Supporting Information

Schulman et al. 10.1073/pnas.1009972107

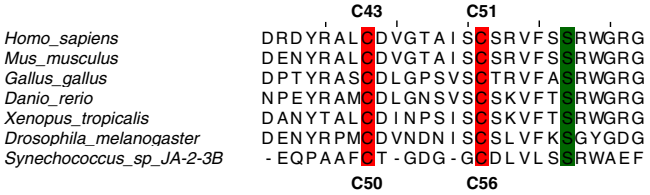

Fig. S1. Loop cysteines Cys43 and Cys51 are conserved across all vitamin K epoxide reductases (VKORs). VKORs from representative organisms were aligned by the CLUSTAL W2 algorithm (1) and truncated to the region shown. The conserved cysteines and serine are shown in red and green, respectively.

1 Larkin MA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948.

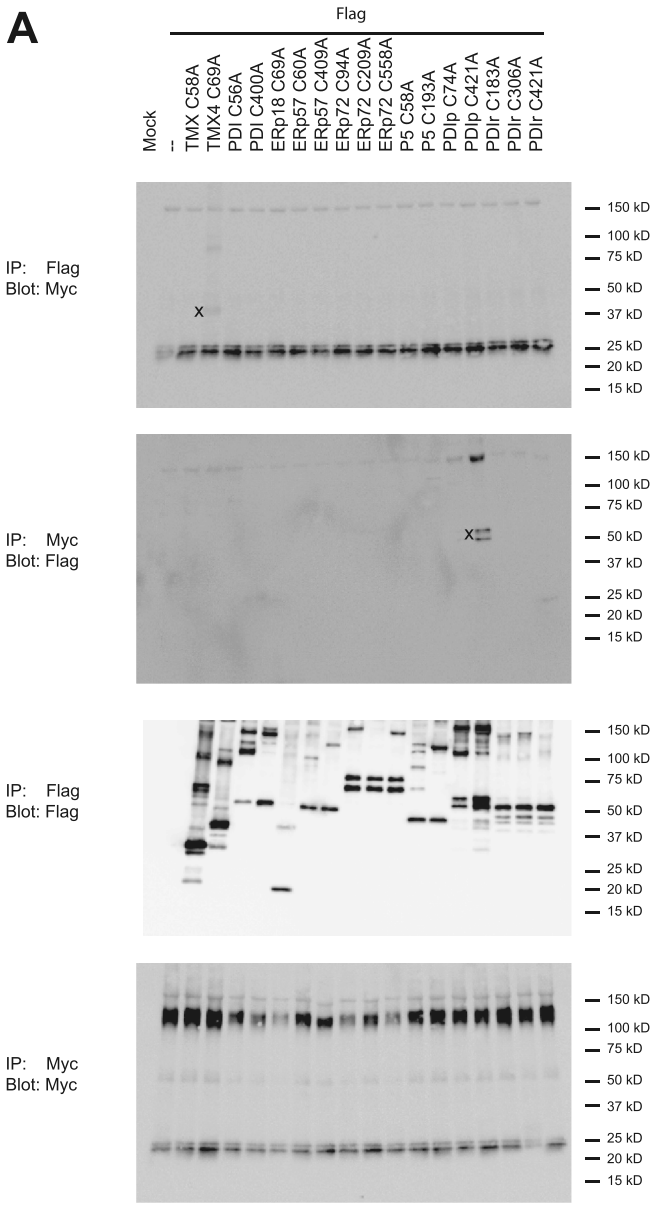

Fig. S2. Thioredoxin (Trx)-like endoplasmic reticulum (ER) proteins do not form adducts in the absence of vitamin K epoxide reductase. (*A*) FLAG-tagged Trx-like ER proteins bearing single CXXA mutations were expressed in COS-7 cells. The cells were treated with NEM and lysates subjected to denaturing immunoprecipitation (IP) with either FLAG or Myc antibodies. Following nonreducing SDS-PAGE, the samples were analyzed by immunoblotting with FLAG or Myc antibodies. The bands indicated by crosses are not cross-linked species; their molecular weight corresponds to non-cross-linked Trx-like protein coprecipitated by the indicated antibody. These bands were not seen in the reciprocal immunoprecipitation.

