

SUPPLEMENTARY INFORMATION

**A Human Antibody against Human Endothelin Receptor
Type A that Exhibits Antitumor Potency**

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Supplementary Figure Legends

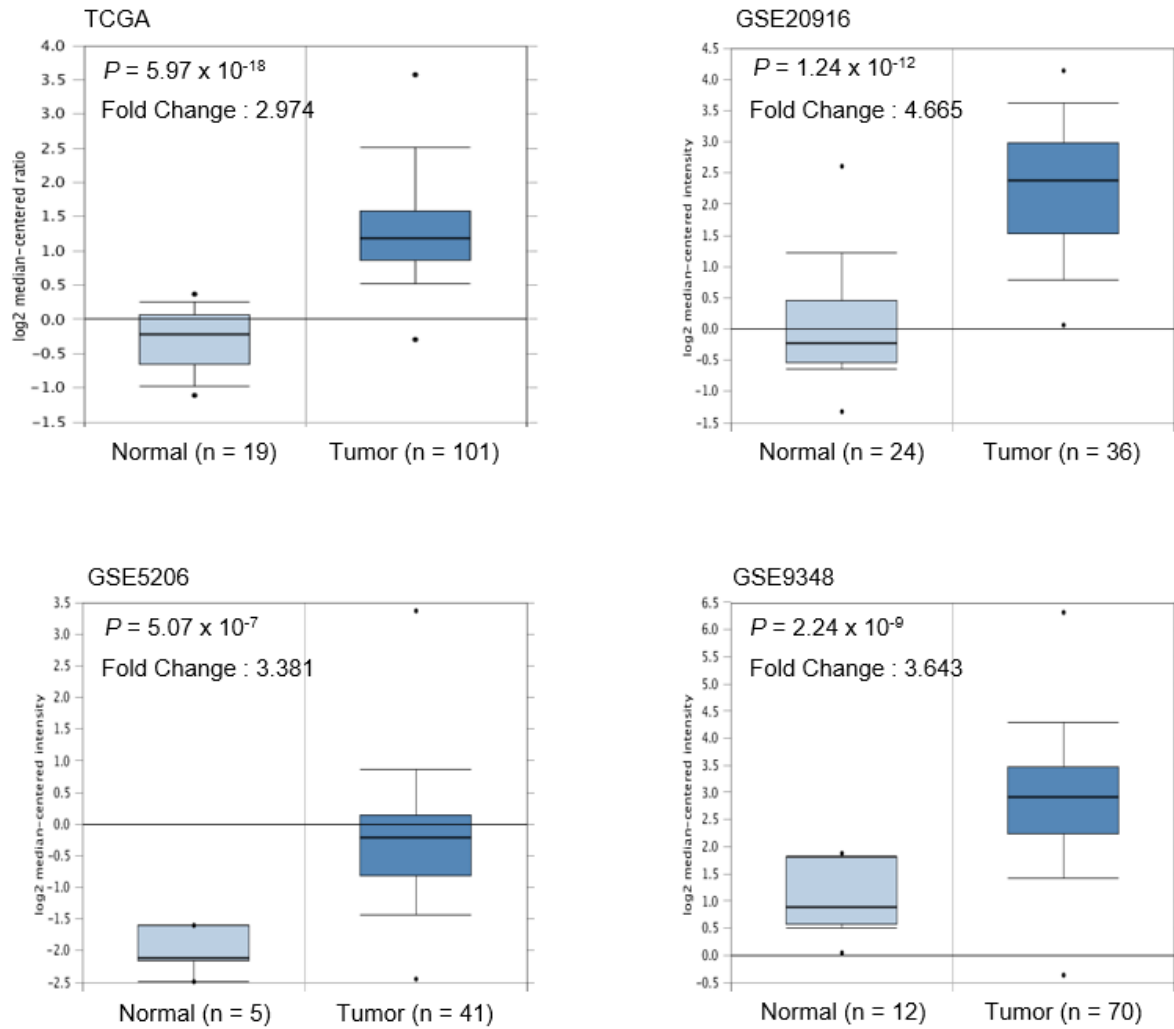
Supplementary Figure 1. Analysis of ET_A mRNA expression in normal colon and tumor colon tissues from the Oncomine database (n is number of samples).

