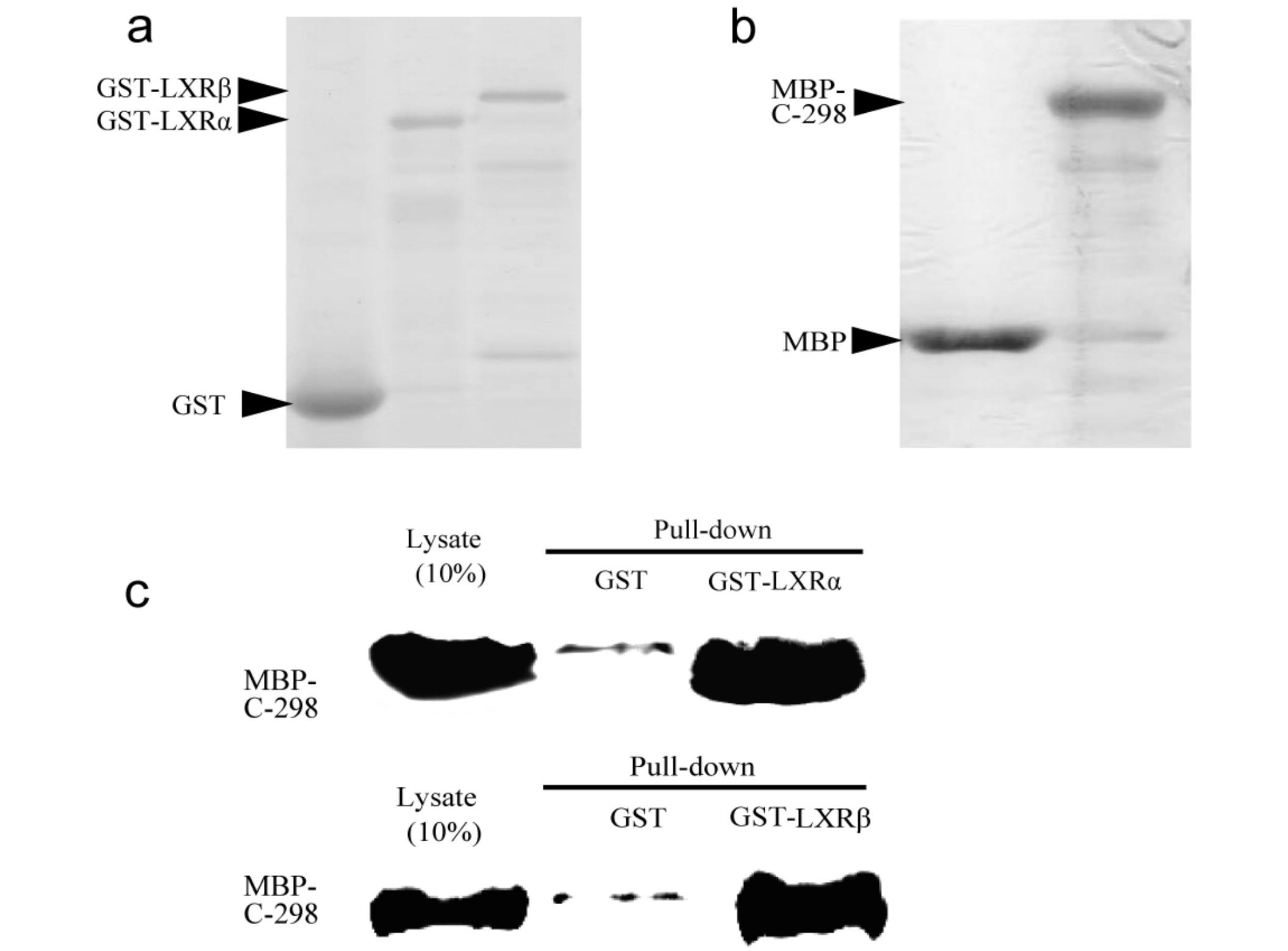
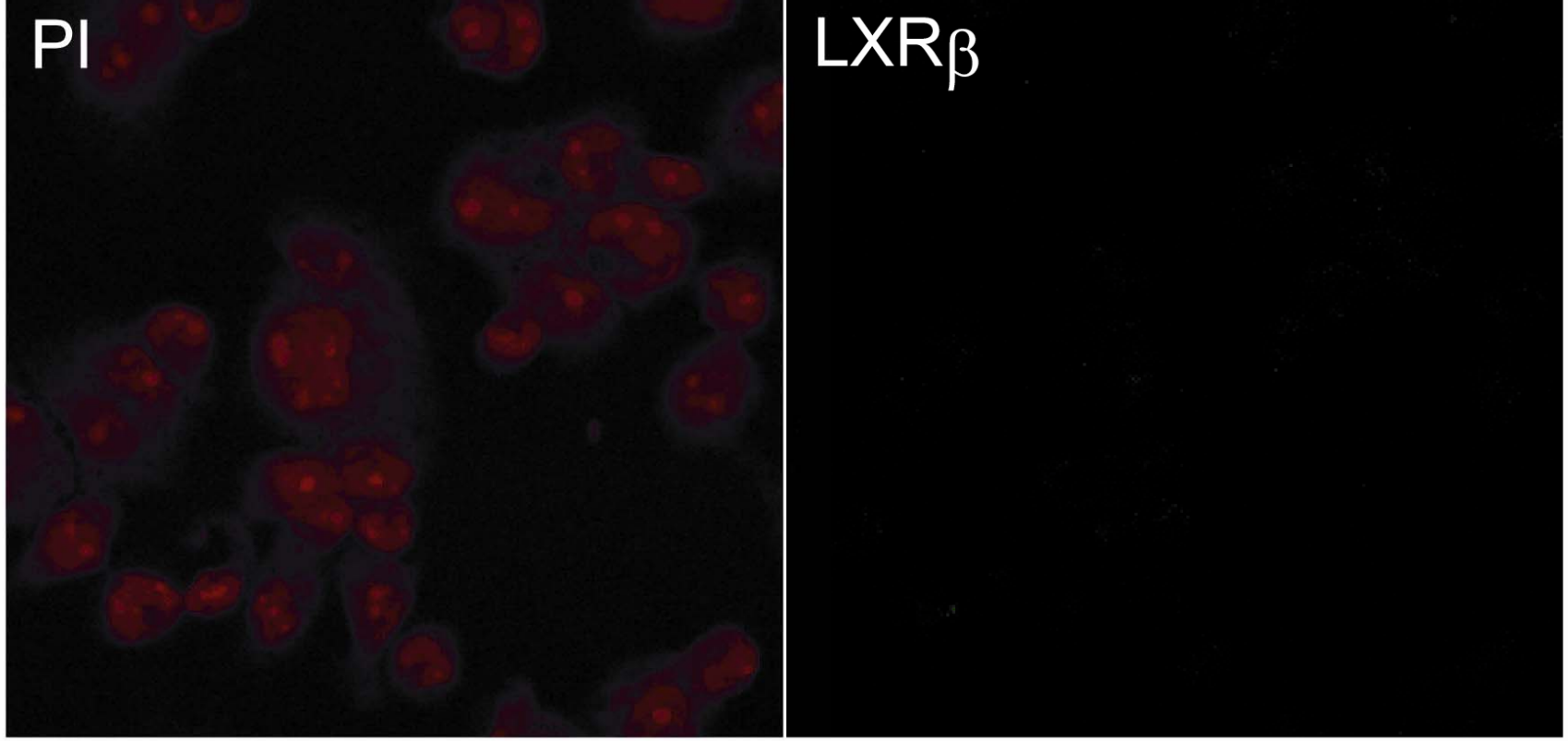
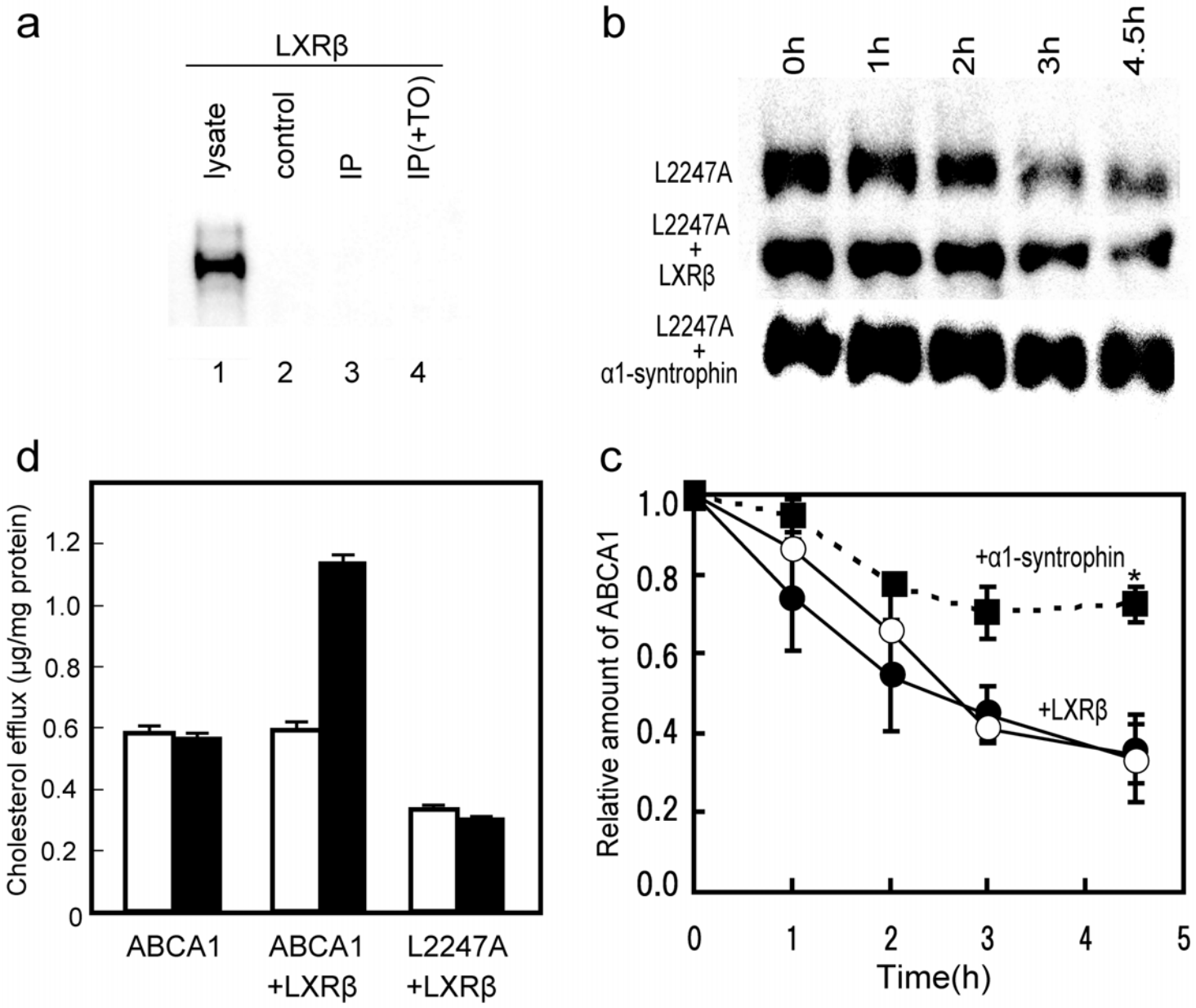
Supplementary Figure 1. Direct interaction between ABCA1 and LXRs.
(a) LXRα and LXRβ fused with glutathione-S-transferase (GST) were expressed in *E. coli* strain BL21, and purified with glutathione beads. (b) C-terminal 298 amino acids of ABCA1 fused with maltose-binding protein (MBP-C-298) were expressed in *E. coli* strain BL21, and purified with amylose resin. (c) MBP-C-298 was mixed with purified GST-fused LXRs and precipitated with glutathione beads.

