

# iCeMS-iTHEMS Joint Workshop on Interdisciplinary Biology

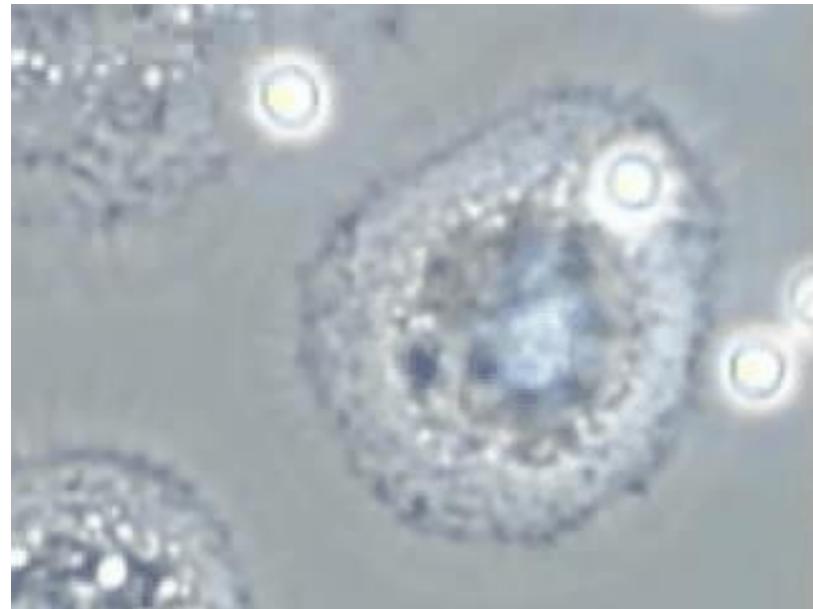
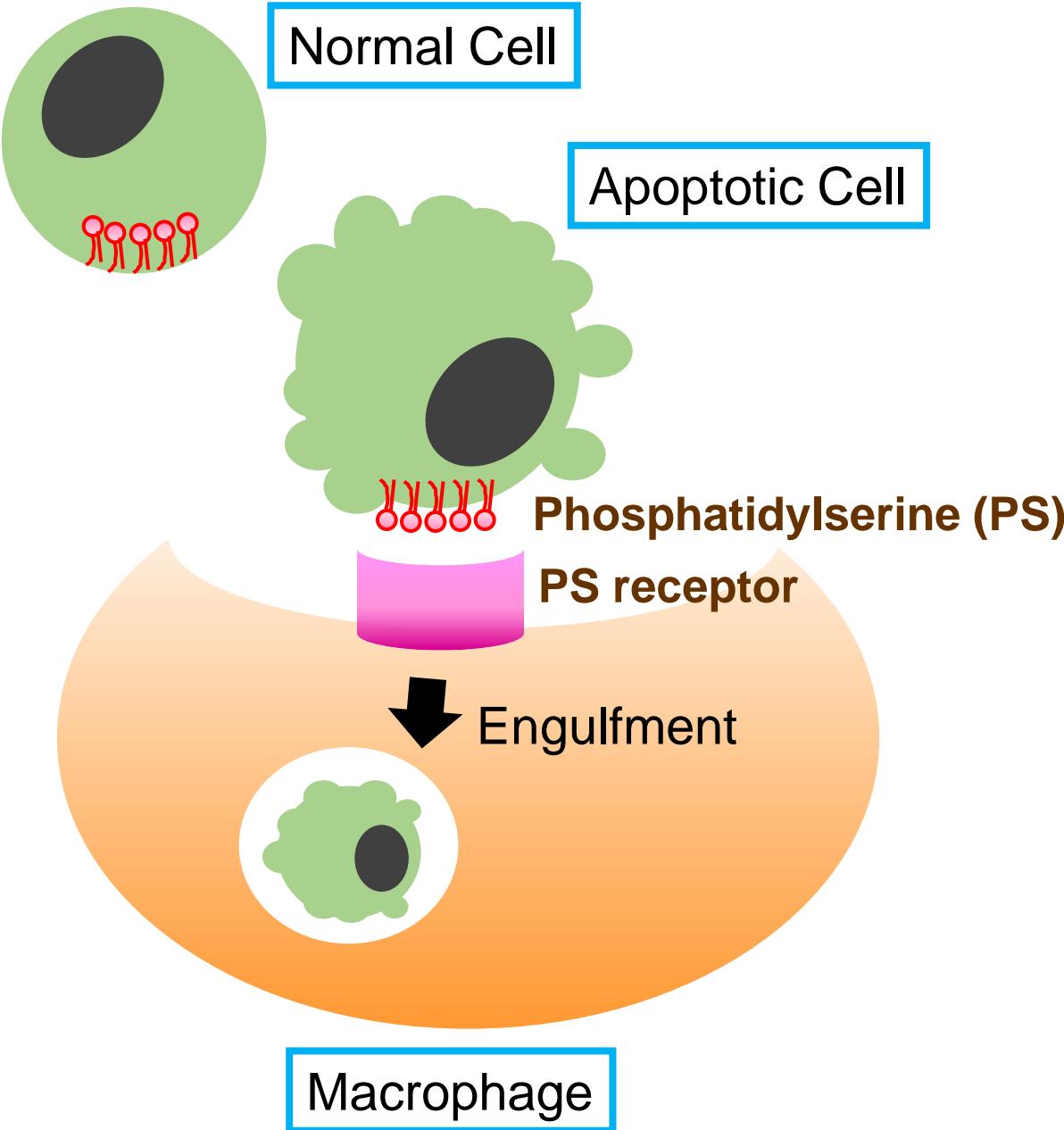
## Phospholipid Scrambling on the Plasma Membrane

Kyoto University, iCeMS

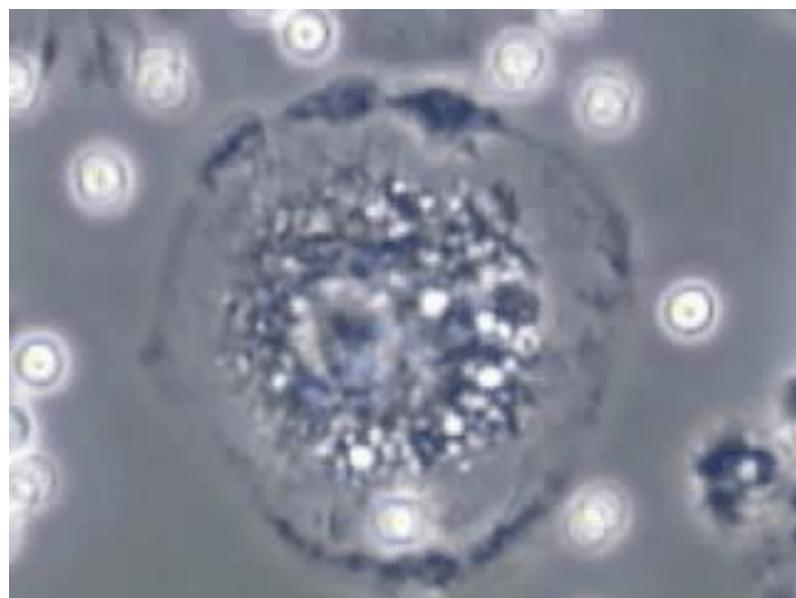
Jun Suzuki, Ph.D.



# Phosphatidylserine: Eat-me signal

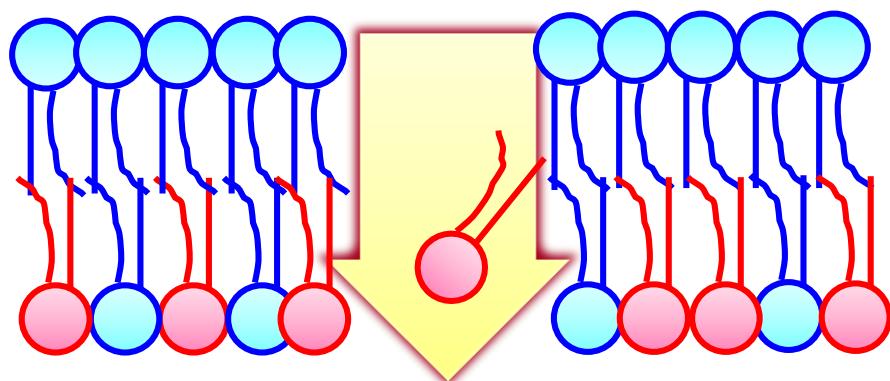


What happens if PS is masked?



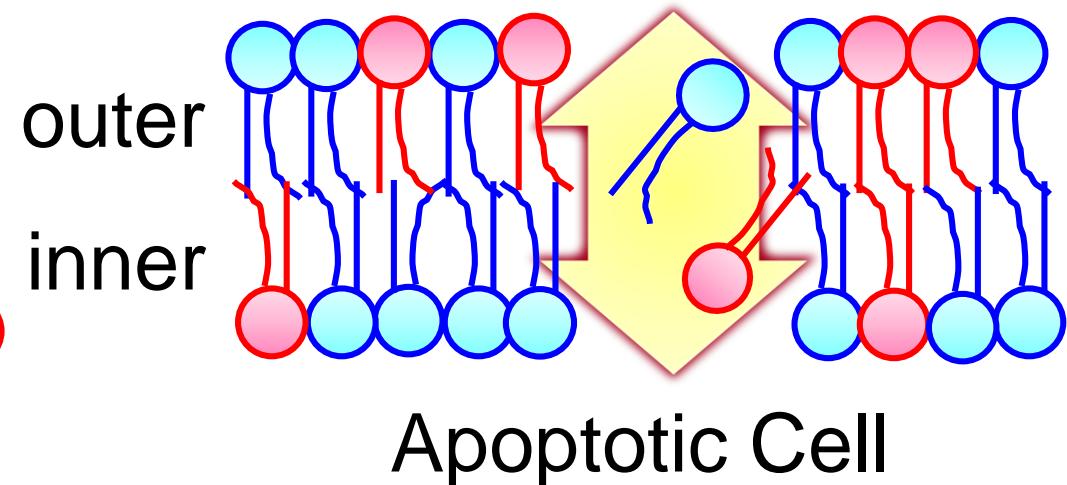
# PS is exposed on the cell surface in dying cells by scramblases

ATP-dependent Flippases  
(P4-type ATPase)



Normal Cell

Calcium-dependent Scramblases  
(not identified)



Apoptotic Cell



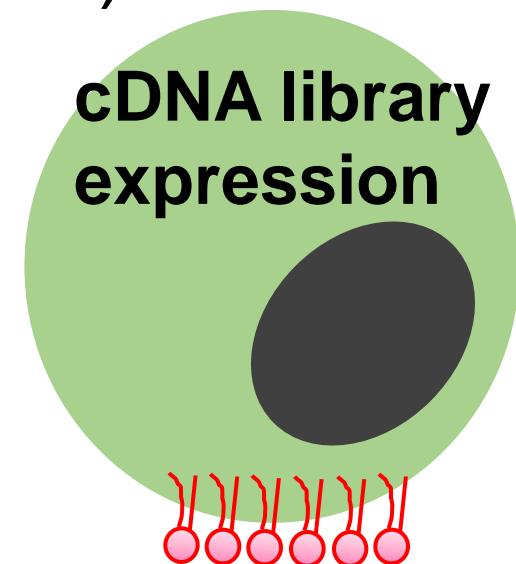
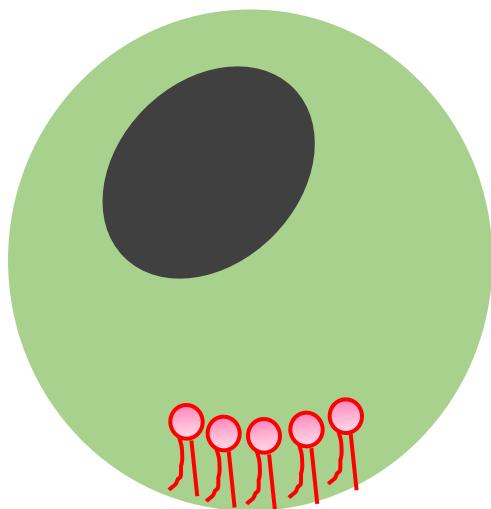
PS

**PLSCR1  
Denied!!**

What is genuine scramblases?

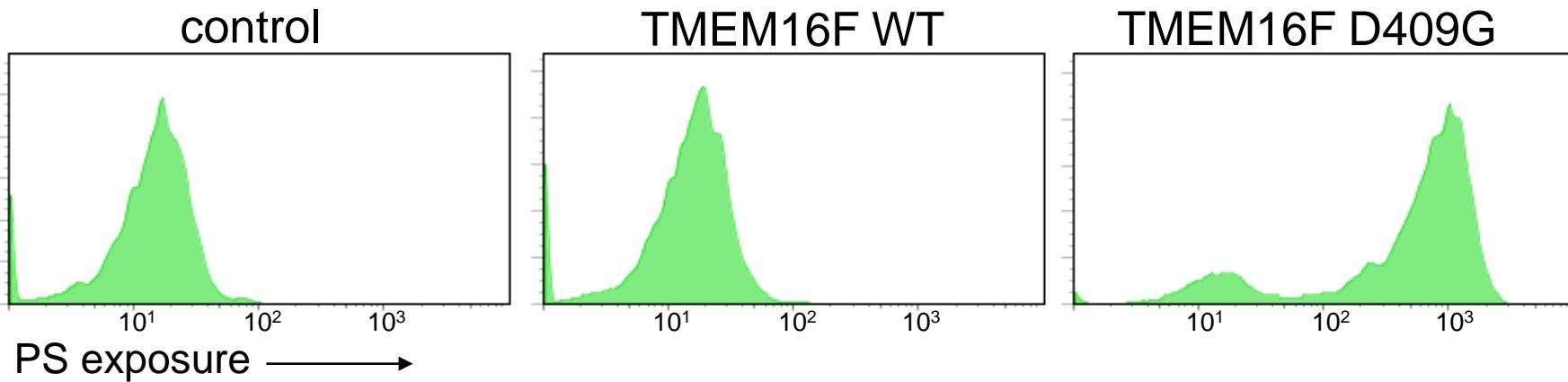
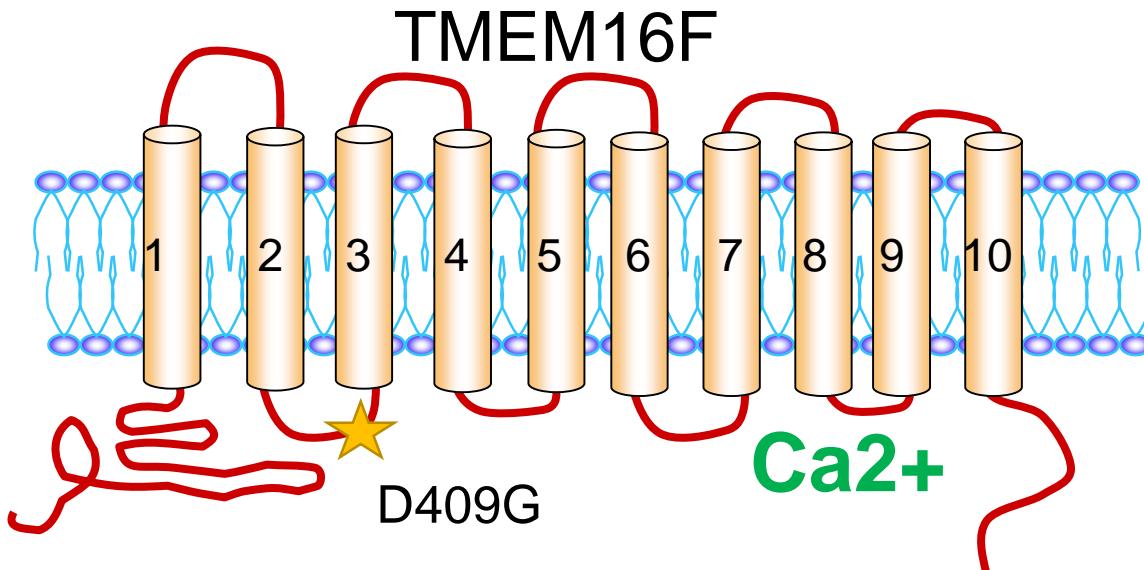
# Approach

1. Living cells can transiently expose PS using the calcium ionophore A23187.
2. Obtain PS exposure-sensitive mutants.  
(19 times repeated sorting, PS19 cells).
3. Expression cloning  
(cDNA library from PS19 cells).

