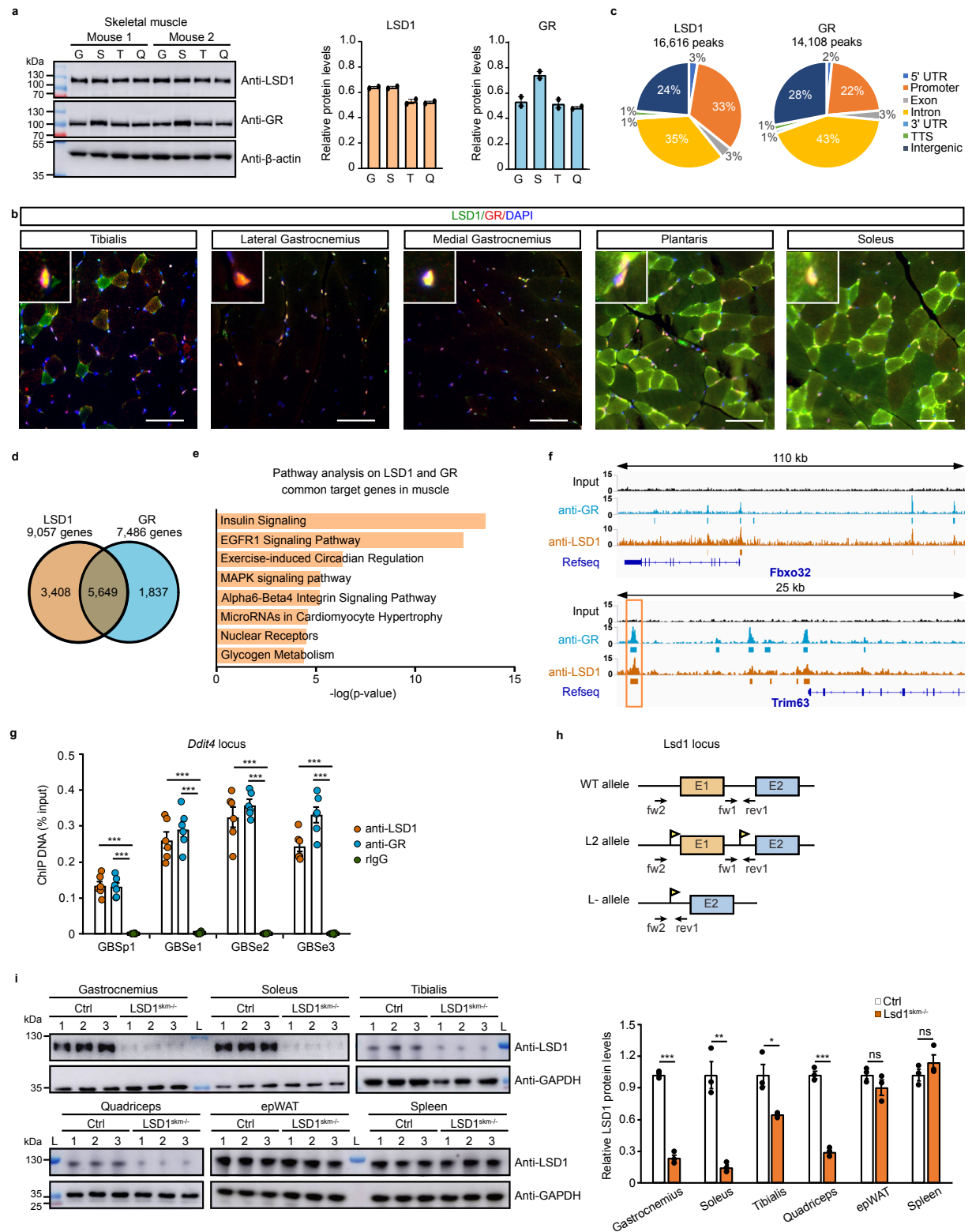


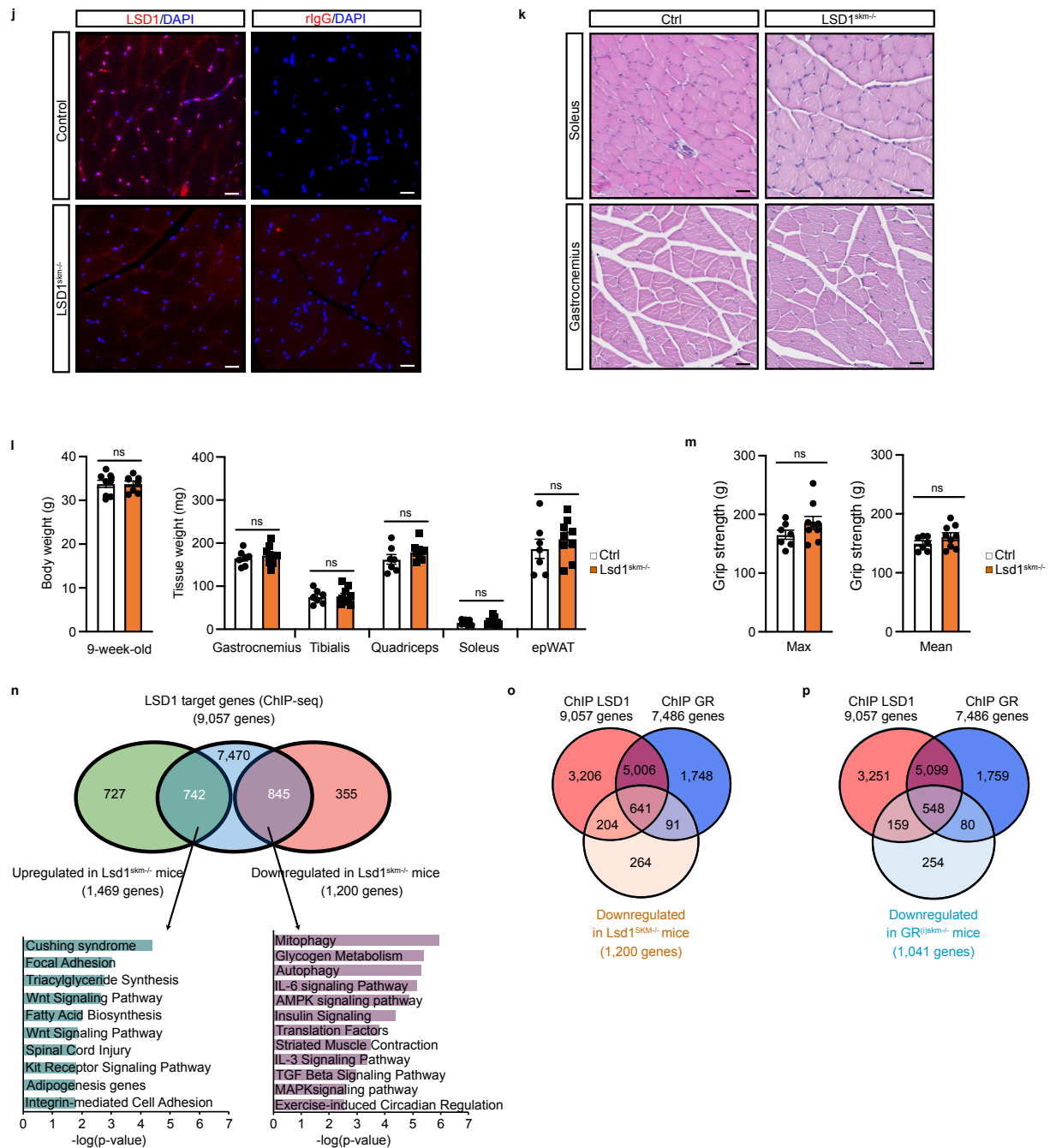
Supplementary Information

LSD1 inhibition circumvents glucocorticoid-induced muscle wasting

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Supplementary figures

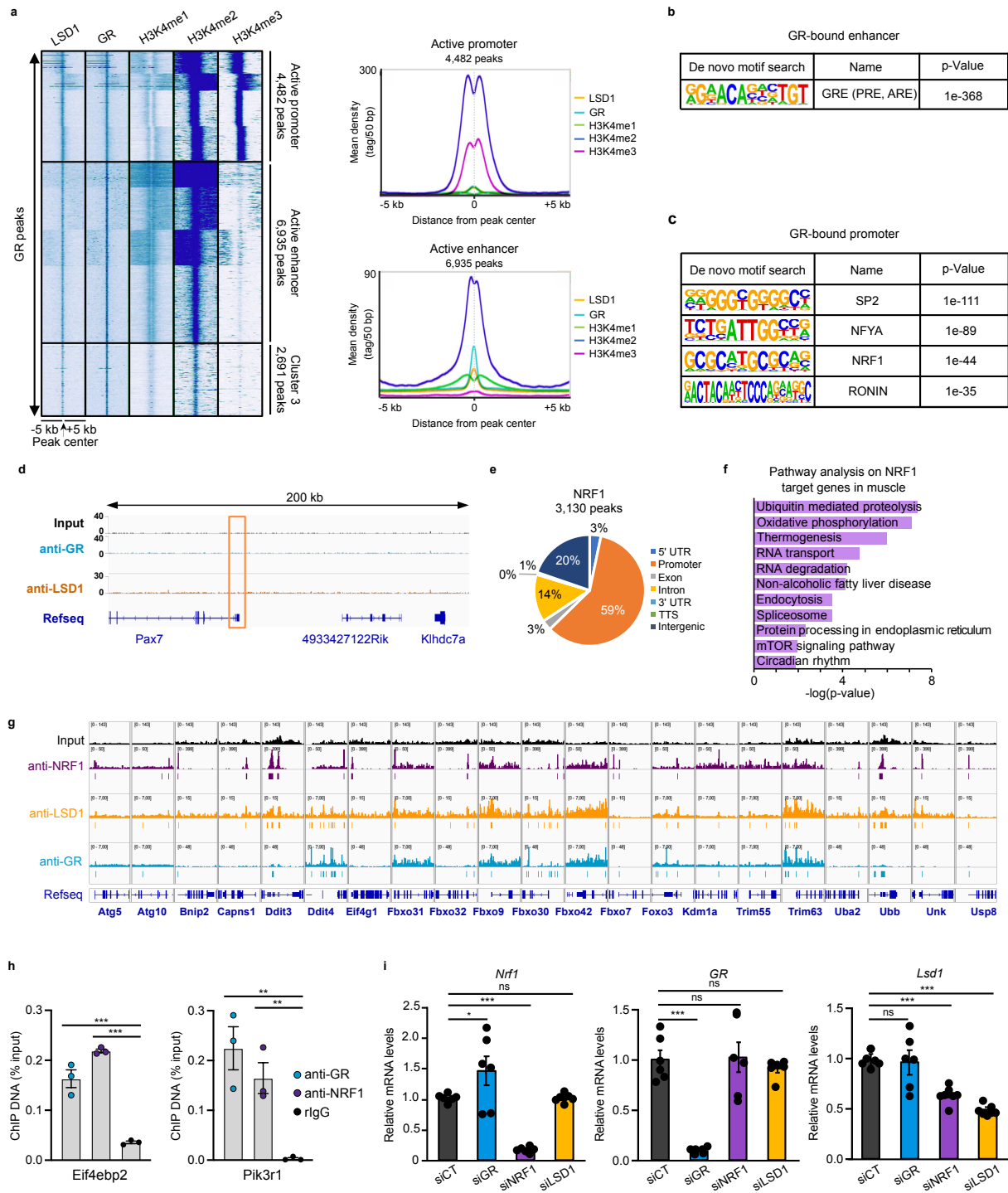




Supplementary Fig. 1. Control of gene expression by GR and LSD1 at physiological glucocorticoid levels.

a. Representative western blot analysis (left) and corresponding quantification (right) of LSD1 and GR protein levels in gastrocnemius (G), soleus (S), tibialis (T) and quadriceps (Q) muscles of 9-week-old wild type mice. β -actin was used as a loading control. **b.** Representative immunofluorescent detection of LSD1 (green) and GR (red) in tibialis, lateral and medial gastrocnemius, plantaris, soleus muscles of 9-week-old wild-type mice. Nuclei were stained with DAPI. Slow-twitch fibers are visible in green due to auto-fluorescence. A zoomed-in view of the confocal observation is shown on the right panel. Scale bar, 100 μ m. N=3. **c.** Pie chart depicting the genomic location of LSD1 (left) and GR (right) binding in mouse skeletal muscles. **d.** Overlap between genes bound by LSD1 or GR in skeletal muscles. **e.** Pathway analysis on LSD1 and GR common target genes in skeletal muscles. **f.** Localization of GR and LSD1 at the *Fbxo32* and *Trim63* loci. The region of *Trim63* that will be hereafter considered for ChIP-qPCR analyses is boxed. **g.** ChIP-qPCR analysis at the indicated locations of the *Ddit4* locus with anti-LSD1 and anti-GR antibodies, or a rabbit IgG in skeletal muscle of wild-type mice. N=6. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ***, $p < 0.001$. **h.** Schematic representation of wild type (WT), floxed (L2) and Cre-mediated exon 1 deleted (L-) *Lsd1* alleles. LoxP sites are shown by arrowheads. Primers used for allele characterization are depicted by arrows and sequences are in Supplementary Table 1. **i.** Representative western

