Supplemental Methods:

Cell cultures, plasmids and adenoviral vectors

HEK293T, COS7 and primary E12.5 mouse embryonic fibrolasts were maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100U/mL penicillin, and 100μg/ml streptomycin. HEK293T Flp-IN T-REX cell (Invitorgen) was maintained in same culture medium for HEK293T plus 10μg/ml blasticidine and 200μg/ml Zeocin. Pancreatic β-cell lines INS-1Flp-In T-REX, a kind gift from Dr. Gerhart U Ryffel **(**Institut für Zellbiologie, Universitätsklinikum Essen), were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 10 mM HEPES, 2 mM L-glutamine and 50 μM β-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin, 10μg/ml blasticidine and 200μg/ml Zeocin. INS-1 and HEK293T stable cell lines were generated according to manufacture's instruction and maintained in respective culturing mediums supplemented with 10 μg/ml blasticidine and 50 μg/ml hygromycin. Neonatal rat ventricular cardiomyocytes were essentially isolated and cultured as previously described (1). Plasmid and adenoviral vectors of *PP2Cm*, *PP2Cm-H129A*, *PP2Cm-R236G*, *PP2Cm-D298A* and *PP2Cm-MD* (PP2Cm truncated mutant with deletion of N-terminal mitochondrial targeting signal (residues 1-30))were generated as previously described (2). Coding sequences of human E1α, E1β, E2 and E3 were sub-cloned from EST clones: BC023983, BC040139, BC016675, BC018696, respectively. Briefly, the coding sequences of individual BCKD subunits were PCR amplified using the following primers, digested with NheI and XhoI and then cloned into pET28a vector (Novagen).

E1α NheI For 5' ATATATGCTAGCCAGCAGCAGCAGTTTTCATC 3',

E1α XhoI Rev 5’ AGCACGCTCGAGGTCTCACTTATCGAAGTGAT 3’;

E1β NheI For 5’ ATATATGCTAGCTTTTTGCACCCCGCCGCGAC 3’,

E1β XhoI Rev 5’ AGCACGCTCGAGTGGTCAATAGTTGATCATTT 3’;

E2 NheI For 5’ ATATATGCTAGCCAGGTTGTTCAGTTCAAGCT 3’,

E2 XhoI Rev 5’ AGCACGCTCGAGTCTTCATTTCAGATCTAGTA 3’;

E3 NheI For 5’ ATATATGCTAGCGCAGATCAGCCGATTGATGC 3’,

E3 XhoI Rev 5’ AGCACGCTCGAGAATTCAAAAGTTGATTGATT 3’.

E1α-S293A, E1α-S303A as well as E1α-S293A/S303A mutants were generated via overlapping PCR using the following primers and confirmed by sequencing:

E1A-S293A For 5’CAGGATCGGGCACCACGCCACCAGTGACGACAGT 3’

E1A-S293A Rev 5’ ACTGTCGTCACTGGTGGCGTGGTGCCCGATCCTG 3’

E1A-S303A For 5’CAGTTCAGCGTACCGCGCGGTGGATGAGGTCAA 3’

E1A-S303A Rev 5’ TTGACCTCATCCACCGCGCGGTACGCTGAACTG 3’

Immunoblotting and immunoprecipitation

Cells were washed twice with ice cold PBS, and harvested in Buffer A (50mM HEPES [pH7.4], 150mM NaCl, 1% NP-40, 1mM EDTA, 1mM EGTA, 1mM glycerophosphate, 2.5mM sodium pyrophosphate 1mM Na3VO4, 20mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL of aprotinin, leupeptin, and pepstatin). Total cell lysates were separated on 4-12% Bis-Tris gels (Invitrogen), transferred onto nitrocellulose blot (Amersham). The blot was probed with the indicated primary antibodies. Protein signals were detected using HRP conjugated secondary antibodies and enhanced chemiluminescence (ECL) western blotting detection regents (Pierce) or IRDye™ 800 conjugated secondary

