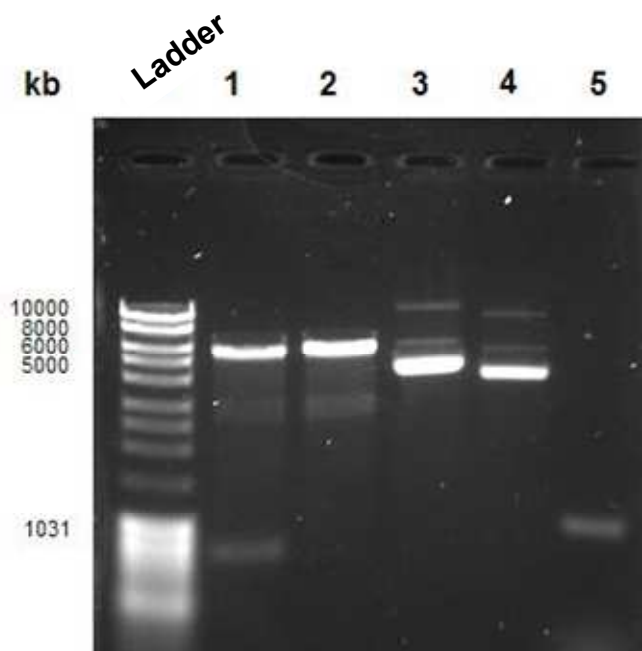


## Supporting Information Figure 1



**Constructed pET-LinkCD plasmid for the expression of the LinkCD fusion protein.** The insert size is 885 bp. 1) Double digestion of pET-LinkCD with NdeI and BamHI restriction enzymes. 2) double digestion of empty plasmid, 3) uncut pET-LinkCD, 4) uncut pET15, 5) PCR amplified LinkCD fragment.

## Supporting Information Figure 2

**A**

```

reference      GGCTACAAGGAGGGAGGGGTCCCAATTGGTGGCTGCCTCATCAACAACAAGGATGGCAGT 540
mutant         GGCTACAAGGAGGGAGGGGTCCCAATTGGTGGCTGCCTCATCAACAACAAGGATGGCAGT 540
                *****

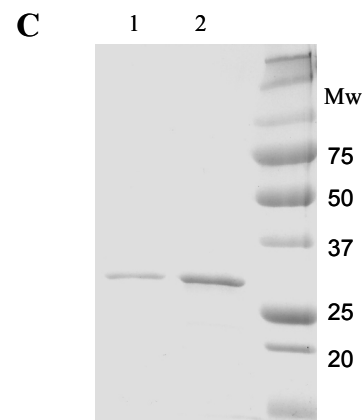
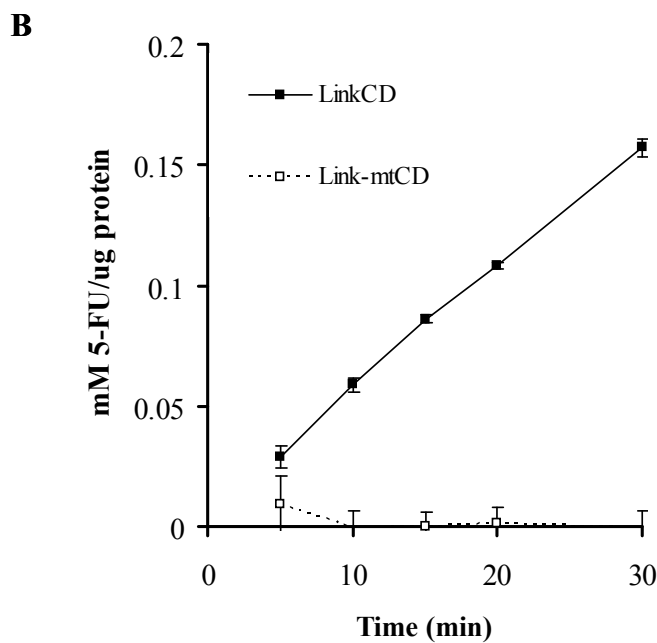
reference      GTCCTGGGCAGGGGCCACAACATGAGGTTCCAGAAGGGCAGTGCCACCCTGCATGGGGAG 600
mutant         GTCCTGGGCAGGGGCCACAACATGAGGTTCCAGAAGGGCAGTGCCACCCTGCATGGGGCG 600
                *****

reference      ATCAGCACCTGGAGAACTGTGGCAGGCTGGAGGGCAAGGTCTACAAGGACACCACTCTG 660
mutant         ATCAGCACCTGGAGAACTGTGGCAGGCTGGAGGGCAAGGTCTACAAGGACACCACTCTG 660
                *****

reference      TACACCACCTCAGCCCTTGTGACATGTGCACAGGGGCCATCATCATGTATGGCATTCCC 720
mutant         TACACCACCTCAGCCCTTGTGACATGTGCACAGGGGCCATCATCATGTATGGCATTCCC 720
                *****

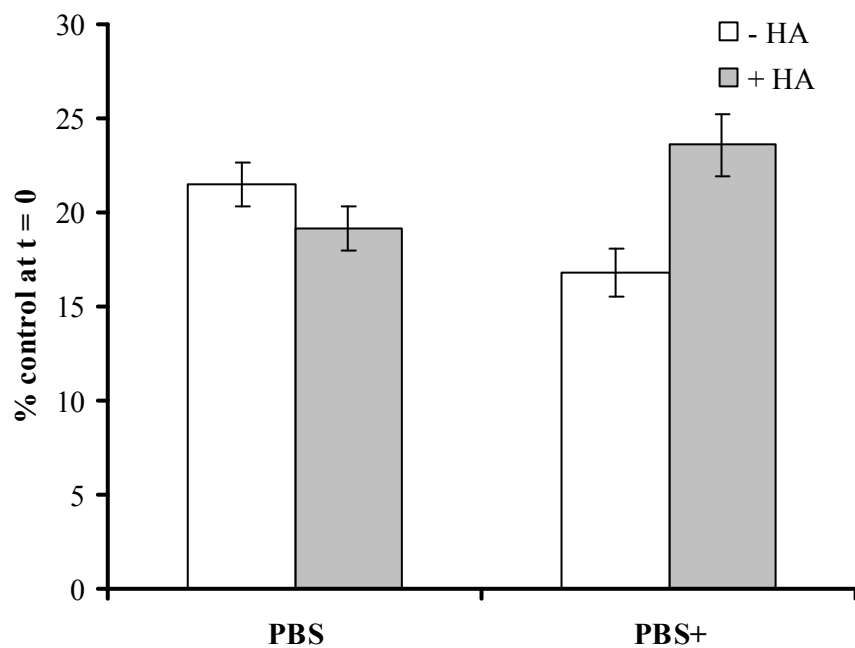
```

\*\*\*\*\*



**Link-mtCD: a functional mutant that lacks the enzyme activity.** **A.** A sequence alignment between the pET-Link-mtCD (mutant) and pET-LinkCD (reference) showing the point mutation of A to C, indicated by the arrow. **B.** Enzyme assay shows that Link-mtCD does not convert 5-FC to 5-FU. **C)** SDS-PAGE gel showing purified LinkCD (lane 1) and Link-mtCD (lane 2).

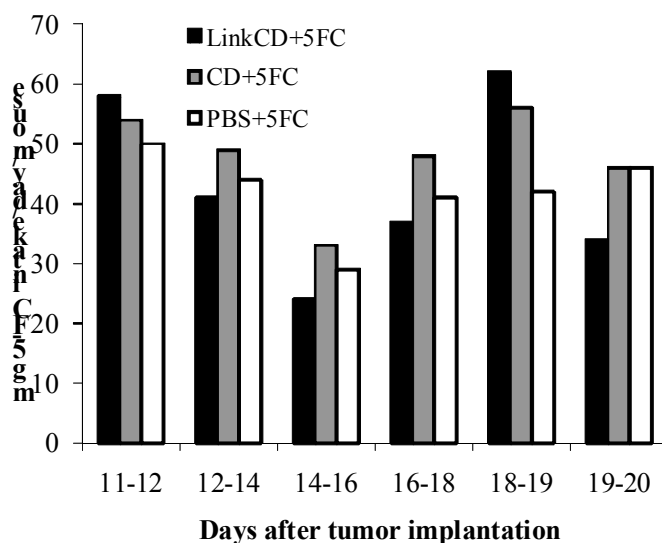
## Supporting Information Figure 3

**Cytosine deaminase activity of the LinkCD fusion protein after 24 hr incubation at 37°C in PBS.**

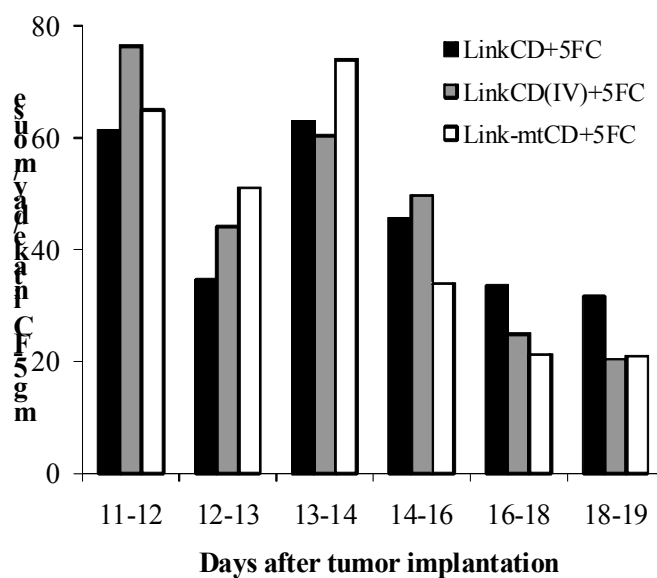
Enzyme activity of LinkCD in the presence of 0.1 mg/ml hyaluronan (HA). PBS+ contained  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  whereas PBS did not contain these divalent cations.

## Supporting Information Figure 4

A



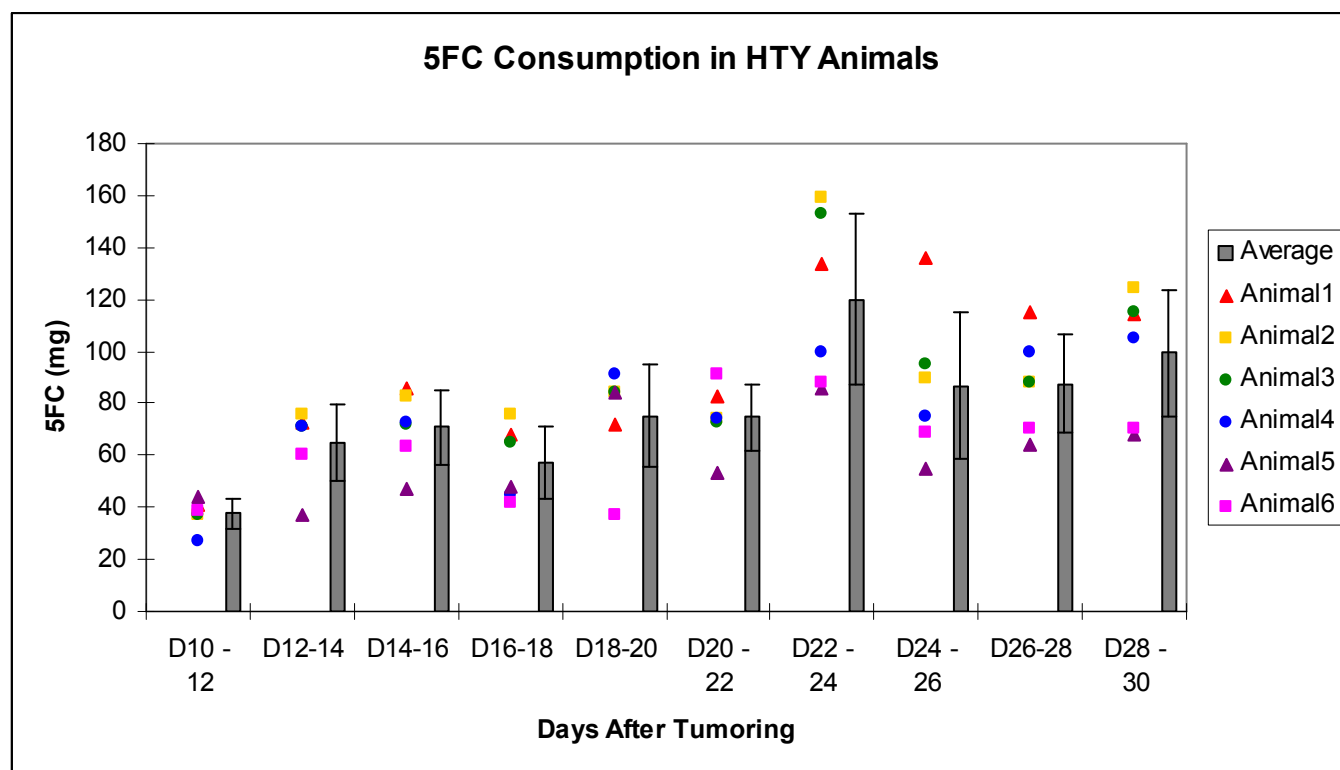
B



**5-FC consumption by animals in therapeutic experiments.** The amount of 5-FC consumption was estimated by weighing water bottles containing 5-FC (10mg/mL). **A.** Anti-tumor experiment with LinkCD + 5FC, CD + 5FC, and PBS + 5FC groups. **B.** Second experiment with LinkCD + 5FC, LinkCD (IV) + 5FC, and Link-mtCD + 5FC groups.

## Supporting Information Figure 5

	5-FC intake (mg)/day									
	Day 10-12	Day 12-14	Day 14-16	Day 16-18	Day 18-20	Day 20-22	Day 22-24	Day 24-26	Day 26-28	Day 28-30
Animal 1	41	73	86	68	72	83	134	136	115	114
Animal 2	37	76	83	76	84	74	159	90	88	124
Animal 3	37	71	72	65	84	73	153	95	88	115
Animal 4	27	71	73	45	91	74	100	75	100	105
Animal 5	44	37	47	48	84	53	86	55	64	68
Animal 6	39	60	63	42	37	91	88	69	70	70
Mean	38	65	71	57	75	75	120	87	88	99
stdev	6	15	14	14	20	13	33	28	19	24



**5-FC consumption by individual animal that received LinkCD treatment.** Each animal was housed separately and the daily intake of 5-FC by individual animal was monitored. The survival of animals showed similar results as shown in Figure 4 and 5.

**Supporting Information Table 1: Summary of SPR binding kinetics data**

pH	$k_a$ ( $M^{-1}S^{-1}$ )	$k_d$ ( $S^{-1}$ )	$K_D$ (M)	$\chi^2$
6.0	246.06	0.0013	5.32E-6	0.168
7.4	49.35	0.0027	5.50E-5	0.705

Summary of the binding kinetics between LinkCD and 12-mer hyaluronan measured by SPR. The  $\chi^2$  value represents the sum of squared deviation of the binding model from the experiment data. The model fits well for the interaction between LinkCD and hyaluronan at pH 7.4.