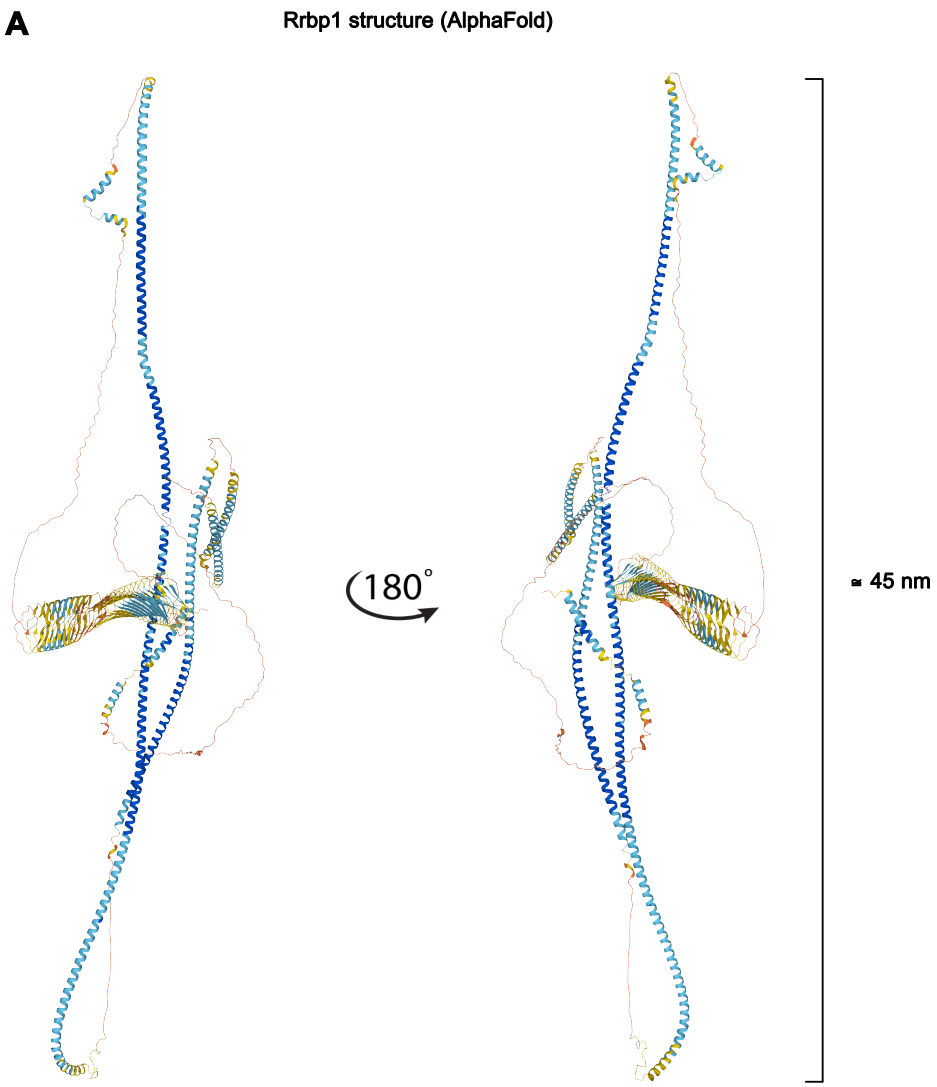
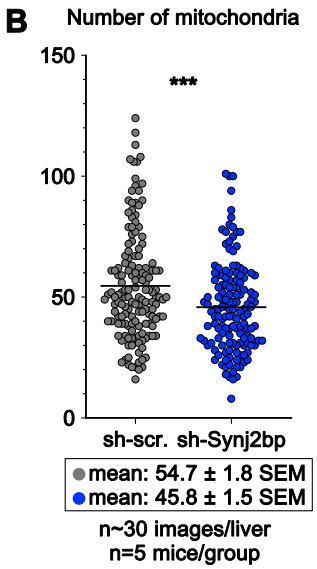

