

# Supplementary Materials for

## Substrate and product complexes reveal mechanisms of Hedgehog acylation by HHAT

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Movie S1 MDAR Reproducibility Checklist (PDF)

#### **Materials and Methods**

Cloning, expression and purification of HHAT

The cDNA encoding human HHAT (synthesized by IDT Inc.) was cloned into a mammalian expression vector with an N terminal EGFP tag and an intervening PreScission protease recognition site (24, 25). Wild type HHAT and all mutants were transiently expressed in HEK293T suspension cells (Invitrogen) that were cultured in Expi 293 media (Invitrogen) at  $37^{\circ}$ C with 8% CO<sub>2</sub> and 80% humidity. For transient transfection, cells were expanded into 1 liter cultures to a density of  $2 \sim 3 \times 10^{6}$  cells/ml, at which point approximately 1 mg of plasmid was combined with 3 mg PEI25K (Polysciences, Inc. Cat # 23966) in 100 ml OptiMEM media (Invitrogen, and incubated at room temperature for 30 min) and the mixture was added to the cell culture. 5 mM sodium butyrate (final concentration) was added after 12 hours and the cells were cultured for an additional 48 hours before harvesting.

For purification of HHAT, the pellet from 1 liter of cells was resuspended in 50 ml extraction buffer (20 mM Tris, 300 mM NaCl, 10 mM lauryl maltose neopentyl glycol [LMNG, Anatrace], 40 µg/ml DNaseI, 1.5 µg/ml leupeptin, 1.5 µg/ml pepstatin A, 1 mM 4benzenesulfonyl fluoride [AEBSF] and 1 mM benzamidine, pH 8.0) and solubilized by stirring at 4°C for 2 hours. The insoluble fraction was removed by centrifugation (60,000 g, 4°C, 45 min) and the supernatant was filtered through a 0.22 µm Millipore polystyrene membrane. 1.5 ml GFP nanobody resin was added and the sample was agitated at 4 °C for 2 hr (26). The beads were then washed with 40 ml of wash buffer (20 mM Tris, 300 mM NaCl, 1 mM LMNG, 10 µg/ml 1palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine [POPS, Avanti], pH 8.0) by gravity flow. HHAT was eluted by adding PreScission protease (0.1 mg, 6 hours at 4 °C) in elution buffer (20 mM Tris, 150 mM NaCl, 1 mM LMNG, 10 µg/ml POPS, 25 µM palmitoyl-CoA[preparing 25] mM stock in water, Sigma, Cat#P9716], 1 mM DTT, pH 8.0). The eluate was concentrated (100 kDa cutoff, to  $OD_{280} \sim 1$ ) and flash frozen in liquid nitrogen (for enzymatic analysis) or further purified by size-exclusion chromatography (SEC, Superose-6 Increase, 10/300 GL column, GE Healthcare) in buffer (20 mM Tris, 150 mM NaCl, 0.06% [w/vol] digitonin [Cayman chemical], pH 8.0) prior to combining with Fab antibody fragments for cryo-EM studies, described below. Antibody generation and purification of HHAT-Fab complexes

Monoclonal antibodies (designated 1C06 and 3H02) of isotype IgG1 were raised in mice by the Monoclonal Antibody Core Facility of the Memorial Sloan Kettering Cancer Center. Two antigens were used: HHAT that was purified in LMNG detergent, which yielded 1C06, and HHAT that had been reconstituted into nanodiscs (mMSP1D1, Cube Biotech Inc.), which yielded 3H02. The antibody selection process included ELISA, western blot, and fluorescencedetection size exclusion chromatography (FSEC) analysis (27) to identify antibodies that preferentially bound to native HHAT in comparison to SDS-denatured protein. IgG1 proteins were purified from hybridoma supernatants using affinity chromatography (HiTrap<sup>TM</sup> Protein G HP, Invitrogen) and Fab fragments were generated by ficin proteolysis (Pierce<sup>TM</sup> Mouse IgG1 Fab and F(ab')2 Preparation Kit, Thermo Scientific, Cat#44980), following the manufacturer's protocols. Fab fragments were further purified by anion exchange chromatography (Resource S column, GE Healthcare, in buffer 20 mM Sodium Acetate, 10 mM to 500 mM NaCl, pH 5.0) and then by SEC (Superdex 200 10/300 column, in 20 mM Tris, 150 mM NaCl, pH 8.0). For generation of HHAT-Fab complexes, purified antibodies were combined with purified  $\sim 0.1$ mg/ml HHAT (using a molar ratio of 1:1.4, HHAT:Fab) and incubated at room temperature for 20 min. The samples were then concentrated to approximately 250 µl (using a 50 kDa Vivaspin 2 concentrator) and further purified by SEC (Superose-6 Increase, 5/10 column, GE Healthcare) in

20 mM Tris, 150 mM NaCl, 0.06% w/v Digitonin, pH 8.0. The elution fractions containing the HHAT-Fab complexes were pooled and concentrated (using a 50 kDa Vivaspin 2 concentrator).

Nanodisc reconstitution (used for antibody generation) was performed using an on-bead method analogous to that previously described (25). GFP-HHAT protein (from 500 ml of cell culture, corresponding to approximately 250 µg of purified HHAT) bound to 1 ml GFPnanobody resin was combined with 80 µl lipid/DDM mixture (20 mM Tris, 150 mM NaCl, 14 mM DDM, 7.5 mg/ml POPC, 2.5 mg/ml POPE, 50 µg/ml POPS, 1 mg/ml lipid A, pH 8.0) and 100 µl mMSP1D1 (Cube Biotech Inc., 5 mg/ml, in buffer 20 mM Tris-HCl, 100 mM NaCl, 5 mM sodium cholate, pH8.0). This yielded a molar ratio of approximately 1:4:160 (protein:mMSP1D1:lipid). After 1 h agitation at 4 °C, 500 mg of Bio-Beads (Bio-Rad, SM2, wet) were added to the resin, and the sample was rotated at 4 °C for detergent removal. Another 500 mg of Bio-Beads was added and the sample was incubated for 16 h (at 4 °C with rotation). The resin was then washed with 40 ml buffer (20 mM Tris, 150 mM NaCl, pH 8.0). The HHATnanodisc sample was eluted by adding 0.1 mg PreScission protease (6 h incubation at 4 °C in 3 ml of 20 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0), further purified by SEC (Superose 6 increase, 10/300 GL column, GE Healthcare, equilibrated in 20 mM Tris, 150 mM NaCl, pH 8.0), concentrated to 1 mg/ml (50 kDa Vivaspin-2 concentrator), and used for immunization. Cryo-EM sample preparation and data acquisition

For the palmitoyl-CoA substrate complex, 4  $\mu$ l of freshly purified HHAT-1C06Fab-3H02Fab sample (at A<sub>280</sub>=18.2) was loaded onto glow-discharged (10 s) Quantifoil R 1.2/1.3 carbon grids (Au 400, Electron Microscopy Sciences) and plunge-frozen in liquid nitrogencooled liquid ethane using a Vitrobot Mark IV plunge-freezing device (FEI, with 2.5 s blotting time at 4 °C, 0 blot force, and 100% humidity). Images were collected using SerialEM (*28*) using a Titan Krios microscope (Thermo, at MSKCC) at 300 kV that was equipped with a K3 summit direct electron detector (Gatan). Super-resolution movies (1.5 s exposure time; 60 frames per movie) were collected using a dose rate of 20 e<sup>-</sup>/pixel/s at a nominal magnification of 22500× (super-resolution pixel size of 0.532 Å) and with defocus ranging from -0.7  $\mu$ m to -2.5  $\mu$ m.

For preparing the palmitoylated product complex, 1 mM DTT was added to the SECpurified HHAT-3H02Fab sample after concentrating it to  $A_{280}$ =7.6. 4 µl of the complex was then supplemented with 0.5 µl of 25 mM palmitoyl-CoA (in water) and 0.5 µl of 20 mM Shh peptide (sequence: CGPGRGFGKRRK-biotin, synthesized by Peptide 2.0 Inc., 20 mM stock solution in 20 mM HEPES, 150 mM NaCl, 20 mM TCEP, pH 7.3) and the mixture was incubated on ice for 1 hour. The complete sample (5 µl) was used for grid preparation following the method described above. Images were collected using a Titan Krios3 microscope (Thermo, located at the New York Structural Biology Center) at 300 kV with a K3 electron detector (Gatan) and energy filter (BioQuantum, 20 eV filter width). Super-resolution movies (3 s exposure, 60 frames per movie) were collected with a dose rate of 17 e<sup>-</sup>/pixel/s at a nominal magnification of 20,250× (super-resolution calibrated pixel size = 0.541 Å) with defocus ranging from -1.0 µm to -2.8 µm. Structure determination and model building

Cryo-EM data processing workflows for the two datasets are summarized in figure S3 and followed the same scheme. Raw movies were motion-corrected using MotionCor2 (29). Micrograph defocus was estimated using CTFFIND-4.1 (30). Micrographs with estimated contrast transfer function (CTF) fits worse than 6 Å were discarded. Particles were autopicked in Relion 3.0 and extracted with 2x binning (31) and imported into CryoSPARC V2 for further processing (32). Particles that did not resemble HHAT antibody complexes were removed by reference-free 2D classification. Initial 3D models were generated ab initio. Particles were

selected by several rounds of heterogenous refinement. Classes yielding the highest resolution, as judged by density inspection and Fourier shell correlation (FSC), were selected for non-uniform refinement. Selected particles were then re-extracted without binning using Relion 3.0 and imported back to CryoSPARC V2 for additional rounds of heterogenous refinement. When the resolution no longer improved with rounds of heterogenous and non-uniform refinements, the particles were imported into Relion for Bayesian polishing, and then back into CryoSPARC V2 for non-uniform and CTF refinements. A second round of Bayesian polishing followed by non-uniform and CTF refinements yielded the final reconstructions at 2.68 Å or 3.20 Å resolutions for the palmitoyl-CoA and product complexes, respectively. All resolution estimates are based on gold-standard FSC calculations.

The atomic models were manually built and refined in real space using COOT (33). Further refinements were carried out by iterative cycles of real space refinement in PHENIX (34) and building in COOT. Structural figures were prepared with ChimeraX and Chimera (35, 36). Model quality was assessed using PHENIX and Molprobity (37). Electrostatic calculations used the APBS (38) plugin in Pymol (pymol.org).

#### Molecular dynamics simulation

The CHARMM-GUI (http://www.charmm-gui.org) *Membrane Builder* was used to generate the simulation system and GROMACS input files (*39-43*). The atomic model of HHAT (including the heme but without additional ligands) was uploaded to CHARMM-GUI via the "Bilayer Builder." HHAT was oriented in the lipid bilayer using the Orientations of Proteins in Membranes (OPM) PPM server (https://opm.phar.umich.edu/ppm\_server)(*44*). The system size is approximately  $103 \times 103 \times 108$  Å<sup>3</sup> and ~ 108,000 atoms, including 21,537 water molecules, 99 sodium ions, 58 chloride ions, and 260 lipid molecules. The lipid bilayer is composed of 60% POPC, 15% POPE, 10% POPI, 10% cholesterol, and 5% POPS. GROMACS input and parameter files were created using the CHARMM36m force field (*45*), automatically-generated grid information for Particle-mesh Ewald (PME) fast Fourier transform (FFT) long-range interaction calculations, and the NPT equilibration ensemble (i.e., constant particle number, pressure and temperature) at 1 atm and 310 K (*45, 46*).

