Supplementary figure and figure legends

Figure S1. Cumulated spike duration (CSD) correlates with nuclear translocation of NFκB in histamine-stimulated single cell(s).

Cells transfected with RelA-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and NFκB-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 112 out of a total of 164 cells examined in 61 separate experiments. Cells with $[Ca^{2+}]_i$ oscillations were separated by their frequencies. For each of the 0.1, 0.2 and 0.3 min^{-1} frequent $[Ca^{2+}]_i$ oscillations, CSD dynamically regulated NFκB translocation for cells with the same frequency (four parameter logistic regression, n=22, 27 and 22 cells, $p < 0.05$ for 0.1, 0.2 and 0.3 min^{-1} frequency as **a**i, ii and iii, respectively). Cells with $[Ca^{2+}]_i$ oscillations were also separated by their CSD in the narrow ranges that did not significantly affect NFκB translocation. For each of the 90-190, 208-385, 394-445, 481-578, 635-812 and 853-1326 sec CSD, frequency did not correlate with NFκB translocation for cells with the same CSD range (two parameter power regression, n=13, 16, 12 ,12, 11 and16 cells, $p > 0.05$ for 90-190, 208-385, 394-445, 481-578, 635-812 and 853-1326 sec as **b**i, ii, iii, iv, v and vi, respectively).

Figure S2. Cumulated spike duration (CSD) correlates with nuclear translocation of STAT3 in histamine-stimulated single cell(s).

Cells transfected with STAT3-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and STAT3-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 157 out of a total of 271 cells examined in 40 separate experiments. Cells with $[Ca^{2+}]_i$ oscillations were separated by their frequencies. For each of the 0.1, 0.2 and 0.3 min^{-1} frequent $[Ca^{2+}]_i$ oscillations, CSD dynamically regulated STAT3 translocation for cells with the same frequency (two parameter power regression, n=32, 34 and 17 cells, $p < 0.05$ for 0.1, 0.2 and 0.3 min^{-1} frequency as **a**i, ii and iii, respectively). Cells with $[Ca^{2+}]_i$ oscillations were also separated by their CSD in the narrow ranges that did not significantly affect STAT3 translocation. For each of the 21-100, 102-195, 204-297, 301-389 and 405-537 sec CSD, frequency did not correlate with with STAT3 translocation for cells with the same CSD range (two parameter power regression ,n=30, 21, 21, 12 and 13 cells, $p > 0.05$ for 21-100, 102-195, 204-297, 301-389 and 405-537 sec as **b**i, ii, iii, iv and v, respectively).

Figure S3. Frequency does not correlate with cumulated spike duration (CSD) in agonist-stimulated $[Ca^{2+}]_i$ oscillations.

Cells transfected with RelA-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and NFκB-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 112 out of a total of 164 cells examined in 61 separate experiments. CSD at the $[Ca^{2+}]_i$ threshold level of 180 nM for NFκB activation in this type of cells was determined individually and plotted agonist the frequency for each of the 112 cells ($p > 0.05$, **a**). Cells transfected with STAT3-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and STAT3-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 157 out of a total of 271 cells examined in 40 separate experiments. CSD at the $[Ca^{2+}]_i$ threshold level of 350 nM for STAT3 activation in this type of cells was determined individually and plotted agonist the frequency for each of the 157 cells ($p > 0.05$, **b**).

Figure S4. Frequency correlates with cumulated spike duration (CSD) and nuclear translocation of NFκB and STAT3 in cells showing $[Ca^{2+}]_i$ oscillations with the amplitude of each spike reaching the Ca^{2+} threshold for NFκB or STAT3 activation in response to agonist stimulation.

Cells transfected with RelA-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and NFκB-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 112 out of a total of 164 cells examined in 61 separate experiments. CSD at the $[Ca^{2+}]_i$ threshold level of 180 nM for NFκB activation in this type of cells was determined individually. The frequency were plotted agonist the CSD from 42 cells each with the amplitude of each spike reaching 180 nM and agonist the ratio of nucleus/cytosol NFκB-GFP fluorescence intensity, as **a**i and **a**ii respectively (hyperbola regression, $p < 0.05$ for both). Cells transfected with STAT3-GFP vector were loaded with fura-red and then subjected to a stimulation of 10 μM histamine for 60 min, alterations in $[Ca^{2+}]_i$ and STAT3-GFP translocation for each cell were simultaneously monitored. Histamine stimulated $[Ca^{2+}]_i$ oscillations in 157 out of a total of 271 cells examined in 40 separate experiments. CSD at the $[Ca^{2+}]_i$ threshold level of 350 nM for STAT3 activation in this type of cells was determined individually. The frequency were plotted agonist the CSD from 24 cells each with the amplitude of each spike reaching 350 nM and agonist the ratio of nucleus/cytosol STAT3-GFP fluorescence intensity, as **b**i and **b**ii respectively (hyperbola regression, $p < 0.05$ for both).

