

**Experimental Protocol LRRK2, Ageing and Lowgrade Inflammation (10 month age-group) Row Data**

- A. Sacrifice, tissue dissection and identification of tissue samples of 10 M age-group**
- B. Genotype (tail samples)**
- C. WB (row data)**

# A. Sacrifice, tissue dissection and identification of tissue samples of 10 M age-group

**1° SACRIFICIO PROTOCOLLO AGING DEL 28 e 29 OTTOBRE 2020**

**RANGE 10 MESI**

Numero Identificativo	Genotipo	Somministrazioni	Organi Prelevati
1	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
2	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
3	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
4	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
5	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
6	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
7	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
8	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, coda, milza, timo, intestini
<b>TOTALE</b>		2 WT SALINA 2 WT LPS 2 TG SALINA 2 TG LPS	

**2° SACRIFICIO PROTOCOLLO AGING DEL 3 e 5 NOVEMBRE**

**2020 RANGE 10 MESI**

Numero Identificativo	Genotipo	Somministrazione	Organi Prelevati
9	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
10	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
11	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
12	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
13	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
14	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
15	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
16	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
17	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
18	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
19	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
20	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
21	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza

22	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
23	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
24	WT	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
25	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
26	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
27	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
28	WT	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
29	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
30	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
31	TG	SALINA	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
32	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
33	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
34	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza
35	TG	LPS	Mesencefalo, Striato, Cortex, cervelletto, ½ milza

## Experimental Protocol LRRK2, Ageing and Lowgrade Inflammation (10 months)-Row Data

VM, Str, CX, Spleen, tails, samples snap frozen for proteins

A. Genotype (tail samples)

B. WB (central/peripheral tissues)

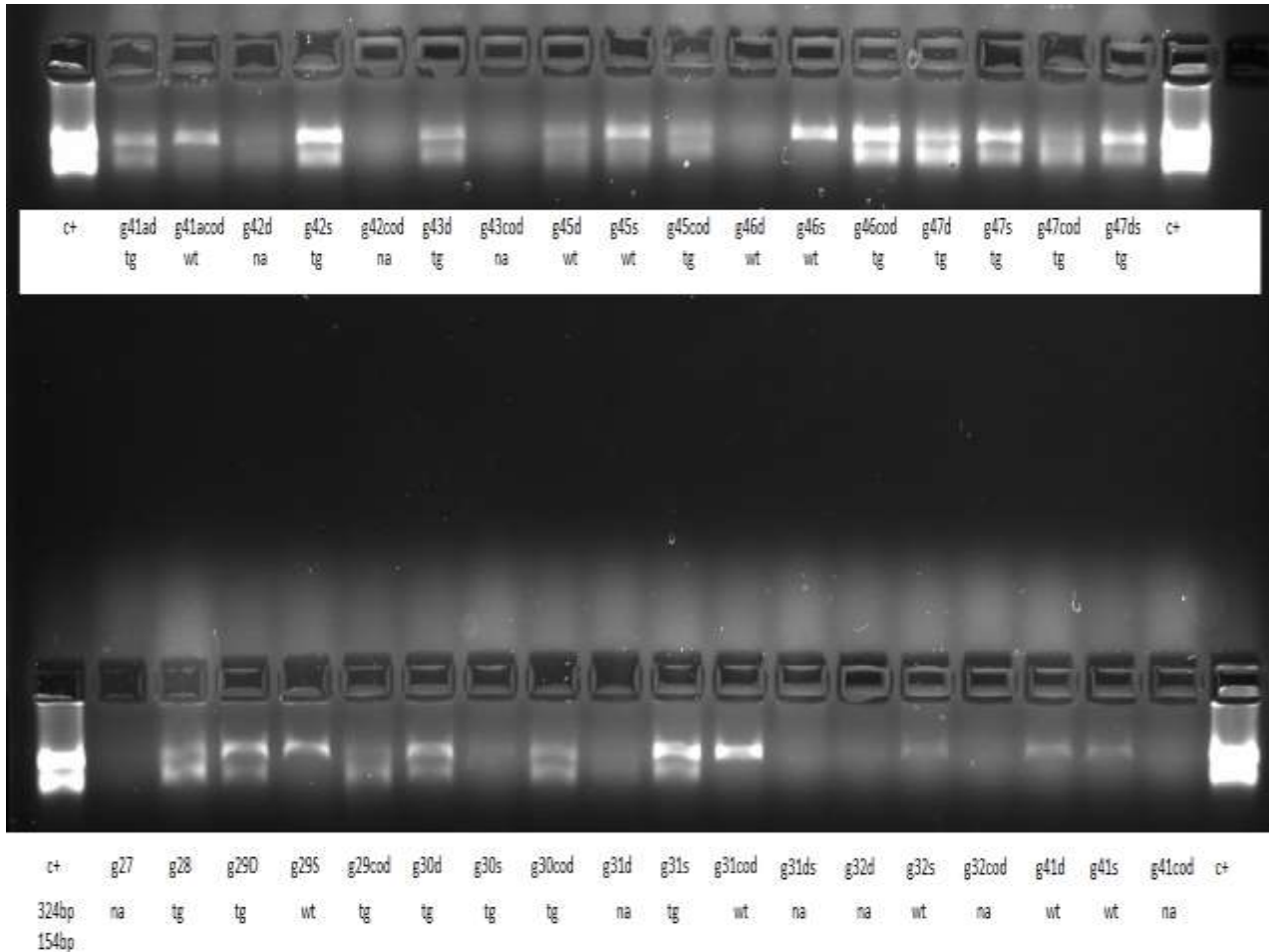
### A. Genotype (tail samples)

Mouse strain: C57BL/6J-TG(LRRK2\*-G2019S)2AMjff/j

Expected Results: transgene 154 bp

internal positive control: 324 bp

21157	CTC CCA ACC CCA GAG GTA GT	Internal Positive Control Forward	Reaction A	
21225	AGA CCC CAG ATC CAG AAA GG	Internal Positive Control Reverse	Reaction A	
8752	TGA TTC TCG TTG GCA CAC AT	Transgene Forward	Reaction A	LRRK2
8753	GCC AAA GCA TCA GAT TCC TC	Transgene Reverse	Reaction A	LRRK2



## Agarose gel electrophoresis

the tails collected from each mouse were genotyped through PCR followed by electrophoretic analysis, as described in the manufacturer's instructions (Jackson Laboratory).