antibodies and Odyssey infrared imaging system (LI-COR). Rabbit polyclonal antisera against the E1 and E2 subunits of BCKD complex is a kind gift from Dr. Yoshiharu Shimomura (Nagoya Institute of Technology). For anti-FLAG immunoprecipitation assay, total cell lysates were incubated with anti-FLAG M2 bead (sigma) for 2 hours at 4^{o}C and wished with Buffer A for 6 times, followed by elution with FLAG peptide (200ug/mL). For anti-E1a immunoprecipitation, rabbit anti-E1α polyclonal antibody was crosslinked with nProtein A Sepharose beads (Amersham) using dimethyl Pimelimidate (Sigma). Then, total cell lysates precleared with nProtein A Sepharose beads were incubated with the anti-E1α beads for 6 hours at 4^{o}C and washed with Buffer A for six times, followed by elution with low PH buffer (0.1M glycine, PH2.5).

GST pull down assay

GST-tagged and HIS-tagged recombinant proteins were expressed in E.coli BL21(DE3) and purified with glutathione Sepharose 4B beads (Amersham) and TALON resins (Clontech), respectively . 1μg purified GST-PP2Cm recombinant protein was incubated with 0.5μg HIS-tagged recombinant proteins in 500μL Buffer B [50 mM Tris-HCl (pH 7.4), 150mM NaCL, 0.5% NP-40, 1 mM EDTA, 1mM DTT, 5mM $MgCl_2$, 1mM phenylmethylsulfonyl fluoride, 1 μg/mL of aprotinin, leupeptin, and pepstatin] at 4°C for 2 hours. The GST-PP2Cm and its interacting proteins were captured by using glutathione beads. After washed with Buffer B for 6 times, glutathione beads were boiled in SDS loading buffer, followed by SDS-PAGE analysis.

Electron spin resonance detection of superoxide production in MEFs:

Gently collected cells were suspended in modified Kreb's/HEPEs buffer. The specific superoxide ($O_2^{\bullet-}$) spin trap methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH, 1mmol/L, Alexis) solution was prepared freshly in nitrogen gas bubbled Krebs/HEPEs buffer containing diethyldithiocarbamic acid (DETC, 5 μmol/L Sigma) and deferoxamine (25 μmol/L, Sigma). Approximately $1x10^5$ cells were then mixed with spin trap solution in the presence or absence of SOD (100 U/ml, manganese containing). The cell mixture loaded in glass capillaries was immediately analyzed for $O_2^{\bullet-}$ production kinetically for 10 min by using e-Scan electron spin resonance (ESR) spectrophotometer (Bruker Biospin, Germany). The ESR settings used were static-field, 3484 sweep width, 9.00 G (1 G = 0.1 mT); microwave frequency, 9.748660 GHz; microwave power 21.02 mW; modulation amplitude, 2470 mG; resolution in X, 512, number of X-scan, 10; and receiver gain, 1000. . The SOD-inhibitable $O_2^{\bullet-}$ signals at 10 min time point, normalized by protein concentrations, were compared among different experimental groups.

Electron spin resonance measurement of superoxide production in tissues

Freshly isolated aortas were placed into chilled modified Krebs/HEPES buffer (composition in mmol/l: 99.01 NaCl, 4.69 KCl, 2.50 $CaCl_2$, 1.20 $MgSO_4$, 1.03 KH_2PO_4, 25.0 $NaHCO_3$, 20.0 Na-HEPES, and 5.6 glucose [pH 7.4]), cleaned of excessive adventitial tissue, with care taken not to injure the endothelium. The homogenates from other tissues were prepared by homogenizing with pestle (50 strokes) in fresh homogenization buffer (50 mmol/L of Tris-HCl, [pH 7.4] 0.1 mmol/L of EDTA, 0.1 mmol/L of EGTA) containing protease inhibitor cocktail and centrifuged at 800 g for 10 min. After centrifugation, supernatants were collected and then subjected for protein

