Supporting Information

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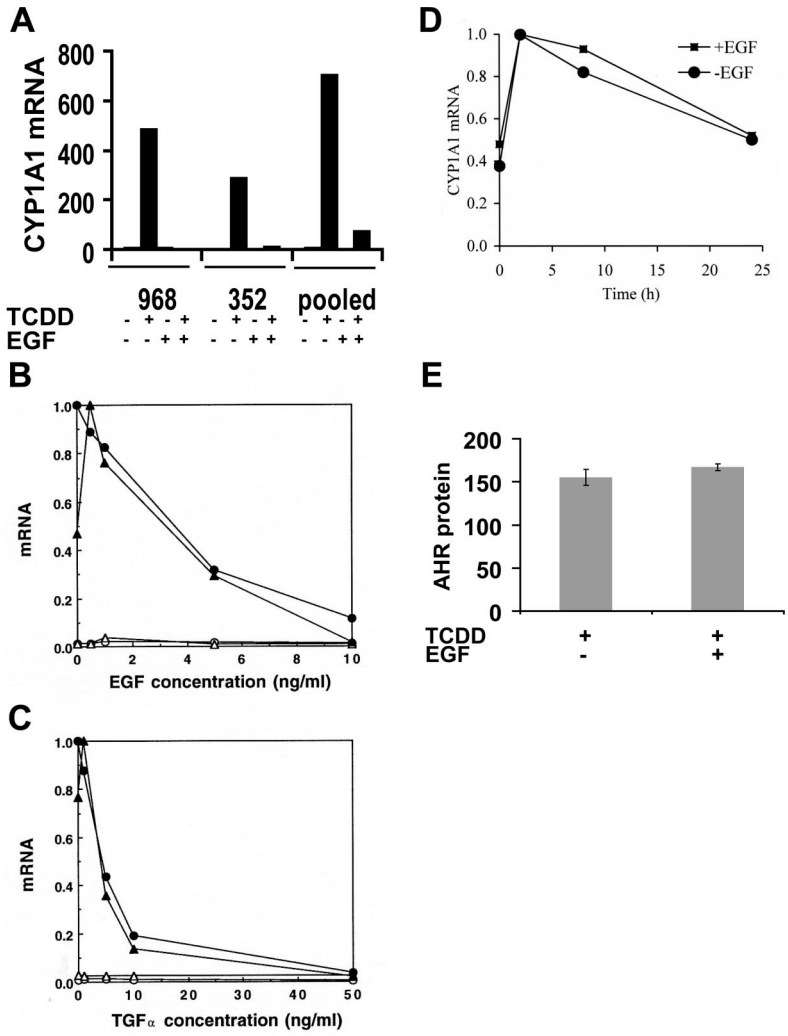

Fig. S1. EGF and TGFα dose-dependently repress TCDD-mediated increases in CYP1 mRNA, in multiple individuals, without affecting CYP1A1 messenger RNA half-life or aryl hydrocarbon receptor (AHR) protein. (*A*) Normal human epidermal keratinocytes (NHEKs) were grown to confluence before basal medium, or medium with EGF (10 ng/mL) was added 24 h before treatment. Messenger RNA was isolated after treatment with either 0.1% DMSO or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (10 nM) for 24 h. NHEK lines 968 and 352 were isolated from separate individuals, and the pooled sample was from 3 additional individuals. Levels of mRNA ($n = 1$) are expressed in units relative to the minimum, given a value of 1. (*B* and *C*) NHEKs were grown to confluence before basal medium, or medium with the indicated amount of EGF, or TGFα was added 24 h before treatment. Cells were treated with either control vehicle or TCDD (10 nM) for 24 h. Levels of mRNA (*y* axis) are expressed in units relative to the maximum observed density, given a value of 1. (*B*) Concentration effect of EGF (0, 0.5, 1, 5, and 10 ng/mL). (*C*) Concentration effect of TGFα (0, 1, 5, 10, and 50 ng/mL). CYP1A1: control (○), TCDD (●); CYP1B1: control (△), TCDD (▲). (*D*) Medium was replaced with basal medium containing TCDD (10 nM) for 12 h. EGF (10 ng/mL) was added to one set of cells for 4 h, and all of the cells were then treated with actinomycin D (5 μg/mL). RNA was isolated 0, 2, 8, and 24 h after the addition of actinomycin D. Levels of RNA (*y* axis) are expressed in units relative to the maximum observed density for each sample set, given a value of 1. Circles, without EGF; squares, with EGF. (*E*) NHEKs were grown to confluence before basal medium, or medium with EGF (10 ng/mL) was added 24 h before treatment. Nuclear extracts were isolated after treatment with either TCDD (10 nM) or TCDD plus EGF (10 ng/mL) for 30 min. Densitometric units of AHR protein from immunoblots [mean ($n = 2$) $\pm$ SD] are plotted relative to value obtained from untreated controls.