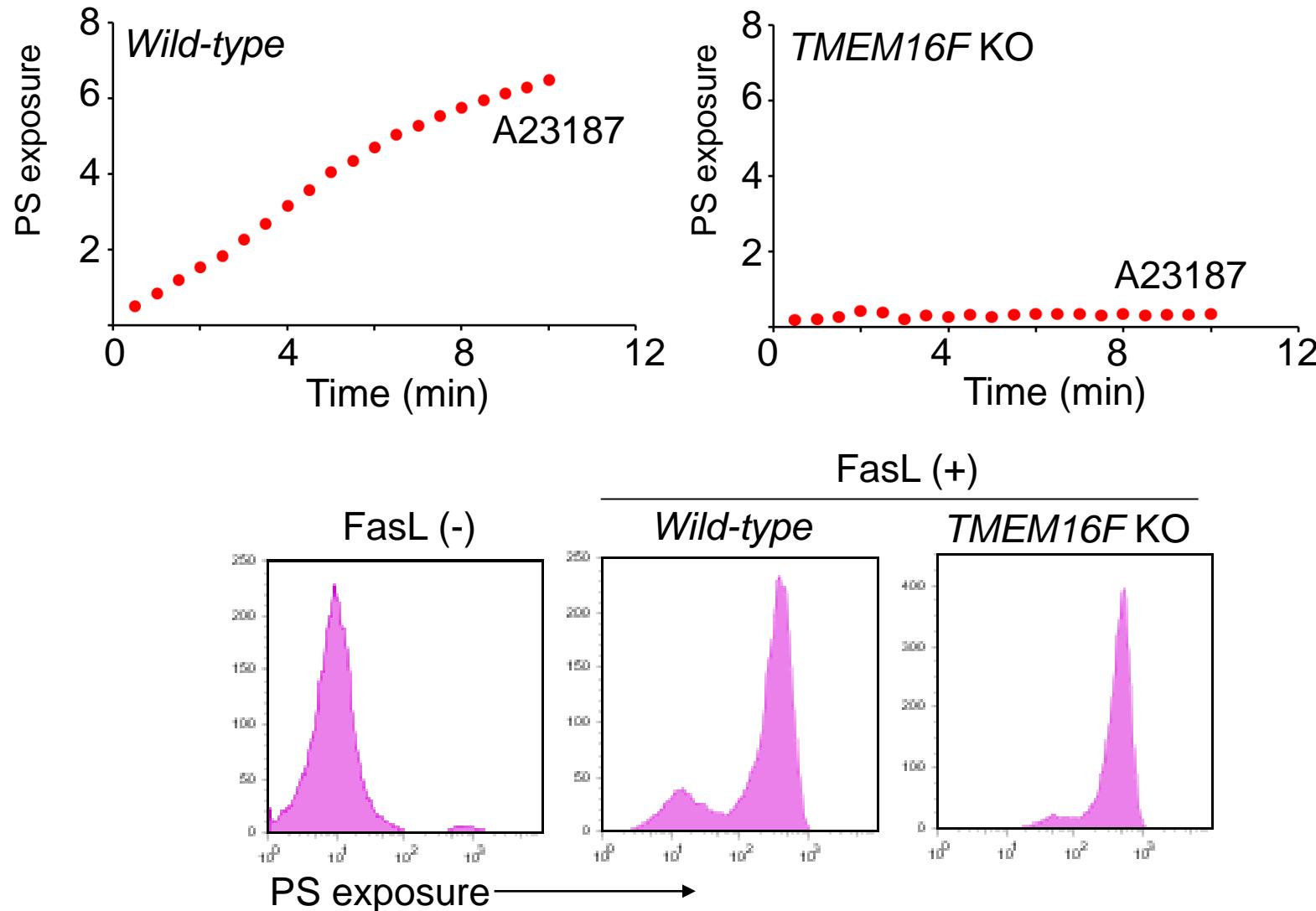


**Continuous PS exposure**

# TMEM16F is essential for calcium-dependent PS exposure

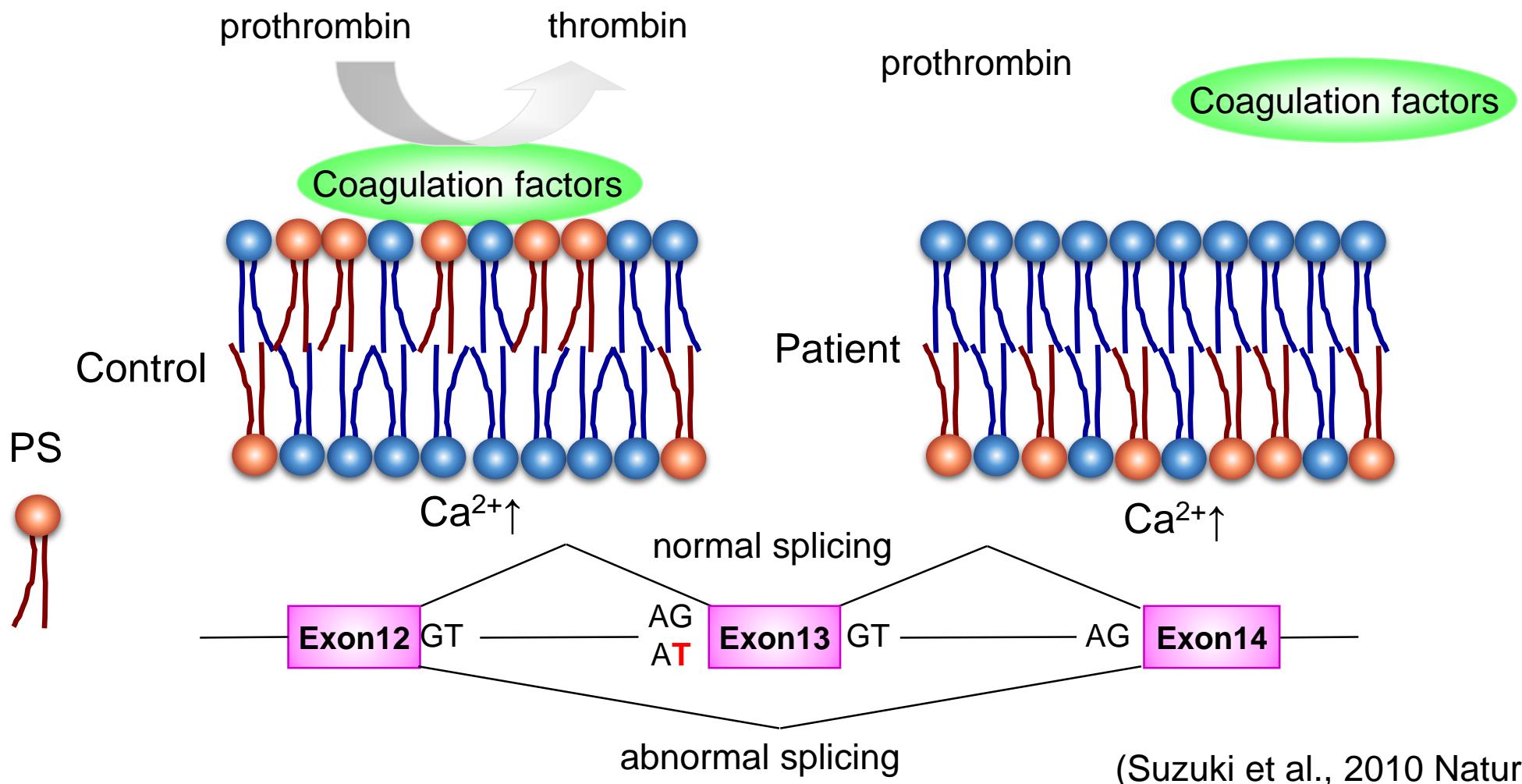


# TMEM16F is not involved in apoptotic PS exposure



# Calcium-dependent PS exposure is defective in Scott syndrome patient

Scott syndrome: Mild bleeding disorder



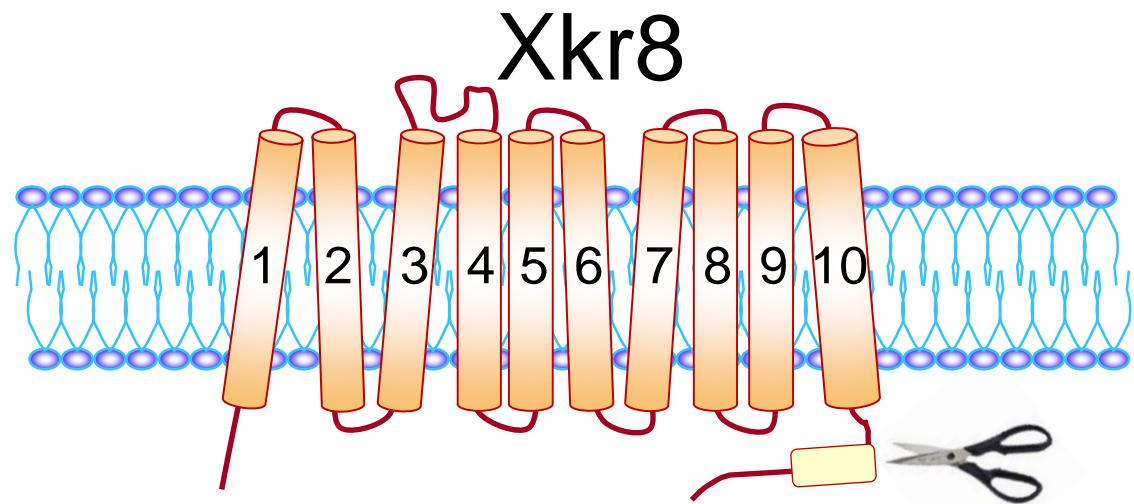
# Summary of TMEM16 family

	Cl <sup>-</sup> channel	Lipid scrambling	Cellular localization	Tissue distribution	Human disease
TMEM16A	O	-	PM	Most	
TMEM16B	O	-	PM	eye	
TMEM16C	-	O	PM	brain	Craniocervical dystonia
TMEM16D	-	O	PM	ovary / uterus	
TMEM16E	-	O	intracellular	muscle bone	Muscle dystrophy Gnathodiaphyseal dysplasia
TMEM16F	-	O	PM	Ubiquitous	Scott syndrome
TMEM16 G	-	O	PM	stomach	
TMEM16H	-	-	intracellular	ubiquitous	
TMEM16J	-	O	PM	intestine	
TMEM16K	-	-	intracellular	ubiquitous	Cerebellar ataxia

