Figure S1

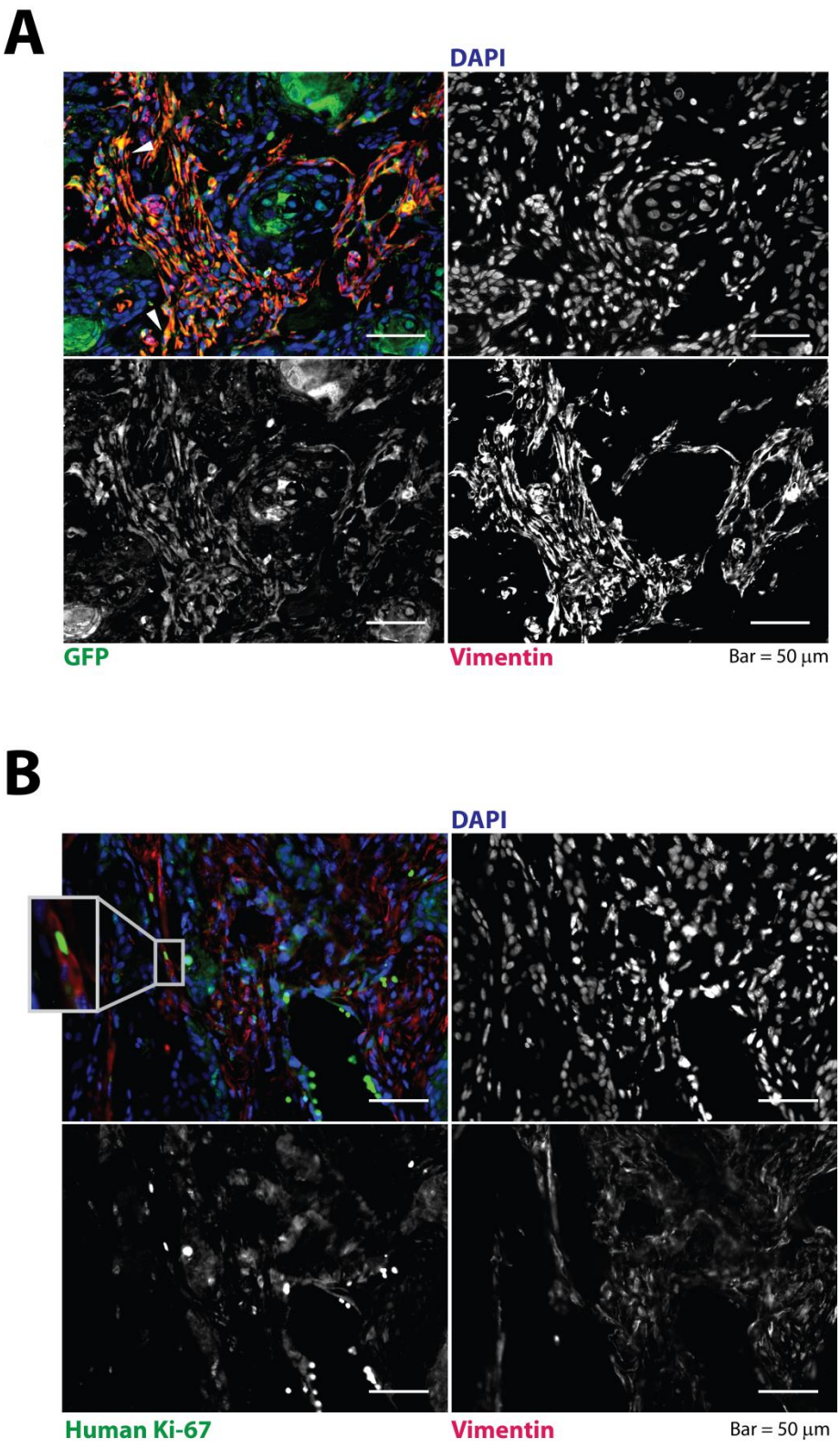


Figure S1: Human CAFs persist within the subcutaneous xenograft tumors. Related to Figure 1.

Representative images of TE11+FEF3303 co-implanted tumors co-stained for DAPI, vimentin, and GFP (A) or human Ki-67 (B) by immunofluorescence.

Figure S2

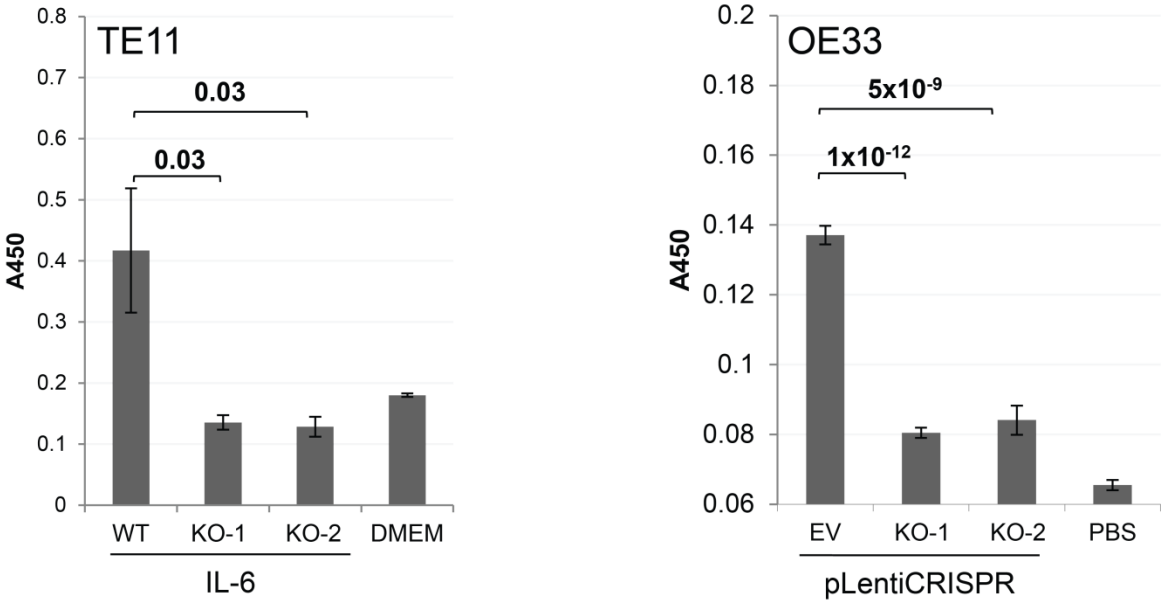


Figure S2: Quality-control of the IL-6-knockout subclones of TE11 ESCC cell line and OE33 EAC cell line. Related to Figure 3.

Levels of IL-6 in cell culture conditioned media were measured by ELISA and compared to wild-type (TE11) or empty-vector control (OE33) conditioned media, as well as to DMEM (TE11) or PBS (OE33) (negative control). Results shown are for the subclones at passage 3 post-transfection with the CRISPR/Cas9 construct. The stability of knockout was confirmed by ELISA for up to passage 12 post-transfection.

Figure S3

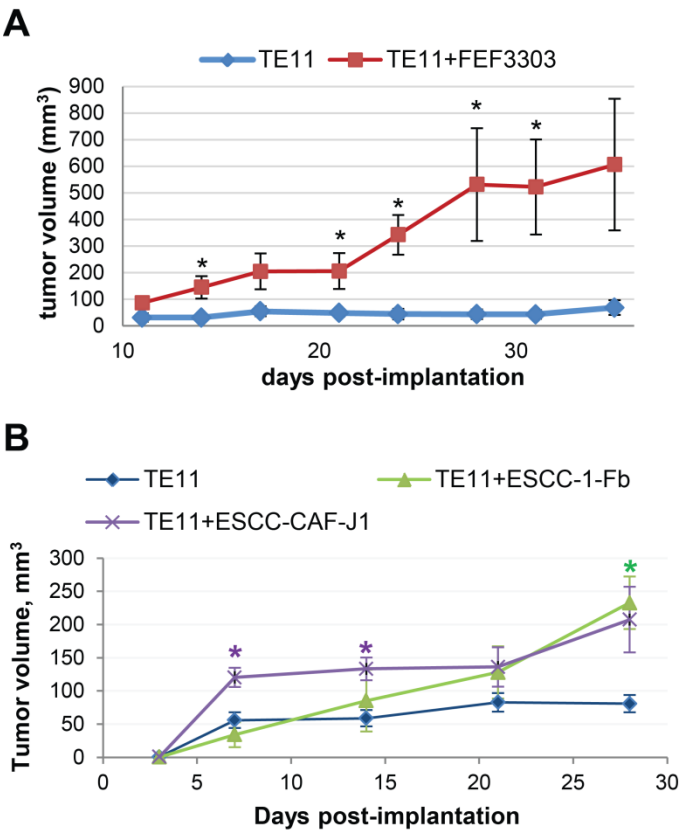


Figure S3: Co-implantation TE11 cells with CAFs enhances growth of ESCC xenografts. Related to Figure 1.

