Supplementary Methods

Amplicon Construction

All PCR reactions were carried out using Pfu polymerase (Promega, Madison, WI) to minimize errors. Final amplicons were sequenced for verification. 11βHSD: The 11βHSD type 2 gene (Genbank accession number U14631) and polyadenylation sequence was subcloned from plasmid 11βHSD2 into the HindIII site downstream of the α4 promoter of plasmid pα22-eGFP, creating a functional amplicon plasmid.

dnGR: Rat GR (Genbank M14053) was PCR amplified from plasmid pGRGR via a two-step process. In the first round, primers amplified base pairs 61 to 2303 corresponding to amino acids 1 — 745 (out of 795 total). The 3' primer included the first 24 base pairs of the GRβ alternate sequence (bases 2314-2338 of GRβ: Genbank X03348), resulting in a truncated product with half of the necessary GRβ sequence attached to the 3' end of the product. PCR product was purified and re-amplified with the same 5' primer and a new 3' primer containing the remaining bases coding for GRβ's aberrant C-terminus. The product was purified, digested, and ligated into plasmid pG310-HIND that contains a cytomegalovirus polyadenylation sequence. The gene and poly-A were then subcloned into the HindIII site in plasmid pα22-eGFP, creating a functional amplicon plasmid. ER/GR: Bases 293-1024 of the human ERα (Genbank M12674) were PCR-amplified with primers containing known restriction sites. PCR product was purified, digested, and ligated into pG310-HIND. A similar process was applied to bases 1580-2459 of the rat GR, which was ligated directly downstream of the ER fragment in plasmid pG310-HIND-ER. The ER/GR chimera and polyadenylation sequence were subcloned into the HindIII site of pα22-eGFP, creating a functional amplicon plasmid.

eGFP Fusions: dnGR and ER/GR genes were PCR-amplified from the intermediate pG310 backbone with primers designed to provide an in-frame XhoI site directly upstream of the initial methionine codon in the gene. PCR product was purified and digested with XhoI and ligated into the plasmid vector pα22-eGFP-XhoI (Figure 2a), which provides an in-frame XhoI restriction site directly upstream of the termination codon of the eGFP reporter gene. These ligations created eGFP-Gene of Interest fusions.

Generation and use of herpes simplex virus-1 amplicon vectors

This subject is covered in detail in46. Vectors are generated by transfection of plasmids into E5 cells using lipofectamine and superinfecting 24 hours later with the helper virus d120 at a multiplicity of infection (MOI) of 0.3. d120 has a deletion mutation in the α4 gene, requiring propagation in E5 cells, which have been stably transformed with α4. Amplicons are harvested by freeze-thaw lysis followed by sucrose cushion centrifugation at 20,000 X g overnight. Pelleted viruses are resuspended in ddH2O and frozen at —80°C. Superinfection gives rise to vector titers of 2-3 X10^6 infectious particles/ml and d120 helper virus titers of 0.4 - 1.0 X 10^7 plaque forming units (PFU)/ml.

Supplementary Reference: