



Suppl. fig. 1. Schematic of the experimental timeline. **A.** Mice (aged P50--55) were injected (I.P) with either VCD (130 mg/kg) or oil for 15 days. Mice were weighed at the beginning of each week. **B.** At pre-, peri- and post-AOF timepoints mice were implanted with osmotic minipumps containing AngII (delivered at rate of 600 ng/kg/min) or saline. Estrous cycles were assessed using vaginal smear cytology 8--10 days prior to implanting osmotic minipumps. Blood pressure was assessed using tail-cuff plethmography 1 day prior to mini-pump implantation (baseline) and every 3--4 days after minipump implantations. Mice were euthanized 14 days following minipump implantations and their brains were collected for electron microscopic or ROS experiments.

Suppl. fig. 1. Schematic of the experimental timeline. **A.** Mice (aged P50-55) were injected (I.P) with either VCD (130 mg/kg) or oil for 15 days. Mice were weighed at the beginning of each week. **B.** At pre-, peri- and post-AOF timepoints mice were implanted with osmotic minipumps containing AngII (delivered at rate of 600 ng/kg/min) or saline. Estrous cycles were assessed using vaginal smear cytology 8-10 days prior to implanting osmotic minipumps. Blood pressure was assessed using tail-cuff plethmography 1 day prior to minipump implantation (baseline) and every 3-4 days after minipump implantations. Mice were euthanized 14 days following minipump implantations and their brains were collected for electron microscopic or ROS experiments.

Suppl. table 3. Systolic Blood Pressure measurements from AOF mice

Group	N	Pre-implant	Post implant 2 days	Post-implant 5 days	Post implant 9 days
Pre-AOF:					
Oil-saline	9	107.0 ± 5.0	109.4 ± 5.0	112.0 ± 5.3	106.7 ± 6.8
Oil-AngII	7	109.3 ± 2.4	121.0 ± 6.1	111.3 ± 7.3	110.0 ± 3.7
VCD-saline	5	105.6 ± 3.2	107.7 ± 2.4	110.7 ± 8.4	112.5 ± 4.2
VCD-AngII	8	111.2 ± 2.7	111.4 ± 5.4	109.2 ± 4.4	107.6 ± 4.8
Peri-AOF:					
Oil-saline	6	103.0 ± 4.6	103.2 ± 5.2	103.4 ± 5.5	104.6 ± 5.8
Oil-AngII	4	110.6 ± 8.8	109.4 ± 8.0	108.8 ± 6.8	119.8 ± 9.6
VCD-saline	4	114.7 ± 2.9	116.8 ± 5.8	105.0 ± 12.5	107.9 ± 4.0
VCD-AngII	5	113.5 ± 2.5	114.4 ± 7.0	115.0 ± 2.8	121.6 ± 3.8
Post-AOF:					
Oil-saline	6	107.4 ± 5.9	108.2 ± 6.2	112.1 ± 5.5	109.7 ± 7.6
Oil-AngII	9	107.5 ± 3.5	116.3 ± 4.5	112.1 ± 4.8	116.9 ± 6.8
VCD-saline	8	104.5 ± 5.6	104.1 ± 3.1	105.9 ± 5.6	112.8 ± 6.1
VCD-AngII	7	108.7 ± 4.9	116.0 ± 4.2	111.0 ± 7.8	120.0 ± 7.8

Suppl. table 2. Estrous Cycle Stages for mice not used for EM experiments

PRE AOF TIMEPOINT (~2.7 months old) *

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
62-1	M	D	E		M	M	PE	E	M		saline	E
62-2	M	M	PE		M	M	M	E	M		saline	E
62-3	E	PE	E		M	M	M	M	M		saline	MD
70-2											saline	
64-1	E	E	EM		M	M	PE	PE	M		AngII	MD
64-2	M	DE	M		M	M	PE	PE	E		AngII	PE
64-3	M	M	P		E	M	M	PE	E		AngII	E

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
61-1	PE	E	E		M	D	PE	E	M		saline	MD
61-3	E	M	D		E	M	M	MD	M		saline	E
61-2	E	M	M		M	M	M	M	D		AngII	E
68-1	MD	MD	D	PE	E	MD	PE	E			AngII	MD
68-2	PE	E	EM	MD	MD	MD	E	E			AngII	E
68-3	PE	E	E	MD	MD	E	E	E			AngII	

