

**Table S1. The assembly statistics of RNA-sequencing**

RNA source	Number of pairs (x 10 <sup>6</sup> )			CEGMA referring to CVG		
	Before QC	After QC	Number of contigs	Number of predicted genes*	N50 length (bp)	Complete (%)
Kidney from SW No.2	14.2	13.8	151,720	128,754	1,207	71.24
Kidney from SW No.3	15.2	14.9	179,734	135,902	1,271	77.25
Kidney from FW No.5	16.3	14.3	189,356	161,324	1,201	81.12
Kidney from FW No.6	15.8	14.9	196,057	168,624	1,256	83.69
Merged	-	-	305,524	268,917	1,468	91.42
						97.42

\*Genes were defined as clusters of the contigs based on the sequence overlapping

Note. QC: Quality control. CVG is referred from Hara et al., 2015.

**Table S2. Primer sets used in the present study**

Gene name		Primer sequence (5' to 3')
For bull shark		
Primer sets for cDNA cloning and <i>in situ</i> hybridization		
NKAc1	Sense	TTGTGCAACCGTGCCGTC
	Antisense	AGATGTCGTCTAGCTGTTCT
NKCC2	Sense	GCACTTATCAACTTTCATCT
	Antisense	TTTCAGTGAACGGATTCTTC
NCC	Sense	GGGTACGGGAAGAATAACGAGC
	Antisense	CTTCCTTCCTCATCCACACGAT
UT	Sense	CAGAATCCATGGTGGGCACT
	Antisense	ACAGGACTGTACCGTCTGAG
EF1 $\alpha$ 1	Sense	AAACGATACGACGAAATCACAAA
	Antisense	CATCTCCAGACTTCACAAATTG
Primer sets for qPCR		
NKAc1	Sense	CTGTTGCCCTGACGACACA
	Antisense	ACAAAGCATAGATCAATGAGG
NKCC2	Sense	CAGGCCACAGTGTATCGTAC
	Antisense	GTAAATACTTGACTACAGATGCAG
NCC	Sense	TGATTGCGAAGTGGAACACA
	Antisense	ATCACAAGTACGTTGGGCTTCA
UT	Sense	TGGCTGCAACAGGACACAAT
	Antisense	TTGCAATGAAGTAACTGGTCAA
EF1 $\alpha$ 1	Sense	AGACGGTAAGGTCACAGGACACA

Antisense      GCTTATAGGCCTCTGGAGGTA

For houndshark

Primer sets for standard cDNAs in qPCR

NKA $\alpha$ 1	Sense	CTCAAAGACCTAACCGCAGA
	Antisense	CCTCAGTGCTATATCAGTACCGG
NKCC2	Sense	CATCGGTATTGCTAACTTTT
	Antisense	CTTGCCAAAGAAATACAGC
NCC	Sense	GTTGGCTTCCTCTGGCT
	Antisense	CAACAGCACTGGAGTCCAC
UT	Sense	ACTGGCTGAAAGAGCAGAAC
	Antisense	AGCACTACAGGCCAAAACGC
	Antisense	CAGGGTAGGTCACTTCAGATAGA
EF1 $\alpha$ 1	Sense	AAACGATACGACGAAATCACAAA
	Antisense	CATCTCCAGACTTCACAAATTG