assay. The specific superoxide ($O_2^{\bullet-}$) spin trap methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH, 1mmol/L, Alexis) solution was prepared freshly in nitrogen gas bubbled Krebs/HEPEs buffer containing diethyldithiocarbamic acid (DETC, 5 μmol/L Sigma) and deferoxamine (25 μmol/L, Sigma). Aortic segment (~3 mm) or homogenates (15 μg protein) was then mixed with the spin trap solution in presence or absence of 100 units of SOD (manganese containing enzyme, Sigma) and loaded into glass capillary (Fisher Scientific). For analysis of $O_2^{\bullet-}$ signal ($CM^{\bullet}$ formed after trapping $O_2^{\bullet-}$), capillary was immediately loaded in e-Scan electron spin resonance (ESR) spectrophotometer (Bruker Biospin, Germany). The ESR settings used were static-field, 3484 sweep width, 9.00 G (1 G = 0.1 mT); microwave frequency, 9.748660 GHz; microwave power 21.02 mW; modulation amplitude, 2470 mG; resolution in X, 512, number of X-scan, 10; and receiver gain, 1000.

DCF-DA staining

The immortalized MEF Cells were grown in 10% FBS DMEM for 24 hour to near confluence in glass-bottom plates coated with poly-d-lysine (MatTek). Cells were treated with or without BCKAs (5mM each) together with 10 μM DCF-DA for 30 min at 37°C in dark. Cells were then washed with Dulbecco's phosphate buffer to remove excess CM-H_2DCF-DA. The fluorescent images were visualized by a Nikon Eclipse TE2000U microscope equipped with a FITC filter, and then captured using a CCD digital camera controlled by SPOT program. The imaging parameters were kept constant for each plate.

RT-PCR primers:

PP2Cm Exon 1:
For 5' GGAAATACACTCCTCCGGG 3'
Rev 5' CTTGTGATGGGTAGGTGTCTGTAA 3'

PP2Cm Exon 2:
For 5' TTAAGCTCCATCCTCCTGCA 3'
Rev 5' CCCACGTTCTCCAGGCTAAT 3'

PP2Cm Exon 3:
For 5' GCTTCCTCGGGAGAAAGACT 3'
Rev 5'AGACAGGTGGGCATAACTCG 3'

PP2Cm Exon Exons 6&7:

For 5' GACGACAGTTTCCTGGTCCT 3'
Rev 5' GAGGCAAAGCTTCTGCTGA 3'

BCKDK:
For 5' TGGCGCTACATGAAGACAAG 3'
Rev 5' TTATGAGCGATTCCTCCACC 3'

BCKA mixture:

BCKA mixture was prepared with the following reagents purchased from Sigma: ketovaline sodium salt: Fluka (68253) 97% Ketoleucine sodium salt: Aldrich (499137) 98% Ketoisoleucine sodium salt: Sigma (k7125).

Supplemental Figure Legends:

Supplemental Figure 1. Identification of PP2Cm interacting partners by mass spectrometry. INS-1 stable cell line expressing PP2Cm tagged with both FLAG and HA at its C-terminus was sequentially purified with anti-FLAG and anti-HA column. The HA peptide elute was resolved on 1D SDS-PAGE gel, following by silver staining. To identify interacting partners of PP2Cm, bands were excised from the PAGE gel and digested with trypsin. The digested proteins were analyzed by reverse phase liquid chromatography and tandem mass spectrometry on a Thermo Orbitrap in data-dependent mode. Representative spectra used to identify PP2Cm (**A**) and three interacting proteins, BCKDHA (E1α) (**B**), BCKDHB (E1β) (**C**), and Dbt (E2) (**D**) are shown; b- (red) and y- (blue) ions used to match the sequences are labeled in the individual spectra. Bottom panels indicate the total coverage of each region of the protein in red (based on tandem MS data); the underlined peptides shown were identified on the basis of the spectra in the top panels.

