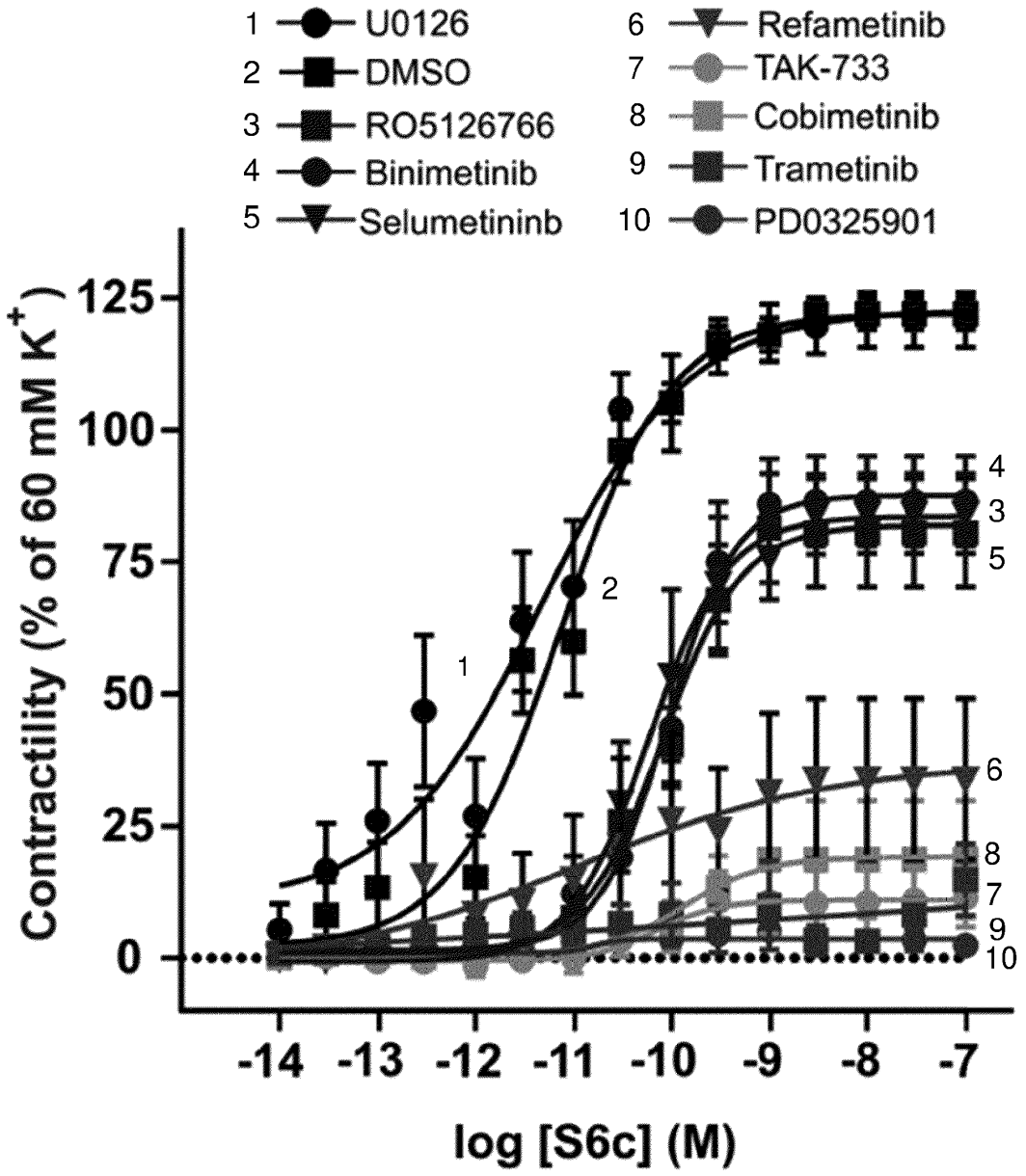

1 U0126
2 DMSO
3 RO5126766
4 Binimetinib
5 Selumetininb
6 Refametinib
7 TAK-733
8 Cobimetinib
9 Trametinib
10 PD0325901
Contractility (% of 60 mM K+)
125
100
75
50
25
0
-14 -13 -12 -11 -10 -9 -8 -7
log [S6c] (M)

Fig. 1A

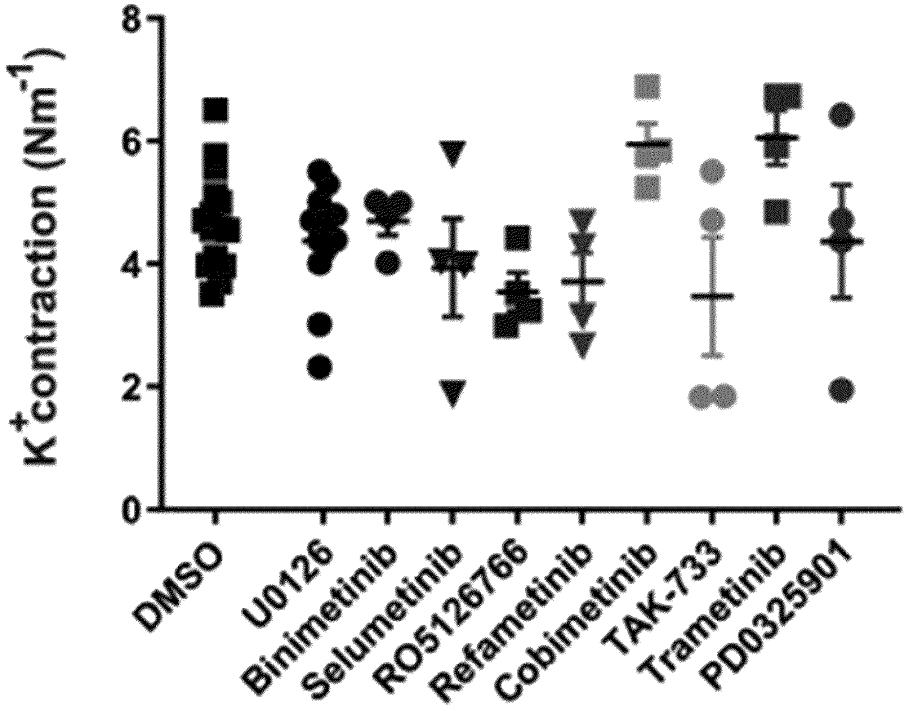

K+contraction (Nm-1)
8
6
4
2
0
DMSO
U0126
Binimetinib
Selumetinib
RO5126766
Refametinib
Cobimetinib
TAK-733
Trametinib
PD0325901

Fig. 1B

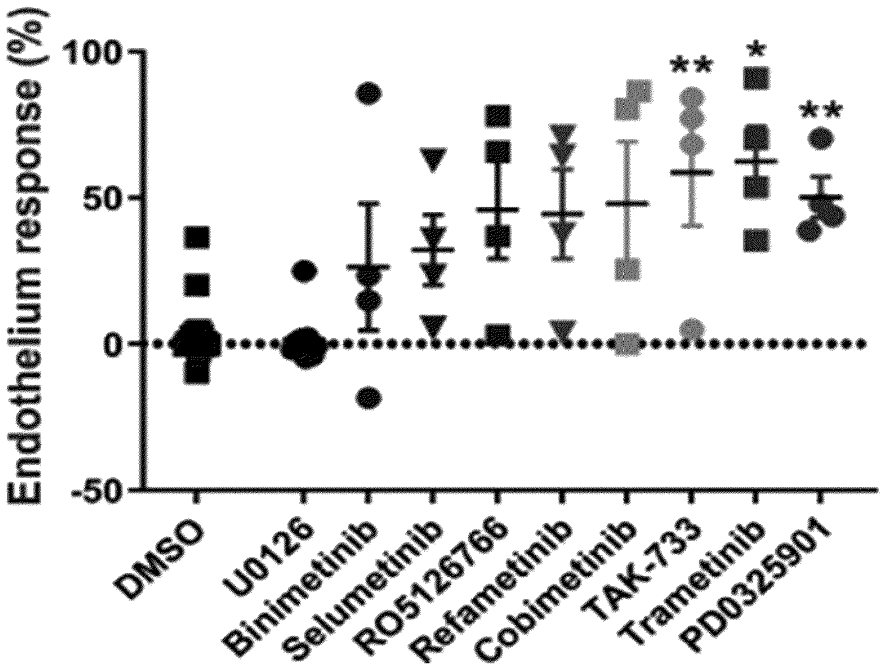

Endothelium response (%)
100
50
0
-50
**
*
**
DMSO
U0126
Binimetinib
Selumetinib
RO5126766
Refametinib
Cobimetinib
TAK-733
Trametinib
PD0325901

Fig. 1C

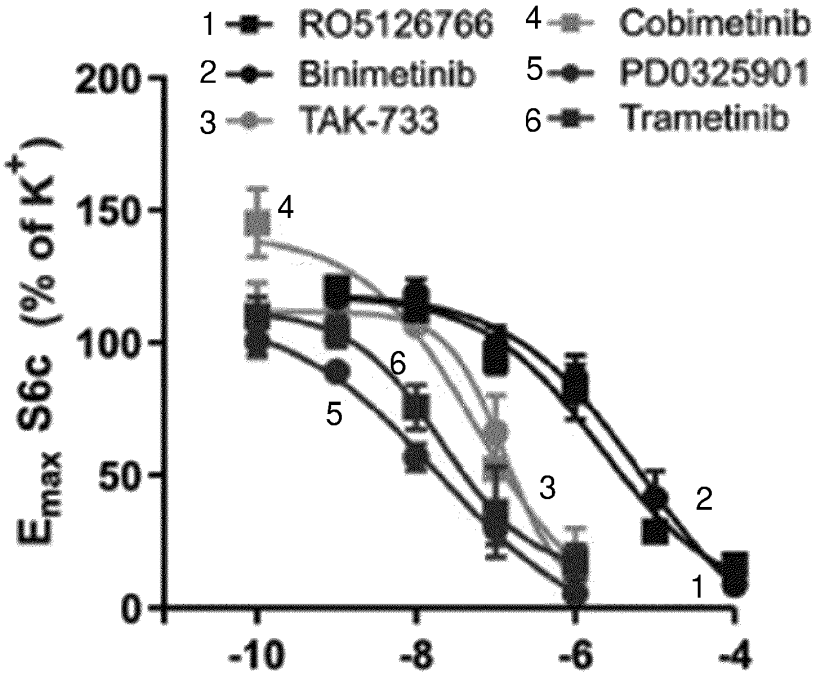

1 RO5126766
2 Binimetinib
3 TAK-733
4 Cobimetinib
5 PD0325901
6 Trametinib
E_{max} S6c (% of K^+)
200
150
100
50
0
-10
-8
-6
-4

Fig. 2A

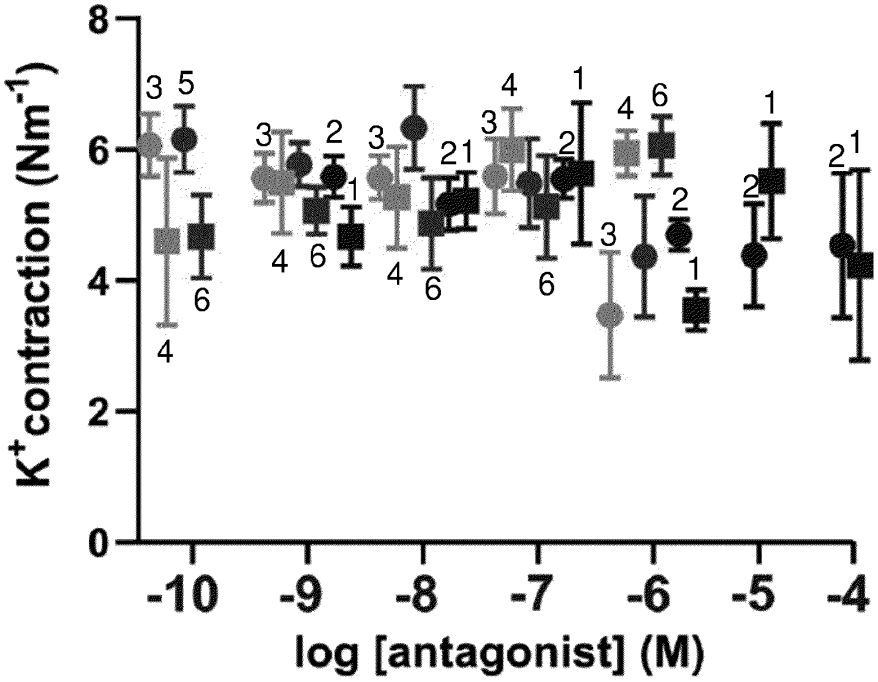

K^+contraction (Nm^{-1})
8
6
4
2
0
-10
-9
-8
-7
-6
-5
-4
log [antagonist] (M)

