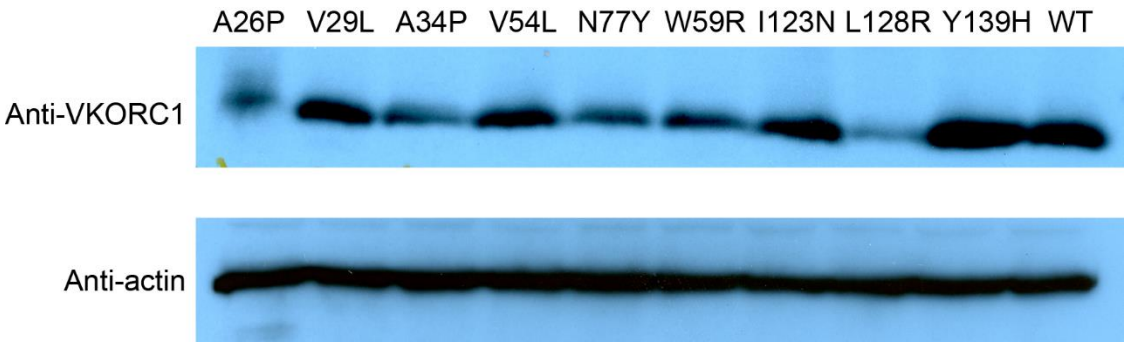
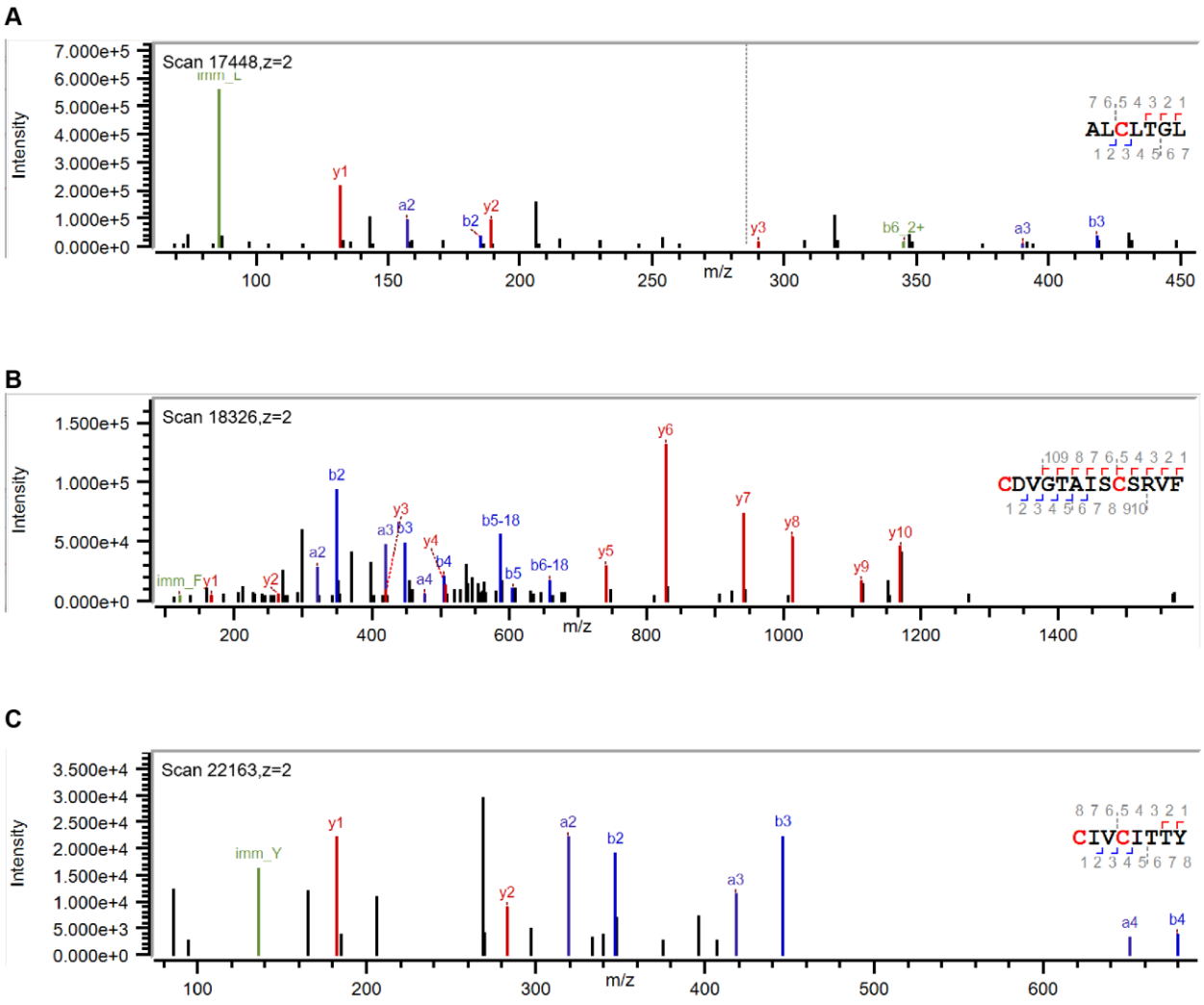


Supplemental Figure 1. Protein expression levels of wildtype VKORC1 and WR mutants.

The western blots were carried out using the anti-VKORC1 antibody (*top*) and anti-actin antibody (*bottom*).



Supplemental Figure 2. Representative product-ion (MS/MS) spectrum of VKORC1 peptides containing different cysteines and NEM isotopic modifications. A, A peptide containing Cys16 labeled by NEM. **B,** A peptide containing Cys43 and Cys51, and both cysteines are labeled by NEM. **C,** A peptide containing Cys132 and Cys135, one labeled by NEM and another by NEM-d_5.

Movie S1. Severe WR mutations expose VKORC1 active site for GSH reduction.

Movements during the 100 ns simulation are shown for WT (*left*), Ala28Pro (*middle*) and Trp59Arg (*right*). Compared to WT, Ala26Pro and Trp59Arg mutation (yellow sphere) induces aberrant movement of TM1 extension and cap region, respectively. The movements expose the active site, allowing GSH to gain access (arrow) and reduce the active site (Cys132 and Cys125 in green spheres), generating the increased R state that cannot be inhibited by warfarin. The movie used MD models of every 5 ns during the 100 ns simulation, and superimposition and interpolation of these models were generated by Chimera.