PWD/Ph-encoded genetic variants modulate the cellular Wnt/β-Catenin response to suppress *Apc*^{Min}-triggered intestinal tumor formation

Supplementary Figures

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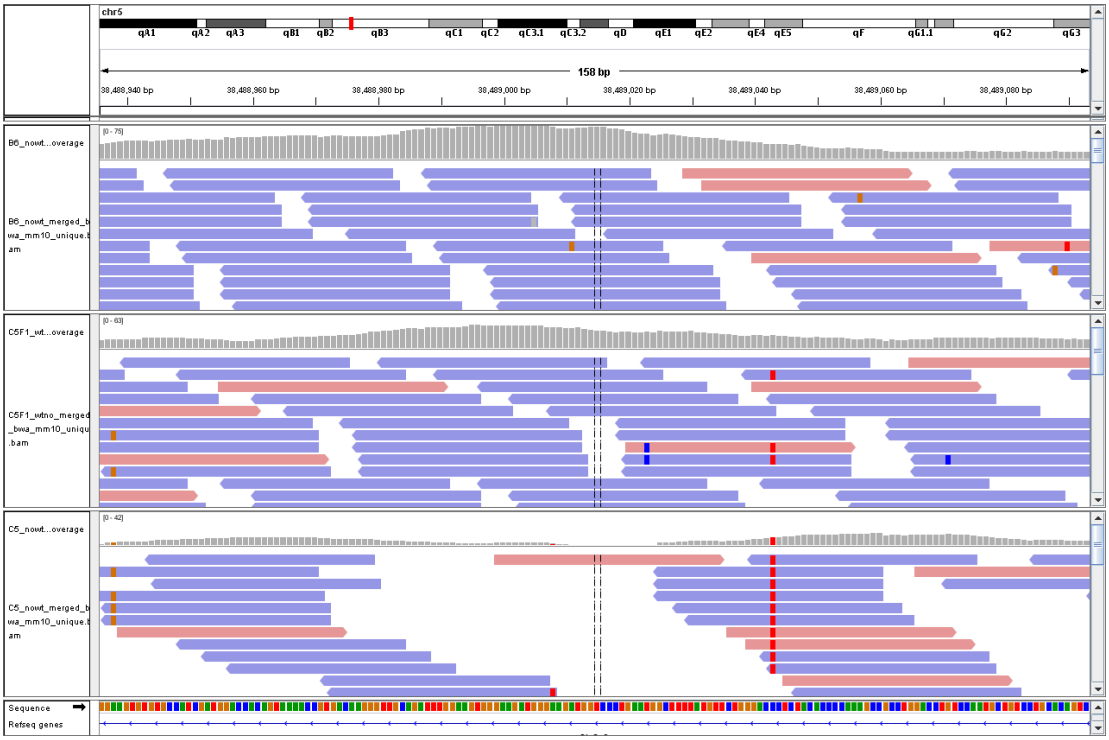
A

	Contigs	Scaffolds	Superscaffods	Chr5 Superscaffolds
Total length	2,351,352,542 bp	2,535,550,058 bp	2,570,436,817 bp	147,715,539
Max length	103,731 bp	11,514,174 bp	131,840,581 bp	78,939,632
N50 length	10,741 bp	2,459,518 bp	62,404,610 bp	78,939,632
N50 count	66,739	296	15	1

