**Fig.1** **Pearson correlation between RNA-seq samples.** R2: the square of the Pearson correlation coefficients.

**Fig. 2 Number (A) and Venn (B, C) of DEGs in different tissues of two cultivars.**

**Fig. 3** **Heatmap of the major differentially expressed genes (DEGs) in the exocarp (1), mesocarp (2), and endocarp (3) of the two cultivars of cucumber, including ‘H105’(A) and ‘H28’ (B).** The tree structures were generated separately for the two cultivars based on the similarity of the gene expression patterns of the three tissue samples. A1\_1-A1\_3: exocarp of ‘H105’, A2\_1-A2\_3: mesocarp of ‘H105’, A3\_1-A3\_3: endocarp of ‘H105’, B1\_1-B1\_3: exocarp of ‘H28’, B2\_1-B2\_3: mesocarp of ‘H28’, B3\_1-B3\_3: endocarp of ‘H28’.

**Fig. 4 Significantly enriched GO terms in cultivars H105 (A) and H28 (B).** The GO terms of the most significantly enriched genes in their three GO categories, Molecular function (MF), Biological process, and Cellular component.

**Fig. 5 The KEGG enrichment classification of DEGs in H105 (A) and H28 (B).** Rich Factor was the ratio of the differentially expressed number of genes located in the pathway. The higher the Rich Factor, the higher the degree of enrichment. The closer to zero, the more significant the enrichment.

**Fig. 6 Heatmap of DEGs involved in VB1 biosynthesis (A) and VE(B) biosynthesis in the cucumber fruit tissues, namely the exocarp (1), mesocarp (2), and endocarp (3) in the two tested cultivars of cucumber, ‘H105’and ‘H28’.** AIR, 5-aminoimidazole ribonucleotide; HMP-P: 4-aminp-2-methyl-5-hydroxymethylpyrimidine monophosphate; HMP-PP: 4-aminp-2-methyl-5-hydroxymethylpyrimidine diphosphate; HET-P, 4-methyl-5-(2-hydroxyethyl) thiazole phosphate; TMP: thiamin monophosphate; TDP, thiamine diphosphate; PDP: phytyl diphosphate; MPBQ: 2-methyl-6-phytyl-1,4-benzoquinol; DMPBQ: 2,3-dimethyl-6-phytyl-1,4-benzoquinone.

**Fig. 7 (A) Schematic diagram of different tissues in cucumber fruit (B) Ascorbic acid (AsA), dehydroascorbic acid (DHA), and Total ascorbate (T-AsA) contents in different tissues in the two cucumber cultivars of cucumber.** ‘H105’(A) and ‘H28’(B). Exocarp (1), Mesocarp (2), and endocarp (3) Numbers above the bar chart columns represent the AsA/DHA ratio. Error bars represent the mean ±SD of the three corresponding replicates. Different lowercase letters represent significantly different AsA content in the different tissues. A1: exocarp of ‘H105’, A2: mesocarp of ‘H105’, A3: endocarp of ‘H105’, B1: exocarp of ‘H28’, B2: mesocarp of ‘H28’, B3: endocarp of ‘H28’.

**Fig. 8** Heatmap of DEGs involved in AsA biosynthesis and the recycling pathways in the cucumber fruit tissues, namely the exocarp (1), mesocarp (2), and endocarp (3) in the two tested cultivars of cucumber, ‘H105’and ‘H28’.

**Fig. 9 Expression profiles of the genes involved in the L-galactose pathway in the different tissues of cucumber fruit, the exocarp (1), mesocarp (2), and endocarp (3), in the two cultivars of cucumber that were studied, ‘H105’(A) and ‘H28’ (B).** Error bars represent standard errors of the mean from three independent replicates. The broken line represents the relative expression level of genes measured by transcriptome. The column chart represents the relative expression level of genes by qRT-PCR.

**Fig. 10 Expression profiles of the genes involved in the AsA recycling pathway in the different tissues of cucumber, the exocarp (1), mesocarp (2), and endocarp (3), in the two cultivars of cucumber that were studied, ‘H105’(A) and ‘H28’ (B).** Error bars represent standard errors of the mean from three independent replicates.

**Table 1. The enrichment analysis of DEGs in GO.**

**Table 2. The enrichment analysis of DEGs in pathways.**

**Supplemental material**

**Supplemental Table S1. Sequences of specific primers used for quantitative real-time PCR.**

**Supplemental Table S2. MIQE checklist for qPCR methods and analysis.**

**Supplemental Table S3. RNA Quality assessment.**

**Supplemental Table S4. Overview of the transcriptome sequencing.**

**Supplemental Table S5. Power analysis between different groups.**

### Supplemental Table S6. FPKM of 33 validated genes in all samples in H105.

**Supplemental Table S7. FPKM of 33 validated genes in all samples in H28.**

**Supplemental Table S8. Raw qPCR data of 21 validated genes in all samples.**

**Supplemental Table S9. Raw data of AsA, DHA and T-AsA content in all samples.**

**Supplemental Figure 1 Top 20 of GO Enrichment in H105 (A) and H28 (B).**