

Supplementary Material

The deubiquitinase OTUB1 is a key regulator of energy metabolism

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Genotyping PCR primers

Otub1^{fl/fl}:

Ef_5272: 5'-TCCACCCCTTCATCCTGCTTTCT-3'

Er_5277: 5'-CAGACCAGAGCAGGATTAAGAAGCCTA-3'

UBC-Cre^{ERT2}:

UBCre 25285: 5'-GACGTCACCCGTTCTGTTG-3'

UBCre-olMR7338: 5'-CTAGGCCACAGAATTGAAAGATCT-3'

UBCre-olMR7339: 5'-GTAGGTGGAAATTCTAGCATC-3'

UBCre-olMR9074: 5'-AGGCAAATTTTGGTGTACGG-3'

Otub1 iKO:

L3r_5274: 5'-TCTACCCATCCCAACACCAGCAAG-3'

Ef_5273: 5'-GAAGGACAAAGGGCGTGTCTCAGT-3'

loxP:

Primer 1307 forw: 5'-GGCAGAAGCACGCTTATCG-3'

Primer 1307 rev: 5'-GACAAGCGTTAGTAGGCACAT-3'

Supplementary Figures

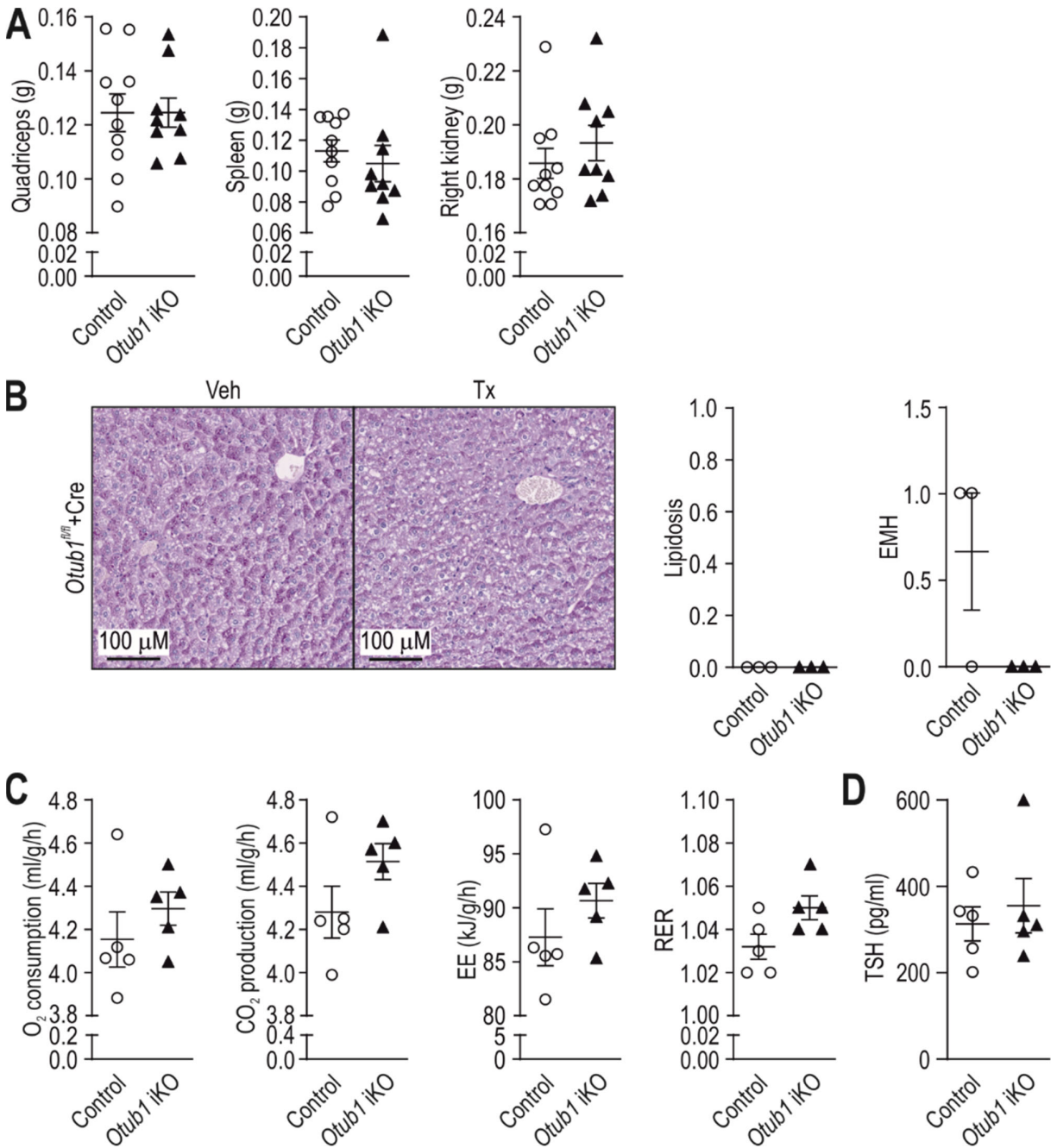


Figure S1. Energy metabolism in mice with induced *Otub1* ablation. **(A)** Weight of additional organs of the same mice shown in Figure 1C. **(B)** Representative periodic acid-Schiff (PAS) staining of transversal liver slices with scored lipidosis levels and extramedullary hematopoiesis (EMH) (n=3). **(C)** Averaged O₂ consumption, CO₂ production, energy expenditure (EE) and respiratory exchange ratio (RER) of 3 consecutive days during the night cycle (n=5). **(D)** Thyroid-stimulating hormone (TSH) plasma levels (n=5). All analyses were performed 5 months after *Otub1* deletion in 6.5 months old mice. Data are shown as mean ± SEM.