Supplemental Figure 2. Genetic targeting of PP2Cm allele in mouse ES cells and intact animals. A). Southern blot analysis of Hind III digested genomic DNA of different recombinant embryonic stem (ES) cell clones identified. Clones No. 17 and 66 were used to establish the founder chimera. **B)** Southern blot analysis of Hind III digested tail genomic DNA of *PP2Cm*+/+, +/- and -/- mice.

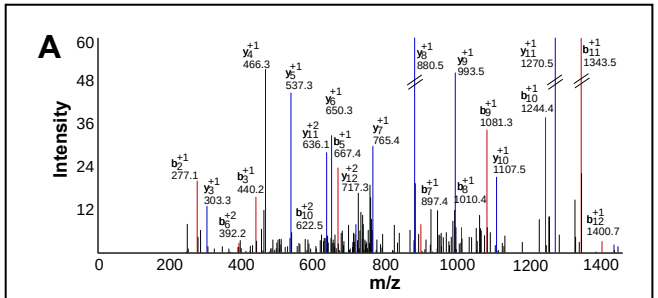
Supplemental Figure 3. *LacZ* expression in *PP2Cm -/-* adult mouse brain, heart, liver and diaphragm. Whole mount staining of β-galactosidase activity in adult mouse tissues as indicated and recorded under stereo dissection microscope.

Supplemental Figure 4. Superoxide induction in PP2Cm tissues. Electron spin resonance measurement of superoxide production in aorta (A) and lung (B) from random fed animals. *, $p<0.05$, Student's *t*-test.

Supplemental Figure 5. Human SNPs identified in human PP2Cm (PPM1k) coding region. SNP 1-5 are identified from Pubmed and labeled on aligned human, mouse and zebrafish amino acid sequences, the changes in amino acid sequence and the reference numbers for each SNP are listed below.

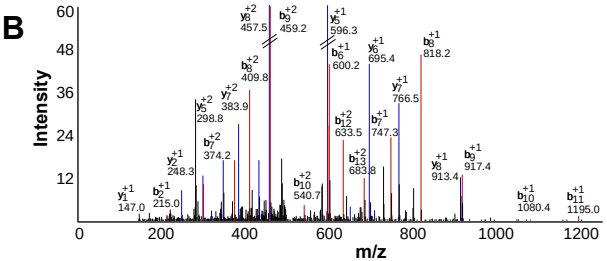
Supplemental Reference:

1. Lu, G., Kang, Y.J., Han, J., Herschman, H.R., Stefani, E., and Wang, Y. 2006. TAB-1 modulates intracellular localization of p38 MAP kinase and downstream signaling. *J Biol Chem* 281:6087-6095.
2. Lu, G., Ren, S., Korge, P., Choi, J., Dong, Y., Weiss, J., Koehler, C., Chen, J.N., and Wang, Y. 2007. A novel mitochondrial matrix serine/threonine protein phosphatase regulates the mitochondria permeability transition pore and is essential for cellular survival and development. *Genes Dev* 21:784-796.



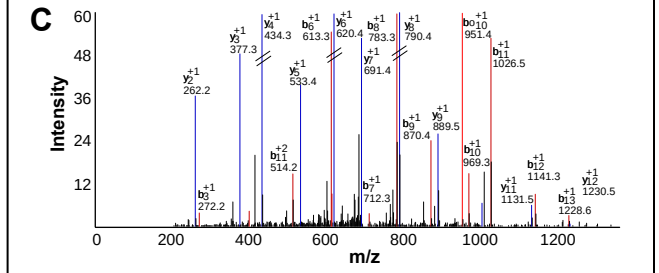
PP2Cm; Accession Number – IPI00189308.2
24% Sequence Coverage