B



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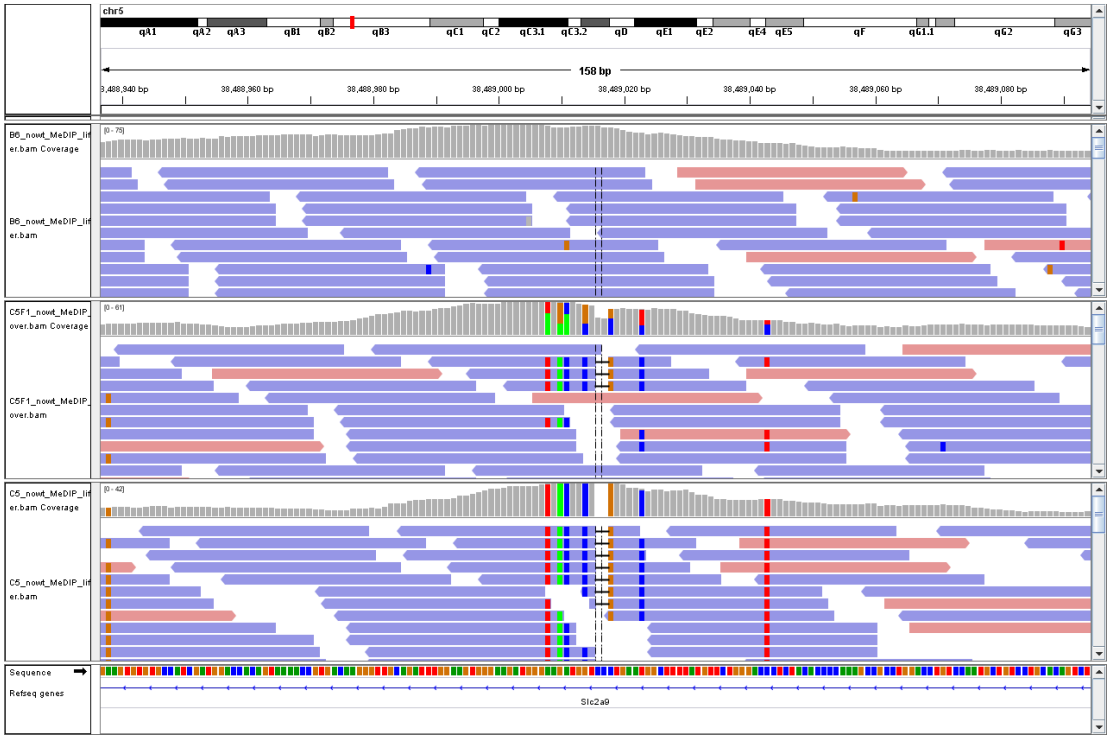
