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Supplemental information

Characterization and prognostic value of LXR splice variants in triple-negative breast cancer

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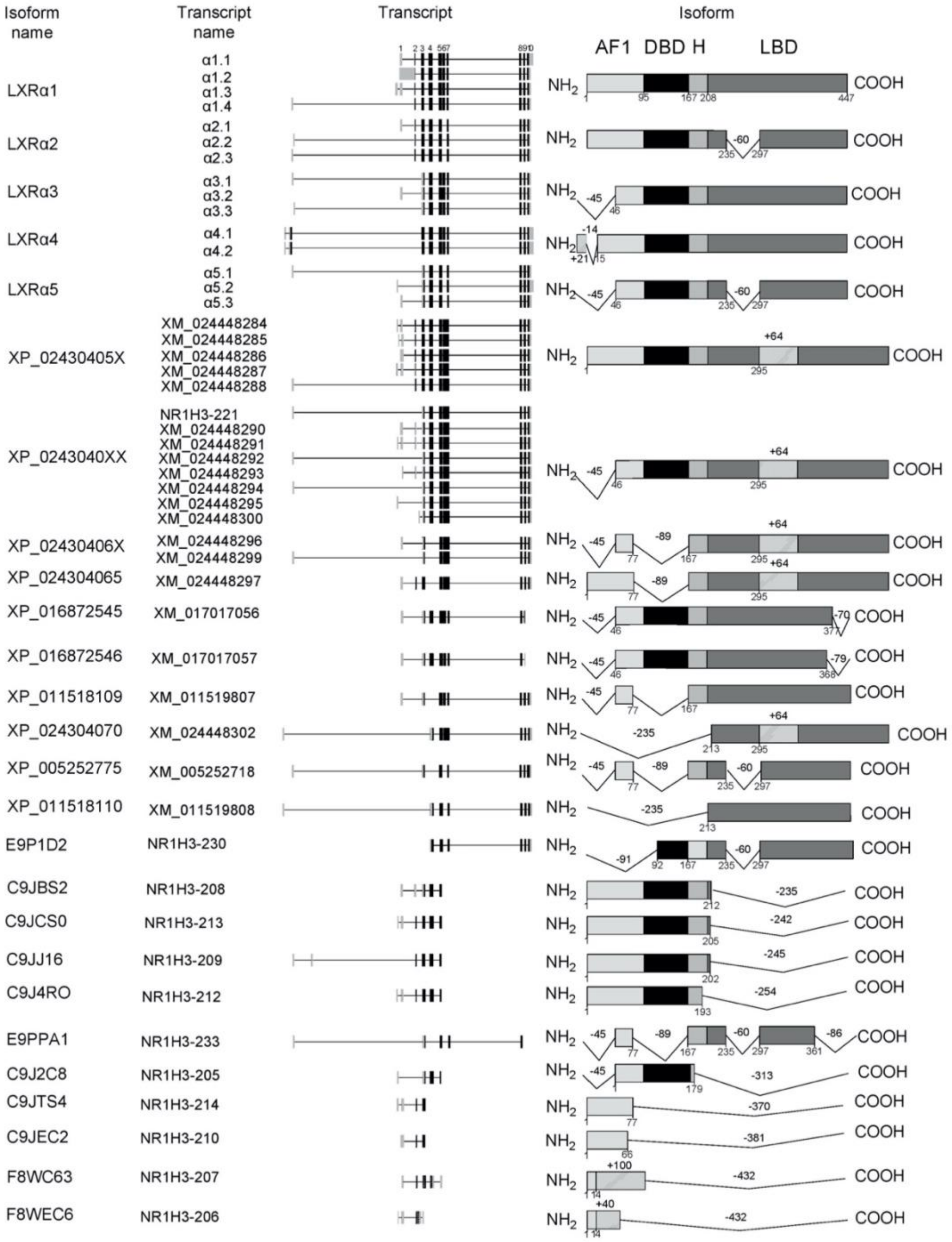
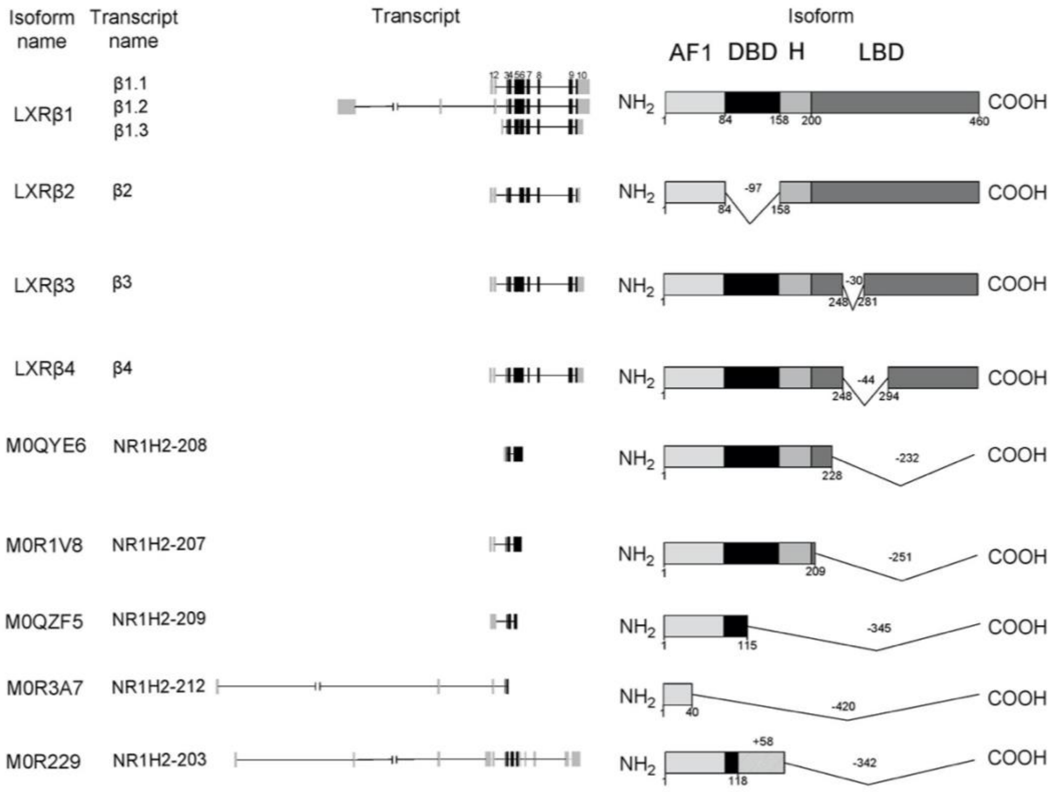
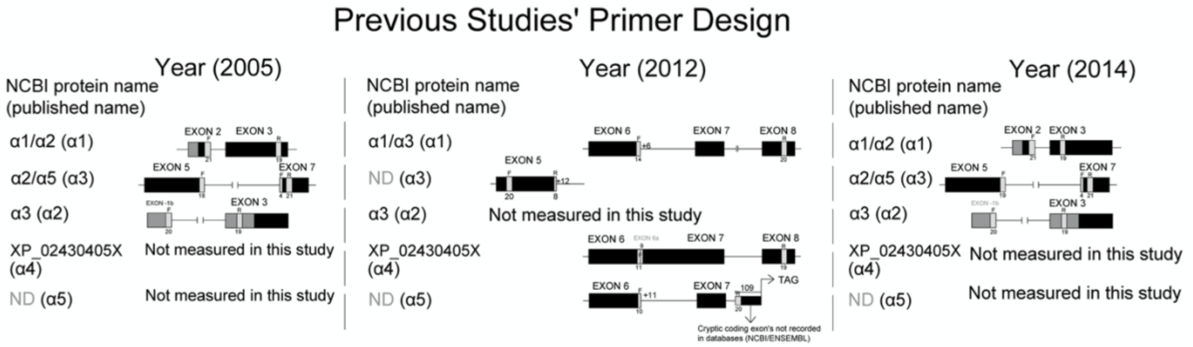


Supplementary Figure 1. Schematic diagram of LXRα splice variants. Related to Figure 1. LXRα splice variants reported in NCBI, ENSEMBL, and/or UNIPROT databases. For transcript, black boxes represent translated exons, grey boxes represent untranslated exons, lines joining exons represent intronic regions. Silver boxes show Activation Function 1 (AF1), black boxes are represented DNA Binding Domain (DBD), light grey are represented hinge (H), and dark grey boxes are represented Ligand Binding Domain (LBD). The numbers below the isoform domain boxes represent the position of amino acids. The hatched boxes represent protein domain derived from later-discovered coding exon. The numbers above connecting lines represent missing amino acids.