MLSTAFITLVRSGRNQVKKRVLLSSILLQDHRQMTPACYCSISEPRCSRF
DPDGSGQPATWDNFGIWDNR**IDEPILLPPSIK**YGKPIPK**ISLENVGCASL**
IGKRKENEDRFGFAQLTEEVLYFAVYDGHGGPAAADFCHTHMEKCVTD
LLPR**EKDLETVLTLAFLEIDK**AFSSYAHLSADASLLTSGTTATVALLR**DG**
VELVVASVGDSRALLCRKGKPMKLTTDHTPERKDEKERIKKCGGFVAW
NSLGQPHVNGRLAMTR**SIGDLDLKASGVIAEPETTR**IKLYHADDSFLVLT
TDGINFMVNSQEICDFVNQCHDPKEAAHAVTEQAIQYGTEDNSTAVVVP
FGAWGKYK**NSEITFSFSR**SFASSGRWA



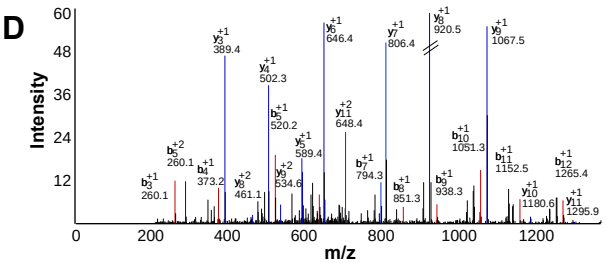
BCKDHA(E1a); Accession Number – IPI00365663.1
20% Sequence Coverage

MAVAMSAAKIWRPSRGLRQAALLLLGRPGARGLARFHPSRQQQQQFP
SLDDKPQFPGASAEFVDKLEFIQPNVISGIPIYRVMDR**QGQIINPSEDPH**
LPQEEVLKLYRSMTLLNTMDRILYESQRQGRISFYMTNYGEEGTHVGS
AAALER**TDLVFGQYR**EAGVLMYRDYPLELFMAQCYGNVSDPGKGRQM
PVHYGCKER**HFVTISSPLATQIPQAVGAAYAAK**RANANQIVICYFGEGA
ASEGDAHAGFNFAATLECPIIFFCR**NNGYAISTPTSEQYR**GDGIAARGP
GYGIMSIR**VDGNDVFAVYNATK**EARRRAVAENQPFLIEAMTYRIGHHST
SDDSSAYRSVDEVNYWDKQDHPISRLRQYLLNQGWWDEEQEKAWRK
QSRKKVMEAFEQAERKLKPNPSLLFSDVYQEMPAQLR**RQQESLAR**HL
QTYGEHYPLDHFDK



BCKDHB(E1b); Accession Number – IPI00201636.3
14% sequence coverage

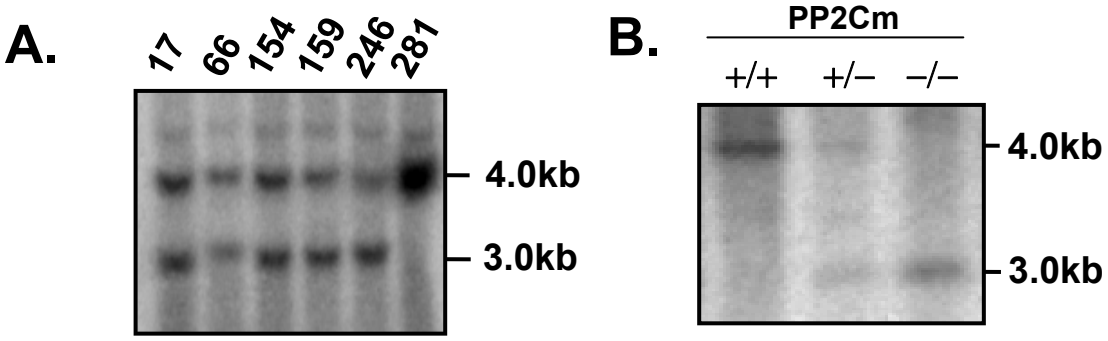
MAAVAARAGGLLRLGAAGAERRRRGLRCAALVQGFLQPAVDDASQKR
RVAHFTFQPDPESLQYGQTQKMNLFQSITSALDNSLAKDPTAVIFGEDV
AFGGVFRCTVGLRDKYGKDRVFNTPLCEQGIVGFGIGIAVTGATAIAEIQ
FADYIFPAFDQIVNEAAKYRYR**SGDLFNCGSLTIR**APWGCVGHGALYHS
QSPEAFFAHCPGIKVVIPRSPFQAK**GLLLSCIEDKNPCIFFEPK**ILYR**AAV**
EQVPVEPYKIPLSQAEVIQEGSDVTLVAWGTQVHVIREVASMAQEK**LGV**
SCEVIDLRTIVPWDVDTVCKSVIKTGRLLISHEAPLTGGFASEISSTVQEE
CFLNLEAPISRVCGYDTPFPHIFEPFYIPDKWKCYDALRKMINY



