

An Epigenomic and Transcriptional Basis for Insulin Resistance

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ENCODE Research Applications and Users Meeting
2015



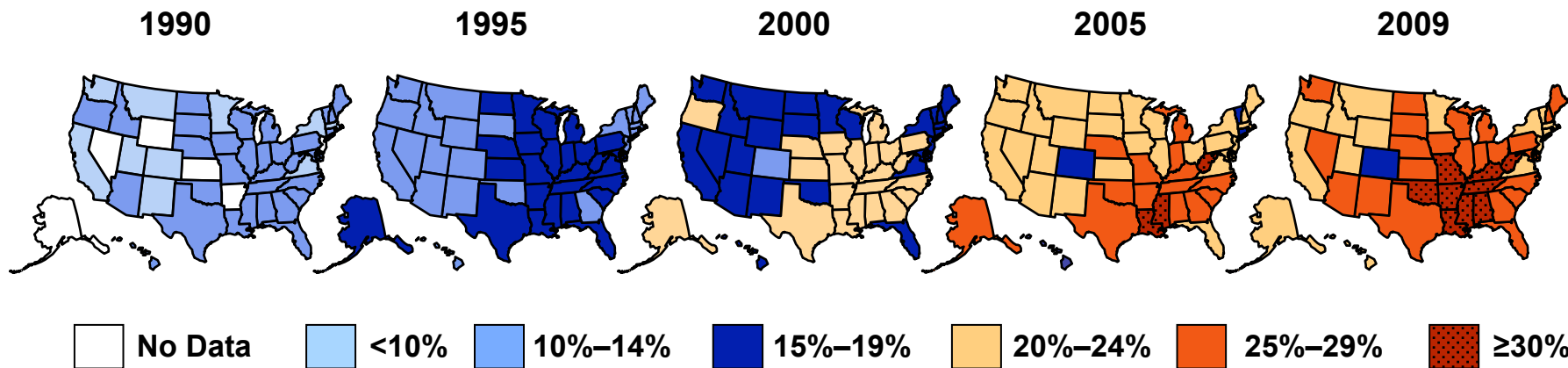
Beth Israel Deaconess
Medical Center



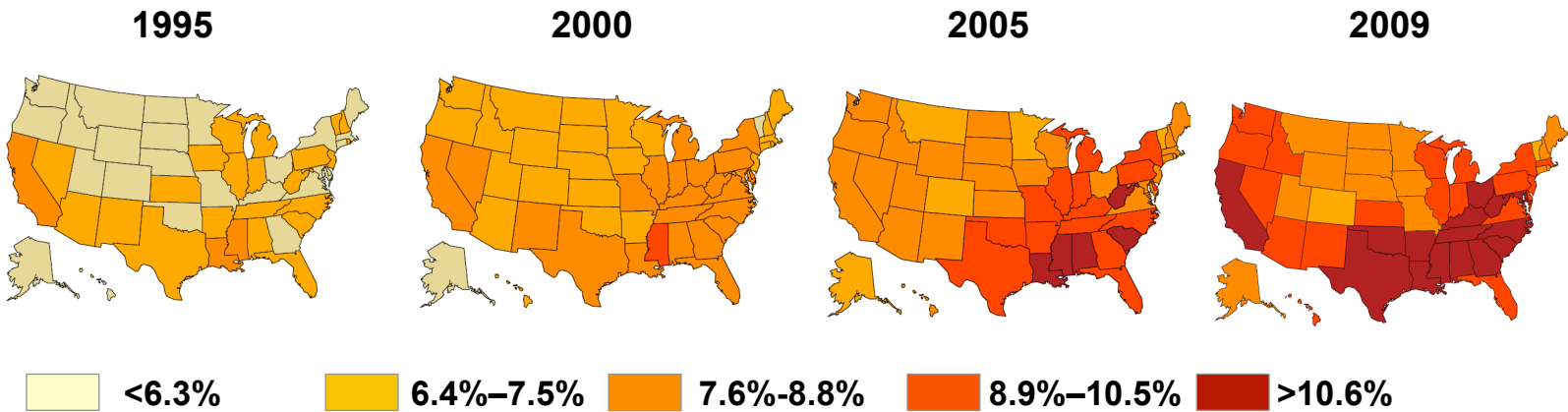
A teaching hospital
of Harvard
Medical School

Obesity and diabetes trends among US adults

Obesity



Diabetes



Obesity is one of the top three social burdens generated by human beings.

Impact on global GDP¹

\$2.1 trillion



Smoking

\$2.1 trillion



Armed violence,
war, and terrorism

\$2.0 trillion



Obesity

\$1.4 trillion

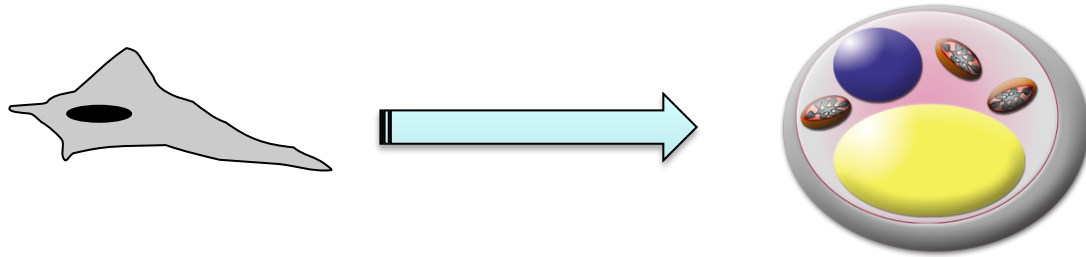


Alcoholism

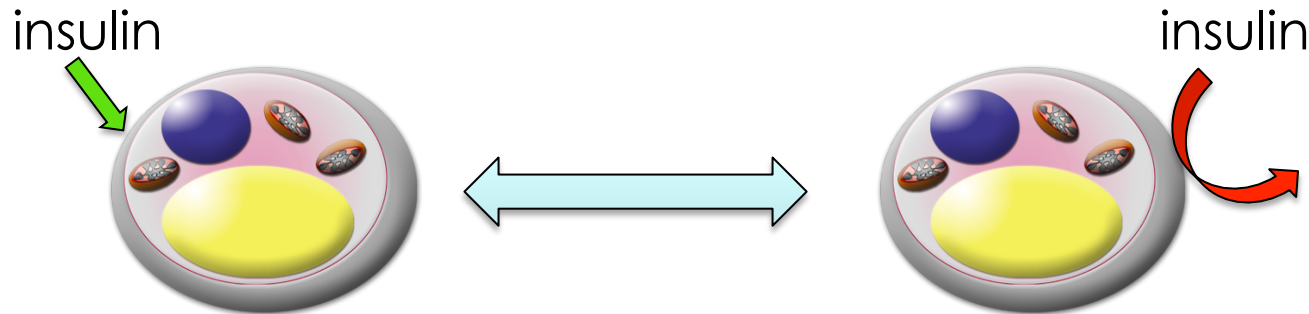
¹In 2014 dollars at purchasing-power parity.

Source: Literature review; World Health Organization global burden of disease (GBD) database; McKinsey Global Institute analysis

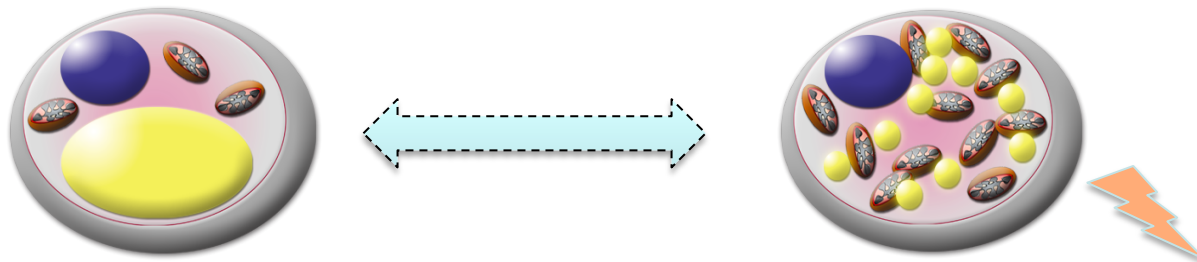
What are the critical transcriptional pathways that underlie key transitions or distinctions in adipose biology?



