Expanded View Figures

Figure EV1. Glucocorticoid treatment during skeletal muscle regeneration and signaling through AMPK in macrophages is independent of p38.

A WT and AMPKα1$^{-/-}$ Bone marrow-derived macrophages (BMDMs) were treated with Dexamethasone (Dex) for 1 h before analysis by Western blot for p38 phosphorylation and quantified.

B Representation of GSVA analysis from Fig 1F with gene sets labeled.

C–E Experimental procedure of mice treatment with Dex. After cardiotoxin injection to damage the muscle, AMPKα1$^{fl/fl}$ (WT) and LysM-α1$^{-/-}$ mice were treated with (C) a single dose of Dexamethasone (Dex) intra-peritoneal (i.p.) (0.1 mg/kg) at day 3 (D3), or (D) multiple doses of Dex i.p. (0.1 mg/kg) on D3 to D7 after CTX, or (E) a single dose of Dex i.p. (1 mg/kg) at D3, and *Tibialis Anterior* (TA) muscles were harvested at day 8 (D8) and 14 (D14) after injury. Myofiber cross sectional area was then quantified.

F The number of immune cells (CD45^{pos}, left panel) and macrophages (CD45^{pos}CD64^{pos}, right panel) per milligram of muscle tissue (TA muscle) was assessed by flow cytometry in AMPKα1$^{fl/fl}$ (WT) and LysM-α1$^{-/-}$ mice at day 2, 4 and 8 after cardiotoxin injury.

G Muscles and cells were treated as described in the legend of Fig 2D. Plot on top shows the whole population of macrophages stained with CD64 and Ly6C. Plots on bottom show proportion of macrophages that have phagocytosed fluorescent dead myoblasts in the Ly6C^{pos} (green population), Ly6C^{int} (orange population) and Ly6C^{neg} (red population) macrophages in the various conditions.

Data information: results are means ± SEM (A) or box and whiskers in which the central band represents the median of three biological replicates (A) or 7 (D), 8–10 (C, E), 2–6 (F–G) animals. $^{\$}P < 0.05$, $^{\$\$\$}P < 0.001$ vs untreated WT, using ANOVA tests.
Source data are available online for this figure.