A
Rrbp1 structure (AlphaFold)
180°
≈ 45 nm


B
Number of mitochondria
150
100
50
0
***
sh-scr.
sh-Synj2bp
mean: 54.7 ± 1.8 SEM
mean: 45.8 ± 1.5 SEM
n~30 images/liver
n=5 mice/group

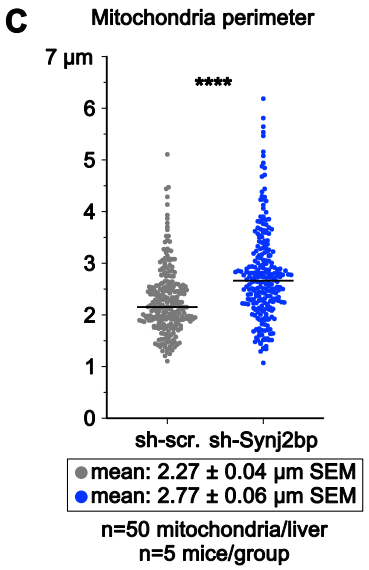
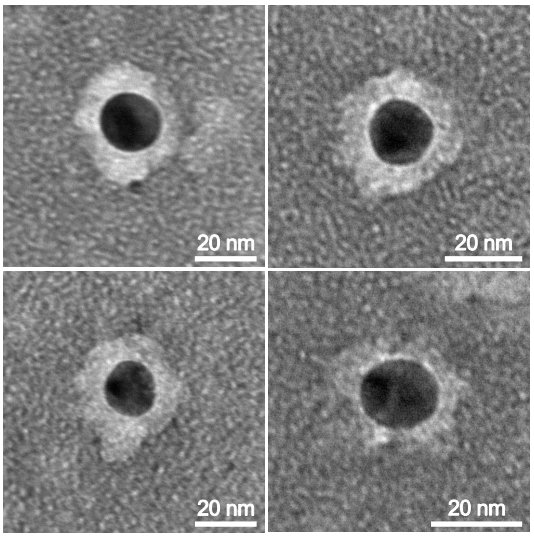

C
Mitochondria perimeter
7 µm
6
5
4
3
2
1
0
****
sh-scr.
sh-Synj2bp
mean: 2.27 ± 0.04 µm SEM
mean: 2.77 ± 0.06 µm SEM
n=50 mitochondria/liver
n=5 mice/group

Fig. S1 Synj2bp silencing in the mouse liver alters mitochondrial morphology.

A AlphaFold-predicted 3D structure of mouse Rrbp1, which regulates wrappER-mitochondria contact distance [1]. **B**,**C** Quantitative EM morphometric analyses of the number and size (perimeter) of mitochondria present in control and Synj2bp-silenced livers. In **B**, each dot represents the number of mitochondria counted in a single low-magnification EM image of livers collected from mice injected with control and AAV8-shSynj2bp vectors. In **C**, each dot represents the length of the perimeter of the mitochondria imaged in high-magnification EM images. This study shows that Synj2bp-silenced livers have fewer but larger mitochondria (mice at 3 h postprandial). The Student's *t* test was used to calculate *p* values.

A Negative staining EM + anti-ApoB immunogold labelling



anti-ApoB immunolabelled lipoparticles floated
from liver WAM fraction
(gold particles diameter = 15nm)

B Morphometric analysis of ApoB-containing lipoparticles

diameter of anti-ApoB immunolabelled lipoparticles floated from liver WAM fractions

