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**Title**: Elucidating the Physiological Functions of the Cullin-RING Ubiquitin Ligase CRL5-SPSB

**Background and Purpose**: CRL5-SPSB is one of about 600 ubiquitin ligases that tag proteins for degradation. It targets proteins such as iNOS and the transcriptional regulator FOG-2, which are then rapidly broken down by the proteasome. Degradation of FOG-2 by CRL5-SPSB is essential for adipocyte differentiation, and blocking this process prevents preadipocytes from maturing.

Our laboratory is investigating how CRL5-SPSB regulates adipocyte differentiation through the FOG-2 degradation pathway. We focus on its roles not only in white adipocytes but also in brown and beige adipocytes. By clarifying this pathway's importance in producing fully functional fat cells, we aim to lay the groundwork for new therapeutic approaches to metabolic syndrome.

**Research Outline**: In our laboratory, we use Nif (N-terminal iNOS fragment)**,** a strong inhibitor of CRL5-SPSB, to create preadipocytes in which FOG-2 cannot be degraded. By stimulating these cells to undergo differentiation and then examining their morphological and functional changes, we aim to clarify the role of the FOG-2 degradation pathway in adipocyte differentiation. Our research proceeds as follows:

1. Gene introduction – Introduce the Nif gene into preadipocytes using a viral vector.
2. Differentiation induction – Stimulate the modified cells to undergo differentiation.
3. Cellular analysis – Assess cell morphology, functions, and degree of differentiation; identify molecules linked to observed changes.
4. Pathological relevance – Characterize "incompletely differentiated adipocytes" caused by blocking FOG-2 degradation and examine their link to metabolic syndrome.

**Future Perspectives**: If we can clarify the role of the FOG-2 degradation pathway in adipocyte differentiation and its connection to the development of metabolic syndrome, we expect that the importance of the ubiquitin–proteasome system in metabolic regulation will be more widely recognized. Such findings could open the way to the development of novel therapeutic drugs that target this pathway for the prevention and treatment of metabolic syndrome.

## References

1. Regulation of inducible nitric-oxide synthase by the SPRY domain- and SOCS box-containing proteins. *J Biol Chem.* doi: 10.1074/jbc.M110.190678.
2. The ECS(SPSB) E3 ubiquitin ligase is the master regulator of the lifetime of inducible nitric-oxide synthase. *Biochem Biophys Res Commun* doi: 10.1016/j.bbrc.