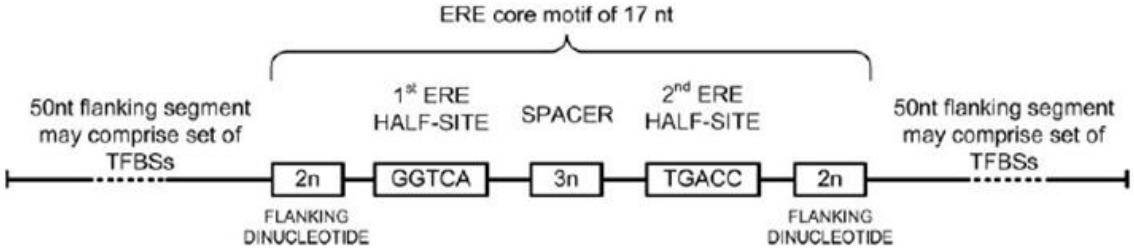

Promoter region of human *NDRG2* gene (-1455/+274). Boxed site is the putative initiation site (+1). Sequence flanking the first exon of NDRG2 (NT_026437) were applied to Promoter prediction assay with online Promoter Scan service (http://thr.cit.nih.gov/molbio/proscan/). -254/-5 region was predicted as putative promoter region. In agreement with the prediction in part, reporter gene assay located the promoter region at -1455/+274.

GAGCTGCCCTGACATGGAAGAACCATATCTTAGGGGAGAACCAGCCTTACTTCTGCCTGCAGCAGGCTGGGTGGGACCCCAAGGTCTAGGAAG
CCAATGGATGTCAGTGGAAATTTCCCATCTCCTGCCTGCCCTGCGCCCCTGCGTAGGCCCCTTATCAGGTTCCCCCTGAGTCATCCTGACCCAGA
GACGAATTTAGATGCTGGAACCTTCAGGTCCTTCCCTCCCCCACTCCAAGTAAGACGACTTTTGACAAAGAAAAGCCTGCAGGCAAAAGTTCTC
ACTCGAGGCTCTCCCTCTCCCCAGGTTCCTCTGGTGCTGATGAGATCTCTCCTGTCTTGCGCACCTTTGGCTGTGACCCTTATTCCAGCAGGCTTT
TCCTTGTCCAGGAGAGTGTTAAACTGGCATCCAGAGGGAAGAGGGGACTAGGAGAGGAGCCAGGGTGCCCAGCTTCTGTGCCAGTTTCTGGGG
AAAAAGTTATAAAGGGAGTGGGAGAAAGGAACAGAAGAAAAGTTGGGTGCTCCAAATTGGGGGATGTAGGGGGAGCTCCTTAGATTTGGGGTT
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GGATGAAAGGGGCATTGATGTTCCTTGGATGGGAGGGGTATTGCGGGTGGTGAGGGTGGTGGTGGGAGGGTGGTTCCCAGGCAGGAGGGGGCA
GGGAAACTTGGGGGTCTTGGGGGGGCAGTCGGTGCTGGTGTTGCAGTTGGCAGCTCAAGTTCCATTGGCCATTGTTCCTGACTGGCCTGGCGCC
CAGCCCCCGAAATGGCAACTCAGGAACCAGTCTGAAGGGGGTTGGGGTGGATATGATCCTGGGATTGGGGGAGCTACAAGGGGGGCAGGTGGG
GAGAAATTCAGTCAGGTTTCCTTTGCCTAAACAACTTCGAATTCCCTTAAGCCACATTTTTAATCAACTAACTCATTCCAGGGAGCCTTCCTCCG
AGGGGCACAAGGAGAGCTCATTCCAGGGTGTGTGTGTGTGTGTGCGTGTGCGTGTGTGTGTAAAGTCTACAGTGGCAAACCCACTCGGGCACC
GAGAGGGACGCGGCAGACCCTGAGACCATCCCCCGGGCATTGCGATTCAGCGGGCCTGAGCTGGTCTCCTATTCTGGGCCGGACTGGGAGGGG
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CTGCGCCTGTTTACCCAGGAGTCAGAGCCTCTGGGGGTCCCCGGCCCCTCCCCCGCGCCCCTTGAGGCATTGACCCCAGAGTCCCTGTTGGCCA
TCCTCTCCCCACCCCCCGCCTGCCCGCGATCGCCCTCTATTTATAGCGGCCCCCCGCATTCCCCGCCCCCACCTCCCCGCCTTGTTGTCCAACTTC
TCCCGGAGCAGCCGGAGAGCAGGCGTCGGGACGCAGCAAAGAGAGGAGAGGTACCAGGACTCTGGGCACGAGGGAGTCGGAACTCCTGCGC
CCTCGACGTTCCCCCGTGCCCCCTTTCGGCGCCAGCACACCTGCCCCTGCCAGCCGGCGAGACCCCCGCCTCCTCAGCGGCGGTCCCGCCCACC
TCCTCCTTTCTTAATCTAGTTCTCTGTGTTCGGGCGGGG.

