

Supplementary material to

Original article:

DEXMEDETOMIDINE INHIBITS INFLAMMATION IN MICROGLIA CELLS UNDER STIMULATION OF LPS AND ATP BY C-FOS/NLRP3/CASPASE-1 CASCADES

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Figure 1A: Cell viability tests

Groups	Test1*	Test2	Test3	Average	Compared to control
A	0.466	0.423	0.522	0.470333333	1
B	0.362	0.274	0.307	0.314333333	0.651266766
C1	0.309	0.281	0.295	0.295	0.627214741
C2	0.367	0.333	0.355	0.351667	0.747696669
C3	0.394	0.403	0.419	0.405333	0.861800142

*The tested value is absorbance. Test (1-3) indicates that one experiment was repeated for 3 times.
 Note: The absorbance in the table is equal to original test values deducting the average background value (0.023). For example in the group A of Test1, $0.489-0.023=0.466$

Figure 1B: Extracellular concentrations of inflammatory factors

IL-1 β concentration

Groups	Test1*	Test2	Test3	Average	Concentrations (pg/ml)
A	0.735	0.648	0.607	0.663333	101.7796667
B	2.012	2.676	2.935	2.541	423.49024
C1	2.096	2.327	2.658	2.360333333	392.4806133
C2	1.867	1.543	1.526	1.645333333	269.7580133
C3	1.314	1.029	1.005	1.116	178.90324

*The tested value is absorbance.
 Note: a, b, c, d, e, f, g and h indicate the test values of the standard substance of IL-1 β at 500, 250, 125, 62.5, 31.25, 15.625, 7.8, and 0 pg/ml, respectively.
 The absorbance in the table is equal to original test values deducting the average background value (0.028). For example in the group A of Test1, $0.763-0.028=0.735$

IL-18 concentration

Groups	Test1*	Test2	Test3	Average	Concentrations (pg/ml)
A	0.463	0.408	0.577	0.482666667	147.4943467
B	0.846	0.748	0.971	0.855	281.5269
C1	0.893	0.764	0.781	0.812666667	266.2877467
C2	0.767	0.645	0.708	0.706666667	228.1298667
C3	0.502	0.536	0.592	0.543333333	169.3331333

*The tested value is absorbance.
 Note: a, b, c, d, e, f, g and h indicate the test values of the standard substance of IL-18 at 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 0 pg/ml, respectively.
 The absorbance in the table is equal to original test values deducting the average background value (0.028). For example in the group A of Test1, $0.491-0.028=0.463$

Figure 1C: Relative protein levels

NLRP3 protein level

Groups	Test1*	Test2	Test3	Average
A	0.036	0.079	0.022	0.045667
B	0.206	0.269	0.324	0.266333
C1	0.178	0.153	0.177	0.169333
C2	0.049	0.083	0.103	0.078333
C3	0.044	0.071	0.095	0.07

*The value is the ratio of optical density of NLRP3 to that of GAPDH.

Noncleaved caspase-1

Groups	Test1*	Test2	Test3	Average
A	0.275	0.179	0.237	0.230333
B	0.07	0.01	0.03	0.036667
C1	0.09	0.05	0.02	0.053333
C2	0.08	0.03	0.04	0.05
C3	0.31	0.28	0.21	0.266667

*The value is the ratio of optical density of noncleaved caspase-1 to that of GAPDH.

Cleaved caspase-1

Groups	Test1*	Test2	Test3	Average
A	0.09	0.06	0.03	0.06
B	0.32	0.24	0.29	0.283333
C1	0.175	0.195	0.226	0.198667
C2	0.082	0.176	0.123	0.127
C3	0.097	0.074	0.076	0.082333

*The value is the ratio of optical density of cleaved caspase-1 to that of GAPDH.

Figure 2A: Relative protein expression

Groups	Test1*	Test2	Test3	Average
A1	0.155	0.136	0.093	0.128
A2	0.133	0.104	0.092	0.109667
A3	0.081	0.056	0.072	0.069667
A4	0.043	0.024	0.031	0.032667

*The value is the ratio of optical density of c-Fos to that of histone.

Figure 2B: Relative luciferase activity

Groups	Test1*	Test2	Test3	Average
A	0	0.12	0.25	0.123333
B	11.75	13.52	16.71	13.99333
C	2.61	3.86	1.43	2.633333
D	7.83	6.32	6.54	6.896667
E	13.21	11.32	10.57	11.7

*The value is relative luciferase activity.

Figure 3A: c-Fos protein levels in cell nucleus

Groups	Test1*	Test2	Test3	Average
A	0.146	0.109	0.137	0.130667
B	0.045	0.037	0.021	0.034333
C	0.246	0.225	0.199	0.223333
D	0.016	0.006	0.012	0.011333
E	0.088	0.033	0.069	0.063333

*The value is the ratio of optical density of c-Fos to that of histone.