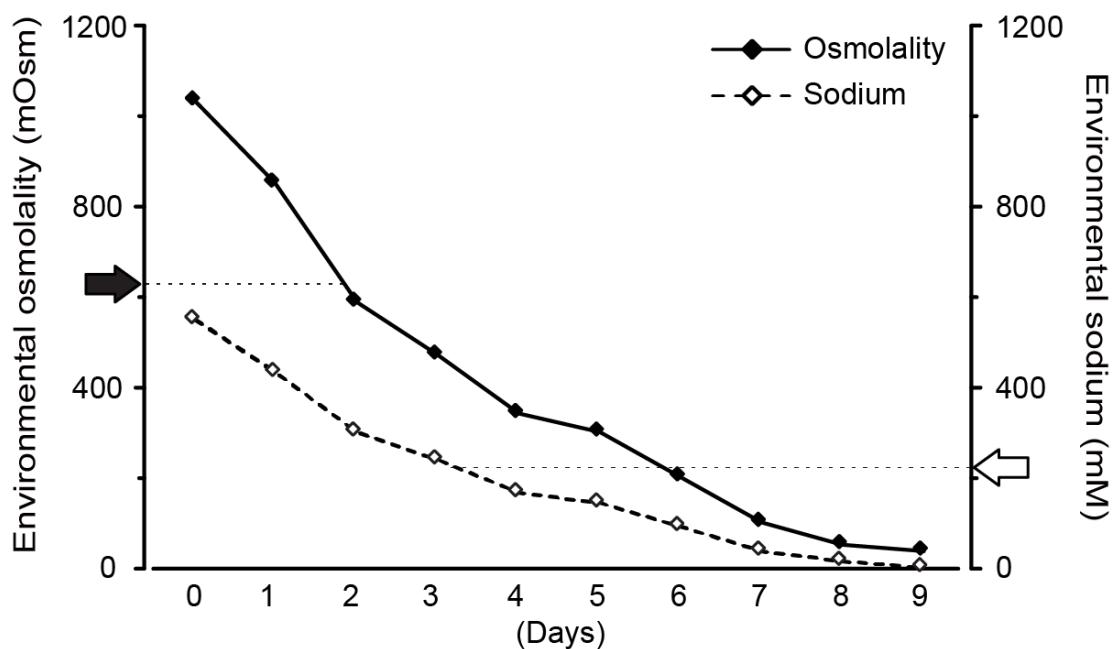
Primer sets for *in situ* hybridization

NKA $\alpha$ 1	Sense	GGTGCATTGTAGCTGTGAC
	Antisense	TATAAGGGAAGGCGCAGAACCA
NKCC2	Sense	ACGGTTGCGGGGATGGAGTGGGA
	Antisense	TGCCACCAGTTATTACGAACAT
NCC	Sense	GGGTACGGGAAGAACACAGA
	Antisense	CTTACGCTCTCGTCCATCC
UT	Sense	(Same as the cloning primer)