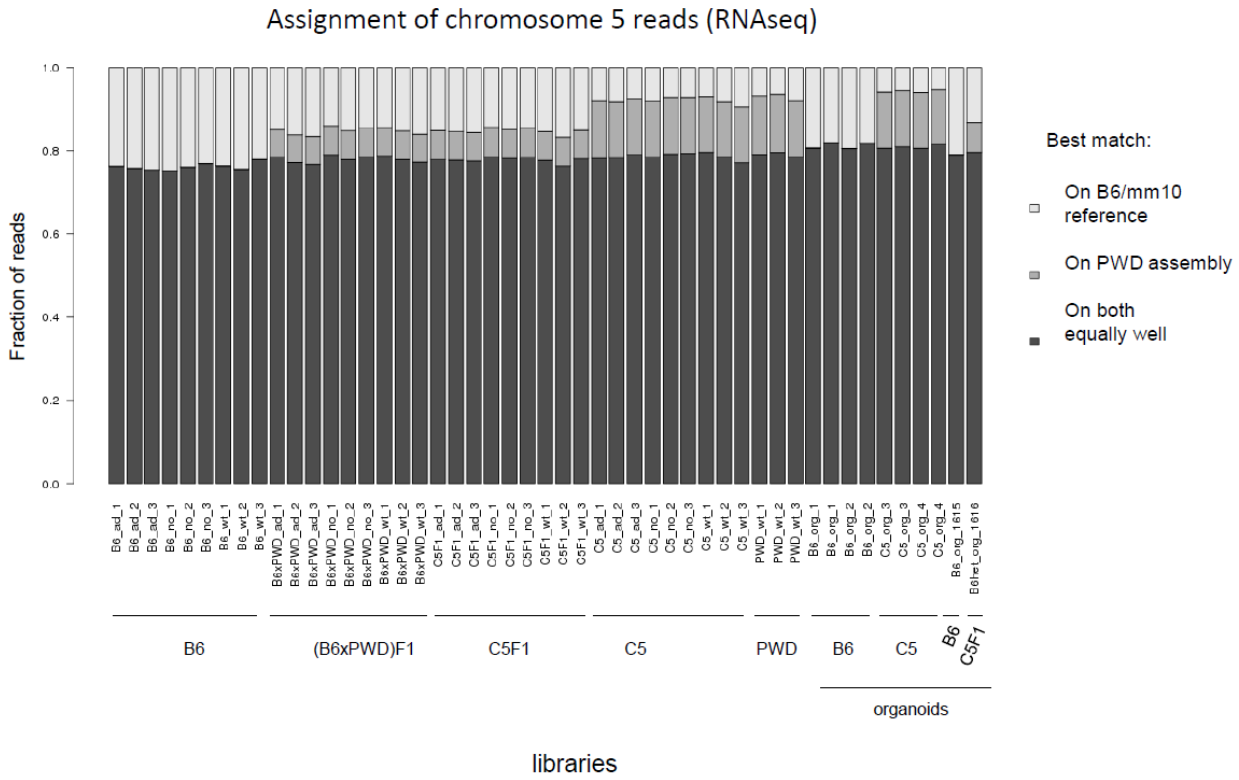
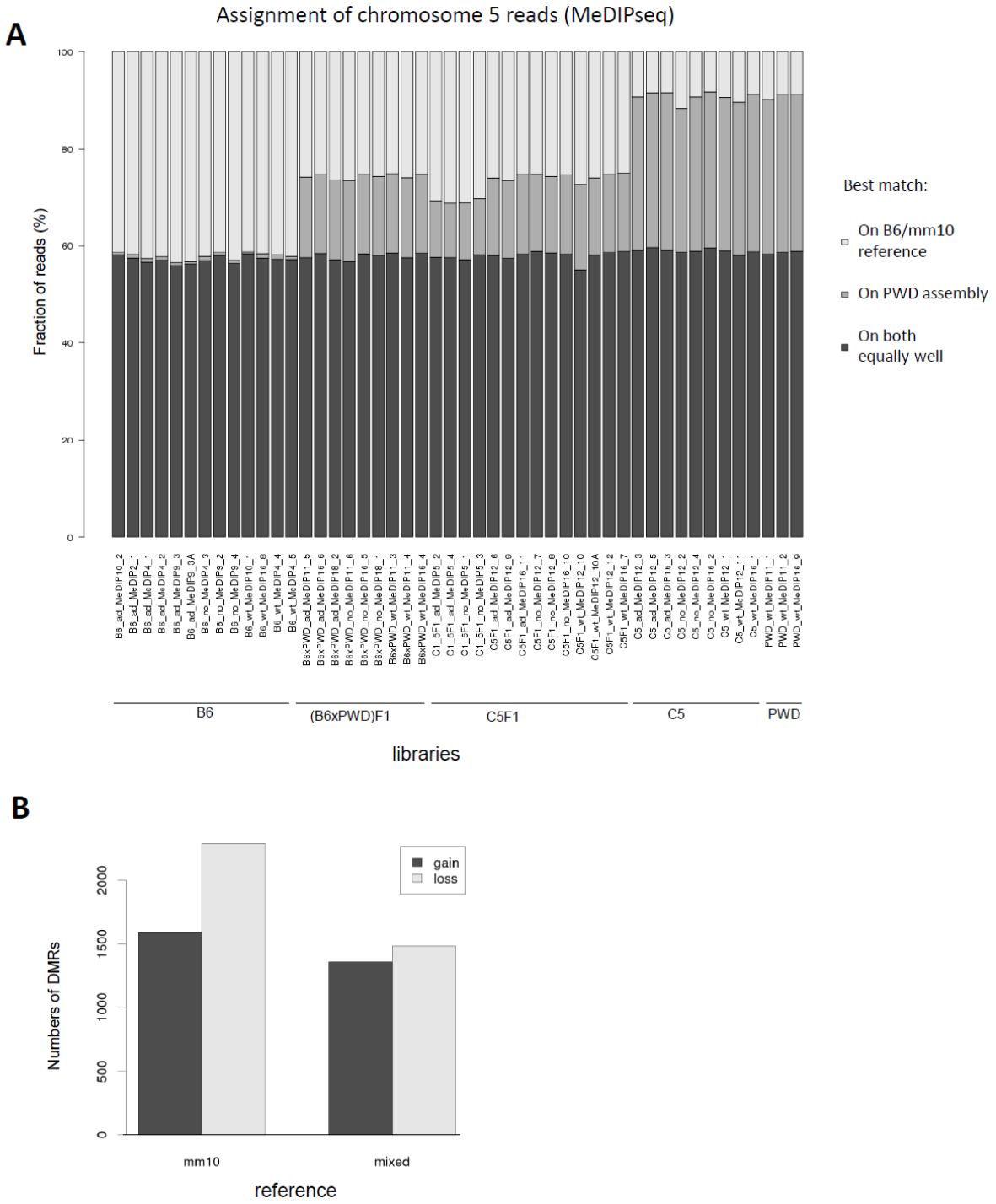


