Supplementary materials

Supplementary methods

Hematoxylin and eosin (H&E) staining

At the end of experiment, the kidney and liver were excised from euthanized mice. The tissues were fixed with formaldehyde solution, embedded in paraffin block, and sectioned with microtome. The sections were deparaffinized, rehydrated, and with H&E solution for histopathological examination.

KEGG mapping

After GSEA analysis, KEGG mapping analysis was conducted for visualizing the significantly changed genes in the pathways, including P53 signaling pathway, apoptosis, chemokine signaling pathway, ECM-receptor interaction, and cell adhesion molecules. The maps were visualized by KEGG mapper color tool (https://www.genome.jp/kegg/mapper/color.html) according to recent report [1].

Supplementary Table list.

Table S1. The possible targets of Fr were collected from TCMID database.

Table S2. The KEGG pathway analysis was conducted by Cytoscape with JEPPETO plugin using possible target genes of Fr.

Table S3. The GO_cellular components analysis was conducted by Cytoscape with JEPPETO plugin using possible target genes of Fr.

Table S4. The GO_molecular function analysis was conducted by Cytoscape with JEPPETO plugin using possible target genes of Fr.

Table S5. The GO_biological pathway analysis was conducted by Cytoscape with JEPPETO plugin using possible target genes of Fr.

Table S6. The genes related with endometriosis in DisGeNet database.

The supplementary tables were uploaded as separated Microsoft excel file.

Supplementary Figures

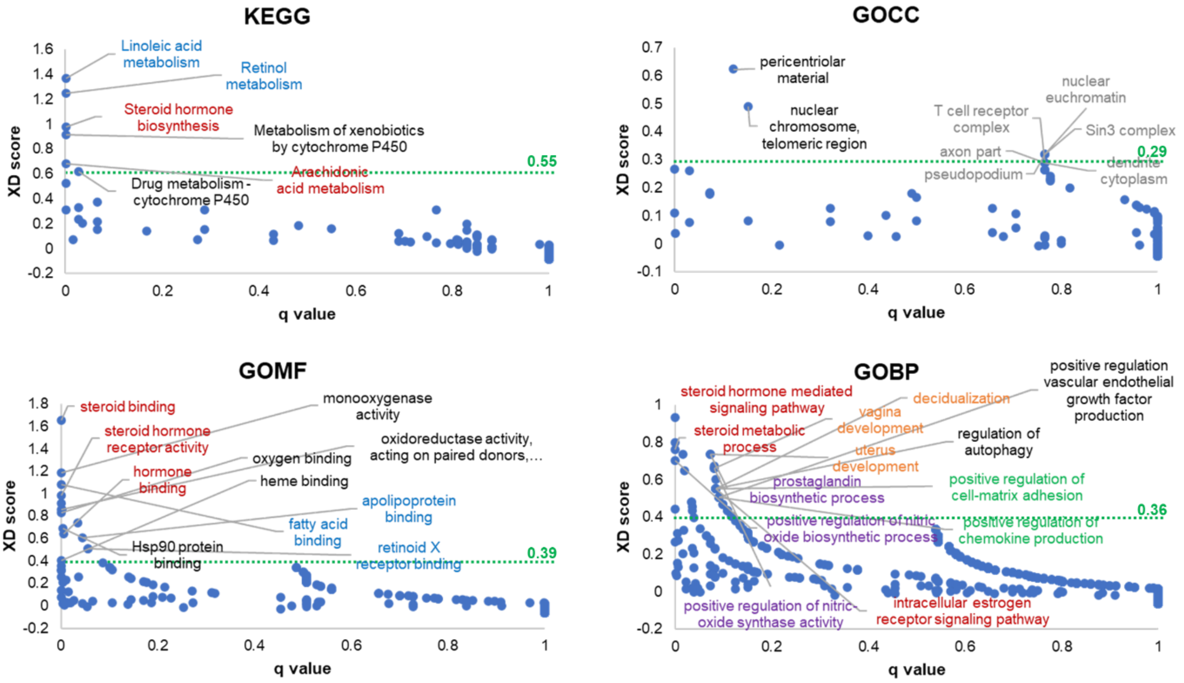


Fig. S1. Network pharmacological GO analysis suggested the potential therapeutic effect of Fr on endometriosis. The TCMID database was used to gather the potential target genes for Fr. With the aid of the JEPPETO plugin, Cytoscape performed network studies on these target genes.

