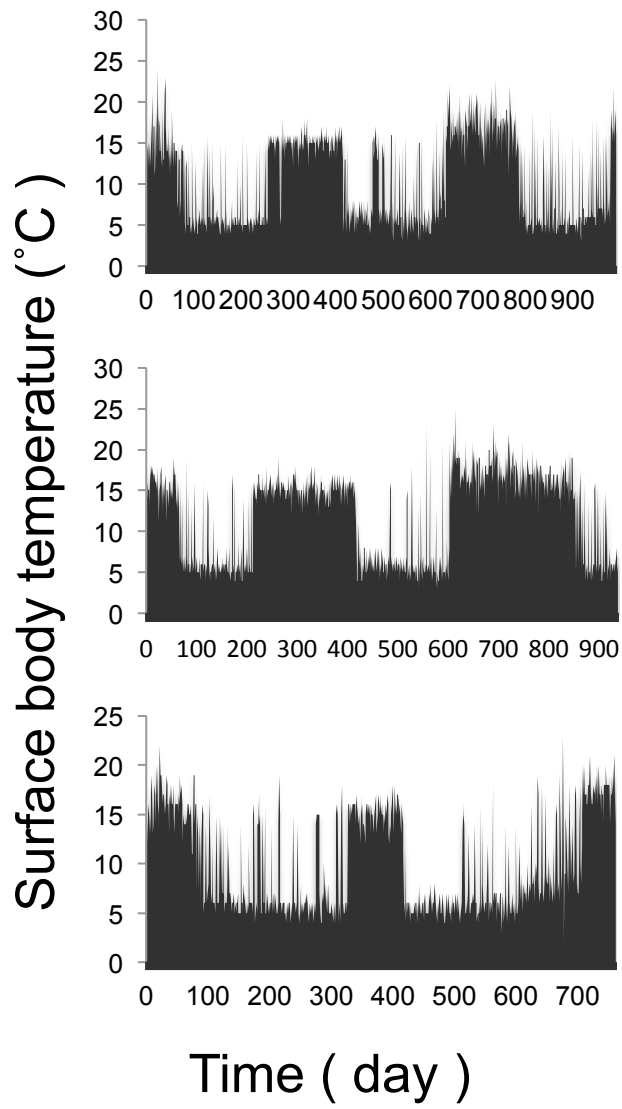


Supplemental Figures

AMPK activation, eEF2 inactivation, and reduced protein synthesis in the cerebral cortex of hibernating chipmunks

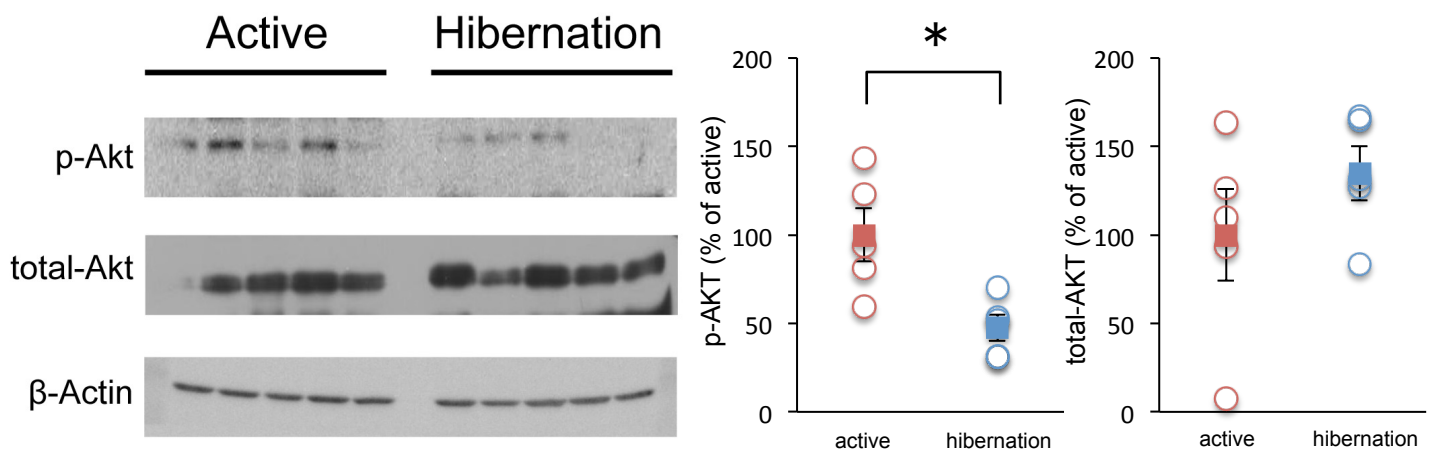
Shintaro Yamada, Taito Kamata, Hiroyuki Nawa, Tsuneo Sekijima
and Nobuyuki Takei



