A Distinct Mechanism for Coactivator versus Corepressor Function by Histone Methyltransferase G9a in Transcriptional Regulation*

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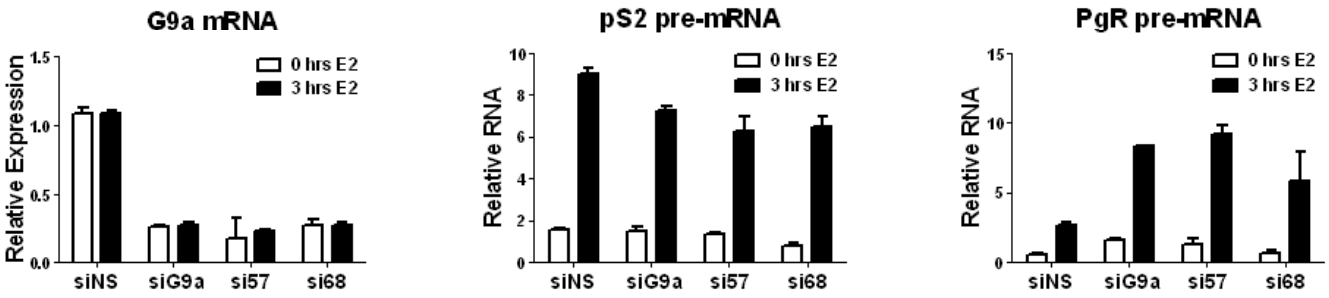
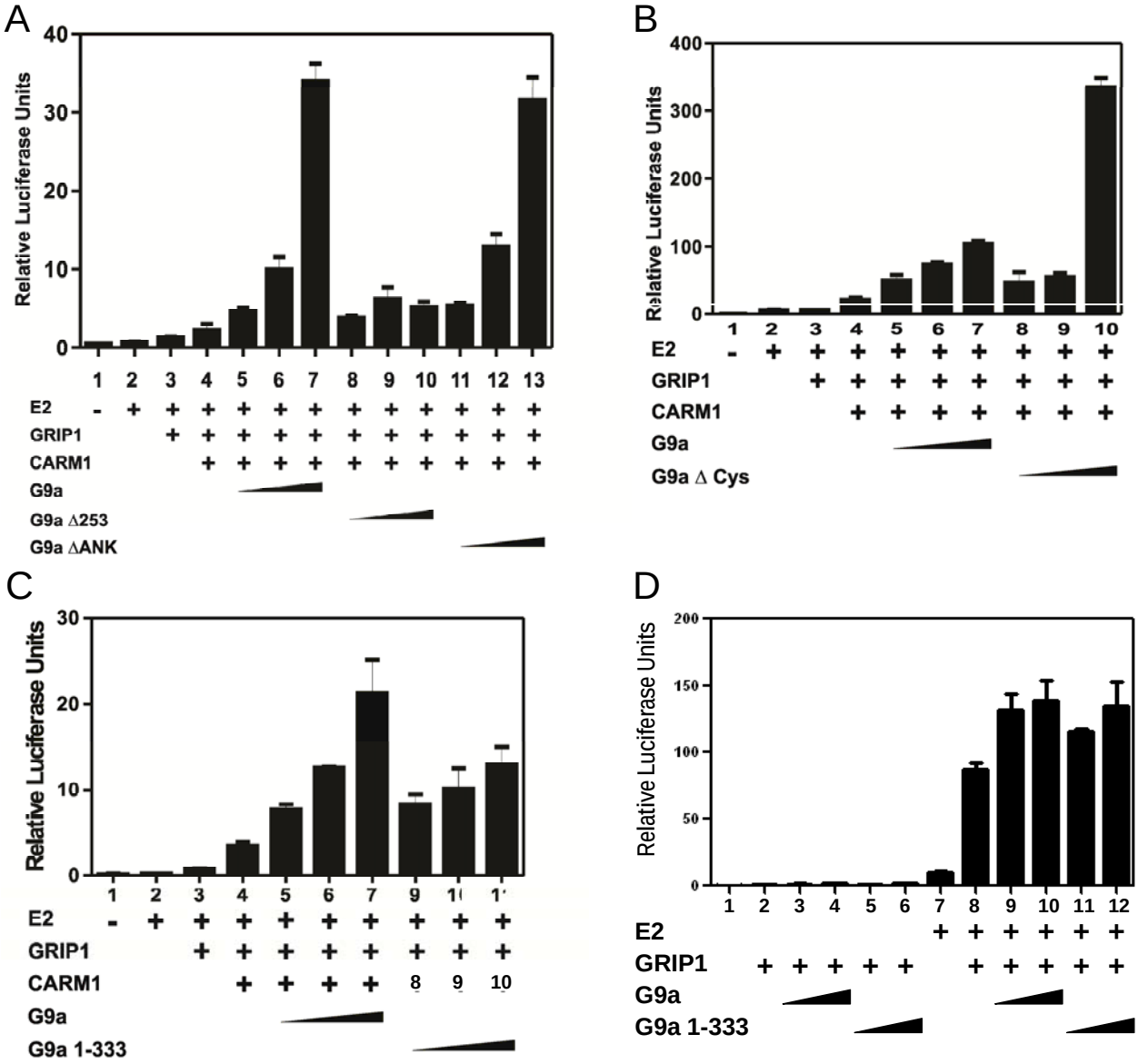
SUPPLEMENTARY INFORMATION

