Electronic Supplementary Material (ESI) for Toxicology Research.

Perturbation of microRNA signalling by doxorubicin in spermatogonial, Leydig and Sertoli cell lines *in vitro*

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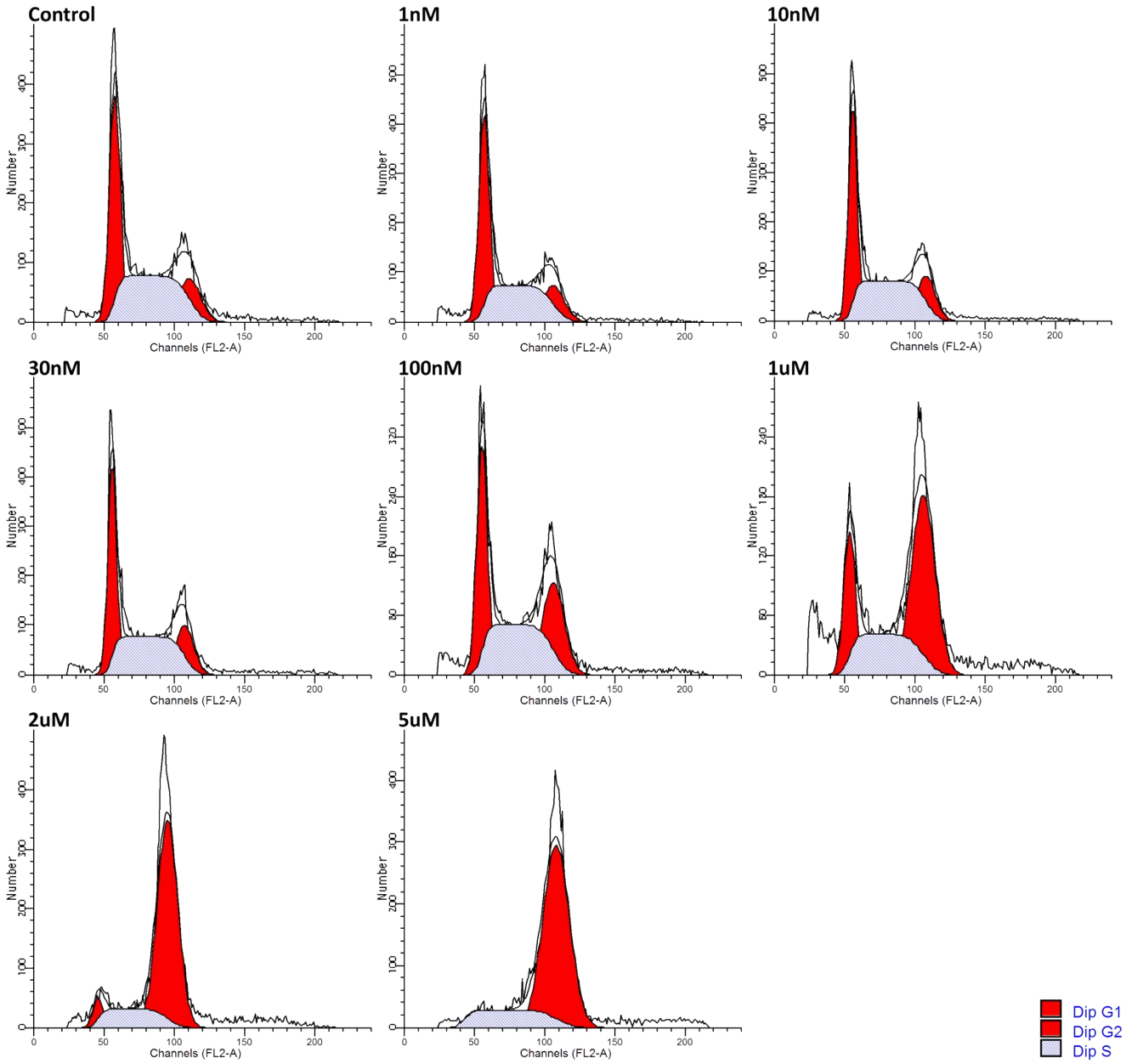
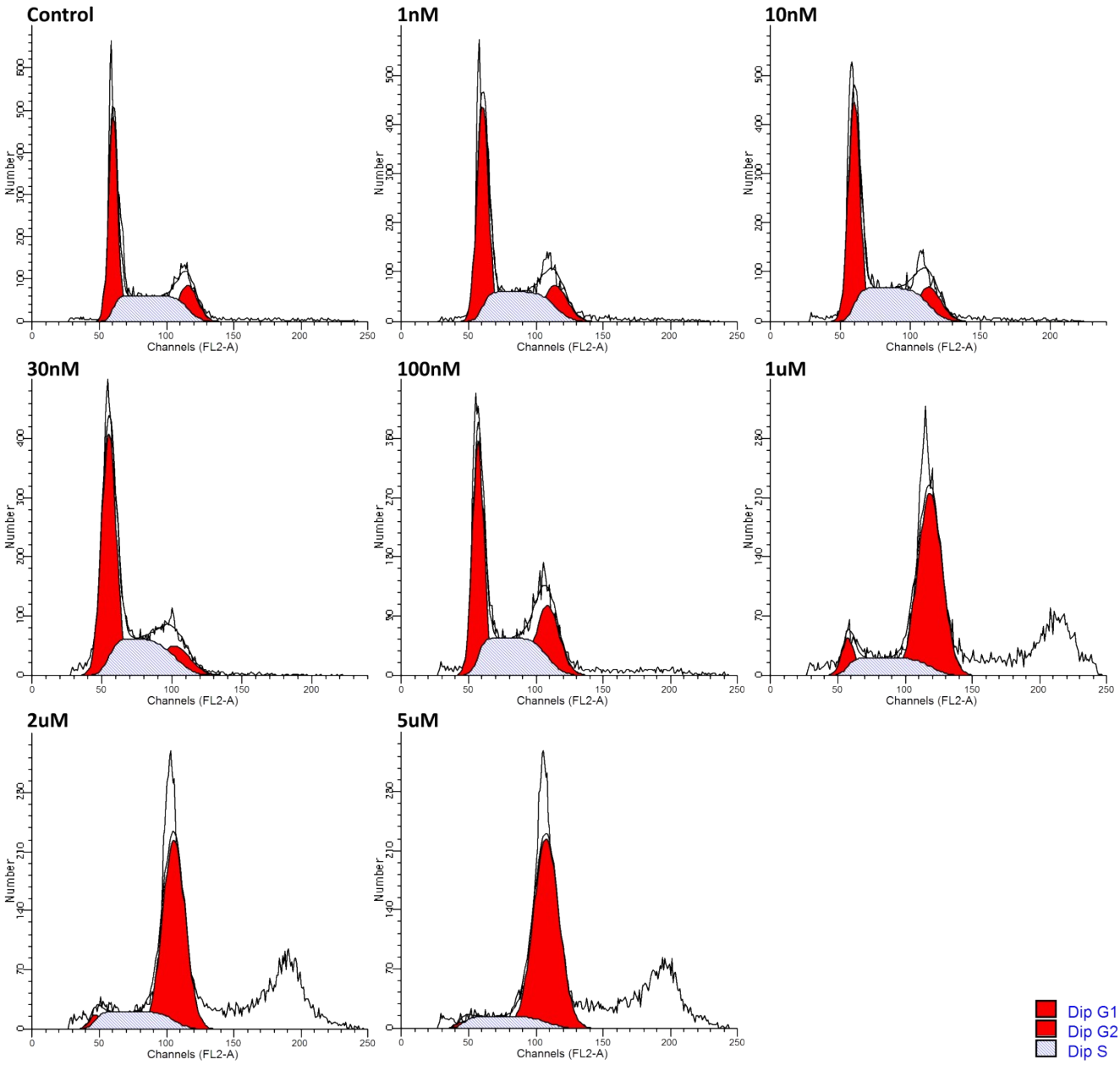
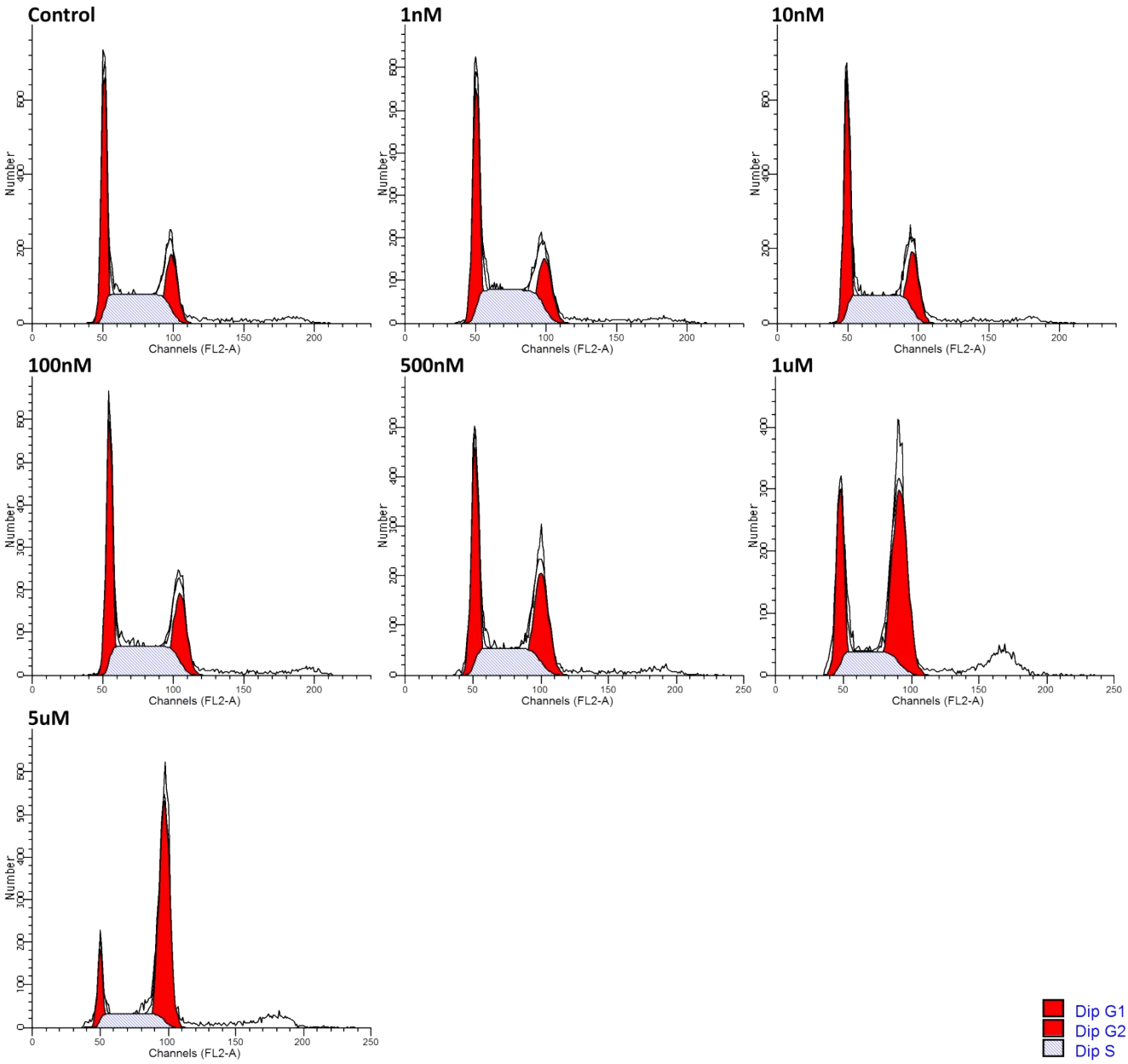