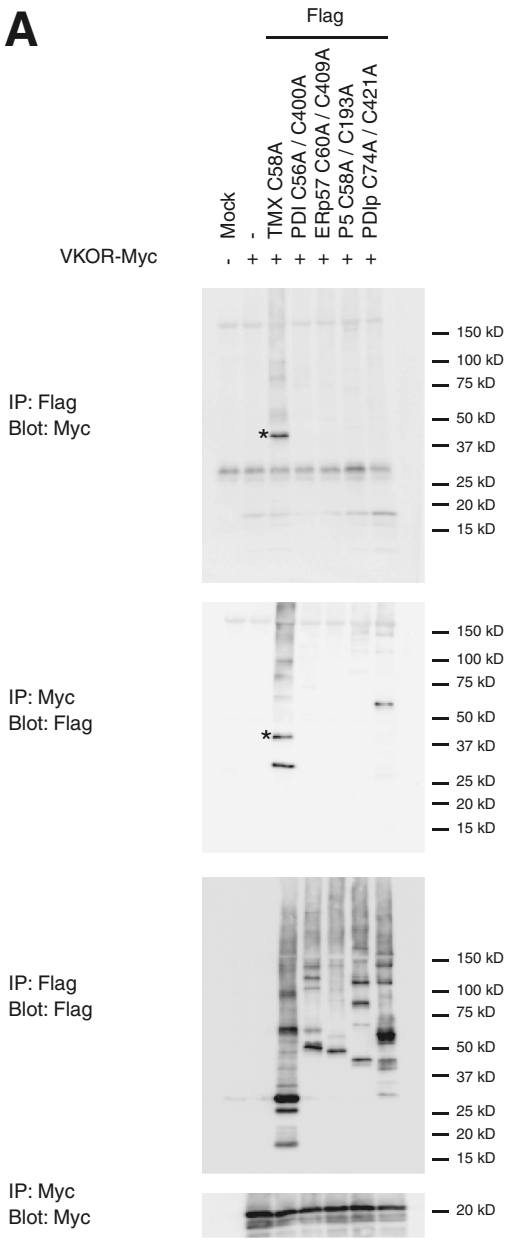

Fig. S3. Thioredoxin (Trx)-like endoplasmic reticulum (ER) proteins with two CXXA mutations do not form adducts with vitamin K epoxide reductase (VKOR). To exclude the possibility that transient cross-links between VKOR and multidomain Trx-like proteins are resolved by a neighboring CXXC motif, we mutated both CXXC motifs to CXXA in each of the candidate redox partners containing two catalytic Trx folds. FLAG-tagged Trx-like ER proteins bearing two CXXA mutations were expressed in COS-7 cells together with Myc-tagged VKOR. TMX (C58A) was used as a positive control. The cells were treated with NEM and lysates subjected to denaturing immunoprecipitation (IP) with either FLAG or Myc antibodies. Following nonreducing SDS-PAGE, the samples were analyzed by immunoblotting with FLAG or Myc antibodies.

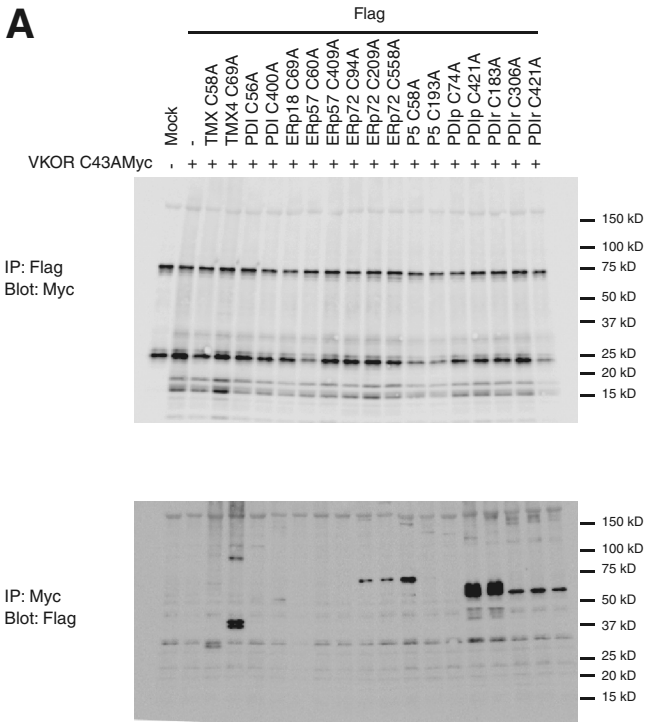

Fig. S4. Vitamin K epoxide reductase (VKOR) (Cys43Ala) does not form disulfide bridges with thioredoxin (Trx)-like ER proteins. (*A*) FLAG-tagged Trx-like endoplasmic reticulum (ER) proteins bearing single CXXA mutations were coexpressed in COS-7 cells with Myc-tagged human VKOR carrying the Cys43Ala mutation. The cells were treated with NEM and lysates subjected to denaturing immunoprecipitation (IP) with either FLAG or Myc antibodies. Following nonreducing SDS-PAGE, the samples were analyzed by immunoblotting with FLAG or Myc antibodies.

