Supplementary Material

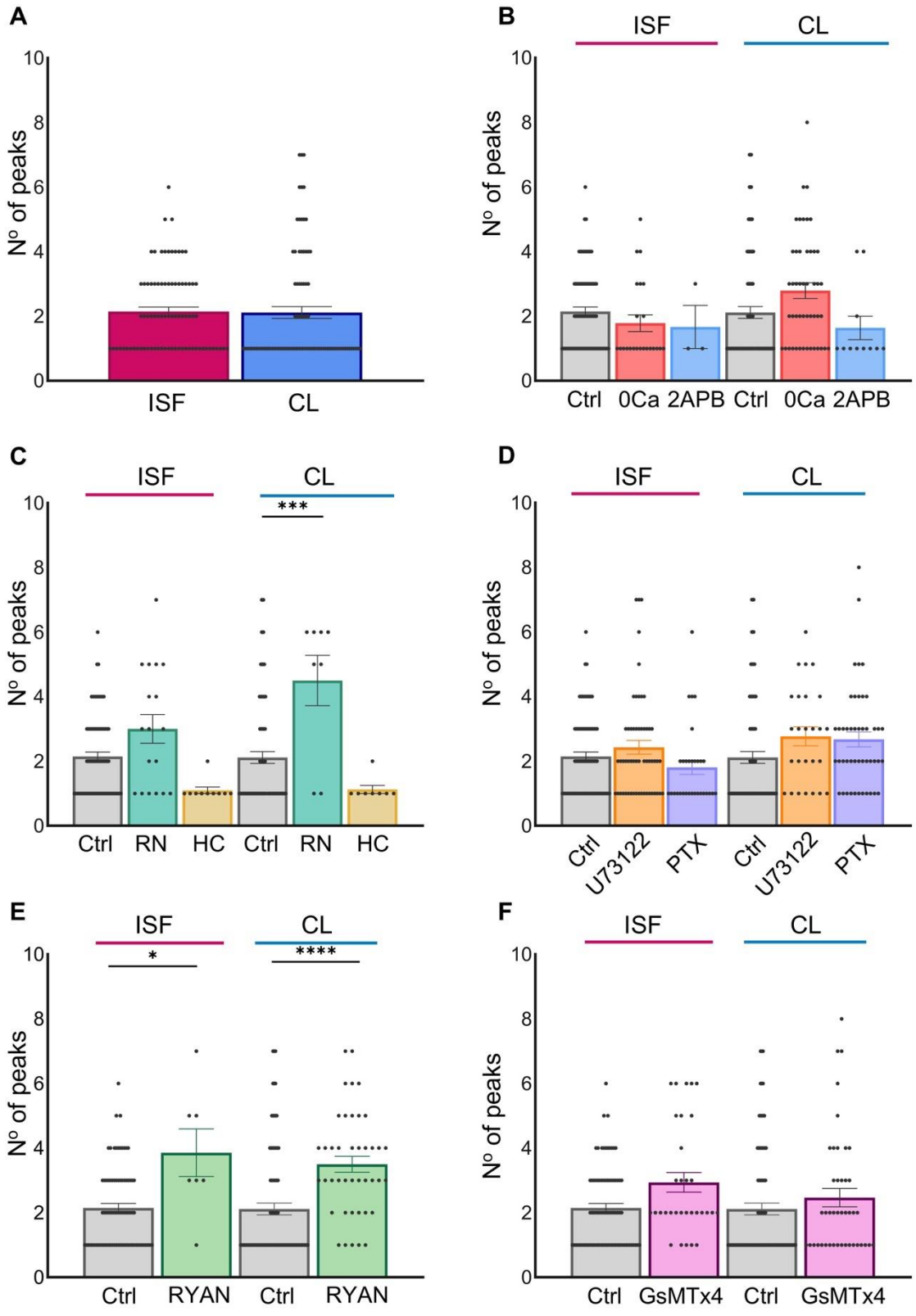

Figure S 1 Histograms of the number of Ca^{2+} transient peaks per cell measured by calcium imaging in in-vitro astrocytes following ISF or continuous-light stimulation. Distributions are shown for each condition;

