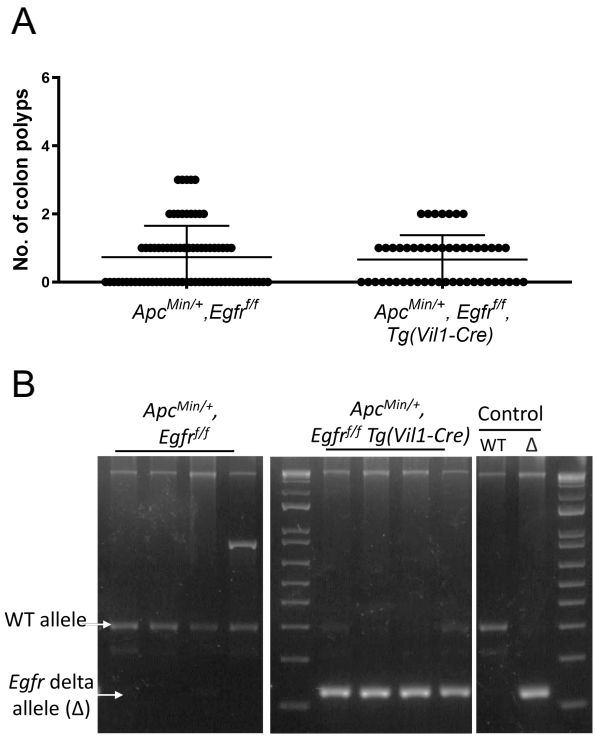
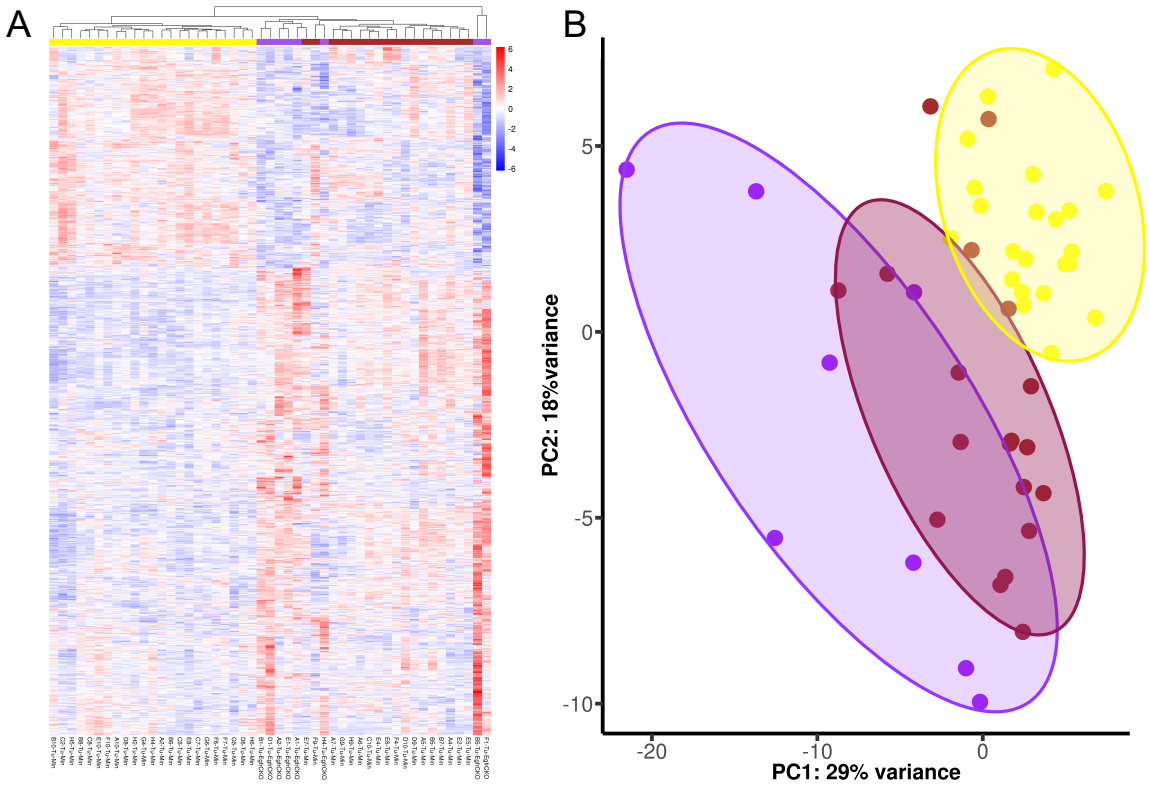
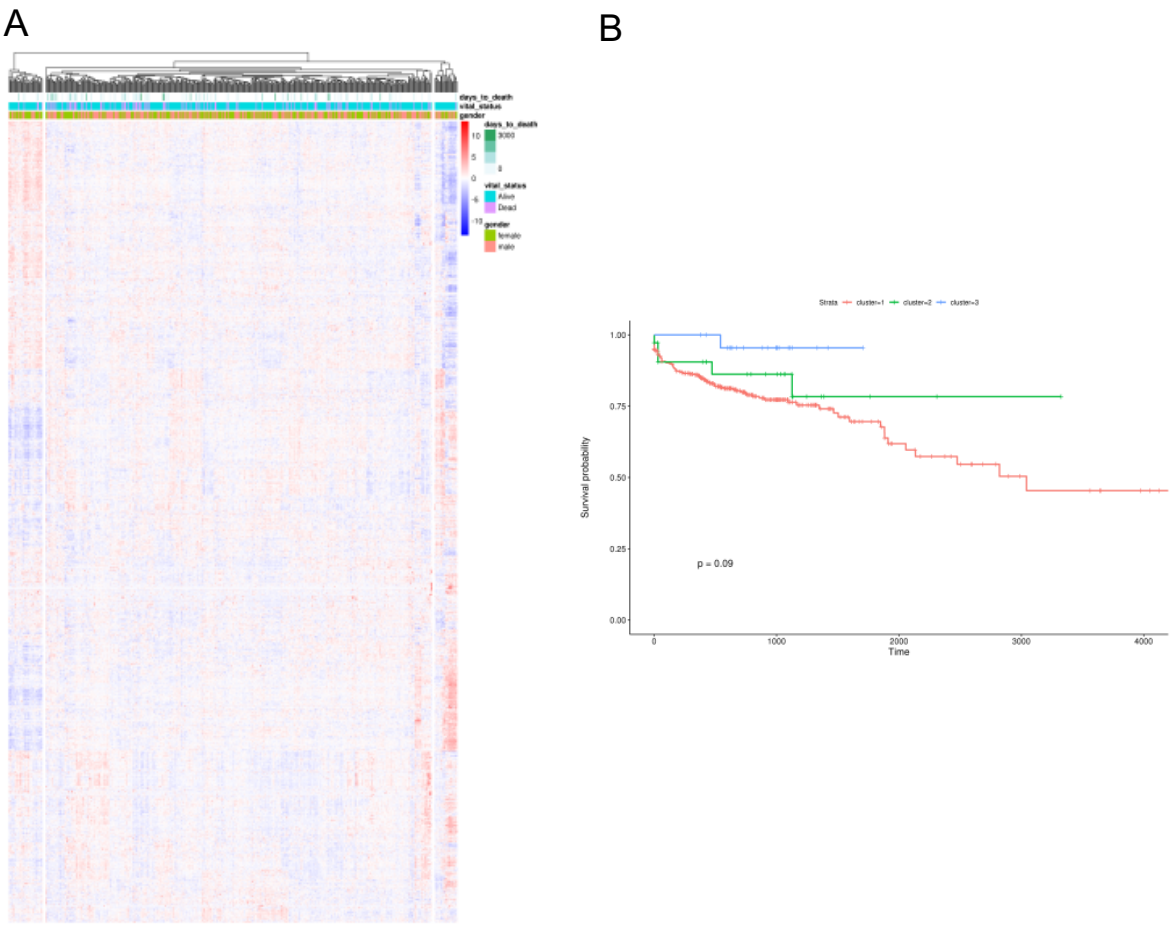
Supplementary Figures