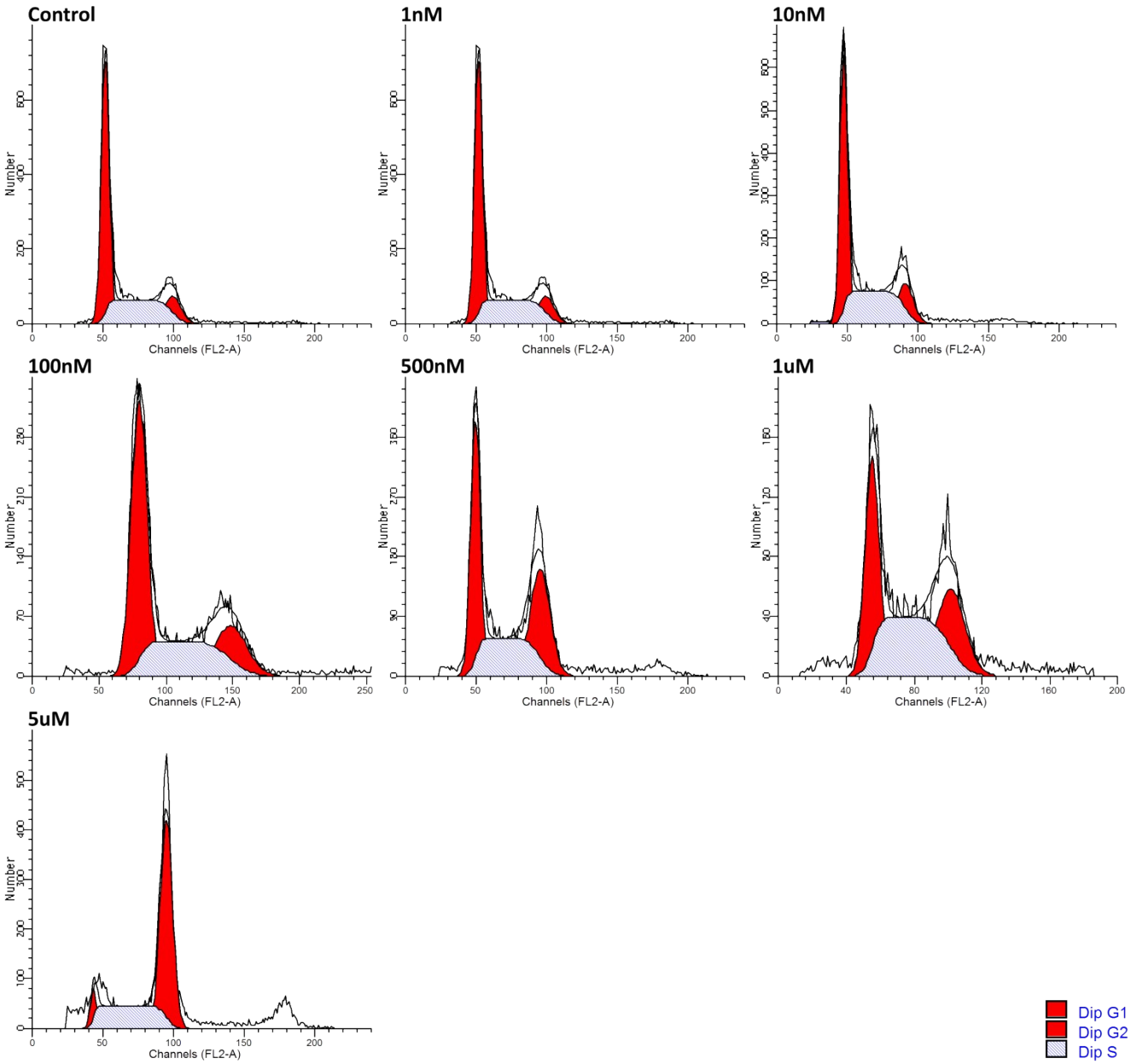
ESI Fig. 1 Changes in the cell cycle distribution of the GC1 spermatogonial cell line following 24 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 μM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