(A) Growth kinetics of subcutaneous xenograft tumors formed by TE11 cells alone or co-injected with FEF3303 fibroblasts (n=4; *p<0.05). (B) Growth kinetics of subcutaneous xenograft tumors formed by TE11 cells alone or co-injected with two ESCC CAF cell lines, ESCC-1-Fb or ESCC-CAF-J1 (n=4 (TE11 mono) or 8 (TE11+CAF); *p<0.05).

Figure S4

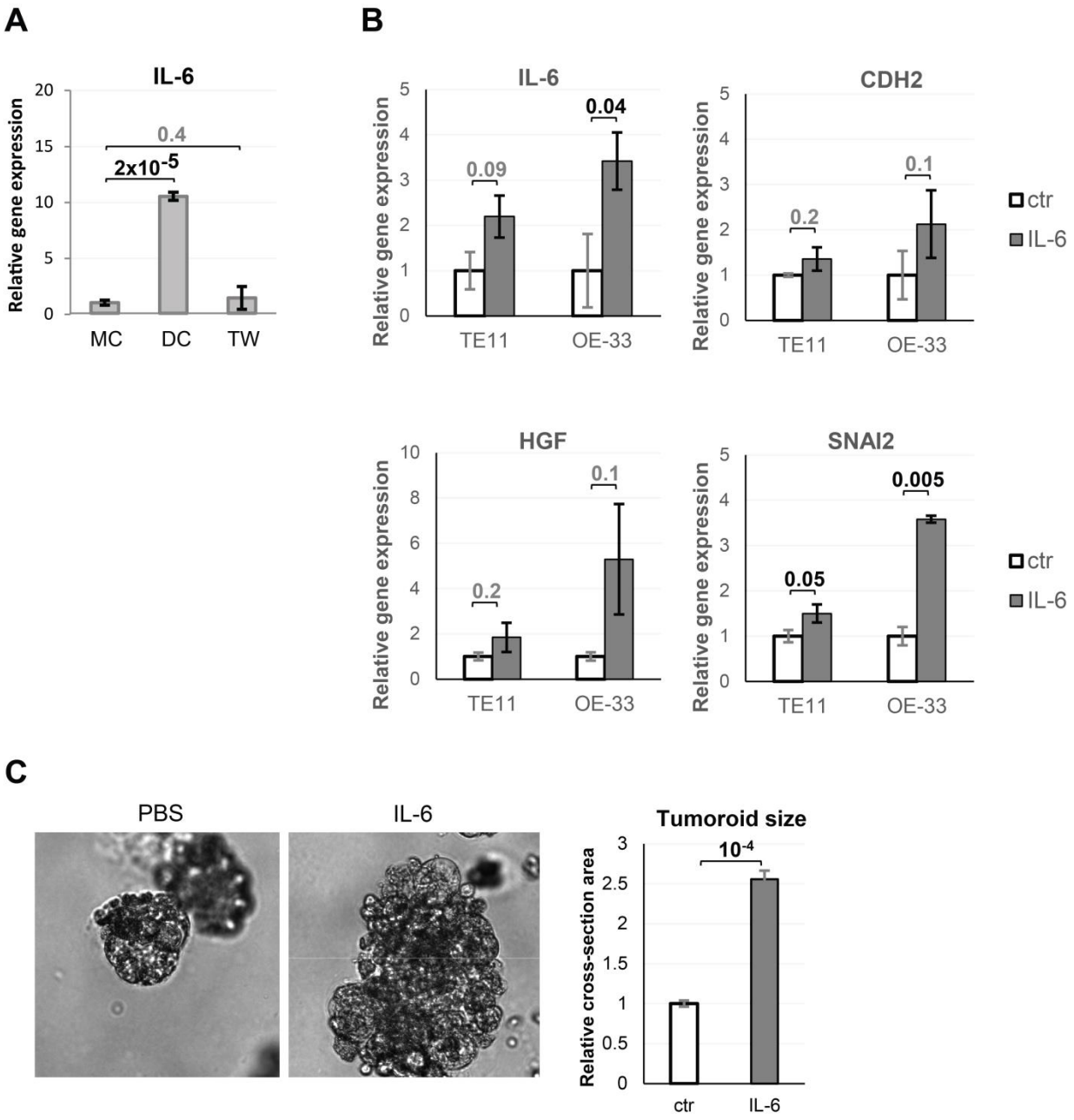


Figure S4: IL-6 is a mediator of the interaction between tumor cells and CAFs in esophageal cancer. Related to Figure 2.

(A) qPCR analysis of IL-6 gene expression in TE11 cells in mono-culture (MC), compared to either direct co-culture (DC) or transwell co-culture (TW) (n=3/cohort). (B) qPCR analysis of gene expression in ESCC or EAC 3D tumoroids (TE11 or OE-33, respectively), treated with either recombinant human IL-6 or PBS (ctr=control) (n=2/cohort; results of one of 2 independent experiments are shown). (C) Representative brightfield images of 3D tumoroids formed from OE-33 cells, treated with either recombinant human IL-6 or PBS (ctr). Tumoroid size is quantified as relative cross-sectional area; tumoroid shape quantified as deviation from elliptical shape (n=2/cohort; results of one of two independent experiments are shown).

Figure S5

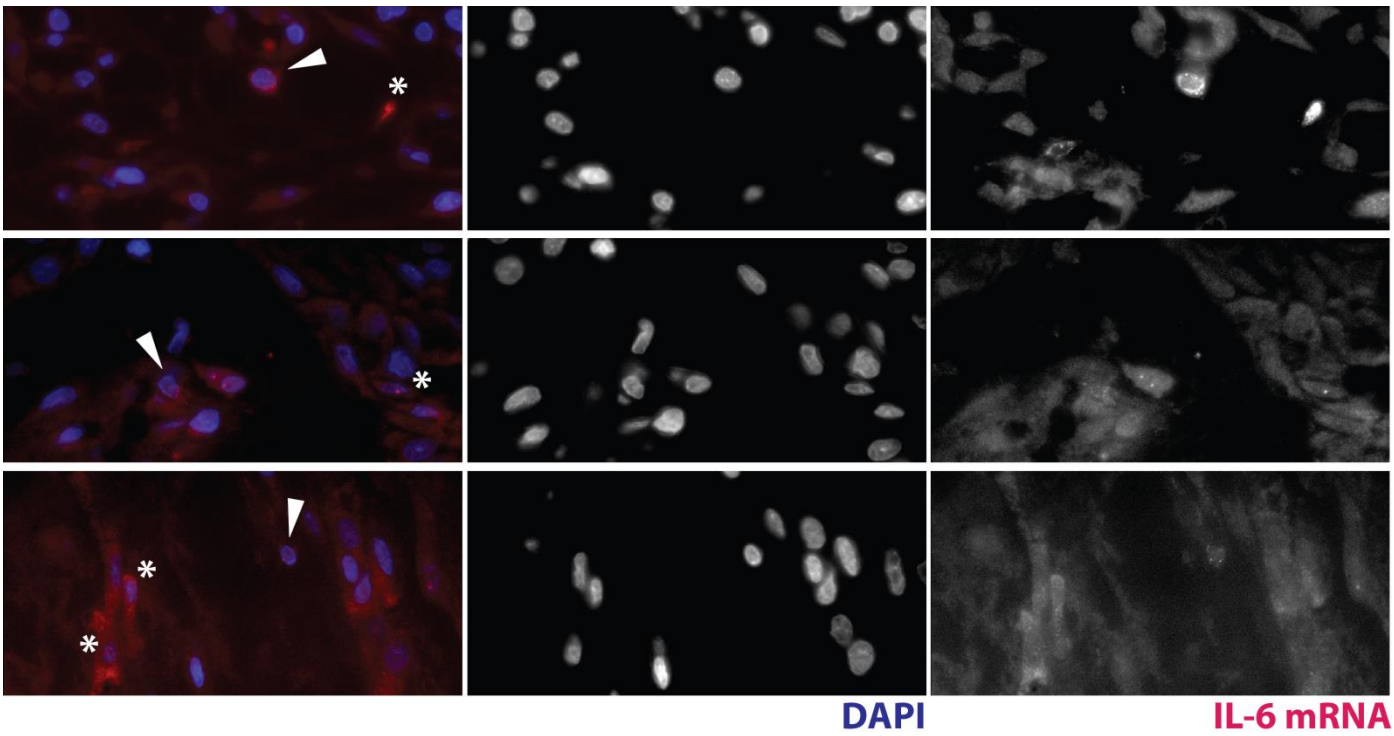


Figure S5: IL-6 is produced by both tumor cells and CAFs in patient tissue. Related to Figure 2.