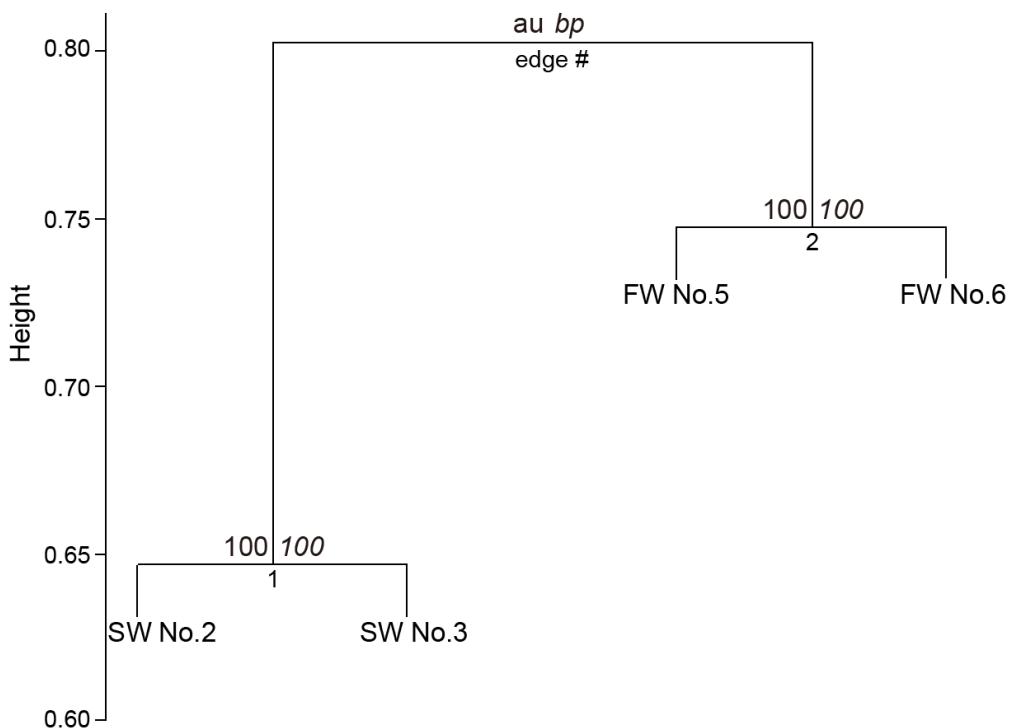
Antisense CAGGGTAGGTCACTTCAGATAGA

Primer sets for qPCR

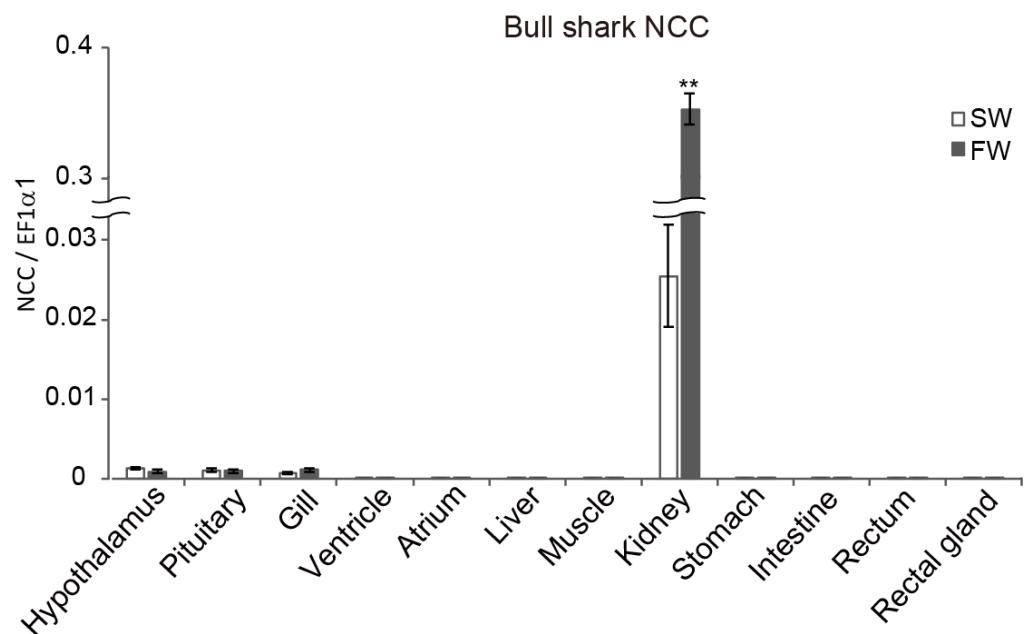
NKA $\alpha$ 1	Sense	TGCTTACACTTAACCAGCAATATCC
	Antisense	GGCTGTCTCTTCATTATATCACTTTC
NKCC2	Sense	ATCAATGATACTATAAGTTCTCGCTGAA
	Antisense	TATGATTCTCTACCTGTAATGCACAGG
NCC	Sense	GGGAACGTGACCAAAAAACCAC
	Antisense	CTGAGATATGCTCTAGTGGACACATCA
UT	Sense	TGGCTGCAACAGGACACAAT
	Antisense	TGCGATGAAGTAACTGGTCAA
EF1 $\alpha$ 1	Sense	CCTCCAGAACGCCCTGTAAG
	Antisense	GTACCAATACCAACCAATCTTAGACA



