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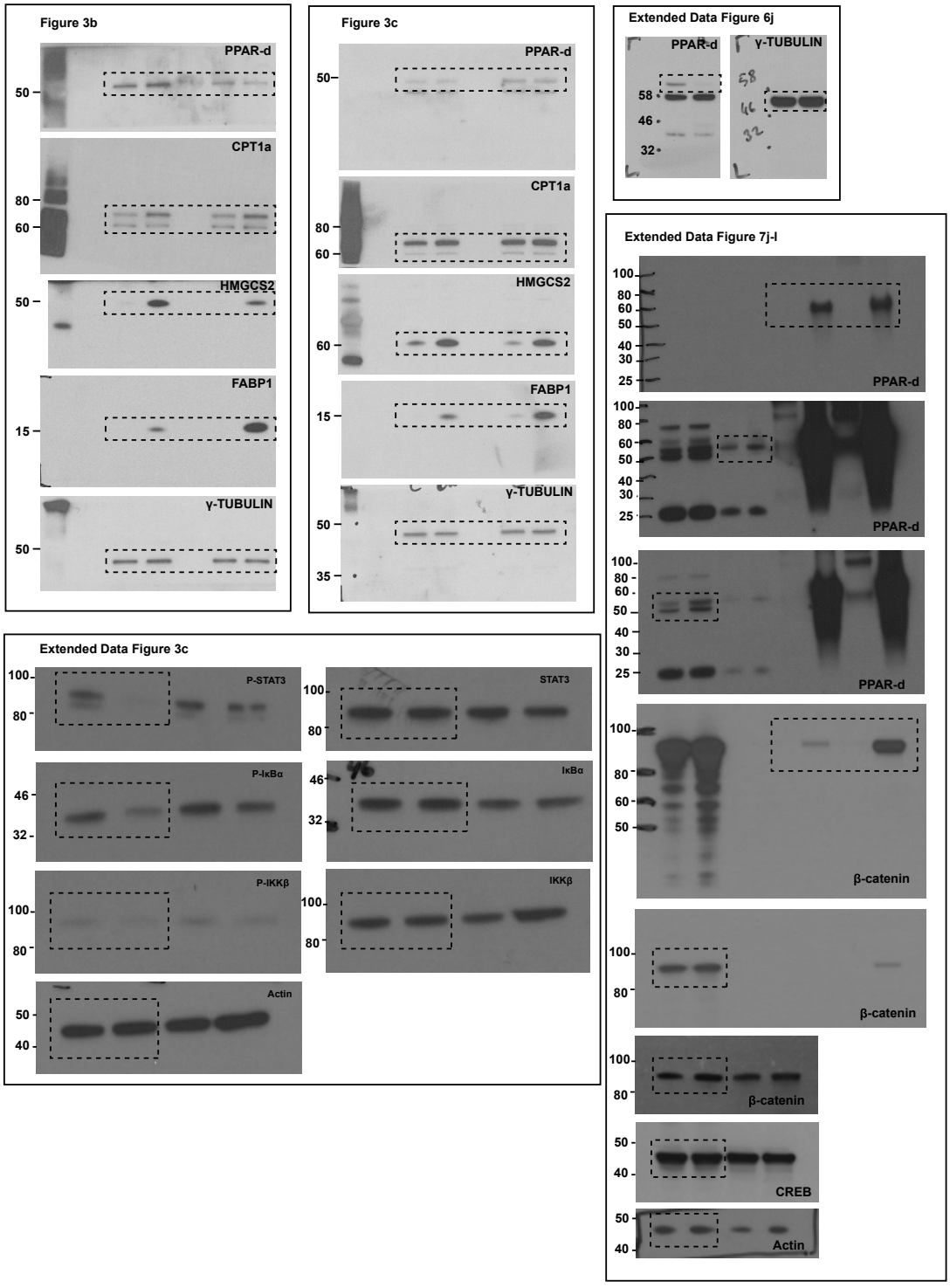

Supplementary Figure 1. Original blots from figures. Dashed box indicates cropped images shown in the corresponding figures.

