

Supplemental Material for

**Myeloid-specific deficiency of pregnane X receptor decreases atherosclerosis
in LDL receptor-deficient mice**

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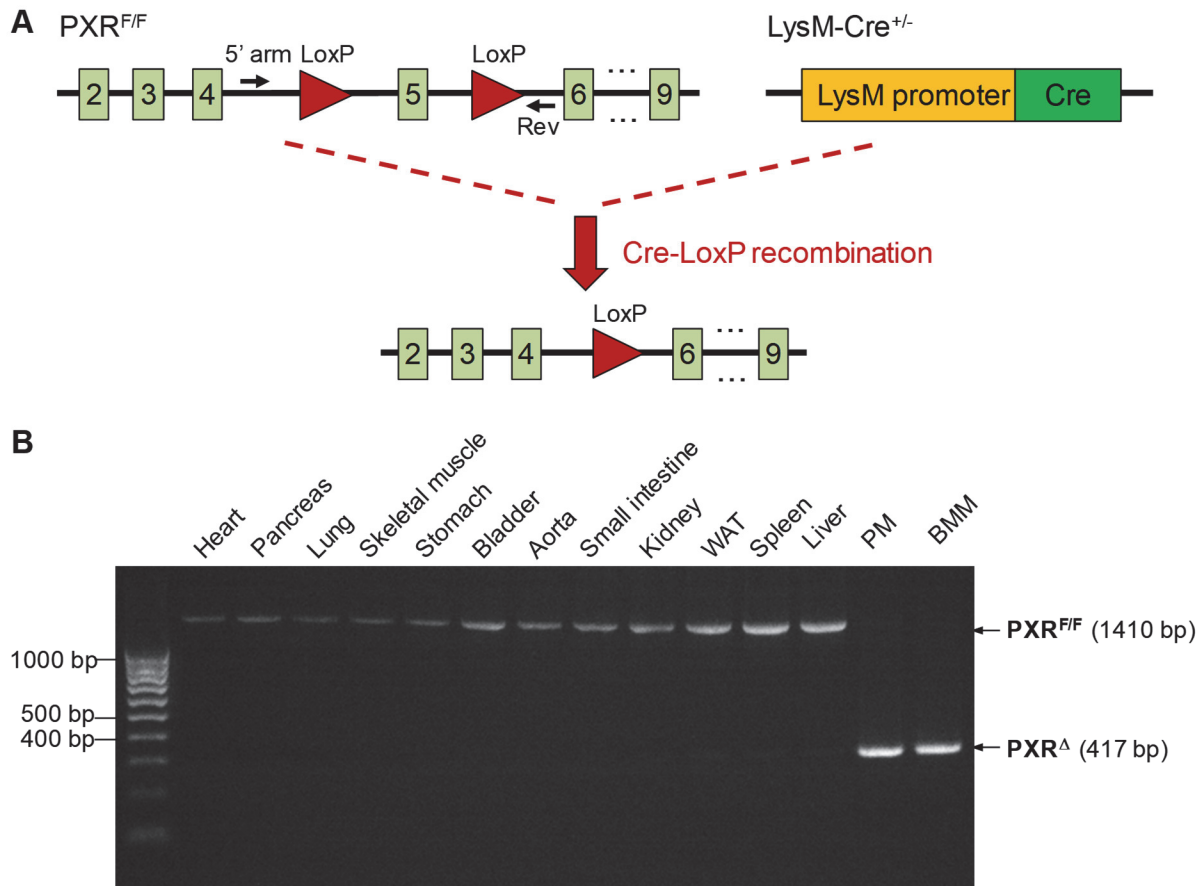
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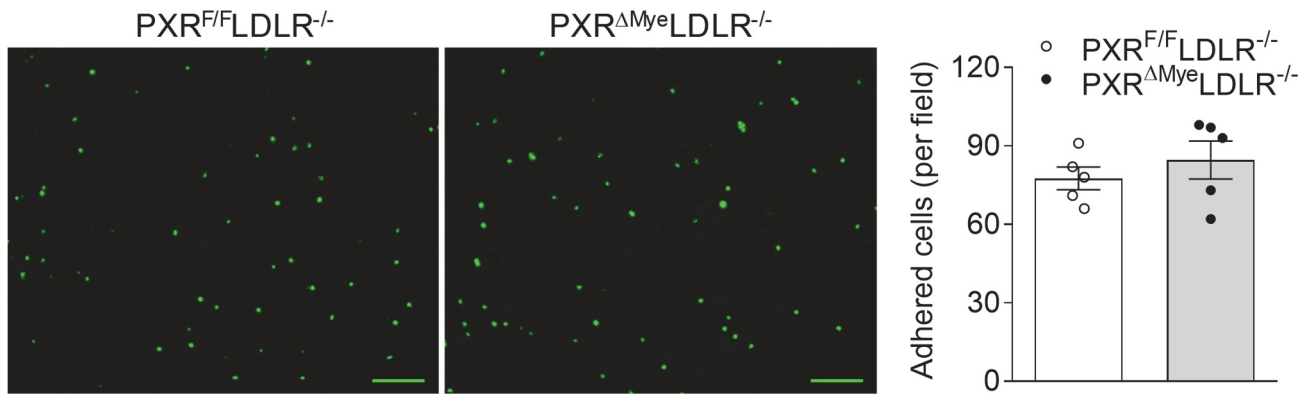
Supplemental Figure S1-S4

Supplemental Table S1-S2

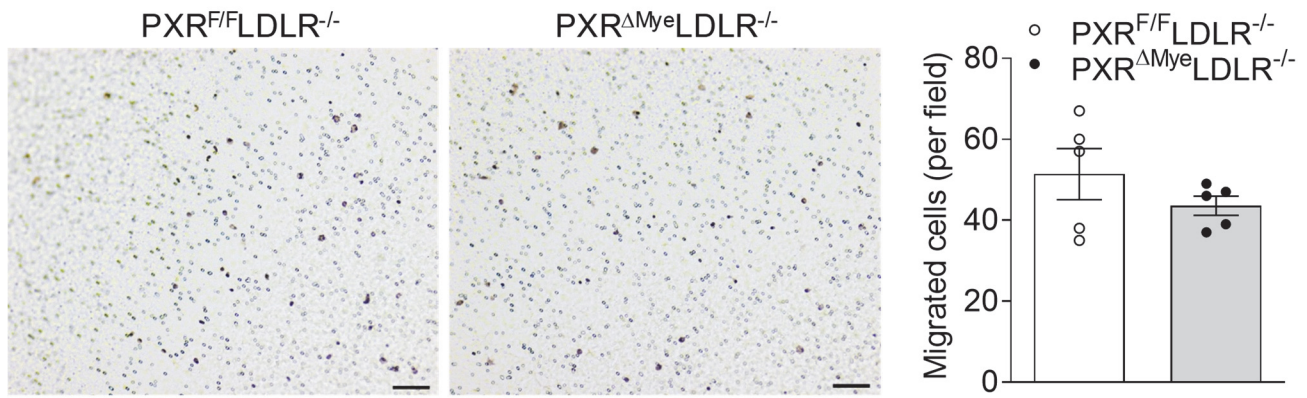


Supplemental Figure S1. Generation of myeloid-specific PXR knockout mice.

(A) Schematic diagram of generating myeloid-specific PXR knockout mice. (B) PCR analysis of genomic DNA from major tissues and macrophages showing that LysMCre-mediated recombination was specific to macrophages. WAT, white adipose tissue; PM, peritoneal macrophages; BMM; bone marrow-derived macrophages.

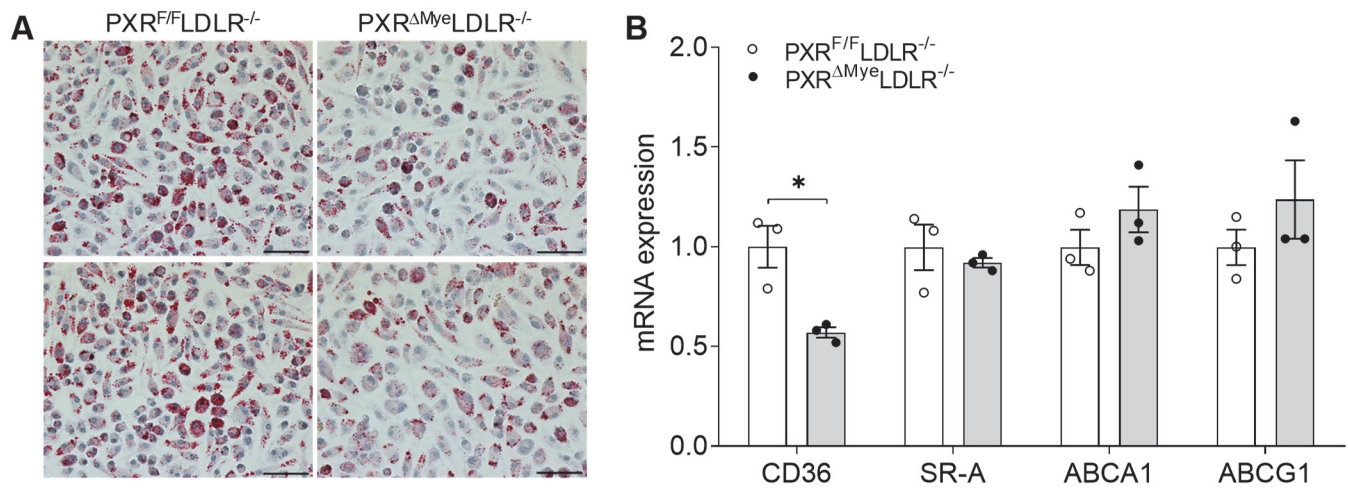


Supplemental Figure S2. Ablation of PXR does not affect macrophage adhesion properties. Peritoneal macrophages isolated from PXR^{F/F}LDLR^{-/-} and PXR^{ΔMye}LDLR^{-/-} mice were labeled with calcein acetoxymethyl and cultured with primary porcine endothelial cell monolayer for 30 minutes. Adhered cells were then counted under a fluorescence microscope, and quantification of adhered cells is presented on the right panel (n=5) (Scale bars; 100 μM).



Supplemental Figure S3. Deficiency of PXR does not affect migration of macrophages of $PXR^{\Delta Mye}LDLR^{-/-}$ mice.

Peritoneal macrophages isolated from $PXR^{F/F}LDLR^{-/-}$ and $PXR^{\Delta Mye}LDLR^{-/-}$ mice were seeded on the matrigel-coated transwell filters. Cells that infiltrated and migrated to the underside of transwell were stained with hematoxylin and counted under the microscope (n=5) (Scale bars; 100 μ M).



Supplemental Figure S4. Deficiency of PXR decreases CD36 expression and reduces foam cell formation in bone marrow-derived macrophages.

(A) Bone marrow-derived macrophages from $PXR^{F/F}LDLR^{-/-}$ and $PXR^{\Delta Mye}LDLR^{-/-}$ littermates were incubated with oxLDL (100 $\mu\text{g}/\text{mL}$) for 24 hr, and stained with Oil-red-O and hematoxylin (Scale bars, 50 μm). (B) The expression levels of indicated genes in bone marrow-derived macrophages were analyzed by QPCR (n=3, *P<0.05).

