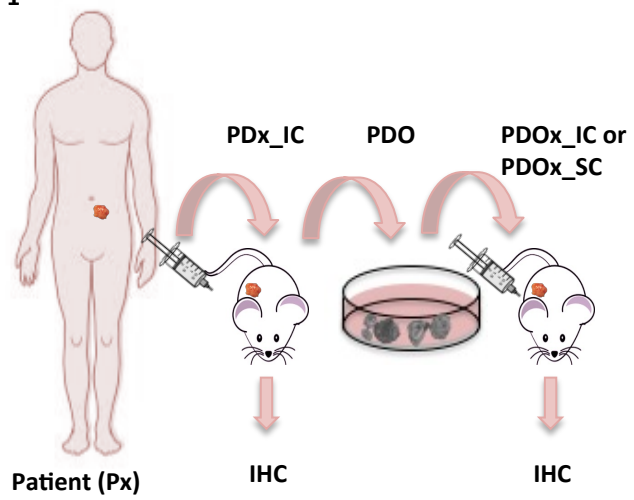
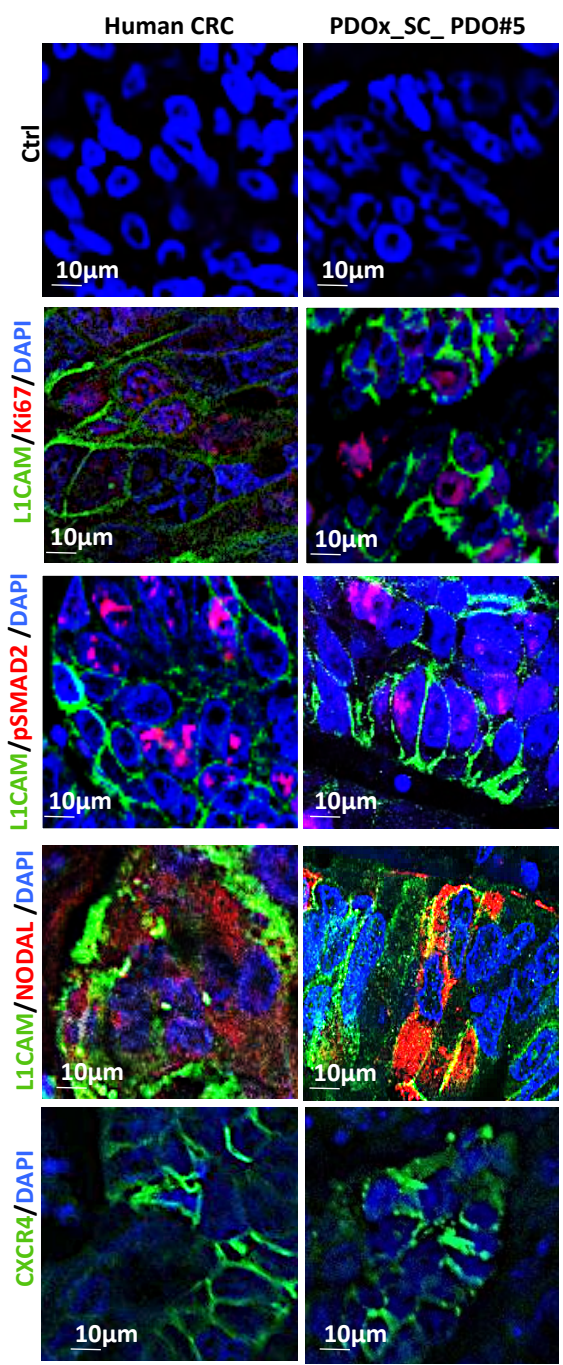


