Supplementary Material

Large DNA fragment deletion in lncRNA77580 regulates neighboring gene expression in soybean (*Glycine max*)

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Supplementary Material

Table S1. Sequences of the primers used in this study

Primer Name	Forward Primer(5′-3′)	Reverse Primer(5′-3′)
77580-5'I1		TCTGCCCTTGTGGACTCCTTTTGA
77580-3'I2	ACAAAGAAACACAAACCACCAAGA GA	
C3	AAGATGAAGACAAGGT	ATAGGGATAGGCAAAC
G2	CTCCAGCCAACAAGCA	GGAAATATACCCGTCGTA
P1	TGCAGCTACATTCGGGTGAA	ATGACACGTCACAGGCAACT
P2	ATCTTCCTCTACCTCAAAC	GACTACATCCTCCTTCCAT
P3	TTTACTCCTCCCCGCCTTTG	AACAAAAAGGCTATCGCCGC
P4	GCCTTTTCACCTAGGGTGCT	AATCCGGCTCAAATCCACCT
Cons15	TAAAGAGCACCATGCCTATCC	TGGTTATGTGAGCAGATGCAA
GLYMA_20G224300	CCCACTGAGTTGTTATCTGCG	CTCGTCCATCACCGTCCAAA
GLYMA_20G224400	CCTCAAGACAGAGGGGCATT	AAAACCCATCCCACCCATGT
GLYMA_20G224500	ATCTCAAGACCCGTCCCCTT	GGCCTAATTGCAACAGCAGG
GLYMA_20G224600	AGGCTCTAACTTGCAACGCA	AATGGTTTTGCCGATGGAGC
LOC100807037	AGGCTCTAACTTGCAACGCA	AATGGTTTTGCCGATGGAGC
GLYMA_20G224700	TGCACCAATCACCGATAGGG	TGTGGCATAGCAGCCATTGA
GLYMA_20G224800	AACGTATGAAGCCCTCAGGC	TGCGCTAGTTTCGTGAAGGT
GLYMA_20G224900	CACACACCATGAACCTCCAC	TGGGATTTGGGTCTGATTGAGG
GLYMA_20G225000	TGCGCTGGCTAAGATGTTCA	GCGAAGACGCTTGCATGATT
GLYMA_20G225100	CTCCCACATCACTCCAACCC	GGAAAGAGTGTCGTCCGTGT
GLYMA_20G225200-1	GTGAGTGGTGAGAGTGCGAA	GCATCCCATGTAGCGCAGTA
GLYMA_20G225200-2	GAACTGGGACTGAGGCTACG	TCAGCGCAATTGACGAAACG
GLYMA_20G225300-1	GGTGTGTGTACTTGCTTTGGC	ATCCCAAGTGGCGCAATAGT
GLYMA_20G225300-2	ATGCAAAAATGGGGCGTTGG	TGGCCTTGAGCATTTCCACT
GLYMA_20G225400	CGAGCCAGGTGGTGAAAGTA	CCTGCAAAAGGTGTGAAGGC
GLYMA_20G225500	TTGGAGGATGCAAGCAACGA	TTCAGCCATCTGAGCAAGCA
GLYMA_20G225600	CCAAACCCTCGACGACAGAA	CGAGAACCACCGAGACGATT
GLYMA_20G225700-1	GTTGTCGATGTGCGAGAAGC	CGGGCAAGGTGTTACAGAGT
GLYMA_20G225700-2	TGCCCGCACTTGACCTTTAT	TCTAGGGCTTCCAAACGACG
GLYMA_20G225800-1	TGTTGGGGTGTGGATTAGGC	TCGGAGGTAAAAACGCGTGA
GLYMA_20G225800-2	AGTTCCCCGTTAAACCCGAC	AGGGCTTGGCATGTGATTGA
GLYMA_20G225900-1	CAGCCCAAGGCCAAGAAAAC	TCCTTGTGCTTGCCTTCGAT
GLYMA_20G225900-2	CAAGGAGTTGCCTCCAACCT	CTTAGTCGGTGCGAGCTTGA
GLYMA_20G226000	GAACGCGCAGAGAAACGTAG	CTCAACTGCTTGATGGCTGC
GLYMA_20G226100	ACGTCAATCTCGCCAAGAGG	TCTTCTCAGTGGCAAGTCCA

