Supporting Information

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SI Methods

Plasmid Construction. The pJG4–5-GRIP1 NID-RD bait, pCDNA6(B)-GRIP1 NID, 2-RD, 3-RD, 3-RDmt and pCDNA6(A)-GRIP1 RD were described previously (1–3). pJG4–5 GRIP1-NID was constructed by excising NID from the pSG424-GRIP1 NID-RD (2) with EcoRI-XhoI and subcloning it into the EcoRI-XhoI sites of pJG4–5. pGex4T1-Suv4-20h1.1 795–885 (795C) was created by excising the 795C cDNA isolate from pEG202 with EcoRI-XhoI and subcloning it into the EcoRI-XhoI sites of pGex4T1.

Full-length Suv4-20h1.1 was reverse-transcribed from LNCaP cell total mRNA using the 3′UTR-specific primer 5′GGCTTTACCTCTAAC, PCR-amplified (5′ATGAAGTGGTTGGGAGAATC and 5′CCTCTAACTCAGAATTGCAC) and introduced into the pCR-Blunt II-TOPO vector using Zero Blunt-TOPO kit (Invitrogen). The insert was excised with BamHI-XhoI and introduced into pCDNA3 and pCDNA6 vectors for mammalian expression and into a bacterial vector pET30a (Novagene). Full-length Suv4-20h1.1 was then excised from pET30a with BglII-XhoI and subcloned into the BglII-SalI sites of pCMV2FLAG (Sigma) for mammalian expression. The Suv4-20h1.1 C-terminal deletion mutant N409 was created by excising the HindIII-EcoRI fragment from pCDNA3-Suv4–20h1 and subcloning it into the HindIII-EcoRI sites of pCMV2FLAG. Point mutations in the SET domain were created using the QuikChange (Stratagene) protocol and the following primers (mutated bases italicized): 5′gGGTCCTGCTGCGTTTATAAAC*ATC*GATTGCAGACCTAATTGTaag and 5′CTTACAATTAGGTCTGCAATC*GAT*GTTTATAAACGCAGCAGGACCC (for H264I); and 5′gaGA-CATTGAACCTGGAGAAG*CG*ATTTCTTGTTATTATGGAGatg and 5′CATCTCCATAATAACAAGAAAT*CG*CTTCTCCAGGTTCAATGTCTC (for E293A). pSG424-GRIP1 expressing Gal4-DBD-fused full-length GRIP1 was a gift of M. Stallcup (USC). pCMV2FLAG-Suv4-20h1.1 ΔSET (lacking amino acids 183–271) was created by excising PciI-PsiI fragment, filling ends with Klenow fragment and religating. pSG424-GRIP1ΔRD was created by cloning BspEI-BsrGI fragment from pCDNA3-GRIP1ΔRD (lacking amino acids 765-1007) into pSG424-GRIP1. pBIND-GRIP1 NID and 3RD were generated by excising the GRIP1 fragments from pCDNA6(B) with BamHI-XbaI and subcloning them into the BamHI-XbaI sites of pBIND (Promega) in-frame with Gal4 DBD. VP16-Suv4-20h1.1 418–885 (418C) was generated by excising the 418C fragment from pCDNA3-Suv4-20h1.1 with EcoRI, subcloning it into the EcoRI site of pGex4T1, reexcising it with BamHI-NotI and subcloning it into the BamHI-NotI sites of pAct (Promega) in-frame with VP16 AD. The fragments encoding Suv.505–828 and Suv.505–795 were PCR-amplified with primers incorporating the BamHI site (5′TCGGGATCCATGGCCAGCGGGTGCTTGACTAGACAC and 5′CTGGGATCCATCATCTGTACTTTCTTCCTC; and 5′TCGGGATCCATGGCCAGCGGGTGCTTGACTAGACAC and 5′CAGGGATCCAAGCCCCTCTGTATAAGACCC, respectively) and subcloned into the BamHI site of pAct. VP16–795C was created by subcloning the EcoRI-XhoI fragment from pGex4T1-Suv4-20h1.1 795C into pAct. MMTV-LTR-Luc and β-actin-LacZ mammalian reporters were as described in ref. 13. pEYFP-Suv4-20h1.1 was created by subcloning the BamHI-XhoI fragment from pCDNA3-Suv4-20h1.1 into the BglII-SalI sites pEYFP-C1 (Clontech).

