Supporting information

Figure S1. The light micrographs of cultured L6 cells were enlarged by 10*10. The myoblasts were grown in DMEM supplemented with 10% FBS before differentiation initiation (A), then the myoblasts were cultured in DMEM with 2% FBS for 6 days, and the giant elongated multinucleated myotubes were clearly seeable (B).

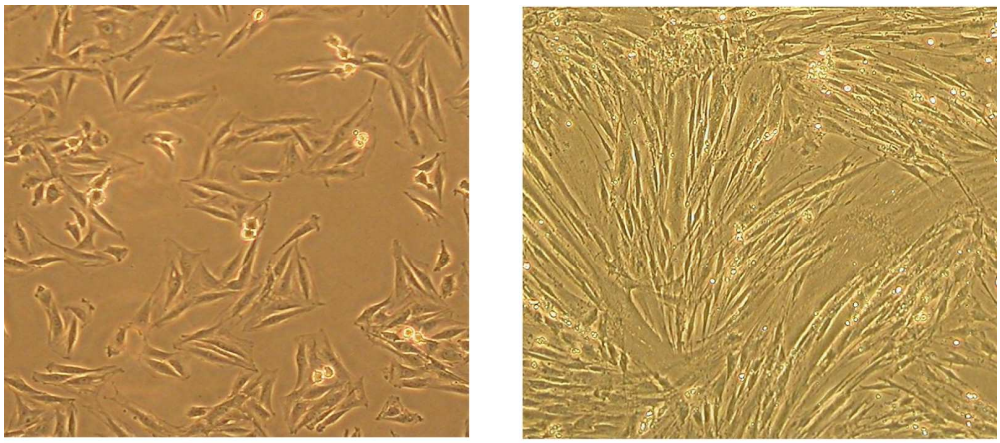
Figure S2. Sketch of our strategy for the large-scale phosphorylation analysis of rat L6 myotubes.

Figure S3. The percentage of phosphorylated (phos) and non-phosphorylated peptides (non-phos) identified in four experiments.

Figure S4. The MAPK signaling pathway derived from KEGG pathway database[1]. Red and green rectangles represent the phosphoproteins whose genes are observed and unobserved in our study, respectively.

Figure S5. The network diagram of signaling pathways associated with cell-to-cell communication and cell fusion including adherens junction, focal adhesion, gap junction, tight junction and regulation of actin cytoskeleton, using Cytoscape[2].

Figure S1



(A) (B)

