Article title: **Induction of auxin biosynthesis and *WOX5* repression mediate changes in root development in Arabidopsis exposed to chitosan**

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Figure S1. Chitosan induces reactive oxygen species accumulation in Arabidopsis roots.

Figure S2. Chitosan provokes the accumulation autofluorescent compounds in Arabidopsis primary roots.

Figure S3. Chitosan reduces expression of WOX5*:*GFP in secondary roots of Arabidopsis.

Figure S4. Chitosan induces *AQC1* gene expression at low chitosan doses.

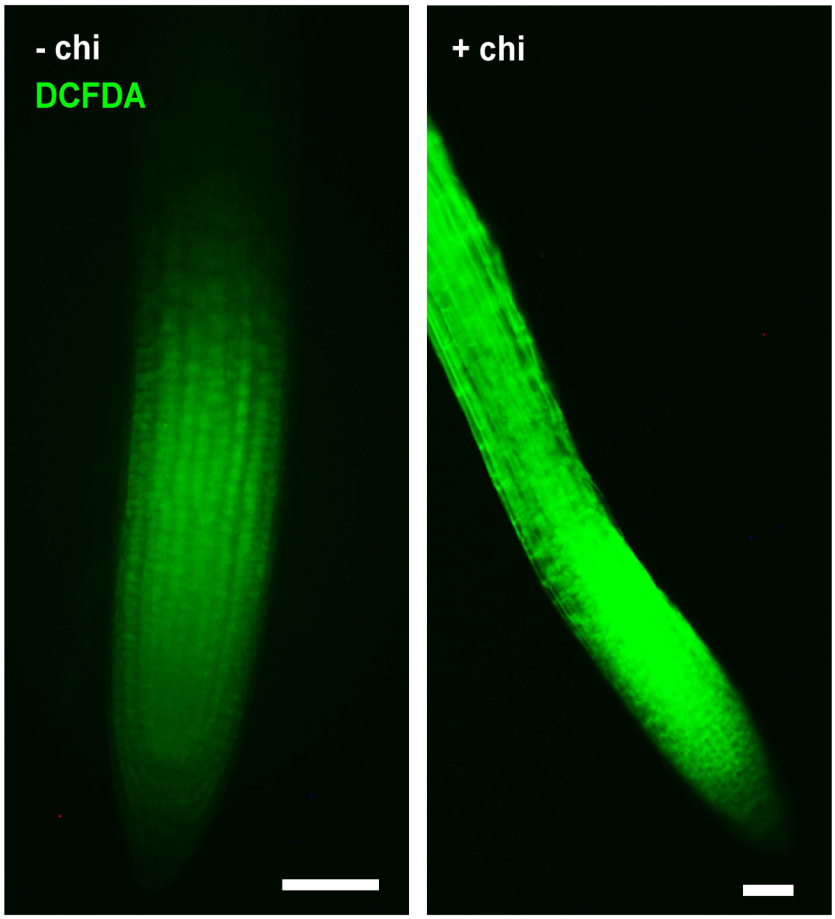
Figure S5. Chitosan induces expression of genes involved in SA and JA biosynthesis.

Figure S6. Reduction of Arabidopsis root length by combined exogenous application of hormones induced by chitosan (IAA+SA+JA).

Figure S7. Chitosan induces accumulation of violaceous compounds in the abaxial side of tomato leaves.

Figure S8. Chitosan irrigation arrests growth of barley roots.

Table S1. Primers used to quantify expression of genes related with auxin metabolism using qRT-PCR.



FigureS1

Figure S1. Chitosan induces reactive oxygen species accumulation in Arabidopsis roots. Exposure of Arabidopsis plants to chitosan induces accumulation of ROS in roots measured by H2DCFDA staining. (Scale bar 25 µm).

