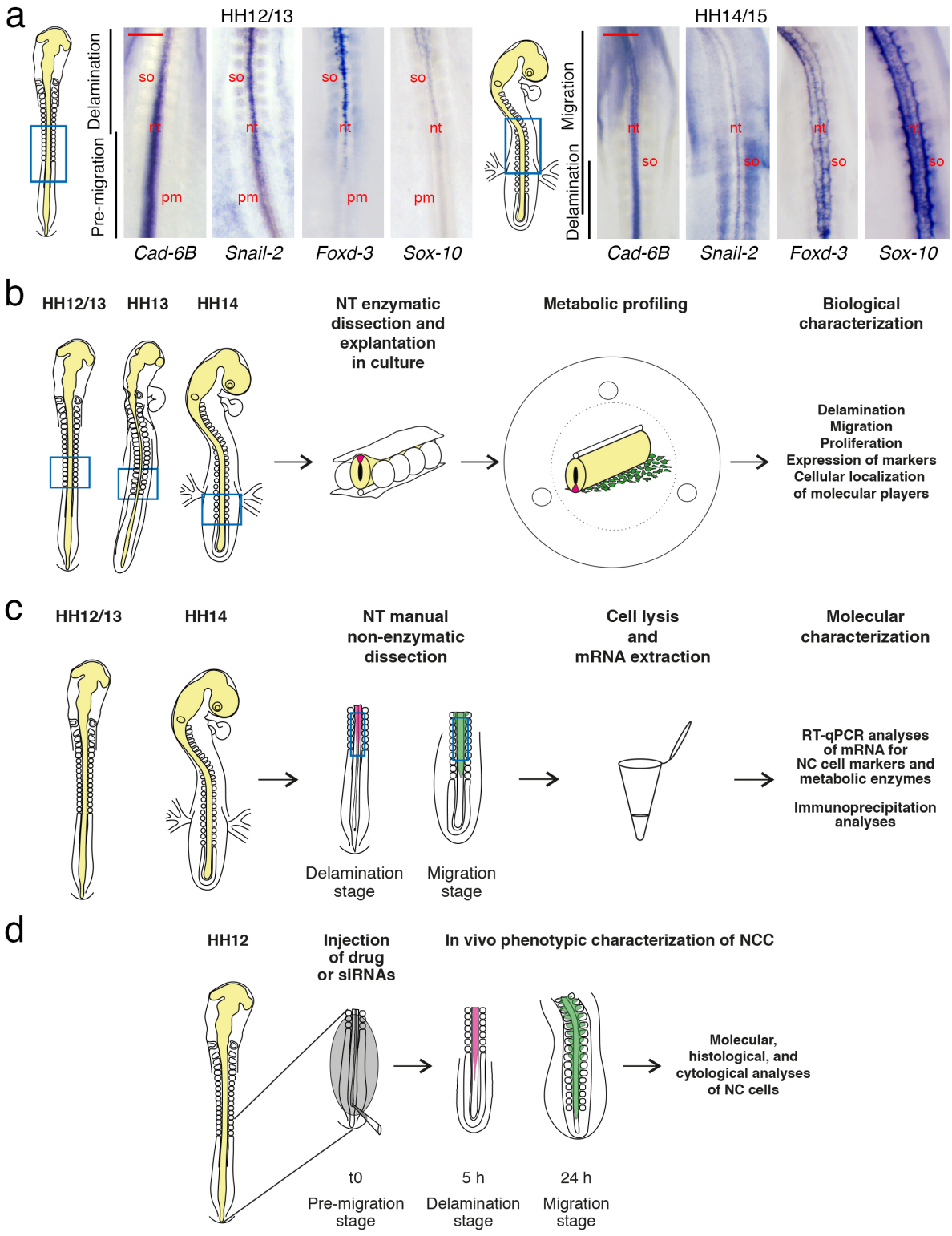
Supplementary Data to the Article entitled
"Non-canonical nuclear function of glutaminase cooperates with Wnt signaling to drive EMT during neural crest development"
by Nioosha Nekooie Marnany et al.