summary values are reported as mean ± SEM. A) Data values corresponding to ISF in control conditions and CL in control conditions are colored in magenta and dark blue respectively. Asterisks denote significant difference: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$), **** ($p<0.0001$), One-way ANOVA with Bonferroni Correction. ISF N=82, n=7; CL N=88, n=7 **B)** Data values corresponding to control are colored in grey, $0[Ca^{2+}]_o$ in red, and 2-APB in blue. ISF control N=82, n=7; ISF $0[Ca^{2+}]o$ N=23, n=5; ISF 2-APB N=3, n=4; CL control N=88, n=7; CL $0[Ca^{2+}]o$ N=48, n=5; CL 2-APB N=11, n=4 **C)** Data values corresponding to control are colored in grey, to RN-1734 (RN) in teal and HC-030031 (HC) in gold. ISF control N=82, n=7; ISF RN-1734 N=18, n=4; ISF HC-030031 N=10, n=3; CL control N=88, n=7; CL RN-1734 N=8, n=4; CL HC-030031 N=8, n=3. **D)** Data values corresponding to control standard extracellular solution are colored in grey, to G_q–PLC–IP_3 inhibitor U73122 in orange, and $G_{i/o}$ inhibitor PTX in purple. **E)** Data values corresponding to the control standard extracellular solution are colored in grey, and to RYAN in green. ISF control N=82, n=7; ISF ryanodine N=14, n=3; CL control N=88, n=7; CL ryanodine N=42, n=3. **F)** Data values corresponding to the control standard extracellular solution are colored in grey, and those for MSCs inhibitor GsMTx4 (1 μM) are colored in pink. ISF control N=82, n=7; ISF GsMTx4 N=56, n=3; CL control N=88, n=7; CL GsMTx4 N=50, n=3. *N*, number of peaks per cell/ROI, and *n*, number of experiments for each panel, are reported in Tables S1–S5, and p-values in Tables S6–S10.

