SUPPLEMENTARY INFORMATION

AEG-1 regulates retinoid X receptor and inhibits retinoid signaling

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SUPPLEMENTARY MATERIAL AND METHODS

Generation of Alb/AEG-1 and AEG-1 KO mice:

Generation and characterization of a hepatocyte-specific AEG-1 transgenic mouse (Alb/AEG-1) have been described previously. To generate both conventional and conditional *AEG-1* knockout (KO) mice, an *AEG-1-KO* targeting vector was constructed. Contiguous *AEG-1* genomic regions were generated by high-fidelity PCR from 129/SvEv embryonic stem (ES) cell DNA and sequentially cloned into the NDTV targeting vector, which contains both a floxed neomycin phosphotransferase (*neo*) cassette for positive selection of homologous recombinants with the neomycin analog G418, and a diphtheria toxin A (DT-A) cassette for negative selection of non-homologous recombinants. The final *AEG-1-KO* targeting vector possesses a 5′ homology arm containing both the *AEG-1* promoter and exon 1, and a 3′ homology arm containing part of intron 1. The 5′ arm also has a *loxP* site inserted just 5′ of a 257-bp region of homology (71%) between the human and mouse promoters. The *AEG-1-KO* targeting vector was linearized with *Nhe* I and electroporated into 129/SvEv ES cells. ES cell clones resistant to G418 were screened for homologous recombination by Southern blot analysis using both 3′-flanking genomic and *neo* cassette probes. Retention of the introduced *loxP* site in homologous recombinants was verified by

PCR using a 5′-flanking primer in combination with a unique *loxP* primer. Two ES cell clones possessing the targeted *AEG-1* KO allele were injected into C57BL/6 blastocysts, which were then implanted into pseudopregnant CD-1 recipients. To generate the final conventional and conditional *AEG-1* KO alleles, chimeric males were bred to homozygous EIIa-*cre* females (Jackson Laboratory, stock number 003724). Agouti offspring were screened for the conventional KO allele with a 3′-primer PCR protocol using a common anti-sense primer (5′-CTTGAGATAGACATCTCTCTCTAC-3′) in combination with two sense primers: (5′-GAAGAATCTCAGAAGCGAGGAGC-3′) and (5′-GCTTAGCAAGAGTAGCTTATTCG-3′), specific for the wild-type and conventional KO alleles and generating PCR products of 397 bp and 577 bp, respectively. The conditional KO allele was detected with a 2-primer PCR protocol utilizing the sense primer (5'-GAAGAATCTCAGAAGCGAGGAGC-3') and the anti-sense primer (5′-CCTACTGGTTCTTACACTTTCCG-3′), generating PCR products of 176 bp and 223 bp for the wild-type and conditional KO alleles, respectively.

All animal studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University, and were conducted in accordance with the Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

Antibodies, Retinoids, Rexinoids, Inhibitors and other reagents:

Antibodies were obtained from the following companies: RXRα (D20), RXRαΔN (ΔN197), p-serine, Nucleolin (C23) and GAPDH from Santa Cruz Biotechnology; RXRβ and EF1α from Millipore; RXRγ, Myc-tag, HA-tag, HDAC3, Acetyl-Histone H3, SRC-1 and α-tubulin from Cell Signaling; β-actin from Abnova; and RXRβ from Thermo Scientific. The polyclonal antibody against AEG-1 was generated in-house. Eight retinoids and rexinoids, and eight different phospho-kinase inhibitors were used as listed in Table S2. They were prepared as per the manufacturer's instructions, and their details along with

concentrations are provided in the respective legends and Supplementary Table S2. Trichostatin A (TSA) (Cell Signaling) was used as per the manufacturer's instructions.

Plasmids and generation of deletion constructs for RXRα:

RARE and luciferase reporter plasmid (pGL3.*RARE*.luc) and expression constructs for SMRT and SRC-1 were kind gifts from Dr. Yoshitaka Hayashi, Nagoya University, Japan. Myc-tagged expression constructs for RXRα and RXRβ, were obtained from Origene. Expression constructs for full-length AEG-1, AEG-1 deletion mutants and AEG-1shRNA have been described before (1, 2). LXXLL mutant construct was generated using GeneArt site-directed mutagenesis kit (Invitrogen) with AEG-1 expression construct and primer 5′CTGCGGGAAAAGCAC3′ (mutated bases are underlined). For deletion constructs of RXRα, primers were designed flanking the AF-1, DBD and LBD domains separately to amplify these domains by PCR. PCMV6-Myc tag-RXRα expression plasmid was cut with SgfI-MluI and each domain was cloned into this site, respectively and was checked for desired protein size by western blotting.

Immunoprecipitation and Western Blotting:

Experiments were performed as described previously (1, 2). Whole cell lysates were prepared in cell lysis buffer (Cell Signaling) according to manufacturer's protocol. The lysates were subjected to immunoprecipitation using anti-RXRα, anti-RXRαΔN (rabbit polyclonal; 1:200) and anti-p-serine antibodies (mouse monoclonal; 1:200) from Santa Cruz Biotechnology; anti-RXRβ (Millipore; rabbit polyclonal 1:200); anti-Myc (Cell Signaling, rabbit monoclonal, 1:500) and anti-HA (Covance, mouse monoclonal, 1:500), and immunoblotting using anti-AEG-1 antibody as described. In reverse experiment for IPs, anti-AEG-1 antibody was used at 1:500-1000. Five percent or equal quantity of total cell lysates was used as inputs.

For western blots, the primary antibodies were AEG-1 (chicken polyclonal, 1:3000), RXRα (Santa Cruz

Biotechnology, rabbit polyclonal, 1:200); RXRβ (Millipore, rabbit polyclonal, 1:400); RXRαΔN (Santa Cruz Biotechnology, rabbit polyclonal, 1:400), p-serine (Santa Cruz Biotechnology, mouse monoclonal; 1:200); Myc-tag, HDAC3 and α-tubulin (Cell Signaling; rabbit monoclonal; 1:1000); HA-tag (Covance, mouse monoclonal, 1:1000), Nucleolin (Santa Cruz Biotechnology, mouse monoclonal, 1:200) and GAPDH (Santa Cruz Biotechnology, goat polyclonal, 1:400), β-actin (Abcam, mouse monoclonal, 1:2000); EF1α (Millipore, mouse monoclonal, 1:1000).

Transient transfection and luciferase reporter assays:

Transfections were carried out using lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol for human HCC cells as described elsewhere (1-3). For transfections in primary hepatocytes, $1X10^5$ mouse hepatocytes were plated in 24-well collagen coated plates and the next day transfected using Promofectin-Hepatocyte transfection reagent in 9:1 ratio of respective luciferase reporter plasmid containing responsive elements (pGL3.luc and pGL3.*RARE*.luc) and renilla luciferase reporter plasmid (pGL3.renilla.luc) with and without transcription factors. After 24-48 hours, cells were incubated in presence or absence of respective ligands for another 24 hours. Luciferase assays were measured using Dual Luciferase Reporter Assay kit (Promega) following manufacturer's protocol and firefly luciferase activity was normalized by renilla luciferase activity. Each experiment was performed in triplicates and three times to calculate means and standard deviations.