adipogenesis

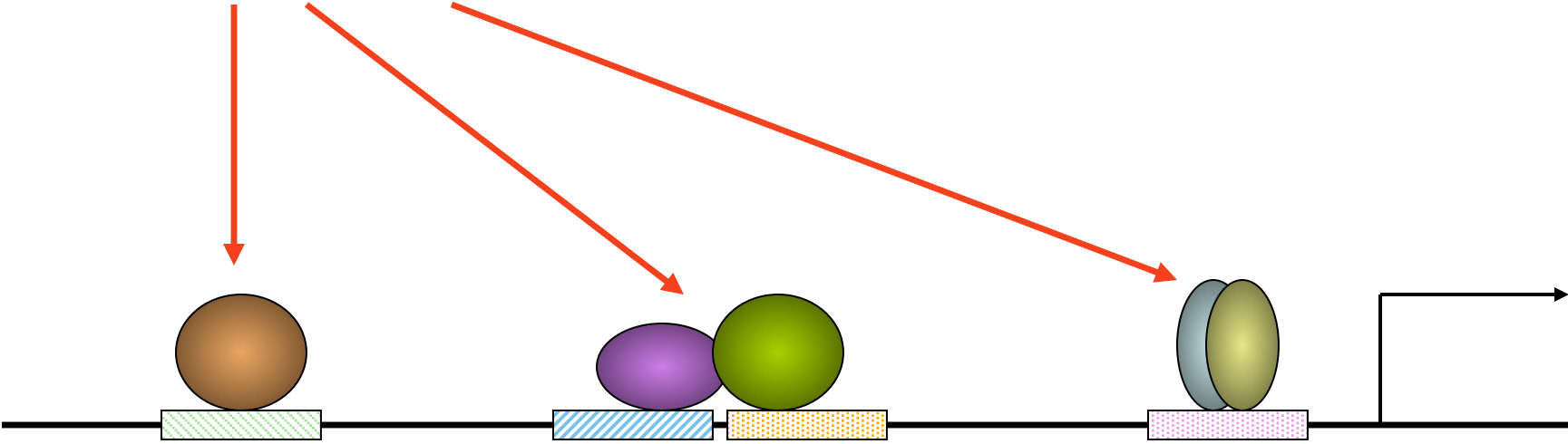


insulin resistance



thermogenesis

Find candidate TFs → Identify target genes → Function

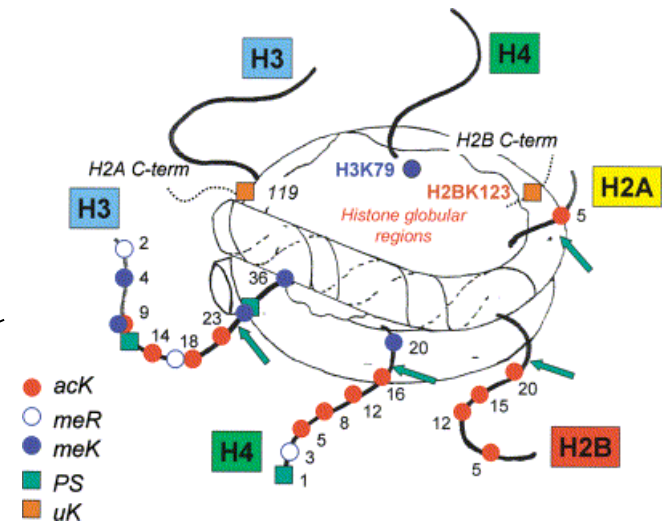


Find *cis* motifs → Identify cognate TFs → Function

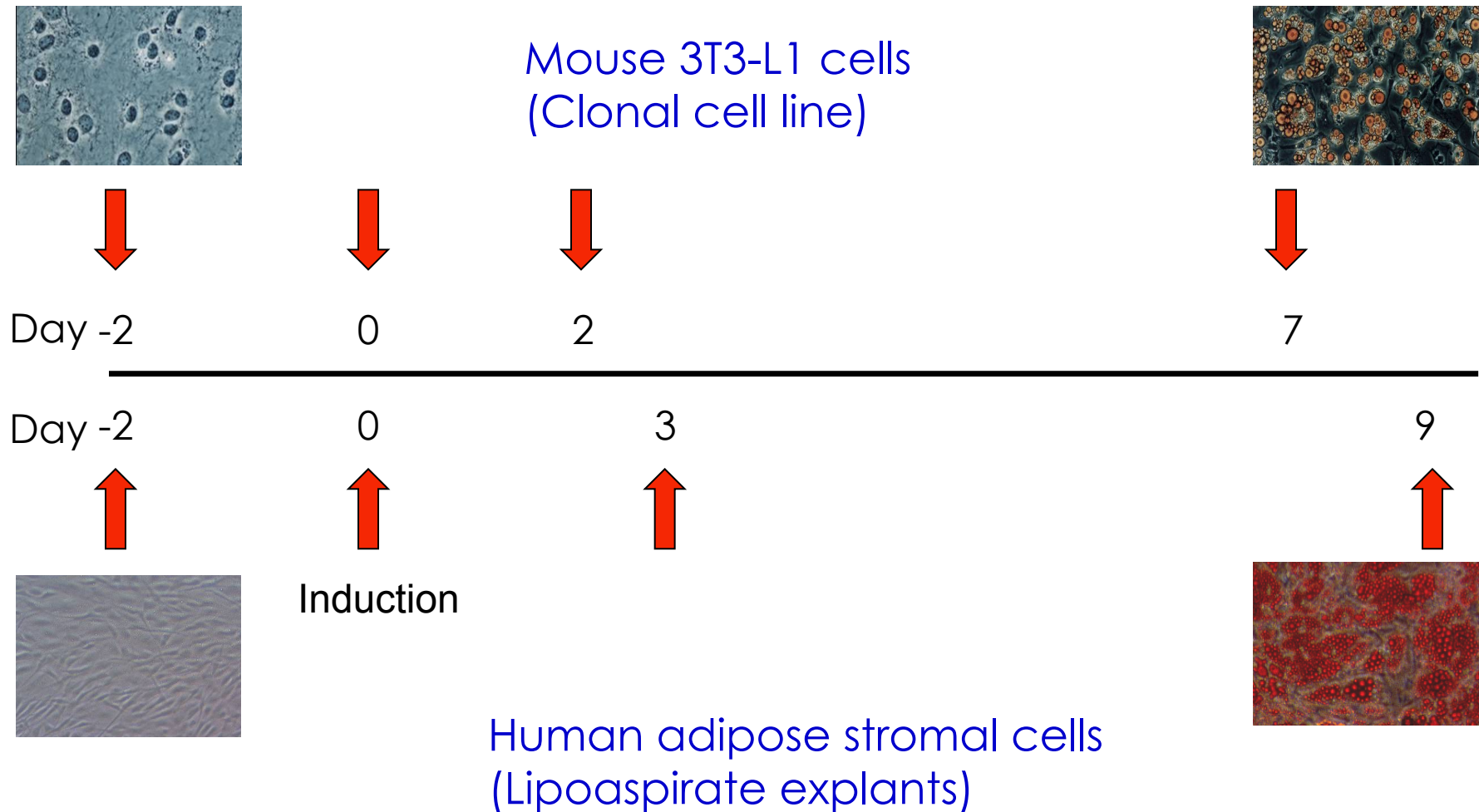
The epigenome



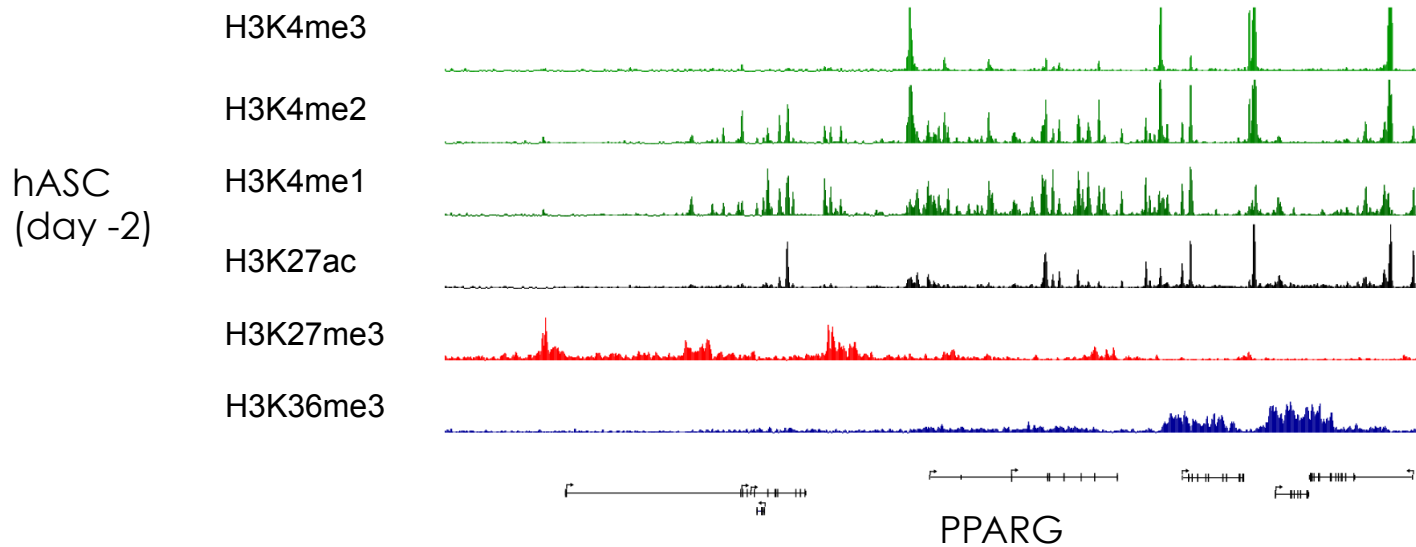
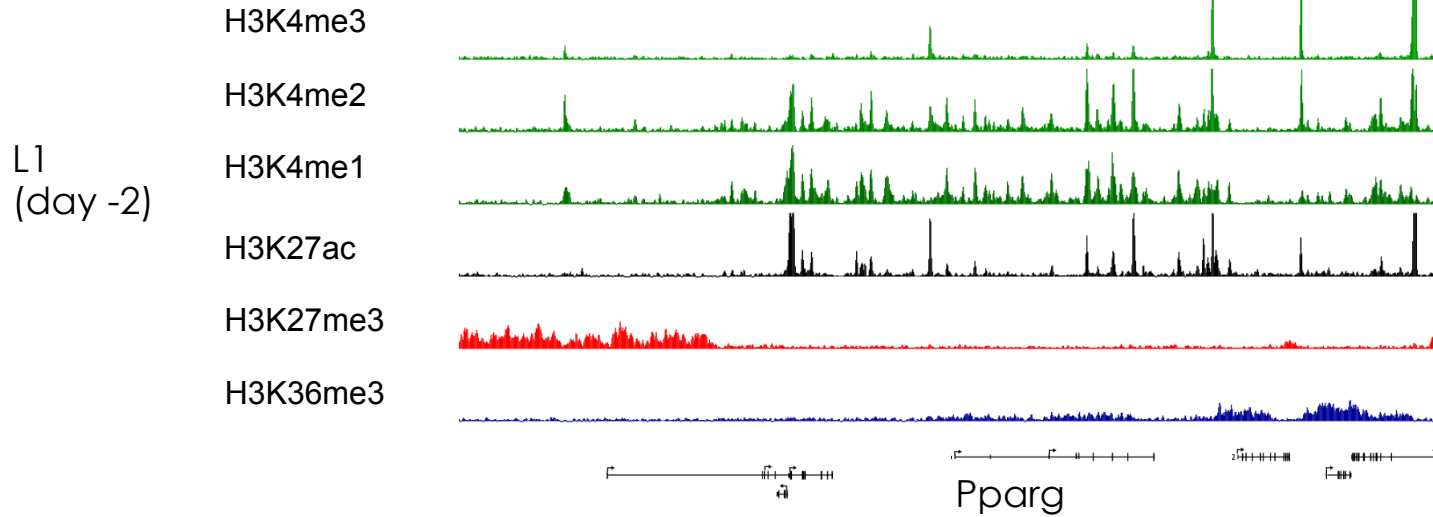
Covalent histone modifications



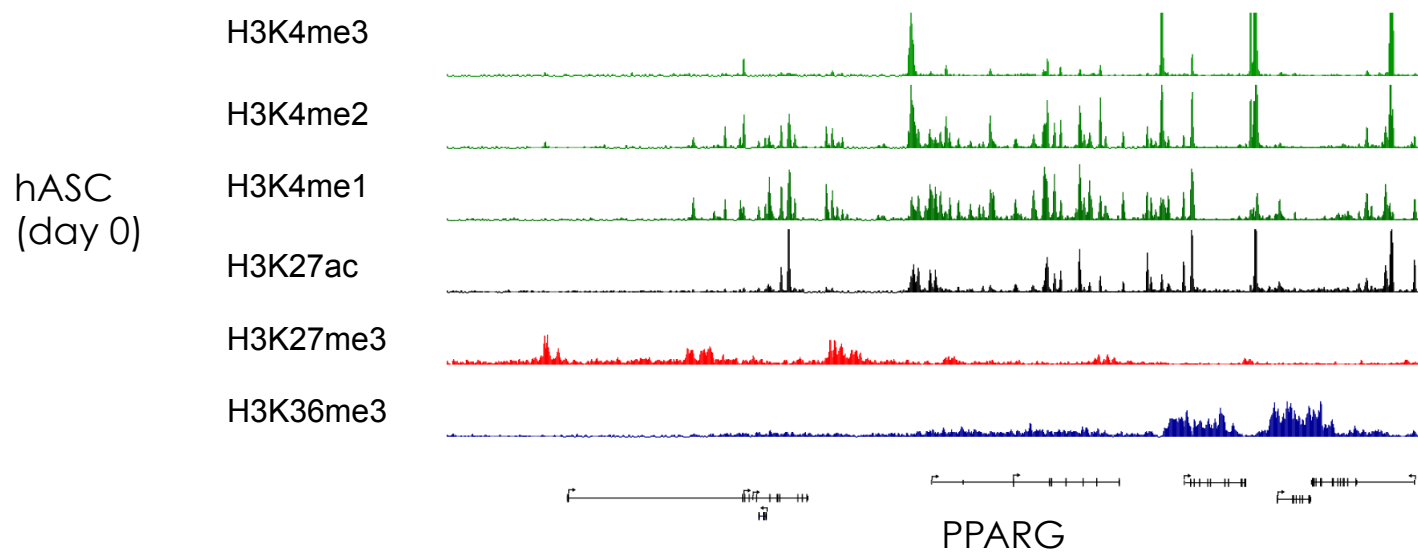
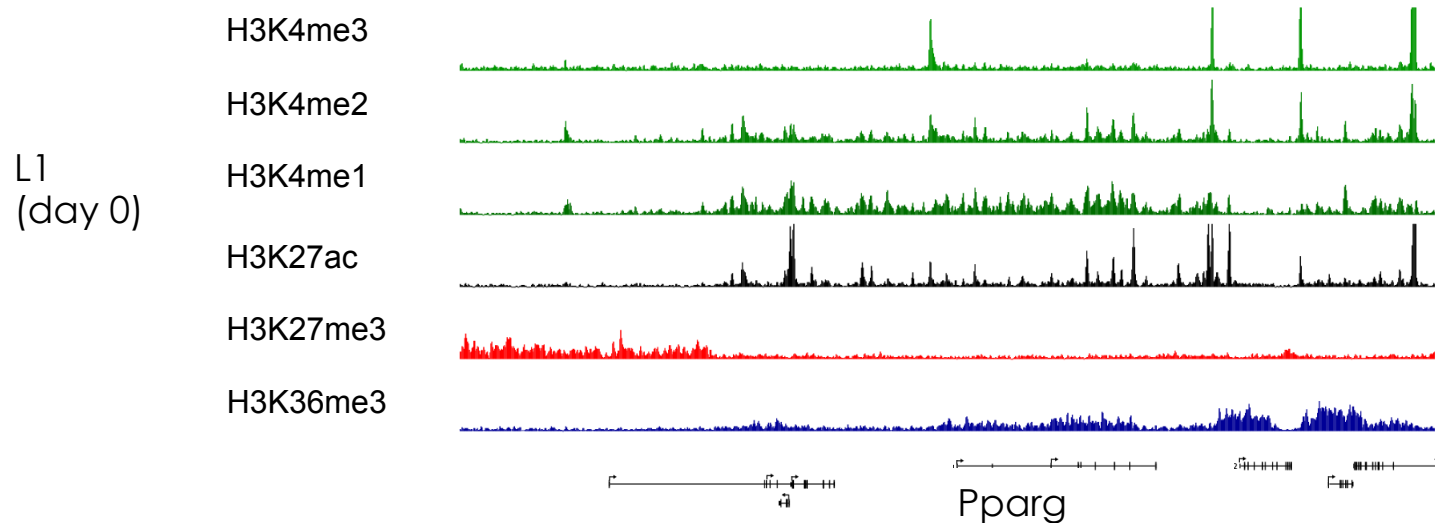
Comparative epigenomic analysis of L1 and hASC adipogenesis



Comparative epigenomic analysis of L1 and hASC adipogenesis

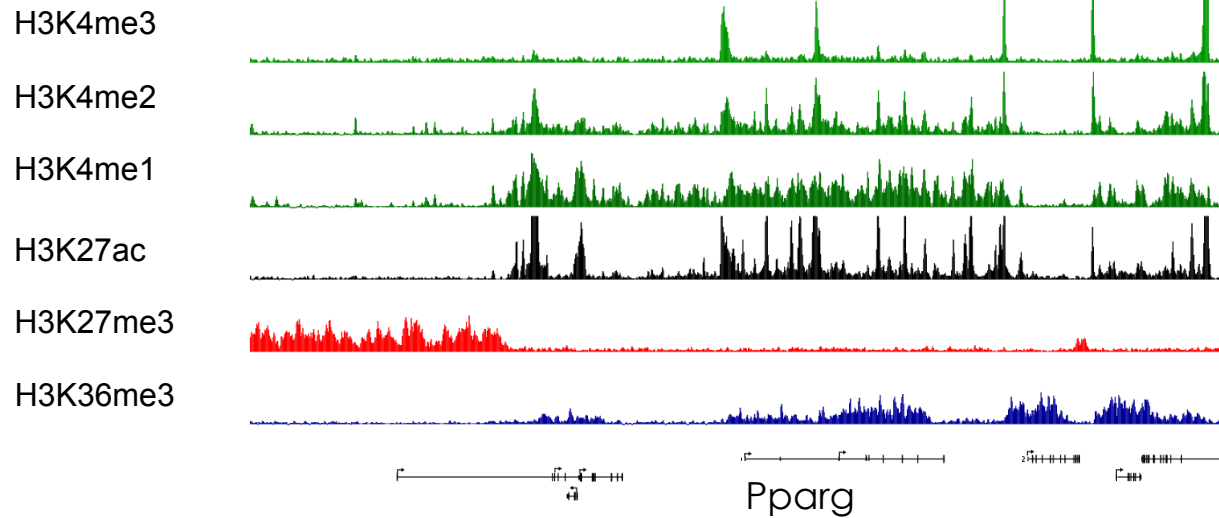


Comparative epigenomic analysis of L1 and hASC adipogenesis

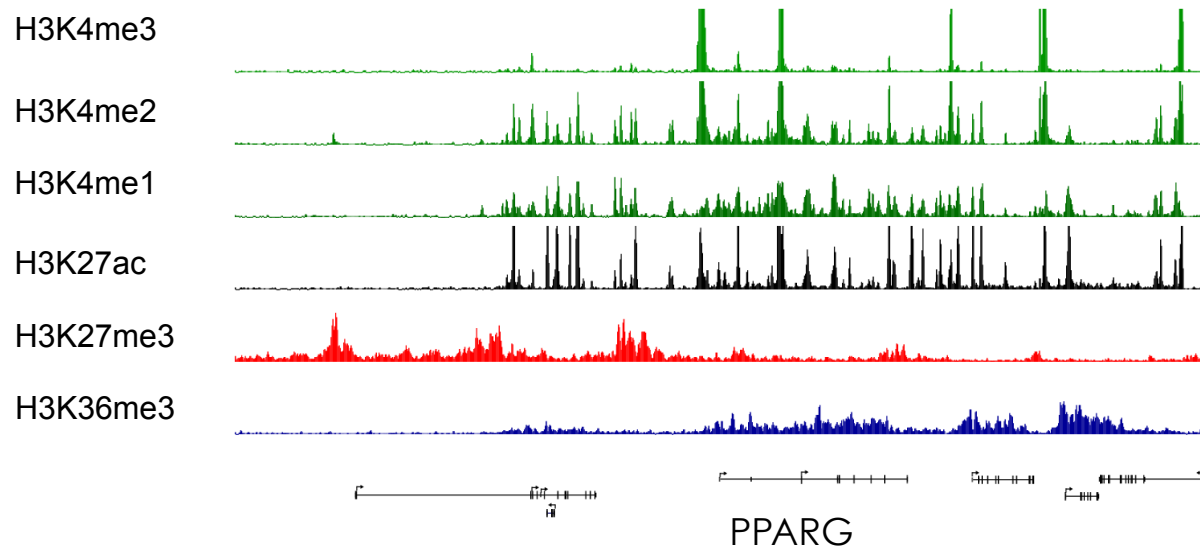


Comparative epigenomic analysis of L1 and hASC adipogenesis

L1
(day 2)



hASC
(day 3)



Comparative epigenomic analysis of L1 and hASC adipogenesis

L1
(day 7)

H3K4me3

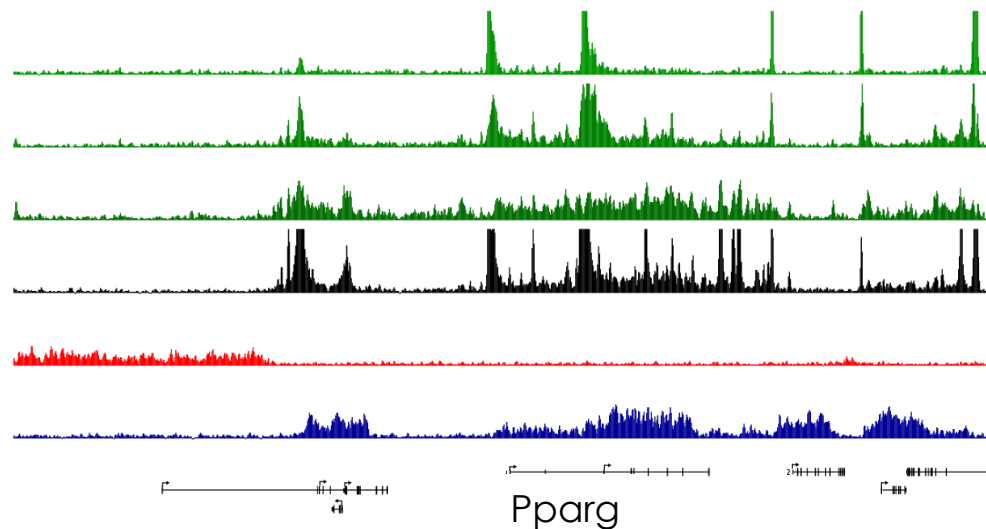
H3K4me2

H3K4me1

H3K27ac

H3K27me3

H3K36me3



hASC
(day 9)

