Table S1. **Effects of oligomycin and 2-DG on the concentration of ATP in HEK293T cells co-transfected with NCX1 and the various individual CK isozymes.** HEK293T cells were transfected with pGFP and pNCX1 together with either empty vector or a plasmid encoding one of the various CK isozymes. The concentration of ATP was then measured after treatment with oligomycin and 2-DG to induce energy-compromised conditions.

Co-transfection	Control	Oligomycin + 2-DG
NCX +	[ATP] (pmol/10^4 cells)	
Vector	5.27 ± 0.44	1.87 ± 0.47
uMiCK	4.72 ± 0.02	1.92 ± 0.32
sMiCK	5.18 ± 0.50	1.65 ± 0.55
CKB	5.16 ± 0.45	0.99 ± 0.19
CKM	5.12 ± 0.21	1.33 ± 0.23

Figer S1. **Characterization of rabbit anti-NCX1IL antibodies.** (A) NCX1IL detected by rabbit anti-NCX1IL antibodies with an apparent molecular weight of 70 kDa (left lane). The band was competed out by NCX1IL (right lane). (B) Cell lysates of HEK293T cells transfected with the empty vector (Vector), pNCX1 (NCX), psMiCK (sMiCK), or pCKM (CKM), and mouse heart extract (Heart) were subjected to Western blot using rabbit anti-NCX1IL antibodies (left panel). In cells transfected with pNCX1, two major bands with apparent molecular weight of 120 and 140 kDa and a minor band of 160 kDa were detected. In mouse heart extract, two bands of 140 and 160 kDa were detected. In some blots, the 160 kDa band was not detected. Addition of NCX1IL competes out the bands recognized by the antibodies (right panel).

Figure S2. **Alignment of the amino acid sequence of human uMiCK, sMiCK, CKB, and CKM.** The amino acid residues in the frame are the shortest region of the human sMiCK required to interact with NCX1IL based on the results obtained from yeast two-hybrid assays.

Figure S3. **Only the C-terminus of sMiCK but not that of uMiCK, CKB, and CKM interacts with NCX1IL in yeast two-hybrid assays.** pNCX1IL or pLamin and puMiCK(225-378), psMiCK(226-380), pCKB(231-381), or pCKM(231-381) were co-transformed into yeasts by the lithium acetate method. (a) Five-fold serial dilutions of transformed yeasts were spotted on −KLUT or −KLUTH plate and incubate at 30°C for 3 days. (b) The ONPG assay was used to determine the β−galactosidase activity in the different yeast strains. The unit was defined as $(OD_{420}/OD_{600}/ml/min)\times10^3$.

Figure S4. **NCX activity in NCX1-overexpressing HEK293T cells.** HEK293T cells were co-transfected with pGFP and empty vector (A) or pNCX1 (B). Cells with GFP fluorescence were selected to measure the reverse mode NCX activity. NCX1 activity in individual cells was measured by puffing with a Na^+-free buffer for 30 s as indicated. (C) Average NCX activity in control and NCX1-overexpressing HEK293T cells. Data were mean ± S.E.M. values from three individual experiments (empty vector, n=20; NCX1, n=29 cells).

Fig. S1_Yang et al.

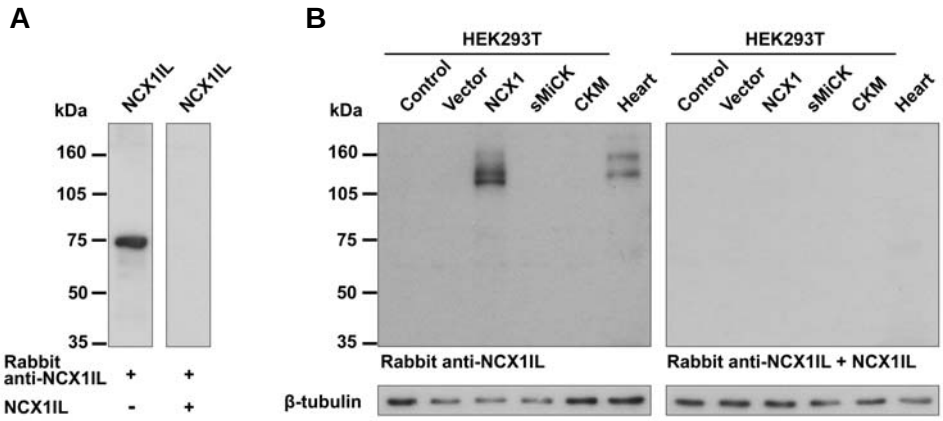

Fig. S2 _Yang et al.

