

Cell type-specific proteomics uncovers a RAF15-SnRK2.6/OST1 kinase cascade in guard cells

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SUPPORTING INFORMATION

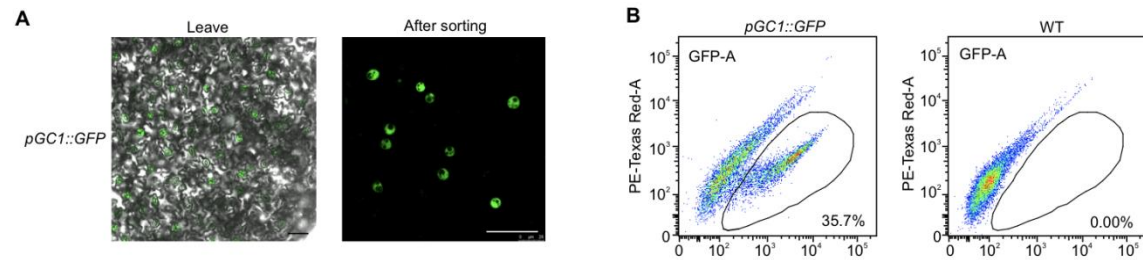


Figure S1. The cell-sorting of guard cell protoplasts

(A) Fluorescent images showing the GFP fluorescence of *pGC1::GFP* transgenic leaves (left, Bar=50 μ m) and sorted GCPs (right, Bar=50 μ m). (B) Representative flow cytometry scatter plots for *pGC1::GFP* (left) and wild type control (WT, right).

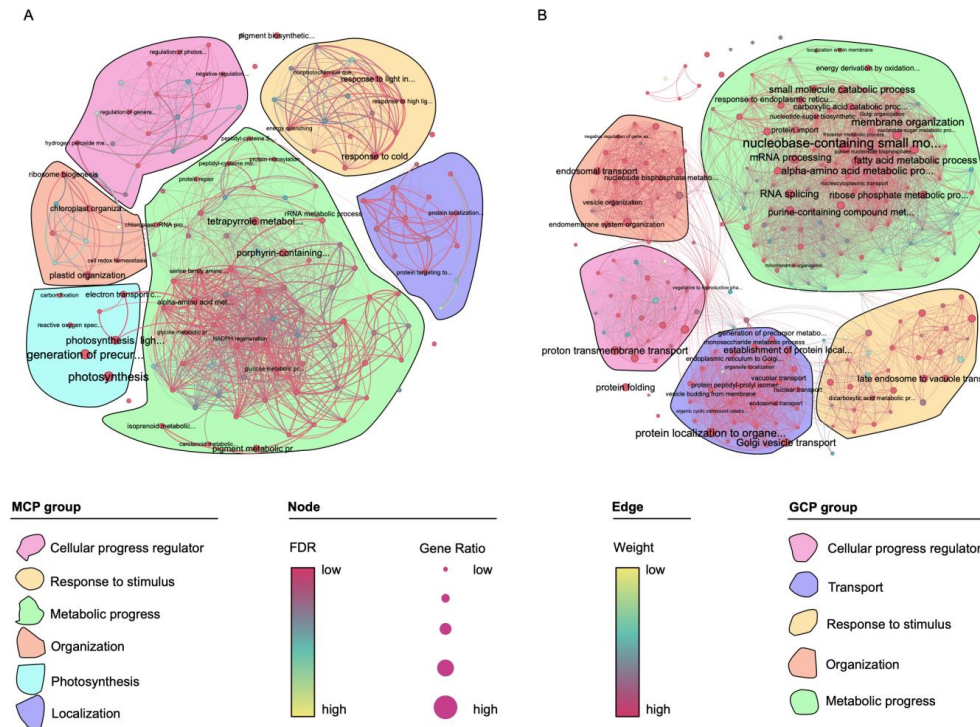


Figure S2. GO enrichment analysis of proteins enriched in GCPs and MCPs

(A) GO enrichment analysis of proteins enriched in MCPs. (B) GO enrichment analysis of proteins enriched in GCPs. The original GO enrichment results are filtered by REVIGO (Supek et al., 2011) to remove semantic redundancy. The circular dots represent GO terms, and the lines between the dots represent the connection between GO terms. The bubble size is determined by the significance of enrichment (number of proteins in corresponding GO term/total number of input). The bubble color is decided by FDR (BH-method). Independent networks or sub-networks are manually circled with irregular graphics. (C) The expression pattern of *RAF15* in various tissues and developmental stages. The expression data for *RAF15* was retrieved from the Arabidopsis eFP browser website (<http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>).

