Table SI. Evidence grading for proposed enzyme-substrate relationships in S-palmitoylation.

Substrate and modified site	**Proposed ZDHHC enzyme/depalmitoylase**	**Cell type and disease context**	**Palmitoylation detection method**	**Endogenous loss-of-function evidence**	**Rescue using wild-type versus catalytically inactive ZDHHC**	**Functional rescue using a palmitoylation-deficient substrate mutant**	***In vivo* immune validation**	**Current therapeutic development stage**
Fyn (Cys3, Cys6; Cys3 predominant)	DHHC21/not reported	$CD4^+$ T cells; TCR signaling	ABE	ZDHHC21 knockdown decreased Lck and Fyn palmitoylation in EL-4 murine T cells. Zdhhc21 conditional knockout mice (Zdhhc21 cKO) shows loss of Fyn palmitoylation and mislocalization.	Not reported	Not reported	Not reported	Broad mechanistic tool
Lck (Cys3, Cys5)	DHHC21/not reported	$CD4^+$ T cells; TCR signaling	Acyl-RAC	ZDHHC21 knockdown decreased Lck palmitoylation; ZDHHC21 knockdown inhibited Fas-mediated phosphorylation of Lck, ZAP-70 and LAT.	Not reported	Not reported	Not reported	Broad mechanistic tool
ZAP-70 (Cys564)	Not identified/not reported	$CD4^+$ Jurkat T cells $CD4^+$ Jurkat T cells and primary human $CD4^+$ T cells; TCR signaling	ABE	Not reported	Not reported	WT ZAP-70 rescued TCR signaling in P116 ZAP-$70^{-/-}$ Jurkat cells; C564R/C564S failed. C564R	Not reported	Broad mechanistic tool

						retained *in vitro* SLP-76 kinase activity but showed prolonged Lck interaction and increased Y319/Y493 phosphorylation.		
LAT (Cys26, Cys29; Cys26 dominant)	Not identified/not reported	$CD4^+$ Jurkat T cells; TCR signaling	ABE	Lack of LAT palmitoylation leads to anergic T cells.	Not reported	Not reported	Not reported	Broad mechanistic tool
CD4 (Cys396, Cys399)	Not identified/not reported	$CD4^+$ T cells; TCR signaling	Not reported	Palmitoylation of membrane-proximal Cys396 and Cys399 of CD4 and association with Lck contribute to CD4 enrichment in lipid rafts.	Not reported	Not reported	Not reported	Broad mechanistic tool
CD8β (cytoplasmic tail)	Not identified/not reported	$CD8^+$ T cells (human and murine); TCR signaling	Not reported	CD8 is palmitoylated at the cytoplasmic tail of CD8β; this allows partitioning of CD8αβ, but not CD8αα, in lipid rafts. Cytoplasmic portion of CD8β, mainly due to its palmitoylation, mediates partitioning of CD8 in lipid rafts where it associates	Not reported	Not reported	Not reported	Broad mechanistic tool