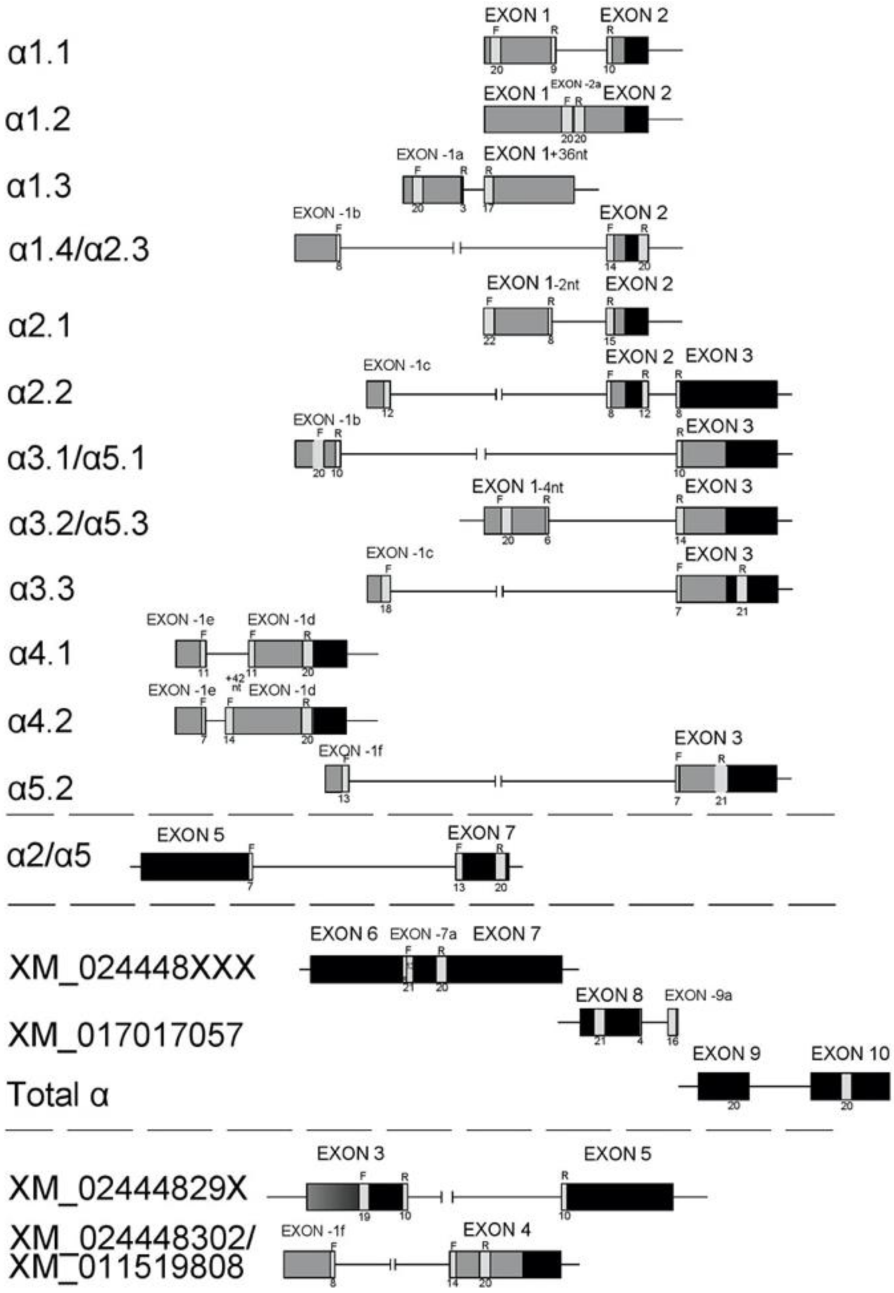


Supplementary Figure 2. Schematic diagram of LXRβ splice variants. Related to Figure 1. LXRβ splice variants reported in NCBI, ENSEMBL, and/or UNIPROT databases. For transcript, black boxes represent translated exons, grey boxes represent untranslated exons, lines joining exons represent intronic regions. For isoform, silver boxes are represented Activation Function 1 (AF1), black boxes represent DNA Binding Domain (DBD), light grey represent hinge (H), and dark grey boxes represent Ligand Binding Domain (LBD). The numbers right below the isoform domain boxes represent the position of amino acids. The hatched boxes are represented protein domain derived from later-discovered coding exon. The numbers above connecting lines represent missing amino acids.



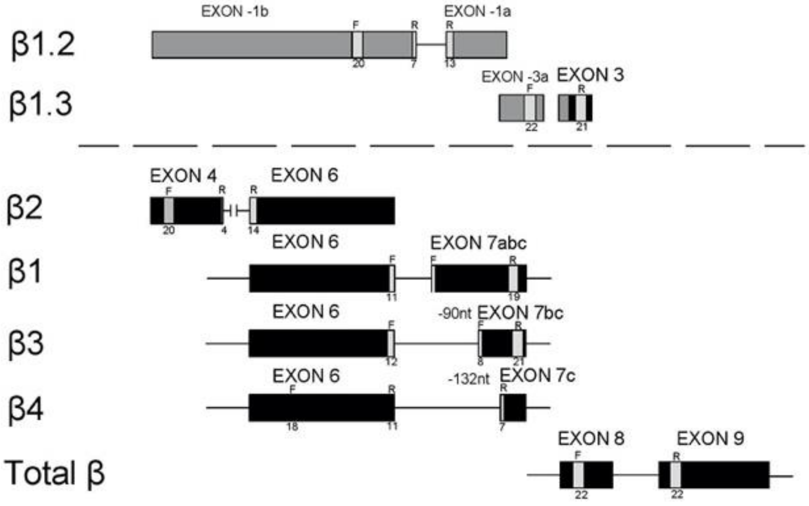
Supplementary Figure 3: Schematic diagrams illustrating the location of primer pairs for detecting LXR splice variants in previous studies. Related to Figure 1. ND = transcript not documented in any database.

LXRα



Supplementary Figure 4: Schematic diagrams illustrating the location of primer pairs for detecting LXRα splice variants in the current study. Related to Figure 1. The grey boxes show non-coding exons; the black boxes show coding exons; the lines connecting boxes denote introns; the grey text (e.g., for exon 3 of XM_024448XXX isoforms) indicated that exon 7a can be either coding or non-coding exon. The numeral X in transcript names (e.g. XM_0244829X or XM_024448XXX) denotes multiple variants share these features in NCBI as Ref. Seq. ID. Location of primers is denoted by grey boxes with F (forward) and R (reverse) above and base number below. Primers split between exons have number of bases to which the primers bind on each side of the boundary.

LXRβ



Supplementary Figure 5: Schematic diagrams illustrating the location of primer pairs for detecting LXRβ splice variants in the current study. Related to Figure 1. The grey boxes show non-coding exons; the black boxes show coding exons; the lines connecting boxes denote introns. Location of primers is denoted by grey boxes with F (forward) and R (reverse) primers above and size in bases below. Primers split between exons have number of bases to which the primers bind on each side of the boundary. ND = transcript not documented in any database.

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CLUSTAL O(1.2.4) multiple sequence alignment


α1(NP_005684.2)                           MSLWLGAPVPDIPPDSAVELWKPGAQDASSQAQGGSSCILREEARMPHSAGGTAGVGLEA    60
αx5(XP_011518107.1,XP_005252763.1)        MSLWLGAPVPDIPPDSAVELWKPGAQDASSQAQGGSSCILREEARMPHSAGGTAGVGLEA    60
                                          ************************************************************

α1(NP_005684.2)                           AEPTALLTRAEPPSEPTEIRPQKRKKGPAPKMLGNELCSVCGDKASGFHYNVLSCEGCKG    120
αx5(XP_011518107.1,XP_005252763.1)        AEPTALLTRAEPPSEPTEIRPQKRKKGPAPKMLGNELCSVCGDKASGFHYNVLSCEGCKG    120
                                          ************************************************************

α1(NP_005684.2)                           FFRRSVIKGAHYICHSGGHCPMDTYMRRKCQECRLRKCRQAGMREECVLSEEQIRLKKLK    180
αx5(XP_011518107.1,XP_005252763.1)        FFRRSVIKGAHYICHSGGHCPMDTYMRRKCQECRLRKCRQAGMREECVLSEEQIRLKKLK    180
                                          ************************************************************

α1(NP_005684.2)                           RQEEEQAHATSLPPRASSPPQILPQLSPEQLGMIEKLVAAQQQCNRRSFSDRLRVTPWPM    240
αx5(XP_011518107.1,XP_005252763.1)        RQEEEQAHATSLPPRASSPPQILPQLSPEQLGMIEKLVAAQQQCNRRSFSDRLRVTPWPM    240
                                          ************************************************************

α1(NP_005684.2)                           APDPHSREARQQRFAHFTELAIVSVQEIVDFAKQLPGFLQLSREDQIALLKTSAIEVMLL    300
αx5(XP_011518107.1,XP_005252763.1)        APDPHSREARQQRFAHFTELAIVSVQEIVDFAKQLPGFLQLSREDQIALLKTSAIEVMLL    300
                                          ************************************************************

α1(NP_005684.2)                           ETSRRYNPGSESITFLKDFSYNREDFAKAGLQVEFINPIFEFSRAMNELQLNDAEFALLI    360
αx5(XP_011518107.1,XP_005252763.1)        ETSRRYNPGSESITFLKDFSYNREDFAKAGLQVEFINPIFEFSRAMNELQLNDAEFALLI    360
                                          ************************************************************

