Supplementary Material Metabolic reprogramming of mammary epithelial cells during TGF-β-induced epithelial-to-mesenchymal transition

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Supplementary Figures

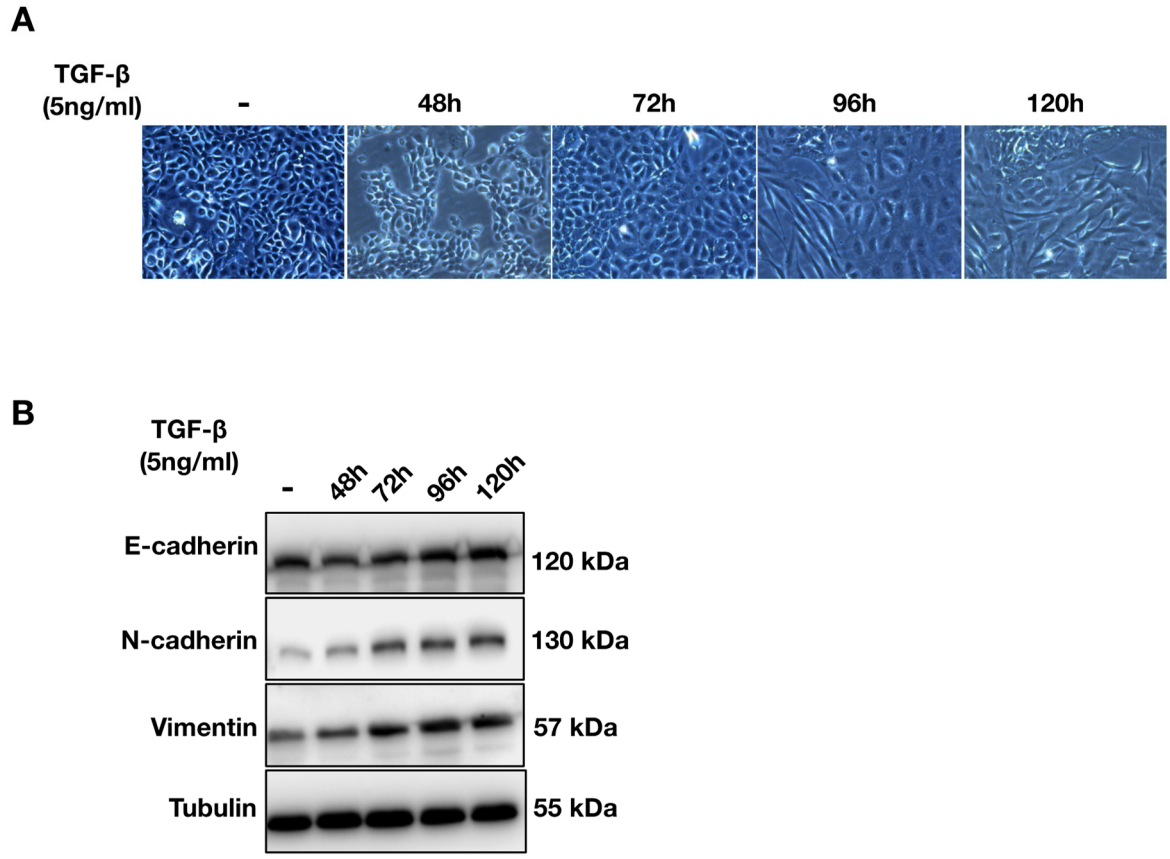

Figure S1. TGF-β induces EMT of MCF10A-Ras cells. A. TGF-β induced a morphological change in MCF10A-Ras cells from an epithelial to a spindle-shaped fibroblast-like mesenchymal phenotype. B. TGF-β induced an increase in expression of N-cadherin and Vimentin in MCF10A-Ras cells for 48 h, 72 h, 96 h and 120 h.