These CHARMM-GUI-generated input and parameter files were used for energy minimization, NPT equilibration, and molecular dynamics (MD) production simulation using GROMACS version 2020.2 (10.5281/zenodo.3773799) (47). Equilibration included 6 cycles of 50–100 ps each (1 fs time step) with reducing force constants with each subsequent cycle. The MD production was run for 90 ns (2 fs time step) using the Verlet cutoff scheme, Nose-Hoover temperature coupling, and Parrinello-Rahman pressure coupling. In the final trajectory, HHAT was re-centered and molecules were re-wrapped within the unit cell. For visualization, individual frames were least-squares fit to the protein backbone. Visual Molecular Dynamics (VMD; https://www.ks.uiuc.edu/Research/vmd/) was used to visualize the simulation and generate figures (48). Snapshots and the movie were rendered using Tachyon in VMD (49). HHAT activity assay

In the standard assay, a biotin-labeled peptide (<sup>24</sup>CGPGRGFGKRRK<sup>35</sup>-biotin) corresponding to the N-terminal region of human Sonic Hedgehog after signal peptide removal was chemically synthesized (Peptide 2.0) and resuspended in buffer (20 mM stock in 20 mM HEPES, 150 mM NaCl, 20 mM TCEP, pH 7.3). For experiments with the R28A mutant peptide, a <sup>24</sup>CGPGAGFGKRRK<sup>35</sup>-biotin peptide was used. Radiolabeled [<sup>3</sup>H]palmitoyl-CoA was used to detect acyl transfer. The standard reaction mixture (50 µl) contains 10 nM HHAT (protein concentration determined from A<sub>280</sub> for purified protein, or by GFP fluorescence for

microsomes), 50 µM palmitoyl-CoA (Sigma Aldrich, 25mM stock in H<sub>2</sub>O), 40 nM <sup>3</sup>H]palmitoyl-CoA (60 Ci/mmol, American Radiolabeled Chemicals, Inc.) and 50 µM peptide in reaction buffer (150 mM MES at pH 6.5, 150 mM NaCl, 1 mM DTT, with 1 mM LMNG for detergent-solubilized HHAT or without detergent for microsomes). 5 nM HHAT (in detergent) was used for Fab inhibition studies. For substrate response curves the peptide and palmitoyl-CoA concentrations were varied as indicated in figure legends. Reactions were carried out at 37°C for 20 min and stopped by adding 400 µl ice-cold quench buffer (20 mM HEPES, 150 mM NaCl, 0.2% v/v Tween-20, pH 7.5). The peptide was captured using 20 µl streptavidin beads (Streptavidin Sepharose High Performance, GE Healthcare) with rotation at 4°C for 1 hour. The beads were then collected by centrifugation (using a Costar® Spin-X centrifuge tube filter, 3000 g, 2 min, at room temperature) and washed twice with 400 µl buffer (20 mM HEPES, 150 mM NaCl, pH 7.5). The beads were then resuspended with 100 µl of the same buffer and transferred into scintillation vials containing 5 ml scintillation fluid (Ultima Gold, PerkinElmer Life Sciences). [<sup>3</sup>H]palmitoyl incorporation was determined by scintillation counting. Background levels were subtracted using reaction mixture controls without enzyme (when assaying purified protein) or with microsomes from untransfected cells. Enzymatic data are mean  $\pm$  s.e.m. and derived from three or more independent experiments. Initial velocity curves were fitted to a Michaelis-Menten model:  $Y = V_{max}X / (K_M + X)$ , where Y is the initial velocity, X is the substrate concentration,  $V_{\text{max}}$  is the maximum enzyme velocity, and  $K_{\text{M}}$  is the concentration of halfmaximal velocity. For substrate response curves, the concentration of the fixed substrate was 50 µM. For assaying the activity of HHAT mutants shown in Figure 4G, proteins were purified using GFP-nanobody affinity chromatography as described above and the protein concentration was estimated by  $A_{280}$  and by comparative western blot (using the 1C06 antibody).

To assess the ability of purified HHAT to palmitoylate Hedgehog protein, amino acids 24-197 of human Sonic Hedgehog were expressed and purified from *E. coli* as previously described (8) and the incorporation of [<sup>3</sup>H]palmitate was measured using a similar assay. Reaction mixtures (50 µl) contained 10 nM purified HHAT, 50 µM palmitoyl-CoA, 40 nM [<sup>3</sup>H]palmitoyl-CoA, and 40 µM Hedgehog protein in the standard reaction buffer (150 mM MES at pH 6.5, 150 mM NaCl, 1 mM DTT, 1 mM LMNG). Reactions were carried out at 37°C for 30 min. Reactions were stopped by the addition of 200 µl 5% w/v SDS and proteins were precipitated by the addition of 200 µl 37.5% w/v trichloroacetic acid (TCA). Following a 45 minute incubation on ice, precipitates were collected on filters by centrifugation (Costar<sup>®</sup> Spin-X centrifuge tube filters, 4000 g, 1 min, at room temperature). The filters were then washed twice with 300 µl of Solution 1 (2% SDS, 6% TCA) and once with 300 µl Solution 2 (6% TCA) to remove free palmitoyl-CoA. The filters were then transferred into scintillation vials containing 4 ml scintillation fluid (Ultima Gold, PerkinElmer Life Sciences) and [<sup>3</sup>H]palmitate incorporation was determined by scintillation counting.

To assay the activity of HHAT in cell membranes, microsomes were prepared in the following manner. Cells from 200 ml of cell culture were pelleted (900 g), resuspended in 10 ml resuspension buffer (20 mM Tris, 300 mM NaCl, pH 8.0), and lysed by sonication on ice (20% nominal converter amplitude, 20 duty cycle, 4 min. using a Branson Sonifier 250A). Cell debris was removed by centrifugation (15,000 g, 4 °C, 30 min). Microsomes were then pelleted by centrifugation (70,000 g, 4 °C, 45 min), resuspended in 5 ml of resuspension buffer, aliquoted, frozen at -80 °C, and used in the enzyme assay. To quantify the amount of HHAT protein (in the form of a GFP-HHAT fusion protein) present in the microsomes, an aliquot of microsomes was solubilized in buffer containing detergent (20 mM Tris, 300 mM NaCl, 10 mM LMNG, pH 8.0).

The detergent-solubilized sample was then applied to an SEC column (Superose-6 Increase, 10/300 GL GE Healthcare, equilibrated in 20 mM Tris, 150 mM NaCl, 1 mM LMNG, 10  $\mu$ g/ml POPS, pH 8.0) and the amount of GFP-HHAT protein was assessed from the fluorescence signal of the eluted GFP-HHAT peak (normalized using purified GFP as described (*50*)). For measuring the activity of archway mutants relative to the wild type enzyme (fig. S8), 10 nM HHAT, 20  $\mu$ M palmitoyl-CoA and 50  $\mu$ M peptide were used in the assay. Thermostability, Fab binding, and heme assays

To assess the thermostability of wild type and C324V HHAT, 50 nM concentrations of purified proteins (in 20 mM Tris, 150 mM NaCl, 1 mM LMNG, 10 µg/ml POPS, pH 8.0) were incubated at temperatures ranging from 25 °C to 51 °C for 20 min and then analyzed by SEC (Superdex-200 10/300 GL column, GE Healthcare, equilibrated in the same buffer). The fraction of folded protein for each condition was quantified from the area under the elution peak (at ~13.7 ml, using tryptophan fluorescence:  $\lambda_{excitation}=280$  nm,  $\lambda_{emission}=340$  nm) in comparison to the peak of at 25 °C control sample.

To assess the approximate binding affinity of Fab 3H02, 2 nM of purified Fab was incubated (30 min at 20 °C) with concentrations of purified HHAT ranging from 0 to 100 nM in buffer (20 mM Tris, 150 mM NaCl, 1 mM LMNG, pH 8.0). Samples (500 µl) were then analyzed by SEC (Superdex-200 10/300 GL, GE Healthcare) in the same buffer. The fraction of unbound Fab was quantified from the area under the elution peak corresponding to free Fab (elution volume ~16.6 ml, using tryptophan fluorescence), which is well separated from the peaks for HHAT alone (~13.7 ml) and the HHAT-3H02Fab complex. The curve corresponds to fits of: fraction of Fab bound=[HHAT]<sup>*h*</sup>/( $K_D^h$ +[HHAT]<sup>*h*</sup>),  $K_D$ : dissociation constant, *h*: Hill coefficient, [HHAT]: concentration of HHAT.

A heme assay (Sigma-Aldrich, MAK316) was used to determine the amount of heme present in the purified HHAT sample according to the manufacturer's protocol. The assay detects the amount of heme from absorbance at 400 nm following addition of a Heme Reagent supplied with the assay. We generated a standard curve using the Heme Calibrator solution, which is also supplied with the assay. The amount of heme present in a 15  $\mu$ M sample of purified HHAT was determined to be approximately 18  $\mu$ M, consistent with a stoichiometric amount with the protein. As a control, 15  $\mu$ M bovine serum albumin (Sigma-Aldrich, A9647) was measured to contain less than 0.1  $\mu$ M heme.



**Figure S1. Hedgehog processing and HHAT biochemistry.** (**A**) Sonic Hedgehog processing scheme. (**B**) Purification scheme for HHAT. (**C**) Size-exclusion chromatography (SEC) profile of purified HHAT in digitonin. Elution volumes of other purified membrane proteins from our laboratory are shown for comparison, with the total mass of these membrane proteins in parentheses. (**D**) Topology of HHAT determined from the structure, with coloring as in Figure 1C. Bars represent *a*-helices.



Figure S2. Enzymatic activity of HHAT and characterization of antibodies. (A and B) Enzymatic activities of purified wild type (WT) HHAT (or HHAT with a E59A mutation, as indicated) as functions of palmitoyl-CoA concentration (A) or wild type or E59A mutant Hedgehog peptide concentration (B). Michaelis-Menten fits are shown, mean  $\pm$  s.e.m., with three independent experiments. (C) Palmitoylation of human Sonic Hedgehog protein (amino acids 24-197) catalyzed by HHAT. The amount of palmitate transferred to purified Sonic Hedgehog during a 30 min reaction per pmol of HHAT enzyme is shown. A control without enzyme is also indicated. Three independent experiments were performed. Details can be found in the methods. The right panel shows Coomassie-stained SDS-PAGE analysis of the purified Sonic Hedgehog protein, along with molecular weight markers. (D) Relative catalytic activities of HHAT (5 nM) in microsomes and in purified form, with and without 20 nM concentrations of the 1C06 and 3H02 antibodies. (E) Binding characterization indicates that 3H02 antibody binds tightly to the enzyme even though has minimal effect on catalytic activity.



Figure S3. Cryo-EM data processing workflows for HHAT with palmitoyl-CoA (left) and with the palmitoylated peptide product complex (right). (A) Example cryo-EM micrographs, with picked particles circled. (B) 2D class averages of particles used for the final reconstructions. (C) Flow charts outlining the cryo-EM processing workflows. Details may be found in Methods.



Figure S4. Density for the structure of HHAT with palmitoyl-CoA. (A) Densities (semi-transparent surface renderings) for indicated regions are shown in the context of the atomic model (sticks). (B) Angular distribution of particles used in the final cryo-EM reconstruction.
(C) Gold-standard FSC curve of the final reconstruction. (D) Map-to-model correlation curve.
(E) Estimation of the local resolution of the map, colored as indicated (calculated using CryoSPARC V2).