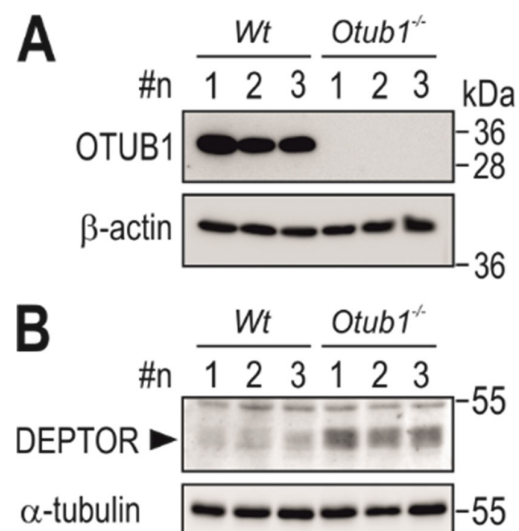


Figure S2. Generation of *Otub1*^{-/-} mouse embryonic fibroblasts. (**A-B**) Immunoblotting of the indicated proteins in wildtype (*Wt*) and *Otub1*^{-/-} cell lysates; #n, n-number; exp., exposure. Full-length immunoblots are shown in Figure S9.

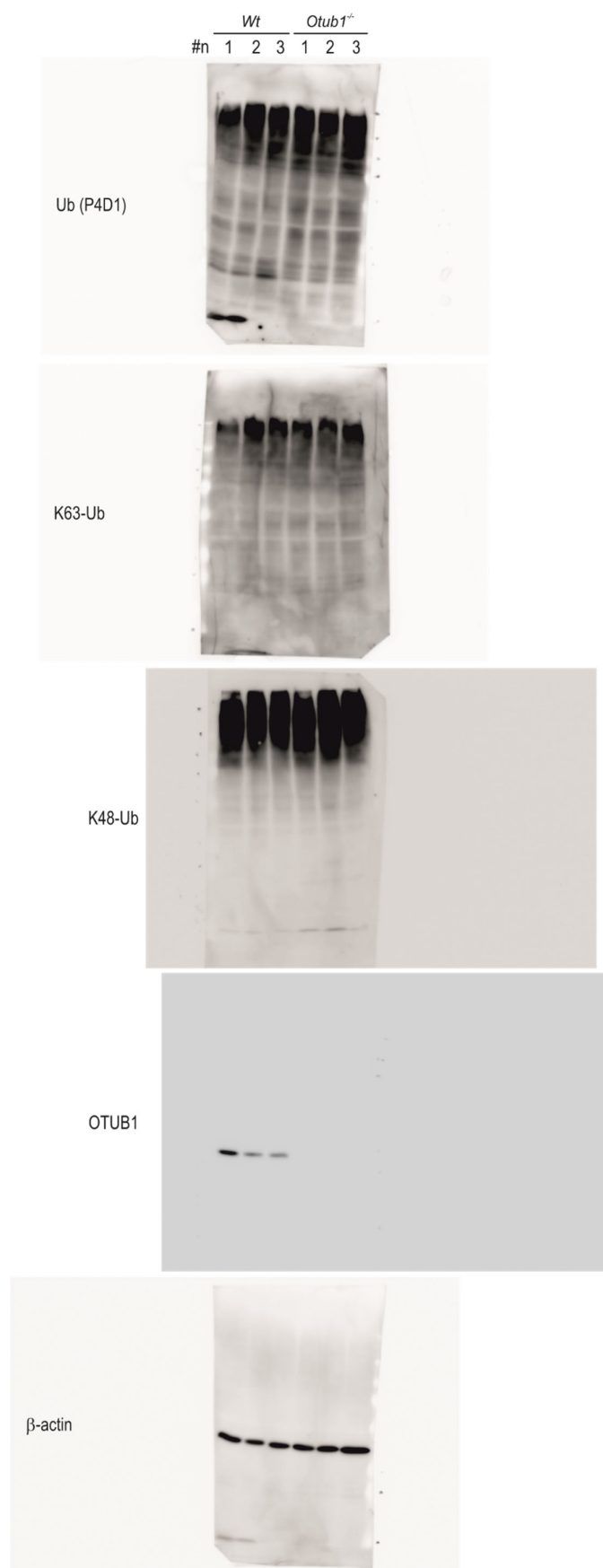


Figure S3. Full-length immunoblots of results shown in Figure 3A.

