# Epigenetic Control of ENaC Transcription, $Na^+$ Metabolism and Blood Pressure

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International Conference on
Geriatrics & Gerontology

Chicago, July 08-10, 2014

# Epithelial Na Channel (ENaC)

- Consists of 3 partially homologous subunits $\alpha$, $\beta$ and $\gamma$.
- Expressed in the apical membrane of salt-absorbing epithelia of kidney, colon, and lung.
- Critical for $Na^+$ absorption in the lung, colon, and kidney.
- Mutations associated with two genetic diseases: Liddle's syndrome (Hypertension) and pseudohypoaldosteronism type 1 (PHA-1, Hypotension).
- Tightly regulated by aldosterone at multiple levels (mRNA, protein, and subcellular distribution).

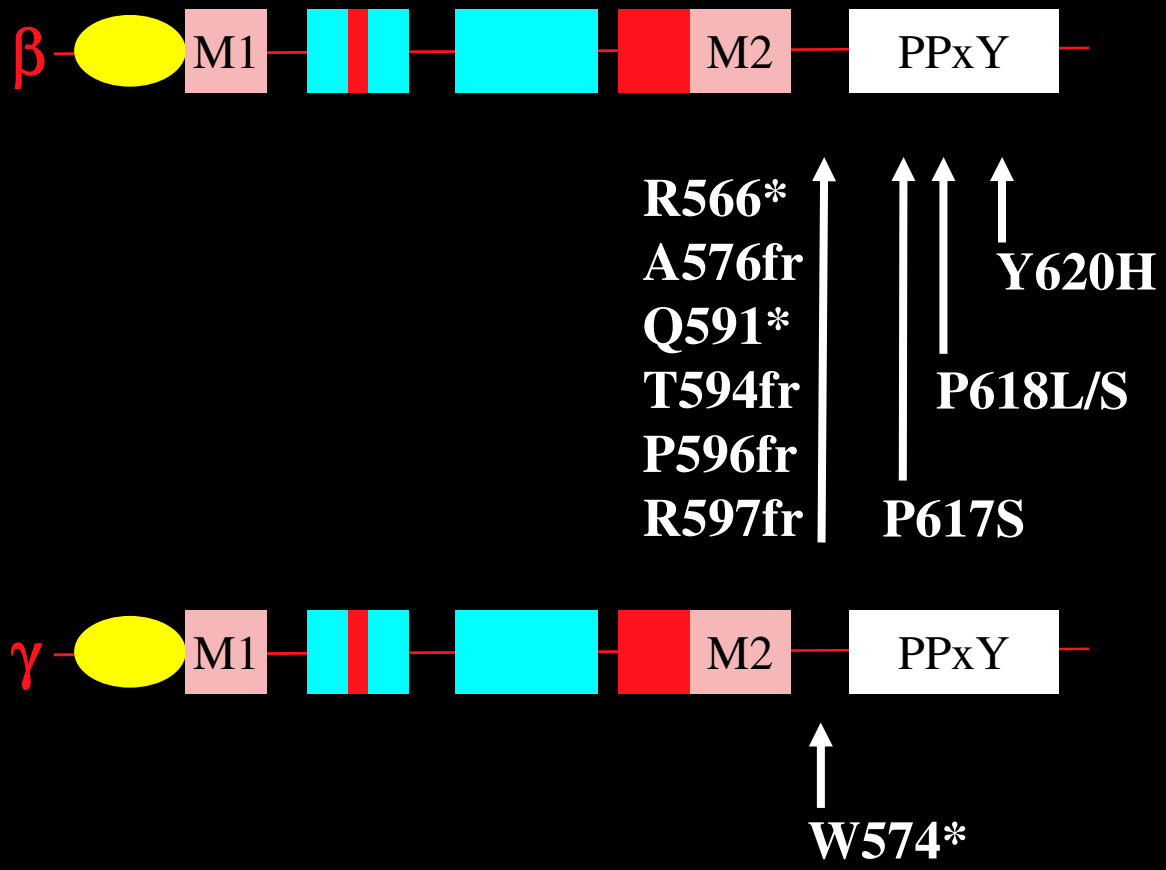
# Liddle's Syndrome (Hypertension)

- Described by G. Liddle in 1963
- Autosomal dominant fashion
- Early and severe hypertension
- Hypokalemia
- Metabolic alkalosis
- Low plasma aldosterone concentration

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Constitutive hyperactivity of ENaC

Bonny et al *Kidney International* (2000) 57, 1313–1318



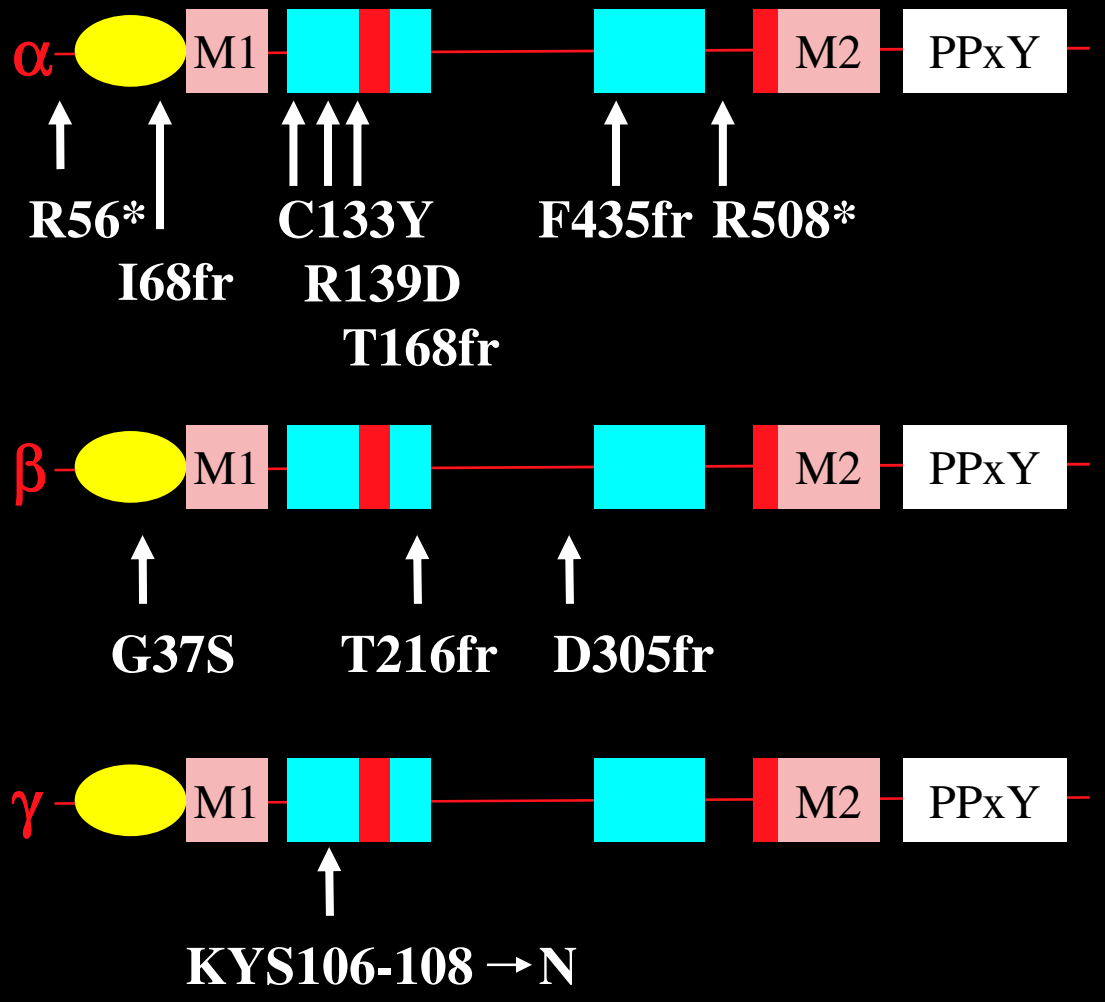
# Pseudohypoaldosteronism type 1 (PHA-1, Hypotension)

- Two forms:
  Autosomal dominant renal form by mutations in MR
  Autosomal recessive form by mutations in ENaC subunits
- Salt wasting
- Hyperkalemia
- Metabolic acidosis
- High plasma aldosterone concentration

