Figure S1. Human proteome arrays of cells in which SPTAN1 and MLH1 were knocked down. Human proteome arrays were used to analyse the expression levels of 119 different chemokines and soluble receptors in the MLH1- or SPTAN1-knockdown SW620 and MLH1- or SPTAN1-knockdown SW480 cell lines. Protein expression levels of (A) shSPTAN1-transduced SW480 cells, (B) shMLH1-transduced SW480 cells, (C) shSPTAN1-transduced SW620 cells, and (D) shMLH1-transduced SW620 cells were compared to the pLKO.1 transduced control. IL-8 expression levels are indicated by black arrows. (E) The differential expression levels of IL-8 of shSPTAN1- or shMLH1-transduced SW480 or SW620 are shown in comparison to the pLKO.1 transduced control, respectively. The arrays are based on antibodies which have been spotted in duplicate on nitrocellulose membranes. These duplicates were quantified using Multi Gauge version 3.2 and resulting mean values are shown. sh, short hairpin; SPTAN1, non-erythroid spectrin αII; MLH1, MutL homologue 1.

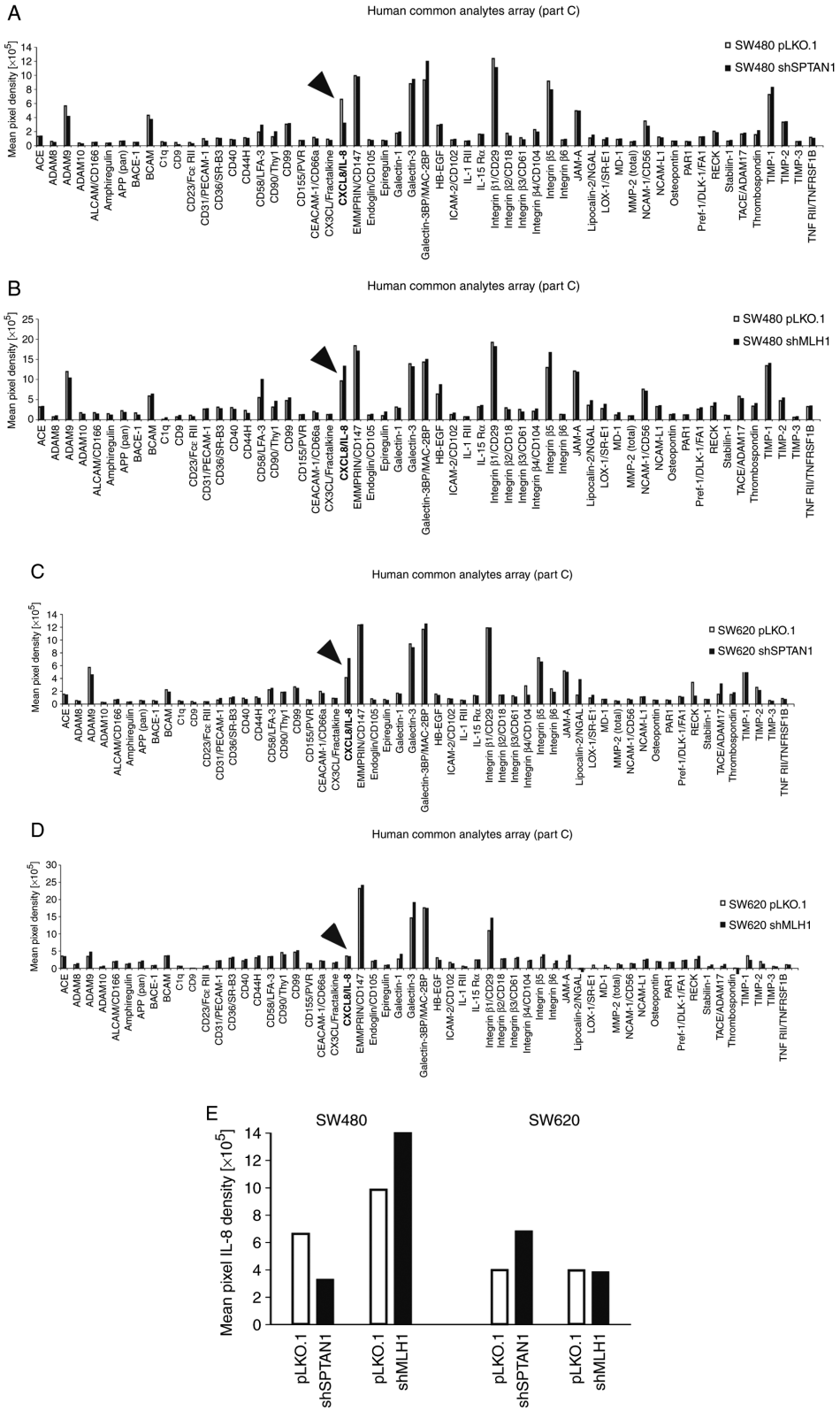

Figure S2. Analysis of MSI in single cell clones of SW480 or SW620 cells. SW480 and SW620 cells were stably transfected with shSPTAN1 and shMLH1 using lentiviral transduction. Following the verification of successful