表1. PCR Arrayにスポットされた84遺伝子

AMH	BDNF	BMP1	BMP10	BMP2	BMP3	BMP4	BMP5	BMP6	BMP7	BMP8B	CECR1
CLC	CSF1	CSF2	CSF3	CSPG5	CXCL1	DKK1	ERAP1	EREG	FGF1	FGF11	FGF13
FGF14	FGF17	FGF19	FGF2	FGF22	FGF23	FGF5	FGF6	FGF7	FGF9	FIGF	GDF10
GDF11	GDNF	GPI	HBEGF	IGF1	IGF2	IL10	IL11	IL12B	IL18	IL1A	IL1B
IL2	IL3	IL4	INHA	INHBA	INHBB	JAG1	JAG2	LEFTY1	LEFTY2	LIF	LTBP4
MDK	MSTN	NDP	NGF	NODAL	NRG1	NRG2	NRG3	NRTN	NTF3	OSGIN1	PDGFC
PGF	PSPN	PTN	SLCO1A2	SPP1	TDGF1	TGFB1	THPO	TNNT1	TYMP	VEGFA	VEGFC
ACTB	B2M	GAPDH	HPRT1	RPLP0	HGDC	RTC	RTC	RTC	PPC	PPC	PPC

AMH：anti-Mullerian hormone，BDNF：brain derived neurotrophic factor
BMP：bone morphogenetic protein
CECR：cat eye syndrome chromosome region candidate
CLC：Charcot-Leyden crystal galectin
CSF：colony stimulating factor，CSPG5：chondroitin sulfate proteoglycan
CXCL：C-X-C motif chemokine ligand，DKK：dickkopf WNT signaling pathway inhibitor
ERAP：endoplasmic reticulum aminopeptidase，EREG：epiregulin
FGF：fibroblast growth factor，FIGF（VEGFD）：vascular endothelial growth factor D
GDF：growth differentiation factor，GDNF：glial cell derived neurotrophic factor
GPI：glucose-6-phosphate isomerase，HBEGF：heparin binding EGF like growth factor
IGF：insulin like growth factor，IL：interleukin，INH：inhibin，JAG：jagged
LEFTY：left-right determination factor，LIF：leukemia inhibitory factor
LTBP：latent transforming growth factor beta binding protein，MDK：midkine，MSTN：myostatin
NDP：NDP norrin cystine knot growth factor，NGF：nerve growth factor
NODAL：nodal growth differentiation factor，NRG：neuregulin，NRTN：neurturin
NTF3：neurotrophin，OSGIN：oxidative stress induced growth inhibitor
PDGF：platelet derived growth factor，PGF：placental growth factor，PSPN：persephin
PTN：pleiotrophin，SLCO：solute carrier organic anion transporter family member
SPP：secreted phosphoprotein，TDGF：teratocarcinoma-derived growth factor
TGFB：transforming growth factor beta，THPO：thrombopoietin
TNNT：troponin T slow skeletal type，TYMP：thymidine phosphorylase
VEGF：vascular endothelial growth factor，ACTB：beta actin，B2M：beta 2 microgrobulin
GAPDH：glyceraldehyde 3 phosphate dehydrogenase
HPRT1：Hypoxanthine-guanine phosphoribosyltransferase，RPLP0：ribosomal protein large P0
HGDC：Human Genomic DNA Contamination，RTC：Reverse Transcription Control
PPC：Positive PCR Control

