Signaling pathways related to decreased influx of Ca^{2+} and increased cAMP concentration in epithelial cells of polycystic kidney disease (PKD)

多発性嚢胞腎(PKD)上皮細胞における Ca^{2+}輸送低下および 細胞内cAMP増加に関わるシグナル伝達

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Today's subject

1. Polycystic kidney disease (PKD)
2. Decreased influx of Ca^{2+} concentration
3. Increased cAMP concentration
4. Clinical trial
5. Signaling pathways in epithelial cells of PKD

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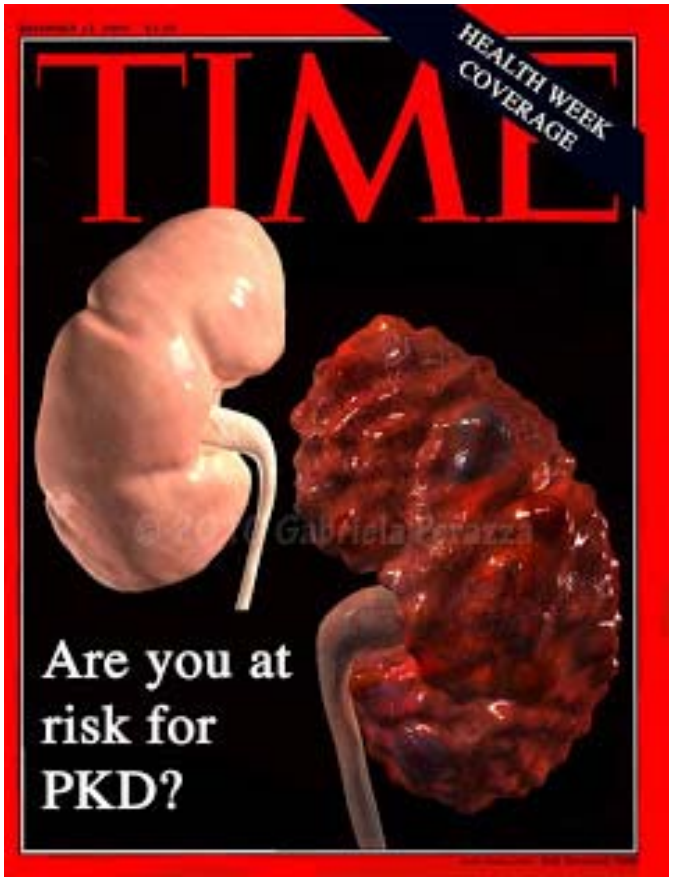
PKD is characterized by the presence of numerous cysts that originate from the renal tubules.



November 19, 2012

http://www.thekidneydoctor.org/

- Cell proliferation
- Fluid secretion
- Fibrosis

Polycystic kidney disease (PKD)

ADPKD : incidence of 1:500-1000

Autosomal Dominant

PKD1 (human chromosome 16) 85%

PKD2 (human chromosome 4) 15%

ARPKD : incidence of 1:20,000-40,000

Autosomal recessive

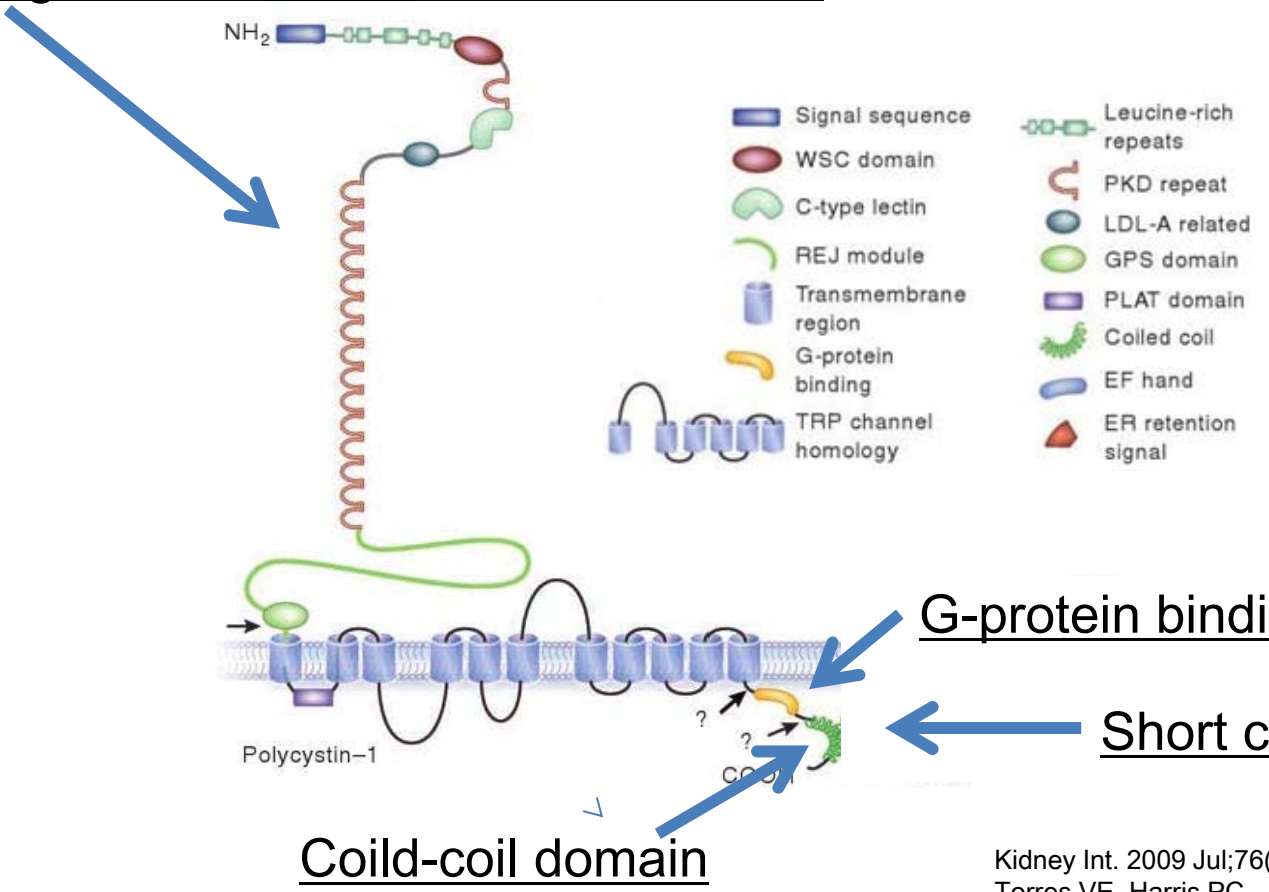
PKHD1 (human chromosome 6)

ADPKD

PKD1 Gene product:

polycystin-1 (PC1): a large receptor-like protein

Long N-terminus extracellular domain



G-protein binding

Short cytoplasmic C-terminus

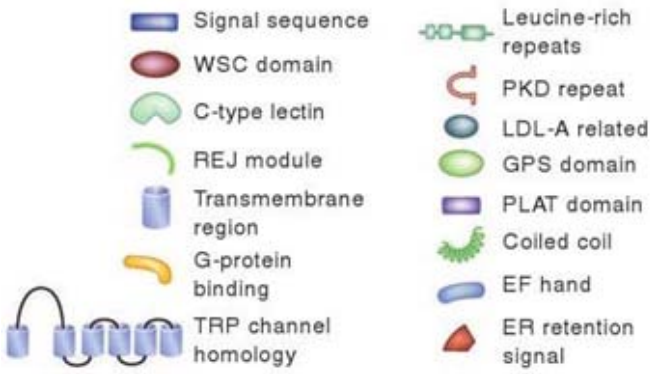
Coild-coil domain

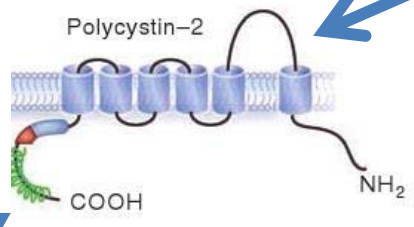
Kidney Int. 2009 Jul;76(2):149-68.
Torres VE, Harris PC.

ADPKD

PKD2 Gene product:

polycystin-2 (PC2): calcium (Ca^{2+}) channel

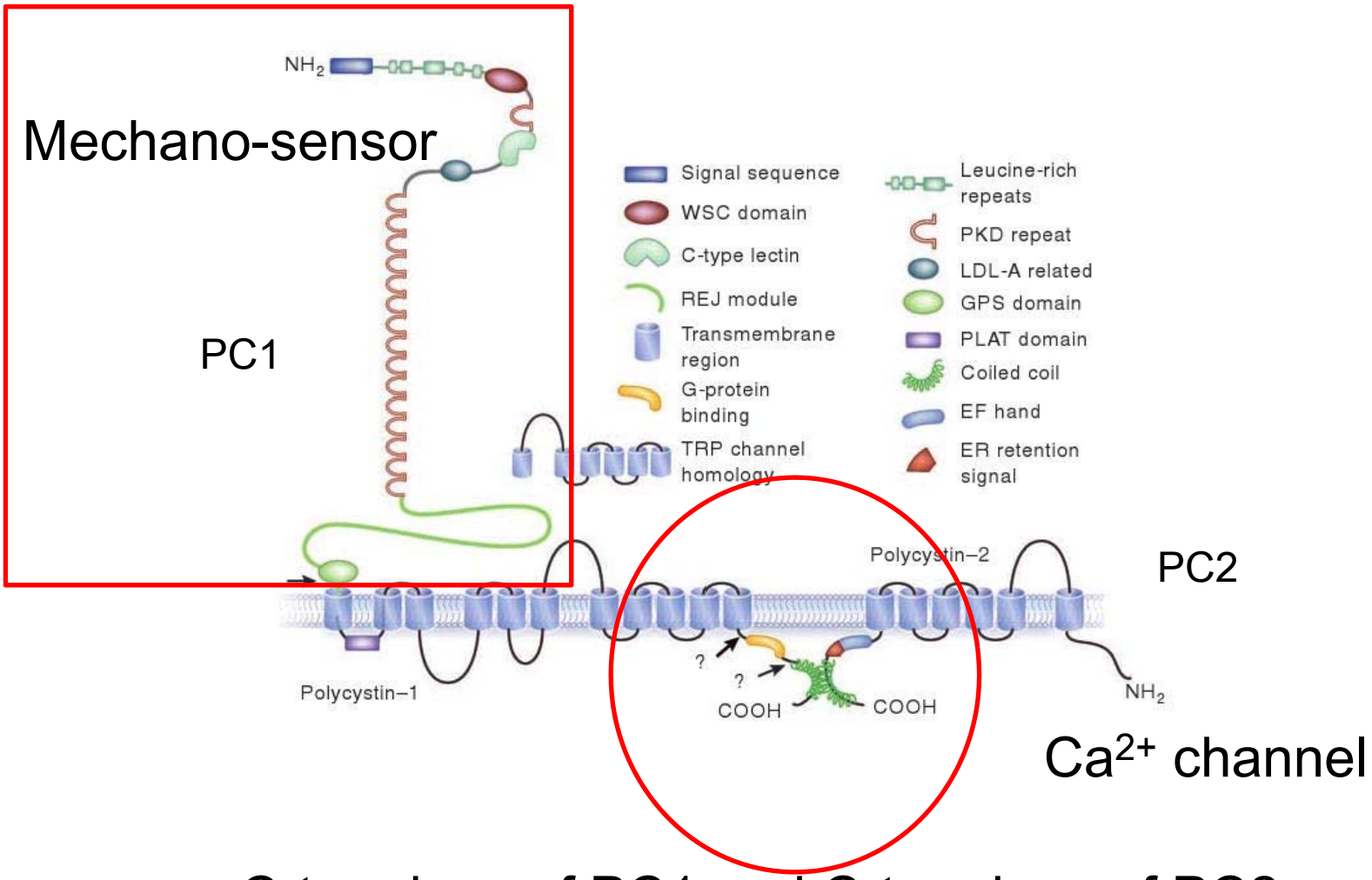




TRP channel homology
…Ca^{2+} channel

Coild-coil domain

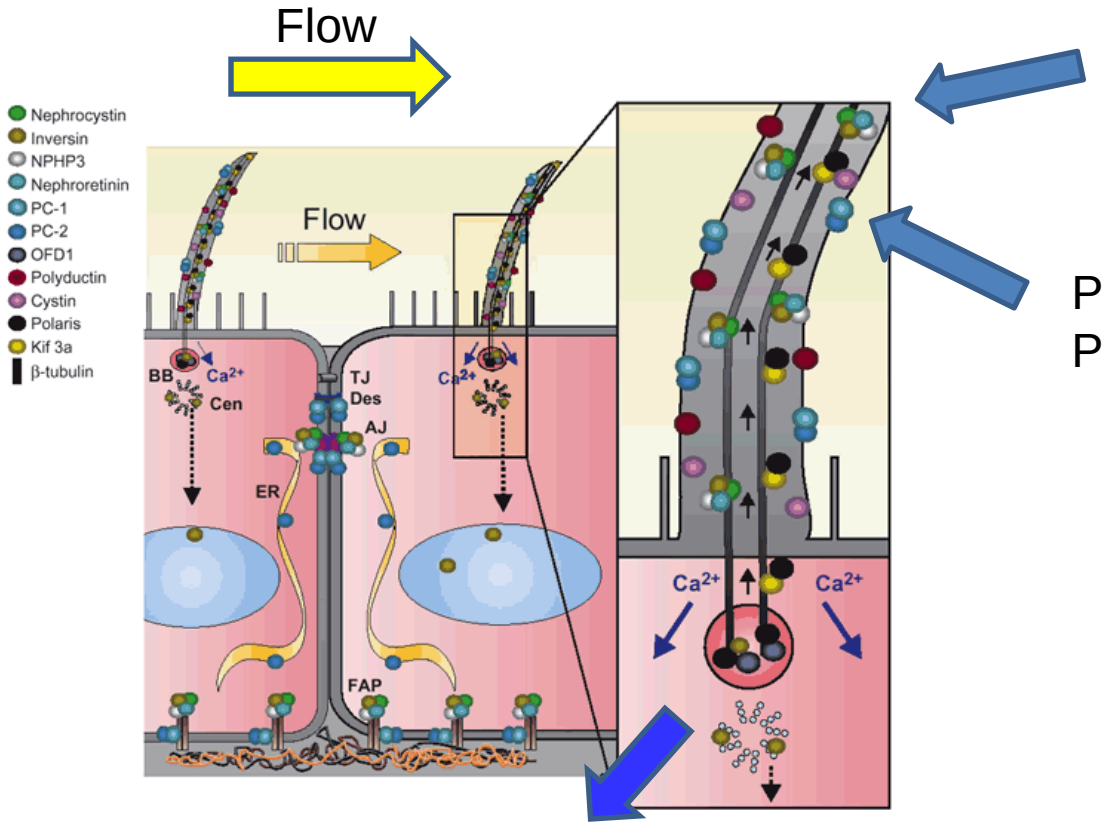
Kidney Int. 2009 Jul;76(2):149-68.
Torres VE, Harris PC.



C-terminus of PC1 and C-terminus of PC2 interact by coiled-coil domain.

Kidney Int. 2009 Jul;76(2):149-68.
Torres VE, Harris PC.

Polycystins locate in primary cilia of epithelia cells.



