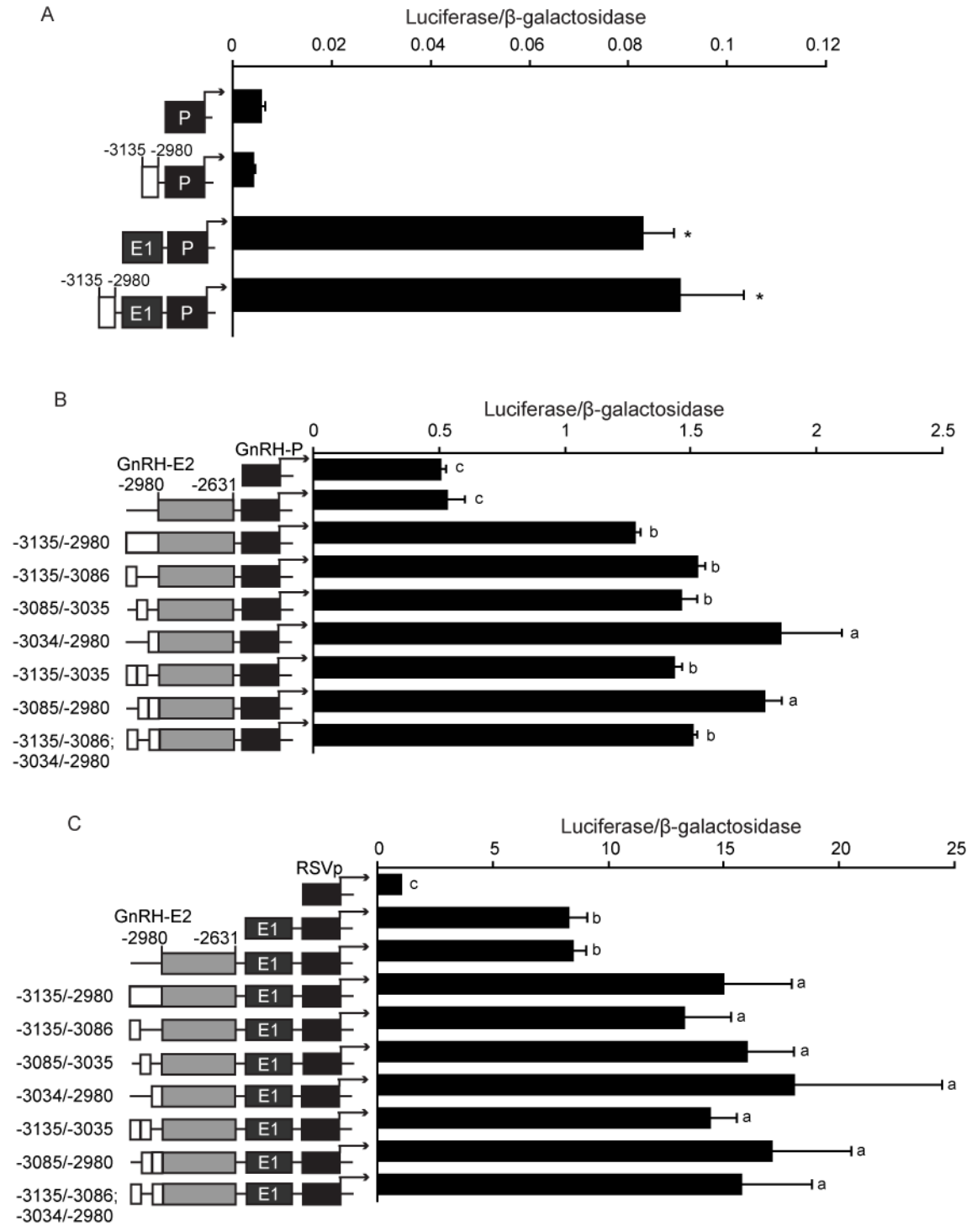
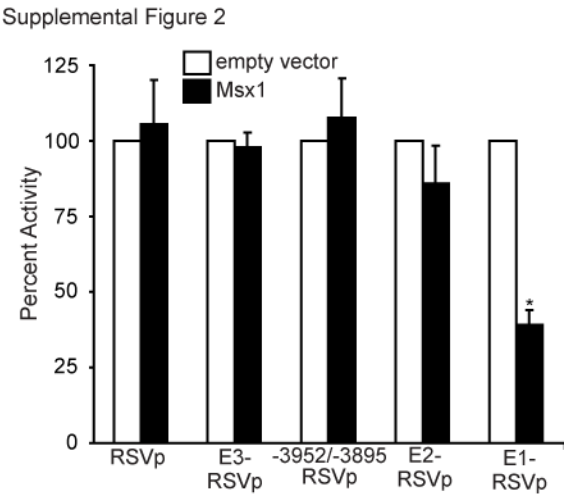
SUPPLEMENTAL MATERIAL

