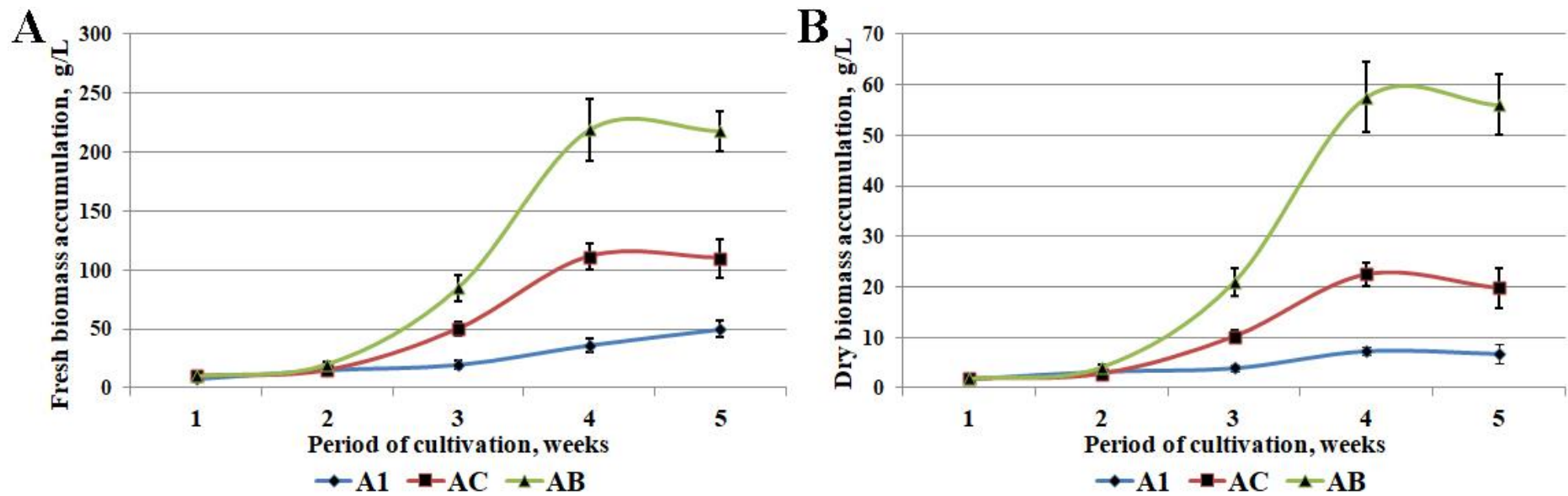
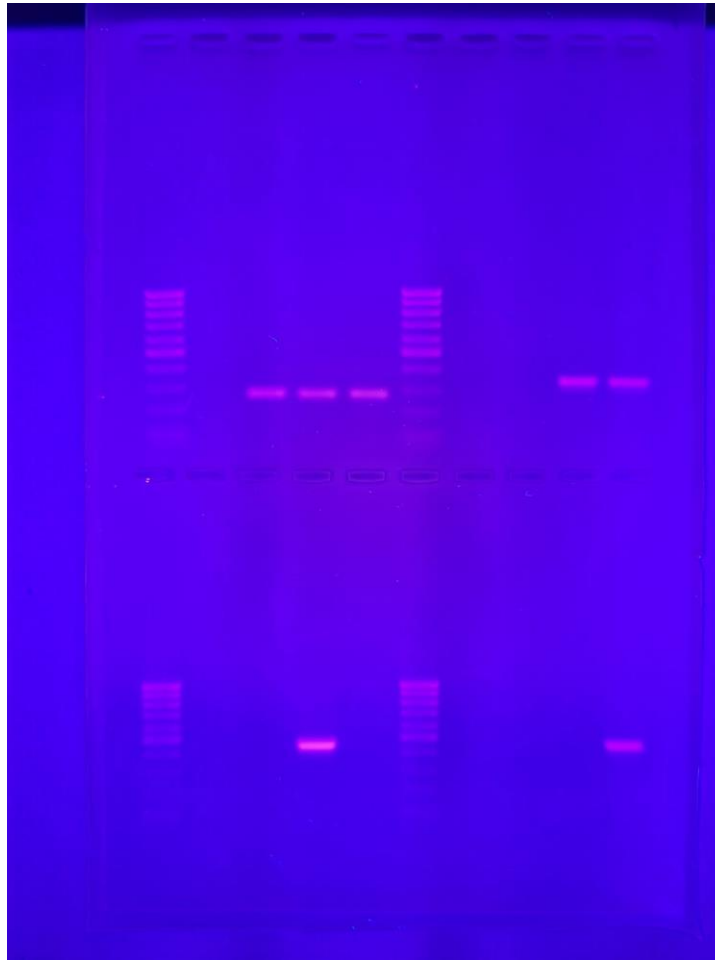