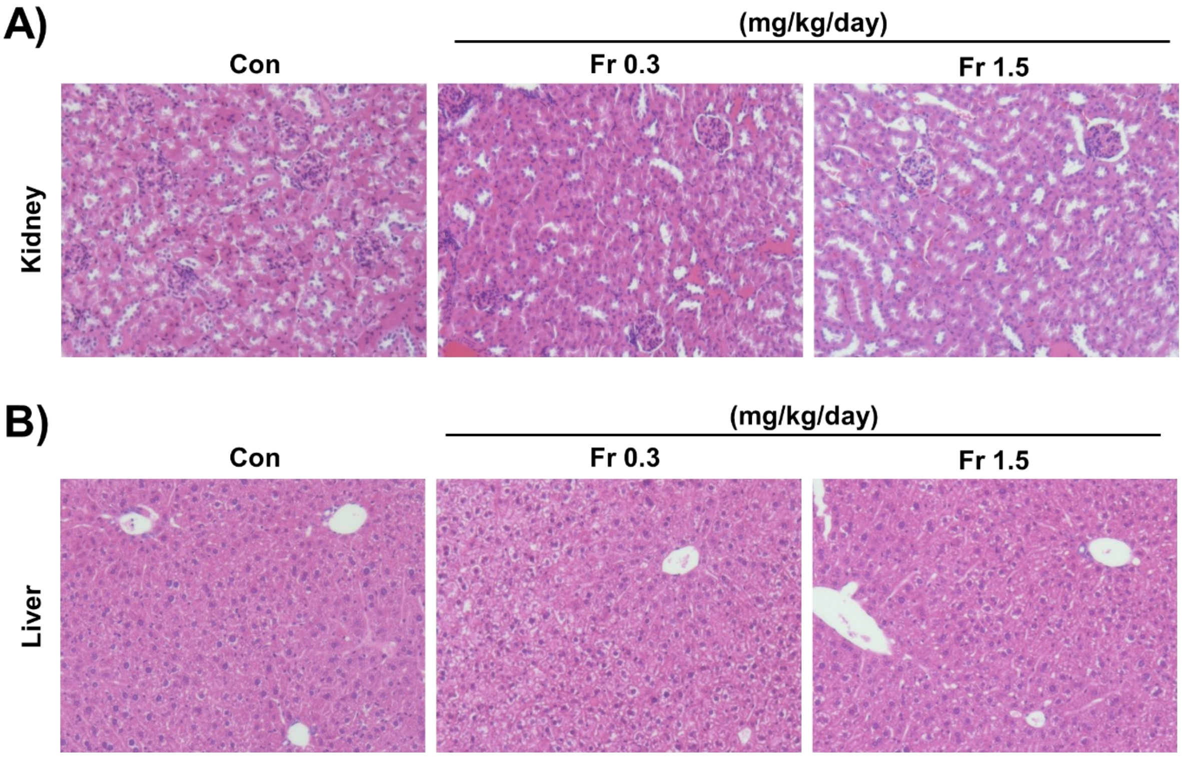


Fig. S2. The mice were treated indicated dose of Fr (0.3 or 1.5 mg/kg/day) after induction of endometriosis. The microphotographic images of H&E stained- kidney and liver sections were presented (×100).

