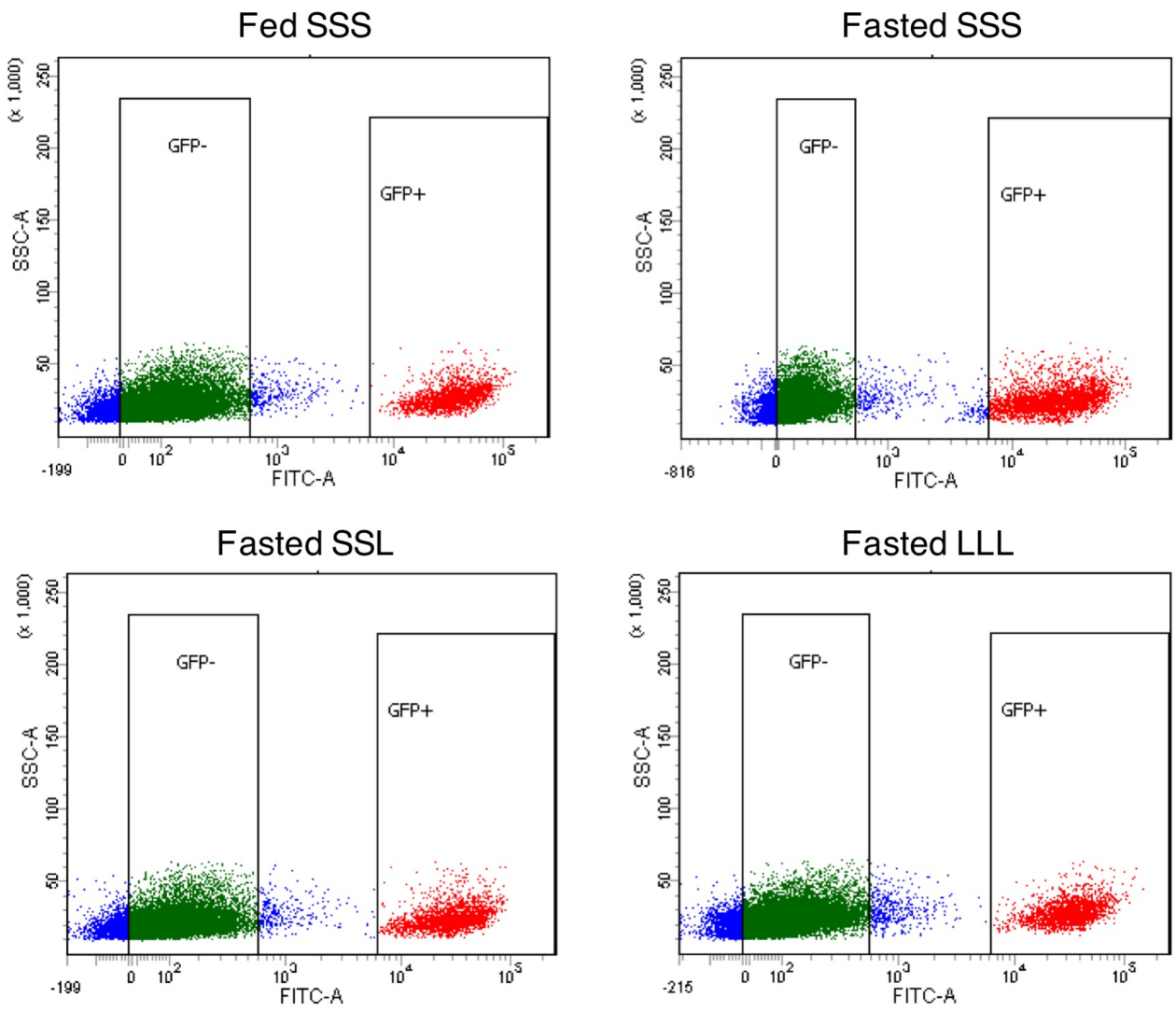
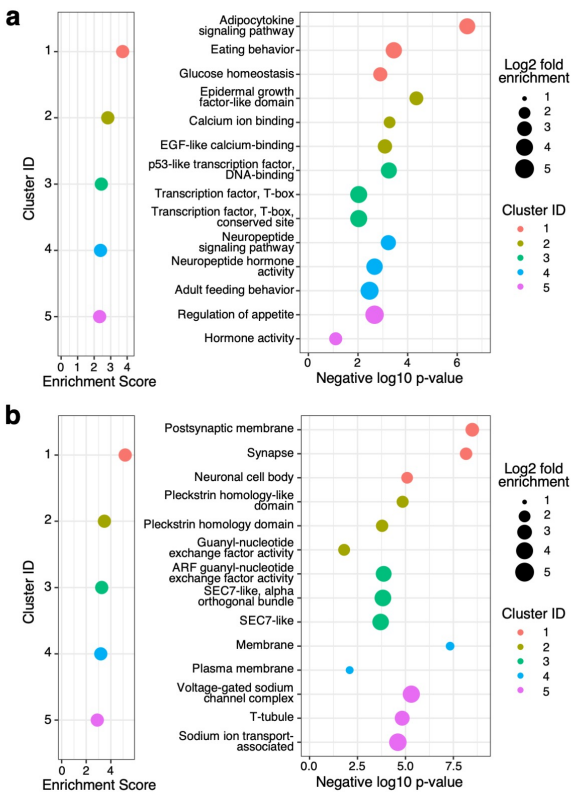
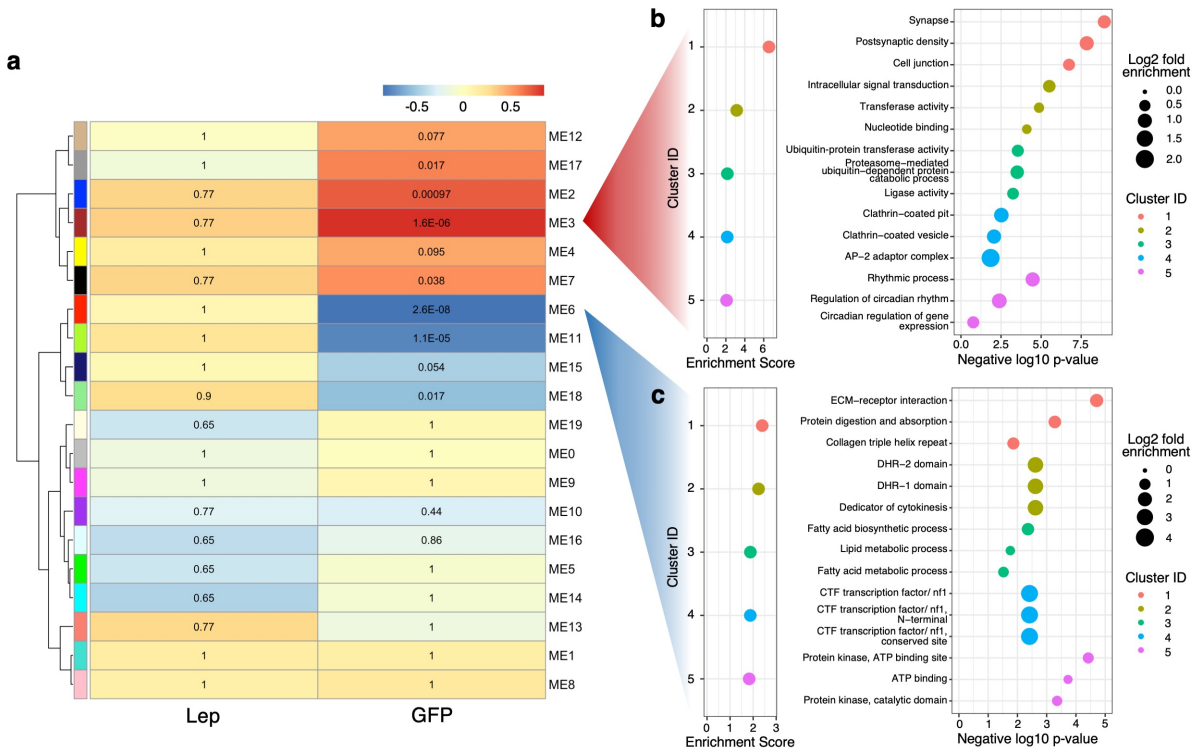