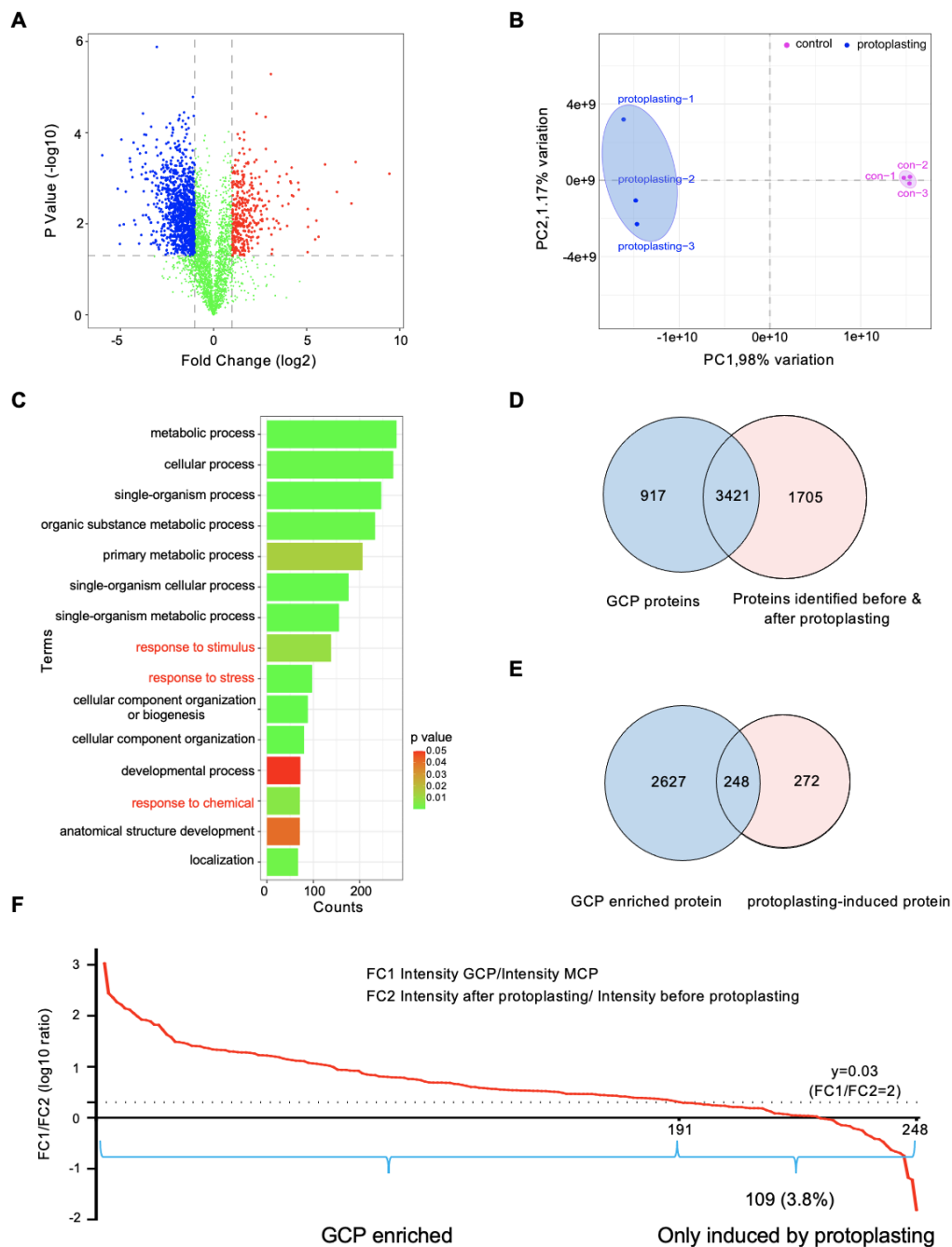


Figure S3. Quantitative proteomics of epidermal peels before and after protoplating

(A) Volcano plot of proteins before and after protoplating. On the y-axis, the negative log₁₀ values are plotted. On the x-axis, the log₂ values of the fold change are seen in the comparison. (B) PCA analysis of proteome of the 6 samples

before and after protoplasting. **(C)** GO enrichment analysis of protoplasting-induced proteins. **(D)** Venn diagrams showing the overlap between the GCP proteome and proteins identified before and after protoplasting. **(E)** Venn diagrams showing the overlap between the GCP-enriched protein and protoplasting-induced proteins. **(F)** The relative changes of the 248 overlapping proteins in two sets of proteomics.

