FOR IMMEDIATE RELEASE

February 2, 2016

Sharpin emerges from the pack as a regulator of inflammation
Study reveals an unanticipated mechanism governing survival of immunosuppressive T cells

LA JOLLA, CA-- It is normal—in fact *necessary*—for our immune system to occasionally fly into an inflammatory rage to defend the host (us) against pathogens or even tumor cells. Problems arise when the rage persists or is re-directed against one's self, as occurs in autoimmune disease. The cellular peacemakers assigned to block this unruly behavior are a class of immunosuppressive T cells called "Tregs" (short for "regulatory T cells"). Factors required for Treg well-being are therefore of great interest to immunologists and clinicians alike.

One of those factors headlines a new paper published in the February 1, 2016, issue of *Nature Immunology* by La Jolla Institute for Allergy & Immunology (LJI) researcher Yun-Cai Liu, Ph.D. In it, Liu's team deduces a surprising activity of a once humble protein called Sharpin, based on analysis of T cell populations in mutant mice that lack it.

They, as others had before, report that Sharpin-deficient mice develop inappropriate inflammation. What is new is Liu's discovery that Sharpin-deficient mice harbor too few immunosuppressive "Tregs" due to loss of a previously uncharacterized cell survival mechanism orchestrated by Sharpin. That finding is significant to researchers seeking ways to manipulate immune responses, either down or up, as treatment for autoimmunity or cancer.

"Abnormal function of T lymphocytes called regulatory T cells is associated with self-destruction of multiple organs or tissues in mice and humans," says Liu, a professor in LJI's Division of Cellular Biology. "Previously, we knew that Sharpin loss was linked to autoimmune or inflammatory disease in mice. Here, we found that Sharpin is essential for generation of regulatory T cells, providing a mechanistic explanation for pathologies we see in Sharpin-mutant mice."

Soon after birth, Sharpin-mutant mice develop serious skin lesions and inflammation in multiple organs—even in the absence of infection. Using an experimental approach called flow cytometry to distinguish and count T cell subtypes in tissues like lung or gut, Liu's group observed a paucity of Tregs in all tissues from Sharpin-deficient mice, including the thymus, where Tregs originate.

The team then undertook a "rescue" experiment by transferring Tregs from normal mice (that is, mice with intact Sharpin) into mutant mice and observed that the manipulation

reduced severity of the inflammation. According to the study's co-author Hyung-seung Jin, Ph.D., formerly a postdoctoral fellow in the Liu lab and now a research scientist at MilliporeSigma, inflammation was prevented confirming that decreased Treg counts account for why inflammation emerges and goes unchecked.

"This likely happens in human autoimmune disease as well," says Jin, citing the human syndrome called IPEX (for immune dysregulation, polyendocrinopathy, enteropathy, and X-linked), which is marked by a constellation of autoimmune symptoms and caused by a different mutation that halts Treg development. "Treg-based intervention has in fact been proposed as a novel therapeutic means to treat various inflammatory diseases, meaning that knowing how Treg function is regulated could speed development of effective treatments."

Ubiquitination is a biochemical process whereby proteins are chemically decorated with small ubiquitin peptides as a way to change their activity. Sharpin was previously known to participate in a multi-protein complex that attached ubiquitin peptides to downstream targets, some of which controlled high-profile proteins notorious for fomenting inflammation, such as NF kappa B.

The new study provides biochemical evidence that Sharpin itself can itself be a "ubiquitination," albeit in a different manner. When that happens, Sharpin directly controls Treg activity by interacting with cell-surface signaling proteins called T cell receptors and preventing them from becoming overactive.

That brake on receptor activity is essential for Treg survival. "Our work shows that uncontrolled T cell receptor activation following Sharpin loss kills Tregs as they develop in the thymus," says Yoon Park, Ph.D., a former Liu lab post-doctoral fellow who, with Jin, is a co-first author. "This selectivity is fascinating because all T cells express the T cell receptor, but strong receptor activation has a particularly skewed effect in eliminating Tregs."

In short, the new paper shows that Sharpin controls inflammation in an unexpected way. In revealing this, it elevates Sharpin from auxiliary to player status in showing that, once ubiquitinated, Sharpin acts on its own to protect a specific class of immunosuppressive T cells from being overwhelmed by their inflammatory T cell counterparts.

Park, who now works on cancer immunotherapies at Pfizer, finds these discoveries potentially relevant to multiple fields. "Tregs are getting a lot of attention in both autoimmunity and cancer," she says, explaining that while sluggish Tregs allow unchecked inflammation, overprotective Tregs may block tumor recognition and eradication by inflammatory cells. "Right now, cancer researchers are trying to inhibit Treg function in tumor cells as immunotherapy. Sharpin's specific impact on Treg regulation suggests a way to target this T cell subtype."

Liu's laboratory at LJI has a long-term interest in how ubiquitination regulates immune signaling through the T cell receptor. The new work illustrates not only the complexity but the profound importance of these mechanisms.

"Our body's immune system is equipped with machinery to keep infectious pathogens at bay, and Tregs ensure that in doing so we will not harm ourselves," says Liu. "Loss of such a control would lead to disastrous consequences such as the development of self-attacking, or autoimmune, disease. This study addresses the means to keep such a balance."

Other contributors include Justine Lopez, Jeeho Lee, Ph.D., Lujian Liao, Ph.D., and Chris Elly.

The study was supported by National Institute of Allergy and Infectious Diseases of the National Institutes of Health (RO1AI62969, RO1AI78272 and PO1AI089624).

DOI: **10.1038/ni.3352**

La Jolla Institute for Allergy and Immunology is dedicated to understanding the intricacies and power of the immune system so that we may apply that knowledge to promote human health and prevent a wide range of diseases. Since its founding in 1988 as an independent, nonprofit research organization, the Institute has made numerous advances leading towards its goal: life without disease®.

###