

ESM Tables

ESM Table 1. Fluorescent immunohistochemistry primary antibodies

Protein Target	Name of Antibody	Manufacturer	Catalog Number	Species Rised (Monoclonal or Polyclonal)	Dilution buffer	Dilution used
BDNF	anti-BDNF	Abcam, Cambridge, MA, USA	108319	rabbit-monoclonal	1x PBS, 20mM NA-azid, 0,3% Triton X-100	1:500
GFAP	anti-GFAP	Synaptic Systems, Goettingen, Germany	173006	chicken-polyclonal		
ICAM-1	anti- ICAM-1/CD54	R&D Systems, Minneapolis, MN, USA	#AF796	mouse-polyclonal		
Iba1	anti-AIF-1/Iba1	Novusbio, Littleton, CO, USA	NB100-1028	goat-polyclonal		
IL-1α	anti-IL-1 alpha /IL-1F1	R&D Systems, Minneapolis, MN, USA	AF-400-NA	goat-polyclonal		
P2Y12	anti-P2Y12	AnaSpec, Fermont, CA, USA	55043A	rabbit-polyclonal		
p-CREB	anti-pCREB	Cell Signaling Technology Inc., Danvers, MA, USA	87G3	goat-monoclonal		
PGP9.5	anti-PGP9.5	Abcam, Cambridge, MA, USA	72910	chicken-polyclonal		

ESM Table 2. Fluorescent immunohistochemistry secondary antibodies

Protein target	Name of Antibody	Manufacturer	Catalog Number	Species Rised	Dilution buffer	Dilution used
Chicken IgG	Alexa Fluor® 488 AffiniPure F(ab') ₂ Fragment Donkey Anti-Chicken IgY (IgG)	Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK	703-546-155	donkey-polyclonal		
Goat IgG	Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Thermo Fisher Scientific Waltham, MA USA	A11058	donkey-polyclonal	1xPBS, 20mM NA-azid, 0,3% Triton X-100	1:200
Mouse IgG	Alexa Fluor® 647 AffiniPure Donkey Anti-Mouse IgG	Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK	715-605-150	donkey-polyclonal		
Rabbit IgG	Alexa Fluor® 488 AffiniPure F(ab') ₂ Fragment Donkey Anti-Rabbit IgG	Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK	711-546-152	donkey-polyclonal		

ESM Table 3. Immunoblot primary antibodies

Protein Target	Name of Antibody	Manufacturer	Catalog Number	Species Rised (Monoclonal or Polyclonal)	Dilution buffer	Dilution used
BDNF	anti-BDNF	Abcam, Cambridge, MA, USA	108319	rabbit-monoclonal	1x TBS 0.05% wt/vol Tween 20	1:1000
Endothelin-1	anti-endothelin-1	Abcam, Cambridge, MA, USA	117757	rabbit-polyclonal	5% wt/vol non-fat milk, 1x TBS 0.05% wt/vol Tween 20	1:1000
Furin	anti-furin	Abcam, Cambridge, MA, USA	183495	rabbit-monoclonal	5% wt/vol non-fat milk, 1x TBS 0.05% wt/vol Tween 20	1:2000
MMP3	anti-MMP3	Abcam, Cambridge, MA, USA	52915	rabbit-monoclonal	5% wt/vol non-fat milk, 1x TBS 0.05% wt/vol Tween 20	1:1000
NF-κB	anti-NF- κ B p65	Cell Signaling Technology Inc., Danvers, MA, USA	C22B4	rabbit-monoclonal	5% wt/vol bovine serum albumin, 1x TBS 0.05% wt/vol Tween 20	1:1000
p75Ntr	anti-p75NGF	Abcam, Cambridge, MA, USA	52987	rabbit-monoclonal	1x TBS 0.05% wt/vol Tween 20	1:500
p-CREB	anti-pCREB	Cell Signaling Technology Inc., Danvers, MA, USA	87G3	goat-monoclonal	1x TBS 0.05% wt/vol Tween 20	1:500
p-eNOS	anti-peNOS	Cell Signaling Technology Inc., Danvers, MA, USA	9571	rabbit-polyclonal	5% wt/vol bovine serum albumin, 1x TBS 0.05% wt/vol Tween 20	1:1000
p-ERK	anti-pERK	Santa Cruz Biotechnology, Santa Cruz CA, USA	sc-7383	mouse-monoclonal	1x TBS 0.05% wt/vol Tween 20	1:500
p-JNK	anti-pJNK	Cell Signaling Technology Inc., Danvers, MA, USA	9251	rabbit-polyclonal	5% wt/vol bovine serum albumin, 1x TBS 0.05% wt/vol Tween 20	1:1000
TPA	anti-TPA	Abcam, Cambridge, MA, USA	157469	rabbit-monoclonal	5% wt/vol non-fat milk, 1x TBS 0.05% wt/vol Tween 20	1:1000
TrkB	anti-TrkB	Santa Cruz Biotechnology, Santa Cruz CA, USA	sc-8316	rabbit-polyclonal	1x TBS 0.05% wt/vol Tween 20	1:500

