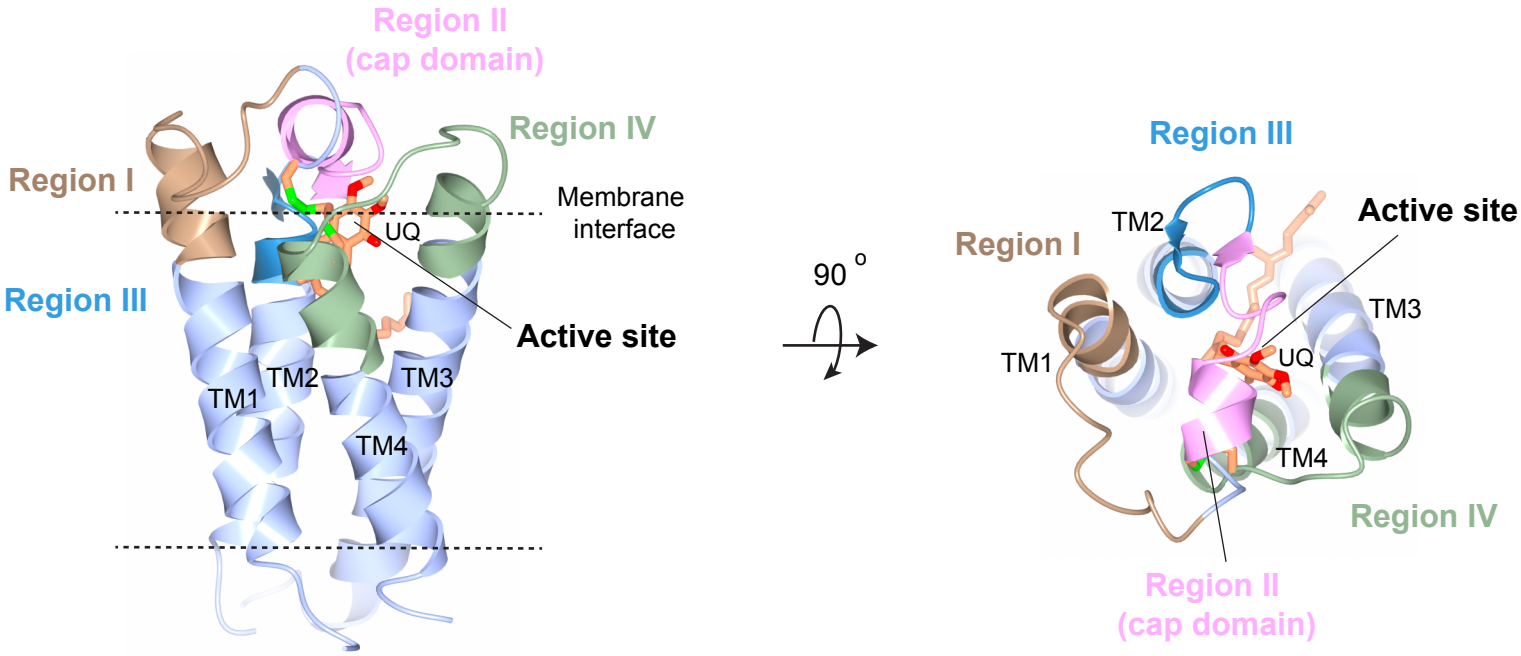

Figure S1. Structure of the bacterial VKOR showing the locations of active site and regions I–IV. These regions (same color as in Fig. 3D) are mapped onto the bacterial structure (PDB: 4NV5). The active sites of VKORC1 and VKORL1 are expected to be at the same location as in this bacterial VKOR homolog, which is defined by the catalytic cysteines (green bonds) and the bound quinone molecule (UQ). The rest of the bacterial VKOR structure (excluding TM1−TM4) are omitted for clarity.

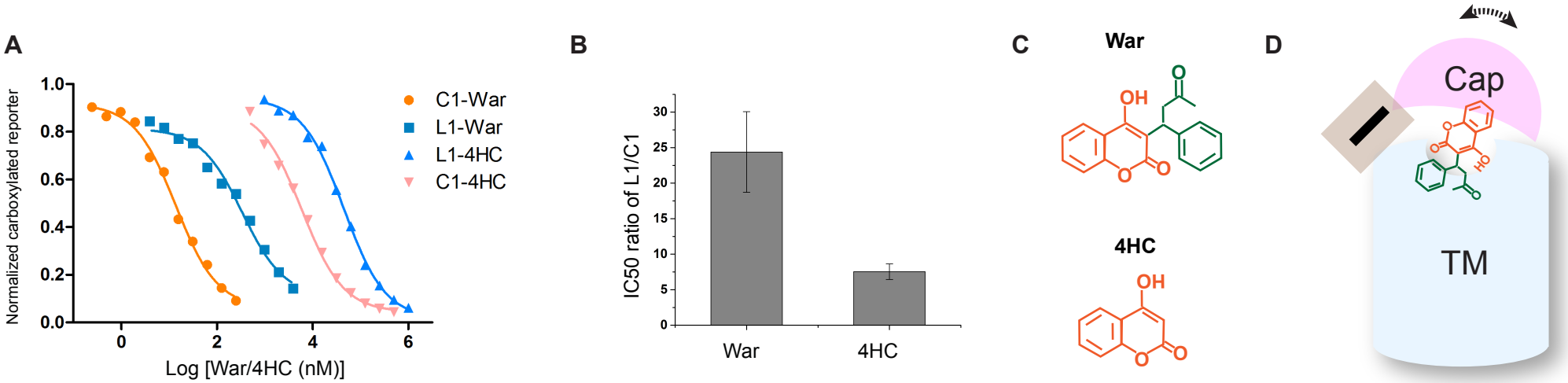

Figure S2. VKORL1 and VKORC1 show relatively different response to warfarin and 4-hydroxycoumarin (4HC). The relative inhibition level of warfarin (War) and 4-hydroxycoumarin (4HC) is an indicator of the cap domain stability. (A) Inhibition curves of warfarin and 4-HC against VKORC1 and VKORL1. (B) Ratio of IC50 between VKORL1 and VKORC1, inhibited by warfarin or 4HC. (C) Chemical structures of warfarin and 4-hydroxycoumarin. (D) Cartoon shows that warfarin binds VKORC1 with its 4HC ring facing the cap domain and its phenyl butanone side group facing TM domain. This binding topology is deduced from the comparison of warfarin and the 4HC compound by their MS footprinting pattern and by their relative IC50s of inhibiting WR mutants [15]. These data also suggest that warfarin is sensitive to the relative movement (dashed arrow) between the cap domain and the TM domain, a movement that is primarily determined by the stability and conformation of the cap domain. In contrast, the 4HC binding should be less affected by this movement because it interacts more with the cap domain [15]. Consistent with this model, here we find that the difference of 4HC response between VKORC1 and VKORL1 is only 5–6 fold, in contrast to the 25-fold difference of their warfarin response. Because VKORL1 shows less difference in respond to 4HC and warfarin than VKORC1 does, the cap domain of VKORL1 appears to undergo less movement.