				with p56(Lck).				
CD80 (Cys261, Cys262, Cys266, Cys271)	ZDHHC20/not reported	293T, CHO-K1, Raji B-lymphoma; T cell co-stimulation	Metabolic labeling, BioID, co-IP, CHX chase, click chemistry, lysosomal/proteasomal inhibition	Not reported for zDHHC20 KO/KD; 2-BP and tunicamycin reduce CD80 palmitoylation/membrane localization.	WT zDHHC20 increases CD80 palmitoylation; C156S mutant fails (overexpression assay).	CD80 4CS mutant: Loss of palmitoylation, reduced stability, impaired surface localization, loss of costimulatory function.	Not reported	*In vitro* pharmacological validation
H-Ras, N-Ras (not reported)	Not identified/not reported	293T; Ras signaling pathway	Metabolic labeling/click chemistry, FRET, live-cell imaging	Not reported	Not reported	Not reported	Not reported	Broad mechanistic tool
TRAF6 (not directly palmitoylated; ZDHHC11 interacts)	ZDHHC11/not reported	293T, MEF, BMDM; NF-κB activation, inflammation	Not reported	DHHC11 knockdown or knockout decreases IL-1β/LPS/HSV-1-induced NF-κB activation; Zdhhc11$^{-/-}$ mice show lower serum IL-6 after LPS/D-gal or HSV-1.	WT ZDHHC11 re-expression rescues ZDHHC11 knockdown; catalytically inactive mutants still activate NF-κB, indicating palmitoyltransferase activity is dispensable.	Not applicable (TRAF6 not palmitoylated)	Zdhhc11$^{-/-}$ mice infected with HSV-1 or treated with LPS/D-gal	Genetic proof of concept
STAT3 (Cys108)	DHHC7, DHHC3/APT2	293T, mouse splenocytes; Th17 differentiation, IBD	Click chemistry; ABE	DHHC7 KO decreases STAT3 palmitoylation; Zdhhc7 KO reduces Th17 differentiation	WT DHHC7 but not DHHS7 mutant restores STAT3 palmitoylation in	C108S mutant reduces phosphorylation and Th17 differentiation	DSS-induced colitis in C57BL/6J mice; human IBD PBMCs	Genetic proof of concept; *in vitro* pharmacological validation

				and STAT3 phosphorylation; APT2 inhibition increases palmitoylation but impairs nuclear translocation.	DHHC7-KO cells			
FoxP3 (Cys204, Cys218, Cys280, Cys281, Cys424)	DHHC2, DHHC3, DHHC7/not reported	293T, mouse Tregs; Treg function, melanoma	Mass spectrometry, ABE, Acyl-RAC, co-IP	$Dhhc2^{-/-}$, $Dhhc3^{-/-}$, $Dhhc7^{-/-}$; T cell-specific Dhhc2 KO decreases Foxp3 in Tregs and suppresses tumor growth.	Not reported	Not reported	YUMM3.3 melanoma syngeneic model with T cell-specific Dhhc2 deletion	Genetic proof of concept; immune-competent animal validation
PD-1 (Cys192)	Not identified/not reported	Human colorectal cancer RKO, HCT116; melanoma A375; liver cancer HepG2	Click-iT , co-IP	siDHHC9 decreases PD-1 palmitoylation and protein level	Not reported	C192S disrupts PD-1-Rab11 binding and accelerates degradation. Replacing Cys192 with serine blocks PD-1 palmitoylation .	Not reported	*In vitro* pharmacologic al validation
TIM-3 (Cys296)	DHHC9/not reported	Human T cells; T cell exhaustion	ABE/metabolic labeling	DHHC9 knockdown attenuates CAR-T cell exhaustion; ZDHHC9 inhibitors restore cytotoxic activity of CAR-T cells and NK cells.	Not reported	Not reported	Clinical analysis showed that ZDHHC9 expression positively correlates with TIM-3	*In vitro* pharmacologic al validation

							levels in tumor-infiltrating $CD8^+$ T cells and NK cells in patients with HCC, and simultaneous high expression of both is associated with poorer patient prognosis.	
TNFR1 (Cys248)	Not identified/APT2	Human monocytic U937 cells; TNF signaling	Acyl-RAC; 17-ODYA metabolic labeling + click chemistry	APT2 knockdown inhibits TNF-R1 depalmitoylation, increases surface expression, enhances apoptosis and blocks NF-κB activation.	Not reported	C248S impairs receptor delivery and signaling. C248 mutation interferes with TNFR1 localization to the plasma membrane, thereby affecting normal signal transduction.	Not reported	Broad mechanistic tool
Fas (Cys199)	DHHC7/not reported	Human colorectal adenocarcinoma SW480,	ABE assay, co-IP, siRNA knockdown	DHHC7 knockdown reduces Fas S-palmitoylation. Knockdown of	WT DHHC7 overexpression increases Fas palmitoylation;	C199V mutant reduces stability. Mutation of	Not reported	Broad mechanistic tool

