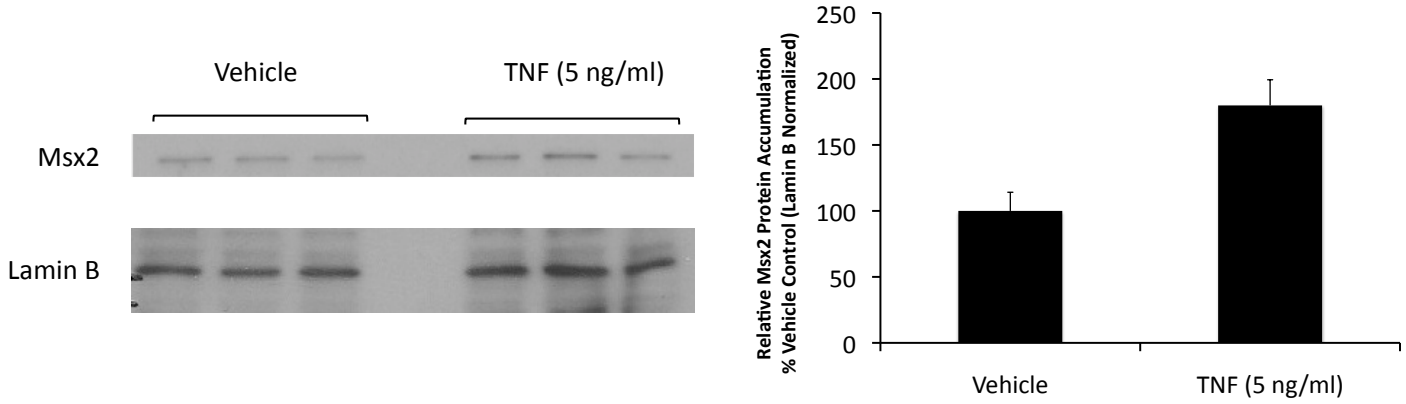
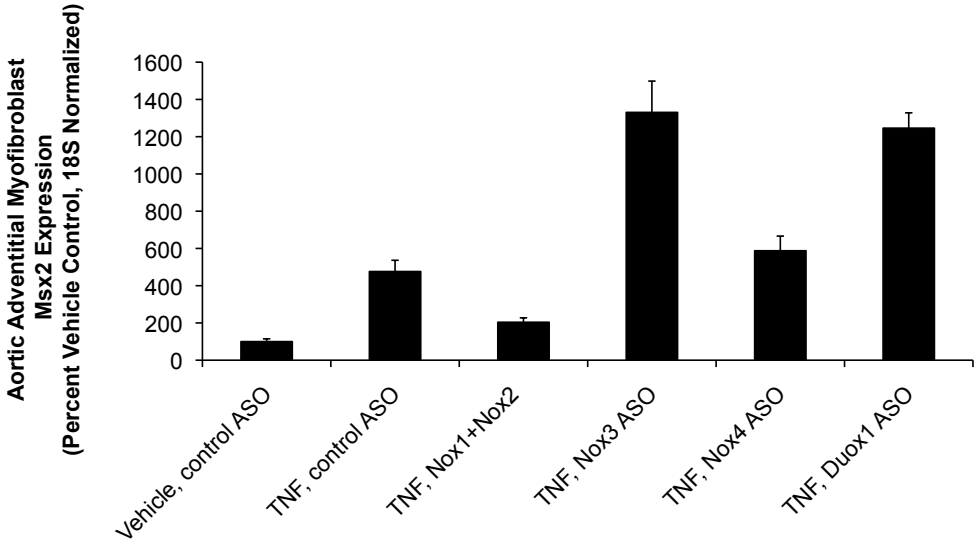
Data Supplement, Lai et al:
"TNFR1 -Activated Reactive Oxidative Species Signals Upregulate Osteogenic Msx2 Programs In Aortic Myofibroblasts"

Figure S1



Supplemental Figure S1: Independent replicates of western blot analyses for Msx2 protein accumulation in myofibroblasts after 4 hours of TNF treatment.