Nuclear and Cytoplasmic Fractionation:

Nuclear and cytoplasmic extractions were performed using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) as per the manufacturer's protocol.

Immunohistochemistry and Immunofluorescence:

Immunohistochemistry was performed using formalin-flixed paraffin embedded (FFPE) sections as described previously (3-5). The sections were blocked in PBST using 10% normal goat serum for rabbit

polyclonal antibody, 10% normal horse serum for mouse monoclonal antibody and 10% normal rabbit serum for goat polyclonal antibody. Primary antibodies were diluted in PBST containing 5% corresponding blocking serum. The primary antibodies used were: AEG-1 (chicken polyclonal; 1:500), PCNA and Cleaved caspase-3 (Cell signaling; mouse and rabbit monoclonal, respectively; 1:300). Secondary antibodies were diluted in PBST containing corresponding 2.5% blocking serum. The signals were developed by avidin-biotin-peroxidase complexes with a DAB substrate solution (Vector laboratories).

Immunofluorescence was performed as described (2, 6, 7). For Immunostaining, human HCC cells were grown on four chambered non-coated as well as collagen-coated slides (BD BioCoat collagen type I, BD Biosciences), whereas mouse hepatocytes were always plated on the collagen-coated slides. Afterwards, cells were fixed with 4% paraformaldehyde, and incubated with primary antibodies AEG-1 (chicken polyclonal; 1:500), RXRα and RXRαΔN (Santa Cruz, rabbit polyclonal, 1:50); RXRβ (Millipore, rabbit polyclonal, 1:100); RXRβ (Thermo scientific, mouse polyclonal, 1:200); and the secondary antibodies were Alexa flour 488-conjugated anti-mouse IgG and Alexa flour 568-conjugated anti-chicken IgG (Molecular Probes; 1:400). The slides were mounted in VectaShield fluorescence mounting medium containing DAPI (Vector Laboratories). Images were analyzed using a Zeiss confocal laser-scanning microscope.

SUPPLEMENTARY REFRENCES

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LEGENDS FOR SUPPLEMENTARY FIGURES AND TABLES

Supplementary Fig. S1: Construction of RXRα deletion constructs.

(A) Schematic of deletion constructs of RXRα. Different domains are illustrated and deletion constructs harboring particular domain and respective amino acids have been shown. (B) Authenticity of the generated constructs was checked by transfecting all the constructs into HEK293 cells, and the expression of the protein products was analyzed in the cell lysates by western blotting using anti-Myc antibody.

Supplementary Fig. S2: AEG-1 overexpression inhibits while AEG-1 knockdown increases RARE reporter activity.

(A) Hep-PC-4 (PC-4) and Hep-AEG-1-14 (AEG1-14) cells were transfected with RARE luciferase reporter plasmid (*RARE*.Luc) and after 24 h treated with indicated doses of ATRA, 9CRA, TTNPB and BT for 24 h at which point, cells were harvested for luciferase assays.

(B) RARE activity was measured after 48 hours of co-transfections of pcDNA3.1 vector, AEG-1FL and siAEG-1 constructs in HepG3 and HEK-293 cells in the presence or absence of the indicated ligands.

(D) RARE activity was measured 48 hours after co-transfection of pcDNA3.1, AEG-1FL, AEG-1 deletion mutants N1 (71-582) and N2 (101-582), and AEG-1 LXXLL-mut constructs in HEK-293 cells.

9CRA abbreviates 9-cis Retinoic Acid, ATRA, All-trans Retinoic Acid and BT, Bexarotene. All data represent mean ± SEM of three independent experiments with significant *p*-values mentioned in their respective panels or *:$p<0.04$ and **: $p<0.001$.

Supplementary Fig. S3: AEG-1 upregulation provides protection whereas AEG-1 inhibition sensitizes the cells to retinoids and rexinoids-induced cell death.

(A-H) MTT assays were performed to determine cell viability in Hep-PC-4, Hep-AEG1-14 and Hep-AEG-1si cells after treatment with the indicated retinoic (RA) and rexinoic acids (RXA) at the indicated concentrations and durations from 48 to 96 h. The graphs are represented for 9CRA (A), ATRA (B), Docosahexaenoic acid or DH (C), LG100268 or LG (D), SR11237 or SR (E), Fenretinide or FEN (F), Bexarotene or BT (G), and TTNPB (H).

(I-P) Cell viability was assessed in WT and AEG1-KO mice hepatocytes upon treatment with different

RA and RXA at the indicated doses doses and time points between 48 to 96 h. Graphs are shown for 9CRA (I), ATRA (J), DH (K), LG (L), SR (M), FEN (N), BT (O), and TTNPB (P).

(Q) QGY-7703 and HepG3 cells were transiently transfected with control and AEG-1 siRNA and treated with the indicated ligands for 48 hours.

Cell viability of the control untreated cells was considered as 100%. All data represent mean ± SEM of three independent experiments. *:$p< 0.02$.

Supplementary Fig. S4: AEG-1 downregulates RXR target genes by modulating RXR binding.

(A-C) qPCRs were performed to assess the mRNA levels of RAR/RXR downstream genes upon 24 h of 2μM 9CRA or 5μM ATRA treatment. *CYP26A1* and *CRABP2* mRNA levels were measured in Hep-PC-4 and Hep-AEG1-14 cells (A), *Hoxa1* and *Tll1* in WT and Alb/AEG-1 hepatocytes (B), and *Tll1* in WT and AEG-1KO mice hepatocytes (C).

(D) Quantification of *RARB* mRNA in five different sets of WT and Alb/AEG-1 mice hepatocytes. Average expression level is provided in the right panel.

(E) ChIP assays were performed using anti-RXRα, anti-Acetyl Histone H3 (AHH3) and anti-SRC-1 antibodies in PC-4, AEG1-14, AEG-1si cells treated or not with 5μM 9CRA and 2μM ATRA, and PCR primers amplifying the promoter fragments. Micrographs in the right panel represented the chromatin-bound *HOXA1* after normalization with respective inputs.

For A-D, all the genes were normalized using *GAPDH* as an endogenous control. All data represents mean ± SEM of three independent experiments. where *:$p<0.02$ and **: $p <0.001$.

Supplementary Fig. S5: AEG-1 controls localization of RXRs.

(A) Representative confocal photomicrographs to analyze co-localization of AEG-1 and RXRs using anti-AEG-1 and anti-RXRαΔN antibodies in PC-4/ AEG1-14 cells with or without treatment with 9CRA for 2 hours.

(B) Confocal images showing respective negative controls using RXRα, RXRβ and RXRαΔN antibodies but no secondary antibody in Hep-PC-4 and Hep-AEG-1-14 cells.

Supplementary Fig. S6: RXR localization is determined by the expression level and localization of AEG-1.

