Fig. 1

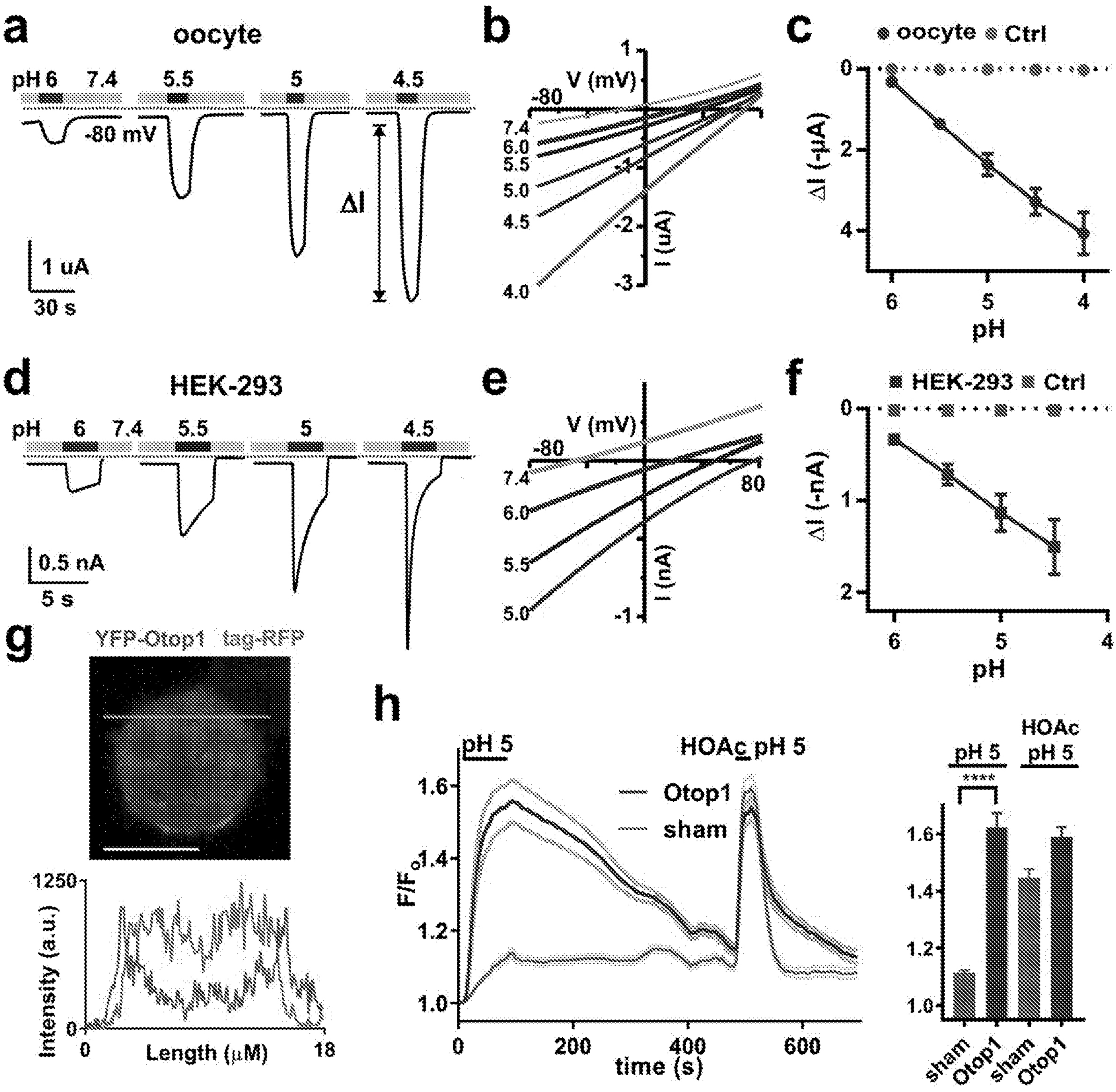

a
oocyte
pH 6
7.4
5.5
5
4.5
-80 mV
ΔI
1 uA
30 s
b
V (mV)
-80
1
7.4
6.0
5.5
5.0
4.5
4.0
-2
-3
I (uA)
c
oocyte
Ctrl
0
2
4
ΔI (-μA)
6
5
4
pH
d
HEK-293
pH
6
7.4
5.5
5
4.5
0.5 nA
5 s
e
V (mV)
-80
80
7.4
6.0
5.5
5.0
-1
I (nA)
f
HEK-293
Ctrl
0
1
2
ΔI (-nA)
6
5
4
pH
g
YFP-Otop1
tag-RFP
1250
0
Intensity (a.u.)
0
Length (μM)
18
h
pH 5
HOAc pH 5
Otop1
sham
1.6
1.4
1.2
1.0
F/F0
0
200
400
600
time (s)
HOAc
pH 5
pH 5
****
1.6
1.4
1.2
1.0
sham
Otop1
sham
Otop1

