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Supplemental Materials and Methods

Proteomic analysis

Sperm from the caudae epididymides of C57BL/6 mice were collected in PBS containing complete protease inhibitor cocktail (Roche Applied Sciences; Indianapolis, IN), and washed two times at 500 g for 5 min at 4°C. Sperm pellets were collected by centrifugation at13,700 g for 15 min at 4°C, lysed in 1% Triton X-100 buffer (TX) (50 mM Tris-HCl, (pH 9.0), 1 mM EDTA, 1 mM EGTA, 2mM phenylmethylsulfonyl fluoride (PMSF), 2 mM DTT, one tablet of EDTA-free complete proteinase inhibitor cocktail (Roche Applied Sciences)] or 80 mM n-Octyl-b-D-glucopyranoside buffer (NOG) (10 mM Tris-HCl (pH 7.2), one tablet of complete protease inhibitor cocktail (Roche Applied Sciences)], and incubated for 2 h at 4°C. The sperm solutions were centrifuged at 13,700 g for 15 min at 4°C, and the supernatants were used as samples.

Sperm lysate samples (100 µg) from *Akap4*$^{-/-}$ (Miki et al., 2004) and WT mice were added to 200 µl of DeStreak rehydration solution and loaded on 11-cm Immobiline DryStrip (GE Healthcare Life Sciences, Piscataway NJ), pH3-11NL with IPG buffer pH3-11NL. The first dimension-isoelectric focusing (IEF) was run by IPGphor Isoelectric Focusing System (GE Healthcare Life Sciences) overnight, and the samples were resolved by rehydration for 12 h, at 500 volts/h for 1 h, at 1000 volts/h for 1 h, and at 16,000 volts/h for 2 h. The IPG sample strips were equilibrated twice for 15 min at room temperature (RT) in SDS equilibration buffer (50 mM Tris-Cl, (pH 8.8), 6 M urea, 30 % glycerol (v/v), 2 % SDS, bromophenol blue) containing freshly added 1 % DTT, followed by equilibration in the buffer containing 2.5 % iodoacetamide for 15 min at RT. The equilibrated IPG strips were placed on 8-16 % Criterion Tris-HCl precast gels (Bio-Rad, Hercules, CA), and subjected to second dimensional electrophoresis. After running, gels were fixed and stained overnight using SYPRO Ruby Protein Stains (Life Technologies

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Corp., Carlsbad, CA) according to the supplier's directions. The experiments were performed in triplicate.

Gel images were captured (Fluor-S Multimager, Bio-Rad) for analysis and comparison using Image J and PDQuest software. Differentially expressed proteins with 1.5 fold changes in the density level of the spot (Supplemental Fig. S1) were excised manually from gels, digested with trypsin (Promega, Madison, WI) for 8 hour in a Progest In-gel Digester (Genomics Solutions, Bath, UK), and analyzed by MALDI mass spectrometry (MALDI/MS). Samples were lyophilized to dryness and resuspended in 50:50 (v/v) 0.2 % formic acid:acetonitrile, and then 0.3 µl samples were spotted onto a 192-sample stainless steel MALDI plate and mixed with 0.3 µl of 33 % saturated α-cyano-hydroxycinnamic acid. Mass spectrometric analyses were performed with an AB 4700 Proteomics Analyzer (Life Technologies) in the positive ion and reflector modes. The MS was internally calibrated using autolytic tryptic peptides and the MS/MS calibrated externally using the fragment ions of the angiotensin I M+H ion (m/z 1296.68). A focus mass of m/z 2000 was used for the MS acquisition. For the MS/MS, 1000 volts were used for the collision energy and argon used as the collision gas with a recharge threshold set at 1.0×10^{-7} torr. Protein identification was performed by interrogating both the MS and the MS/MS using MASCOT and the entire NCBI non-redundant database. Search parameters included an allowance of two missed tryptic cleavages, a 0.06 Da mass tolerance for the MS data, a 0.1 Da mass tolerance for the MS/MS data, and an allowance for variable oxidation of methionine residues.

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Supplemental Table S1: PCR Primer Sequences

MGI Gene name	Chromosome (strand; location)	Primer Name (Amplified Region)	Primer (upper: forward; lower: reverse)	Amplified size (bp)	Primer position (upper: forward; lower: reverse)
Eno1 (Enolase 1, alpha non-neuron)	4 (+; 149610830-149622988	*Eno1* (5'UTR+ORF)	5'- GTG GGA GGC GCT TAG TGC TGC -3' 5'- GAG GCA GCC ACA TCC ATG CCA A -3'	869	31-51 898-878
Gm5506 (Predicted gene 5506)	18 (+; 48204989-48208032)	*Gm5506_v1* (5'UTR+ORF)	5'- GAA GGT GTG GCT GTT TAT TTT AC -3' 5'- CAC CGC AGC TCG GAA GAG ACC -3'	264	1-23 261-241
Eno4 (Enolase 4)	19 (+; 59017915-59045909)	*Eno4-5'ORF (ORF)*	5'- GAG AAC GAC GTC CCG CGC AA -3' 5'- TGG TGG AGG TGC GGG AAC CA -3'	489	117-136 605-586
		Eno4-3'ORF (ORF)	5'- AGC ATG GCC ATA GGC GCT GT -3' 5'- AGC TGC GCT TTT GTG GGT GC -3'	561	741-760 1301-1282
		Eno4-3'UTR (3'UTR)	5'- AGG GCA CAG CCA CTC CAC CA -3' 5'- GGT GCG TGA CTG GGC GTG AA -3'	297	1915-1934 2211-2192
		Eno4-full length (ORF+3'UTR)	5'- GTC CCG CGC AAG CTG GAA GAT -3' 5'- TCC AGA ACC GGG AGG GAG GC -3'	1784	126-146 1909-1890
		Eno4-3'ORF-ex13 (ORF including exon 13 nucleotides)	5'- CCG CTT CCG GGA GTG TCT CT -3' 5'- GCC CTG GGA GTC CAG AAC CG -3'	476	1444-1463 1919-1900
Rn18S		*Rn18S*	5'- GAC CCG GGG AGG TAG TGA CGA-3' 5'- GGA GCT GGA ATT ACC GCG GCT-3'	141	

NCBI Reference sequences: Eno4: NM_178689.4; Eno1:NM_023119.2; Gm5506: see Supplemental Fig. S4
ORF positions of each gene: Eno4: 33-1889; Eno1:154-1458

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Supplemental Table S2. qPCR Primer Sequences

