

Figure S1

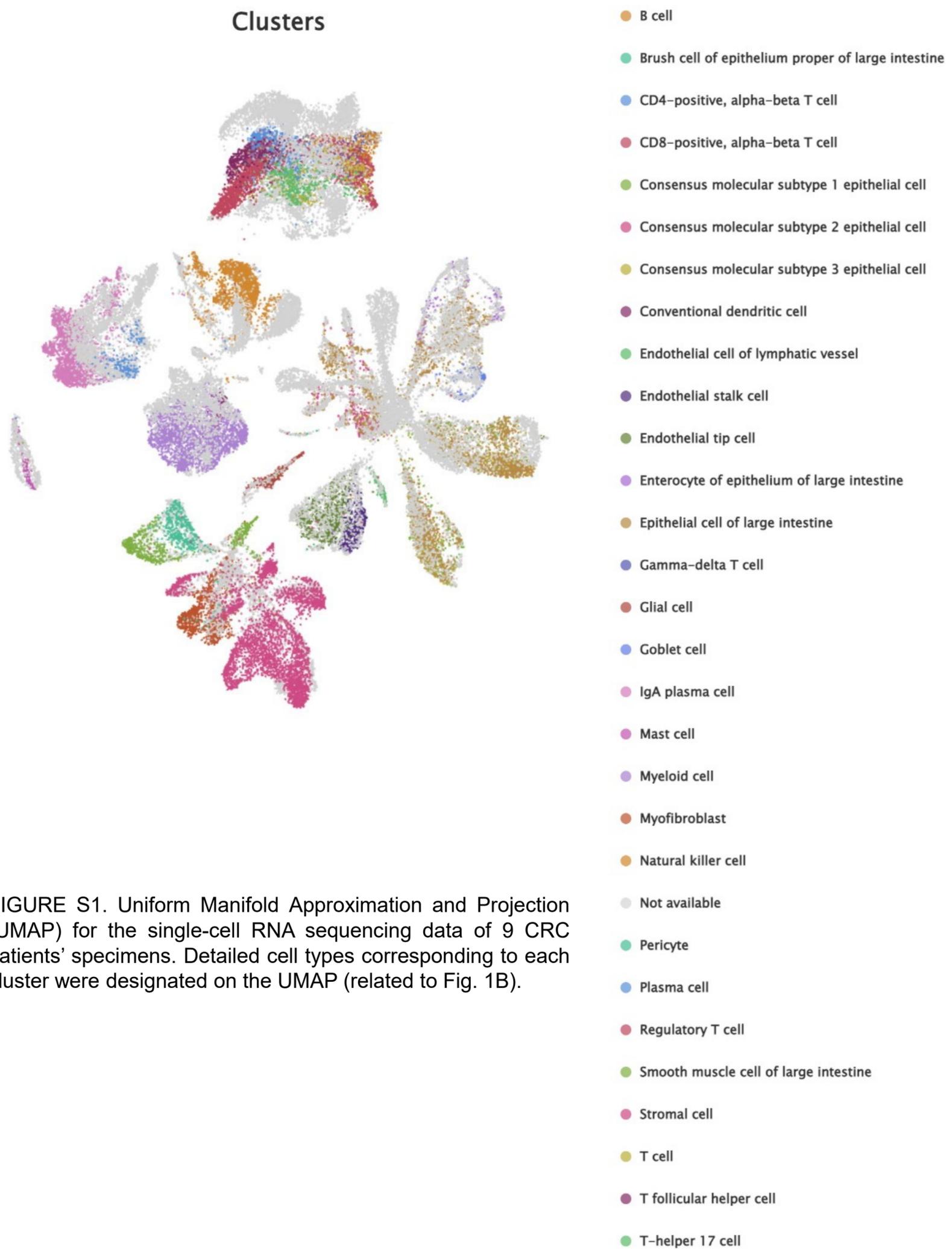


FIGURE S1. Uniform Manifold Approximation and Projection (UMAP) for the single-cell RNA sequencing data of 9 CRC patients' specimens. Detailed cell types corresponding to each cluster were designated on the UMAP (related to Fig. 1B).