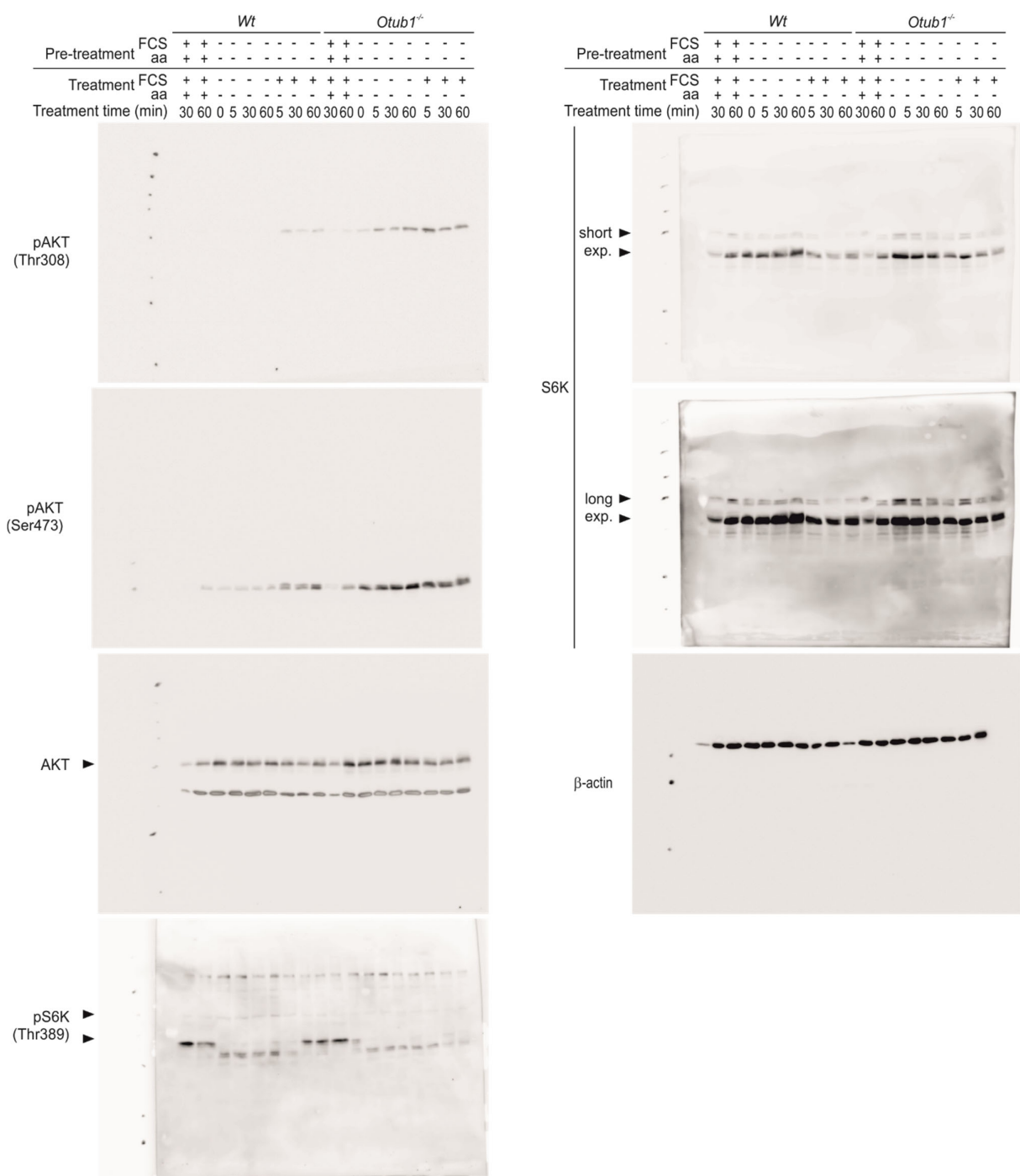


Figure S4. Full-length immunoblots of results shown in Figure 3B.