Supplemental Figure 1

(a)

(b)



Supplemental Figure. 2

	5	15	25	35	45	55
M.musc	IGHYILGDTL	GVGTFGKVKV	GKHELTGHKV	AVKILNRQKI	RSLDVVGKIR	REIQNLKLF
R.norv	IGHYILGDTL	GVGTFGKVKV	GKHELTGHKV	AVKILNRQKI	RSLDVVGKIR	REIQNLKLF
O.cuni	IGHYILGDTL	GVGTFGKVKV	GKHELTGHKV	AVKILNRQKI	RSLDVVGKIR	REIQNLKLF
H.sapi	IGHYILGDTL	GVGTFGKVKV	GKHELTGHKV	AVKILNRQKI	RSLDVVGKIR	REIQNLKLF
U.parr	IGHYILGDTL	GVGTFGKVKV	GKHELTGHKV	AVKILNRQKI	RSLDVVGKIR	REIQNLKLF
I.trid	-----	-----	-----	-----	-----	-----

	65	75	85	95	105	115
M.musc	HPHIKLYQV	ISTPSDIFMV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR
R.norv	HPHIKLYQV	ISTPSDIFMV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR
O.cuni	HPHIKLYQV	ISTPSDIFMV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR
H.sapi	HPHIKLYQV	ISTPSDIFMV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR
U.parr	HPHIKLYQV	ISTPSDIFMV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR
I.trid	-----	-----MV	MEYVSGGELF	DYICKNGR--	-----	---LDEKESR

	125	135	145	155	165	175
M.musc	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP
R.norv	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP
O.cuni	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP
H.sapi	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP
U.parr	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP
I.trid	RLFQQILSGV	DYCHRHMVVH	RDLKPENVLL	DAHMNAKIAD	FGLSNMMSDG	EFLRTSCGSP

	185	195	205	215	225	235
M.musc	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL
R.norv	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL
O.cuni	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL
H.sapi	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL
U.parr	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL
I.trid	NYAAPEVISG	RLYAGPEVDI	WSSGVILYAL	LCGTLPFDDD	HVPTLFKKIC	DGIFYTPQYL

	245	255	265	.		
M.musc	NPSVISLLKH	MLQVDPMKRA	AIKDIREHEW	F		
R.norv	NPSVISLLKH	MLQVDPMKRA	TIKDIREHEW	F		
O.cuni	NSSVISLLKH	MLQVDPMKRA	TIKDIREHEW	F		
H.sapi	NPSVISLLKH	MLQVDPMKRA	TIKDIREHEW	F		

