SUPPLEMENTARY INFORMATION

Heterogeneity in ROS levels in antibiotics-exposed mycobacterial subpopulations confer differential susceptibility

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Short title: ROS heterogeneity in mycobacterial subpopulations

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Table S1. Bacterial strains and plasmids used in the present study

Bacterial strains or plasmid	Purpose	Reference
Bacterial strains		
Mycobacterium smegmatis mc^2155	Experimental system	[1]
Escherichia coli JM109	Cloning host	[2]
Plasmids		
pAKMN2	Integrating vector	[3]
pMV762-Mrx1-roGFP2	Redox sensor	[4]
pAKMN2-Mrx1-roGFP2	Redox sensor	This study
pBS-KS	Cloning vector	[5]
pBS-KS-Mrx1-roGFP2	Sequencing vector	This study

References

1. Snapper SB, Melton RE, Mustafa S, Kieser T, Jacobs Jr WR. Isolation and characterisation of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. *Mol Microbiol* 1990;4:1911-1919. https://doi.org/10.1111/j.1365-2958.1990.tb02040.x.

2. Yanisch-Peron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 1985;33:103-119. https://doi.org/10.1016/0378-1119(85)90120-9.

3. Roy S, Narayana Y, Balaji KN, Ajitkumar P. Highly fluorescent GFPm2+- based genome integration-proficient promoter probe vector to study *Mycobacterium tuberculosis* promoters in infected macrophages. *Microbial Biotechnology* 2012;5:98-105. doi: 10.1111/j.1751-7915.2011.00305.x.

4. Bhaskar A, Chawla M, Mehta M, Parikh P, Chandra P *et al*. Reengineering redox sensitive GFP to measure mycothiol redox potential of *Mycobacterium tuberculosis* during infection. *PLoS Pathog* 2014;10:e1003902. https://doi.org/10.1371/journal.ppat.1003902.

5. Alting-Mees MA, Short JM. pBluescript II: gene mapping vectors. *Nucleic Acids Res* 1989;17:9494. https://doi.org/10.1093/nar/17.22.9494.

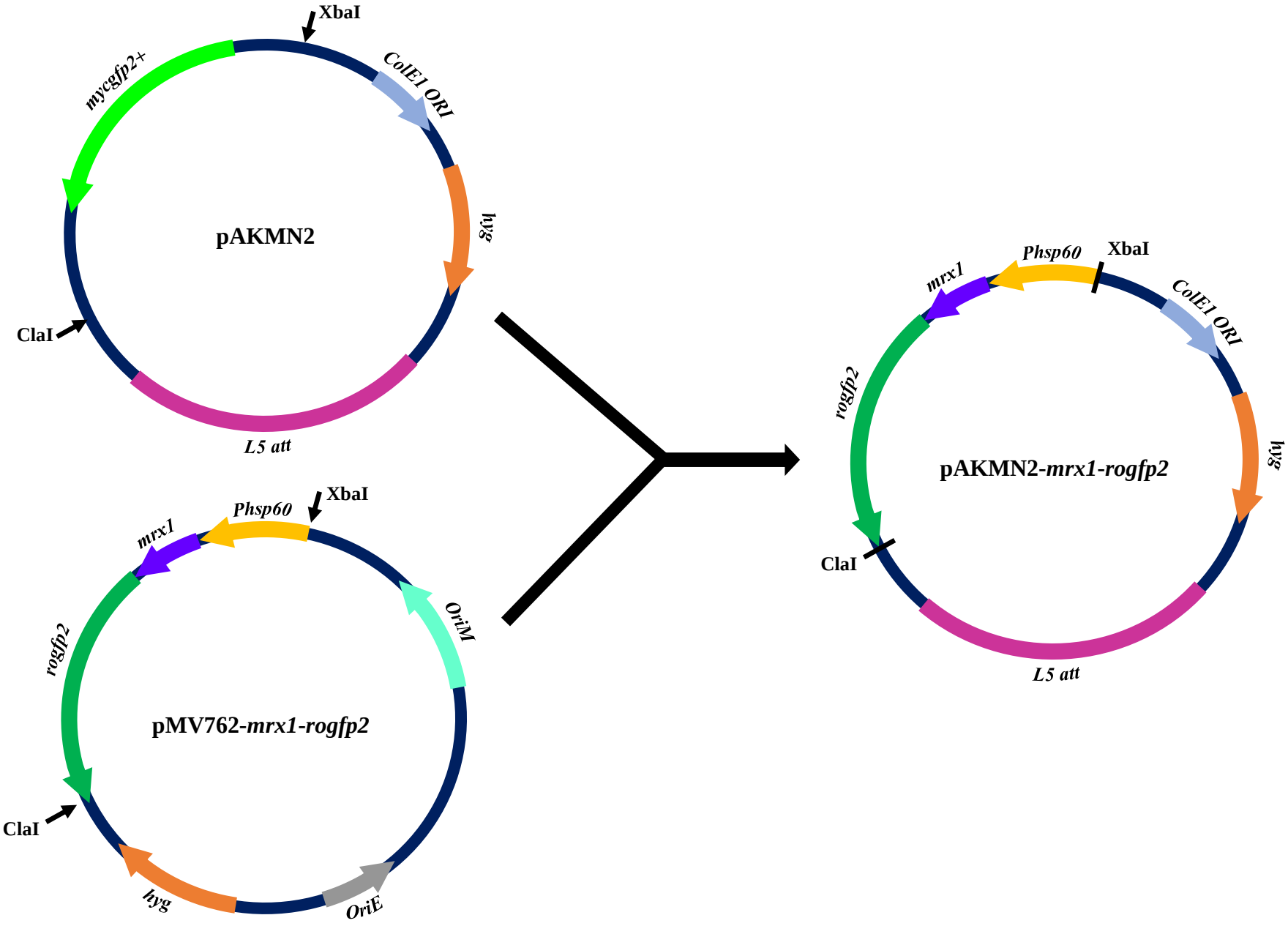


Fig. S1. Schematic representation of pAKMN2-*mrx1-rogfp2* generation. *mrx1-rogfp2* under *hsp60* promoter was obtained from pMV762-*mrx1-rogfp2* using XbaI and ClaI restriction enzymes and this segment was subcloned into pAKMN2.

(a)

	SCF/Mrx1-roGFP2 + RIF 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	314	808	0.39
2.	501	1,620	0.31
3.	231	859	0.27
	Avg ± SD = 0.32 ± 0.06		
	SCF/Mrx1-roGFP2 + RIF 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	466	584	0.80
2.	121	139	0.87
3.	261	400	0.65
	Avg ± SD = 0.77 ± 0.11		
	p < 0.01		

(b)

	NCF/Mrx1-roGFP2 + RIF 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	200	438	0.46
2.	467	776	0.60
3.	42	82	0.51
	Avg ± SD = 0.52 ± 0.07		
	NCF/Mrx1-roGFP2 + RIF 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	179	331	0.54
2.	539	407	1.32
3.	150	169	0.89
	Avg ± SD = 0.92 ± 0.39		
	ns		

(c)

	SCF/Mrx1-roGFP2 + RIF + TU 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	297	794	0.37
2.	243	478	0.51
3.	117	317	0.37
	Avg ± SD = 0.42 ± 0.08		
	SCF/Mrx1-roGFP2 + RIF + TU 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	381	789	0.48
2.	177	377	0.47
3.	138	310	0.45
	Avg ± SD = 0.47 ± 0.02		
	ns		

(d)

	NCF/Mrx1-roGFP2 + RIF + TU 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	129	239	0.54
2.	212	361	0.59
3.	236	444	0.53
	Avg ± SD = 0.55 ± 0.03		
	NCF/Mrx1-roGFP2 + RIF + TU 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	149	281	0.53
2.	646	386	1.67
3.	129	119	1.08
	Avg ± SD = 1.10 ± 0.57		
	ns		

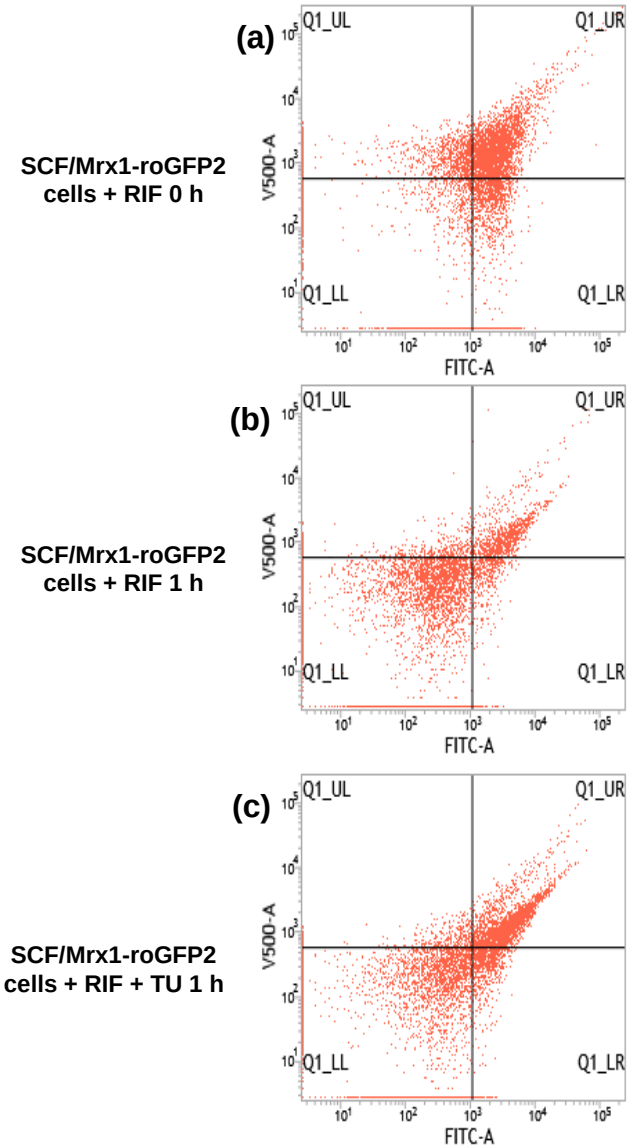
(e)

Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF/Mrx1-roGFP2 + RIF	2.43 ± 0.38	p < 0.05
SCF/Mrx1-roGFP2 + RIF + TU	1.14 ± 0.19	

(f)

Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
NCF/Mrx1-roGFP2 + RIF	1.71 ± 0.51	ns
NCF/Mrx1-roGFP2 + RIF + TU	1.96 ± 0.94	

Fig. S2. Quantitation of V500-A:FITC-A ratios of RIF-exposed Mrx1-roGFP2-integrated *Msm* SCF and NCF cells. Redox status is given as the ratio of the median fluorescence at V500-A and FITC-A. Median fluorescence of RIF-exposed: **(a)** SCF/Mrx1-roGFP2 cells and **(b)** NCF/Mrx1-roGFP2 cells at V500-A (405 nm) and FITC-A (488 nm) at 0 h (immediately upon addition of RIF) and after 1 h of exposure to RIF. Median fluorescence at V500-A (405 nm) and FITC-A (488 nm) of: **(c)** SCF/Mrx1-roGFP2 and **(d)** NCF/Mrx1-roGFP2 cells in the combined presence of RIF and TU at 0 h (immediately upon addition of RIF and TU) and after 1 h of exposure to RIF and thiourea. **(e, f)** Fold change in the V500-A:FITC ratios of the RIF-exposed cells, with and without TU, at 1 h, after normalisation with its respective 0 h value: **(e)** SCF/Mrx1-roGFP2 cells and **(f)** NCF/Mrx1-roGFP2 cells. Statistical significance was calculated using the paired *t* test between 0 h and 1 h of the respective samples and Students' *t* test for normalised values. ns, not significant.



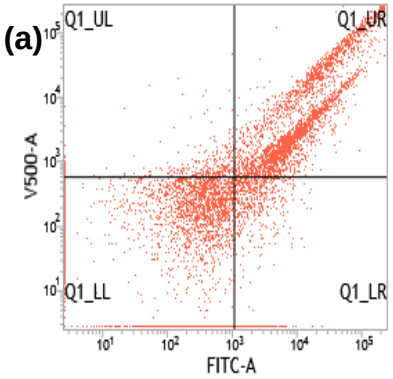
SCF/Mrx1-roGFP2 + RIF 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	14.66	296	1181	3.99
Q1_UR	**32.14**	2822	1554	**0.55**
Q1_LL	24.10	186	-256	-1.38
Q1_LR	29.10	2293	-161	-0.07

SCF/Mrx1-roGFP2 + RIF 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	3.74	207	712	3.44
Q1_UR	9.59	3,970	1,129	0.28
Q1_LL	**81.40**	45	57	**1.27**
Q1_LR	5.28	2,056	351	0.17

SCF/Mrx1-roGFP2 + RIF + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	2.73	565	810	1.43
Q1_UR	24.04	4,095	1,141	0.28
Q1_LL	**61.99**	57	24	**0.42**
Q1_LR	11.24	1,958	357	0.18

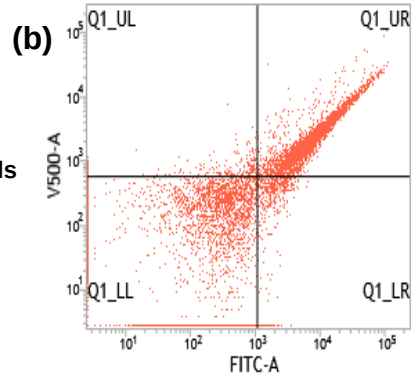
Fig. S3. Scatter plot of SCF/Mrx1-roGFP2 cells during RIF exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** SCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of RIF). **(b)** SCF/Mrx1-roGFP2 cells at the end of 1 h of RIF exposure. **(c)** SCF/Mrx1-roGFP2 cells at the end of 1 h exposure to RIF + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red colour in bold in the table.

NCF/Mrx1-roGFP2 cells + RIF 0 h



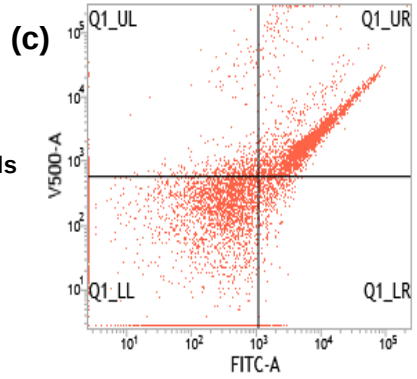
NCF/Mrx1-roGFP2 + RIF 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	2.58	397	746	1.88
Q1_UR	28.94	12421	3439	0.28
Q1_LL	**61.40**	56	15	**0.27**
Q1_LR	7.08	2303	230	0.10

NCF/Mrx1-roGFP2 cells + RIF 1 h



NCF/Mrx1-roGFP2 + RIF 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	1.76	412	740	1.80
Q1_UR	27.37	7,440	1,808	0.24
Q1_LL	**65.40**	36	11	**0.31**
Q1_LR	5.46	2,000	359	0.18

NCF/Mrx1-roGFP2 cells + RIF + TU 1 h



NCF/Mrx1-roGFP2 + RIF + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	3.61	402	796	1.98
Q1_UR	21.49	6,490	1,916	0.30
Q1_LL	**70.6**	63	16	**0.25**
Q1_LR	4.3	1,809	321	0.18

Fig. S4. Scatter plot of NCF/Mrx1-roGFP2 cells during RIF exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** NCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of RIF). **(b)** NCF/Mrx1-roGFP2 cells at the end of 1 h of RIF exposure. **(c)** NCF/Mrx1-roGFP2 cells at the end of 1 h exposure to RIF + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red in bold colour in the table.

(a)

SCF/Mrx1-roGFP2 + INH 0 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	306	749	0.41
2.	667	2,085	0.32
3.	200	924	0.22
	Avg ± SD = 0.31 ± 0.10		
SCF/Mrx1-roGFP2 + INH 1 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	163	171	0.95
2.	201	318	0.63
3.	175	355	0.49
	Avg ± SD = 0.69 ± 0.24		
	p < 0.04		

(b)

NCF/Mrx1-roGFP2 + INH 0 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	466	793	0.59
2.	253	338	0.75
3.	199	306	0.65
	Avg ± SD = 0.66 ± 0.08		
NCF/Mrx1-roGFP2 + INH 1 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	261	676	0.39
2.	230	275	0.84
3.	241	423	0.57
	Avg ± SD = 0.60 ± 0.23		
	ns		

(c)

SCF/Mrx1-roGFP2 + INH + TU 0 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	287	735	0.39
2.	249	423	0.59
3.	139	407	0.34
	Avg ± SD = 0.44 ± 0.13		
SCF/Mrx1-roGFP2 + INH + TU 1 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	357	1,028	0.35
2.	185	234	0.79
3.	171	439	0.39
	Avg ± SD = 0.52 ± 0.24		
	ns		

(d)

NCF/Mrx1-roGFP2 + INH + TU 0 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	305	411	0.74
2.	155	185	0.84
3.	51	91	0.56
	Avg ± SD = 0.71 ± 0.14		
NCF/Mrx1-roGFP2 + INH + TU 1 h			
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	662	1,488	0.44
2.	133	168	0.79
3.	64	146	0.44
	Avg ± SD = 0.56 ± 0.20		
	ns		

(e)

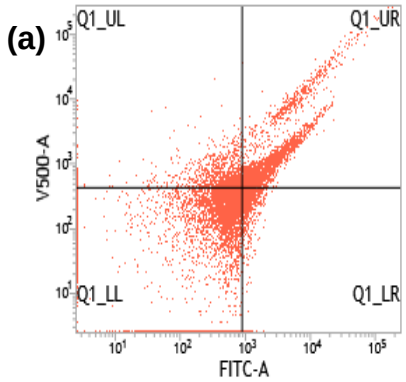
Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF/Mrx1-roGFP2 + INH	2.20 ± 0.19	p < 0.05
SCF/Mrx1-roGFP2 + INH + TU	1.12 ± 0.23	

(f)

Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
NCF/Mrx1-roGFP2 + INH	0.88 ± 0.23	ns
NCF/Mrx1-roGFP2 + INH + TU	0.78 ± 0.17	

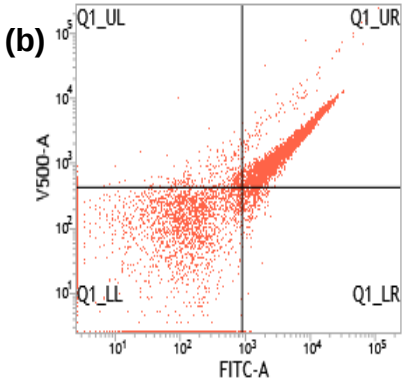
Fig. S5. Quantitation of V500-A:FITC-A ratios of INH-exposed Mrx1-roGFP2-integrated *Msm* SCF and NCF cells. Redox status is given as the ratio of the median fluorescence at V500-A and FITC-A. Median fluorescence of INH-exposed: **(a)** SCF/Mrx1-roGFP2 cells and **(b)** NCF/Mrx1-roGFP2 cells at V500-A (405 nm) and FITC-A (488 nm) at 0 h (immediately upon addition of INH) and after 1 h of exposure to INH. Median fluorescence at V500-A (405 nm) and FITC-A (488 nm) of: **(c)** SCF/Mrx1-roGFP2 and **(d)** NCF/Mrx1-roGFP2 cells in the combined presence of INH and TU at 0 h (immediately upon addition of INH and TU) and after 1 h of exposure to INH and TU. **(e, f)** Fold change in the V500-A:FITC ratios of the INH-exposed cells, with and without TU, at 1 h after normalisation with its respective 0 h value: **(e)** SCF/Mrx1-roGFP2 cells and **(f)** NCF/Mrx1-roGFP2 cells. Statistical significance was calculated using the paired *t* test between 0 h and 1 h of the respective samples and Students' *t* test for normalised values. ns, not significant.