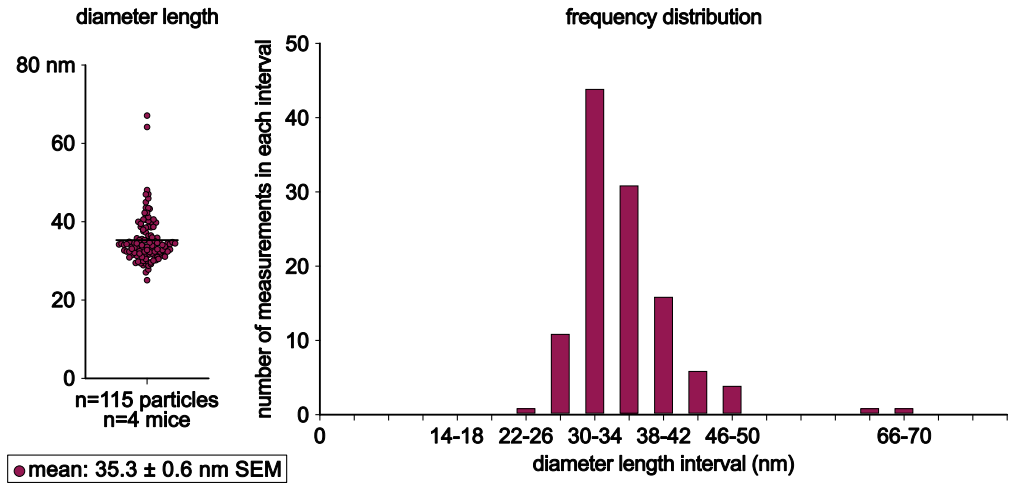


Fig. S2 The wrappER contains ApoB-containing lipoparticles.

A,B Negative staining EM coupled to immunogold labeling of anti-ApoB lipoparticles floated from mouse liver WAM-enriched fractions. **B** Size of ApoB-containing lipoparticles isolated from WAM fractions. The value of this parameter is expressed in nm and refers to the diameter of the lipoparticle. The right graph shows the frequency distribution of lipoparticle sizes. Gold particles diameter = 15 nm.

WAM: wrappER-associated mitochondria.