Supplementary Figure 1 (previous page): Assembly of the PWD genome and alignment to the C57BL/6 genome. A Assembly statistics for the PWD genome. See Material and Methods for details. **B-C** Mapping of short reads to polymorphic sites in B6 and PWD correspondence maps. Genetic differences between the analyzed mouse strains C57Bl/6J (B6)and PWD/Ph (PWD) may lead to bias in the analysis of sequencing experiments, such as mRNA sequencing (RNA-seq) and sequencing of immunoprecipitated methylated DNA (MeDIP-seq), where local depth of coverage is the quantitative readout of the experiment. To overcome this issue, we assembled a PWD specific reference sequence to support analysis of the samples with PWD genetic background. **B** Exemplary alignment to B6 reference. **C** Alignment to a mixed reference. In this case, PWD reads were transferred to the exact corresponding position of mm10, along with the B6 reads. Colored positions depict alternative bases. The mapping strategy enabled us to assign reads from polymorphic sites to the originating strain, while reads equally aligning to both references are considered of ambiguous origin. Homozygous samples served as quality check. For samples derived from intercross animals, the strategy directly enables allele-specific analysis.

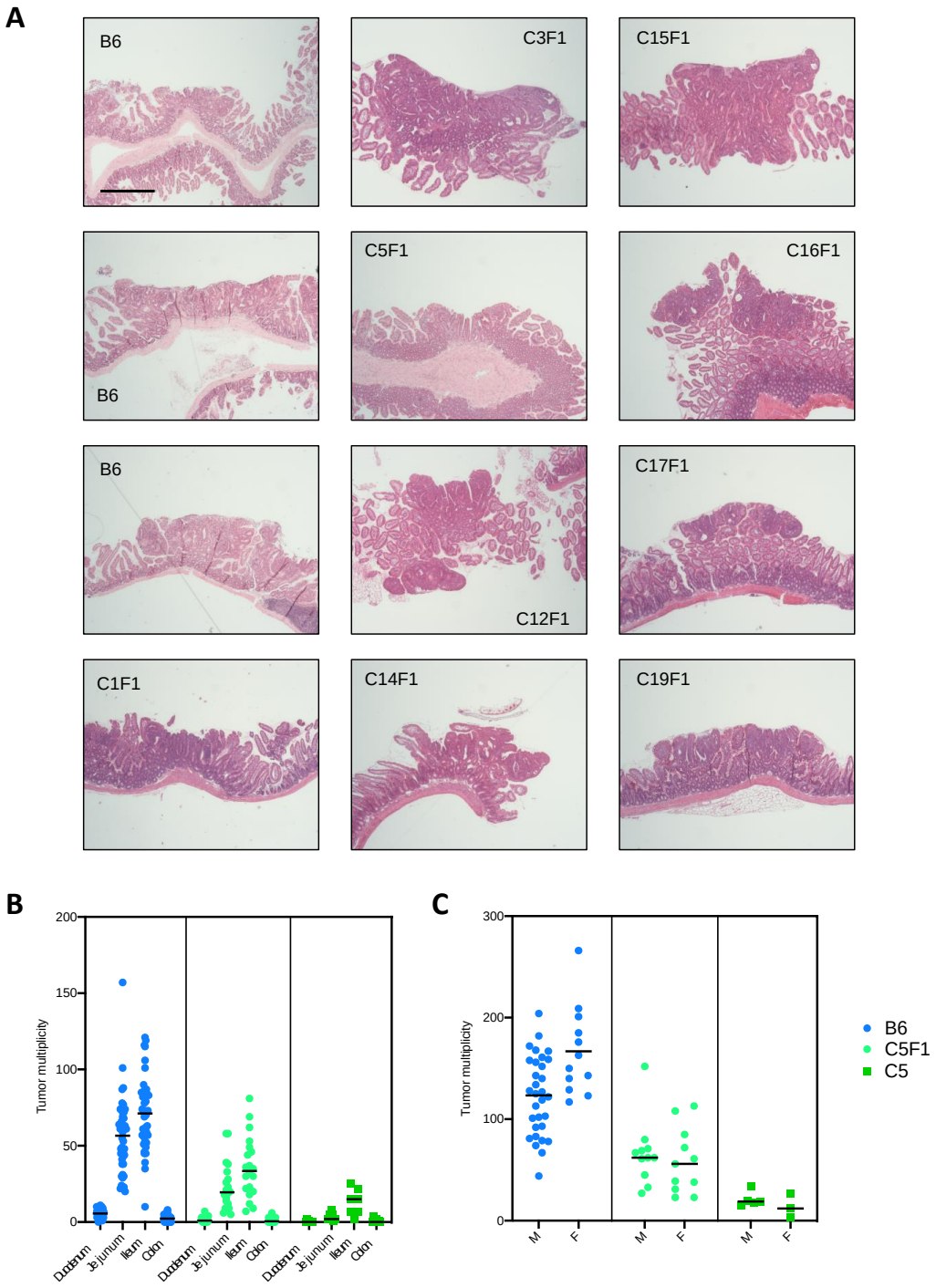


Supplementary Figure 2: RNA-seq read assignment. Graph shows assignment of reads on chromosome 5 for RNAseq for all libraries used in the study. Genotypes of sample is given below the column, as first letters of library name. Primary tissue libraries are to the left, libraries derived from organoids are to the right. Approx. 78% of RNA-seq reads mapped to chr5 were strain-ambiguous (i.e. aligning to B6 and PWD references). Yet, of 793 genes on chr5 with more than 10 reads in total for all samples, 574 genes have more than 10 strain-specific reads. Reads derived from B6 animals showed less than 0.3% misassignment to PWD, and strain-specific reads derived from heterozygous C5F1 mice were assigned approximately a third to PWD and two thirds to B6. 64% of allele-specific reads from C5 homozygous mice were correctly assigned to PWD reference, and approx. 36% (8% of total reads) were incorrectly assigned to B6, probably due to PWD genome assembly gaps and errors.