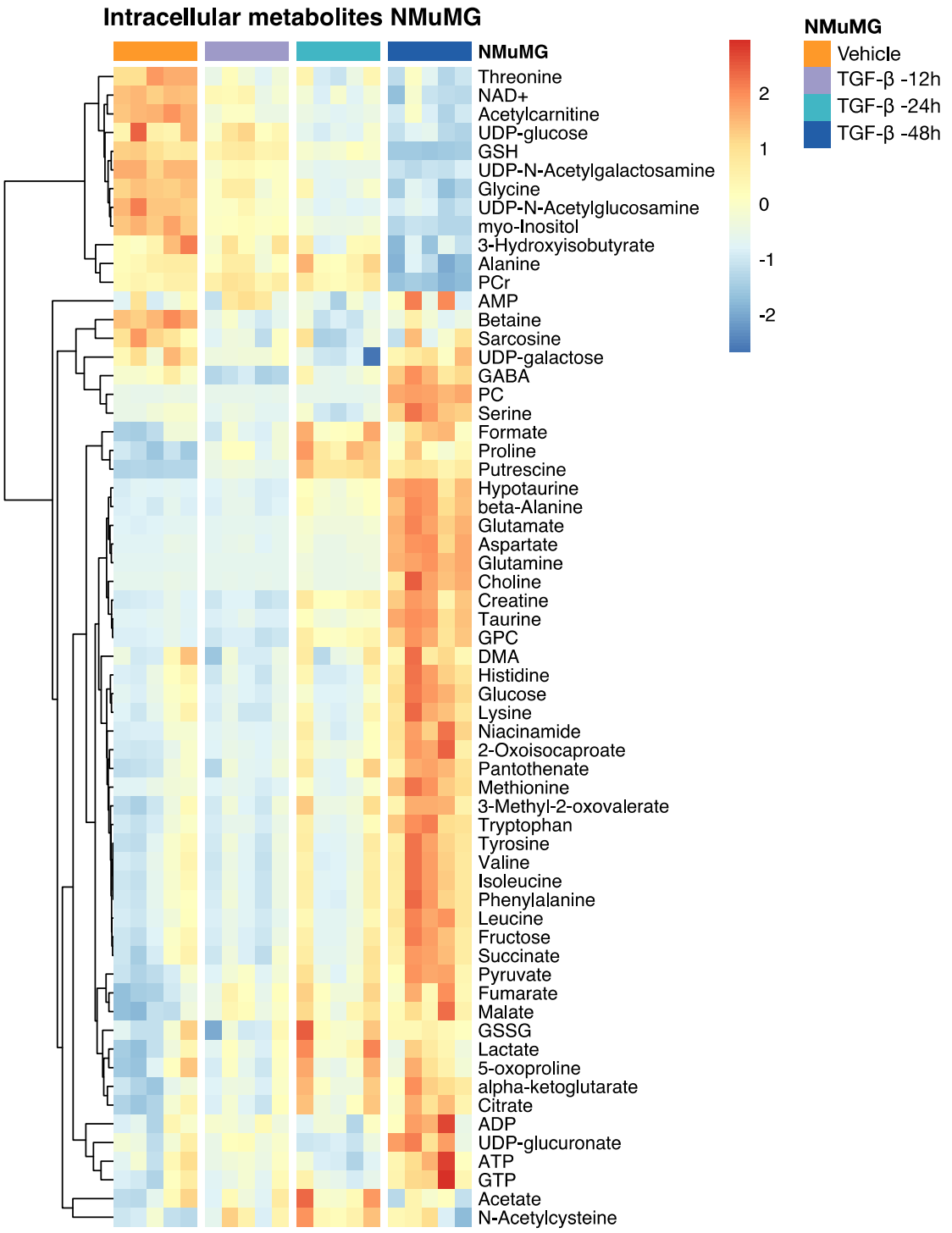

Figure S2. TGF-β induced metabolic kinetic changes in intracellular metabolites in NMuMG cells. The treatment-induced changes in intracellular metabolites are compared to vehicle control-treated cells. Results from n = 5 independent experiments are shown.