表2. IL-6関連遺伝子発現に(+)-terreinが及ぼす影響

Conditions				(-) vs rIL-6	(-) vs (+)-terrein	rIL-6 vs rIL-6 + (+)-terrein
#	Description	Refseq	Symbol	Fold Regulation	Fold Regulation	Fold Regulation
1	Brain-derived neurotrophic factor	NM_001709	BDNF	9.1343	-3.0509	-7.7316
2	Bone morphogenetic protein 1	NM_006129	BMP1	7.9222	-1.2963	-8.8046
3	Bone morphogenetic protein 4	NM_130851	BMP4	4.3678	-1.2073	-5.433
4	Colony stimulating factor 1 (macrophage)	NM_000757	CSF1	6.6821	-1.2033	-11.0331
5	Dickkopf homolog 1 (Xenopus laevis)	NM_012242	DKK1	8.5309	-1.7486	-4.5982
6	Endoplasmic reticulum aminopeptidase 1	NM_016442	ERAP1	7.5491	-1.1127	-5.8798
7	Fibroblast growth factor 1 (acidic)	NM_000800	FGF1	4.8878	-3.2819	-4.6511
8	Fibroblast growth factor 5	NM_004464	FGF5	2.6533	1.063	-2.3793
9	Fibroblast growth factor 7	NM_002009	FGF7	2.5121	1.2977	-2.4602
10	Growth differentiation factor 11	NM_005811	GDF11	-1.0837	3.4593	-2.4271
11	Glial cell derived neurotrophic factor	NM_000514	GDNF	3.1468	1.9254	-5.2903
12	Heparin-binding EGF-like growth factor	NM_001945	HBEGF	3.1052	-1.7615	-1.1679
13	Insulin-like growth factor 2	NM_000612	IGF2	2.2732	-1.3274	-1.0172
14	Inhibin, beta A	NM_002192	INHBA	1.9203	-1.0286	-1.1618
15	Jagged 1	NM_000214	JAG1	2.0782	-1.6035	1.3033
16	Leukemia inhibitory factor (cholinergic differentiation factor)	NM_002309	LIF	3.7203	-1.0707	-3.0476
17	Nerve growth factor (beta polypeptide)	NM_002506	NGF	-1.0128	1.0489	1.2568
18	Neuregulin 1	NM_013957	NRG1	1.6703	1.2052	-1.9125
19	Neurotrophin 3	NM_002527	NTF3	2.7074	1.166	-2.5689
20	Oxidative stress induced growth inhibitor 1	NM_182981	OSGIN1	4.8848	6.2341	-32.6398
21	Pleiotrophin	NM_002825	PTN	-1.5881	3.5337	-2.4369
22	Transforming growth factor, beta 1	NM_000660	TGFB1	2.6625	1.7452	-6.0606
23	Thymidine phosphorylase	NM_001953	TYMP	2.1167	10.65	-17.701
24	Vascular endothelial growth factor A	NM_003376	VEGFA	15.4325	-2.7824	-7.894
25	Beta-2-microglobulin	NM_004048	B2M	-1.8898	1.7729	-3.4185
26	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	GAPDH	3.0479	5.5635	-22.5689

HGFs（5.0×10^4 cells/cm^2 ）を(+)-terrein（10 μM）で30分間前処理した後に，rIL-6/rsIL-6R（各50 ng/ml）を添加し，12時間培養後に回収したmRNAから逆転写反応で得られたcDNA中の遺伝子発現量をPCR Arrayにて調べた。mRNA発現量はGAPDHのmRNA量を内部対照とした相対発現量として示した。RT^2 Profiler PCR Array Data Analysis（http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php）にて解析を行い，その精度が高いと判断された遺伝子の相対発現量を示した。

表3. リアルタイムPCR法における標的遺伝子とプライマー塩基配列，および増幅産物長

標的遺伝子	プライマーの塩基配列	増幅産物長 (bp)
VEGF-A	5'-AGGGCAGAATCATCACGAAGT-3' (forward)	75
	5'-AGGGTCTCGATTGGATGGCA-3' (reverse)	
CSF1	5'-AGACCTCGTTGCCAAATTACATT-3' (forward)	249
	5'-AGGTGTCTCATAGAAAGTTCGGA-3' (reverse)	
BDNF	5'-AATCAGTTGCGCGTTCTGAA-3' (forward)	185
	5'-TAGCCATGATTTACCCAAATG-3' (reverse)	
DKK1	5'-ATAGCACCTTGGATGGGTATTCC-3' (forward)	96
	5'-CTGATGACCGGAGACAAACAG-3' (reverse)	
BMP1	5'-GGGGTGAAACCTCCCATTGG-3' (forward)	170
	5'-CACACGCAGTGCATGTGAG-3' (reverse)	
ERAP1	5'-GGCAATCTTTCGGAGACTTTC-3' (forward)	141
	5'-GAAGGCAGGTTCATCAAAGC-3' (reverse)	
GAPDH	5'-TGGCAAATTCCATGGCA-3' (forward)	164
	5'-CCTTCTCCATGGTGGT-3' (reverse)	