SCF/Mrx1-roGFP2 cells + INH 0 h



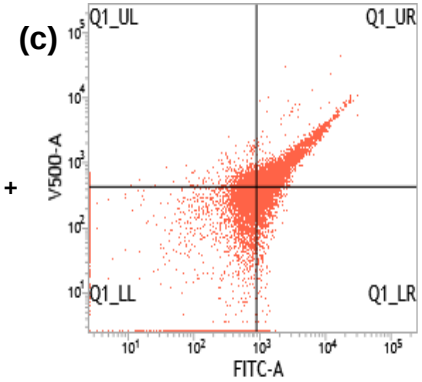
SCF/Mrx1-roGFP2 + INH 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	9.39	547	589	1.08
Q1_UR	26.16	2441	925	0.38
Q1_LL	**52.33**	469	148	**0.32**
Q1_LR	12.12	1172	287	0.24

SCF/Mrx1-roGFP2 cells + INH 1 h



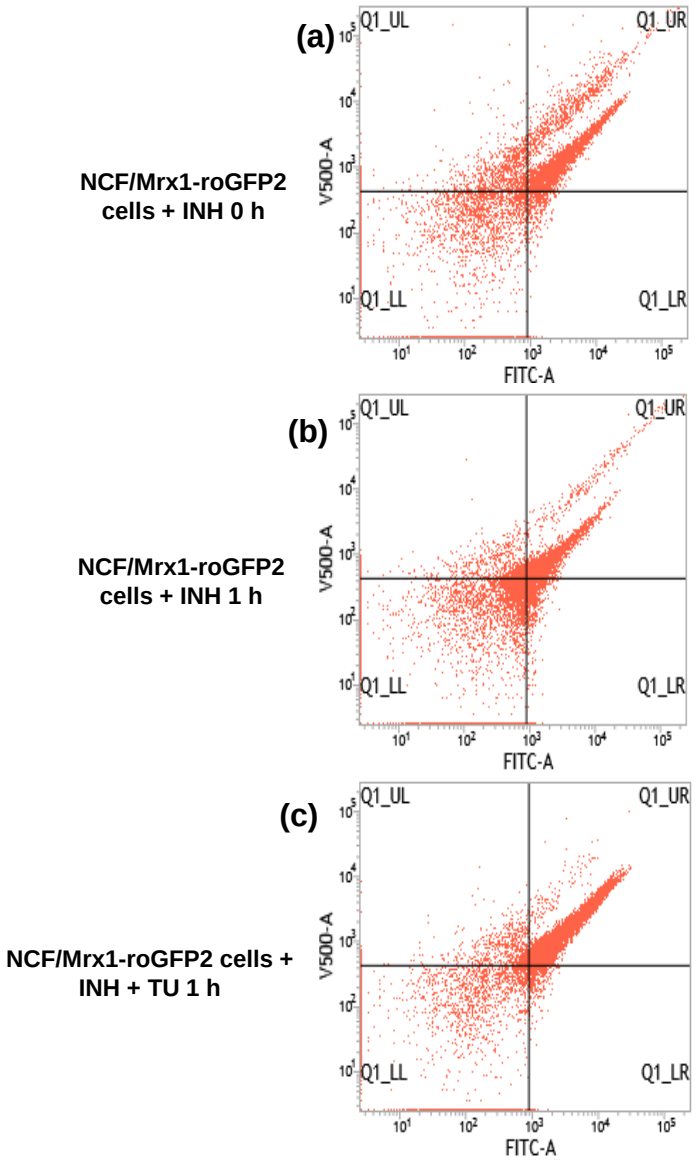
SCF/Mrx1-roGFP2 + INH 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	2.77	412	571	1.39
Q1_UR	31.57	3,717	1,318	0.35
Q1_LL	**62.16**	32	19	**0.59**
Q1_LR	3.50	1,354	304	0.22

SCF/Mrx1-roGFP2 cells + INH + TU 1 h



SCF/Mrx1-roGFP2 + INH + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	6.21	739	525	0.71
Q1_UR	33.9	2,043	720	0.35
Q1_LL	**38.17**	622	178	**0.29**
Q1_LR	21.72	1,199	273	0.23

Fig. S6. Scatter plot of SCF/Mrx1-roGFP2 SCF cells during INH exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** SCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of INH). **(b)** SCF/Mrx1-roGFP2 cells at the end of 1 h of INH exposure. **(c)** SCF/Mrx1-roGFP2 cells at the end of 1 h exposure to INH + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red colour in bold in the table.



NCF/Mrx1-roGFP2 + INH 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	9.23	438	743	1.70
Q1_UR	42.51	3530	1536	0.44
Q1_LL	**43.20**	62	47	**0.76**
Q1_LR	5.06	1352	313	0.23

NCF/Mrx1-roGFP2 + INH 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	7.40	506	583	1.15
Q1_UR	24.72	2,222	786	0.35
Q1_LL	**54.83**	155	98	**0.63**
Q1_LR	13.05	1,194	284	0.24

NCF/Mrx1-roGFP2 + INH + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	6.22	506	709	1.40
Q1_UR	**54.08**	3,328	1,363	**0.41**
Q1_LL	34.64	56	52	0.93
Q1_LR	5.07	1,268	312	0.25

Fig. S7. Scatter plot of NCF/Mrx1-roGFP2 cells during INH exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** NCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of INH). **(b)** NCF/Mrx1-roGFP2 cells at the end of 1 h exposure to INH. **(c)** NCF/Mrx1-roGFP2 cells at the end of 1 h exposure to INH + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red colour in bold in the table.

