Supplementary Information

Materials and Methods

Quantitative RT-PCR

To analyze the expression of CYP1A1, real time PCR analysis using TaqMan probes was performed. The following primers were used: CYP1A1 sense 5'-TCAAAGAGCACTACAGGACATTTG-3' and antisense 5'-GGGTTGGTTACCAGGTACATGAG-3'; β-Actin sense 5'-GACAGGATGCAGAAGGAGATTACTG-3' and antisense 5'-GCTGATCCACATCTGCTGGAA-3'. The sequences of TaqMan probes to detect the PCR products were: CYP1A1 (labeled with FAM) 5'-AAGGGCCACATCCGGGACATCA-3'; β-Actin (labeled with VIC) 5'-ATCAAGATCATTGCTCCTCCTGAG-3'. The reaction was performed at 95°C for 3 min followed by 50 cycles of two-step PCR at 95°C for 15 sec (annealing) and 55°C for 60 sec (extension). PCR products were analyzed by LightCycler 480 (Roche diagnostics).

Cloning of AIP and *in vitro* binding assay

AIP was cloned using a yeast two-hybrid screening kit (Matchmaker III, Clontech) with $G\alpha_{13}$Q226L as bait. To perform an *in vitro* binding assay, recombinant proteins of Gα subunits were produced in *Sf*9 cells and purified as described before (Kozasa, 2004). GST-AIP was expressed in *E. coli* and purified with glutathione Sepharose 4B (GE healthcare). An *in vitro* binding assay was performed as described before (Meigs *et al.*, 2001). One hundred pmol of $G\alpha_{13}$ was pretreated with 10 μM GTPγS or 100 μM GDP for 2 hr at 30^0C. Then, 10 pmol of GST-AIP-Full, GST-AIP-N or GST-AIP-C bound to

glutathione Sepharose 4B was incubated with the aliquots of GTPγS- or GDP-loaded recombinant Gα13 in the absence or presence of 100 pmol of Gβγ in 200 μl of binding buffer (50 mM Hepes-NaOH (pH 8.0), 1 mM EDTA, 3 mM DTT, 0.05% Lubrol-PX, 10 mM $MgSO_4$, 1 mM PMSF, 1 μg/ml leupeptin) for 3 hr at 4 ^{0}C, followed by washing three times with binding buffer. The precipitates were resolved by SDS-PAGE and immunoblotted with each anti-Gα antibody or anti-GST antibody. In the case of other Gα families, all of experiments were done with GDP-loaded Gα subunits.

Immunoprecipitation and immunofluorescence analysis

HEK293T cells were transfected with the indicated combinations of plasmids including pCMV5-Myc-AIP, pCMV5-HA-AhR, and pCMV5-FLAG-Gα_{13}Q226L. Forty-eight hr after transfection, cells were lysed in a lysis buffer (20 mM HEPES-NaOH (pH 7.5), 3 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, 1 mM Na_3VO_4, 10 mM NaF, 20 mM β-glycerophosphate, 1 mM PMSF, 1 μg/ml leupeptin, 1 mM EGTA, and 0.5% Lubrol-PX), and cell lysates were used for immunoprecipitation with 1 μg of the anti-HA antibody (12CA5, Roche diagnostics) and protein-G Sepharose (GE healthcare), and the immunoprecipitates were analyzed by immunoblot with the anti-Myc (9E10, Babco) and anti-AhR (SA-210, Biomol) antibodies. For the immunofluorescence analysis, transfected COS-7 cells were stimulated by 1 μM 3-MC and/or 10 μM LPA for 24 hr and then fixed with 4% PFA in PBS for 20 min. After fixation, cells were permeabilized and sequentially stained with the anti-HA antibody and Alexa Flour 488-conjugated goat anti-mouse antibody (Molecular Probes).

GST pull-down assay

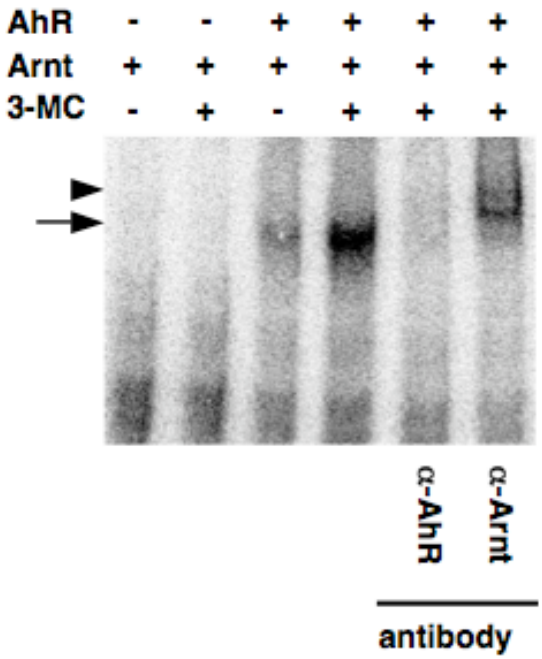
COS-7 cells were transfected with the indicated combinations of plasmids encoding HA-AhR, GST-Arnt, and Gα_{13}Q226L. Cells were starved for 12 hr and stimulated with

