Supplementary Materials

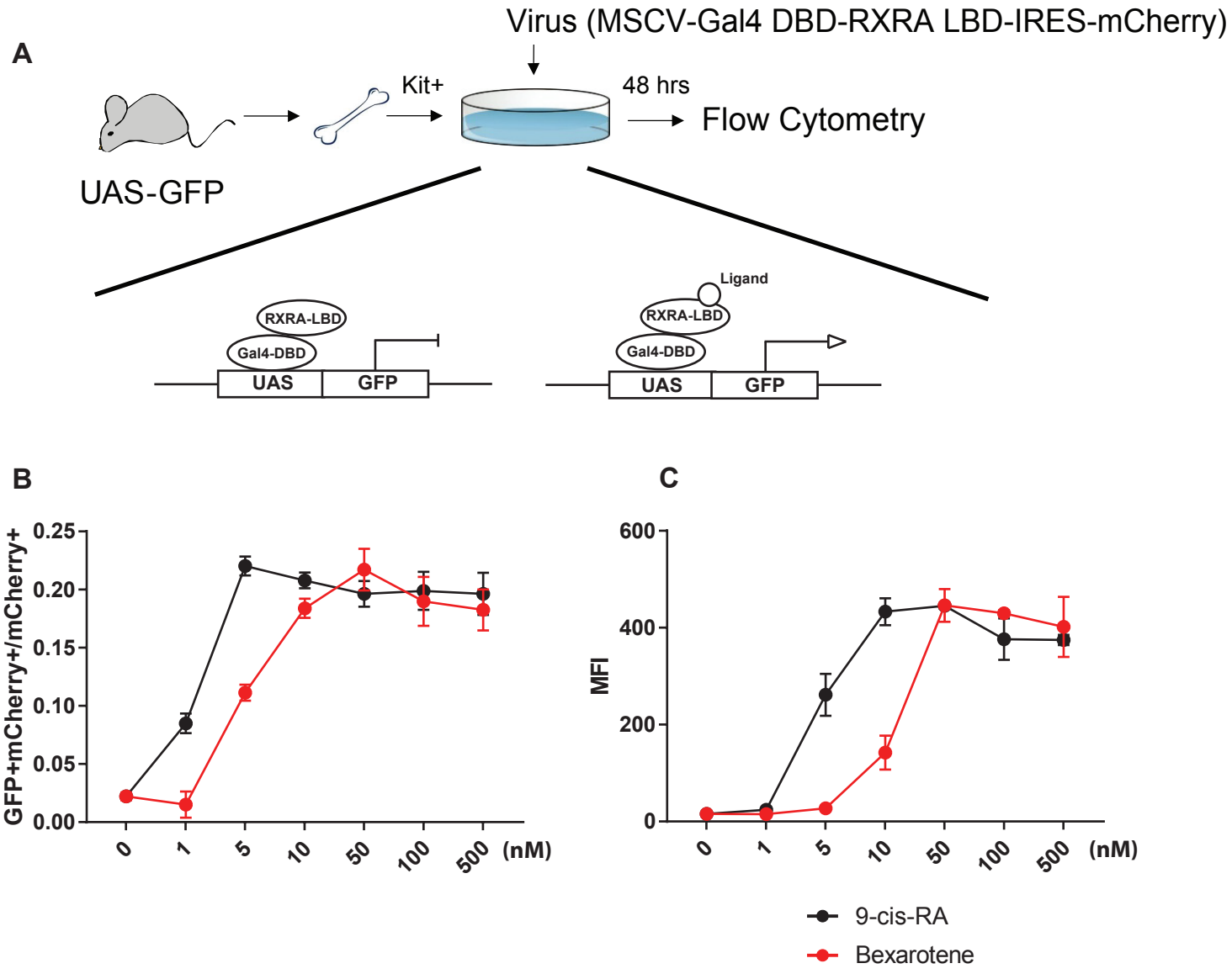

Figure S1. Ex vivo validation UAS-GFP reporter transgene. (A) Schema describes activation of GFP reporter by RXRA ligands. Bone marrow cells were collected from UAS-GFP mice. Kit+ cells were isolated by MACS. The Gal4-DNA Binding Domain and RXRA-Ligand Binding Domain fusion protein is expressed in UAS-GFP bone marrow Kit+ cells by retroviral transduction (Gal4-RXRA). Gal4-DBD recognizes and binds to UAS sequence. If there are no RXRA ligands, the GFP reporter is not activated. If there are RXRA ligands, they bind to RXRA-LBD, and the GFP reporter is activated. An IRES mCherry cassette is used for normalization purposes. (B and C) UAS-GFP bone marrow Kit+ cells, which were transduced with Gal4-RXRA retrovirus were treated with RXRA agonists 9-cis-retinoic acid (9-cis-RA) and bexarotene. (B) The ratio of GFP+mCherry+ cells to total mCherry+ cells. (C) The mean fluorescence intensity (MFI) of GFP+mCherry+ cells. Error bars represent standard deviation of triplicate experiments.