HFD increases ISC proliferation

The fact that HFD boosted the numbers of ISCs and crypt depth in the small intestine suggested that it augments the proliferation of ISCs and their more differentiated progeny (i.e. progenitors), which reside in intestinal crypts. To address this possibility, we assessed incorporation of 5-bromodeoxyuridine (BrdU) into ISCs and progenitors. After a 4-hour pulse of BrdU, crypts of HFD mice had more $BrdU^+$ stem cells compared to controls (Fig. 1b, Extended Data 1h, i; and for the colon, Extended Data 3f). Furthermore, HFD augmented the number of $BrdU^+$ cells in the larger pool of progenitors (Fig. 1b, Extended Data 1i), indicating that output and migration from this compartment into villi may also increase. Indeed, HFD mice 24 hour after a single dose of BrdU had more absolute numbers of BrdU-labeled cells in the villi (Extended Data 1j, k) and a greater percentage of villus enterocytes that were $BrdU^+$ (Extended Data 1j, k). These findings demonstrate that the increase in stem and progenitor cell proliferation induced by HFD translates into enhanced output and migration of crypt cells into the villus.

A HFD and PPAR-d signaling increase β-catenin activity

Single cell analyses of β-catenin target gene expression revealed heterogeneity in control and HFD-derived ISCs and progenitors (Extended Data 8a). t-Distributed stochastic neighbor embedding (t-SNE) analysis of single cell gene expression data of all β-catenin target genes demonstrated clustering of HFD-derived ISCs and progenitors (Extended Data 8d) from their control-fed counterparts, consistent with the notion that HFD induces β-catenin target gene expression in intestinal stem and progenitor cells. Furthermore, we found that while HFD-induced β-catenin signature genes (HFD signature; Extended Data 8c) were heterogeneously

expressed in control ISCs and progenitors, they formed a distinct cluster upon HFD as shown in the t-SNE analysis (Extended Data 8f) and by qRT-PCR in the colon (Extended Data 3i).

Analysis of leptin receptor deficient db/db obese mice

In contrast to controls, small intestinal mass, length, and villi length increased, while crypt depth, ISC and Paneth cell numbers decreased in *db/db* mice (Extended Data 9a-h). Although organoid-forming capacity of *db/db* crypts trended higher relative to controls (Extended Data 9i), there was no increase in HFD-induced PPAR-d or β-catenin target gene expression in *db/db* intestinal progenitors (Extended Data 9j). Overall, these findings are mostly opposite to what we observed in HFD mice and highlight that, even in obesity, diet impacts intestinal stem and progenitor biology.

Enforced PPAR-d signaling permits non-*Lgr5*$^{+}$ cells to initiate intestinal adenomas

The finding that *Apc-loss* after enforced PPAR-d signaling does not have additive effects on tumor initiation indicates that in *Apc-wild type* stem cells enforced PPAR-d signaling drives many of its effects (e.g. reduced Paneth cell dependence in the assay) through beta-catenin signaling (Fig. 5g, h). In the *Apc*-null state, it is interesting to note that enforced PPAR-d signaling differentially affects the adenoma-initiating capacity of stem and progenitor cells upon *Apc* loss (Fig. 5g, h). One possible explanation for this divergence may be that ISCs, in contrast to progenitors, are in a cellular state that is facilitative for β-catenin signaling. Progenitors, however, are in a more differentiated cellular state that is less responsive to β-

catenin signaling, and perhaps enforced PPAR-d signaling in a subset of progenitors augments this responsiveness—thus enabling them to initiate tumors upon *Apc-loss*.

