Cerebrospinal Fluid Steroidomics: Are Bioactive Bile Acids Present in Brain?

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Supplementary Figure 2. Analysis of the nuclear receptor activational capacity of acidic intermediates of bile acid biosynthesis.
(a) Analysis of luciferase activity in SN4741 cells transfected with an LXR-responsive luciferase reporter construct (LXRE) and LXRα as indicated, and stimulated for 24 h with 22R-hydroxycholesterol (10 µM) or the acidic compounds indicated.
(b) Analysis of luciferase activity in SN4741 cells transfected with an FXR-responsive luciferase reporter construct (FXRE) and FXR as indicated, and stimulated for 24h with chenodeoxycholic acid (CDCA; 10 µM) or the acidic compounds indicated.
(c) Analysis of luciferase activity in SN4741 cells transfected with a DR5-responsive luciferase reporter construct and NURR1 as indicated, and stimulated for 24h with 9-cis-retinoic acid (9-cis-RA; 10 µM) or the acidic compounds indicated.

For all (a), (b), and (c) firefly luciferase activity was normalized to Renilla luciferase activity, and the values are expressed as fold activation over the normalised basal LXRE-Luc activity (or FXRE-Luc or DR5-Luc) activity set to 1. Data are means ± SEM (n=3), *P<0,05, **P<0,01 compared to vehicle treatment.

