

Title: Novel complex of HAT protein TIP60 and nuclear receptor PXR promotes cell migration and adhesion

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Figure legends

Supplementary Figure S1: Intracellular localization of RFP-TIP60 & GFP-PXR in various cell lines. Graphs show the percentage intracellular localization of RFP-TIP60 and GFP-PXR proteins in various cell lines as indicated. Cells were transfected with RFP-TIP60 and GFP-PXR plasmids alone or together and at least 100 transfected cells were counted in each case. Graphs represent the average values of three independent experiments with \pm SD

Supplementary Figure S2: Expression and purification of recombinant proteins from bacteria. *E.coli* BL21 DE3 codon plus cells were transformed with pET28a-TIP60 or pGEX6P2-PXR plasmids. IPTG was used to induce the production of recombinant proteins followed by affinity purification method as mentioned in material and method section. Coomassie gel shows the expression and purification profile of recombinant proteins. Asterisk shows the purified protein band of recombinant proteins.

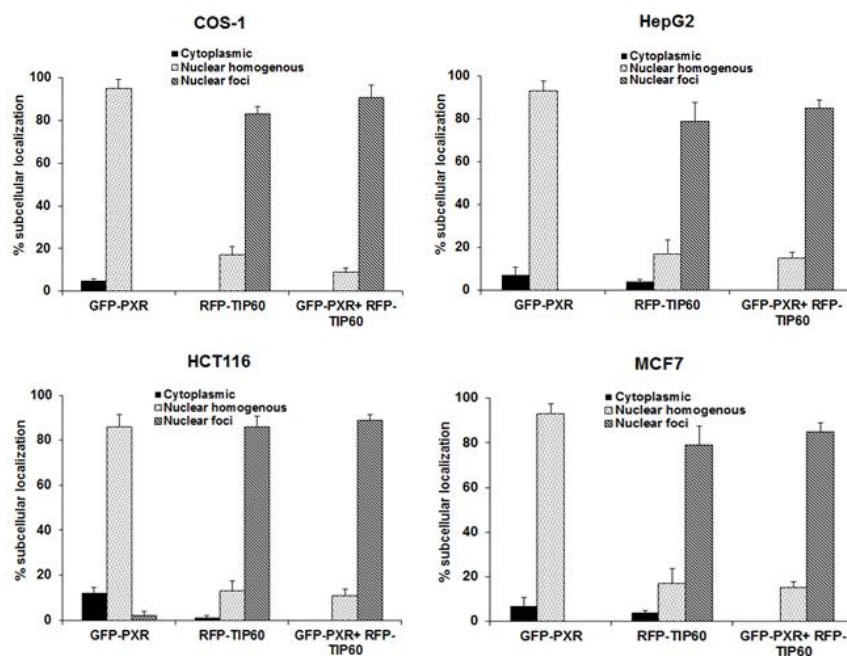
Supplementary Figure S3: Western blot for TIP60 knockdown. Cells were transfected with siTIP60 or siGL2 followed by transfection with GFP-PXR. Immunoblotting was performed using anti-TIP60 or anti- β actin antibody for the samples as indicated.

Supplementary Figure S4: Multiple sequence alignment of different members of class II NRs. Clustal omega analysis was performed to identify sequence similarity between different class II NRs protein sequence. Red highlighted residue shows the corresponding amino acids to PXR lysine 170 and yellow highlighted residue shows the corresponding amino acids to PXR 174-210 region in other class II NRs.

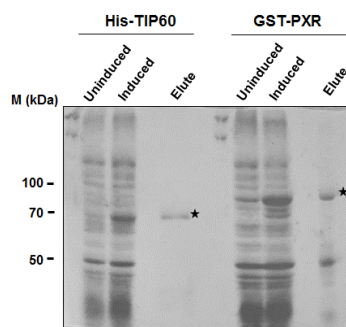
Supplementary Figure S5: Immunoblots showing expression of tagged proteins. (A) For promoter reporter assay cells were transfected with plasmids as indicated. Lysates of the transfected cells were resolved followed by Western blotting with the indicated antibody. (B), (C), (D) & (E) shows the expression of tagged proteins by immunoblotting by indicated antibody in scratch assay, cell adhesion assay, cell proliferation assay and cell invasion assay respectively.