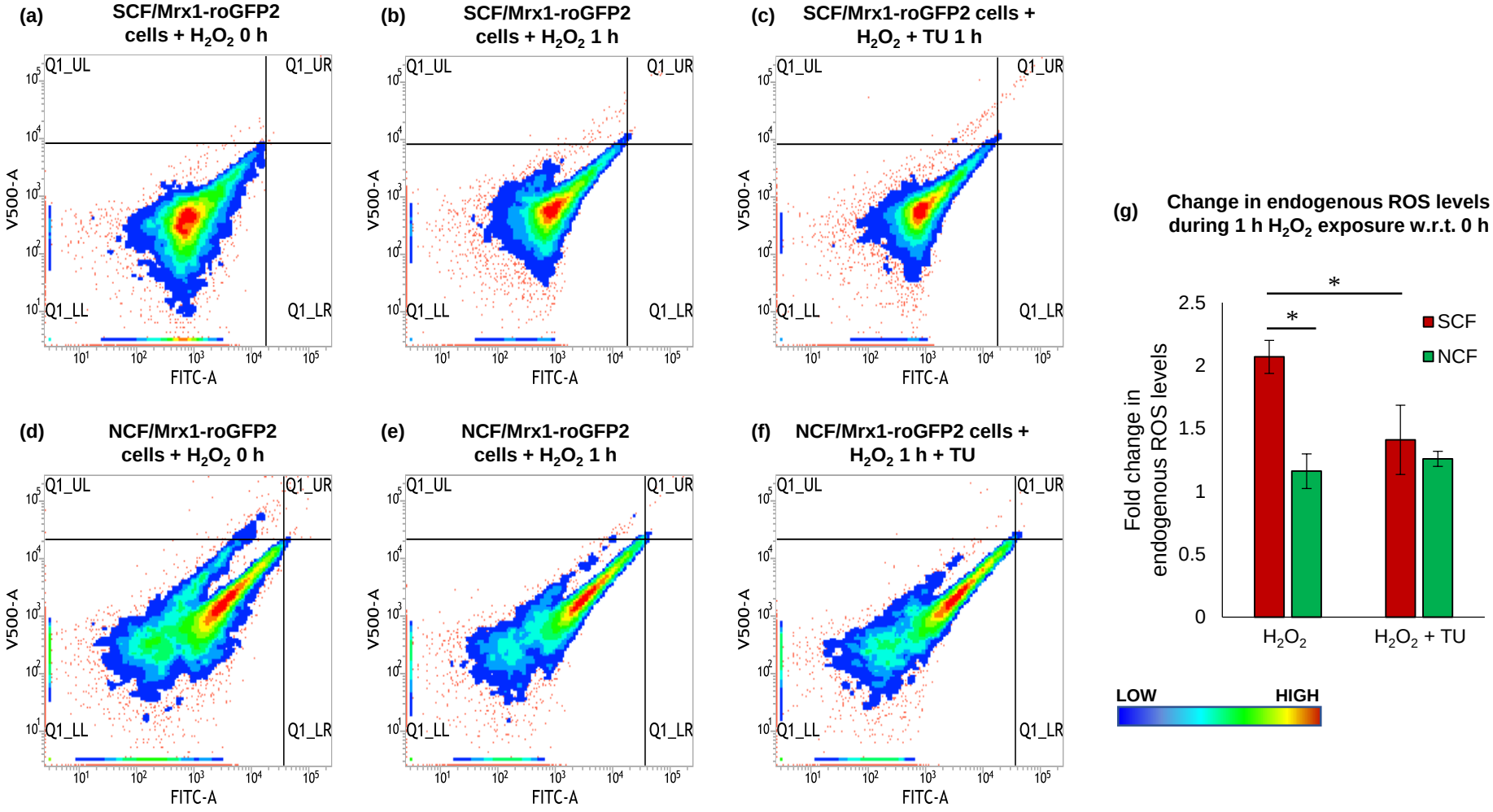


Fig. S8. Flow cytometry profile of Mrx1-roGFP2-integrated *Msm* SCF and NCF cells exposed to H_2O_2. Density plots of the SCF/Mrx1-roGFP2 and NCF/Mrx1-roGFP2 cells exposed to 0.8 mM H_2O_2. **(a, d)** for 0 h; **(b, e)** for 1 h; **(c, f)** in the presence of 5 µM TU for 1 h, respectively. A decrease in the fluorescence at FITC (488 nm) or an increase in the fluorescence at V500 (405 nm) indicates an increased endogenous ROS levels in the cell. **(g)** Quantitation of the fold-change in the endogenous ROS levels in SCF/Mrx1-roGFP2 and NCF/Mrx1-roGFP2 cells exposed to H_2O_2 for 1 h with respect to (w.r.t.) the respective 0 h value (considering 0 h value as 1). 10^4 cells/ml was used in each case (n = 3). * indicates $p \leq 0.05$. Statistical significance was calculated using Students' *t* test. The scale with the colours in the density plot represents the gradation in the population density.

(a)

	SCF/Mrx1-roGFP2 + H_2O_2 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	202	733	0.28
2.	944	2,050	0.46
3.	242	1,063	0.23
	Avg ± SD = 0.32 ± 0.12		
	SCF/Mrx1-roGFP2 + H_2O_2 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	487	806	0.60
2.	215	245	0.88
3.	189	394	0.48
	Avg ± SD = 0.65 ± 0.20		
	p < 0.01		

(b)

	NCF/Mrx1-roGFP2 + H_2O_2 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	432	648	0.67
2.	132	284	0.46
3.	322	840	0.38
	Avg ± SD = 0.50 ± 0.15		
	NCF/Mrx1-roGFP2 + H_2O_2 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	556	666	0.83
2.	155	320	0.48
3.	300	709	0.42
	Avg ± SD = 0.58 ± 0.22		
	ns		

(c)

	SCF/Mrx1-roGFP2 + H_2O_2 + TU 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	283	744	0.38
2.	196	412	0.48
3.	242	984	0.25
	Avg ± SD = 0.37 ± 0.12		
	SCF/Mrx1-roGFP2 + H_2O_2 + TU 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	489	743	0.66
2.	160	301	0.53
3.	223	657	0.34
	Avg ± SD = 0.51 ± 0.16		
	ns		

(d)

	NCF/Mrx1-roGFP2 + H_2O_2 + TU 0 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	277	317	0.87
2.	108	262	0.41
3.	59	177	0.33
	Avg ± SD = 0.54 ± 0.29		
	NCF/Mrx1-roGFP2 + H_2O_2 + TU 1 h		
S. No.	V500-A (405 nm)	FITC-A (488 nm)	V500-A/FITC-A
1.	416	389	1.07
2.	137	249	0.55
3.	58	143	0.41
	Avg ± SD = 0.68 ± 0.35		
	ns		

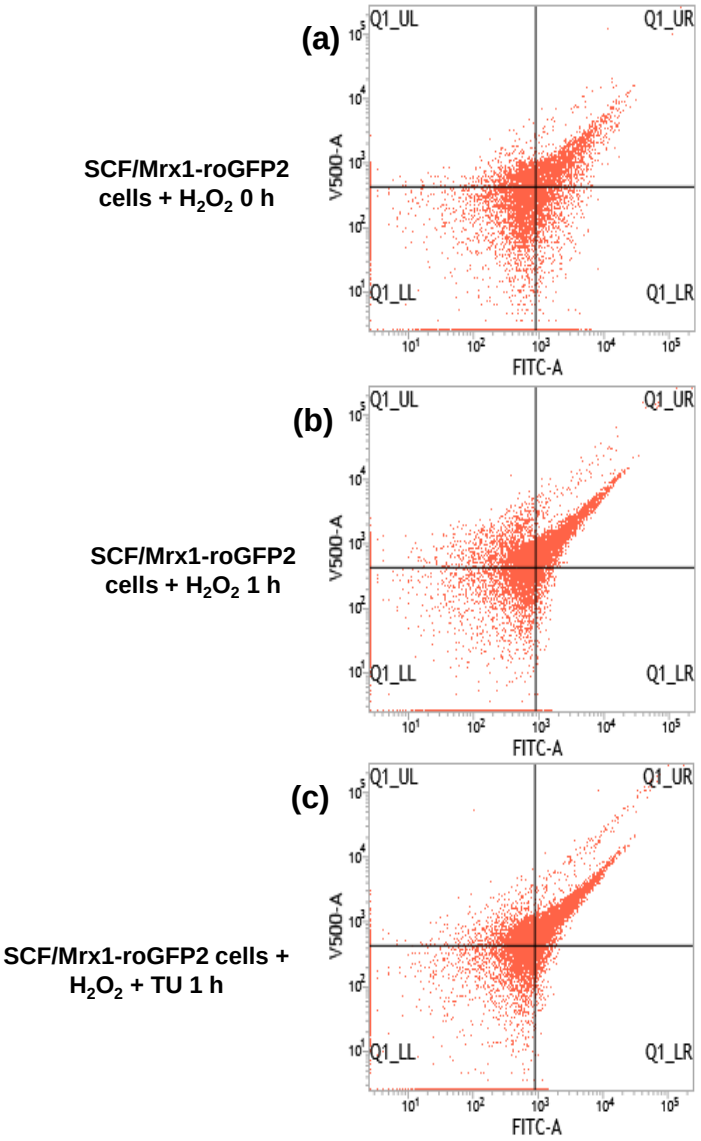
(e)

Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF/Mrx1-roGFP2 + H_2O_2	2.07 ± 0.15	p < 0.05
SCF/Mrx1-roGFP2 + H_2O_2 + TU	1.41 ± 0.31	

(f)

Fold change of Mrx1-roGFP2 405/488 ratio in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
NCF/Mrx1-roGFP2 + H_2O_2	1.13 ± 0.11	ns
NCF/Mrx1-roGFP2 + H_2O_2 + TU	1.26 ± 0.07	

Fig. S9. Quantitation of V500-A:FITC-A ratios of H_2O_2-exposed Mrx1-roGFP2-integrated *Msm* SCF and NCF cells. Redox status is given as the ratio of the median fluorescence at V500-A and FITC-A. Median fluorescence of H_2O_2-exposed: **(a)** SCF/Mrx1-roGFP2 and **(b)** NCF/Mrx1-roGFP2 cells at V500-A (405 nm) and FITC-A (488 nm) at 0 h (immediately upon addition of H_2O_2) and after 1 h of exposure to H_2O_2. Median fluorescence at V500-A (405 nm) and FITC-A (488 nm) of: **(c)** SCF/Mrx1-roGFP2 and **(d)** NCF/Mrx1-roGFP2 cells in the combined presence of H_2O_2 and TU at 0 h (immediately upon addition of H_2O_2 and TU) and after 1 h of exposure to H_2O_2 and TU. **(e, f)** Fold change in the V500-A:FITC ratios of the H_2O_2-exposed cells, with and without TU, at 1 h after normalisation with its respective 0 h value: **(e)** SCF/Mrx1-roGFP2 cells and **(f)** NCF/Mrx1-roGFP2 cells. Statistical significance was calculated using the paired *t* test between 0 h and 1 h of the respective samples and Students' *t* test for normalised values. ns, not significant.



SCF/Mrx1-roGFP2 cells + H_2O_2 0 h

SCF/Mrx1-roGFP2 + H_2O_2 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	9.75	605	577	0.95
Q1_UR	18.97	2412	924	0.38
Q1_LL	**52.65**	462	58	**0.13**
Q1_LR	18.63	1304	123	0.09

SCF/Mrx1-roGFP2 cells + H_2O_2 1 h

SCF/Mrx1-roGFP2 + H_2O_2 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	22.67	568	657	1.16
Q1_UR	32.27	1,820	1,028	0.56
Q1_LL	**39.29**	331	143	**0.43**
Q1_LR	5.76	1,136	288	0.25

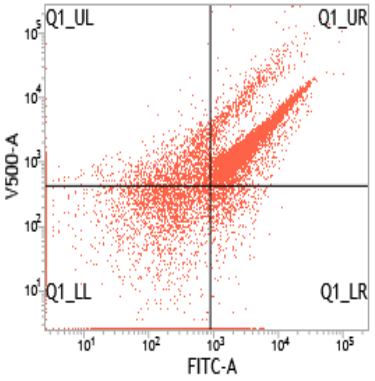
SCF/Mrx1-roGFP2 cells + H_2O_2 + TU 1 h

SCF/Mrx1-roGFP2 + H_2O_2 + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	19.51	632	610	0.97
Q1_UR	35.43	1,884	1,090	0.58
Q1_LL	**38.56**	398	154	**0.39**
Q1_LR	6.49	1,120	268	0.24

Fig. S10. Scatter plot of SCF/Mrx1-roGFP2 cells during H_2O_2 exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** SCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of H_2O_2). **(b)** SCF/Mrx1-roGFP2 cells at the end of 1 h exposure to H_2O_2. **(c)** SCF/Mrx1-roGFP2 cells at the end of 1 h exposure to H_2O_2 + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red colour in bold in the table.