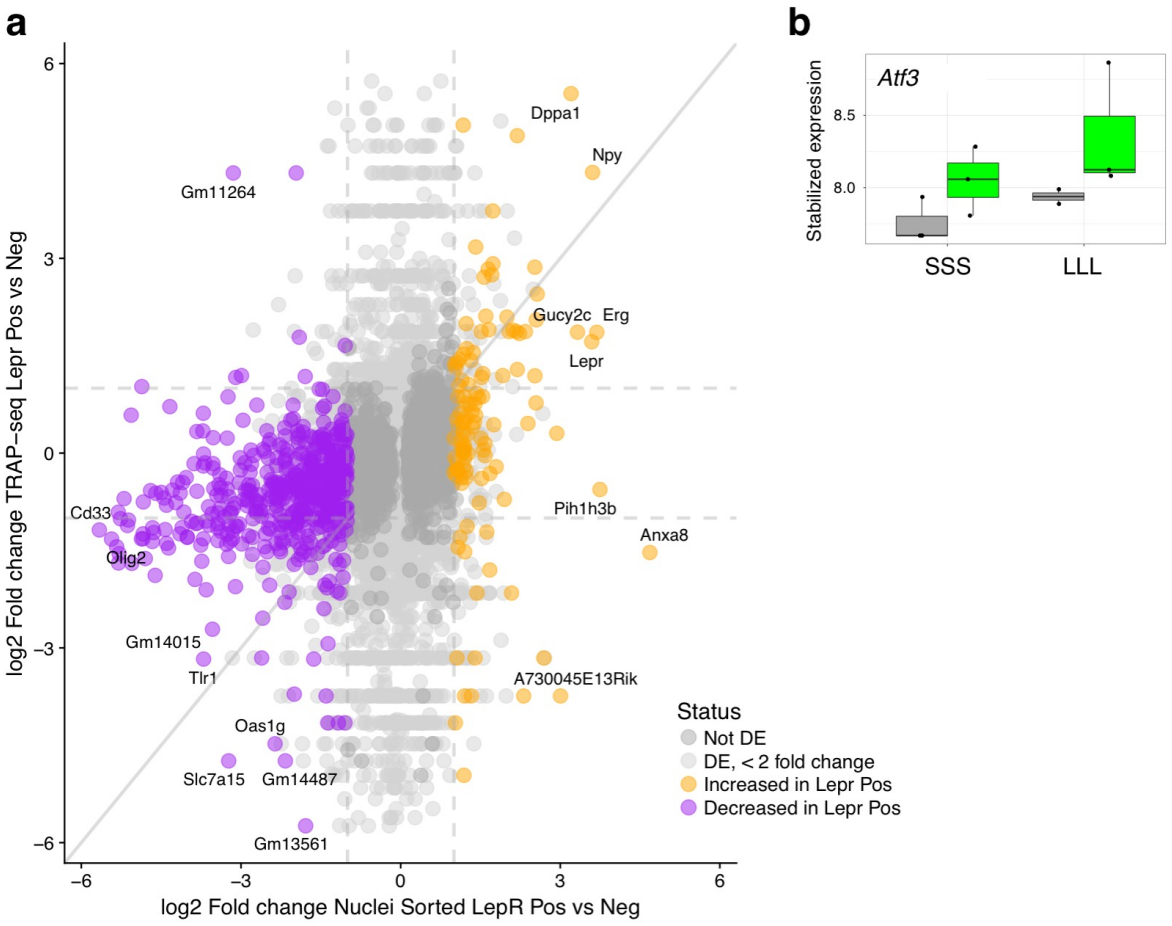
Supplementary Figure 1. Gating strategy for flow cytometry. a-d Cell nuclei obtained from mice with four leptin conditions including fed and injected with saline three times (Fed SSS; **a**) fasted and injected with saline three times (Fasted SSS; **b**), saline at 12 and 24 hours and leptin at 34 hours (Fasted SSL; **c**), and fasted and injected with leptin three times at 12, 24 and 34 hours (Fasted LLL; **d**). Cell nuclei with fluorescence activity between zero to 600 were defined as GFP negative, and those with more than 6,000 as GFP positive.



