

Transcriptomics insights into the genetic regulation of root apical meristem exhaustion and determinate primary root growth in *Pachycereus pringlei* (Cactaceae)

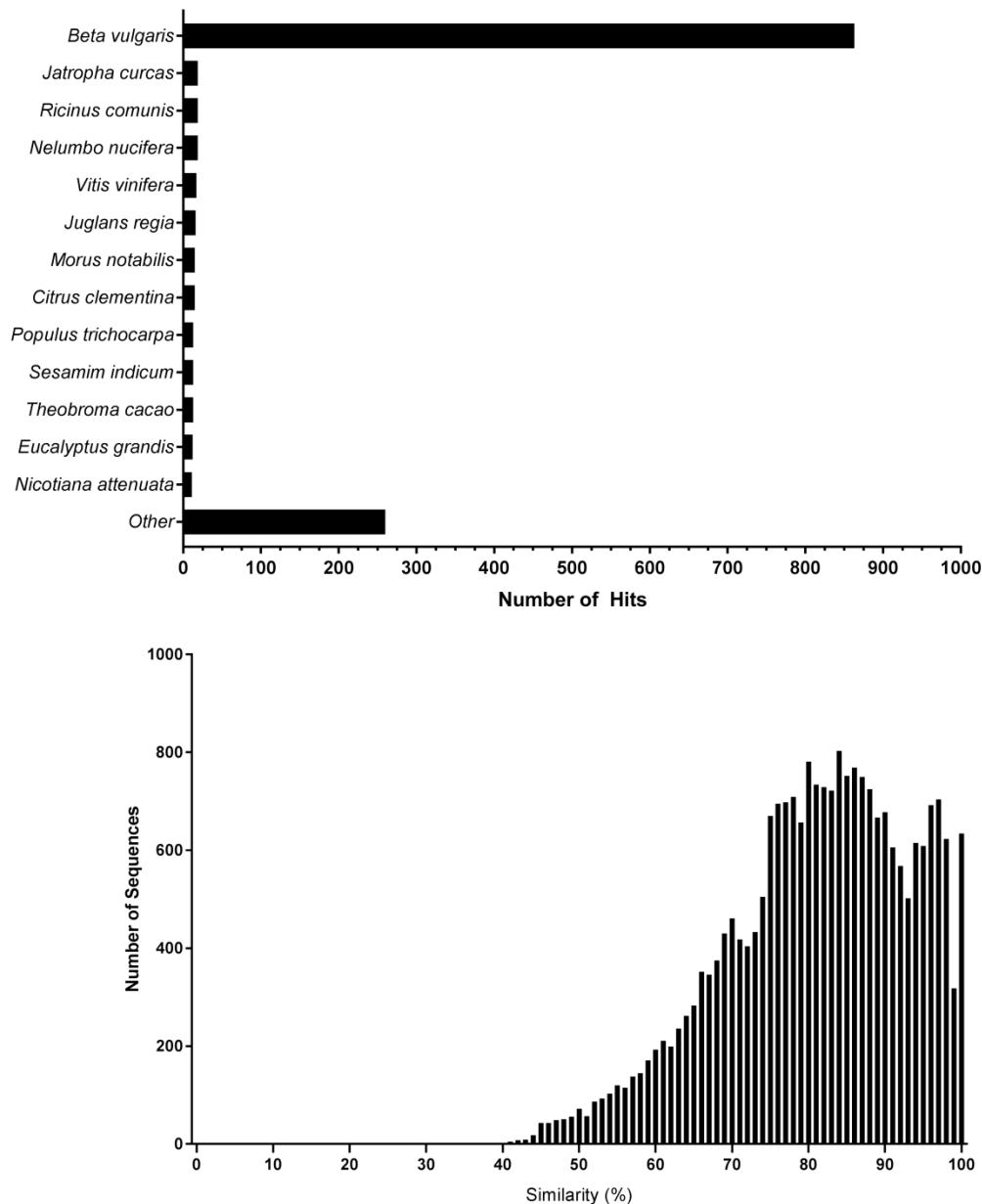
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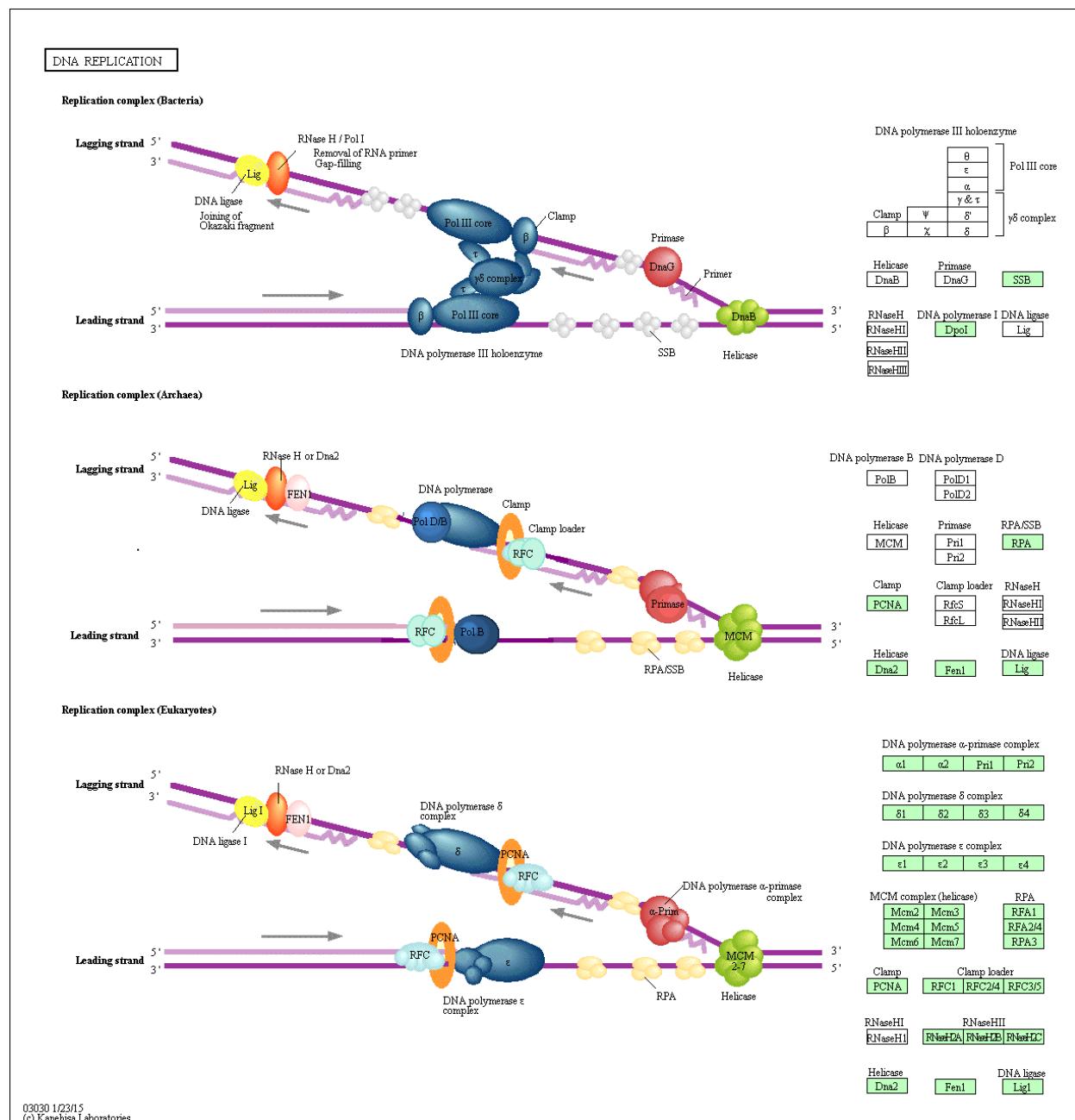
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Supplementary Figure S1. B2GO annotation statistics for the *de novo* assembled transcriptome of the *Pachycereus pringlei* primary root apex. Note: the following stats are from a representative subset of the total contigs. Upper panel: top hit species during the B2GO annotation; lower panel: distribution of similarity values between *P. pringlei* translated contigs and the top hit sequences during B2GO annotation.

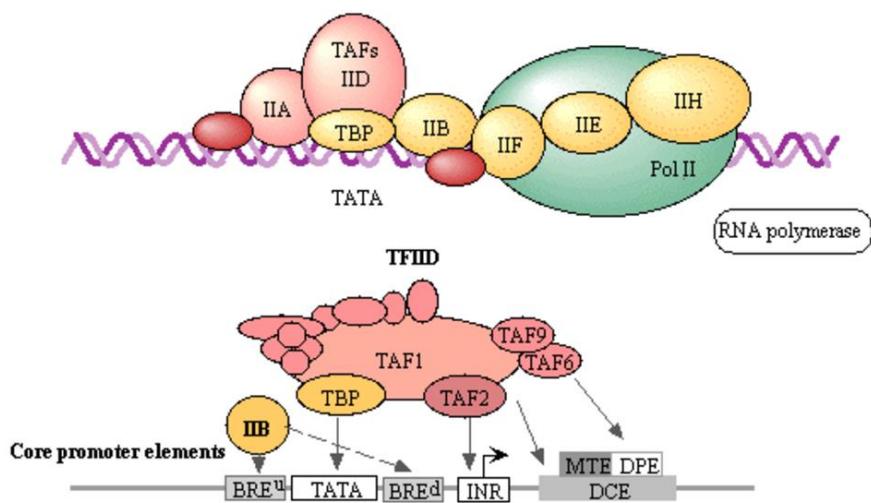


Supplementary Figure S2. The *Pachycereus pringlei* transcriptome is comprehensive.

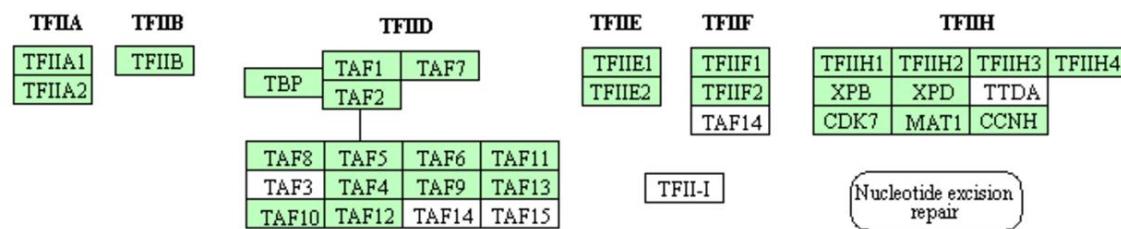
KEGG Automatic Annotation Server mapping of the *de novo* assembled primary root apex transcriptome of *P. pringlei* to some biological processes and pathways. The boxes in each process or pathway represent enzymes or structural proteins. Green boxes: at least one *P. pringlei* putative ortholog was identified in the transcriptome; white boxes: no *P. pringlei* putative ortholog was identified. Permission to use the schemes was kindly granted by Kanehisha Laboratories. Schemes were generated by the KAAS mapping tool (Moriya *et al.*, 2007, DOI:10.1093/nar/gkm321). Reference: Kanehisa M., Furumichi M., Tanabe M., Sato Y., Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nuc. Ac. Res. 45, D353-D361 (2017).