FigureS2

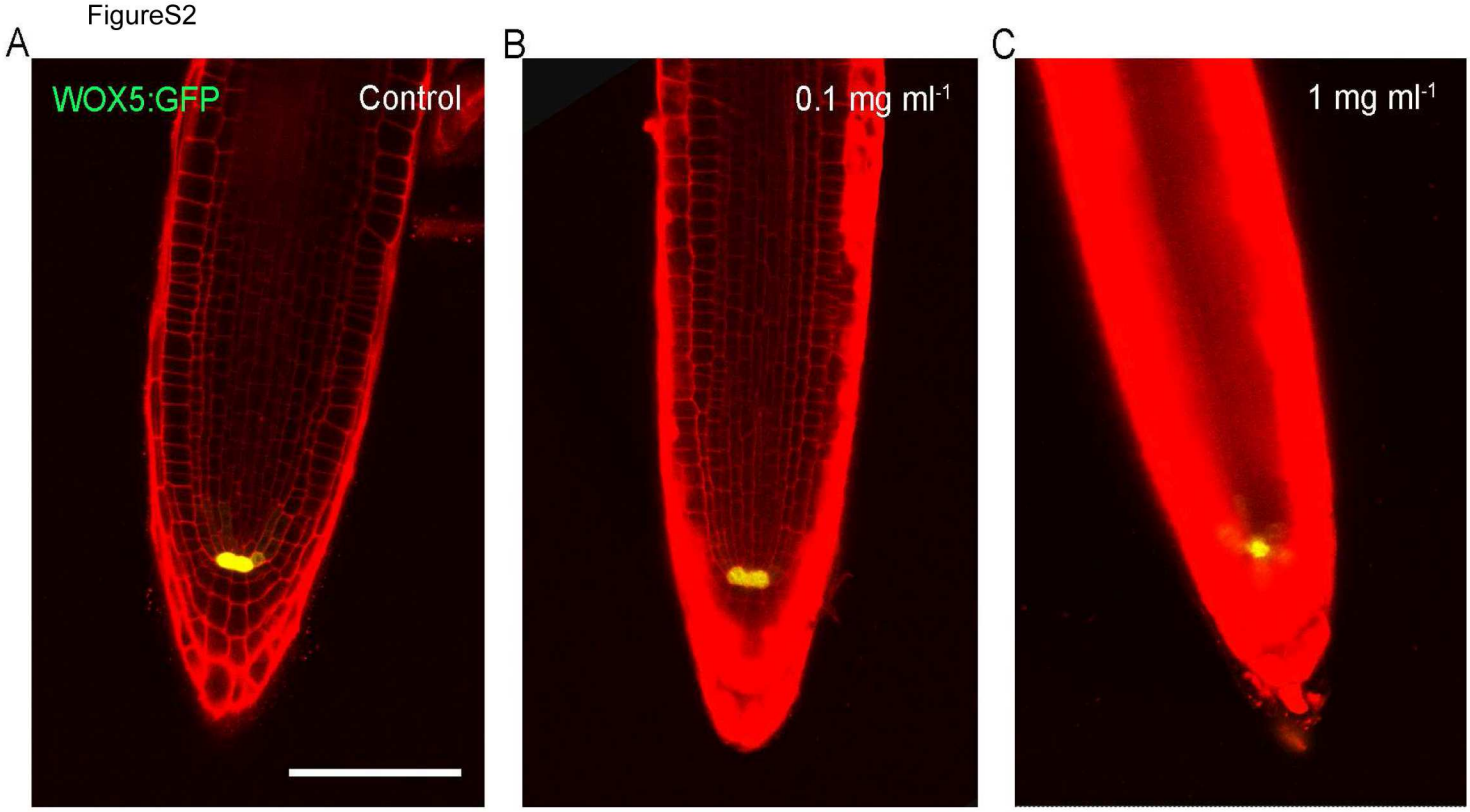
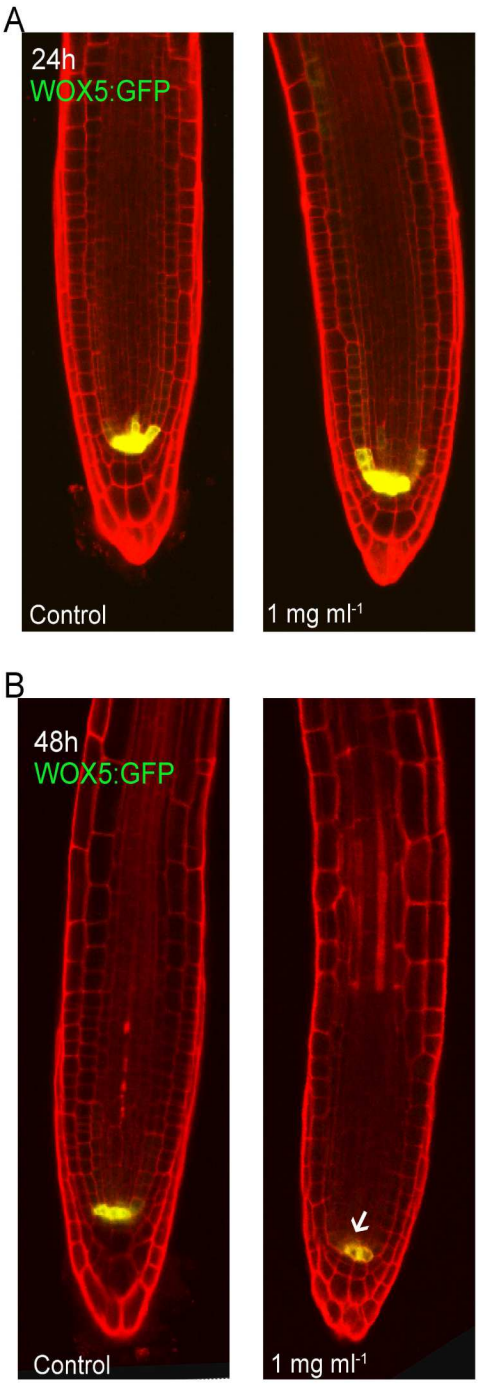


