Supplemental Figures and Legends

Figure S1 (related to Figure 1). Additional data: BN-APAGE analysis of PER complexes.

(A) Longer exposures of immunoblots in Figure 1D. Circadian profiles of nuclear protein complexes containing the core circadian negative feedback proteins. Nuclear extracts from mouse livers collected at the indicated circadian times (CT) were analyzed by BN-APAGE, and immunoblots of the gels were probed for the proteins indicated at the right of each panel. Asterisks, monomer.

(B) From the experiment in Figure 1D and Figure S1A, SDS-PAGE immunoblots showing circadian temporal profiles of the individual clock proteins indicated at right. SAP155, loading control.

(C) Representative positive control BN-APAGE immunoblot showing observation of the expected spectrum complexes labeled by an antibody against the large subunit of RNA Polymerase II (RNAP II). Each blot from the experiment in Figure 1D and S1A was stripped and re-probed for RNAP II to confirm correct operation of BN-APAGE.

(A-C).

Figure S2 (related to Figure 2). Known CLOCK-BMAL1 interacting proteins not detected in the ~750-kDa CLOCK-BMAL1 complex.

(A) Control BN-APAGE immunoblots of immuno-purified CLOCK-BMAL1 complex (eluted from anti-CLOCK antibody beads) from mouse liver nuclear extract (CT4) probed for CLOCK and BMAL1, as indicated.

(B) BN-APAGE immunoblots of the immuno-purified complex (plus respective input controls) probed for the CLOCK-BMAL1-associated proteins indicated at bottom.

(C) SDS-PAGE immunoblots of mouse liver nuclear extract (Input) or immunoprecipitates (IP) with IgG control or anti-CLOCK antibody, as labeled, probed for the proteins indicated at right. IgG-HC, IgG heavy chain, control for immunoprecipitation.

Figure S3 (related to Figure 3). Scheme for purification and analysis of nuclear PER complexes from mouse liver.

Figure S4 (related to Figure 3). Class averages of negatively stained nuclear PER complex.

(A) Class averages obtained from classifying 10,866 particles into 200 classes. Boxes, 78.3 nm.

(B) Class averages obtained from classification of the same data set into 500 classes.

Figure S5 (related to Figure 4). Additional data: CK1δ action in PER complexes.

(A) Identification of the targets of CK1δ action in the PER complex. SDS-PAGE autoradiogram. Left two lanes (also shown in Figure 4B) show control (WT) or affinity-

purified nuclear PER complex (PER2-FH) from mouse liver after incubation *in vitro* with γ-^{32}P-ATP (25ºC, 1 h). IP lanes show the PER2-FH sample, denatured after incubation with γ-^{32}P-ATP, and immunoprecipitated with an IgG control antibody or antibodies against the indicated proteins. Size markers at left.

(B) Nuclear PER complex binds to CLOCK-BMAL1 E-box DNA site. BN-PAGE immunoblot probed for PER2 showing PER complex from wildtype mouse liver nuclear extract (CT18) (input) or after the extract was incubated with a synthetic control oligonucleotide containing three copies of a scrambled E-box sequence (Mut) or a synthetic oligonucleotide containing three copies of the E-box sequence CACGTG (E-box), followed by release of the oligonucleotide from the beads by restriction enzyme cleavage. There was a slight change in migration of the E-box bound PER complex compared to the PER complex in the input, which might be due to the residual negatively charged DNA bound to CLOCK-BMAL1 in the complex.

(C) Control experiment showing no evident loss of PER2, CLOCK, or BMAL1 from FLAG-immunopurified nuclear PER complex (PER2-FH mouse; CT18) after treatment with buffer alone (mock), lambda phosphatase (PPase), or lamba phosphatase treatment followed by washing and treatment with recombinant CK1δ (PPase, CKδ).