α1(NP_005684.2)                           AISIFSADRPNVQDQLQVERLQHTYVEALHAYVSIHHPHDRLMFPRMLMKLVSLRTLSSV    420
αx5(XP_011518107.1,XP_005252763.1)        AISIFSADRPNVQDQLQVERLQHTYVEALHAYVSIHHPHDRLMFPRMLMKLVSLRTLSSV    420
                                          ************************************************************

α1(NP_005684.2)                           HSEQVFALRLQDKKLPPLLSEIWDVHE     447
αx5(XP_011518107.1,XP_005252763.1)        HSEQVFALRLQDKKLPPLLSEIWDVHE     447
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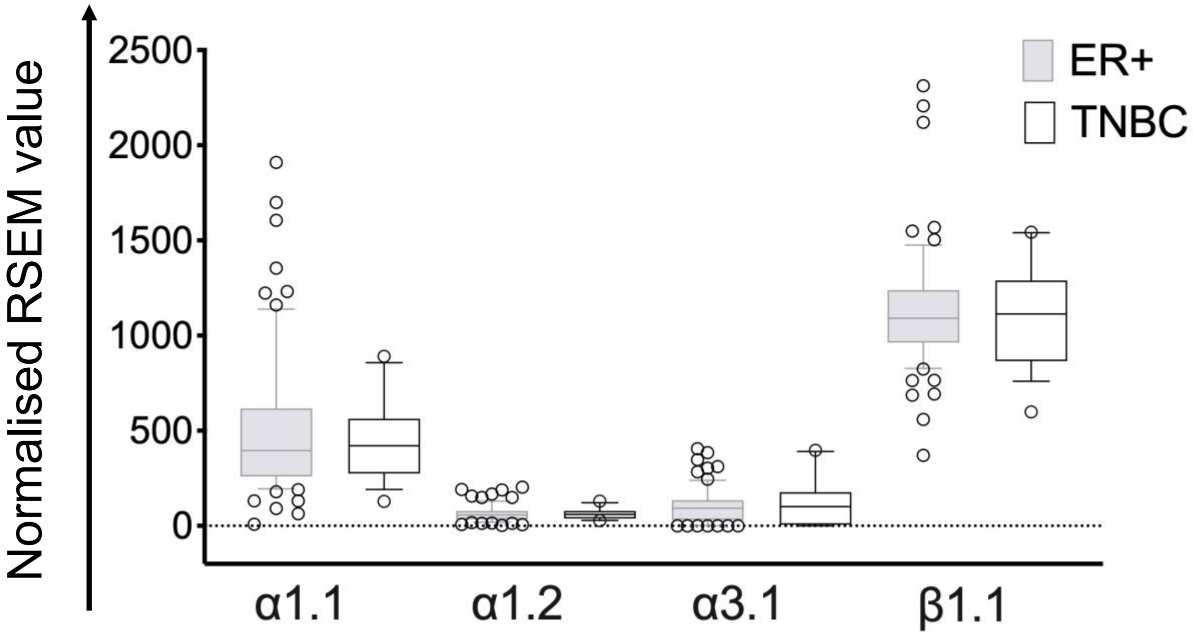
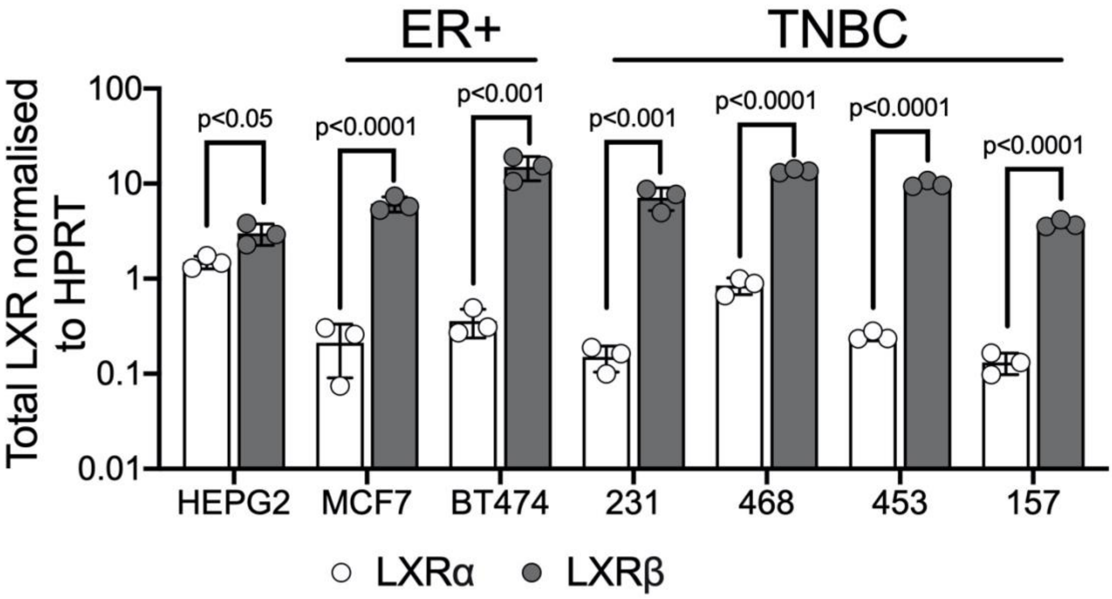
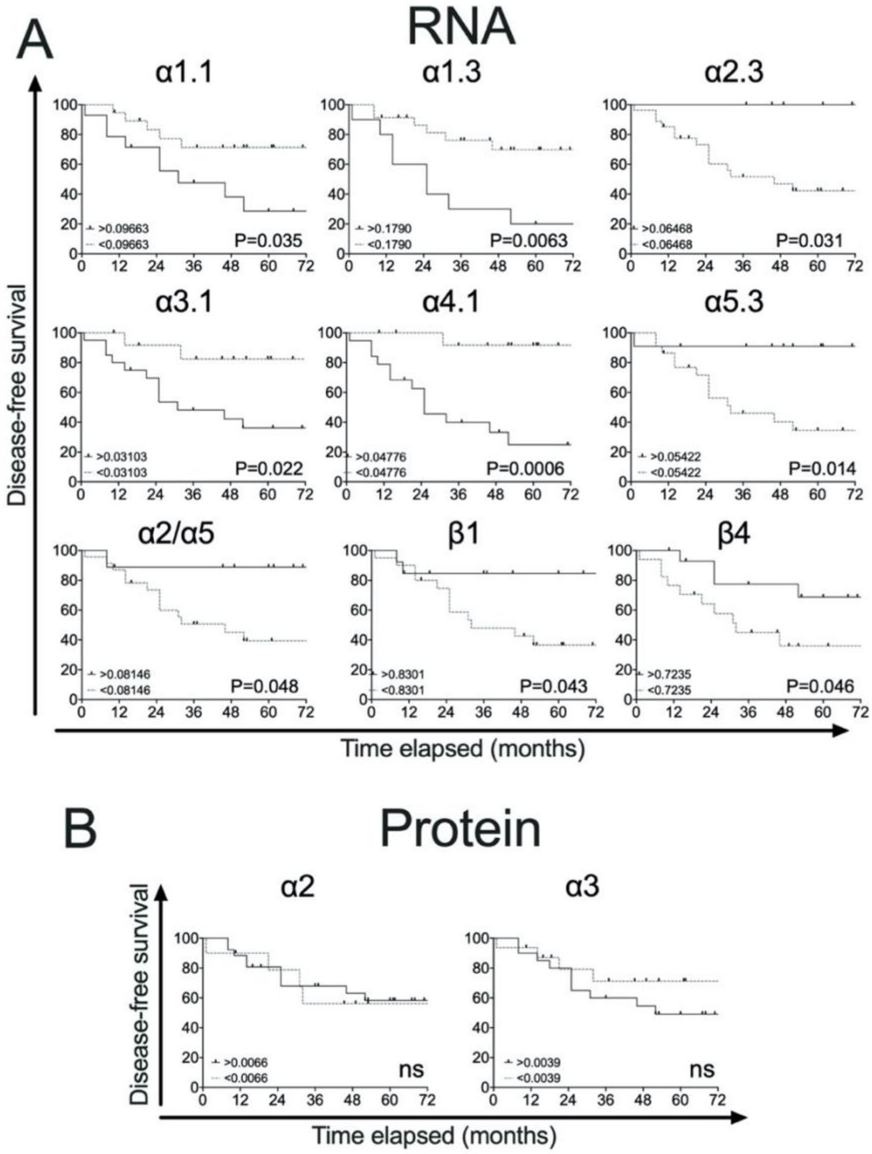
Supplementary Figure 6. NCBI predicted (α1) and curated (ax5) isoforms are fully homologous. Related to Figure 1.



