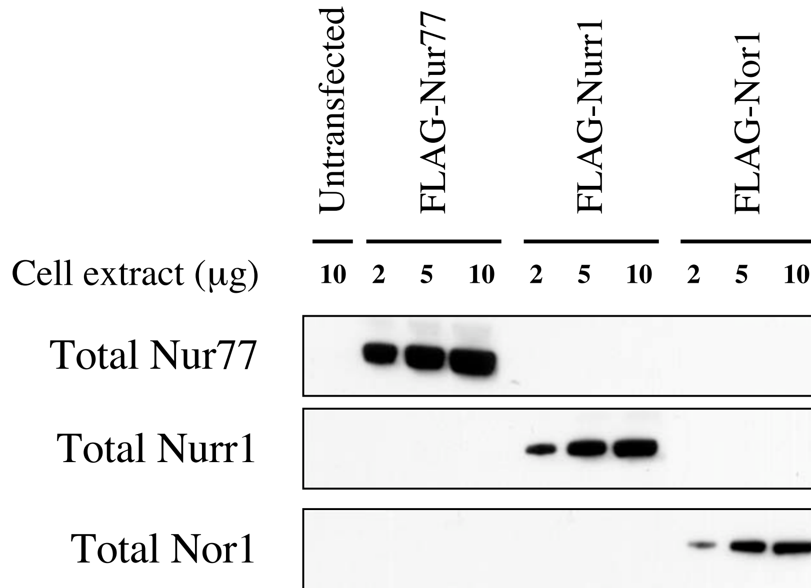
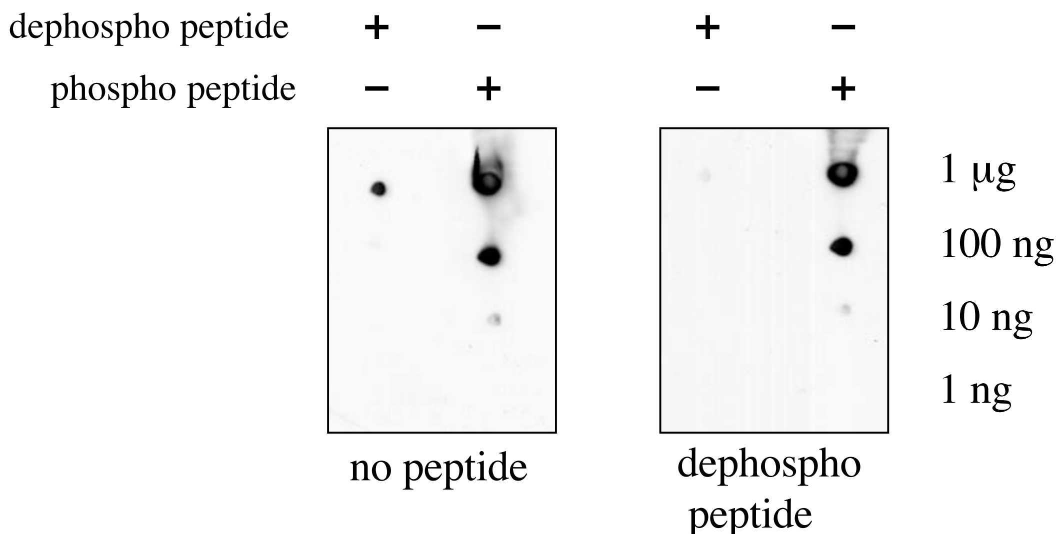
Supplementary Fig 1 Specificity of NR4A antibodies

