

Supplementary data to:

A-NGR FUSION PROTEIN INDUCES APOPTOSIS IN HUMAN CANCER CELLS

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Percentage of cell viability after treatment with A-NGR fusion protein for 48 and 72 h

Table 1: Viability of HT1080 cells after treatment with A-NGR fusion protein for 48 h

Concentration	Percentage of cell viability		
0.5 µg/ml	67.33	69.14	84.72
1 µg/ml	63.16	64.83	66.50
2 µg/ml	53.56	64.41	74.43
4 µg/ml	60.52	64.27	57.60
8 µg/ml	56.07	57.73	55.93
16 µg/ml	51.06	53.84	52.03
32 µg/ml	46.19	48.69	52.73
40 µg/ml	50.92	44.52	48.69

Table 2: Viability of HT1080 cells after treatment with A-NGR fusion protein for 72 h

Concentration	Percentage of cell viability		
0.5 µg/ml	62.37	62.20	73.84
1 µg/ml	61.39	55.18	56.67
2 µg/ml	57.54	56.67	52.42
4 µg/ml	58.50	58.17	48.38
8 µg/ml	56.15	45.24	47.05
16 µg/ml	40.09	47.72	50.27
32 µg/ml	46.32	45.27	41.15
40 µg/ml	43.52	39.64	45.02

Table 3: Viability of U937 cells after treatment with A-NGR fusion protein for 48 h

Concentration	Percentage of cell viability		
0.5 µg/ml	98.04	98.01	97.95
1 µg/ml	97.06	96.21	97.03
2 µg/ml	103.04	90.29	88.92
4 µg/ml	90.49	87.65	81.27
8 µg/ml	87.23	77.75	77.75
16 µg/ml	58.82	57.35	56.96
32 µg/ml	49.08	46.43	41.23
40 µg/ml	40.99	43.54	42.56

Table 4: Viability of U937 cells after treatment with A-NGR fusion protein for 72 h

Concentration	Percentage of cell viability		
0.5 µg/ml	96.41	98.66	97.66
1 µg/ml	77.46	73.09	81.84
2 µg/ml	56.73	56.50	72.65
4 µg/ml	63.90	51.57	56.28
8 µg/ml	50.67	51.79	51.23
16 µg/ml	46.64	57.85	45.07
32 µg/ml	38.52	45.91	40.09
40 µg/ml	38.64	38.19	36.39

Table 5: Viability of HT-29 cells after treatment with A-NGR fusion protein for 48 h

Concentration	Percentage of cell viability		
0.5 µg/ml	100.82	99.02	99.65
1 µg/ml	99.74	99.40	98.54
2 µg/ml	99.41	92.82	99.20
4 µg/ml	97.99	94.36	97.99
8 µg/ml	87.91	96.19	91.39
16 µg/ml	78.44	100.11	89.28
32 µg/ml	93.32	88.22	85.42
40 µg/ml	77.70	65.89	70.74

Table 6: Viability of HT-29 cells after treatment with A-NGR fusion protein for 72 h

Concentration	Percentage of cell viability		
0.5 µg/ml	100.43	100.48	100.38
1 µg/ml	92.09	93.13	104.59
2 µg/ml	95.82	100.72	88.38
4 µg/ml	88.53	94.62	90.11
8 µg/ml	93.30	73.58	95.72
16 µg/ml	93.01	67.39	80.58
32 µg/ml	87.88	69.45	70.70
40 µg/ml	80.87	59.56	54.47

Table 7: Viability of MRC-5 cells after treatment with A-NGR fusion protein for 48 h

Concentration	Percentage of cell viability		
0.5 µg/ml	109.09	109.70	107.07
1 µg/ml	106.01	108.27	105.81
2 µg/ml	100.50	103.07	105.05
4 µg/ml	99.17	102.90	103.53
8 µg/ml	97.74	100.77	103.37
16 µg/ml	95.98	95.27	98.59
32 µg/ml	96.87	94.56	96.36
40 µg/ml	90.09	94.27	99.12

Table 8: Viability of MRC-5 cells after treatment with A-NGR fusion protein for 72 h

Concentration	Percentage of cell viability		
0.5 µg/ml	104.78	108.85	105.25
1 µg/ml	105.22	101.05	100.24
2 µg/ml	97.82	94.85	96.41
4 µg/ml	97.65	95.51	95.25
8 µg/ml	96.55	95.86	94.00
16 µg/ml	93.36	97.47	90.56
32 µg/ml	89.51	97.25	93.61
40 µg/ml	85.67	80.13	80.79

Data was analyzed with Graph Pad Prism 5 software.

Table 9: Identification of apoptosis by annexin-V/PI staining

Treatment	The percentage of early and late apoptotic cells after treatment		
Control	1.43	4.52	3.48
72 h-1/2IC ₅₀	12.83	18.02	18.63
72 h-IC ₅₀	14.95	23.77	16.64

Control: The percentage of early and late apoptotic cells after treatment with PBS.

72 h-1/2 IC₅₀: The percentage of early and late apoptotic cells after treatment with A-NGR fusion protein at 72 h-1/2 IC₅₀ concentration for 48 h.

72 h-IC₅₀: The percentage of early and late apoptotic cells after treatment with A-NGR fusion protein at 72 h-IC₅₀ concentration for 48 h.

Table 10: The analysis of mRNA expression of caspase 8, caspase 9 and caspase 3 by real time RT-PCR

	Fold change		
Caspase 8	1.49	1.24	1.15
Caspase 9	3.39	3.46	4.06
Caspase 3	1.77	1.91	1.99

The relative expression of each gene was analyzed by comparative threshold cycle (Ct). Ct value was normalized using the formula $\Delta Ct = Ct(\text{apoptotic genes}) - Ct(\text{GAPDH})$. Then formula $\Delta\Delta Ct = \Delta Ct(\text{treated}) - \Delta Ct(\text{control})$ was used. Finally, the formula $2^{-\Delta\Delta Ct}$ was used for estimating relative expression of each gene.

Figure 1 was extracted from Tables 1 to 8.

Figure 2 is a representative of percentage of Vital cells, Early apoptosis and Late apoptosis when treated with PBS, A-NGR fusion protein.

Figure 3 was extracted from Table 9.

Figure 4 was extracted from Table 10.