Figure S1. Fibroblast characterization. IHC staining for vimentin, α-SMA, FAP, CD31 and CK19 in primary fibroblasts. Scale bar, 100 μm. All experiments were performed in triplicate. IHC, immunohistochemistry; α-SMA, α-smooth muscle actin; fibroblast activation protein; CAFs, cancer-associated fibroblasts; NFs, named adjacent-normal fibroblasts.

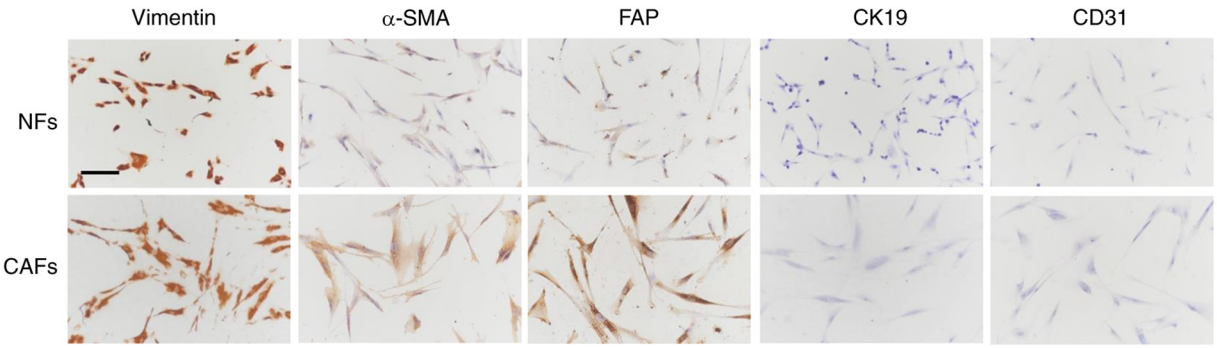

Figure S2. METTL3, NCALD and miR-181d-5p expression in CAFs and CRC cells. (A and B) Expression of METTL3 in METTL3-silencing or METTL3-overexpressing lentivirus-transduced CAFs. (C) Expression of miR-181d-5p in CRC cells transfected with miR-181d-5p inhibitor, mimic, or corresponding NC. (D) Expression of NCALD in HT29 and HCT116 cells transduced with NCALD-overexpressing lentivirus. All experiments were performed in triplicate. ***P<0.001. METTL, methyltransferase like; NACLD, neurocalcin δ; miR/miRNA, microRNA; CAFs, cancer-associated fibroblasts; CRC, colorectal cancer; sh, short hairpin; NC, negative control.