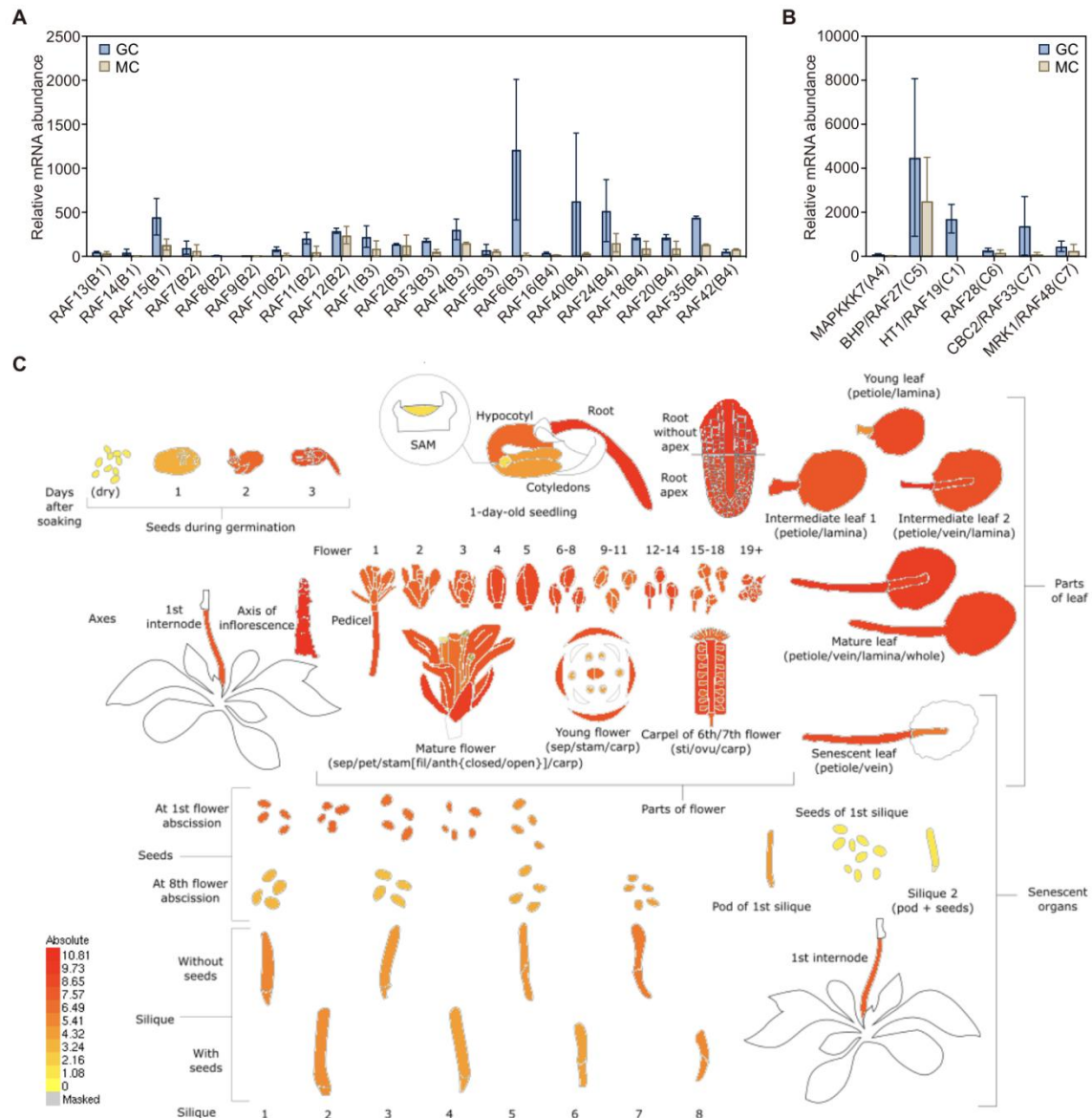


Figure S4. The relative expression of *RAF* genes in guard cells
(A) The relative abundance of B1, B2, B3, and B4 *RAF* transcripts in GC and MC. **(B)** The relative abundance of A and C subgroup *RAF* transcripts in GC and MC. **(C)** The tissue specific expression (Klepikova atlas) of *RAF15* gene. The expression of *RAFTs* shown in **(A)** to **(C)** was obtained from the Arabidopsis eFP browser web site <http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>.

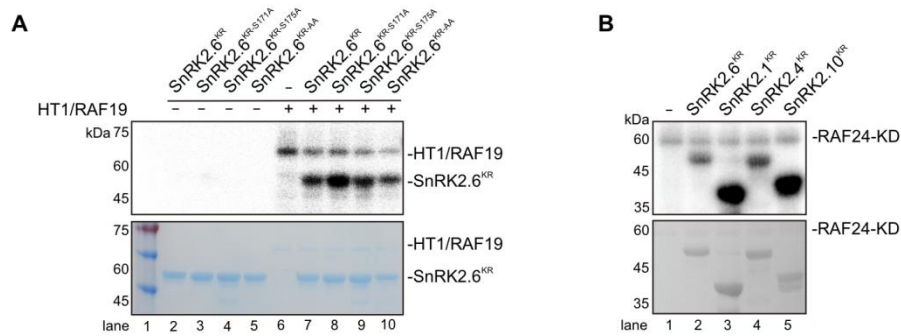


Figure S5. HT1/RAF19 and RAF24 phosphorylate SnRK2s *in vitro*

(A) Ser175Ala and Ser171Ala mutation did not affect RAF19-mediated phosphorylation of SnRK2.6^{KR}. Recombinant full-length HIS-RAF15 and GST-HT1/RAF19 were used to phosphorylate HIS-SnRK2.6^{KR}, SnRK2.6^{KR} with Ser171Ala mutation (SnRK2.6^{KR-S171A}), SnRK2.6^{KR} with Ser175Ala mutation (SnRK2.6^{KR-S175A}), or SnRK2.6^{KR} with Ser171AlaSer175Ala mutations (SnRK2.6^{KR-AA}), in the presence of [γ -³²P]ATP. Autoradiograph (upper) and Coomassie staining (bottom) show phosphorylation and loading, respectively, of purified HIS-RAF15, HIS-SnRK2.6^{KR}, and GST-HT1/RAF19.

(B) Recombinant RAF24 kinase domain was used to phosphorylate SnRK2.6^{KR}, SnRK2.1^{KR}, SnRK2.4^{KR}, SnRK2.10^{KR}, (SnRK2.6^{K50R}, SnRK2.1^{K33R}, SnRK2.4^{K33R}, SnRK2.10^{K33R}, a kinase-dead form of SnRK2.6/1/4/10), in the presence of [γ -³²P]ATP. Autoradiograph (upper) and Coomassie staining (bottom) show phosphorylation and loading, respectively, of purified GST-RAF24-KD, and HIS-SnRK2.6^{KR}, SnRK2.1^{KR}, SnRK2.4^{KR}, SnRK2.10^{KR}.

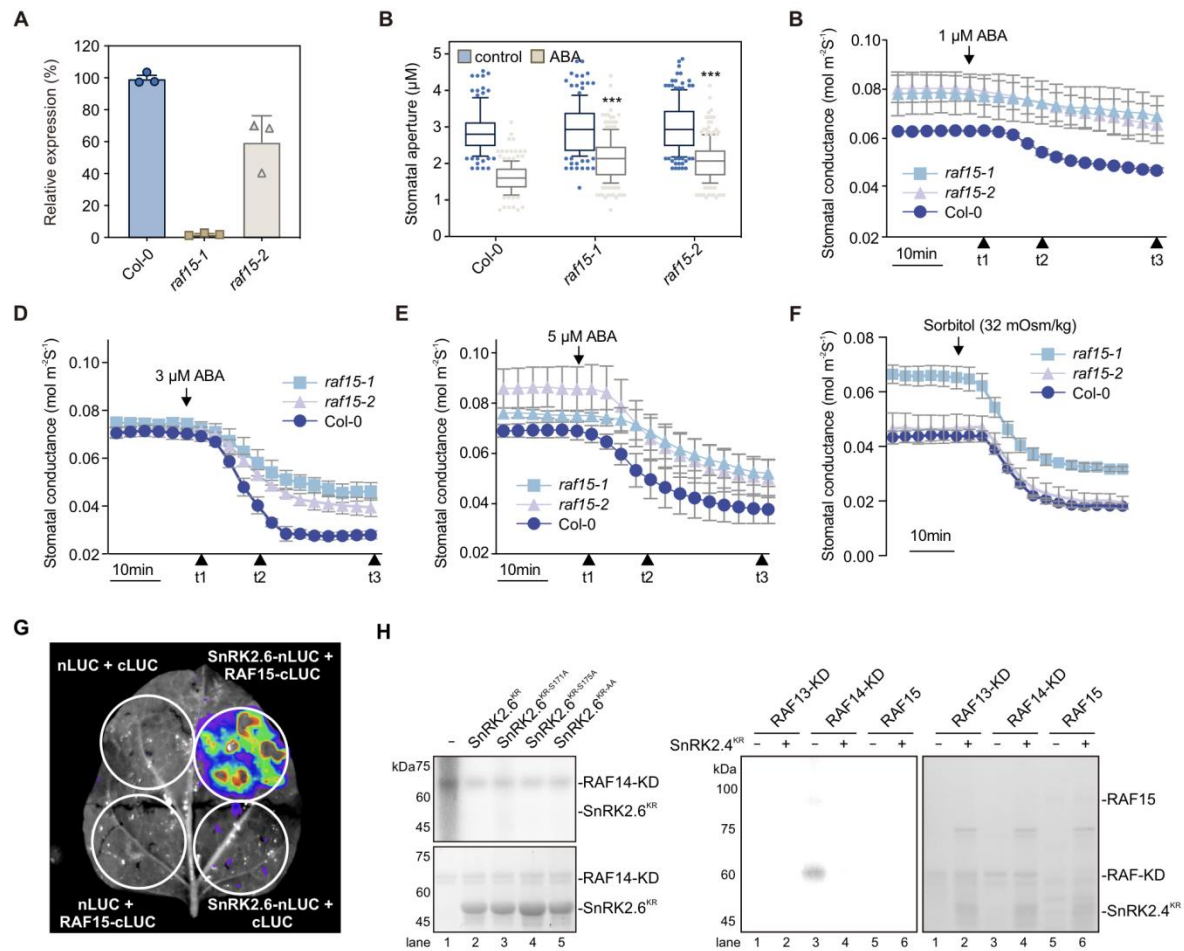


Figure S6. RAF15 and RAF13 in B1 subgroup phosphorylate SnRK2.6/OST1 *in vitro*

(A) Expression of *RAF15* in *raf15-1* and *raf15-2* mutants. (B) Stomatal closure of 4-week-old wild type and *raf15* mutants in response to ABA. Error bars, SD (n = 176 to 210 individual stomates). Unpaired two-tailed *t*-tests, *** *p* < 0.001. Box and whisker: 10–90 percentiles. (C) to (E) Time-resolved stomatal conductance in response to different concentration of ABA in *Col-0* and *raf15* mutants. Error bars, SEM (n = 3 leaves). Data represent one of two independent sets of experiments. (F) Time-resolved stomatal conductance in response to sorbitol in

Col-0 and *raf15* mutants. Error bars, *SEM* (n = 3 leaves). Data represent one of two independent sets of experiments. **(G)** Split-Luciferase assays show the interaction between SnRK2.6 and RAF15 in tobacco leaves. **(H)** Recombinant GST-RAF14-KD were used to phosphorylate wild type and mutated SnRK2.6^{KR} in the presence of [γ -³²P]ATP. Autoradiograph (upper) and Coomassie staining (bottom) show phosphorylation and loading, respectively, of purified GST-RAF14-KD and HIS-SnRK2.6^{KR}. **(I)** Recombinant GST-RAF13-KD, GST-RAF14-KD, and HIS-RAF15, were used to phosphorylate SnRK2.4^{KR} in the presence of [γ -³²P]ATP. Autoradiograph (left) and Coomassie staining (right) show phosphorylation and loading, respectively, of purified RAFs and HIS-SnRK2.4^{KR}.

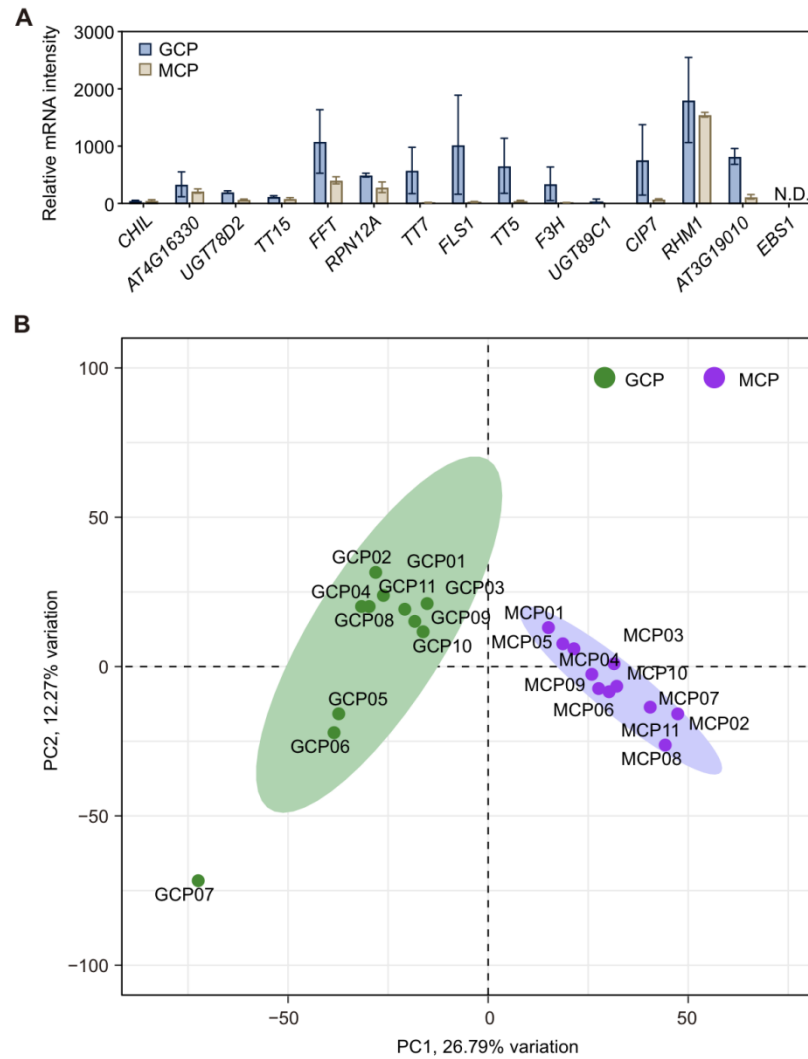


Figure S7. Flavonoid compounds specifically accumulate in GCPs

(A) Relative abundance of genes in GC and MC that encoding the proteins showed in Figure 5A. (B) PCA analysis of metabolome of the 11 MCP and 11 GCP samples.

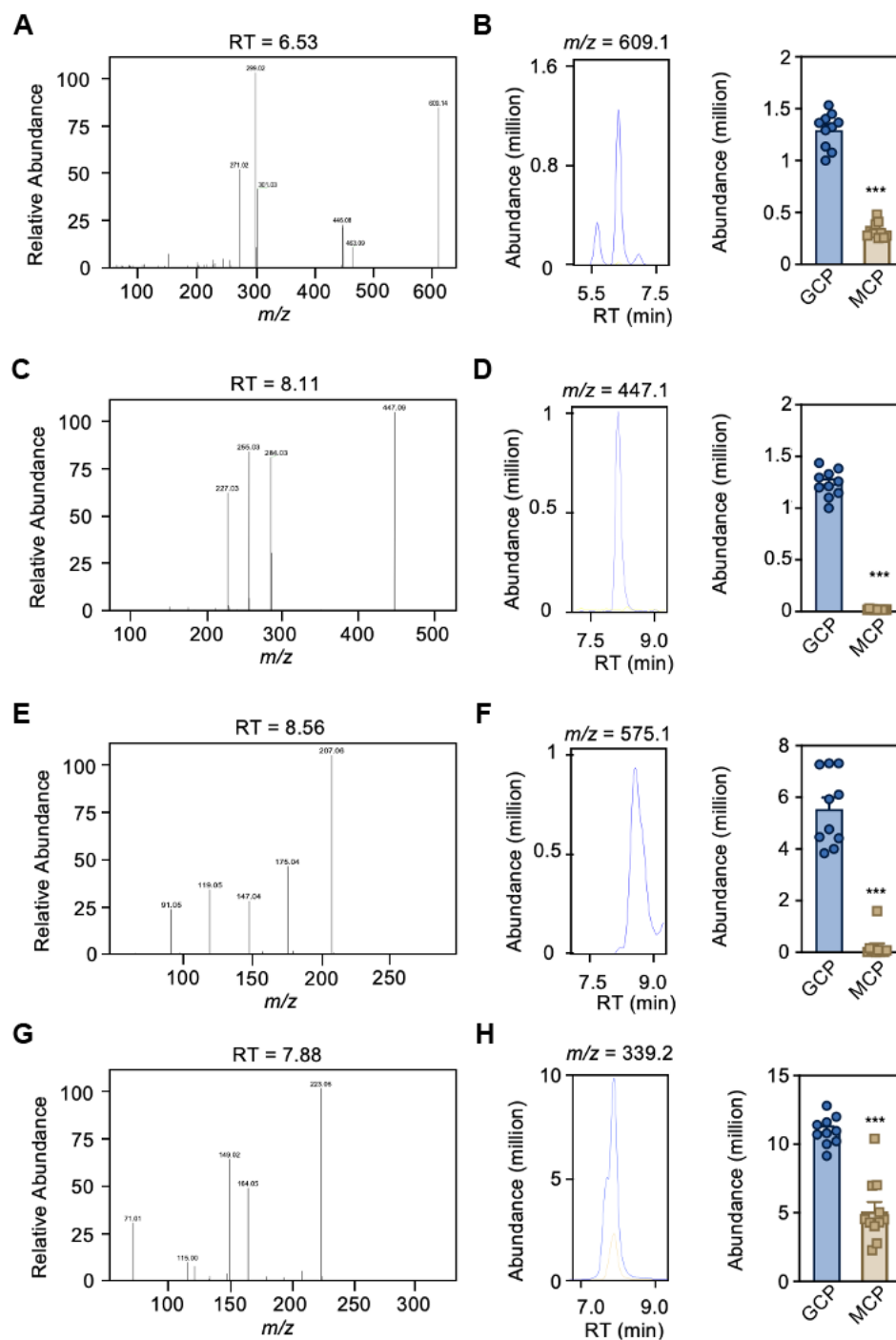


Figure S8. Flavonoid compounds specifically accumulate in GCPs

(A) The tandem MS spectra of the GCP-enriched peaks at 6.51 min. (B) Quantitative comparison of the compound shown in (A) between GCP and MCP. (C) The tandem MS spectra of the GCP-enriched peaks at 8.08 min. (D)

Quantitative comparison of the compound shown in **(C)** between GCP and MCP.
(E) The tandem MS spectra of the GCP-enriched peaks at 8.71 min. **(F)**
Quantitative comparison of the compound shown in **(E)** between GCP and MCP.
(G) The tandem MS spectra of the GCP-enriched peaks at 7.88 min. **(H)**
Quantitative comparison of the compound shown in **(G)** between GCP and MCP.

Dataset S1. All peptides identified in GCP

Dataset S2. This proteomics study compared with previous studies

Dataset S3. Quantitative comparison between GCP and intact GC proteomics

Dataset S4. All peptides identified in MCP

Dataset S5. Quantitative data of proteins identified in GCP and MCP

Dataset S6. GCP and MCP enriched proteins

Dataset S7. GO and KEGG analysis of GCP and MCP.

Dataset S8. Quantitative proteomics of epidermal peels before and after protoplasting

Dataset S9. Quantification and comparative analysis statistics of filtered mass features from untargeted metabolomics data

Dataset S10. Sequences of primers used in this study

Supplemental references

Supek, F., Bošnjak, M., Škunca, N., and Šmuc, T. (2011) REVIGO

summarizes and visualizes long lists of gene ontology terms. PLoS ONE **6**:
e21800.