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GOAT ACAT1 DGAT1	234 350 305	D -  -  ( R	CQQF (QEP MKP	F S J	 А DМГ	- I - F	ECI RVL			ר ד <mark>ע</mark> ר ד / ר ד /	TA NS KL		   L A V	 - P (	G V	L F L I L I	K L L F W I	. T -	- F F F F	-Y FA YW	YS FL LF	H- HC HS	W		) D \	SL - F - V	L H . A E I A E		G F ( R F ( D F (	G P G - G -	E L 	G Q 	S P  	G E - C - C	: E G ) R - ) R -	Y V 	' P D - N - E	A- FY	- 288 K 405 R 362
HHAT	322					PF	2 C V	/ S T	M	= <u>s</u> I	FT	GM	W F	۲Y	FD	VG	LH		FL	I R	Y V	YI	P \	/ G (	5 S (	QHO	GL	LG	T L	FS	TA	мт	FA	FV	/ S Y	WF	IG G	YD	Y 384
GOAT ACAT1	280 289 406		Г L E - N S		PLI  		RT	н я Н Я 	- T	5 V I 5 V I 5 N	FS YS	R K N Y	W- YF	 - 1	· N · N // N	Q S V V	T A V F	R V N R V	V L V L V L	R R Y Y	L V Y A	FQ YK	H- DI	 - L V	VF	- SI FSI	R A V K R	N P F K	L S A -	 - A	- L M L	Q T A V	F A F A	F S V S		/W  - / W  - / V  -	IG F IG L I E Y	HP AL	G 343 A 465
DGAT1 HHAT	363 385	<u>D</u> W <u>W</u> I	NS						- 6	= <u>s</u> \	VТ 	Y F 		2 N \ 	M <mark>N</mark>		V F	IKV LV	vc vc	I R W A	H F	Y K	. P N / L (	и L F G V 1	RR	G S E N (	S K V	N R R	• м•	- А Е Т	R T	G V 	E L	A S	- P	F C	Q D	L V S L	S 420 A 416
PORCN GOAT ACAT1	347 344 466	Q V V C L -	5 F F Y	  P V	  L F \	  /	 	  = F (	 			  - F	  N F		  . N	  D S	F C	 5 F \ 6 K F		A A W A W N	V L V M V L	L S V E MW	L /	4 F -  5 L I		 	 	 		  Y S	 	  WY	  A R	  0 F	  	 	 	  T F	357 - 357   536
DGAT1	421		RMFR	·		LV	VAF	TG	5 M N	AAC		P L	AV	VF	V G	RF	FC	QGN	N Y	G N		V M		S L		GQI				YV	HD	ΥΥ	V L	NY	EA	PA	AE	A -	- 488
PORCN GOAT	358 358	TYV DYL	E H V L I H S F	R K A N	R L A E F I	AR IR S		A C		_ S H = Y F	K R R T	C P L T	 W A	 	. Р Г Q	D C	SH		HR M	L G L A	 	- L - V	GI	/ R <i>A</i> / R 9	A L I 5 L	N L S S		GA- LL(	 C	 - N	 S Y	L A N S	I F V F	H L P N	. A Y 1 V Y			FDLL	V 419 L 427
ACAT1 DGAT1	537	D <mark>Y</mark> V I 	?Р								-				-				-					R -															- 542 -
HHAT PORCN GOAT	485 420 428	A D V D I A K R I	отте Стте Стте	EQ	G Y (	5 M /	 \ Y T	· V H	ικν 	 VSI	 E L	- W SW		2 T Y 5 H \ 	YA NV	T D T F	 G C	 W	- IF	 Y R	 L I	- G																	493 461 435
ACAT1 DGAT1	543										-	s <mark>w</mark>	/т с 	R 1	Y V -	F -			-			-																	550

**Figure S5. Sequence comparison among human MBOATs.** The sequences of human HHAT, Porcupine, GOAT, ACAT1, and DGAT1 are aligned and colored according to sequence identity. Highly conserved polar residues implicated in catalysis (Asp 339 and His 379 in HHAT) are

boxed in red.



**Figure S6. Comparison of HHAT with lipid-modifying MBOATs.** (A-D) Structure of HHAT (A) in comparison to DltB (B) and monomers of ACAT1 (C) and DGAT1 (D) (*15-20*), with MBOAT fold domains in white and other regions in color.



Figure S7. Interactions with palmitoyl-CoA in the substrate binding site. (A) Interacting residues shown as sticks, with hydrogen bonds denoted by dashes. (B) Schematic of the interactions. van der Waals interactions are shown as spiked arcs.

