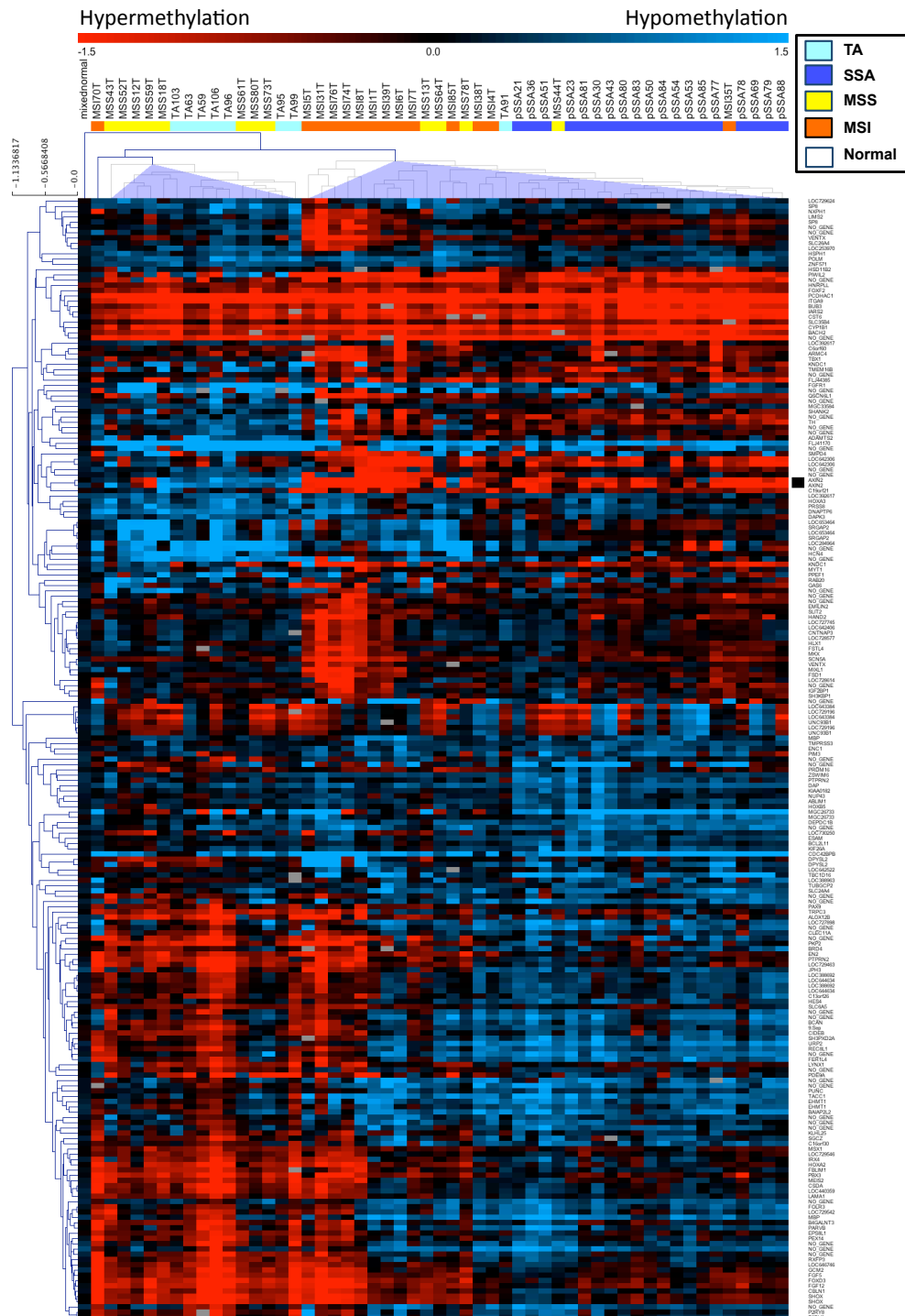
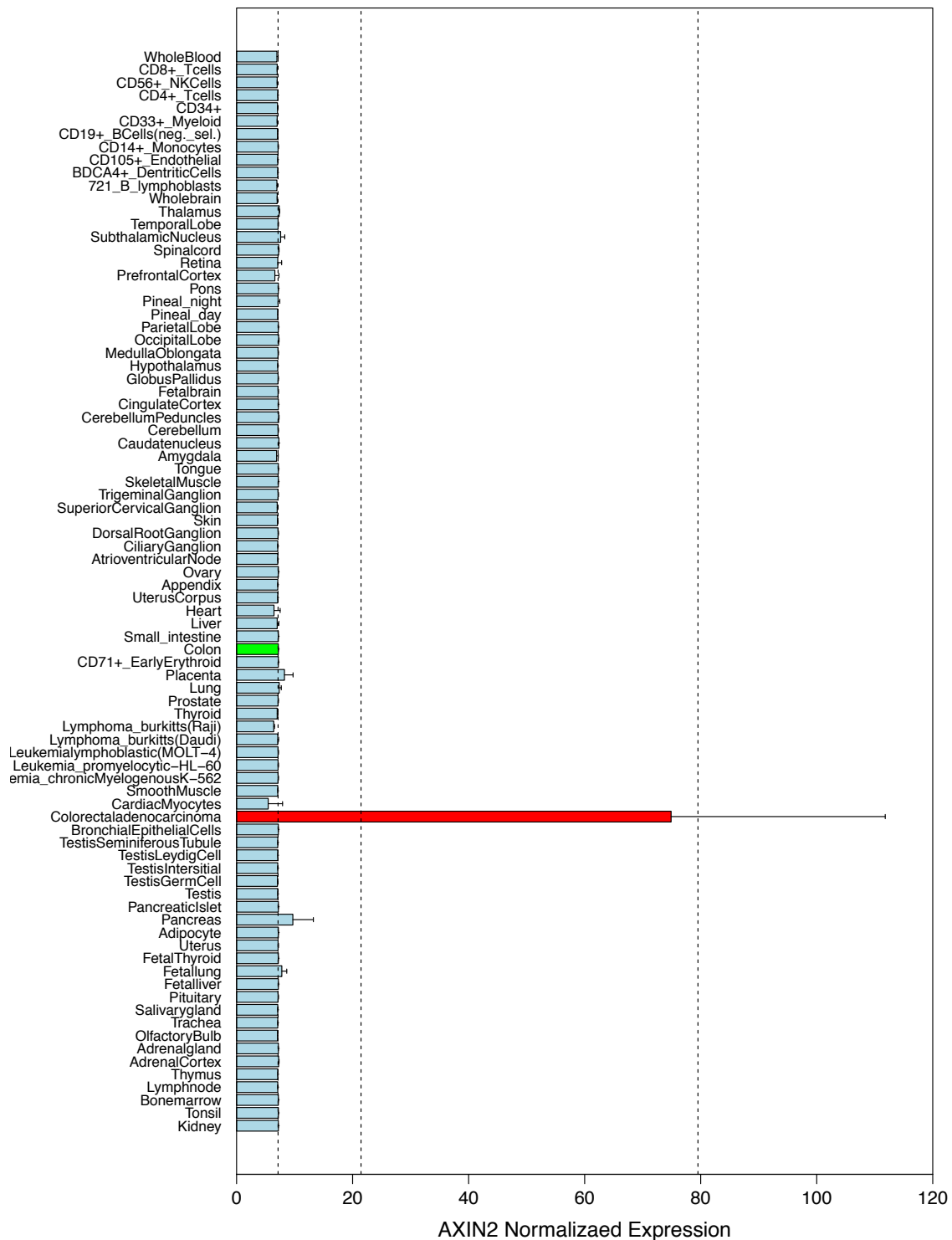