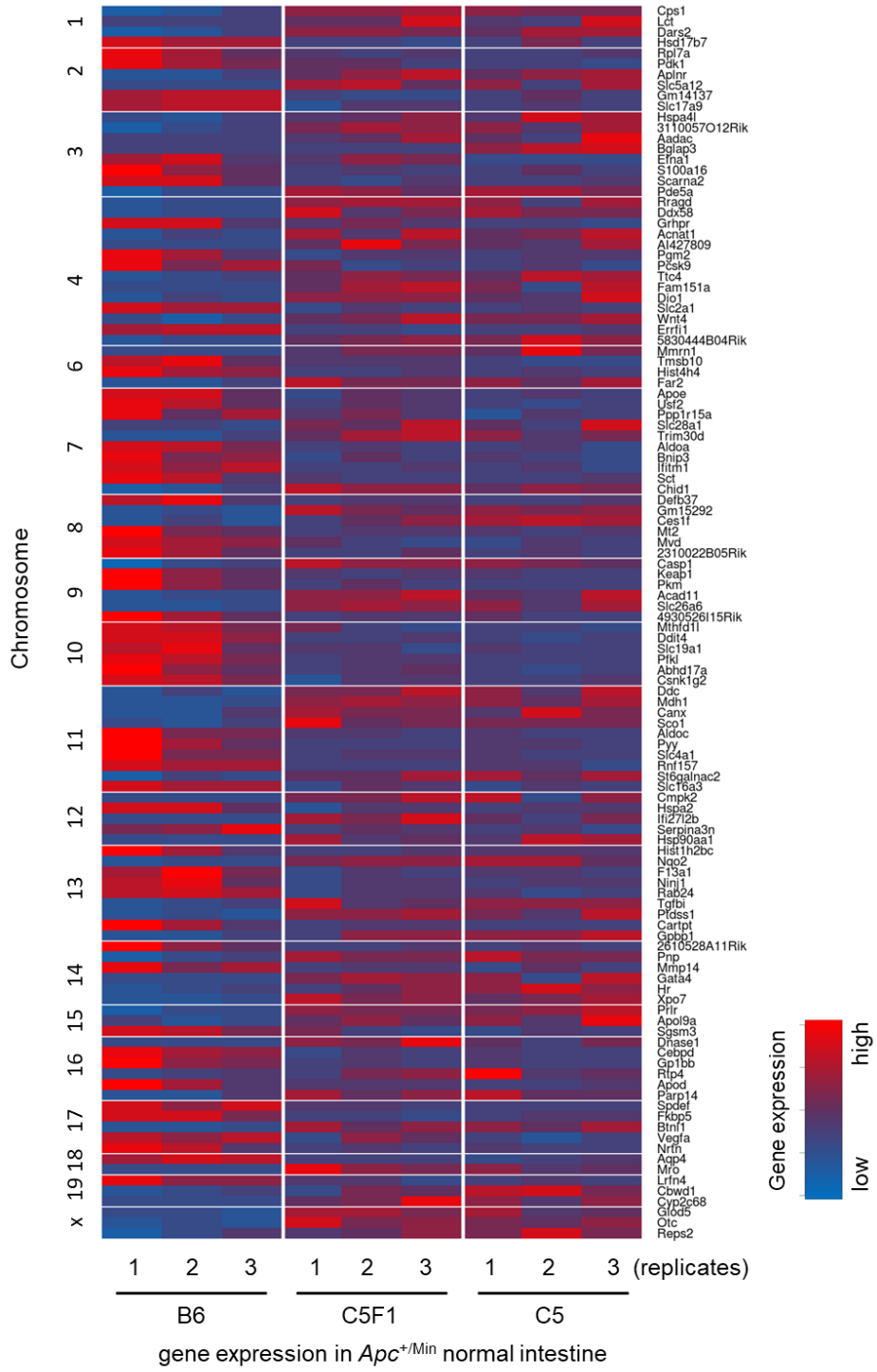
Supplementary Figure 3: MeDIP-seq quality controls. A Assignment of reads on chromosome 5 for MeDIP-seq. All libraries were generated from primary intestinal tissue. 41%-43% of MeDIP reads were identified as strain-specific to B6 or PWD. For B6 samples, more than 99% of reads were assigned to the B6/mm10 reference genome. For

heterozygous C5F1 samples, one-third of the polymorphic reads were assigned to PWD and two thirds to B6, respectively. For homozygous C5 samples, 78% of the reads were assigned to PWD and 22% were assigned to B6, likely due to inaccurate assembly and gaps in the PWD sequence **B** Reference bias in MeDIP-seq analyses. To quantify the impact of the mixed alignment strategy on the number of DMRs, we compared alignment to mm10 to mixed reference alignment. For the mm10 alignment, we found 43% more hypomethylated compared to hypermethylated DMRs on chr5 in PWD vs B6 samples. With the mixed strategy, we found a more balanced ratio of 52:48 for hypo- versus hypermethylated DMRs To the left: mm10 alignment, showing reference bias when comparing PWD to B6 samples. To the right: mixed reference strategy.