ESM Table 4. Immunoblot secondary antibodies

Protein target	Name of Antibody	Manufacturer	Catalog Number	Species Rised	Dilution buffer	Dilution used
Goat IgG	Anti-goat IgG HRP-linked Antibody	Santa Cruz Biotechnology, Santa Cruz CA, USA	sc-2020	donkey		
Mouse IgG	Anti-mouse IgG HRP-linked Antibody	Cell Signaling Technology Inc., Danvers, MA, USA	7076	goat	1x TBS 0.05% wt/vol Tween 20	1:3000
Rabbit IgG	Anti-mouse IgG HRP-linked Antibody	Cell Signaling Technology Inc., Danvers, MA, USA	7074	goat		

ESM Table 5. Sequence of forward and reverse primers for qRT-PCR

Gene	NCBI reference	Primer pairs	Product length
<i>Il1a</i>	NM_017019.1	forward: 5' TCTGCCATTGACCATCTGTCTCTG 3' reverse: 5' ACCACCCGGCTCTCCTTGAA 3'	152 bp
<i>Il6</i>	NM_012589.2	forward: 5' GCCACTGCCTTCCCTACTTC 3' reverse: 5' GCCATTGCACAACCTTTTTCTC 3'	153 bp
<i>Tnf</i>	NM_012675.3	forward: 5' GGGGCCACCACGCTCTTCTGT 3' reverse: 5' CTCCGCTTGGTGGTTTGCTACGAC3'	180 bp
<i>Bcl2</i>	NM_016993.1	forward: 5' ATGGCGCAAGCCGGGAGAACAG 3' reverse: 5' TGGCGACAAGGGGCCGTAGAGG 3'	232 bp
<i>Bax</i>	NM_017059.2	forward: 5' AGCCGCCCCAGGACGCATCCA 3' reverse: 5' CAGCCGCTCCCGGAGGAAGTCCAG 3'	299 bp
<i>Rn18s</i>	NR_046237.1	forward: 5' GCGGTCGGCGTCCCCCAACTTCTT 3' reverse: 5' GCGCGTGCAGCCCCGGACATCTA 3'	105 bp

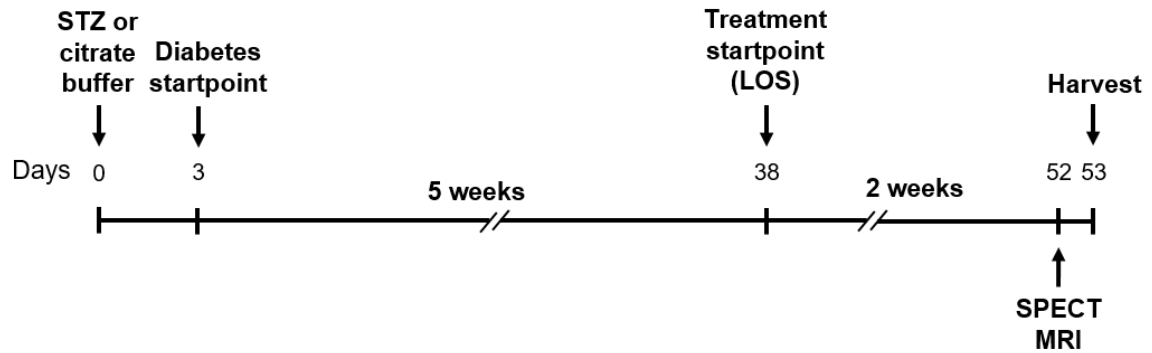
ESM Table 6. Metabolic parameters were measured in control, diabetic (DM) and losartan-treated diabetic (DM+LOS) rats. Data are presented as means±SDs (n=6-8/group). ** p<0.01 or *** p<0.001 vs. C. LOS: losartan.

	Control	DM	DM+LOS
Weight gain (Δg)	223±32.4	79.4±45.4**	80.0±20.0**
Non-fasting blood glucose (mmol/L)	13.2±1.62	44.6±9.13**	43.2±8.09**
Fructosamine (μmol/L)	153±11.9	262±14.4***	249±18.2***

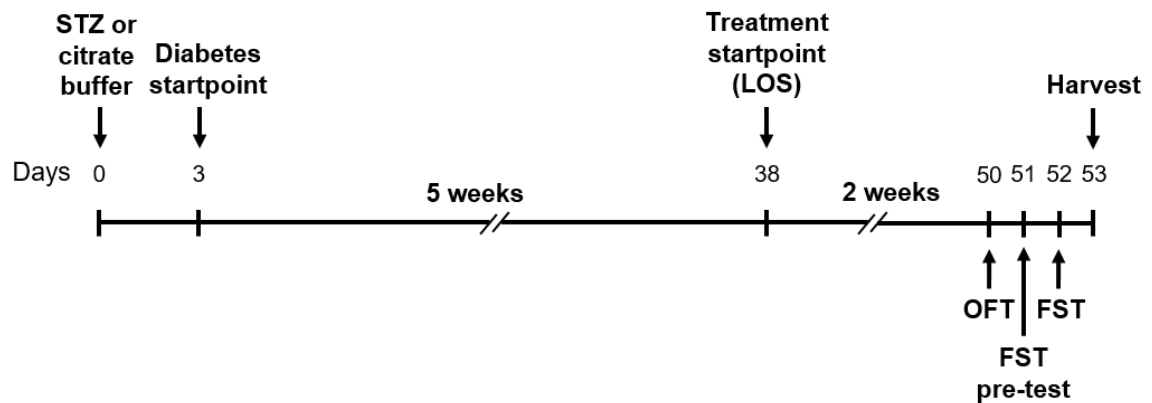
ESM Figures

ESM Figure 1. Study design. STZ: streptozotocin, LOS: losartan, SPECT: Single photon emission computed tomography, MRI: Magnetic Resonance Imaging, OFT: open field test; FST: forced swim test.