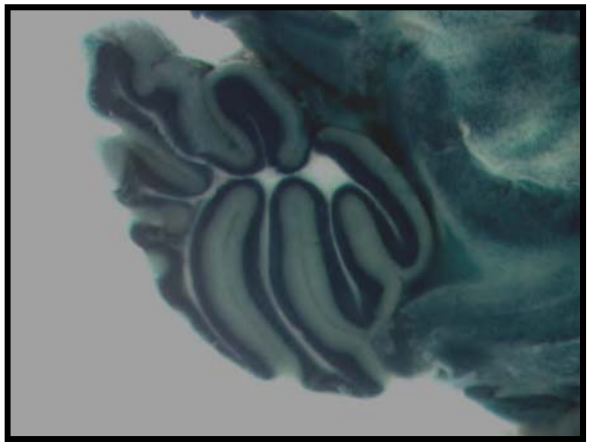
Dbt (E2); Accession Number – IPI00373418.3
36% Sequence Coverage

MAAARVLGTWSRNAVRLTCVRYFQTYNNVHILKPQHVCSVGYPLLKSS
QPRHSLRTAAVLQGQVVQFK**LSDIGEGIR**EVTIKEWYVKEGDTVSQFDS
ICEVQSDKASVTITSRYDGVIKR**LYYNLDDIAYVGKPLIDIETEALK**DSEE
DVVETPAVAHDEHTHQEIKGQKTLATPAVRRLAMENNIK**LSEVVGSGK**D
GR**ILKEDILNFLEK**QTGAILPPSPKSEITPPPPQPRDRPFPTPVSKPPVFL
GK**DRTEPVTGFQK**AMVKTMSAALK**IPHFGYCDEVDLTELVKLREELKP**
VALARGIKLSFMPFFLK**AASLGLLQFPILNASVDENCQSITYK**ASHNIGIA
MDTER**GLIVPNVK**NVQVRSVFEIAMELNRLQK**LGSLGQLSTTDLTGGT**
FTLSNIGSIGGTYAKPVILPPEVAIGALGAIKALPRFDQKGDVYKAQIMN
VSWSADHRVIDGATMSRFSNLWKSYLENPAFMLLDLK