Supplementary Table 1. Primers used in this study (underlining indicates the restriction enzyme sites).

Supplementary Table 2. Plasmids used in this study.

Supplementary Table 3. Output phage titers after each round of screening of the human scFv phage display library against human ET_A.

Supplementary Figure 1. Analysis of ET_A mRNA expression in normal colon and tumor colon tissues from the Oncomine database (n is number of samples).



Supplementary Table 1. Primers used in this study (underlining indicates the restriction enzyme sites).

Primer Name	Primer Nucleotide Sequence (5' → 3')
MSJ#01	GAGCCTGGTGCCGCGCGGCTCCCGGGACAACCTGAGAGATACAGTGCAA
MSJ#02	GGTGATGGTGATGGTGAGAAGCTTCATTCATGGAGTCTTTATGCGACGAA
MSJ#03	GCGGAATTCATATGCTAAAGCTCCTTGACAACCTGGGACAGCGTGACCTCCACCTTCAGC AAGCTGCGCGAACAGCTCGGCCCTGTGACCCAGGAGTTCTGGGATAACCTGGAAAAGG
MSJ#04	CTCCTGCCACTTCTTCTGGAAGTCGTCAGGTAGGGCTGCACCTTGGCCTTACCTCCT CCAGATCCTTGCTCATCTGCCTCAGGCCCTGTCTCCTTTCCAGGTTATCCAGAA
MSJ#05	CCAGAAGAAGTGGCAGGAGGATGGAGCTACCGCCAGAAGGTGGAGCCGTGCGCGC AGAGCTCCAAGAGGGCGCGGCCAGAAGCTGCACGAGCTGCAAGAGAAGCTGAGCCACT
MSJ#06	GGCGCGCGCCAAGCGCTGGCGCAGCTCGTCGCTGTAGGGGGCCAGATGCGTGCGCAGC GCGTCCACATGGGCGCGCGCGGTCGCGCATCTCTCGCCAGTGGGCTCAGCTTCTCTT
MSJ#07	TTGGCCGCGCCTTGAGGCTCTCAAGGAGAACGGCGCGCCAGACTGGCCGAGTACCAC GCCAAGGCCACCGAGCATCTGAGCAGCTCAGCGAGAAGGCCAAGCCGCGCTCGAGGAC
MSJ#08	GAATTCGATCCTCACTGGGTGTTGAGCTTCTAGTGTACTCCTCGAGAGCCTCAGGAAG CTGACCTTGAAGCTCCTCAGCACGGGCAGCAGGCCTTGGCGGAGGTCTCAGCGCGGG
MSJ#09	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGCAGGTGCAGCTGGTGCAGTCTGG
MSJ#10	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGCAGGTCACTTAAGGGAGTCTGG
MSJ#11	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGGAGGTGCAGCTGGTGGAGTCTGG
MSJ#12	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGCAGGTGCAGCTCAGGAGTCTGGG
MSJ#13	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGGAGGTGCAGCTGTTGAGTCTGC
MSJ#14	GCTACCGTGGCCAGCCGGCCATGGCGATGGCGCAGGTACAGCTGCAGCAGTACAG
MSJ#15	CAGATCCACCTCCACCGAACCTCCACCTCCAGAGCTCCCTCCACCTGAGGAGACGGTGACCAGGGTGCC
MSJ#16	CAGATCCACCTCCACCGAACCTCCACCTCCAGAGCTCCCTCCACCTGAAGAGACGGTGACCATTGTCCC
MSJ#17	CAGATCCACCTCCACCGAACCTCCACCTCCAGAGCTCCCTCCACCTGAGGAGACGGTGACCAGGGTTCC
MSJ#18	CAGATCCACCTCCACCGAACCTCCACCTCCAGAGCTCCCTCCACCTGAGGAGACGGTGACCGTGGTTCC
MSJ#19	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGACATCCAGATGACCCAGTCTCC
MSJ#20	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGATGTTGTGATGACTCAGTCTCC
MSJ#21	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGAAATTGTGTTGACGCAGTCTCC
MSJ#22	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGACATCGTATGACCCAGTCTCC
MSJ#23	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGAAACGACACTCAGCAGTCTCC
MSJ#24	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCGAAATTGTGCTGACTCAGTCTCC
MSJ#25	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCCAGTCTGTGTTGACGCAGCCGCC
MSJ#26	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCCAGTCTGCCCTGACTCAGCCTGC
MSJ#27	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCTCCTATGTGCTGACTCAGCCACC
MSJ#28	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCTCTTCTGAGCTGACTCAGGACCC
MSJ#29	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCCAGTTATACTGACTCAACCGCC
MSJ#30	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCCAGGCTGTGCTCACTCAGCCGTC
MSJ#31	GGTCCGGTGGAGGTGGATCTGGTGGAGGTGCTAGCAATTTTATGCTGACTCAGCCCCA
MSJ#32	GGTGCTGGCCCCGAGGCCCGTACGTTTGATTTCCACCTTGGTCCC
MSJ#33	GGTGCTGGCCCCGAGGCCCGTACGTTTGATCTCCAGCTTGGTCCC
MSJ#34	GGTGCTGGCCCCGAGGCCCGTACGTTTGATATCCACTTTGGTCCC
MSJ#35	GGTGCTGGCCCCGAGGCCCGTACGTTTGATCTCCACCTTGGTCCC
MSJ#36	GGTGCTGGCCCCGAGGCCCGTACGTTTAATCTCCAGTCTGTCCC
MSJ#37	GGTGCTGGCCCCGAGGCCCGTACCTAGGACGGTGACCTTGGTCCC
MSJ#38	GGTGCTGGCCCCGAGGCCCGTACCTAGGACGGTGACCTTGGTCCC
MSJ#39	GGTGCTGGCCCCGAGGCCCGTACCTAAAACGGTGAGCTGGGTCCC
MSJ#40	GCTACCGTGGCCAGCCGGCCATGGCG
MSJ#41	GGCCTCGGGGGCCAGCACC
MSJ#42	CCACAGGCGCGCACTCCGAGGTGCAGCTGGTGGAG
MSJ#43	CCTGGTCACCGTCTCCTCAGCAAGCTTCAAGGGCCCA
MSJ#44	TGGGCCCTTGAAGCTTGTGAGGAGACGGTGACCAGG
MSJ#45	CGAGCTICTAGATCATTTACCCGGGGACAGGGAG
MSJ#46	CCACAGGCGCGCACTCCGGACATCGTGATGACCCAGTCTCC
MSJ#47	GGACCAAGGTGGAGATCAAACGTACGGCGGCCGCAC
MSJ#48	GTGCGGCCGCCGTACGTTTGATCTCCACCTTGGTCC
MSJ#49	CGAGCTTCTAGAGCACTCTCCCCTGTTGAAGC
MSJ#50	GGTCCCTTGTGAGCCTTGA
MSJ#51	AGGAAASGTGCCTGTGATGGG