H3K4me3

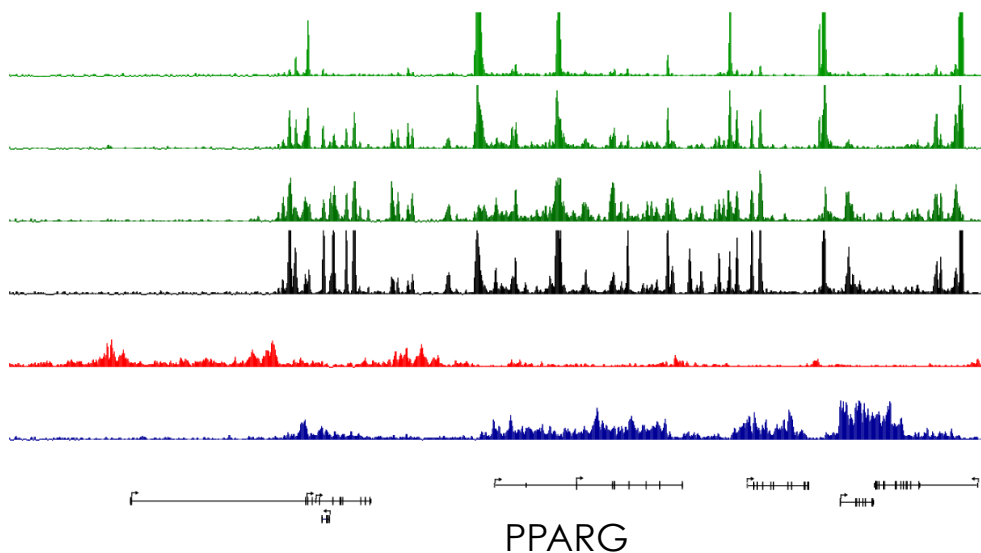
H3K4me2

H3K4me1

H3K27ac

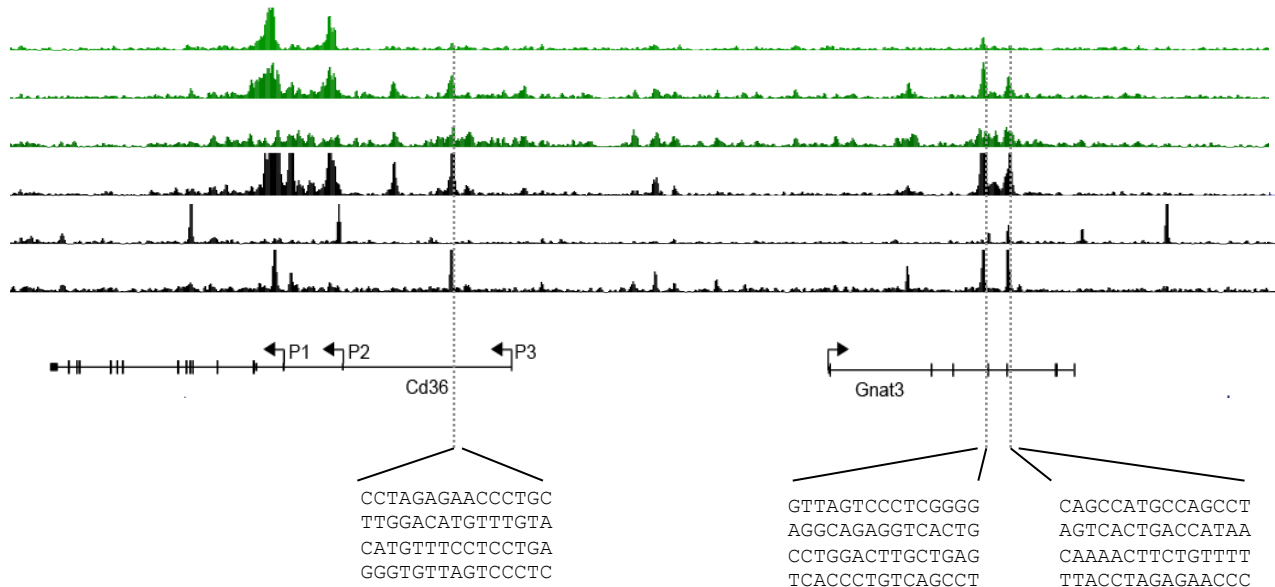
H3K27me3

H3K36me3



Strategy for identification of sequence-specific regulators

Cell type-specific enhancer sequences



Database of TF motifs

	M00539	Max, Myc + others (E box)
	M00651	Nfkb1
	M00040	Atf2

Ranked list of enriched TF motifs

Motif ranks from adipogenesis recover many known regulators

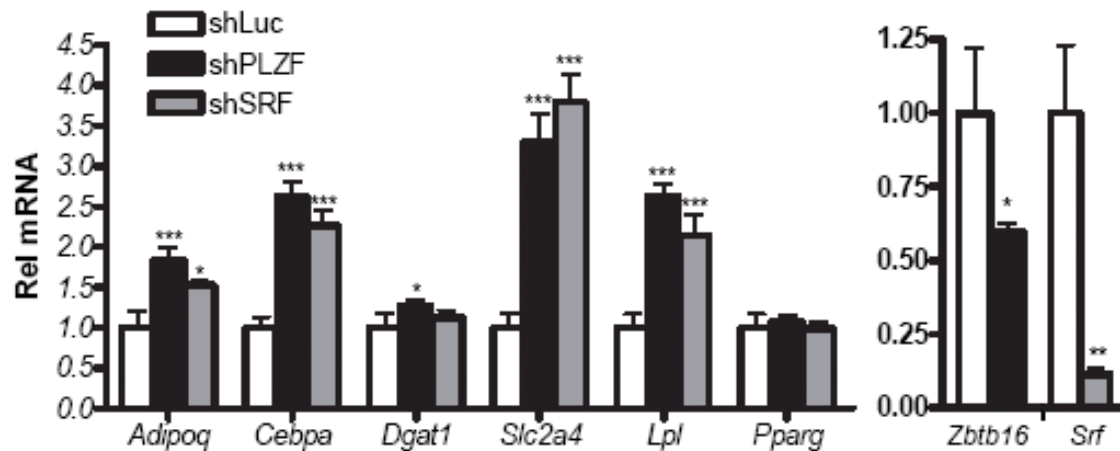
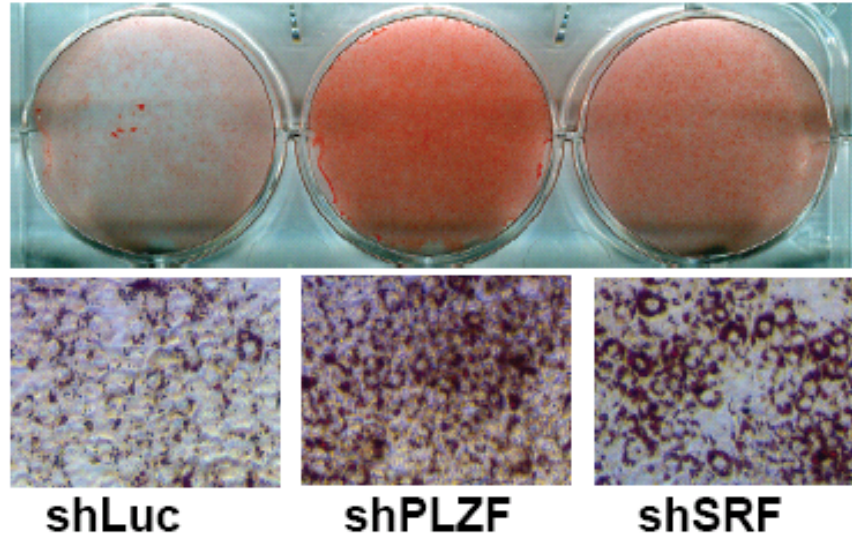
Most enriched in pre-adipocyte-specific enhancers

Motif	ID	Ratio	Candidates
	U_Pou3f3	0.32	?
	M00498	0.63	(Stat half-site) ✓
	U_Cphx	0.34	?
	U_Hoxa6	0.36	Homeobox-family
	M00026	0.41	Mef2a ✓
	U_Tbp	0.44	Tbp
	U_Srf	0.46	Srf ?
	M00495	0.52	Bach1/2
	M00199	0.54	Fos/Jun (AP-1) ✓
	M00987	0.55	Foxp1
	M00795	0.56	Pou2f1 (Octamer motif)
	M01075	0.57	Zbtb16 (PLZF) ?
	M00999	0.60	?
	U_Tcf7	0.62	Tcf7l2, Tcf3, Lef1
	M00747	0.62	Irf1/3 ✓
	M01146	0.62	?
	M00920	0.63	E2f ✓
	U_Evx2	0.65	Homeobox-family
	M00694	0.67	E4f1 ✓
	U_Hoxa13	0.71	Homeobox-family

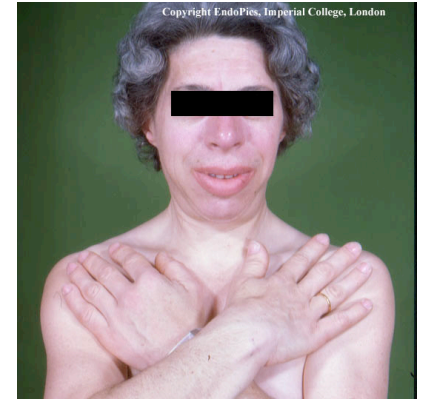
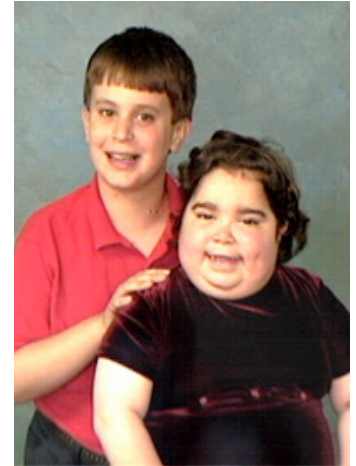
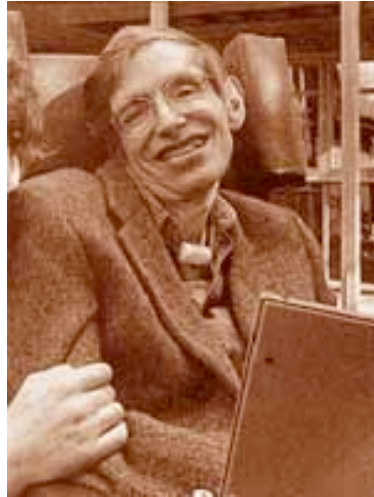
Most enriched in adipocyte-specific enhancers

Motif	ID	Ratio	Candidates
	M00278	2.1	Gata-family ✓
	M01132	2.1	Rxra/b + others (NHR half-site) ✓
	M00240	1.9	? (Nkx2 family)
	M00117	1.8	Cebpa/b/d/gz ✓
	M00526	1.8	Nr6a1
	M00539	1.6	Max, Myc + others (E box)
	U_Zfp161	1.6	Zfp161
	M00191	1.6	Rxra/b + others (NHR half-site) ✓
	M01069	1.6	Gzf1
	U_Irf4	1.6	Irf3/4/5/6 ✓
	M00237	1.6	Ahr:Arnt dimer ✓
	U_Gmeb1	1.6	Gmeb1
	M00105	1.5	Cux1
	M00651	1.5	Nfkb1 ✓
	M00979	1.5	?
	M00963	1.5	Rxra/b + others (NHR half-site) ✓
	M00196	1.4	Sp1 + others (G/C-box)
	M00040	1.4	Atf2
	U_Rxra	1.4	Rxra/b + others (NHR half-site) ✓
	U_Osr2	1.3	Osr1/2

Knockdown of PLZF or SRF enhances adipogenesis



Insulin resistance: is there a common molecular denominator?



Also:

Infection/sepsis

Burn injury

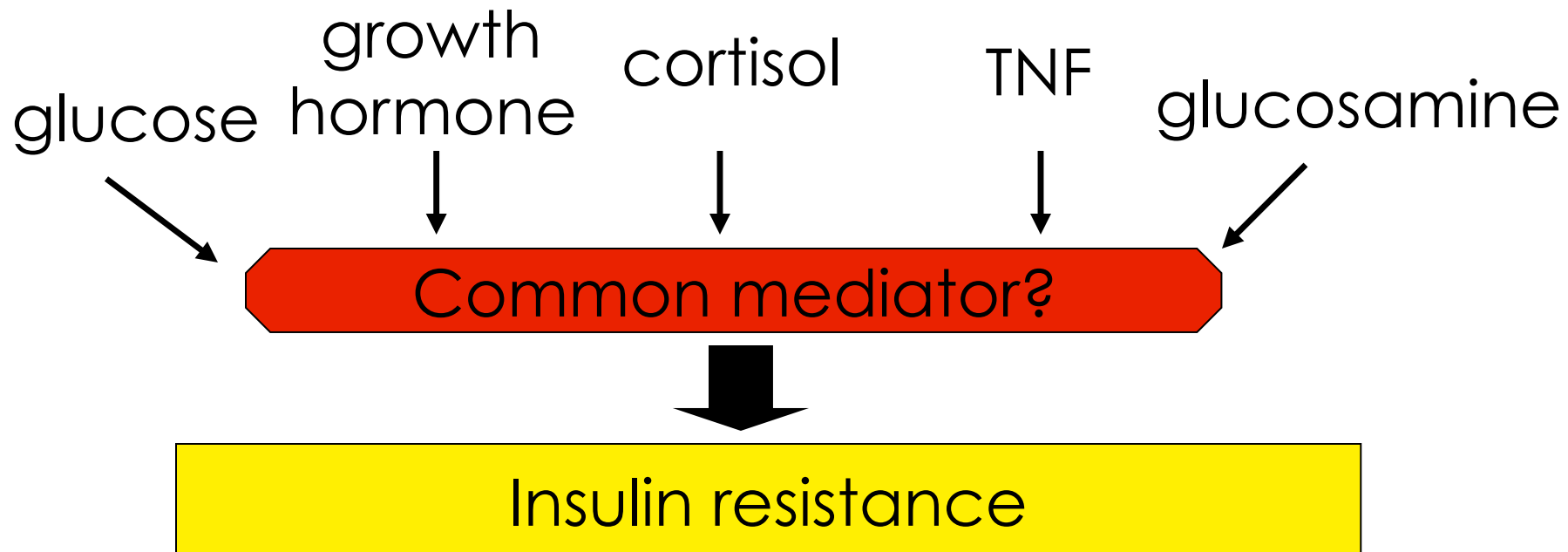
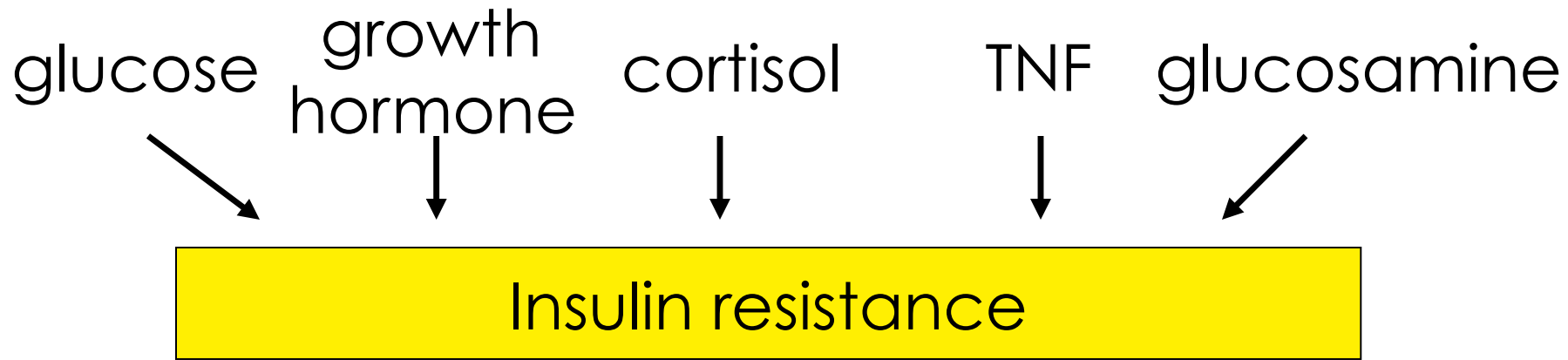
Starvation

Insulin resistance: is there a common molecular denominator?