Fig. 2

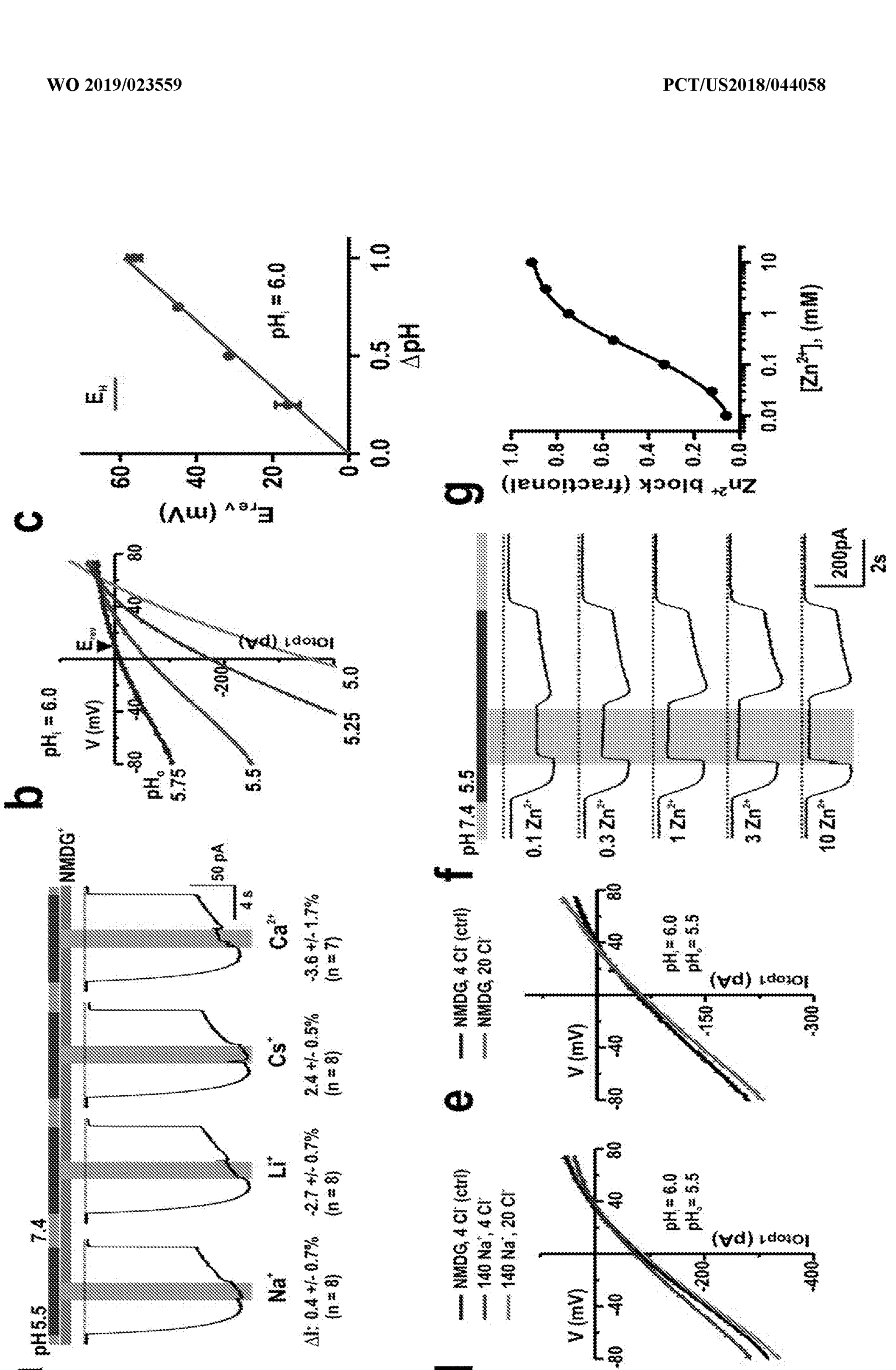

a
pH5.5
7.4
NMDG+
50 pA
4 s
Na+
Li+
Cs+
Ca2+
ΔI: 0.4 +/- 0.7% (n = 8)
-2.7 +/- 0.7% (n = 8)
2.4 +/- 0.5% (n = 8)
-3.6 +/- 1.7% (n = 7)
b
pHi = 6.0
V (mV)
pHo
5.75
5.5
5.25
5.0
Itotp1 (pA)
c
pHi = 6.0
ΔpH
Erev (mV)
d
NMDG, 4 Cl- (ctrl)
140 Na+, 4 Cl-
140 Na+, 20 Cl-
pHi = 6.0
pHo = 5.5
e
NMDG, 4 Cl- (ctrl)
NMDG, 20 Cl-
f
pH 7.4
pH 5.5
0.1 Zn2+
0.3 Zn2+
1 Zn2+
3 Zn2+
10 Zn2+
200pA
2s
g
Zn2+ block (fractional)
[Zn2+]o (mM)

