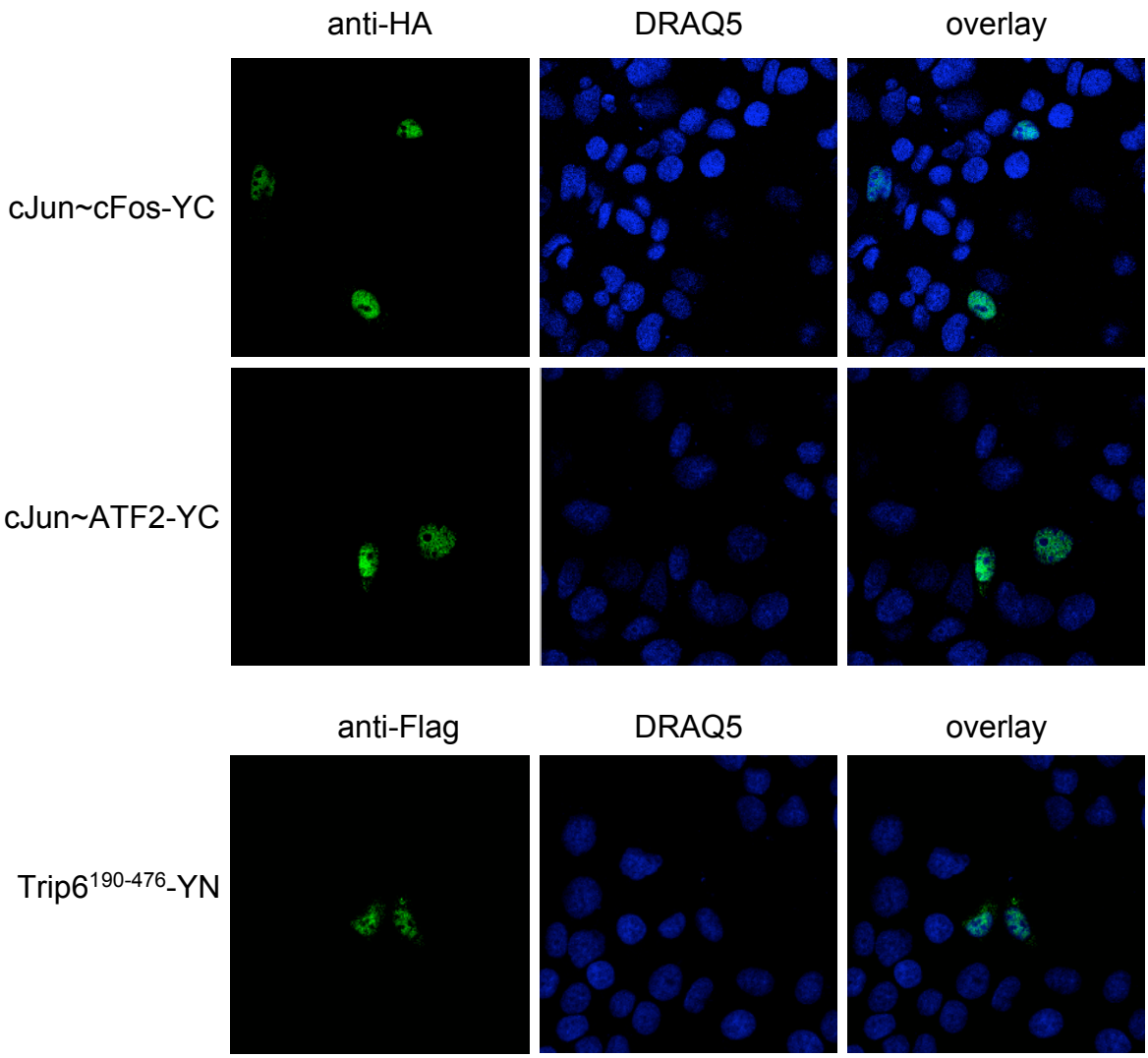
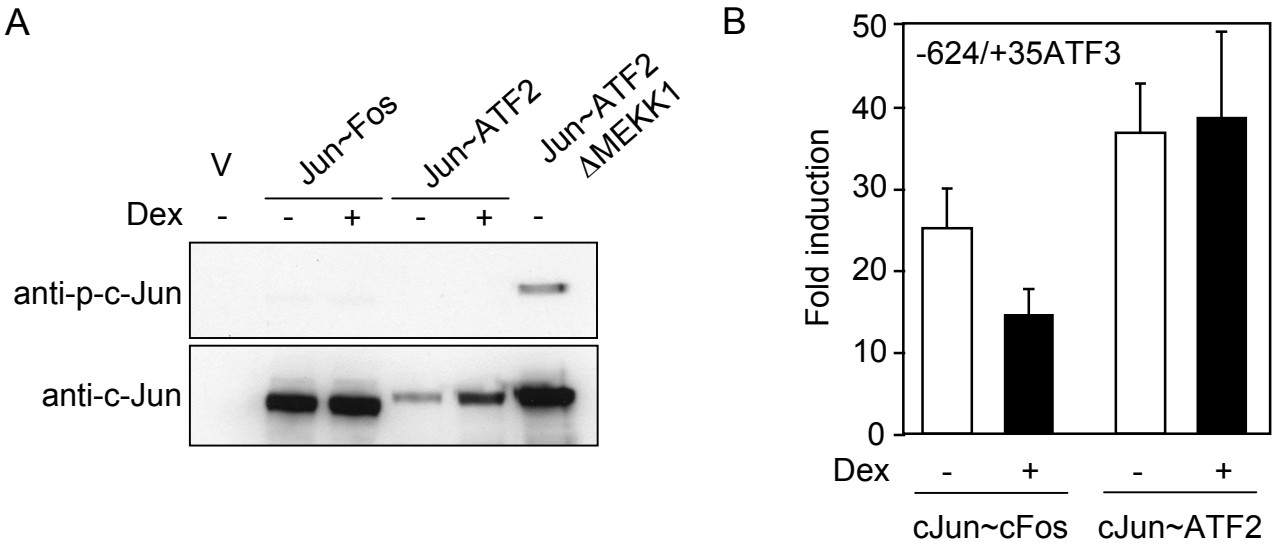