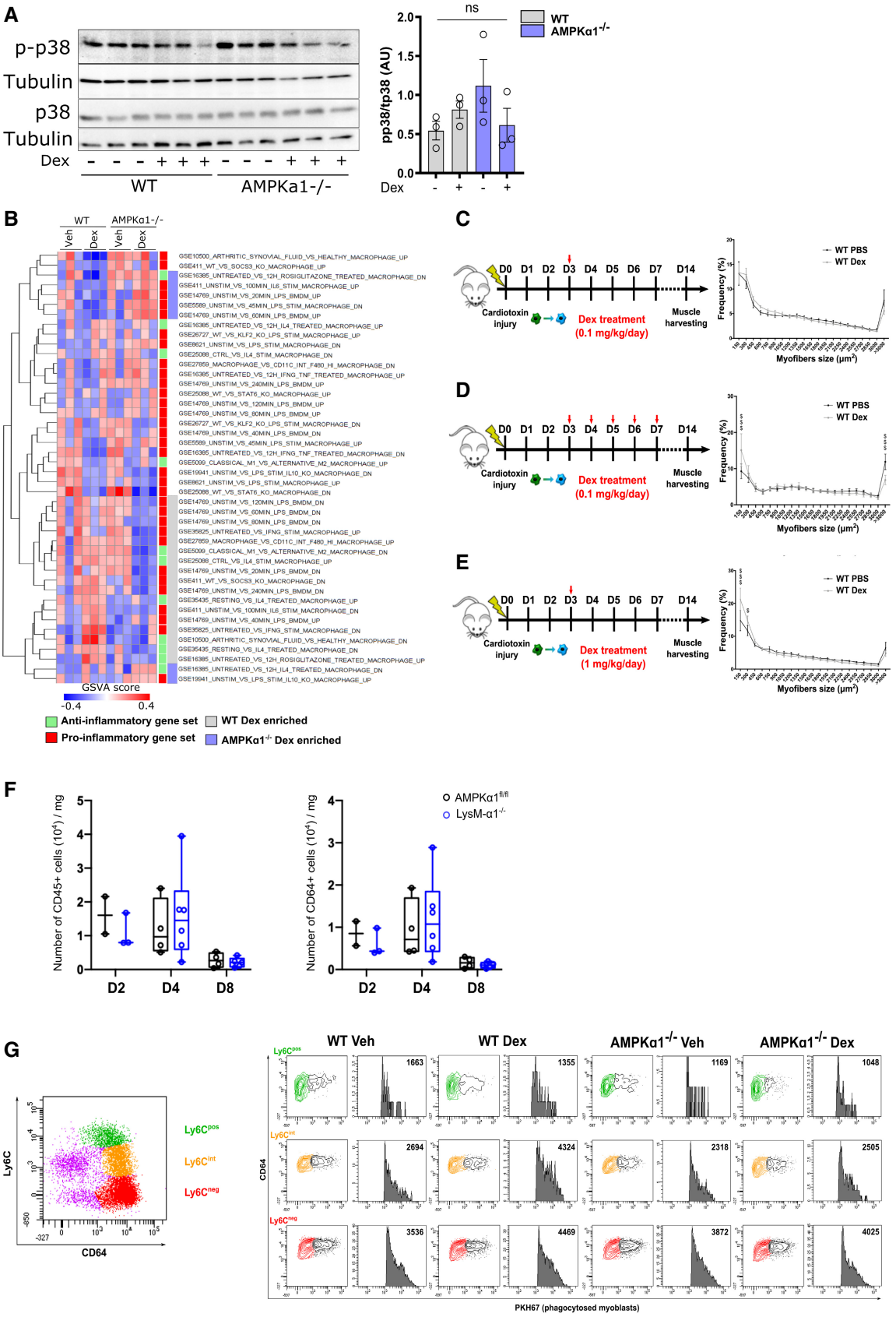


Figure EV1.

Figure EV2. AMPKα1 in macrophages is not required for glucocorticoid-dependent suppression of specific classical inflammatory gene expression and glucocorticoid receptor localization.

A, B AMPKα1$^{fl/fl}$ (WT) and LysM-α1$^{-/-}$ mice were exposed for 24 h to vehicle, lipopolysaccharide (LPS) or LPS + Dex treatment and (A) cytokines and (B) neutrophil numbers were quantified in BAL.

C, D (C) Body mass and (D) body temperature curves for each group during survival experiment in Fig 3E.

E RT–qPCR was performed on AMPKα1$^{fl/fl}$ and LysM-α1$^{-/-}$ macrophages treated with LPS and Dex for 6 h.

F WT and AMPKα1$^{-/-}$ BMDMs were treated with Dex for 1 h and nuclear Glucocorticoid Receptor (GR) intensity determined by immunofluorescence was compared to cytoplasmic GR intensity.

Data information: results are means ± SEM or box and whiskers in which the central band represents the median of 5–9 (B), 13–15 (C, D), 3–7 (A, data below detection threshold were excluded from analysis) animals, and 3 (E, F) biological replicates. Statistical analysis by ANOVA (A, B) or ANOVA with repeated measures (C, D). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ vs untreated WT; $^{£}P < 0.05$ vs untreated LysM-α1$^{-/-}$ or AMPKα1$^{-/-}$, using one-way ANOVA. Bar = 70 μm.