(D) Electrophoretic mobility assay showing that dephosphorylation of one or more proteins in the immunopurified nuclear PER complex (CT18) increases the binding of the complex to the E-box DNA, and subsequent phosphorylation of one or more proteins of the complex by CK1δ returns the E-box DNA binding of the complex to its original lower level. Autoradiogram of native gel showing mobility of ^{32}P-labeled dsDNA

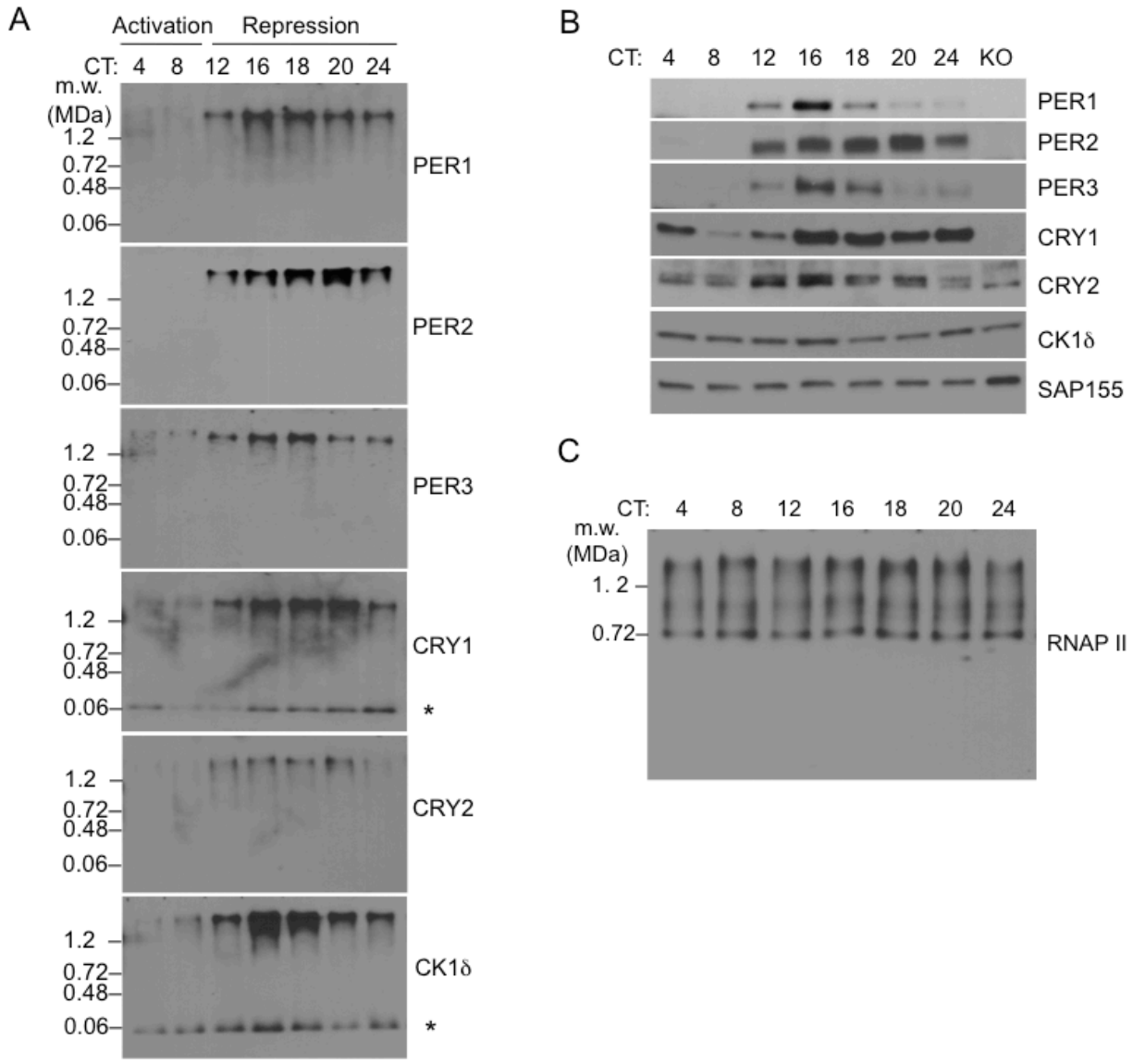
containing three copies of scrambled E-box (M) or wildtype E-box (E) after incubation with different concentrations of immunopurified nuclear PER complex (represented by black boxes) and treatment by buffer (mock), lambda phosphatase (PPase), or lambda phosphatase followed by CK1δ (PPase; CK1δ) as in (C).

Figure S6 (related to Figure 5). Scheme for purification and analysis of cytoplasmic PER complexes from mouse liver.

Figure S7 (related to Figure 7). Class averages of negatively stained lower and upper cytoplasmic PER complexes.

(A) The 108 class averages of negatively stained LC obtained by using the iterative stable alignment and classification (ISAC) procedure and less stringent classification parameters (100 images per group, pixel error threshold of 2). Boxes, 33.4 nm.

