Supplemental Figure 1. Validation of Nup88 silenced clones. Stable clones expressing pSM2-Nup88 and selected in puromycin media were analyzed for Nup88 protein expression after incubation under hypertonic stress. A representative western blot reveals that clones 3 and 6 demonstrate near complete silencing of Nup88 expression. These clones were selected for further experiments regarding Nup88 silencing. B) QPCR for TonEBP target genes (AR and Hsp70) showing an intermediate expression for both genes in a clone partially silenced for Nup88 expression (clone #4). In contrast, no changes were observed in mRNA levels from a non silenced clone (clone #5) and as compared to totally silenced clone (#3). C, D) Protein analysis for AR (C) and Hsp70 (D) in partially (#4) and non silenced (#5) clones demonstrating intermediate and no change in protein expression, respectively, and as compared to the totally silenced clone (#3) and the empty vector control.

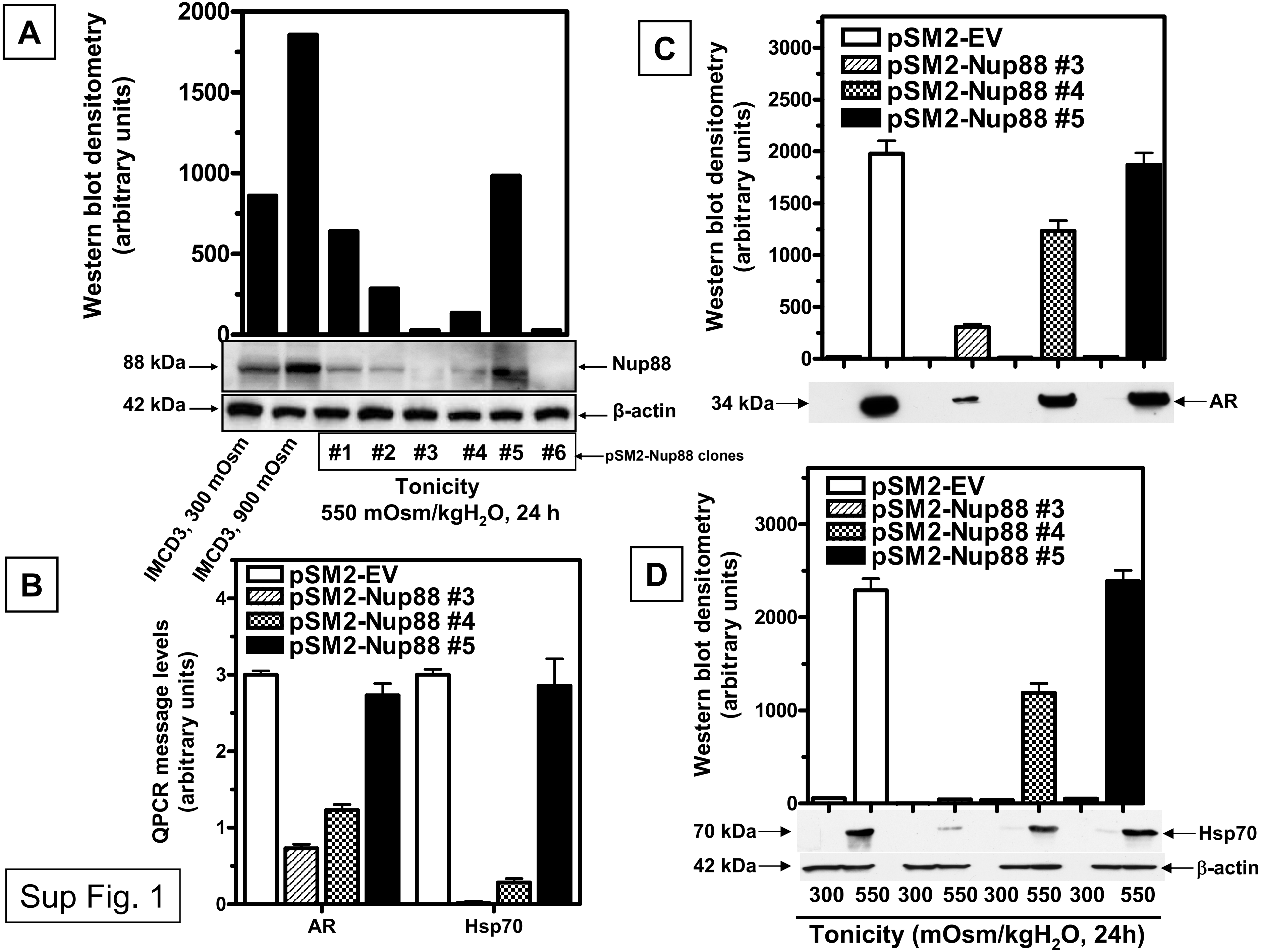
Supplemental Figure 2A. Evaluation of the involvement of CRM1 in TonEBP trafficking in IMCD3 cells under isotonic conditions. IMCD3 cells transfected with the TonEBP-GFP construct were incubated in the presence and absence of 10 ug/ml LMB, a specific inhibitor of CRM1. In the absence of LMB, IMCD3 cells demonstrate substantial TonEBP localization in the cytosol. However, in the presence of the inhibitor LMB, TonEBP is localized mainly in the nucleus, suggesting CRM1 is involved in TonEBP export from the nucleus under isotonic conditions.