Fig. 2B

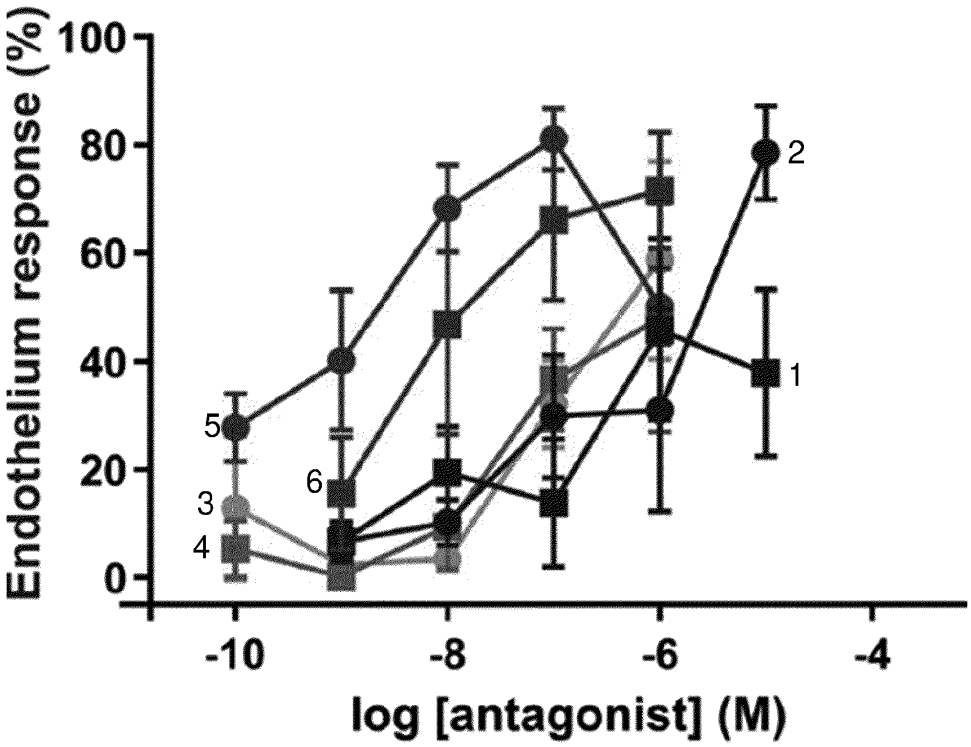

Endothelium response (%)
100
80
60
40
20
0
-10
-8
-6
-4
log [antagonist] (M)
1
2
3
4
5
6

Fig. 2C

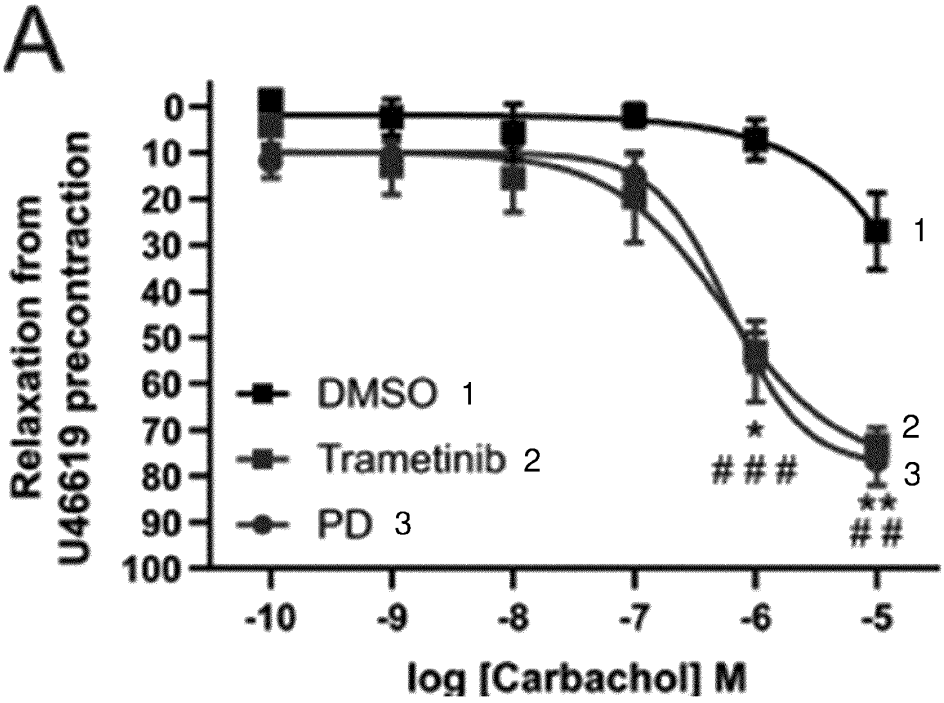

A
Relaxation from U46619 precontraction
0
10
20
30
40
50
60
70
80
90
100
DMSO 1
Trametinib 2
PD 3
1
2
3
*
###
**
##
-10
-9
-8
-7
-6
-5
log [Carbachol] M

Fig. 3A

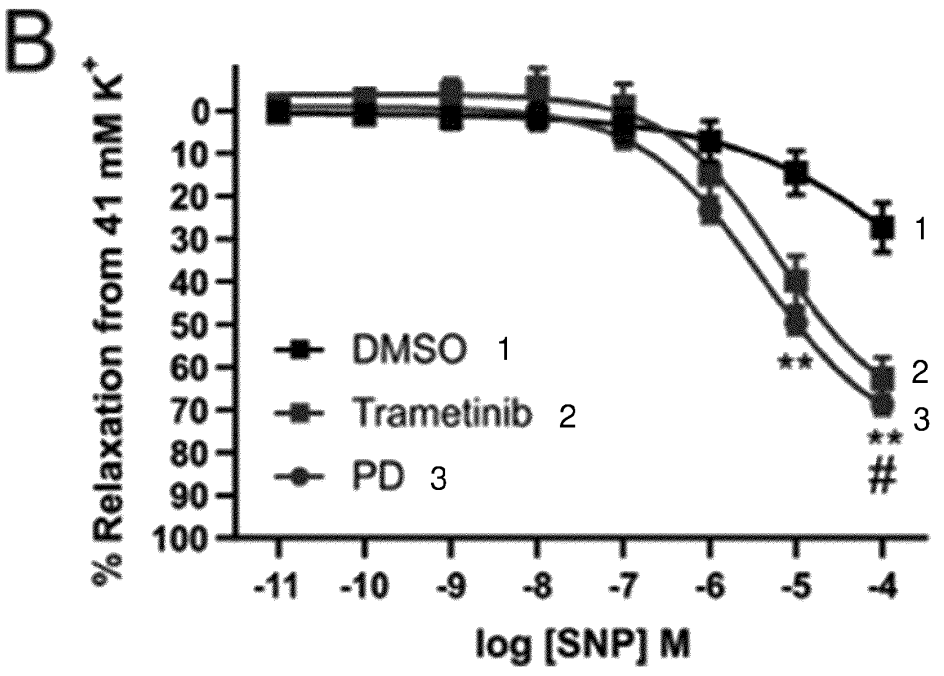

B
% Relaxation from 41 mM K+
0
10
20
30
40
50
60
70
80
90
100
DMSO 1
Trametinib 2
PD 3
1
2
3
**
**
#
-11
-10
-9
-8
-7
-6
-5
-4
log [SNP] M

Fig. 3B

C

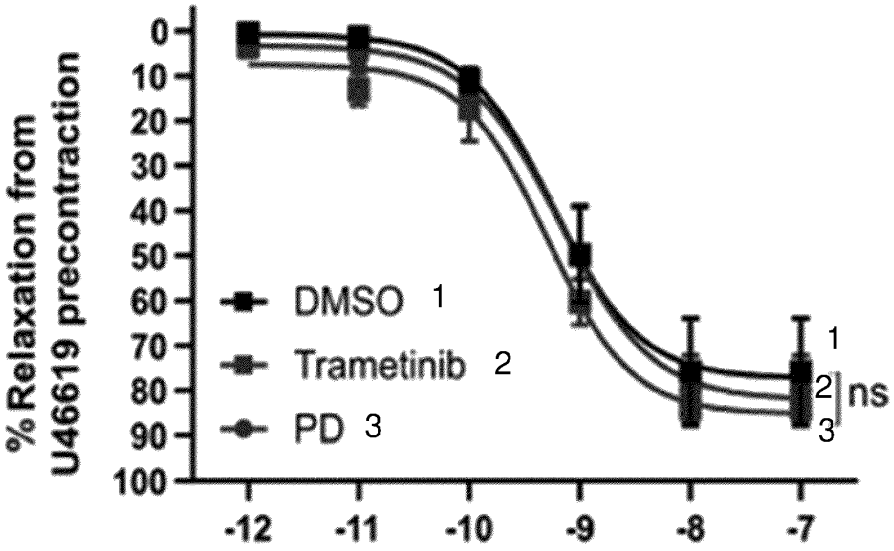


Fig. 3C

D

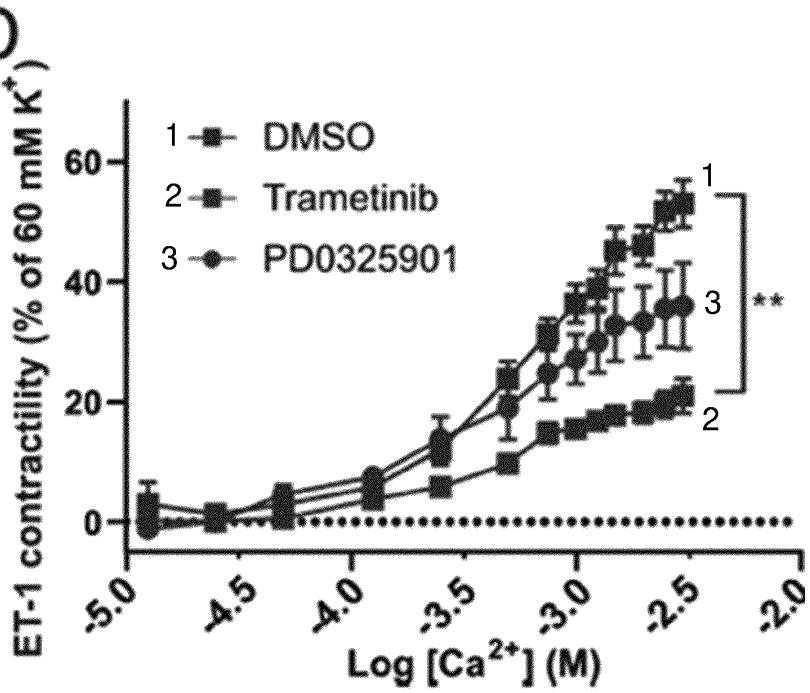


Fig. 3D

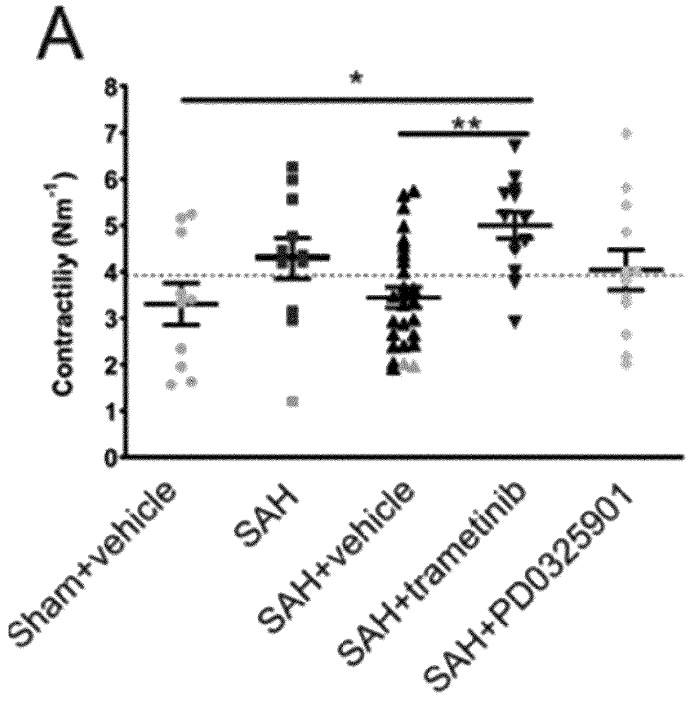

A
Contractiliy (Nm-1)
0
1
2
3
4
5
6
7
8
*
**
Sham+vehicle
SAH
SAH+vehicle
SAH+trametinib
SAH+PD0325901

Fig. 4A

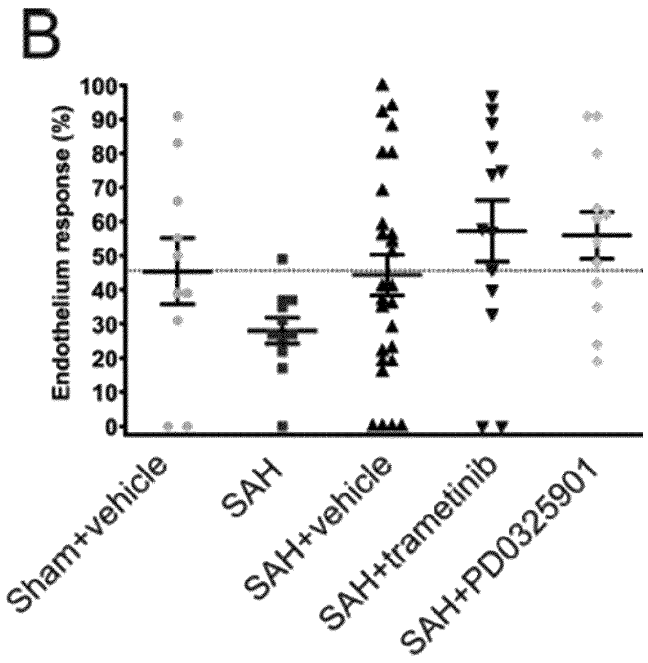

B
Endothelium response (%)
0
10
20
30
40
50
60
70
80
90
100
Sham+vehicle
SAH
SAH+vehicle
SAH+trametinib
SAH+PD0325901

