Agrin in the Muscularis Mucosa Serves as a Biomarker Distinguishing Hyperplastic Polyps from Sessile Serrated Lesions

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要旨

Sessile serrated lesion（SSL）は大腸癌の前駆病変であり，他のポリープ，特に Hyperplastic polyp（HP）との区別は診断上，重要な問題である．筆者らは以前に同定された大腸癌関連細胞外マトリックス（extracellular matrix；ECM）蛋白の大腸ポリープにおける発現パターンを評価し，SSL を他のポリープと区別するバイオマーカーを同定することを目的とした．

バイオマーカー選択に至るアプローチは Figure 1 に要約されている．筆者らは以前に RNA-seq によって分析された SSL，HP，Traditional serrated adenoma（TSA），Tubular adenoma（TA），正常コントロールのデータセットを用いて，大腸癌の肝転移で増加が確認されている 65 の ECM 蛋白について遺伝子発現レベルを分析した．その結果，SSL，HP で発現上昇し，TA で発現低下する 5 つの ECM 蛋白が特定された（Figure 2）．このうち信頼性の高い市販の抗体を持つ 3 つの ECM 蛋白（AGRN, SERPINE2, TIMP1）について免疫組織化学により発現を評価したところ（Figure 1），AGRN のみ正常およびポリープ組織において明確かつ一貫した発現の違いを認めた．

AGRN は正常組織では血管基底膜にのみ発現し，陰窩基底膜には発現していなかったが，ポリープ（SSL，HP，TSA）では陰窩基底膜に発現し，かつ各ポリープでその発現パターンに違いを認めた（Figure 3）．特に，SSL では腫瘍腺管に隣接する粘膜筋板に強い発現を認めており（Figure 4，6），この所見は消化管専門の病理医間でさえ診断が分かれるような症例においても SSL を鑑別する指標として有用であることが示唆された（Figure 5）．

Figure 1.

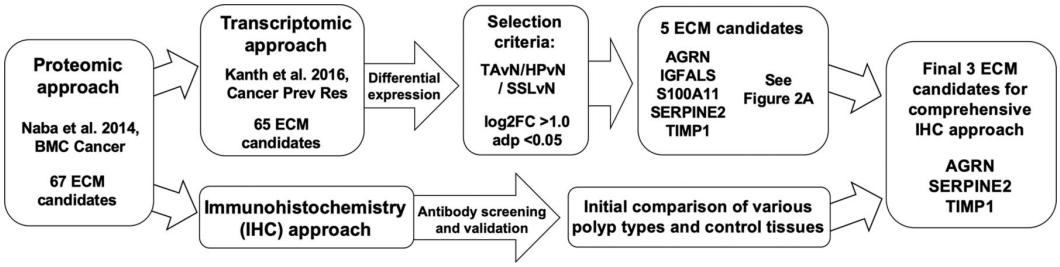
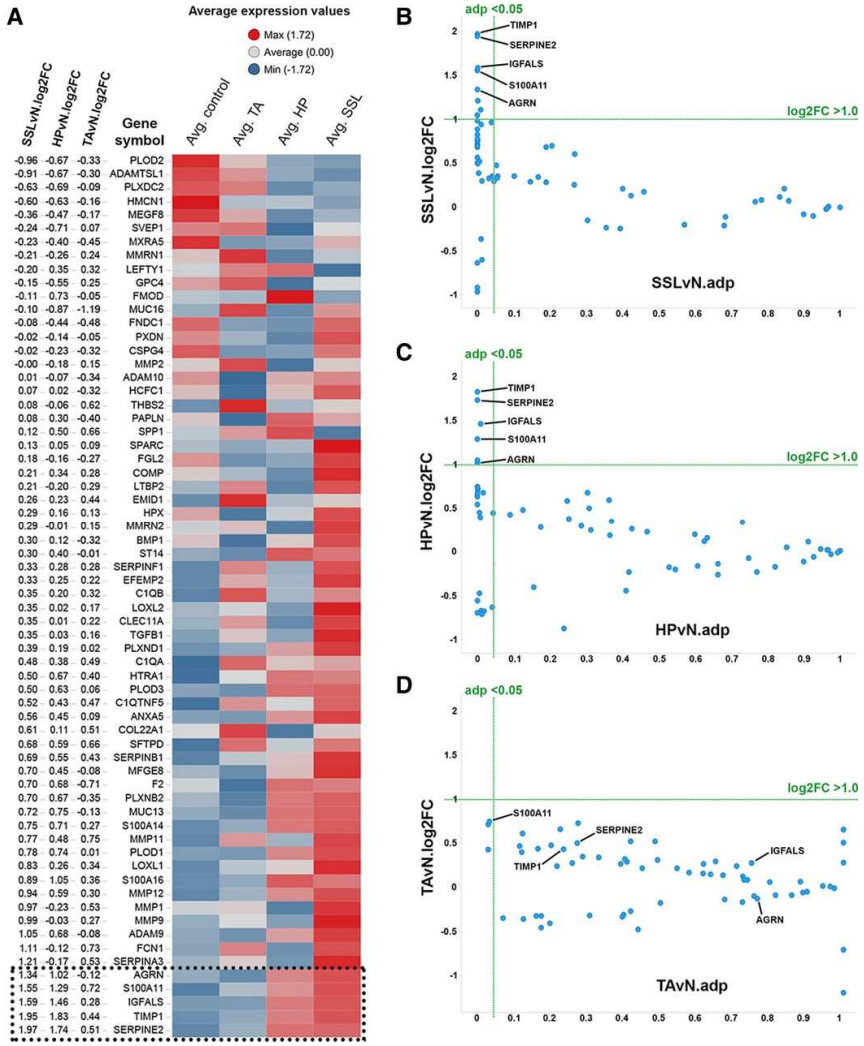