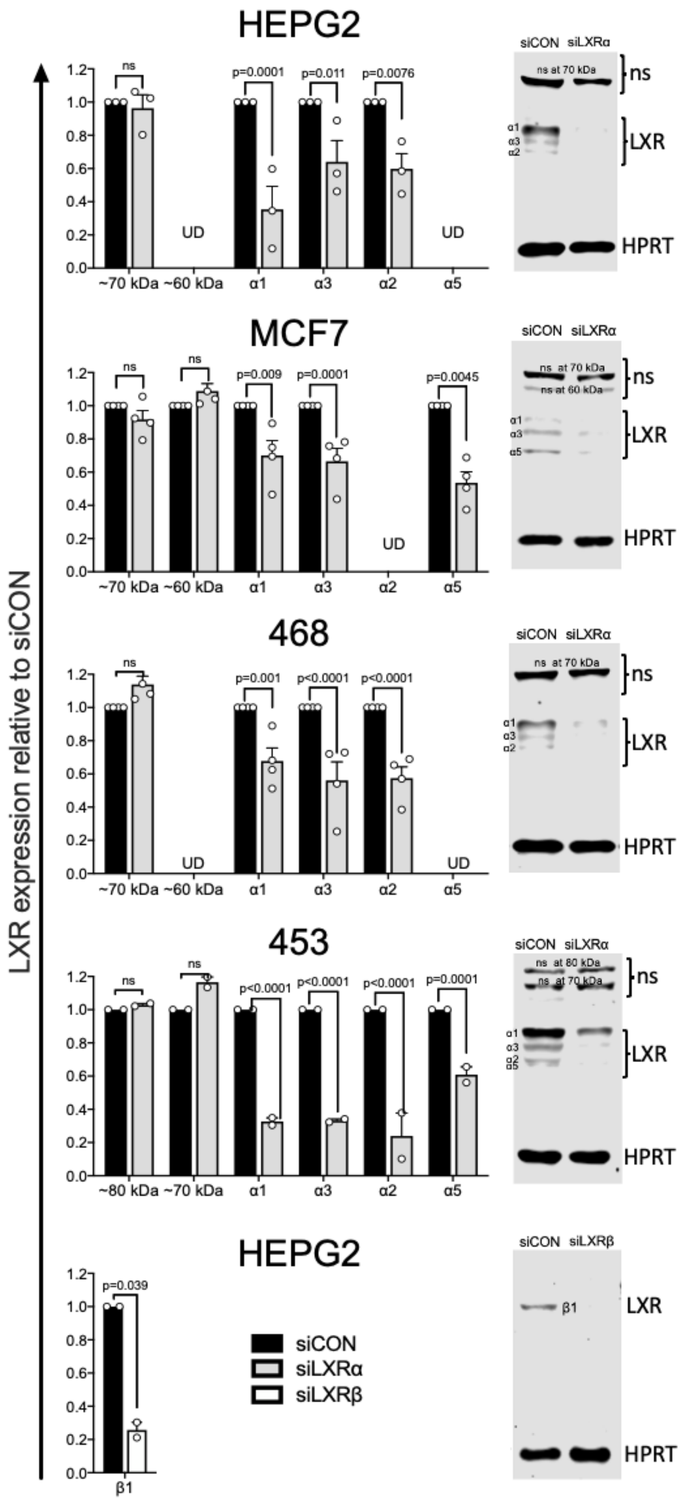
Supplementary Figure 7. **LXR transcript variant expression in ER+ and TNBC tumour samples from the TSVdb TCGA tumour cohort. Related to Figure 2.** Differential expression of LXR transcript variants in ER+ and TNBC tumour samples were plotted in box and whisker charts. The box extends from the 10th to 90th percentile. The line in the middle of the box shows median. The whiskers show minimum and maximum values. Significance was considered when p≤0.05.



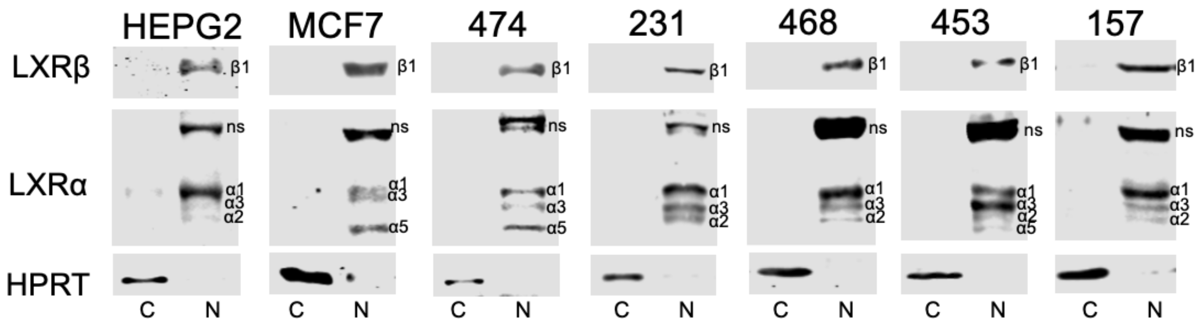
Supplementary Figure 8. LXRβ is expressed at higher levels than LXRα in all breast cancer cell lines. Related to Figure 3. Data showed mean of three independent replicates with SEM. p-values calculated using multiple two-way t-tests. Significance was considered when $p \leq 0.05$.



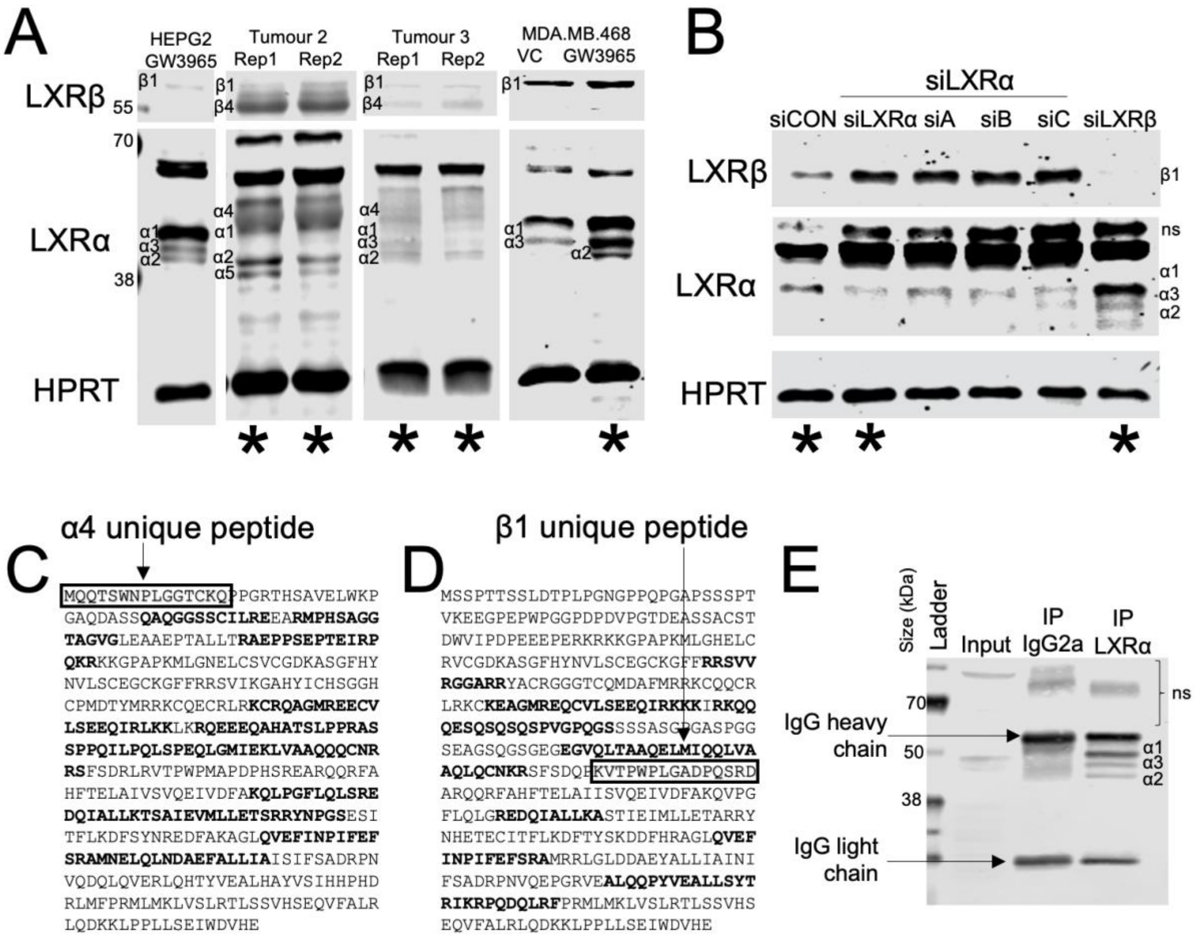
Supplementary Figure 9. Splice variants and TNBC patient survival. Related to Figure 4. (A) All transcript variants are prognostic. (B) LXRα2 and α3 protein variants are not prognostic. TNBC patients were divided into two groups (n=38), no event (n=23) and event (n=15), based on their disease-free survival status. Kaplan-Meier survival curves plotting disease free survival of TNBC patients with high or low variant expression relative to HPRT. Data derived from the mean of two different slices of tumours. Significance determined by the Log-rank (Mantel-cox) test, p≤0.05 was considered significant.



