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Supplementary Information for

Requirement of Xk and Vps13a for the P2X7-mediated phospholipid scrambling and cell lysis in mouse T cells

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Supplementary Figures

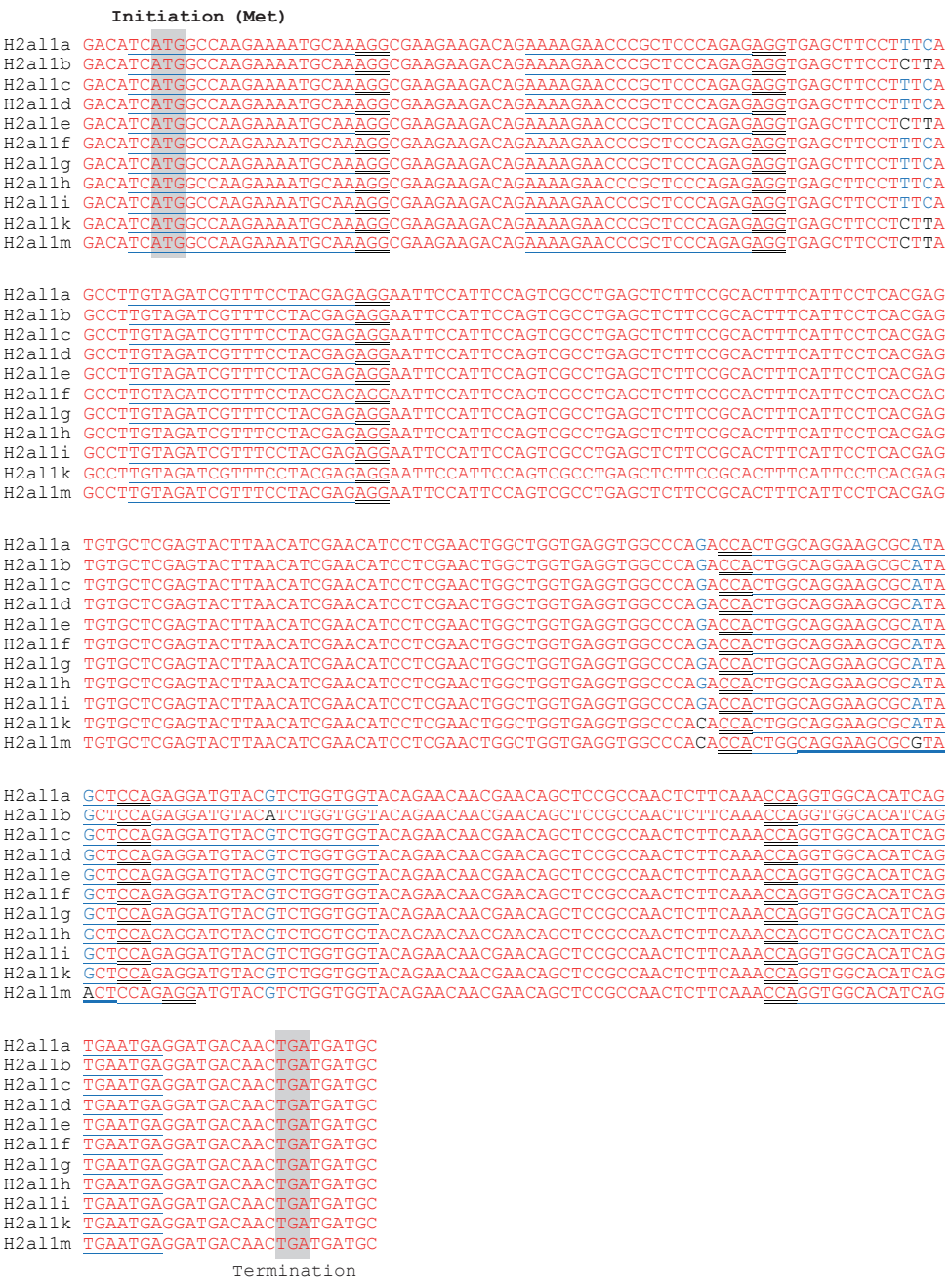

Fig. S1. Sequence alignment of *H2al1* variant genes. The nucleotide sequences of eleven mouse *H2al1* variant genes (GenBank NM_001111037, 001242947, 001242949, 001242950, 001025260, 001242951, 001242952, 001242953, 001242954, 001085537, and 029588) are aligned. The nucleotides conserved in all eleven variants are in red, and those conserved in more than seven variants are in blue. Blue underlines indicate Protospacer sequences, and double underlines protospacer-adjacent motifs (PAM). Initiation (ATG) and termination (TGA) codons are shaded in gray.