Primer Name (Region)	Primer (upper: forward; lower: reverse)	Amplified size (bp)	Primer Position*
Eno4-5'	5'- ATG GGA GAT GAA GAC GGC GG-3'	146	33-52
(*Eno4* -5'ORF region)	5'- GCA GGC TGG AGG TAG AAG GT-3'		178-159
Eno4-3'ORF (exon13)	5'- GGT GCC CGG TTT ATC AAG TT -3'	105	1656-1675
(*Eno4* -exon13 region)	5'- CCA TAC TCC CCT CTG GAT GA -3'		1760-1741
Gapdh	5'- TGC ACC ACC AAC TGC TTA G-3'	176	
	5'- GAT GCA GGG ATG ATG TTC-3'		

*NCBI Reference sequences: *Eno4*: NM_178689.4

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Supplemental Table S3. Sperm Motility

	% motility			% progress motility		
	5 min	60 min	120 min	5 min	60 min	120 min
Eno4$^{+/+}$ (n=2)	45.6 ± 8.2	48.2 ± 10.1	37.4 ± 16.9	26.8 ± 3.0	24.4 ± 4.4	17.3 ± 8.0
Eno4$^{Gt/Gt}$ (n=2)	15.2 ± 2.9	8.0 ± 2.5	1.9 ± 0.2	5.7 ± 1.8[1]	2.5 ± 1.6	1.1 ± 0.3

[1] $p < 0.05$ compared to *Eno4* $^{+/+}$

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. Two-dimensional gel analysis of NOG extract of WT C57BL/6 sperm. The samples were prepared and analyzed as described in Supplemental Methods and Materials. The gel was silver stained and the proteins in the white box were determined by MS/MS analysis to be enolase 4 (ENO4).

Supplemental Fig. S2. Western blotting of kidney, brain, skeletal muscle, testis and sperm, using pan-enolase antibody (ENO) and antibodies to ENO2 and ENO3. Pan-enolase antibody detected a band in all tissues, while antibodies to ENO2 and ENO3 detected a band in brain and skeletal muscle, respectively, but not in sperm. The CBB stained gel for the ENO western blot is shown as a loading control.

Supplemental Fig. S3. Alignment of mouse ENO1, ENO2, ENO3 and ENO4 amino acid sequences. Gaps are show as (-), identities are show as (*), similarities are shown as (:).

Supplemental Fig. S4. Alignment of *Eno4*, *Eno4_v1* and *Eno4_v2* nucleotides and their deduced amino acid sequences. **A**) The underlined ATG in bold in the nucleotide sequences identifies the predicted initiation codons. Gaps are show as (-). **B**) The initiation methionine (**M**) of ENO4 and ENO4_V1 protein sequences is underlined. The predicted initiation methionine (**M**) of ENO4_V2 is shown in bold.

Supplemental Fig. S5. Alignment of *Gm5506* and *Gm5506_v1* nucleotides. Nucleotide sequences in 5'untranslated region of *Gm5506_v1* aligned with *Gm5506*. The underlined ATG identifies the predicted initiation codon. Gaps are show as (-).

Supplemental Fig. S6. Characterization of *Eno4* gene and of *Eno4* gene trap. **A**) Structure of WT *Eno4* and *Eno4* gene trap alleles. The gene trap vector (including a splice acceptor site (diagonal line box) and β-geo coding region integrated 300 base pair downstream of exon 1 of the *Eno4* gene. The relevant Hind 3 sites and probe positions used for Southern and Northern analysis are indicated. Probe 1 was located in the intron region downstream of the gene trap sequence; Probe 2 was located within the β-geo coding region. **B**) Southern analysis. DNA was isolated from testes of WT mice and of mice heterozygous ($Eno4^{+/Gt}$) or homozygous ($Eno4^{Gt/Gt}$) for the gene trap, digested with Hind 3 and hybridized with probe 1 (left panel) and probe 2 (right panel). The 1.6 and 9.0 Kb bands correspond to *Eno4* wild type and *Eno4* gene trap alleles, respectively. **C**) Northern analysis of mRNA isolated from testis of 3-month-old of WT and $Eno4^{Gt/Gt}$ mice using a [^{32}P]-labeled mouse *Eno4* cDNA probe located in the 3' portion of the ORF. **D**) Western blotting analysis of extracts of testis from 3-month-old WT and $Eno4^{Gt/Gt}$ mice with antibodies of ENO4 (left panels) and β-galactosidase (right panels). The antibody to ENO4 detected a band in WT testis, but not in $Eno4^{Gt/Gt}$ testis. The antibody to β-galactosidase did not detect a band in WT testis, but detected a band in $Eno4^{Gt/Gt}$ testis.

Supplemental Fig. S7. TUNEL assay for apoptosis in testis of WT and $Eno4^{Gt/Gt}$ mice. (**A**) TUNEL staining on sections of testis from WT (**A**) and $Eno4^{Gt/Gt}$ mice (**B**-**D**). TUNEL positive cells are shown as a fluorescent signal in the seminiferous tubules. Bars = 25 μm.

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Supplemental Fig. S8. Western blotting with sperm from WT and *Eno4*$^{Gt/Gt}$ mice using ENO4, pan-enolase (ENO) and AKAP4 antisera. ENO4 was detected in sperm from WT mice, but not in *Eno4*$^{Gt/Gt}$ mice (left upper panel). ENO was detected with antibody to pan-enolase in WT and *Eno4*$^{Gt/Gt}$ mice. ; the amount of ENO4 protein detected in *Eno4*$^{Gt/Gt}$ sperm extracts was appreciably lower than in sperm WT mice (left middle panel). AKAP4 was detected in WT and *Eno4*$^{Gt/Gt}$ mice; the amount of band detected with antibody to AKAP4 decreased in *Eno4*$^{Gt/Gt}$ mice. Antibody to α-tubulin was used as a loading control.