Figure S1

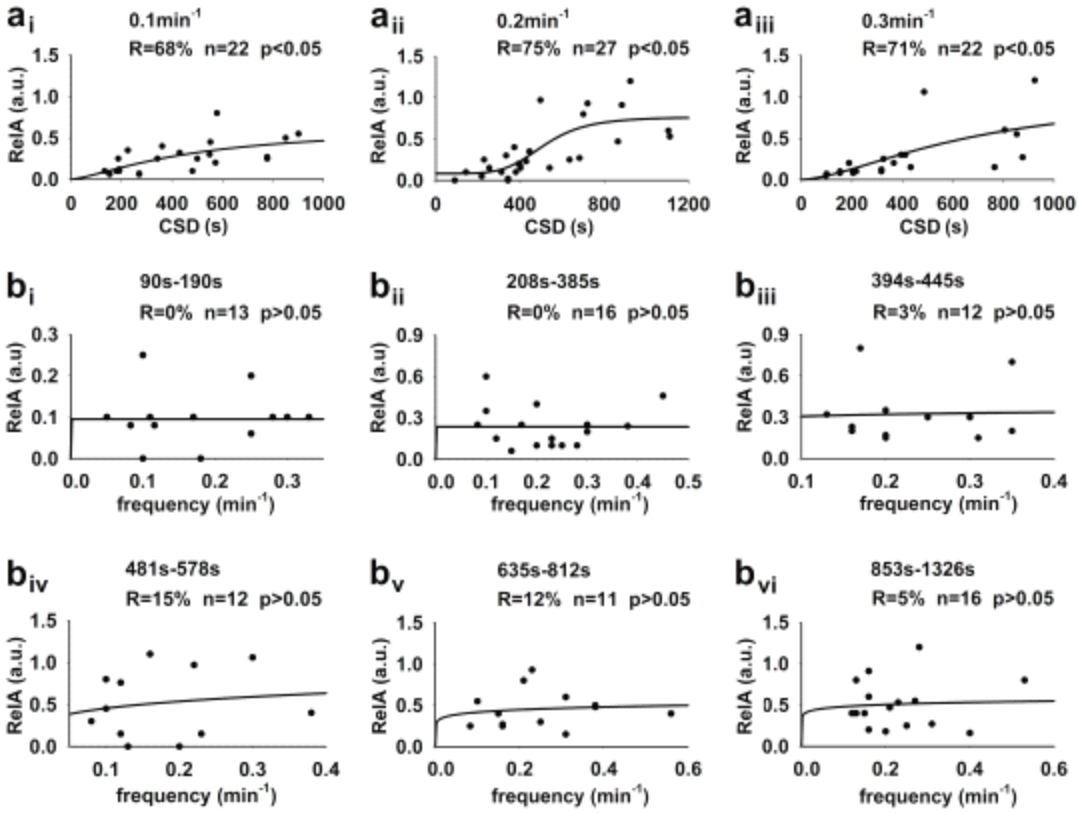

a_i
0.1min-1
R=68% n=22 p<0.05
a_ii
0.2min-1
R=75% n=27 p<0.05
a_iii
0.3min-1
R=71% n=22 p<0.05
RelA (a.u.)
CSD (s)
b_i
90s-190s
R=0% n=13 p>0.05
b_ii
208s-385s
R=0% n=16 p>0.05
b_iii
394s-445s
R=3% n=12 p>0.05
b_iv
481s-578s
R=15% n=12 p>0.05
b_v
635s-812s
R=12% n=11 p>0.05
b_vi
853s-1326s
R=5% n=16 p>0.05
frequency (min-1)

