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Supplemental Information

Full-Length Anion Exchanger 1 Structure and Interactions with Ankyrin-1 Determined by Zero Length Crosslinking of Erythrocyte Membranes

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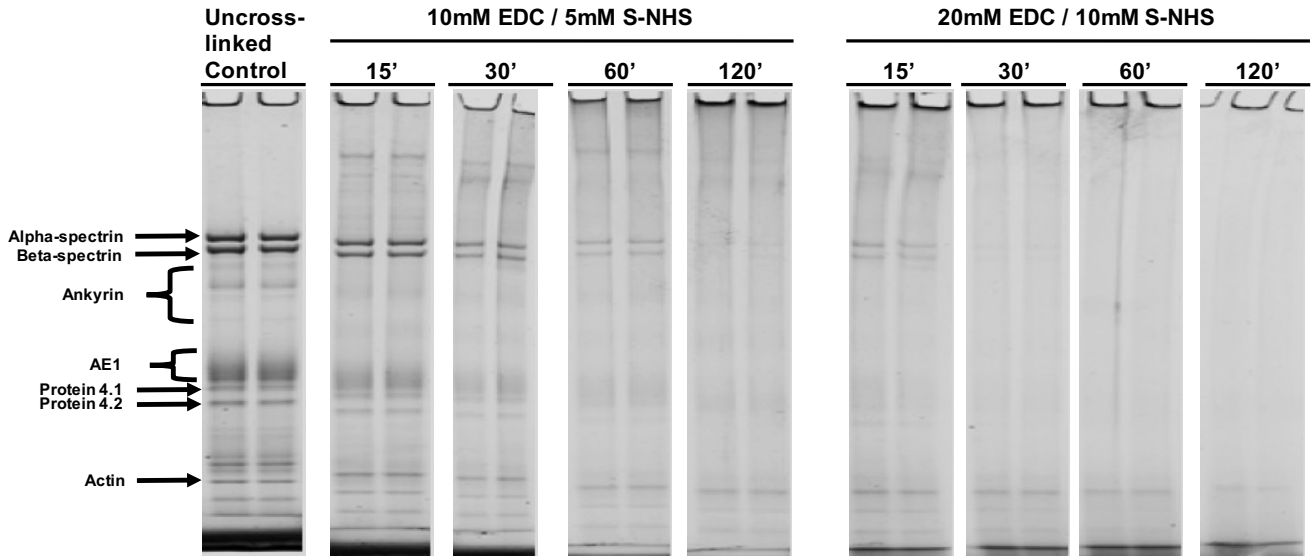

Fig. S1. Related to Experimental Procedures. SDS-PAGE analysis of cross-linking reactions of erythrocyte membranes. EDC and *sulfo*-NHS concentrations and reaction times in minutes are indicated above the relevant cross-linked lanes. The amount of protein loaded in each lane of this gel was 4 μg.