Fig. S1. An illustration of the human *NDRG2* promoter (-1455/+274). Upper panels: an illustration of the ERE frame that contains a 117-bp segment centered by a core of the 17-bp ERE. Down panels: The red sequence indicates the putative ERE.

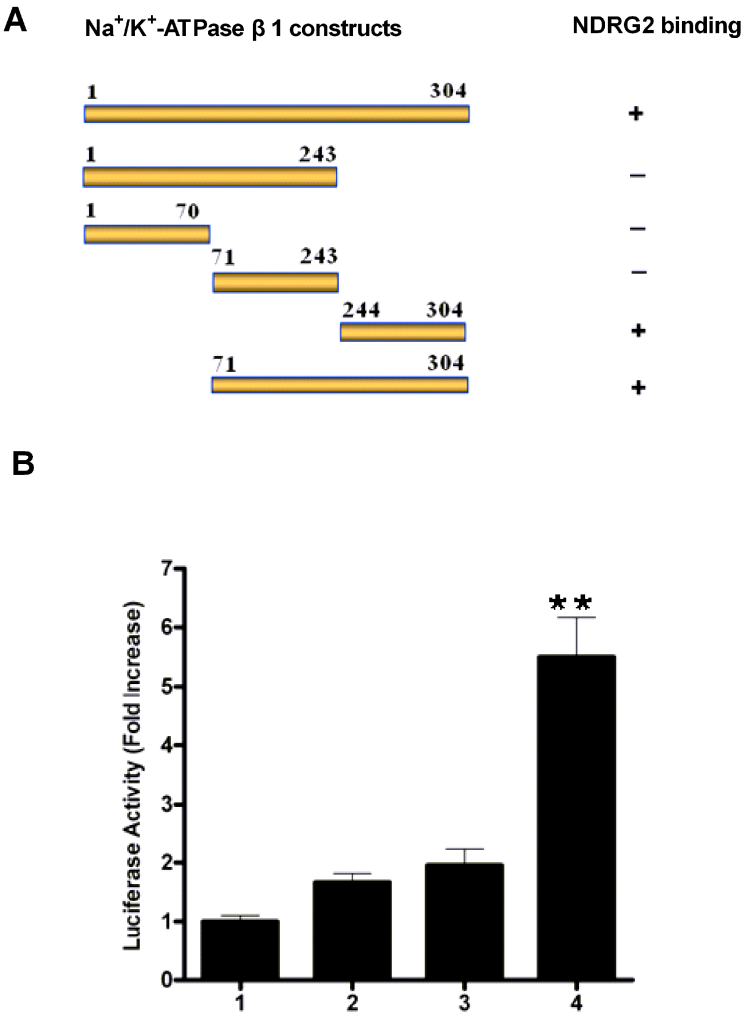

Fig. S2. NDRG2 interacts with the Na^+/K^+-ATPase β1. (A) Schematic of the full-length Na^+/K^+-ATPase β1 and its truncated fragments. The cDNAs encoding them were fused to Gal4-AD in pACT vector, and co-transformed into yeast *AH109* with full-length NDRG2-Gal4-BD vector. The grown clones on SD/-Trp-Leu-His-Ade/X-α-Gal dish were transferred onto nitrocellulose membrane and tested for β-galactosidase activity. The "+" stands for positive interaction, while "-" for negative clones. (B) Mammalian two-hybrid assay. HEK293 cells were co-transfected with 250 ng Na^+/K^+-ATPase β1-AD plasmid, 250 ng NDRG2-Gal4-BD plasmid and 250 ng pG5-*luc* reporter. The luciferase activity was measured and expressed as fold increase relative to the control cells from three experiments. 1. Background control: Gal4-AD vector, Gal4-BD vector, and pG5-*luc* vector; 2. Negative control: Gal4-BD vector, Na^+/K^+-ATPase β1-AD vector, and pG5-*luc* vector; 3. Negative control: NDRG2-Gal4-BD vector, Gal4-AD vector, and pG5-*luc* vector; and 4. Experiment group: NDRG2-Gal4-BD vector, Na^+/K^+-ATPase β1-AD vector, and pG5-*luc* vector. ** $p<0.01$ *versus* background control.