Fig. S2a

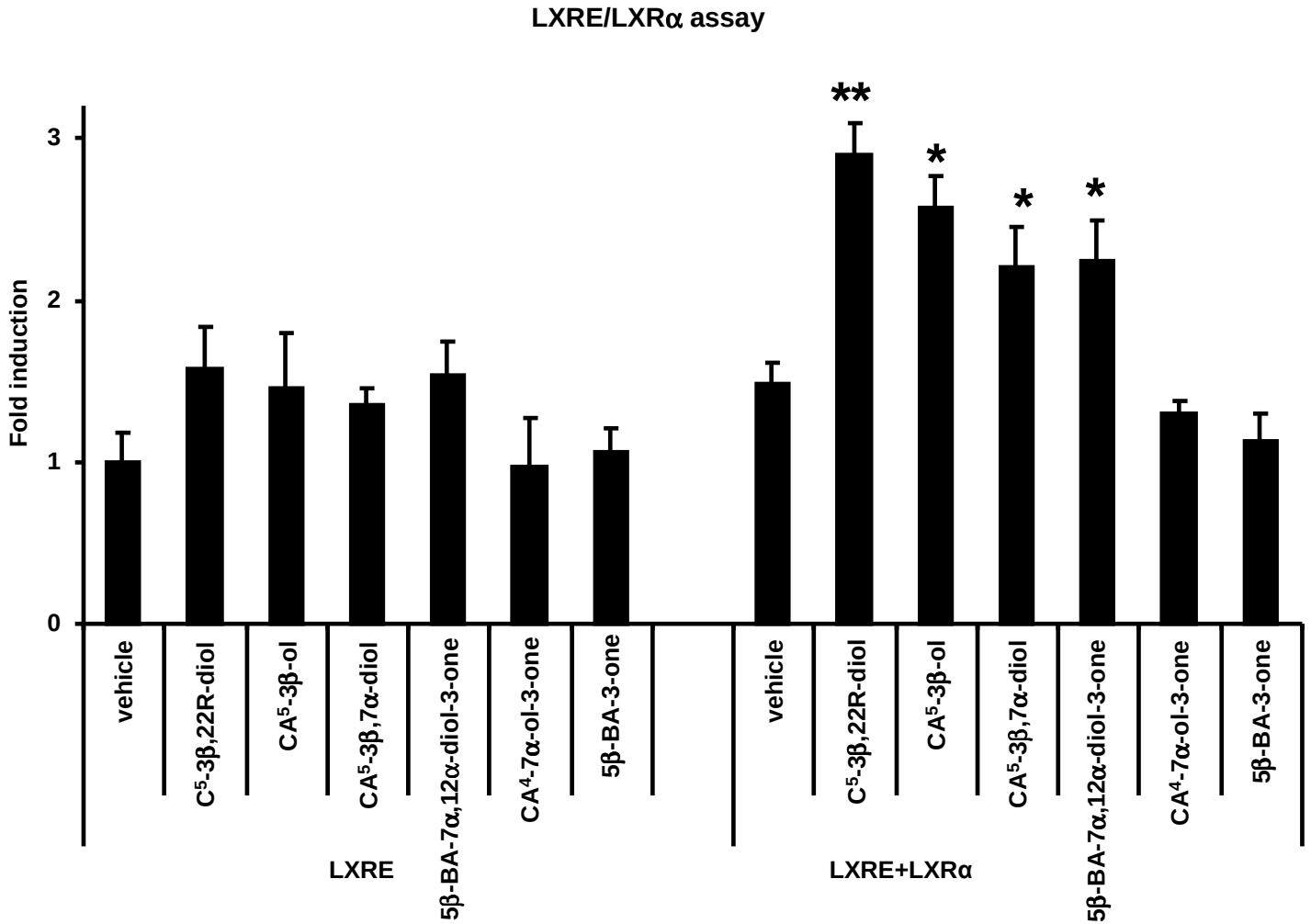

LXRE/LXRα assay
Fold induction
0
1
2
3
**
*
*
*
vehicle
C5-3β,22R-diol
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
vehicle
C5-3β,22R-diol
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
LXRE
LXRE+LXRα