Supplementary Figure 1

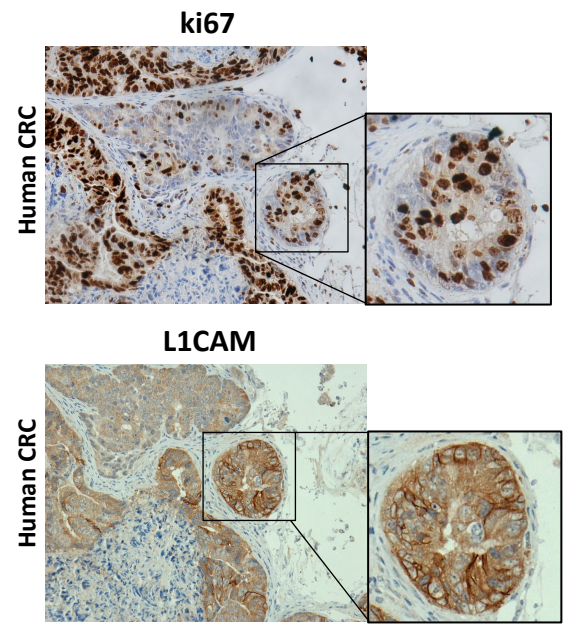
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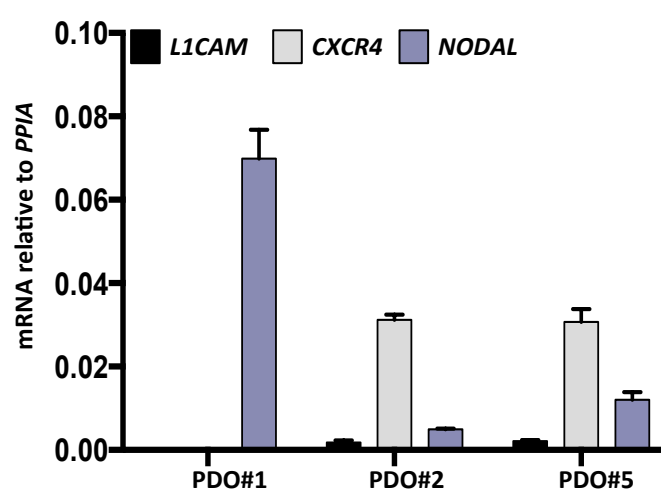
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