3rd International Conference on Nutrition and Food Science
(Nutritional Science-2014)
Track 1: Nutrition and Basic Science
14:10-14:30, September 23, 2014
Committee Room 1-2
Palacio de Congresos de Valencia, Spain

Recent Advances in Vitamin K Metabolism

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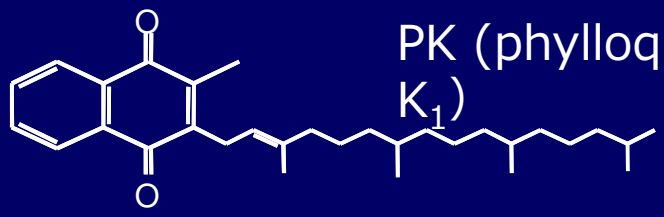
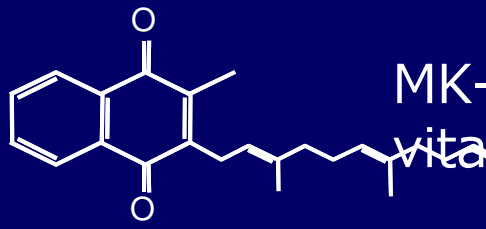
Naturally occurring vitamin K

Plant origin
(Major dietary source)

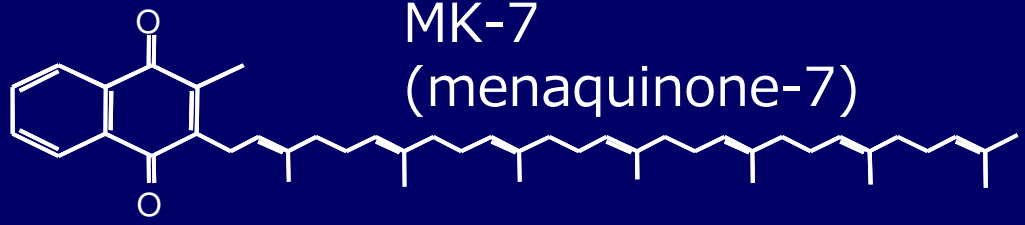


PK (phylloquinone: vitamin K_1)

Bacterial origin
(Minor dietary source)



MK-4 (menaquinone-4: vitamin K_2)



MK-7
(menaquinone-7)

Plasma vitamin K concentrations of healthy Japanese women

phylloquinone (ng/mL)	MK-4 (ng/mL)
1.74±1.29 (0.13～8.83)	0.10±0.19 (n.d.～1.44)

Tsugawa N, Okano T, et al, Am J Clin Nutr, 2006;83:380-386.

Amounts of dietary intake and plasma concentrations

phylloquinone > > MK-4

Tissue concentrations of animals and humans

MK-4 > > phylloquinone

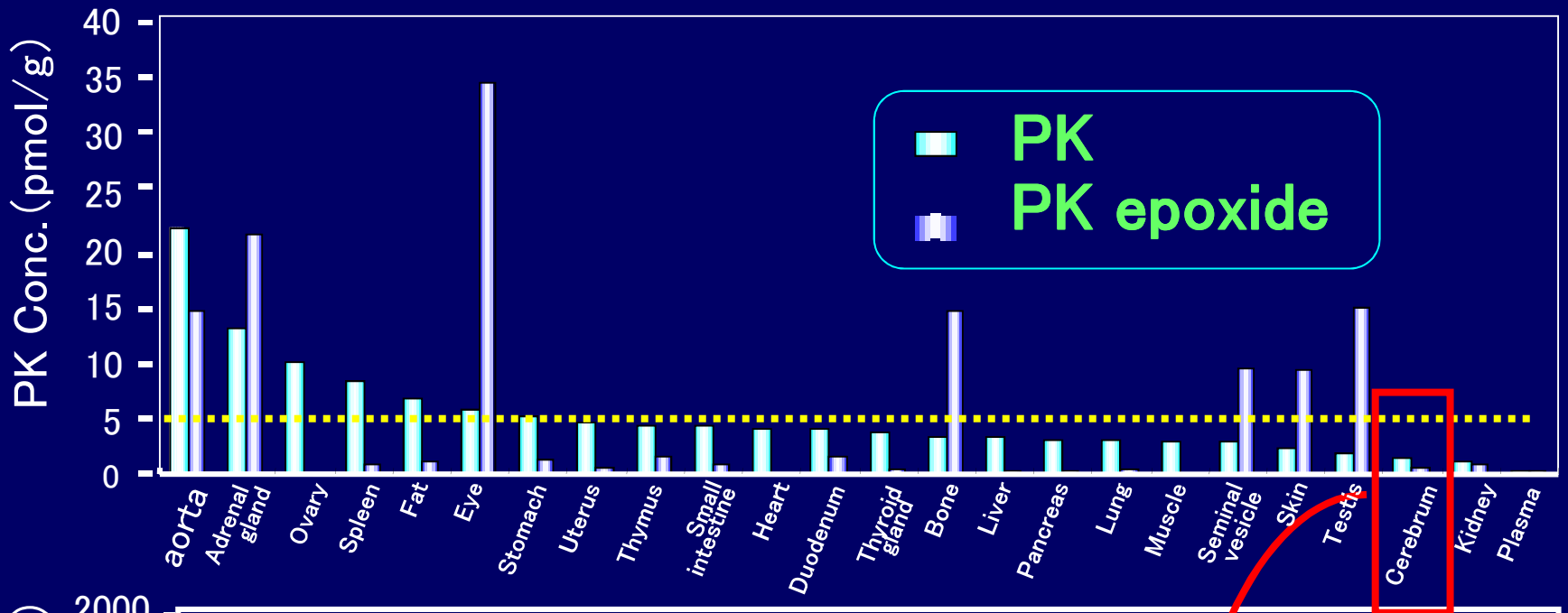
Is phylloquinone converted into MK-4 in the body ?

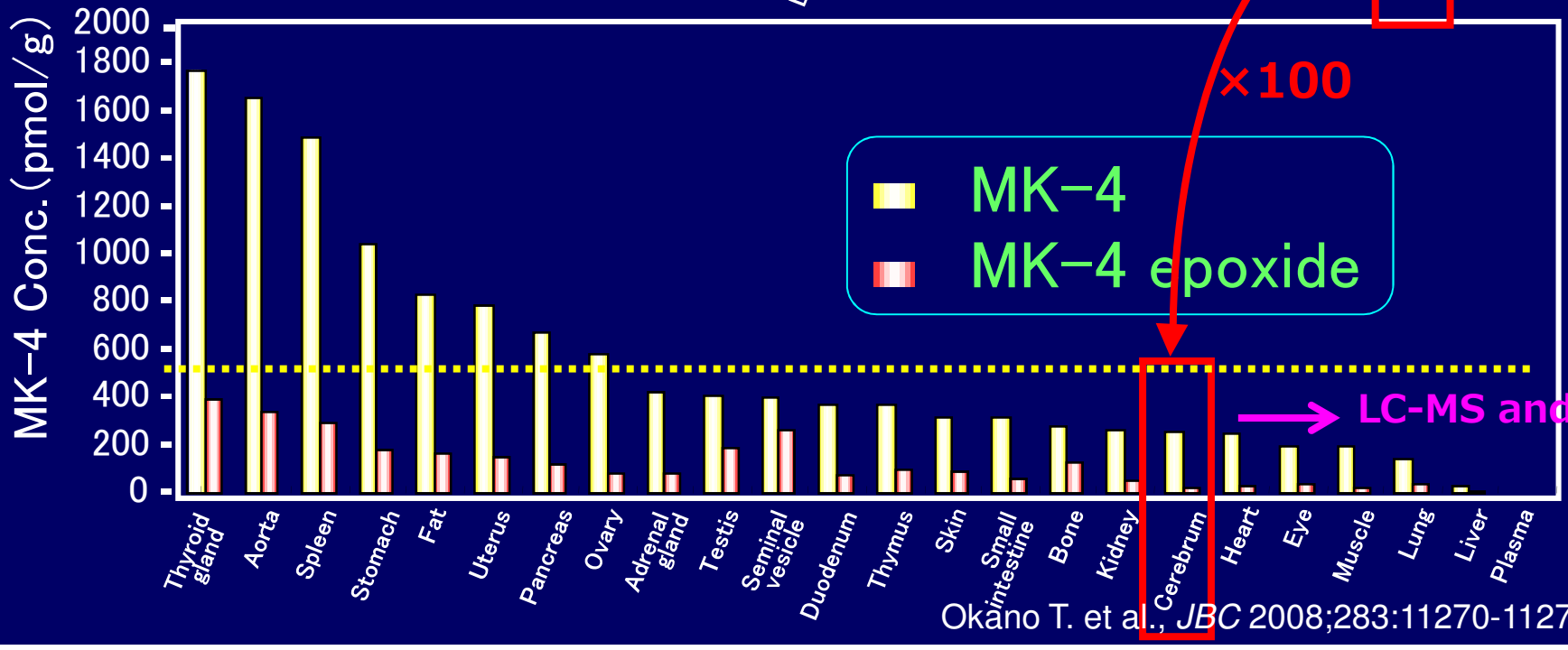
What is the physiological significance of this conversion and what functions dose MK-4 have, and can we develop MK-4 analogues for clinical use ?

Suhara Y, Okano T, et al, *J Med Chem*, 2011;54:4269-4273
Suhara Y, Okano T, et al, *J Med Chem*, 2011;54:4918-4922
Suhara Y, Okano T, et al, *J Med Chem*, 2012;55;1553-1558

The aim of our study is to examine the above issues in animals and humans.

Tissue concentrations of PK and MK-4 in mice fed a conventional laboratory chow diet

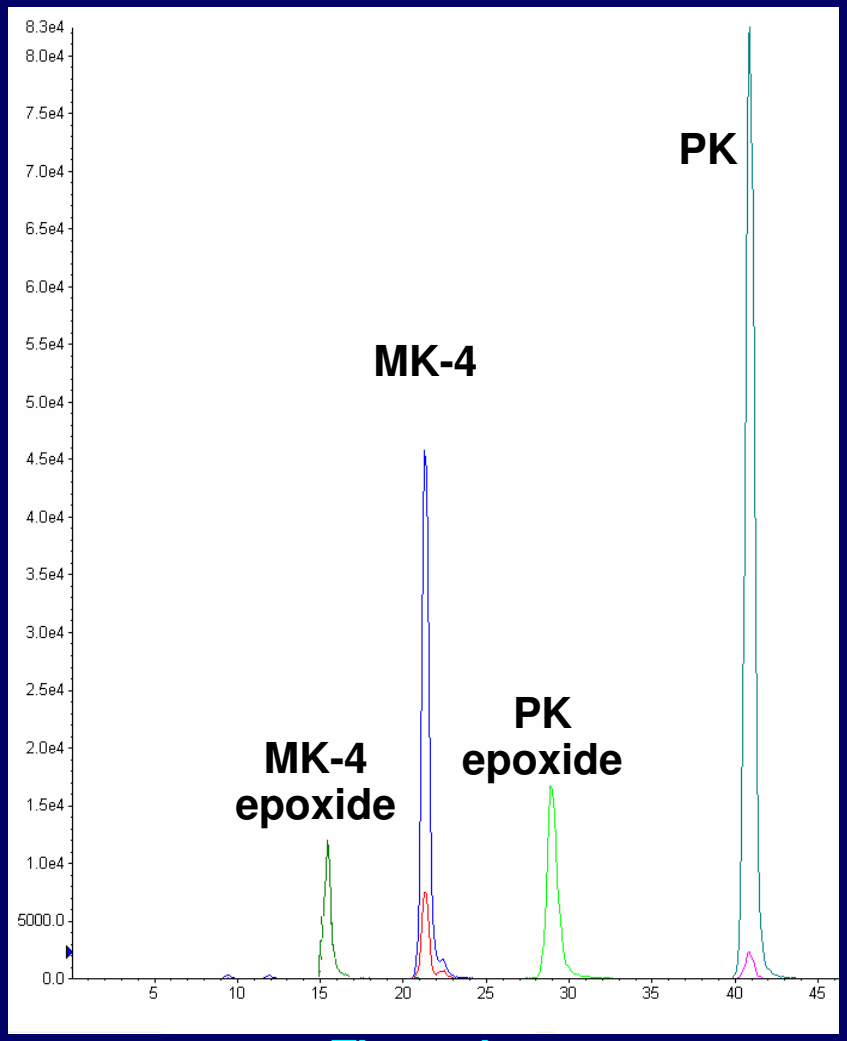




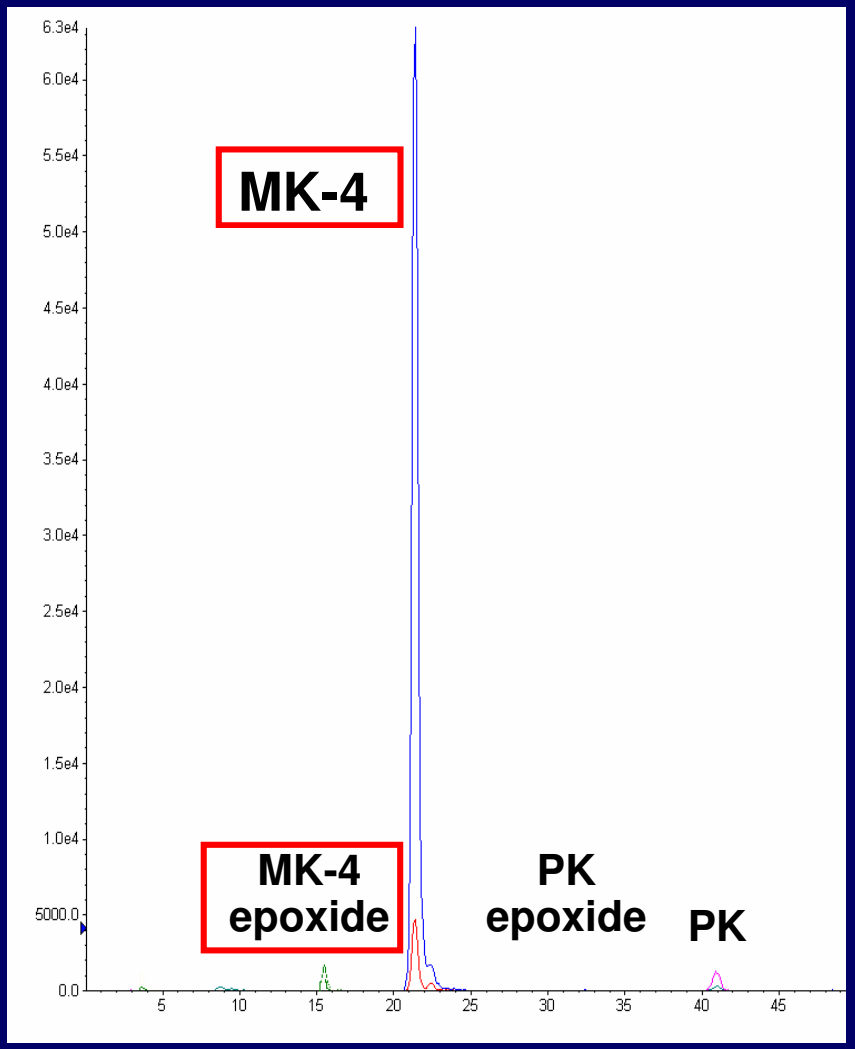
Okano T. et al., *JBC* 2008;283:11270-11279