Supplemental Figure 2B. Evaluation of the involvement of CRM1 in TonEBP trafficking in IMCD3 cells under hypertonic conditions. The left column demonstrates under hypertonic stress and in the presence of Nup88 (empty vector control cells), TonEBP is localized in the nucleus. Comparatively, when Nup88 expression is silenced (pSM2-Nup88) and cells are incubated under hypertonic stress, TonEBP is found in the cytosol (middle column). However, in the presence of the inhibitor LMB, in cells silenced for Nup88, TonEBP is observed in the cytosol indicating that CRM1 (inactive in this setting) is not responsible for shuttling TonEBP out of the nucleus under hypertonic stress (right column).

Supplemental Table 1. Nucleotide sequence alignment of the N-terminus of TonEBP of human, mouse and IMCD3 cells.

Supplemental Table 2. Protein sequence alignment of the N-terminus of TonEBP of human, mouse and IMCD3 cells. Sequence cloned from IMCD3 cells (*top line*) shares the same N-Terminus sequence than human TonEBP (*middle lane*) including the same Auxiliary Export Domain (AED) and Nuclear Export Signal (NES) (depicted in bold and underlined and surrounded by dotted rectangle). Note that the three sequences share the same Nuclear Localization Signal (NLS) (depicted in bold and italic and surrounded by dotted rectangle).