Supplementary Figure S6: Full-length immunoblots. Full-length blot images with indicated antibody.

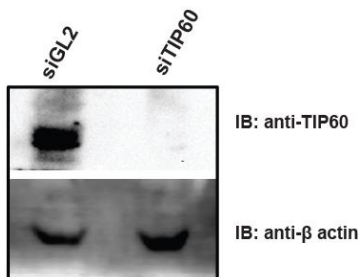
Supplementary Figure S1: Graphs depicting TIP60-mediated nuclear reorganization of unliganded PXR in various cell lines.



Supplementary Figure S2: Expression and purification of His-TIP60 and GST-PXR recombinant proteins from *E. coli*



Supplementary Figure S3: Western blot of TIP60 knockdown



Supplementary Figure S4: Multiple sequence alignment of class II NRs

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CAR human          -----MELLEEDLTCPICCSLFDDP-----
TSH human          -----
PPARα human       ---MVD---TESPLCPLSP-----LEAGDLESPLSEEFLQE-MGNIQ
PPARγ human       -----SSISTPHYED-----
RXR human          -----
PXR human          -----
VDR human          -----
FXR human          MVMQFQGLNPIQISPHCSCTPSGFFMEMMSMKPAKGVLTEQVAGPLGQNLEVEPYSQYS
LXR human          -----MS-SPTTSS---LDTPLPGNGPPQ-----

CAR human          -----RVL---PCSHNFCKKCLEGILEGSVRNSLWRPAPF-KCPTCRKETSATGINSLQV
TSH human          -----MEQ-----KP-----SKVEC--
PPARα human       EISQSI---GEDSSGSFGFTEYQY----LGSCPGSDGSVI-----TDTLSP--
PPARγ human       -IPFTR---TDP---VVADYKY----DLKLEQYQSAI-----KVPE--
RXR human          -----
PXR human          -----MEV-----RPKESWNHAD-----FVH--
VDR human          -----MEA-----
FXR human          NVQFPQVQPQISSSSYSNLGFYP----QQPEEWYSPGIYELRRMPAETLYQGTEV--
LXR human          -----PGAPS---SSPTVKE---EGPEPWPGGPDVPGTDEASSACSTDWV--

CAR human          NYSLKGIVEKYKIKISPKMPVCKGHLG---QPLNIFCLTDMQLICGICATRGE--HTK
TSH human          ---GSDPEENSARSPDGKRKRKNGQCSLKTSMGYIPSYLDKDEQCVVCGDKATGYHYR
PPARα human       ---ASSPSS---VTY--PVVPGS-----VDESPGALNIECRI CGDKASGYHYG
PPARγ human       ---ASPPYY---SEKTQLYNKP-----HEEPSNSLMAIECRVCGDKASGFHYG
RXR human          -----
PXR human          ---CEDTES---VPGKPSVNA-----D---EEVGGPQICRVCGDKATGYHFN
VDR human          ---MAASTS---LP---DPG-----D---FDRNVPRICGVCGDRATGFHFN
FXR human          ---AEM--P---VTKKPRMGAS-----A---GRIKGDLECVVCGDRASGYHYN
LXR human          ---IPDPEE---EPERKRKKGKGP-----A---PKMLGHEL CRVCGDKASGFHYN

