PLGA/BK Microspheres as a Therapeutic Strategy for Delaying Intervertebral Disc Degeneration

Xiaoming Qiu[#1,2,3], Yizhi Zhang[#1,3], Ziyan Wei[#1,3], Zhangbin Luo[1,3], Zhuanping Wang[1,3,4], Xuewen Kang*[1,3]

1. Lanzhou University Second Hospital, Lanzhou, Gansu 730030, PR China

2. The First Affiliated Hospital of Gansu University of Traditional Chinese Medicine, Gansu provincial hospital of TCM, Lanzhou, Gansu 730030, PR China

3. Orthopaedics Key Laboratory of Gansu Province, Lanzhou, Gansu 730030, PR China

4. Department of Endocrinology, Lanzhou University Second Hospital, Lanzhou, Gansu 730030, PR China

* Corresponding author.

E-mail addresses: ery_kangxw@lzu.edu.cn (Xuewen Kang)

[#] Xiaoming Qiu, [#]Yizhi Zhang and [#]Ziyan Wei contributed equally to this work.

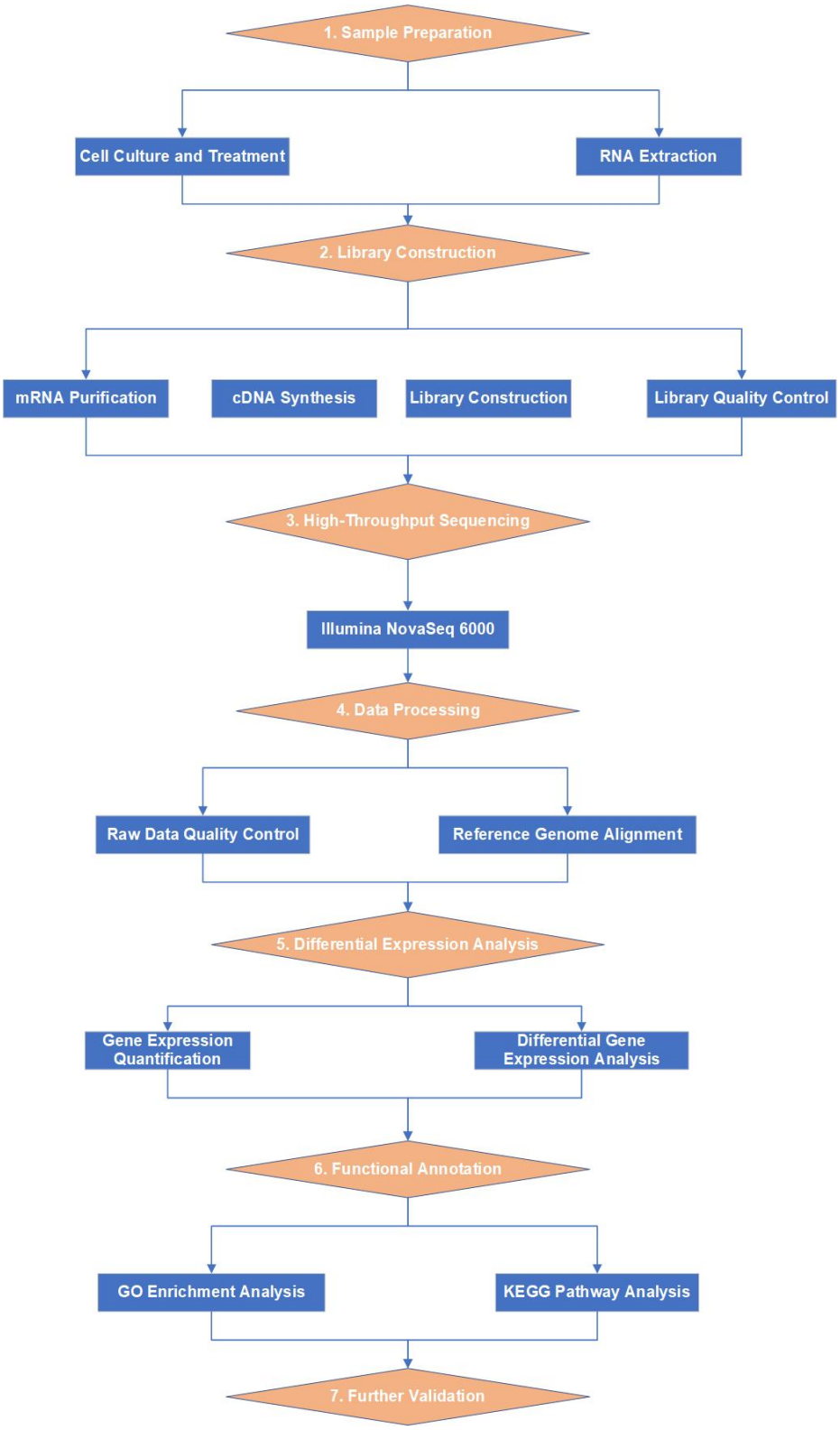

Figure S1: Bulk RNA-seq Workflow for BK-Intervened NPCs

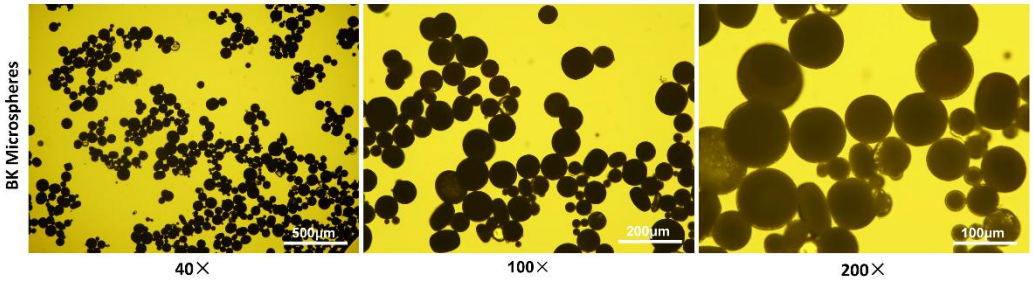

Figure S2: Microsphere morphology under optical microscope

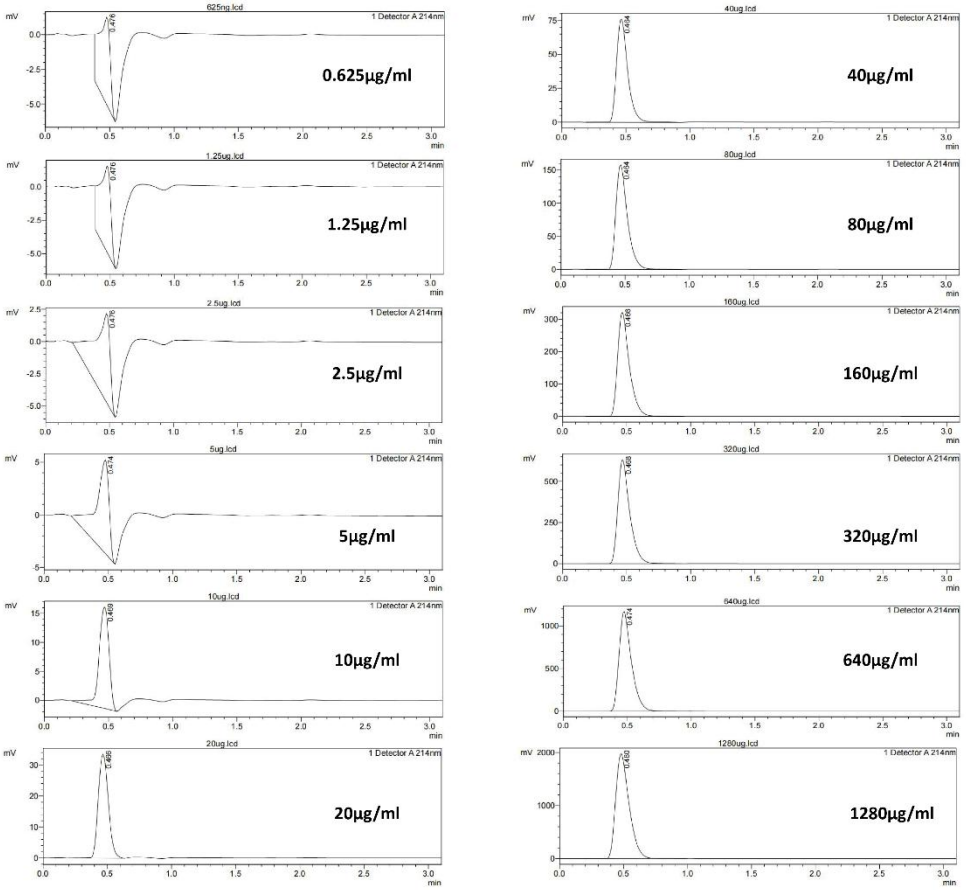

