Dec 7, 2025 11:10-12:25
APCN X TSN 2025
Novel regulation and function in renal Na^+-associated transporters

Role and regulation of ubiquitin ligase Kelch-like 3 in health and disease

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COI Disclosure

Shigeru Shibata

1: Advisory role : none
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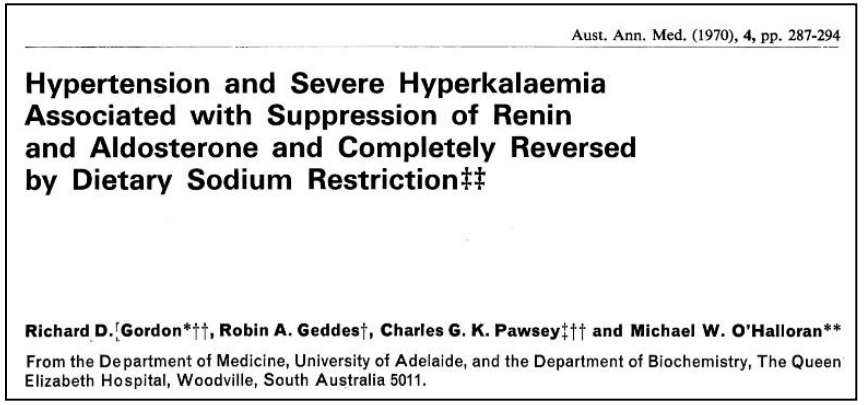
9: Others (e.g. trips, travel, or gifts): none

Mutations in WNK kinases result in increased Na-Cl cotransporter (NCC) activity in pseudohypoaldosteronism II (PHAII), aka Gordon syndrome



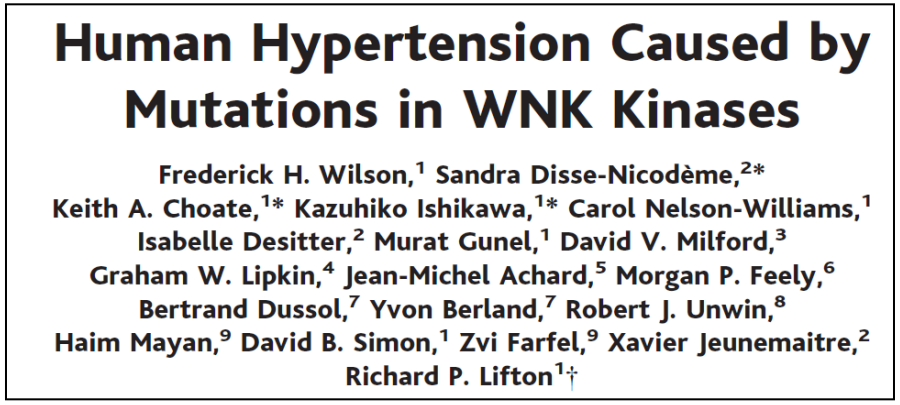
Aust. Ann. Med. (1970), 4, pp. 287-294

Hypertension and Severe Hyperkalaemia Associated with Suppression of Renin and Aldosterone and Completely Reversed by Dietary Sodium Restriction‡‡

Richard D. Gordon*††, Robin A. Geddes†, Charles G. K. Pawsey‡†† and Michael W. O'Halloran**

From the Department of Medicine, University of Adelaide, and the Department of Biochemistry, The Queen Elizabeth Hospital, Woodville, South Australia 5011.

Aust Ann Med 1970



Human Hypertension Caused by Mutations in WNK Kinases

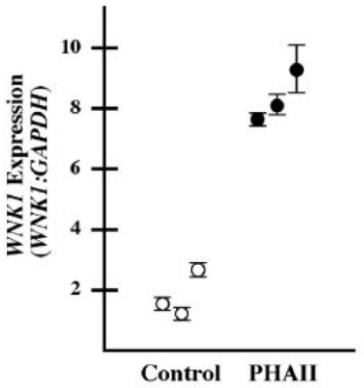
Frederick H. Wilson,[1] Sandra Disse-Nicodème,[2]* Keith A. Choate,[1]* Kazuhiko Ishikawa,[1]* Carol Nelson-Williams,[1] Isabelle Desitter,[2] Murat Gunel,[1] David V. Milford,[3] Graham W. Lipkin,[4] Jean-Michel Achard,[5] Morgan P. Feely,[6] Bertrand Dussol,[7] Yvon Berland,[7] Robert J. Unwin,[8] Haim Mayan,[9] David B. Simon,[1] Zvi Farfel,[9] Xavier Jeunemaitre,[2] Richard P. Lifton[1]†

Science 2001

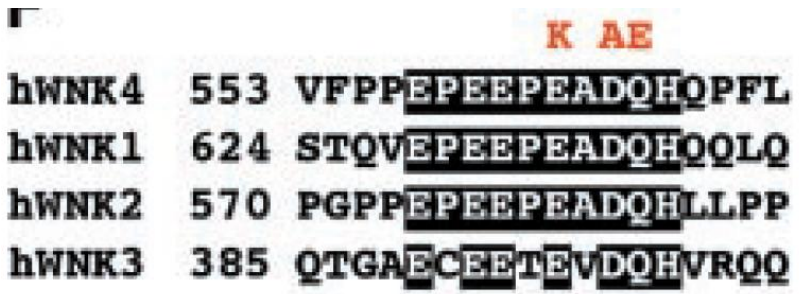
Brief summary of PHAII

- PHAII (aka Gordon syndrome) was first reported by Richard Gordon in 1970.
- Hypertension and hyperkalemia are corrected by thiazides.
- Mutations in WNK1/4 cause PHAII in ~20% of cases (Wilson et al. Science 2001).
- WNK4 activates NCC via SPAK phosphorylation.

WNK1 mutations



WNK4 mutations



Gordon RD et al. Aust Ann Med 1970; Wilson et al. Science 2001; Vitari AC et al. Biochem J 2005; Moriguchi T et al. J Biol Chem 2005

Mutations in cullin-E3 ubiquitin ligase KLHL3/CUL3 explains 80% of cases with pseudohypoaldosteronism II, featuring hypertension and hyperkalemia