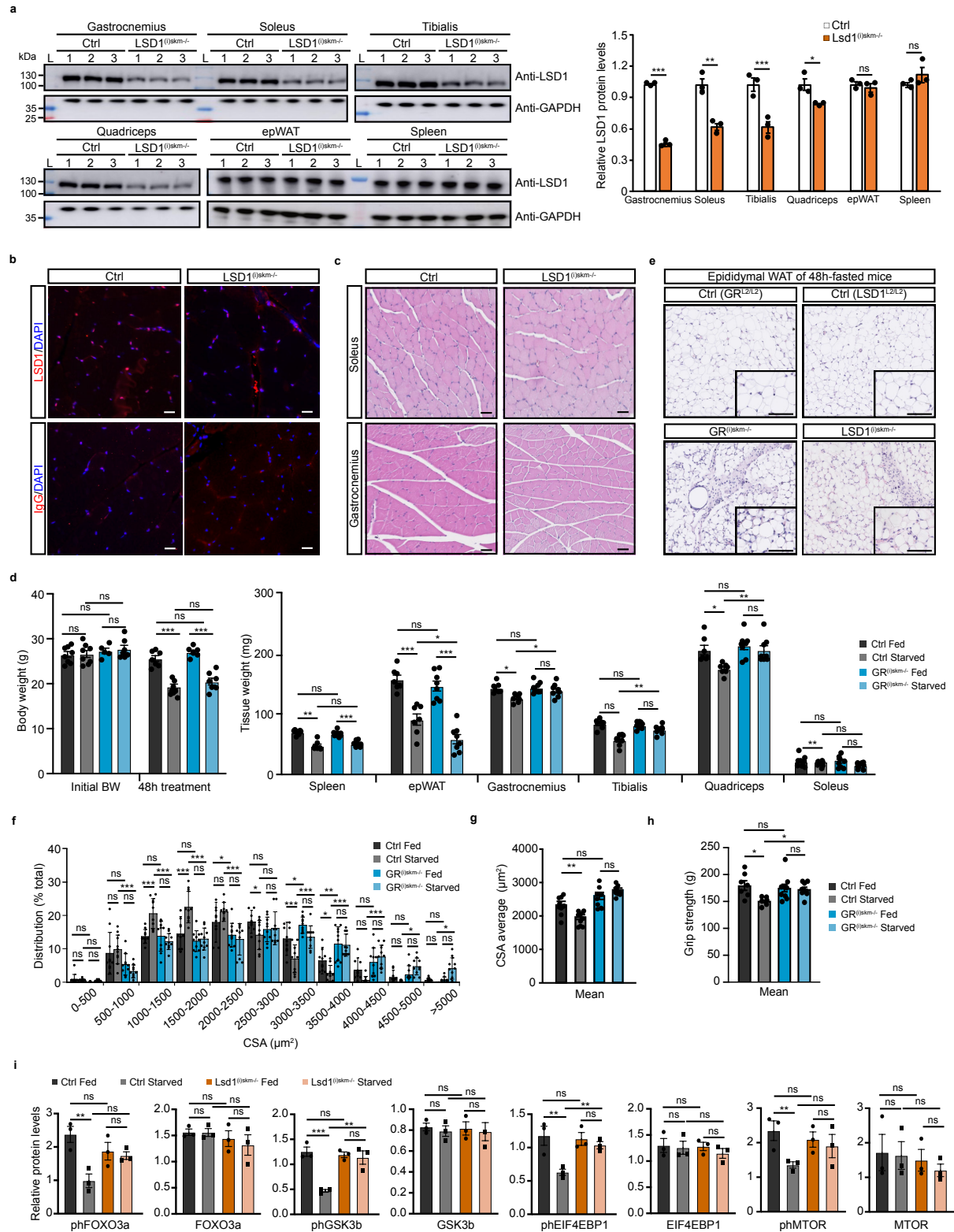
blot analysis (left) and corresponding quantification (right) of LSD1 protein levels in gastrocnemius, soleus, tibialis and quadriceps muscles, epididymal white adipose tissue (epWAT) and spleen of 9-week-old ctrl and LSD1^{skm-/-} mice. GAPDH was used as a loading control. L: ladder. N=3 mice. Mean $\pm$ SEM. Two-tailed t-test. ns: non-significant; *, p<0.05; **, p<0.01; ***, p<0.001. **j.** Representative immunofluorescent detection of LSD1 or rabbit IgG (red) in gastrocnemius of 10-week-old control, LSD1^{skm-/-} mice. Nuclei are stained with DAPI (blue). N=12. Scale bar, 50 μ m. **k.** Representative H&E staining of soleus and gastrocnemius tissue of Ctrl and LSD1^{skm-/-} mice. Scale bar, 50 μ m. N=3. **l.** Body, gastrocnemius, tibialis, quadriceps and soleus muscles, and epWAT mass of 9-week-old Ctrl and LSD1^{skm-/-} mice. N=7 Ctrl and 9 LSD1^{skm-/-} mice. Mean $\pm$ SEM. Two-tailed t-test. ns ns: non-significant. **m.** Maximal (Max) and average (Mean) grip strength of 9-week-old Ctrl and LSD1^{skm-/-} mice. N=7 Ctrl and 9 LSD1^{skm-/-} mice. Mean $\pm$ SEM. Two-tailed t-test. ns: non-significant. **n.** Overlap between genes bound by LSD1 in skeletal muscle, and genes that are either up- or down-regulated in skeletal muscles of LSD1^{skm-/-} mice, and corresponding pathway analysis of direct targets. **o-p.** Overlap between genes bound by GR and LSD1 in skeletal muscle, and down-regulated in skeletal muscles of LSD1^{skm-/-} (o) or GR^{(i)skm-/-} (p) mice. Source data are provided as a Source Data file.