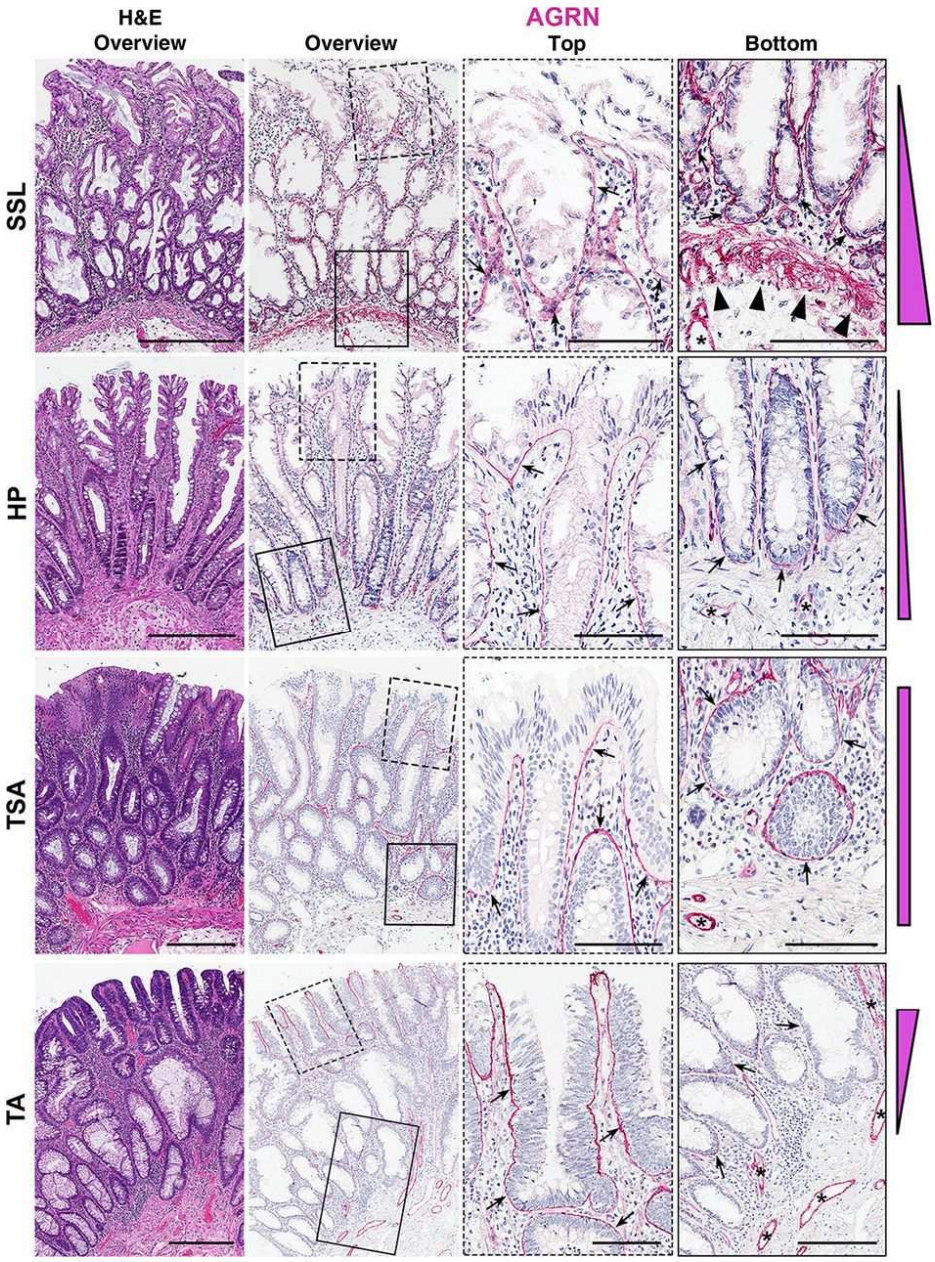
Figure 2.