These supplementary data contains 3 figures and 1 Table.


a
HH12/13
HH14/15
Delamination
Pre-migration
Migration
Delamination
so
nt
pm
Cad-6B
Snail-2
Foxd-3
Sox-10
b
HH12/13
HH13
HH14
NT enzymatic dissection and explantation in culture
Metabolic profiling
Biological characterization
Delamination
Migration
Proliferation
Expression of markers
Cellular localization
of molecular players
c
HH12/13
HH14
NT manual non-enzymatic dissection
Cell lysis and mRNA extraction
Molecular characterization
Delamination stage
Migration stage
RT-qPCR analyses of mRNA for NC cell markers and metabolic enzymes
Immunoprecipitation analyses
d
HH12
Injection of drug or siRNAs
In vivo phenotypic characterization of NCC
t0
Pre-migration stage
5 h
Delamination stage
24 h
Migration stage
Molecular, histological, and cytological analyses of NC cells

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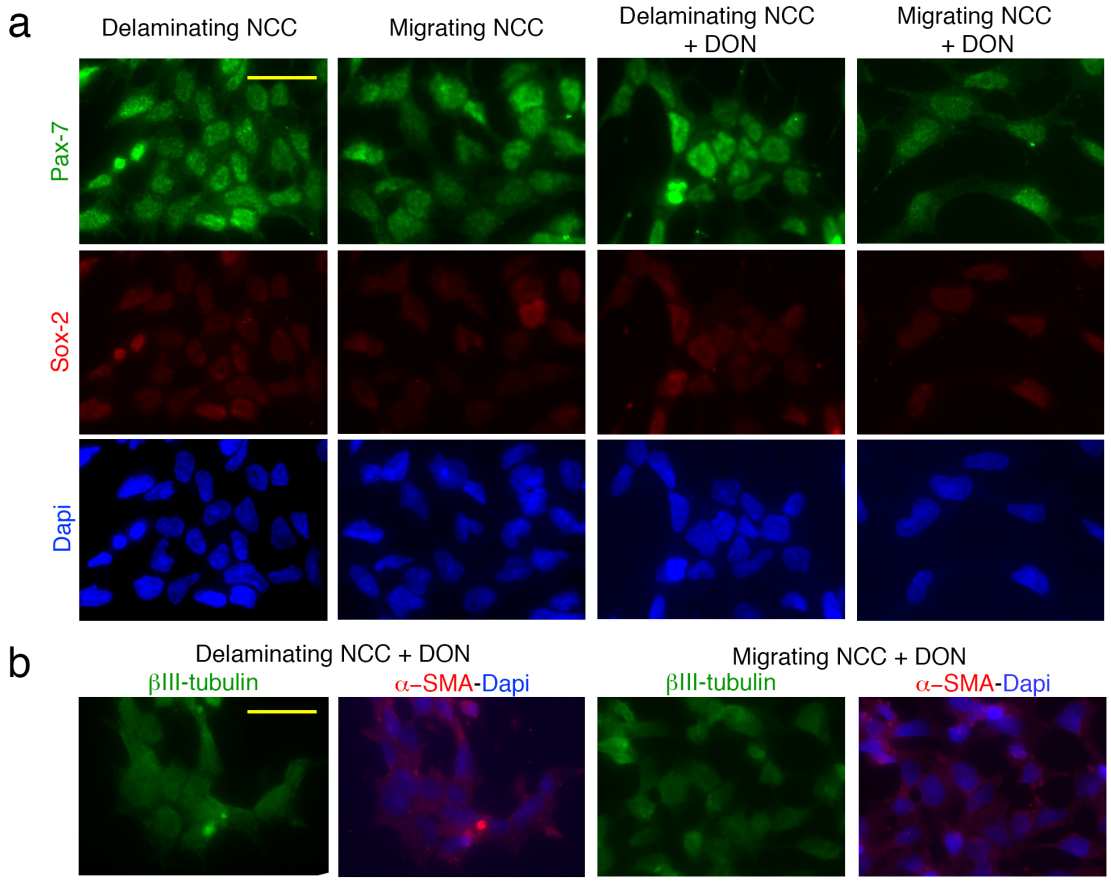
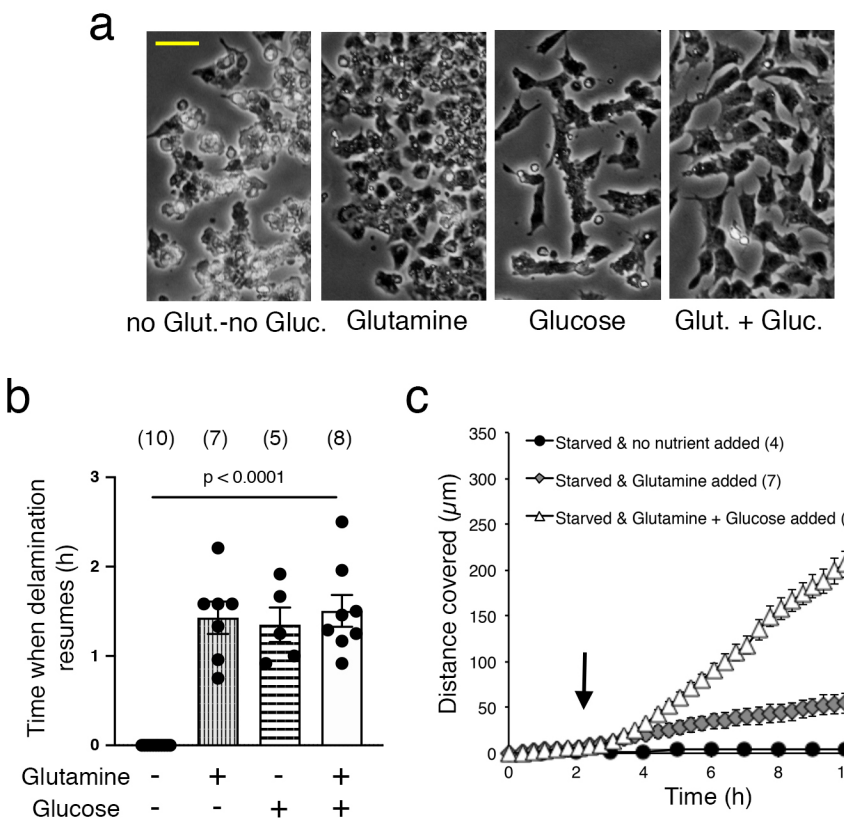
Supplementary Figure 1: Strategies used in the study for investigating metabolic features of trunk NCCs at delamination and migration