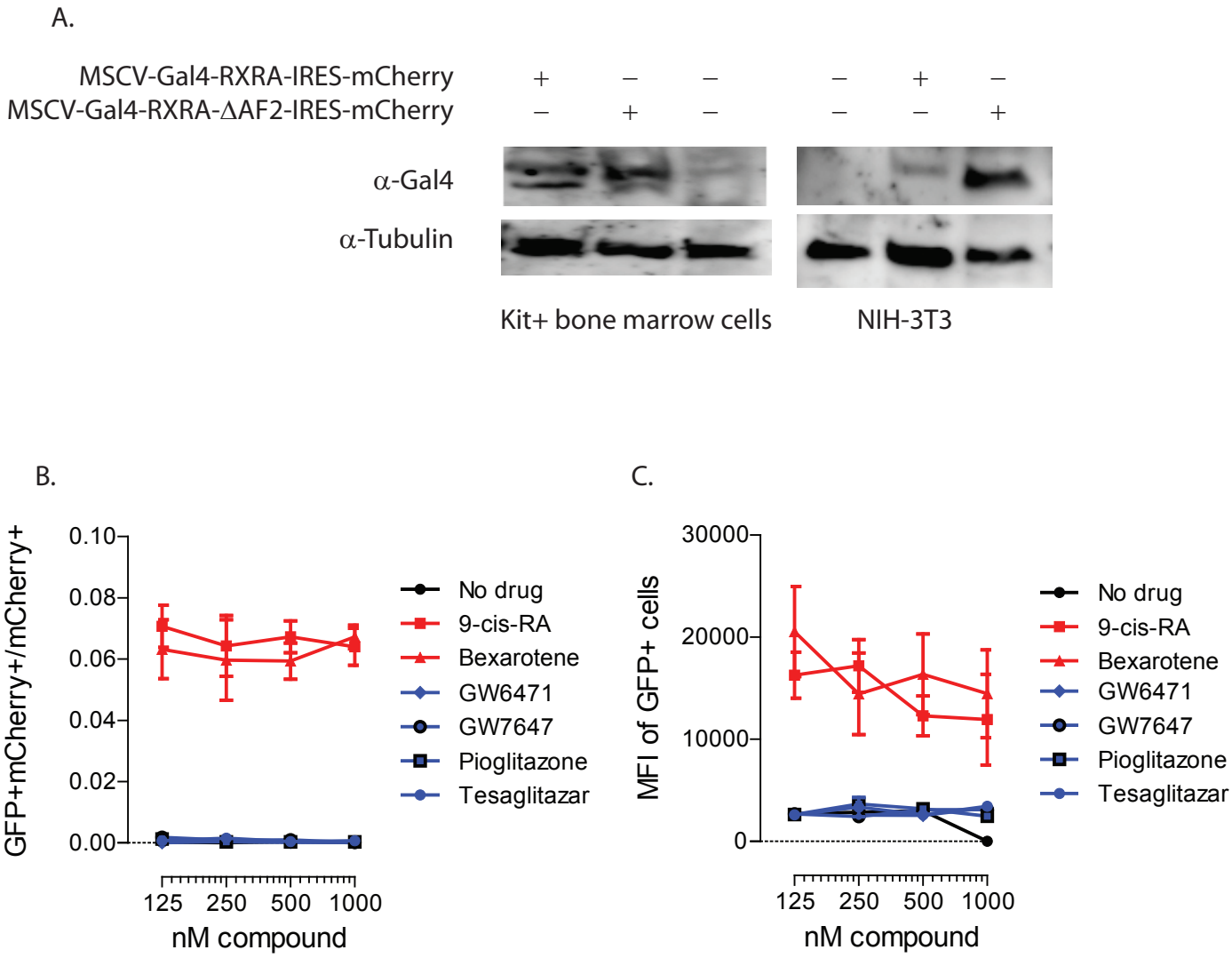

Figure S2. Reporter analysis. A. UAS-GFP Kit+ bone marrow cells or NIH-3T3 cells were transduced with indicated virus and Western blot was performed using anti-Gal4 or anti-Tublin antibodies. B and C. UAS-GFP Kit+ bone marrow cells were transduced with Gal4-RXRA and treated with indicated ligands ex vivo. GW6471 is PPARA agonist with EC50 of 240 nM. GW7647 is a PPARA agonist with EC50 of 6 nM. Pioglitazone is a PPARG agonist with EC50 of 700 nM. Tesaglitazar is a PPARA agonist with EC50 of 300 nM. Error bars indicate standard deviation of biological triplicate measurements.

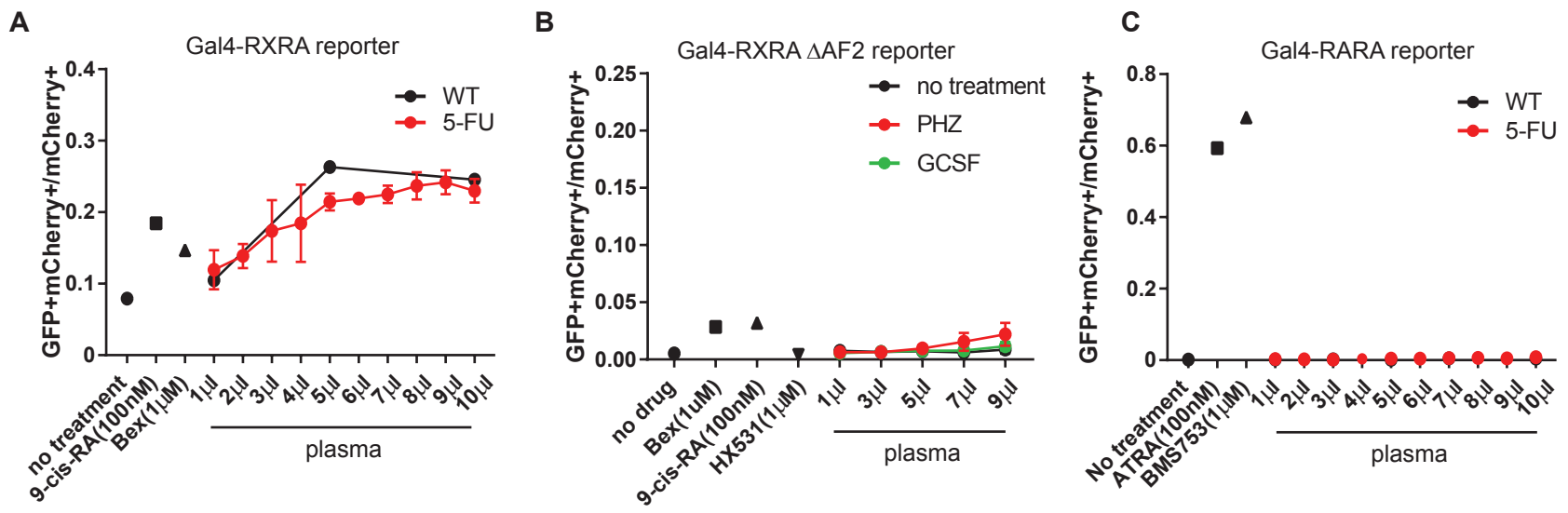

Figure S3. Analysis of RXRA-ΔAF2 and RARA to natural ligands in mouse sera. (A) UAS-GFP bone marrow Kit+ cells were transduced with Gal4-RXRA retrovirus, and treated with bexarotene, 9-cis-RA, HX531 or plasma from mice treated with 5-flurouracil (5-FU) as indicated. (B) UAS-GFP bone marrow Kit+ cells were transduced with Gal4-RXRA-ΔAF2 retrovirus, and treated with bexarotene, 9-cis-RA, HX531 or plasma as indicated. (C) UAS-GFP bone marrow Kit+ cells were transduced with Gal4-RARA retrovirus, and treated with bexarotene, all-trans retinoic acid (ATRA), BMS753 (an RARA-specific synthetic ligand), or the same plasma used in (A). Error bars indicate standard deviation between results of sera from three individual mice or tripicate treatment with indicated drugs.