Supplementary Figure 1



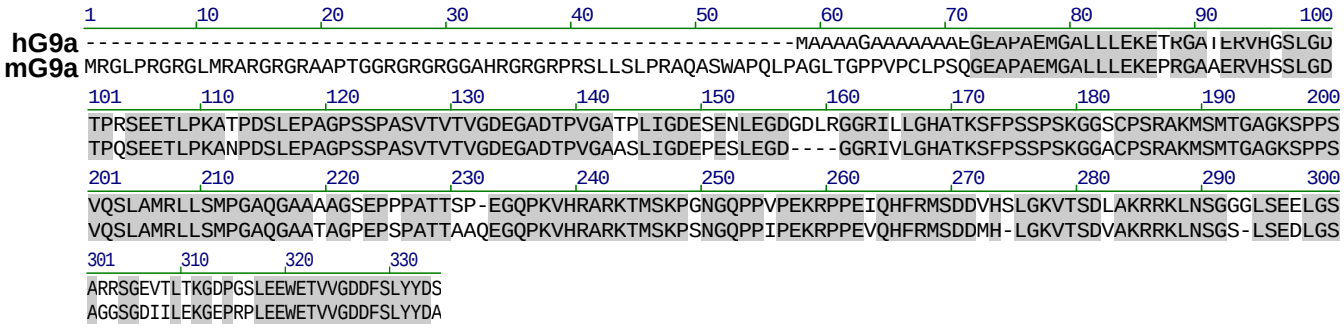
SUPPLEMENTARY FIGURE 1. **G9a affects ERα target gene expression at the level of transcription**. The SMARTpool siRNA against G9a (e.g. Fig. 1) is composed of four siRNAs that target G9a, designated by Dharmacon as #5, #6, #7, and #8. In this experiment MCF-7 cells were transfected with pairs of these siRNAs, either #5 plus #7 (si57) or #6 plus #8 (si68), or with siNS. Transfected cells were cultured for three days in hormone-free medium prior to addition of 100 nM E2 for the indicated time. Total RNA samples were analyzed by qRT-PCR, using primers designed to detect pS2, GREB1, and PgR pre-mRNA, as illustrated in Fig. 2A. Results are presented relative to GAPDH mRNA levels as the mean and SD for three PCR reactions performed with the cDNA samples from a single experiment, and are representative of two independent biological experiments performed on different days.