RNA Isolation and Real-Time PCR. Total RNA isolation, cDNA synthesis and qPCR were as described in ref. 23. Primer pairs for GILZ, IGFBP1, Msx2, SGK, I6PK, Rpl19, Mt2A, VMDL2 and VIPRI were as in (23, 38). Other primer pairs used: actin (5′TTGTTACAGGAAGTCCCTTGCC and 5′ATGCTATCACCTCCCCTGTGTG), Dusp1 (5′CTGAGGTTTTGCCCGGTAC and 5′CCAGGCAAGTCTTTGTTTCC), Fra1 (5′CATCGCAAGAGTAGCAGCAG and 5′AGGAGACATTGGCTAGGGTG), JunB (5′ AACATGGAAGACCAAGAGCG and 5′TCTTCACCTTGTCCTCCAGG), PacI(5′ ATACCTCATGCAGAGTCGCC and 5′ACAGCACCTGGGTCTCAAAC), EphA1 (5′CTTCACACCTTTCACCCTGG and 5′CAGTCACCTTCAACCTCAAGC), IL8 (5′ATGACTTCCAAGCTGGCCGTGGCT and 5′GCGCAGTGTGGTCCACTCTCAATC), cMyc (5′TGAACCAGAGTTTCATCTGCG and 5′TTCTCTGAGACGAGCTTGGC) and Suv4.20h1.1 (C: 5′TACCAGATTTGATGGGTCCC and 5′TCACAACTGAACAACCGACG; or N: 5′GAAGGACACCCTGAAGGCTGGC and 5′CCTTGGCGGACATTCCAGAGGATG).

Cell Imaging. U2OS cells cultured on glass coverslips for 24 h and transfected with pEYFP-C1 or pEYFP-Suv4-20h1.1. Cells were fixed in 2% formaldehyde, permeabilized, mounted in Vectashield mounting media and imaged using Olympus BX51 fluorescent microscope.

Immunoblotting. Protein extracts were prepared as described in ref. 3 except RIPA buffer (1) contained either 150 or 400 mM NaCl, as shown in Fig. 2*A* and *C*. Total cell lysates were prepared by boiling 100 μL of cell suspension in equal amount of 2xSDS sample buffer. Immunoblotting was performed with mouse monoclonal antibodies to GRIP1 (BD Transduction Labs) and FLAG (Sigma) or rabbit polyclonal antibodies to Suv4–20h1 (Genway), GR (N499 (1)) and ERK (Santa Cruz Biotechnology). Blots were developed using horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies (Promega) and the enhanced chemiluminescence substrate (GE Healthcare).

1. Rogatsky I, Zarember KA, Yamamoto KR (2001) Factor recruitment and TIF2/GRIP1 corepressor activity at a collagenase-3 response element that mediates regulation by phorbol esters and hormones. *EMBO J* 20:6071–6083.
2. Rogatsky I, Luecke HF, Leitman DC, Yamamoto KR (2002) Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts. *Proc Natl Acad Sci USA* 99:16701–16706.
3. Reily MM, Pantoja C, Hu X, Chinenov Y, Rogatsky I (2006) The GRIP1:IRF3 interaction as a target for glucocorticoid receptor-mediated immunosuppression. *EMBO J* 25:108–117.