Figure S2

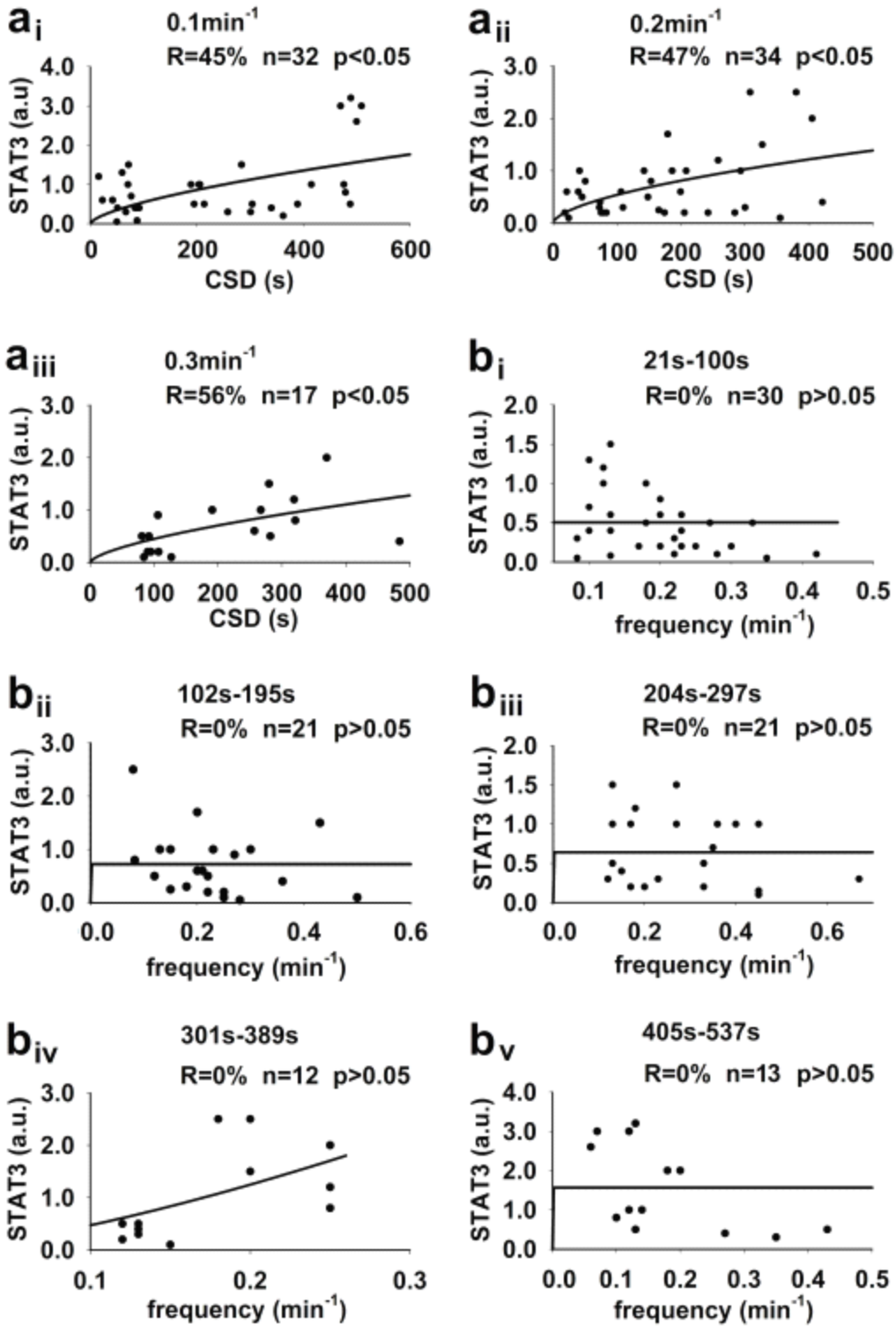

a i
0.1min-1
R=45% n=32 p<0.05
STAT3 (a.u)
CSD (s)
a ii
0.2min-1
R=47% n=34 p<0.05
STAT3 (a.u.)
CSD (s)
a iii
0.3min-1
R=56% n=17 p<0.05
STAT3 (a.u.)
CSD (s)
b i
21s-100s
R=0% n=30 p>0.05
STAT3 (a.u.)
frequency (min-1)
b ii
102s-195s
R=0% n=21 p>0.05
STAT3 (a.u.)
frequency (min-1)
b iii
204s-297s
R=0% n=21 p>0.05
STAT3 (a.u.)
frequency (min-1)
b iv
301s-389s
R=0% n=12 p>0.05
STAT3 (a.u.)
frequency (min-1)
b v
405s-537s
R=0% n=13 p>0.05
STAT3 (a.u.)
frequency (min-1)