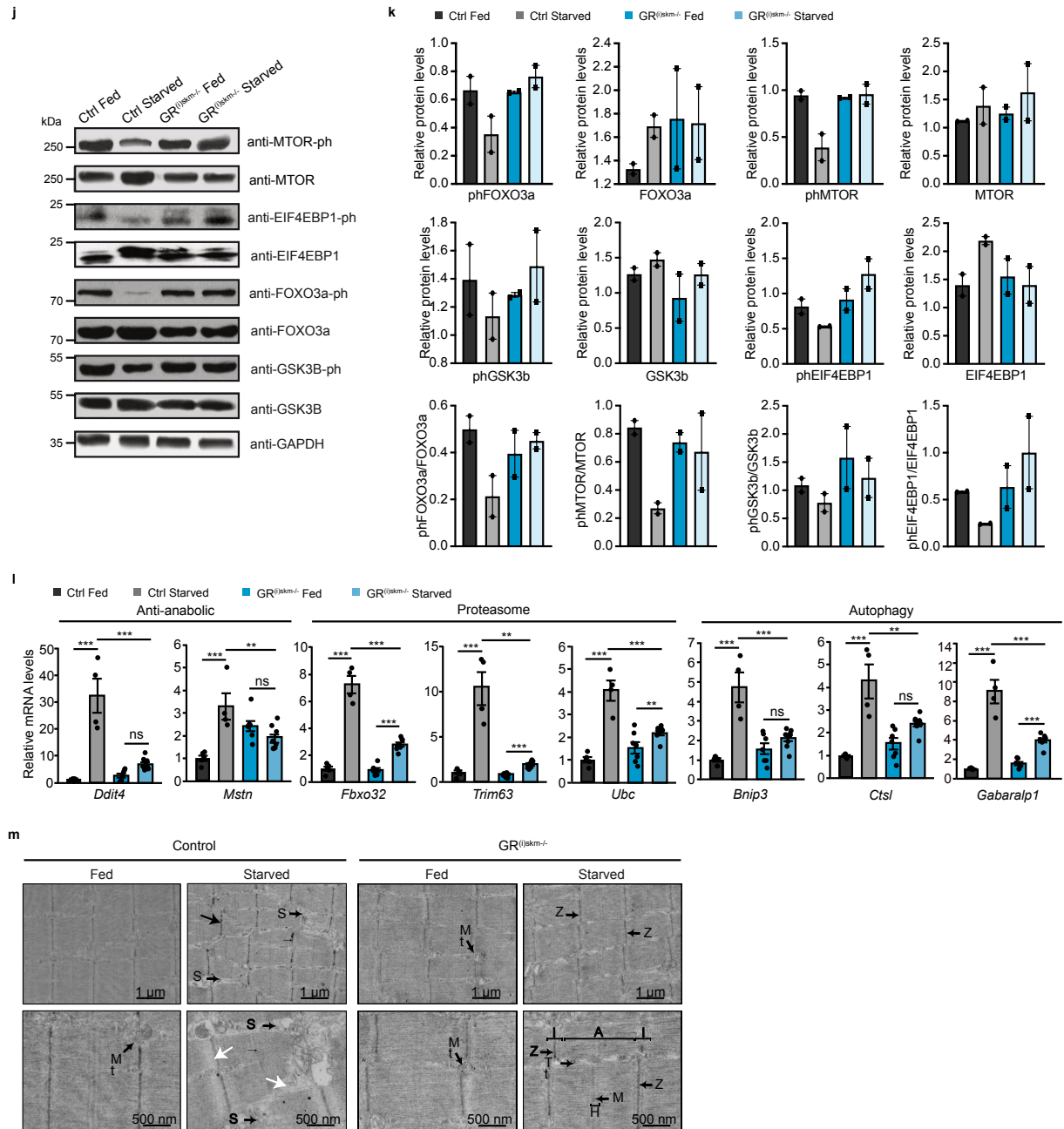


Supplementary Fig. 2. Analysis of LSD1, GR and NRF1 interactions and genomic distribution in mouse skeletal muscle

a. Tag density map of LSD1, GR, H3K4me1, H3K4me2 and H3K4me3 in mouse skeletal muscles, ± 5 kb from the GR peak center and corresponding average tag density profiles. **b-c.** HOMER *de novo* motif analysis of GR binding sites located at enhancer (b) and promoter regions (c). **d.** Localization of GR and LSD1 at the *Pax7* locus. The region considered for ChIP-qPCR analyses is boxed in orange. **e.** Pie chart depicting the genomic location of NRF1 binding in mouse skeletal muscles. **f.** Pathway analysis of NRF1 target genes in skeletal muscle of wild-type mice at 9 weeks. **g.** Localization of GR, LSD1 and NRF1 at the indicated gene loci. **h.** ChIP-qPCR analysis performed with anti-GR and anti-NRF1 antibodies or rabbit IgG at the indicated gene loci. One-way ANOVA with Tukey correction. **, $p < 0.001$; ***, $p < 0.001$. **i.** Relative *Nrf1*, *Gr* and *Lsd1* transcript levels determined in C2C12 myotubes transfected siRNA directed against *Gr*, *Nrf1* and *Lsd1* (siGR, siNrf1 or siLSD1, respectively), or with a

scramble siRNA (siCtrl). Mean $\pm$ SEM. One-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$. ***, $p < 0.001$. Source data are provided as a Source Data file.