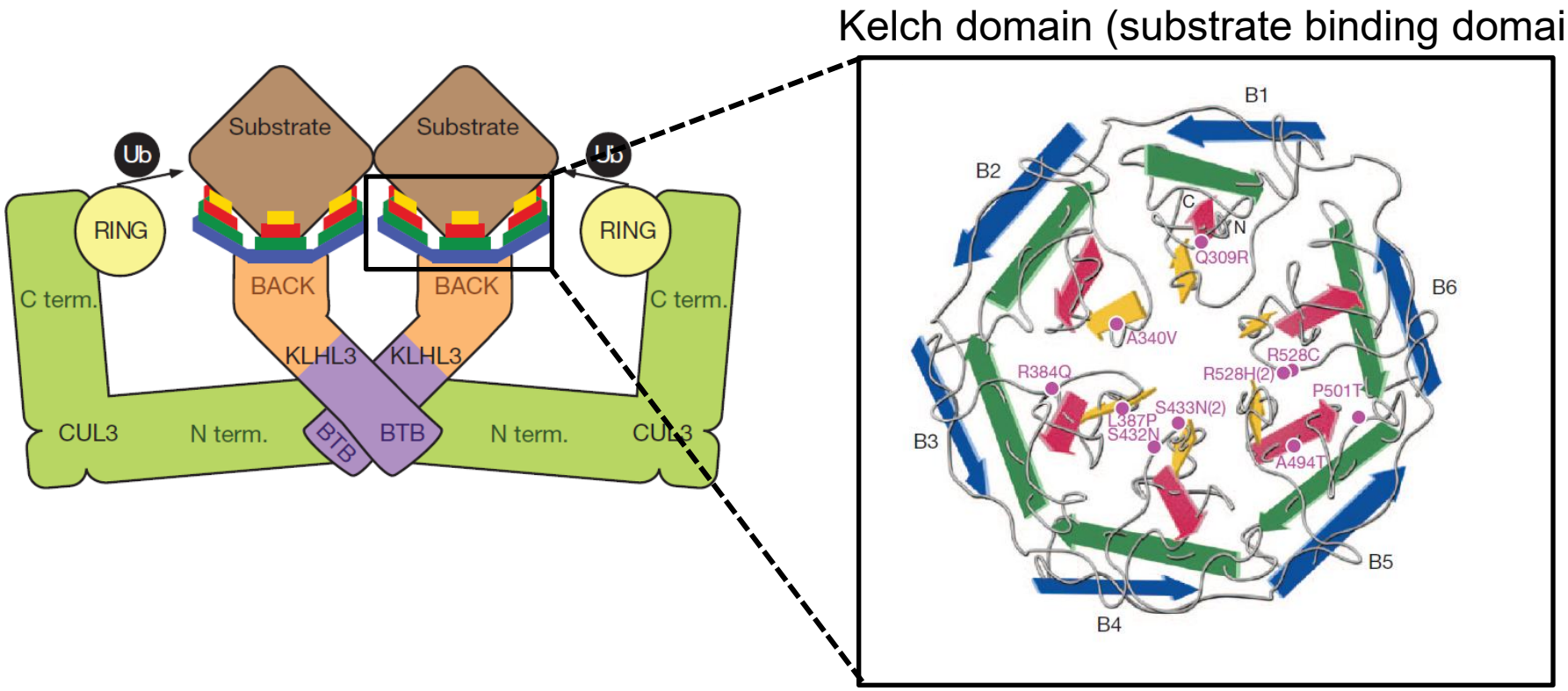


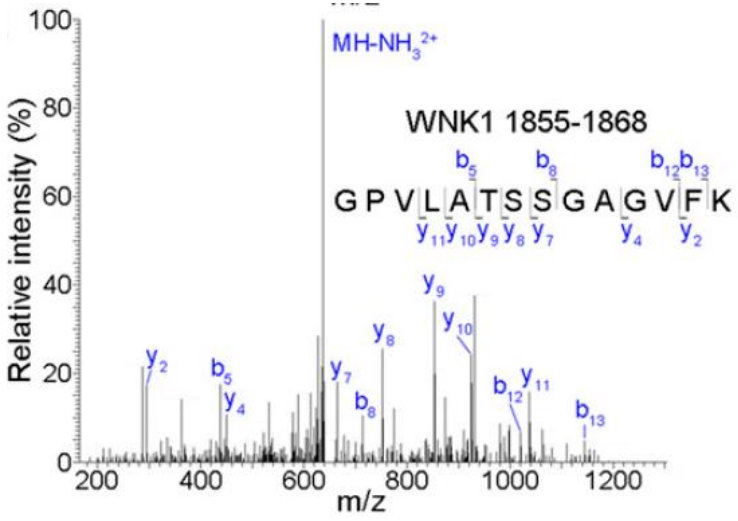
Table 1 | PHAII phenotypes, stratified by genotype.

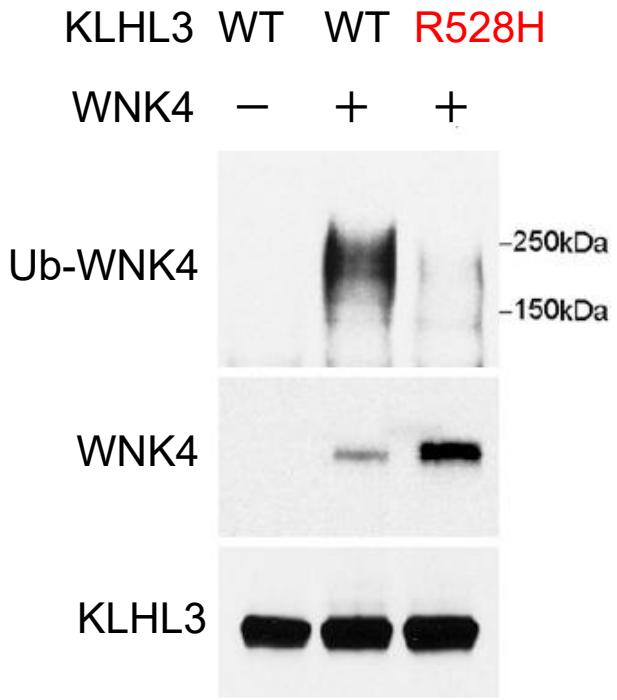
Mutant gene	No. of kindreds	No. of affecteds	Dx/Ref age*	K^+ (mM) (nl 3.5–5.0 mM)†	HCO_3^- (mM) (nl 22–28 mM)†	Hypertension at ≤age 18 (%)†
CUL3	17	21	9 ± 6	7.5 ± 0.9	15.5 ± 2.0	94
KLHL3 recessive	8	14	26 ± 14	6.8 ± 0.5	17.6 ± 1.5	14
KLHL3 dominant	16	40	24 ± 18	6.2 ± 0.6	17.2 ± 2.5	17
WNK4	5	15	28 ± 18	6.4 ± 0.7	20.8 ± 2.3	10
WNK1	2	23	36 ± 20	5.8 ± 0.8	22.4 ± 4.6	13

Boyden LM, Lifton RP, et al. Nature 2012

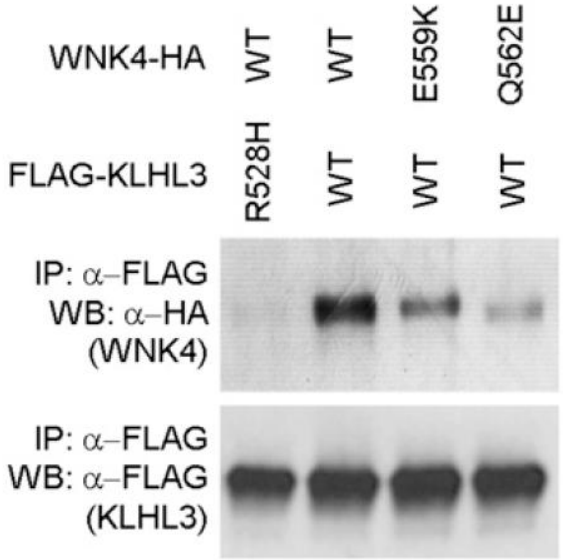
WNK kinases are substrates of KLHL3/CUL3 ubiquitin ligase

LC-MS/MS analysis
(KLHL3-IP)



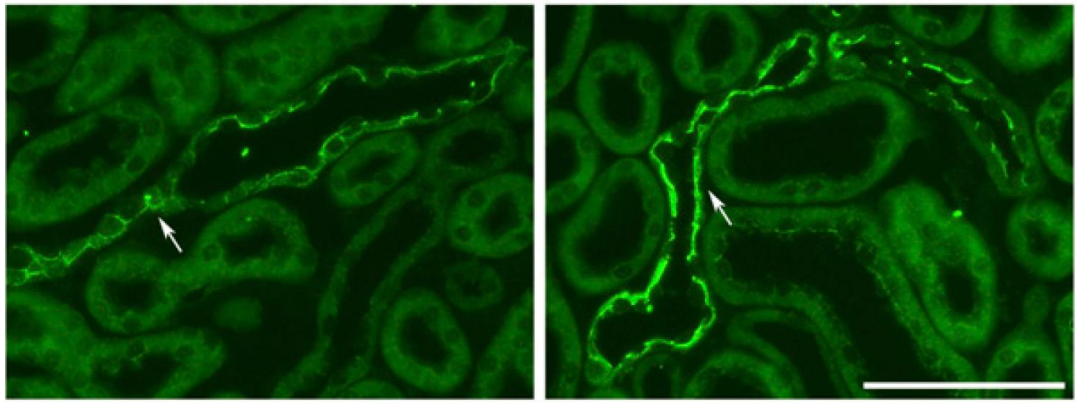


Co-IP and WB
(WT and disease-causing mutations)