Figure S2

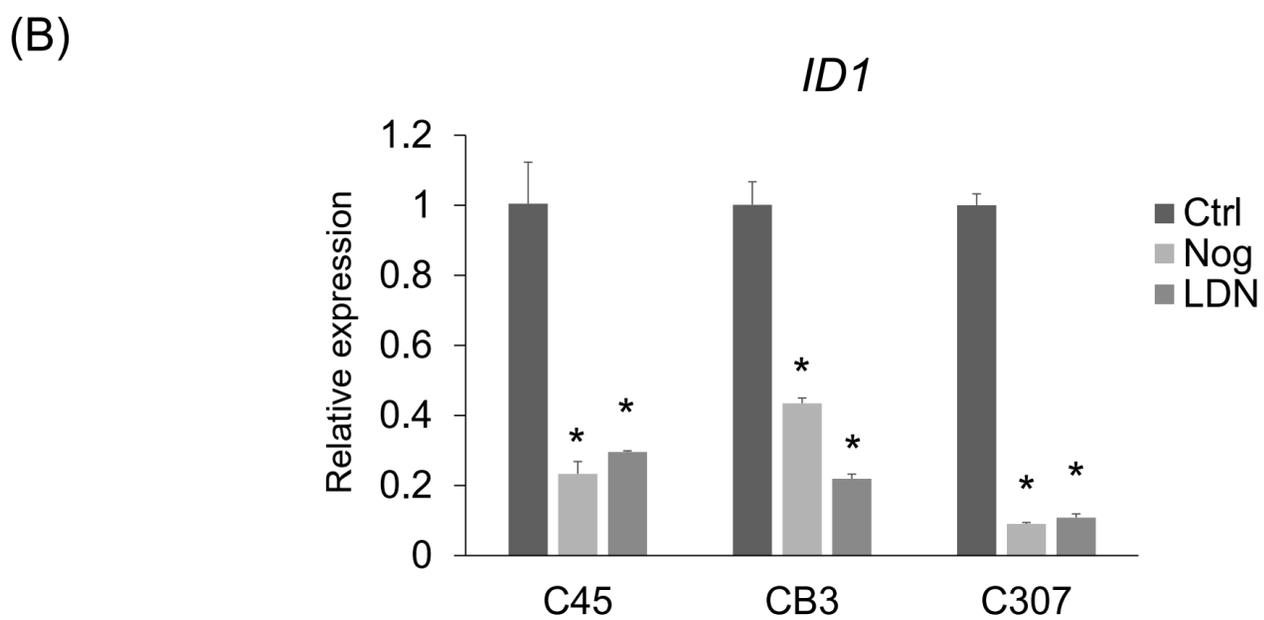
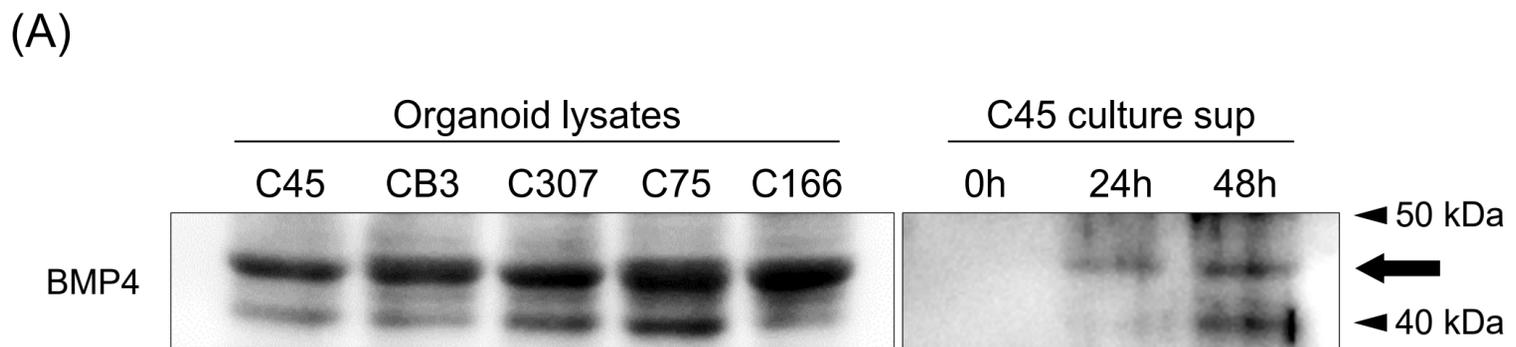