1 μM 3-MC in the presence or absence of 100 nM LPA for 3 hr. Cells were lysed in buffer A (10 mM HEPES-KOH (pH 7.8), 10 mM KCl, 0.1 mM EDTA, 0.1% NP-40, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml aprotinin, 2 μg/ml pepstatin, 2 μg/ml leupeptin). Nuclei were then isolated by centrifugation at 2,000 x g for 2 min. Isolated nuclei were resuspended in buffer C (50 mM HEPES-KOH (pH 7.8), 420 mM KCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 2% glycerol, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml aprotinin, 2 μg/ml pepstatin, 2 μg/ml leupeptin) and rotated at 4^0C for 30 min. Nuclear extracts were prepared by centrifugation at 15,000 x g for 15 min at 4^0C. For GST pull-down, the nuclear extracts were mixed with 10 μl of glutathione Sepharose 4B and rotated for 1 hr. Pulled-down protein complexes were washed with a lysis buffer and then eluted by the addition of the lysis buffer containing 40 mM glutathione. The eluted samples were analyzed by immunoblot using the anti-AhR antibody or anti-GST antibody (B-14, Santa Cruz Biotechnology).

Ubiquitination analysis

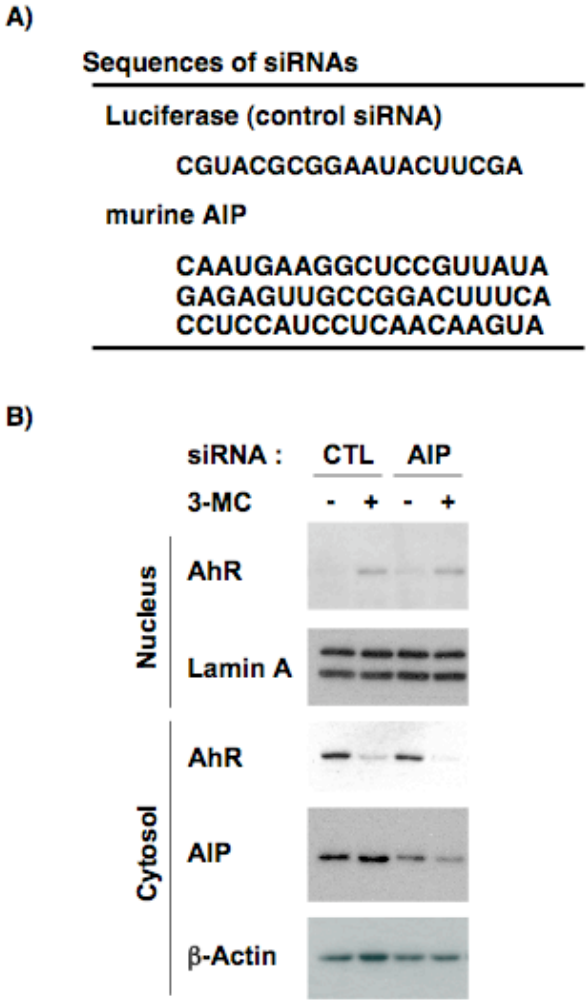
The ubiquitination analysis was performed as described previously (Treier, 1994). HEK293T cells transfected with the indicated combination of plasmids encoding HA-AhR, Myc-AIP, FLAG-$G\alpha_{13}$Q226L, and $[His]_6$-ubiquitin were lysed in 1 ml of urea lysis buffer (8 M urea, 0.1 M NaH_2PO_4, 10 mM Tris-HCl (pH 8.0), 10 mM imidazole, 10% glycerol, 0.1% Triton X-100, 0.5 M NaCl, 10 mM β-mercaptoethanol). The cell lysate was centrifuged at 15,000 rpm at room temperature for 10 min. The supernatants were mixed with Ni-NTA agarose (Qiagen) and rotated for 4 hr. After washing with urea washing buffer (urea lysis buffer including 20 mM imidazole), the precipitated proteins were eluted by the addition of elution buffer (200 mM imidazole, 5% SDS, 0.15 M Tris-HCl (pH 6.7), 30% glycerol, 0.72 M β-mercaptoethanol). Eluted proteins were analyzed by immunoblot using the anti-AhR antibody.

Supplemental Figures



Supplemental Figure 1

COS-7 cells transfected with the indicated combinations of plasmids were stimulated with 1 μM 3-MC for 6 hr. Nuclear extracts were analyzed by EMSA with the radioactively labeled AhR-binding element DNA probe. The arrow indicates the protein-DNA complex including AhR and Arnt. The arrowhead indicates a super-shifted band.



Supplemental Figure 2

(A) Hepa1c1c7 cells were transfected with the mixture of siRNAs to AIP or firefly luciferase. (B) Forty-eight hr after transfection, cells were harvested and lysed with hypotonic buffer (buffer A). After centrifugation, supernatant was analyzed as cytosolic fraction. Remained pellet was utilized as nucleus. The sample of each fraction was resolved by SDS-PAGE and analyzed by immunoblot. For immunoblot analysis, the anti-AhR, AIP, Lamin A, and β-actin antibodies were used respectively.

References

Kozasa T (2004) Purification of G protein subunits from Sf9 insect cells using hexahistidine-tagged α and βγ subunits. *Methods Mol Biol* **237**: 21-38

Meigs TE, Fields TA, McKee DD, Casey PJ (2001) Interaction of $G\alpha_{12}$ and $G\alpha_{13}$ with the cytoplasmic domain of cadherin provides a mechanism for beta-catenin release. *Proc Natl Acad Sci USA* **98**: 519-524

Treier M, Staszewski LM, Bohmann D (1994) Ubiquitin-dependent c-Jun degradation *in vivo* is mediated by the delta domain. *Cell* **78**: 787-798