Supplemental Figure 1

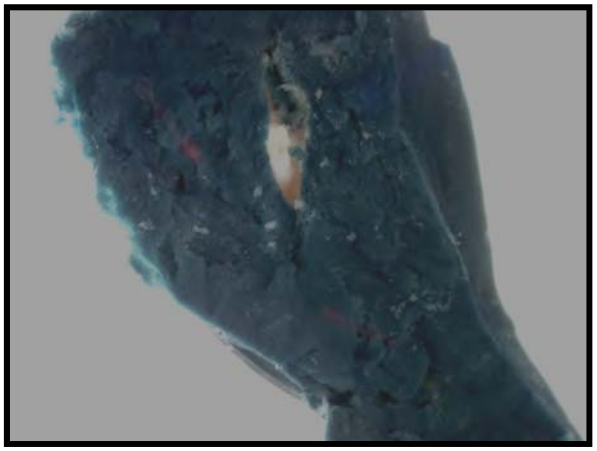


Supplemental Figure 2

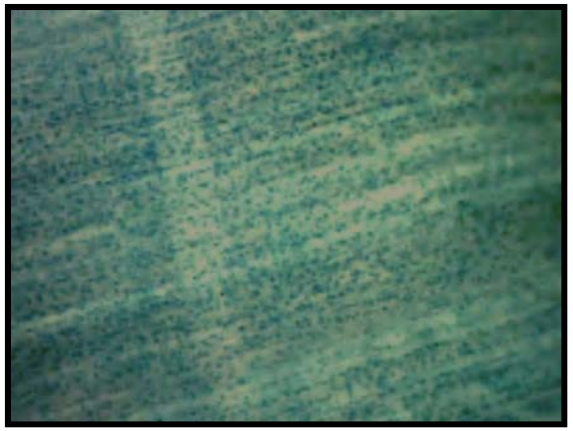


Brian (Cerebellum)