FIGURE S2. **A**, Immunoblotting analysis of CRC organoid lysates (left) and concentrated culture supernatant of C45 (right). Arrow indicates the band size for the BMP4 precursor, a monitoring protein for the secreted mature-BMP4 ligands. Proteins were detected with indicated antibodies. **B**, Gene expression of *ID1* in CRC organoids treated with Noggin (Nog) or LDN. The expression level was normalized to control (Ctrl) in each organoid. N = 3 for each condition. Data are presented as mean + SD. Statistical comparisons were made for each control condition. * P < 0.05, t-test with Bonferroni correction.

Figure S3

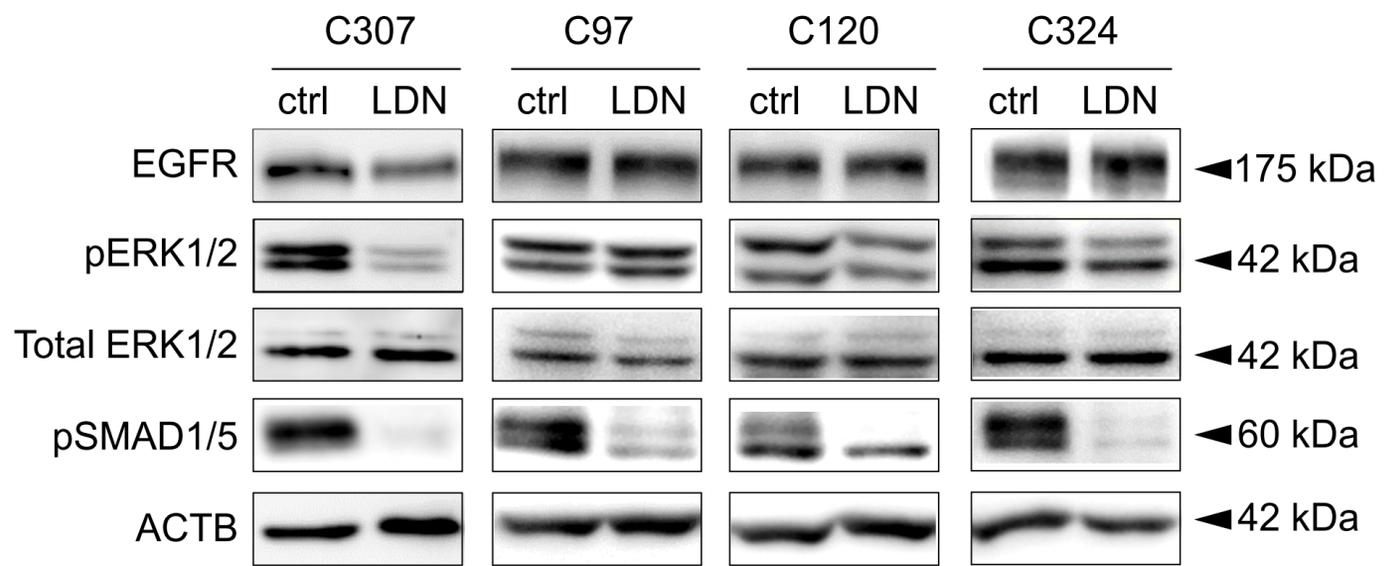


FIGURE S3. Immunoblotting analysis of CRC organoids cultured in the presence (LDN) or absence (ctrl) of 0.1 μ M LDN. Proteins were detected with indicated antibodies.