Figure S3: HPLC analysis at the wavelength of 214 nm

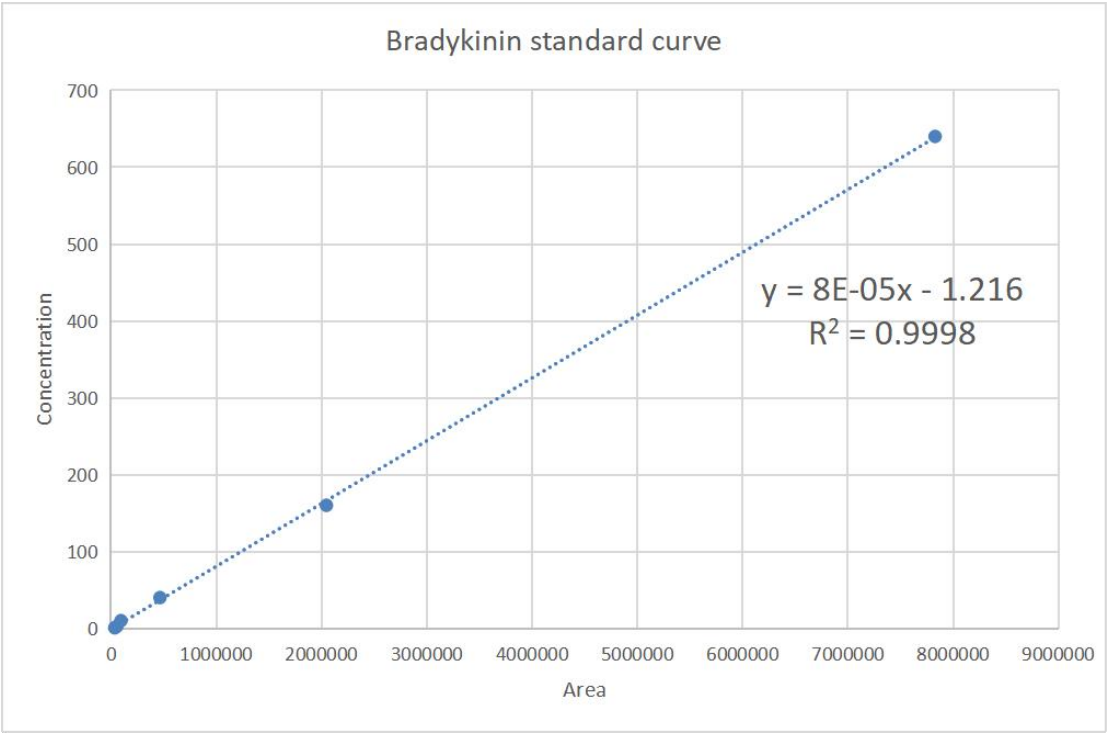

Figure S4: Standard curve of BK

FIG 1

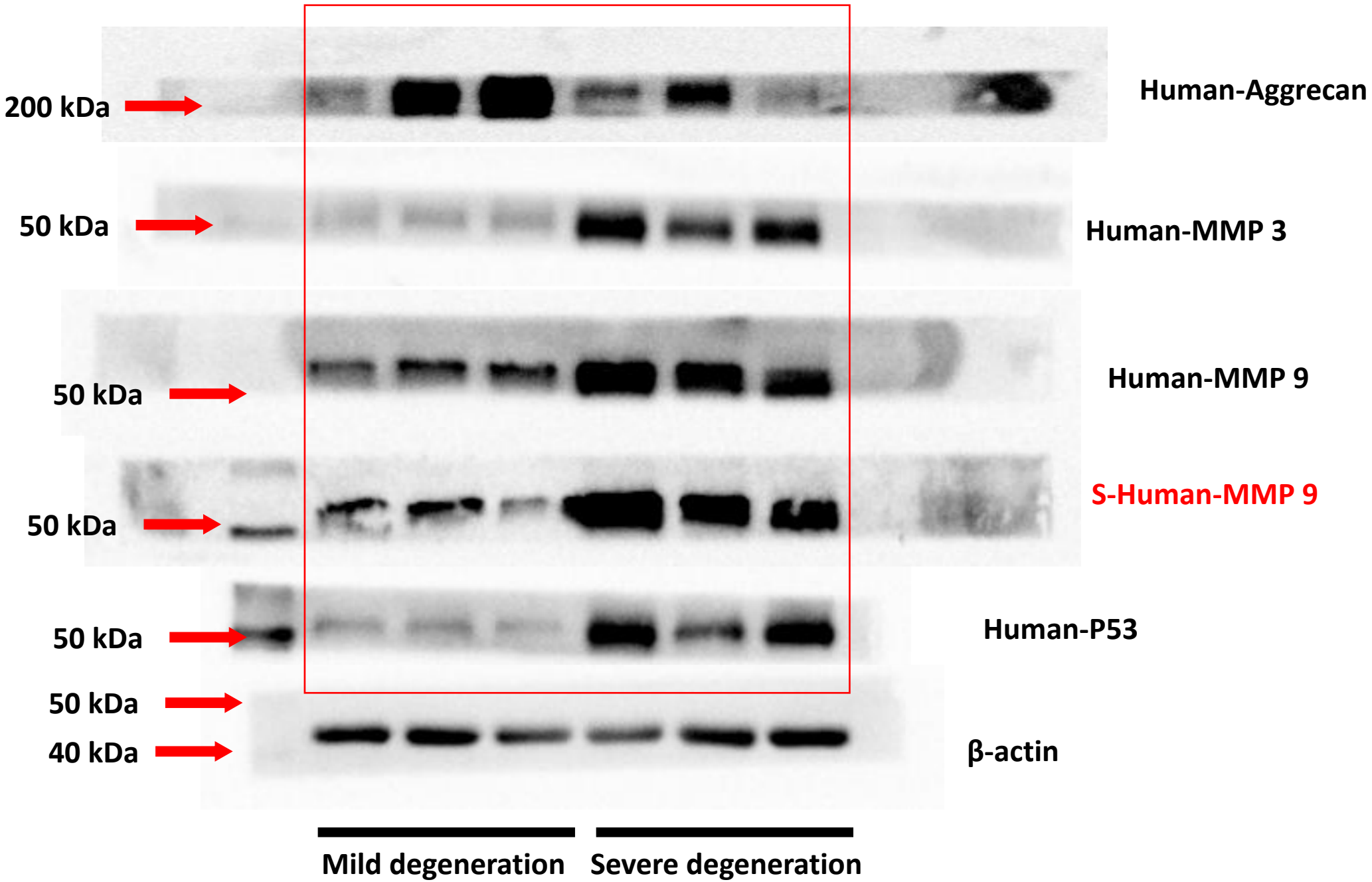
200 kDa
Human-Aggrecan
50 kDa
Human-MMP 3
50 kDa
Human-MMP 9
50 kDa
S-Human-MMP 9
50 kDa
Human-P53
50 kDa
40 kDa
β-actin
Mild degeneration
Severe degeneration

FIG 3C

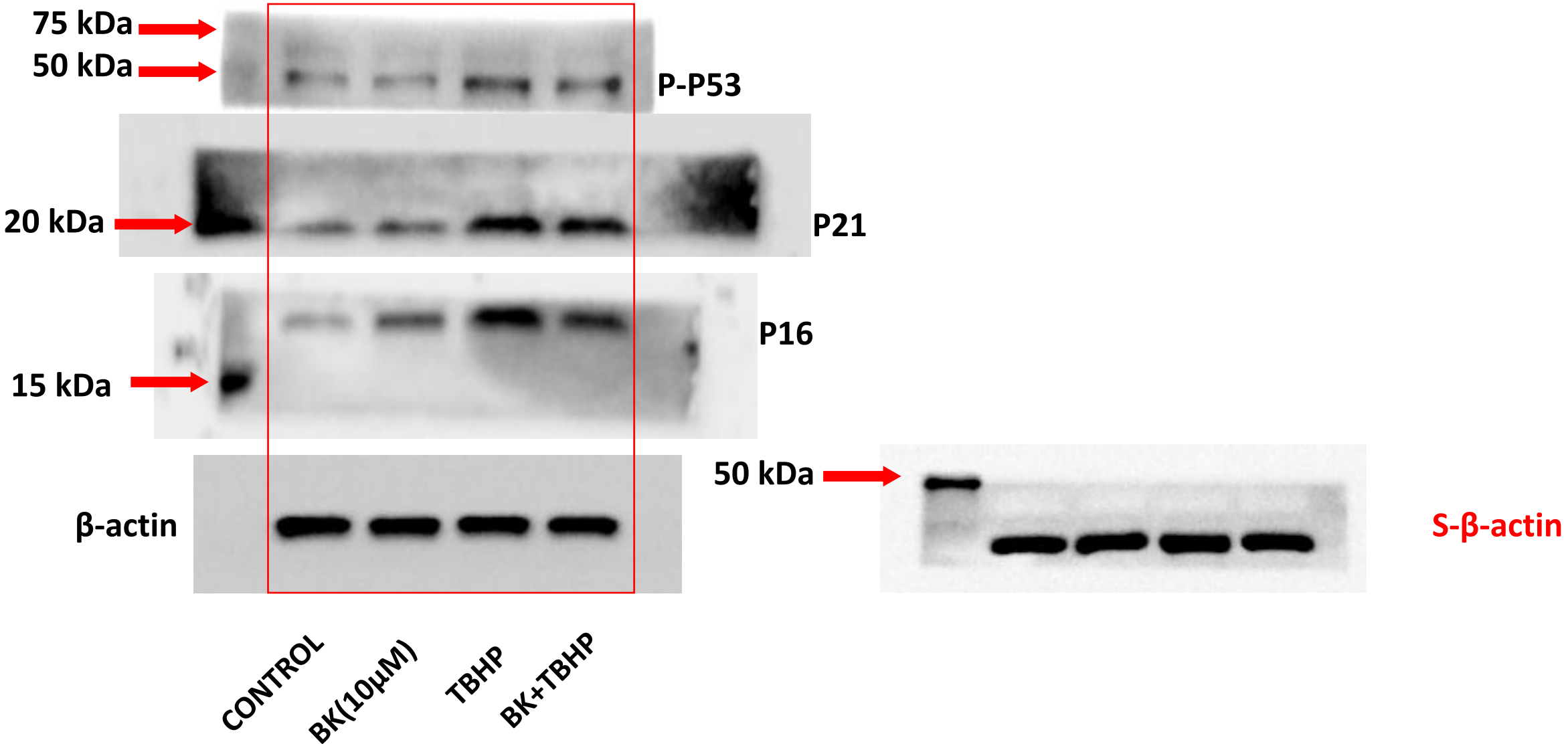
75 kDa
50 kDa
P-P53
20 kDa
P21
P16
15 kDa
β-actin
50 kDa
S-β-actin
CONTROL
BK(10μM)
TBHP
BK+TBHP

