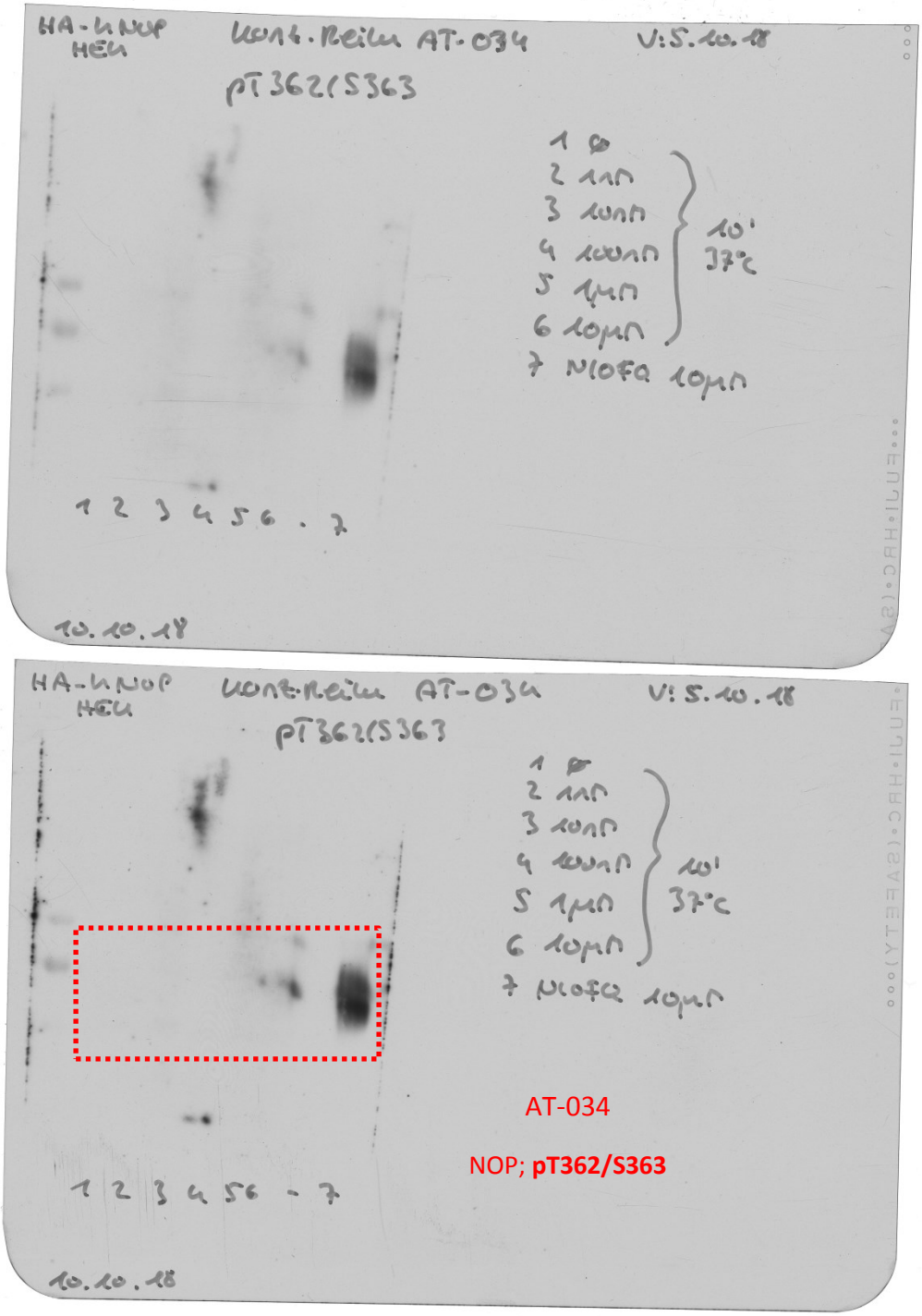
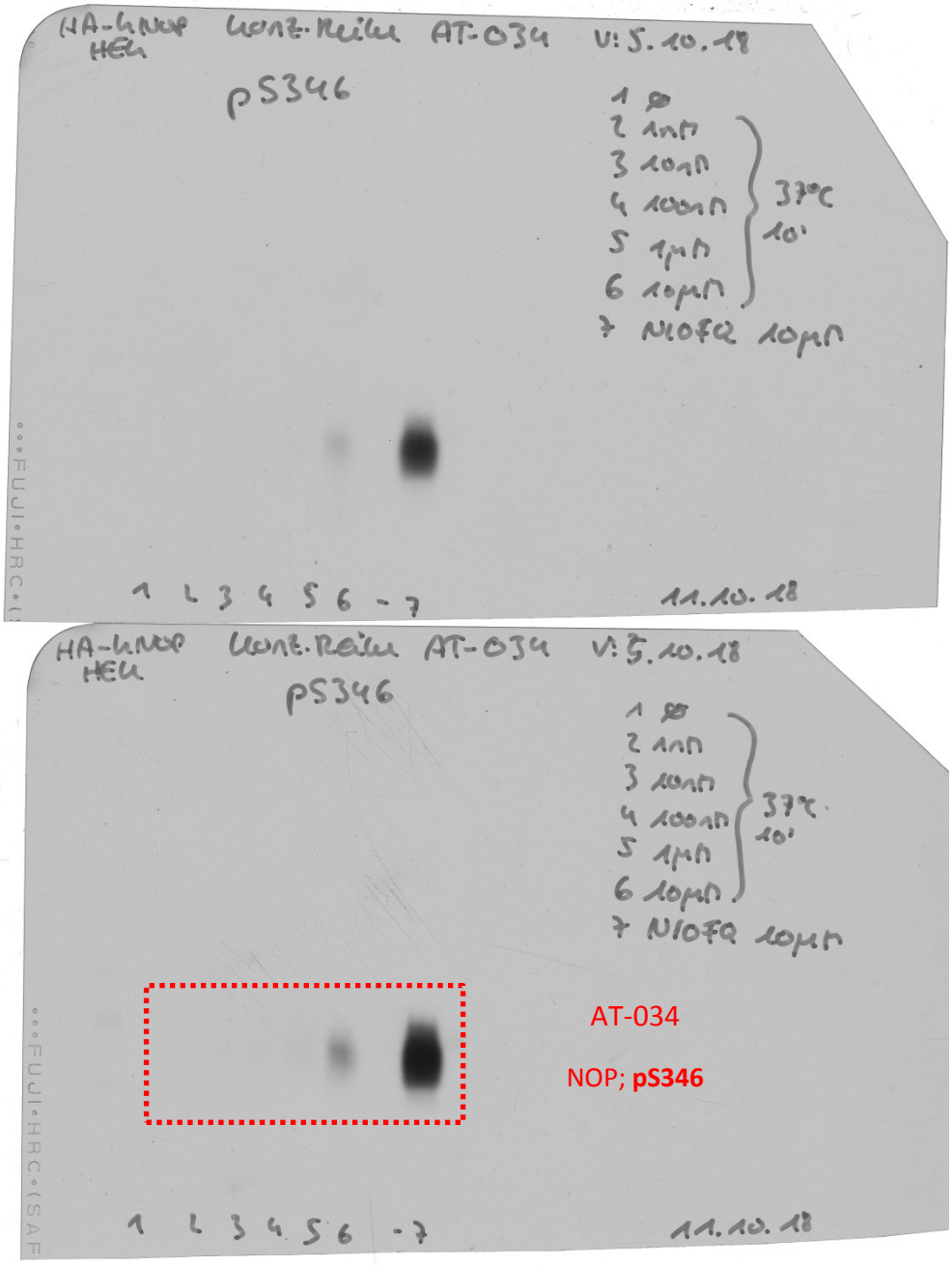
AT-034

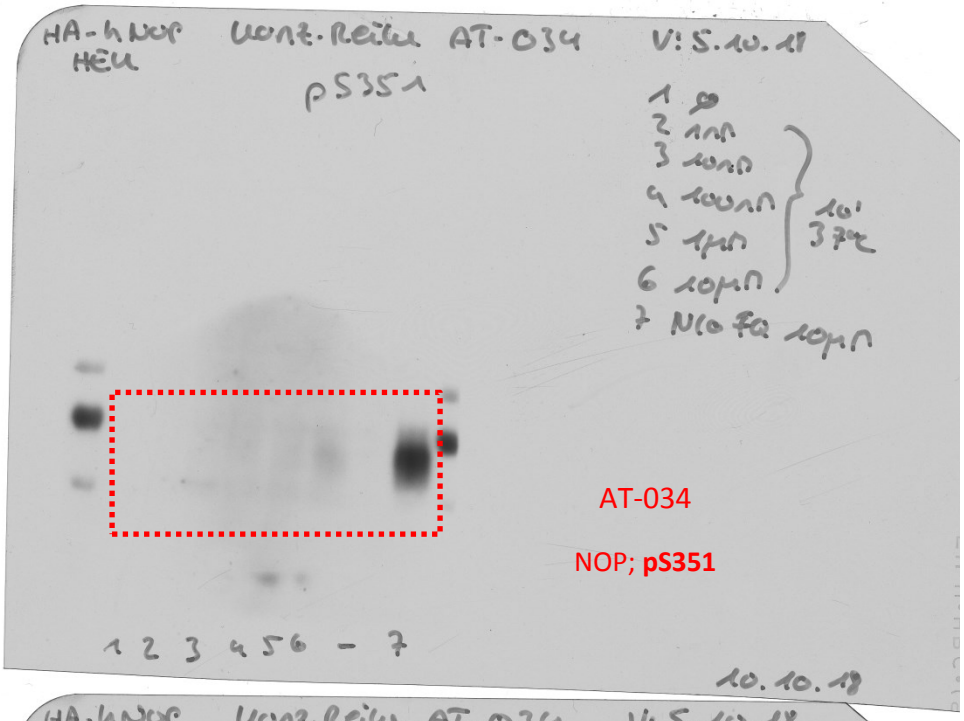
NOP

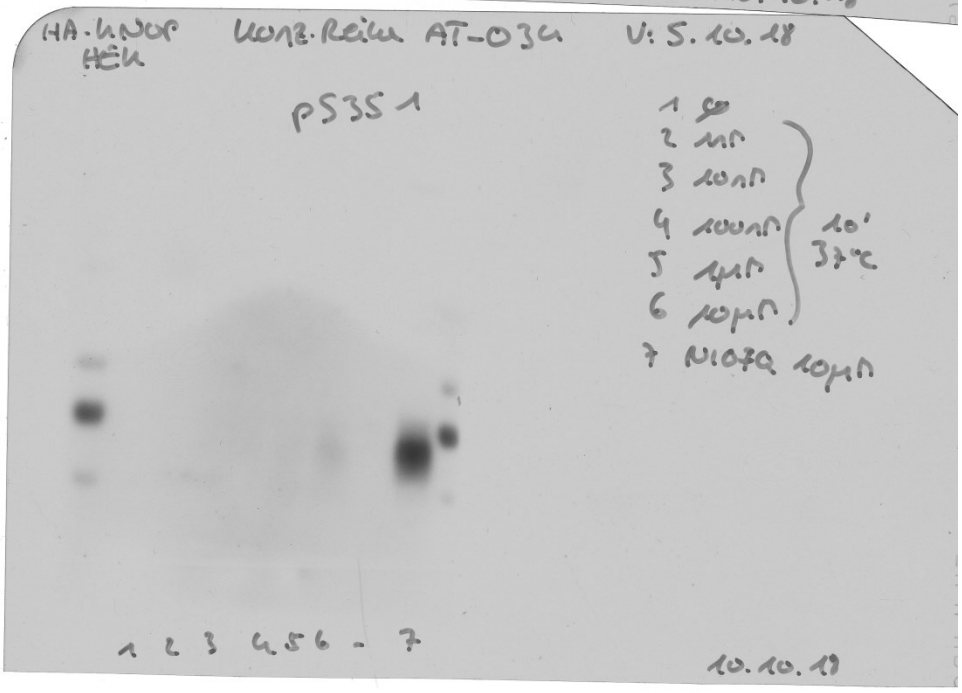


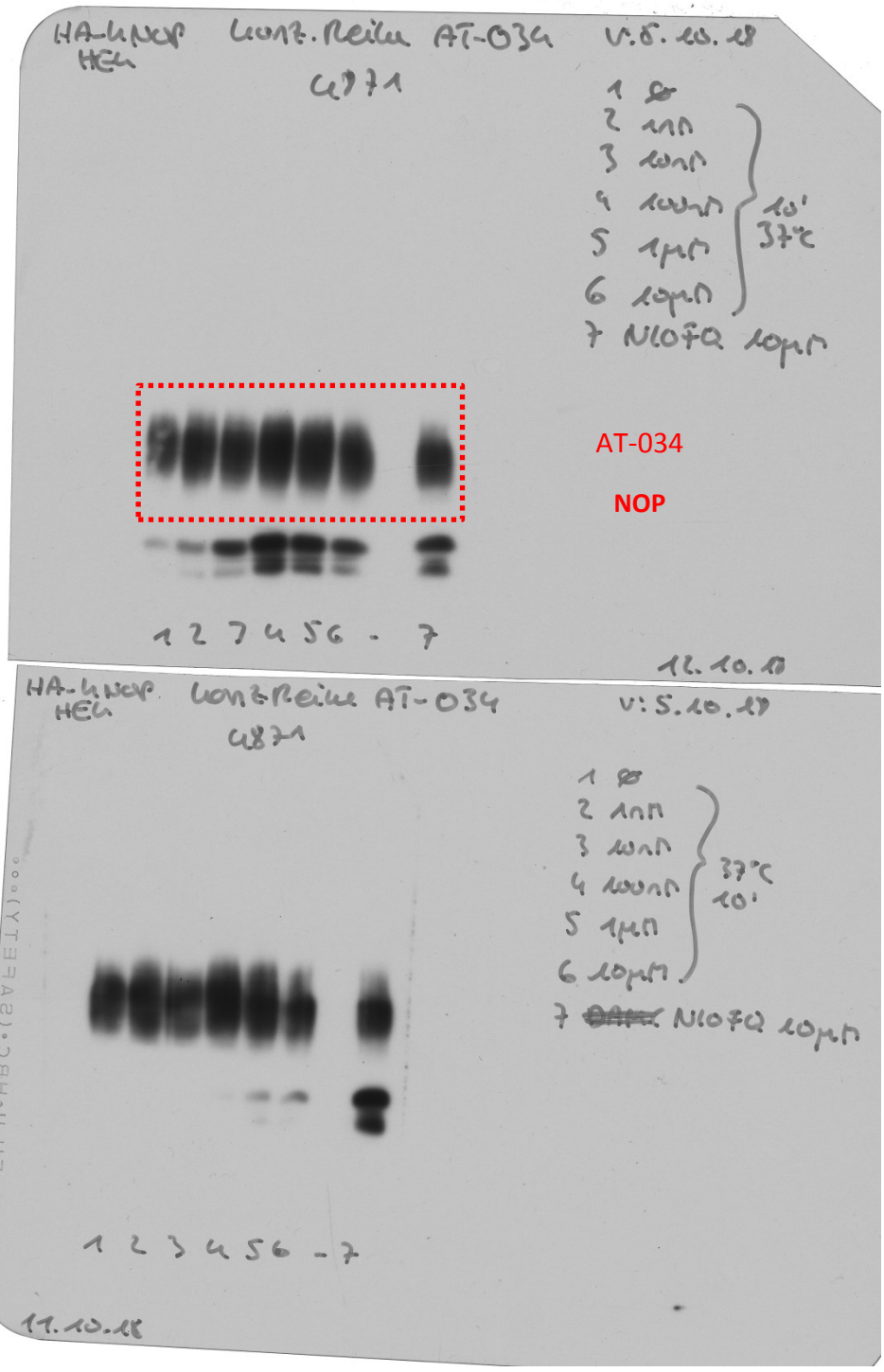
AT-034

NOP; **pT362/S363**


HA-hNOP HEK
pS346
AT-034
V: 5.10.18
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
37°C 10'
7 N1OFQ 10µM
1 2 3 4 5 6 -7
11.10.18
AT-034
NOP; pS346

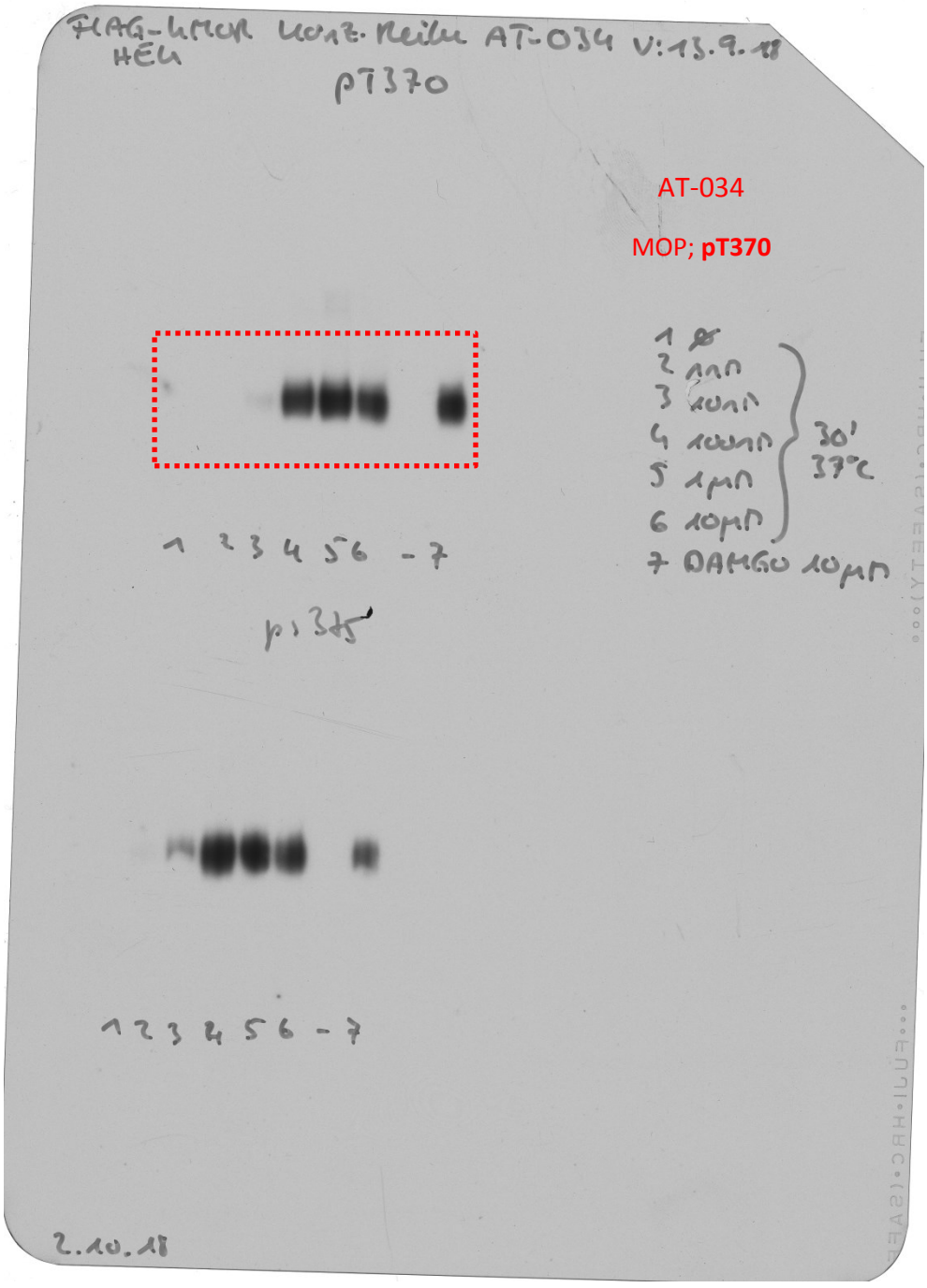

HA-hNOP HEK
pS351
AT-034
NOP; pS351
1 2 3 4 5 6 - 7

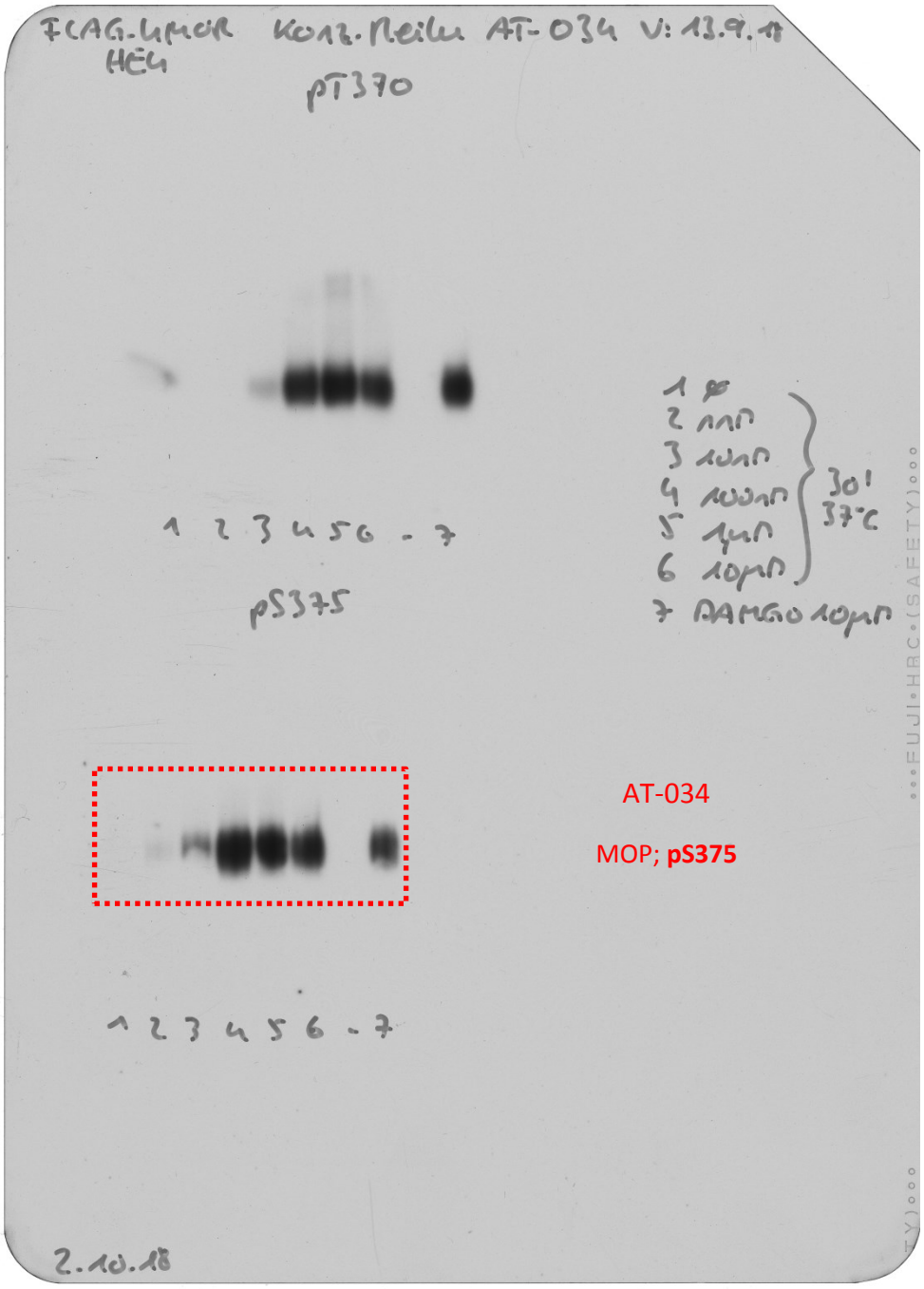

HA-hNOP HEK
pS351
1 2 3 4 5 6 - 7


HA-hNOP HEK
Wdh. Reihe AT-034
v. 5.10.18
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
10' 37°C
7 NIOFQ 10µM
AT-034
NOP
1 2 3 4 5 6 - 7
11.10.18
HA-hNOP HEK
Wdh Reihe AT-034
v: 5.10.18
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
37°C 10'
7 ~~Ø NOP~~ NIOFQ 10µM
1 2 3 4 5 6 - 7
11.10.18