Identification of MK-4 from brain of mice by LC-MS/MS

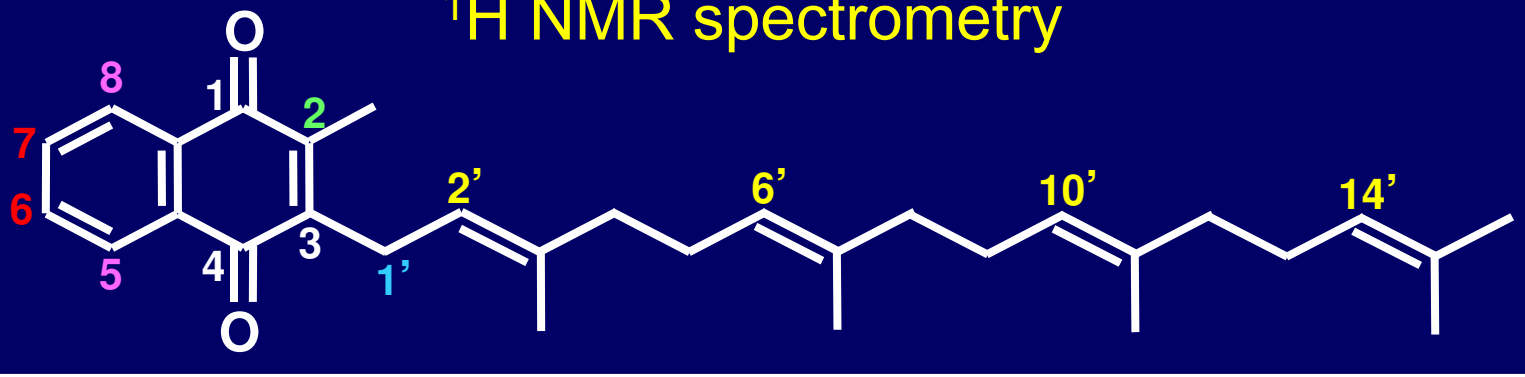
Authentic vitamin Ks

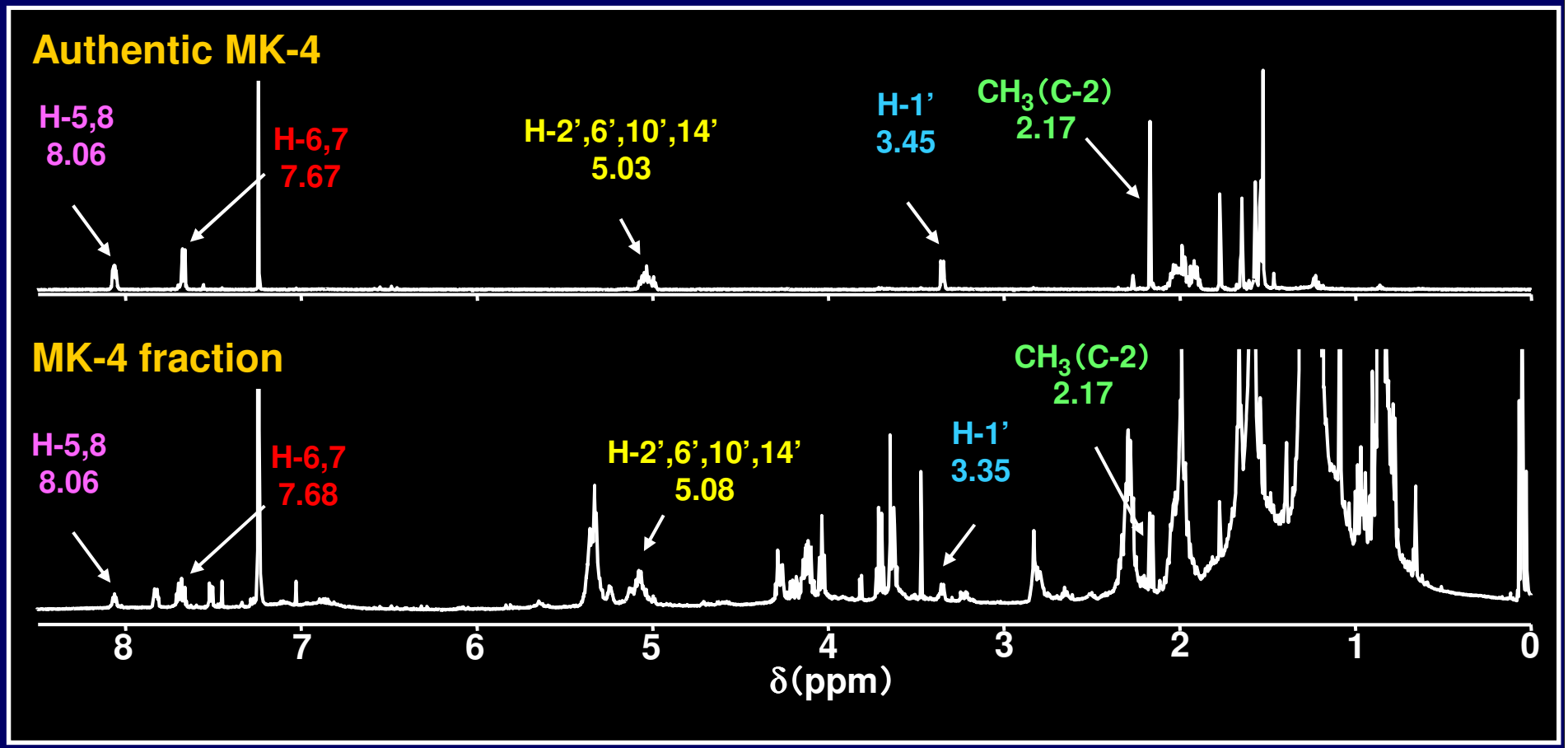


MK-4 fraction



Identification of MK-4 from brain of mice by ^{1}H NMR spectrometry





Experimental design

Experiment

C57BL/6J mice
7-weeks old
n=200

Oral administration

PK-d_7

Fast

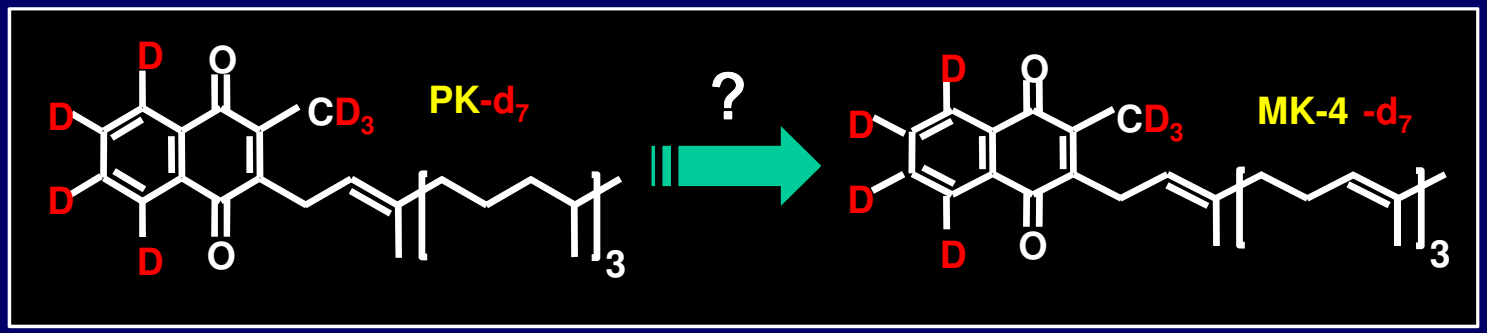
Sacrifice

Cerebra

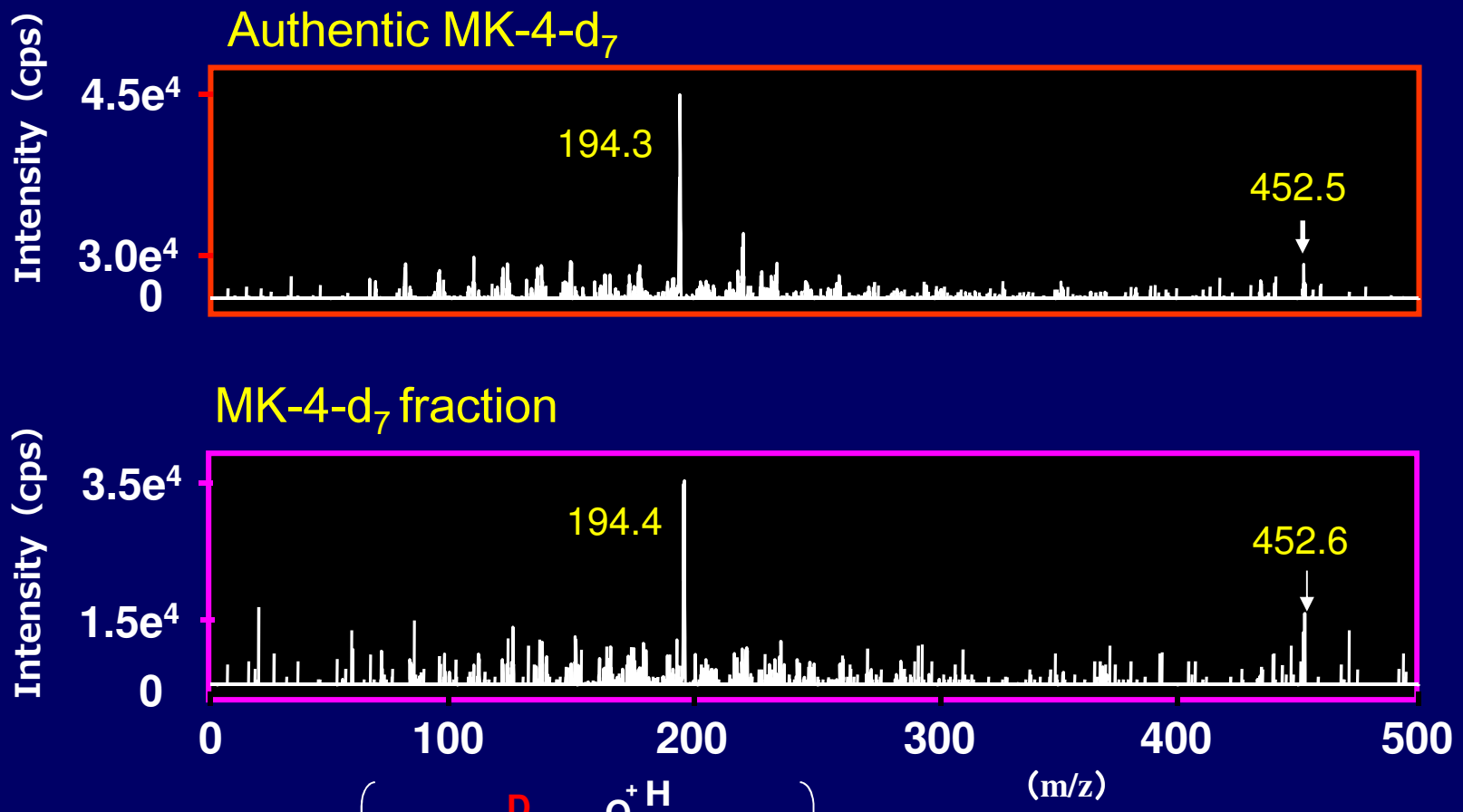
1 w | **12 hr** | **0** | **24 hr**

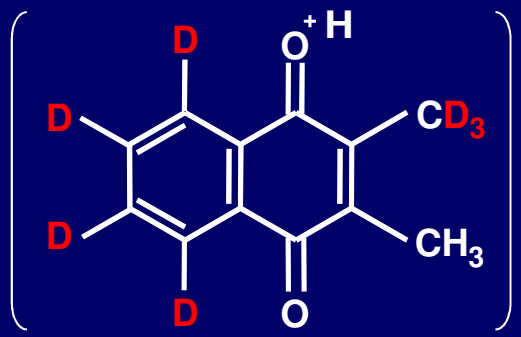
Isolation and purification of MK-4-d_7

Analyses by LC-MS/MS and ^{2}H NMR



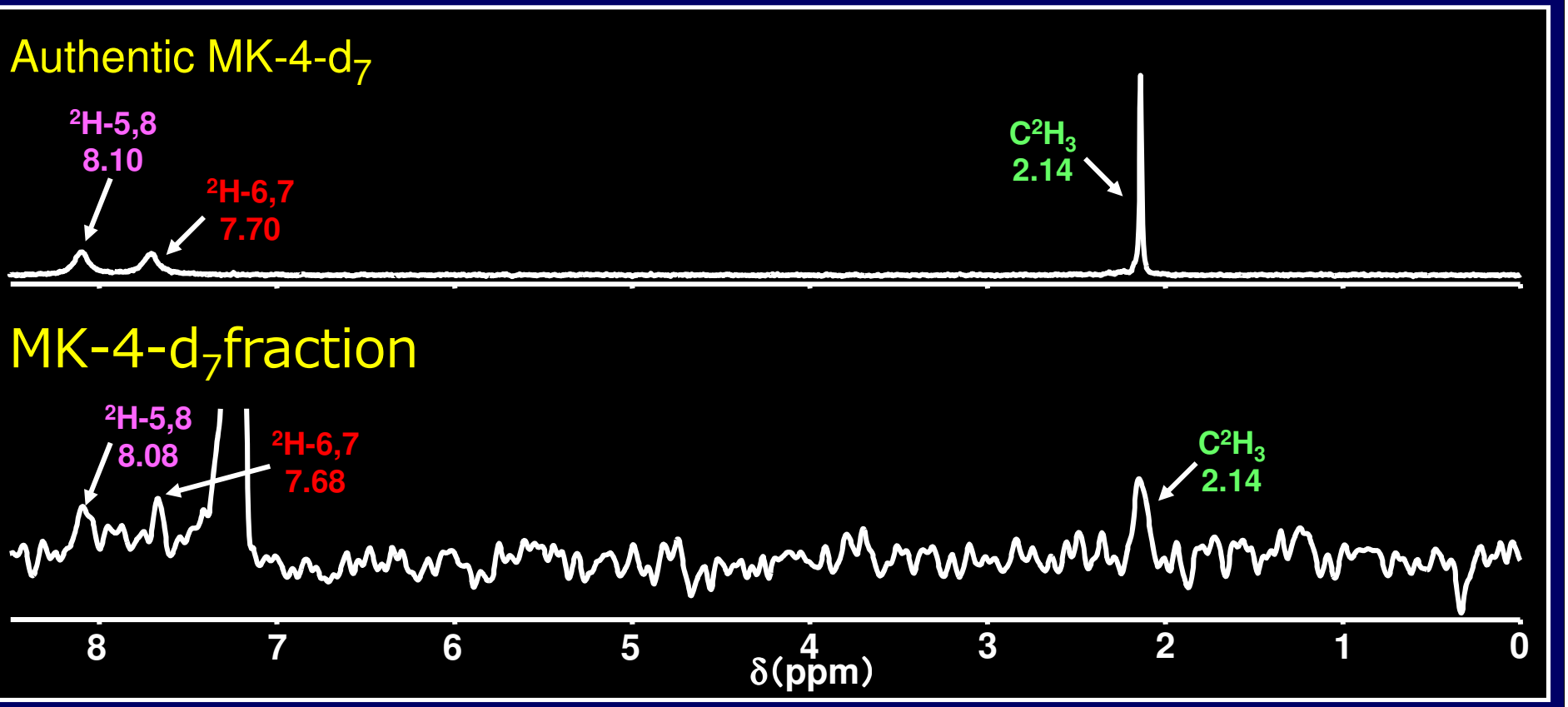
Identification of MK-4-d_7 from brain of mice by LC-MS/MS