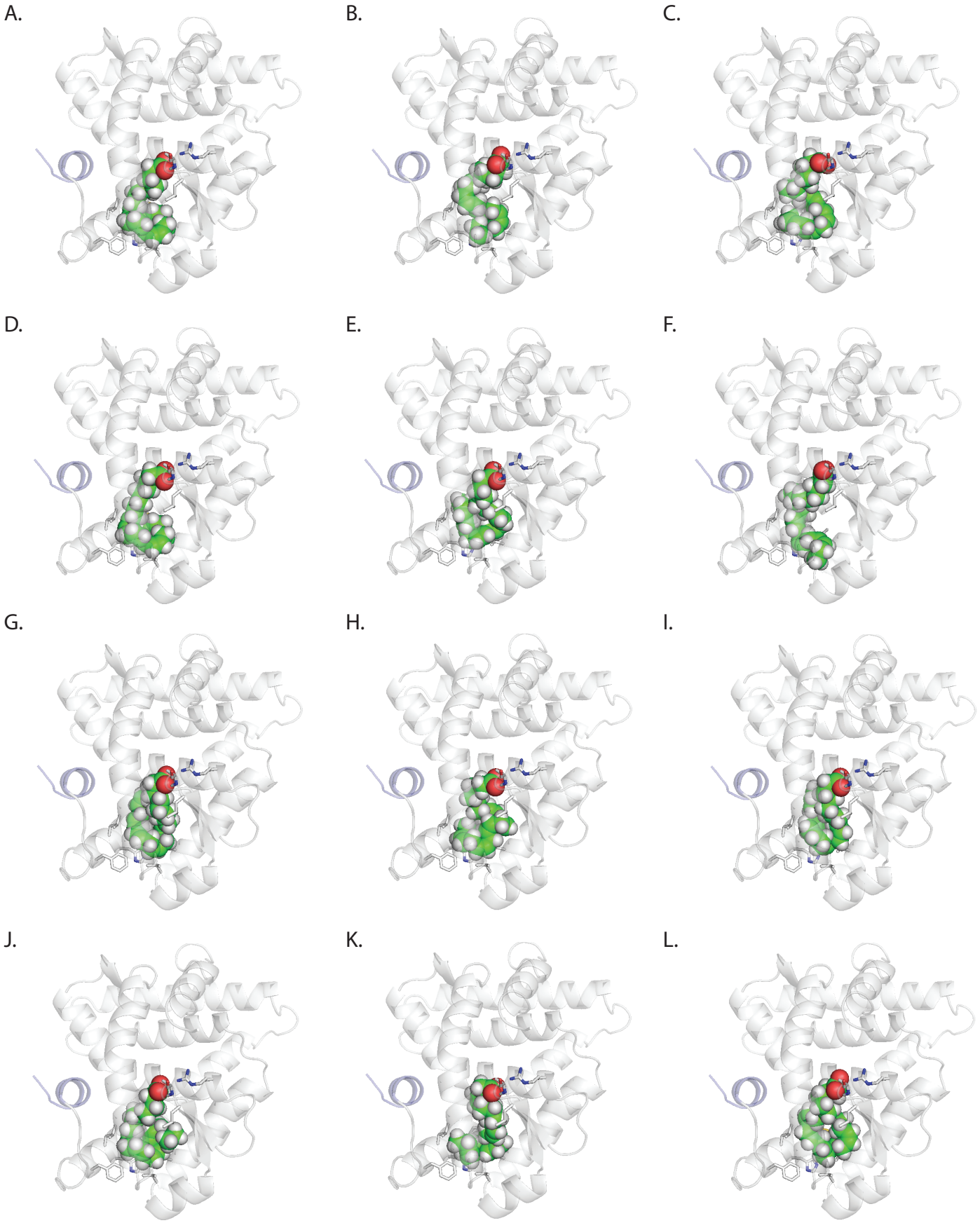

Figure S4. Sixteen different docking configurations of C24:5 in RXRA (1XDK). Note that all configurations retain interaction of the carboxyl end with R321 and the long-chain fatty acid then coils itself into the hydrophobic pocket between H5, H7, and H11. Amino acid side-chains shown are: L331, A332, V347, I350, C437, H440, L441, F444. H12 is highlighted in blue. Colors: hydrogen - grey; nitrogen - blue; oxygen - red; carbon - green (lipid), grey (protein).