Supplemental Fig. S1

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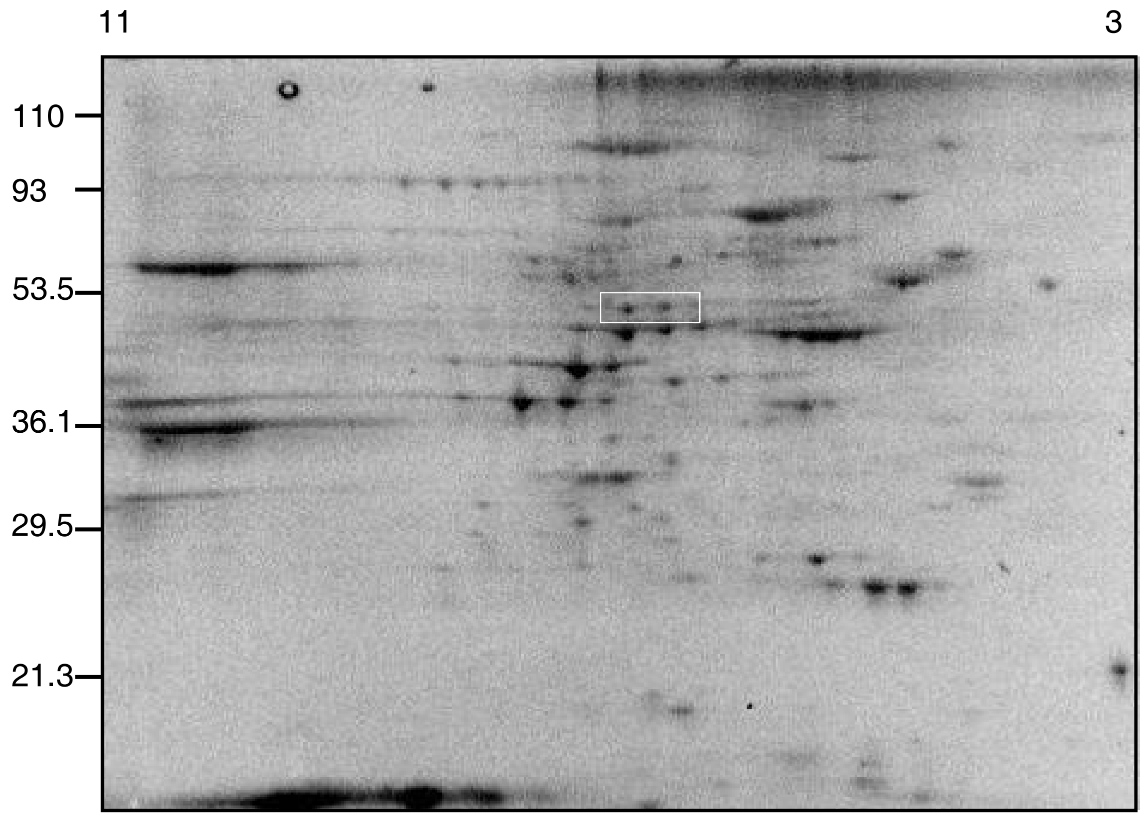

Supplemental Fig. S2

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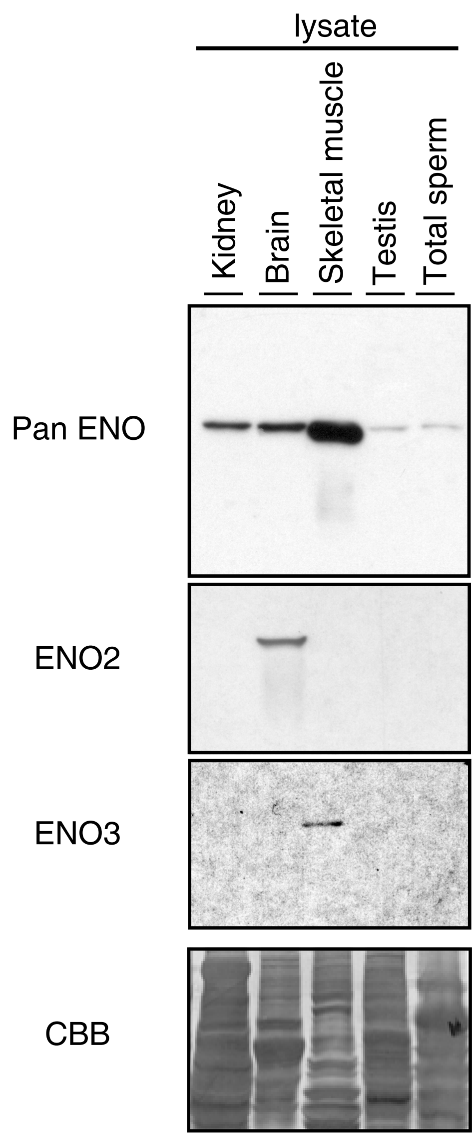