## Supplementary Materials

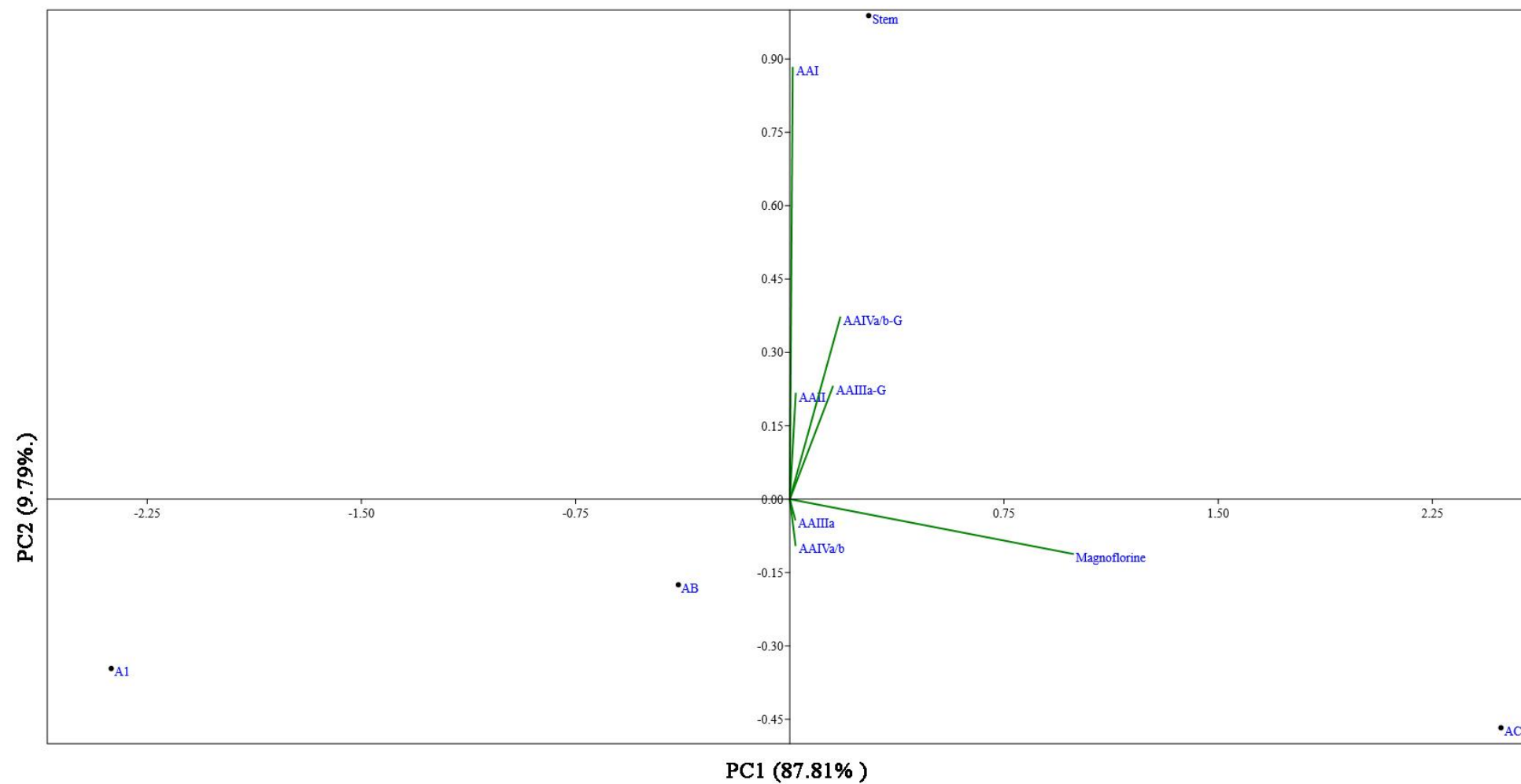
### Enhanced production of nitrogenated metabolites with antioxidant and anticancer potential in *Aristolochia manshuriensis* hairy root cultures