Table S1. Molecular weights of the tested thioredoxin (Trx)-like proteins

	No. of catalytic CXXC motifs	Molecular weight, kD	Molecular weight of the predicted adduct with VKOR, kD
TMX	1	31	49
TMX4	1	39	57
PDI	2	55	73
ERp18	1	18	36
ERp57	2	54	72
ERp72	3	71	90
P5	2	46	64
PDIp	2	55	73
PDIr	3	57	75

Summary of the Trx-like proteins screened for interaction with vitamin K epoxide reductase (VKOR). For each protein tested, the number of catalytic Trx-like domains containing a functional CXXC motif is listed. The native molecular weight is provided for each candidate as well as the expected molecular weight for an adduct with VKOR. PDI, protein disulfide isomerase.

Table S2. Primers used to clone thioredoxin (Trx)-like proteins

Protein	Forward	Reverse
ERp18	CCACCATGgagacgcggcctcgtctcggggcca	tcaTTTATCATCATCATCTTTATAATCcaattcatcttcaagatgtttct
ERp57	CACCATGcgcctccgccgcctagcgctgttc	tcaTTTATCATCATCATCTTTATAATCgagatcctcctgtgccttcttc
ERp72	CACCATGaggccccggaaagccttcctgctc	tcaTTTATCATCATCATCTTTATAATCaagctcttccttggtcctgctca
P5	CACCATGgctctcctggtgctcggtctggtg	tcaTTTATCATCATCATCTTTATAATCcaactcatctttccctaagtcat
PDI	CACCATGctgcgccgcgctctgctgtgcctg	tcaTTTATCATCATCATCTTTATAATCcagttcatctttcacagctttctg
PDIp	CACCATGagccgccagcttctgcctgtactg	tcaTTTATCATCATCATCTTTATAATCcagttcctccttggaccccatag
PDIr*	CACCATGgcgcgggcgtgggggctgctactg	tcaTTTATCATCATCATCTTTATAATCcagctcttccctcctttttctctag
TMX	CACCATGgcgccctccgggagtcttgcagttc	tcaTTTATCATCATCATCTTTATAATCggatttatctgtggccaatgatg
TMX4	CACCATGgcgggtgggcgctgcggcccgc	tcaTTTATCATCATCATCTTTATAATCcagtcccttgtcagcatgctga

Summary of primers used to incorporate a C-terminal FLAG tag into mammalian Trx-like proteins and to facilitate directional TOPO cloning into the pcDNA3.1/V5-His TOPO TA expression vector (Invitrogen). Segments of the primers in lower case are complementary to the coding sequence of the corresponding cDNA. The asterisk indicates use of murine PDIr. PDI, protein disulfide isomerase.

Table S3. Primers used for site-directed mutagenesis

Protein	Mutagenesis primer	Mutation
ERp18	5′-taaatcctggtgtggagctgccaaagctctaaagcccaaa-3′	C69A
ERp57	5′-ccctggtgtggacacgccaagagacttgcacc-3′	C60A
	5′-cccttggtgtggtcatgctaagaacctggagccc-3′	C409A
ERp72	5′-ctccatggtgtggacatgccaagcagtttgctccgg-3′	C94A
	5′-cccatggtgtggacacgccaagaaacttgccccc-3′	C209A
	5′-catggtgcgggcacgccaagcagctagagc-3′	C558A
P5	5′-tgctccatggtgtggtcacgctcaaagattaacaccagaa-3′	C58A
	5′-ctccttggtgtggacacgccaaaaacctagagccag-3′	C193A
PDI	5′-ctggtgtggccacgccaaggctctggcc-3′	C56A
	5′-cccatggtgtggtcacgccaaacagttggctccc-3′	C400A
PDIp	5'-gtggtgtgggcacgcccaggccctggcc-3'	C74A
	5'-cgtggtgcacccacgccaaggagatggccc-3'	C421A
PDIr	5′-ctccctggtgcagcatggccaagagaatcatgccac-3	C183A
	5′cgccccatggtgtggacattgctaagaaaatgaagccagag-3′	C306A
	5′-cccttggtgcccacacgctaagaaggtcatcccc-3′	C427A
TMX	5′-atgccccgtggtgccctgctgctcaaaatcttcaac-3′	C59A
	5′-atagaattttatgccccgtgggcccctgctgctcaaaatcttcaaccggaatg-3′	C56A/C59A
TMX4	5′-cccatggtgtccatccgcccagcagactgattca-3′	C67A
VKORC1	5′ctcatctcagaagaggatctggcatagaatgatatcctggattacaaggat-3′	Stop before FLAG tag
	5′-tcgccatgggctgcacctggggg-3′	S3C
	5′-ttaccgcgcgctcgccgacgtgggcacc-3′	C43A
	5′-gcaccgccatcagcgcttcgcgcgtcttct-3′	C51A
	5′-gtccaagaaccccagtgcaaggctaagaggc-3′	G158C

Summary of primers used to incorporate or eliminate cysteine residues for vitamin K epoxide reductase (VKOR) or the thioredoxin-like proteins.