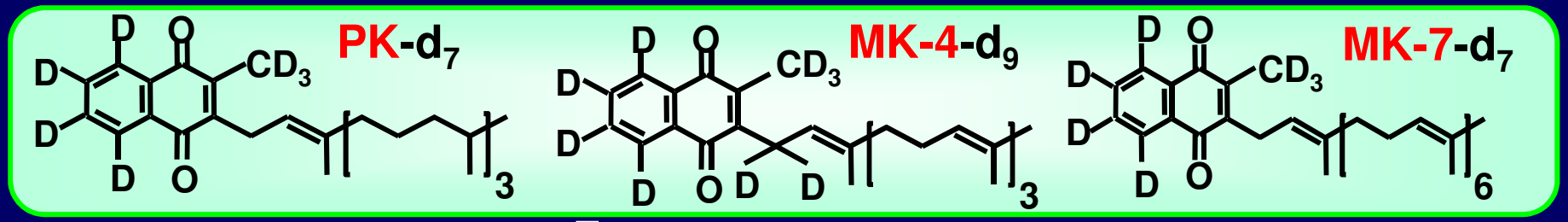


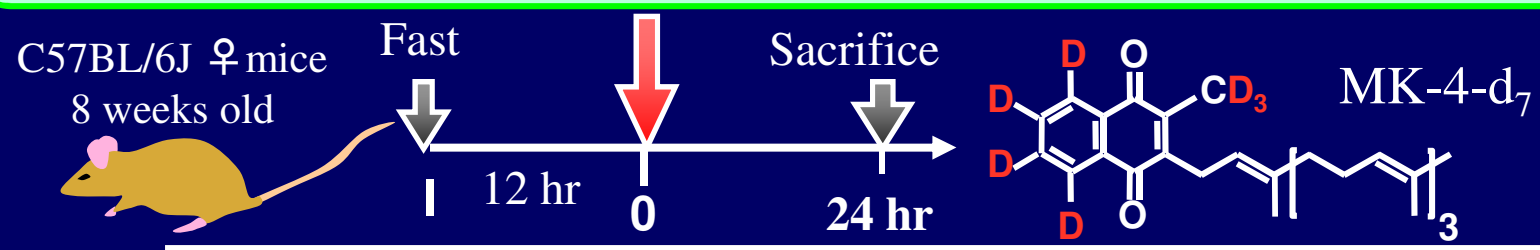
m/z : 194

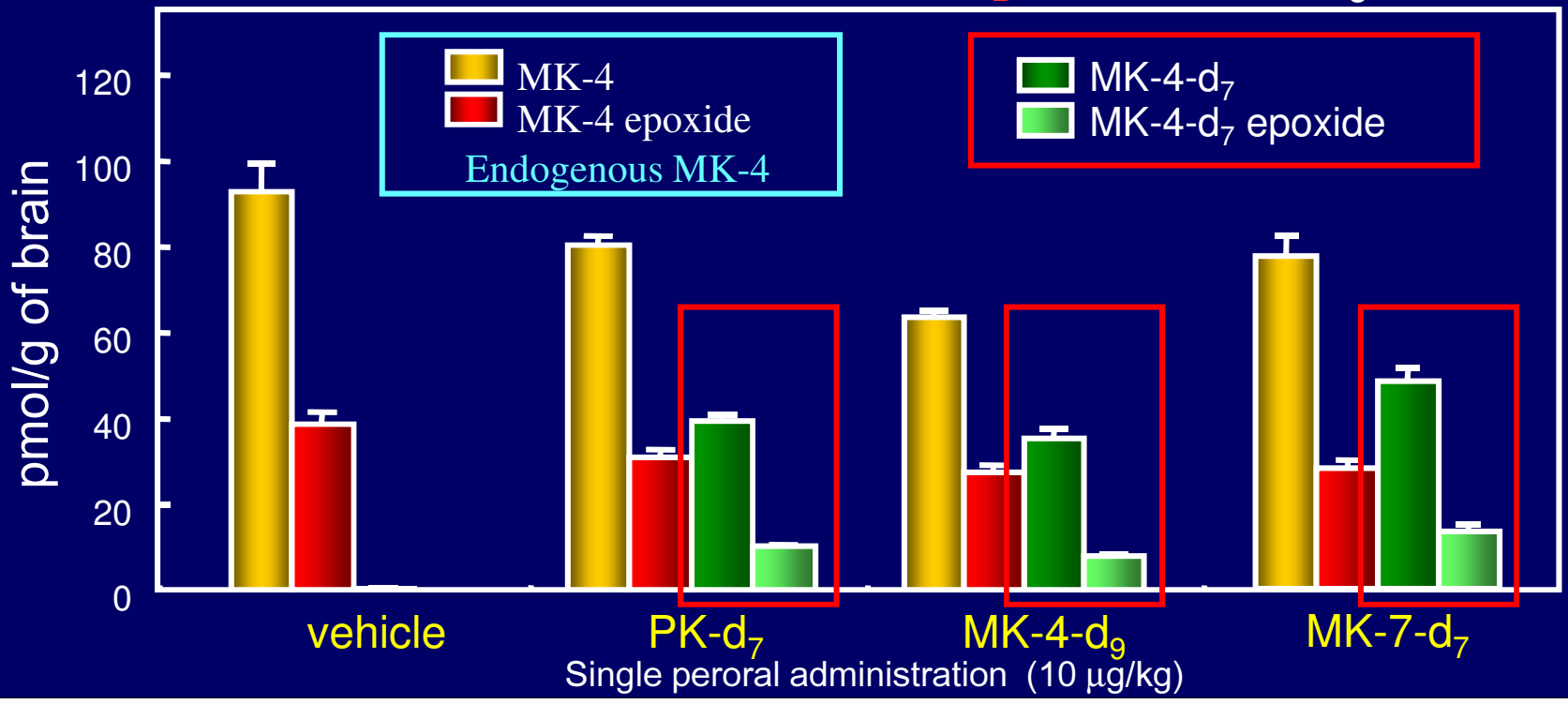
Identification of MK-4-d_7 from brain of mice by ^{2}H NMR spectrometry



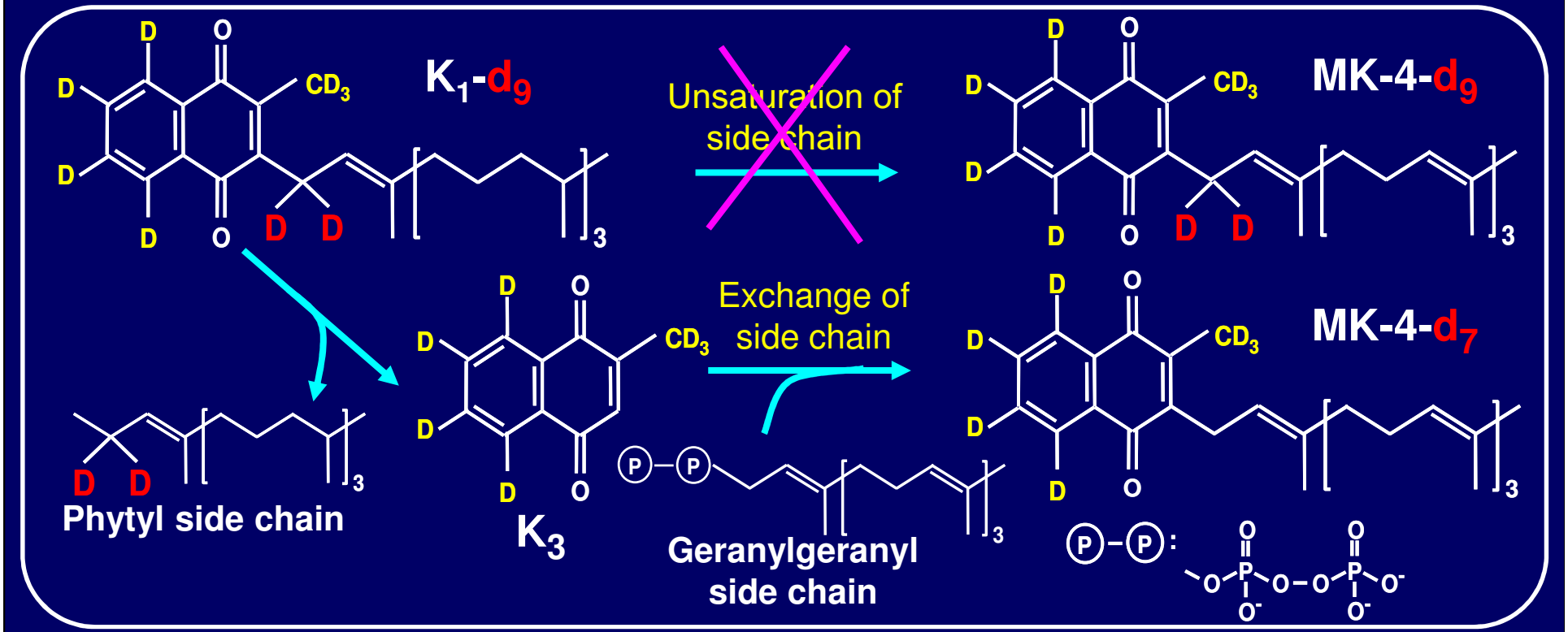
Not only PK-d_7 but also MK-4-d_9 and MK-7-d_7 are converted into MK-4-d_7 and accumulate in brain of mice







Phylloqunone is converted into MK-4 via integral side-chain removal



K vitamins are converted into MK-4 and accumulate in tissues

Where does this conversion take place?

Following four routes for the conversion of PK or K_3 into MK-4,

1. Oral route
2. Enteral route
3. Intravenous route
4. Intra-cerebroventricular route

were examined in mice.

Experimental design

Oral(1), enteral(2), intravenous(3) or intra-cerebroventricular(4) dose

C57BL/6J mice
7 weeks old

Fast

K_1-d_7, K_3-d_8

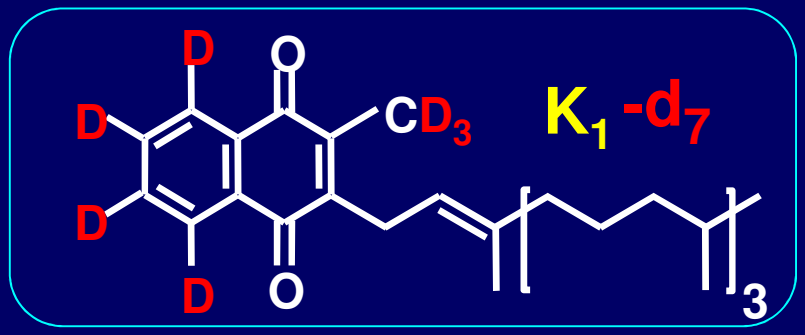
Sacrifice

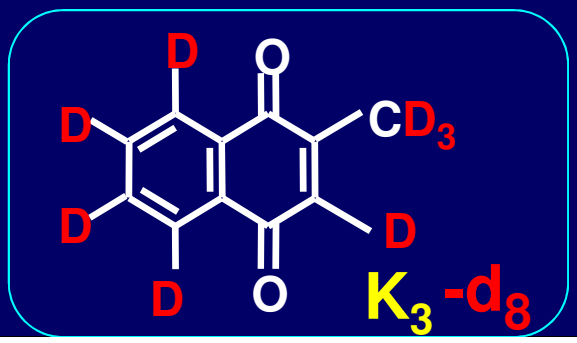
Cerebra

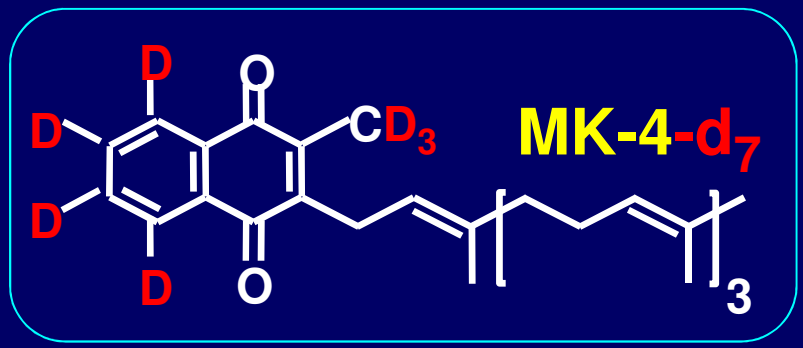
LC-APCI//MS

1 w | **12 hr** | **0** | **24 hr**

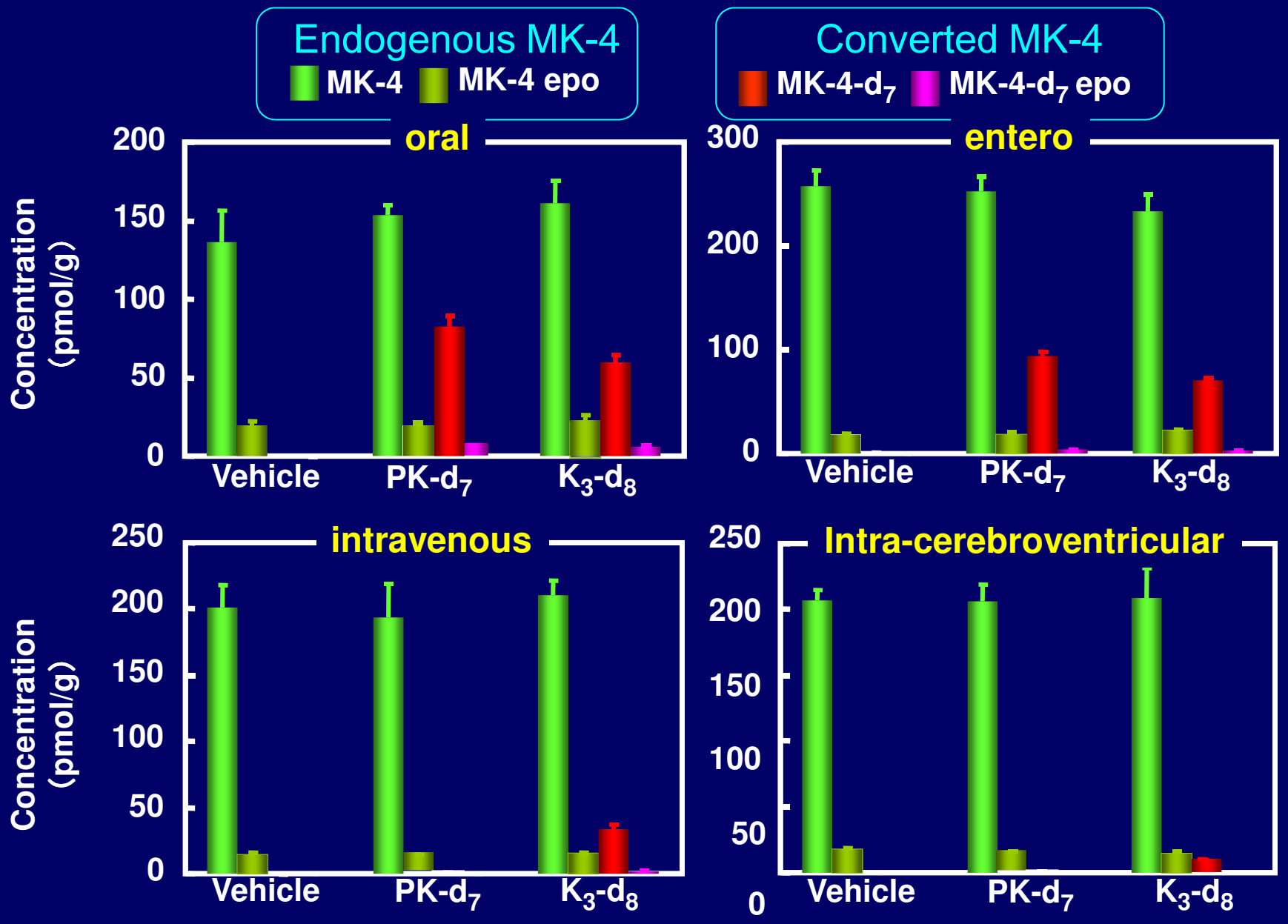
A single dose of 10 μmol/kg BW for (1), (2), (3)
At 0.1 μmol/Kg BW for (4)





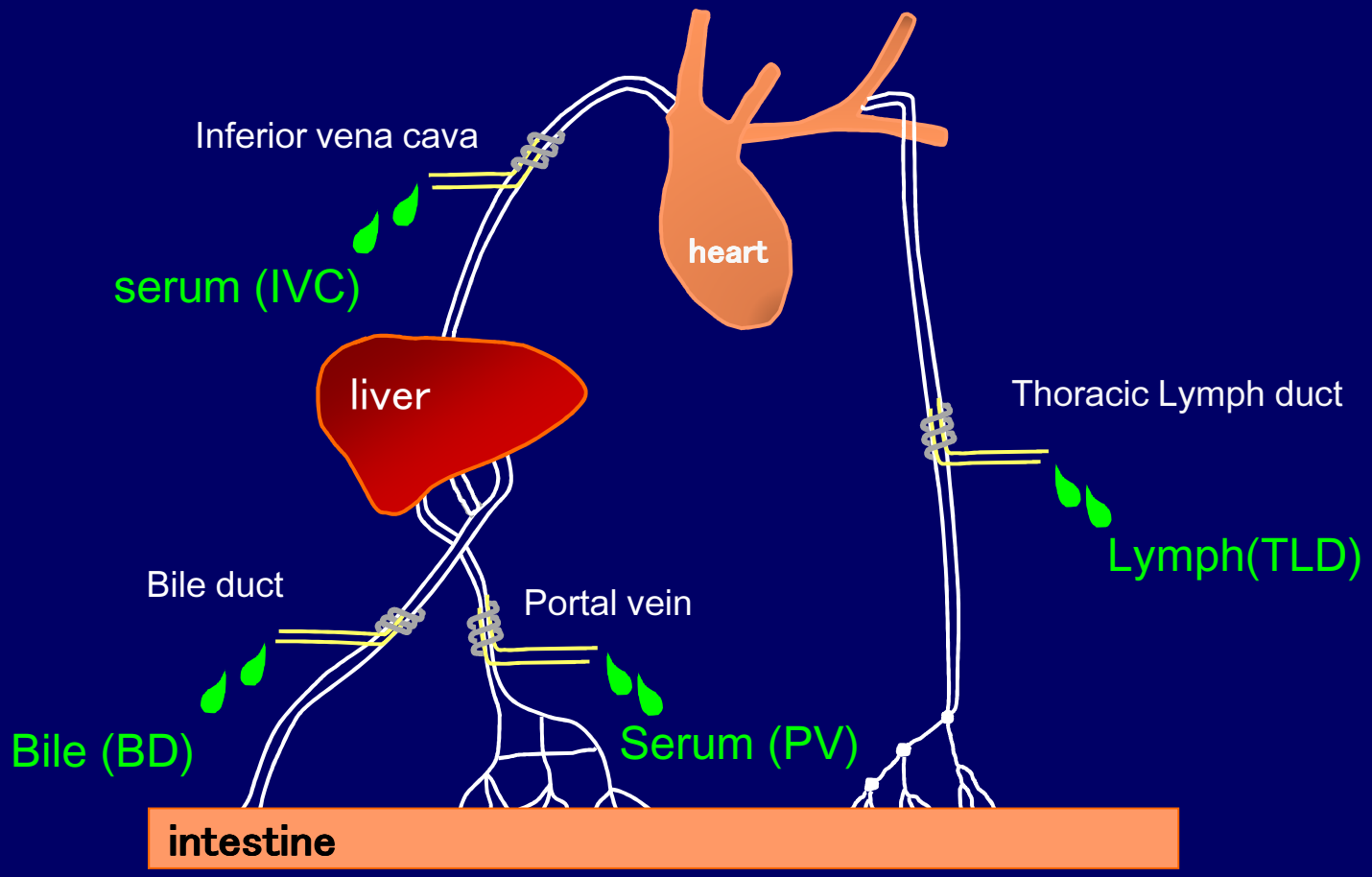


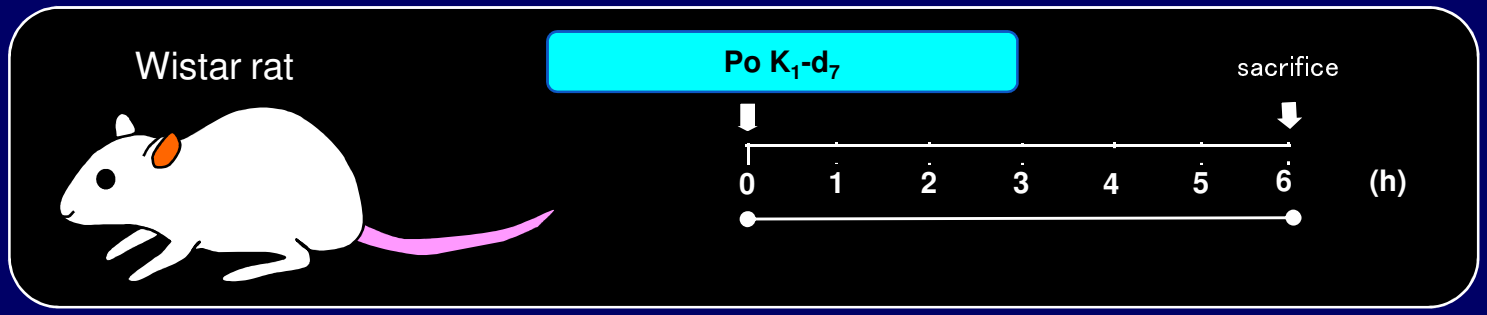
Concentrations of MK-4 in cerebra of mice