Tissue section from an ESCC patient was stained for IL-6 mRNA by fluorescent *in situ* hybridization (FISH). Arrowheads indicate IL-6^{+} tumor cells, asterisks – fibroblasts.

Figure S6

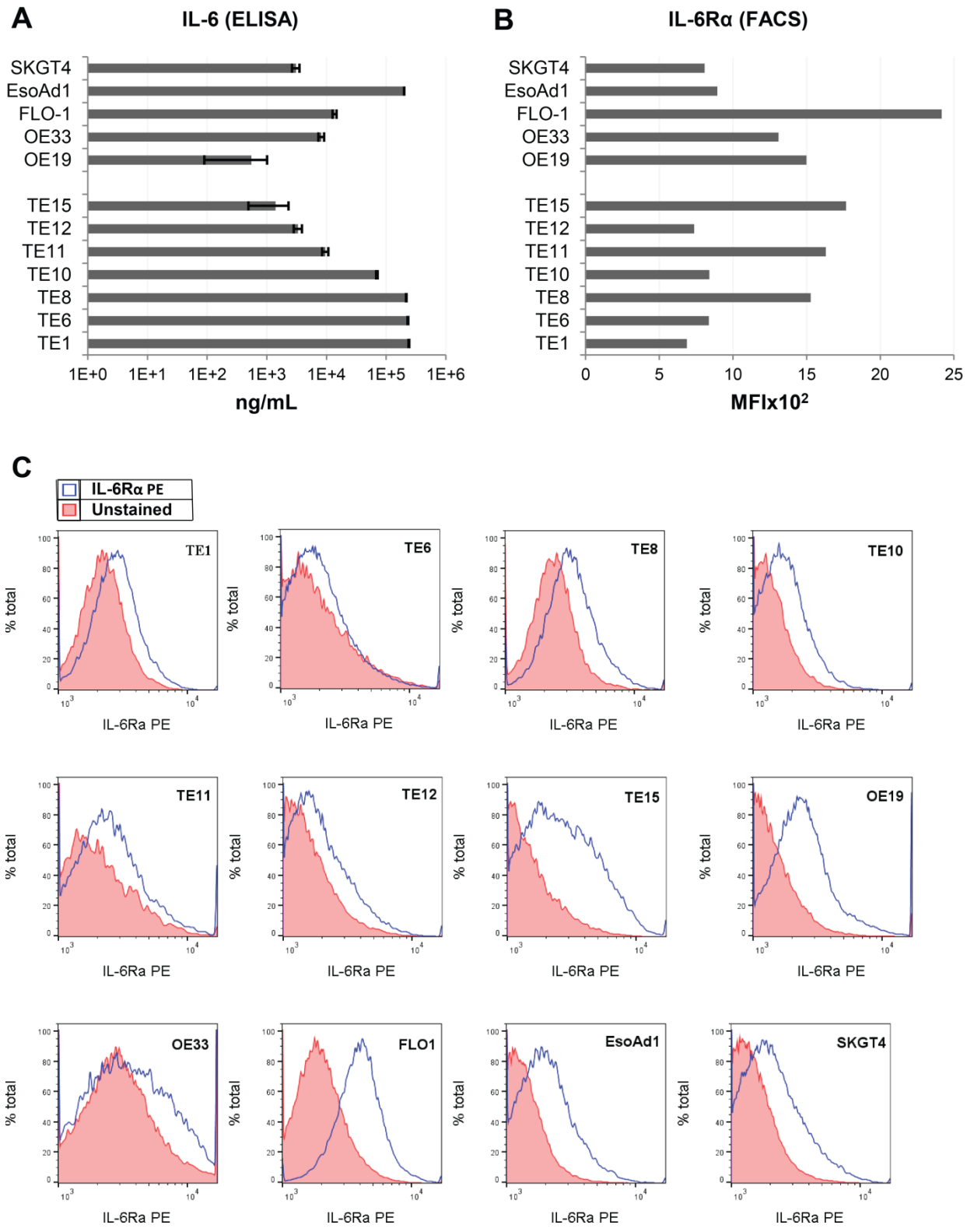


Figure S6: IL-6 and IL-6Rα are expressed by ESCC and EAC cell lines. Related to Figure 2.

(A) Secretion levels of IL-6, measured by ELISA. (B,C) Cell surface levels of IL-6Rα, measured by FACS.

Figure S7

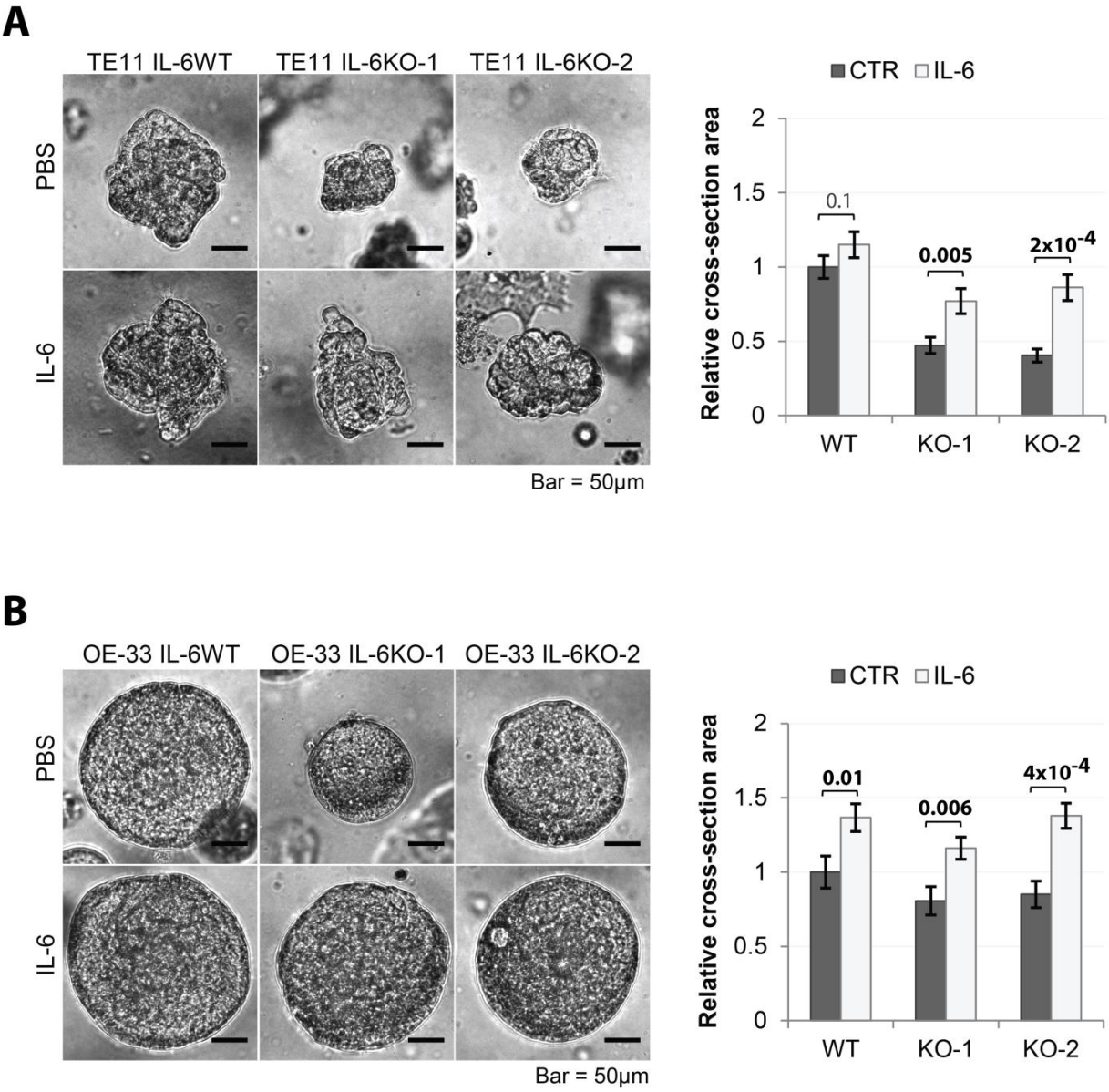


Figure S7: Exogenous IL-6 rescues reduced growth in IL-6 deficient esophageal cancer tumoroids. Related to Figure 3.