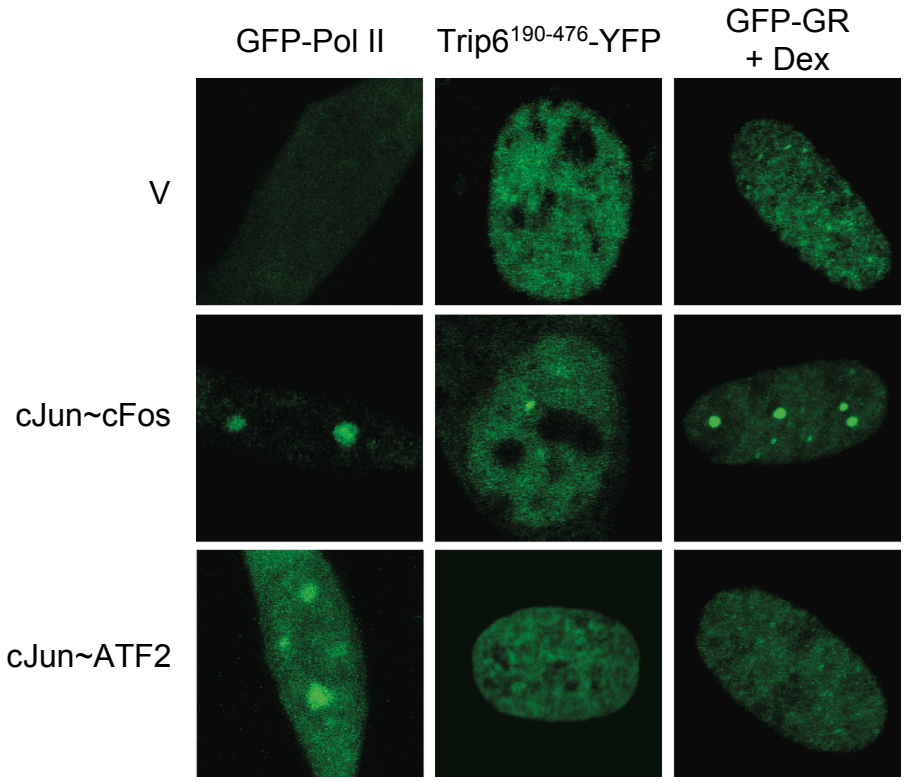
Supplemental Fig. 1. Expression and subcellular localization of c-Jun~c-Fos-YC, c-Jun~ATF2-YC and Trip6$^{190-476}$-YN.