		TM1	IH1	
H.sapines	1 M L P R	W <mark>E                                   </mark>	VYKVSREHEEELDQEFE	LETDTLFGGLKKDATDFE 59
M.musculus	1 M L P G'	W <mark>E</mark>	VYKV SREHEEELDQEFE	LEMDTLFGGLKKDPTDFE 59
S.scofa	1 M L P R	W <mark>E </mark> L  S  F  Y  L  F  A  S  L <mark>G</mark> F  H  F  Y  S  F  Y  E	VFKVSREHEEELDQEFD	LETDSLFGGLKKDPTDFE 59
G.gallus	1 M K N S R A A P R P V V L <mark>P</mark> A '	W <mark>E</mark> M C A Y L F L T I <mark>G</mark> S H F Y S F <mark>Y</mark> E	AYQVSQKYEDEIDRSFG	LEQGTLFWGLKKDALDFE 70
D.rerio	1 MT P K W E L V S L P R '	W <mark>E</mark> M V A Y W I L S F S S H F Y S F <mark>Y</mark> Q	LHSFSKEHEVSLDKEVQ	LERGFLLW <mark>GFK</mark> <mark>KDPTDFE</mark> 67
D.melanogaster	1 M S R L P D R S L L T R	C	IYGLRDHIVKE AKFQ	FPEGWSLYPFSQRRRDDSNDELE 70
		ТМО	TM2	
	IHZ		11115	1114
H.sapines 60	D W S F W M E W G K Q W L V W L	L L G H M V V S Q M A T L L A R K H R P	WILMLY GMWACWCVLGT	PGVAMVLLHTTISFCVAQFRS 132
M.musculus 60	D WN FWM EWGK R R L V W L	F I G H M A V S Q L A T L L T K K H R P	W I V M V Y GMWA C W C V L G A	PGVVMVLLHSTIAFCVAQFRS 132
S.scota 60	D W S F WM E W G K Q R L A W L	LLGHVGVSQVANLLARKHRP	WILAAYGMWACHCALGI	RGIAMVLLHIIISFCVAQFRS 132
G.gallus /1	I WSYWIEWGREYILWL		WCLMVYGMAACWFLLGT	KGFAVILVHIAI SFAVAQFQI 143
D.rerio 68	3 W S F W N E WAWR S L L W S		AV FAMY GLI SAGWILGV	ROVIVLNILHLSISLVVAQLRS 140
D.meianogaster 7	INFGDFIVSFWPFYL-		QCLGFIGVCALALSVNL	DWSSMVLLVILIASYYIVSLLSL 140
	TM5	- IH3[	TM6	
H.sapines 133	3 Q L <mark>L T W L C </mark> S L L L L S T L	R L Q G V E E V K R K WY K T E N	EYYLLQFTLTVRCLYYT	SFSLELCWQQLPA 194
M.musculus 133	8 V L L S W L C <mark>S</mark> L L L L S T L	R L Q S V E E V K R R WY K T E N	EYYLLQFTLTVRCLYYT	SFSLELCRQPPSAQPTPSA 200
S.scofa 133	B LALSWLC <mark>S</mark> LFLLSTL	R L Q D V E E V K R G W Y E T E N	EYYLLQFTLTVRCLYYT	<mark>SFSLEL</mark> CWQQLPA 194
G.gallus 144	F P L L T W L C <mark>S</mark> L I L L S T L	R I P A V E E A <mark>K R</mark> E <mark>W Y</mark> D T E N	EYYLLLFTVSVRCLFNT	<mark>S F S L E Y</mark> C W N G R A 204
D.rerio 141	P V L <mark>S W F C S</mark> L L L L S <mark>T</mark> L	N V S A L Q D L Q R G WY T S E D	Q Y Y L L L F <mark>S</mark> T A V C S L H C I	SFCLEMCWHPLQAGRPKHCSSLQ 212
D.melanogaster 141	K	N <mark>V</mark> MQ <mark>K</mark> N V <mark>WW</mark> T D R V G Y T	EYVLVIVTMSWSVLRGC	<mark>SYSL</mark> SKIG 196
				TM7
100				
H.sapines 195	OCACHEVENUL	AYVFYYPVLHNGPILSFSEF	TRUMUQUEHDSLKASLC	V LA LGLGR LLCWWWLA ELMAHLM 265
Mi.musculus 201			FRUMUUPELNSLUHSLU	I VARGEGREECWWWEAELMVHEM 2/1
C gallus 205		ATVETTPVENOPILSEPER		
O.galius 203 Dirorio 213			SEQUERRUEAF SERTINEV	ILIVGIIINIFFWWCLAELMIHFM 2/3
Direlio 213	AKOEDITRYSIVOVI	GYAMY EPCITY GPLISYOR		GEV GGV L B SA LWWLVMO CAL HV E 264
D.melanogaster 197	ARQEDEIRIJEVQIE			
	- <u>IH7</u>	[	M8	
H.sapines 266	- IH7 7 MHAIYSSIPLLETV	SC WTLG <mark>G</mark> LALAQVLFFYV	M8 <sup>7</sup> K <mark>Y</mark> L V L F <mark>G</mark> V P A L L M R L D G	IH8 LTPPALPRCVSTMFSFTGMWRYF 338
H.sapines 266 M.musculus 272	- IH7 5 YMHAIYSSIPLLETV 2 YMHALYSSAPLLESV	S C WT L G <mark>G L A L A Q V L F F Y V</mark> S C WT L G <mark>G L A L A Q V L F F Y V</mark>	M8 ′KYLVLFGVPALLMRLDG ′KYLVLFGVPALLMRLDG	LT PPALPRCVSTMFSFTGMWRYF338 LTPPPLPRCVSTMFSFTGMWRYF344
H.sapines 266 M.musculus 272 S.scofa 266	IH7       YMHAIYSSIPLLETV       YMHALYSSAPLLESV       YMHAICSSAPLLSAV	SC WT LGGLALAQVLFFY           SC WT LGGLALAQVLFFY           SC WT LGGLALAQVLFFY           SC WT LGGLALAQVLFFY	M8 ' K Y L V L F <mark>G</mark> V P A L L MR L D G ' K Y L V L F G V P A L L MR L D G ' K Y L V L F G V P A L L MR L D G	LTPPALPRCVSTMFSFTGMWRYF338       LTPPPLPRCVSTMFSFTGMWRYF344       LSPPPLPRCVSTMFSFTGMWRYF338
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276	YMHAIYSSIPLLETV       YMHALYSSAPLLESV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YHAICSTSSLEAM	SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SY WA LG G V A LAQ V L F F Y V	M8 'KYLVLFGVPALLMRLDG 'KYLVLFGVPALLMRLDG 'KYLVLFGVPALLMRLDG 'KYLVLYGIAALLLRMDG	LTPPALPRCVSTMFSFTGMWRYF338       LTPPPLPRCVSTMFSFTGMWRYF334       LSPPPLPRCVSTMFSFTGMWRYF338       LSPPPLPRCVSTMFSFTGMWRYF338       LRPPALPRCVSLMHSFTKMWRSF348
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276 D.rerio 285	IH7       YMHAIYSSIPLLETV       YMHALYSSAPLLESV       YMHAICSSAPLLSAV       YHAICSTSSLEAM       YHAIQSNETYLEML	SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SC WT LG G LA LAQ V L F F Y V           SY WA LG G V A LAQ V L F F Y V           P WAMG G LA LA LAQ F F Y V	M8 'K Y L V L F G V P A L LMR L D G 'K Y L V L F G V P A L LMR L D G K Y L V L F G V P A L LMR L D G K Y L V L Y G I A A L L L RM D G 'K Y L V L F G A V S L L V R L D G	IH8         LTPPALPRCVSTMFSFTGMWRYF338         LTPPPLPRCVSTMFSFTGMWRYF334         LSPPPLPRCVSTMFSFTGMWRYF338         LRPPALPRCVSLMHSFTGMWRSF338         LRPPALPRCVSLMHSFTGMWRAF337
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276 D.rerio 285 D.melanogaster 267	YMHAIYSSIPLLETV       YMHAIYSSAPLLESV       YMHAICSSAPLLESV       YHAICSSAPLLESAV       YIHAICSTSSLEAM       YHAIQSNETYLEML       YHHYMSRDVRMVEMM	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV PP - WAMGGLALALVQFFYV DSVFWQH - SAGYFMGQFFFL	M8 'K Y LV L F G V P A L LMR L D G 'K Y LV L F G V P A L LMR L D G K Y LV L F G V P A L LMR L D G K Y LV L F G A V A L L R M D G 'K Y LV L F G A V S L LV R L D G Y Y V V T Y G L G I A F A V Q D G	LTPPALPRCVSTMFSFTGMWRYF338         LTPPPLPRCVSTMFSFTGMWRYF334         LSPPPLPRCVSTMFSFTGMWRYF338         LRPPLPRCVSLMFSFTGMWRFF337         LRPPLPRCVSLMFSFTGMWRFF337         JPPCLPRCVSLMFSFTGMWRF353         JPPCLPRCVSLMFSFTGMWRF334         JPPCLPRCVSLMFSFTGMWRF334
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276 D.rerio 285 D.melanogaster 267	YMHAIYSSIPLLETV       YMHAIYSSAPLLESV       YMHAICSSAPLLESV       YMHAICSSAPLLESAV       YMHAICSTSSLEAM       YMHAIQSNETVLEML       YHHYMSRDVRMVEMM	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV PP - WAMGGLALALVQFFYV DSVFWQH - SAGYFMGQFFT	M8 'KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG 'KYLVLFGVPALLMRLDG 'KYLVLFGAVSLLVRLDG YYVVTYGLGIAFAVQDG	LTPPALPRCVSTMFSFTGMWRYF338         LTPPPLPRCVSTMFSFTGMWRYF334         LSPPPLPRCVSTMFSFTGMWRYF338         LRPPLPRCVSLMHSFTGMWRFF337         LPPTLPRCVSLMHSFTGMWRF5338         LPPTLPRCVSLMHSFTGMWRF333         IPPTLPRCVSLMHSFTGMWRF333         IPPTLPRCVSLMHSFTGMWRF333         IPPTLPRCVSLMHSFTGMWRF333         IPPTLPRCVSLMHSFTGMWRF333         IPPTLPRCVSLMHSFTGMWRF333         IPTM10
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276 D.rerio 285 D.melanogaster 267	IH7       YMHAIYSSIPLLETV       YMHALYSSAPLLESV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV       YMHAINSNAPLLSAV	SC WT LG GLA LAQ V L F F Y V SC WT LG GLA LAQ V L F F Y V SC WT LG GLA LAQ V L F F Y V SY WA LG GVA LAQ V L F F Y V P P WAMG GLA LA L V Q F F Y V D SV F WQ H - SA GY F M GQ F F F L TM9	M8 'K Y L V L F G V P A L L M R L D G K Y L V L F G V P A L L M R L D G 'K Y L V L F G V P A L L M R L D G K Y L V L F G A V S L L V R L D G Y Y V V T Y G L G I A F A V Q D G E V S V U C G C Y D Y L W C WA A	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 338         LT P P L P R C V S T M F S F T G MWR Y F 338         L P R C V S T M F S F T G MWR Y F 338         L P R C V S T M F S F T G MWR Y F 338         L P P A L P R C V S T M F S F T G MWR Y F 344         L P P C V S T M F S F T G MWR Y F 348         L P P C V S T M F S F T G MWR Y F 348         L P P C V S T M F S F T G MWR Y F 340         I P A P N R P C I G R I H F Y S DMWK Y F 340         archway         T M 10         I H 10         I M V P P V F T C C D 0         T M 10         I H 0
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 276 D.rerio 285 D.melanogaster 267 H.sapines 339 M.musculus 34	IH7       YMHAIYSSAPLLETV       YMHALYSSAPLLESV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAICSSAPLLSAV       YMHAIQSNETYLEML       YHAISRVRWEMM       HYMSRVRWEMM       YHANSRVRWEMM	SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SY WA LG G V A LAQ V L F F Y V P P - WAMG G LA LA L V Q F F Y V D S V F WQ H - S A G Y F M G Q F F L MG S Q H G L LG T L F S T A MT F A	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVVTYGLGIAFAVQDG (FVSYWHGGYDYLWCWAA (FVSYWHGSYEDWCWAA	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         LT P P A L P R C V S T M F S F T G MWR Y F 338         L P R C V S T M F S F T G MWR Y F 338         L P R C V S T M F S F T G MWR Y F 338         L P P A L P R C V S T M F S F T G MWR Y F 340         I P A P R C V S I M Y S F T G MWR H F 357         I P A P R C V S I M Y S F T G MWR Y F 340         T M 10         I H Y S D MWK Y F 340         T M 10         I H 10         L NWL G V T V E N G V R R L V E T P C V D C 4 13
H.sapines 266 M.musculus 277 S.scofa 266 G.gallus 276 D.rerio 285 D.melanogaster 267 H.sapines 335 M.musculus 345 S.scofa 333	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YHAICSTSSLEAM         YMHAIQSNETYLEML         YHAICSTSSLEAM         YHAICSTYLEML         YHAISNEY         YHAIN         YHAISNEY         YHAISNEY         YHAISNEY         YHAISNEY         YHAISNEY         YHAISNEY	SC WT LG GLALAQ V L F F Y V SC WT LG GLALAQ V L F F Y V SC WT LG GLALAQ V L F F Y V SY WA LG GV A LAQ V L F F Y V P P - WAMG GLALALV Q F F Y V D S V F WQ H - S A G Y F M G Q F F F L 	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVTYGLGIAFAVQDG (FVSYWHGGYDYLWCWAA (FVSYWHGGYDYLWCWAA (FVSYWHGGYUWCWAA	IH8         LT P P A L P R C V ST M F S F T G MWR Y F 348         LT P P L P R C V ST M F S F T G MWR Y F 348         LS P P L P R C V ST M F S F T G MWR Y F 348         L P R C V ST M F S F T G MWR Y F 348         L P R C V ST M F S F T G MWR Y F 348         L P R C V ST M F S F T G MWR Y F 340         T P A L P R C V ST M Y S F T G MWR Y F 340         T A P N R P R C I G R I H F Y S D MWK Y F 340         T M T O IH 10         IH10         I H 10         L NW L G V T V E N G V R R L V E T P C I Q D 413         NW L G V T V E S G V R R L V E T P C V R E 419         NW L G V T V E S G V R R L V E T P C V R E 410         NW L G V T V E S G V R R L V E T P C V R E 410
H.sapines 266 M.musculus 272 S.scofa 266 G.gallus 277 D.rerio 285 D.melanogaster 267 H.sapines 335 M.musculus 345 S.scofa 335	HT YMHAIYSSIPLLETV YMHALYSSAPLLESV YMHAICSSAPLLESV YIHAICSTSSLEAM YHAIQSNETVLEML YHHYMSRDVRMVEMM H9 DVGLHNFLIRYVYIP DVGLHNFLIRYVYIP DVGLHNFLIRYVYIP DVGLHNFLIRYVYIP DVGLHNFLIRYVYIP	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV PP WAMGGLALALVQFFYV DSVFWQH - SAGYFMGQFFFL TM9 VGGSQHGLLGTLFSTAMTFA VGGSQHGLLGTLFSTATTFA	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YYVVTYGLGIAFAVQDG (YYVVTYGLGIAFAVQDG (FVSYWHGGYDYLWCWAA (FVSYWHGGNDYLWCWAA (FVSYWHGGNDYLWCWAA (FVSYWHGGONYLWCWAA	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 388         LP R C V S T M F S F T G MWR Y F 388         LP R C V S T M F S F T G MWR Y F 388         LP R C V S L M H S F T K MWR S F 348         LP R C V S L M H S F T G MWR H F 357         I P A P N R P R C I G R I H F Y S D MWK Y F 340         TM10         I H 10         L N W L G V T V E N G V R R L V C T P C I Q D 413         L N W L G V T V E S G V R R L L C T P C V R E 419         L N W L G V T V E S G V R R L V Q K P S I Q D 413         L N W L G V T V E S G V R R L L C Q K P S I Q D 413
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         339           M.musculus         345           S.scofa         336           M.musculus         345           S.scofa         333           G.gallus         345           D.rerio         358           D.rerio         358	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YHAICSSAPLLESV         YHAICSNETVEM         YIHAICSNETVEM         YIHYMSRDVRWEMM         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHNFLIRVVI         DVGLHRFLVRVI         DVGLHRFVVI	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV DSVFWQH - SAGYFMGQFFYV DSVFWQH - SAGYFMGQFFFI 	M8 'KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG 'KYLVLFGVPALLMRLDG 'KYLVLFGAVSLLVRLDG 'KYLVLFGAVSLVVRDG 'YVVVTYGLGIAFAVQDG FVSYWHGGYEDUWWAA FVSYWHGGYEDUWWAA FVSYWHGGQSLWYWGV FVSYWHGCQYLWYWGV	IH8         LT P P A L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 338         L P P L P R C V ST M F S F T G MWR Y F 370         L P P L P R C V ST M F S F T G MWR H F 357         I P A P N R P R C I G R I H F Y S DMWK Y F 340         archway         TM10         I H 10         L NWL G V T V E N G V R R L V E T P C I Q D 413         L NWL G V T V E S G V R R L V Q K P S I Q D 413         L NWL G V T V E N G V R R I L S V S P L R H 432         L NWL G V T V E N G V K R I L S V S P L R H 432
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         345           D.rerio         358           D.melanogaster         359           D.melanogaster         345	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSTYSLEAM         YMHAISSAP         YHAYSRDVRWEMM         JUGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHRFLVRYYYP         DVGLHRFLVRYYYP         DVGLHRFLVRYIYYP         DVGLYKWLIRYYYYP         DVGLYKWLIRYYYYA	SC WT LG GLA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SY WA LG GV A LAQ V L F F Y V P WAMG GLA LA V Q F F Y D SV FWQ H - SAG Y FM Q F F F I MG SQ HG LLGT L F ST A WT F A V GG SQ HG LLGT L F ST A VT F A MG G SQ S S L P GM L F ST A UT F A LG G S R HG A F R K LL ST A LA F A LG G S R HG A F R K LL ST A LA F A	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (KYLVLFGAVSLLVRLDG (FVSYWHGGYDYLWCWAA (FVSYWHGGYDYLWCWAA (FVSYWHGGNDYLWCWAA (FVSYWHGCYTVLWSU	LTPPALPRCVSTMFSFTGMWRYF338 LTPPPLPRCVSTMFSFTGMWRYF344 LSPPPLPRCVSTMFSFTGMWRYF338 LRPPALPRCVSLMHSFTKMWRSF348 LEPPTLPRCVSIMYSFTGMWRHF357 PAPNRPRCIGRIHFYSDMWKYF340 archway TM10 LNWLGVTVENGVRRLVETPCIQD413 LNWLGVTVESGVRRLVETPCVRE419 LNWLGVTVESGVRRLVETPCVRE419 LNWLGVTVESGVRRLVETPCVRE419 LNWLGVTVESGVRRLVETPCVRE419 LNWLGVTVESGVRRLVQKPSIQD413 LNWLGVTVESGVRRLVQKPSIQD413 LNWLGVTVESGVRRLVQKPSIQD423 LNWLGVTVENGVKRLSVSPIQD423 LNWLGVIVENGVKRLSVSPIQD423 LNWLGVIVENGVKRLSVSPIQD423 LNWLGVIVENGVKRLSVSPIQD423
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         288           D.melanogaster         267           H.sapines         339           M.musculus         345           S.scofa         339           G.gallus         344           D.rerio         358           D.melanogaster         341	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         YMHAICSYAP         DVGLHNFLIRYVY         DVGLHNFLIRYVY         DVGLHNFLIRYVY         DVGLHRFLVRYVY         DVGLHRFLVRYVY         DVGLHRFLVRYVY         DVGLY         SUMIN	SC WT LG GLA LAQ V L F F Y V SC WT LG GLA LAQ V L F F Y V SC WT LG GLA LAQ V L F F Y V SY - WA LG GV A LAQ V L F F Y V P P - WAMG GLA LA LVQ F F Y D SV FWQ H - SA GY FM GQ F F F VGG SQ HG LLGT LF ST AMT F A LGG SQ HG LLGT LF ST AMT F A VGG SQ HG LLGT LF ST AVT F A MGG SQ SS LP GM LF ST A LT F A LGG SR HG A F K LL ST A LA F G LCG K R SS A A AK F GAT A LT F A	M8 (KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG VYVVTYGLGIAFAVQDG (FVSYMHGGYDYLWCWAA FVSYMHGGQSYLWYWGV FVSYMHGCYTYVLWSI	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         L P R C V S T M F S F T G MWR Y F 344         L P R C V S T M F S F T G MWR Y F 348         L P R C V S T M F S F T G MWR Y F 348         L P R C V S T M F S F T G MWR Y F 348         L P R C V S I M Y S F T G MWR H F 357         T L P R C V S I M Y S F T G MWR Y F 340         I P A P N R P C I G R I H F Y S DMWK Y F 340         T M10         I T M10         I M V E N G V R R L V E T P C I Q D 413         I NW L G V T V E N G V R R L V E T P C V R E 419         L NW L G V T V E S G V R R L V Q K P S I Q D 413         L NW L G V T V E N G V K R I L S V S P I Q D 423         L NW L G V I V E N A F A L L F S S P P L R H 432         L N F L C L A A E K V F K T F T A M P E Y Q R 415