AT-034

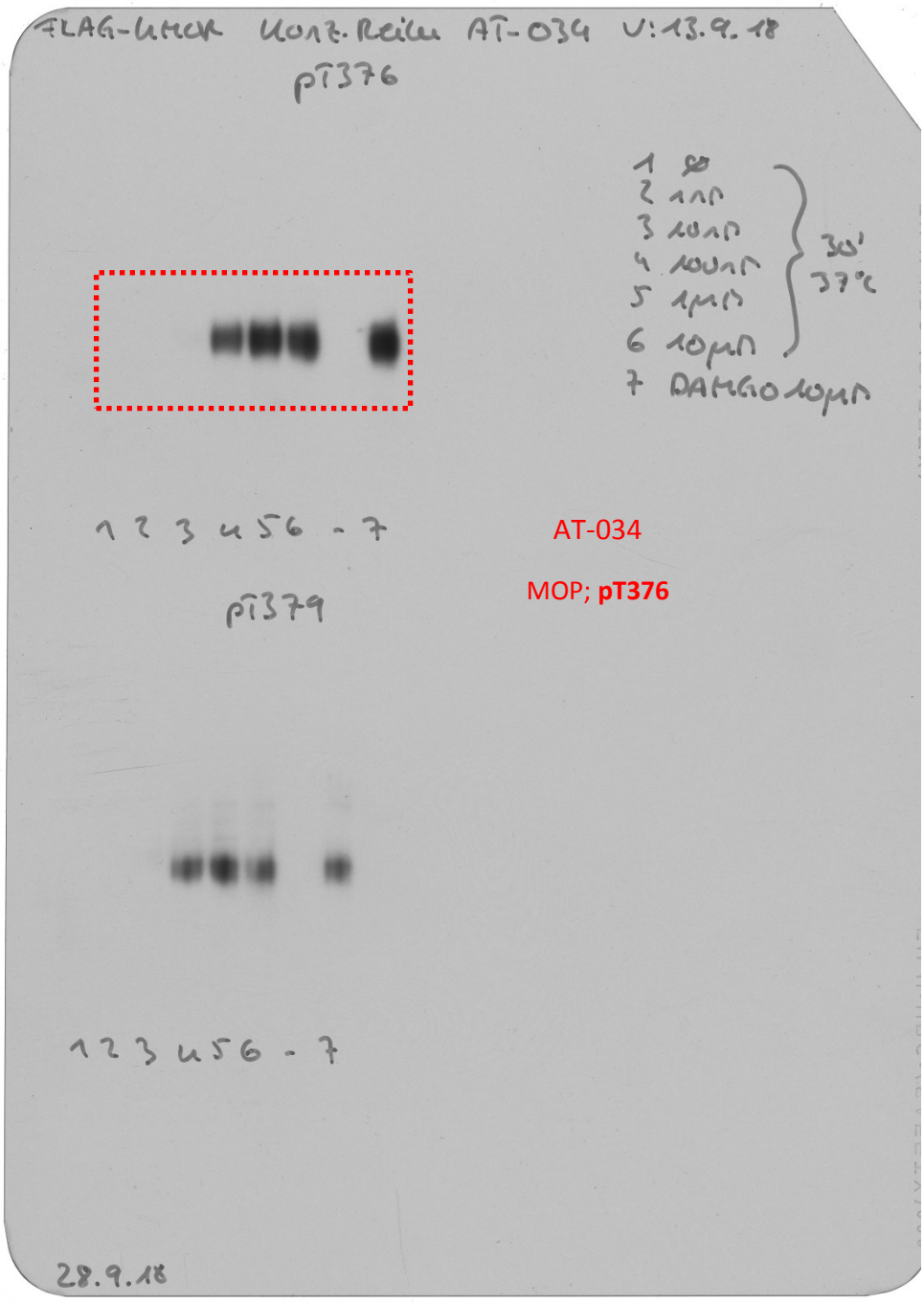
MOP

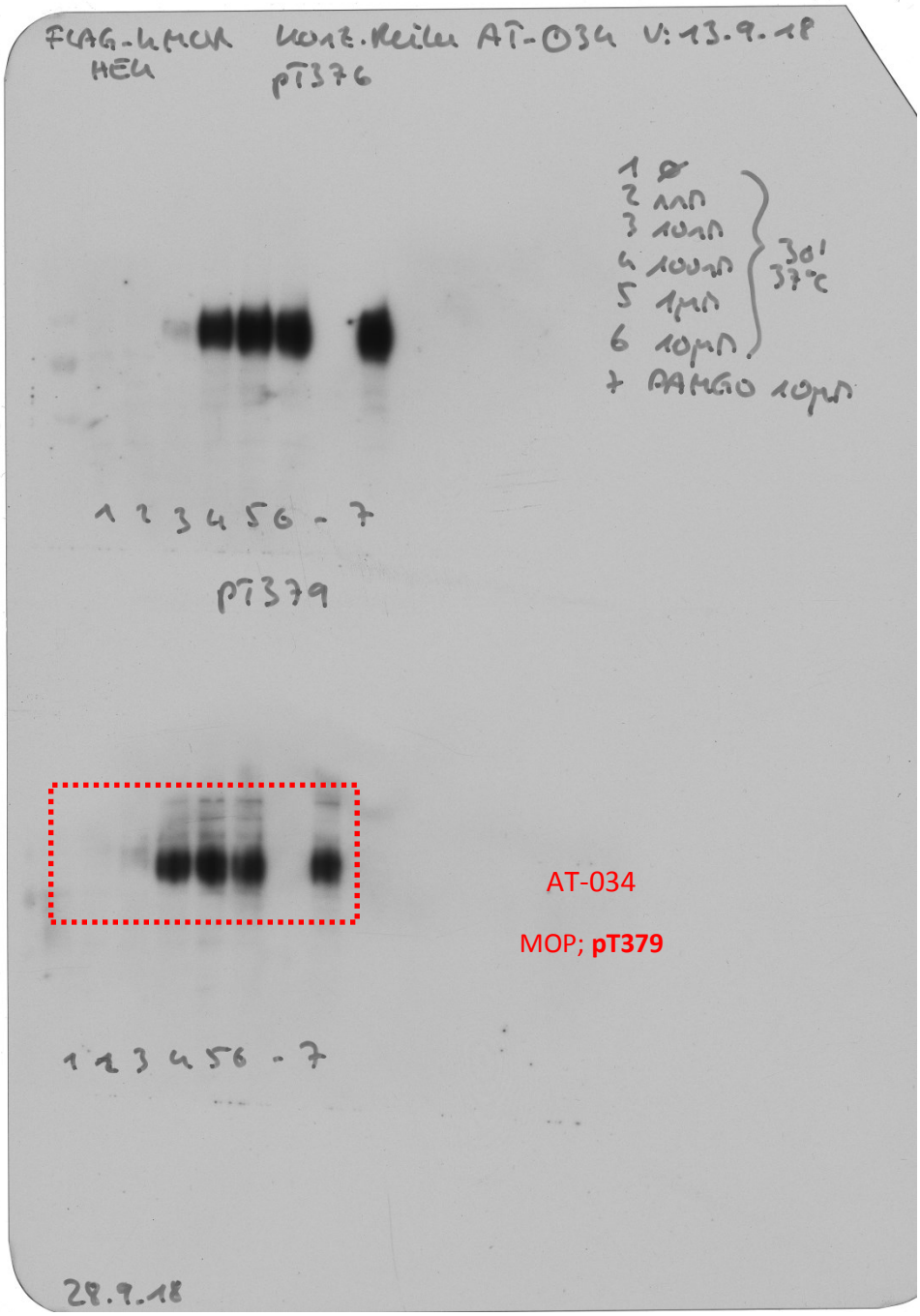

FLAG-hMOR konz. Reihe AT-034 V:13.9.18
HEK
pT370
AT-034
MOP; pT370
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
pT370
1 2 3 4 5 6 - 7
2.10.18



AT-034

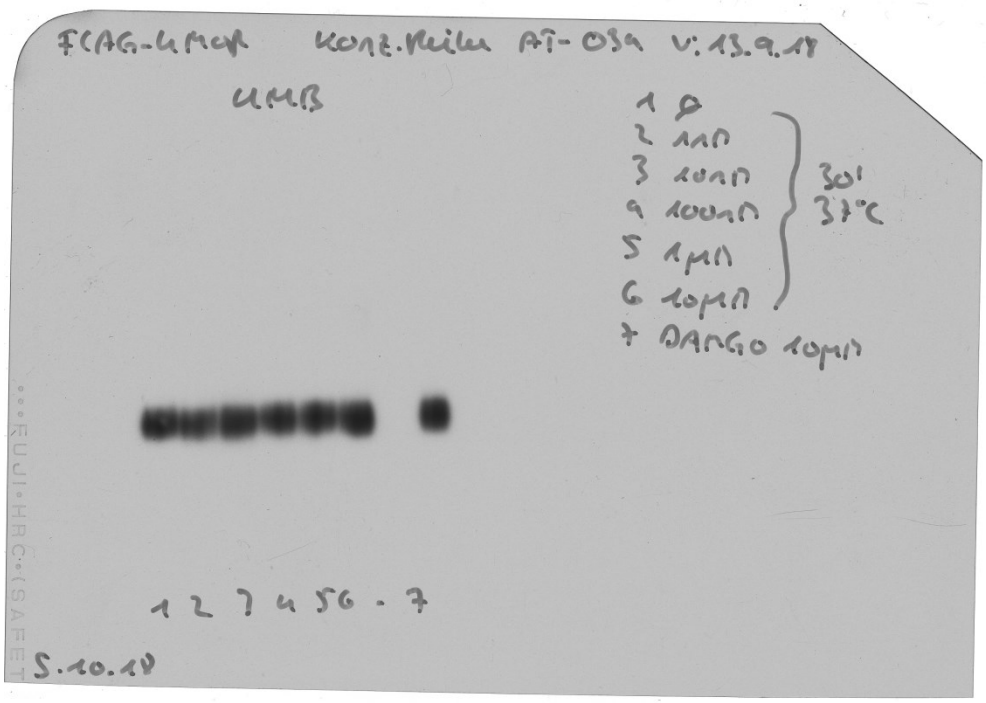
MOP; **pS375**

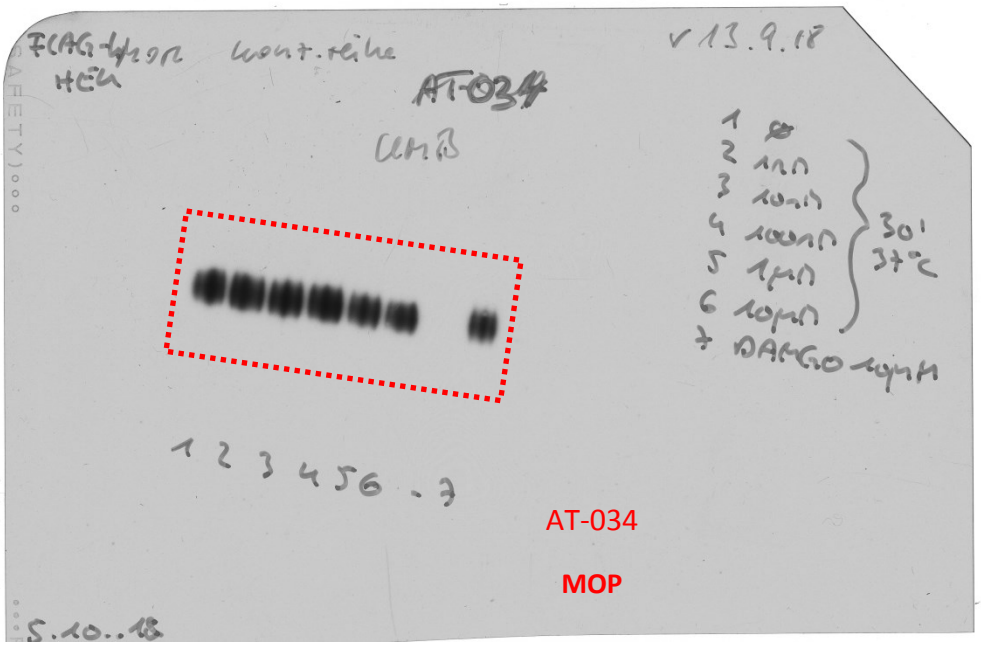

FLAG-uMOR Kon. Reihe AT-034 V: 13.9.18
pT376
1 ∅
2 1µl
3 10µl
4 100µl
5 1µl
6 10µl
7 DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
AT-034
MOP; pT376
pT379
1 2 3 4 5 6 - 7
28.9.18



AT-034

MOP; **pT379**


FLAG-hMOR kont.reihe AT-034 v. 13.9.18
UMB
1 0
2 1µl
3 10µl
4 100µl
5 1µl
6 10µl
+ DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
5.10.18

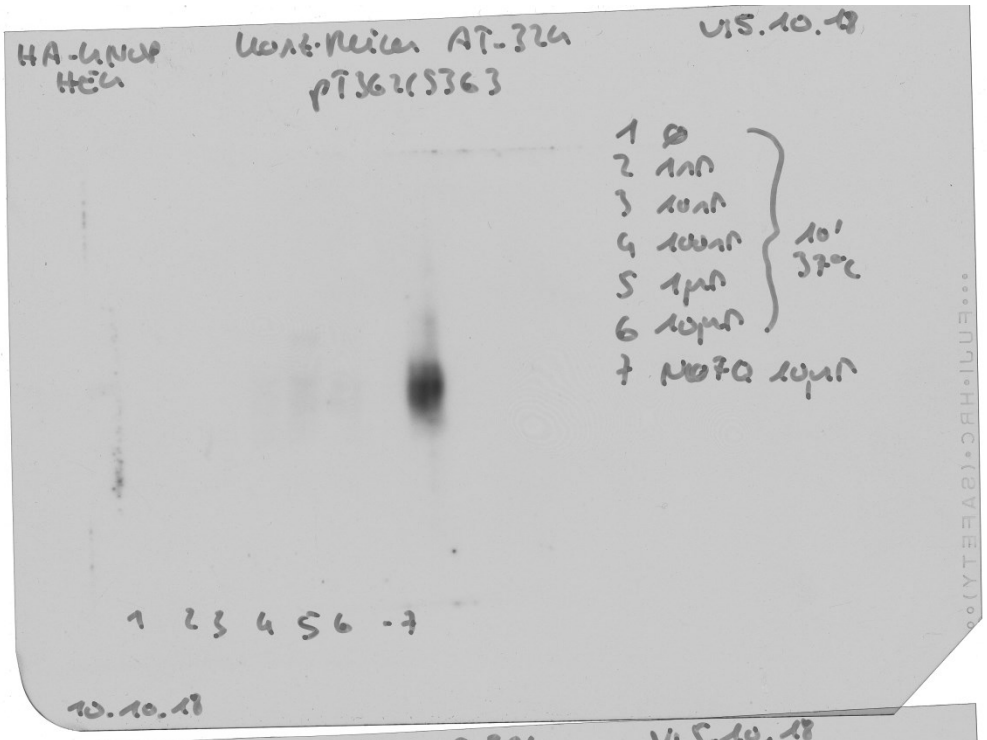

FLAG-hMOR kont.reihe
HEK
AT-034
UMB
v 13.9.18
1 0
2 1µl
3 10µl
4 100µl
5 1µl
6 10µl
+ DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
5.10..18

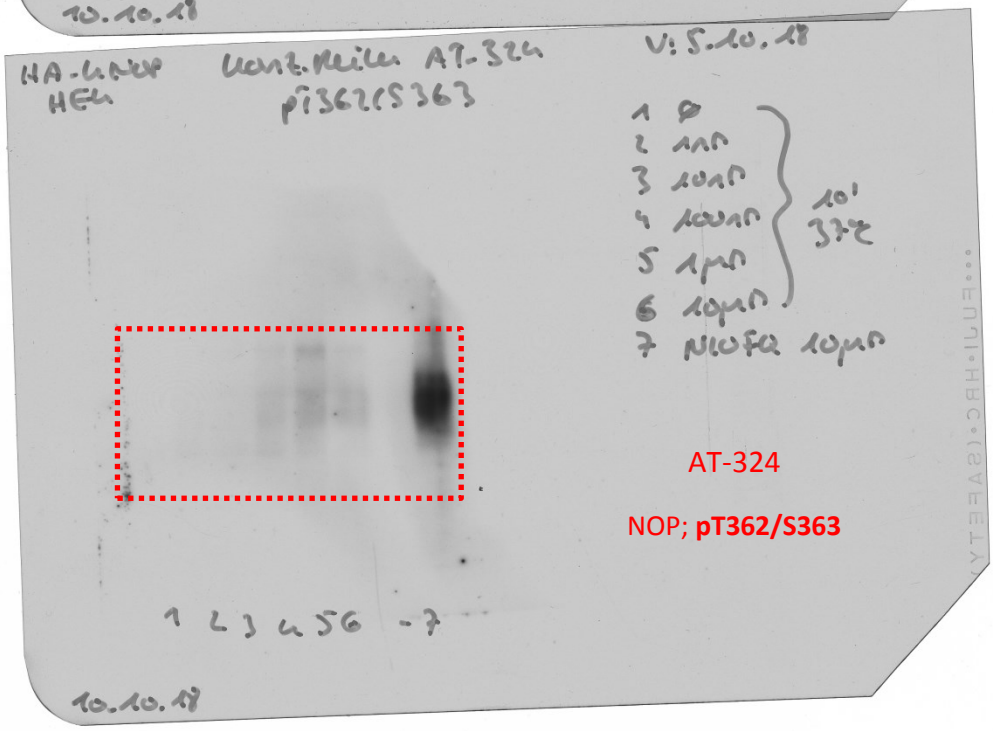
AT-034

MOP

AT-324

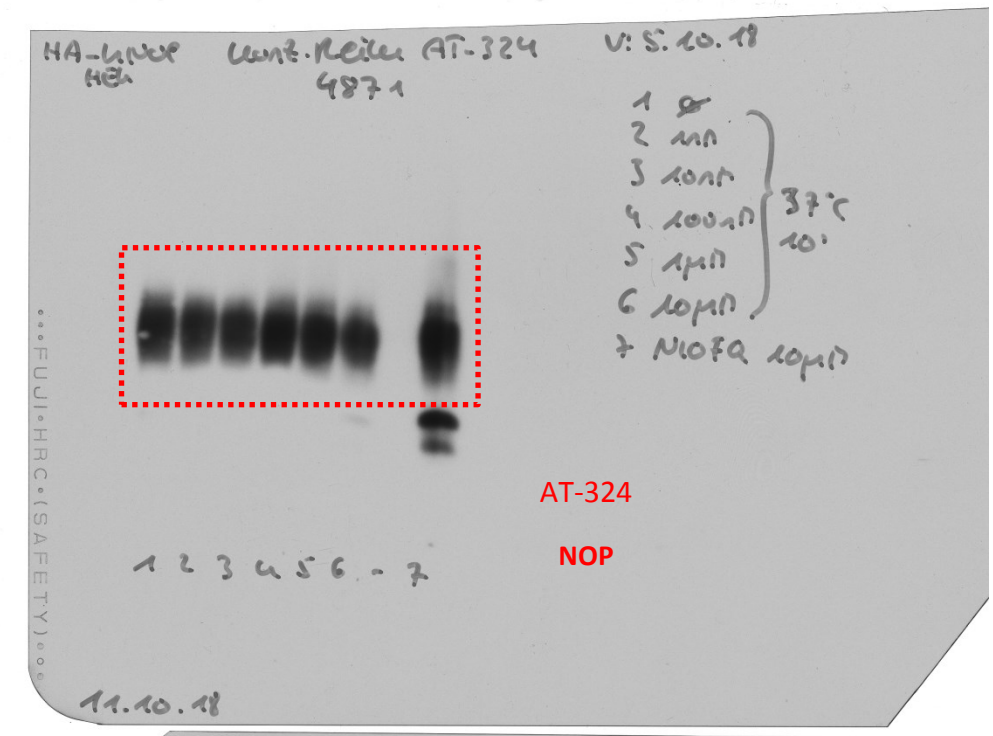
NOP

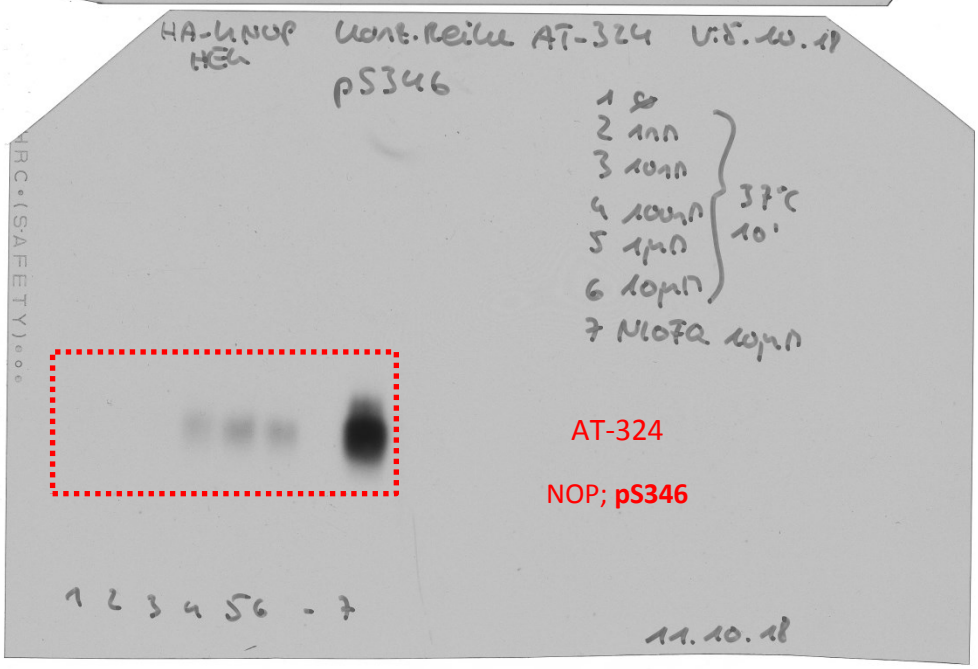


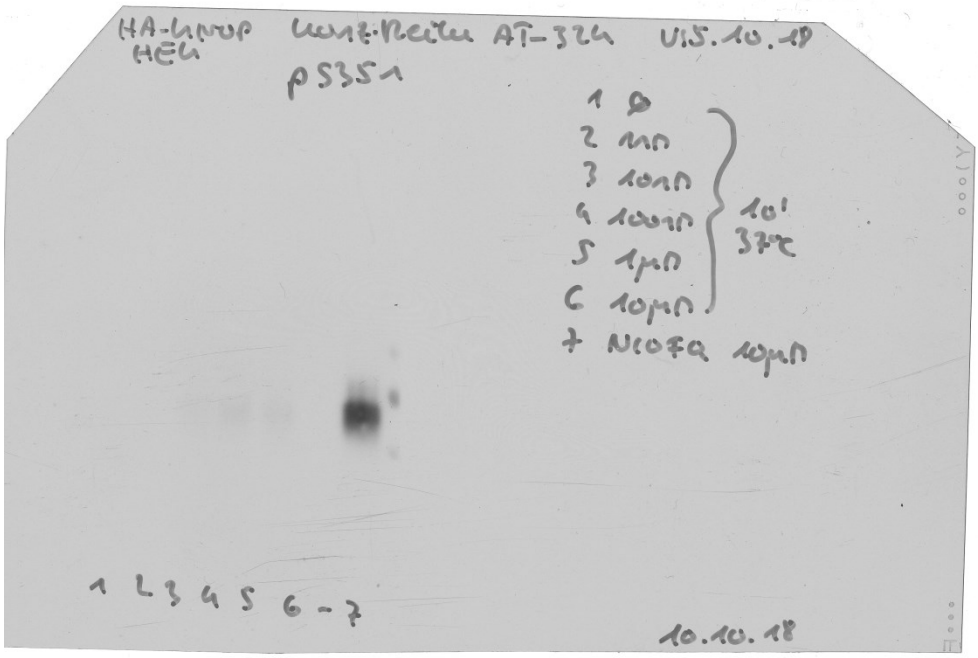


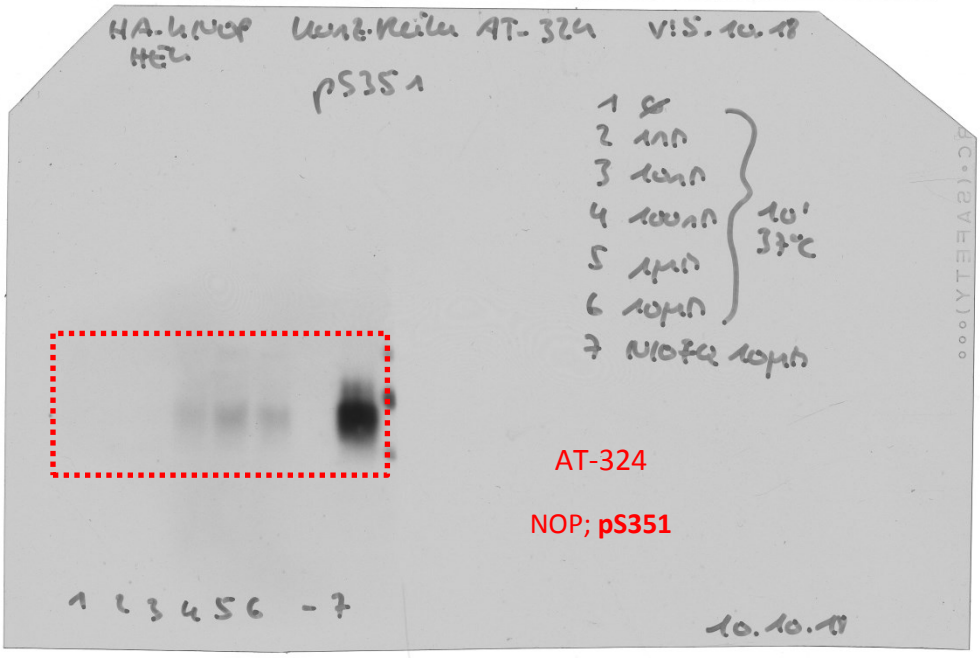
AT-324

NOP; **pT362/S363**


HA-hNOP
HEK
Wdh. Reihe AT-324
4871
V: 5.10.18
1 –
2 1min
3 10min
4 100min
5 1µM
6 10µM
37°C
10'
7 NIOFQ 10µM
AT-324
NOP
1 2 3 4 5 6 - 7
11.10.18