Figure 3A: Relative protein levels of NLRP3

Groups	Test1*	Test2	Test3	Average
A	0.046	0.078	0.084	0.069333
B	0.038	0.023	0.027	0.029333
C	0.214	0.284	0.271	0.256333
D	0.046	0.013	0.036	0.031667
E	0.19	0.11	0.14	0.146667

*The value is the ratio of optical density of NLRP3 to that of GAPDH.

Figure 3B: Fluorescence intensity of c-Fos

Groups	Test1*	Test2	Test3	Average
A	4.02	3.74	3.11	3.623333
B	2.17	2.53	1.94	2.213333
C	7.47	4.88	5.99	6.113333
D	1.76	1.22	2.17	1.716667
E	2.22	1.34	1.52	1.693333

*The value is the fluorescence intensity.

Figure 3C: Relative mRNA levels of *NLRP3*

Groups	Test1*	Test2	Test3	Average
A	1.192	0.916	1.081	1.063
B	0.658	0.595	0.358	0.537
C	2.57	2.91	1.89	2.456667
D	0.247	0.596	0.149	0.330667
E	1.57	0.941	0.879	1.13

*The value is relative mRNA expression level of *NLRP3*.

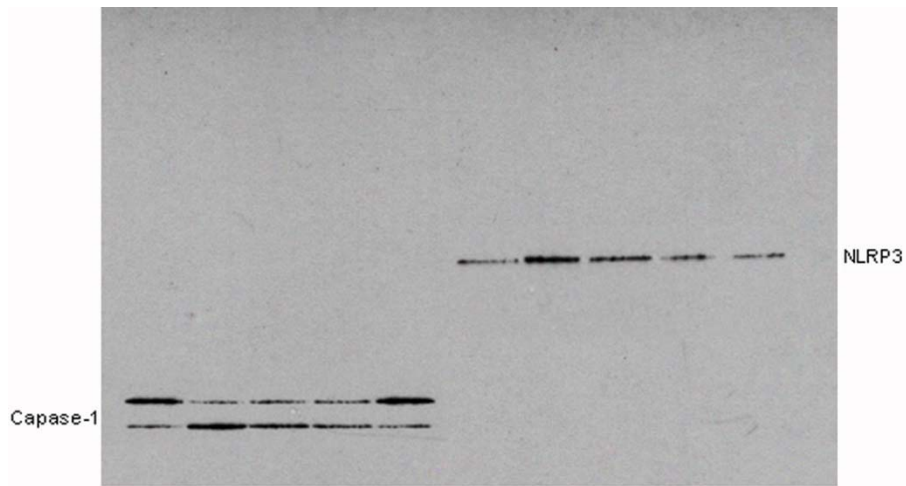


Figure-1C