(a)

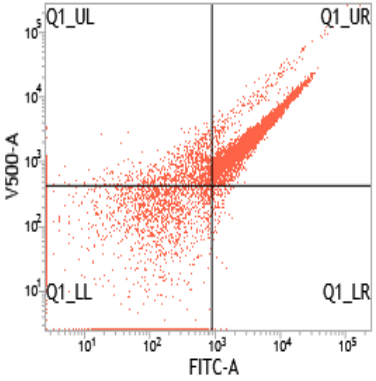
NCF/Mrx1-roGFP2 cells + H_2O_2 0 h



NCF/Mrx1-roGFP2 + H_2O_2 0 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	10.71	307	718	2.34
Q1_UR	39.36	3586	1816	0.51
Q1_LL	**43.41**	50	3	**0.06**
Q1_LR	6.52	1679	183	0.11

(b)

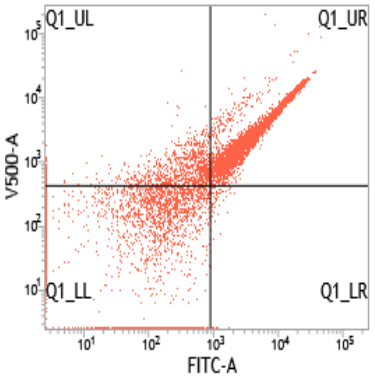
NCF/Mrx1-roGFP2 cells + H_2O_2 1 h



NCF/Mrx1-roGFP2 + H_2O_2 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	9.28	387	672	1.74
Q1_UR	44.57	3,526	2,057	0.58
Q1_LL	**44.60**	43	16	**0.37**
Q1_LR	1.55	1,224	292	0.24

(c)

NCF/Mrx1-roGFP2 cells + H_2O_2 + TU 1 h



NCF/Mrx1-roGFP2 + H_2O_2 + TU 1 h				
Quadrants	% of cells	FITC-A (488 nm)	V500-A (405 nm)	V500-A/ FITC-A
Q1_UL	9.36	370	675	1.82
Q1_UR	39.97	3,459	2,058	0.59
Q1_LL	**49.52**	44	22	**0.50**
Q1_LR	1.14	1,123	299	0.27

Fig. S11. Scatter plot of NCF/Mrx1-roGFP2 cells during H_2O_2 exposure. Percentage of cells in each quadrant with their respective FITC and V500 median fluorescence is given in the table. Redox status of the percentage of cells in each quadrant is given as the ratio of V500 and FITC. **(a)** NCF/Mrx1-roGFP2 cells at 0 h (immediately upon addition of H_2O_2). **(b)** NCF/Mrx1-roGFP2 cells at the end of 1 h exposure to H_2O_2. **(c)** NCF/Mrx1-roGFP2 cells at end of 1 h exposure to H_2O_2 + thiourea. The major proportion of the cells amongst the four quadrants and the respective redox status are given in red colour in bold in the table.

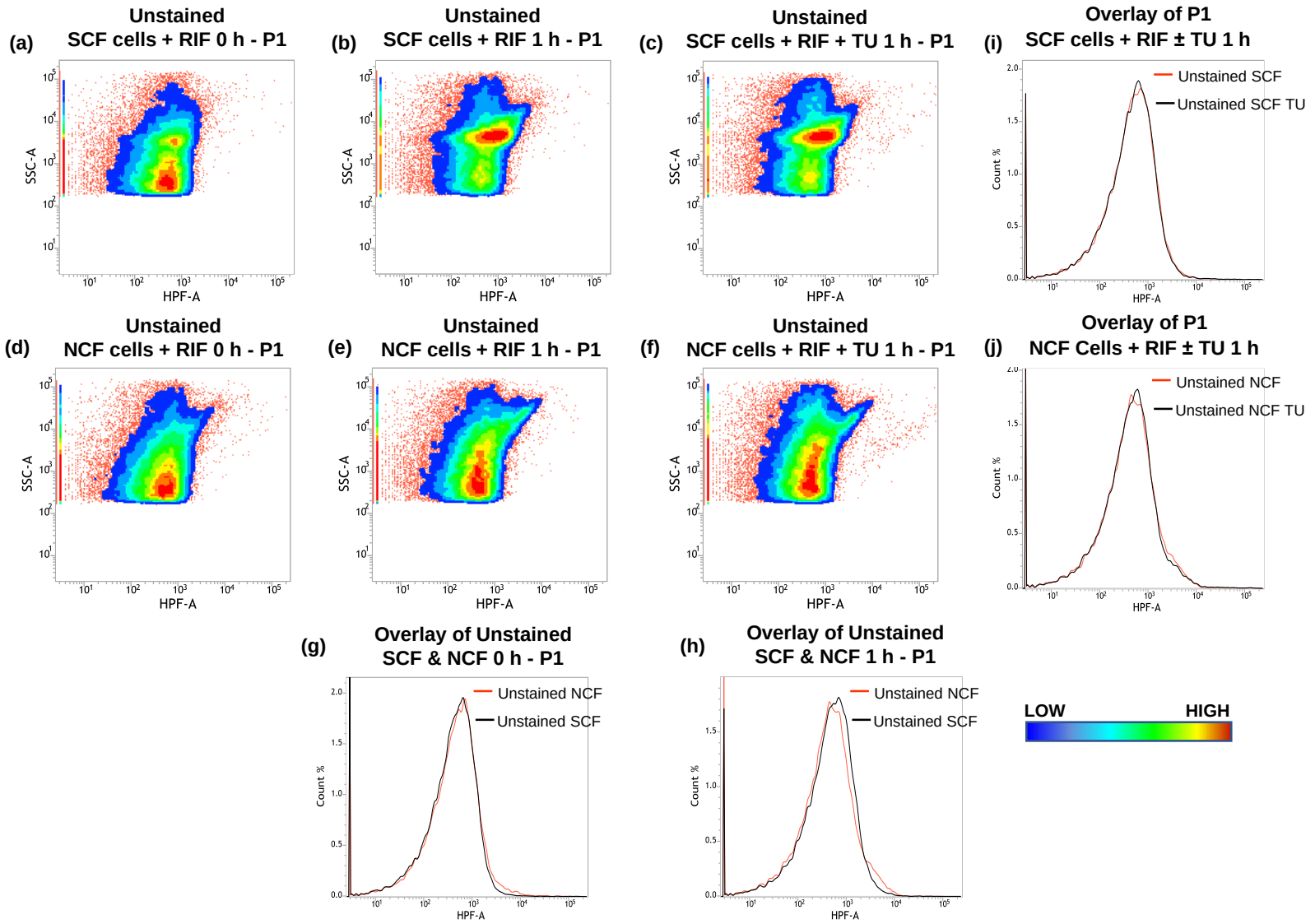


Fig. S12. Flow cytometry profile of unstained 10^4 cells/ml of RIF-exposed SCF and NCF cells. Autofluorescence of unstained control for HPF-stained SCF and NCF cells at 0 h and 1 h of RIF exposure. Density plots of the unstained SCF and NCF cells at: **(a, d)** 0 h, **(b, e)** 1 h of RIF exposure, **(c, f)** in the presence of RIF + TU at 1 h. **(g, h)** Respective histogram overlay of unstained SCF and NCF cells at: **(g)** 0 h and **(h)** 1 h of RIF-exposure. **(i)** Histogram overlay of unstained SCF cells in the absence and presence of TU at 1 h from **(b** and **c)**. **(j)** Histogram overlay of unstained NCF cells in the absence and presence of TU at 1 h from **(e** and **f)**. The scale with the colours in the density plot represents the gradation in population density.

