

Expanded View Figures

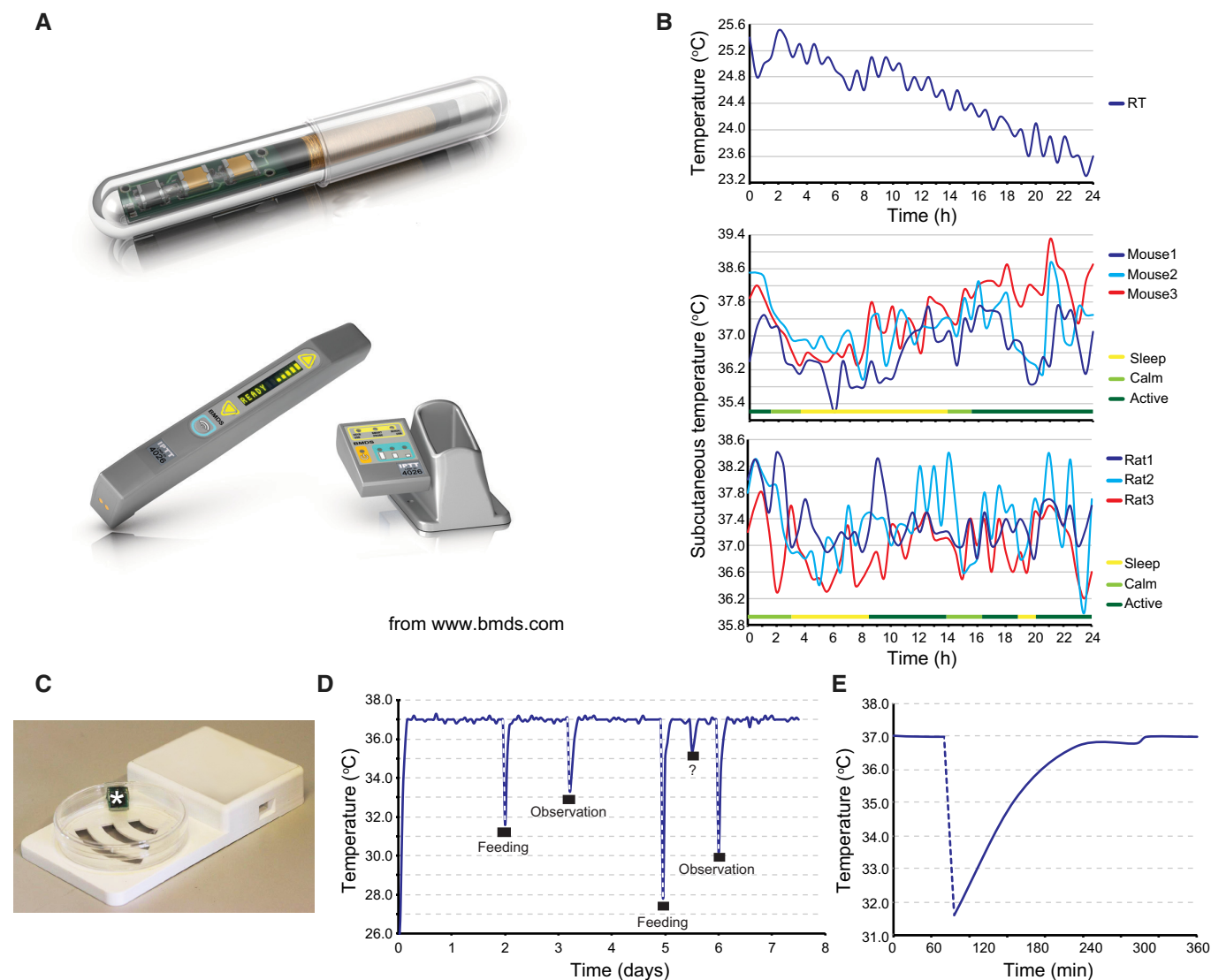


Figure EV1. Keratinocyte stem cells are constantly exposed to temperature variations *in vivo* and *in vitro*.