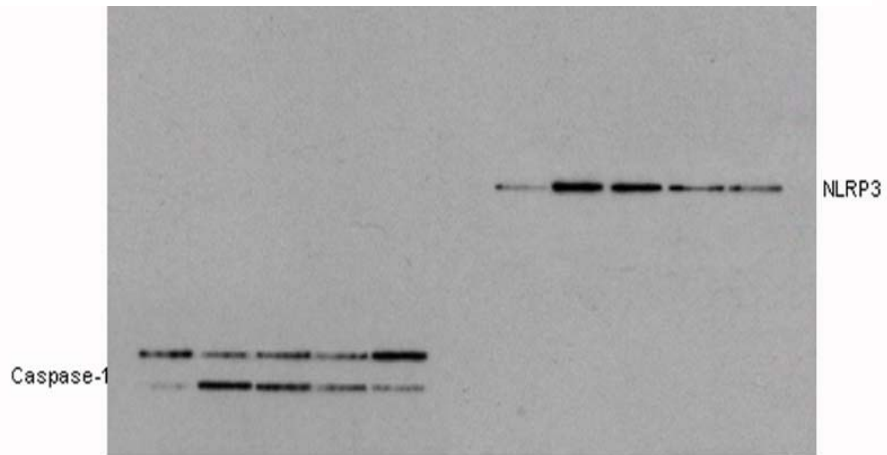


Figure 1C

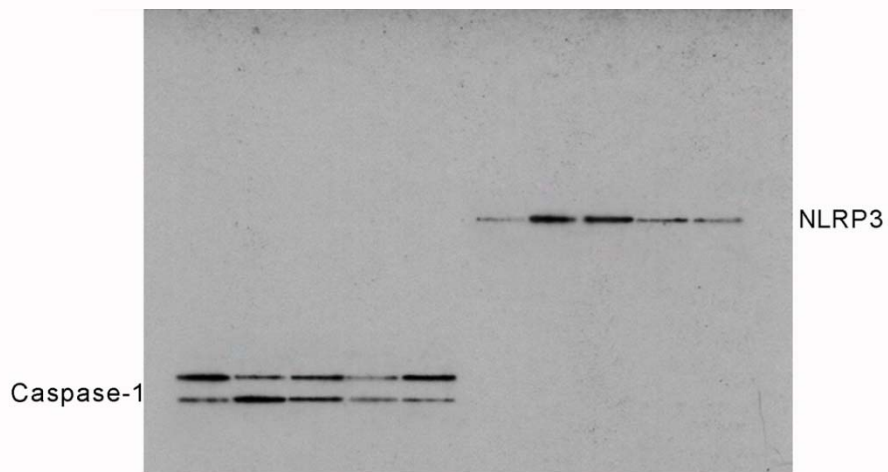


Figure-1C

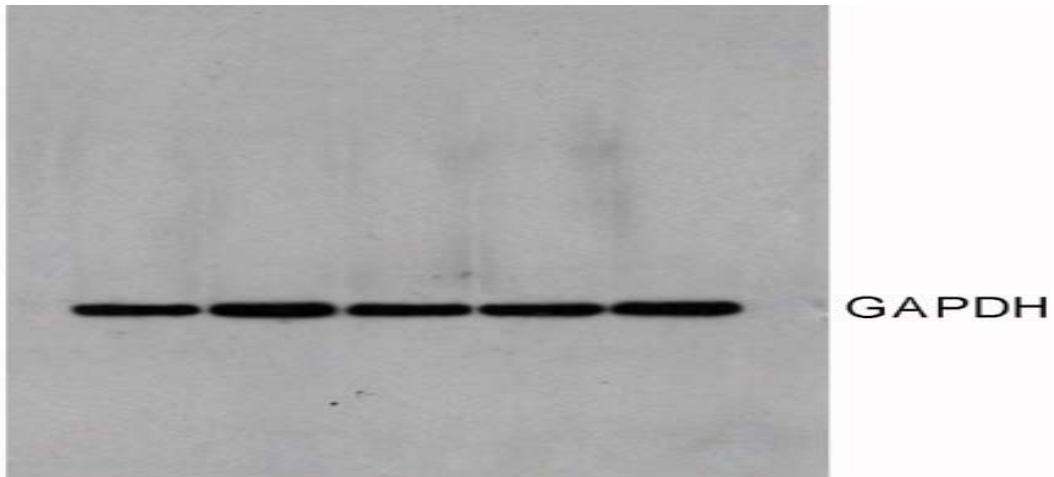


Figure-1C

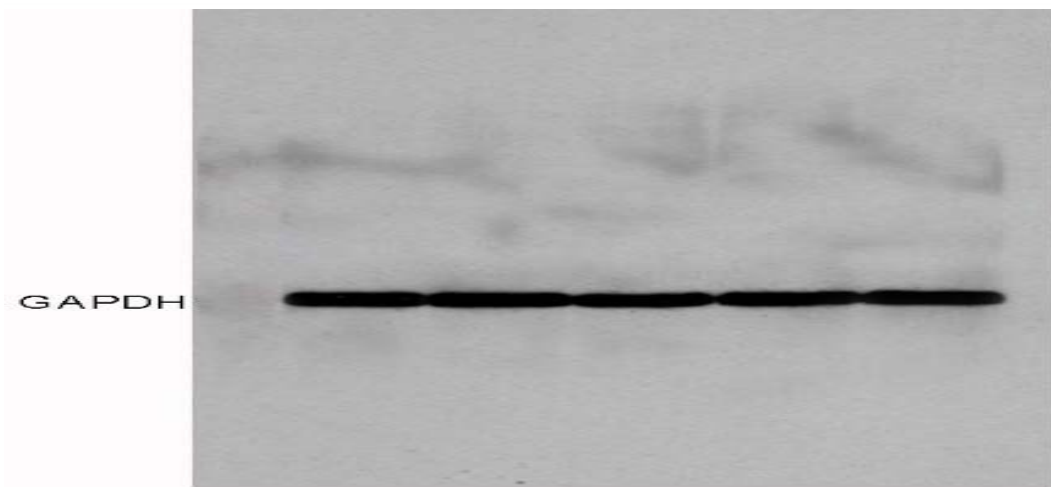


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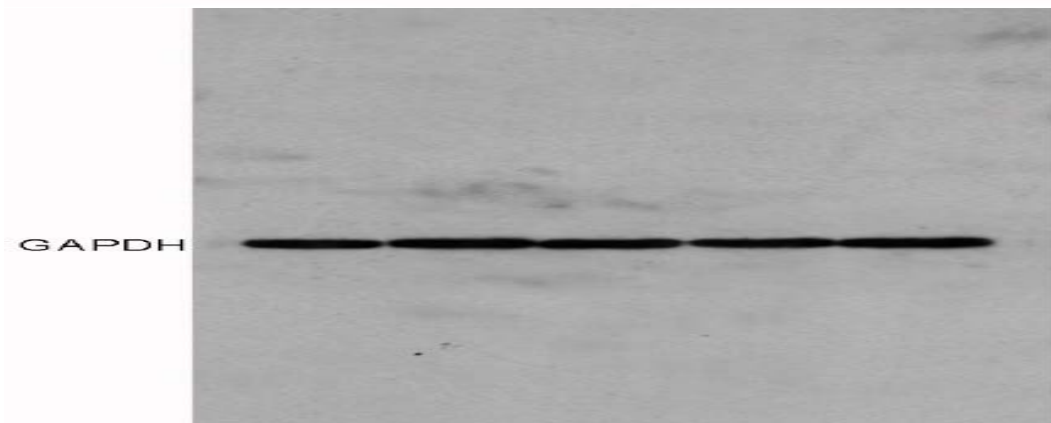


Figure-1C

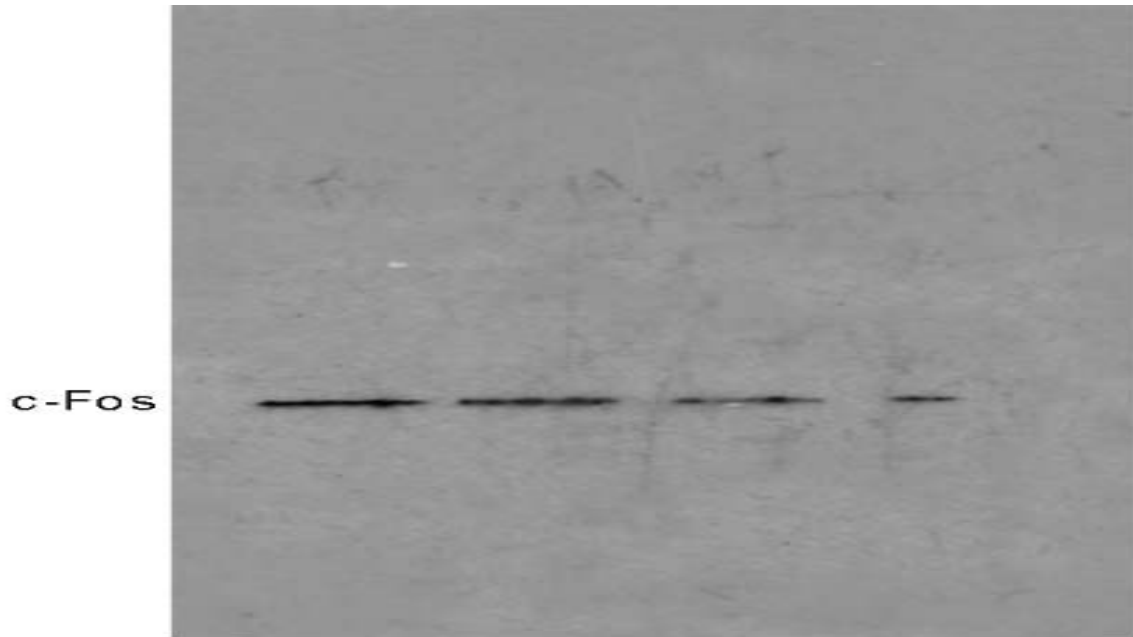


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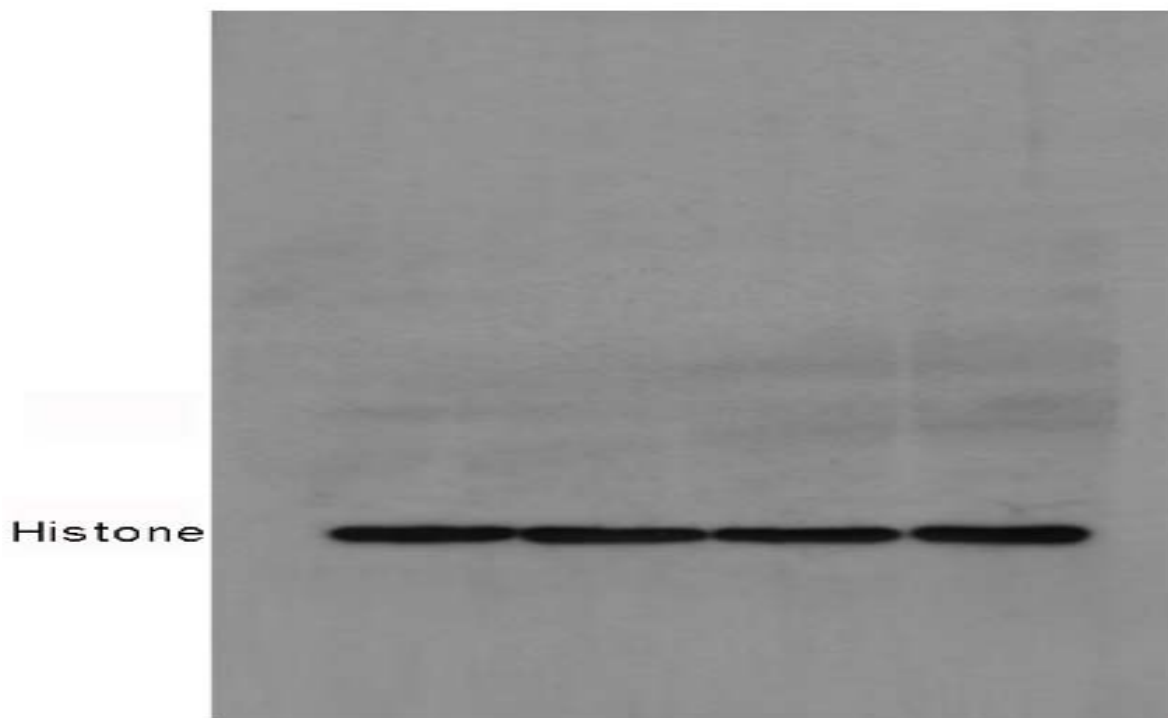