a Expression patterns of markers for NCC delamination and migration at stages HH12/13 (left) and HH14/15 (right). Whole mount views of the thoracic region at mid-trunk level (corresponding to somite pairs 15-22) of quail embryos hybridized with probes for *Cadherin-6B*, *Snail-2*, *Foxd-3*, and *Sox-10*. The diagrams depict the gross morphology of the embryos at the respective stages, and the regions shown in the in situ hybridization images are delineated with blue rectangles. In situ hybridizations for each probe and at each developmental stage were done in triplicate. nt, neural tube; pm, presomitic paraxial mesoderm; so, somite. Bar = 500 µm. **b** Schematic representation of the procedure for analyzing *in vitro* the metabolic profile of individual NT explants at stages HH12/13, HH13 and HH14 and the corresponding molecular and cellular properties of NCCs (see Methods for details of the procedure). The embryonic region extirpated for NT dissection is delineated with a blue rectangle. **c** Schematic representation of the procedure to characterize the molecular features of NTs and associated NCCs collected from embryos at stages HH12/13 and HH14. The embryonic region extirpated for mRNA extraction or for immunoprecipitation analyses is delineated with a blue rectangle. **d** Schematic representation of the procedure for *in-ovo* injection of quail embryos for loss-of-function studies with indication of the timing of NCC development (see Methods for details of the procedure). In **c** and **d**, zones with NCC at the delamination and migration stage are colored in magenta and green, respectively. Images in **a** are from representative experiments.



