Supplementary Figures and Tables

Identification and characterization of DNA sequences that prevent glucocorticoid receptor binding to nearby response elements

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Supplemental figures:

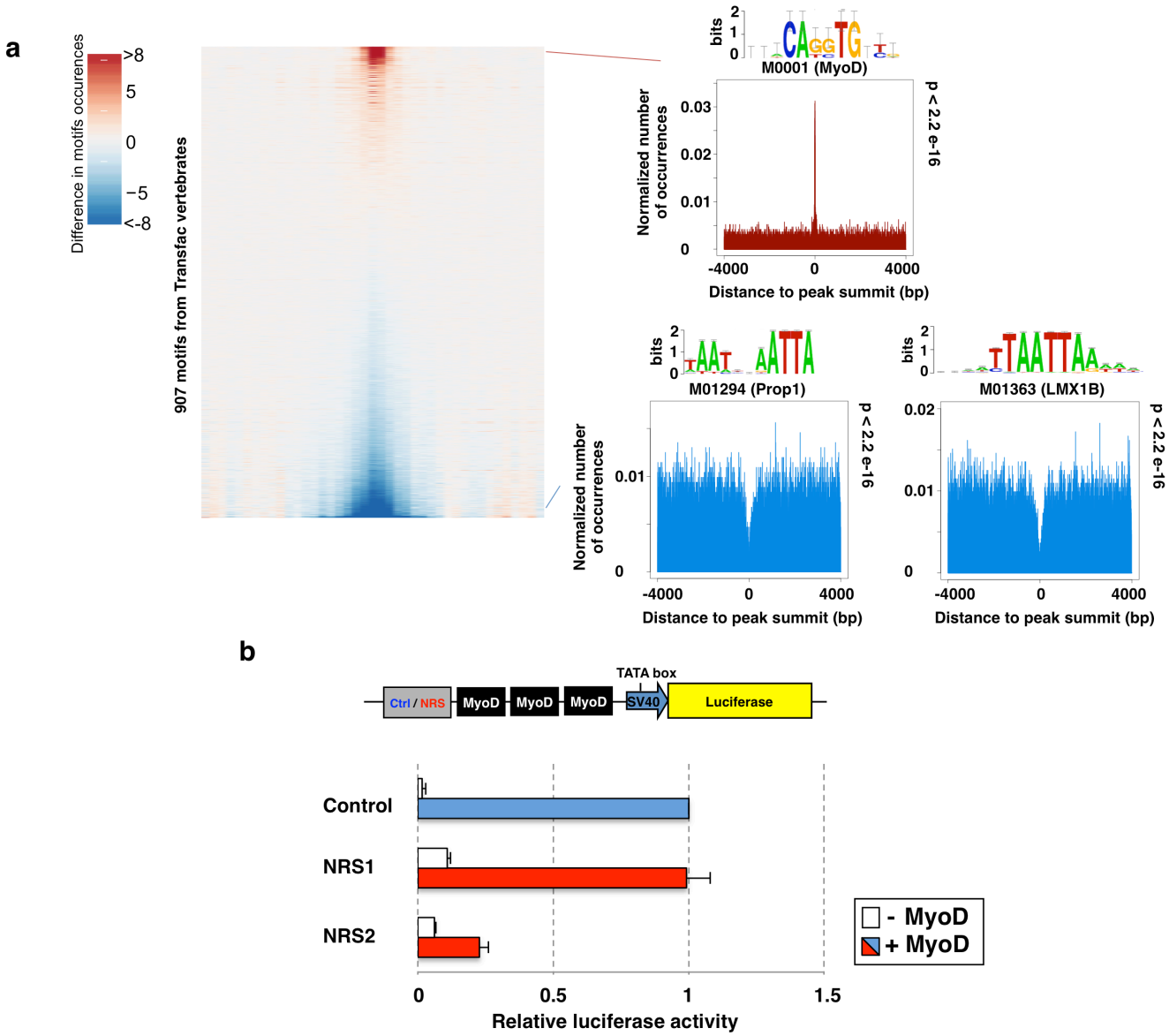

Figure S1. Motif occurrence and functional analysis of depleted sequences around MyoD-bound regions. (a) Occurrence of motif around MyoD-bound regions was analyzed as described for Fig. 1a. (b) Cells were transfected with luciferase reporter constructs with 3 MyoD binding sites, flanked by a single NRS or control sequence as indicated. Along with reporter, U2OS cells were transfected with either an empty, or a MyoD-encoding expression construct. Reporter activity, normalized to control reporter + MyoD, is shown ±SEM (n=3).