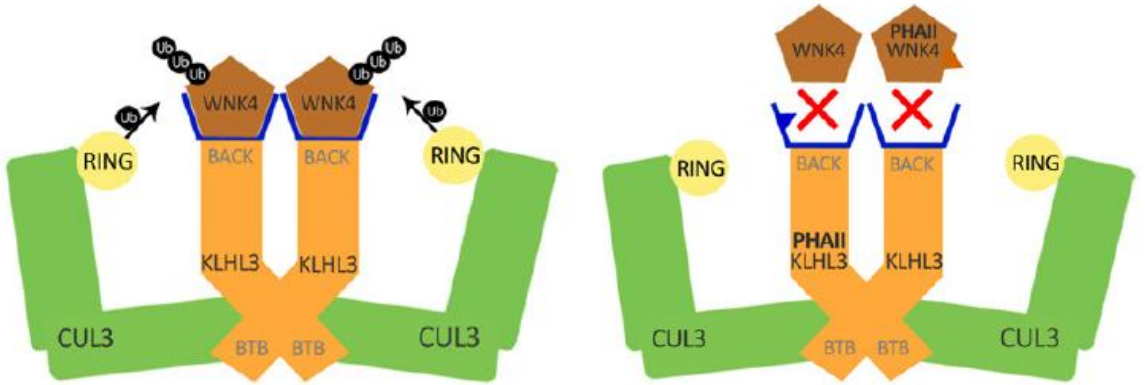
WT-WNK4
Transgenic mice

Mutated WNK4
Transgenic mice



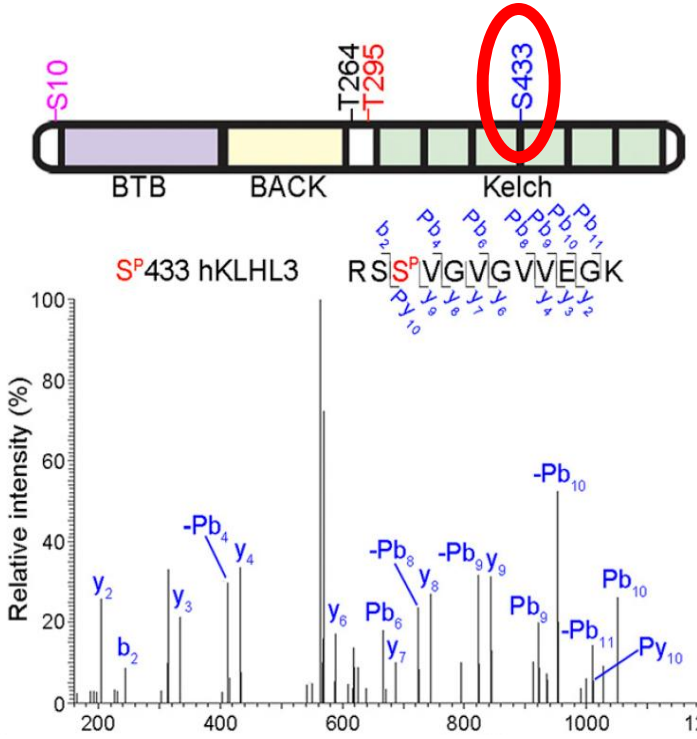
Normal

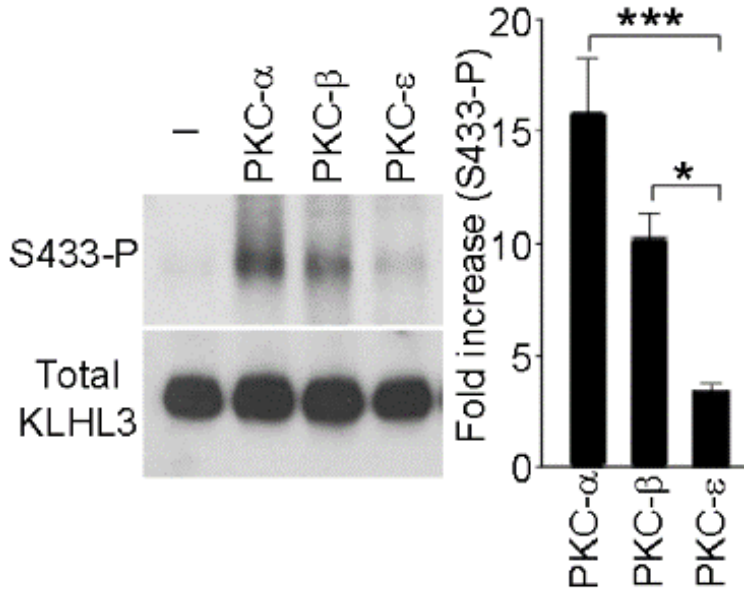
PHAII

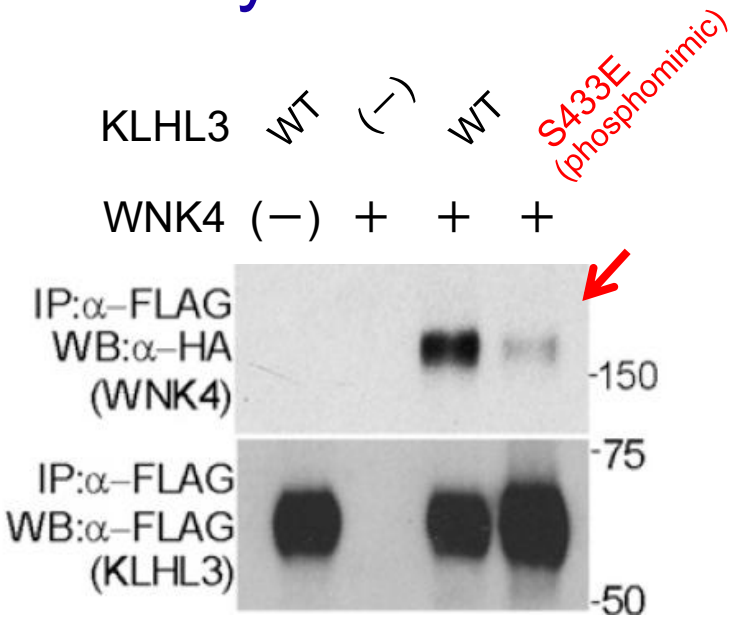


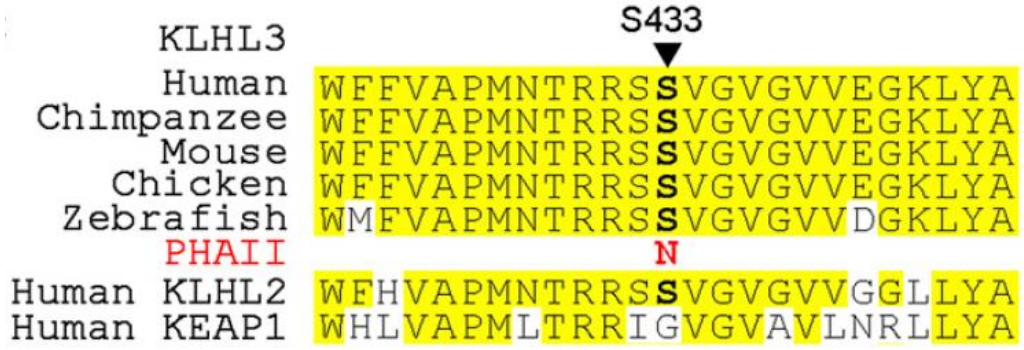
Shibata S, Lifton RP, et al. Proc Natl Acad Sci USA 2013

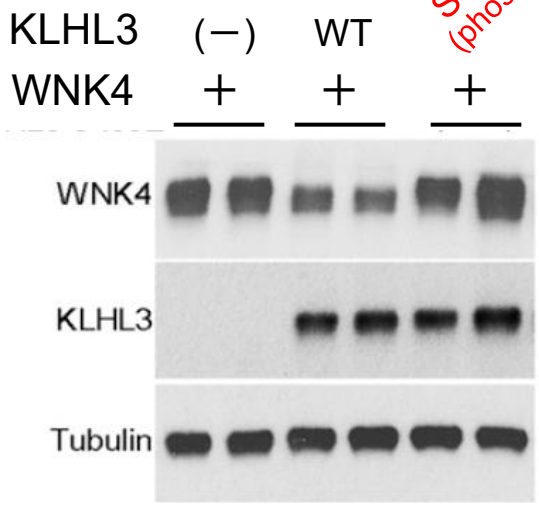
Identification of the key phosphorylation site, at S433 in the substrate-binding domain, that regulates KLHL3 activity