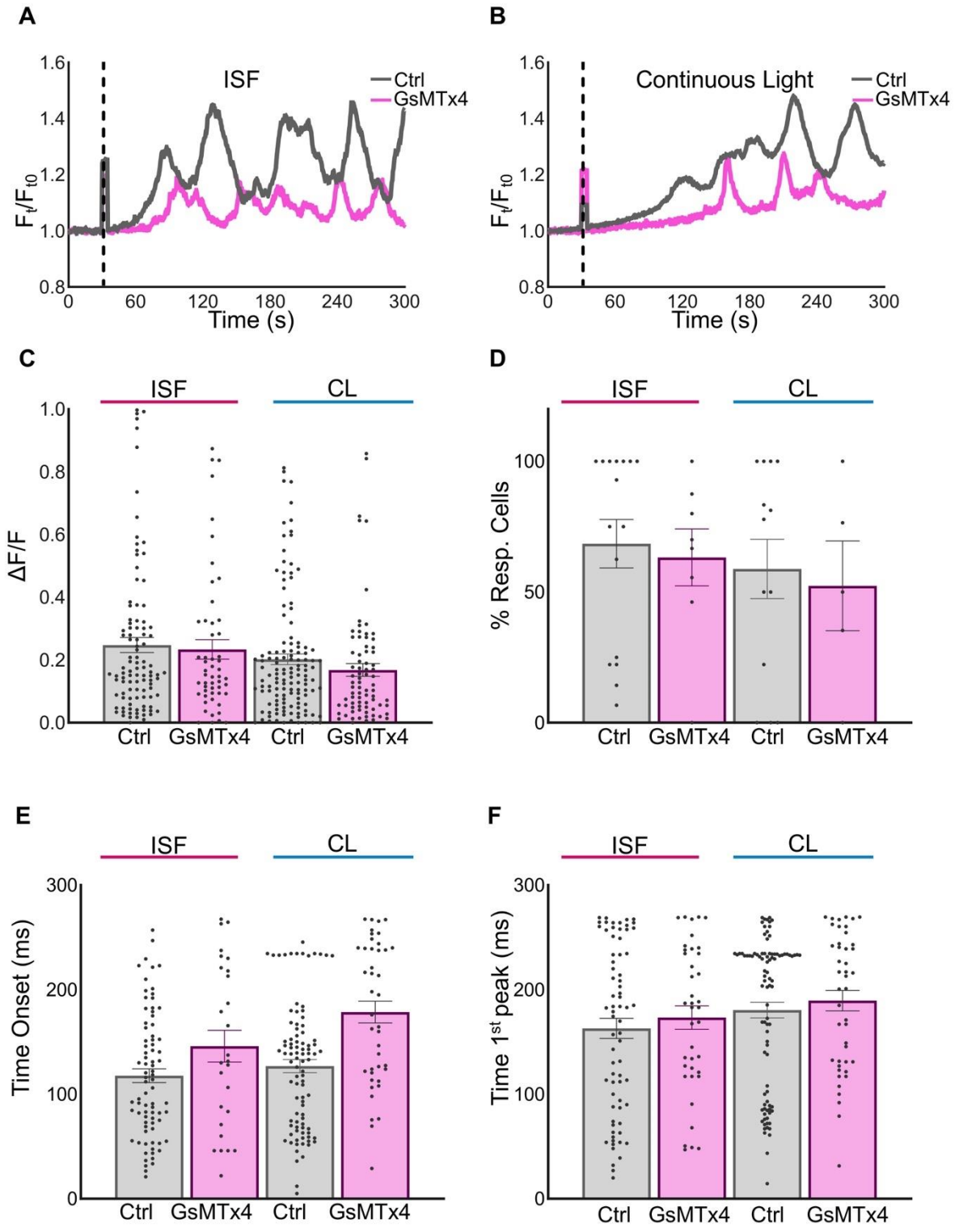

Figure S 2 Involvement of cationic mechanosensitive ion channels (MSCs) in astrocytic responses after ISF and Continuous Light stimulation. A-D) Histogram plots of measurements performed on astrocytes in vitro after ISF and continuous light stimulation. Data values corresponding to the control standard extracellular solution are colored in grey, and those for MSCs inhibitor GsMTx4 (1 µM) are colored in pink. All the results are expressed as mean ± SEM and statistical p-values. **A)** Maximal fluorescence variation, **B)** percentage of responding cells, **C)** Time onset (first fluorescence variation above the cut-off of the baseline-0.1), **D)** Time to first peak. For ΔF/F (all cells, responding and not responding): ISF control N=101, n=7; ISF GsMTx4 N=78, n=3; CL control N=129, n=7; CL GsMTx4 N=81, n=3. *N*, number of analysed cells (regions of interest), *n*, number of experiments. Sample

sizes vary by metric; N (cells/ROIs) and n (experiments) for each panel are reported in Tables S1–S5, and p-values in Tables S6–S10.

Table S 1 Data values of means of maximal fluorescence variation (ΔF/F), ± Standard Error of the Mean (SEM), N=number of cells, n=number of experiments reported in Figures 3-**Error! Reference source not found.** and Figure S .

Condition	**N**	**n**	**Mean**	**±SEM**
No stim Control	106	7	0.0465	0.006891
No stim 0 Ca	100	5	0.1005	0.009628
No stim 2APB	74	4	0.04218	0.006149
No stim RN–1734	95	4	0.03953	0.008092
No stim HC–030031	63	3	0.05744	0.006159
No stim U73122	59	3	0.2633	0.0281
No stim PTX	59	3	0.2966	0.02312
ISF Control	101	7	0.2476	0.024
ISF 0 Ca	55	5	0.1114	0.01669
ISF 2APB	41	4	0.01044	0.002928
ISF RN–1734	80	4	0.06707	0.01033
ISF HC–030031	58	3	0.04645	0.008793
ISF U73122	74	3	0.3509	0.02904
ISF PTX	49	3	0.1974	0.02199
ISF RYAN	61	3	0.05585	0.008570
ISF GsMTx4	53	3	0.2337	0.03115
CL Control	129	7	0.2022	0.01693
CL 0 Ca	75	5	0.2111	0.02449
CL 2APB	59	4	0.04908	0.008915
CL RN–1734	66	4	0.02907	0.007337
CL HC–030031	76	3	0.04352	0.006526

