Figure 1

a

| Glucose Starvation: | +TLR9-HA | -IgG | +IgG |
|---------------------|----------|------|------|
| TLR9-HA             |          |      |      |
| beclin 1            |          |      |      |
| TLR9-HA             |          |      |      |
| beclin 1            |          |      |      |

IP: beclin 1

WCL
Figure 1

- **Figure 1b**

  - **Tissue Lysate**
  - **IP: HA**

  - **WT** 0 5 10 20 30 50 min

  - **Tlr9-HA**
  - **beclin 1**
  - **p-AMPKα**
  - **total AMPKα**
Figure 2

a

|       | WT       | Tlr9⁻/⁻  |
|-------|----------|----------|
|       | Resting  | Exercise | Resting | Exercise |
| p-AMPKα | 1 2 3 1 2 3 |         | 1 2 3 1 2 3 |         |
| total AMPKα |         |         |         |         |
| p-TBC1D1 |         |         |         |         |
| Total TBC1D1 |         |         |         |         |

Exercise Resting
Figure 2

|          | Resting | Exercise 90 min |
|----------|---------|-----------------|
| Recipient: |         |                 |
| WT       | WT      | Tlr9⁻/⁻         |
| Donor:   |         |                 |
| WT       | Tlr9⁻/⁻ | WT              |

1 2 3 1 2 3 1 2 3

**p-AMPKα**

**Total AMPKα**

**h**

| Electrical stimulation | WT |          | Tlr9⁻/⁻ |
|------------------------|----|----------|---------|
| -                      | 1  | 2        | 1       |
| +                      | 2  | 3        | 2       |

1 2 3 1 2 3

**p-AMPKα**

**Total AMPKα**
**Figure 3**

(a) **WT** and **Tlr9**

**IP:**
- beclin 1

**Tissue Lysate**
- UVRAG
- Atg14
- Vps34
- beclin 1

**Tissues:**
- WT
- KO
- KO

**Total AMPKα**
- p-AMPKα
Figure 3

**Panel C**

**Western Blot**
- **IgG**
- **beclin 1**
- **ATG14**
- **VPS34**

**Immunoprecipitation (IP)**
- **beclin 1**

**WCL**
- **UVRAG**
- **ATG14**
- **VPS34**
- **beclin 1**

**Lanes**
- IgG 0 20 40 60 120 min
- 75kd
- 50kd
- 100kd

**Markers**
- 75kd
- 50kd
- 100kd
**Figure 3**

- **p-AMPKα**
- **AMPKα**
- **beclin 1**
- **β-actin**

**gRNA:**

- **Growth**
- **Glucose Medium Starvation**
- ng 1 ng 2 ng 1 ng 2

**Western Blots:**

- **p-AMPKα**
- **AMPKα**
- **beclin 1**
- **β-actin**

**Gel Images:**

- Glucose
- Starvation
- Growth

**Molecular Weights:**

- 75kd
- 50kd
- 75kd
- 50kd
- 37kd
- 100kd
- 75kd
- 50kd
- 37kd
- 25kd

**gRNAs:**

- UVRAG
**Figure 3**

d (continued)

| gRNA:   | Growth Medium | Glucose Starvation |
|--------|---------------|--------------------|
| p-AMPKα| ng 1 2        | ng 1 2             |

![Image of gel electrophoresis showing protein bands for p-AMPKα, AMPKα, ATG14, and β-actin under different conditions.](image-url)

**ATG14 gRNAs**
Extended Data Fig. 1

a

TLR7-HA

Flag-

beclin 1

Flag-

beclin 1

Flag-

beclin 1

TLR9-HA

Flag-

beclin 1

Flag-

beclin 1

b

+ Flag-beclin 1

- +

+ glucose starvation

- +

+ glucose starvation

+ Flag-beclin 1

- + TLR9-HA
Extended Data Fig. 1
Extended Data Fig. 1

e

MBP pull-down

input

beclin 1

MBP-TIR

MBP

beclin 1

MBP-TIR

MBP

50kd

75kd

37kd
Extended Data Fig. 1

 TL9R-HA: FL | FL | 1-868 (ΔTIR)
 Flag-beclin 1: FL | Δ267-284 | FL

TLR9-HA

Flag-beclin 1

TLR9-HA

Flag-beclin 1

IP: Flag

WCL
Extended Data Fig. 1

TLR9-HA

Flag-beclin 1

p-AMPKα

total AMPKβ1/β2

Flag-beclin 1:

- + + + + +

TLR9-HA

- - - - - -

p-AMPKα

- - - - - -

total AMPKβ1/β2

- - - - - -

Control normal CpG OA PTZ α
Extended Data Fig. 2

(a) The diagram shows the time course of beclin 1 expression in wild-type (WT) and Tlr9-HA knockout (KI) mice. The western blot analysis is performed at 0, 10, 20, 30, and 50 minutes. The bands at 75kDa and 50kDa are highlighted, indicating the expression levels of beclin 1 and Tlr9-HA, respectively. The legend for the western blot is provided, indicating the sample types: IP: HA, Tissue, and Lysate.
Extended Data Fig. 2

c  
0  10  20  30  50  90 min of exercise

c-AMPKα

total AMPKα

Tlr9-HA KI

WT 0  10  20  30  50  90 min of exercise

beclin 1

Tlr9-HA

beclin 1

Tissue Lysate

IP: HA
Extended Data Fig. 3

**c**

- p-AMPKa
- total AMPKa

**e**

- p-AMPKa
- total AMPKa
Extended Data Fig. 5

a

|        | WT Resting | WT Exercise | Tlr9−/− Resting | Tlr9−/− Exercise |
|--------|------------|-------------|----------------|-----------------|
| p-ACC  |            |             |                |                 |
| total ACC |           |             |                |                 |
| p-Raptor |           |             |                |                 |
| total Raptor |       |             |                |                 |
| β-actin |           |             |                |                 |
Extended Data Fig. 5