Figure S4

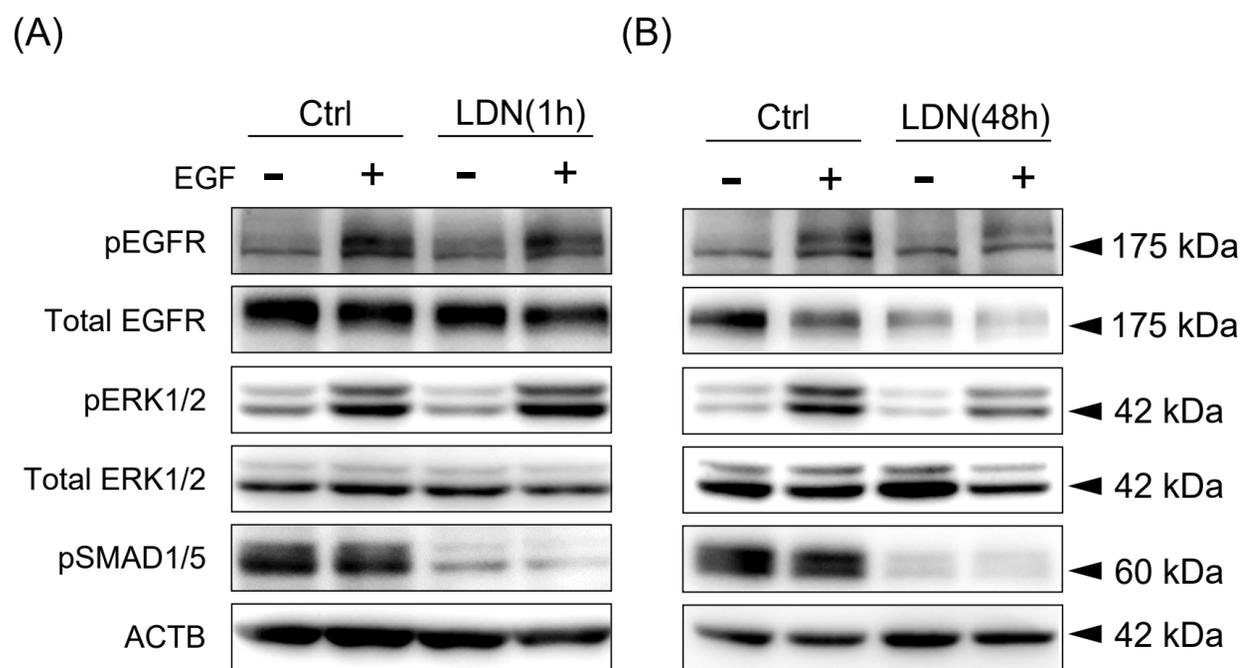


FIGURE S4. Immunoblotting analysis of CRC organoids cultured in the presence (LDN) or absence (Ctrl) of 0.1 μ M LDN for 1 hour (A) or 48 hours (B) followed by 50 ng/ml EGF for 5 minutes. Proteins were detected with indicated antibodies.