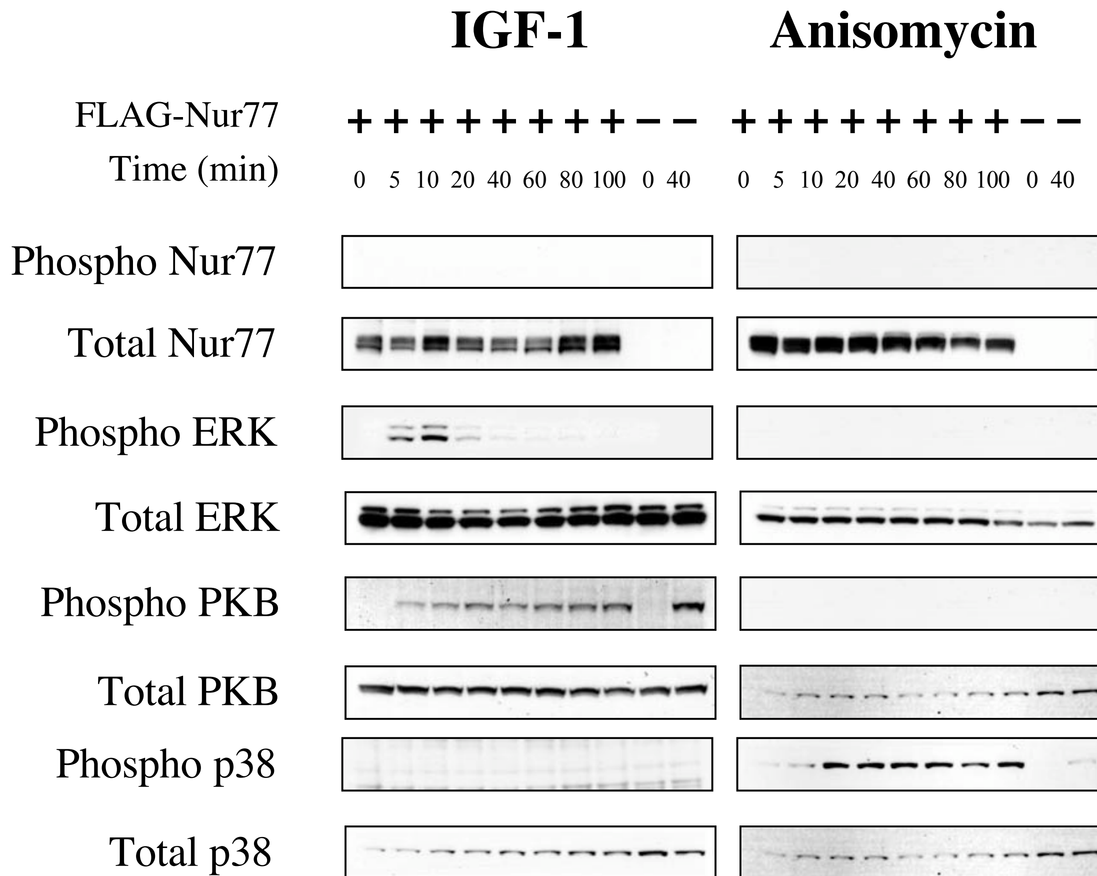




A) Hek293 cells were transfected with expression vectors for Flag nur77, nurr1 or nor1 as indicated. Cell were lysed and 2, 5 or 10 mg of soluble protein from the cell extract was run on 4-12% polyacrylamide gels and blotted with antibodies raised against peptides specific for nur77 (RDHLTGDPLALEFGK), nurr1 (SGEYSSDFLTPEFVK) or nor1 (CPRPLIKMEEGREHG).

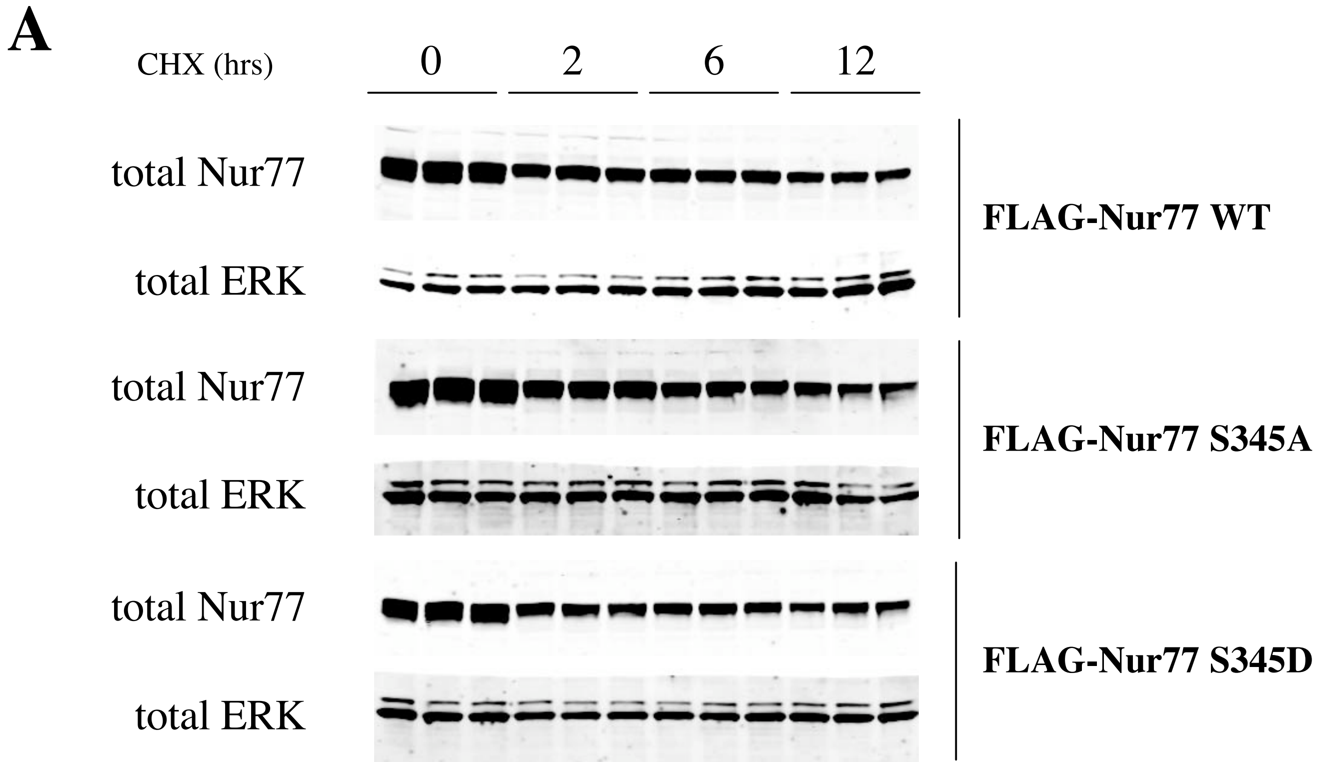
B) A phospho Ser354 nur77 antibody was raised against the peptide CGRLP(phospho-S)KPKQP and tested in dot blots against the indicated amounts of the immunising peptide or its equivalent dephospho peptide. The antibody was used at 1 μg/ml in 2% BSA/TBS either in the presence of no peptide (1st panel) or 10 μg/ml of the dephospho peptide (2nd panel).