Fig. 3

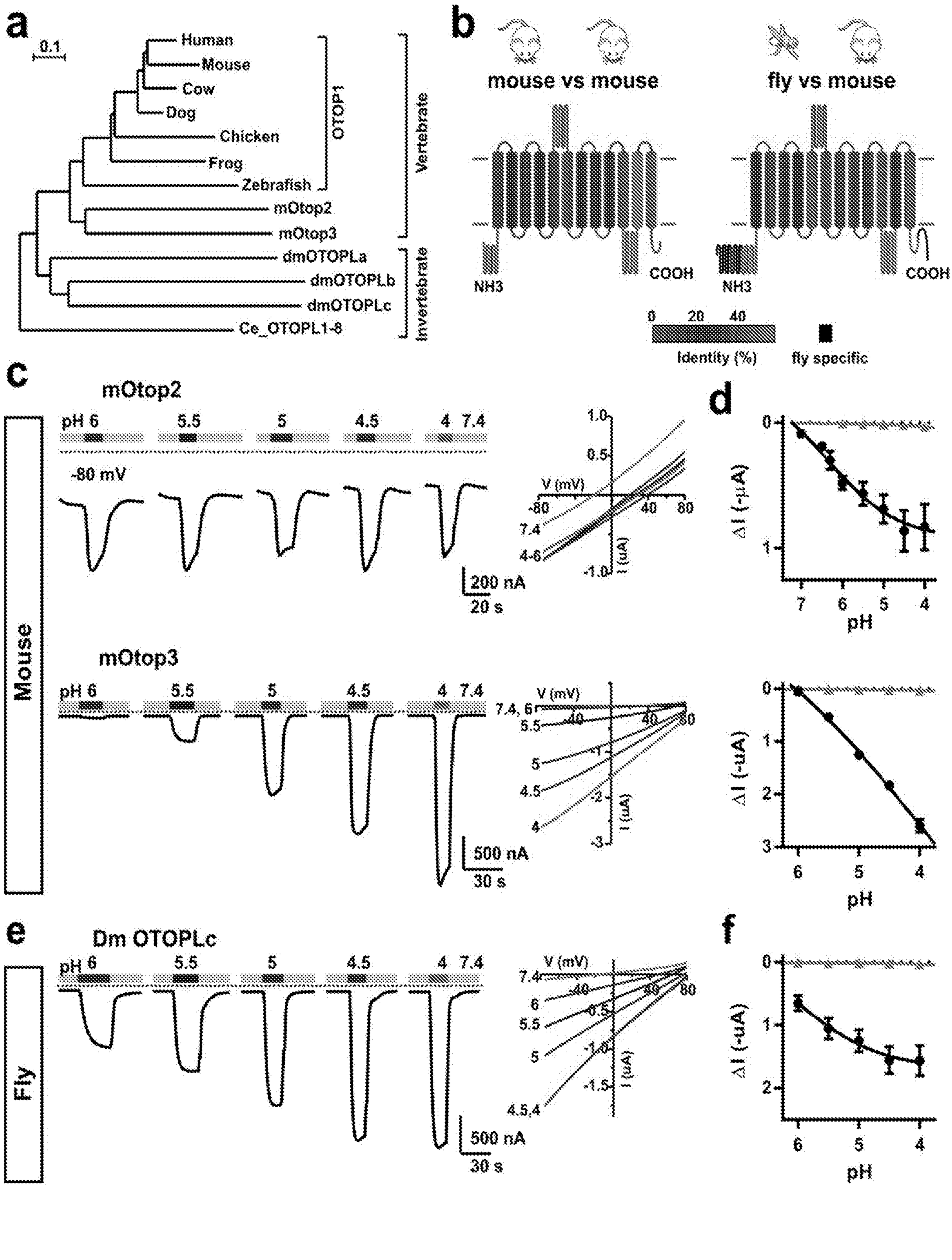

a
0.1
Human
Mouse
Cow
Dog
Chicken
Frog
Zebrafish
mOtop2
mOtop3
dmOTOPLa
dmOTOPLb
dmOTOPLc
Ce_OTOPL1-8
OTOP1
Vertebrate
Invertebrate
b
mouse vs mouse
fly vs mouse
NH3
COOH
NH3
COOH
0 20 40
Identity (%)
fly specific
c
mOtop2
pH 6
5.5
5
4.5
4 7.4
-80 mV
200 nA
20 s
V (mV)
-80
40
80
7.4
4-6
1.0
0.5
-1.0
I (μA)
d
