Antenatal Hypoxia Induces Epigenetic Repression of Glucocorticoid Receptor and Promotes Ischemic-Sensitive Phenotype in the Developing Heart

By

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Short title: Glucocorticoid receptor and heart development.

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Supplementary Table I. Primer sequences.

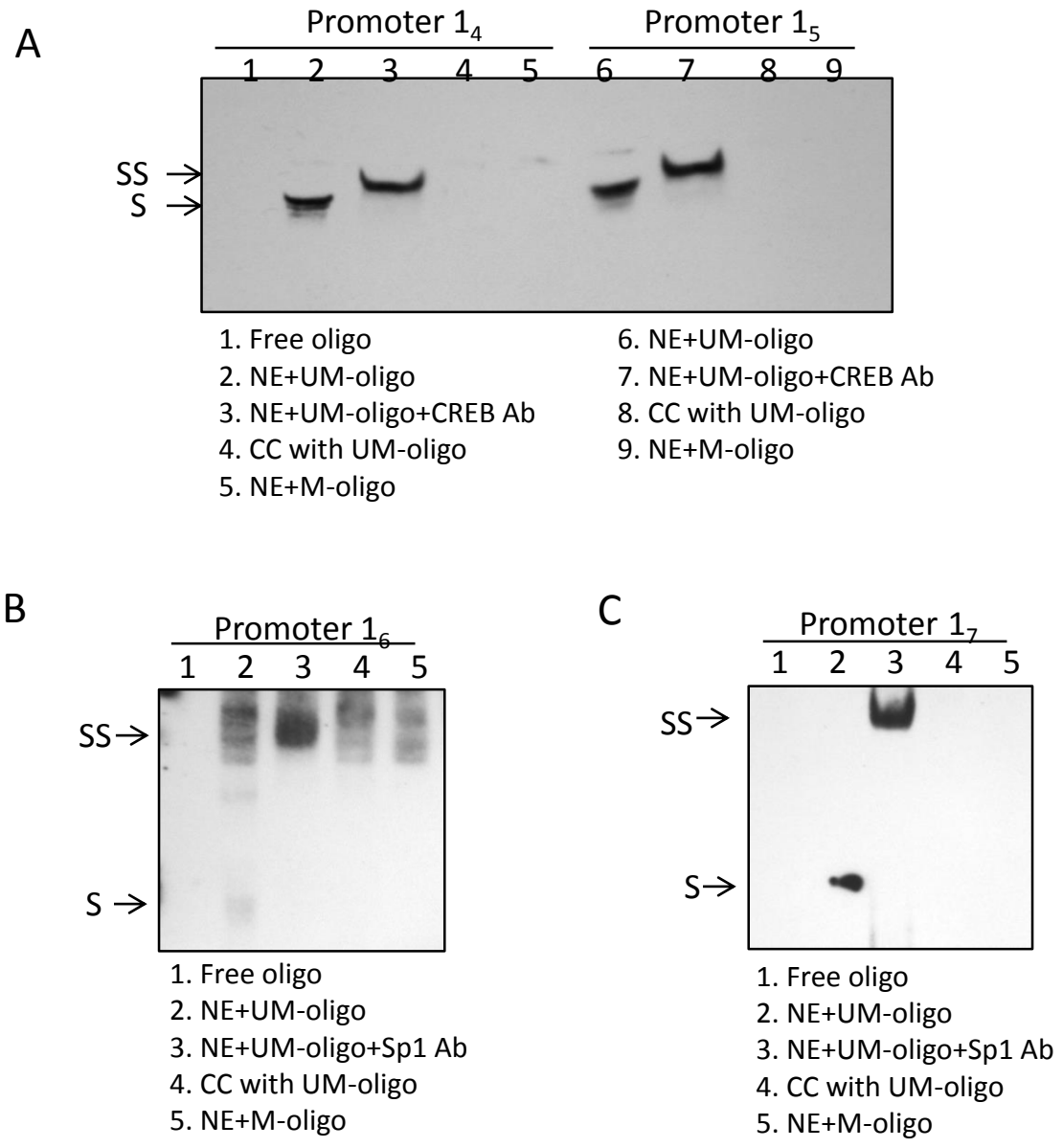
	Forward	Reverse
exon 1_4	AAGCAACACCGTAACACCTT	AGAAGCAGCAGCCACTGA
exon 1_5	CATGCAACTTCCTCCGAGT	
exon 1_7	GGAGCCTGGGAGAAGAGAAA	
exon 1_{11}	GCCGCAGAGAACTCAACAG	
exon 1_{10}	CACGCCGACTTGTTTATC	TCTGCTGCTTGGAATCTG
exon 1_6	ACCTGG CGG CAC GCG AGT	GCAGCCACTGAGGGCGAAGA
exon 1_8	GACAGTCGCCAACAGGTTAA	TGAGAAGCAGCAGCCACT
exon 1_9	GTCAGTGCCTGGAGCCCGAG	AGCAGCCACTGAGGGCGAAG
GR total	AGGTCTGAAGAGCCAAGAGTTA	TGGAAGCAGTAGGTAAGGAGAT
β-Actin	TCAGGTCATCACTATCGGCAAT	ACTGTGTTGGCATAGAGGTCTT
