Supporting Information

Tissue-specific kinase expression and activity regulates flux through the pyruvate dehydrogenase complex.

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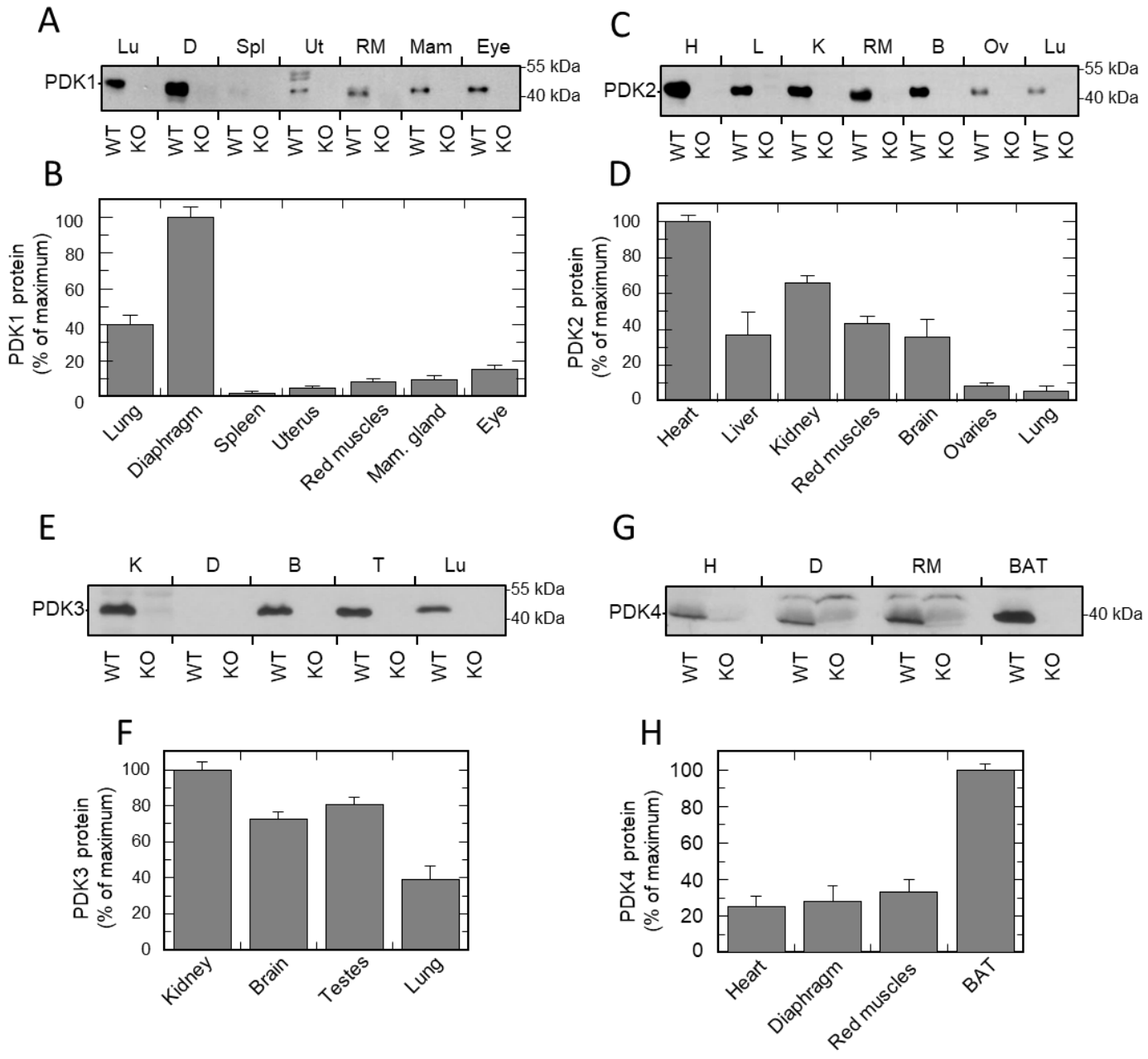

Figure S1. Protein levels of PDK isozymes in mouse tissues. Panels A, C, E, and G, show representative Western blots of PDK1, PDK2, PDK3, and PDK4 protein, respectively. Panels B, D, F, and H illustrate the relative abundance of PDK1, PDK2, PDK3, and PDK4 proteins in mouse tissues calculated based on the results of scanning densitometry. Data are expressed as percent of area of the strongest band. Western blotting was carried out as described under Experimental procedures. Each lane contains 35 µg of total whole-tissue protein. Data points represent means ± SD for three mice in each group. Abbreviations are: Lu – lung, D – diaphragm, Spl – spleen, Ut – uterus, RM – red skeletal muscle, Mam – mammary gland, H – heart, L – liver, K – kidney, B – brain, Ov – ovary, BAT –brawn adipose tissue.