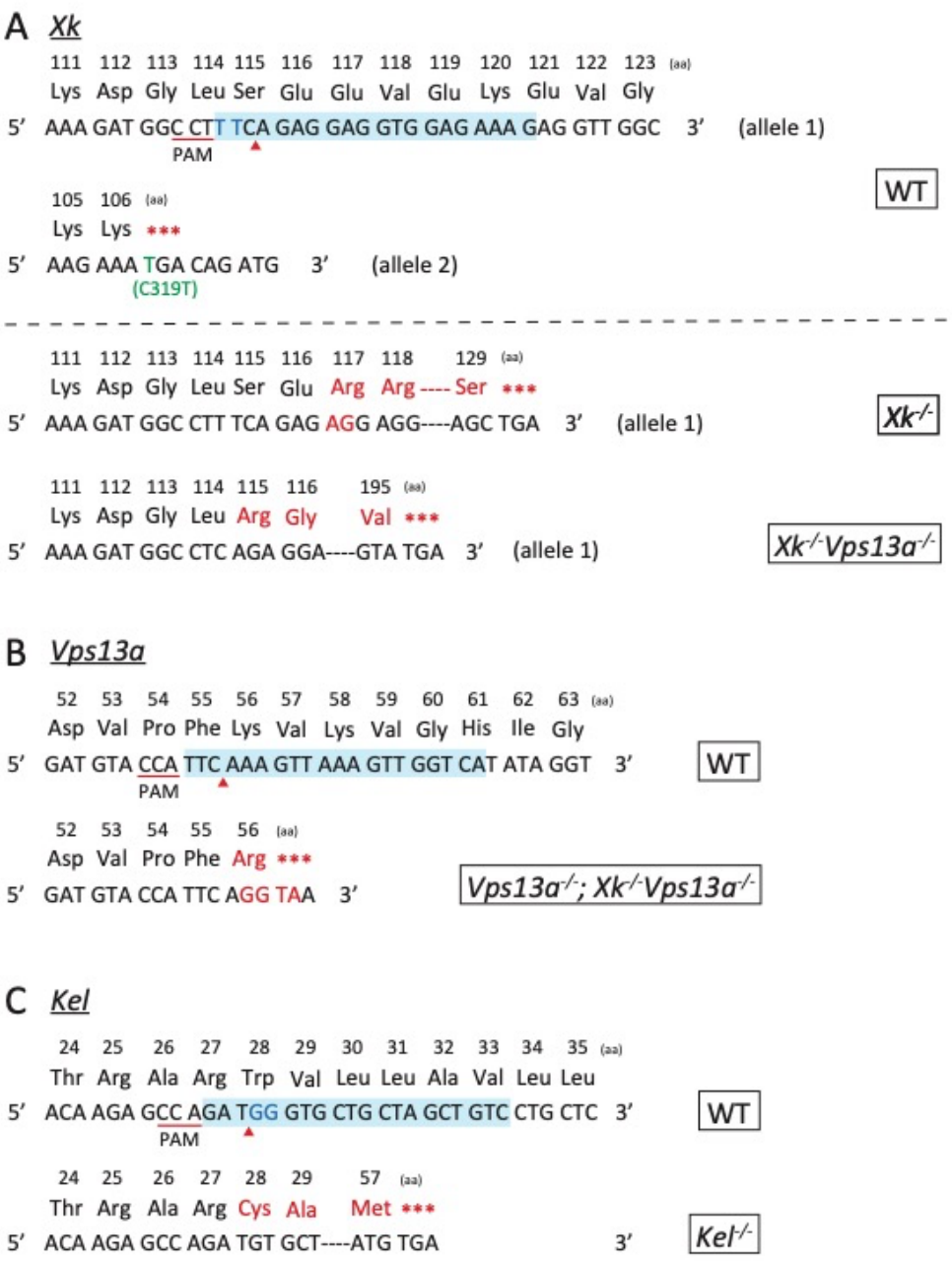

Fig. S2. Gene knock-out in mouse WR19L cells by the CRISPR/Cas9 system. (*A*) The wild-type and mutated allele (allele 1) of the *Xk* gene in *Xk*$^{-/-}$ and *Xk*$^{-/-}$*Vps13a*$^{-/-}$ *DKO*-P2X7 are shown. The other allele (allele 2) of the *Xk* gene in WR19L cells had been inactivated by a nonsense mutation, possibly due to the vulnerability of the inactive X chromosome in cancer cells (1). (*B*) Two alleles of *Vps13a* carried the same 4-bp insertion in *Vps13a*$^{-/-}$ and *Xk*$^{-/-}$*Vps13a*$^{-/-}$ *DKO*-P2X7. (*C*) Two alleles of *Kel* carried the same 2-bp deletion in *Kel*$^{-/-}$ *DKO*-P2X7. Protospacer sequences are highlighted in light blue, protospacer-adjacent motifs (PAM) are underlined in red, and red arrowheads point to cleavage sites. Sequences deleted, inserted, or mutated are shown in blue, red, and green, respectively. The mutated amino acids are shown in red.

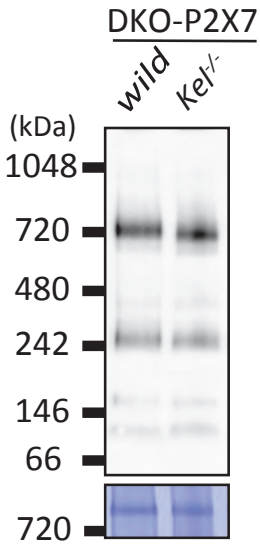

Fig. S3. BN-PAGE analysis of Xk in *Kel*$^{-/-}$ WR19L cells. The solubilized crude membrane fractions (9.1 μg protein) from *DKO-P2X7* and *Kel*$^{-/-}$*DKO-P2X7* (*Kel*$^{-/-}$) were separated by BN-PAGE and analyzed by Western blotting with anti-Xk. The membrane was stained with CBB and shown in the lower panel.