ΔI (-μA)
0
1
7
6
5
4
pH
Mouse
mOtop3
pH 6
5.5
5
4.5
4 7.4
500 nA
30 s
V (mV)
7.4, 6
5.5
5
4.5
4
-40
40
80
-2
-3
I (μA)
ΔI (-uA)
0
1
2
3
6
5
4
pH
e
Dm OTOPLc
Fly
pH 6
5.5
5
4.5
4 7.4
500 nA
30 s
V (mV)
7.4
6
5.5
5
4.5,4
-40
-80
-0.5
-1.0
-1.5
I (μA)
ΔI (-uA)
0
1
2
6
5
4
pH
f

Fig. 4

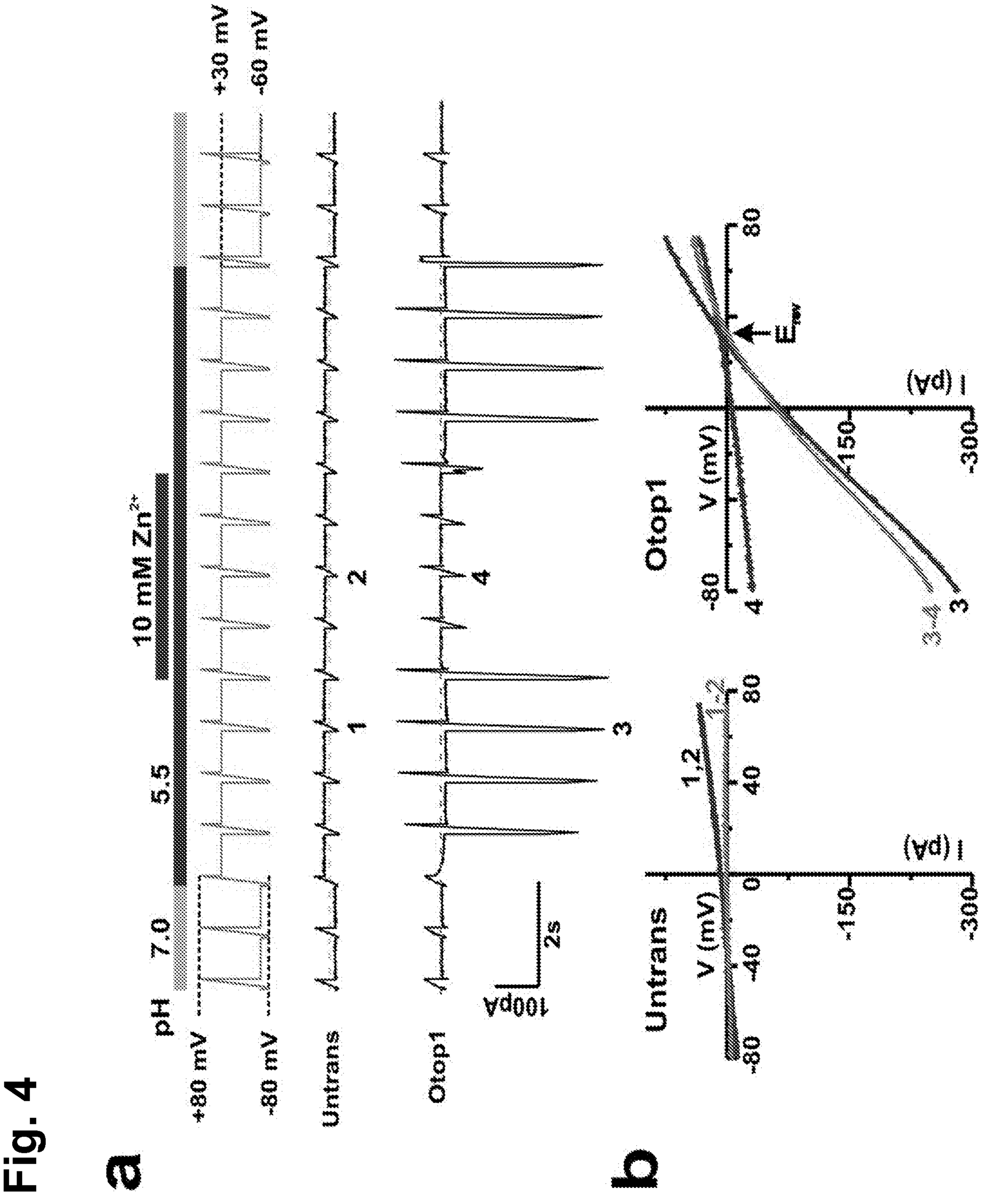
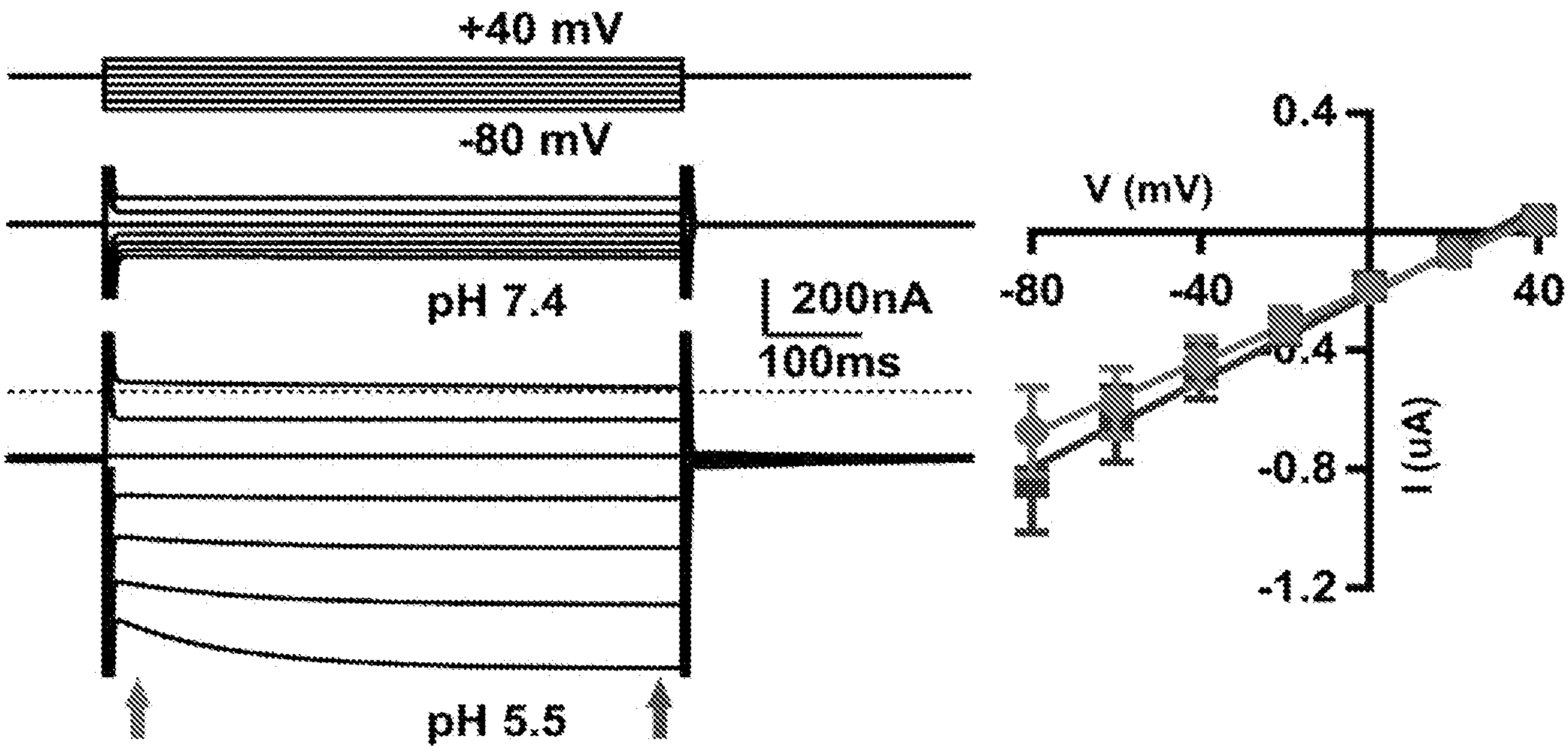