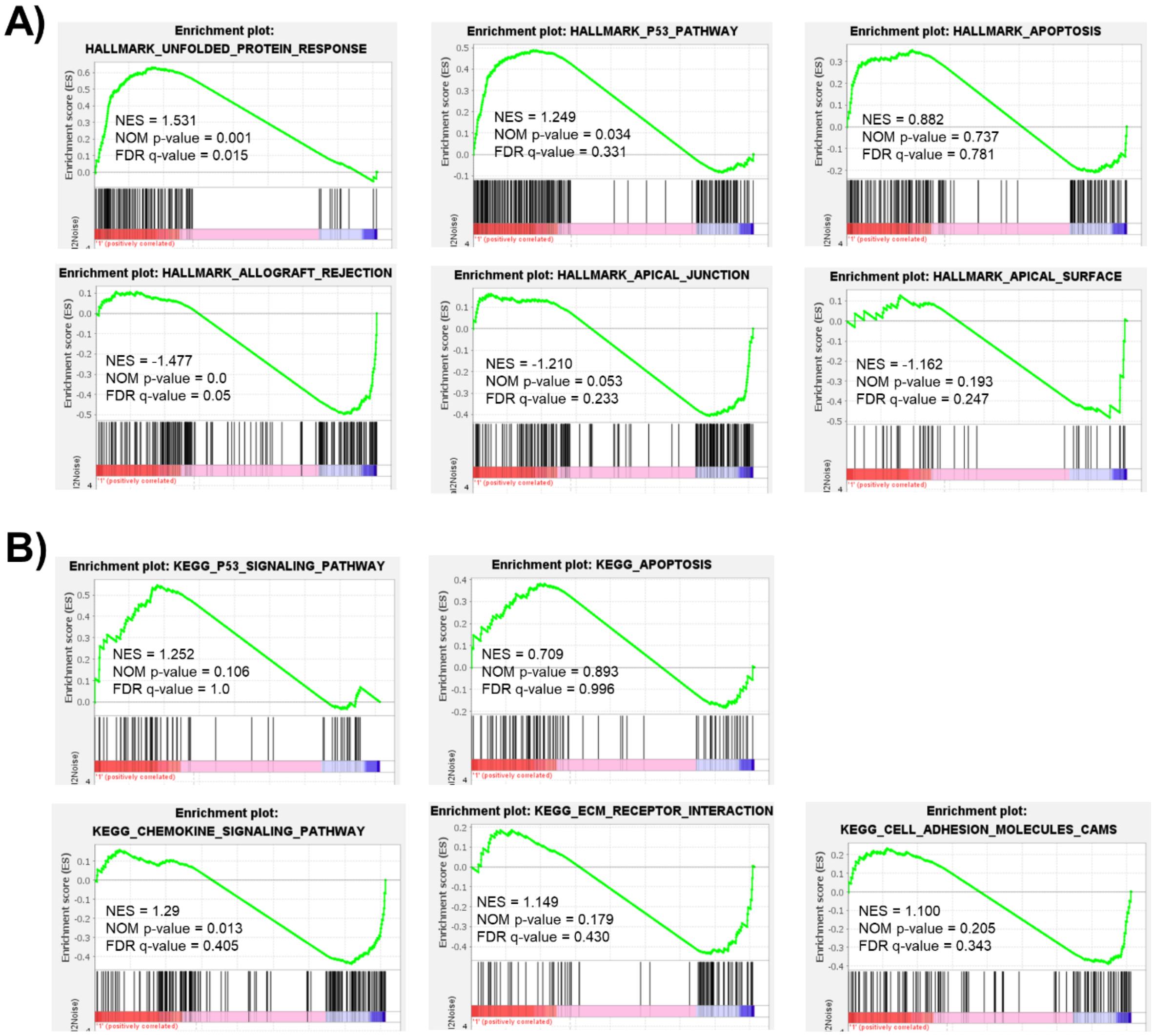
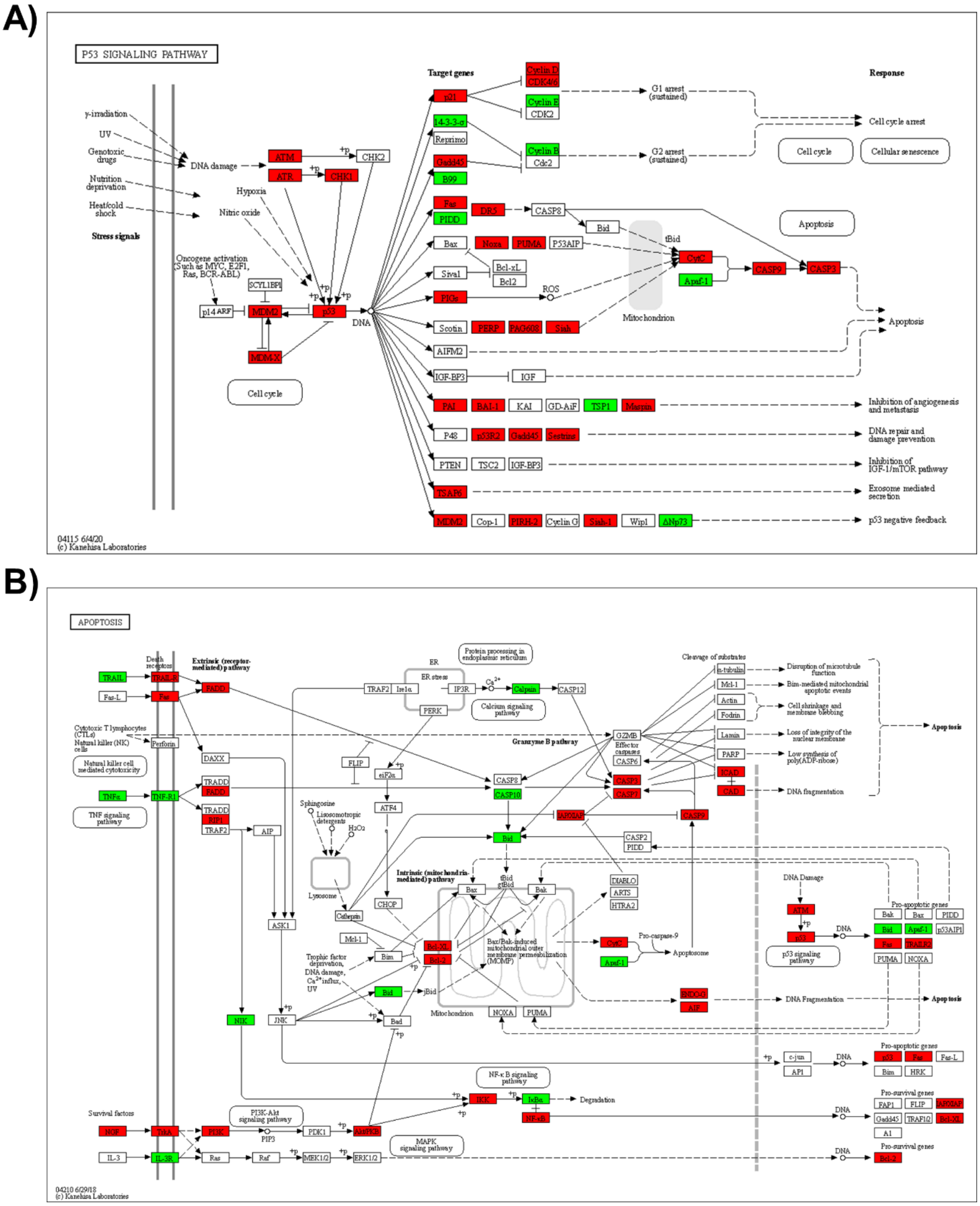
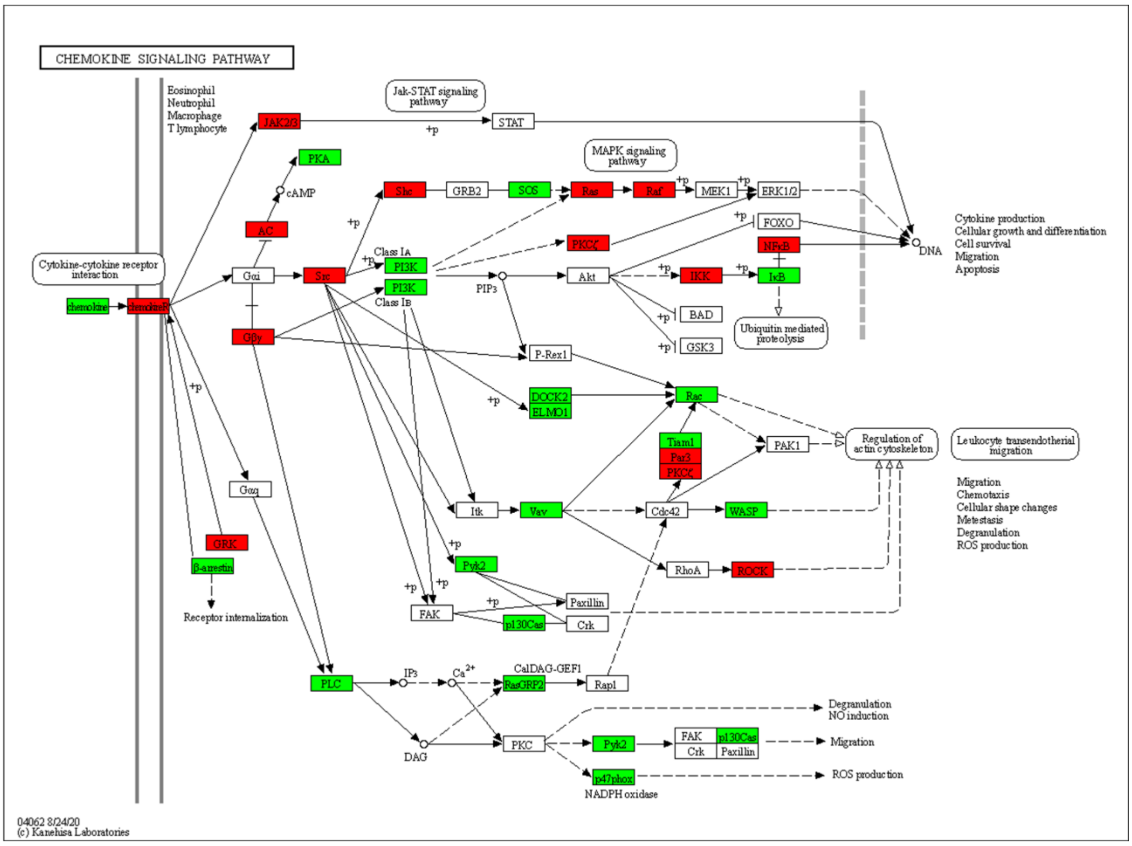


