● *Supplementary material 1*

High-Performance Liquid Chromatography analysis of WTJD

Method: The chemical characterization of WTJD was analyzed using the HPLC system (Shimadzu Corporation, LC-2030C3D, Japan). Accurately weighted 0.5 g of WTJD powder, and added 50 mL of 70% methanol. The sample was weighed, heated, and then refluxed for 30 min. Afterward, the mixture was placed for cooling, weighed, and then compensated for its reduced weight by adding 70% methanol. Afterward, the sample went through a 0.45 μm microporous membrane. Subsequently, 10 μL of the filtrate's supernatant was introduced into the HPLC system.

Chromatographic separations were performed using the Shimadzu InertSustain AQ-C18 (250 mm × 4.6 mm, 5 μm) column. The mobile phase system comprises acetonitrile (A) and 0.3% phosphoric acid solution (B). The flow rate, injection volume, column temperature, and detection wavelength are programmed at 1 mL/min, 5 μL, 35 °C, and 240 nm, respectively. **Table** presents the gradient elution procedure.

Table. HPLC gradient elution procedure

Time（min）	Mobile phase A (%)	Mobile phase B (%)
0~20	7	93
20~50	7→20	93→80

Preparation of reference solution: The reference substance was precisely weighed and dissolved with methanol. Loganin and morroniside were set to a mass concentration of 50 ug/mL; Nystose was set to a mass concentration of 0.2 mg/mL; Curcumin was set to a mass concentration of 2 mg/mL. Subsequently, all reference substances were filtered by 0.22 μm microporous membrane.

Result:

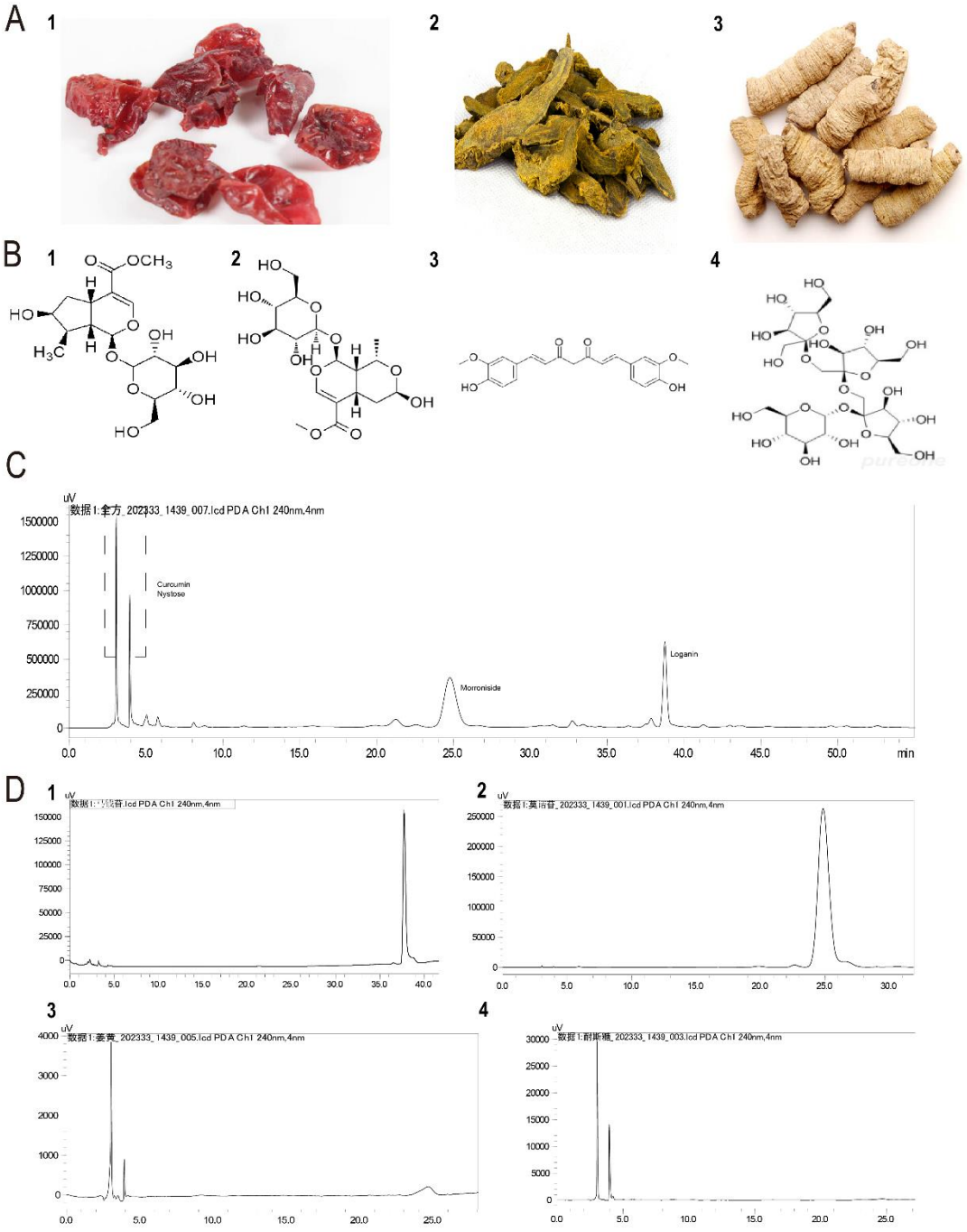

Figure. HPLC chromatography profiles of the major phytochemicals in WTJD. (A) The composition of WTJD: (1) Shanzhuyu; (2) Yujin; (3) Bajitian. (B) The structures of the major phytochemicals: (1) Loganin; (2) Morroniside; (3) Curcumin; (4) Nystose. (C) HPLC chromatogram of WTJD. (D) HPLC chromatograms of standards: (1) Loganin; (2) Morroniside; (3) Curcumin; (4) Nystose.

Conclusion: HPLC characterized the main chemical components of WTJD. Figure shows that the four main monomer components in WTJD were

identified based on the retention time of the standard: Loganin, Morroniside, Curcumin, and Nystose.

● *Supplementary material 2*

Kidney yang deficiency pattern trait score table in rats

		0 score	1 score	2 scores	3 scores
Vital signs	**Temperature（℃）**	38.5～39.5	38.0～38.4	37.5～37.9	≤37.4
	Weight（g）	≥300	275～299	250～274	＜250
Behavioral evaluation (5 min continuous observation, recording time)	**Curl up and bend over（s）**	≤30	31～149	150～299	300
	Get a bunch up（s）	≤30	31～149	150～299	300
Physical features	**Auricle**	The color of the pinna is pale pink	The pinna is pale pink and clear blood vessels are visible	The pinna is pale and clear blood vessels are visible	The color of the pinna is white as paper
	Claw armor	The claw nails are rosy and shiny	The claw nails are pink and not soft	The claw armor is pale in color	The color of the claw armor is white as paper
	Tail color	The tail is pink	The tail is slightly whitish Color lines	The tail is pale white	The tail color is white paper
	Eyes	The eyes are rosy and agile	The eyes are red and slightly dull	The eyes are dull and faint, haggard	Squinting, lethargy

	Tongue color	Purple and red	The color of the tongue darkened	The color of the tongue has become lighter	The tongue is pale in color
	Fluid on the tongue	Moisturizing the tongue	Tongue fluid slightly increased	The fluid on the surface of the tongue has increased, and it feels very slippery	The liquid on the tongue is about to drip down
	Densification of body hair	No change in body hair (uniform and fine distribution)	Loss of body hair (shedding body hair visible at the bottom of the cage)	The body hair visible to the naked eye is fluffy and sparse	Local hair removal is severe
	Body hair gloss	Body hair is white and shiny and smooth	The gloss of the body hair is reduced, and the white is interspersed with yellow	The body hair is dull, slightly messy, yellowish in color, and harder in texture	Body hair is very dull, dry, messy, yellow in color
Pattern evaluation	**Overall score< 12: normal; Total score 12~16: mild kidney yang deficiency pattern; Total score 17~21: moderate kidney yang deficiency pattern; Total score>22: Severe kidney yang deficiency pattern.**				

- ***Supplementary material 3***

Instruction of Prestained Protein Marker II (10-200 kDa）

Servicebio® Prestained Protein Marker II (10-200 kDa)

目录号：G2058

产品信息

产品名称	产品编号	规格
Prestained Protein Marker II (10-200 kDa)	G2058-250UL	250 μL

产品简介

本产品 Prestained Protein Marker II 由 10 种高纯度并预染的重组蛋白质组成，在 Tris-Glycine gel 中指示分子量范围为 10-200 kDa（~10、~18、~23、~30、~42、~55、~75、~110、~140、~200 kDa），其中 75 kDa 为橙红色条带，10 kDa 为玫红色条带，其他均为蓝色条带，方便动态观察蛋白质电泳状态或判断蛋白质转膜效果。适合作为 SDS-PAGE 和 Western blot 的蛋白质分子量标准。

储存与运输

冰袋（wet ice）运输；-20℃保存，12 个月有效。

组成

Component Number	Component	G2058-250UL
G2058	Prestained Protein Marker II	250 μL
说明书		1 份

操作步骤

1. 本产品为即用型，无需加入还原剂或加热。取-20℃保存的 Prestained Protein Marker 于室温下解冻后轻轻充分混匀；
2. 取本产品 3-10 μL 与实验样品同时进行蛋白电泳；建议有条件的实验室在初次使用本产品时可以根据自身的实验条件和实验习惯通过预实验确定合适的上样量，这样既可以节约成本，同时也可获得效果更佳的实验图片；
3. Prestained Protein Marker 使用完后及时保存于-20℃（建议 5-10 μL 分装保存使用）。

注意事项

1. 本预染蛋白分子量标准不能 100℃加热或者煮沸，加热或煮沸会导致蛋白条带发生降解或脱色。
2. 若使用抗 his 标签抗体，不建议使用本产品。
3. 大分子量蛋白 Western blot 时需要延长转膜时间或升高转膜电压。
4. 图中参考预染蛋白分子量大小是根据非预染蛋白分子量进行标定。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

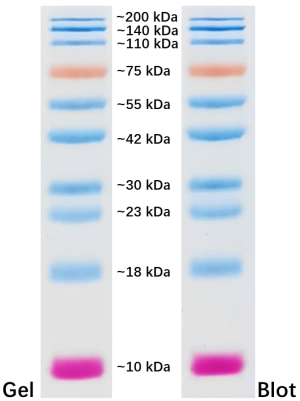

图 1. Prestained Protein Marker II 参考分子量大小

产品仅供科研用途，不用于临床诊断！

武汉赛维尔生物科技有限公司
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电话：027-85714038 网址：www.servicebio.cn 全国免费热线：4006027178

Servicebio® Prestained Protein Marker II (10-200 kDa)

Catalog number： G2058

Product information

Product name	Product number	Specifications
Prestained Protein Marker II (10-200 kDa)	G2058-250UL	250 μL

Product briefing:

The product Prestained Protein Marker II consists of 10 highly purified and pre-stained recombinant proteins in Tris-Glycine gel. The molecular weight range is 10-200kDa (~ 10, ~ 18, ~ 23, ~ 30, ~ 42, ~ 55, ~ 75, ~ 110, ~ 140, ~ 200kDa). The 75 kDa was orange red band, the 10 kDa was rose red band, and the others were blue bands, which was convenient for dynamic observation of protein electrophoresis status or judgment.