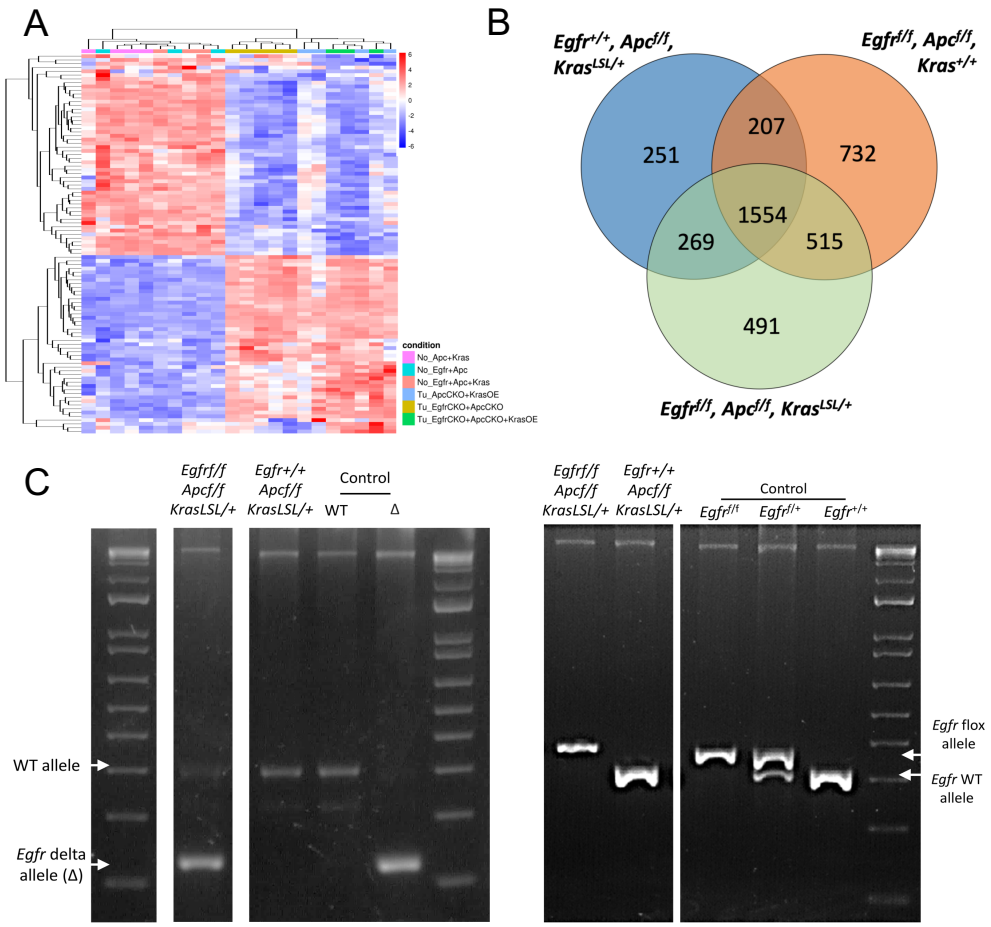
Supplementary Fig. 1 Characterization of EGFR-independent polyps in the *Apc$^{Min/+}$* model. **a** Multiplicity of colon polyps. Dots represents the polyp number in each 100-day-old mice. *Apc$^{Min/+}$*, *Egfr$^{f/f}$* (n = 100) and *Apc$^{Min/+}$*, *Egfr$^{f/f}$, Tg(Vil-Cre)* (n = 75). **b** Validation of *Egfr* deletion (delta) allele in *Apc$^{Min/+}$* polyps. All animals were genotyped and 3 polyps per animal were used to validate the *Egfr* genotype of the polyps.