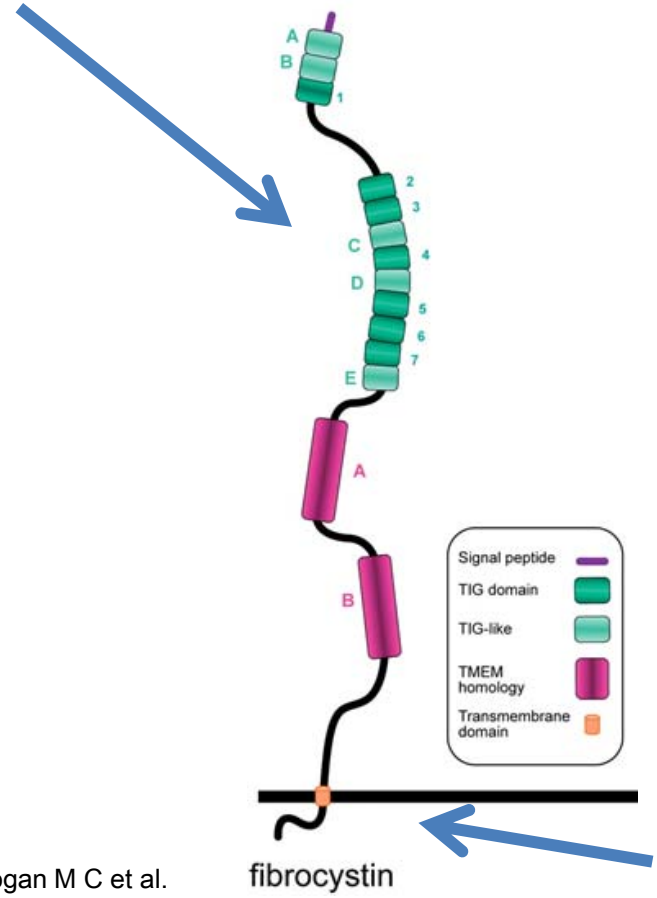
Nature Genetics 34, 355 - 356 (2003)
Terry Watnick & Gregory Germino

ARPKD

PKHD1 Gene product:

Fibrocystin/polyductin (FPC)

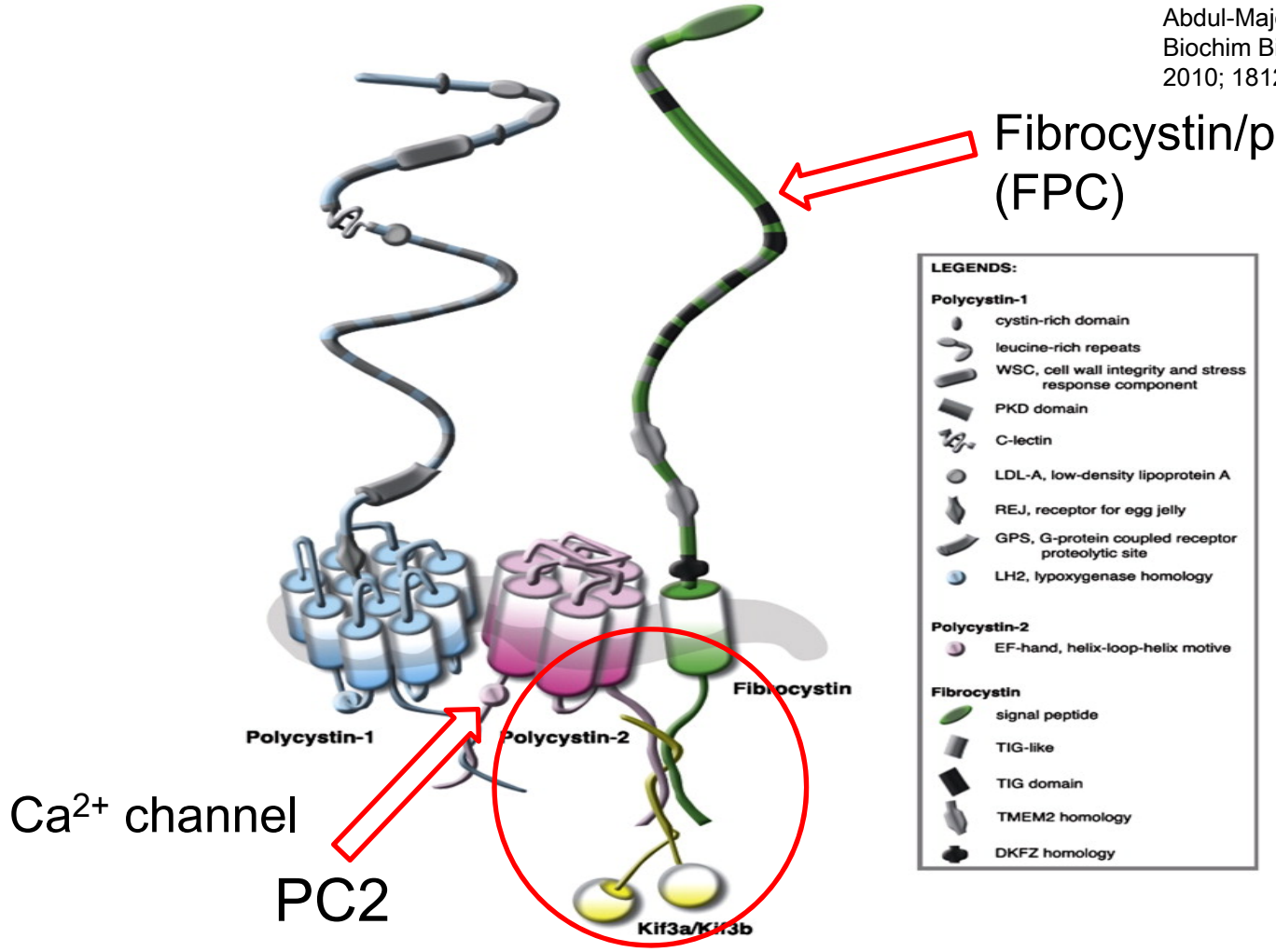
Long N-terminus extracellular domain



Short cytoplasmic C-terminus

Hogan M C et al. Hum. Mol. Genet. 2003;12:685-698

Abdul-Majeed S, Nauli SM.
Biochim Biophys Acta.
2010; 1812:1281-1290.



C-terminus of FPC and N-terminus of PC2 interact.

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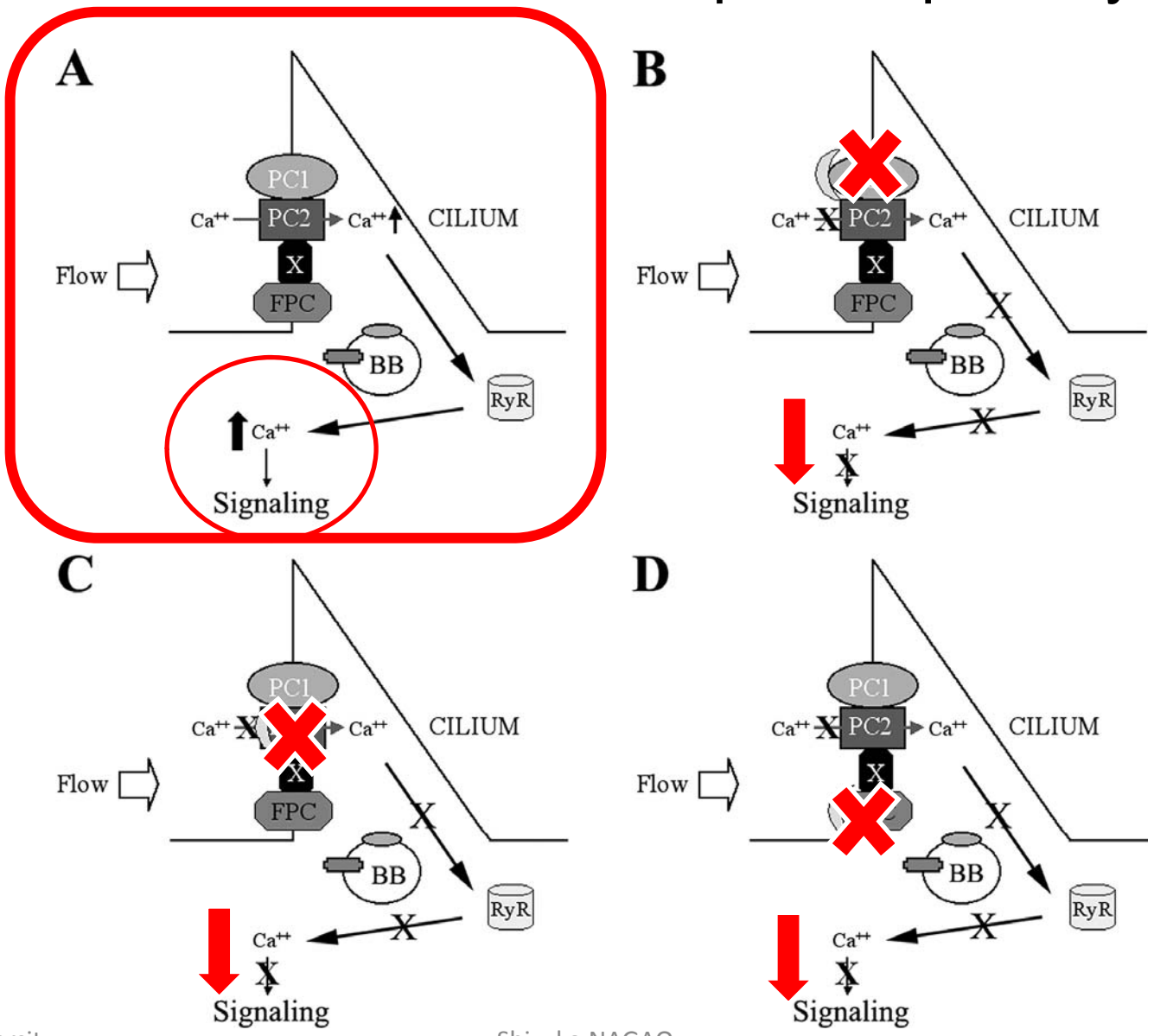
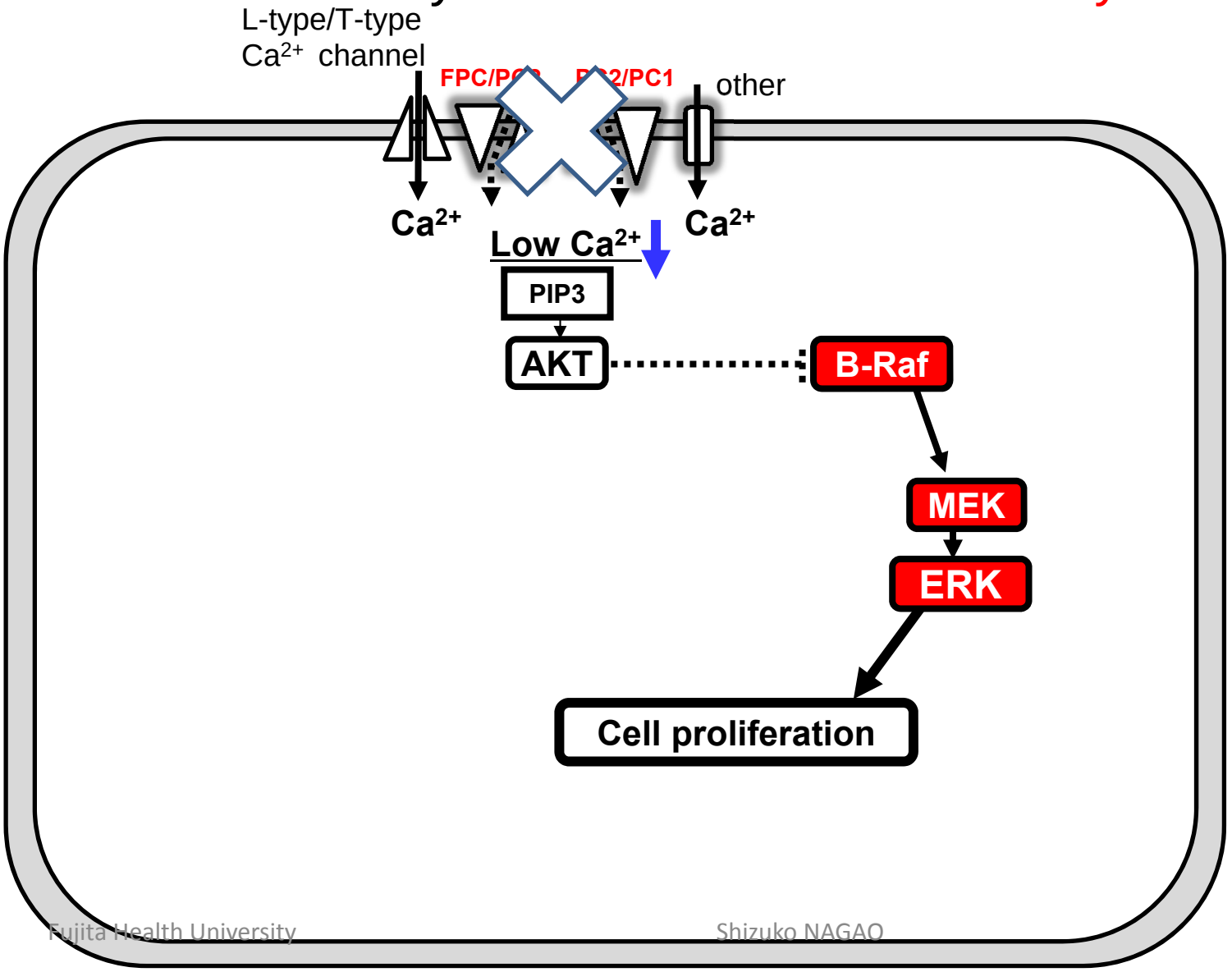
Model of PC1-PC2-FPC complex at primary cilium



Wang S et al. Mol. Cell. Biol. 2007;27:3241-3252

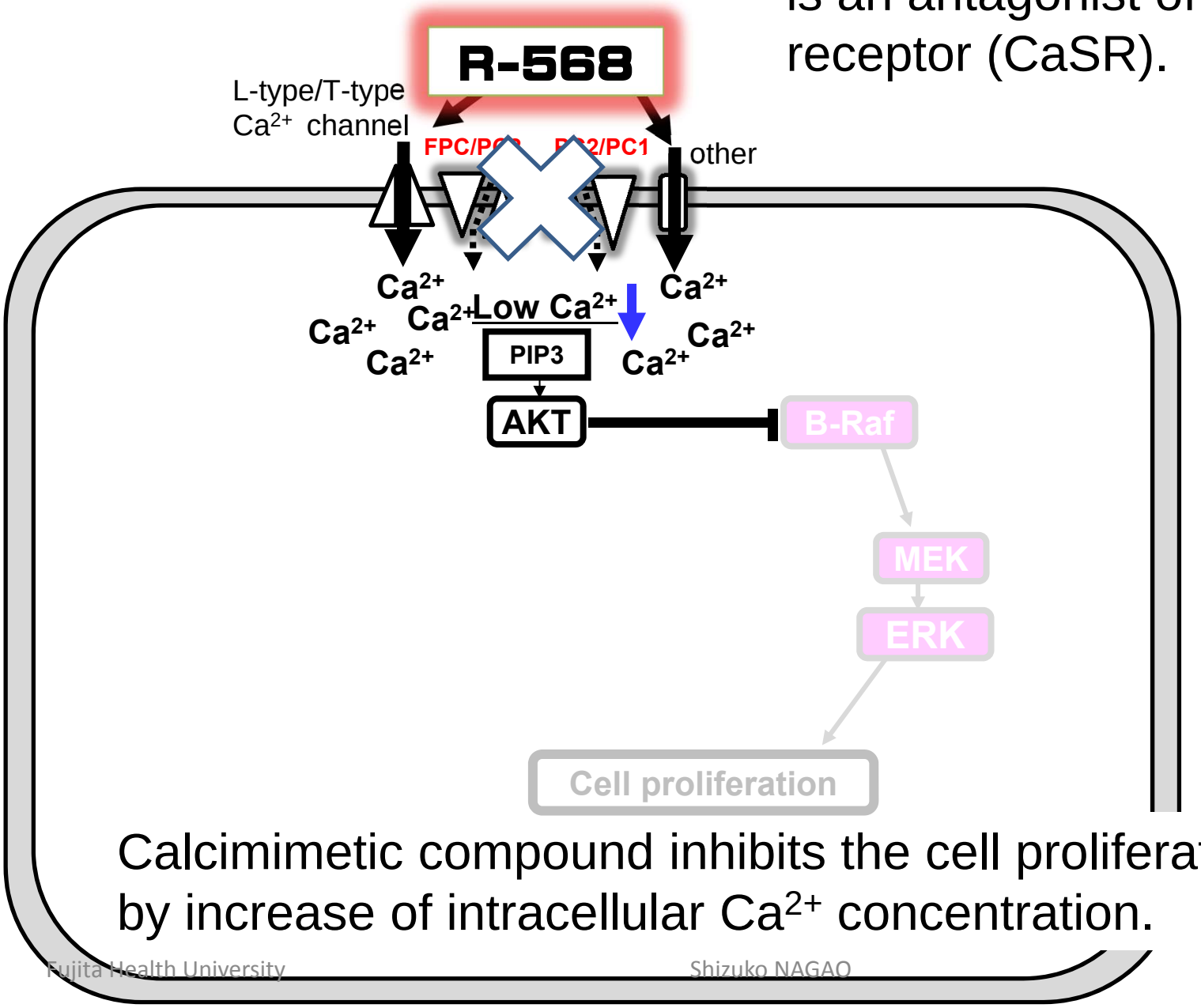
PKD cells

In PKD epithelia cells, decreased influx of intracellular Ca^{2+} induces cell proliferation by the reduction of AKT activity.



PKD cells

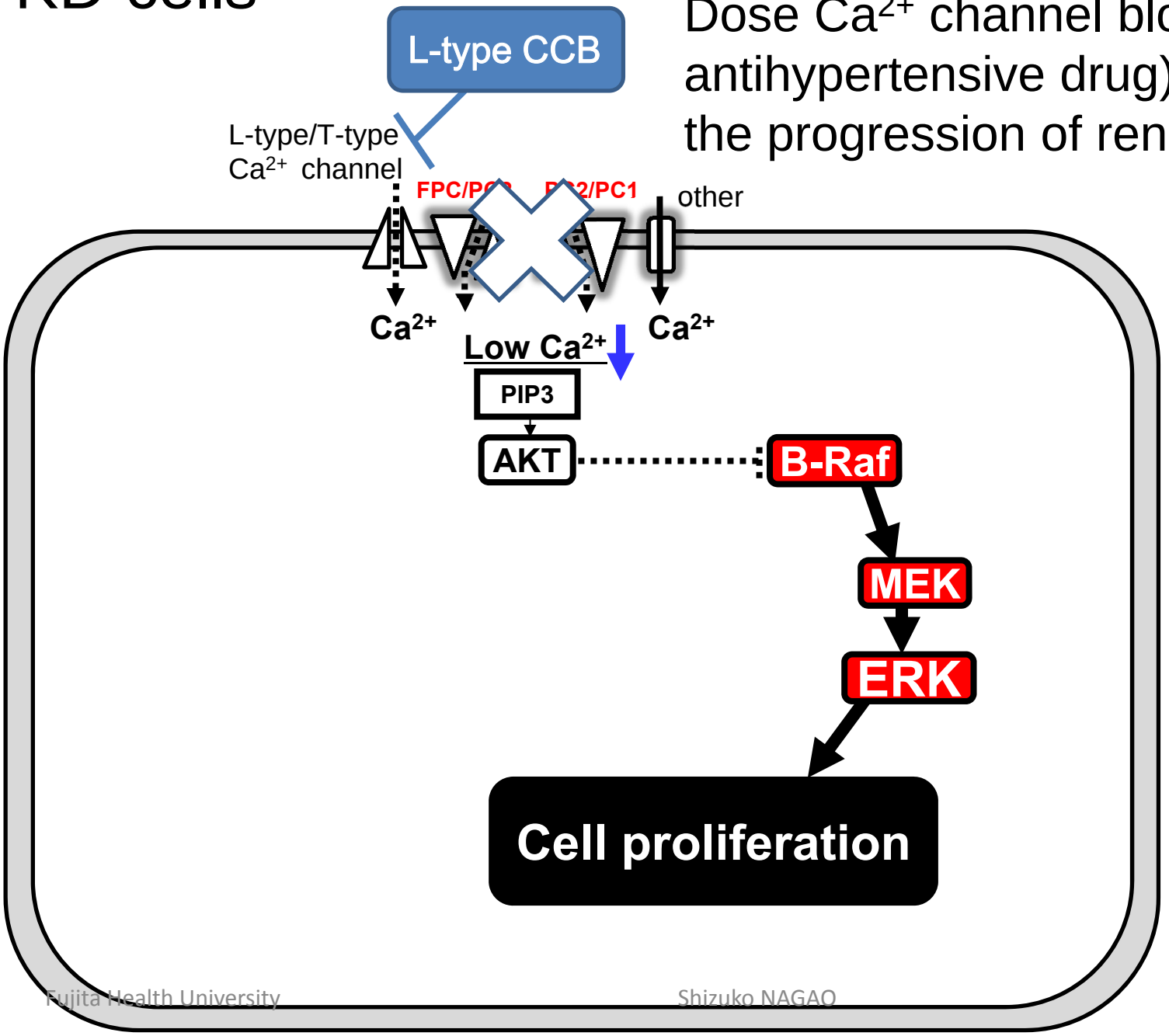
Calcimimetic compound, R-568, is an antagonist of Ca^{2+}sensing receptor (CaSR).



Calcimimetic compound inhibits the cell proliferation by increase of intracellular Ca^{2+} concentration.

PKD cells

Dose Ca^{2+} channel blocker (CCB: antihypertensive drug) accelerate the progression of renal disease?



Outline

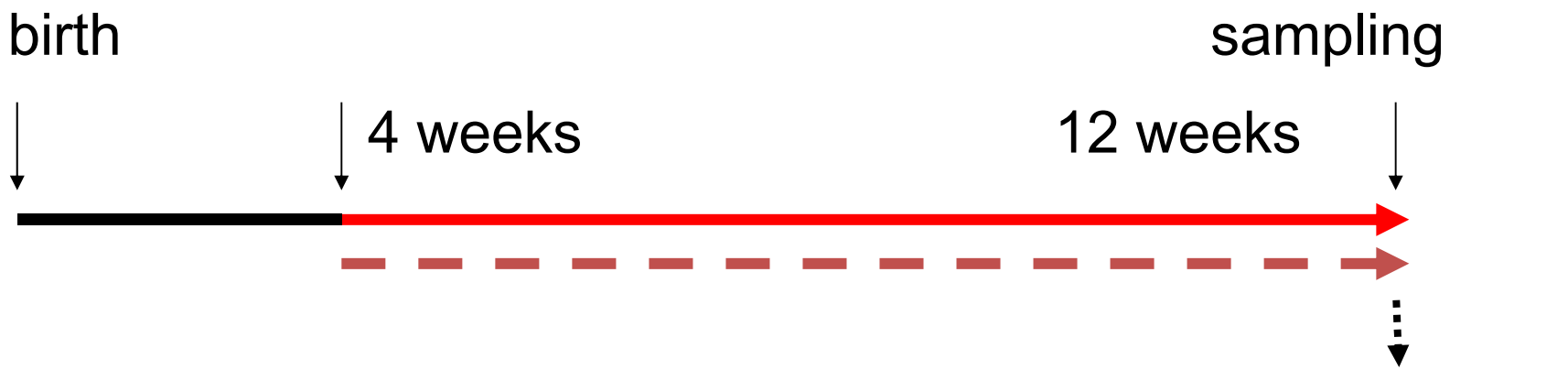
- Treatment with verapamil, a L-type CCB, increased renal activity of B-Raf/MEK/ERK signaling and caused an acceleration in growth of renal cysts in a PKD model, Cy/+ rats.

Nagao et al.
Kidney International (2008) 73, 269–277

Protocol

- Rats were treated with verapamil from 4 to 12 weeks of age.

Cy/+ : polycystic kidney
+/+ : normal kidney



Verapamil : L-type CCB
Antihypertensive drug

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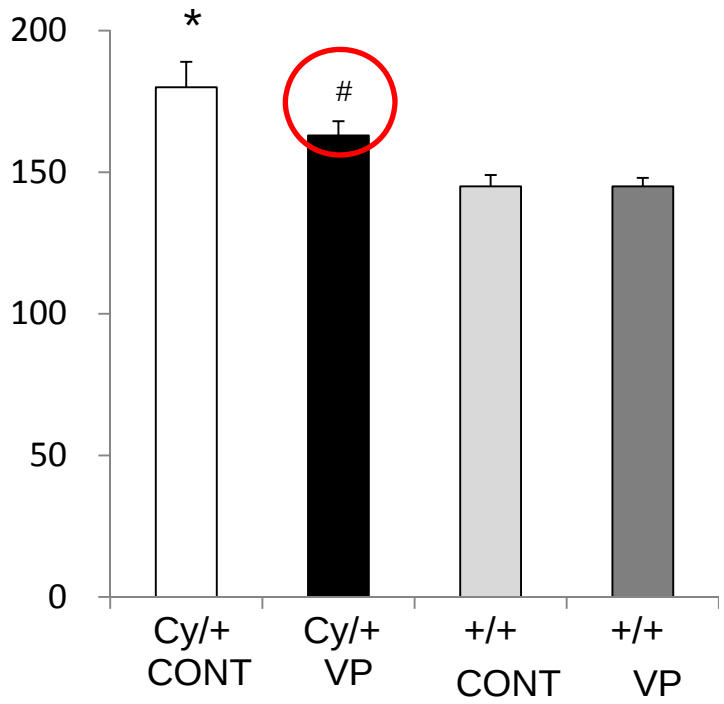
Effect of verapamil (VP) treatment on systolic blood presure



- Systolic BP was significantly elevated in Cy/+ rats compared to +/+ rats ($^{*}P<0.01$).
- Comparison between VP vs. CONT-treated Cy/+ rats (male and female), $^{\#}P<0.001$.
- Non-significant effect of VP on systolic BP was shown in +/+ rats.

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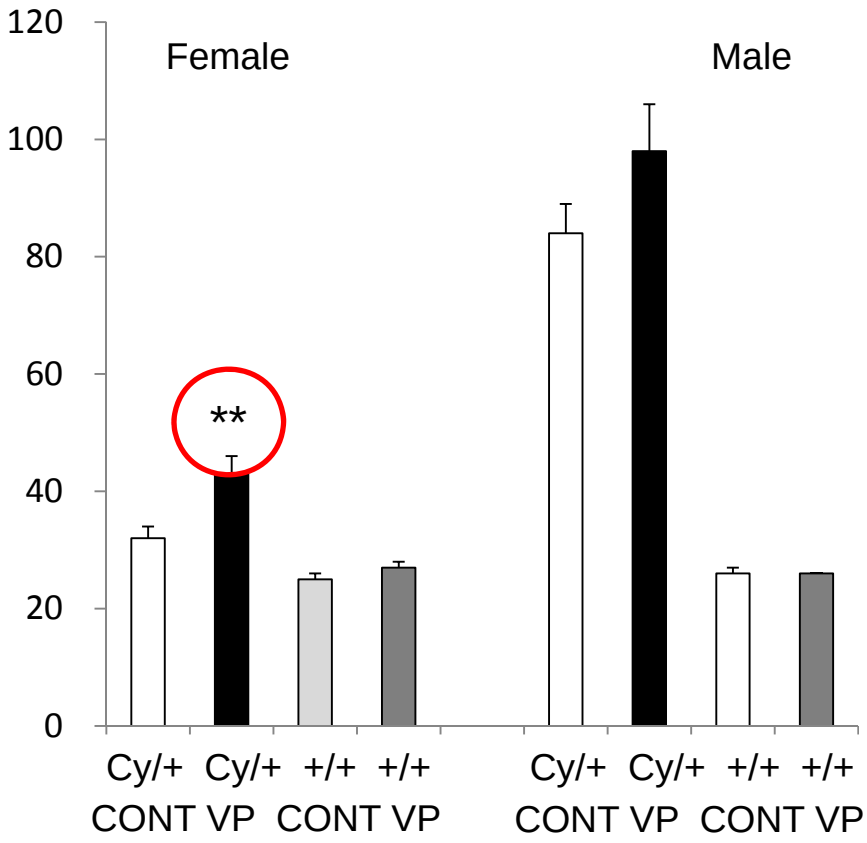
Effect of VP treatment on renal function (serum urea nitrogen: SUN, mg/dl)



- Treatment with VP caused a significant increase in SUN in female Cy/+ animals (n=5), **P<0.01.

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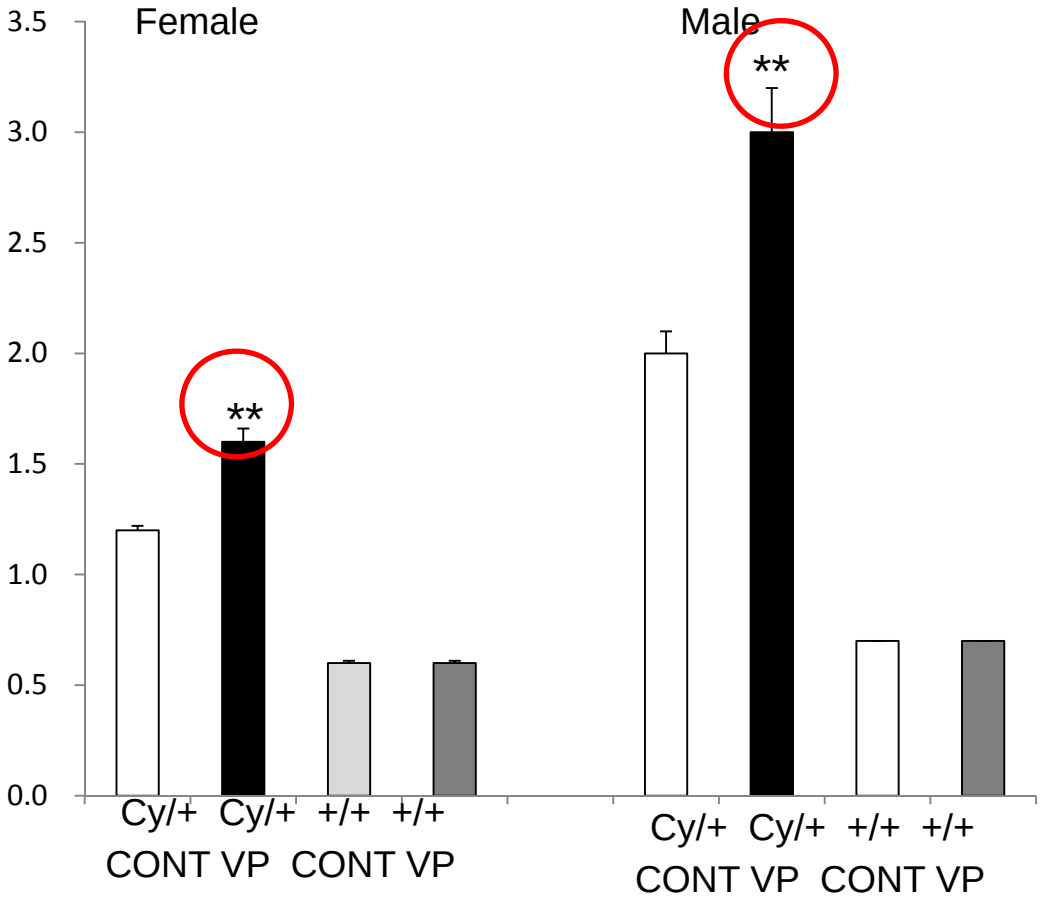
Effect of VP treatment on kidney weight



- Vp treatment caused a significant increase in kidney weight in both genders of Cy/+ rats (**P<0.001), but had no effect on total kidney weight of +/+ rats.

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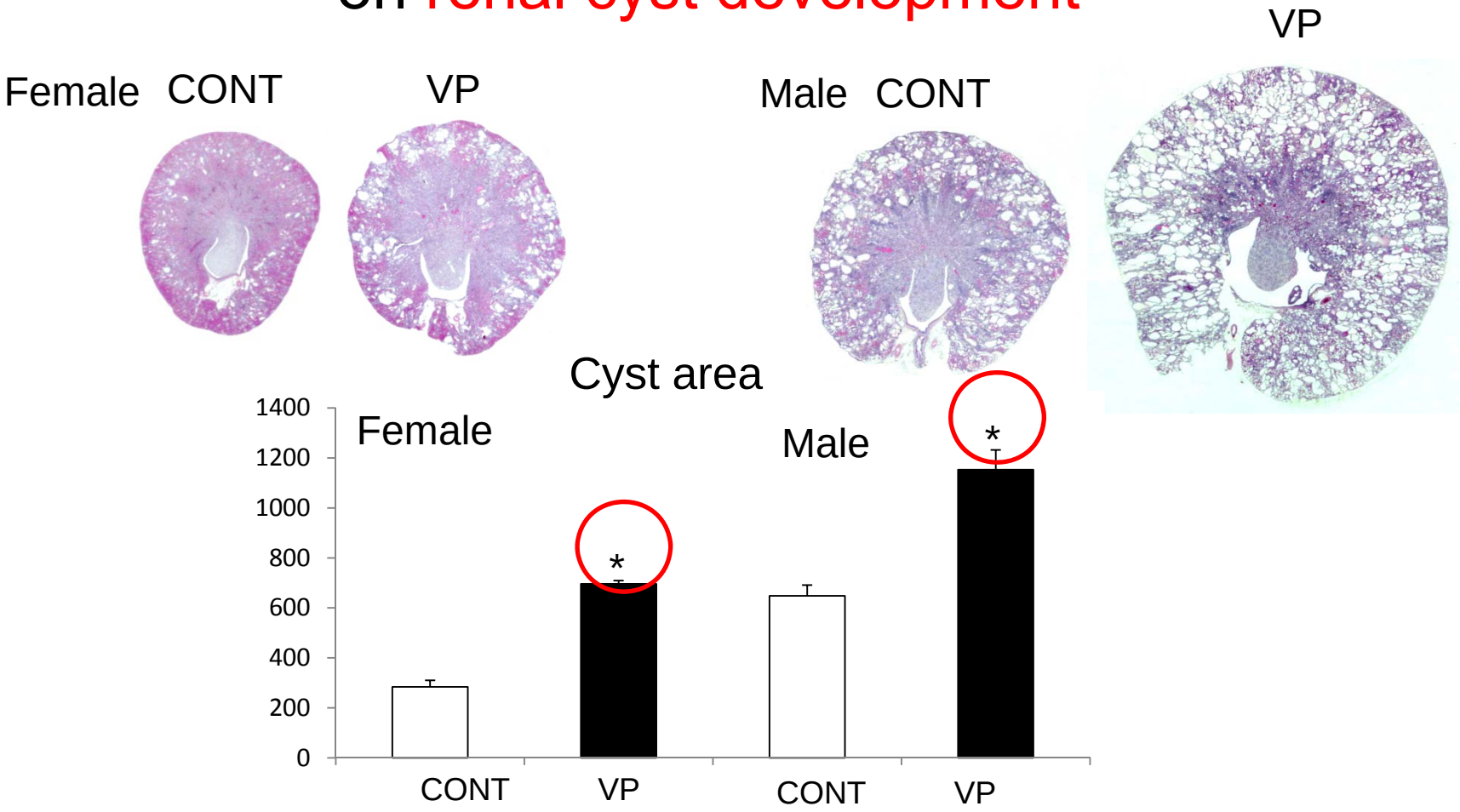
Effect of VP treatment on renal cyst development



- Cross-sectional surface area of cysts (represented as % of total area) from Cy/+ kidneys was measured by morphometric analysis.
- Comparisons between CONT and VP showed that VP increased cyst area 140% in females and 75% in males of Cy/+ rats, *P<0.001.

