Supplementary Figure Legends

Figure S1. Effects of sodium citrate and phenytoin on the membrane potential. (A) V_m in control physiological saline solution, after perfusion with the vehicle for TTX (148 μM sodium citrate, pH = 4.8) and following washout. Solid line, mean; gray shading, SEM (n = 13). (B) Quantification of V_m over the last 5 s in control, sodium citrate, and washout (n = 13). (C) Representative trace showing the inhibitory effect of phenytoin (100 μM) on Na^+ current, and recovery after washout. The cell was held at -120 mV for 250 ms before depolarizing to -10 mV for 50 ms. (D) Expanded view of persistent Na^+ current 40-45 ms following onset of depolarization. (E) Quantification of the normalized transient Na^+ current elicited by depolarizing to -10 mV from a holding potential of -120 mV (n = 9). (F) Quantification of the normalized persistent Na^+ current 40-45 ms after depolarizing to -10 mV from a holding potential of -120 mV (n = 4). (G) Quantification of the normalized transient Na^+ current elicited by depolarizing to -10 mV from a holding potential of -80 mV (n = 4). (H) Quantification of the normalized persistent Na^+ current 40-45 ms after depolarizing to -10 mV from a holding potential of -80 mV (n = 3). (I) V_m in control physiological saline solution, after phenytoin (100 μM) treatment and following washout. Solid line, mean; gray shading, SEM (n = 12). (J) Quantification of V_m over the last 5 s in control, phenytoin, and washout (n = 12). (K) Quantification of V_m over the last 5 s in control, NaOH (75 μM; vehicle for phenytoin) and washout (n = 9). Data are mean and SEM. *P < 0.05; **P < 0.01; ***P < 0.001; repeated measures ANOVA with Tukey test.

Figure S2. Effects of veratridine on Na^+ current and NMDG on membrane potential. (A) I-V relationship for transient Na^+ current in control physiological saline solution and following perfusion of veratridine (100 μM) (n = 6). (B) I-V relationship for persistent Na^+ current (defined as mean current density 45-50 ms following onset of depolarization; n = 6). Data are mean and SEM. Consistent with previous reports [1,2], veratridine caused a small reduction in the transient peak Na^+ current density but increased the peak persistent Na^+

current density. (C) V_m in control physiological saline solution, after extracellular Na^+ replacement with N-methyl-D-glucamine (NMDG) and following washout. Solid line, mean; gray shading, SEM (n = 7). (D) Quantification of V_m over the last 5 s in control, NMDG, and washout (n = 7).

Figure S3. Tetrodotoxin and NS-1619 do not affect the intracellular Ca^{2+} level. (A) Intracellular Ca^{2+} level (340/380 ratio) following perfusion with TTX (30 µM). Solid line, mean; gray shading, SEM (n = 55). (B) 340/380 ratio over the last 30 s in control, TTX and washout (n = 3). (C) Intracellular Ca^{2+} level (340/380 ratio) following pre-treatment with TTX (30 µM) for 48 h (n = 55). (D) 340/380 ratio over the last 30 s of TTX and washout (n = 3). (E) Intracellular Ca^{2+} level (340/380 ratio) following perfusion with NS-1619 (1 µM). Solid line, mean; gray shading, SEM (n = 40). (F) 340/380 ratio over the last 30 s in control, NS-1619 and washout (n = 3). Ionomycin was used at the end of all experiments as a positive control confirming sensitivity of the Ca^{2+} indicator. Data are mean and SEM.

Figure S4. Effect of tetrodotoxin and NS-1619 on proliferation and invasion. (A) Proliferation (quantified as number of cells using the MTT assay) following treatment for 24 h with NS-1619 (1 µM, 40 µM) or vehicle (n = 3). (B) Matrigel invasion ± NS-1619 (1 µM) or TTX (30 µM), normalized to control (n = 4). (C) Matrigel invasion ± NS-1619 (40 µM), normalized to control (n = 3). Data are mean and SEM. *P < 0.05; **P < 0.01; repeated measures ANOVA with Tukey test.