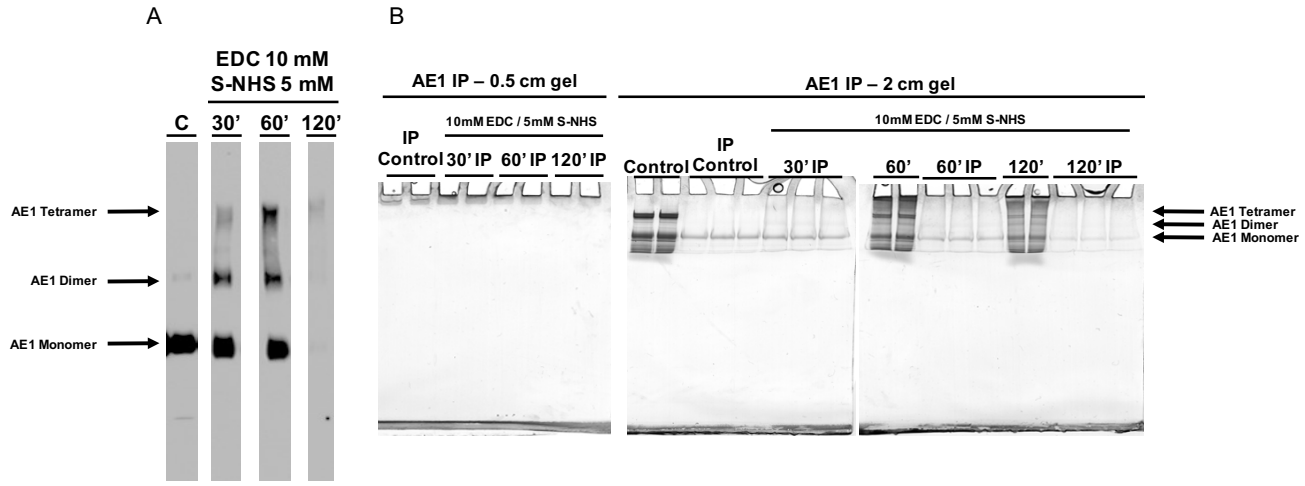

Fig. S2. Related to Experimental Procedures. Analysis of AE1 immunoprecipitation reactions. (A) Western Blots for AE1 IP reactions. EDC and *sulfo*-NHS concentrations and cross-linking reaction times are indicated above the relevant cross-linked bands and C indicates uncross-linked control. Arrows indicate oligomeric forms of AE1. (B) Control and cross-linked samples prior to IP and IP eluants run on an SDS gel for 0.5 cm (left) or 2 cm (right) and stained with Colloidal Coomassie Blue. Arrows indicate oligomeric forms of AE1 on the 2 cm gel. Control, 60' and 120' lanes contained 10 μg of protein, whereas IP Control, 30' IP, 60' IP, and 120' IP from 20 μg of protein.