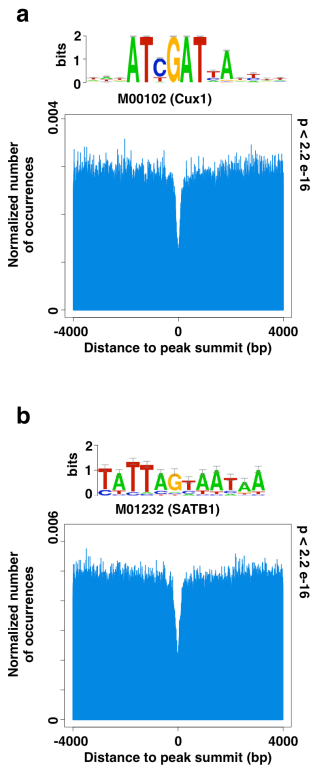

Figure S2. Depletion of NRS sequences identified in previous studies. DNA sequences from GR ChIP-Seq peaks in U2OS cells stably expressing GR were aligned at the peak summit and flanking genomic DNA +/− 4000 bp was sub-divided into 50 bp bins. For each bin, the relative frequency distribution of sequence motifs for (a) Cux1 (M00102) and (b) SATB1 (M01232) was determined by scanning for alignment to these motifs. The normalized number of occurrences for each motif per bin is shown.

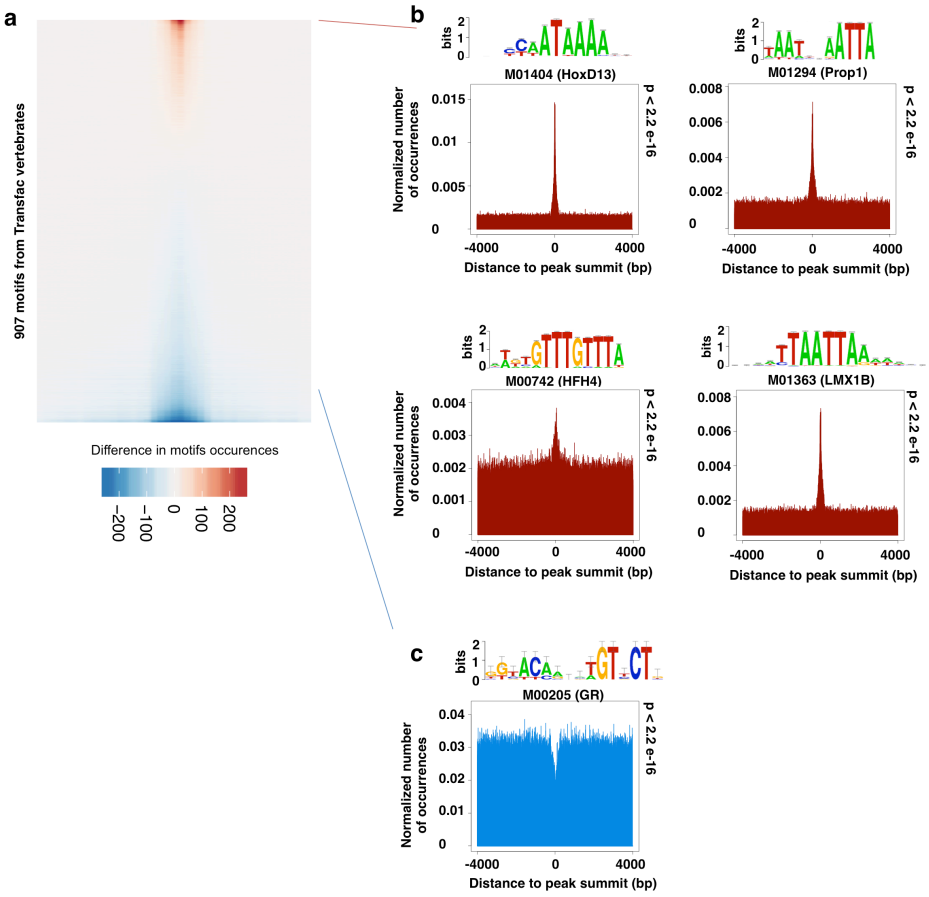

Figure S3. Analysis of motif occurrence around HoxD13-bound regions. (a) Occurrence of motif around HoxD13-bound regions was analyzed as described for Fig. 1a. Examples of motifs that are either (b) enriched, or (c) depleted around the peak summit of HoxD13-bound regions.