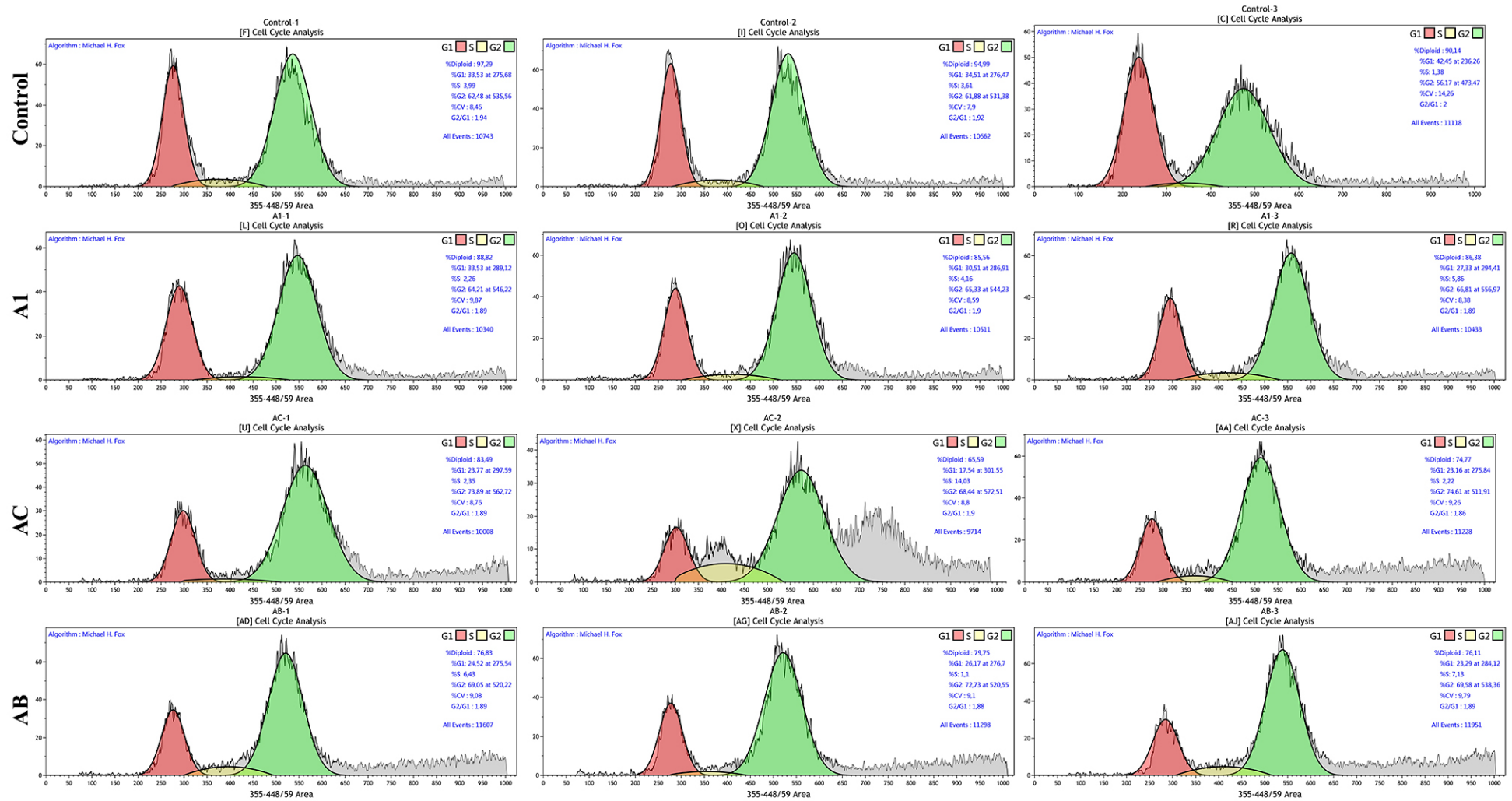
**Figure S1.** Growth profile of *A. manshuriensis* cell cultures at the specified culture times (week) as measured by fresh (A) and dry (B) weight (g/L). Data are presented as the mean  $\pm$  standard errors from three biological replicates.