HA-hNOP
HEK
Wdh. Reihe AT-324
V: 8.10.18
pS346
1 –
2 1min
3 10min
4 100min
5 1µM
6 10µM
37°C
10'
7 NIOFQ 10µM
AT-324
NOP; pS346
1 2 3 4 5 6 - 7
11.10.18



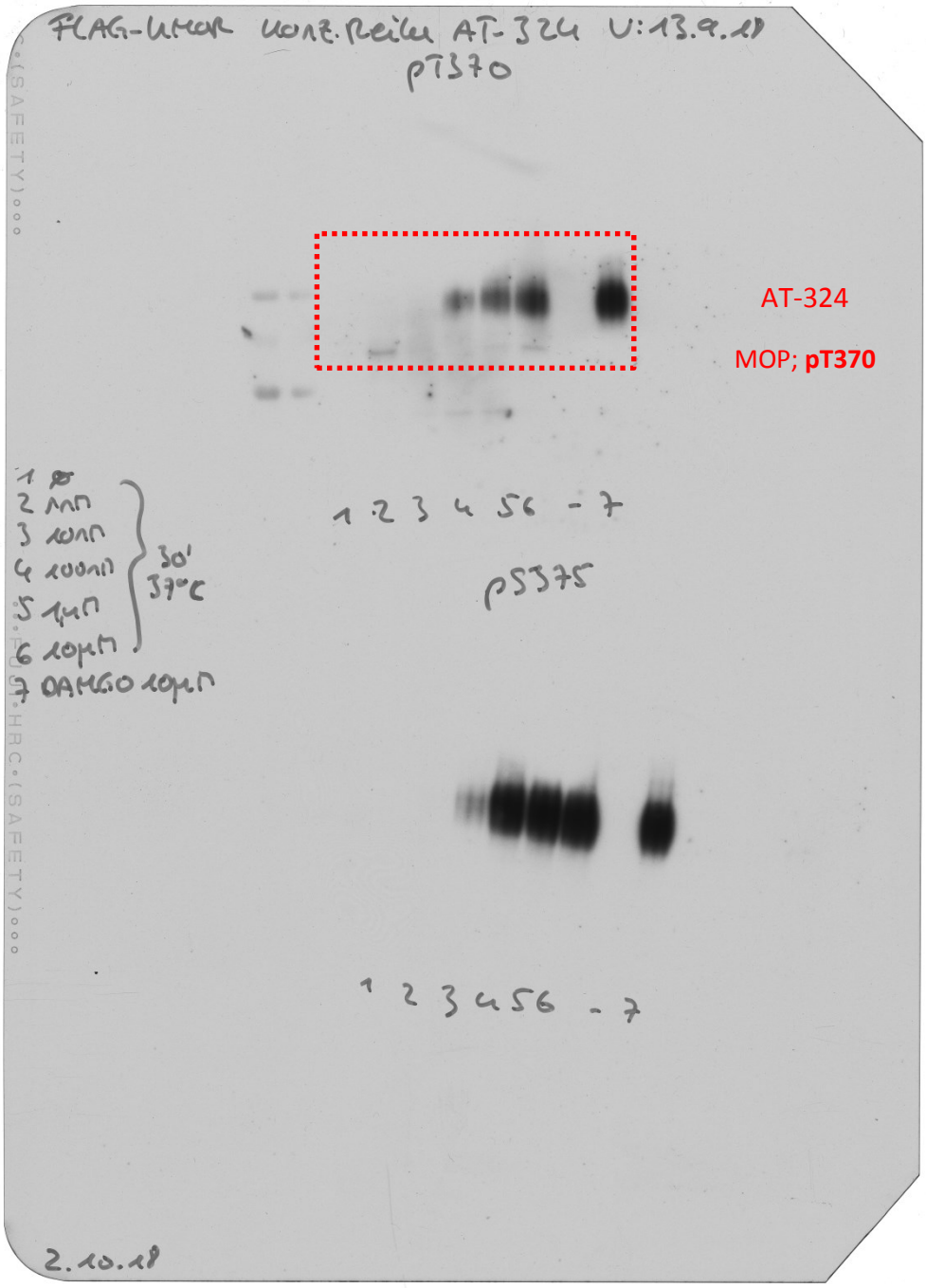


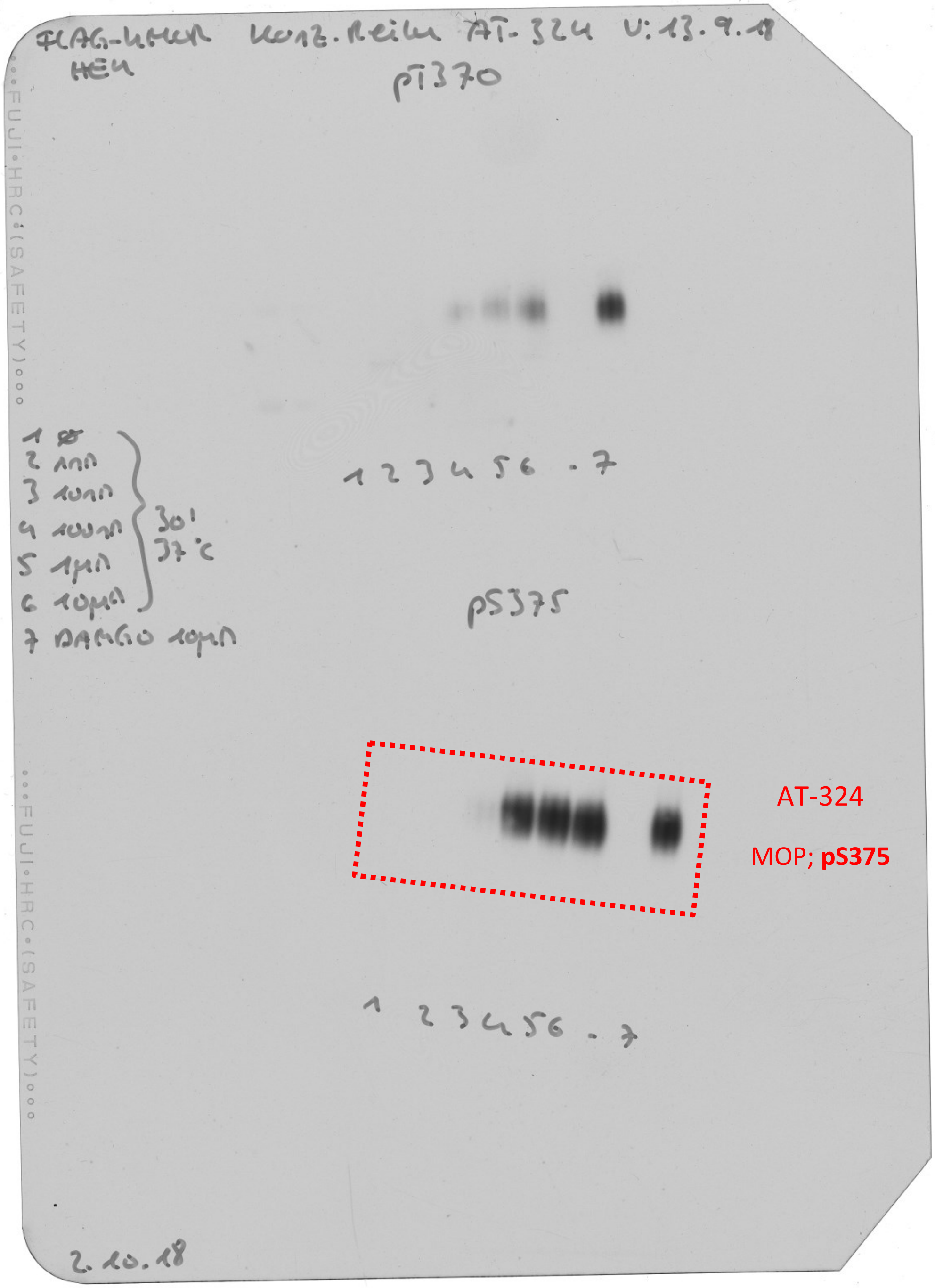
AT-324

NOP; **pS351**

AT-324

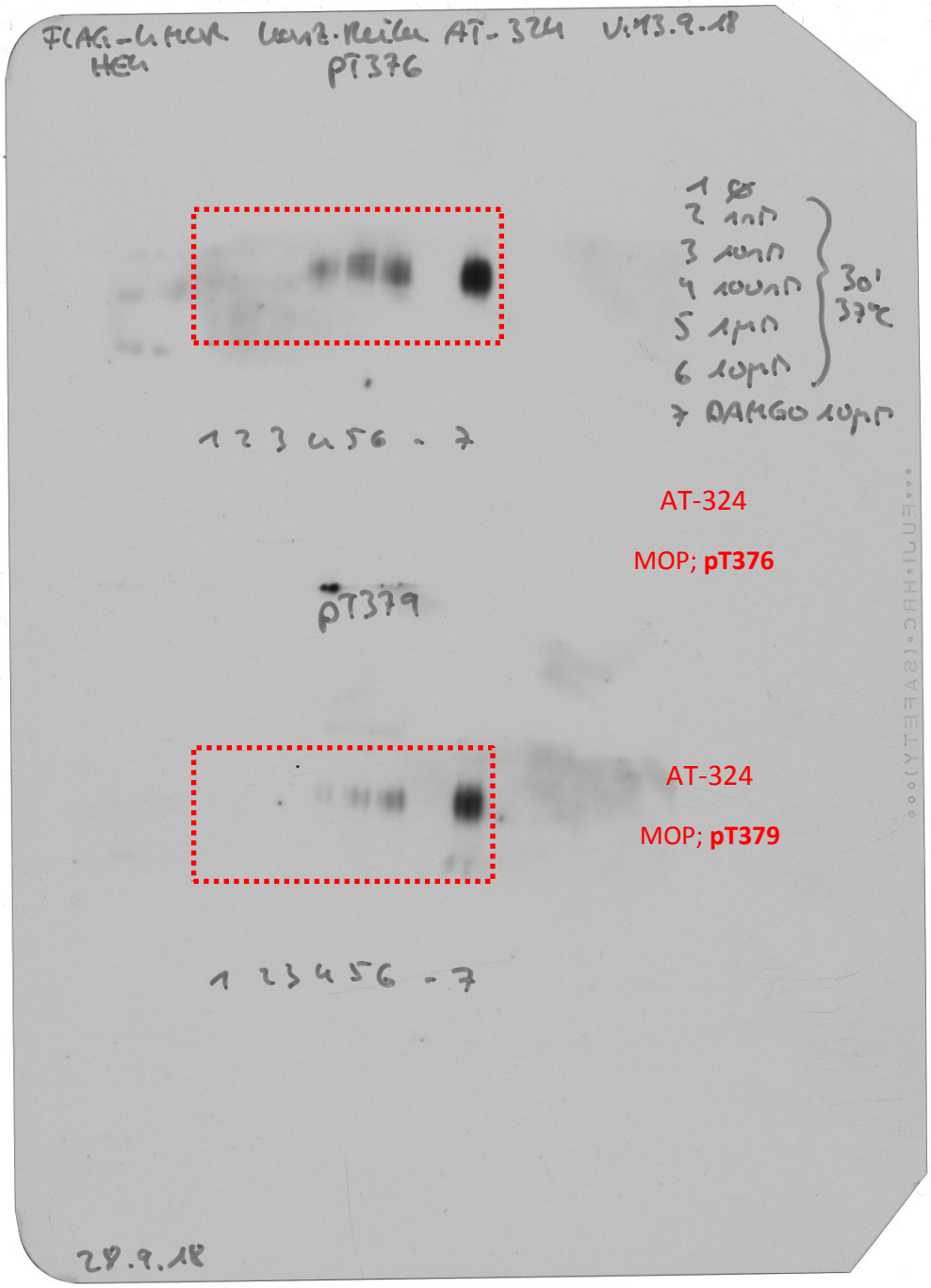
MOP

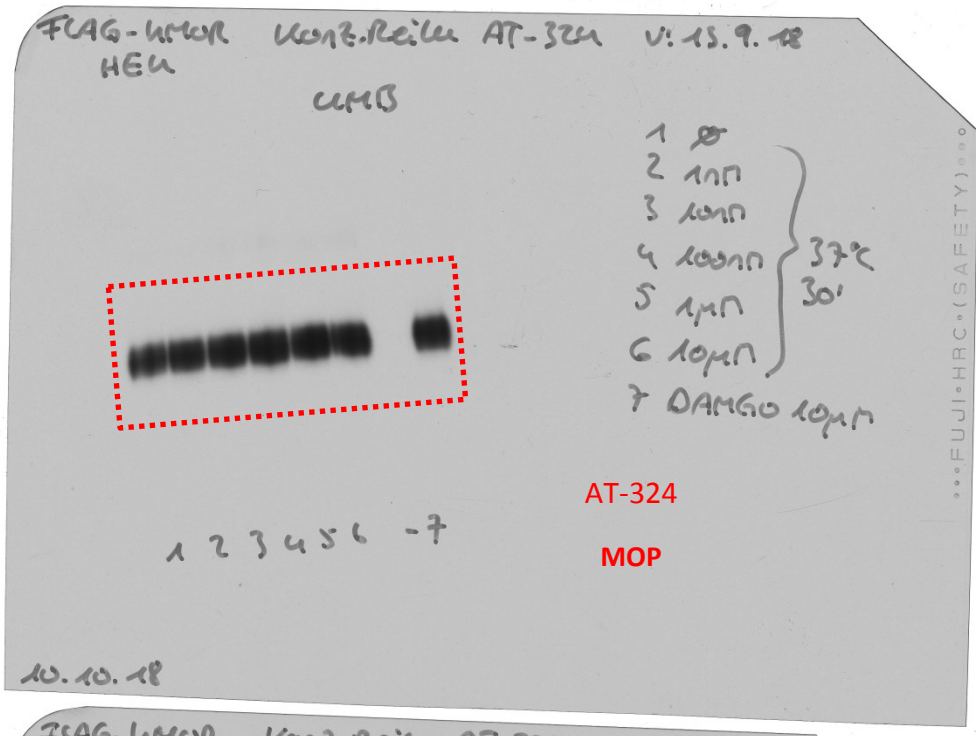

FLAG-MOR Wdh. Reihe AT-324 U:13.9.21
pT370
AT-324
MOP; pT370
1 2 3 4 5 6 - 7
pS375
1 2 3 4 5 6 - 7
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
2.10.21

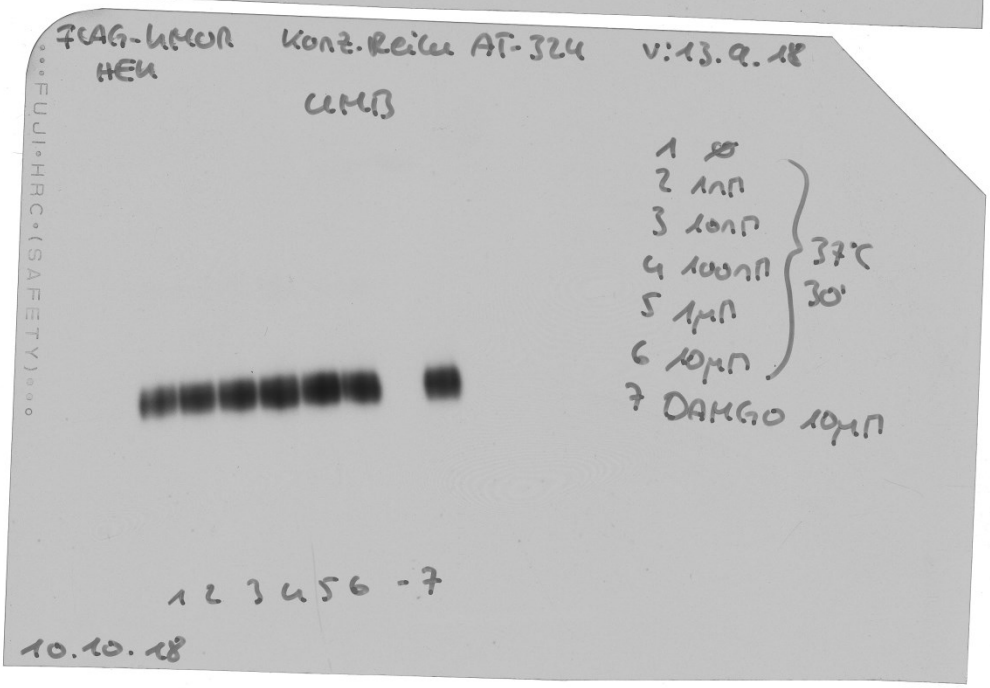


AT-324

MOP; **pS375**

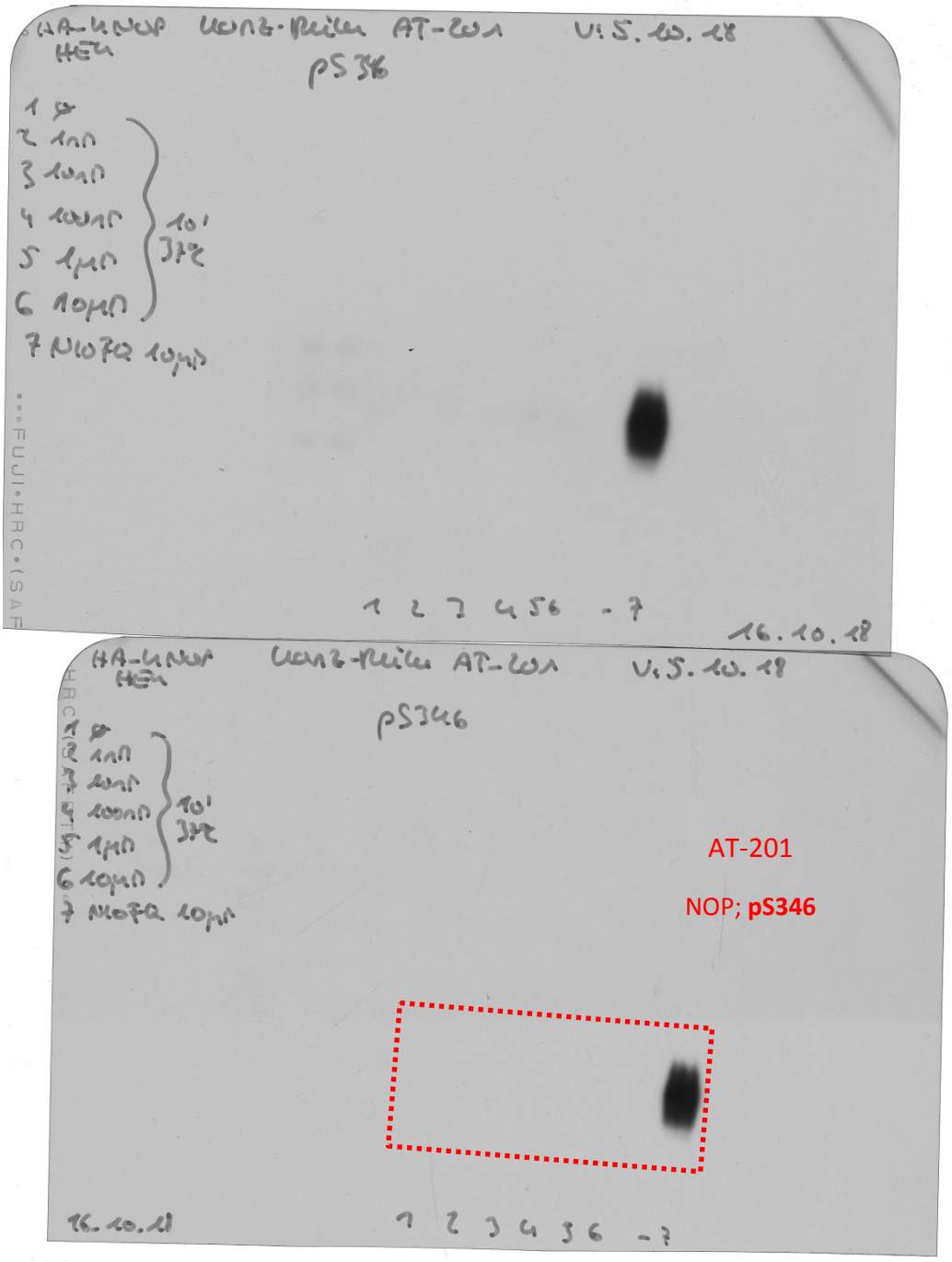

FLAG-MOR
HEK
Verd. Reihe AT-324
pT376
Vi 13.9.18
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
AT-324
MOP; pT376
pT379
AT-324
MOP; pT379
1 2 3 4 5 6 - 7
28.9.18

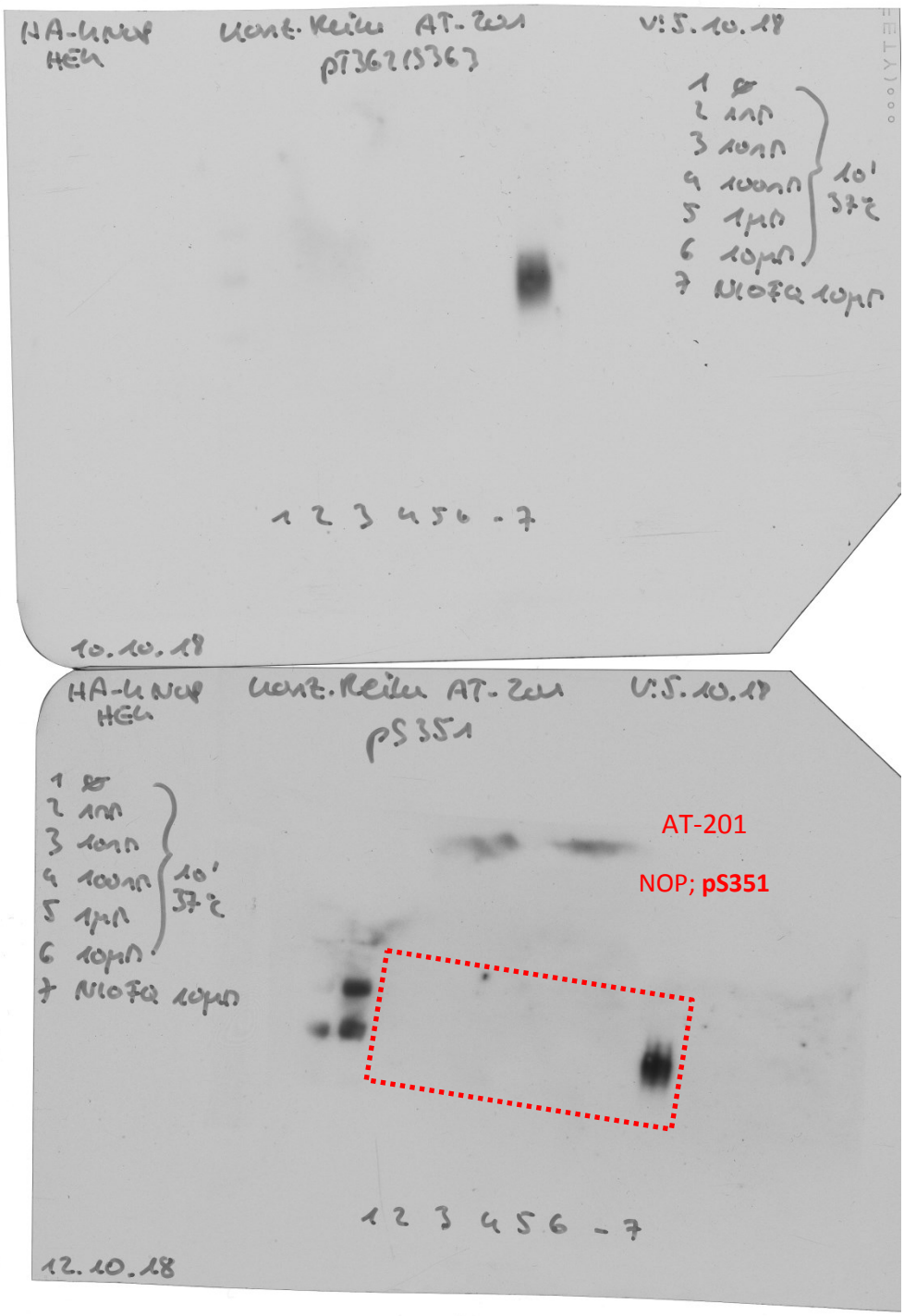

FLAG-hMOR HEK
Konz.Reihe AT-324
V: 13.9.18
CHO
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
37°C
30'
7 DAMGO 10µM
1 2 3 4 5 6 -7
AT-324
MOP
10.10.18
FUJI HRC (SAFETY)