Many molecular mediators have been proposed:

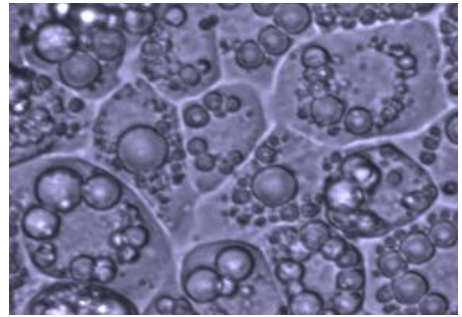
- Cortisol
- TNF- α
- IL-6
- Growth hormone
- Insulin
- Glucose
- Free Fatty Acids
- Glucosamine

To what extent are molecular pathways shared in these conditions?

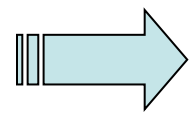


Cellular models of insulin resistance: TNF, dexamethasone

3T3-L1 Adipocytes

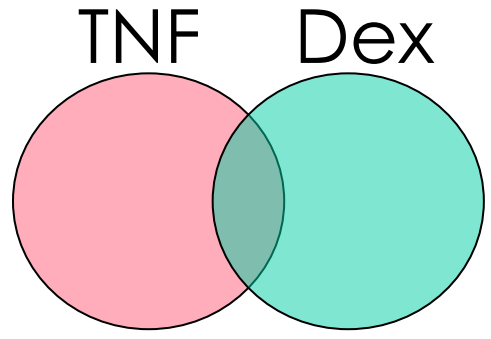
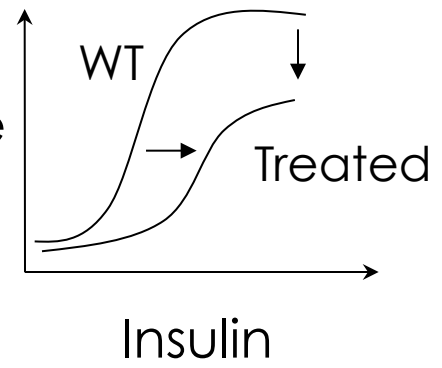


+ TNF, Dex



Glucose uptake

Insulin Resistance



Cellular models of insulin resistance: TNF and Dex

Why Dex and TNF?

- Both GCs and TNF are elevated in multiple insulin resistant states
- Exogenous GCs/TNF induce insulin resistance in vivo
- TNF^{-/-} mice are protected from diet-induced insulin resistance
- Glucocorticoid antagonists block diet-induced insulin resistance in mice

Dex and TNF are very different

- Dex is the prototypical anti-inflammatory agent; acts through a nuclear receptor
- TNF is the prototypical pro-inflammatory agent; acts through a cell-surface receptor

Nuclear mechanisms of insulin resistance?

Virtually all mechanisms proposed for insulin resistance involve signal transduction or mitochondrial pathways

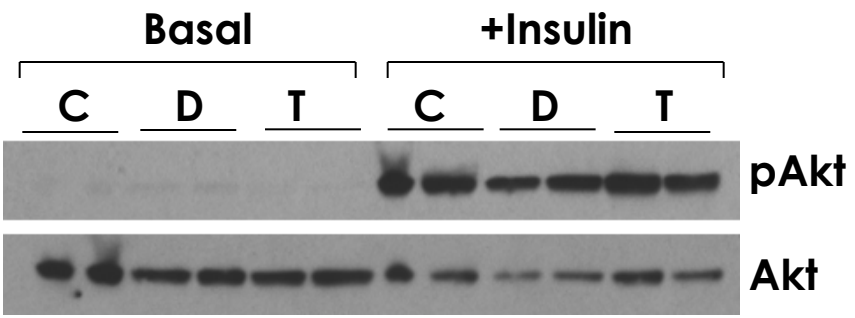
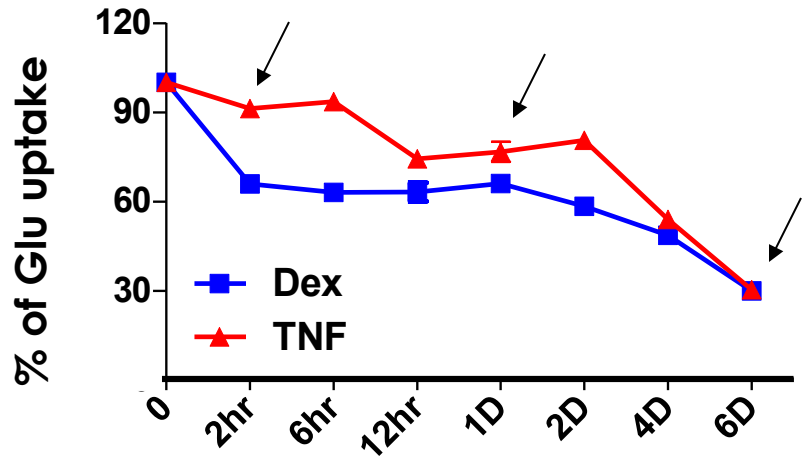
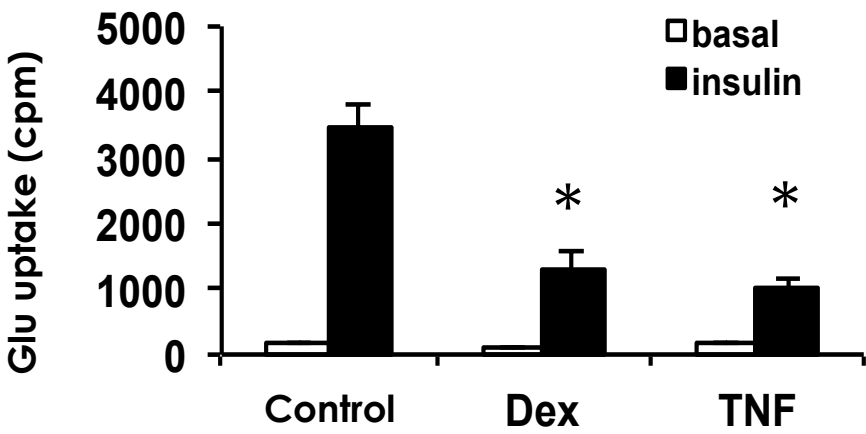
Yet....

- Thiazolidinedione class of insulin-sensitizing drugs work by binding and activating the transcription factor PPAR γ

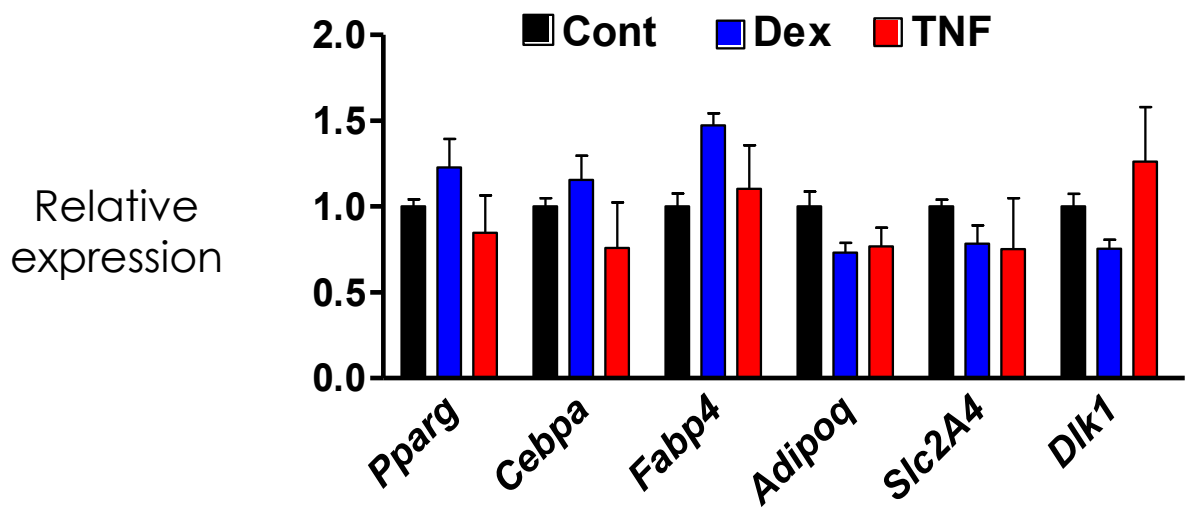
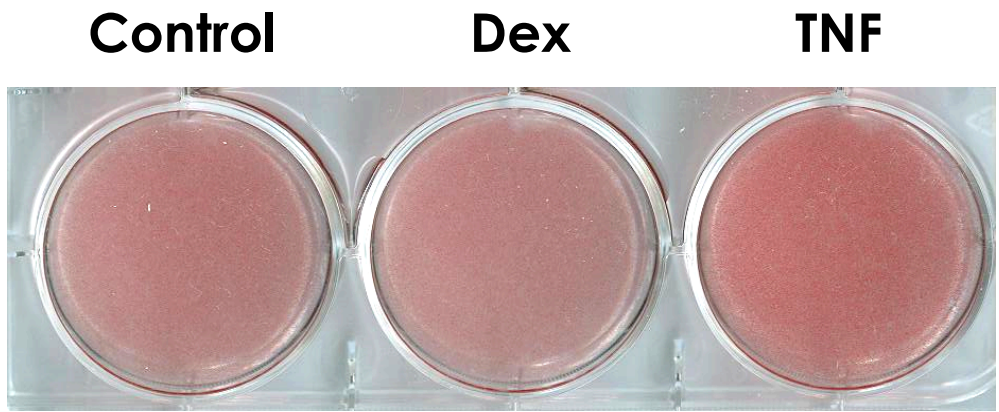
- Cellular models of insulin resistance develop slowly over the course of many days

- There is a wealth of data linking chromatin state to obesity and its complications

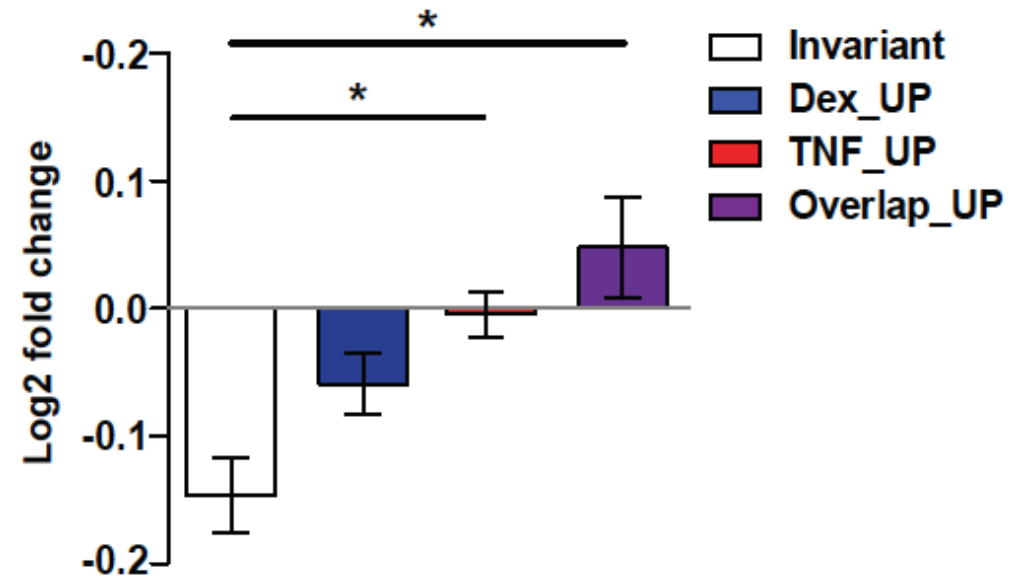
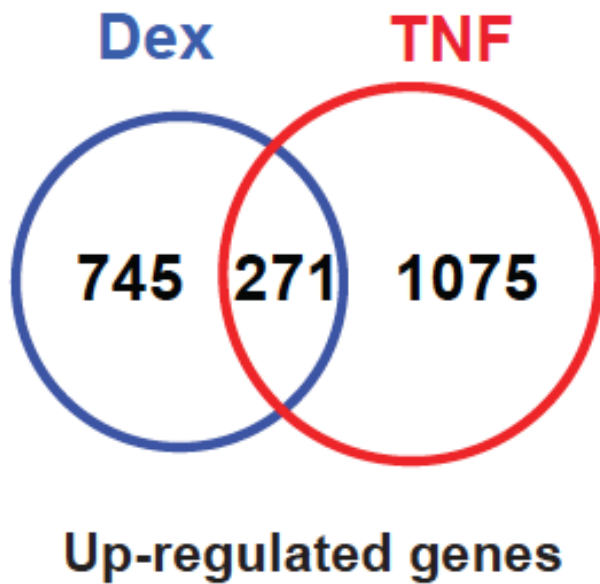
Establishment of the comparative IR model