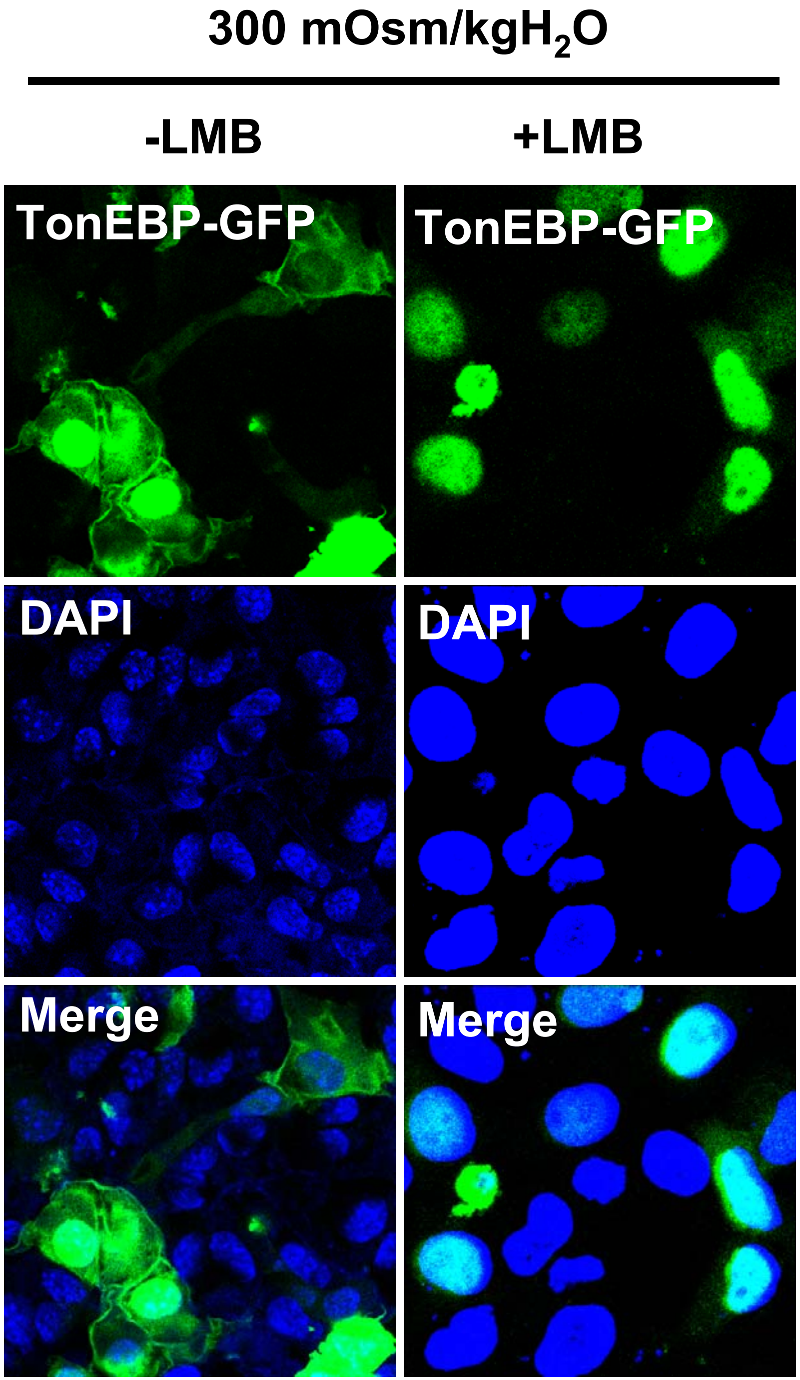
Supplemental Table 3. Primers and accession number of mRNAs used for QPCR experiments.