FIG 3I

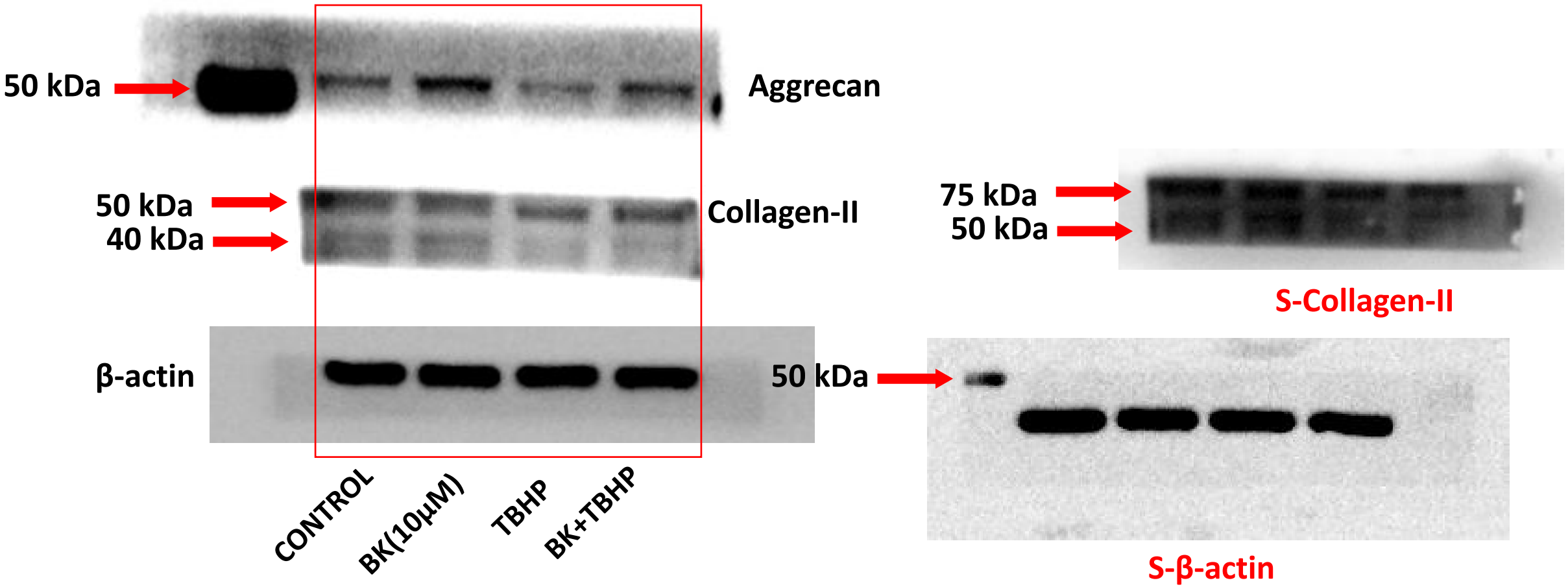
50 kDa
Aggrecan
50 kDa
40 kDa
Collagen-II
β-actin
50 kDa
CONTROL
BK(10μM)
TBHP
BK+TBHP
75 kDa
50 kDa
S-Collagen-II
S-β-actin