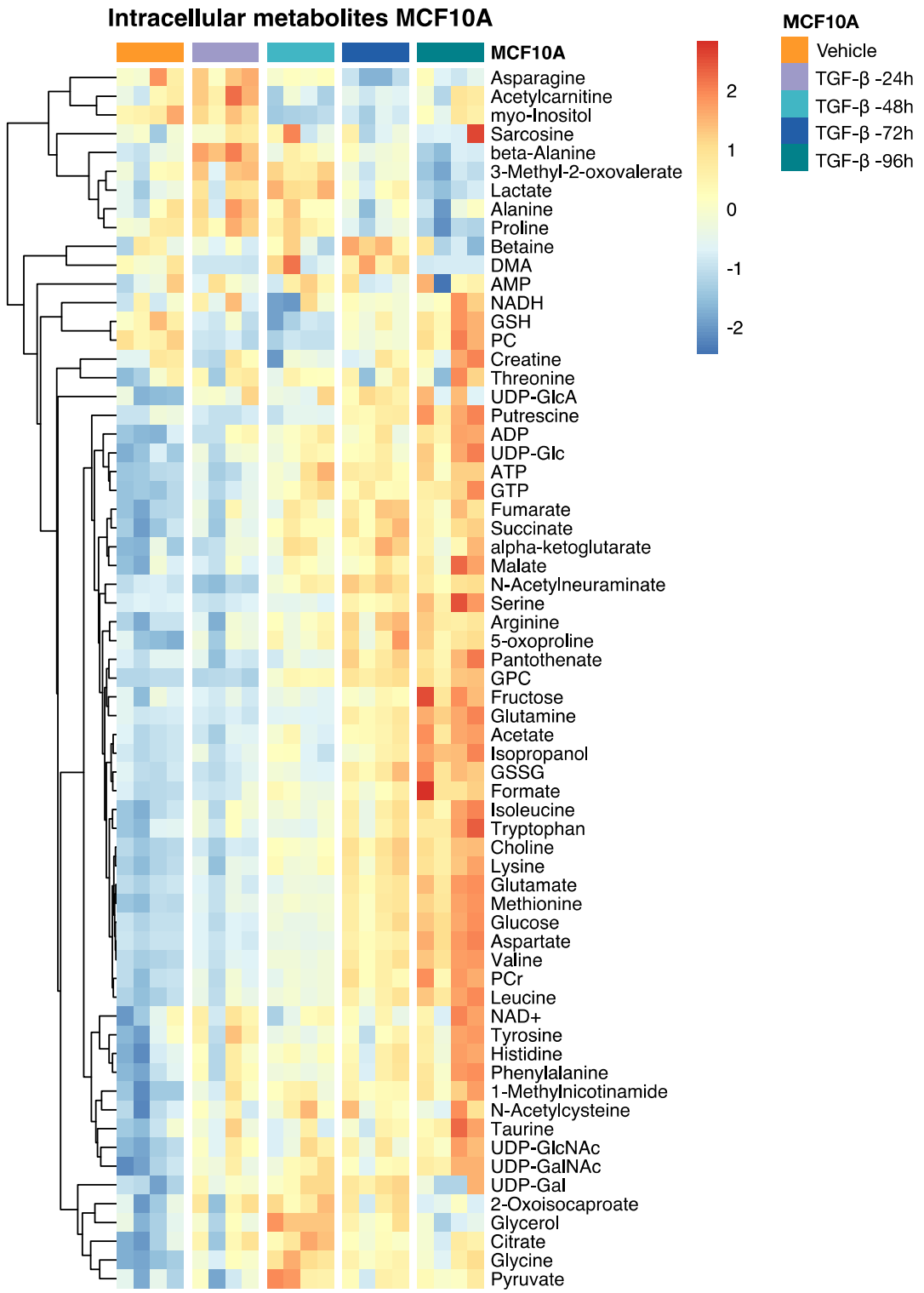

Figure S3. TGF-β induced metabolic kinetic changes in intracellular metabolites in MCF10A-Ras cells.

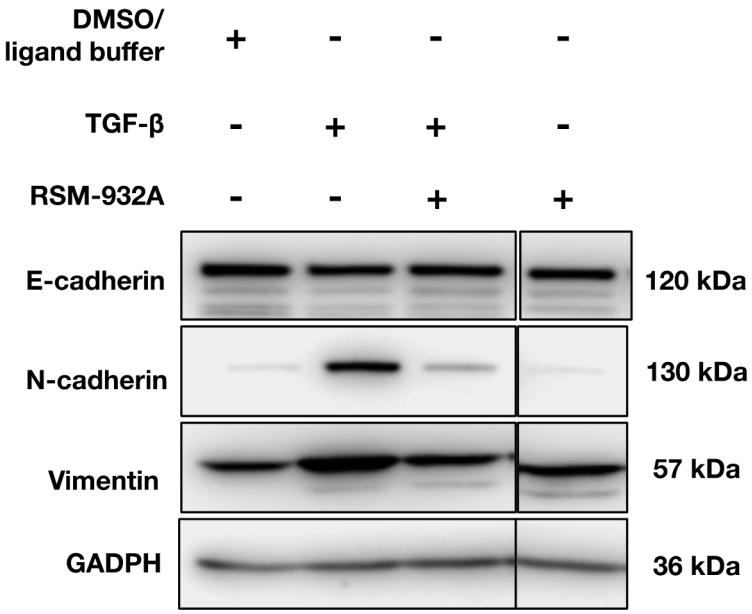

Figure S4. Effect of RSM-932A on TGF-β induced an increase of expression of N-cadherin and Vimentin. RSM-932A was used at 10μM and TGF-β at 5ng/mL (All samples were run on the same gel).