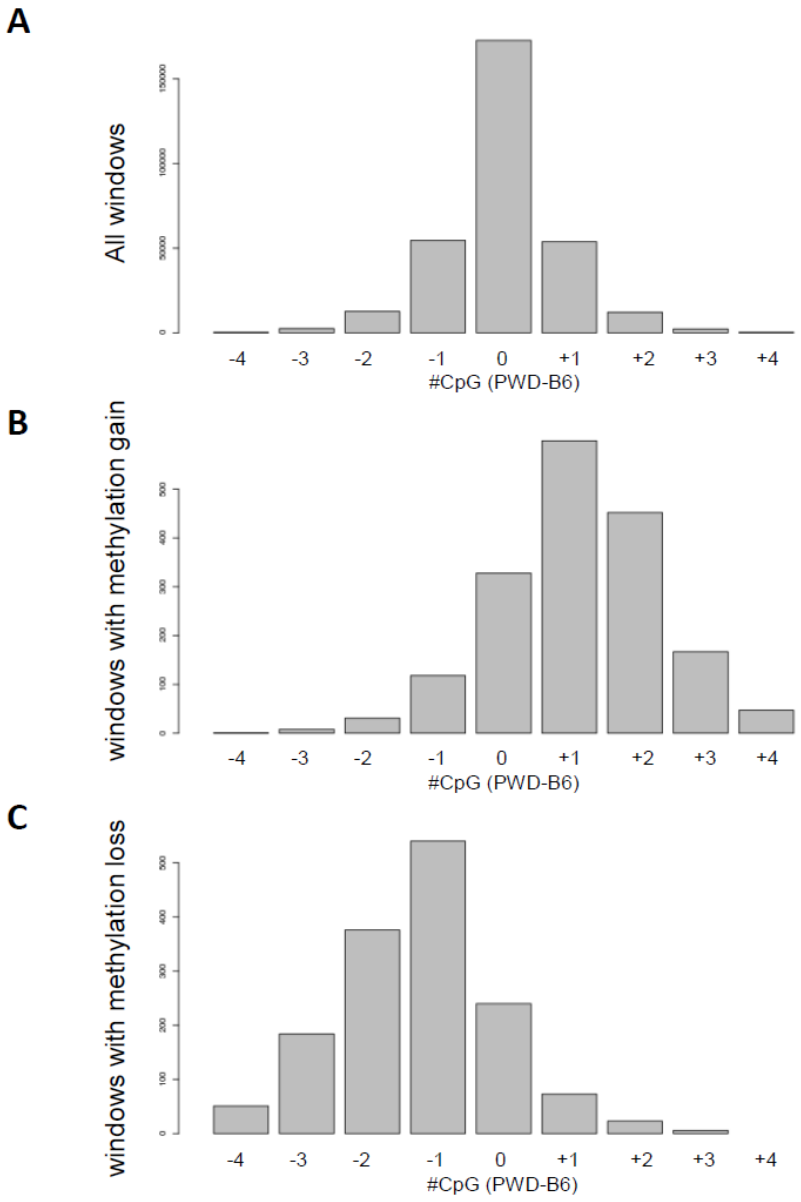


Supplementary Figure 4: Histology and distribution of tumors in B6 x PWD consomic mice