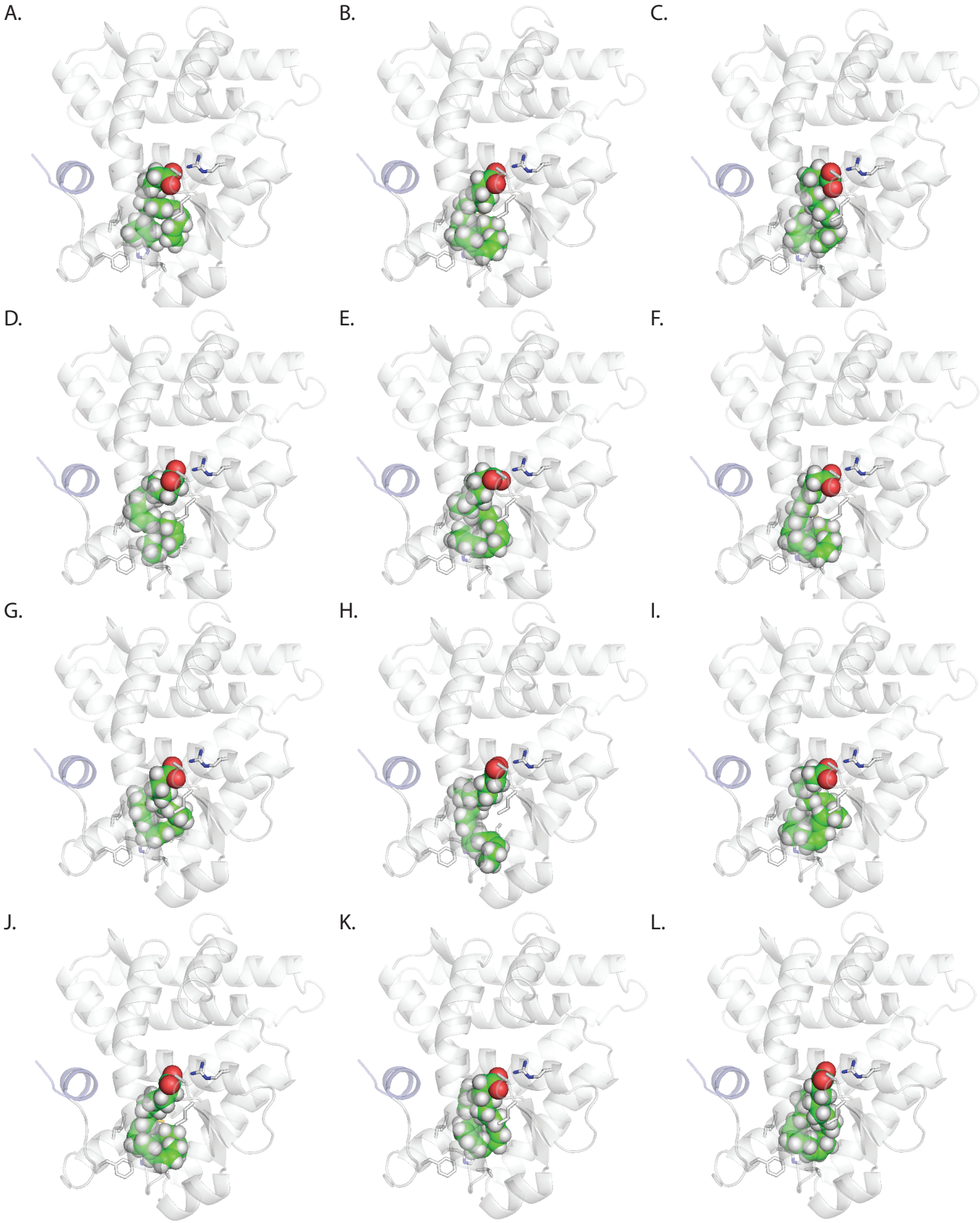

Figure S5. Sixteen different docking configurations of C24:4 in RXRA (1XDK). Note that all configurations retain interaction of the carboxyl end with R321 and the long-chain fatty acid then coils itself into the hydrophobic pocket between H5, H7, and H11. Amino acid side-chains shown are: L331, A332, V347, I350, C437, H440, L441, F444. H12 is highlighted in blue. Colors: hydrogen - grey; nitrogen - blue; oxygen - red; carbon - green (lipid), grey (protein).