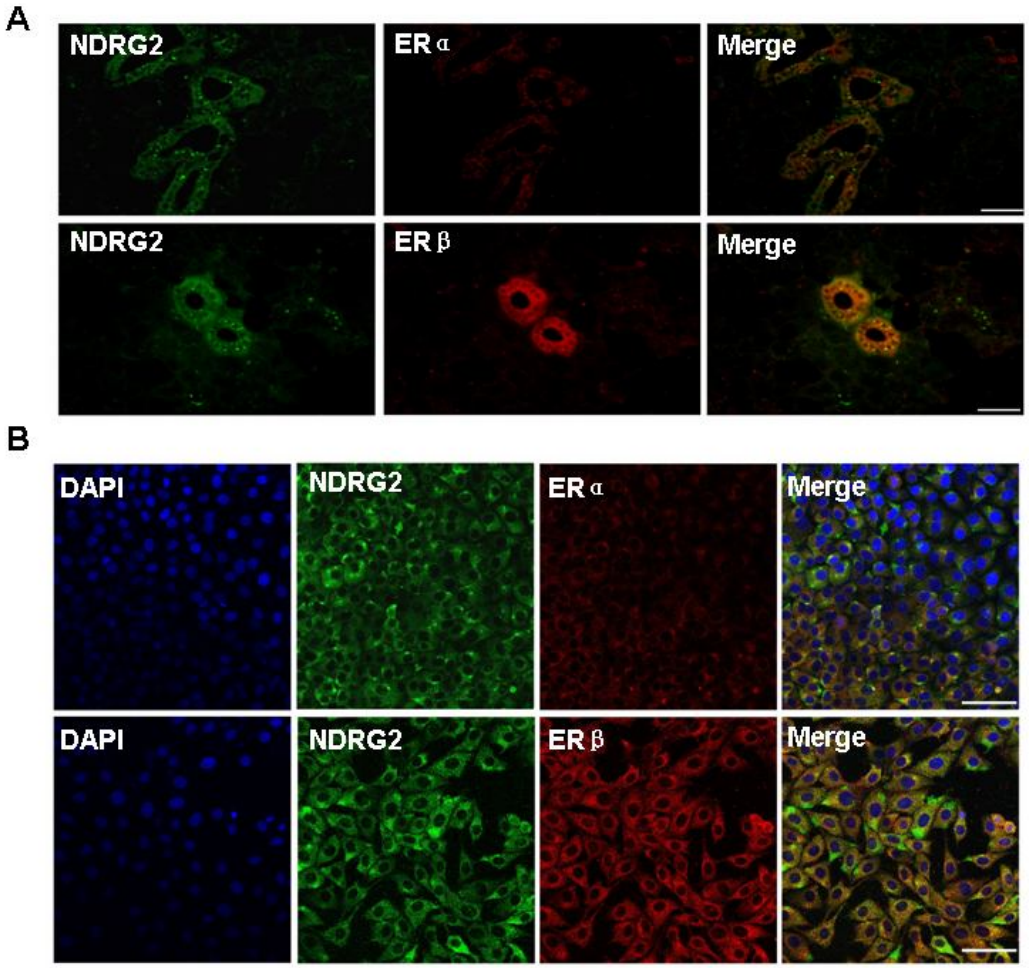

Fig. S3. NDRG2 is co-localized with the ERβ in human salivary gland tissues and cells. (A) The expression of NDRG2 and ER in salivary glands (Scale bars=50 μm). (B) The expression of NDRG2 and ER in HSG cells (Scale bars=20 μm). Salivary gland tissue sections and HSG cells were probed with mAb against NDRG2 antibody and rabbit anti-ERα or anti-ERβ antibodies and the bound antibodies were detected by FITC-goat anti-mouse IgG and Cy3-goat anti-rabbit IgG. The isotype mouse and rabbit IgG were used as negative controls (data not shown). The expression of NDRG2 and ER were examined under a fluorescent microscope. Data shown are representative images from five individual subjects in three independent experiments.