Brightfield images of 3D tumoroids from TE11 (A) and OE-33 (B) cell lines treated with either recombinant human IL-6 or PBS. The histograms depict average tumoroid size (± SEM), quantified as relative cross-sectional area (n=3/condition).

Figure S8

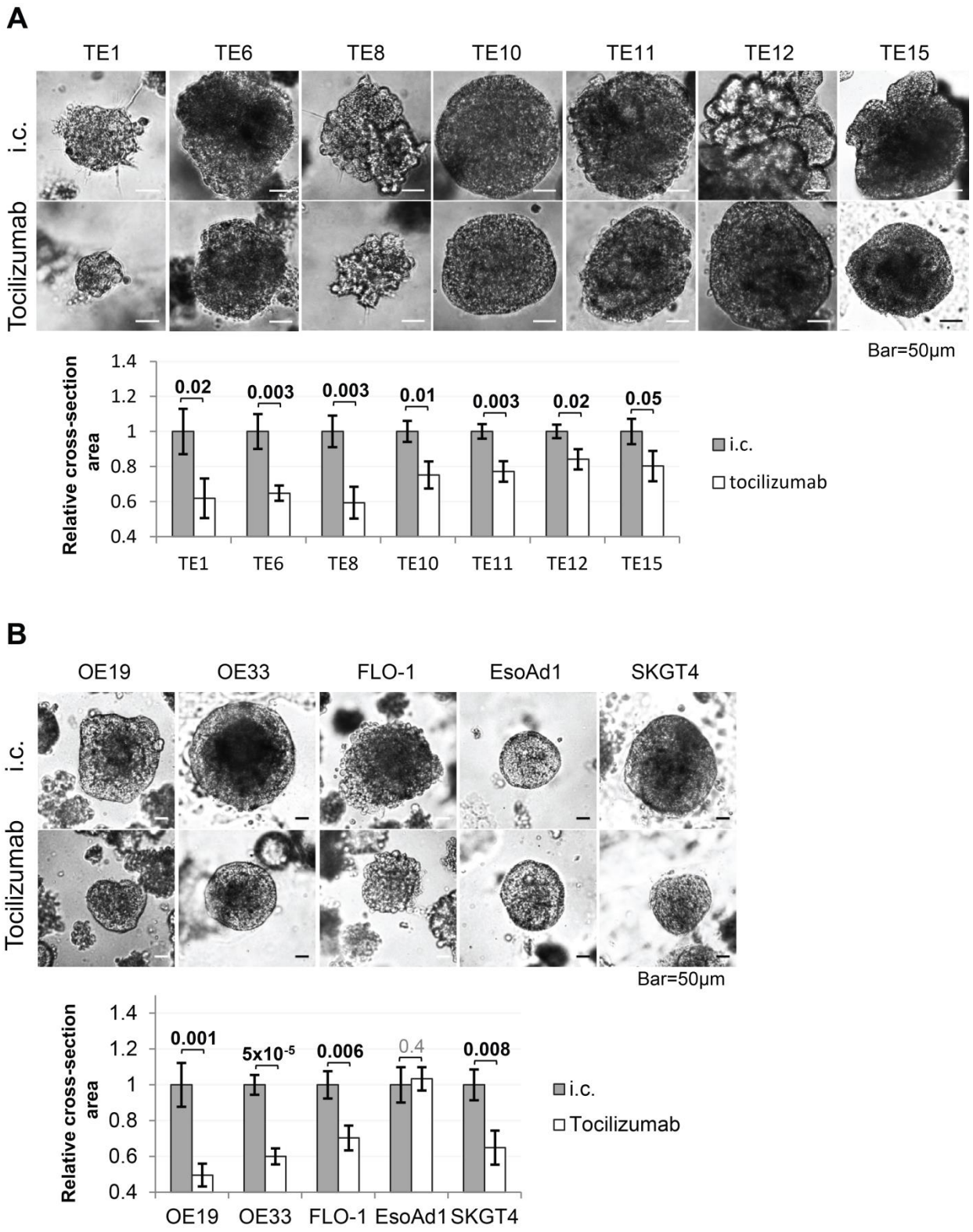


Figure S8: Tocilizumab treatment results in reduced growth of ESCC and EAC tumoroids. Related to Figure 4.

Brightfield images of 3D tumoroids from ESCC (A) and EAC (B) cell lines treated with either tocilizumab or isotype control (i.c.) antibody. The histograms depict average tumoroid size (± SEM), quantified as relative cross-sectional area.

Figure S9

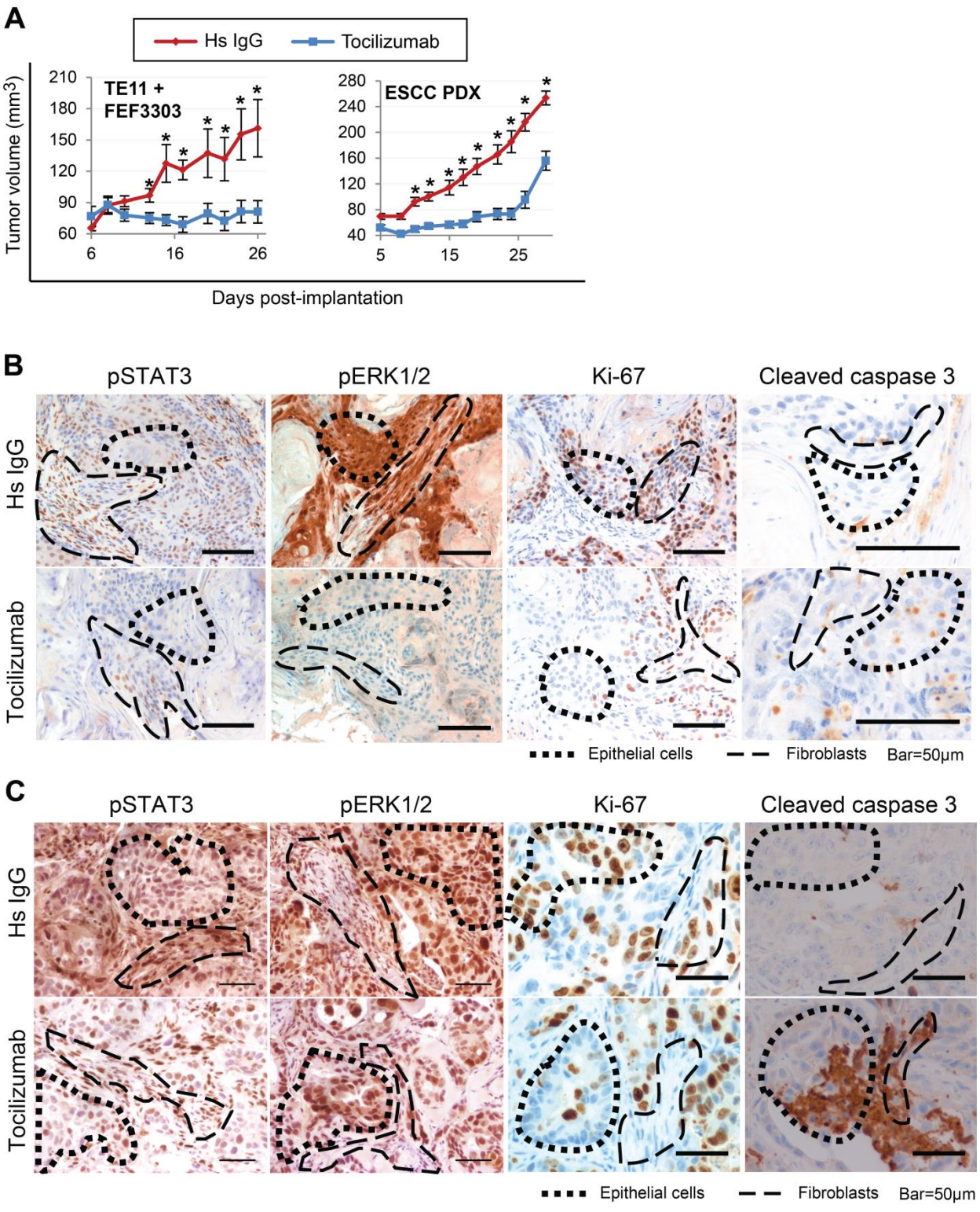
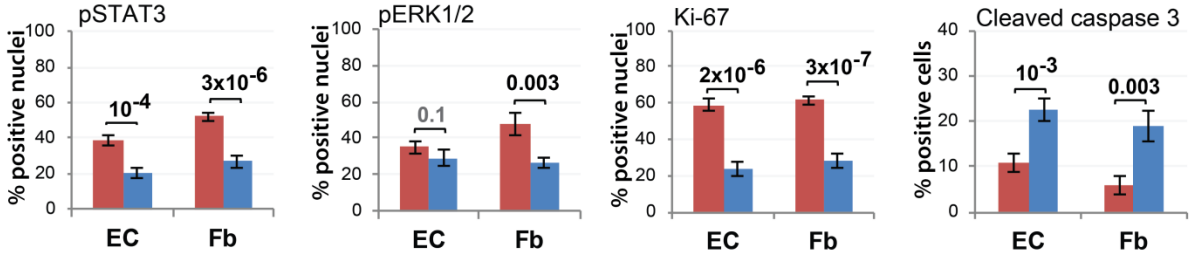