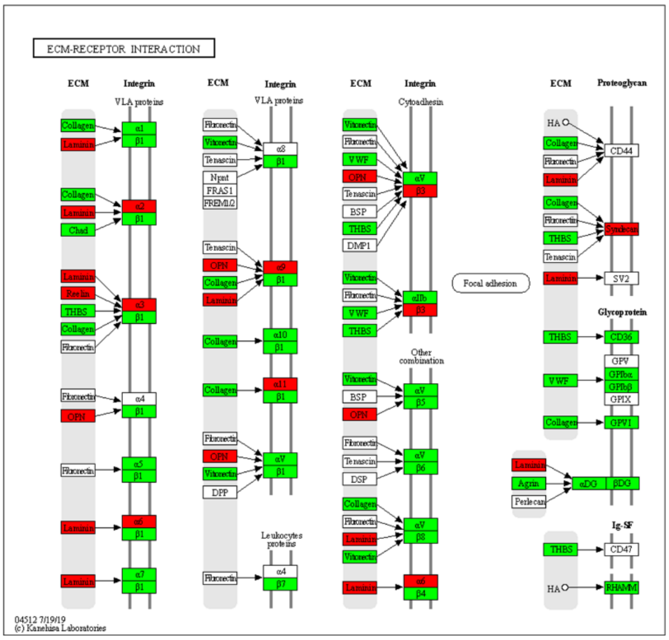
Fig. S3. The enrichment plots of significantly changed Hallmark (A) and KEGG (B) pathways by Fr-treatment were presented.