Table S1: Oligos used for cloning.

Transient NRS-GBS reporters (Fig. 2a, 2b, 2c)

GBS (FKBP5):
CCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCT

Control:
GTACGCAAGCCTACCTCG
CTAGCGAGGTAGGCTTGC

Control #2:
GTACGAGGTTTGTTTGCG
CTAGCGCAAACAAACCTC

NRS1:
GTACGAGGTTAATTAACG
CTAGCGTTAATTAACCTC

NRS2:
GTACGTTAATTCAATTAA
CTAGTTAATTGAATTAAC

Control +5:
GTACGCGAGGTAGGCTTGGCTGA
CTAGTCAGCCAAGCCTACCTCGC

Control #2 +5:
GTACGAGAGGTTTGTTTGGCTGA
CTAGTCAGCCAAACAAACCTCTC

NRS1 +5:
GTACGAGAGGTTAATTAAGCTGA
CTAGTCAGCTTAATTAACCTCTC

NRS2 +5:
GTACGTTAATTCAATTAAGCTGA
CTAGTCAGCTTAATTGAATTAAC

AAAATT:
GTACGAGGAAAAATTGCG
CTAGCGCAATTTTTCCTC

AAAAAAA:
GTACGAGGTTTTTTTGCG
CTAGCGCAAAAAAACCTC

AAAAAAAA:
GTACGAGGAAAAAAAACG
CTAGCGTTTTTTTTCCTC

AAAATTTT:
GTACGAGGAAAATTTTCG
CTAGCGAAAATTTTCCTC

TTTTTAA:
GTACGAGGTTTTTAAGCG
CTAGCGCTTAAAAACCTC

ATTTTTA:
GTACGAGGATTTTTAGCG
CTAGCGCTAAAAATCCTC

TATATATA:
GTACGAGGTATATATACG
CTAGCGTATATATACCTC

TTTTAAAA:
GTACGAGGTTTTAAAACG
CTAGCGTTTTAAAACCTC

Control +10:
GTACAGAGGTAGGCTTGGAGCTGCTGACTAGCCCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCTCCGGGCTAGTCAGCAGCTCCAAGCCTACCTCT

NRS2 +10:
GTACTTAATTCAATTAAGAGCTGCTGACTAGCCCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCTCCGGGCTAGTCAGCAGCTCTTAATTGAATTAA

Control +20:
GTACAGAGGTAGGCTTGCAGTTGGCGAGAGCTGCTGACTAGCCCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCTCCGGGCTAGTCAGCAGCTCTCGCCAACTGCAAGCCTACCTCT

NRS2 +20:
GTACTTAATTCAATTAACAGTTGGCGAGAGCTGCTGACTAGCCCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCTCCGGGCTAGTCAGCAGCTCTCGCCAACTGTTAATTGAATTAA

Integrated NRS-GBS reporters (Fig. 3b)

GBS (FKBP5):
CCGGAGAACAGGGTGTTCT
GATCAGAACACCCTGTTCT

Control:
GTACGAGGTAGGCTTG
CTAGCAAGCCTACCTC

Control #2:
GTACGAGGTTTGTTTG
CTAGCAAACAAACCTC

NRS1:
GTACGAGGTTAATTAA
CTAGTTAATTAACCTC

NRS2:
GTATTAATTCAATTAA
CTAGTTAATTGAATTAA

JT163: CCAGGTCTCAGTACCGTGCCAGAACATTTCTCTATCGATA
JT164: CCAGGTCTCATCGACGGATCCTTATCGATTTTACC

6 GBS-NRS-TagRFP reporters (Fig. 4a)

6 GBS (PacI-cons-fkbp5-tat-fkbp5-cons-tat-AscI):
ggaattaattaaAGAACAaaaTGTACCAGAACAgggTGTTCTAGAACAtcccTGTACAAGAACAgggTGT
TCTAGAACAaaaTGTACCAGAACAtcccTGTACAggcgcgccttcc

Control:
CGCGCAAGCCTACCTCGGCCAAGCCTACCTCG
CGCGCGAGGTAGGCTTGGCCGAGGTAGGCTTG

Control #2:
CGCGCAAACAAACCTCGGCCAAACAAACCTCG
CGCGCGAGGTTTGTTTGGCCGAGGTTTGTTTG

NRS1:
CGCGTTAATTAACCTCGGCTTAATTAACCTCG
CGCGCGAGGTTAATTAAGCCGAGGTTAATTAA

NRS2:
CGCGTTAATTGAATTAAGCTTAATTGAATTAA
CGCGTTAATTCAATTAAGCTTAATTCAATTAA

NRS-3MyoD constructs (Fig. 7)