Condition	N	n	Mean	±SEM
CL U73122	36	3	0.2483	0.03338
CL PTX	56	3	0.3184	0.03469
CL RYAN	81	3	0.1225	0.01008
CL GsMTx4	78	3	0.1682	0.02026

Table S 2 Data values of means of percentage of responding cells (% Resp Cells) ± Standard Error of the Mean (SEM), N=number of cells, n=number of experiments reported in Figures 3–**Error! Reference source not found.** and Figure S .

Condition	N	n	Mean	±SEM
No stim Control	16	7	21.35	7.3
No stim 0 Ca	9	5	38.86	12.78
No stim 2APB	8	4	10.98	5.671
No stim RN–1734	7	4	11.22	7.613
No stim HC–030031	5	3	9.621	5.033
No stim U73122	10	3	78.84	5.772
No stim PTX	4	3	92.73	4.753
ISF Control	16	7	68.48	9.264
ISF 0 Ca	23	5	1.783	0.2589
ISF 2APB	8	4	2.965	2.052
ISF RN–1734	8	4	29.15	13.2
ISF HC–030031	6	3	9.722	9.722
ISF U73122	7	3	72.55	13.55
ISF PTX	6	3	73.48	11.07
ISF RYAN	8	3	19.06	5.427
ISF GsMTx4	8	3	63.23	10.88
CL Control	16	7	58.81	11.36
CL 0 Ca	23	5	65.21	15.12
CL 2APB	8	4	14.32	5.796
CL RN–1734	8	4	9.697	6.749
CL HC–030031	6	3	7.751	3.818

Condition	N	n	Mean	±SEM
CL U73122	7	3	73.75	9.437
CL PTX	6	3	81.98	10.25
CL RYAN	9	3	51.45	11.76
CL GsMTx4	5	3	52.35	17.16

Table S 3 Data values of means of Time Onset (ms) ± Standard Error of the Mean (SEM), N=number of cells, n=number of experiments reported in Figures 3–**Error! Reference source not found.** and Figure S .

Condition	N	n	Mean	±SEM
No stim Control	32	7	169	10.39
No stim 0 Ca	38	5	174.2	10.94
No stim 2APB	11	4	184.5	19.24
No stim RN–1734	4	4	214	17.15
No stim HC–030031	5	3	137.8	34.28
No stim U73122	47	3	108.7	9.218
No stim PTX	54	3	99.74	8.545
ISF Control	80	7	117.7	6.619
ISF 0 Ca	23	5	156.6	19.36
ISF 2APB	3	4	143.3	24.51
ISF RN–1734	21	4	130.5	20.14
ISF HC–030031	10	3	192.1	18.62
ISF U73122	56	3	85.12	6.707
ISF PTX	46	3	128.3	11.17
ISF RYAN	11	3	97.45	10.36
ISF GsMTx4	27	3	145.9	15.21
CL Control	93	7	126.9	6.325
CL 0 Ca	47	5	120.1	8.27
CL 2APB	11	4	173.8	18.33
CL RN–1734	6	4	99.7	39.98
CL HC–030031	10	3	208.4	15.86
CL U73122	28	3	83.82	11.7

Condition	N	n	Mean	±SEM
CL PTX	34	3	92.54	9.361
CL RYAN	52	3	101.1	7.934
CL GsMTx4	42	3	178.5	10.45

Table S 4 Data values of means of Time to first Peak (ms) ± Standard Error of the Mean (SEM), N=number of cells, n=number of experiments reported in Figures 3–**Error! Reference source not found.** and Figure S .