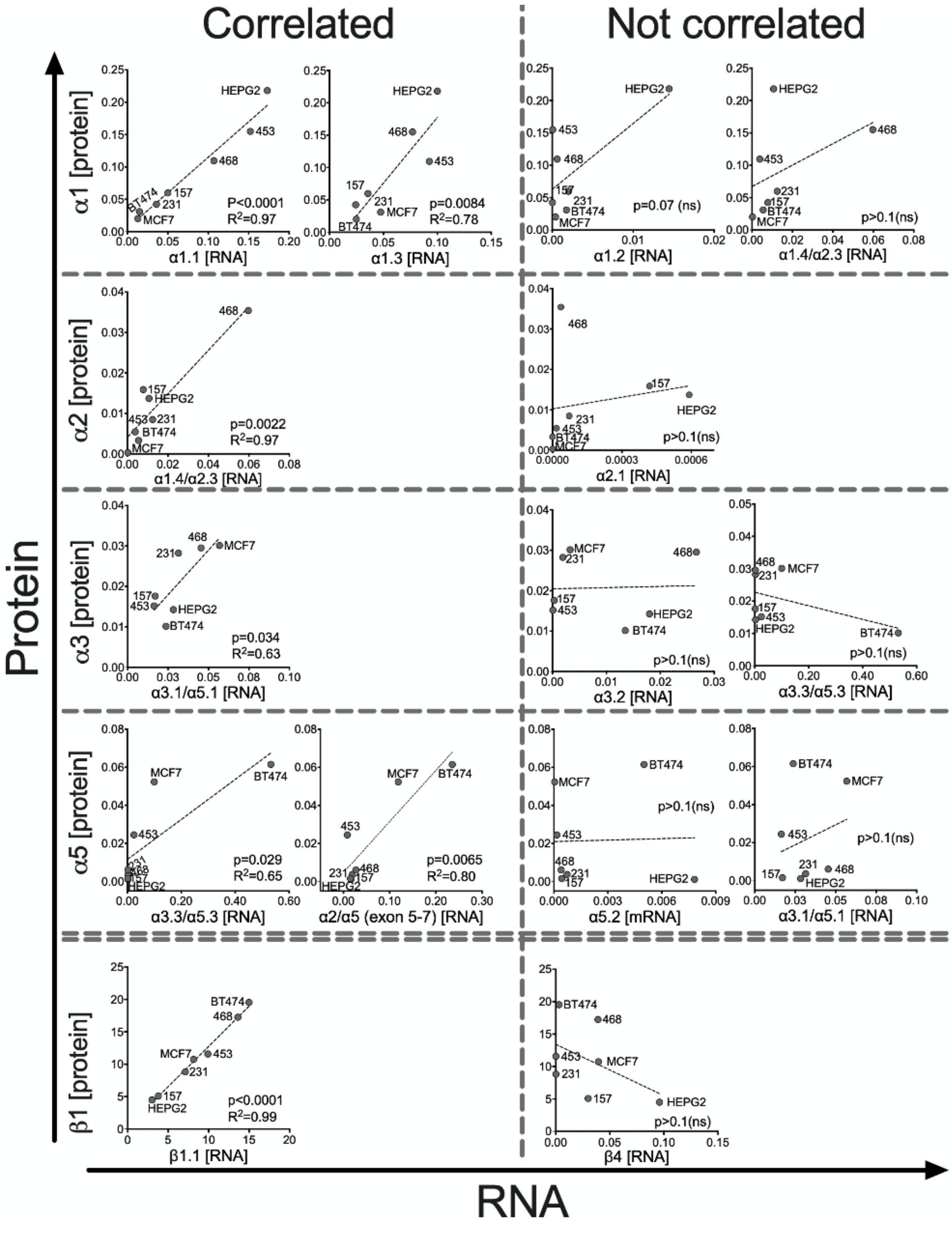
Supplementary Figure 10. Relative expression LXR protein variants in siLXRα relative siRNA control. Related to Figure 3. Protein expression determined in ImageJ. Data represent mean of 2-4 independent replicates with SEM. Additional bands at 60kDa and 70kDa were assumed to be non-specific (ns) as their sizes did not correspond to any of the 48 LXRα transcripts. As expected, non-specific bands were unaffected by siRNA treatment. Statistical significance was measured by two-tailed one-way ANOVA, p-values are given above bars showing significant difference compared to siCON. siCON = siControl.



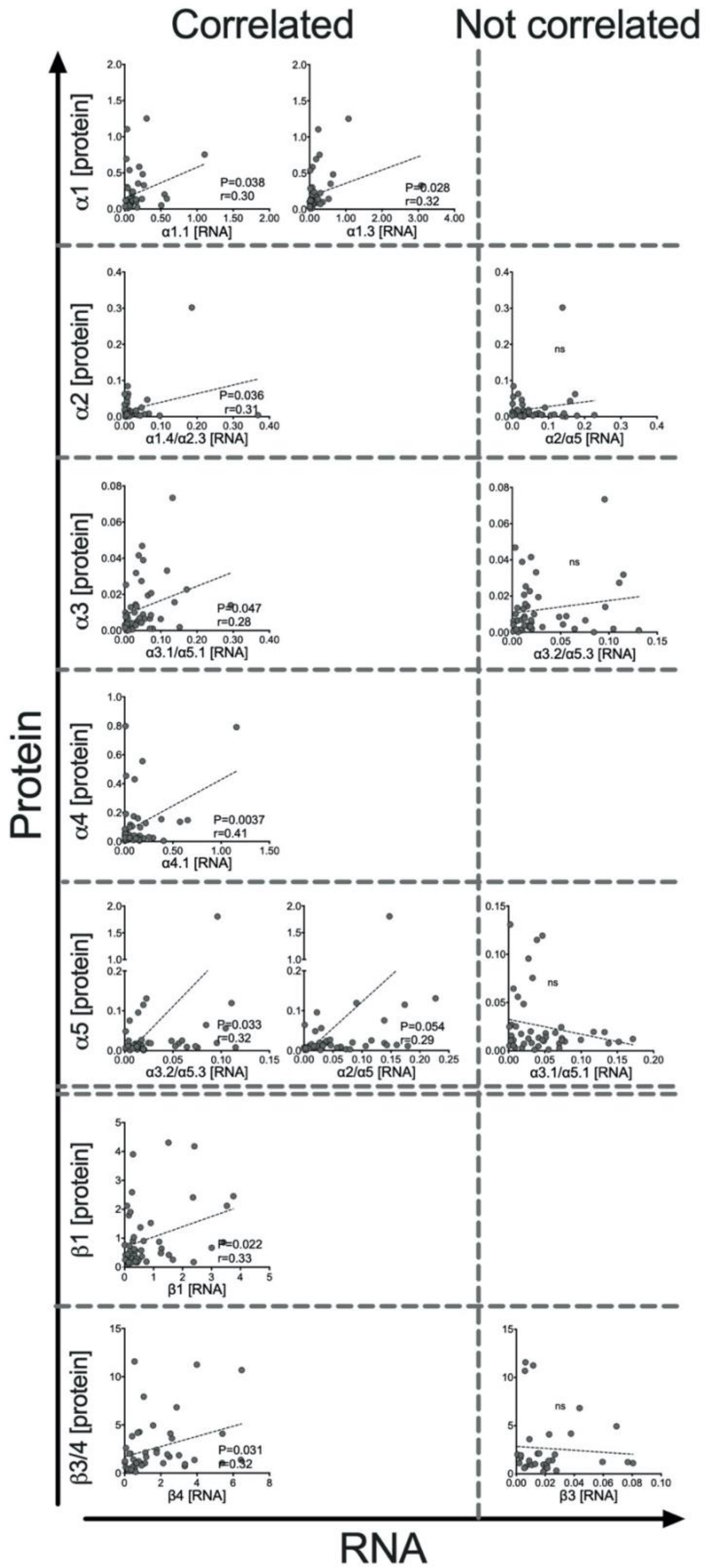
Supplementary Figure 11. All LXR splice variants are localized in nucleus. Related to Figure 3. HPRT is used as cytoplasmic loading control. ns = non-specific binding; C=cytoplasmic; N=nuclear.