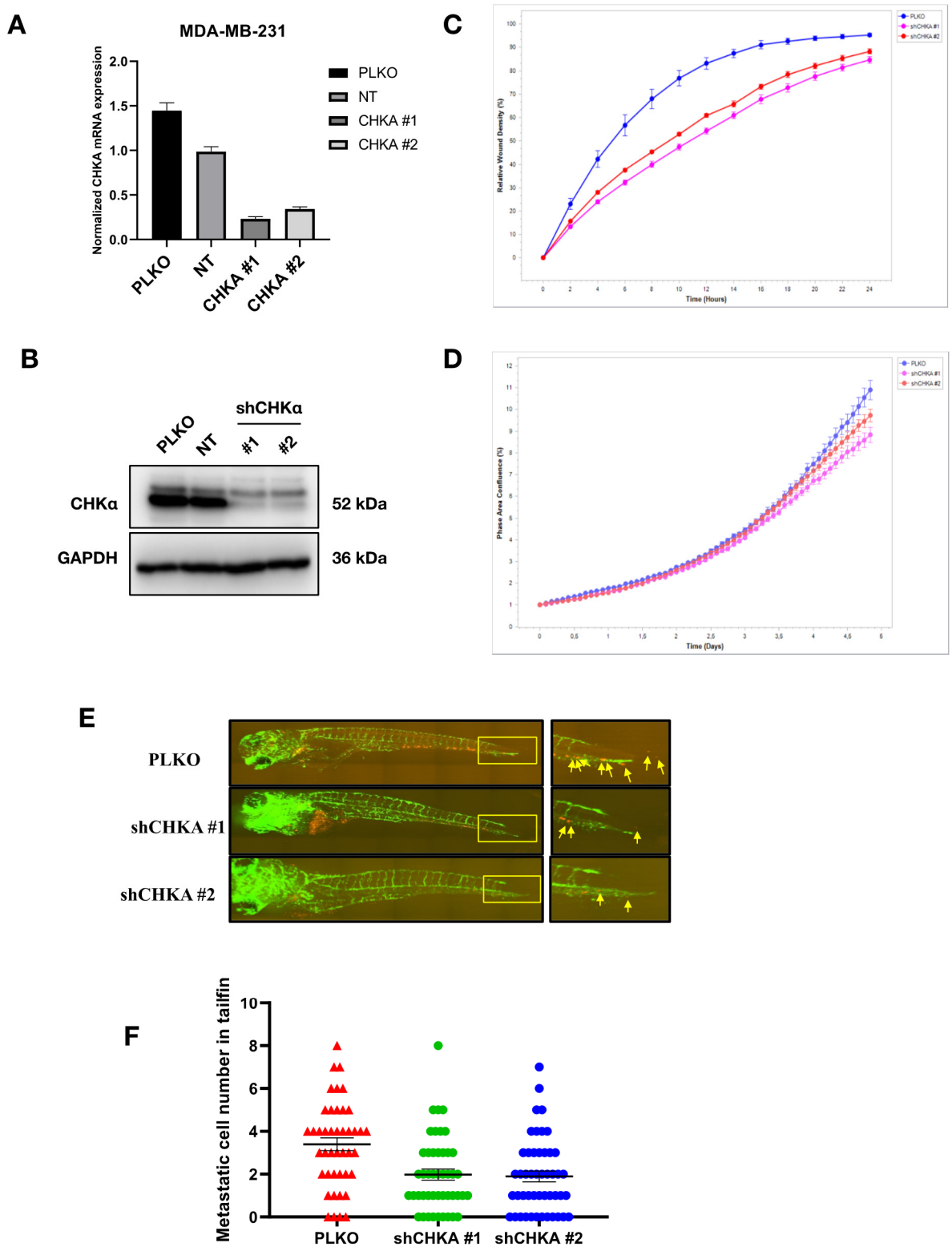

Figure S5. Depletion of CHKα in MDA-MB-231 cancer cells inhibited migration and extravasation. A. shRNA targeting *CHKα* inhibit its mRNA expression. PLKO, empty vector control; NT, non-targeting control. B. shRNA targeting *CHKα* inhibit its encoded protein expression. PLKO, empty vector control; NT, non-targeting control. C. Effect of shRNA-mediated *CHKα* knockdown on MDA-MB-231 cell proliferation was not significantly

affected. The proliferation was measured by analyzing the cell confluency using Incucyte standard module. PLKO-infected cells were used as control. D. Inhibitory effect of shRNA-mediated *CHKα* depletion on MDE-MB-231 cell migration. Migration was measured using Incucyte scratch wound analysis module. PLKO-infected cells were used as control. E. shRNA-mediated depletion of *CHKα* in MDA-MD-231 cells inhibited extravasation. Control (PLKO-infected) cells and shRNA-mediated depleted CHKα MDA-MB-231 cells were injected into the blood circulation of 48 hpf zebrafish. Representative images of the zebrafish at 6 days post-injection are shown. F. Quantification of the results shown in (E). The number of cells that extravasated in the posterior tail fin was calculated at 6 dpi.