Fig. 4B

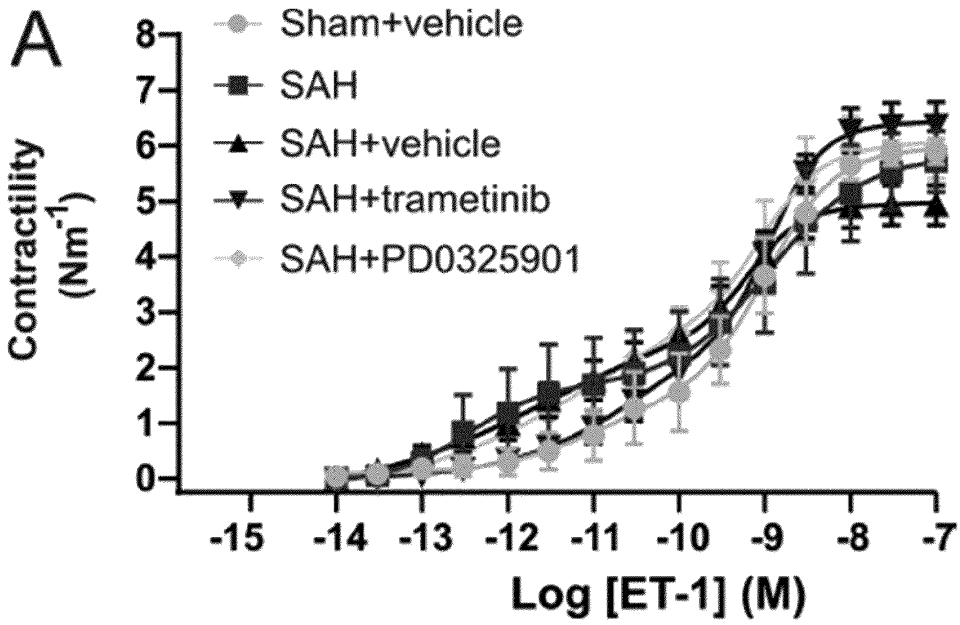

A
Sham+vehicle
SAH
SAH+vehicle
SAH+trametinib
SAH+PD0325901
Contractility (Nm-1)
0
1
2
3
4
5
6
7
8
-15
-14
-13
-12
-11
-10
-9
-8
-7
Log [ET-1] (M)

Fig. 5A

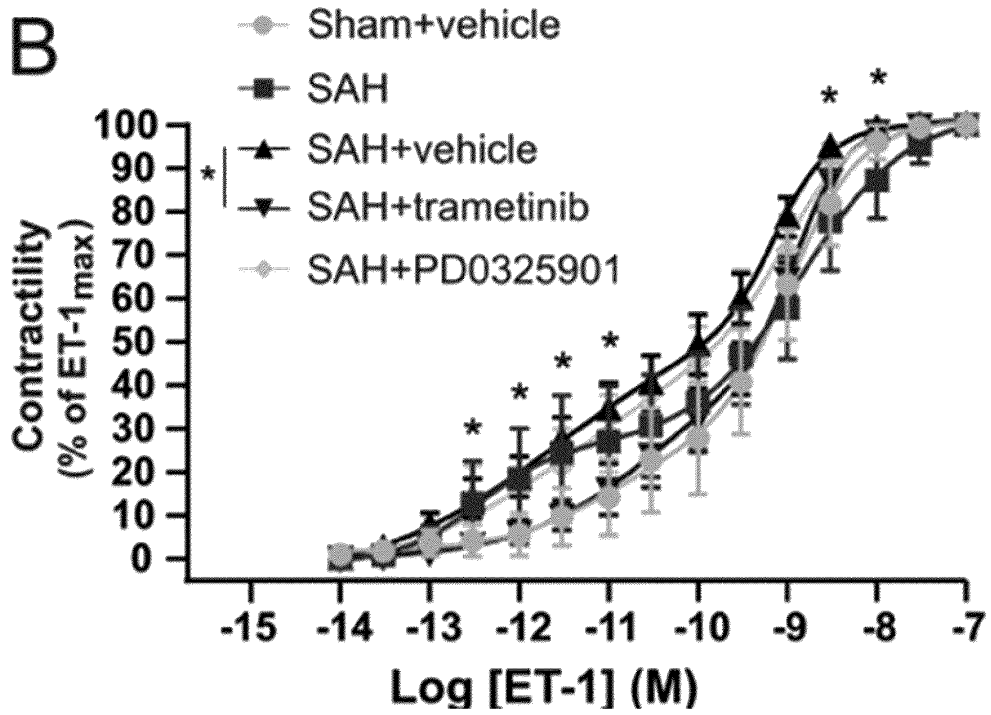

B
Sham+vehicle
SAH
SAH+vehicle
SAH+trametinib
SAH+PD0325901
Contractility (% of ET-1max)
0
10
20
30
40
50
60
70
80
90
100
*
-15
-14
-13
-12
-11
-10
-9
-8
-7
Log [ET-1] (M)

Fig. 5B

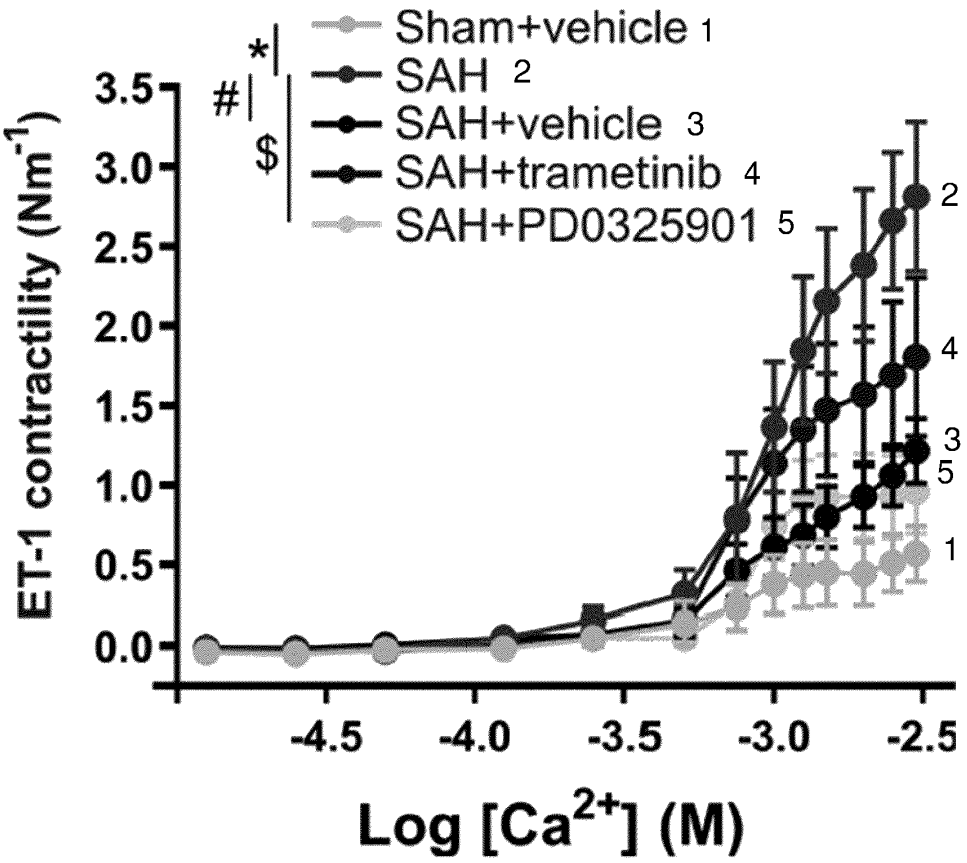

Sham+vehicle 1
SAH 2
SAH+vehicle 3
SAH+trametinib 4
SAH+PD0325901 5
*
#
$
ET-1 contractility (Nm-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-4.5
-4.0
-3.5
-3.0
-2.5
Log [Ca2+] (M)
2
4
3
5
1

Fig. 6

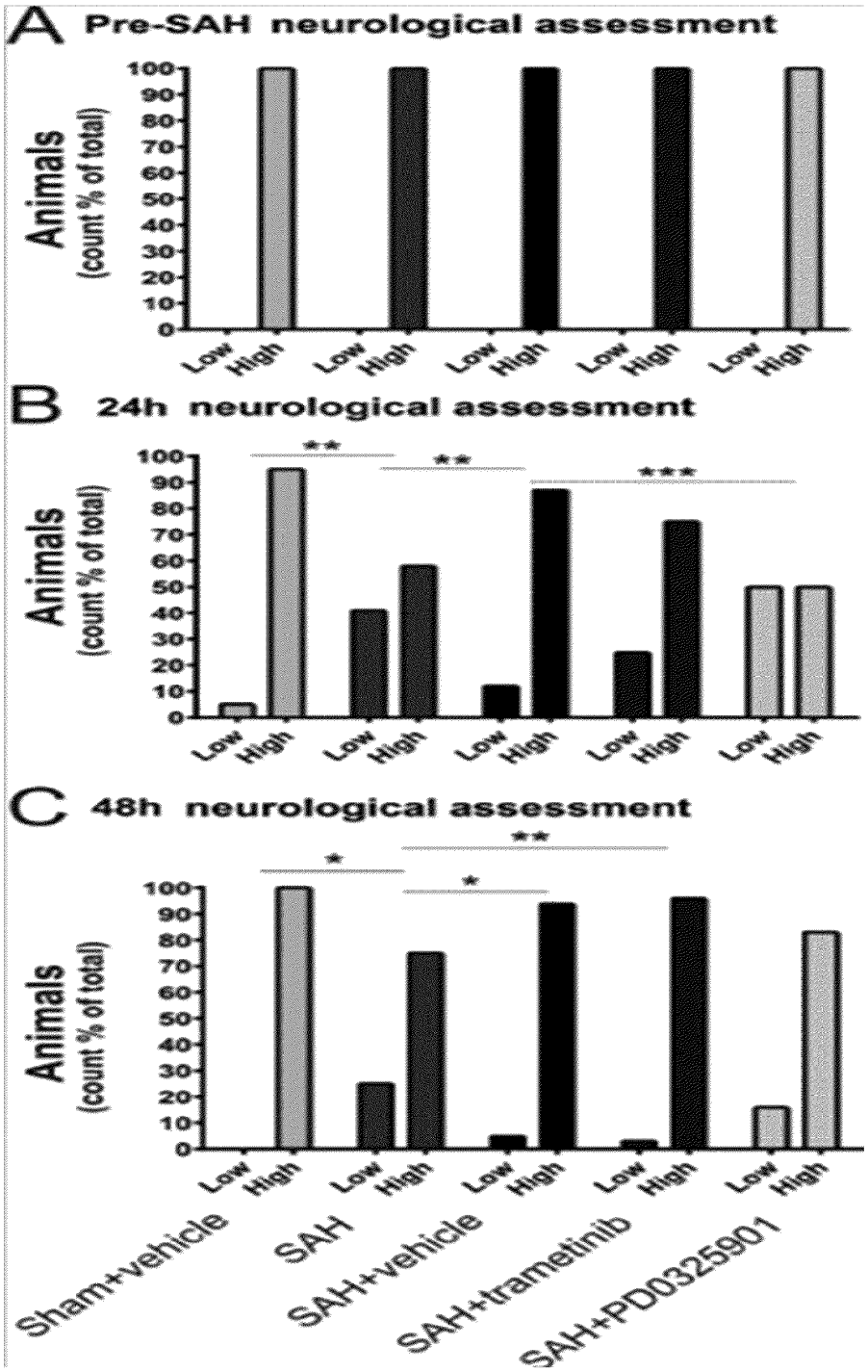

A Pre-SAH neurological assessment
Animals (count % of total)
100
90
80
70
60
50
40
30
20
10
0
Low
High
B 24h neurological assessment
**
**
***
C 48h neurological assessment
*
*
**
Sham+vehicle
SAH
SAH+vehicle
SAH+trametinib
SAH+PD0325901

Fig. 7A-C

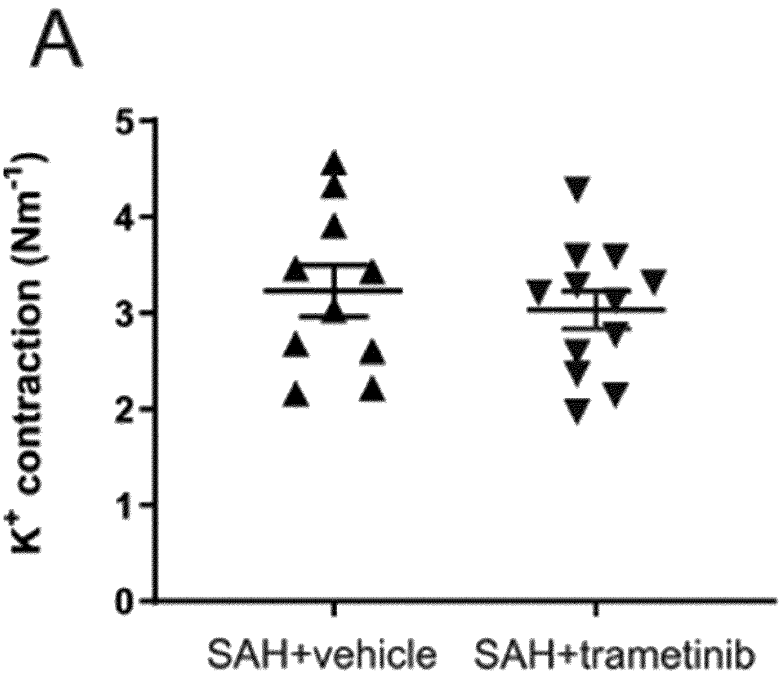

A
K+ contraction (Nm-1)
5
4
3
2
1
0
SAH+vehicle
SAH+trametinib

Fig. 8A

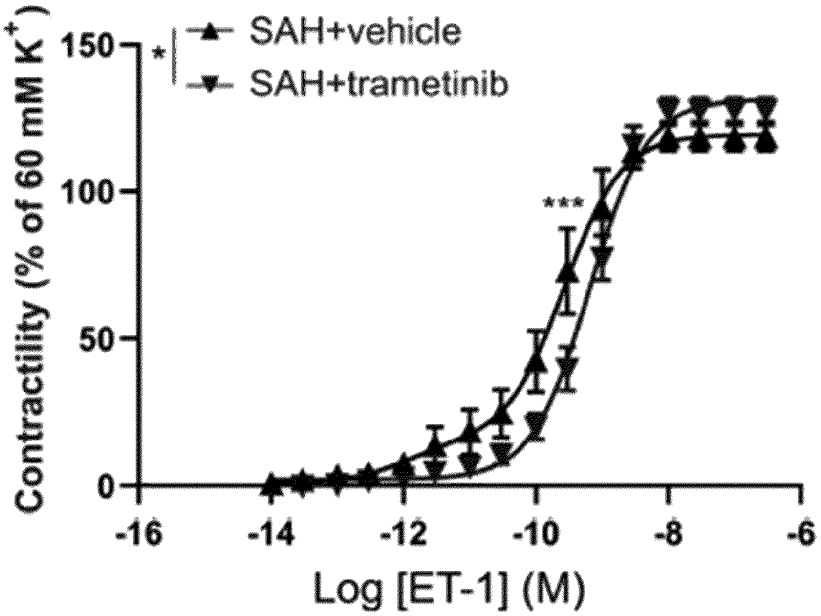

Contractility (% of 60 mM K+)
150
100
50
0
*
SAH+vehicle
SAH+trametinib
***
-16
-14
-12
-10
-8
-6
Log [ET-1] (M)