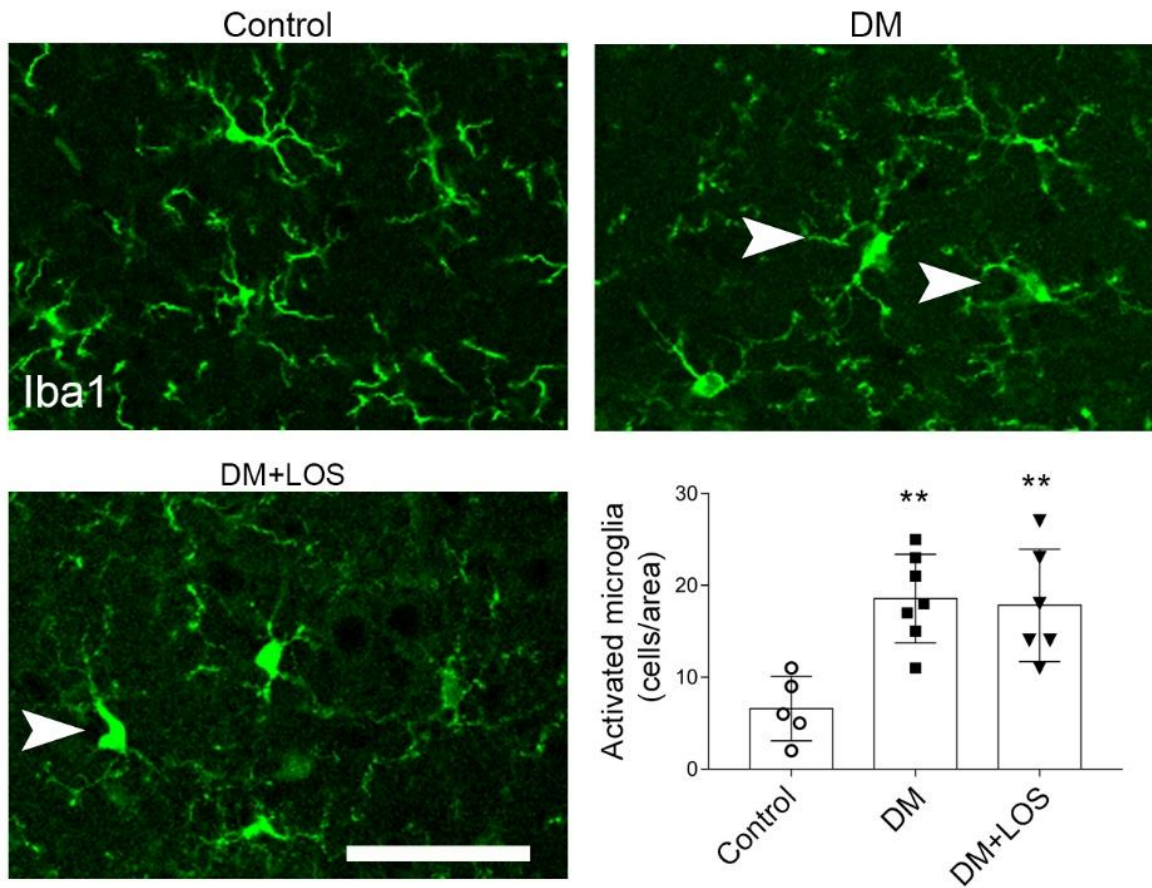
Protocol 1.



Protocol 2.



ESM Figure 2. Microglia were visualized by Iba1 to assess microglial activation in the hippocampus of control, diabetic (DM) and diabetic losartan-treated rats (DM+LOS). Arrowheads showing microglia with increased Iba1 expression, thicker cell bodies and a partial withdrawal of processes, indicative of higher level of microglial activity. Blinded evaluation is showing elevated numbers of activated microglia in the hippocampus of diabetic rats. Data are presented as means±SDs, (n=5-7/group). * $p < 0.05$ vs. C. Scale bar: 50 μ m.



ESM Figure 3. Ponceau S-stained membranes of the representative samples. (a) Ponceau S-stained membranes of the representative examples of Figure 2 and 3. (b) Ponceau S-stained membranes of the representative examples of Figure 4. (c) Ponceau S-stained membranes of the representative examples of Figure 5. Black rectangles show the region of protein of interest. DM: diabetic group; DM+LOS: diabetic losartan-treated group.