Figure S5

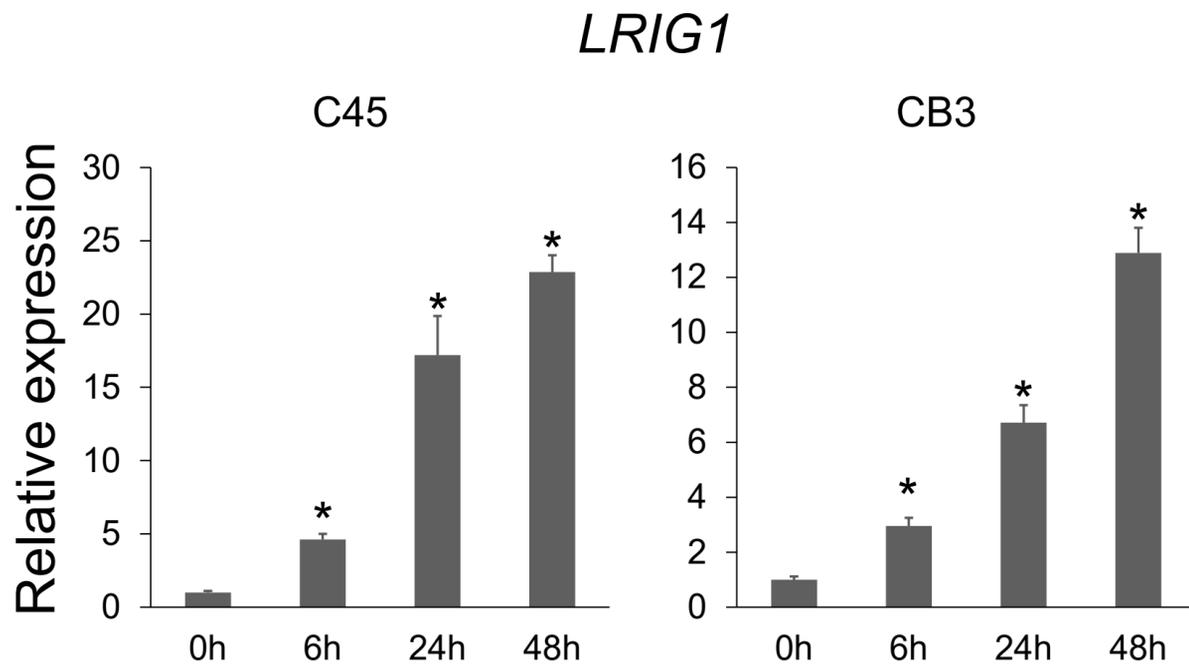


FIGURE S5. Gene expression of *LRIG1* in CRC organoids treated with LDN. The expression level was normalized to control (Ctrl) in each organoid and time point. N = 3 for each condition. Data are presented as mean + SD. Statistical comparisons were made for each control condition. * P < 0.01, t-test with Bonferroni correction.

Figure S6

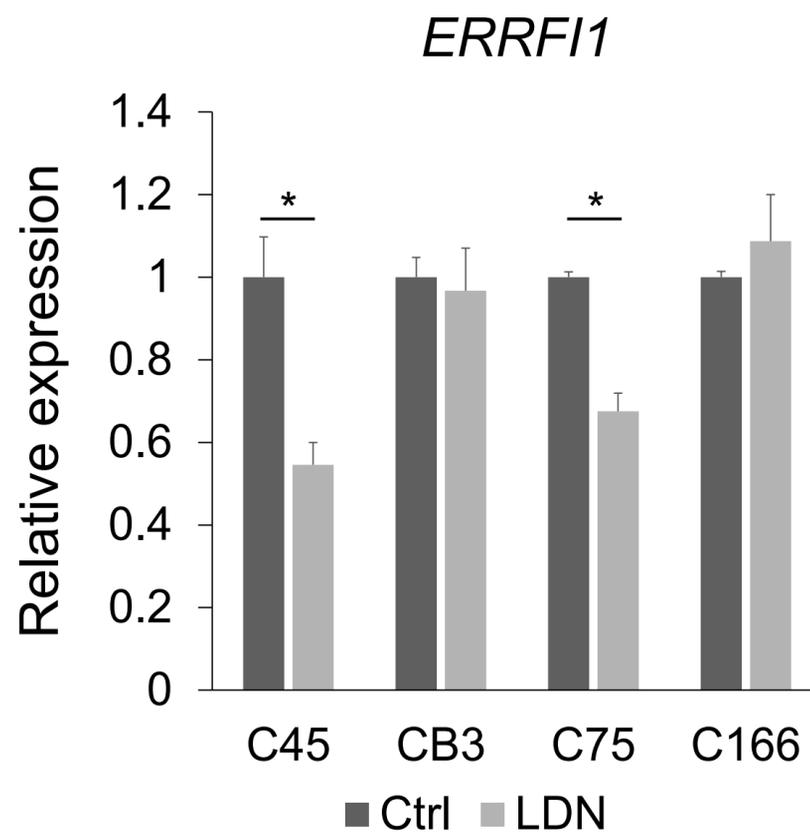


FIGURE S6. Gene expression of *ERRFI1* in CRC organoids treated with LDN. The expression level was normalized to control (Ctrl) in each organoid. N = 3 for each condition. Data are presented as mean + SD. Statistical comparisons were made for each control condition. * P < 0.001, t-test with Bonferroni correction.