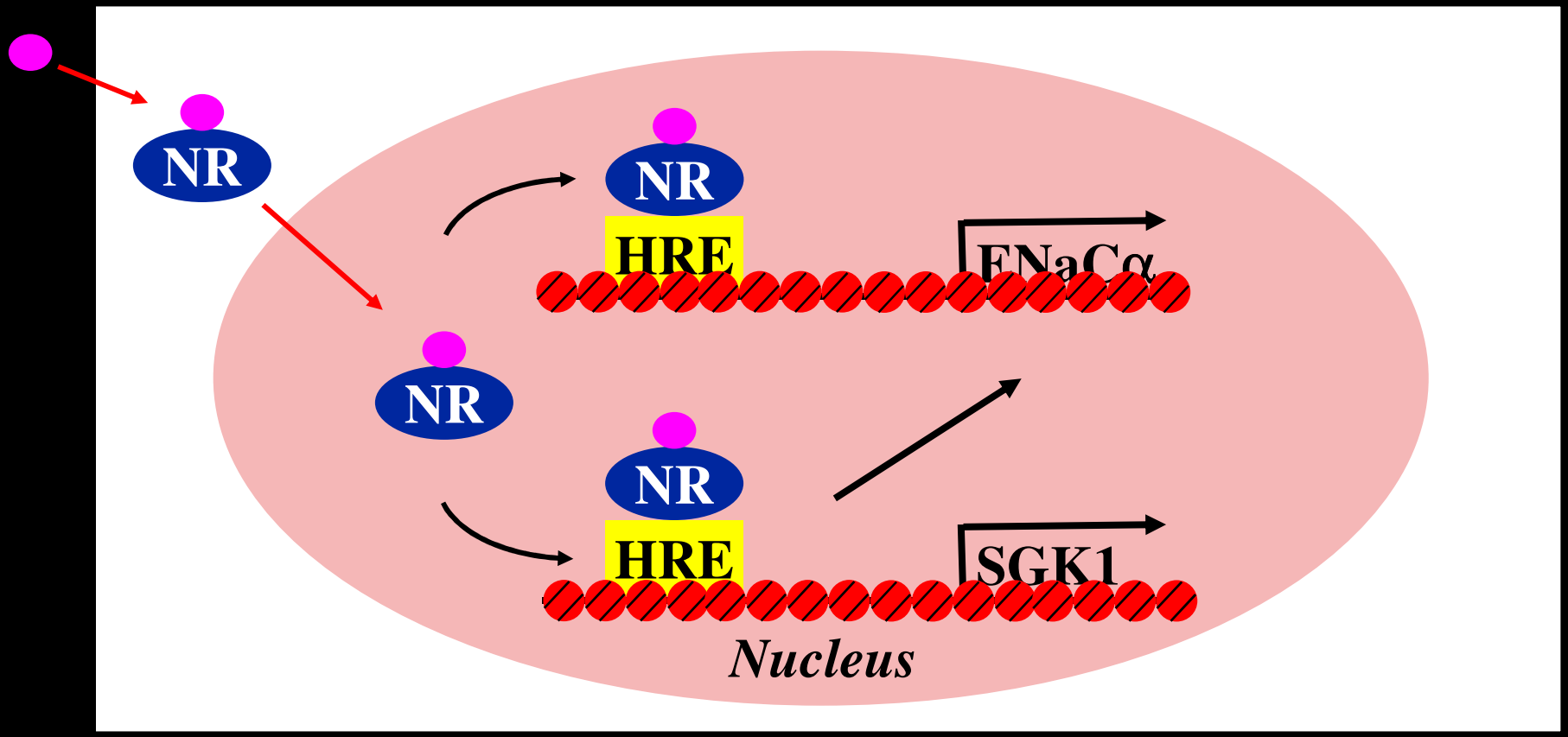
↓

Loss-of-function mutations in ENaC

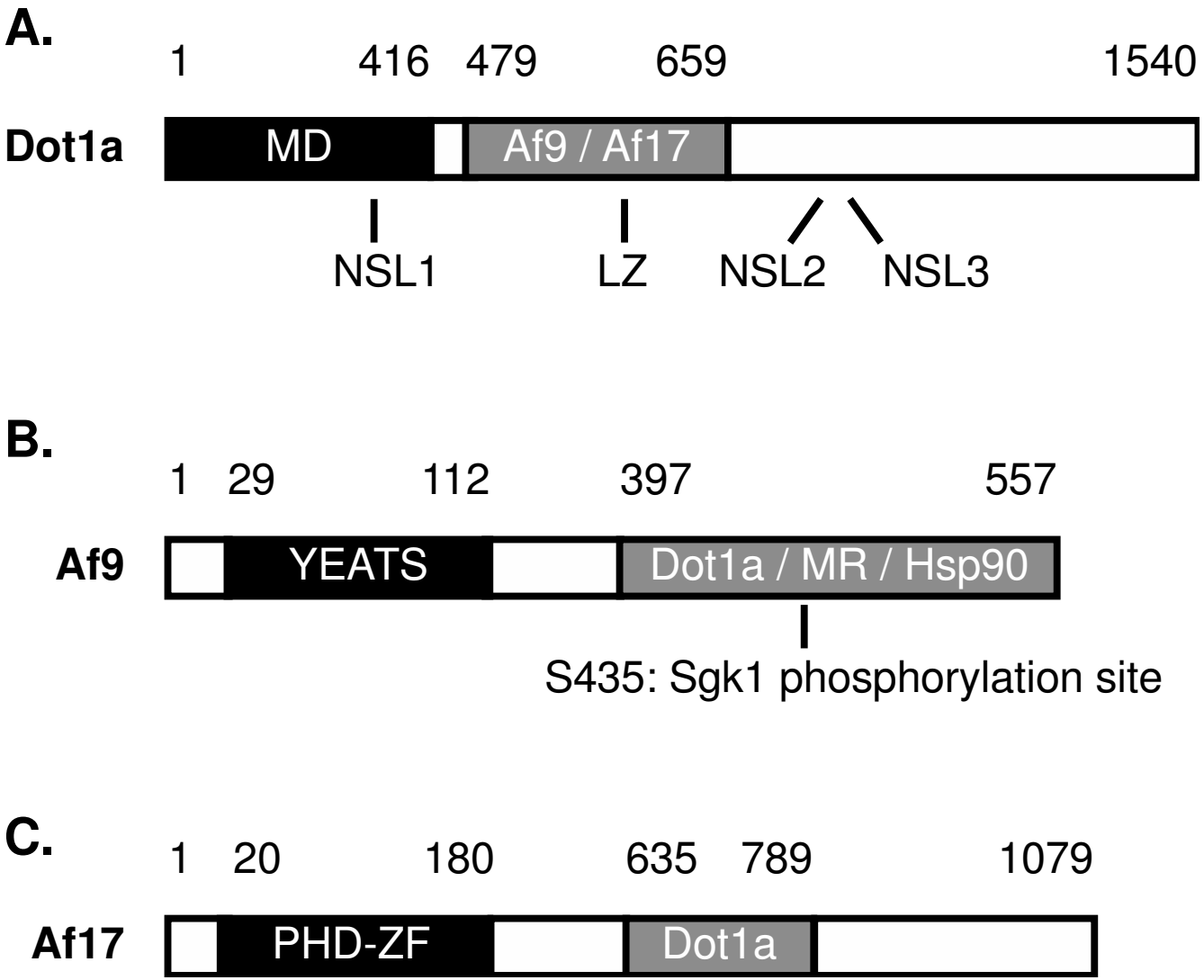
Bonny et al *Kidney International* (2000) 57, 1313–1318



# The Classical model for Molecular Action of Aldosterone



# Novel Epigenetic Regulators of ENaC Transcription



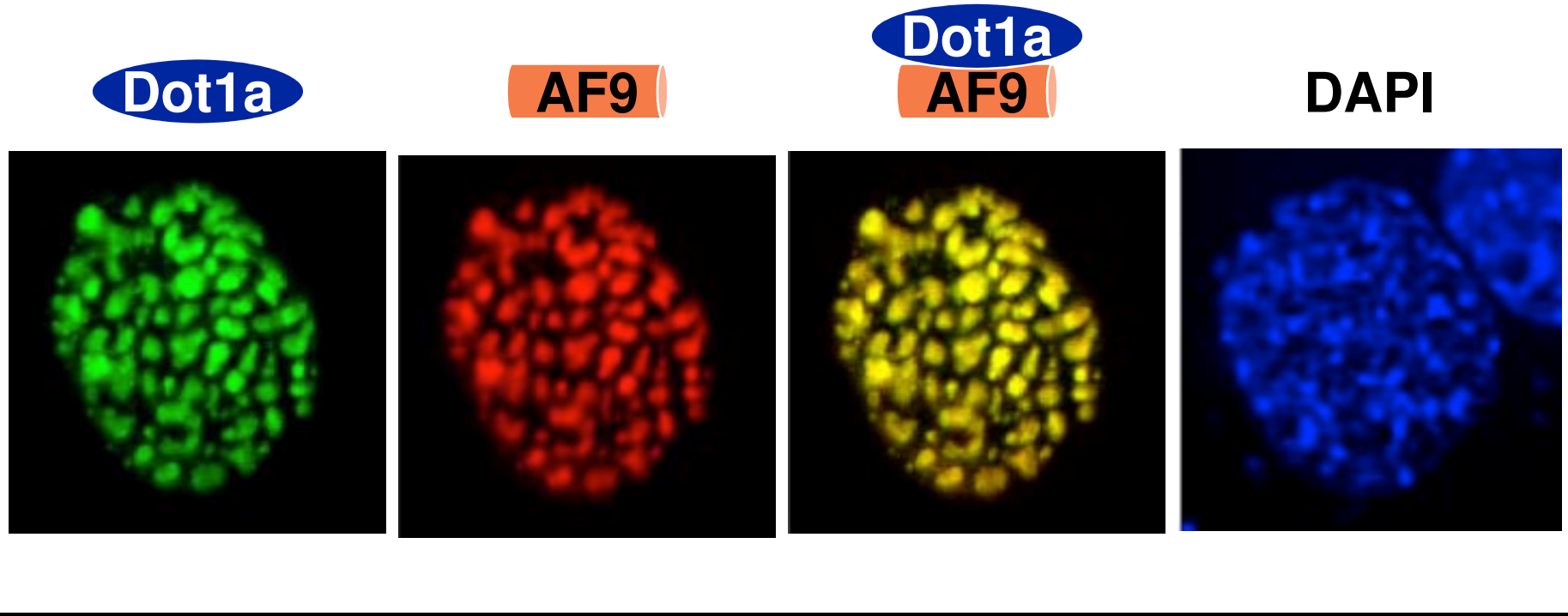
# Histone Methyltransferase Gene Dot1

- Identified in yeast as a disruptor of telomeric silencing.
- Encodes a methyltransferase specific for histone H3 K79.
- Expressed in kidney and other tissues and as early as 2-cell stage.
- Five alternative splicing variants in mouse (Dot1a-e), with Dot1a being the most highly expressed and most closely related to hDot1L.
- Dot1L-deficient mouse embryos show multiple developmental abnormalities, including growth impairment, angiogenesis defects in the yolk sac, and cardiac dilation, and die between 9.5 and 10.5 days post coitum.
- Involved in cell cycle regulation, DNA repair, chromosome remodeling, transcription regulation and leukemogenesis.

# Putative Transcription Factor AF9

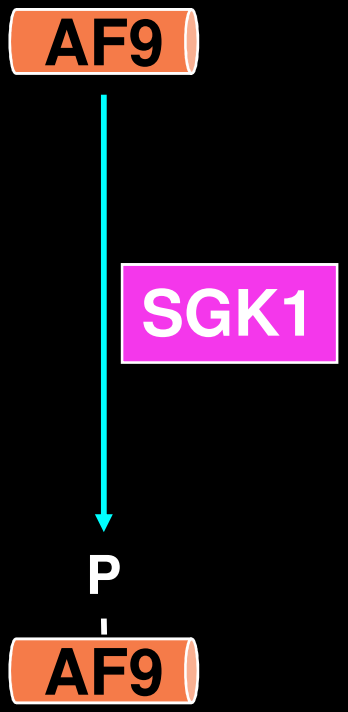
- Identified as one of the most common fusion partners of the mixed-lineage leukemia protein (MLL or ALL-1)
- A putative transcription factor, although, no AF9-regulated genes have been well documented
- Expressed in kidney and other tissues
- AF9 deletion leads to perinatal lethality

# Dot1a and AF9 Colocalize in the Nucleus of mIMCD3 Cells




Zhang et al. JBC. 2006.

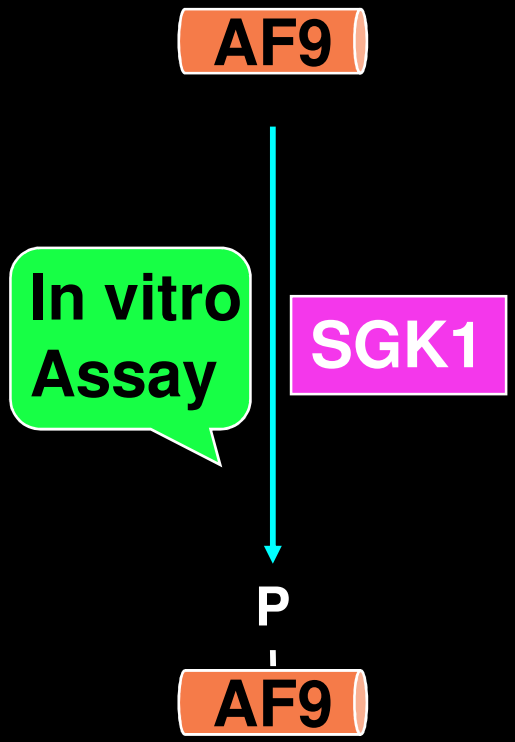
# AF9 Contains a Putative Phosphorylation Site for SGK1