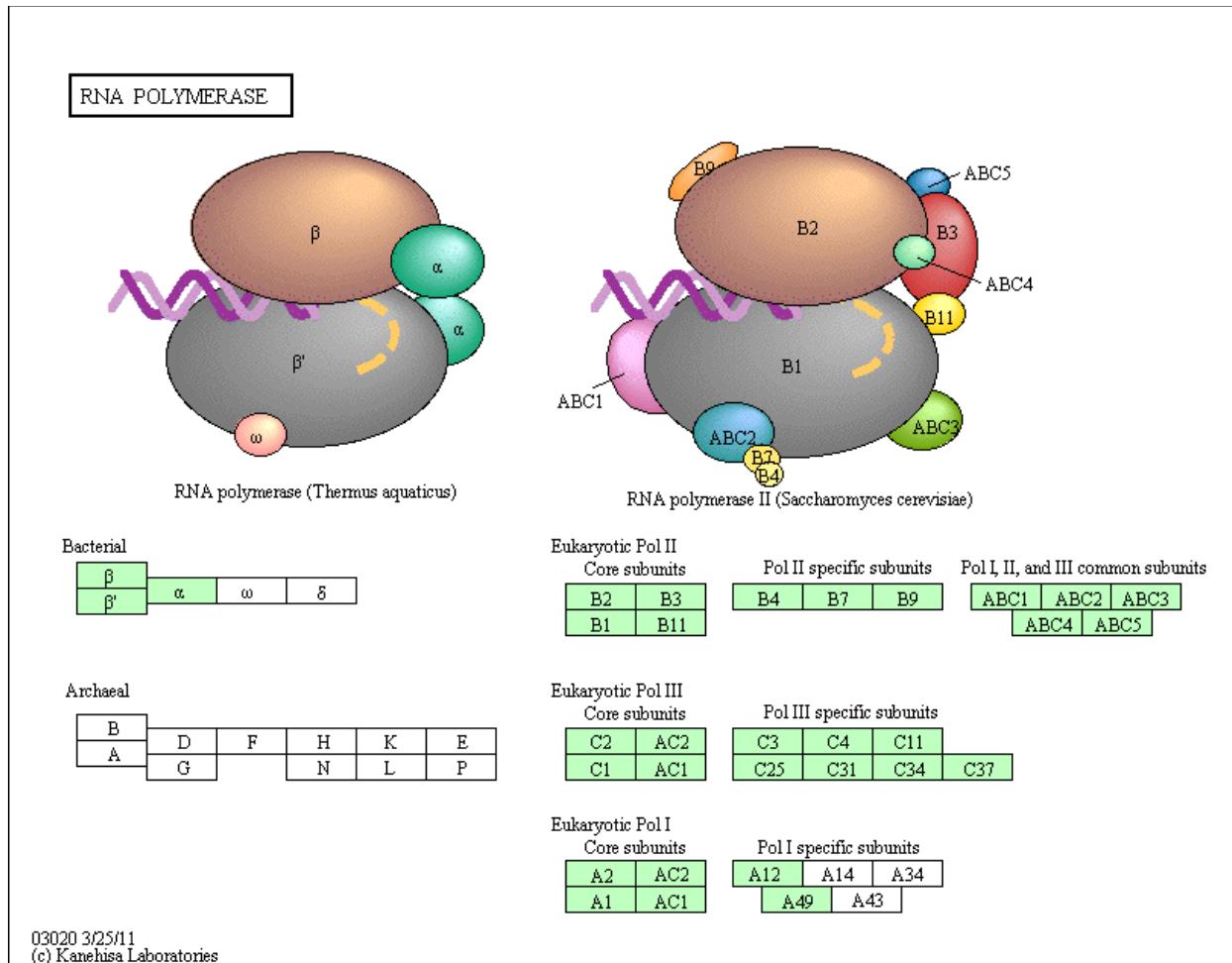
BASAL TRANSCRIPTION FACTORS (EUKARYOTES)

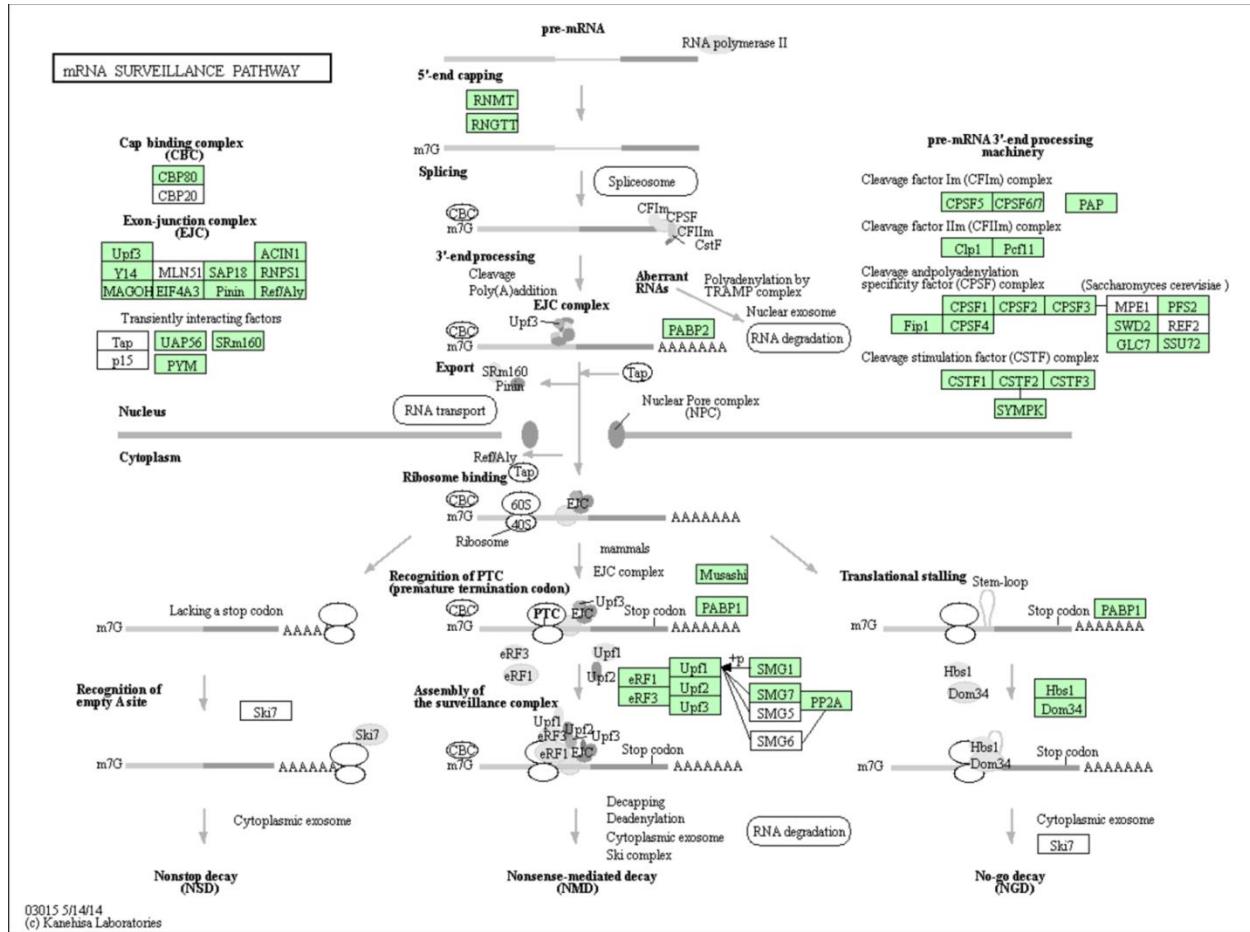


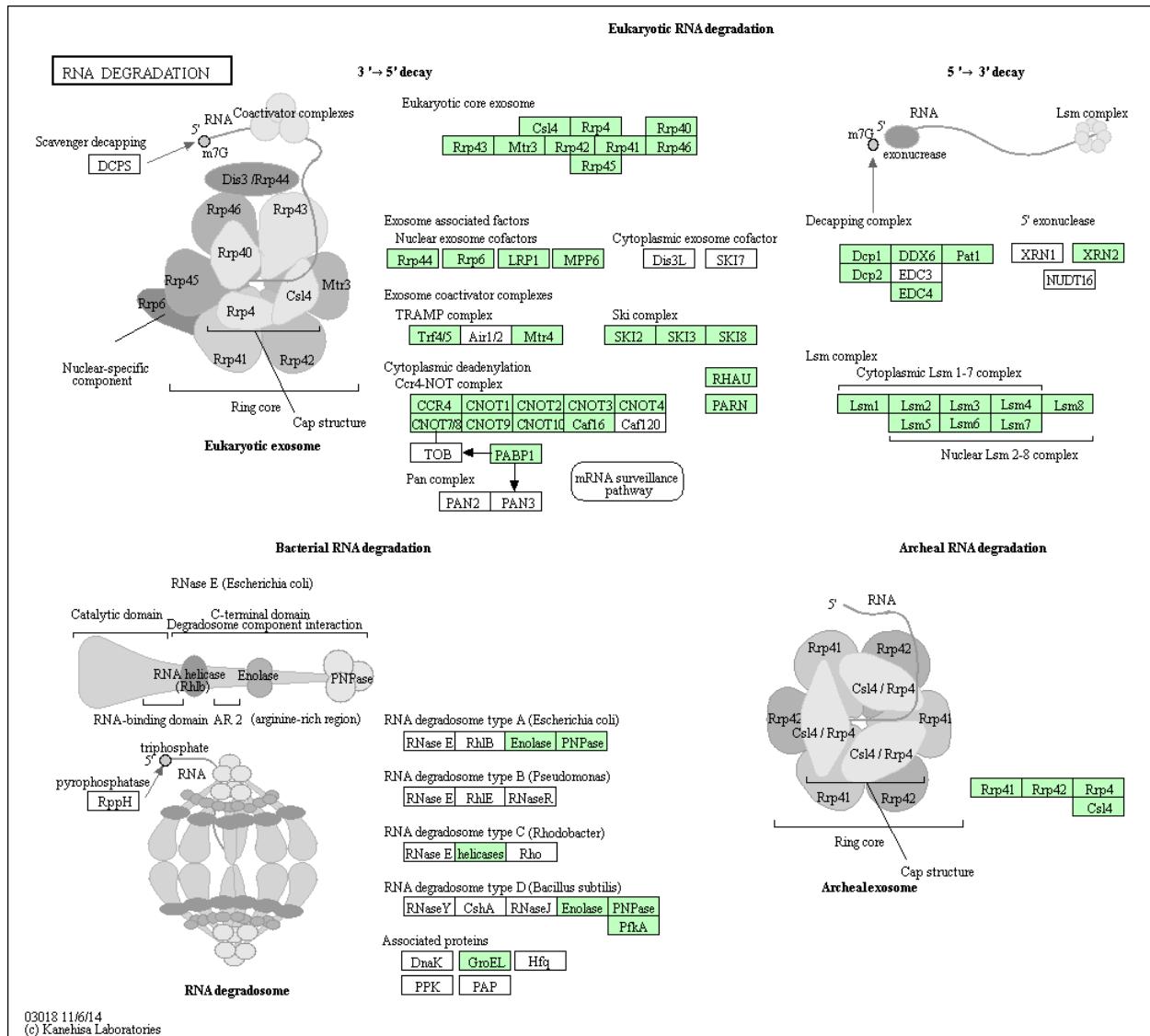
General transcription factors for RNA polymerase II