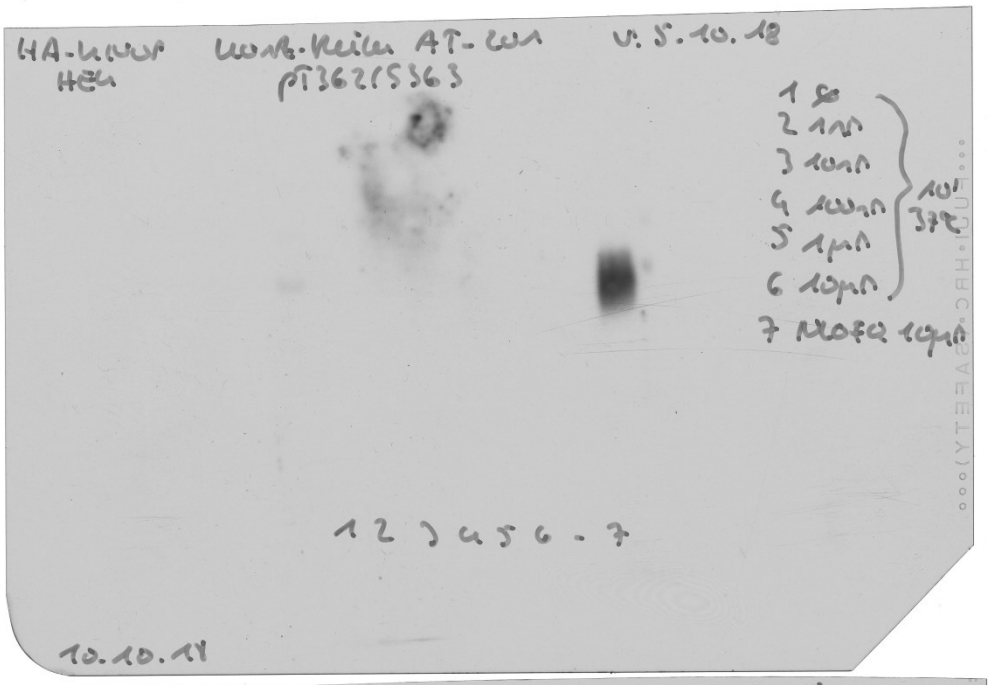

FLAG-hMOR HEK
Konz.Reihe AT-324
V: 13.9.18
CHO
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
37°C
30'
7 DAMGO 10µM
1 2 3 4 5 6 -7
10.10.18
FUJI HRC (SAFETY)

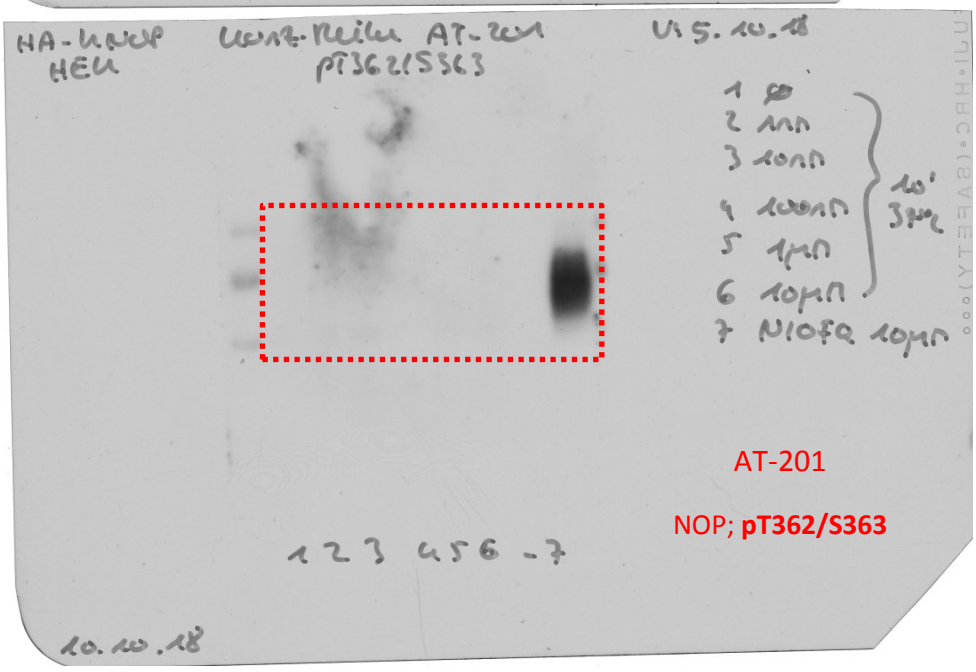
AT-201

NOP


pS346
1 2 3 4 5 6 - 7
16.10.18
pS346
AT-201
NOP; pS346
16.10.18

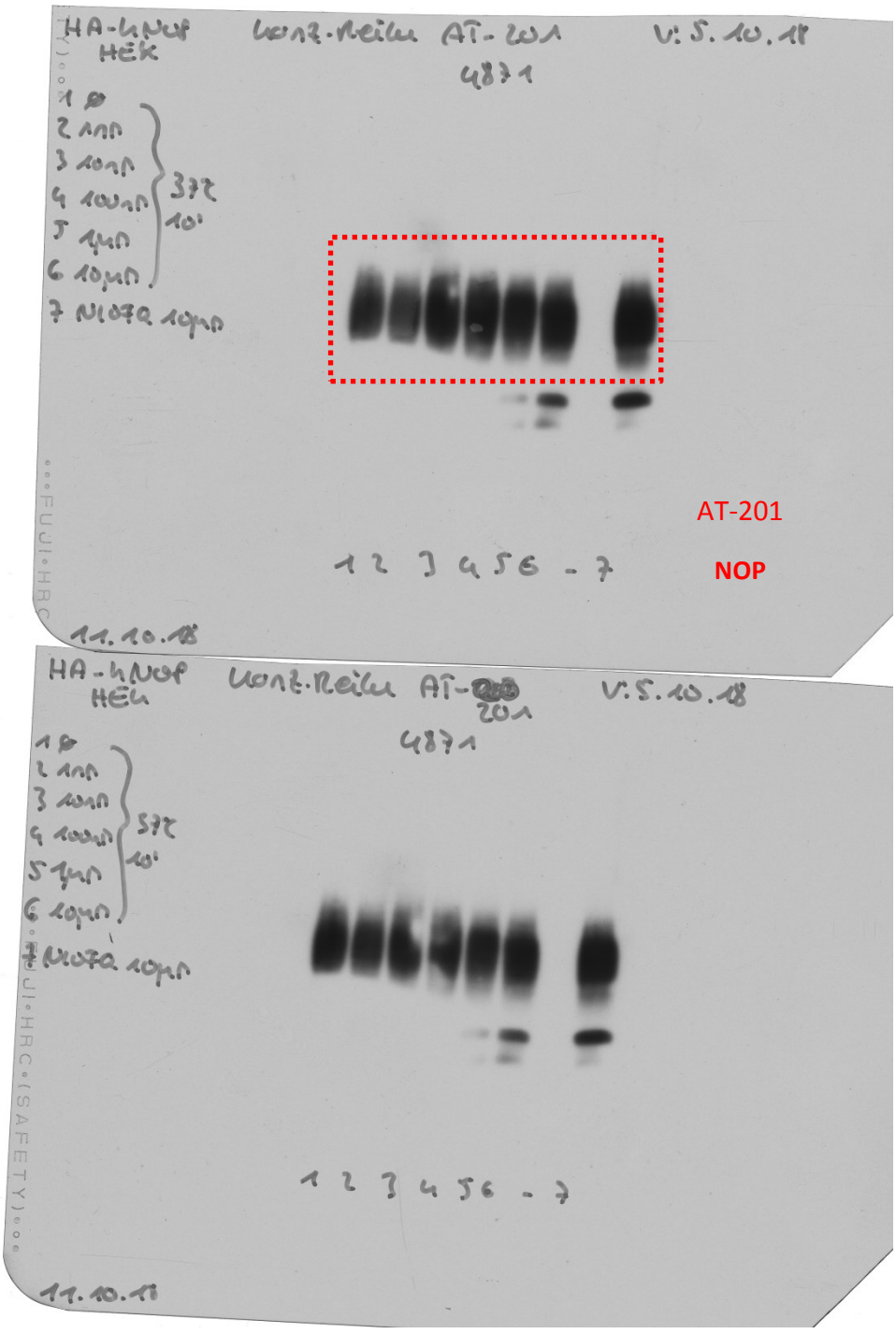

HA-UNOP
HEK
Kont. Reiki AT-201
pT362/S367
V: 5.10.18
1 ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 NIOFQ 10µM
10'
37°C
1 2 3 4 5 6 - 7
10.10.18
HA-UNOP
HEK
Kont. Reiki AT-201
pS351
V: 5.10.18
1 ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 NIOFQ 10µM
10'
37°C
AT-201
NOP; pS351
1 2 3 4 5 6 - 7
12.10.18





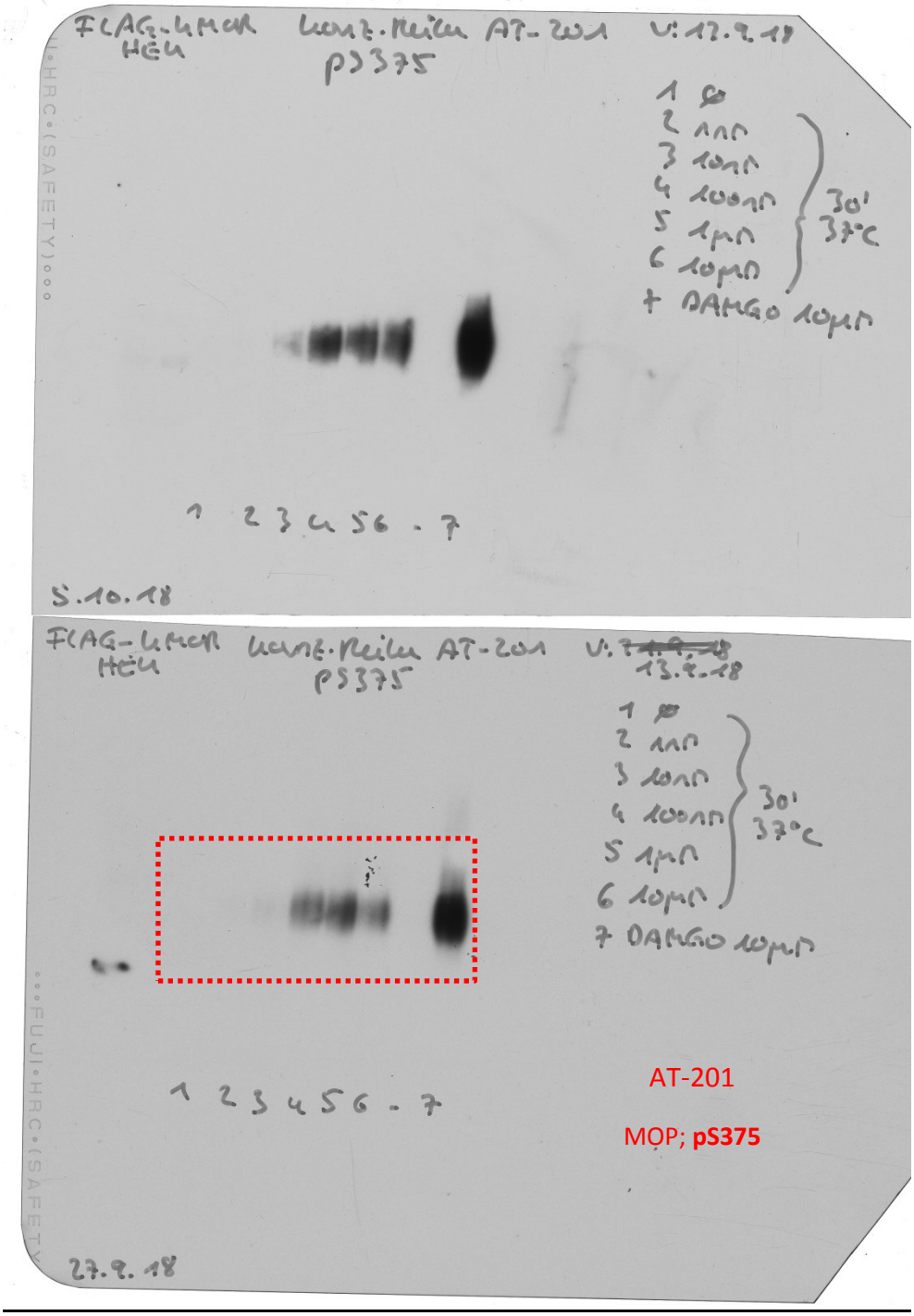
AT-201

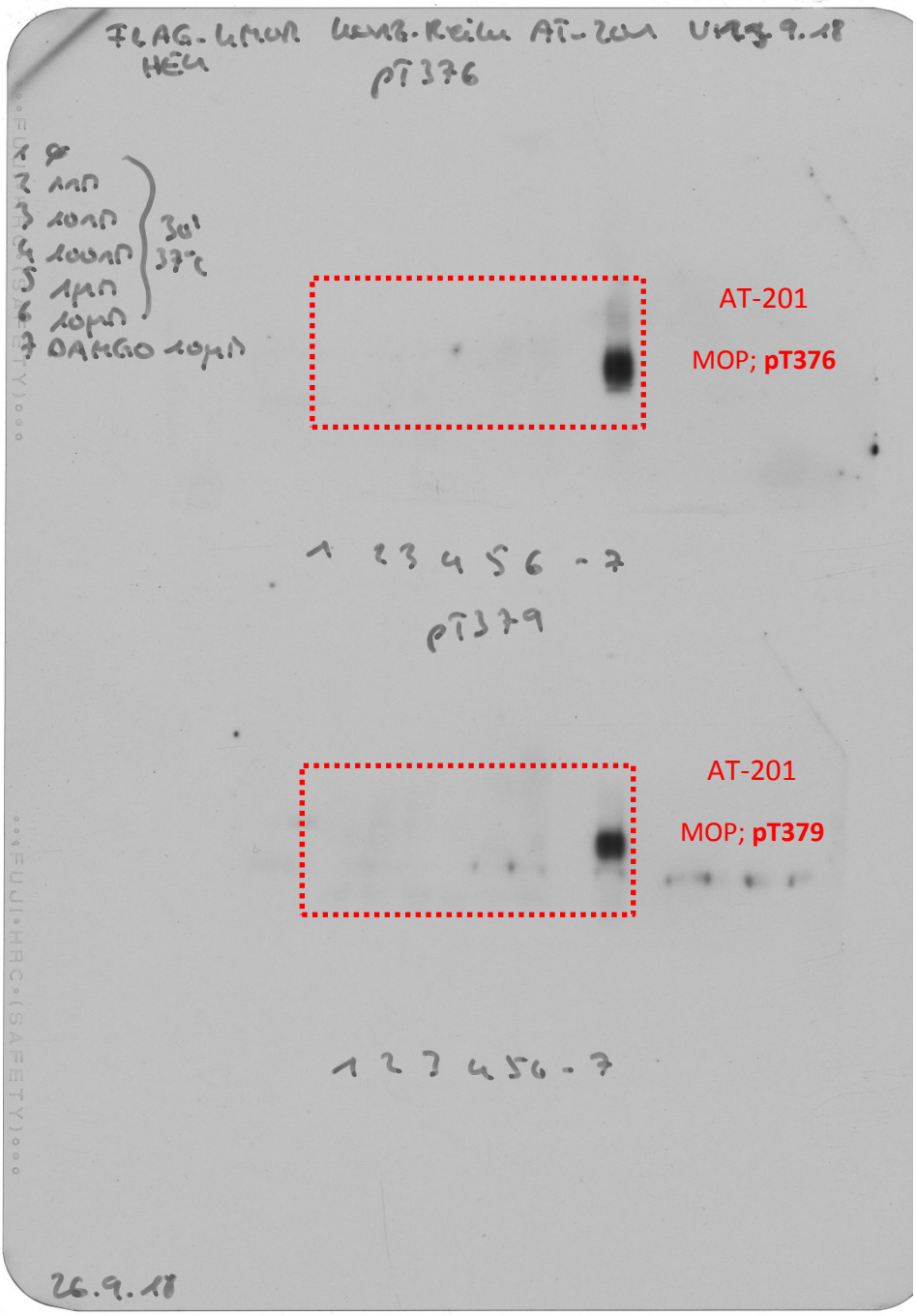
NOP; **pT362/S363**


HA-hNOP
HEK
Wash.Reihe AT-201
V.5.10.18
4871
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 Nl07a 10µM
37°
10'
1 2 3 4 5 6 - 7
AT-201
NOP
11.10.18
HA-hNOP
HEK
Wash.Reihe AT-201
V.5.10.18
4871
1 ∅
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 Nl07a 10µM
37°
10'
1 2 3 4 5 6 - 7
11.10.18

AT-201

MOP


FLAG-hMOR HEK
pS375
AT-201
V: 12.9.18
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
30' 37°C
+ DAMGO 10µM
1 2 3 4 5 6 - 7
5.10.18
FLAG-hMOR HEK
pS375
AT-201
V: 13.9.18
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
30' 37°C
7 DAMGO 10µM
1 2 3 4 5 6 - 7
AT-201
MOP; pS375
27.9.18