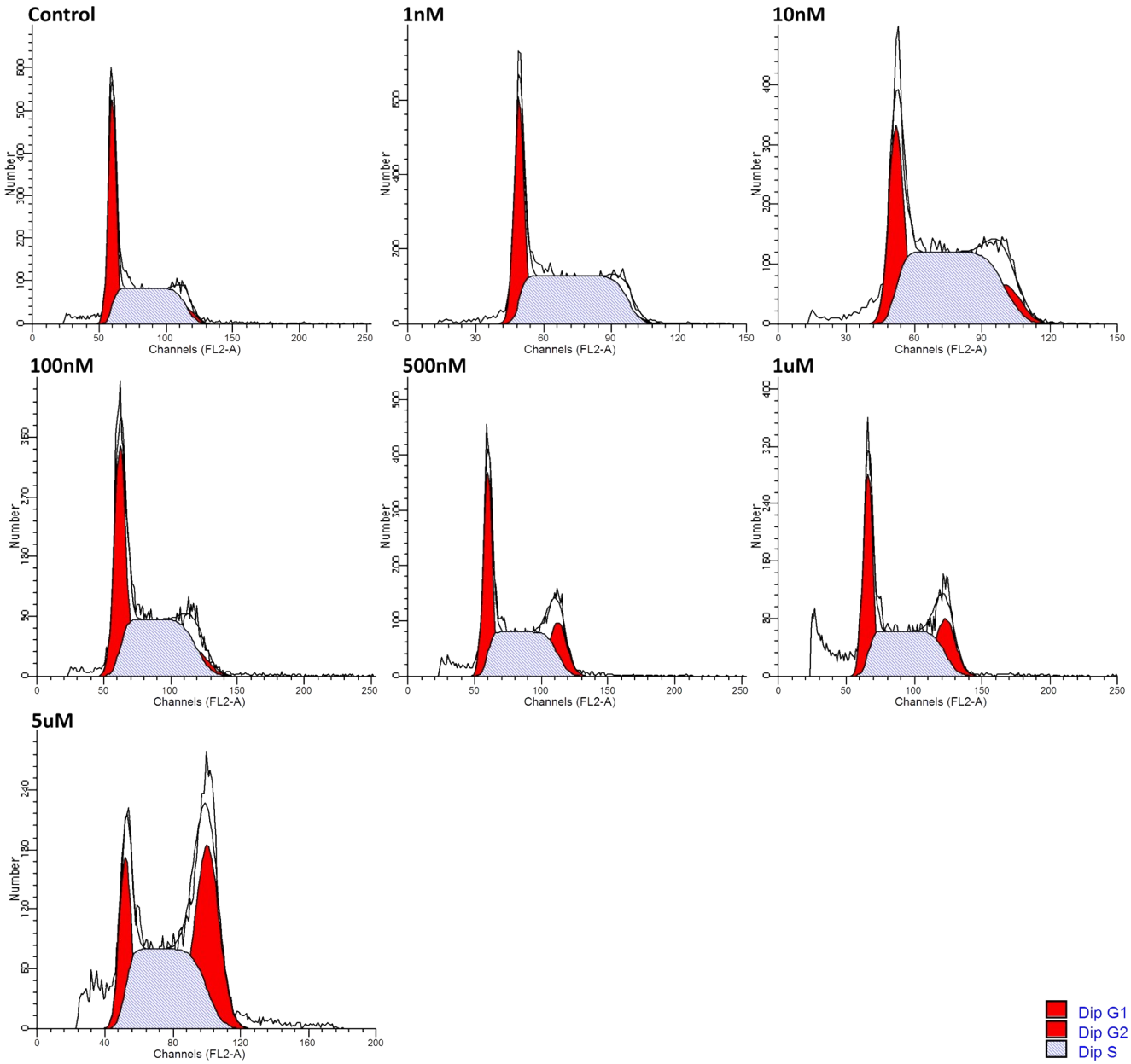
ESI Fig. 2 Changes in the cell cycle distribution of the GC1 spermatogonial cell line following 48 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 μM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



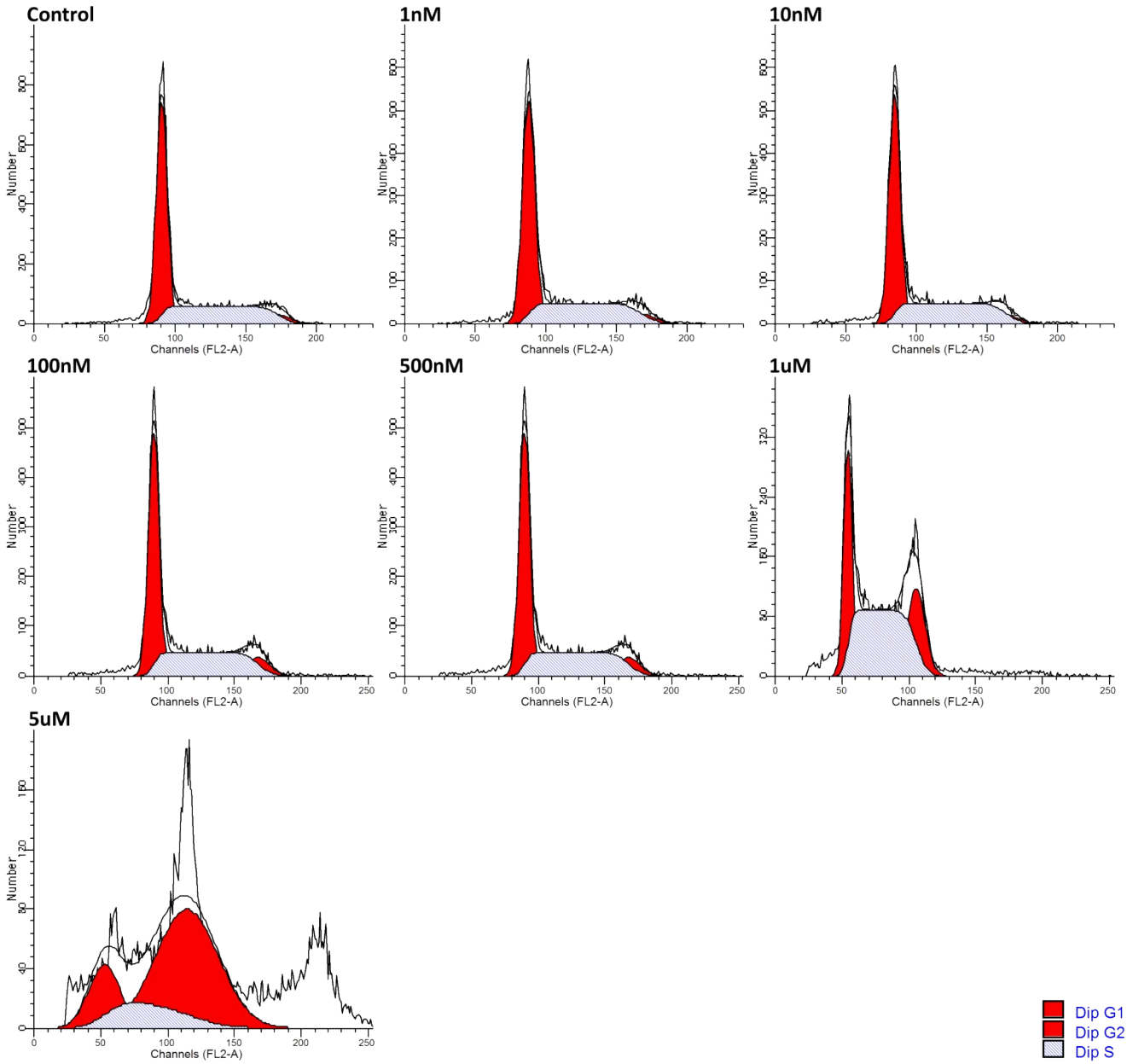
ESI Fig. 3 Changes in the cell cycle distribution of the TM3 Leydig cell line following 24 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 µM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