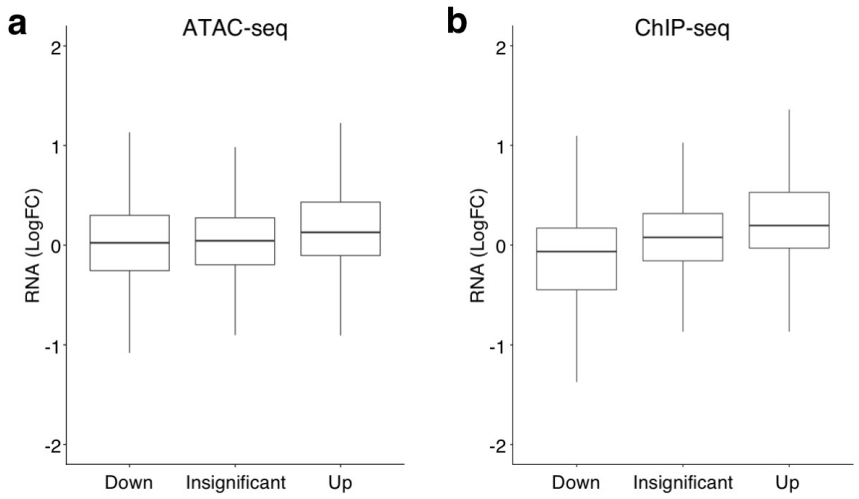
Supplementary Figure 2. Gene ontology analysis. a Gene ontology analysis of differentially expressed genes between LepRb GFP positive versus GFP negative nuclei using functional annotation clustering in DAVID[1]. The panel on the left shows the enriched gene ontology clusters and the right panel the terms associated with these clusters. **b** Functional annotation clustering using DAVID[1] of LepRb GFP positive nuclei of genes that are differentially expressed between fasted mice injected at 12, 24 and 34 hours with saline (SSS) or leptin (LLL). The panel on the left shows the enriched gene ontology clusters and the right panel the terms associated with these clusters. N=3 biologically independent replicates, p-values by modified Fishers Exact test (**a, b**).



