Supplemental Information

Structural conservation of ligand binding reveals a bile acid-like signaling pathway in nematodes

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Cholestenoic acids do not induce recovery of hookworm iL3.

Table S1 Sequence of peptides used in AlphaScreen assays.

Peptide	Sequence
SRC1-2	SPSSHSSLTERHKILHRLLQEGSP
SRC1-4	QKPTSGPQTPQAQQKSLLQQLLTE
PGC1α-1	QEAEEPSLLKKLLLAPANTQ
TRAP-1	GHGEDFSKVSQNPILTSLLQITGN
CBP-1	SGNLVPDAASKHKQLSELLRGGSG
NcoR-2	GHSFADPASNLGLEDIIRKALMGSF
SHP-1	PCQGSASHPTILYTLLSPGP
SHP-2	VAEAPVPSILKKILLEEPNS
SMRT-2	ASTNMGLEAIIRKALMGKYDQ
SRC2-3	QEPVSPKKKENALLRYLLDKDDTKD
SRC3-1	AENQRGPLESKGHKKLLQLLTSS
SRC3-2	TSNMHGSLLQEKHRILHKLLQNG

Table S2 Structural comparison between *Ace*DAF-12 and mammalian nuclear receptors by superposition.

Mammalian nuclear receptor	PDB ID	RMSD
FXR	1OSV	1.611
LXR	1P8D	1.722
VDR	1DB1	1.742
PR	1A28	1.803
GR	1M2Z	1.821
ROR	1N83	1.844
MR	2A3I	1.849
ER	1ERE	1.85
AR	1I37	1.965