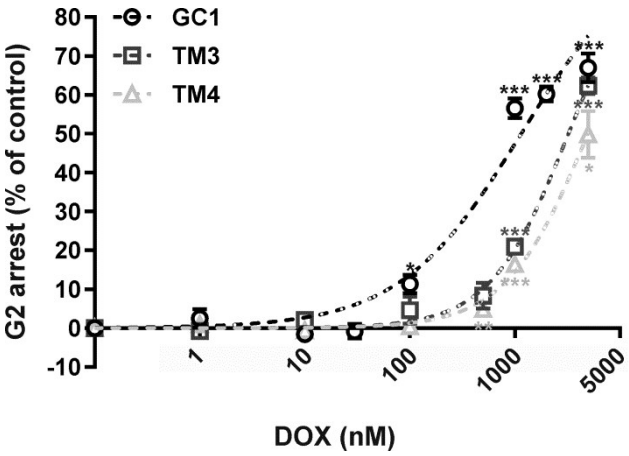
ESI Fig. 4 Changes in the cell cycle distribution of the TM3 Leydig cell line following 48 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 µM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



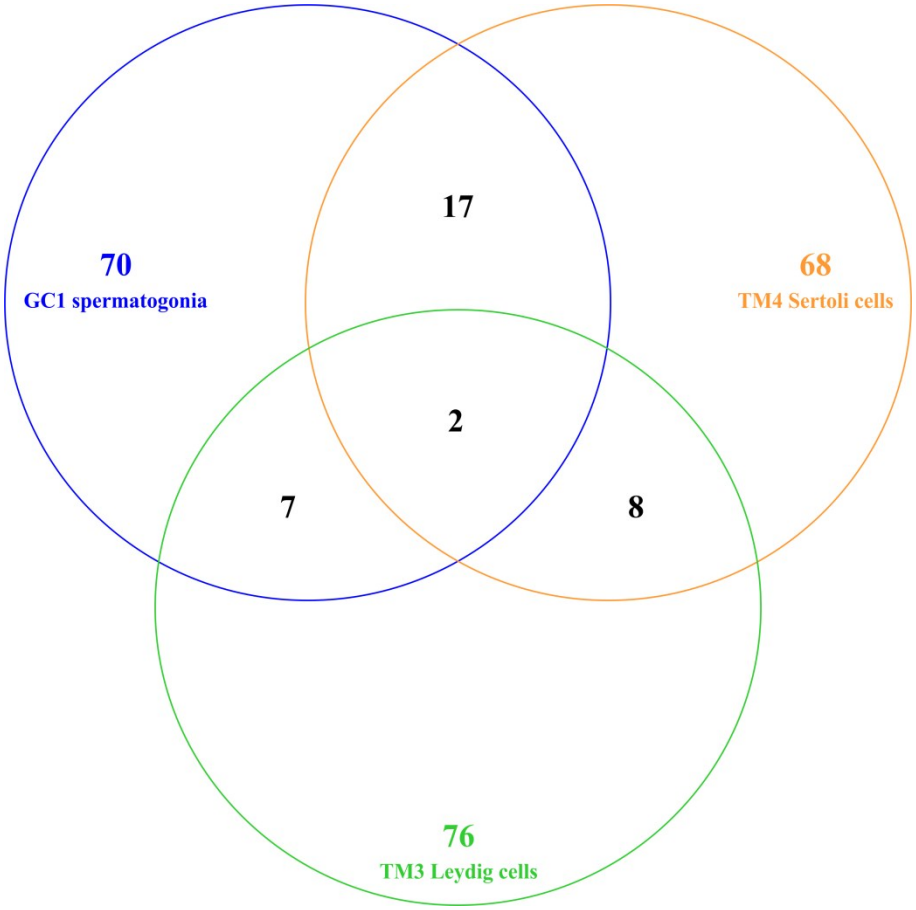
ESI Fig. 5 Changes in the cell cycle distribution of the TM4 Sertoli cell line following 24 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 µM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



