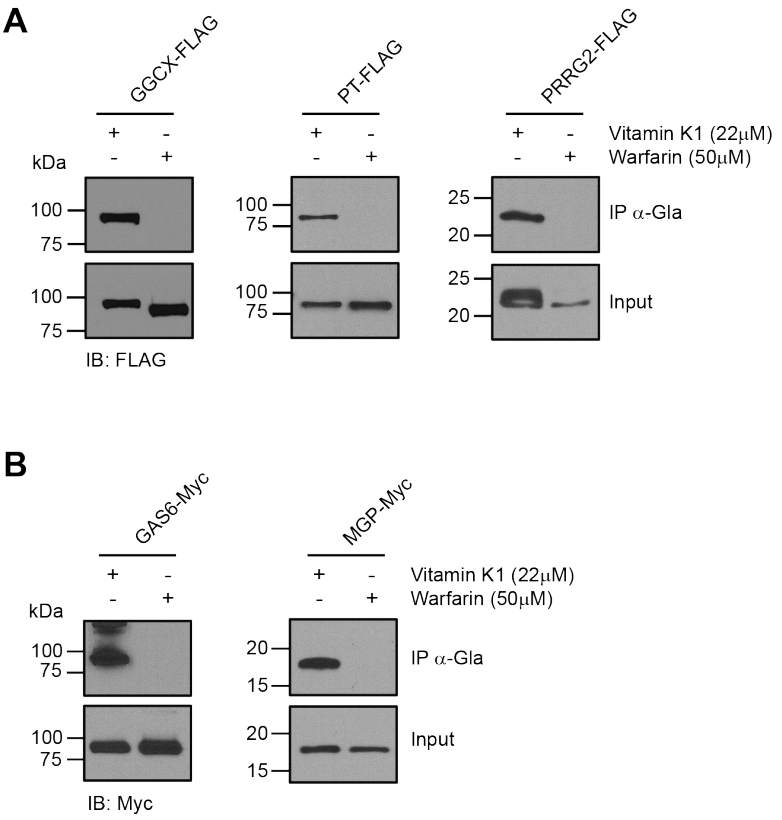
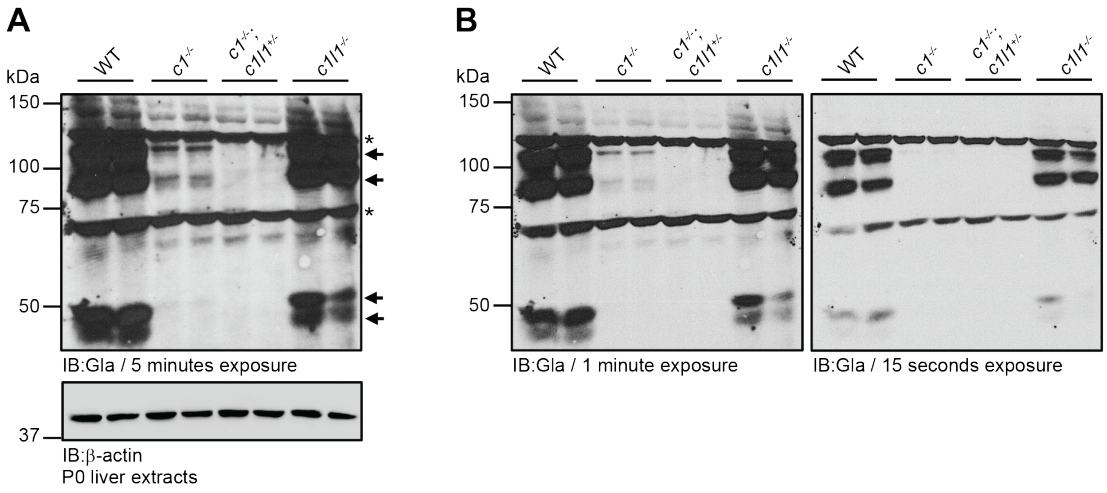
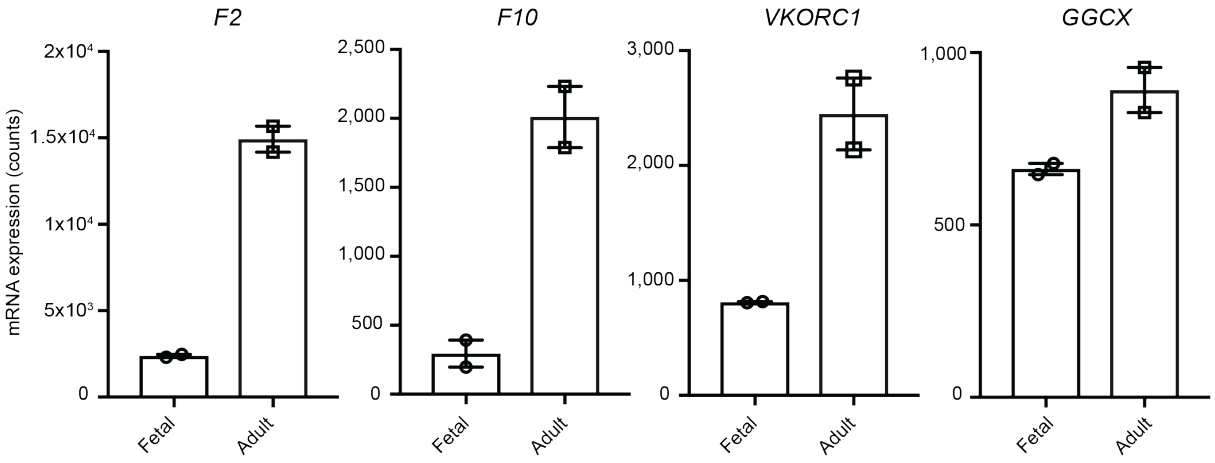