- A Implantable IPTT-300 transponder and its DAS-7006/7 s reader system from BioMedic Data Systems, Seaford DE, USA (www.bmds.com). Actual size of the implantable transponder: length 14 mm and diameter 2 mm.
- B Subcutaneous temperature of mice and rats constantly fluctuates. Three mice and three rats were each implanted subcutaneously with an IPTT-300 transponder (BioMedic Data Systems); the temperature and the behavior (active, calm, sleep) of each animal were then monitored over a 24 h. Room temperature (RT) was simultaneously monitored with a nonimplanted transponder.
- C Picture of an EPFL-designed submersible RFID temperature sensor and its reading base station (Laboratory of Microengineering for Manufacturing). The culture vessel was a 100 mm size Petri dish.
- D Cultured human keratinocytes were subjected to sharp temperature fluctuations when removal from the culture incubator (Nuair 8700E) for medium change (feeding) or microscopic observation. Temperature of the culture medium was recorded over a week using immersed temperature sensors. Note that an unknown event (opening of the incubator door?) occurred between the fifth and sixth day of culture.
- E Zoom of the temperature curve at the first medium change shown in B demonstrates that recovery to the set temperature (37°C) takes more than 2 h.

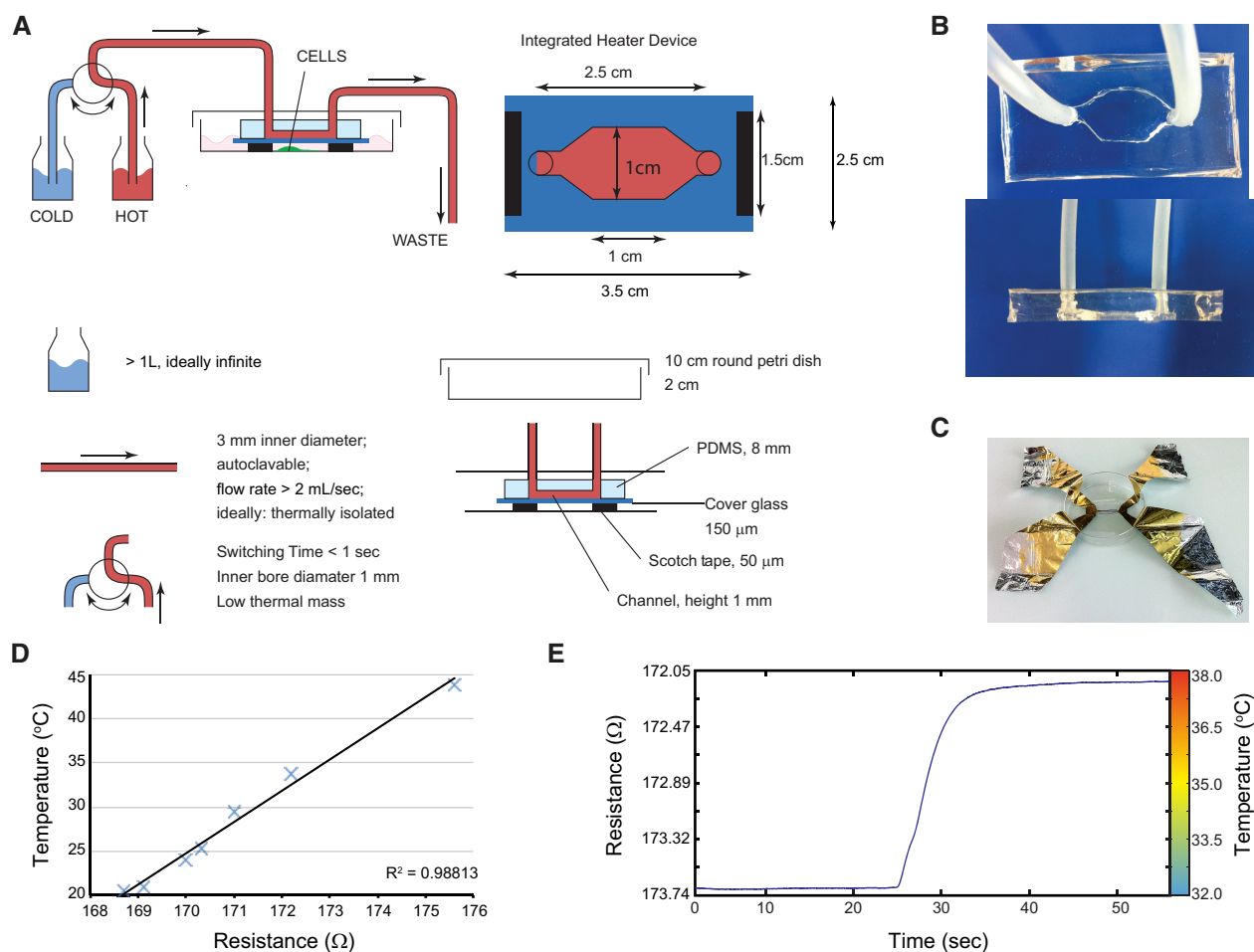


Figure EV2. Custom-made in-house device to control temperature while imaging cultured keratinocytes in real-time.