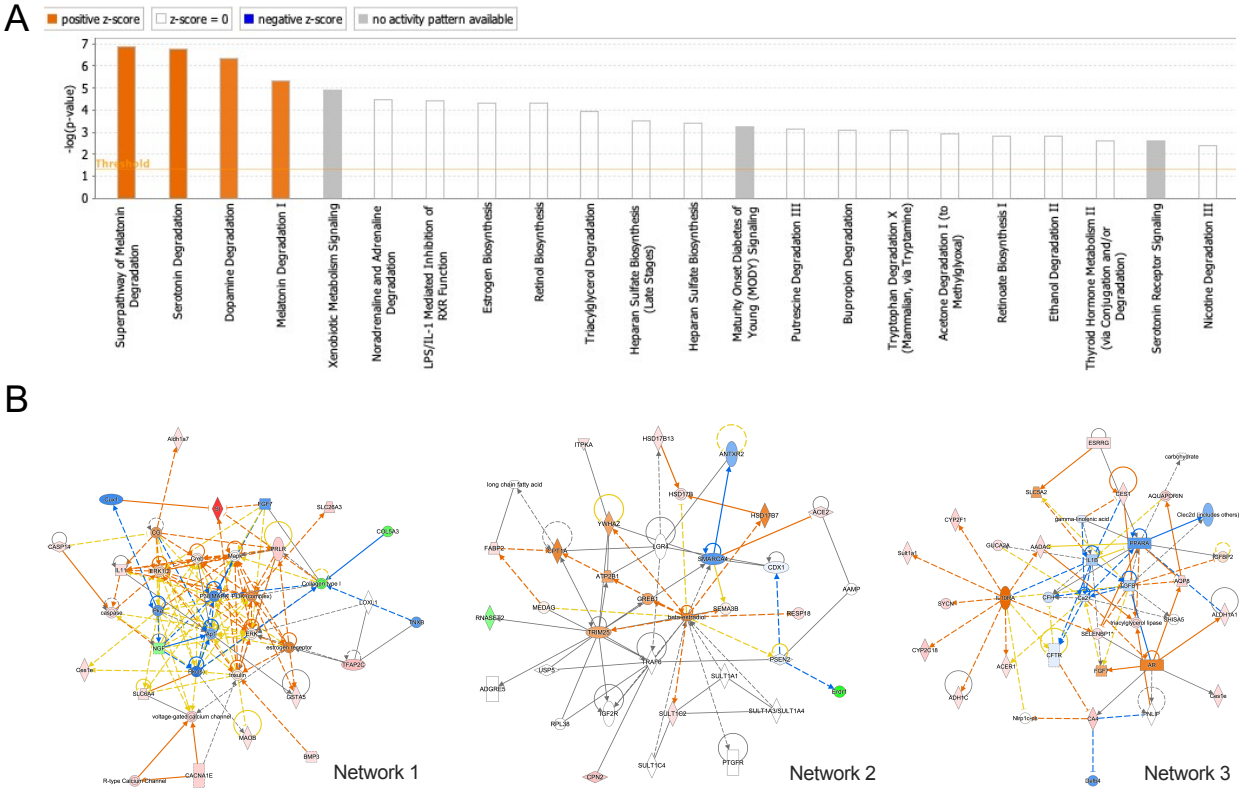
Supplementary Fig. 2 Transcriptomic analysis of intestinal polyps in the *Apc*$^{Min/+}$ mouse model. a Hierarchical clustering of differentially expressed genes. **b** Principal Component Analysis of polyp transcriptomes. Yellow, *Apc*$^{Min/+}$, *Egfr*$^{f/f}$; purple, *Apc*$^{Min/+}$, *Egfr*$^{f/f}$, *Tg(Vil1-Cre)*; and maroon, *Apc*$^{Min/+}$, *Egfr*$^{f/f}$ polyps that clustered more similar to *Apc*$^{Min/+}$, *Egfr*$^{f/f}$, *Tg(Vil1-Cre)*.



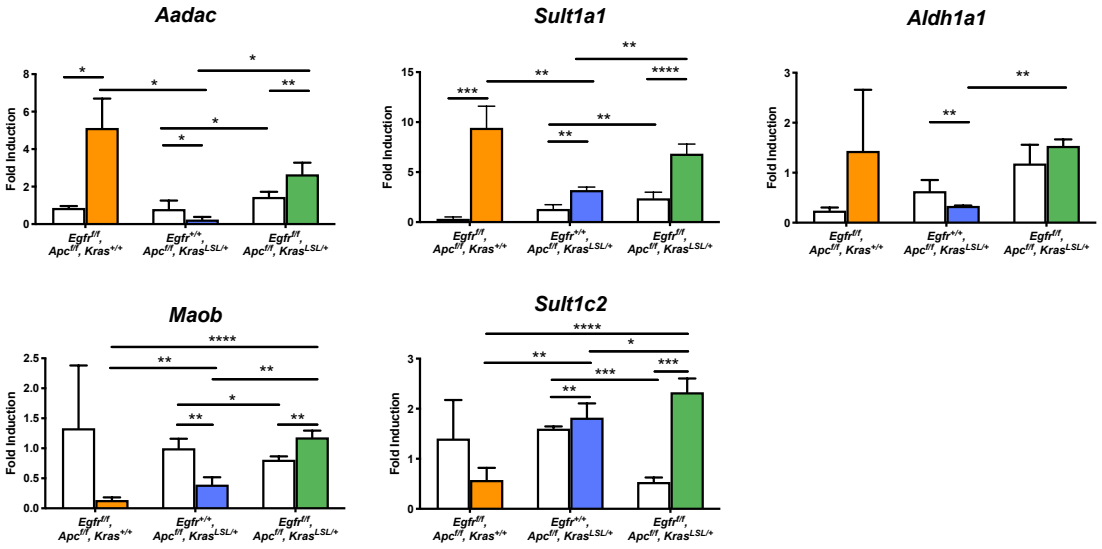
Supplementary Fig. 3 Transcriptomic analysis of human CRCs. a Unsupervised hierarchical clustering of human CRCs using orthologs from the 1,200 gene mouse EGFR-independent signature. **b** Survival curves of the three major classes. Red line is the putative EGFR-independent class based on directionality of gene expression patterns.