Fig. 8B

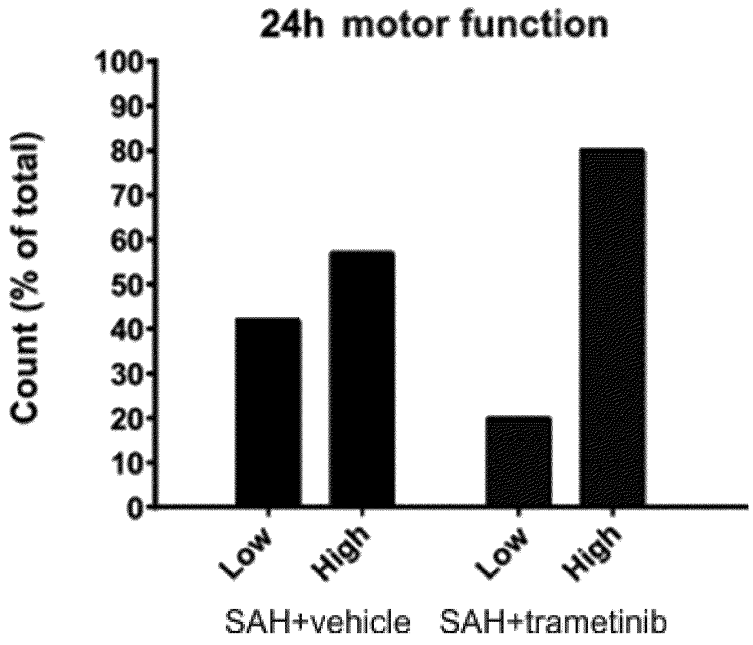

24h motor function
Count (% of total)
100
90
80
70
60
50
40
30
20
10
0
Low
High
Low
High
SAH+vehicle
SAH+trametinib

Fig. 8C

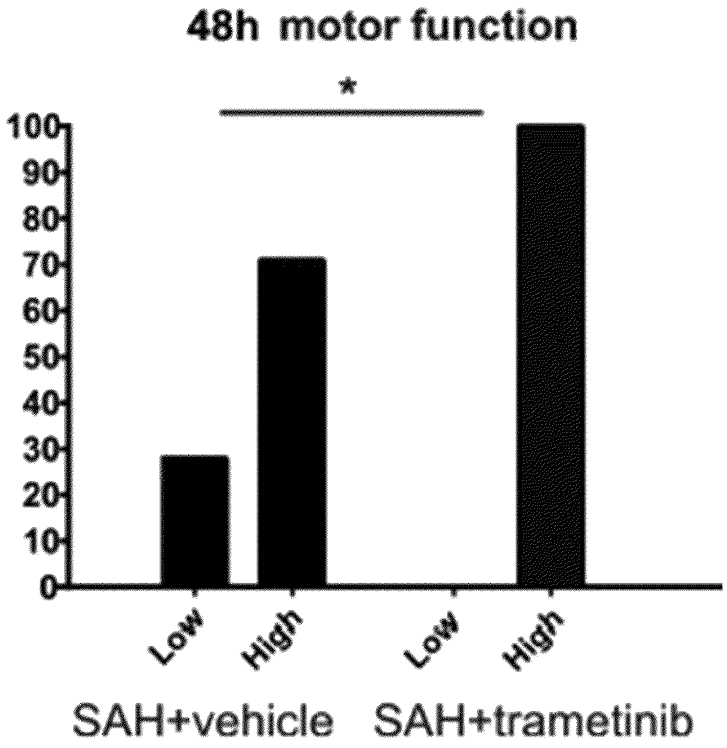
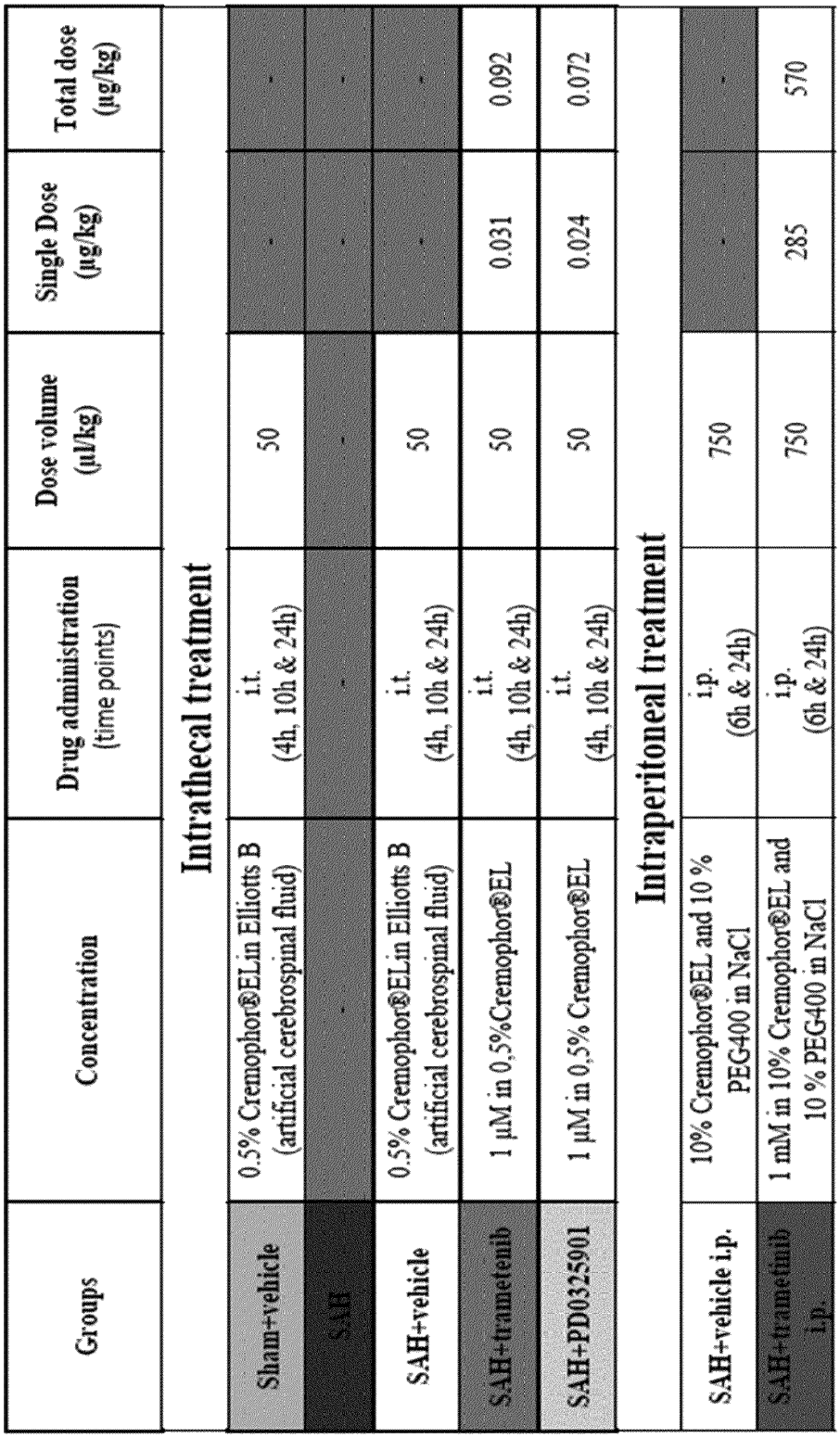
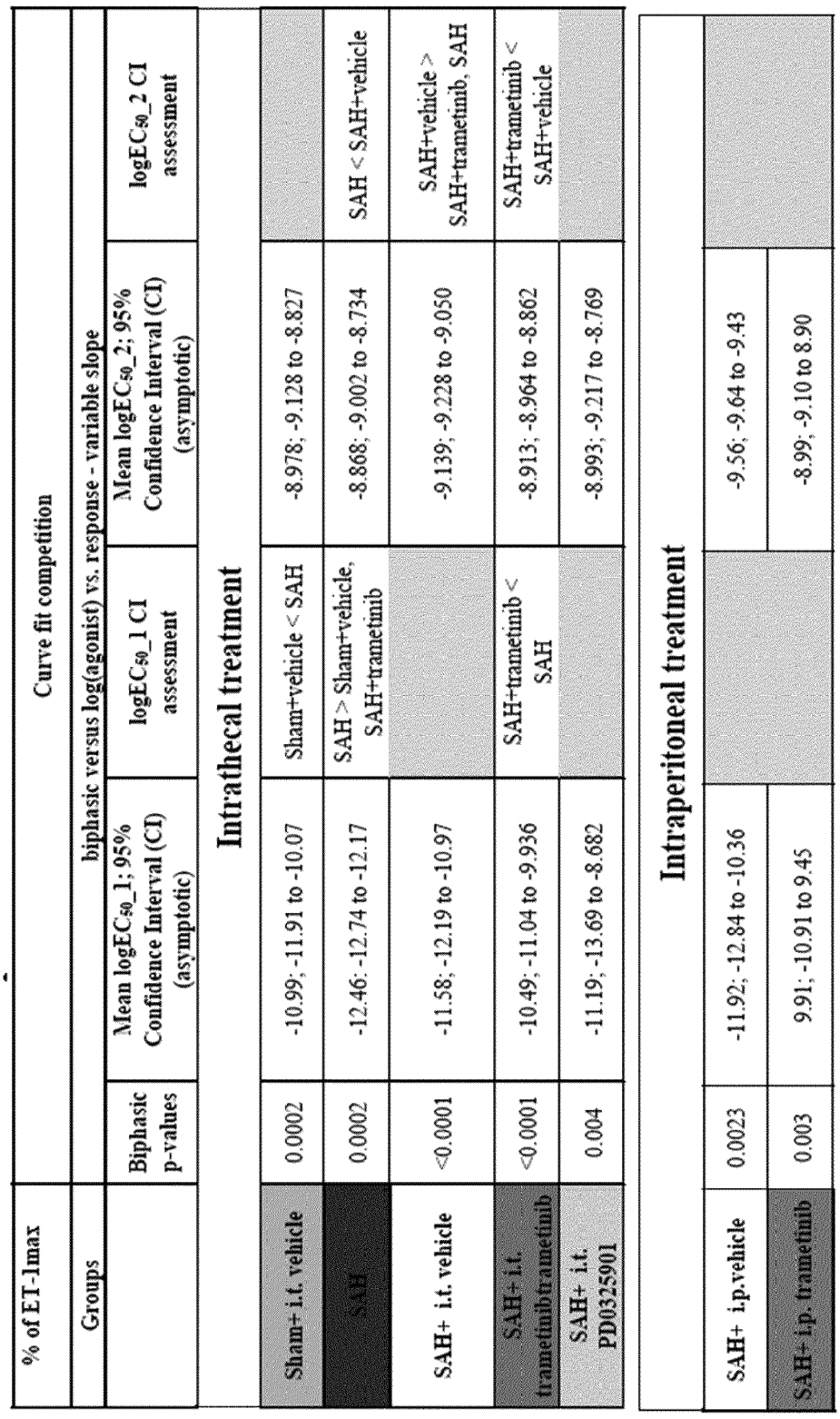

48h motor function
*
100
90
80
70
60
50
40
30
20
10
0
Low
High
Low
High
SAH+vehicle
SAH+trametinib