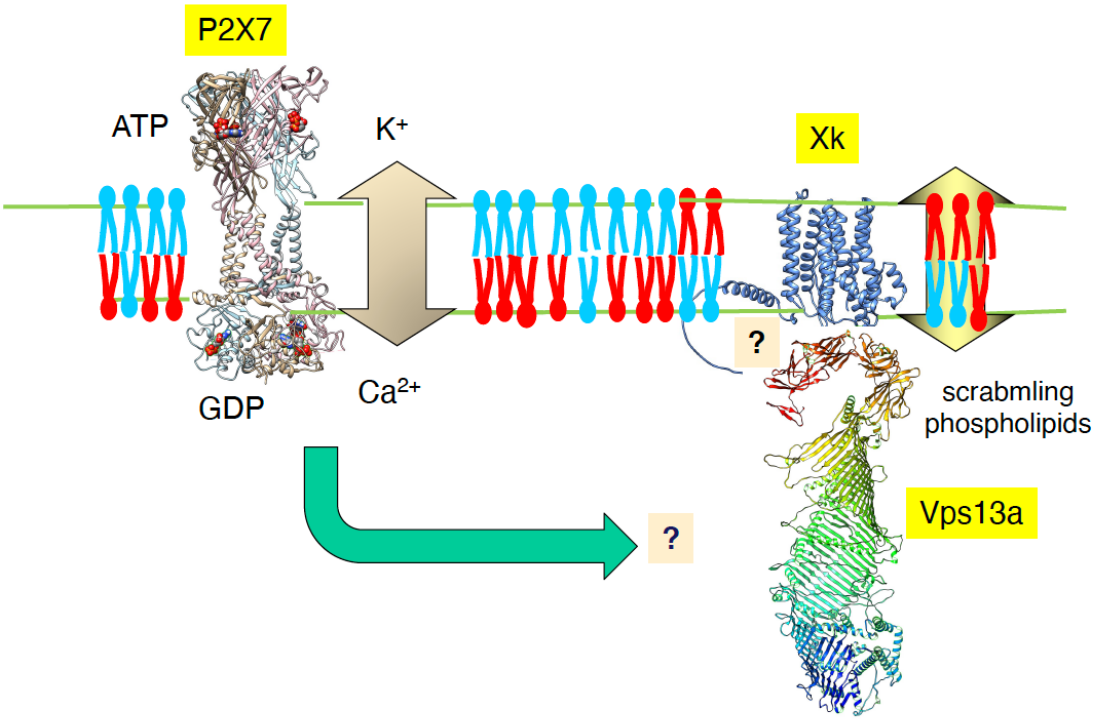

Fig. S4. A working hypothesis for the ATP-induced scrambling of phospholipids. In the resting cells, most of PtdCho is localized in the outer leaflet of plasma membranes, while all PtdSer is confined to the inner leaflet. The binding of ATP to the trimeric P2X7 receptor activates its channel activity, including the K^+-efflux and Ca^{2+}-influx. The Xk is associated with Vps13a via probably the interaction of the Xk's β-hairpin in the cytoplasmic region and the Vps13's N-terminal region. An unidentified signal from the activated P2X7 receptor would potentiate the Xk-Vps13a complex to scramble phospholipids. The ATP-bound open structure of P2X7 is from Mansoor et al. (2) (PDB; 6U9W). AlphaFold (https://alphafold.ebi.ac.uk/) predicted the structures of Xk and Vps13a.