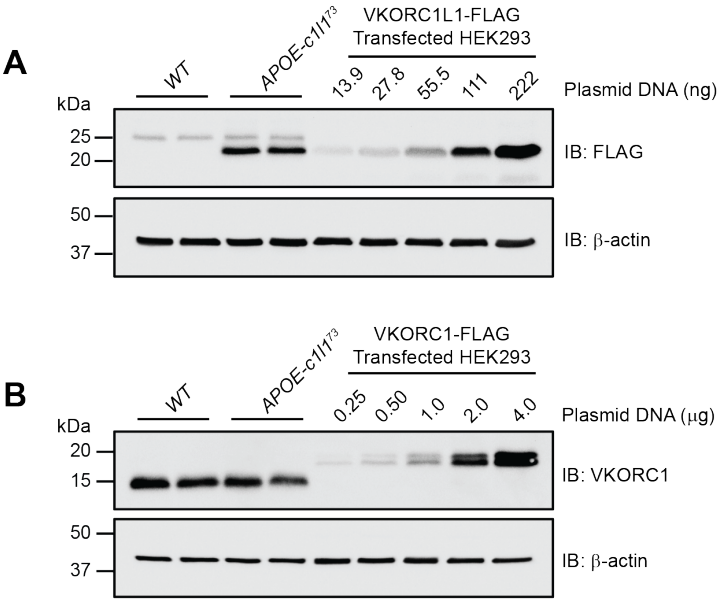
Supplemental Figure 1: Rabbit α-Gla antibodies specifically recognize carboxylated VKD proteins. (A) HEK293 cells were transfected with plasmids encoding GGCX-FLAG, PT-FLAG or tagged proline rich Gla protein 2 (PRRG2-FLAG). Cell media contained either vitamin K_1 (22μM) or warfarin that blocks carboxylation (50μM). Carboxylated proteins in cellular extracts were immunoprecipitated using α-Gla antibody, and proteins were resolved on SDS-PAGE and revealed with α-FLAG antibody. Input represents 2% of total protein extract. **(B)** HEK293 cells were transfected with plasmids encoding tagged growth arrest specific 6 (GAS6-Myc) or tagged matrix Gla protein (MGP-Myc) in the presence of vitamin K_1 (22μM) or warfarin (50μM). Carboxylated proteins in cellular extracts were immunoprecipitated using α-Gla antibody, and then resolved on SDS-PAGE and revealed with α-Myc antibody. Input represents 2% of total protein extract. These immunoprecipitation experiments are representative of at least two independent experiments.