A
Hs IgG
Tocilizumab
TE11 + FEF3303
ESCC PDX
Tumor volume (mm3)
Days post-implantation
B
pSTAT3
pERK1/2
Ki-67
Cleaved caspase 3
Hs IgG
Tocilizumab
Epithelial cells
Fibroblasts
Bar=50μm
C
pSTAT3
pERK1/2
Ki-67
Cleaved caspase 3
Hs IgG
Tocilizumab
Epithelial cells
Fibroblasts
Bar=50μm

Figure S9

D


pSTAT3
% positive nuclei
10-4
3x10-6
EC
Fb
pERK1/2
% positive nuclei
0.1
0.003
EC
Fb
Ki-67
% positive nuclei
2x10-6
3x10-7
EC
Fb
Cleaved caspase 3
% positive cells
10-3
0.003
EC
Fb

E

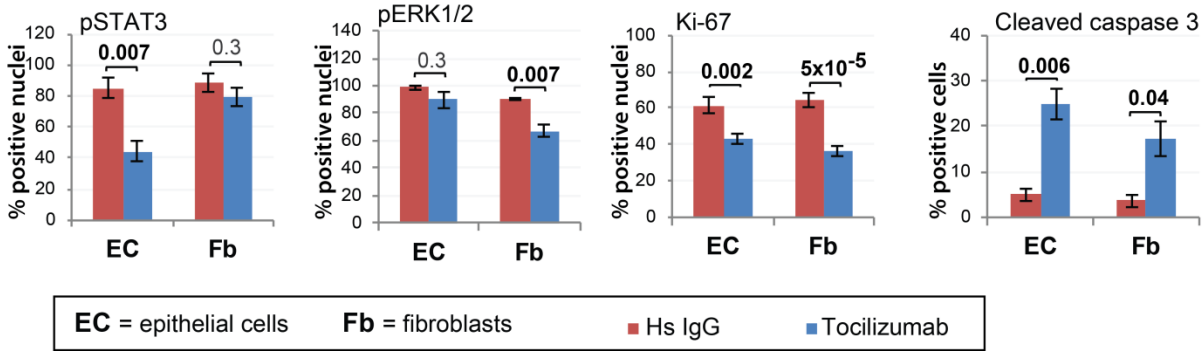

pSTAT3
% positive nuclei
0.007
0.3
EC
Fb
pERK1/2
% positive nuclei
0.3
0.007
EC
Fb
Ki-67
% positive nuclei
0.002
5x10-5
EC
Fb
Cleaved caspase 3
% positive cells
0.006
0.04
EC
Fb
EC = epithelial cells
Fb = fibroblasts
Hs IgG
Tocilizumab

Figure S9

F

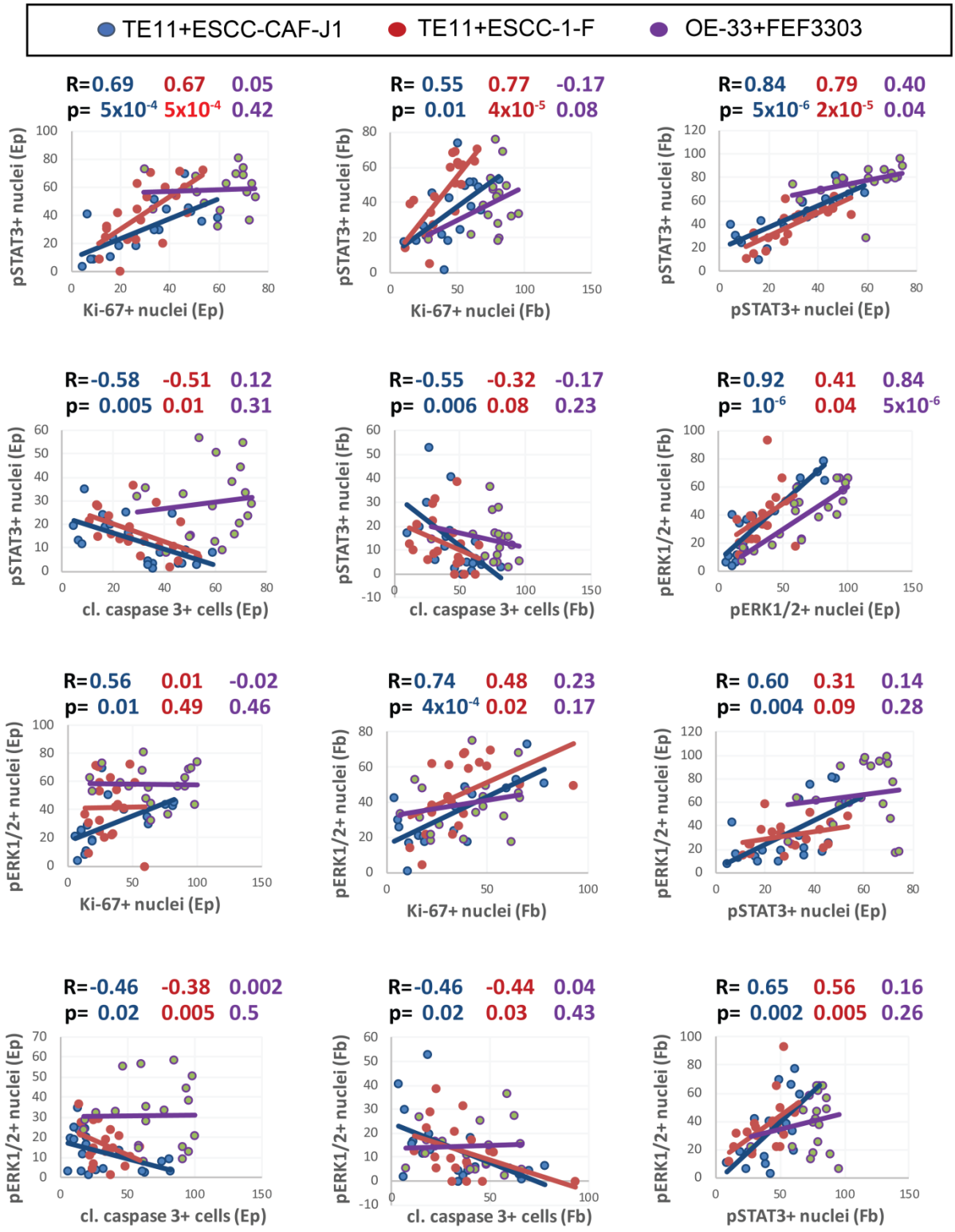

TE11+ESCC-CAF-J1
TE11+ESCC-1-F
OE-33+FEF3303
R=0.69 0.67 0.05
p= 5x10-4 5x10-4 0.42
pSTAT3+ nuclei (Ep)
Ki-67+ nuclei (Ep)
R= 0.55 0.77 -0.17
p= 0.01 4x10-5 0.08
pSTAT3+ nuclei (Fb)
Ki-67+ nuclei (Fb)
R=0.84 0.79 0.40
p= 5x10-6 2x10-5 0.04
pSTAT3+ nuclei (Fb)
pSTAT3+ nuclei (Ep)
R=-0.58 -0.51 0.12
p= 0.005 0.01 0.31
pSTAT3+ nuclei (Ep)
cl. caspase 3+ cells (Ep)
R=-0.55 -0.32 -0.17
p= 0.006 0.08 0.23
pSTAT3+ nuclei (Fb)
cl. caspase 3+ cells (Fb)
R=0.92 0.41 0.84
p= 10-6 0.04 5x10-6
pERK1/2+ nuclei (Fb)
pERK1/2+ nuclei (Ep)
R=0.56 0.01 -0.02
p= 0.01 0.49 0.46
pERK1/2+ nuclei (Ep)
Ki-67+ nuclei (Ep)
R=0.74 0.48 0.23
p= 4x10-4 0.02 0.17
pERK1/2+ nuclei (Fb)
Ki-67+ nuclei (Fb)
R= 0.60 0.31 0.14
p= 0.004 0.09 0.28
pERK1/2+ nuclei (Ep)
pSTAT3+ nuclei (Ep)
R=-0.46 -0.38 0.002
p= 0.02 0.005 0.5
pERK1/2+ nuclei (Ep)
cl. caspase 3+ cells (Ep)
R=-0.46 -0.44 0.04
p= 0.02 0.03 0.43
pERK1/2+ nuclei (Fb)
cl. caspase 3+ cells (Fb)
R= 0.65 0.56 0.16
p= 0.002 0.005 0.26
pERK1/2+ nuclei (Fb)
pSTAT3+ nuclei (Fb)

Figure S9: Tocilizumab treatment results in histological changes. Related to Figure 4.