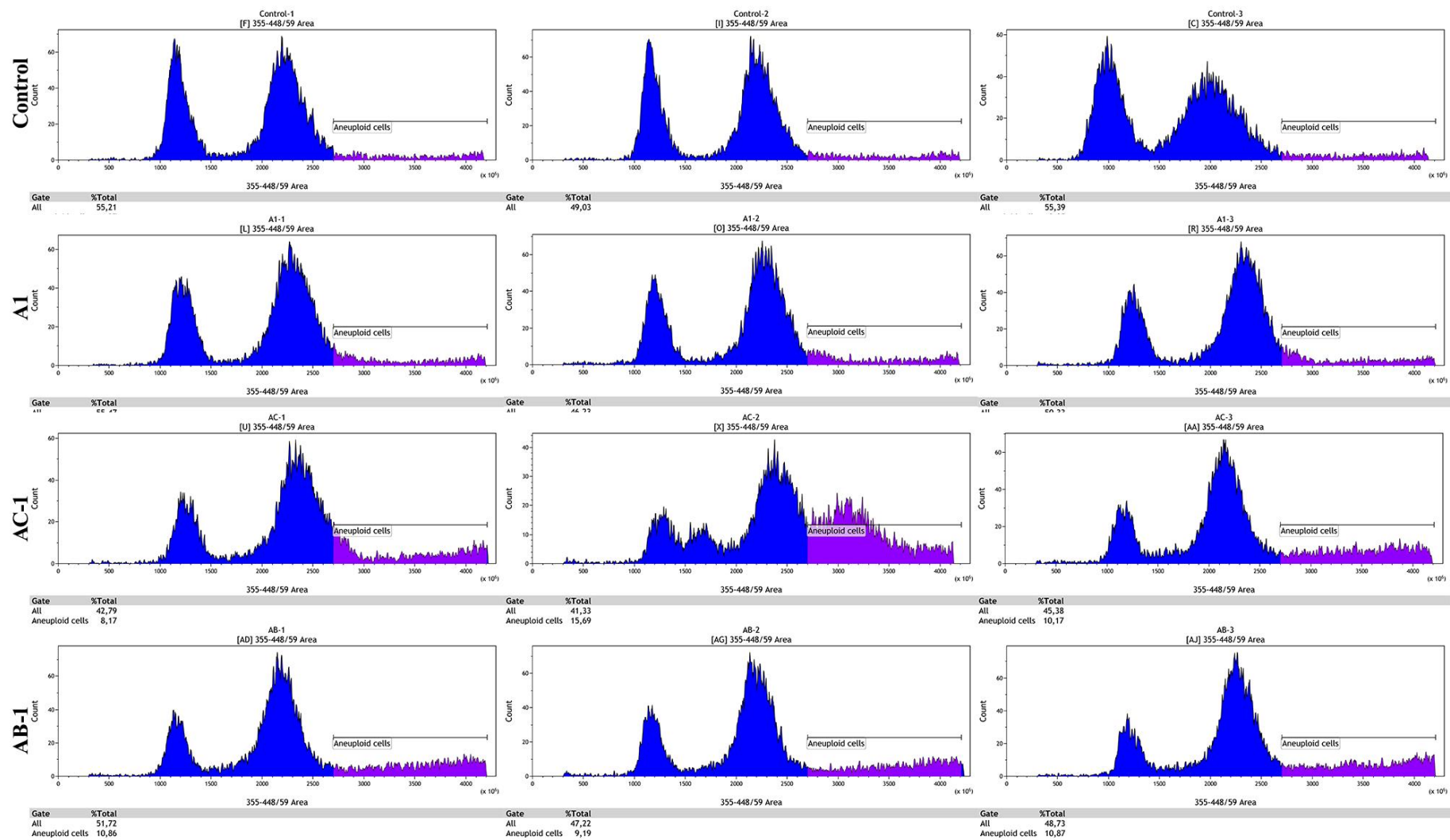
**Figure S2.** Unprocessed and uncropped image of the agarose gel electrophoresis reported in Figure 1.