標的遺伝子とプライマー塩基配列，および増幅産物長を表に示す

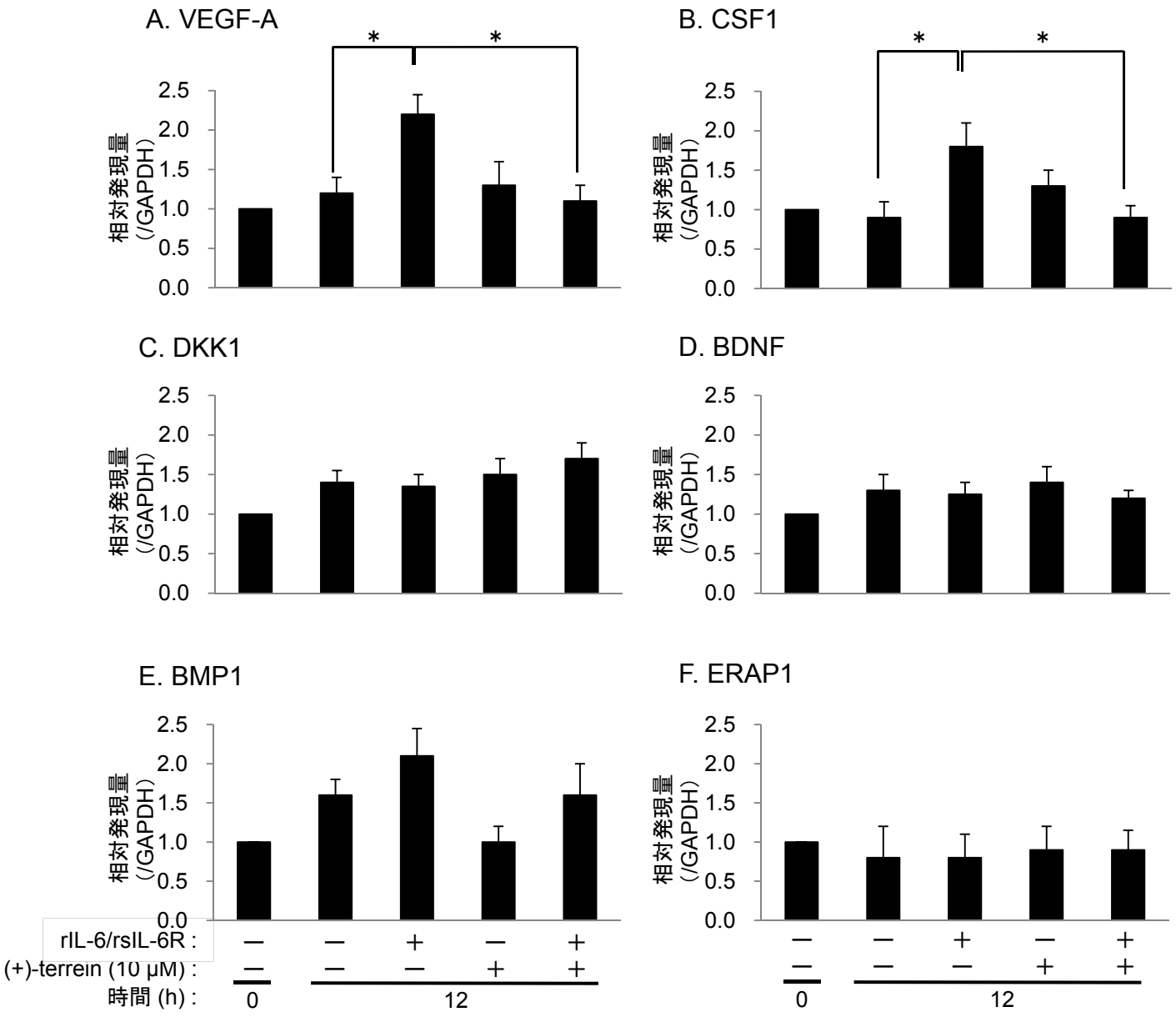
A. VEGF-A
B. CSF1
C. DKK1
D. BDNF
E. BMP1
F. ERAP1
相対発現量
(/GAPDH)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
rIL-6/rsIL-6R :
(+)-terrein (10 μM) :
時間 (h) :
0
12
−
+