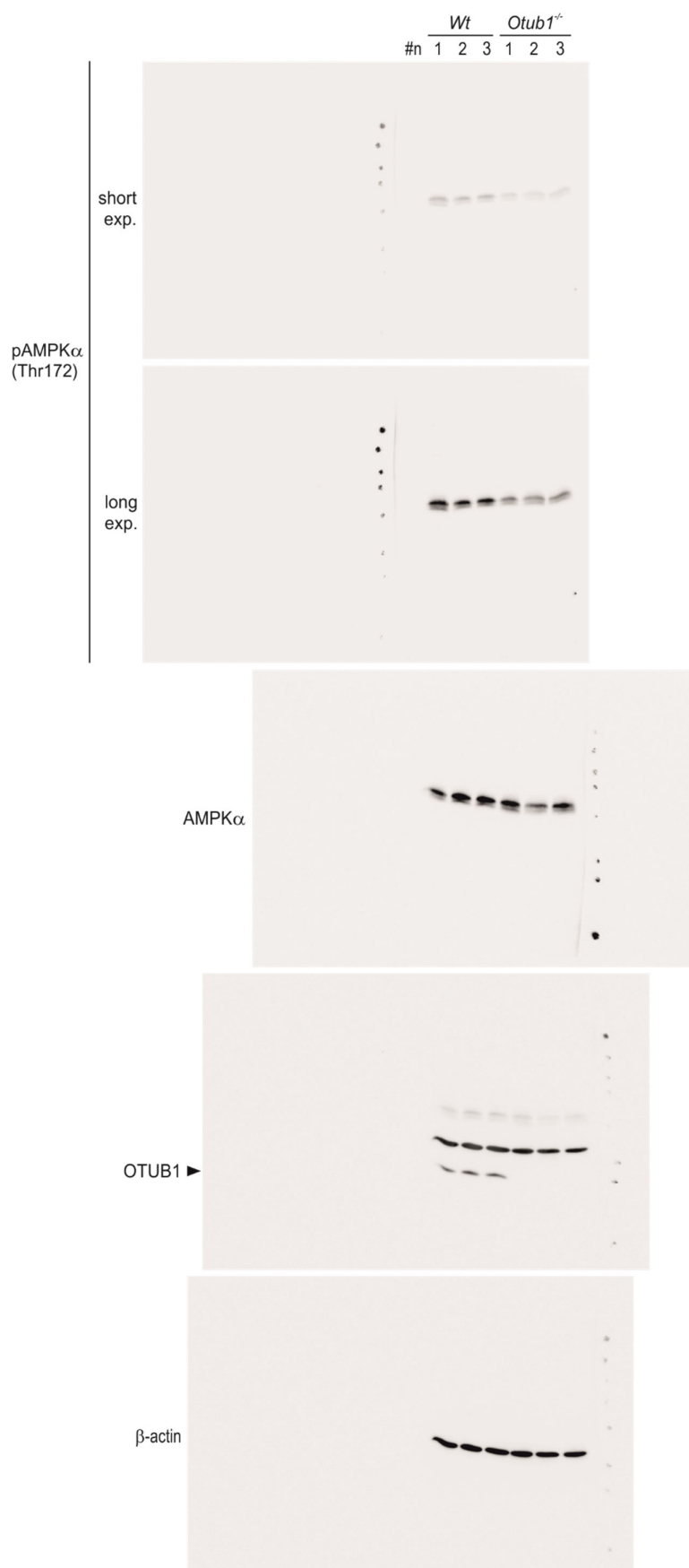


Figure S5. Full-length immunoblots of results shown in Figure 3F.