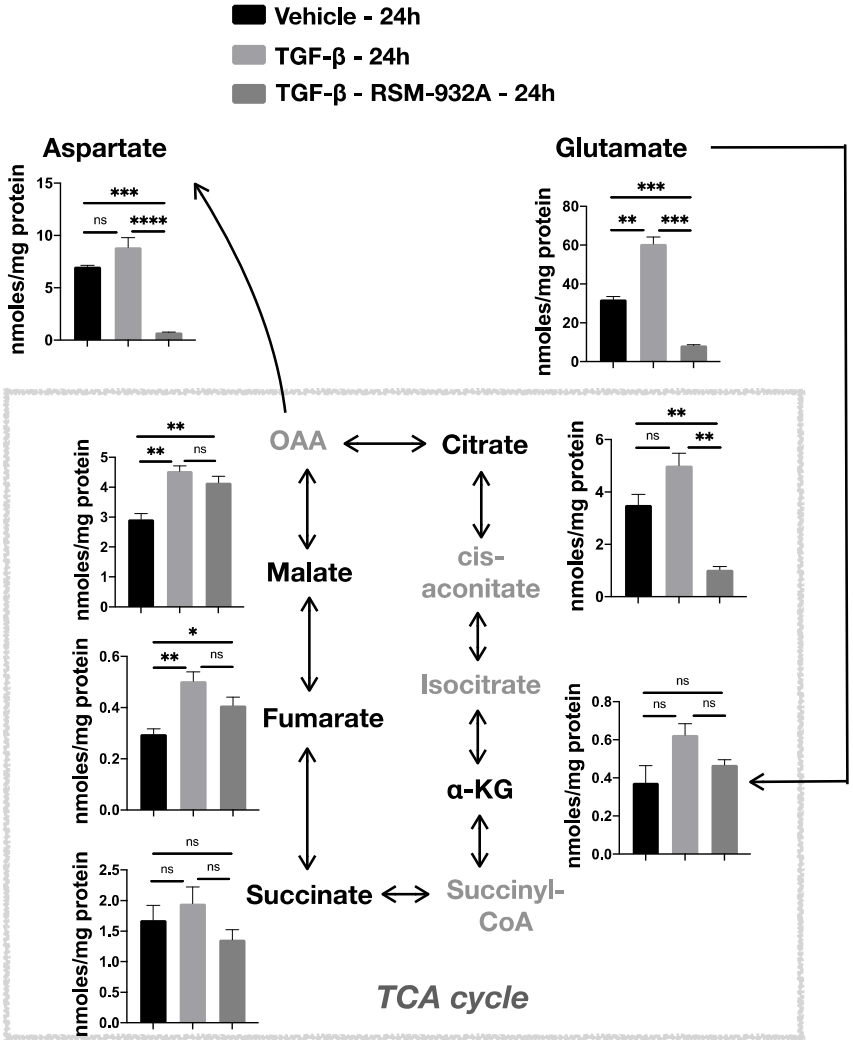

Figure S6. Effect of TGF-β in the absence and presence of RSM-932A on TCA cycle intermediates in MCF10A-Ras cells. TGF-β induced an increase in TCA cycle intermediates in MCF10A-Ras, and this was decreased by

RSM-932A (10μM). Results are shown as mean ± s.d; Results are shown as mean ± s.d; *p< 0.05; **p< 0.01; ***p<0.001; ****p< 0.0001; ns, non-significant. αKG, α-ketogluturate; TCA, Tricarboxylic acid cycle.