Fig. 8D



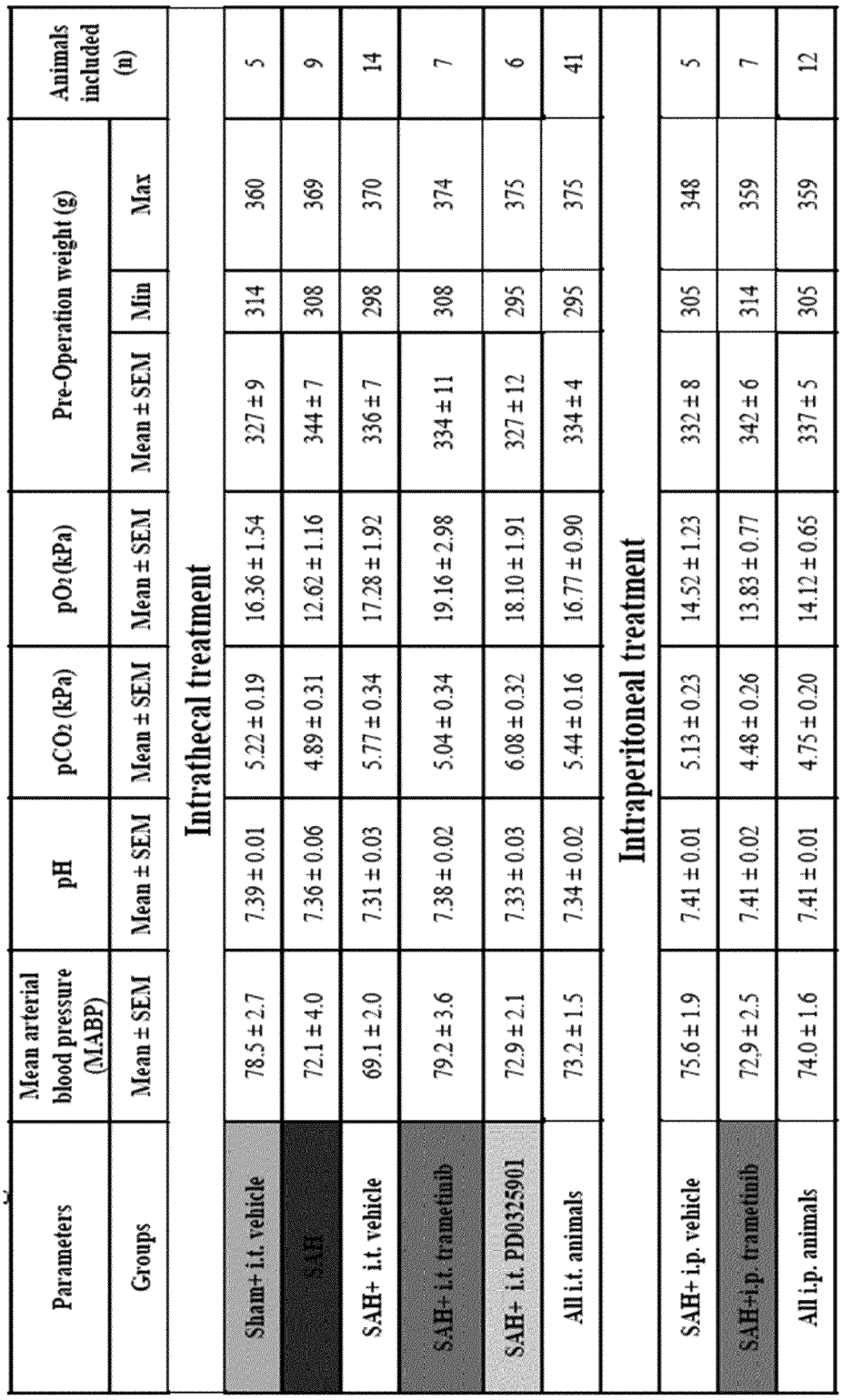
Groups	Concentration	Drug administration (time points)	Dose volume (µl/kg)	Single Dose (µg/kg)	Total dose (µg/kg)
Intrathecal treatment					
Sham+vehicle	0.5% Cremophor®ELin Elliotts B (artificial cerebrospinal fluid)	i.t. (4h, 10h & 24h)	50	-	-
SAH	-	-	-	-	-
SAH+vehicle	0.5% Cremophor®ELin Elliotts B (artificial cerebrospinal fluid)	i.t. (4h, 10h & 24h)	50	-	-
SAH+trametenib	1 µM in 0,5%Cremophor®EL	i.t. (4h, 10h & 24h)	50	0.031	0.092
SAH+PD0325901	1 µM in 0,5% Cremophor®EL	i.t. (4h, 10h & 24h)	50	0.024	0.072
Intraperitoneal treatment					
SAH+vehicle i.p.	10% Cremophor®EL and 10 % PEG400 in NaCl	i.p. (6h & 24h)	750	-	-
SAH+trametinib i.p.	1 mM in 10% Cremophor®EL and 10 % PEG400 in NaCl	i.p. (6h & 24h)	750	285	570

Fig. 9



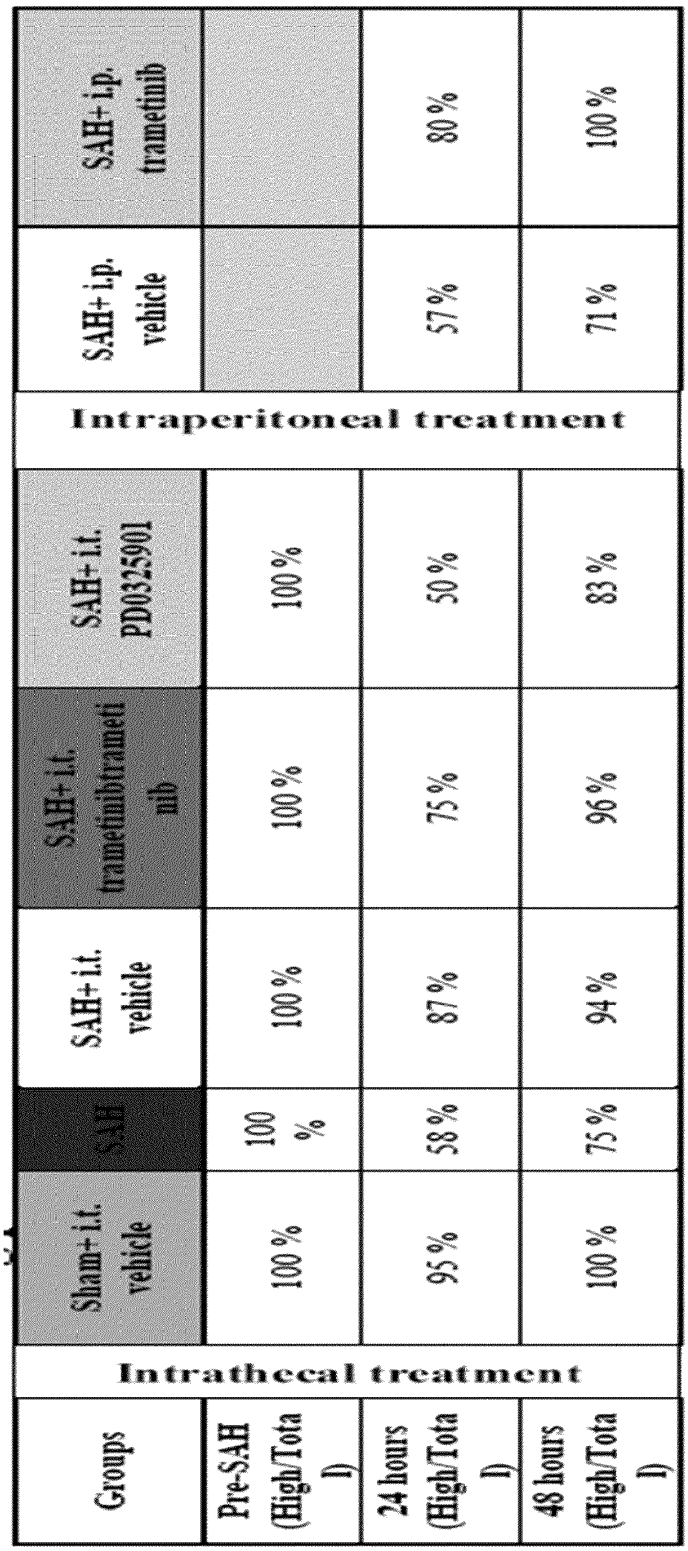
% of ET-1max	Curve fit competition				
Groups	biphasic versus log(agonist) vs. response - variable slope				
	Biphasic p-values	Mean $logEC_{50}$_1; 95% Confidence Interval (CI) (asymptotic)	$logEC_{50}$_1 CI assessment	Mean $logEC_{50}$_2; 95% Confidence Interval (CI) (asymptotic)	$logEC_{50}$_2 CI assessment
Intrathecal treatment					
Sham+ i.t. vehicle	0.0002	-10.99; -11.91 to -10.07	Sham+vehicle < SAH	-8.978; -9.128 to -8.827	
SAH	0.0002	-12.46; -12.74 to -12.17	SAH > Sham+vehicle, SAH+trametinib	-8.868; -9.002 to -8.734	SAH < SAH+vehicle
SAH+ i.t. vehicle	<0.0001	-11.58; -12.19 to -10.97		-9.139; -9.228 to -9.050	SAH+vehicle > SAH+trametinib, SAH
SAH+ i.t. trametinibtrametinib	<0.0001	-10.49; -11.04 to -9.936	SAH+trametinib < SAH	-8.913; -8.964 to -8.862	SAH+trametinib < SAH+vehicle
SAH+ i.t. PD0325901	0.004	-11.19; -13.69 to -8.682		-8.993; -9.217 to -8.769	
Intraperitoneal treatment					
SAH+ i.p.vehicle	0.0023	-11.92; -12.84 to -10.36		-9.56; -9.64 to -9.43	
SAH+ i.p. trametinib	0.003	9.91; -10.91 to 9.45		-8.99; -9.10 to 8.90	

Fig. 10



Parameters	Mean arterial blood pressure (MABP)	pH	pCO_2 (kPa)	pO_2(kPa)	Pre-Operation weight (g)			Animals included (n)
Groups	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Min	Max	
Intrathecal treatment								
Sham+ i.t. vehicle	78.5 ± 2.7	7.39 ± 0.01	5.22 ± 0.19	16.36 ± 1.54	327 ± 9	314	360	5
SAH	72.1 ± 4.0	7.36 ± 0.06	4.89 ± 0.31	12.62 ± 1.16	344 ± 7	308	369	9
SAH+ i.t. vehicle	69.1 ± 2.0	7.31 ± 0.03	5.77 ± 0.34	17.28 ± 1.92	336 ± 7	298	370	14
SAH+ i.t. trametinib	79.2 ± 3.6	7.38 ± 0.02	5.04 ± 0.34	19.16 ± 2.98	334 ± 11	308	374	7
SAH+ i.t. PD0325901	72.9 ± 2.1	7.33 ± 0.03	6.08 ± 0.32	18.10 ± 1.91	327 ± 12	295	375	6
All i.t. animals	73.2 ± 1.5	7.34 ± 0.02	5.44 ± 0.16	16.77 ± 0.90	334 ± 4	295	375	41
Intraperitoneal treatment								
SAH+ i.p. vehicle	75.6 ± 1.9	7.41 ± 0.01	5.13 ± 0.23	14.52 ± 1.23	332 ± 8	305	348	5
SAH+i.p. trametinib	72,9 ± 2.5	7.41 ± 0.02	4.48 ± 0.26	13.83 ± 0.77	342 ± 6	314	359	7
All i.p. animals	74.0 ± 1.6	7.41 ± 0.01	4.75 ± 0.20	14.12 ± 0.65	337 ± 5	305	359	12

Fig. 11



Groups	Intrathecal treatment			Intraperitoneal treatment			
	Sham+ i.t. vehicle	SAH	SAH+ i.t. vehicle	SAH+ i.t. trametinibtrametinib	SAH+ i.t. PD0325901	SAH+ i.p. vehicle	SAH+ i.p. trametinib
Pre-SAH (High/Total)	100 %	100 %	100 %	100 %	100 %		
24 hours (High/Total)	95 %	58 %	87 %	75 %	50 %	57 %	80 %
48 hours (High/Total)	100 %	75 %	94 %	96 %	83 %	71 %	100 %

Fig. 12

Tradename	Name	Formula	Mol. weight	MEK IC_{50}	Target
PD0325901	PD0325901	$C_{16}H_{14}F_3IN_2O_4$	482.19 g/mol	0.33 nM	MEK
Trametinib	GSK1120212	$C_{26}H_{23}FIN_5O_4$	615.39 g/mol	0.92 nM /1.8 nM	MEK1/2
RO5126766	CH5126766	$C_{21}H_{18}FN_5O_5S$	471.46 g/mol	160 nM	MEK1 (also RAF)
Selumetinib	AZD6244	$C_{17}H_{15}BrClFN_4O_3$	457.68 g/mol	14 nM	MEK1
Binimetinib	ARRY-162	$C_{17}H_{15}BrF_2N_4O_3$	441.23 g/mol	12 nM	MEK1/2
Cobimetinib	GDC-0973	$C_{21}H_{21}F_3IN_3O_2$	531.31 g/mol	4.2 nM	MEK1
TAK-733	TAK-733	$C_{17}H_{15}F_2IN_4O_4$	504.23 g/mol	3.2 nM	MEK1
Refametinib	RDEA119	$C_{19}H_{20}F_3IN_2O_5S$	572.34 g/mol	19 nM / 47 nM	MEK1/2
U0126 - EtOH	U0126 - EtOH	$C_{18}H_{16}N_6S_2.C_2H_6O$	426.56 g/mol	0.07 µM / 0.06 µM	MEK1/2

Fig. 13

	Sham	SAH
Weight (gram)	264±4.8	259±4.8
MABP (mmHg)	105±4.1	95±3.4
MABP post-SAH (mmHg)	-	93±4.6
pH	7.4±0.01	7.4±0.01
pH post-SAH	-	7.3±0.01
pO_2 (kPa)	17.1±1.3	16.2±0.9
pCO_2 (kPa)	4.8±0.2	4.9±0.3

