



POSTER #16

## FOLLOWING THE DYNAMICS OF THE Hippo/YAP PATHWAY IN SINGLE CELLS

Michal Shreberk-Shaked<sup>1</sup>, Jacob Stewart-Orenstein<sup>2</sup>, Nir Drayman<sup>3</sup>, Yael Aylon<sup>1</sup>, Galit Lahav<sup>2</sup>, Uri Alon<sup>1</sup> and Moshe Oren<sup>1</sup>

<sup>1</sup> Molecular Cell Biology Department, Weizmann Institute of Science, Rehovot, Israel

<sup>2</sup> System Biology Department, Harvard Medical School, Boston, MA, USA

<sup>3</sup> Institute of Molecular Engineering, University of Chicago, IL, USA

Control of organ size and tissue regeneration is crucial in animal development and tissue homeostasis. The Hippo signaling pathway is a key regulator of these processes through cell proliferation and apoptosis [1]. Moreover, recent studies indicate that Hippo pathway deregulation can induce tumors in model organisms, occurs in a broad range of human carcinomas, and may contribute to anti-cancer drug resistance [2, 3]. The mammalian Hippo pathway consists of a large network of proteins, the core of which is a kinase cascade that limits tissue growth by facilitating phosphorylation and inhibition of the transcriptional co-factors Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). This phosphorylation causes YAP/TAZ nuclear exclusion and cytoplasmic sequestration [4].

Despite the critical role of Hippo signaling in regulating cell fate, tissue homeostasis and tumorigenesis, the mechanisms involved in the dynamic control of its activity in response to stimuli or following perturbation have not been thoroughly elucidated. Given that within the tissue context the behavior of individual cells needs to be coordinated in order to maintain proper development and homeostasis, it will be interesting to compare the temporal patterns of the Hippo response among individual cells within a cell population. These issues were never addressed before with regard to the Hippo pathway, and understanding its dynamics and the extent of cell-to-cell heterogeneity in its response may shed light on the pathway's dynamic regulation and robustness.

Employing the human lung cancer cell line H1299 with CRISPR-based technology, we generated a stable cell line that expresses YAP fused to YFP (yellow fluorescent protein). In this system, protein level and localization can be tracked over several days at a resolution of minutes using time-lapse microscopy. A major advantage of this approach is the ability to follow endogenous protein levels under their native regulation. In addition, this system enables the detection of changes in protein nuclear localization over time. As a proof of concept, the YAP-YFP clone was followed upon treatment with one of its known activators, lysophosphatidic acid (LPA) [5] using time-lapse microscopy. Custom software was used to output the level of the tagged protein in each individual cell over time, as well as its subcellular localization as a function of time (*e.g.* its relative nuclear/cytoplasmic levels). Then, we generated quantitative patterns of behavior and deduced the extent of heterogeneity between single cells. First, as expected, we observed an increase in YAP nuclear/cytoplasmic intensity in response to LPA addition. In addition, we found that cells show variability in their YAP dynamic response upon LPA stimulation (Figure 1). Thus, we intend to further explore the different properties of this variability and its source. Hopefully, understanding the mechanisms that control the extent of variability between cells will shed light on the Hippo pathway robustness.

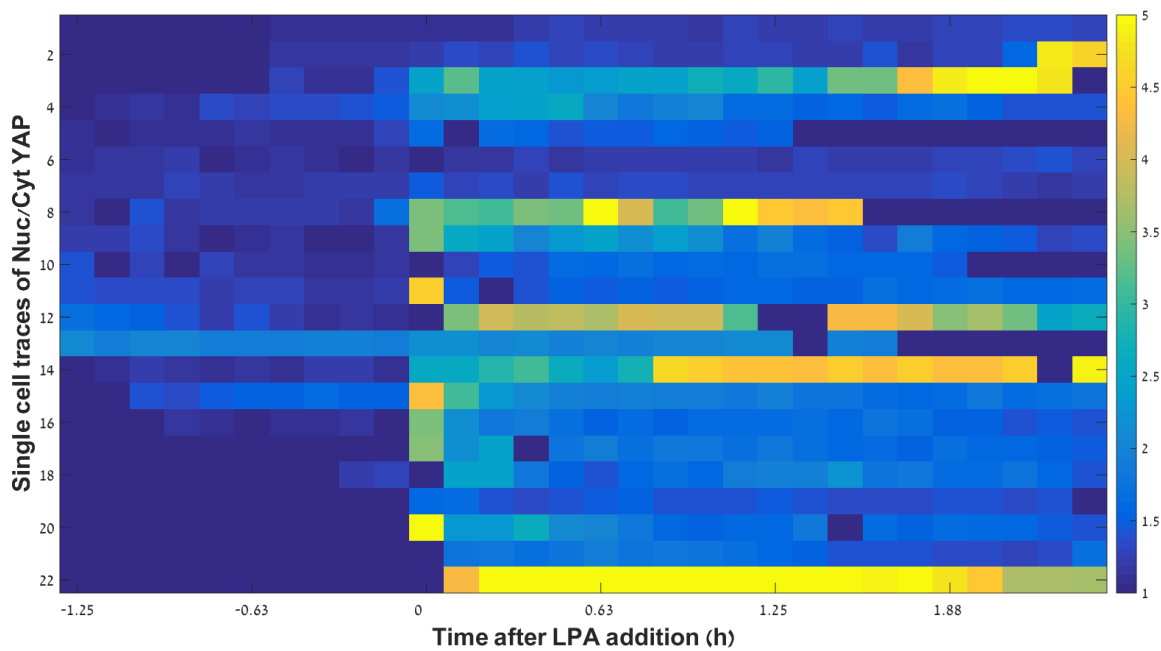


Figure 1. Variability in YAP-YFP dynamics in response to LPA. Single cell traces of the ratio between YFP intensity in the nucleus and the YFP intensity in the cytoplasm over time following LPA.

References:

1. Yu, F.X. and K.L. Guan, *The Hippo pathway: regulators and regulations*. *Genes Dev*, 2013. **27**(4): p. 355-71.
2. Harvey, K.F., X. Zhang, and D.M. Thomas, *The Hippo pathway and human cancer*. *Nat Rev Cancer*, 2013. **13**(4): p. 246-57.
3. Lai, D., et al., *Taxol resistance in breast cancer cells is mediated by the hippo pathway component TAZ and its downstream transcriptional targets Cyr61 and CTGF*. *Cancer Res*, 2011. **71**(7): p. 2728-38.
4. Hong, W. and K.L. Guan, *The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway*. *Semin Cell Dev Biol*, 2012. **23**(7): p. 785-93.
5. Yu, F.X., et al., *Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling*. *Cell*, 2012. **150**(4): p. 780-91.