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Supplemental Fig. S3

```
ENO1            M-----------------------------------------------------------
ENO2            M-----------------------------------------------------------
ENO3            M-----------------------------------------------------------
ENO4            MGDEDGGRRGGITRDLQKLKQQAMAYYQENDVPRKLEDLLNSTFYLQPADVYGHLKANYF
                *

ENO1            -------SILRIHAREIFDSRGNPTVEVDLYTA-----KGLFRAAVPSG---ASTGIYEA
ENO2            -------SIEKIWAREILDSRGNPTVEVDLYTA-----KGLFRAAVPSG---ASTGIYEA
ENO3            -------AMQKIFAREILDSRGNPTVEVDLHTA-----KGRFRAAVPSG---ASTGIYEA
ENO4            SKLAKPPSICKIVGKTILDGLGLPTLQVEISCTIQNFPKYICAVAIPTHFEVVENALPEA
                       :: :* .: *:*. * **::*::  :     *    .*:*:    ....: **

ENO1            LELRDNDKTRFMGKGVSQAVEHINKTIAPAL----------VSKKVNVVEQEKI--DKLM
ENO2            LELRDGDKQRYLGKGVLKAVDHINSRIAPAL----------ISSGISVVEQEKL--DNLM
ENO3            LELRDGDKARYLGKGVLKAVEHINKTLGPAL----------LEKKLSVVDQEKV--DKFM
ENO4            LDAEDSERA----QAVNTAVQWINQSITEELWGLVPSNQAEVDHRLRTFFEHKVQEDKER
                *: .*.::     :.*  **: **. :   *          :.  : .. :.*:  *:

ENO1            IEMDGTE-----------------------NKSK-------------------------F
ENO2            LELDGTE-----------------------NKSK-------------------------F
ENO3            IELDGTE-----------------------NKSK-------------------------F
ENO4            KELEKSQEELVPAPPPVTLPPPPPPPPPPPSKKKGQKAGRRDTLLEKPVSPPEPPEPVLH
                 *:: ::                       .*.*                         .

ENO1            GANAILGVSLAVCKAGAVEKGVPLYRHIADLAGNPE----VILPVPAFNVINGGSHAGNK
ENO2            GANAILGVSLAVCKAGAAERDLPLYRHIAQLAGNSD----LILPVPAFNVINGGSHAGNK
ENO3            GANAILGVSLAVCKAGAAEKGVPLYRHIADLAGNPD----LVLPVPAFNVINGGSHAGNK
ENO4            GSMAIGAVSLAVAKASATLASDPLYLTLASLKHDQEQPSTFSMPLLMGSVLSCGKSSPGK
                *: ** .*****.**.*.   . ***  :*.*  : :    . :*:   .*:. *. : .*

ENO1            L-AMQEFMILPVGASSFREAMRIGAEVYHNLKNVI---------KEKYGKDAT--------
ENO2            L-AMQEFMILPVGAESFRDAMRLGAEVYHTLKGVI---------KDKYGKDAT--------
ENO3            L-AMQEFMILPVGASSFKEAMRIGAEVYHHLKGVI---------KAKYGKDAT--------
ENO4            LHLMKEVICIPSPGLTAKQSVELLLEIQKQVNRAMETLPPPKQETKKGHNGSKRAQPPIT
                *  *:*.: :*  . : ::::.:  *: : :: .:         : * *::.:

ENO1            -NVGDEGGFAPNILENKEALELLKTAIAKAGYTDQVV--IGMDVAASEF--YRSGKYDLD
ENO2            -NVGDEGGFAPNILENSEALELVKEAIDKAGYTEKMV--IGMDVAASEF--YRDGKYDLD
ENO3            -NVGDEGGFAPNILENNEALELLKTAIQAAGYPDKVV--IGMDVAASEF--YRNGKYDLD
ENO4            GKVSHLGCLTINYDAIEQPLLLLQGICSNLGLELGVNFHLAINCAGHELMDYSKGKYEVM
                 :*.. * :: *    .:.* *::       *    :   :.:: *. *:  * .***::

ENO1            FKSPDDPSRYITPDQLADLYKSFVQNYP-VVSIEDPFDQDDWGAWQKFTASAGIQ---VV
ENO2            FKSPADPSRYITGDQLGALYQDFVRNYP-VVSIEDPFDQDDWAAWSKFTANVGIQ---IV
ENO3            FKSPDDPARHISGEKLGELYKNFIQNYP-VVSIEDPFDQDDWATWTSFLSGVDIQ---IV
ENO4            VGTHKSAA------EMVELYVDLINKYPSIIALIDPFRKEDAEQWDSLYAALASRCYLIA
                . :  ..:       ::  ** .::.:** :::: *** ::*    * .: :     :   :.

ENO1            GDDLTVTNPKRIAKAASEKSCNCLLLKVNQIGSVTESLQACKLAQSNGWGVMVSHRSGET
ENO2            GDDLTVTNPKRIERAVEEKACNCLLLKVNQIGSVTEAIQACKLAQENGWGVMVSHRSGET
ENO3            GDDLTVTNPKRIAQAVEKKACNCLLLKVNQIGSVTESIQACKLAQSNGWGVMVSHRSGET
ENO4            GAASGSVSKLLECRNISTLKSHGLIIKHTNQTTMSDLVEITHLINGKKLLAVFGSTDSES
                *      ..      :  .   .: *::* .:  :::: ::  :* : :    .:..  ..*:

ENO1            EDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQILRIEEELGSKAKFAGRSFRN------
ENO2            EDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLMRIEEELGDEARFAGHNFRN------
ENO3            EDTFIADLVVGLCTGQIKTGAPCRSERLAKYNQLMRIEEALGDKAVFAGRKFRN------
ENO4            SDDSLVDLAVGFGARFIKLGGLSRGERMTKYNRLLAIEEELIQRGVWGFSEEHNFSFFQE
                .*  :.**.**: :  ** *. .*.**::***::: *** * ... :.  . :*
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```
ENO1            -------------------------PLAK
ENO2            -------------------------PSVL
ENO3            -------------------------PKAK
ENO4            DATATMAEETLGLLDSIFPTEVIEESAKT
```