(A-C) Representative confocal micrographs of double immunofluorescence analysis showing co-localization of AEG-1 and RXRs in Hep-AEG1-14 cells using RXRα, RXRβ and RXRΔN antibodies. Panel A shows cells where AEG-1 is present in both nucleus and cytoplasm though dominant in cytoplasm. RXRs showed immunostaining in both the compartments with more in nucleus. Panel B shows cluster of cells where AEG-1 is present either in both compartments or exclusively in the cytoplasm, and RXRs are following the same pattern. Panel C shows cluster of cells where AEG-1 is predominantly or completely cytoplasmic, so is RXRα and RXRβ.

(D-E) Representative confocal micrographs of double immunofluorescence analysis for AEG-1 and RXRs co-localization in confluent cultures of Hep-PC-4 and Hep-AEG1-14 cells demonstrating that even at the confluent to sub-confluent condition, AEG-1 and RXRs remain in the nucleus.

(F-G) AEG-1 and RXRα subcellular localization upon maintaining the cells on collagen-fibronectin matrix or after synchronization.

Note: Here, we demonstrated that RXRs localization depends upon the AEG-1 expression level and culture conditions. Even though Hep-AEG-1-14 cells stably overexpress AEG-1, the distribution of AEG-1 varies among these cells. In some cells, where AEG-1 is more in cytoplasm along with presence in the nucleus as well, RXRα and RXRβ are equally distributed in both the compartments (Supplementary Fig. S7A). In some clusters of cells, most cells show exclusive cytoplasmic localization of AEG-1, while others show both cytoplasmic and nuclear localization and RXRs follow the same pattern (Supplementary Fig. S7B). In other clusters, both AEG-1 and RXRs are almost completely cytoplasmic (Supplementary

Fig. S7C). Besides, RXRs' subcellular localization is also regulated by cell culture conditions, but only in an AEG-1-dependent manner. When Hep-AEG-1-14 cells are non-confluent, only 20-35% cells showed dominant cytoplasmic co-localization of AEG-1 and RXRs (data not shown). As Hep-AEG-1-14 cells become sub-confluent to confluent, ~60% cells show complete to complete co-localization of AEG-1 and RXRs in the cytoplasm (Supplementary Fig. S7D-E). In contrast, such effects of cell density were not observed in control Hep-PC-4 cells even at confluent conditions (Supplementary Fig. S7D-E).

Supplementary Fig. S7: AEG-1 induces RXR phosphorylation.

(A) Western blotting was conducted to detect the expression levels of RXRα, RXRβ, RXRγ and AEG-1 in two other sets of WT and Alb/AEG-1 mice hepatocytes.

(B-C) Relative mRNA levels in four different sets of WT and Alb/AEG-1 mice hepatocytes for *Rxra* (B) and *Rxrb* (C).

(D) Nuclear and cytoplasmic extracts were obtained from PC-4 and AEG1-14 cells and phosphorylation levels of RXRα and RXRβ were determined by immunoprecipitating cell lysates with anti-RXRα and anti-RXRβ antibodies followed by WB with anti-phospho-serine (p-serine) antibody. Five percent of extracts were used as inputs.

(E) Inhibition of RXRα and RXRβ phosphorylation in Hep-PC-4 and Hep-AEG-1-14 cells upon treatment with inhibitors of various phospho-kinases such as PI3K, PKA, PKC, JNK, ERK and p38MAPK in DMEM containing 1% FBS. To assess the phospho-RXRs, respective lysates were subjected to immunoprecipitation (IP) with RXRΔN, RXRα and RXRβ antibodies followed by western blotting (WB) with p-serine antibody. Five percent of extracts were used as inputs.

(F-G) Graphical representations of the densitometric analysis of the inhibited levels of phospho-RXRα (E) and phospho-RXRβ (F) shown for Hep-PC-4 and Hep-AEG-1-14 cells

Selective inhibitors for PI3K, ERK, JNK, TK were used at 10 μM concentration and PKA, PKC and p38 MAPK at 1 μM for 16 hours in 1% charcoal striped FBS containing DMEM. Inhibited fold levels were

compared with untreated samples within Hep-PC-4 and Hep-AEG-1-14 cells and then also compared with each other. Data represents mean ± SEM of three independent experiments. *:p-<0.02 and **:p-<0.001.

Supplementary Fig. S8: Inhibition of multiple kinases reciprocates RXR phosphorylation retrieves the RARE promoter activity.

(A-B) HepG3 cells were transfected with *RARE*.luc luciferase construct along with pcDNA or AEG-1FL. Twenty four hours post-transfections, cells were treated with various phospho-kinase inhibitors for additional 24 hours and luciferase assay was performed. Basal luciferase activity (A) and luciferase activity after inhibitor treatment (B) are shown.

(C) RARE reporter activity in Hep-PC-4 and Hep-AEG1-14 cells after treatment with two increasing concentrations of ERK inhibitor, PD98059 showing maximum retrieval of activity.

(D) Expression of representative RXR/RAR target genes, *CYP26A1* and *FOXA1* upon treatment with different phospho-kinase inhibitors.

(E) RARE activity was measured in HepG3 and QGY-7703 cells after transient co-transfections of pcDNA3.1 vector, AEG-1FL, siAEG-1 or hRXRα constructs in the presence of indicated ligands

Selective inhibitors for PI3K, ERK, JNK, TK were used at 15 μM concentration and PKA, PKC and p38 MAPK at 2 μM. Firefly luciferase readings were normalized with respective renilla luciferase, and respective untreated cells with no inhibitors were considered as one. Data represents mean ± SEM of three independent experiments. *:p-<0.02 and **:p-<0.001.

Supplementary Table S1: Yeast-two hybrid screening unravels potential AEG-1 interacting partners.

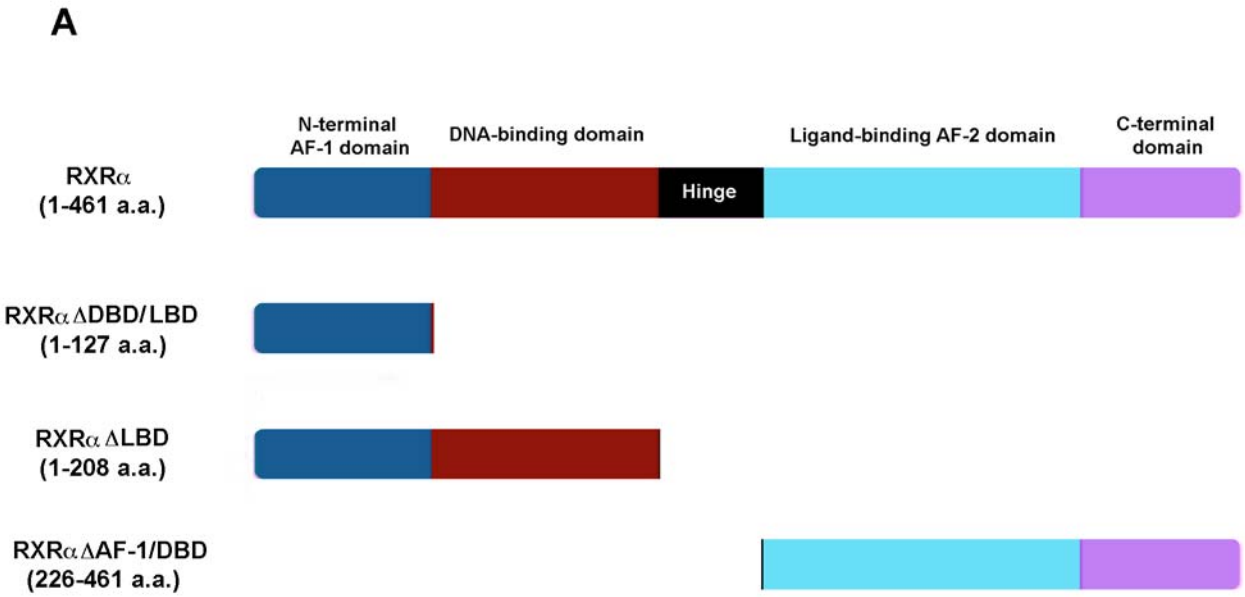
First fifty-seven amino acids of AEG-1 were used to screen the yeast two-hybrid library to search for possible AEG-1 interacting proteins.