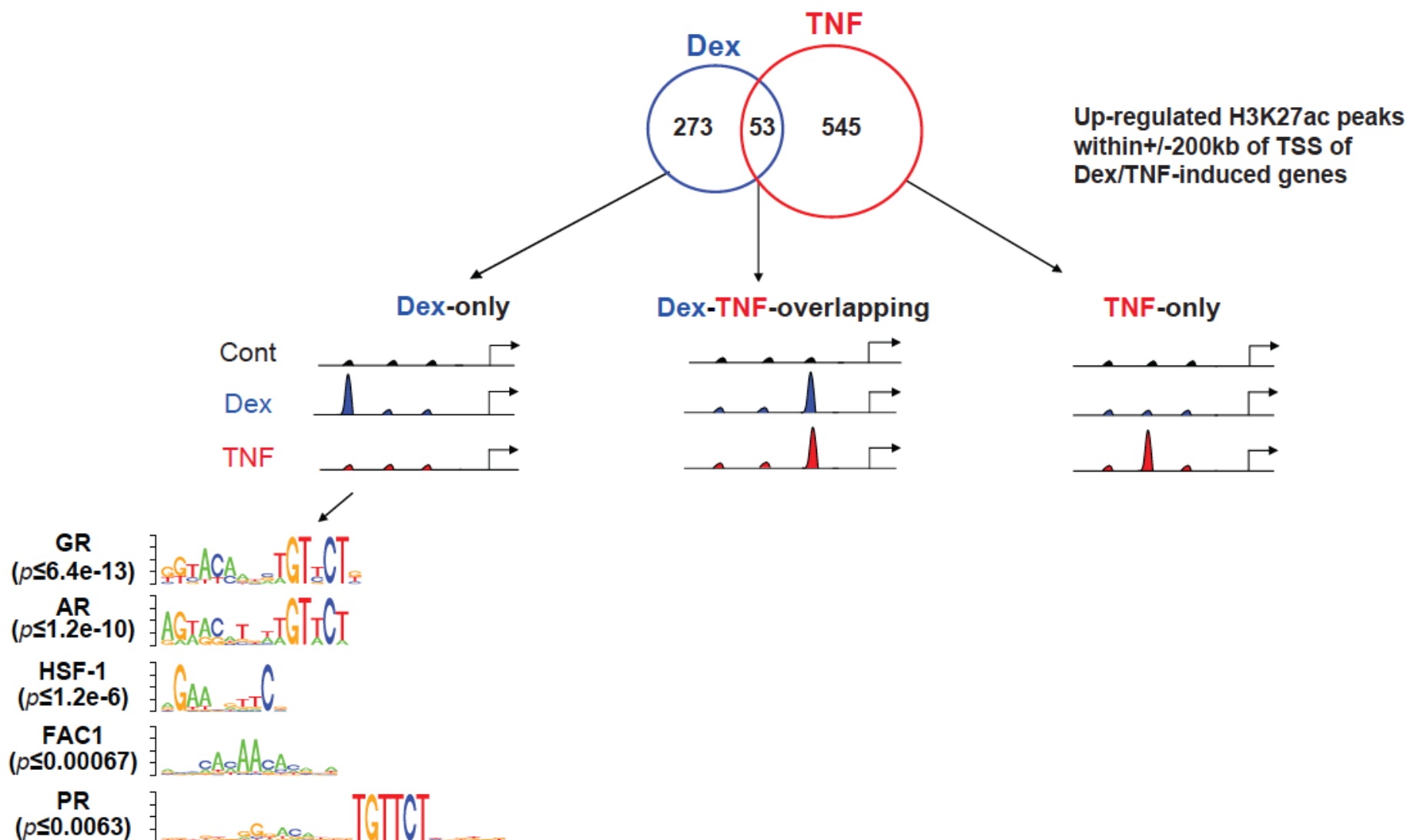


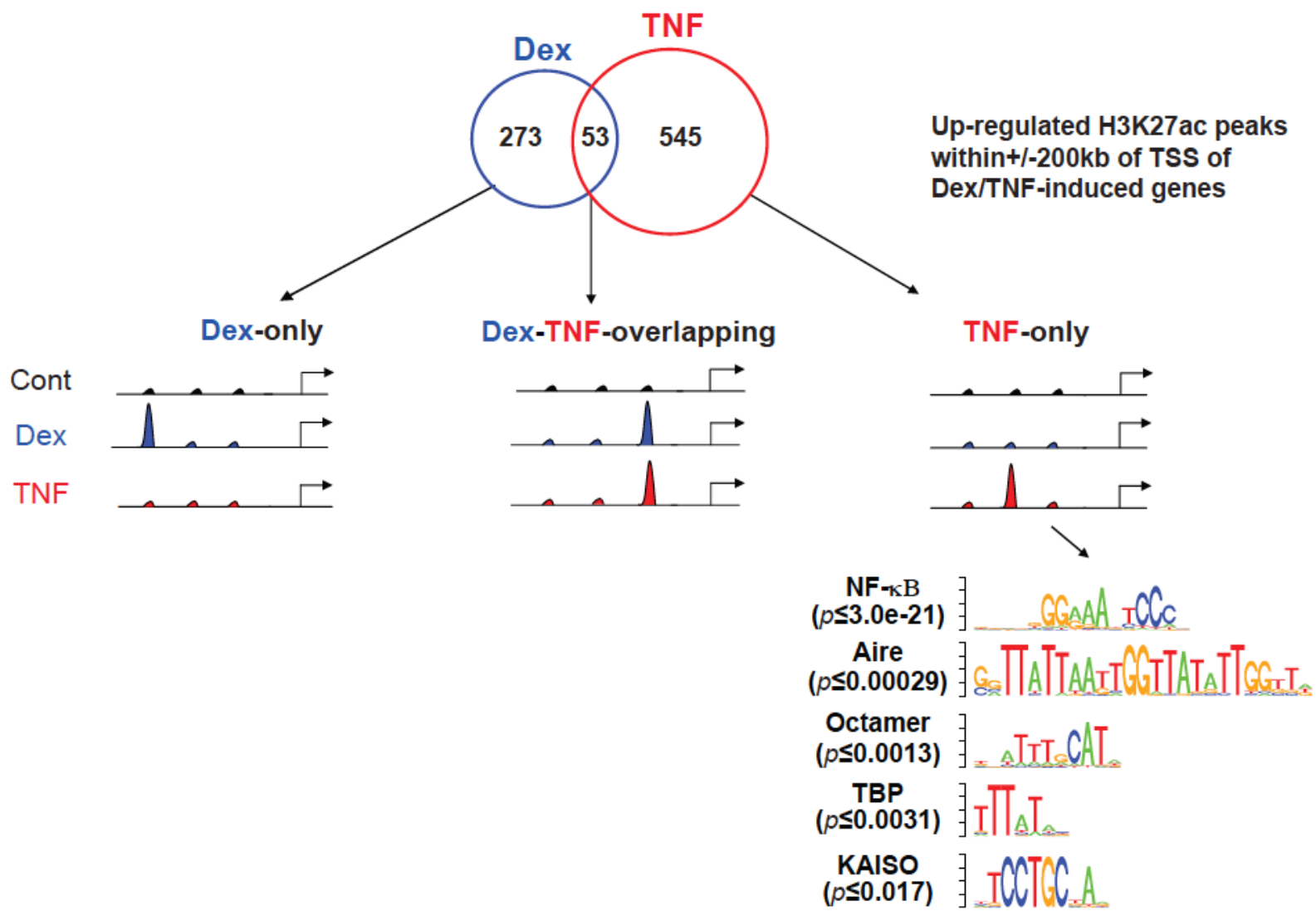
Dex and TNF do *not* cause de-differentiation

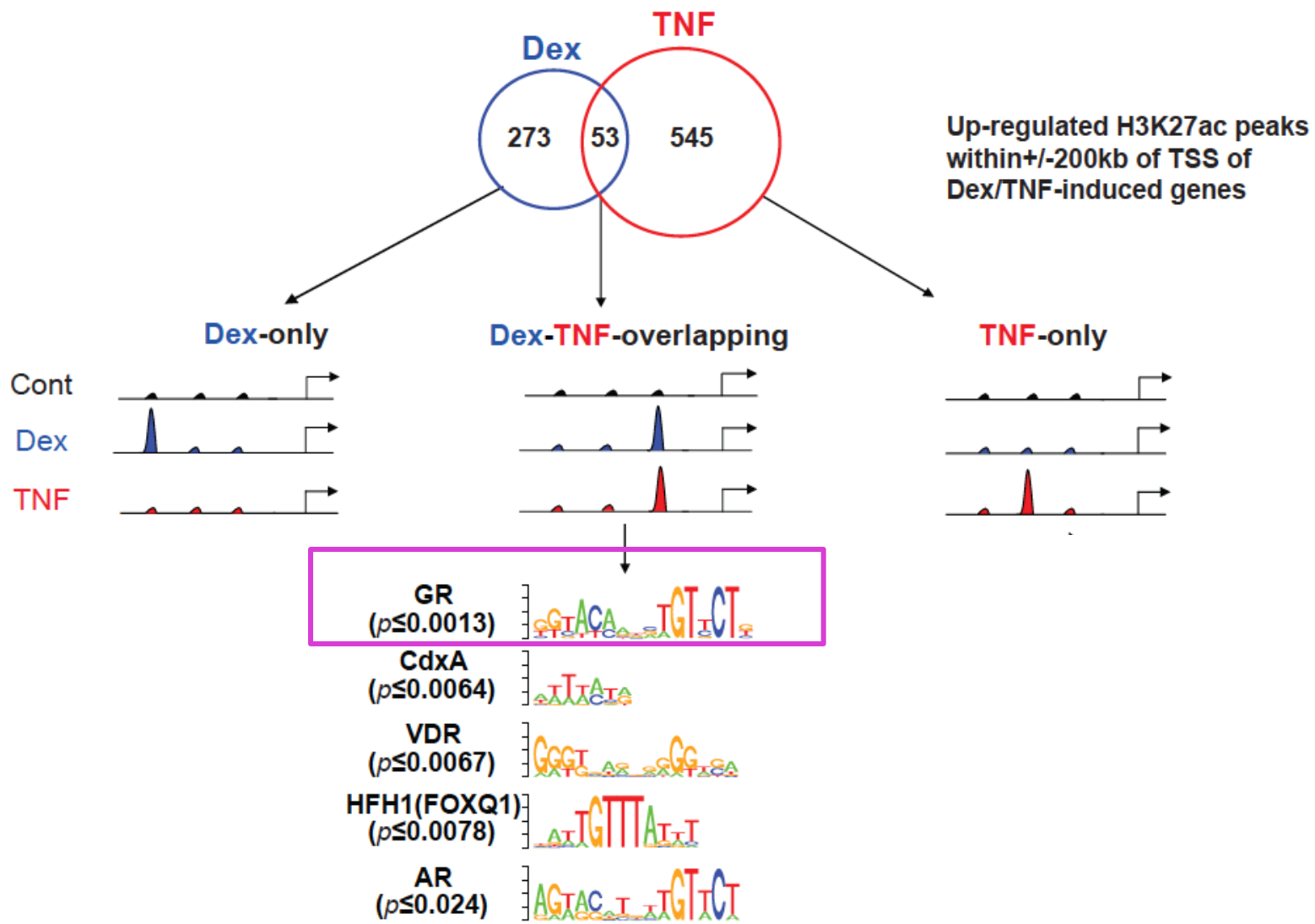


The overlapping gene set affected by Dex and TNF is altered in obesity

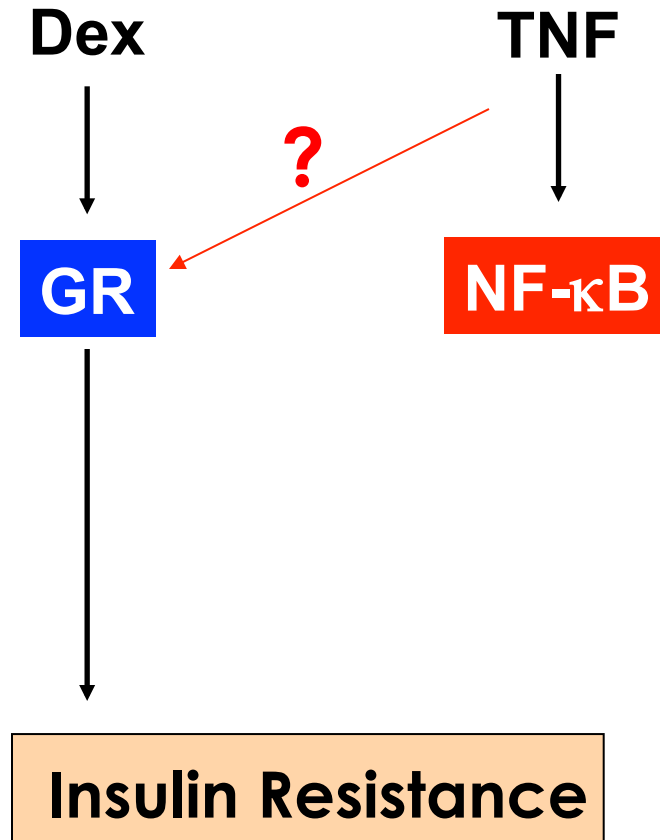




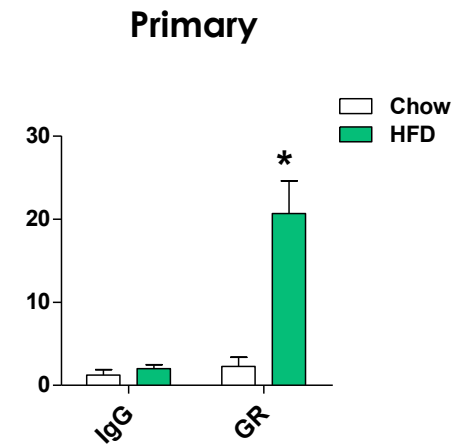
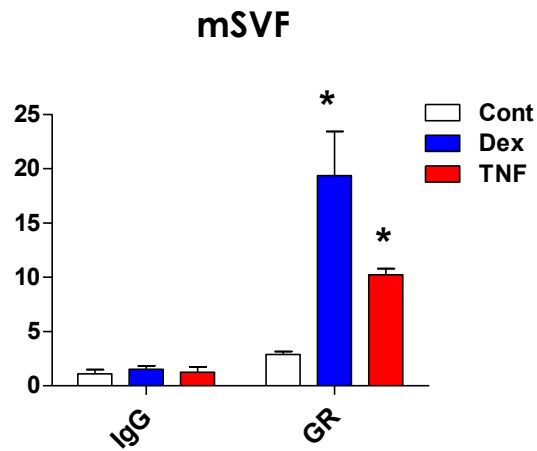
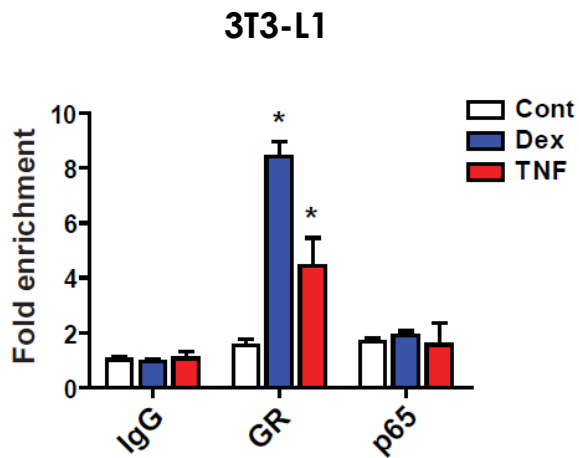
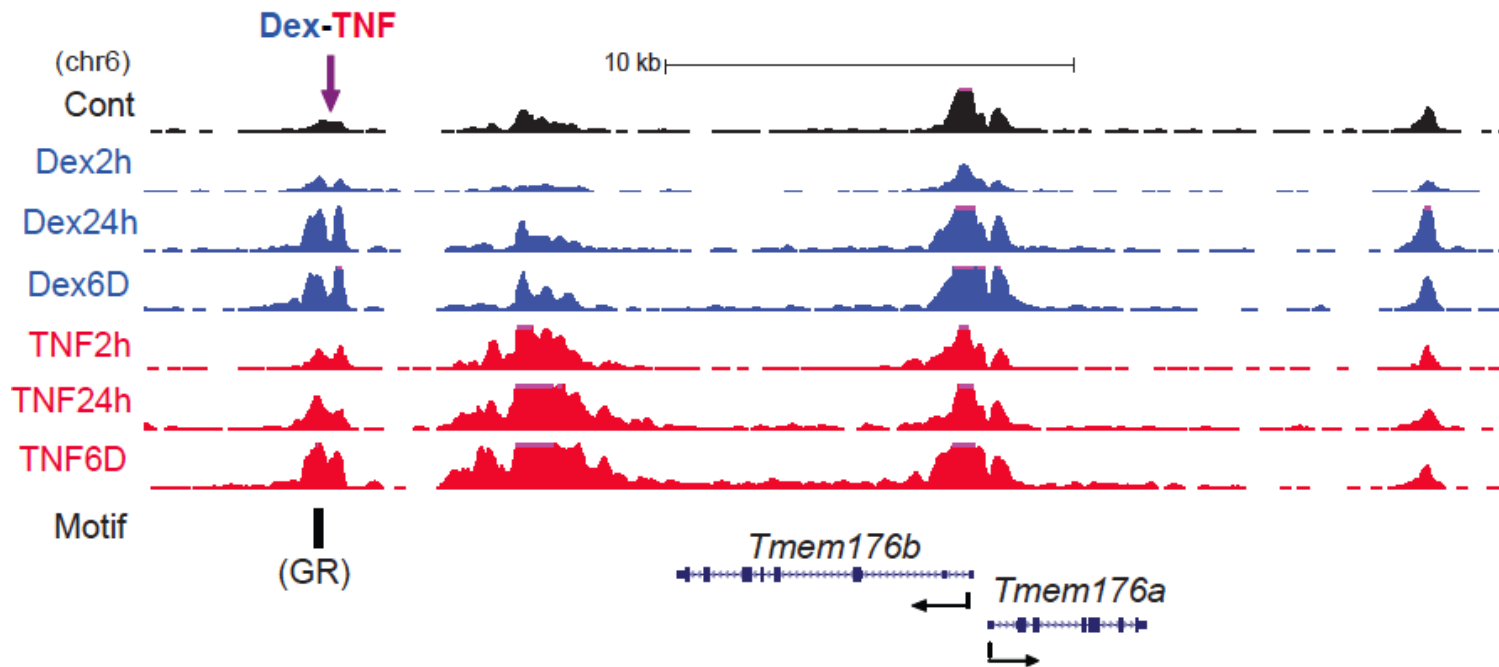