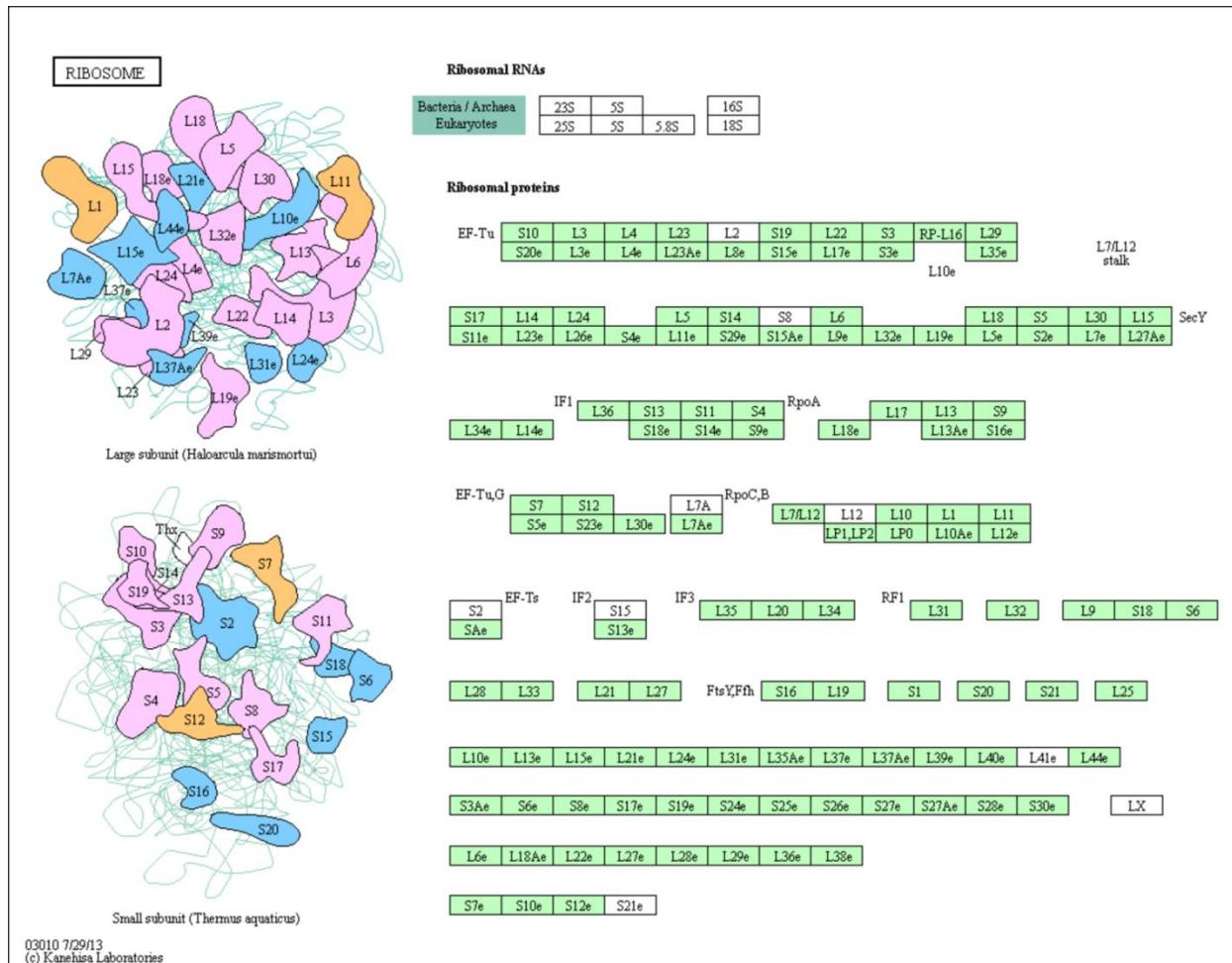
03022 3/15/11
(c) Kanehisa Laboratories



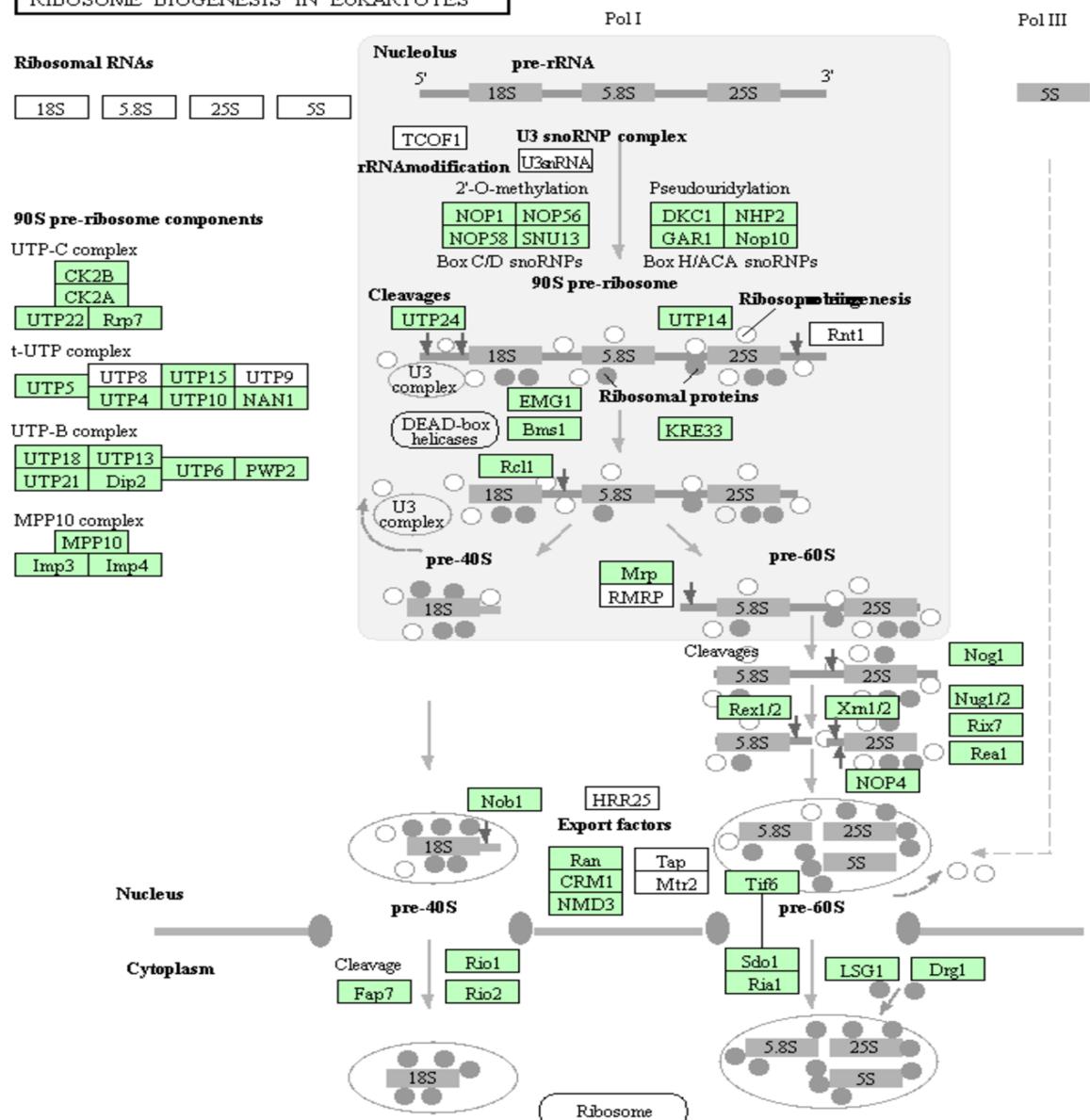




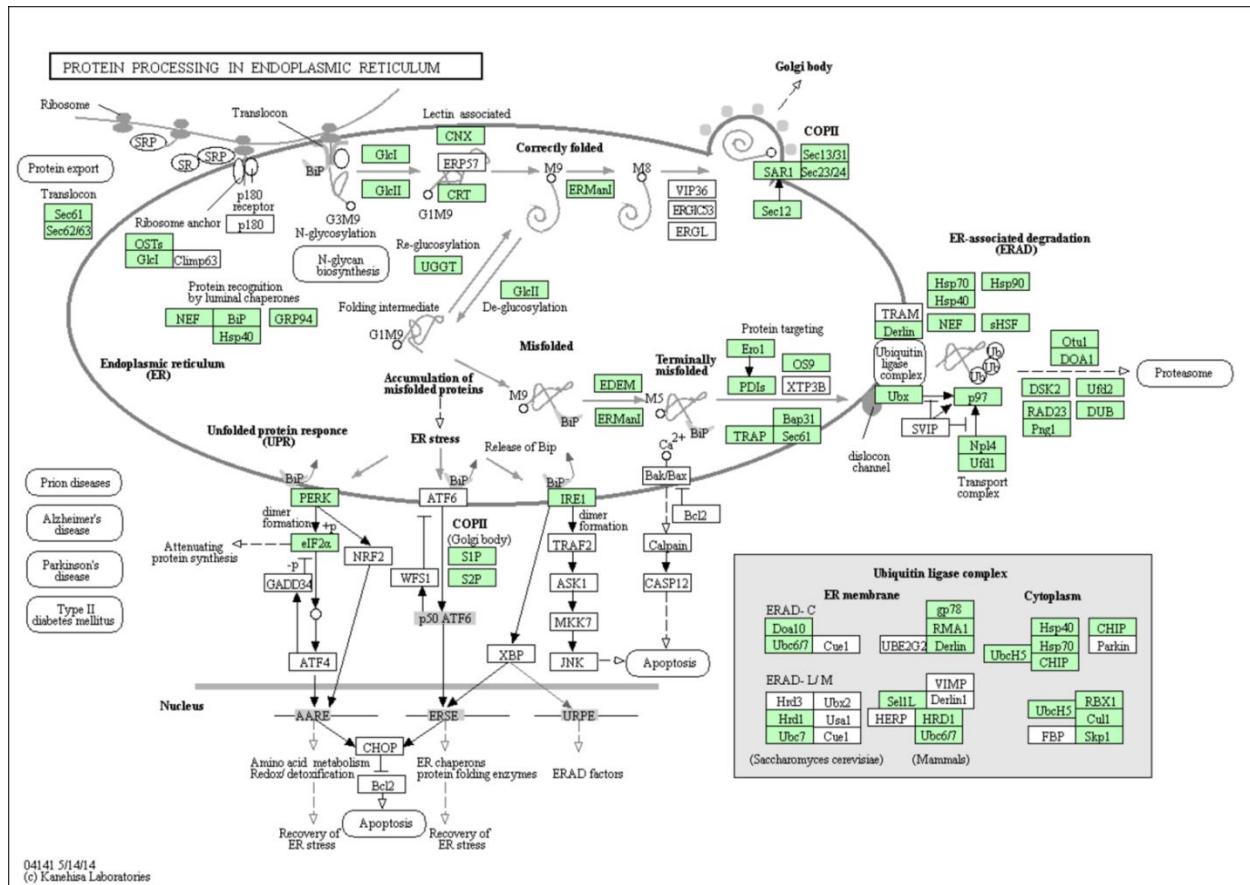
03018 11/6/14
(c) Kanehisa Laboratories

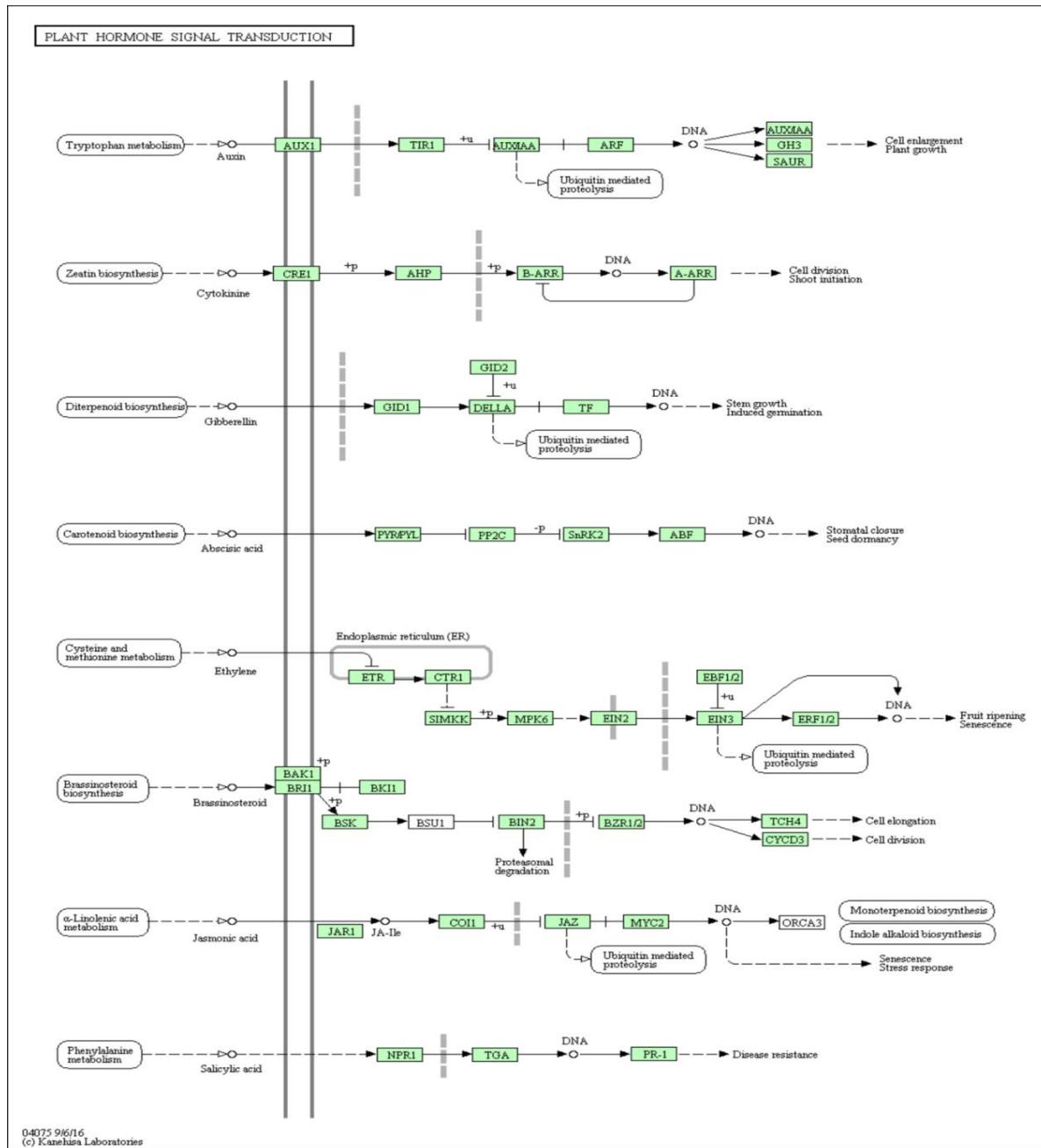


RIBOSOME BIOGENESIS IN EUKARYOTES

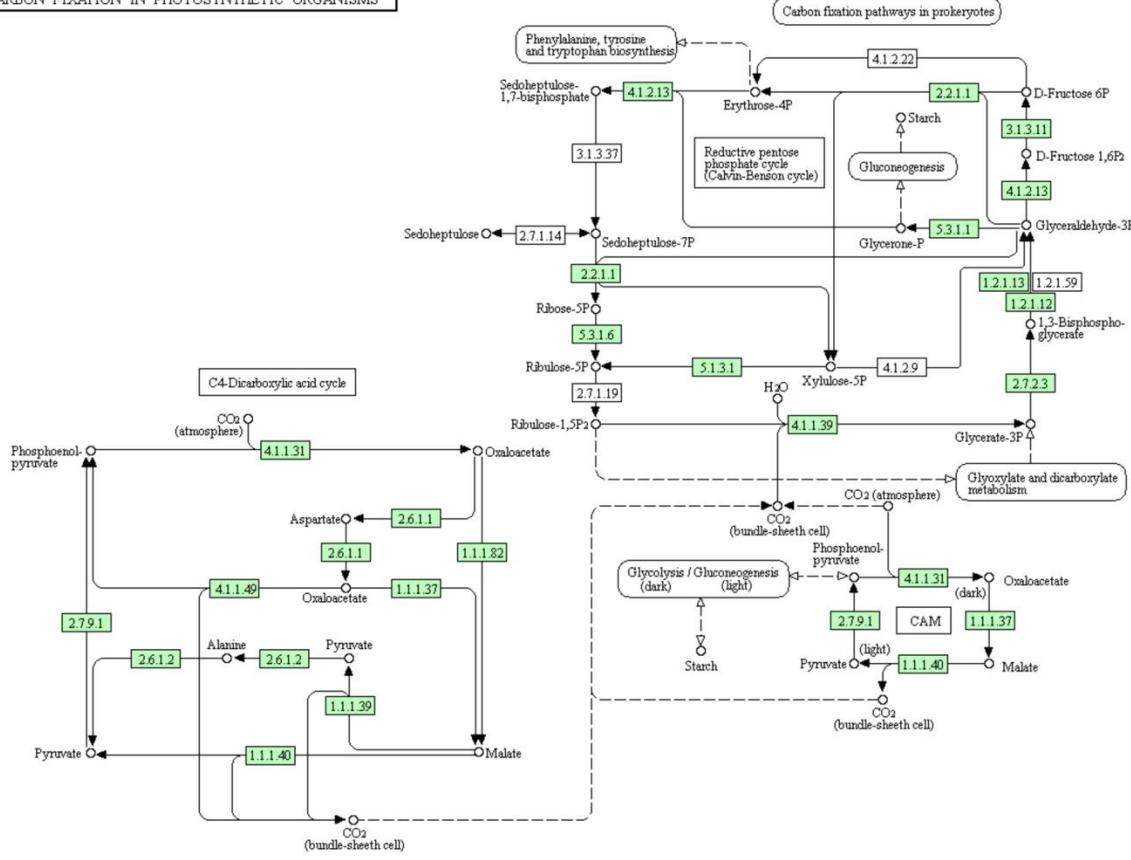


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 (c) Kanelusa Laboratories