Figure S2



Supplemental Figure S2: Inhibition of *Msx2* was specific for *Nox1* and *Nox2* ASO, other Nox family ASOs did not inhibit TNF induction of Msx2. The *Nox1* and *Nox2* ASO concentrations used were each at 0.25 uM, and all others including the control ASO were 0.5 uM.

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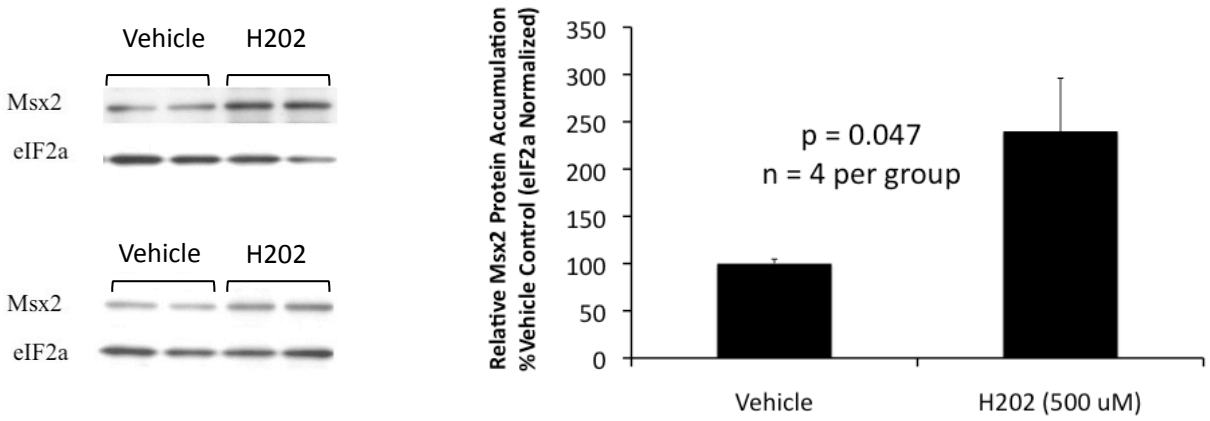
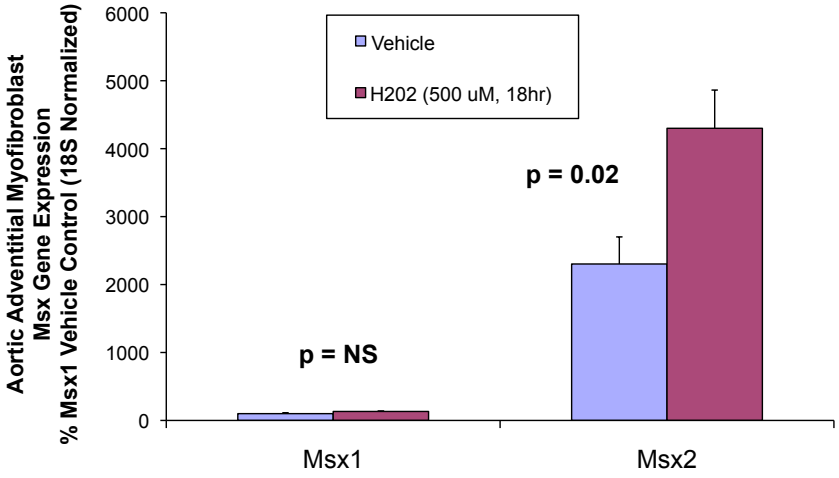
Figure S3



Supplemental Figure S3: Independent replicates of western blot analyses for Msx2 protein accumulation in aortic adventitial myofibroblasts after 4 hours of H202 treatment.