Supplementary Table 1 | Primer sequences for qRT-PCR

Gene	Forward Primer	Gene	Reverse Primer
Pparg_F	CCACCAACTTCGGAATCAGCT	Pparg_R	TTTGTGGATCCGGCAGTTAAGA
Ppara_F	AGAGCCCCATCTGTCCTCTC	Ppara_R	ACTGGTAGTCTGCAAAACCAAA
Ppard_F	TCCATCGTCAACAAAGACGGG	Ppard_R	ACTTGGGCTCAATGATGTCAC
Mgll_F	CGGACTTCCAAGTTTTTGTCAGA	Mgll_R	GCAGCCACTAGGATGGAGATG
Slc22a3_F	CGTTTCTGCTCTTTCGGCTG	Slc22a3_R	TGCAACTGTGAACTGCCAAG
Fabp1_F	GGGAAGAAAATCAAACTCACCATC	Fabp1_R	AGTTGTCACCATTTTATTGTCACC
Pdk4_F	AGGGAGGTCGAGCTGTTCTC	Pdk4_R	GGAGTGTTCACTAAGCGGTCA
Hmgcs2_F	ATACCACCAACGCCTGTTATGG	Hmgcs2_R	CAATGTCACCACAGACCACCAG
Cpt1a_F	CCATGAAGCCCTCAAACAGATC	Cpt1a_R	ATCACACCCACCACCACGATA
Actb_F	GGCTGTATTCCCCTCCATCG	Actb_R	CCAGTTGGTAACGCCATGT
mtCytB_F	CATTTATTATCGCGGCCCTA	mtCytB_R	TGTTGGGTTGTTTGATCCTG
bglobin_F	GAAGCGATTCTAGGGAGCAG	bglobin_R	GGAGCAGCGATTCTGAGTAGA
Il6_F	TAGTCCTTCCTACCCCAATTTCC	Il6_R	TTGGTCCTTAGCCACTCCTTC
Il1b_F	GCAACTGTTCCTGAACTCAACT	Il1b_R	ATCTTTTGGGGTCCGTCAACT
Tnfa_F	CCCTCACACTCAGATCATCTTCT	Tnfa_R	GCTACGACGTGGGCTACAG
Ifng_F	ATGAACGCTACACACTGCATC	Ifng_R	CCATCCTTTTGCCAGTTCCTC
Apc-Int13Flox_F	GAGAAACCCTGTCTCGAAAAAA	Apc-Int13Flox_R	AGTGCTGTTTCTATGAGTCAAC
Apc-Int13F2_del_F	GAGAAACCCTGTCTCGAAAAAA	Apc-Int14R4_del_R	TTGGCAGACTGTGTATATAAGC
qCD24a_F	TTCTGGCACTGCTCCTACC	qCD24a_R	GCGTTACTTGGATTTGGGGAA
qChga_Fwd	CCAAGGTGATGAAGTGCGTC	qChga_Rev	GGTGTCGCAGGATAGAGAGGA
Ppard_del_F	TGTGCAGACCTCTCCCAGAA	Ppard_del_R	CCGACATTCCATGTTGAGGC
Lyz_F	GAGACCGAAGCACCGACTATG	Lyz_R	CGGTTTTGACATTGTGTTCGC
Muc2_F	ATGCCCACCTCCTCAAAGAC	Muc2_R	GTAGTTTCCGTTGGAACAGTGAA
human_Cpt1a_F	TCCAGTTGGCTTATCGTGGTG	human_Cpt1a_R	TCCAGAGTCCGATTGATTTTTGC
human_Pdk4_F	GGAGCATTTCTCGCGCTACA	human_Pdk4_R	ACAGGCAATTCTTGTCGCAAA
human_Hmgcs2_F	GACTCCAGTGAAGCGCATTCT	human_Hmgcs2_F	CTGGGAAGTAGACCTCCAGG
human_Fabp1_F	GTGTCGGAAATCGTGCAGAAT	human_Fabp1_F	GACTTTCTCCCCTGTCATTGTC