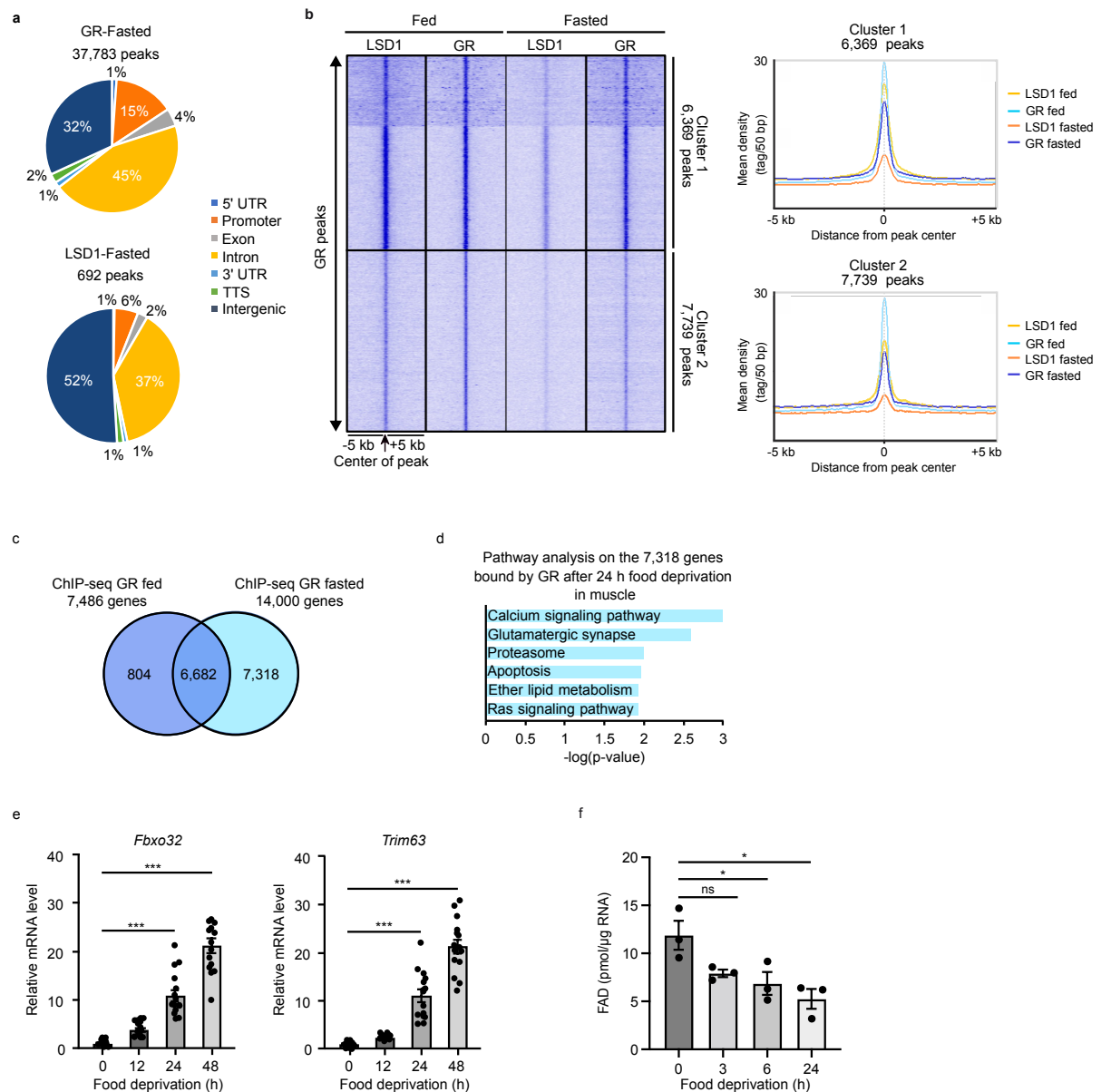




Supplementary Fig. 3. Role of GR and LSD1 in starvation-induced muscle wasting.

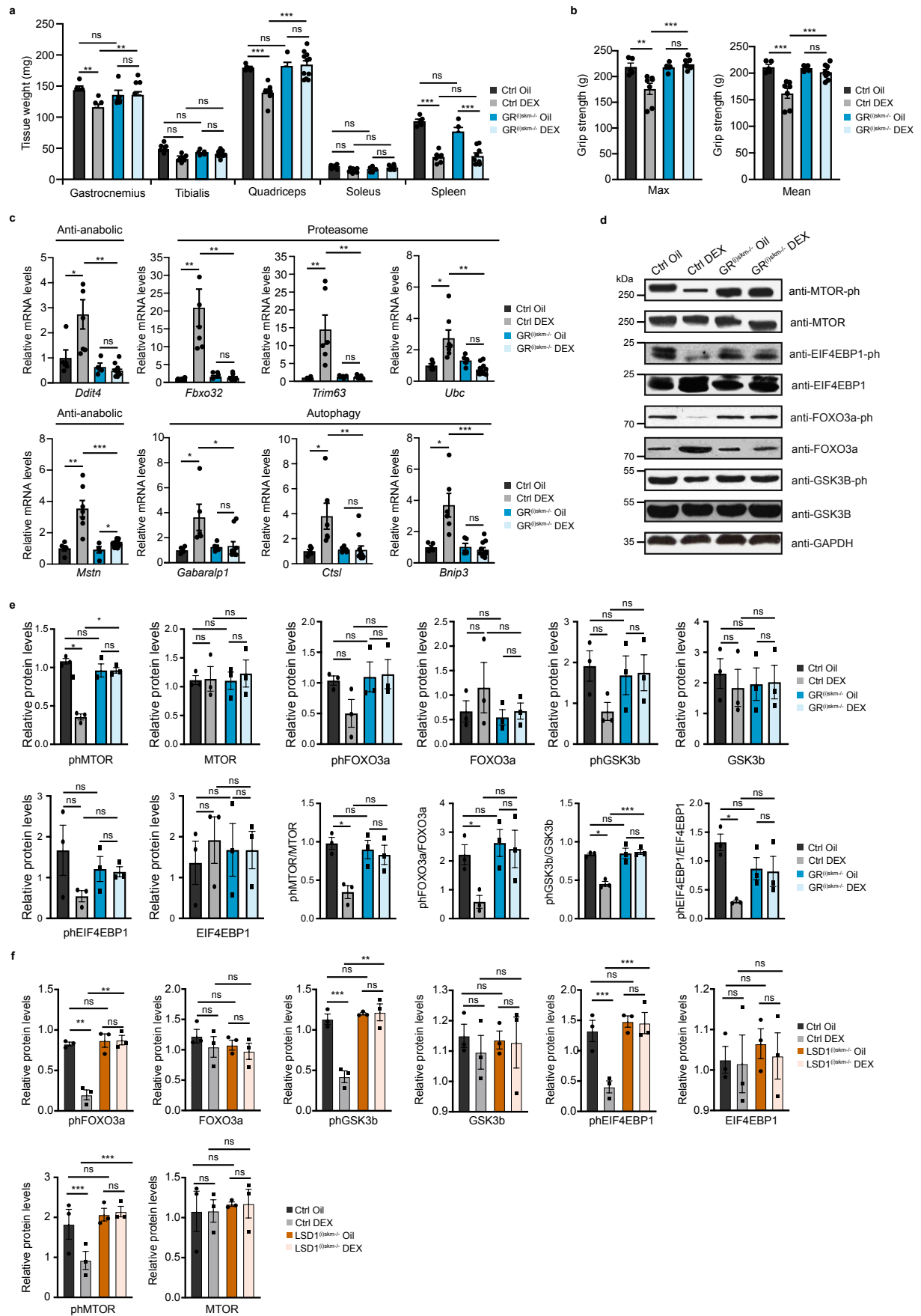
a. Representative western blot analysis (left) and corresponding quantification (right) of LSD1 protein levels in gastrocnemius, soleus, tibialis and quadriceps muscles, epWAT and spleen of 12-week-old ctrl and LSD1^{(i)skm/-} mice. GAPDH was used as a loading control. L: Ladder. N=3 mice. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **b.** Representative immunofluorescent detection of LSD1 or rabbit IgG (red) in gastrocnemius of 10-week-old Ctrl and LSD1^{(i)skm/-} mice. Nuclei are stained with DAPI (blue). N=12. Scale bar, 50 μ m. **c.** Representative H&E staining of soleus and gastrocnemius muscles of Ctrl and LSD1^{(i)skm/-} mice. Scale bar, 50 μ m. N=3. **d.** Body, spleen, epWAT gastrocnemius, tibialis, quadriceps and soleus muscle mass of 12-week-old Ctrl and GR^{(i)skm/-} mice fed or starved for 48 h. N=7 Ctrl Fed, 7 Ctrl Starved, 8 GR^{(i)skm/-} Fed and 8 GR^{(i)skm/-} Starved mice. Mean $\pm$ SEM. Two-way ANOVA. ns: non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **e.** Representative H&E staining of epWAT of 12-week-old Ctrl, GR^{(i)skm/-} and LSD1^{(i)skm/-} mice starved for 48 h. Scale bar, 50 μ m. N=3. **f-h.** Distribution of fiber CSA (f) and average CSA (g) in gastrocnemius, and average (mean) grip strength (h) of 12-week-old Ctrl and GR^{(i)skm/-} mice fed or starved for 48h. N=9. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **i.** Relative levels of the indicated proteins in quadriceps muscle of 12-week-old Ctrl and LSD1^{(i)skm/-} mice fed or starved for 48 h. N = 3 mice. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant;