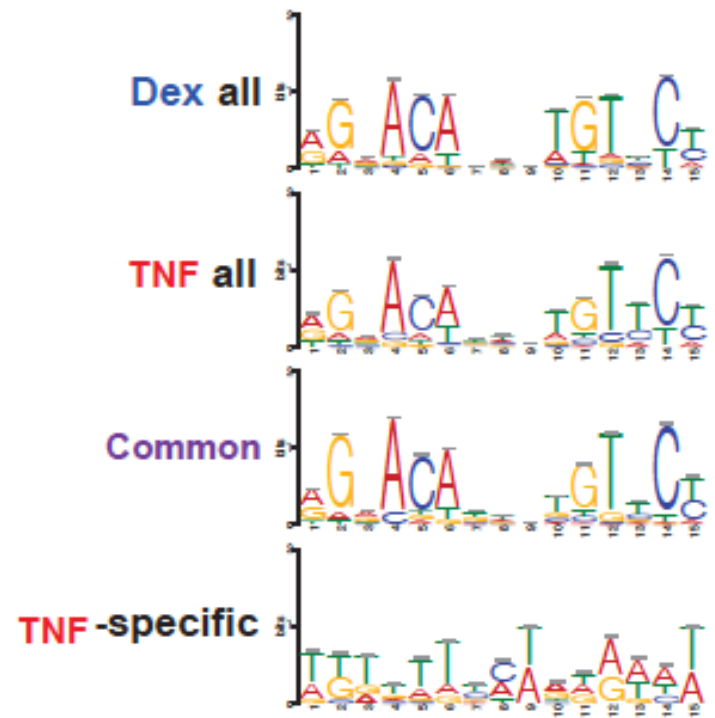
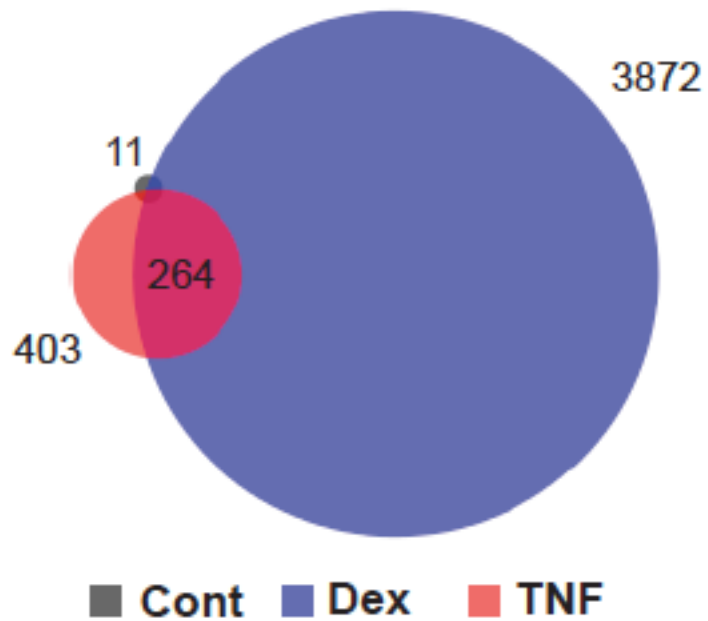
Is the GR required for TNF to induce insulin resistance?



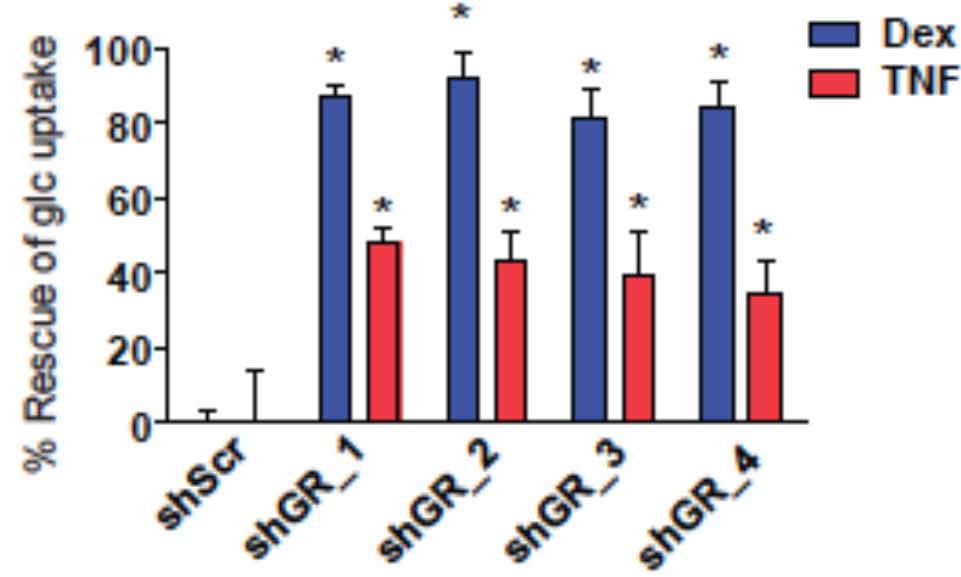
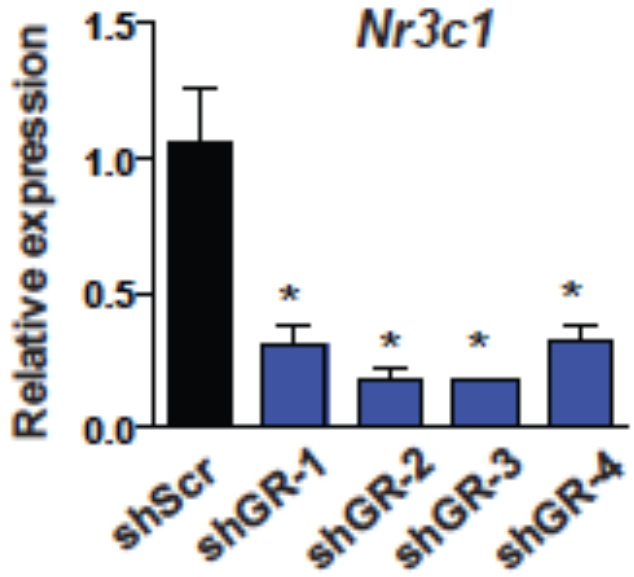
TNF causes GR binding to predicted motifs