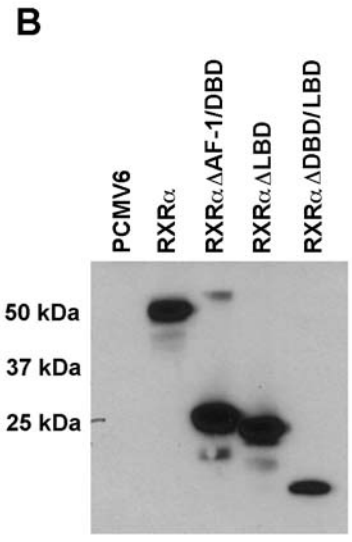
Supplementary Table S2: Details of retinoids, Rexinoids and phospho-kinase inhibitors used in this study.

The names and ligands for RAR, RXR, and agonists for both the receptors along with the concentrations used in this study have been given in this table. The bottom panel provides the details of phospho-kinases, respective ligands and concentrations.

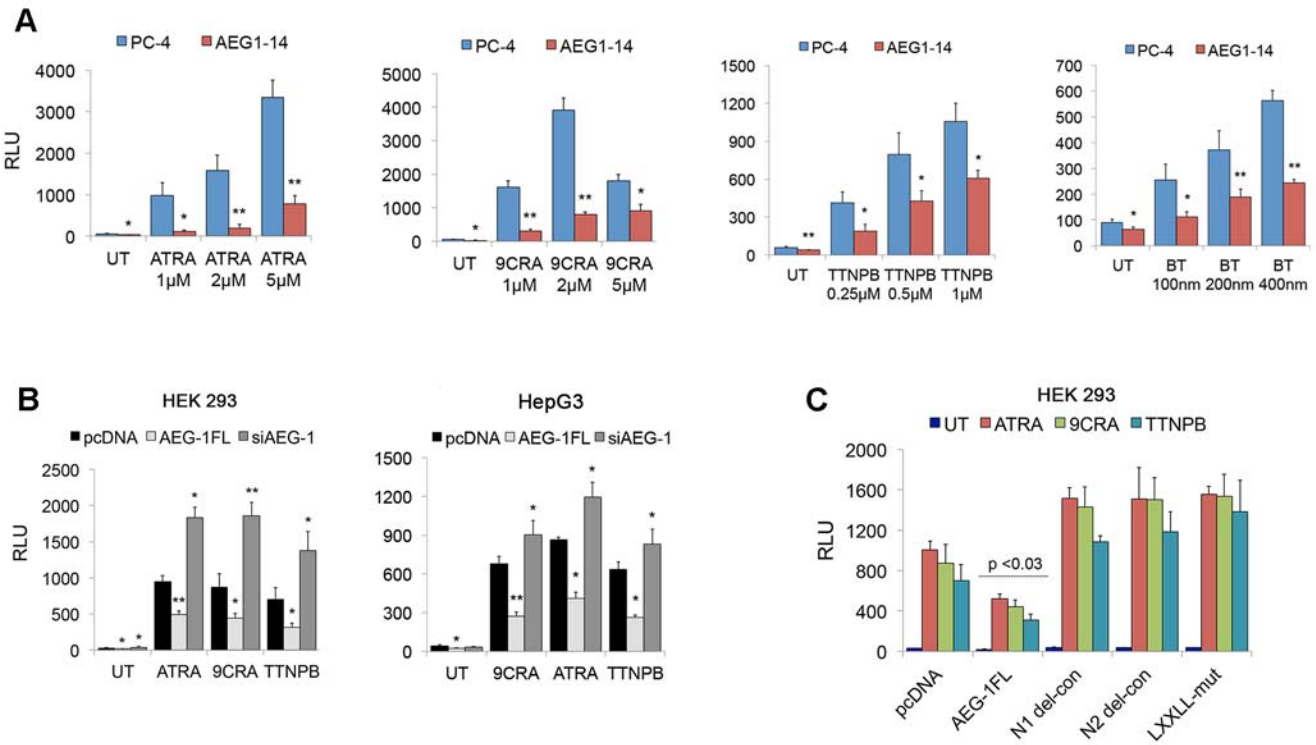
Supplementary Table S3: Details of primer sequences used to amplify the domain-specific deletion constructs of RXRα.

Supplementary Fig. S1





Supplementary Fig. S2



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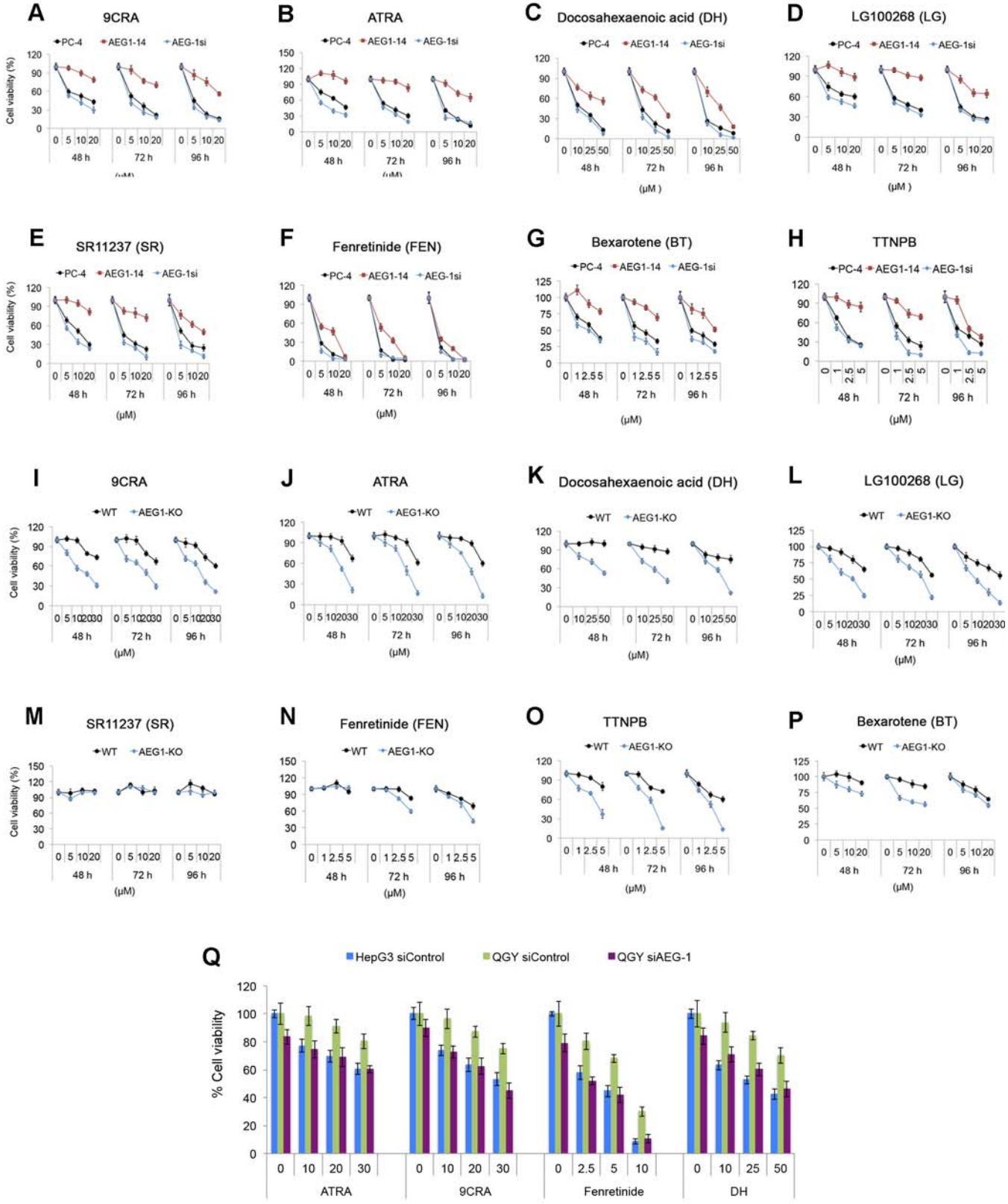