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Supplemental Fig. S4

A

```
Eno4      ACCATGGGAGATGAAGACGGCGGCCGCAGAGGCGGGATCACTAGGGACCTGCAGAAGCTG    60
Eno4_v1   ACCATGGGAGATGAAGACGGCGGCCGCAGAGGCGGGATCACTAGGGACCTGCAGAAGCTG    60
Eno4_v2   ------------------------------------------------------------

Eno4      AAGCAGCAGGCGATGGCGTACTACCAGGAGAACGACGTCCCGCGCAAGCTGGAAGATCTG    120
Eno4_v1   AAGCAGCAGGCGATGGCGTACTACCAGGAGAACGACGTCCCGCGCAAGCTGGAAGATCTG    120
Eno4_v2   ------------------------------------------------------------

Eno4      CTCAACTCCACCTTCTACCTCCAGCCTGCGGATGTCTACGGGCACCTGAAGGCAAACTAC    180
Eno4_v1   CTCAACTCCACCTTCTACCTCCAGCCTGCGGATGTCTACGGGCACCTGAAGGCAAACTAC    180
Eno4_v2   ------------------------------------------------------------

Eno4      TTTTCTAAACTTGCGAAGCCTCCCTCCATATGCAAAATAGTAGGGAAAACCATACTGGAT    240
Eno4_v1   TTTTCTAAACTTGCGAAGCCTCCCTCCATATGCAAAATAGTAGGGAAAACCATACTGGAT    240
Eno4_v2   ------------------------------------------------------------

Eno4      GGCCTGGGACTTCCTACTCTACAGGTGGAAATCTCCTGCACCATTCAAAATTTCCCCAAG    300
Eno4_v1   GGCCTGGGACTTCCTACTCTACAGGTGGAAATCTCCTGCACCATTCAAAATTTCCCCAAG    300
Eno4_v2   ------------------------------------------------------------

Eno4      TACATCTGTGCCGTGGCGATCCCTACGCACTTTGAAGTCGTGGAGAACGCTTTGCCAGAG    360
Eno4_v1   TACATCTGTGCCGTGGCGATCCCTACGCACTTTGAAGTCGTGGAGAACGCTTTGCCAGAG    360
Eno4_v2   ------------------------------------------------------------

Eno4      GCGTTGGATGCAGAGGACTCAGAAAGGGCCCAGGCCGTGAACACAGCGGTGCAGTGGATC    420
Eno4_v1   GCGTTGGATGCAGAGGACTCAGAAAGGGCCCAGGCCGTGAACACAGCGGTGCAGTGGATC    420
Eno4_v2   ------------------------------------------------------------

Eno4      AATCAGTCCATCACAGAGGAGCTGTGGGGTCTGGTCCCCTCCAACCAGGCAGAGGTGGAC    480
Eno4_v1   AATCAGTCCATCACAGAGGAGCTGTGGGGTCTGGTCCCCTCCAACCAGGCAGAGGTGGAC    480
Eno4_v2   ------------------------------------------------------------

Eno4      CACTGGCTCAGGACCTTCTTTGAACATAAAGTACAAGAAGATAAAGAGAGAAAAGAATTG    540
Eno4_v1   CACTGGCTCAGGACCTTCTTTGAACATAAAGTACAAGAAGATAAAGAGAGAAAAGAATTG    540
Eno4_v2   ------------------------------------------------------------

Eno4      GAAAAGAGCCAGGAGGAGCTGGTTCCCGCACCTCCACCAGTAACGCTGCCGCCGCCGCCG    600
Eno4_v1   GAAAAGAGCCAGGAGGAGCTGGTTCCCGCACCTCCACCAGTAACGCTGCCGCCGCCGCCG    600
Eno4_v2   ------------------------------------------------------------

Eno4      CCGCCGCCTCCCCCACCTCCCTCCAAAAAGAAAGGGCAGAAGGCAGGACGGAGGGATACG    660
Eno4_v1   CCGCCGCCTCCCCCACCTCCCTCCAAAAAGAAAGGGCAGAAGGCAGGACGGAGGGATACG    660
Eno4_v2   ---------------------------------------------GGACGGAGGGATACG    15

Eno4      CTTTTGGAGAAACCTGTTTCACCCCCGGAGCCCCCTGAACCAGTCCTCCATGGTAGCATG    720
Eno4_v1   CTTTTGGAGAAACCTGTTTCACCCCCGGAGCCCCCTGAACCAGTCCTCCATGGTAGCATG    720
Eno4_v2   CTTTTGGAGAAACCTGTTTCACCCCCGGAGCCCCCTGAACCAGTCCTCCATGGTAGCATG    75

Eno4      GCCATAGGCGCTGTGTCCCTGGCAGTGGCCAAAGCCAGCGCAACCTTGGCCAGCGATCCT    780
Eno4_v1   GCCATAGGCGCTGTGTCCCTGGCAGTGGCCAAAGCCAGCGCAACCTTGGCCAGCGATCCT    780
Eno4_v2   GCCATAGGCGCTGTGTCCCTGGCAGTGGCCAAAGCCAGCGCAACCTTGGCCAGCGATCCT    135
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Eno4      CTCTACTTAACCCTGGCGTCACTGAAGCATGATCAGGAGCAGCCGTCAACATTCTCTATG    840
Eno4_v1   CTCTACTTAACCCTGGCGTCACTGAAGCATGATCAGGAGCAGCCGTCAACATTCTCTATG    840
Eno4_v2   CTCTACTTAACCCTGGCGTCACTGAAGCATGATCAGGAGCAGCCGTCAACATTCTCTATG    195

Eno4      CCTTTGCTGATGGGATCTGTACTGAGCTGTGGGAAGTCGTCACCTGGGAAGCTACATTTA    900
Eno4_v1   CCTTTGCTGATGGGATCTGTACTGAGCTGTGGGAAGTCGTCACCTGGGAAGCTACATTTA    900
Eno4_v2   CCTTTGCTGATGGGATCTGTACTGAGCTGTGGGAAGTCGTCACCTGGGAAGCTACATTTA    255

Eno4      ATGAAAGAAGTGATTTGTATTCCCAGTCCTGGATTAACAGCCAAACAAAGTGTCGAGTTG    960
Eno4_v1   ATGAAAGAAGTGATTTGTATTCCCAGTCCTGGATTAACAGCCAAACAAAGTGTCGAGTTG    960
Eno4_v2   ATGAAAGAAGTGATTTGTATTCCCAGTCCTGGATTAACAGCCAAACAAAGTGTCGAGTTG    315

Eno4      CTTCTGGAAATTCAGAAACAAGTTAACAGAGCGATGGAGACGCTTCCACCTCCAAAACAA    1020
Eno4_v1   CTTCTGGAAATTCAGAAACAAGTTAACAGAGCGATGGAGACGCTTCCACCTCCAAAACAA    1020
Eno4_v2   CTTCTGGAAATTCAGAAACAAGTTAACAGAGCGATGGAGACGCTTCCACCTCCAAAACAA    375

Eno4      GAGACCAAAAAAGGGCATAATGGGAGCAAAAGAGCTCAGCCACCGATCACTGGCAAGGTA    1080
Eno4_v1   GAGACCAAAAAAGGGCATAATGGGAGCAAAAGAGCTCAGCCACCGATCACTGGCAAGGTA    1080
Eno4_v2   GAGACCAAAAAAGGGCATAATGGGAGCAAAAGAGCTCAGCCACCGATCACTGGCAAGGTA    435

Eno4      TCTCACCTTGGCTGTTTAACCATCAACTACGACGCCATAGAGCAGCCACTGCTGCTGCTT    1140
Eno4_v1   TCTCACCTTGGCTGTTTAACCATCAACTACGACGCCATAGAGCAGCCACTGCTGCTGCTT    1140
Eno4_v2   TCTCACCTTGGCTGTTTAACCATCAACTACGACGCCATAGAGCAGCCACTGCTGCTGCTT    495

Eno4      CAGGGGATCTGCTCAAACCTGGGGTTGGAACTGGGAGTGAACTTCCATCTAGCCATCAAC    1200
Eno4_v1   CAGGGGATCTGCTCAAACCTGGGGTTGGAACTGGGAGTGAACTTCCATCTAGCCATCAAC    1200
Eno4_v2   CAGGGGATCTGCTCAAACCTGGGGTTGGAACTGGGAGTGAACTTCCATCTAGCCATCAAC    555

Eno4      TGTGCTGGGCATGAGCTGATGGACTACAGTAAAGGGAAGTACGAAGTGATGGTGGGCACC    1260
Eno4_v1   TGTGCTGGGCATGAGCTGATGGACTACAGTAAAGGGAAGTACGAAGTGATGGTGGGCACC    1260
Eno4_v2   TGTGCTGGGCATGAGCTGATGGACTACAGTAAAGGGAAGTACGAAGTGATGGTGGGCACC    615

Eno4      CACAAAAGCGCAGCTGAGATGGTGGAGCTCTACGTGGACCTGATCAACAAGTATCCTTCC    1320
Eno4_v1   CACAAAAGCGCAGCTGAGATGGTGGAGCTCTACGTGGACCTGATCAACAAGTATCCTTCC    1320
Eno4_v2   CACAAAAGCGCAGCTGAGATGGTGGAGCTCTACGTGGACCTGATCAACAAGTATCCTTCC    675

Eno4      ATAATCGCCTTAATTGATCCTTTCAGGAAGGAGGACGCCGAGCAGTGGGACAGCCTCTAT    1380
Eno4_v1   ATAATCGCCTTAATTGATCCTTTCAGGAAGGAGGACGCCGAGCAGTGGGACAGCCTCTAT    1380
Eno4_v2   ATAATCGCCTTAATTGATCCTTTCAGGAAGGAGGACGCCGAGCAGTGGGACAGCCTCTAT    735

Eno4      GCTGCTCTGGCTTCCCGGTGCTACCTAATTGCAGGCGCCGCTTCCGGGAGTGTCTCTAAA    1440
Eno4_v1   GCTGCTCTGGCTTCCCGGTGCTACCTAATTGCAGGCGCCGCTTCCGGGAGTGTCTCTAAA    1440
Eno4_v2   GCTGCTCTGGCTTCCCGGTGCTACCTAATTGCAGGCGCCGCTTCCGGGAGTGTCTCTAAA    795

Eno4      CTCCTAGAGTGCAGAAATATAAGCACCCTGAAATCCCACGGACTGATCATAAAGCACACA    1500
Eno4_v1   CTCCTAGAGTGCAGAAATATAAGCACCCTGAAATCCCACGGACTGATCATAAAGCACACA    1500
Eno4_v2   CTCCTAGAGTGCAGAAATATAAGCACCCTGAAATCCCACGGACTGATCATAAAGCACACA    855

Eno4      AACCAAACCACAATGTCTGACTTGGTGGAAATAACCCATCTTATCAACGGTAAGAAGCTC    1560
Eno4_v1   AACCAAACCACAATGTCTGACTTGGTGGAAATAACCCATCTTATCAACGGTAAGAAGCTC    1560
Eno4_v2   AACCAAACCACAATGTCTGACTTGGTGGAAATAACCCATCTTATCAACGGTAAGAAGCTC    915

Eno4      CTGGCCGTCTTTGGAAGCACAGACTCGGAGTCCTCTGATGACAGCCTTGTCGATTTGGCT    1620
Eno4_v1   CTGGCCGTCTTTGGAAGCACAGACTCGGAGTCCTCTGATGACAGCCTTGTCGATTT----    1616
Eno4_v2   CTGGCCGTCTTTGGAAGCACAGACTCGGAGTCCTCTGATGACAGCCTTGTCGATTTGGCT    975

Eno4      GTTGGATTCGGTGCCCGGTTTATCAAGTTGGGGGGTCTTTCTCGGGGTGAACGGATGACC    1680
```