Sample Numbers (10 M for WB)

**WT-NaCl**

1,5,22,23,24

**WT-LPS**

2,6,25,26,27,28

**TG-NaCl**

3,7,29,30,31

**TG-LPS**

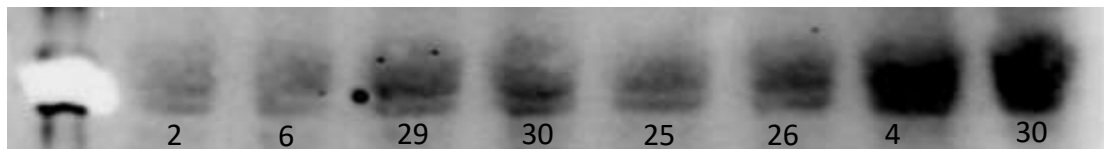
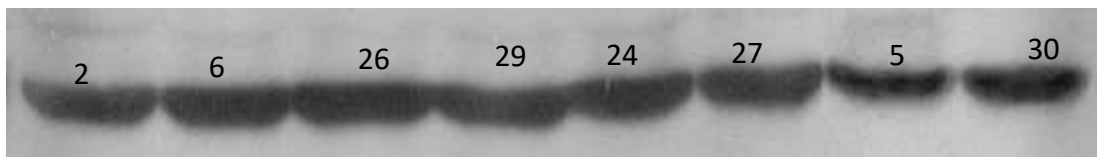
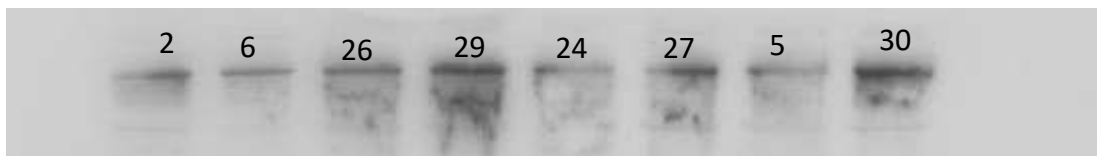
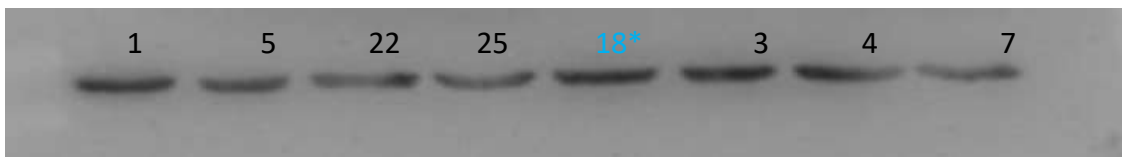
4,8,32,33,34,35

Agarose gel electrophoresis of 35 tail samples from 10 M-NaCl and LPS-treated WT and G2019S (TG) mice carried out using primers and indications of Jackson Laboratories. Positive controls (C+) in right and left hand sides. In this representative gel, 15 Tg, 9 WT and 7 non amplified (n.a.) are show, n.a. samples were re-run For protein quantification, Western blot analyses of central (VM, Str, CX) and peripheral (spleen) tissues were carried out in the 10 M NaCl- and LPS-treated WT/TG groups in a total of 11 WT and 11 TG .

# Anti-LRRK2- MJFF2 (c41-2) Ab133474 286 KDa- rabbit 1:1000



VM



Summary blots of anti-LRRK2 Ab in WT and TG G2019S mice ventral midbrain (VM). A series of 4 TG and 3 age- and strain-matched WT were used. The rabbit monoclonal antibodies c41-2 (MJFF2) was used for the detection of endogenous LRRK2 in mouse tissues. VM from NaCl-treated ~10 months old TG and WT mice were rapidly dissected and protein extracted, equal volumes and protein were loaded onto the gel. Molecular masses are indicated in kDa. The antibody detected lower-molecular-mass bands, besides the band of ~280 kDa. Resolution of cross-reactive bands was achieved under differential extraction conditions (<http://www.biochemj.org/bj/453/bj4530101add.htm>).

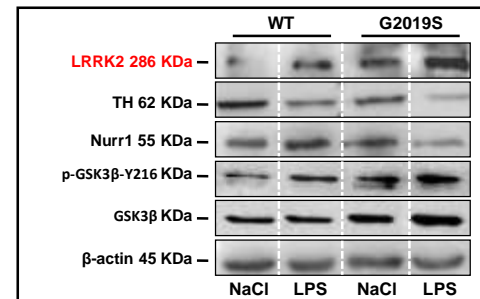
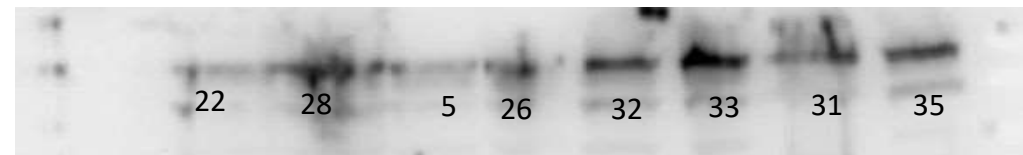