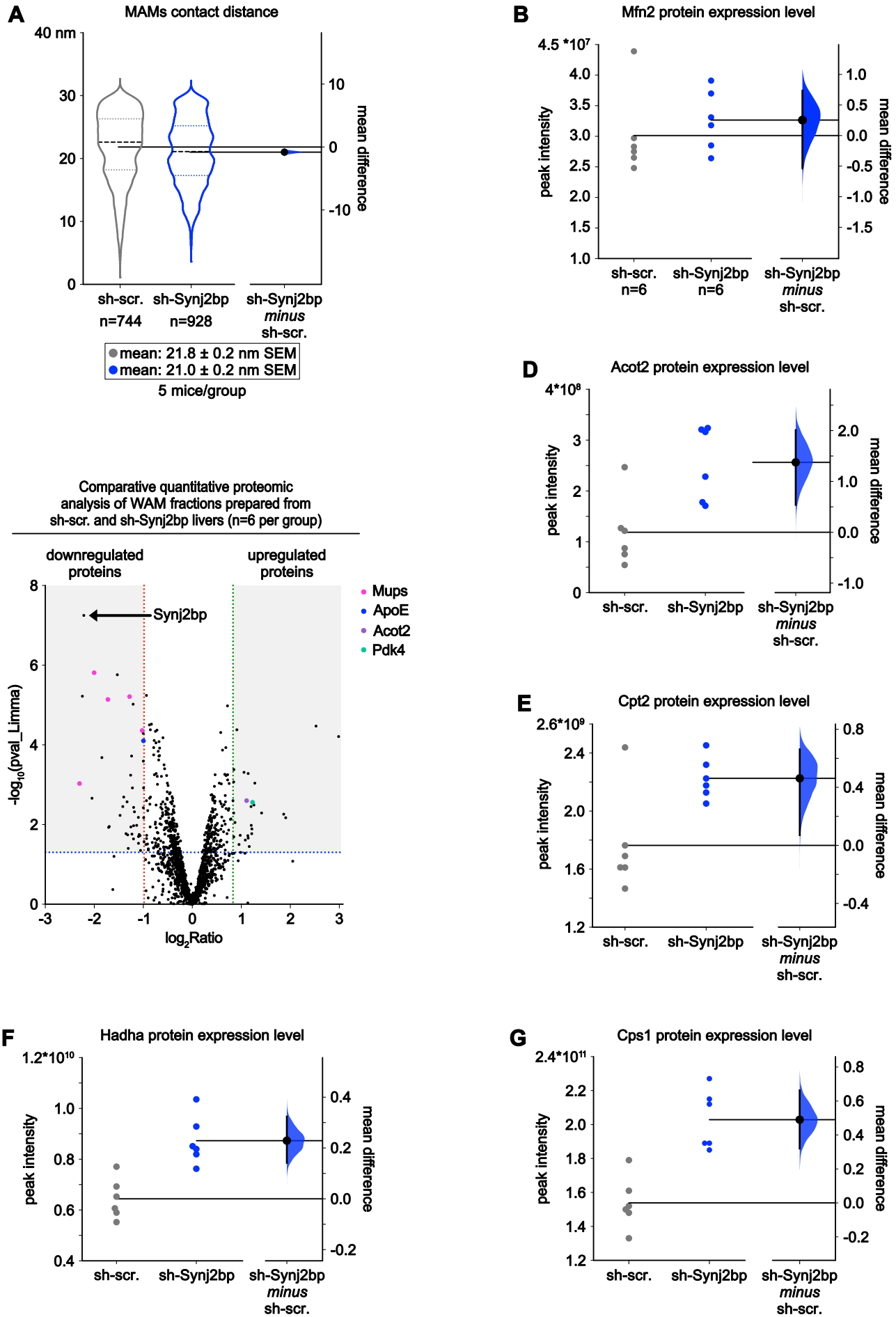

A
MAMs contact distance
40 nm
30
20
10
0
10
0
-10
mean difference
sh-scr.
n=744
sh-Synj2bp
n=928
sh-Synj2bp
minus
sh-scr.
mean: 21.8 ± 0.2 nm SEM
mean: 21.0 ± 0.2 nm SEM
5 mice/group
B
Mfn2 protein expression level
4.5 *10⁷
4.0
3.5
3.0
2.5
2.0
1.5
1.0
peak intensity
1.0
0.5
0.0
-0.5
-1.0
-1.5
mean difference
sh-scr.
n=6
sh-Synj2bp
n=6
sh-Synj2bp
minus
sh-scr.
C
Comparative quantitative proteomic
analysis of WAM fractions prepared from
sh-scr. and sh-Synj2bp livers (n=6 per group)
downregulated
proteins
upregulated
proteins
Synj2bp
Mups
ApoE
Acot2
Pdk4
-log10(pval_Limma)
8
6
4
2
0
-3
-2
-1
0
1
2
3
log2Ratio
D
Acot2 protein expression level
4*10⁸
3
2
1
0
peak intensity
2.0
1.0
0.0
-1.0
mean difference
sh-scr.
sh-Synj2bp
sh-Synj2bp
minus
sh-scr.
E
Cpt2 protein expression level
2.6*10⁹
2.4
2.0
1.6
1.2
peak intensity
0.8
0.4
0.0
-0.4
mean difference
sh-scr.
sh-Synj2bp
sh-Synj2bp
minus
sh-scr.
F
Hadha protein expression level
1.2*10¹⁰
1.0
0.8
0.6
0.4
peak intensity
0.4
0.2
0.0
-0.2
mean difference
sh-scr.
sh-Synj2bp
sh-Synj2bp
minus
sh-scr.
G
Cps1 protein expression level
2.4*10¹¹
2.2
2.0
1.8
1.6
1.4
1.2
peak intensity
0.8
0.6
0.4
0.2
0.0
-0.2
mean difference
sh-scr.
sh-Synj2bp
sh-Synj2bp
minus
sh-scr.

Fig. S3 Synj2bp silencing in the mouse liver upregulates mitochondria fatty acid respiration capacity while down-regulating ApoE and Mups expression at the WAM.

A Electron microscopy morphometric analyses of the MAMs contact distance in control and Synj2bp-silenced mouse livers. Data were processed by estimation statistics analysis **B** Estimation statistics analysis of the WAM proteomics data of the MAM tether Mfn2. **C** Comparative quantitative proteomic analysis. The volcano plot shows the proteins that are down- and up-regulated in WAM-enriched fractions isolated from Synj2bp-silenced livers compared to control AAV8-shScrambled injected mice. **D**-**G** Estimation statistics analysis of the WAM proteomics data related to the indicated proteins. **D-F** Enzymes that participate in mitochondrial FA respiration; their up-regulation indicates the activation of this catabolic process in Synj2bp-silenced livers. **G** Cps1 is the rate-limiting step of the urea cycle; its increased expression suggests a possible upregulation of this pathway.

A

Synj2bp orthologs sequence alignment

PDZ domain

```
Q7ZV59_Fish              MNGSAH-SAPSDVEFTLKRGPAGLGFNIVGGVDQQYMMNDSGIYVAKIKENGAAALDGRLQEG
Q9D6K5_Mouse             MNGRVD-YLVTEEEINLTRGPSGLGFNIVGGTDQQYVSNDSGIYVSRIKEDGAAAQDGRLQEG
P57105_Human             MNGRVD-YLVTEEEINLTRGPSGLGFNIVGGTDQQYVSNDSGIYVSRIKENGAAALDGRLQEG
XP_029454621_Amphibia    MNGSVD-YLAFDEEIQLKRGPSGLGFNIVGGTDQQYICNDSGIYVSRIKEDGAAAVDGRLQEG
XP_025951563_Bird        MNGSVAGGGLAEEEISLTRGPSGLGFNIVGGTDQQYISNDSSIYVSRIKKDGAAYLDGRLQEG
                         *** .       *: *.***:*********.****: ***.***::**::***  *******
```

predicted

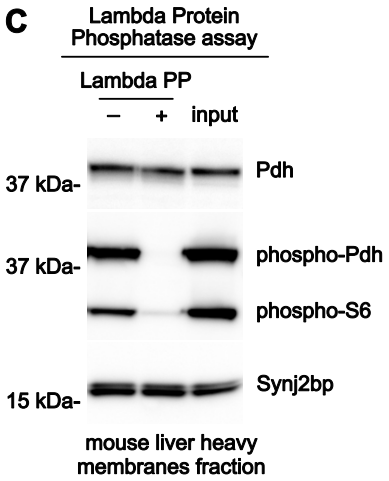
```
Q7ZV59_Fish              DKILAINGRKLDNLSHGAAVELFRSAGEDVHLCIQQRPVLQ-NGPTSSRADGESSSALGTWTL
Q9D6K5_Mouse             DKILSVNGQDLKNLLHQDAVDLFRNAGCAVSLRVQHRLPVQ-NGPIVHRGEGEPSGVPVAMVL
P57105_Human             DKILSVNGQDLKNLLHQDAVDLFRNAGYAVSLRVQHRLQVQ-NGPIGHRGEGDPSGIPIFMVL
XP_029454621_Amphibia    DQILAVNDQALKNLLHKEVVNLFRNAGEDVKLKVQHKAQVQPNGPSGHRGDGESGGLSLSTVL
XP_025951563_Bird        DKILAVNGRDLKDLRHKDAVELFRNAGYDVSLKIQRRLQPQ-NGPVSHRGDGESGGLPLAAIL
                         *:**::*.: *.:* *  .*:***.**  * * :*:    * ***   *.:*:...     *
```

transmembrane domain

```
Q7ZV59_Fish              FAV-VTLAVMTA-SFIAYKRFH--PRGPRGPF
Q9D6K5_Mouse             LPV-FALTMVAVWAFVRYRKQL----------
P57105_Human             VPV-FALTMVAAWAFMRYRQQL----------
XP_029454621_Amphibia    VPVLLACTAAAIWLAMKYRHRH----------
XP_025951563_Bird        VPG-LALAVAAVWIVLRYRQRM----------
                          .   : :  :    : *::
```