FIG 3N

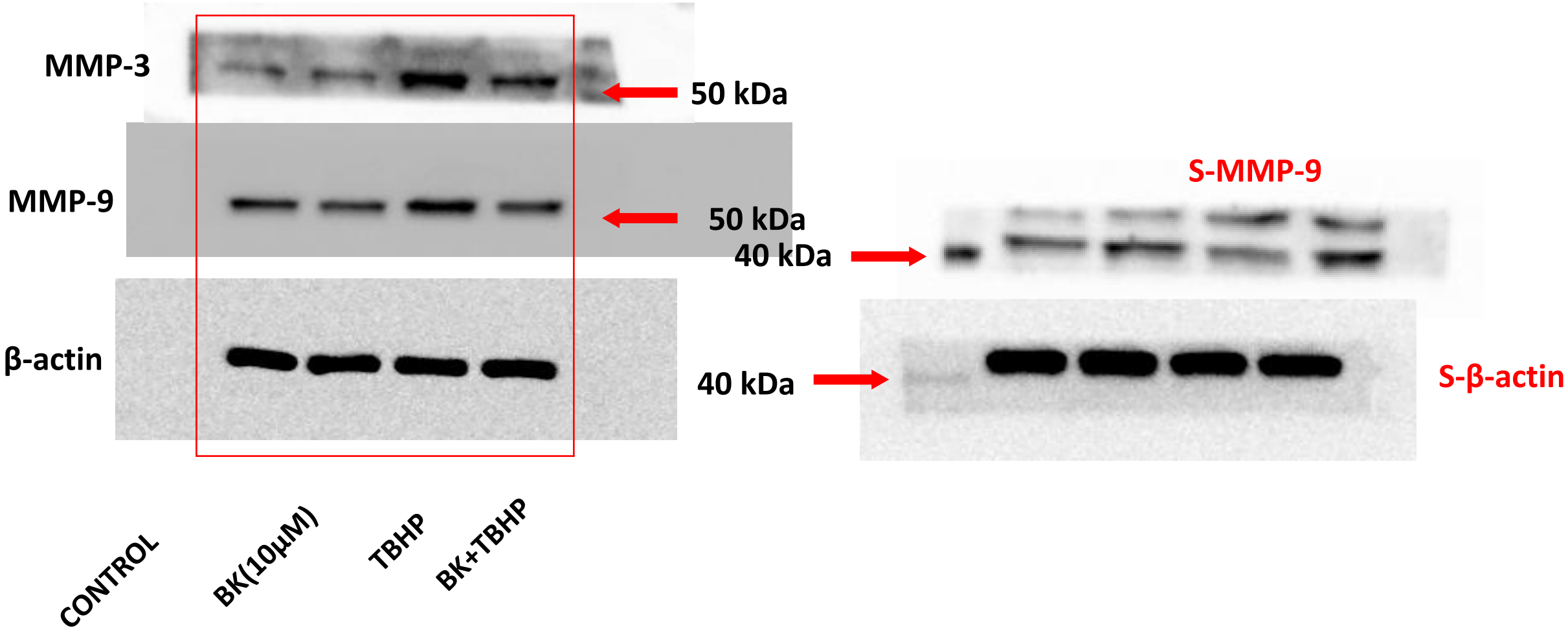
MMP-3
50 kDa
MMP-9
50 kDa
40 kDa
S-MMP-9
β-actin
40 kDa
S-β-actin
CONTROL
BK(10μM)
TBHP
BK+TBHP

FIG 3Q

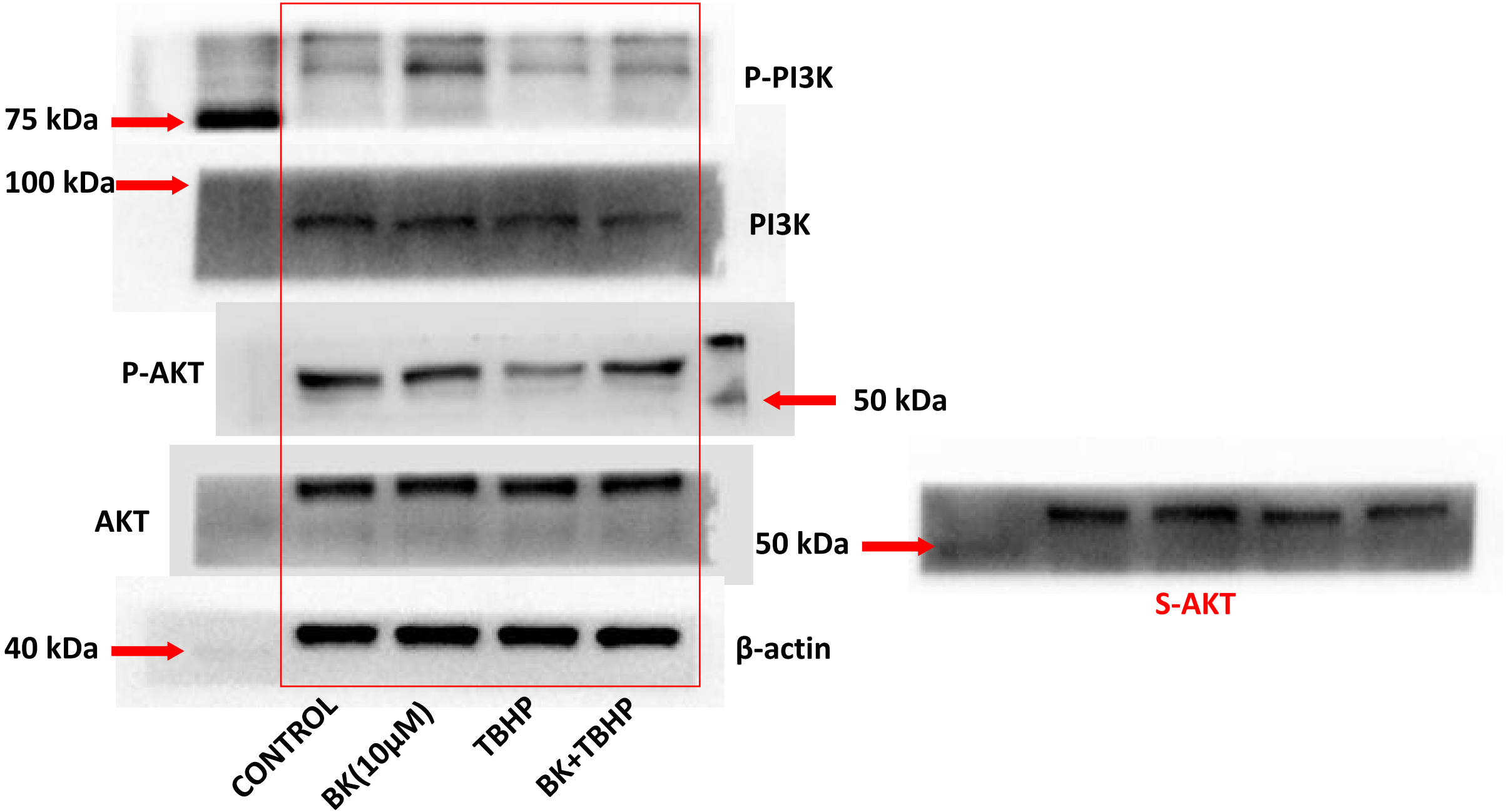
P-PI3K
75 kDa
100 kDa
PI3K
P-AKT
50 kDa
AKT
50 kDa
S-AKT
40 kDa
β-actin
CONTROL
BK(10μM)
TBHP
BK+TBHP