Storage and transportation :

Ice bag (wetice) transport ; stored at-20 ℃ for 12 months.

Component：

Component Number	Component	G2058-250UL
G2058	Prestained Protein Marker II (10-200 kDa)	250 μL
Specification		1 sample

Operation steps:

1.This product is ready-to-use, no need to add reducing agent or heating. Prestained Protein Marker stored at -20 ℃ was thawed at room temperature and then gently and fully mixed.

2.Take 3-10μL of this product and the experimental sample for protein electrophoresis at the same time ; it is suggested that conditional laboratories can determine the appropriate loading amount through pre-experiment according to their own experimental conditions and experimental habits when using this product for the first time, which can not only save costs, but also obtain better

experimental pictures .

3.Prestained Protein Marker is stored at-20 ℃ in time after use (5-10μL is recommended for storage).

Precautions:

1. This prestained protein ladder cannot be heated or boiled at 100°C, as heating or boiling will cause degradation or decolorization of protein bands.

2. This product is not recommended if anti-HIS-tag antibody is used.

3. In Westernblot, large molecular weight proteins need to extend the transfer time or increase the transfer voltage.

4. The molecular weight of the reference prestained protein in the figure is calibrated according to the molecular weight of the unstained protein.

5. For your safety and health, please wear a lab coat and disposable gloves to operate.

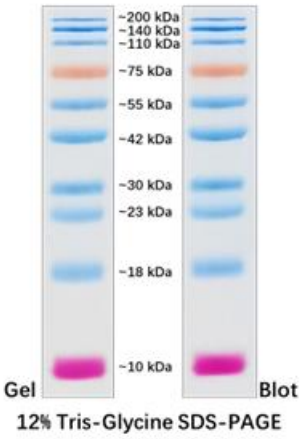


Figure.Prestained Protein Marker II Reference Molecular Weight

●*Supplementary material 4*

Western blot raw data

Grayscale value											
	No.		GAPDH 36KD		ROR 54KD		FOXP3 54KD		T-BET 58KD		GATA3 47KD
Hippoca-mpus tissues	C1	1	24739.49	1	4858.69	1	28551.85	1	8971.933	1	27021.32
	M1	2	25071.49	2	25788.66	2	18868.13	2	27701.63	2	6423.761
	F1	3	25243.44	3	21364.42	3	21571.49	3	22557.32	3	9953.418
	WTJD-H1	4	27376.73	4	14521.64	4	22704.61	4	15017.47	4	20103.37
	WTJD-M1	5	25218.61	5	17928.42	5	22842.49	5	15644.37	5	17476.61
	C2	1	23421	1	14625.88	1	29452.02	1	11627.75	1	26954.15
	M2	2	24037.44	2	28426.44	2	15514.08	2	24644.49	2	5903.225
	F2	3	25305.08	3	24006.32	3	17490.9	3	19684.54	3	12580.54
	WTJD-H2	4	24400.59	4	19236.54	4	17928.49	4	15232.76	4	20190.9
	WTJD-M2	5	23894.35	5	24067.37	5	15697.61	5	21223.54	5	16401.66
	C3	1	23339.25	1	7546.974	1	24667.61	1	8106.397	1	23115.15
	M3	2	24456.2	2	35103.58	2	13271.54	2	24169.13	2	9842.296
	F3	3	25460.44	3	26721.48	3	14317.25	3	21077.65	3	15019.78
	WTJD-H3	4	26370.49	4	18426.27	4	23582.78	4	16569.82	4	20508.94
	WTJD-M3	5	25848.9	5	27115.97	5	21252.95	5	18563.41	5	17685.08
	C4	1	23458.42	1	3305.134	1	26726.85	1	17010.66	1	21169.15
	M4	2	24808.9	2	33410.89	2	6590.054	2	27801.56	2	5664.175
	F4	3	25974.15	3	25233.61	3	11958.37	3	26225.9	3	14011.37
	WTJD-H4	4	24962.02	4	15739.71	4	26222.44	4	15897.83	4	18520.43
	WTJD-M4	5	23888.2	5	25601.2	5	17034.78	5	18881.66	5	16108.39
Gray value of target protein/grayscale value of reference protein											
	No.		GAPDH 36KD		ROR 54KD		FOXP3 54KD		T-BET 58KD		GATA3 47KD
Hippoca-mpus tissues	C1		1		0.196394		1.1541		0.362656		1.092234
	M1		1		1.028605		0.752573		1.104906		0.256218
	F1		1		0.846335		0.854538		0.893591		0.394297
	WTJD-H1		1		0.530437		0.82934		0.548549		0.734323
	WTJD-M1		1		0.71092		0.905779		0.62035		0.693004
	C2		1		0.624477		1.257505		0.496467		1.150854
	M2		1		1.18259		0.645413		1.025254		0.245585
	F2		1		0.948676		0.691201		0.777889		0.497155
	WTJD-H2		1		0.788364		0.734756		0.624278		0.827476
	WTJD-M2		1		1.007241		0.656959		0.888224		0.686424
	C3		1		0.32336		1.056915		0.347329		0.990398
	M3		1		1.435366		0.542666		0.988262		0.402446
	F3		1		1.049529		0.562333		0.827859		0.589926
	WTJD-H3		1		0.698746		0.894287		0.628347		0.777723
	WTJD-M3		1		1.049018		0.822199		0.718151		0.684171
	C4		1		0.140893		1.139329		0.725141		0.902411
	M4		1		1.34673		0.265633		1.120628		0.228312
	F4		1		0.971489		0.460395		1.009693		0.539435
	WTJD-H4		1		0.630546		1.050493		0.636881		0.741944
	WTJD-M4		1		1.071709		0.713105		0.790418		0.674324

RORγt

Tukey multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
Normal vs. Model	-0.927	-1.274 to -0.5801	Yes	****	<0.0001	A-B
Normal vs. Fluoxetine	-0.6327	-0.9797 to -0.2858	Yes	***	0.0004	A-C
Normal vs. WTJD-M	-0.6384	-0.9854 to -0.2915	Yes	***	0.0004	A-D
Normal vs. WTJD-H	-0.3407	-0.6877 to 0.006187	No	ns	0.0554	A-E
Model vs. Fluoxetine	0.2943	-0.05261 to 0.6412	No	ns	0.1164	B-C
Model vs. WTJD-M	0.2886	-0.05833 to 0.6355	No	ns	0.1271	B-D
Model vs. WTJD-H	0.5863	0.2394 to 0.9332	Yes	***	0.0008	B-E
Fluoxetine vs. WTJD-M	-0.005715	-0.3526 to 0.3412	No	ns	>0.9999	C-D
Fluoxetine vs. WTJD-H	0.292	-0.05494 to 0.6389	No	ns	0.1207	C-E
WTJD-M vs. WTJD-H	0.2977	-0.04923 to 0.6446	No	ns	0.1105	D-E

Test details	Mean1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Normal vs. Model	0.3213	1.248	-0.927	0.1124	4	4	11.67	15
Normal vs. Fluoxetine	0.3213	0.954	-0.6327	0.1124	4	4	7.964	15
Normal vs. WTJD-M	0.3213	0.9597	-0.6384	0.1124	4	4	8.036	15
Normal vs. WTJD-H	0.3213	0.662	-0.3407	0.1124	4	4	4.289	15
Model vs. Fluoxetine	1.248	0.954	0.2943	0.1124	4	4	3.705	15
Model vs. WTJD-M	1.248	0.9597	0.2886	0.1124	4	4	3.633	15
Model vs. WTJD-H	1.248	0.662	0.5863	0.1124	4	4	7.38	15
Fluoxetine vs. WTJD-M	0.954	0.9597	-0.005715	0.1124	4	4	0.07193	15
Fluoxetine vs. WTJD-H	0.954	0.662	0.292	0.1124	4	4	3.675	15
WTJD-M vs. WTJD-H	0.9597	0.662	0.2977	0.1124	4	4	3.747	15

FOXP3

Tukey multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
Normal vs. Model	0.6004	0.2774 to 0.9234	Yes	***	0.0003	A-B
Normal vs. Fluoxetine	0.5098	0.1868 to 0.8329	Yes	**	0.0016	A-C
Normal vs. WTJD-M	0.3775	0.05442 to 0.7005	Yes	*	0.0186	A-D
Normal vs. WTJD-H	0.2747	-0.04829 to 0.5978	No	ns	0.1151	A-E
Model vs. Fluoxetine	-0.09055	-0.4136 to 0.2325	No	ns	0.905	B-C
Model vs. WTJD-M	-0.2229	-0.5460 to 0.1001	No	ns	0.2577	B-D
Model vs. WTJD-H	-0.3256	-0.6487 to -0.002617	Yes	*	0.0477	B-E
Fluoxetine vs. WTJD-M	-0.1324	-0.4554 to 0.1906	No	ns	0.7149	C-D
Fluoxetine vs. WTJD-H	-0.2351	-0.5581 to 0.08793	No	ns	0.2156	C-E
WTJD-M vs. WTJD-H	-0.1027	-0.4257 to 0.2203	No	ns	0.8593	D-E

