Figure S1

A

High L1CAM Low L1CAM

N = 16

P = 0.015
HR = 2.52 (95% CI, 1.19-5.34)

0 16 33 50 66 83 100
Months

Overall Survival (%)

GSE16125

High L1CAM Low L1CAM

N = 16

P = 0.05
HR = 2.96 (95% CI, 0.96-9.16)

0 16 33 50 66 83 100
Months

Overall Survival (%)

GSE17537

High L1CAM Low L1CAM

N = 26

P = 0.02
HR = 2.26 (95% CI, 1.17-4.39)

0 16 33 50 66 83 100
Months

Overall Survival (%)

Combined gene expression: L1CAM/CXCR4

GSE39582

High L1CAM Low L1CAM

N = 286

P = 0.03
HR = 1.22 (95% CI, 1.01-1.46)

0 16 33 50 66 83 100
Months

Overall Survival (%)

B

Transcripts Per Million (TPM)

0 0.8 1.6 2.4 3.2 4

NODAL

Combined gene expression: L1CAM/CXCR4/NODAL

Combined gene expression: L1CAM/CXCR4/ALK4

N (n = 41) T (n = 272)
**Figure S1 - Detection of L1CAM/CXCR4 subpopulation in human CRC**

(A) Kaplan-Meier curves showing overall survival of CRC patients, stratified according to the median value of *L1CAM* and *CXCR4* expression. A Median Group cut-off (50% High vs 50% Low) was used for L1CAM and (25% High vs 75% Low) was used for CXCR4. (B) Expression levels of *L1CAM/CXCR4/ALK4* in a series of CRC (T, n = 272) and adjacent normal colon (N, n = 41) tissue samples using GEPIA (Gene Expression Profiling Interactive Analysis) webserver.
Supplementary Figure 2

A

Patient (Px)

PDO

PDOx_IC or PDOx_SC

IHC

IHC

C

ki67

Human CRC

L1CAM

D

E

mRNA relative to PPIA

L1CAM

CXCR4

NODAL

Supplementary Figure 2

Human CRC

PDOx_SC_PDO#5

Ctr

10µm

10µm

10µm

10µm

10µm

10µm

10µm

10µm

CXCR/DAPI

L1CAM/NODAL/DAPI

L1CAM/pSMAD2/DAPI

L1CAM/Ki67/DAPI

E

mRNA relative to PPIA

PDO#1

PDO#2

PDO#5

L1CAM

CXCR4

NODAL
Figure S2 - Detection of L1CAM/CXCR4 subpopulation in human CRC

(A) Schematic representation of PDOs generation. PDx were isolated from fresh patient biopsies and injected in the caecum (IC) of immunocompromised mice. Then, the cells obtained from enzymatic digestion of PDx_IC were cultured in matrigels PDO and then subcutaneously (PDOx_SC) or intracaeicum (PDOx_IC) injected in nude mice. (B) Confocal images for L1CAM (green), Ki67 (red), pSMAD2 (red), NODAL (red), CXCR4 (green) and nuclei (blue, DAPI) in the human CRC and PDOx-SC_PDO#5. (C) Representative immunohistochemistry for Ki67 and L1CAM (brown) in tissue section from human CRC patients. (D) qPCR analysis of L1CAM, CXCR4 and NODAL in PDO#5 human organoids. Data are normalized to PPIA expression. Data are mean ± SD, n ≥ 6. (E) qPCR analysis for L1CAM, CXCR4 and NODAL in PDO#1, PDO#2 and PDO#3. Data are normalized to PPIA expression. N ≥ 6.
Figure S3