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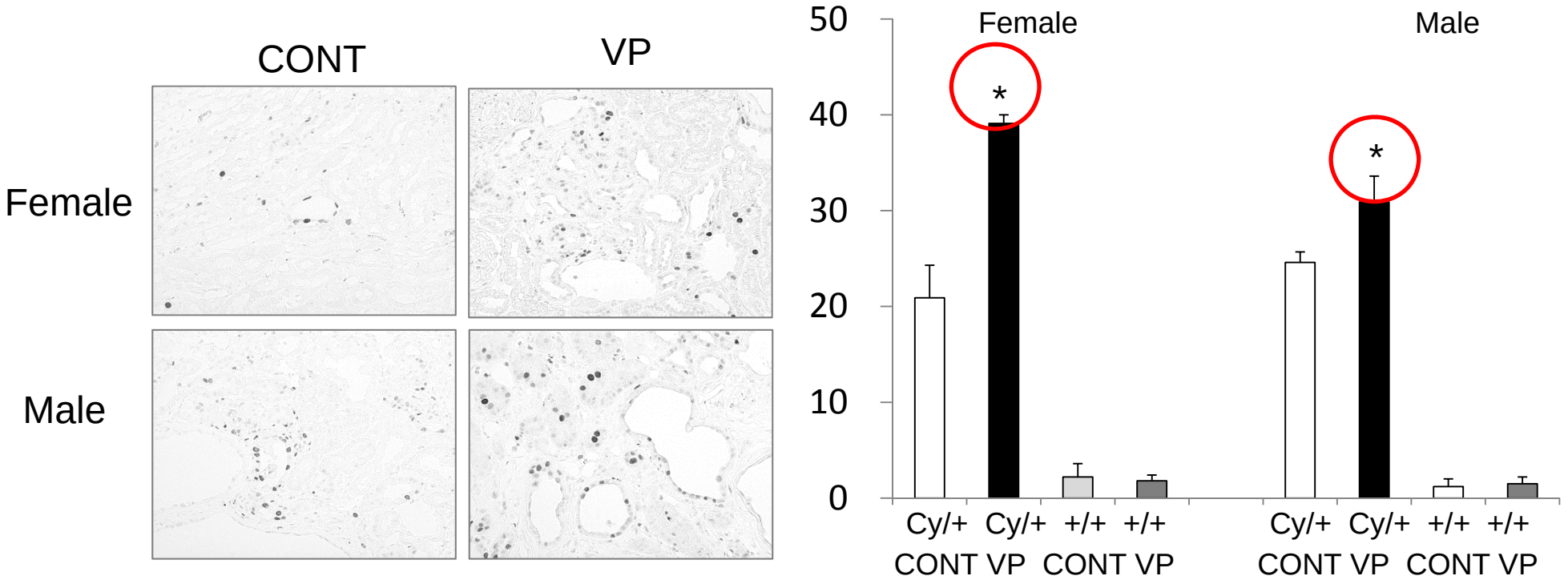
Effect of VP treatment on cell proliferation (PCNA)



- Comparison between VP and CONT-treated Cy/+ rats (female or male), *P<0.01.
- By contrast, few PCNA-positive cells were shown in +/+ kidney sections and VP had no effect on the proliferative index (data not shown).

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Effect of VP treatment on the activity of B-Raf/MEK/ERK signaling

Genotype	Treatment	*N*	P-BRaf/B-Raf	B-Raf	P-ERK/ERK	ERK
Female						
+/+	CONT	5	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
+/+	VP	5	1.15 ± 0.11	0.76 ± 0.17	1.04 ± 0.08	0.98 ± 0.11
Cy/+	CONT	5	1.81 ± 0.07‡	1.16 ± 0.05	2.07 ± 0.21	1.16 ± 0.10
Cy/+	VP	5	2.16 ± 0.17$^{‡, **}$	1.41± 0.11*	3.75 ± 0.63$^{‡,\#}$	1.03 ± 0.05
Male						
+/+	CONT	5	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
+/+	VP	5	1.00 ± 0.08	0.94 ± 0.03	1.62 ± 0.50	0.98 ± 0.08
Cy/+	CONT	5	1.88 ± 0.23†	1.65 ± 0.24*	4.77 ± 0.58†	1.13 ± 0.14
Cy/+	VP	5	1.96 ± 0.27†	1.86 ± 0.27*	6.50 ± 0.98‡	1.09 ± 0.12

- Comparison between Cy/+ and +/+ kidneys (* $P<0.05$, † $P<0.01$, ‡ $P<0.001$)
- Comparison between CONT and VP treatment either in Cy/+ or +/+ kidneys (** $P<0.05$, $^{\#}$ $P<0.01$).

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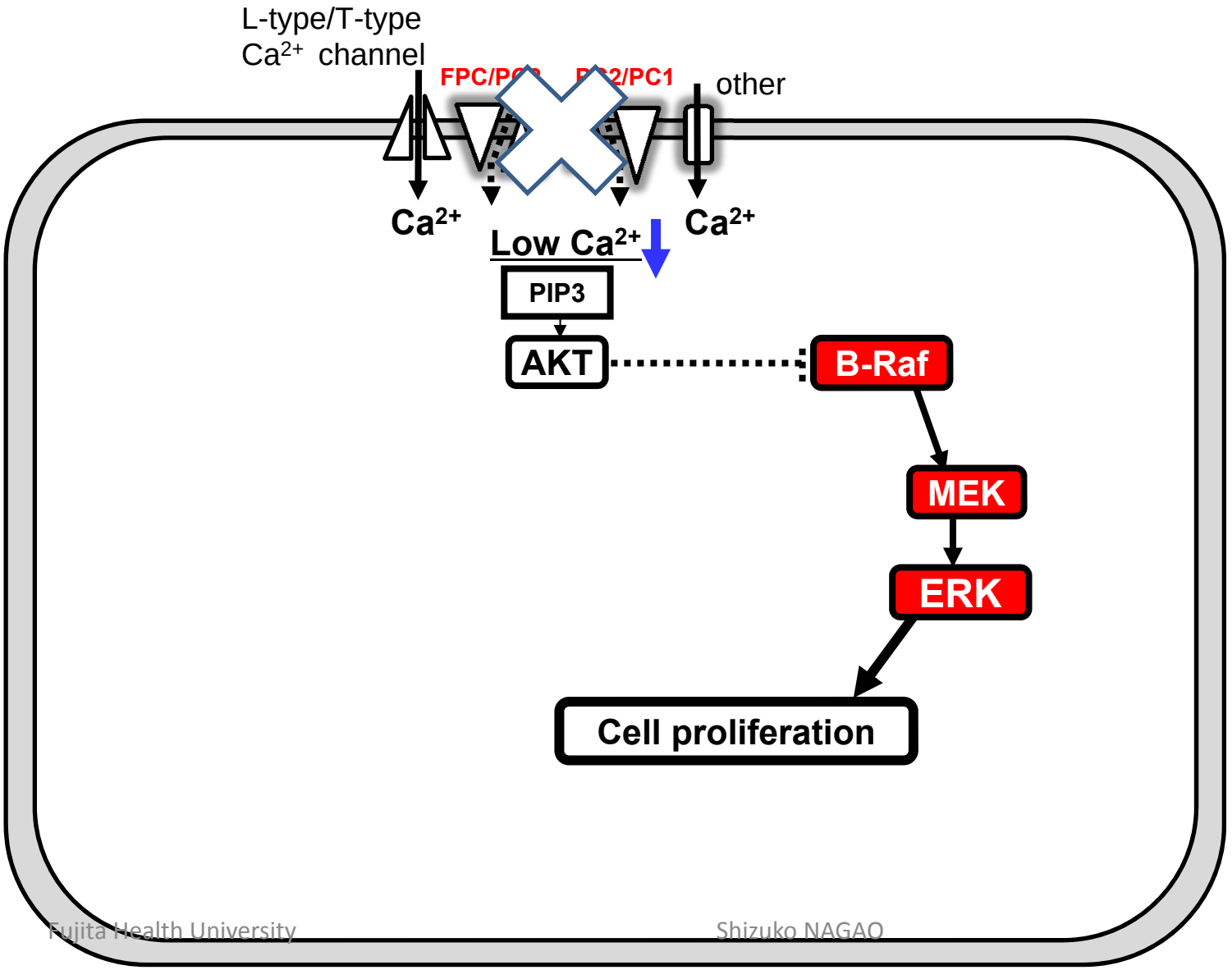
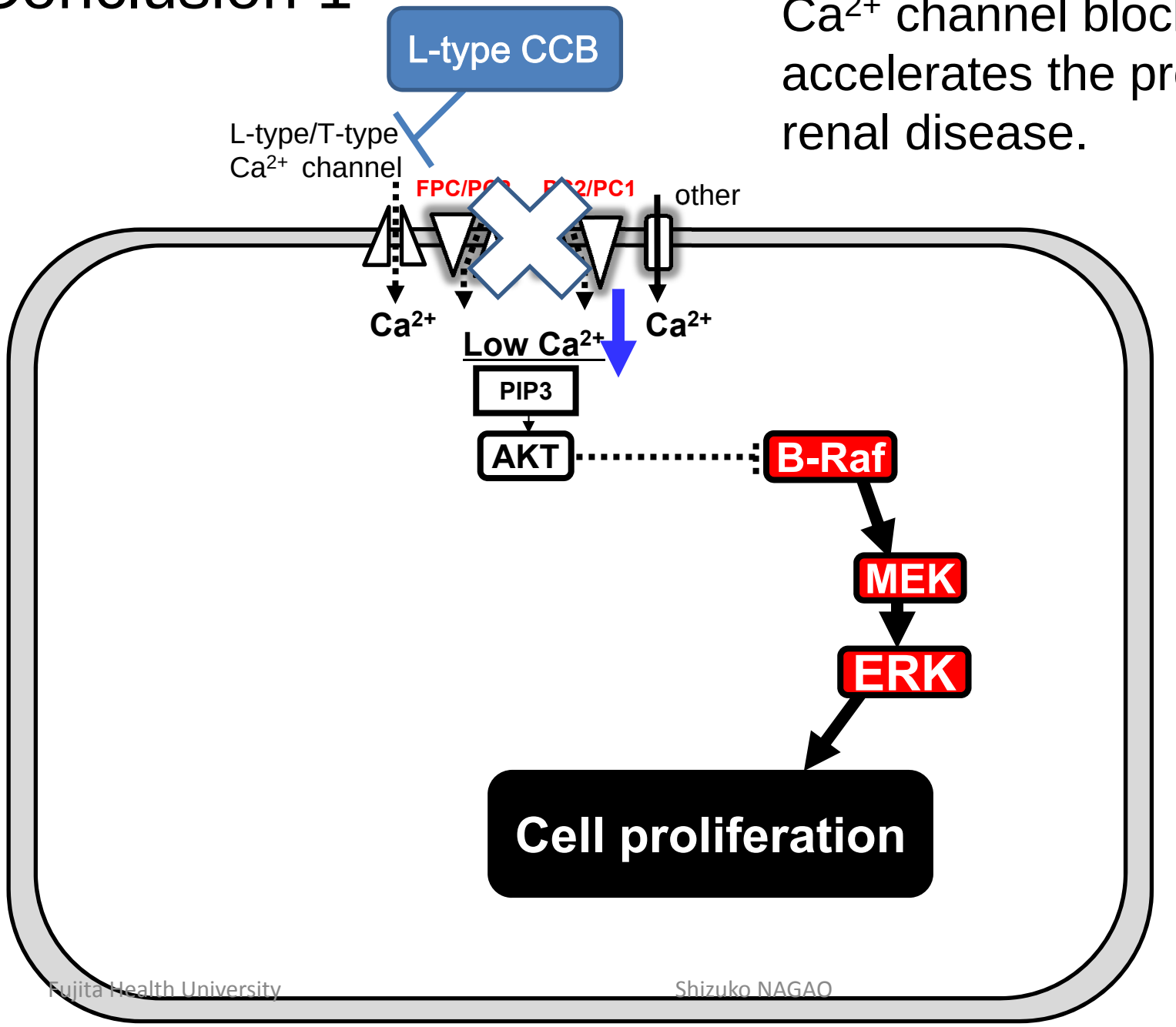
Conclusion 1



Conclusion 1

Ca^{2+} channel blocker (CCB) accelerates the progression of renal disease.