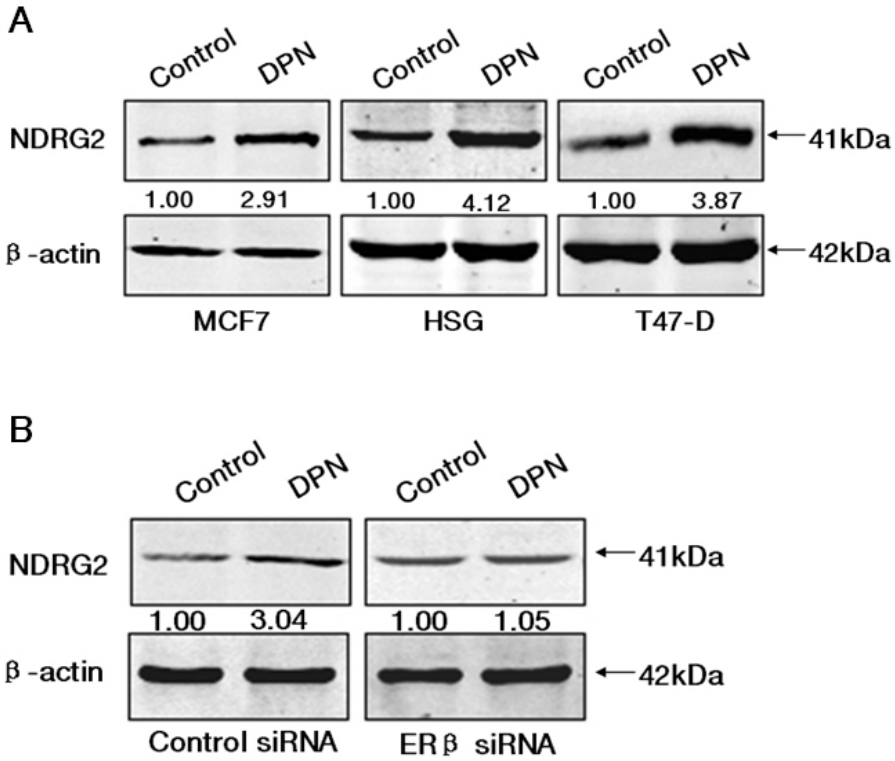

Fig. S4. DPN enhances endogenous NDRG2 protein expression. (A) MCF7, HSG and T-47D cells that express endogenous ERβ were treated with ERβ selective agonist, 10 nM DPN, or control ethanol for 24 h. The NDRG2 protein expression was determined by immunoblotting. (B) Silence of ERβ expression reduces their responses to DPN. HSG cells were transfected with 100 pmol ERβ specific siRNA or control siRNA for 48 h and treated with DPN for 24 h. The NDRG2 protein expression was determined by immunoblotting. Data are representative images of each group of cells from three separated experiments.