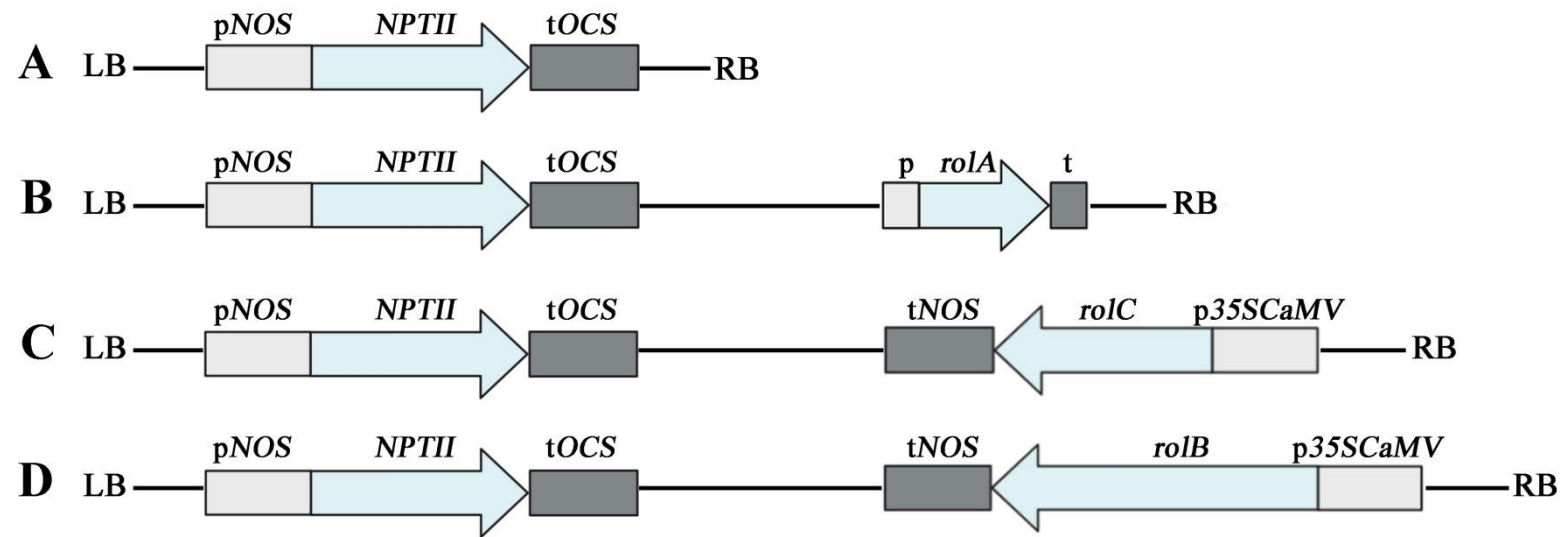
**Figure S3.** Principal component analysis (PCA) of HPLC-UV data from samples of *A. manshuriensis*.



**Figure S4.** Cell cycle analysis of RKO cell culture using the Michael H. Fox algorithm with Kaluza 2.1 software after treatment with methanol (Control), A1, AC, and AB extracts.



**Figure S5.** DNA ploidy analysis of RKO cell culture using Kaluza 2.1 software after treatment with methanol (Control), A1, AC, and AB extracts.



**Figure S6.** Schematic representation of the T-DNA region from the empty pPCV002 (A), pPCV002-A (B), pPCV002-CaMVC (C), and pPCV002-CaMVB (D) binary vectors.

**Table S1.** List of phenanthroic acid derivatives identified in cell cultures and stems of *A. manshuriensis* measured by using HPLC-MS.