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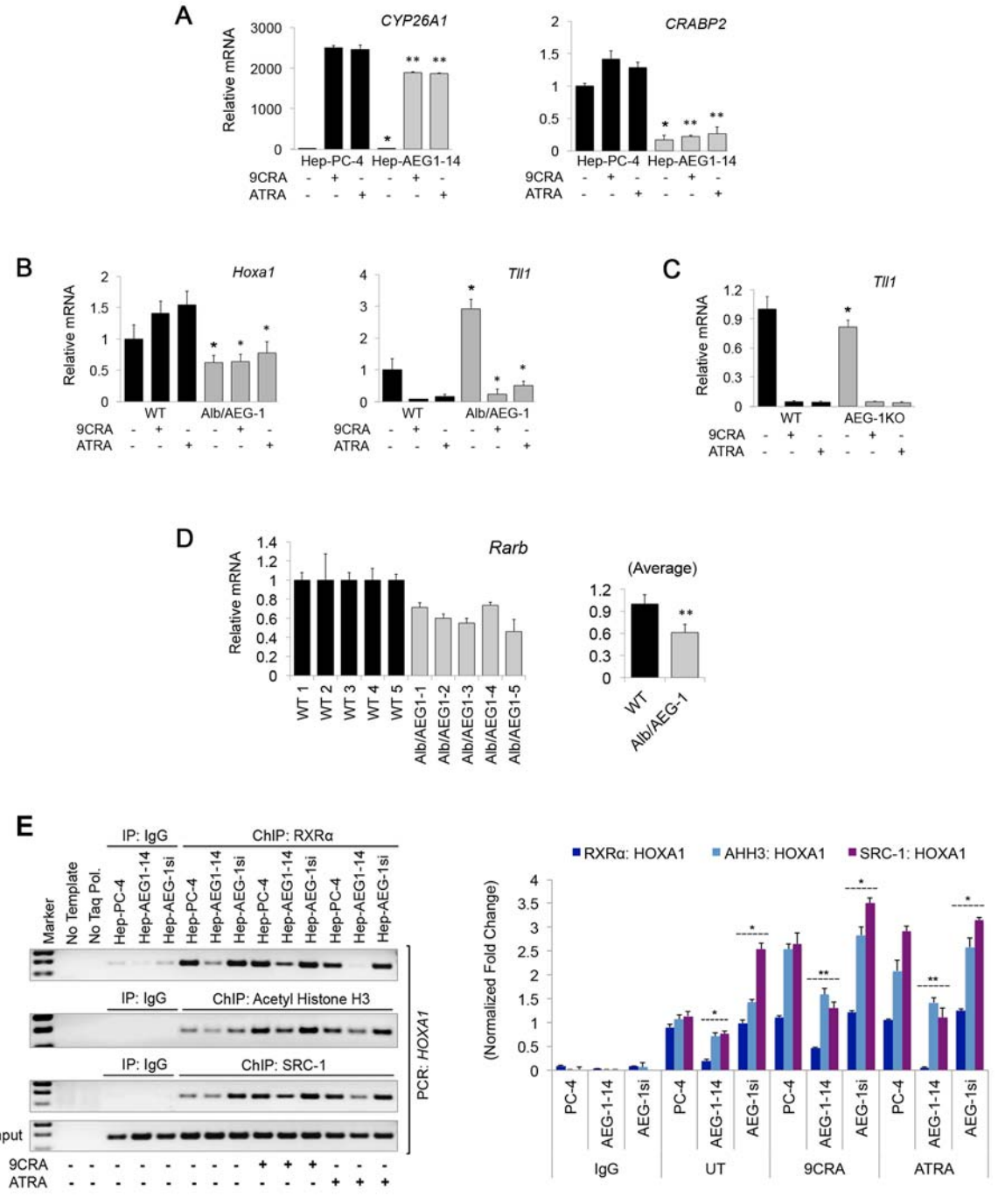

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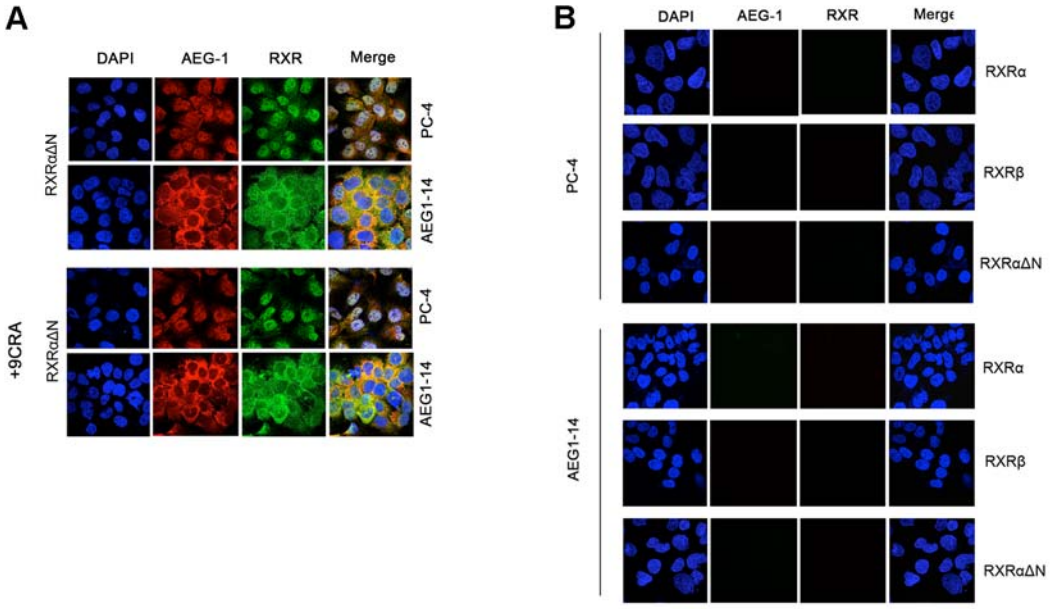