図1.

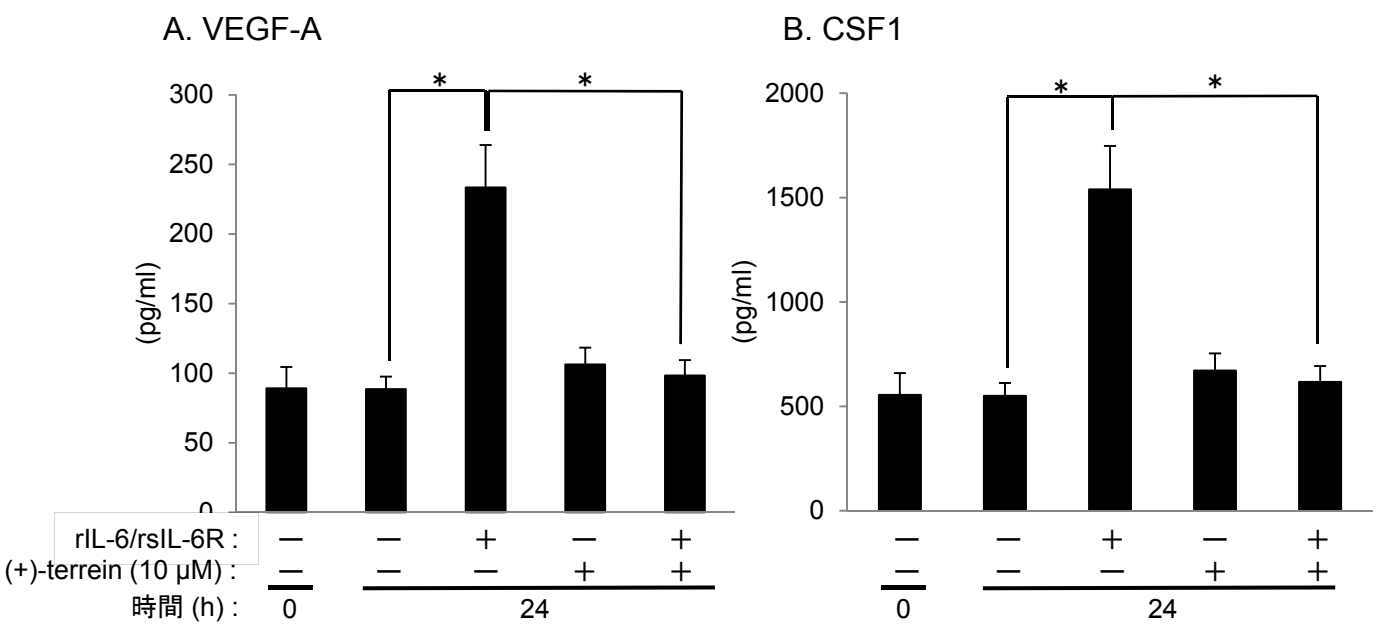
A. VEGF-A
B. CSF1
(pg/ml)
300
250
200
150
100
50
0
2000
1500
1000
500
*
*
rIL-6/rsIL-6R :
(+)-terrein (10 μM) :
時間 (h) :
0
24
−
+

図2.

