

## **Cellular mechanotransduction of human osteoblasts in microgravity**

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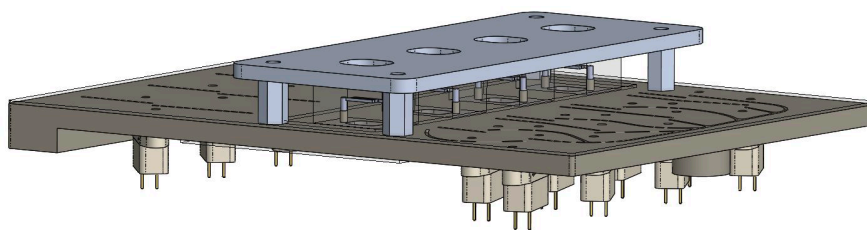
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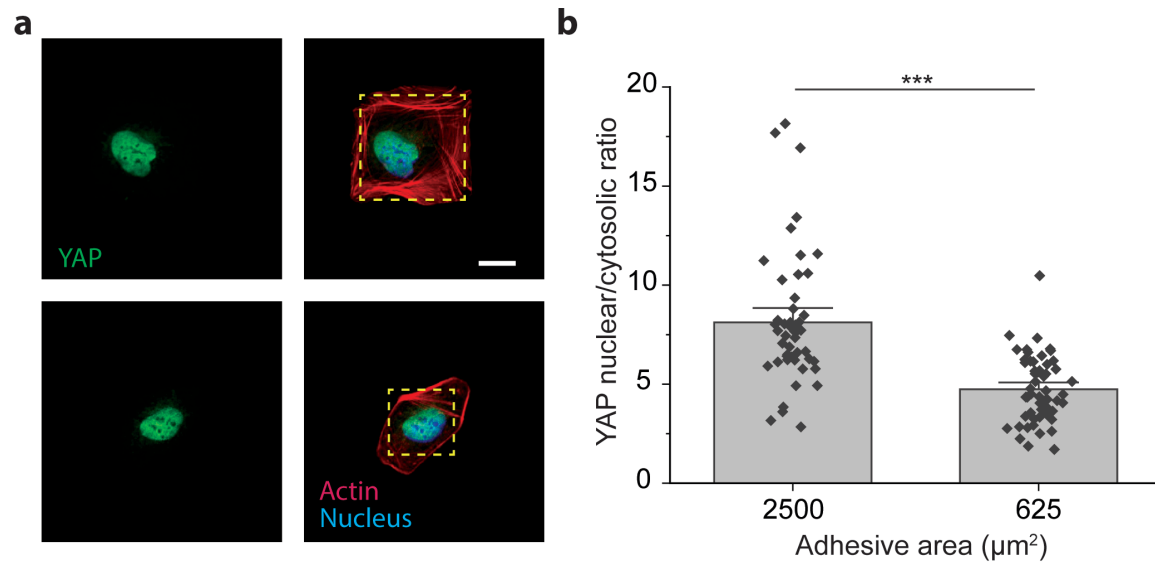
# Equal contribution

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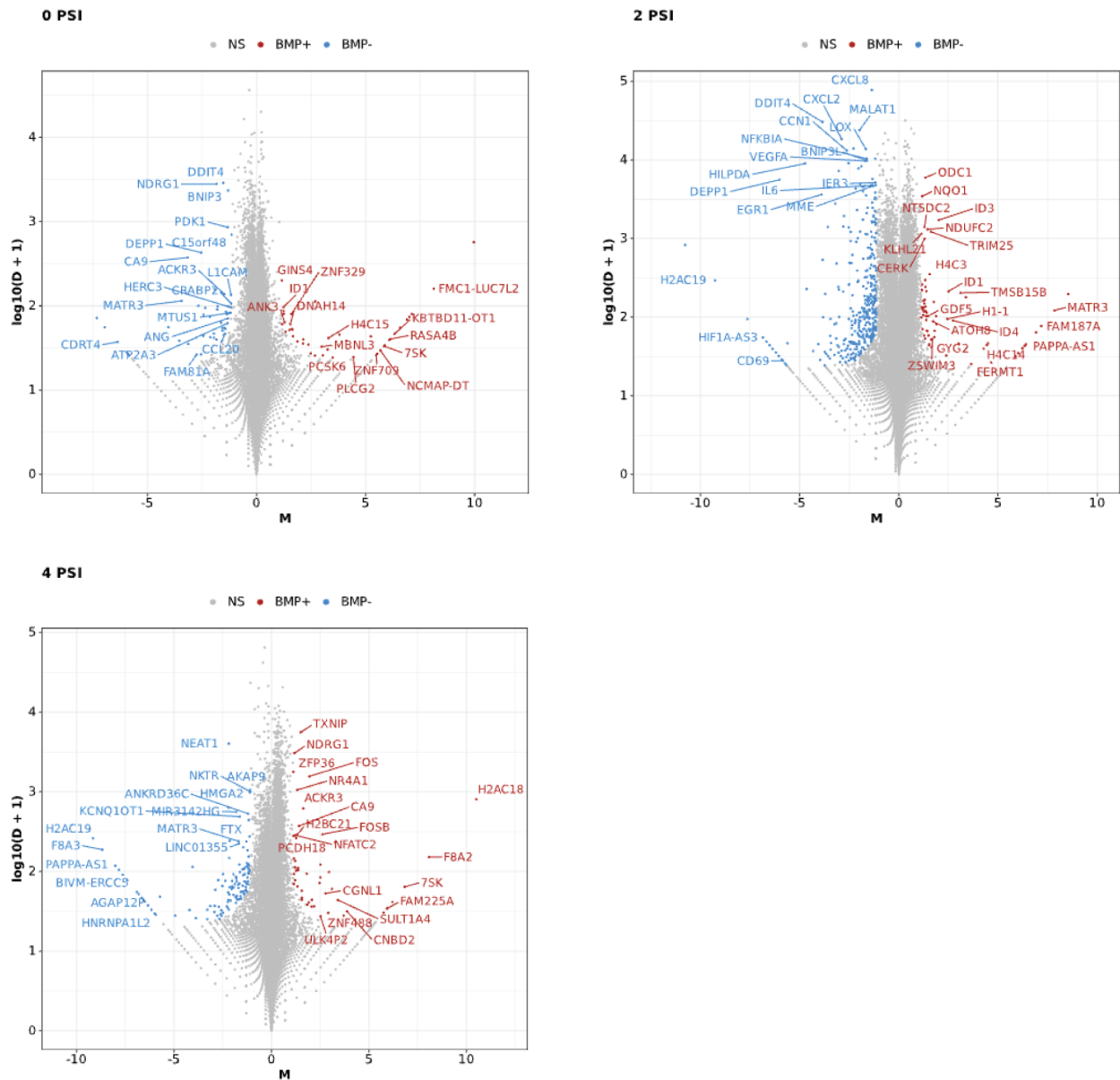
## Supplemental Figures