a
+80 mV
-80 mV
Untrans
Otop1
100pA
2s
1
2
3
4
pH 7.0 5.5
10 mM Zn2+
b
Otop1
V (mV)
-80
80
-150
-300
I (pA)
Untrans
1,2
40
0
-40

Fig. 5



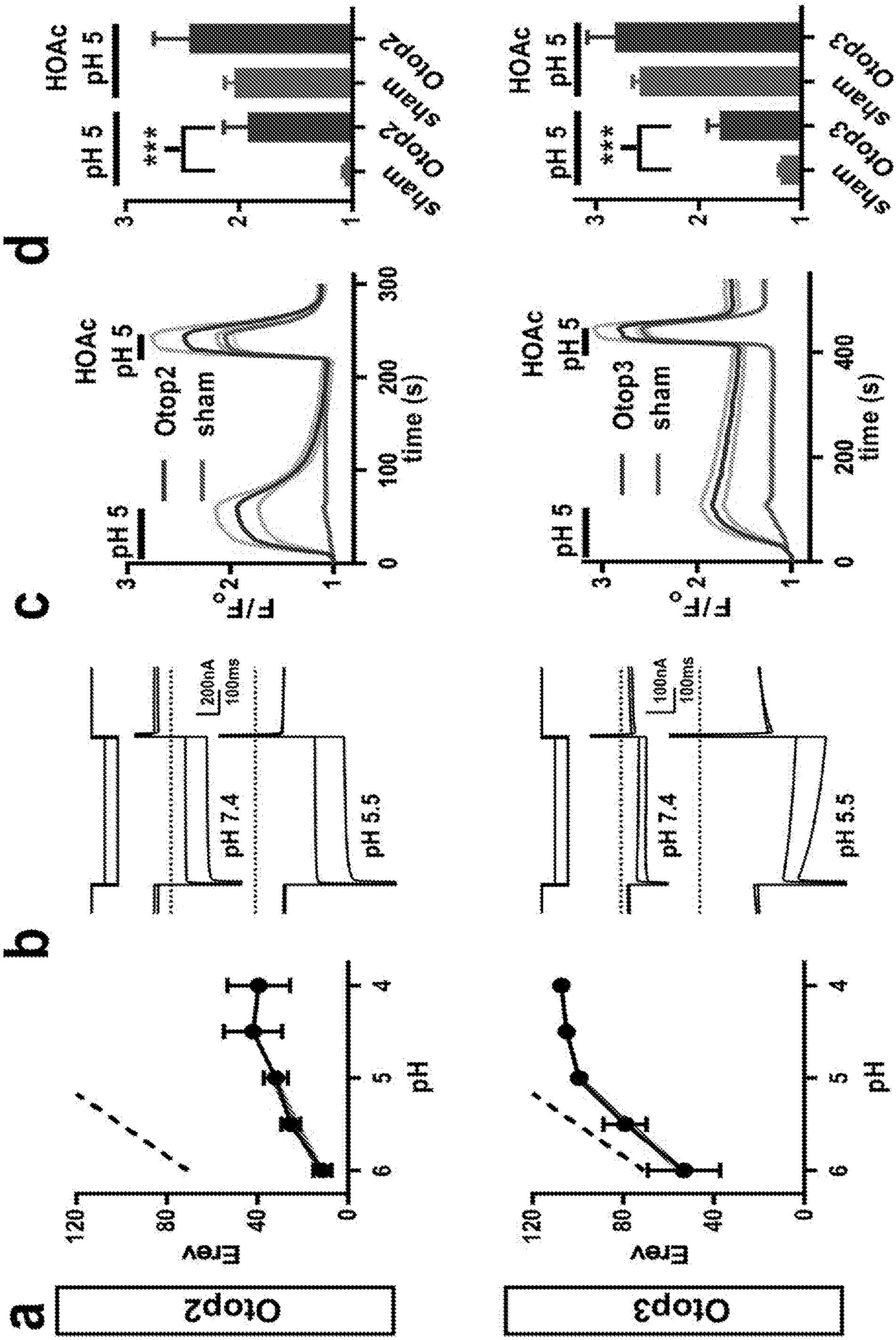

a
Otop2
Otop3
Erev
pH
b
pH 7.4
pH 5.5
200nA
100ms
c
HOAc
pH 5
Otop2
sham
F/F0
time (s)
d
Otop3
***