Figure S2

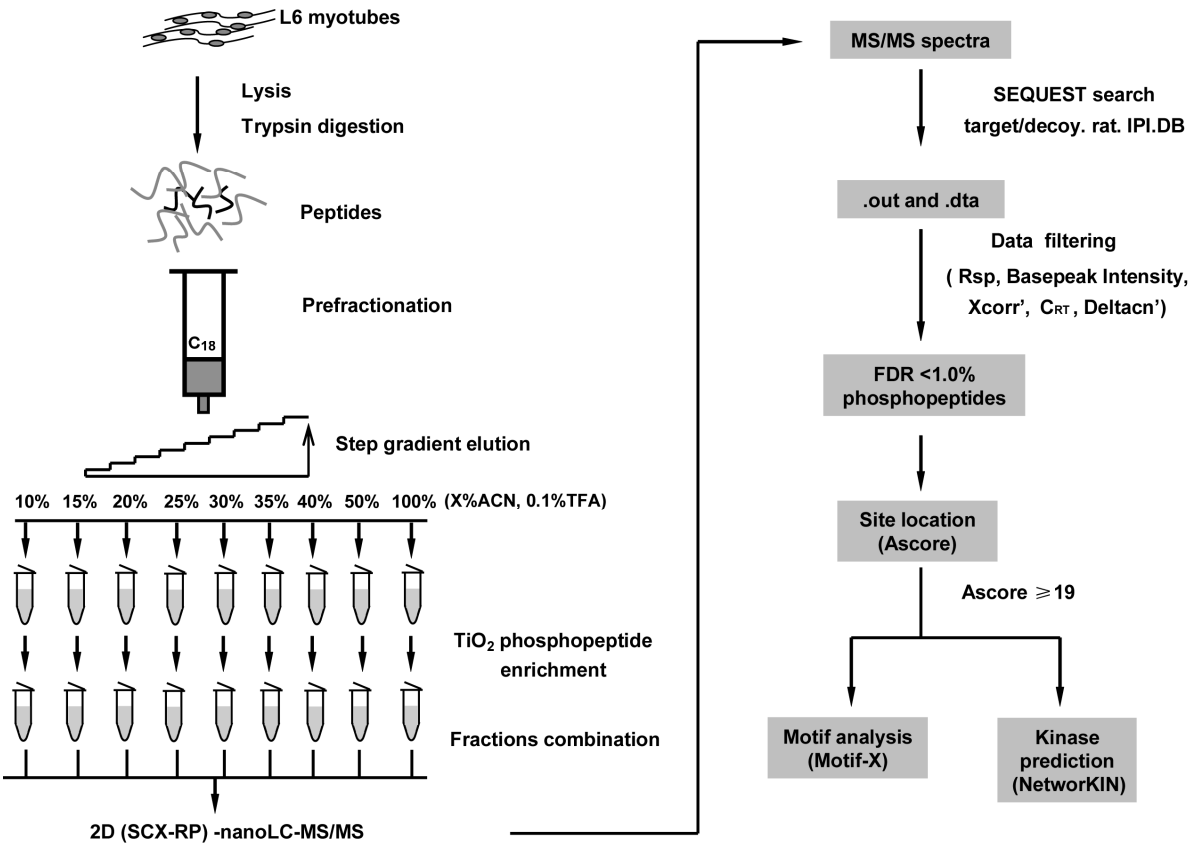

L6 myotubes
Lysis
Trypsin digestion
Peptides
Prefractionation
C18
Step gradient elution
10% 15% 20% 25% 30% 35% 40% 50% 100% (X%ACN, 0.1%TFA)
TiO2 phosphopeptide enrichment
Fractions combination
2D (SCX-RP) -nanoLC-MS/MS
MS/MS spectra
SEQUEST search
target/decoy. rat. IPI.DB
.out and .dta
Data filtering
(Rsp, Basepeak Intensity,
Xcorr', CRT, Deltacn')
FDR <1.0%
phosphopeptides
Site location
(Ascore)
Ascore ⩾19
Motif analysis
(Motif-X)
Kinase
prediction
(NetworKIN)