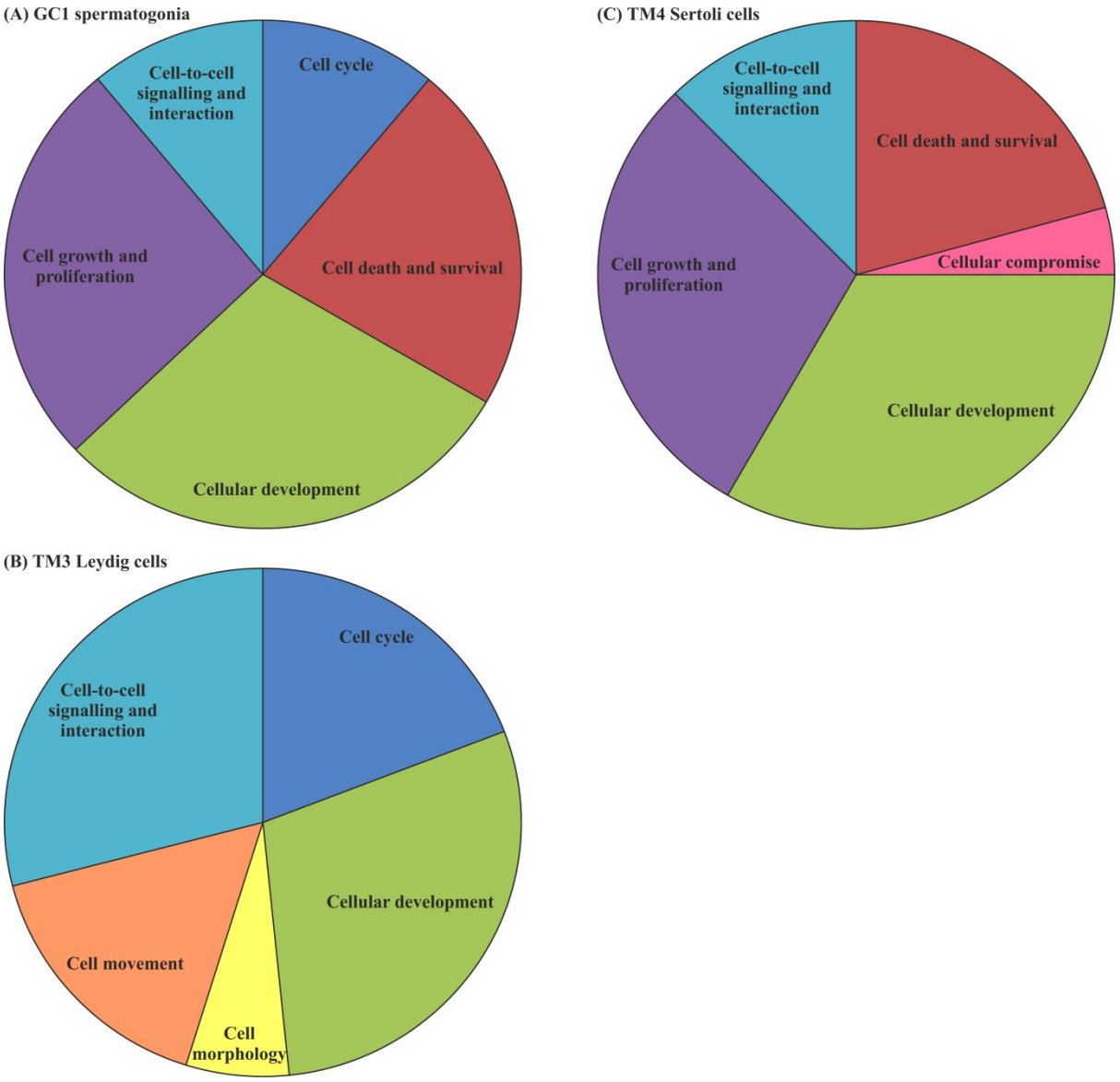
ESI Fig. 6 Changes in the cell cycle distribution of the TM4 Sertoli cell line following 48 hour treatment with increasing concentrations of DOX. Cells were treated with 1nM – 5 µM DOX. Controls received vehicle only (PBS). Cell cycle distribution was measured following propidium iodide staining and FACS analyses to determine DNA content. Each histogram is a representative plot (n=3). Dip G1 (first red peak) represents cells in the G0/G1 phase, Dip G2 (second red peak) represents cells in the G2/M phase, Dip S represents cells in the S phase and cells undergoing apoptosis are present in small peaks before Dip G1 (sub G1).



ESI Fig. 7 Dose-response of G2/M arrest following treatment with DOX for 48 hours. Cellular DNA content was analysed by FACS, and normalised by subtracting control values (percentage of cells in G2/M phase in the absense of DOX) from treated values. Values represent mean ± SD (n=3). *p<0.05, **p<0.01 and ***p<0.001 (T-test compared to vehicle only).



ESI Fig. 8 Venn diagram of DOX induced miRNAs in the three testicular cell lines highlighting the numbers present in one or more cell line.



ESI Fig. 9 Pie chart representation of the molecular and cellular functions associated with up to the top 100 significantly differentially expressed miRNAs following treatment with DOX in **(A)** GC1 spermatogonia, and **(B)** TM3 Leydig and **(C)** TM4 Sertoli cells. IPA® software was used to identify the different functional categories (p<0.05).

ESI Table 1 DOX-induced miRNAs present in two or more of the three testicular cell lines

miRNA	GC1 spermatogonia						TM3 Leydig cells						TM4 Sertoli cells					
	0.1μM			1μM			0.5μM			5μM			0.5μM			5μM		
mmu-miR-1187	-1.1/-2.2	-1.3/-2.5	-0.9/-1.9	-1.2/-2.3	-0.9/-1.9	-1.1/-2.1	1.0/2.0	1.2/2.2	0.9/1.8	2.0/4.0	1.0/2.0	2.2/4.7						
mmu-miR-128-2-5p							0.1/1.1	0.0/1.0	0.0/1.0	0.3/1.2	0.2/1.1	0.2/1.2	-0.6/-1.5	-0.6/-1.5	-1.1/-2.1	-1.3/-2.5	-1.0/-2.1	-1.1/-2.2
mmu-miR-1902	1.0/1.9	0.6/1.5	1.0/1.9	2.9/7.4	2.5/5.6	1.2/2.3	-0.3/-1.2	-0.5/-1.4	-0.5/-1.4	-0.9/-1.9	-0.8/-1.7	-0.6/-1.6						
mmu-miR-191-3p	-1.0/-2.0	-1.1/-2.1	-1.5/-2.8	-0.7/-1.6	-1.1/-2.2	-0.9/-1.8	0.1/1.1	0.1/1.1	0.3/1.2	0.5/1.4	0.5/1.4	0.5/1.4						
mmu-miR-1934	0.9/1.9	1.5/2.8	1.8/3.5	0.5/1.4	0.9/1.9	0.5/1.5							-0.8/-1.7	-0.6/-1.5	-0.4/-1.3	-1.4/-2.6	-1.1/-2.2	-1.2/-2.2
mmu-miR-1945	-1.4/-2.6	-1.0/-2.0	-0.8/-1.8	-0.7/-1.6	-0.9/-1.9	-0.7/-1.6	-0.8/-1.8	-0.8/-1.8	-0.5/-1.4	-0.7/-1.6	-0.9/-1.8	-1.1/-2.2						
mmu-miR-199b-5p							0.0/1.0	-0.2/-1.2	-0.2/-1.2	0.5/1.4	0.5/1.4	0.4/1.3	-0.4/-1.4	-1.4/-2.6	-0.9/-1.8	0.2/1.1	0.0/1.0	0.0/1.0
mmu-miR-206	0.5/1.4	0.5/1.4	0.3/1.2	0.8/1.7	0.8/1.7	1.4/2.7	0.7/1.6	0.3/1.2	0.7/1.7	2.0/4.1	1.6/3.1	2.1/4.2						
mmu-miR-208b-5p	-0.4/-1.3	-0.7/-1.6	-0.5/-1.1	-0.2/-1.1	-0.3/-1.2	0.0/1.0	0.2/1.1	0.5/1.4	0.3/1.3	0.4/1.3	0.6/1.5	0.3/1.3						
mmu-miR-2136	1.0/2.0	1.4/2.6	1.3/2.4	1.1/2.1	1.2/2.3	1.5/2.8	-1.3/-2.4	-0.9/-1.8	-0.8/-1.8	-0.6/-1.6	-0.9/-1.8	-0.8/-1.8						
mmu-miR-218-1-3p	-0.5/-1.4	-0.5/-1.4	-0.6/-1.5	-0.3/-1.3	-0.4/-1.4	-0.1/-1.1							-0.4/-1.3	-0.6/-1.5	-0.9/-1.9	0.2/1.2	0.6/1.5	0.3/1.2
mmu-miR-25-5p							0.8/1.8	0.6/1.5	0.7/1.6	0.7/1.6	0.7/1.6	0.8/1.7	0.4/1.3	0.3/1.2	0.4/1.3	0.9/1.9	1.2/2.3	0.9/1.9
mmu-miR-297c	-1.7/-3.3	-1.8/-3.4	-2.0/-4.0	-1.6/-3.0	-1.1/-2.1	-2.4/-5.4	0.5/1.4	0.8/1.8	-0.2/-1.2	1.6/3.1	2.4/5.4	1.8/3.6						
mmu-miR-29c	-0.6/-1.5	-0.4/-1.3	-0.2/-1.2	-0.5/-1.4	-0.8/-1.7	-0.8/-1.7							0.2/1.1	-0.1/-1.1	-0.2/-1.1	1.0/1.9	1.2/2.2	0.6/1.6
mmu-miR-300-5p	-0.6/-1.5	-0.7/-1.7	-0.7/-1.6	-0.6/-1.5	-0.6/-1.5	-0.8/-1.8	-1.5/-2.9	-0.9/-1.9	-1.6/-3.0	-0.9/-1.9	-1.3/-2.5	-0.7/-1.6						
mmu-miR-3072	2.0/4.0	1.2/2.3	1.3/2.4	0.8/1.7	0.7/1.6	0.8/1.7	-2.3/-4.8	-1.7/-3.3	-1.9/-3.8	-1.2/-2.2	-1.7/-3.3	-1.7/-3.2	0.6/1.5	0.0/1.0	-0.3/-1.2	-3.3/-9.6	-2.0/-4.0	-2.0/-4.1
mmu-miR-3084-5p							0.3/1.2	0.7/1.7	0.6/1.5	0.7/1.6	1.6/3.0	1.2/2.4	0.9/1.9	0.5/1.4	0.0/1.0	-1.9/-3.7	-1.3/-2.5	-1.6/-3.0
mmu-miR-30c	-0.2/-1.1	-0.6/-1.5	-0.5/-1.4	-0.7/-1.6	-0.8/-1.7	-0.8/-1.8	-0.1/-1.1	-0.4/-1.4	-0.3/-1.2	-0.5/-1.4	-1.0/-2.0	-0.6/-1.5						
mmu-miR-34a-3p	-2.7/-6.7	-1.6/-2.9	-1.7/-3.2	-1.2/-2.3	-1.6/-3.0	-1.6/-3.0	0.4/1.4	0.4/1.3	0.0/1.0	0.8/1.7	0.7/1.6	0.6/1.5						
mmu-miR-376c							0.0/1.0	0.6/1.5	0.6/1.5	0.9/1.9	1.7/3.3	1.1/2.2	-0.1/-1.1	0.9/1.9	1.2/2.3	-0.9/-1.9	-1.8/-3.5	-1.6/-3.0
mmu-miR-378-5p	-0.7/-1.6	-0.4/-1.3	-0.7/-1.6	-0.7/-1.6	-0.6/-1.6	-1.0/-2.0	-0.7/-1.6	-0.8/-1.8	-0.4/-1.4	-0.6/-1.5	-0.7/-1.6	-0.8/-1.7						
mmu-miR-450a-1-3p	0.5/1.4	0.4/1.3	0.6/1.5	0.8/1.8	1.3/2.4	1.0/2.0							-0.6/-1.6	0.1/1.0	-0.8/-1.7	-1.9/-3.8	-2.2/-4.7	-1.4/-2.7
mmu-miR-466d-3p							0.1/1.1	0.1/1.1	0.3/1.2	0.2/1.1	0.2/1.1	0.2/1.1	-1.9/-3.6	-1.5/-2.9	-1.2/-2.2	-1.0/-1.9	-1.1/-2.2	-1.2/-2.4
mmu-miR-466i-5p	0.1/1.0	0.5/1.4	0.5/1.4	0.7/1.7	0.8/1.7	0.8/1.7	0.9/1.9	1.4/2.7	0.9/1.8	0.9/1.8	1.4/2.6	1.5/2.8						
mmu-miR-467b-3p							1.4/2.6	0.9/1.9	0.8/1.7	0.2/1.2	0.3/1.3	0.7/1.6	-0.8/-1.7	-1.7/-3.2	-1.3/-2.5	-2.0/-4.0	-2.0/-3.9	-2.1/-4.4

mmu-miR-467d-3p	-0.2/-1.2	-0.6/-1.6	-0.7/-1.7	-1.0/-2.1	-1.5/-2.8	-1.1/-2.1							0.0/1.0	0.1/1.0	0.6/1.5	0.8/1.7	0.9/1.8	0.7/1.7
mmu-miR-489	-0.9/-1.9	-0.9/-1.8	-1.3/-2.4	-0.7/-1.6	-1.1/-2.1	-1.2/-2.3	0.5/1.4	1.0/2.0	0.9/1.8	0.3/1.2	0.7/1.6	0.5/1.4						
mmu-miR-551b							0.4/1.3	0.5/1.4	0.5/1.4	0.4/1.3	0.5/1.4	0.4/1.3	0.1/1.1	-0.1/-1.1	-0.4/-1.4	0.5/1.4	0.6/1.5	0.6/1.5
mmu-miR-592-3p	1.0/1.9	0.5/1.4	0.6/1.6	1.0/2.0	1.0/2.0	0.7/1.6	-1.6/-3.0	-1.5/-2.8	-1.2/-2.2	-1.6/-3.1	-1.6/-3.1	-1.7/-3.2						
mmu-miR-683	0.2/1.1	0.3/1.2	0.1/1.1	0.5/1.4	0.5/1.4	0.4/1.3							-0.4/-1.3	-0.4/-1.3	-0.4/-1.3	-0.4/-1.3	-0.7/-1.6	-0.4/-1.3
mmu-miR-710	-0.7/-1.6	-0.6/-1.6	-0.6/-1.5	-0.9/-1.9	-0.5/-1.4	-0.5/-1.5	-1.6/-3.1	-1.6/-3.0	-1.6/-3.1	-1.8/-3.4	-1.5/-2.9	-1.5/-2.8						
mmu-miR-721	-0.9/-1.9	-0.7/-1.6	-0.6/-1.6	-0.6/-1.5	-0.8/-1.7	-0.7/-1.6	-1.8/-3.6	-1.7/-3.2	-1.2/-2.3	-0.9/-1.9	-1.2/-2.3	-2.0/-3.9						
mmu-miR-767	-0.4/-1.4	-0.6/-1.5	-0.5/-1.4	-0.3/-1.2	-0.1/-1.1	-0.5/-1.4	-0.8/-1.7	-0.6/-1.5	-0.5/-1.4	-0.1/-1.1	-0.3/-1.3	-0.3/-1.2	0.0/1.0	0.0/1.0	-0.1/-1.1	-0.9/-1.9	-1.5/-2.8	-1.0/-2.0
mmu-miR-874-5p	1.2/2.3	0.9/1.9	1.0/2.0	1.1/2.1	1.0/1.9	1.0/2.1							0.1/1.1	-0.4/-1.4	-0.9/-1.8	-4.5/-22.1	-3.1/-8.5	-4.2/-18.4

miRNAs were selected due to their significant differential expression ($p<0.05$) in one or more of the cell lines following treatment with DOX. Values represent mean $\log_2$[DOX/Control] / fold change[DOX/Control] (n=3). Black boxes represent absence of significant differential expression.