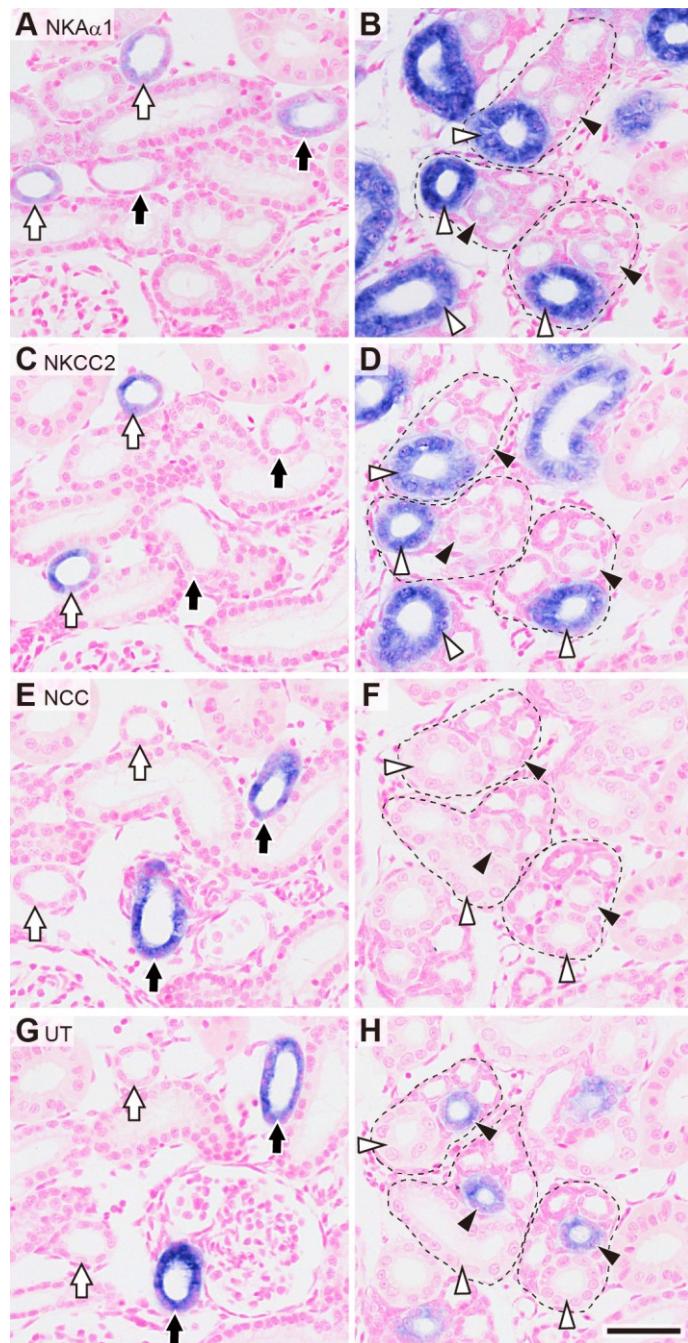
**Figure S1. Changes in osmolality and  $\text{Na}^+$  concentration of the aquarium tank water during the FW-acclimation experiment.** Filled circle and solid line show the osmolality of tank water and open circle and broken line show the  $\text{Na}^+$  concentration of that. Note that osmolality and  $\text{Na}^+$  concentration of the tank water fell below than the plasma values of FW-acclimated individuals after day 2 (filled arrow) and day 4 (open arrow), respectively.



**Figure S2. Clustering analysis based on the expression profiles from transcriptome analysis.** Two values of each node represent  $P$  values of approximately unbiased test (au) and bootstrapping probability (*bp*, *in italics*) for supporting the bifurcation. See the manual for Pvclust for details (<https://cran.r-project.org/web/packages/pvclust/>).



**Figure S3. Tissue distribution of NCC mRNA in the SW-acclimated (open bars) and FW-acclimated (filled bars) bull sharks.** Data are expressed as the mean  $\pm$  SEM. (SW,  $n = 4$ ; FW,  $n = 3$ ). Statistical analysis was performed using *t* test (except for rectal gland) or Mann-Whitney *U* test (rectal gland). Statistically significant differences are shown with asterisks. \*\*,  $P < 0.001$  (kidney,  $P = 0.000004$ ).



**Figure S4. Co-localization of mRNA signals in the kidney of houndshark in SW.**  
NKA $\alpha$ 1 (A, B), NKCC2 (C, D), NCC (E, F) and UT (G, H) mRNAs in the sinus zone (A, C, E, G) and bundle zone (B, D, F, H). Open arrows and filled arrows in the sinus zone represent the LDT and the transitional segment from the LDT to the CT, respectively. Filled arrowheads and open arrowheads represent the CT and the EDT, respectively. Sections were counterstained with Nuclear Fast Red. Bar = 50  $\mu$ m.