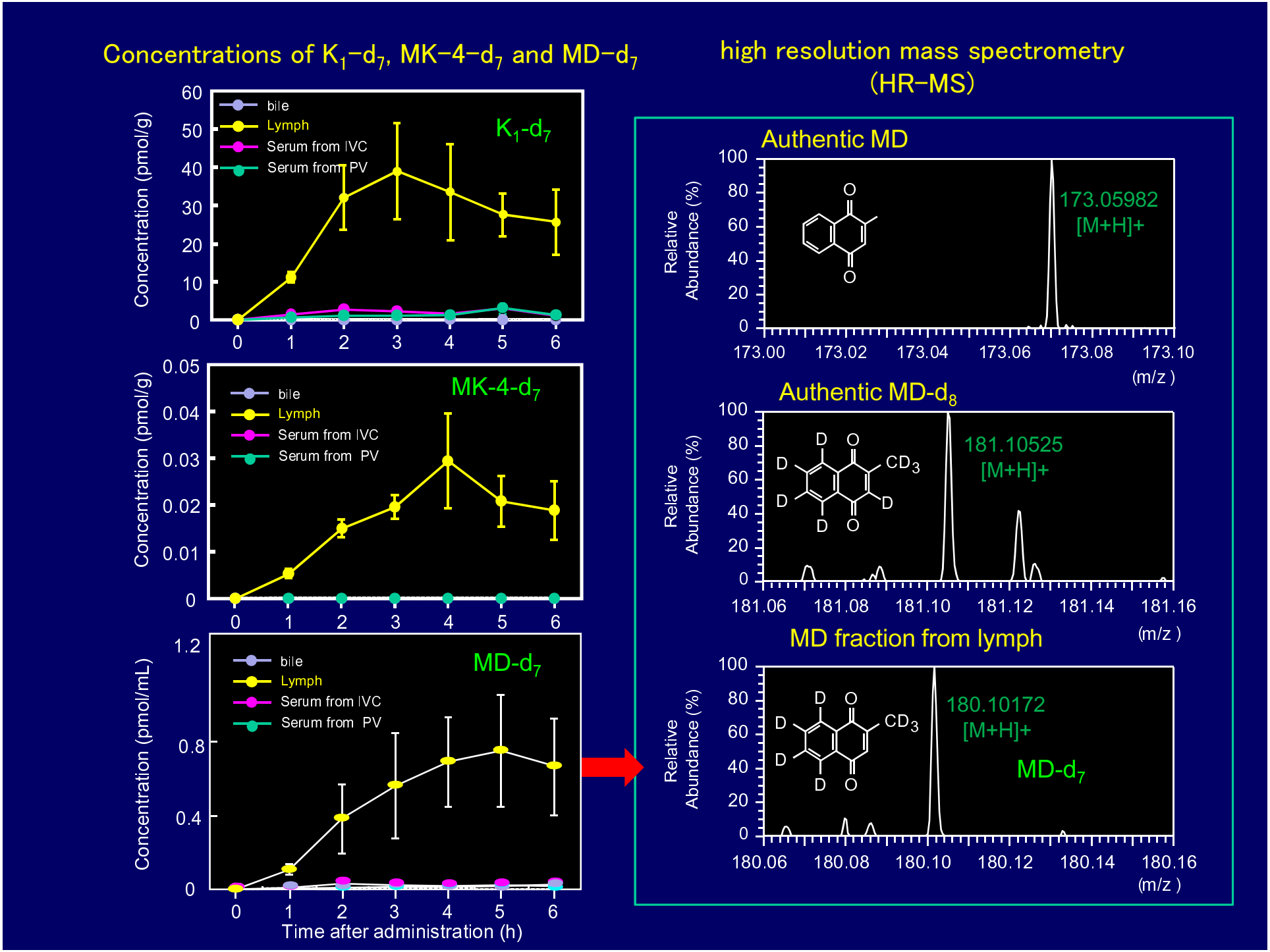


Okano T, et al, J Biol Chem, 2008; 283:11270-11279

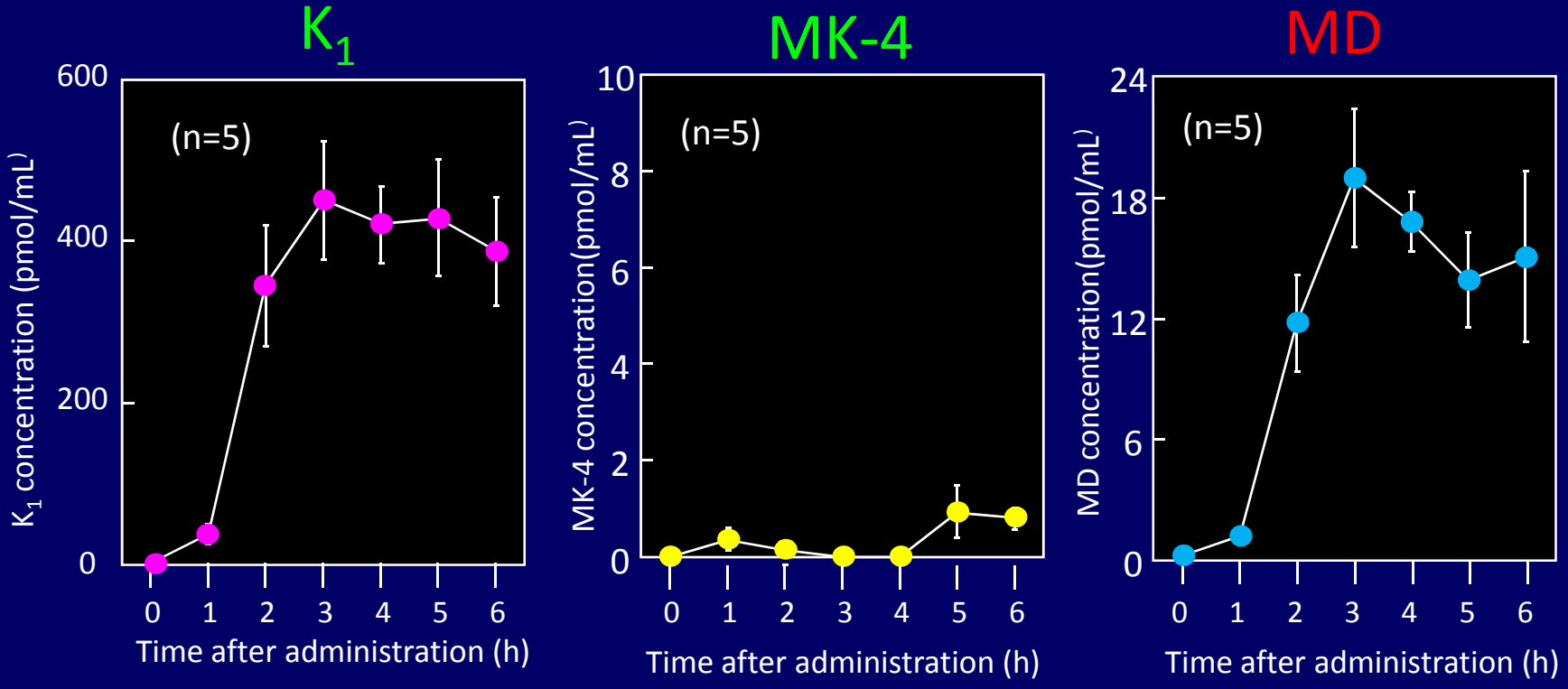
Four Sites Cannulation experiments



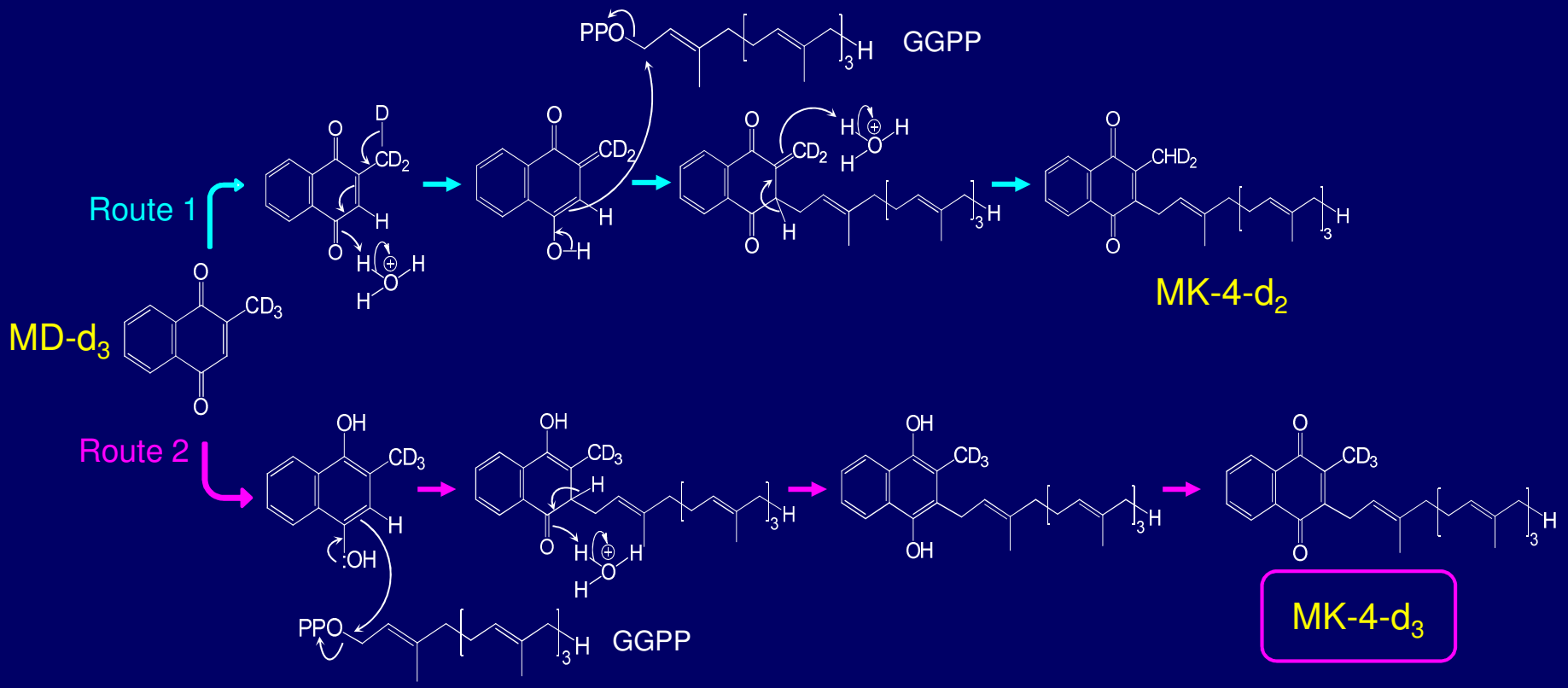



Concentrations of K1-d7, MK-4-d7 and MD-d7
high resolution mass spectrometry (HR-MS)
Concentration (pmol/g)
bile
Lymph
Serum from IVC
Serum from PV
K1-d7
MK-4-d7
Concentration (pmol/mL)
MD-d7
Time after administration (h)
Authentic MD
Relative Abundance (%)
173.05982
[M+H]+
(m/z)
Authentic MD-d8
181.10525
[M+H]+
MD fraction from lymph
180.10172
[M+H]+
MD-d7

Time course changes in serum concentrations of K_1, MK-4 and MD in humans orally given K_1 capsules (40 mg)

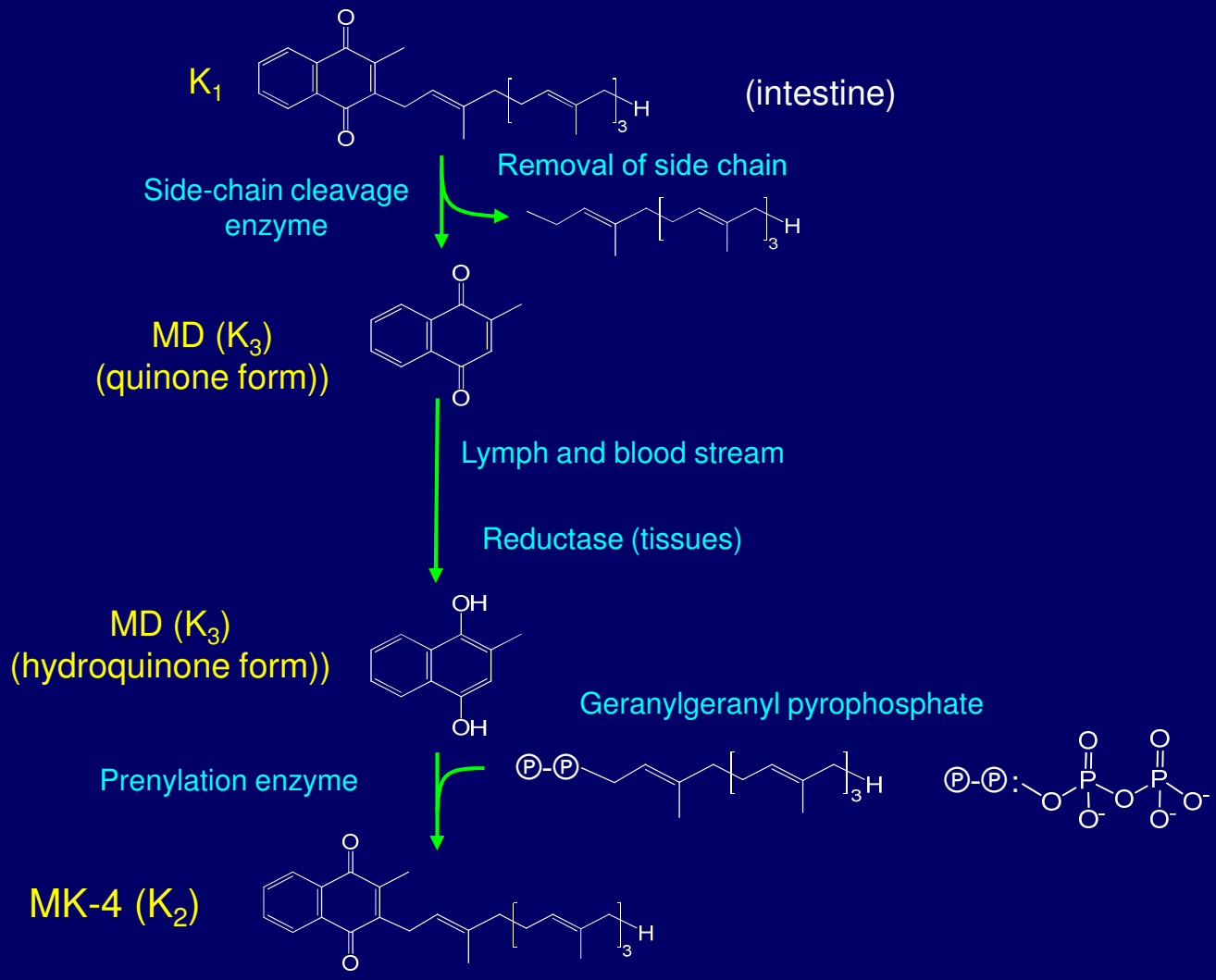


Conversion pathway of MD-d_3 to MK-4-d_3 demonstrated by HR-MS and ^{1}H NMR analyses (Route 2)



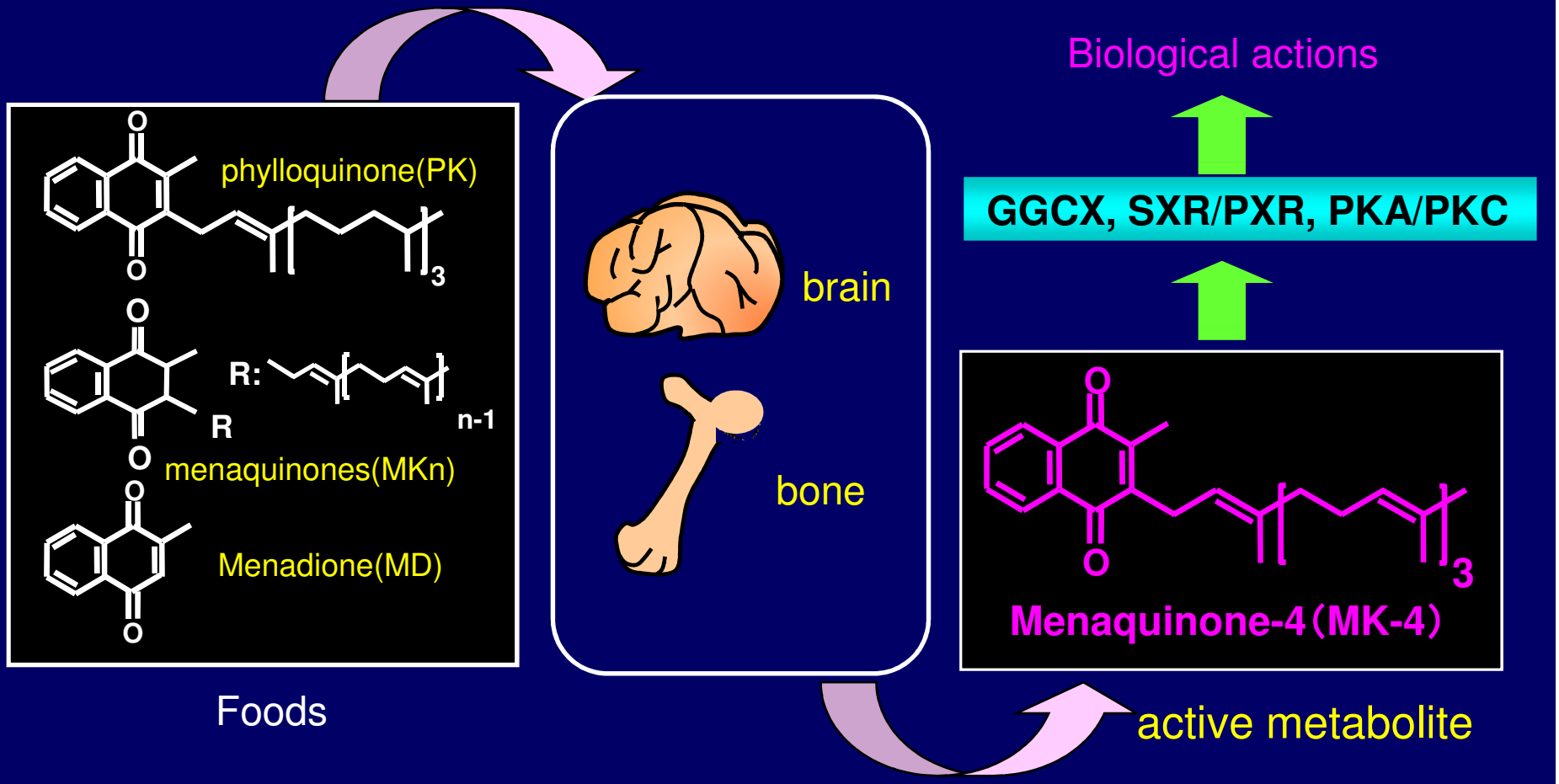
Hirota Y, Okano T, et al, J Biol Chem, 2013; 288:33071-33080

MD(K_3) is a catabolic product of oral phylloquinone (K_1) in the intestine and a circulating precursor of tissue MK-4 (K_2) in mammals

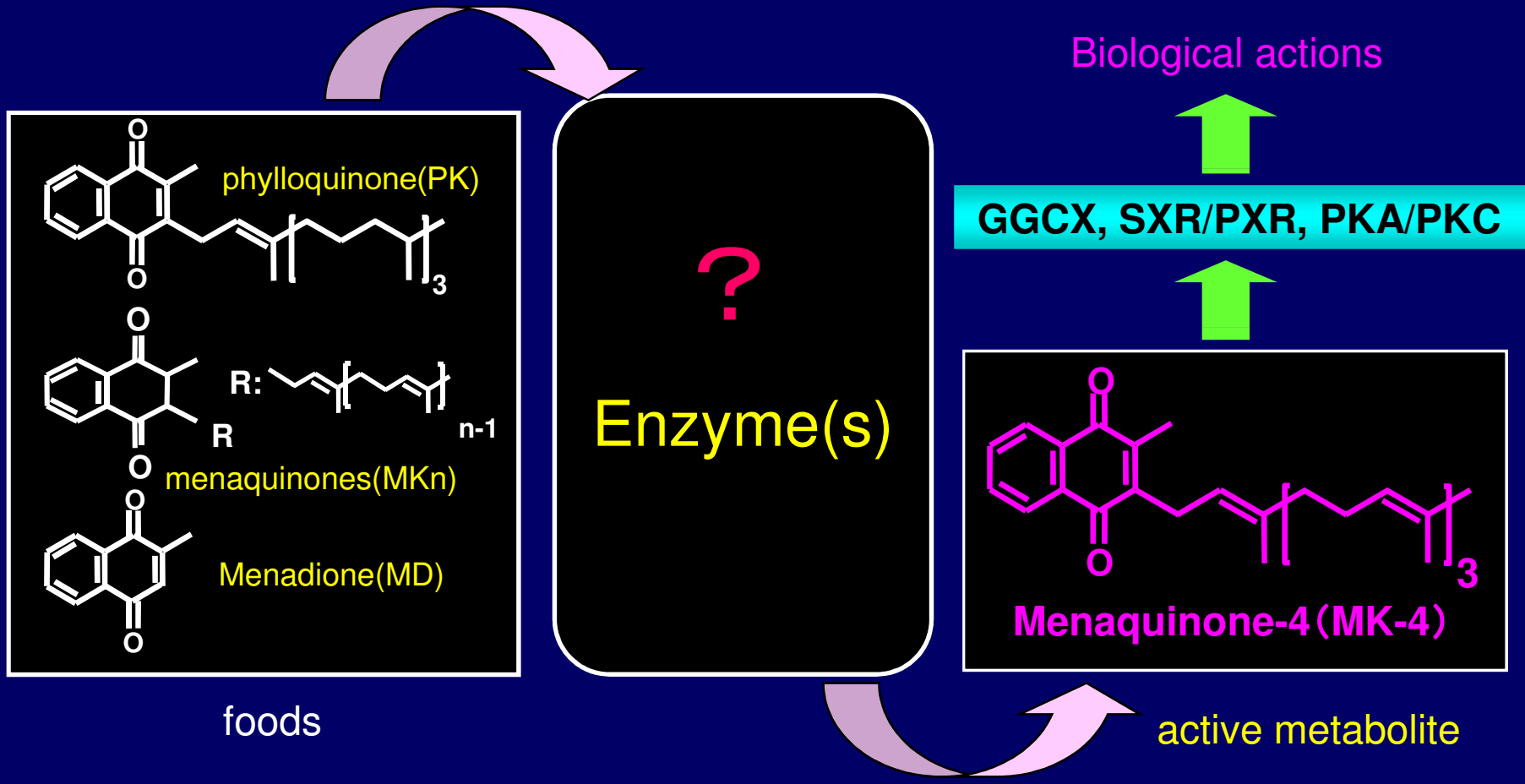


Hirota Y, Okano T, et al, J Biol Chem, 2013; 288:33071-33080

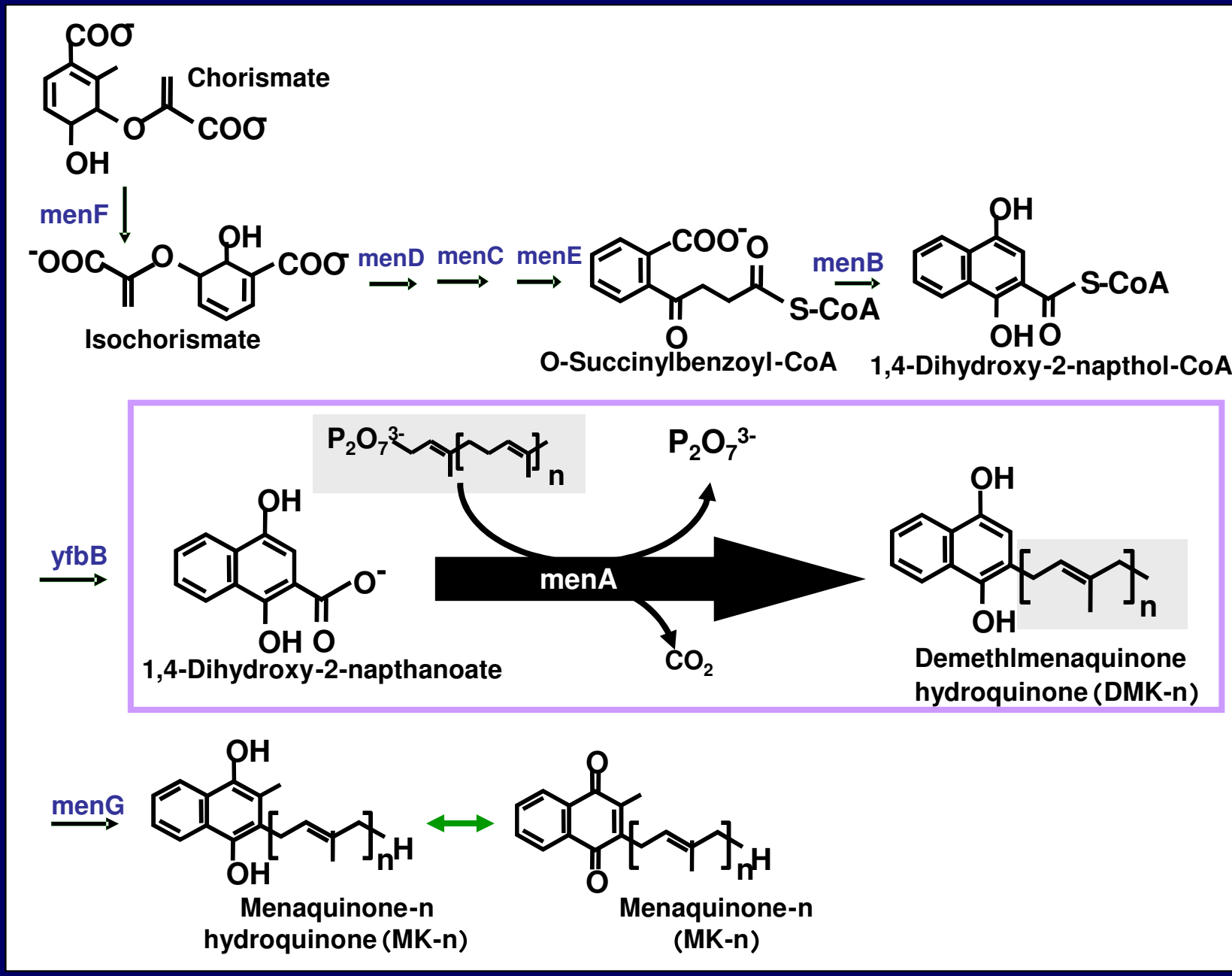
New paradigm of the metabolic activation of vitamin K in brain and bone



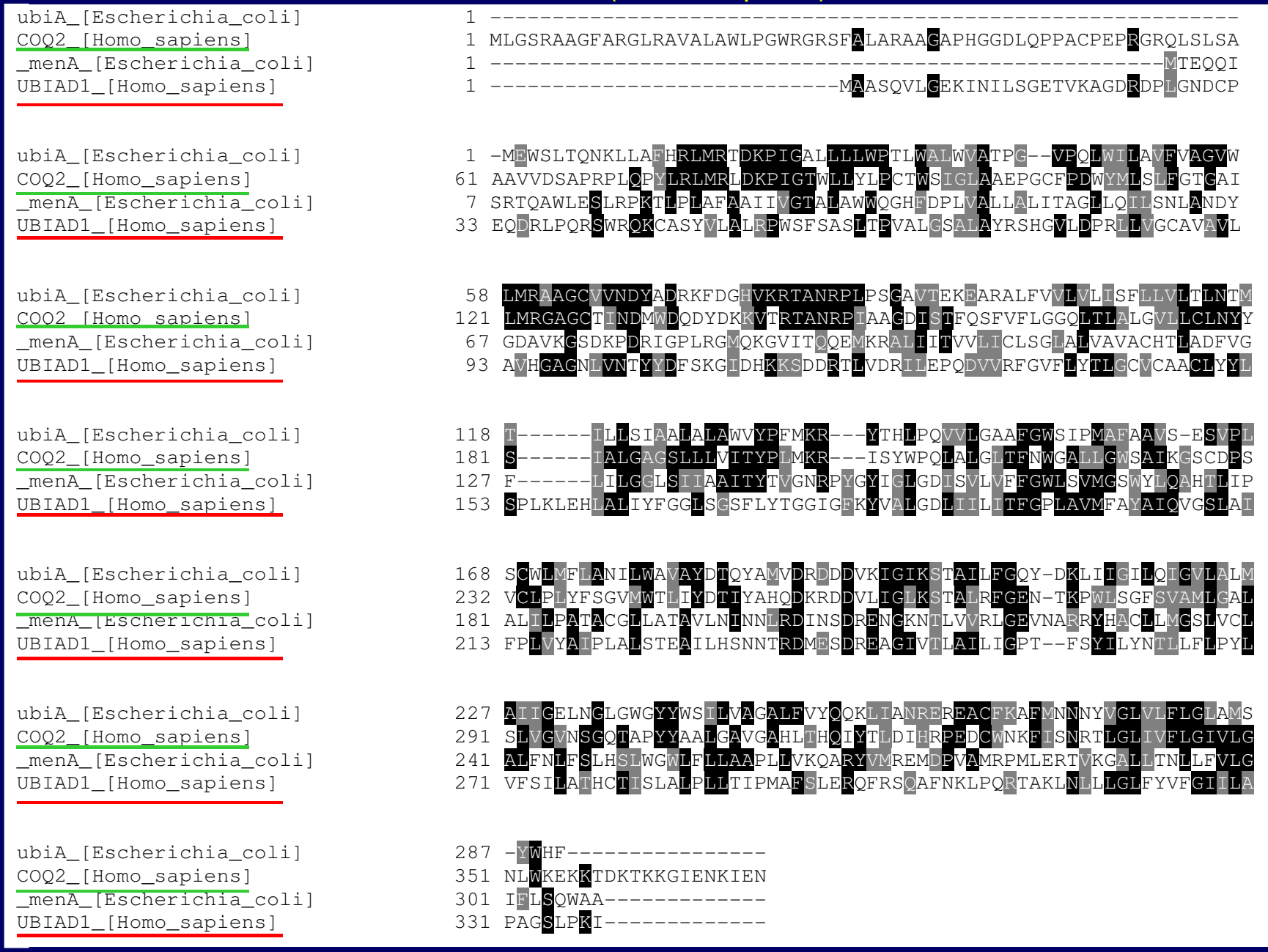
What is the enzyme(s) involved in MK-4 biosynthesis in mammals ?