(B) ISAC analysis of negatively stained UC did not yield stable averages. Shown are all 276 candidate class averages resulting from ISAC analysis specifying 50 images per group. Boxes, 52.2 nm. Asterisk marks the class average image enlarged in Figure 7G.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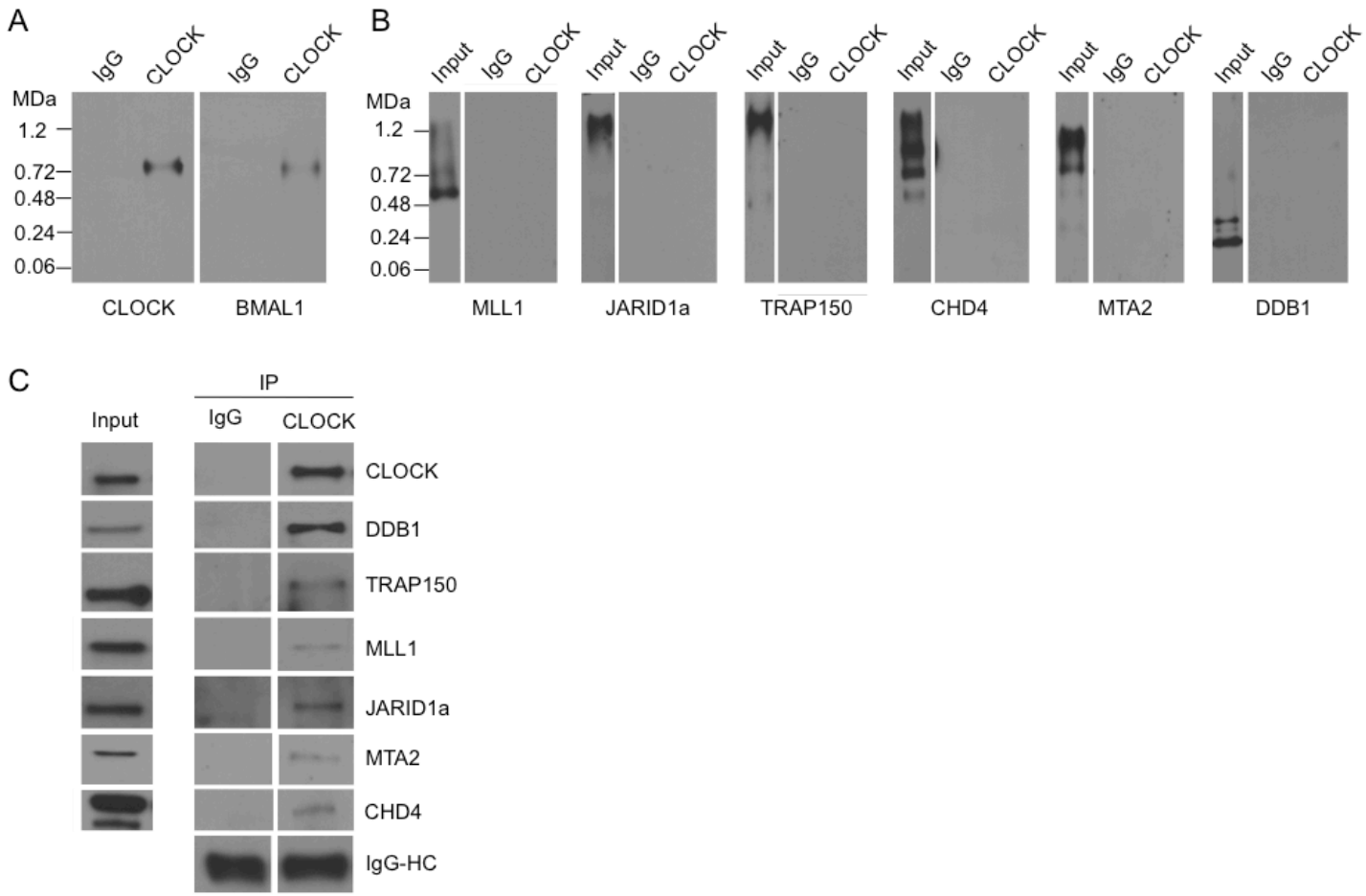
Activation
Repression
CT: 4 8 12 16 18 20 24
m.w.
(MDa)
1.2
0.72
0.48
0.06
PER1
PER2
PER3
CRY1
CRY2
CK1δ
*
B
CT: 4 8 12 16 18 20 24 KO
PER1
PER2
PER3
CRY1
CRY2
CK1δ
SAP155
C
CT: 4 8 12 16 18 20 24
m.w.
(MDa)
1. 2
0.72
RNAP II