Today's subject

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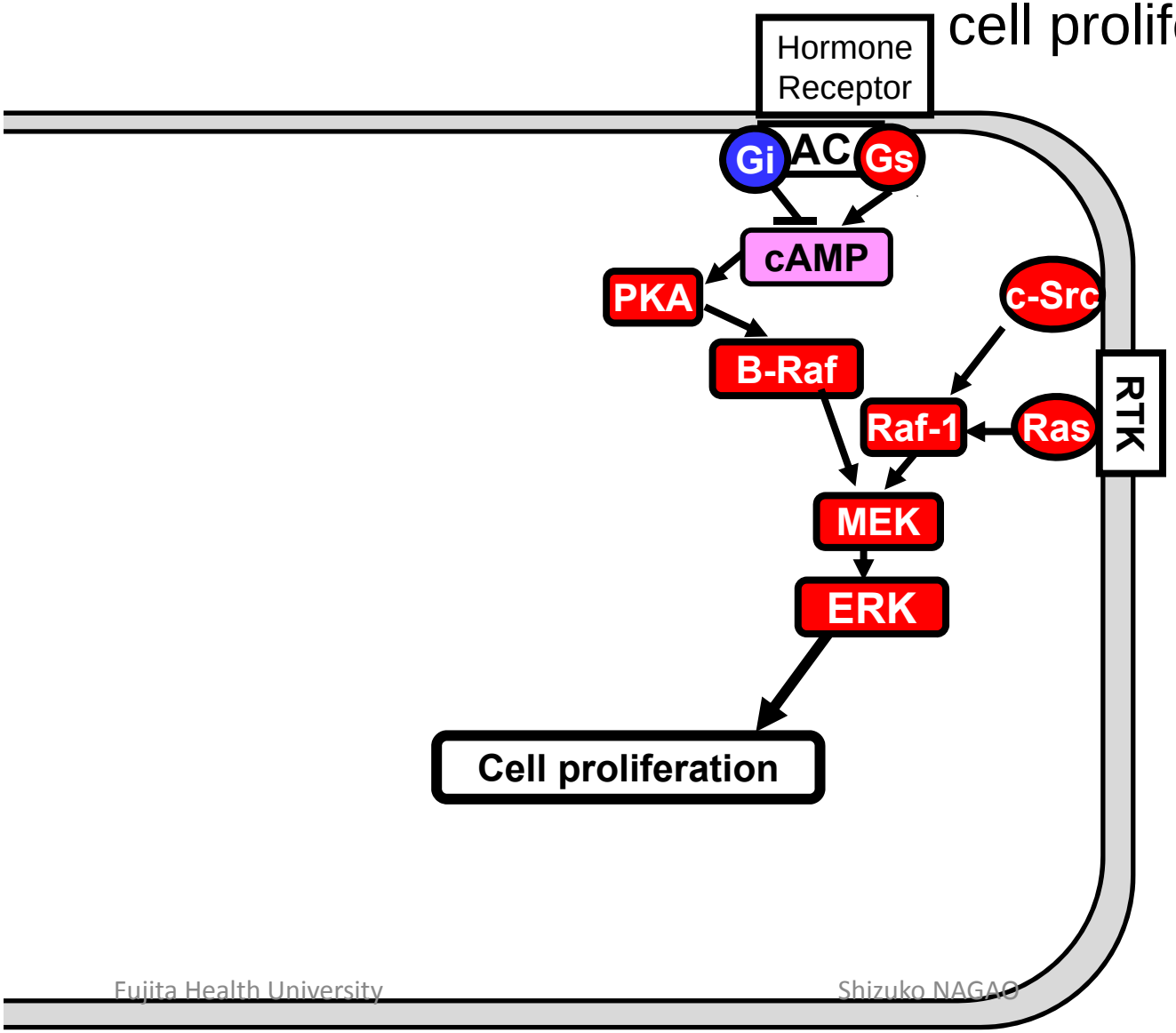
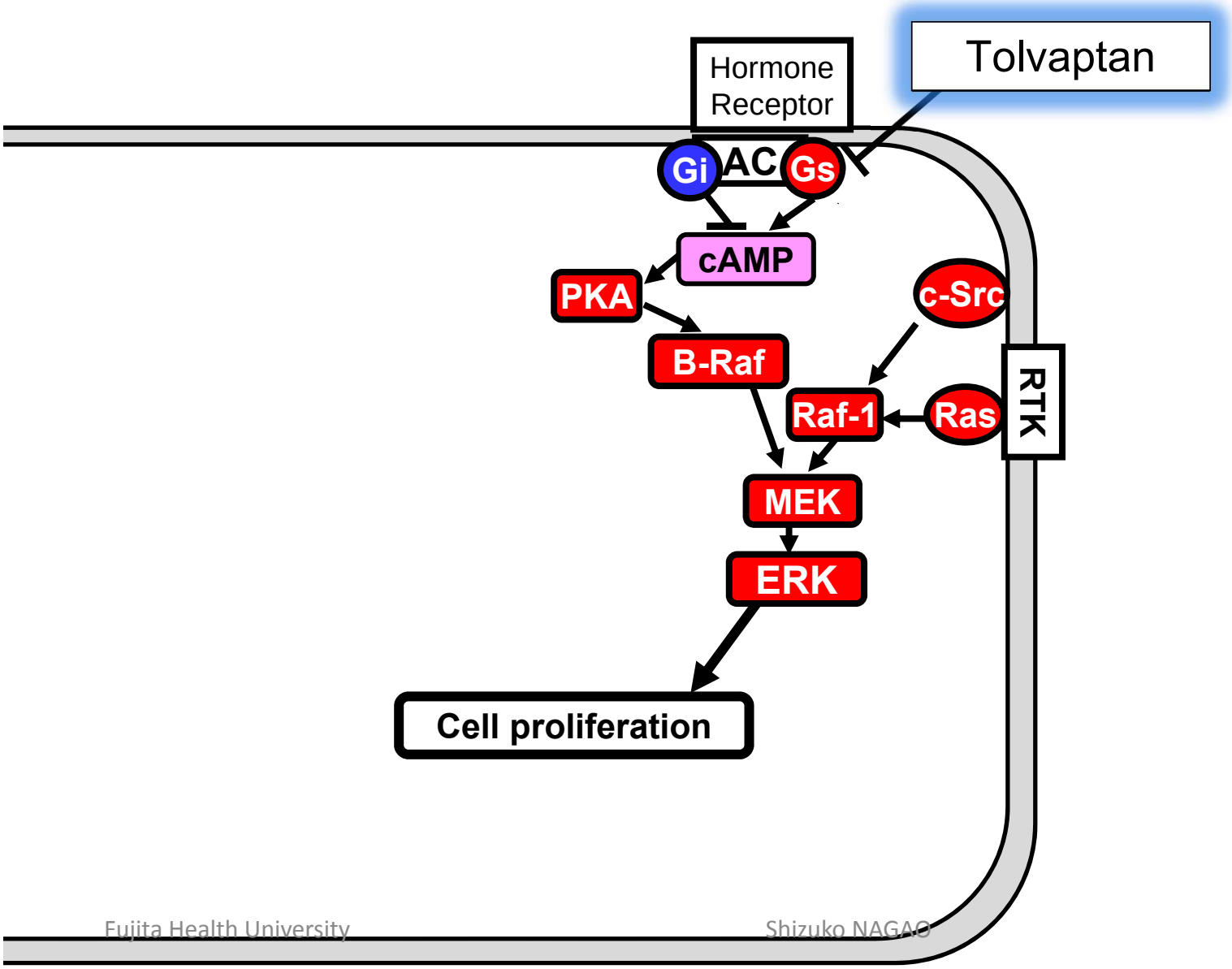
5. Signaling pathways in epithelial cells of PKD

PKD cells

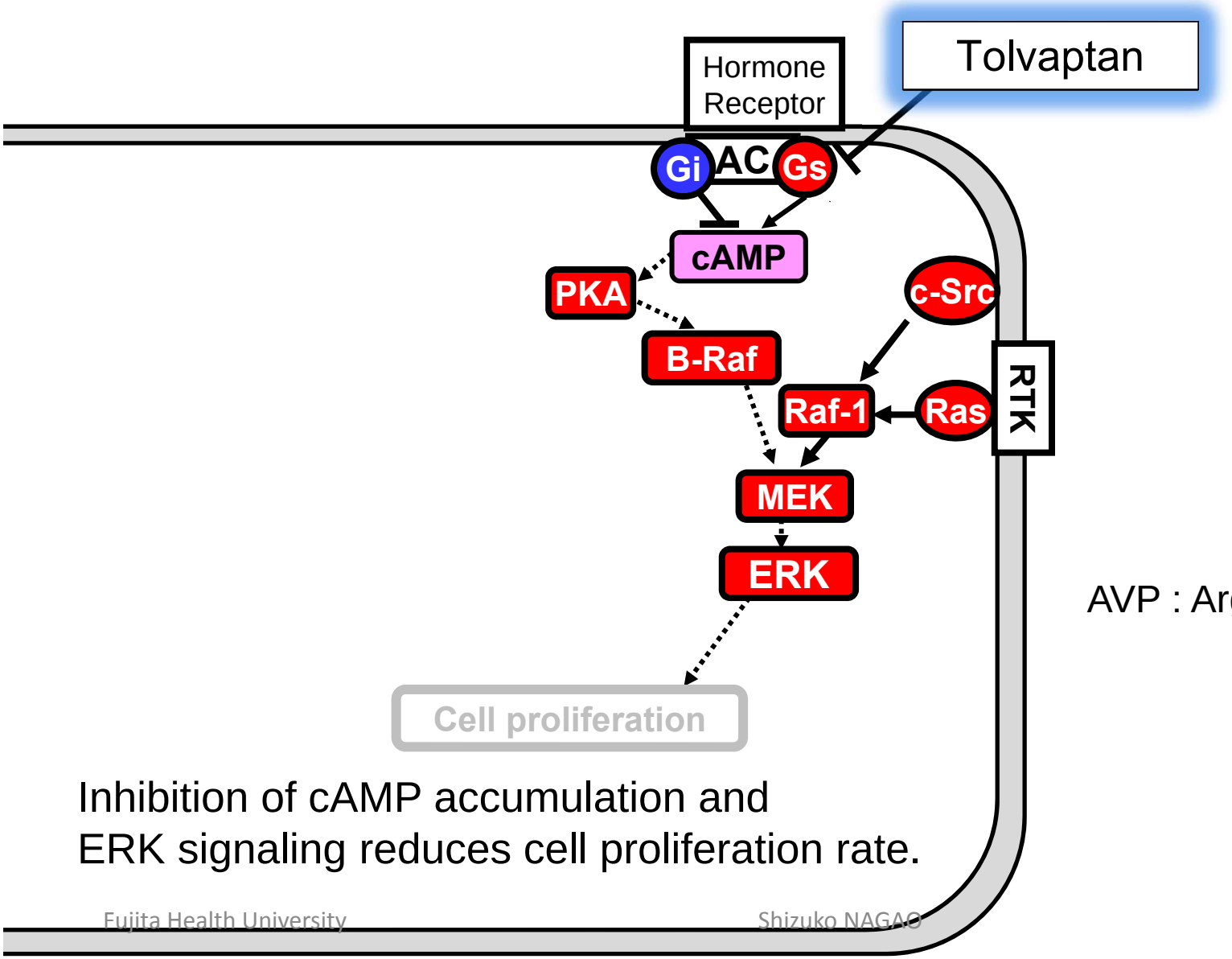
In PKD renal epithelial cells, intracellular cAMP is increased, ERK signaling is up-regulated and cell proliferation rate is elevated.



Tolvaptan suppresses arginine vasopressin (AVP)-activated Gs protein.



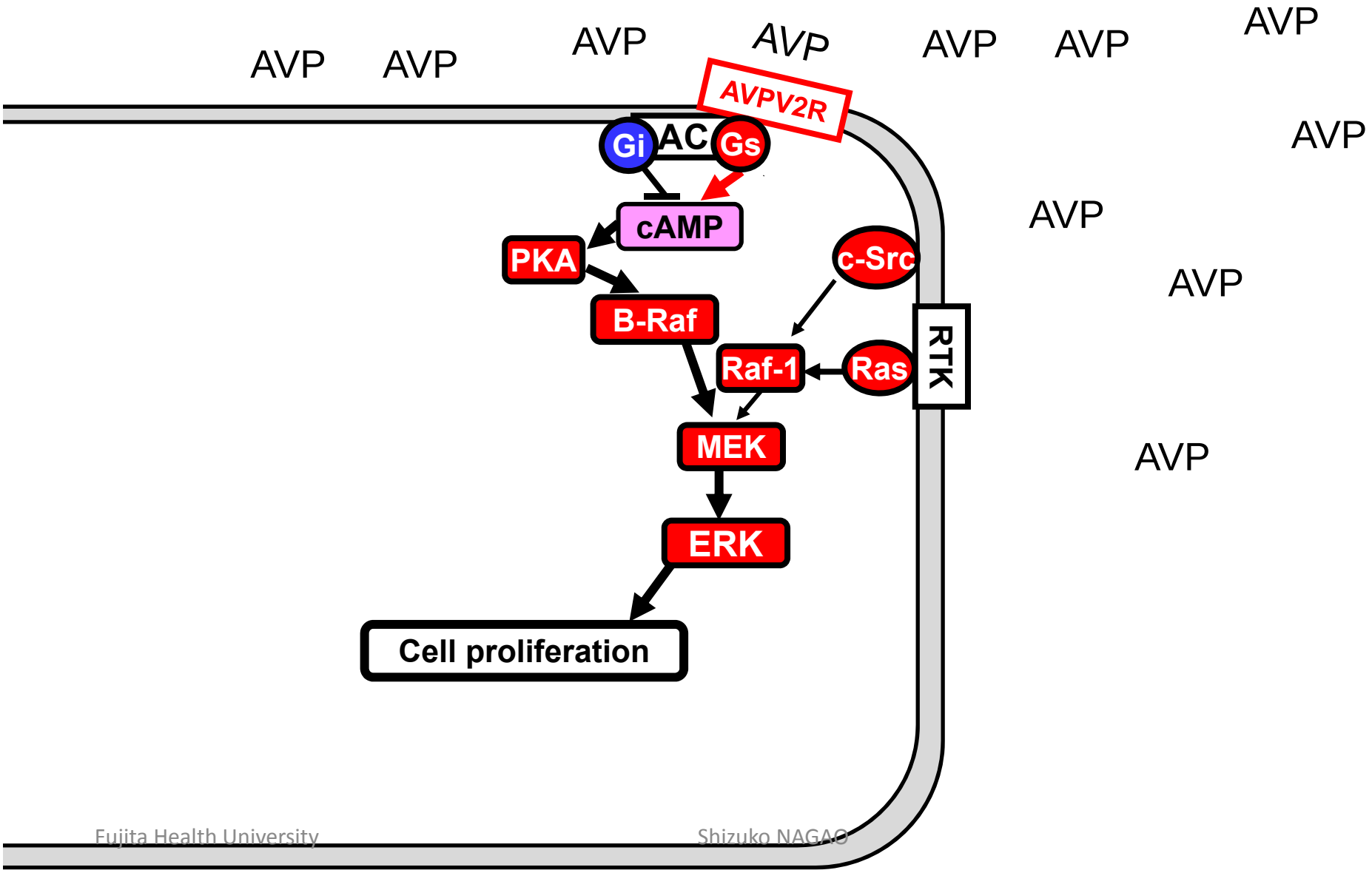
Tolvaptan suppresses arginine vasopressin (AVP)-activated Gs protein.
Tolvaptan reduces the concentration of intracellular cAMP in PKD cells.



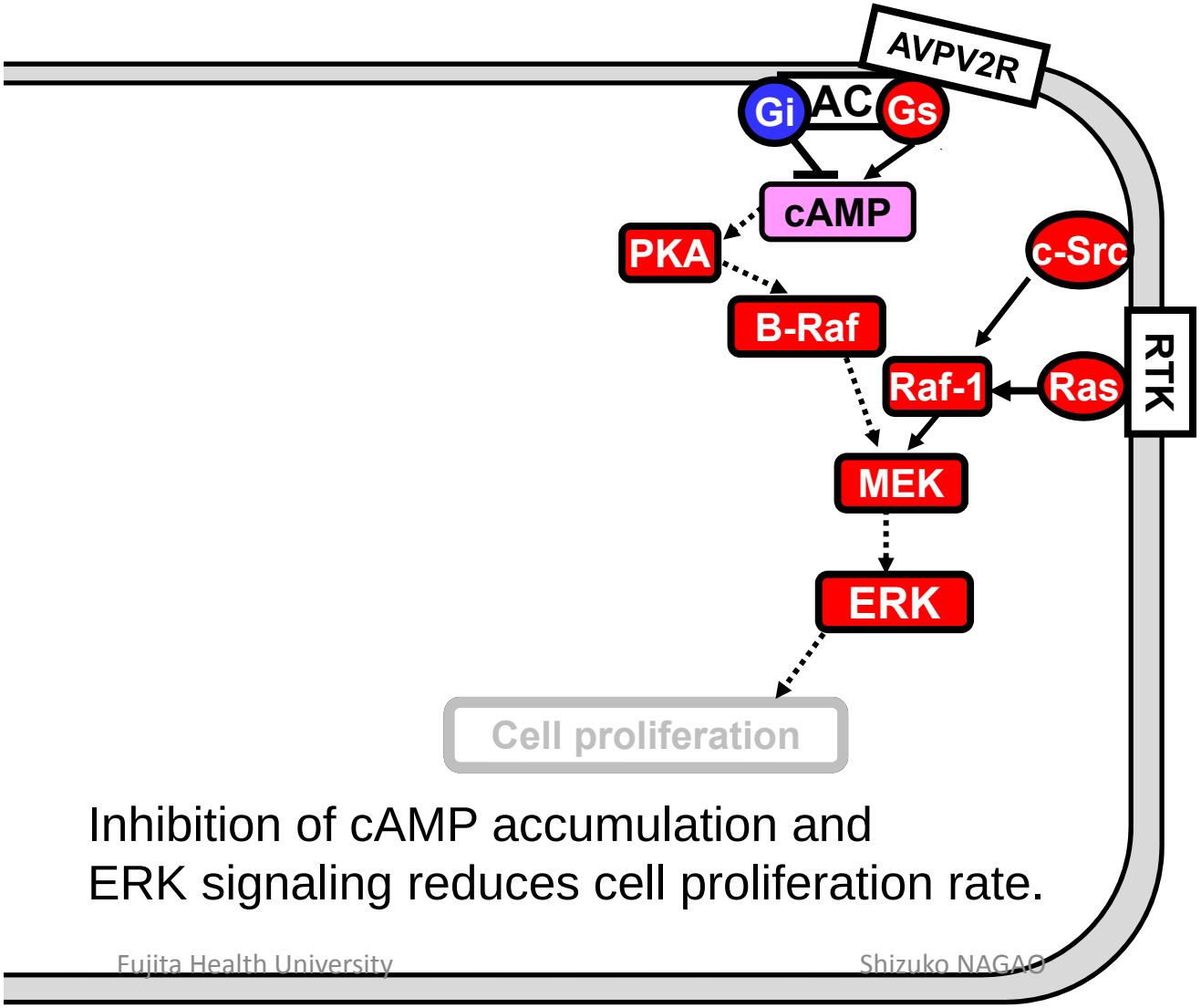
AVP : Arginine vasopressin

Inhibition of cAMP accumulation and
ERK signaling reduces cell proliferation rate.

AVP bands to a hormone receptor, AVPV2R.
AVP increases intercellular cAMP.