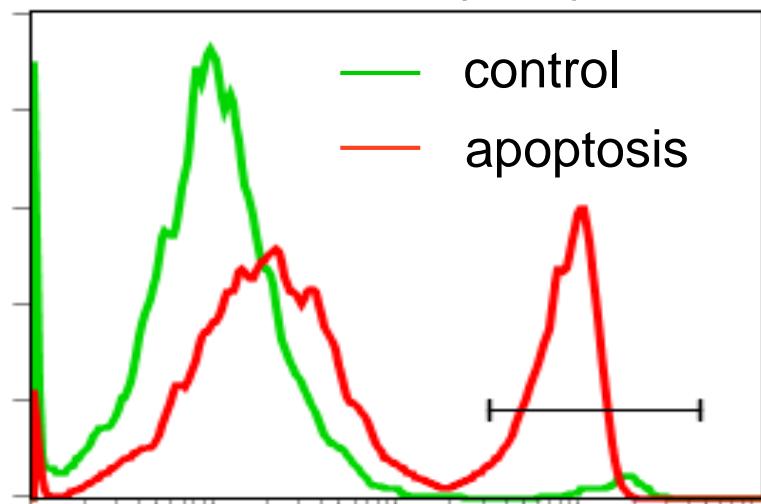
(Suzuki et al., 2013 J Biol Chem; Gyobu et al., 2015 Mol Cell Biol)

**What is a **scramblase**  
during apoptosis?**

# Xkr8 exposes PS during apoptosis

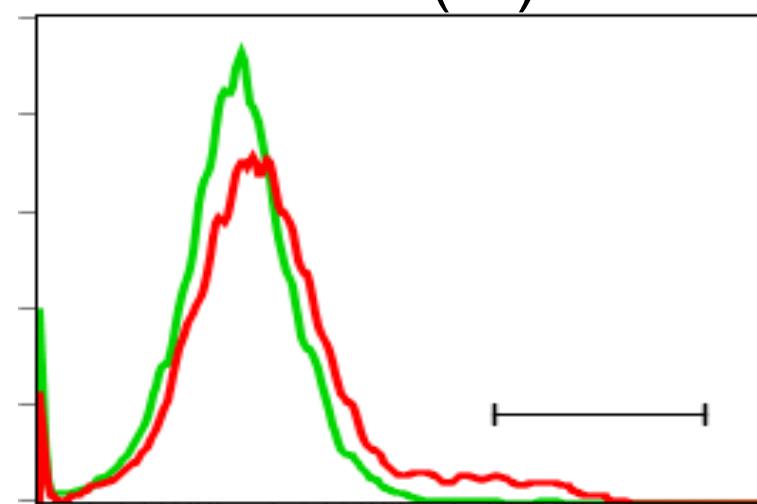


*Xkr8 (+/+)*



**Caspase3**

*Xkr8 (-/-)*



PS exposure →

(Suzuki et al., 2013 Science)

# Summary of Xkr family

	Phospholipid Scrambling	Caspase Cleavage	Tissue
Xkr1	-	-	Many Tissues
Xkr2	-	-	Skin, Lung, Intestine
Xkr4	○	○	Brain, Eye
Xkr5	-	-	Many Tissues
Xkr6	-	-	Brain
Xkr7	-	○	Testis
Xkr8	○	○	Ubiquitous
Xkr9	○	○	Intestine

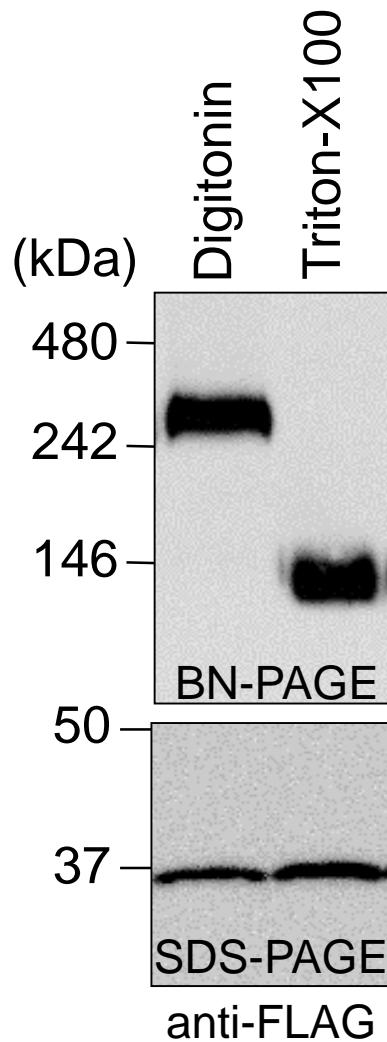
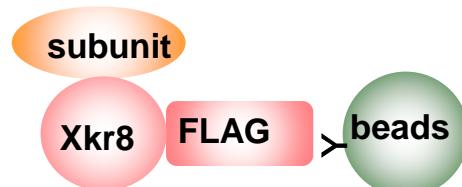
Do Xkrs require subunits for their function?

(Suzuki et al., 2014 J Biol Chem)

# Isolation of subunits in Xkr8 complex

Solubilize membrane of  
Xkr8 (-/-) cells expressing  
Xkr8-FLAG by detergents

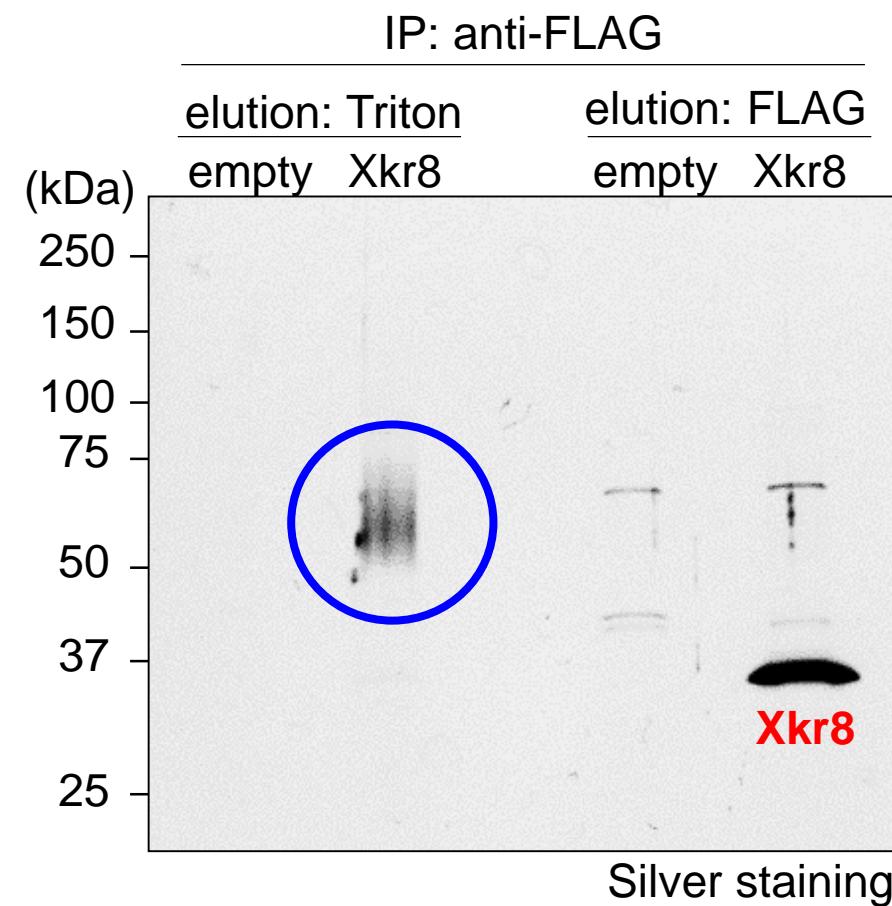
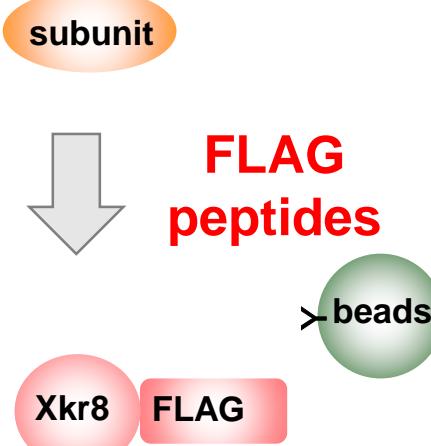
**Solubilize membrane  
with Digitonin and IP**



