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**The circadian clock influences T cell responses to vaccination by regulating dendritic cell antigen processing**

- I. Supplementary table
- II. Supplementary figures

24 I. Supplementary table

25 Supplementary Table 1. Primer sequences

Primer name	Primer Sequence	
		26
<i>Bmal1</i> forward	TGCAATGTCCAGGAAGTTAGAT	
<i>Bmal1</i> reverse	GTTTGCTTCTGTGTATGGGT	27
<i>Per2</i> forward	ATGCTCGCCATCCACAAGA	28
<i>Per2</i> reverse	GCGGAATGGAATGGGAGAAT	29
<i>Mcub</i> forward	TCACAAGAAAGGTCAAAGCTGC	
<i>Mcub</i> reverse	CCAGGAGTACACCCACCAAG	30
<i>Emre</i> forward	GACGACGATTAACAGGGCAC	31
<i>Emre</i> reverse	CAGGACTCTGGGCTCTTGTC	32
<i>Nr1d1</i> forward	GAGAGGCCATCACAACCTCC	
<i>Nr1d1</i> reverse	ACACCACCTGTGTTGTTATTGG	33
<i>Fis1</i> forward	AGGCTCTAAAGTATGTGCGAGG	34
<i>Fis1</i> reverse	GGCCTTATCAATCAGGCGTTC	35
<i>Opa1</i> forward	CTGCAGGTCCCAAATTGGTT	
<i>Opa1</i> reverse	TCTTTGTCTGACACCTTCCTGT	36
<i>Mfn1</i> forward	CCTACTGCTCCTTCTAACCCA	37
<i>Mfn1</i> reverse	AGGGACGCCAATCCTGTGA	
<i>Rps18</i> forward	CCCTCTATGGGCTCGAATTT	38
<i>Rps18</i> reverse	GGATGTGAAGGATGGGAAGT	39