Figure S2. Chitosan provokes the accumulation autofluorescent compounds in Arabidopsis primary roots. Autofluorescent zones are usually associated with deposition of phenolic and calose depositions. Green fluorescence indicates WOX5:GFP expression in roots exposed to different chitosan concentrations. (Scale bar 50 μm).



FigureS3

Figure S3. Chitosan reduces expression of WOX5:GFP in secondary roots of Arabidopsis. WOX5:GFP expression at 24h (A) and 48h (B). Arrows show WOX5:GFP repression in plants treated with chitosan in comparison with untreated controls.

FigureS4

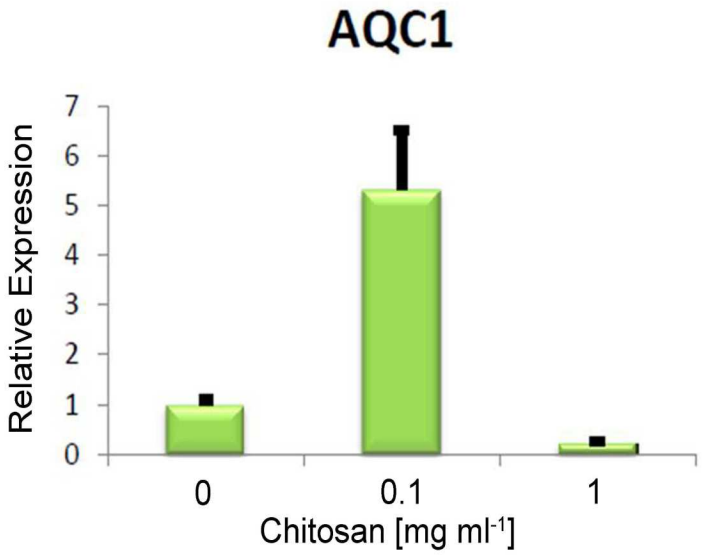


Figure S4. Chitosan induces *AQC1* gene expression at low chitosan doses. Low chitosan doses (0.1 mg ml^{-1}) propitiate an overexpression of *AQC1* gene related to cell division regulation in the root meristem. High doses of chitosan (1 mg ml^{-1}) repress gene expression of those genes affecting cell division and reducing root elongation.