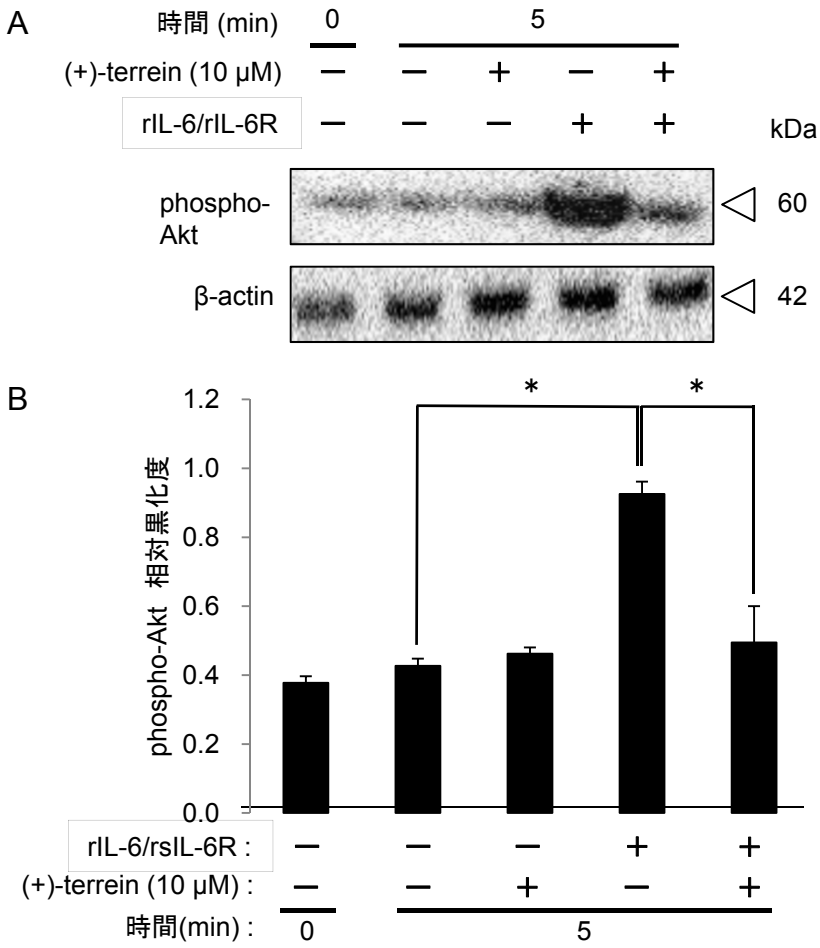
A
時間 (min)
0
5
(+)-terrein (10 μM)
rIL-6/rIL-6R
kDa
phospho-Akt
60
β-actin
42
B
phospho-Akt 相対黒化度
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
rIL-6/rsIL-6R :
(+)-terrein (10 μM) :
時間(min) :
0
5

図3.