Supplementary Table 2. Plasmids used in this study.

Plasmid	Relevant characteristics	Reference or source
pP9	Ap _r , T7 promoter, thrombin cleavage site, P9 gene in pRSET _A	20
pP9-mET _A	Ap _r , T7 promoter, thrombin cleavage site, P9 gene, mouse ET _A in pP9	This study
pP9-hET _A	Ap _r , T7 promoter, thrombin cleavage site, P9 gene, human ET _A in pP9	20
pET28a(+)	Kan _r , T7 promoter, thrombin cleavage site, polyhistidine tag	Novagen
pET28-MSP1	Kan _r , T7 promoter, thrombin cleavage site, N-terminal polyhistidine tag	This study
pMAZ-IgH-GlycoT	Trastuzumab H chain gene in pMAZ-IgH-H23	21
pMAZ-IgL-GlycoT	Trastuzumab L chain gene in pMAZ-IgL-H23	21
pComb3X	Ap _r , lac promoter, polyhistidine tag, HA tag in phagemid	24
pEL3X	Modified <i>Sfi</i> I restriction nucleotide sequence in pComb3X	This study
pET28a-G _{α3}	Kan _r , T7 promoter, thrombin cleavage site, N-terminal polyhistidine tag, human G _{α3} gene in pET28a(+)	25

Supplementary Table 3. Output phage titers after each round of screening of the human scFv phage display library against human ET_A.

Round	1	2	3	4	5
Number of washes	2	3	3	6	10
Output phage titer (cfu/ml)	2×10^6	1.5×10^6	2×10^6	1.8×10^5	1×10^6