Fig. 2D

β-actin 45 KDa



LRRK2 286KDa



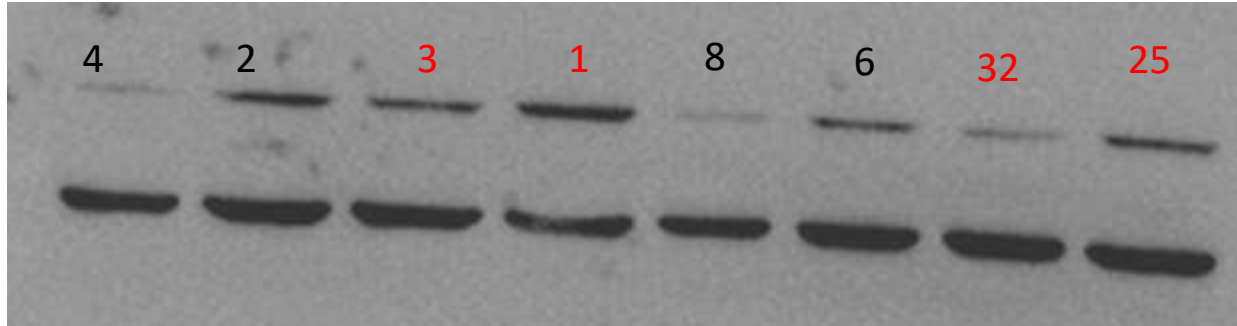
β-actin 45 KDa

P-S935-LRRK2  
286 KDa

18\* discarded

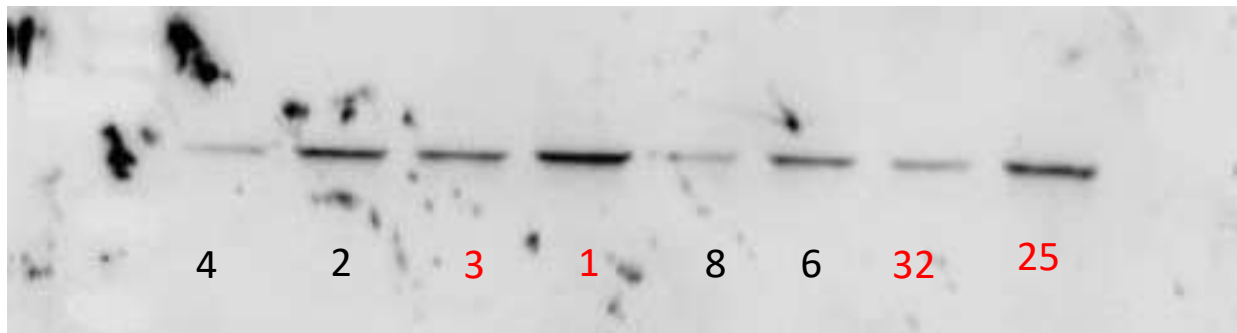
**Anti-TH-Ab ( rabbit, Millipore, 1:1000 ) (62 Kda)**

**Anti-β-actin (Cell signaling, 1: 1000 (45 KDa)**

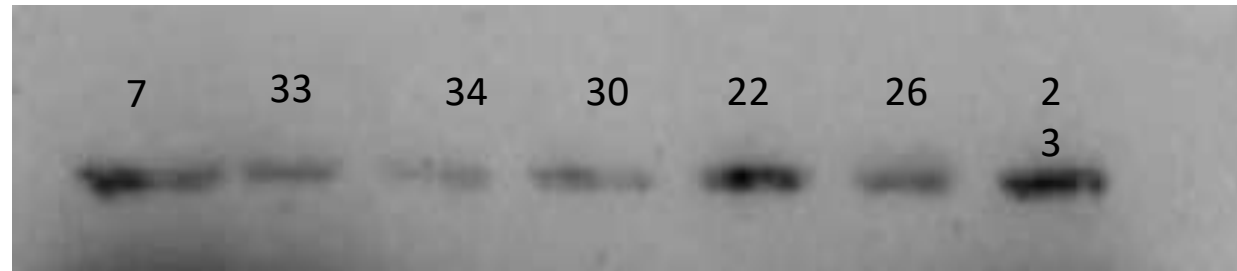


TH 62 KDa

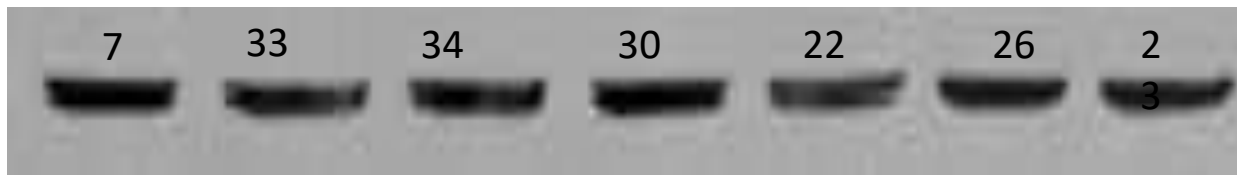
β-actin 45 KDa



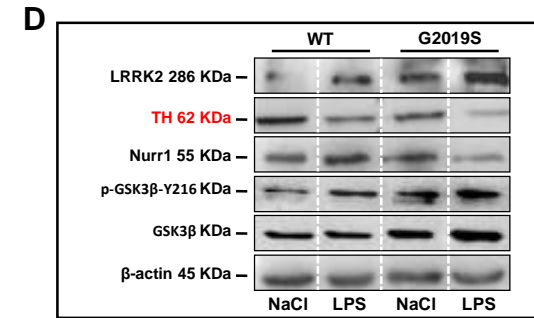
TH 62 KDa



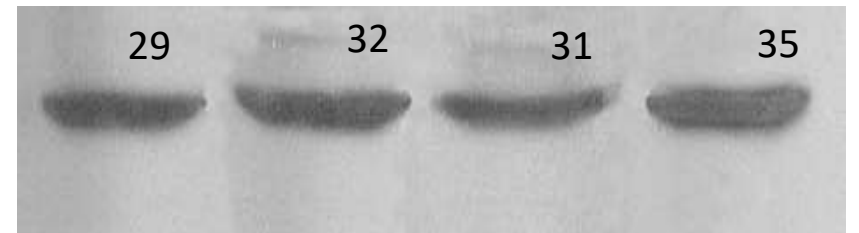
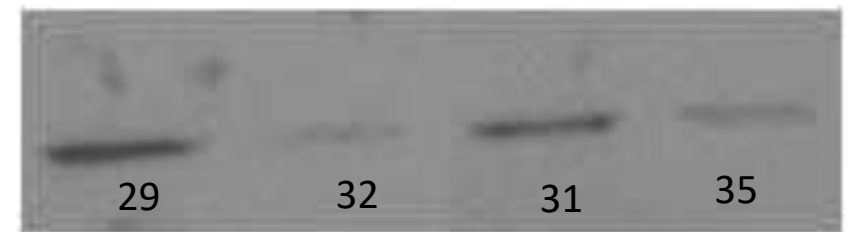
TH 62 KDa



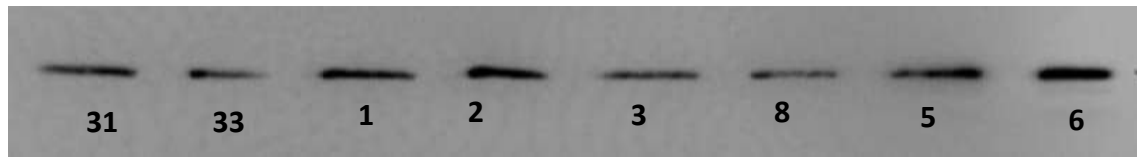
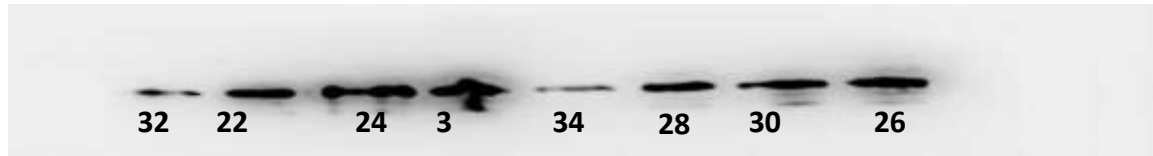
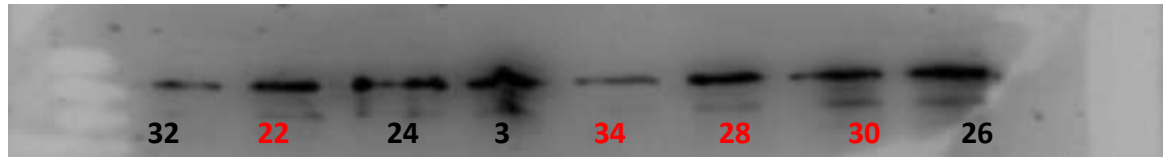
β-actin 45 KDa