Peak no.	Rt (min)	UVmax (nm)	Detected ions composition	Detected ions (m/z)	Diff (ppm)	Diff (mDa)	Molecular Formula	MS2 fragmentation, main diagnostic ions (m/z)	Compound assignment	References
1	13.3	258, 305 350-380	[M+NH <sub>4</sub> ] <sup>+</sup>	507.1257	2.3	1.15	C <sub>22</sub> H <sub>19</sub> NO <sub>12</sub>		Aristolochic acid IIIa O-glucoside*	56
			[M+H-Hex-H <sub>2</sub> O] <sup>+</sup>	310.0336	3.3	1.01		266, 208		57
			[M+H-Hex-NO <sub>2</sub> ] <sup>+</sup>	282.0542	6.8	1.93				58
			[M-H] <sup>-</sup>	488.0844	1.4	0.95		279		
			[M+HCOO] <sup>-</sup>	534.0882	1.5	0.73		488		
2	14.2	228, 273, 305	M <sup>+</sup>	342.1683	4.9	1.69	C <sub>20</sub> H <sub>24</sub> NO <sub>4</sub> <sup>+</sup>	311, 297, 279, 265	Magnoflorine**	St.
			[M-2H] <sup>-</sup>	340.1551	1.0	0.33		325		56
			[M-H+HCOO] <sup>-</sup>	386.1608	0.3	0.11		340		57
3	15.5	255, 325, 402	[M+NH <sub>4</sub> ] <sup>+</sup>	537.1330	4.1	2.12	C <sub>23</sub> H <sub>21</sub> NO <sub>13</sub>	474, 312	Aristolochic acid IVa/IVb O-glucoside*	56
			[M+H-NO <sub>2</sub> ] <sup>+</sup>	474.1169	2.6	1.24		312		58
			[M+H-Hex-H <sub>2</sub> O] <sup>+</sup>	340.0432	5.8	1.98		296, 281, 253		
			[M+H-Hex-NO <sub>2</sub> ] <sup>+</sup>	312.0614	4.6	1.44		297, 269, 267		
			[M-H] <sup>-</sup>	518.0961	4.0	2.09		309		
			[M+HCOO] <sup>-</sup>	564.0996	0.2	0.11		518, 309		
4	19.3	257, 304, 350-380	[M+H-H <sub>2</sub> O] <sup>+</sup>	310.0338	2.6	0.81	C <sub>16</sub> H <sub>9</sub> NO <sub>7</sub>	266, 238	Aristolochic acid IIIa*	56
			[M+H-NO <sub>2</sub> ] <sup>+</sup>	282.0520	1.0	0.28				58
			[M-H] <sup>-</sup>	326.0294	3.7	1.23		280, 236		
			[M-H-NO <sub>2</sub> ] <sup>-</sup>	280.0364	4.7	1.32		236		
5	21.6	255, 325, 405	[M+NH <sub>4</sub> ] <sup>+</sup>	375.0816	1.9	0.69	C <sub>17</sub> H <sub>11</sub> NO <sub>8</sub>	358, 312	Aristolochic acid IVa/IVb*	56
			[M+H-H <sub>2</sub> O] <sup>+</sup>	340.0434	5.2	1.78		296, 281		58

			[M+H-NO <sub>2</sub> ] <sup>+</sup>	312.0618	3.3	1.04		297, 269		
			[M-H] <sup>-</sup>	356.0399	3.6	1.29		310, 266		
			[M-H-NO <sub>2</sub> ] <sup>-</sup>	310.0467	5.1	1.59		266		
6	24.5	250, 303, 350-380	[M+NH <sub>4</sub> ] <sup>+</sup>	329.0760	2.6	0.81	C <sub>16</sub> H <sub>9</sub> NO <sub>6</sub>	294, 268	Aristolochic acid II**	St.
			[M+Na] <sup>+</sup>	334.0310	3.9	1.21				56
			[M+H-H <sub>2</sub> O] <sup>+</sup>	294.0382	5.1	1.50		279, 250, 222, 192		57
			[M+H-CO <sub>2</sub> ] <sup>+</sup>	268.0615	4.0	1.07		238		58
			[M-H+HCOONa] <sup>-</sup>	378.0245	4.1	1.36		266		
			[2M-2H+Na] <sup>-</sup>	643.0615	1.3	0.86		379, 266		
7	26.4	249, 319, 395	[M+NH <sub>4</sub> ] <sup>+</sup>	359.0860	4.0	1.38	C <sub>17</sub> H <sub>11</sub> NO <sub>7</sub>	342, 324, 298	Aristolochic acid I**	St.
			[M+Na] <sup>+</sup>	364.0410	5.2	1.77		318		56
			[M+H-H <sub>2</sub> O] <sup>+</sup>	324.0491	3.6	1.16		280, 265, 237, 222		57
			[M+H-CO <sub>2</sub> ] <sup>+</sup>	298.0698	4.0	1.20		281, 268, 252, 222		58
			[M+H-NO <sub>2</sub> ] <sup>+</sup>	296.0665	4.8	1.43		281		
			[M-H+HCOONa] <sup>-</sup>	408.0348	3.0	1.10		296		
			[2M-2H+Na] <sup>-</sup>	703.0807	1.5	1.07		296		

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\* The compound was identified by comparison with the literature data.

\*\* The compound was identified by comparison with reference standard (St.).



**Table S2.** List of primer sequences used in this study.

Gene name (GenBank accession No.)	Forward (5' to 3')	Reverse (5' to 3')
<i>nptII</i> (AY818371)	TGATATTCGGCAAGCAGGCA	TTGTCACTGAAGCGGGAAGG
<i>rolC</i> (K03313)	GAGCGTAAACCCTTGATCGA	CCGATTGCAAACCTTGCACTC
<i>rolB</i> (K03313)	TTGTCTATCTTTCTCGCGAG	CTTCAGGTTTACTGCAGCAG
<i>AmAct1</i> (OQ676410)	AGATTCCGATGCCCTGAGGT	TCCTCCAATCCAAACGCTGT