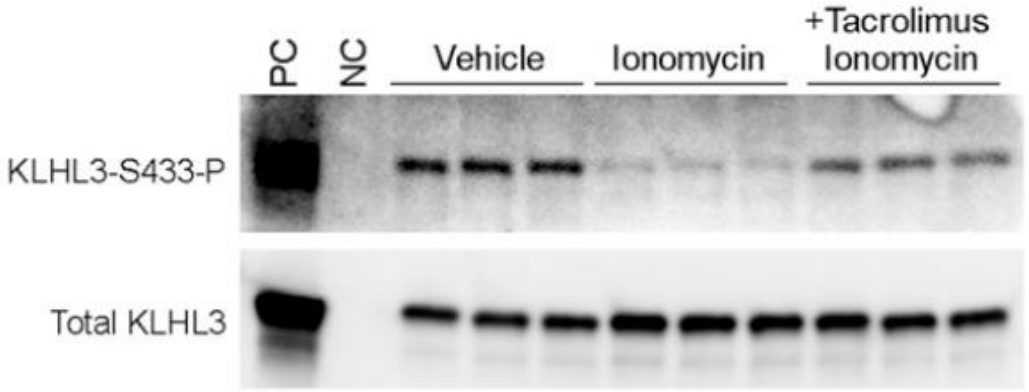


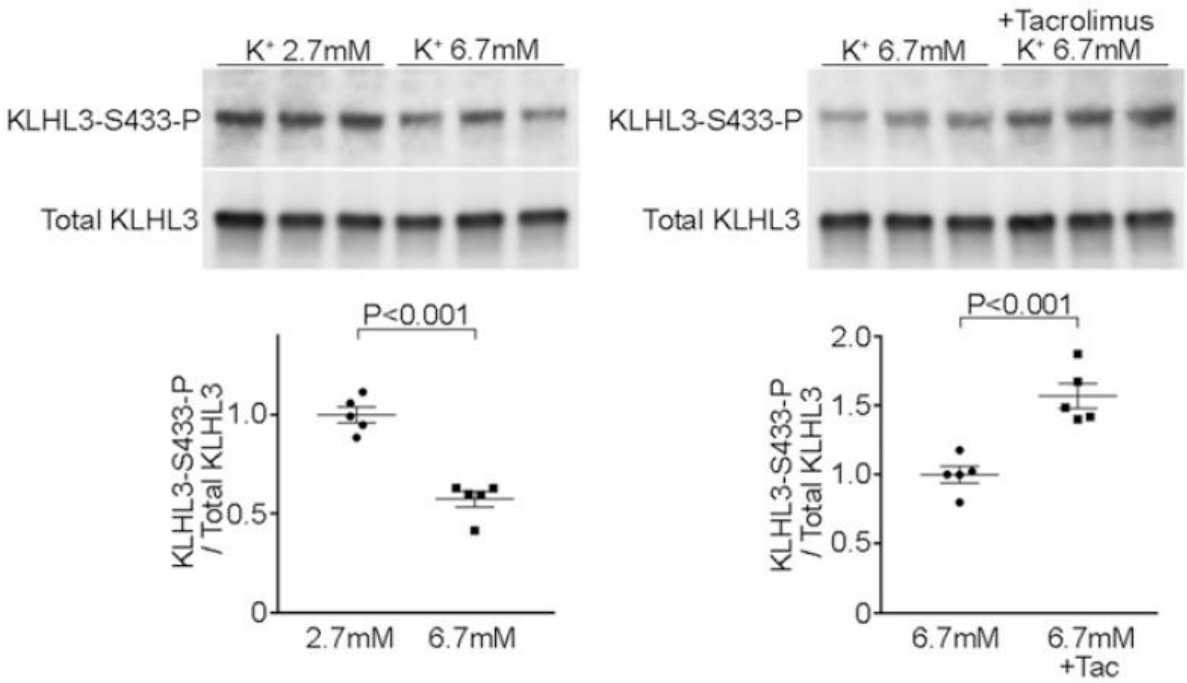


Shibata et al. PNAS 2014

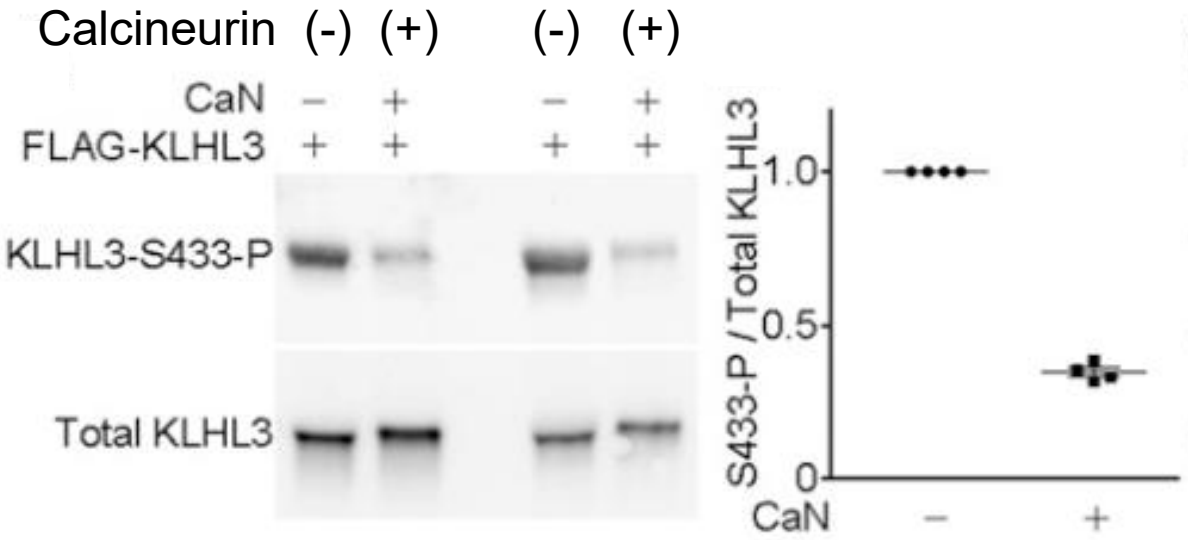
Ca^{2+} signaling and calcineurin dephosphorylates phospho-KLHL3