**Fig. 2D**



# Anti-Nurr1 (Rabbit, Santa Cruz) 55-64 kDa (1:200)



Nurr1 55 KDa

Nurr1 55 KDa

Nurr1 55 KDa

Nurr1 55 KDa

beta-actin 45 KDa

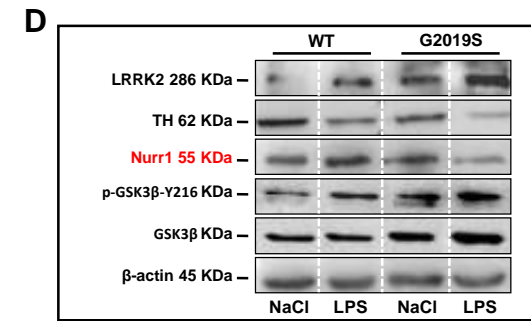
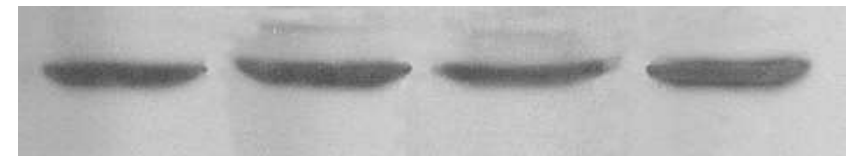
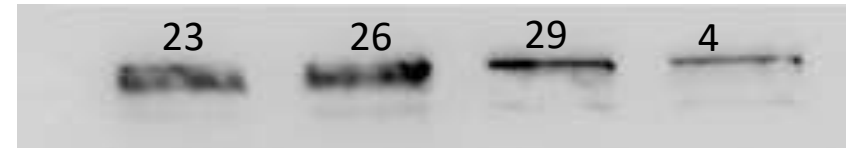
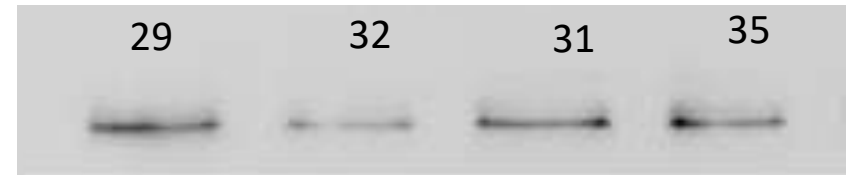
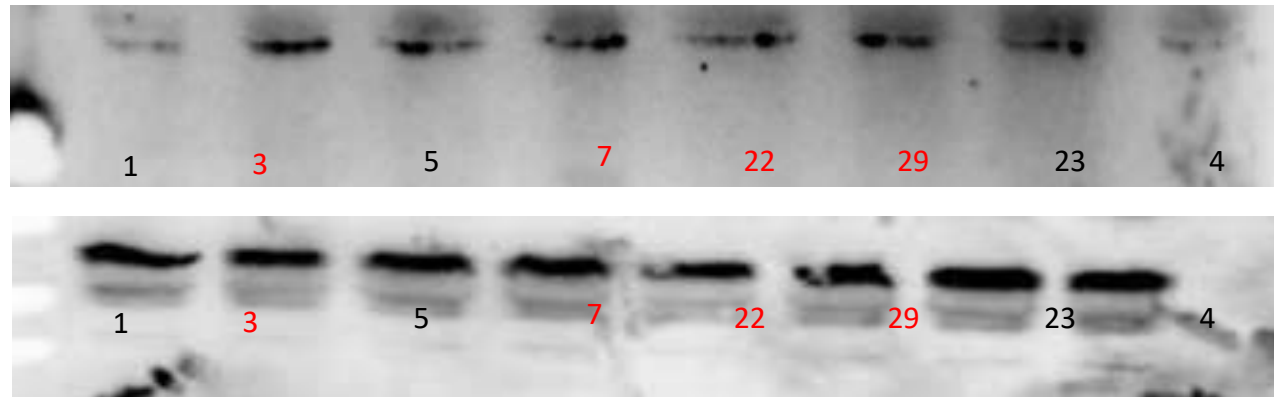


Fig. 2D

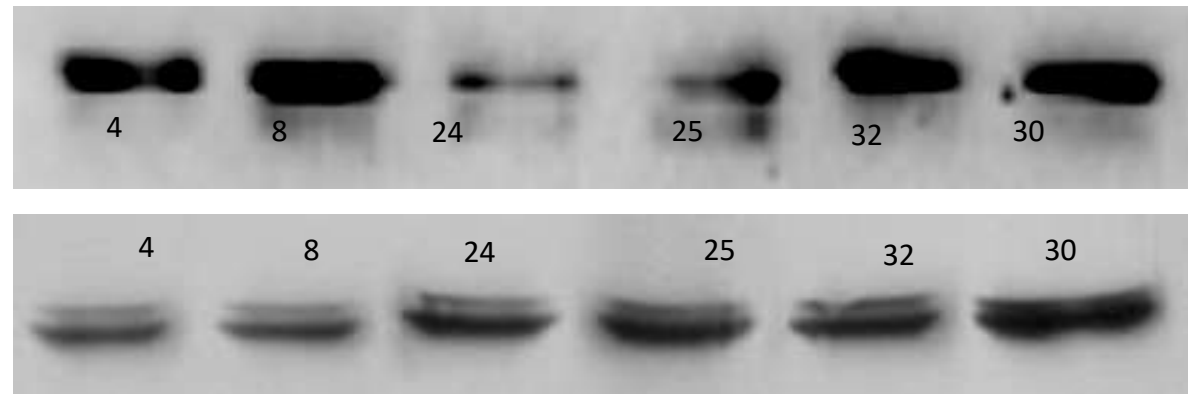
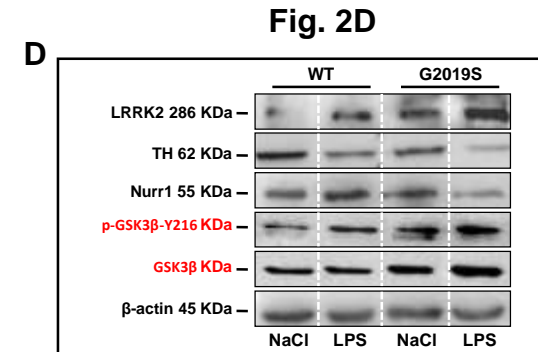