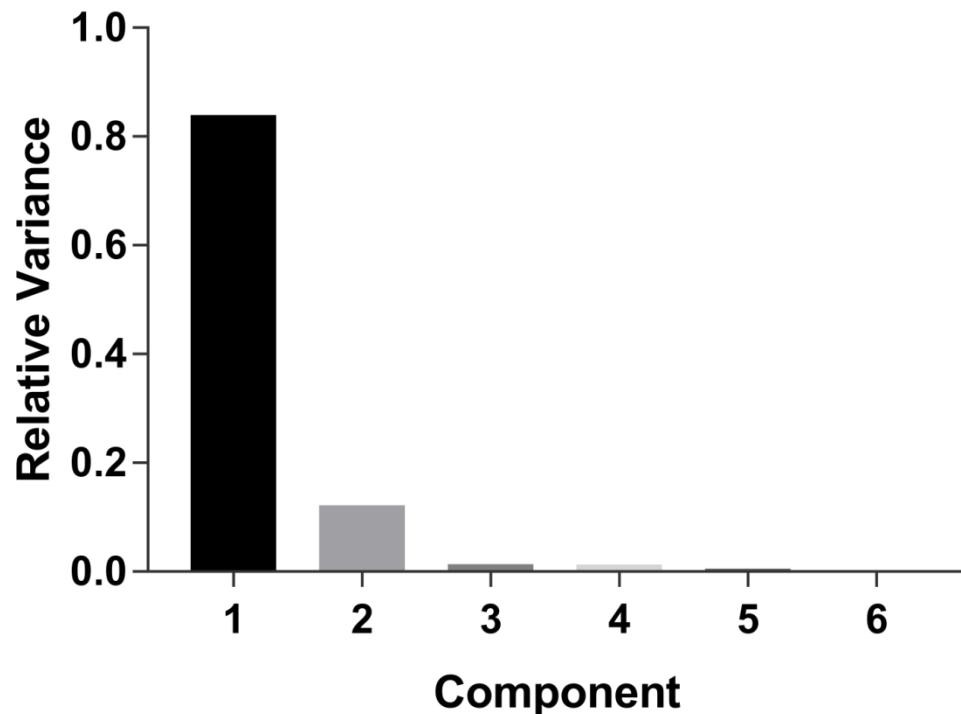




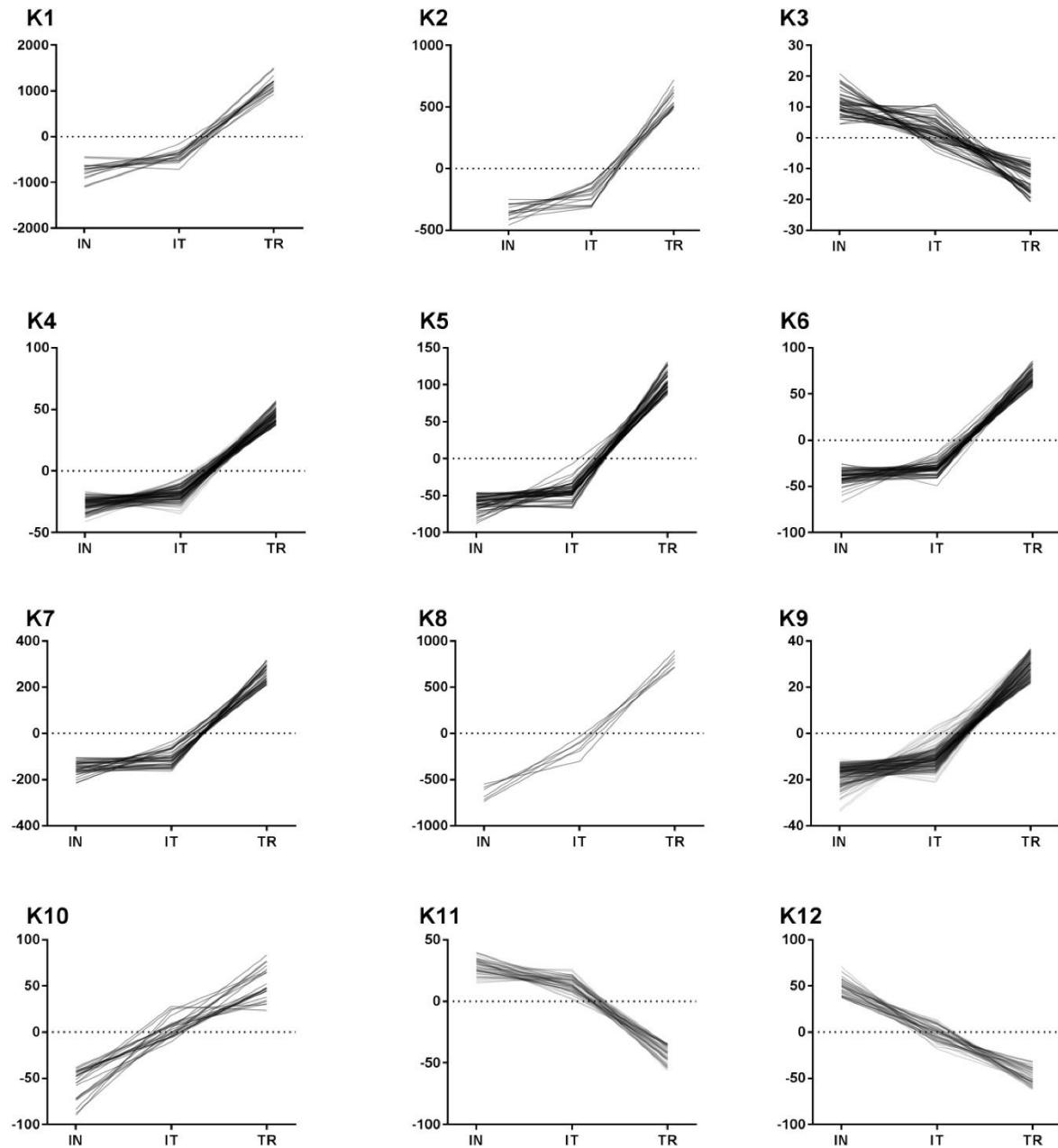
CARBON FIXATION IN PHOTOSYNTHETIC ORGANISMS

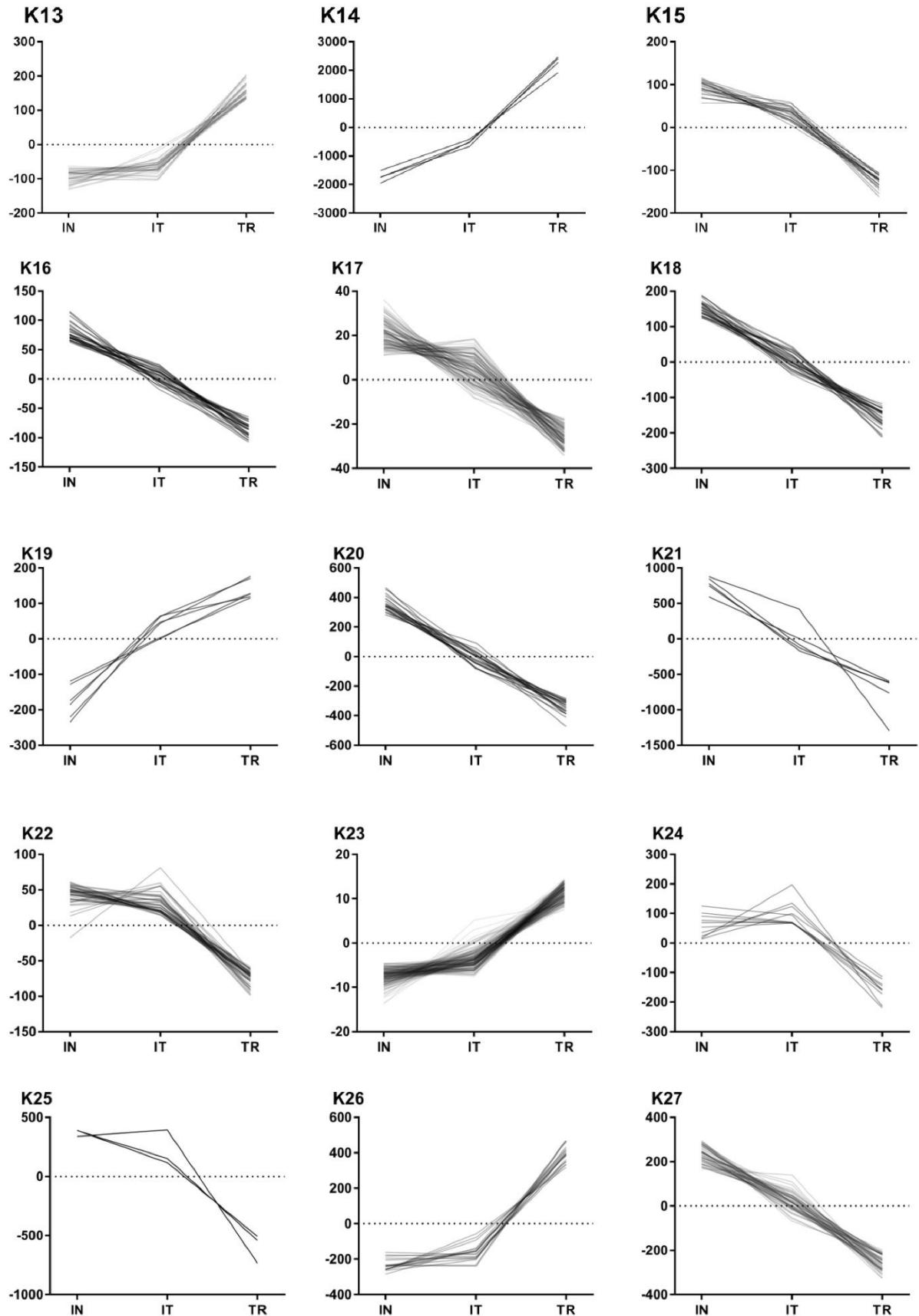


Supplementary Figure S3. Scree plot of the principal components for the *Pachycereus pringlei* samples used in this study. The cumulative variance of the two first principal components is 96.2%.

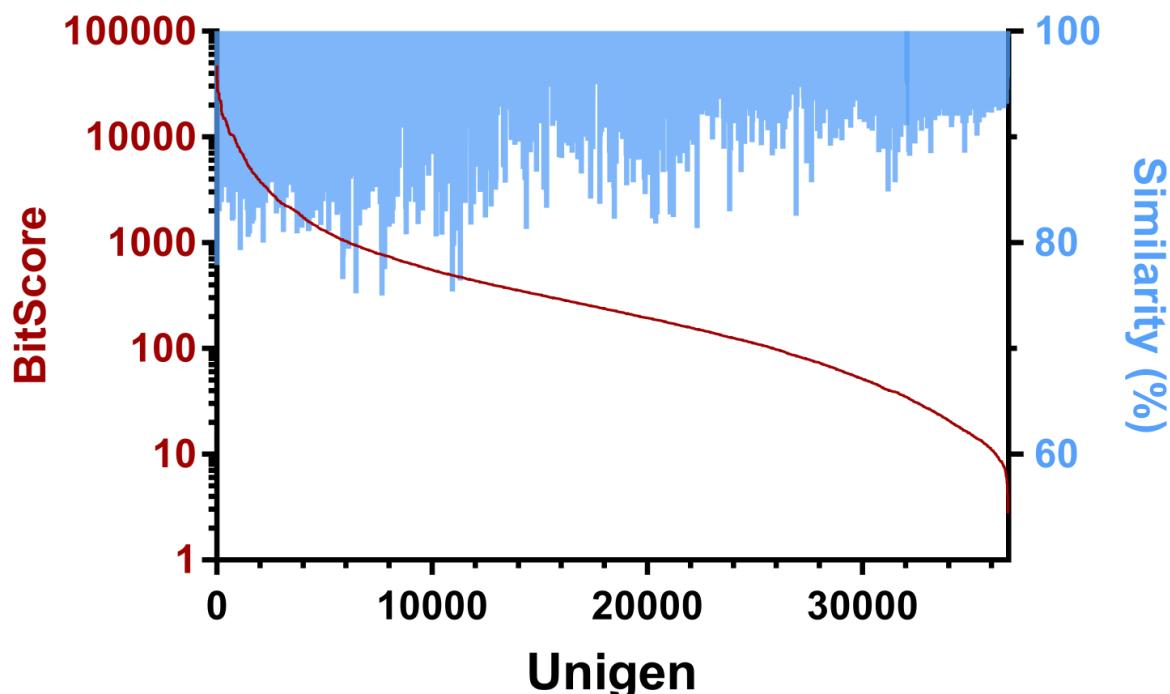


Supplementary Figure S4. Transcriptional profiles identified by k -mean clustering of the differentially expressed transcripts (DETs). Each plot depicts the normalized and centered expression level of the DETs at the three developmental stages of the *Pachycereus pringlei* primary root apex: IN, initial; IT, intermediate; TR, terminal.

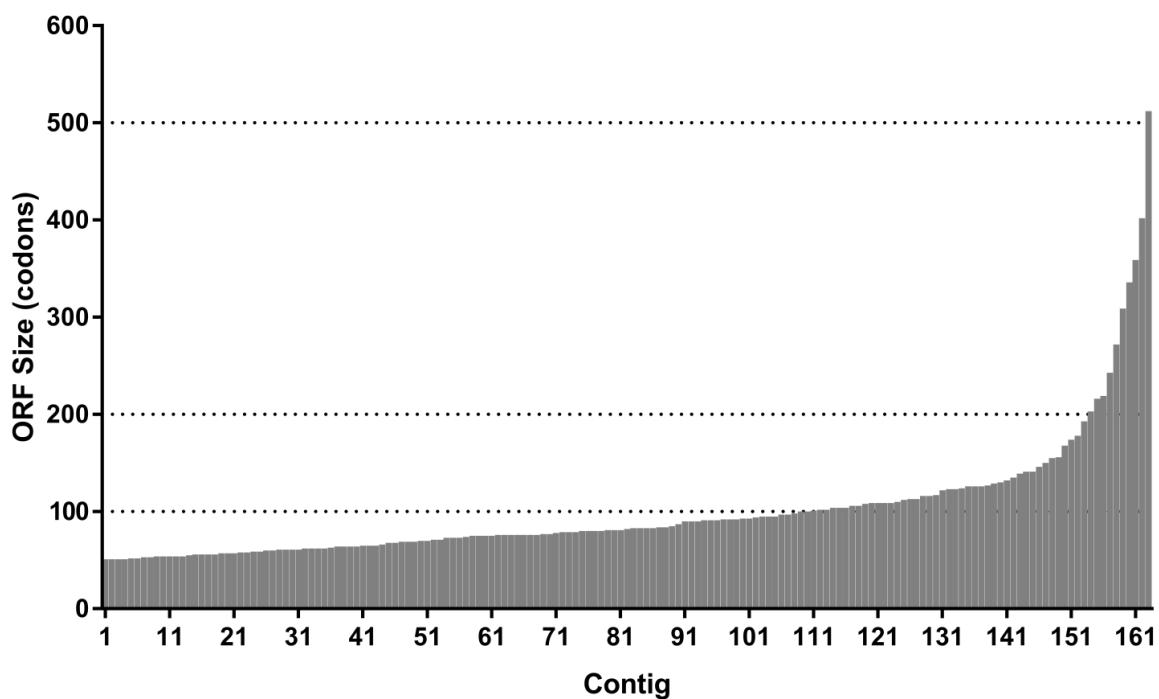




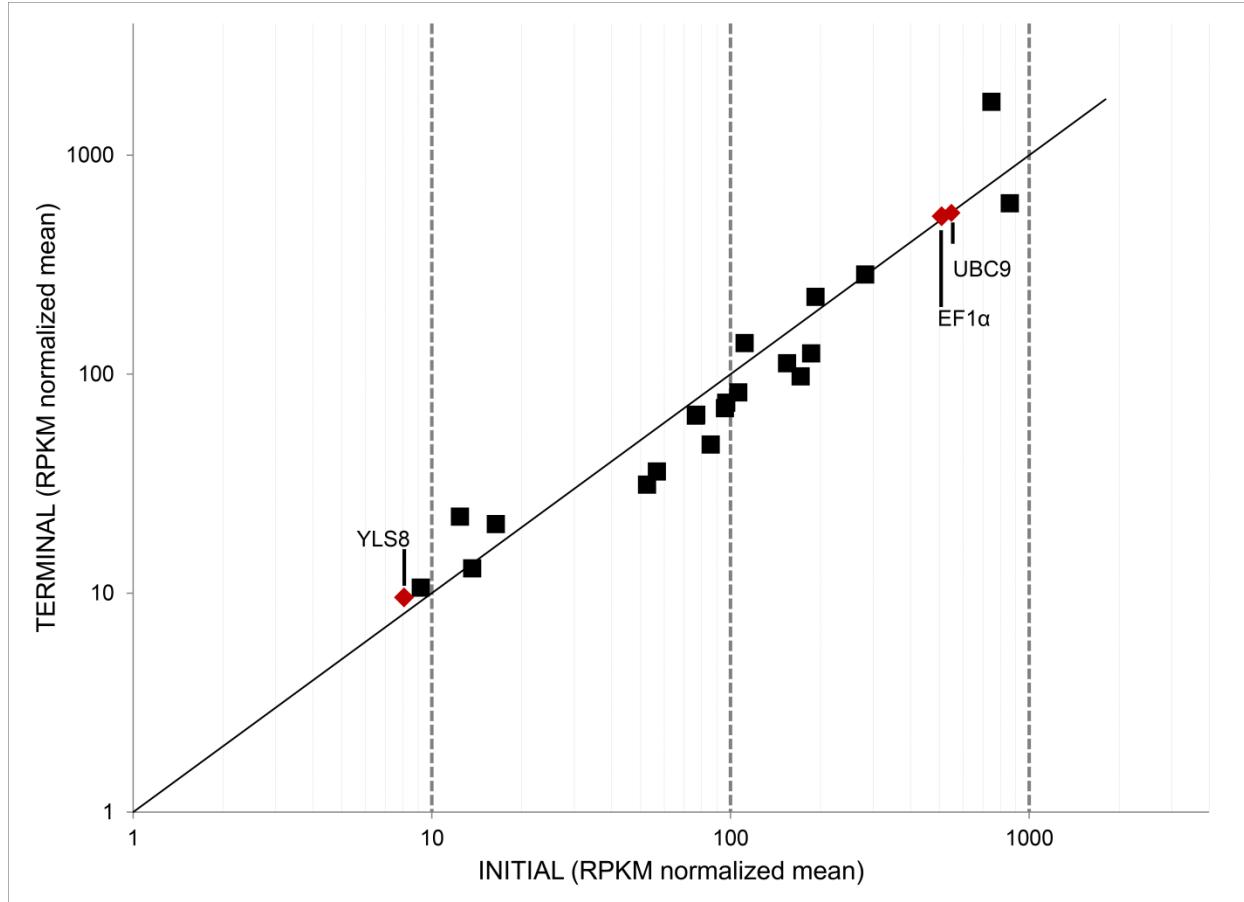
Supplementary Figure S5. Summary plot of the BlastN of the *Lophophora williamsii* shoot+root transcriptome against the *de novo* assembled transcriptome of the primary root apex of *Pachycereus pringlei*.



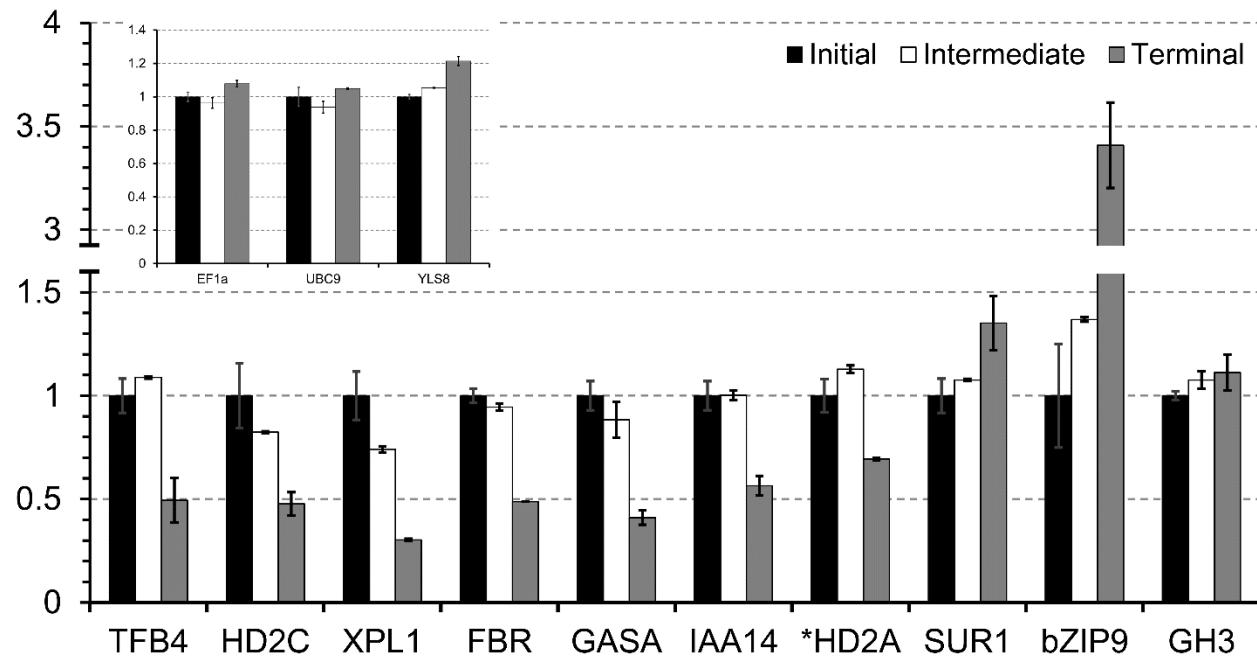
Supplementary Figure S6. Length distribution of the putative open reading frames identified in the un-annotated *Pachycereus pringlei* differentially expressed transcripts.



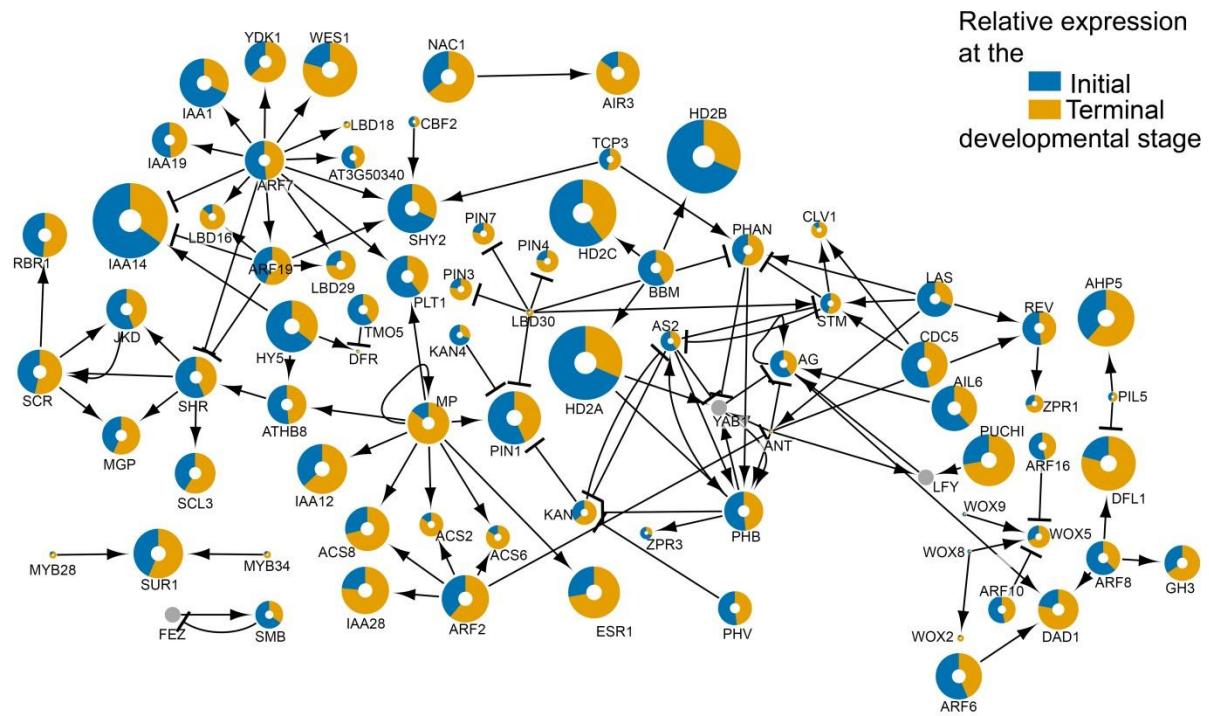
Supplementary Figure S7. Expression levels (RPKM values) of the *Pachycereus pringlei* candidate reference genes in the primary root apex at the initial and terminal developmental stages. Red diamonds correspond to the selected reference genes for this study.



Supplementary Figure S8. Average RPKM values of the *Pachycereus pringlei* transcripts quantified by RT-qPCR. * the trend for RPKM values and transcript abundance evaluated by qRT-PCR between initial and terminal development stages was different for this transcript.



Supplementary Figure S9. Inferred transcriptional regulatory network for the *Pachycereus pringlei* primary root apex. The node size is proportional to the average expression level ($\log_2(\text{RPKM})$) of the transcript in the *P. pringlei* transcriptome.



Supplementary Table S1. Number of differentially expressed genes for developmental zones of the primary root of six angiosperm species.

Species	EDGE MZ vs EZ		EDGE MZ vs DZ	
	MZ	EZ	MZ	DZ
<i>Arabidopsis thaliana</i>	2,683	4,432	3,734	8,947
<i>Cucumis sativus</i>	601	380	1,341	301
<i>Glycine max</i>	1,357	4,159	3,866	7,685
<i>Oryza sativa</i>	772	1,655	3,084	7,743
<i>Solanum lycopersicum</i>	2,905	3,542	4,125	5,661
<i>Zea mays</i>	2,018	3,285	8,012	8,563

*With the exception of *Cucumis sativus*, the number of up-regulated genes at the meristematic zone is lower in comparison to the up-regulated genes at the elongation or differentiation zone. This exception may arise from the fact that in *C. sativus*, unlike all the other species included in this table, new lateral root primordia are developed within meristematic zone (moreover, *C. sativus* develops the first lateral root primordia since the embryo development) (Dubrovsky & Rost, 2003).

Row data taken from Huang & Schiefelbein, 2015.

Dubrovsky J.G. & Rost T.L. 2003. Lateral root initiation. Encyclopedia of Applied Plant Sciences. Ed. by Thomas B., Murphy D.J., and Murray B.G. Academic Press (ISBN:0122270509) pp: 1101-1107.

Supplementary Table S2. Comparison of *Lophophora williamsii* and *Pachycereus pringlei* *de novo* assembled transcriptomes.

		<i>Lophophora</i> *	<i>Pachycereus</i>
Sequencing	Platform	IonTorrent / 454	Illumina HiSeq
	Reads:	Single End	Paired End
	Total Reads	6×10^6	280×10^6
	Read Size (nt)	50-150	100 (x2)
Assembly	Min. contig Length (nt)	200	400
	Max. contig Length (nt)	4,170	14,583
	Av. contig Length (nt)	572	1,081
	Median contig Length (nt)	454	728
	Total contigs	40,436	49,045

* Data taken from Ibarra-Laclette *et al.*, 2015.

Supplementary Table S3. BlastN statistics from the comparison of *Lophophora williamsii* shoot+root transcriptome and *Pachycereus pringlei* root tip transcriptome.

	Minimum	Maximum	Average	Median
Identity	75	100	99.26	100
Bit Score	20.02	47,628.06	1,054.18	261.38
HSP*	1	270	21.6	21
E-value	0	9.75	0.02	0

* HSP: High-scoring significant pair.

Supplementary Table S4. *Pachycereus pringlei* putative orthologs of the *Arabidopsis* superior reference genes (Czechowski *et al.*, 2005). The less variable contigs are included in the box. The asterisks indicate the reference genes used in this study.

Arabidopsis name	<i>P. pringlei</i> Contig	INITIAL		INTERMEDIATE		TERMINAL	
		RPKM 1	RPKM 2	RPKM 1	RPKM 2	RPKM 1	RPKM 2
AcylCoA BP	03269	73.21	58.6	66.48	66.63	38.02	38.08
PP2A	05248	142.42	121.42	123.2	123.98	77.23	78.74
AcylCoA BP	02079	43.93	36.76	39.08	40.8	24.55	25.43
CLA	09503	44.57	42.57	45.06	40.6	26.73	30.67
PP2A	09902	149.25	136.93	142.45	131.55	99.33	99.38
E2F α	05001	717.61	604.83	684.04	727.78	545	415.74
PP2A	08913	112.74	124.83	112.59	100.72	88.43	90.68
CLA	12003	76.83	70.33	70.34	74.13	51.9	55.87
PP2A	07793	73.44	75.34	66.15	55.63	53.3	65.22
PP2A	04543	84.65	78.54	76.9	77.21	61.8	70.07
UBC	22624	59.02	58.65	56.54	54.67	48.15	55.25
KOR1	03527	58.84	59.27	64.18	72.66	54.75	49.39
KOR1	22902	10.52	10.46	14.94	17.09	10.13	10.62
UBC	24691	414.88	427.47	396.49	377.1	402.53	470.79
UBC9*	15035	229.12	204.45	210.96	195.78	226.4	228.57
E1Fα*	06759	401.27	379.59	363.6	388.22	428.72	413.51
GalaOx	03600	7.44	6.67	8.34	8.38	9.1	7.8
YLS8*	17282	145.98	150.19	156.32	155.68	183.81	175.92
AGL31	07058	5.9	6.49	5.29	7.16	7.85	7.45
UBC	17222	80.47	90.87	89.78	90.51	100.3	121.37
PTB	48438	12.67	12.51	16.71	20.22	16.55	16.46
YLS8	30952	9.48	9.62	7.07	7.67	19.24	16.42
UBC	35678	525.63	622.83	655.12	672.13	1,546.79	1,249.75

Supplementary Table S5. Interaction of the *Arabidopsis* nodes used for the *Pachycereus pringlei* transcriptional regulatory network.

Node A	Interaction type	Node B
AT1G30330	Activation	AT2G44810
AT1G30490	Repression	AT5G16560
AT5G16560	Repression	AT1G65620
AT5G16560	Repression	AT1G73590
AT1G09770	Activation	AT1G62360
AT1G09770	Activation	AT1G75820
AT1G62360	Repression	AT2G37630
AT1G62360	Repression	AT1G65620
AT1G62360	Activation	AT1G75820
AT2G45190	Repression	AT4G18960
AT2G45190	Activation	AT2G34710
AT4G18960	Activation	AT2G44810
AT4G18960	Activation	AT4G18960
AT2G34710	Activation	AT1G65620
AT2G34710	Repression	AT5G16560
AT2G34710	Activation	AT2G45190
AT2G34710	Activation	AT3G52770
AT2G37630	Activation	AT2G34710
AT2G37630	Repression	AT2G45190
AT1G65620	Repression	AT2G45190
AT1G65620	Activation	AT2G34710
AT1G65620	Repression	AT1G62360
AT1G65620	Repression	AT5G16560
AT4G37750	Activation	AT5G61850
AT4G37750	Activation	AT5G60690
AT4G37750	Repression	AT4G18960
AT4G37750	Activation	AT2G45190
AT4G37750	Activation	AT2G34710
AT5G42630	Repression	AT1G73590
AT5G45980	Activation	AT5G59340
AT5G45980	Activation	AT3G11260
AT5G62000	Activation	AT1G01480
AT5G62000	Activation	AT4G11280
AT5G62000	Repression	AT4G37750
AT5G62000	Activation	AT4G37770
AT5G62000	Activation	AT5G25890
AT2G28350	Repression	AT3G11260
AT5G61850	Activation	AT4G18960

AT5G60690	Activation	AT2G45450
AT3G44750	Activation	AT2G34710
AT3G44750	Activation	AT2G45190
AT1G55580	Activation	AT1G62360
AT1G55580	Activation	AT2G37630
AT1G55580	Activation	AT5G60690
AT1G55580	Activation	AT4G37750
AT1G79580	Repression	AT1G26870
AT1G26870	Activation	AT1G79580
AT1G56010	Activation	AT2G04160
AT3G25710	Repression	AT5G42800
AT4G30080	Repression	AT3G11260
AT4G25470	Activation	AT1G04240
AT4G37650	Activation	AT5G03150
AT4G37650	Activation	AT3G54220
AT4G37650	Activation	AT1G03840
AT4G37650	Activation	AT1G50420
AT5G03150	Activation	AT3G54220
AT3G54220	Activation	AT1G03840
AT3G54220	Activation	AT3G12280
AT3G54220	Activation	AT5G03150
AT5G11260	Activation	AT4G32880
AT5G11260	Activation	AT4G14550
AT5G11260	Activation	AT5G42800
AT4G32880	Activation	AT4G37650
AT5G10510	Activation	AT4G18960
AT1G19850	Activation	AT3G20840
AT1G19850	Activation	AT4G11280
AT1G19850	Activation	AT4G32880
AT1G19850	Activation	AT4G37770
AT1G19850	Activation	AT1G04550
AT1G19850	Activation	AT1G12980
AT1G19850	Activation	AT1G19850
AT1G19850	Activation	AT1G73590
AT1G19850	Activation	AT1G01480
AT5G61420	Activation	AT2G20610
AT2G33880	Activation	AT3G11260
AT1G53230	Activation	AT2G37630
AT1G53230	Activation	AT1G04240
AT1G19220	Repression	AT4G14550
AT1G19220	Repression	AT4G37650
AT1G19220	Activation	AT2G42430
AT1G19220	Activation	AT3G58190