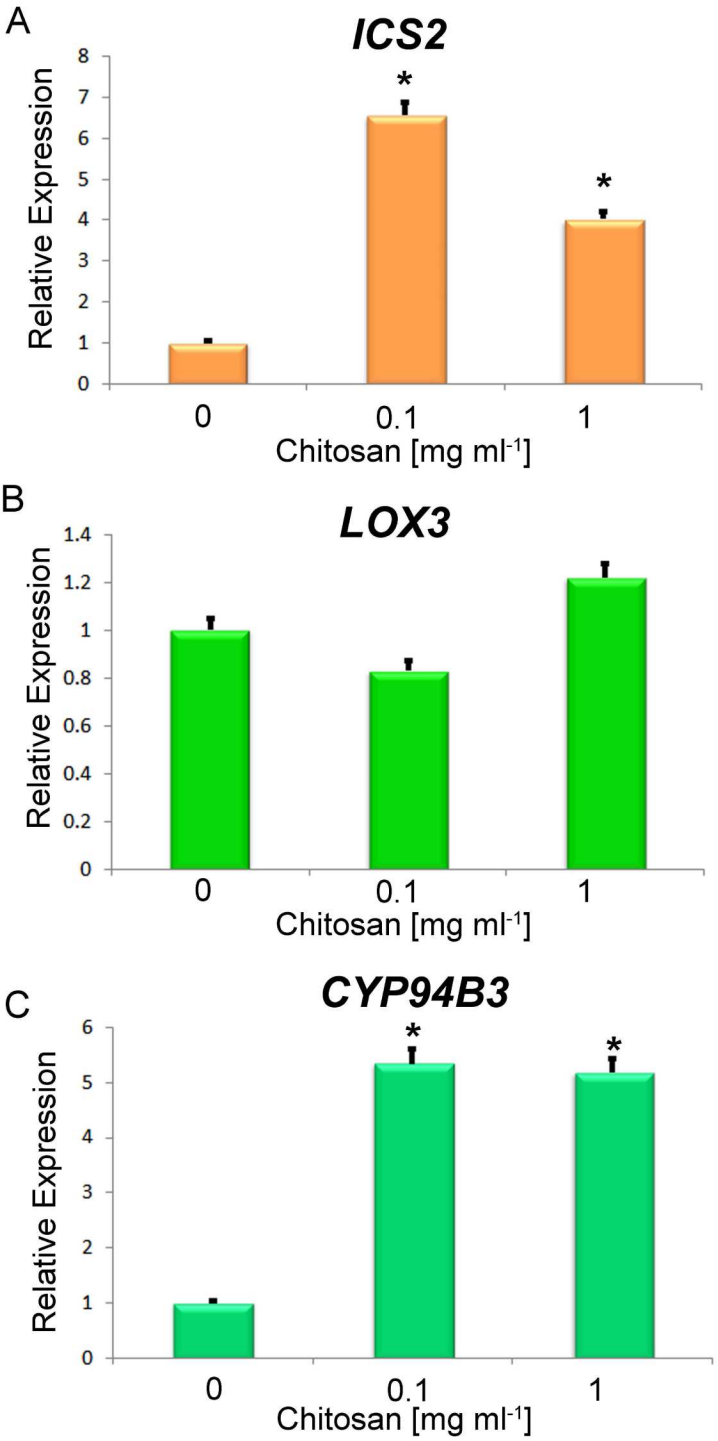


Figure S5

Figure S5. Chitosan induces expression of genes involved in SA and JA biosynthesis. **A)** *ICS2* (SA biosynthesis), **B)** *LOX3* (JA biosynthesis) and **C)** *CYP94B3* (JA biosynthesis).

