LIST OF TEN BEST PUBLICATIONS FROM THE LAB

(In order of importance, *denotes corresponding author)

1. Chakraborty, A., **Kamat, S. S.*** (2024) Lysophosphatidylserine: a signaling lipid with implications in human diseases, ***Chemical Reviews*** 124 (9), 5470-5504.
   Featured on the May 2024 issue front cover of Chemical Reviews.

   Importance: Chemical Reviews invites very limited exhaustive articles on topics that are considered very contemporary and of interest to the chemistry and/or chemical biology community. Additionally, the lead author invited to contribute an article, is considered to be a leader of the field and/or should have made substantial contribution to the field in which the review is being written. Given our lab's contributions towards mapping the biological pathways associated with lysophosphatidylserine (lyso-PS) metabolism and signaling over the past decade, we were invited to write an up to date, comprehensive and authoritative review on lyso-PS lipids. In this review, we discuss our lab's contributions towards annotating functions to lipases associated with lyso-PS metabolism, and various pharmacological studies that have led to the identification of signaling pathways modulated by lyso-PS in different immunological contexts.

2. Kelkar, D. S., Ravikumar, G., Mehendale, N., Singh, S., Joshi, A., Sharma, A. K., Mhetre, A., Rajendan, A., Chakrapani, H., **Kamat, S. S.*** (2019) A chemical genetic screen identifies ABHD12 as an oxidized phosphatidylserine lipase, ***Nature Chemical Biology*** 15, 169-178.

   Importance: Oxidative stress is an imbalance between cellular oxidants and antioxidants in favor of the oxidants, resulting in increased cellular ROS levels, that disrupts redox signaling pathways, and is implicated in several human diseases. Phosphatidylserine (PS), an inner membrane leaflet localized phospholipid has several critical functions in mammalian biology, especially its role in ROS sensing and apoptosis. In response to elevated cellular ROS, PS flips its membrane orientation, and while a lot is known of the externalization of PS under oxidative stress, nothing was known of the chemical structure of oxidized PS lipids and enzymes that metabolize such lipids. In this paper, using pharmacological tools in conjunction with genetic mouse models, we report for the first time the chemical structures of oxidized PS lipids, and by performing a chemical genetic screen, showed that the enzyme ABHD12 is a major oxidized PS lipase that metabolizes these oxidatively damaged PS lipids under oxidative stress conditions. Following our findings, inhibitors to ABHD12 as clinical candidates for killing cancer cells by the ferroptosis pathway, and ABHD12's role in resolving (neuro)inflammation are being assessed.

3. Khandelwal, N., Shaikh, M., Mhetre, A., Singh, S., Sajeevan, T., Joshi, A., Balaji, K. N., Chakrapani, H., **Kamat, S. S.*** (2021) Fatty acid chain length drives lysophosphatidylserine dependent immunological outputs, ***Cell Chemical Biology*** 28, 1169-1179.
   Featured on August 2021 issue front cover of Cell Chemical Biology

   Importance: The lysophosphatidylserines (lyso-PSs) are immunomodulatory signaling lipids that regulate many facets of mammalian physiology. However, studying their biological effects has been relatively challenging given their commercial paucity and limited synthetic routes in making them. To overcome these challenges, in this paper, we report a facile synthetic method in making lyso-PS lipids, and assessed the biological pathways that they are known to regulate as a function of the lipid tail esterified to them. We found very-long chain lyso-PS are preferred substrates for the lyso-PS lipase ABHD12, and they robustly elicit pro-inflammatory responses via a TLR2 dependent pathway. Interestingly, we also found that long chain lyso-PSs signal through GPCRs, and modulate signaling

events that are important for macrophage activation and mast cell degranulation. This is the first rigorous structure activity relationship study performed for this important lysophospholipid class, particularly in the context of important immunological processes. Our synthetic strategy has now paved the way for making biorthogonal multi-functional probes for the identification of the receptors and/or protein ligands for lyso-PS lipids.

4. Mehdiratta, K., Singh, S., Sharma, S., Bhosale, R. S., Choudhary, R., Masal, D. P., Manocha, A., Dhamale, B. D., Khan, N., Vivekanand, A., Sharma, P., Ikeh, M., Brown A. C., Parish, T., Ojha, A., Michael, J. S., Faruq, M., Medigeshi, G. R., Mohanty, D., Reddy, D. S., Natarajan, V. T., **Kamat, S. S.***, Gokhale, R. S.* (2022) Kupyaphores are zinc homeostatic metallophores required for colonization of *Mycobacterium tuberculosis*, ***PNAS*** 119(8), e2110293119.