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         346           D.rerio         358           D.rerio         358           D.nelanogaster         341	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YMHAICSNAP         YUNGLHAR         YIHYNSRAP         DVGLHNFLIRYVYIP         DVGLHRFLVRYVYIP         DVGLHRFLVRYVYIP         DVGLHRFLVRYVYIP         DVGLHRFLVRYVY         YVP         BOVGLYKWLIRYIYYP         DEGLYEFLFQNIYAE         archway         H10	SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SY WA LG G V A LAQ V L F F Y V P P WAMG G LA LA L V Q F F Y D SV F WQ H - SA G Y F M G Q F F L C G SQ HG L LG T L F ST A MT F A V GG SQ HG L LG T L ST A T T F A V GG SQ HG L LG T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG SQ HG L G T L ST A T T F A V GG S T HG A F K L ST A L T F A	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVTYGLGIAFAVQDG (FVSYWHGGYDYLWCWAA (FVSYWHGGSYEDWCWAA (FVSYWHGGQSYLWYWGV (FVCFWHGCYTYVLIWSI (FVFVWHGCYTYVLIWSI (FVFVWHGCYTYVLIWSI	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 338         LR P A L P R C V S T M F S F T G MWR Y F 348         L P R C V S T M F S F T G MWR Y F 348         L P R C V S T M F S F T G MWR H F 357         IP A P N R P R C I G R I H F Y S DMWK Y F 340         T M10         L NWL G V T V E N G V R R L V E T P C I Q D 413         L NWL G V T V E N G V R R L L E T P C V R E 419         L NW L G V T V E N G V R R L L E T P C V R E 419         L NW L G V T V E N G V R R L L G V K P S I Q D 413         L NW L G V T V E N G V R R L L G S P P L R H 432         L NW U G V L V E N A F A L L F S S P P L R H 432         TM12
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         345           S.scofa         345           S.scofa         345           D.rerio         358           D.melanogaster         3414           H.sapines         414	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YHAICSTSSLEAM         YHAIQSNETYLEML         YUGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHRELVRYVYVP         DVGLHRELVRYVYVP         DVGLHRELVRYVYVP         DVGLHRELVRYVYVP         BUSGLHRELFQNIYAE         archway         HIO         SLARYFSPQARR	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WALGGVALAQVLFFYV PP WAMGGLALALVQFFYV DSVFWQH - SAGYFMGQFFFU TM9 VGGSQHGLLGTLFSTAMTFA LGGSQHGLLGTLFSTATTFA VGGSQSLGLLGTLFSTATTFA LGGSRHGAFRKLLSTALAFC LCGKRSSAAAKFGATALTFA	M8 (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRDG (KYLVLFGAVSLLVRDG (KYVVTYGLGALLVRDG (FVSYWHGGYDYLWCWAA (FVSYWHGGYDYLWCWAA (FVSYWHGGQSYLWYWGV (FVCFMHGCHDYLLYWAL (FVFVWHGCYTYVLIWS) (FVFVWHGCYTYVLIWS) (FVFVWHGCYTYVLIWS)	IH8         LT P P A L P R C V S T M F S F T G MWR Y F       338         LT P P L P R C V S T M F S F T G MWR Y F       344         LS P P L P R C V S T M F S F T G MWR Y F       338         LP P C V S T M F S F T G MWR Y F       338         LP P C V S L M F S F T G MWR Y F       338         LP P C V S L M F S F T G MWR H F       357         I P A P N R P R C I G R I H F Y S D MWK Y F       340         TM10       II T N O         I M U G V T V E N G V R R L V T P C I Q D       413         L NW L G V T V E S G V R R L V Q K P S I Q D       413         L NW L G V T V E S G V R R L V Q K P S I Q D       413         L NW L G V T V E S G V R R L V Q K P S I Q D       413         L NW L G V T V E N G V K R I L S V S P I Q D       432         I M W G V I V E N G V K R I L S V S P I Q D       432         I M W G V L V E N A F A L L F S S P P L R H       432         I M F L C L A A E K V F K T F T A M P E Y Q R       415         T M 12         P W V T L S V L G F L Y C Y S H V
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         288           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         345           D.rerio         358           D.melanogaster         341           M.musculus         414           M.musculus         422	H7 YMHAIYSSIPLLETV YMHALYSSAPLLESV YMHAICSSAPLLESV YHAICSSAPLLESAV YHAICSTSSLEAM YMHAIQSNETYLEML YIHYMSRDVRMVEMM OVGLHNFLIRYVYIP DVGLNFR	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV DSVFWQH-SAGYFMGQFFYV DSVFWQH-SAGYFMGQFFF UTM9 VGGSQHGLLGTLFSTAMTFA VGGSQHGLLGTLFSTATFFA VGGSQHGLLGTLSTALTFA TMGSQSSHGAFKLSTALTFA TM1 AALASCSTSMLILSNLVFLG	M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F G V S A LLMR MD G KY LV L F G AV S LLV R L D G F V S Y WH G G Y D Y LWC WA A F V S Y WH G S Y E D LWC WA A F V S Y WH G C Y L V L WC WA A F V S Y WH G C Y L V L WC WA A F V S Y WH G C Y L V L WC WA A F V S Y WH G C Y L V L WS I F V F V WH G C Y T Y V L I WS I F V S W H G C Y T Y V L I WS I F V S W H G C Y T Y W R I F I Q G W G I Q V G K T Y WN R I F I Q G W	IH8         LTPPPL       PRCVSTMFSFTGMWRYF         338       SPPL         LSPPL       PRCVSTMFSFTGMWRYF         338       SFTGMWRYF         LPPTL       PRCVSLMFSFTGMWRYF         338       SFTGMWRYF         JPAPALPRCVSLMHSFTGMWRHF       357         IPAPALPRCVSLMHSFTGMWRHF       357         IPAPNRPRCVSLMHSFTGMWRHF       357         IPAPNRFRCVSLMHSFTGMWRHF       357         IPAPNRFRCVSLMHSFTGMWRHF       340         archway       archway         TM10       IH10         LNWLGVTVESGVRRLVCTPCVRE       413         LNWLGVTVESGVRRLVQKPSIQD       413         LNWLGVTVESGVRRLVQKPSIQD       413         LNWLGVTVENAFALLFSVL9       414         LNWLGVTVENAFALLFSVRL4       415         LNWLGVTVENAFALLFSVRL4       414         LNWLGVTVENAFALLFSVRL4       415         LNWLGVTVENAFALLFSVR1       414         LNWLGVTVENAFALLFSVR1       414         LNWLGVTVENAFALLFSVR1       415         LNWLGVTVENAFALLFSVR1       414         LNWLGVTVENAFALLFSVR1       415         LNWLGVTVENAFALLFSVR1       414         LNWLGVTVENAFALLFSVR1       415         LNVL
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         345           D.rerio         358           D.renologaster         341           M.musculus         414           M.musculus         420           S.scofa         414           M.musculus         420           S.scofa         414           M.musculus         420           S.scofa         414	H7 YMHA IYSSIPLLETV YMHA LYSSAPLLESV YMHA ICSSAPLLSAV YHA ICSTSSLEAM YMHA IQSNETYLEML YIHYMSR DVRMVEMM OUT OF CONTACT OF CONTACT OUT OF CONTACT OUT OF CONTACT OUT OF CONTACT OUT OUT OUT OF CONTACT OUT OUT OUT OF CONTACT OUT OUT OUT OUT OF CONTACT OUT OUT OUT OUT OUT OUT OUT OUT OUT OUT	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SY WALGGVALAQVLFFYV DSVFWQH- SAGYFMGQFFYV DSVFWQH- SAGYFMGQFFYV OSVFWQH- SAGYFMGQFFY 	M8 'K Y LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G 'K Y LV L F GV P A LLMR L D G 'K Y LV L F GAV S L V R L D G 'Y V V Y G L G I A F A V Q D G Y Y V V Y G L G I A F A V Q D G F V S Y WH G G Y E D L W C WA A F V S Y WH G G Q S Y L W W W A F V S Y WH G C Y Y V L I W S I F V C F W H G C Y T Y V L I W S I F V C F W H G C Y T Y V L I W S I G N E V G K T Y W N R I F I Q G W G N Q V G R I Y W N R I F I Q G W	IH8         LT P P A L P R C V ST M F S F T G MWR Y F 338         LT P P L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 338         LR P P L P R C V ST M F S F T G MWR Y F 338         LP P L P R C V ST M F S F T G MWR Y F 338         LP P L P R C V ST M F S F T G MWR H F 357         IP A P N R P R C I G R I H F Y S DMWK Y F 340         archway         TM10         I F N V S T G N R L V E T P C I Q D 413         L NWL G V T V E N G V R R L V C T P C V R E 419         L NWL G V T V E N G V R R L V C Y P C V R E 419         L NWL G V T V E N G V R R L L S V S P I D A 433         L NW L G V T V E N G V R A L L F S S P P L R H 432         L NW G V L V E N A F A L L F S S P P L R H 432         L N F L C LA A E K V F K T F T A M P E Y Q R 415         TM12         P W V T L S V L G F L Y C Y S H V G I A WA - 493         P W V T L S V L G F L Y C Y S H V G I A WA - 493         P W V T L S V L G F L Y C Y S H V G I A WA - 497
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         339           G.gallus         345           D.melanogaster         341           M.musculus         424           M.musculus         424	H7 Y MHA I Y SS I P L L ET V Y MHA L Y SS AP L L ES V Y MHA I C SS AP L L SAV Y MHA I C SS AP L SAV Y M SS AP L SAV Y MHA I SS AP L SAV Y M SS AP L SAV Y MHA I C SS AP L SAV Y MHA I SS AP L SAV Y M SS AP	SC WT LG GLA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SC WT LG G LA LAQ V L F F Y V SC WT LG G V A LAQ V L F F Y V SY WA LG G V A LAQ V L F F Y V D SV FWQ H- SAG Y FM GQ F F I O SV FWQ H- SAG Y FM GQ F F F I MG SQ HG LLGT L F ST A WT F A V GG SQ HG LLGT L F ST A WT F A V GG SQ HG LLGT L F ST A VT F A V GG SQ HG LLGT L F ST A VT F A V GG SQ HG L GT L S T A T T F A V GG SQ S S L P GM L F ST A LT F A LGG SR HG A F RK L L ST A T T F A V GG SQ S S L P GM L F ST A LT F A LGG SR HG A F RK L L ST A T T F A LGG SR HG A F RK L L ST A V F L A L A SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A V A SV C T SM L I L S N L I F L	M8 (KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG YVVTYGLAALLLRMDG VYVVTYGLGIAFAVQDG FVSYMHGGYDYLWCWAA FVSYMHGGQSYLWYWAA FVSYMHGGQSYLWYWAA FVSYMHGCYTYVLWSI FVCFWHGCYTYVLWSI FVCFWHGCYTYVLWSI FVCFWHGCYTYVLWSI GNEVGKTYWNRIFLQGW GNQVGRIYWNRIFLQGW GNHVGKYYWNRIFMEGK	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 348         LS P P L P R C V S T M F S F T G MWR Y F 348         LS P P L P R C V S T M F S F T G MWR Y F 348         LS P P L P R C V S T M F S F T G MWR Y F 348         L P R C V S I M Y S F T G MWR H F 357         P A P N R P C I G R I H F Y S DMWK Y F 340         archway         TM10         I M V C V E N G V R L V E T P C I Q D 413         I NW L G V T V E N G V R R L V E T P C I Q D 413         L NW L G V T V E N G V R R L V C K P S I Q D 413         L NW L G V T V E N G V R R L L S V S P I Q D 423         L NW L G V T V E N G V R R L L S V S P I Q D 423         L NW L G V L V E N A F A L L F S S P P L R H 432         L N W L G V L V E N A F A L L F S S P P L R H 432         L N W L G V L V E N G F L Y C Y S H V G I AWA - 487         P W V T L S V L G F L Y C Y S H V G I AWA - 487         P W V T L S V L G F L Y C Y S H V D I AWA - 487         F I L C A L         V C I S V L G F L Y C Y S H V G I AWA - 487         F I L C A L
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         277           D.rerio         285           D.melanogaster         267           H.sapines         339           M.musculus         344           S.scofa         356           G.gallus         344           D.rerio         358           D.melanogaster         341           H.sapines         414           M.musculus         424           S.scofa         414           M.musculus         424           D.rerio         414           M.cosculus         424           D.rerio         414	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YHAICSTSSLEAM         YHAIQSNETYLEML         YHAIN         YHAINN	SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WT LGGLALAQVLFFYV SC WALGGVALAQVLFFYV PP WAMGGLALALVQFFYV DSVFWQH - SAGYFMGQFFFU TM9 VGGSQHGLLGTLFSTAMTFA LGGSQHGLLGTLFSTATTFA VGGSQSSLPGMLFSTALTFA LGGSRHGAFRKLLSTALAFC LCGKRSSAAAKFGATALTFA TM11 AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AVLASVCTSMLILSNLVFLC	M8 (KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVTYGLGALLVRLDG (YVVTYGLGALVRLDG (YVVTYGLGAVSLLVRLDG (SVMHGGVDYLWCWAA FVSYMHGGVDYLWCWAA FVSYMHGGQSYLWYWGV FVCFWHGCVTYVLWSI FVCFWHGCYTYVLWSI FVFVHGCYTYVLWSI FVFVHGCYTYVLWSI GUQVGRTYWNRIFLQGW GNUVGRIYWNRIFLQGW GNUVGRIYWNRIFMEGK GTYVGRIFWKKLFVQDW	IH8         LT P P A L P R C V S T M F S F T G MWR Y F       338         LT P P L P R C V S T M F S F T G MWR Y F       344         LS P P L P R C V S T M F S F T G MWR Y F       338         LP R C V S T M F S F T G MWR Y F       338         LP R C V S L M F S F T G MWR H F       357         I P A L P R C V S L M F S F T G MWR H F       357         I P A P N R P R C I G R I H F Y S D MWK Y F       340         TM10       II I N W L G V T V E N G V R R L V E T P C I Q D       413         L NW L G V T V E S G V R R L V C Y P S I Q D       413         L NW L G V T V E S G V R R L V C Y S P I Q D       423         I NW L G V T V E S G V R R L V C Y S P I Q D       431         I NW L G V L V E N A F A L L F S S P P L R H       432         I N W C V L V E N A F A L L F S S P P L R H       432         I M W C V L V E N A F A L L F S S P P L R H       432         I M W C V L V E N A F A L L F S S P P L R H       432         I M W T L S V L G F L Y C Y S H V G I A WA - 487         P W V T L S V L G F L Y C Y S H V G
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         345           S.scofa         346           G.gallus         345           S.scofa         349           D.rerio         358           D.melanogaster         3414           M.musculus         424           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           S.rerio         433           D.rerio         433           D.rerio         433           D.rerio         433           D.rerio         433           D.melanogaster         414	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YHAICSTSSLEAM         YHAICSTVEW         YHO         DVGLHNFLIRY         YYH         DVGLHNFLIRY         YYH         YHAICSTVEW         YYH         YHAICSTVEW         YYH         YHAICSTVEW         YYH         YHHAINENT         YH	SC WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SY - WALGGVALAQVLFFYV SY - WANGGLALAQVLFFYV DSVFWQH-SAGYFMGQFFFU TM9 VGGSQHGLLGTLFSTAMTFA LGGSQHGLLGTLFSTATFA VGGSQSSLFGALFSTATFA LGGSRHGAFRKLLSTALAFC LCGKRSSAAAKFGATALTFA TM11 AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTAFLLSNLFFLC	M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G Y Y LV L F GA V S LLW R L D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G Y Y V T Y G LG I A F A V Q D G G N Y Y K Y W N R I F I Q G W G N V G K T Y W N R I F I Q G W G N V G K Y Y W N R I F I Q G W G N Y G K Y Y W N R I F I Q G W G N Y G K Y Y W N R I F I Q G W G N Y G K Y Y W N R I F I Q G W G N Y G R I F W K K L F Y Q D W G Q E I G D F LM R G A Y L S G V	IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLPRCVSTMFSFTGMWRYF       338         LRPPLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         IPAPNRPCVSLMYSFTGMWRHF357       1         IPAPNRFCVSLMYSFTGMWRYF       340         archway         TM10       III10         LNWLGVTVESGVRRLVQKPSLQD433       LNWLGVTVESGVRRLVQKPSLQD433         LNWLGVTVESGVRRLVQKFSLQD433       LNWLGVTVESGVRRLFFTAMPEYQR4155         TM10         TM10         TM10         TM10         TM10         TM10         TM10         TM10         TM12