Test details	Mean1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Normal vs. Model	1.152	0.5516	0.6004	0.1046	4	4	8.117	15
Normal vs. Fluoxetine	1.152	0.6421	0.5098	0.1046	4	4	6.892	15
Normal vs. WTJD-M	1.152	0.7745	0.3775	0.1046	4	4	5.103	15
Normal vs. WTJD-H	1.152	0.8772	0.2747	0.1046	4	4	3.714	15
Model vs. Fluoxetine	0.5516	0.6421	-0.09055	0.1046	4	4	1.224	15
Model vs. WTJD-M	0.5516	0.7745	-0.2229	0.1046	4	4	3.014	15
Model vs. WTJD-H	0.5516	0.8772	-0.3256	0.1046	4	4	4.402	15
Fluoxetine vs. WTJD-M	0.6421	0.7745	-0.1324	0.1046	4	4	1.79	15
Fluoxetine vs. WTJD-H	0.6421	0.8772	-0.2351	0.1046	4	4	3.178	15
WTJD-M vs. WTJD-H	0.7745	0.8772	-0.1027	0.1046	4	4	1.388	15

T-bet

Tukey multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
Normal vs. Model	-0.5769	-0.8143 to -0.3394	Yes	****	<0.0001	A-B
Normal vs. Fluoxetine	-0.3944	-0.6318 to -0.1569	Yes	***	0.001	A-C
Normal vs. WTJD-M	-0.2714	-0.5088 to -0.03393	Yes	*	0.0216	A-D
Normal vs. WTJD-H	-0.1266	-0.3641 to 0.1108	No	ns	0.4927	A-E
Model vs. Fluoxetine	0.1825	-0.05495 to 0.4200	No	ns	0.1762	B-C
Model vs. WTJD-M	0.3055	0.06802 to 0.5429	Yes	**	0.0092	B-D
Model vs. WTJD-H	0.4502	0.2128 to 0.6877	Yes	***	0.0003	B-E
Fluoxetine vs. WTJD-M	0.123	-0.1145 to 0.3604	No	ns	0.5198	C-D
Fluoxetine vs. WTJD-H	0.2677	0.03029 to 0.5052	Yes	*	0.0237	C-E
WTJD-M vs. WTJD-H	0.1448	-0.09268 to 0.3822	No	ns	0.367	D-E

Test details	Mean1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Normal vs. Model	0.4829	1.06	-0.5769	0.0769	4	4	10.61	15
Normal vs. Fluoxetine	0.4829	0.8773	-0.3944	0.0769	4	4	7.253	15
Normal vs. WTJD-M	0.4829	0.7543	-0.2714	0.0769	4	4	4.991	15
Normal vs. WTJD-H	0.4829	0.6095	-0.1266	0.0769	4	4	2.329	15
Model vs. Fluoxetine	1.06	0.8773	0.1825	0.0769	4	4	3.356	15
Model vs. WTJD-M	1.06	0.7543	0.3055	0.0769	4	4	5.618	15
Model vs. WTJD-H	1.06	0.6095	0.4502	0.0769	4	4	8.28	15
Fluoxetine vs. WTJD-M	0.8773	0.7543	0.123	0.0769	4	4	2.262	15
Fluoxetine vs. WTJD-H	0.8773	0.6095	0.2677	0.0769	4	4	4.924	15
WTJD-M vs. WTJD-H	0.7543	0.6095	0.1448	0.0769	4	4	2.662	15

GATA3

Tukey multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
Normal vs. Model	0.7508	0.5894 to 0.9122	Yes	****	<0.0001	A-B
Normal vs. Fluoxetine	0.5288	0.3674 to 0.6902	Yes	****	<0.0001	A-C
Normal vs. WTJD-M	0.3495	0.1881 to 0.5109	Yes	****	<0.0001	A-D
Normal vs. WTJD-H	0.2636	0.1022 to 0.4250	Yes	**	0.0012	A-E
Model vs. Fluoxetine	-0.2221	-0.3835 to -0.06067	Yes	**	0.0054	B-C
Model vs. WTJD-M	-0.4013	-0.5627 to -0.2399	Yes	****	<0.0001	B-D
Model vs. WTJD-H	-0.4872	-0.6486 to -0.3258	Yes	****	<0.0001	B-E
Fluoxetine vs. WTJD-M	-0.1793	-0.3407 to -0.01788	Yes	*	0.0262	C-D
Fluoxetine vs. WTJD-H	-0.2652	-0.4266 to -0.1038	Yes	**	0.0011	C-E
WTJD-M vs. WTJD-H	-0.08589	-0.2473 to 0.07551	No	ns	0.4946	D-E

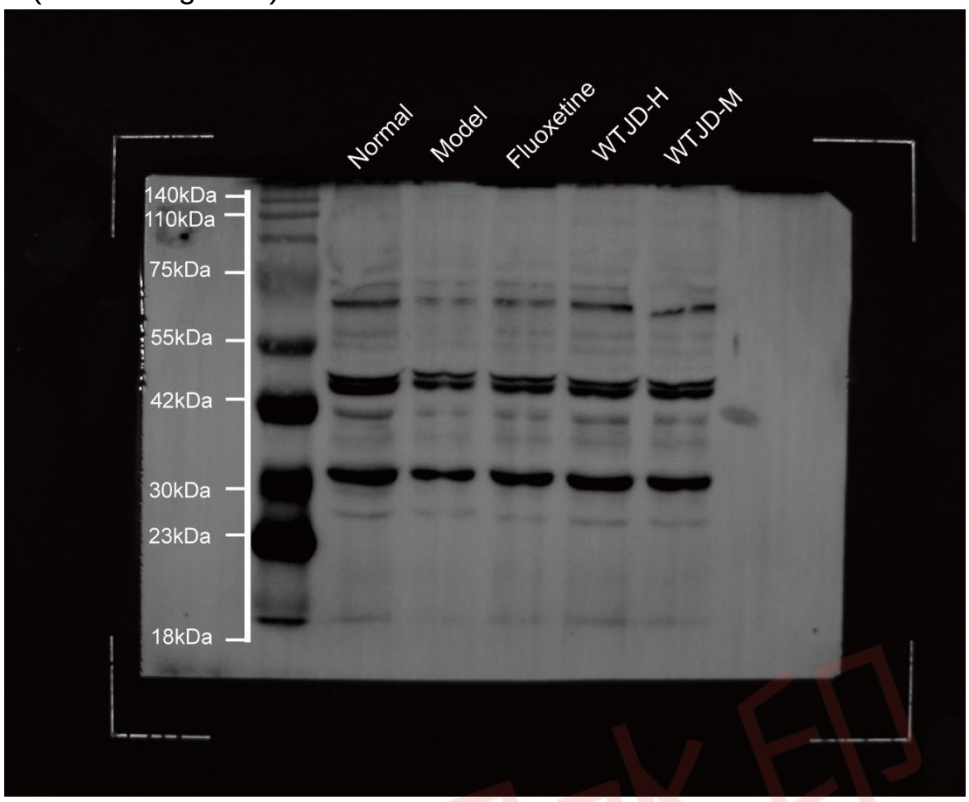
Test details	Mean1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Normal vs. Model	1.034	0.2831	0.7508	0.05227	4	4	20.32	15
Normal vs. Fluoxetine	1.034	0.5052	0.5288	0.05227	4	4	14.31	15
Normal vs. WTJD-M	1.034	0.6845	0.3495	0.05227	4	4	9.456	15
Normal vs. WTJD-H	1.034	0.7704	0.2636	0.05227	4	4	7.133	15
Model vs. Fluoxetine	0.2831	0.5052	-0.2221	0.05227	4	4	6.008	15
Model vs. WTJD-M	0.2831	0.6845	-0.4013	0.05227	4	4	10.86	15
Model vs. WTJD-H	0.2831	0.7704	-0.4872	0.05227	4	4	13.18	15
Fluoxetine vs. WTJD-M	0.5052	0.6845	-0.1793	0.05227	4	4	4.851	15
Fluoxetine vs. WTJD-H	0.5052	0.7704	-0.2652	0.05227	4	4	7.175	15
WTJD-M vs. WTJD-H	0.6845	0.7704	-0.08589	0.05227	4	4	2.324	15

● *Supplementary material 5*

Western blot pictures

FOXP3

1.(For the Figure 5)



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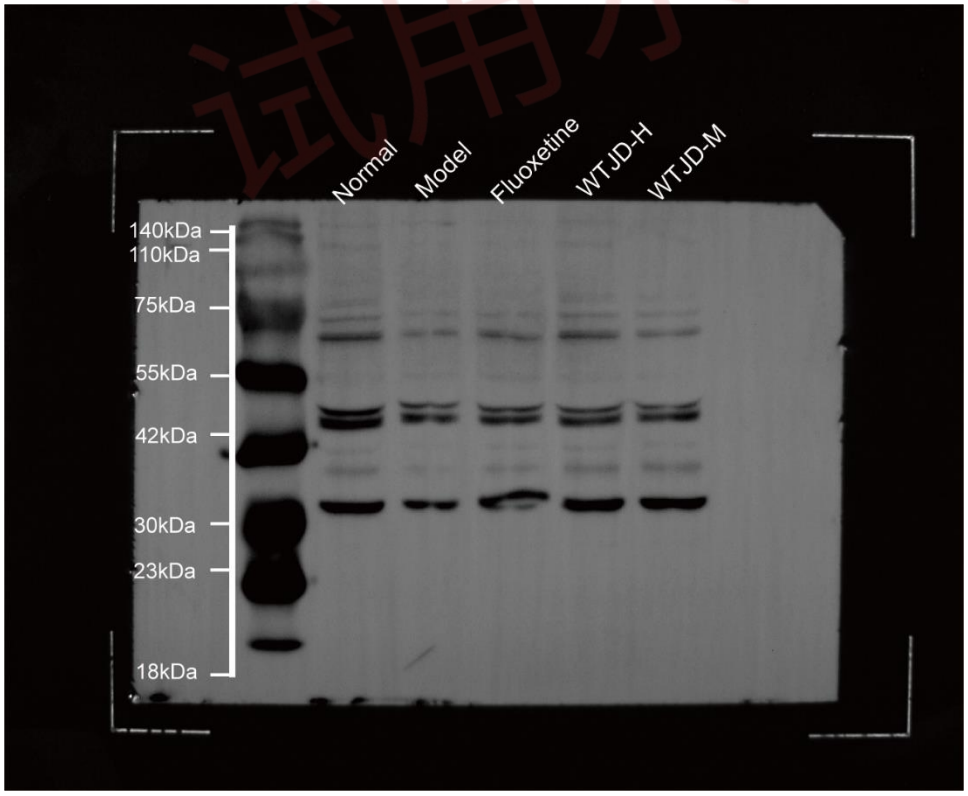