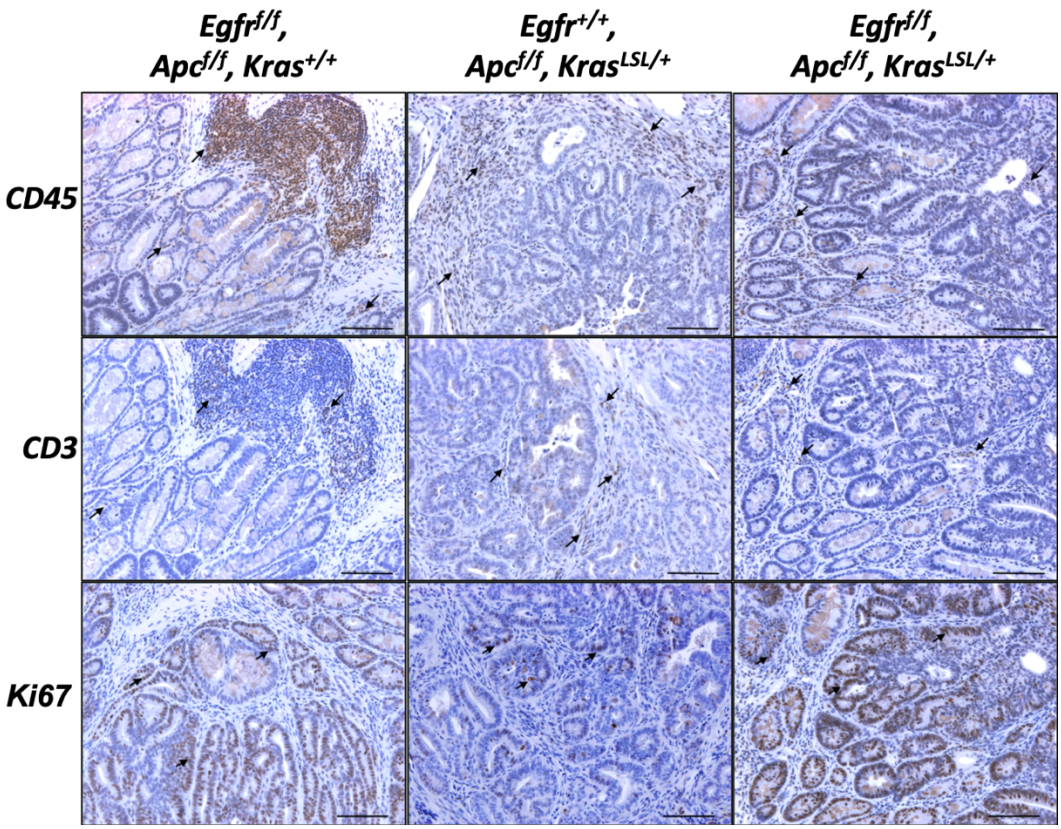
Supplementary Fig. 4 Transcriptomic analysis from induced colon polyps with different EGFR and KRAS levels. a Hierarchical clustering of differentially expressed genes between colon polyps. N, adjacent normal epithelia; T, tumor**. b** Venn diagram of differentially expressed genes between normal colon and polyp tissue. **c** Validation of *Egfr* delta allele present in EGFR-independent colon polyps.



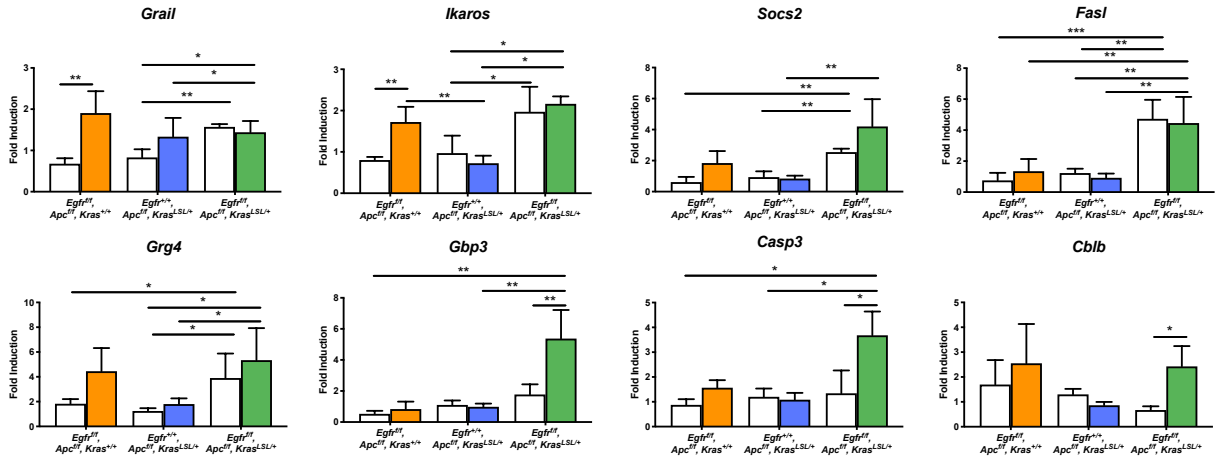
Supplementary Fig. 5 Ingenuity Pathway Analysis of EGFR-independent colon polyps (*Egfr*$^{f/f}$, *Apc*$^{f/f}$, *Kras*^{LSL}). a Significant canonical pathways identified from IPA analysis. **b** Top three enriched signaling networks.