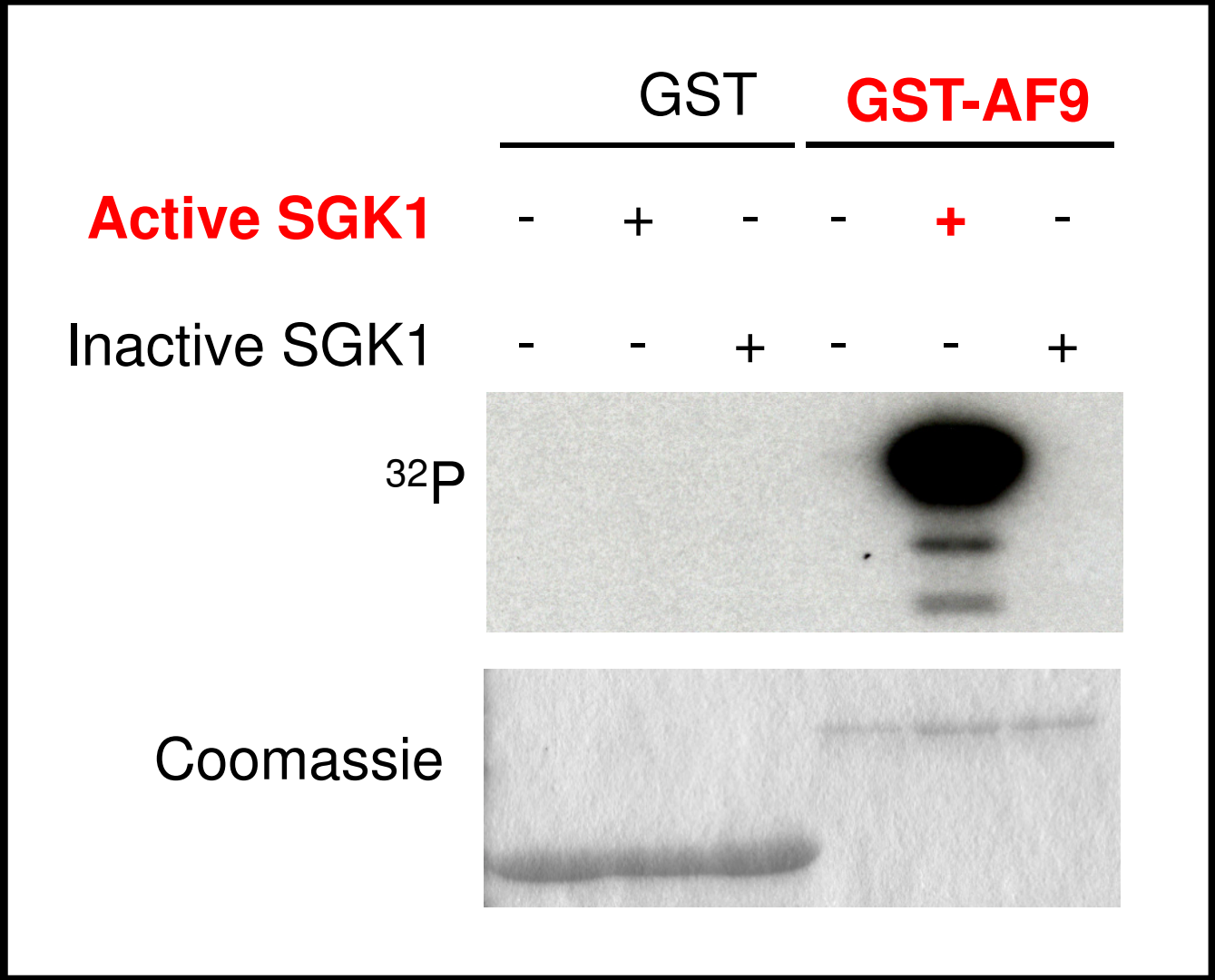



| | |
|---|---|
| Human AF9 | 441…RSRRV**S**…446 |
| **Mouse AF9** | 430…RSRRV**S**…**435** |
| Rat AF9 | 243…RSRRV**S**…248 |
| Horse AF9 | 35…RSRRV**S**…. 40 |
| Consensus | RXRXX(**S/T**) |

Zhang et al. JCI. 2007.

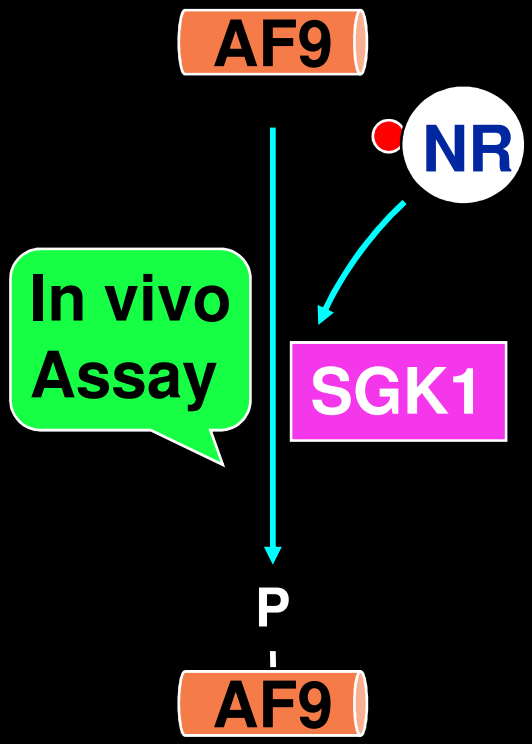
# SGK1 Phosphorylates AF9 in vitro

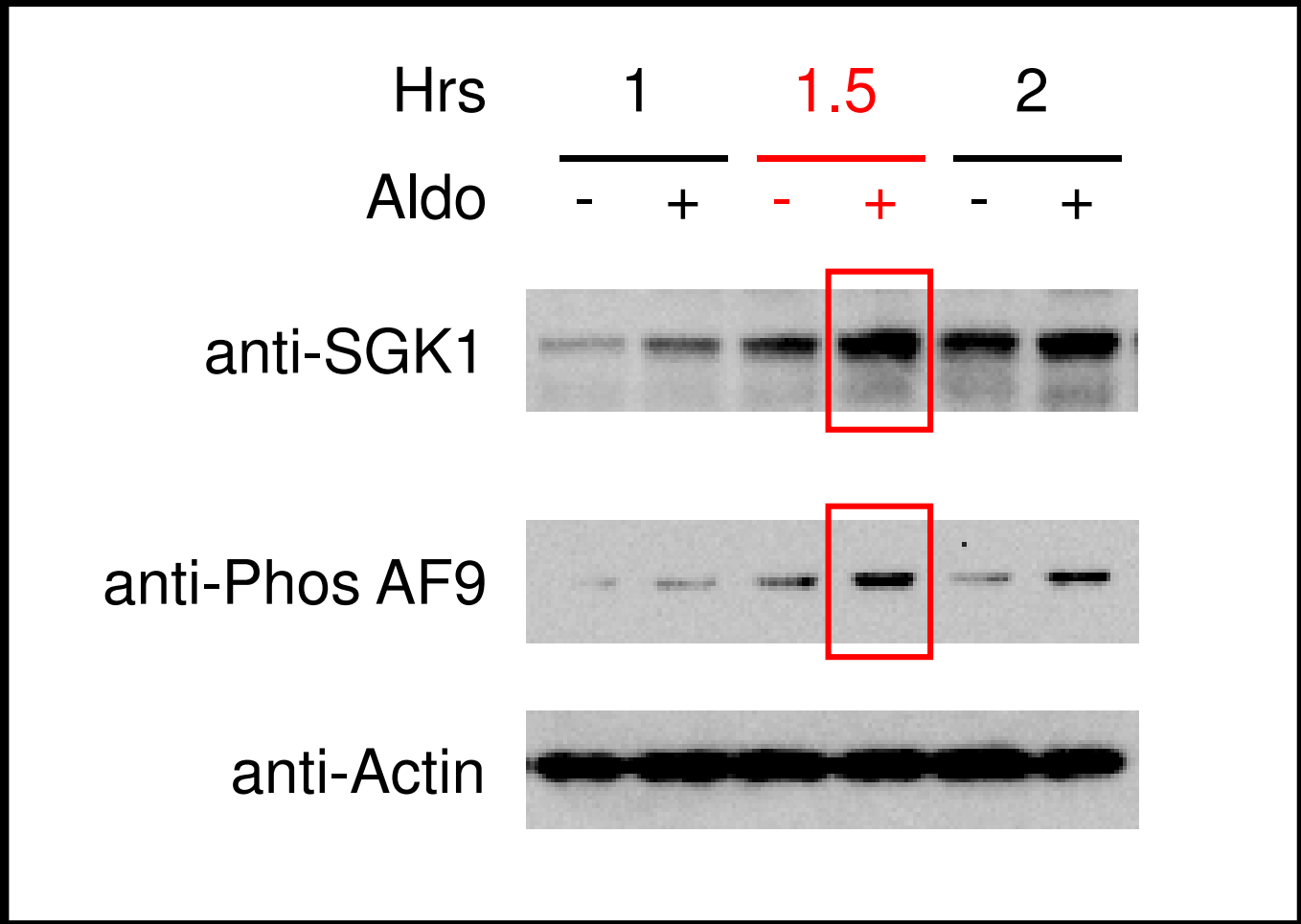



Zhang et al. JCI. 2007.

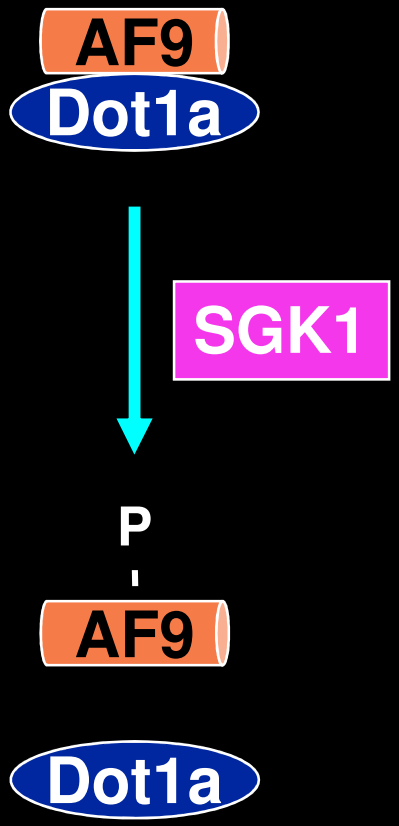
# Aldosterone Induces SGK1 and Phos-AF9 in mIMCD3 cells

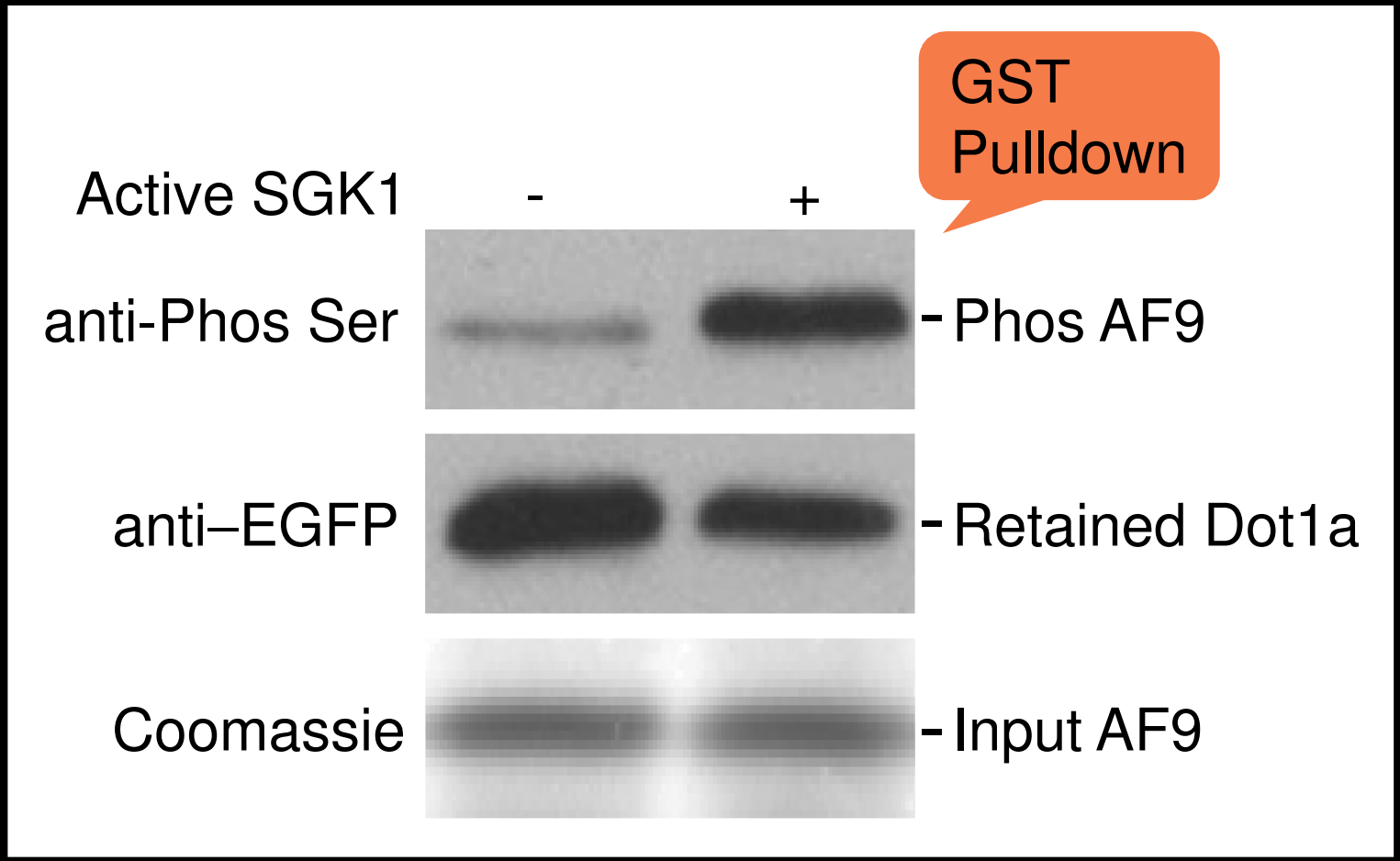



Zhang et al. JCI. 2007.