**Triton  
treatment**



**FLAG  
peptides**

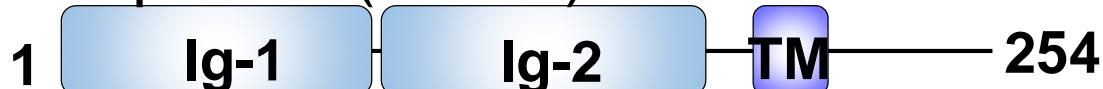


# Identification of BSG & NPTN as Xkr8 subunits

Basigin (BSG)

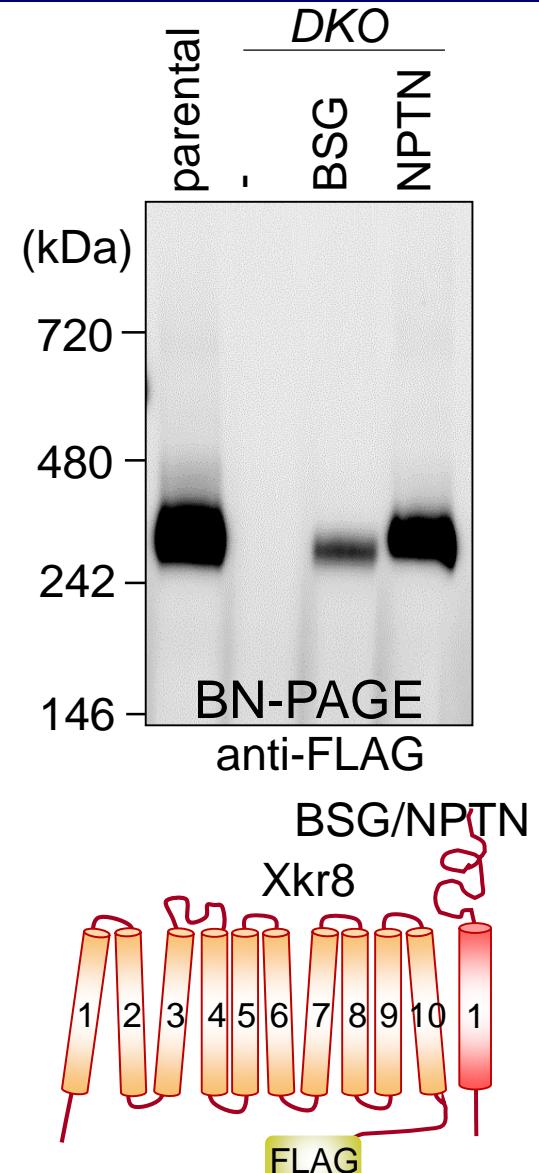


Neuroplastin (NPTN)

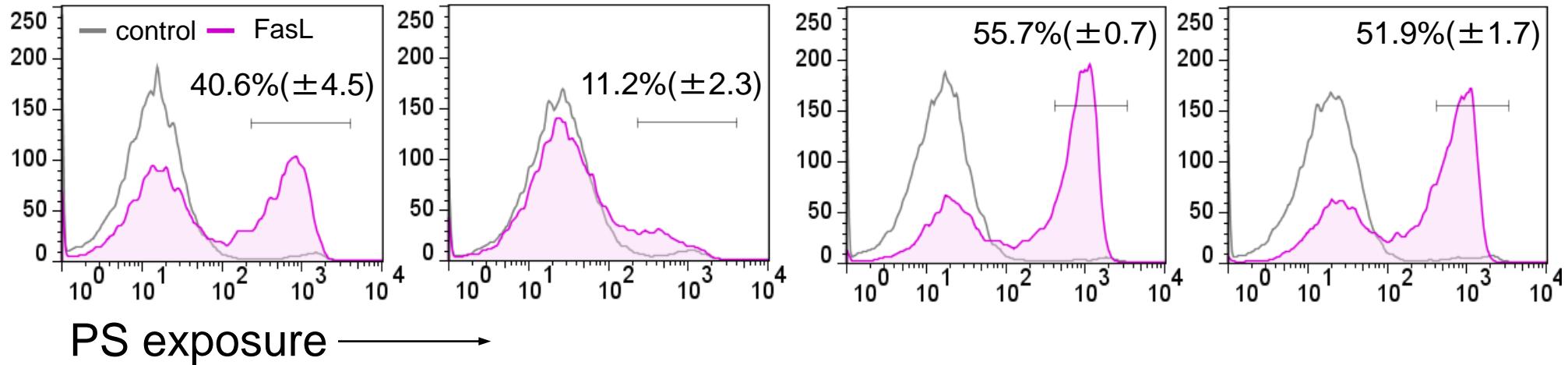
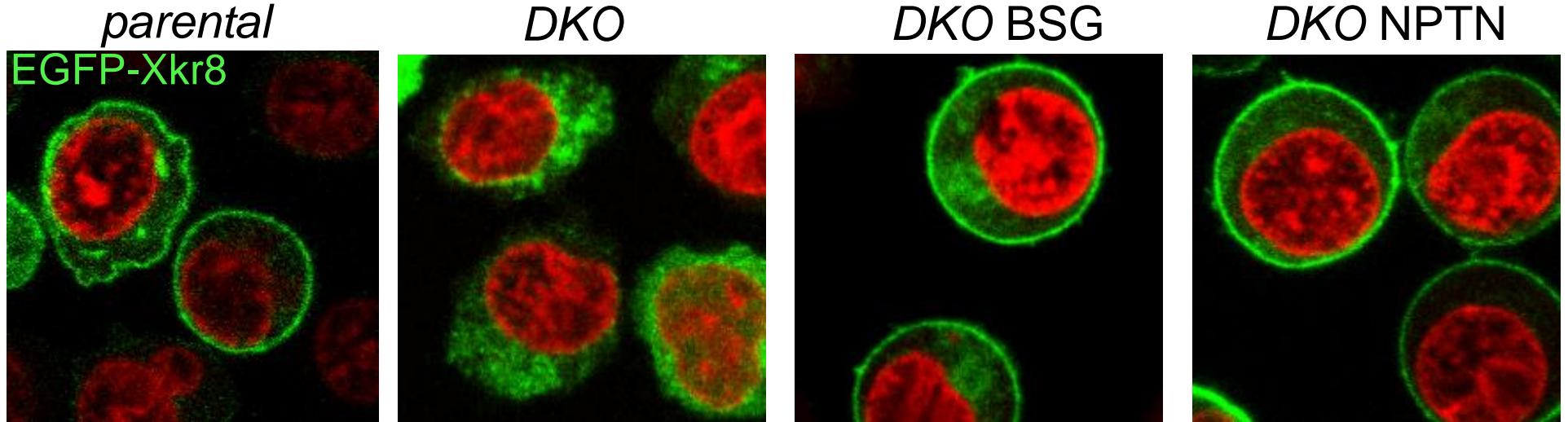