Supplemental Figure 3

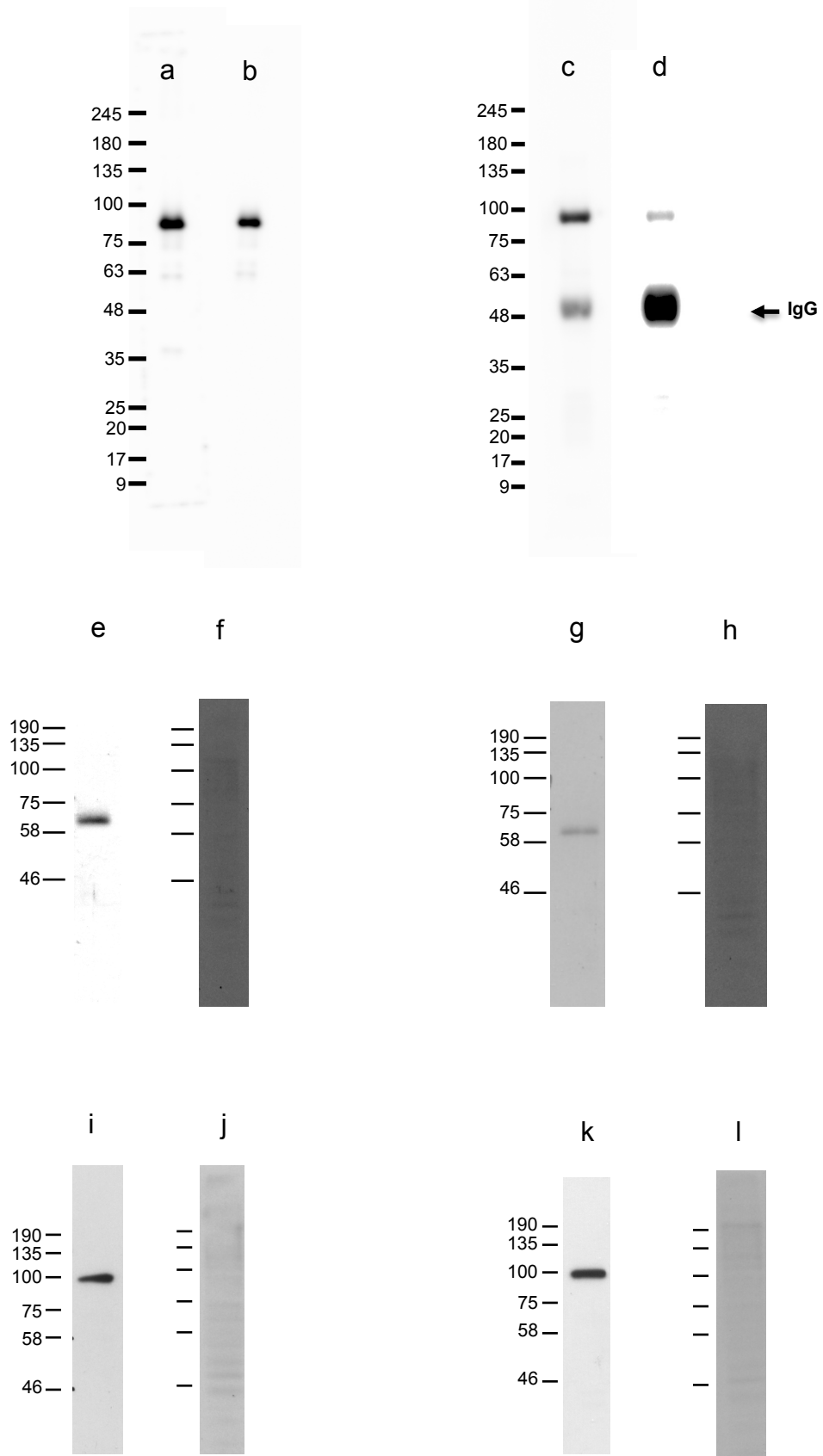
Legends to Supplemental Figures

Supplemental Figure 1. Examples of active-hibernation rhythms of surface body temperature of chipmunks. Note some of the individuals looks free-running of rhythm over a year.

Supplemental Figure 2. Phosphorylation of Akt in cerebral cortex of chipmunks during active and hibernation periods. Panel b shows quantitation of the blots by Image J. Each circle represents the each band. Squares represent mean \pm SE (n=5). * p <0.05 (Student t -test)

Supplemental Figure 3. Comparison of amino acid sequences of different species. M. musc: mouse, R. norv: rat, O. cuni: rabbit, H. sapi: human, U. parr: arctic ground squirrel, I. trid: thirteen-lined ground squirrel. Red "T" indicates phosphorylation amino acid residue.

Supplemental Figure 4. Recombinant antigens were prepared as described in materials& methods. Rat recombinant GST-AMPK α (a, b) and Flag-eEF2 (c, d) were applied to SDS-PAGE and transferred to the PVDF membrane. Western blotting was performed using anti-total AMPK α (a), anti-P-AMPK α (b), anti-total eEF2 (c) and anti-P-eEF2 (d). Purified antigens were used for absorption of each antibody. Antibody specificity was checked as follows. Same amount (40 μ g) of the lysates of newly sacrificed chipmunk brain were applied to Western blotting using anti-AMPK α (e, f), anti-P-AMPK α (g, h) , anti-total eEF2 (i, j) and anti-P-eEF2 (k,l) antibodies. f, h, j, l were blotted with antigen-absorbed antibodies and images were obtained in longer exposure time.



Supplemental Figure 4