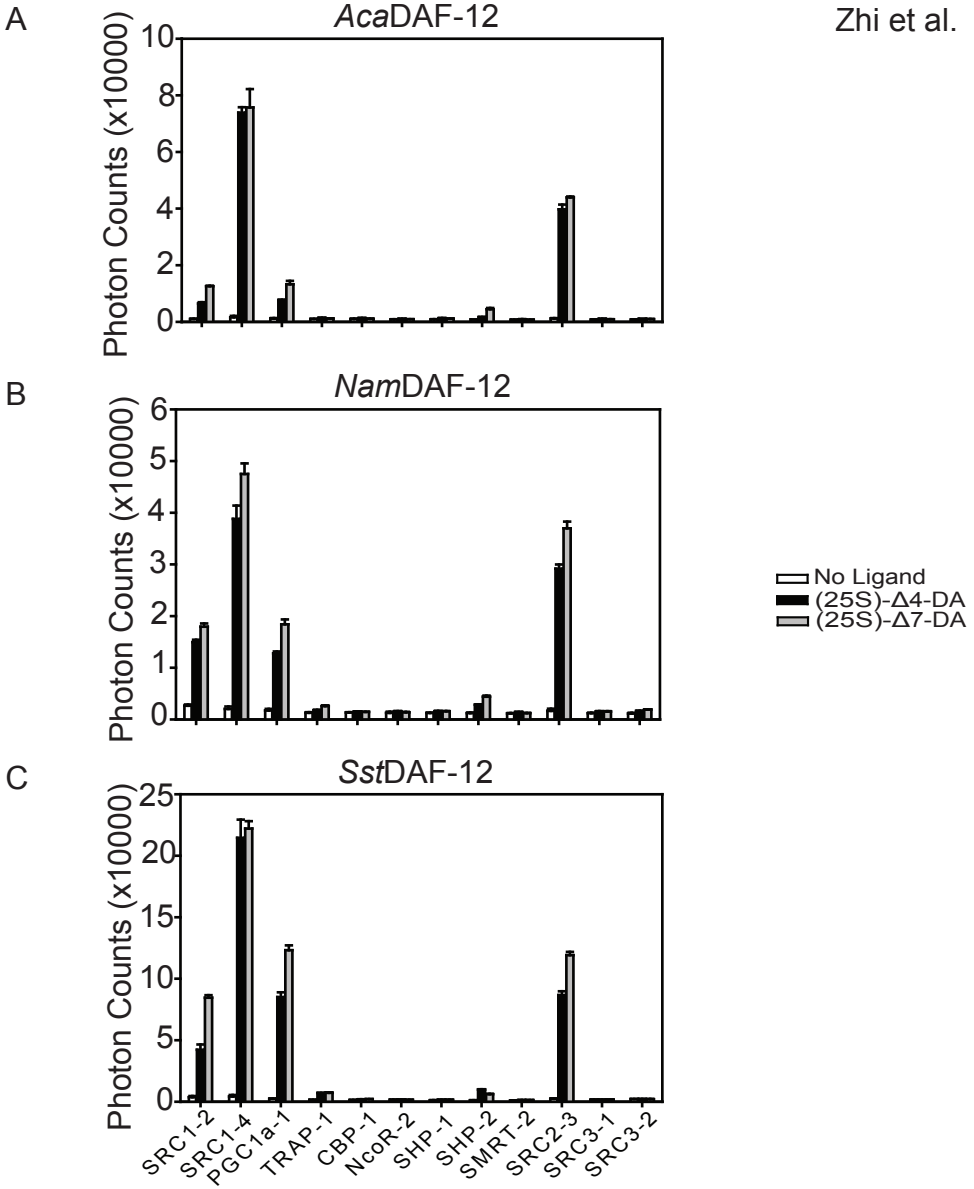

Fig. S1
AlphaScreen assays to search for *Aca*, *Nam*, and *Sst*DAF-12 interacting peptides in the presence and absence of 1 μM (25S)-DAs.