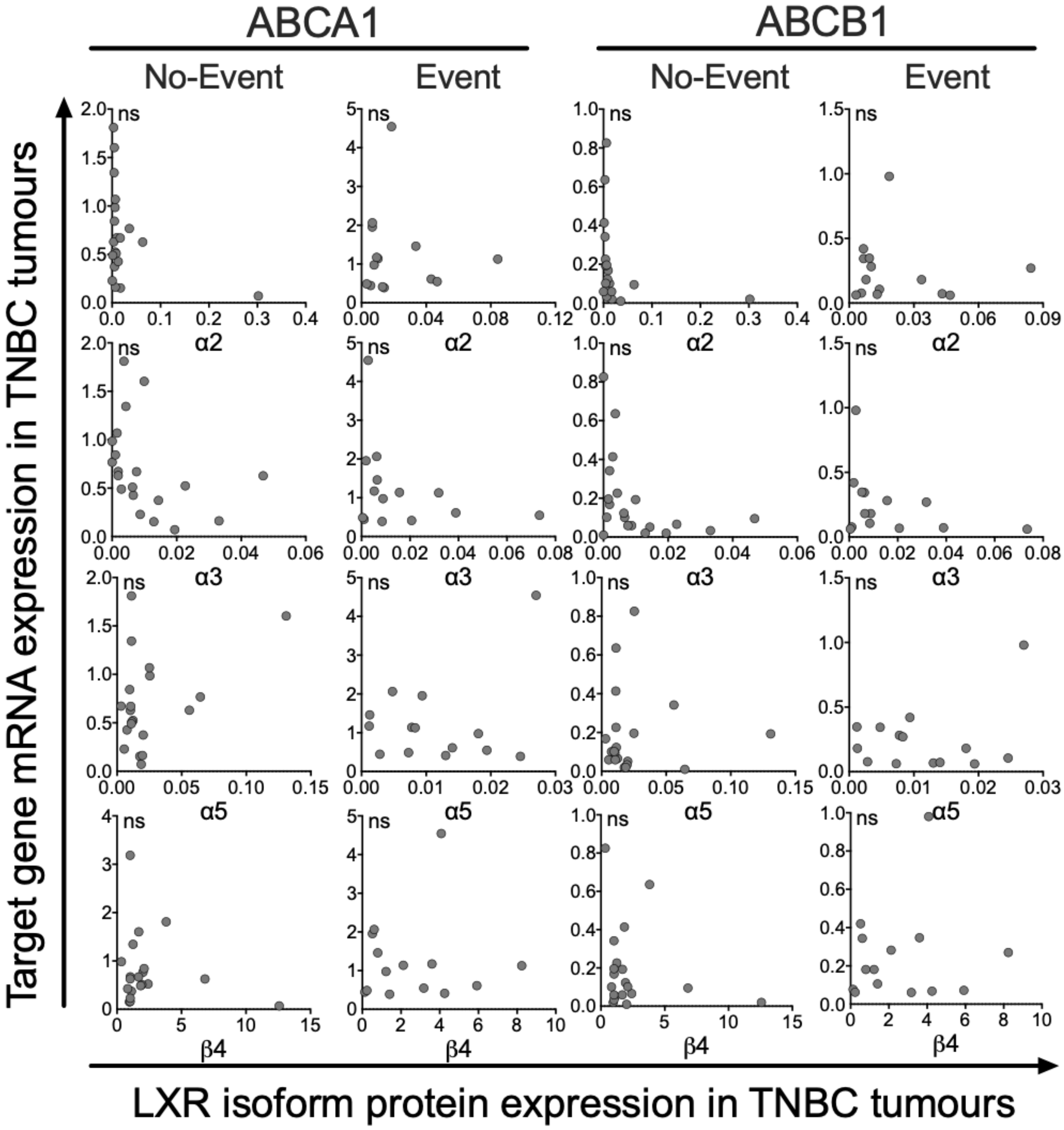
Supplementary Figure 12. Representative blots showing the LXR splicing protein expression in samples subjected to Mass-Spectrometry (MS). Related to Table 1, Table 2, and Table 3. (A) TNBC tumours and in MDA.MB.468 cell line samples treated with GW3965, siLXRα or siLXRβ treated MDA.MB.468 cells. Samples marked with asterix were subjected to mass spectrometry (MS) analysis. Sequence coverage of unique (boxes) or homologous (bold) peptides LXRα (C) and LXRβ (D) peptides identified by proteomic analysis. Homologous peptides are generated by multiple LXR splice variants and can't be used to confirm the presence or individual variants. (E) MDA.MB.468 subjected to immunoprecipitation. VC = vehicle control; T = tumour; siA = siExon4 of LXRα, siB = siExon9-10 (coding exon) of LXRα, siC = siExon10 (non-coding 3'UTR) of LXRα, siLXRα = siA+siB+siC. ns = non-specific binding.



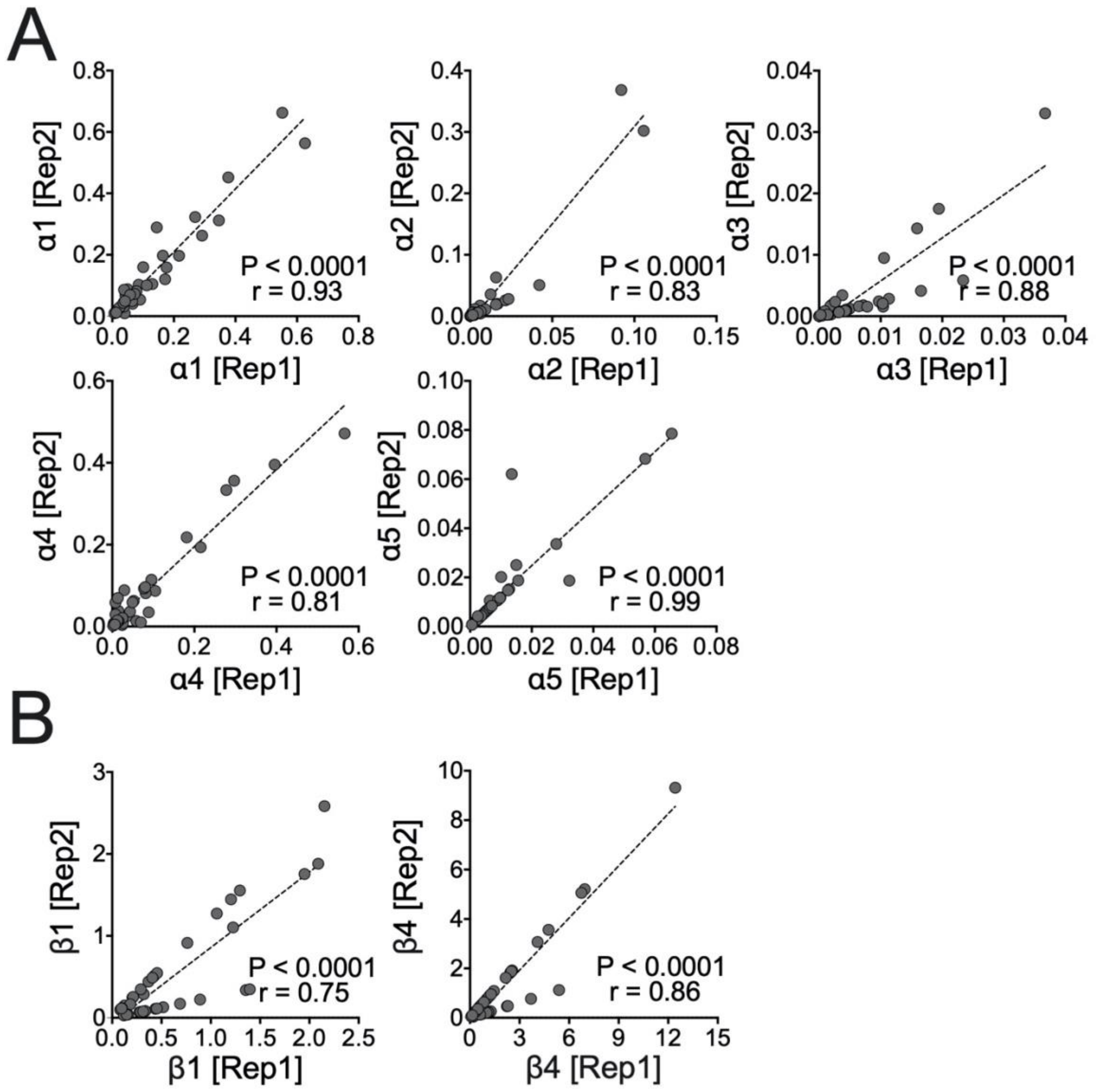
Supplementary Figure 13. Correlation of LXR transcripts with LXR protein variants in cell lines. Related to Figure 1. RNA and protein normalised to HPRT levels, and significance tested for using linear regression. Circles represent mean of 3-5 separate passage of each cell line. P-values and R-squared (R^2) are shown in each graph. NS = not significant.



Supplementary Figure 14. Correlation of LXR transcripts with LXR protein variants in TNBC tumours. Related to Figure 1. RNA and protein normalised to HPRT levels, and significance tested for using linear regression. Circles represent mean of two different tumour slices from one TNBC tumour samples. P-values and R-squared (R^2) are shown in each graph. NS = not significant.



Supplementary Figure 15. LXRα2, α3, α5, and β4 protein were not correlated to target genes. Related to Figure 5. TNBC patients (n=38) were divided into two groups, no event (n=23) and event (n=15), based on their disease-free survival status. LXRα or LXRβ protein variants at x axis versus (A) ABCA1 or (B) ABCB1 gene expression levels at y axis in TNBC tumours were normalised for HPRT levels and correlated using linear regression. Circles represent individual TNBC samples from the mean of two different tumour slices. Significance levels were set at p-values ≤ 0.05.



Supplementary Figure 16. Correlation between two slices of each tumour sample when measuring LXR protein variant expression. Related to Figure 4. LXRα (A) or LXRβ (B) protein variant expression of tumour replicate 1 (x axis) is plotted against replicate 2 (y axis). Replicate 1 and 2 were assigned randomly. Samples were derived from the same TNBC tumour sample, normalised by HPRT expression level, and expression correlated using Interclass correlation coefficient through Spearman's rank [1]. Circles represented individual TNBC samples patients. Significance was considered when p≤0.05.

Table S1. The summary information of the different name used for LXRα spice variants. Related to Figure 1.

This study: protein designation	NCBI protein designation	This study: transcript designation	NCBI nucleotide Ref. Seq. Hyperlinked	NCBI protein Ref. Seq. Hyperlinked	ENSEMBL ID	TSVdb ID	UNIPROT ID	Year of publication (doi): hyperlinked	Previous designation	Total amino acids	Size (kDa)	Notes
LXRα1	LXRα isoform 1	**α1.1**	NM_005693.4	NP_005684.2	NR1H3-211	uc009ylm	Q13133-1	1995 (10.1101/gad.9.9.1033); 2005 (10.1194/jlr.M500157-JLR200); 2012 (10.1124/mol.111.077206); 2014 (10.1016/j.fertnstert.2014.04.033)	**LXRα1**	447	50.41	Detected by PCR and immunoblotting X5 shares 100% amino acid homology
		α1.2			NR1H3-217	uc001nem						
	LXRα isoform X5*	**α1.3**	XM_005252706.1	XP_005252763.1								
	LXRα isoform X5*	**α1.4**	XM_011519805.2	XP_011518107.1								
LXRα2	LXRα isoform 2	**α2.1**	NM_001130101.3	NP_001123573.1	NR1H3-203	uc001nen	Q13133-2	2005 (10.1194/jlr.M500157-JLR200); 2012 (10.1124/mol.111.077206); 2014 (10.1016/j.fertnstert.2014.04.033)	**LXRα3**	387	43.56	Detected by PCR and immunoblotting X11 shares 100% amino acid homology
		α2.2			NR1H3-204							
	LXRα isoform X11*	**α2.3**	XM_005252713.3	XP_005252770.1								
LXRα3	LXRα isoform 3	**α3.1**	NM_001130102.3	NP_001123574.1	NR1H3-201	uc001nek	Q13133-3	2005 (10.1194/jlr.M500157-JLR200); 2012 (10.1124/mol.111.077206); 2014 (10.1016/j.fertnstert.2014.04.033)	**LXRα2**	402	45.69	Detected by PCR and immunoblotting X2 shares 100% amino acid homology
	LXRα isoform X2*	**α3.2**	XM_024448289.1	XP_024304057.1								
	LXRα isoform X2*	**α3.3**	XM_024448298.1	XP_024304066.1								
LXRα4	LXRα isoform 4	**α4.1**	NM_001251934.1	NP_001238863.1	NR1H3-235	uc010rhk	B4DXU5			435	51.11	Detected by PCR, immunoblotting and MS by unique peptide
	LXRα isoform 4	**α4.2**	NM_001251935.1	NP_001238864.1		uc009yll						
LXRα5	LXRα isoform 5	**α5.1** **α5.2**	NM_001363595.2	NP_001350524.1	NR1H3-202		B5MBY7			342	38.85	Detected by PCR and immunoblotting X7 shares 100% amino acid homology
	LXRα isoform X7*	**α5.3**	XM_011519806.1	XP_011518108.1								
XP_02430405X	LXRα isoform X1	XM_02444828X	XM_024448284.1 XM_024448285.1 XM_024448286.1 XM_024448287.1 XM_024448288.1	XP_024304052.1 XP_024304053.1 XP_024304054.1 XP_024304055.1 XP_024304056.1				2012 (10.1124/mol.111.077206)	**LXRα4**	511	57.53	Not detected
XP_0243040XX	LXRα isoform X3	XM_024448XXX	XM_024448290.1 XM_024448291.1 XM_024448292.1 XM_024448293.1 XM_024448294.1 XM_024448295.1 XM_024448300.1	XP_024304058.1 XP_024304059.1 XP_024304060.1 XP_024304061.1 XP_024304062.1 XP_024304063.1 XP_024304068.1	NR1H3-221		E9PLL4			466	52.81	Not detected
XP_02430406X	LXRα isoform X4	XM_02444829X	XM_024448296.1 XM_024448299.1	XP_024304064.1 XP_024304067.1						377	42.64	Not detected
XP_024304065.1	LXRα isoform X6	XM_024448297.1	XM_024448297.1	XP_024304065.1						422	47.34	Not detected
XP_016872545.1	LXRα isoform X8	XM_017017056.1	XM_017017056.1	XP_016872545.1						332	37.25	Not measured (no unique exon-exon boundaries)
XP_016872546.1	LXRα isoform X9	XM_017017057.1	XM_017017057.1	XP_016872546.1						323	36.26	Detected by PCR
XP_011518109.1	LXRα isoform X10	XM_011519807.1	XM_011519807.1	XP_011518109.1						313	35.52	Not measured (no unique exon-exon boundaries)
XP_024304070.1	LXRα isoform X12	XM_024448302.1	XM_024448302.1	XP_024304070.1						299	34.44	Detected by PCR
XP_005252775.1	LXRα isoform X13	XM_005252718.3	XM_005252718.3	XP_005252775.1						253	28.68	Not measured (no unique exon-exon boundaries)
XP_011518110.1	LXRα isoform X14	XM_011519808.2	XM_011519808.2	XP_011518110.1						235	27.32	Detected by PCR

E9P1D2	NR1H3-230	NR1H3-230	E9P1D2	296	34.14	Not measured (no unique exon-exon boundaries)
C9JBS2	NR1H3-208	NR1H3-208	C9JBS2	212	23.10	Not measured (no unique exon-exon boundaries)
C9JCS0	NR1H3-213	NR1H3-213	C9JCS0	205	22.38	Not measured (no unique exon-exon boundaries)
C9JJ16	NR1H3-209	NR1H3-209	C9JJ16	202	22.04	Not measured (no unique exon-exon boundaries)
C9J4RO	NR1H3-212	NR1H3-212	C9J4RO	193	21.10	Not measured (no unique exon-exon boundaries)
E9PPA1	NR1H3-233	NR1H3-233	E9PPA1	167	18.55	Not measured (no unique exon-exon boundaries)
C9J2C8	NR1H3-205	NR1H3-205	C9J2C8	134	14.79	Not measured (no unique exon-exon boundaries)
C9JTS4	NR1H3-214	NR1H3-214	C9JTS4	77	7.82	Not measured
C9JEC2	NR1H3-210	NR1H3-210	C9JEC2	68	6.86	Not measured
F8WC63	NR1H3-207	NR1H3-207	F8WC63	114	12.29	Not measured (non-sense mediated decay)
F8WEC6	NR1H3-206	NR1H3-206	F8WEC6	54	5.71	Not measured (non-sense mediated decay)
		NR1H3-215 NR1H3-218 NR1H3-219 NR1H3-226 NR1H3-228 NR1H3-229 NR1H3-231 NR1H3-232 NR1H3-234 NR1H3-216 NR1H3-220 NR1H3-222 NR1H3-223 NR1H3-224 NR1H3-225 NR1H3-227	Processed transcripts [no protein produced]			

Table S2. The summary information of the different name used for LXRβ spice variants. Related to Figure 1.

This study: protein designation	NCBI protein designation	This study: transcript designation	NCBI nucleotide Ref. Seq. Hyperlinked	NCBI protein Ref. Seq. Hyperlinked	ENSEMBL ID	TSVdb ID	UNIPROT ID	Year of publication (doi): hyperlinked	Previously published designation	Total amino acids	Size (kDa)	Notes
LXRβ1	LXRβ isoform 1	**β1.1**	NM_007121.7	NP_009052.4	NR1H2-201	uc010enw	P55055-1	1995(10.1101/gad.9.9.1033); 2014(10.1016/j.fertnstert.2014.04.033)	LXRβ	460	50.97	Detected by PCR and immunoblotting
		β1.2			NR1H2-214							Not detected
		β1.3	XM_005252706.1	XP_005252763.1	NR1H2-204							
LXRβ2	LXRβ isoform 2	**β2**	NM_001256647.3	NP_001243576.2	NR1H2-202	uc002psa	P55055-2			363	39.92	Not detected
LXRβ3		**β3**			NR1H2-210		M0R0K3			430	47.56	Detected by PCR
LXRβ4		**β4**			NR1H2-211		M0R2F9			416	45.98	Detected by PCR and immunoblotting
M0QYE6		**NR1H2-208**			NR1H2-208		M0QYE6			228	24.13	Not measured (no unique exon-exon boundaries)
M0R1V8		**NR1H2-207**			NR1H2-207		M0R1V8			209	22.24	Not measured (no unique exon-exon boundaries)
M0QZF5		**NR1H2-209**			NR1H2-209		M0QZF5			115	12.13	Not measured (no unique exon-exon boundaries)
M0R3A7		**NR1H2-212**			NR1H2-212		M0R3A7			40	3.97	Not measured (no unique exon-exon boundaries)
M0R229 (non-sense mediated decay)		**NR1H2-203**			NR1H2-203		M0R229 (non-sense mediated decay)			176	18.73	Not measured (non-sense mediated decay)
					NR1H2-213 NR1H2-206 NR1H2-205		Processed transcripts [no protein produced]					

Table S3. TNBC patient tumour characteristics. Related to Figure 4 and Figure 5.

Characteristic	Category	Leeds Breast Research Tissue Bank No. of patients = 38 (%)
Invasive Tumour grade	1	0 (0)
	2	3 (8)
	3	35 (92)
Tumour size	≤35 mm	29 (76)
	>35 mm	9 (24)
Survival status	Alive	28 (74)
	Deceased	10 (26)
Recurrence/metastasis	None	23 (60)
	Local and/or distal	15 (40)

Table S4. LXRα peptides detected by S-trap column coupled with MS in MDA.MB.468 cell line control samples. Related to Table 1, Table 2, and Table 3.

Amino acids position number based on LXRα1. Amino acid numbering position with "-" indicates the additional amino acid(s) coming before α1 and/or β1's amino acid position number 1.

Sample	Total identified peptides	-10lgP [LXRα]	Coverage LXRα peptides	Supporting peptides [LXRα]	Unique	Amino acids position	
						start	end
MDA.MB.468 siCON	4512	42.09	12%	KC (+57.02)RQAGMR.E	No	158	164
				R.EEC(+57.02)VLSEEQIR.L	No	165	175
				W.PMAPDPHSREAR.Q	No	239	250
				K.TSAIEVMLLETSR.R	No	292	304
				YN (+.98)PGSESITFLK.D	No	307	317
MDA.MB.468 siLXRα	3726	37.87	8%	R.ASSPPQILPQLSPEQ(+.98)LGMIEK.L	No	196	216
				G.MIEKLVAAQ(+.98)Q(+.98)QC(+57.02)NR.R	No	213	226
				R.AMNELQLN(+.98)DAEFA.L	No	345	357
				R.M(+15.99)LMKLVSLR.T	No	407	415
MDA.MB.468 siLXRβ	3641	44.90	12%	A.QGGSSC(+57.02)ILR.E	No	33	41
				H.SAGGTAGVGLEAA.E	No	49	61
				L.LTRAEPPSEPTEIRPQ(+.98)K.R	No	67	83
				R.KC(+57.02)RQ(+.98)AGMREEC(+57.02)VLSEEQ.I	No	157	173
				R.ASSPPQILPQLSPEQ(+.98)LGMIEK.L	No	196	216
				G.MIEKLVAAQ(+.98)Q(+.98)QC(+57.02)NR.R	No	213	226
				K.LVAAQQQ(+.98)C(+57.02)N(+.98)R.R	No	217	226
				K.AGLQ(+.98)VEFINPIFE.F	No	329	341
				K.AGLQVEFINPIFEFSR.A	No	329	344
				Q.VEFINPIFEFSR.A	No	333	344
				R.M(+15.99)LMKLVSLR.T	No	407	415
MDA.MB.468 treated with GW3965	4013	58.14	19%	R.ASSPPQILPQLSPEQ(+.98)LGMIEK.L	No	196	216
				R.AMNELQLN(+.98)DAEFA.L	No	345	357
				R.EDQ(+.98)IALLK.T	No	284	291
				R.EDQ(+.98)IALLKTSAIE.V	No	284	296
				R.VTPWPMAPDPHSREARQQ(+.98)R.F	No	235	253
				K.LVAAQQQC(+57.02)NR.R	No	217	226
				F.TELAIVS.V	No	258	264

Table S5. LXRβ peptides detected by S-trap column coupled with MS in MDA.MB.468 cell line control samples. Related to Table 1, Table 2, and Table 3.

Amino acids position numbers based on LXRβ1. The grey highlight indicated unique peptides of LXR variant detected by MS.

Sample	Total identified peptides	-10lgP [LXRβ]	Coverage LXRβ peptides	Supporting peptides [LXRβ]	Unique	Amino acids position	
						start	end
MDA.MB.468 siCON	4512	44.58	17%	R.RSVVRGGAR.R	No	113	121
				R.YAC(+57.02)RGGGTC(+57.02)QMDAFM(+15.99)RR.K	No	123	139
				S.EAGSQGSGEGEGVQ(+.98)LTAAQEL.M	No	205	225
				A.QELMIQ(+.98)Q(+.98)LVAAQLQC(+57.02)NKR.S	No	223	240
				L.QVEFIN(+.98)PIFEFSRAM(+15.99)R.R	No	345	360
MDA.MB.468 siLXRα	3726	50.87	18%	G.NGPPQPGAPSSSPTVK.E	No	16	31
				R.RSVVRGGAR.R	No	113	121
				K.RSFSDQPKVTPWPLGADPQ(+.98)SR.D	No	240	260
				K.QVPGFLQLGREDQ(+.98) IA	No	287	300
				A.KQ(+.98)VPGFLQLGREDQIALLK.A	No	286	304
				A.LQQ(+.98)PYVEALLS.Y	No	394	404
				R.M(+15.99)LMKLVSLR.T	No	420	428
MDA.MB.468 siLXRβ	3641	-	0%	-	-	-	-
MDA.MB.468 treated with GW3965	4013	58.60	15%	G.EGVQLTAAQELMIQ.Q	No	215	228
				V.QLTAAQ(+.98)ELMIQ(+.98).Q	No	218	228
				G.EGVQ(+.98)LTAAQELMIQ(+.98)Q(+.98)LVAAQLQ(+.98)C(+57.02)NK.R	No	215	239
				Q.Q(+.98)LVAAQLQ(+.98)C(+57.02)N(+.98)KR.S	No	229	240
				R.QQRFAHFTELAIISVQ(+.98)E.I	Yes [β1]	264	280
				K.QVPGFLQ(+.98)LGR.E	No	287	296
				R.EDQ(+.98)IALLK.A	No	297	304
				K.RPQDQ(+.98)LR.F	No	410	416

Table S6. Primer Sequences of LXR transcripts for qPCR. Related to Figure 3 and Figure 5. *denotes potential ambiguity in amplicon.

LXR transcript	Forward	Reverse
α1.1	TGCTCAGCTCCAGCTCACTG	AGGCACTGTCCAAATCCCCA
α1.2	TCTGGGGAGAAGTGAGGGGT	CACTTTCCAGGGTCCCAGCA
a1.3	CCTATGGAGGGGAGGGAACA	TGAGCACAAGCAGGACCCAG
α1.4/α2.3	AGGAGCATAAGAAGGACAGTGC	GAGGAATGTCAGGCACAGGG
α2.1	CTCAGCCTTTCCCCAAATTGCT	TACCAAGGCACTGTCCAAATCC
α2.2	GAGCATAAGAAG-GACAGTGC	CGCAGAGTCAGGAGGAATGT
α3.1/α5.1*	GAAAAGGCGCAGTCTCGGTG	ACCGCAGAGTCTTCTTATGCT
α3.2/α5.3*	CACCGAGACTTCTGGACAGG	CCACCGCAGAGTCAAATCCC
α3.3	GGAGAAATCCCTTACCAAGACTCTG	GCATCCTGGCTTCCTCTCTGA
α4.1	GCCAAGGTACAGGTAACGAAGC	TCCTTCTCGGCGTGAACCTG
α4.2	AGGTACAGCTTCAGGGAAGTC	CTCCTTCTCGGCGTGAACCT
α5.2	GCTAAGAGCGCTGGACTCTG	GCATCCTGGCTTCCTCTCTGA
α2/α5*	GAGTCACGGTGATGCTTCTG	TGGCAAAGTCTTCCCGGTTA
XM_024448XXX	GATCGAGGTGGCTGGAGAAG	AACCTCAAACGGGGACTAGG
XM_02444829X	CACTCTGCTGGGGGTACTG	GACAGGACACCTGTGGGTTC
XM_017017057	CATCTTCGAGTTCTCCAGGGC	GCCAAGCTCTCTCATCCTGC
XM_024448302/ XM_011519808	AGGAGCATAAGAAGGTGTCCTG	CAAGGATGTGGCATGAGCCT
Total α	TGGAAGCCCTGCATGCCTAC	ACTTGCTCTGAGTGGACGCT
β1.2	CAGTGGGTCCTGTGATGAGG	GGACAGAGCAAGACTTCGTG
β1.3	TTAAAGGAGAATGGCCCTACC	GGTATCCAGGGAACTCGTGGT
β1	CCCAAAGTCACGCCCTGG	CTTCAGGAGGGCGATCTGG
β2	CCCCTTCTTCTTCACCCACT	CTTCAGAAAGGACGCCCC
β3	CCCAAAGTCACGGAGATCGT	CGATAGTGGATGCCTTCAGGA
β4	AGGAGTCACAGTCACAGTCG	CGGCCCAGCGTGACTTTG
Total β	CGCTACAACCACGAGACAGAGT	GCGAGAACTCGAAGATGGGGTT
HPRT	AGGCGAACCTCTCGGCTTTC	TCACTAATCACGACGCCAGGG