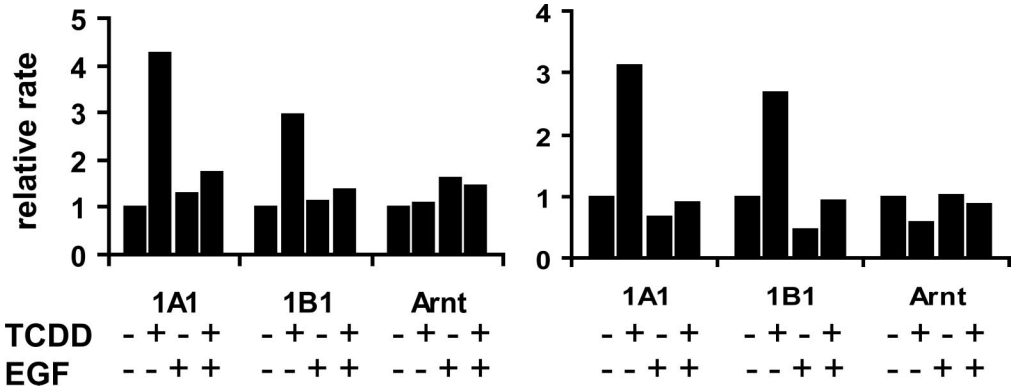

Fig. S2. The rates of transcription of CYP1A1 and CYP1B1 are decreased by EGF. Replicate nuclear run-on experiments are shown. Basal medium, or medium with EGF (10 ng/mL) was added 24 h before treatment. Cells were treated with either 0.1% DMSO or TCDD (10 nM) for 4 h. Hybridization signals were normalized to β-actin and plotted relative to treatment without EGF and TCDD (*x* axis, −/−), which was given a value of 1 (*y* axis, relative rate of transcription).

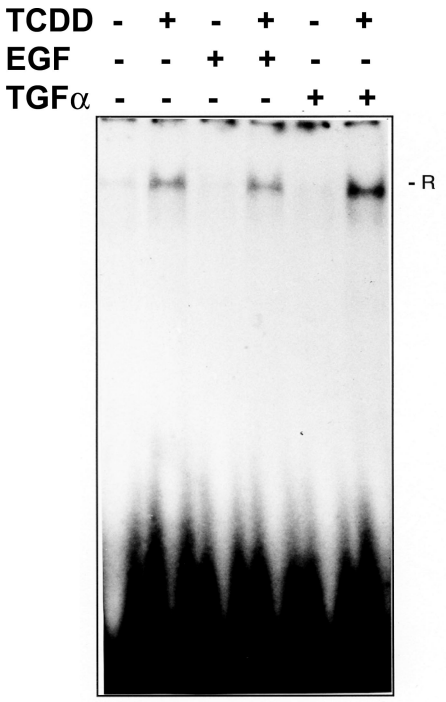

Fig. S3. Lack of effect of EGF or TGFα on the DNA binding activity of the AHR or *Ah* receptor nuclear translocator (ARNT). AHR–DNA binding activity (− R) was detected by EMSA under the indicated conditions. Basal medium or medium with EGF (10 ng/mL) or TGFα (50 ng/mL) was added 24 h before treatment. Cells were treated with either 0.1% DMSO or TCDD (10 nM) for 2 h.

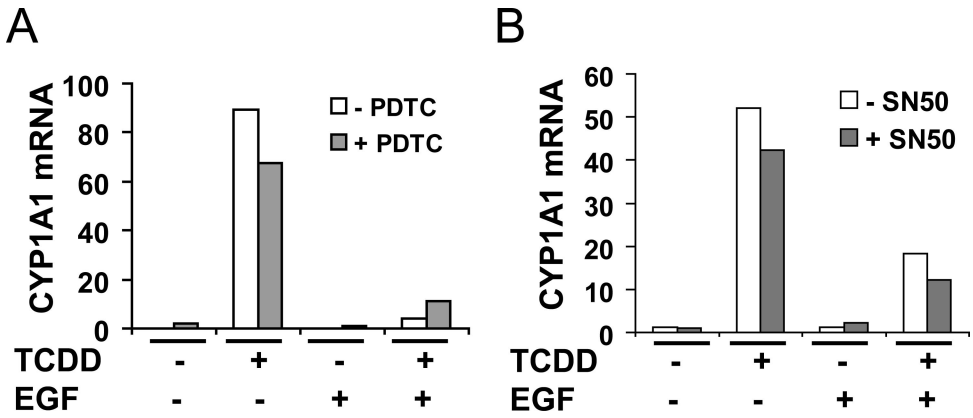

Fig. S4. Inhibitors of NF-kB do not prevent EGF-mediated repression of CYP1A1 transcription. (*A*) NHEKs were grown to confluence before basal medium was added for 24 h. Ammonium pyrrolidinedithiocarbamate (PDTC, 100 μM) was then added 30 min prior to EGF (10 ng/mL). After 1 h, the cells were treated with either 0.1% DMSO or TCDD (10 nM) for 16 h. Real-time PCR was used to determine the relative expression of CYP1A1. (*B*) NHEKs were grown to confluence before the inhibitory peptide SN50 (50 μg/mL) was added to the basal medium. After 15 min EGF (10 ng/mL) was added. After 26 h, the cells were treated with either 0.1% DMSO or TCDD (10 nM) for 13 h. Real-time PCR was used to determine the relative expression of CYP1A1.