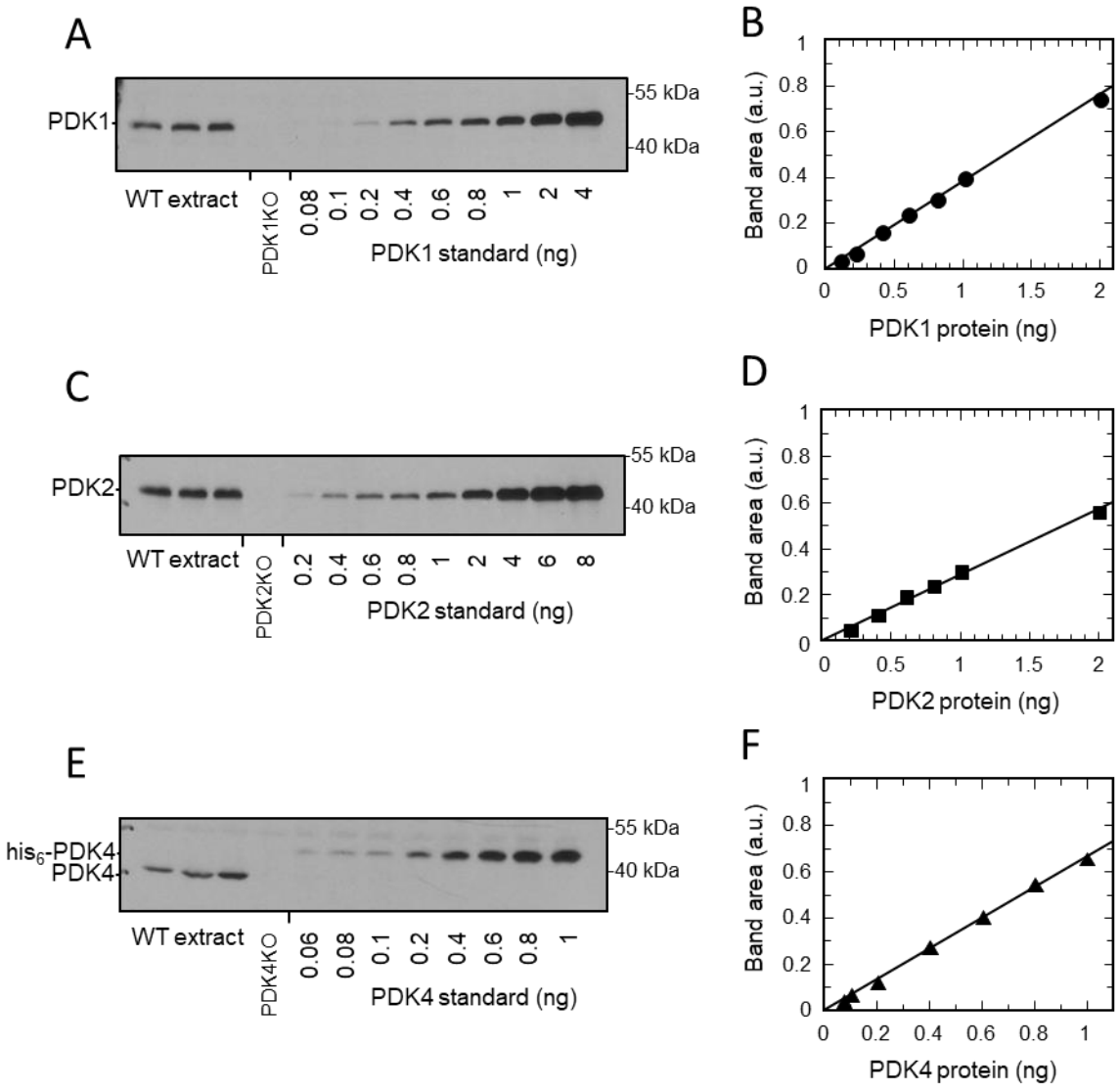

Figure S2. Quantitative Western blot analysis of PDK1, PDK2, and PDK4 in heart muscle. Panels A, C, and E - representative Western blots showing protein levels of PDK1, PDK2, and PDK4 in heart muscle from three animals along with the corresponding protein standards. Samples were prepared as described under Experimental procedures. For PDK1 and PDK2 staining, each lane contains 10 µg of total protein extracted from hearts of wild-type mice. The lanes with protein standards contain 10 µg of total protein extract from the appropriate PDK knockout mice. For PDK4, each lane contains 40 µg of total protein extracted from heart of wild-type mice. The lanes with protein standards contain 40 µg of total protein extract from PDK4 knockout mice. Panels B, D, and F – calibration curves for measuring protein levels of PDK1, PDK2, and PDK4 in heart muscle constructed based on the results of scanning densitometry of Western blots.

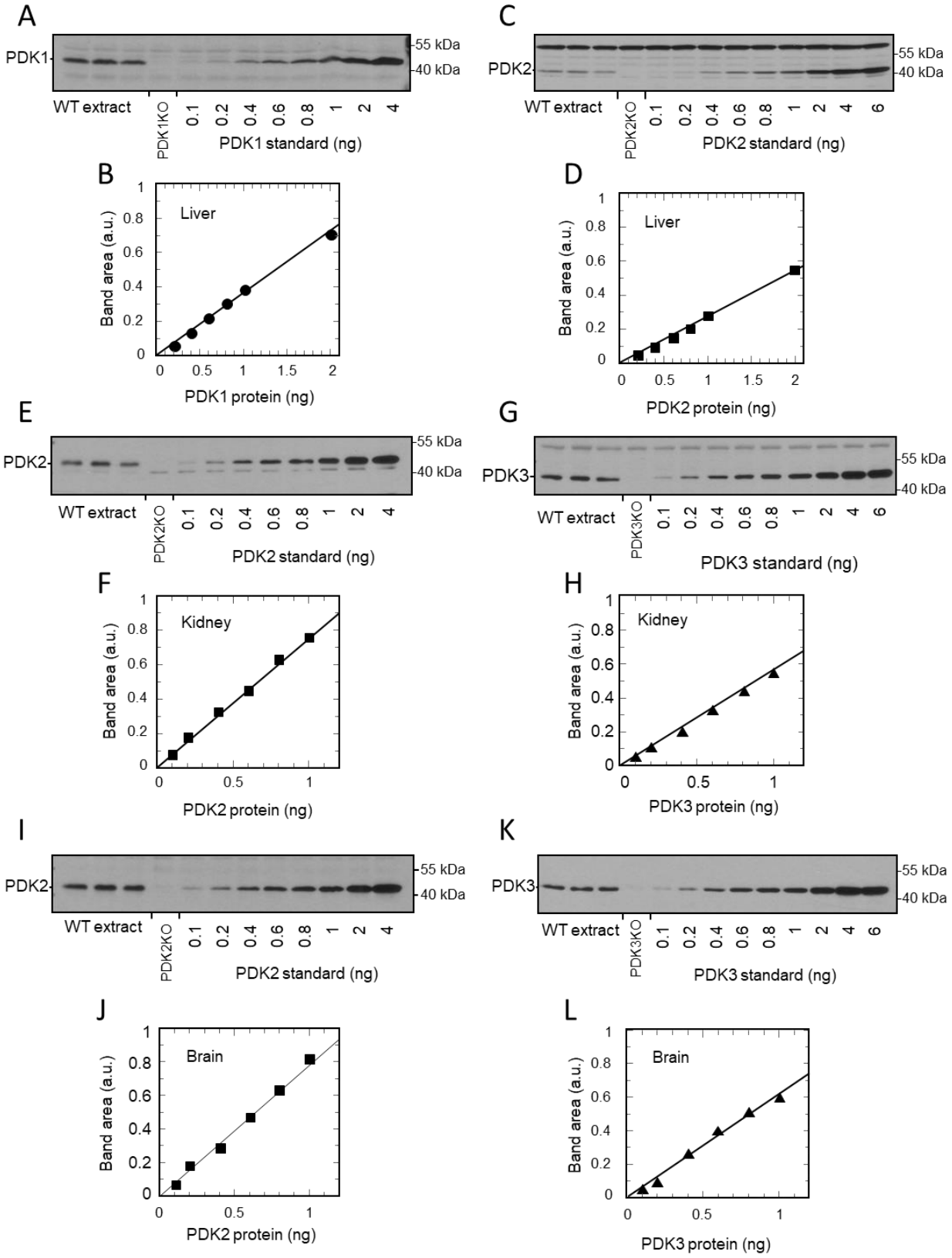
A
PDK1
55 kDa
40 kDa
WT extract
PDK1KO
0.1 0.2 0.4 0.6 0.8 1 2 4
PDK1 standard (ng)
B
Liver
Band area (a.u.)
PDK1 protein (ng)
C
PDK2
55 kDa
40 kDa
WT extract
PDK2KO
0.1 0.2 0.4 0.6 0.8 1 2 4 6
PDK2 standard (ng)
D
Liver
Band area (a.u.)
PDK2 protein (ng)
E
PDK2
55 kDa
40 kDa
WT extract
PDK2KO
0.1 0.2 0.4 0.6 0.8 1 2 4
PDK2 standard (ng)
F
Kidney
Band area (a.u.)
PDK2 protein (ng)
G
PDK3
55 kDa
40 kDa
WT extract
PDK3KO
0.1 0.2 0.4 0.6 0.8 1 2 4 6
PDK3 standard (ng)
H
Kidney
Band area (a.u.)
PDK3 protein (ng)
I
PDK2
55 kDa
40 kDa
WT extract
PDK2KO
0.1 0.2 0.4 0.6 0.8 1 2 4
PDK2 standard (ng)
J
Brain
Band area (a.u.)
PDK2 protein (ng)
K
PDK3
55 kDa
40 kDa
WT extract
PDK3KO
0.1 0.2 0.4 0.6 0.8 1 2 4 6
PDK3 standard (ng)
L
Brain
Band area (a.u.)
PDK3 protein (ng)

Figure S3. Quantitative Western blot analysis of PDK isozymes in liver, kidney, and brain. Panels A and C - representative Western blots showing protein levels of PDK1 and PDK2 in liver from three animals along with corresponding protein standards For PDK1 staining, each lane contains 60 µg of total protein extracted from heart of wild-type mice. The lanes with protein standards contain 60 µg of total protein extract from PDK1 knockout mice. For PDK2, each lane contains 30 µg of total protein extracted from heart of wild-type mice. The lanes with protein standards contain 30 µg of total protein extract from PDK2 knockout mice. Panels B and D - calibration curves for measuring protein levels of PDK1 and PDK2 in liver constructed based on the results of scanning densitometry. Panels E and G - representative Western blots depicting protein levels of PDK2 and PDK3 in kidney from three animals along with corresponding protein standards. Each lane contains 20 µg of total protein extracted from kidney of wild-type mice or, for lanes containing protein standards, from appropriate PDK knockout mice. Panels F and H - calibration curves for measuring protein levels of PDK2 and PDK3 in kidney constructed based on the results of scanning densitometry. Panels I and K - representative Western blots illustrating protein levels of PDK2 and PDK3 in brain from three animals along with corresponding protein standards. Each lane contains 20 µg of total protein extracted from brain of wild-type mice or, for lanes containing protein standards, from appropriate PDK knockout mice. Panels J and L - calibration curves for measuring protein levels of PDK2 and PDK3 in brain constructed based on the results of scanning densitometry.