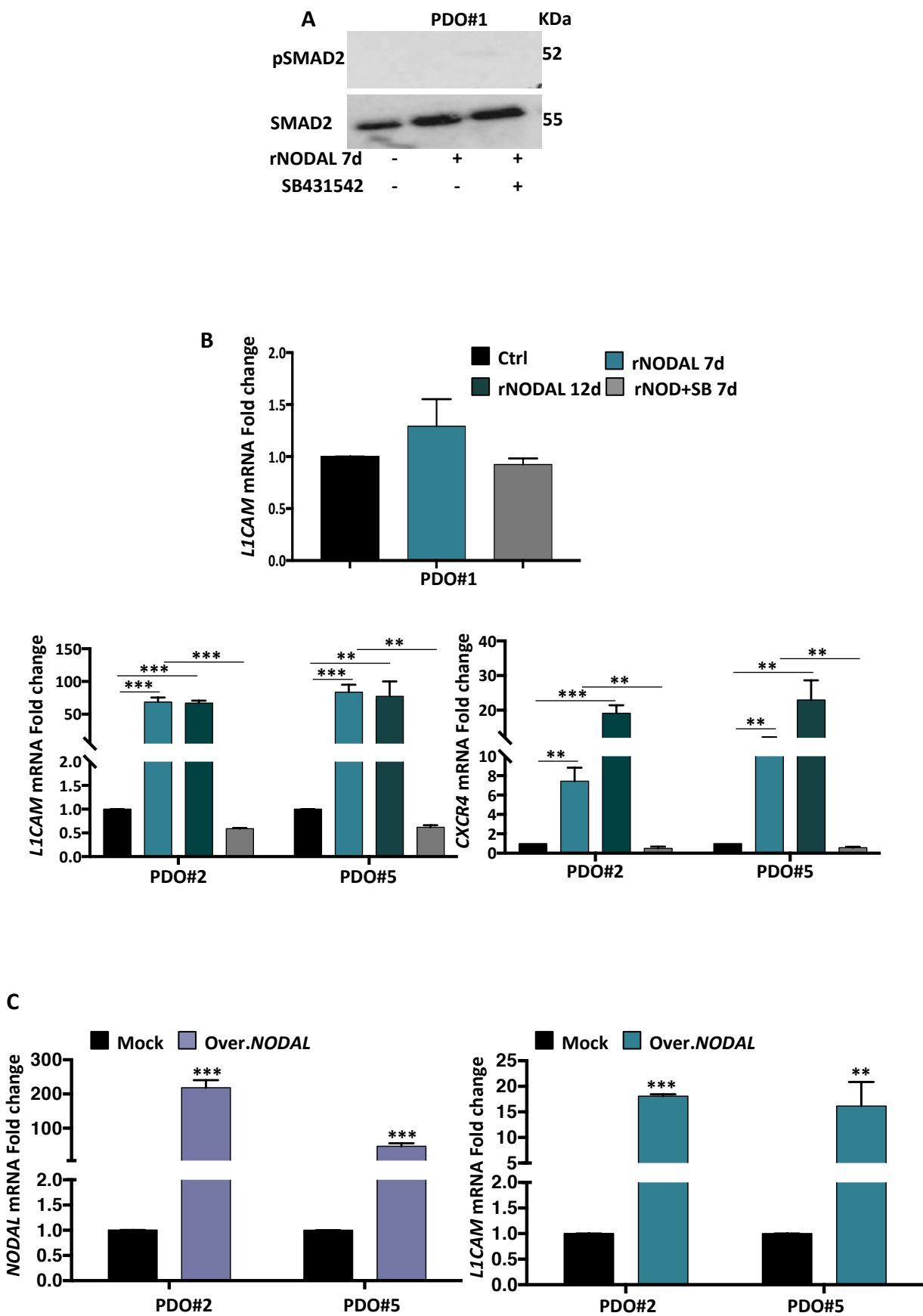
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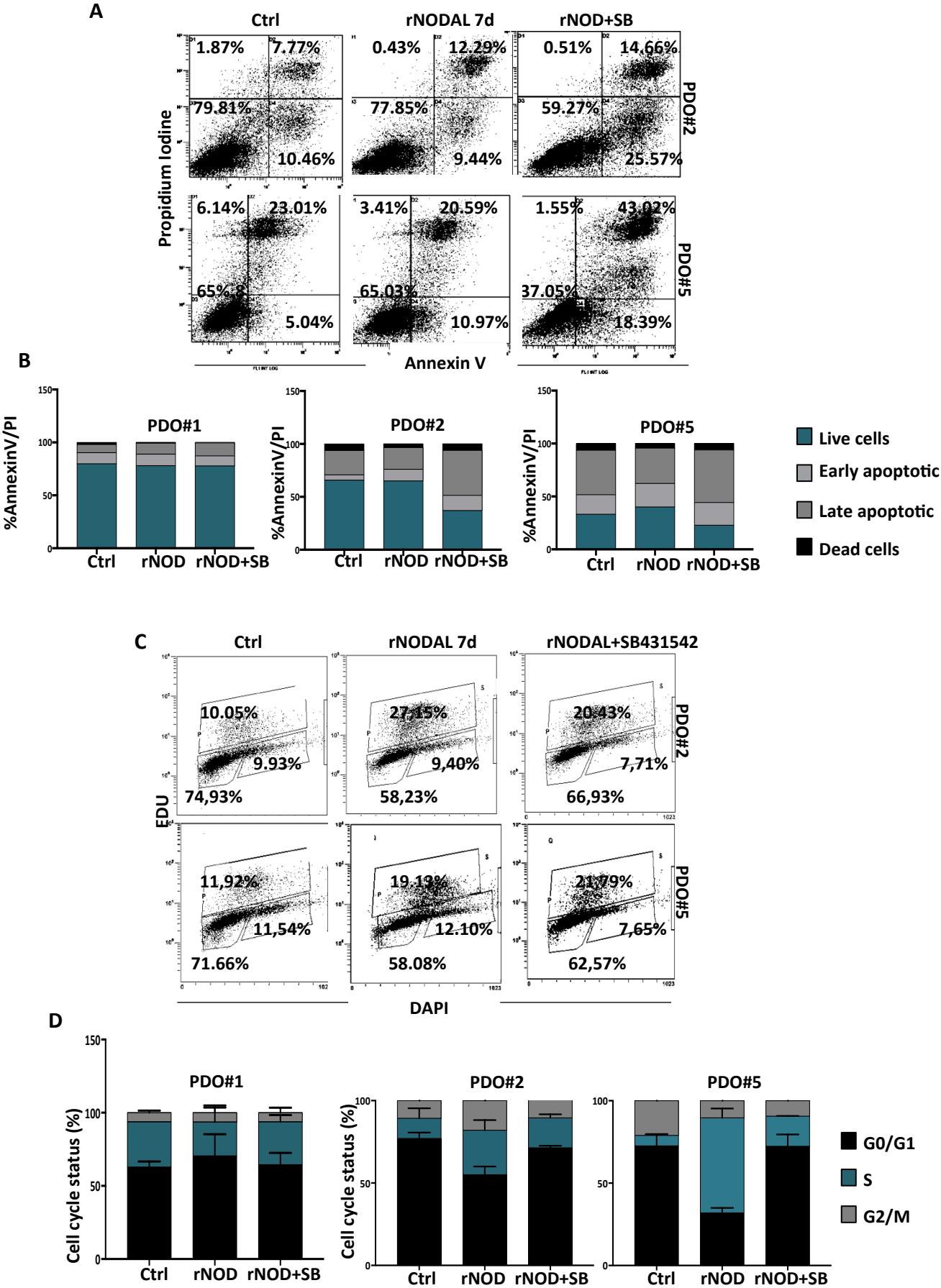
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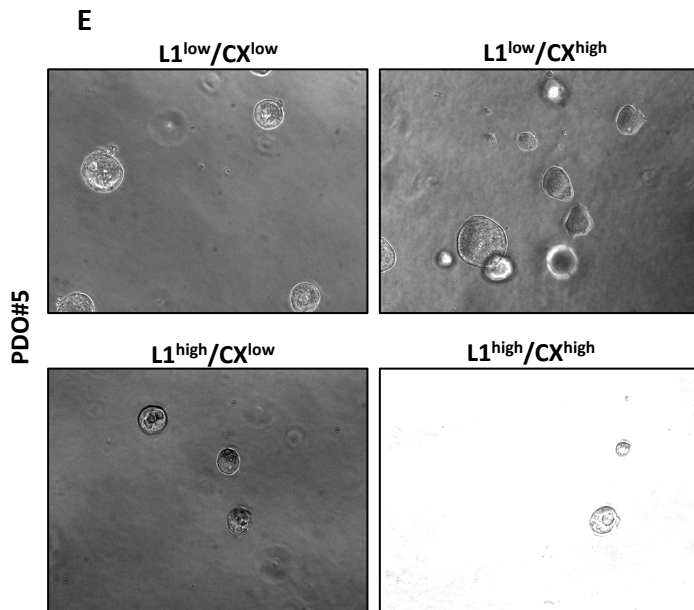
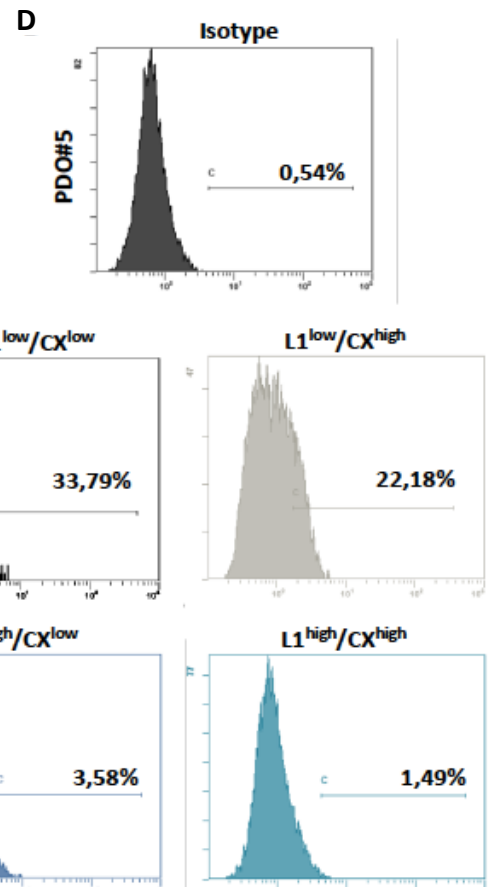
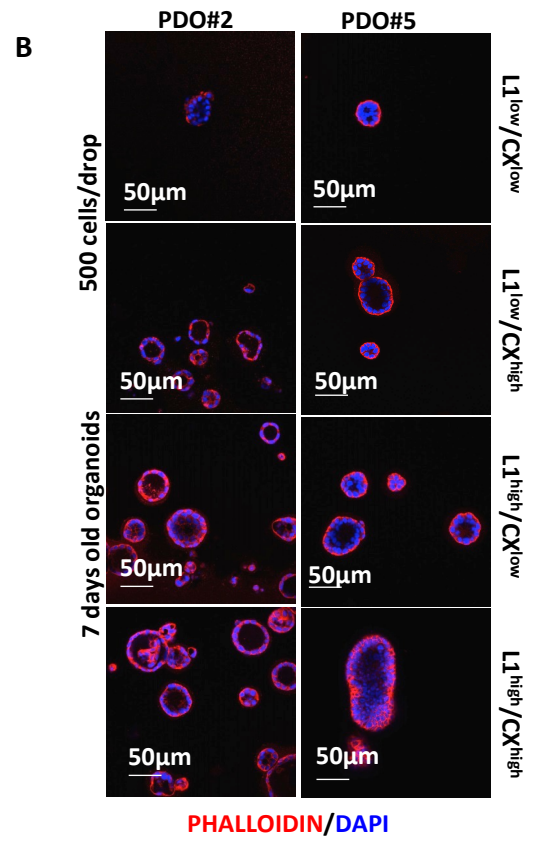
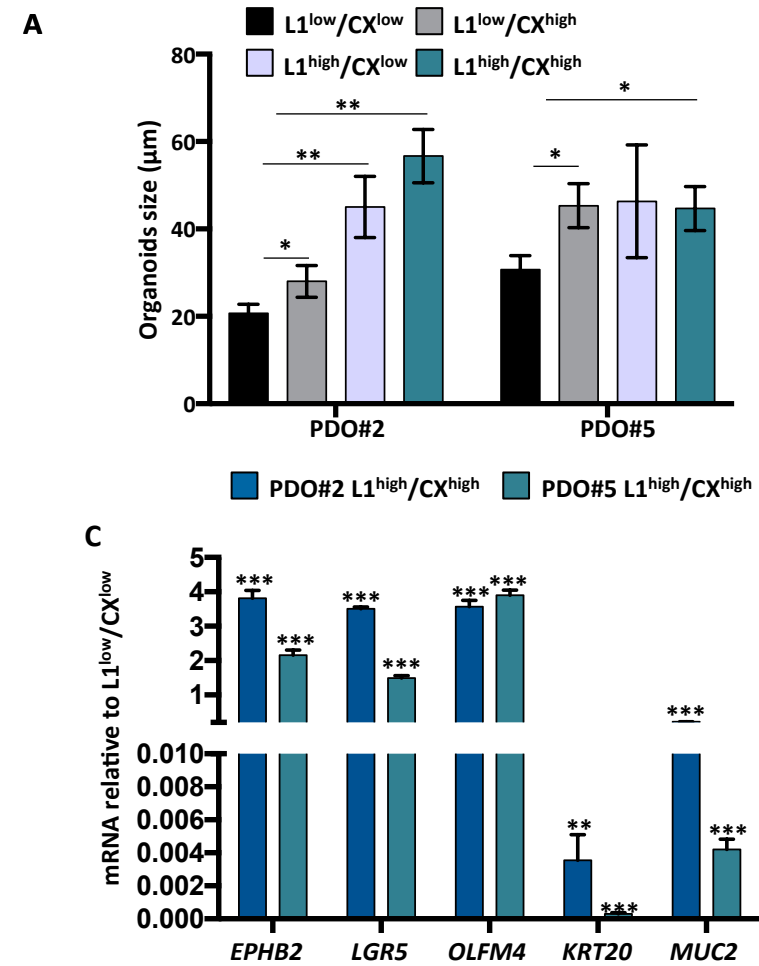
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 5