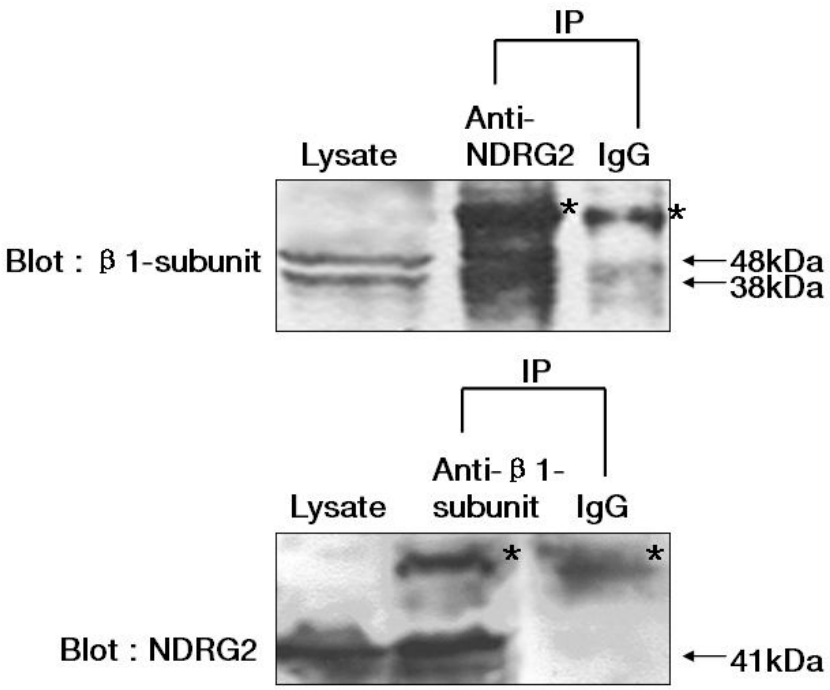

Fig. S5. The interaction of endogenous NDRG2 and endogenous Na^+/K^+-ATPase β1. IPs were performed with whole cell lysates of HSG cells pretreated with protein A conjugated sepharose beads. Whole cell lysates were probed for input. The antibodies for immunoprecipitation and western blot are as indicated. The location of various proteins is indicated with arrowhead. Blot, western blot; IP, immunoprecipitation; *, Ig heavy chain.

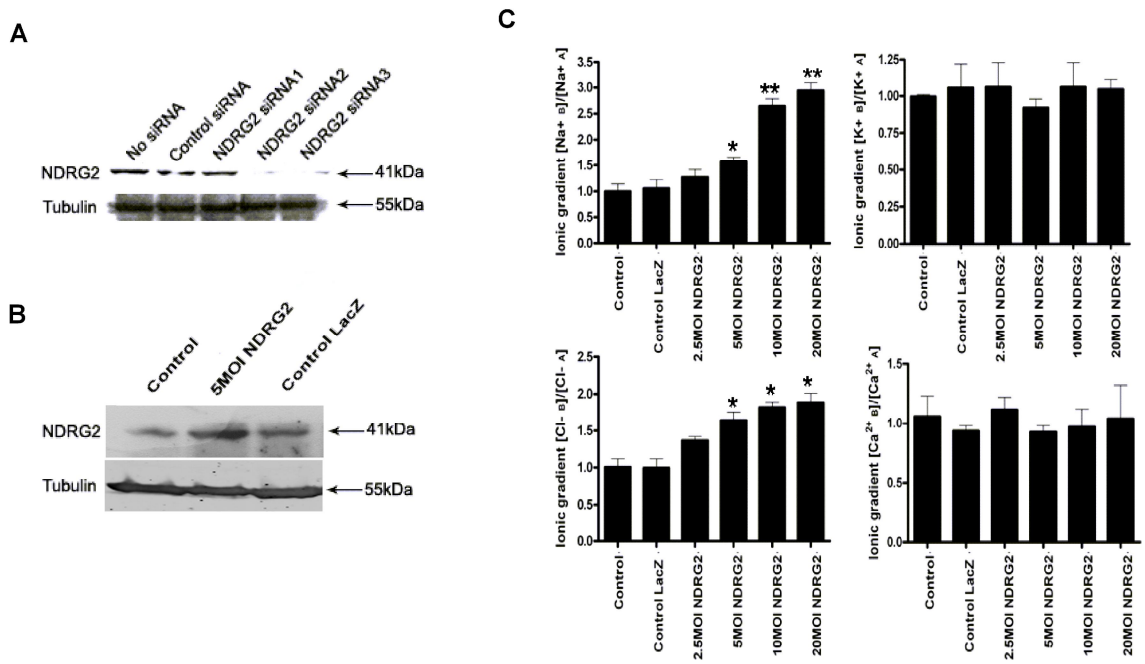

Fig. S6. NDRG2 stimulate ionic transport in HSG cells. (A) Silence of NDRG2 expression by siRNA. HSG cells were transfected with, or without, control siRNA or individual *NDRG2*-specific siRNAs, and the NDRG2 expression was determined by western blotting. The *NDRG2*-specific siRNA 2 was used for the following experiments. (B) Induction of NDRG2 expression by NDRG2 adenovirus. HSG cells were transfected with NDRG2 or LacZ adenovirus (5MOI) for 48 h, and the NDRG2 expression was determined by western blotting. (C) Induction of NDRG2 over-expression modulates the Na^+ and Cl^- gradients. HSG cells were cultured on collagen I-coated Transwell-Col filters for monolayer and infected with NDRG2 or control LacZ adenovirus (2.5-20 MOI) for 48 h. The concentrations of Na^+, K^+, Cl^- and Ca^{2+} in the harvested media were measured. Data are expressed as the mean ± SD of the relative ratios of individual electrolyte in the basolateral compartment to that in the apical compartment from three independent experiments. A, apical; B, basolateral. *p<0.05; ** p<0.01 *versus* control.