- A Schematic representation of the heating/cooling system. The device was designed to perfectly fit a 100 mm Petri dish and consists of a transparent polydimethylsiloxane (PDMS) chamber cast on a microscope slide cover glass. Two holes are pierced at each extremity of the chamber to allow connection of the chamber with the help of tubing to two separate bottles filled with water, immersed in individual water baths set at different temperatures. An external valve allows temperature variation in the chamber by switching the water flow between water baths.
- B Top and side views of the PDMS chamber.
- C Four-point measurement of the electrical resistance of a thin aluminum foil was used to calibrate the device.
- D The relation between temperature and resistance was always linear in the experimental temperature range.
- E Example of the calibration curve of the device used for the imaging experiments is presented in Fig 2D and E.

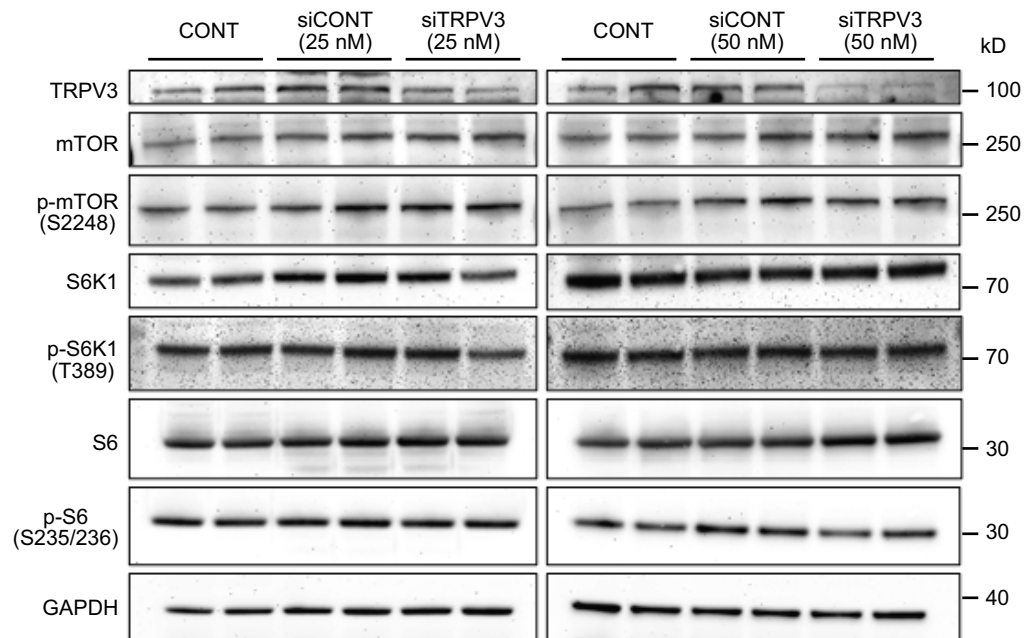


Figure EV3. Downregulation of *TRPV3* gene expression does not affect mTORC1 kinase activity.

Human keratinocytes were treated with siRNA targeting *TRPV3* or control siRNA, and the expression and phosphorylation of mTOR, S6K1, and S6 were examined by western blotting. Keratinocytes were independently transfected with 25 or 50 nM siRNA targeting *TRPV3* and decreased expression of *TRPV3* was also confirmed by western blotting. Notably, decreased expression of *TRPV3* did not affect the mTORC1 kinase activity.

Source data are available online for this figure.