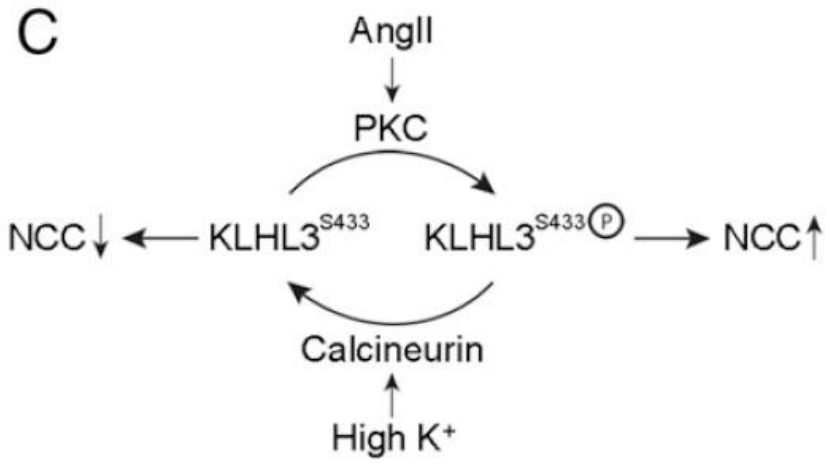
HEK cells





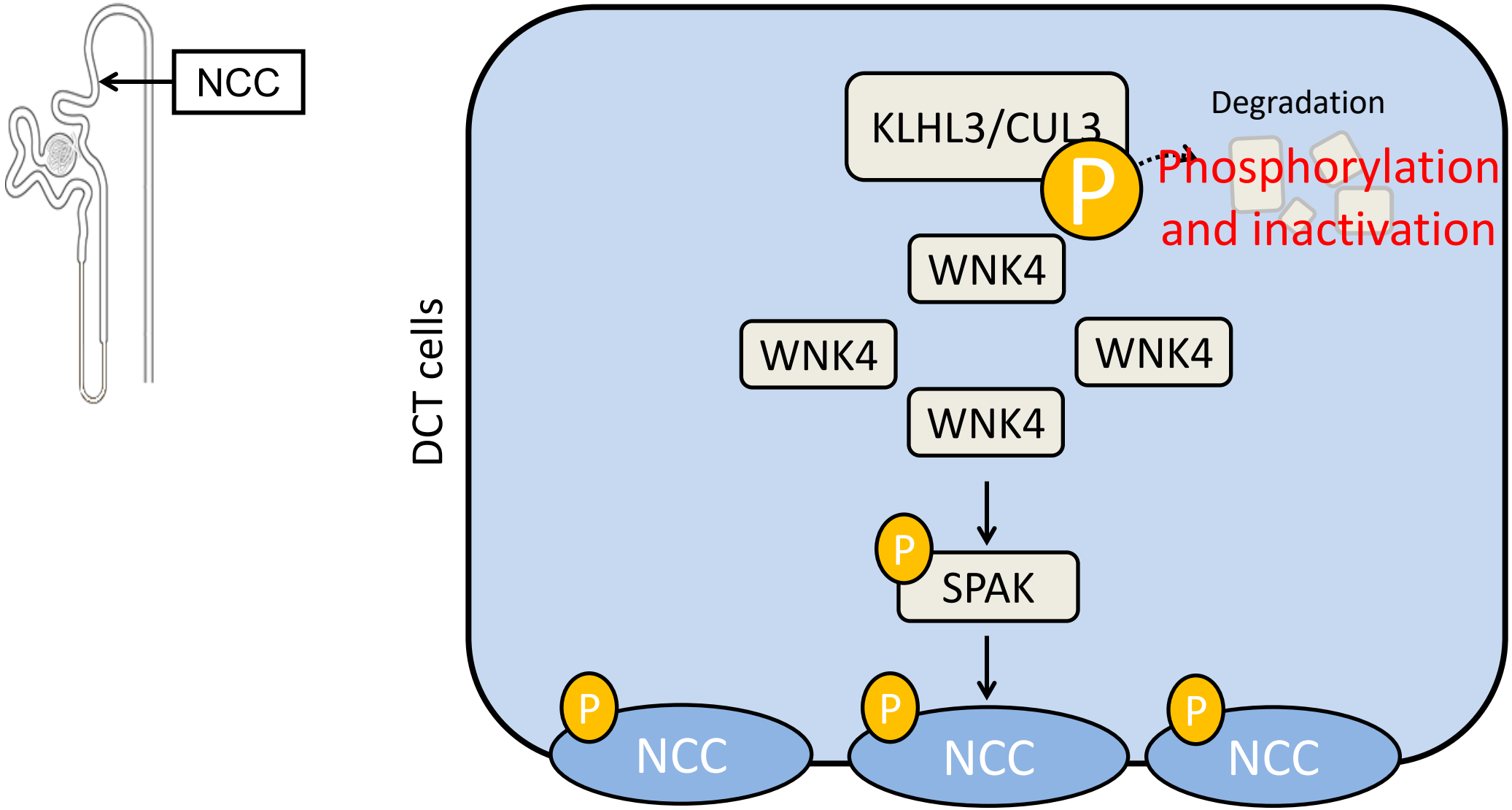
Phosphatase assay



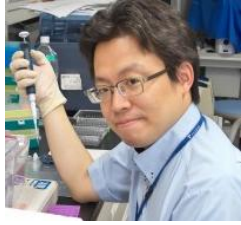


Ishizawa, Wang, et al. PNAS 2019

Regulation of WNK/SPAK/NCC by KLHL3/CUL3 and pathogenesis of PHAII



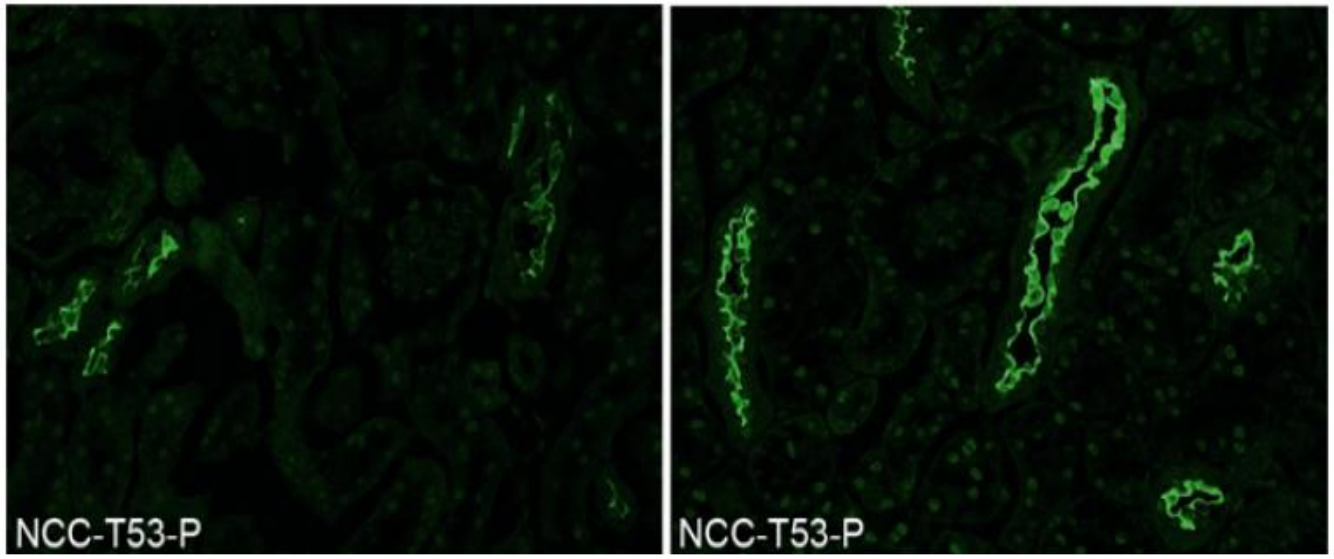
NCC abundance is increased in type 2 diabetic mouse model (db/db mice)