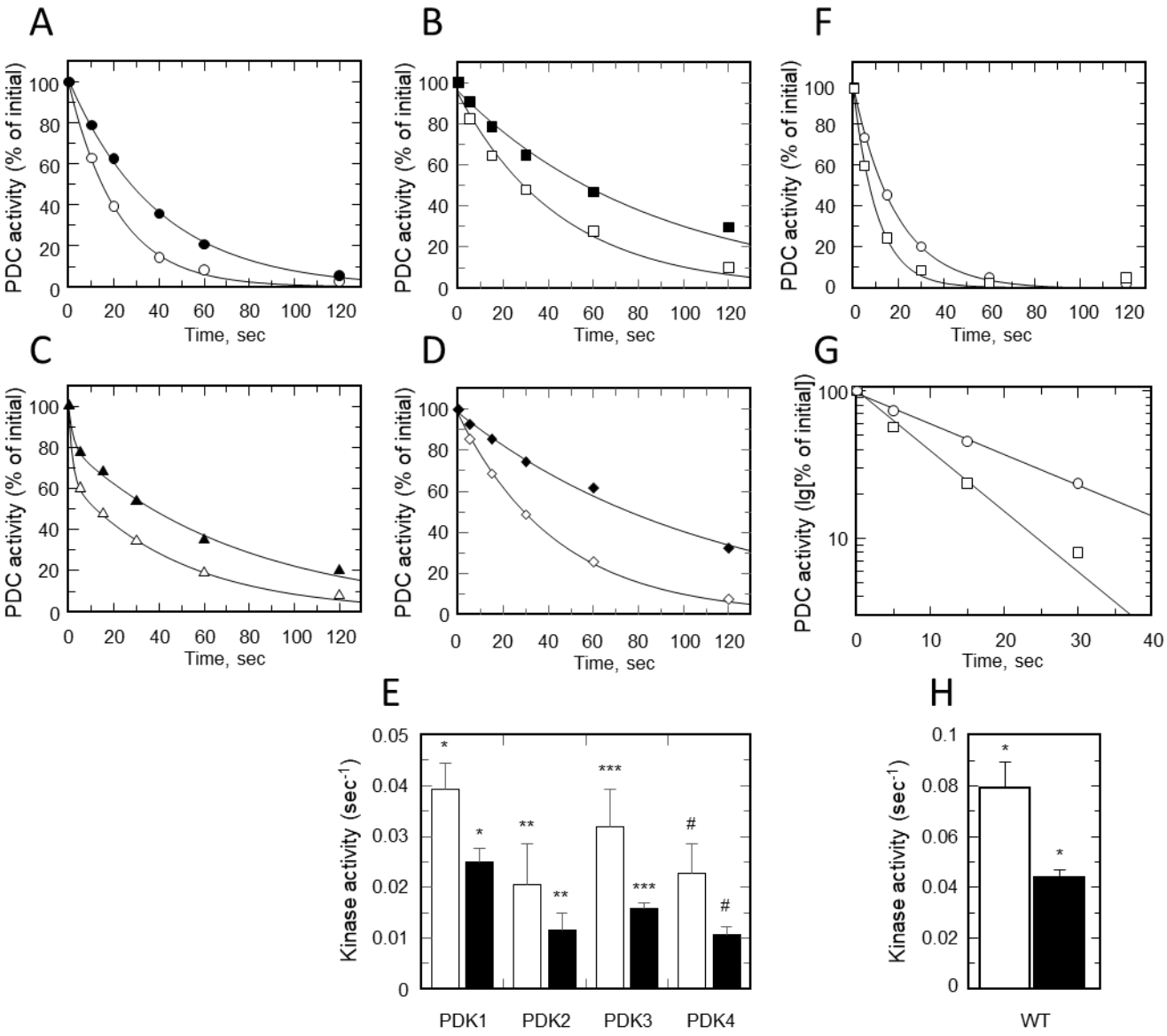

Figure S4. Kinase activity measurements utilizing ATP or ATPγS as nucleotide substrate. Panels A, B, C, and D - representative ATP- and ATPγS-dependent inactivation curves (open and closed symbols, respectively) of highly purified, recombinant PDC catalyzed by highly purified, recombinant PDK1, PDK2, PDK3, and PDK4, respectively. Experiments were carried out as described under Experimental procedures. Each phosphorylation reaction received highly purified recombinant PDC at the final protein concentration of 0.5 mg/ml and appropriate isozyme of kinase at the final protein concentration of 3 μg/ml. Inactivation reactions were initiated by the addition of ATP or ATPγS at saturating concentration of 0.4 mM. Panel E – kinase activities of recombinant PDK isozymes measured with ATP (open bars) or ATPγS (closed bars). Activities are expressed in terms of pseudo-first order rate inactivation constants

(sec^{-1}). Data points represent means ± SD for three independent experiments. $P<0.05$ between groups is considered statistically significant. Groups showing statistically significant differences are indicated by *, **, ***, and # symbols, respectively. Panel