Fig. S2b

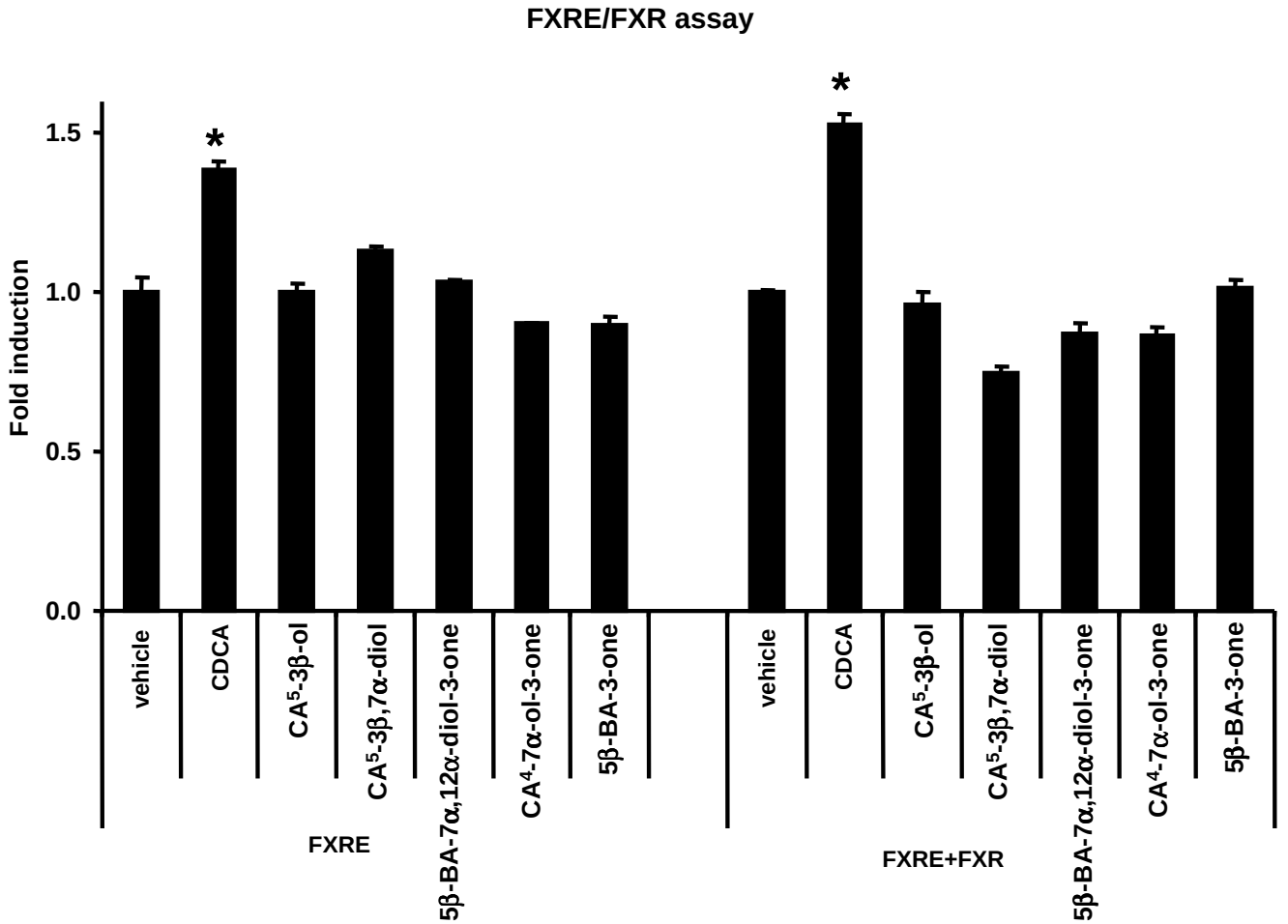
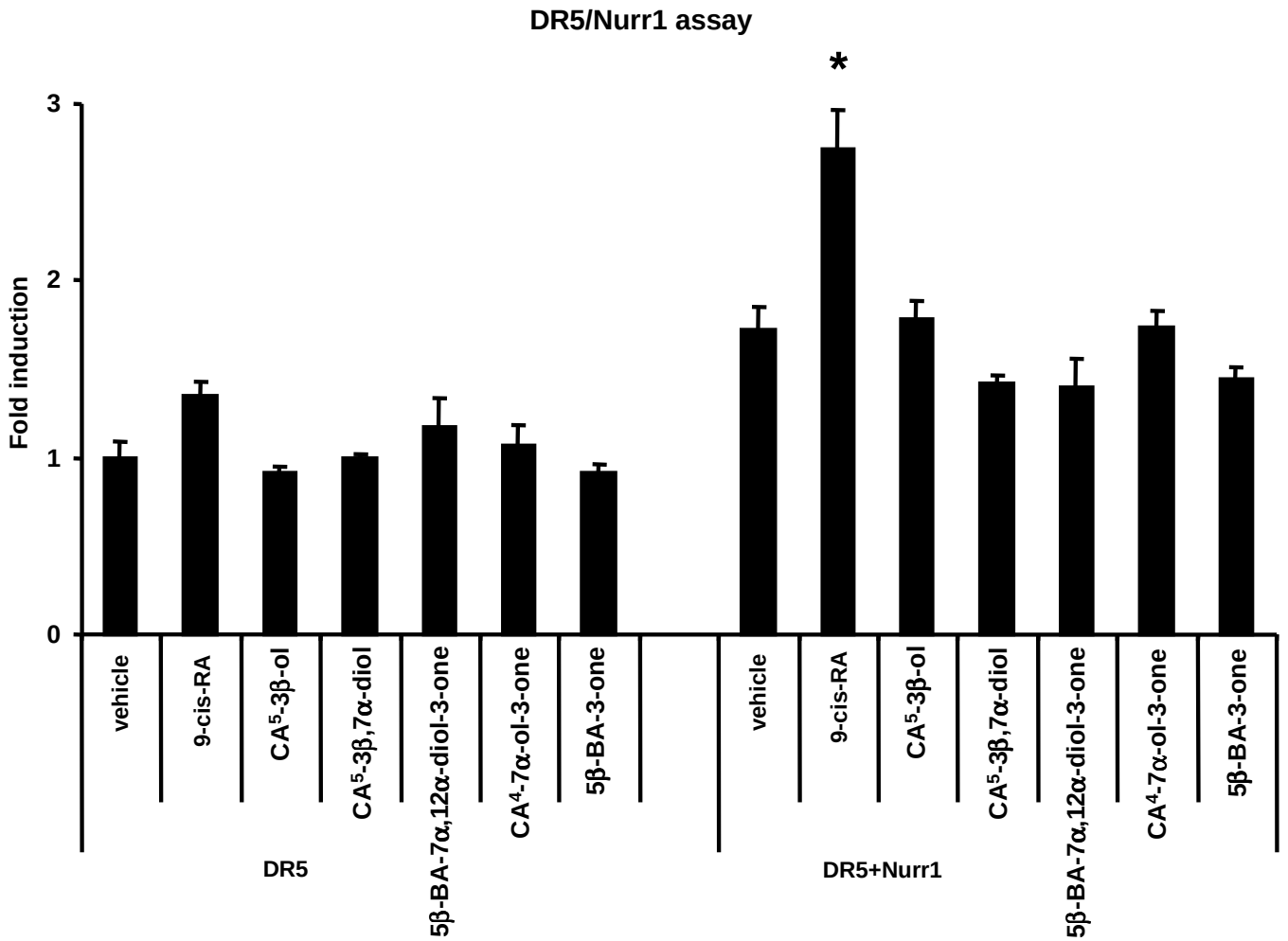

FXRE/FXR assay
Fold induction
1.5
1.0
0.5
0.0
*
*
vehicle
CDCA
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
vehicle
CDCA
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
FXRE
FXRE+FXR

Fig. S2c


DR5/Nurr1 assay
Fold induction
3
2
1
0
*
vehicle
9-cis-RA
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
vehicle
9-cis-RA
CA5-3β-ol
CA5-3β,7α-diol
5β-BA-7α,12α-diol-3-one
CA4-7α-ol-3-one
5β-BA-3-one
DR5
DR5+Nurr1