

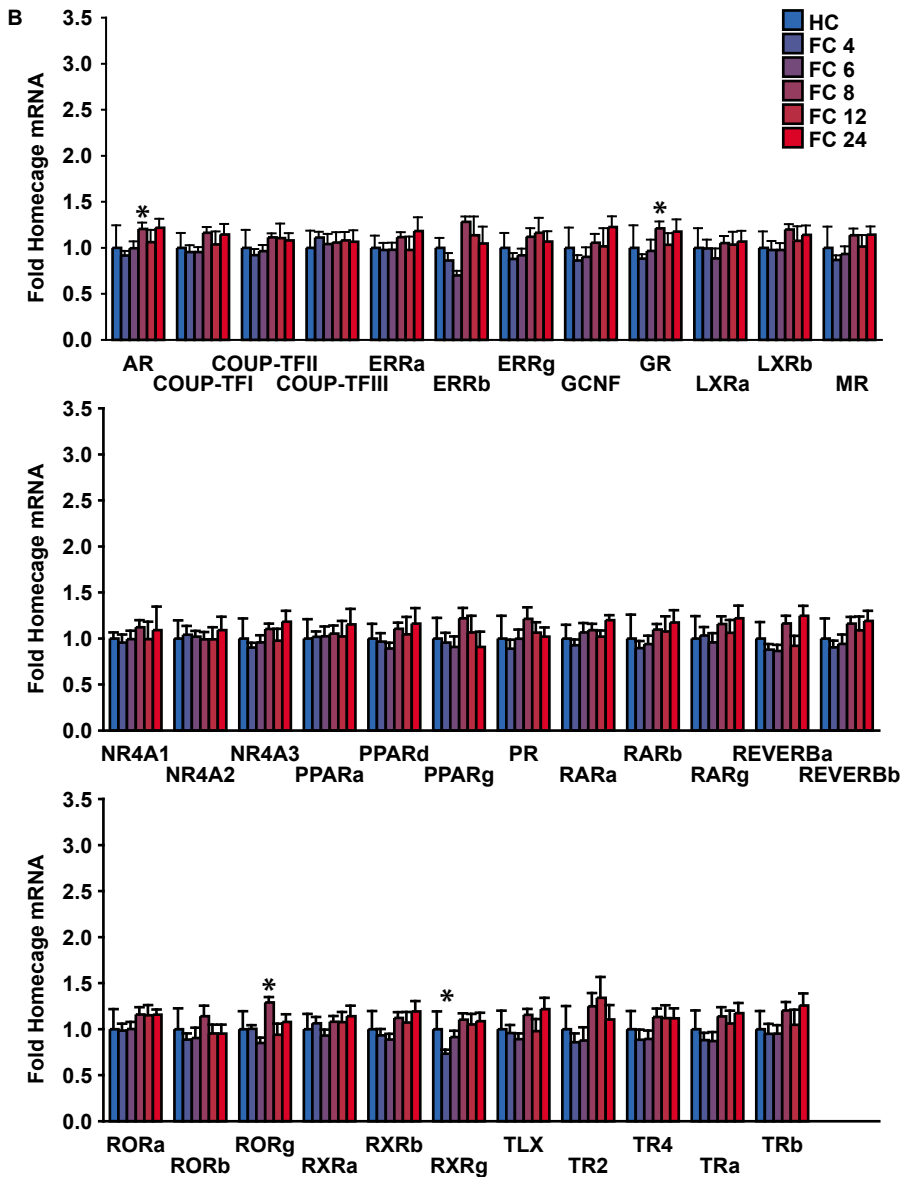
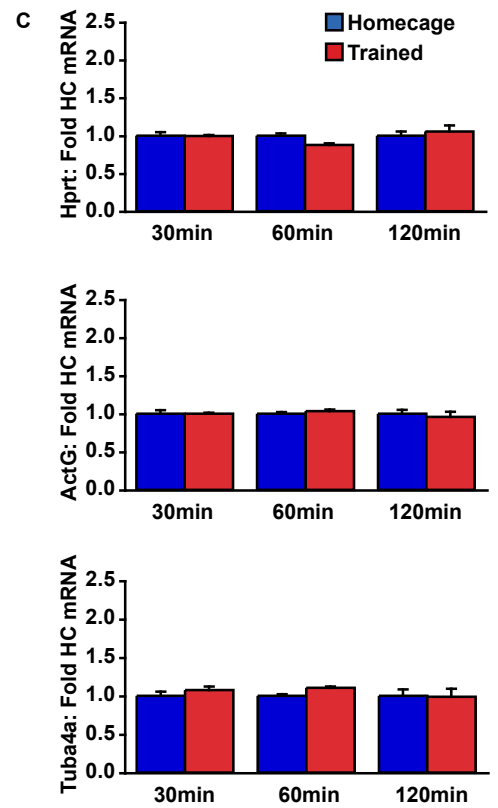
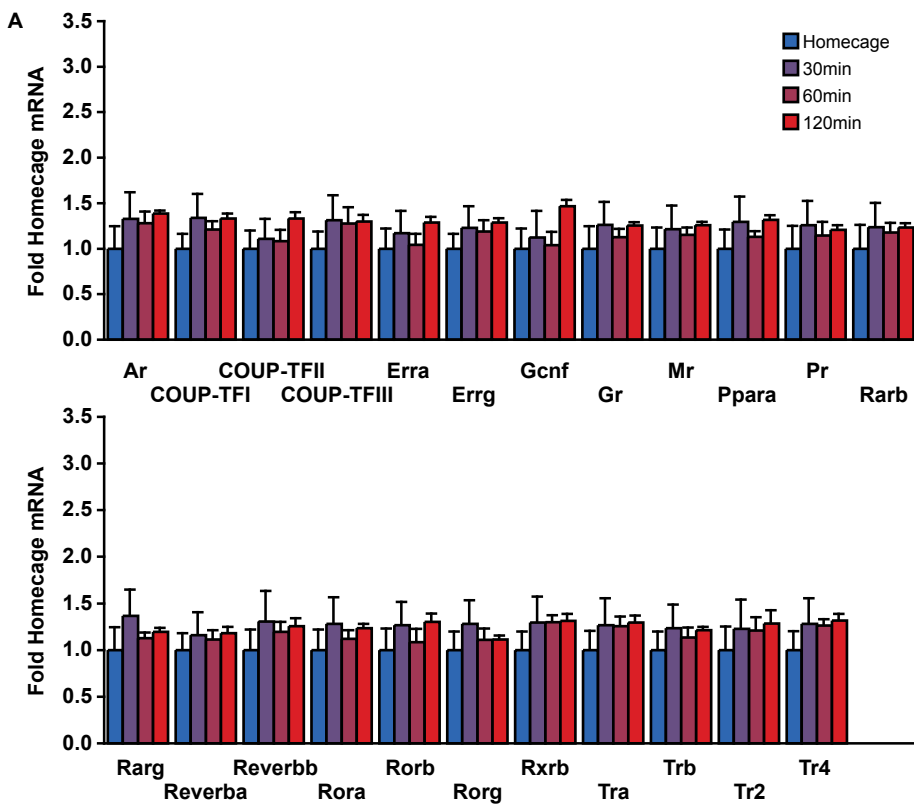
## Supplementary Figure Legends