Supplemental Table S1. Detailed RNA-Seq data of CD36 and DEGs shown in Figure 9C

Gene Symbol	Gene name	PXR ^{F/F} LDLR ^{-/-} (PCN vs. Control)				PXR ^{ΔMyo} LDLR ^{-/-} (PCN vs. Control)				Control (PXR ^{ΔMyo} LDLR ^{-/-} vs. PXR ^{F/F} LDLR ^{-/-})				PCN (PXR ^{ΔMyo} LDLR ^{-/-} vs. PXR ^{F/F} LDLR ^{-/-})			
		Log2FC	P-value	FDR	FC	Log2FC	P-value	FDR	FC	Log2FC	P-value	FDR	FC	Log2FC	P-value	FDR	FC
Jazf1	JAZF zinc finger 1	5.243372	0.000167	0.003051	37.8802	-0.301	0.697198	1	0.811689	6.20123265	1.87E-17	1.54E-15	73.57953	0.64709	0.597268	0.918560	1.56600
Npc111	NPC1 like intracellular cholesterol transporter 1	4.522761	0.004124	0.041872	22.98724	0.053716	0.96031	1	1.037935	3.18437217	0.05206486	0.22036	9.090579	-1.34774	0.178194	0.437915	0.39290
Itgb6	Integrin subunit beta 6	2.311186	0.004387	0.04391	4.96291	-0.86338	0.036651	0.414918	0.549662	1.81581544	0.00420528	0.03729	3.520586	-1.36100	0.010827	0.059137	0.38931
Epha2	Eph receptor A2	0.83212	0.001071	0.014368	1.7803	0.421321	7.87E-06	0.002683	1.339153	2.05033393	1.62E-33	3.56E-31	4.142019	1.64527	1.90E-33	3.15E-31	3.12807
Il1m	Interleukin 1 receptor antagonist	0.779372	1.27E-21	5.05E-19	1.716383	0.352363	6.01E-05	0.012857	1.27665	-0.47223731	4.90E-07	1.30E-05	0.720846	-0.89334	6.45E-21	5.65E-19	0.53836
Ccl2	C-C motif chemokine ligand 2	0.763592	0.000735	0.010515	1.697713	0.391191	0.001441	0.094205	1.311475	1.20044398	2.80E-10	1.17E-08	2.298103	0.83437	7.49E-09	2.03E-07	1.78308
Ptgs2	Prostaglandin- endoperoxide synthase 2	0.735459	0.000235	0.004038	1.664928	0.339475	1.36E-05	0.004011	1.265296	2.62922943	1.56E-87	1.55E-84	6.18695	2.23854	1.73E-85	1.22E-82	4.71921
Aasdh	Aminoacidpate- semialdehyde dehydrogenase	0.716535	0.001907	0.023244	1.643231	-0.09512	0.391782	0.890723	0.936194	0.53067146	0.00485243	0.04158	1.44460	-0.27568	0.0581692	0.206017	0.82605
Ccl3	C-C motif chemokine ligand 3	0.705128	3.73E-30	4.08E-27	1.630289	0.202955	0.001059	0.079412	1.151054	0.35080864	2.89E-06	6.41E-05	1.27528	-0.14477	0.024650	0.110890	0.90451
Il1a	Interleukin 1 alpha	0.697222	5.37E-19	1.56E-16	1.62138	0.373905	2.84E-07	0.000188	1.295855	0.26485349	0.00076973	0.00941	1.20151	-0.05227	0.543455	0.867580	0.96441
Gde1	Glycerophosphodiester phosphodiesterase 1	0.673234	5.20E-31	7.10E-28	1.594643	0.247306	0.000128	0.020704	1.186989	0.05630782	0.45739369	0.79349	1.03980	-0.36316	3.48E-08	8.54E-07	0.77745
Sphk1	Sphingosine kinase 1	0.673085	2.65E-05	0.000632	1.594479	0.132075	0.307191	0.816837	1.095869	0.1062022	0.51734512	0.85290	1.076390	-0.42990	0.001495	0.012013	0.74231
Rgcc	Regulator of cell cycle	0.658112	5.06E-06	0.000149	1.578016	0.119611	0.355059	0.860107	1.086442	-0.28201087	0.07282951	0.27603	0.822443	-0.81466	8.67E-11	2.93E-09	0.56854
Clec4e	C-type lectin domain family 4, member e	0.639011	1.03E-29	1.02E-26	1.557262	0.232268	0.000969	0.074756	1.17468	0.11857425	0.11159824	0.35719	1.08566	-0.28197	0.000180	0.002005	0.82246
Plcx2	Phosphatidylinositol specific phospholipase C X domain containing 2	0.625189	3.38E-09	2.19E-07	1.542413	0.335943	0.002227	0.116634	1.262202	-0.17900189	0.14849904	0.41744	0.88331	-0.46444	6.49E-05	0.000821	0.72475
Hsd17b1	Hydroxysteroid 17-beta dehydrogenase 1	0.596867	0.001759	0.021789	1.512429	0.231677	0.070118	0.517141	1.174199	0.02826638	0.85871415	1	1.01978	-0.33103	0.029750	0.127424	0.79496
Pla2g7	Phospholipase A2 group	-0.59314	4.89E-24	2.67E-21	0.662898	-0.22833	0.000312	0.037332	0.853623	-0.65847653	7.33E-19	6.90E-17	0.63354	-0.28690	6.35E-06	0.000103	0.81965

VII																	
Pik3cd	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta	-0.59701	1.66E-16	3.32E-14	0.661122	-0.11067	0.09874	0.590446	0.926156	-0.27008078	0.00091392	0.01079	0.82927	0.22180	0.003634	0.024796	1.16619
Sor1	Sortilin related receptor 1	-0.6107	1.18E-18	3.25E-16	0.654878	-0.07164	0.487967	0.966506	0.951557	-0.4823263	2.43E-05	0.000443	0.71582	0.06136	0.548471	0.87309	1.04345
Tlr7	Toll-like receptor 7	-0.61524	1.89E-13	2.34E-11	0.652822	-0.26341	0.00022	0.029868	0.833117	-0.1917413	0.02115727	0.121772	0.87554	0.16593	0.049113	0.18261	1.12189
Ncf1	Neutrophil cytosolic factor 1	-0.62006	3.83E-22	1.71E-19	0.650644	-0.32538	6.45E-08	5.41E-05	0.798089	0.29821444	1.76E-05	0.00033	1.22962	0.59897	6.79E-17	4.30E-15	1.51463
Tnfrsf14	TNF receptor superfamily member 14	-0.62348	0.004882	0.047857	0.649102	-0.19215	0.102497	0.590446	0.875303	-0.01442185	0.91603598	1	0.99005	0.42104	0.02702	0.118668	1.33889
Naaa	N-acyl ethanolamine acid amidase	-0.63979	1.08E-17	2.52E-15	0.641807	-0.2174	0.000556	0.054452	0.860114	0.19740743	0.00946029	0.06863	1.14663	0.62625	3.76E-17	2.46E-15	1.54355
Scd2	Stearoyl-Coenzyme A desaturase 2	-0.6596	0.001216	0.015901	0.633056	-0.53979	3.04E-09	3.68E-06	0.687869	1.74238203	4.74E-41	1.44E-38	3.34587	1.86727	2.56E-37	4.94E-35	3.64842
Hadh	Hydroxyacyl-CoA dehydrogenase	-0.66352	1.08E-09	7.60E-08	0.631337	-0.44162	3.14E-06	0.001345	0.736308	0.24786732	0.02751094	0.14496	1.18745	0.47798	4.30E-06	7.28E-05	1.39280
Pld4	Phospholipase D family, member 4	-0.71348	2.83E-15	4.51E-13	0.609847	-0.35327	3.72E-06	0.001563	0.782807	-0.25693676	0.00441531	0.03866	0.83686	0.109846	0.197135	0.470012	1.07911
Cpt1a	Carnitine palmitoyl transferase	-0.71568	5.31E-44	1.45E-40	0.608919	-0.38218	4.21E-11	8.36E-08	0.767277	-0.58208153	2.59E-15	1.79E-13	0.66799	-0.24323	4.82E-05	0.000637	0.84484
Themis2	Thymocyte selection associated family member 2	-0.72416	1.63E-08	9.02E-07	0.605349	-0.26016	0.002182	0.115597	0.834994	0.06733668	0.53194097	0.86727	1.04778	0.536526	2.07E-06	3.76E-05	1.45047
Tlr1	Toll like receptor 1	-0.77989	2.39E-07	1.00E-05	0.58241	-0.06221	0.568783	1	0.957793	-0.23376197	0.06769941	0.26391	0.85041	0.49020	0.000318	0.003253	1.404647
Panx1	Pannexin 1	-0.8433	8.43E-05	0.00171	0.557366	-0.15375	0.420915	0.913509	0.898913	-0.05853875	0.7210404	1	0.96023	0.63648	0.015080	0.076263	1.554541
Ccr2	C-C motif chemokine receptor 2	-0.87194	0.004813	0.047348	0.546411	-0.51637	0.007815	0.219847	0.699127	-0.642375	0.00380271	0.03453	0.64065	-0.28045	0.306658	0.62010	0.823331
Fgr	FGFR proto-oncogene, Src family tyrosine kinase	-0.87237	3.23E-21	1.18E-18	0.546248	-0.35594	3.01E-06	0.001315	0.781358	0.08963778	0.28691985	0.61590	1.06410	0.61326	7.44E-11	2.54E-09	1.529712
Idi1	Isopentenyl-diphosphate delta isomerase 1	-0.87306	3.08E-08	1.61E-06	0.545987	-0.87603	1.18E-17	1.29E-13	0.544864	0.43467458	5.46E-05	0.00092	1.35160	0.43861	0.004195	0.02793	1.355304

Dhcr7	7-dehydrocholesterol reductase	-0.90783	4.30E-09	2.75E-07	0.532986	-0.53794	0.000196	0.028562	0.688752	-0.0036516	0.97944434	1	0.99747	0.37374	0.039569	0.15735	1.29570
Acaa2	Acetyl-CoA acyltransferase 2	-0.91972	2.10E-25	1.31E-22	0.528611	-0.43943	3.90E-10	6.08E-07	0.737425	-0.32288455	3.95E-05	0.00069	0.79946	0.16331	0.066277	0.22660	1.11985
Tlr3	Toll like receptor 3	-1.02255	0.001514	0.019117	0.492245	-0.1905	0.266884	0.767534	0.8763	0.25938144	0.20289351	0.50953	1.19696	1.09964	4.09E-05	0.00055	2.14302
Cysl1r1	Cysteinyl leukotriene receptor 1	-1.23801	2.64E-08	1.40E-06	0.423958	-0.36054	0.007416	0.215675	0.778871	-0.25352295	0.09114727	0.31832	0.83884	0.62951	0.001604	0.01273	1.54704
Tlr8	Toll like receptor 8	-1.55591	6.60E-29	5.77E-26	0.340114	-0.66465	1.95E-12	5.32E-09	0.630842	-0.46466632	6.73E-05	0.00111	0.72463	0.43214	0.0007432	0.00671	1.34923
C5ar1	Complement component 5a receptor 1	-1.585	1.49E-14	2.20E-12	0.333324	-0.56592	6.66E-06	0.002385	0.675523	-0.34239846	0.00435239	0.03823	0.78872	0.68187	0.001436	0.011635	1.60422
Hmgcs2	3-hydroxy-3-methylglutaryl-CoA synthase 2	-1.72607	0.000284	0.004747	0.302274	-1.10641	0.000119	0.019986	0.464447	-0.67113462	0.0192111	0.11359	0.62801	-0.03992	0.93219	1	0.97270
Dhcr24	24-dehydrocholesterol reductase	-2.35744	5.27E-07	2.04E-05	0.195138	-1.23679	3.12E-15	2.27E-11	0.424315	1.32015157	4.82E-12	2.41E-10	2.49692	2.44145	4.11E-11	1.47E-09	5.43190
Plch2	Phospholipase C eta 2	-4.60753	0.004074	0.041557	0.04102	-0.51817	0.60683	1	0.698256	-0.3498288	0.72009704	1	0.78467	3.767311	0.026373	0.11658	13.6167
Tlr11	Toll-like receptor 11	-5.05975	0.00431	0.043298	0.029982	-0.5449	0.409618	0.907456	0.685439	-0.851068	0.33354015	0.67515	0.55437	3.68177	0.019122	0.09105	12.83293
Cd36	Cd36 molecule	0.05624	0.24770	0.64325	1.03975	0.022825	0.711007	1	1.015947	-0.41125804	7.24E-09	2.52E-07	0.75196	-0.43835	3.58E-13	1.64E-11	0.73797

FC: Fold change; FDR: False discovery rate

Supplemental Table S2. Primer sequences for QPCR

Genes	Primer sequences	Gene	Primer sequences
PXR	5'- GACGCTCAGATGCAAACCTT -3' 5'- TCTTCTCCGCGCAGCTGCA -3'	ABCA1	5'-CCGAGGAAGACGTGGACACCTTC-3' 5'-CCTCAGCCATGACCTGCCTTGTAG-3'
CD36	5'-CAGTCGGAGACATGCT-3' 5'-CTCGGGGTCCTGAGTT-3'	ABCG1	5'-AGGTCTCAGCCTTCTAAAGTTCCTC-3' 5'-TCTCTCGAATGAAATTTATCG-3'
SR-A	5'-GGAGTGTAGGCGGATC-3' 5'-GTCAATGGAGGCCCCA-3'	GAPDH	5'-AACTTTGGCATTGTGGAAGG-3' 5'-GGATGCAGGGATGATGTTCT-3'