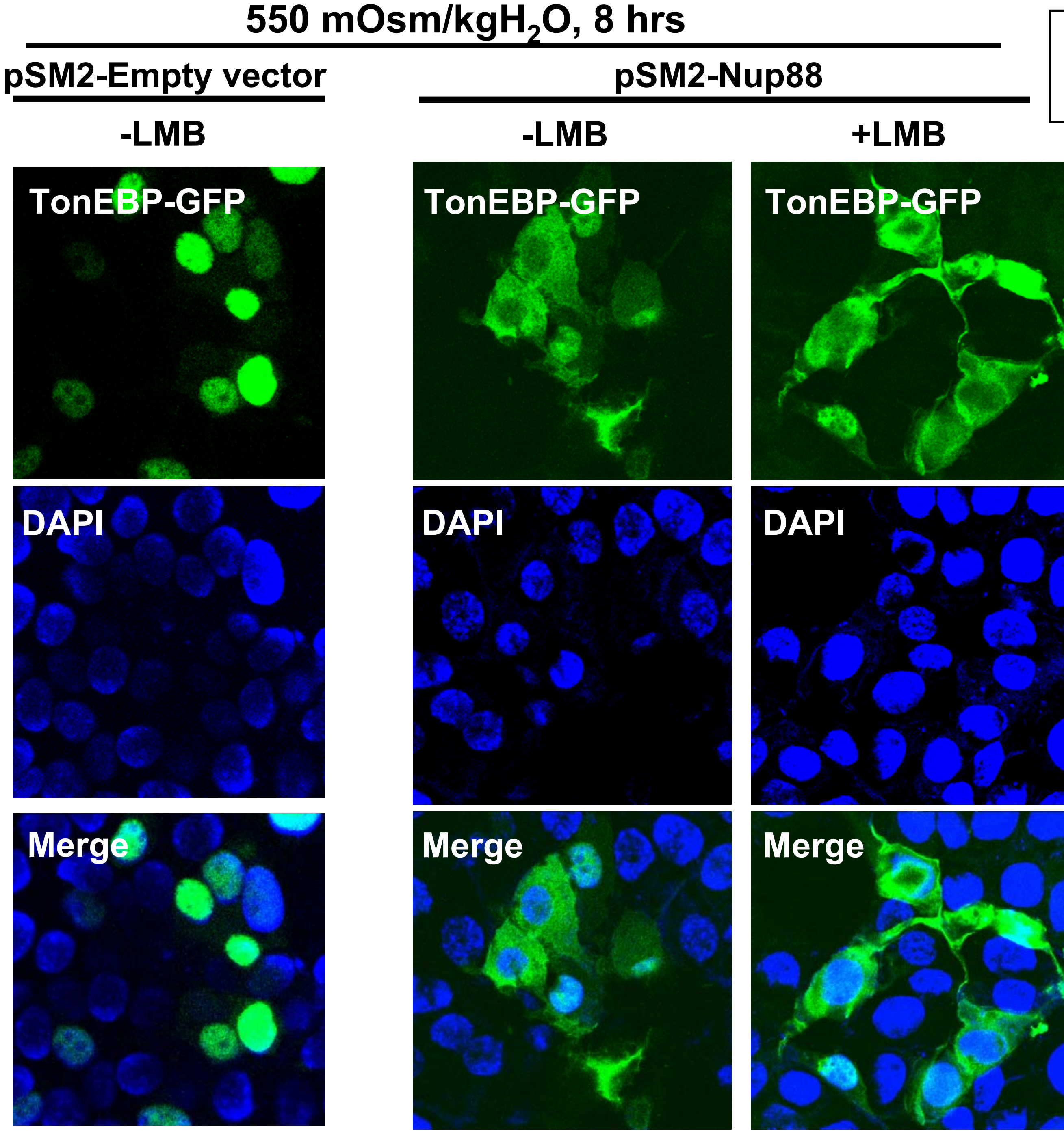


A
Western blot densitometry (arbitrary units)
2000
1500
1000
500
0
88 kDa
Nup88
42 kDa
β-actin
IMCD3, 300 mOsm
IMCD3, 900 mOsm
#1 #2 #3 #4 #5 #6
pSM2-Nup88 clones
Tonicity
550 mOsm/kgH2O, 24 h
B
QPCR message levels (arbitrary units)
pSM2-EV
pSM2-Nup88 #3
pSM2-Nup88 #4
pSM2-Nup88 #5
4
3
2
1
0
AR
Hsp70
C
Western blot densitometry (arbitrary units)
pSM2-EV
pSM2-Nup88 #3
pSM2-Nup88 #4
pSM2-Nup88 #5
3000
2500
2000
1500
1000
500
0
34 kDa
AR
D
Western blot densitometry (arbitrary units)
pSM2-EV
pSM2-Nup88 #3
pSM2-Nup88 #4
pSM2-Nup88 #5
3000
2000
1000
0
70 kDa
Hsp70
42 kDa
β-actin
300 550 300 550 300 550 300 550
Tonicity (mOsm/kgH2O, 24h)