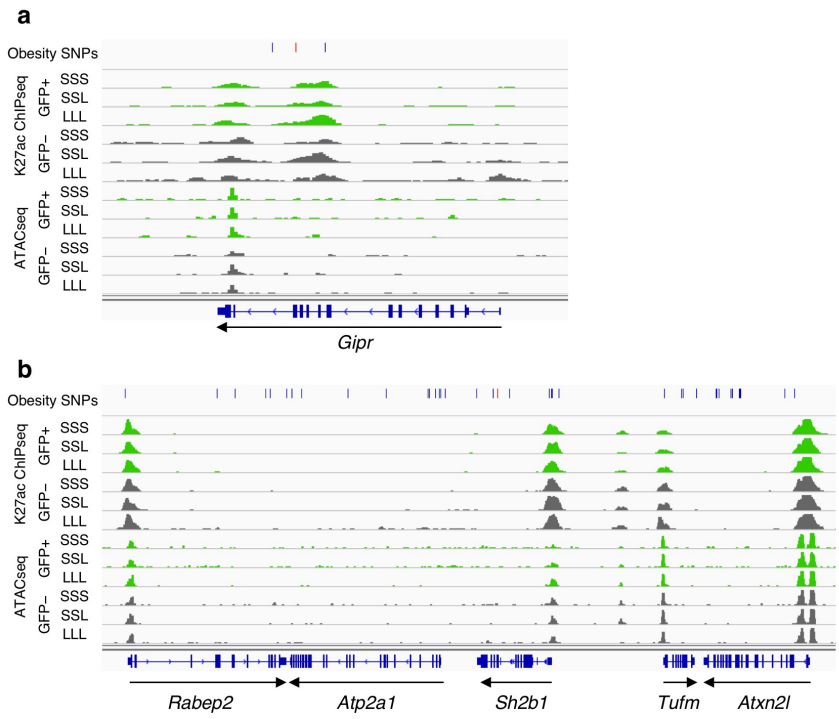
Supplementary Figure 3. WGCNA analysis. a WGCNA analysis found 10 modules to be highly correlated with GFP-positive and GFP-negative cell states. **b-c** Gene ontology analysis using DAVID[1] for two of the most significantly correlated modules, ME3 (**b**) and ME6 (**c**). N=3 biologically independent replicates, p-values calculated by Student's asymptotic test and corrected for multiple testing using Benjamini & Hochberg method (**b, c**).



