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Supplemental Data

A CpG Mutational Hotspot in a ONECUT Binding Site

Accounts for the Prevalent Variant of Hemophilia B Leyden

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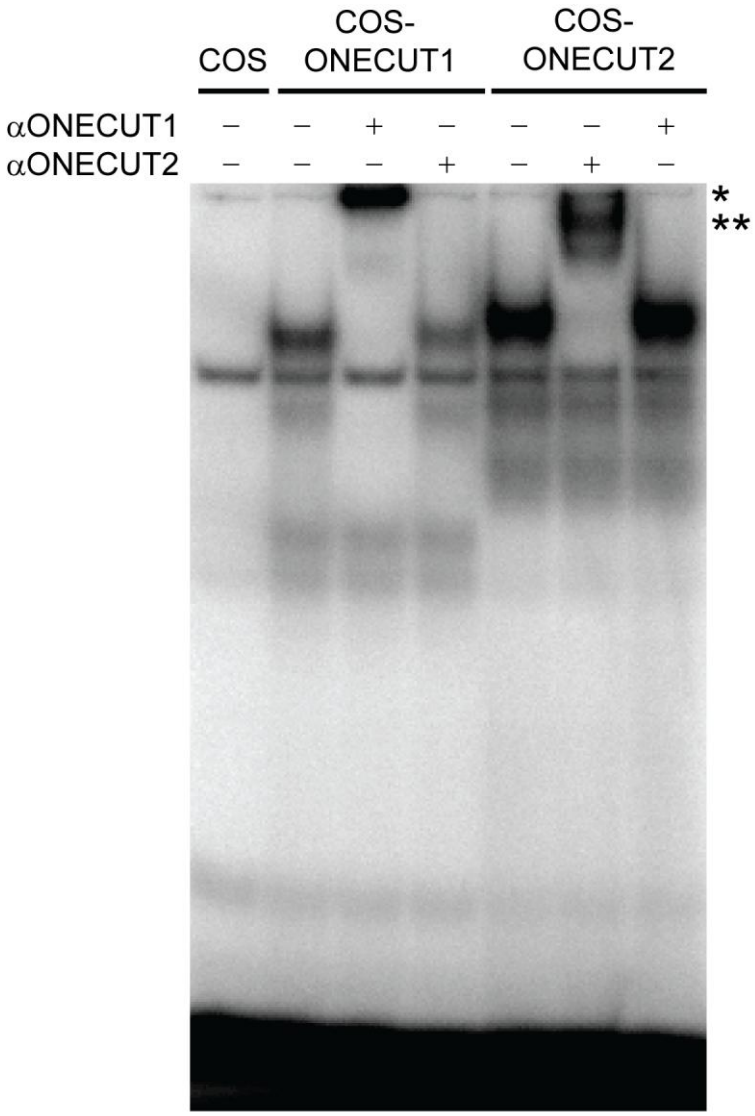

Figure S1. Confirmation of ONECUT1 and ONECUT2 DNA-Binding Complexes by Antibody Supershifts

Nuclear extracts from COS cells transfected with full-length ONECUT1 and ONECUT2 were mixed with wild-type *F9* promoter probe and antibodies specific for ONECUT1 and ONECUT2 respectively. Supershifted complexes are denoted by asterisks.