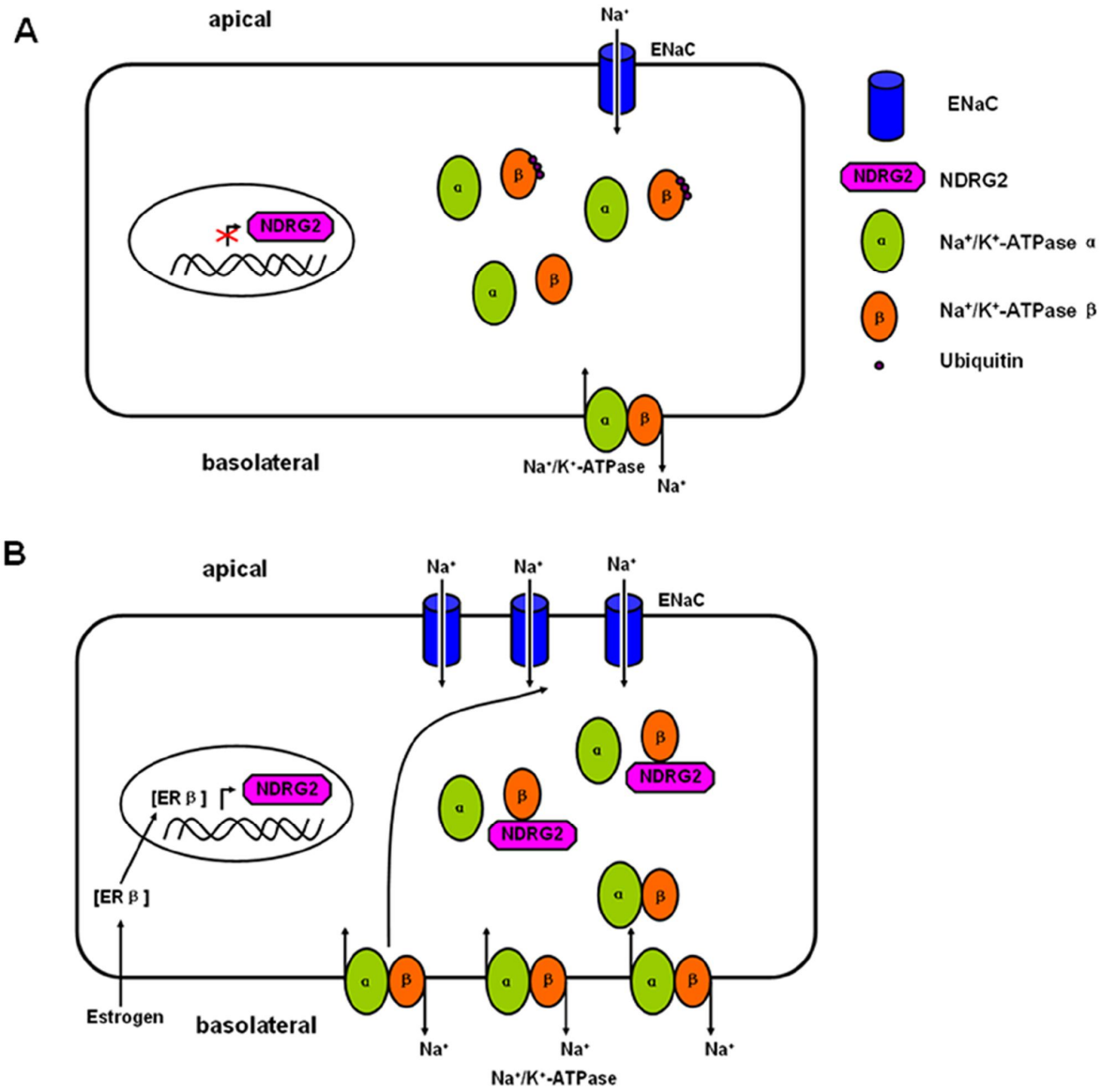

Fig. S7. Model for the mechanism of NDRG2 participates in estrogen-mediated Na^+/K^+-ATPase regulation in epithelial cells. (A) In the absence of estrogen, NDRG2 transcripts at low levels. Na^+/K^+-ATPase β1 is ubiquitinated and naturally degraded in cytosol. Na^+/K^+-ATPase and ENaC mediate Na^+ transport on basolateral and apical sides of plasma membrane at low levels, respectively. (B) In the presence of estrogen, estrogen binds to ERβ, forming transcription factor, which activates the *NDRG2* transcription. Increased NDRG2 protein binds and stabilizes Na^+/K^+-ATPase β1 protein by inhibiting its ubiquitination and degradation. A greater accumulation of Na^+/K^+-ATPase promotes Na^+ pumping across the basolateral plasma membrane, leading to a low cytosolic concentrations of Na^+, which stimulates the sensors of cytosolic Na^+ and activates the ENaC in the apical plasma membrane, facilitating the reabsorption of Na^+ in epithelial cells.

Table S1. The sequences of primers. The restricted endonuclease sites were underlined, and the mutant bases were framed. * For vector construction.

pCMV-Myc-Na^+/K^+-ATPase β *	Forward: ACGCGTCGACCATCGCCATGGCCCGCGGGAAAGCCAAG
	Reverse: CCGCTCGAGGTGGGAAAGATTTGTGCTTGTG
NDRG2 siRNA1	ACATCCTGGCGAGATATGCTCTTAA
NDRG2 siRNA2	GCTCTCTGGAAATTCTGAGTTGATA
NDRG2 siRNA3	GCACCCAACCTGGATAACATTGAAT
Control siRNA	GGCCGCAAAGACCTTGTCCTTAGAA