**pGSK3- $\beta$ Tyr216, (rabbit, 1:200, sc135653) Santa Cruz**  
**GSK3- $\beta$  (H76)-sc 9166, 47 KDA (rabbit, 1:200)**



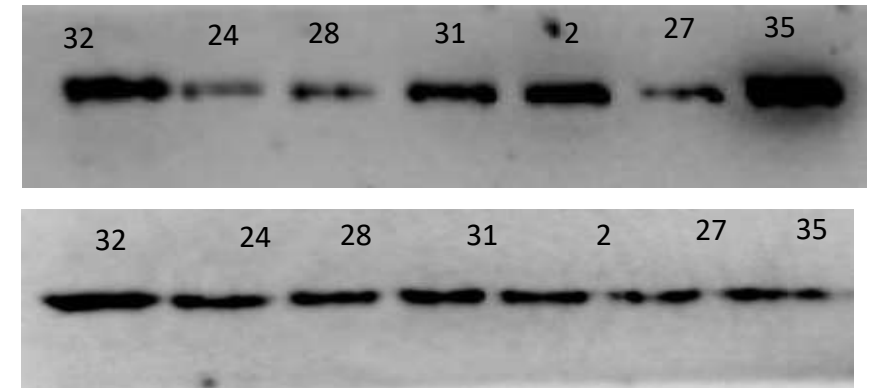
**p-Tyr<sup>216</sup> GSK3 $\beta$  47 KDa**

**GSK3 $\beta$  47 KDa**

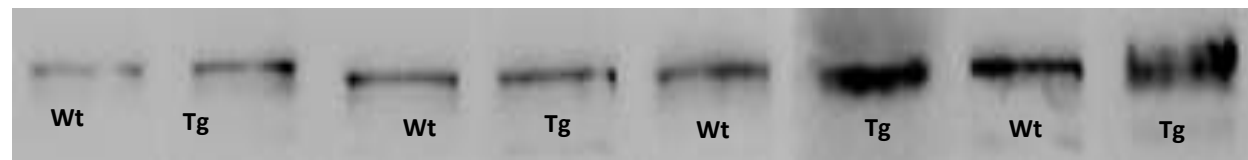


**p-Tyr<sup>216</sup> GSK3 $\beta$  47 KDa**

**GSK3 $\beta$  47 KDa**



**GSK3 $\beta$  47 KDa**



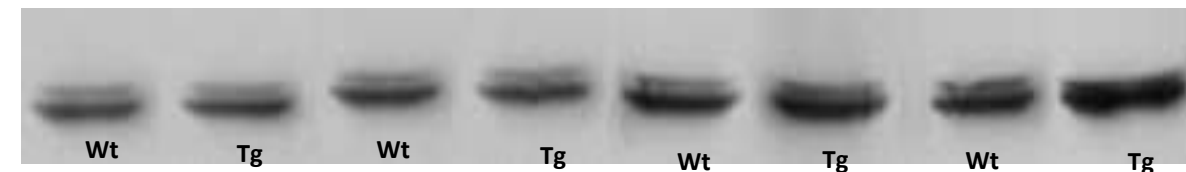
**p-Tyr<sup>216</sup> GSK3 $\beta$  47 KDa**