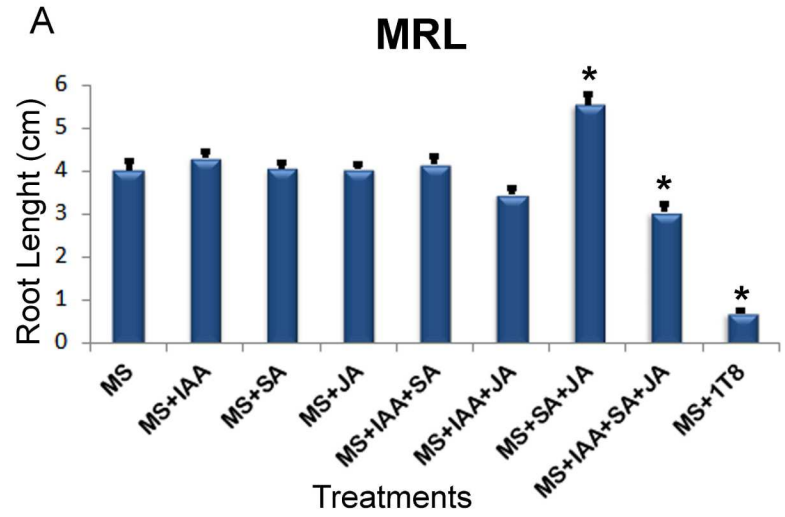


Figure S6

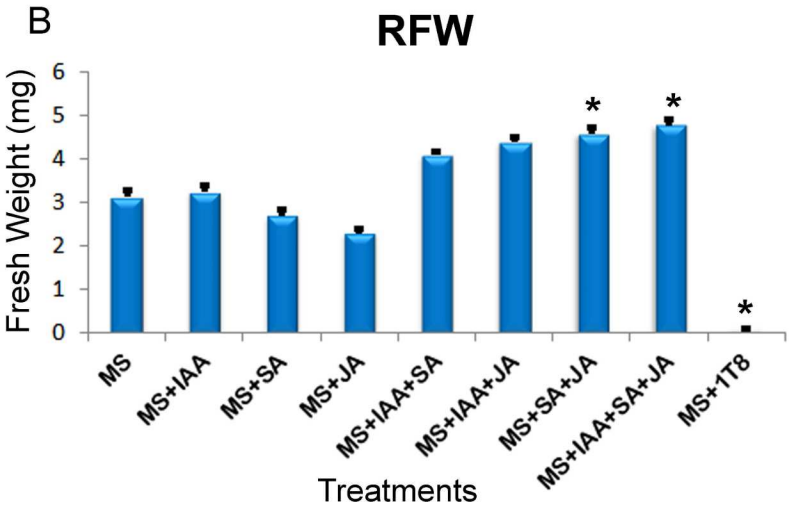
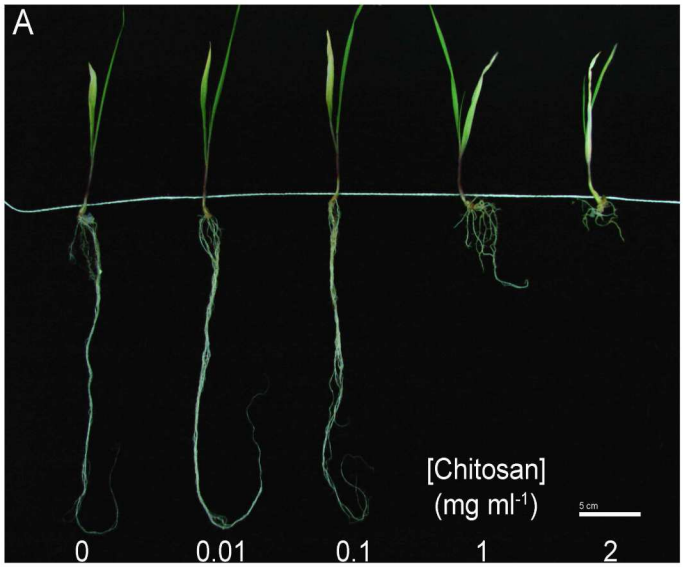


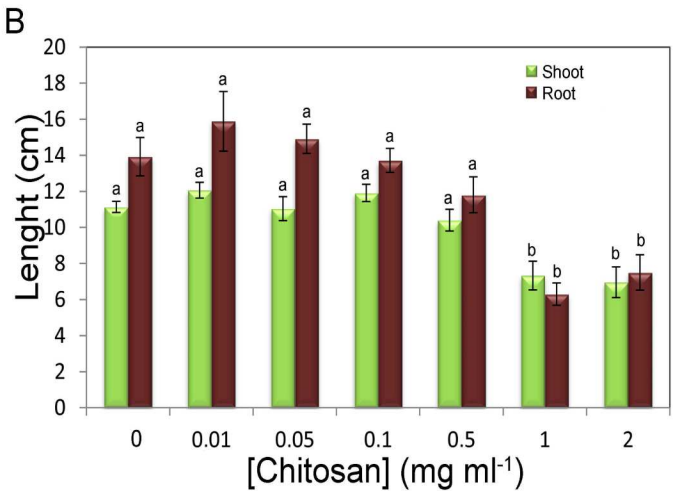
Figure S6. Reduction of Arabidopsis root length by combined exogenous application of hormones induced by chitosan (IAA+SA+JA). **A)** Maximum root length (MRL). **B)** Fresh root weight (FRW). **C)** Example of plants grown on MS medium amended with hormones combinations and controls. Hormones were amended to MS at the same amount (+10%) quantified in plants exposed by chitosan (Table 1). Abbreviations: MS: Murashige and Skoog medium; IAA: indole acetic acid; SA: salicylic acid; JA: jasmonic acid. 1T8: 1 mg ml^{-1} chitosan.