Cos7 cells were transfected with either the HA tagged single chain AP-1 c-Jun~c-Fos or c-Jun~ATF2, fused to the C-terminal half of YFP (YC), or Flag tagged Trip6$^{190-476}$ fused to the N-terminal half of YFP (YN). Expression of the constructs was detected by immunofluorescence using anti-HA or anti-Flag antibodies. Nuclei were counterstained with DRAQ5.



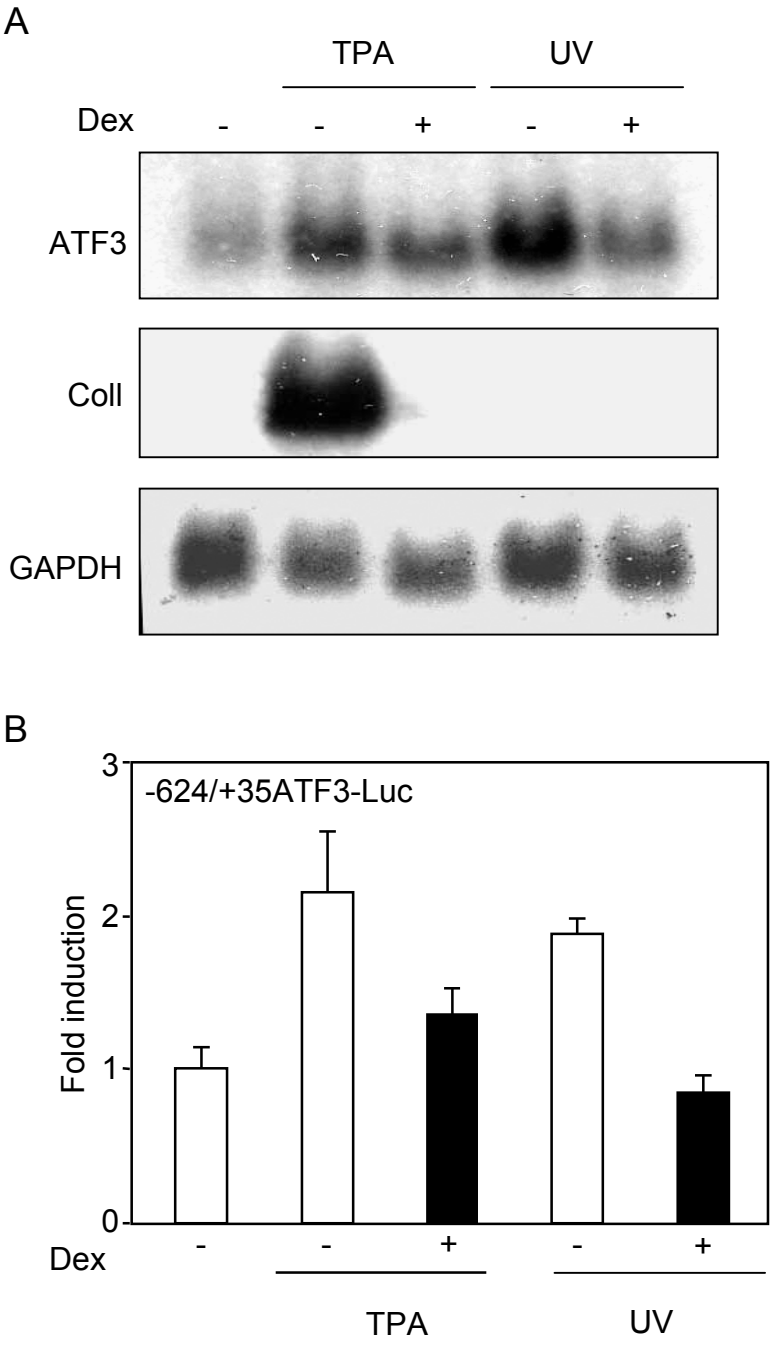
Supplemental Fig. 2. cJun:ATF2 is not trans-repressed by GR.