Fig. 6

Fig. 7

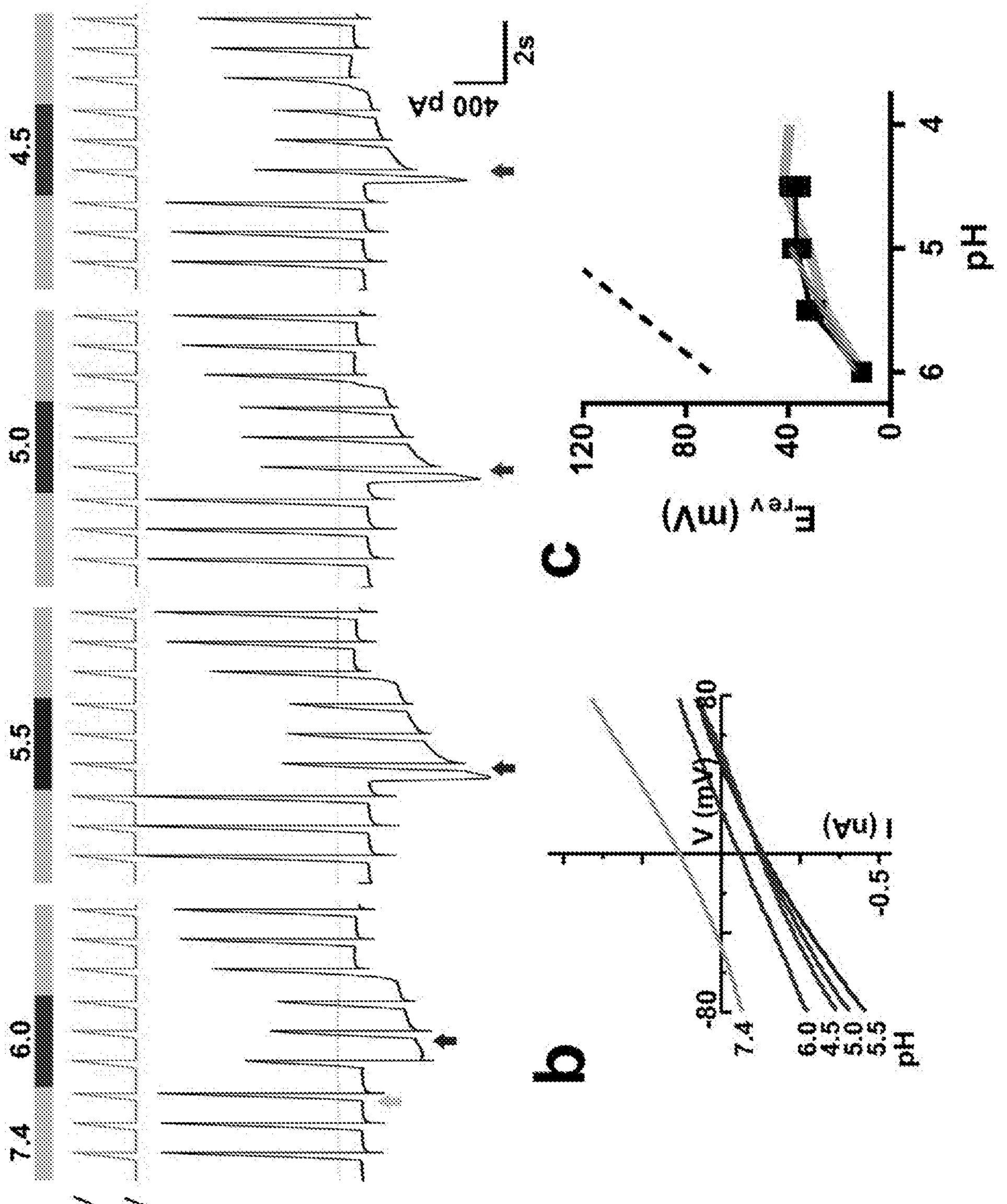
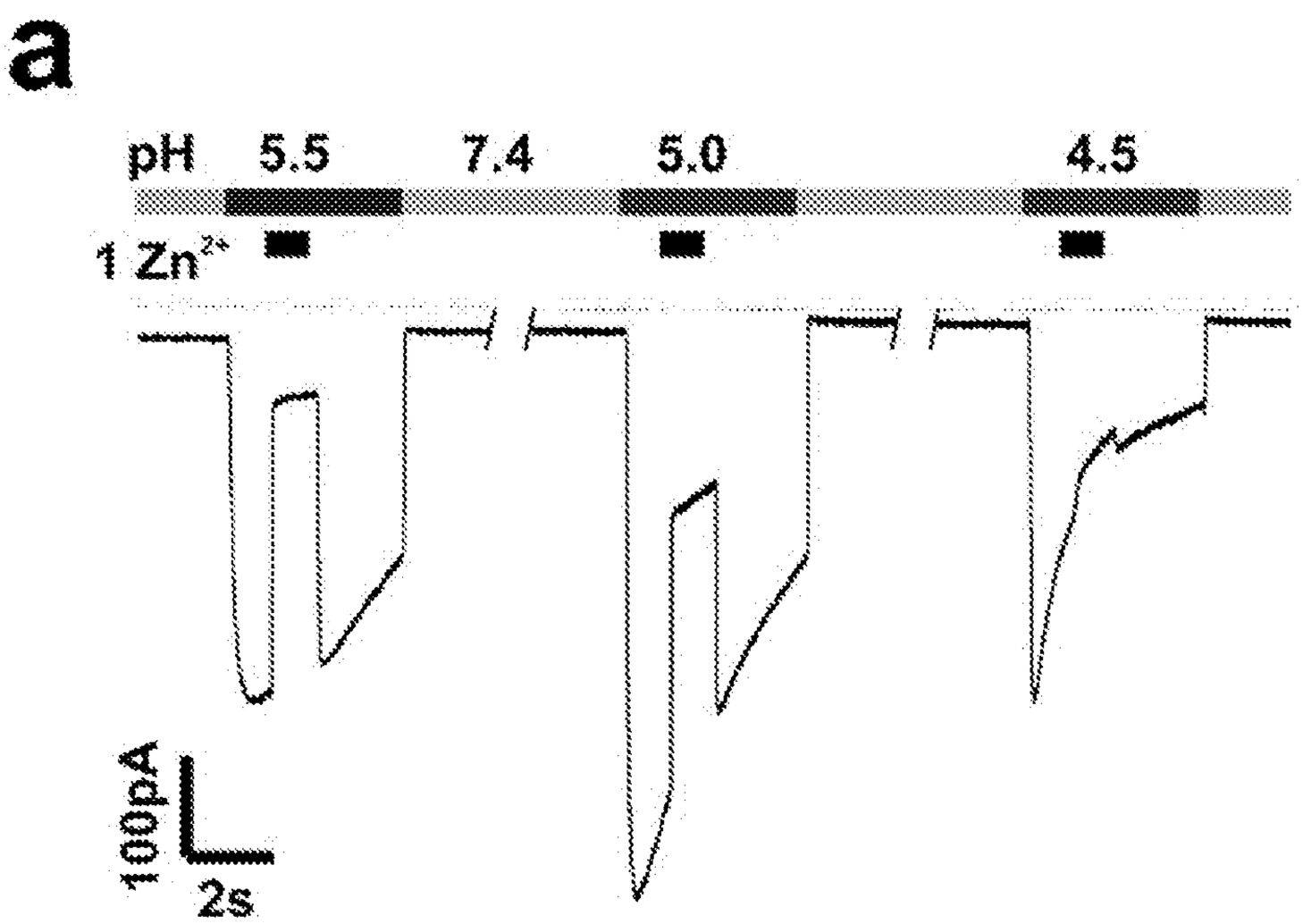