Condition	N	n	Mean	±SEM
No stim Control	34	7	202.4	8.776
No stim 0 Ca	40	5	175.6	10.06
No stim 2APB	9	4	201.2	20.93
No stim RN–1734	4	4	231.4	15.04
No stim HC–030031	5	3	157.6	41.22
No stim U73122	47	3	139.6	10.53
No stim PTX	56	3	129.6	10.97
ISF Control	71	7	163	9.553
ISF 0 Ca	23	5	156.6	19.36
ISF 2APB	3	4	144.2	24.63
ISF RN–1734	17	4	132.3	23.22
ISF HC–030031	10	3	207.3	20.52
ISF U73122	56	3	122.8	8.804
ISF PTX	32	3	174.1	13.98
ISF RYAN	11	3	106.1	10.72
ISF GsMTx4	39	3	173.3	11.20
CL Control	93	7	180.4	7.528
CL 0 Ca	49	5	127.1	8.416
CL 2APB	11	4	173.8	18.33
CL RN–1734	3	4	103.1	39.11
CL HC–030031	13	3	217	18.71
CL U73122	27	3	121.3	15.64
CL PTX	46	3	139.7	9.987

Condition	N	n	Mean	±SEM
CL RYAN	47	3	119.9	10.01
CL GsMTx4	44	3	189.5	9.814

Table S 5 Data values of means of Number of Peaks ± Standard Error of the Mean (SEM), N=number of cells, n=number of experiments reported in Figures 3–**Error! Reference source not found.** and Figure S .

Condition	N	n	Mean	±SEM
No stim Control	30	7	1.367	0.1312
No stim 0 Ca	36	5	2.583	0.25
No stim 2APB	9	4	1.444	0.3379
No stim RN–1734	4	4	2	0.7071
No stim HC–030031	7	3	1.571	0.202
No stim U73122	45	3	1.689	0.145
No stim PTX	53	3	2.774	0.2296
ISF Control	82	7	2.146	0.1368
ISF 0 Ca	23	5	1.783	0.2589
ISF 2APB	3	4	1.667	0.6667
ISF RN–1734	18	4	3	0.4428
ISF HC–030031	10	3	1.1	0.1
ISF U73122	56	3	2.429	0.213
ISF PTX	31	3	1.806	0.2196
ISF RYAN	7	3	3.857	0.7377
ISF GsMTx4	32	3	2.938	0.3043
CL Control	88	7	2.114	0.1846
CL 0 Ca	48	5	2.792	0.2436
CL 2APB	11	4	1.636	0.3636
CL RN–1734	8	4	4.5	0.7792
CL HC–030031	8	3	1.125	0.125
CL U73122	26	3	2.769	0.2951
CL PTX	46	3	2.674	0.2306
CL RYAN	42	3	3.500	0.2463
CL GsMTx4	43	3	2.465	0.2831

Table S 6 Data values referred to the histogram plots in Figures 3–**Error! Reference source not found.** and Figure S , regarding maximal fluorescence variation (ΔF/F). Here are reported the means difference between different conditions, the statistical significance and the p–values. One–way ANOVA was performed with Bonferroni's test for means comparisons.