Figure S4



Supplemental Figure S4: Even following 18 hour of treatment, induction of *Msx* gene expression by peroxide was selective for *Msx2* vs. *Msx1* in cultured aortic myofibroblasts.

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Figure S5

Cold Competitor Oligo Sequences

Oligo A:
WT: [-69/-40]: 5'-GTG GGA GCT TTA TAA ATG TTC CAT GCC CTC-3'

M1: [-69/-40]: 5'-GTG GGA GCT TTG ATC ATG TTC CAT GCC CTC-3'

M2: [-69/-40]: 5'-GTG GGA GCT TTA TAA ATG TTG TAC GCC CTC-3'

OCTA26: 5'-GAT CAG TAC TAA TTA GCA TTA TAA AG-3'

Oligo E:
WT: [-11/+26]: 5'- GCG TGT GGG ATC GGC ACC AGA AAC ACT TTA AAG AGG G -3'

M3: [-11/+26]: 5'- GCG CAC TTT ATC GGC ACC AGA AAC ACT TTA AAG AGG G -3'

M4: [-11/+26]: 5'- GCG TGT GGG ATC GGC CAC TAC AAC ACT TTA AAG AGG G -3

M5: [-11/+26]: 5'- GCG TGT GGG ATC GGC ACC AGA AAC ACT CTC GCT AGG G -3'

Supplemental Figure S5: Upper strand sequences of Msx2 promoter annealed duplex oligonucleotides used in cold competition analyses. The A/T-rich OCTA26 homeobox cognate has been previously described (Towler DA, Bennett CD, Rodan GA. Activity of the rat osteocalcin basal promoter in osteoblastic cells is dependent upon homeodomain and CP1 binding motifs. Mol Endocrinol. 1994 May;8(5):614-24).

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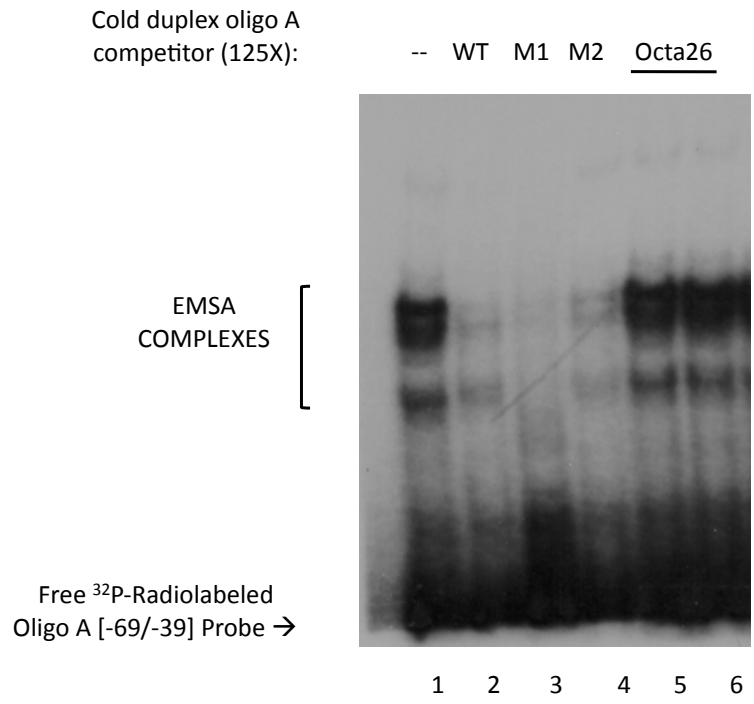
Figure S6



Supplemental Figure S6: The C3H10T1/2 factors specifically binding duplex Oligo A, the Msx2 promoter region -69/-39, do not recognize intact A/T rich TATA- or homeo-box cognates. Lane 1, no cold competitor. Lane 2, 125-fold molar excess of unlabeled cold homologous oligonucleotide completely inhibits binding to radiolabeled probe. Lanes 3-4, mutation of TATA and POU homeodomain binding cognates in Oligo A (M1, M2) does not impair binding, as demonstrated in cold competition assay. Lanes 5-6, the OCTA26 A/T rich homeodomain binding cognate does not compete for factor binding to radiolabeled duplex OligoA. See Figure 5B and S5 for sequences.