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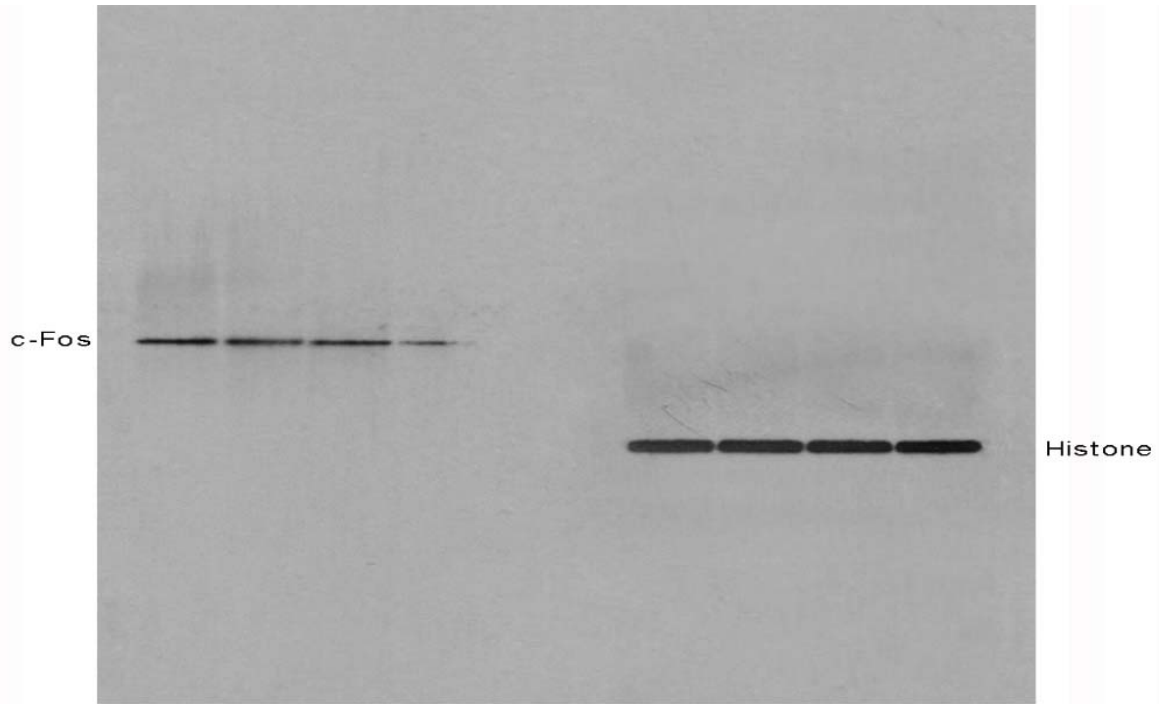