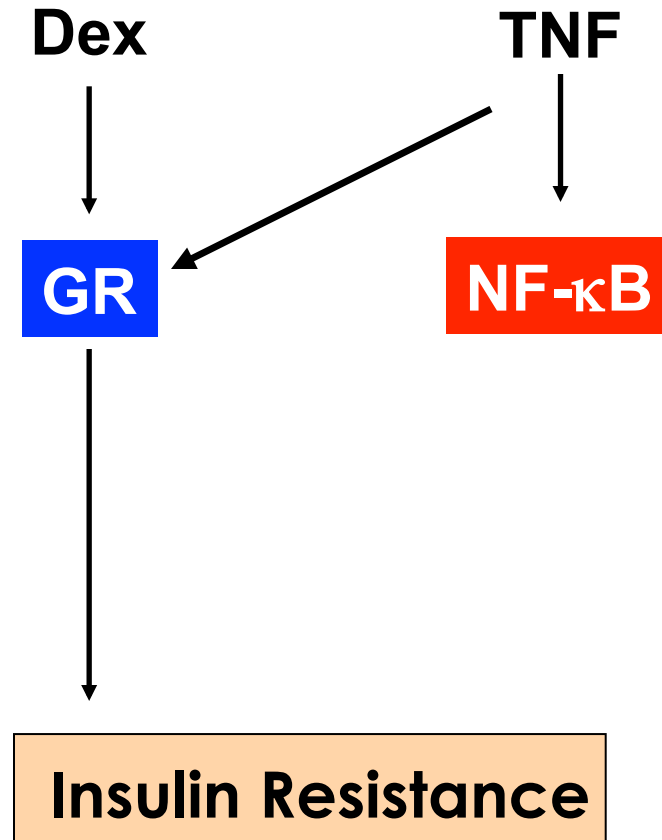
TNF induces genome-wide GR binding

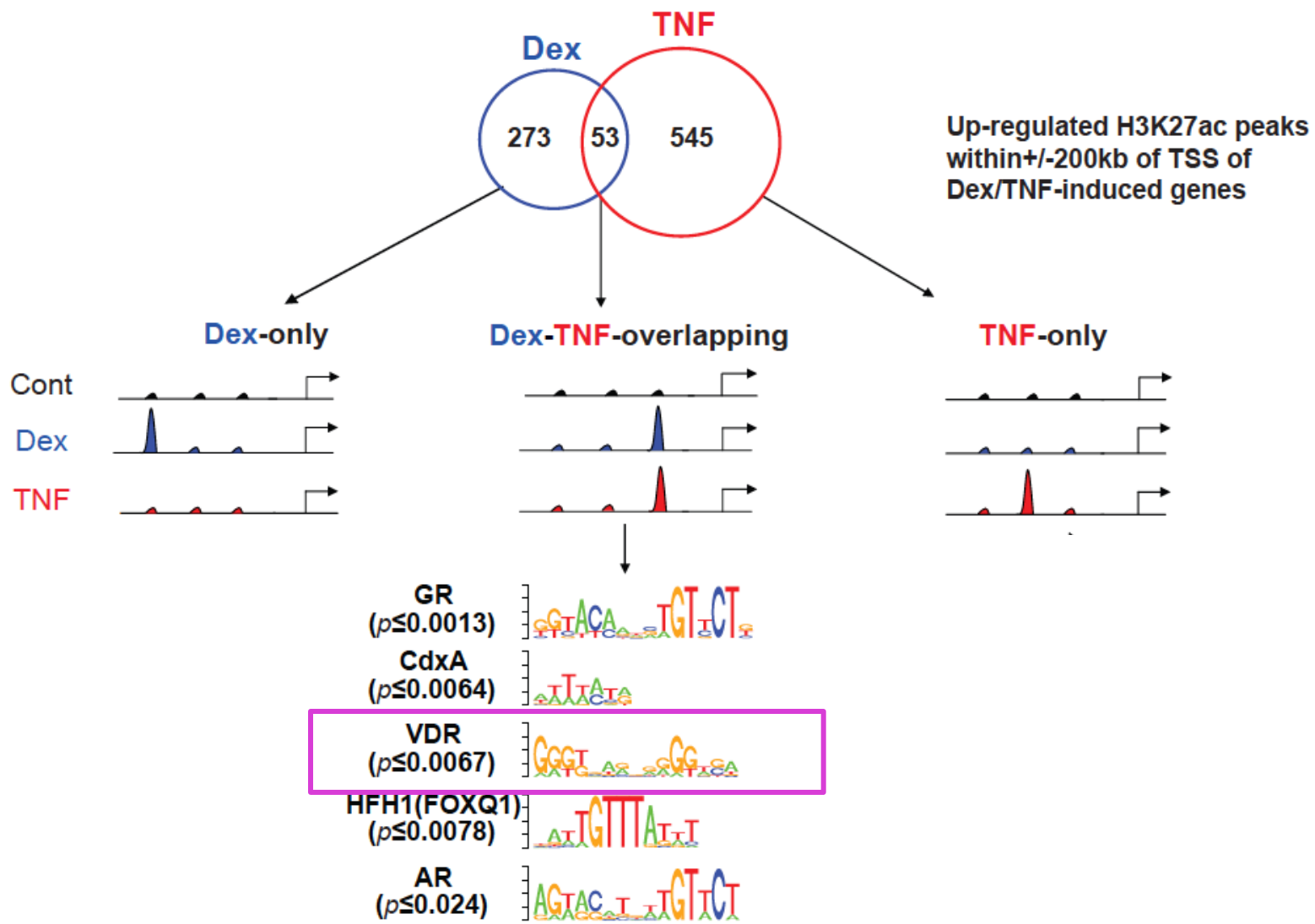


GR is required for TNF to fully induce insulin resistance

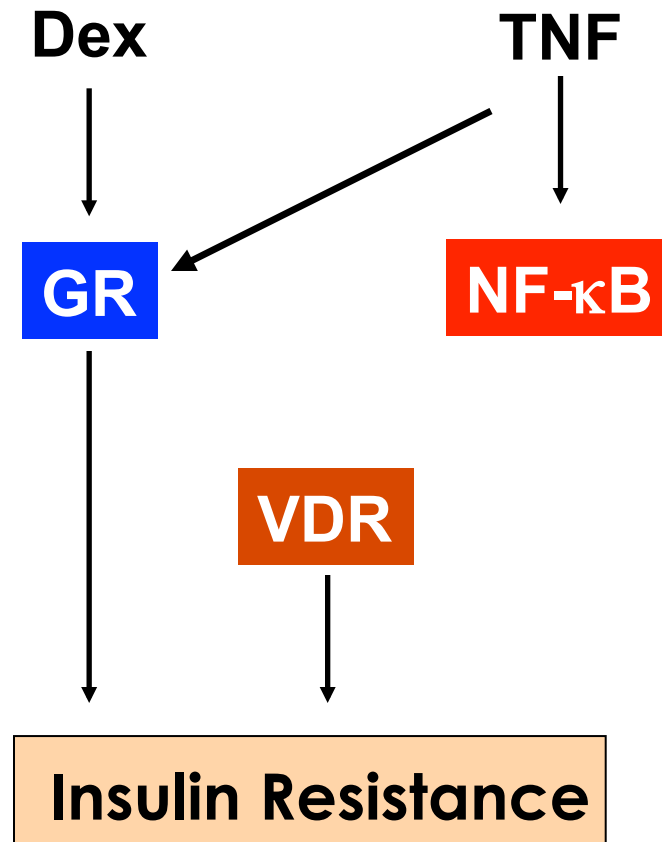


GR is required for TNF to fully induce insulin resistance

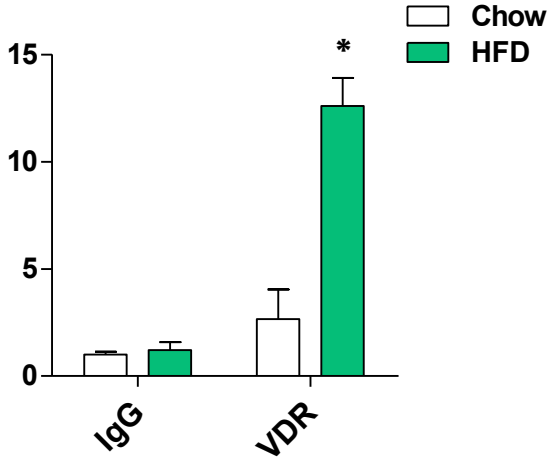
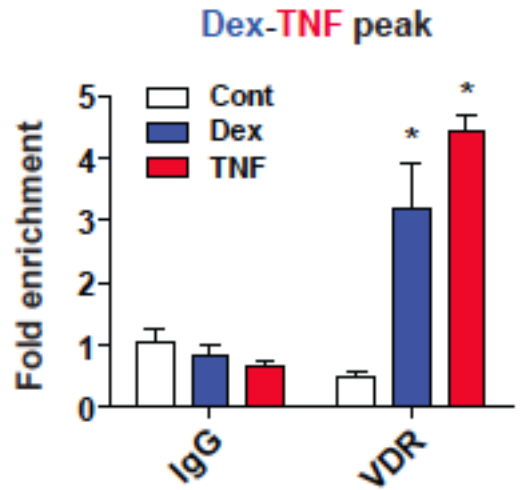
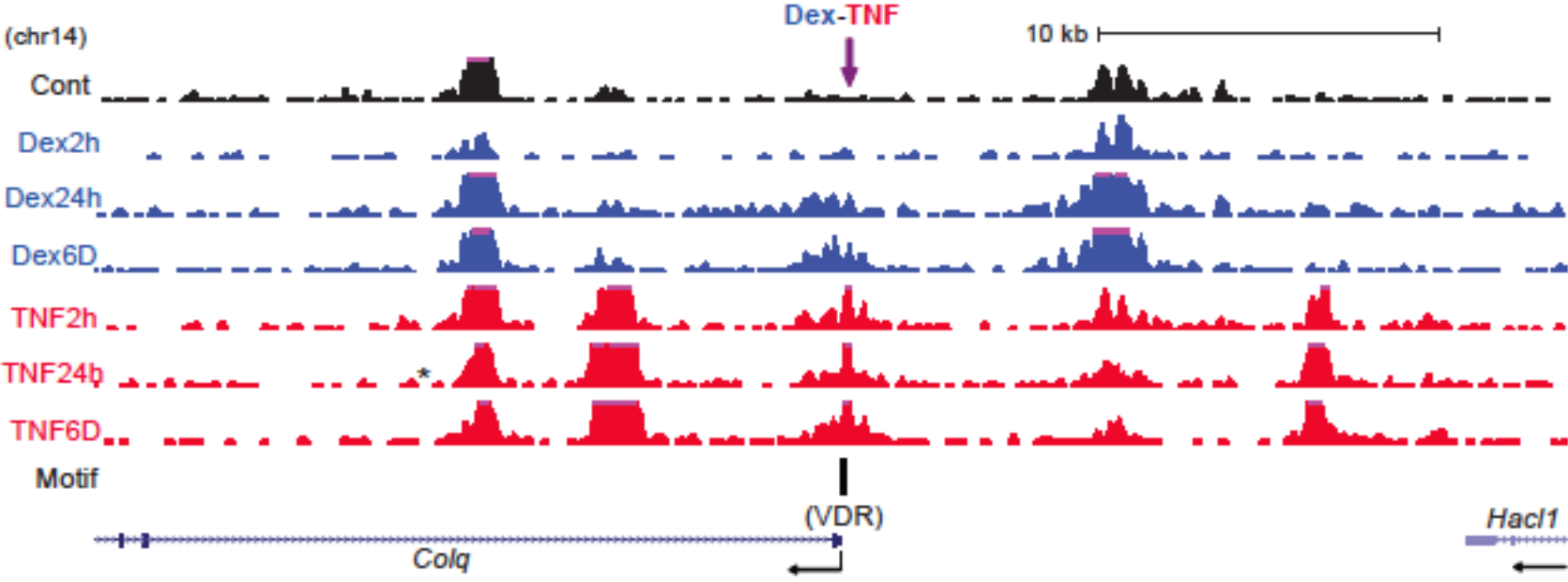




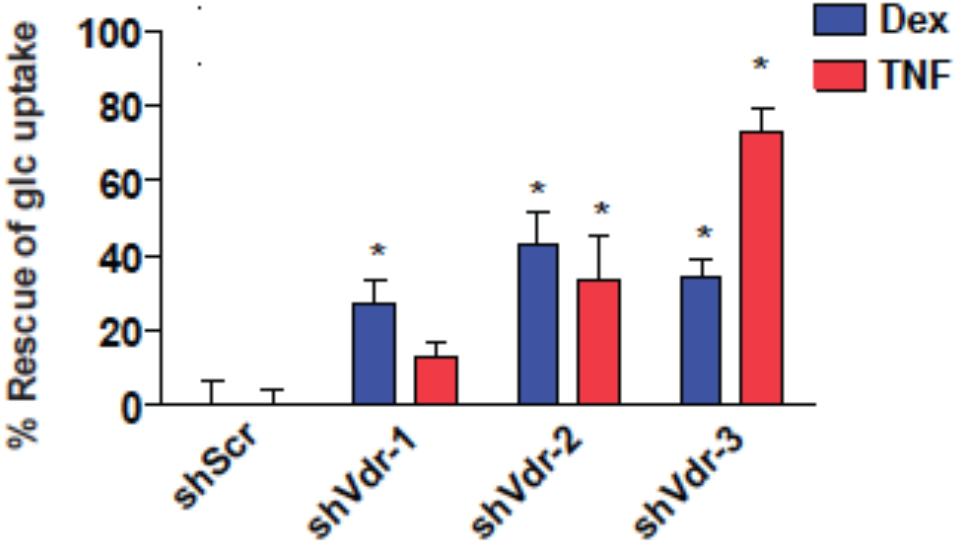
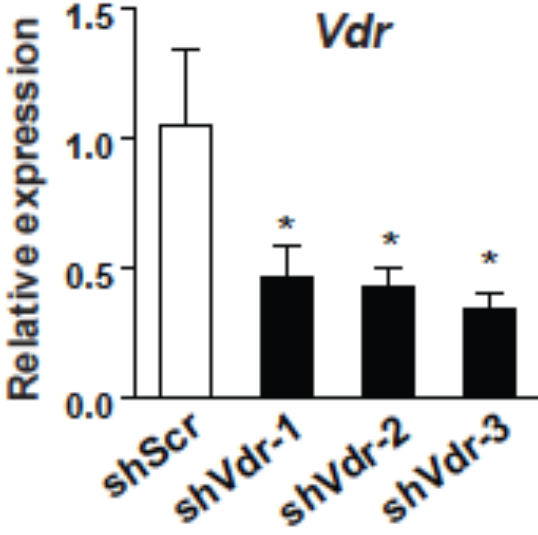
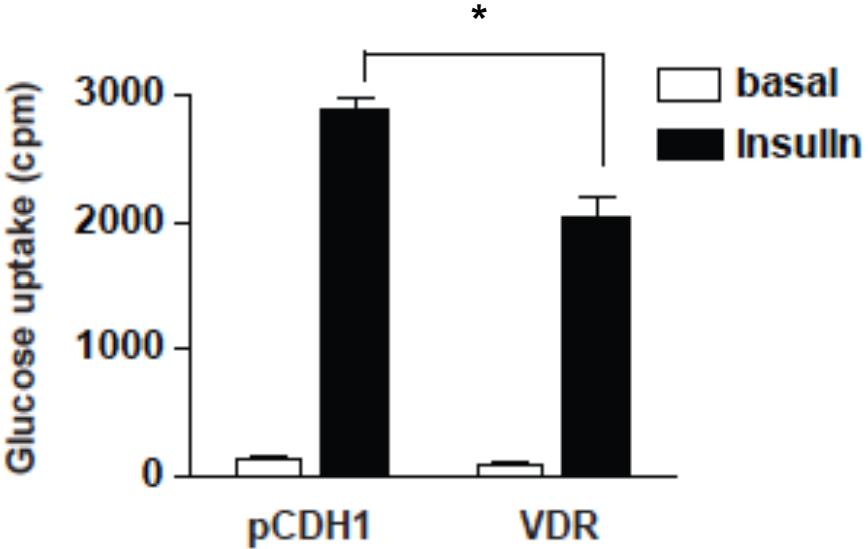
Is the VDR a mediator of insulin resistance?



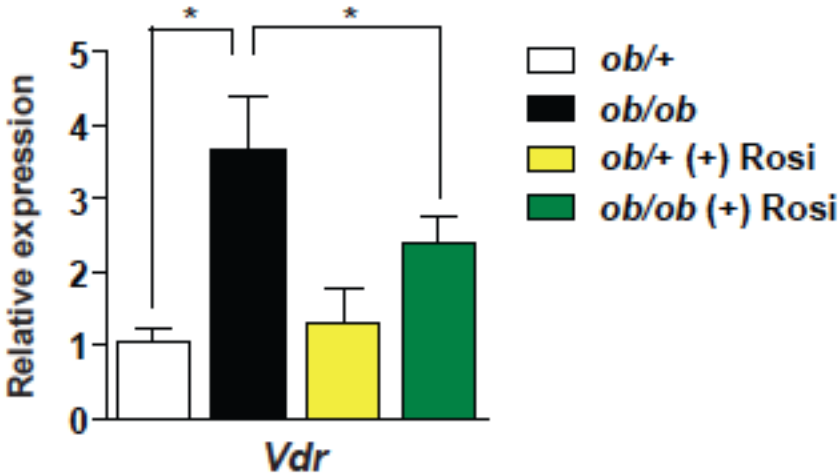
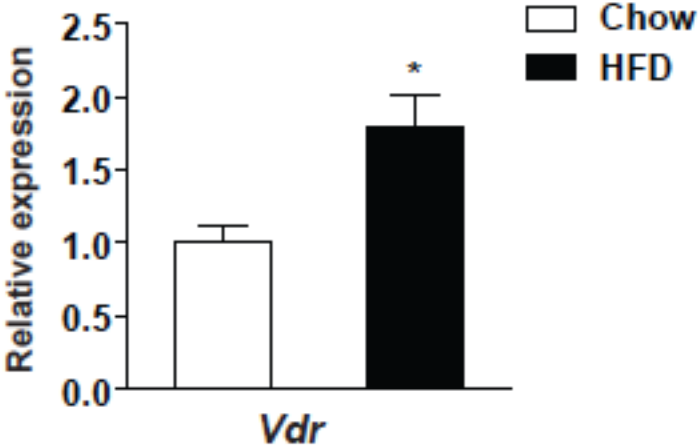
Dex and TNF increase *Vdr* binding to predicted motifs



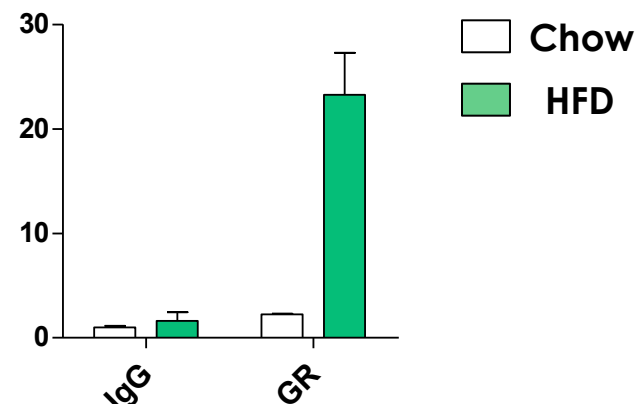
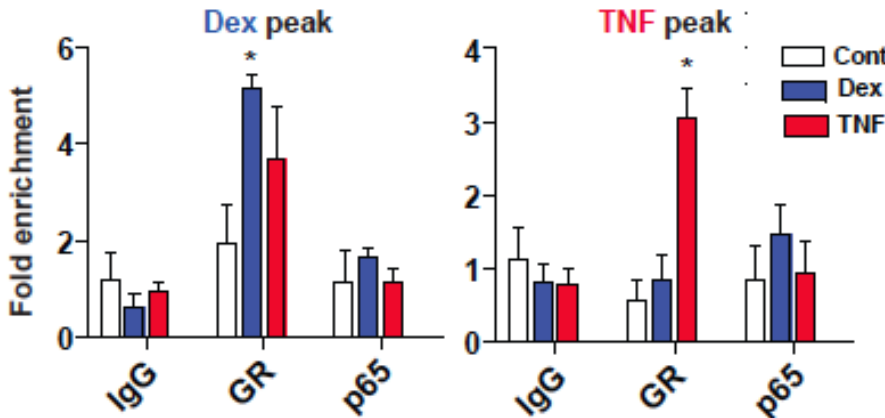
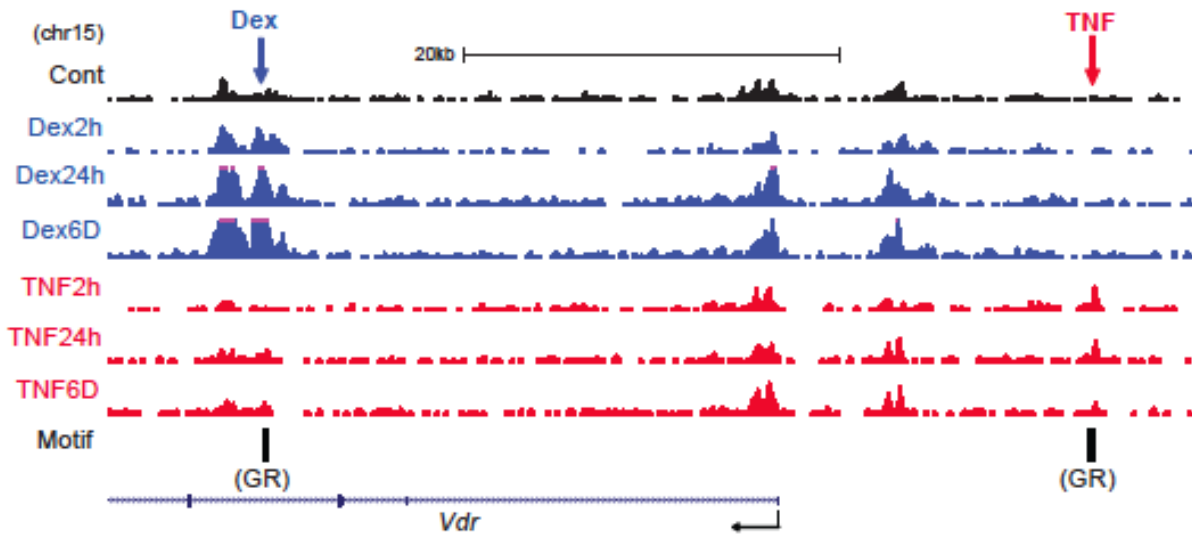
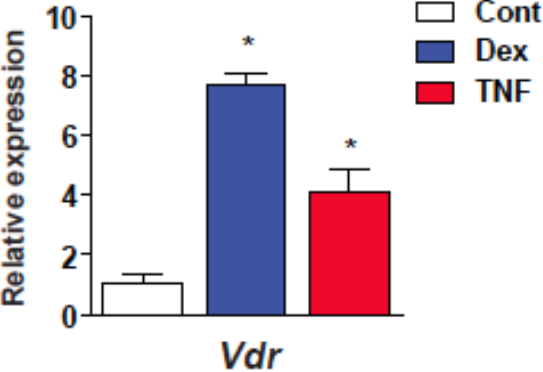
VDR causes insulin resistance