Supplementary Table 2 | Primer sequences for single cell analysis

SEQUENCE NAME	Forward Primer	SEQUENCE NAME	Reverse Primer
ABCB1F	CAGCAGTCAGTGTGCTTACAA	ABCB1R	ATGGCTCTTTTATCGGCCTCA
ADAM10F	ATGGTGTTGCCGACAGTGTTA	ADAM10R	GTTTGGCACGCTGGTGTTTTT
ALEX1F	CTGGTGCCTGCTACTGTGTAT	ALEX1R	CCCCTACCCCAACATTAGTCT
ASCL2F	AAGCACACCTTGACTGGTACG	ASCL2R	AAGTGGACGTTTGCACCTTCA
AXIN2F	TGACTCTCCTTCCAGATCCCA	AXIN2R	TGCCCACACTAGGCTGACA
BAMBIF	GATCGCCACTCCAGCTACTTC	BAMBIR	GCAGGCACTAAGCTCAGACTT
BCL2L2F	GCGGAGTTCACAGCTCTATAC	BCL2L2R	AAAAGGCCCCTACAGTTACCA
BCL2L1F	GACAAGGAGATGCAGGTATTGG	BCL2L1R	TCCCGTAGAGATCCACAAAAGT
BIRC5F	GAGGCTGGCTTCATCCACTG	BIRC5R	CTTTTTGCTTGTTGTTGGTCTCC
BMI1F	ATCCCCACTTAATGTGTGTCCT	BMI1R	CTTGCTGGTCTCCAAGTAACG
BMP4F	TTCCTGGTAACCGAATGCTGA	BMP4R	CCTGAATCTCGGCGACTTTTT
CCND1F	GCGTACCCTGACACCAATCTC	CCND1R	CTCCTCTTCGCACTTCTGCTC
CD44F	CACCATTGCCTCAACTGTGC	CD44R	TTGTGGGCTCCTGAGTCTGA
CDKN2AF	CGCAGGTTCTTGGTCACTGT	CDKN2AR	TGTTCACGAAAGCCAGAGCG
CDX1F	GGACGCCCTACGAATGGATG	CDX1R	GTACCGGCTGTAGTGAAACTC