**Extended Data Fig. 5**

**c**

|          | WT |          | Tlr9<sup>−/−</sup> |
|----------|----|----------|------------------|
|          | Resting | Exercise | Resting | Exercise |
| p-AMPKα  | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |
| Total AMPKα | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |
| p-TBC1D1 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |
| Total TBC1D1 | 1 2 3 | 1 2 3 | 1 2 3 | 1 2 3 |

**e**

|          | WT |          | Tlr9<sup>−/−</sup> |
|----------|----|----------|------------------|
|          | 1 2 3 4 5 6 | 1 2 3 4 5 6 | 1 2 3 4 5 6 |
| GLUT4    | 1 2 3 4 5 6 | 1 2 3 4 5 6 | 1 2 3 4 5 6 |
| β-actin  | 1 2 3 4 5 6 | 1 2 3 4 5 6 | 1 2 3 4 5 6 |
Extended Data Fig. 6

g

|          | WT |          |          |
|----------|----|----------|----------|
|          |    | Resting | Exercise |
|          | 1  | 2  | 3  | 1  | 2  | 3  | 1  | 2  | 3  | 1  | 2  | 3  |
| p-LKB1 Ser428 |    |    |    |    |    |    |    |    |    |    |    |    |
| p-AMPK Thr172 |    |    |    |    |    |    |    |    |    |    |    |    |
| total LKB1    |    |    |    |    |    |    |    |    |    |    |    |    |
| total AMPK    |    |    |    |    |    |    |    |    |    |    |    |    |
Extended Data Fig. 6

|       | WT | Tlr9⁻ |       |
|-------|----|-------|-------|
| PF-739| -  | +     | -    | +     |
|       | 1  | 2     | 1    | 2     |

- **p-AMPKα**
  - WT: -
  - Tlr9⁻: +

- **total AMPKα**
  - WT: -
  - Tlr9⁻: -

- **p-TBC1D1**
  - WT: -
  - Tlr9⁻: +

- **total TBC1D1**
  - WT: -
  - Tlr9⁻: -
Extended Data Fig. 7

**c**

| Recipient | Donor | WT | WT | Tlr9−/− | Tlr9−/− |
|-----------|-------|----|----|---------|---------|
| WT        | WT    | 1  | 2  | 3       | 1       |
| WT        | Tlr9−/− | 2 | 3 | 1       | 2       |
| Tlr9−/−   | WT    | 3 | 1 | 2       | 3       |
| Tlr9−/−   | Tlr9−/− | 1 | 2 | 3       | 1       |

**p-TBC1D1**

**total TBC1D1**

**e**

| Electrical stimulation | WT | Tlr9−/− |
|------------------------|----|---------|
| -                      | -  | +       |
| +                      | +  | +       |

**p-TBC1D1**

**total TBC1D1**
Extended Data Fig. 8

**c**

|          | WT; Tlr9-HA KI | BCL2 AAA; Tlr9-HA KI |
|----------|----------------|----------------------|
| WT 0     | 10 20 30       | 0 10 20 30 min       |

**beclin 1**

**Tlr9-HA**

**beclin 1**

**Tissue Lysate**

**p-AMPKα**

**total AMPKα**

**BCL2**

**IP: HA**
**Extended Data Fig. 8**

| Flag-beclin 1 | IgG | Myc-BCL2 WT | Myc-BCL2 AAA |
|---------------|-----|-------------|--------------|
| Glucose starvation: | - + + + + + | - + + + + + |

**IP:**
- Myc

**WCL**

Flag-beclin 1

Myc-BCL2
Extended Data Fig. 8

Flag-beclin 1

TLR9-HA

Glucose starvation:

Flag-beclin 1: + + + +

TLR9-HA: - + + +

Myc-BCL2 WT:  -  +  +  +

Myc-BCL2 AAA:  +  +  +  +

IP: HA

WCL
Extended Data Fig. 9

a

Extended Data Fig. 9a shows a Western blot experiment with IgG levels measured at different time points (0, 10, 20, 30, 50, 90 minutes) of exercise. The blot shows protein bands for UVRAG and beclin 1, with molecular weight markers at 100kDa and 75kDa.

b

Extended Data Fig. 9b presents a similar Western blot experiment for UVRAG and beclin 1 in tissue lysates with electrical stimulation. The blot includes protein bands before and after stimulation, showing changes in the expression levels of these proteins.

c

Extended Data Fig. 9c demonstrates the effects of electrical stimulation on WT and Tlr9-/- mice. The blot illustrates the changes in UVRAG and beclin 1 expression levels, highlighting the impact of Tlr9 on these proteins.

Extended Data Fig. 10

a

Extended Data Fig. 10a shows the expression levels of p-LKB1 Ser428, LKB1, and AMPKβ1/β2 under different conditions. The gRNAs used were beclin 1, LKB1, and UVRAG. The samples were grown in either Growth Medium or Glucose starvation.

b

Extended Data Fig. 10b illustrates the expression profiles of p-LKB1 Ser428, LKB1, and AMPKβ1/β2 when UVRAG gRNA was used. The conditions were Growth Medium or Glucose starvation.

c

Extended Data Fig. 10c presents the expression data for p-LKB1 Ser428, LKB1, and AMPKβ1/β2 with the use of ATG14 gRNA. The samples were evaluated under Growth Medium or Glucose starvation.