A

Isotype

PDO#2

L1CAM

0.087%

0.087%

99.7%

13.0%

20% O2

L1CAM

2.0%

19.1%

79.8%

1% O2

CXCR4

0.13%

23.3%

73.9%


sh scramble 20% O2

sh scramble 1% O2

shNODAL 20% O2

shNODAL 1% O2

shNODAL 1% O2 rescued

#1

0.41%

3.11%

0.18%

0.25%

2.02%

#2

0.84%

8.74%

0.27%

0.42%

7.75%

B

C

PDO#1

KDa

pSMAD2

52

SMAD2

55

rNODAL 7d

- + +

SB431542

- - +

D

PDO#1

L1CAM mRNA Fold change

Ctrl

rNODAL 12d

rNODAL 7d

rNOD+SB 7d

E

PDO#2

PDO#5

Mock

Over.NODAL

PDO#2

PDO#5

PDO#2

PDO#5

Mock

Over.NODAL

PDO#2

PDO#5

PDO#2

PDO#5

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**Figure S3** - Nodal induces L1CAM and CXCR4 expression in human CRC organoids

(A) Representative flow cytometry for L1CAM and CXCR4 in PDO#2 growth in normoxia or hypoxia. All cytometry gates were established based on isotype controls. N ≥ 3. (B) Representative flow cytometry analysis for L1CAM in PDO#2 sh scramble or shNODAL#1 and #2 growth in normoxia or hypoxia. The shNODAL #1 and #2 growth in hypoxia were rescued with rNODAL. N ≥ 3. (C) Western blot analysis of pSMAD2 in PDO#1 treated or untreated with rNODAL for 7 days in presence or absence of SB431542. Parallel SMAD2 immunoblotting was performed. (D) qPCR analysis of L1CAM and CXCR4 in PDO#1, PDO#2 and PDO#5 treated or untreated with rNODAL for 7 and 12 days in presence or absence of SB431542. Data are normalised to PPIA expression and are presented as fold change (FC) in gene expression relative to control cells. **p<0.005, ***p<0.0005 compared with Ctrl. N ≥ 6. (E) qPCR analysis of NODAL and L1CAM in PDO#2 and PDO#5 transfected with control vector (mock) or NODAL overexpressing vector. Data are normalised to PPIA expression and are presented as fold change in gene expression relative to mock-transfected cells. **p<0.005, ***p<0.0005. n ≥ 6.
**Figure S4**

**A**

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**B**

Annexin V

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EDU

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Cell cycle status (%)

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**Figure S4 - Nodal induces L1CAM and CXCR4 expression in human CRC organoids**

(A) Representative flow cytometry plots for apoptotic cells as determined by AnnexinV/PI staining in PDO#2 and PDO#5 treated or untreated with rNODAL for 7 days in presence or absence of SB431542. 

(B) Percentage of apoptotic cells as determined by AnnexinV/PI staining in control PDO#1, PDO#2 and PDO#5 treated or untreated with rNODAL for 7 days in presence or absence of SB431542.

(C) Representative flow cytometry plots for cell cycle analysis performed with EDU incorporation in PDO#2 and PDO#5 treated or untreated with rNODAL for 7 days in presence or absence of SB431542.

(D) Percentage of PDO#1, PDO#2 and PDO#5 derived cells in each phase of cell cycle treated or untreated with rNODAL for 7 days in presence or absence of SB431542.
Figure S5

A. rNODAL 12d

PDO#2

PDO#5

50 µm

50 µm

B. Ctrl

rNODAL 12d

E-CADHERIN

VIMENTIN

PDO#2

PDO#5

45.52

22.45

7.82

11.20

17.65

5.22

3.16

11.25

E-CADHERIN

VIMENTIN

C. Number of cells

L1CAM

GAPDH

PDO#2

sh scramble

sh L1#1

sh L1#2

2×10⁶

5×10⁵

D. PDO#2

Mean tumor volume (mm³)

Days after tumor xenograft

sh scramble n=8/8

sh L1#1 n=4/8

sh L1#2 n=6/8

***

***

***

***

D. PDO#2

Mean tumor volume (mm³)

Days after tumor xenograft

sh scramble n=8/8

sh L1#1 n=4/8

sh L1#2 n=6/8

***

***

***

***

E. mRNA relative to sh scramble

L1CAM

EPHB2

OLFM4

sh scramble

sh L1#1

sh L1#2

***

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***
Figure S5 - Identification of metastatic L1CAM$^{\text{high}}$/CXCR4$^{\text{high}}$ subpopulation in CRC organoids

