

**Table 3** Alteration of biological functions related to cell signaling in MACO model

Biological process	Number of gene	FDR	Genes
Positive regulation of ERK1 and ERK2 cascade	56	2.62E-08	ARHGAP8, FLT4, ITGB3, HTR2B, HTR2C, FGF2, CX3CL1, ICAM1, PYCARD, C1QTNF3, EDNRA, CYSLTR2, GLIPR2, CD36, LGALS9, NPY5R, DCC, IL1B, RARA, CHI3L1, CD44, CCL12, C5AR2, C5AR1, NOD2, C3, CCL9, THPO, GPNMB, CCL7, CCL6, ERBB4, NPY, PDGFD, CCL4, GCNT2, CCL3, CCL2, CCL19, NTSR2, CCR1, JUN, CCL22, TGFB1, CCL20, CFLAR, SCIMP, MT3, ESR1, EPOR, IL6, GPR183, TRPV4, FGF18, F2RL1
Positive regulation of MAPK cascade	38	1.04E-06	ITGB1, CD40, ITGB3, FLT4, TNFRSF11B, NOD2, KNG2, KNG1, LEPR, RIPK1, CD36, RELT, IL6R, NTSR2, TBX1, NGFR, IL11, GPR37, KSR1, IGFBP3, PRKCD, OSM, IGF2, LIF, GPR37L1, IGF1, TNFRSF18, SORBS3, AGT, AR, IL6, PTPRC, ADRB3, FAS, CD27, LTBR, TNFRSF26, FGFR1
Cellular response to tumor necrosis factor	41	3.59E-06	REG3B, CCL12, CD40, CALCA, THBS1, CX3CL1, ICAM1, PYCARD, ZFP36, CCL9, ADAMTS13, MUC2, CCL7, CCL6, CCL4, ZC3H12A, CCL3, CCL2, CYP1B1, HAS2, RIPK1, CCL19, ADAMTS7, VCAM1, CCL22, NOS2, NOS3, CCL20, ERBIN, ACOD1, CYBA, MMP9, OCLN, IL6, FAPB4, IRF1, GPD1, LCN2, FAS, CHI3L1, BIRC3
Integrin-mediated signaling pathway	27	6.25E-04	ITGB1, LAMA5, ITGAM, ITGB3, PLEK, ITGB2, ITGAL, PRAM1, ADAMTS2, ADAMTS1, ITGAX, ITGB8, ITGB7, ADAMTS9, FCER1G, ITGA2, ITGA1, ADAM11, VAV1, FGR, COL3A1, TYROBP, ZYX, PLP1, ITGA5, CDH17, FERMT3
Positive regulation of protein kinase B signaling	26	0.0012	CSF3, TNF, THBS1, FGF2, C1QTNF3, THPO, CCL3, CPNE1, GCNT2, CCL19, NGFR, TGFB1, MYOC, F10, IGF2, IGF1, F3, F7, IL6, MC1R, RARA, CD28, HCLS1, CHI3L1, HBEGF
Positive regulation of I-kappaB kinase/NF-kappaB signaling	38	0.0016	CD40, ECM1, EDA, HTR2B, NOD2, ZC3HAV1, TNF, LITAF, MALT1, GJA1, LGALS1, CASP8, S100A13, TNFSF10, HMOX1, FLNA, RIPK1, CD36, LGALS9, CCL19, TGM2, SECTM1B, NEK6, IRAK4, CFLAR, TICAM1, RHOC, TNFRSF1A, GPRC5B, TNIP2, CTH, IL1B, REL, F2RL1, S100A4, LTBR, MYD88, CARD11