BSG A**LWPFLGIVAEVLV**LVTIIIFIY

NPTN P**LWPFLGILAE**III**LVVIIWVY**

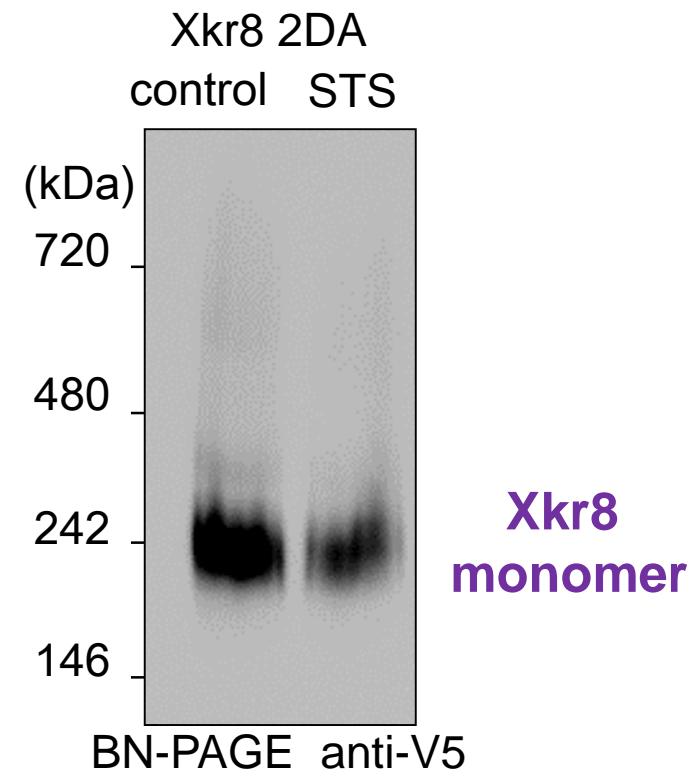
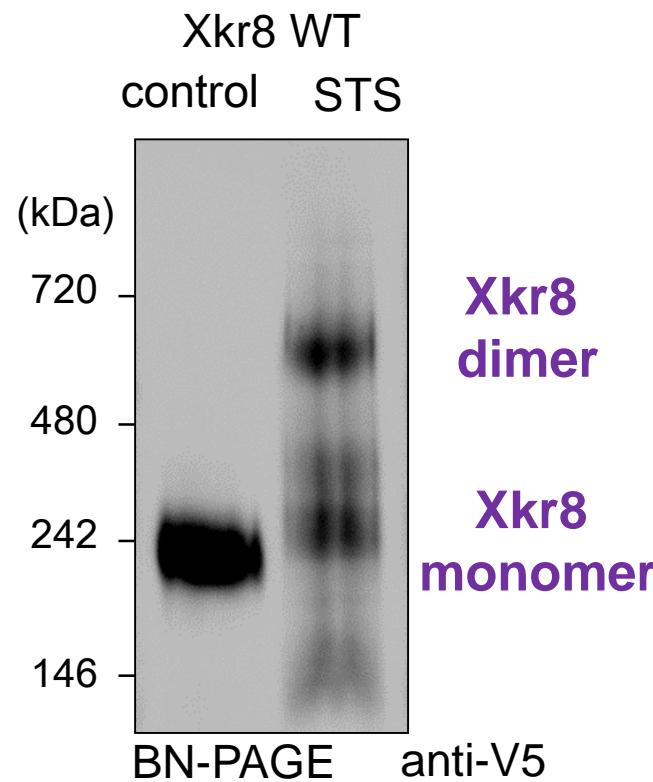
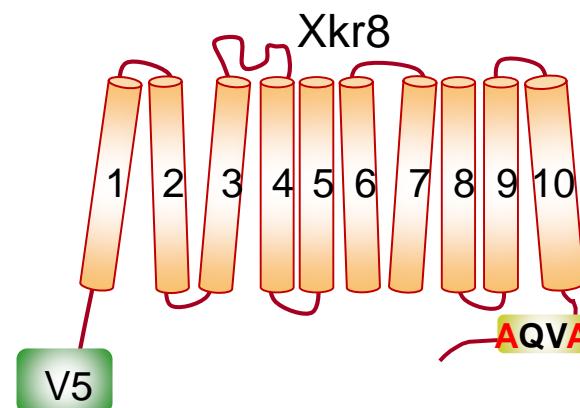
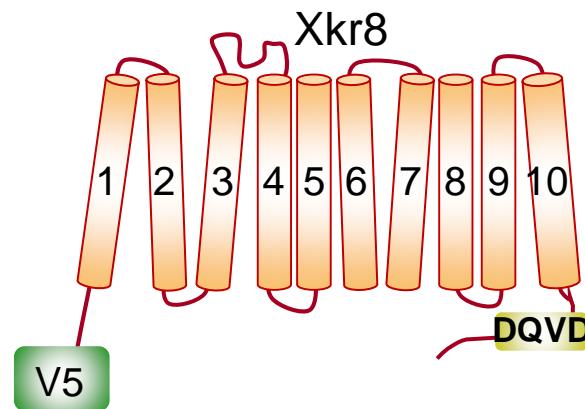


# BSG & NPTN are essential for apoptotic PS exposure



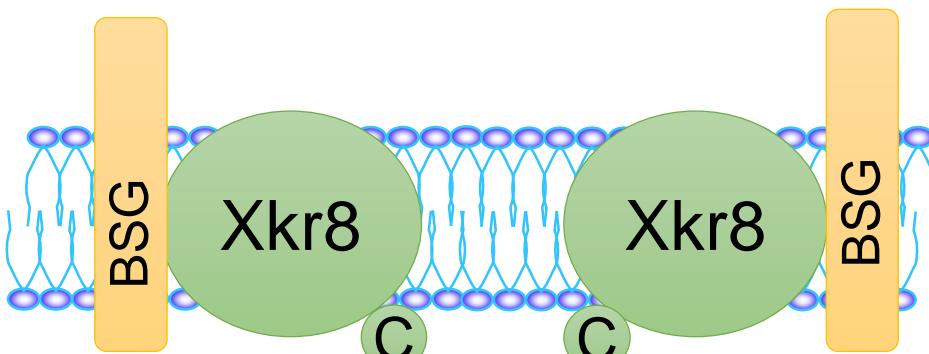
(Suzuki et al., 2016 PNAS)

# Xkr8 forms dimer during apoptosis

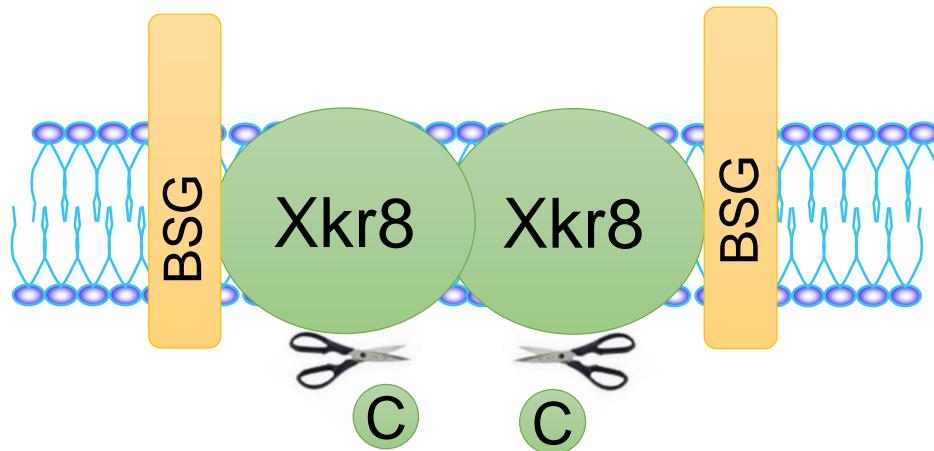


# Activation of Xkr8 and TMEM16F

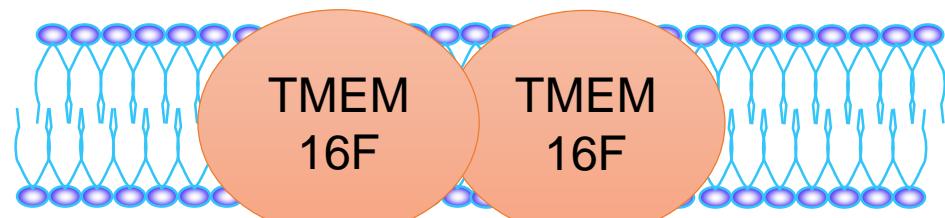
## Activation of Xkr8



Apoptotic stimuli  
(C-terminal cleavage of Xkr8)