A)
P53 SIGNALING PATHWAY
Stress signals
γ-irradiation
UV
Genotoxic drugs
Nutrition deprivation
Heat/cold shock
DNA damage
Hypoxia
Nitric oxide
Oncogene activation (Such as MYC, E2F1, Ras, BCR-ABL)
ATM
ATR
CHK2
CHK1
SCYL1BP1
p14ARF
MDM2
MDM-X
p53
DNA
Cell cycle
Target genes
p21
14-3-3-σ
Reprimo
Gadd45
B99
Fas
PIDD
DR5
CASP8
Bid
tBid
Bax
Noxa
PUMA
P53AIP
Bcl-xL
Bcl2
Siva1
PIGs
ROS
Scotin
PERP
PAG608
Siah
AIFM2
IGF-BP3
IGF
CytC
Apaf-1
CASP9
CASP3
Mitochondrion
Cyclin D
CDK4/6
Cyclin E
CDK2
Cyclin B
Cdc2
G1 arrest (sustained)
G2 arrest (sustained)
Response
Cell cycle arrest
Cell cycle
Cellular senescence
Apoptosis
Apoptosis
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
Inhibition of angiogenesis and metastasis
P48
p53R2
Gadd45
Sestrins
DNA repair and damage prevention
PTEN
TSC2
IGF-BP3
Inhibition of IGF-1/mTOR pathway
TSAP6
Exosome mediated secretion
MDM2
Cop-1
PIRH-2
Cyclin G
Siah-1
Wip1
ΔNp73
p53 negative feedback
04115 6/4/20
(c) Kanehisa Laboratories
B)
APOPTOSIS
Death receptors
Extrinsic (receptor-mediated) pathway
TRAIL
TRAIL-R
Fas-L
Fas
FADD
Cytotoxic T lymphocytes (CTLs)
Natural killer (NK) cells
Perforin
Natural killer cell mediated cytotoxicity
DAXX
TNFα
TNF-R1
TRADD
FADD
TRADD
RIP1
TRAF2
TNF signaling pathway
AIP
ASK1
Protein processing in endoplasmic reticulum
ER
ER stress
TRAF2
Ire1α
IP3R
Ca2+
Calpain
CASP12
PERK
Calcium signaling pathway
FLIP
eIF2α
ATF4
CHOP
Sphingosine
Lysosomotropic detergents
H2O2
Lysosome
Cathepsin
Granzyme B pathway
GZMB
Effector caspases
CASP6
CASP8
CASP10
CASP7
CASP3
XIAP
CASP9
Bid
CASP2
PIDD
Intrinsic (mitochondria-mediated) pathway
tBid
gtBid
Bax
Bak
DIABLO
ARTS
HTRA2
Mcl-1
Bim
Bcl-XL
Bcl-2
Bad
jBid
Bax/Bak-induced mitochondrial outer membrane permeabilization (MOMP)
CytC
Apaf-1
Pro-caspase-9
Apoptosome
Mitochondrion
NOXA
PUMA
ENDOG
AIF
DNA Fragmentation
Trophic factor deprivation, DNA damage, Ca2+ influx, UV
NIK
JNK
Cleavage of substrates
α-tubulin
Mcl-1
Actin
Fodrin
Lamin
PARP
ICAD
CAD
Disruption of microtubule function
Bim-mediated mitochondrial apoptotic events
Cell shrinkage and membrane blebbing
Loss of integrity of the nuclear membrane
Low synthesis of poly(ADP-ribose)
DNA fragmentation
Apoptosis
DNA Damage
ATM
p53
DNA
p53 signaling pathway
Pro-apoptotic genes
Bak
Bax
PIDD
Bid
Apaf-1
p53AIP1
Fas
TRAIL-R
PUMA
NOXA
c-jun
AP1
DNA
Pro-apoptotic genes
p53
Fas
Fas-L
Bim
HRK
NF-κB signaling pathway
IKK
IκBα
NF-κB
Degradation
Pro-survival genes
FAP1
FLIP
XIAP
Gadd45
TRAF1/2
Bcl-XL
A1
Pro-survival genes
Bcl-2
Survival factors
NGF
TrkA
PI3K
PI3K-Akt signaling pathway
PIP3
PDK1
Akt/PKB
IL-3
IL-3R
Ras
Raf
MEK1/2
ERK1/2
MAPK signaling pathway
+p
04210 6/29/18
(c) Kanehisa Laboratories