PERI AOF TIMEPOINT (~4 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
36-2	M	D	E	M	E	E	M	MD			saline	MD
38-1	M	M	M	M	PE	E	M	M	PE		saline	E
38-2	M	E	M	M	M	E	M	M	PE		saline	MD
985-5	MD	MD	D	PE	ED	E	ED	MD	EP		AngII	DP

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
30-1	E	MD	MD	E	MD	MD	MD	D			saline	MD
38-4	M	PE	E	E	M	M	PE	PE	E		AngII	E
43-2	MD	PE	E	MD	MD	MD	MD	PE	E	E	AngII	MD

POST AOF TIMEPOINT (~6 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
02-2	MD	MD	E	EM	P	E	E	MD	MD		saline	MD
16-1	M	D	D	D	E	E	M	M	E	M	saline	
16-2	E	M	M	P	E		M	PE	E	M	saline	
02-1	MD	PE	E	MD	MD	D	DP	MD	MD		AngII	MD
32-1	E	M	M	M	M	E	E	E	M	M	AngII	E
33-1	E	M	M	PE	M	M	PE	E	E		AngII	E
33-2	EM	MD	M	PE	M	M	M	M	M		AngII	MD
33-3	PE	E	M	PE	MD	E	E	M	M		AngII	E

VCD – Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
02-3	MD	MD	MD	MD	MD	E	E	E	E		saline	E
17-1	M	M	M	M	M	M	M	M	M	M	saline	
17-2	M	M	M	M	M	M	M	M	M	E	saline	
17-3	E	M	M	M	M	M	M	E	E	M	saline	
29-3	MD	MD	MD	D	D	D	EM	E	MD		saline	
92-2	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	AngII	MD
95-1	E	MD	MD	E	MD	MD	D	MD	MD		AngII	MD
29-1	E	E	E	E	E	MD	D	E	MD		AngII	
29-2	MD	MD	MD	D	E	MD	MD	E	MD		AngII	

D = diestrus

E = estrus

M = metestrus

P = proestrus

Black – mice used for ROS studies

Suppl. table 2. Estrous Cycle Stages for mice not used for EM experiments

PRE AOF TIMEPOINT (~2.7 months old) *

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
62-1	M	D	E		M	M	PE	E	M		saline	E
62-2	M	M	PE		M	M	M	E	M		saline	E
62-3	E	PE	E		M	M	M	M	M		saline	MD
70-2											saline	
64-1	E	E	EM		M	M	PE	PE	M		AngII	MD
64-2	M	DE	M		M	M	PE	PE	E		AngII	PE
64-3	M	M	P		E	M	M	PE	E		AngII	E

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
61-1	PE	E	E		M	D	PE	E	M		saline	MD
61-3	E	M	D		E	M	M	MD	M		saline	E
61-2	E	M	M		M	M	M	M	D		AngII	E
68-1	MD	MD	D	PE	E	MD	PE	E			AngII	MD
68-2	PE	E	EM	MD	MD	MD	E	E			AngII	E
68-3	PE	E	E	MD	MD	E	E	E			AngII	

PERI AOF TIMEPOINT (~4 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
36-2	M	D	E	M	E	E	M	MD			saline	MD
38-1	M	M	M	M	PE	E	M	M	PE		saline	E
38-2	M	E	M	M	M	E	M	M	PE		saline	MD
985-5	MD	MD	D	PE	ED	E	ED	MD	EP		AngII	DP

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
30-1	E	MD	MD	E	MD	MD	MD	D			saline	MD
38-4	M	PE	E	E	M	M	PE	PE	E		AngII	E
43-2	MD	PE	E	MD	MD	MD	MD	PE	E	E	AngII	MD

POST AOF TIMEPOINT (~6 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
02-2	MD	MD	E	EM	P	E	E	MD	MD		saline	MD
16-1	M	D	D	D	E	E	M	M	E	M	saline	
16-2	E	M	M	P	E		M	PE	E	M	saline	
02-1	MD	PE	E	MD	MD	D	DP	MD	MD		AngII	MD
32-1	E	M	M	M	M	E	E	E	M	M	AngII	E
33-1	E	M	M	PE	M	M	PE	E	E		AngII	E
33-2	EM	MD	M	PE	M	M	M	M	M		AngII	MD
33-3	PE	E	M	PE	MD	E	E	M	M		AngII	E