Fig. 14

			Biphasic Curve				Sigmoidal Curve	
Group (log M)	n	K^+ response	$E_{max(1)}$ (%)	$E_{max(2)}$ (%)	$pEC_{50(1)}$ (log M)	$pEC_{50(2)}$ (log M)	pEC_{50}	E_{max} (%)
5-CT BA								
Sham	2	4.95±0.95	28.00±21.00	40.50±8.50	-7.87±0.46	-4.06±7.51		
SAH (250µl)	4	4.90±0.47	31.50±8.59	50.00±12.81	-8.16±0.34	-4.84±0.97		
Sham	4	5.18±0.65	11.50±4.94	57.75±14.41	-5.04±0.32	-7.92±0.30		
SAH (300µl)	7	6.13±0.52	38.00±8.68	77.29±10.14	-8.41±0.25	-5.22±0.42		
5-CT MCA								
Sham	3	2.40±0.72	6.00±1.53	35.33±6.96	-8.12±0.55	-4.02±0.84		
SAH (250µl)	4	3.10±0.47	44.25±8.47	80.75±20.79	-7.63±0.29	-4.81±0.26		
Sham	5	2.32±0.34	15.80±6.11	53.80±5.19	-8.09±0.33	-4.69±0.28		
SAH (300µl)	7	3.81±0.57	42.57±8.36	75.00±7.82	-8.41±0.25	-4.44±0.82		
ET-1 BA								
Sham	3	6.27±1.07					121.7±10.37	-9.13±0.07
SAH (250µl)	7	5.60±0.31					117.0±5.18	-9.66±0.10
Sham	4	5.00±0.44					107.8±12.31	-9.27±0.17
SAH (300µl)	7	5.64±0.67	48.00±11.49	114.7±10.17	-12.41±0.36	-9.23±0.28		
ET-1 MCA								
Sham	4	2.35±0.23					148.5±15.89	-8.67±0.09
SAH (250µl)	6	2.90±0.50					156.0±15.33	-9.32±0.14
Sham	4	1.86±0.31					179.8±24.85	-8.91±0.08

Fig. 15

	Sham	SAH
ICP (mmHg)	4.0±0.2	4.1±0.3
ICP during SAH (mmHg)	-	149.0±11.5
ICP 30 mins post-SAH (mmHg)	-	7.1±0.8
CBF (% baseline)	100	100
CBF post-SAH (% drop of pre-SAH)	-	89±0.03
CBF 30 mins post-SAH (% of pre-SAH)	-	41±0.06

Fig. 16

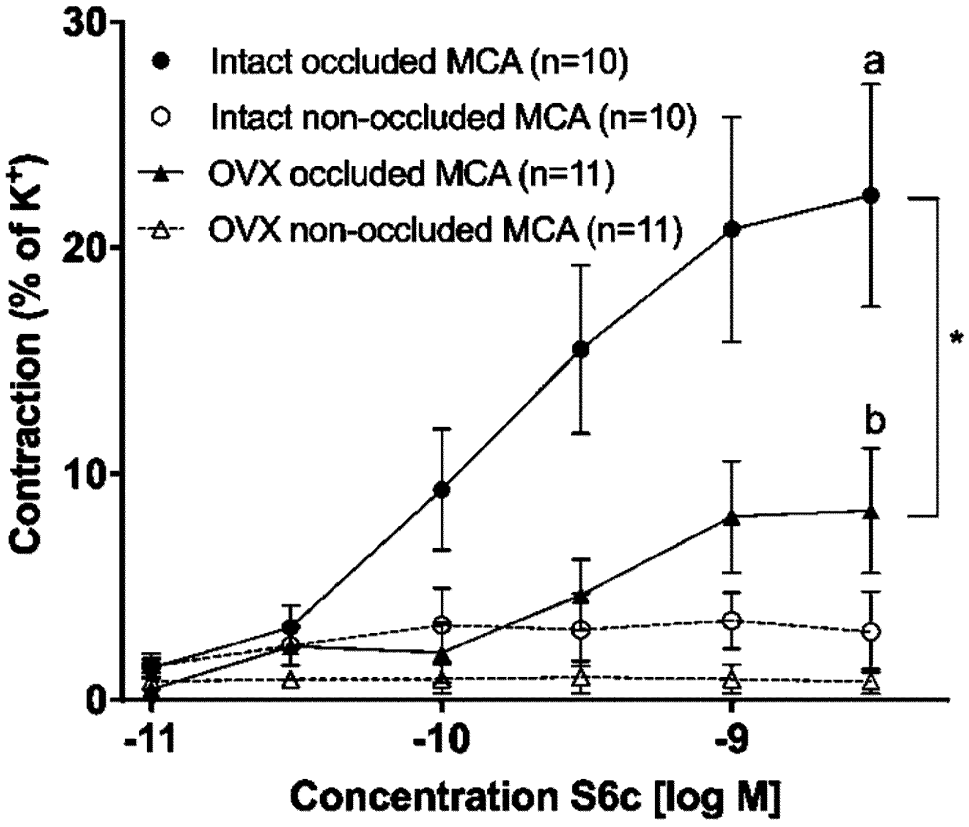

30
20
10
0
Contraction (% of K+)
Intact occluded MCA (n=10)
Intact non-occluded MCA (n=10)
OVX occluded MCA (n=11)
OVX non-occluded MCA (n=11)
a
b
*
-11
-10
-9
Concentration S6c [log M]

Fig. 17A

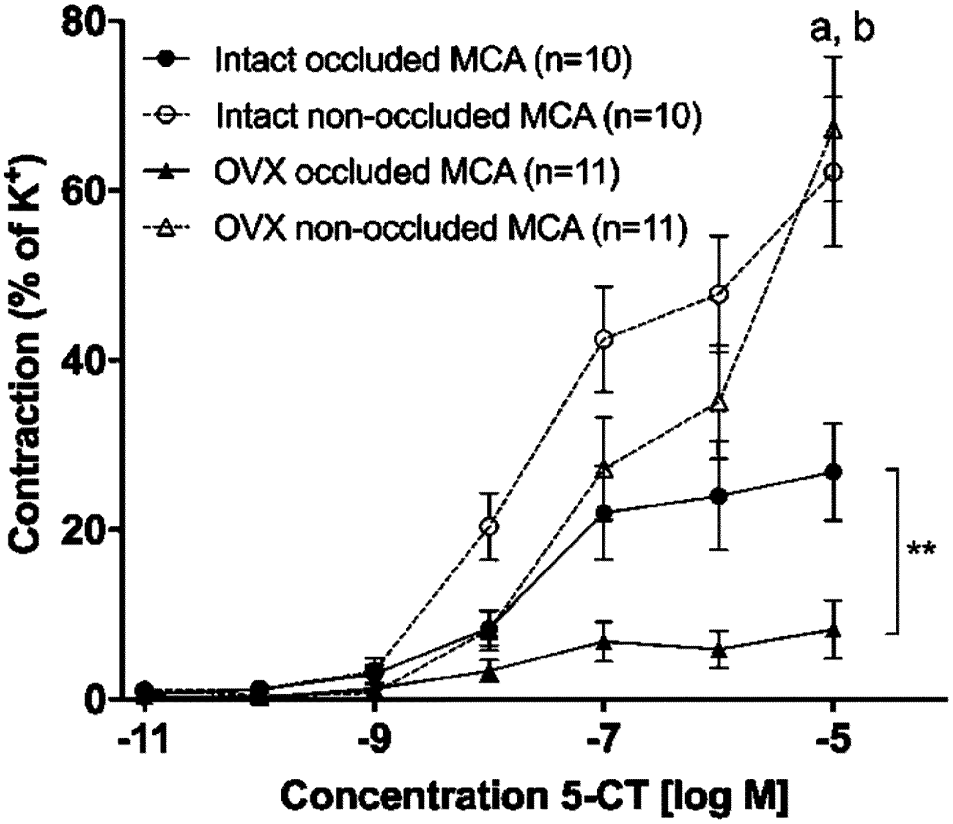

80
60
40
20
0
Contraction (% of K+)
a, b
Intact occluded MCA (n=10)
Intact non-occluded MCA (n=10)
OVX occluded MCA (n=11)
OVX non-occluded MCA (n=11)
**
-11
-9
-7
-5
Concentration 5-CT [log M]

Fig. 17B

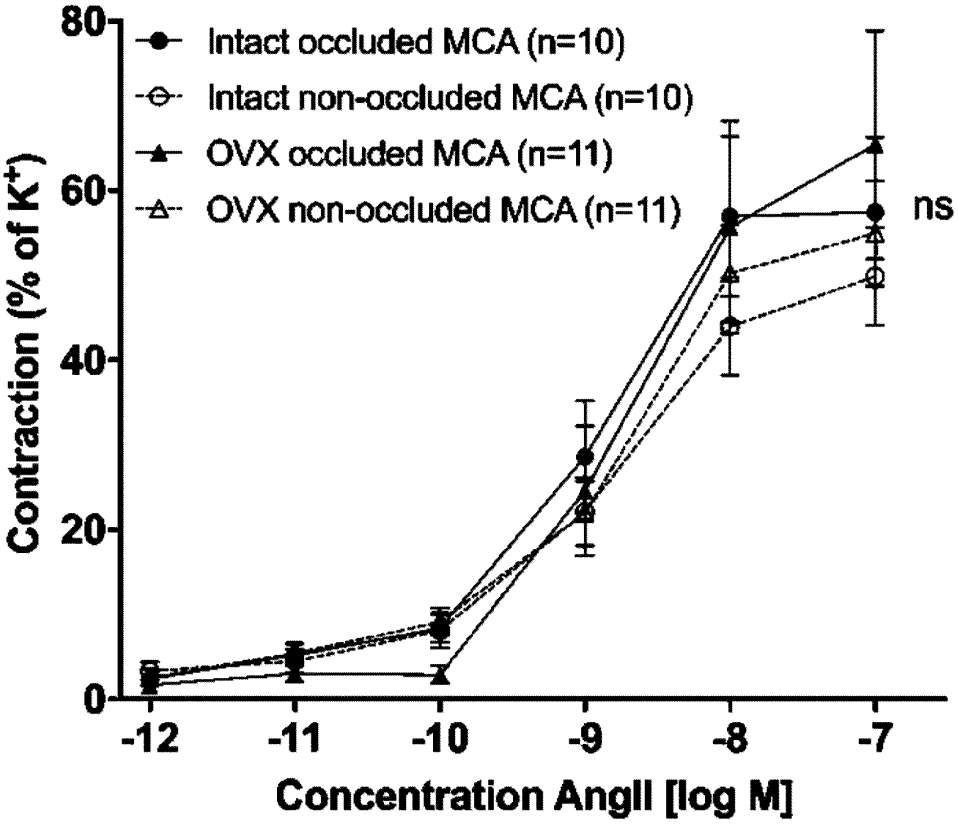

Intact occluded MCA (n=10)
Intact non-occluded MCA (n=10)
OVX occluded MCA (n=11)
OVX non-occluded MCA (n=11)
ns
Contraction (% of K+)
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Concentration AngII [log M]

Fig. 17C

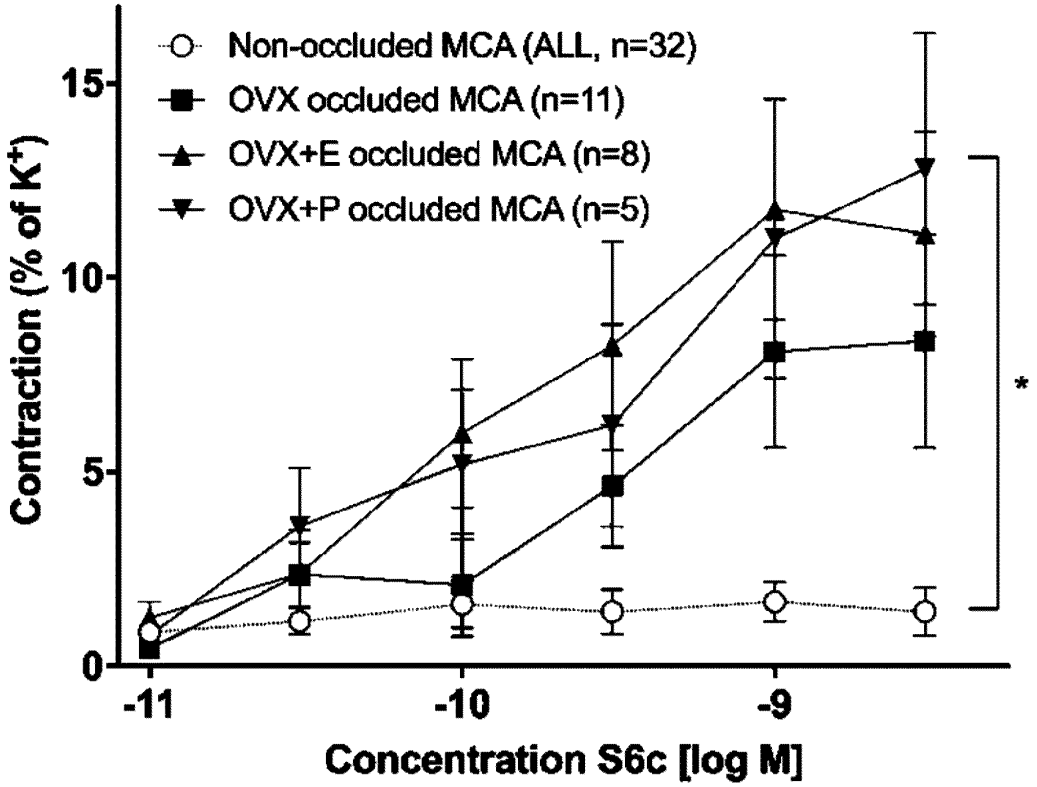

Non-occluded MCA (ALL, n=32)
OVX occluded MCA (n=11)
OVX+E occluded MCA (n=8)
OVX+P occluded MCA (n=5)
Contraction (% of K+)
15
10
5
0
-11
-10
-9
Concentration S6c [log M]
*