CAR human          HVFCSIEDAYAQERDA-----FESLF---QSFETWRRGDALSRLDT-LETSKRKSLO
TSH human          CITCEGCKGFFRRTIQKNLHPTYSCY-DSCCVIDKITRNQCQLCRFKKCIAVGMAMDLV
PPARα human       VHACEGCKGFFRRTIRLK--LVYDKC--DRSCKIQKKNRNCQYCRFHKCLSVGMSHNAI
PPARγ human       VHACEGCKGFFRRTIRLK--LIYDRC--DLNCR IHKKS RNKCQYCRFQKCLAVGMSHNAI
RXR human          -----
PXR human          VMTCEGCKGFFRRAMKRN--ARLRCPFRKGACEITRKTRQCQACRLRKCLES GMKKEMI
VDR human          AMTCEGCKGFFRRSMKRK--ALFTCPFN-GDCRITKDNRRHCQACRLKRCVDIGMMKEFI
FXR human          ALTCEGCKGFFRRSITKN--AVYKCKN-GGNCVMDMYMRKQCERLRKCKEMGMLAECM
LXR human          VLSCEGCKGFFRRSVVRGGARRYACRG-GGTCQMDAFMRRKQCQRLRKC KEAGMREQCV

CAR human          LLTKDSDK--VKEFF-EKL-----QHTLD-----Q
TSH human          LDDSKRVA--KRKLIEQNRERRRKEEMIRSL-----Q
PPARα human       RFGMRPRS--EKAKLKAEILTC---EHDIED-----
PPARγ human       RFGRMPQA--EKEKLLAEISS---DIDQLN-----
RXR human          -----
PXR human          MSDEAVEE--RRALIKRKSERT---GTQPLGV-----
VDR human          LTDEEVQR--KREMILKRKEEEA---LKDSL-R-----
FXR human          YTGLLTEIQCKSKRLRKNVKQHADQ-TV---NE---DSEGRDLRQ---VTSTTKSCR
LXR human          LSEE---QIRKKKIRKQQESQSQ-SQSPVGPQGSSSSASGPGASPGGSEAGSQSGEG

CAR human          KKNEILSDFETMKLAVMQAYDPEINKLNTILQEQRFMAFNIAEAFKDVSEPIVFLQQMQEF
TSH human          QRPEPTPEEWDLIHIATEA-----HRSTNAQGSHWQ-----RKF
PPARα human       ---SETADLKS LAKRIYEA-----YLKNFNM--NKVK-----ARVI
PPARγ human       ---PESADLRALAKHLYDS-----YIKSFPL--TKAK-----ARAI
RXR human          -----
PXR human          --QGLTEEQRMMIRELMDA-----QMKTFTD--TFSH-----FKNF
VDR human          --PKLSEEQRRI IAILLDA-----HHKTYDP--TYS-----FCQF
FXR human          EKTELTDPDQQTLLHFIMD-----SYNK-----QR--
LXR human          EGVQLTAAQELMIQQLVAA-----QLQCNKR--SFSD-----QPKV

CAR human          REKIKVIKETPLPP-----SNLPASPLMKNFDTSQWE
TSH human          -LPDD-----IGQS
PPARα human       ---L-SGKA-----SNNPPFVIHDMETLCMA-----EKT LVAK
PPARγ human       ---L-TGKT-----TDKSPFVIYDMNSLMMG-----EDKIKFK
RXR human          -----

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FXR human RLPGLVSSGCELPESLQAPSREEAAKWSQVRKDLCSLKVSLQL-----RGEDGSVW-
VDR human RPPVRVNDGGGSHPSR--PNSRHTPSFSGDSSSSCSDHCITSSDMMSSSFSNLDLSEE-
FXR human -----MPQ-
LXR human -----TPW-