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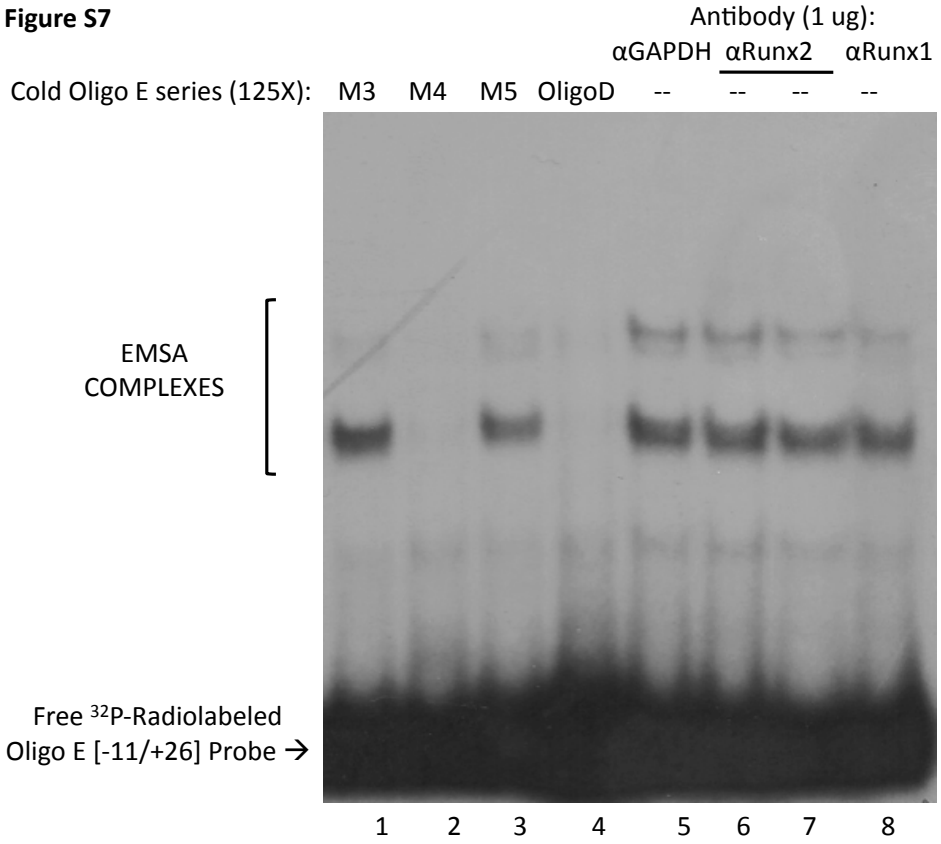
Figure S7



Supplemental Figure S7: The C3H10T1/2 factors specifically binding duplex OligoE, *Msx2* promoter region -11/+26, following H202 treatment. While Oligo E mutants M3 (lane 1) and M5 (lane 3) lacking intact TGTGGG and C(A/T)6G motifs, respectively, were inactive in cold competition binding assays, OligoE mutant M4 lacking the ACCAGA Runx motif was still active (lane 2). Antibodies to Runx2 (Santa Cruz sc-12488/lane 6 and sc-8566/lane 7) and Runx1 (sc-8563) also did not disrupt binding (lanes 6 -8). Note that OligoD, Msx2 -27/+3, a fragment that retains the intact TGTGGG motif at *Msx2* promoter region -8 to -3, successfully competes for complex formation on the radiolableled OligoE (Msx2 -11 /+26) (lane 4). See Figure 5B and S5 for sequences.