A, Cell lysates from the experiment shown in Fig. 2C were subjected to Western blotting and c-Jun phosphorylation (p-c-Jun) was assessed using a phospho-specific antibody. The membrane was stripped and reprobed with a phosphorylation state-independent anti-c-Jun antibody. The positive control in the last lane consists in a lysate of cells transfected with c-Jun~ATF2 together with a constitutively active mutant of the MEK kinase 1 (ΔMEKK1), a direct activator of SEK1, which in turn activates JNK, leading to c-Jun phosphorylation. B, HeLa cells were transiently cotransfected with the -624/+35ATF3-luciferase reporter gene together with expression vectors for either the single chain AP-1 c-Jun~c-Fos or the single chain AP-1 c-Jun~ATF2. Cells were treated with dexamethasone (Dex) as indicated. Normalized luciferase activities are plotted as fold induction (mean ± S.D. of one representative experiment performed in triplicates).



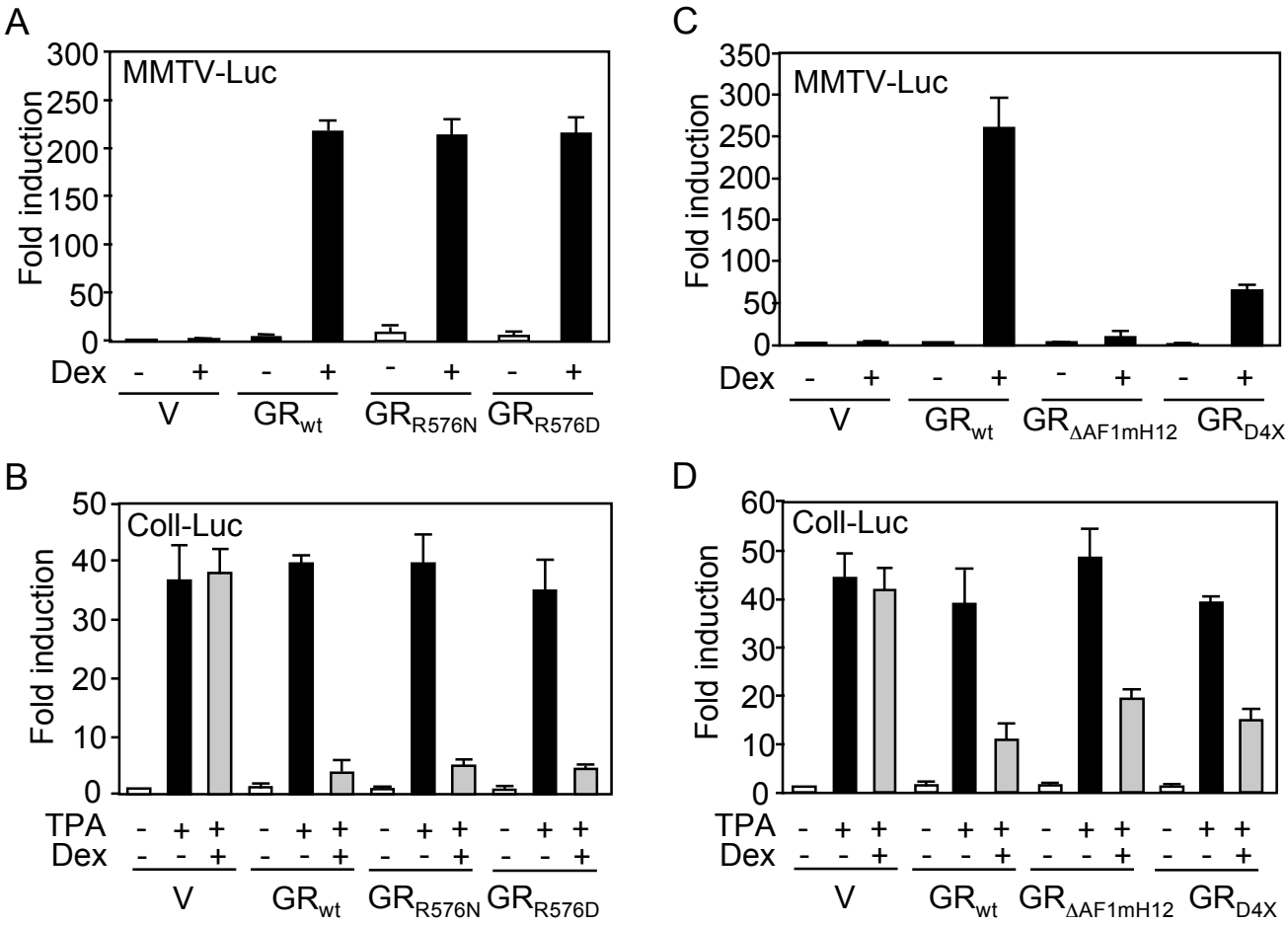
Supplemental Fig. 3. nTrip6 and GR are not tethered to c-Jun:ATF2 occupied promoter.