****p < 0.01; ***p < 0.001. j-k.** Representative western blot analysis (j) and relative quantification (k) of the indicated proteins in quadriceps muscle of 12-week-old Ctrl and GR^{(i)skm-/-} mice fed or starved for 48 h. GAPDH was used as a loading control. **l.** Relative transcript levels of the indicated genes in gastrocnemius muscle of 12-week-old Ctrl and GR^{(i)skm-/-} mice fed or starved for 48 h. N=5 Ctrl fed, 4 Ctrl starved, 6 GR^{(i)skm-/-} fed and 8 GR^{(i)skm-/-} starved mice. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; **, p<0.01, ***, p<0.001. **m.** Ultrastructure analysis of gastrocnemius muscles of 12-week-old Ctrl and GR^{(i)skm-/-} mice fed or starved for 48 h. A, A band; I, I band; H, H band; M, M line; Mt, mitochondria; Tt, T-tubule; S, sarcoplasm; Z, Z line. Black arrow indicates Z line disruption; white arrows indicate loss of myofilaments. Source data are provided as a Source Data file.



Supplementary Fig. 4. Analysis of LSD1 and GR binding dynamics in skeletal muscle during food deprivation.

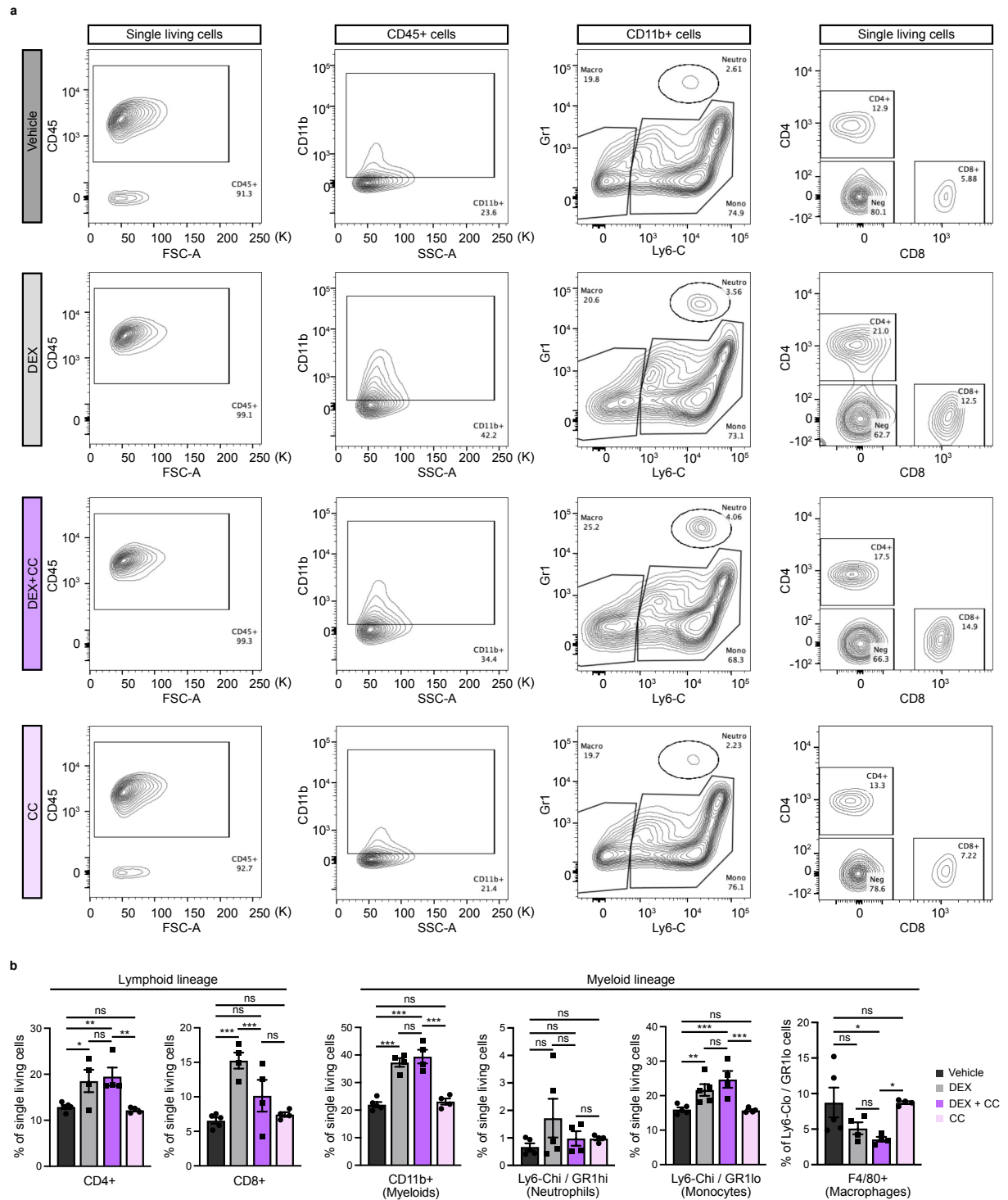
a. Pie chart depicting the genomic location of LSD1 and GR binding in skeletal muscles of wild-type mice fasted for 24 h. **b.** Tag density map of LSD1 and GR in skeletal muscles of mice fed or fasted for 24 h, ± 5 kb from the GR peak center and corresponding average tag density profiles. **c.** Overlap between genes bound by GR fed and fasted in skeletal muscle. **d.** Pathway analysis on the genes targeted by GR only in skeletal muscles 24 h after food deprivation. **e.** Relative transcript levels of *Fbxo32* and *Trim63* in gastrocnemius of wild-type mice fed or food deprived for 12, 24 or 48 h. N=13-15. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ***, $p < 0.001$. **f.** FAD levels in quadriceps muscles of wild-type mice after 0, 3, 6 and 24 h of food deprivation. N=3. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$. Source data are provided as a Source Data file.