Response to tumor necrosis factor	15	0.0020	NOS2, GCH1, MMP3, PTGS2, MMP9, CXCL16, ADAMTS13, CASP8, CHI3L1, CCL2, RIPK1, MBP, CD14, JAK2, GGT1
Intracellular signal transduction	74	0.0021	ITK, GUCY1B2, MAST3, DGKB, PLEK, HSPB1, PREX2, NRBP2, ZFP36, GRB14, CASP3, STK32A, JAK2, JAK3, GUCY1A2, CISH, PRKCD, MASTL, DGKZ, LAX1, VAV1, TIAM2, LAT2, ZAP70, DEPDC1B, MELK, TYROBP, RASA4, NRG4, SPATA13, DCX, ARHGEF2, PLCB1, SHC4, LOC100911548, GUCY2D, RGS14, SHC1, ADCY4, ASB11, NOD2, KALRN, ADCY7, RGD1562638, SOCS3, NIAK2, SOCS1, IRAK2, CHN2, CHN1, HMOX1, PLEK2, SH2B2, SOCS4, LYN, NOS2, MCF2, ECEL1, DCLK3, DCDC2, SMAD7, MOS, ADCY10, SPSB2, SPSB1, MC1R, CAMK4, STAC2, GPR182, PTPN6, PLCH2, ASB2, PLCD4, PLCD1
Tumor necrosis factor-mediated signaling pathway	16	0.0024	TNFSF18, NGFR, CD40, TNFRSF10B, TNFRSF11B, TNFRSF1B, TNF, TNFRSF1A, PYCARD, FAS, CD27, LTBR, TNFRSF26, JAK2, RELT, CARD14
Cell surface receptor signaling pathway	44	0.0024	CD63, CD274, LOC103690020, VIPR1, VIPR2, TNFRSF13B, GIPR, ITGAL, MCHR1, ADGRG3, GHRHR, UPK1B, ADGRG5, CXCR2, PRLHR, CD36, FCGR1A, CD53, LAG3, FCER1G, ANXA1, ADGRV1, TRPA1, EDN3, NPY5R, GCGR, GLP2R, TACR1, F2, TNFRSF1B, AGT, TNFRSF1A, ADGRF2, FCGR2A, MAPKAPK3, CD8A, CLCF1, TSPAN18, ADGRF4, COL4A3, CD9, OSTN, FCGR2B, MYD88
Positive regulation of tumor necrosis factor production	19	0.0039	FCER1G, CYBA, NOD2, TICAM1, NFATC4, TNFRSF1A, PYCARD, CCL4, CCL3, CCL2, RIPK1, CD36, LGALS9, CCL19, ARHGEF2, LBP, CD14, JAK2, MYD88
Positive regulation of NF-kappaB transcription factor activity	30	0.0052	CD40, EDA, ITGB2, CAMK2A, NOD2, TNF, MALT1, ICAM1, PYCARD, IRAK2, NLRP3, RIPK1, LGALS9, IL6R, TNFSF18, TGFB1, RIPK3, SPHK1, CFLAR, TICAM1, AR, IL6, CTH, IL1B, ARHGEF2, RAB7B, CARD14, MYD88, HSPA1B, CARD11
Adenylate cyclase-activating G-protein coupled receptor signaling pathway	18	0.0090	PTGFR, PTGIR, CALCA, GPR26, GCGR, PTGER2, PTGER3, ADCY4, ADRB1, HTR4, ADCY7, RXFP1, RXFP2, GHRHR, ADRB3, GALR2, UCN2, ADM2