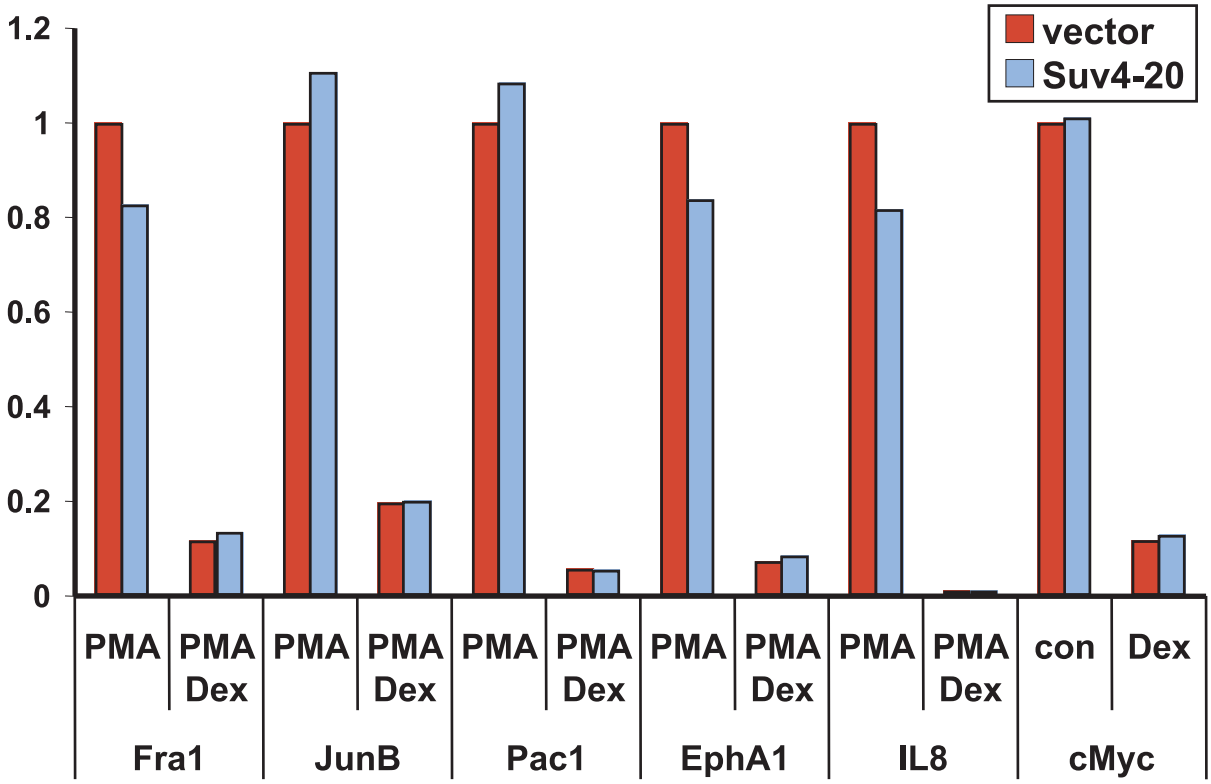

Fig. S1. Overexpression of Suv4-20h1.1 does not affect Dex-dependent downregulation of several glucocorticoid-sensitive genes. U2OS-GR cells were transfected with pCDNA3 vector or pCDNA3-Suv4-20h1.1 (200 ng per 3×10^5 cells) and 18 h later, treated with vehicle or 100 nM Dex for 2 h. For all genes except cMyc, treatments were performed in the presence of 25 ng/ml phorbol ester, PMA, as indicated. Total RNA was isolated, reverse-transcribed and mRNA levels were measured by qPCR using β-actin mRNA as normalization control. Shown is a representative of 3 or more independent experiments done in duplicates.