Figure S7

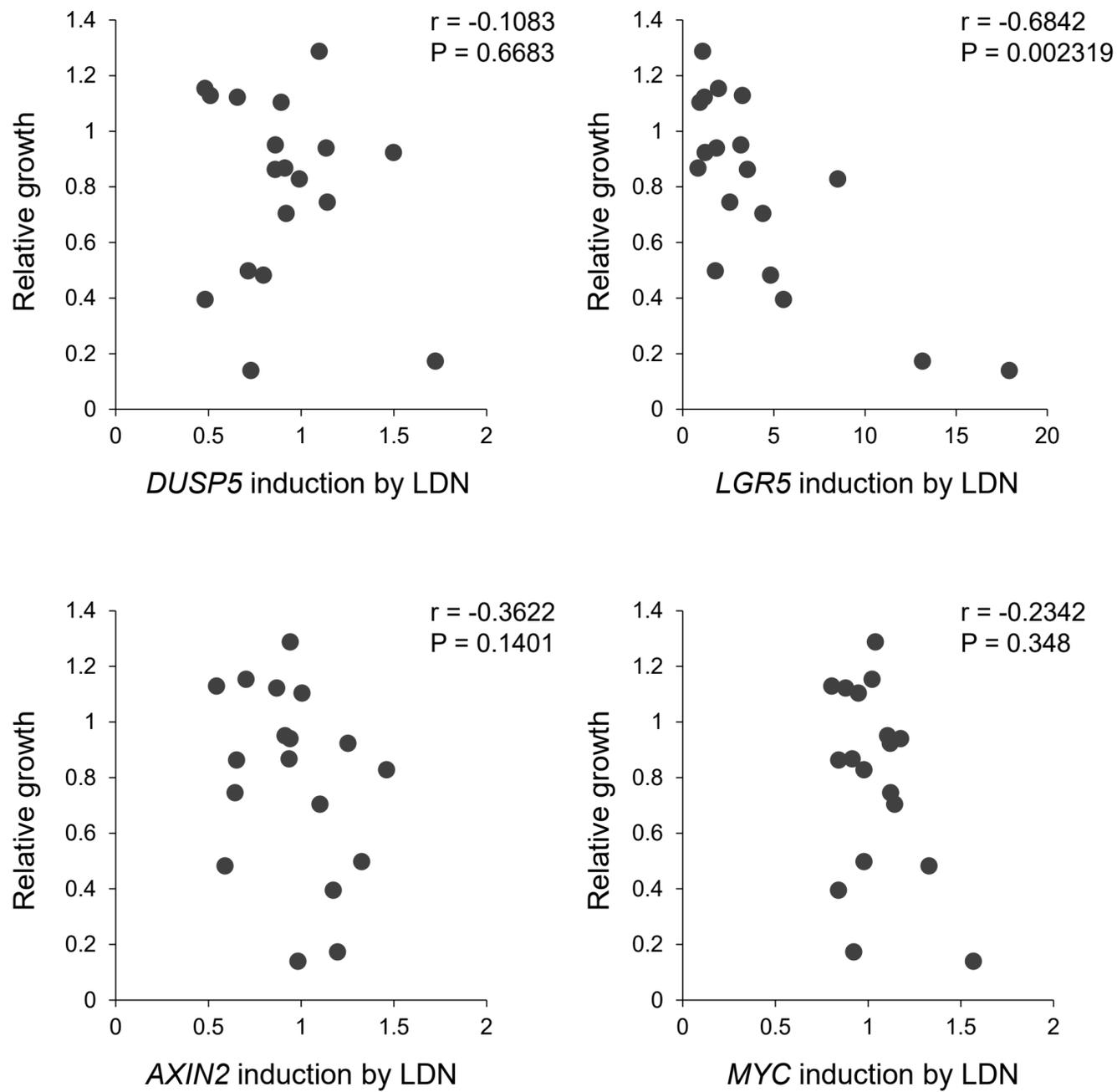


FIGURE S7. Scatter plot showing the correlation of organoid growth and *DUSP5*, *LGR5*, *AXIN2*, and *MYC* induction in the presence of 0.1 μ M LDN relative to the LDN-free condition. Each dot represents an individual CRC organoid line (N=18). r, Spearman's rank correlation coefficient; P value, Spearman's rank correlation test.