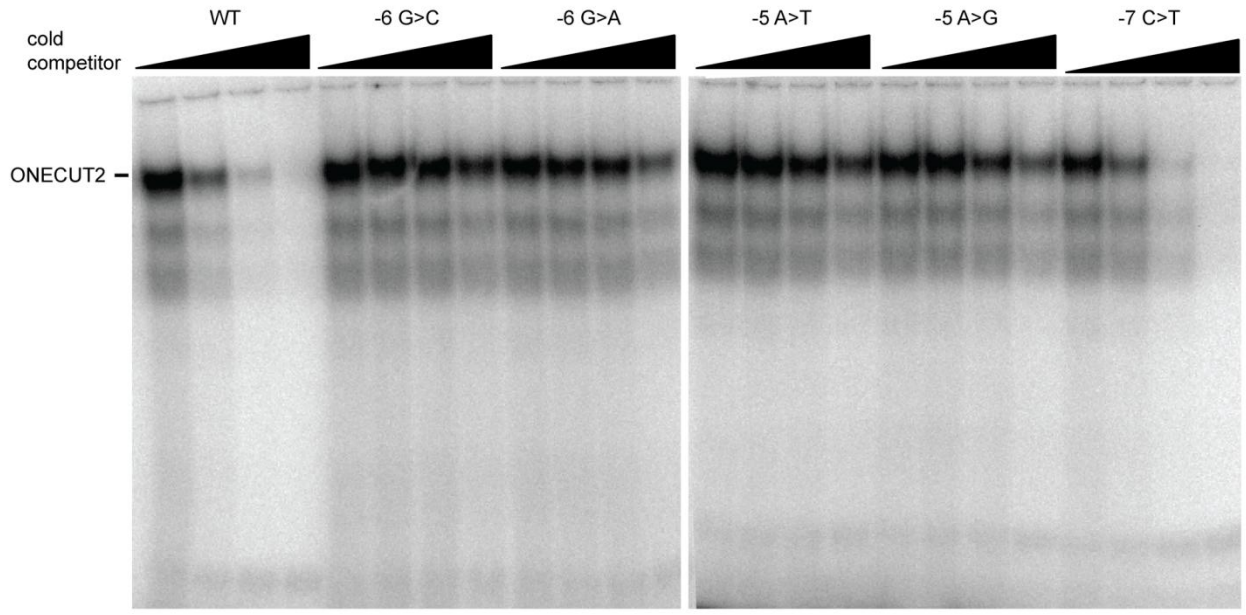

Figure S2. ONECUT2 Binds to the Wild-Type and -7C<T *F9* Promoter Probes but Only Weakly to Probes Containing the -5/-6 Leyden Mutations

Shown is a competition assay using radiolabeled wild-type probe (1.6 ng per lane) and cold competitor probes (0 ng, 16 ng, 160 ng and 1.6 μg, that is, 0x, 10x, 100x and 1000x excesses) comparing the ability of the different mutants to compete for binding by full-length ONECUT2. 2 μl nuclear extracts harvested from COS cells expressing ONECUT2 were used per lane.

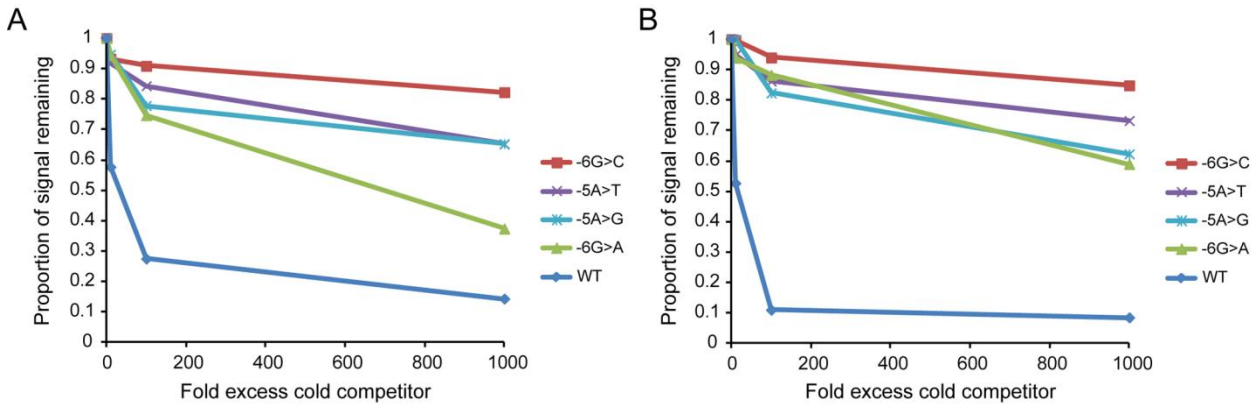

Figure S3. Quantification of ONECUT1 and ONECUT2 Binding to the Wild-Type and -5/-6 Leyden Mutant *F9* Promoter Sequences

Quantification was performed by densitometric analysis of competition assays shown in Figure 2F and Figure S2 using radiolabeled probe containing the wild-type sequence and 0x, 10x, 100x and 1000x excesses of cold competitors as listed. Both ONECUT1 (A) and ONECUT2 (B) bind more strongly to the wild-type sequence than to the -5/-6 Leyden mutant sequences.