Supplemental Figure 2: *Vkorc1* and *Vkorc1l1* gene dosage affect VKD protein carboxylation. (A-B) Global carboxylation profile in livers from WT, *Vkorc1*$^{-/-}$, *Vkorc1*$^{-/-}$*;Vkorc1l1*$^{+/-}$ and *Vkorc1l1*$^{-/-}$ P0 pups was analyzed by Western blot using α-Gla antibodies. β-actin was used as a loading control. Arrows show VKD proteins, while stars indicate non-specific bands. Western blot was exposed for 5 minutes **(A)**, 1 minute and 15 seconds **(B)**. Duplicates represent biological replicates and the image is representative of two independent experiments.



Supplemental Figure 3: Gene expression in fetal and adult livers. Shown are *F2*, *F10*, *VKORC1* and *GGCX* gene expression levels in fetal and adult human livers (n=2; mean ± SEM). Each sample was hybridized to the HG-U133A array (Su et al., 2004), and normalized data sets were downloaded from the Gene Expression Omnibus website (www.ncbi.nlm.nih.gov/geo). Graphs represent the data obtained with the following probe sets: *F2* (205754_at), *F10* (205620_at), *VKORC1* (217949_s_at) and *GGCX* (205351_at).



Supplemental Figure 4: VKORC1L1-FLAG and VKORC1 expression in WT and *APOE-Vkorc1l1*^{73} mice. (A) VKORC1L1-FLAG expression in WT and *APOE-Vkorc1l1*^{73} livers, and in protein extracts from HEK293 cells transfected with increasing amount of the pCMV14-VKORC1L1-FLAG plasmid was determined by Western blot using α-FLAG antibody. β-actin was used as a loading control. Densitometry units from the VKORC1L1-FLAG band in HEK293 cell extracts and the corresponding transfected DNA quantity were used as standards to quantify the level of VKORC1L1-FLAG protein in WT and *APOE-Vkorc1l1*^{73} livers. **(B)** VKORC1 expression in WT and *APOE-Vkorc1l1*^{73} livers, and in protein extracts from HEK293 cells transfected with increasing amount of the pCMV14-VKORC1-FLAG plasmid was determined by Western blot using α-VKORC1 antibody. Densitometry units from the VKORC1 band in HEK293 cell extract and the corresponding transfected DNA quantity were used as standards to quantify the level of VKORC1 protein in WT and *APOE-Vkorc1l1*^{73} livers. The standards used in **(A)** and **(B)** differ since VKORC1L1-FLAG and VKORC1 expression levels are not in the same range. Duplicates represent biological replicates and the image is representative of two independent experiments. The results of the quantification are shown in **Figure 5E**.

Supplemental Table 1: Expected and observed genotype frequencies following *Vkorc1*$^{+/-}$*;APOE-Vkorc1l1*^{70} X *Vkorc1*$^{+/-}$ intercrosses.

Genotype		**Observed frequency** P21		**Expected frequency**
Vkorc1	***APOE-Vkorc1l1***	**%**	**(n)**	**%**
+/+	*WT*	12.2	(5)	12.5
+/+	*Tg*	12.2	(5)	12.5
+/-	*WT*	34.1	(14)	25.0
+/-	*Tg*	36.6	(15)	25.0
-/-	*WT*	0.0	(0)	12.5
-/-	*Tg*	4.9	(2)	12.5
	Total mice number	**41**		
	Chi-square *P value*	***0.160***		

