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Optogenetic experiments with iLID system

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ABSTRACT

This protocol details experiments with the iLID optogenetic system as performed to acutely recruit Miro to mitochondria in https://doi.org/10.1083/jcb.202010004.

ATTACHMENTS

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Optogenetic experiments with iLID system 3d 12h

1 Plate the COS-7 cells for imaging and transfect each MatTek dish (see Cell Culture, Transfection and Imaging Protocol) with the plasmids containing the bait (construct encoding iLID) and the prey (construct encoding SspB peptide).

As an example, in the case of Guillen-Samander A, Leonzino M et al 2021 (https://doi.org/10.1083/jcb.202010004), the plasmids and amounts transfected were:

- **0.25 ug** mCh-Miro1ΔTM-SspB
- **0.5 ug** Venus-Mito-iLID
- **0.8 ug** VPS13D^Halo

Suggestion: When dealing with an optogenetic pair of constructs encoding a membrane anchored protein and a cytosolic one, we recommend transfecting them in a ratio of 2:1 for membrane: cytosolic proteins.

2 3d 12h

After adding the transfection mix, incubate the dishes at **37 °C** in the dark for **36:00:00** - **48:00:00** .

3 Just before imaging, remove the growth medium and replace with Live Cell Imaging solution (Life technologies).

4 Image the cells at **37 °C** and 5% CO_2.

Warning: Remember to keep the cells in the dark while handling them and as much as possible while changing the medium. Exposure to light could preactivate the optogenetic system which could affect the interpretation of results.

Step 4 includes a Step case.
For whole cell activation:
For localized activation:

step case

For whole cell activation:

5

Carry out imaging in an Andor Dragonfly system equipped with a PlanApo objective (63X, 1.4NA, Oil) and a Zyla sCMOS camera.

6 Identify the cells to be imaged by scanning the dish looking for red fluorescence in the 564nm channel.

Remember that looking for green fluorescence would preactivate your sample.

7

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Image the cells at a rate of 0.5Hz. Acquire the 5 frames before the activation, and achieve the activation with a single 200ms pulse of the 488nm laser. Acquire the 90 frames after activation.

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