(A) Representative images of PDO#2 and PDO#5 treated with rNODAL for 12 days. (B) Representative flow cytometry plots of E-CADHERIN and VIMENTIN in Ctrl vs 12-days NODAL treated organoids. (C) Western blot analysis of L1CAM in PDO#2 sh scramble and shL1. Parallel GAPDH immunoblotting was performed. Cells growth curves for PDO#2 sh scramble and shL1. Each data point represents the mean ± SD of three independent experiments. **p < 0.005; ***p < 0.0005 compared to sh scramble. n ≥ 6. (D) In vivo tumor growth of subcutaneously injected PDO#2 control (sh scramble) and shL1. Tumor size was measured every 2–5 days and tumor volume was calculated. Data are shown as mean (points) ± s.d. ***p<0.0005 compared to sh scramble. N = 8. (E) qPCR analysis for L1CAM, EPHB2 and OLFM4 in sh scramble and shL1 PDO#2 sh scramble and shL1. Data are normalised to PPIA expression. **p<0.005 ***p<0.0005. n ≥ 6.

Figure S6
Figure S6 - Nodal stimulation does not cause an L1CAM and CXCR4 dependent YAP nuclear localization

(A) Confocal images for YAP (red) and nuclei (blue, DAPI) of SW480 and SW620 L1CAM or CXCR4 sorted cells treated or untreated with rNODAL (short treatment). (B) qPCR analysis of L1CAM, CXCR4, CTGF, CYR61, ANKRD1 and ITGB2 in SW480, SW620 and PDO#2 sorted for L1CAM or CXCR4. Data are normalized to GAPDH expression and are presented as fold change in gene expression relative to untreated cells. *p<0.05, **p<0.005. n ≥ 6.
Figure S7

A

**Figure S7**

**A**

- Organoids size (µm)
- PDO#2
- PDO#5

**B**

- PDO#2
- PDO#5

**C**

- mRNA relative to L1<sub>low</sub>/CX<sub>low</sub>
- L1<sub>high</sub>/CX<sub>high</sub>

**D**

- E-CADHERIN
- PDO#5

**E**

- PDO#5
- L1<sub>low</sub>/CX<sub>low</sub>
- L1<sub>low</sub>/CX<sub>high</sub>
- L1<sub>high</sub>/CX<sub>low</sub>
- L1<sub>high</sub>/CX<sub>high</sub>
Figure S7 - Identification of metastatic L1CAM\textsuperscript{high}/CXCR4\textsuperscript{high} subpopulation in CRC organoids

(A) Quantification of organoids size in the four sorted indicated populations from PDO\#2 and PDO\#5. (B) Confocal images for phalloidin (red) and nuclei (blue, DAPI) of PDO\#2 and PDO\#5. (C) qPCR analysis for EPHB2, LGR5, OLFM4, KRT20 and MUC2 in the L1\textsuperscript{low}/CX\textsuperscript{low} and L1\textsuperscript{high}/CX\textsuperscript{high} populations sorted from PDO\#2 and PDO\#5. Data are normalised to PPIA expression. **p<0.005 ***p<0.0005. n ≥ 6. (D) Representative flow cytometry plots of E-CADHERIN in the four sorted indicated populations. (E) Representative invasion assay videos of PDO\#5 human organoids expressing different L1CAM and CXCR4 levels.

SUPPLEMENTARY METHODS

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**TABLE S1** - List of primary antibodies used.
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**TABLE S2-** List of TaqMan probes used for qPCR.

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**TABLE S3-** List of primers used for qPCR (SYBR green method).
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<td>L1CAM</td>
<td>CCGGCCACCTTGTTTAAAGGAGGATCTCGAGATCTCTCCTAACAAGTGGTTTTTG</td>
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<tr>
<td>L1CAM</td>
<td>CCGGGCTAACCTGAAGGTTAAAGATCTCGAGATCTTTACCTTCAGGTTAGCTTTTTG</td>
</tr>
<tr>
<td>SCRAMBLE</td>
<td>CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTTTTCATCTTGTTTTTTT</td>
</tr>
</tbody>
</table>

**TABLE S4** - List of nucleotide sequence of shRNA used.