Supplementary Figure 4. MM plot of TRAP-seq versus our RNA-seq and Atf3 expression levels. a Comparison of the log2 fold changes between LepRb GFP positive and negative sorted nuclei in this data set (x-axis) to the log2 fold change between LepRb positive verus negative cells in the TRAP-seq assay[2] (y-axis). **b** Boxplot showing the expression level of *Atf3* in LepRb GFP-positive (green) or negative (grey) nuclei within the two conditions. The y-axis shows stabilized expression which is log2 scale for sequencing depth normalized counts determined by DESeq2[3]. Box represents 25th to 75th percentiles and the middle line indicates the median. TRAP-seq N=1 per group, Nuclei Sorted RNA-seq N=3 per group, P-values given by Wald test and corrected for multiple testing by the Benjamini & Hochberg procedure.



Supplementary Figure 5. ATAC-seq or ChIP-seq to RNA-seq comparisons. a-b Comparison between differentially enriched ATAC-seq (**a**) or ChIP-seq (**b**) peaks of leptin-responsive neurons that were upregulated, insignificant or downregulated and nearest gene fold change. Boxes represent 25th to 75th percentiles and the midline indicates the median. ATAC-seq down (N=3745), Insignificant (N=48330), Up (N=3767). ChIP-seq down (N=9903), Insignificante (N=121669), Up (N=7726).



Supplementary Figure 6. Obesity GWAS loci. a-b Genomic snapshots of *Gipr* (**a**) and *Sh2b1* (**b**) loci showing H3K27ac ChIP-seq and ATAC-seq signals in leptin-responsive neurons (green) compared to GFP-negative nuclei (grey). Leptin conditions include mice treated with saline (SSS) or leptin (LLL) injections at 12, 24 and 34 hours (LLL) or saline injections at 12 and 24 hours followed by a single leptin injection at 34 hours (SSL). The obesity SNPs track shows the obesity-associated lead SNPs as a red line and in addition SNPs that are in linkage disequilibrium (r^2>0.8) as black lines. Each of two replicates for ChIP-seq and ATAC-seq presented comparable signals as shown (**a**, **b**).

Supplementary Table 1. Number of mice and nuclei used for experiments.

