

Supplemental

**Supplemental
Figure 1**

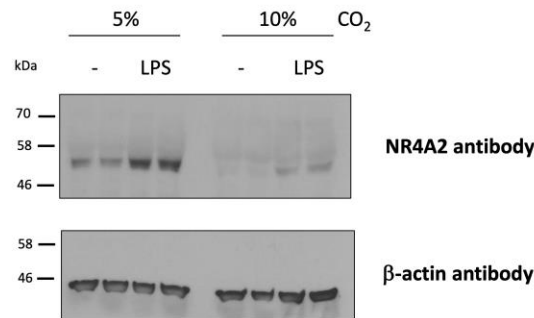


Figure S1. NR4A2 protein is reduced in hypercapnia. Western blot analysis of whole cell lysates from THP-1 monocytes exposed to 5% or 10% CO₂ for 90 mins +/- LPS 2.5μg/ml for an additional 4h. Primary antibodies for NR4A2 and β-actin were used followed by species specific HRP-conjugated secondary antibodies and visualisation using enhanced chemiluminescence. NR4A2 data is representative of $n=3$ separate experiments.

**Supplemental
Figure 2**

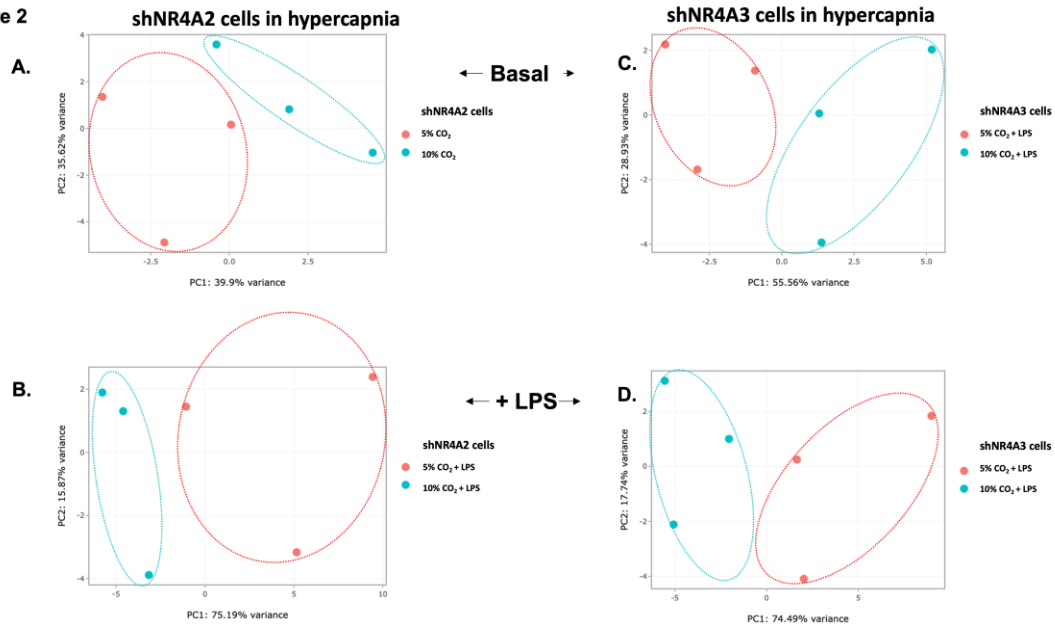


Figure S2. RNA-seq analysis of shNR4A2 and shNR4A3 monocytes exposed to buffered hypercapnia for 4h in the presence of LPS. Principal component analysis of shNR4A2 and shNR4A3 cells exposed to 5% CO₂ (red) or 10% CO₂ (blue) (4h) in the basal (A, C) and LPS stimulated state (2.5μg/ml for 2h) (B, D). The x-axis (PC1) is the vector that displays the most variance between samples and the y-axis (PC2) displays the second most. The percentage of total variance per principal component is shown on the axis label. Data is representative of $n = 3$ individual experiments.

Supplemental
Figure 3

Significant DEGs			Significant DEGs		
shNR4A2 cells			shNR4A2 cells		
5% CO ₂ Vs 10% CO ₂			5% CO ₂ +LPS Vs 10% CO ₂ + LPS		
Upregulated genes	LOG2FC	p-adj	Upregulated genes	LOG2FC	p-adj
AL158166.1	1.513133682	0.01789332	IGF2	1.886242783	0.00506764
LINC00638	1.289175828	0.01289527	GPR141	1.73612091	0.0046409
MT-ND4	0.901831302	9.21E-06	ANO5	1.608909746	2.61E-06
MTCO1P12	0.876481578	0.04456635	FGF2	1.571824613	0.02591089
MANEAL	0.866500856	0.04456635	SLC16A9	1.536446884	8.76E-06
SERPINB2	0.82893373	0.01789332	LINC00452	1.534786415	0.00312442
MT-ND4L	0.792455979	0.00295093	PI15	1.534361143	0.032785
ZNF74	0.758192403	0.01789332	CD80	1.512017558	0.00411919
ALKBH2	0.757463503	0.01789332	NR5A2	1.500300244	0.04000766
MT-CO1	0.750010367	0.00427708	AL133330.1	1.500081302	0.01510462
Downregulated genes	LOG2FC	p-adj	Downregulated genes	LOG2FC	p-adj
RHBDL3	-1.23736269	0.02645631	KCNK13	-1.816902654	0.01039153
PBX4	-1.230849402	0.04079731	B3GNT7	-1.662201722	1.94E-05
TNF	-1.111081376	0.00186637	TMEM37	-1.514588968	0.00033047
RFX8	-1.056672607	0.04079731	PDK4	-1.462895076	0.02544185
ATP2A1	-1.029901667	0.01289527	SMIM3	-1.448126654	3.66E-05
MIR22HG	-0.917155435	0.0230187	RHBDL3	-1.419928648	0.00428612
CXCL8	-0.869862187	0.01904809	WNT7B	-1.407503797	3.56E-05
REC8	-0.868430727	0.0354228	F2RL3	-1.26111394	0.0021081
A4GALT	-0.840387807	0.04456635	ST7-AS1	-1.243265224	0.04401988
AL034397.3	-0.820840095	9.21E-06	GPRC5C	-1.242618146	0.00013872
Basal			+ LPS		

Figure S3. Top 10 differentially regulated genes in response to buffered hypercapnia in shNR4A2 cells. List of the 10 most differentially expressed genes cells (up (green) and down (red)) in shNR4A2 in hypercapnia in the basal (A) and LPS stimulated state (2.5µg/ml for 2h) (B). Genes are ranked by Log2 fold change. All genes have a *p*-adj<0.05. Data is representative of *n* = 3 individual experiments.

Supplemental
Figure 4

Significant DEGs			Significant DEGs		
shNR4A3 cells			shNR4A3 cells		
5% CO ₂ Vs 10% CO ₂			5% CO ₂ +LPS Vs 10% CO ₂ + LPS		
Upregulated genes	LOG2FC	p-adj	Upregulated genes	LOG2FC	p-adj
RN7SL2	1.93945221	0.011400512	SLAMF1	1.8364275	0.00215068
SLC16A9	1.51550278	0.000224193	SPATA31C1	1.77899834	0.00014948
THNSL1	1.15787347	0.024101935	CRIM1	1.767316	0.03020285
LINC00638	1.09600166	0.005308471	IGKC	1.76586563	0.00023239
SLC46A1	0.99804242	0.014229174	C6orf58	1.64356511	0.00070974
HSPH1	0.9498798	3.26257E-07	EVA1A	1.59751902	6.47E-05
RIOX2	0.87844398	4.30557E-06	HSPA1A	1.5590445	0.01313429
SERPINB2	0.84603628	6.61351E-06	RPA4	1.48803857	0.0010438
TBC1D9	0.83792635	0.003106565	PSTPIP2	1.43967736	0.00963072
HSPA4L	0.80506854	0.000224193	TP63	1.43036218	0.00322725
Downregulated genes	LOG2FC	p-adj	Downregulated genes	LOG2FC	p-adj
C2orf48	-1.2339701	0.003001251	AC116351.2	-1.7062145	0.00023526
ATP2A1	-1.2063019	0.000154311	B3GNT7	-1.5215211	0.03358237
MIR22HG	-1.1571433	0.000734399	F2RL3	-1.4876489	0.00011359
AP001347.1	-1.1266397	0.040724478	AC132872.3	-1.4788048	0.00160728
AC114490.1	-1.1070416	0.041340928	AC132872.4	-1.4588767	6.47E-05
RFX8	-1.092369	0.00102008	C2orf48	-1.4494994	0.00084597
ARTN	-1.0088844	0.00060915	OCLN	-1.3251797	0.04307551
MAFF	-1.0008972	0.010459013	FAM241B	-1.2868822	0.0097268
OSM	-0.952871	0.00533492	ADAMTS15	-1.277873	0.00363948
RAB43	-0.9289324	0.017810107	SLC6A6	-1.2532132	2.52E-10
Basal			+ LPS		

Figure S4. Top 10 differentially regulated genes in response to buffered hypercapnia in shNR4A3 cells. List of the 10 most differentially expressed genes cells (up (green) and down (red)) in shNR4A3 in hypercapnia in the basal (A) and LPS stimulated state (2.5µg/ml for 2h) (B). Genes are ranked by Log2 fold change. All genes have a $p\text{-adj}<0.05$. Data is representative of $n = 3$ individual experiments.

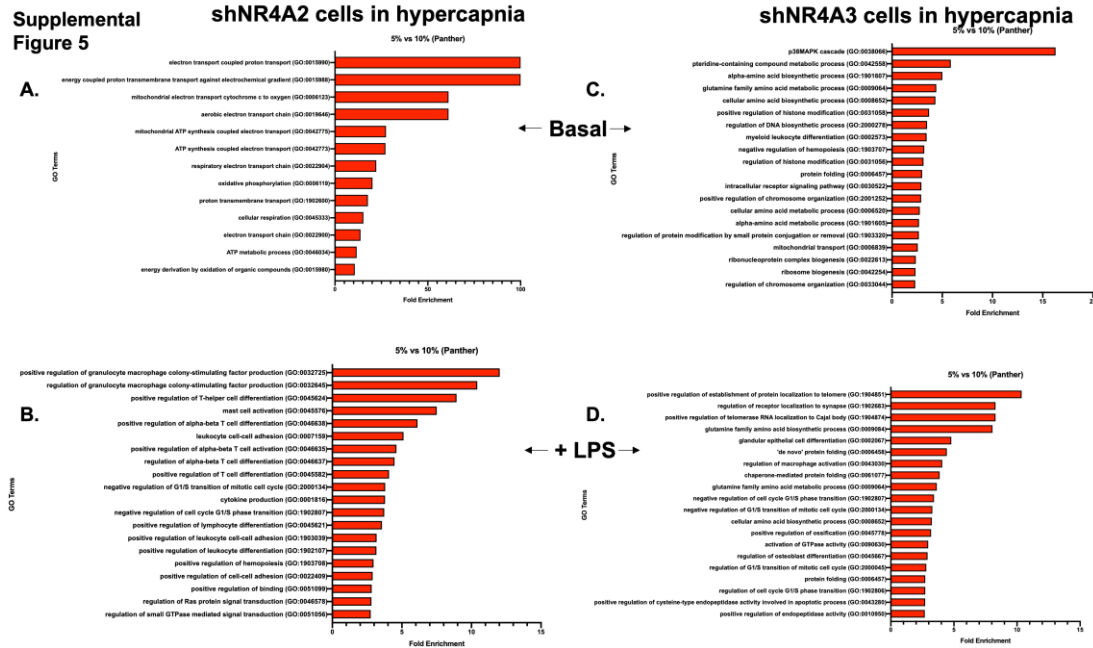
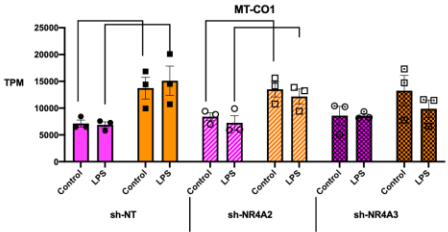


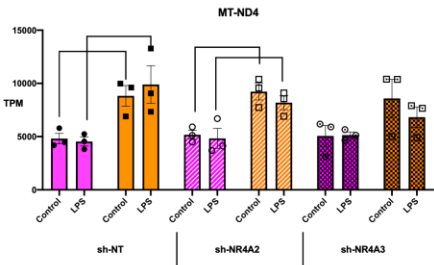
Figure S5. GO analysis of shNR4A2 and shNR4A3 cells exposed to hypercapnia in the basal and LPS-stimulated state. List of the top gene ontology terms associated with significantly differentially expressed genes in hypercapnia in the basal (A, C) and LPS stimulated state (2.5µg/ml for 2h) (B, D) in shNR4A2 and shNR4A3 cells, using a cut off value for significance of $p\text{-adj}<0.05$. Terms are ranked by fold enrichment. Data is representative of $n = 3$ individual experiments.

**Supplemental
Figure 6**

A.



B.



C.

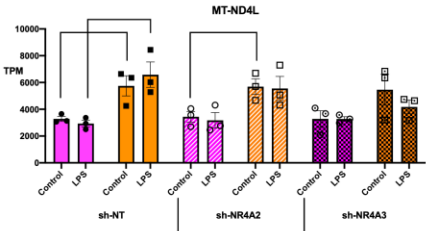


Figure S6. RNA-seq analysis of mitochondrial genes in shNT, shNR4A2 and shNR4A3 cells exposed to hypercapnia in the basal and LPS-stimulated state. Raw TPM values extracted from RNA-seq data for (A) *MT-CO1*, (B) *MT-ND4*, and (C) *MT-ND4L* in shNT, shNR4A2 and shNR4A3 THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5µg/ml for 2h) state in both 5% (pink) and 10% (orange) CO₂ conditions (4h). CO₂ comparisons that had a $p\text{-adj} < 0.05$ are indicated by the brackets. Data shown as mean \pm SEM for $n=3$ independent experiments.

Supplemental Figure 7

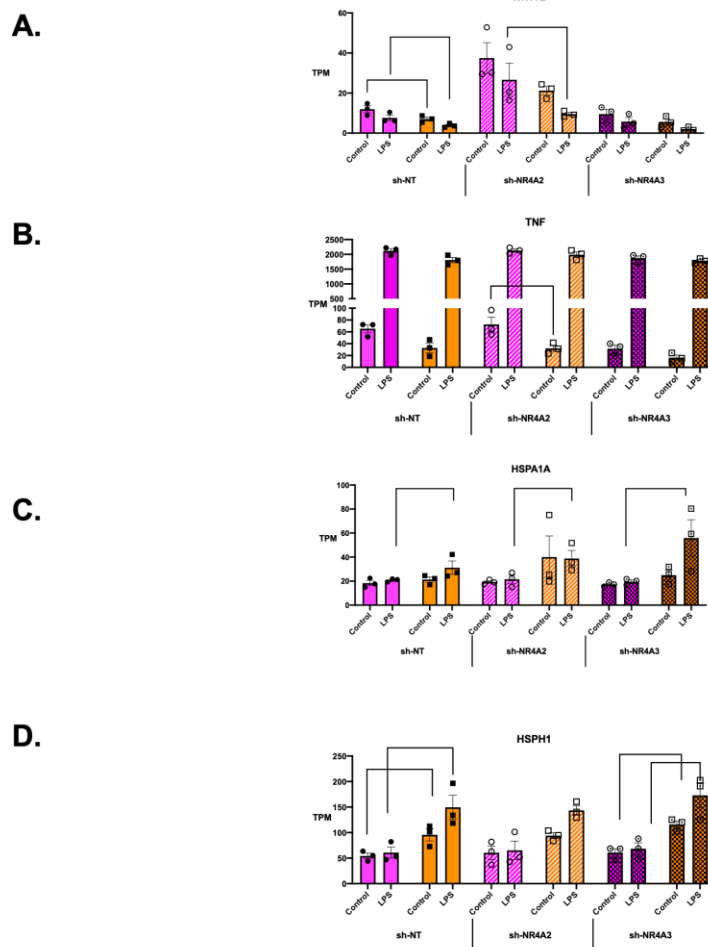


Figure S7. RNA-seq analysis of inflammation and heat-shock associated genes in shNT, shNR4A2 and shNR4A3 cells exposed to hypercapnia in the basal and LPS-stimulated state. Raw TPM values extracted from RNA-seq data for (A) *WNT7B*, (B) *TNF*, (C) *HSPA1A* and (D) *HSPH1* in shNT, shNR4A2 and shNR4A3 THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5 μ g/ml for 2h) state in both 5% (pink) and 10% (orange) CO₂ conditions (4h). CO₂ comparisons that had a $p\text{-adj} < 0.05$ are indicated by the brackets. Data shown as mean \pm SEM for $n=3$ independent experiments.

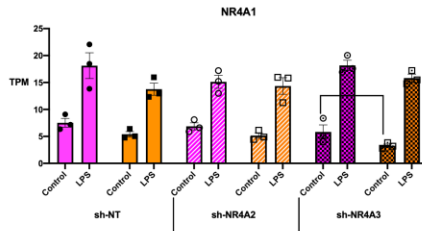
Supplemental
Figure 8

	GO Term	shNT	shNR4A2	shNR4A3	
Basal	electron transport coupled proton transport (GO:0015990)	✓	✓		Lost in shNR4A3 cells
	ATP synthesis coupled electron transport (GO:0042773)	✓	✓		Lost in shNR4A3 cells
	cellular respiration (GO:0045333)	✓	✓		Lost in shNR4A3 cells
	alpha-amino acid biosynthetic process (GO:1901607)	✓		✓	Lost in shNR4A2 cells
	protein folding (GO:0006457)*	✓		✓	Lost in shNR4A2 cells
	ribosome biogenesis (GO:0042254)	✓		✓	Lost in shNR4A2 cells
	ribonucleoprotein complex biogenesis (GO:0022613)	✓		✓	Lost in shNR4A2 cells
+ LPS					
	positive regulation of establishment of protein localization to telomere (GO:1904851)	✓		✓	Lost in shNR4A2 cell
	positive regulation of telomerase RNA localization to Cajal body (GO:1904874)	✓		✓	Lost in shNR4A2 cells
	'de novo' protein folding (GO:0006458)	✓		✓	Lost in shNR4A2 cells
	negative regulation of G1/S transition of mitotic cell cycle (GO:2000134)		✓	✓	Gained in shNR4A2/A3 cells
	negative regulation of cell cycle G1/S phase transition (GO:1902807)		✓	✓	Gained in shNR4A2/A3 cells

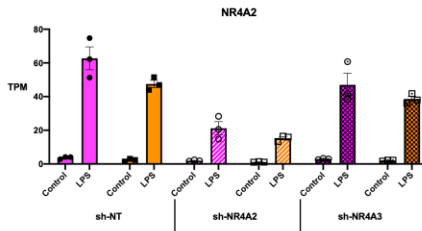
Figure S8. Comparison of common CO₂-responsive GO terms between shNT, shNR4A2 and shNR4A3 cells exposed to hypercapnia in the basal and LPS-stimulated state. Analysis reveals enriched GO terms that are lost in shNR4A3 cells (green text), lost in shNR4A2 cells (orange text) and gained in shNR4A2/3 cells (black text).

Supplemental
Figure 9

A.



B.



C.

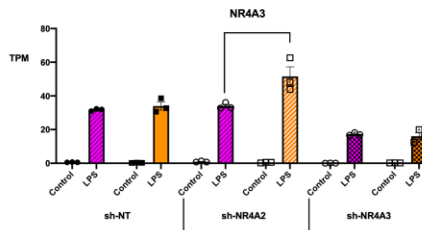


Figure S9. RNA-seq analysis of orphan nuclear receptor family 4A genes in shNT, shNR4A2 and shNR4A3 cells exposed to hypercapnia in the basal and LPS-stimulated state. Raw TPM values extracted from RNA-seq data for (A) *NR4A1*, (B) *NR4A2*, and (C) *NR4A3* in shNT, shNR4A2 and shNR4A3 THP-1 cells in the basal (unstimulated) and LPS stimulated (2.5µg/ml for 2h) state in both 5% (pink) and 10% (orange) CO₂ conditions (4h). CO₂ comparisons that

had a $p\text{-adj}<0.05$ are indicated by the brackets. Data shown as mean \pm SEM for $n=3$ independent experiments.

**Supplemental
Figure 10**

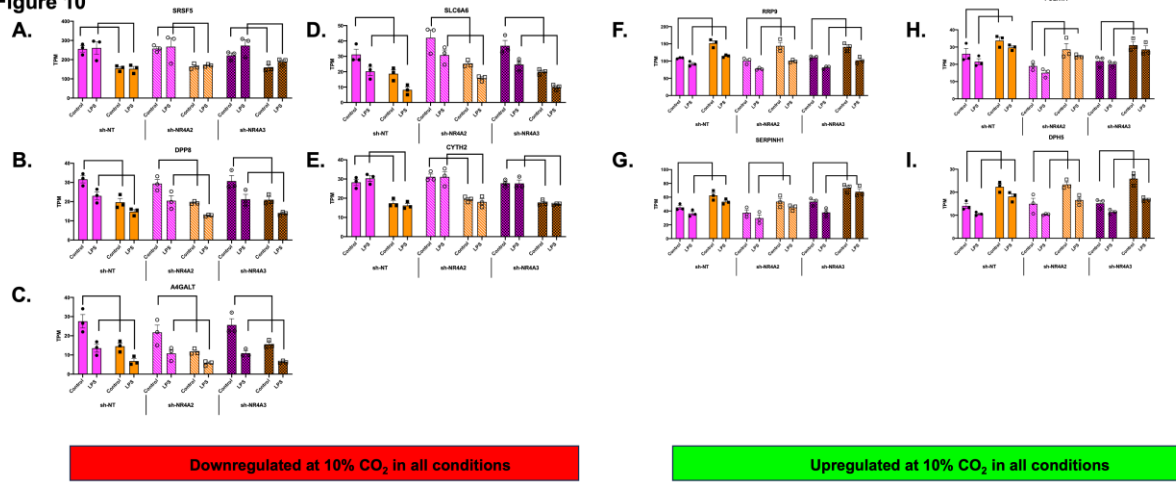


Figure S10. Hypercapnia consistently regulates genes in shNT, shNR4A2 and shNR4A3 THP-1 monocytes in the presence and absence of LPS. Raw TPM values extracted from RNA-seq data for (A) *SRSF5*, (B) *DPP8*, (C) *A4GALT*, (D) *SLC6A6*, (E) *CYTH2* (F) *RRP9*, (G) *SERPINH1*, (H) *POLR1A* and (I) *DPH5* in shNT/shNR4A2/shNR4A3 THP-1 cells in the basal and LPS stimulated (2.5 μ g/ml for 2h) state in both 5% and 10% CO₂ conditions (4h). All CO₂ comparisons had a $p\text{-adj}<0.05$ as indicated by the brackets. Data shown as mean \pm SEM for $n=3$ independent experiments.