

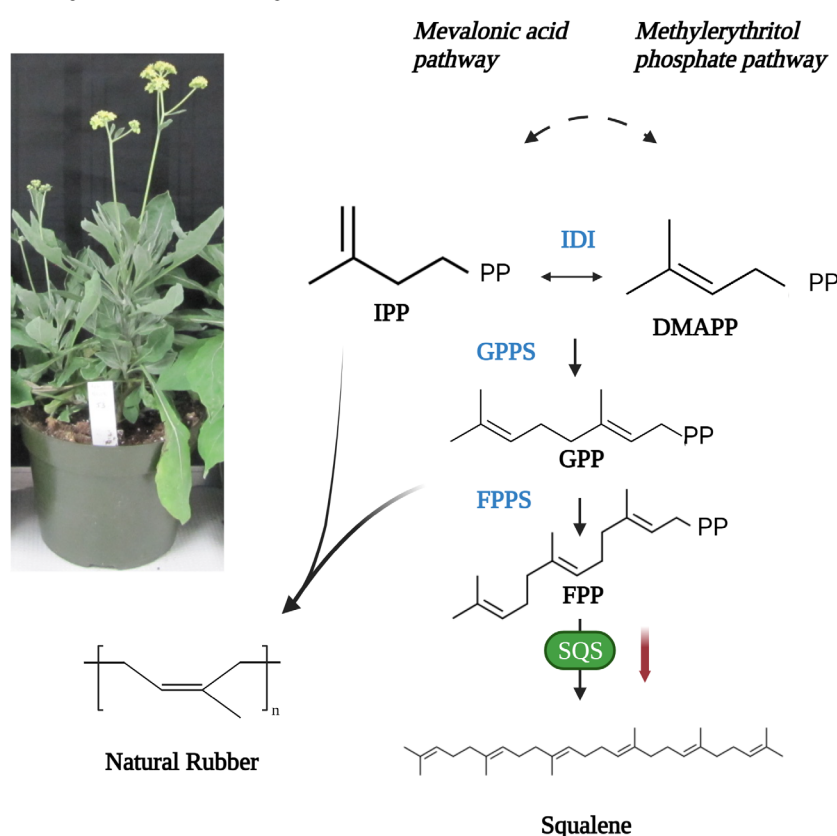
Downregulation of squalene synthase broadly impacts isoprenoid biosynthesis in guayule

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Supplementary Materials:

Natural Rubber and Squalene

Biosynthetic Pathway



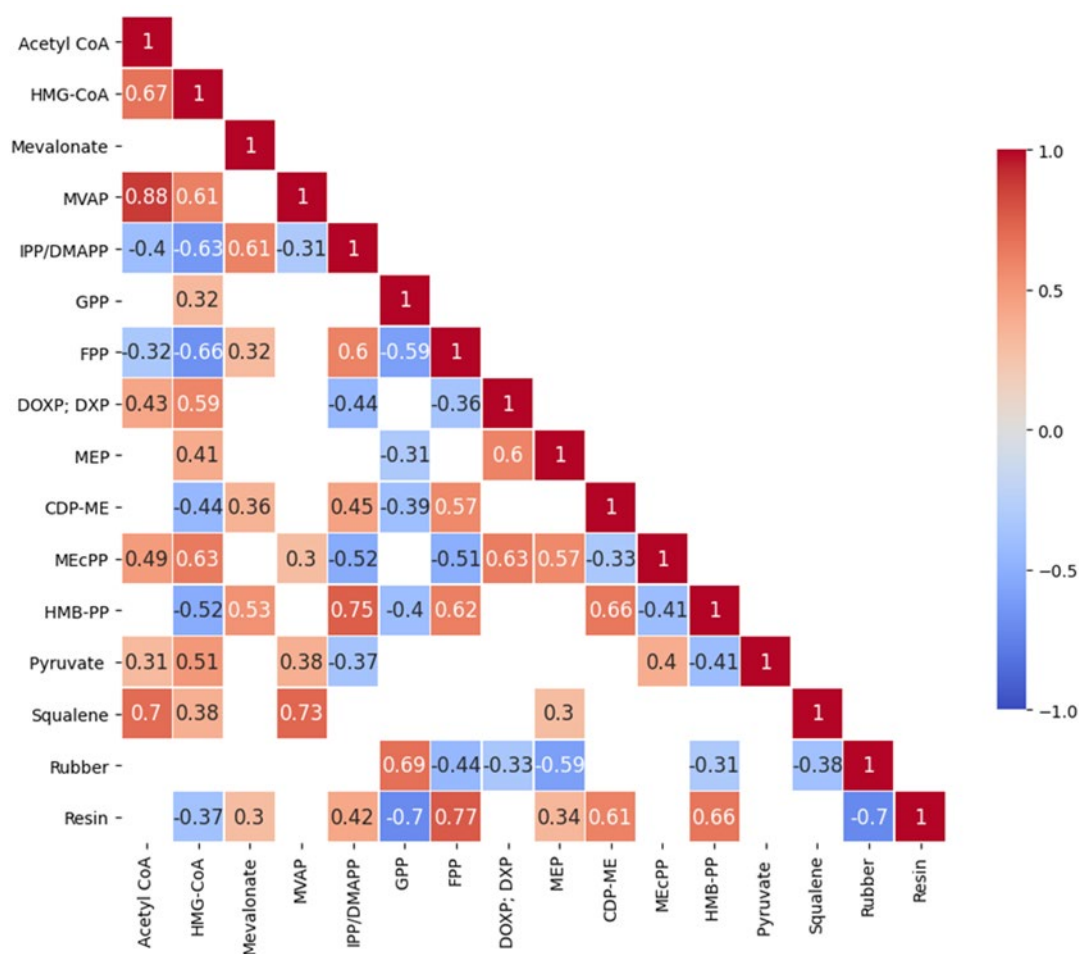
Supplementary Figure S1: Isoprenoid pathway schematic with squalene synthase downregulation: 1) the cytosolic Mevalonic acid pathway produces the 5 carbon isopentenyl pyrophosphate (IPP). The Methylerythritol phosphate pathway (chloroplast) produces dimethylallyl diphosphate (DMAPP), which can be isomerized by isopentenyl isomerase (IDI) to IPP. Other key components include: geranyl diphosphate (GPP) and its synthase (GPPS), farnesyl diphosphate (FPP) and its synthase (FPPS), squalene and natural rubber. Downregulation of squalene synthase (SQS) by RNAi technology is represented by the red arrow.



B.

Genotype	Plant phenotype characteristics						
	Height (cm)	Width (cm)	Stembark thickness (mm)	Number of branches	Number of flowering buds	SPAD reading	Dry Weight (g)
WT	22 ± 2	30 ± 3	2.1 ± 0.4	8 ± 1	59 ± 8	49 ± 3	11 ± 1
VC	22 ± 3	35 ± 4	1.7 ± 0.1	5 ± 1 ^a	5 ± 4 ^a	51 ± 1	13 ± 1
SQSi-J	25 ± 2	30 ± 2	1.6 ± 0.2	8 ± 2	50 ± 7	57 ± 4	12 ± 1
SQSi-L	23 ± 2	30 ± 3	1.9 ± 0.4	7 ± 1	33 ± 19	47 ± 1	11 ± 2
SQSi-M	23 ± 3	28 ± 2	1.3 ± 0.1 ^a	6 ± 1	17 ± 6 ^a	53 ± 6	9 ± 1

Supplementary Figure S2. Growth phenotypes of WT guayule and SQS transgenic lines. **A.** Images of 5-month-old plants, **B.** Phenotype characteristics: all values average of 3 biological replicates, +/- one standard deviation. 'a' indicates significantly different than WT control per student T-test at $p > 0.05$.



Supplementary Figure S3. Correlation matrix heatmap of precursor and intermediate compounds involved in natural rubber and squalene biosynthesis across all control lines and SQSi lines, with leaf, stem and root tissue. Negative correlations in blue, positive in red. Color intensity are proportional to the correlation coefficients, which are the numbers displayed for each pair. Correlations with p-value > 0.05 are considered as insignificant and did not show in the matrix.