Supplementary Fig. 5. LSD1 and GR are required for dexamethasone-induced muscle wasting

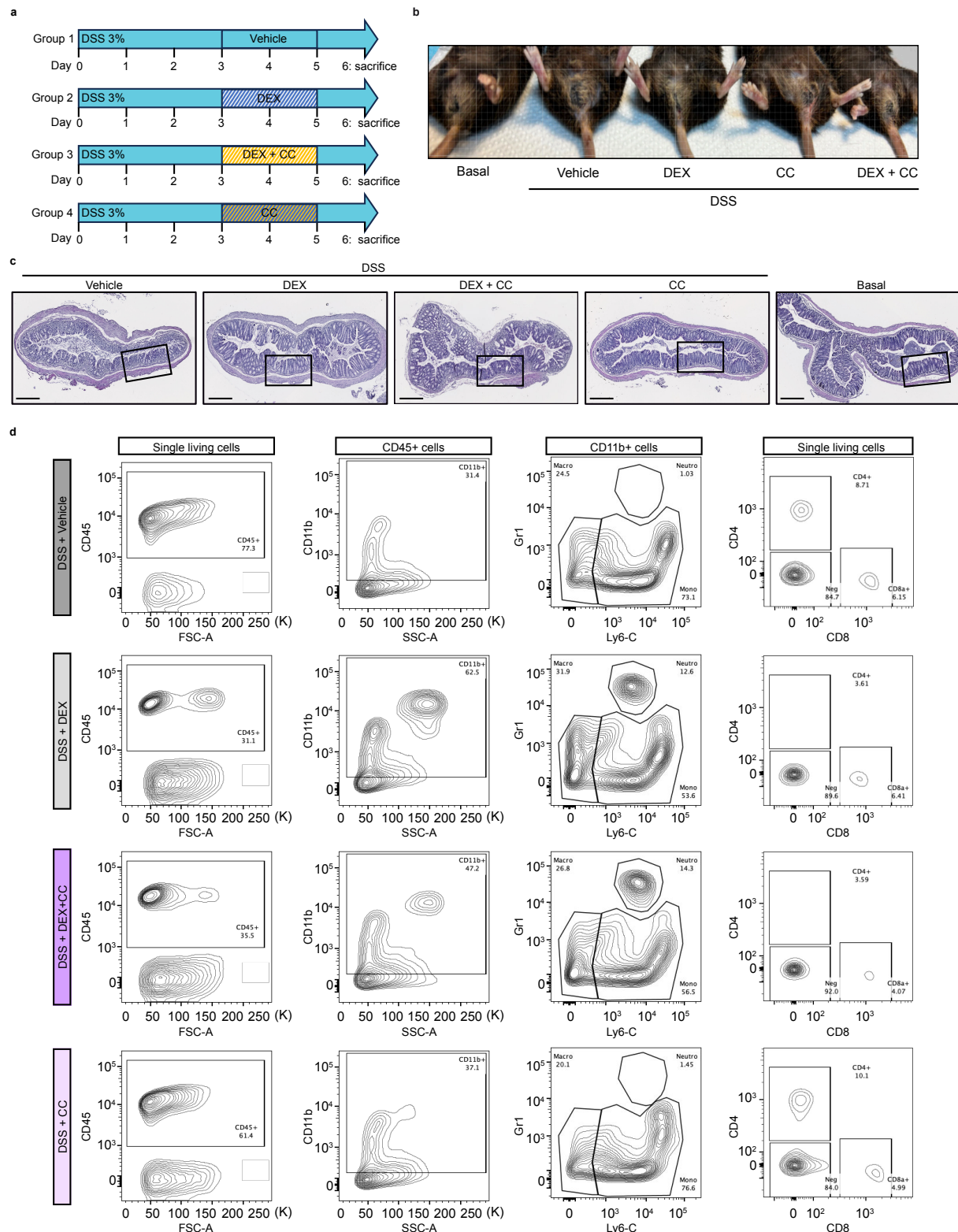
a. Gastrocnemius, tibialis, quadriceps, soleus and spleen mass of Ctrl and $GR^{(i)skm-/-}$ mice treated with dexamethasone (DEX) or vehicle (Oil) for 72 h. N=5 Ctrl Oil, 6 Ctrl DEX, 5 $GR^{(i)skm-/-}$ Oil and 11 $GR^{(i)skm-/-}$ DEX mice.

Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; **, $p < 0.01$; ***, $p < 0.001$. **b.** Maximal (Max) and average (Mean) grip strength of Ctrl and GR^{(i)skm-/-} mice treated with DEX or Oil for 72 h. N=5 Ctrl Oil, 7 Ctrl DEX, 5 GR^{(i)skm-/-} Oil and 9 GR^{(i)skm-/-} DEX mice. Mean $\pm$ SEM. One-way ANOVA with Tukey correction. ns: non-significant; **, $p < 0.01$; ***, $p < 0.001$. **c.** Relative transcript levels of the indicated genes in gastrocnemius of Ctrl and GR^{(i)skm-/-} mice treated with DEX or Oil for 72 h. N=5 Ctrl Oil, 7 Ctrl DEX, 5 GR^{(i)skm-/-} Oil and 8 GR^{(i)skm-/-} DEX mice. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. **d-e.** Representative western blot analysis (d) and corresponding quantification (e) of the indicated proteins in quadriceps muscle of 12-week-old Ctrl and GR^{(i)skm-/-} mice treated with DEX or Oil for 72h. GAPDH was used as a loading control. n = 3 mice. Mean $\pm$ SEM. Two-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$. **, $p < 0.01$; ***, $p < 0.001$. **f.** Relative levels of the indicated proteins in quadriceps muscle of 12-week-old Ctrl and LSD1^{(i)skm-/-} mice treated with DEX or Oil for 72h. Source data are provided as a Source Data file.



Supplementary Fig. 6. Effect of LSD1 inhibition on dexamethasone anti-inflammatory activities.

a-b. Flow cytometry analysis of the lymphoid and the myeloid lineages in spleen of wild-type mice treated with a vehicle, DEX, DEX with CC-90011 (DEX+CC) or CC-90011 (CC) for 72 h (a) and corresponding quantification (b). Ly6-Chi: high levels of Ly6-C, Ly6-Clo: low levels of Ly6-C, GR1lo: low levels of GR1, GR1hi: high levels of GR1. N=5 vehicle, 4 DEX, 4 DEX+CC, 4 CC mice. Mean $\pm$ SEM. One-way ANOVA with Tukey correction. ns: non-significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Source data are provided as a Source Data file.



Supplementary Fig. 7. Effect of LSD1 inhibition on dexamethasone-induced amelioration of experimental colitis.

a. Flow chart illustrating study design. Mice were treated with 3% DSS in drinking water over a six-day period to induce colitis, DEX and/or CC-90011 were administered from the 3rd to the 5th day of colitis. N=5 mice per condition. **b.** Representative colitis syndrome with diarrhea, and/or hematochezia in mice from indicated groups. **c.** Representative H&E staining of colons in mice from indicated groups. Scale bars, 200 μ m. This experiment has been performed on 3 mice per group. **d.** Flow cytometry analysis of the lymphoid and the myeloid lineages in spleen of mice from indicated groups.

Supplementary Tables

Supplementary Table 1. List of the primers used for genotyping.