Fig. 18A

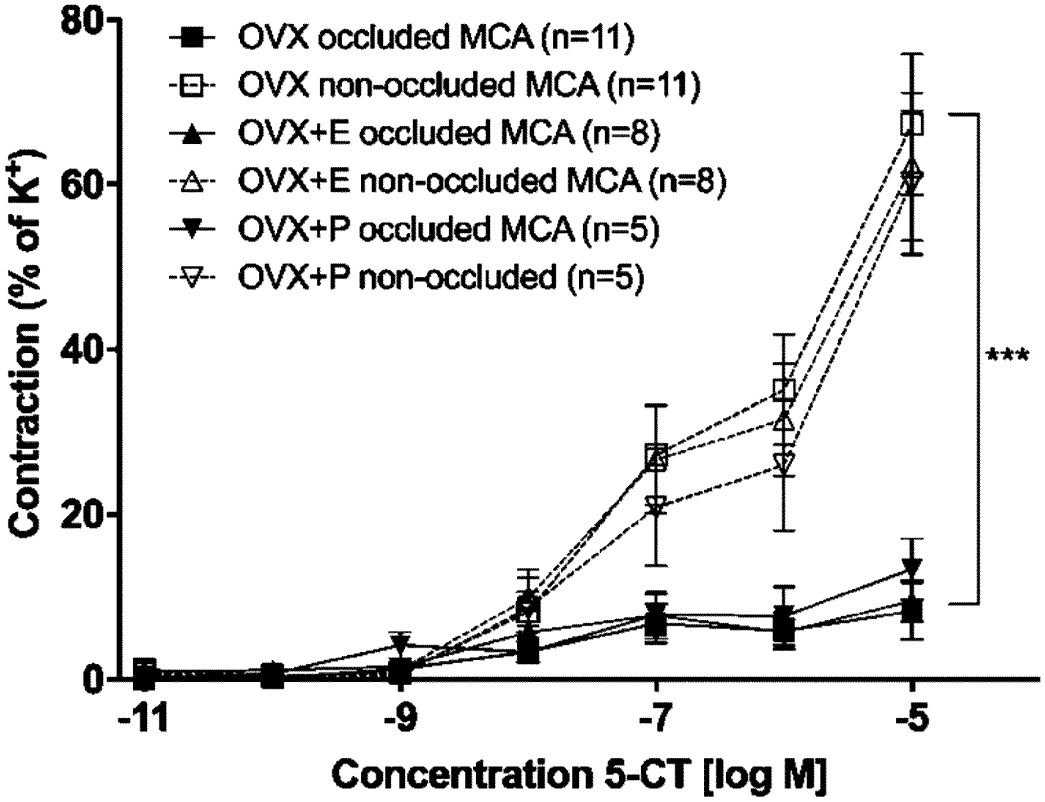

OVX occluded MCA (n=11)
OVX non-occluded MCA (n=11)
OVX+E occluded MCA (n=8)
OVX+E non-occluded MCA (n=8)
OVX+P occluded MCA (n=5)
OVX+P non-occluded (n=5)
Contraction (% of K+)
80
60
40
20
0
-11
-9
-7
-5
Concentration 5-CT [log M]
***

Fig. 18B

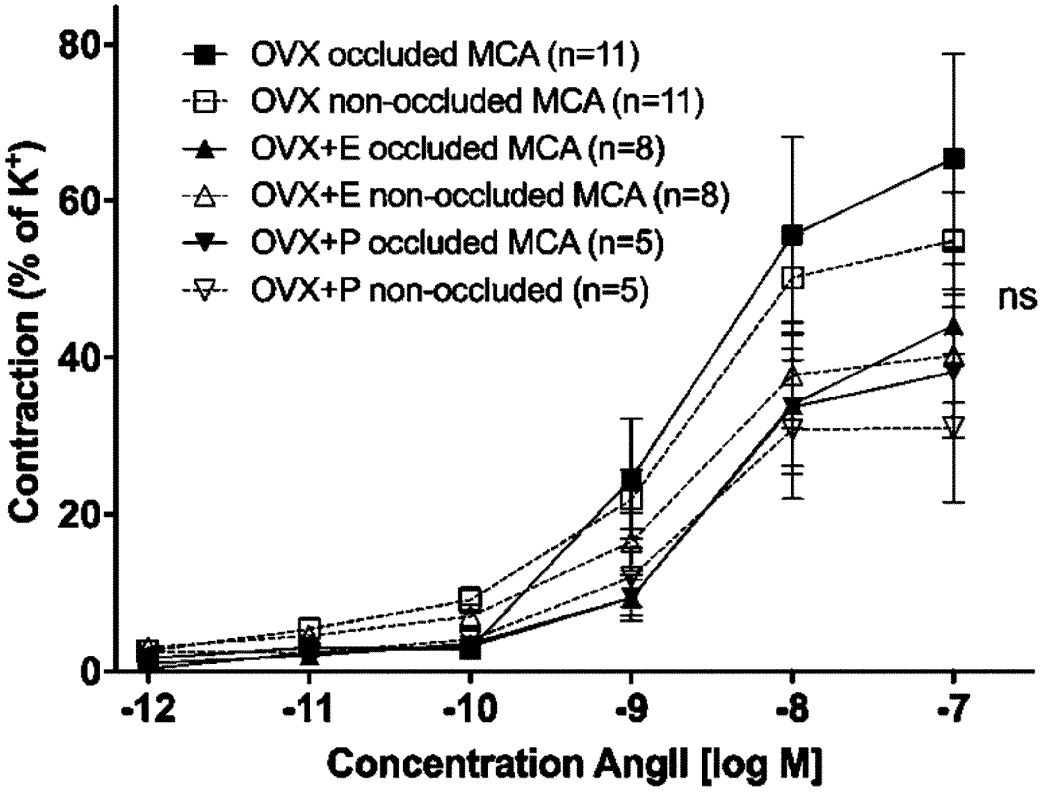

OVX occluded MCA (n=11)
OVX non-occluded MCA (n=11)
OVX+E occluded MCA (n=8)
OVX+E non-occluded MCA (n=8)
OVX+P occluded MCA (n=5)
OVX+P non-occluded (n=5)
ns
Contraction (% of K+)
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Concentration AngII [log M]

Fig. 18C

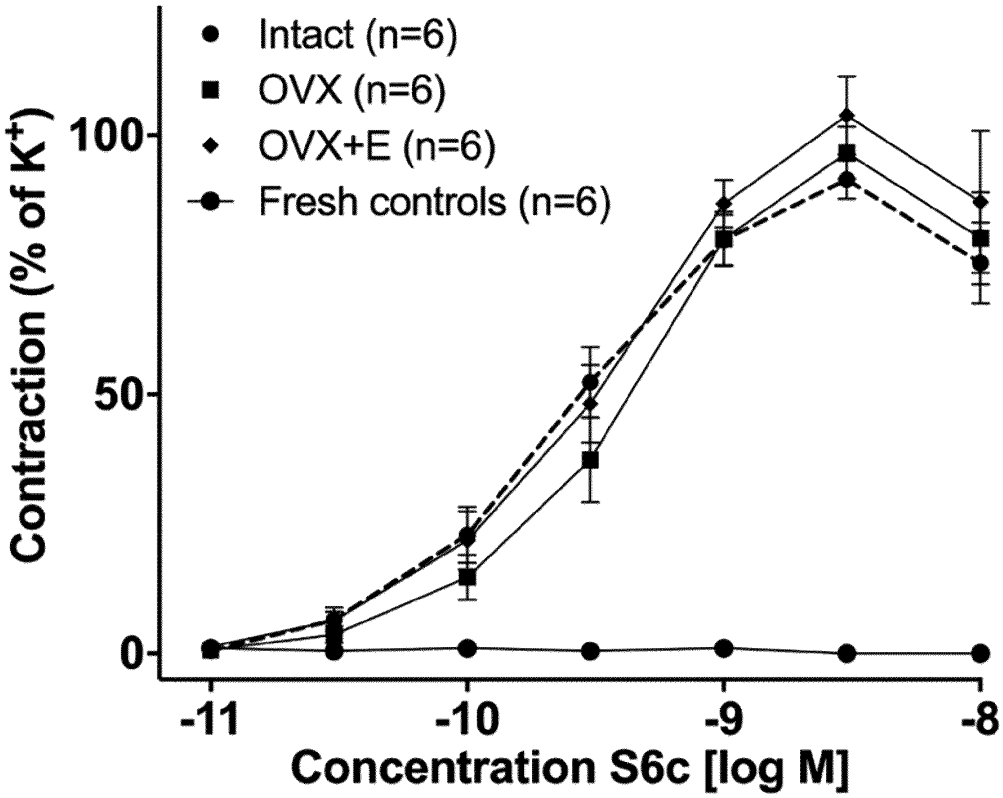

Intact (n=6)
OVX (n=6)
OVX+E (n=6)
Fresh controls (n=6)
Contraction (% of K+)
100
50
0
-11
-10
-9
-8
Concentration S6c [log M]

Fig. 19

	Females (intact)			Females (OVX)			Males		
	Non-occluded	Occluded	N	Non-occluded	Occluded	N	Non-occluded	Occluded	N
S6c	3 ± 6% (0.1 ± 0.2 mN)	22 ± 16%* (0.9 ± 0. 7 mN)	10	1 ± 2 % (0.04 ± 0.1 mN)	8 ± 9%*[a] (0.3 ± 0.4 mN)	11	0%[1] (0 mN)	68%[1] (6.9 mN)	7
5-CT	62 ± 28% (2.6 ± 3.2 mN)	27 ± 18%** (1.1 ± 0.8 mN)	10	67 ± 28% (2.8 ± 2.2 mN)	8 ± 11%**[b] (0.3 ± 0.5 mN)	11	41%[2] (6.1 mN)	13%[2] (1.9 mN)	6
Ang II	49 ± 19% (2.1 ± 0.8 mN)	57 ± 28%[ns] (2.4 ± 1.2 mN)	10	55 ± 20% (2.3 ± 0.8 mN)	65 ± 43%[ns] (2.7 ± 1.8 mN)	11	15%[3] (2.3 mN)	65%[3] (9.7 mN)	8

Fig. 20

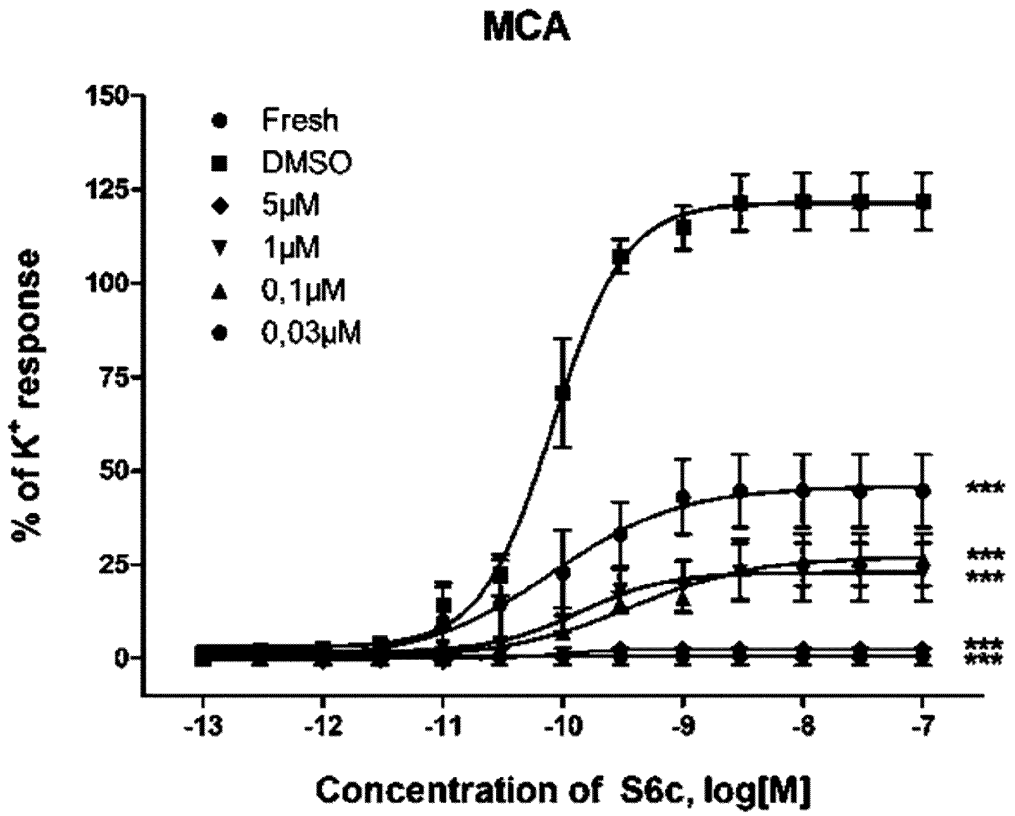

MCA
Fresh
DMSO
5µM
1µM
0,1µM
0,03µM
% of K+ response
150
125
100
75
50
25
0
-13
-12
-11
-10
-9
-8
-7
Concentration of S6c, log[M]
***
***
***
***
***