**6 M NaCl**

**6 M LPS**

**10 M LPS**

**16 M LPS**



**6 M NaCl**

**6 M LPS**

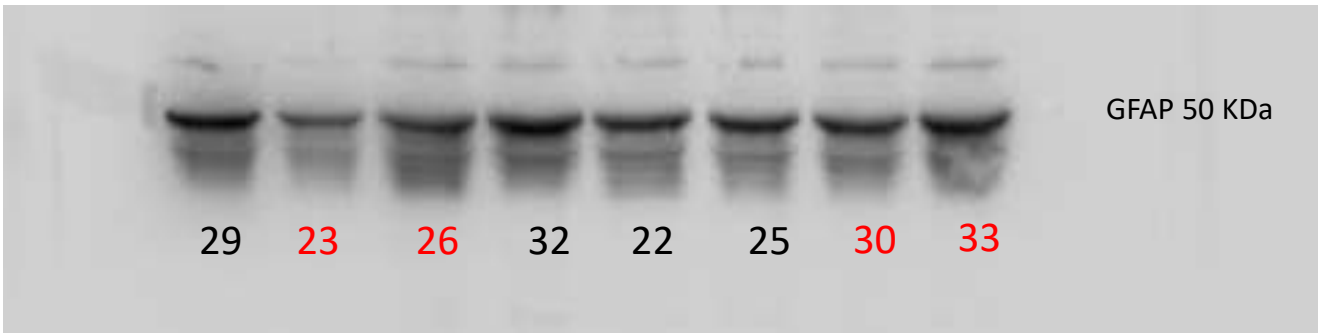
**10 M LPS**

**16 M LPS**



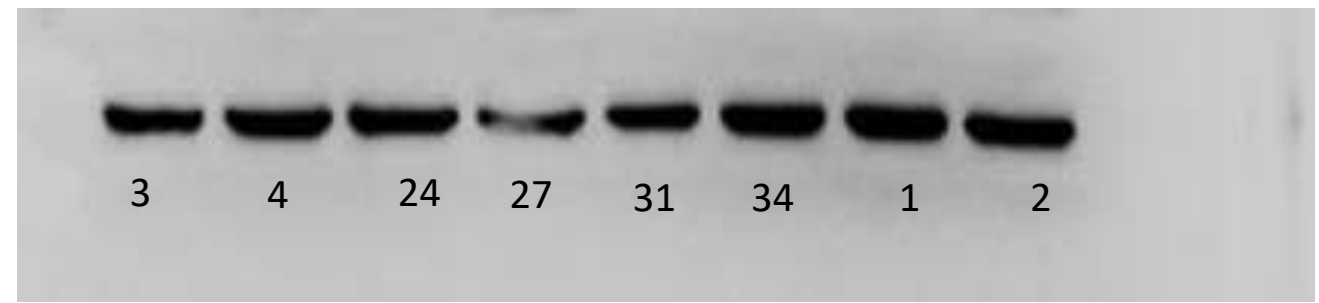
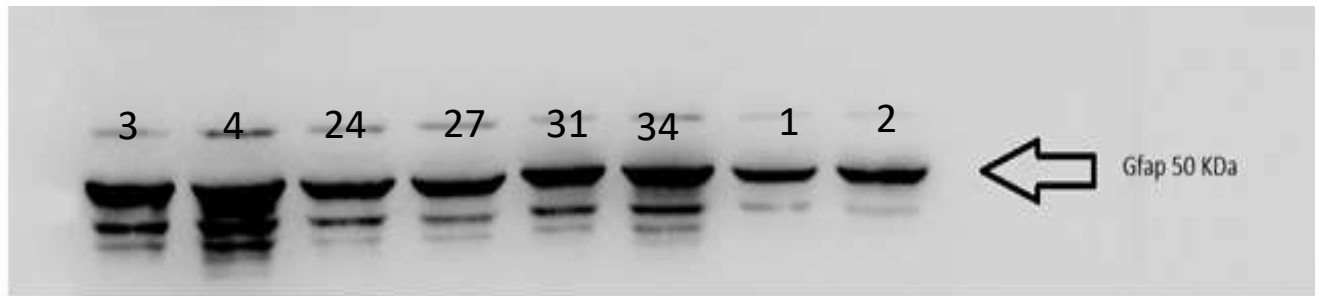
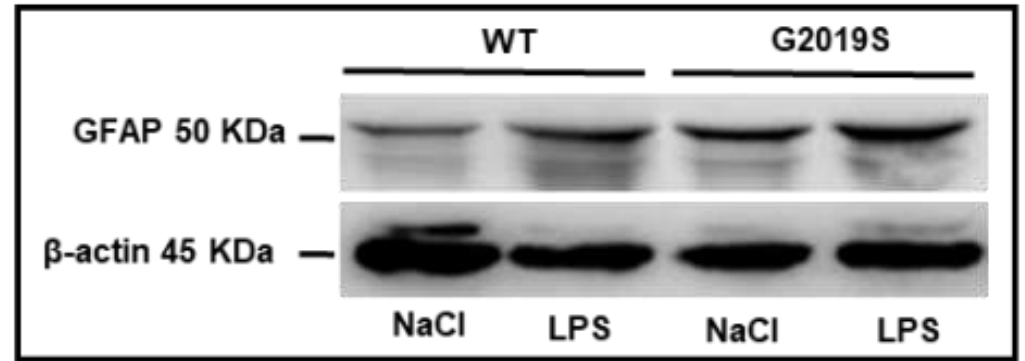
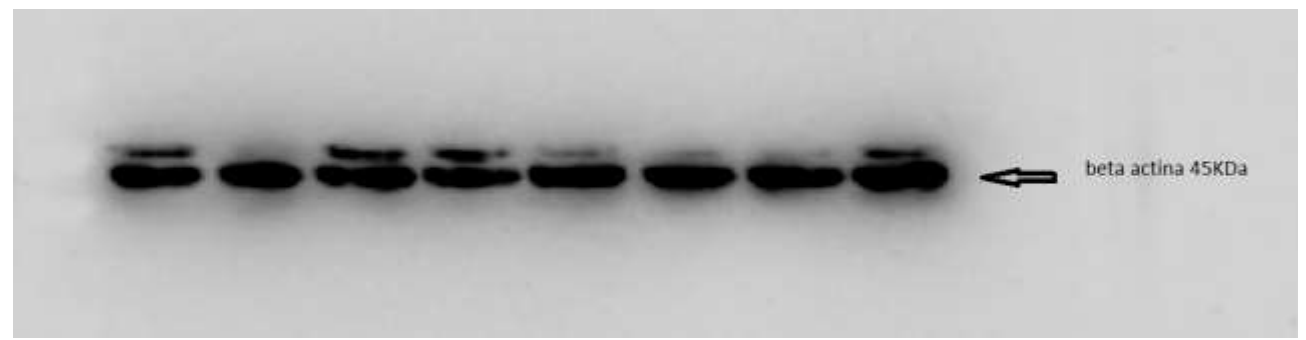






**Anti-GFAP (rabbit, DAKO, 20334) 50 KDa rabbit 1 : 200**

**Anti-β-actin Cell signaling 1: 1000 (45 KDa)**



**β-actin 45 KDa**

**IBA1 goat polyclonal, Abcam, 1:200**

**NfKb (p65)-60-64 KDa, rabbit (Abcam 16502, 1:1000)**

**iNOS mouse Santa Cruz 1:200**

**NOX2/gp91phox (Abcam, EPR6991, 1:500)**

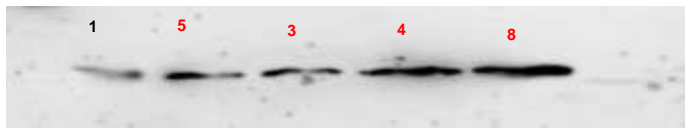
**IL-1 beta (,goat 1367, SIGMA) 17-31 KDa**

**CASPASI-1 (AB1871- Chemicon) 45 KDa**

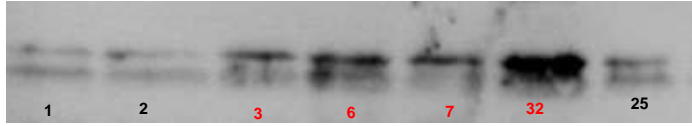
**CCR2-(goat-polyclonal, Thermofisher 1:500/ Abcam, EPR 19698,  
ab 203138), rabbit, 1: 1000 43 Kda**

**CCl2, goat polyclonal (1/200; R&D),**

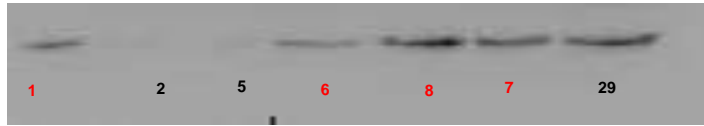
**Mac2/Gal3 (1:500; CL8942AP Cedarlane)**