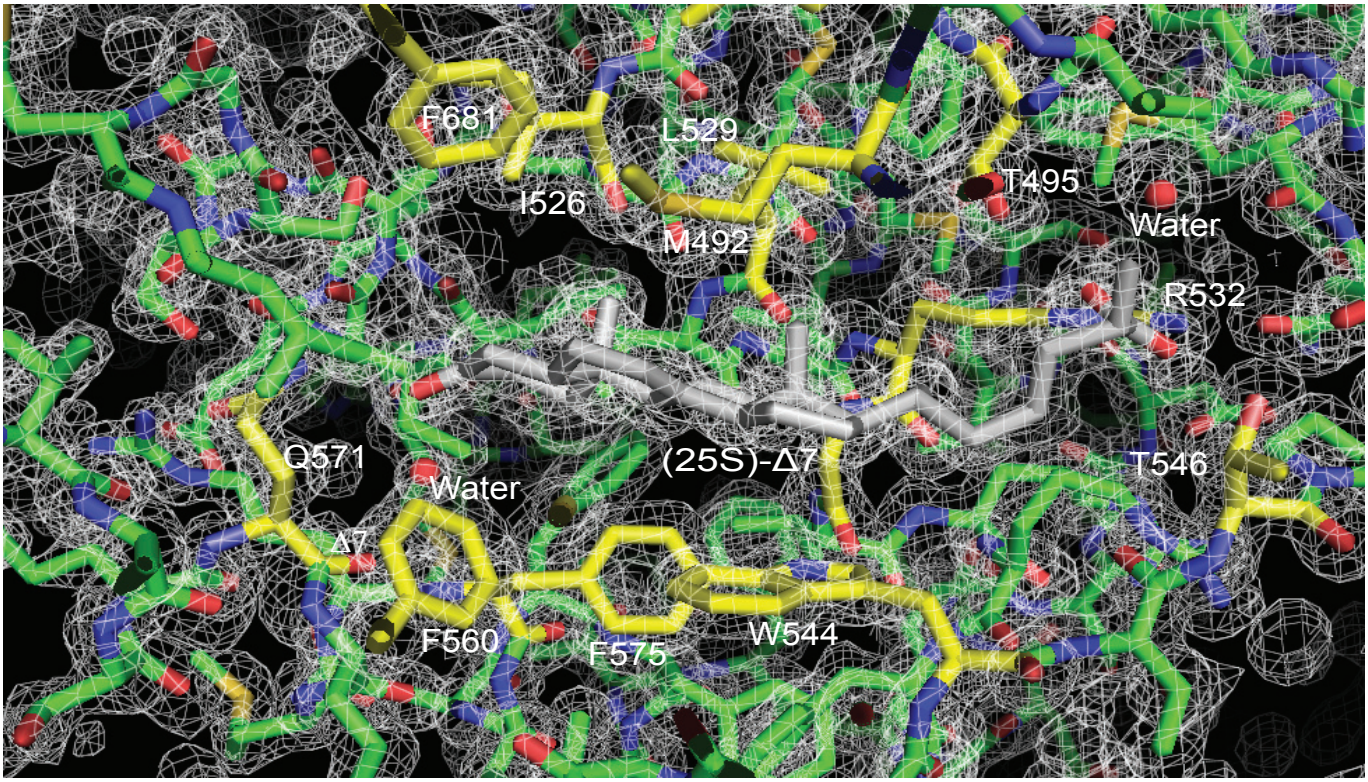

FIG. S2
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Fig. S2
The electron density map of the *Ace*DAF-12 *b* chain ligand binding pocket bound to (25S)-Δ7-DA. The surrounding amino acids are highlighted in yellow.

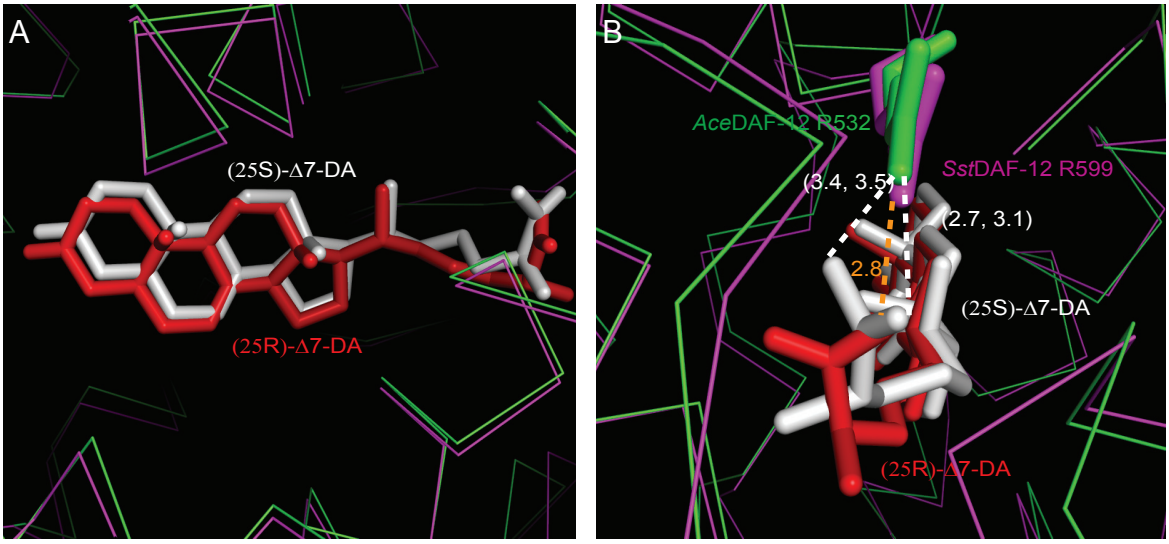

FIG. S3
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Fig. S3
Structrual comparison of complexes *Ace*DAF-12 LBD/(25S)-Δ7-DA and *Sst*DAF-12 LBD/(25R)-Δ7-DA. (A) Superposition of the *Ace*DAF-12 LBD complex *a* (green) onto the *Sst*DAF-12 LBD (magenta) reveals that the only noticeable difference is their ligand conformation, which is probably due to the opposite stereochemistry at the C25 position of ligands. (25R)-Δ7-DA in red is more streched than (25S)-Δ7-DA in white. (B) (25R)-Δ7-DA's extended C27 end forms a single H-bond with R599 (2.8 Å , orange dashed line). (25S)-Δ7-DA in the *Ace*DAF-12 LBD complex *a* or *b* makes a slight turn at the C27 end, allowing it to form two H-bonds with R532, one stronger (2.7 Å in *a* and 3.1 Å in *b*, white dashed line) and one weaker (3.4 Å in *a* and 3.5 Å in *b*).