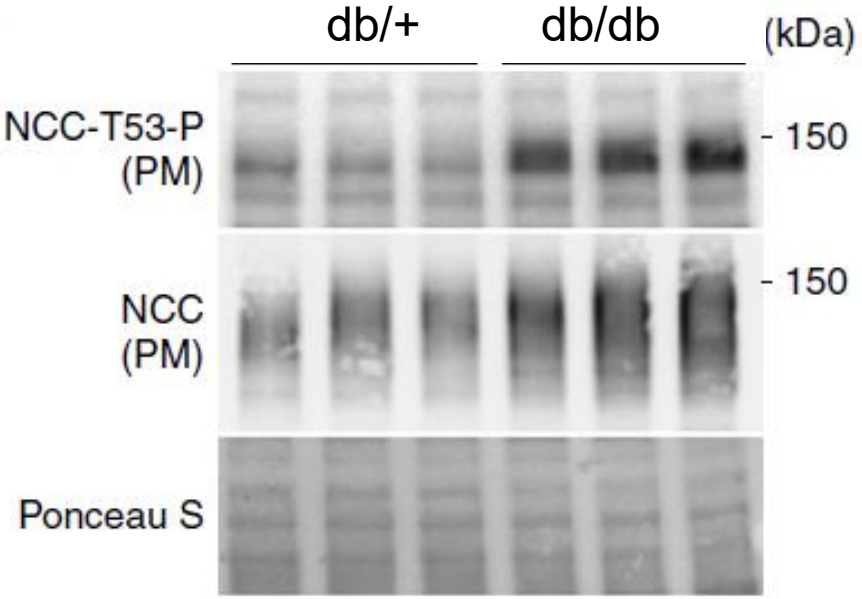


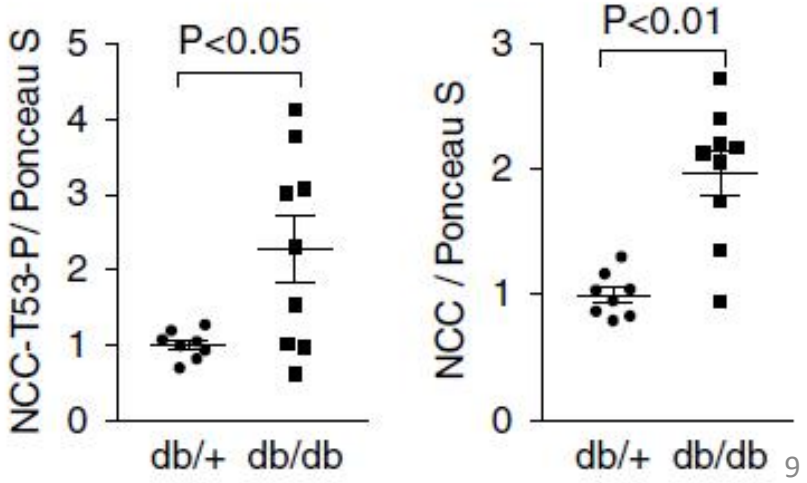
Phospho-NCC staining

db/+ mice

db/db mice

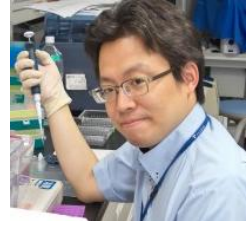






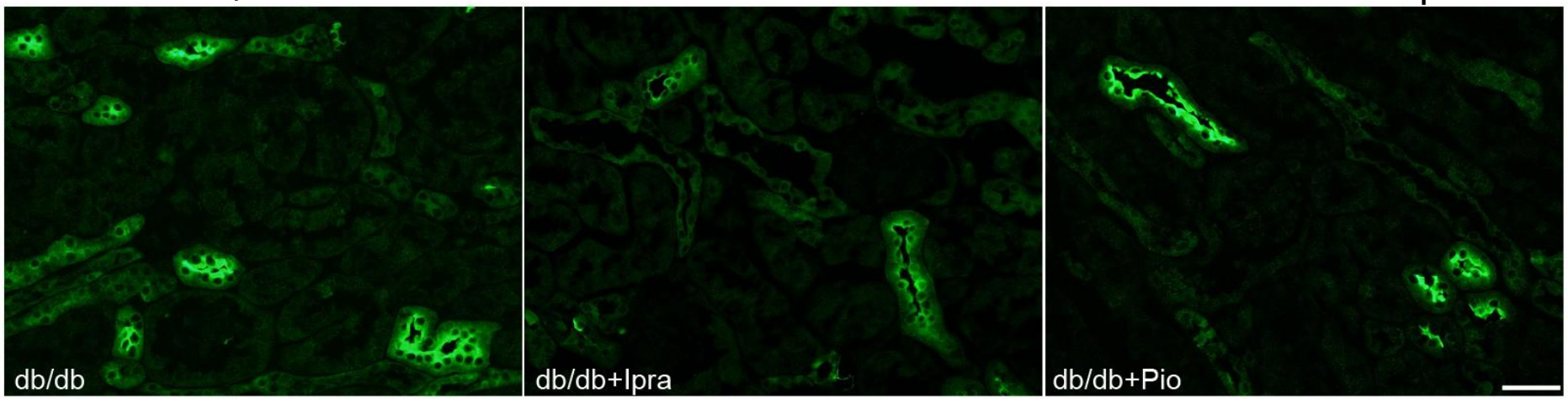
Ishizawa K, Shibata S, et al. JASN 2019

SGLT2 inhibition attenuates phospho-KLHL3 and NCC in db/db mice

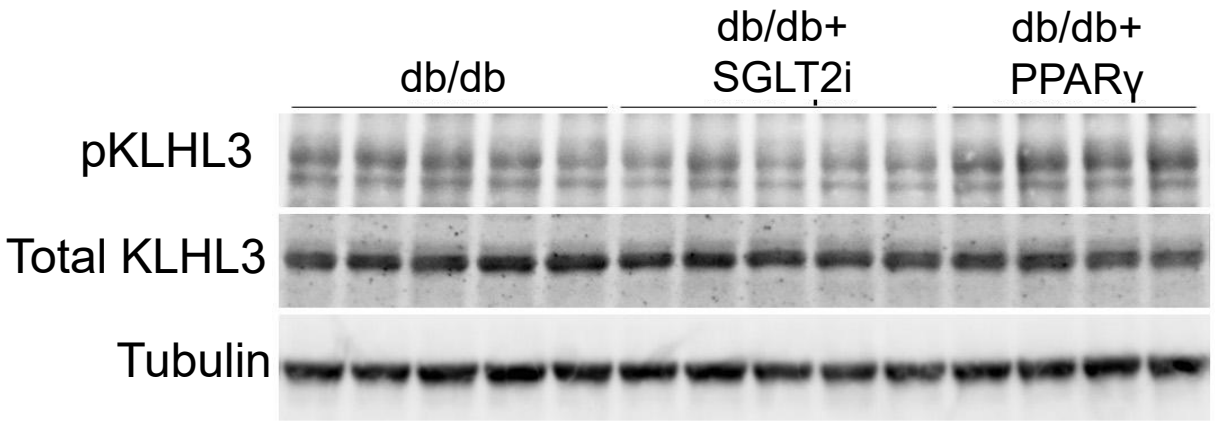


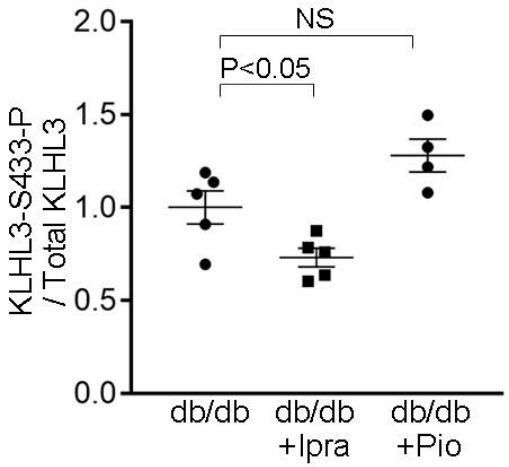
NCC

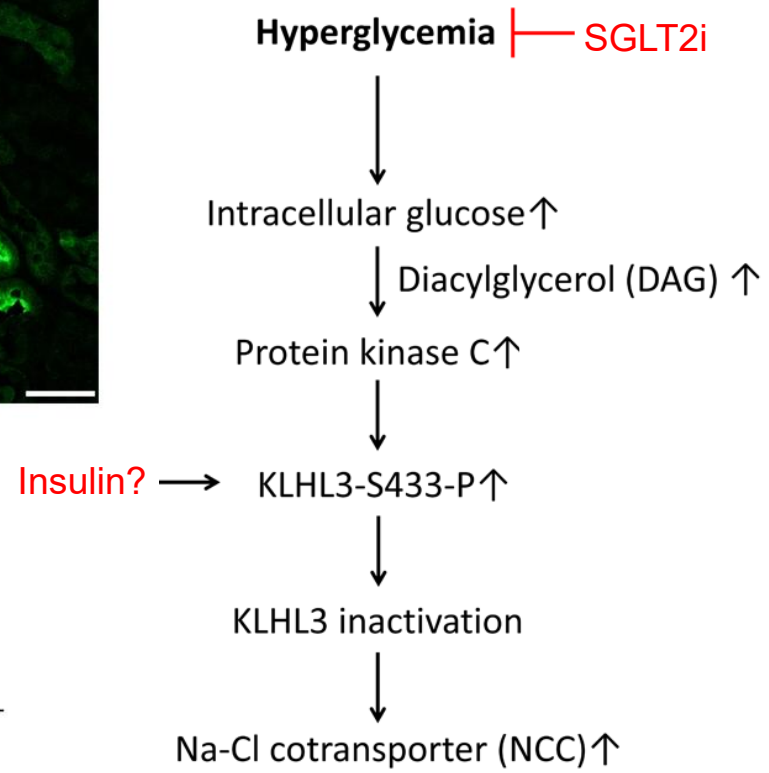
db/db | db/db+SGLT2i | db/db+PPARγ



リン酸化KLHL3

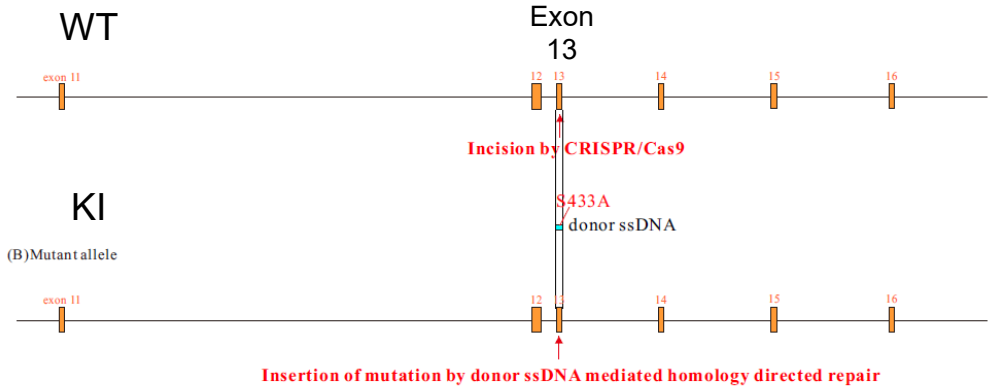


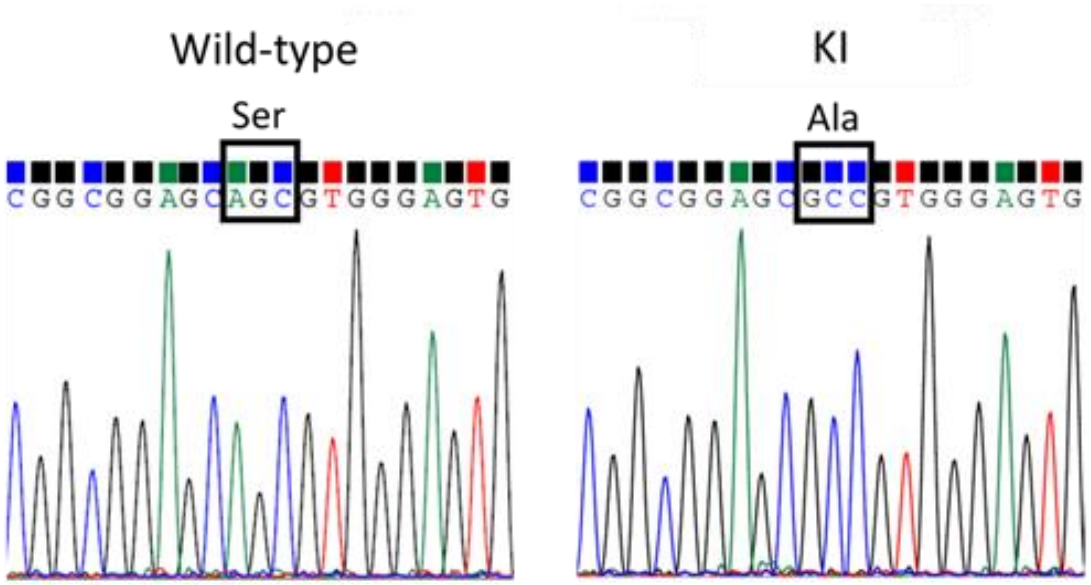


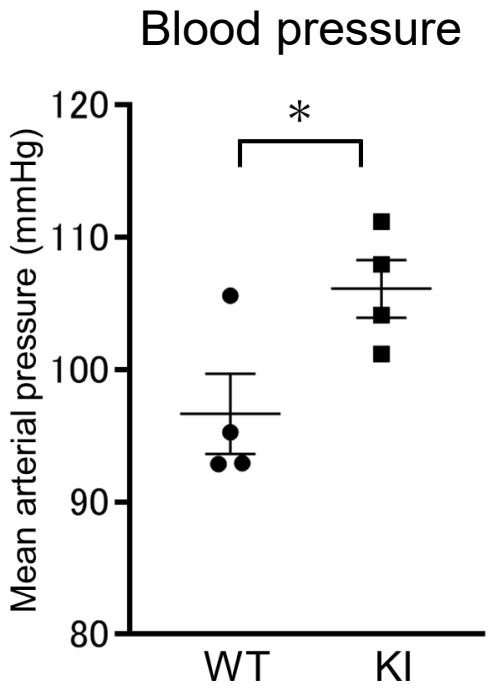


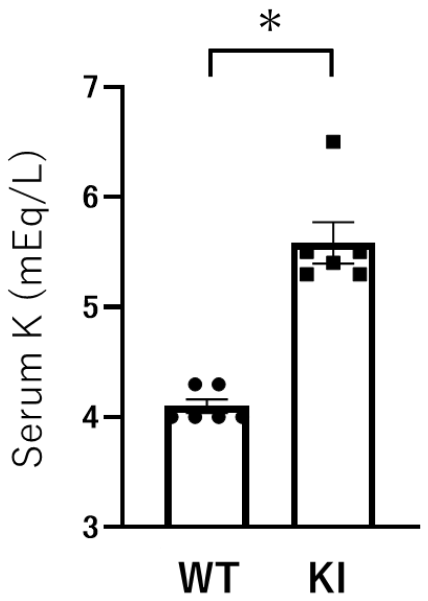
Ishizawa K et al. J Am Soc Nephrol 2019

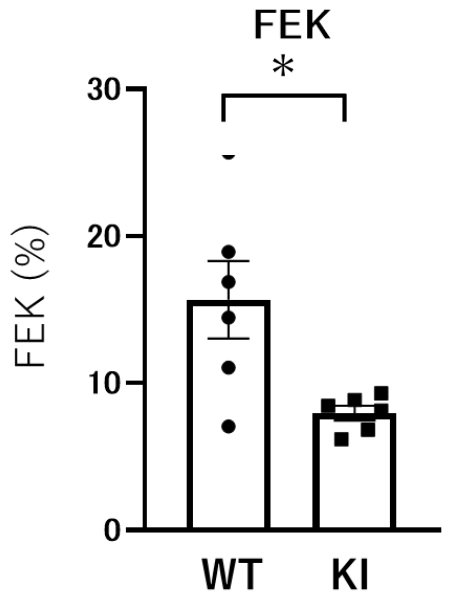
KLHL3-S433A knock-in mice show hypertension and hyperkalemia





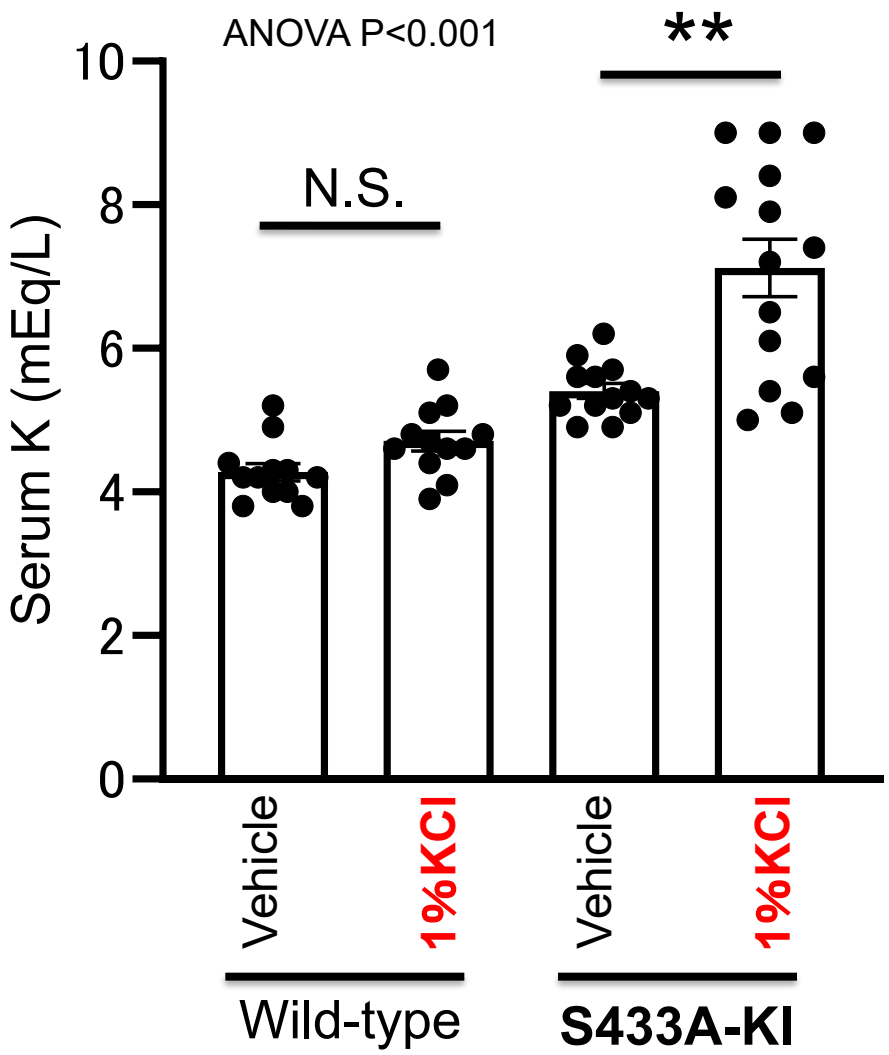


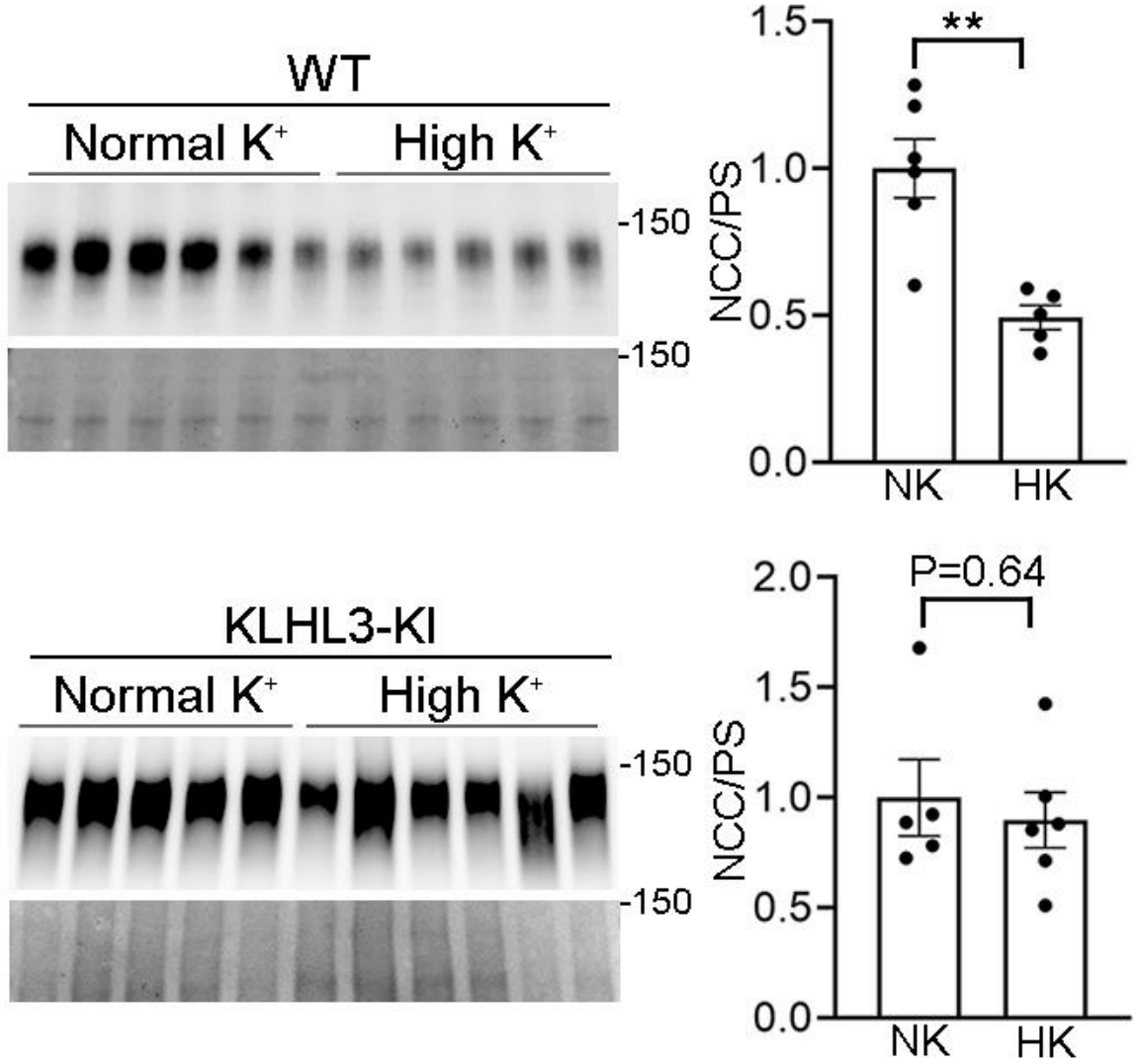




Ishizawa et al. presented in ISH 2022

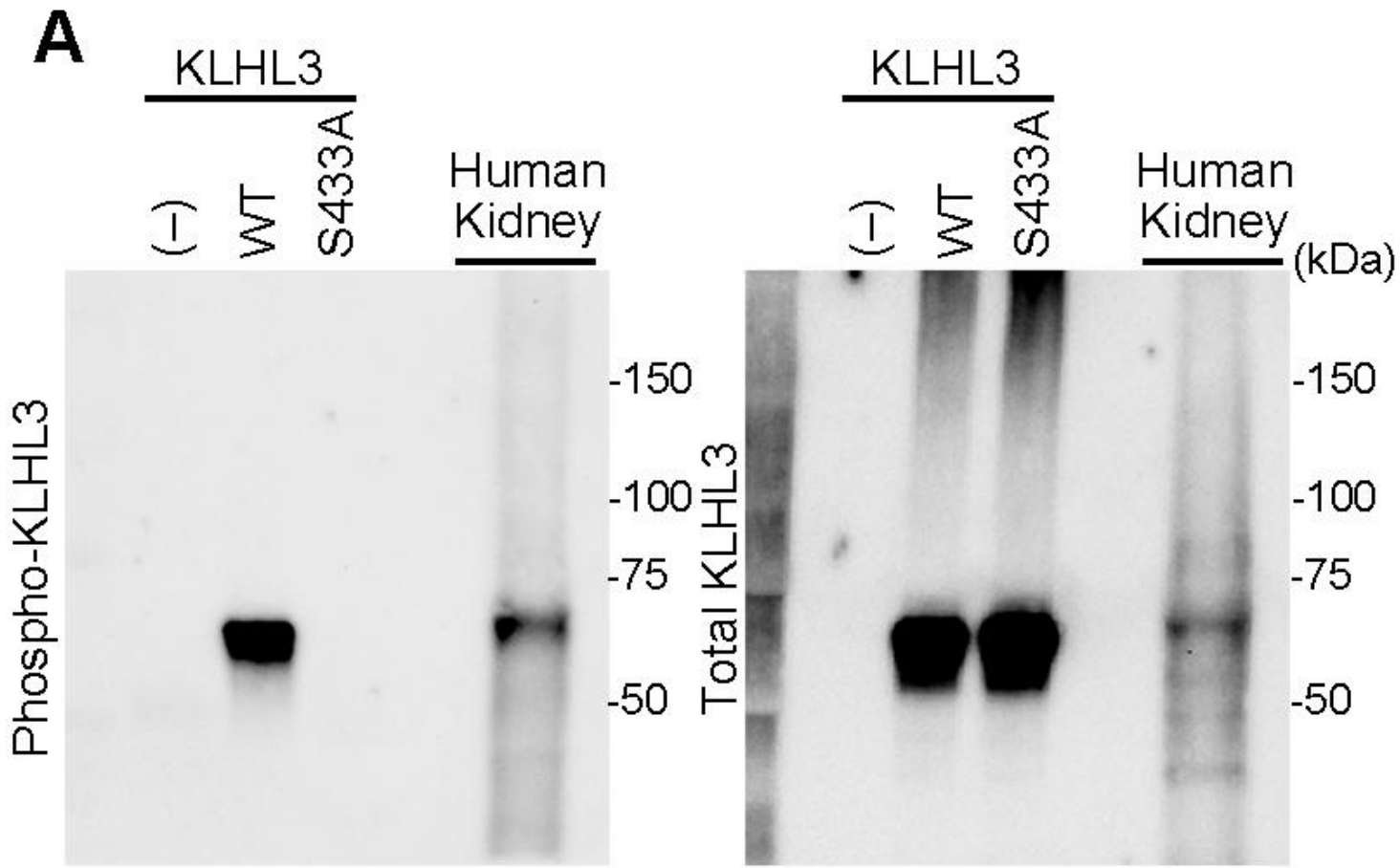
NCC reduction by potassium is impaired in KLH3-S433A-KI, resulting in significant elevation in serum K^+ levels





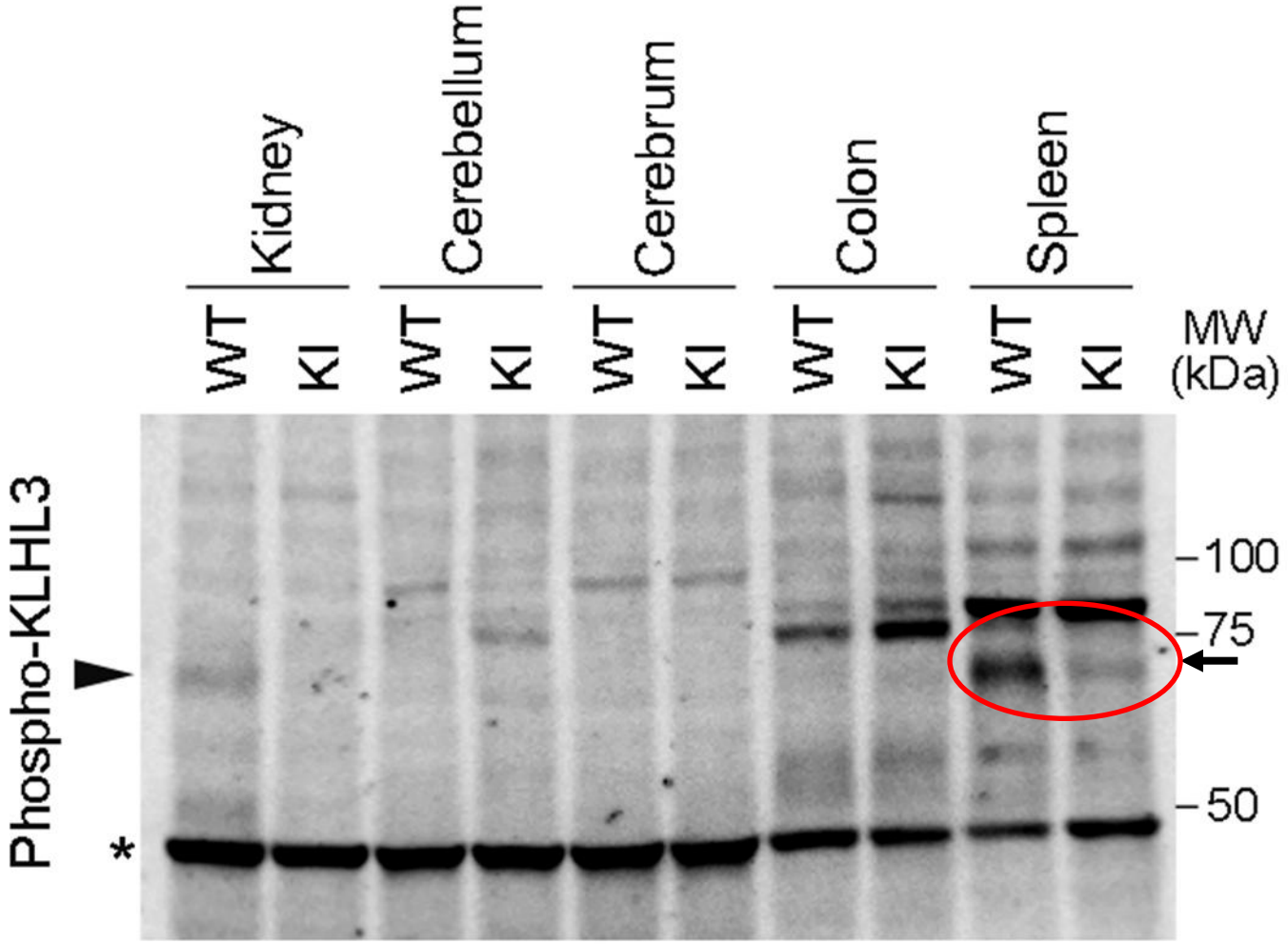
Ishizawa et al. unpublished data

KLHL3 is phosphorylated *in vivo* in human kidney



Ishizawa et al. unpublished data

Creation of KLHL3-S433-KI mice combined with tissue survey with phospho-KLHL3 antibody suggests a role of KLHL3 in the spleen



Ishizawa et al. unpublished data

Summary

- The ubiquitin ligase component KLHL3 regulates blood pressure and potassium homeostasis by binding and promoting degradation of WNK kinases.
- Substrate-binding ability of KLHL3 is regulated by phosphorylation at S433, which is counter-regulated by AGC kinases and Calcineurin.
- Analysis using KLHL3-S433A Knock-in mice confirm the physiological importance of S433 modification in regulating fluid homeostasis.

Acknowledgments

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