GR-IP-1_4	AAAGAACGACTCGGGTTTGA	CTCTGCCTGACCTCTTGGAG
GR-IP-1_5	ACAGCTGGACGGAGCTAAAA	CCCGAATCTTGACATTTGCT
GR-IP-1_6	GGGTTCTGCTTTGCAACTTC	GAGAGGGTCAGCGCATACAT
GR-IP-1_7	GACACACTTCGCGCAACTC	CACCCAAGGAACGAGAAAAA
EMSA 1_4-CREUM	CGACTCGGGTTTGACGCCAAAGAGCAC	GTGCTCTTTGGCGTCAAACCCGAGTCG
EMSA 1_4-CREM	CGACTCGGGTTTGA$_m$CGCCAAAGAGCAC	GTGCTCTTTGG$_m$CGTCAAACCCGAGTCG
EMSA 1_5-CREUM	GAGCTAAAAGCTGACGTTTTAAAGATG	CATCTTTAAAACGTCAGCTTTTAGCTC
EMSA 1_5-CREM	GAGCTAAAAGCTGA$_m$CGTTTTAAAGATG	CATCTTTAAAA$_m$CGTCAGCTTTTAGCTC
EMSA 1_6-Sp1UM	GATCGGGGCGCGGGGGAGGGTGGGT	ACCCACCCTCCCCCGCGCCCCGATC
EMSA 1_6-Sp1 M	GAT$_m$CGGGG$_m$CG$_m$CGGGGGAGGGTGGGT	ACCCACCCTCCCC$_m$CG$_m$CGCCC$_m$CGATC
EMSA 1_7-Sp1UM	ACCCACGGGGCGGGCTCCCGAGCGG	CCGCTCGGGAGCCCGCCCCGTGGGT
EMSA 1_7-Sp1 M	ACCCA$_m$CGGGG$_m$CGGGCTCC$_m$CGAG$_m$CGG	C$_m$CGCT$_m$CGGGAGCC$_m$CGCCC$_m$CGTGGGT