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Eno4_v1   ------------------------------------------------------------
Eno4_v2   GTTGGATTCGGTGCCCGGTTTATCAAGTTGGGGGGTCTTTCTCGGGGTGAACGGATGACC     1035

Eno4      AAATACAACCGCCTTCTTGCTATAGAGGAAGAACTCATCCAGAGGGGAGTATGGGGTTTC     1740
Eno4_v1   ------------------------------------------------------GGTTTC     1622
Eno4_v2   AAATACAACCGCCTTCTTGCTATAGAGGAAGAACTCATCCAGAGGGGAGTATGGGGTTTC     1095

Eno4      AGTGAAGAACACAATTTTTCTTTCTTTCAAGAGGATGCTACTGCCACAATGGCTGAGGAA     1800
Eno4_v1   AGTGAAGAACACAATTTTTCTTTCTTTCAAGAGGATGCTACTGCCACAATGGCTGAGGAA     1682
Eno4_v2   AGTGAAGAACACAATTTTTCTTTCTTTCAAGAGGATGCTACTGCCACAATGGCTGAGGAA     1155

Eno4      ACTCTTGGGCTCCTGGACTCCATCTTCCCCACAGAGGTGATAGAGGAATCGGCTAAAACA     1880
Eno4_v1   ACTCTTGGGCTCCTGGACTCCATCTTCCCCACAGAGGTGATAGAGGAATCGGCTAAAACA     1742
Eno4_v2   ACTCTTGGGCTCCTGGACTCCATCTTCCCCACAGAGGTGATAGAGGAATCGGCTAAAACA     1215