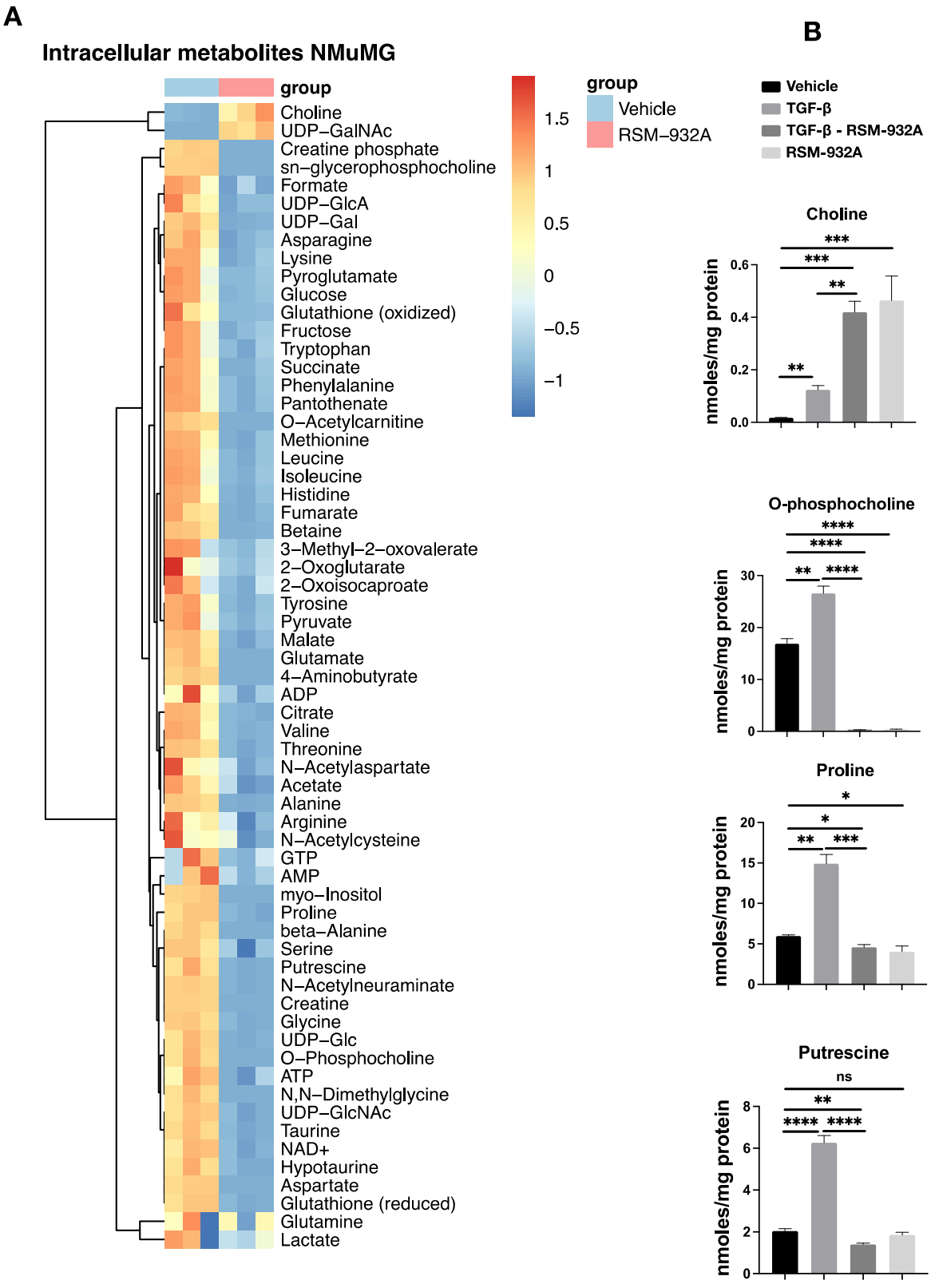

Figure S7. Analysis of RSM-932A and/or TGF-β treatment in NMuMG cells on choline metabolism. A. Analysis of RSM-932A (10μM) -induced intracellular metabolic changes in NMuMG after 24 h treatment. B. Analysis of changes in choline, O-phosphocholine, proline and putrescine levels in response to RSM-932A (10μM) and/or TGF-β (5ng/mL) treatment in NMuMG cells. Results of three biological replicates are shown as mean ± s.d; *p<0.05; **p< 0.01; ****p< 0.0001; ns, non-significant.

Supplementary Table S1~~:~~ . List of RT-qPCR primers.

Gene		Sequence (5' to 3')
CPT	Forward	ACCGGAACAGGTTCAAGTTCTTT
	Reverse	TGAAACAGCAGCAGCAAGAGA
PCYT1A	Forward	TGACCCCCGGAGCAGTAAAT
	Reverse	CTTTTAATCGGTCATGGTCCCGT
PCYT1B	Forward	GAGACGAAAGAAAGTTGGGGC
	Reverse	GTCAGGGTCTTGCGTGAGAA
CHKA	Forward	GCCTACCTGTGGTGTAAGGAA
	Reverse	ACCACCCCTGATGACACTGA
SLC44A1	Forward	AAGGGAAGGAAAACGCTTGTG
	Reverse	GCTCTGCTCCAGGCTTTCAA
SLC44A4	Forward	AGACTGAGCCATGGGGAGAA
	Reverse	GCAGCCCCTGTTTTTGATGG
SLC5A7	Forward	TGCTGTCTTTTGCAACCCAG
	Reverse	TCCCAAGTCAAGTCGTCTGC
CHAT	Forward	AAATGGCGTCCAACGAGGAT
	Reverse	CCCGGTTGGTGGAGTCTTTT
PEMT	Forward	AGGCTTCGGCAATATCGACT
	Reverse	CCATCTCGCTACCACATTCCA
ALDOA	Forward	GGCAGTGGGAGGCAATATCT
	Reverse	GCAGTGCTTTCCGGTCTTTAG
PGI	Forward	TACGAACACGGCCAAAGTGA
	Reverse	AGTCCAATGGCTGACCACAG
GLUT1	Forward	GGGTCTTAAGTGCGTCAGGG
	Reverse	TCACCTTCTTGCTGCTGGG
HK2	Forward	TCGGTTTCTCTATTTGGCCCC
	Reverse	GGTCAACCTTCTGCACTTGG
PFKFB3	Forward	AGAGGTCAGAGAACATGAAGAGC
	Reverse	TTGGCCTCGAGAAGATGAGC
PHGDH	Forward	ATGTCATCAGCTGTCCCCAC
	Reverse	GCGTTCACAACGCCTGTTAG
GAPDH	Forward	GGCAAATTCAACGGCACAGT
	Reverse	TAGGGCCTCTCTTGCTCAGT
LDHA	Forward	GATGGCAACCCTCAAGGACC
	Reverse	TCATCCGCCAAGTCCTTCATT
LDHB	Forward	GGTGGACAGGGAATGTACGG
	Reverse	GAGCGACCTCATCGTCCTTC
LDHC	Forward	CGGAGTCAGCAGTAAGGCTC
	Reverse	GCCATGCCCACATTTCCAAC
MPC1	Forward	GACTCCCACGGTAGATGCAC
	Reverse	ACAGAGGGCGAAAGTCATCC

Supplementary Table S1~~:~~ . List of RT-qPCR primers (*continued*).