Supplementary Table II. Pre-ischemic baseline values of left ventricle functional parameters

Animal groups	HR (beat/min)	LVDP (mmHg)	dP/dt_{max} (mmHg/s)	dP/dt_{min} (mmHg/s)	CF (ml/min/g)
N_{-Aza} (n=8)	317±9.5	126.7±5.1	4468±116	2692±86	10.5±0.9
H_{-Aza} (n=9)	320±13.5	125.4±8.8	4432±150	2573±66	9.6±0.7
N_{+Aza} (n=8)	298±11.0	133.2±1.9	4543±183	2681±79	8.8±0.6
H_{+Aza} (n=8)	295±17.6	130.6±4.1	4698±111	2549±58	8.3±0.7

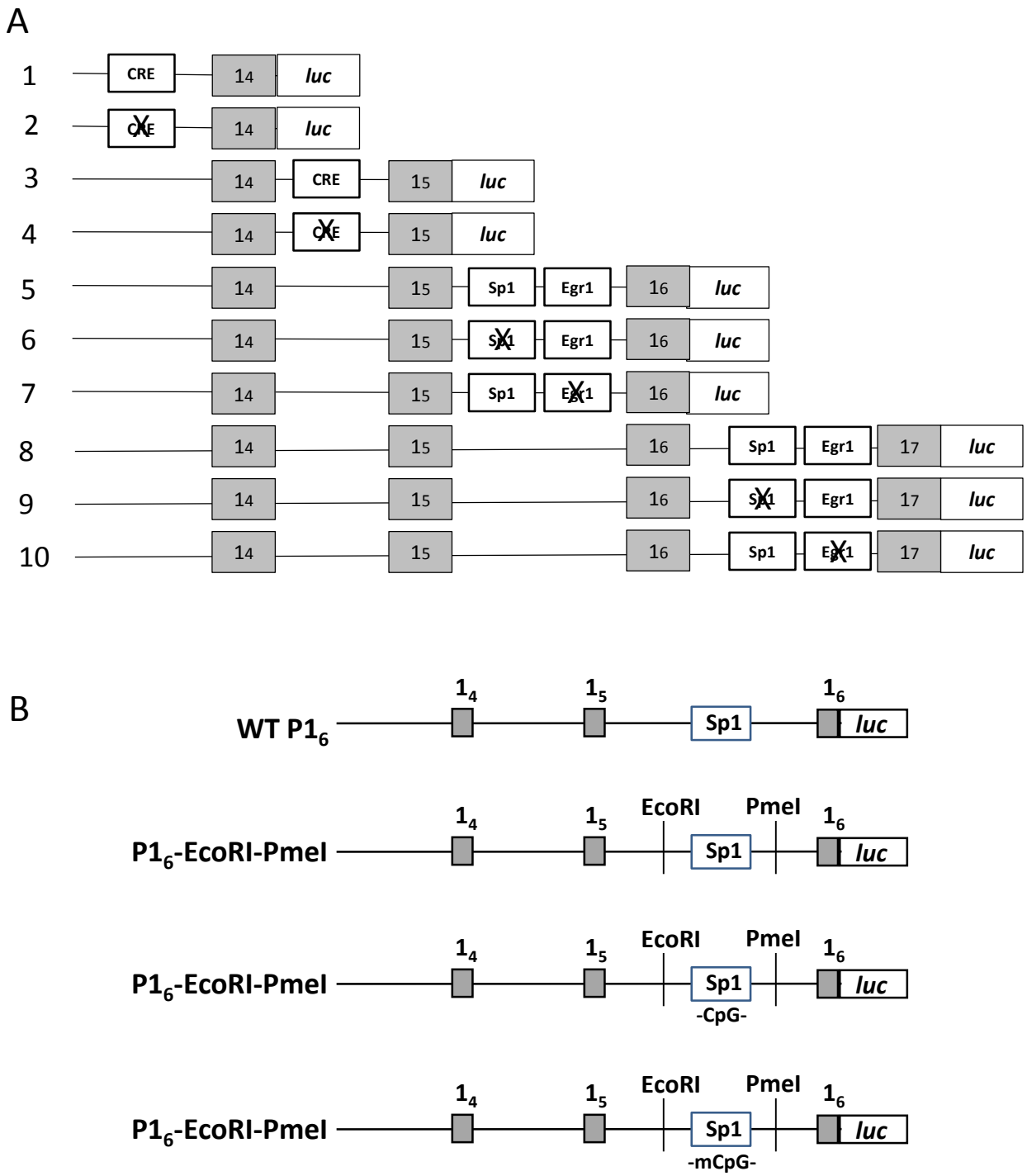
Pregnant rats were exposed to normoxia (N) or hypoxia (H) at 10.5% O_2 from day 15 to day 21 of gestation. Newborn rats were injected with saline (-AZA) or 5-aza-2'-deoxycytidine (+AZA) (1 μg/g/day, *i.p.*) at postnatal day 1 and day 3. Hearts were isolated from 4 week old offspring and subjected to 45 minutes of ischemia and 30 minutes of reperfusion in a Langendorff preparation. HR, heart rate; LVDP, left ventricular developed pressure; dP/dt_{max}, maximal rate of contraction; dP/dt_{min}, maximal rate of relaxation; CF, coronary flow.

