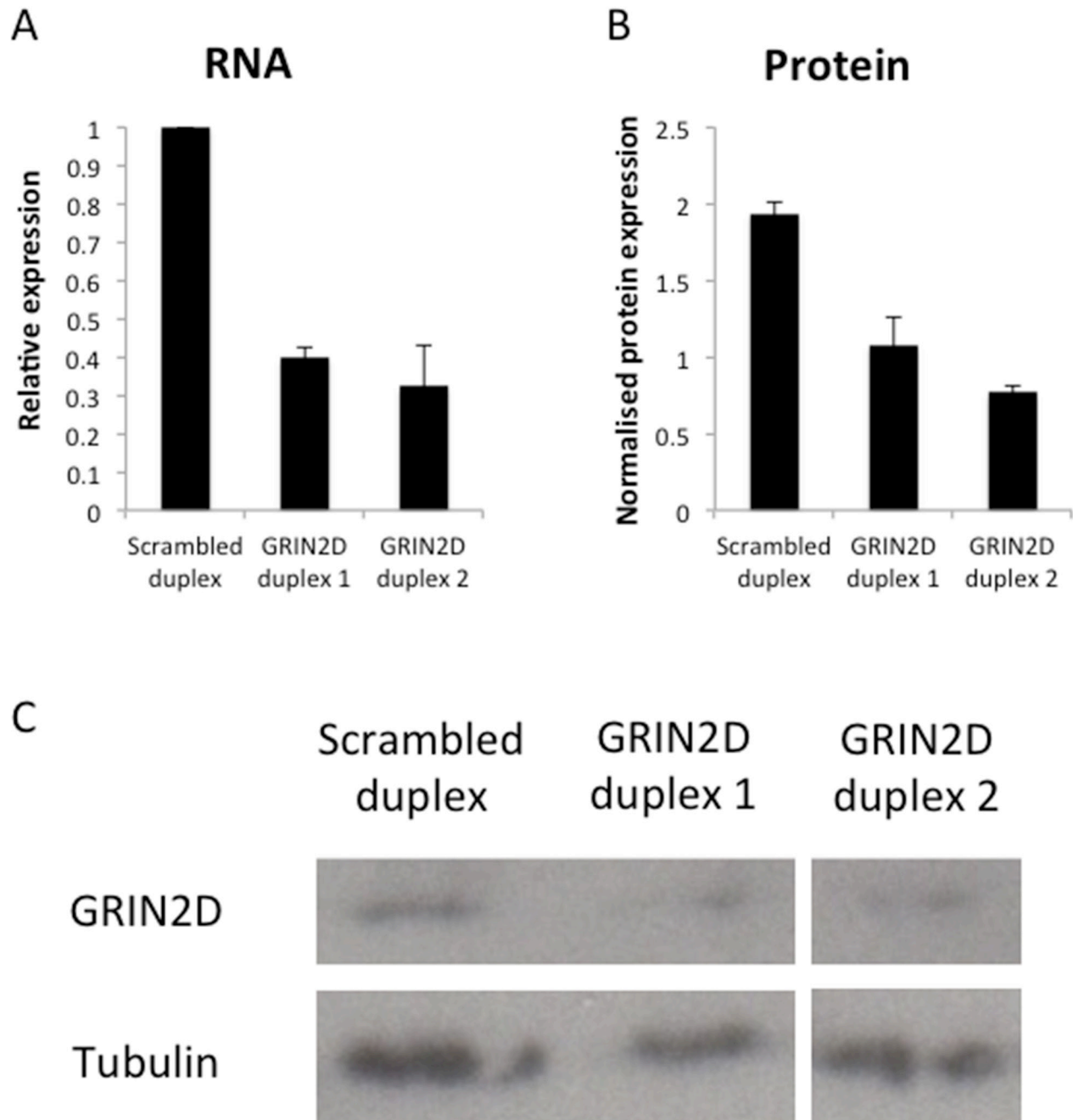


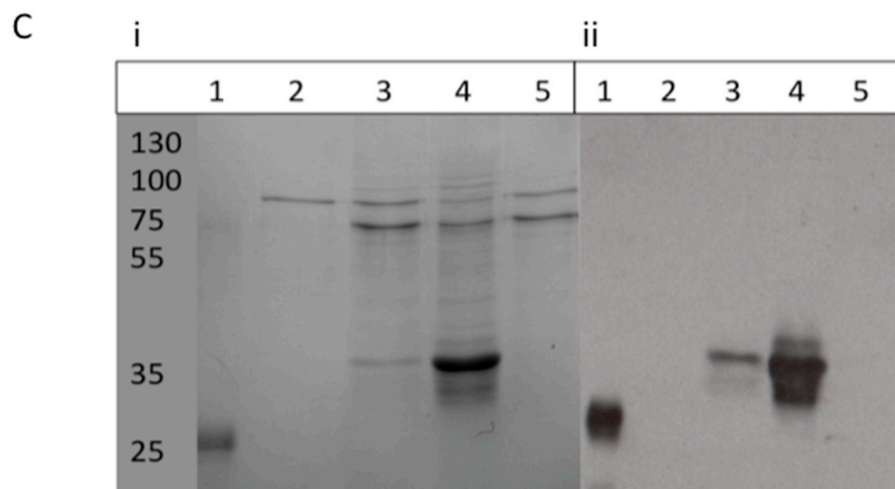
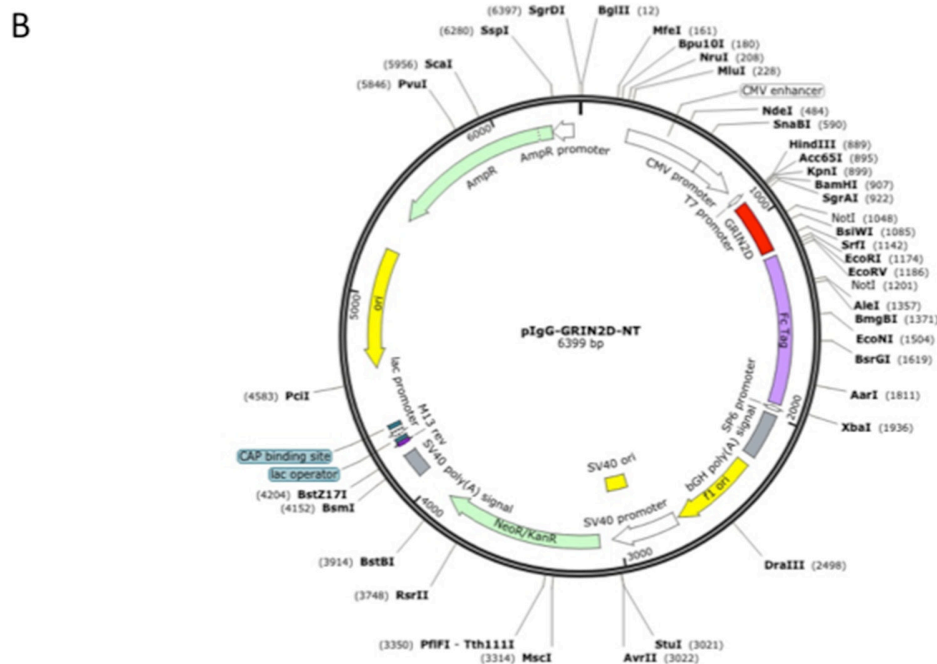
SUPPLEMENTARY FIGURES AND TABLES



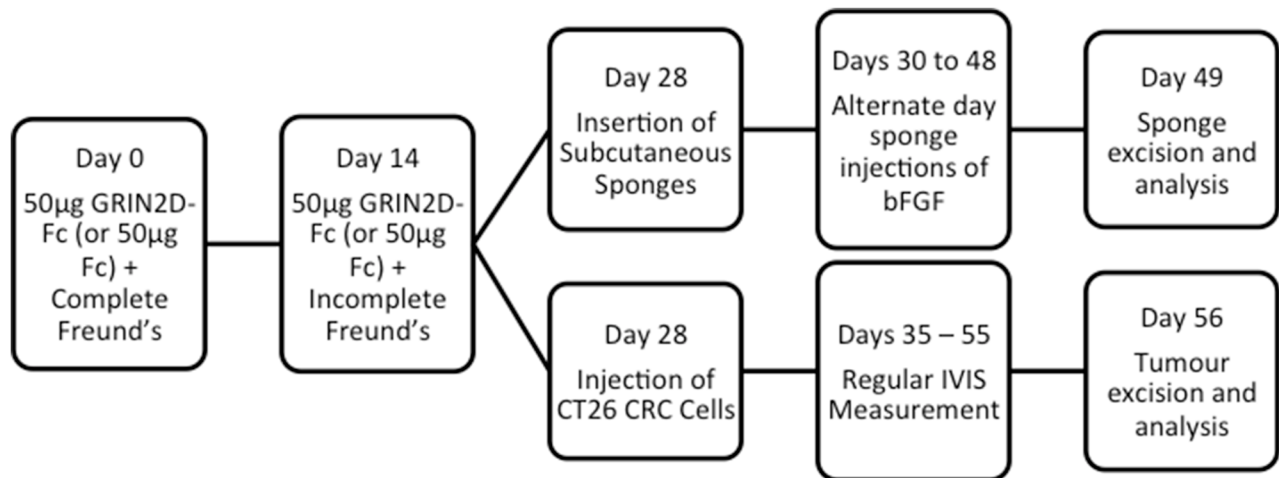
Supplementary Figure S1: siRNA duplexes successfully knockdown GRIN2D expression on the RNA and protein level. Analysis of GRIN2D expression in three cords of HUVEC treated with a scrambled duplex or two GRIN2D targeting duplexes, by **A.** RTqPCR (normalised to flotillin-2) or **B.** western blot (normalised to Tubulin) (n=3, average expression \pm SEM). **C.** representative western blot images (GRIN2D band - 70 kDa, Tubulin band - 55 kDa).

A

| | | |
|--------|--|----|
| | 1 | 60 |
| GRIN2D | MRGAGGPRGPRGPAKMLLLLALACASPFPEEVPGPGAAGGGTGGARPLNVALVFS--GPA | |
| GRIN2A | -----MGR LGYWTLLVLPALL-----VWHGPAQNAAAEKGTPALNI AVLLG-HSHD | |
| GRIN2B | ----MKPSAECCSPKFWLVLAVLAV-----SGSKARSQKSAPSIGI AVILVGTSD E | |
| GRIN2C | ---MGGA---LGPALLTSL-----LGAWAGLGAGQGEQAVTVAVVFGSSGPL | |
| | 78 | |
| GRIN2D | YAAEAARLGPVAAA VRSPG | |
| GRIN2A | VTERE-LRNLWGPEQATGLP | |
| GRIN2B | VAI----KDAHEKDDFH HLS | |
| GRIN2C | QAQARTRLTPQ---NFLDLP | |



Supplemental Figure S2: Construction and production of GRIN2D-Fc fusion protein. A. The N-terminal sequence (bp 1-78) was identified as unique and specific to GRIN2D. B. This peptide was ligated into the pIgG (pcDNA3.1) vector for onward production in HEK-293T cells. Secreted media was collected and affinity purified using a protein A column. C. Corresponding Coomassie Stain (i) and anti-Fc Western Blot (ii) of 12% SDS-PAGE Gel. Lane 1 – Purified Human Fc. Lane 2 – Optimem. Lane 3 – Secreted Media. Lane 4 – Purified GRIN2D-Fc. Lane 5 – Depleted Media. Predicted size of hGRINDFc = 34 kDa; Fc = 26 kDa.



Supplementary Figure S3: Protocols for murine vaccination model followed by either subcutaneous sponge or subcutaneous CT26-luc tumour implantation.

Supplementary Table S1: Matrix metalloproteinases enriched in colorectal cancer vessels

| Gene ID | Gene Symbol | GeneBank accession no. | LogFC | P-value |
|---|-------------|------------------------|-------|---------|
| matrix metalloproteinase 3 (stromelysin 1, progelatinase) | MMP3 | NM_002422 | 5.72 | 0.01 |
| matrix metalloproteinase 7 (matrilysin, uterine) | MMP7 | NM_002423 | 5.54 | 0.00 |
| matrix metalloproteinase 12 (macrophage elastase) | MMP12 | NM_002426 | 4.86 | 0.00 |
| matrix metalloproteinase 1 (interstitial collagenase) | MMP1 | NM_002421 | 4.49 | 0.04 |
| matrix metalloproteinase 9 (gelatinase B) | MMP9 | NM_004994 | 4.10 | 0.00 |
| matrix metalloproteinase 11 (stromelysin 3) | MMP11 | NM_005940 | 3.25 | 0.01 |
| matrix metalloproteinase 10 (stromelysin 2) | MMP10 | NM_002425 | 2.54 | 0.10 |
| matrix metalloproteinase 14 (membrane-inserted) | MMP14 | NM_004995 | 1.94 | 0.04 |
| matrix metalloproteinase 2 (gelatinase A) | MMP2 | NM_004530 | 1.51 | 0.16 |
| matrix metalloproteinase 13 (collagenase 3) | MMP13 | NM_002427 | 1.18 | 0.11 |

Supplementary Table S2: Collagens enriched in colorectal cancer vessels

| Gene ID | Gene Symbol | GeneBank accession no. | LogFC | P-value |
|-------------------------------|-------------|------------------------|-------|---------|
| collagen, type I, alpha 1 | COL1A1 | NM_000088 | 4.18 | 0.00 |
| collagen, type XII, alpha 1 | COL12A1 | NM_004370 | 3.13 | 0.00 |
| collagen, type IV, alpha 1 | COL4A1 | NM_001845 | 2.86 | 0.00 |
| collagen, type I, alpha 2 | COL1A2 | NM_000089 | 2.79 | 0.01 |
| collagen, type XV, alpha 1 | COL15A1 | NM_001855 | 2.56 | 0.01 |
| collagen, type XVIII, alpha 1 | COL18A1 | NM_030582 | 2.42 | 0.00 |
| collagen, type IV, alpha 2 | COL4A2 | NM_001846 | 2.36 | 0.00 |
| collagen, type V, alpha 1 | COL5A1 | NM_000093 | 2.30 | 0.04 |
| collagen, type VIII, alpha 1 | COL8A1 | NM_001850 | 2.28 | 0.02 |
| collagen, type V, alpha 2 | COL5A2 | NM_000393 | 2.10 | 0.03 |
| collagen, type VII, alpha 1 | COL7A1 | NM_000094 | 2.00 | 0.17 |
| collagen, type V, alpha 3 | COL5A3 | NM_015719 | 1.79 | 0.01 |
| collagen, type X, alpha 1 | COL10A1 | NM_000493 | 1.69 | 0.06 |
| collagen, type III, alpha 1 | COL3A1 | NM_000090 | 1.66 | 0.10 |
| collagen, type XIV, alpha 1 | COL14A1 | NM_021110 | 1.54 | 0.08 |
| collagen, type VI, alpha 1 | COL6A1 | NM_001848 | 1.45 | 0.14 |
| collagen, type VI, alpha 3 | COL6A3 | NM_004369 | 1.44 | 0.08 |
| collagen, type IX, alpha 1 | COL9A1 | NM_001851 | 1.08 | 0.11 |

Supplementary Table S3: Key clinical data for CRC cohort showing overall survival (OS)

| Characteristics | N of patients | Median OS | Mean OS |
|------------------------|---------------|-----------|---------|
| All | 90 | 87.5 | 112.8 |
| Gender | | | |
| Male | 65 | 86 | 107.2 |
| Female | 25 | 122 | 127.3 |
| Age | | | |
| above 58 | 47 | 122 | 130.2 |
| below or equal to 58 | 43 | 67 | 93.8 |
| Stage | | | |
| I/II | 44 | 157.5 | 148.5 |
| III/IV | 46 | 38 | 78.6 |
| Tumour location | | | |
| Ascending colon | 21 | 27 | 63.5 |
| Descending colon | 6 | 194 | 183 |
| Sigmoid colon | 31 | 84 | 96.6 |
| Transverse colon | 9 | 186 | 127.1 |
| Cecum | 4 | 102.5 | 109 |
| Rectum | 19 | 186 | 165.6 |
| T-stage | | | |
| T2 | 8 | 187.5 | 160.5 |
| T3 | 75 | 86 | 105.5 |
| T4 | 7 | 122 | 136.3 |
| N-stage | | | |
| N0 | 50 | 129.5 | 138.3 |
| N1+ | 40 | 33 | 80.1 |
| M-stage | | | |
| M0 | 75 | 116 | 126.3 |
| M1 | 15 | 42 | 45.3 |
| GRIN2D staining | | | |
| Positive | 21 | 154 | 137.6 |
| Negative | 69 | 86 | 109.8 |