(A) Growth kinetics of subcutaneous ESCC (TE11+FEF3303) xenograft tumors and ESCC PDX-1 tumors treated with Tocilizumab or isotype control (i.c.) antibody (n=10/cohort, *p≤0.01). (B) Representative images of TE11+ESCC-CAF-J1 tumor sections stained (IHC) for pSTAT3, pERK1/2, Ki-67 and cleaved caspase 3, with outlined examples of epithelial cells (dotted line) and fibroblasts (dashed line). (C) Representative images of OE-33+FEF3303 tumor sections stained (IHC) for pSTAT3, pERK1/2, Ki-67 and cleaved caspase 3, with outlined examples of epithelial cells (dotted line) and fibroblasts (dashed line). Quantification of IHC staining for pSTAT3, pERK1/2, Ki-67 and cleaved caspase 3 of TE11+ESCC-1-F and TE11+FEF3303 tumors is presented in panels (D) and (E), respectively. (F) Correlation analysis of activation of STAT3 or ERK1/2 and downstream targets (Ki-67, cleaved caspase 3). Correlation coefficients (R) and p-values are listed above corresponding graphs.

Figure S10

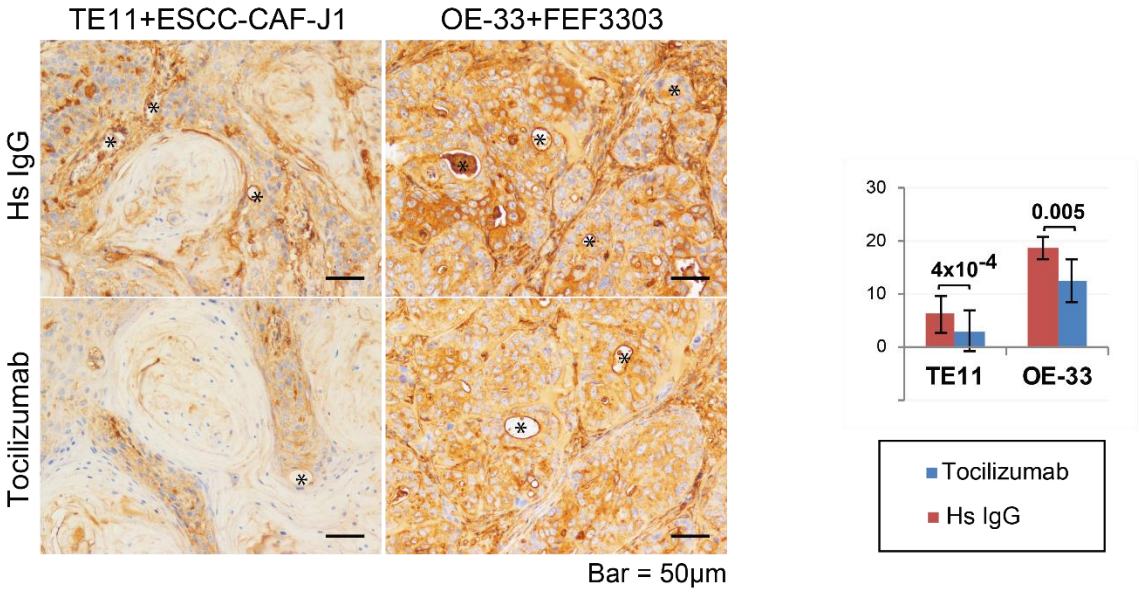


Figure S10: Tocilizumab treatment reduces angiogenesis. Related to Figure 4.

To visualize the endothelium, histological sections of xenograft tumors were stained with Lycopersicon Esculentum (Tomato) Lectin (Vector Labs). Number of open blood vessels (*) per high-power field was used to quantify angiogenesis.

Figure S11

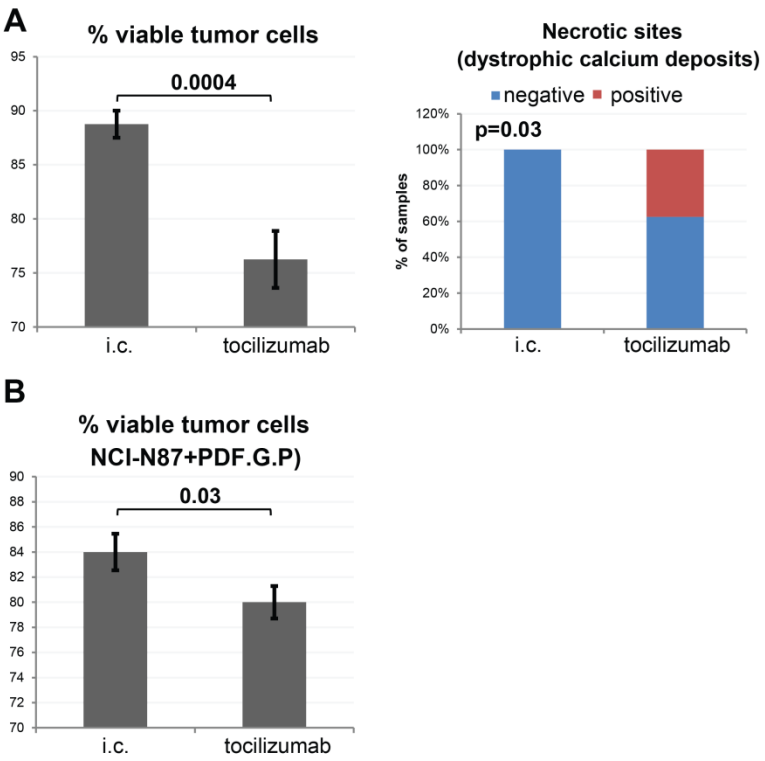


Figure S11: Tocilizumab treatment results in histological changes. Related to Figure 6.

(A) Quantification (based on H&E staining) of the frequency of viable tumor cells and amount of necrosis (presented as dystrophic calcium deposits) within LNT14 PDX tumors, treated with isotype control antibody (i.c.) or tocilizumab (n=8/each cohort). (B) Quantification (based on H&E staining) of the frequency of viable tumor within NCI-N87+PDF.G.P xenograft tumors, treated with isotype control antibody (i.c.) or tocilizumab (n=6/i.c. or 8/tocilizumab cohort).