Supplemental Fig. 1. A, Conserved region -3135/-2980 alone does not enhance GnRH expression. Transient transfections were performed in GT1-7 cells using luciferase reporter plasmids containing -3135/-2980 upstream of GnRH-P or GnRH-E1/GnRH-P as indicated in schematic diagrams. Data represent luciferase/β-galactosidase values normalized to RSVe/RSVp-luc and are shown as mean ± SD. *, significantly different from GnRH-P by one-way ANOVA and post-hoc Tukey Kramer HSD ($P<0.05$). B and C, Redundancy within the -3135/-2980 region. B, Transient transfections were performed in GT1-7 cells using luciferase reporter plasmids containing portions of the -3135/-2980 region of GnRH-E2 on GnRH-P. Previously characterized region -2980/-2631 is colored in gray, -3135/-2980 is colored in white. Positions of subregions studied are left of schematic diagrams. Data represent luciferase/β-galactosidase values normalized to empty pGL3 vector and are shown as mean ± SD. Levels not assigned the same letter are significantly different by one-way ANOVA and by post-hoc Tukey-Kramer HSD ($P<0.05$). C, Transient transfections were performed in GT1-7 cells using luciferase reporter plasmids containing portions of the -3135/-2980 region of GnRH-E2 upstream of GnRH-E1/RSVp. Data represent luciferase/β-galactosidase values normalized to RSVp and are shown as mean ± SD. Levels not assigned the same letter are significantly different by one-way ANOVA and by post-hoc Tukey-Kramer HSD ($P<0.05$).

Supplemental Figure 1





Supplemental Fig. 2. Msx1 does not change GnRH-E2 or GnRH-E3 expression. Luciferase reporters with RSVp alone, or with GnRH-E3, the GnRH-E3 critical region (-3952/-3895), GnRH-E2, or GnRH-E1 on RSVp were co-transfected into GT1-7 cells with either empty or Msx1 expression plasmid. Data are presented as percent luciferase/β-galactosidase activity is relative to empty expression vector control, mean ± SD. *, significantly different from empty expression vector control by Student's t test.

Supplemental Table 1: ChIP and EMSA Oligonucleotide Primer Sequences

Name/Region	Location	*	Sequence
ChIP mGnRH-P	Genomic (mouse) -173/+53	F R	CAGCAGGTGTTGCAATTACATTCACCATTAAG CCTGTTTGGATGTGAAAGTCAAAGGGATCTC
ChIP mGnRH-E1	Genomic (mouse) -1814/-1653	F R	GCCAAACACCACAGTCTTCTCTTGAGTGAC CTGGCACAAAGAGCAAAAGAACCTCCTCTC
ChIP mGnRH-E2	Genomic (mouse) -3579/-3427	F R	CTACAGGCTGGTCGGCTTGAGGCAGTGAATC TGCTCTCCTCCCCATGTAAGCCCTTTACTGTG
ChIP mGnRH-E3	Genomic (mouse) -4706/-4533	F R	TTCCCCTCATTGGGACTGTAACAGAAGGAC TTGAACCAAGATGGCACTTCCACACAATGC
ChIP mGnRH-Intron 3	Genomic (mouse) +3623/+3820	F R	CGGTGACTTCAATTTCCACACCCAATGGAC CATTAGCCGCGTAAAGGATGACGCTGTGAG
EMSA** -3927	Genomic (rat) -3927/-3903	F	TGTTCGGGTTTATAAATAGCTTTAG
EMSA -3927 m1	Genomic (rat) -3927/-3903	F	GTGGCGGGTTTATAAATAGCTTTAG
EMSA -3927 m2	Genomic (rat) -3927/-3903	F	TGTTATTTTTTATAAATAGCTTTAG
EMSA -3927 m3	Genomic (rat) -3927/-3903	F	TGTTCGGGCCGCTAAATAGCTTTAG
EMSA -3927 m4	Genomic (rat) -3927/-3903	F	TGTTCGGGTTTAGCCCTAGCTTTAG
EMSA -3927 m5	Genomic (rat) -3927/-3903	F	TGTTCGGGTTTATAAAGCTATTTAG
EMSA -3927 m6	Genomic (rat) -3927/-3903	F	TGTTCGGGTTTATAAATAGCGGGCT
EMSA -3927 mutAT	Genomic (rat) -3927/-3903	F	TGTTCGGGTTGCGCCCGAGCTTTAG
EMSA -2913	Genomic (rat) -2913/-2889	F	TTTTGAAAATGAATTATTTTCTTTG
EMSA -2913 mOct	Genomic (rat) -2913/-2889	F	TTTTGAAACGTCCGGCTTTTCTTTG
EMSA -2913 m4a	Genomic (rat) -2913/-2889	F	TTTTGAAACGTCATTATTTTCTTTG
EMSA -2913 m4b	Genomic (rat) -2913/-2889	F	TTTTGAAAATGACGGCTTTTCTTTG

* F indicates forward primer and R indicates reverse primer

** Only top strand (sense) is shown; mutations are underlined