C)


CHEMOKINE SIGNALING PATHWAY
Eosinophil
Neutrophil
Macrophage
T lymphocyte
Jak-STAT signaling pathway
JAK2/3
STAT
+p
PKA
cAMP
AC
MAPK signaling pathway
Shc
GRB2
SOS
Ras
Raf
MEK1
ERK1/2
FOXO
PKCδ
NFκB
IκB
IKK
Akt
Class IA
PI3K
PI3K
Class IB
PIP3
BAD
GSK3
Ubiquitin mediated proteolysis
DNA
Cytokine production
Cellular growth and differentiation
Cell survival
Migration
Apoptosis
Cytokine-cytokine receptor interaction
chemokine
chemokineR
Gαi
Src
Gβγ
P-Rex1
DOCK2
ELMO1
Rac
Tiam1
Par3
PKCζ
PAK1
Regulation of actin cytoskeleton
Leukocyte transendothelial migration
Migration
Chemotaxis
Cellular shape changes
Metestasis
Degranulation
ROS production
Gαq
Itk
Vav
Cdc42
WASP
RhoA
ROCK
GRK
β-arrestin
Receptor internalization
Pyk2
Paxillin
FAK
p130Cas
Crk
PLC
IP3
Ca2+
CalDAG-GEF1
RasGRP2
Rap1
Degranulation
NO induction
DAG
PKC
Pyk2
FAK
p130Cas
Crk
Paxillin
Migration
p47phox
NADPH oxidase
ROS production
04062 8/24/20
(c) Kanehisa Laboratories

D)