Fig. S1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IgG
CLOCK
IgG
CLOCK
MDa
1.2
0.72
0.48
0.24
0.06
CLOCK
BMAL1
B
Input
IgG
CLOCK
MDa
1.2
0.72
0.48
0.24
0.06
MLL1
JARID1a
TRAP150
CHD4
MTA2
DDB1
C
IP
Input
IgG
CLOCK
CLOCK
DDB1
TRAP150
MLL1
JARID1a
MTA2
CHD4
IgG-HC

Fig. S2

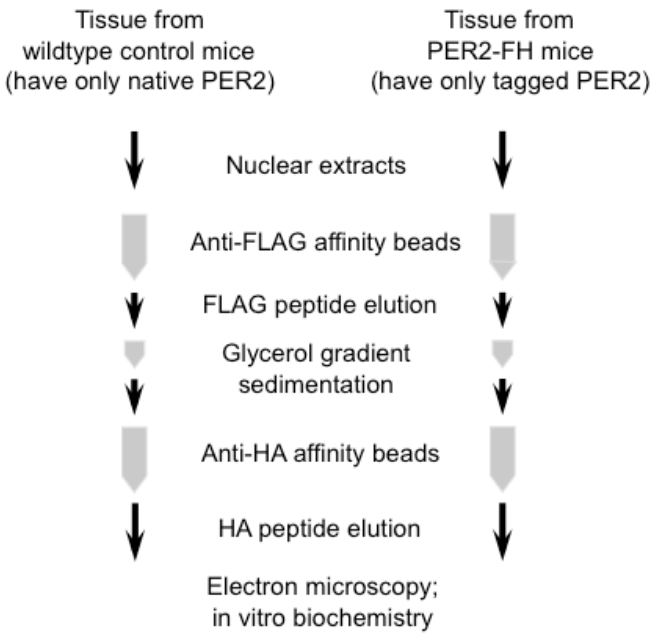
Tissue from
wildtype control mice
(have only native PER2)
Tissue from
PER2-FH mice
(have only tagged PER2)
Nuclear extracts
Anti-FLAG affinity beads
FLAG peptide elution
Glycerol gradient
sedimentation
Anti-HA affinity beads
HA peptide elution
Electron microscopy;
in vitro biochemistry