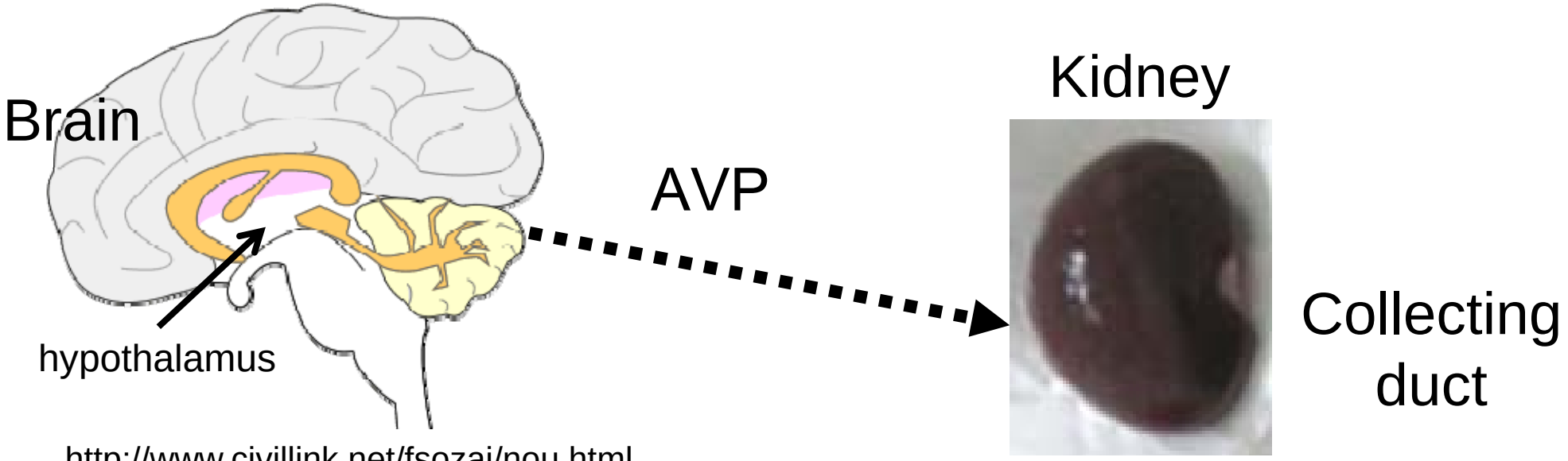
Does decreased release of AVP cause a reduction of intercellular cAMP concentration in PKD epithelial cells and ameliorate renal disease progression?



Inhibition of cAMP accumulation and ERK signaling reduces cell proliferation rate.

Outline

- Decreased plasma AVP by high water intake (HWI) suppresses B-Raf/MEK/ERK signaling activity in PKD kidneys and slows the progression of cystic disease in PCK rats.

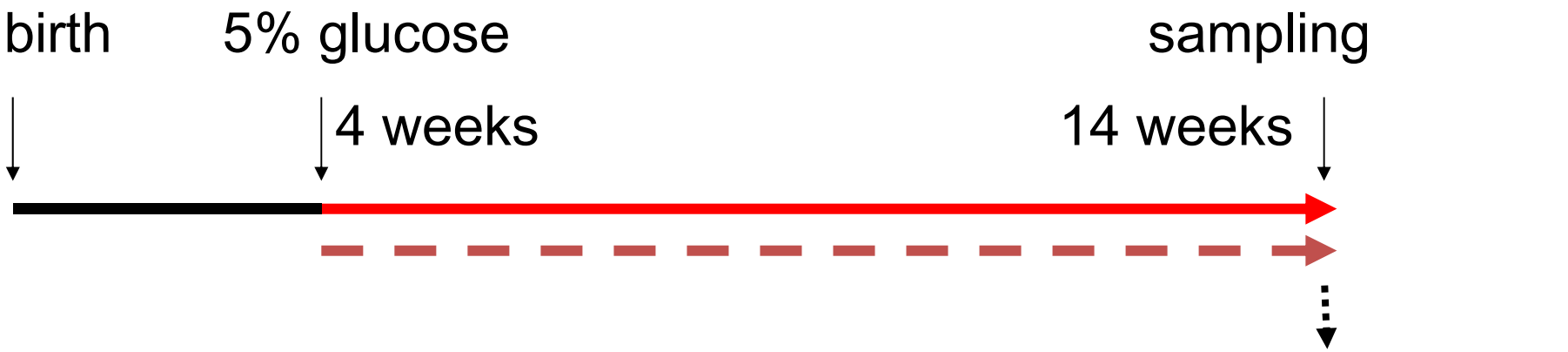


http://www.civillink.net/fsozai/nou.html

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

Protocol

- PCK rats were allowed free access to water and food throughout the study.
- Animal on high water intake (HWI) were offered water containing 5% glucose from 4 to 14 weeks of age.



PCK : polycystic kidney
+/+ : normal kidney

Kidney weight, SUN, Urinary AVP, Urine osmolality,
Western blot, Immunohistochemistry

NAGAO et al. J Am Soc Nephrol
17: 2220–2227, 2006.

Renal effects of high water intake (HWI)

Genotype	Treatment	n	Water Intake (mL)		Urine Volume (mL)		Urine Osmolarity (mOsmol/kg H2O)		Urinary AVP (pg/mg creatinine)	
Male					3.8 hold					
PCK	CONT	8	18 ± 1		18 ± 2		1088 ± 96		257 ± 22	
PCK	HWI	10	68 ± 5	**	58 ± 5	**	232 ± 47	**	114 ± 46	**
+/+	CONT	8	10 ± 5		9 ± 1		1498 ± 130		160 ± 19	
+/+	HWI	8	94 ± 26	*	91 ±10	**	197 ± 73	**	88 ± 38	
Female					3.5 hold					
PCK	CONT	10	18 ± 3		16 ± 2		982 ± 74		435 ± 83	
PCK	HWI	8	63 ± 9	**	49 ± 8	**	284 ± 52	**	111 ± 59	**
+/+	CONT	7	13 ± 5		12 ± 1		1559 ± 211		335 ± 49	
+/+	HWI	10	107 ± 10	**	80 ±11	**	131 ± 25	**	31 ± 4	**

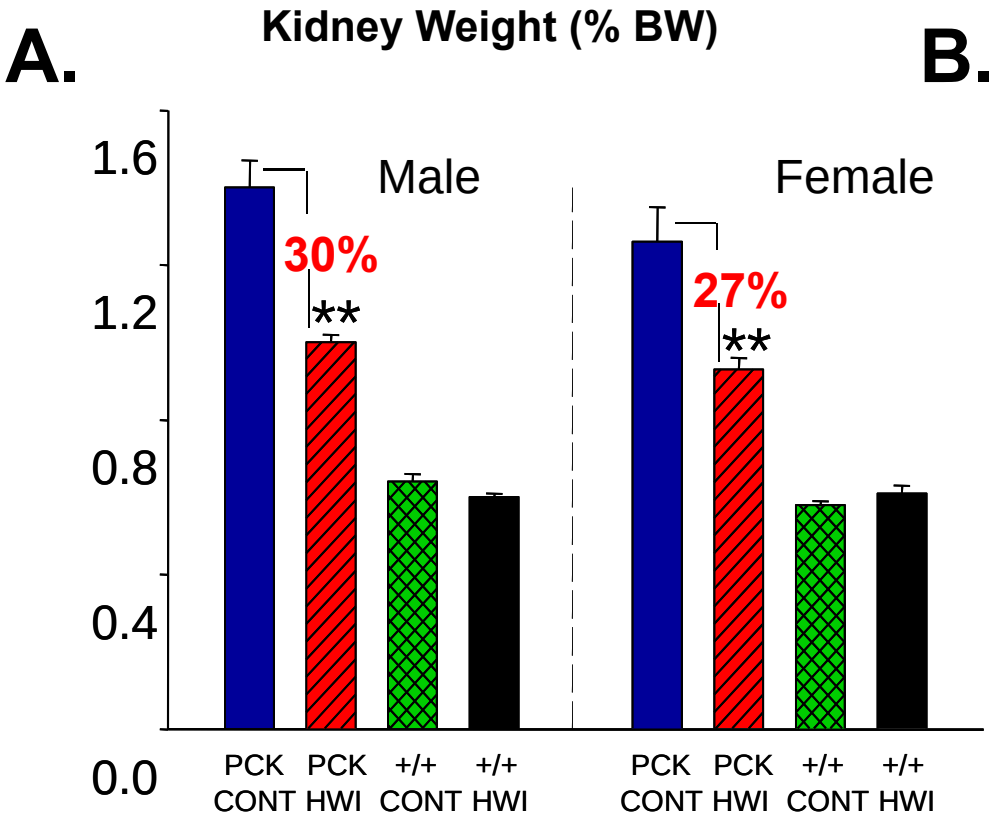
56%

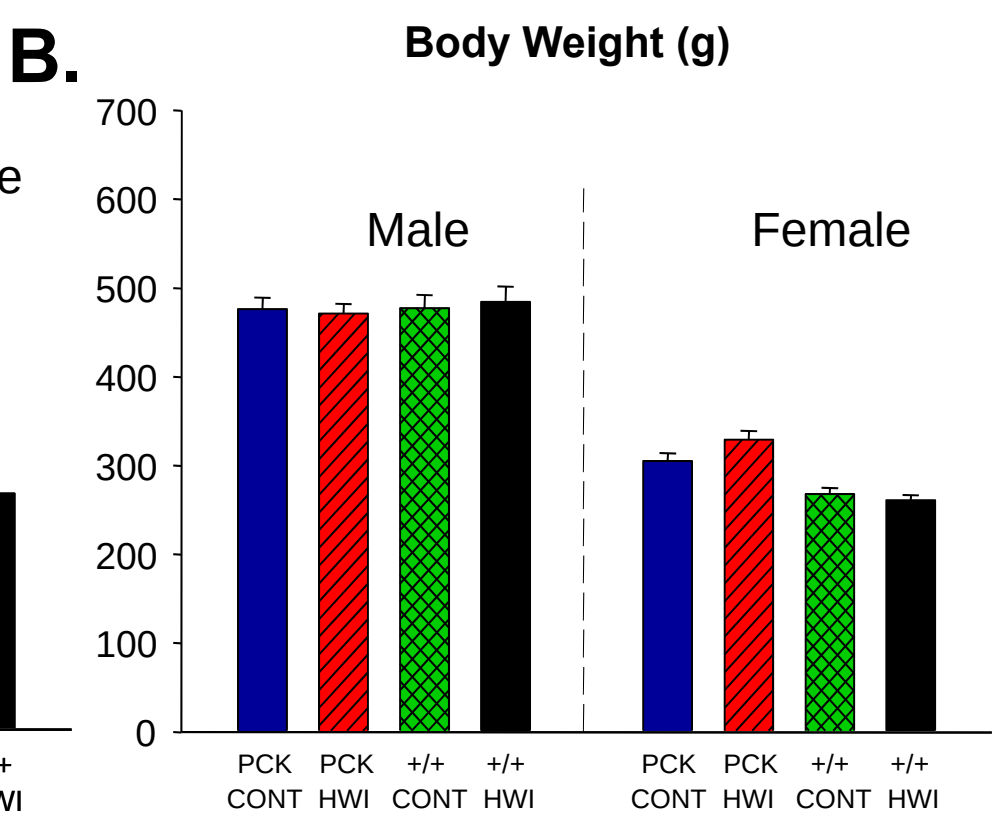
74%

- The rate of urine AVP excretion, an indicator of plasma AVP levels, was decreased 56% in male and 74% in female, respectively, by HWI
- Suppression of the renal effects of AVP decreases intracellular cAMP levels and reduces the water permeability of collecting ducts.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

Effect of HWI on kidney weight (%BW)



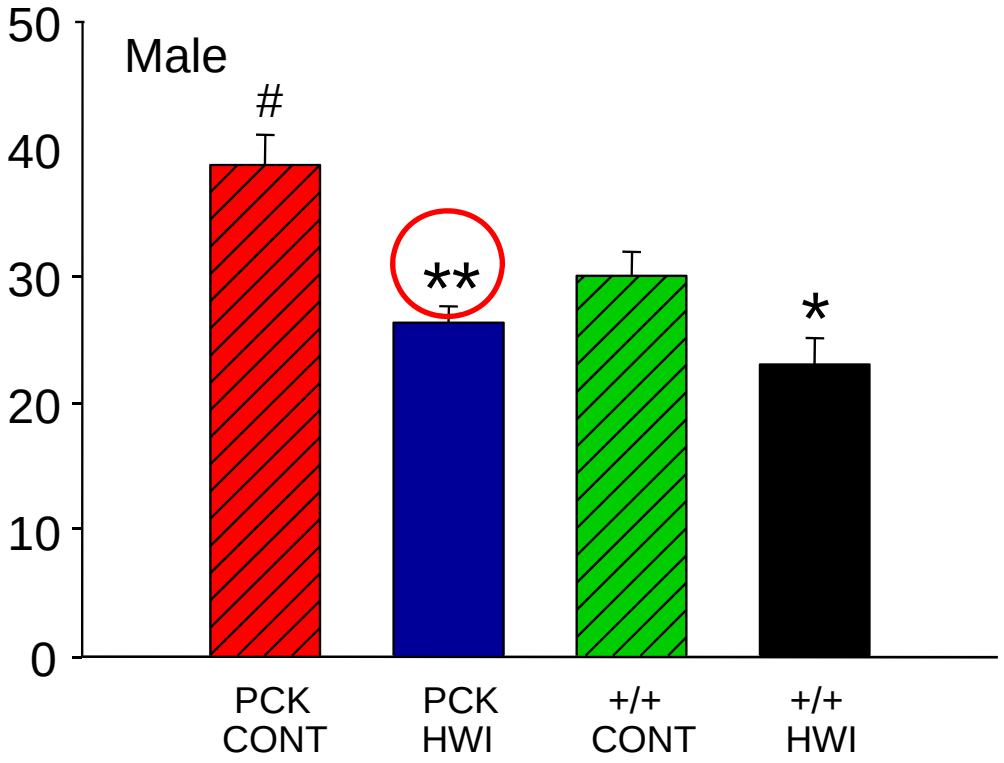


- Kidney weight (% body weight) significantly decreased 30 and 27% in PCK male and female rats, respectively.

- Body weight was unaffected by water intake either in +/+ or PCK rats.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

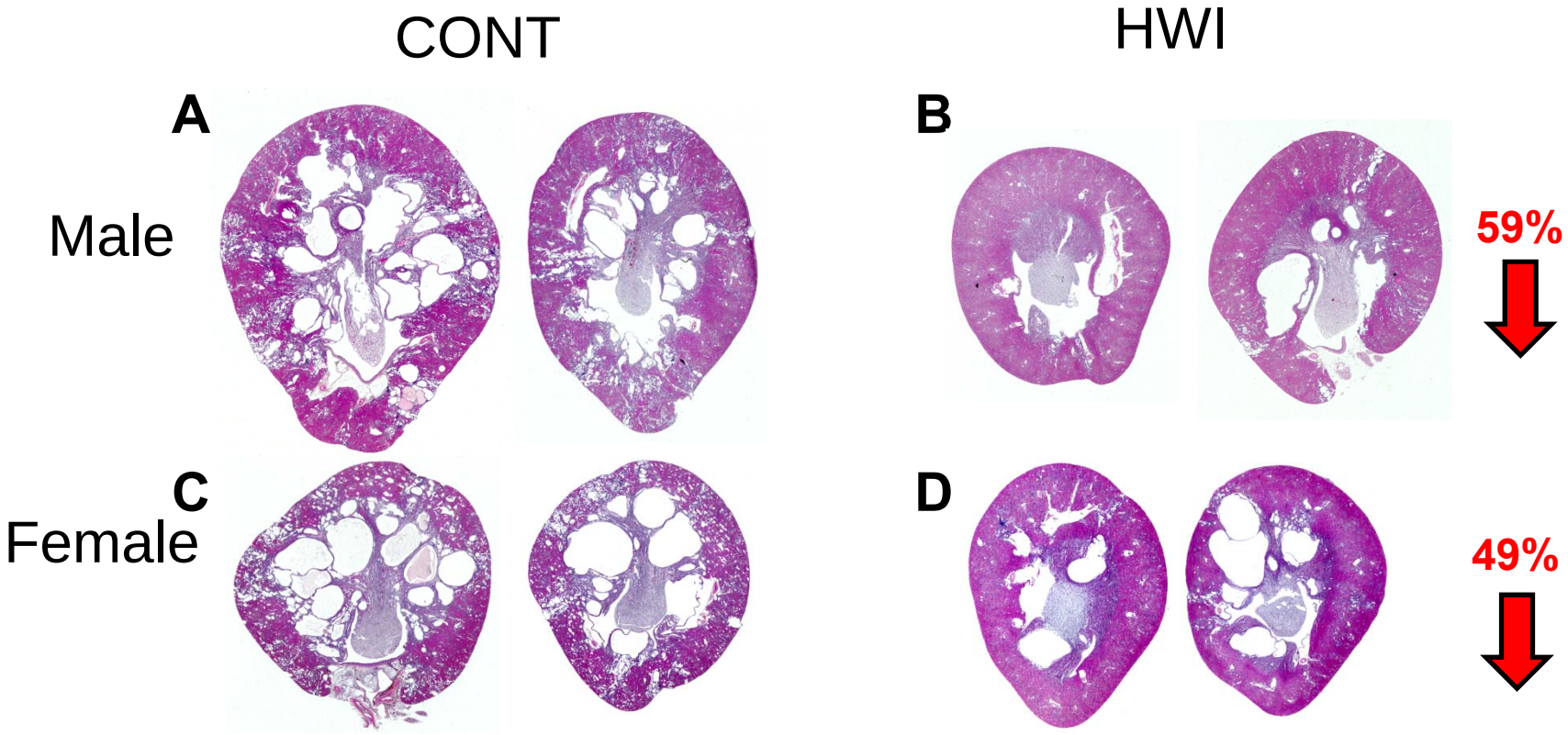
Effect of HWI
on renal function (serum urea nitrogen : SUN, mg/dl)