**Table 3** (continued)

Biological process	Number of gene	FDR	Genes
Phospholipase C-activating G-protein coupled receptor signaling pathway	19	0.010	CASR, C5AR2, PTGER3, C5AR1, HTR2B, HTR2C, GPR84, ESR1, AGTR1A, GNG13, ANO1, CYSLTR2, GALR2, P2RY2, CXCR2, C3AR1, PLCE1, TGM2, NTSR2
Positive regulation of phosphatidylinositol 3-kinase signaling	19	0.019	CSF3, TGFB2, MYOC, IGF1, F2, AGT, FGR, SELP, GH1, ERBB4, PDGFD, RARA, CD28, F2RL1, HCLS1, PLXNB1, PTPN6, JAK2, HCST
Lipopolysaccharide-mediated signaling pathway	12	0.028	LYN, TGFB1, IRAK2, NOS3, IL1B, CCL3, CCL2, LBP, CD14, TICAM1, TNF, MYD88

( $p < 0.05$ ). The dysregulation of oxygen could lead to alteration of metabolic processes, including the response to the lipopolysaccharide and retinoic acid biosynthetic process, retinol metabolic process (Fig. 2C & Table 2) ( $p < 0.05$ ), and alteration of cell signaling pathways, such as one involved in the regulation of the extracellular signal-regulated kinase 1 and extracellular signal-regulated kinase 2 cascades, the mitogen-activated protein kinase cascade, protein kinase B signaling, I-kappaB kinase/NF-kappaB signaling, tumor necrosis factor production, NF-kappaB transcription factor activity, phosphatidylinositol 3-kinase signaling, and lipopolysaccharide-mediated signaling pathway in the brain tissue of the stroke model (Fig. 2D and Table 3) ( $p < 0.05$ ). These alterations might result in immune and inflammatory responses (Fig. 2E and Table 4) ( $p < 0.05$ ) and alteration of cell proliferation and cell apoptosis (Fig. 2F and Table 5) ( $p < 0.05$ ). The outcomes would be neurological disorders and diseases such as aging, axon injury, disruption of membrane potential, neuron differentiation, myeloid dendritic cell differentiation, and memory (Fig. 2G and Table 6) ( $p < 0.05$ ).

In the GO analysis of molecular function, we also found the structural alteration of myelin sheath and alteration of many functions related to ion channels such as calcium ion binding, potassium channel activity, extracellular ligand-gated ion channel activity, and chloride channel activity (Fig. 3A) ( $p < 0.05$ ). Additionally, immune and inflammatory-related activity, such as chemokine activity, cytokine receptor activity, and C-C motif chemokine receptor binding, was highlighted in our results. In the GO analysis of cellular components, we observed the involvement of postsynaptic membrane, dendritic spine, synapse, immunological synapse, postsynaptic density, neuronal cell body, neuronal cell body membrane, and cortical actin cytoskeleton (Fig. 3B) ( $p < 0.05$ ). Taken together, our data suggested the contribution of the dysregulated gene in the pathology of the stroke model.

#### Alteration of gene network involved in brain disorders in the MCAO model

To delineate the molecular mechanisms and gene network involved in brain disorders in the MCAO model,

IPA was conducted. In the diseases and biological functions of IPA, our results highlighted many neurological diseases and abnormalities, especially cerebrovascular dysfunction, and stroke (Fig. 4A) ( $p < 0.05$ ). There were 107 DEGs that were closely associated with stroke (Table 7) ( $q < 0.05$ ). Moreover, we further looked at the altered canonical pathways in the stroke model. We found that activation of cell signaling was related to immune and inflammatory response pathways such as the neuroinflammation signaling pathway, inflammasome pathway, integrin-linked kinase (ILK) signaling, and Th1 pathway (Fig. 4B) ( $p < 0.05$ ). Additionally, neuronal functions-and disorders-related signaling pathways, including cAMP response element-binding protein signaling in neurons, glioblastoma multiform signaling, and neuregulin signaling, were also highlighted (Fig. 4B) ( $p < 0.05$ ). The network analysis showed the involvement of many ion channels, enzymes, cytokines, and complexes in these activations (Fig. 4C) ( $p < 0.05$ ). On the other hand, the IPA analysis showed the inhibition of signaling was related to brain functions such as neurovascular coupling signaling pathway, semaphorin neuronal repulsive signaling pathway, endocannabinoid neuronal synapse pathway, dopamine-DARPP32 feedback in cAMP signaling, dopamine degradation, oxytocin in spinal neurons signaling pathway (Fig. 4D) ( $p < 0.05$ ). Moreover, some important cell signaling pathways, including WNT/ $\text{Ca}^{2+}$ , protein A signaling, p53 signaling, Janus kinases (JAK1 and JAK2), tyrosine kinase 2 in interferon signaling, WNT/ $\beta$ -catenin signaling, and phosphatase and tensin homolog signaling, were also found to be inhibited in the stroke model. The gene network construction further demonstrated the contribution of ion channels, enzymes, and transcriptional factors in controlling these inhibitions (Fig. 4E) ( $p < 0.05$ ).

#### Validation of transcriptome finding

To validate the findings from transcriptome sequencing, immunohistochemistry staining was used to evaluate the induction of the 2 identified biomarkers of ischemia stroke. The results of IHC well-matched with the findings of transcriptomic analysis that both *Angpt2* and *Lepr* were induced in MACO model (Fig. 5A & B) ( $p < 0.05$ ).