Fig. S3

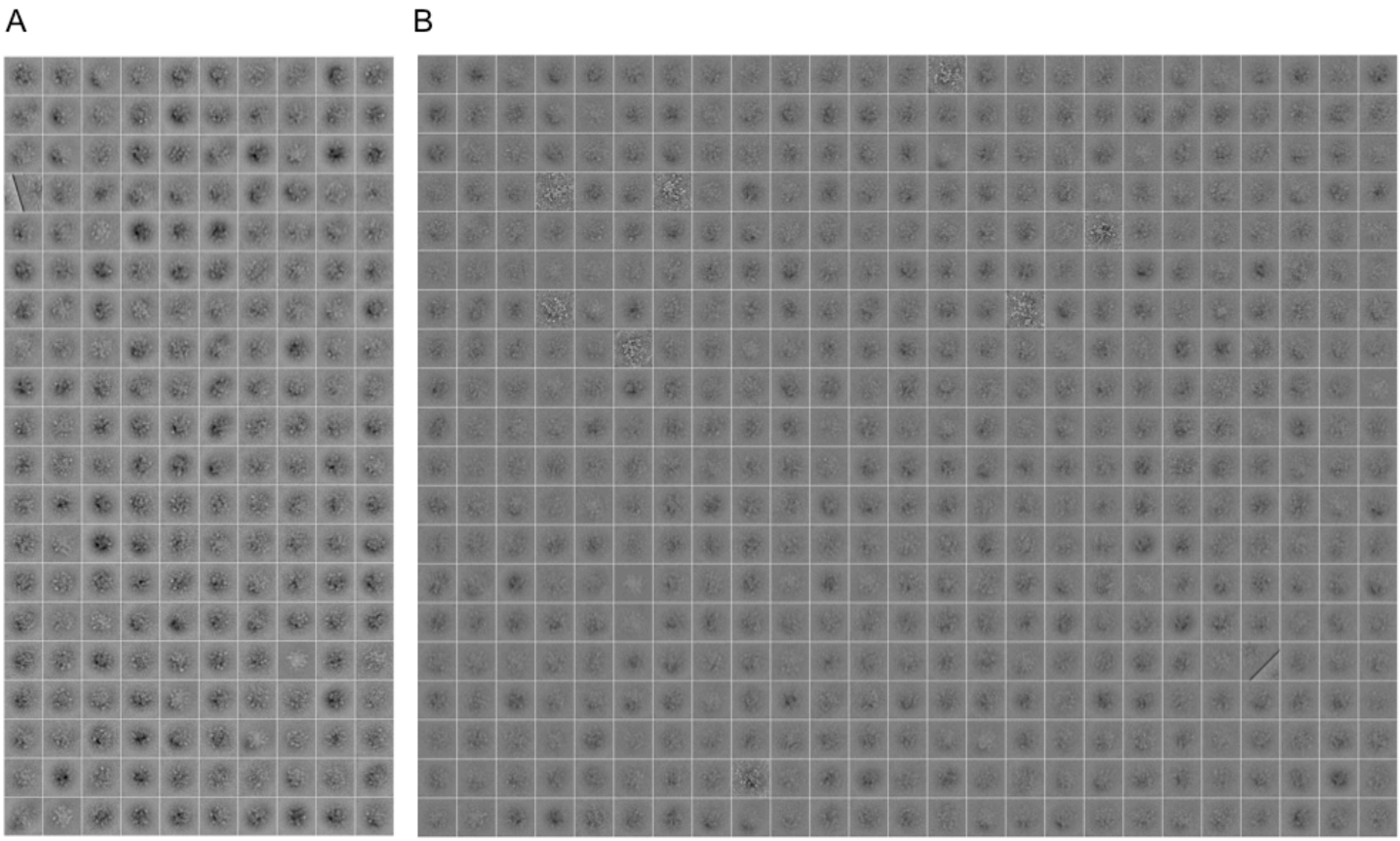

Fig. S4

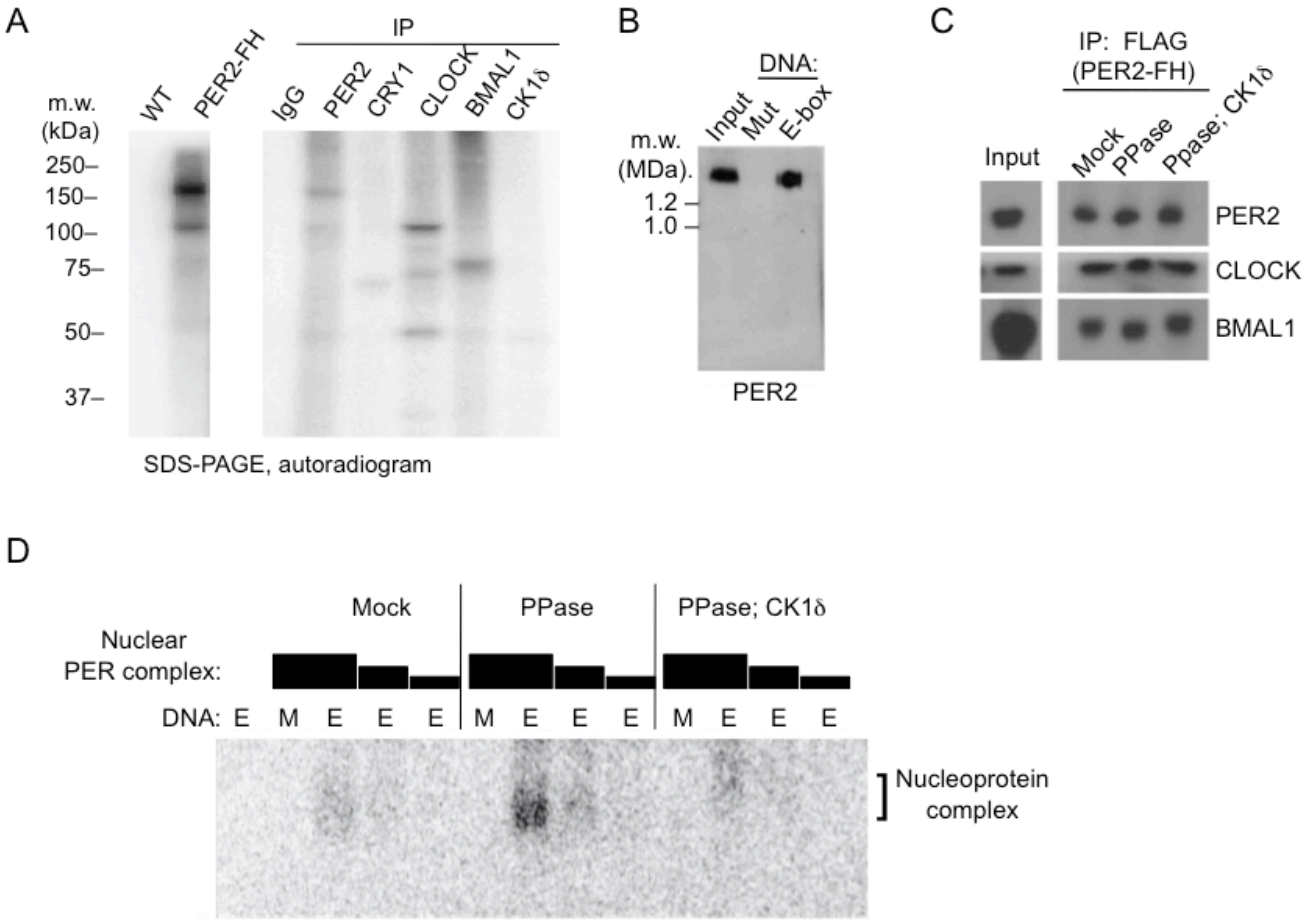
A
IP
WT
PER2-FH
IgG
PER2
CRY1
CLOCK
BMAL1
CK1δ
m.w.
(kDa)
250–
150–
100–
75–
50–
37–
SDS-PAGE, autoradiogram
B
DNA:
Input
Mut
E-box
m.w.
(MDa).
1.2
1.0
PER2
C
IP: FLAG
(PER2-FH)
Input
Mock
PPase
Ppase; CK1δ
PER2
CLOCK
BMAL1
D
Mock
PPase
PPase; CK1δ
Nuclear
PER complex:
DNA: E M E E E M E E E M E E E
Nucleoprotein
complex