- HWI for 10 weeks decreased SUN from 38.7 to 26.3 mg/dl in the PCK male rats, a level that was similar to that of normal rats that drank increased water.
- HWI also caused a small but significant decrease in SUN in +/+ rats.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

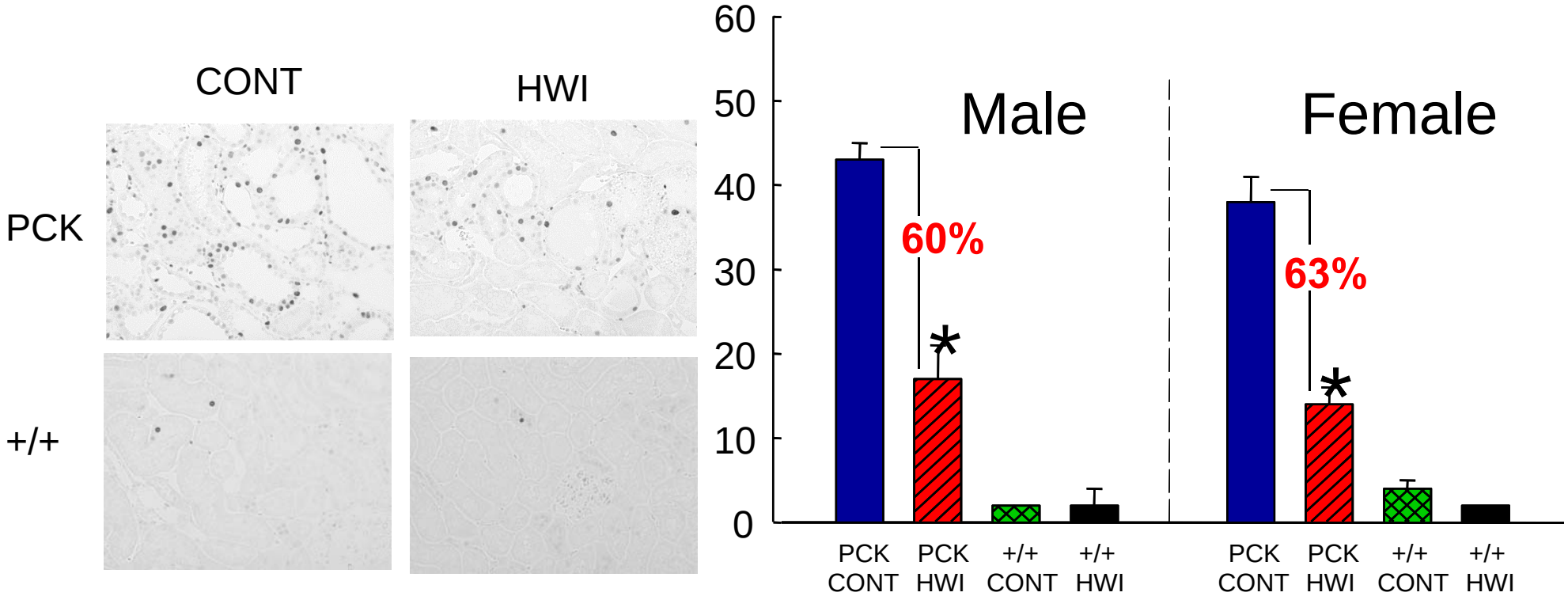
Effect of High Water Intake (HWI) on renal cyst development



- HWI treatment diminished cystic area 59% in male and 49% in female PCK rats, compared with rats that drank tap water.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

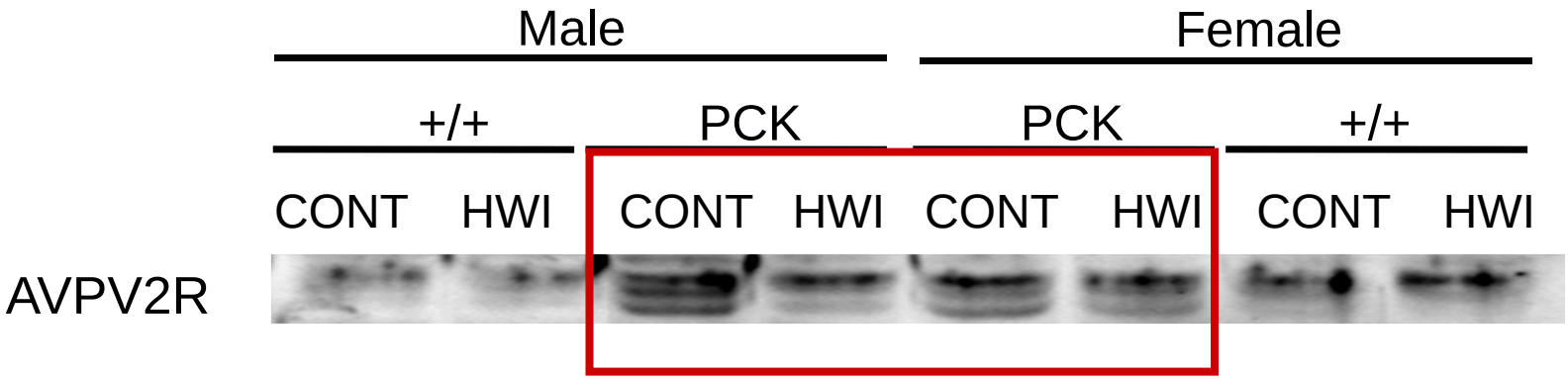
Effect of HWI
on cell proliferation (PCNA)



- Consistent with a reduction in cyst area and kidney weight, HWI decreased cell proliferation of cyst epithelial cells (PCNA-positive cells decreased approximately 60%).

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

Effect of HWI on V2R expression

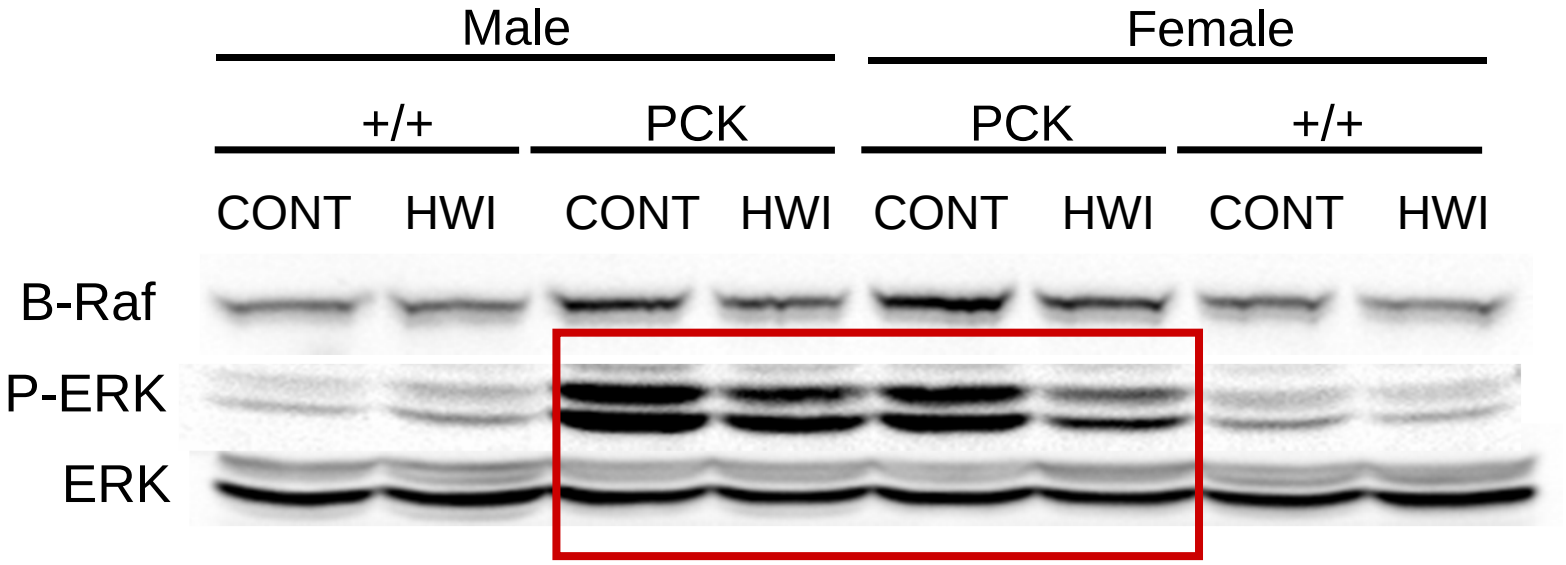


- Overexpression of AVPV2R in the epithelial cells of collecting duct cysts could contribute to persistently high levels of cAMP.

- HWI normalized AVPV2R expression in the PCK kidneys.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

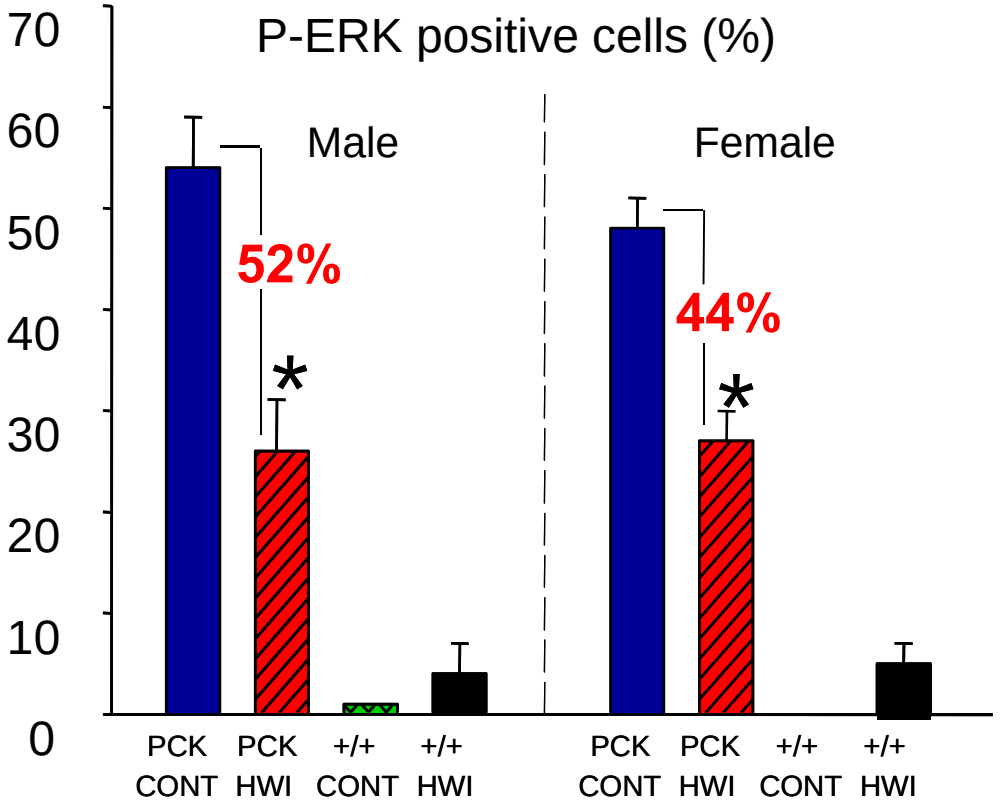
Effect of HWI
on renal activity of B-Raf/MEK/ERK signaling



- HWI decreased the level of P-ERK 33% in male and 41% in female rats, but had no effect on P-ERK levels in +/+ rats.

NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

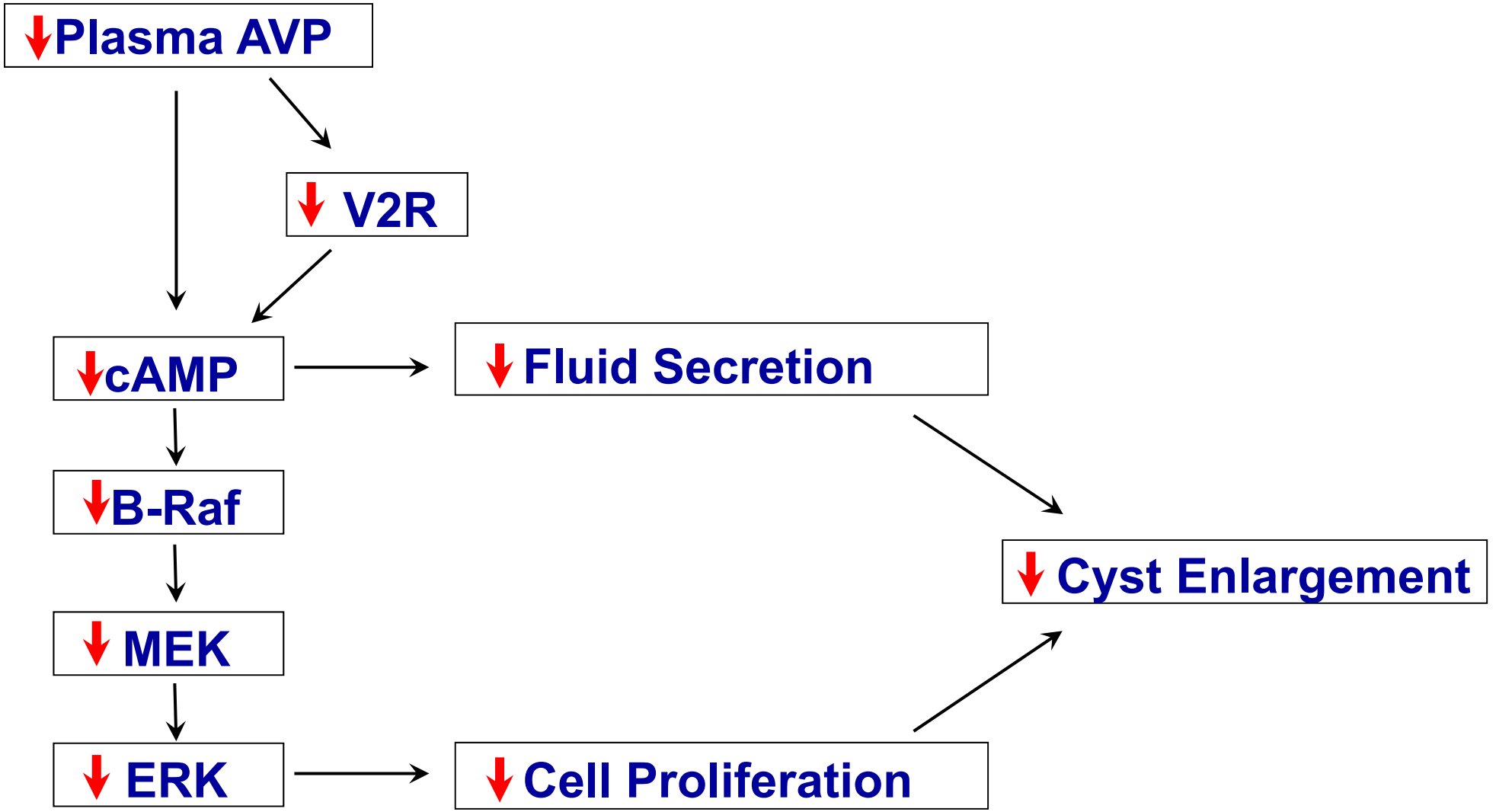
Effect of HWI
on number of cells staining for P-ERK



- The number of cells (% of cells per section) that stained positive for P-ERK was reduced 52% in PCK male rats and 44% in PCK female rats, confirming the observations made by immunoblot analysis.