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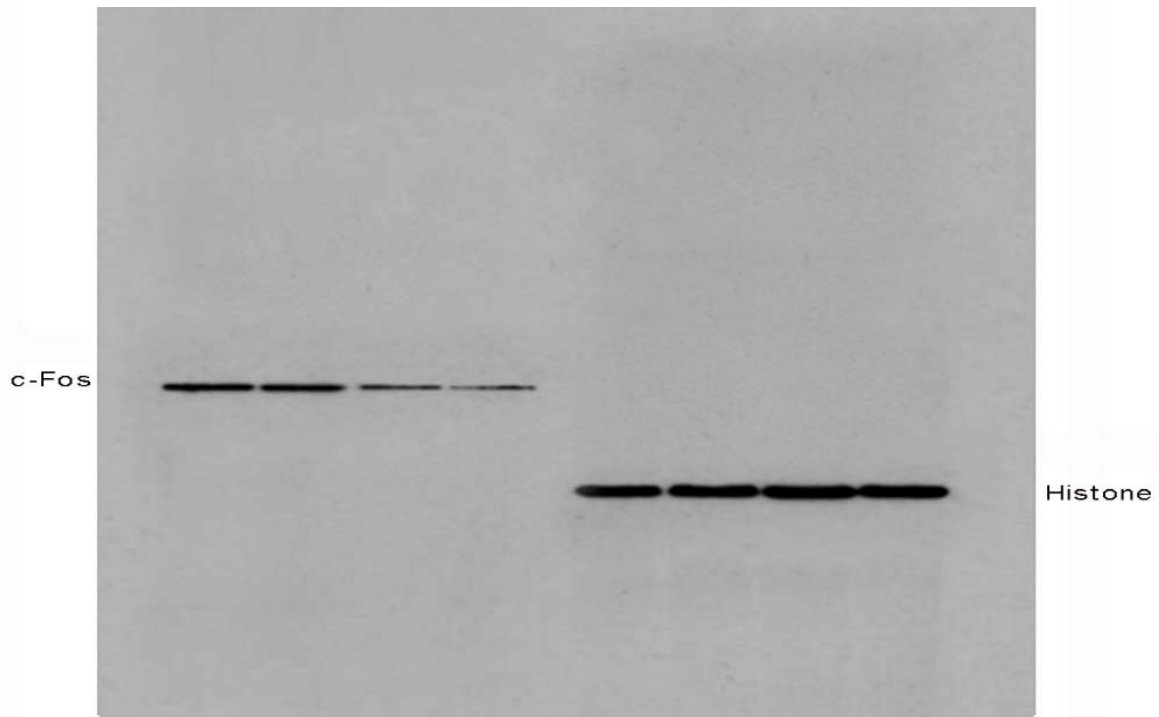


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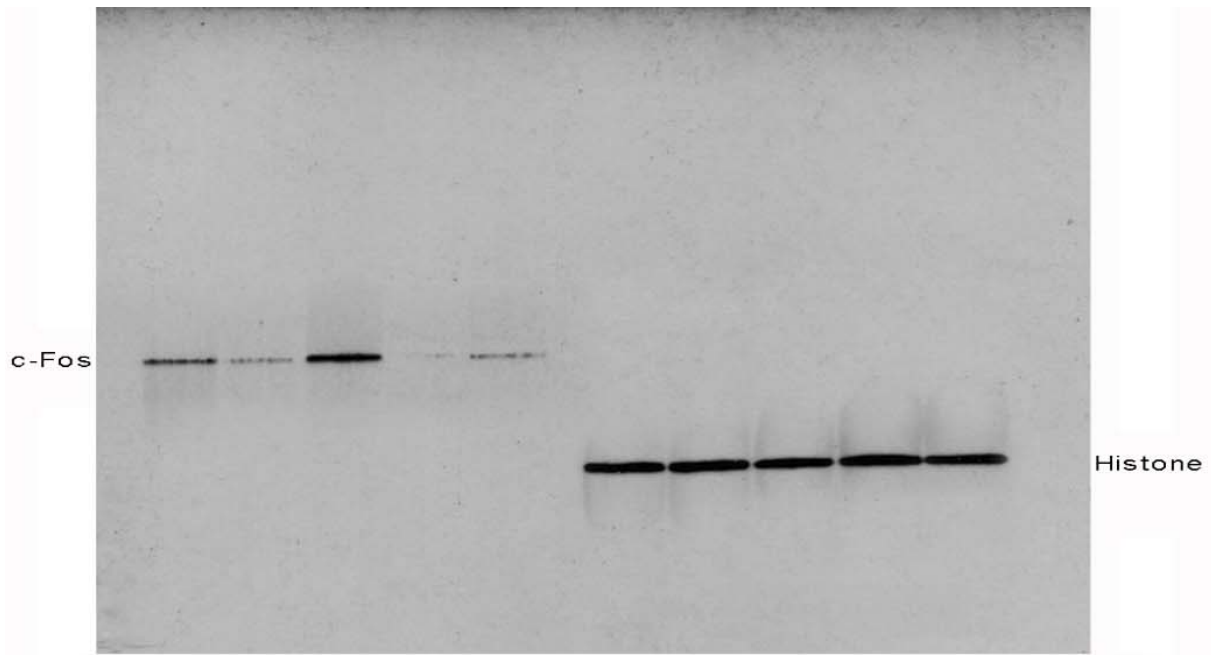