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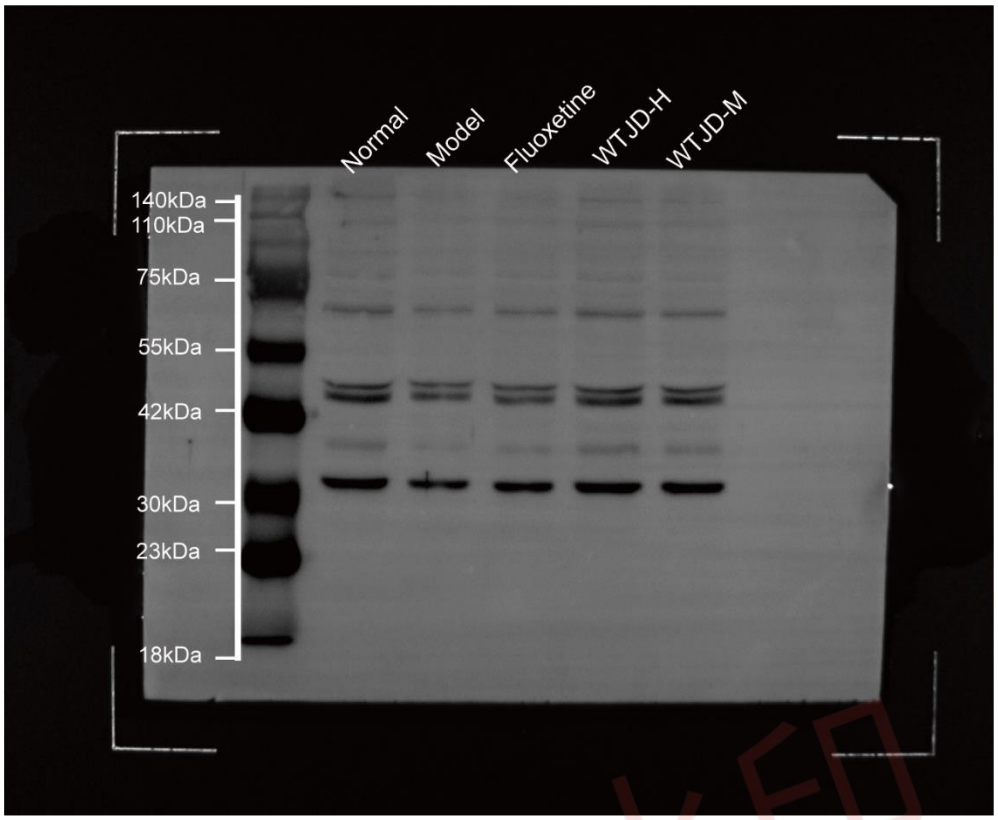
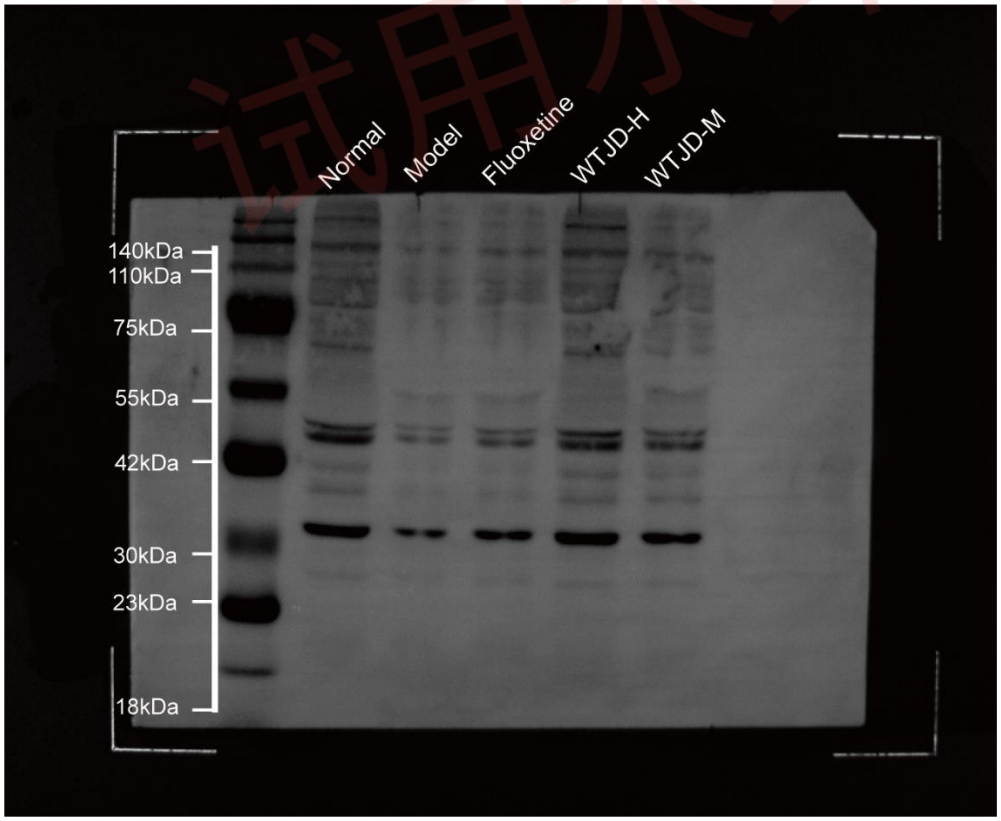
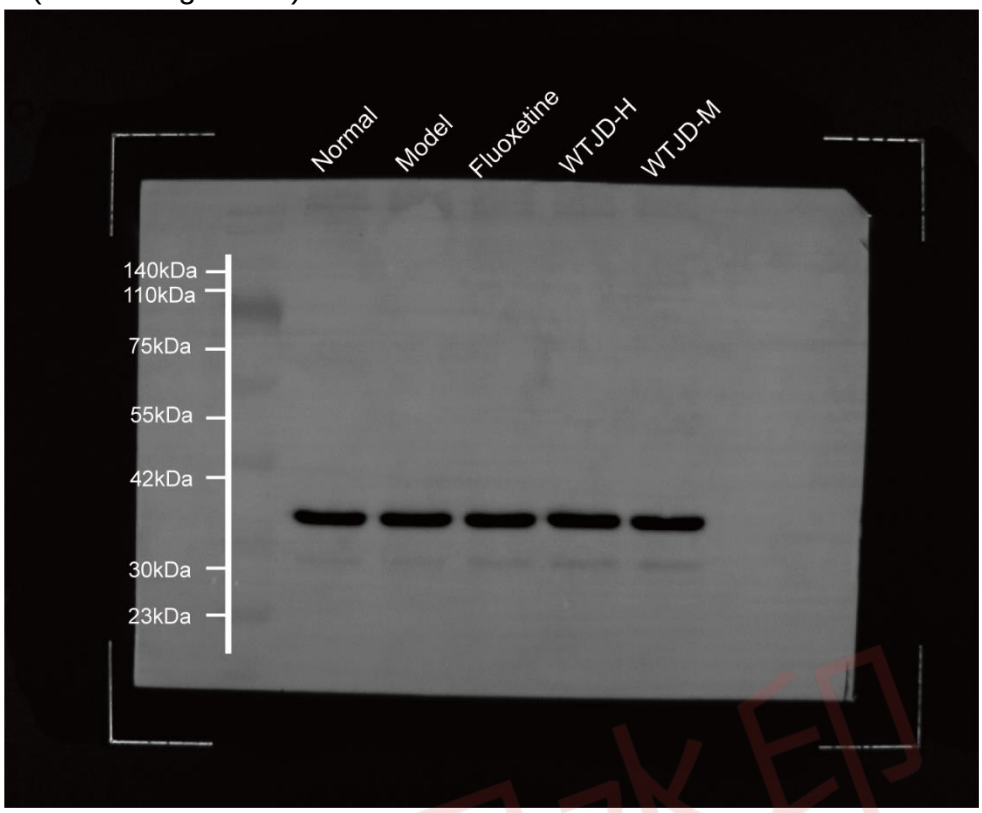

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa
18kDa

4.