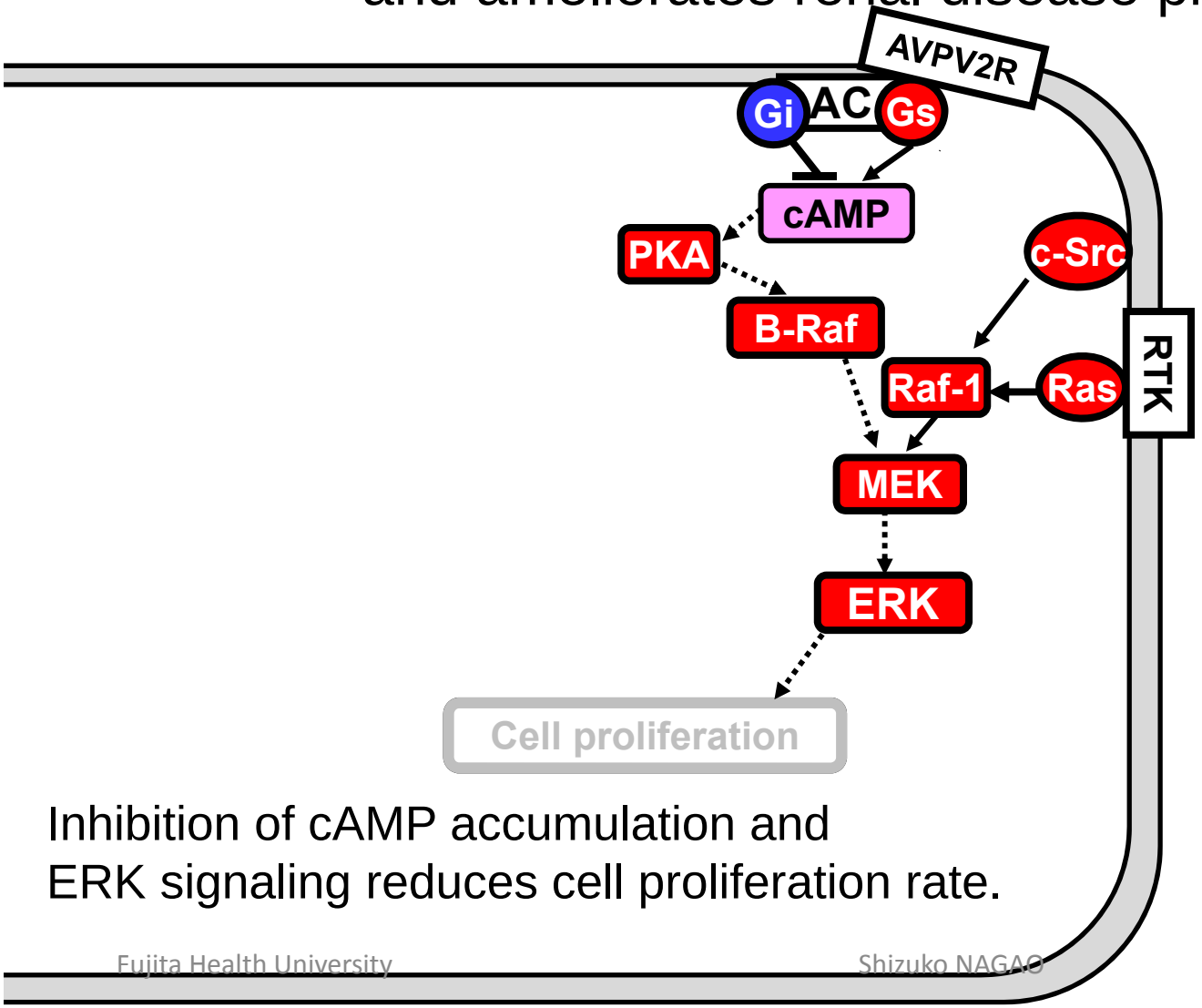
NAGAO et al. J Am Soc Nephrol 17: 2220–2227, 2006.

Effects of HWI on cyst enlargement



Conclusion 2

Decreased release of AVP by HWI causes a reduction of intercellular cAMP concentration in PKD epithelial cells and ameliorates renal disease progression.



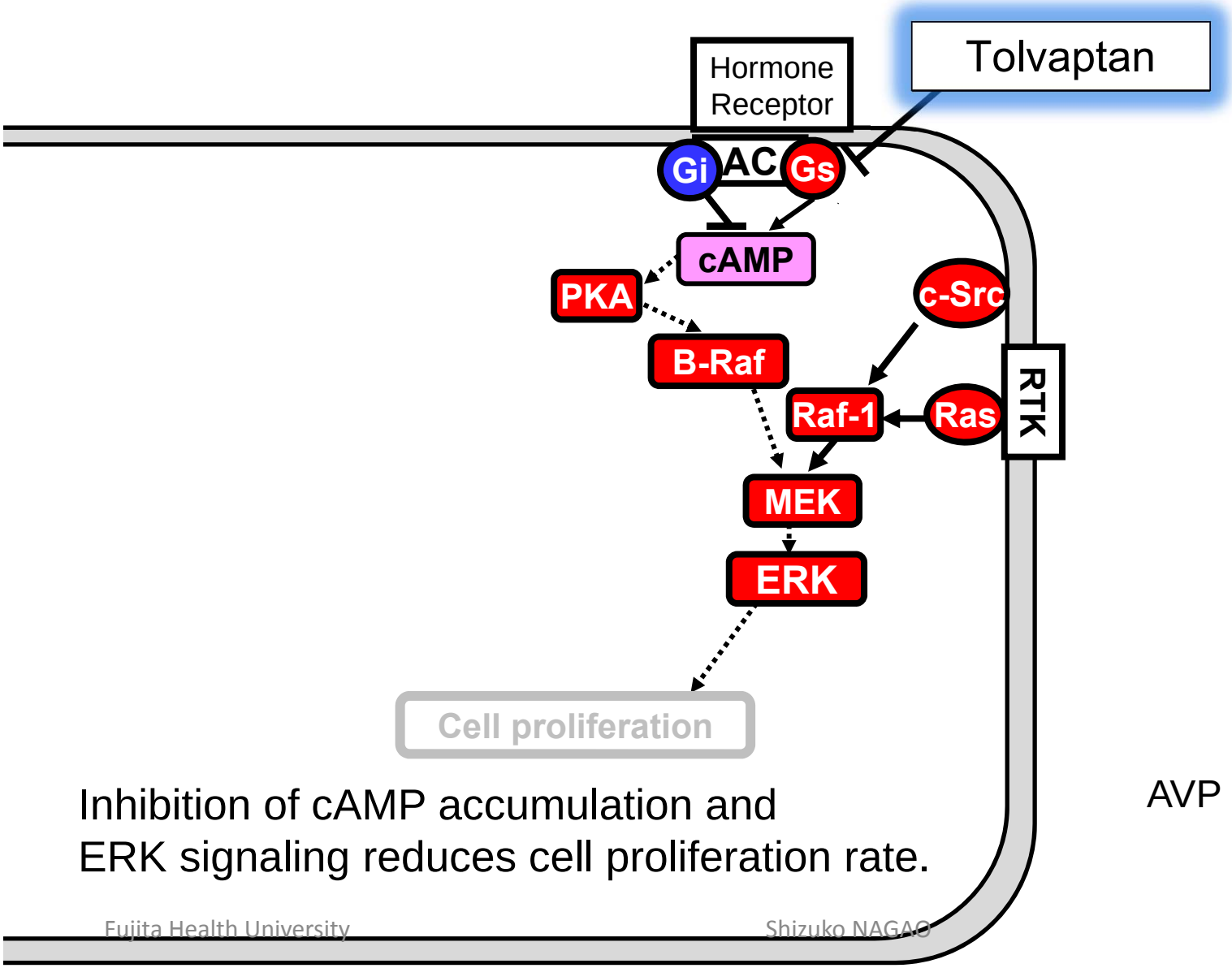
Inhibition of cAMP accumulation and ERK signaling reduces cell proliferation rate.

Today's subject

1. Polycystic kidney disease (PKD)
2. Decreased influx of Ca^{2+} concentration
3. Increased cAMP concentration
4. Clinical trial
5. Signaling pathways in epithelial cells of PKD

PKD cells

Tolvaptan suppresses AVP-activated Gs protein, and reduces the concentration of intracellular cAMP.



Inhibition of cAMP accumulation and ERK signaling reduces cell proliferation rate.

AVP : Arginine vasopressin

Effect of Tolvaptan (reduction of cAMP) in PKD patients

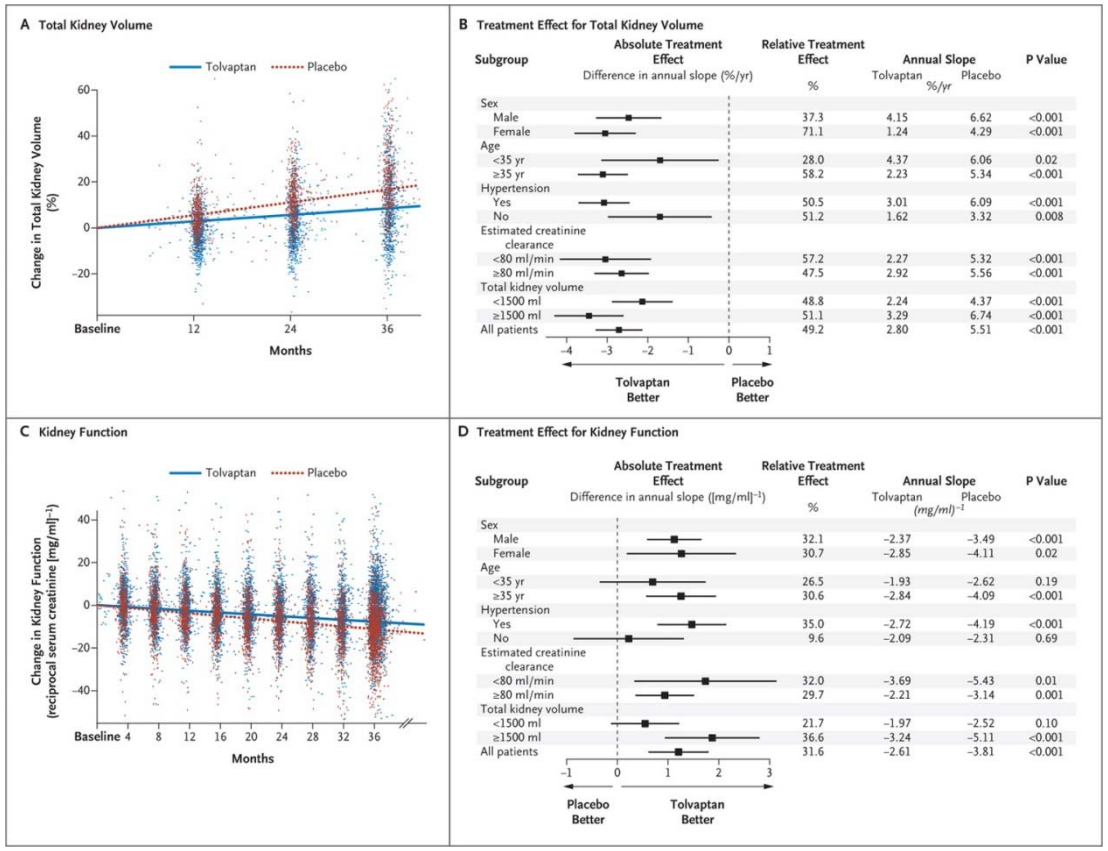
Growing Ratio of Total Kidney Volume

Tolvaptan	Placebo
2.8%/year	5.5%/year

- 50%

Decline of Kidney Function (serum creatinine)

Tolvaptan	Placebo
-2.61 mg/mL/year	-3.81 mg/mL/year



Torres VE et al. N Engl J Med 2012. DOI: 10.1056/NEJMoa1205511

Today's subject

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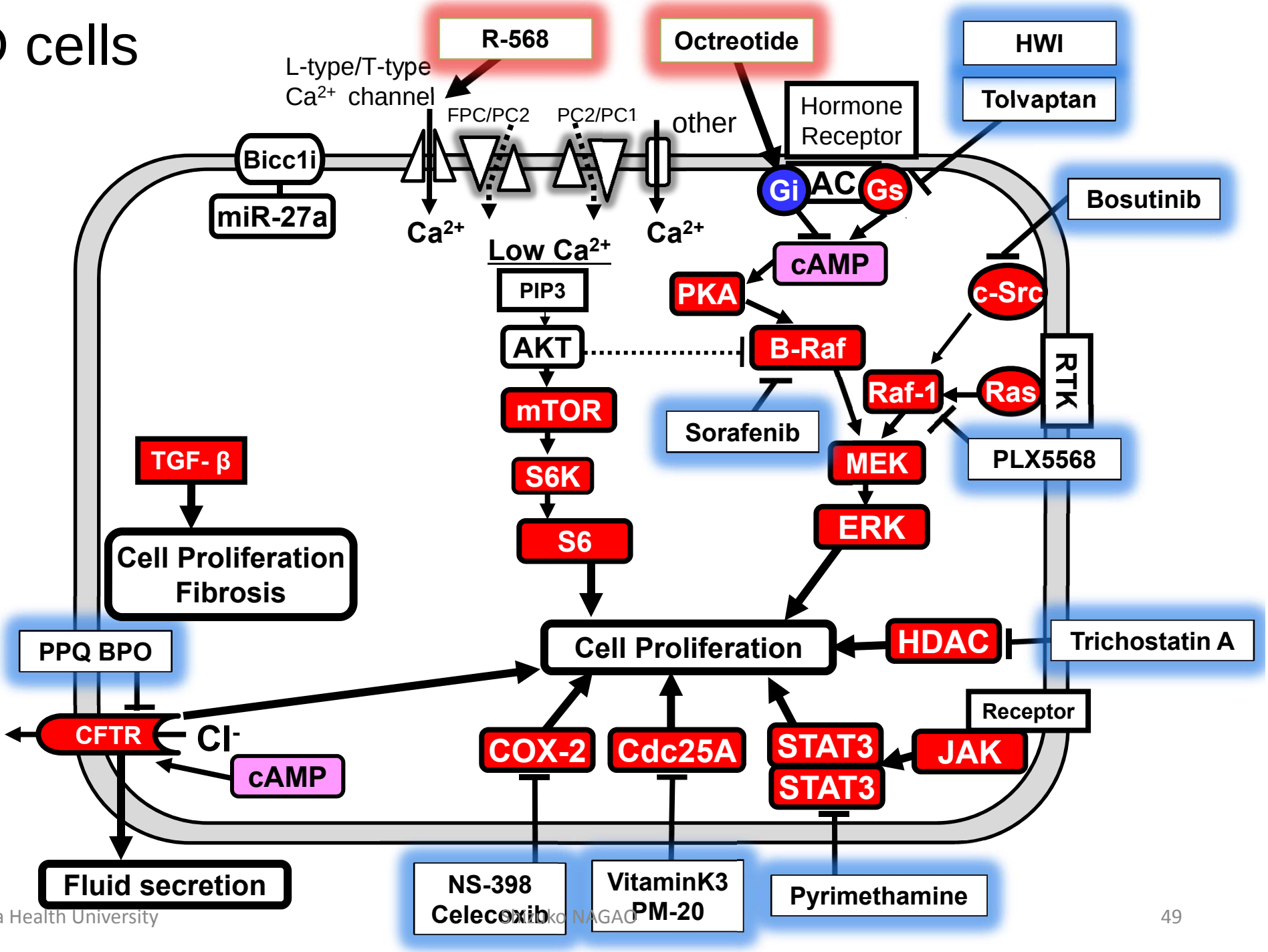
R-568
Octreotide
HWI
Tolvaptan
L-type/T-type
Ca2+ channel
FPC/PC2
PC2/PC1
other
Hormone
Receptor
Bicc1i
miR-27a
Ca2+
Ca2+
Gi
AC
Gs
Bosutinib
Low Ca2+
cAMP
PIP3
PKA
c-Src
AKT
B-Raf
RTK
Raf-1
Ras
mTOR
Sorafenib
MEK
PLX5568
TGF- β
S6K
ERK
Cell Proliferation
Fibrosis
S6
PPQ BPO
Cell Proliferation
HDAC
Trichostatin A
Receptor
CFTR
Cl-
cAMP
COX-2
Cdc25A
STAT3
STAT3
JAK
Fluid secretion
NS-398
Celecoxib
VitaminK3
PM-20
Pyrimethamine

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- Curr Mol Pharmacol. 2012 Jun;5(2):292-300.
- Am J Physiol Renal Physiol. 2011 Feb;300(2):F465-74.