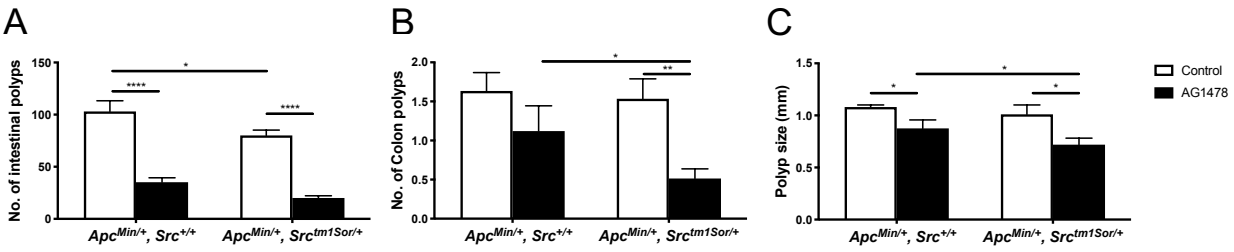
Supplementary Fig. 6 Validation of differentially expressed genes in EGFR-independent colon polyps. White bars, mean transcript levels in the adjacent normal tissue; colored bars, mean transcript levels in colon polyps. Data are represented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



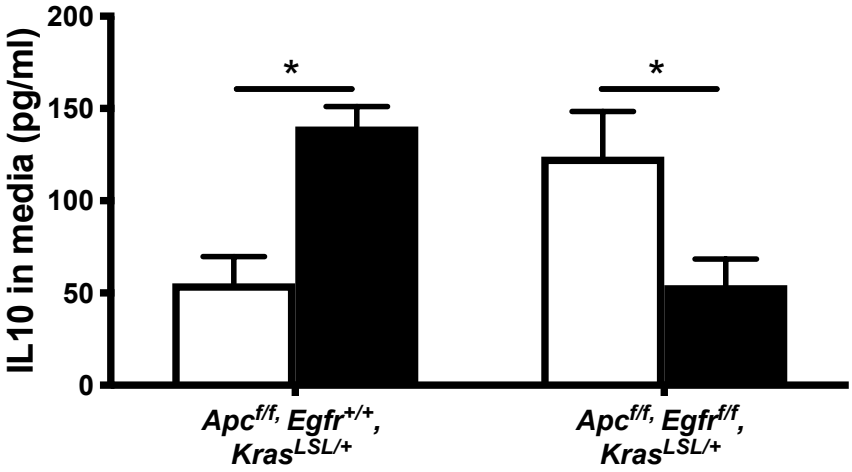
Supplementary Fig. 7 Histological evaluation of colon polyps. Immunostaining of leukocytes (CD45), T-cell (CD3), and proliferation (Ki67). All scale bars represent 100um.



Supplementary Fig. 8 Up-regulation of anergy-associated genes in EGFR-independent colon polyps. White bars, mean transcript levels in adjacent normal tissue; colored bars, transcript levels in colon polyps. Data are represented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Fig. 9 Effect of *Src*^{tm1Sor} targeted mutation and small molecule EGFR inhibitor AG1478 on small intestinal and colonic polyp number and size in *Apc*$^{Min/+}$ mice. a Intestinal polyp multiplicity. **b** Colon polyp multiplicity. **c** Colon polyp size. Data are represented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <$ 0.0001.



Supplementary Fig. 10 IL10 levels in media from organoid cultures. White bars, control; black bars, AG1478-treated organoids**.** * $p < 0.05$.

Supplementary Tables

Supplementary Table 1 Differentially expressed genes between EGFR-dependent and EGFR-independent induced colon polyps.

Gene name	Gene symbol	Expr Fold Change	Expr p-value	Location	Type(s)
Sucrase-isomaltase	Sis	22.642	1.07E-14	Cytoplasm	Enzyme
Mucosal pentraxin 1	Mptx1	7.296	4.76E-08	Other	Other
Transcription factor AP-2 gamma	Tfap2c	7.117	2.35E-05	Nucleus	Transcription regulator
Carboxypeptidase N subunit 2	Cpn2	6.738	1.07E-05	Extracellular Space	Peptidase
Carbonic anhydrase 4	Car4	6.032	1.03E-07	Plasma Membrane	Enzyme
Cytochrome P450 family 2 subfamily C member 18	Cyp2c55	5.902	1.91E-05	Cytoplasm	Enzyme
Prolactin receptor	Prlr	5.447	0.000151	Plasma Membrane	Transmembrane receptor
Solute carrier family 26 member 3	Slc26a3	5.433	9.05E-05	Plasma Membrane	Transporter
RIKEN cDNA 2010106E10 gene	2010106E10Rik	5.412	2.13E-05	Other	Other
Cytochrome P450 family 2 subfamily F member 1	Cyp2f2	5.05	0.000103	Cytoplasm	Enzyme
Aquaporin 8	Aqp8	5.015	0.000152	Plasma Membrane	Transporter
Calcium voltage-gated channel subunit alpha1 E	Cacna1e	4.884	4.04E-05	Plasma Membrane	Ion channel
Syncollin	Sycn	4.87	1.13E-05	Extracellular Space	Other
Carboxylesterase 1E	Ces1e	3.932	6.73E-07	Cytoplasm	Enzyme
Regulated endocrine specific protein 18	Resp18	3.916	4.47E-06	Extracellular Space	Other
Arylacetamide deacetylase	Aadac	3.865	3.62E-05	Cytoplasm	Enzyme
Carboxylesterase 1	Ces1d	3.8	9.69E-05	Cytoplasm	Enzyme
Sulfotransferase family 1A, phenol-preferring, member 1	Sult1a1	3.693	3.7E-08	Cytoplasm	Enzyme
Solute carrier family 6 member 4	Slc6a4	3.645	1.97E-05	Plasma Membrane	Transporter
Fatty acid binding protein 2	Fabp2	3.574	0.000108	Cytoplasm	Transporter
Inositol-trisphosphate 3-kinase A	Itpka	3.413	1.02E-05	Cytoplasm	Kinase
RIKEN cDNA 1810063I02 gene	1810063I02Rik	3.41	0.000145	Other	Other
Hydroxysteroid 17-beta dehydrogenase 13	Hsd17b13	3.374	2.27E-05	Extracellular Space	Enzyme