Supplementary Figure I



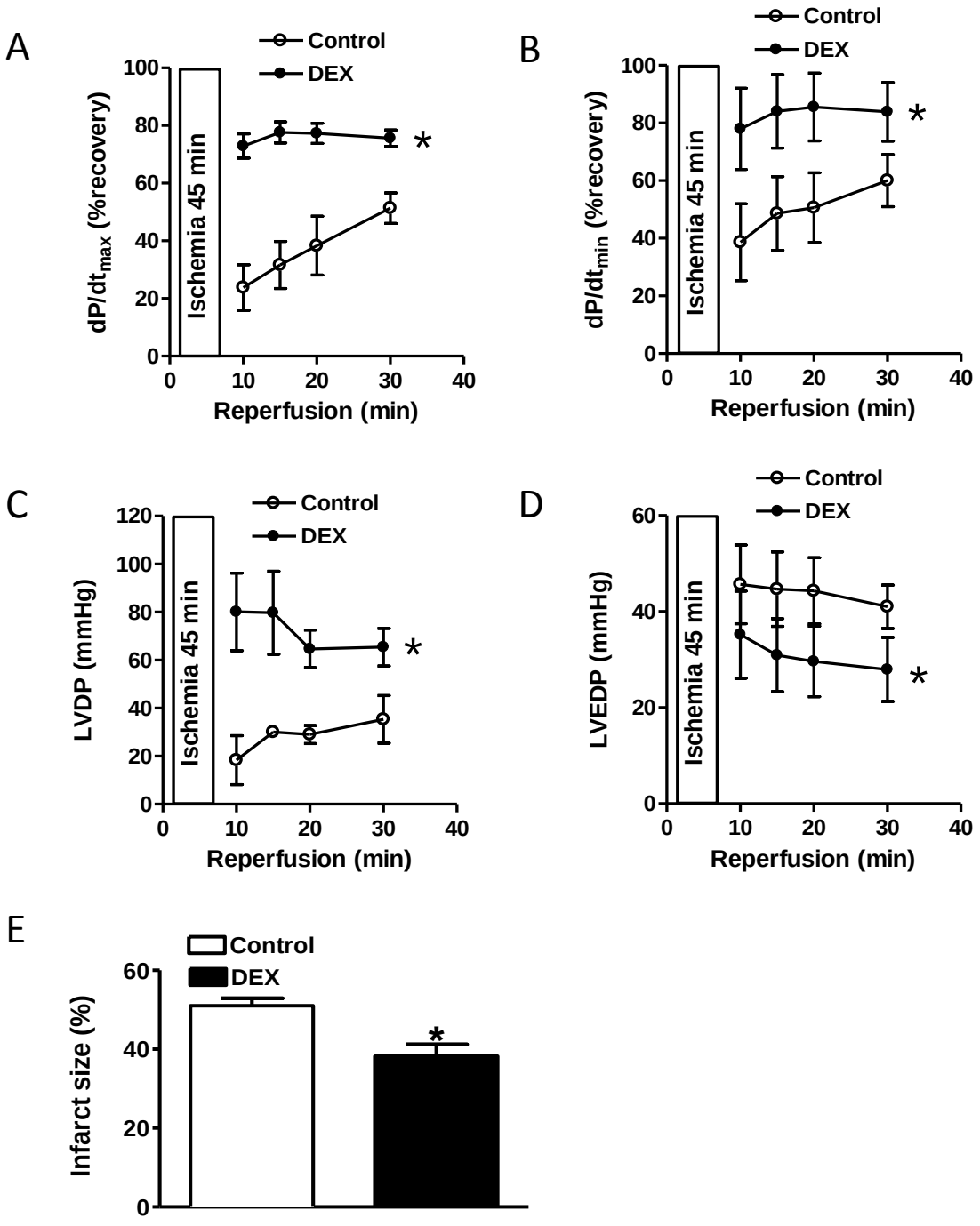
Supplementary Figure I. Effects of methylation on binding of CREB and Sp1 to GR promoter. Nuclear extracts (NE) from fetal hearts were incubated with double-stranded oligonucleotide probes containing unmethylated (UM) or methylated (M) CRE at GR promoter 1_4 and 1_5 (**A**), or Sp1 binding site at GR promoter 1_6 (**B**) and 1_7 (**C**) in the absence or presence of antibodies (Ab) against CREB or Sp1. Cold competition (CC) was performed with unlabeled competitor oligonucleotide at a 100-fold molar excess. Free oligo: no nuclear extracts were added. S, shift; SS, super-shift.

Supplementary Figure II



Supplementary Figure II. Diagrammatic representation of GR promoter constructs. Panel A shows site-directed deletion of CRE_{-4408}, CRE_{-3896}, $Sp1_{-3425}$, $Egr\text{-}1_{-3361}$, $Sp1_{-3034}$ and $Egr\text{-}1_{-2996}$ in GR promoter 1_4, 1_5, 1_6 and 1_7 constructs. Panel B shows wild type GR promoter 1_6 reporter construct (WT-$P1_6$) and constructs with a dual insertion of EcoR1 and PmeI site ($P1_6$-EcoRI-PmeI), an insertion of CpG methylation at Sp1 (Sp1-mCpG), and unmethylation at Sp1 (Sp1-CpG).

Supplementary Figure III



Supplementary Figure III. Dexamethasone protected the heart from ischemia and reperfusion injury in 4 week old rats. Four week old rats were injected with either saline (control) or dexmethasone (DEX, 1 mg/kg/day, *i.p.*) 24 hours prior to the heart isolation. Hearts were subjected to 45 minutes of ischemia and 30 minutes of reperfusion in a Langendorff preparation. LVDP, left ventricle developed pressure; LVEDP, left ventricle end-diastolic pressure. Data are mean ± SEM, $n = 5$. * $P < 0.05$, DEX *vs*. control.