Fig. S5

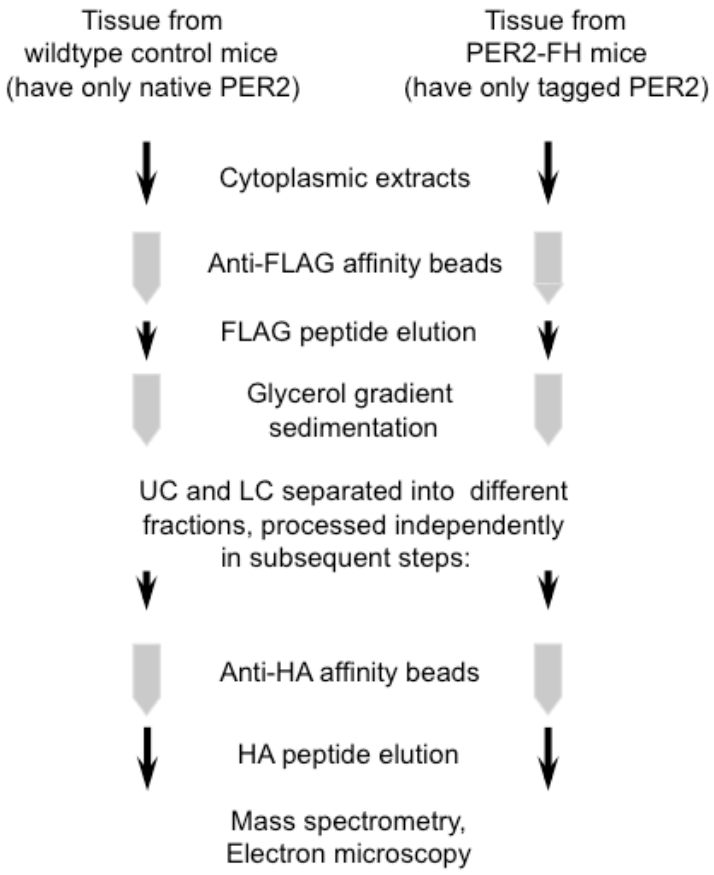
Tissue from
wildtype control mice
(have only native PER2)
Tissue from
PER2-FH mice
(have only tagged PER2)
Cytoplasmic extracts
Anti-FLAG affinity beads
FLAG peptide elution
Glycerol gradient
sedimentation
UC and LC separated into different
fractions, processed independently
in subsequent steps:
Anti-HA affinity beads
HA peptide elution
Mass spectrometry,
Electron microscopy