FIG 4C

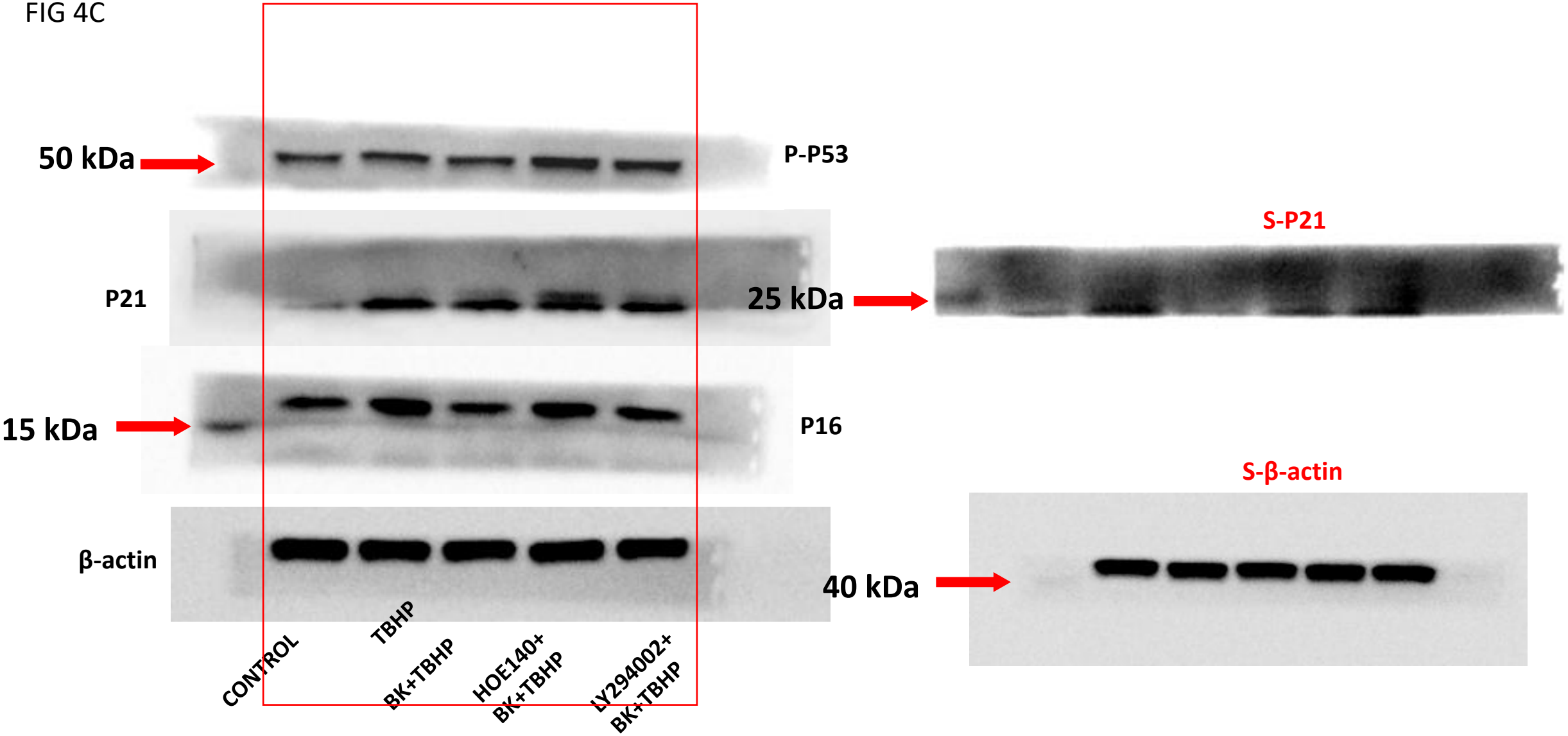
50 kDa
P-P53
P21
25 kDa
S-P21
15 kDa
P16
β-actin
S-β-actin
40 kDa
CONTROL
TBHP
BK+TBHP
HOE140+ BK+TBHP
LY294002+ BK+TBHP