Name	Forward (5'-3')	Reverse primer (5'-3')
Cre and CreER ^{T2} recombinase alleles	TTCCCGCAGAACCTGAAGATGTTCG	GGGTGTTATAAGCAATCCCCAGAAATGC
GR WT/L2 allele	AGATCATTTGCCTAGCAGGCATGAG	GTCAACACATGATCACCTTGCAGTC
GR L- allele	CCAGAGAACTAATTGGCTCTTGAC	GTCAACACATGATCACCTTGCAGTC
<i>Lsd1</i> WT/L2 allele (fw1-rev1)	CCTCAGTAGGCCTGGTTTGT	TTGGTTTTGGTTGACCTTC
<i>Lsd1</i> L- allele (fw2-rev1)	CCGTGGAAATTCGTGCACTC	GCAGGCGGTTTGAAATGTATTC

Supplementary Table 2. List of the primers used for RT-qPCR analyses.

Gene name	Forward (5'-3')	Reverse primer (5'-3')
<i>m18S</i>	TCGTCTTCGAAACTCCGACT	CGCGGTTCTATTTGTTGGT
<i>m36b4</i>	AGATTCGGGATATGCTGTTGG	AAAGCCTGGAAGAAGGAGGTC
<i>mAtg7</i>	CCACTGAGGTTACCATCCT	TCCGTTGAAGTCCTCTGCTT
<i>mBecn1</i>	GAGCCATTTATTGAACTCGCCA	CCTCCCCGATCAGAGTGAA
<i>mBnip3</i>	TTCCACTAGCACCTTCTGATGA	GAACACCGCATTTACAGAACAA
<i>mCtsl</i>	GTGGACTGTTCTCACGCTCAAG	TCCGTCCTTCGCTTCATAGG
<i>mDdit4</i>	TGGTGCCACCTTTCAGTTG	GTCAGGGACTGGCTGTAACC
<i>mFbxo32</i>	TCAGCCTCTGCATGATGTTT	TCAGCCTCTGCATGATGTTT
<i>mGabaralp1</i>	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCATGGTAG
<i>mHprt</i>	GTTGGATACAGGCCAGACTTTGTTG	GATTCAACTTGCCTCATCTTAGGC
<i>mMstn</i>	GGCCATGATCTTGCTGTAAC	TTGGGTGCGATAATCCAGTC
<i>mTrim63</i>	TGAGGTGCCTACTTGCTCCT	GTGGACTTTTCCAGCTGCTC
<i>mUbc</i>	TCTTCGTGAAGACCTGACC	CAGGTGCAGGGTTGACTCTT
<i>mIL-6</i>	TCTGCAAGAGACTTCCATCCA	AGTCTCTCTCCGGACTTGT

Gene name	Forward (5'-3')	Reverse primer (5'-3')
<i>hATG7</i>	TCCCATGTGTTTCACTTCAACC	AACAGCCATTTTGCCACTAATC
<i>hCTSL</i>	GTGGACATCCCTAAGCAGGA	CACAATGGTTTCTCCGGTC
<i>hDDIT4</i>	TGTTTAGCTCCGCCAACTC	TTCTTGATGACTCGGAAGCC
<i>hFBXO32</i>	GACTTCTCAACTGCCATTC	TCGTCTCCATCCGATACAC
<i>hGABARALP1</i>	TGAGACCTGAGGACGCCTT	GGGCTTCCAACCACTCATTTT
<i>hGADD45A</i>	CGTTTTGCTGCGAGAACGAC	GAACCCATTGATCCATGTAG
<i>hMSTN</i>	GATGACGATTATCACGCTAC	GCACAAACACTGTTGTAGGA
<i>hRPLP0</i>	CGTCCTCGTGGAAGTGACAT	TAGTTGGACTTCCAGGTCGC
<i>hTRIM63</i>	CGTGGAGATGAAAACCGACC	GGCCCACTCTAGACTTGGTT

Supplementary Table 3. List of the primers used for ChIP-qPCR experiments.

Locus	Forward (5'-3')	Reverse primer (5'-3')
<i>Ddit4_GBSp1</i>	AGGCGCTAAGAATCGGAGTC	CACGCTGTCTTTGCTTCTGA
<i>Ddit4_GBSe1</i>	AAGAAGGCAGGTCTCCACTC	GTTACCCTGGGCTGAAGTCT
<i>Ddit4_GBSe2</i>	TAGAACACACCCAGCAGAGG	AGCCAGAGGTAGGAGAGACA
<i>Ddit4_GBSe3</i>	CTGTGGGTGGAAGGATGCT	ACTCCCTGTTTACACGGTGT
<i>Eif4ebp2_GBSe2</i>	ACAACCTTGACATCCACCCA	GGTGCATCTGGTGGAAATGTG
<i>Eif4ebp2_GBSp2</i>	AGAGCAGGCGAGTTGAGAG	GTAGTCCTGAGGTAGCTGCG
<i>Pax7</i>	CCCCAGGCTAGAACCATTG	ATAGAGCTGCCTTGTTCCC
<i>Pik3r1_GBSe3</i>	TTCTTTCTCCCGTCTGTGCT	TGGGCAGTGGGAATAAAGGT
<i>Murf1_5kb</i>	GCTGAGTTCCTGGGACACTC	GGGTTCTGGGTCATGATGCA
<i>Unrelated region (Eif4ebp2 intron)</i>	CGGATTTGGAGTTCAGCCTG	CCCCTTCCTGTTTGTTGG

Supplementary Table 4. List of the antibodies used for spleen FACS analysis.

Antigen	Conjugation	Company	Cat. number	Dilution
CD45	Alexa Fluor 700	BioLegend	103128	1:100
CD11b	PerCP-Cy5.5	eBioscience	45-0112-82	1:100
Ly-6G (GR-1)	FITC	FISHER SCIENTIFIC SAS	11-5931-82	1:100
Ly-6C	PE-CF594	BD Biosciences	562728	1:100
F4/80	APC eFluor 780	Invitrogen	47-4801-80	1:100
CD3ε	PerCP-Cy5.5	BioLegend	100328	1:50
CD4	APC-H7	BD Pharmingen™	560181	1:100
CD8a	Alexa Fluor 700	eBioscience	56-0081-80	1:100

Supplementary Table 5. List of the antibodies used for Th cells isolation and differentiation.

Antigen	Conjugation	Company	Cat. number	Dilution
CD16/CD32	purified	BioLegend	101302	1:100
CD4	AlexaFluor 700	BioLegend	100536	1:100
CD8a	PerCP-Cy5.5	BioLegend	100734	1:100
CD44	PE-Cy7	eBioscience	25-0441	1:500
CD25	PE	BioLegend	102008	1:300
Nk1.1	PE	BioLegend	108708	1:300
TCR gd	PE	BD Biosciences	553178	1:100
CD3ε	Ultra-leaf	BioLegend	100359	1:50
CD28	Ultra-leaf	BioLegend	102121	1:100
IFN-g	Ultra-leaf	BioLegend	505847	1:100
IL-4	Ultra-leaf	BioLegend	504135	1:100
IL-17	APC	eBioscience	17-7177	1:100
IFN-g	PE-Dazzle 594	BioLegend	505846	1:100