Figure S3

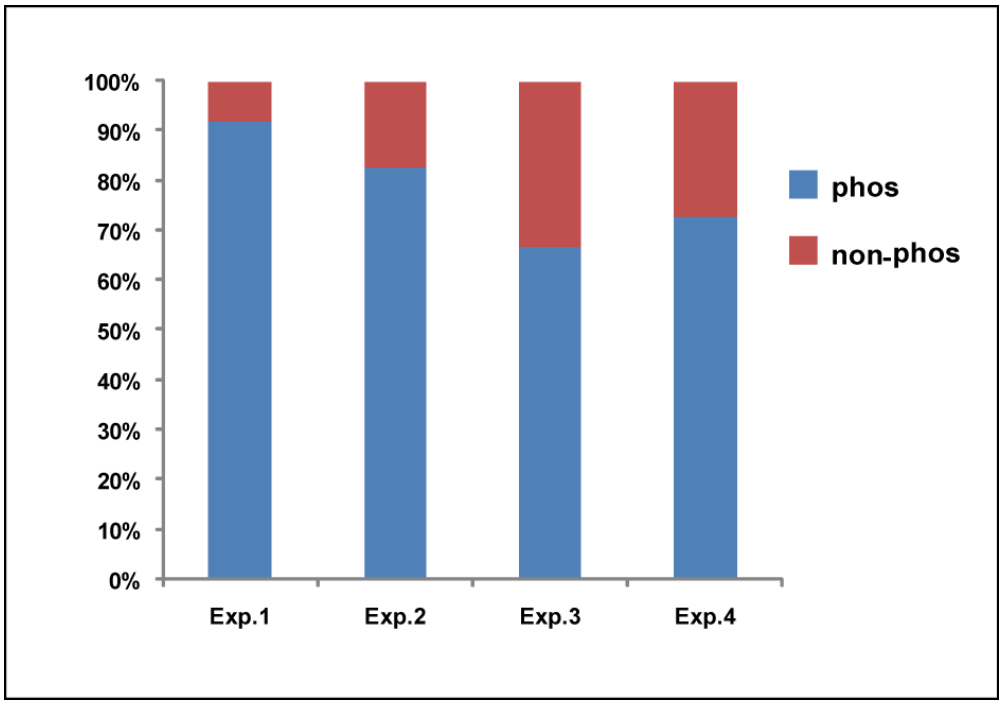


Figure S4

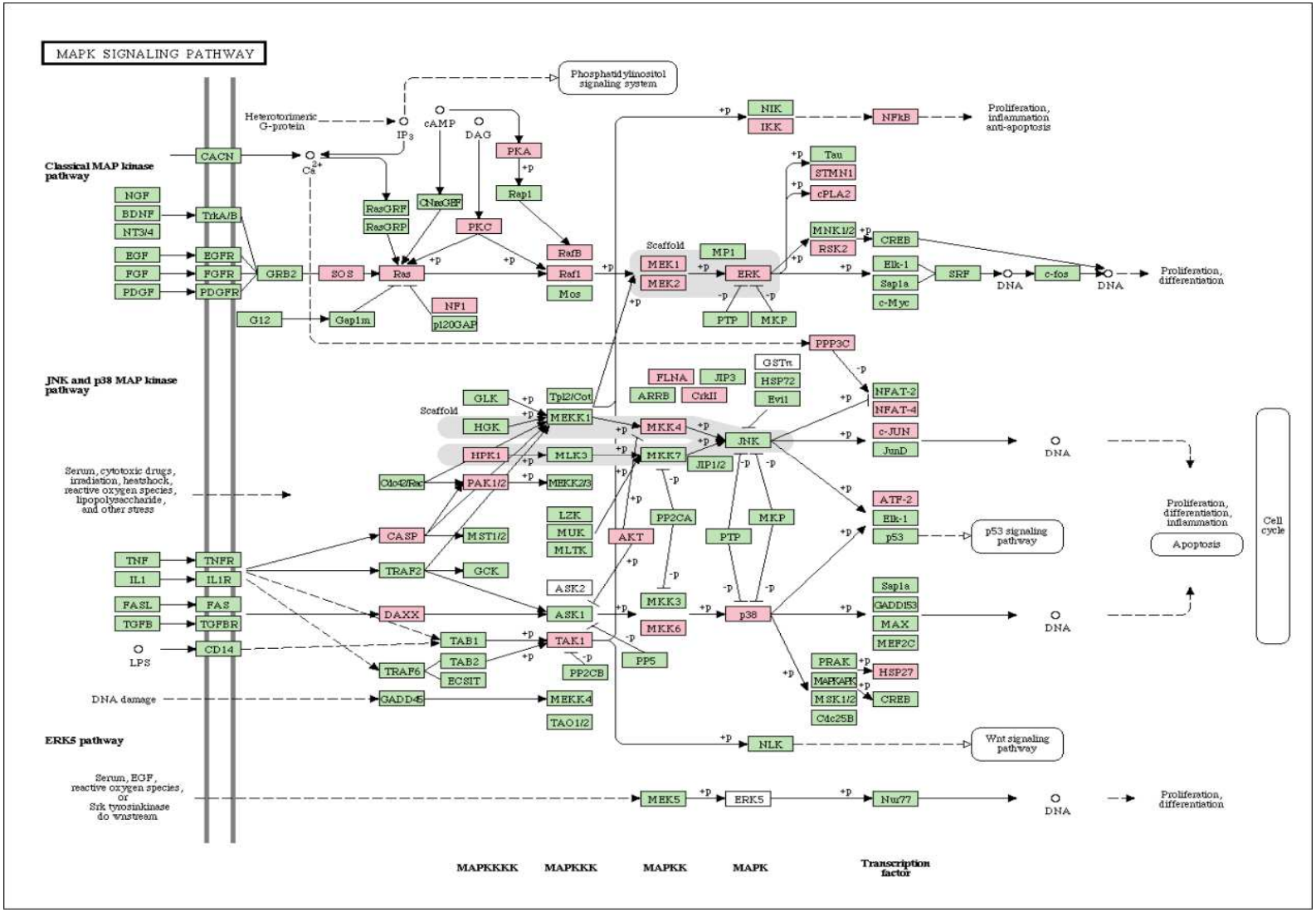
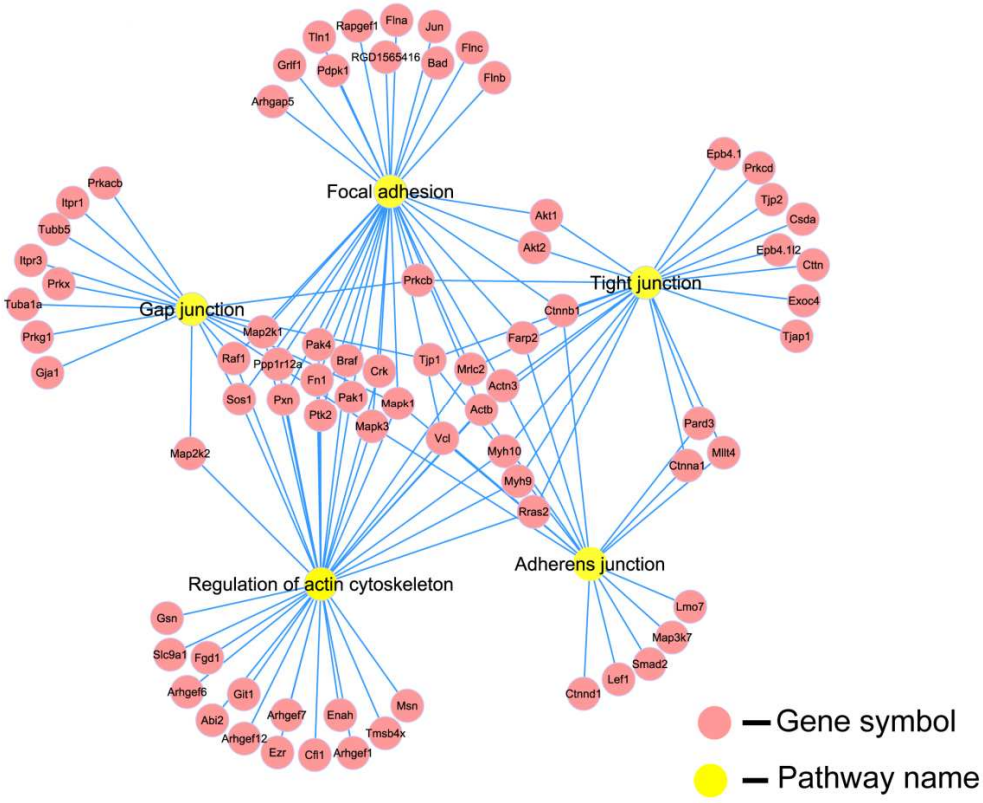

