Supplementary Table I: Summary of the Effects of LTβR Activation on the Growth of Various Human Tumor Cell Lines

Tumor Line	In vitro Growth Inhibition Short term[a]	Soft Agar[b]	In vivo Tumor Inhibition[c]
Colorectal			
WiDr	+++	nd[d]	+++
HT29	+++	+++	+++
KM20L2	+++	nd	+++
LS174T	-	nd	-
HCT-15	-	nd	-
DLD-1	-	+	-
HCT-116	-	nd	-
Geo	-	nd	-
KM12	-	nd	-
Colo205	-	nd	-
Cervical			
HT-3	-[e]	nc [f,g]	+++
ME180	-	++	nd
Melanoma			
A375	-	++	+/-
Breast			
DU445	-	+++	-
MCF7	-	-	-
MDA-MB-468	-	nc[f]	-
MDA-MB-231[h]	-	++	-
Lung			
A549	-	-	-
Rhabdomyosarcoma			
RD	+/-	nd	nd
A673	-	nd	nd
RMS13	-	nd	nd

[a] Effects on a 3-5 day MTT proliferation assay either with or without added INFγ.
[b]Colony formation in soft agar in the presence and absence of CBE11p
[c]Effects of CBE11 in a xenograft model
[d]nd, not done
[e]Altered cell morphology, flattened cells
[f]nc, no colonies
[g]HT3 cells showed +++ inhibition when grown in long term cultures on a collagen gel.
[h]NCI version of this line was tested in vivo.

Supplementary Table. II: Samples used to develop the predictor gene panel

CELL LINE	FORMAT	SAMPLES		
DLD1	culture	3	RESISTANT	TRAINING SET
DLD1	xenograft	10		
HCT116	culture	5		
HCT116	xenograft	2		
HCT15	culture	3		
HCT15	xenograft	2		
LoVo	culture	1		
LoVo	xenograft	2		
SW480	culture	1		
SW480	xenograft	3		
HT29	culture	3	SENSITIVE	
WiDr	culture	3		
WiDr	xenograft	8		
Colo205	*culture*	*1*	UNKNOWN	TEST SET
Colo205	*xenograft*	*1*		
Geo	*xenograft*	*3*		
KM12	*culture*	*1*		
KM12	*xenograft*	*2*		
KM20L2	*culture*	*1*		
KM20L2	*xenograft*	*6*		
LS174T	*culture*	*1*	KNOWN RESISTANT	
LS174T	*xenograft*	*5*		
SW620	*xenograft*	*1*		

Supplementary Table II
Listing of the samples used to generate the predictor panel of genes. Xenograft samples were harvested at 21 and 32-35 days after implantation (0.5-2 cm3 tumor volume). Cell culture samples were prepared from cells grown to near-confluence under standard conditions as specified by ATCC.

Gene expression profiles from those tumors and cell lines listed in the training set were used to develop a predictor gene panel. The gene predictor panel was then applied to a set of four tumor lines with unknown sensitivity along with the known resistant LS174T and SW620 lines (not included in the original training set) as positive controls. KM20L2 was defined as a potential responder and in vivo and in vitro testing (supplementary figure 1) revealed that this line did respond to CBE11. The other three lines, Geo, KM12 and Colo205, were not responsive both in vivo and in vitro.

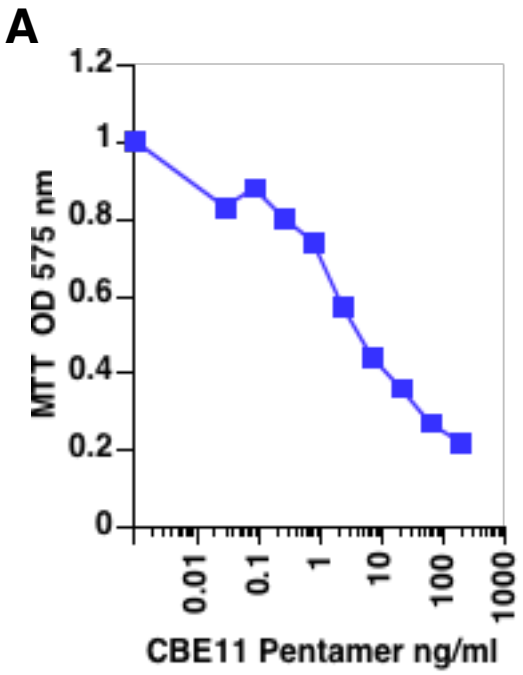
Table III: Summary of the Effects of CBE11 on Low Passage Colorectal Tumors in an Orthotopic Xenograft Study

		50% Survival Time (Days)		
Tumor ID[a]	n	PBS	1E6[b]	CBE11
AC3717-1	10	21	30	121[c]
AC3717-2	20	nd	170	>347[c]
AC3609	10	113	113	>113[d]
AC3603	10	24	45	24
AC3557-1	10	97	14	128
AC3557-2	20	nd	311	326
AC3528-1	10	90	76	111
AC3528-2	10	284	314	266
AC3445	10	53	23	23

[a]ID- Expt number
[b]1E6 was a matched control binding anti-LFA3 mAb.
[c] $p < 0.05$ vs. saline or control mAb, log-rank analysis.
[d]A precise survival value was not obtained, however, tumor take rates were reduced by 50% by CBE11 and growth was strongly reduced.

Patient tumors were passaged directly as small pieces on the cecum in mice.

Supplementary Figure 1



Legend

The human colorectal tumor cell line KM20L2 is sensitive to LTβR activation. A). Soluble pentameric CBE11 (CBE11p) inhibits the growth of KM20L2 cells in an in vitro culture (5 day MTT proliferation assay with 80 U/ml hIFNγ included). B). Effects of murine CBE11 on the growth of established KM20L2 tumors grown in nude mice (n = 30 saline vehicle, otherwise n = 10). CBE11 was administered ip on days 8 and 22 and chemotherapeutic agents were injected on days 8, 12 and 16 (cytoxan, ip) and 8, 10, 12, 14, 16 and18 (cis-platinum, sc). Red squares and circles are largely coincident.