			hnRNA-seq		ATAC-seq		H327ac ChIP-seq	
			# mice used (Male, Female)	# nuclei used (thousands)	# mice used (Male, Female)	# nuclei used (thousands)	# mice used (Male, Female)	# nuclei used (thousands)
GFP positive	Fed Saline x3	rep1	(3, 2)	50	(3, 2)	11.2	(10, 10)	568
		rep2	(3, 2)	58	(2, 3)	14.4	(10, 10)	536
		rep3	(3, 2)	44				
	Fas Saline x3	rep1	(3, 2)	50	(3, 2)	11.2	(10, 10)	497
		rep2	(3, 2)	56	(2, 3)	14.4	(10, 10)	540
		rep3	(3, 2)	74				
	Fas Leptin x1	rep1	(3, 2)	50	(3, 2)	11.2	(10, 10)	435
		rep2	(3, 2)	55	(2, 3)	14.4	(10, 10)	454
		rep3	(3, 2)	61				
	Fas Leptin x3	rep1	(3, 2)	50	(3, 2)	11.2	(10, 10)	477
		rep2	(3, 2)	50	(2, 3)	18.0	(10, 10)	540
		rep3	(3, 2)	71				
GFP negative	Fed Saline x3	rep1	(3, 2)	70	(3, 2)	18.0	(10, 10)	300
		rep2	(3, 2)	66	(2, 3)	18.0	(10, 10)	500
		rep3	(3, 2)	70				
	Fas Saline x3	rep1	(3, 2)	70	(3, 2)	18.0	(10, 10)	300
		rep2	(3, 2)	66	(2, 3)	18.0	(10, 10)	500
		rep3	(3, 2)	70				
	Fas Leptin x1	rep1	(3, 2)	70	(3, 2)	18.0	(10, 10)	300
		rep2	(3, 2)	66	(2, 3)	18.0	(10, 10)	500
		rep3	(3, 2)	70				
	Fas Leptin x3	rep1	(3, 2)	70	(3, 2)	18.0	(10, 10)	300
		rep2	(3, 2)	66	(2, 3)	18.0	(10, 10)	500
		rep3	(3, 2)	70				

Supplementary Table 4. Sequences of primers used for genotyping, cloning and qPCR and sgRNAs used for CRISPRi.

Mouse genotyping for LepRcre and SUN1-sfGFP	
Lepr WT.F	GCCCTCATTAATCTAGTAATGTAGAT
Lepr WT.R	GCAAAAAAAGTAGTTAACCTATTCCT
Cre.F	CGATGCAACGAGTGATGAGG
Cre.R	GCATTGCTGTCACTTGGTCGT
INTACT.F	GCACTTGCTCTCCCAAAGTC
INTACT WT.R	CATAGTCTAACTCGCGACACTG
INTACT MT.R	GTTATGTAACGCGGAACTCC
Socs3 enhancer cloning	
Socs3_1.F	TGGCCTAACTGGCCGGTACCTCCCACACAGGATCAGCTTCCAAG
Socs3_1.R	TCTAGTGTCTAAGCTTAGCAGGTCCCAAGCTAGGTATGAG
Socs3_2.F	TGGCCTAACTGGCCGGTACCCTAAGTAAATAGTCACCGACCATCTG
Socs3_2.R	TCTAGTGTCTAAGCTTTTGCCTCTGGACCATTGCACCCAC
Socs3_3.F	TGGCCTAACTGGCCGGTACCGGCCATCTGGATTCAACAGGATCC
Socs3_3.R	TCTAGTGTCTAAGCTTCTCTGATCAAGATTTCTTGTCTGGG
sgRNA cloning. sgRNA sequences are underlined.	
Socs3_sgRNA_1.F	CCCTTGGAGAACCACCTTGTTGGTGACATTGTCACAGAAGTGGTTTAAGAGCTAAGCTGGAAACAGCA
Socs3_sgRNA_2.F	CCCTTGGAGAACCACCTTGTTGGAGGCTGATGTTCCTGTGAGGTTTAAGAGCTAAGCTGGAAACAGCA
pLG1.R	GATCCTAGTACTCGAGAAAAAAAGCACCGAC
qPCR	
Socs3.F	ACCTCGCAGATCCCTTGCACC
Socs3.R	TCTGCCTCCCTTCGGTGTTGG
Pgs1.F	GCTTCATCTCAGCCTTCTCAA
Pgs1.R	TTACACAGGAGGGATCAGGAA
Tha1.F	TAGCAGGATTGCCTAGTGTGC
Tha1.R	GCTTCTAATCCCCTAGCATGG

Supplementary References

1. Huang da, W., Sherman, B.T. & Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* **4**, 44-57 (2009).
2. Allison, M.B. *et al.* TRAP-seq defines markers for novel populations of hypothalamic and brainstem LepRb neurons. *Mol Metab.* **4**, 299-309. doi: 10.1016/j.molmet.2015.01.012. eCollection 2015 Apr. (2015).
3. Love, M.I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550. (2014).