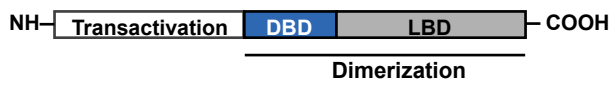
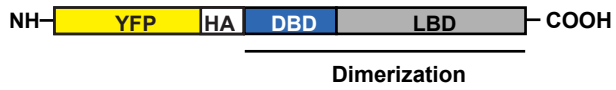
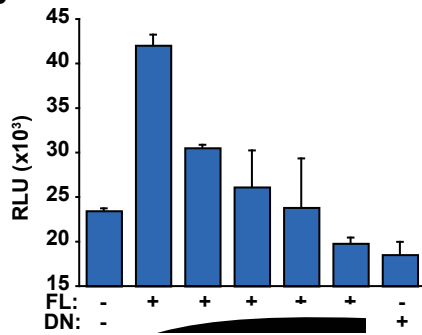
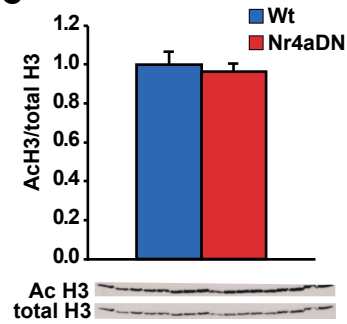
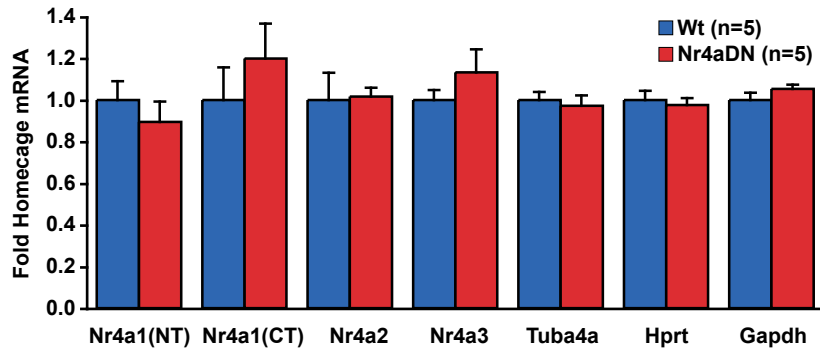
**Figure S1.** Out of 49 NRs, 13 showed significant changes in gene expression after contextual fear conditioning. (A) The expression levels for these 13 NRs are illustrated relative to expression under untrained conditions (“Homecage”). For each of these 13 genes, expression is significantly increased at the 2hr timepoint. All three *Nr4a* genes show increased expression within this time window. (B) Expression levels of 23 NRs was unchanged in the 2hr window after learning. (C) mRNA levels for the house-keeping genes *Hprt*, *ActG*, and *Tuba4a* were not impacted by fear conditioning. Error bars denote s.e.m. and \* signifies  $p < 0.05$