Figure S7

Figure S7. Chitosan induces accumulation of violaceous compounds in the abaxial side of tomato leaves. Yellow arrows indicate accumulation of anthocyanins related to the response of tomato plants to stress when they were irrigated with chitosan (2 mg ml^{-1}) for 20d. Root and shoot growth were arrested by chitosan in comparison with untreated controls.

Figure S8





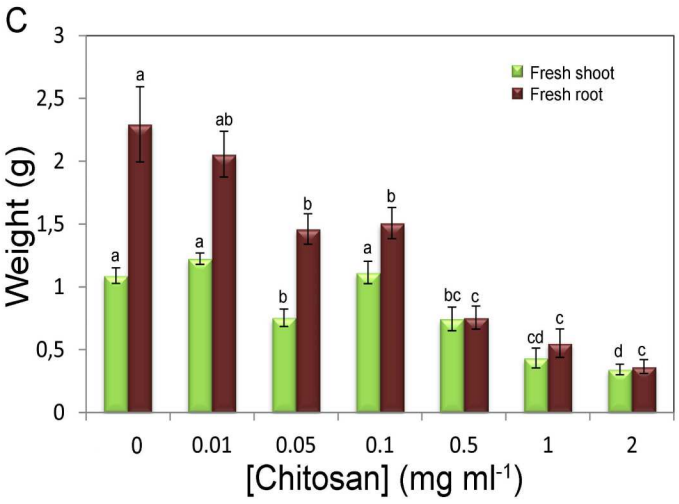


Figure S8. Chitosan irrigation arrests growth of barley roots. **A)** Overview of the inhibitory effect of chitosan on development of barley plantlets after 21d treatment. (Scale bar 5 cm). **B)** Effect of chitosan on barley root and shoot development. **C)** Effect of chitosan on fresh biomass of barley shoot and root. Different letters indicate significant differences ($p<0.05$) between treatments.

Table S1. Primers used for quantification hormone-related gene expression by qRT-PCR

Primer Name	**5'→3'**
HK_ACT2_fw	TCCCTCAGCACATTCCAGCAGAT
HK_ACT2_rev	AACGATTCCTGGACCTGCCTCATC
HK_OTC_fw	TGAAGGGACAAAGGTTGTGTATGTT
HK_OTC_rev	CGCAGACAAAGTGGAATGGA
WOX5_fw	CTATTGGTTTCAGAATCATAAGGCTA
WOX5_rev	TGACAATCTTCTTCGCTTATTTCA
AQC1_fw	GGAGCGACTTTTCAGATTGC
AQC1_rev	ATTGGACGGAACCACTTGAG
AMI1_IAM_fw	CCAGGTCCTCCACCGCAT C
AMI1_IAM_rev	TTCGTGTAGCCCCAATGGTATG
PIN1_fw	ATCTCCGAGCAGTTTCCAGA
PIN1_rev	CGCAGATAAGCCTTGAGACC
YUC2_fw	TAGCGTCACTATGGCAGCAC
YUC2_rev	CCTCCACGGTCTGGTTAAAA
AAO1_fw	AGAGCGTCAAGCTTGGTTGT
AAO1_rev	GCATTGTGTGGCATGAAAAC
ARF1_fw	TGAATGGCATTTTAGGCACA
ARF1_rev	ATGTGCCGCCTAACACCTAC
ICS2F1	ATTGGCAGGACAGCTAAAAAGAG
ICS2R1	AGCTGACTCATGTTCAAGTGACTT
ICS1F	AGTGAATTTGCAGTCGGGATCAG
ICS1R	TCGCCTGTAGAGATGTTGTTGC
NPR1F	GGACACAAGAAGAAGGCCAAGA
NPR1R	TCCATCACCGTTGACTTCAACATC
AOC3-1F	CGGTCCAGATTTCTCCTCCCGAT
AOC3-1R	CACCGCTCTTTTCAGGAACGTGT
CYP9-1F	GCCATGAGGCTATATCCTCCAGTT
CYP9-1R	CCGGCACAATCTCAAACCGACTCA
LOX3-1F	TCGCACGTCAAGCCATAGCTGGA
LOX3-1R	GCGCTCAATTGCCTGTGTGCAGCT
MYC2-S	CAAGGAGGAGTGTTTGGGATGC
MYC2-AS	GTCGAAAAATTAAGTTCTCGGGAG

HK: indicates primers used to amplify genes used as a housekeeping