Interleukin 11	Il11	3.373	3.49E-05	Extracellular Space	Cytokine
Estrogen related receptor gamma	Esrrg	3.336	0.000136	Nucleus	Ligand-dependent nuclear receptor
Glutathione S-transferase alpha 5	Gsta1	3.315	7.16E-05	Cytoplasm	Enzyme
RIKEN cDNA 0610005C13 gene	0610005C13Rik	3.212	5.22E-06	Other	Other
Angiotensin I converting enzyme 2	Ace2	3.1	0.000141	Plasma Membrane	Peptidase
Monoamine oxidase B	Maob	3.056	2.26E-07	Cytoplasm	Enzyme
Selenium binding protein 1	Selenbp1	2.989	2.5E-08	Cytoplasm	Other
Caspase 14	Casp14	2.969	6.18E-06	Cytoplasm	Peptidase
Sulfotransferase family 1C member 2	Sult1c2	2.906	9.97E-05	Cytoplasm	Enzyme
Alcohol dehydrogenase 1C (class I), gamma polypeptide	Adh1	2.853	3.73E-05	Cytoplasm	Enzyme
Bone morphogenetic protein 3	Bmp3	2.817	6.46E-07	Extracellular Space	Growth factor
Predicted gene 5485	Gm5485	2.709	7.16E-05	Other	Other
Alkaline ceramidase 1	Acer1	2.65	8.92E-06	Cytoplasm	Enzyme
Aldehyde dehydrogenase 1 family member A1	Aldh1a1	2.244	1.05E-06	Cytoplasm	Enzyme
Aldehyde dehydrogenase family 1, subfamily A7	Aldh1a7	2.212	1.34E-05	Cytoplasm	Enzyme
Guanylate cyclase activator 2A	Guca2a	2.156	2.92E-05	Extracellular Space	Other
Nerve growth factor	Ngf	-2.089	1.18E-06	Extracellular Space	Growth factor
Ribonuclease T2	Rnaset2b	-2.277	2.19E-06	Cytoplasm	Enzyme
Collagen type V alpha 3 chain	Col5a3	-3.155	0.000041	Extracellular Space	Other
Erythroid differentiation regulator 1	Erdr1	-4.192	0.000037	Other	Other

Supplementary Table 2 Prediction of top activated and down-regulated upstream regulators involved in progression of EGFR-independent intestinal polyps.

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	p-value of overlap
TRIM24	Transcription regulator	Activated	2.000	$2.40x10^{-04}$
Epigallocatechin-gallate	Chemical drug	Activated	1.987	$5.06x10^{-03}$
SOCS1	Other	Activated	1.982	$7.16x10^{-04}$
IL10RA	Transmembrane receptor	Activated	1.664	$3.73x10^{-04}$
IL10	Cytokine	-	1.412	$2.50x10^{-02}$
SIM1	Transcription regulator	Inhibited	-2.433	$4.63x10^{-05}$
ARNT2	Transcription regulator	Inhibited	-2.433	$4.05x10^{-05}$
MYC	Transcription regulator	Inhibited	-2.393	$5.08x10^{-02}$
TP53	Transcription regulator	Inhibited	-2.049	$1.11x10^{-01}$
Beta-estradiol	Chemical	Inhibited	-2.017	$1.30x10^{-01}$

TRIM24, Tripartite Motif Containing 24; SOCS1, Suppressor of cytokine signaling 1; IL10RA, Interleukin 10 Receptor Subunit Alpha; IL10, Interleukin 10; SIM1, Single Minded homolog 1; ARNT2, Aryl Hydrocarbon Receptor Nuclear Translocator 2; MYC, Myelocytoma Proto-Oncogene; TP53, Tumor Protein p53.

Supplementary Table 3 Prediction of top activated and down-regulated upstream regulators involved in progression of *Egfr*$^{f/f}$, *Apc*$^{f/f}$, *Kras*$^{+/+}$ colon polyps.

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	p-value of overlap
IL10RA	Transmembrane receptor	Activated	6.466	5.53×10^{-35}
Dexamethasone	Chemical drug	Activated	6.568	1.78×10^{-43}
Alpha catenin	Group	Activated	4.579	1.42×10^{-12}
DKK1	Growth factor	Activated	4.579	5.02×10^{-10}
PPARGC1A	Transcription regulator	Activated	4.385	1.46×10^{-13}
TNF	Cytokine	Inhibited	-6.440	3.01×10^{-56}
ERK	Group	Inhibited	-5.980	1.47×10^{-12}
PDGF BB	Complex	Inhibited	-5.724	6.59×10^{-21}
EGF	Growth factor	Inhibited	-5.697	1.41×10^{-22}
MYD88	Other	Inhibited	-5.641	1.34×10^{-14}

IL10RA, Interleukin 10 Receptor Subunit Alpha; DKK1, Dickkopf WNT Signaling Pathway Inhibitor 1; PPARGC1A, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha; TNF, Tumor Necrosis Factor; ERK, Extracellular-signal-regulated Kinase; PDGF BB, Platelet-derived growth factor BB; EGF, Epidermal Growth Factor; MYD88, Myeloid differentiation primary response 88.