knockdown of SPTAN1 and MLH1, single cell clones were cultivated from these cells. DNA from 7-15 different cell clones was isolated and tested for MSI using a pentaplex PCR. PCR results were compared to pLKO.1 transduced control cells. No MSI was detectable in the tested single cell clones. (A-C) Typical allele profiles of NR-21, BAT-25, BAT-26, NR-24 and NR-22 fragment analysis with no MSI shown. MSI could not be detected in all tested single cell clones. Representative data are shown for (A) SW480 pLKO.1, (B) SW480 shSPTAN1 and (C) SW480 shMLH1. MSI, microsatellite instability; sh, short hairpin.

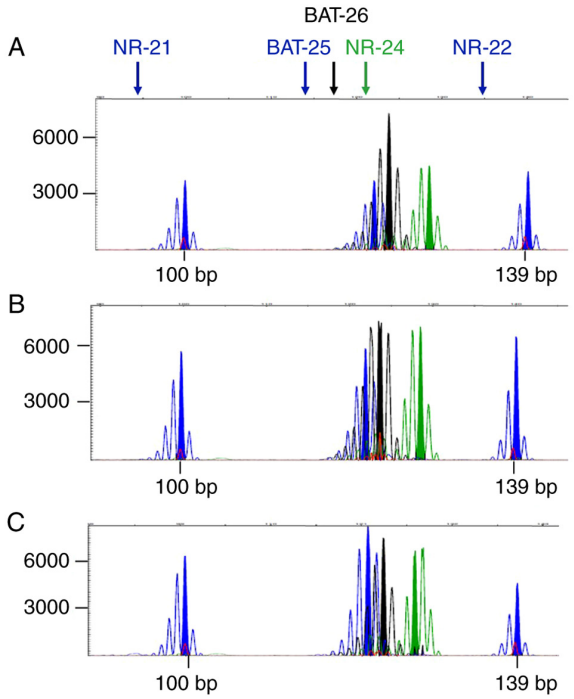

Figure S3. Full-length western blots of stably transfected shMLH1 and shSPTAN1 SW480, SW620, HT29, 293 and HCT116 mlh1-2. SW480, SW620, HT29, 293 and HCT116 mlh1-2 were stably transduced with shSPTAN1 and shMLH1 using lentiviral transduction and the successful knockdown of SPTAN1 and MLH1 was verified using western blotting. (A) Corresponding full-length western blot from Fig. 1A, (B and C) corresponding full length western blots from Fig. 1C, which were cropped from different gels. (D) Corresponding full length western blot from Fig. 4A. sh, short hairpin; SPTAN1, non-erythroid spectrin αII; MLH1, mutL homologue 1.