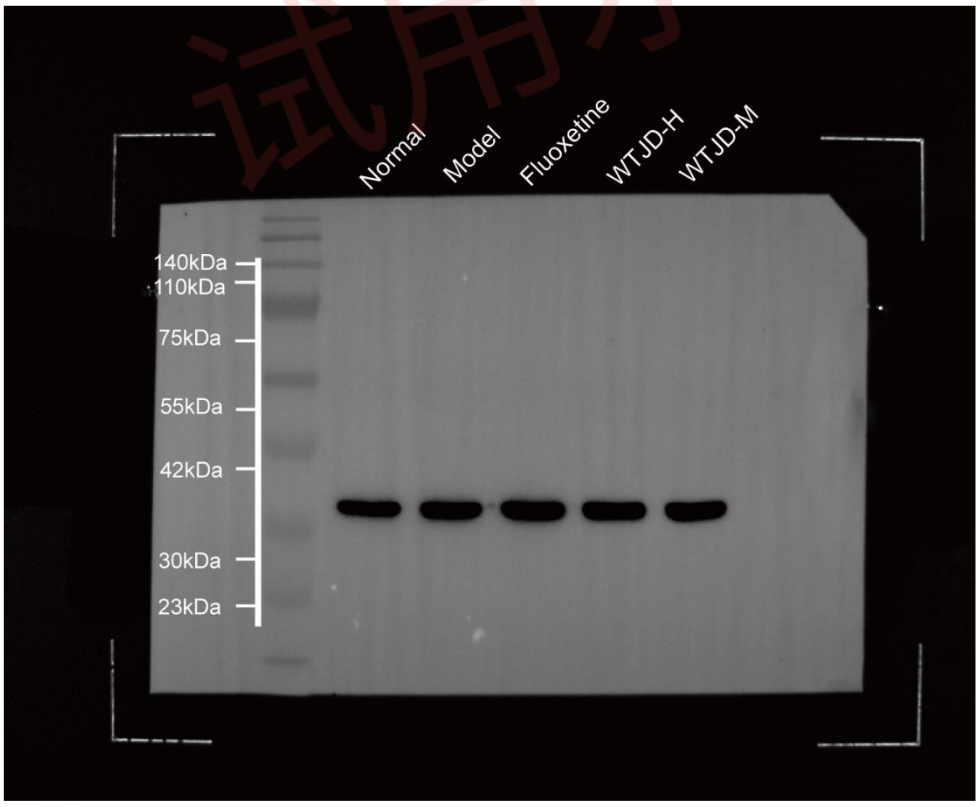

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa
18kDa

GAPDH

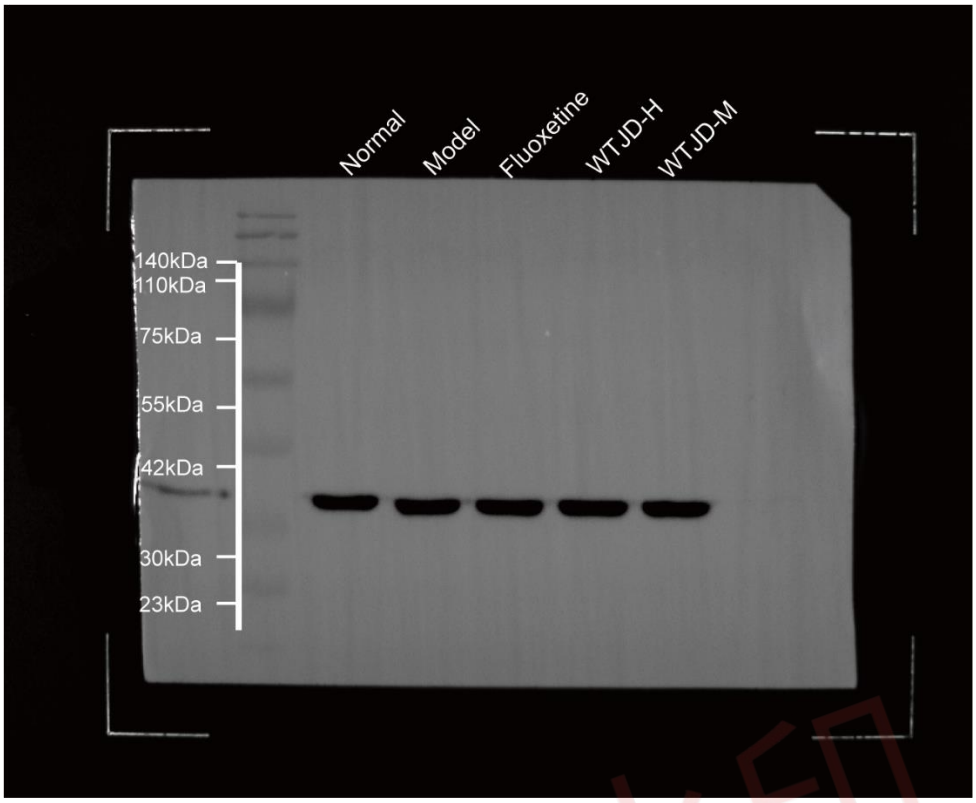
1.(For the Figure 5A)



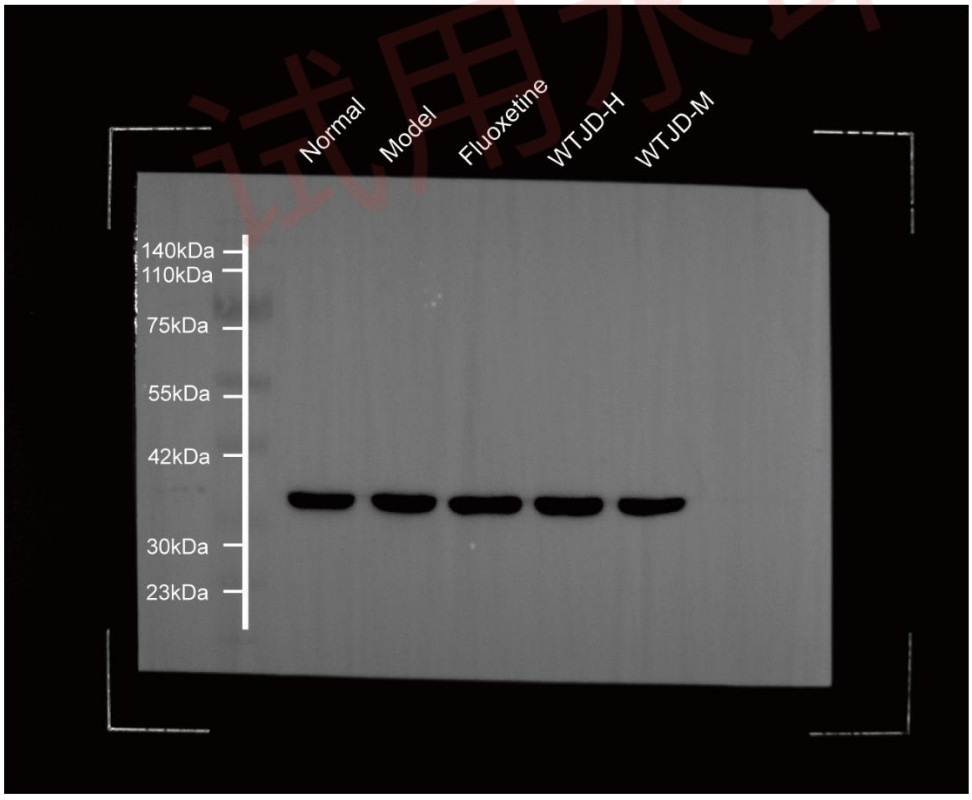
2.(For the Figure 5B)



3.(For the Figure 5C)

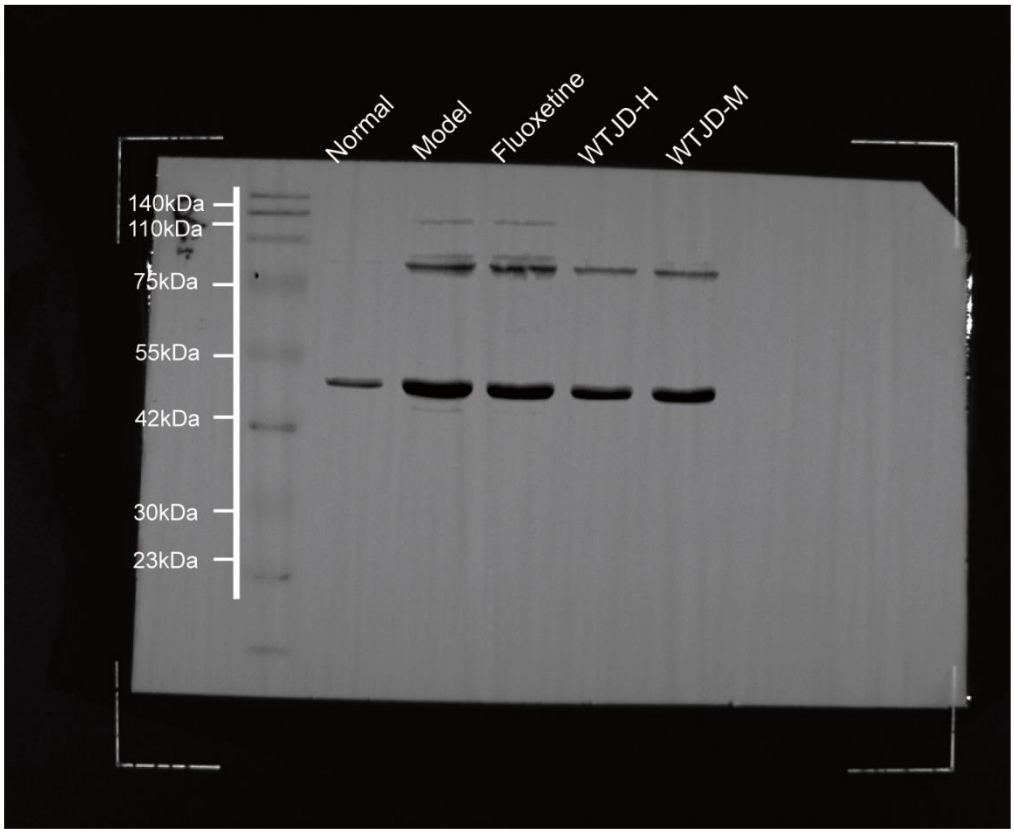


4.(For the Figure 5D)



ROR γ t

1.(For the Figure 5)