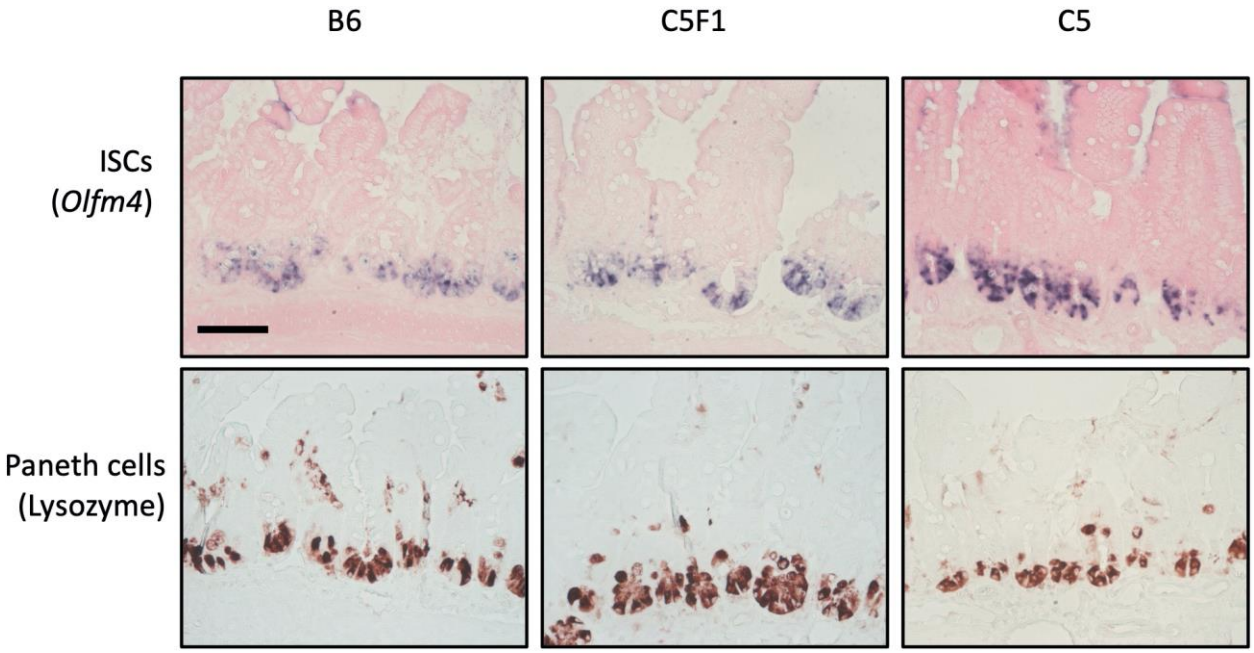
A Hematoxilin and eosin stainings of adenoma sections of B6 *Apc*$^{Min/+}$ mice, and B6 x PWD consomic crosses, as indicated. Scale bar: 1mm. **B** Tumor distribution across the cephalocaudal axis of the intestine, in C5F1 *Apc*$^{Min/+}$ mice. **C** Tumor multiplicity in male (M) and female (F) C5F1 *Apc*$^{Min/+}$ mice.



Supplementary Figure 5: Transcriptome analyses identify modifier candidate genes on other chromosomes than chromosome 5. Gene expression heat map of modifier candidate genes differentially expressed in the normal intestine of B6 versus C5 and not located on mouse chromosome 5 (see main Fig. 4B for genes located on chromosome 5).



Supplementary Figure 6: Strain-specific DNA methylation on chromosome 5 is correlated to CpG availability. Graphs display gains and losses of CpGs on chromosome 5 between PWD and B6 genomes per analysis window in MeDIP-seq. **A**. All analysis windows on chromosome 5 with nucleotide differences ≤ 10 between B6 and PWD genome in the window. **B** Genome windows on chromosome 5 with a gain of methylation in PWD compared to B6. **C** Genome windows on chromosome 5 with a loss of methylation in PWD compared to B6.



Supplementary Figure 7: Assessment of cell types in the intestinal crypt. Upper row: RNA *in situ* hybridization for the stem cell marker *Olfm4* on intestinal sections of *Apc*$^{Min/+}$ B6, C5F1 and C5 mice, as indicated. Lower row: Immunohistochemistry for the Paneth cell marker Lyz on intestinal sections of *Apc*$^{Min/+}$ B6, C5F1 and C5 mice, as indicated. Scale bar: 100µm.