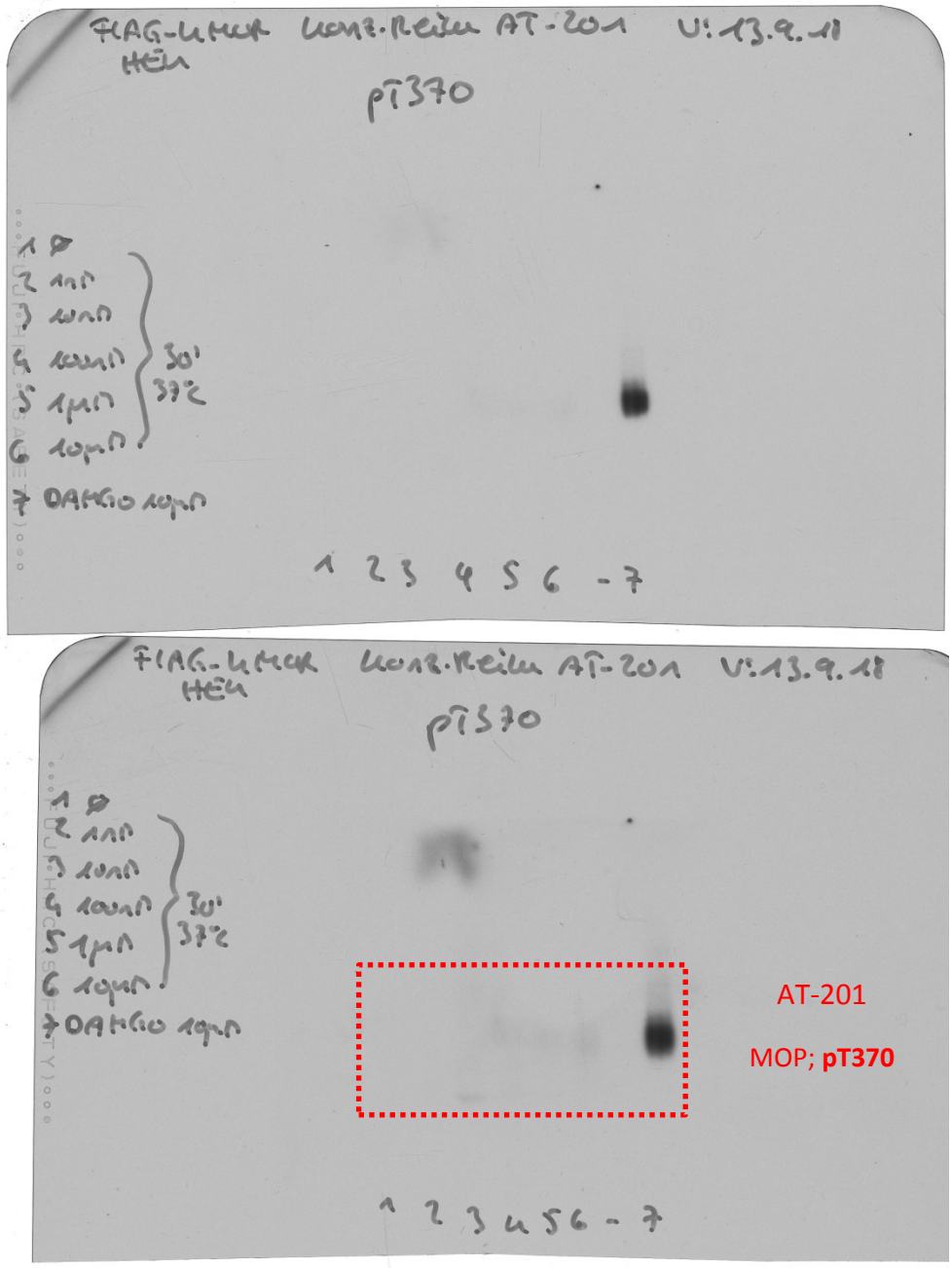


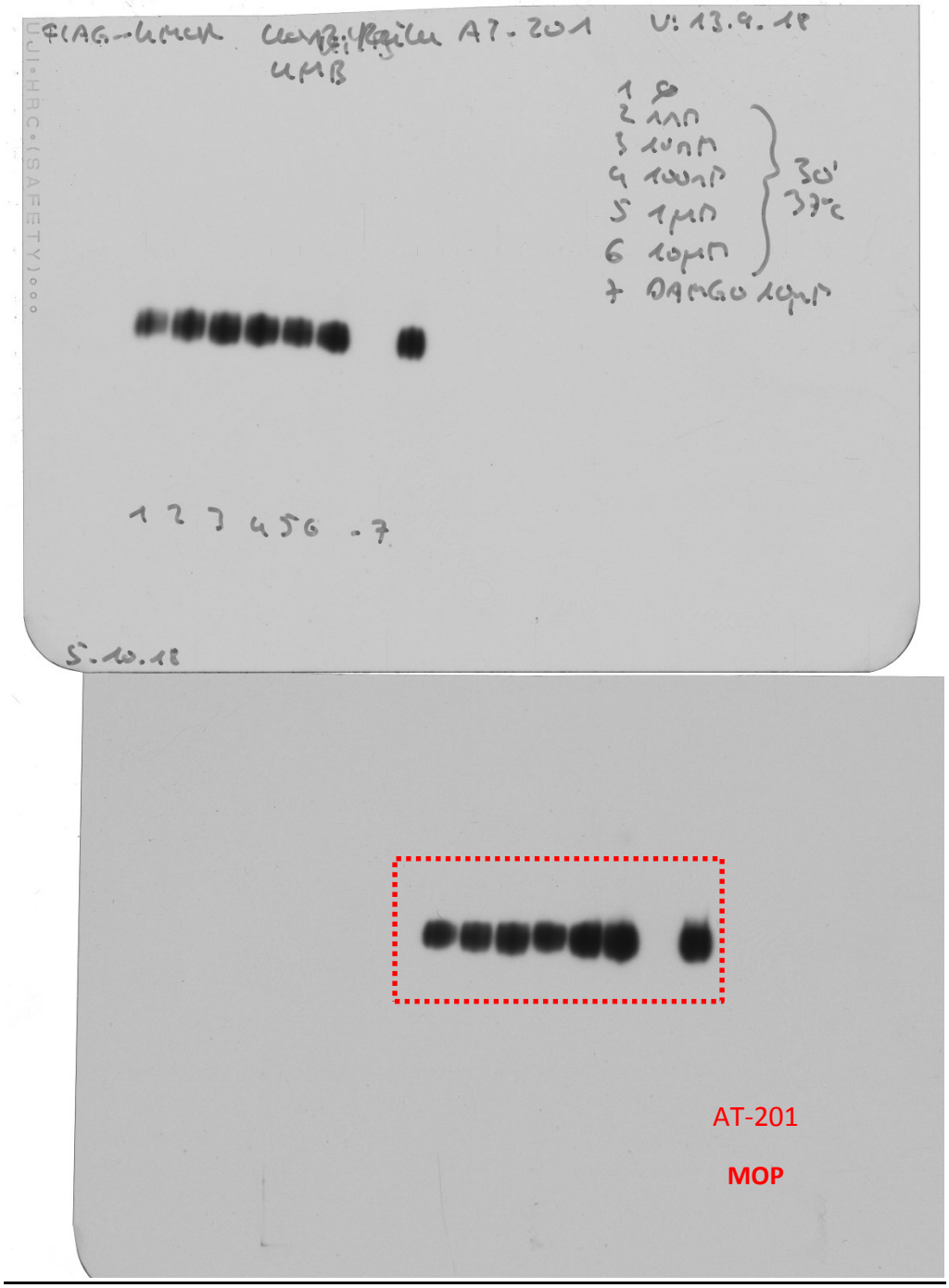
AT-201

MOP; **pT376**

AT-201

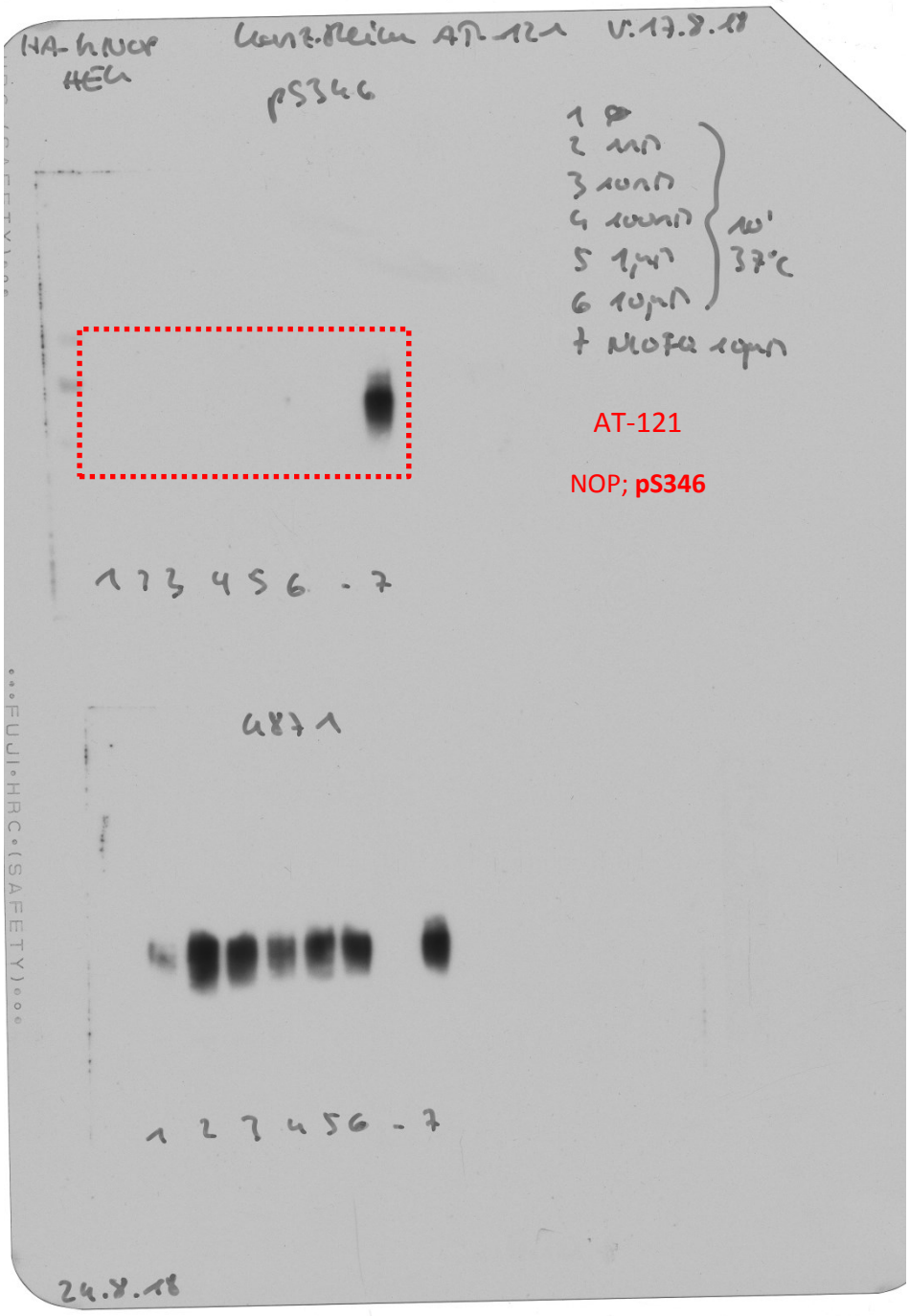
MOP; **pT379**

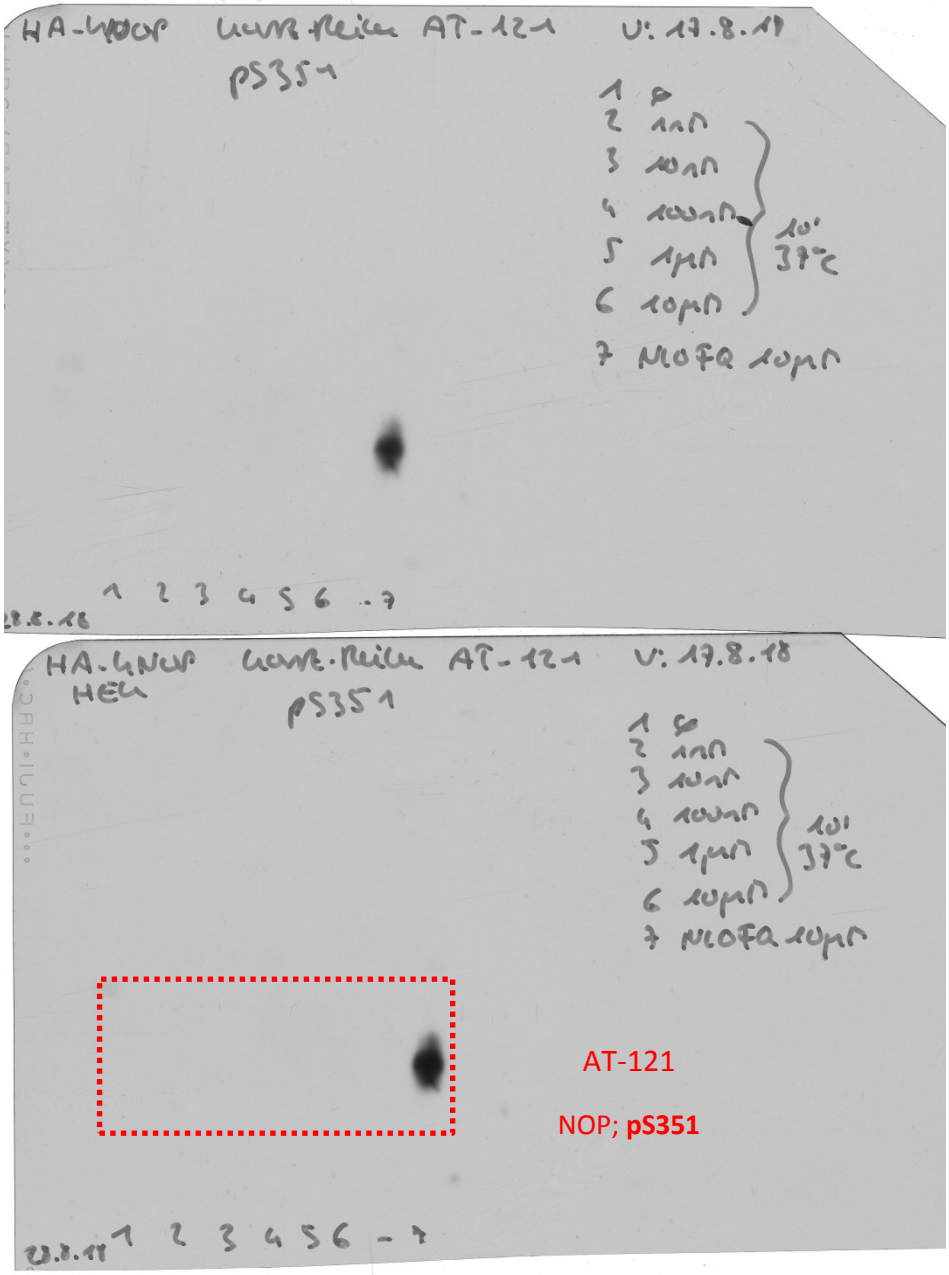

FLAG-hMOR Kont.Reihe AT-201 V:13.9.11
pT370
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
FLAG-hMOR Kont.Reihe AT-201 V:13.9.11
pT370
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
1 2 3 4 5 6 - 7
AT-201
MOP; pT370

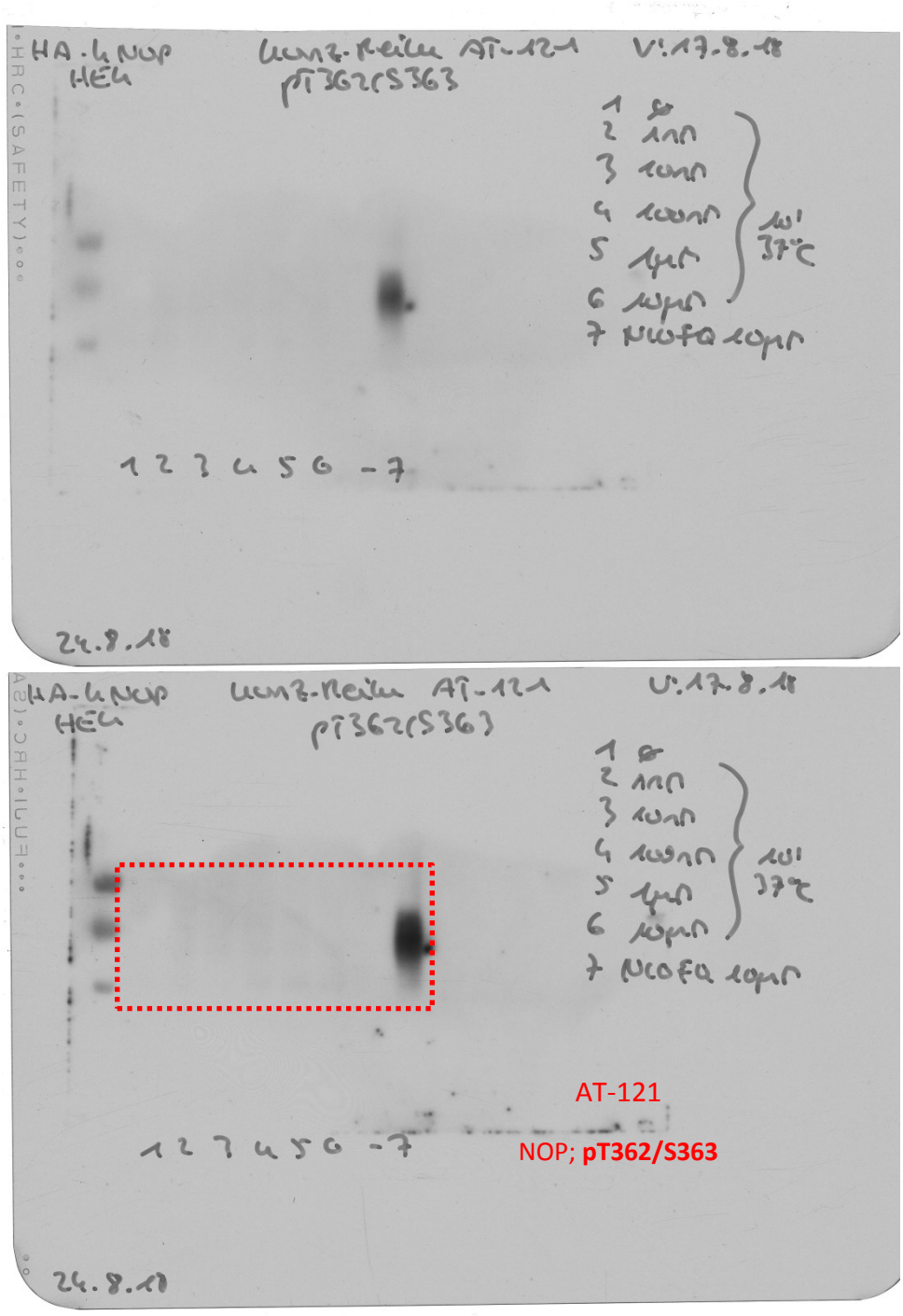

FLAG-hMOR
AT-201
U: 13.9.18
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
37°C
127456-7
5.10.18
AT-201
MOP