Sup Fig. 1

Supplemental Figure 2A





Supplemental Figure 2B

Sup Table 1

HUMAN	ATGCCCTCGG ACTTCATCTC ATTGCTCAGC GCGGACCTAG ACCTGGAATC GCCCAAGTCC CTCTACTCGC GAGA------ ----------
MOUSE	---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
IMCD	ATGCCCTCGG ACTTCATCTC ATTGCTCAGC GCGGACCTAG ACCTGGAATC GCCCAAGTCC CTGTACTCGC GAGATTCTCT GAAGTTACAC
HUMAN	---------- ---------- ---------- --------AT CTGTCTATGA TCTTCTCCCA AAGGAGTTAC AGTTACCTCC ATCTAGAGAA
MOUSE	---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
IMCD	CCATCACAGA CTTTTCATAG AGCTGGACTA TTGGAAGAAT CTGTCTATGA TCTTCTCCCA AAGGAGTTAC AGTTACCTCC ACCTAGAGAA
HUMAN	ACATCTGTAG CATCAATGAG TCAGACAAGC GGTGGTGAGG CAGGCTCGCC TCCTCCAGCT GTTGTTGCTG CTGATGCTTC TTCAGCTCCC
MOUSE	---------- -----ATGAG TCAGACAAGC GGTGGTGAGG CAGGCTCGCC TCCTCCAGCT GTAGTTGCTG CTGATGCTTC TTCAGCTCCC
IMCD	ACATCTGTAG CATCAATGAG TCAGACAAGC GGTGGTGAGG CAGGCTCGCC TCCTCCAGCT GTAGTTGCTG CTGATGCTTC TTCAGCTCCC
HUMAN	TCCTCTTCCT CCATGGGCGG TGCTTGCAGC TCCTTTACCA CCTCTTCCAG CCCTACCATT TATTCTACCT CAGTCACCGA CAGCAAGGCT
MOUSE	TCCTCTTCCT CCATGGGCGG TGCTTGCAGC TCCTTTACCA CCTCTTCCAG CCCTACCATT TATTCTACCT CAGTCACCGA CAGCAAGGCT
IMCD	TCCTCTTCCT CCATGGGCGG TGCTTGCAGC TCCTTTACCA CCTCTTCCAG CCCTACCATT TATTCTACCT CAGTCACCGA CAGCAAGGCT
HUMAN	ATGCAAGTGG AGAGCTGCTC CTCAGCCGTG GGGGTAAGTA ACAGAGGGGT AAGTGAAAAG CAGTTAACCA GTAACACAGT TCAGCAGCAT
MOUSE	ATGCAAGTGG AGAGCTGCTC CTCAGCCGTG GGGGTAAGTA ACAGAGGGGT AAGTGAAAAG CAGTTAACCG GTAACACAGT TCAGCAGCAT
IMCD	ATGCAAGTGG AGAGCTGCTC CTCAGCCGTG GGGGTAAGTA ACAGAGGGGT AAGTGAAAAG CAGTTAACCG GTAACACAGT TCAGCAGCAT
HUMAN	CCATCAACAC CGAAGAGGCA CACAGTCTTG TACATCTCAC CACCACCTGA GGACTTGCTG GATAACAGTC GGATGTCCTG CCAGGATGAG
MOUSE	CCATCAACCC CGAAGAGGCA CACAGTTTTG TACATCTCAC CACCACCTGA GGACTTGCTG GATAACAGTC GGATGTCCTG CCAGGATGAG
IMCD	CCATCAACCC CGAAGAGGCA CACAGTTTTG TACATCTCAC CACCACCTGA GGACTTGCTG GATAACAGTC GGATGTCCTG CCAGGATGAG
HUMAN	GGGTGTGGAT TGGAATCTGA GCAGAGCTGC AGTATGTGGA TGGAGGATTC CCCCTCCAAC TTCAGTAACA TGAGCACCAG TTCCTACAAT
MOUSE	GGGTGTGGAT TGGAATCTGA GCAGAGCTGC AGTATGTGGA TGGAGGATTC CCCCTCCAAC TTCAGTAACA TGAGCACCAG TTCCTACAAT
IMCD	GGGTGTGGAT TGGAATCTGA GCAGAGCTGC AGTATGTGGA TGGAGGATTC CCCCTCCAAC TTCAGTAACA TGAGCACCAG TTCCTACAAT
HUMAN	GATAACACTG AGGTACCTCG TAAATCACGA AAACGAAATC CAAAGCAGAG GCCGGGGGTC AAACGACGAG ATTGTGAAGA ATCTAATATG
MOUSE	GATAACACTG AGGTACCTCG TAAATCACGA AAACGAAATC CAAAGCAGAG GCCGGGGGTC AAACGACGAG ATTGTGAAGA ATCTAATATG
IMCD	GATAACACTG AGGTACCTCG TAAATCACGA AAACGAAATC CAAAGCAGAG GCCGGGGGTC AAACGACGAG ATTGTGAAGA ATCTAATATG
HUMAN	GATATATTTG ATGCCGACAG TGCCAAAGCA CCTCACTATG TGCTTTCTCA GCTTACCACG GACAACAAAG GCAACTCAAA AGC
MOUSE	GATATATTTG ATGCCGACAG TGCCAAAGCA CCTCACTATG TGCTTTCTCA GCTTACCACG GACAACAAAG GCAACTCAAA AGC
IMCD	GATATATTTG ATGCCGACAG TGCCAAAGCA CCTCACTATG TGCTTTCTCA GCTTACCACG GACAACAAAG GCAACTCAAA AGC