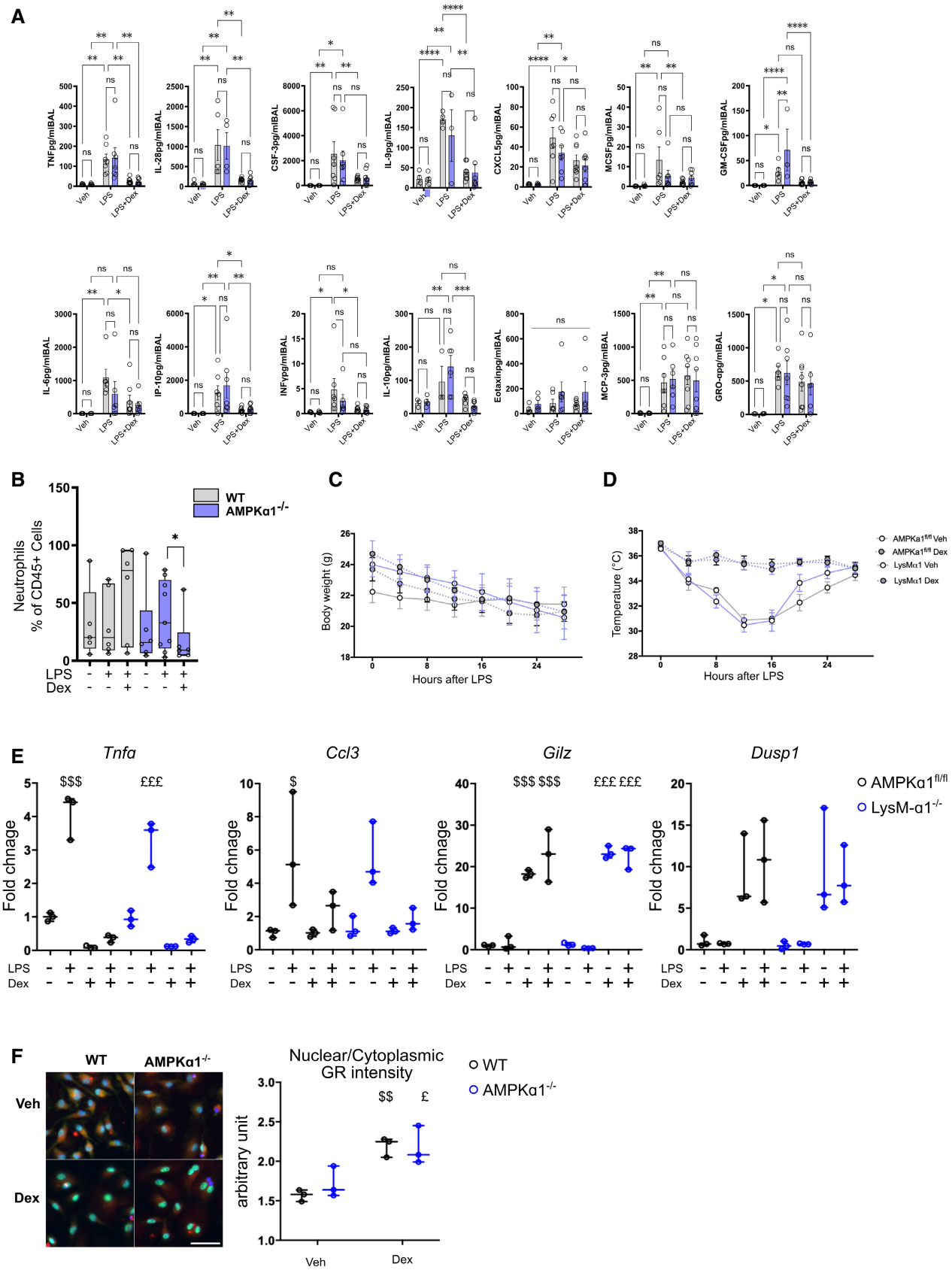


Figure EV2.

Figure EV3. Transcription factor binding sites enriched in Dexamethasone-activated genes.

Top 15 transcription factor binding sites enriched in Dexamethasone (Dex)-activated genes commonly in WT and AMPK$\alpha 1^{-/-}$ macrophages (left panel), in WT cells only (middle panel) and in AMPK$\alpha 1^{-/-}$ cells only (right panel), ranked by decreasing *Z*-score. The consensus binding motif for each transcription factor is shown from the JASPAR database, and its associated *Z*-score.