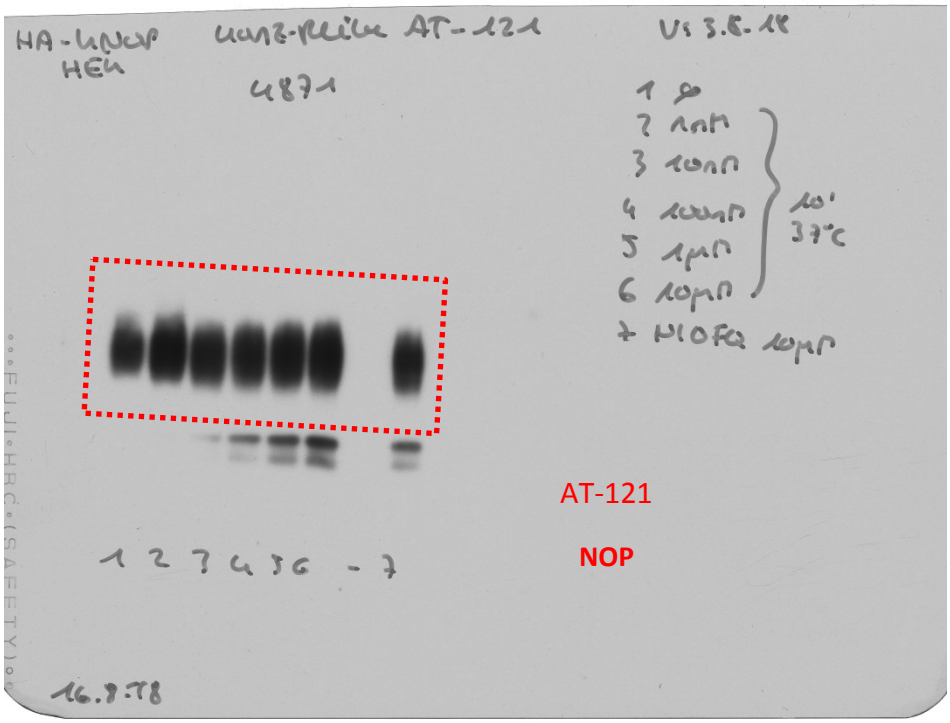
AT-121

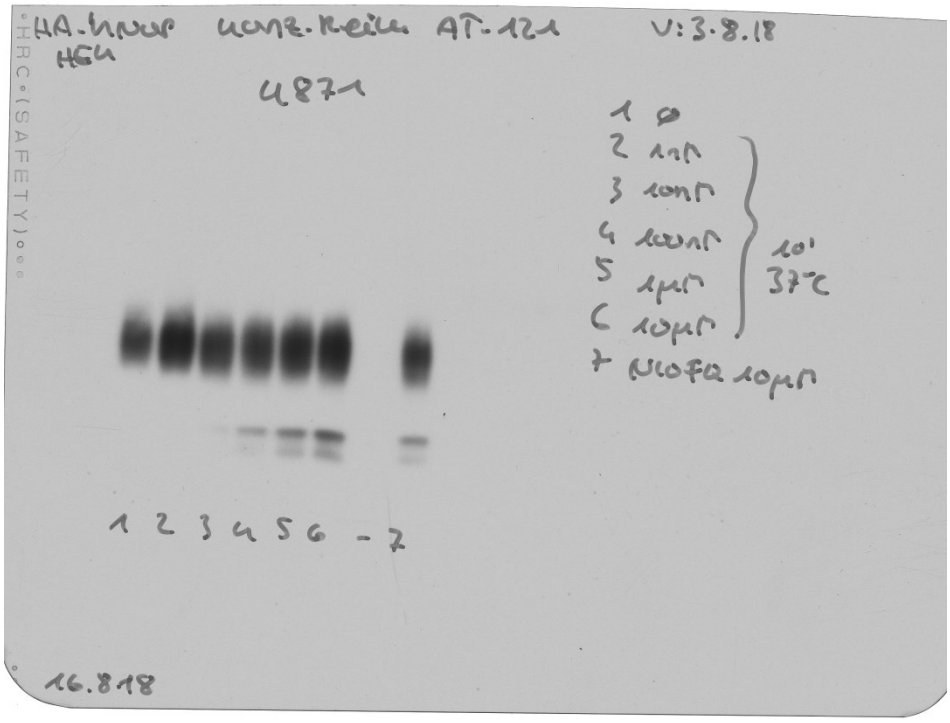
NOP


HA-hNOP
HEK
pS346
AT-121
NOP; pS346
1 2 3 4 5 6 - 7
1 2 3 4 5 6 - 7
24.8.18


AT-121
NOP; pS351

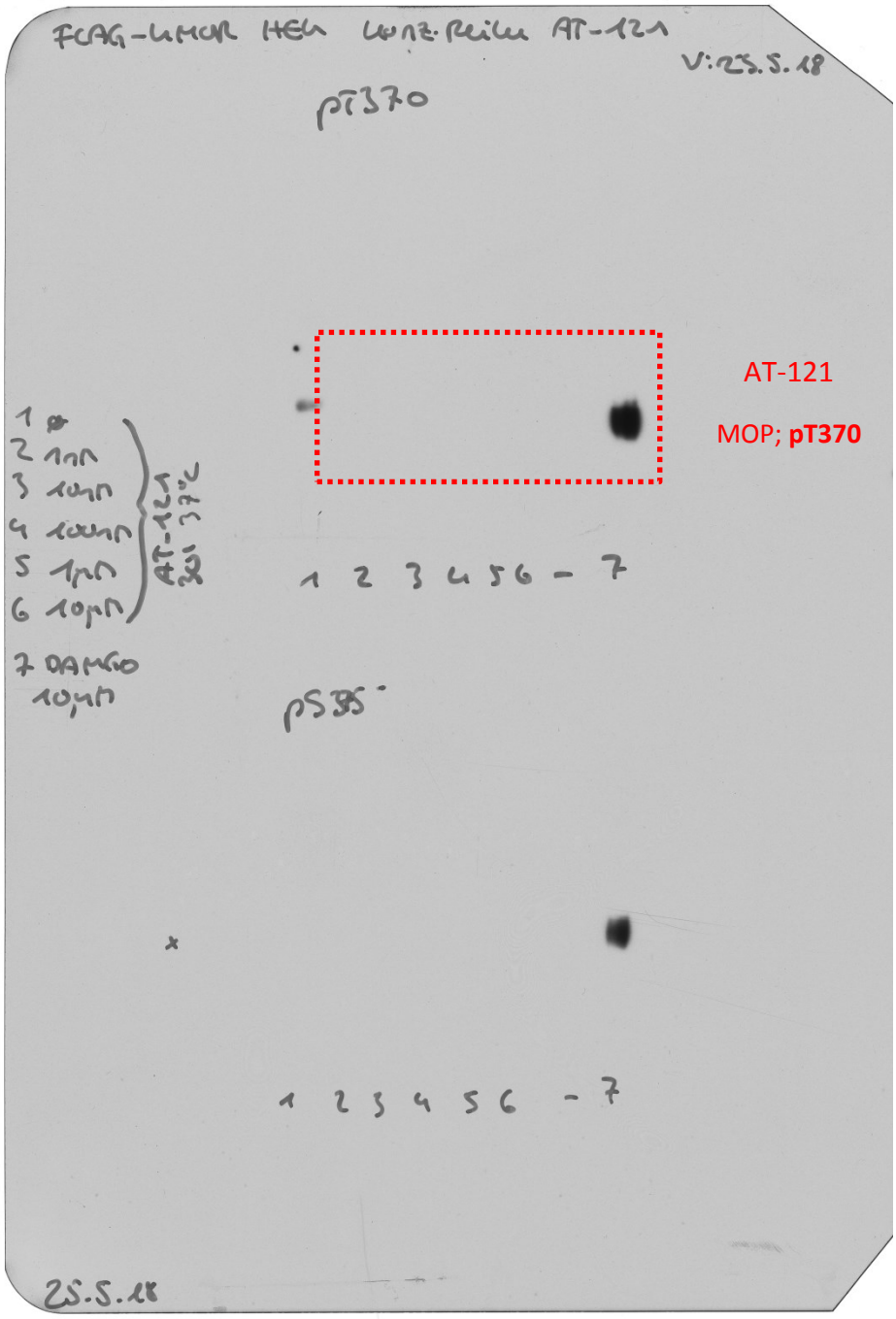

HA-hNOP
HEK
pT362/S363
24.8.18
AT-121
NOP; pT362/S363


AT-121
NOP



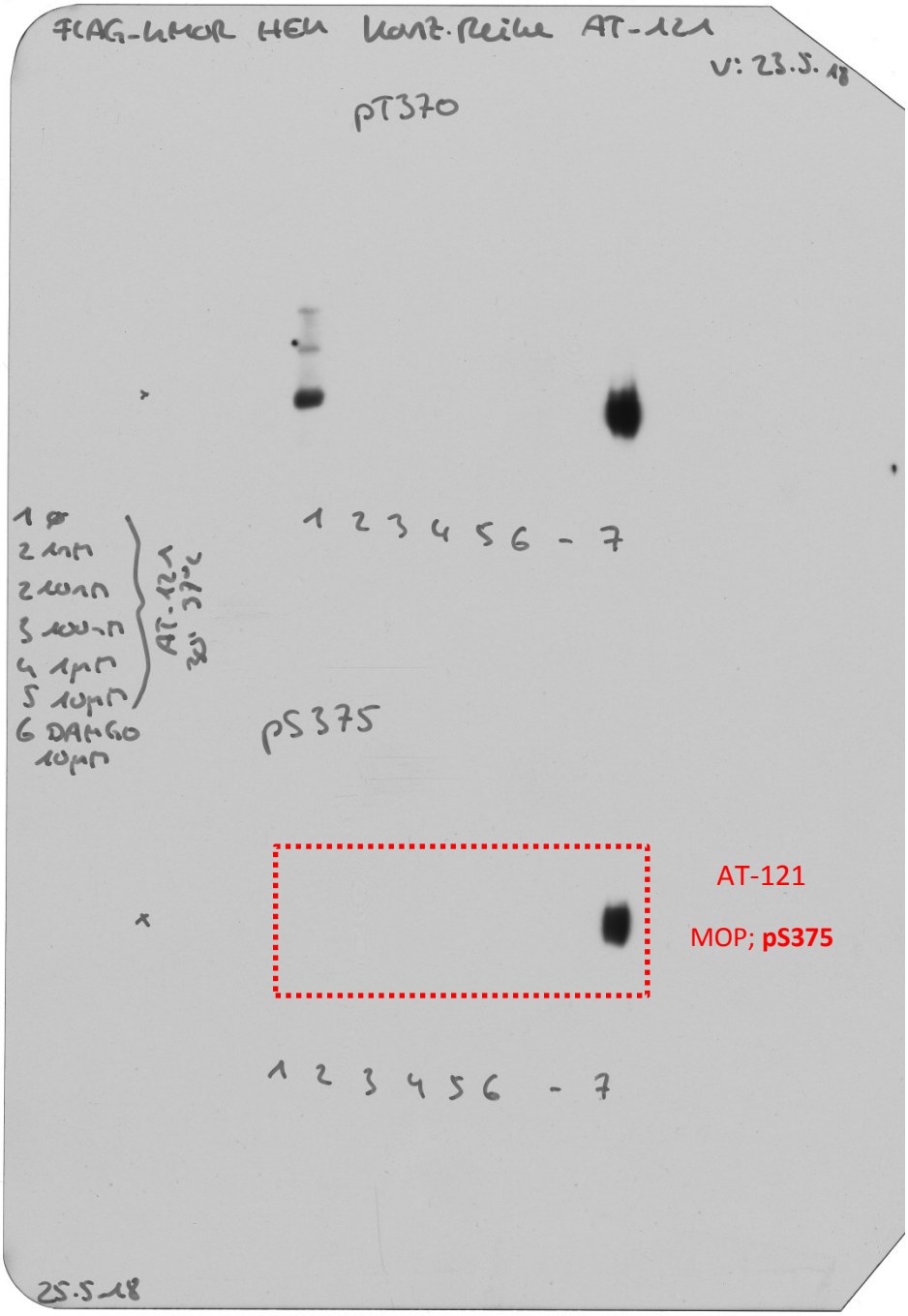
AT-121

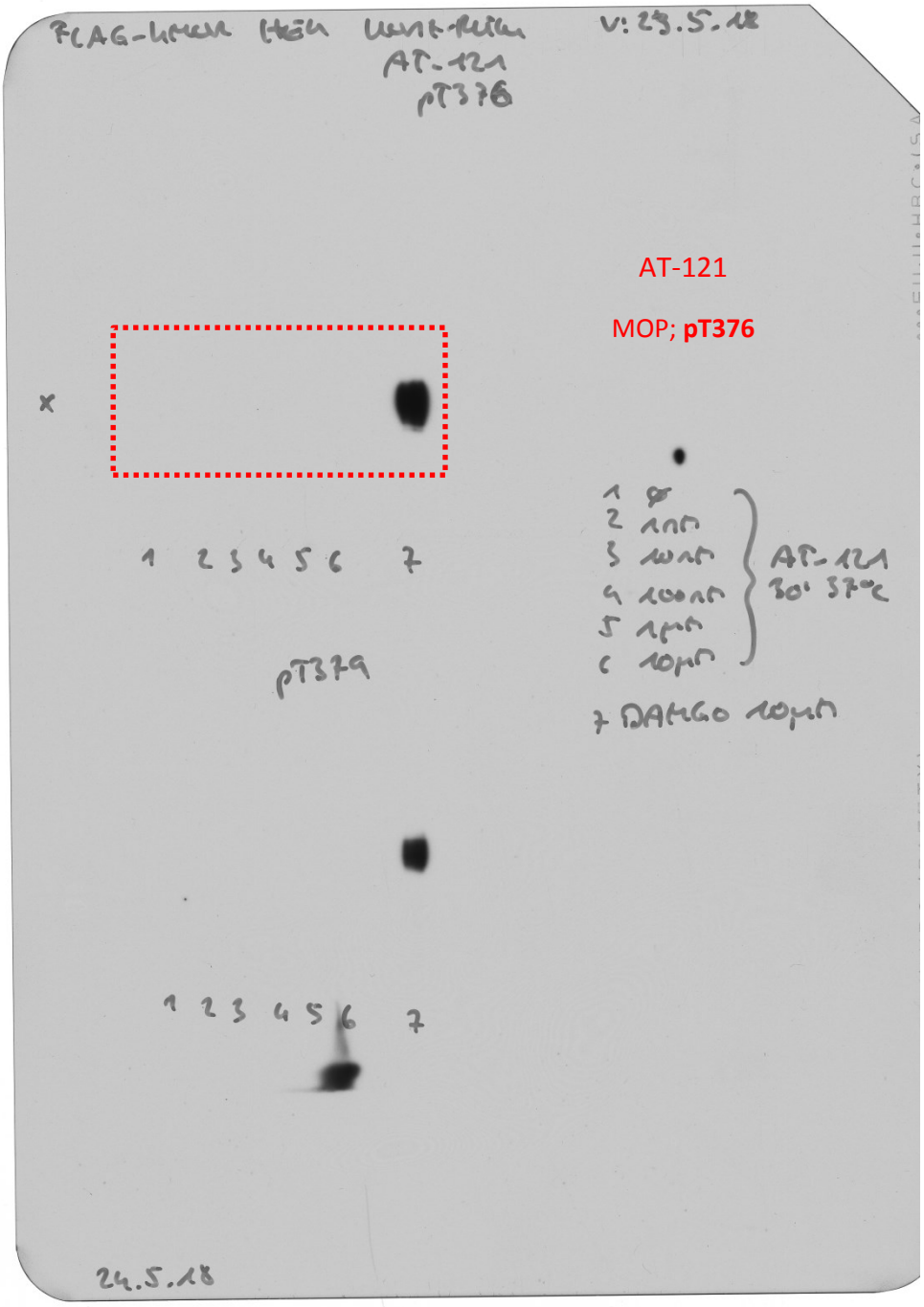
MOP

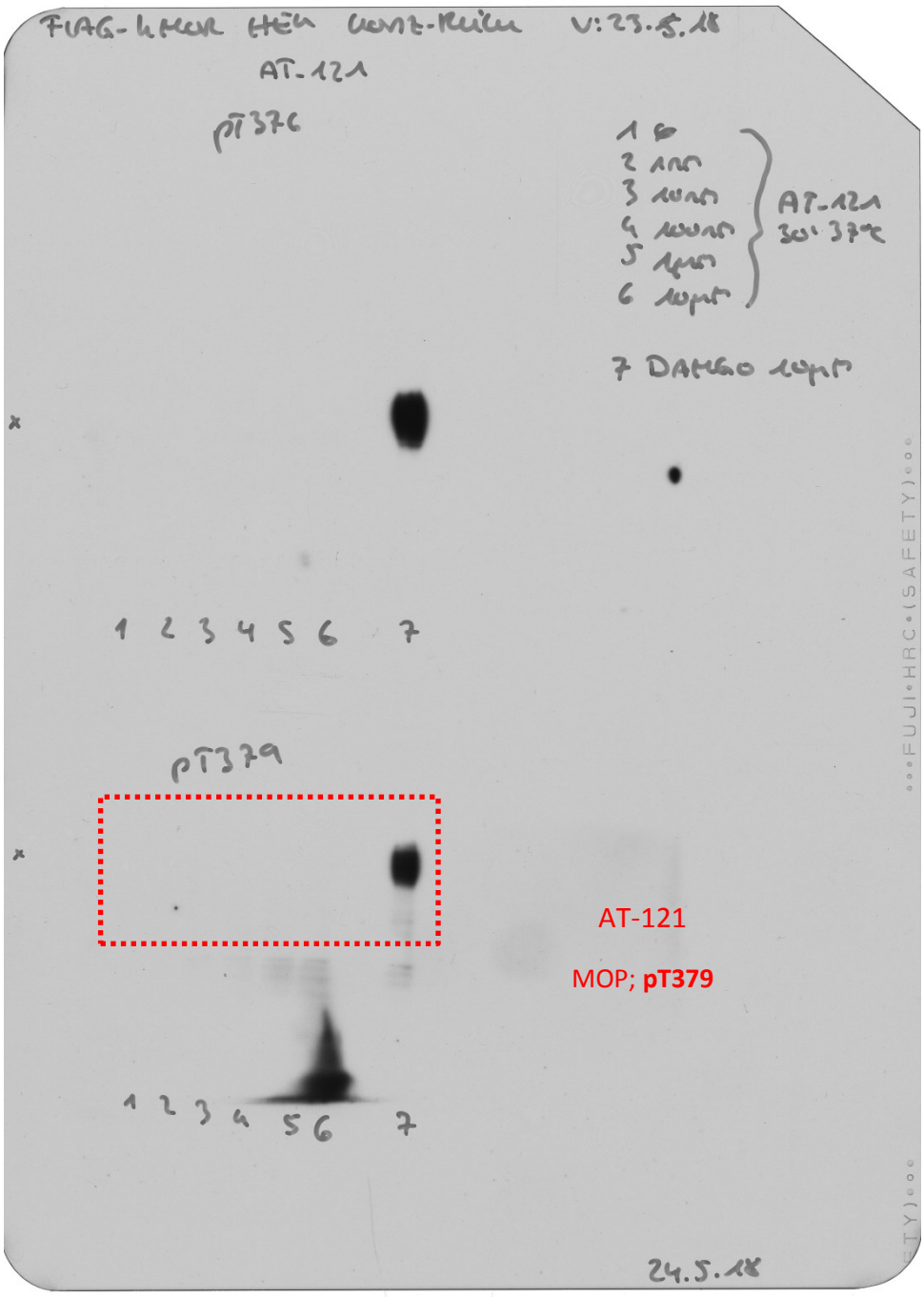


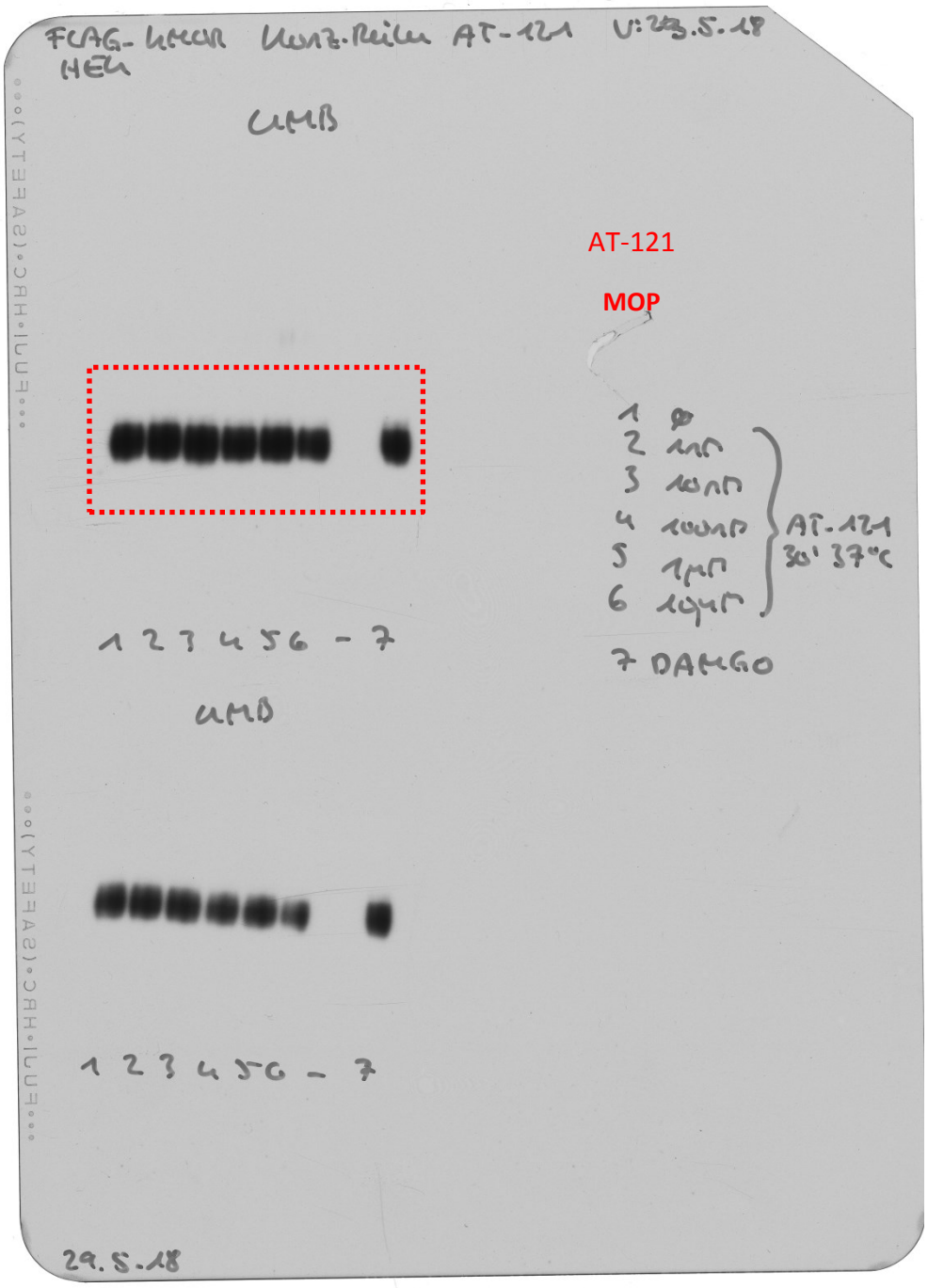
AT-121

MOP; **pT370**


FLAG-LMOR HEK Konz.Reihe AT-121
V: 23.5.18
pT370
1 2 3 4 5 6 - 7
1 ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
DAMGO 10µM
AT-121 30' 37°C
pS375
1 2 3 4 5 6 - 7
25.5.18
AT-121
MOP; pS375

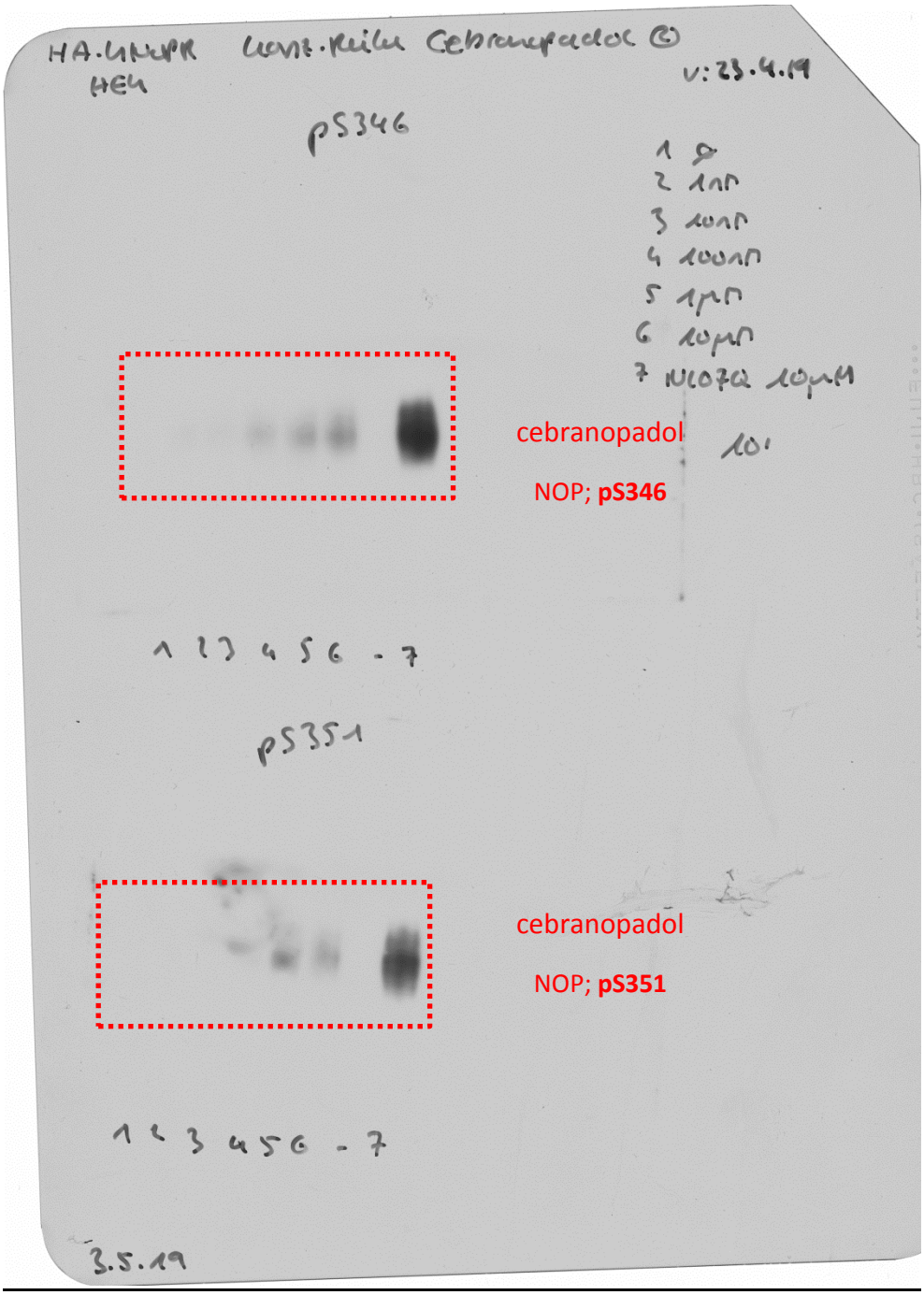

AT-121
MOP; pT376
1 2 3 4 5 6 7
pT379
1 2 3 4 5 6 7
24.5.18

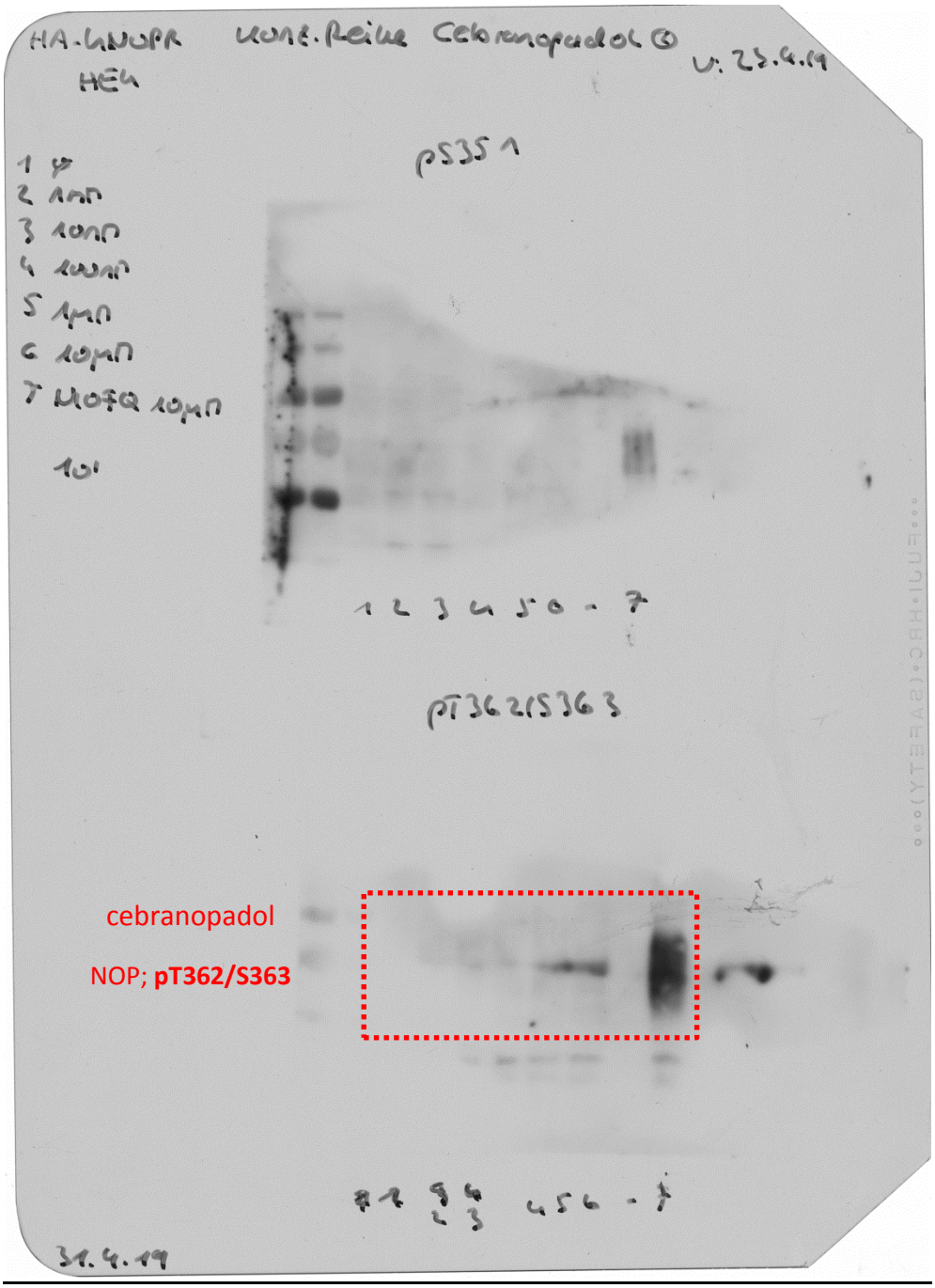

AT-121
pT376
1
2
3
4
5
6
AT-121
30' 37°C
7 DAMGO 10µM
1 2 3 4 5 6 7
pT379
AT-121
MOP; pT379
1 2 3 4 5 6 7
24.5.18

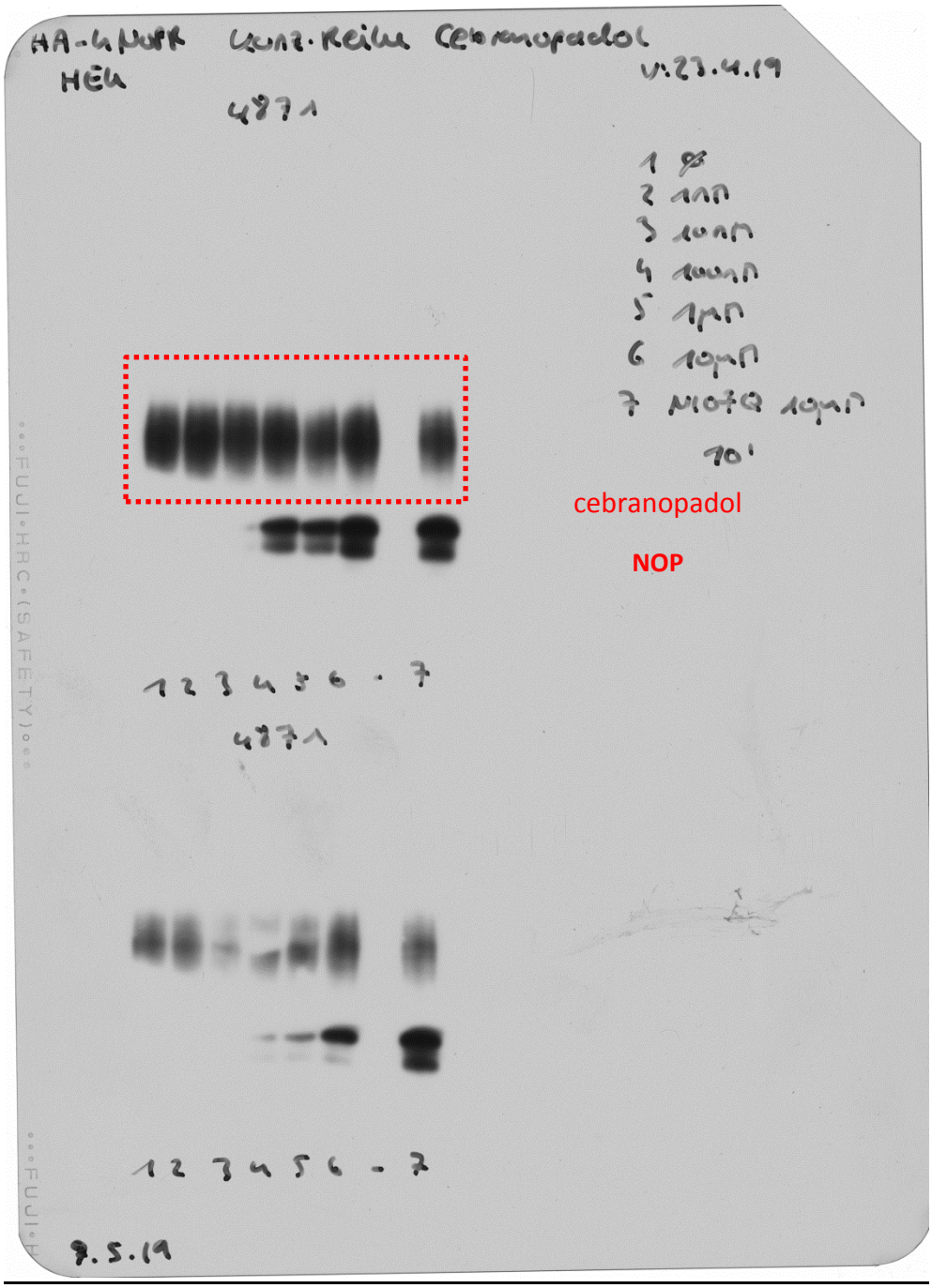

FLAG-hMOR Mutz.Reihe AT-121 V: 23.5.18
HEK
cAMP
AT-121
MOP
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
AT-121
30' 37°C
7 DAMGO
1 2 3 4 5 6 - 7
cAMP
1 2 3 4 5 6 - 7
29.5.18

Cebranopadol

NOP

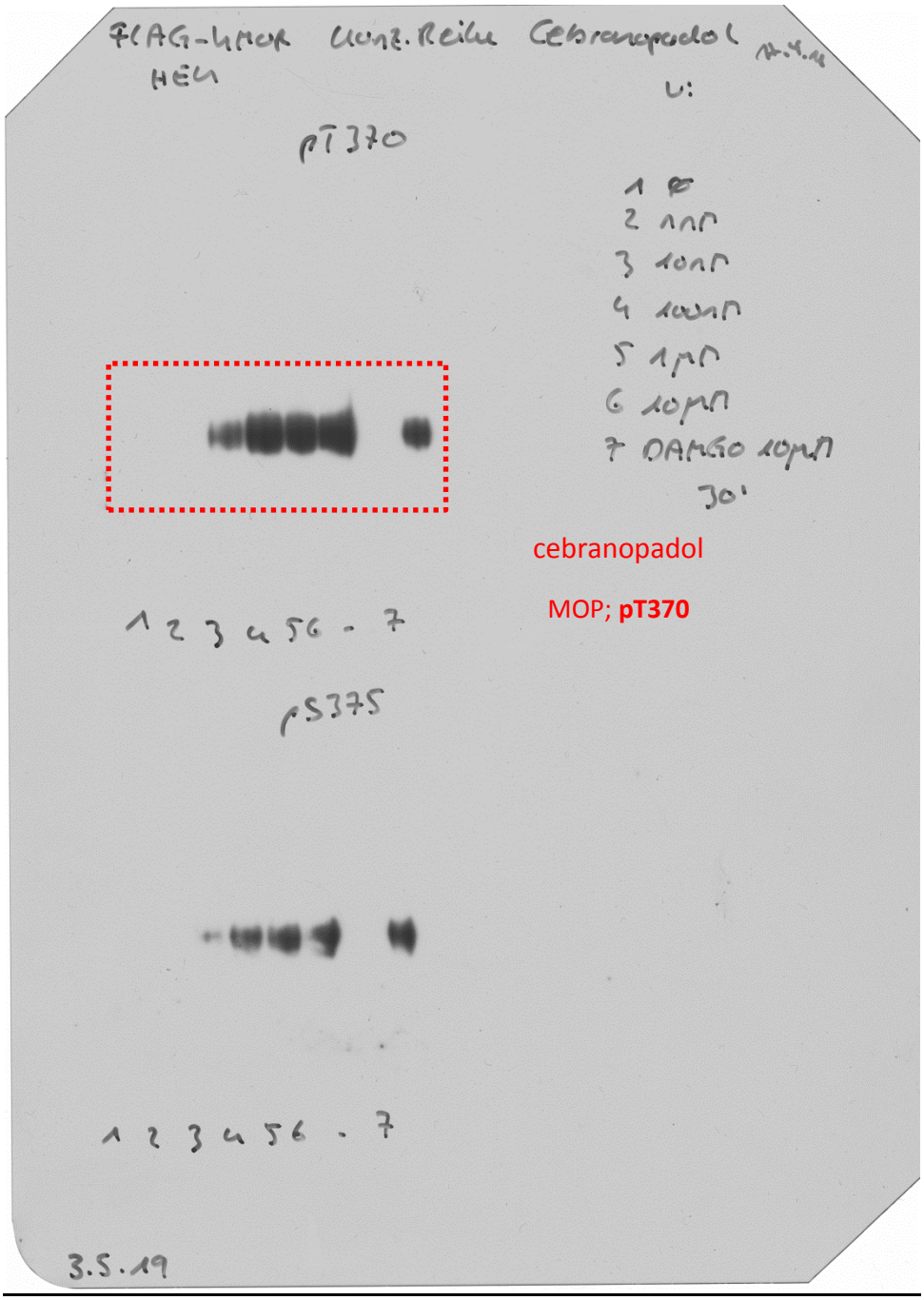

pS346
cebranopadol
NOP; pS346
1 2 3 4 5 6 - 7
pS351
cebranopadol
NOP; pS351
1 2 3 4 5 6 - 7