Biosynthesis of menaquinones in *Escherichia*. coli

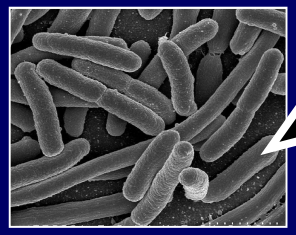


Alignment of the amino acid sequence of Ubia(E.coli), COQ2(Homo-sapiens), Men A(E.coli) and UBIAD1(Homo-sapiens)

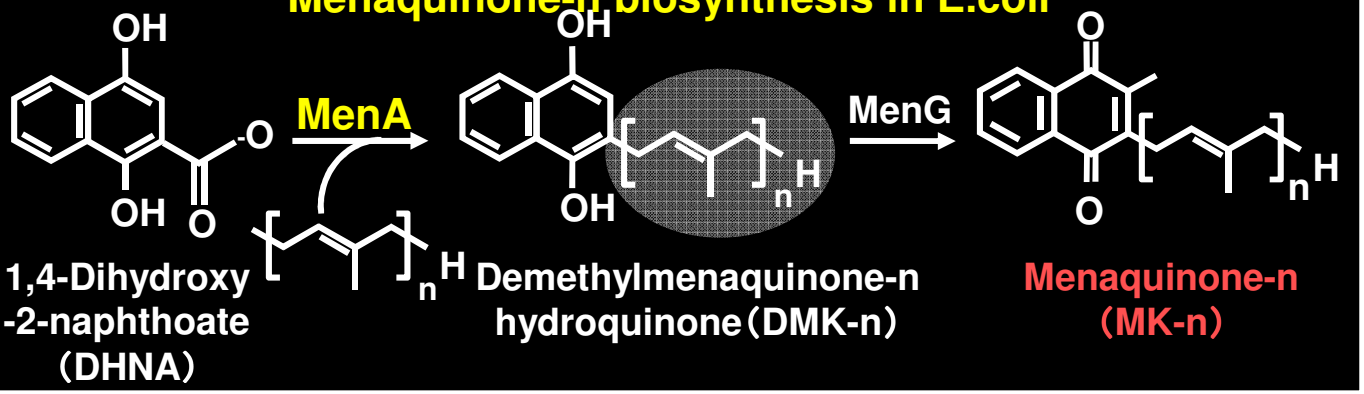


Search for the enzyme(s) responsible for the MK-4 synthesis

E.coli



Menaquinone-n biosynthesis in E.coli

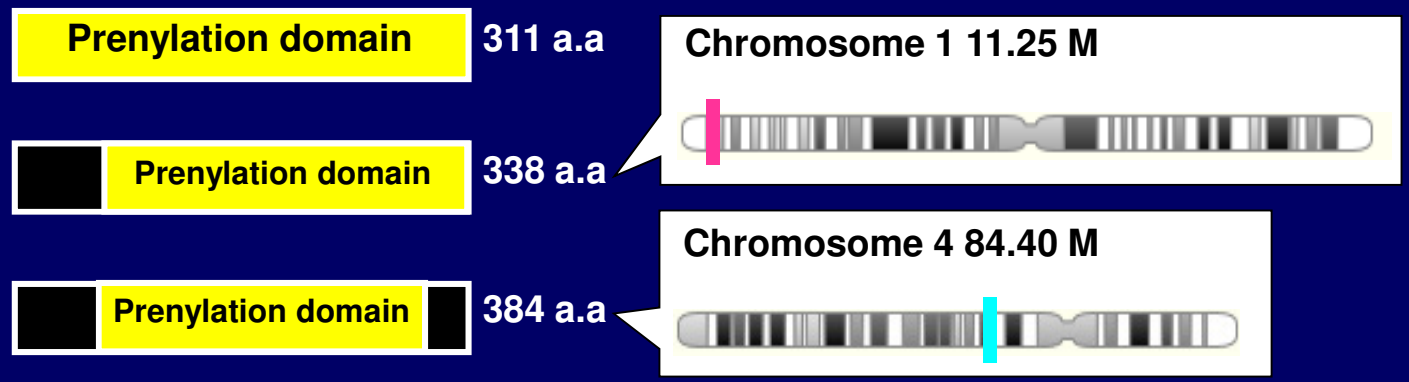


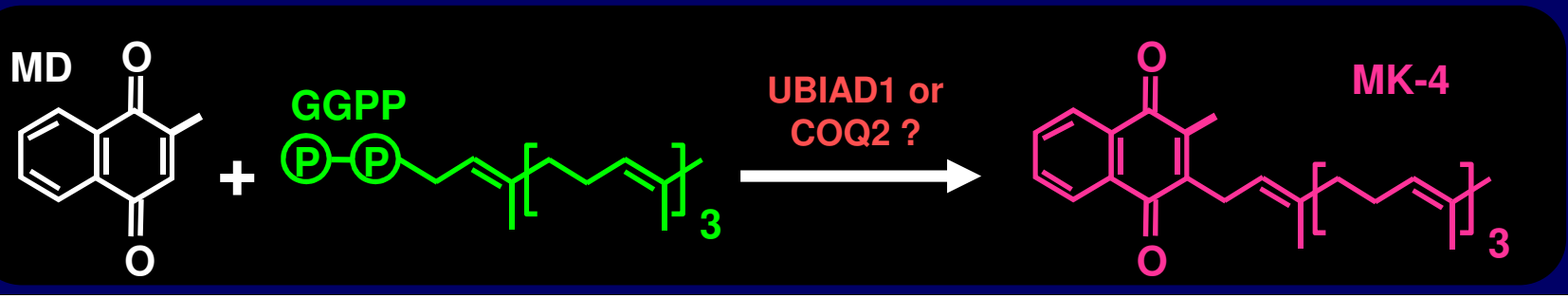
MenA human homologs

MenA

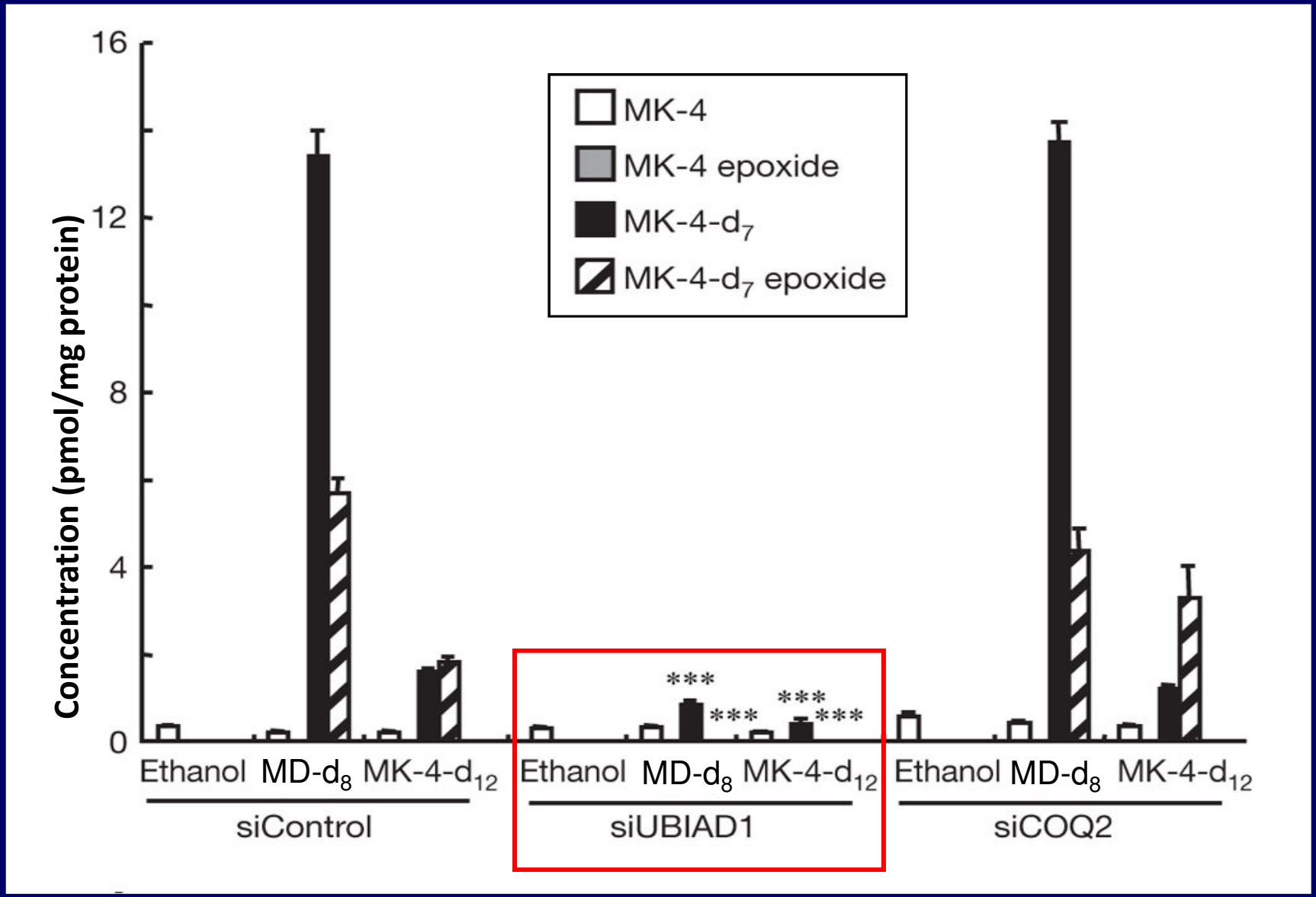
UbiA prenyltransferase domain containing 1 (UBIAD1)

CoenzymeQ2 homolog, prenyltransferase (COQ2)

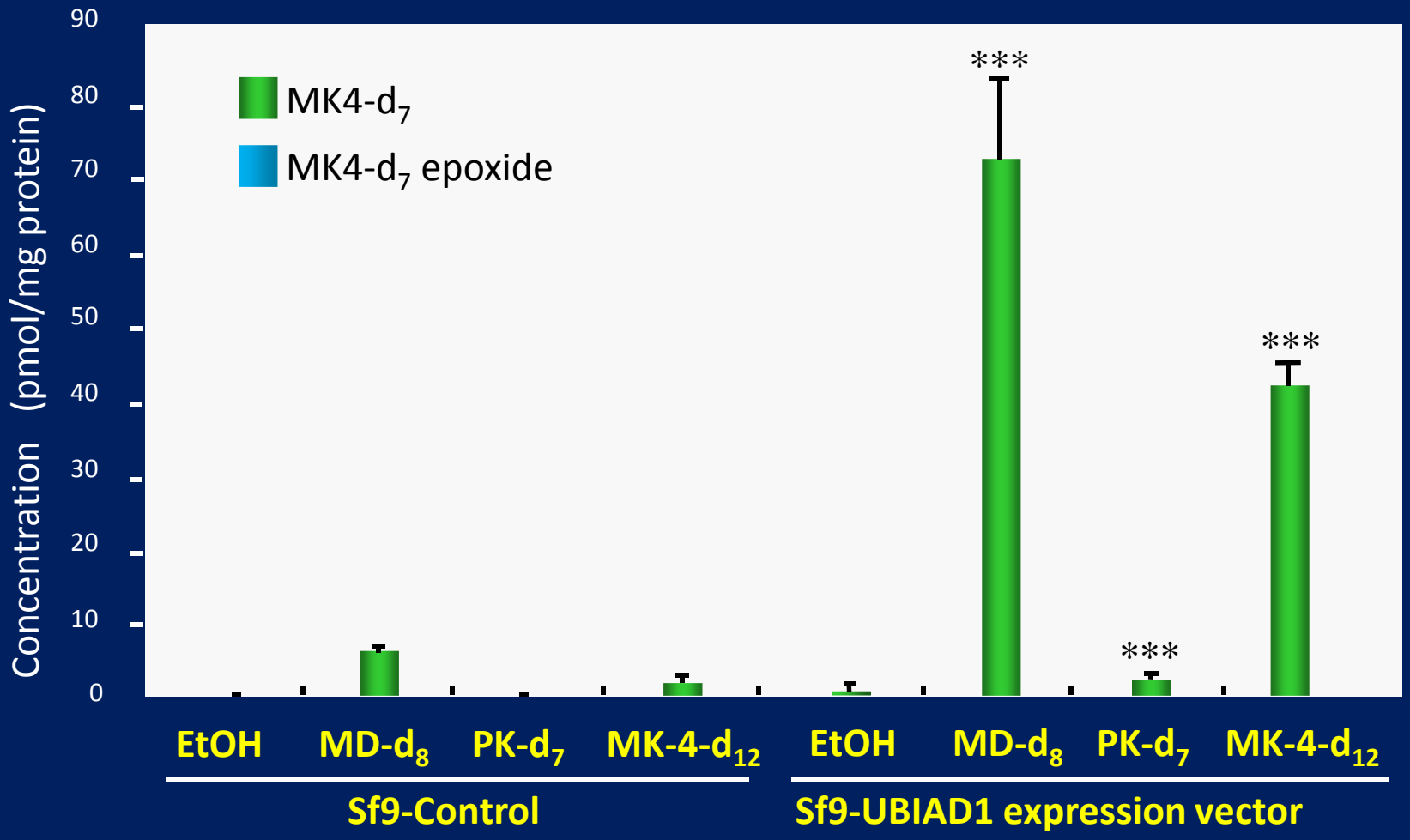




Conversion of K_3-d_8 or MK-4-d_{12} to MK-4-d_7 in siControl-, siUBIAD1- or siCOQ2-transfected MG-63 cells