CAR human DIKLVVDVKLSLPQDTGTGTFISKI PWSFYKFLFLILLGLVIVFG--PTMFLEWSLFDDLA
TSH human PI-VSMPD-----GDKVDLEAFSEFTK-IITPAITRVVDFAKKLPMFSELPCEDQII
PPAR α human L-VANGIQ-----NKEAEVRI FHCCQC-TSVETVTELEFAKAI PGFANLDLNDQVT
PPAR γ human HITPLQEIQ-----SKEVAIRIFQGCQF-RSVEAVQEITEYAKSIPGFVNLDLNDQVT
RXR human -----PLDDQVI
FXR human NYKPPADS-----GGKEIFSLPHMAD-MSTYMFKGIISFAKVISYFRDLPIEDQIS
VDR human DSDDPSVT-----LELSQLSMLPHLAD-LVYSIQKVI GF AKMI PGFRDLTSEDQIV
FXR human EITNKILK-----EEFSAEENFILTE-MATNHVQVLFVFTKLPGFQTLDHEDQIA
LXR human PLGA-DPQ-----SRDARQQRFAHFTE-LAIISVQEI VDFAKQVPGFLQLGREDAQIA
*::

CAR human TWKGCLSNFSSYLTKTADFIEQSVFYWEQVTDGFFIFNERF--KNFTLVVLNNVAEFVCK
TSH human LLKGCCMEIMSLR-AAVRYDP-ESDTLTLIS-GEMAVKREQLKNGG-LGVVSDAIFELGKS
PPAR α human LLKYGVYEAIFAM-LSSVMN-KDGLMVAYG-NGFITREFLKS LRKPFCDIMEPKFDFAMK
PPAR γ human LLKYGVHEI IYTM-LASLMN-KDGVLI SEG-QGFMTREFLKS LRKPFDFMEPKFEFAVK
RXR human LLRAGWNELLIAS-FSHRSI AVK DGI--LLATGLHVHRNSAHSAGVGAIFDRVLTSLVSK
FXR human LLKGA AFELCQLR-FNTVFN-AETGTWECGRLSYCLE---DTAGGFQQLLEPMLKFHYM
VDR human LLKSSAIEVIMLR-SNESFT-MDDMSWTCGNQDYKYRVSVDVTKAGHSLELIEPLIKFQVG
FXR human LLKGS AVEAMFLR-SAEIFN-KKLP SG----HSDLLEERIRNSGISDEYITPMFSFYKS
LXR human LLKASTIEIMLLE-TARRYN-HETECITFL-KDFTYSKDDFHRAGLQVEFINPIFEFSRA
: : . : :

CAR human YKLL-----
TSH human LSAFNLDDEVALLQAVLLMSTDRSGLLCVVKIEKSQEAYLLAFEHYVNRKHNI--PHF
PPAR α human FNALELDDSDISLFVAAI ICCGDRPGLLN VGHIEKMQEGIVHVLRHLQSNHPDD--IFL
PPAR γ human FNALELDDSDLAIFIAVILS GDRPGLLN VKPIEDI QDNLLQALELQLKLNHPES--SQL
RXR human MRDMQMDKTELGCLRAIVL FNPDSKGLSNPAEVEALREKVYASLEAYCKHKYPEQ--PGR
FXR human LKKLQLHEEEYVLMQAISLFS PDRPGVLQHRVVDQLQEQAITLKS YIECNRPQPAHRFL
VDR human LKKLNLHEEEHVLLMAICIVSPDRPGVQDAALIEAI QDRLSNTLQTYIRCRHPPPGSHLL
FXR human IGELKMTQEEYALLTAIVILS PDRQYIKDREAVEKLQEPLLDVLQKLCCKIHQPEN--PQH
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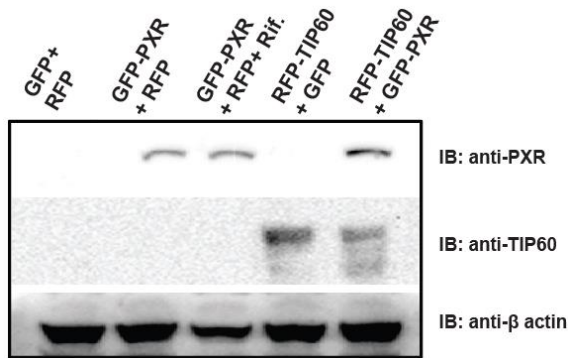
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TSH human WPKLLMKEREVQSSILYKGA AAAE---GRPGGSLGVHPEGQQLLGMHVVGPPQVRQLEQQL
PPAR α human FPKLLQKMDLRQLVTEHAQLVQI IKK-TESDAALHPLLQEIYRDMY-----
PPAR γ human FAKLLQKMTDLRQIVTEHVQLLQVIKK-TETDMSLHPLLQEIYKDLY-----
RXR human FAKLLRLPALRSIGLKCLEHLFFFKLIGD--TPIDTFMEMLEAPH-----
FXR human FLKIMAMTELRSINAQHTQRLLRIQD---IHPFATPLMQELFGITG-----
VDR human YAKMIQKLADLRSLNEEHSKQYRCLSFQPECSMKLTPLVLEVFGEI-----
FXR human FACLLGRLELRTFNHHHAEMLSWRV---NDHKFTPLLCEIWDVQ-----
LXR human FPRMLMKLVSLRTLSSVHSEQVFA LRL---QDKKLPPLLSEIWDVHE-----