CLDN1F	GGGGACAACATCGTGACCG	CLDN1R	AGGAGTCGAAGACTTTGCACT
COX2F	TGAGCAACTATTCCAAACCAGC	COX2R	GCACGTAGTCTTCGATCACTATC
DKK1F	CTCATCAATTCCAACGCGATCA	DKK1R	GCCCTCATAGAGAACTCCCG
DKK4F	GTACTGGTGACCTTGCTTGGA	DKK4R	CCGTTCATCGTGAAACGCTAAG
DNMT1F	AAGAATGGTGTTGTCTACCGAC	DNMT1R	CATCCAGGTTGCTCCCCTTG
EDN1F	GCACCGGAGCTGAGAATGG	EDN1R	GTGGCAGAAGTAGACACACTC
EFNB1F	TGTGGCTATGGTCGTGCTG	EFNB1R	CCAAGCCCTTCCCACTTAGG
ENC1F	CTGTTTCATAAGTCCTCCTACGC	ENC1R	CACCACTGAACATGGCTTCG
EPHB2F	GCGGCTACGACGAGAACAT	EPHB2R	GGCTAAGTCAAAATCAGCCTCA
EPHB3F	CATGGACACGAAATGGGTGAC	EPHB3R	GCGGATAGGATTCATGGCTTCA
FGF18F	CCTGCACTTGCCTGTGTTTAC	FGF18R	TGCTTCCGACTCACATCATCT
FGFBP1F	CTAAATCTCTGACGCATGGCA	FGFBP1R	AACTCCTGATCGGCTTGTGTG
FGFBP3F	GGTCGCTTCGTGAGTCCAG	FGFBP3R	AGCAGCCGTCTCCAGTAGT
FRA1F	ATGTACCGAGACTACGGGGAA	FRA1R	CTGCTGCTGTCGATGCTTG
FSCN1F	GACTGCGAAGGTCGCTACC	FSCN1R	CTGATCGGTCTCTTCATCCTGA
GASTF	CGCTCCCAGCTACAGGATG	GASTR	GGTCTGCTATGAAGTGTTGAGG
HATH1F	GAGTGGGCTGAGGTAAAAGAGT	HATH1R	GGTCGGTGCTATCCAGGAG
NEDD9F	ATGTGGGCGAGGAATCTTATGG	NEDD9R	TTCCCTGGGACAATGCCTTG
HES1F	CCAGCCAGTGTCAACACGA	HES1R	AATGCCGGGAGCTATCTTTCT
ID2F	ATGAAAGCCTTCAGTCCGGTG	ID2R	AGCAGACTCATCGGGTCGT
TCF4F	CGAAAAGTTCCTCCGGGTTTG	TCF4R	CGTAGCCGGGCTGATTCAT
JAG1F	CCTCGGGTCAGTTTGAGCTG	JAG1R	CCTTGAGGCACACTTTGAAGTA
JUNF	CCTTCTACGACGATGCCCTC	JUNR	GGTTCAAGGTCATGCTCTGTTT
L1CAMF	AAAGGTGCAAGGGTGACATTC	L1CAMR	TCCCCACGTTCCTGTAGGT
LAMC2F	CAGACACGGGAGATTGCTACT	LAMC2R	CCACGTTCCCCAAAGGGAT
LEF1F	TGTTTATCCCATCACGGGTGG	LEF1R	CATGGAAGTGTCGCCTGACAG
LGR5F	CCTACTCGAAGACTTACCCAGT	LGR5R	GCATTGGGGTGAATGATAGCA
MENAF	GCCCAGAGCAAGGTTACTG	MENAR	GCCCACAGAAAATACATCGCAA
METF	GTGAACATGAAGTATCAGCTCCC	METR	TGTAGTTTGTGGCTCCGAGAT
MMP14F	CAGTATGGCTACCTACCTCCAG	MMP14R	GCCTTGCCTGTCACTTGTAAA
MMP7F	CTGCCACTGTCCCAGGAAG	MMP7R	GGGAGAGTTTTCCAGTCATGG
MYBF	AGACCCCGACACAGCATCTA	MYBR	CAGCAGCCCATCGTAGTCAT
MYCF	ATGCCCCTCAACGTGAACTTC	MYCR	CGCAACATAGGATGGAGAGCA
MYCBPF	GCTGGACACGCTGACGAAA	MYCBPR	TCTAGGCGAAGCAGCTCTATTT
NOS2F	GTTCTCAGCCCAACAATACAAGA	NOS2R	GTGGACGGGTCGATGTCAC
NOTCH2F	ATGTGGACGAGTGTCTGTTGC	NOTCH2R	GGAAGCATAGGCACAGTCATC
NRCAMF	AAAGGGAAACCTCCCCCAAG	NRCAMR	TGTTGATGACAAGGGTTCCTGA
PLAUF	GCGCCTTGGTGGTGAAAAAC	PLAUR	TTGTAGGACACGCATACACCT
PLAURF	CAGAGCTTTCCACCGAATGG	PLAURR	GTCCCCGGCAGTTGATGAG
PPARDF	TCCATCGTCAACAAAGACGGG	PPARDR	ACTTGGGCTCAATGATGTCAC
S100A4F	TCCACAAATACTCAGGCAAAGAG	S100A4R	GCAGCTCCCTGGTCAGTAG
SGK1F	CTGCTCGAAGCACCCTTACC	SGK1R	TCCTGAGGATGGGACATTTTCA
SMC3F	CGAAGTTACCGAGACCAAACA	SMC3R	TCACTGAGAACAAACTGGATTGC

SOX9F	AGTACCCGCATCTGCACAAC	SOX9R	ACGAAGGGTCTCTTCTCGCT
SP5F	TGGGTTCACCCTCCAGACTTT	SP5R	CCGGCGAGAACTCGTAAGG
SRSF3F	GCGCAGATCCCCAAGAAGG	SRSF3R	ATCGGCTACGAGACCTAGAGA
SUZ12F	AACTCGAAATCTTATCGCACCAA	SUZ12R	TGCAAATGTGCAGACAAGCTAT
TCF1F	AGGAGTGTAATAGGGCGGAGT	TCF1R	GAGGTCCGTTATAGGTGTCCA
TIAM1F	GAAGCACACTTCACGCTCC	TIAM1R	CTCCAGGCCATTTTCAGCCA
TNCF	ACGGCTACCACAGAAGCTG	TNCR	ATGGCTGTTGTTGCTATGGCA
VEGFF	GCCAGACAGGGTTGCCATAC	VEGFR	GGAGTGGGATGGATGATGTCAG
YAP1F	TACTGATGCAGGTACTGCGG	YAP1R	TCAGGGATCTCAAAGGAGGAC
CCND3F	CGAGCCTCCTACTTCCAGTG	CCND3R	GGACAGGTAGCGATCCAGGT
FZD1F	CAGCAGTACAACGGCGAAC	FZD1R	GTCCTCCTGATTCGTGTGGC
FZ7F	GCCACACGAACCAAGAGGAC	FZ7R	CGGGTGCGTACATAGAGCATAA
BTRCPF	AAGACTGTAATAATGGCGAACCC	BTRCPR	TCTCTTGGTTTATGCAAAGCCTG
BCATENINF	ATGGAGCCGGACAGAAAAGC	BCATENINR	CTTGCCACTCAGGGAAGGA
NEMOF	AAGCACCCCTGGAAGAACC	NEMOR	CCTGCTCTGAAGGCAGATGTA
NAKED1F	AGGAAAGGCATCGAGGAGTG	NAKED1R	TCGCTCAGTCTCTCCATTCTC
RNF43F	TCCGAAAGATCAGCAGAACAGA	RNF43R	GGACTGCATTAGCTTCCCTTC
LRP6F	TTGTTGCTTTATGCAAACAGACG	LRP6R	GTTCGTTTAATGGCTTCTTCGC
LRP5F	AAGGGTGCTGTGTACTGGAC	LRP5R	AGAAGAGAACCTTACGGGACG
OLFM4F	CAGCCACTTTCCAATTTCACTG	OLFM4R	GCTGGACATACTCCTTCACCTTA
PREX1F	TTTAACCAGGTCGATTCCATCCA	PREX1R	CGGACCGTGCATTCCTCTTT
RAC1F	GAGACGGAGCTGTTGGTAAAA	RAC1R	ATAGGCCCAGATTCACTGGTT
Cldn15F	ATGTCGGTAGCTGTGGAGAC	Cldn15R	GGACGGAAAGTCCCAGCAG
Edn3F	CCCTGGTGAGAGGATTGTGTC	Edn3R	CCTTGTCCTTGTAAGTGAAGCAC
EFNB2F	ATTATTTGCCCCAAAGTGGACTC	EFNB2R	GCAGCGGGGTATTCTCCTTC
EPHB4F	TATGCCACGATACGCTTCACC	EPHB4R	AGCTTCGCTCTCGTAATAGAAGA
FGFR4F	GCTCGGAGGTAGAGGTCTTGT	FGFR4R	CCACGCTGACTGGTAGGAA
NEDD8F	GGAGCGAATCAAGGAGCGT	NEDD8R	ACGGAACCACCTAGAATCTTGT
TCF7F	AGCTTTCTCCACTCTACGAACA	TCF7R	AATCCAGAGAGATCGGGGGTC
JAG2F	CAATGACACCACTCCAGATGAG	JAG2R	GGCCAAAGAAGTCGTTGCG
SMC2F	GGCTGGGATTACCAAAGCCTC	SMC2R	CACCAATAACCACCTGTCTTGT
Sox9F	GAGCCGGATCTGAAGAGGGA	Sox9R	GCTTGACGTGTGGCTTGTTC
NcadherinF	ATGTGCCGGATAGCGGGAGC	NcadherinR	TACACCGTGCCGTCCTCGTC
EcadherinF	CACCTGGAGAGAGGCCATGT	EcadherinR	TGGGAAACATGAGCAGCTCT
ACTINBF	GGCTGTATTCCCCTCCATCG	ACTINBR	CCAGTTGGTAACAATGCCATGT
GAPDHF	AGGTCGGTGTGAACGGATTTG	GAPDHR	TGTAGACCATGTAGTTGAGGTCA