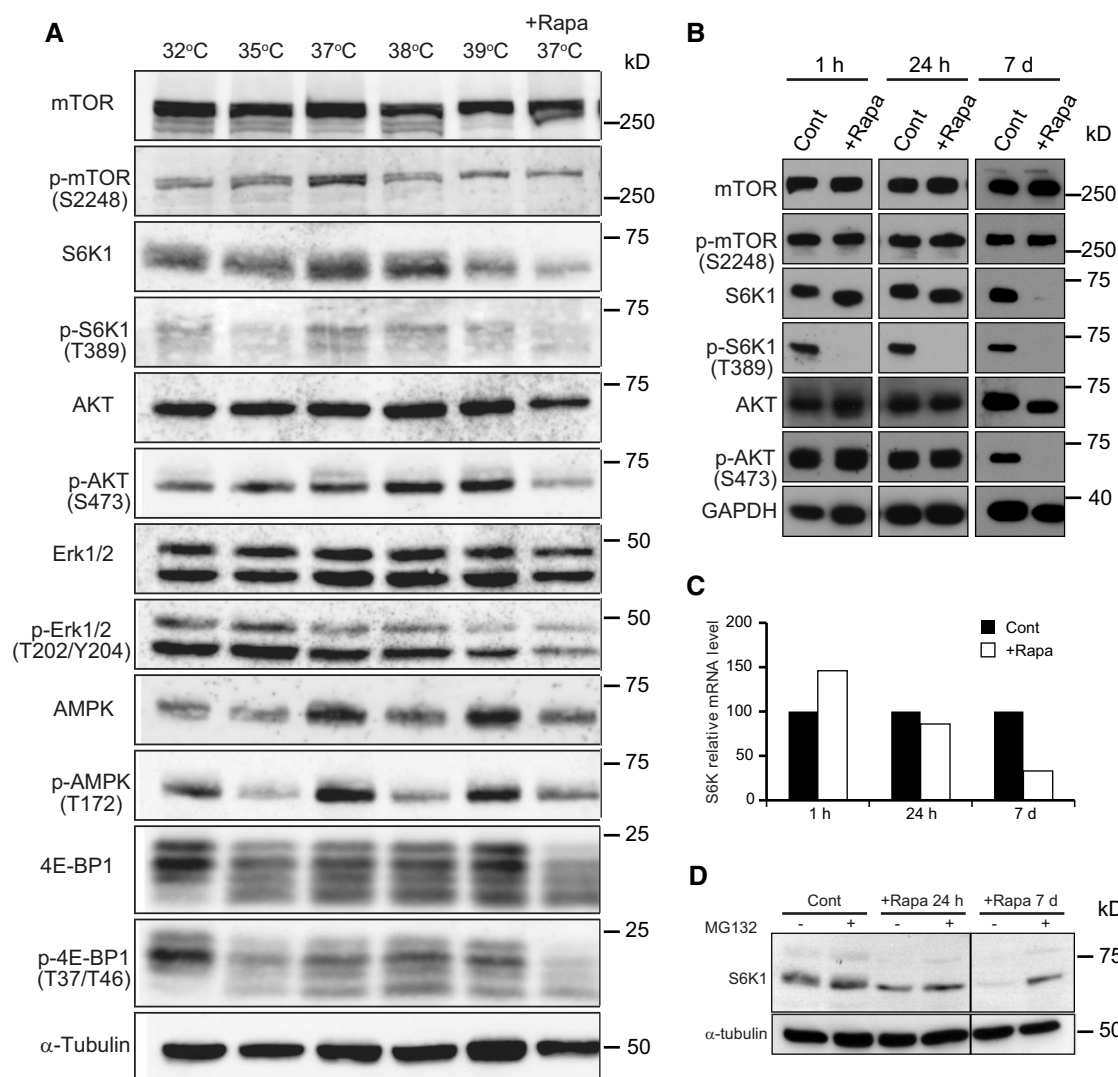


Figure EV4. Long-term exposure of human keratinocytes to temperatures below 37°C decreases mTORC1 kinase activity.

A YF29 keratinocytes were cultured for 7 days at 32, 35, 37, and 37°C in the presence of rapamycin (100 nM). Note that phosphorylation levels of ERK are completely independent of temperature, demonstrating that the decrease in phosphorylated S6K1 is not linked to a general effect of temperature on enzyme activity. Thermo Scientific Heracell 150 incubators with temperatures set at 32, 35, 36, 37, and 38°C.

B–D YF29 keratinocytes were cultured for 7 days before proteins were extracted and expression of S6K1 was analyzed by western blotting and qPCR. Rapamycin (100 nM) was added either for the entire duration of the culture (7 days) or the last 24 h, or for the last 1 h. (B) As expected, a short rapamycin exposure inhibited S6K1 phosphorylation without affecting S6K1, whereas a long rapamycin exposure (7 days) significantly affected the detection of S6K1. (C) qPCR experiments indicate that the level of expression of S6K1 mRNAs decreases with long rapamycin exposure. (D) MG132 (1 μM), a specific proteasome inhibitor, was added to YF29 cells grown in the absence for the presence of rapamycin for a day (24 h) or for 7 days (7 d) before proteins were extracted. These experiments demonstrate that a decrease in expression and increase in protein degradation are responsible for the low levels of S6K1 after 7 days of rapamycin exposure to rapamycin.