Vdr expression is elevated in obesity

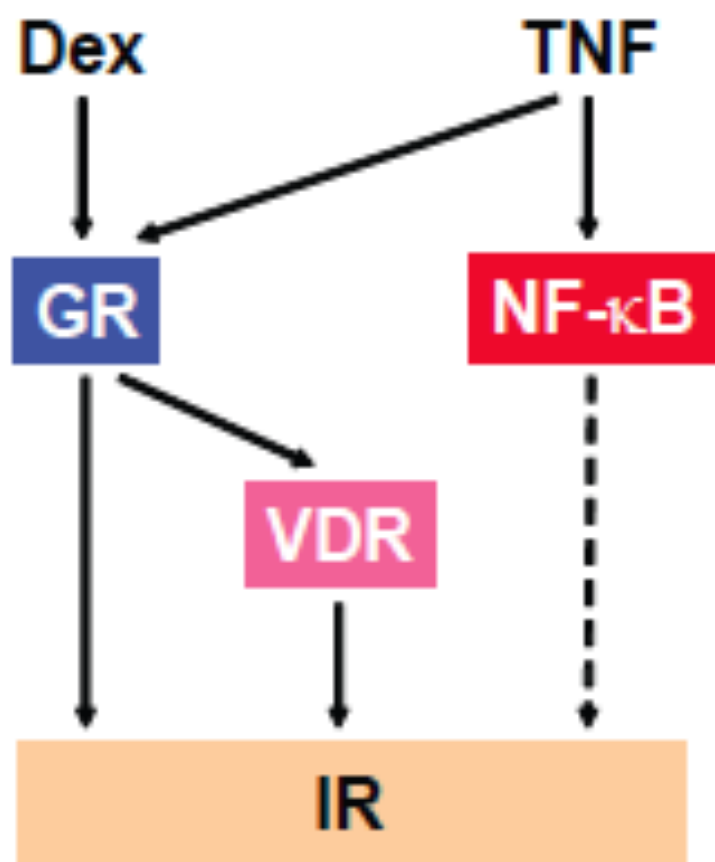


Dex and TNF increase *Vdr* expression



L1

Primary

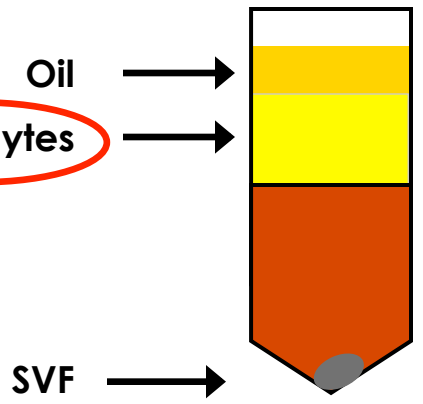


What about humans?

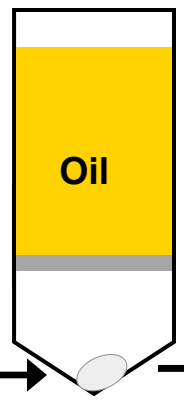


Collagenase
Low-speed centrifugation

Oil
Adipocytes



RNA for
RNA-seq

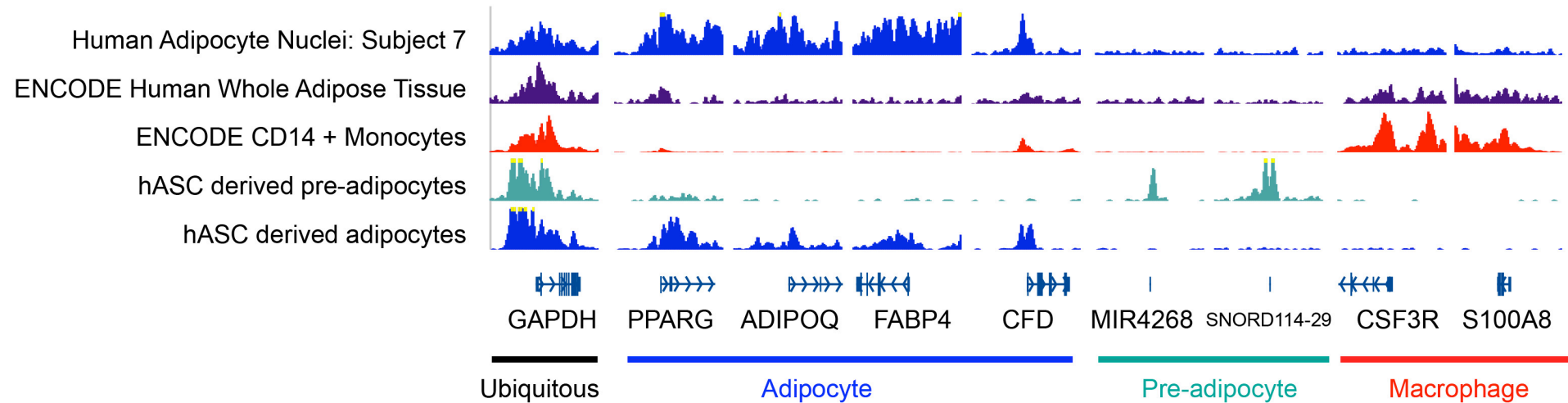


DNA for
Methylation

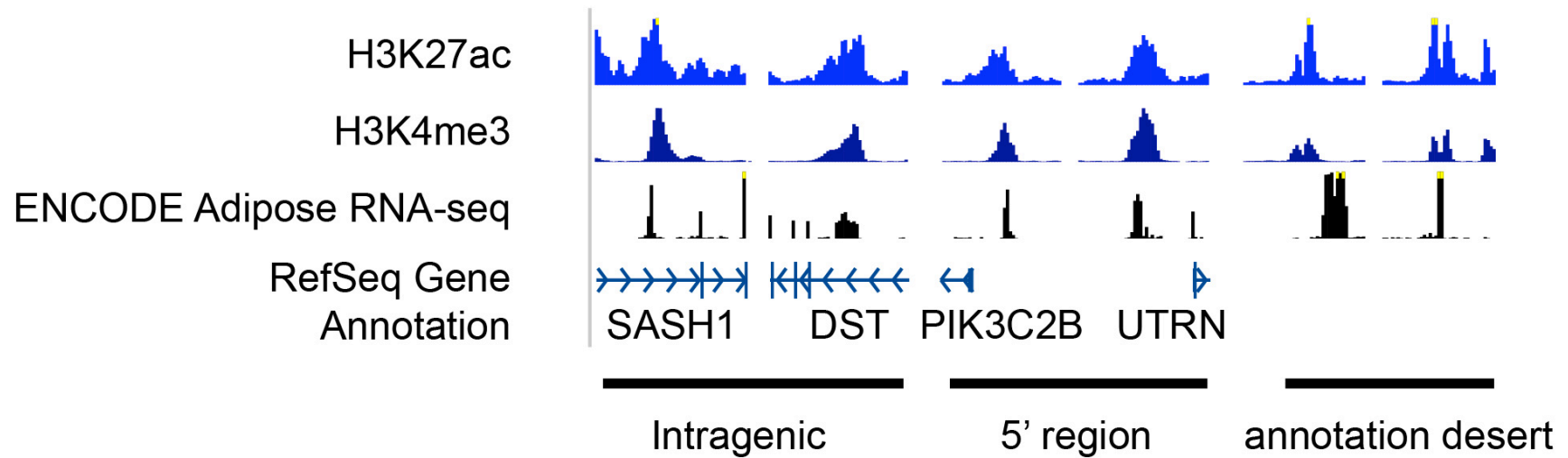
Crosslinked
nuclei for
ChIP-seq

- Also:
- Serum
 - Buffy Coat Layer
 - SVF pellets
 - Whole Fat

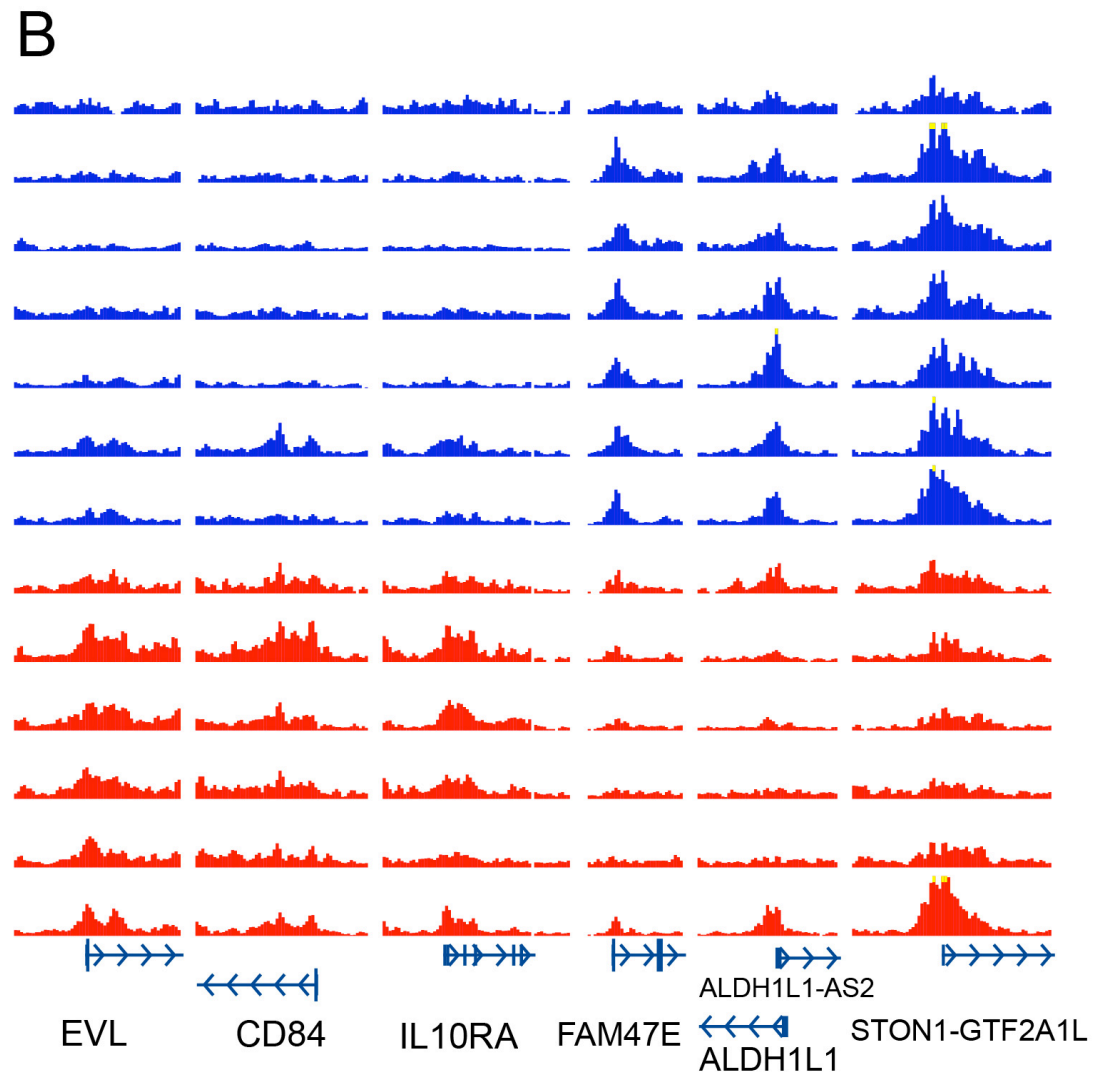
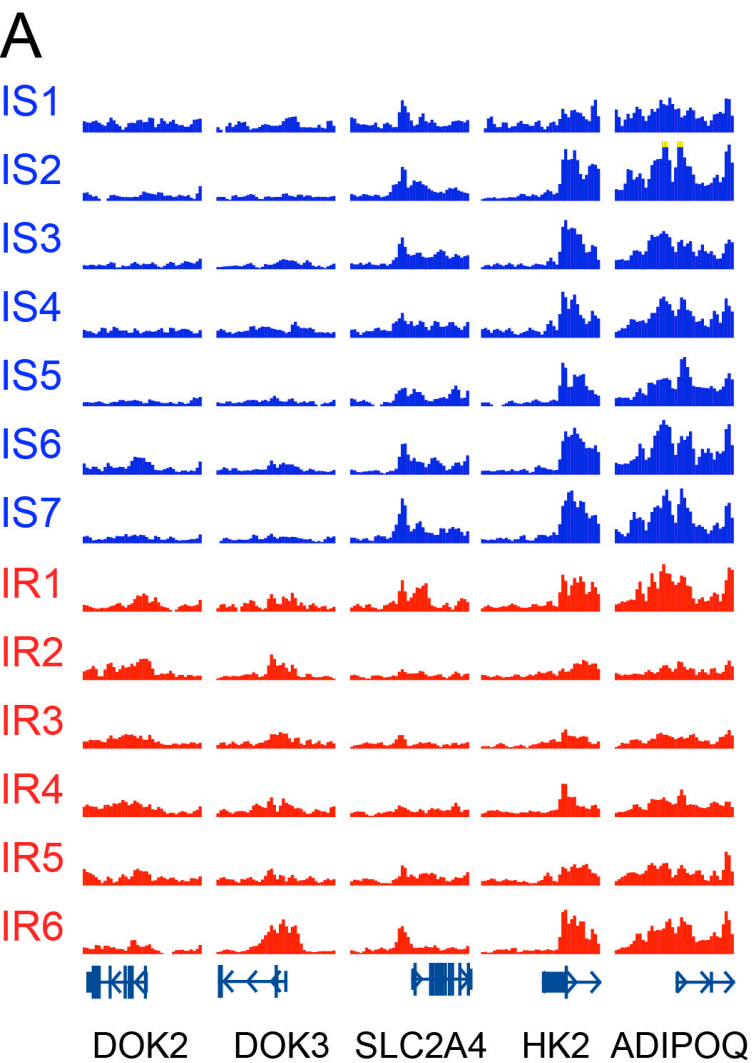
Our isolated adipocytes yield excellent ChIP-seq profiles free from evidence of stroma or immune cells



Histone profiles suggest the presence of novel transcripts and alternative promoters in human adipocytes



We can identify *cis*-elements that differ between IR and IS subjects



Summary

1. Dex and TNF causes discrete changes in epigenome of L1 cells that associate with IR.
2. Motif finding in differentially regulated regions can identify novel pathways leading to IR.
3. TNF causes IR, in part, through ligand-independent activation of the GR.
4. The VDR is a GR target that further induces downstream IR genes.
5. *Tmem176a*, *Colq*, *Lcn2* and *Serpina3n* are part of an IR-inducing gene network downstream of GR and VDR.
6. Human studies are underway to confirm and extend these results.

Acknowledgements

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Chad Cowan

Ray Camahort

Penn/Princeton

Adam Evertts

Ben Garcia

Broad Institute

Tarjei Mikkelsen

Chuck Epstein

Noam Shores

Robbyn Issner

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