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         345           S.scofa         345           D.rerio         358           G.gallus         349           D.rerio         358           D.melanogaster         341           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.gallus         422           S.scofa         414           G.gallus         422           S.scofa         414           J.melanogaster         416           G.gallus         424           S.scofa         414           S.scofa         414           J.melanogaster         414	IH7         YMHAIYSSIPLLETV         YMHAIYSSAPLLESV         YMHAICSSAPLLESV         YHAICSTSSLEAM         YHAICSTVEW         YHAISTVEW         YHAISTVEW         YHONGLHNELINGVEW         YHNNELINGVEW         YHNNELINGVEW         YHONGLHNELINGVEW         YHONGLHNELINGVEW         YHONGLHNELINGVEW         YHONGLHNELINGVEW         YHON	SC WT LG G L A LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SY WA LG G V A LA Q V L F F Y V D S V F WQ H - SA G Y F M G Q F F F L 	M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G YY LV L F G V P A LLMR L D G YY LV L F G A V S LLWR L D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G F V S Y WH G C Y T Y V L I W S I F V S Y WH G C Y T Y V L I WS I F V S Y WH G C Y T Y V L I WS I F V S Y WH G C Y T Y V L I WS I F V S Y WH G C Y T Y V L I WS I F V S Y WH G C Y T Y V L I WS I G N V G K T Y WN R I F I Q G W G N V G K I Y WN R I F I Q G W G N V G R I Y WN R I F I Q G W G N V G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y G R I Y WN R I F I Q G W G N Y Y G Y Y Y G Y Y Y Y Y Y Y Y Y Y Y	IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLPRCVSTMFSFTGMWRYF       338         LRPPLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       340         archway         TM10       III0         LNWLGVTVESGVRRLVQKPSLQ       413         LNWLGVTVESGVRRLVQKPSLQ       413         LNWLGVTVESGVRRLUETPCVRE       419         LNWLGVTVESGVRRLUQKPSLQ       413         LNWLGVTVESGVRRLUQKPSLQ       413         LNWLGVTVESGVRRLUGVR       1         TM10         TM10         TM10         TM10         TM10         TM10         NRFRETGONGR         INFRETGONGR         TM10         TM12         TM12         TM12         TM12         TM12         TM12
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         345           D.rerio         358           D.melanogaster         341           M.musculus         342           D.rerio         358           D.melanogaster         341           M.musculus         420           S.scofa         414           M.musculus         420           S.scofa         414           G.gallus         424           D.rerio         433           D.rerio         433           D.rerio         433           D.rerio         433           M.sapines         414           G.gallus         424           H.sapines         438           H.sapines         488	H7 YMHAIYSSIPLLETV YMHAIYSSAPLLESV YMHAICSSAPLLESV YMHAICSSAPLLESAV YHAICSSAPLLESAV YHAICSSAPLLESAV YHAICSSAPLLESAV YHAIQSNETVLEML YHHYMSRDVRMVEMM OUTHOUSSAP DVGLHNFLIRYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLHNFLIRVYVYIP DVGLNAVYVIP	SC WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SY - WALGGVALAQVLFFYV DSVFWQH-SAGYFMGQFFYV DSVFWQH-SAGYFMGQFFYV VGGSQHGLLGTLFSTAMTFA LGGSHGLLGTLFSTATFA VGGSQHGLLGTLSTATFA VGGSQHGLLGTLSTATFA TM1 AALASCSTSMLILSTALTFA TM1 AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSALLSNLVFLC AALASCSTSALLSNLFLC AALASCSTAFLLSNLFFLC AMLATQLFIPAAFSNVYFIC	M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GAV S LLWR L D G KY LV L F GAV S LLWR L D G KY LV L F G A V S L V R L D G KY LV L F G A V S L V R L D G KY LV L F G A V S L V R L D G KY LV L F G A V S L V R L D G F V S Y MH G G Y D Y L V C WA A F V S Y MH G G Y D Y L V C WA A F V S Y MH G G Y D Y L V C WA A F V S Y MH G G Y C L V L V C WA A F V S Y MH G C Y T Y L U W S I F V F V MH G C Y T Y V L I W S I F V F V MH G C Y T Y V L I W S I F V F V W H G C Y T Y V L I W S I F V S Y W S Y W N R I F L Q G W G N H V G K T Y W N R I F L Q G W G N H V G R I F W W R I F M E G K G T H V G R I F W W R I F M E G K G T H V G R I F W K L F V Q W G Q E I G D F LMR G A Y L S G V	IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLPRCVSTMFSFTGMWRYF       338         LRPPLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRFF       357         IPAPRCVSLMYSFTGMWRFF       378         IPAPRCVSLMYSFTGMWRFF       370         IPAPNRFC       IGRIHFYSDMWKYF         MUGVTVESGVRRLVCTPC       IQD 413         LNWLGVTVESGVRRLVQKPSIQD       413         INWLGVTVESGVRRLVQKPSIQD       413         NWLGVTVESGVRRLVQKPSIQD       430         INWLGVTVESGVRRLVQKPSIQD       413         NWLGVTVESGVRRL       SPLRH432         INVLGVTVESGVRRL       415         INVLGVTVESGVRRL       5         INVLGVTVESGVRRL       415         INVLGVTVESGVRAL       487         INVLGVTVESQUE       415         INVLGVTVESVLGFL       487         INVLGVTVESVLGFL       487         INVLGVTVESVLGFL       487         INVLGVTVESVLGFL       487         INVLGVTVESVLGFL       487         INVLCLCLAAE
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         336           G.gallus         349           D.rerio         358           D.melanogaster         341           M.musculus         420           S.scofa         414           M.musculus         420           S.scofa         414           G.gallus         420           S.scofa         414           G.gallus         420           S.scofa         414           G.gallus         420           S.melanogaster         414           G.gallus         420           S.melanogaster         414           M.musculus         420           S.scofa         414           G.gallus         424           M.musculus         424	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSNETVEM         YHAICSNETVEM         YHYIHYMSRDVEM         YHAICSNETVEM	SC WT LG GLALAQ V L F F Y V SC WT LG GLALAQ V L F F Y V SC - WT LG GLALAQ V L F F Y V SY - WALG GV ALAQ V L F F Y V D SV FWQ H- SAG Y FM GQ F F F I 	M8 KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG KYLVLFGAVSLLVRLDG YVVVTYGLGIAFAVQDG FVSYMHGGYDYLWCWAA FVSYMHGGNDYLWCWAA FVSYMHGCNDYLWCND FVSYMHGCNDY FVSYMHGCND	IH8         LT P P A L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 344         LS P P L P R C V ST M F S F T G MWR Y F 388         LR P P A L P R C V S L M H S F T K MWR S F 348         LP P A L P R C V S L M H S F T K MWR S F 348         LP P A L P R C V S L M H S F T K MWR S F 348         LP P A L P R C V S L M H S F T K MWR S F 348         LP P A L P R C V S L M Y S F T G MWR H F 357         I P A P N R P R C I G R I H F Y S DMWK Y F 340         archway         TM10         I H O         I M V C V E N G V R R L V E T P C I Q D 413         L NW L G V T V E N G V R R L V C K P S I Q D 413         L NW L G V T V E N G V R A L L F S S P I L R H 42         TM12         TM12         PWVT L S V L G F L Y C Y S H V G I AWA - 487         F M T S V L G F L Y C Y S H V G I AWA - 487         F W V T L S V L G F L Y C Y S H V G I AWA - 487         F W V T L S V L G F L Y C Y S H V G I AWA - 487         F W V T L S V L G F L Y C Y S H V G I AWA - 487         F W V T L S V L G F L Y C Y S H V G I AWA - 487         F W V T L S V L G F L Y C F A Q V
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         358           O.rerio         358           D.melanogaster         341           M.musculus         342           S.scofa         414           M.musculus         424           D.rerio         358           D.melanogaster         414           M.musculus         424           D.rerio         432           D.melanogaster         414           M.musculus         424           M.musculusculus         424	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YHYNSRDVRWEMM         POVGLHNFLIRYVYIEM         DVGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHRFLVRYIYVP         DVGLYKWLIRYYYYP         DVGLYKWLIRYYYYP         DVGLYKWLIRYYYYP         DEGLYEFLFQNIYAE         archway         HIO         SLARYFSPQARR         SLAQVLSPQARR	SC WT LG G L A LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SC WT LG G V A LA Q V L F F Y V SY - WA LG G V A LA Q V L F F Y V D S V FWQ H- SA G Y FM G Q F F I U G S Q HG LLGT L F ST AMT F A LG G SQ HG LLGT L F ST A WT F A V GG SQ HG LLGT L F ST A VT F A MG G SQ S S L P GM LF ST A VT F A LG G SR HG A F R K LL ST A LA F G LC G K R S A A A K F G A T A LT F A LG G S C ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N L V F L A A LA SC ST SM L I L S N V Y F L C A M LA T Q L F I P A A F S N V Y F I G	M8 (KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG (KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVTYGLGAALLLRMDG (YVVTYGLGAALLLRMDG (FVSYMHGGYDYLWCWAA (FVSYMHGGYDYLWCWAA (FVSYMHGGNDYLWCWAA (FVSYMHGCYTYVLWSI (FVCFWHGCHDYLLYWSI (FVFVHGCYTYVLWSI (FVFVHGCYTYVLWSI (FVFVHGCYTYVLWSI (FVCFWHGCYTYVLWSI (FVFVHGCYTYVLVLWSI (FVFVHGCYTYVLVLWSI (FVFVHGCYTYVLVLWSI (FVFVHGCYTYVLVLWSI (FVFVHGCYTYVLVLWSI (FVFVHGCYTYVLVLVLWSI (FVFVHGCYTYVLVLVLVLVLVLVLVLVLVLVLVLVLVLVLVLVLVLV	IH8         LT P P A L P R C V S T M F S F T G MWR Y F 344         LS P P L P R C V S T M F S F T G MWR Y F 348         LS P P L P R C V S T M F S F T G MWR Y F 388         LP R C V S T M F S F T G MWR Y F 388         LP R C V S T M F S F T G MWR Y F 388         LP P L P R C V S I M Y S F T G MWR H F 357         P A L P R C V S I M Y S F T G MWR H F 377         T M C I G R I H F Y S DMWK Y F 340         archway         T M 10         I M V C V E N G V R I V E T P C I Q D 413         I M V G V T V E N G V R R L V C Y P S I Q D 413         I M V G V T V E N G V R R L V C Y P V C I Q D 413         I M V G V I V E N G V R R L V C Y S P I Q D 423         I M V G V I V E N G V R I L S V S P I Q D 423         I M V G V I V E N G V K R I L S V S P I Q D 423         I M V G V I V E N G V K R I L S V S P I Q R 432         I M V G V I V E N G V K R I L S V S P I Q R 432         I M V I S V L G F L Y C Y S H V G I AWA - 487         P W V T L S V L G F L Y C Y S H V G I AWA - 487         F U C A L - C F C S Y C F F Q C S E L L L T 489         S V A V P M V A F L Y C F A Q V G I E W D - 506         G N Y A L - C F C S Y C F F
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         272           D.rerio         285           D.melanogaster         267           H.sapines         345           S.scofa         346           O.gallus         345           S.scofa         349           D.rerio         358           D.melanogaster         3414           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           S.scofa         414           M.musculus         422           D.rerio         433           D.melanogaster         416           H.sapines         486           M.musculus         424           S.scofa         414           S.scofa         448           S.scofa         448           G.gallus         424           S.scofa         448           G.gallus         424	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSTSSLEAM         YHAICSTSSLEAM         YHAICSTVEM         YHAICSTVEM         YHAICSTVEM         YHAICSTVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHANSRDVRMVEMM         DVGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHNFLIRYVYIP         DVGLHNFLIRYIYVYP         DVGLYELFFQNIY         NYGLHOFLVRYVY         DVGLYELFFQNIY         SLARYFSPQARR         YHAICSNET         YHAICSNET <tr< td=""><td>SC WT LG G L A LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SY WA LG G V A LA Q V L F F Y V P WAMG G LA LA LV Q F F Y V D S V F WQ H - SA G Y F M G Q F F F L </td><td>M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G YY LV L F GV P A LLMR L D G YY LV L F GA V S LLWR L D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G F V S Y WH G C Y T Y V L I WS I H11 G N E V G K T YWNR I F I Q GW G N E V G K T YWNR I F I Q GW G N H V G K V YWNR I F I Q GW G N H V G K V YWNR I F M E G K G T H V G R I F W K K L F V Q DW G Q E I G D F LMR G A Y L S G V</td><td>IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLRCVSTMFSFTGMWRYF       338         LSPPLRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       340         archway         TM10       III0         LNWLGVTVESGVRRLVCFPCVR       419         LNWLGVTVESGVRRLVQKPSIQD       430         INWLGVTVESGVRRLVQKPSIQD         INWLGVTVESGVRRLVQKPSIQD         TM10         III0         LNWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWTLSVLGFLYCSHVGIAWA-</td></tr<>	SC WT LG G L A LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SC WT LG G LA LA Q V L F F Y V SY WA LG G V A LA Q V L F F Y V P WAMG G LA LA LV Q F F Y V D S V F WQ H - SA G Y F M G Q F F F L 	M8 KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G KY LV L F GV P A LLMR L D G YY LV L F GV P A LLMR L D G YY LV L F GA V S LLWR L D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G YY VV T Y G LG I A F A V Q D G F V S Y WH G C Y T Y V L I WS I H11 G N E V G K T YWNR I F I Q GW G N E V G K T YWNR I F I Q GW G N H V G K V YWNR I F I Q GW G N H V G K V YWNR I F M E G K G T H V G R I F W K K L F V Q DW G Q E I G D F LMR G A Y L S G V	IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLRCVSTMFSFTGMWRYF       338         LSPPLRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRYF       340         archway         TM10       III0         LNWLGVTVESGVRRLVCFPCVR       419         LNWLGVTVESGVRRLVQKPSIQD       430         INWLGVTVESGVRRLVQKPSIQD         INWLGVTVESGVRRLVQKPSIQD         TM10         III0         LNWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVRRLLETPCVRE         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWLGVTVESGVR         INWTLSVLGFLYCSHVGIAWA-
H.sapines         266           M.musculus         272           S.scofa         266           G.gallus         276           D.rerio         285           D.melanogaster         267           H.sapines         335           M.musculus         345           S.scofa         345           O.gallus         345           J.musculus         345           D.rerio         358           D.melanogaster         3414           M.musculus         420           S.scofa         414           M.musculus         420           S.scofa         414           G.gallus         420           S.scofa         414           G.gallus         420           S.scofa         414           G.gallus         420           D.melanogaster         416           M.musculus         424           S.scofa         488           M.musculus         494           S.scofa         486           G.gallus         D.rerio         507	IH7         YMHAIYSSIPLLETV         YMHALYSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSSAPLLESV         YMHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAICSNETVEM         YHAISNEW         YHAISNEW         YHAISNEW         YHAISNEW         YIHYNSRDVRMVEM         YHAISNEW         YHAISNEW      <	SC WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SC - WT LGGLALAQVLFFYV SY - WALGGVALAQVLFFYV DSVFWQH-SAGYFMGQFFYV DSVFWQH-SAGYFMGQFFY LGGSQHGLLGTLFSTAMTFA VGGSQHGLLGTLFSTAMTFA GGSQHGLLGTLLSTATTFA VGGSQHGLLGTLLSTATTFA TM1 AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSMLILSNLVFLC AALASCSTSALISNLVFLC AALASCSTSALISNLVFLC AALASCSTSALISNLVFLC AALASCSTAFLISNLFLC AALASCSTAFLISNLFLC	M8 (KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG KYLVLFGVPALLMRLDG (KYLVLFGAVSLLVRLDG (YVVVYGIGALLVRLDG YVVVYGIGALLVRLDG FVSYMHGGYDYLWCWAA FVSYWHGGYDYLWCWAA FVSYWHGCHYLVVLWWAA FVSYWHGCHYLVVLIWSI FVFVFHGCHYLLYWAL FVFVHGCYYVLIWSI GNEVGKTYWNRIFIQGW GNEVGKTYWNRIFIQGW GNHVGRIFWNRIFIQGW GNHVGRIFWNRIFIQGW GONFGRIFWKLFVQDW GQEIGDFLMRGAYLSGV	IH8         LTPPALPRCVSTMFSFTGMWRYF       338         LTPPPLPRCVSTMFSFTGMWRYF       338         LRPPLPRCVSLMFSFTGMWRYF       338         LPPTLPRCVSLMFSFTGMWRFF       357         IPAPRCVSLMFSFTGMWRFF       378         IPAPRCVSLMFSFTGMWRFF       378         IPAPNRFCUGR       IFF CMWRFF         IPAPNRFRCUGR       IFF CMWRFF         IPAPNRFRCUGR       IFF CMWRFF         IPAPNRFRCUGR       IFF CMWRFF         INWLGVTVESGVRRLUETPCURE       4110         LNWLGVTVESGVRRLUQKPSIQD       413         INWLGVTVESGVRRLUQKPSIQD       413         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVREUQE         INWLGVTVE       SOVE         INWLGVTVE       SOVE         INWLGVTVE       SOVE         INWLGVTVE       SOVE         INWLGVTVE       SOVE