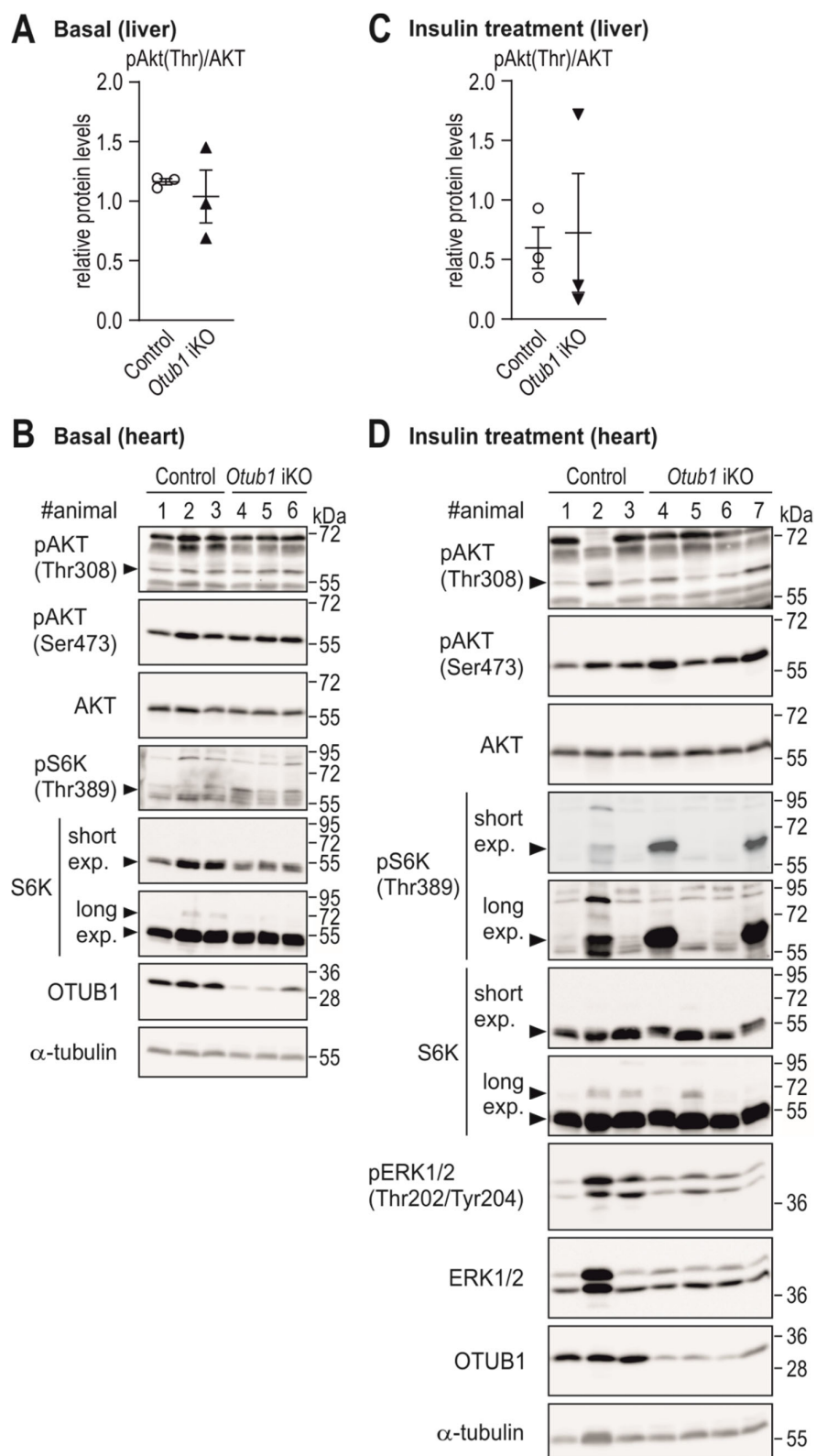


Figure S6. Protein levels in hearts of *Otub1* iKO mice. (**A, C**) Quantification of the indicated immunoblots shown in Figure 5 (normalized to loading control and the indicated protein). (**B**) Immunoblotting of the indicated proteins in heart lysates from control (with/without Cre and vehicle/tamoxifen treatment) and *Otub1* iKO mice (6 months after *Otub1* deletion; 8.5 months old; $n = 3$). (**D**) 10 days after *Otub1* KO (2 months old), mice were starved overnight (16 h) followed by insulin IP application (0.75 mU insulin/g) 15 min prior to tissue harvest (control, $n = 3$; *Otub1* iKO, $n = 4$). Heart lysates were analysed by immunoblotting. #animal, animal number. Full-length immunoblots are shown in Figures S10 and S11.

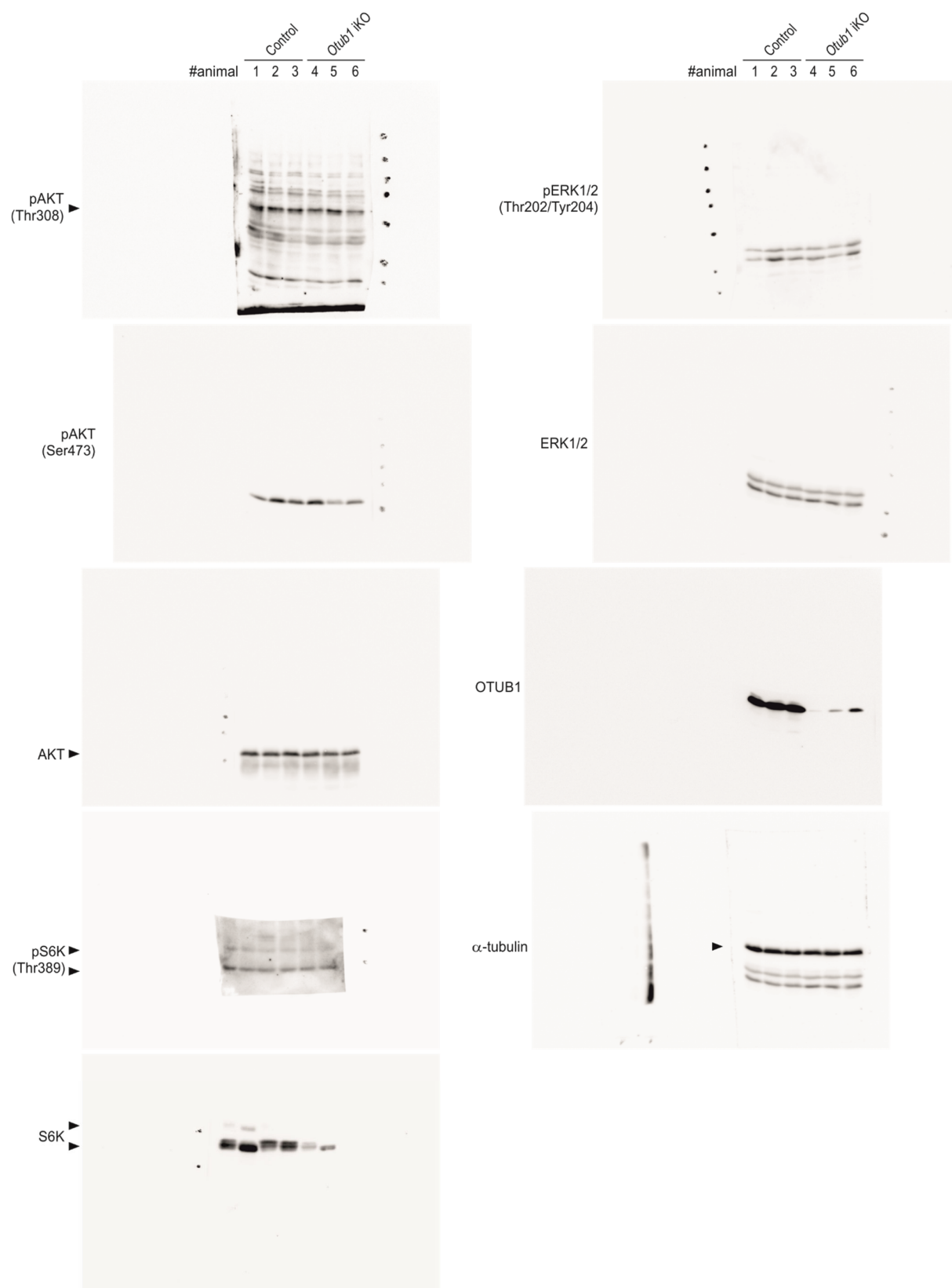


Figure S7. Full-length immunoblots of results shown in Figure 5A.

