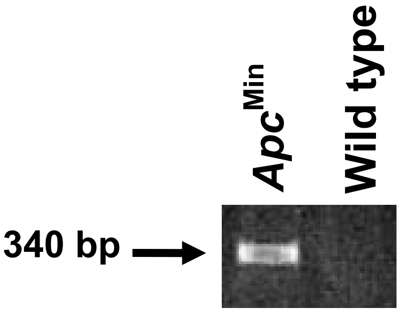
Supplemental material

Supplementary Figure 1: Genotyping of *Apc*$^{\text{Min/+}}$ mice:

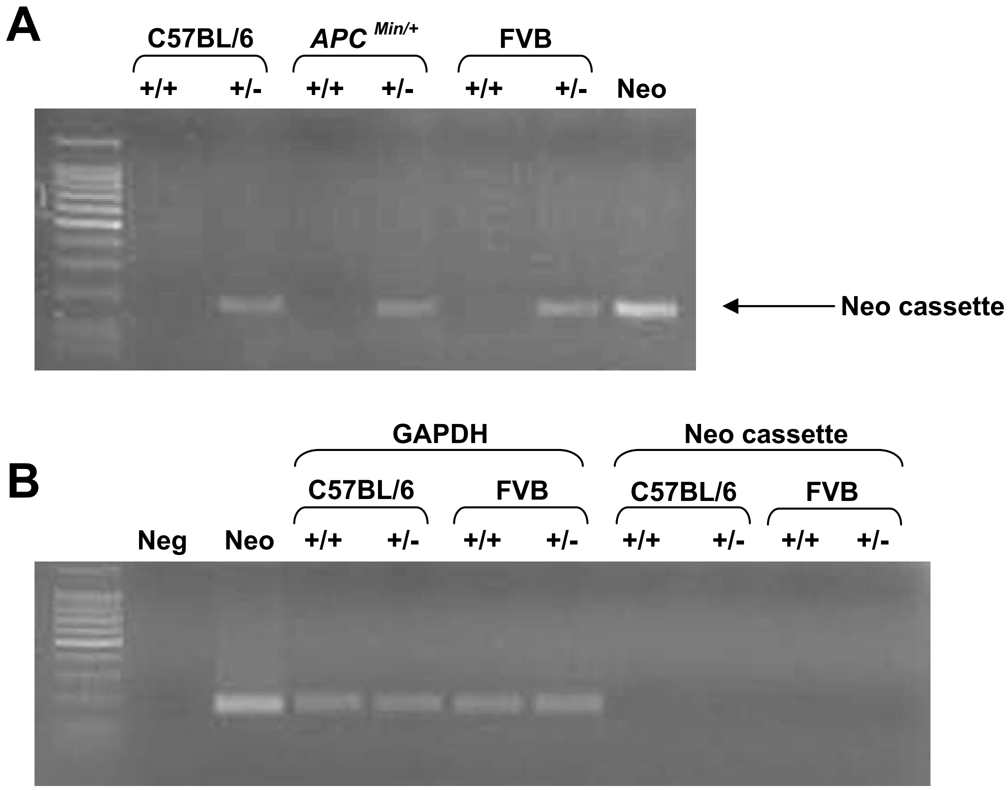
1: Genotyping of mouse tail DNA by PCR amplification identifies carriers of the *Apc*$^{\text{Min/+}}$ (presence of 340 bp band) alleles. The *Apc*$^{\text{Min/+}}$ locus was detected using the following primers: 5'-TTCCACTTTGGCATAAGGC-3‘, 5'-TTCTGAGAAAGACAGAAGTTA-3’.