F – representative ATP- and ATPγS-dependent inactivation curves of partially purified PDC from heart (open squares or circles, respectively). Panel G - representative ATP- and ATPγS-dependent inactivation curves from Panel F re-plotted in semi logarithmic coordinates. Panel H - kinase activity in preparation of partially purified PDC obtained from heart measured with ATP or ATPγS as nucleotide substrate (open or closed bars, respectively). Data points represent means ± SD for three independent experiments. $P<0.05$ between groups is considered statistically significant. Groups showing statistically significant differences are indicated by * symbol.

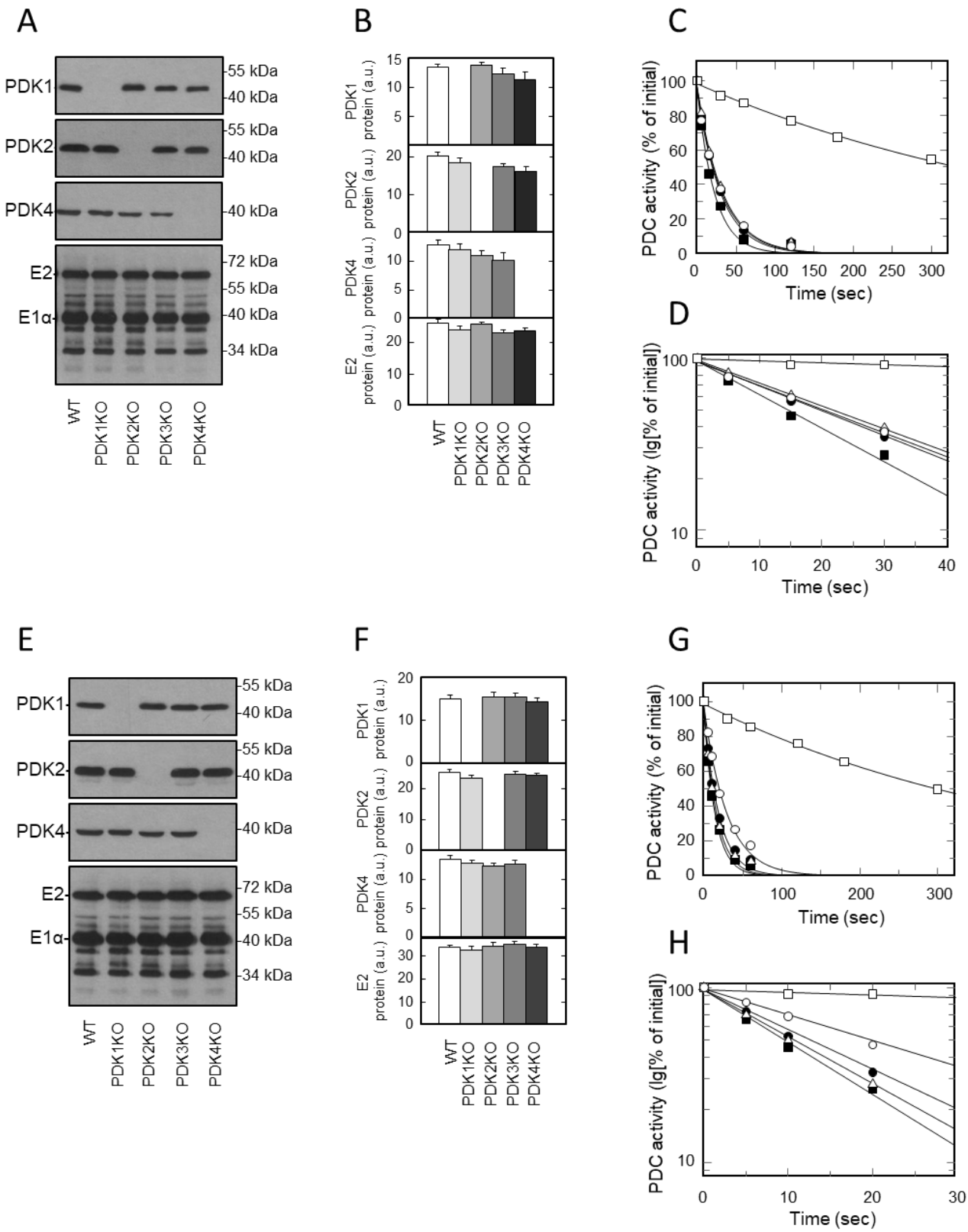
A
PDK1
PDK2
PDK4
E2
E1α
55 kDa
40 kDa
72 kDa
34 kDa
WT
PDK1KO
PDK2KO
PDK3KO
PDK4KO
B
PDK1 protein (a.u.)
PDK2 protein (a.u.)
PDK4 protein (a.u.)
E2 protein (a.u.)
C
PDC activity (% of initial)
Time (sec)
D
PDC activity (lg[% of initial])
Time (sec)
E
F
G
H

Figure S5. Isozymic composition of PDK and regulation of activity of PDC isolated from diaphragm and skeletal muscle. Panels A and E – representative Western blots illustrating protein levels of PDK1, PDK2, PDK4, as well as E2 and E1α proteins of PDC in skeletal muscle and diaphragm, respectively. Panels B and F – relative abundance of PDK1, PDK2, PDK4 and E2 proteins in skeletal muscle and diaphragm, respectively, based on the results of scanning densitometry (data points represent means ± SD for three mice in each group). White, light grey, grey, dark grey and black bars represent samples obtained from wild-type, PDK1 knockout, PDK2 knockout, PDK3 knockout, and PDK4 knockout mice, respectively. Isolation of PDC and Western blotting were carried out as described under Experimental procedures. Each lane contains 15 or 6 μg of partially purified PDC protein for kinase or PDC staining, respectively. Panels C and G – representative ATPγS-dependent inactivation curves of partially purified PDC from skeletal muscle and diaphragm of wild-type (dark squares), PDK1 knockout (dark circles), PDK2 knockout (open squares), PDK3 knockout (open triangles), and PDK4 knockout mice (open circles), respectively. ATP-dependent inactivation assay was carried out as described under Experimental procedures. Panels D and H - representative ATPγS -dependent inactivation curves from Panels C and G re-plotted in semi logarithmic coordinates.