M1	Forward: CAGGTTCCCCCTGA**T**TCATCCTGACCCAGAGACGAATTTAG
	Reverse: CTAAATTCGTCTCTGGGTCAGGATGA**A**TCAGGGGGAACCTG
M2	Forward: CAGGTTCCCCCTGAGTCATCCT**C**ACCCAGAGACGAATTTAG
	Reverse: CTAAATTCGTCTCTGGGT**G**AGGATGACTCAGGGGGAACCTG
M3	Forward: CAGGTTCCCCCTGA**T**TCATCCT**C**ACCCAGAGACGAATTTAG
	Reverse: CTAAATTCGTCTCTGGGT**G**AGGATGA**A**TCAGGGGGAACCTG
ERβΔE（7-8）*	Forward: 5'-CCGCTCGAGATAATGACCTTTGTGCCTC-3'
	Reverse: 5'-CGGGATCCTCAGGAATTGAGCAGGATCATG-3'
ERβ siRNA	GCTACAAATCAGTGTACAATT
Control siRNA	GGCCGCAAAGACCTTGTAATT
ChIP (-1378/-1240 NDRG2 promoter)	Forward: CCAAGGTCTAGGAAGCCAATG
	Reverse: CCTGAAGGTTCCAGCATCTAA
ChIP (-75/+50 NDRG2 promoter)	Forward: GGCATTGACCCCAGAGTCCCTG
	Reverse: GAAGTTGGACAACAAGGCGGGG
EMSA wt ERE NDRG2 promoter	Forward: CAGGTTCCCCCTGAGTCATCCTGACCCAGAGACGAATTTAG
	Reverse: CTAAATTCGTCTCTGGGTCAGGATGACTCAGGGGGAACCTG
EMSA mutant ERE NDRG2 promoter	Forward: CAGGTTCCCCCTGA**T**TCATCCT**C**ACCCAGAGACGAATTTAG
	Reverse: CTAAATTCGTCTCTGGGT**G**AGGATGA**A**TCAGGGGGAACCTG
NDRG2-Gal4-BD *	Forward: ACGCGTCGACCCATGGCGGAGCTGCAGGAGG
	Reverse: CGGGGTACCAAGGGCCATTCAACAGGAGAC
Na^+/K^+-ATPase β1-AD *	Forward: CGCGGATCCTCGCCATGGCCCGCGGGCCC
	Reverse: CGGGGTACCGTGGGAAAGATTTGTGCTTGTG
Na^+/K^+-ATPase β1(real-time PCR)	Forward: TAAGGCGTACGGTGAGAACA
	Reverse: AATACTTAGGGGCCAGAAACA
NDRG2 (real-time PCR)	Forward: GAGATATGCTCTTAACCACCCG
	Reverse: GCTGCCCAATCCATCCAA
β-actin (real-time PCR)	Forward: TGGCACCCAGCACAATGAA
	Reverse: CTAAGTCATAGTCCGCCTAGAAGCA