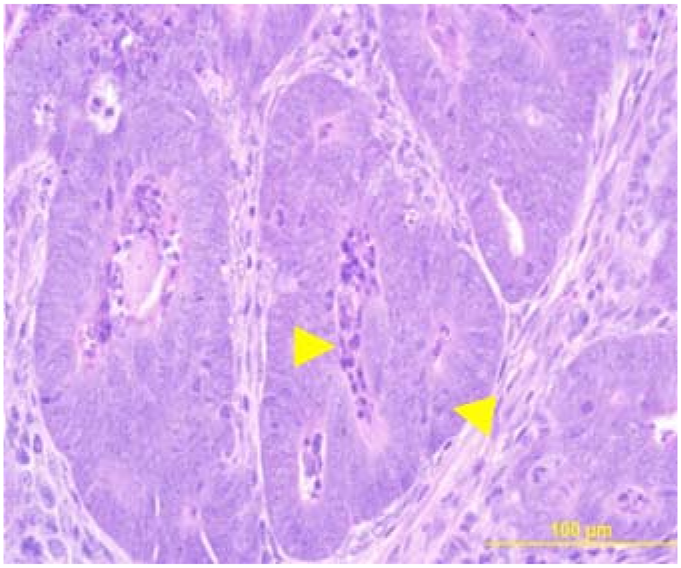
Supplementary Fig 2: PCR amplification of the neomycin cassette:

A) The presence of the neomycin insertion sequence in the genomic DNA of *Tgfbr1*$^{+/-}$ mice from C57BL/6, *Apc*$^{Min/+}$, and FVB backgrounds is confirmed by neomycin-specific PCR. PCR performed from genomic DNA of *Tgfbr1*$^{+/+}$ mice produced no bands. This result confirms the presence of the neomycin sequence in the genomic DNA of haploisufficient mice. (Neo, pIRES vector containing neomycin insertion sequence positive control)

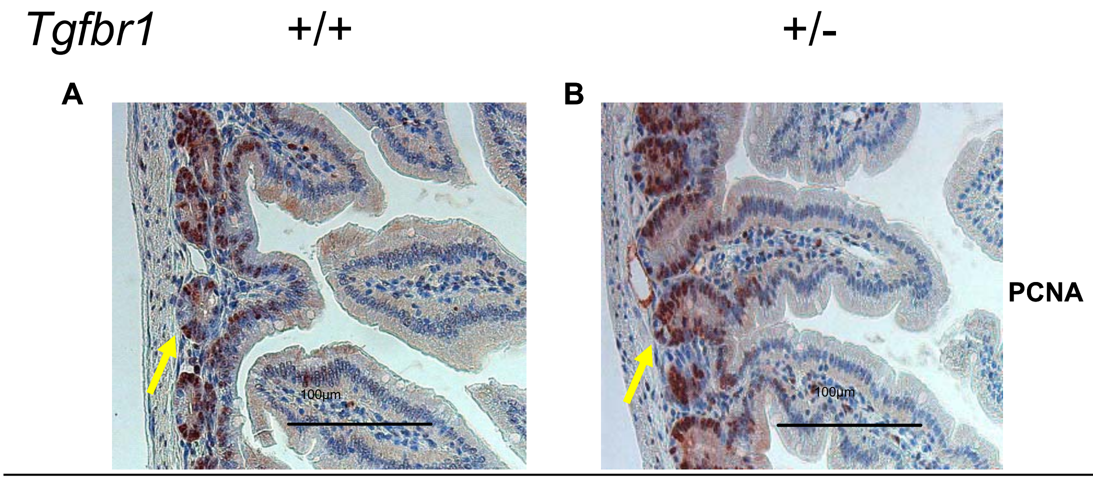
B) Neomycin-specific PCR performed on cDNA from *Tgfbr1*$^{+/+}$ and *Tgfbr1*$^{+/-}$ mice from both C57BL/6 and FVB backgrounds shows that the inserted neomycin sequence is not transcribed, providing evidence that the disrupted *Tgfbr1* allele is not expressed. GAPDH-specific PCR confirms cDNA quality is sufficient. (Neg, reaction with no template DNA; Neo, pIRES/neomycin positive control)