Supplementary Figure 1. Frequency of hypermethylation (left) and hypomethylation (right) of the different tumor groups, estimated by MS-AFLP arrays. TA, in light blue: tubular adenomas. SSA, in dark blue: sessile serrated adenomas. MSS, in yellow: microsatellite stable carcinomas. MSI, in orange: microsatellite unstable carcinomas. Frequencies were calculated as the percentage of probes with log₂ ratio value below -1.5 (for hypermethylation) or above 1.5 (for hypomethylation), after filtering the 30% lower-intensity probes from each array. Top graphs, results of probes in intergenic regions (range: 2,495-2,571 probes per array, depending on the sample). Middle graphs, probes inside gene body, defined as the region from 2.5Kb downstream the 5' end and the 3' end (range: 2,069-2,127 probes per array). Bottom graphs, probes within the 2.5Kb downstream the 3' of genes (range: 893-935 probes per array). P-values were calculated by one-way ANOVA followed by Tukey's HSD multi-hypothesis testing correction. Only p-values below 0.05 are shown.



Supplementary Figure 2. Clustering of the samples according to their methylation profile. Hypermethylation is indicated in red. Hypomethylation is indicated in blue. Samples are shown on top of the heatmap. In white, the normal tissue DNA mix used as reference. TA, in light blue: tubular adenomas. SSA, in dark blue: sessile serrated adenomas. MSS, in yellow: microsatellite stable carcinomas. MSI, in orange: microsatellite instable carcinomas. On the right side of the heatmap, the genes associated to MS-AFLP probes. *AXIN2* probes are indicated in black. Clustering was performed by complete linkage using Pearson's correlation on a subset of MS-AFLP probes previously selected by t-test.