Figure S5. Dose-dependent effect of EHT1864 on cell morphology. (A) Circularity of cells after treatment with EHT1864 (0.5-10 µM) or vehicle for 3 h (n ≥ 277). (C) Feret's diameter (µm) of cells after treatment with EHT1864 (0.5-10 µM) or vehicle for 3 h (n ≥ 277). Data are mean and SEM.

References

1. Ulbricht W (1998) Effects of veratridine on sodium currents and fluxes. Rev Physiol Biochem Pharmacol 133:1-54
2. Ulbricht W (1969) The effect of veratridine on excitable membranes of nerve and muscle. Ergeb Physiol 61:18-71

Figure S1

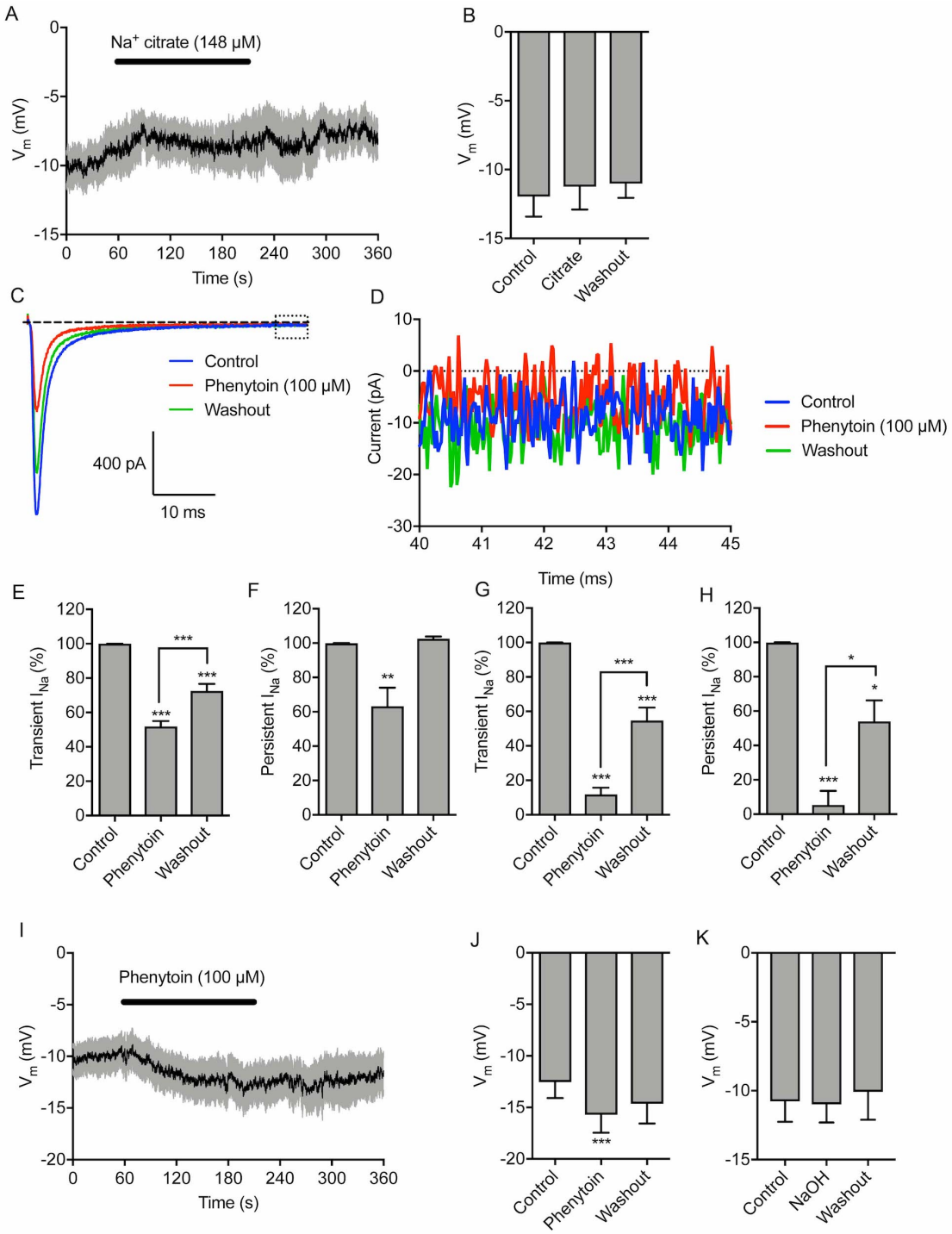

A
Na+ citrate (148 µM)
Vm (mV)
Time (s)
B
Vm (mV)
Control
Citrate
Washout
C
Control
Phenytoin (100 µM)
Washout
400 pA
10 ms
D
Current (pA)
Time (ms)
Control
Phenytoin (100 µM)
Washout
E
Transient INa (%)
F
Persistent INa (%)
G
Transient INa (%)
H
Persistent INa (%)
Control
Phenytoin
Washout
I
Phenytoin (100 µM)
Vm (mV)
Time (s)
J
Vm (mV)
K
Vm (mV)
NaOH