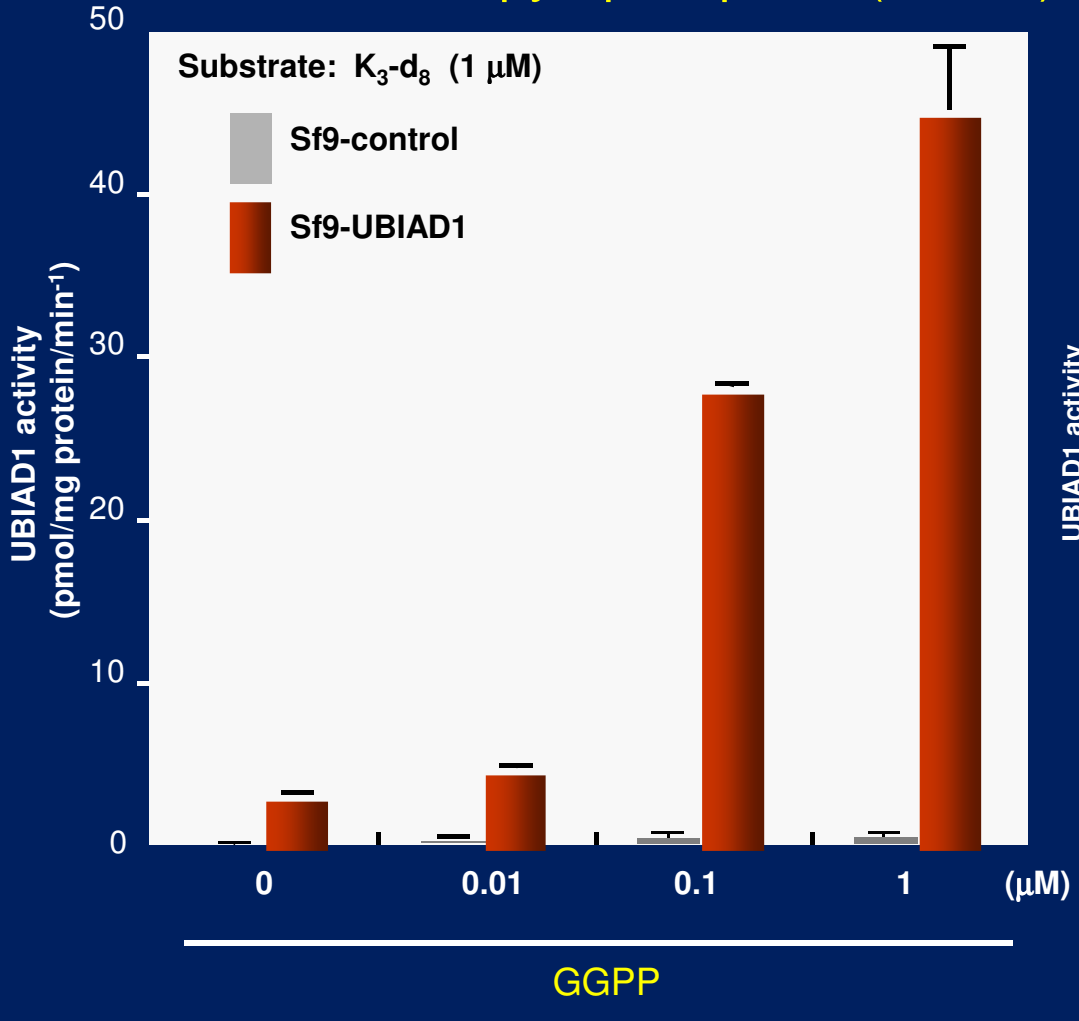


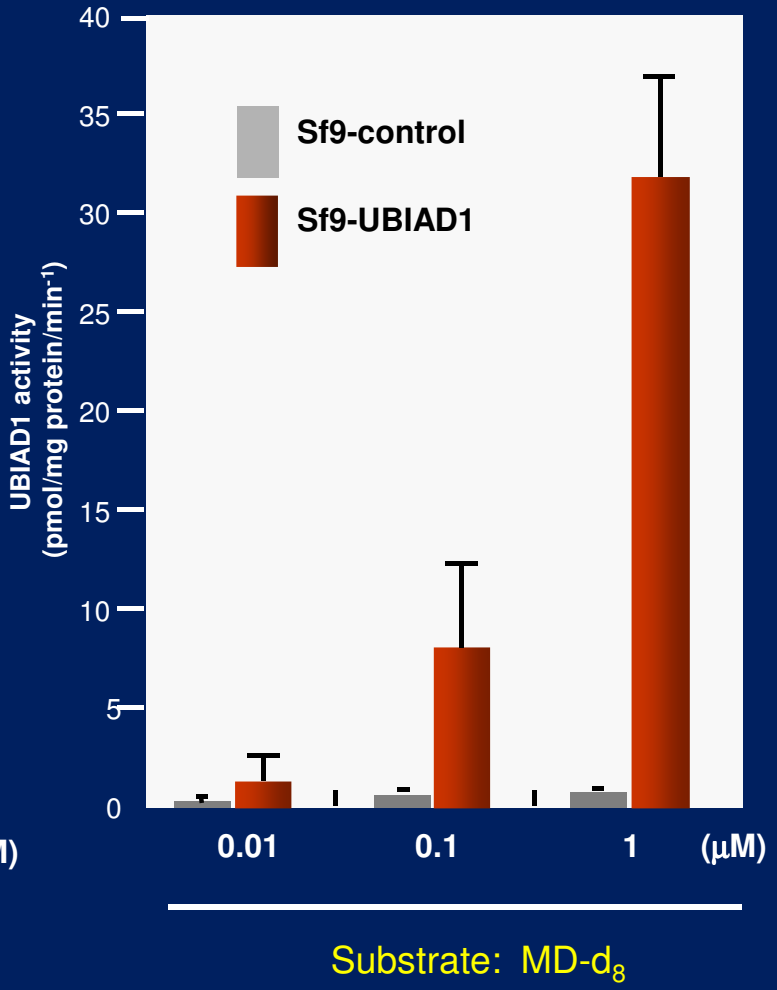
Conversion of PK-d_7 and MK-4-d_{12} into MK-4-d_7 in Sf9 cells transfected with siControl or pcDNA3.3-UBIAD1(UBIAD1 expression vector)



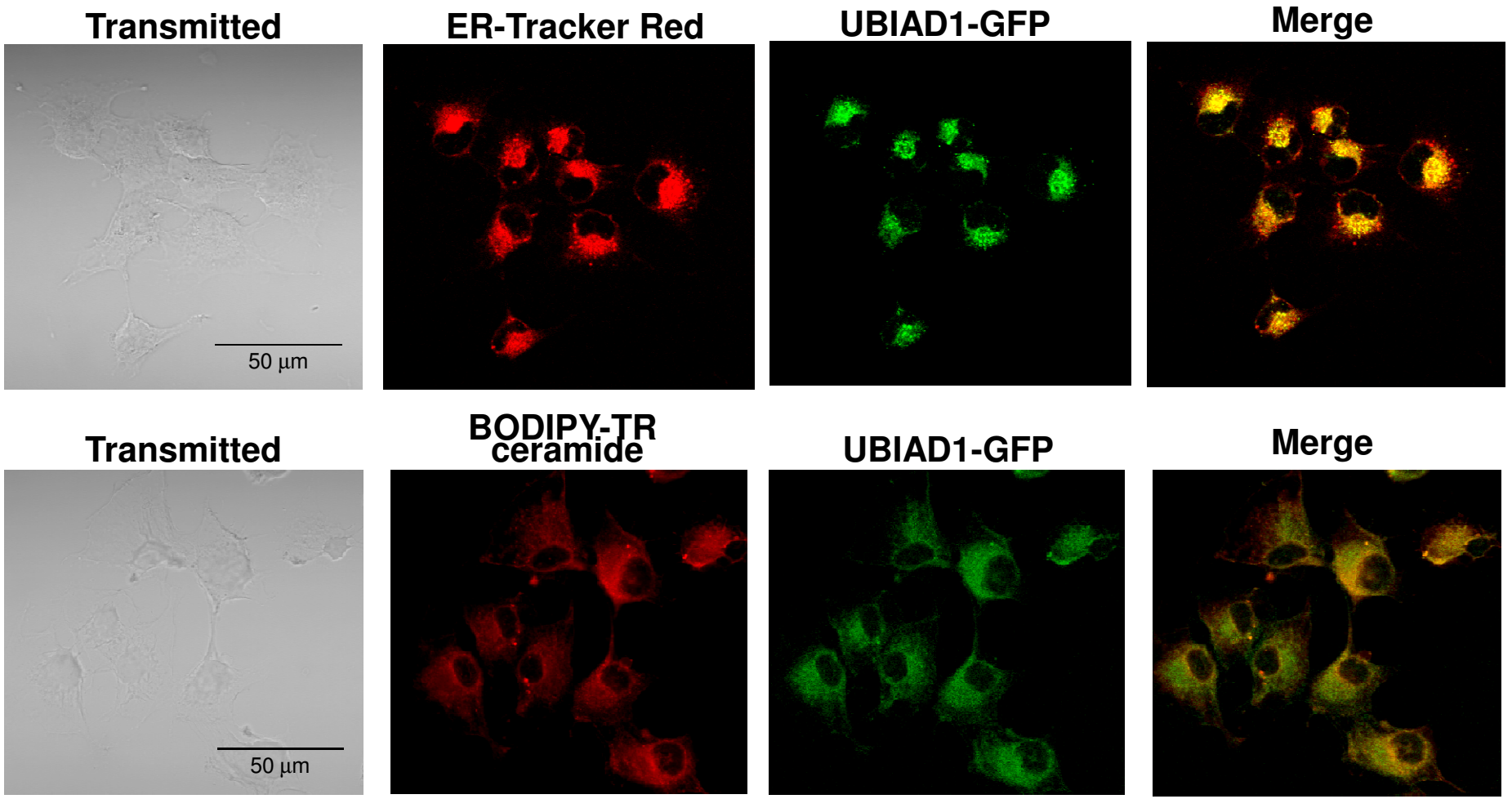
Values are means and s.e.m. (*n*=6). Three asterisks, P<0.001 versus control-infected Sf9 cells with the same compound treatment.

MK-4 biosynthetic activity of UBIAD1 in microsomes prepared from *UBIAD1* baculovirus-infected Sf9 cells with geranylgeranyl pyrophosphate (GGPP) and MD





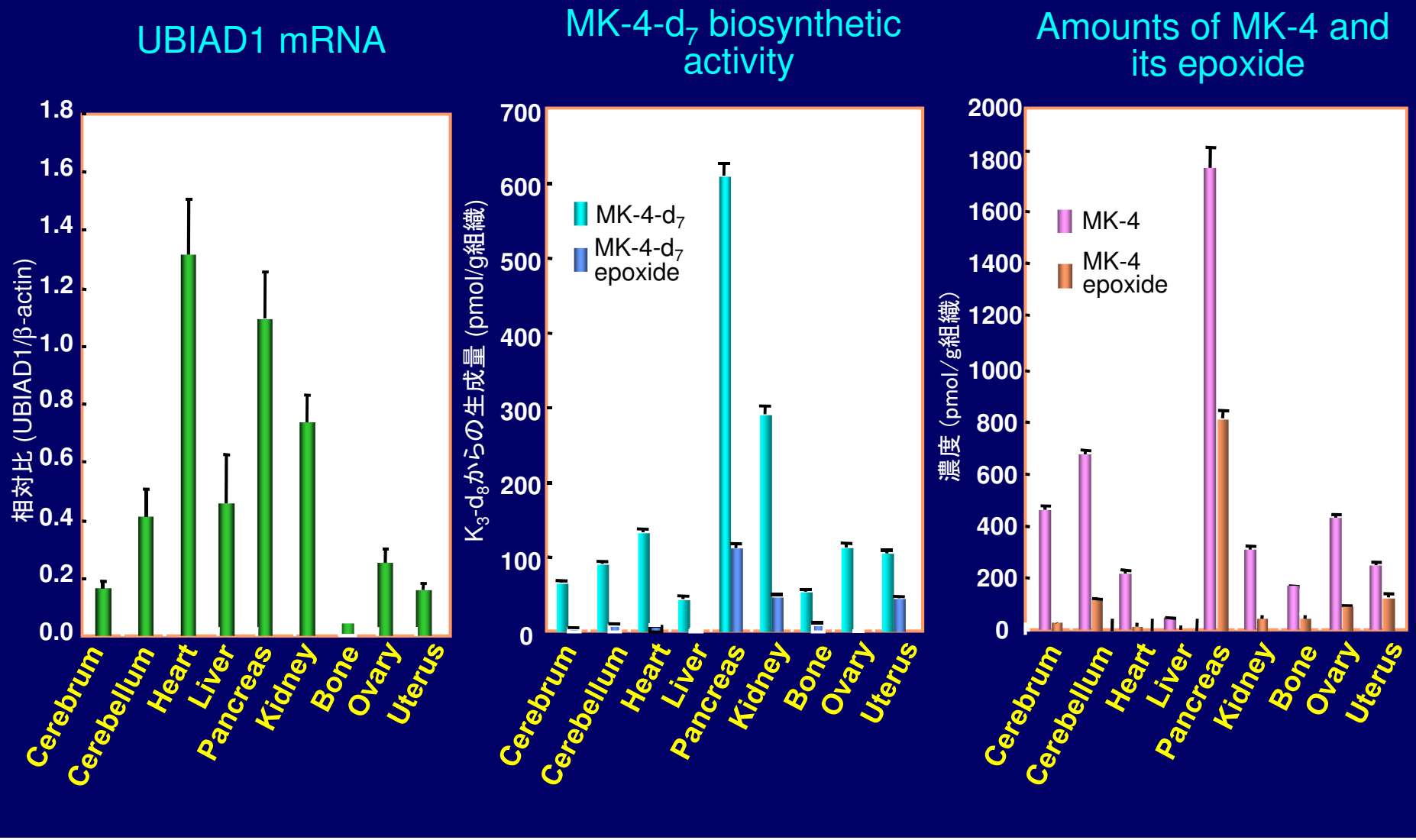
Subcellular localization of UBIAD1 in MG-63 cells



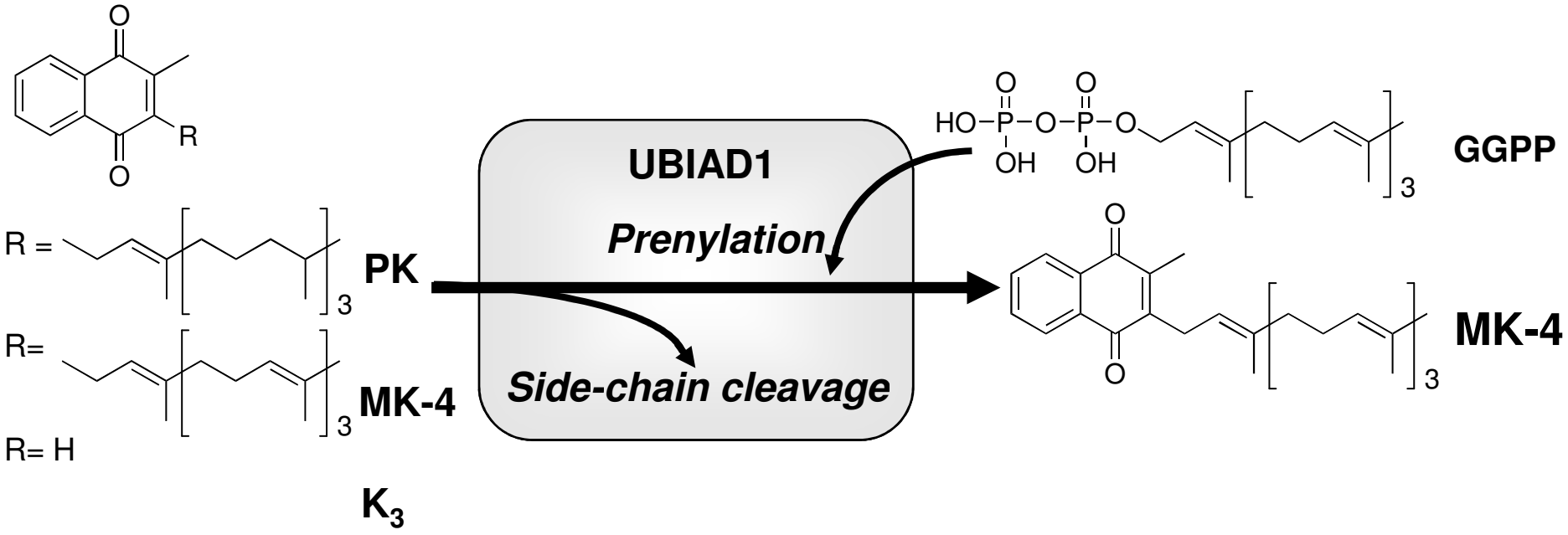
MG-63 cells stably transfected with a UBIAD1–GFP expression vector were stained with ER-tracker Red or BODIPY-TR ceramide (red) and were detected by GFP fluorescence (green). Merged images of GFP fluorescence and by ER-marker or Golgi-marker fluorescence are shown at the right. The control construct (mock-GFP) showed a diffuse fluorescence throughout the cytoplasm.

Nakagawa K. et al., Nature 2010; 468:117-121.

UBIAD1 mRNA expression, MK-4 biosynthetic activity, concentrations of MK-4 and its epoxide in tissues of female mice



UBIAD1 is a novel biosynthetic enzyme for MK-4 that may have both side-chain cleavage and prenylation activities



Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme

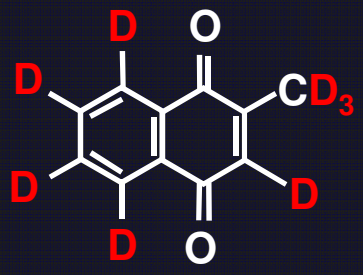
Nature 2010; 468:117-121.