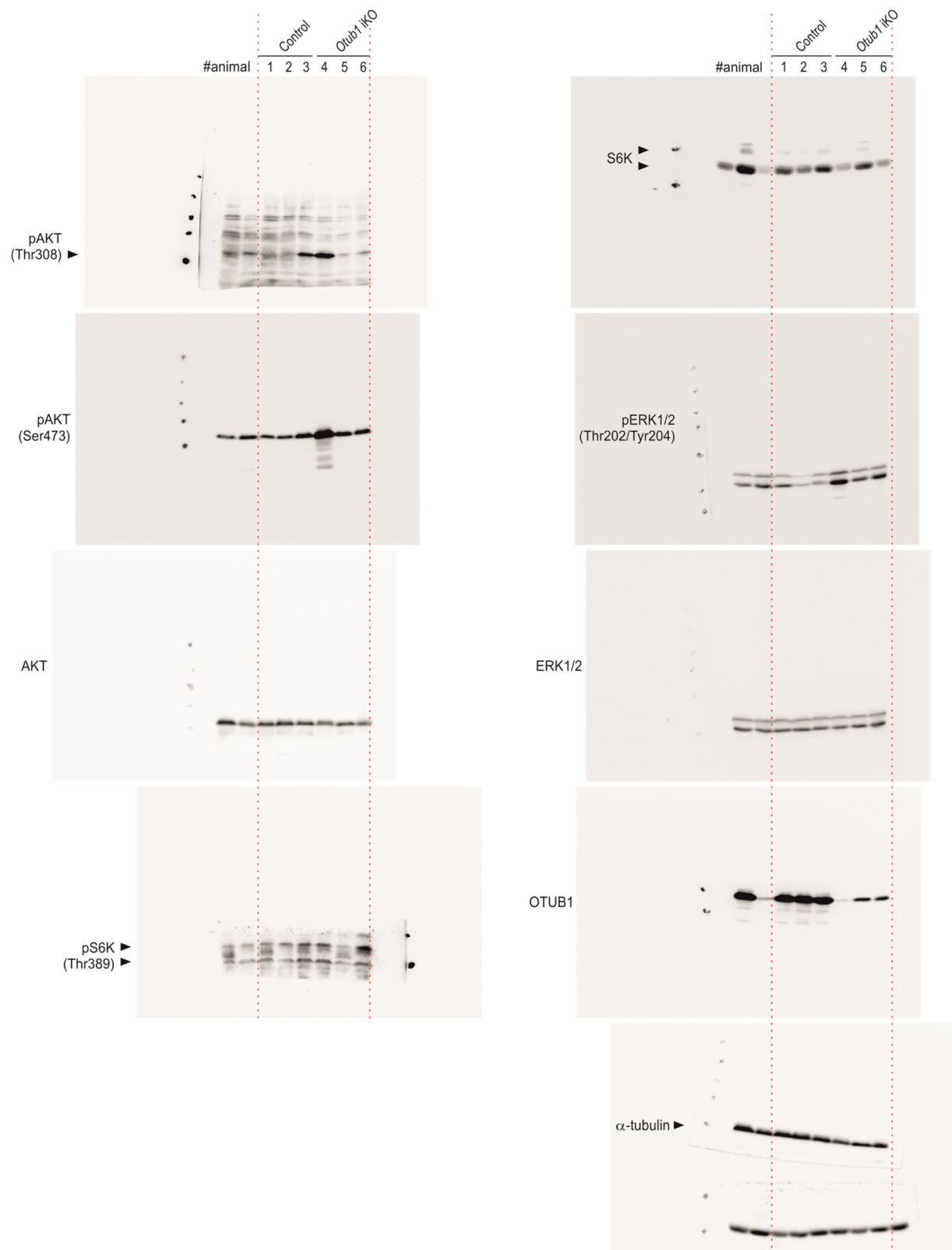


Figure S8. Full-length immunoblots of results shown in Figure 5B.