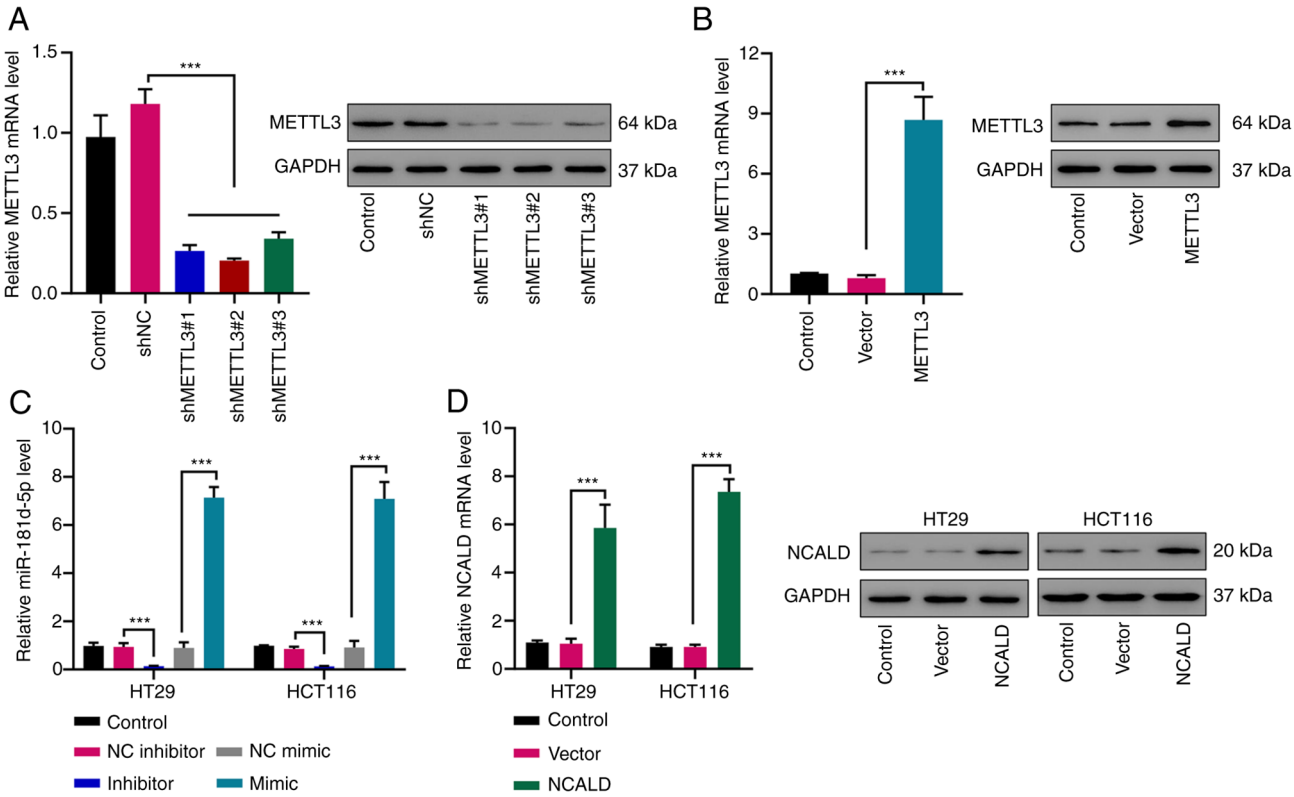

Figure S3. Percentage of EdU positive (A) HT29 and (B) HCT116 cells co-cultured with METTL3-overexpressing or METTL3-silencing lentivirus-transduced CAFs. ***P<0.001. EdU, Ethynyl-2-deoxyuridine; sh, short hairpin; NC, negative control; METTL, methyltransferase like; CAFs, cancer-associated fibroblasts.

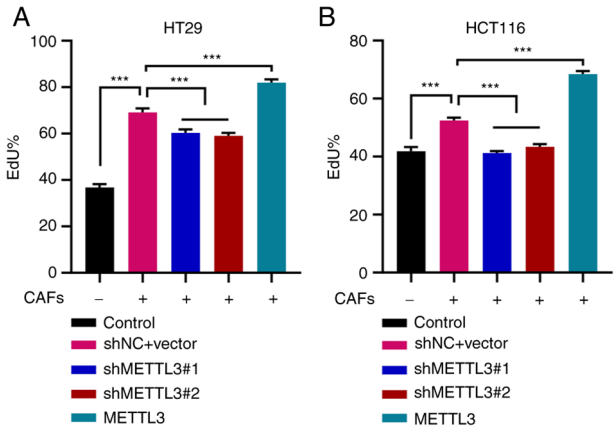

Figure S4. Characterization of exosomes from CAFs. (A) Transmission electron microscopy observation of CAFs-derived exosomes. Scale bar, 100 nm. (B) Particle size distribution of exosomes from CAFs measured by Flow Nano Analyzer. CAFs, cancer-associated fibroblasts.

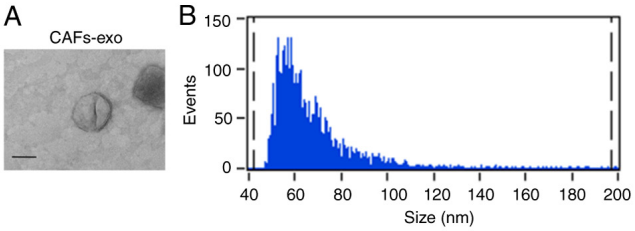

Figure S5. Schematic representation of the regulation of 5-FU sensitivity in CRC through the METTL3/miR-181d-5p/NCALD axis. Briefly, CRC cells uptake cancer CAF-secreting exosomes, leading to an increase of miR-181d-5p and a decrease of 5-FU sensitivity in CRC cells. Mechanistically, miR-181d-5p inhibits 5-FU sensitivity by directly binding to the 3'UTR of NCALD and suppressing its expression in CRC cells. METTL3-dependent m^6A modification regulates the processing of miR-181d-5p by DGCR8 in CRC cells. 5-FU, 5-Fluorouracil; CRC, colorectal cancer; METTL, methyltransferase like; miR/miRNA, microRNA; NACLD, neurocalcin δ; CAFs, cancer-associated fibroblasts; m^6A, RNA N6-methyladenosine; pri, primary; pre, precursor.