Eno4      TGA                                                              1883
Eno4_v1   TGA                                                              1745
Eno4_v2   TGA                                                              1218
```

B

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ENO4      MGDEDGGRRGGITRDLQKLKQQAMAYYQENDVPRKLEDLLNSTFYLQPADVYGHLANYFS     60
ENO4_v1   MGDEDGGRRGGITRDLQKLKQQAMAYYQENDVPRKLEDLLNSTFYLQPADVYGHLANYFS     60
ENO4_v2   ------------------------------------------------------------

ENO4      KLAKPPSICKIVGKTILDGLGLPTLQVEISCTIQNFPKYICAVAIPTHFEVVENALPEAL     120
ENO4_v1   KLAKPPSICKIVGKTILDGLGLPTLQVEISCTIQNFPKYICAVAIPTHFEVVENALPEAL     120
ENO4_v2   ------------------------------------------------------------

ENO4      DAEDSERAQAVNTAVQWINQSITEELWGLVPSNQAEVDHRLRTFFEHKVQEDKERKELEK     180
ENO4_v1   DAEDSERAQAVNTAVQWINQSITEELWGLVPSNQAEVDHRLRTFFEHKVQEDKERKELEK     180
ENO4_v2   ------------------------------------------------------------

ENO4      SQEELVPAPPPVTLPPPPPPPPPPPSKKKGQKAGRRDTLLEKPVSPPEPPEPVLHGSMAI     240
ENO4_v1   SQEELVPAPPPVTLPPPPPPPPPPPSKKKGQKAGRRDTLLEKPVSPPEPPEPVLHGSMAI     240
ENO4_v2   -------------------------------------------------------GSMAI     5

ENO4      GAVSLAVAKASATLASDPLYLTLASLKHDQEQPSTFSMPLLMGSVLSCGKSSPGKLHLMK     300
ENO4_v1   GAVSLAVAKASATLASDPLYLTLASLKHDQEQPSTFSMPLLMGSVLSCGKSSPGKLHLMK     300
ENO4_v2   GAVSLAVAKASATLASDPLYLTLASLKHDQEQPSTFSMPLLMGSVLSCGKSSPGKLHLMK     65

ENO4      EVICIPSPGLTAKQSVELLLEIQKQVNRAMETLPPPKQETKKGHNGSKRAQPPITGKVSH     360
ENO4_v1   EVICIPSPGLTAKQSVELLLEIQKQVNRAMETLPPPKQETKKGHNGSKRAQPPITGKVSH     360
ENO4_v2   EVICIPSPGLTAKQSVELLLEIQKQVNRAMETLPPPKQETKKGHNGSKRAQPPITGKVSH     125

ENO4      LGCLTINYDAIEQPLLLLQGICSNLGLELGVNFHLAINCAGHELMDYSKGKYEVMVGTHK     420
ENO4_v1   LGCLTINYDAIEQPLLLLQGICSNLGLELGVNFHLAINCAGHELMDYSKGKYEVMVGTHK     420
ENO4_v2   LGCLTINYDAIEQPLLLLQGICSNLGLELGVNFHLAINCAGHELMDYSKGKYEVMVGTHK     185

ENO4      SAAEMVELYVDLINKYPSIIALIDPFRKEDAEQWDSLYAALASRCYLIAGAASGSVSKLL     480
ENO4_v1   SAAEMVELYVDLINKYPSIIALIDPFRKEDAEQWDSLYAALASRCYLIAGAASGSVSKLL     480
ENO4_v2   SAAEMVELYVDLINKYPSIIALIDPFRKEDAEQWDSLYAALASRCYLIAGAASGSVSKLL     245
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ENO4     ECRNISTLKSHGLIIKHTNQTTMSDLVEITHLINGKKLLAVFGSTDSESSDDSLVDLAVG    540
ENO4_v1  ECRNISTLKSHGLIIKHTNQTTMSDLVEITHLINGKKLLAVFGSTDSESSDDSLVDLVSV    540
ENO4_v2  ECRNISTLKSHGLIIKHTNQTTMSDLVEITHLINGKKLLAVFGSTDSESSDDSLVDLAVG    305

ENO4     FGARFIKLGGLSRGERMTKYNRLLAIEEELIQRGVWGFSEEHNFSFFQEDATATMAEETL    600
ENO4_v1  KNTIFLSFKRMLLPQWLRKlLGSWTPSSPQR-----------------------------    572
ENO4_v2  FGARFIKLGGLSRGERMTKYNRLLAIEEELIQRGVWGFSEEHNFSFFQEDATATMAEETL    365

ENO4     GLLDSIFPTEVIEESAKT                                              618
ENO4_v1  ------------------                                              572
ENO4_v2  GLLDSIFPTEVIEESAKT                                              388
```

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Supplemental Fig. S5

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Gm5506     -----------------------GAGGTCCTGATACCAACACAGACACTGCTAGAAACTAT   38
Gm5506_v1  GAAGGTGTGGCTGTTTATTTTACAGTTCTGTTAGAAAAAAACAGAGAGTGCTGCTCCGGT   60

Gm5506     TGTGGTTTGGTCTAATGCTTGAATTATGTTAATCGGGTTCCTCAAATTGCATGAGAATCT   98
Gm5506_v1  ACAGGGTCGCGCTCGCGTCTGTCCTTAAGGCTCTCCTCGGTGTCCACGGCACCCTCTTTC   120

Gm5506     GCATGTAAGAAGCGATCCTACTGCCAGAAATTCGCCATGTCTATTCTCAGGATCCACGCC   158
                     ::::::::::::::::::::::::::::::::::::::::::::::::::
Gm5506_v1  CTTGCTTTGCAGCGATCCTACTGCCAGAAATTCGCCATGTCTATTCTCAGGATCCACGCC   180

Gm5506     AGAGAGATCTTTGACTCCCGTGGGAATCCCACTGTTGAGGTCGATCTGTACACCGCAAAA   218
           ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Gm5506_v1  AGAGAGATCTTTGACTCCCGTGGGAATCCCACTGTTGAGGTCGATCTGTACACCGCAAAA   240

Gm5506     GGTCTCTTCCGAGCTGCGGTGCCCAGCGGTGCGTCCACTGGCATCTACGAGGCCCTAGAA   278
           ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Gm5506_v1  GGTCTCTTCCGAGCTGCGGTGCCCAGCGGTGCGTCCACTGGCATCTACGAGGCCCTAGAA   300

Gm5506     CTCCGAGACAATGATAAGACCCGCTTCATGGGGAAGGGTGTCTCACAGGCTGTTGAGCAC   338
           ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Gm5506_v1  CTCCGAGACAATGATAAGACCCGCTTCATGGGGAAGGGTGTCTCACAGGCTGTTGAGCAC   360

Gm5506     ATCAATAAAACTATTGCGCCTGCTCTGGTTAGCAAGAAAGTGAATGTTGTGGAGCAAGAG   398
           ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Gm5506_v1  ATCAATAAAACTATTGCGCCTGCTCTGGTTAGCAAGAAAGTGAATGTTGTGGAGCAAGAG   420