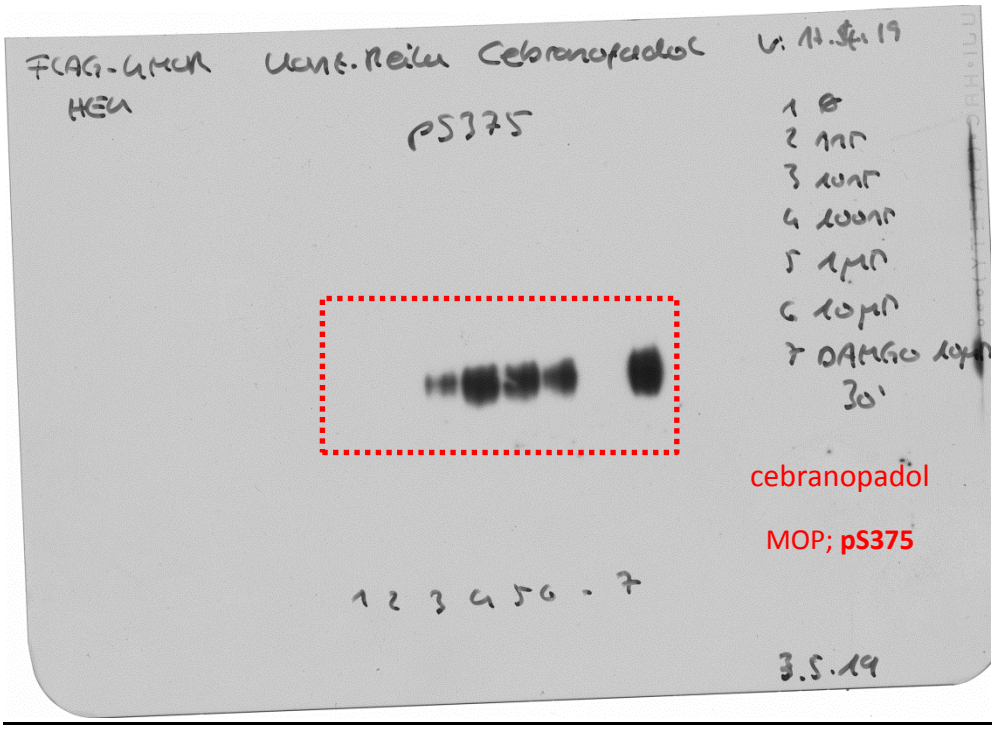

HA-hNOPR
HEK
cont. Reihe Cebranopadol
U: 23.4.19
1 -
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 Nociceptin 10µM
10'
pS35
1 2 3 4 5 6 - 7
pT362/S363
cebranopadol
NOP; pT362/S363
1 2 3 4 5 6 - 7
31.4.19


HA-hNOP HEK
Cont. Reihe Cebranopadol
V. 23.4.19
4871
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 Nal 10µM
90'
cebranopadol
NOP
1 2 3 4 5 6 - 7
4871
1 2 3 4 5 6 - 7
9.5.19
FUJI HRC (SAFETY)
FUJI

Cebranopadol

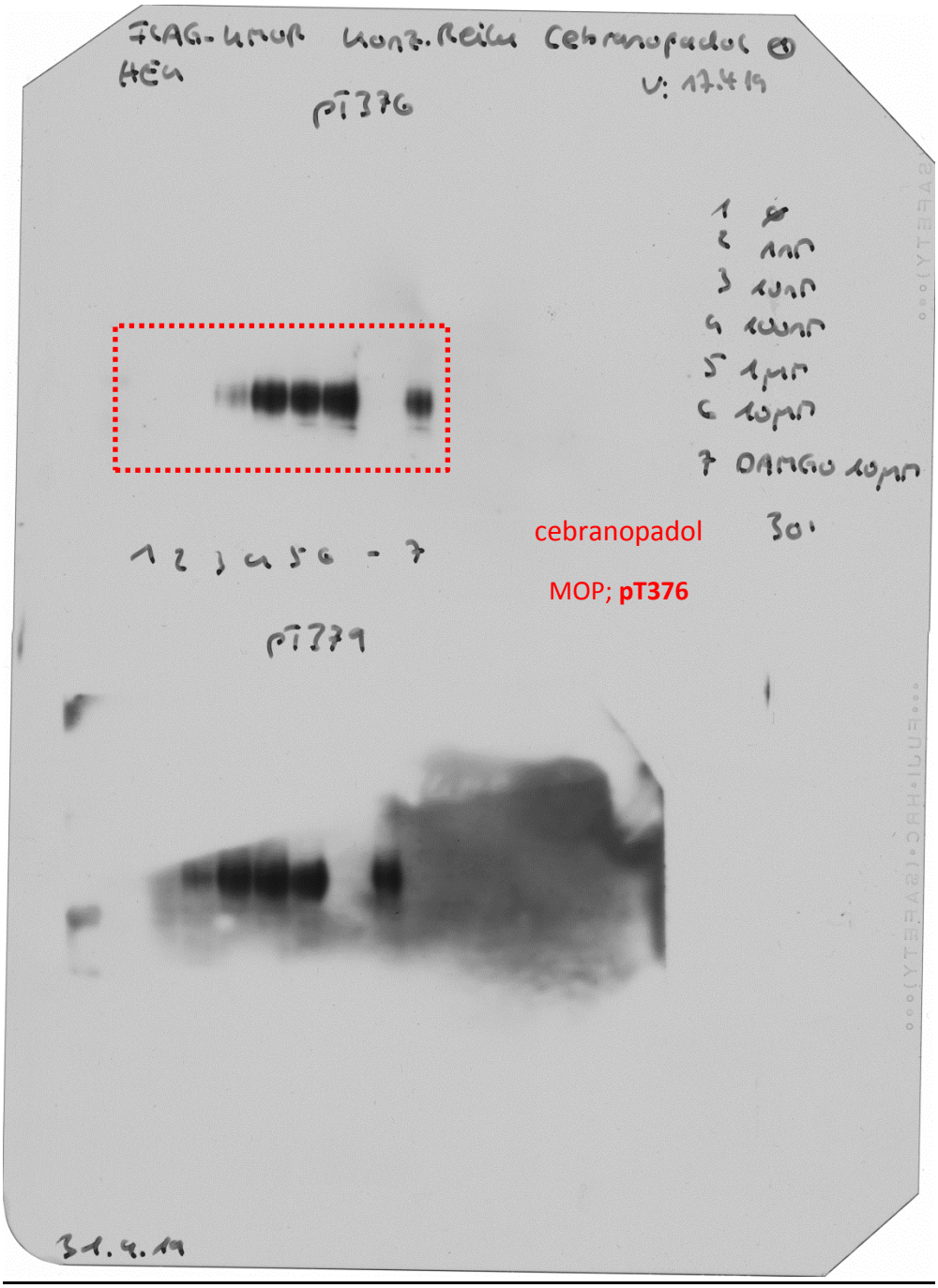
MOP

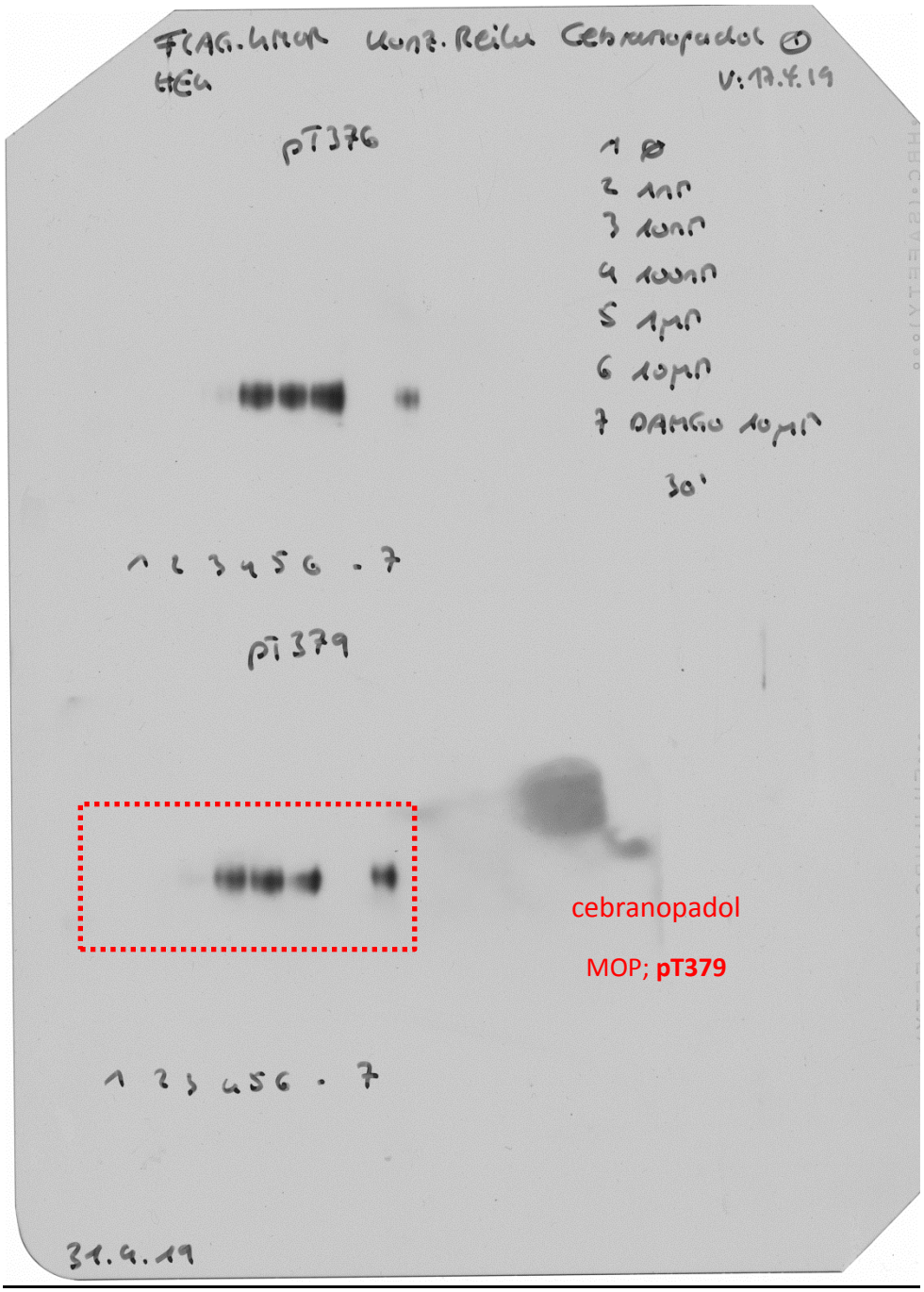

FLAG-hMOR HEK
Konz. Reihe Cebranopadol
pT370
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
7 DAMGO 10µM
30'
cebranopadol
MOP; pT370
1 2 3 4 5 6 - 7
pS375
1 2 3 4 5 6 - 7
3.5.19

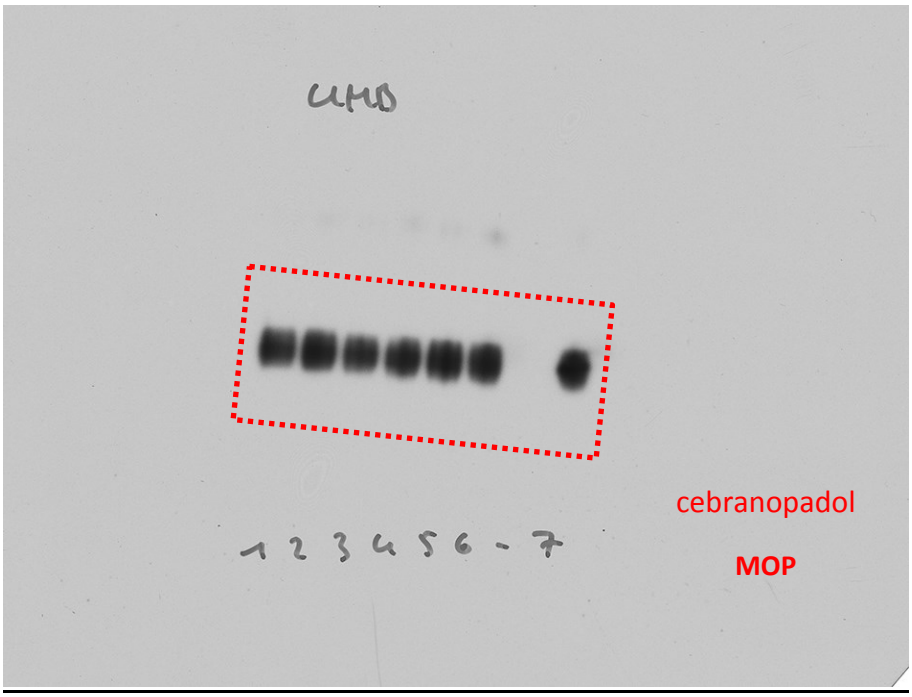


cebranopadol

MOP; **pS375**

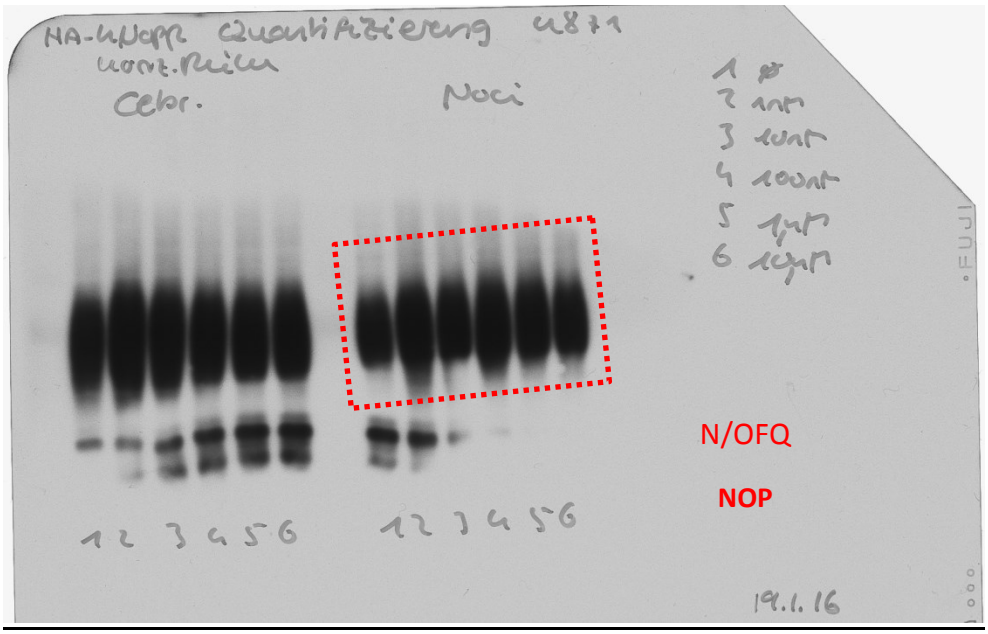

cebranopadol
MOP; pT376

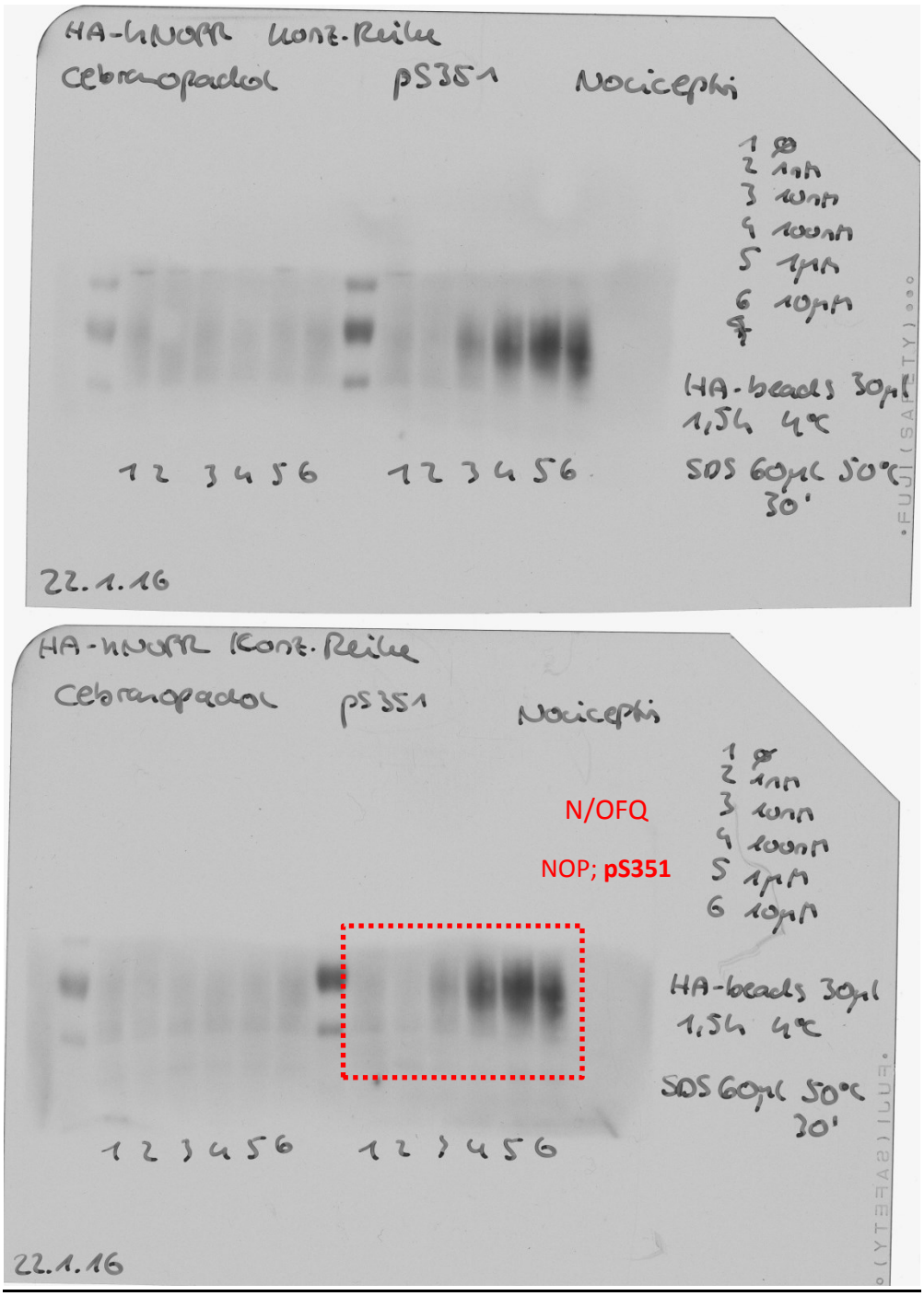

pT376
pT379
cebranopadol
MOP; **pT379**

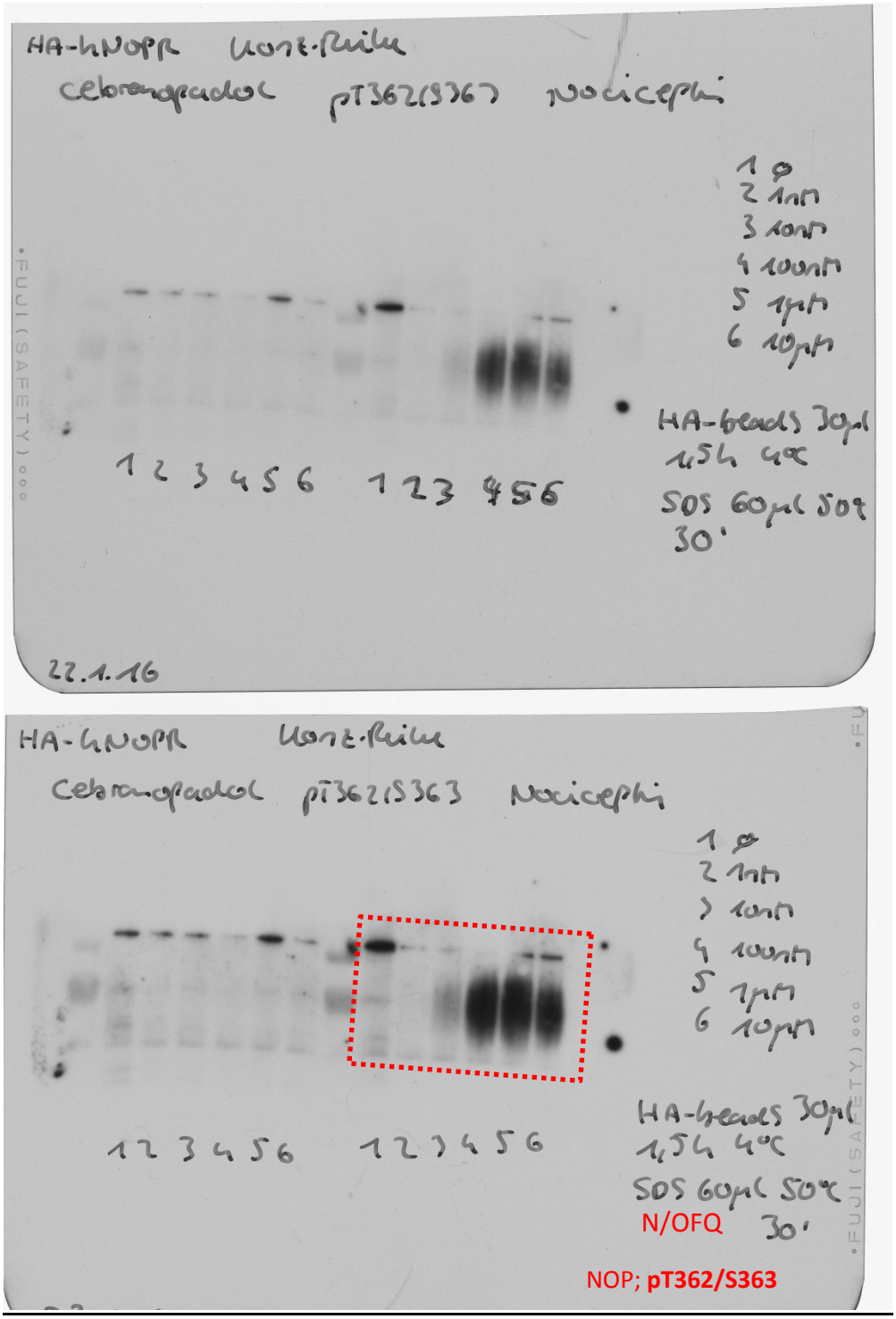

UHS
1 2 3 4 5 6 - 7
cebranopadol
MOP

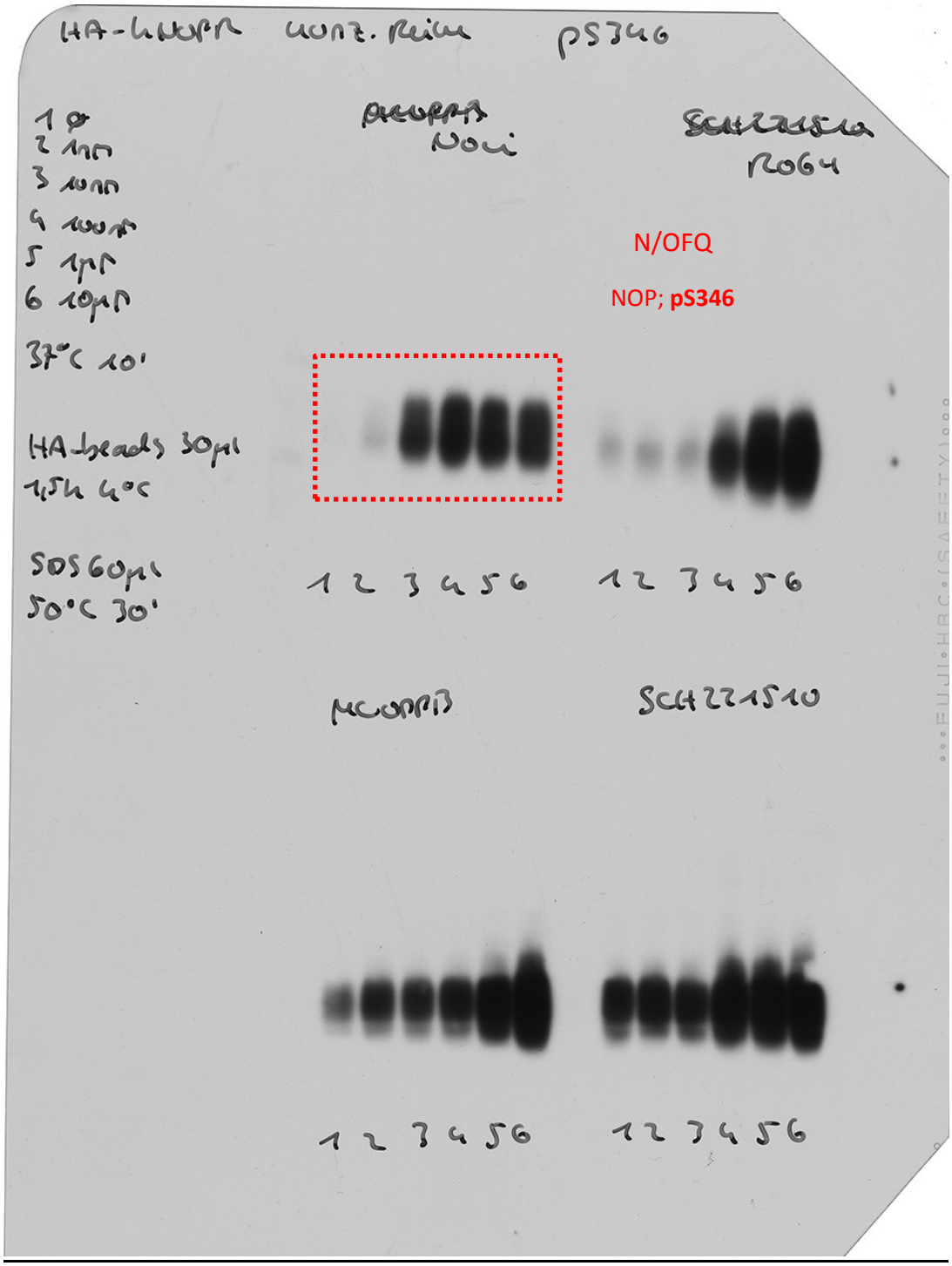
N/OFQ

NOP


HA-hNOPR Quantifizierung 4871
Cebr.
Noci
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
1 2 3 4 5 6
1 2 3 4 5 6
N/OFQ
NOP
19.1.16