		SW620; colorectal cancer		ZDHHC7 by siRNA promotes colorectal cancer cell lines to evade FasL-induced cell death.	C160S mutant fails to do so; no classical knockdown-rescue performed.	the Fas palmitoylation site reduces its lipid raft association and impairs apoptotic signaling.		
FasL (Cys82)	Not identified/not reported	Human WSU B cells, COS-7; T cell apoptosis	Fluorescence correlation spectroscopy, ABE	Not reported	Not reported	FasL C82S impairs cell death induction, ADAM10 cleavage and raft localization.	Not reported	Broad mechanistic tool
PD-L1 (Cys272)	ZDHHC9 (breast cancer), ZDHHC3 (colorectal cancer)/not reported	Human breast cancer cells; colorectal cancer RKO, HCT116; breast cancer, colorectal cancer	Acyl-RAC, ABE	ZDHHC9 KO reduces mPD-L1 palmitoylation in 4T1; PD-L1 C272A abolishes palmitoylation and reduces stability; ZDHHC3-mediated PD-L1 S-palmitoylation prevents its monoubiquitination and blocks degradation via the ESCRT pathway and lysosomes.	Not reported	PD-L1 C272A: abolishes palmitoylation, reduces stability/surface expression, enhances T-cell killing, impairs tumor growth in mice.	BALB/c mice with 4T1 tumors: C272A impairs tumor growth; mZDHHC9 KO inhibits tumor growth and improves anti-mPD-L1 therapy; MC38 tumor-bearing C57BL/6	Immune-competent animal validation

							mice with benzosceptrin C.	
B7-H3 (Cys496)	ZDHHC24/not reported	Human CRC (RKO, SW480), murine colon cancer (CT26, MC38), 293T; MSS CRC; CAC mouse model	ABE, CHX chase, co-IP	Zdhhc24$^{\Delta IEC}$ mice (CAC model). Knockout of Zdhhc24 in a CAC mouse model enhances CD8^{+} T cell responses.	Not reported	C496S mutant restores autophagic degradation (abolishes p62-disrupting effect)	CAC mouse model; subcutaneous CT26/MC38 in BALB/c and C57BL/6J	Immune-competent animal validation
B7-H4 (Cys130)	ZDHHC3/not reported	Human breast cancer cells (MDA-MB-468, T-47D, SK-BR-3, MCF7), mouse breast cancer cells (4H11, Py230, MMTV-PyMT), human ovarian cancer OVCAR3, mouse colorectal cancer CT26, 293T; Breast cancer, ovarian cancer, colorectal	ABE, Click-iT, LC-MS/MS	shZDHHC3 in 4H11 reduced B7-H4, inhibited tumor growth, enhanced CD8^{+} T cells	Not reported	The C130A mutant (palmitoylation-deficient) showed markedly lower input protein levels than the wild-type group, confirming C130 as a B7-H4 palmitoylation site.	B7-H4 KO and ZDHHC3 KD in immune-competent mice; abemaciclib in MMTV-PyMT and CT26-B7-H4-OE; CD8 depletion confirmed	Immune-competent animal validation

