Figure 1

Epitrochlearis AS160

Epitrochlearis pAS160 Thr^{+/-}

EDL Memcode

EDL AS160

EDL pAS160 Thr^{+/-}

Gastrocnemius Memcode

Gastrocnemius AS160

Gastrocnemius pAS160 Thr^{+/-}

WAT Memcode

WAT AS160

WAT pAS160 Thr^{+/-}

Soleus Memcode

Soleus AS160

Soleus pAS160 Thr^{+/-}

Heart Memcode

Heart AS160

Heart pAS160 Thr^{+/-}

W: wild type
K: AS160 Knock-out

All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Figure 4 (All samples are from the hyperinsulinemic euglycemic clamp)

W: wild type
K: AS160 Knock-out
X: value excluded from statistical analysis because of technical problems with the hyperinsulinemic euglycemic clamp

All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Figure 5 (All samples are from the hyperinsulinemic euglycemic clamp)

- **Epitrochlearis TBC1D1**
  - W K W K W K W K W K
  - 150 kDa

- **EDL TBC1D1**
  - W K W K W K W K W K W K
  - 150 kDa

- **Gastrocnemius TBC1D1**
  - W K W K W K W K W K W K
  - 150 kDa

- **Soleus TBC1D1**
  - W K W K W K W K W K W K
  - 150 kDa

- **WAT TBC1D1**
  - W K W K W K W K W K W K
  - 150 kDa

- **Heart TBC1D1**
  - W K W K W K W K W K W K
  - 150 kDa

W: wild type
K: AS160 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Figure 6 (All samples are from the hyperinsulinemic euglycemic clamp)

Epitrochlearis GLUT4

50 kDa
37 kDa

Soleus GLUT4

75 kDa
50 kDa
37 kDa

W: WT
K: AS160 Knock-out

EDL GLUT4

50 kDa
37 kDa

WAT GLUT4

50 kDa
37 kDa

Gastrocnemius GLUT4

50 kDa
37 kDa

Heart GLUT4

50 kDa
37 kDa

All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple.
Figure 7 (All samples are from the hyperinsulinemic euglycemic clamp)

Heart pAkt2\textsubscript{Ser\textgreek{374}}

|    | W | K | W | K | W | K | W | K |
|----|----|----|----|----|----|----|----|----|
| 75 kDa |    |    |    |    |    |    |    |    |
| 50 kDa |    |    |    |    |    |    |    |    |

Heart SERCA2

|    | W | K | W | K | W | K | W | K |
|----|----|----|----|----|----|----|----|----|
| 150 kDa |    |    |    |    |    |    |    |    |
| 100 kDa |    |    |    |    |    |    |    |    |

Heart SGLT1

|    | W | K | W | K | W | K | W | K |
|----|----|----|----|----|----|----|----|----|
| 75 kDa |    |    |    |    |    |    |    |    |
| 50 kDa |    |    |    |    |    |    |    |    |

Heart CD36

|    | W | K | W | K | W | K | W | K |
|----|----|----|----|----|----|----|----|----|
| 100 kDa |    |    |    |    |    |    |    |    |
| 75 kDa |    |    |    |    |    |    |    |    |

W: wild type
K: AS160 Knock-out
All images were captured under the ChemiImmunoscopy channel by FluorChemE by Proteinsimple
X: value excluded from statistical analysis because of technical problems with the hyperinsulinemic euglycemic clamp

Denotes quantified band at expected molecular weight
Figure 8 (All samples are from the hyperinsulinemic euglycemic clamp)

Heart pAMPK<sup>Thr172</sup>

|   | W | K | W | K | W | K | W | K |
|---|---|---|---|---|---|---|---|---|
| 75 kDa |   |   |   |   |   |   |   |   |
| 50 kDa |   |   |   |   |   |   |   |   |

Heart pACC<sup>Ser79</sup>

|   | W | K | W | K | W | K | W | K |
|---|---|---|---|---|---|---|---|---|
| 250 kDa |   |   |   |   |   |   |   |   |

Heart pTBC1D1<sup>Ser237</sup>

|   | W | K | W | K | W | K | W | K |
|---|---|---|---|---|---|---|---|---|
| 150 kDa |   |   |   |   |   |   |   |   |

W: wild type
K: AS160 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Figure 9 (All samples are from the hyperinsulinemic euglycemic clamp)

Heart GLUT8

75 kDa — W K W K W K W K
50 kDa —
37 kDa —

Heart LDH

37 kDa — W K W K W K W K

W: wild type
K: AS180 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Denotes quantified band at expected molecular weight
Figure 11

A Ex Vivo pAS160 Thr\(^{642}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

150 kDa
150 kDa

B Ex Vivo pAS160 Thr\(^{642}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

150 kDa
150 kDa

C Ex Vivo pAkt Ser\(^{473}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

75 kDa
50 kDa

D Ex Vivo pAkt Ser\(^{473}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

75 kDa
50 kDa

E Ex Vivo pAkt Thr\(^{308}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

75 kDa
50 kDa

F Ex Vivo pAkt Thr\(^{308}\) ± Insulin

| Genotype | W | K | W | K | W | K | W | K |
|----------|---|---|---|---|---|---|---|---|
| Insulin  | - | + | - | + | - | + | - | + |

75 kDa
50 kDa

W: wild type
K: AS160 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple

— Denotes quantified band at expected molecular weight
S1 (All samples are from the hyperinsulinemic euglycemic clamp)

Epitrochlearis GLUT1

W K W K W K W K W K
50 kDa 37 kDa

Soleus GLUT1

W K W K W K W K W W
50 kDa 37 kDa

EDL GLUT1

W K W K W K W K W K
50 kDa 37 kDa

WAT GLUT1

W K W K W K W K W K
50 kDa 37 kDa

Gastrocnemius GLUT1

W K W K W K W K W K
50 kDa 37 kDa

Heart GLUT1

W K W K W K W K W K
50 kDa 37 kDa

W: wild type
K: AS160 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple
Denotes quantified band at expected molecular weight
S2 (All samples are from the hyperinsulinemic euglycemic clamp)

W: wild type
K: AS160 Knock-out

All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple

Denotes quantified band at expected molecular weight
S3 (All samples are from the hyperinsulinemic euglycemic clamp)

W: wild type
K: AS160 Knock-out
All images were captured using the Chemiluminescence channel by FluorChemE by Proteinsimple

Denotes quantified band at expected molecular weight