**Supplemental Figure 1.** CAD drawing of the fluidic manifold with a compression plate for holding down the microfluidic chips.



**Supplemental Figure 2. YAP translocates to cytosol at low cell tension. A)** Representative immunofluorescence images of human osteoblasts (hFOBs), on large ( $2500 \mu\text{m}^2$ ) and small ( $625 \mu\text{m}^2$ ) micropatterns, stained for F-actin, DNA, and YAP. Scale bar is  $20 \mu\text{m}$ . **B)** Quantification of YAP nuclear/cytoplasmic ratio of individual hFOBs from three independent experiments. Error bars denote standard error of the means. \*\*\*  $p < 0.001$ .



**Supplemental Figure 3. Summary M-D plots showing differences in expression between BMP2 treated and untreated samples within each psi group.** M values indicate log2 ratios between conditions. D values indicate the difference in counts between conditions. Significant genes are identified as those that are >90% likely to be differentially expressed compared to a null distribution.

## Supplemental Tables

**Supplemental Table 1.** Timeline of the ISS experiment on microfluidic aspiration of human osteoblast (flown on SpX-27)

| Date (2023)                                                         | Mission time days | Time from loading (hours) | Event (EST)                                             |
|---------------------------------------------------------------------|-------------------|---------------------------|---------------------------------------------------------|
| March 13                                                            | 0                 |                           | 1:00 pm: final loading into CubeLab                     |
| March 14                                                            | 1                 | 28.5                      | 5:30 pm: Handover to NASA                               |
| March 15                                                            | 2                 | 55.5                      | 8:30 pm: Launch                                         |
| March 16                                                            | 3                 | 66.5                      | 7:30 am: Dock with ISS                                  |
| March 17                                                            | 4                 | 90.5                      | 7:50 am: Installed on ISS                               |
| March 17                                                            | 4                 | 95                        | 10:30 am: Chip 2 seeding, pressurization, imaging/video |
| March 17                                                            | 4                 | 97                        | 12:50 pm: Chip 3 seeding, pressurization, imaging/video |
| March 18                                                            | 5                 | 124.5                     | 4:25 pm: Chip 4 seeding, pressurization, imaging/video  |
| Payload remained powered to perform potential additional activities |                   |                           |                                                         |
| March 24                                                            | 11                | 149                       | 5:30 pm: Payload turned off                             |

**Supplemental Table 2.** Well plate layout for the osteoblast spheroids pressurization experiment.

| Well             | 1   | 2    | 3   | 4    | 5   | 6    | 7         | 8         | 9         | 10        | 11        | 12        |
|------------------|-----|------|-----|------|-----|------|-----------|-----------|-----------|-----------|-----------|-----------|
| Pressure (psi)   | 0   | 0    | 2   | 2    | 4   | 4    | 0         | 0         | 2         | 2         | 4         | 4         |
| Ligand condition | BMP | None | BMP | None | BMP | None | BMP       | None      | BMP       | None      | BMP       | None      |
| Fixative         | PFA | PFA  | PFA | PFA  | PFA | PFA  | RNA Later | RNA Later | RNA Later | RNA Later | RNA Later | RNA Later |

**Supplemental Table 3.** Timeline of the ISS experiment on osteoblast spheroid pressurization (flown on NG-18)

| Date (2022) | Mission time days | Time from loading (hours) | Event (EST)                                                                                                                 |
|-------------|-------------------|---------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| November 4  | 0                 |                           | 12:00 pm: Final loading into CubeLab                                                                                        |
| November 5  | 1                 | 17                        | 5:00 am: Handover to NASA                                                                                                   |
| November 6  | 2                 | 41                        | 5:05 am: Launch attempt 1                                                                                                   |
| November 7  | 3                 | 66                        | 5:30 am: Launch                                                                                                             |
| November 8  | 4                 | 95                        | 11:18 am: Power shut off due to Cygnus solar panel malfunction                                                              |
| November 9  | 5                 | 117                       | 9:00 am Docked with ISS                                                                                                     |
| November 9  | 5                 | 123                       | 3:30 pm: Power turned back on                                                                                               |
| November 10 | 6                 | 139<br>151                | 7:04 am: Installed on to ISS<br>7:00 pm: Start serum starvation                                                             |
| November 11 | 7                 | 162<br>164<br>165<br>168  | 5:45 am: Start BMP treatment<br>7:36 am: Start pressurization<br>8:45 am: Fixation start<br>11:33 am: Moved to cold stowage |