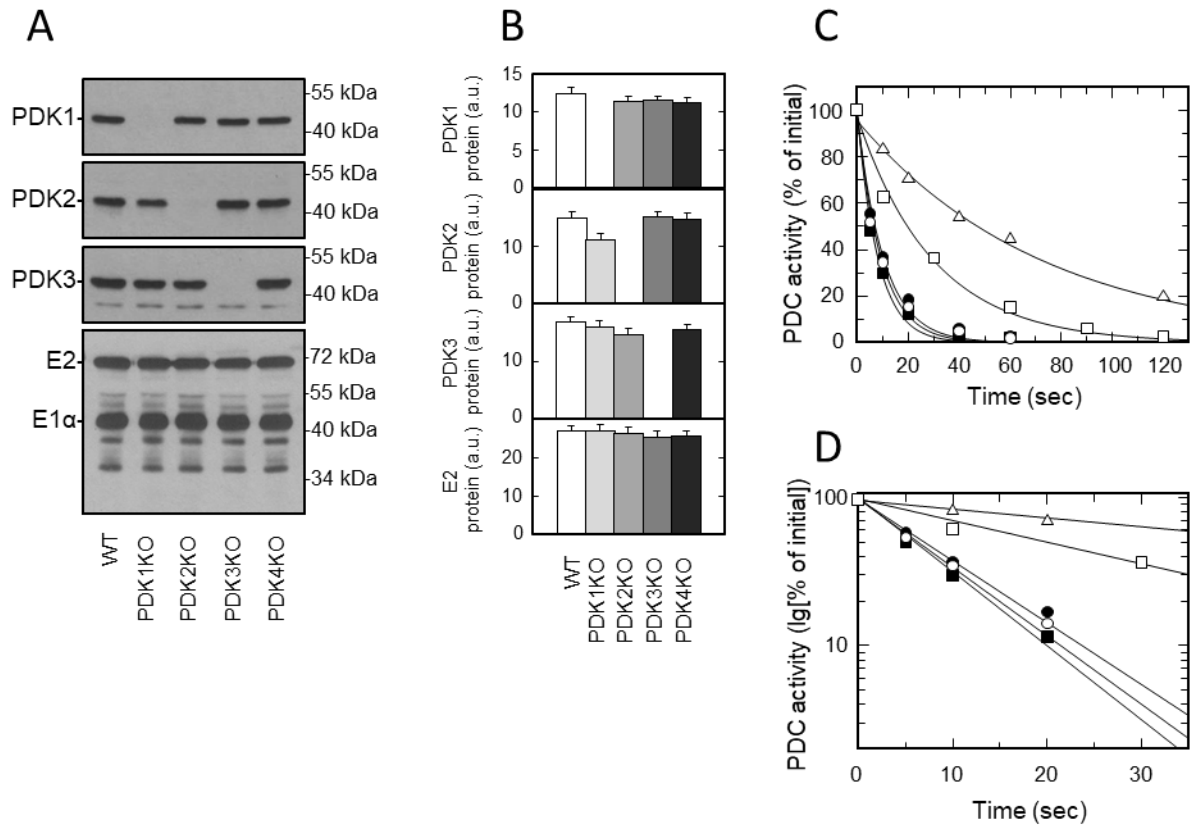

Figure S6. Isozymic composition of PDK and regulation of activity of PDC isolated from brain. Panel A – representative Western blots illustrating protein levels of PDK1, PDK2, PDK3, as well as E2 and E1α proteins of PDC in brain. Panel B – relative abundance of PDK1, PDK2, PDK3, and E2 proteins based on the results of scanning densitometry (data points represent means ± SD for three mice in each group). White, light grey, grey, dark grey and black bars represent samples obtained from wild-type, PDK1 knockout, PDK2 knockout, PDK3 knockout, PDK4 knockout mice, respectively. Each lane contains 15 or 6 μg of partially purified PDC protein for kinase or PDC staining, respectively. Panel C – representative ATP-dependent inactivation curves of partially purified PDC from brain of wild-type (dark squares), PDK1 knockout (dark circles), PDK2 knockout (open squares), PDK3 knockout (open triangles), and PDK4 knockout mice (open circles), respectively. Panel D - representative ATP-dependent inactivation curves from Panel C re-plotted in semi logarithmic coordinates.