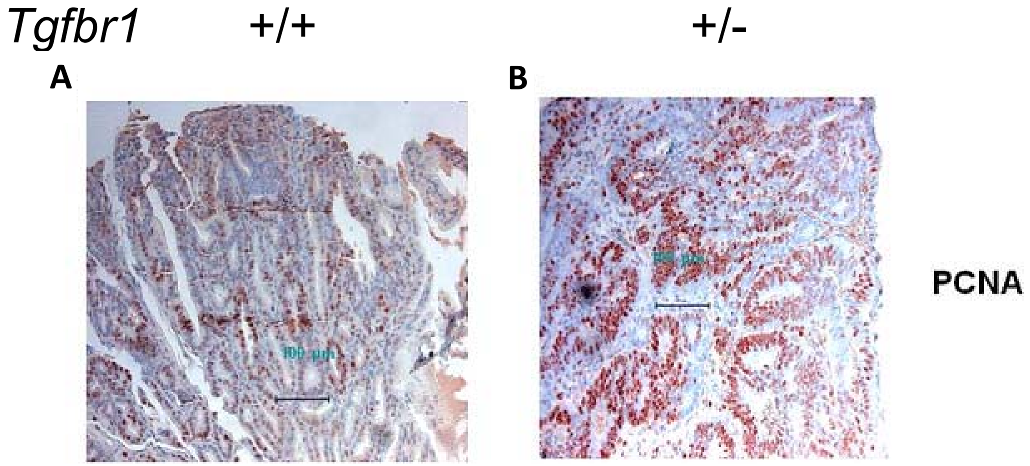
Supplementary Figure 3: Tumorigenesis of *Apc*$^{Min/+}$;*Tgfbr1*$^{+/+}$ and *Apc*$^{Min/+}$;*Tgfbr1*$^{+/-}$ mice: Enlarged view from part of the colonic polypoid tumor shown in Fig. 2 D. Left arrow: central luminal necrosis. Right arrow: cribriform architecture with highly pleomorphic nuclei. Scale bar, 100 µM.



Supplementary Figure 4: Immunohistochemistry staining patterns of normal appearing small bowel tissues from *Apc*^{Min}/$^{+}$;*Tgfbr1*$^{+/+}$ and *Apc*$^{Min/+}$; *Tgfbr1*$^{+/-}$ mice: Levels of proliferating cell nuclear antigen (PCNA) expression were significantly higher in *Apc*$^{Min/+}$;*Tgfbr1*$^{+/-}$ intestinal epithelial crypts (F, 62.2 ± 2.2% of positive staining) (arrow) than in their wild type counterpart (E, 44.4 ± 2.8% positive staining) (arrow) (p = 0.008).



Supplementary Figure 5: Characterization of *Tgfbr1* haploinsufficiency on molecular signaling within tumors: Levels of proliferating cell nuclear antigen (PCNA) expression are significantly higher in $Apc^{Min/+}$; $Tgfbr1^{+/-}$ tumors (A, 82.0 ± 2.9% of positive staining) than in their wild type counterpart (B, 48.2 ± 3.8% positive staining) (p = 0.0003) indicating increased cellular proliferation in vivo.



Supplementary Table 1: Backcrossing of *Tgfbr1*$^{+/-}$ mice into C57BL6 background

F3 *Tgfbr1*$^{+/-}$ mice described in the manuscript were backcrossed into the C57BL6/J background using speed congenics markers. Briefly, a minimum of 8 *Tgfbr1*$^{+/-}$ animals from each generation of backcrossing were genotyped for 152 markers by the Jackson Laboratory (Bar Harbor, ME). Mice with the highest percentage of the host genome were backcrossed to the host for next generation. Two fully congenic F6 males (99.9% C57BL6/J) were confirmed using a full genome wide panel of 150 SNP markers (Jackson laboratory, Bar Harbor, ME). These two males were crossed with C57BL6/J females. Their female progeny was mated with *Apc*$^{Min/+}$ male mice (C57BL/6J) to generate pure C57BL6/J animals.