CAR human -----
TSH human GEAGSLQGPVLQHQS PKSPQORLELLHRSGILHARAVCGEDDSSEADSPSSSEEEPEVC
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PPAR γ human -----
RXR human ---QMT-----
FXR human ---S-----
VDR human ---S-----
FXR human -----
LXR human -----

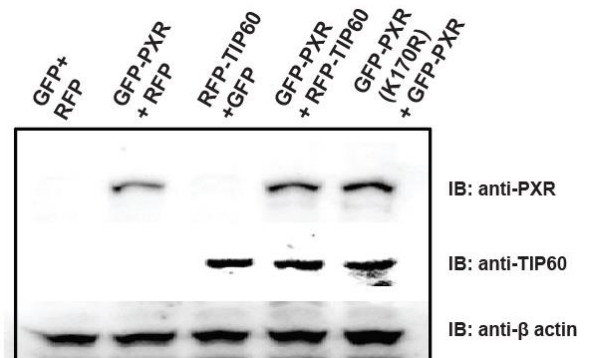
CAR human -----
TSH human EDLAGNAASP
PPAR α human -----
PPAR γ human -----
RXR human -----
FXR human -----
VDR human -----
FXR human -----
LXR human -----

Supplementary Figure S5: Immunoblots showing expression of tagged proteins.

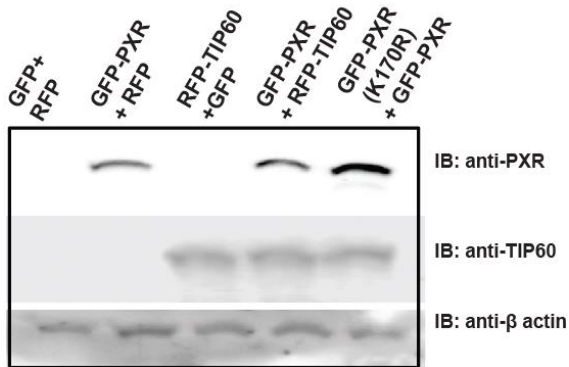
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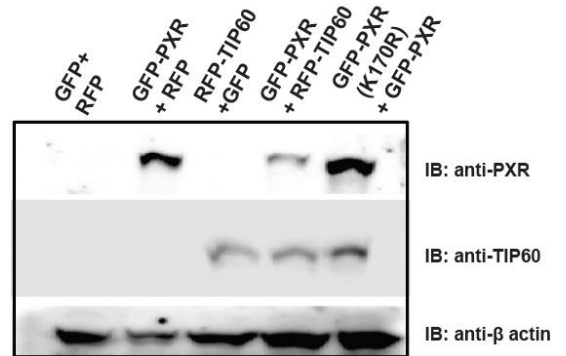
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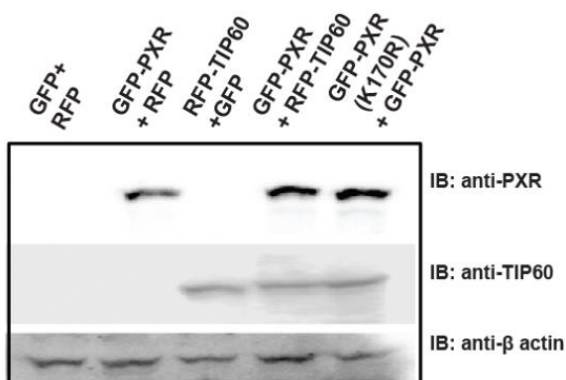
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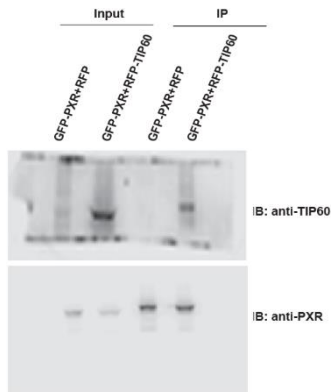