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GLYMA_20G226200	GAACATGGGACTGGCTGCG	GGGTACCACAAGCTGTGACT
GLYMA_20G226300	CAAGACTGCAGTTGCACCAC	TCGTGAGGCACACCAAACAT
GLYMA_20G226400	GGGTCGCTACACGAAGATGT	TTGCACGTGGCCTCTTTGTA
LOC102668810-1	ATCGAGCCGTTCGTGAGTTT	GACCATCTCTGGTCGGTGTC
LOC102668810-2	CGAGCCGTTCGTGAGTTTGA	AACTGGCGCATGACCATCTC
GLYMA_20G226500	AGGCTCAGCAGATGTCACAA	TTCCATTGGAGCTCATGCCA
GLYMA_20G226600-1	ACTTGCATGAGCCTGGAGTC	CAACTGCAAACACGTGAGGG
GLYMA_20G226600-2	TTGAAGAAGCACCTCCCGTC	CGCTACAAACTTGGGCTTCG
GLYMA_20G226700	GCTGGTGTGGTGACCTCATT	CCCGTGGAAACAGATCCCTC
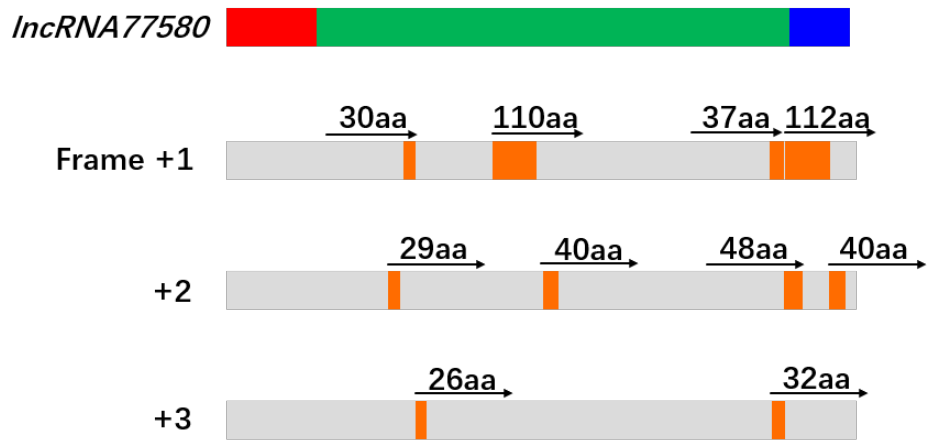
Figure S1. Analysis of the open reading frames (ORFs) in *lncRNA77580*.

All frames (orange boxes) were identified in the three forward frames. The two longest ORFs encoded 112 and 110 amino acids (aa).
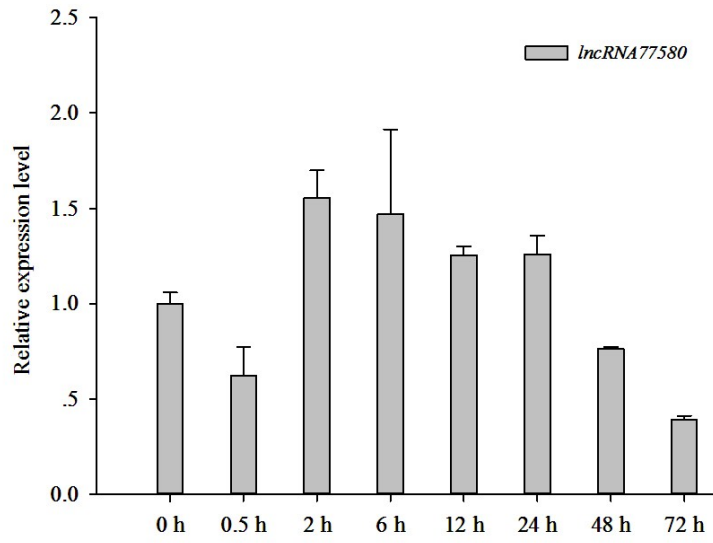
Figure S2. Expression levels of *lncRNA77580* in soybean roots treated with tap water at different time points.
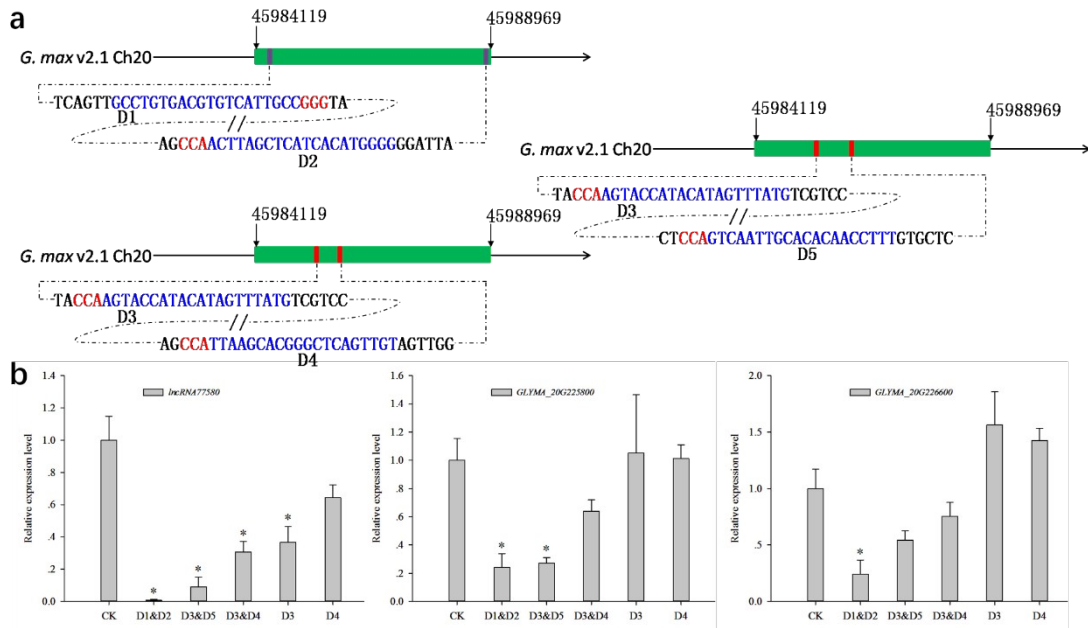
Figure S3. DNA fragment deletion of different lengths in *lncRNA77580* has different effects on the expression levels of *lncRNA77580* itself and neighboring protein-coding genes.