Figure S8

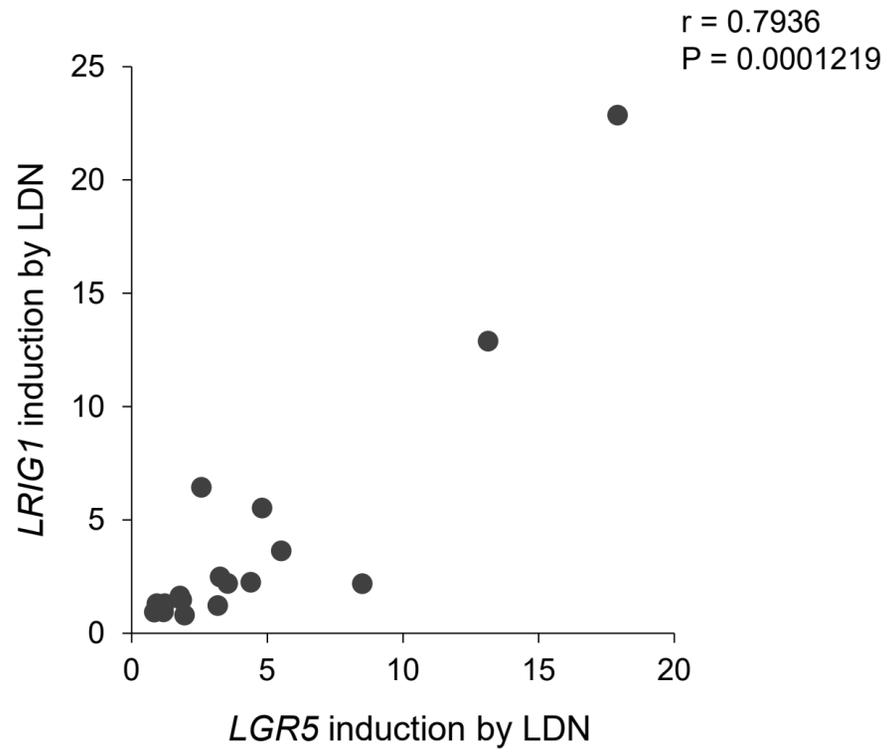


FIGURE S8. Scatter plot showing the correlation of *LRIG1* and *LGR5* induction in the presence of 0.1 μ M LDN relative to the LDN-free condition. Each dot represents an individual CRC organoid line (N=18). r , Spearman's rank correlation coefficient; P value, Spearman's rank correlation test.

Figure S9

Sample ID	LDN (0.1µM)		Mutational status of organoids										
	growth ratio	<i>LRIG1</i> induction	APC	TP53	KRAS	BRAF	PIK3CA	AKT1	SMAD4	CTNNB1	LTN1	MLH1	FBXW7
KUC19	1.287525597	0.999455014	Mut	WT	Mut	WT	WT	WT	Mut	WT	WT	WT	WT
C75	1.153373842	0.804953166	Mut	WT	Mut	WT	WT	Mut	WT	WT	WT	WT	WT
C132	1.128608715	2.487417965	Mut	WT	WT	WT	Mut	WT	WT	WT	Mut	WT	WT
KUC16	1.122026864	0.951780056	Mut	Mut	WT	WT	WT	WT	Mut	WT	WT	WT	WT
C120	1.103893588	1.311911342	WT	WT	WT	Mut	Mut	WT	WT	WT	WT	WT	WT
KUC17	0.950358478	1.228127066	Mut	Mut	Mut	WT	WT	WT	Mut	WT	Mut	WT	WT
C166	0.939583878	1.473004254	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	Mut	Mut
C191	0.923064563	1.312232253	WT	Mut	WT	WT	WT	WT	Mut	Mut	WT	WT	WT
C138	0.867100823	0.939548321	Mut	Mut	Mut	WT	WT	WT	Mut	WT	WT	WT	WT
C111	0.862624559	2.19993762	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	WT	WT
KUC21	0.828055279	2.189574955	Mut	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	WT
KUC22	0.745287972	6.438410824	Mut	Mut	WT	WT	Mut	WT	WT	WT	WT	WT	WT
C97	0.704131701	2.256117353	WT	Mut	Mut	WT	Mut	WT	WT	WT	WT	WT	WT
KUC3	0.497852501	1.646142092	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	WT	WT
C324	0.482312897	5.523302072	Mut	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
C307	0.394814617	3.634832505	Mut	Mut	Mut	WT	Mut	WT	WT	WT	WT	WT	WT
CB3	0.173005112	12.88706856	Mut	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	WT
C45	0.139401236	22.85927219	Mut	Mut	Mut	WT	WT	WT	WT	WT	WT	WT	WT

FIGURE S9. Growth ratio and *LRIG1* expression under treatment with 0.1 µM LDN relative to vehicle control for each of 18 CRC organoid lines are presented with colors indicating lowest (blue) to highest (red), along with the status of detected mutation. The list is sorted by growth ratio.