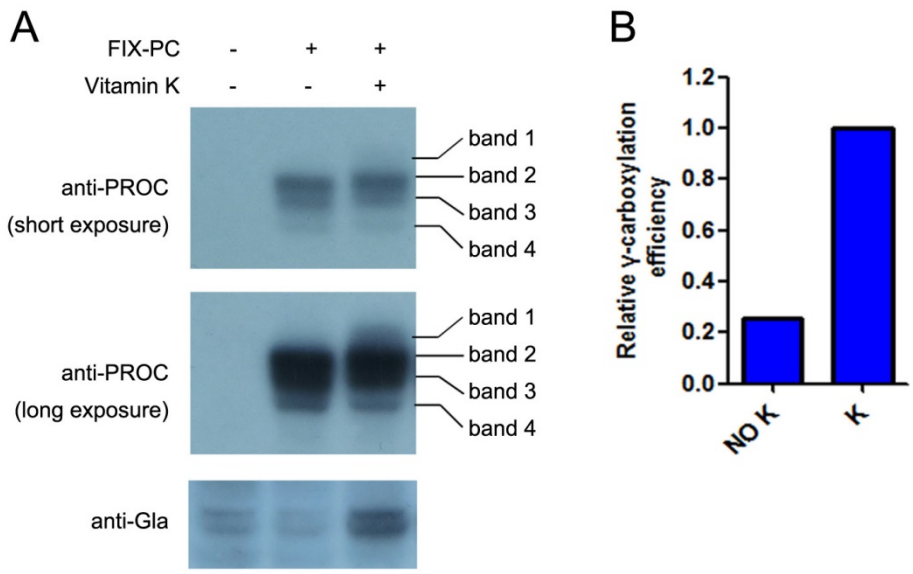
Supplementary figure 1. The principle and scheme of the cell-based reporter assay. A. Chimera reporter FIX-PC with N-terminus domain of FIX and followed C-terminus domain of protein C. B. Chimera reporter gene *FIX-PC* is sub-cloned into EF1α promoter multi-cloning site of the pBud CE4.1 vector, and metridia luciferase cDNA is sub-cloned into the CMV promoter multi-cloning site of the pBud CE4.1 vector. C. A scheme flow of experiment to detect and analyze secreted reporter in the medium.



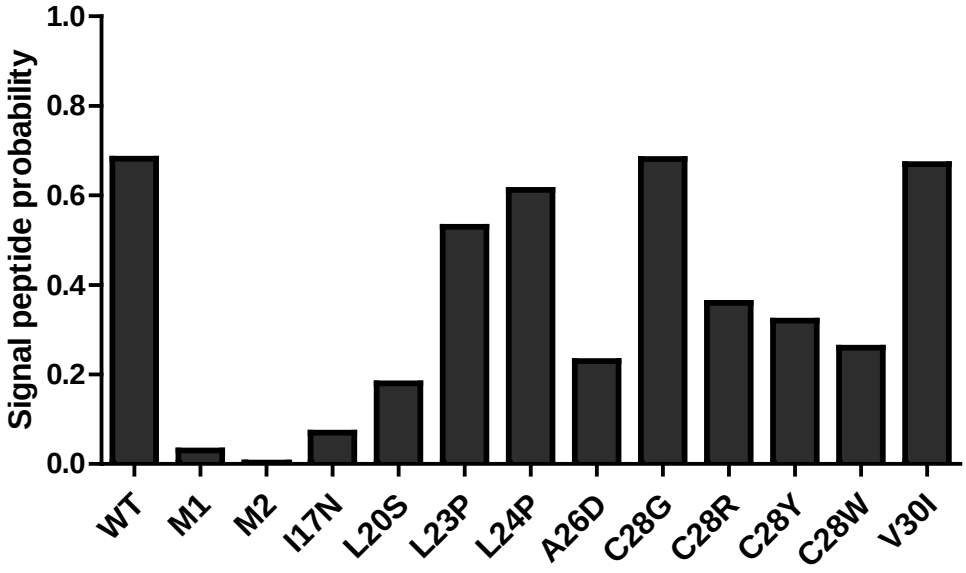
Supplementary figure 2. Analysis of patients with missense mutations in the database. A. The distribution of patients with residues mutations in signal peptide and propeptide. The residues of C28 and R43 are two hotspot mutations in signal peptide and propeptide, respectively. B. Types and percentage of unique missense mutations in the database. C. Number and percentage of patients with different mutations in the database.