Fig. 21A

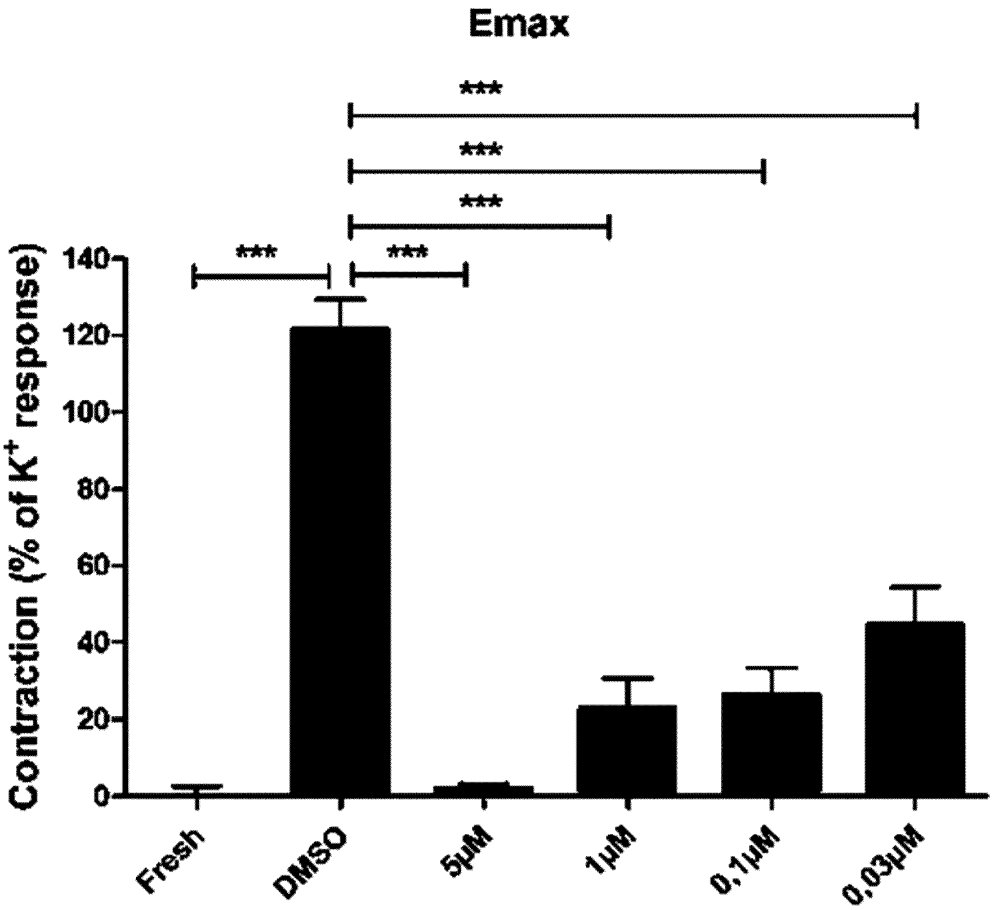

Emax
***
***
***
***
***
Contraction (% of K+ response)
140
120
100
80
60
40
20
0
Fresh
DMSO
5µM
1µM
0,1µM
0,03µM

Fig. 21B

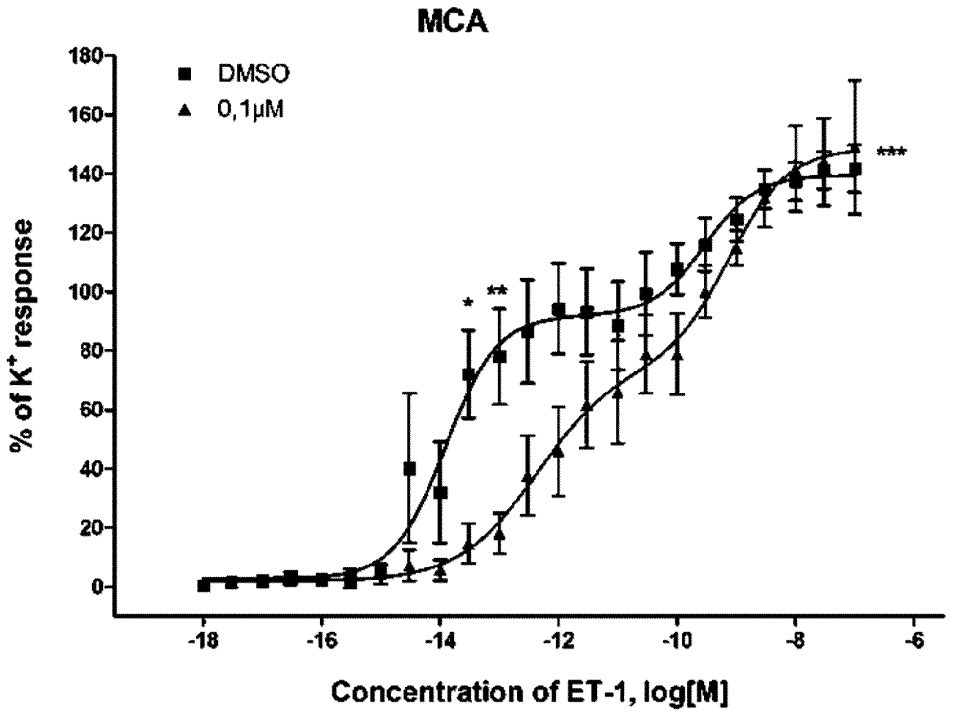

MCA
DMSO
0,1µM
% of K+ response
Concentration of ET-1, log[M]
180
160
140
120
100
80
60
40
20
0
-18
-16
-14
-12
-10
-8
-6
*
**
***

Fig. 21C

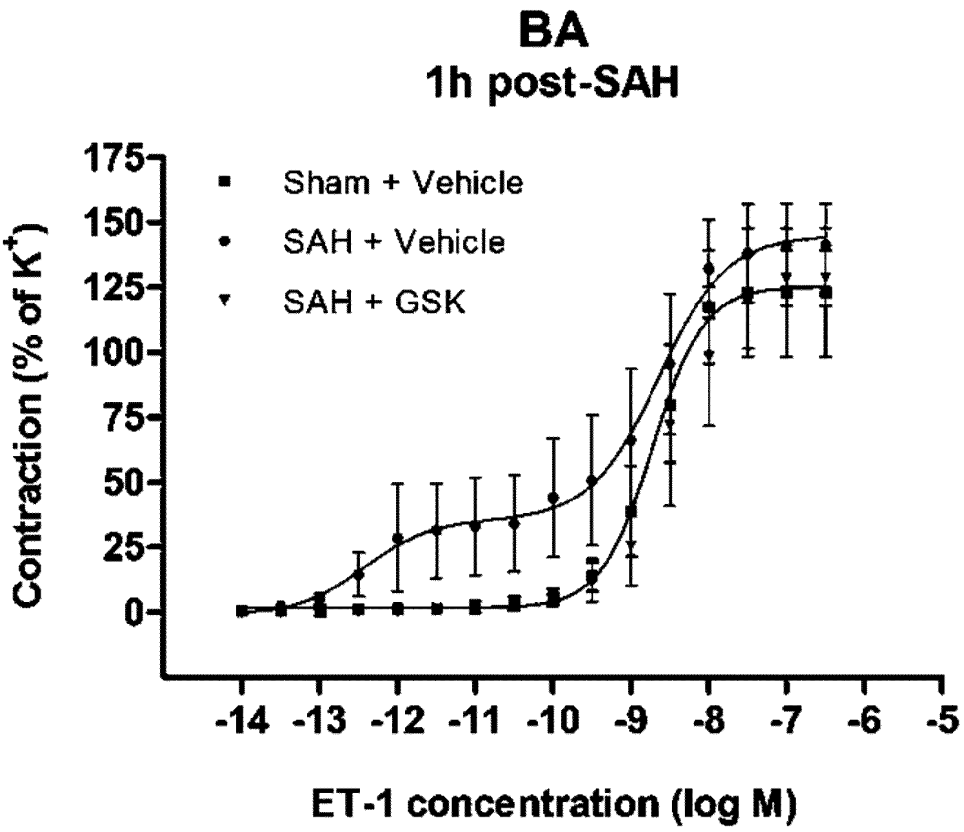

BA
1h post-SAH
Sham + Vehicle
SAH + Vehicle
SAH + GSK
Contraction (% of K+)
175
150
125
100
75
50
25
0
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5
ET-1 concentration (log M)

Fig. 22A

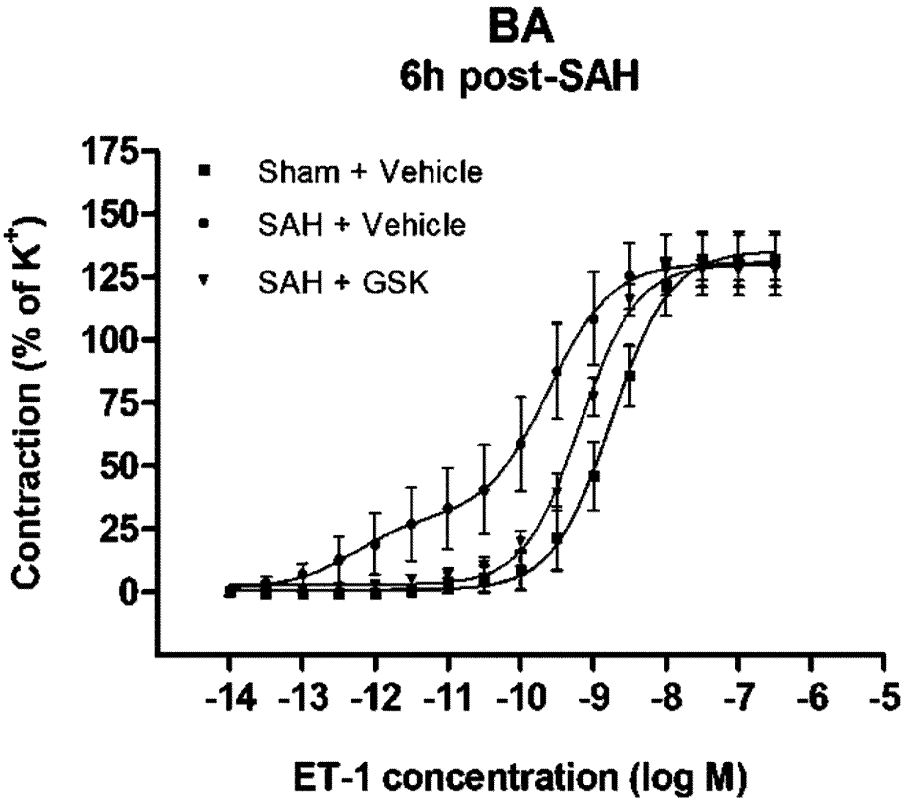

BA
6h post-SAH
Sham + Vehicle
SAH + Vehicle
SAH + GSK
Contraction (% of K+)
175
150
125
100
75
50
25
0
-14 -13 -12 -11 -10 -9 -8 -7 -6 -5
ET-1 concentration (log M)

Fig. 22B

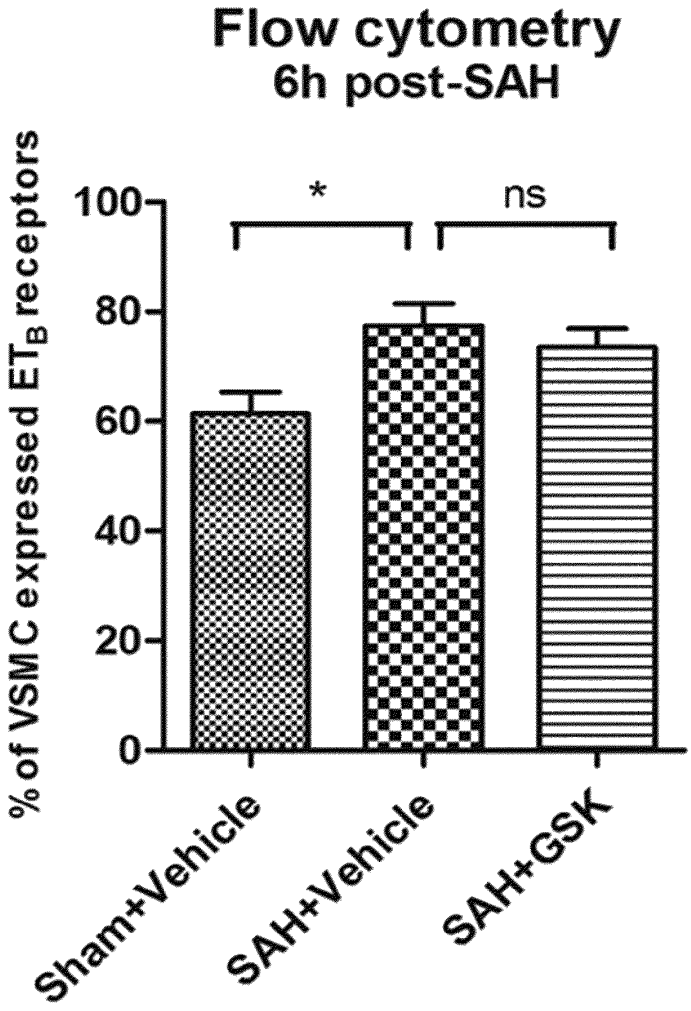

Flow cytometry
6h post-SAH
% of VSMC expressed ET_B receptors
100
80
60
40
20
0
*
ns
Sham+Vehicle
SAH+Vehicle
SAH+GSK

Fig. 22C