(a)

RIF Exposed			
SCF 0 h		**NCF 0 h**	
S. No.	HPF	S. No.	HPF
1	79	1	68
2	129	2	185
3	49	3	57
Avg ± SD = 85.66 ± 36.14		Avg ± SD = 103.33 ± 63.44	
SCF 1 h		**NCF 1 h**	
S. No.	HPF	S. No.	HPF
1	245	1	86
2	317	2	163
3	180	3	79
Avg ± SD = 247.33 ± 61.29		Avg ± SD = 109.33 ± 41.68	
p < 0.01		ns	

(b)

INH Exposed			
SCF 0 h		**NCF 0 h**	
S. No.	HPF	S. No.	HPF
1	89	1	183
2	80	2	80
3	181	3	231
Avg ± SD = 116.66 ± 49.99		Avg ± SD = 164.33 ± 69.00	
SCF 1 h		**NCF 1 h**	
S. No.	HPF	S. No.	HPF
1	200	1	187
2	189	2	141
3	318	3	88
Avg ± SD = 235.66 ± 63.96		Avg ± SD = 138.66 ± 44.31	
p < 0.005		ns	

(c)

H_2O_2 Exposed			
SCF 0 h		**NCF 0 h**	
S. No.	HPF	S. No.	HPF
1	181	1	87
2	114	2	67
3	155	3	286
Avg ± SD = 150 ± 30.21		Avg ± SD = 146.66 ± 108.29	
SCF 1 h		**NCF 1 h**	
S. No.	HPF	S. No.	HPF
1	468	1	155
2	252	2	106
3	451	3	475
Avg ± SD = 390.33 ± 107.42		Avg ± SD = 245.33 ± 179.24	
p < 0.04		ns	

(d)

SCF TU 0 h		NCF TU 0 h	
S. No.	HPF	S. No.	HPF
1	74	1	100
2	162	2	115
3	31	3	83
Avg ± SD = 89 ± 59.72		Avg ± SD = 99.33 ± 14.32	
SCF TU 1 h		**NCF TU 1 h**	
S. No.	HPF	S. No.	HPF
1	115	1	109
2	182	2	65
3	57	3	84
Avg ± SD = 118 ± 55.94		Avg ± SD = 86 ± 19.73	
ns		ns	

(e)

SCF TU 0 h		NCF TU 0 h	
S. No.	HPF	S. No.	HPF
1	161	1	365
2	81	2	76
3	226	3	85
Avg ± SD = 156 ± 64.96		Avg ± SD = 175.33 ± 146.97	
SCF TU 1 h		**NCF TU 1 h**	
S. No.	HPF	S. No.	HPF
1	247	1	203
2	124	2	109
3	226	3	200
Avg ± SD = 199 ± 58.84		Avg ± SD = 170.66 ± 47.78	
ns		ns	

(f)

SCF TU 0 h		NCF TU 0 h	
S. No.	HPF	S. No.	HPF
1	67	1	93
2	98	2	124
3	225	3	202
Avg ± SD = 130 ± 74.88		Avg ± SD = 139.66 ± 50.23	
SCF TU 1 h		**NCF TU 1 h**	
S. No.	HPF	S. No.	HPF
1	92	1	81
2	145	2	33
3	388	3	292
Avg ± SD = 208.33 ± 141.17		Avg ± SD = 135.33 ± 123.23	
ns		ns	

(g)

Fold change of HPF in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF + RIF	3.08 ± 0.54	p < 0.05
SCF + RIF + TU	1.51 ± 0.32	
NCF + RIF	1.18 ± 0.24	p < 0.05
NCF + RIF + TU	0.89 ± 0.25	

(h)

Fold change of HPF in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF + INH	2.12 ± 0.29	p < 0.05
SCF + INH + TU	1.36 ± 0.27	
NCF + INH	1.06 ± 0.62	ns
NCF + INH + TU	1.45 ± 0.80	

(i)

Fold change of HPF in 1 h w.r.t. 0 h		
Sample	Avg ± SD	Significance
SCF + H_2O_2	2.57 ± 0.31	p < 0.05
SCF + H_2O_2 + TU	1.53 ± 0.16	
NCF + H_2O_2	1.67 ± 0.09	ns
NCF + H_2O_2 + TU	0.86 ± 0.53	

Fig. S13. Quantitation of the HPF median fluorescence of RIF/INH/H_2O_2 exposed SCF/NCF cells. HPF median fluorescence of SCF and NCF cells at 0 h (immediately upon stress exposure) and after 1 h of exposure, in the absence and presence of TU, to: **(a,d)** RIF, **(b,e)** INH and **(c,f)** H_2O_2. **(g, h, i)** Fold change in the HPF median fluorescence at 1 h, after normalisation with its respective 0 h value, of SCF/NCF cells exposed to: **(g)** RIF, **(h)** INH, **(i)** H_2O_2, in the absence or presence of TU. Statistical significance was calculated using the paired *t* test between 0 h and 1 h of the respective samples and Students' *t* test for normalised values. ns, not significant.

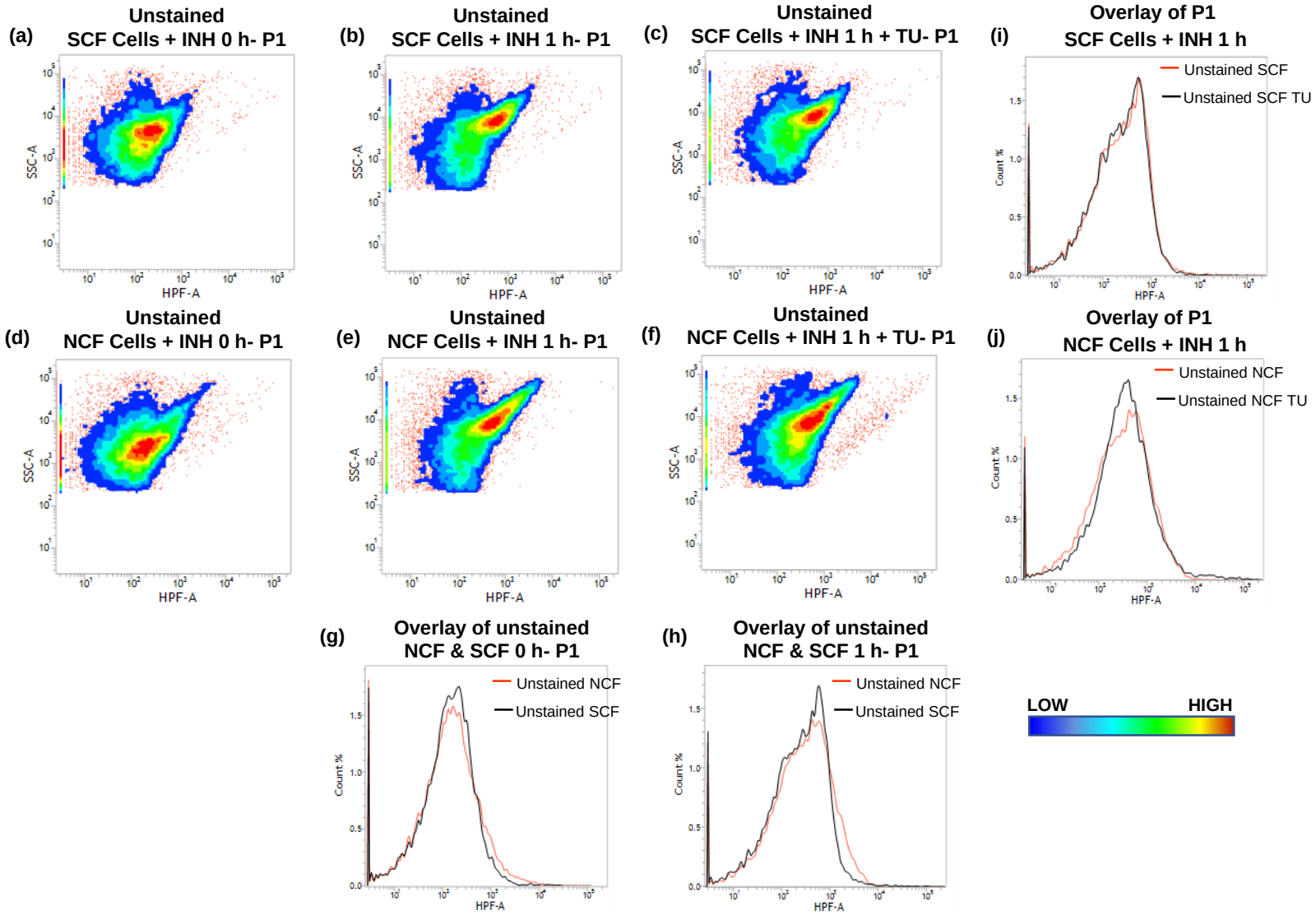


Fig. S14. Flow cytometry profile of unstained 10^4 cells/ml of INH-exposed SCF and NCF cells. Autofluorescence of unstained control for HPF-stained SCF and NCF cells during 0 hr and 1 hr of INH exposure. Density plots of unstained SCF and NCF cells at: **(a,d)** 0 h, **(b, e)** 1 h of INH exposure, **(c, f)** in the presence of INH + TU at 1 h. **(g, h)** Respective histogram overlay of unstained SCF and NCF cells at: **(g)** 0 h and **(h)** 1 h post INH exposure. **(i)** Histogram overlay of unstained SCF cells in the absence and presence of TU at 1 h from **(b** and **c)**. **(j)** Histogram overlay of unstained NCF cells in the absence and presence of TU at 1 h from **(e** and **f)**. The scale with the colours in the density plot represents the gradation in population density.