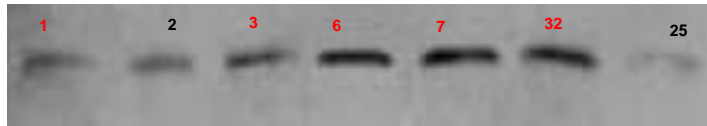
**Iba1 17 KDa**



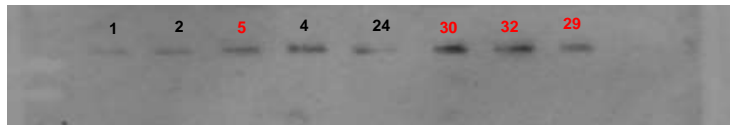
**NFKb 64 KDa**



**iNOS 130 KDa**



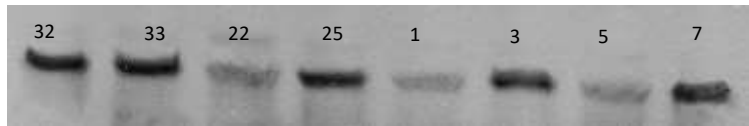
**gp91-Phox 60 KDa**



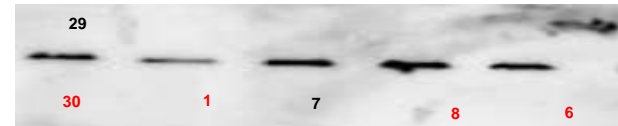
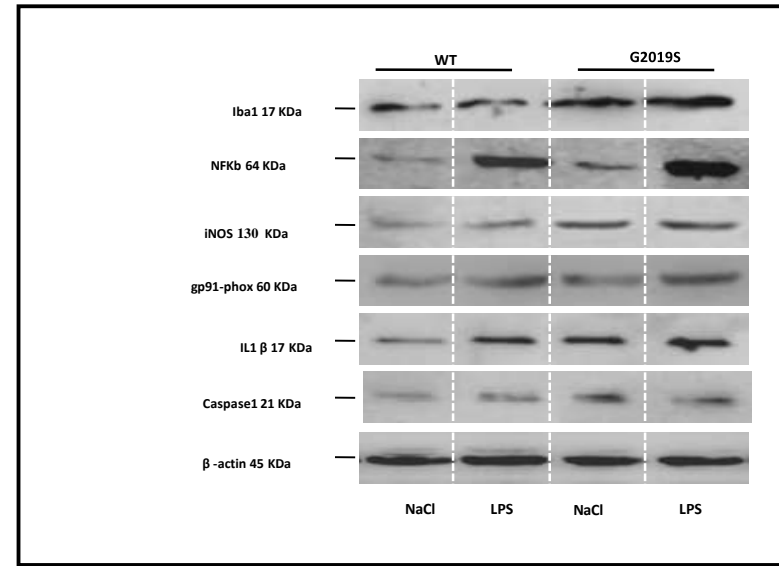
**Caspase1 45 KDa**



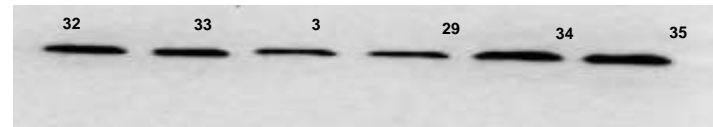
**Caspase1 45 KDa**



**gp91-Phox 60 KDa**



**IL1β 17 KDa**

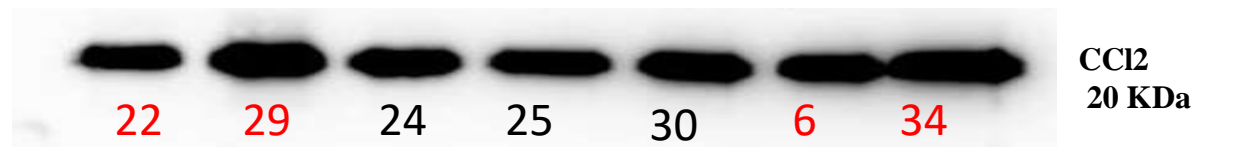
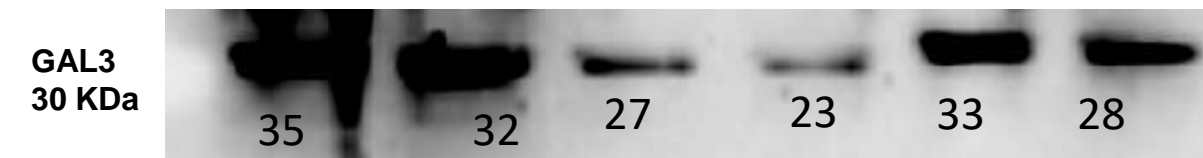
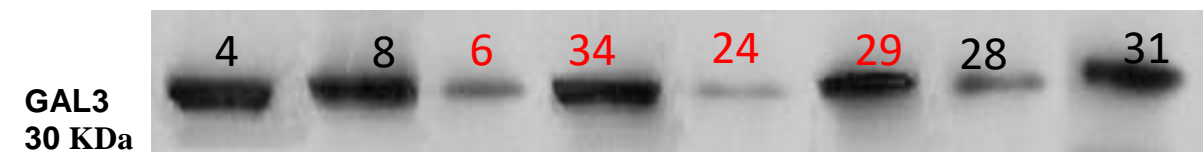
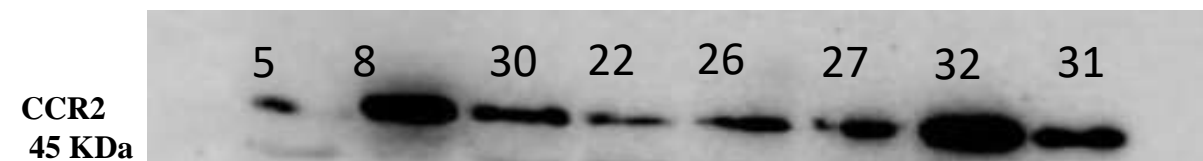
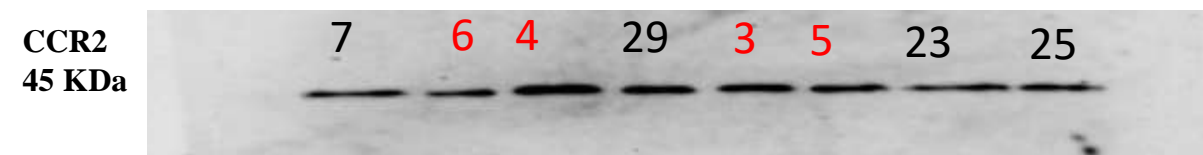
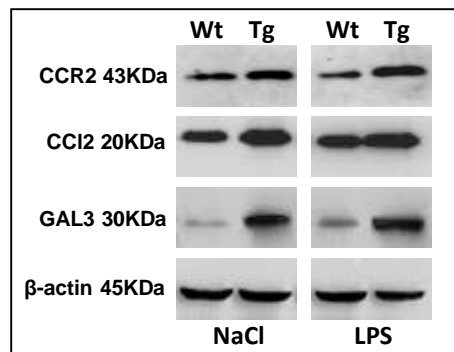