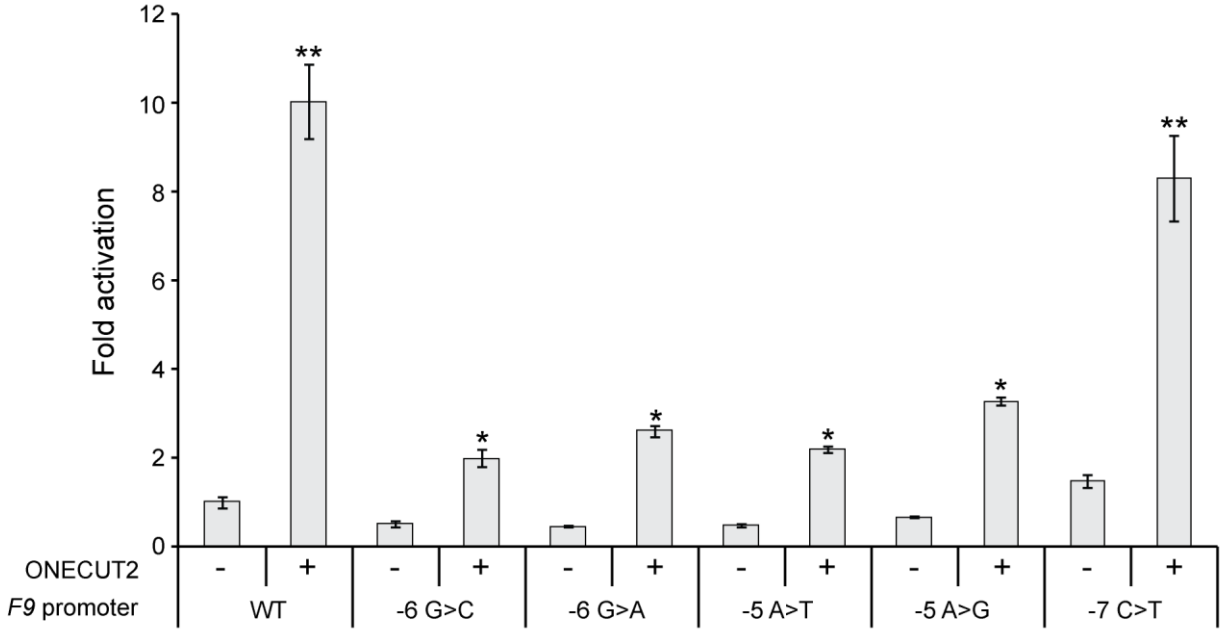

Figure S4. ONECUT2 Transactivates the Wild-Type and -7C<T *F9* Promoter Sequences but Displays Reduced Transactivation at the -5/-6 Leyden Mutant Promoters

Luciferase reporter assay in HepG2 cells. Wild-type (WT) and mutant *F9* promoter fragments were tested in the presence or absence of ONECUT2. n=3 for each data point using independent DNA preparations for promoter constructs and error bars represent standard error of the mean. Firefly levels have been normalized using Renilla for all samples and the value for the wild-type promoter in the absence of ONECUT2 has been set to 1.0. * = $p < 0.04$ for -5/-6 mutant promoters relative to both the wild-type and -7C<T promoters and ** = $p < 0.02$ for the wild-type and -7C<T promoters in the presence compared to the absence of ONECUT2 (two-tailed t-tests).

Table S1. Primers Used for Murine Liver ChIP Quantitative Real-Time PCR

Gene	Forward	Reverse
F9 TSS1	5′-CAGCTTGCACTTTGGAACGAT-3′	5′-TCAGGTGCTTCATGACCTTTGT-3′
F9 TSS2	5′-CAGCTTGCACTTTGGAACGAT-3′	5′-TGCTTCATGACCTTTGTTAGGAGAT-3′
F9 TSS3	5′-CGAGGGAGATGGACAACAATTT-3′	5′- TCAGGTGCTTCATGACCTTTGT-3′
F9 +1.0 kb	5′-AGGTGCCAACCCTGAGCAT-3′	5′-TTCAGCTTTTAAGTCTGATTCCACAGT-3′
F9 -1.5 kb	5′-CCAGTAAAGACCTTTAGGAAGCAGTACC-3′	5′-GGTTAACAGTGACCCATCACTCAG-3′
F7 TSS1	5′-GGTGGGTGAGCTCTGTTCACA-3′	5′-TCCAGGGAAAGAGGAAAGCTAAG-3′
F7 TSS2	5′-GCCAGAAGCCACAGTCTCATC-3′	5′-GGAGCAGAAAGCAGAGAAGCA-3′
F10 TSS	5′-TTCAGGACCAAGGGAAATGG-3′	5′-TGCAGAGCATGTGGCTCATAC-3′

Table S2. Primers Used for Murine Liver Quantitative Real-Time RT-PCR

Gene	Forward	Reverse
18S rRNA	5′-CACGGCCGGTACAGTGAAAC-3′	5′-AGAGGAGCGAGCGACCAA-3′
F9	5′-CACTCGAGTTGTTGGTGGAGAA-3′	5′-CACCTCCACAGAATGCCTCAA-3′
Onecut1	5′-CTGCAGGAGCCGGAGTTC-3′	5′-TTCCCGTGTTCTTGCTCTTTC-3′
Onecut2	5′-GGCAGCTGGAAGAGATCAACAC-3′	5′-TTGTTTGGTTCTTGCTCTTTGC-3′
Hnf4a	5′-CCTGCAGGTTTAGCCGACAA-3′	5′-ATCCGGTCCCGCTCATTT-3′
Cebpa	5′-TCTATAGACATCAGCGCCTACATC-3′	5′-CTCCCGGGTAGTCAAAGTCAC-3′
F7	5′-CACTGCTTCGATAATATCCGCTACT-3′	5′-GCTCATCCCCATCCTTCTCA-3′
F10	5′-GTGGCCGGGAATGCAA-3′	5′-GAACCCTTCATTGTCTTCGTTAATG-3′
F11	5′-CGGATGATCCTACCAAATGGTTT-3′	5′-CGCGCCTGTCATGTTTACC-3′