SNP_ID	**CHR**	**Current_Position**	**MB_Position**	**RS_ID**	**Expected SNPs**	
JAX Stock #					B6	129
01-005230167-M	1	5,215,199	5.22	rs3708040	C	T
01-026072256-M	1	25,731,275	25.73	rs3717497	G	T
01-046003967-M	1	45,504,098	45.50	rs3716368	A	C
01-065042402-M	1	64,459,042	64.46	rs3024048	G	A
01-086182722-M	1	87,687,980	87.69	rs4222507	A	G
01-102073421-M	1	103,425,685	103.43	rs3684654	G	C
01-122978406-M	1	124,137,123	124.14	rs3704842	G	T
01-143006008-M	1	143,991,782	143.99	rs3710036	A	G
01-162977516-M	1	163,725,177	163.73	rs3714825	G	A
01-178701443-M	1	179,305,169	179.31		A	G
01-193173300-M	1	193,617,817	193.62	rs4137557	G	A
02-003179310-M	2	3,151,175	3.15	rs13476360	G	A
02-020551513-M	2	20,293,400	20.29	rs13459063	A	G
02-041042651-M	2	40,450,275	40.45	rs3666514	C	G
02-061161692-M	2	60,302,386	60.30	rs4223249	T	C
02-078062303-M	2	77,028,245	77.03	rs3670874	T	G
02-101074874-M	2	99,962,752	99.96	rs3686811	A	G
02-119931810-M	2	118,811,005	118.81	rs13476789	A	T
02-139603599-M	2	138,397,679	138.40	rs13476861	T	A
02-161858323-M	2	160,792,944	160.79	rs4223706	C	A
02-179086475-M	2	180,489,599	180.49	rs3698991	G	T

03-011350737-M	3	11,297,853	11.30	rs3659585	G	C
03-032431792-M	3	32,477,107	32.48	rs6246988	G	A
03-053161686-M	3	52,508,729	52.51	rs3676567	T	C
03-073214161-M	3	72,409,948	72.41	rs3688731	A	T
03-088155864-N	3	87,174,449	87.17	rs3708141	T	C
03-110268748-M	3	109,572,245	109.57	rs3659645	C	T
03-131286062-N	3	130,432,706	130.43	rs3677005	C	T
03-151125653-M	3	150,231,659	150.23	rs13477603	A	T
03-160287200-M	3	159,329,523	159.33	rs3706812	G	A
04-003163167-M	4	3,649,668	3.65	rs3703981	G	T
04-022875524-M	4	23,212,481	23.21	rs3654495	C	T
04-043070534-M	4	44,245,673	44.25	rs3726519	A	G
04-065107790-M	4	67,443,152	67.44	rs3672377	G	C
04-084241978-M	4	86,563,736	86.56	rs13477946	T	C
04-106314110-M	4	108,237,002	108.24	rs3724911	C	A
04-120122875-M	4	122,489,120	122.49	rs13478101	G	A
04-141084977-M	4	142,898,789	142.90	rs3693853	A	G
04-151168886-M	4	155,091,989	155.09	rs13478208	G	T
05-003188964-M	5	10,671,078	10.67	rs3671776	C	T
05-023890459-M	5	24,922,231	24.92	rs3666143	C	G
05-044010971-M	5	45,444,992	45.44	rs3657487	T	G
05-065734378-N	5	67,053,321	67.05	rs3719351	A	G
05-084120220-M	5	84,999,770	85.00	rs3141573	G	T
05-106058057-M	5	108,807,704	108.81	rs4225658	T	C
05-125054661-M	5	127,277,828	127.28	rs13478656	T	C
05-139966164-M	5	141,533,535	141.53	rs3699842	T	C
05-149044358-M	5	150,598,041	150.60	rs4225867	C	A
06-003167392-M	6	3,416,870	3.42	rs3023069	C	T
06-020075608-M	6	20,086,996	20.09	rs3671401	T	G
06-045126062-M	6	44,823,195	44.82	rs3691330	A	G
06-062599520-M	6	62,010,344	62.01	rs3684245	A	G
06-083216741-M	6	82,284,691	82.28	rs8268650	G	T
06-103313512-M	6	102,435,179	102.44	rs3714392	C	T
06-122941044-M	6	121,990,121	121.99	rs13479217	T	A
06-135955068-M	6	135,557,845	135.56	rs13479279	C	A
06-149052281-M	6	148,260,469	148.26	rs3679035	C	G
07-004201219-N	7	4,796,809	4.80	rs3686423	A	G
07-022997618-M	7	31,508,107	31.51	rs3710857	C	T
07-044964980-M	7	62,576,551	62.58	rs3684285	G	C
07-065183517-M	7	82,850,465	82.85	rs3700241	G	T
07-085095955-M	7	102,503,409	102.50	rs3023177	C	T
07-106153798-M	7	123,671,703	123.67	rs3653751	G	T
07-135345950-N	7	152,082,670	152.08	rs3696502	A	G
08-003089774-M	8	3,189,168	3.19	rs3699199	T	C
08-025034424-M	8	27,414,184	27.41	rs13479965	C	T
08-045080531-M	8	47,006,288	47.01	rs4227429	T	C