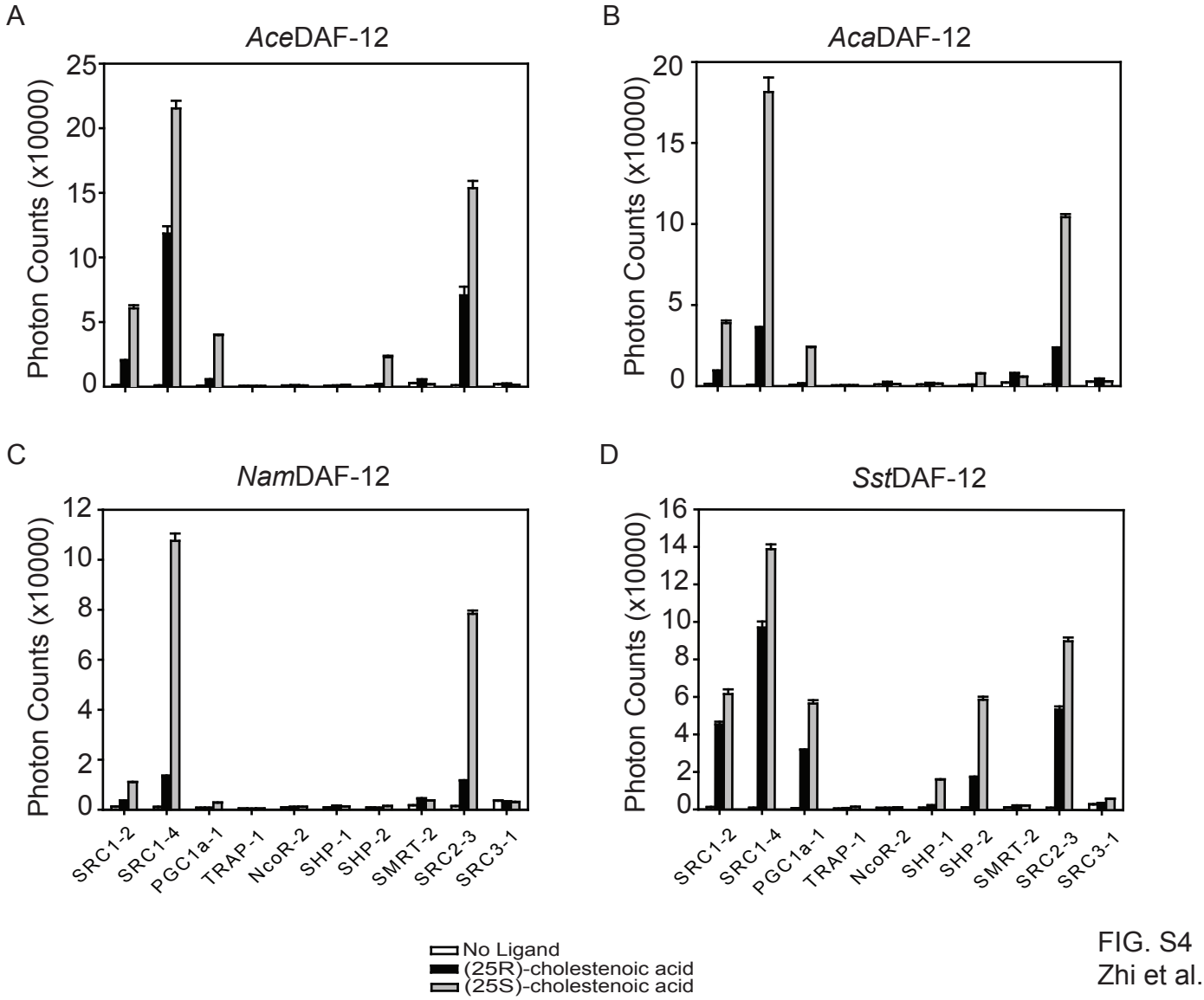

FIG. S4
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Fig. S4
AlphaScreen assays to search for *Ace*, *Aca*, *Nam*, and *Sst*DAF-12 interacting peptides in the presence and absence of 10 μM cholestenoic acids.