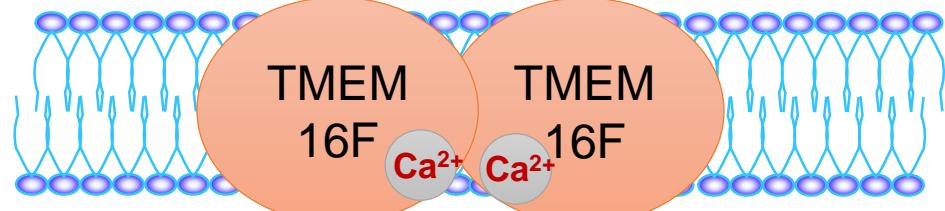


## Activation of TMEM16F

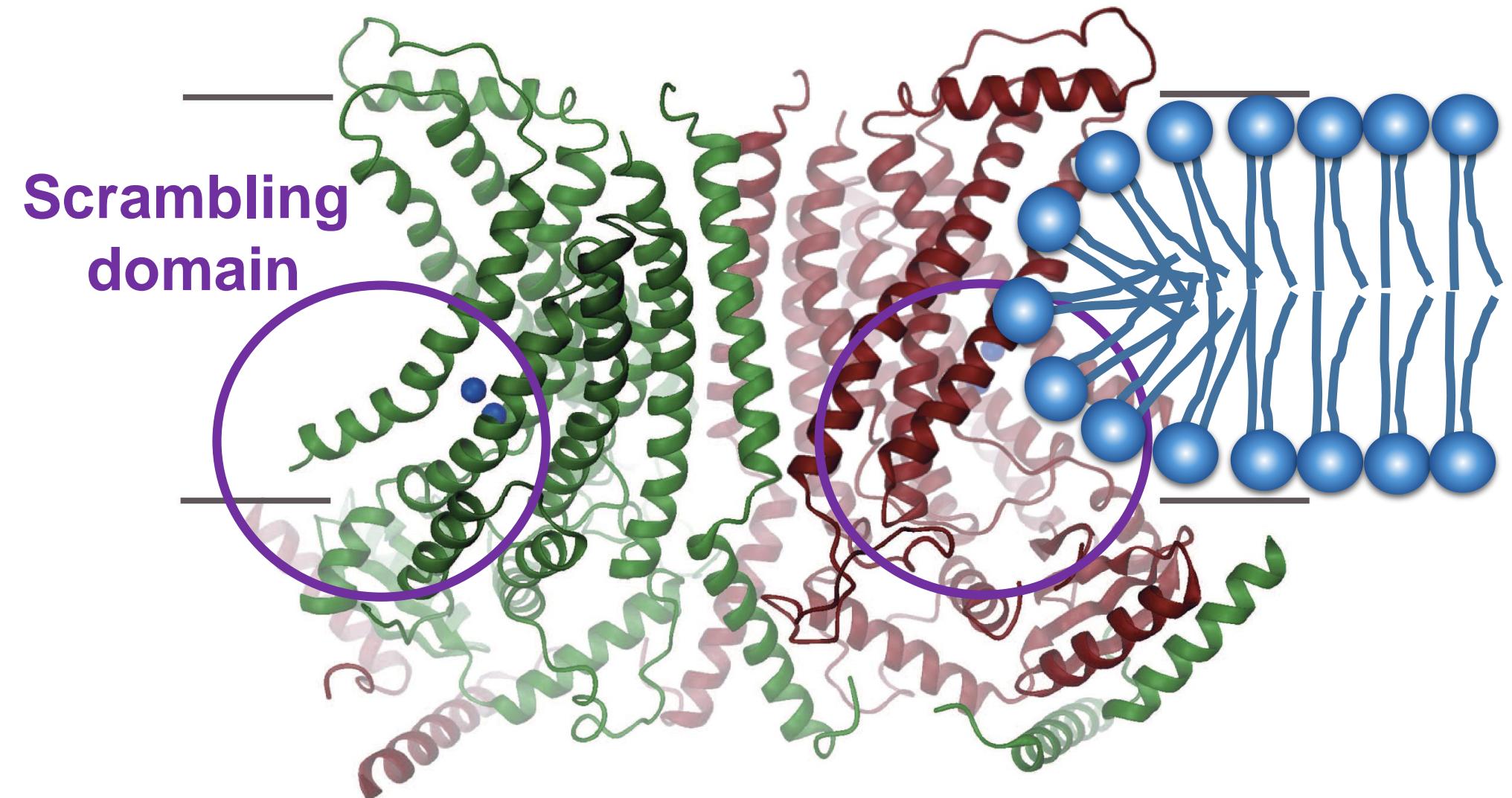


(Suzuki T et al., 2014; Ishihara et al., 2016)

Calcium stimulation  
(Direct binding of  $\text{Ca}^{2+}$  to TMEM16F)

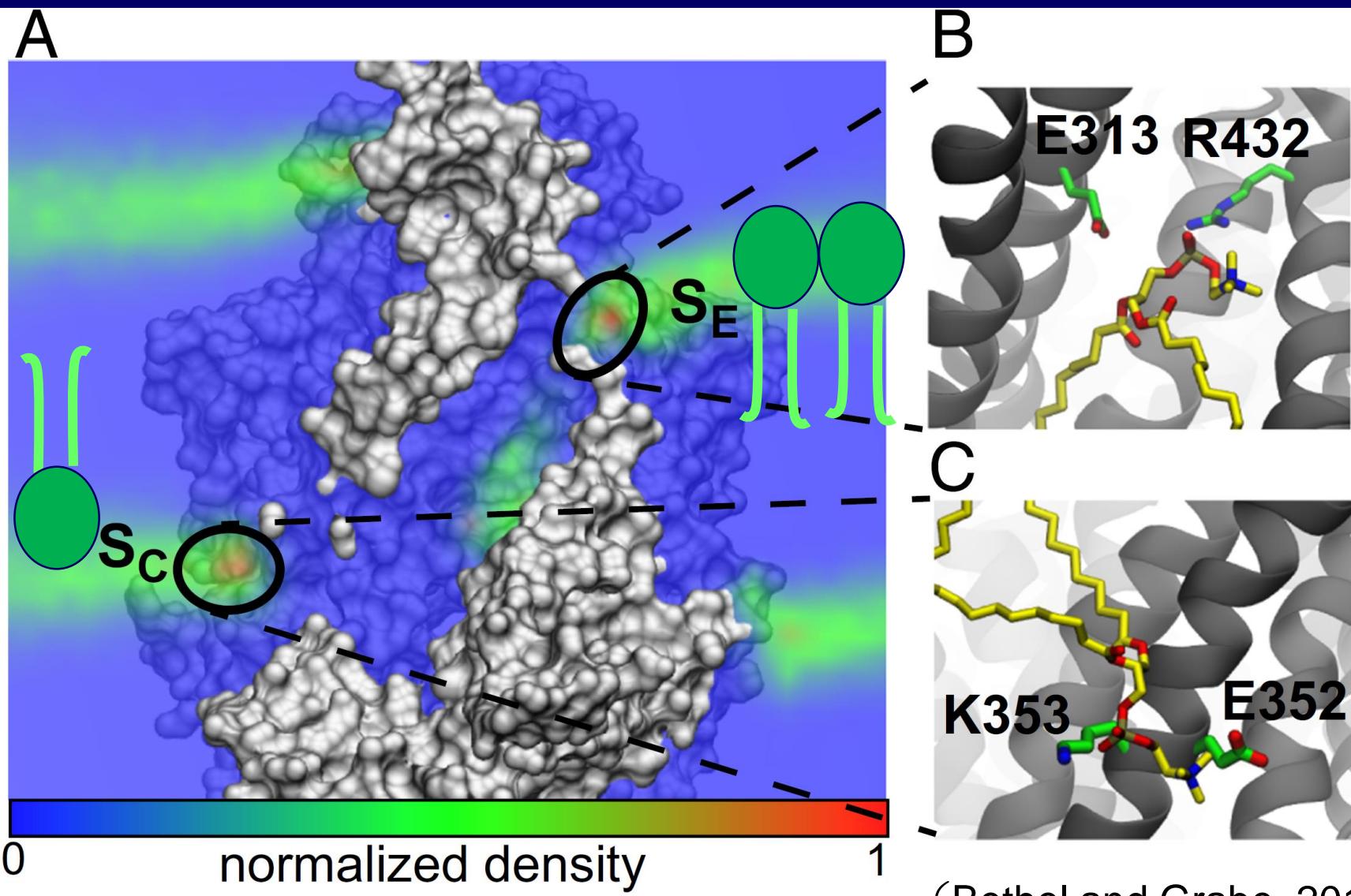


# Model of lipid scrambling by TMEM16



(Brunner et al., 2014 Nature; Yu et al., 2015 eLIFE; Gyobu et al., 2017 PNAS)

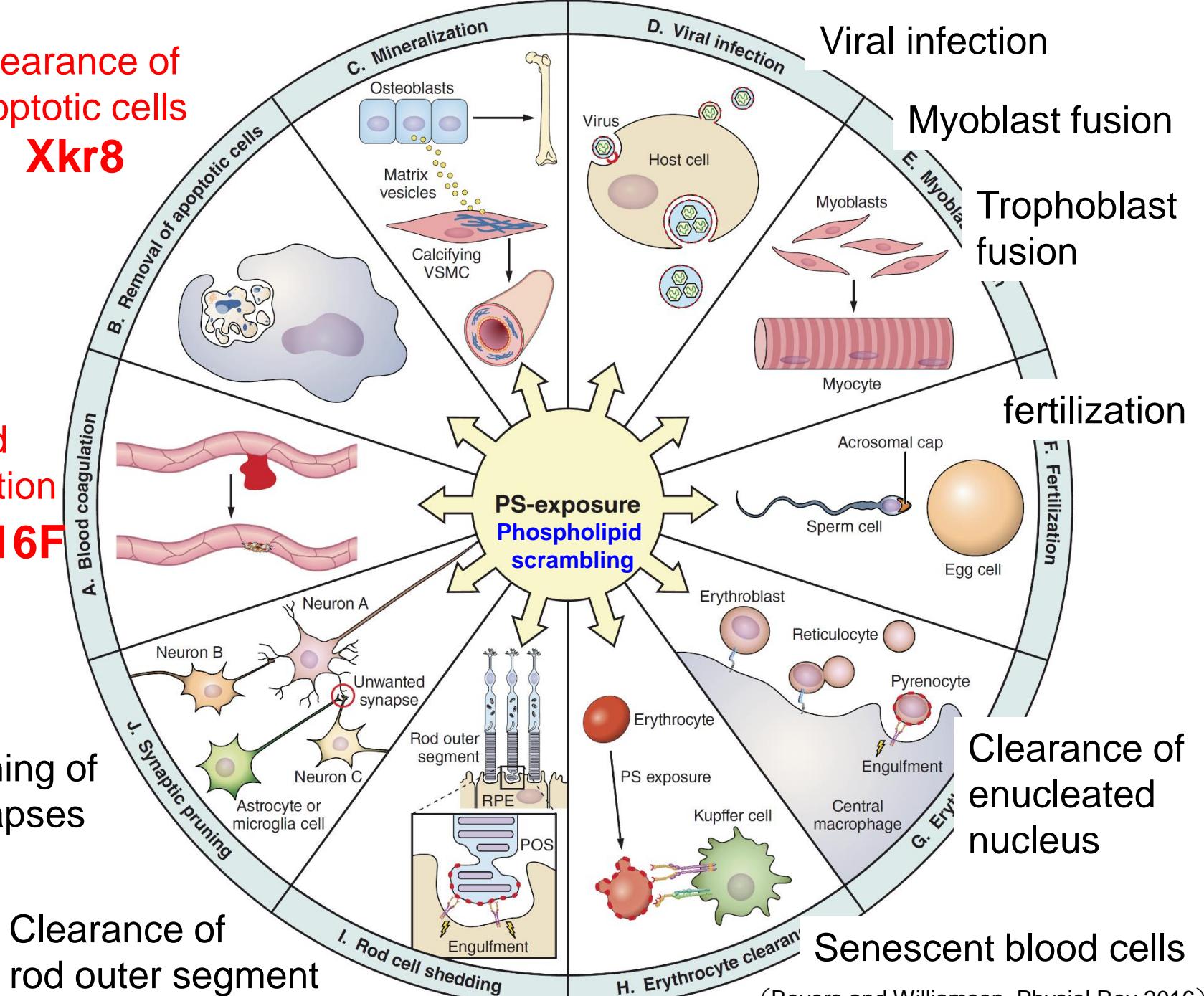
# Phosphate density computed from MD simulations