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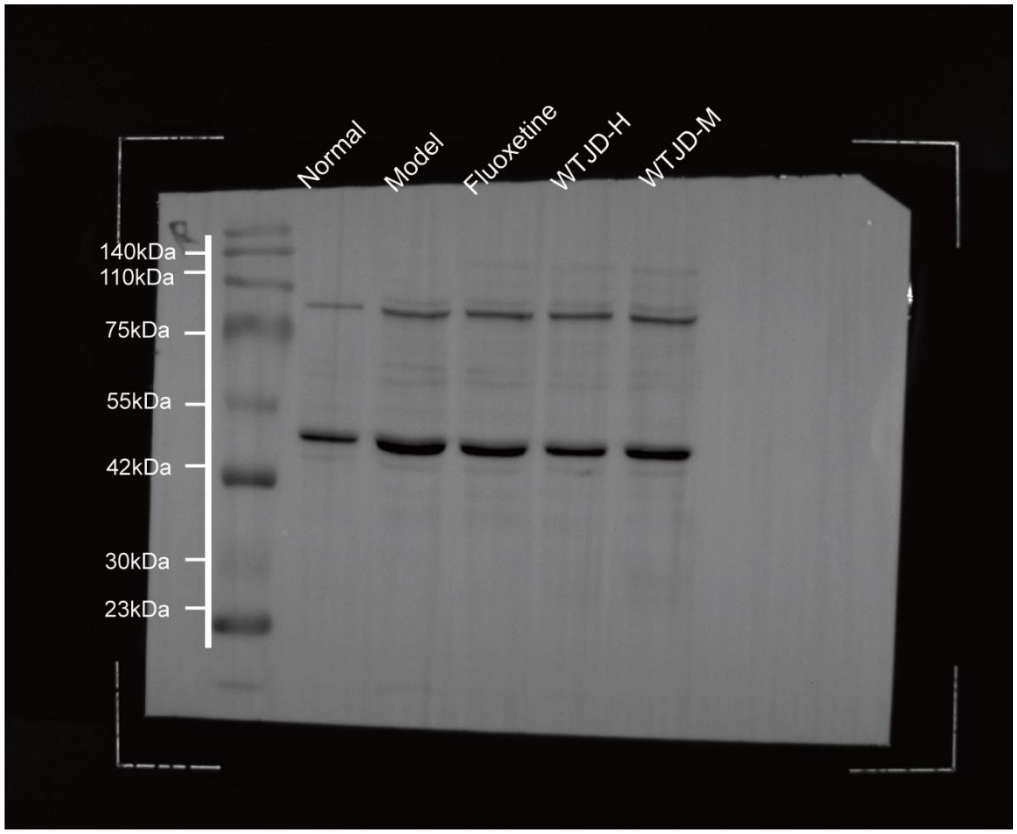

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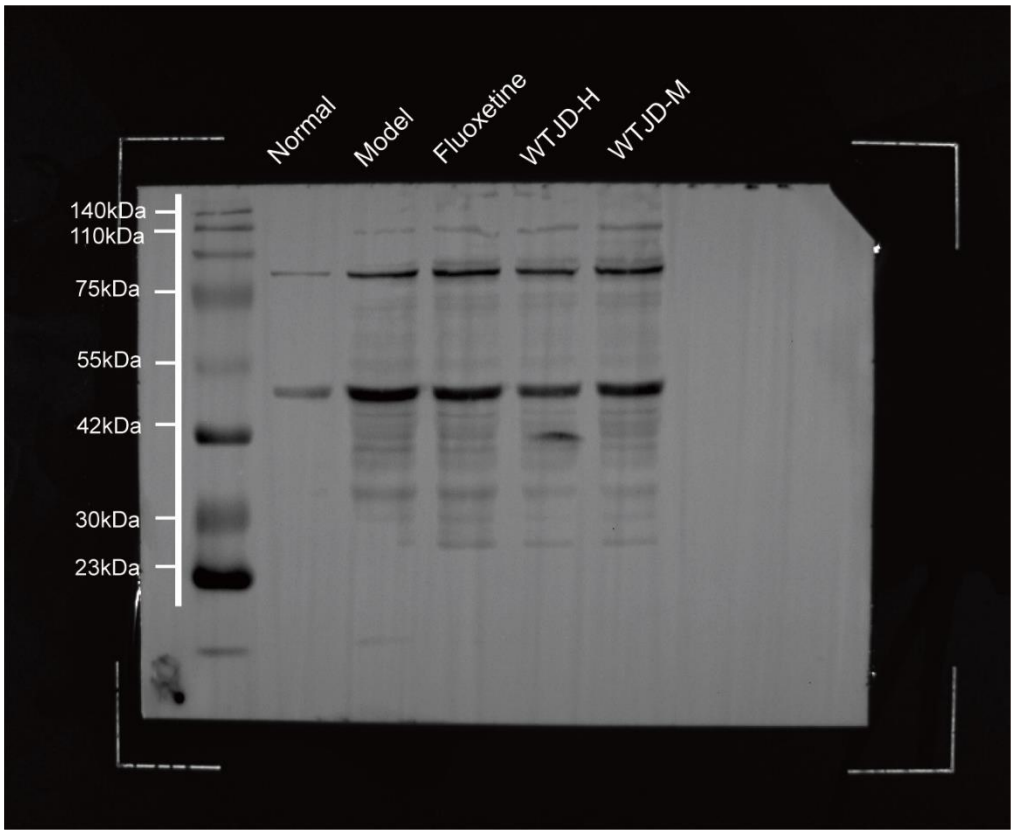
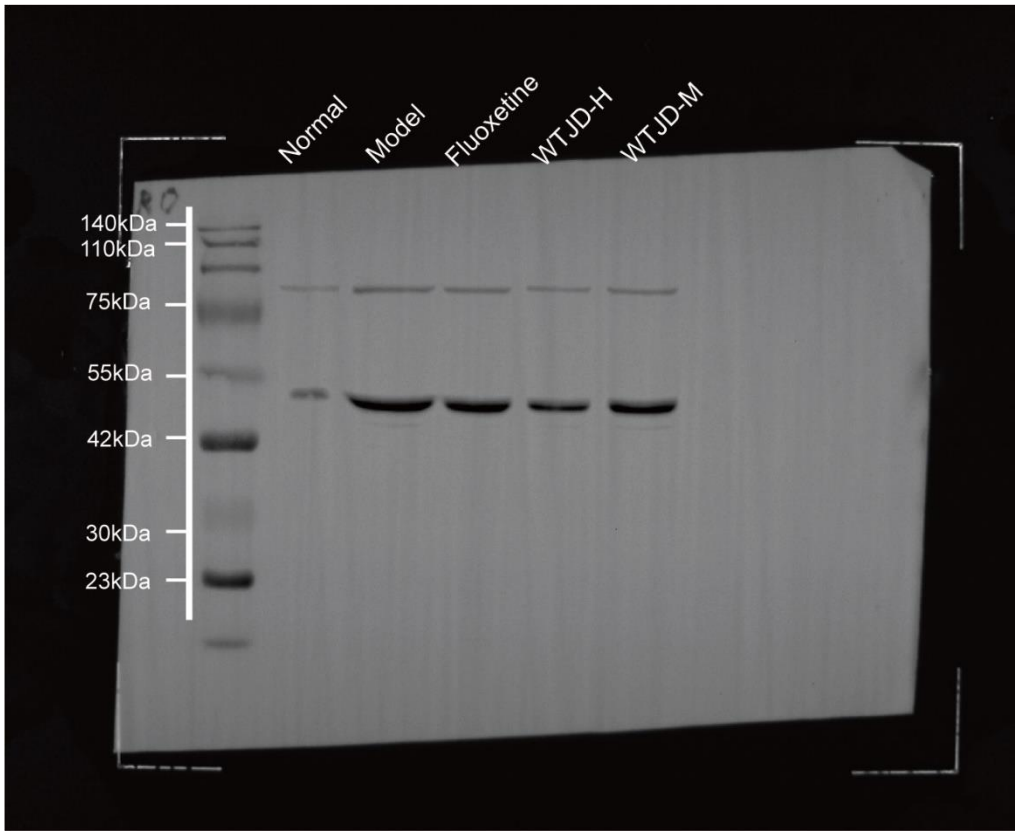
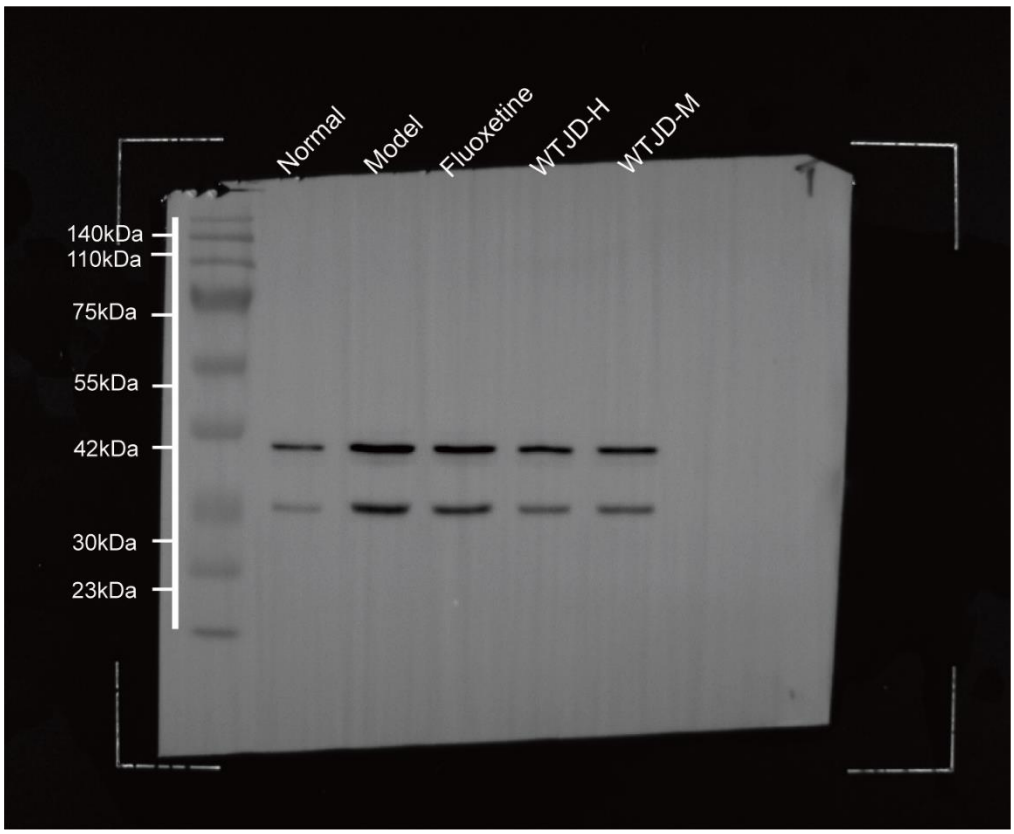

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa

4.