Control:
CTAGCGAGGTAGGCTTGGGGCAGCTGCTGAAGGGCAGCTGCTGAAGGGCAGCTGCTGTGCA
CAGCAGCTGCCCTTCAGCAGCTGCCCTTCAGCAGCTGCCCCAAGCCTACCTCG

Control #2:
CTAGAGAGGTTTGTTTGGGGCAGCTGCTGAAGGGCAGCTGCTGAAGGGCAGCTGCTGTGCA
CAGCAGCTGCCCTTCAGCAGCTGCCCTTCAGCAGCTGCCCCAAACAAACCTCT

NRS1:
CTAGAGAGGTTAATTAAGGGCAGCTGCTGAAGGGCAGCTGCTGAAGGGCAGCTGCTGTGCA
CAGCAGCTGCCCTTCAGCAGCTGCCCTTCAGCAGCTGCCCTTAATTAACCTCT

NRS2:
CTAGTTAATTCAATTAAGGGCAGCTGCTGAAGGGCAGCTGCTGAAGGGCAGCTGCTGTGCA
CAGCAGCTGCCCTTCAGCAGCTGCCCTTCAGCAGCTGCCCTTAATTGAATTAA

Table S2: Primers used for qPCR analysis.

Gene/Locus:	**Fw primer:**	**Rev. Primer:**
h*FKBP5*	GCATGGTTTAGGGGTTCTTG	TAACCACATCAAGCGAGCTG
h*RPL19*	ATGTATCACAGCCTGTACCTG	TTCTTGGTCTCTTCCTCCTTG
Integr. GBS	GCAGATCGCAGATCAGAACA	TATGGTACCGTGCCAGAACA
h*IGFBP1*	ACGTCCTGGATACAGTATGTGC	TCATGTTCTTAGGGGGCAAC
h*GAPDH*-TSS	AAAAGCGGGGAGAAAGTAGG	GGTCTTGAGGCCTGAGCTAC
h*GAPDH* +1nuc	CCCCGGTTTCTATAAATTGAGC	AAAGAAGATGCGGCTGACTG
h*NONO*	ACAGCAGGAAGGATTCAAGG	GCATGGCACCTCTGTTGTT
h*SFPQ*	GAGGAGAAGATCTCGGACTCG	CGACATCGCTGTGTGTAAGTTT
ECFP	ACGTAAACGGCCACAAGTTC	GCAGATGAACTTCAGGGTCAG
z*FKBP5*	CAAAAGGGGGAATGCTGTT	TTCTTTTCTGCCCTCTTTGC
TagRFP	GCTGGGAGGCCAACACCGAG	CAGGGCCATGTCGCTTCTGC

h: human
z: zebrafish

Table S3: Identification of NRS-associated proteins by affinity purification and subsequent mass spectrometry analysis. Shown are only proteins with a ratios >2 between NRS experiments versus controls and identified in at least two out of 3 experiments for one or both of the NRS sequences. Amount of unique peptides, sequence coverage in % and a posterior error probability of the identifications are shown.

Protein name:	Gene:	NRS1/control ratio >2	NRS2/control ratio >2	Unique peptides	Sequence coverage [%]	PEP
Splicing factor, proline- and glutamine-rich	SFPQ	3	2	29	43.6	0
Non-POU domain-containing octamer-binding protein	NONO	3	3	20	49.3	0
DNA-3-methyladenine glycosylase	MPG	2	3	14	63.3	1.55E-139
RNA-binding protein 14	RBM14	2	2	18	29.1	5.07E-80
Heterogeneous nuclear ribonucleoprotein D-like	HNRPDL	2	2	7	39.3	1.01E-92
60S ribosomal protein L4	RPL4	2	1	12	34.9	2.78E-75
ATP-dependent RNA helicase A	DHX9	2	1	28	28.4	6.89E-118
Nucleolin	NCL	2	0	21	30.8	7.79E-193
60S ribosomal protein L31	RPL31	2	0	3	26.4	5.97E-31
THO complex subunit 4	ALYREF	1	2	2	48.6	0
PC4 and SFRS1-interacting protein	PSIP1	1	2	13	24.5	5.95E-112