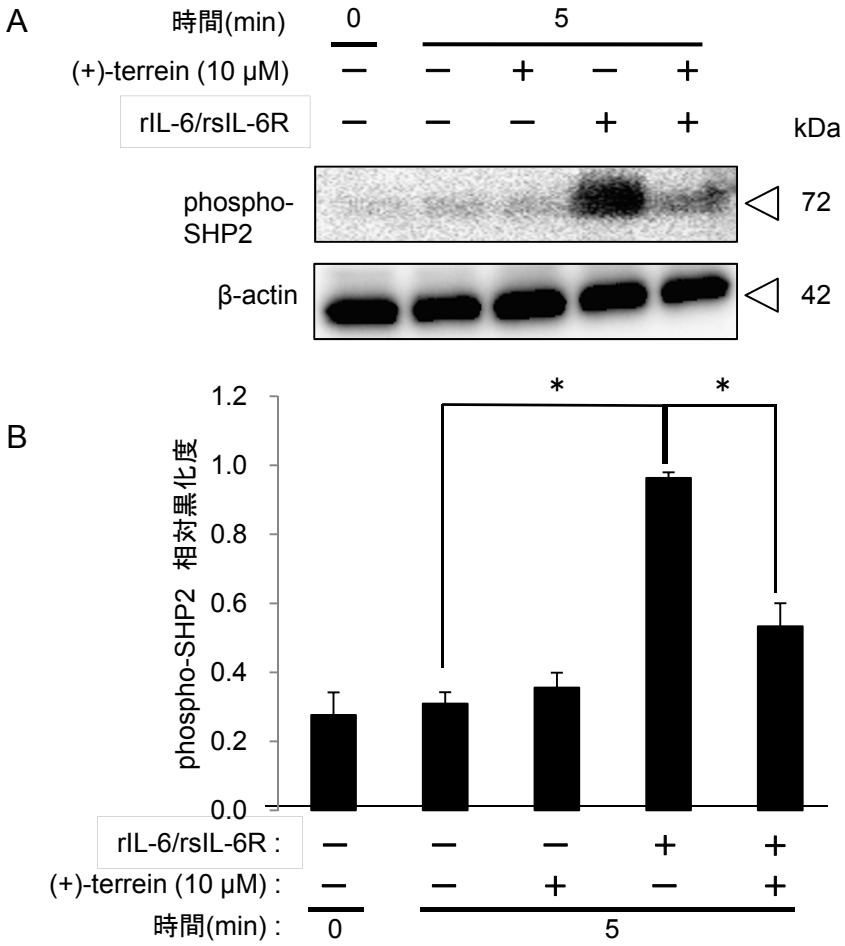
A
時間(min)
0
5
(+)-terrein (10 μM)
rIL-6/rsIL-6R
kDa
phospho-SHP2
72
β-actin
42
B
phospho-SHP2 相対黒化度
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
rIL-6/rsIL-6R :
(+)-terrein (10 μM) :
時間(min) :
0
5

図4.

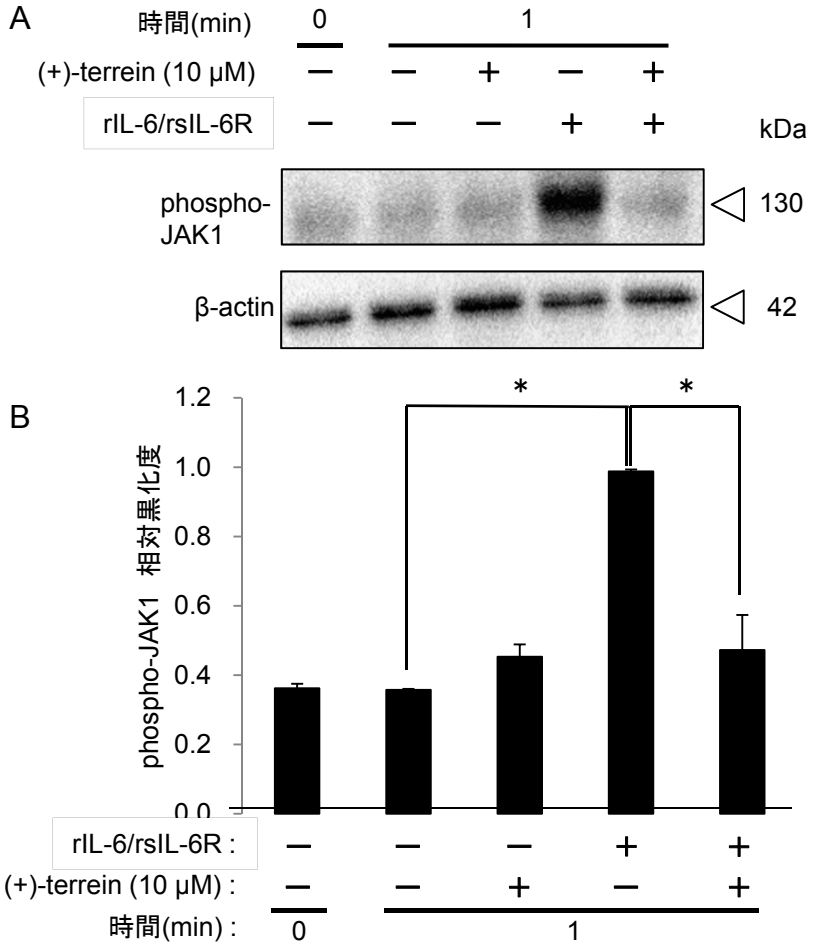
A
時間(min)
0
1
(+)-terrein (10 μM)
rIL-6/rsIL-6R
kDa
phospho-JAK1
130
β-actin
42
B
phospho-JAK1 相対黒化度
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
rIL-6/rsIL-6R :
(+)-terrein (10 μM) :
時間(min) :
0
1

図5.