MAPK SIGNALING PATHWAY
Classical MAP kinase pathway
JNK and p38 MAP kinase pathway
ERK5 pathway
Phosphatidylinositol signaling system
Heterotorimeric G-protein
cAMP
DAG
PKA
Rap1
RasGRF
CNrasGEF
RasGRP
PKC
RafB
Raf1
Mos
Ras
SOS
GRB2
EGFR
FGFR
PDGFR
EGF
FGF
PDGF
NGF
BDNF
NT3/4
TrkA/B
G12
Gap1m
NF1
p120GAP
CACN
Ca2+
IP3
NIK
IKK
NFkB
Proliferation, inflammation anti-apoptosis
Tau
STMN1
cPLA2
MNK1/2
RSK2
CREB
Elk-1
Sap1a
c-Myc
SRF
c-fos
DNA
Proliferation, differentiation
Scaffold
MP1
MEK1
MEK2
ERK
PTP
MKP
PPP3C
GSTn
FLNA
JIP3
HSP72
ARRB
CrkII
Evi1
NFAT-2
NFAT-4
c-JUN
JunD
JNK
GLK
HGK
HPK1
Cdc42/Rac
PAK1/2
Tpl2/Cot
MEKK1
MLK3
MEKK2/3
MKK4
MKK7
JIP1/2
Serum, cytotoxic drugs, irradiation, heatshock, reactive oxygen species, lipopolysaccharide, and other stress
CASP
MST1/2
TRAF2
GCK
LZK
MUK
MLTK
ASK2
ASK1
DAXX
TAB1
TAB2
ECSIT
TAK1
TRAF6
GADD45
MEKK4
TAO1/2
PP2CA
PP2CB
PP5
AKT
MKK3
MKK6
p38
MKP
PTP
ATF-2
Elk-1
p53
p53 signaling pathway
Sap1a
GADD153
MAX
MEF2C
PRAK
MAPKAPK
MSK1/2
HSP27
CREB
Cdc25B
TNF
IL1
FASL
TGFB
LPS
TNFR
IL1R
FAS
TGFBR
CD14
DNA damage
Proliferation, differentiation, inflammation
Apoptosis
Cell cycle
NLK
Wnt signaling pathway
Serum, EGF, reactive oxygen species, or Src tyrosinkinase do wnstream
MEK5
ERK5
Nur77
MAPKKKK
MAPKKK
MAPKK
MAPK
Transcription factor

Figure S5


Focal adhesion
Gap junction
Tight junction
Regulation of actin cytoskeleton
Adherens junction
Rapgef1
Flna
Jun
Tln1
RGD1565416
Flnc
Grlf1
Pdpk1
Bad
Flnb
Arhgap5
Prkacb
Itpr1
Tubb5
Itpr3
Prkx
Tuba1a
Prkg1
Gja1
Akt1
Akt2
Prkcb
Epb4.1
Prkcd
Tjp2
Csda
Epb4.1l2
Cttn
Exoc4
Tjap1
Ctnnb1
Farp2
Map2k1
Raf1
Ppp1r12a
Pak4
Braf
Crk
Tjp1
Mrlc2
Actn3
Actb
Fn1
Sos1
Pxn
Pak1
Mapk1
Ptk2
Mapk3
Vcl
Myh10
Myh9
Rras2
Map2k2
Pard3
Mllt4
Ctnna1
Gsn
Slc9a1
Fgd1
Arhgef6
Git1
Abi2
Arhgef7
Enah
Msn
Arhgef12
Ezr
Cfl1
Arhgef1
Tmsb4x
Lmo7
Map3k7
Smad2
Lef1
Ctnnd1
Gene symbol
Pathway name

Reference

(1) Kanehisa, M., Goto, S., KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000, *28*, 27-30.

(2) Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003, 13(11):2498-2504.