Differential expression analysis to identify candidate ECM genes in human colon polyps. Differential expression analysis of 65 genes encoding ECM proteins previously identified to be upregulated in patients with colorectal cancer (26) using RNA-seq data (29) from a variety of colonic polyps, including SSLs (n = 21), HP (n = 10), TA (n = 10), and normal colon controls (N; n = 20). A, Row-centered average expression values for all three polyp types and normal controls are plotted in the heatmap. The rows are rank-ordered according to SSLvN log2 fold changes (log2FC) as shown in the first three columns. Five ECM genes that meet the threshold [log2FC >1.0 and adjusted P value (adp) <0.05] in SSL and HP samples are indicated at the bottom of the heatmap. See Supplementary Fig. S1 for gene expression data of the individual samples. B–D, Scatterplots showing the distribution of expression levels of the 65 ECM-protein genes and

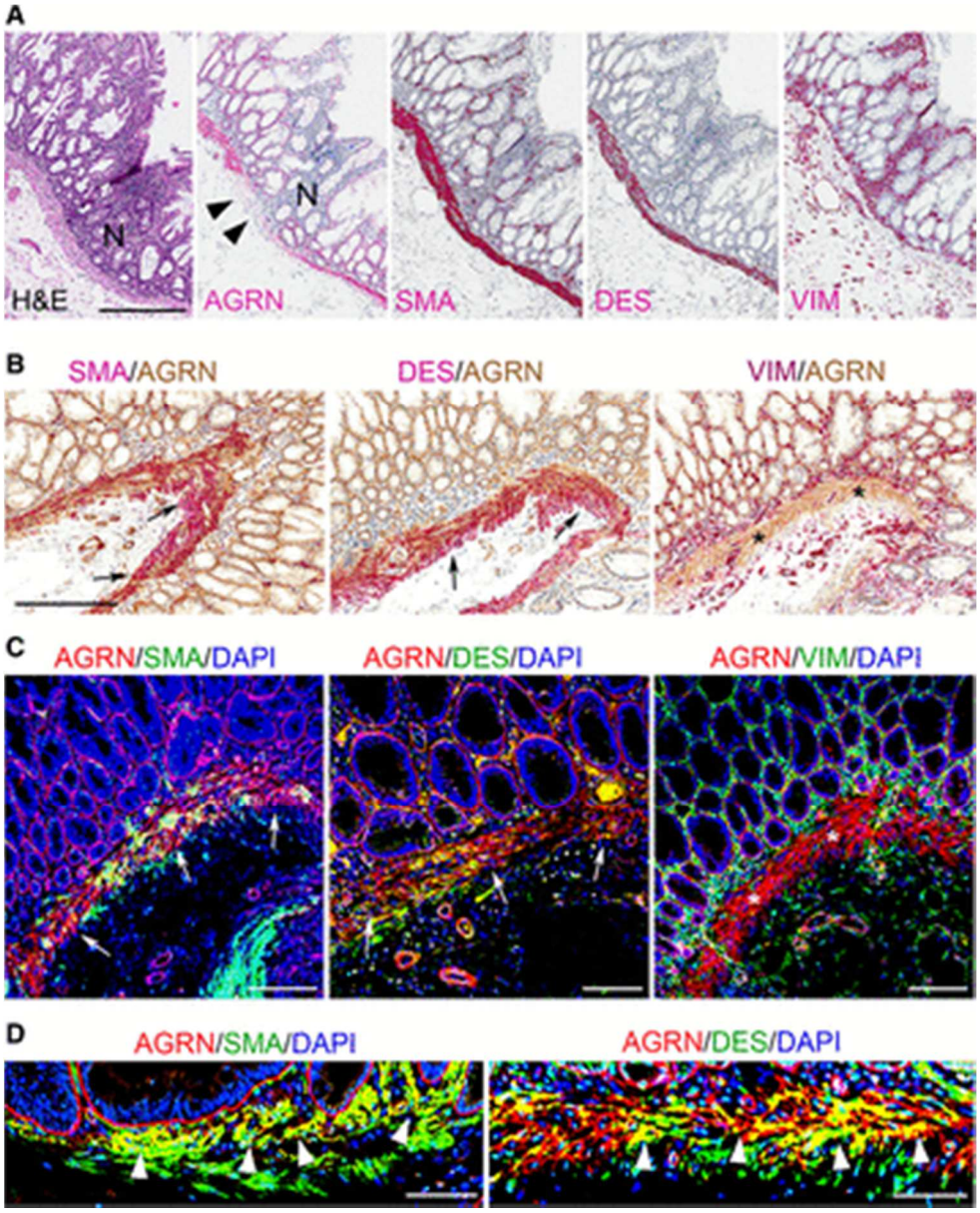
selection criteria; log2FC >1.0 and adp <0.05 (green lines) among SSL (B), HP (C), and TA (D) samples analyzed. Highlighted are the five genes (AGRN, IGFALS, S100A11, SERPINE2, TIMP1) that meet the selection threshold and are overexpressed in SSLs and HPs but not TAs.