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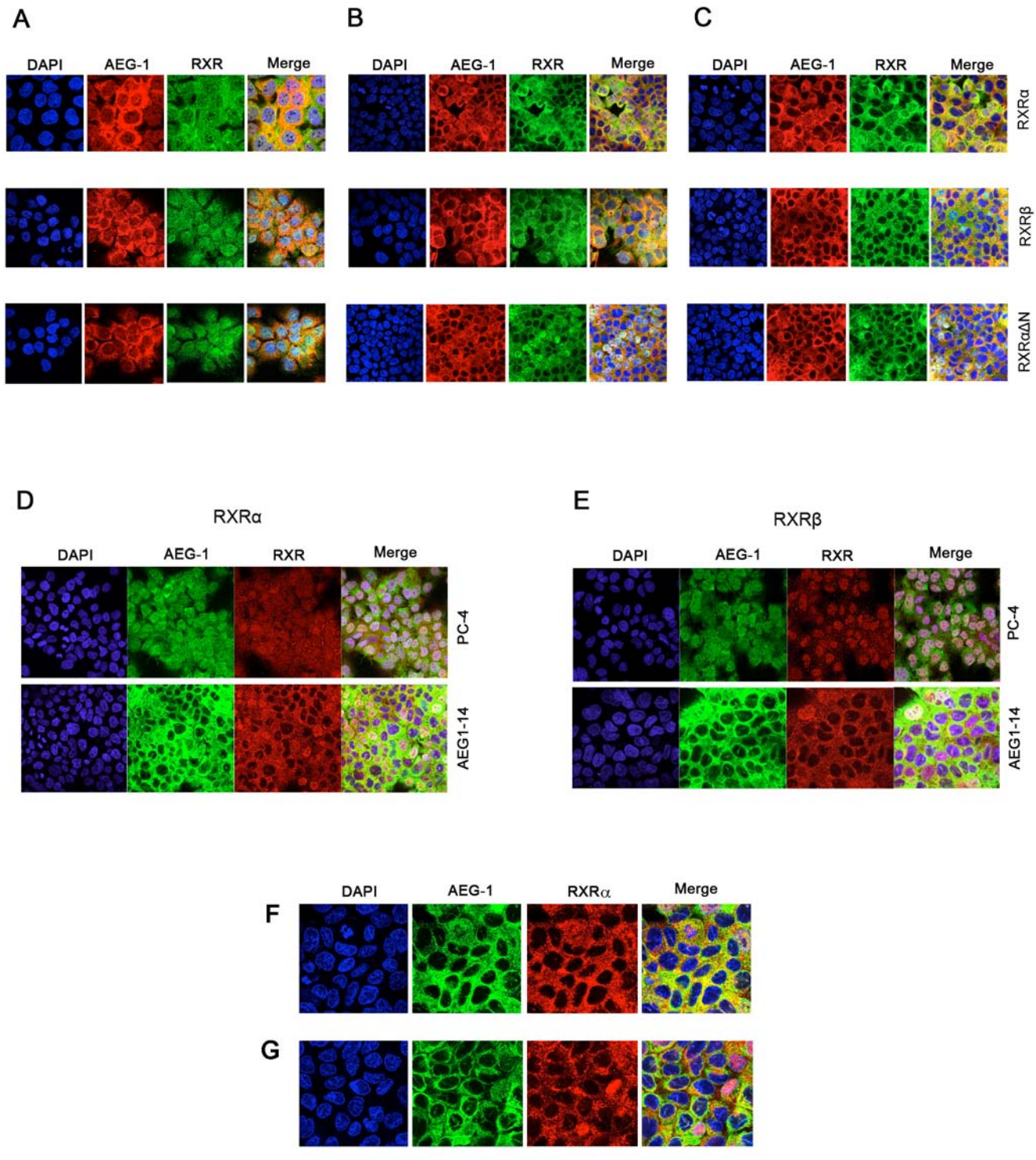