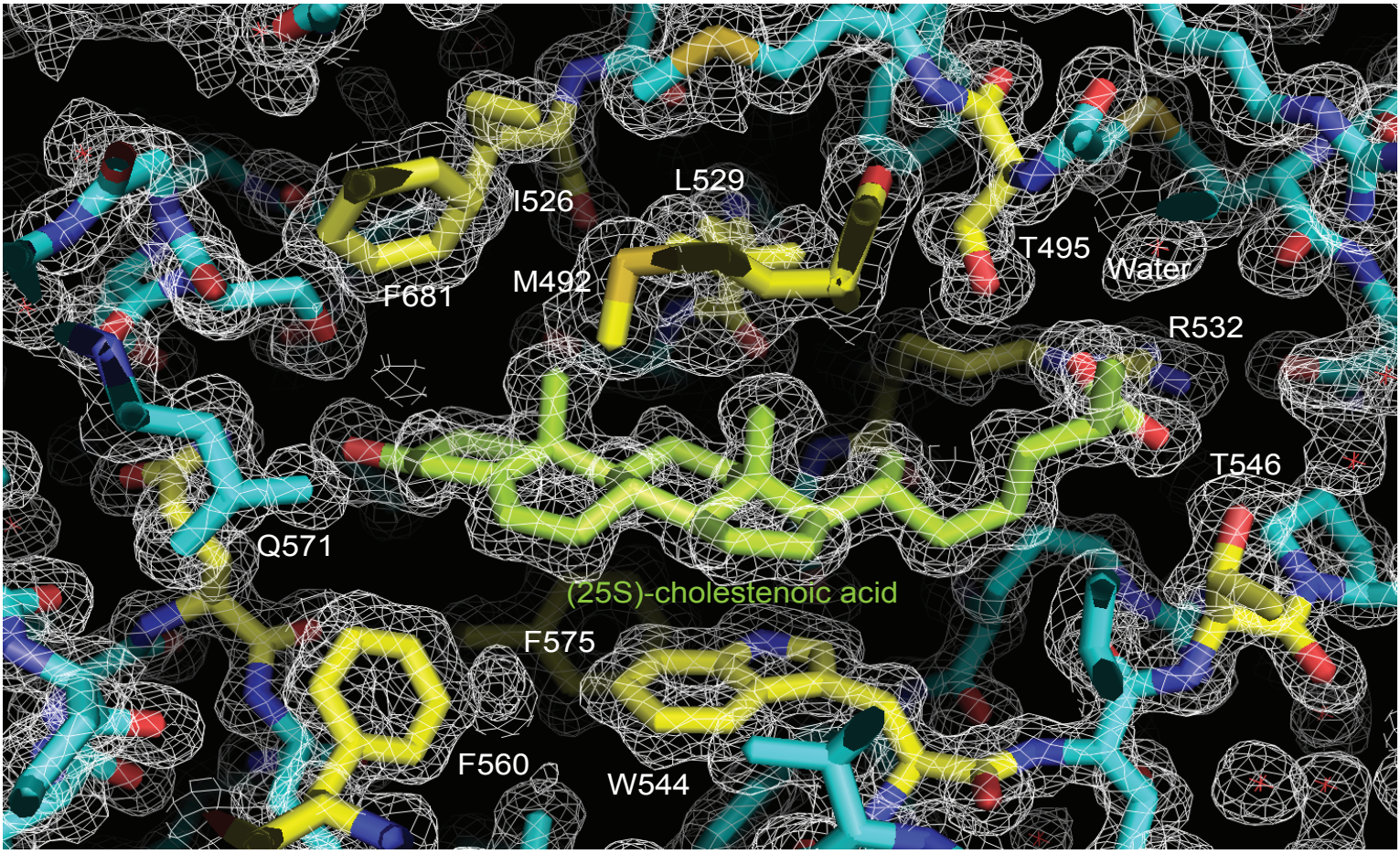

FIG. S5
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Fig. S5
The electron density map of the *Ace*DAF-12 ligand binding pocket bound to (25S)-cholestenoic acid. The surrounding amino acids are highlighted in yellow.