Fig. S6

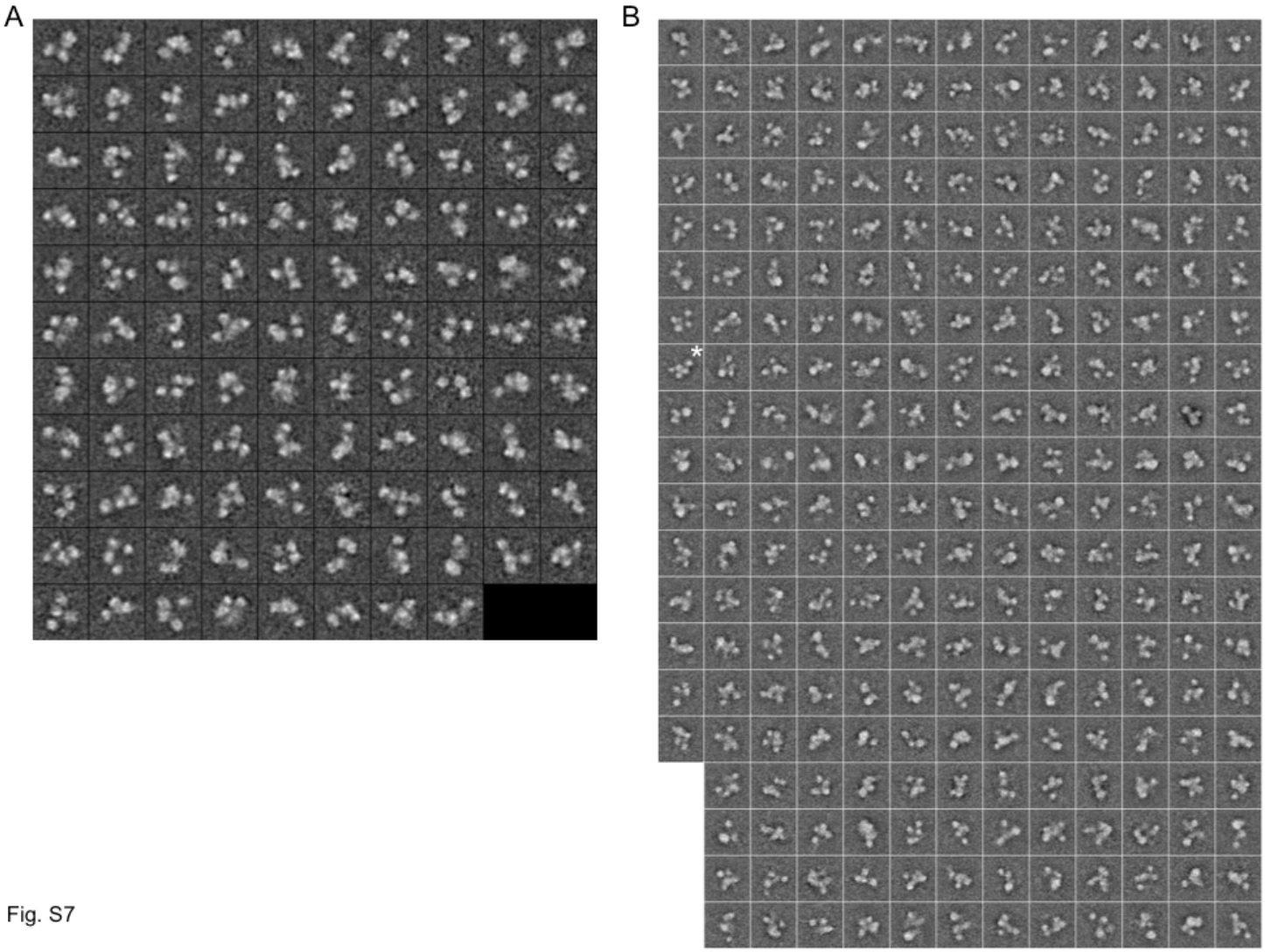

Fig. S7