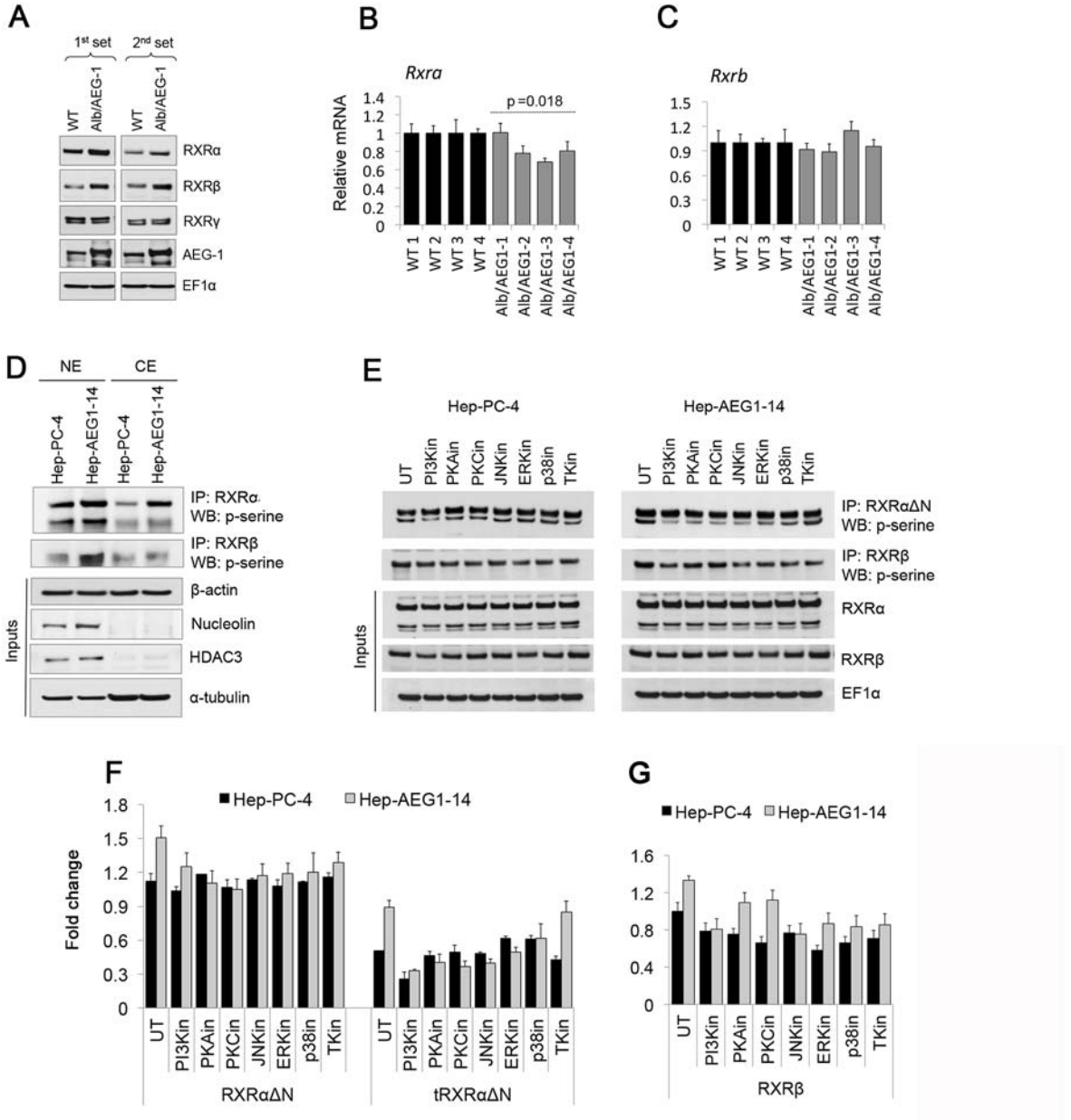
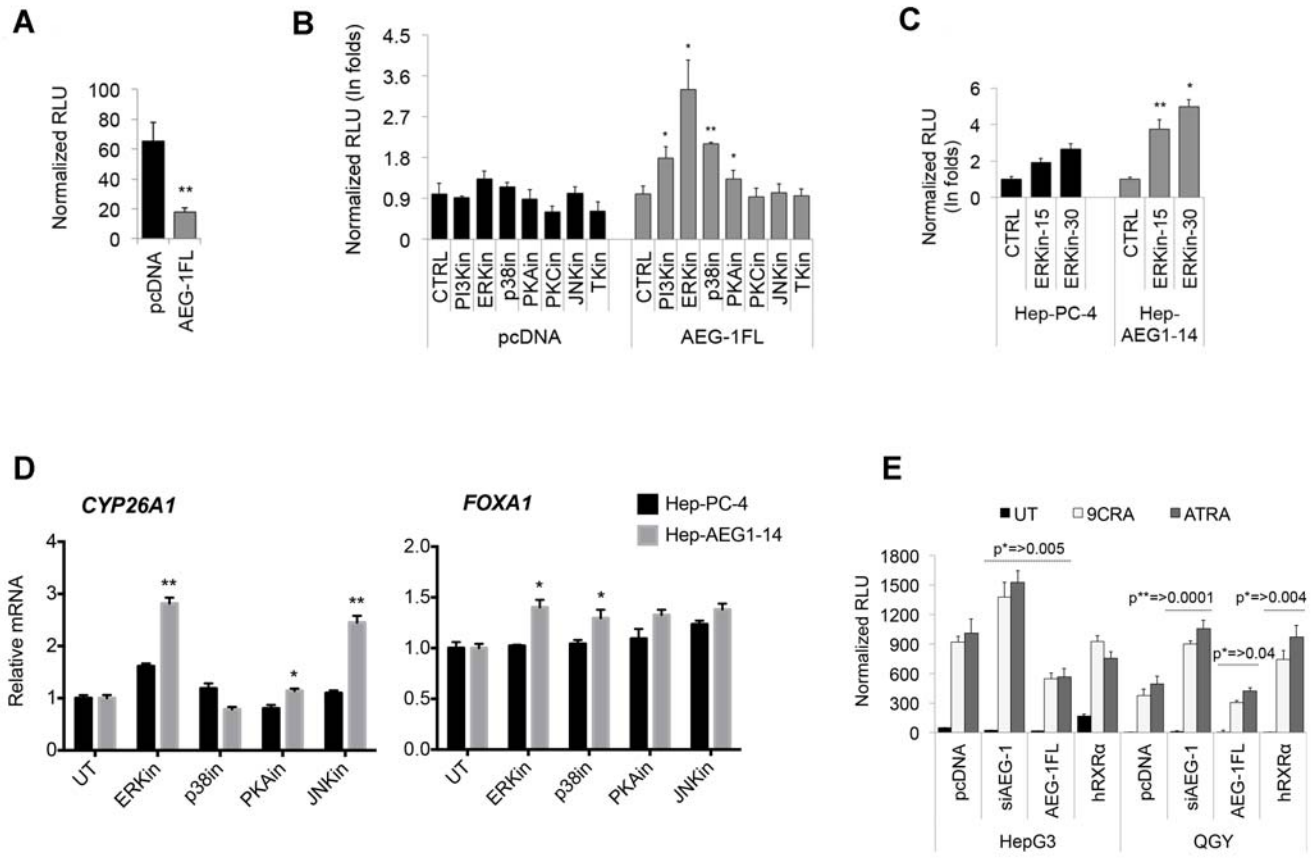