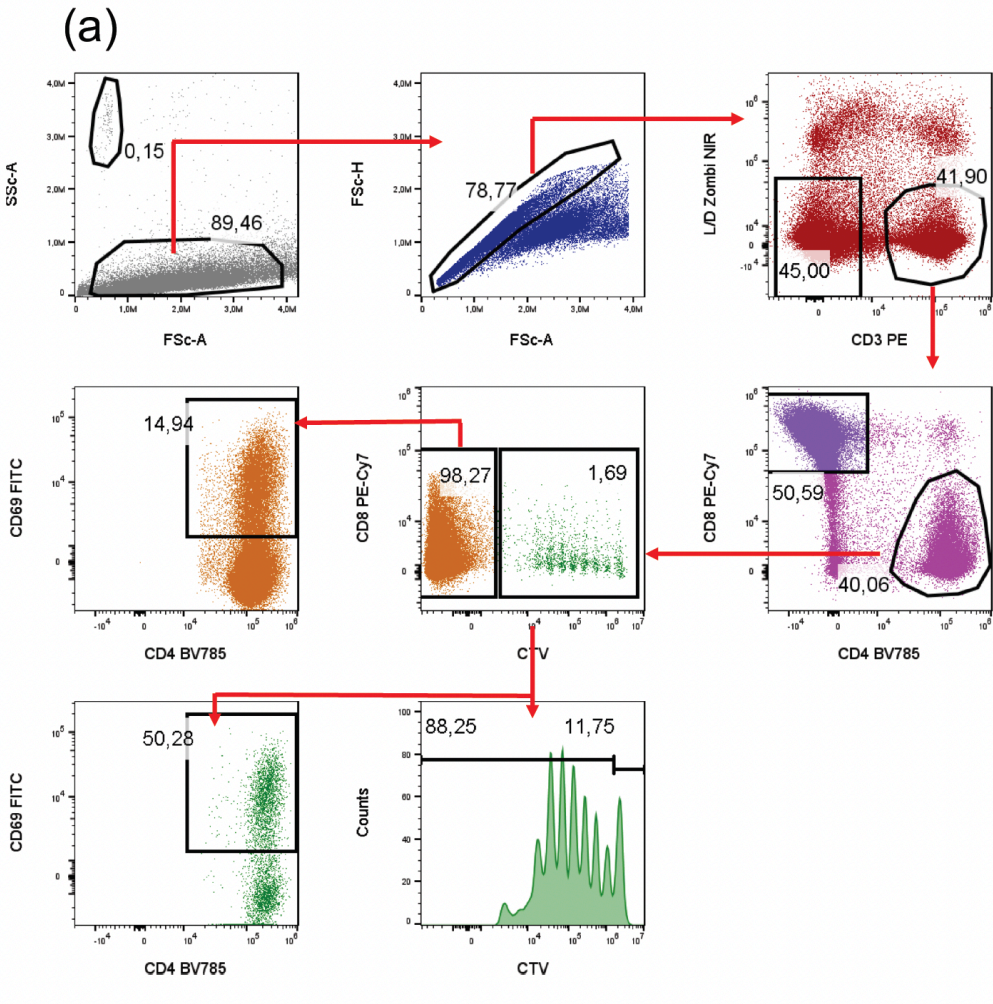
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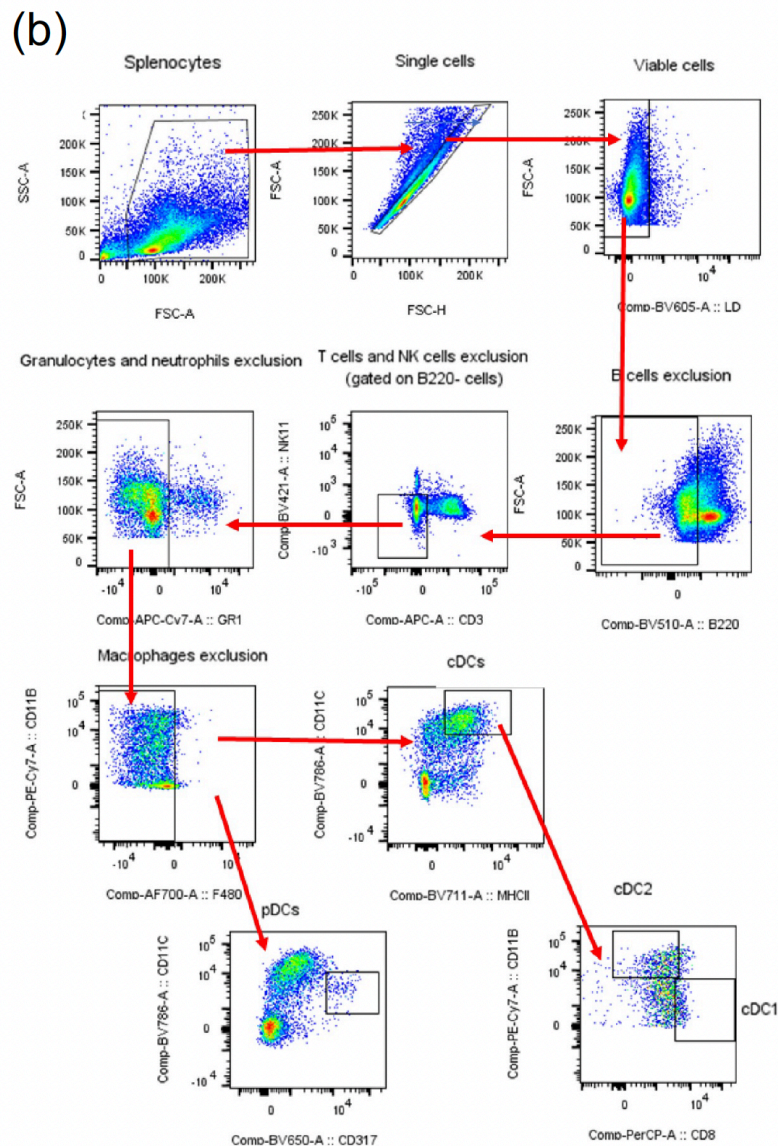
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II. Supplementary Figures