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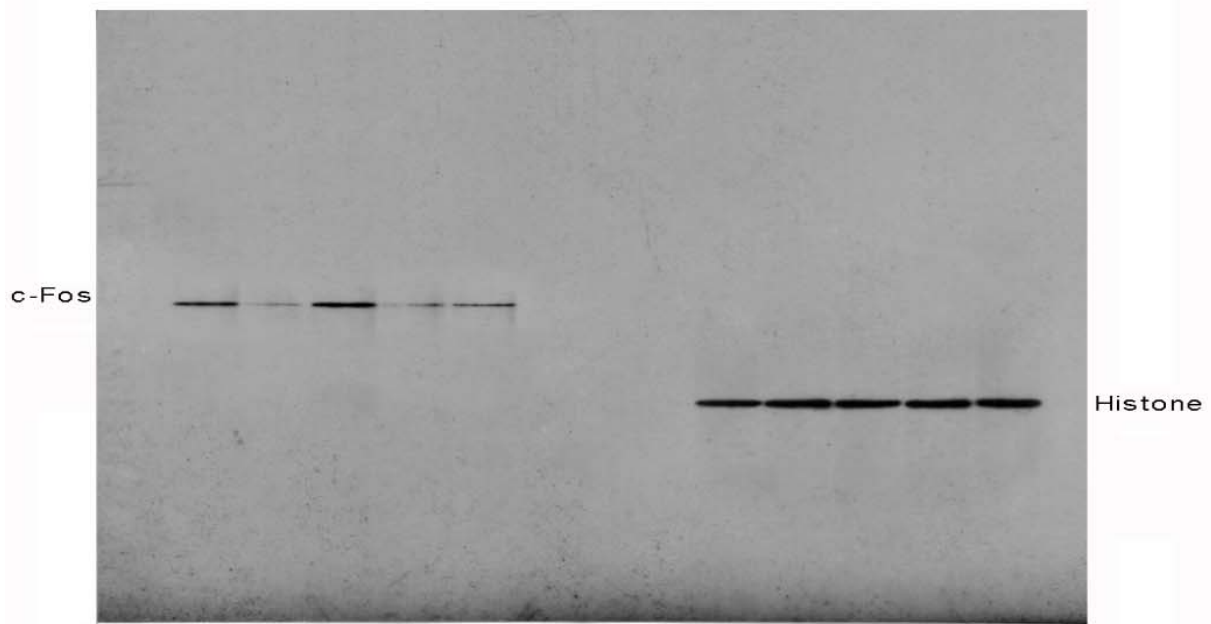