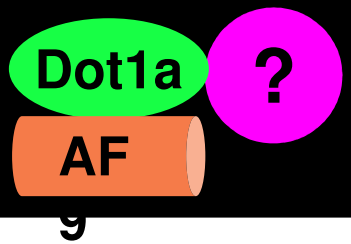
# AF9 Phosphorylation by SGK1 Impairs AF9-Dot1a Interaction in vitro

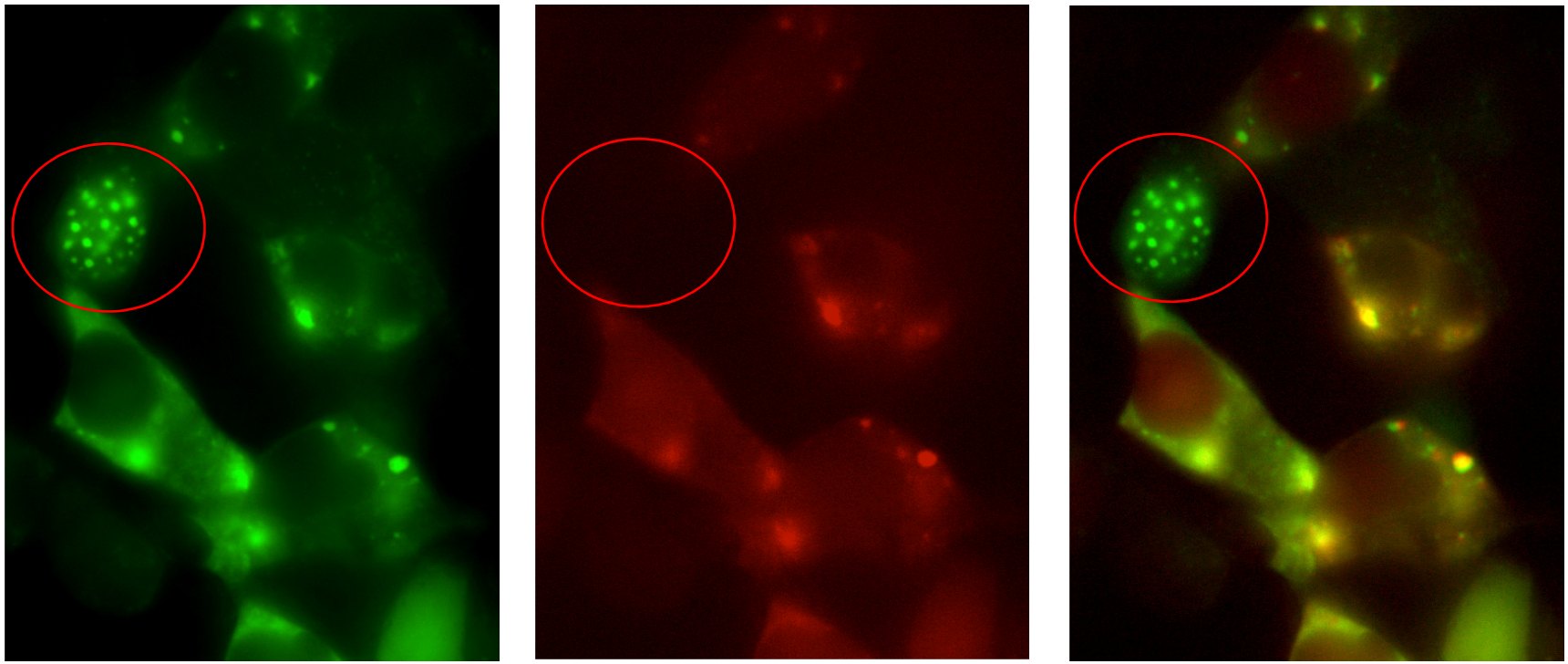



Zhang et al. JCI. 2007.