**Figure S8. Sequence alignment of HHAT enzymes from various species.** Secondary structural elements determined from the structure are positioned above the alignment, with helices shown as bars and loop regions as lines. Amino acids that when mutated to alanine dramatically reduced activity (Glu59, Cys324, Asp339 and His379) are denoted with black rectangles. The archway is labeled with brackets.



**Figure S9. Relative enzymatic activity of the indicated mutants**. His428 and Arg403 are located on the walls of the archway and contact palmitoyl-CoA there. Arg404 faces away from palmitoyl-CoA.



Figure S10. The heme is exposed to the membrane and a component of purified HHAT. (A) The molecular surface of HHAT shown from the side and colored according to electrostatic potential (light grey regions are neutral; red,  $-10 \text{ kT e}^{-1}$ ; blue,  $+10 \text{ kT e}^{-1}$ ). The heme is shown in stick representation. Approximate boundaries of the membrane are indicated by horizontal bars. The porphyrin ring of the heme is nearly co-planar with the central iron atom, indicative of a Fe(III) oxidation state (*51*). Its two propionate groups are oriented toward the cytosolic side where they form direct and water-mediated hydrogen bonds with Arg 250 and Arg 323, respectively (Fig. 3B). Although the heme does not contact the palmitoyl-CoA substrate, only a thin wall of protein separates it from the end of its acyl chain. (**B**) Biochemical assay showing that 15  $\mu$ M purified HHAT contains approximately 18  $\mu$ M heme (refer to Methods). 15  $\mu$ M bovine serum albumin (BSA), used as a control, contains less than 0.1  $\mu$ M heme. (**C**) Snapshot of an atomistic molecular dynamics simulation of HHAT in a lipid membrane, showing that the heme (red sticks) is in contact with lipid molecules (acyl chains shown as semi-transparent sticks; phosphate moieties as spheres). Regions of HHAT within the membrane's hydrophobic core are cyan; solvent-exposed regions are magenta. Two orthogonal perspectives are shown.



**Figure S11. Thermal stability analyses of wild type and C324V HHAT.** Purified wild type HHAT (**A**) or C324V HHAT (**B**) enzymes were incubated at the indicated temperatures and analyzed by SEC, measuring tryptophan fluorescence. The elution volume for properly folded HHAT is indicated by an arrow. Vertical lines indicate the region used to calculate the areas under the curves. (**C**) Quantification of folded protein following incubation at the indicated temperatures, determined from the areas under the curves indicated in (A) and (B).