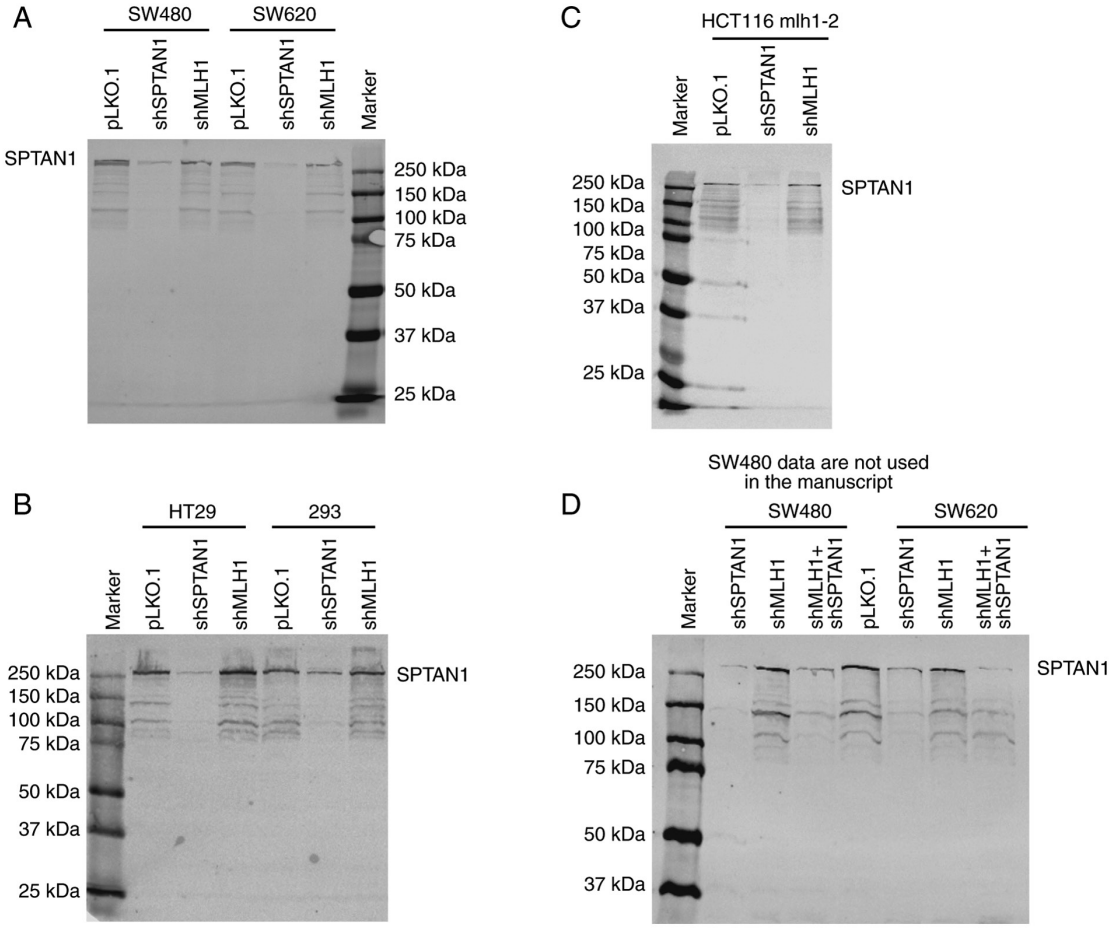

Table SI. Length of analysed microsatellites.

	Size (bp)								
	MSI			SW480			SW620		
Locus	Size (bp)	Colour	Delta (bp)	pLKO.1	shSPTAN1	shMLH1	pLKO.1	shSPTAN1	shMLH1
NR21	99	Blue	≥3	**99.92**	**99.72**	**99.64**	**99.21**	**99.78**	**99.47**
NR-22	139	Blue	≥3	**140.08**	**139.99**	**139.95**	**139.91**	**140.04**	**139.89**
NR-24	128	Green	≥3	**128.47**	**128.01**	**127.55**	**127.39**	**128.50**	**128.43**
BAT-25	123	Blue	≥3	**122.73**	**123.29**	**121.81**	**121.63**	**122.00**	**121.72**
BAT-26	124	Black	≥4	**123.77**	**124.04**	**123.66**	**123.50**	**123.76**	**123.55**

Numbers in bold font indicate microsatellite stability (MSS) and no loci shortened. SPTAN1, non-erythroid spectrin αII; MLH1, mutL homologue 1.