FIG 4I

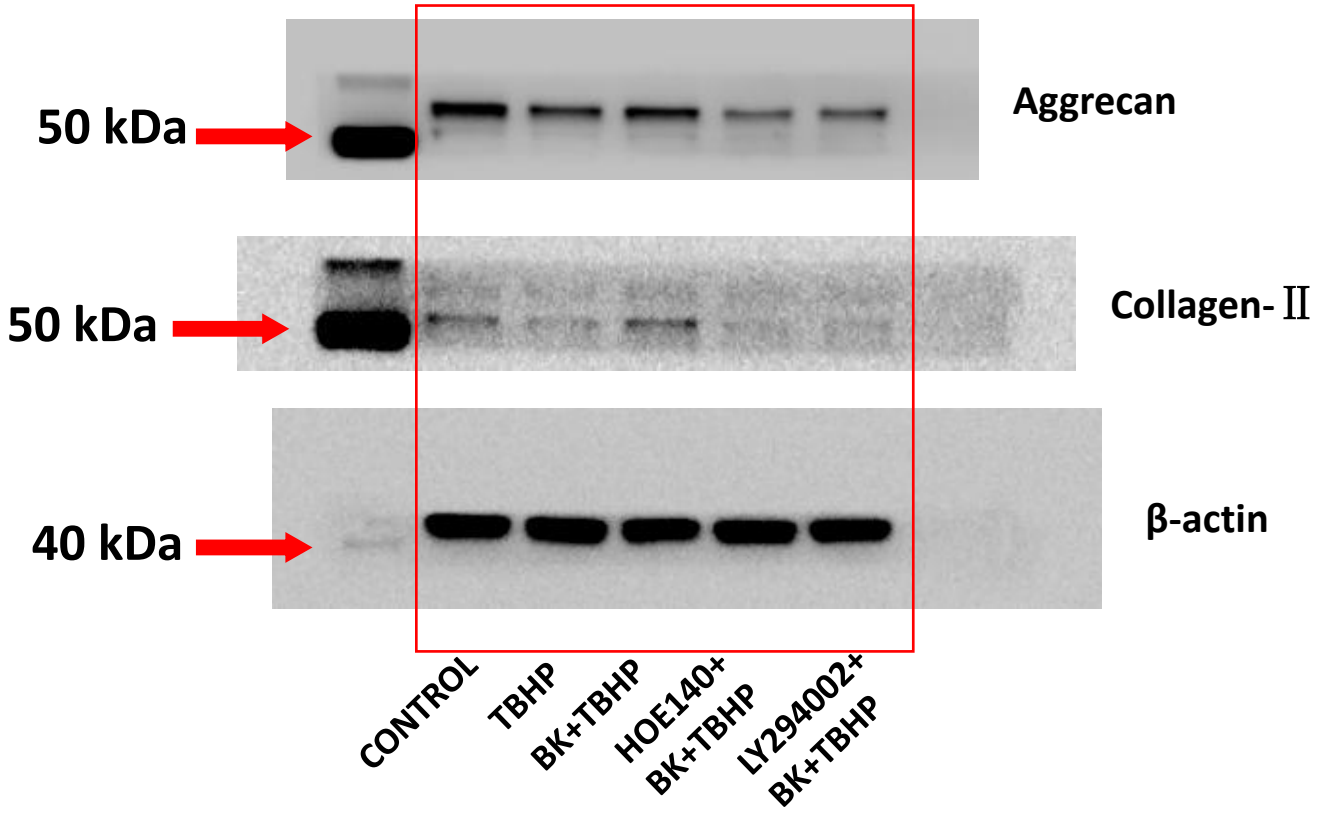
50 kDa
Aggrecan
50 kDa
Collagen- II
40 kDa
β-actin
CONTROL
TBHP
BK+TBHP
HOE140+ BK+TBHP
LY294002+ BK+TBHP

FIG 4N

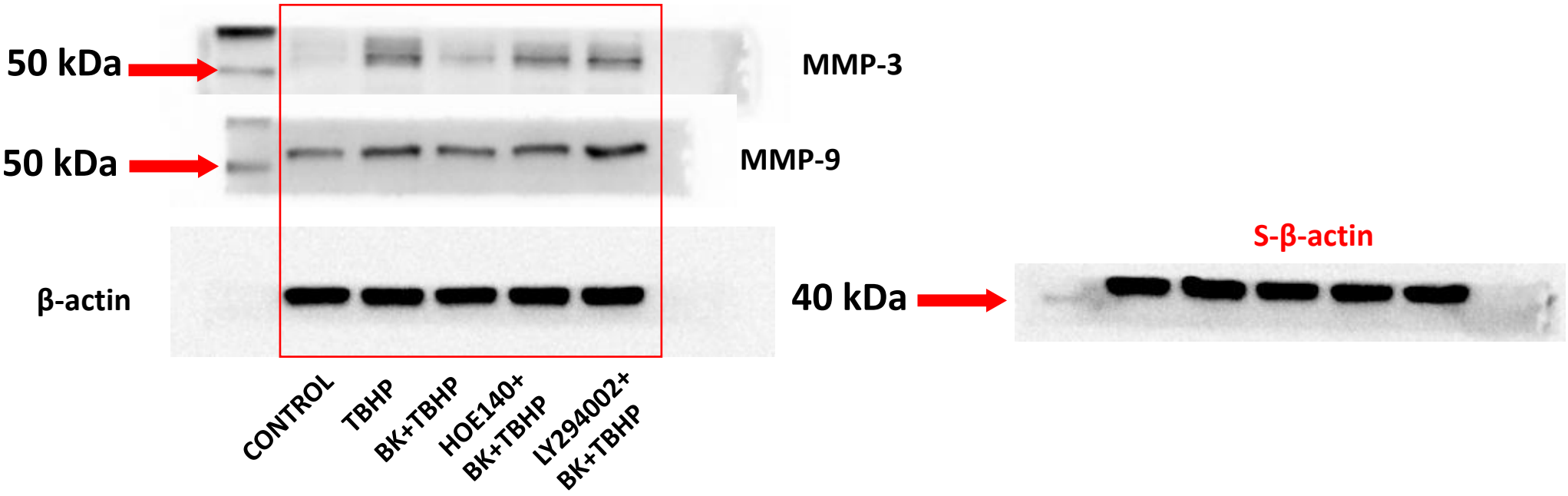
50 kDa
MMP-3
50 kDa
MMP-9
β-actin
40 kDa
S-β-actin
CONTROL
TBHP
BK+TBHP
HOE140+ BK+TBHP
LY294002+ BK+TBHP