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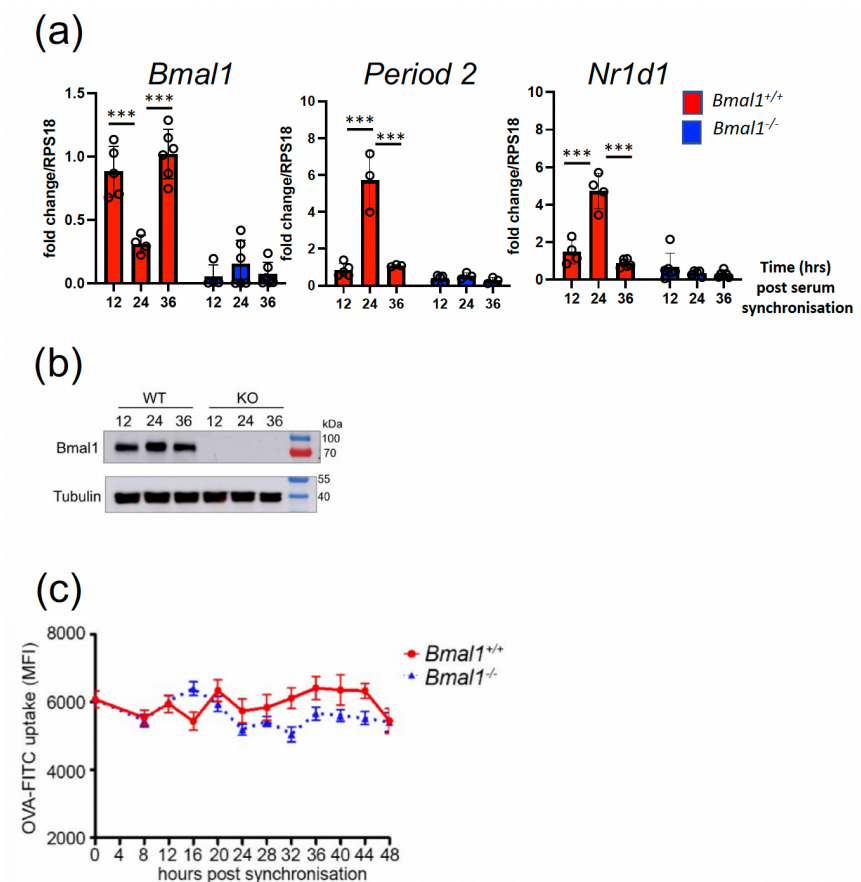
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## 57 Supplementary Fig. 1. Gating strategies

58 (a) Gating strategy for CTV<sup>+</sup> stained OT-II CD4 T cells obtained by lymph node harvesting and  
 59 analysed via flow cytometry (**Fig. 1**) (b) Gating strategy for splenic DCs populations obtained  
 60 by spleen isolation and analysed via flow cytometry (**Fig. 2**).

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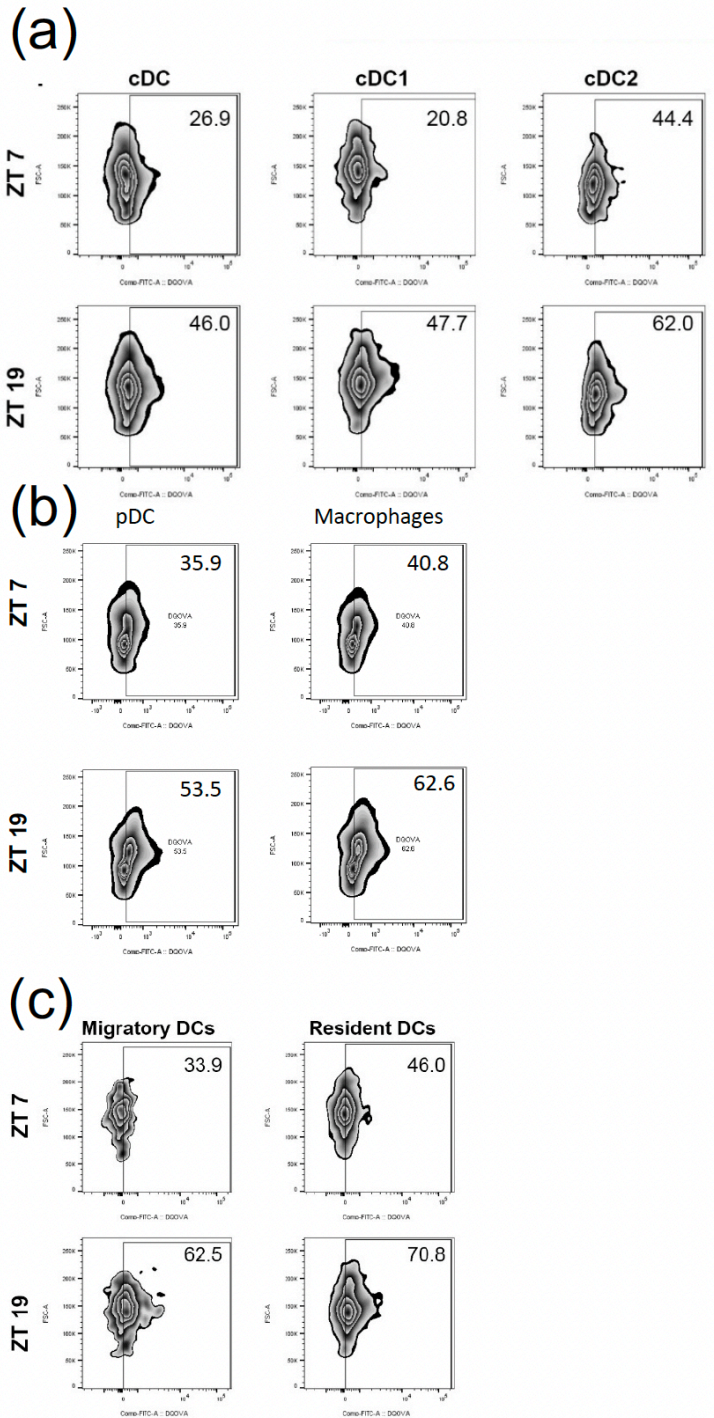
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## 64 **Supplementary Fig. 2. Synchronised DCs produce rhythms in clock gene expression** 65 **but antigen uptake is not affected by the clock**