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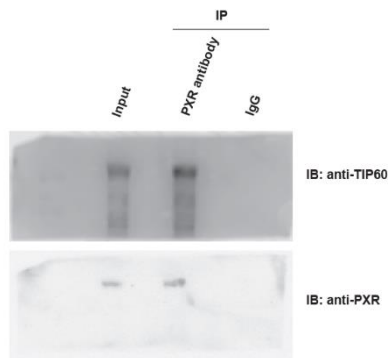


Supplementary Figure S6: Full-length immunoblots showing expression of proteins.

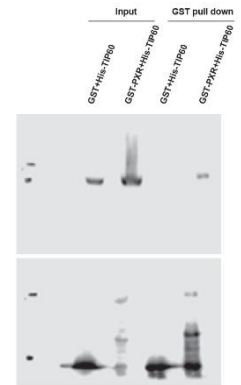
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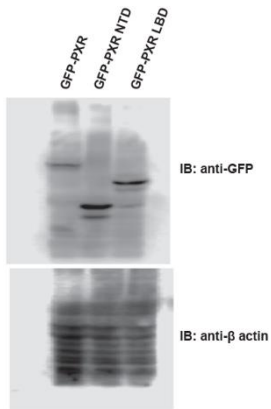
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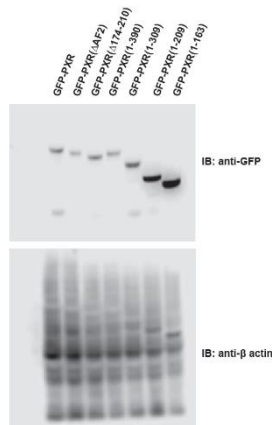
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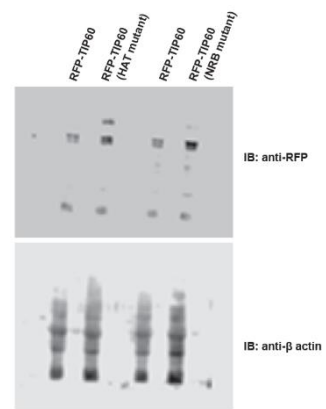
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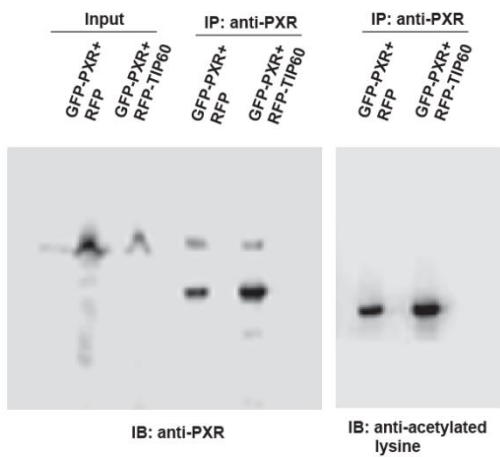
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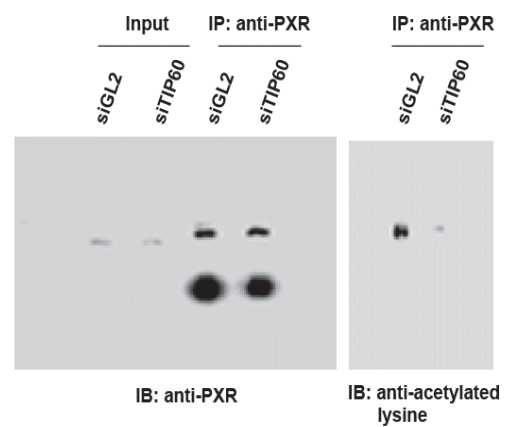
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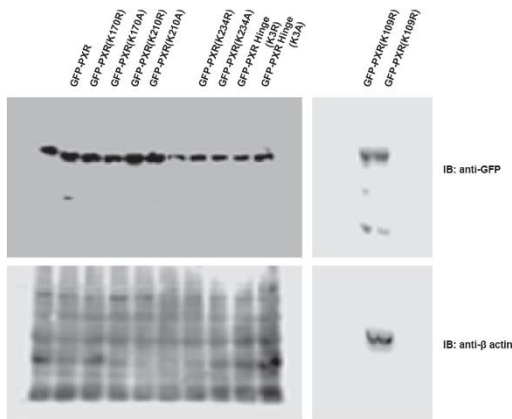
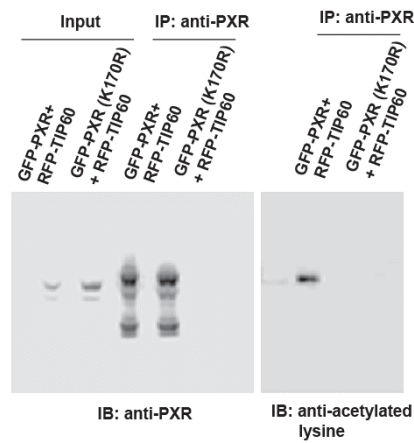
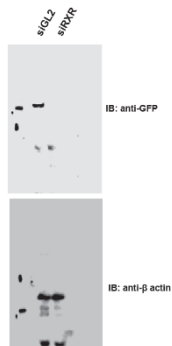