08-067470102-N	8	70,607,462	70.61	rs13480101	G	C
08-086985036-M	8	89,791,885	89.79	rs3705734	C	T
08-107088585-M	8	109,771,826	109.77	rs3685575	A	G
08-123340602-N	8	125,792,617	125.79	rs3705676	C	T
09-003938578-M	9	4,020,321	4.02	rs3698994	T	C
09-016198772-M	9	16,021,041	16.02	rs3696932	G	T
09-037129176-M	9	36,759,904	36.76	rs4227968	T	C
09-057114504-M	9	56,434,767	56.43	rs4228121	C	T
09-077792491-M	9	76,938,604	76.94	rs13480582	T	A
09-098865563-M	9	98,249,448	98.25	rs13480625	T	C
09-115037423-M	9	113,824,128	113.82	rs3717445	G	A
09-125082704-M	9	123,551,657	123.55	rs3713441	G	A
10-003233934-M	10	6,998,239	7.00	rs4228405	G	C
10-023127952-M	10	22,906,845	22.91	rs3151132	A	G
10-043132794-M	10	42,763,592	42.76	rs3685856	T	G
10-053898997-M	10	53,462,955	53.46	rs4228622	G	C
10-092128703-M	10	91,386,428	91.39	rs3673679	G	A
10-115062433-M	10	114,294,228	114.29	rs3663879	T	C
10-129117100-M	10	127,890,188	127.89	rs3090050	G	A
11-004367508-M	11	4,408,733	4.41	rs13481176	G	A
11-024515722-M	11	24,386,229	24.39	rs3726373	G	A
11-047224208-M	11	46,666,864	46.67	rs3719031	C	A
11-070331570-M	11	69,560,965	69.56	rs3665793	T	C
11-090199967-M	11	89,314,273	89.31	rs3707048	T	C
11-111226923-M	11	110,246,168	110.25	rs6288403	A	C
11-122195777-M	11	121,116,553	121.12	rs3692977	C	T
12-003567042-M	12	3,701,820	3.70	rs13481630	G	T
12-022232159-M	12	28,835,162	28.84	rs13481675	C	G
12-041270227-M	12	48,020,093	48.02	rs13481736	A	G
12-061271925-M	12	67,818,222	67.82	rs3090002	A	T
12-084289638-M	12	91,408,395	91.41	rs3677220	C	A
12-107536049-N	12	114,496,728	114.50	rs3695655	A	G
12-113315003-M	12	120,327,443	120.33	rs13481991	C	G
13-003966099-M	13	4,160,601	4.16	rs13482000	A	G
13-023105764-M	13	23,788,316	23.79	rs3689508	A	G
13-043995023-N	13	44,496,918	44.50	rs13482109	T	G
13-065339791-N	13	67,941,214	67.94	rs3659053	C	T
13-084819885-M	13	87,813,907	87.81	rs4230429	G	A
13-105849719-M	13	109,214,086	109.21	rs3666178	A	G
13-117094028-M	13	120,254,901	120.25	rs3712154	G	A
14-003066925-M	14	8,613,747	8.61	rs13482383	T	C
14-020426806-M	14	26,162,806	26.16	rs3681808	A	G
14-040017031-M	14	48,792,639	48.79	rs3091175	G	C
14-060626617-N	14	70,097,369	70.10	rs4230721	T	C
14-077156430-M	14	86,443,660	86.44	rs3088560	C	T
14-097042967-M	14	106,254,320	106.25	rs3707104	A	G