Figure S12

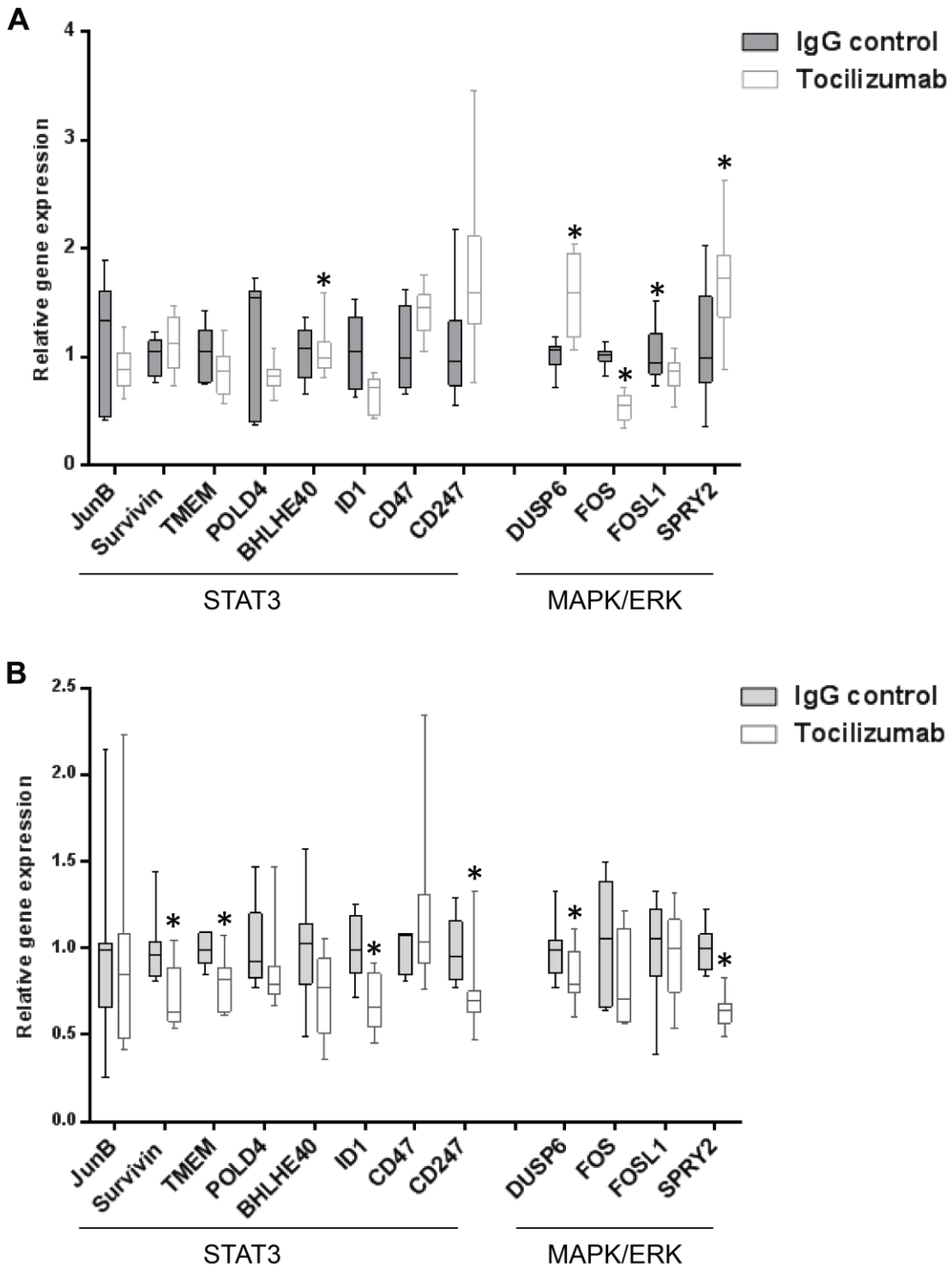


Figure S12: Tocilizumab treatment results in decreased expression of STAT3 and MAPK/ERK1/2 target genes. Related to Figure 6.

Changes in expression of select STAT3 and MAPK/ERK genes were assessed by qPCR analysis of mRNA from tocilizumab- or control IgG-treated tumor tissue. (A) LNT14 PDX model of HNSCC, n=3/control and 6/tocilizumab cohorts. (B) NCI-N87+PDF.G.P xenograft model of gastric cancer, n=7/cohort. *Changes that were statistically significant by the linear mixed models test.

Table S1: qPCR primer sequences.

Table S2: Cytokine array results. Related to Figure 2.

Table S3: Antibodies used in IHC.

Antibody	Vendor	Cat. number	Ag retrieval	Dilution
IL-6	Abcam	ab6672	pH6.0	1:600
IL-6	R&D	MAB2061-SP	pH6.0	1:100
pSTAT3 (Y705)	Cell Signaling	9145	pH9.0	1:100
pERK1/2 (T202/Y204)	Cell Signaling	4370	pH6.0	1:100
Ki-67	Abcam	ab16667	pH6.0	1:500
Cl. caspase 3	Cell Signaling	9661	pH6.0	1:100

Table S4A: Linear regression analysis of therapeutic study of tocilizumab in TE11+ESCC-CAF-J1 ESCC xenograft. Related to Figure 4A.

lnsize	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
tx						
toc	-.2514668	.1237806	-2.03	0.042	-.4940722	-.0088614
tx#c.day						
control	.0415469	.0073385	5.66	**0.000**	.0271637	.0559301
toc	.0221502	.0072715	3.05	**0.002**	.0078983	.0364022
_cons	3.999032	.08869	45.09	0.000	3.825202	4.172861

Table S4B: Linear regression analysis of therapeutic study of tocilizumab in TE11+ESCC-1-F ESCC xenograft. Related to Figure 4A.

size	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
tx						
toc	-1.197863	11.31737	-0.11	0.916	-23.37951	20.98378
tx#c.day						
control	5.097738	.9371529	5.44	**0.000**	3.260952	6.934523
toc	1.66651	.9317234	1.79	**0.074**	-.1596342	3.492655
_cons	40.8954	8.134156	5.03	0.000	24.95275	56.83806

Table S4C: Linear regression analysis of therapeutic study of tocilizumab in TE11+FEF3303 ESCC xenograft. Related to Figure S7A.

size_	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
day						
6	0	(base)				
8	21.99	11.78	1.87	0.0621	-1.111	45.08
10	25.69	11.78	2.18	0.0293	2.59	48.78
13	31.42	11.78	2.67	0.0077	8.32	54.51
15	61.99	11.78	5.26	0.0000	38.89	85.08

17	55.85	11.78	4.74	0.0000	32.75	78.94
20	71.84	11.78	6.10	0.0000	48.74	94.93
22	66.15	11.78	5.61	0.0000	43.05	89.24
24	89.89	11.78	7.63	0.0000	66.8	113
26	95.72	11.78	8.12	0.0000	72.63	118.8
txx#day						
TOCILIZUMAB# 6	7.746	18.52	0.42	0.6758	-28.56	44.05
TOCILIZUMAB# 8	-3.387	18.52	-0.18	0.8549	-39.69	32.91
TOCILIZUMAB#10	-16.58	18.22	-0.91	0.3630	-52.29	19.14
TOCILIZUMAB#13	-25.95	17.98	-1.44	0.1489	-61.2	9.288
TOCILIZUMAB#15	-58.42	17.98	-3.25	**0.0012**	-93.66	-23.17
TOCILIZUMAB#17	-52.36	17.78	-2.95	**0.0032**	-87.21	-17.52
TOCILIZUMAB#20	-57.79	17.78	-3.25	**0.001**2	-92.63	-22.94
TOCILIZUMAB#22	-59.21	17.78	-3.33	**0.0009**	-94.06	-24.37
TOCILIZUMAB#24	-74.05	17.78	-4.17	**0.0000**	-108.9	-39.2
TOCILIZUMAB#26	-80.12	17.78	-4.51	**0.0000**	-115	-45.27
_cons	65.44	13.25	4.94	0.0000	39.47	91.41

Table S4D: Linear regression analysis of therapeutic study of tocilizumab in EAC xenograft. Related to Figure 4A.

size_	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
day						
5	0	(base)				
7	34.29	18.11	1.89	0.0583	-1.201	69.79
10	84.18	18.11	4.65	0.0000	48.68	119.7
12	104.4	18.11	5.77	0.0000	68.95	139.9
14	113.6	18.11	6.27	0.0000	78.11	149.1
17	154.8	18.11	8.55	0.0000	119.3	190.3
19	174.6	18.11	9.64	0.0000	139.1	210
txx#day						
TOCILIZUMAB# 5	-12.96	43.92	-0.30	0.7680	-99.03	73.12
TOCILIZUMAB# 7	-17.99	43.68	-0.41	0.6804	-103.6	67.62
TOCILIZUMAB#10	-77.29	43.68	-1.77	0.0768	-162.9	8.316
TOCILIZUMAB#12	-94.57	43.68	-2.17	**0.0304**	-180.2	-8.963
TOCILIZUMAB#14	-97.86	43.68	-2.24	**0.0251**	-183.5	-12.25
TOCILIZUMAB#17	-106.3	43.68	-2.43	**0.0150**	-191.9	-20.68
TOCILIZUMAB#19	-108.2	43.68	-2.48	**0.0133**	-193.8	-22.58
_cons	73.26	30.89	2.37	0.0177	12.73	133.8

Table S4E: Linear regression analysis of therapeutic study of tocilizumab in ESCC PDX. Related to Figure 4A.

size	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
1.tx	-4.5	12.23702	-0.37	0.713	-28.48412	19.48412
daynum						
3	1.25	9.479698	0.13	0.895	-17.32987	19.82987
5	9.3	9.479698	0.98	0.327	-9.279867	27.87987
8	9.3	9.479698	0.98	0.327	-9.279867	27.87987