ECM-RECEPTOR INTERACTION
ECM
Integrin
VLA proteins
Collagen
Laminin
α1
β1
Collagen
Laminin
Chad
α2
β1
Laminin
Reelin
THBS
Collagen
Fibronectin
α3
β1
Fibronectin
OPN
α4
β1
Fibronectin
α5
β1
Laminin
α6
β1
Laminin
α7
β1
ECM
Integrin
VLA proteins
Fibronectin
Vitronectin
Tenascin
Npnt
FRAS1
FREM1/2
α8
β1
Tenascin
OPN
Collagen
Laminin
α9
β1
Collagen
α10
β1
Collagen
α11
β1
Fibronectin
OPN
Vitronectin
DPP
αV
β1
Leukocytes proteins
Fibronectin
α4
β7
ECM
Integrin
Cytoadhesin
Vitronectin
Fibronectin
VWF
OPN
Tenascin
BSP
THBS
DMP1
αV
β3
Vitronectin
Fibronectin
VWF
THBS
αIIb
β3
Other combination
Vitronectin
BSP
OPN
αV
β5
Fibronectin
Tenascin
DSP
αV
β6
Collagen
Fibronectin
Laminin
Vitronectin
αV
β8
Laminin
α6
β4
Focal adhesion
ECM
Proteoglycan
HA
Collagen
Fibronectin
Laminin
CD44
Collagen
Fibronectin
THBS
Tenascin
Syndecan
Laminin
SV2
Glycoprotein
THBS
CD36
VWF
GPV
GPIbα
GPIbβ
GPIX
Collagen
GPVI
Laminin
Agrin
Perlecan
αDG
βDG
Ig-SF
THBS
CD47
HA
RHAMM
04512 7/19/19
(c) Kanehisa Laboratories

E)

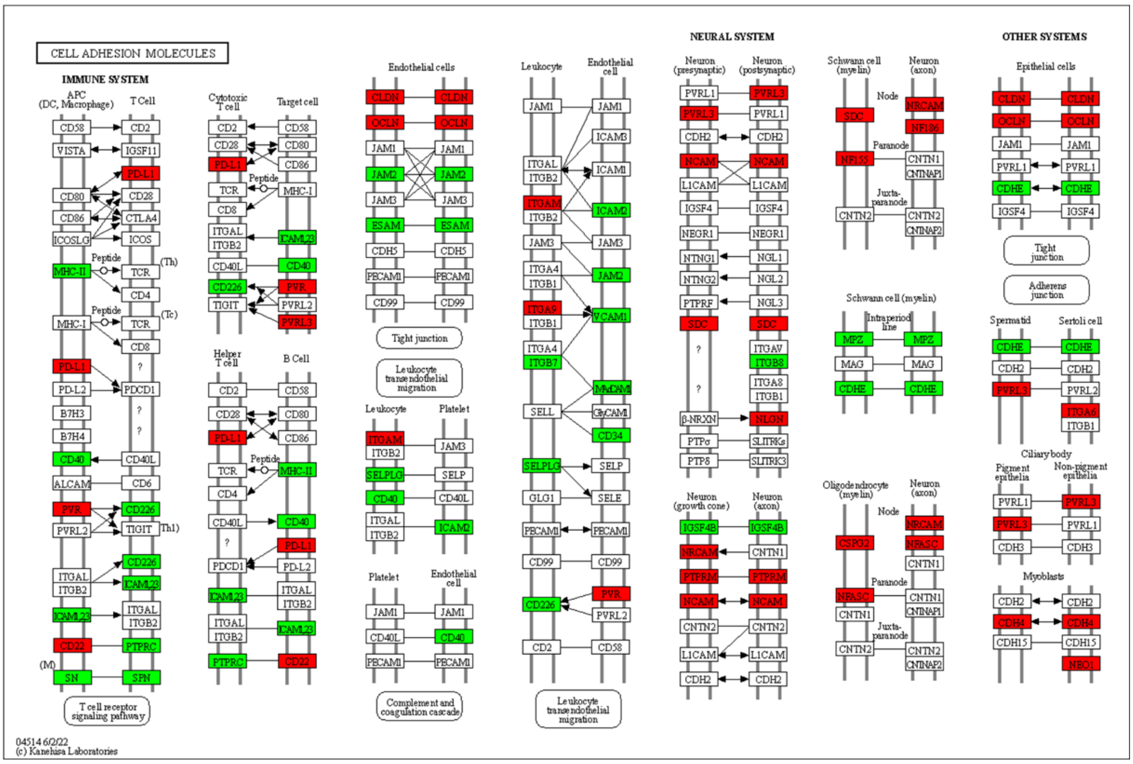


Fig. S4. The KEGG pathway map for P53 signaling pathway (A), apoptosis (B), chemokine signaling pathway (C), ECM-receptor interaction (D), and cell adhesion molecules (E) are presented. The maps were visualized by KEGG mapper color tool. The genes significantly upregulated or downregulated by Fr-treatment were indicates by red or green background, respectively.