**IL1β 17 KDa**

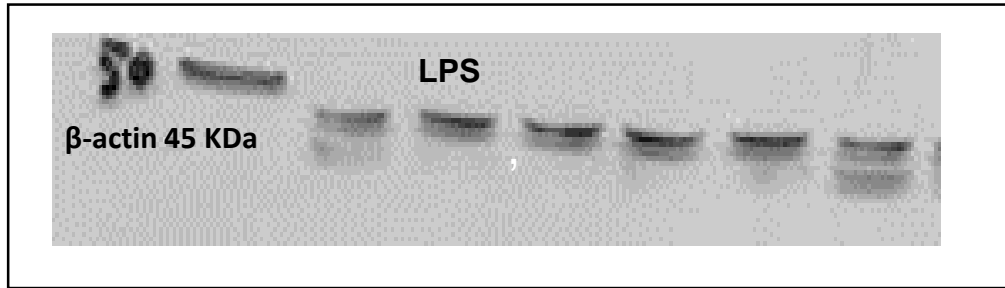
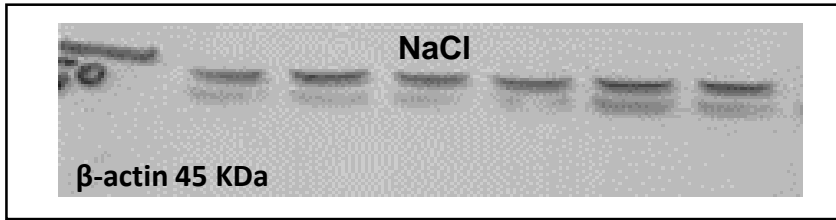
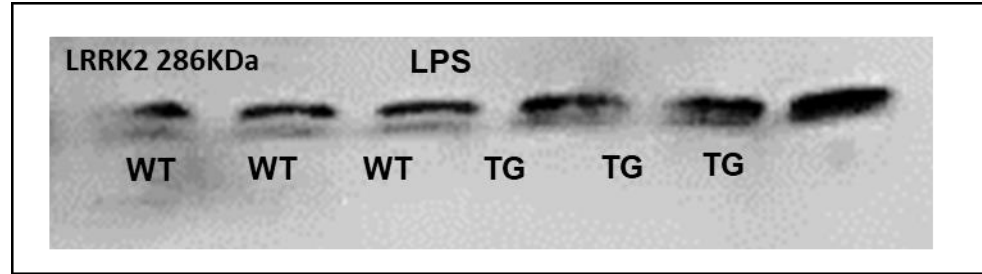
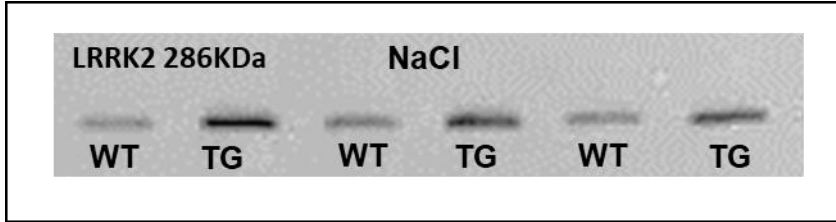


**β-actin 45 KDa**

Suppl Fig 7 E

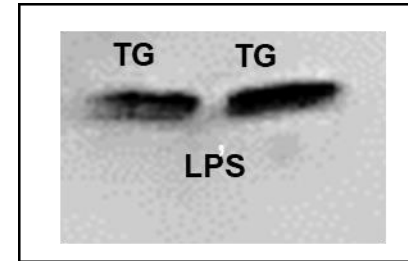
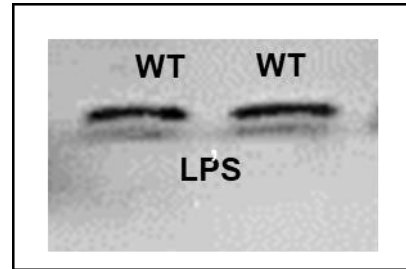
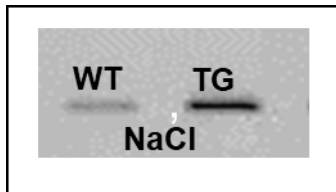
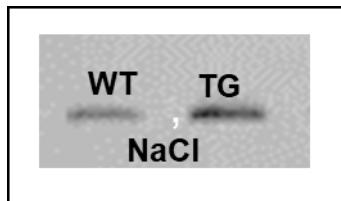


# Uncropped WB spleen

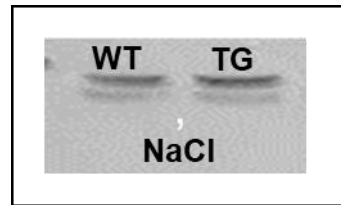
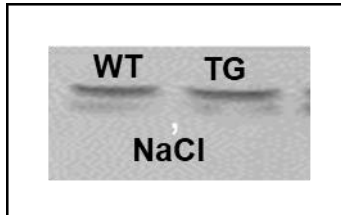


LRRK2 286KDa

LRRK2 286KDa

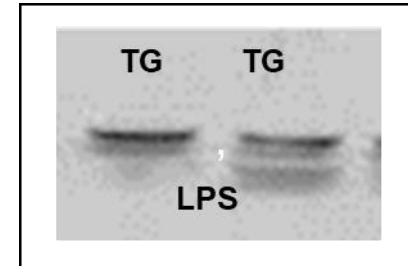
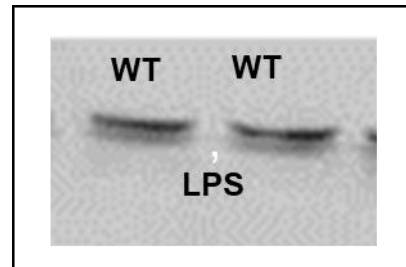


LRRK2 286KDa



beta-actin 45 KDa

beta-actin 45 KDa



beta-actin 45 KDa