C

Lambda Protein Phosphatase assay



mouse liver heavy membranes fraction

B

Synj2bp phylogenetic tree

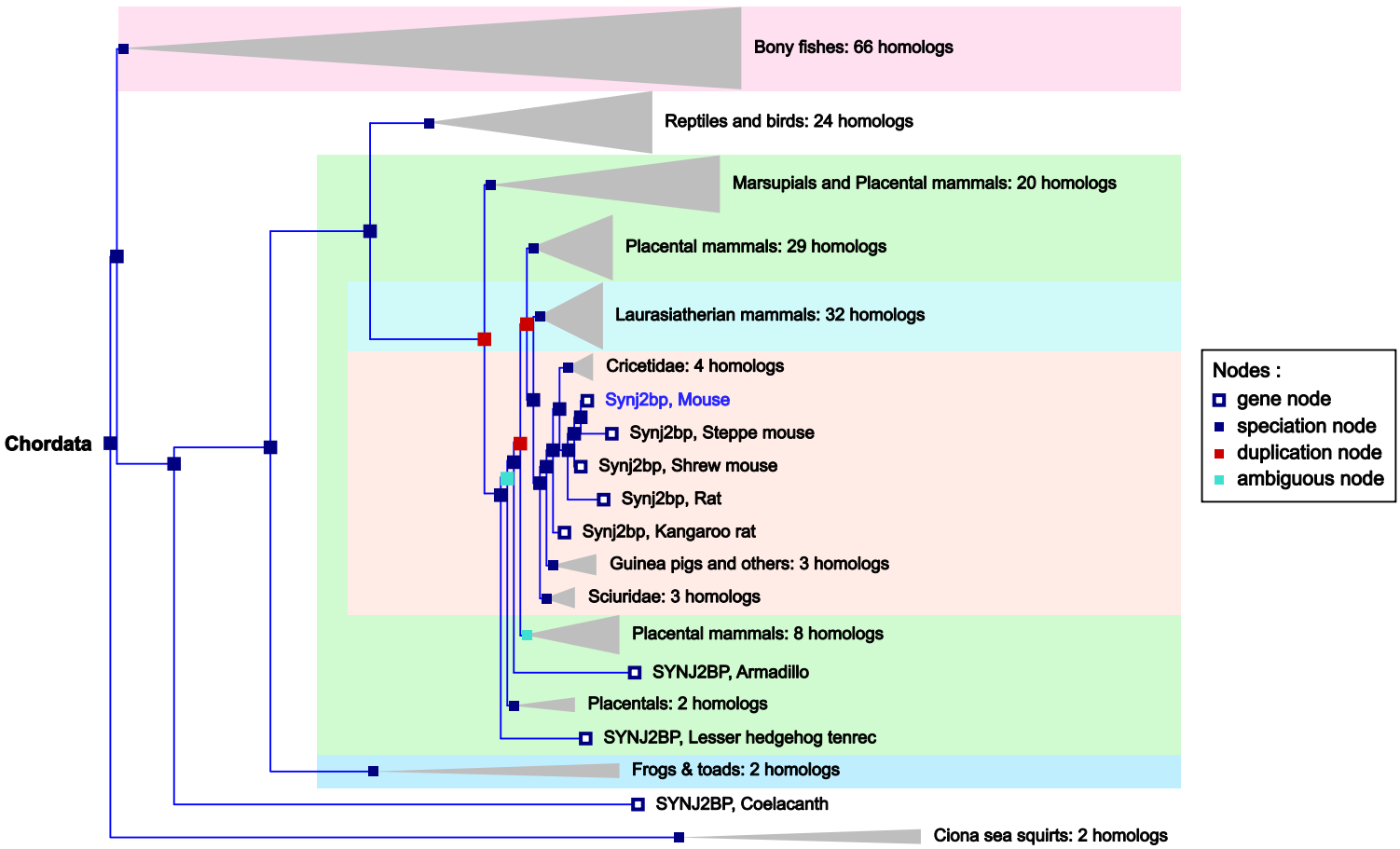


Fig. S4 Synj2bp evolutionary analysis.

A Multiple sequence alignment of representative Synj2bp orthologs from the indicated vertebrate groups. **B** Schematic representation of the mouse Synj2bp phylogenetic tree. **C** Lambda Protein Phosphatase assay on mouse liver heavy membranes fraction. The image shows the immunoblot analysis done using a mix of four antibodies that recognize Synj2bp, Pdh, phosphorylated Pdh (Ser293), and phosphorylated S6 (Ser240/244), respectively. Note that phosphatase treatment abolishes the signal generated by phospho-specific antibodies, used here as positive controls. This data indicates that the two forms of Synj2bp are likely not the result of Ser/Thr/Tyr phosphorylation.