Supplementary Table 4 Prediction of top activated and downregulated upstream regulators involved in progression of *Egfr*$^{+/+}$, *Apc*$^{f/f}$, *Kras*$^{LSL/+}$ colon polyps.

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	p-value of overlap
Alpha catenin	Group	Activated	6.293	1.87×10^{-25}
DUSP1	Phosphatase	Activated	4.277	2.27×10^{-09}
DKK1	Growth factor	Activated	4.256	1.46×10^{-09}
NR3C1	Ligand-dependent nuclear receptor	Activated	4.029	1.81×10^{-09}
SFTPA1	Transporter	Activated	3.928	2.75×10^{-08}
TNF	Cytokine	Inhibited	-7.804	3.24×10^{-40}
Ige	Complex	Inhibited	-6.882	9.11×10^{-14}
PDGF BB	Complex	Inhibited	-6.508	9.71×10^{-15}
MYD88	Other	Inhibited	-6.410	1.41×10^{-11}
IL1B	Cytokine	Inhibited	-6.320	3.75×10^{-27}

DUSP1, Dual Specificity Phosphatase 1; DKK1, Dickkopf WNT Signaling Pathway Inhibitor 1; NR3C1, Nuclear Receptor Subfamily 3 Group C Member 1; SFTPA, Surfactant Protein A; TNF, Tumor Necrosis Factor; Ige, Immunoglobulin E; PDGF BB, Platelet-derived growth factor BB; MYD88, Myeloid differentiation primary response 88; IL1B, Interleukin 1B.

Supplementary Table 5 Prediction of top activated and down-regulated upstream regulators involved in progression of *Egfr*$^{f/f}$, *Apc*$^{f/f}$, *Kras*$^{+/+}$ colon polyps.

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	p-value of overlap
Alpha catenin	Group	Activated	6.763	$5.58x10^{-34}$
IL10RA	Transmembrane receptor	Activated	6.568	$1.78x10^{-43}$
SFTPA1	Transporter	Activated	4.579	$1.42x10^{-12}$
DKK1	Growth factor	Activated	4.579	$5.02x10^{-10}$
PPARGC1A	Transcription regulator	Activated	4.385	$1.46x10^{-13}$
TNF	Cytokine	Inhibited	-6.440	$3.01x10^{-56}$
ERK	Group	Inhibited	-5.980	$1.47x10^{-12}$
PDGF BB	Complex	Inhibited	-5.724	$6.59x10^{-21}$
EGF	Growth factor	Inhibited	-5.697	$1.41x10^{-22}$
MYD88	Other	Inhibited	-5.641	$1.34x10^{-14}$

IL10RA, Interleukin 10 Receptor Subunit Alpha; SFTPA, Surfactant Protein A; DKK1, Dickkopf WNT Signaling Pathway Inhibitor 1; PPARGC1A, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha; TNF, Tumor Necrosis Factor; ERK, Extracellular-signal-regulated Kinase; PDGF BB, Platelet-derived growth factor BB; EGF, Epidermal Growth Factor; MYD88, Myeloid differentiation primary response 88.

Supplementary Table 6 Prediction of top activated and down-regulated upstream regulators involved in progression of *Egfr*$^{f/f}$, *Apc*$^{f/f}$, *Kras*$^{LSL/+}$ colon polyps.

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	p-value of overlap
IL10RA	Transmembrane receptor	Activated	3.148	1.04×10^{-10}
HNF1A	Transcription regulator	Activated	2.392	1.55×10^{-05}
Beta-estradiol	Chemical drug	-	1.367	1.74×10^{-02}
Glucocorticoid	Chemical drug	-	0.762	1.71×10^{-03}
Gentamicin	Chemical drug	-	-1.342	6.39×10^{-05}
Methylprednisolone	Chemical drug	-	-1.192	2.30×10^{-03}
AGT	Growth factor	-	-1.103	1.08×10^{-03}
Dexamethasone	Growth factor	-	-0.781	7.78×10^{-03}

IL10RA, Interleukin 10 Receptor Subunit Alpha; HNF1A, Hepatocyte nuclear factor 1 homeobox A; AGT, Angiotensinogen.

Supplementary Table 7 PCR primers used for qPCR analysis.

Gene symbol	Gene name	Gene function	Forward (sequence 5' → 3')	Reverse (sequence 5' → 3')
Anergy associated genes				
Gbp3	Guanylate Binding Protein 3	Cell signaling	AGGAAACCCTCACTGTTTGG	AGTGAGCCGAGGAATTTCAG
Ikzf1	Ikaros family zinc finger protein	Zinc protein -Transcription factor	CGGGATCCCTTTGAGTGTAA	AGCTCAGGTGGTAACGATGC
Casp3	Caspase 3	Apoptosis-related cysteine peptidase	ACGCGCACAAGCTAGAATTT	CTTTGCGTGGAAAGTGGAGT
Dgka	Diacylglycerol	Diacylglycerol kinase	CTGCCAATCTCAATTGCAC	AGTGCGGCCAAAATAATCAC
Socs2	Suppressor of Cytokine Signaling 2	Negative regulator in the growth hormone/IGF1 signaling pathway.	GTGCAAGGATAAACGGACAG	TCGACAGAAATGCTGCAGAG
FasL	Fas ligand gene	Triggers apoptosis through Fas	GCAAATAGCCAACCCCAGTA	ATTCCAGAGGGATGGACCTT
Grg4	Groucho related gene 4	Groucho transcription factor	TCACTCAAGTTTGCCCACTG	CACAGCTAAGCACCGATGAG
Grail	Gene Related to Anergy in Lymphocytes	Inhibitor of cytokine gene transcription	ATGCAAGAGCTCAAAGCAGGAAGC	GTGCGCAGCTGAAGCTTTCCAATA
Cbl-b	Casitas B-lineage Lymphoma - b	E3 ubiquitin-protein ligase	GCAGCATCATTGACCCTTTCAGCA	ATGTGACTGGTGAGTTCTGCCTGT
ErbB family members				
Egfr	Epidermal Growth Factor Receptor	ErbB tyrosine kinase receptor	GCATCATGGGAGAGAACAACA	CTGCCATTGAACGTACCCAGA