Supplementary Figure 2, related to Fig. 3: Inhibition of glutamine metabolism has no short term effect on trunk NCC identity and fate *in vitro*

a Immunofluorescence stainings for Pax-7 (green) and Sox-2 (red) with Dapi visualization of nuclei (blue) in delaminating and migrating NCCs from HH12/13 or HH14 trunk NT explants after 5 h in culture in the presence or not of DON at 60 µM. **b** Immunofluorescence stainings for βIII-tubulin (green) and α-smooth muscle actin (red) with Dapi visualization of nuclei (blue) in HH12/13 or HH14 trunk NT explants after 5 h in culture in the presence of DON at 60 µM. In **a** and **b** the NT was removed before immunostaining to visualize delaminating NCCs. Images are from a representative experiment out of 4 independent experiments. Bars = 50 µm.



Supplementary Figure 3, related to Fig. 4: Glutamine and glucose play additive and complementary roles for trunk NCC delamination and migration

a Phase contrast images of NCCs from HH12/13 or HH14 trunk NT explants cultured first for 3 h in medium deprived of glucose and glutamine, then for 2 h in the same medium or after addition of glutamine, glucose, neither or both. Bar = 20 µm. **b** Scatter dot plots of the recovery of NCC delamination in the presence of glutamine, glucose, neither or both after nutrient deprivation as determined by video microscopy. **c** Graph of the distance covered by the NCC population over time in the presence of glutamine, glutamine and glucose or neither, after deprivation. Time 0 corresponds to the onset of recording 2-3 h after initiation of culture in medium deprived of both glucose and glutamine and the arrow indicate the time when the nutrients were added to the medium. Images in panels **a** are from a representative experiment out of 3 independent experiments. In **b** and **c** (n) indicate the number of explants analyzed. In **b** data were analyzed by two-way ANOVA relative to the condition without addition of glutamine and glucose. ns, not significantly different, P>0.05.

Snail2fwd	GATGCGCTCGCAGTGATAGT
Snail2rev	AGCTTTCATACAGGTATGGGGATA
Cad6Bfwd	ACCGTGAAAACAGGGAGCAA
Cad6Brev	TGAATTGGTAGGTGCTCTGGG
cMycfwd	GCAGCGACTCGGAAGAAGAACAAGAA;
cMycrev	GGTGGGGCTTACAGTGCTCC
Sox10fwd	CGGAGCACTCTTCAGGTCAG
Sox10rev	CCCTTCTCGCTTGGAGTCAG
Foxd3fwd	TCTGCGAGTTCATCAGCAAC
Foxd3rev	TTCACGAAGCAGTCGTTGAG
Glut1fwd	AAGATGACAGCTCGCCTGATG
Glut1rev	AGTCTTCAATCACCTTCTGCGG
PFKfwd	TTGGAATTGTCAGCTGCCCG
PFKrev	TGCAGACAACTTTCATAGGCATCAG
LDHfwd	GAGCGGAGTGAATGTTGCTG
LDHrev	ACCTCATAGGCACTGTCCAC
PDHXfwd	CTGCTGCTGACTGTGACATTG
PDHXrev	TCACATCTGGCATTTGCTTCA
PRPSfwd	GCATCTCAGATTCAGGGCTTT
PRPSrev	GTCACTCTCTTGGCTCCACC
GLSfwd	GTCGCCTCCTTTGGACAAGA
GLSrev	GCACAGAGAAACCAGATCATGACA
GDHfwd	GGAGATGTCCTGGATCGCTG
GDHrev	CCCACGTTACCAAATCCCTGA
GLULfwd	TGGAGGTCTCAAGCACATCG
GLULrev	AGCCTTTCTTCTCATGGCCC
SNAT2fwd	AGCAACGACTTCAGCTACCC
SNAT2rev	GGAAGTGGTACCCGGATGATAC
CyclinEfwd	GCTCCTGAAGACTTGCACAAT
CyclinErev	TTTGCTTGAGCTTTGTCCAGC
βActinfwd	CTGTGCCCATCTATGAAGGCTA
βActinrev	ATTTCTCTCTCGGCTGTGGTG

Supplementary Table 1: List of oligonucleotide primers used in the study for the quantitative RT-PCR analyses.