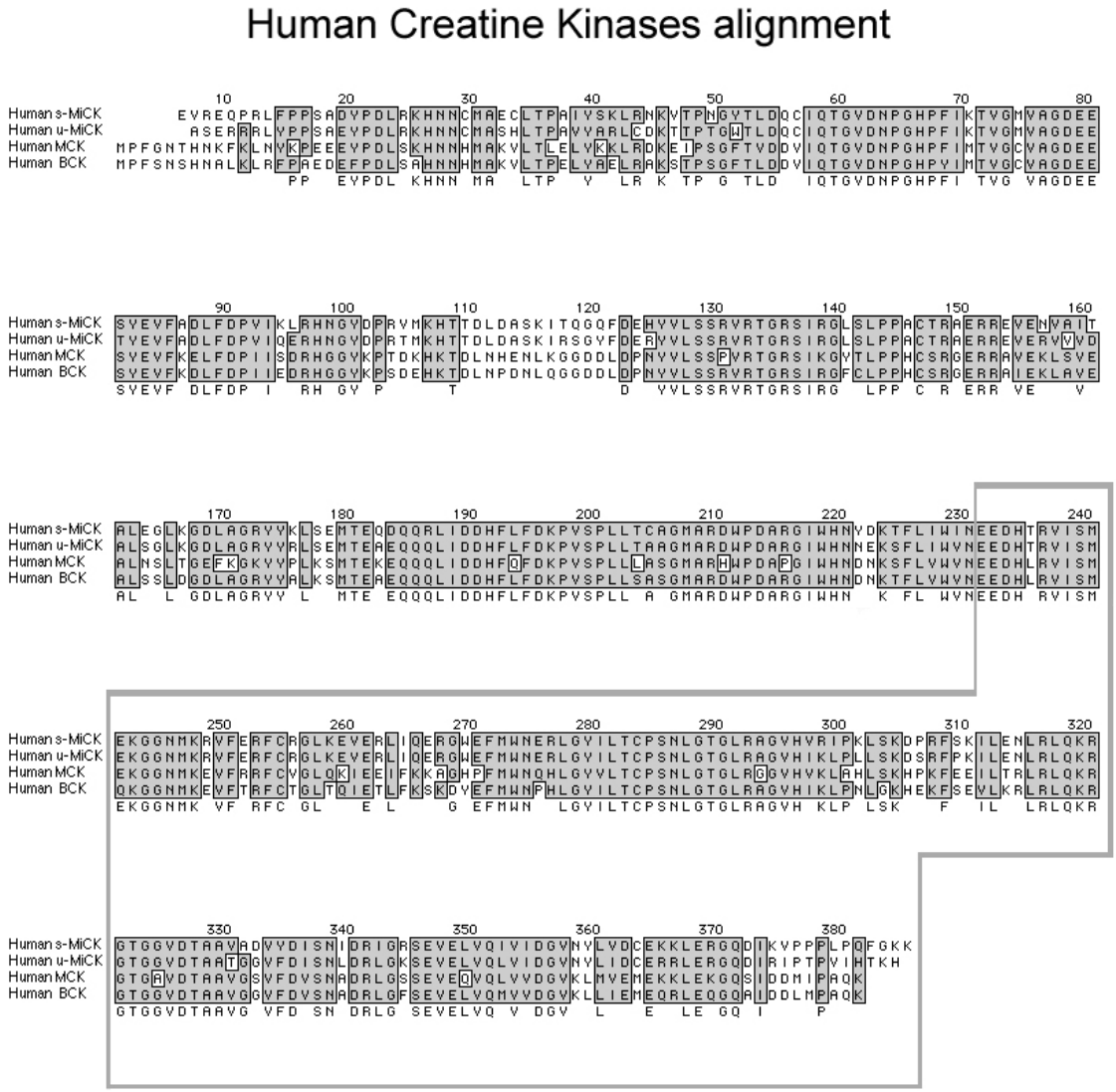

Fig. S3 _Yang et al.

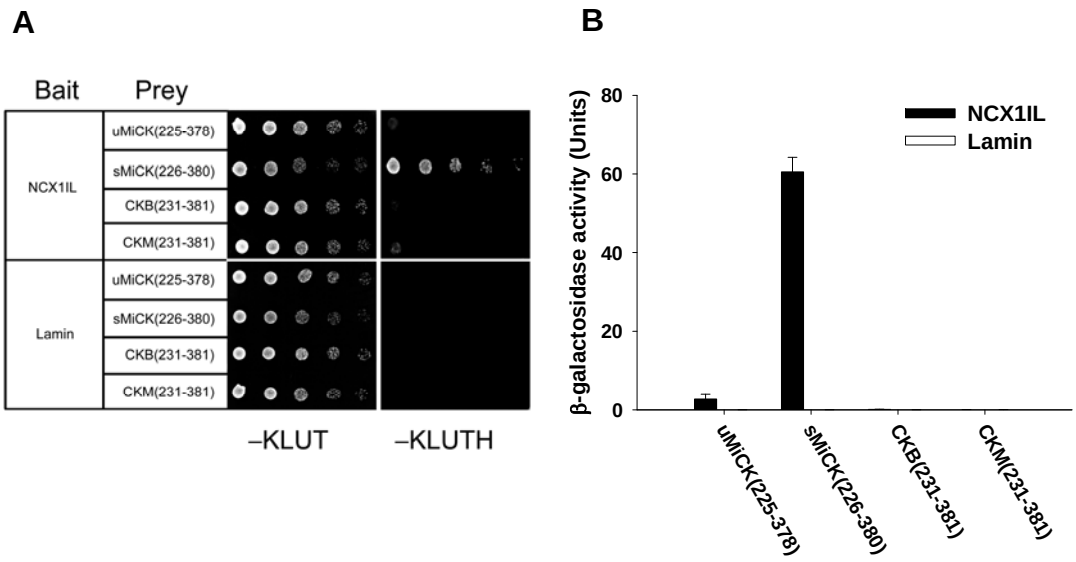

Fig. S4_Yang et al.