Supplementary Figure 2. Specificity of the anti-LXRβ monoclonal antibody. When LXRβ was knocked down by siRNA, no signal for LXRβ was observed.

Supplementary Figure 3. Effect of L2247A substitution on the interaction with LXRβ. (a) HEK293 cells were co-transfected with ABCA1-L2247A and LXRβ. Cells were lysed and immunoprecipitated with anti-LXRβ antibody in the absence (lanes 2, 3) or presence (lane 4) of TO901317. Cell lysates (10%) and immunocomplexes were subjected to immunoblotting with anti-ABCA1 monoclonal antibody KM3110. (b, c) Turnover of ABCA1-L2247A. HEK293 cells were co-transfected with ABCA1-L2247A and vector (mock, ○), LXRβ (●), or α1-syntrophin (■). At 48 h after transfection, 100 μg/ml of cycloheximide was added to block protein synthesis. Cell lysates were subjected to immunoblotting with KM3110. Values are expressed as the fold increase relative to the amount of ABCA1 just before cycloheximide was added. (d) ApoA-I-dependent cholesterol efflux. HEK293 cells were co-transfected with 3 μg of ABCA1 or ABCA1-L2247A and 1 μg of LXRβ. Fresh medium was added at 28 h after transfection, and apoA-I-dependent cholesterol efflux was measured during a 2-h incubation in the presence (filled bars) or absence (open bars) of 100 nM TO901317. *$p<0.05$, significantly different from control.


a
GST-LXRβ
GST-LXRα
GST
b
MBP-
C-298
MBP
c
Lysate
(10%)
Pull-down
GST
GST-LXRα
MBP-
C-298
Lysate
(10%)
Pull-down
GST
GST-LXRβ
MBP-
C-298


PI
LXRβ


a
LXRβ
lysate
control
IP
IP(+TO)
1
2
3
4
b
0h
1h
2h
3h
4.5h
L2247A
L2247A + LXRβ
L2247A + α1-syntrophin
d
Cholesterol efflux (μg/mg protein)
0
0.2
0.4
0.6
0.8
1.0
1.2
ABCA1
ABCA1 +LXRβ
L2247A +LXRβ
c
Relative amount of ABCA1
0.0
0.2
0.4
0.6
0.8
1.0
+α1-syntrophin
*
+LXRβ
0
1
2
3
4
5
Time(h)