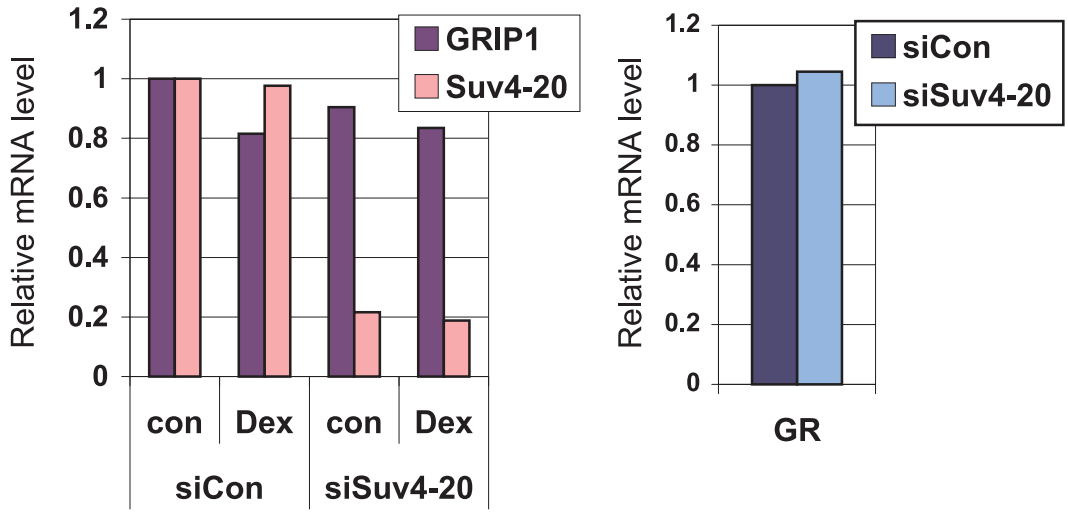

Fig. S2. Suv4-20h1.1 knockdown in U2OS-GR cells does not affect GRIP1 or GR expression. U2OS-GR cells were transfected with siCon or siSuv4–20 using 1 μg of annealed RNA duplex and 8 μL of RNAiFect reagent (Qiagen) per 20,000 cells as per manufacturer's instructions. 24 h later, cells were treated with Dex for 2 h, as indicated, and the expression of Suv4-20h1.1, GRIP1 and GR mRNA was assessed by qPCR using Rpl19 expression as normalization control.

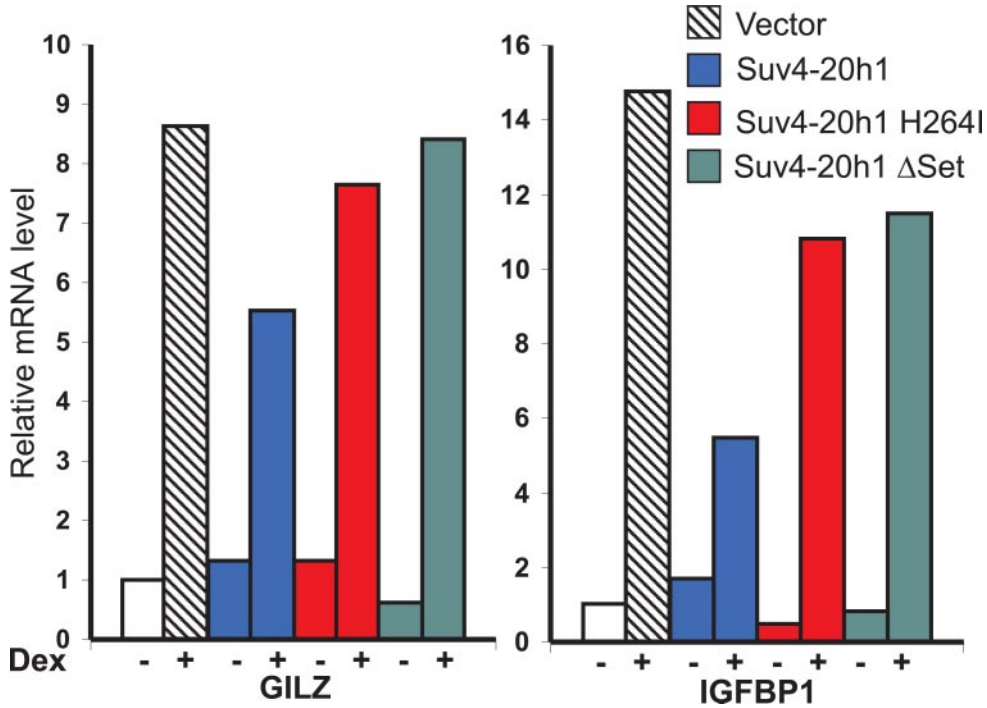

Fig. S3. Suv4-20h1.1 SET domain is required for its ability to affect GR-mediated activation. U2OS-GR cells were transfected with an empty vector, wt Suv4-20h1.1, or its H264I or ΔSET mutant, lacking amino acids 183–271 of the SET domain, as indicated. 20 h later, cells were treated with Dex for 2 h as shown, and the expression of indicated GR target genes were assessed as in Fig. 4C.