Kimie Nakagawa, Yoshihisa Hirota, Natsumi Sawada, Naohito Yuge, Masato Watanabe, Yuri Uchino, Naoko Okuda, Yuka Shimomura, Yoshitomo Suhara & Toshio Okano
Department of Hygienic Sciences, Kobe Pharmaceutical University,

MK-4 biosynthesis in tissues is decreased by the treatments with statins and bisphosphonates

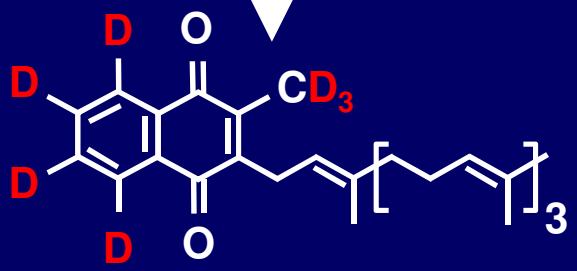
Vitamin K homologues

K_1-d_7



K_3-d_8

UBIAD1



MK-4-d_7

Mevalonate pathway

Acetyl-CoA

Statin

HMG-CoA

HMG-CoA reductase

Mevalonic acid

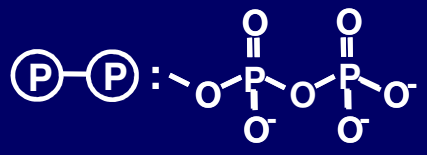
Bisphosphonate

Geranyl pyrophosphate (GPP)

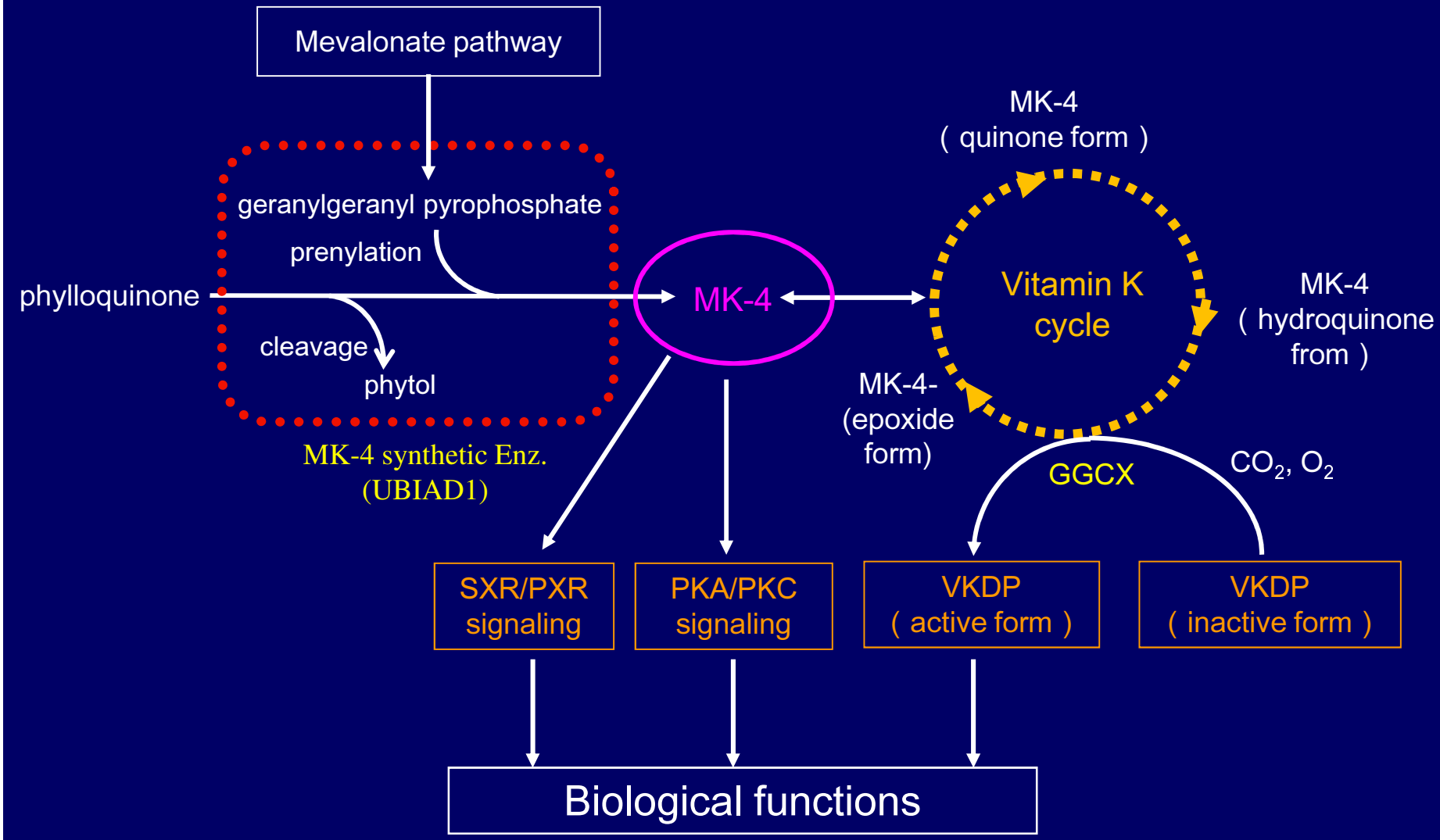
FPP synthase

Farnesyl pyrophosphate(FPP)

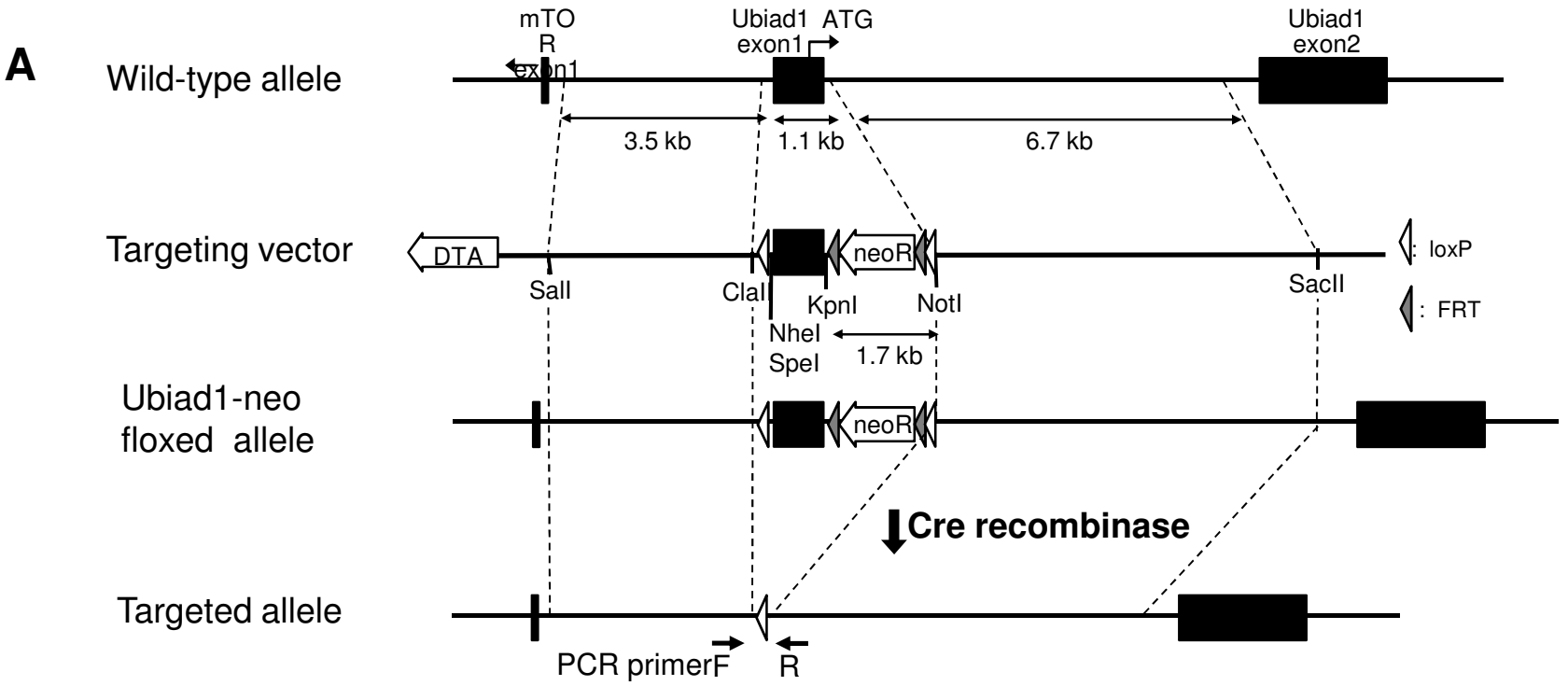
Geranylgeranyl pyrophosphate(GGPP)

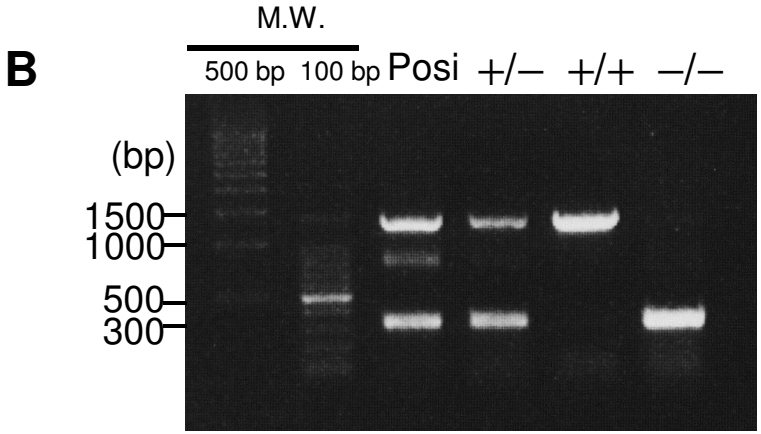


Possible interaction of MK-4 biosynthesis, vitamin K cycle and vitamin K action



Generation of *Ubiad1* knockout mice

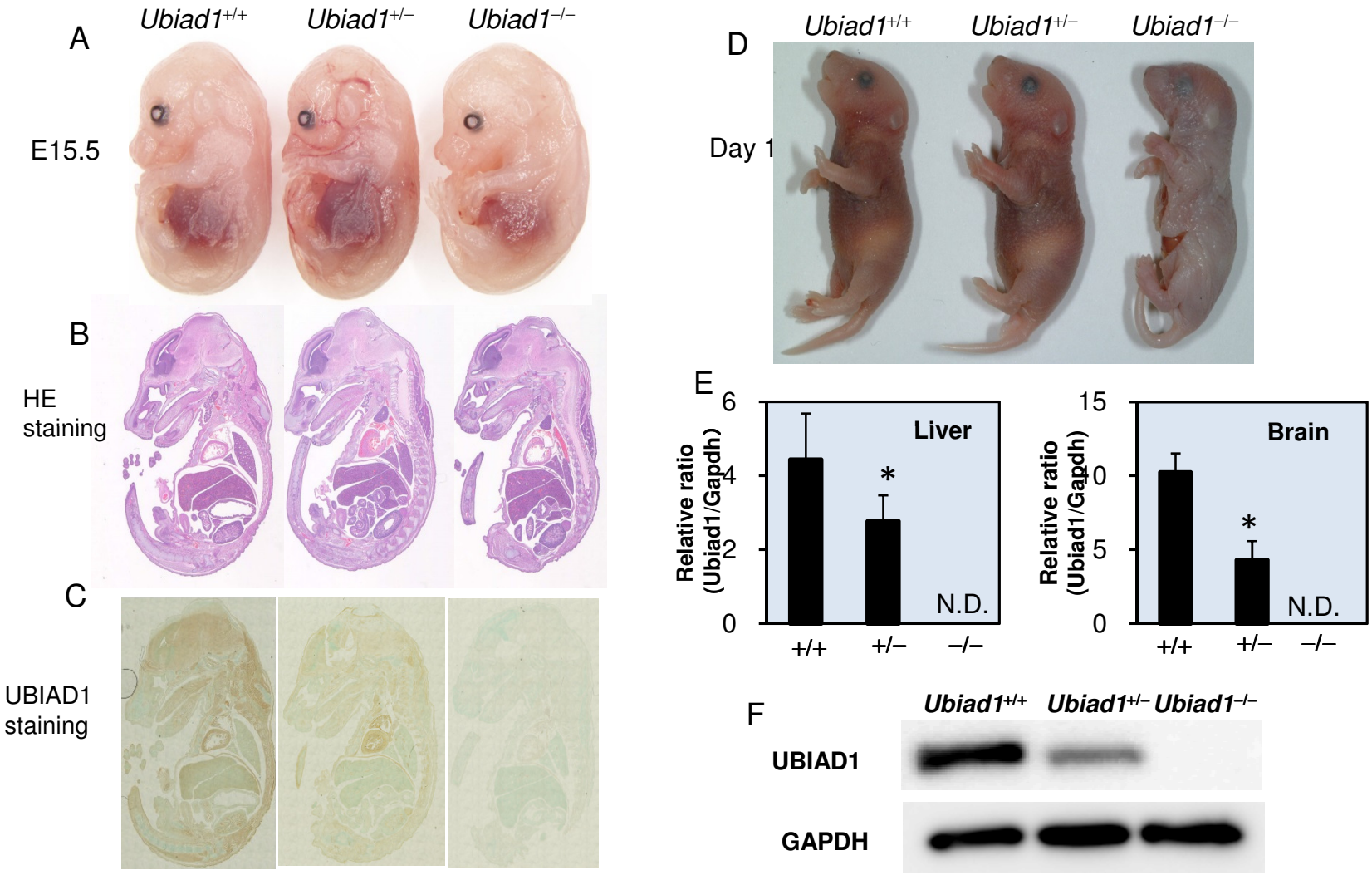




(A) Schematic presentation of ubiad1 genome, targeting vector and disrupted *Ubiad1* genome. (B) PCR genotyping of *Ubiad1*$^{+/+}$, *Ubiad1*$^{+/-}$ and *Ubiad1*$^{-/-}$ embryos. PCR genotyping of tail DNA of *Ubiad1*$^{+/+}$, *Ubiad1*$^{+/-}$ and *Ubiad1*$^{-/-}$ embryos. Lane 1, positive controls for *Ubiad1*$^{+/-}$ allele. Lane 2, PCR bands of *Ubiad1*$^{+/-}$ embryos. Lane 3, PCR bands of *Ubiad1*$^{+/+}$ embryos. Lane 4, PCR bands of *Ubiad1*$^{-/-}$ embryos.

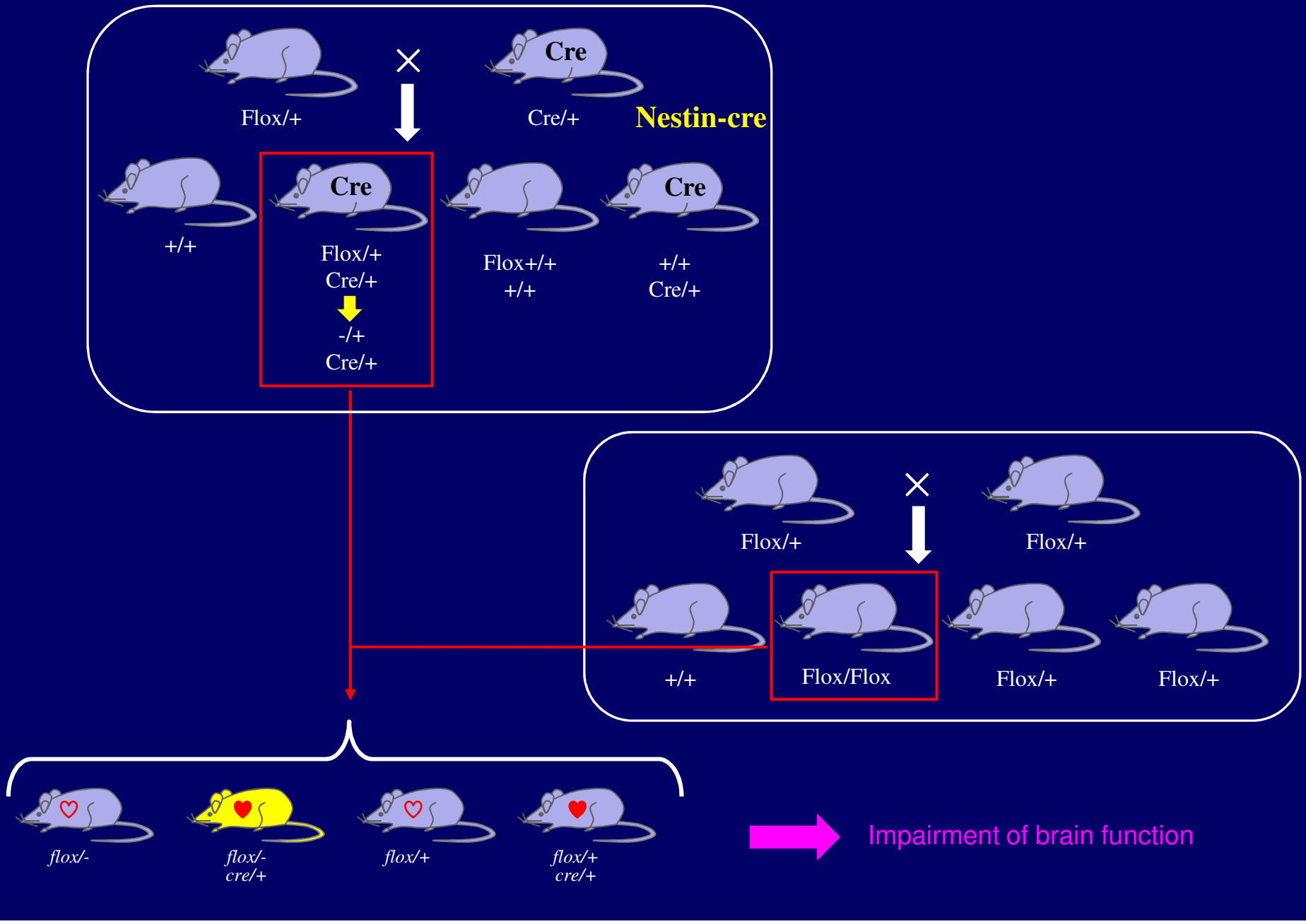
Nakagawa K, Okano T, et al, PLOS ONE 2014; 9: 1-12, e104078

Morphological examination of *Ubiad1*$^{+/+}$, *Ubiad1*$^{+/-}$ and *Ubiad1*$^{-/-}$ embryos and weanling mice (postnatal day 1) from pregnant *Ubiad1*$^{+/-}$ mice orally administered CoQ10



Ubiad1-deficient mouse embryos failed to survive beyond embryonic day 7.5 exhibiting small-sized body and gasturation arrest !!!

Generation of a neural cell specific *Ubiad1*$^{-/-}$ mouse




Thank you for your attention!