AXIN2 Expression in Human Tissues



Supplementary Figure 3. Gene expression pattern of the *AXIN2* gene in human normal and cancerous tissues. *AXIN2* is dramatically overexpressed in colorectal adenocarcinomas (red bar) compared to normal colon tissue (green bar). Vertical dashed lines represent the 1x, 3x and 10x mean expression values. Diagram created for the gnf1h00059_at probe of the dataset GeneAtlas U133A, gcrrna, obtained from BioGPS (<http://biogps.org/dataset/1/>). [1]

Supplementary Table 1. *Loci* with differential methylation alterations in the tumor groups.

Gene	Description	Function	Chrom.
ATP2A3	TPase, Na ⁺ /K ⁺ transporting, alpha 3 polypeptide	ATPase, Na ⁺ /K ⁺ transporting	17
AXIN2	AXIN2	inhibitor of the Wnt signalling pathway	17
C13orf26	Chromosome 13 open reading frame 26	unknown	13
CBLN1	Cerebellin 1 precursor	a cerebellum-specific precursor protein	16
CSDA	DNA-binding protein A	Shoch domain protein	9
DMRT1	Doublesex and mab-3 related transcription factor 1	transcription factor	9
DOCK8	Dedicator of cytokinesis 8	interact with guanine exchange factor (GEF)	12
EN2	Engrailed homeobox 2	Homeobox	7
EPS8L1	EPS8-like 1, transcript variant 1,	related to EGFR pathway substrate 8	19
FBLIM1	Filamin binding LIM protein 1, transcript variant 1	cell adhesion structures to the actin cytoskeleton	1
FER1L4	Fer-1-like 4 (C. elegans), non-coding RNA	non-coding RNA	20
FGF5	Fibroblast growth factor 5, transcript variant 1	member of the fibroblast growth factor (FGF) family	4
FOXD3	Forkhead box D3	forkhead family of transcription factors	1
FSD1	Fibronectin type III and SPRY domain containing	associates with a subset of microtubules	19
FSTL1	Follistatin-related protein 1	autoantigen associated with rheumatoid arthritis	3
GRIP1	Glutamate receptor interacting protein 1	glutamate receptor interacting protein family	12
HOXA2	Homeobox A2	Homeobox	7
KIAA1303	KIAA1199 (KIAA1303),	Regulatory-associated protein of mTOR	17
OSR1	Odd-skipped related 1	oxidative-stress responsive	2
PCBP1	Poly(rC) binding protein 1	retrotransposition of a fully processed PCBP-2	2
PCSK2	Proprotein convertase subtilisin/kexin type 2	subtilisin-like proprotein convertase family	20
PDE9A	High affinity cGMP-specific phosphodiesterase 9A	catalyzes the hydrolysis of cAMP and cGMP	21
PKP2	Plakophilin-2	may regulate beta-catenin signaling	12
PLEKHA7	Pleckstrin homology domain containing, family A member 7	pleckstrin homology domain containing	11
POLM	DNA polymerase mu	Involved in NHEJ pathway of DNA repair	7
PRDM16	PR domain containing 16	a zinc finger transcription factor	1
PTPRZ1	Protein tyrosine phosphatase, receptor-type, Z polypeptide 1	the receptor protein tyrosine phosphatase family	7
REC8L1	Human homolog of rad21	structural maintenance of chromosome)	14
SEPT9	Septin-9 (methylated in colorectal cancer)	essential for tumor cell migration and invasion	17
SH3PXD2A	SH3 and PX domains 2A	SH3 and PX domains 2A	10
SHOX	The paired homeobox family	Homeobox	X, Y
SLIT2	Slit homolog 2	interact with Glypican 1	4
TACC1	Transforming acidic coiled-coil-containing protein 1	breast cancer candidate gene	8
URP2	Fermitin family homolog 3 (FERMT3)	regulation of hemostasis and thrombosis	11
VENTX	Vent family of homeodomain proteins	Homeobox	10

The table lists in alphabetical order the *loci* found differentially methylated among the four different groups of tumors after ANOVA analysis. Of note the relative high frequency of cell growth signaling and transcription factors, including homeobox related loci, in consonance with our original prediction of high frequency of methylation alterations in homeobox genes [2].

Supplementary References

1. Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW, 3rd, Su AI: **BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources.** *Genome Biol* 2009, **10**:R130.
2. Perucho M, Tokino T, Nakamura Y: **Cancer genomics and molecular diagnosis--The Nineteenth International Symposium of Sapporo Cancer Seminar.** *Jpn J Cancer Res* 1999, **90**:1273-1276.