Supplementary Figure 1-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 matrigel as PDO and then subcutaneously (PDOx_SC) or intracaecum (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 for *L1CAM*, *CXCR4* and *NODAL* in PDO#1, PDO#2 and PDO#3. Data are normalized to *PPIA* expression. $n \geq 6$.

Supplementary Figure 2-Nodal induces L1CAM and CXCR4 expression in human CRC organoids

(A) 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. (B) 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. $n \geq 6$. (C) 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. $**p < 0.005$, $***p < 0.0005$. $n \geq 6$.

Supplementary Figure 3-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.

Supplementary Figure 4-Identification of metastatic L1CAM^{high}/CXCR4^{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 sh*L1*. Parallel GAPDH immunoblotting was performed. Cells growth curves for PDO#2 sh scramble and sh*L1*. Each data point represents the mean $\pm$ SD of three independent experiments. $**p < 0.005$; $***p < 0.0005$ compared to sh scramble. $n \geq 6$. (D) In

in vivo tumor growth of subcutaneously injected PDO#2 control (sh scramble) and sh*L1*. Tumor size was measured every 2–5 days and tumor volume was calculated. Data are shown as mean (points) $\pm$ s.d. *** $p < 0.0005$ compared to sh scramble. $n = 8$. (E) qPCR analysis for *L1CAM*, *EPHB2* and *OLFM4* in sh scramble and sh*L1* PDO#2 sh scramble and sh*L1*. Data are normalised to *PPIA* expression. ** $p < 0.005$ *** $p < 0.0005$. $n \geq 6$.

Supplementary Figure 5-Identification of metastatic *L1CAM*^{high}/*CXCR4*^{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*^{low}/*CX*^{low} and *L1*^{high}/*CX*^{high} populations sorted from PDO#2 and PDO#5. Data are normalised to *PPIA* expression. ** $p < 0.005$ *** $p < 0.0005$. $n \geq 6$. (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.