Supplementary Fig. S7



Supplementary Fig. S8



Mon Aug 18 13:31:09 CEST 2008 HYBRIGENICS CONFIDENTIAL Program = HLV_RP1 Screen = hgx1955v2 Vector = pB29 (N-bait-LexA-C fusion)

hgx001955 - Human AEG-1 (1-57) vs Human Liver RP1

Clone Name	Type	Seq	Contig(s) Name	Gene Name (I	GID	Global PBS	Additional Ge	Start	Stop	Frame	Orientation	UTR Inclusion	% Id 5p/3p
HLV_RP1_hg	5p		30589501	Homo sapiens	GID: 1094713	B	ARG1; [gi|10	141	No Data	IF	Sense		96.4
HLV_RP1_hg	5p	3p	30589501	Homo sapiens	GID: 1094713	B	ARG1; [gi|10	201	887	IF	Sense		99.4 / 98.9
HLV_RP1_hg	5p	3p	30589501	Homo sapiens	GID: 1094713	B	ARG1; [gi|10	201	887	IF	Sense		99.9 / 99.1
HLV_RP1_hg	5p	3p	30589501	Homo sapiens	GID: 1094713	B	ARG1; [gi|10	201	887	IF	Sense		96.2 / 100.0
HLV_RP1_hg	5p	3p	30589481	Homo sapiens	GID: 5833122	D	ATP11C; [gi|5	1896	2622	IF	Sense		99.9 / 99.9
HLV_RP1_hg	5p	3p	30589481	Homo sapiens	GID: 5833122	D	ATP11C; [gi|5	1896	2622	IF	Sense		99.9 / 100.0
HLV_RP1_hg	5p		30589524	Homo sapiens	GID: 9899177	D	CYP2C8; [gi|9	846	1430	IF	Sense		100
HLV_RP1_hg	5p	3p	30589477	Homo sapiens	GID: 1141551	B	DHCR24; [gi|	294	992	IF	Sense		99.3 / 100.0
HLV_RP1_hg	5p	3p	30589477	Homo sapiens	GID: 1141551	B	DHCR24; [gi|	363	1001	IF	Sense		100.0 / 99.7
HLV_RP1_hg	5p	3p	30589477	Homo sapiens	GID: 1141551	B	DHCR24; [gi|	363	1001	IF	Sense		100.0 / 99.7
HLV_RP1_hg	5p	3p	30589484	Homo sapiens	GID: 1339258	D	ITIH3; [gi|133	825	1083	IF	Sense		100.0 / 100.0
HLV_RP1_hg	5p	3p	30589484	Homo sapiens	GID: 1339258	D	ITIH3; [gi|133	825	1083	IF	Sense		100.0 / 100.0
HLV_RP1_hg	5p	3p	30589494	Homo sapiens	GID: 8699043	D	MARK2; [gi|8	1797	2161	IF	Sense		100.0 / 100.0
HLV_RP1_hg	5p	3p	30589494	Homo sapiens	GID: 8699043	D	MARK2; [gi|8	1797	2161	IF	Sense		100.0 / 100.0
HLV_RP1_hg	5p	3p	30589494	Homo sapiens	GID: 8699043	D	MARK2; [gi|8	1797	2161	IF	Sense		100.0 / 100.0
HLV_RP1_hg	5p	3p	30589487	Homo sapiens	GID: 3422209	C	PTGR1; [gi|34	204	974	IF	Sense		98.7 / 99.8
HLV_RP1_hg	5p	3p	30589487	Homo sapiens	GID: 3422209	C	PTGR1; [gi|34	216	926	IF	Sense		99.7 / 99.3
HLV_RP1_hg	5p	3p	30589519	Homo sapiens	GID: 4506358	D	QDPR; [gi|45	-31	274	IF	Sense		98.7 / 99.0
HLV_RP1_hg	5p		30589519	Homo sapiens	GID: 4506358	D	QDPR; [gi|45	-31	No Data	IF	Sense		98.7
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1546	IF	Sense		94.2 / 83.2
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.9 / 97.9
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.9 / 97.8
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		96.7 / 97.9
HLV_RP1_hg	5p		30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	No Data	IF	Sense		97.4
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.7 / 97.5
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.9 / 97.7
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.3 / 97.9
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.5 / 92.7
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.7 / 97.5
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.6 / 98.1
HLV_RP1_hg	5p	3p	30589506	Homo sapiens	GID: 2743694	D	RXRB; [gi|27	861	1547	IF	Sense		97.7 / 97.5
HLV_RP1_hg	5p	3p	30589498	Homo sapiens	GID: 6298832	D	TNN; [gi|629	2238	2648	IF	Sense		99.5 / 99.0
HLV_RP1_hg	5p	3p	30589498	Homo sapiens	GID: 6298832	D	TNN; [gi|629	2238	2648	IF	Sense		99.5 / 99.5
HLV_RP1_hg	5p	3p	30589490	Homo sapiens	GID: 1485969	D	TRIM8; [gi|14	267	964	IF	Sense		99.7 / 99.7
HLV_RP1_hg	5p		30589490	Homo sapiens	GID: 1485969	D	TRIM8; [gi|14	267	No Data	IF	Sense		99.4
HLV_RP1_hg	5p		30589490	Homo sapiens	GID: 1485969	D	TRIM8; [gi|14	267	No Data	IF	Sense		96.6
HLV_RP1_hg	5p	3p	30589526	Homo sapiens	GID: 2279789	D	unknown; [pr	-1	172	IF	Sense		99.4 / 100.0
HLV_RP1_hg	5p		30589522	Homo sapiens	GID: 6286548	D	unknown; [pr	-1	601	IF	Sense		100
HLV_RP1_hg	5p	3p	30589475	Homo sapiens	GID: 2195402	D	unknown; [pr	-1	678	IF	Sense		100.0 / 100.0

Summary of PBS categories

A : Very high confidence in the interaction

B : High confidence in the interaction
C : Good confidence in the interaction
D : Moderate confidence in the interaction
This category is the most difficult to interpret because it mixes two classes of interactions :
  * False-positive interactions
  * Interactions hardly detectable by the Y2H technique (low representation of the mRNA in the library, prey folding, prey toxicity in yeast...)
E : Interactions involving highly connected prey domains, warning of non-specific interaction. The threshold for high connectivity is 10 for screens with Human, Mouse, Drosophila and Arabidopsis and 6 for all other organisms. They can be classified in different categories:
  * Prey proteins that are known to be highly connected due to their biological function
  * Proteins with a prey interacting domain that contains a known protein interaction motif or a biochemically promiscuous motif
F : Experimentally proven technical artifacts
N/A : The PBS is a score that is automatically computed through algorithms and cannot be attributed for the following reasons :
  * All the fragments of the same reference CDS are antisens
  * The 5p sequence is missing
  * All the fragments of the same reference CDS are either all OOF1 or all OOF2
  * All the fragments of the same reference CDS lie in the 5' or 3' UTR

	Retinoids and Rexinoids	Ligand(s)	Source	Concentration used	
				For luciferase assays	For viability assays
1	9-cis retinoic acid (9CRA)	RXR, RAR	Sigma, Tocris Biosciences	2 μM	5-30 μM
2	All-trans retinoic acid or Tretinoin (ATRA)	RAR	Sigma	5 μM	5-30 μM
3	TTNPB or Arotinoid acid	RAR	Sigma	1 μM	0.25-5 μM
4	Bexarotene or Targretin (BT)	RXR	Sigma	0.4 μM	0.25-5 μM
5	Docosahexaenoic acid (DH)	RXR	Tocris Biosceinces		10-50 μM
6	Fenretinide (FEN)	RAR, RXR	Tocris Biosceinces		5-20 μM
7	SR11237 or BMS 649 (SR)	RXR	Sigma		5-20 μM
8	LG100268 or LG268 (LG)	RXR	Sigma		5-30 μM

	Phospho-kinase inhibitors	Inhibits		Concentration used
1	PD98059	ERK	Sigma	10-20 μM
2	U0126 monoethanolate	ERK or p44/42	Sigma	10-20 μM
3	LY294002	PI3K/AKT	Sigma	10-20 μM
4	SB 203580	p38 MAPK	Sigma	1-2 μM
5	H-89	PKA	Sigma	1-2 μM
6	Go 6983	PKC	Sigma	1-2 μM
7	SP 600125	JNK	Sigma	10-20 μM
8	Genistein	Tyrosine Kinase (TK)	Sigma	10-20 μM

Supplementary Table 3: Primer sequences used to amplify the domain-specific deletion constructs of RXRα

(1) RXRα ΔDBD/LBD:

F 5′ GCGATCGCCATGGACACCAAACAT 3′

R 5′ ACGCGTAGCCATGTTTCCTGAGGGGTG 3′

(2) RXRα ΔLBD:

F 5′ GCGATCGCCATGGACACCAAACAT 3′

R 5′ ACGCGTCTGCCGCTCCTCCTGCACGGC 3′

(3) RXRα ΔAF-1/DBD:

F 5′ GCGATCGCCATGGAGGACATGCCGGTG 3′

R 5′ ACGCGTAGTCATTTGGTGCGGCGC 3′