10	32.05	9.479698	3.38	0.001	13.47013	50.62987
12	40.2	9.479698	4.24	0.000	21.62013	58.77987
15	54.15	9.479698	5.71	0.000	35.57013	72.72987
17	69.85	9.479698	7.37	0.000	51.27013	88.42987
19	86.8	9.479698	9.16	0.000	68.22013	105.3799
22	105.55	9.479698	11.13	0.000	86.97013	124.1299
24	125.4	9.479698	13.23	0.000	106.8201	143.9799
26	155.6	9.479698	16.41	0.000	137.0201	174.1799
29	193.35	9.479698	20.40	0.000	174.7701	211.9299
tx#daynum						
1 3	-2.05	13.40632	-0.15	0.878	-28.3259	24.2259
1 5	-13.15	13.40632	-0.98	0.327	-39.4259	13.1259
1 8	-22.95	13.40632	-1.71	0.087	-49.2259	3.3259
1 10	-38.15	13.40632	-2.85	0.004	-64.4259	-11.8741
1 12	-41.8	13.40632	-3.12	0.002	-68.0759	-15.5241
1 15	-53.25	13.40632	-3.97	0.000	-79.5259	-26.9741
1 17	-67.7	13.40632	-5.05	0.000	-93.9759	-41.4241
1 19	-73.75	13.40632	-5.50	0.000	-100.0259	-47.4741
1 22	-87.95	13.40632	-6.56	0.000	-114.2259	-61.6741
1 24	-107.8	13.40632	-8.04	0.000	-134.0759	-81.5241
1 26	-116.05	13.40632	-8.66	0.000	-142.3259	-89.7741
1 29	-93.05	13.40632	-6.94	0.000	-119.3259	-66.7741
_cons	60.25	8.65288	6.96	0.000	43.29067	77.20933

Table S4F: Linear regression analysis of therapeutic study of tocilizumab in ESCC PDX-1. Related to Figure S7A.

size	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
1.tx	-4.5	12.23702	-0.37	0.713	-28.48412	19.48412
daynum						
3	1.25	9.479698	0.13	0.895	-17.32987	19.82987
5	9.3	9.479698	0.98	0.327	-9.279867	27.87987
8	9.3	9.479698	0.98	0.327	-9.279867	27.87987
10	32.05	9.479698	3.38	0.001	13.47013	50.62987
12	40.2	9.479698	4.24	0.000	21.62013	58.77987
15	54.15	9.479698	5.71	0.000	35.57013	72.72987
17	69.85	9.479698	7.37	0.000	51.27013	88.42987
19	86.8	9.479698	9.16	0.000	68.22013	105.3799
22	105.55	9.479698	11.13	0.000	86.97013	124.1299
24	125.4	9.479698	13.23	0.000	106.8201	143.9799
26	155.6	9.479698	16.41	0.000	137.0201	174.1799
29	193.35	9.479698	20.40	0.000	174.7701	211.9299
tx#daynum						
1 3	-2.05	13.40632	-0.15	0.878	-28.3259	24.2259
1 5	-13.15	13.40632	-0.98	0.327	-39.4259	13.1259
1 8	-22.95	13.40632	-1.71	0.087	-49.2259	3.3259
1 10	-38.15	13.40632	-2.85	0.004	-64.4259	-11.8741
1 12	-41.8	13.40632	-3.12	0.002	-68.0759	-15.5241
1 15	-53.25	13.40632	-3.97	0.000	-79.5259	-26.9741
1 17	-67.7	13.40632	-5.05	0.000	-93.9759	-41.4241
1 19	-73.75	13.40632	-5.50	0.000	-100.0259	-47.4741
1 22	-87.95	13.40632	-6.56	0.000	-114.2259	-61.6741
1 24	-107.8	13.40632	-8.04	0.000	-134.0759	-81.5241
1 26	-116.05	13.40632	-8.66	0.000	-142.3259	-89.7741

1 29	-93.05	13.40632	-6.94	0.000	-119.3259	-66.7741
_cons	60.25	8.65288	6.96	0.000	43.29067	77.20933

Table S5A: Linear regression analysis of therapeutic study of tocilizumab in HNSCC PDX. Related to Figure 6B.

size_	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
day						
13	0	(base)				
15	104.2	58.4	1.78	0.0745	-10.31	218.6
18	268.6	58.4	4.60	0.0000	154.2	383.1
20	441.7	58.4	7.56	0.0000	327.2	556.2
22	577	58.4	9.88	0.0000	462.5	691.4
25	733.7	58.4	12.56	0.0000	619.2	848.2
27	866.2	58.4	14.83	0.0000	751.8	980.7
29	1064	58.4	18.22	0.0000	949.6	1179
32	1406	58.4	24.08	0.0000	1292	1521
txx#day						
TOCILIZUMAB#13	-3.035	86.01	-0.04	0.9719	-171.6	165.5
TOCILIZUMAB#15	-77.4	86.01	-0.90	0.3682	-246	91.17
TOCILIZUMAB#18	-190.1	86.01	-2.21	**0.0271**	-358.7	-21.54
TOCILIZUMAB#20	-308.5	86.01	-3.59	**0.0003**	-477	-139.9
TOCILIZUMAB#22	-391.2	86.01	-4.55	**0.0000**	-559.8	-222.7
TOCILIZUMAB#25	-493.6	86.01	-5.74	**0.0000**	-662.2	-325
TOCILIZUMAB#27	-578.2	86.01	-6.72	**0.0000**	-746.8	-409.7
TOCILIZUMAB#29	-710.7	86.01	-8.26	**0.0000**	-879.3	-542.2
TOCILIZUMAB#32	-953.5	86.01	-11.09	**0.0000**	-1122	-785
_cons	25.84	60.82	0.42	0.6710	-93.36	145

Table S5B: Linear regression analysis of therapeutic study of tocilizumab in gastric cancer xenograft. Related to Figure 6D.

size	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
day						
6	0	(base)				
8	51.78	42.46	1.22	0.2226	-31.43	135
10	96.71	42.46	2.28	0.0227	13.49	179.9
13	157.1	42.46	3.70	0.0002	73.89	240.3
15	207.9	42.46	4.90	0.0000	124.7	291.1
17	316.7	42.46	7.46	0.0000	233.5	399.9
20	403.9	42.46	9.51	0.0000	320.6	487.1
23	428.9	42.46	10.10	0.0000	345.7	512.1
tx#day						

tocilizumab# 8	-15	59.1	-0.25	0.7997	-130.8	100.8
tocilizumab#10	-60.58	59.1	-1.03	0.3054	-176.4	55.26
tocilizumab#13	-69.03	58.42	-1.18	0.2374	-183.5	45.48
tocilizumab#15	-89.41	58.42	-1.53	0.1259	-203.9	25.1
tocilizumab#17	-151.5	58.42	-2.59	**0.0095**	-266	-37.02
tocilizumab#20	-186.4	58.42	-3.19	**0.0014**	-301	-71.93
tocilizumab#23	-209.1	58.42	-3.58	**0.0003**	-323.6	-94.55
_cons	88.01	47.42	1.86	0.0634	-4.928	181

Table S6A: Mixed effect linear mixed model regression analysis of expression of select STAT3 and ERK1/2 genes in HNSCC PDX tumors treated with tocilizumab or control human IgG. Related to Figure 6C.

	gene	pv	chi2
1.	BHLHE40	.9301642	.0076805
2.	CD47	.0674267	3.344579
3.	DUSPY6	**.0165673**	5.741646
4.	FOS	**3.46e-10**	39.39601
5.	FOSL	**.0079808**	7.03777
6.	ID1	**.0221816**	5.231485
7.	JunB	.3442537	.8945261
8.	PDL1	.0722302	3.231598
9.	PDL2	.2611776	1.262504
10.	POLD4	.1782313	1.812311
11.	SPRY2	**.0231374**	5.158157
12.	TMEM	.2055054	1.60281
13.	survivin	.5158613	.4221597

Table S6B: Mixed effect linear mixed model regression analysis of expression of select STAT3 and ERK1/2 genes in gastric cancer xenograft tumors treated with tocilizumab or control human IgG. Related to Figure 6F.

gene	pv	chi2
BHLHE40	.0697476	3.288951
CD47	.309842	1.031349
DUSPY6	**.0529207**	3.746421
FOS	.2098802	1.572253
FOSL	.857074	.0324361
ID1	**.000531**	12.00358
JunB	.8245311	.0491602
PDL1	**.0259944**	4.956394
POLD4	.3232606	.9757084
SPRY2	**7.00e-10**	38.02061
TMEM	**.0002402**	13.48735
survivin	**.0043027**	8.151422