Gm5506     AAGATTGACAAGCTGATGAT                                           418
           ::::::::::::::::::::
Gm5506_v1  AAGATTGACAAGCTGATGAT                                           440
```

Supplemental Fig. S6

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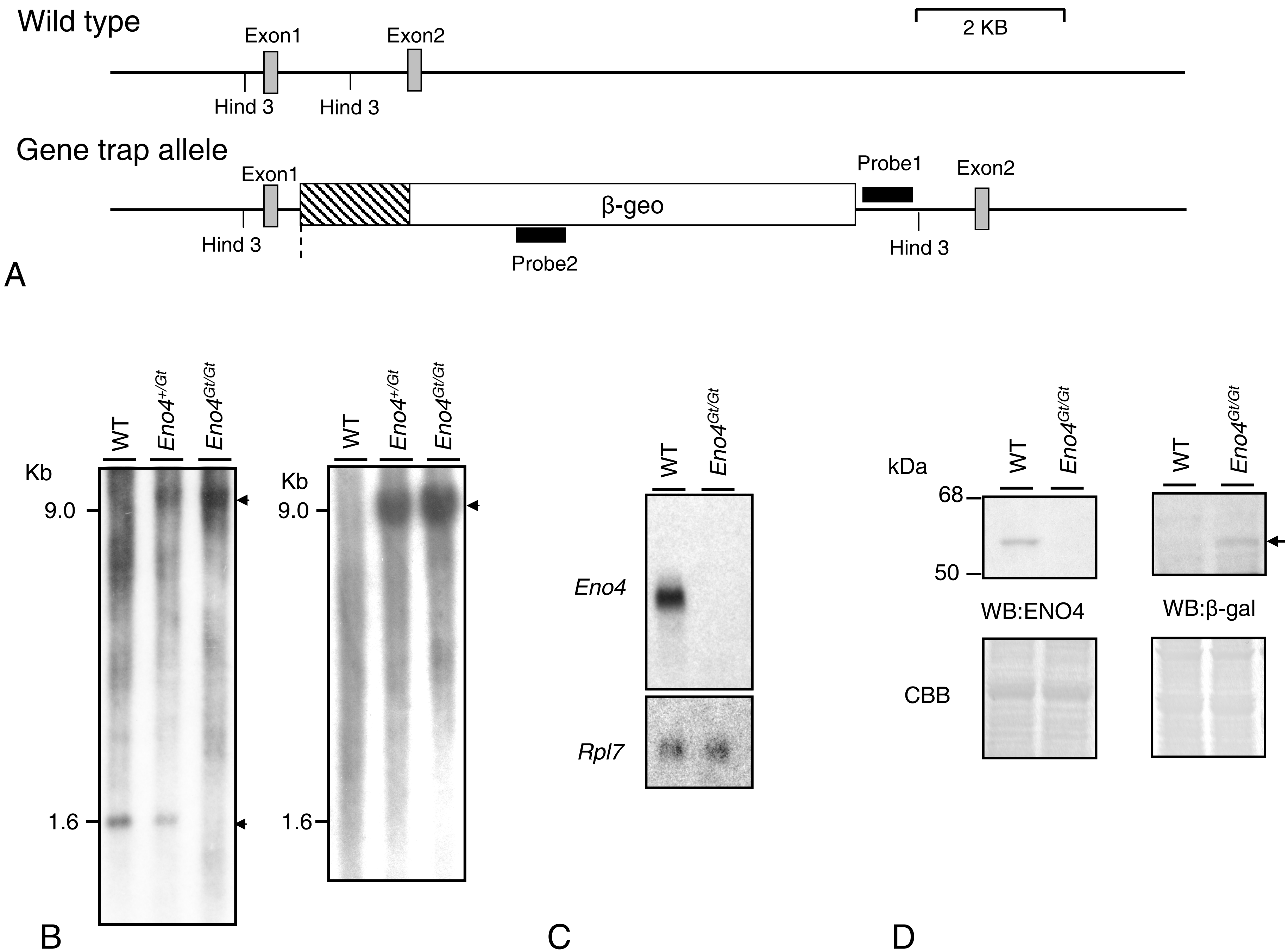

Supplemental Fig. S7

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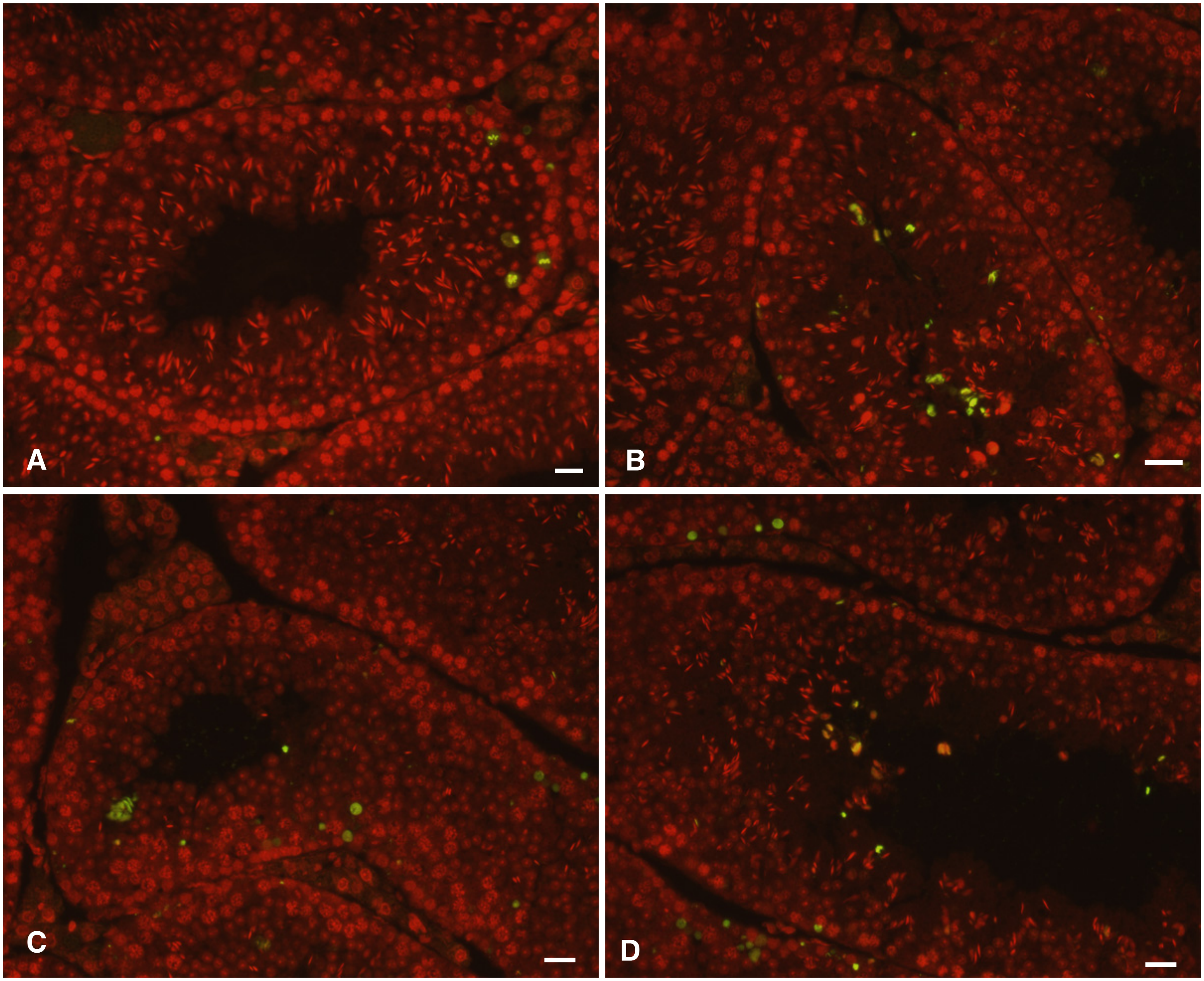

Supplemental Fig. S8

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