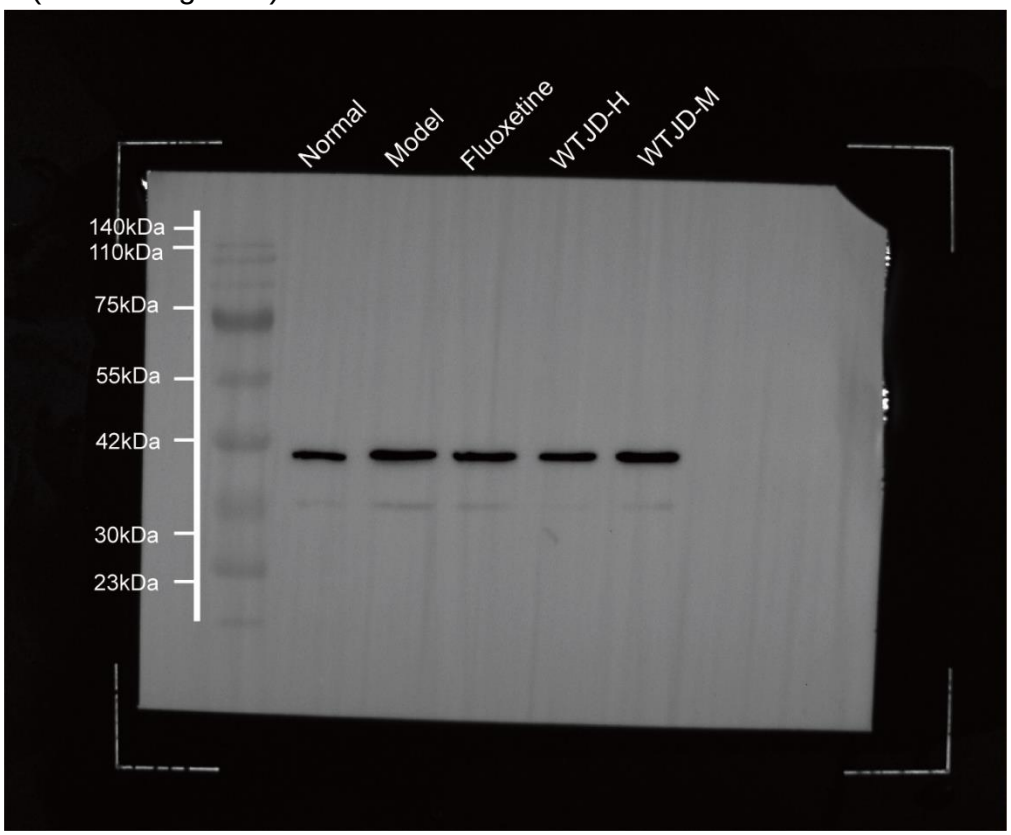

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa

Tbet

1.



2.(For the Figure 5)



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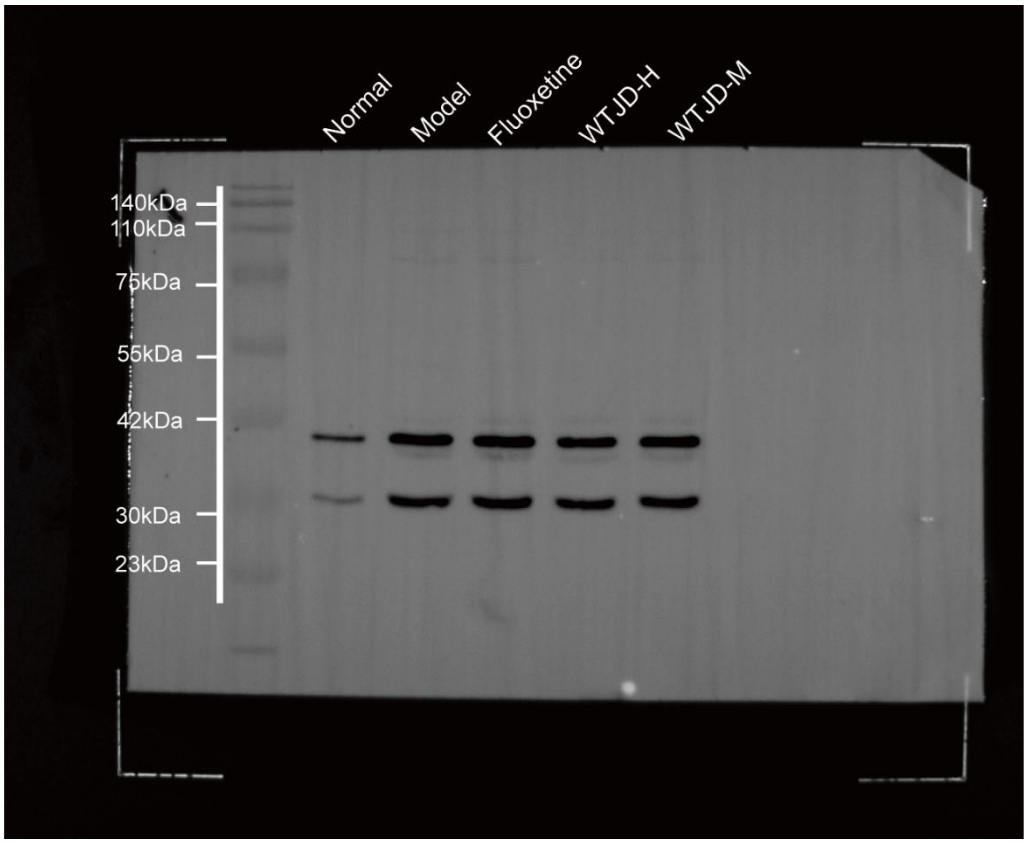
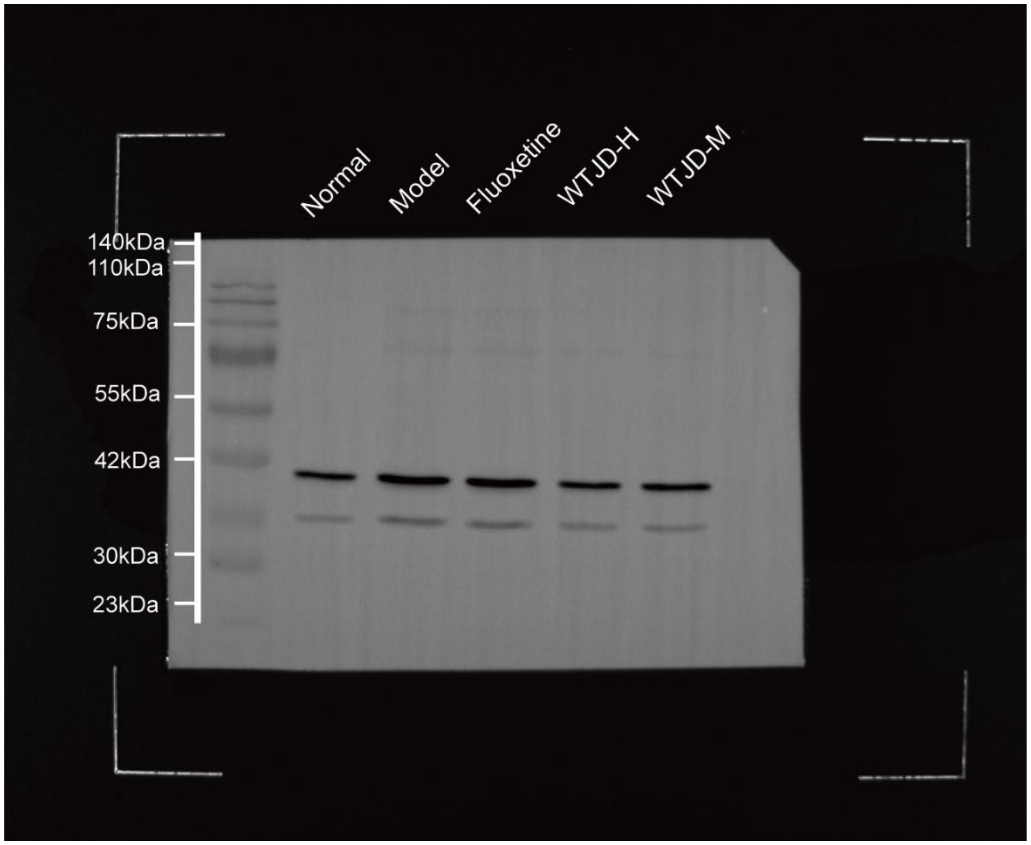
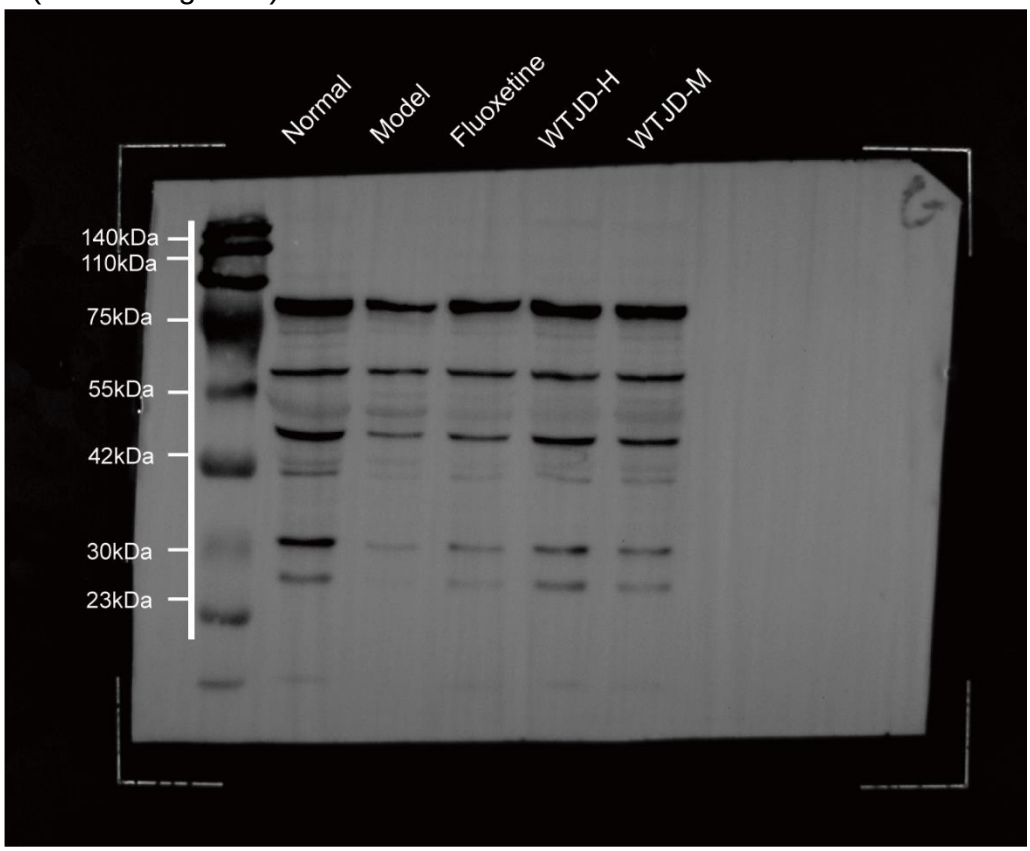

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa

4.


Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa

GATA3

1.(For the Figure 5)



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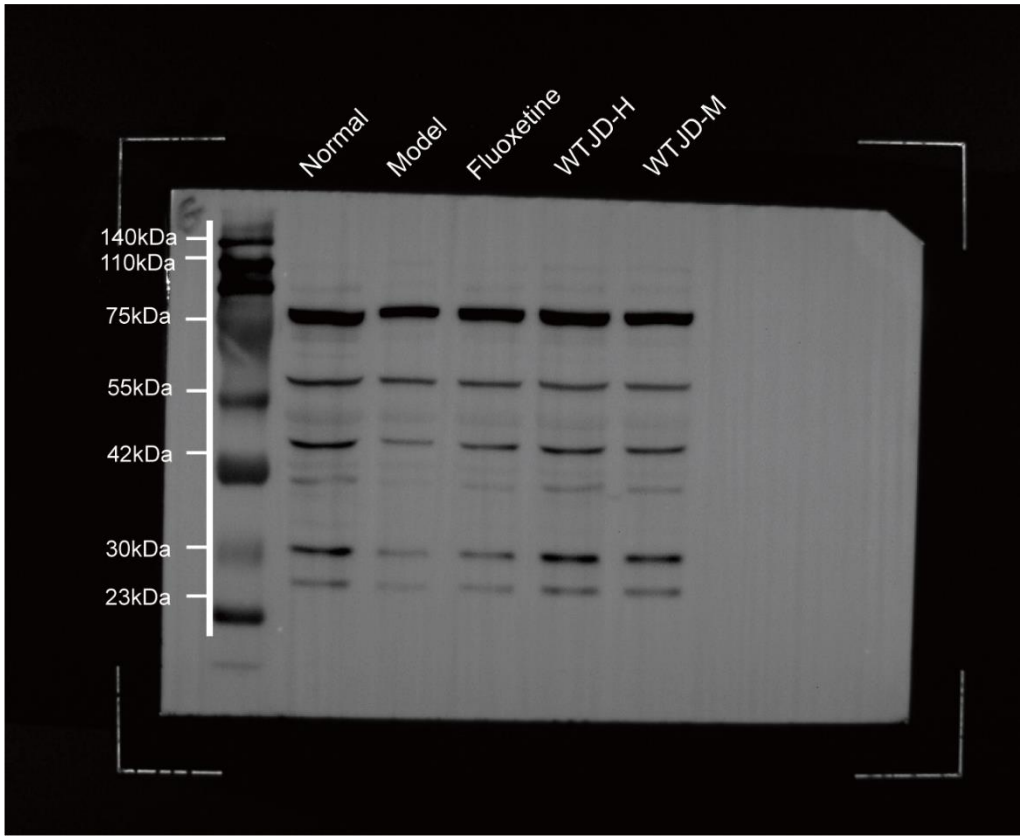

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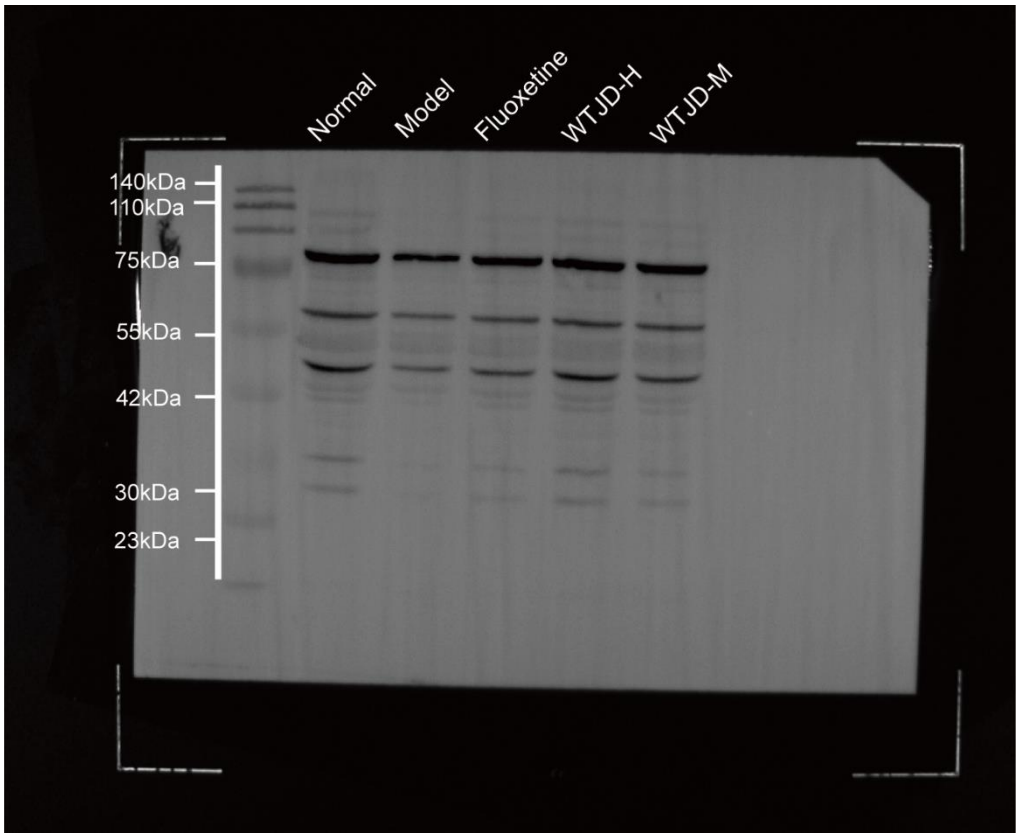
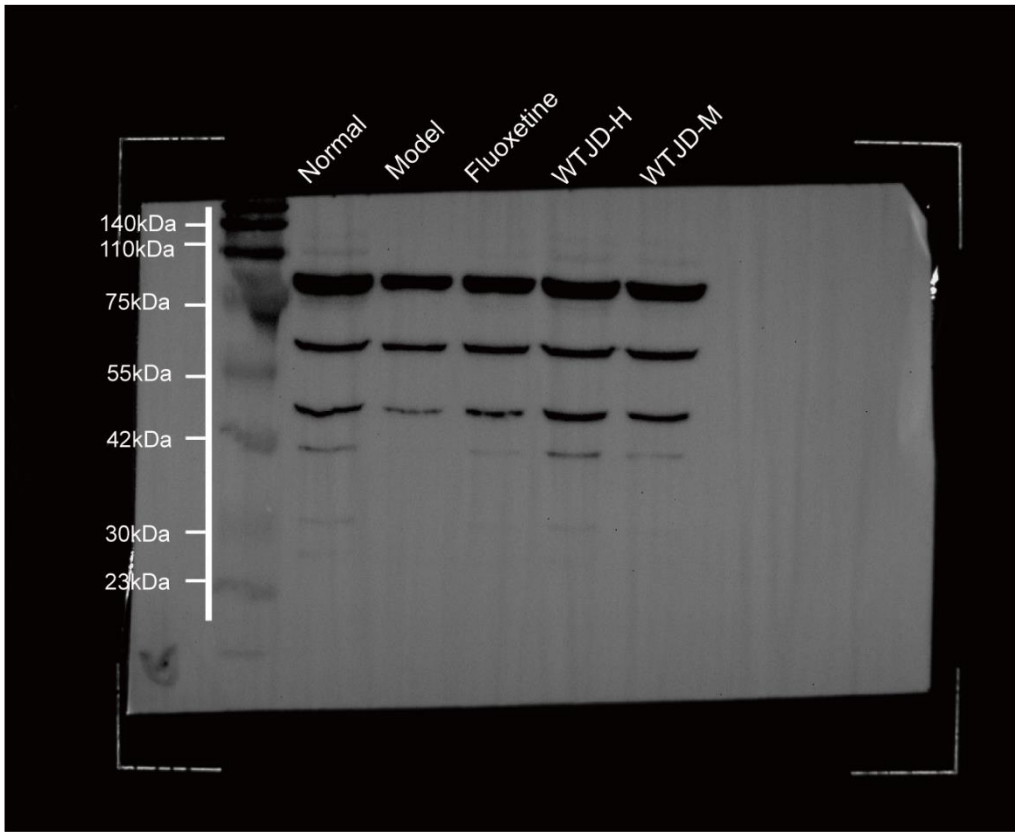

Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa

4.


Normal
Model
Fluoxetine
WTJD-H
WTJD-M
140kDa
110kDa
75kDa
55kDa
42kDa
30kDa
23kDa