66 (a) *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup> BMDCs were synchronised and mRNA levels of *Bmal1*, *Nr1d1* and  
67 *Per2* were analysed by qPCR at designated time points post synchronisation (n=3 biologically  
68 independent samples). (b) *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup> BMDCs were synchronised and subjected to  
69 immunoblot analysis for Bmal1 protein at designated time points post synchronisation (n=1  
70 biologically independent sample). (c) *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup> BMDCs were synchronised and  
71 OVA uptake was measured using FITC-OVA at designated time points post synchronisation  
72 and quantified by confocal microscopy. (n=20 biologically independent samples)

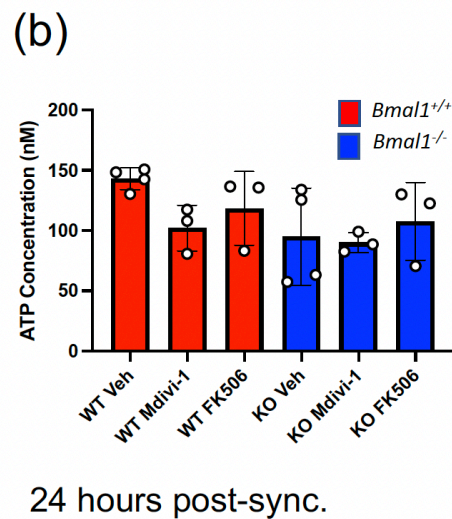
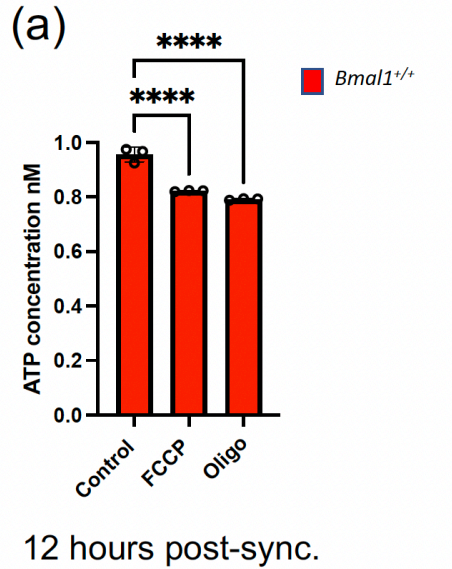
73 Data shown is mean with error bars representing  $\pm$  SEM. Data were compared by one-way  
74 ANOVA with Tukey's post-hoc test for multiple comparisons. \*\*\* p<0.001