Supplementary Figure 2



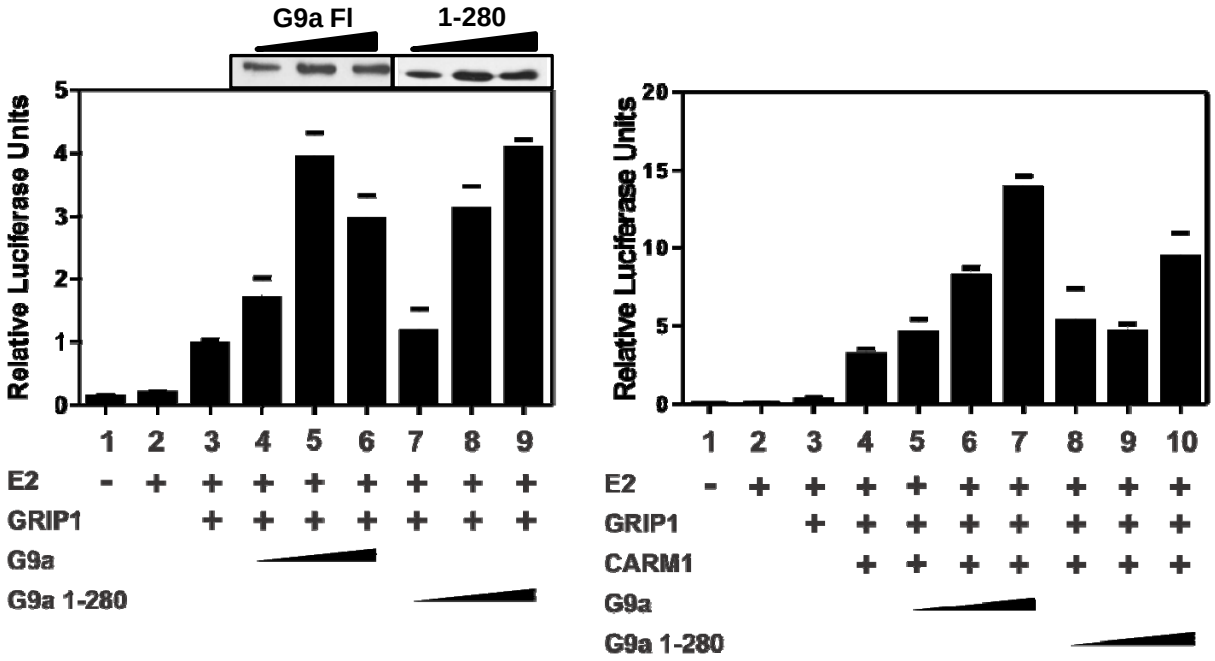
SUPPLEMENTARY FIGURE 2. **Enhancement of E2-dependent reporter gene expression by G9a and G9a protein fragments in cooperation with GRIP1 and CARM1.** *A*-C, CV-1 cells in 12-well dishes were transfected with MMTV(ERE) LUC reporter plasmid (200 ng) and expression vectors encoding ERα (0.02 ng); GRIP1 (100 ng); CARM1 (200 ng); HA-tagged mG9a full length (FL), mG9aΔ252, mG9aΔANK, and mG9aΔCys (100, 200 or 400 ng); and mG9a 1-333 (50, 100, or 200 ng) as indicated. Cells were grown in the presence or absence of E2 for 48 h before measurement of luciferase activity and immunoblot analysis with antibodies against HA. Results shown are mean and SD from triplicate transfected cultures for each condition, and each experiment shown is representative of three or more independent experiments performed on different days. *D*, CV-1 cells in 12-well dishes were transfected with MMTV(ERE) LUC reporter plasmid (200 ng) and expression vectors encoding ERα (2 ng); GRIP1 (50 ng); and mG9a full length (100 or 200 ng) or mG9a 1-333 (50 or 100 ng) as indicated. Cells were grown in the presence or absence of E2 for 48 h before measurement of luciferase activity. Results shown are mean and SD from triplicate transfected cultures for each condition, and each experiment shown is representative of two or more independent experiments performed on different days.

Supplementary figure 3



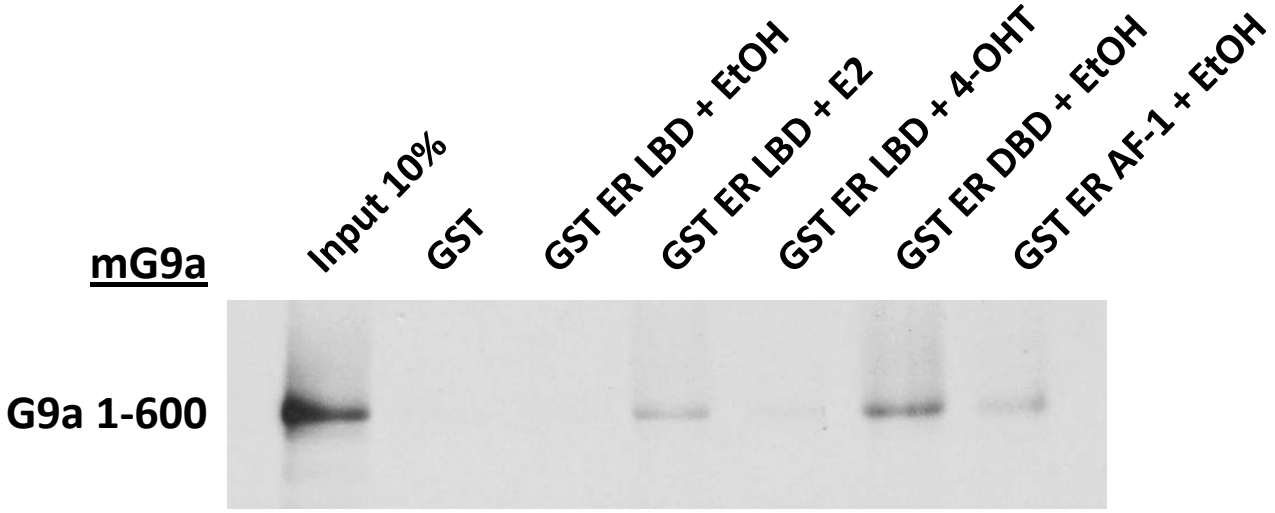
SUPPLEMENTARY FIGURE 3. **Amino acid homology of the N-terminal regions of mouse and human G9a**. The N-terminal mouse and human G9a sequences are aligned, and identical amino acids are indicated in the shaded areas.