Figure S3

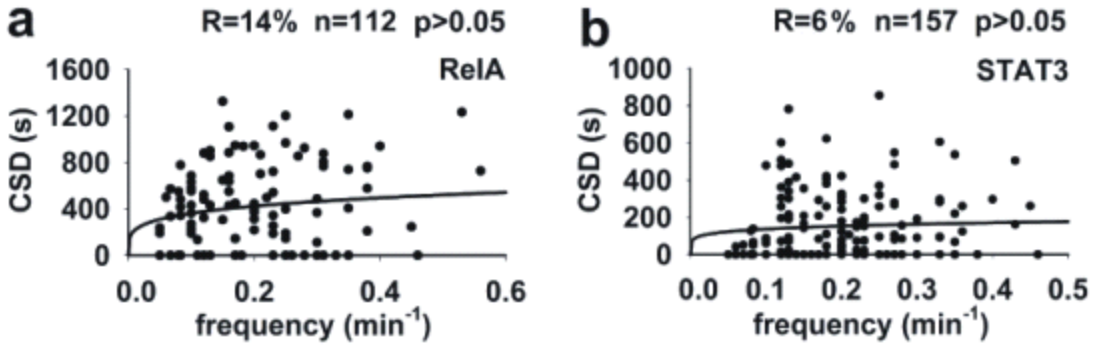
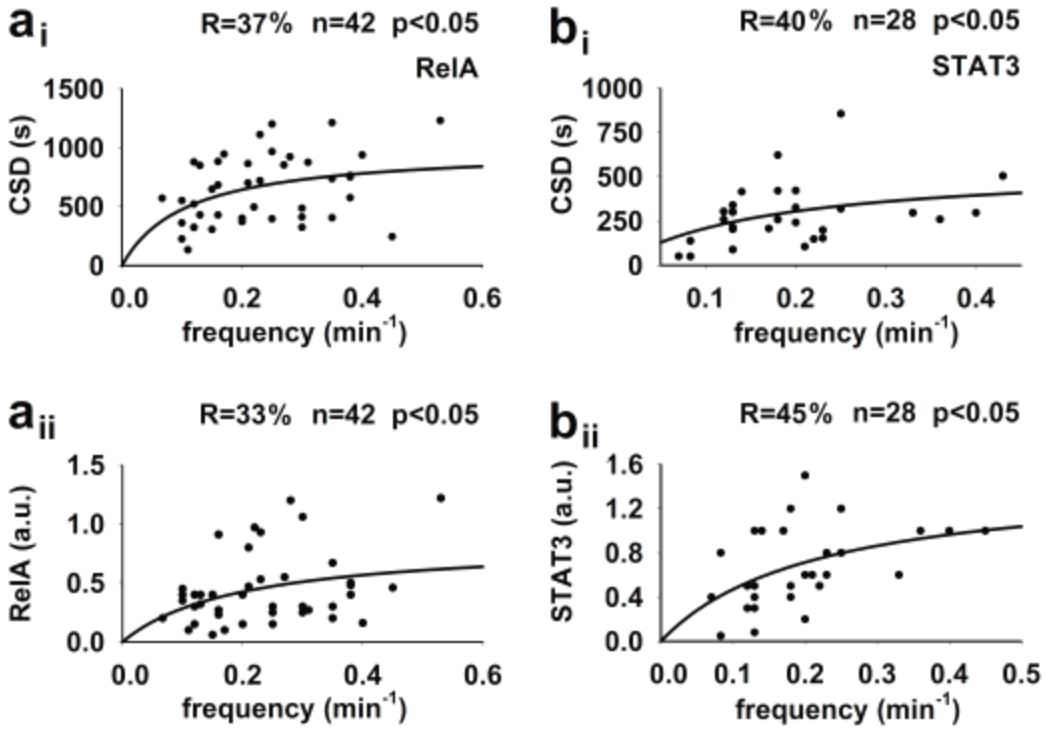

a
R=14% n=112 p>0.05
RelA
CSD (s)
1600
1200
800
400
0
0.0
0.2
0.4
0.6
frequency (min-1)
b
R=6% n=157 p>0.05
STAT3
CSD (s)
1000
800
600
400
200
0
0.0
0.1
0.2
0.3
0.4
0.5
frequency (min-1)

Figure S4


a i
R=37% n=42 p<0.05
RelA
CSD (s)
1500
1000
500
0
0.0
0.2
0.4
0.6
frequency (min-1)
b i
R=40% n=28 p<0.05
STAT3
CSD (s)
1000
750
500
250
0
0.1
0.2
0.3
0.4
frequency (min-1)
a ii
R=33% n=42 p<0.05
RelA (a.u.)
1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
frequency (min-1)
b ii
R=45% n=28 p<0.05
STAT3 (a.u.)
1.6
1.2
0.8
0.4
0.0
0.0
0.1
0.2
0.3
0.4
0.5
frequency (min-1)