75 Source data are provided as a source data file.



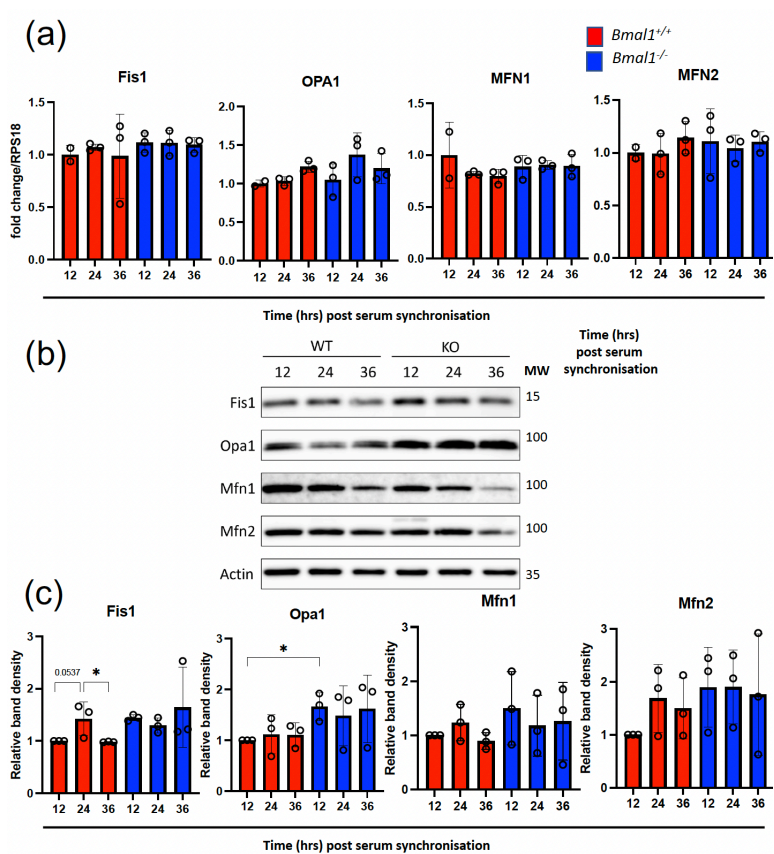
### Supplementary Fig. 3. Flow cytometry analysis of DC subsets for antigen processing

(a-c) Splenic DCs were expanded by B16-FLT3l cells and spleens were isolated from mice at ZT7 and ZT19. Single cell suspensions were generated, then incubated with DQ-OVA (1  $\mu\text{g/mL}$ ) for 60 min prior to staining and characterisation by flow cytometry.



#### Supplementary Fig. 4. Effect of Oligomycin, FCCP, Mdivi-1 and FK506 on ATP levels

(a) *Bmal1*<sup>+/+</sup> BMDCs were synchronised and pretreated with FCCP (10  $\mu$ M), Oligomycin (10  $\mu$ M) or vehicle for 3 hours and then harvested at 12 hours post synchronisation and ATP measured (n=3 biologically independent samples). (b) *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup> BMDCs were synchronised and pre-treated with Mdivi-1 (10  $\mu$ M), FK506 (10  $\mu$ M) for 12 hours and then harvested at 24 hours post synchronisation and ATP measured. (n=3-4 biologically independent samples). Data shown is mean with error bars representing  $\pm$  SEM. Data were compared by one-way ANOVA with Tukey's post-hoc test for multiple comparisons. \*\*\*\* $p < 0.0001$ . Source data are provided as a source data file.



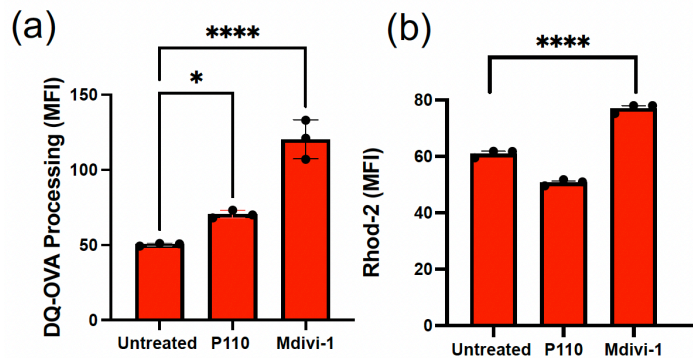
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96 **Supplementary Fig. 5. Genes involved in mitochondrial morphology do not display**  
97 **circadian rhythms at mRNA and protein level except for *Fis1***