FIG 4Q

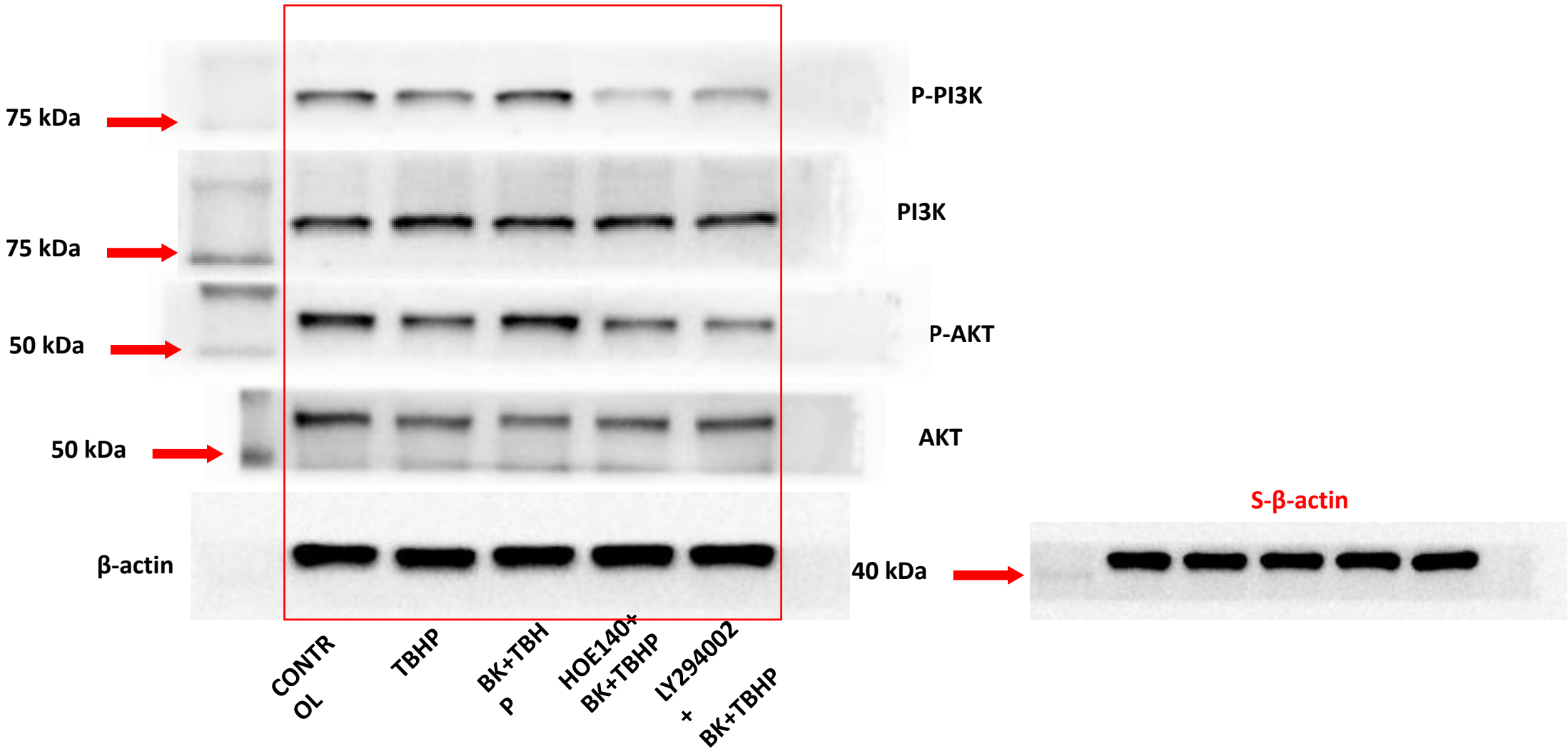
75 kDa
P-PI3K
75 kDa
PI3K
50 kDa
P-AKT
50 kDa
AKT
β-actin
40 kDa
S-β-actin
CONTROL
TBHP
BK+TBHP
HOE140+ BK+TBHP
LY294002 + BK+TBHP

Figure S5: Uncropped and Unedited Western Blot/Gel Images Supporting Quantitative Analyses

Table S1 Orthogonal factor level table

Level	Factor			
	A (mg)	B (mg)	C （%）	D （r/min）
1	200	1	0.5	400
2	100	0.5	1	800
3	50	0.25	1.5	1200

Table S2 Orthogonal experimental design table

Test number	Factor				Result
	A	B	C	D	S
1	1	1	1	1	
2	1	2	2	2	
3	1	3	3	3	
4	2	1	2	3	
5	2	2	3	1	
6	2	3	1	2	
7	3	1	3	2	
8	3	2	1	3	
9	3	3	2	1	

Table S3 Postoperative observation items

	X-ray	MR	Histopathology	IHC
On the day after surgery	√	√	√	
One month after surgery	√	√	√	

Two month after surgery	√	√	√	√

Table S4 Orthogonal table of PLGA/BK microsphere preparation

Number	Factor				Result
	A	B	C	D	S_1/%
1	1	1	1	1	23.97
2	1	2	2	2	14.17
3	1	3	3	3	45.90
4	2	1	2	3	36.74
5	2	2	3	1	62.57
6	2	3	1	2	21.86
7	3	1	3	2	20.89
8	3	2	1	3	5.02
9	3	3	2	1	7.31
K1	28.01	27.003	16.950	31.283	
K2	40.39	27.253	19.407	18.973	
K3	11.07	25.023	43.120	29.220	
R	29.31	2.230	26.170	12.310	

A) oil phase (mass of PLGA added to dichloromethane, 4.5 ml); B) inner water phase (mass of BK in aqueous solution, 0.5 ml); C) outer aqueous phase (PVA solution concentration, 40 ml); and D) stirring speed.

Table S5 Orthogonal table of PLGA/BK microsphere preparation

Number	Factor				Result
	A	B	C	D	S_2/%
1	1	1	1	1	0.120
2	1	2	2	2	0.035
3	1	3	3	3	0.057
4	2	1	2	3	0.367
5	2	2	3	1	0.313
6	2	3	1	2	0.055
7	3	1	3	2	0.418
8	3	2	1	3	0.050
9	3	3	2	1	0.037
K1	0.071	0.302	0.075	0.157	
K2	0.245	0.133	0.146	0.169	
K3	0.168	0.050	0.263	0.158	
R	0.174	0.252	0.188	0.012	

A) oil phase (mass of PLGA added to dichloromethane, 4.5 ml); B) inner water phase (mass of BK in aqueous solution, 0.5 ml); C) outer aqueous phase (PVA solution concentration, 40 ml); and D) stirring speed.

Table S6 Table of parallel experiments on the effect of different weights of PLGA on microsphere size, drug loading rate, and encapsulation efficiency

A（mg）	B（mg）	C（%）	D（r/min）	Encapsulation efficiency（%）	Drug loading rate（%）	Particle size（μm）
200	0.5	1.5	400	15.43±1.29	0.039±0.003	95.06±1.49
100	0.5	1.5	400	74.10±9.17	0.370±0.046	87.44±0.05
50	0.5	1.5	400	5.08±0.07	0.051±0.001	60.33±0.29

A) oil phase (mass of PLGA added to dichloromethane, 4.5 ml); B) inner water phase (mass of BK in aqueous solution, 0.5 ml); C) outer aqueous phase (PVA solution concentration, 40 ml); and D) stirring speed.