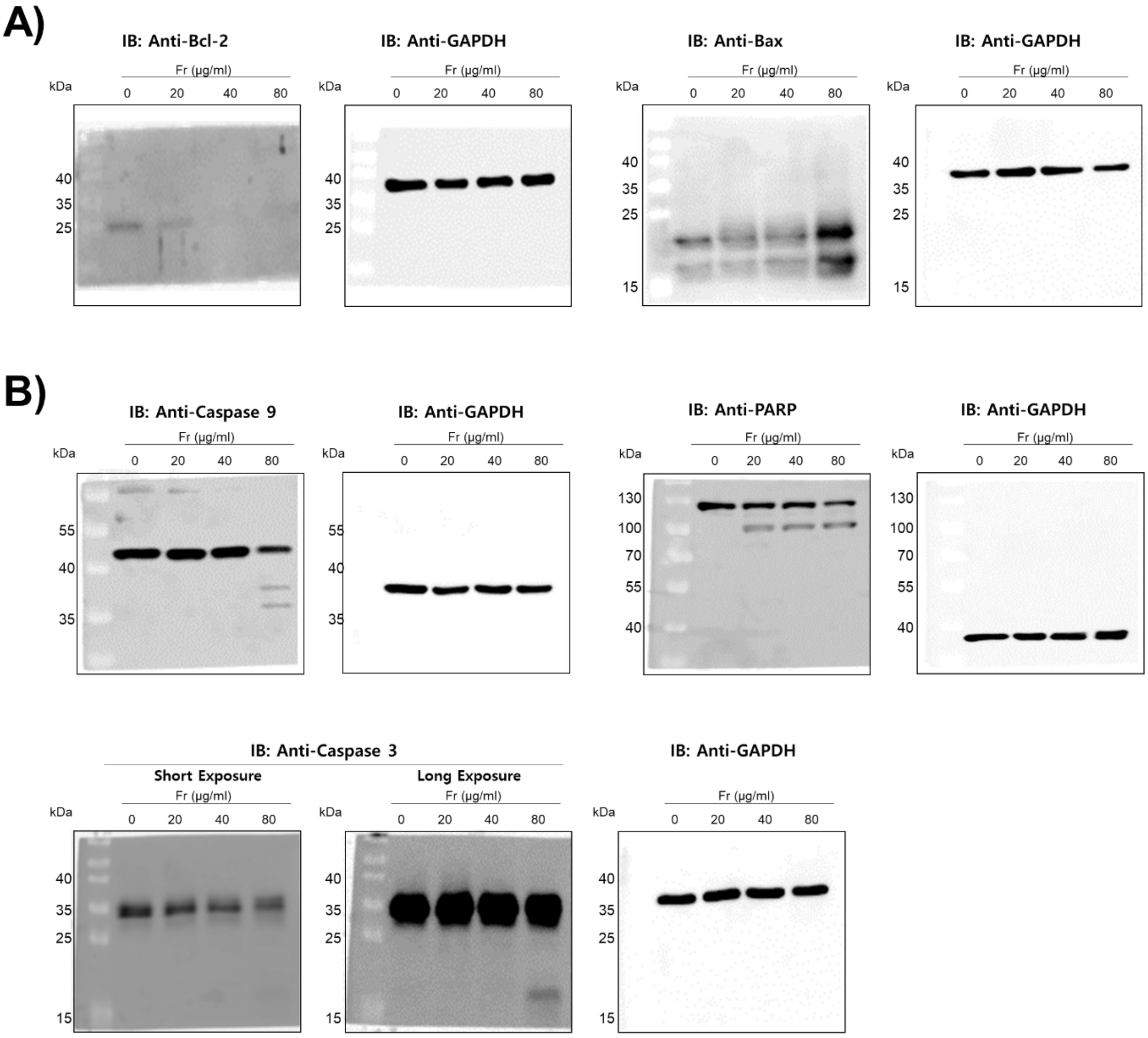


Fig. S4. The whole blot used in this study. (A), Figure 5C; (B), Figure 5D.

Reference

1. Kanehisa, M., Y. Sato, and M. Kawashima, *KEGG mapping tools for uncovering hidden features in biological data.* Protein Sci, 2022. **31**(1): p. 47-53.