Sup Table 2

NES **AED**

```
IMCD3  MPSDFISLLS ADLDLESPKS LYSRDSLKLH PSQTFHRAGL LEESVYDLLP KELQLPPPRE TSVASMSQTS GGEAGSPPPA VVAADASSAP
HUMAN  MPSDFISLLS ADLDLESPKS LYSRE..... .......... ...SVYDLLP KELQLPPSRE TSVASMSQTS GGEAGSPPPA VVAADASSAP
MOUSE  .......... .......... .......... .......... .......... .......... .....MSQTS GGEAGSPPPA VVAADASSAP

IMCD3  SSSSMGGACS SFTTSSSPTI YSTSVTDSKA MQVESCSSAV GVSNRGVSEK QLTGNTVQQH PSTPKRHTVL YISPPPEDLL DNSRMSCQDE
HUMAN  SSSSMGGACS SFTTSSSPTI YSTSVTDSKA MQVESCSSAV GVSNRGVSEK QLTSNTVQQH PSTPKRHTVL YISPPPEDLL DNSRMSCQDE
MOUSE  SSSSMGGACS SFTTSSSPTI YSTSVTDSKA MQVESCSSAV GVSNRGVSEK QLTGNTVQQH PSTPKRHTVL YISPPPEDLL DNSRMSCQDE

IMCD3  GCGLESEQSC SMWMEDSPSN FSNMSTSSYN DNTEVPRKSR KRNPKQRPGV KRRDCEESNM DIFDADSAKA PHYVLSQLTT DNKGNSK
HUMAN  GCGLESEQSC SMWMEDSPSN FSNMSTSSYN DNTEVPRKSR KRNPKQRPGV KRRDCEESNM DIFDADSAKA PHYVLSQLTT DNKGNSK
MOUSE  GCGLESEQSC SMWMEDSPSN FSNMSTSSYN DNTEVPRKSR KRNPKQRPGV KRRDCEESNM DIFDADSAKA PHYVLSQLTT DNKGNSK
```

NLS

Sup Table 3

	Accesion number	Forward primer 5'→3'	Reverse primer 5'→3'
Nucleoporin 88 (Nup88)	NM_172394	CCATTACTCTCAGTGCCTACC	AGCCTTCAAGTTATGTTCATTCC
Aldose Reductase (AR)	NM_009658	CTATTTCCCACTGGATGCCTCAG	TTTCACCAAACCTTCATCCACTAG
Sodium-myoinositol transporter (SMIT)	NM_017391	CCTACCGTGCTCCTGAGTGTG	GTGGGAGGTGGTGTGAGAAGAC
Betaine/GABA transporter (BGT1)	NM_133661	AAGGCAAGGACCAGGTGAAGG	CACACTCCCCTGGCTGCTATAC
Taurine transporter (TauT)	NM_009320	CGCATCCATCGTCATTGTGTC	GTGTCCTCCATGCACTGTGG
Heat shock protein 70 (Hsp70)	NM_010479	GGTCTAAACGTGCTGCGGATC	AGTCCTCCCCTCCCAGGTG
α_1-subunit Na/K-ATPase	NM_144900	GGATTGTTGGCTCTGATG	CGGAATTGTTACTGGTTAGG
β-Actin	NM_007393	CTCTCCCTCACGCCATCC	GTAACAGTCCGCCTAGAAGC