Figure-3A



Figure-3A

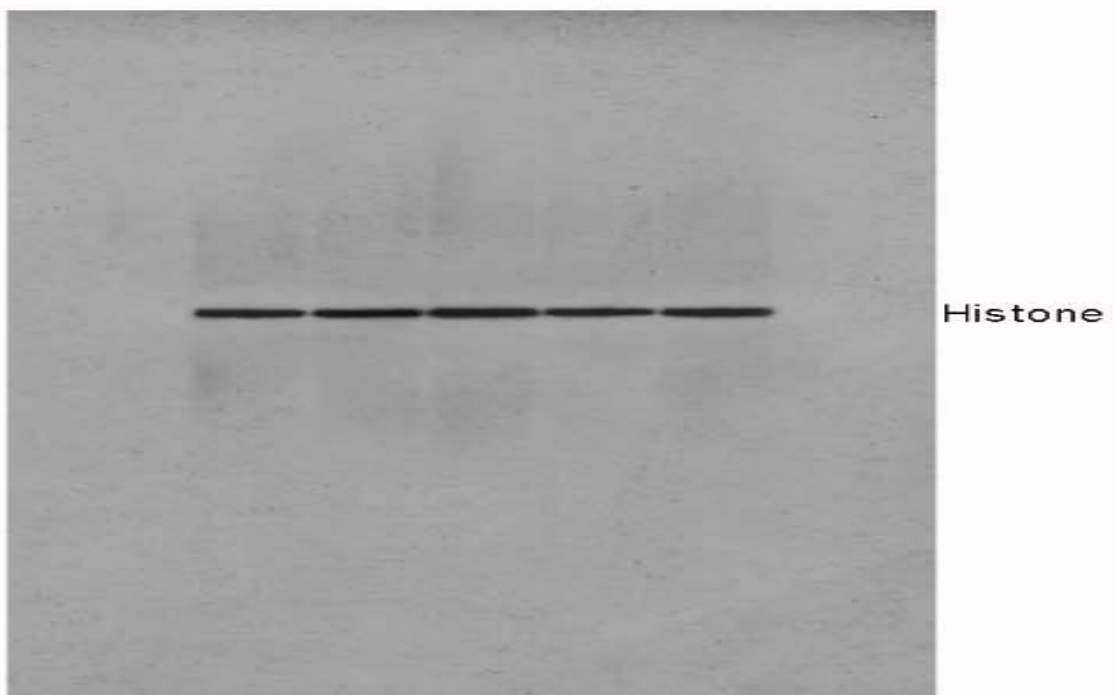


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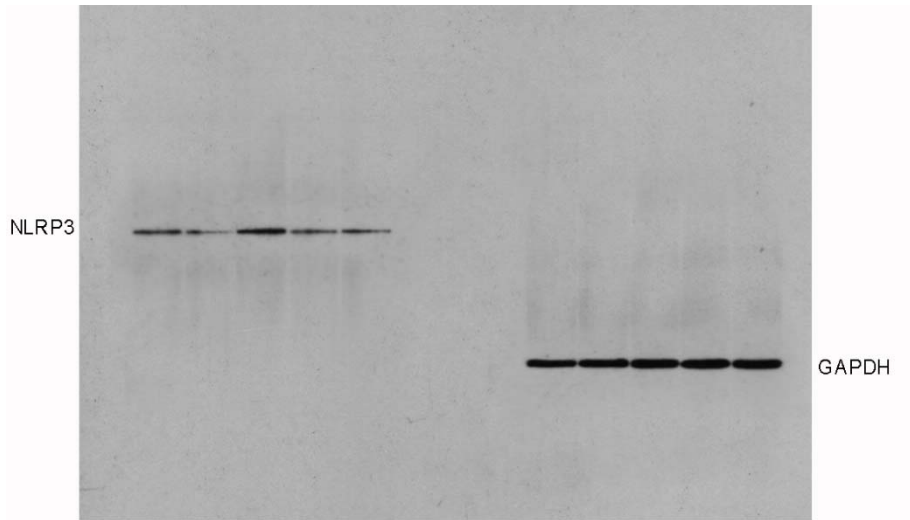


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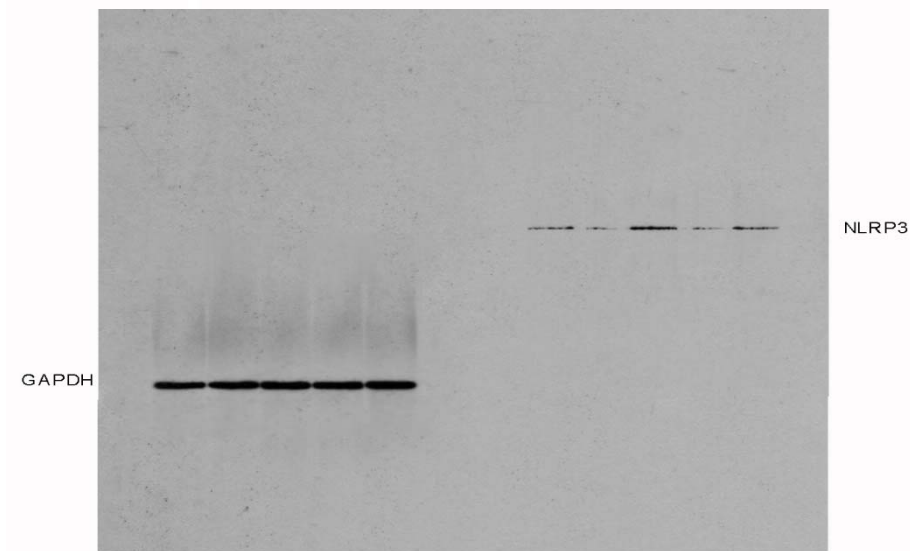


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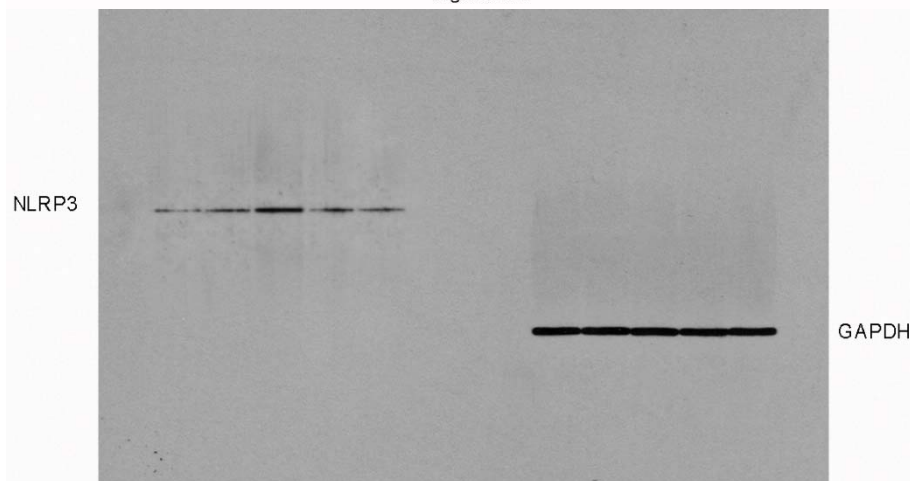


Figure 3A