Figure S2

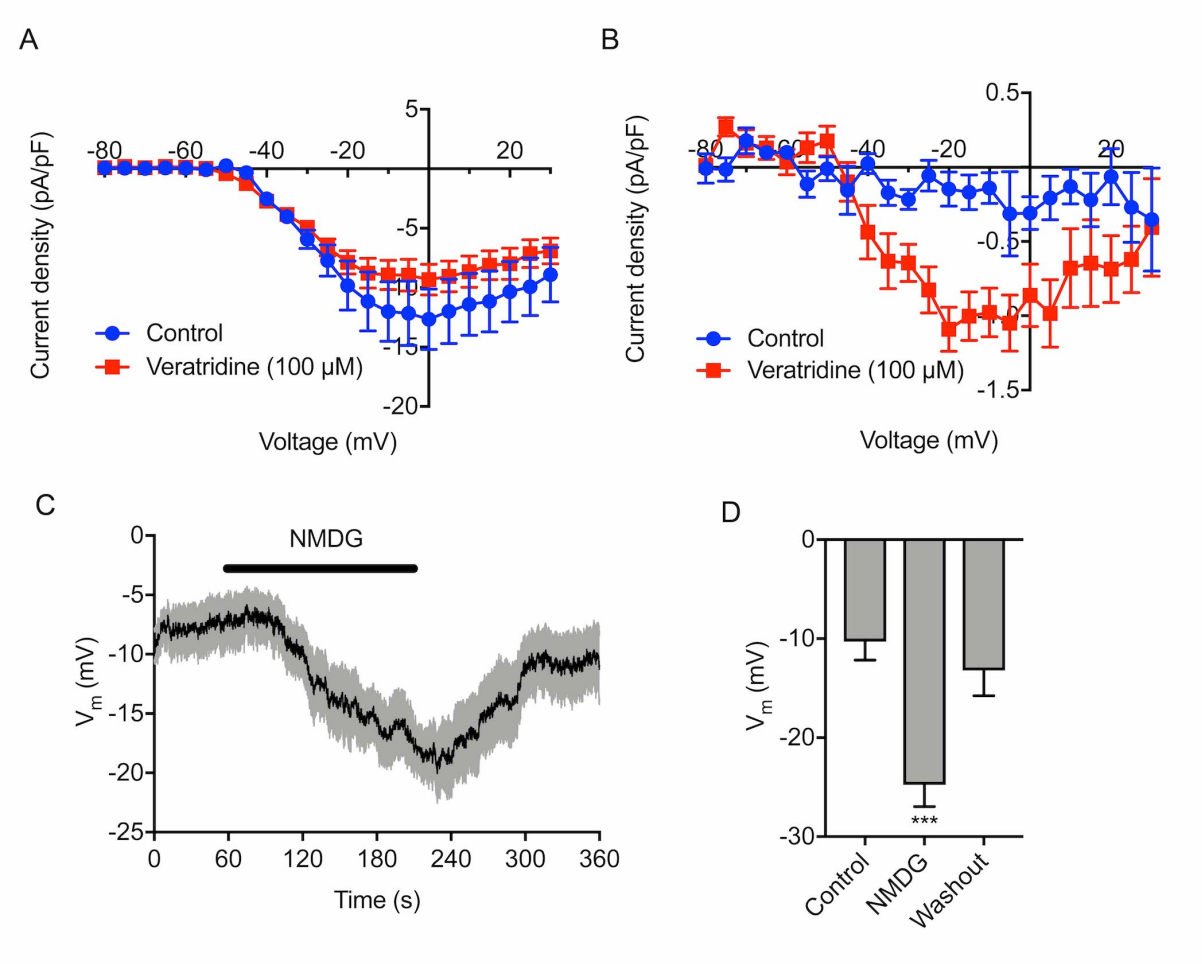

A
Current density (pA/pF)
5
-5
-15
-20
-80
-60
-40
-20
20
Control
Veratridine (100 μM)
Voltage (mV)
B
Current density (pA/pF)
0.5
-0.5
-1.5
-80
-40
-20
20
Control
Veratridine (100 μM)
Voltage (mV)
C
NMDG
V_m (mV)
0
-5
-10
-15
-20
-25
0
60
120
180
240
300
360
Time (s)
D
V_m (mV)
0
-10
-20
-30
***
Control
NMDG
Washout