Source data are available online for this figure.

Figure EV5. Analysis of mRNA expression in cultured human keratinocytes.

- A, B Venn diagrams of upregulated (A) or downregulated (B) genes in keratinocytes cultured at 32°C or treated with 100 nM rapamycin compared with gene expression in the control culture (37°C).
- C, D Gene ontology (GO) analysis of genes upregulated (C) or downregulated (D) genes under both conditions in keratinocytes cultured at 32°C or treated with 100 nM rapamycin compared with gene expression in the control culture (37°C).

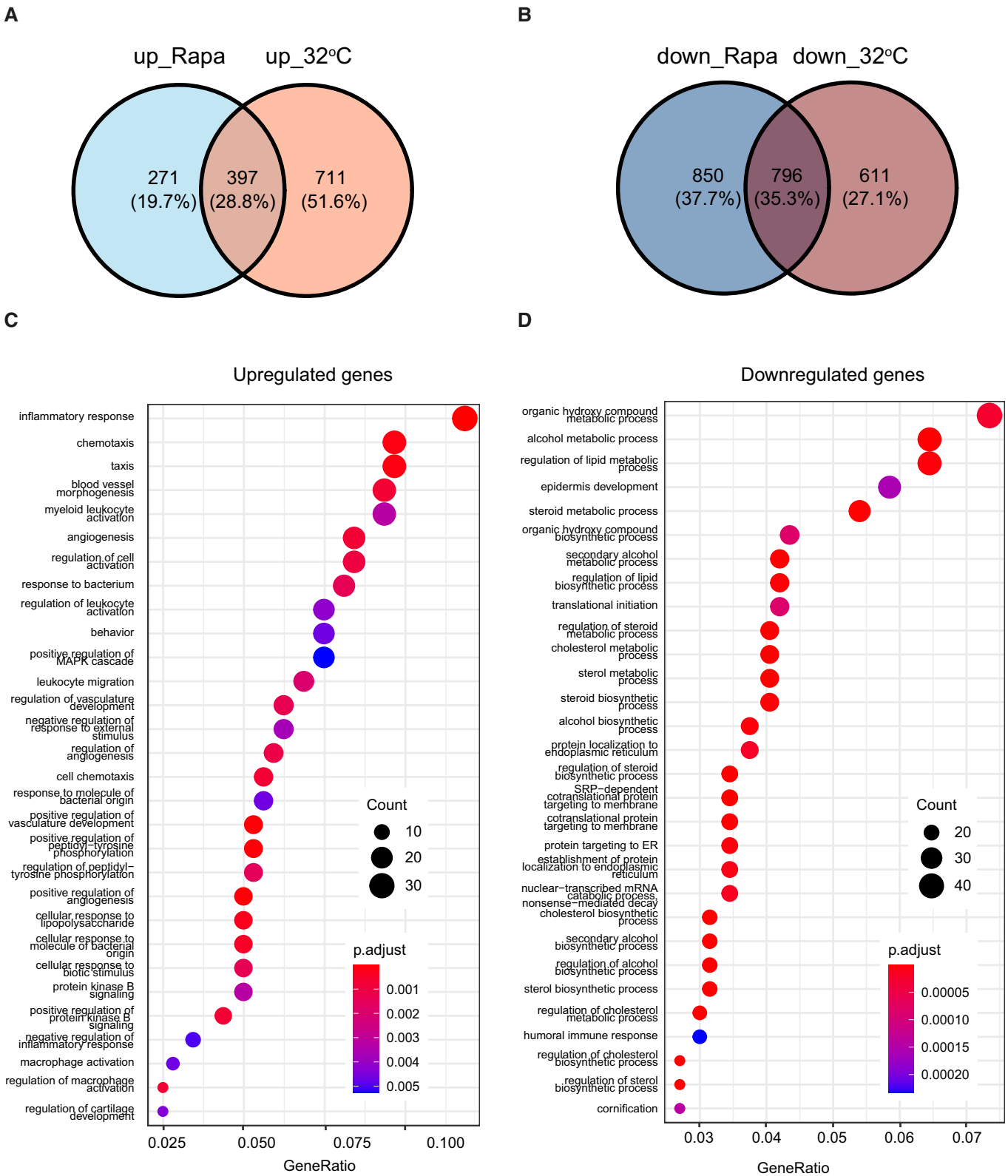


Figure EV5.