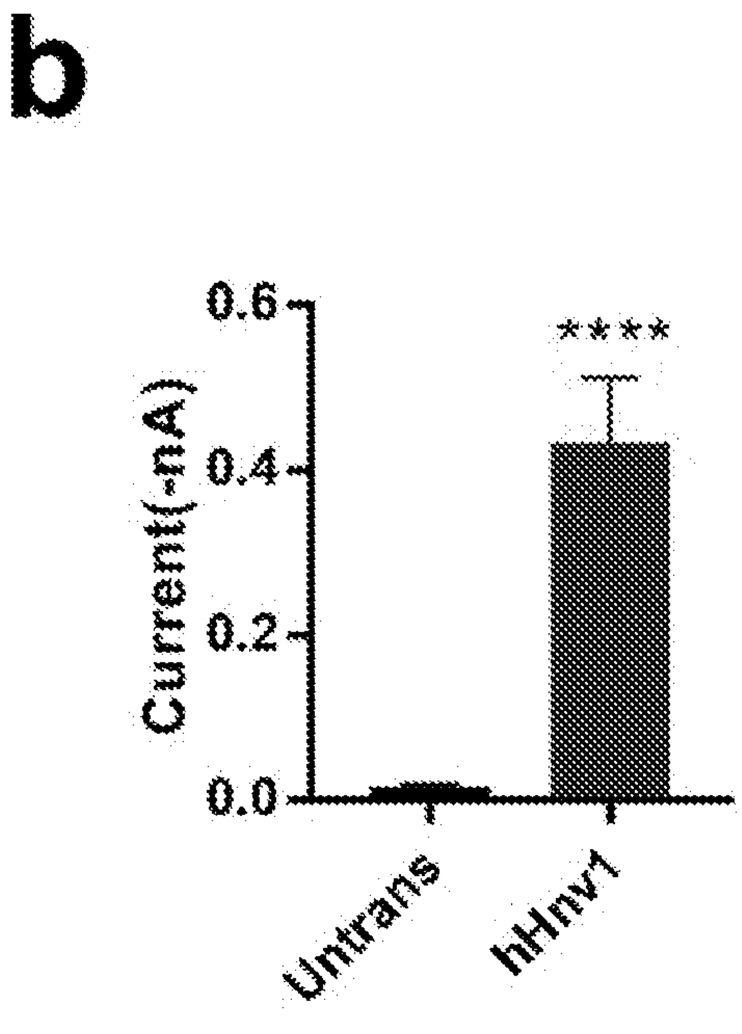
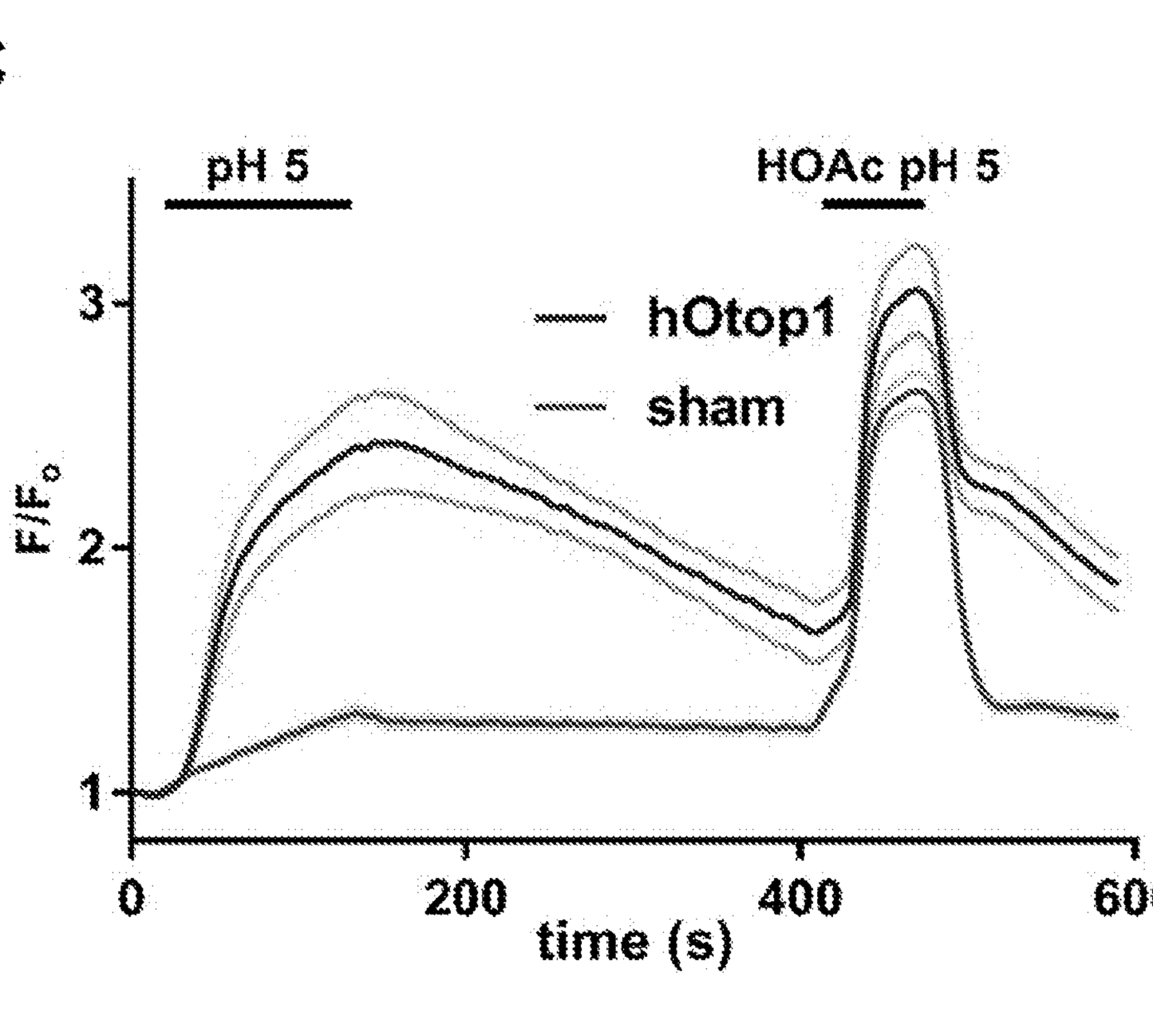
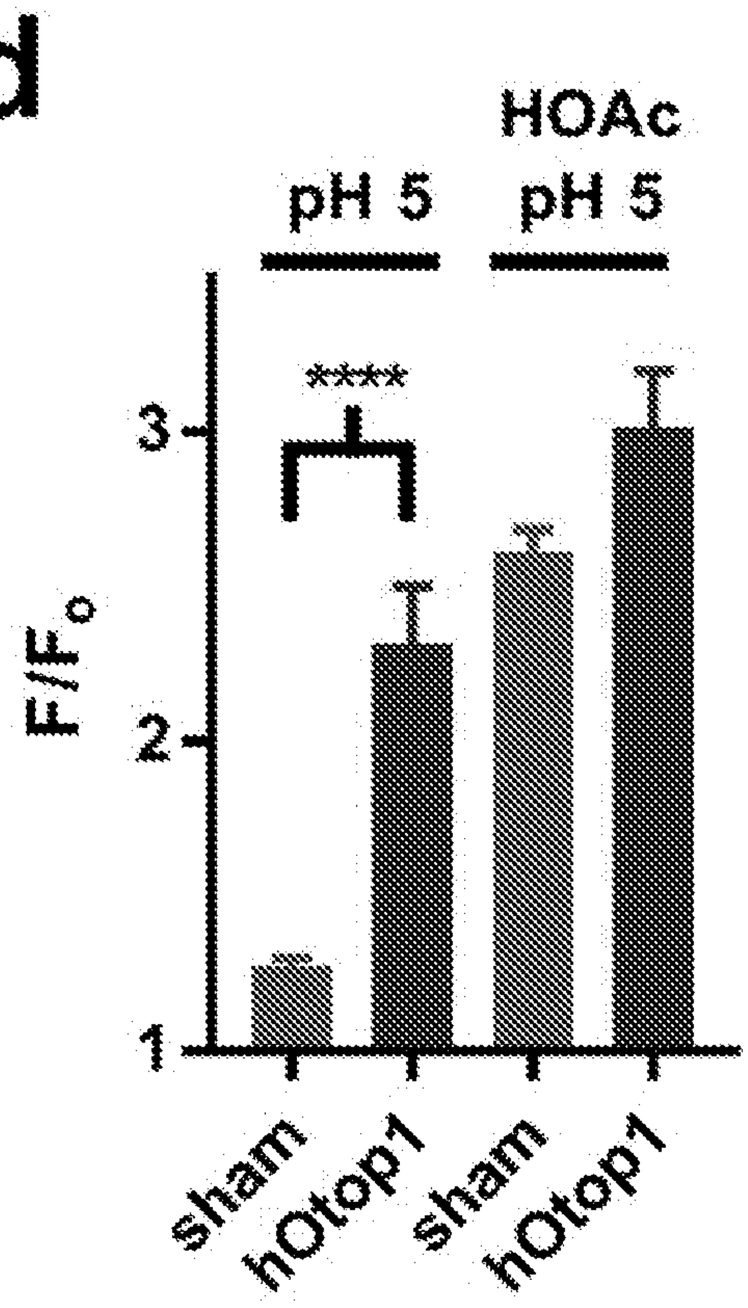