Supplementary Figure 4



SUPPLEMENTARY FIGURE 4. **Coactivator activity of the N-terminal region of hG9a**. *A*, CV-1 cells in 12-well dishes were transfected with MMTV(ERE) LUC reporter plasmid (200 ng) and expression vectors encoding ERα (0.2 ng); GRIP1 (100 ng); and HA-tagged hG9a full length (Fl) (50, 100, or 200 ng) or hG9a 1-280 (20, 50, or 100, ng). Cells were grown in the presence or absence of E2 for 48 h before measurement of luciferase activity and immunoblot analysis with antibodies against HA. Results shown are mean and SD from triplicate transfected cultures for each condition, and each experiment shown is representative of two independent experiments performed on different days. *B*, Transfections and luciferase assays were performed as in A, except that plasmid amounts were: ERα (0.02 ng); GRIP1 (100 ng); CARM1 (200 ng); HA-tagged hG9a full length (50, 100, or 200 ng) or hG9a 1-280 (20, 50, or 100 ng).

Supplementary figure 5



SUPPLEMENTARY FIGURE 5. **Binding of G9a to various domains of ERα**. GST pull-down assays were performed as in Fig. 6, using bead-bound GST fusion proteins encoding the indicated regions of ERα (1).

SUPPLEMENTARY TABLE 1. **Primers for PCR**.

qRT-PCR

pS2
5’-GAACAAGGTGATCTGCG-3’ (forward)
5’-TGGTATTAGGATAGAAGCACCA-3’ (reverse)

pS2 pre-mRNA
5’-ACCATGGAGAACAAGGTGATC-3’ (forward)
5’-TAAAACAGTGGCTCCTGGCG-3’ (reverse)

GREB1
5’-CAAAGAATAACCTGTTGGCCCTGC-3’ (forward)
5’-GACATGCCTGCGCTCTCATACTTA-3’ (reverse)

GREB1 pre-mRNA
5’-GATAAAAGCAACGTGCGTCTC-3’ (forward)
5’-TCTTGCACAATTCCATCGAG-3’ (reverse)

CathepsinD
5’-GTACATGATCCCCTGTGAGAAGGT-3’ (forward)
5’-GTCACCGGAGTCCATCACGATG-3’ (reverse)

SGK3
5’-TGAGGCCAGGAGTGAGTCTT-3’ (forward)
5’-TATCATCTGGTCCAGCAACA-3’ (reverse)

c-Myc
5’-CTCTCAACGACAGCAGCTCG-3’ (forward)
5’-CAACATCGATTTCTTCCTCATCTTC-3’ (reverse)

PKIB
5’-CCAATTTTGCATCTTCAGCA-3’ (forward)
5’-GGCTTTTCCAATTGGTCTTG-3’ (reverse)

CyclinD1
5’-AAGCTCAAGTGGAACCT-3’ (forward)
5’-AGGAAGTTGTTGGGGC-3’ (reverse)

CyclinD1 pre-mRNA
5’-CCTACTTCAAATGTGTGCAGAAG-3’ (forward)
5’-CAACAAGTTGCAGGGAAGTC-3’ (reverse)

CXCL12
5’-TCAGCCTGAGCTACAGATGC-3’ (forward)
5’-CTTTAGCTTCGGGTCAATGC-3’ (reverse)

b-actin
5’-ACCCCATCGAGCACGGCATCG-3’ (forward)
5’-GTCACCGGAGTCCATCACGATG-3’ (reverse)

GAPDH
5’-TCTGGTAAAGTGGATATTGTTG-3’ (forward)
5’-GATGGTGATGGGATTTCC-3’ (reverse)

PGR
5’-GTG CCT ATC CTG CCT CTC AAT C-3’ (forward)
5’-CCC GCC GTC GTA ACT TTC G-3’(reverse)

PGR pre-mRNA
5’-CAG GTC TAC CCG CCC TAT CT-3’(forward)
5’-TAT ATT CTG CGC CCA CCT TC-3’(reverse)
G9a
5’-ATGGGTGAAGCCGTCTCGGA-3’ (forward)
5’-ATCTTGGGTGCCTCCATGCG-3’ (reverse)

qPCR
Primers for Q-PCR analysis of ChIP assays were previously described for pS2 (2) and GREB1 (3)

SUPPLEMENTARY REFERENCES

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