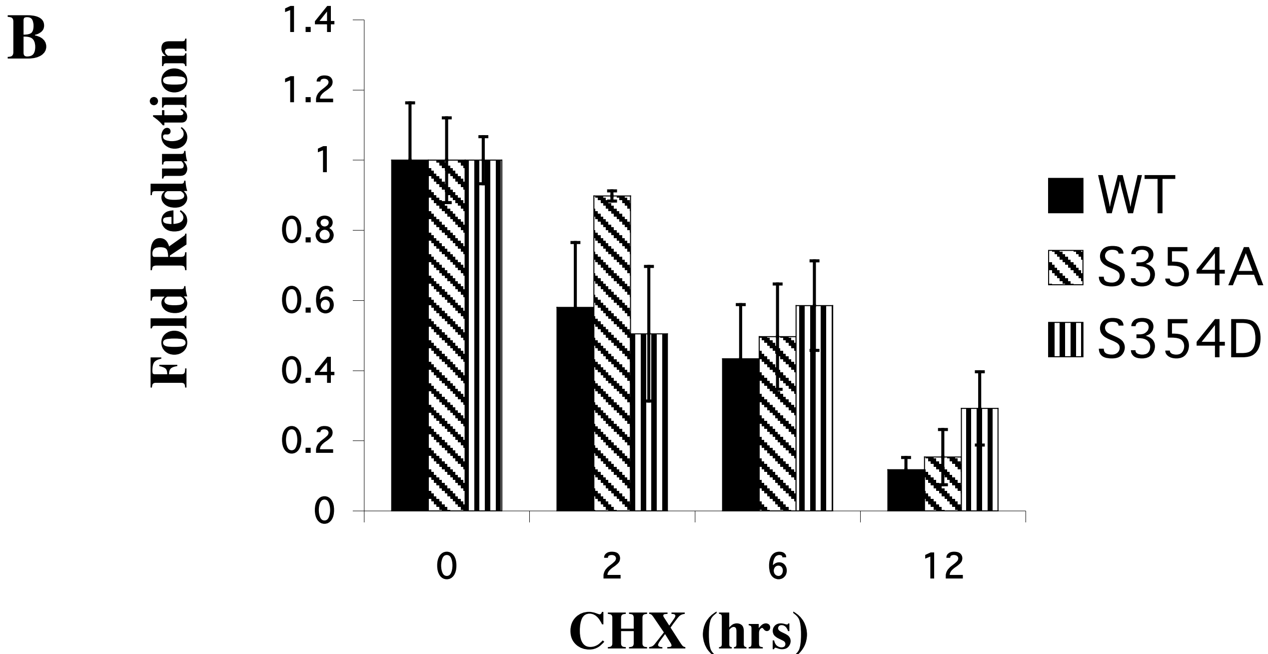
Supplementary Fig 2 Effect of IGF and anismomycin on nur77



Hek293 cells were transfected with an expression plasmid for Flag-nur77. Cells were then starved for 16 hours and stimulated with either 10 ng/mlIGF or 10 μg/ml anisomycin for the times indicated. Cells were then lysed and 30 μg of soluble protein lysate run on 4-12% gradient polyacrylamide gels. The levels of phospho Ser354 nur77, total nur77, phospho ERK1/2, total ERK1/2, phospho Thr 308 PKB, total PKB, phospho p38 and total p38 were then examined by immunoblotting.

Supplementary Fig 3 Degradation of nur77





A Wild type, S354A and S354D Flag-nur77 was transfected into Hek293 cells. Cyclohexamide (CHX)was then added to the cells to block protein synthesis for 0, 2, 6 or 12 hours. The amount of nur77 remaining in the cells after these times was determined by immunoblotting with an antibody against the Flag peptide, lysates were also blotted for total ERK1/2 to control for loading. Representative blots are shown in A, and quantification of these blots in B.