Figure S3

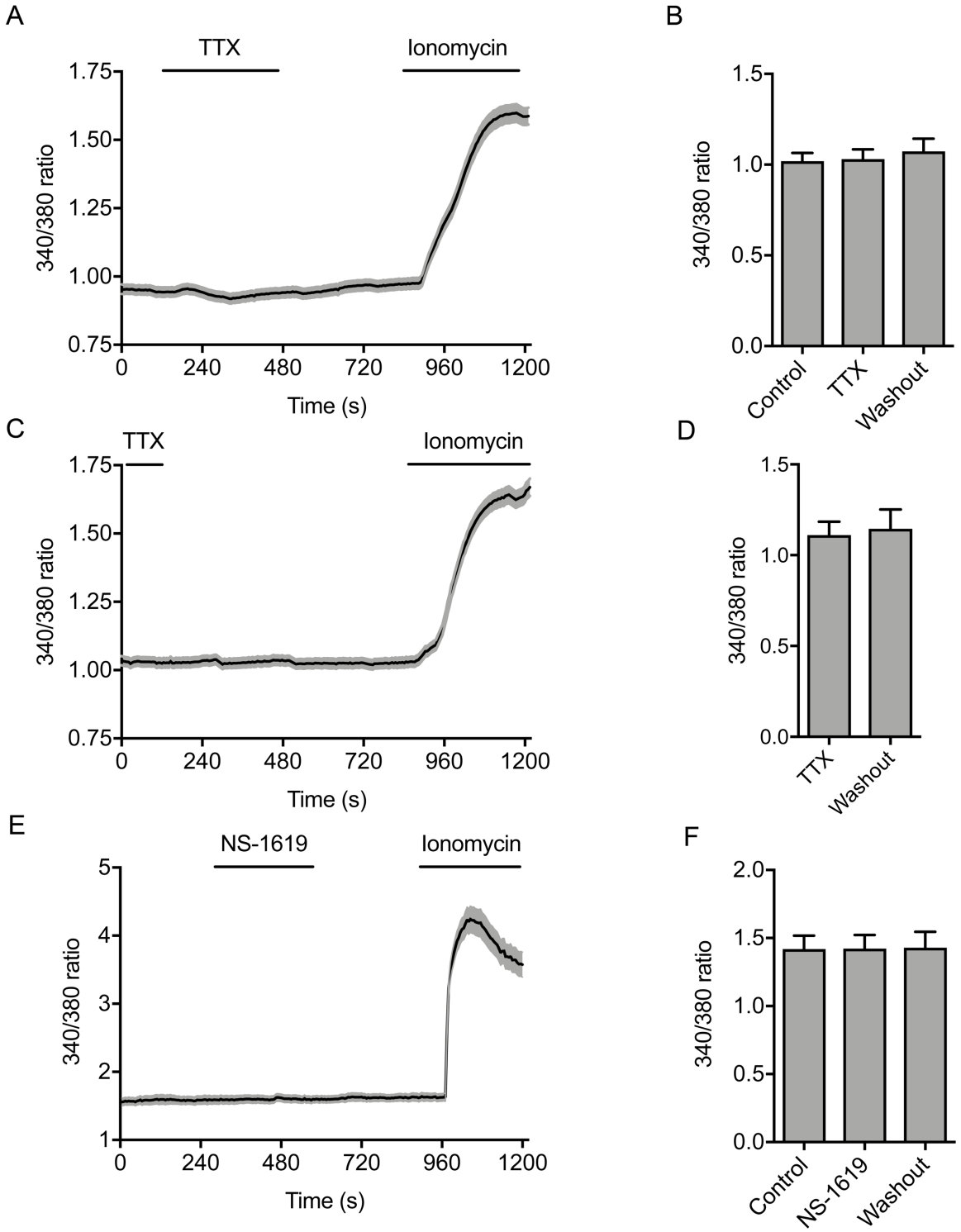

A
TTX
Ionomycin
340/380 ratio
1.75
1.50
1.25
1.00
0.75
0
240
480
720
960
1200
Time (s)
B
340/380 ratio
1.5
1.0
0.5
0.0
Control
TTX
Washout
C
TTX
Ionomycin
340/380 ratio
1.75
1.50
1.25
1.00
0.75
0
240
480
720
960
1200
Time (s)
D
340/380 ratio
1.5
1.0
0.5
0.0
TTX
Washout
E
NS-1619
Ionomycin
340/380 ratio
5
4
3
2
1
0
240
480
720
960
1200
Time (s)
F
340/380 ratio
2.0
1.5
1.0
0.5
0.0
Control
NS-1619
Washout