Heart

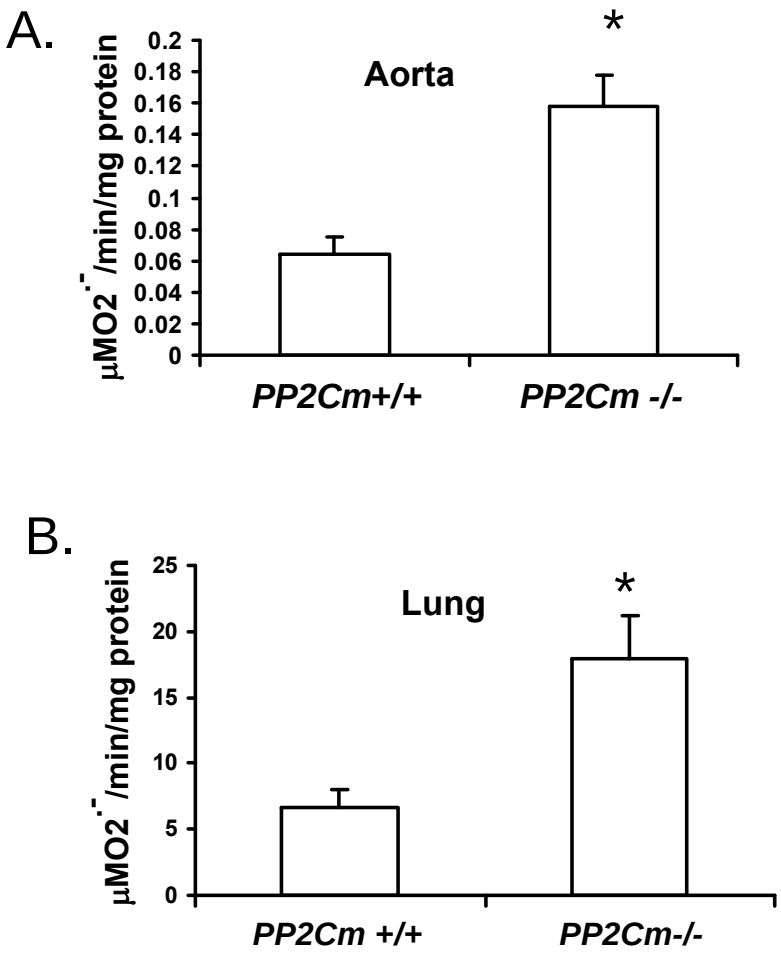


Liver

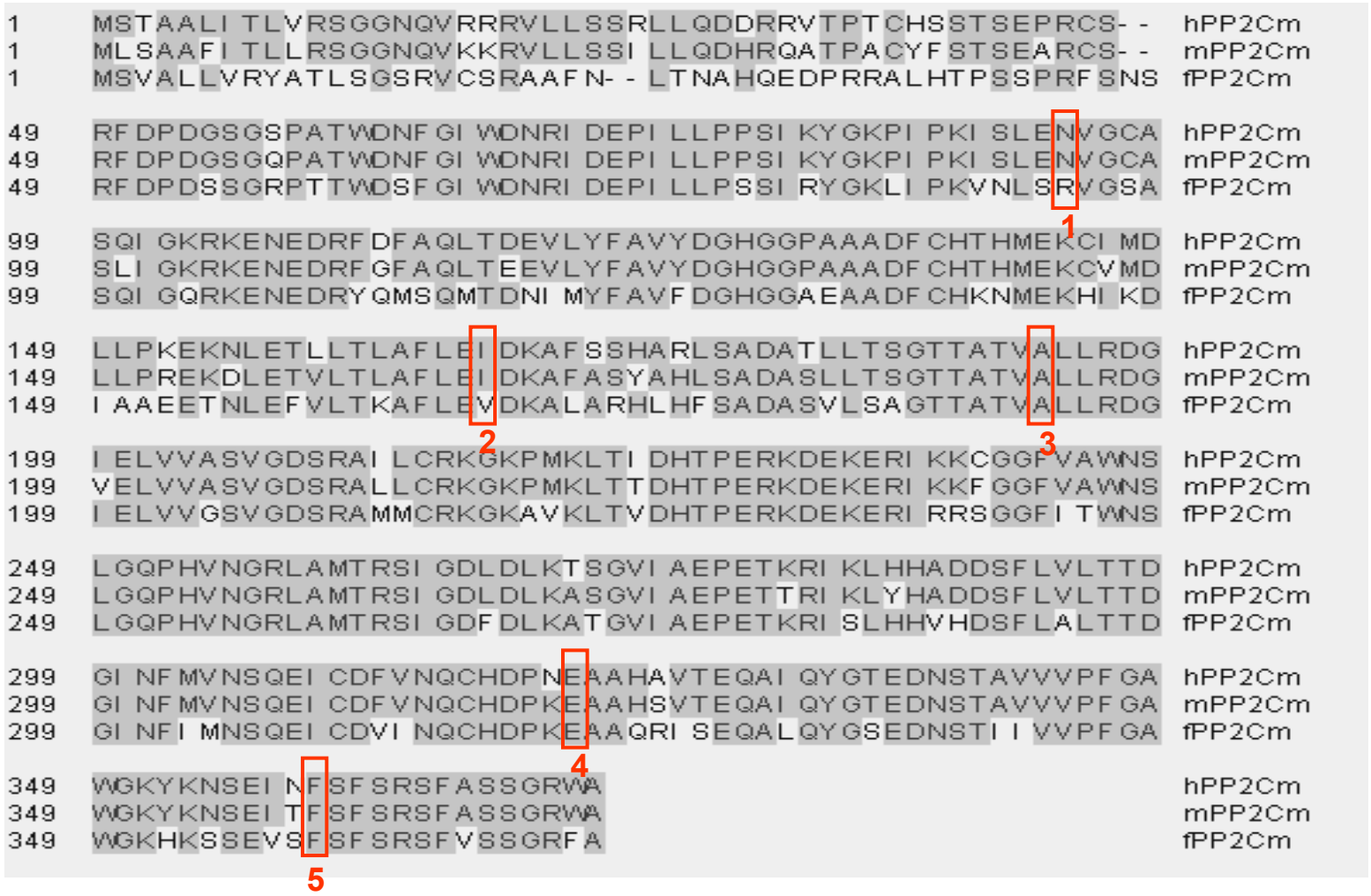


Diaphragm

Supplemental Figure 3



Supplemental Figure 4



1. Missense, N94K, dbSNP rs#17853762
2. Missense, I167T, dbSNP rs#11557705
3. Frame shift, A193, dbSNP rs#35696062
4. Missense, E321K, dbSNP rs#35523553
5. Frame shift, F359, dbSNP rs#36114972

Supplemental Figure 5

Table S1. The number of viable offspring of different PP2Cm genotypes and their percent distribution from *PP2Cm*+/- breeder mice

PP2Cm	+/+	+/–	–/–
Total	**19**	**35**	**25**
%	**23.5**	**45.7**	**30.9**