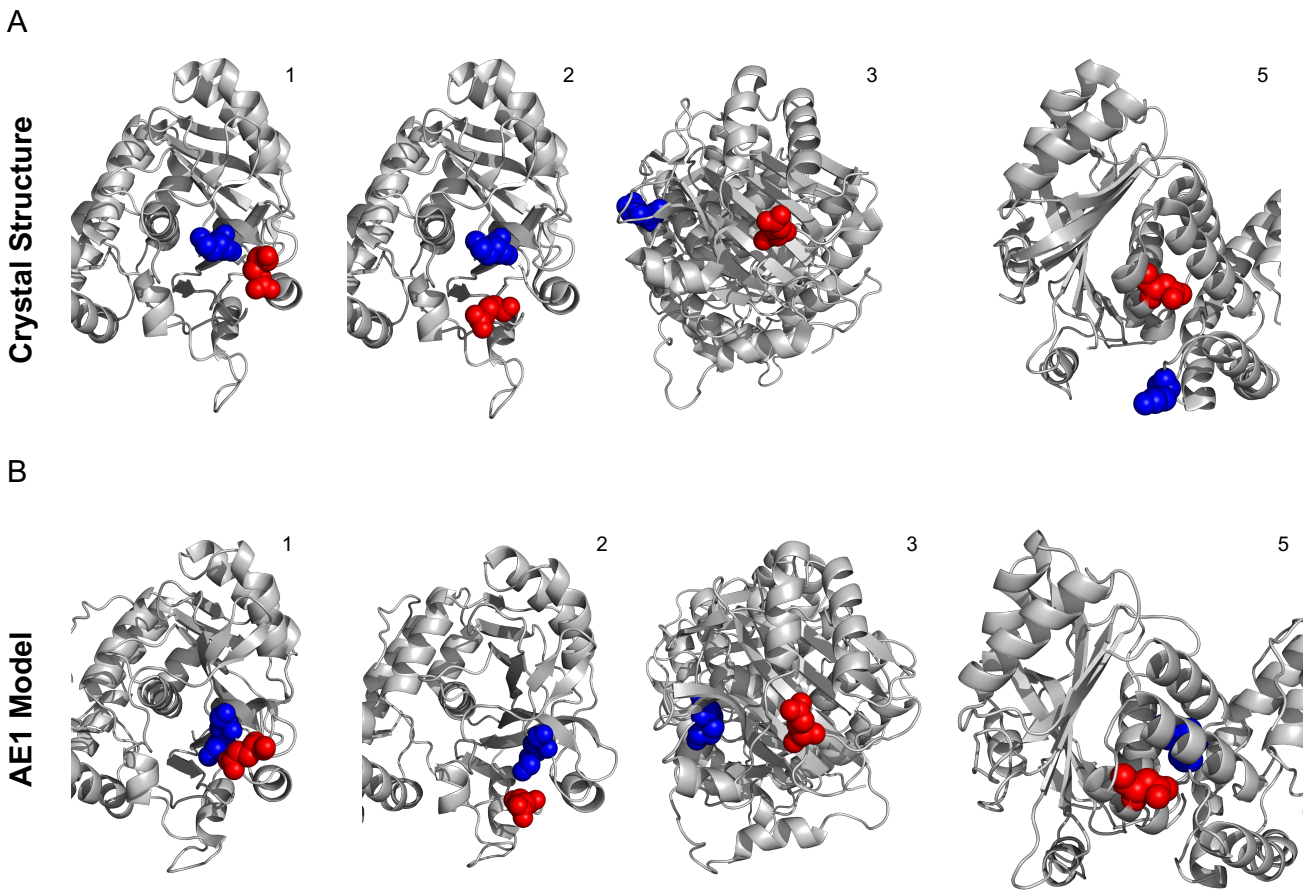

Fig. S3. Related to Table 1. Comparative schematic of cross-links involving the AE1 N-terminal domain in the crystal structure and our final model. (A) Cartoon depictions of identified AE1 cross-links involving N-terminal domain residues in the AE1 N-terminal domain structure published by Zhang et al. (Zhang et al., 2000). Cross-links shown are labeled according to the corresponding cross-link group in Table 1, and identities are as follows: (1) E68-K174; (2) E90-K174; (3) K174-E254; (5) D297-K353. Cross-link group 4 is an inter-chain cross-link, and is not displayed. See Table 1 for distances. Acidic residues are shown in red spheres, and basic residues are shown in blue spheres. (B) Cartoon depictions of identified AE1 cross-links involving N-terminal domain residues in our full-length AE1 model. Cross-link groups, residues, and color-coding are as in panel A.