Figure S4

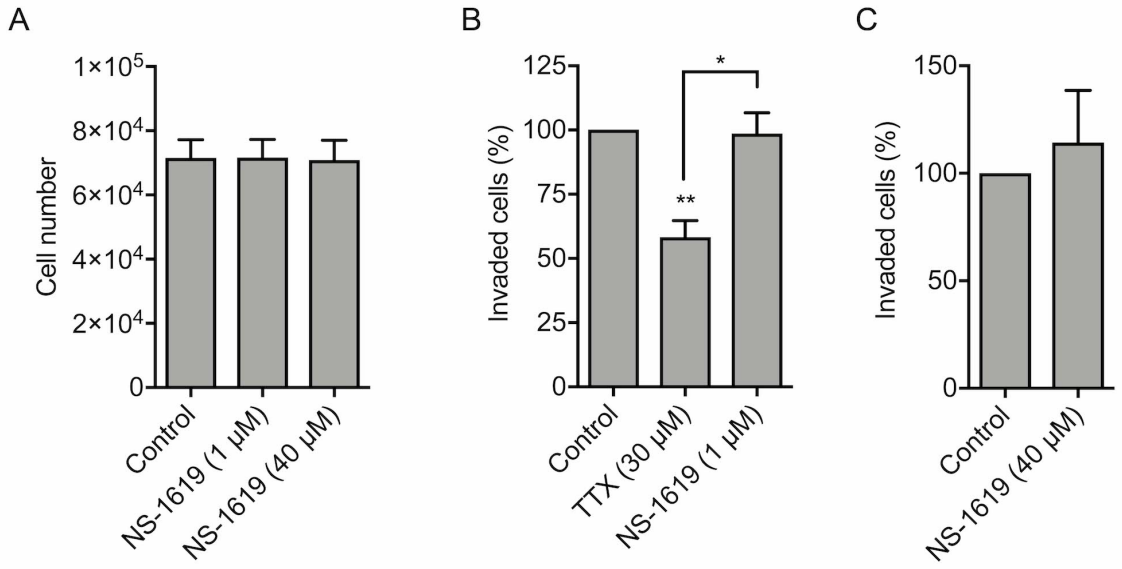
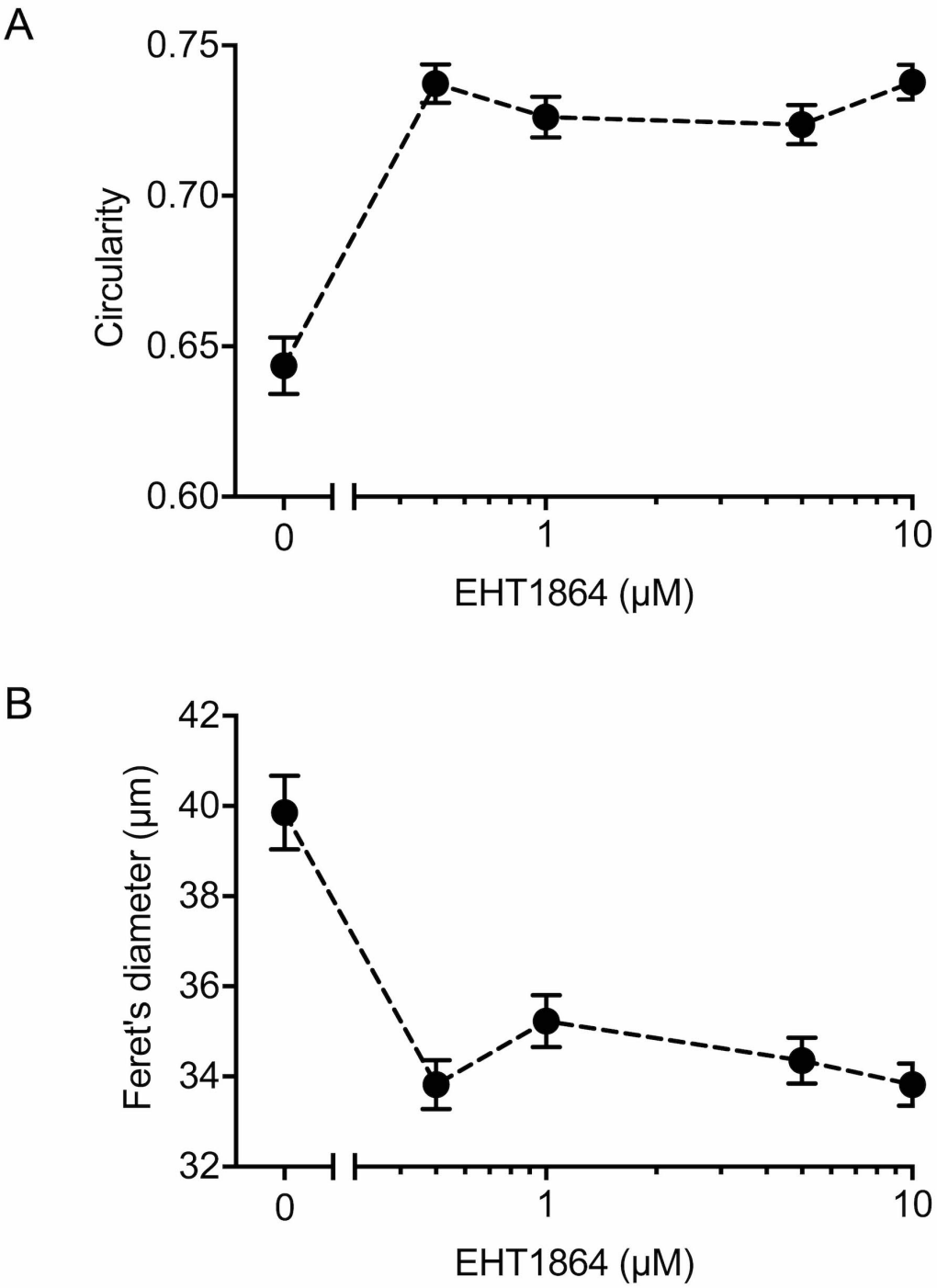

A
Cell number
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
Control
NS-1619 (1 μM)
NS-1619 (40 μM)
B
Invaded cells (%)
125
100
75
50
25
0
**
*
Control
TTX (30 μM)
NS-1619 (1 μM)
C
Invaded cells (%)
150
100
50
0
Control
NS-1619 (40 μM)

Figure S5


A
0.75
0.70
0.65
0.60
Circularity
0
1
10
EHT1864 (μM)
B
42
40
38
36
34
32
Feret's diameter (μm)
0
1
10
EHT1864 (μM)