98 (a) *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup> BMDCs were synchronised by serum shock and mRNA levels of  
99 genes associated with mitochondrial morphology were analysed by qPCR at 12 hr, 24 hr and  
100 36 hr post synchronisation (n= 2-3 biologically independent samples). *Bmal1*<sup>+/+</sup> and *Bmal1*<sup>-/-</sup>  
101 BMDCs were synchronised by serum shock and protein levels of genes associated with  
102 mitochondrial morphology were analysed by (b) immunoblot at 12 hr, 24 hr and 36 hr post  
103 synchronisation and (c) bands quantified (n=3 biologically independent samples). Source data  
104 are provided as a source data file. Data shown is mean with error bars representing  $\pm$  SEM.  
105 Data were analysed by one-way ANOVA with Tukey's post-hoc test for multiple comparisons.  
106 \* $p<0.05$ . Source data are provided as a source data file.

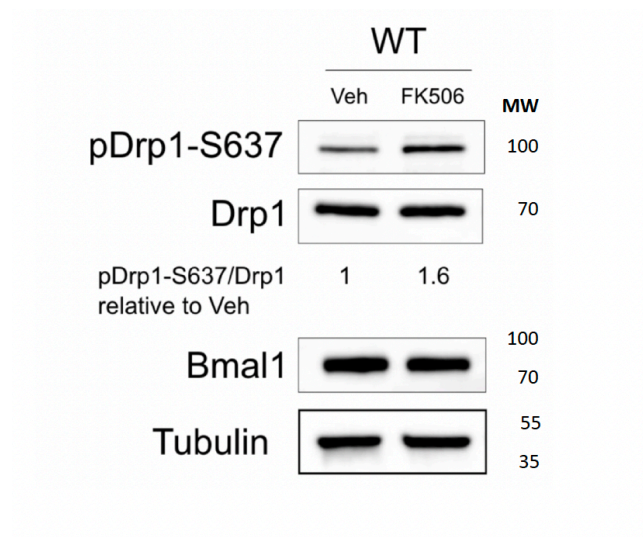
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**Supplementary Fig. 6. Mitochondrial fission inhibitor P110 does not promote mitochondrial  $\text{Ca}^{++}$  uptake and is less efficient than Mdivi-1 at promoting antigen processing**

*Bmal1*<sup>+/+</sup> BMDCs were synchronised by serum shock. (a) Antigen processing and (b) mitochondrial calcium uptake was quantified at 24 hr post synchronisation in the presence and absence of P110 (1  $\mu\text{M}$ ) or Mdivi-1 (10  $\mu\text{M}$ ) (n=3 biologically independent samples). Data shown is mean with error bars representing  $\pm$  SEM. Data were analysed by one-way ANOVA with Tukey's post-hoc test for multiple comparisons. \*\*p<0.05 and \*\*\*\* p<0.0001. Source data are provided as a source data file.



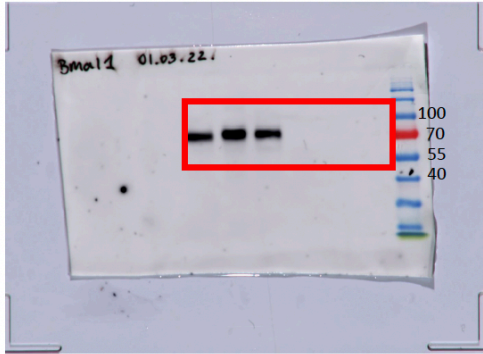


# Supplementary Fig. 7. FK506 prevents the dephosphorylation of S637 on DRP1

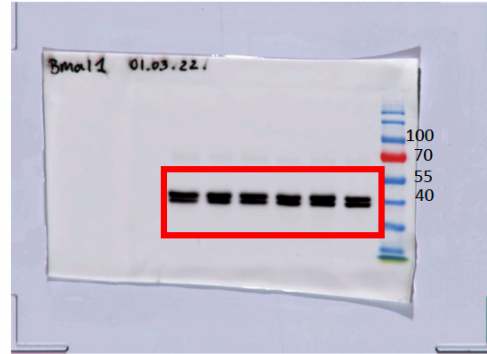
*Bmal1*<sup>+/+</sup> BMDCs were synchronised by serum shock and pre-treated with FK506 (1 µM) at 21 hours post-synchronisation. Cells were lysed for immunoblot analysis at 24 hours post-synchronisation and probed for pDrp1-S637 and BMAL1 (n=1 biologically independent sample).