		cancer.						
IFNGR1 (Cys122)	Not identified/not reported	Human colorectal cancer LS174T, DLD1; murine MC38, CT26; Colorectal cancer	Click-iT	No ZDHHC loss-of-function. Optineurin deficiency increases IFNGR1 palmitoylation and accelerates lysosomal degradation.	Not reported	IFNGR1 C122A abolished palmitoylation, reduced AP3D1 interaction, enhanced stability, decreased lysosomal localization.	Syngeneic MC38/CT26; AOM/DSS model with optineurin$^{\Delta IE C}$	Immune-competent animal validation
TLR9 (not specified)	ZDHHC3 / PPT1	Murine RAW264.7, 293T, Flt3L-derived pDCs, BMDM; SLE	Click chemistry , ABE, mass spectrometry	PPT1 deficiency or HDSF inhibition	Not a classical rescue. WT DHHC3 palmitoylates TLR9; catalytic mutant C157S does not in overexpression. No DHHC3-deficient rescue with WT vs mutant.	TLR9 C258A/C265A (Mut2) reduces palmitoylation and weakens cleaved TLR9; no full functional rescue reported.	B6.Sle1yaa SLE mouse model; human SLE pDCs *ex vivo*. PPT1 aggravates disease by removing TLR9 palmitoylation.	Immune-competent animal validation
TLR2 (Cys609)	Not identified/not reported	Dendritic cells	Metabolic labeling	Not reported	Not reported	Not reported	Not reported	Broad mechanistic tool
CD36 (not specified)	DHHC5/APT1	Murine 3T3-L1 adipocytes, primary adipocytes; high-fat diet	Metabolic labeling	Not reported	Not reported	Not reported	High-fat-diet-induced obesity models	Broad mechanistic tool
FASN	ZDHHC20/not	Human HCC	LC-MS/MS, ABE,	Zdhhc20$^{-/-}$ mice show	Not reported	FASN	DEN/CCl4-	Genetic proof

(Cys1471, Cys1881)	reported	Huh7, HCCLM3, Hep3B, HepG2; DEN/CCl4 mouse HCC	IP-ABE	reduced hepatocarcinogenesis; ZDHHC20 knockdown decreases FASN palmitoylation and protein stability, increases ubiquitination.	C1471S/C1881S mutants show decreased stability, increased ubiquitination, reduced proliferation and tumor growth.	induced HCC mouse models	of concept

ZDHHC, zinc finger DHHC-type palmitoyltransferase; CD, cluster of differentiation; TCR, T cell receptor; ABE, acyl-biotin exchange; cKO, conditional knockout; WT, wild-type; Acyl-RAC, acyl-resin-assisted capture; ZAP-70, zeta-chain-associated protein kinase 70; LAT, linker for activation of T cells; BioID, proximity-dependent biotin identification; co-IP, co-immunoprecipitation; CHX, cycloheximide; 2-BP, 2-bromopalmitate; KO, knockout; KD, knockdown; FRET, fluorescence resonance energy transfer; 4CS, quadruple cysteine-to-serine mutant; TRAF6, TNF receptor-associated factor 6; NF-κB, nuclear factor κB; MEF, mouse embryonic fibroblast; BMDM, bone marrow-derived macrophage; HSV-1, herpes simplex virus 1; LPS, lipopolysaccharide; D-gal, D-galactosamine; IL, interleukin; Th17, T helper 17; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; PBMCs, peripheral blood mononuclear cells; APT2, acyl-protein thioesterase 2; DHHC, aspartate-histidine-histidine-cysteine; TNFR1, tumor necrosis factor receptor 1; TNF, tumor necrosis factor; 17-ODYA, 17-octadecynoic acid; HCC, hepatocellular carcinoma; NK, natural killer; siRNA, small interfering RNA; FasL, Fas ligand; FCS, fluorescence correlation spectroscopy; ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; PD-L1, programmed death-ligand 1; ESCRT, endosomal sorting complex required for transport; CAC, colitis-associated cancer; MSS CRC, microsatellite stable colorectal cancer; LC-MS/MS, liquid chromatography-tandem mass spectrometry; shRNA, short hairpin RNA; OE, overexpression; IFNGR1, interferon γ receptor 1; AP3D1, adaptor related protein complex 3 subunit δ1; AOM/DSS, azoxymethane/dextran sulfate sodium; optineurinΔIEC, intestinal epithelial cell-specific optineurin deletion; TLR9, Toll-like receptor 9; PPT1, palmitoyl-protein thioesterase 1; Flt3L, FMS-like tyrosine kinase 3 ligand; pDCs, plasmacytoid dendritic cells; SLE, systemic lupus erythematosus; Mut2, double mutant.