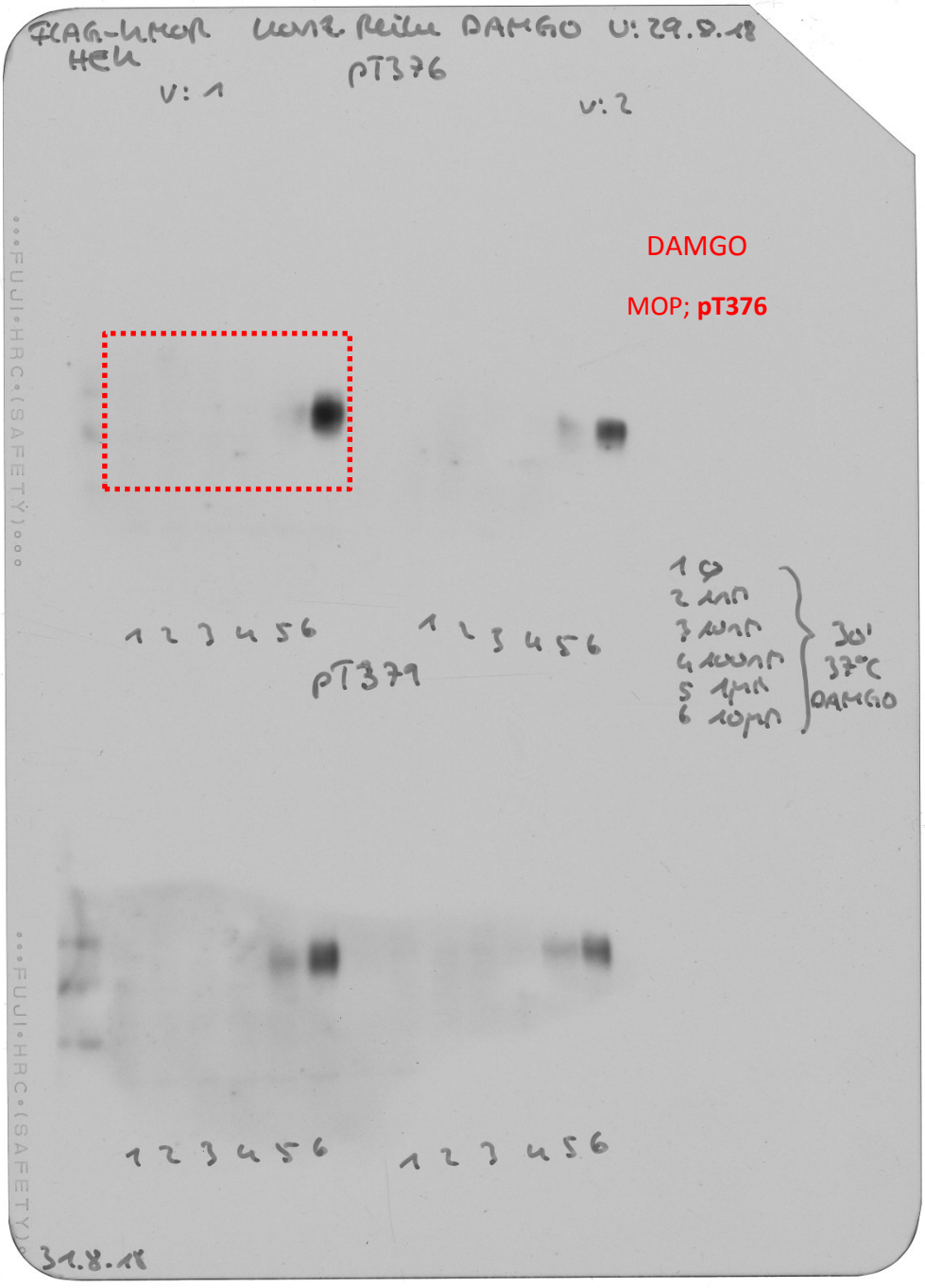

HA-hNOPR Kont. Reihe
Cebranopadol
pS351
Nociceptin
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
HA-beads 30µl
1,5h 4°C
1 2 3 4 5 6
1 2 3 4 5 6
SDS 60µl 50°C
30'
22.1.16
HA-hNOPR Kont. Reihe
Cebranopadol
pS351
Nociceptin
N/OFQ
NOP; pS351
1 Ø
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
HA-beads 30µl
1,5h 4°C
SDS 60µl 50°C
30'
1 2 3 4 5 6
1 2 3 4 5 6
22.1.16

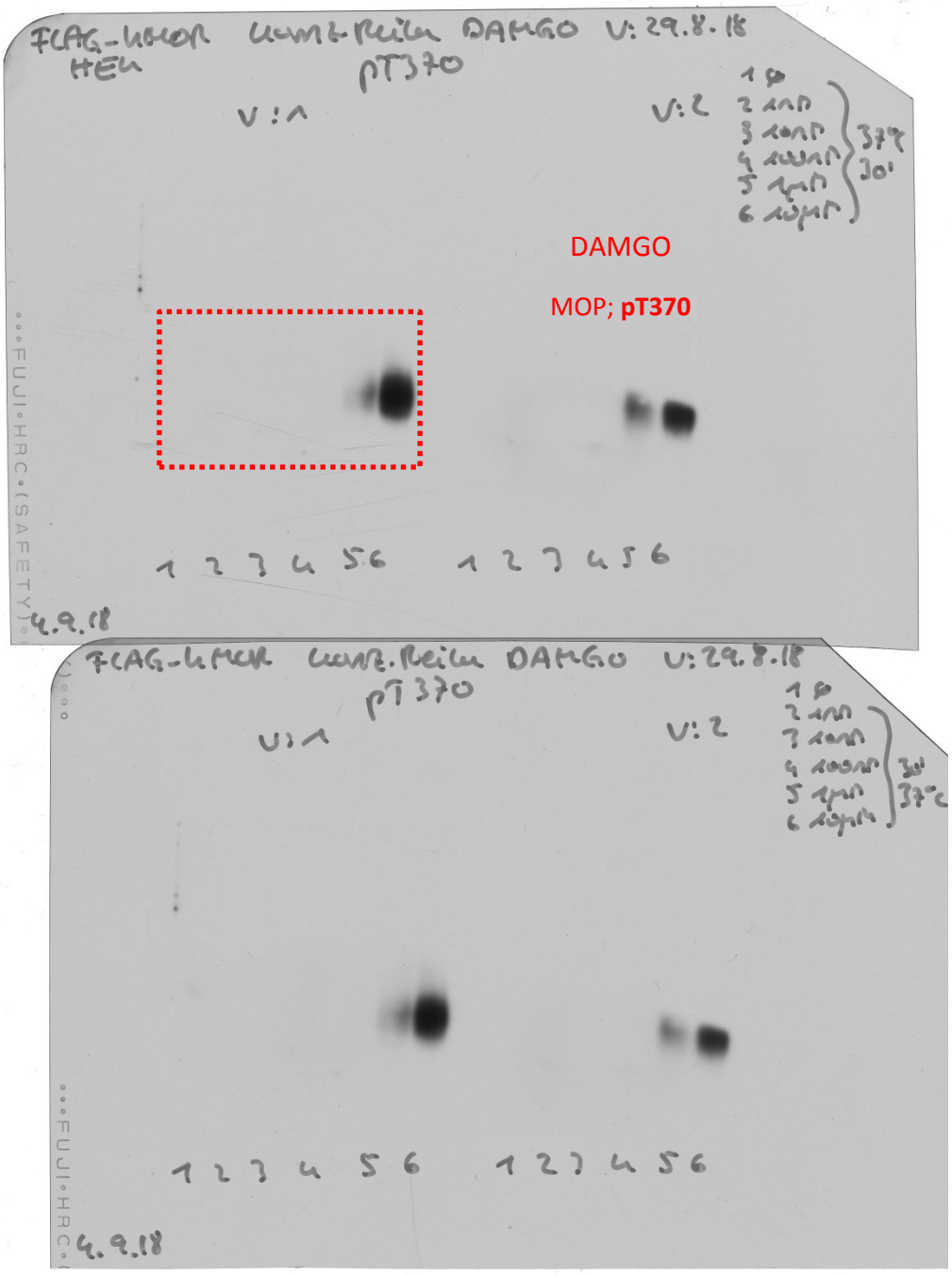

HA-hNOPR
Cebranopadol
pT362/S363
Nociceptin
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
HA-beads 30µl
1,5h 4°C
SDS 60µl 50°C
30'
1 2 3 4 5 6 1 2 3 4 5 6
22.1.16
HA-hNOPR
Cebranopadol
pT362/S363
Nociceptin
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
HA-beads 30µl
1,5h 4°C
SDS 60µl 50°C
N/OFQ 30'
1 2 3 4 5 6 1 2 3 4 5 6
NOP; pT362/S363

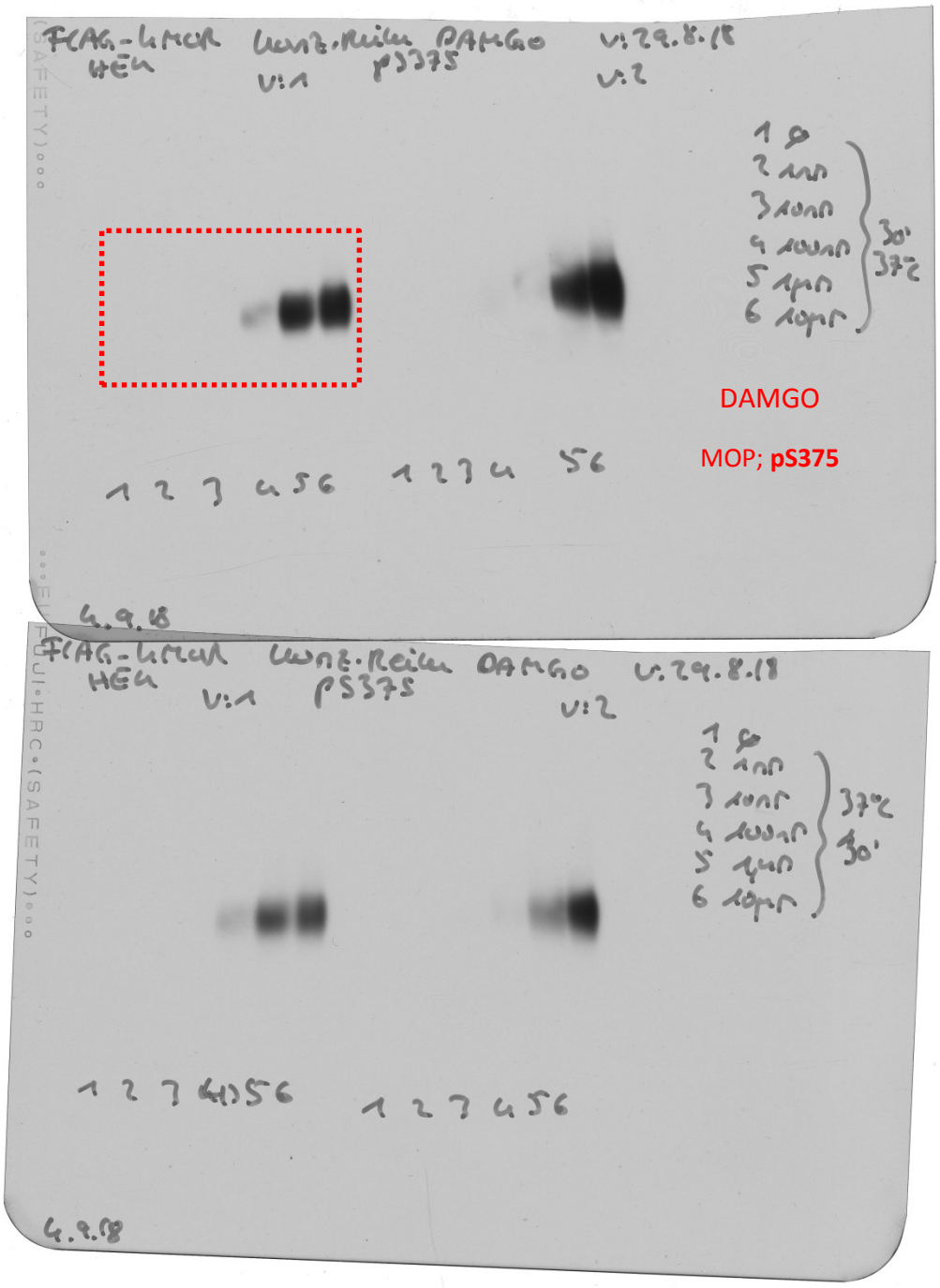

N/OFQ
NOP; pS346
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6

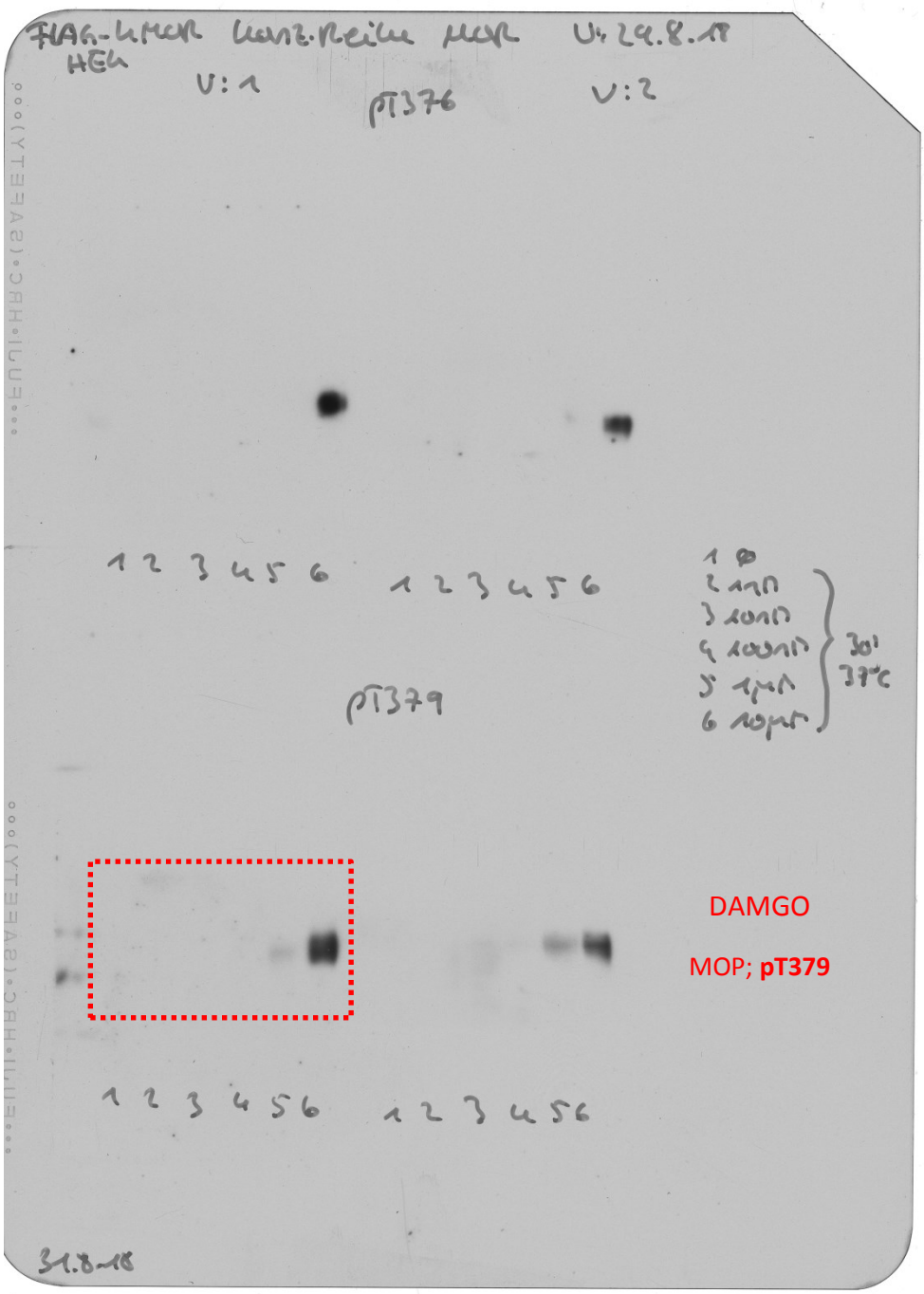
DAMGO

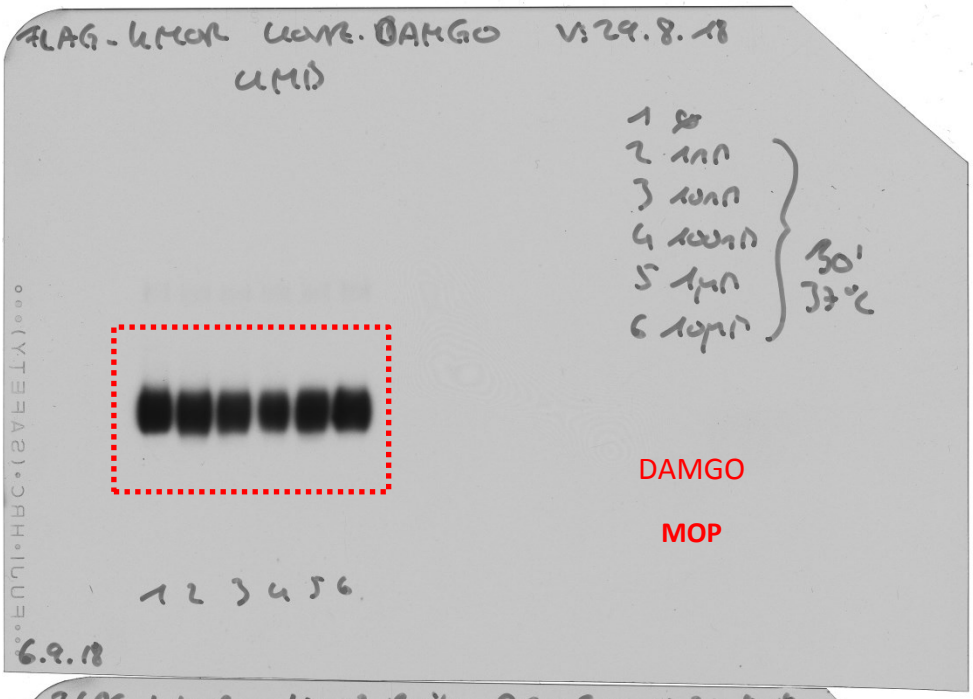
MOP

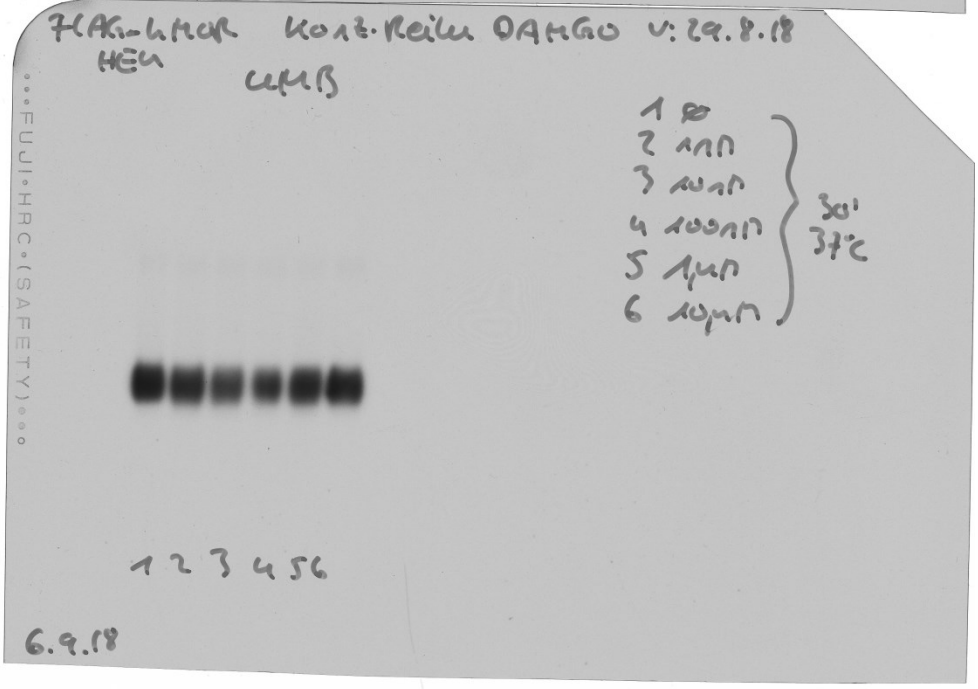

FLAG-hMOR HEK
V: 1
pT376
V: 2
DAMGO
MOP; pT376
1 2 3 4 5 6
1 2 3 4 5 6
pT379
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
30'
37°C
DAMGO
1 2 3 4 5 6
1 2 3 4 5 6
31.8.18


FLAG-hMOR HEK
pT370
DAMGO
MOP; pT370
1 2 3 4 5 6
1 2 3 4 5 6
4.9.18
FLAG-hMOR HEK
pT370
DAMGO
1 2 3 4 5 6
1 2 3 4 5 6
4.9.18


DAMGO
MOP; pS375
30'
37°C
37°C
30'


pT376
pT379
DAMGO
MOP; pT379


FLAG-hMOR HEK. DAMGO V: 29.8.18
HMB
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
30'
37°C
DAMGO
MOP
1 2 3 4 5 6
6.9.18


FLAG-hMOR HEK Konz.Reihe DAMGO V: 29.8.18
HMB
1 0
2 1nM
3 10nM
4 100nM
5 1µM
6 10µM
30'
37°C
1 2 3 4 5 6
6.9.18

Supplementary figure 1. Uncropped images of western blots depicted in figure 4. For NOP, the rabbit polyclonal phosphosite-specific anti-S346 7TM0320A (previously known as 5034), anti-pS351 7TM0320B (previously known as 4876) and anti-pT362/S363 7TM0320C (previously known as 4874) as well as the phosphorylation-independent anti-NOP 7TM0320N (previously known as 4871) antibodies were used. The monoclonal phosphorylation-independent anti-MOP receptor antibody 7TM0319N (previously known as UMB-3) and phosphosite-specific antibodies anti-pT370 7TM0319B1 (previously known as 3196), anti-pS375 7TM0319C (previously known as 2493), anti-pT376 7TM0319D (previously known as 3723) and anti-pT379 7TM0319E (previously known as 3686) were used for MOP. The cropped parts of the blots used in figure 4 are depicted in red boxes.