# Uncropped scans of blots and gels

## Supplementary Figure 2b



anti-Bmal1 rabbit (Cell Signaling Technology Cat# 14020, RRID:AB\_2728705) 1:1000



anti-Tubulin mouse (Cell Signaling Technology Cat# 3873, RRID:AB\_1904178) 1:1000

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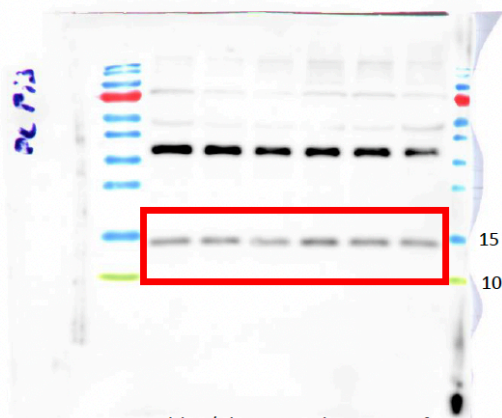
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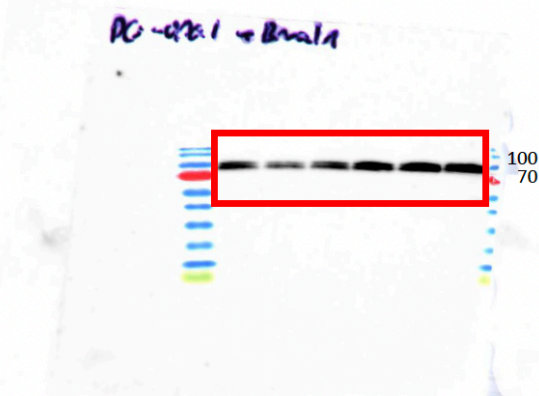
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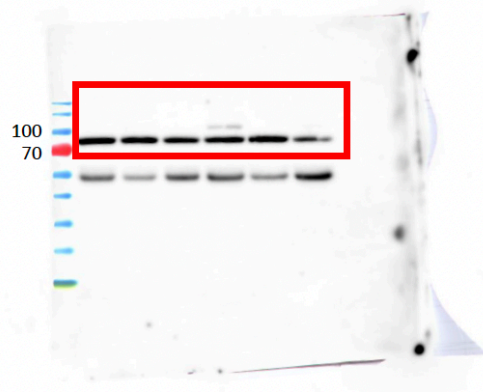
## Supplementary Figure 5b



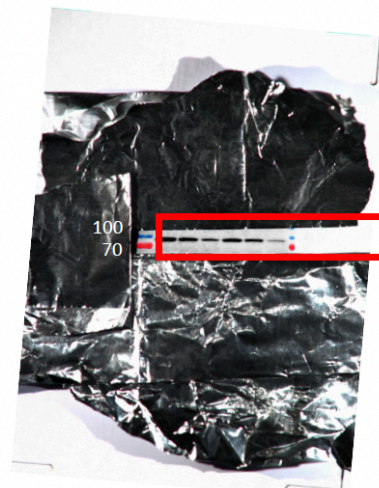
anti-Fis1 rabbit (Thermo Fisher Scientific Cat# PA5-22142, RRID:AB\_11152577) 1:1000



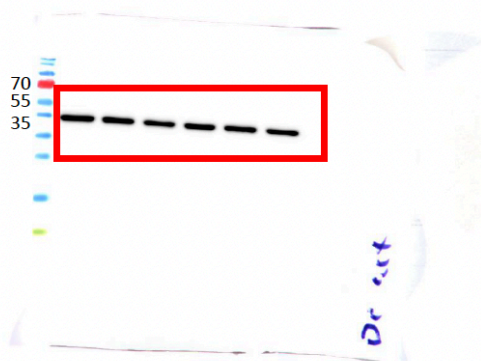
anti-Opa1 mouse (Cell Signaling Technology Cat# 80471, RRID:AB\_2734117) 1:1000



anti-Mfn2 rabbit (Cell Signaling Technology Cat# 9482, RRID:AB\_2716838) 1:1000



anti-Mfn1 mouse (Abcam Cat# ab126575, RRID:AB\_11141234) 1:500



anti- $\beta$  actin mouse (Millipore Cat# MAB1501, RRID:AB\_2223041) 1:10000

## Supplementary Figure 7