(a) Five target sites (D1, D2, D3, D4, and D5) in the *lncRNA77580* sequence were designed for CRISPR/Cas9-induced large DNA fragment deletion. The red letters shown in D1, D2, D3, D4, and D5 indicate protospacer adjacent motifs. (b) Expression levels of *lncRNA77580*, *GLYMA_20G225800*, and *GLYMA_20G226600* in soybean hairy roots with large DNA fragment deletions of different length in *lncRNA77580*, as determined by RT-qPCR. * indicates significant differences $P < 0.05$ and fold change > 2.

Table S2. The predicted protein products of neighboring coding genes affected by *lncRNA77580*

Gene Name (US DOE)	Sequence Name (NCBI:gene ID)	Sequence Length	Position (NCBI)	Strand	Product (NCBI)
GLYMA_20G225200	LOC100500393	753	Chr20:45955984-45956736	positive	pathogenesis-related protein PR-4-like
GLYMA_20G225300	LOC100815211	731	Chr20:45962212-45962942	positive	pseudo wound-induced protein-like
GLYMA_20G225700	LOC100305652	1062	Chr20:45983181-45984242	negative	uncharacterized LOC100305652
GLYMA_20G225800	LOC100815737	3484	Chr20:45989786-45993269	positive	receptor-like protein kinase ANXUR1
GLYMA_20G225900	LOC100810590	880	Chr20:45998596-45999475	positive	histone H1.2-like
	LOC102668810	1409	Chr20:46042520-	negative	protein MAIN-LIKE 2-like

| GLYMA_20G226600 | LOC100816792 | 17149 | 46043928
Chr20:46064196-
46081344 | positive | myosin-11 isoform X2(X1) (myosin-24) traffic |
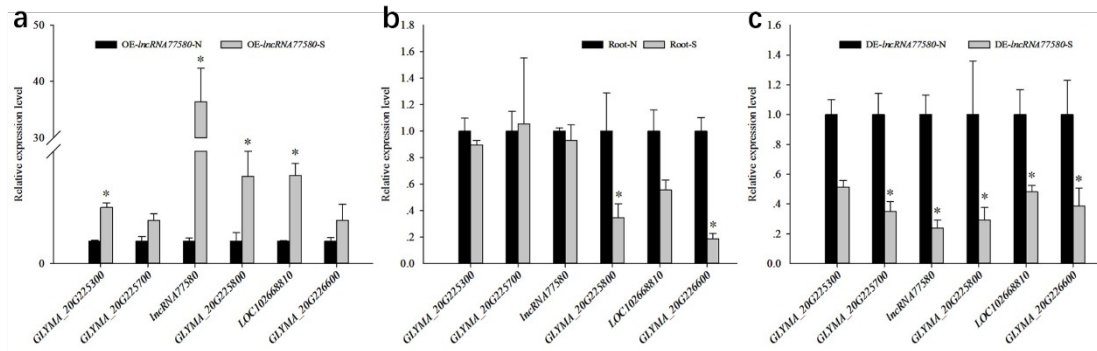
Figure S4. Expression levels of *lncRNA77580* and five neighboring genes in salt-stressed soybean hairy roots.

(a) Expression levels of *lncRNA77580* and five neighboring genes in *lncRNA77580*-overexpressing hairy roots treated with tap water or 170 mM NaCl for 5 d. **(b)** Expression levels of *lncRNA77580* and five neighboring genes in wild-type treated with tap water or 170 mM NaCl for 5 d. **(c)** Expression levels of *lncRNA77580* and five neighboring genes in *lncRNA77580*-deleted hairy roots treated with tap water or 170 mM NaCl for 5 d. * indicates significant differences *P* < 0.05 and fold change > 2.