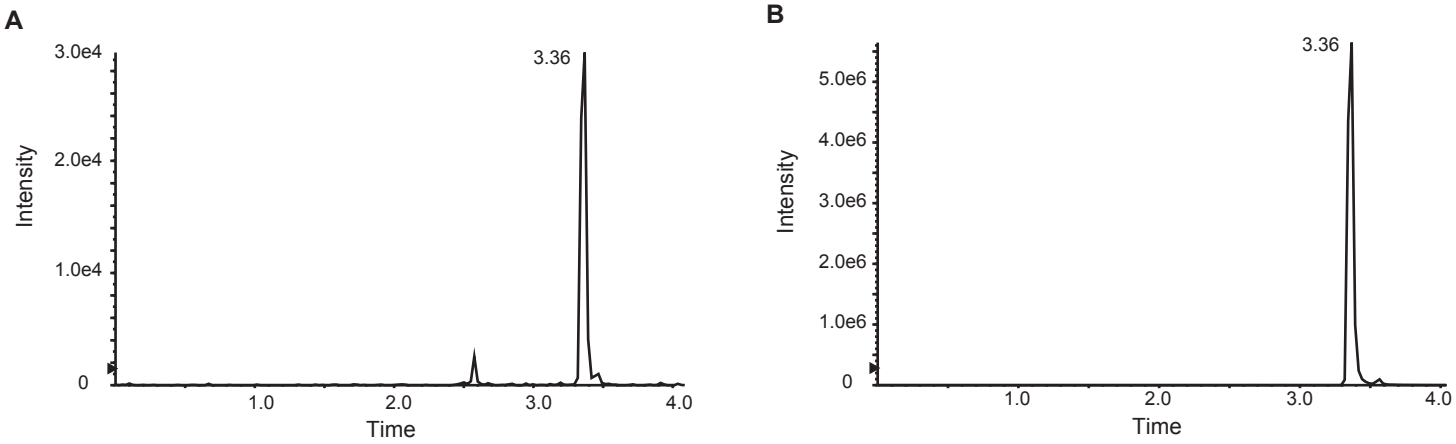

Figure S6. Comparison of LC-MS/MS peaks of C24:5. (A). LC-MS/MS chromatographic spectra of endogenous C24:5 from mouse plasma after DMAPA derivatization. (B) LC-MS/MS chromatographic spectra of synthesized C24:5 after DMAPA derivatization.

Table S1. Analysis of long chain fatty acids in serum samples by mass spectrometry

	Concentration in mouse serum vs. goat serum at least 3 fold greater	Concentration in methanol:H2O 9:1 vs. 5:5 plasma at least 5 fold greater	Concentration in VAD+PHZ vs. VAD plasma at least 1.5 fold greater	Concentration in NFC vs. NF plasma at least 1.5 fold greater	Concentration in NFC-PHZ vs. NF plasma at least 2 fold greater	Time-dependent binding during Flag-Gal4-RXRA pull-down	Binding during pull-down using mouse serum vs. goat serum at least 3 fold greater
C18:1	yes	no	no	ND	ND	ND	no
C18:3	no	yes	no	yes	yes	ND	ND
C20:2	yes	yes	yes	yes	no	no	no
C20:3	yes	yes	no	yes	no	no	no
C20:5	yes	yes	no	yes	no	no	no
C22:0	no	no	yes	no	yes	no	no
C22:1	yes	yes	no	no	yes	no	yes
C22:2	yes	yes	yes	no	ND	ND	ND
C22:3	yes	yes	no	no	no	yes	no
C22:4	yes	yes	no	yes	ND	yes	yes
C22:5	no	yes	no	yes	yes	ND	no
C22:6	yes	yes	yes	yes	no	yes	yes
C24:0	no	no	no	no	yes	no	no
C24:1	no	yes	no	no	no	no	no
C24:2	no	yes	no	no	yes	ND	ND
C24:3	yes	yes	no	no	no	no	ND
C24:4	yes	yes	yes	yes	yes	yes	yes
C24:5	yes	yes	yes	yes	yes	yes	yes
C24:6	yes	yes	no	yes	no	yes	no
C26:0	no	no	yes	no	no	no	no
C26:1	no	yes	yes	no	yes	ND	no
C26:2	no	yes	yes	no	yes	ND	ND
C26:3	no	no	ND	ND	no	ND	ND
C26:4	yes	yes	no	yes	yes	no	ND
C26:5	yes	yes	no	yes	yes	no	yes
C26:6	yes	yes	ND	ND	no	ND	ND
C26:7	ND	ND	yes	no	no	ND	ND