ΔF/F Means Comparison	**Mean Difference**	**Significance**	**P Value**
No stim Control vs. ISF Control	-0.2011	****	<0.0001
No stim Control vs. CL Control	-0.1557	****	<0.0001
ISF Control vs. CL Control	0.04540	ns	0.1857
ISF Control vs. ISF 0 Ca	66.70	****	<0.0001
ISF Control vs. ISF 2APB	65.52	****	<0.0001
ISF Control vs. CL Control	9.671	ns	>0.9999
ISF 0 Ca vs. CL 0 Ca	-63.43	****	<0.0001
ISF 2APB vs. CL 2APB	-11.35	ns	>0.9999
CL Control vs. CL 0 Ca	-6.399	ns	>0.9999
CL Control vs. CL 2APB	44.50	**	0.0058
ISF Control vs. ISF RN	0.1805	****	<0.0001
ISF Control vs. ISF HC	0.2011	****	<0.0001
ISF Control vs. CL Control	0.04540	ns	0.1887
ISF RN vs. CL RN	0.03800	ns	0.9692
ISF HC vs. CL HC	0.002934	ns	>0.9999
CL Control vs. CL RN	0.1731	****	<0.0001
CL Control vs. CL HC	0.1587	****	<0.0001
ISF Control vs. ISF U73122	-0.1033	*	0.0164
ISF Control vs. ISF PTX	0.05019	ns	>0.9999
ISF Control vs. CL Control	0.04540	ns	0.8543
ISF U73122 vs. CL U73122	0.1026	ns	0.1574
ISF PTX vs. CL PTX	-0.1210	*	0.0368
CL Control vs. CL U73122	-0.04607	ns	>0.9999
CL Control vs. CL PTX	-0.1162	**	0.0075
ISF Control vs. ISF RYAN	0.1917	****	<0.0001
CL Control vs. CL RYAN	0.07974	**	0.0063
ISF RYAN vs. CL RYAN	-0.06661	ns	0.1067

ISF Control vs. ISF GsMTx4	0.01390	ns	>0.9999
CL Control vs. CL GsMTx4	0.03404	ns	0.4792
ISF Piezo 1 vs. CL GsMTx4	0.06553	ns	0.2497

Table S 7 Data values referred to the histogram plots in Figures 3–**Error! Reference source not found.** and Figure S , regarding the percentage of responding cells. Here are reported the means difference between different conditions, the statistical significance and the p–values. One–way ANOVA was performed with Bonferroni's test for means comparisons.

% Resp. cells Means Comparison	**Mean Difference**	**Significance**	**P Value**
No stim Control vs. ISF Control	-52.37	***	0.0003
No stim Control vs. CL Control	-42.70	**	0.0055
ISF Control vs. CL Control	9.671	ns	>0.9999
ISF Control vs. ISF 0 Ca	66.70	****	<0.0001
ISF Control vs. ISF 2APB	65.52	****	<0.0001
ISF Control vs. CL Control	9.671	ns	>0.9999
ISF 0 Ca vs. CL 0 Ca	-63.43	****	<0.0001
ISF 2APB vs. CL 2APB	-11.35	ns	>0.9999
CL Control vs. CL 0 Ca	-6.399	ns	>0.9999
CL Control vs. CL 2APB	44.50	**	0.0058
ISF Control vs. ISF RN	39.33	ns	0.0650
ISF Control vs. ISF HC	58.76	**	0.0043
ISF Control vs. CL Control	9.671	ns	>0.9999
ISF RN vs. CL RN	19.45	ns	>0.9999
ISF HC vs. CL HC	1.971	ns	>0.9999
CL Control vs. CL RN	49.12	*	0.0325
CL Control vs. CL HC	51.06	*	0.0234
ISF Control vs. ISF U73122	-4.062	ns	>0.9999
ISF Control vs. ISF PTX	-5.000	ns	>0.9999
ISF Control vs. CL Control	9.671	ns	>0.9999
ISF U73122 vs. CL U73122	-1.203	ns	>0.9999
ISF PTX vs. CL PTX	-8.490	ns	>0.9999
CL Control vs. CL U73122	-14.94	ns	>0.9999

CL Control vs. CL PTX	-23.16	ns	0.9497
ISF Control vs. ISF RYAN	49.42	**	0.0095
CL Control vs. CL RYAN	7.364	ns	>0.9999
ISF RYAN vs. CL RYAN	9.671	ns	>0.9999
ISF Control vs. ISF GsMTx4	5.250	ns	>0.9999
CL Control vs. CL GsMTx4	6.461	ns	>0.9999
ISF Piezo 1 vs. CL GsMTx4	10.88	ns	>0.9999

Table S 8 Data values referred to the histogram plots in Figures 3–**Error! Reference source not found.** and Figure S , regarding the time onset. Here are reported the means difference between different conditions, the statistical significance and the p–values. One–way ANOVA was performed with Bonferroni's test for means comparisons.

