Protocol
PAPC/ATP/Oxidized PAPC
Costimulation Time course (Ishita Shah)

Overview

1. One large experiment will be completed to provide samples to all the lipidomic cores.
2. Cells will be pre-treated for 4hrs with either PAPC (40 µM) or oxPAPC (40 µM) followed by subsequent stimulation with ATP (2 mM) for 4hr, and 20hr. As controls, untreated cells will be harvested at every time point.
3. The experiment will be done in duplicates. 12 aliquots will be generated from each replicate. Cores G, and J will receive one aliquot, cores I, H, E and K will receive two aliquots and core G will receive 15 mL of medium from each treatment.
4. Data will be normalized to cell counts. Cell count data will be generated by core G using Invitrogen Countess.

Setup

1. Seed T-75 flasks for each treatment with 1.2×10^7 cells in 15 ml dye-free DMEM (Dulbecco's Modified Eagle's Medium)-(SG1201170571)
2. Incubate for 16-18 hours at 37^0C

Reagent Preparation

1. ATP (200mM solution) (SG1201170569)
2. PAPC (10mM solution) (SG1203020572)
3. oxPAPC (10mM solution) (SG1203020573)

Treatment

1. Treat each flask as follows:
    a. Pretreatment (priming): 4hr PAPC or OxPAPC (40µM final concentration , 60 µl of 10mM PAPC or oxPAPC in 15ml media). In control experiments, cells will receive 60 µl of medium.
    b. Stimulation with ATP: after 4hr of priming with PAPC or oxPAPC, ATP is added (2mM final concentration, 150 µl of 200mM ATP in 15ml media). In control experiments, cells will receive 150 µl of PBS (RG0000001143).
2. The flasks are incubated at 37^0C

Harvest

1. At appropriate time points remove the flasks from the incubator.
2. Collect medium in 50ml conical polypropylene tubes for Core G.
3. Wash the cells twice with 10ml of PBS.
4. Add 12 ml of PBS and harvest the cells with cell scraper.

5. Transfer the cells to a 50ml conical tube. Gently suspend cells by swirling or lightly vortexing for 10 secs.
6. Remove 100ul of cells suspension for cell counting
7. Make 12 aliquots from the remaining cell suspension.
8. Dispense 1ml into 13 x 100mm glass tubes (Kimble Chase Part #45066A-13100) with Teflon-lined screw caps.
9. Freeze and store cells in -80^{0}C

Figure 1

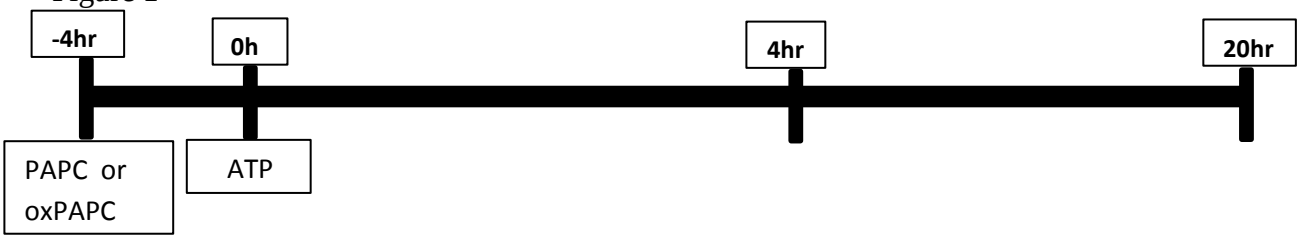

Table 1

Sample #	Pretreatment hour	Treatment hour	Treatment
1	4	0	-PAPC-ATP
2	4	0	-PAPC-ATP
3	4	0	+PAPC-ATP
4	4	0	+PAPC-ATP
5	4	0	+oxPAPC-ATP
6	4	0	+oxPAPC-ATP
7	4	4hr	-PAPC-ATP
8	4	4hr	-PAPC-ATP
9	4	4hr	+PAPC-ATP
10	4	4hr	+PAPC-ATP
11	4	4hr	+oxPAPC-ATP
12	4	4hr	+oxPAPC-ATP
13	4	4hr	+ATP
14	4	4hr	+ATP
15	4	4hr	+PAPC+ATP
16	4	4hr	+PAPC+ATP
17	4	4hr	+oxPAPC+ATP
18	4	4hr	+oxPAPC+ATP
19	4	20hr	-PAPC-ATP
20	4	20hr	-PAPC-ATP
21	4	20hr	+PAPC-ATP
22	4	20hr	+PAPC-ATP
23	4	20hr	+oxPAPC-ATP
24	4	20hr	+oxPAPC-ATP
25	4	20hr	+ATP
26	4	20hr	+ATP
27	4	20hr	+PAPC+ATP
28	4	20hr	+PAPC+ATP
29	4	20hr	+oxPAPC+ATP
30	4	20hr	+oxPAPC+ATP