VCD – Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
02-3	MD	MD	MD	MD	MD	E	E	E	E		saline	E
17-1	M	M	M	M	M	M	M	M	M	M	saline	
17-2	M	M	M	M	M	M	M	M	M	E	saline	
17-3	E	M	M	M	M	M	M	E	E	M	saline	
29-3	MD	MD	MD	D	D	D	EM	E	MD		saline	
92-2	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	AngII	MD
95-1	E	MD	MD	E	MD	MD	D	MD	MD		AngII	MD
29-1	E	E	E	E	E	MD	D	E	MD		AngII	
29-2	MD	MD	MD	D	E	MD	MD	E	MD		AngII	

D = diestrus

E = estrus

M = metestrus

P = proestrus

Black – mice used for ROS studies

Suppl. table 1. Estrous Cycle Stages for electron microscopy experiments

PRE AOF TIMEPOINT (~2.7 months old) (tissue banked for future experiments)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
994-1	M	MD	D	DP	MD	E	E	E	MD		saline	M
600-2		MD	M	MD	PE	E	EM	MD	MD		saline	MD
932-1	E	MD	M		PE	E	E	M	M	M	saline	MD
994-2	MD	PE	PE	E	MD	PE	E	E	MD		AngII	D
600-1		MD	PE	E	MD	MD	D	MD	PE		AngII	MD
932-2	E	M	M		M	PE	E	E	M	MD	AngII	MD

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
985-2	MD	MD	MD	PE	E	D	PE	EM	PE		saline	P
600-3		PE	PE	E	EM	MD1	D	MD	D		saline	MD
932-4	E	M	M		PE	E	E	E	M	MD	saline	MD
985-3	MD	DP	PE	MD	E	E	E	MD	MD		AngII	E
985-4	MD	E	E	E	PE	MD	MD	MD	MD		AngII	E
932-5	E	M	M		PE	E	E	M	M	PE	AngII	PE

PERI AOF TIMEPOINT (~4 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
990 1	E	MD	D	D	PE	D	E	MD	MD	PE	saline	PE
09 2	D	P	E	EM	MD	P	E	EM	MD		saline	MD
36 1	M	D	PE	M	E	MD	PE	E			saline	MD
34 1	E	EM	MD	D		MD	MD	P	PE		AngII	P
30 3	MD	PE	E	EM	MD	EP	E	E			AngII	E
943 1	E	E	E	MD	MD	PE	E	E	MD	MD	AngII	PE

VCD - Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
09 4	MD	MD	PE	E	E	MD	P	E	E		saline	E
34 3	EM	E	MD	MD	MD	E	MD	MD	PE		saline	MD
36 4	E	M	P	E	M	M	E	M			saline	E
34 4	MD	E	E	E	MD	MD	MD	PE	E		AngII	E
36 3	D	M	PE	E	D	P	M	E			AngII	MD
36 5		M	D	D	M	PE	E	E			AngII	E

POST AOF TIMEPOINT (~6 months old)

Oil – Injected (Control)

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
85 2	E	EM	MD	MD	MD	D	E	D	D		saline	D
79 3	PE	MD	MD	MD	E	D	MD	PE	E		saline	E
40 1	MD	MD	PE	PE	E	EM	P	MD	MD	D	saline	D
04 2	MD	PE	E	MD	P	E	MD	D	PE		AngII	PE
04 3	D	PE	E	MD	D	D	MD	MD	PE		AngII	PE
85 1	E	MD	MD	PE	E	PE	E	MD	MD		AngII	MD

VCD – Injected

Animal #	Estrous cycle stage 8-10 days prior to pump implant										Pump contents	terminal estrous stage
	1	2	3	4	5	6	7	8	9	10		
95 2	MD	MD	MD	MD	MD	MD	MD	MD	MD		saline	MD
40 3	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	saline	MD
53 2	MD	MD	PE	E	MD	MD	MD	E	MD	MD	saline	MD
02 4	MD	MD	MD	MD	MD	MD	MD	MD	MD		AngII	MD
79 2	MD	MD	MD	MD	MD	MD	MD	MD	MD		AngII	MD
50 2	E	M	E	M	E	M	M	M	M		AngII	E

D = diestrus
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