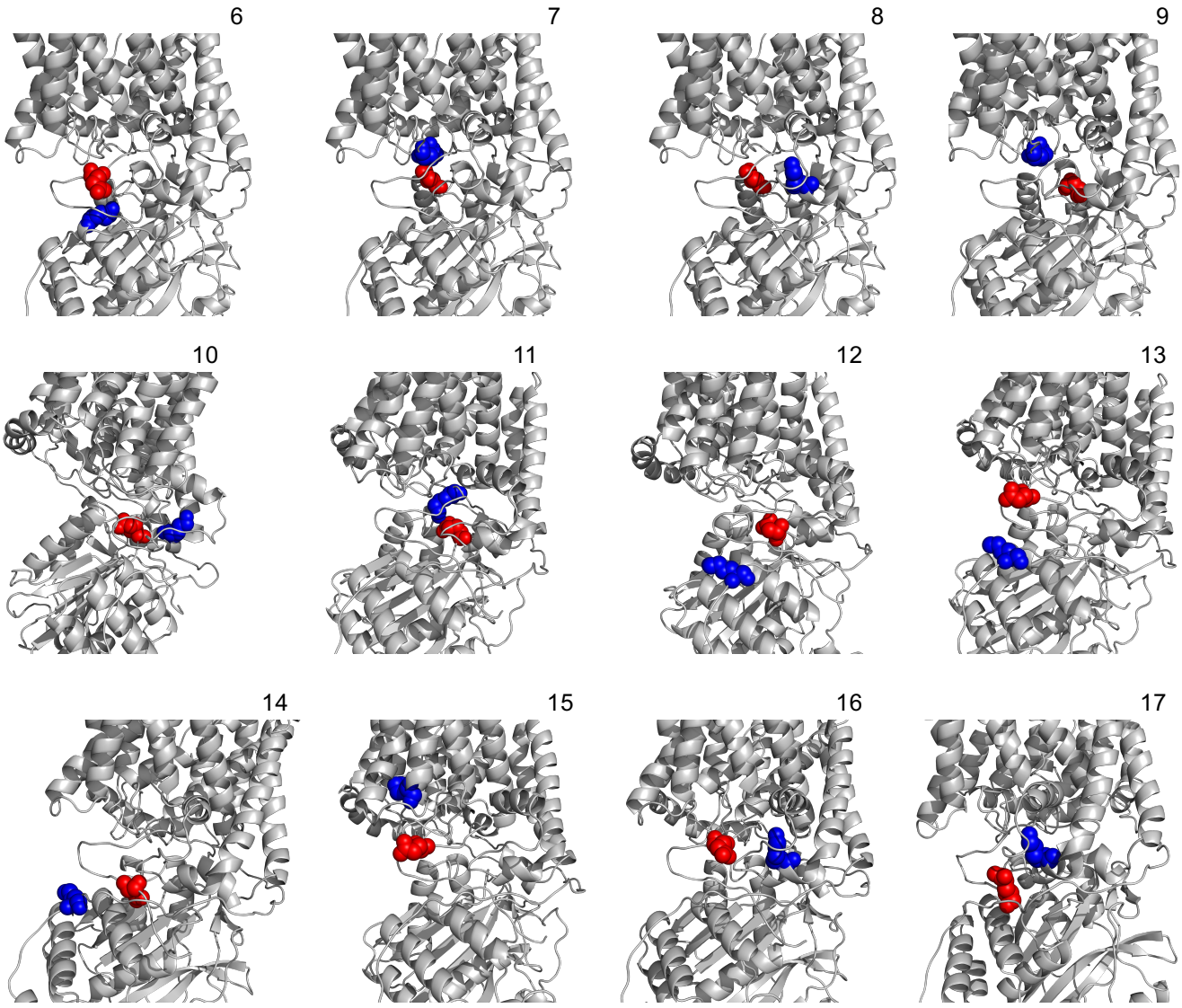

Fig. S4. Related to Table 1. Close-ups of cross-links involving the AE1 cytoplasmic loops. Cross-links shown are labeled according to the corresponding cross-link group in Table 1, and identities are as follows: (6) K116-D897; (7) D143-K743; (8) D143-K826; (9) E238-K743; (10) E238-K826; (11) E238-K892; (12) K353-D821; (13) K353-E899; (14) K353-D905; (15) K592-E899; (16) K826-E897; (17) K826-E906. See Table 1 for distances. Acidic residues are shown in red spheres, and basic residues are shown in blue spheres.

Movie S1. Related to Fig. 3. 360-degree cartoon view for the full-length AE1 model. Color-coding is the same as Fig. 1: N-terminal cytoplasmic domain (wheat), previously uncharacterized linker domain (brown), and C-terminal ion channel (grey). Also, the following segments are shown using "sticks": the cytoplasmic region between transmembrane spans 6 and 7, also referred to as C1 (orange), the cytoplasmic region between transmembrane spans 10 and 11, also referred to as C2 (green), the cytoplasmic region between transmembrane spans 12 and 13, also referred to as C3 (red), and the C-terminal tail following transmembrane span 14 also referred to as C-tail (blue).