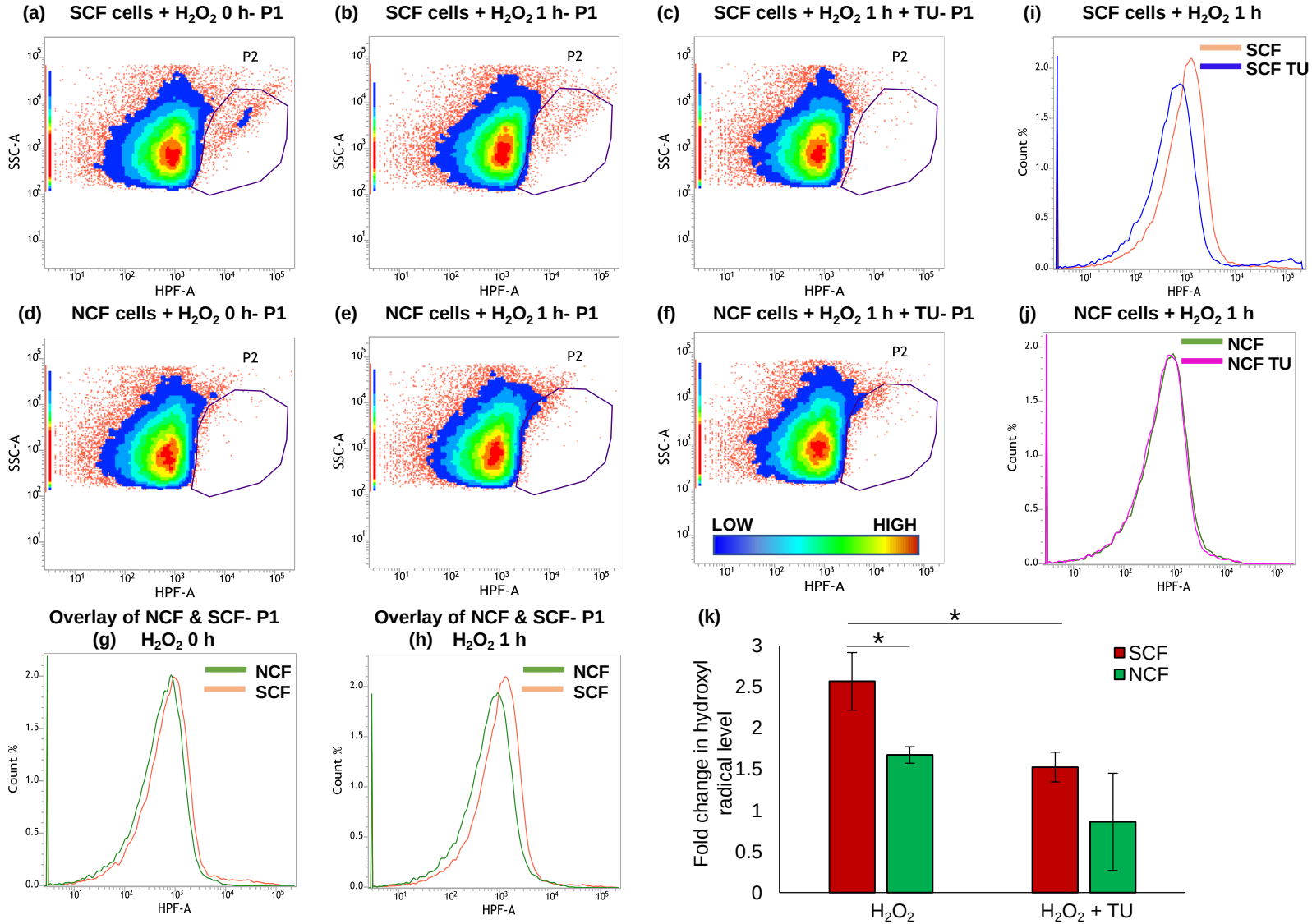


Fig. S15. Flow cytometry profile of HPF-stained H_2O_2-exposed *Msm* SCF and NCF cells. Density plots of SCF cells: **(a)** at 0 h, **(b)** after 1 h H_2O_2 exposure, **(c)** after 1 h H_2O_2 exposure to H_2O_2 + TU. Density plots of *Msm* NCF cells: **(d)** at 0 h, **(e)** after 1 h H_2O_2 exposure, **(f)** after 1 h exposure to H_2O_2 + TU. Respective histogram overlay of NCF and SCF cells: **(g)** at 0 h, **(h)** after 1 h H_2O_2 exposure. **(i)** Histogram overlay of H_2O_2-exposed SCF cells in the absence and presence of TU at 1 h from **(b, c)**. **(j)** Histogram overlay of H_2O_2-exposed NCF cells in the absence and presence of TU at 1 h from **(e, f)**. 10^4 cells/ml was exposed to H_2O_2 in each case. **(k)** Quantitation of the fold-change in the hydroxyl radical levels in SCF and NCF cells exposed to H_2O_2 for 1 h with respect to (w.r.t.) the respective 0 h value (considering 0 h value as 1), n = 3. Statistical significance was calculated using the Students' *t* test. The scale with the colours in the density plot represents the gradation in population density.

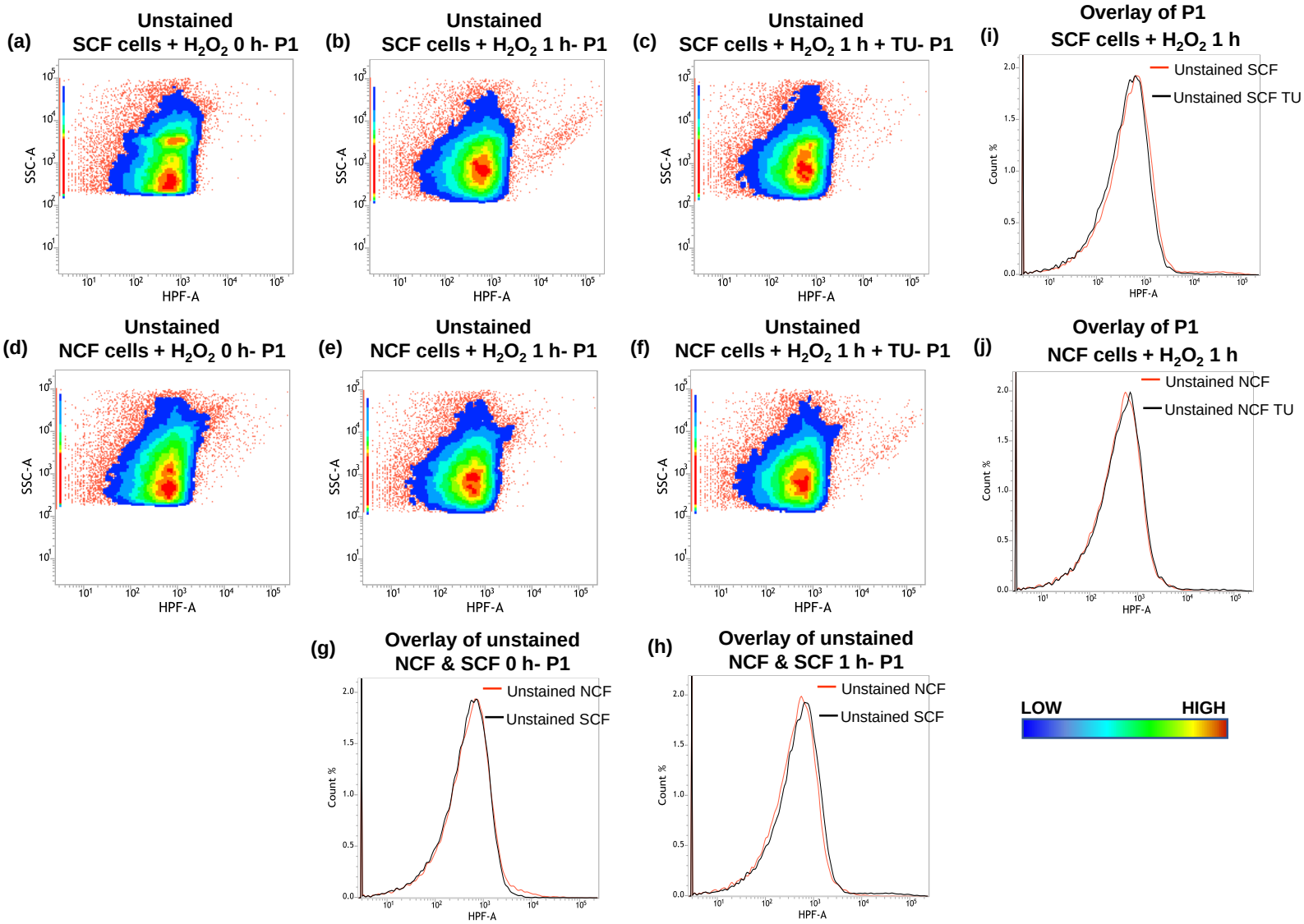


Fig. S16. Flow cytometry profile of unstained 10^4 cells/ml of H_2O_2-exposed *Msm* SCF and NCF cells. Autofluorescence of unstained control for HPF-stained SCF and NCF cells during 0 h and 1 h H_2O_2 exposure. Density plots of unstained SCF and NCF cells: **(a, d)** at 0 h, **(b, e)** after 1 h of H_2O_2 exposure, **(c, f)** in the presence of H_2O_2 + TU at 1 h. **(g, h)** Respective histogram overlay of unstained SCF and NCF cells at: **(g)** 0 h and **(h)** 1 h post H_2O_2 exposure. **(i)** Histogram overlay of unstained SCF cells in the absence and presence of TU at 1 h from **(b** and **c)**. **(j)** Histogram overlay of unstained NCF cells in the absence and presence of TU at 1 h from **(e** and **f)**. The scale with the colours in the density plot represents the gradation in population density.