**Figure S12. Density for the structure of HHAT with the palmitoylated peptide product.** (A) Densities (semi-transparent surface renderings) for indicated regions are shown in the context of the atomic model (sticks). (B) Angular distribution of particles used in the final cryo-EM reconstruction. (C) Gold-standard FSC curve of the final reconstruction. (D) Map-to-model correlation curve. (E) Local map resolution estimation, colored as indicated (calculated using CryoSPARC V2).



**Figure S13. Rearrangement of Trp335 and concomitant rearrangements of Met334 and Asn392 between the substrate and product complexes.** (**A**, **B**) Close up views of the HHATsubstrate complex (A) and HHAT-palmitoylated product complex (B) in the vicinity of Trp335, showing the conformations of Met334 and Asn392. A dashed line indicates a hydrogen bond.

A		Signal Peptide	
H.sapines M.musculus	1	MLLLARC	
S.scrofa G.gallus	1	I M D EM L L L A <mark>R</mark> C L L V M L I S S L L C 22 I	
D.rerio D.melanogaster	1 1	I	
H.sapines M.musculus S.scrofa G.gallus D.rerio D.melanogaster	20 21 23 23 20 81	Shhn — → 9 SG LAC GP GR GF GK R H P K K L T P LA Y K Q F I P N V A EKT L G A S GR Y EG K I S R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 99 9 G L A C G P G R G F G K R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 102 5 G L A C G P G R G F G K R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 102 5 S G L A C G P G R G F G K R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 102 5 S G L T C G P G R G I G K R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 102 5 S G L A C G P G R G Y G R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 102 9 S G L A C G P G R G Y G R R H P K K L T P L A Y K Q F I P N V A EKT L G A S G R Y EG K I T R N S E R F K E L T P N Y N P D I I F K D E ENT G A D R L M T 199 P A H S C G P G R G L G R H P - A R N L Y P L V L K Q T I P N L S EY T N S A S G P L EG V I R H D S P K F K D L V P N Y N R D I L F R D E G T G A D R L M S 155 1 S S L A C G P G R G L G R H R - A R N L Y P L V L K Q T I P N L S EY T N S A S G P L EG V I R H D S P K F K D L V P N Y N P D I L F R D E G T G A D R L M S 155 1 S S L A C G P G R G L G R H R - A R N L Y P L V L K Q T I P N L S EY T N S A S G P L EG V I R H D S P K F K D L V P N Y N P D I L F R D E G T G A D R L M S 155 1 S S L A C G P G R G L G R H R - A R N L Y P L V L K Q T I P N L S EY T N S A S G P L EG V I R R D S P K F K D L V P N Y N P D I L F R D E G T G A D R L M S 155 1 S S L A C G P G R G L G R H R - A R N L Y P L V L K Q T I P N L S EY T N S A S G P L EG V I R R D S P K F K D L V P N Y N P D I L F R D E G T G A D R L M S 155 1 S S L A C S P S S S S S S S S S S S S S S S S S	) 22
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H.sapines M.musculus S.scrofa G.gallus D.rerio D.melanogaster	260 261 263 263 260 320	P R E R LL LT A A H L L F V A P HND S A T G E P E A S S G S G P P S G G A L G P R A L F A S R V R P G Q R V Y V V A E R D G D R R L L P A A V H S V T L S E 33 P R E R L L LT A A H L L F V A P HND S G P T P G G P S A L F A S R V R P G Q R V Y V V A E R G G D R R L P A A V H S V T L R E 32 P R E R L L LT A A H L L F V A P HNN S A AWE P E A L S G A G R P P G G A P G R R A L F A S R V R P G Q HV Y V A E R G G D R R L P A A V H S V T L R E 342 P R A R L L LT A A H L L F V A P HNN S A AWE P E A L S G A G R P P G G A P G R R A L F A S R V R P G Q HV Y V A E R G G D R R L P A A V H S V T L R E 342 P R A R L L LT A A H L L F V A P HNN S A WE P E A L S G A G R P T G G A G A G A F A S R V R P G Q R VY V L G E - G G Q Q L L P A S V H S V T L R E 342 P V E K I T LT A A H L L F V A P ( HN S T E D L T G S T S G Q A L F A S N K P G Q R VY V L G E - G G Q Q L L P A S V H S V S L R E 322 P V E K I T LT A A H L L F V L D N ST E D L H T MT A A Y A S S V R A G Q K V M V V D S G Q L K S V I V Q R I Y T E 319 	) ; ; ; ; ; ; ; ; ; ; ; ;
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<b>B</b> Sonic hedgehog Indian hedgehog Desert hedgehog	1 1 2 1	I MLLLAR CLLLVLVSSLLVCSGLACGPGRG- FG- KRRHPKKLTPLAYKQFIPNVAEKTLGASGRYEGKISPNSER 72 I MSPARLRPRLHFCLVLL LLLVVPAAWGCGPGRV- VGSRRRPPRKLVPLAYKQFSPNVPEKTLGASGRYEGKIARSSER 77 I MALLTNLLPLCCLA LLALPAQSCGPGRGPVGRRRYARKQLVPLLYKQFVPGVPERTLGASGPAEGRVARGSER 73	
Sonic hedgehog Indian hedgehog Desert hedgehog	73 378 374	EFKELTPNYNPDIIFKDEENTGADRLMTQRCKDKLNALAISVMNQWPGVKLRVTEGWDEDGHHSEE <mark>SLHYEGR</mark> AVDI <mark>TTS</mark> D 15 EFKELTPNYNPDIIFKDEENTGADRLMTQRCKDRLNSLAISVMNQWPGVKLRVTEGWDEDGHHSEE <mark>SLHYEGR</mark> AVDI <mark>TTS</mark> D 15 EFRDLVPNYNPDIIFKDEENSGADRLMTERCKERVNALAIAVMNMWPGVRLRVTEGWDEDGHHAQD <mark>SLHYEGR</mark> ALDITTSD 15	273
Sonic hedgehog Indian hedgehog Desert hedgehog	153 158 154	R D R S K Y GM L A R L A V E A G F D W V Y Y E S K A H I H C S V K A E N S VA A K S G G C F P G S A T V H L E Q G G T K L V K D L S P G D R V L A A D D Q G R 23 R D R N K Y G L L A R L A V E A G F D W V Y Y E S K A H V H C S V K S E H S A A A K T G G C F P A G A Q V R L E S G A R V A L S A V R P G D R V L A M G E D G S 23 R D R N K Y G L L A R L A V E A G F D W V Y Y E S R N H V H V S V K A D N S L A V R A G G C F P G N A T V R L W S G E R K G L R E L H R GD W V L A A D A S G R 23	273
Sonic hedgehog Indian hedgehog Desert hedgehog	233 238 234	L L Y <mark>S</mark> D F L T F L <mark>D R</mark> D G A K K V F Y V I <b>E T</b> R E <mark>P</mark> R E <mark>R</mark> L L L <mark>T</mark> A A H L L F V A P H N D S A T <mark>G</mark> E P E A S S G S G P P S G G A L G P R A L F A S <mark>R V R P G</mark> 31 P T F S D V L I F L D R E P H R L R A F Q V I E T Q D P P R L A L T P A H L L F T A D N H T E P A A R F R A T F A S H V Q P G 30 V V P T P V L L F L D R D L Q R R A S F V A V E T E W P P R K L L L T P WH L V F A A R G P A P A P G	2
Sonic hedgehog Indian hedgehog Desert hedgehog	313 302 3298	Q R V Y V V A ER DG DR R L L <mark>P</mark> A A V H S V T L S E EA A <mark>G</mark> A Y A P L T A Q <mark>G T I</mark> L I N R V L A <mark>S</mark> C Y A V I E E H SWA H R A F A P F R L A H A L L A A L A P 39 Q Y V L V A <mark>G</mark> V P G L Q P A R V A A - V S T H V A L G A Y A P L T K H GT L V V E D V V A <mark>S C</mark> F A A V A D H H L A Q L A F WP L R L F H S L A WG 37 D S V L A P <mark>G</mark> G D A L R <mark>P</mark> A R V A R - V A R E E A V <u>G</u> V F A P L T A H GT L L V N D V L A <mark>S C</mark> Y A V L E S H Q WA H R A F A P L R L H A L G A L L P G 37	23
Sonic hedgehog Indian hedgehog Desert hedgehog	393 374 373	ART DR GGD S GGGD R GG GG G R V A LT AP GA A DA P GA GA T A G I HWY SQ LLYQ I G T W LL D S E A LH P L GM A V K S 5         46	2

**Figure S14. Sequence alignments of Hedgehog proteins.** (A) Sequence alignment of Sonic Hedgehog proteins from the indicated species. (B) Alignment of human Sonic, Indian, and Desert Hedgehog proteins. A black rectangle indicates the N-terminal CGPGR sequence motif that is crucial for substrate recognition by HHAT.



**Figure S15. Proposed mechanism of HHAT. (A)** Palmitoyl-CoA enters the reaction chamber from the cytosolic leaflet of the membrane by passing through the archway and then binds as a substrate deep within the enzyme. The amino terminal end of Hedgehog enters from the aqueous environment of the ER lumen. After palmitoyl transfer, the CoA byproduct is released into the cytosol and fully mature Hedgehog is released to the lumen. (**B**) Proposed chemical mechanism. Asp339 acts as a general base to activate the amino terminal end of Hedgehog for nucleophilic attack on the carbonyl carbon, thereby releasing CoA as the leaving group and forming the amide linkage of the fully mature Hedgehog product.



**Figure S16. Substrate access routes for HHAT compared with lipid-modifying MBOATs.** (A-C) Overall structure of HHAT (A) in comparison to the dimeric forms of ACAT1 (B) and DGAT1 (C) (*16-20*). TM9-TM11 of HHAT (A) is colored blue; the palmitoyl-CoA molecule in the archway is indicated. ACAT1 (B) and DGAT1 (C) do not possess an analogous archway. Rather, openings between transmembrane helices denoted by dashed triangles may serve as entry routes for their acyl-CoA substrates (*16-20*). (**D-G**) The active site of HHAT in comparison to the active sites of lipid-modifying MBOAT enzymes (*15-20*). Slices of the molecular surfaces highlight differences in their substrate binding cavities. Arrows indicate the location of the hallmark histidine residue in each enzyme. Monomers of ACAT1 and DGAT1 are shown.

	Palmitoyl-CoA substrate	Hedgehog peptide product
	complex	complex
Data Collection		
Microscope	FEI Titan Krios-MSKCC	FEI Titan Krios-NYSBC(3)
Camera	Gatan K3	Gatan K3
Magnification	22,500	20,250
Voltage (kV)	300	300
Electron expose (e <sup>-</sup> /Ų)	26	53
Defocus range (μm)	-1.0 to -1.5	-1.2 to -2.2
Pixel size (Å)	1.064	1.083
Symmetry imposed	C1	C1
Initial particle images (No.)	1,870,224	3,579,529
Final particle images (No.)	174,058	142,121
Map Resolution (Å)	2.7	3.2
FSC threshold (0.143)		
Refinement		
Initial model used	De novo	Pal-CoA substrate complex
Model Resolution (Å)	2.8	3.5
FSC threshold (0.5)		
Model composition		
Non-hydrogen atoms	7904	6189
Protein residues	912	723
Ligands	18	15
Waters	15	0
Mean <i>B</i> factor (Ų)		
Protein	43.9	110.64
Ligands	61.2	134.91
Water	32.1	-
R.m.s. deviation		
Bond lengths (Å)	0.007	0.004
Bond angles (°)	0.843	0.668
Validation		
Clashscore	8.09	8.12
Rotamers outlier (%)	0.54	0
Ramachandran plot		
Favored (%)	96.7	96.6
Allowed (%)	3.3	3.4
Disallowed (%)	0	0

Table S1. Cryo-EM data collection and model refinement statistics.

**Movie S1. Molecular dynamics simulation of HHAT in a lipid membrane.** The depiction is as in fig. S10C, showing the heme (red sticks) in direct contact with lipid molecules. Lipid molecules are represented as semi-transparent sticks for their acyl chains and spheres for their phosphate moieties. Regions of HHAT that reside within the hydrophobic core of the membrane are colored cyan whereas solvent-exposed regions are magenta.

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