Supplemental Table 2: List of oligonucleotides used in this study

PCR primers for genotyping	Sequence (5'→3')
ApoE-Fw	AAGGCTAACCTGGGGTGAGG
Vkorc1l1-Rv	TGG CTG TCA TGC CAA GTA ATA AC
Il-2-Fw	CTA GGC CAC AGA ATT GAA AGA TCT
Il-2-Rv	GTA GGT GGA AAT TCT AGC ATC ATC C

qPCR primers for gene expression	Sequence (5'→3')
F2-Fw	TGGAAGGTCGCTGTGCTATG
F2-Rv	CAGAGCGAGGAGTCATCACC
F7-Fw	GAGGACTACACGCTACAGCC
F7-Rv	CGGTCACTATCCATCTGGCG
F9-Fw	GCAAAACCGGGTCAAATCCC
F9-Rv	AGACAGTGGGCAGCAGTTAC
F10-Fw	CACTGCCGTCCTTGACCAC
F10-Rv	TTGGCACGTTCCCGGTTAAT
Ggcx-Fw	TTGACCCTCGTGTGGACATC
Ggcx-Rv	AATCTGCAATGAAGACCACC
Vkorc1-Fw	ATTACCGCGCGCTCTGCGA
Vkorc1-Rv	AAGAACAGGATCCAGGCCAG
Vkorc1l1-Fw	CGAGGATTTGGTCTTTTGGGTTC
Vkorc1l1-Rv	TGGCTGTCATGCCAAGTAATAAC
Actb-Fw	GACCTCTATGCCAACACAGT
Actb-Rv	AGT ACT TGC GCT CAG GAG GA

PCR primers for DNA constructs and mutagenesis	Sequence (5'→3')	Cloning vector or mutation	Restriction site included
Vkorc1l1 FLAG EcoRI-Fw	TTTTTGAATTCGACATGGCGGCGCCCGTCCTG	p3xFLAG-CMV14	EcoRI
Vkorc1l1 FLAG XbaI-Rv	TTTTTTCTAGAGTCTTCCTTAGGCTGCAG	p3xFLAG-CMV14	XbaI
Vkorc1l1 479A/T-Fw	TTACAAACGACTTGTTTATTTGAAT	Silent mutation 479 A/T (Spe I site)	
Vkorc1l1 479A/T-Rv	ATTCAAATAAACAAGTCGTTTGTAA	Silent mutation 479 A/T (Spe I site)	
Vkorc1l1 KpnI-Fw	TTATTGGTACCGCCACCATGGCGGCGCCCGTCCTG	pLIV.7	KpnI
Vkorc1l1 FLAG XhoI-Rv	TTTATCTCGAGCTACTTGTCATCGTCATC	pLIV.7	XhoI
GGCX FLAG HindIII-Fw	TATAAAGCTTGCCACCATGGCTGTGCACCGCGGCTC	p3xFLAG-CMV14	HindIII
GGCX FLAG BamHI-Rv	TTTTTGGATCCGAACTCAGAGTGAACATG	p3xFLAG-CMV14	BamHI
F2 FLAG HindIII-Fw	AATTAAGCTTGCCACCATGTCGCACGTCCGCGGC	p3xFLAG-CMV14	HindIII
F2 FLAG BamHI-Rv	AATTGGATCCTCCAAATTGATCAATGAC	p3xFLAG-CMV14	BamHI
PRRG2 FLAG EcoRI-Fw	TTTTTGAATTCGCCACCATGAGGGGCCGTCCTTCC	p3xFLAG-CMV14	EcoRI
PRRG2 FLAG KpnI-Rv	TTTT TGG TAC CGA GTG AGG CCT TCT GAG GCT	p3xFLAG-CMV14	KpnI
MGP Myc EcoRI-Fw	TTTTTGAATTCGCCACCATGAAGAGCCTGCTCCCT	pCDNA3.1/myc-His B	EcoRI
MGP Myc XbaI-Rv	TTTTTTCTAGAATATTTGGCTCCTCGGCG	pCDNA3.1/myc-His B	XbaI
Gas6 Myc EcoRI-Fw	AATTGAATTCGCCACCATGCCGCCACCGCCCGGG	pCDNA3.1/myc-His B	EcoRI
Gas6 Myc XbaI-Rv	TTAATCTAGAGGGGTGGCATGCTCCACAGG	pCDNA3.1/myc-His B	XbaI

SUPPLEMENTAL REFERENCE

Su, A.I., T. Wiltshire, S. Batalov, H. Lapp, K.A. Ching, D. Block, J. Zhang, R. Soden, M. Hayakawa, G. Kreiman, M.P. Cooke, J.R. Walker, and J.B. Hogenesch. 2004. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci U S A* 101:6062-6067.