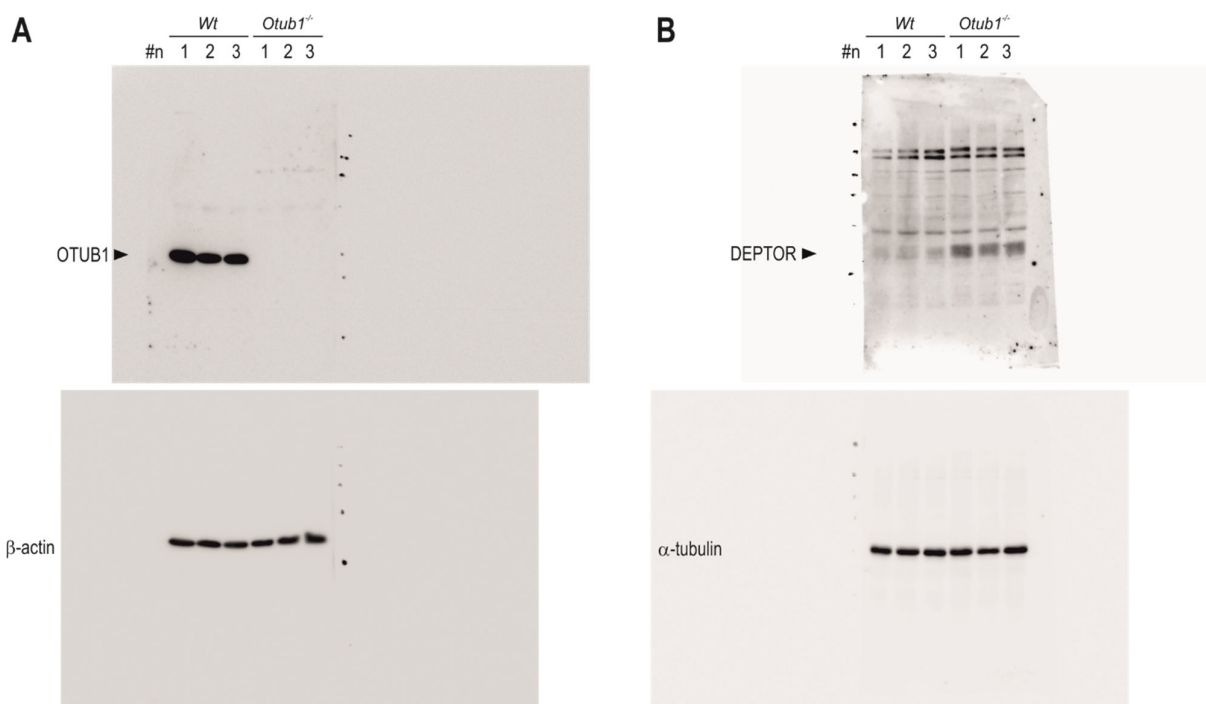


Figure S9. Full-length immunoblots of results shown in Figure S2.