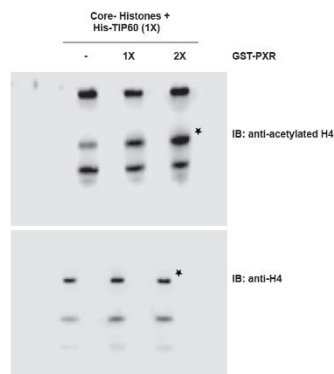


G



H



I**J****K****L****Supplementary Table 1: List of primers used in the study.**

S.No.	Primer Name	Sequence (5' to 3')
1	hPXR(K109R)Fw	GAGCGGCATGAAGAGGGAGATGATCATG
2	hPXR(K109R)Rv	CATGATCATCTCCCTCTTCATGCCGCTC
3	hPXR(164-210)del Fw	ATGAAAACCTTTGACGTCTCTCTGCAGCTGCGG
4	hPXR(164-210)del Rv	ATGAAAACCTTTGACGTCTCTCTGCAGCTGCGG
5	hPXR(174-210)del Fw	TTCAAGAATTTCCGGGTCTCTCTGCAGCTGCGG
6	hPXR(174-210)del Rv	CTGCAGAGAGACCCGGAAATTCTTGAAAT
7	hPXRLBDdel1BamHIRv	CGGGATCCTCAAAGGTTTTTCATCTGAGCGTC
8	hPXRLBDdel2BamHIRv	CGGGATCCTCAAGAGCACAGATCTTTCCGGA
9	hPXRLBDdel3BamHIRv	CGGGATCCTCACAAGCAGTAGGACAGCCG
10	hPXRLBDdel4BamHIRv	CGGGATCCTCACAAGAACCTATGAGCAGGCT
11	hPXRfWkpnI	GGGGTACCATGCTGGAGGTGAGACCCA
12	hTIP60RvEcoRI	CGGAATTCCACTTCCCCCTCTTGCT
13	hPXRdelAF2RvBamHI	CGGGATCCTCACGTAGCAAAGGGGTGT

