Commensal-infected macrophages induce dedifferentiation and reprogramming of epithelial cells during colorectal carcinogenesis

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: *Ctnnb1* expression in YAMC cells exposed to uninfected and *E. coli*-infected macrophages. (A), *Ctnnb1* expression in YAMC cells transiently increases after 48 hrs treatment with uninfected macrophages compared to untreated control. (B), *Ctnnb1* expression increases after 24 - 72 hrs treatment with *E. coli* DH5 α -infected macrophages compared to untreated control. NS, not significant. Data represent mean \pm SD for 3 independent experiments.



Supplementary Figure 2: MIBE-induced phosphorylation of Gsk3 β (Ser⁹). (A), Phosphorylated Gsk3 β (Ser⁹) is transiently increased in YAMC cells exposed to *E. faecalis*-infected macrophages for 5 hrs and decreased afterwards. (B), p-Gsk3 β (Ser9) is transiently increased 24 hrs after 1 hr treatment with 4-HNE. (C), Increased p-Gsk3 β is also noticed for cells treated with TNF α for 24 hrs and subsequently decreased.



Supplementary Figure 3: Activation of β -catenin by *E. coli*-infected macrophages. Western blots show increased active β -catenin and Tcf4 (*upper*) in YAMC cells exposed to *E. coli*-infected macrophages (100 multiplicity of infection) after normalization to β -actin and untreated controls (*lower*). Data represent mean \pm SD for 3 independent experiments.

Genes	Primer sequence (bp)	PCR product	
Human ACTB		148	
forward	5'- ACCTTCTACAATGAGCTGCG -3'		
reverse	5'- CCTGGATAGCAACGTACATGG -3'		
Human CTNNB1		150	
forward	5'- GTTCAGTTGCTTGTTCGTGC -3'		
reverse	5'- GTTGTGAACATCCCGAGCTAG -3'		
Human DCLK1		118	
forward	5'- AAAGTCTGCAGCTCGATGG -3'		
reverse	5'- CATCTCCTATTGTTCTTCCGACT -3'		
Murine Actb		147	
forward	5'- ACCTTCTACAATGAGCTGCG -3'		
reverse	5'- CTGGATGGCTACGTACATGG -3'		
Murine Ctnnb1		135	
forward	5'- GCTATTCCACGACTAGTTCAGC -3'		
reverse	5'- AGCTCCAGTACACCCTTCTAC -3'		
Murine Dclk1		83	
forward	5'- TGCCGACTGAACTGTATCTTG -3'		
Reverse	5'- TGTGTATTTGCTAGTGGAGGTG -3'		

Supplementary Table 1: Primers for qRT-PCR

Application and antigen	Vendor	Catalogue Number	Host	Clonality	Dilution
Western bloting					
Active β -catenin	Cell Signaling Technology	4270	rabbit	Poly	1:1,000
β-actin	Santa Cruz Biotechnology	sc-81178	Mouse	Mono	1:2,000
c-Myc	Santa Cruz Biotechnology	sc-40	mouse	Mono	1:500
CD44	Cell Signaling Technology	5640	Mouse	Mono	1:500
Dclk1	Abgent	AP7219b	Rabbit	Poly	1:1,000
Klf4	Millipore	09-821	Rabbit	Poly	1:500
Oct3/4	Santa Cruz Biotechnology	sc-9081	Rabbit	Poly	1:200
p-Gsk3β (Ser ⁹)	Santa Cruz Biotechnology	sc-11757	Goat	Poly	1:200
Sox2	Abgent	AP2048D	Rabbit	Poly	1:1,000
Tcf4	Santa Cruz Biotechnology	sc-8631	Goat	Poly	1:200
Wif1	Cell Signaling Technology	2064	Rabbit	Poly	1:1,000
Wnt3a	R&D Systems MAB1324	MAB1324	Rat	Mono	1:400
IHC & IF staining					
Active β -catenin	Millipore	05-665	Mouse	Mono	1:250
c-Myc	Abgent	AP1985B	rabbit	Poly	1:25
Dclk1	Abgent	AP7219b	Rabbit	Poly	1:50
Klf4	Millipore	09-821	Rabbit	Poly	1:100
Oct3/4	Santa Cruz Biotechnology	sc-9081	Rabbit	Poly	1:100
Sox2	Cell Signaling Technology	3728	Rabbit	Mono	1:50

Supplementary Table 2: Primary antibodies and dilution used in this study