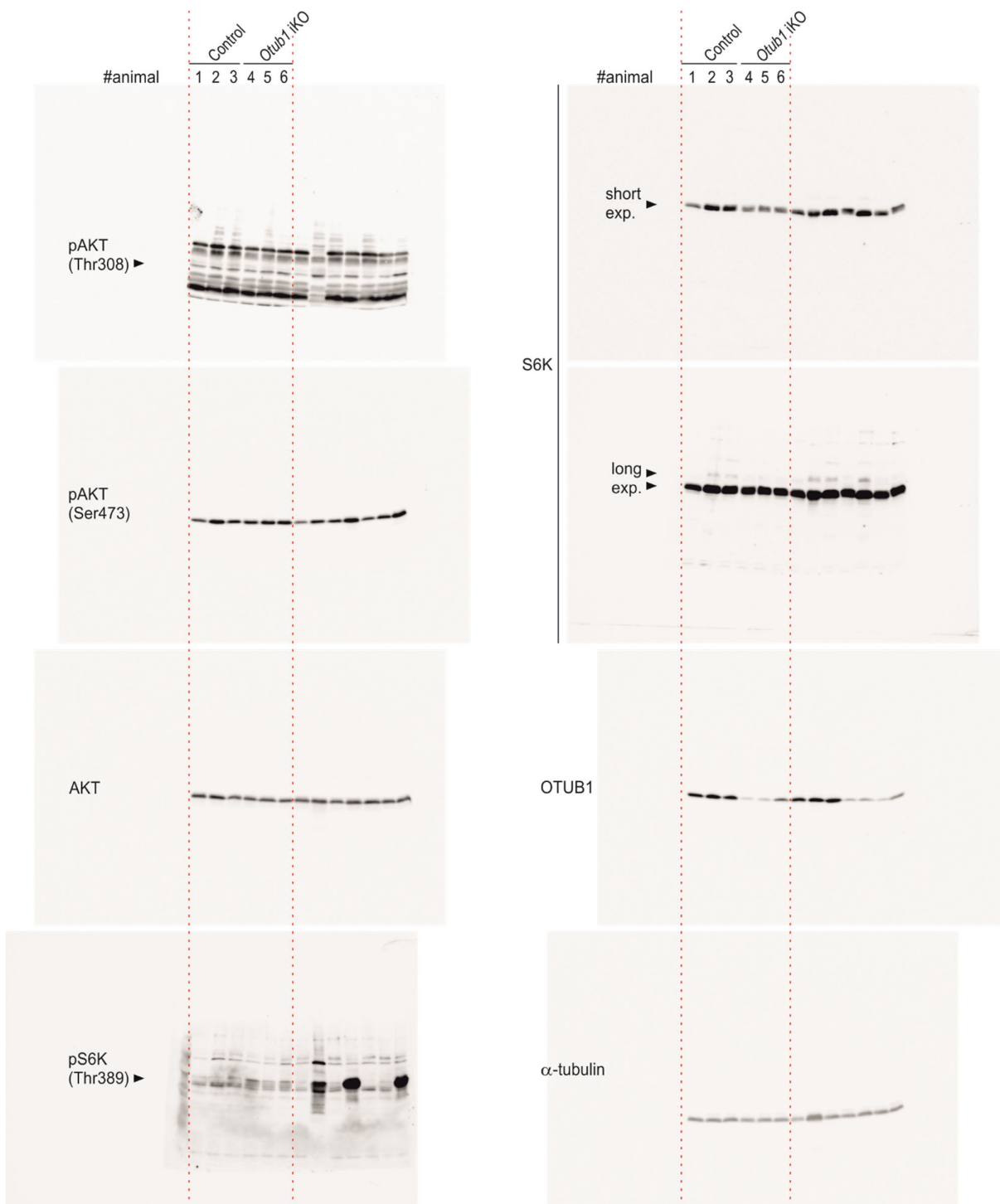


Figure S10. Full-length immunoblots of results shown in Figure S6A.

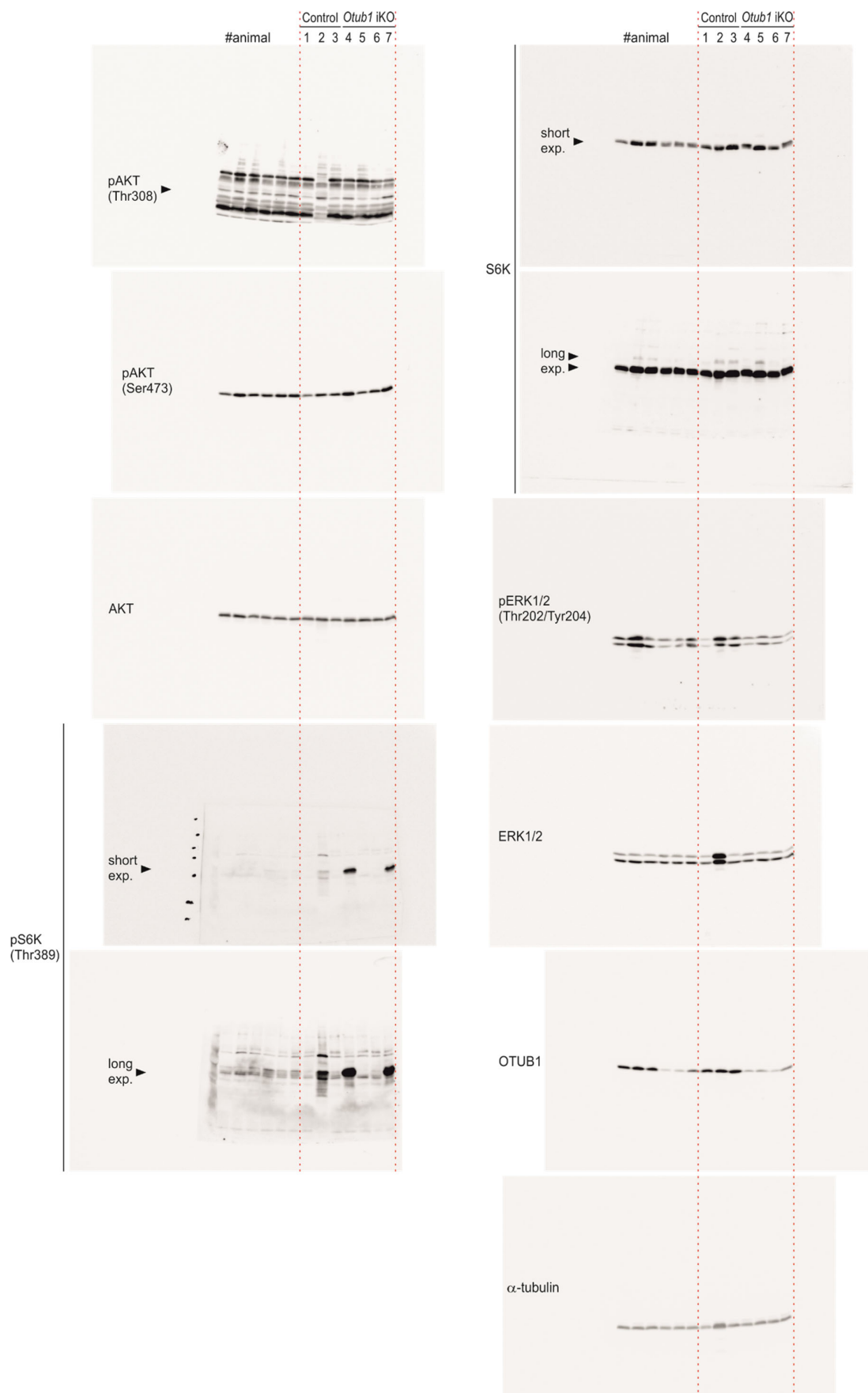


Figure S11. Full-length immunoblots of results shown in Figure S6B.