Time Onset Means Comparison	**Mean Difference**	**Significance**	**P Value**
No stim Control vs. ISF Control	51.34	***	0.0002
No stim Control vs. CL Control	42.11	**	0.0022
ISF Control vs. CL Control	-9.232	ns	0.9415
ISF Control vs. ISF 0 Ca	-38.92	ns	0.0672
ISF Control vs. ISF 2APB	-25.65	ns	>0.9999
ISF Control vs. CL Control	-9.232	ns	>0.9999
ISF 0 Ca vs. CL 0 Ca	36.48	ns	0.1665
ISF 2APB vs. CL 2APB	-30.44	ns	>0.9999
CL Control vs. CL 0 Ca	6.792	ns	>0.9999
CL Control vs. CL 2APB	-46.85	ns	0.1437
ISF Control vs. ISF RN	-12.79	ns	>0.9999
ISF Control vs. ISF HC	-74.36	**	0.0046
ISF Control vs. CL Control	-9.232	ns	>0.9999
ISF RN vs. CL RN	30.78	ns	>0.9999
ISF HC vs. CL HC	-16.35	ns	>0.9999
CL Control vs. CL RN	27.22	ns	>0.9999
CL Control vs. CL HC	-81.48	**	0.0012
ISF Control vs. ISF U73122	32.57	*	0.0156
ISF Control vs. ISF PTX	-10.64	ns	>0.9999

Time Onset Means Comparison	Mean Difference	Significance	P Value
ISF Control vs. CL Control	-9.232	ns	>0.9999
ISF U73122 vs. CL U73122	1.295	ns	>0.9999
ISF PTX vs. CL PTX	35.78	ns	0.0665
CL Control vs. CL U73122	43.10	**	0.0076
CL Control vs. CL PTX	34.38	*	0.0348
ISF Control vs. ISF RYAN	20.23	Ns	>0.9999
CL Control vs. CL RYAN	25.82	*	0.0466
ISF RYAN vs. CL RYAN	-3.642	Ns	>0.9999
ISF Control vs. ISF GsMTx4	-28.26	ns	0.1368
CL Control vs. CL GsMTx4	-51.63	****	<0.0001
ISF Piezo 1 vs. CL GsMTx4	-32.60	ns	0.1138

Table S 9 Data values referred to the histogram plots in Figures 3–**Error! Reference source not found.** and Figure S , regarding the time to the first peak. Here are reported the means difference between different conditions, the statistical significance and the p–values. One–way ANOVA was performed with Bonferroni's test for means comparisons.

Time Peak Means Comparison	Mean Difference	Significance	P Value
No stim Control vs. ISF Control	39.42	*	0.0295
No stim Control vs. CL Control	21.94	ns	0.3982
ISF Control vs. CL Control	-17.48	ns	0.3829
ISF Control vs. ISF 0 Ca	6.356	ns	>0.9999
ISF Control vs. ISF 2APB	18.80	ns	>0.9999
ISF Control vs. CL Control	-17.48	ns	0.9453
ISF 0 Ca vs. CL 0 Ca	29.50	ns	0.8118
ISF 2APB vs. CL 2APB	-29.61	ns	>0.9999
CL Control vs. CL 0 Ca	53.33	***	0.0004
CL Control vs. CL 2APB	6.674	ns	>0.9999
ISF Control vs. ISF RN	30.70	ns	0.9947
ISF Control vs. ISF HC	-44.34	ns	0.6328
ISF Control vs. CL Control	-17.48	ns	>0.9999