MPC2	Forward	TTGAGGCCGCTTTACAACCA
	Reverse	CAGCACACACCAATCCCCAT
PDHA1	Forward	AGATGCTTGCCGCTGTATCC
	Reverse	GCCGATGAAGGTCACATTTCTTAAT
PKLR	Forward	AAAATTGGCCCAGAGGGACT
	Reverse	CCGAAGCGCAGATCCAAAAG
PKM1	Forward	AGCACCTGATAGCTCGGGA
	Reverse	GAGGTCTGTGGAGTGACTGG
LDHAL6B	Forward	GAGAAATGGGGCGATGTCCA
	Reverse	GCCAATGGCCCAGGAAGTAT
PDK1	Forward	GCAGACAGCTTACCTGAATCCT
	Reverse	ACTGACCCGGTCTATAAAGTAATCC
PDK2	Forward	GGAGGTGCTAACAGGGGAAC
	Reverse	TGCTGGATCCTCATGGTAAGTT
PDK3	Forward	TAGGGAGTATCGACCCCACC
	Reverse	TTTGGCGCTTTGGCATTGAA
PDK4	Forward	GCTGCTGGACTTTGGTTCAGA
	Reverse	TTCAGGATATTGGCCAGGCG
ACO2	Forward	CATGTGGCCTCTGTCCTGTG
	Reverse	GTTCAACCGTTTACGGACAATGTTA
MDH1	Forward	ATGATGGGTGTTCTGGACGG
	Reverse	GTCCGTTGCAATGACATCCTG
MDH2	Forward	AGAGTGGAGGTCGTTGGAGT
	Reverse	CATTGTTCTGGGCCGAAGTG
CS	Forward	TGGGAGCCAAGAACTCATCC
	Reverse	TGTCTTCCCATGTTGCTGCT
FH	Forward	ATGCCAGGAAAGGTGAACCC
	Reverse	ACAGCAACGTGATTCCCCAT
IDH1	Forward	GCCAGTCGCTGTTACCGTAT
	Reverse	TCAATAACCCTCTTCACTCTGGAC
IDH2	Forward	GACAGTCACCCGCCATTACC
	Reverse	AGCGTCTGTGCAAACCTGATA
OGDH	Forward	CCCTGGGGATTTTGGATGCT
	Reverse	TGTAGGCCATAGAACCCTCCT
SUCLA2	Forward	ACGGGTCGGACTCAGAAATG
	Reverse	ACATCCTTTGATCCTGGGCG
SUCLG1	Forward	TACGGAAGGTATTCCGCAGC
	Reverse	TTCTCCAGGGTTGATGACGC
GDH1	Forward	AGGGTTTTATTGGTCCTGGCA
	Reverse	ATGGTGCTGGCATAGGTGTC
GLS2	Forward	CAGCACTCGGATCATGACG
	Reverse	CAGTCCAGTGGCCTTCAGAG
GOT1	Forward	CACCAACCTGGGAGAACCAT

Supplementary Table S1~~:~~ . List of RT-qPCR primers (*continued*).

	Reverse	CTCCGCATCCCAGTAGCAAT
GOT2	Forward	GGGGCCAGTTTTCTGCAAAG
	Reverse	AGATGGGCGTGTGATTTCCC
SLC1A5	Forward	TCGTCTTTGGTGTGGCTCTG
	Reverse	AACGGGTGCGTACCACATAA
SLC7A5	Forward	CTGGTCTTCGCCACCTACTT
	Reverse	GCCTTTACGCTGTAGCAGTTC
GLS1	Forward	CCGCGGGCGACAATAAAATA
	Reverse	GGGATCAGATGTTCGCAATCC
6PGDH	Forward	CTCCTCGACTCTGCTTCGTC
	Reverse	TGAAAGCACAGACCACAAATCC
6PGL	Forward	ACTCAAGATACAAGGGCCGC
	Reverse	CGTTGAAGCAGATCCTTGCG
G6PD2	Forward	GGTCGTGGGGGCTATTTTGA
	Reverse	TCTGAATCTGTGGTAGCAGGC
G6PDX	Forward	GAACGGTGGGACGGGGTA
	Reverse	CTGCCACGTCTCGGAACTG
RPE	Forward	CCTGGATGCACCCTGCTC
	Reverse	CTAGCTGCTTTCGGAGGCTT
RPI	Forward	CCCAGAGATTGACCTTGCCA
	Reverse	ACTTGCATAACCAGCCACGA
TALDO	Forward	CAACGAAGACCAAATGGCCG
	Reverse	CATTCGTTCCGTGAGCATCC
TKT	Forward	TGTCCCGAAACAAGCCTTCA
	Reverse	ACTCGGTAGCTGGCTTTGTC
GNPNAT1	Forward	CCGCTCCAGTGCGACTTTA
	Reverse	GGATGCGTTGGAGAAATGGC
OGT	Forward	AGCTATCGAACTGCAACCCC
	Reverse	TTCAGCAACACTGCCCTTCT
PGM3	Forward	TTATTCAACCAGGTCGCGGA
	Reverse	AGAGGGCCGTACAAAAGCTC
GFPT1	Forward	TCTGATGCAAGTGCCGTGAT
	Reverse	GATGGTCTCCTGCAGTTCGT
GFPT2	Forward	ATCCTAGCCGGAGAGCTGAA
	Reverse	GTATGATTGGGCGACCCTGG
UAP1	Forward	CATCCCCCGCAGTGCTAC
	Reverse	AGGCCCTCTCCAGCATAAGA
HAS2	Forward	CGTGGATTATGTACAGGTGTGTG
	Reverse	TCCTCCAACACCTCCAACCA
B3GNT9	Forward	CGTCACCCTGTCCAGACTTTG
	Reverse	CATCCACTCTGTCGACCTCC
BCAT1-1	Forward	ACCCGCCACGCCAAAC
	Reverse	TTACTGCAGTCCTGGCGG

Supplementary Table S1~~:~~ . List of RT-qPCR primers (*continued*).

BCAT1-2	Forward	GATACAAATCACTGGAGGGG
	Reverse	GGAAGATGGAGAGCTGGG
BCAT2	Forward	TATGGACCCACTGTGGCTGT
	Reverse	AGCTCCAGTACTCCGTCTTCA
BHMT	Forward	GAGGCAGTTCGTCAGCTTCA
	Reverse	TCGGGGTTCCAATCCAAAGG
MTHFD1	Forward	CTGACCCAGACAGACAAGGC
	Reverse	ATGCCTAGCCTCCGTAACCT
MTHFD1L	Forward	TTTGGTGGGAACGATGAGCA
	Reverse	CCAGCCTTGACCGCATCTT
MTHFR	Forward	GAGCAGATCGGGATGAACCA
	Reverse	GATTCCTGCTGATAGAGGGTGG
MTR	Forward	AGGAATACTTGCTGGCTGGAG
	Reverse	GTTCATCCGGTAGGCCAAGT
SHMT1	Forward	CCACGCTCCTAATACAAGGCA
	Reverse	TGCTGTAAACCTCGGCATCA
SHMT2	Forward	ACCCCGGTACTACACCGATA
	Reverse	AGCTGACCACATCTCCGAGT
DHFR	Forward	AGGATTTTATCCCCGCTGCC
	Reverse	GTACTTGAACTCGTTCCTGAGC
CA9	Forward	AAGACAGGATGGAGGAGTCCC
	Reverse	TGGCCACCCCCTTTTTCATC
CA12	Forward	TGGATGATCCTACCCCCAGA
	Reverse	GGATGATACCTTGTCGGAAGGAG
ODC	Forward	TCCCTGTCACATCGAGAACC
	Reverse	CCGCAACATAGAACGCATCC
DDAH1	Forward	TGCGTGTTCGTGGAGGAC
	Reverse	ATGTCAACCTCCTTCCTGCG
MICAL2	Forward	CCCCCAGTACGTTTCAGCC
	Reverse	TTGCCAAGAAAATCAGTCACCTT
PLPP3	Forward	CCTGGCGATCATTACAGGGG
	Reverse	TGCCACATACGGGTTCTGAG
PLPP4	Forward	ACCTACCCGCCTCATGTTTG
	Reverse	GAGCCAAGGACACTGCTAAGA
PLCB4	Forward	AAAGAGTTCCGAACCCGCAT
	Reverse	TCAGGCAGGATCACCTTTCG
SULF1	Forward	ATTAAGAGAAGGAAGGCTGCCC
	Reverse	CTCAGCACCTGAAAATACTGCG
GPX8	Forward	AAGGCAAAGCTTCCCTGGTT
	Reverse	GATAGGGCCCGAACTCCTTG
SPHK1	Forward	TCCTGGGCAACACCGATAAG
	Reverse	CGAGGGCATTCTACTGGTTCC
AOX1	Forward	AGAACGGAAGCTGGAGTGTG

Supplementary Table S1~~:~~ . List of RT-qPCR primers (*continued*).

	Reverse	CCGCCTATGTGTATTTCACCTTC
CYP1B1	Forward	TGTGCCTGCCACTATTACGG
	Reverse	CTGAACATCCGGGTATCTGGT

Supplementary Table S2: Changes in extracellular metabolites in the absence or presence of TGF-β or RSM-932A, and TGF-β-induced in intracellular metabolites in MCF10A-Ras cells. This Table is provided in an excel sheet format.

Supplementary Table S3: TGF-β-induced kinetic effects on gene expression encoding metabolic enzymes in NMuMG cells. This Table is provided in an excel sheet format.