Endogenous IP for HK2 cells

- 15x 15cm confluent dishes of HK2 cells per condition were starved for 48 hours for induction of ciliiogenesis.
- Remove medium
- Wash once with PBS
- Harvest on ice by scraping in 6ml ice-cold PBS.
- Centrifuge 10 min @ 4^0C 1500 rpm
- Discard supernatant from all the falcons and resuspend the pellet in 1 ml IP-buffer (+PIM and Na_3VO_4) (for 5 ml IP buffer add 100 µl Na_3VO_4 and 200µl PIM)
- Cell suspensions are incubated for 15 minutes @ 4^0C o/h shaker, centrifuge 30 minutes @ 4^0C full speed in a table top centrifuge
- Transfer 900ul of the supernatant (if not possible less, avoid carryover of the pellet) carefully to a 1.5 ml UZ-Tube
- Centrifuge for 30 minutes @ 4^0C 41000 rpm. (Wait until centrifuge reaches the max. speed because sometimes it stops in between)
- Transfer 850µl of the supernatant to a 1.5 ml eppi
- Take 30µl as lysate, add 30µl 2x laemmeli (containing DTT) and boil at 95^0C for 5 minutes (input)
- To the remaining lysate add anti-FLCN antibody (cell signaling, dilution 1:100, i.e. 8µl) and 2µg of anti-V5 antibody (rabbit) to other eppie containing 850µl lysate and incubate for 1 hour at 4^0C o/h shaker
- Add 50µl protein G beads (µMacs protein G beads, milteney)
- Incubate for 1 hour at 4^0C o/h shaker
- Set up µMacs column in the cold room in the magnet
- Preclear column with 200µl of lysis buffer. Let it run through and add lysate antibody bead mixture onto the column.
- Collect the flow through (keep 30µl flow through + 30µl 2x laemmli (containing DTT), boil for 5 minutes at 95^0C)
- Wash beads 3 times with 200µl of ice-cold buffer A (containing 50mM Tris (pH 7.5), 150mM NaCl, 5% glycerol, 0.05% IGPAL-CA630 or Triton)
- Wash beads 5 times with ice cold buffer B (50mM Tris (pH 7.5) and 150mM NaCl)
  Note: Inform Markus and he will take care of the elution for mass spec)

Cell splitting protocol for IP

For mass spec, 15cm dishes were used. 15 dishes per condition were taken. Cells were initially cultured in 10 cm dishes (protocol mentioned below). When the cells were 80% confluent, each dish was transferred to a 15 cm dish (1:1) in 14 ml fresh medium. After 2-3 days when cells were 80% confluent, starvation was done by keeping cells in medium without growth factor. Cells were incubated for 48 hours to allow ciliogenesis and then cells were harvested for endogenous IP.

Seeding cells on coverslips to estimate percentage ciliation:

While transferring cells from 10 cm dishes to big 15 cm dishes, some cells were also seeded on coverslips to estimate ciliogenesis.

Collagen I coating on coverslips: Put coverslips in 12 well dishes. Coverslips were washed twice with 1 ml PBS. Coverslips were then immersed in 250µl-300µl collagen I followed by 1 hour incubation @ RT. Suck off collagen I after 1 hour and let the plates dry. Trypsinize 10 cm dish of HK2 cells in 1 ml trypsin. Add 5 ml medium to the cells and collect in 15 ml falcon. Centrifuge cells @ 1500 rpm for 10 minutes. Suck off supernatant and resuspend cell pellet in 6 ml fresh medium. Seed them on coverslips with 1 ml each (in 6 wells). While inducing cells for ciliiogenesis for mass spec (for 48 hours), starve 3 wells in 12 wells as well and in rest 3 wells, add fresh medium with growth factors. After 48 hours, fix the cells and perform immunofluorescence using gold standard protocol.

Number of replicates taken: n=5

N=1

14 dishes were taken per condition

Passage number: 24

Confluency: 80%

N=2 and 3

14 dishes were taken per condition

Passage number: 24-29 (forgot to write in labbook)

Confluency: 60%

N=4 and 5

15 dishes were taken per condition

Passage number: 29

Confluency: 80%

While doing endogenous IP and testing on western:

After washing 5 times with wash buffer B

- Apply 20µl of pre-heated (95^{0}C) 1x laemmli (containing DTT) onto the column matrix and incubate for 5 minutes. If a drop of the wash buffer is present on the column tip, then it should be removed by contacting the column tip with the waste tube or by using a fresh pipette tip (discard this drop)
- Place a fresh collection tube under the µ column
- Apply 50µl of pre-heated (95^{0}C) 1x laemmli (or elution buffer, provided with miltney kept in coldroom on right hand side) onto the column matrix. If a drop is present onto the column tip, it should be collected using a pipette tip → this drop is added to the eluate

Note:

If frozen, before loading the samples on gel, boil the samples again at 95^{0}C for 1 minute and spin them down at maximum speed for 2 minutes. Load 20µl of the supernatant (as well as input and flowthrough) onto the gel.

Culturing HK2 cells

Note: split max 1:2 or 1:3 since they are slow growing.

Medium: Kerotinocyte medium

Cell storage: box 474 in Idefix

- Thaw the cells by resuspending in 5ml HK2 medium.
- Centrifuge at 1500 rpm for 5 minutes 2 @ 4^{0}C.
- Discard supernatant.
- Resuspend the pellet in 6 ml HK2 medium and seed on 10cm dish.
- Do a mycoPCR after two days of thawing.

While culturing cells in 10 cm dishes:

- Wash cells 1x PBS 5ml.

- Add 1ml trypsine to the cells, followed by 3-4 minutes incubation @ 37^0C.
- Harvest cells in 5ml PBS and collect in 15ml faclcon.
- Centrifuge at 1500 rpm for 5 minutes.
- Discard supernatant.
- Resuspend the pellet in 6ml HK2 medium and seed on 10cm dish.

Note: the cells are sensitive therefore care must be taken while culturing them.

When you have plenty of dishes, always pool 6 dishes together for splitting further to avoid the chances of contamination.

Always split them after 2-3 days. If they are not confluent, you can also change the medium after 2 days and split them 2 days after changing medium.