**Figure S2.** NR4A dominant negative mouse line. (A) The dominant negative construct contains sequences encoding the DNA-binding domain (DBD) and the defunct C-terminal ligand-binding domain (LBD) of NR4A1, but the N-terminal transactivation domain was replaced by yellow-fluorescent protein (YFP) and hemagglutinin (HA) tags. (B) Co-transfecting increasing amounts of Nr4aDN (DN) into HEK293 cells with constant amounts of full length *Nr4a2* (FL) and the NR4A family luciferase reporter (NBRE-Luc) impairs luciferase production ( $p = 0.007$ ), confirming that this Nr4aDN construct indeed acts as a dominant negative within the NR4A family. (C) Expression of the dominant negative form of NR4A1 does not cause gross changes in histone acetylation. (D) Additionally, dominant negative expression does not alter mRNA levels for *Nr4a* genes in whole hippocampal extracts. A qPCR primer set spanning the region encoding the N-terminal domain of NR4A1, which is deleted in the dominant negative construct, shows no change in levels in the Nr4aDN mice. Primers assaying a region common to both endogenous and mutant *Nr4a1* transcripts suggest a modest increase that would be consistent with the sparse expression of the dominant negative construct. Neither *Nr4a2* nor *Nr4a3* mRNA levels were impacted by Nr4aDN expression. (E) Mice were reared on standard rodent chow and then placed on a doxycycline-containing diet at four weeks of age. Nr4aDN protein was undetectable after 4 weeks of doxycycline treatment. Suppression of transgene expression is shown by comparison to immunohistochemical staining for the HA tag in 2 month old Nr4aDN mice never on a doxycycline diet, “No Dox,” versus Nr4aDN mice after 4 weeks of doxycycline treatment, “4 wks Dox”. Error bars denote s.e.m. and \* signifies  $p < 0.05$ .

**Figure S3.** HDAC inhibitor treatment increases Nr4a expression. (A) Full western blots containing data illustrated in Figure 3. Lanes marked are marked according to treatment with TSA “T” or vehicle “V”. Lanes excluded from analysis because of bubbles in either control or experimental bands are designated with “X”. Periodically, the monoclonal tubulin antibody detects a more slowly migrating form. The more rapidly migrating form was used in all analysis, as it is possible that the slowly migrating form reflects post-translational modifications sensitive to TSA. (B) Data showing increased expression of *Nr4a* genes 1 hour after TSA treatment (Fig. 3) is illustrated with data from three control genes, showing that a universal increase in gene expression is not observed with HDAC inhibitor treatment. (C) In contrast to *Nr4a2*, gene expression for *CaMKII-alpha* is unaffected by TSA treatment, suggesting that CaMKII promoter-driven transgenes, such as the dominant negative, are not regulated by TSA. Error bars denote s.e.m. and \* signifies  $p < 0.05$ .

**Figure S4.** The OpenArray high-throughput qPCR platform was used to survey gene expression in Nr4aDN mice. To reduce the likelihood of missing activity-dependent gene expression defects, a four-way experimental design was chosen for this study including 1hr after fear conditioning. The four groups were: homecage wildtype mice, homecage Nr4aDN mice, fear-conditioned wildtype mice, and fear-conditioned Nr4aDN mice. ANOVAs were performed to detect effects of genotype, training and interactions. (A) Eight genes showed evidence of being affected by the Nr4aDN genotype. There was no interaction between genotype and training for these potential Nr4aDN target genes. (B) Thirty-six genes were identified as induced after fear conditioning, consistent with previous studies. All three of the *Nr4a* genes were induced by fear conditioning without any impact of the genotype, which is consistent with the action of the dominant negative protein being downstream of *Nr4a* gene expression.



**A****Nr4a Family Members****Nr4a Dominant Negative****B****C****D****E**