# AF17 facilitates Dot1a Nuclear Export

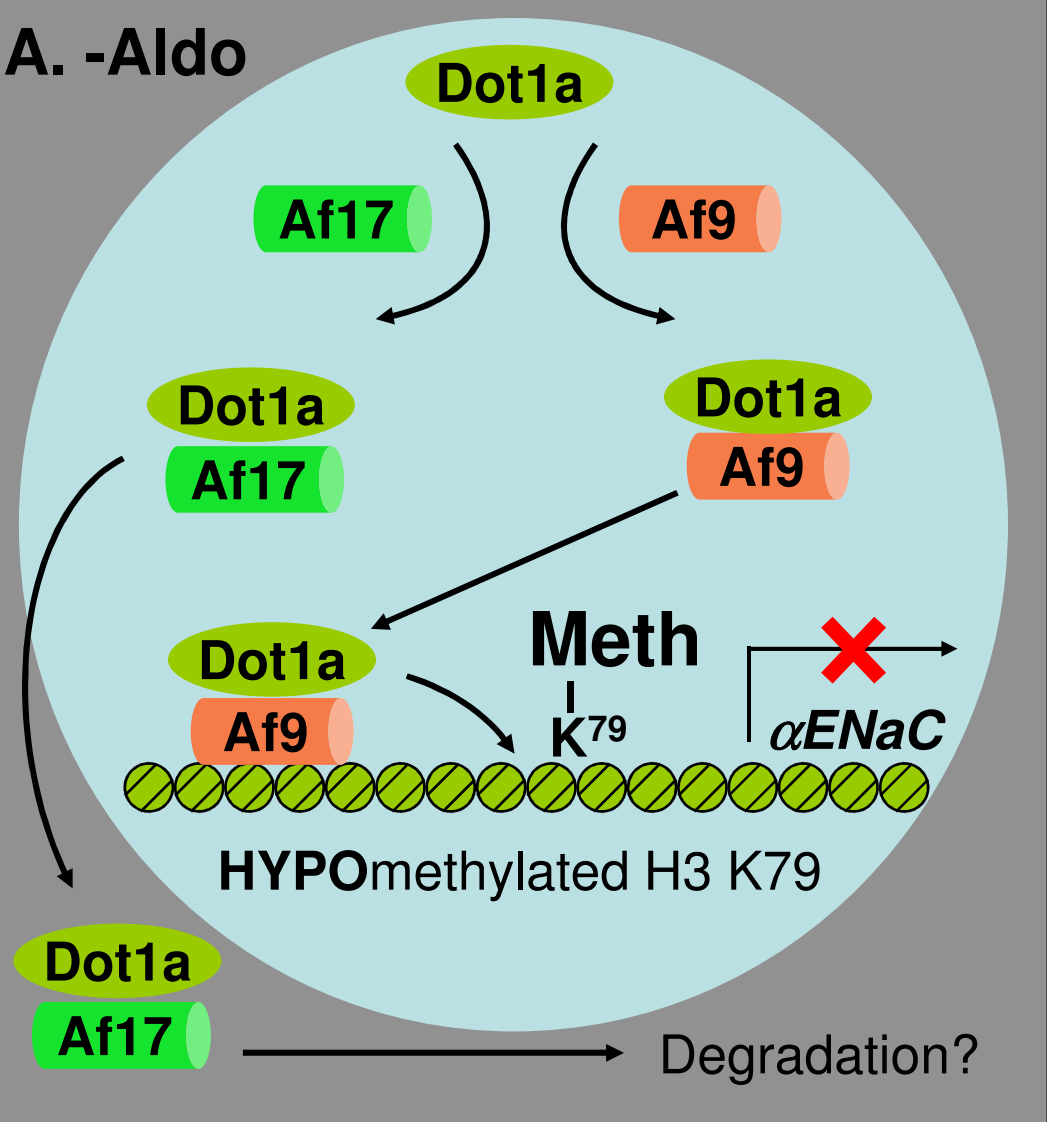


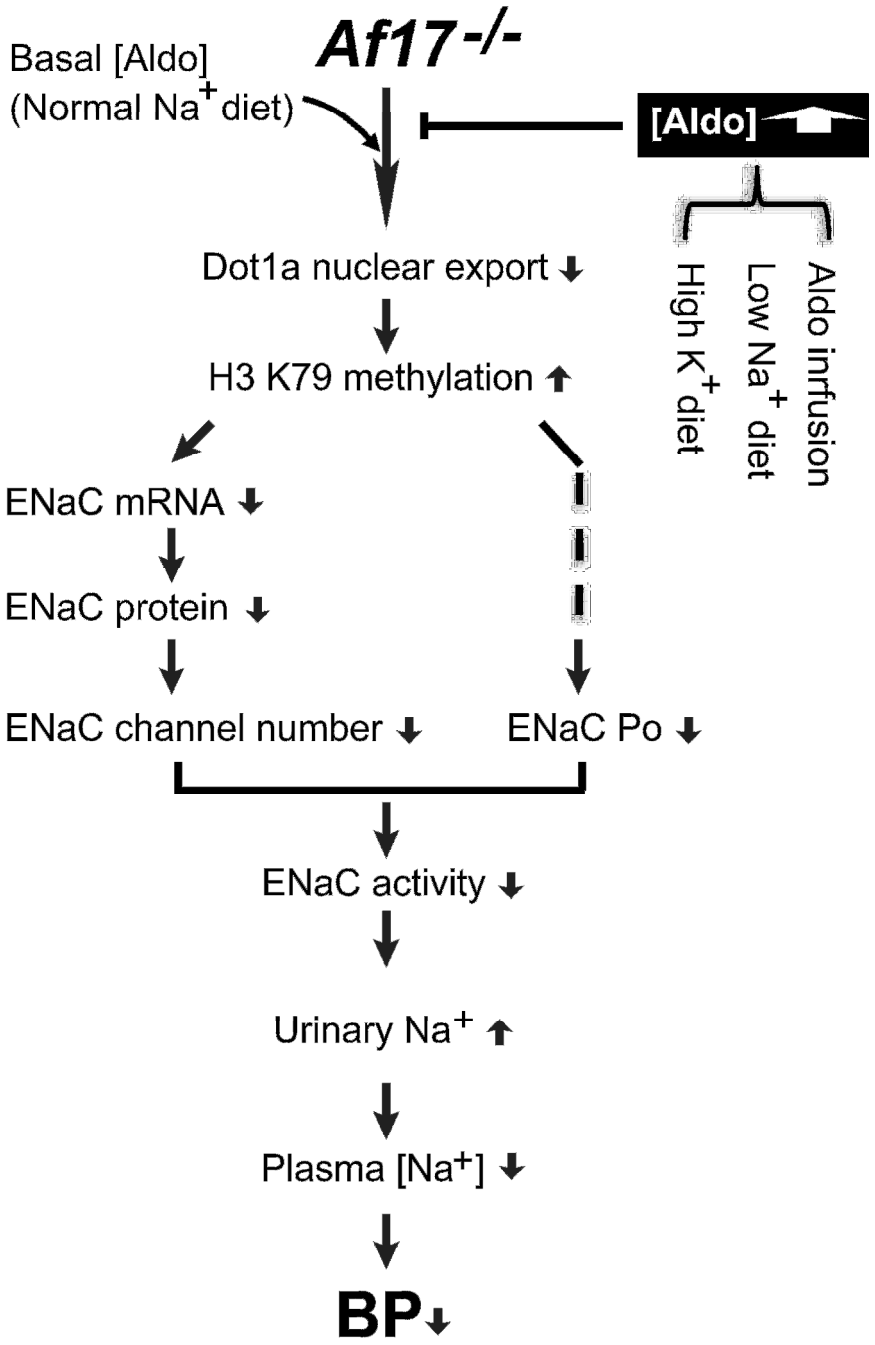



GFP-Dot1a | RFP-AF17 | Merge

Reisenauer et al JBC 2009

# Predicted *Af17*$^{-/-}$ Phenotype



# Characterization of AF17$^{-/-}$ Phenotype







Animal Holders



VPR Cuffs



Occlusion Cuffs

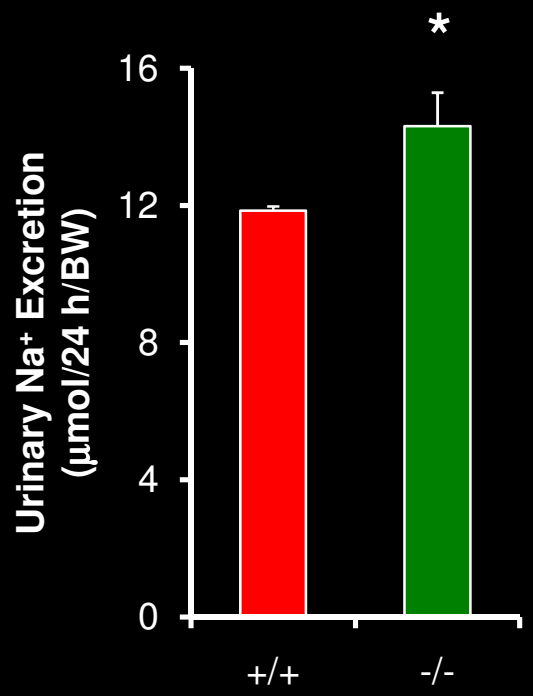


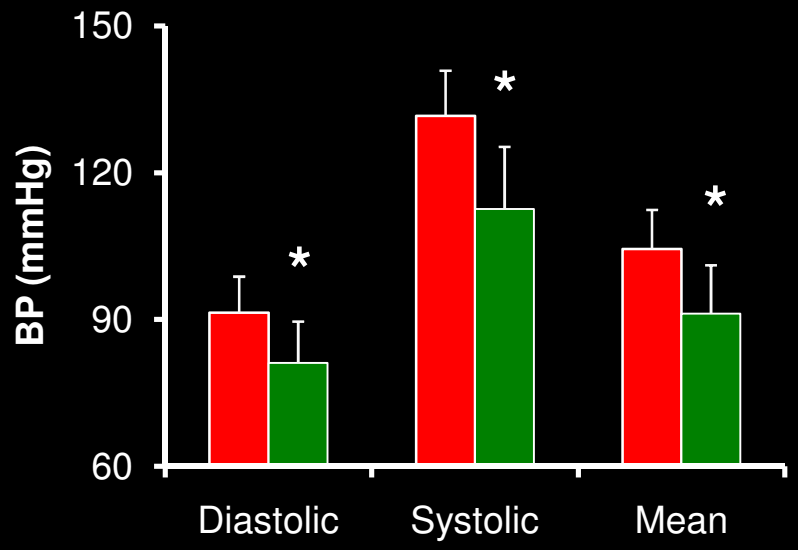

Tissue Collection and Analyses

# Increased $Na^+$ Excretion and Decreased Blood Pressure in *Af17*$^{-/-}$ Mice

+/+ n= 57 mice

-/- n= 65 mice



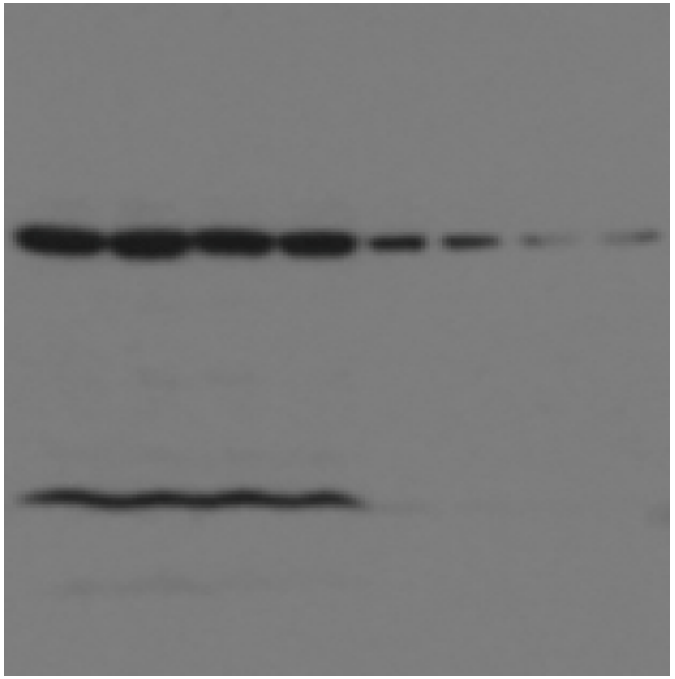




Chen et al JASN 2011

# Decreased ENaC Expression in *Af17*$^{-/-}$ Mice

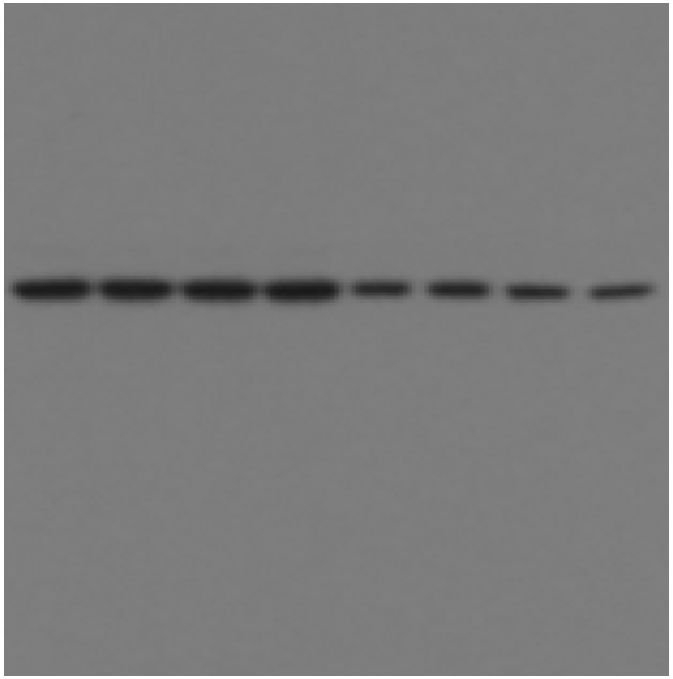
αENaC

+/+ -/-



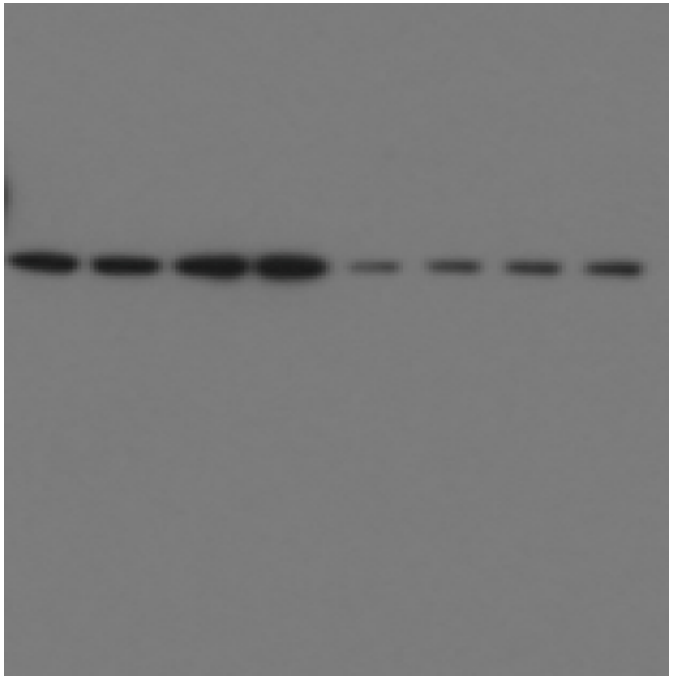
βENaC

+/+ -/-



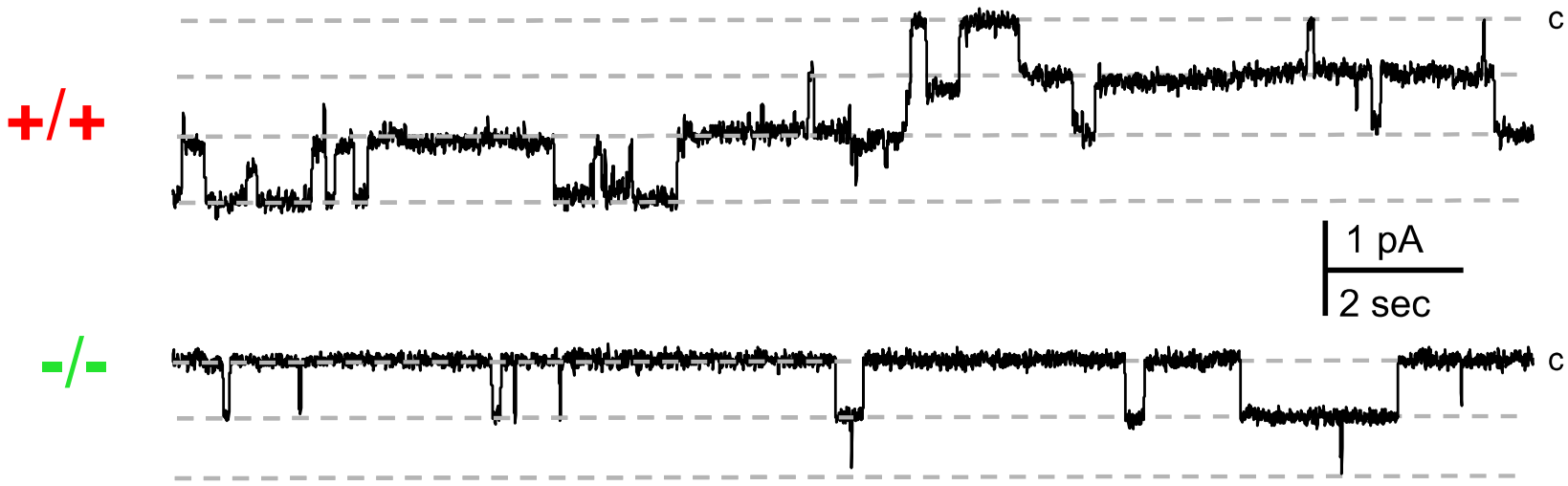
γENaC

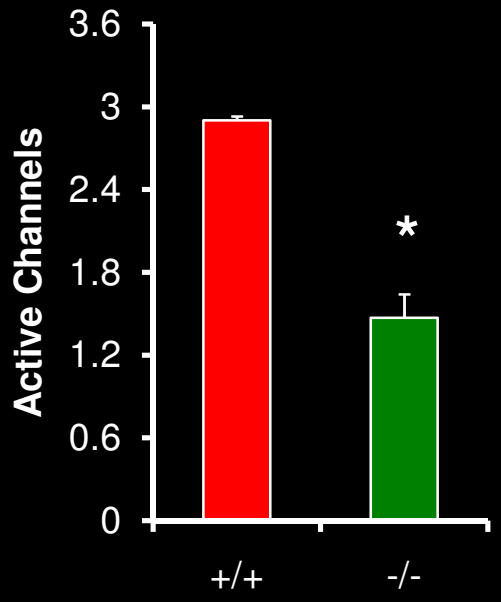
+/+ -/-

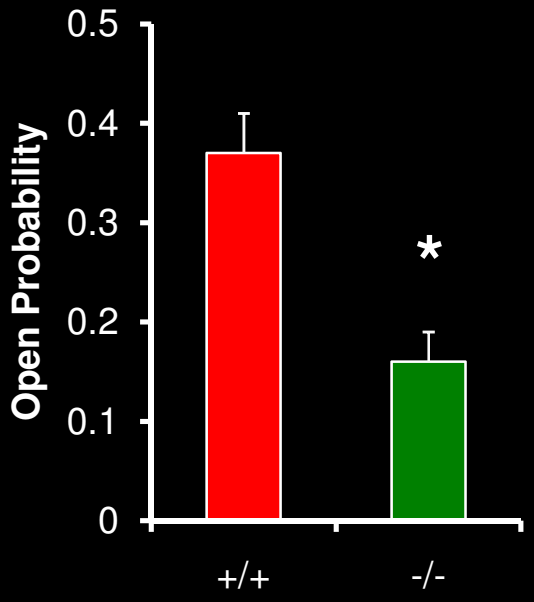


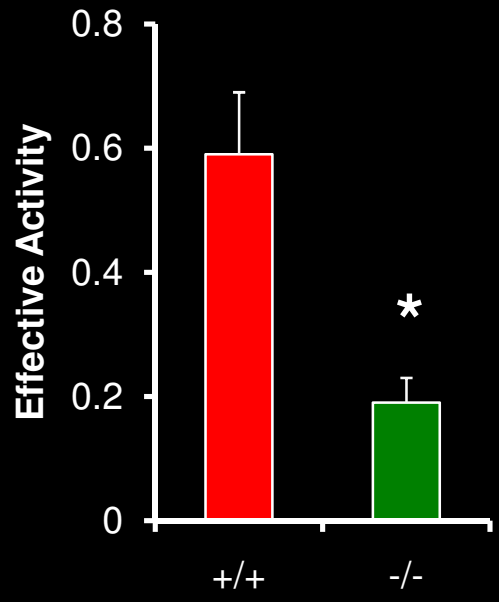
Chen et al JASN 2011

# Decreased ENaC Activity in *Af17*$^{-/-}$ Mice



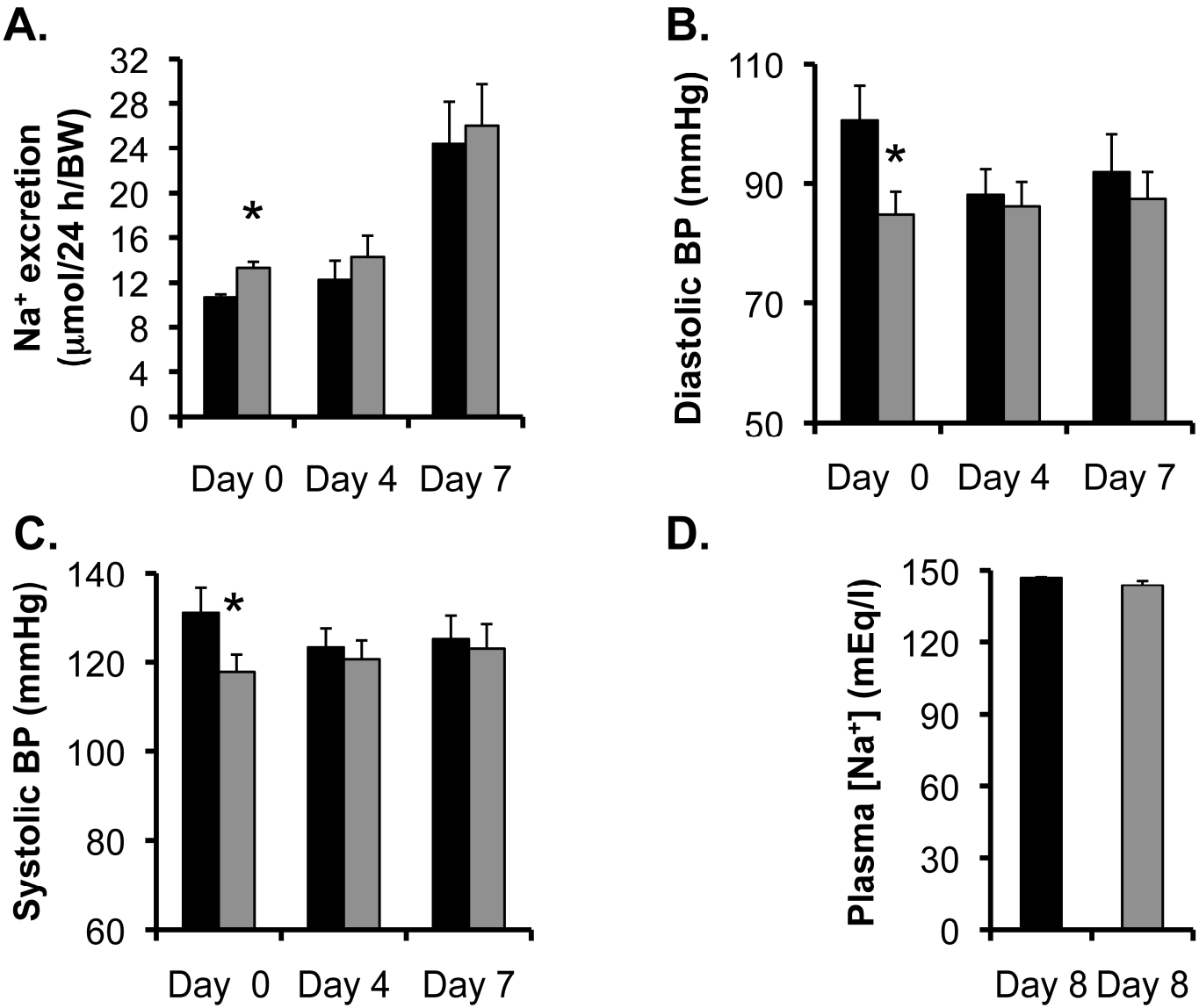



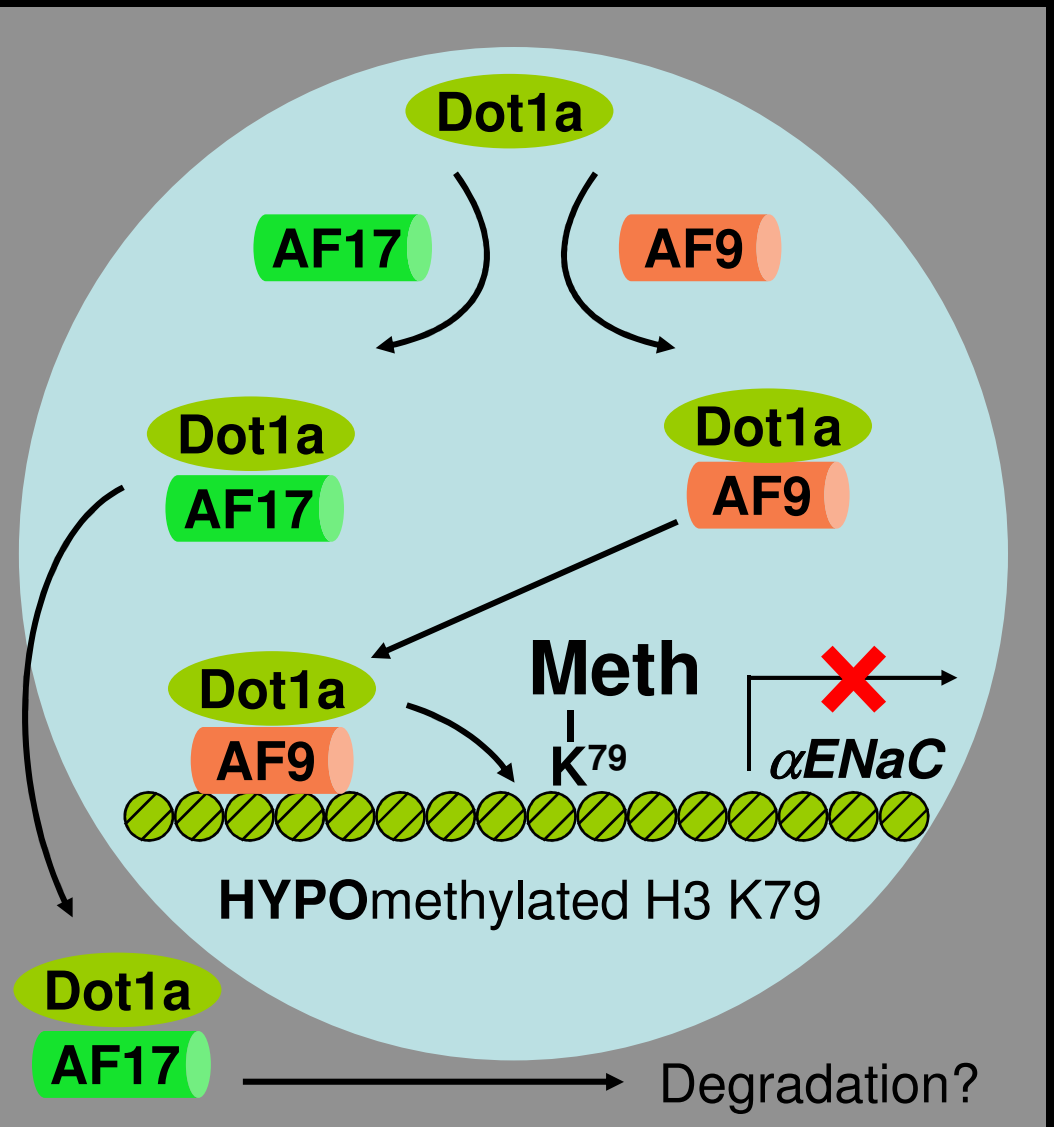

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# Aldosterone Infusion Rescues *Af17*$^{-/-}$ Phenotype



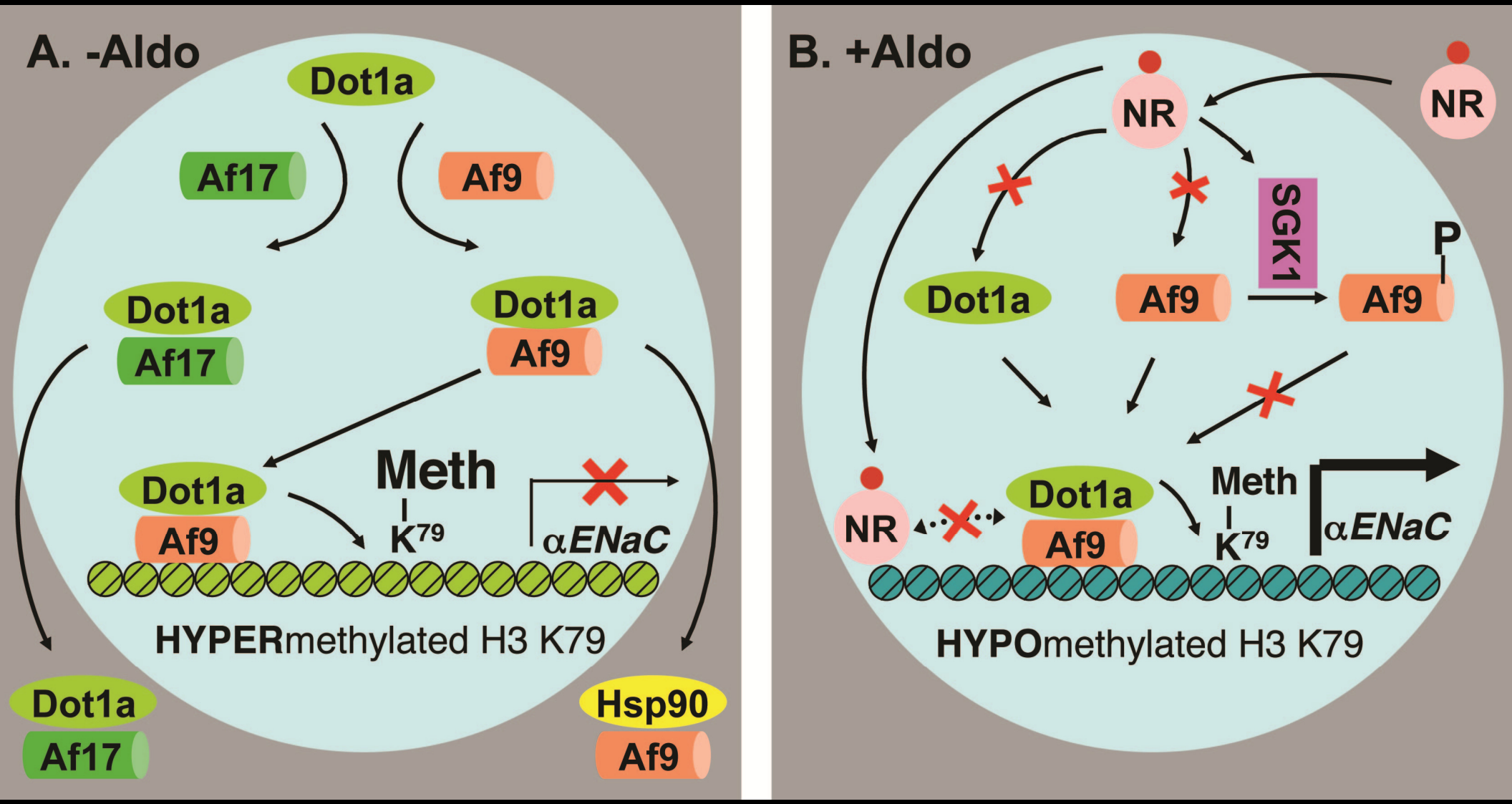

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# Observed AF17 Phenotype




- ↑ H3 m2K79 in the cells and at the αENaC promoter ✓
- ↓ α, β, γENaC expression at mRNA and protein levels ✓
- ↑ $Na^+$ excretion in urine ✓
- ↓ [$Na^+$] in blood ✓
- ↓ Blood pressure ✓

# Working Model



Duarte et al. Journal of Translational Medicine 2012, 10:56
http://www.translational-medicine.com/content/10/1/56

JOURNAL OF TRANSLATIONAL MEDICINE

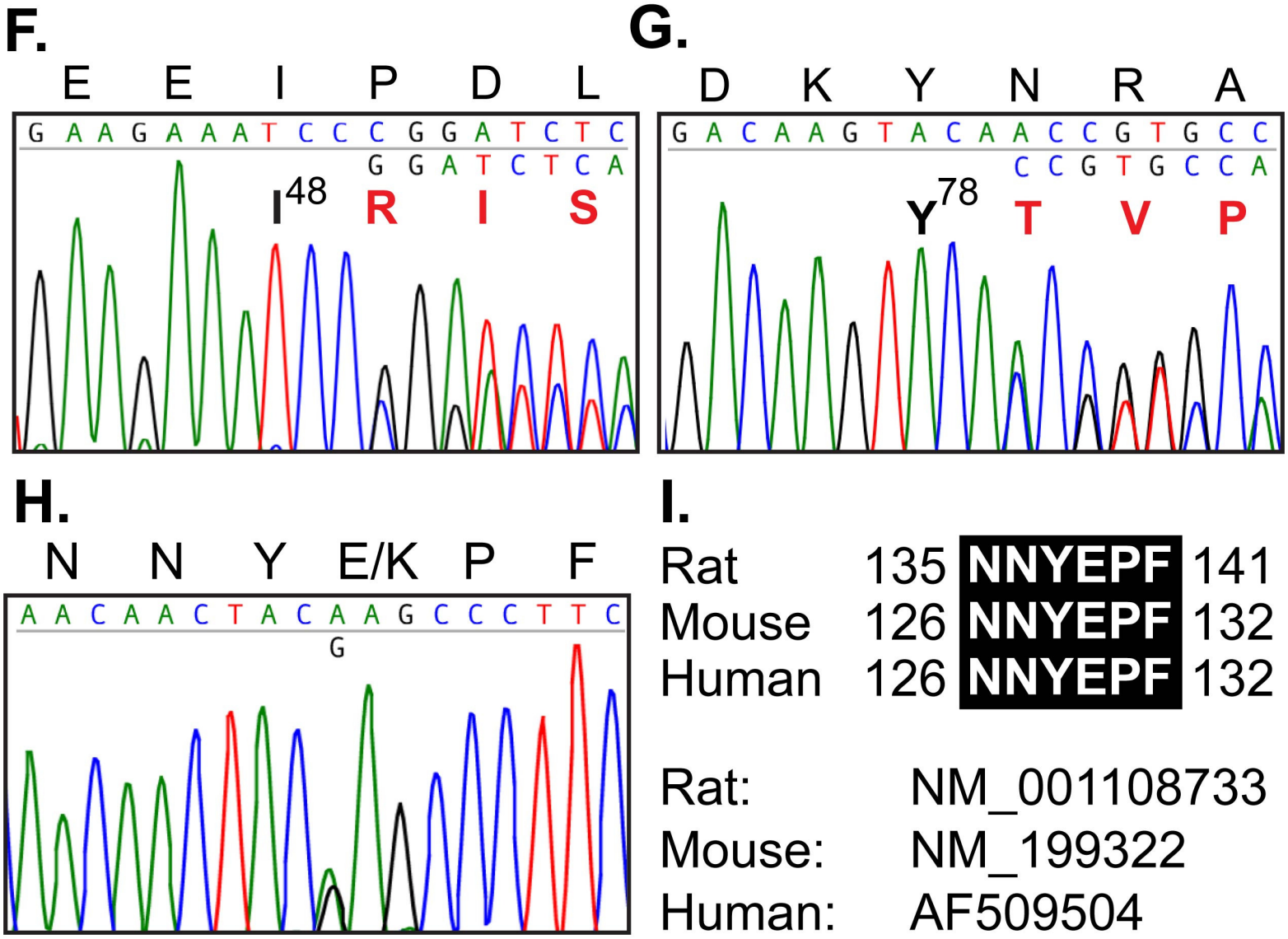
Open Access

# Effects of genetic variation in H3K79 methylation regulatory genes on clinical blood pressure and blood pressure response to hydrochlorothiazide

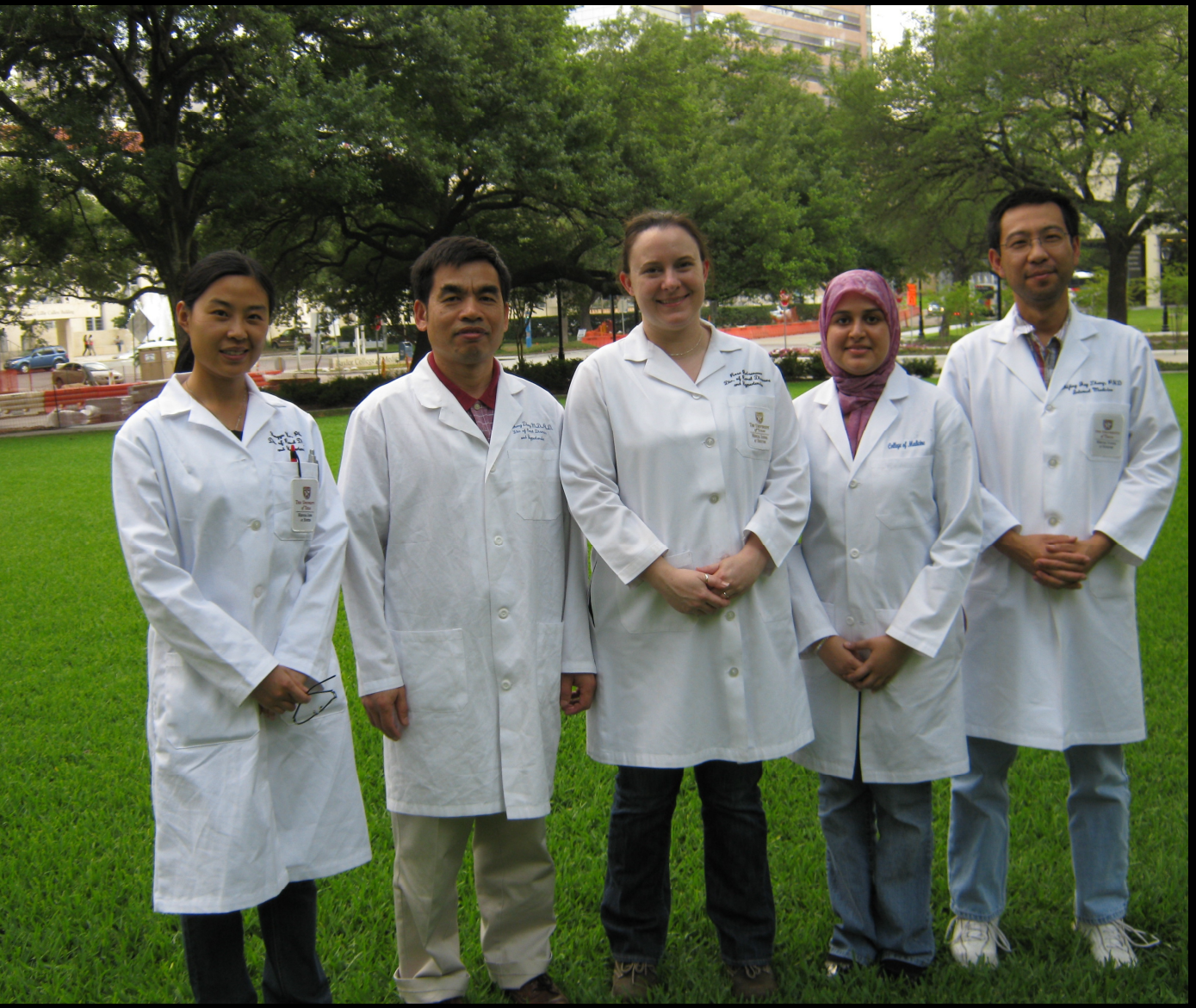
Julio D Duarte[1,2*], Issam Zineh[1,3], Ben Burkley[1], Yan Gong[1], Taimour Y Langaee[1], Stephen T Turner[4], Arlene B Chapman[5], Eric Boerwinkle[6], John G Gums[1], Rhonda M Cooper-DeHoff[1], Amber L Beitelshees[7], Kent R Bailey[8], Roger B Fillingim[9], Bruce C Kone[10,11] and Julie A Johnson[1]

**In one cohort, a polymorphism in DOT1L (rs2269879) was strongly associated with greater systolic (P = 0.0002) and diastolic (P = 0.0016) blood pressure response to hydrochlorothiazide in Caucasians. However, this association was not replicated in the other cohort. When untreated blood pressure levels were analyzed, we found directionally similar associations between a polymorphism in MLLT3 (rs12350051) and greater untreated systolic (P< 0.01 in both cohorts) and diastolic (P < 0.05 in both cohorts) blood pressure levels in both cohorts.**

# Mutated Dot1a in Kidney Biopsies from CKD Patients



# Acknowledgment





# Acknowledgment

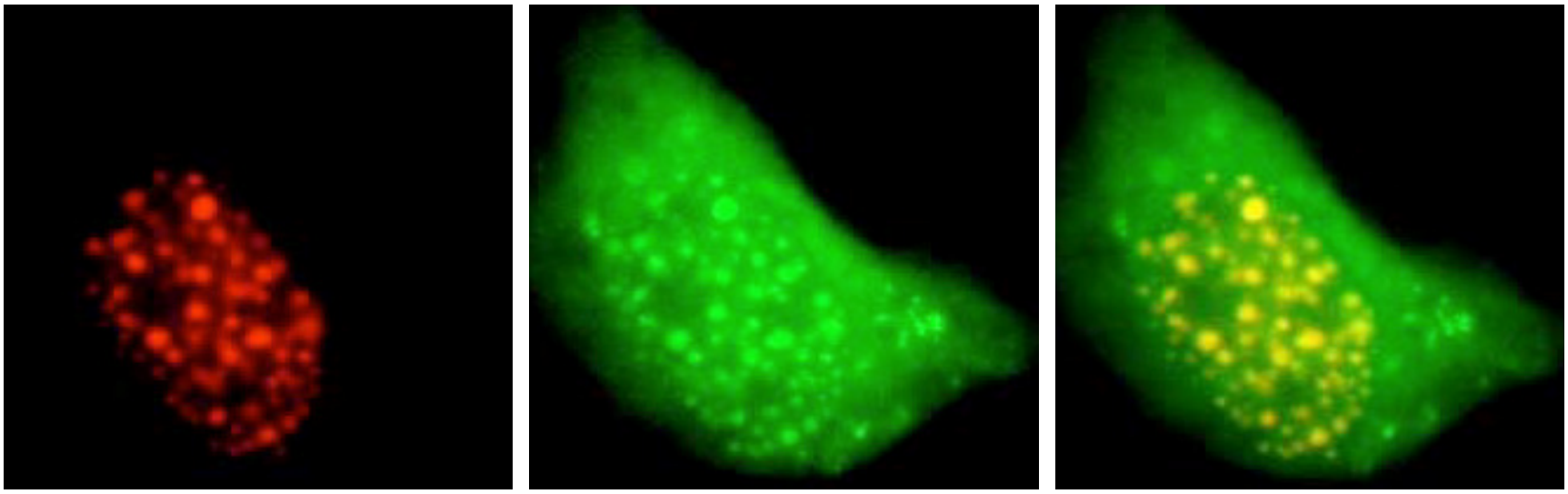
Bruce C. Kone (University of Florida)
Charles C. Hemenway (Loyola University)
David Pearce (UCSF)
Volker Vallon (UCSD)
Florian Lang (Free University, Germany)
Dietmar Khul (Free University, Germany)
Günther Schütz (University of Freiburg, Germany)

Funding:

NIH, AHA, ASN

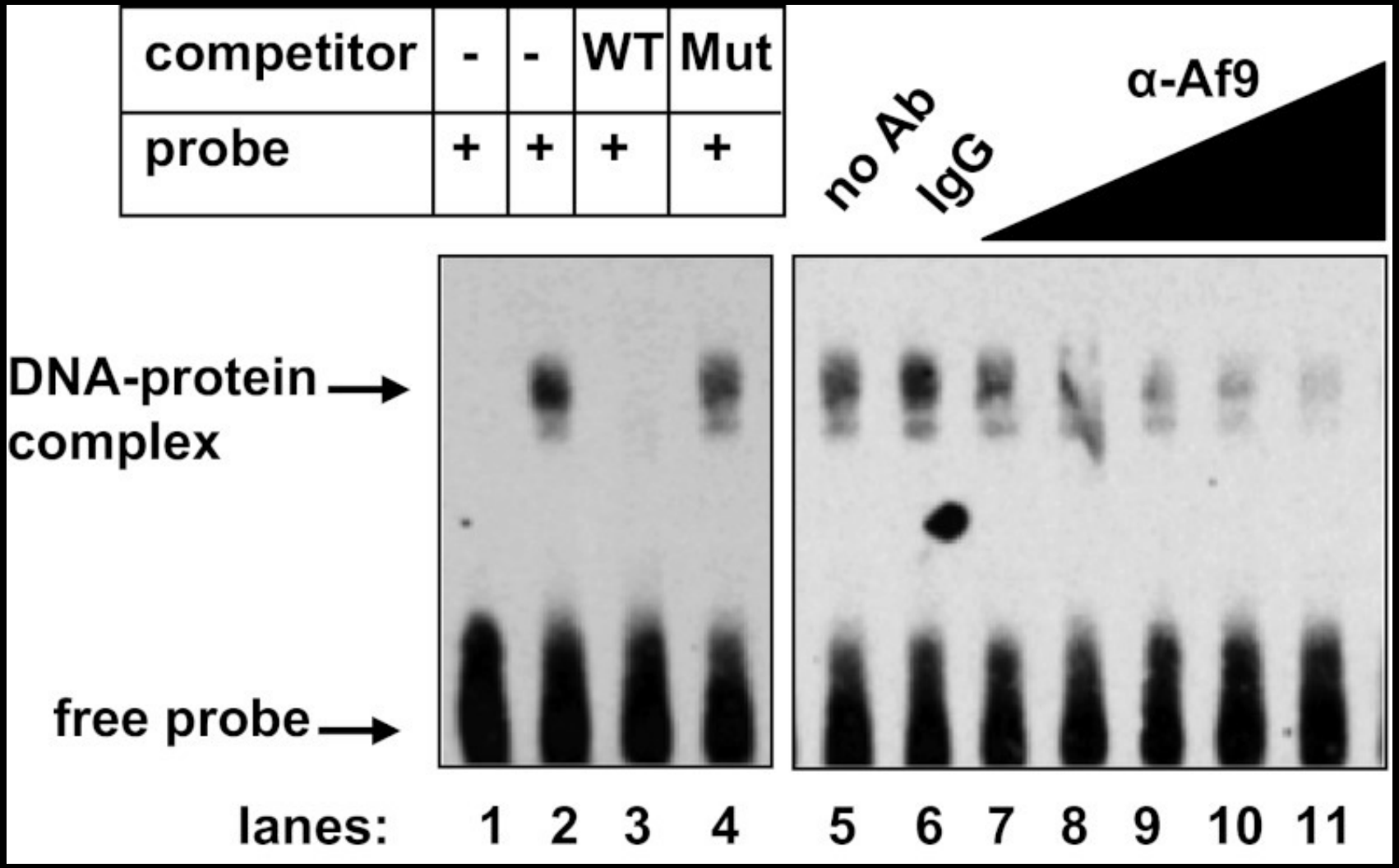
# SGK1 and AF9 Colocalize in mIMCD3 Cells



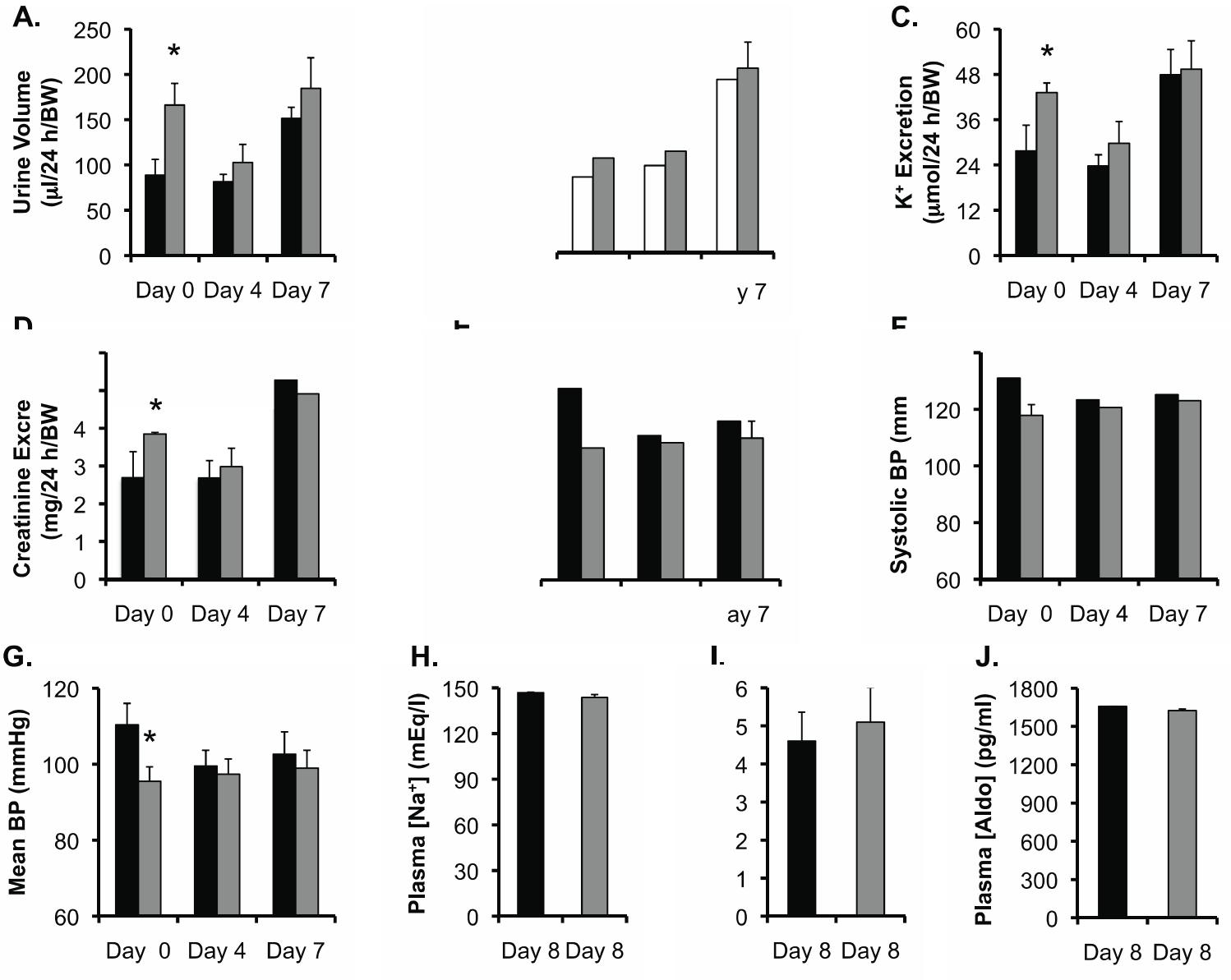

Unpublished.

# Af9 binds to +78/+92 of αENaC in vitro




Zhang et al. Am J Physiol Renal Physiol. 2013.

# Aldosterone Infusion Rescues *Af17*$^{-/-}$ Phenotype




Chen et al JASN 2011