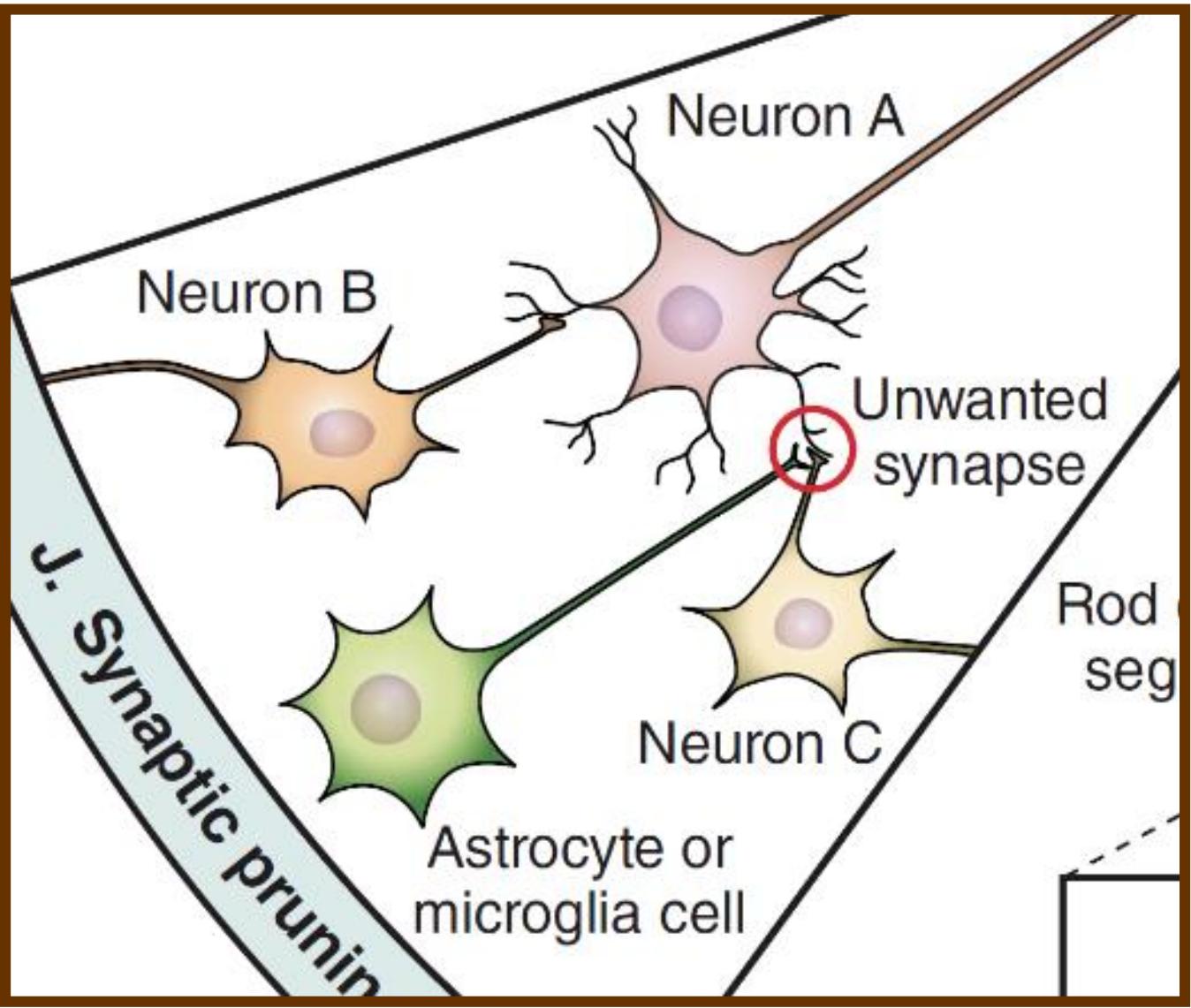
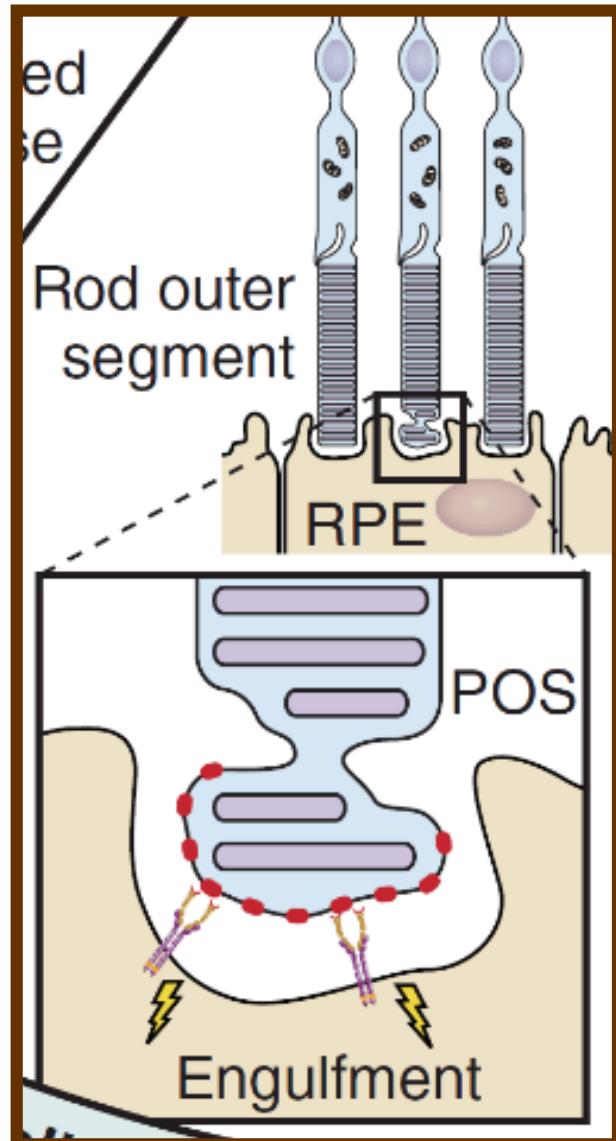
**Blood coagulation**  
**TMEM16F**

**Clearance of apoptotic cells**  
**Xkr8**

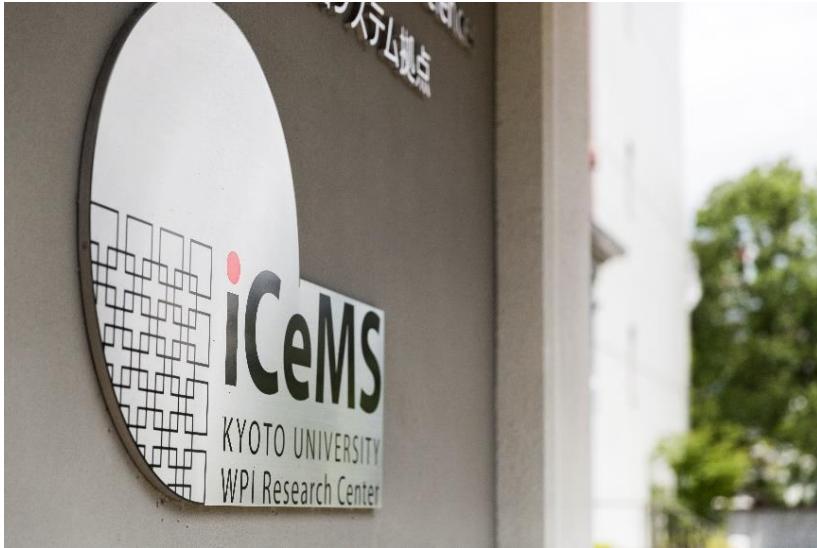
**Clearance of rod outer segment**



# Engulfment of ‘compartments’ in living cells



# Summary of Research in Suzuki Lab



**Lipid scrambling at  
plasma membranes  
and intracellular  
membranes.**

## Approaches

Screening to identify scramblases  
and related molecules

Molecular mechanisms of lipid  
scrambling



# Acknowledgement

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