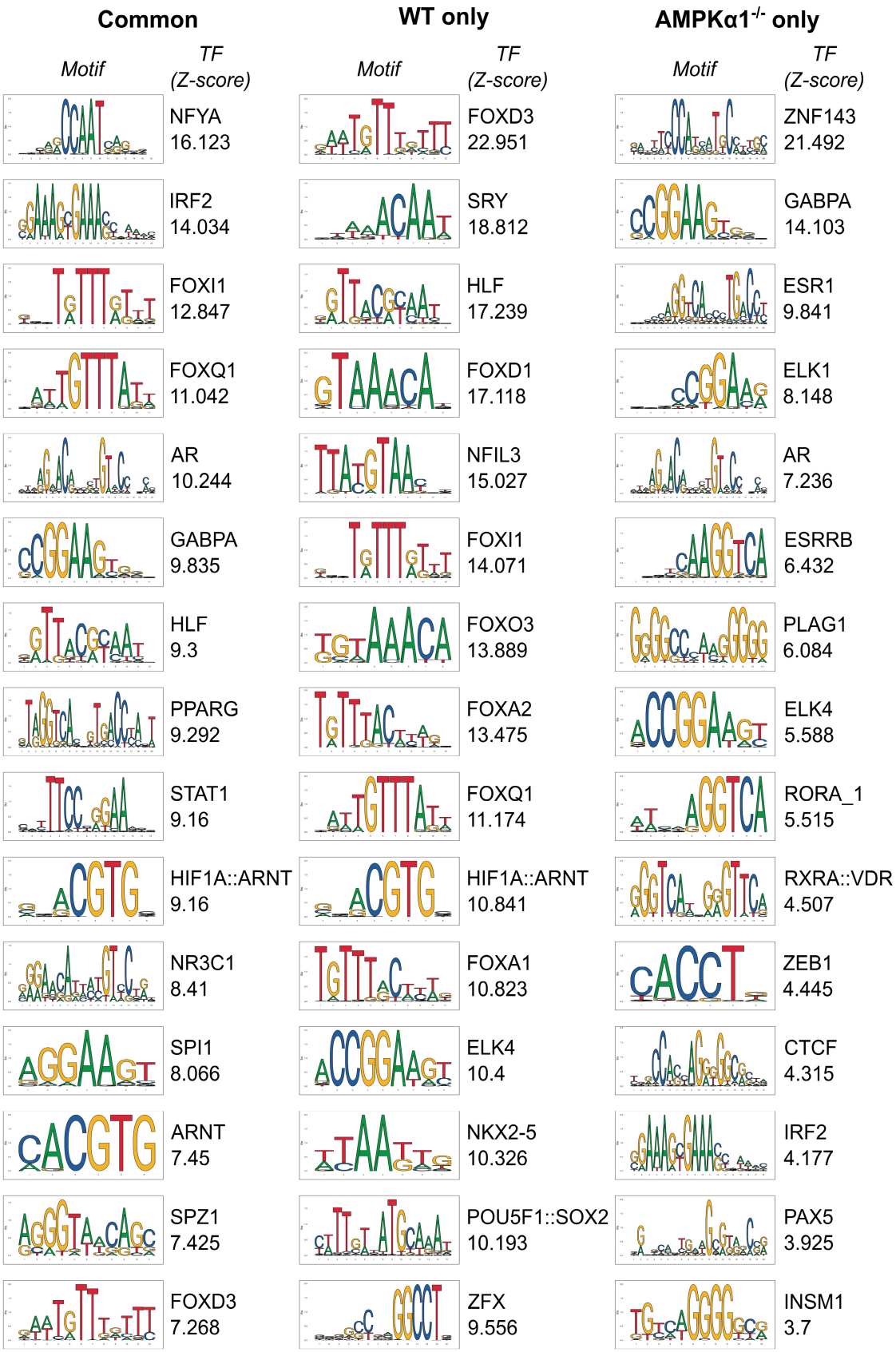


Figure EV3.

Figure EV4. Transcription factor binding sites enriched in Dexamethasone-repressed genes.

Top 15 transcription factor binding sites enriched in Dexamethasone (Dex)-repressed genes commonly in WT and AMPK$\alpha1^{-/-}$ macrophages (left panel), in WT cells only (middle panel) and in AMPK$\alpha1^{-/-}$ cells only (right panel), ranked by decreasing *Z*-score. The consensus binding motif for each transcription factor is shown from the JASPAR database, and its associated *Z*-score.