uPA gene array cells form a clone containing about 3000 copies of the -1977/-1858-uPA-luciferase reporter gene integrated at least 3 different loci, were co-transfected with either empty vector, an expression vector for c-Jun~c-Fos single chain, or an expression vector for c-Jun~ATF2 single chain, together with an expression vector for GFP-Pol II, an expression vector for Trip6$^{190-476}$ fused to YFP (YFP-Trip6$^{190-476}$), or an expression vector for GR fused to GFP. Cells were treated with dexamethasone for 30 min and imaged by confocal microscopy. Nuclei of representative cells are shown.



Supplemental Fig. 4. UV-induced ATF3 expression is repressed by GR.

A, HeLa cells were either treated with TPA or UV irradiated in the presence or absence of dexamethasone (Dex). Total RNA was prepared and subjected to Northern blotting using probes specific for ATF3, collagenase I (Coll), or GAPDH. B, HeLa cells were transfected with the -624/+35ATF3-luciferase reporter gene, and either treated with TPA or UV irradiated in the presence or absence of dexamethasone (Dex). Normalized luciferase activities are plotted as fold induction (mean ± S.D. of one representative experiment performed in triplicates).



Supplemental Fig. 5. Transactivation and trans-repression potential of the different GR mutants used.

Cos7 cells were co-transfected with luciferase reporter genes driven by either (A, C) the GRE-containing mouse mammary tumor virus long terminal repeat (MMTV-Luc) or (B, D) the c-Jun:c-Fos-dependent collagenase I promoter (Coll-Luc), together with either empty vector, expression vectors for wild type GR (GR_{wt}), (A, B) two GR mutants unable to interact with and inhibit JNK (GR_{R576N}, GRR_{576D}), or (C, D) two GR mutants unable to transactivate ($GR_{\Delta AF1mH12}$, GRR_{D4X}). Cells were treated with (A, C) dexamethasone (Dex), or (B, D) TPA in the presence or absence of Dex, as indicated. Normalized luciferase activities are plotted as fold induction (mean ± S.D. of one representative experiment performed in triplicates).