14	hPXRfWbamHI	CGGGATCCATGCTGGAGGTGAGACCCA
15	hPXRrvEcoRI	CGGAATTCTCAGCTACCTGTGATGCCG
16	hTIP60HATmutFw	ACCCTGCCTCCCTACGAGCGCCGGGAGTACGGCAAG
17	hTIP60HATmutRv	CAGCAGCTTGCCGTACTCCCGGCGCTCGTAGGGAGG
18	hPXRNLsmutK66Fw	TGCAAGGGCTTTTTTCGCGGCGGCCATGAAACG
19	hPXRNLsmutK66Rv	GTTGCGTTTCATGGCCGCCGCGAAAAAGC
20	hPXRNLsmutK91Fw	ATCACCCGGAAGACCGCGGCACAGTGCCAG
21	hPXRNLsmutK91Rv	GCAGGCCTGGCACTGTGCCGCGGTCTTCCG
22	HsPXRNtermFwEcoRI	CGGAATTCATGCTGGAGGTGAGACCCA
23	HsPXRNtermRvBamHI	CGGGATCCTCAACTTTTCTTCCGCTTGATC
24	HsPXRLBDFwEcoRI	CGGAATTCATGACAGAGGAGCAGCGGA
25	HsPXRLBDRvBamHI	CGGGATCCTCAGCTACCTGTGATGCCG
26	hPXRHingeK3RmutFw	AGGCGGGCCTTGATCAGGCGGAGGAGGAGTGAACG
27	hPXRHingeK3RmutRv	CCCTGTCCGTTCACTCCTCCTCCGCCTGATCAAGG
28	hPXRK170RmutFw	ACCTTCTCCCATTTTCAGGAATTTCCGG
29	hPXRK170RmutRv	TGGCAGCCGGAAATTCCTGAAATGGGA
30	hPXRK210RmutFw	GATCTGTGCTCTTTGAGGGTCTCTCTG
31	hPXRK210RmutRv	CAGCTGCAGAGAGACCCTCAAAGAGCA
32	hPXRK234RmutFw	GCCGACAGTGGCGGGAGGGAGATCTTC
33	hPXRK234RmutRv	CAGGGAGAAGATCTCCCTCCCGCCACT
34	hPXRHingeK3AmutFw	AGGCGGGCCTTGATCGCGCGGGCGGCAAGTGAACG
35	hPXRHingeK3AmutRv	CCCTGTCCGTTCACTTGCCGCCCGCGCGATCAAGG
36	hPXRK170AmutFw	ACCTTCTCCCATTTTCGCGAATTTCCGG
37	hPXRK170AmutRv	TGGCAGCCGGAAATTCGCGAAATGGGA
38	hPXRK210AmutFw	GATCTGTGCTCTTTGGCGGTCTCTCTG
39	hPXRK210AmutRv	CAGCTGCAGAGAGACCGCCAAAGAGCA
40	hPXRK234AmutFw	GCCGACAGTGGCGGGGCAGAGATCTTC
41	hPXRK234AmutRv	CAGGGAGAAGATCTCTGCCCCGCCACT
42	hTIP60FwBamHI	CGGGATCCATGGCGGAGGTGGGGGAG
43	hTIP60NRBmutRvEcoRI	CGGAATTCTCACCCTTCCCCCTCTTGCTCCAGTCCCTTGGGAGTC GTGCAGACACTTGAGTTCGATCCGCGCGGCCCGCTTGAGCAT
44	hRXRFw	CGGAATTCATGGACACCAAACATTTCTCTG
45	hRXRRv	GGGGTACCAGTCATTTGGTGCGGGC