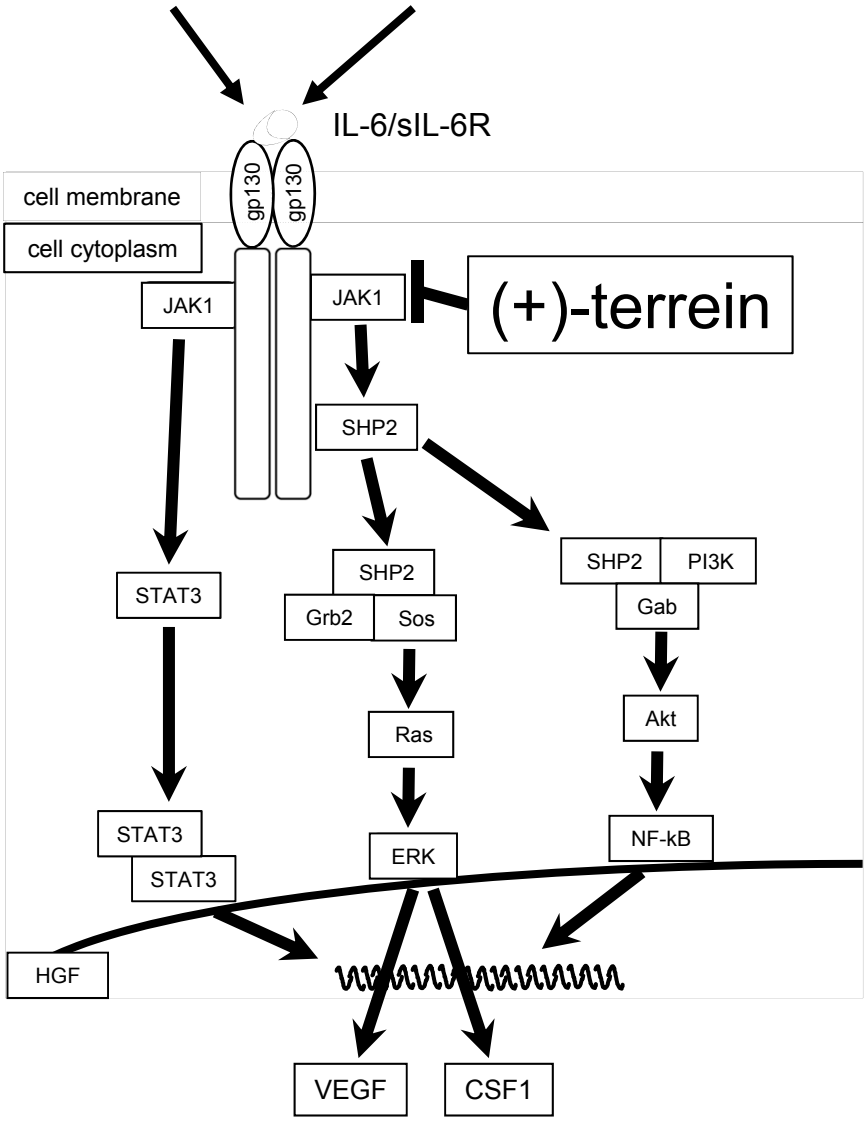
sIL-6R
IL-6
IL-6/sIL-6R
gp130
gp130
cell membrane
cell cytoplasm
JAK1
JAK1
(+)-terrein
SHP2
STAT3
SHP2
Grb2
Sos
SHP2
PI3K
Gab
Ras
Akt
STAT3
STAT3
ERK
NF-kB
HGF
VEGF
CSF1

図6.