14-114403652-M	14	123,545,419	123.55	rs4161857	C	T
15-003094890-M	15	3,219,262	3.22	rs4163058	A	G
15-020280219-M	15	20,138,467	20.14	rs4171393	G	T
15-041051689-M	15	40,708,090	40.71	rs4190619	G	C
15-057561875-M	15	56,992,041	56.99	rs4205765	A	G
15-083914758-M	15	82,956,832	82.96	rs13482876	A	G
15-102788257-N	15	101,641,254	101.64	rs4135869	G	T
16-005053446-C	16	5,596,485	5.60	rs3690712	G	T
16-025179119-C	16	25,407,388	25.41	rs3710986	G	C
16-045078790-C	16	45,037,164	45.04	rs3688141	G	A
16-066862514-C	16	66,406,352	66.41	rs3023460	T	G
16-090091107-C	16	89,451,584	89.45	rs3714233	C	A
16-096070057-C	16	95,329,654	95.33	rs4231786	C	T
17-003335010-M	17	5,885,430	5.89	rs3683436	G	C
17-023157746-M	17	24,105,207	24.11	rs3719627	A	T
17-045146746-M	17	46,262,961	46.26	rs3656292	A	T
17-065545592-M	17	66,770,664	66.77	rs4232042	T	C
17-084615613-M	17	85,718,538	85.72	rs3704503	G	A
17-092673068-N	17	93,598,958	93.60	rs3679049	T	G
18-005066417-M	18	5,207,638	5.21	rs3160376	T	C
18-022777507-M	18	22,828,204	22.83	rs13483720	G	T
18-043081750-M	18	43,083,464	43.08	rs13483769	C	T
18-065947946-M	18	65,769,364	65.77	rs3675552	T	G
18-078813035-M	18	78,443,851	78.44	rs13483915	A	C
18-090060367-M	18	89,629,192	89.63	rs3653678	G	T
19-018239318-M	19	18,669,375	18.67	rs3697198	T	G
19-038092479-M	19	38,517,304	38.52	rs3716904	T	C
19-049914266-M	19	50,050,644	50.05		G	T
19-060823449-N	19	60,842,385	60.84		A	G
X-016114535-G	X	17,889,948	17.89	rs13483721	C	A
X-025132696-G	X	36,252,886	36.25	rs13483729	C	G
X-052681018-M	X	66,761,135	66.76	rs3707564	A	C
X-060478145-M	X	74,709,915	74.71	rs3663719	A	T
X-068494838-M	X	82,748,657	82.75	rs3664154	C	T
X-087817653-G	X	100,102,202	100.10	rs13483918	A	T
X-100413449-M	X	114,022,081	114.02	rs3690903	T	G
X-115587169-N	X	132,749,158	132.75	rs4151956	T	C
X-133535206-G	X	150,081,739	150.08	rs13484074	T	C
X-143466659-M	X	162,753,251	162.75	rs3698078	A	C

Supplementary Table 2: Analysis of *Tgfbr1* LOH in tumors

Normalized LOH ratio values calculated after analysis by SNaPshot of six colonic tumors from three *Apc*$^{Min+}$;*Tgfbr1*$^{+/-}$ mice. The values shown in the Table below represent the averages of the ratios obtained with two different SNPs located within the *Tgfbr1* gene: rs32739456 and rs32428765, and their corresponding standard deviations (SD).

Mean LOH ratio	S.D.
0.89	0.03
0.88	0.13
1.07	0.03
1.09	0.05
0.97	0.08
1.01	0.05