AT1G19220	Activation	AT1G04240
AT2G20180	Activation	AT1G03430
AT2G20180	Repression	AT5G54510
AT5G20730	Activation	AT3G20840
AT5G20730	Activation	AT3G15540
AT5G20730	Activation	AT3G58190
AT5G20730	Activation	AT3G50340
AT5G20730	Activation	AT1G19220
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AT5G20730	Activation	AT2G42430
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AT5G20730	Repression	AT4G14550
AT5G20730	Activation	AT4G37390
AT5G20730	Activation	AT4G27260
AT5G17430	Activation	AT3G44750
AT5G17430	Activation	AT5G03740
AT5G17430	Activation	AT5G22650
AT5G18560	Activation	AT5G61850
AT5G37020	Activation	AT1G28130
AT5G37020	Activation	AT2G44810
AT5G37020	Activation	AT5G54510
AT5G60890	Activation	AT2G20610
AT4G00220	Repression	AT2G37630
AT4G00220	Repression	AT2G01420
AT4G00220	Repression	AT1G73590
AT4G00220	Repression	AT1G70940
AT4G00220	Activation	AT1G62360
AT4G00220	Repression	AT1G23080

Supplementary Table S6. Properties of the nodes included in the *Pachycereus pringlei* transcriptional regulatory network of the primary root apex. NSH=No significant hit; NHF=No hit found; Y= Yes; N=No; Interm.=Intermediate.

Node	Arabidopsis name	P. pringlei contig	P. pringlei annotation	E-value	BBH	RPKM Initial	RPKM Interm.	RPKM Term.
AT1G30330	ARF6	0001038	auxin response factor 6-like phospholipase a1-chloroplastic-like homeobox-leucine zipper protein revoluta probable transcription factor kan2-like cell division cycle 5-like isoform x1 homeobox protein knotted-1-like 6 receptor protein kinase clavata1-like	0 2.52E-75	Y N	24.12 7.27	27.34 9.93	18.65 25.76
AT2G44810	DAD1	0005103						
AT1G30490	PHV	0001618						
AT5G16560	KAN	0005141						
AT1G09770	CDC5	0000721						
AT1G62360	STM	0009327						
AT1G75820	CLV1	0001781						
AT2G45190	YAB1	NSH						
AT4G18960	AG	0032278						
AT2G34710	PHB	0000849						
AT2G37630	PHAN	0008775	transcription factor as1 lob domain-containing protein 6 auxin efflux carrier component 1-like PREDICTED: uncharacterized protein LOC101514572 ap2-like ethylene-responsive transcription factor ant-like probable transcription factor kan2-like wuschel-related homeobox 11-like wuschel-related homeobox 4 wuschel-related homeobox 5-like auxin response factor 2 1-aminocyclopropane-1-carboxylate synthase-	1.94E-118	Y	12.14	14.87	11.42
AT1G65620	AS2	0027369						
AT1G73590	PIN1	0002874						
AT3G52770	ZPR3	0030394						
AT4G37750	ANT	0014765						
AT5G42630	KAN4	0013106						
AT5G45980	WOX8	0045416						
AT5G59340	WOX2	0033393						
AT3G11260	WOX5	0019483						
AT5G62000	ARF2	0001054						
AT1G01480	ACS2	0006764						

AT5G10510	AIL6	0003496	ap2-like ethylene-responsive transcription factor ail6-like transcriptional factor b3 family protein auxin-responsive factor aux	5.36E-115	Y	23.18	27.88	14.55
AT1G19850	MP	0000685	iaa-related ovule development	0	Y	5.84	8.35	33.42
AT3G20840	PLT1	0004605	family protein auxin-responsive	0	Y	15.14	23.61	9.93
AT1G04550	IAA12	0009934	protein iaa11-like ethylene-responsive transcription factor 4-like	6.86E-58	N	22.94	32.12	39.07
AT1G12980	ESR1	0012720	transcription factor myb35	1.54E-16	N	21.46	33.81	55.6
AT5G61420	MYB28	0035249	tyrosine aminotransferase-like	3.00E-68	N	0.41	0.73	1.62
AT2G20610	SUR1	0007195	retinoblastoma-related	7.98E-165	N	27.15	29.93	35.17
AT3G12280	RBR1	0001201	protein wuschel-related	0	Y	18.44	21.99	19.38
AT2G33880	WOX9	0045416	homeobox 11-like transcription factor	1.43E-22	N	1.15	0.48	0.54
AT1G53230	TCP3	0003653	tcp4-like auxin response factor	4.30E-70	N	2.99	3.01	3.47
AT1G19220	ARF19	0000777	19-like lob domain-containing	0	Y	9.5	13.97	12.13
AT2G42430	LBD16	0023003	protein 16 lob domain-containing	4.33E-65	Y	1.6	1.25	9.77
AT3G58190	LBD29	0010011	protein 29-like transcription factor pif3-like	9.93E-63	N	3.23	4.69	9.43
AT2G20180	PIL5	0021486	histidine-containing phosphotransfer	1.29E-26	N	0.85	1.33	1.16
AT1G03430	AHP5	0018863	indole-3-acetic acid-amido synthetase	2.51E-56	N	47.56	43.54	74.89
AT5G54510	DFL1	0006889	auxin response factor 19-like	0	Y	22.05	32.66	83.56
AT5G20730	ARF7	0000777	auxin-induced protein aux22	0	Y	12.14	14.87	11.42
AT3G15540	IAA19	0015499	PREDICTED: uncharacterized protein	3.88E-50	Y	8.92	10.69	8.52
AT3G50340		0010064	LOC100262755 lob domain-containing	3.12E-165	Y	3.36	4.24	2.85
AT2G45420	LBD18	0044113	protein 18-like auxin-induced protein	3.58E-67	N	0.36	0.68	1.73
AT4G14560	IAA1	0014302	22b probable indole-3-acetic acid-amido	6.76E-53	N	35.46	36.07	16.64
AT4G37390	YDK1	0004749	synthetase	0	Y	10.5	18.51	17.66
AT4G27260	WES1	0006889	indole-3-acetic acid-amido synthetase	0	Y	22.05	32.66	83.56

AT5G17430	BBM	0002613	ap2-like ethylene-responsive transcription factor bbm-like histone deacetylase	1.34E-122	Y	9.55	13.02	6.82
AT5G03740	HD2C	0009674	hdt1-like histone deacetylase isoform 3	1.02E-24	N	139.84	161.42	92.97
AT5G22650	HD2B	0027114	ethylene-responsive transcription factor 4-like auxin response factor 8-like	8.21E-26	N	286.77	270.64	131.72
AT5G18560	PUCHI	0012720	indole-3-acetic acid-amido synthetase -like transcription factor myb34	3.71E-23	N	21.46	33.81	55.6
AT5G37020	ARF8	0001138	lob domain-containing protein 18-like auxin efflux carrier component 3-like	0	Y	9.71	9.76	5.8
AT1G28130	GH3	0004939	auxin efflux carrier component 3-like auxin efflux carrier component 3-like	0	Y	7.5	7.25	14.31
AT5G60890	MYB34	0035249	myb34	1.30E-67	N	0.41	0.73	1.62
AT4G00220	LBD30	0044113	lob domain-containing protein 18-like auxin efflux carrier component 3-like	5.91E-59	Y	0.36	0.68	1.73
AT2G01420	PIN4	0002917	auxin efflux carrier component 3-like auxin efflux carrier component 3-like	0	Y	1.66	2.17	5.65
AT1G70940	PIN3	0002917	auxin efflux carrier component 3-like auxin efflux carrier component 3-like	0	Y	1.66	2.17	5.65
AT1G23080	PIN7	0002917	auxin efflux carrier component 3-like auxin efflux carrier component 3-like	0	Y	1.66	2.17	5.65

Supplementary Table S7. List of primers used in this work.

Primer Name	Sequence
PpUBC9_fw	GGTGTCCAGGTATTGAGCTG
PpUBC9_rev	AGCCATCCTCACAAAAGCAACC
PpYLS8_fw	ACCGTTCAGGTTGAGGCGAAG
PpYLS8_rev	GATACCCCAAACTAACGCATAGGC
PpEF1a_fw	GAGGTCTGAAGTTATGGTCGTG
PpEF1a_rev	AGCACACCGACGCTACTAATG
* PpTFB4_fw	GCAATGCCTATAGCTCCTCCG
* PpTFB4_rev	GTTGTTGCCCGAGTCACCTTG
* PpHD2C_fw	GGTGGAAAGACTCCTGGTGGAG
* PpHD2C_rev	GTGCTTGGCCTTGTGTGAGAC
* PpXPL1_fw	GGCCAGATGCTTAGAGATGCTG
* PpXPL1_rev	CTCGGATGAAGTGCAGTCC
* PpFBR_fw	CTGCAGCAAGACCAGTTCAAGC
* PpFBR_rev	CTGCTTCTTAGGCATGCGGTAAC
* PpGASA_fw	GCTCTCATTGCCATTCCATGC
* PpGASA_rev	GAAGGCTTGACGCTGCCAGG
* PpHD2A_fw	GGTTTGAGTCTAGCGAGGAATG
* PpHD2A_rev	TTCCCTGTCCTGCCTGTTCTGTTTC
PpSUR1_fw	TGTTAGATGTCACCCGTTGCTG
PpSUR1_rev	CATGACCATTGTGCCTGATTAGC
PpZIP9_fw	TCTGAACGTGGTCTGGGAATC
PpZIP9_rev	TCACAATCAACTCAACTAGCACC
PpIAA14_fw	CTGGGCAAGATGTTCAGTTCT
PpIAA14_rev	AAGATCAGCGGAACAAACATCAGC
PpGH3_fw	TCAACCAAACACCCCCAAAGAGC
PpGH3_rev	TCAACCAAACACCCCCAAAGAGC

* primers reported in Shishkova *et al.* (2013), designed for regions with 100% homology between *Stenocereus gummosus* and *Pachycereus pringlei* genes.