Time Peak Means Comparison	Mean Difference	Significance	P Value
ISF RN vs. CL RN	29.16	ns	>0.9999
ISF HC vs. CL HC	-9.700	ns	>0.9999
CL Control vs. CL RN	77.35	ns	0.2108
CL Control vs. CL HC	-36.55	ns	0.7779
ISF Control vs. ISF U73122	40.15	*	0.0183
ISF Control vs. ISF PTX	-11.10	ns	>0.9999
ISF Control vs. CL Control	-17.48	ns	0.9459
ISF U73122 vs. CL U73122	1.516	ns	>0.9999
ISF PTX vs. CL PTX	34.37	ns	0.3124
CL Control vs. CL U73122	59.15	**	0.0021
CL Control vs. CL PTX	40.75	*	0.0172
ISF Control vs. ISF RYAN	56.83	ns	0.0697
CL Control vs. CL RYAN	60.60	****	<0.0001
ISF RYAN vs. CL RYAN	-13.71	ns	>0.9999
ISF Control vs. ISF GsMTx4	-10.37	ns	>0.9999
CL Control vs. CL GsMTx4	-9.088	ns	>0.9999
ISF Piezo 1 vs. CL GsMTx4	-16.20	ns	0.9484

Table S 10 Data values referred to the histogram plots in Figures 3–**Error! Reference source not found.** and Figure S , regarding the number of peaks. Here are reported the means difference between different conditions, the statistical significance and the p–values. One–way ANOVA was performed with Bonferroni's test for means comparisons.

Number of Peaks Means Comparison	Mean Difference	Significance	P Value
No stim Control vs. ISF Control	-0.7797	*	0.0333
No stim Control vs. CL Control	-0.7470	*	0.0420
ISF Control vs. CL Control	0.03271	ns	>0.9999
ISF Control vs. ISF 0 Ca	6.356	ns	>0.9999
ISF Control vs. ISF 2APB	18.80	ns	>0.9999
ISF Control vs. CL Control	-17.48	ns	0.9453
ISF 0 Ca vs. CL 0 Ca	29.50	ns	0.8118

Number of Peaks Means Comparison	Mean Difference	Significance	P Value
ISF 2APB vs. CL 2APB	-29.61	ns	>0.9999
CL Control vs. CL 0 Ca	53.33	***	0.0004
CL Control vs. CL 2APB	6.674	ns	>0.9999
ISF Control vs. ISF RN	30.70	ns	0.9947
ISF Control vs. ISF HC	-44.34	ns	0.6328
ISF Control vs. CL Control	-17.48	ns	>0.9999
ISF RN vs. CL RN	29.16	ns	>0.9999
ISF HC vs. CL HC	-9.700	ns	>0.9999
CL Control vs. CL RN	77.35	ns	0.2108
CL Control vs. CL HC	-36.55	ns	0.7779
ISF Control vs. ISF U73122	40.15	*	0.0183
ISF Control vs. ISF PTX	-11.10	ns	>0.9999
ISF Control vs. CL Control	-17.48	ns	0.9459
ISF U73122 vs. CL U73122	1.516	ns	>0.9999
ISF PTX vs. CL PTX	34.37	ns	0.3124
CL Control vs. CL U73122	59.15	**	0.0021
CL Control vs. CL PTX	40.75	*	0.0172
ISF Control vs. ISF RYAN	-1.711	*	0.0214
CL Control vs. CL RYAN	-1.386	****	<0.0001
ISF RYAN vs. CL RYAN	0.3571	ns	>0.9999
ISF Control vs. ISF GsMTx4	-0.7912	ns	0.0567
CL Control vs. CL GsMTx4	-0.3515	ns	0.7221
ISF Piezo 1 vs. CL GsMTx4	0.4724	ns	0.6269

Table S 11 Temperature measurements of the cell bath contained in a petri dish measured with a $NiCr^{-}/NiAl^{+}$ K–type thermocouple (RS) for 10 min of ISF, and CL stimulation.

Time (min)	Temperature (C°)	
	ISF	CL
0	20	20
1	20	20
2	20	20
3	20.5	20
4	20	20
5	20.5	20
6	20	20
7	20	20
8	20	20
9	20.5	20
10	20	20