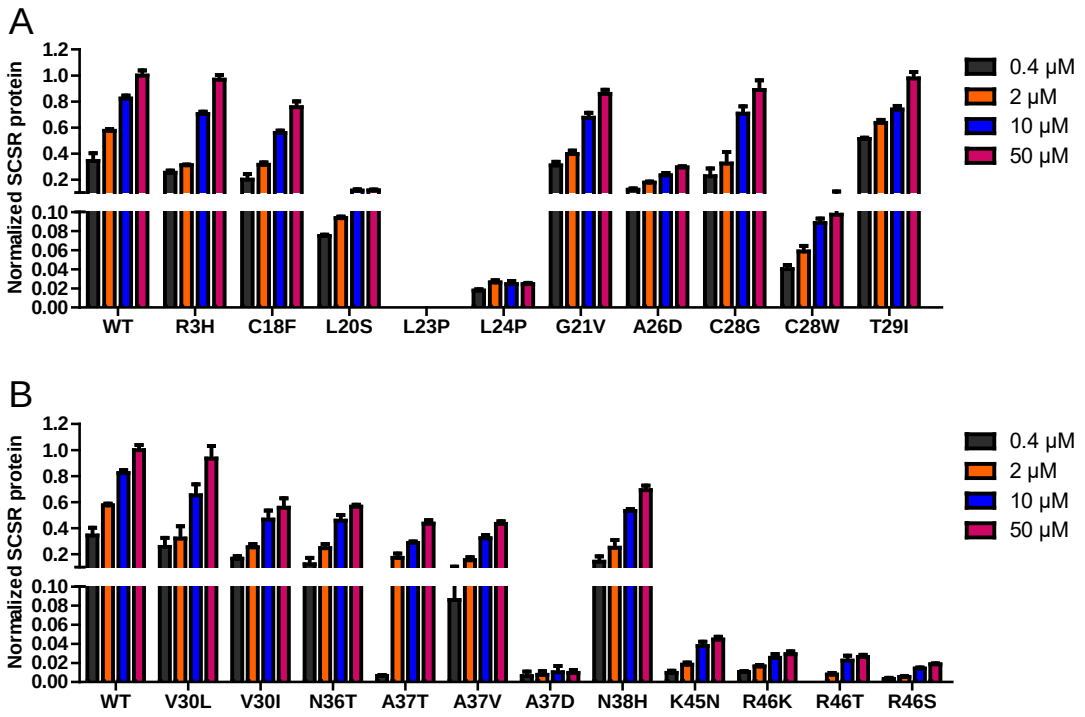
Supplementary figure 3. Intracellular reporter expression profile of the missense mutations. A. Western blot showing the intracellular reporter expression of the mutations detected by HRP-conjugated anti-human protein C, and β-actin was used as an internal control. B. The graph shows the quantification of western blot bands of relative reporter expression, and data is normalized to β-actin and expressed as relative to wildtype FIX-PC.



Supplementary figure 4. Effect of vitamin K on intracellular reporter maturation and γ-carboxylation. A. Western blot showing intracellular reporter protein processing detected by using anti-protein C and anti-Gla antibodies. Long exposure shows 4 bands with vitamin K treatment by anti-protein C antibody, and bands 1-4 represent at least four status during maturation of the reporter. B. The graph shows the relative γ-carboxylation efficiency of the reporter in the presence or the absence of vitamin K. In the absence of vitamin K, unspecific bands (～20%) belong to the background using an anti-Gla antibody.



Supplementary figure 5. Online prediction for signal peptide probability of different constructs.



Supplementary figure 6. The SCSR protein is vitamin K concentration-dependent in most mutations. The SCSR protein was normalized to metridia luciferase, and expressed as relative to 50µM vitamin K of wildtype construct. The error bars indicate the standard deviation from three biological replicates.