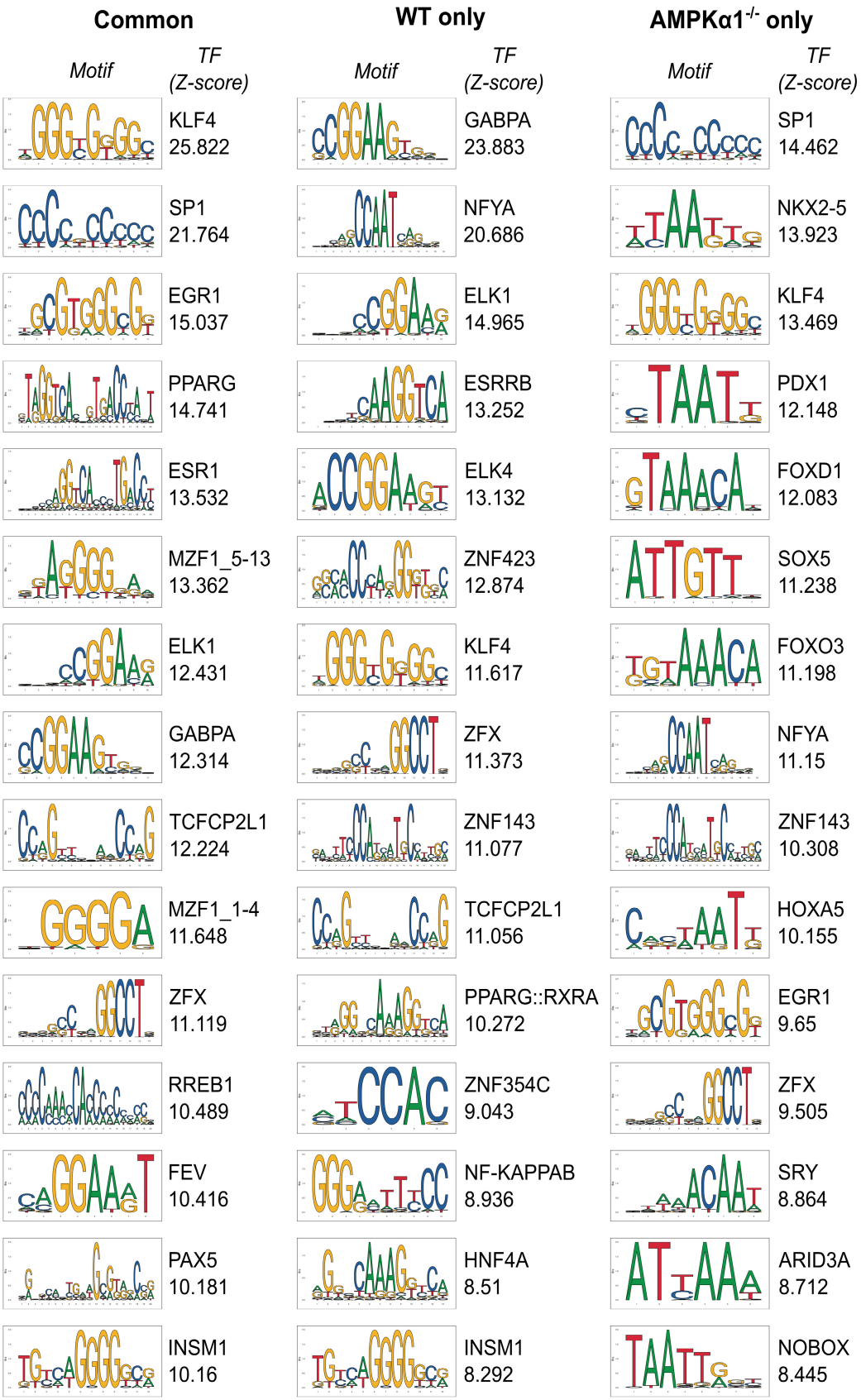


Figure EV4.

Figure EV5. AMPK-GC signaling required FOXO3.

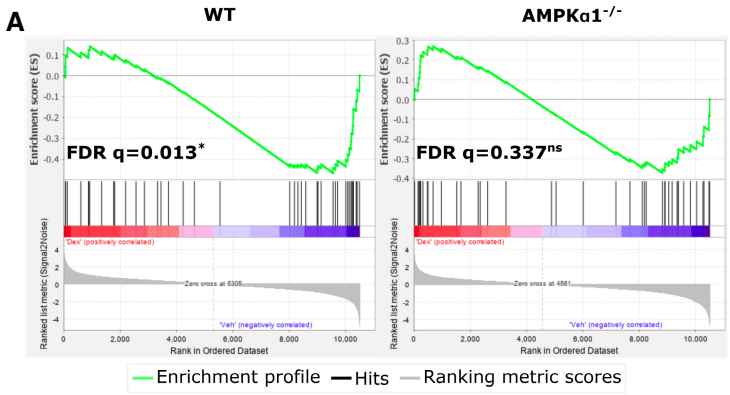
A GSEA analysis of Dexamethasone (Dex)-regulated genes in WT and AMPKα1$^{-/-}$ Bone marrow-derived macrophages (BMDMs) for FOXO3-dependent targets.