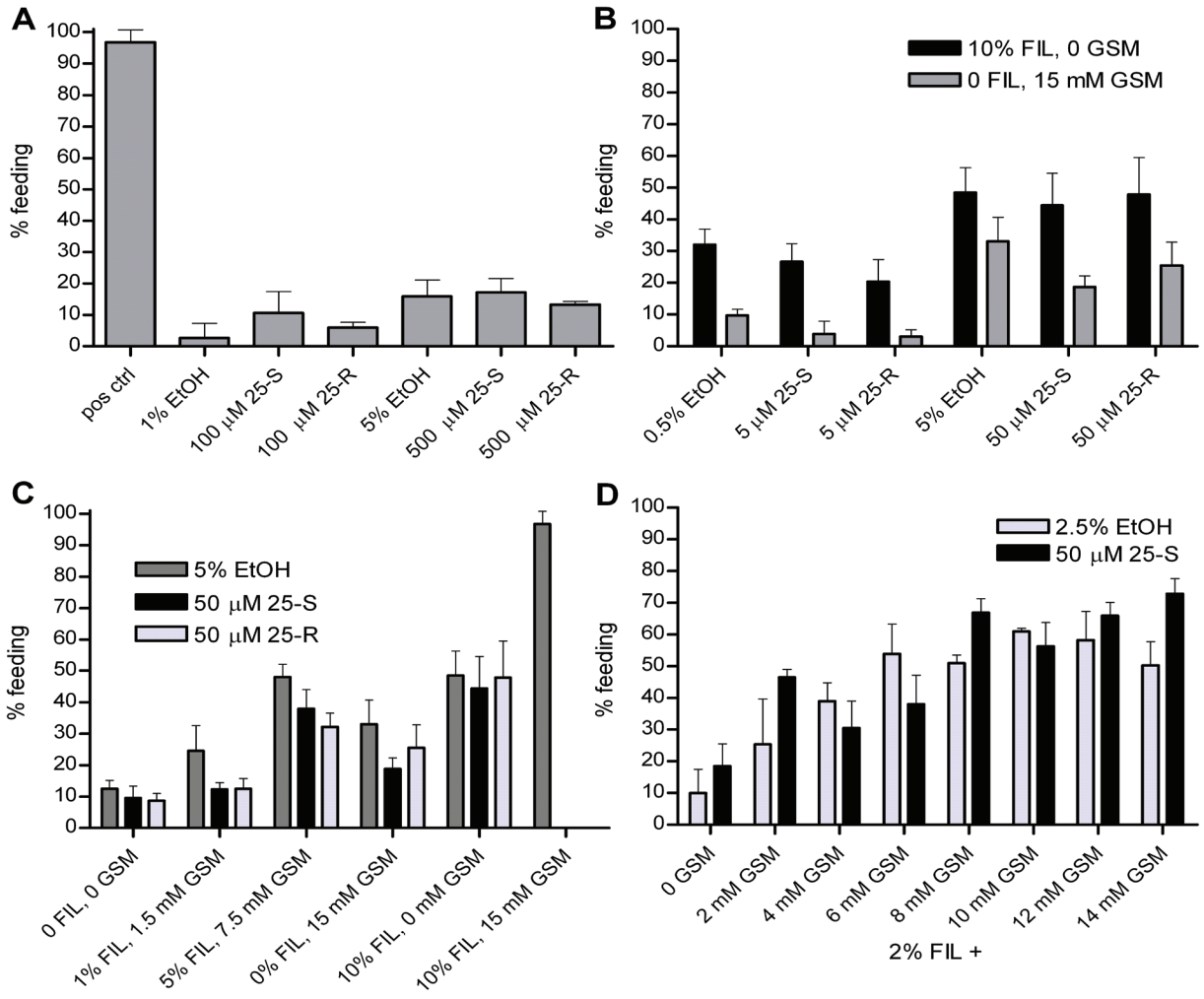

Fig. S6
Cholestenoic acids do not induce recovery of hookworm iL3. (25S)- and (25R)-cholestenoic acids in the indicated concentrations were tested for their ability to stimulate feeding in infectious *A. caninum* L3 larvae (n= 150). Incubation at host-like temperature (37°C) in medium supplemented with 15 mM S-methyl glutathione (GSM) and 10% canine serum filtrate (FIL) is known to stimulate feeding of approximately 95% (A, positive control). (25S)-cholestenoic acid in concentrations of up to 500 µM was unable to induce feeding (A, C). Co-stimulation with GSM and/or FIL in the indicated concentration did not increase the feeding in the iL3 population compared to controls containing the solvent ethanol (EtOH).