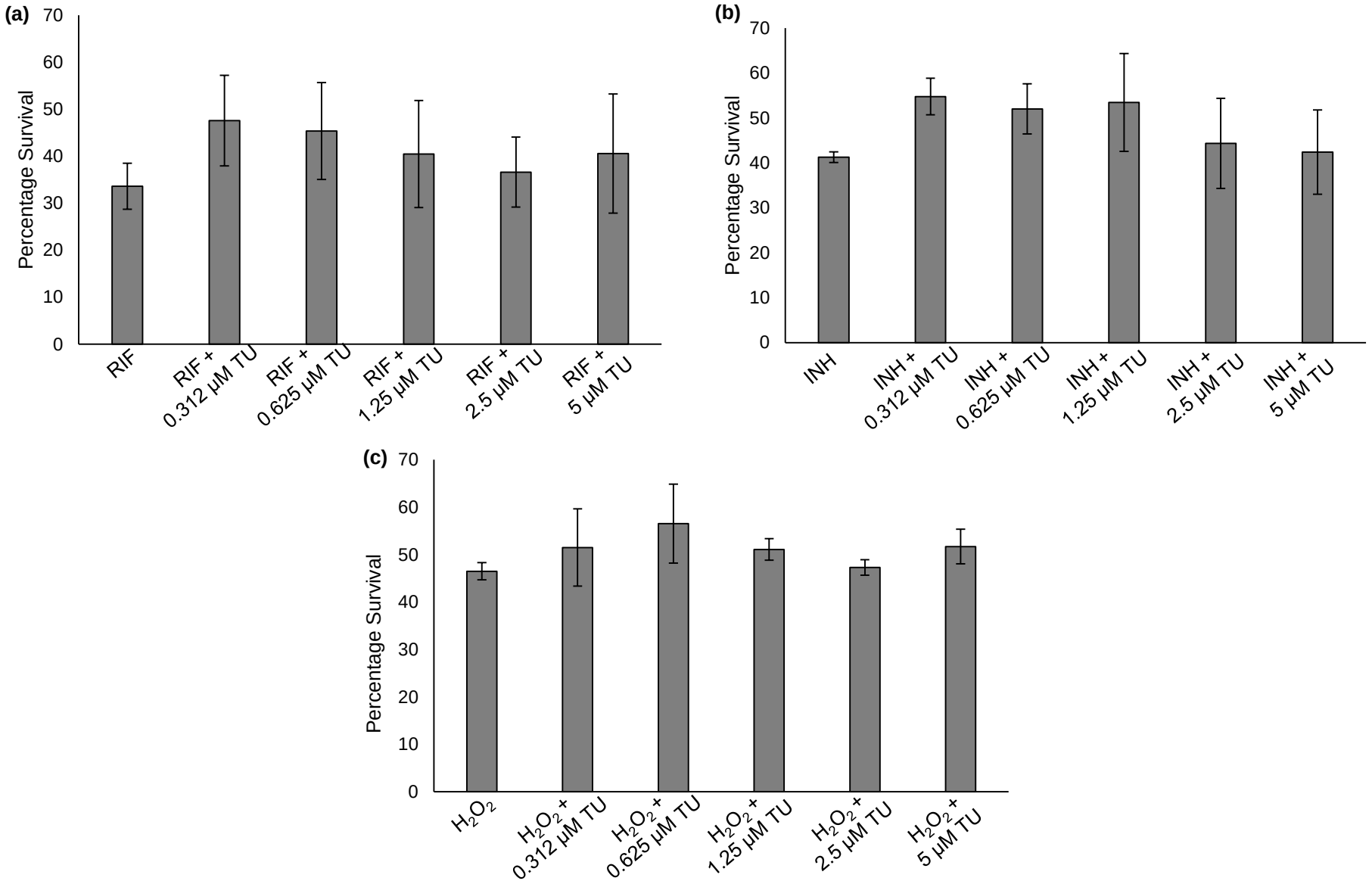


Fig. S17. Percentage survival of *Msm* MLP cells (10^4 cells/ml) when exposed to RIF, INH, and H_2O_2 in the presence of different concentrations of thiourea. **(a)** Exposure of cells to 25 µg/ml RIF in the presence of different concentrations of thiourea (TU for 4 h. **(b)** Exposure of the cells to 2.5 µg/ml INH in the presence of different concentrations of thiourea for 6 h. **(c)** Exposure of the cells to 0.8 mM H_2O_2 in the presence of different concentrations of thiourea for 1 h (n = 3).

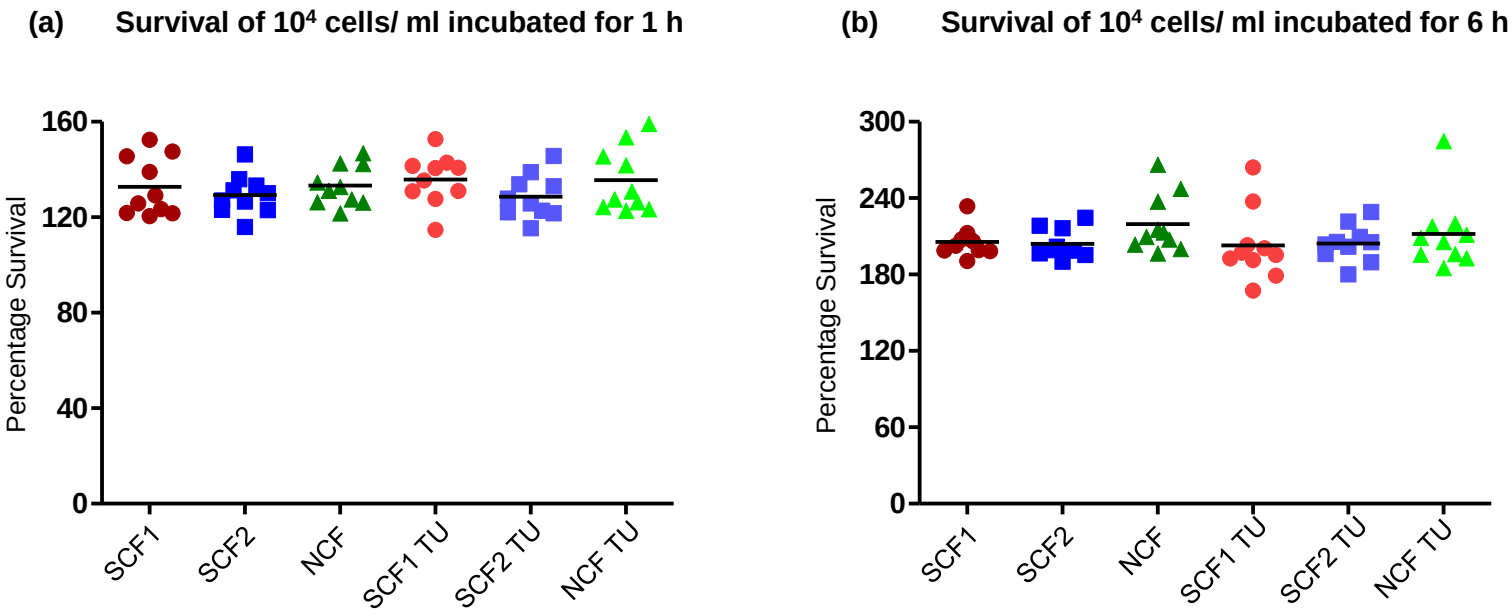


Fig. S18. Percentage survival of *Msm* SCF1, SCF2 and NCF cells (10^4 cells/ml) when incubated under untreated condition in the presence or absence of thiourea for different time durations. **(a)** Survival of SCF1, SCF2 and NCF cells when incubated under untreated condition in the presence or absence of 5 µM thiourea (TU) for 1 h. **(b)** Survival of SCF1, SCF2 and NCF cells when incubated under untreated condition in the presence or absence of 5 µM thiourea for 6 h (n = 10).

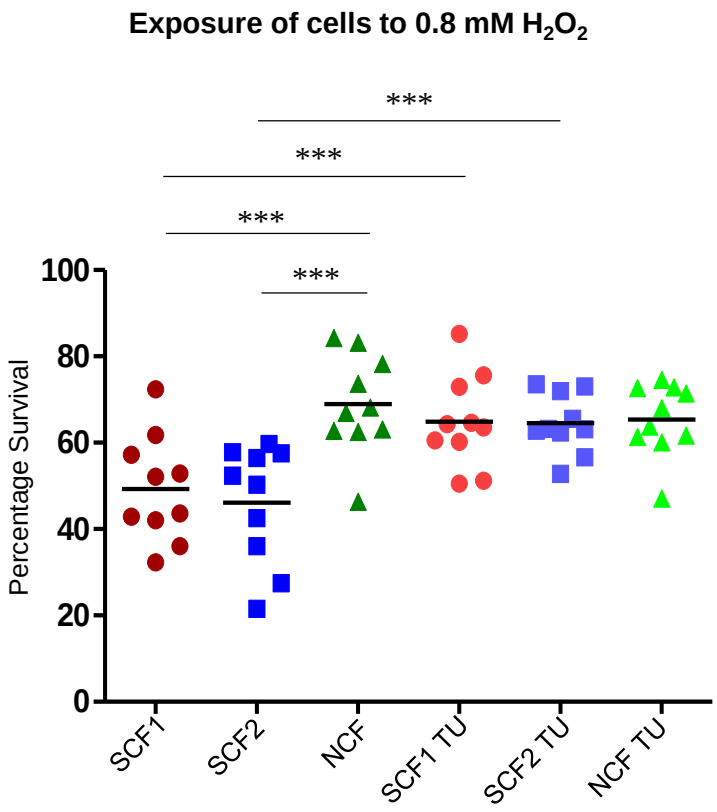


Fig. S19. Exposure of *Msm* SCF1, SCF2 and NCF cells to H_2O_2 in the absence and presence of thiourea. Percentage survival of SCF1 and SCF2 cells in comparison to the survival of NCF cells when exposed to 0.8 mM H_2O_2 for 1 h in the absence and presence of 5 µM thiourea (TU). 10^4 cells/ml was exposed to H_2O_2 in each case (n = 10 samples). *** indicates $p < 0.001$. Statistical significance was calculated using paired *t* test.