a
pH
160mV
-80mV
7.4
6.0
5.5
5.0
4.5
400 pA
2s
b
V (mV)
80
-80
I (nA)
-0.5
7.4
6.0
4.5
5.0
5.5
pH
c
E_rev (mV)
120
80
40
0
4
5
6
pH

Fig. 8


a
pH 5.5
7.4
5.0
4.5
1 Zn2+
100pA
2s


b
0.6
0.4
0.2
0.0
Current(-nA)
****
Untrans
hHnv1


c
pH 5
HOAc pH 5
hOtop1
sham
F/F0
3
2
1
0
200
400
600
time (s)


d
HOAc
pH 5
pH 5
****
F/F0
3
2
1
sham
hOtop1
sham
hOtop1

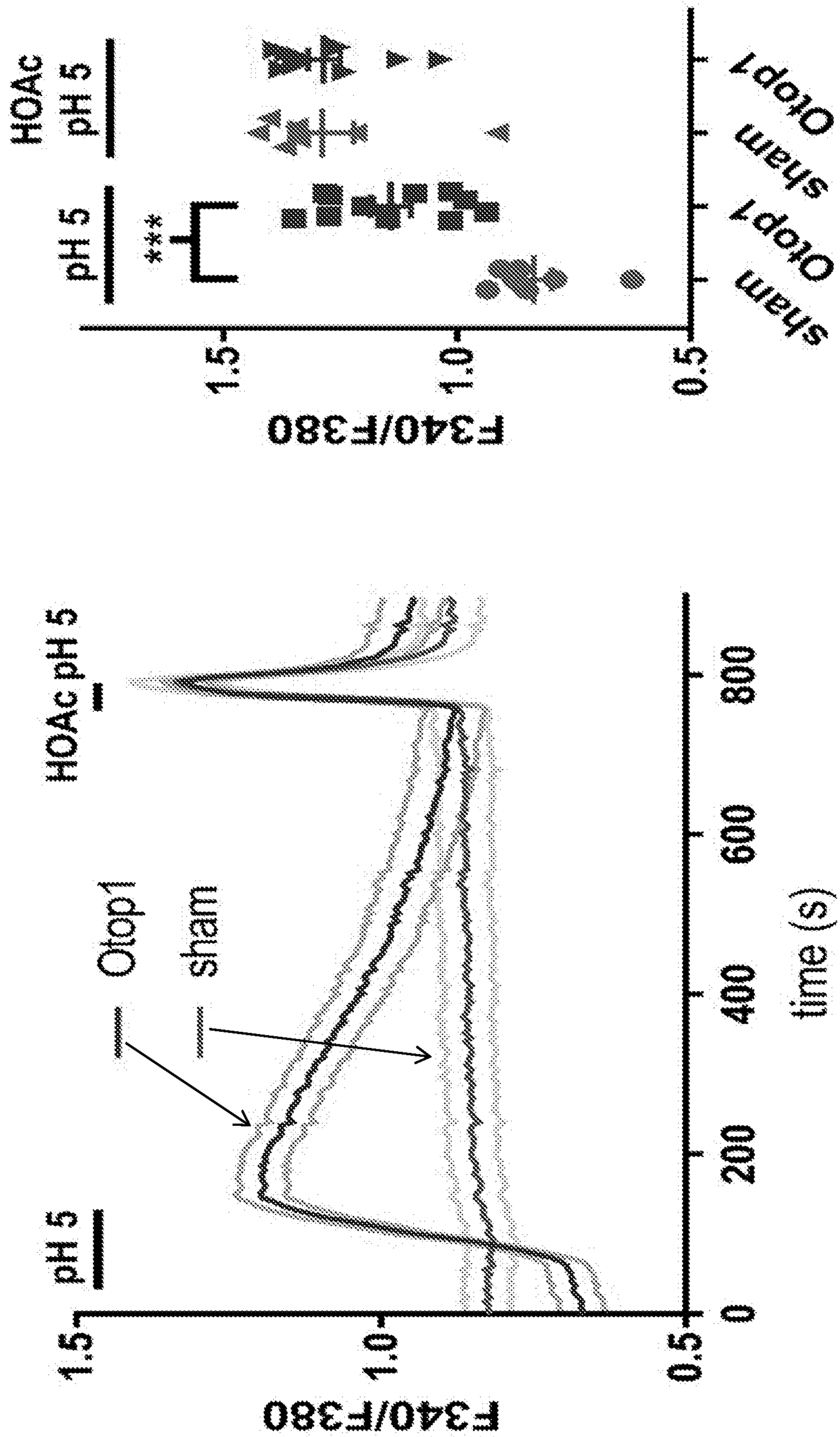

HOAc pH 5
pH 5
***
sham
Otop1
1.5
1.0
0.5
F340/F380
HOAc pH 5
Otop1
sham
800
600
400
200
0
time (s)
pH 5
1.5
1.0
0.5
F340/F380

Fig. 9

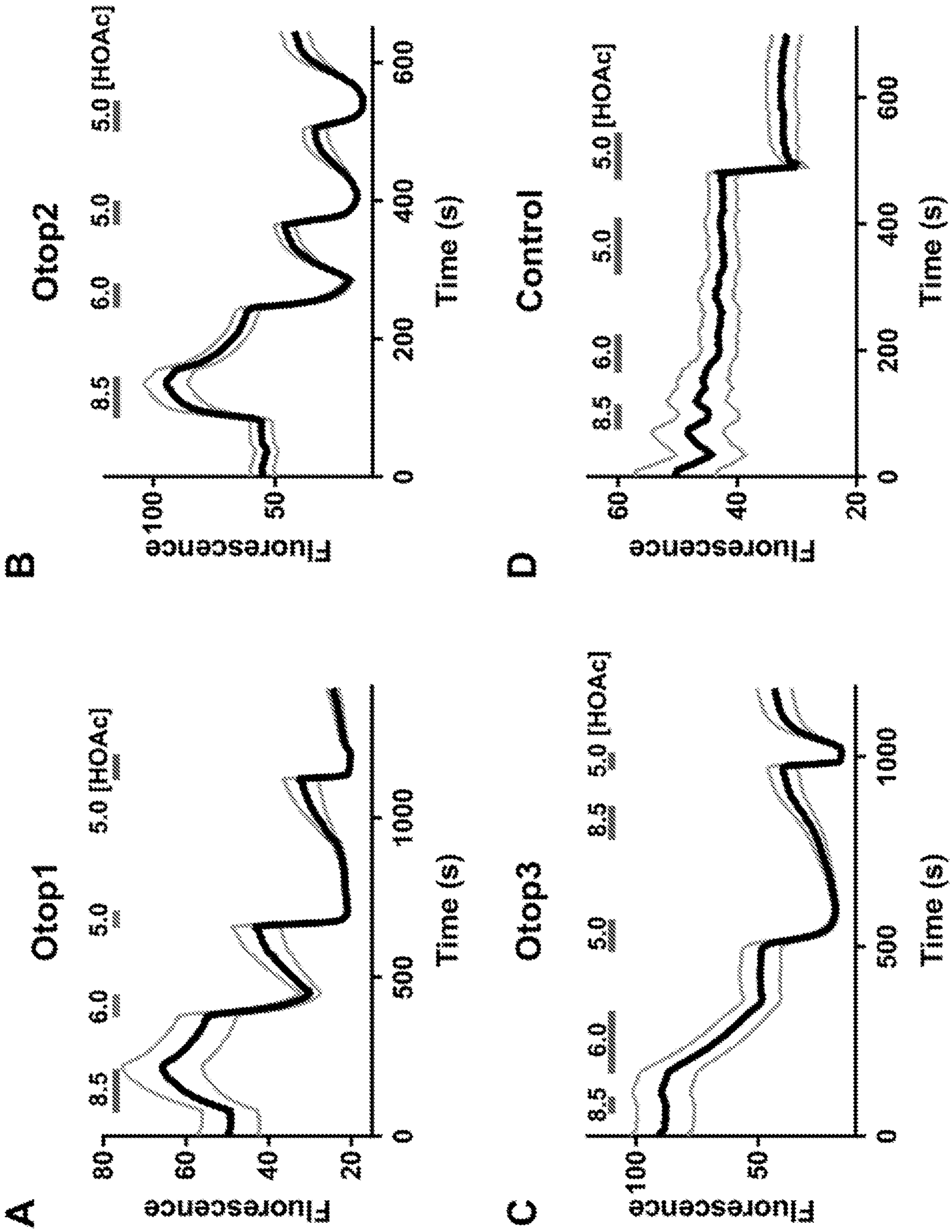

A
Otop1
5.0
5.0 [HOAc]
Fluorescence
Time (s)
B
Otop2
8.5
6.0
5.0
5.0 [HOAc]
Fluorescence
Time (s)
C
Otop3
5.0
8.5
5.0 [HOAc]
Fluorescence
Time (s)
D
Control
8.5
6.0
5.0
5.0 [HOAc]
Fluorescence
Time (s)

Fig. 10