   Importance: It has been known for many years that *Mycobacterium tuberculosis* (MTB) needs optimal concentrations of metals such as iron, zinc, for survival under stressful conditions. However, the pathways and/or secondary metabolites that regulate this, remained unknown till recently. Using a combination of genetic models and stress conditions developed by Rajesh Gokhale' lab, and leveraging advanced lipidomics/metabolomics technologies, our lab identified and elucidated the chemical structures of a set of secondary metabolites, we termed "kupyaphores", that are able to regulate zinc homeostasis in MTB. This study shows for the first time, a need for zinc by MTB, and uncovers a previously unknown zinc acquisition strategy by pathogenic microbes that can modulate host-pathogen interactions and disease outcomes.

5. Joshi, A., Shaikh, M., Singh, S., Rajendran, A., Mhetre, A., **Kamat, S. S.*** (2018) Biochemical characterization of the PHARC associated serine hydrolase ABHD12 reveals its preference for long chain lipids, ***J. Biological Chemistry*** 293, 16953-16963.
   Featured on the November 2018 issue front cover of JBC

   Importance: Deleterious (null) mutations to the ABHD12 gene results in the human neurological disorder PHARC, where very long chain (VLC) lipids, particularly lysophosphatidyserine lipids, accumulate in the brain. In this paper, we synthesize a library of mono-acyl-glycerol lipids with varying lipid tails, and show for the first time, that the lipase ABHD12 strongly prefers VLC lipids as substrates. Additionally, we also show that ABHD12 localizes to the endoplasmic reticulum membrane, where is regulates the secretion of VLC lipids, and that glycosylation of this lipase is important for its biochemical activities. Together our findings reported in this paper, provide the first explanation for why VLC lipids accumulate in the brains of human PHARC subjects, and result in the pathology observed in this neurodegenerative disease.

6. Rajendran, A., Soory, A., Khandelwal, N., Ratnaparkhi, G. S., **Kamat, S. S.*** (2022) A multi-omics analysis reveals that the lysine deacetylase ABHD14B influences glucose metabolism in mammals, ***J. Biological Chemistry*** 298 (7), 102128, 1- 14.
   [Please also refer to companion paper: Rajendran, A., Vaidya, K., Mendoza, J., Bridwell-Rabb, J., **Kamat, S. S.*** (2020) Functional annotation of ABHD14B, an orphan serine hydrolase enzyme, ***Biochemistry*** 59 (2), 183-196. Featured on the January 2020 issue front cover of Future of Biochemistry- Asia Pacific Issue]

   Importance: Until recently, the lysine deacetylase (KDAC) family was thought to comprise only of the sirtuins and the histone deacetylases. Using elegant biochemical assays along with chemical

proteomics and metabolomics approaches, we functionally annotate the orphan serine hydrolase enzyme ABHD14B as a novel KDAC, that is exclusively localized in mammalian liver, and plays an important role in central metabolism. Our annotation of ABHD14B has since expanded the repertoire of the KDAC family (*Biochemistry 2020*). Following up on this discovery, we have since shown that ABHD14B plays in systemic glucose metabolism during feeding-fasting cycles in animal and cell models. Further, we show that hepatic ABHD14B in particular is responsible for this systemic glucose metabolism, and it does so by regulating levels of key enzymes and transcriptional factors that as associated with central metabolism. Our studies designate ABHD14B as an important pharmacological target in the treatment of diabetes, metabolic syndrome and general obesity (*JBC 2022*).

7. Pathak, D., Mehendale, N., Singh, S., Mallik, R. M., **Kamat, S. S.*** (2018) Lipidomics suggests a new role for ceramide synthase in phagocytosis, ***ACS Chemical Biology***, 13, 2280-2287.
Featured on the August 2018 issue front cover of ACS Chemical Biology

   Importance: Phagocytosis is an evolutionary conserved innate immunological process, critical for clearing pathogens and fighting infections in higher organisms, including humans. While significant information is available on the proteomic content of the phagosomes as they mature, little remains known about their lipid composition. In this paper, using our in-house LC-MS based lipidomics platforms, we performed an exhaustive quantitative analysis of the different lipid classes during phagocytosis, and found that as phagosomes mature, they accumulate cholesterol and very-long chain (VLC) ceramides. We further showed that the accumulation of VLC ceramides is orchestrated by the enzyme ceramide synthase 2, and pharmacological inhibition of this enzyme hampers phagocytosis. This finding has tremendous physiological implications, as cholesterol and VLC ceramides form stable lipid rafts, that recruit dynein motors, which produce enough forces on these stable membranes to drive the unidirectional motion of maturing phagosomes to the lysosome for eventual degradation.

8. Mehendale, N., Mallik, R. M., **Kamat, S. S.*** (2021) Mapping sphingolipid metabolism pathways during phagosomal maturation, ***ACS Chemical Biology*** 16(12), 2757-2765.
Featured on the December 2021 issue front cover of ACS Chemical Biology

   Importance: Previously, we have shown that ceramides are important lipids during phagosomal maturation, and disruption in their metabolism can result in hampered phagocytosis. In this study, we specifically show that along with ceramide synthases, the various isoforms of ceramidases also have an important role to play in phagosomal maturation by regulating levels of ceramides during this process. Further, we discover that glucosylceramides, a kind of glycosylated ceramide, is also important during phagocytosis, and that its levels of this lipid are regulated by the enzyme glucosylceramide synthase during phagosomal maturation. Additionally, we show that all the ceramide biosynthetic pathways are “hot” targets for invading microbes, that have developed secondary metabolites to inhibit these enzymes, and by doing so, allows these microbes alternative strategies to evade our immune system.

9. Singh, S., Joshi, A., **Kamat, S. S.*** (2020) Mapping the neuroanatomy of ABHD16A-ABHD12 & lysophosphatidylserines provides new insights into the pathophysiology of the human neurological disorder PHARC, ***Biochemistry*** 59 (24), 2299-2311.

Importance: The human neurological disorder PHARC is marked by cerebellar dystrophy, and the accumulation of lysophosphatidylserine (lyso-PS) lipids are thought to cause this pathology. In this paper, we map the anatomical localization of ABHD16A (major lyso-PS biosynthetic enzyme in the brain) and ABHD12 (major lyso-PS degradation enzyme in the brain), and show that both these enzymes along with lyso-PS lipids are significantly enriched in the mammalian brain. Further, we show that ABHD16A and ABHD12 are localized to distinct neurons in the brain, and that lyso-PS acts as a hormone like signaling molecule that likely coordinates various cerebellar functions. Together, this study provides the first evidence, as to why the cerebellum is the most susceptible anatomical region in the brain and why it undergoes atrophy in human PHARC subjects.

10. Chandramouli, A., **Kamat, S. S.*** (2024) A facile LC-MS method for profiling cholesterol and cholesteryl esters in mammalian cells and tissues, ***Biochemistry*** (In Press, Online, https://doi.org/10.1021/acs.biochem.4c00160)
    To feature on the September 2024 issue front cover of Biochemistry

    Importance: Dysregulation in cholesterol metabolism is linked to numerous human diseases, and therefore, in vivo, the concentrations and flux of cholesterol and cholesteryl esters (fatty acid esters of cholesterol) are tightly regulated. While mass spectrometry has been an analytical method of choice for detecting cholesterol and cholesteryl esters in biological samples, the hydrophobicity, chemically inert nature, and poor ionization of these neutral lipids have often proved a challenge in developing lipidomics compatible liquid chromatography-mass spectrometry (LC-MS) methods to study them. To overcome this problem, in this paper, we report a reverse-phase LC-MS method that is compatible with existing high-throughput lipidomics strategies and capable of identifying and quantifying cholesterol and cholesteryl esters from mammalian cells and tissues. Using this sensitive yet robust LC-MS method, we profiled different mammalian cell lines and tissues and provide a comprehensive picture of cholesterol and cholesteryl esters content in them. Specifically, among cholesteryl esters, we find that mammalian cells and tissues largely possess monounsaturated and polyunsaturated variants. Taken together, our lipidomics compatible LC-MS method to study this lipid class opens new avenues in understanding systemic and tissue-level cholesterol metabolism under various physiological conditions.