

1 **Supplemental Table S1 :Summary of predicted and identified novel miRNAs targeting nicotine pathway genes**

| MiRNA accession | Mature sequences (5'-3') | Mature length | Precursor length | MFE/ kcal/ mol | Predicted target | Expression abundance (RPM*) | | RT-qPCR analysis |
|--------------------|--------------------------|------------------|---------------------|----------------------|---------------------|--------------------------------|------|---------------------|
| | | | | | | root | leaf | |
| nta-miRX13 | AGTGTATAGCTATAAATAGGGACC | 24 | 167 | -90.8 | PMT2 | 0.3 | 0.2 | No |
| nta-miRX17 | CCCCACTTGTGGATTATACTGGG | 24 | 153 | -75.2 | QPT1 | 0.5 | 2.5 | Yes |
| nta-miRX19 | CGGATCTCTGGCGGAAAGAC | 21 | 85 | -40.6 | NAC_148 | 4.5 | 5.6 | Yes |
| nta-miRX20 | CGTTGAAGTGGCAAGGATGAC | 21 | 169 | -76.4 | CYP82E4 | 1.0 | 0.3 | Yes |
| nta-miRX27 | TATTTTGAGAGTCGAGCA | 21 | 131 | -65.5 | QPT2 | 0.5 | 22.0 | Yes |

2 **Supplemental Table S1.Summary of predicted and identified novel miRNAs targeting nicotine pathway genes.***RPM: Repeat normalized
3 reaSummary of predictSummary of predicted and identified novel miRNAs targeting nicotine pathway geneed and identified novel miRNAs

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