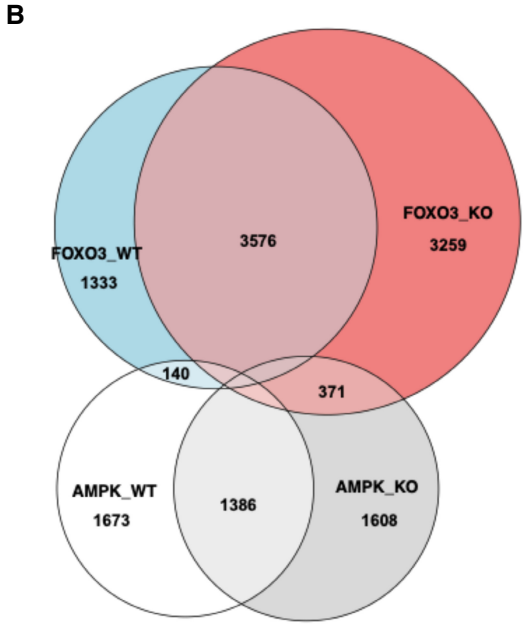
B Venn diagram corresponding to the heatmap shown in Fig 4B.

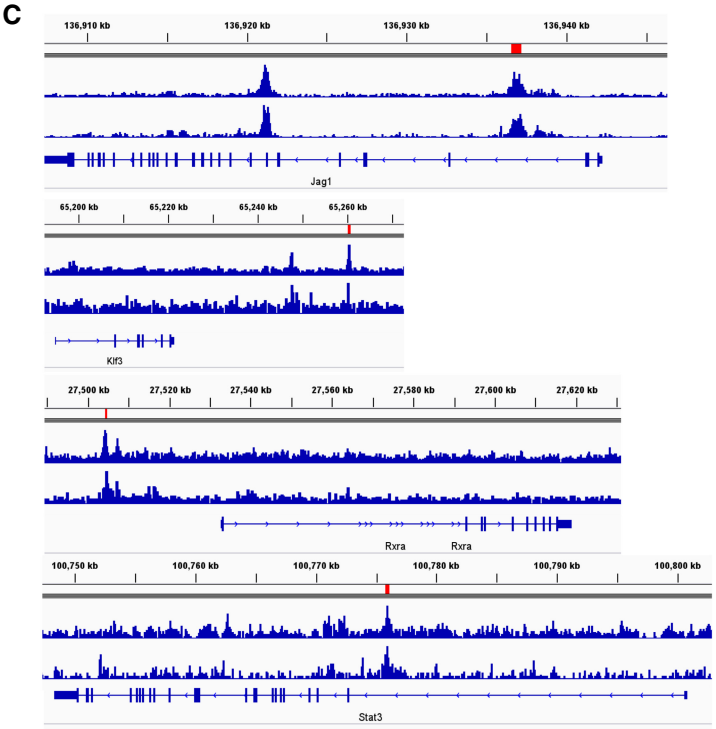
C Screenshot of Integrative Genomics Viewer from publicly available GR ChIP-seq data showing GR DNA binding sites on *Klf3*, *Rxra*, *Stat3* and *Jag1* genes, which are upregulated by Dex specifically in wildtype macrophages (primer position is highlighted in red). Data from GSE110279 (Sacta *et al*, 2018).

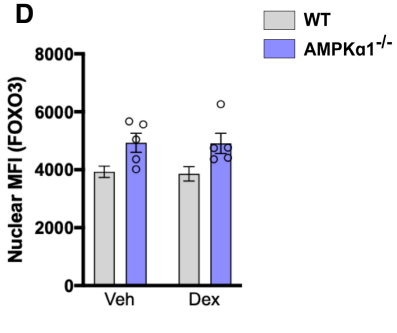
D WT and AMPKα1$^{-/-}$ BMDMs were treated with Dex for 1 h and nuclear FOXO3 intensity was determined by immunofluorescence (results are means ± SEMs of five biological replicates, ANOVA test, no statistical differences).

E AMPKα1$^{-/-}$ BMDMs were treated with lipopolysaccharide (LPS) for 24 h and then incubated with Dex for 6 h in the presence or not of the AKT inhibitor MK-2206. *Rxra* and *Jag1* mRNA levels were analyzed by RT–qPCR (results are means ± SEMs of four biological replicates, Student's *t*-test with $*P < 0.05$, $**P < 0.01$).









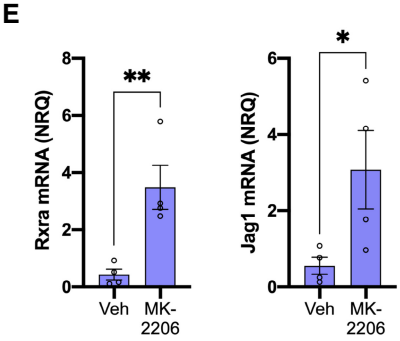


Figure EV5.