Erbb2	Epidermal growth factor receptor 2	ErbB tyrosine kinase receptor	GAGACAGAGCTAAGGAAGCTGA	ACGGGGATTTTCACGTTCTCC
Erbb3	Epidermal growth factor receptor 3	ErbB tyrosine kinase receptor	TCTGCATTAAAGTCATCGAGGAC	CAGCCGTACAATGTGGGCAT
Erbb4	Epidermal growth factor receptor 4	ErbB tyrosine kinase receptor	TCCCCCAGGCTTTCAACATAC	GCACCCTGAGCTACTGGAG
Egf	Epidermal Growth Factor	Growth Factor ligand	TTCTCACAAGGAAAGAGCATCTC	GTCCTGTCCCGTTAAGGAAAAC
Tnfa	Transforming growth factor –a	Growth Factor ligand	CACTCTGGGTACGTGGGTG	CACAGGTGATAATGAGGACAGC
Areg	Amphiregulin	Growth Factor ligand	GCCTCCGAAGTGTGGTATCC	CCTGGTACTGTCCAAACGCA
Epgn	Epigen	Growth Factor ligand	GGGGGTTCTGATAGCAGTCTG	TCGGTGTTGTTAAATGTCCAGTT
Btc	Betacellulin	Growth Factor ligand	AATTCTCCACTGTGTGGTAGCA	GGTTTTCACTTTCTGTCTAGGGG
Hbegf	Heparin-binding Egf	Growth Factor ligand	CGGGGAGTGCAGATACCTG	TTCTCCACTGGTAGAGTCAGC
Ereg	Epiregulin	Growth Factor ligand	CTGCCTCTTGGGTCTTGACG	GCGGTACAGTTATCCTCGGATTC
Nrg1	Neuregulin1	Growth Factor ligand	TCAGCAAGTTAGGAAACGACAG	ACATAGGGTCTTTCAGTTGAGGC
Nrg2	Neuregulin2	Growth Factor ligand	GGATGGCAAGGAACTCAACC	TCGGCCTCACAGACGTACT
Nrg3	Neuregulin3	Growth Factor ligand	TTACGCTGTAGCGACTGCATC	GCCTACCACGATCCATTTAAGC
Nrg4	Neuregulin4	Growth Factor ligand	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
IL10 signaling				
Il10Ra	Interleukin 10 receptor alpha	Cell surface receptor	GCCCTTCCTATGTGTGGTTTG	TTGAGTTTCCGTACTGTTTGAGG
Il10	Interleukin 10	Anti-inflammatory cytokine	AGTGGAGCAGGTGAAGAGTG	TTCGGAGAGAGGTACAAACG

Socs3	Suppressor of cytokine signaling 3	Negative regulation of cytokines that signal through the JAK/STAT pathway	ATGGTCACCCACAGCAAGTTT	CTGGAGGCGGCATGTAGTG
RNA-sequencing validation				
Aadac	Arylacetamide deacetylase	Hydrolase activity and triglyceride lipase activity	TACCGCTTCCAGATGCTATTGA	ACTGATTCCCAAAAGTTCACCAA
Aldh1a1	Aldehyde Dehydrogenase 1 Family Member A1	Oxidoreductase activity and acyl-CoA dehydrogenase activity	ATACTTGTCGGATTTAGGAGGCT	R GGGCCTATCTTCCAAATGAACA
Maob	Monoamine Oxidase B	Protein homodimerization activity and electron transfer activity	ATGAGCAACAAAAGCGATGTGA	TCCTAATTGTGTAAGTCCTGCCT
Sult1c2	Sulfotransferase Family 1C Member 2	Sulfotransferase activity	ATGGCCTTGACCCCAGAAC	TCGAAGGTCTGAATCTGCCTC
Sult1a1	Sulfotransferase Family 1A Member 1	Sulfotransferase activity and flavonol 3-sulfotransferase activity	CAACATGGAGCCCTTGCGTAA	ATGAGCACATCATCAGGCCAG
IL17Ra	Interleukin 17 Receptor A	Proinflammatory cytokine signaling	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
Ndrg4	NDRG Family Member 4	Enhance growth factor signaling	TCCGGGGCTCTCCCAAAGGG	GGCATCCACGTGGCACACCA
Reference genes				
Actb	Beta Actin	Cytoskeletal structural protein	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Tbp	TATA-box-binding protein	General transcription factor	ACCGTGAATCTTGGCTGTAAAC	GCAGCAAATCGCTTGGGATTA

Gusb	Glucuronidase, Beta	Lysosomal exoglycosidase	GGCTGGTGACCTACTGGATTT	GGCACTGGGAACCTGAAGT
Eef2	Eukaryotic Translation Elongation Factor 2	Protein Synthesis	TGTCAGTCATCGCCCATGTG	CATCCTTGCGAGTGTCAGTGA
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase	Glycolysis pathway enzyme	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA