Supplementary information

Biofortified tomatoes provide a new route to vitamin D sufficiency

In the format provided by the authors and unedited



Supplementary Figure 1 a. Vitamin D deficiency by geographical area. Cutoffs refer to serum concentrations of 25(OH)D used to define vitamin D deficiency. 25(OH)D=25-hydroxyvitamin D. Serum concentrations of 25(OH)D lower than 25 (or 30) nmol/L and 50 nmol/L are usually regarded as vitamin D deficiency and insufficiency, respectively. * Percentage for women of reproductive age; NA, data not available. b. Synthesis and activation of vitamin D₃ in human. Figure adapted from Jäpelt et al. (ref¹⁰). c. Adult plants of wild type and SI7-DR2 mutants. Scale bar, 20 cm. d. Yield of WT and SI7-DR2 mutant plants in g per plant (mean ± s.e.m, n=5 plants per line). Statistical significance between WT and mutants was assessed using two-tailed t-tests. No significant differences were detected. e.Stigmasterol content of leaves from wild-type and SI7-DR2 knock-out lines (mean ± s.e.m, n=5 biologically independent leaf samples per line). Statistical significance between WT and mutants was assessed using two-tailed t-tests. No significant differences were detected. f. MALDI images of 7-DHC (m/z 367.33) and its laser-induced derivative ions (m/z 365.32, m/z 363.31), cholesterol (m/z 369.35) and α-tomatine (m/z 1034.55). Scale bar, 2 mm. The HotMetal2 colour scale indicates the range of total ion current (TIC) -normalised intensity. The same metabolite is shown with identical scale intensity for wild type and mutant samples. More details can be found in the online methods. g. a-tomatine content of immature green fruit of wild-type and SI7-DR2 knock-out lines (mean ± s.e.m, n=6, 6, 8, 9, 12 and 5 biologically independent fruit samples). Statistical significance between WT and mutants was assessed using two-tailed t-tests (*P≤0.05, **P≤0.01). h. Cholesterol content of wild-type (WT) and SI7-DR2 knock-out tomato fruit during fruit ripening (IMG, immature green; MG, mature green; Breaker: fruit turning ripe; B+7, seven days after and 15 biologically independent fruit samples. Statistical significance between WT and mutants at each stage was assessed using two-tailed t-tests (* $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; **** $P \le 0.0001$). See source data for P values where relevant.



Supplementary Figure 2 a. Relative expression levels of genes in the cholesterol and phytosterol biosynthetic pathways in leaves of wild type and *SI7-DR2* mutants (mean ± s.e.m). *SIActin* was used as an internal standard. WT, n=5; *SI7-DR2* KO, n=15 (a combination of 5 samples each from Mut#1, Mut#2 and Mut#3, which carry the same mutation in *SI7-DR2*). **b.** Relative expression levels of *SIC5-SD1* in leaves of wild type and *SI7-DR2* mutants. *SIActin* was used as an internal standard (mean ± s.e.m, n=5 biologically independent leaf samples for each line). Statistical significance between WT and mutants was assessed using two-tailed t-tests (* $P \le 0.05$, ** $P \le 0.01$). See source data for *P* values where relevant. **c.** Representative LC-MS spectra of 7-DHC, vitamin D₃ and cholesterol from analysed samples and corresponding authentic standards. **d.** Overlayed chromatograms of extracts from Mut#1 leaf tissues with and without 1h UV irradiation (shown in light blue and black, respectively), as well as standard mix (10 µM of 7-DHC, vitamin D₃ and cholesterol), *m*/*z*= 385.3442-385.3480. Vitamin D₃ was present primarily in the UV irradiated sample.

Consensus Coverage	1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 ATGGGTGGAGAAACAAGTTGGTACACTCTCCCATTAATCACTTATGGATCAATGCTCAGTCTTTCTACACCACCTTTTGTAATCCTCATGTGGGTATACAAATGTGCACGCTGATGGGTCTATCTTGAAAACGTTCAATGTGGAAAATGGTTT 21
SI7-DR1_CDS	1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 Atggtggagaacaagtiggtacactetecattaateacttatggateaatgeteagtetatetteacaceaecttitgtaateeteatgtggtatacaaatgtgeteatgggtetatettgaaaaegtetatettgaaaaegteaatgegaaaatggttt
WT_SI7-DR1_CDS_F	
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Mut#3_SI7-DR1_CDS_R	
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Consensus Coverage1SI7-DR1_CDSWT_SI7-DR1_CDS_FWT_SI7-DR1_CDS_RMut#1_SI7-DR 1_CDS_FMut#2_SI7-DR 1_CDS_F	
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Consensus Coverage1SI7-DR1_CDS.WT_SI7-DR1_CDS_FWT_SI7-DR1_CDS_RMut#1_SI7-DR 1_CDS_RMut#2_SI7-DR1_CDS_RMut#2_SI7-DR1_CDS_RMut#3_SI7-DR1_CDS_RMut#3_SI7-DR1_CDS_RMut#4_SI7-DR 1_CDS_RMut#4_SI7-DR 1_CDS_RMut#5_SI7-DR 1_CDS_R	

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SI7-DR1_CDS	320 GGCTAATGGCA	330 Tggcagcatato	340 3 CAG <mark>TGACACTGA</mark>	350 360 TCACATATATCAG	370 TCTCTGGTGGTT	380 T <mark>ggaatattta</mark>	390 ATCCTGCAA1	400	410	420 GAGATTTTCT	430 TACACTTATT	440	450	460 TGTTTTATTGTAC
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Mut#2_SI7-DR1_CDS_F	GGCTAATGGCA		GCAGTGACACTGA			MMMM tggaatattta	мтсстбсал							
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Mut#4_SI7-DR 1_CDS_F	GGCTAATGGCA		GCAGTGACACTGA											
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WT_SI7-DR1_CDS_F	
WT_SI7-DR1_CDS_R	
Mut#1_SI7-DR 1_CDS_F	
Mut#1_SI7-DR 1_CDS_R	
Mut#2_SI7-DR1_CDS_F	CAGTTETTEETETECACETATTECATAGAAGAATATGGGCGTGTATETGATTECCATGCTTGTGAAATACGATATTGATGTCGTGAAAATTCTTTTGGTGGGAAGCTGGGAAGCTGGGGGGGAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAAGCTGGGAAGCTGGGGAAGCTGGGGAAGCTGGGGAGGGA
Mut#2_SI7-DR1_CDS_R	
Mut#3_SI7-DR1_CDS_F	CASTIFETTEETETEALESTATTECATAAAAAAGEATGATGATGEGEGEGETETATETEGATGETEGETAATTECATGETTAGTGTTAGTGTTAGTGTTAGTGTAAAAAAAATTECTTTTEGTGGGAAAGETGGGGAA
Mut#3_SI7-DR1_CDS_R	
Mut#4_SI7-DR 1_CDS_F	
Mut#4_SI7-DR 1_CDS_R	
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Consensus 1 Coverage	780 790 800 810 820 830 840 850 860 870 880 890 900 910 920 ACAGAGACTGGCTTTATATATGTTGGGGATGCTTGGTGGGGTTCCAAGCATATATACGTCTCCTGGAATGTTCTTGTTAAACAACCTGTGAATCTTGGGCTTCAGCTTTACATACTAGTGGCTGGC
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Consensus Coverage1SI7-DR1_CDS_F1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_R1Mut#2_SI7-DR1_CDS_R1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1	
Consensus Coverage1SI7-DR1_CDS_F1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_R1Mut#2_SI7-DR1_CDS_R1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_F1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR 1_CDS_R1Mut#4_SI7-DR 1_CDS_R1	
Consensus Coverage1SI7-DR1_CDS_F1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#4_SI7-DR1_CDS_R1Mut#5_SI7-DR1_CDS_R1	

Consensus 11 Coverage	930 940 AACTATGATTGTGACAG 21	950 gcagaggcaad	960 Sagttccggag	970 Saacaaatgga	980 AAAATGCACT	990 gtctggggaa/	1,000 Agactccatct	1,010 FAAGATAGTC	1,020 GCCGCATACAC	TACTACCTCI	GGTGAGAAAA	1,050 AAGACCAGCC	1,060 FTCTGCTTACA	1, 070 .tcaggatggtgg
SI7-DR1_CDS	930 940 AACTATGATTGTGACAG	950 GCAGAGGCAAC	960 SAG TTCCGGAG	970 SAACAAATGGA	980 AAAA <mark>TGCACT</mark>	990 g tct ggggaa/	1,000 AGACTCCATCT	1,010 TAAGATAGTC	1,020 GCCGCATACAC	1,030 TACTACCTC1	1,040 GGTGAGAAAA	1,050	1,060	1,070 TCAGGATGGTGG
WT_SI7-DR1_CDS_F														
WT_SI7-DR1_CDS_R						мурия атстададаа								
Mut#1_SI7-DR 1_CDS_F														
Mut#1_SI7-DR 1_CDS_R						му ММ					GGTGAGAAAA			
Mut#2_SI7-DR1_CDS_F										•				M
Mut#2_SI7-DR1_CDS_R				MMM GAACAAAT GGA	MAAATGCACT	<u>му</u>					GGTGAGAAAA		ITCTGCTTACA	
Mut#3_SI7-DR1_CDS_F			A	A.A						A . A			٨	1
Mut#3_SI7-DR1_CDS_R		GCAGAGGCAAG	GAGTTCCGGAG	MWWW Gaacaaatgga	WWWM AAAATGCACT	муруун Стстбеббаал	AGACTCCATCT	FAAGATAGTC	GCCGCATACAC		GGTGAGAAAA		TTCTGCTTACA	TCAGGATGGTGG
Mut#4_SI7-DR 1_CDS_F			NA	AAA	ΔΛΛ		1			٨	. ^ ^^^/	1. 1. 1. 1. 1.		
Mut#4_SI7-DR 1_CDS_R		GCAGAGGCAAG	GAGTTCCGGAG	GAACAAATGGA	MMMM AAAATGCACT	GTCTGGGGAA	AGACTCCATCT		GCCGCATACAC		GGTGAGAAAA		TCTGCTTACA	
Mut#5_SI7-DR 1_CDS_F	A. A. A. A.A.		N	NA 1010			1 00/00	1		٨		1.0 0.0. 0.0.		11
Mut#5_SI7-DR 1_CDS_R		GCAGAGGCAAG	GAGTTCCGGAG	<u>aacaaatgg</u> a	WWW/WM	GTCTGGGGAA/	AGACTCCATCT	FAAGATAGTC	<u>vwww</u> gccgcatacad		GGTGAGAAAA	WWWWW Agaccagcc	ITCTGCTTACA	TCAGGATGGTGG
									1 170	4 4 9 9				
Consensus 1: Coverage	1,080 1,090 GGCTTAGCTCGCCATT 21	1,100 ГССАСТАТБТТ	1,110 	1,120 TAGCGGCATT	1,130 TTTCTGGAG	1,140 rgtaccagctc	1,150 TTTTCAACCA	1,160 .cttcattccc	TACTTCTACG	1,180 тсатстттст	1,190 ААТААТССТС	1,200 CTTCTCGATC	1,210 GAGCTAAAAG	1,220 ggacgatgaccga
Consensus Coverage	1,080 1,090 GGCTTAGCTCGCCATT 1,080 1,090 GGCTTAGCTCGCCATT	1,100 гссастатотт 1,100 гссастатотт	1,110 CCAGAAATAT 1,110 CCAGAAATAT	1,120 TAGCGGCATT 1,120 TAGCGGCATT	1,130 TTTCTGGAG 1,130 TTTCTGGAG	1,140 fgtaccagete 1,140 fgtaccagete	1,150 TTTTCAACCA 1,150 TTTTCAACCA	1,160 CTTCATTCCC 1,160 CTTCATTCCC	1,170 TACTTCTACG	1,180 TCATCTTTCT 1,180 TCATCTTTCT	1,190 AATAATCCTC 1,190 AATAATCCTC	1,200 CTTCTCGATC 1,200 CTTCTCGATC	1,210 gagctaaaagu 1,210 gagctaaaagu	1,220 GGACGATGACCGA 1,220 GGACGATGACCGA
Consensus Coverage		1,100 rccactatgtt 1,100 fccactatgtt	1,110 (CCAGAAATAT) 1,110 (CCAGAAATAT	1,120 TAGCGGCATT 1,120 TAGCGGCATT	1,130 TTTCTGGAG 1,130 TTTCTGGAG	1,140 IGTACCAGCTC 1,140	1,150 TTTTCAACCA 1,150 TTTTCAACCA	1,160 CTTCATTCCC 1,160 CTTCATTCCC	1,170	1,180 TCATCTTTCT 1,180 TCATCTTTCT	1,190 AATAATCCTC 1,190 AATAATCCTC	1,200 CTTCTCGATC 1,200 CTTCTCGATC	1,210 gagctaaaagu 1,210 gagctaaaagu	1,220 56ACGATGACCGA 1,220 56ACGATGACCGA
Consensus Coverage 12 SI7-DR1_CDS WT_SI7-DR1_CDS_F WT_SI7-DR1_CDS_R			1,110 ССАБАЛАТАТ 1,110 ССАБАЛАТАТ	1,120 TAGCGGCATT 1,120 TAGCGGCATT	1,130 TTTCTGGAG							1,200 CTTCTCGATC 1,200 CTTCTCGATC	1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG	
Consensus Coverage 12 SI7-DR1_CDS WT_SI7-DR1_CDS_F WT_SI7-DR1_CDS_R Mut#1_SI7-DR 1_CDS_F			1,110 ССАБАЛАТАТ 1,110 ССАБАЛАТАТ	1,120 TAGCGGCATT	1,130 1,130 1,130 1,130 1,130 1,130 1,110 1,130						1,190 1,190 1,190 AATAATCCTC	1,200 1,200 1,200 CTTCTCGATC	1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG	
Consensus 12 Coverage 12 SI7-DR1_CDS 12 WT_SI7-DR1_CDS_F 12 WT_SI7-DR1_CDS_R 12 Mut#1_SI7-DR 1_CDS_F 12 Mut#1_SI7-DR 1_CDS_R 12				1,120 TAGCGGCATT 1,120 TAGCGGCATT TAGCGGCATT TAGCGGCATT	1,130 1,130 1,130 1,130 1,130 1,130 1,130 1,150 1,130 1,150						1,190 1,190 латалтсстс		1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG GAGCTAAAAGG GAGCTAAAAGG	
Consensus Coverage12SI7-DR1_CDS12WT_SI7-DR1_CDS_F12WT_SI7-DR1_CDS_R12Mut#1_SI7-DR 1_CDS_F12Mut#2_SI7-DR1_CDS_F12				1,120 1,120 1,120 TAGCGGCATT TAGCGGCATT	1,130 1,130 1,130 1,130 1,130 1,130 1,130 1,150								1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG GAGCTAAAAGG GAGCTAAAAGG	
Consensus Coverage1SI7-DR1_CDS1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_R1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_R1				1,120 1,120 TAGCGGCATT 1,120 TAGCGGCATT TAGCGGCATT									1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG GAGCTAAAAGG GAGCTAAAAGG	
Consensus Coverage12 CoverageSI7-DR1_CDS1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_R1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1				1,120 TAGCGGCATT 1,120 TAGCGGCATT	1,130 1,150 1,									
Consensus Coverage12 CoverageSI7-DR1_CDS_F1WT_SI7-DR1_CDS_F1Wt_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_R1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#3_SI7-DR1_CDS_F1Mut#3_SI7-DR1_CDS_F1				1,120 TAGCGGCATT 1,120 TAGCGGCATT TAGCGGCATT TAGCGGCATT	1,130 1,130 1,130 1,130 TTTCTCGAG								1,210 GAGCTAAAAGG 1,210 GAGCTAAAAGG GAGCTAAAAGG GAGCTAAAAGG GAGCTAAAAGG	
Consensus Coverage1 2SI7-DR1_CDS_F1WT_SI7-DR1_CDS_F1Wt_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#3_SI7-DR1_CDS_F1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR 1_CDS_R1				1,120 1,120 TAGCGGCATT 1,120 TAGCGGCATT TAGCGGCATT TAGCGGCATT										
Consensus Coverage1SI7-DR1_CDS1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR 1_CDS_R1Mut#4_SI7-DR 1_CDS_R1				1,120 TAGCGGCATT 1,120 TAGCGGCATT										
Consensus Coverage1SI7-DR1_CDS1WT_SI7-DR1_CDS_F1WT_SI7-DR1_CDS_R1Mut#1_SI7-DR 1_CDS_F1Mut#2_SI7-DR1_CDS_F1Mut#2_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#3_SI7-DR1_CDS_R1Mut#4_SI7-DR 1_CDS_R1Mut#4_SI7-DR 1_CDS_R1Mut#5_SI7-DR 1_CDS_R1				1,120 TAGCGGCATT 1,120 TAGCGGCATT TAGCGGCATT TAGCGGCATT TAGCGGCATT										



Supplementary Figure 3 Sequencing results of coding sequences (CDS) of *SI7-DR1* amplified from cDNA of wildtype and five independent SI7-DR2 knock-out lines. No mutations of *SI7-DR1* were detected in any of the mutants.

Supplementary Table 1. Primers used in RT-qPCR.

Gene	Oligo	Sequence	Product Size		
	ACT-q_F	GGGGGCTATGAATGCACGGT			
SIActin (Solyc03g078400)	ACT-q_F	GGCAATGCATCAGGCACCTC	110bp		
	SSR2_q_F1	TGTCATTCAAGATCTCCTTGTTCCT			
SISSR2 (Solyc02g069490)	SSR2_q_R1	CAAATTGGATATACCTCCATCTCGC	87bp		
	7-DR1_q_F1	TTGGGCTTCAGCTTTCCCTT			
SI7-DR1 (Solyc01g009310)	7-DR1_q_R1	AACTCTTGCCTCTGCCTGTC	88bp		
	SMO4_q_F1	GTCCATCATGAGTACGCGACA			
SISMO4 (Solyc06g005750)	SMO4_q_R1	ATTGCAGGACCAATGACCGT	101bp		
	SMO3_q_F1	TCACAGCGGGTATGAATTTCCA			
SISMO3 (Solyc01g091320)	SMO3_q_R	ACTGTGACAACTTTCCCCAACA	106bp		
SI3βHSD2	3βHSD2_q_F	TAAAGGCCTGCTGACATGCT			
(Solyc02g081730)	3βHSD2_q_R	GATTTTCCAGCCCTTGCAGC	102bp		
	SDR_q_F	GCACGCGGCTAAAGTTATGG			
SISDR (Solyc01g073640)	SDR_q_R	GGCCCCAGCTAAAATCCCTT	85bp		
	C5-SD2_q_F	GGAAGCCTTATGGACGGCTA			
SIC5-SD2 (Solyc02g086180)	C5-SD2_q_R	TGGTATGATAACCGGCACCC	80bp		
	CPI_q_F	CCCTAGCAAGAGATGGGGTG			
SICPI (Solyc12g098640)	CP1_q_R	TGGGACCACAATACCAAGGC	82bp		
	CYP51_q_F	CGTACAGGCAAGGCAGAGAA			
SICYP51 (Solyc01g008110)	CYP51_q_R	TCTCACCCTCTGTTGTTGGC	85bp		
	SMO1_q_F	GGCCAAAGCCAGAGCAATTT			
SISMO1 (Solyc08g079570)	SMO1_q_R	AGCACCCTTTAACTGCTGGA	111bp		
	SMO2_q_F	AGAAAGGGCAGGATGGTTGAG			
SISMO2 (Solyc06g076410)	SMO2_q_R	ATACAGCAACAGGCGAGTGA	94bp		
	8,7 SI_q_F	TTGCCTGGTTTTGTTCCCCT			
SI8,7 SI (Solyc06g082980)	8,7 SI_q_R	AACACCACAACCAGTACGGA	83bp		
	7-DR2_q_F	GAGAATGGCCTGCAAGGACTA			
SI7-DR2 (Solyc06g074090)	7-DR2_q_R	AGGCAACAAAAGCTGAAGTGT	111bp		
	C-14R_q_F	CGGTGTTCAGAGGAGCCAAT			
SIC14-R (Solyc09g009040)	C-14R_q_R	GAAGCAAGCAACTTTCCCCC	106bp		
	CAS_q_F	GTATTGCTAAAGCTGCCGCC			
SICAS (Solyc04g070980)	CAS_q_R	GGTGAAGCAAACTGCCCAAG	86bp		
	C5-SD1_q_F	ACTCTCCCCATTTGCTGGTT			
SIC5-SD1 (Solyc02g063240)	C5-SD1_q_R	GTGTGTGGTGAAATGCACAGG	106bp		

Supplementary Table 2. Detailed information for the identification of cholecalciferol (vitamin D₃), 7-DHC and cholesterol by MALDI.											
	Standards spot WT tis	ted side by sid sue section	le on		Stand	dards spotted on glass slides separa	itely		m/z Obser	ved in mut localisatio	ants with same typical on pattern
m/z	normalised	drift time	colocalised	cholcal		7dh chol Mut#1		#1	Mut#5		

by MAL	y MALDI.															
Standards spotted side by side on WT tissue section Standards spotted on glass slides separately								m/z Observed in mutants with same typical localisation pattern								
m/2	normalised	malised drift time	colocalised	cholcal				7dh		chol			Mut#1 Mut#5			#5
11/2	intensity	(dt) (bins)	ins) with	m/z	intensity	dt bins	m/z	intensity	dt bins	m/z	intensity	dt bins	m/z	dt bins	m/z	dt bins
360.3638	0.06069	12.30	7dh and chol	360.3530	1708	12.27				360.3619	607	12.26				
361.3667	0.01652	12.28					361.2889	2285	10.88							
*363.3063	0.06303	11.00	7dh	*363.305	682	10.97	*363.3047	17699	10.92				*363.3043	10.93	*363.3058	11.03

*363.3063	0.06303	11.00	7dh	*363.305	682	10.97	*363.3047	17699	10.92				*363.3043	10.93	*363.3058	11.03
							364.3088	6183	10.95							
*365.321	0.0324	10.99	7dh	*365.3204	4144	11.08	*365.3192	15508	10.92				*365.319	10.93	*365.3182	11.01
366.3024	0.0083	11.25	7dh	366.3253	1884	11.24	366.3237	6149	11.01							
				*367.3357	11782	11.23	*367.3301	7997	11.14	*367.3384	1366	11.22	*367.3336	11.27	*367.3242	11.43
				368.3391	3171	11.24	368.3340	2587	11.18	368.3438	1037	11.39				
#369.3471	0.2623	11.74	chol	#369.3192	1713	11.39	#369.3162	4587	11.04	#369.3546	14248	11.54	#369.3381	11.78	#369.3350	11.82
370.2974	0.01066	11.27	7dh and chol							370.3576	3924	11.55				
							373.3100	1862	10.91							
^376.3227	0.02078	11.56	cholcal													
^377.3254	0.01356	11.55	cholcal													
							379.2997	6491	11.20							
380.3334	0.25962	10.42					380.3202	5738	11.33							
*381.3328	0.08153	10.68		*381.3138	1098	11.48	*381.3159	13342	11.20				*381.3266	10.79	*381.3273	11.00
382.3397	0.0398	10.80		382.3255	906	11.69	382.3328	7426	11.07				382.3340	10.77	382.3319	11.21
				383.3272	3248	11.71	383.3342	4302	11.20				383.3567	11.62	383.3497	11.78
				384.3326	1615	11.86	384.3433	3929	11.47							
				385.3326	2458	11.69										
388.3957	0.04975	13.18	7dh and chol	388.3934	931	13.26				388.3950	1023	13.19				
389.3983	0.03727	13.17	7dh and chol													

*cholcal: Cholecalciferol, vitamin D₃; 7dh: 7-dehydrocholesterol (7-DHC); chol: Cholesterol. Representative mass for 7-DHC, cholesterol and vitamin D₃ are highlighted and marked as *, # and ^, respectively.

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