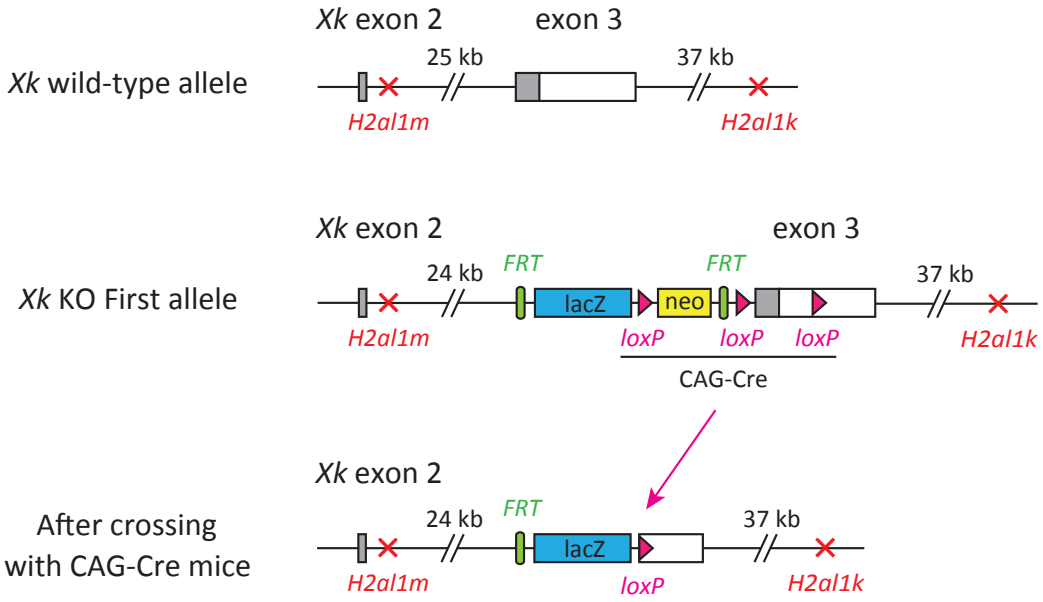

Fig. S5. The wild-type and knock-out alleles of mouse *Xk* gene. In the knock-out first (Xk KO First) allele, a DNA fragment carrying a splice acceptor site, *FRT*, *lacZ*, *loxP*, *neo*, *FRT*, *loxP*, a part of exon 3, and loxP, was inserted in mouse *Xk* gene. Two *H2al1* genes in the flanking region of exon 3 of the *Xk* gene are indicated. Crossing the mice carrying the KO first allele with CAG-*Cre* mice removes the neo and a part of the exon 3 including the coding region (gray).

SI References

1. N. Jäger *et al.*, Hypermutation of the inactive X chromosome is a frequent event in cancer. *Cell* **155**, 567-581 (2013).
2. A. E. McCarthy, C. Yoshioka, S. E. Mansoor, Full-length P2X7 structures reveal how palmitoylation prevents channel desensitization. *Cell* **179**, 659-670 (2019).