Figure 3.



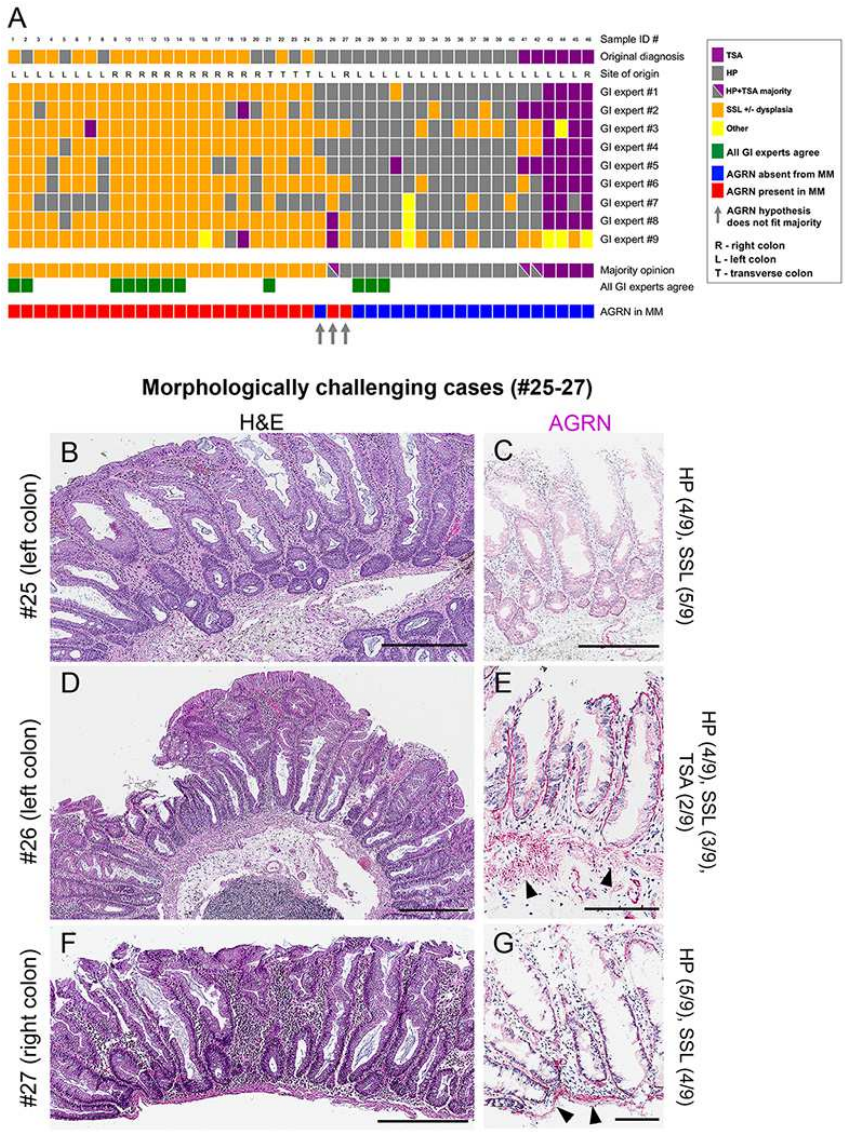
Differential localization of AGRN as a biomarker to distinguish colorectal polyps. Representative H&E and agrin (AGRN) IHC images of colonic polyps. Presented are overview images (left two columns) and enlarged images for AGRN IHC (boxed areas) from the top and the bottom of the crypt (right two columns) for the individual polyp types. Note the positive stain in the BL of all blood vessels (*) and the differential localization patterns of AGRN in the BL (indicated by arrows) of the different types of polyps (also presented schematically at the right edge of each polyp panel). AGRN reactivity is consistently observed as follows: SSLs and HPs (basal crypt predominance), TSAs (top-high and bottom-high), and TAs (top-high-to-bottom-low). Also note the presence of AGRN in the MM exclusively in SSL (arrowheads). Scale bars: 300 μm (overviews) and 100 μm (magnifications).

Figure 4.



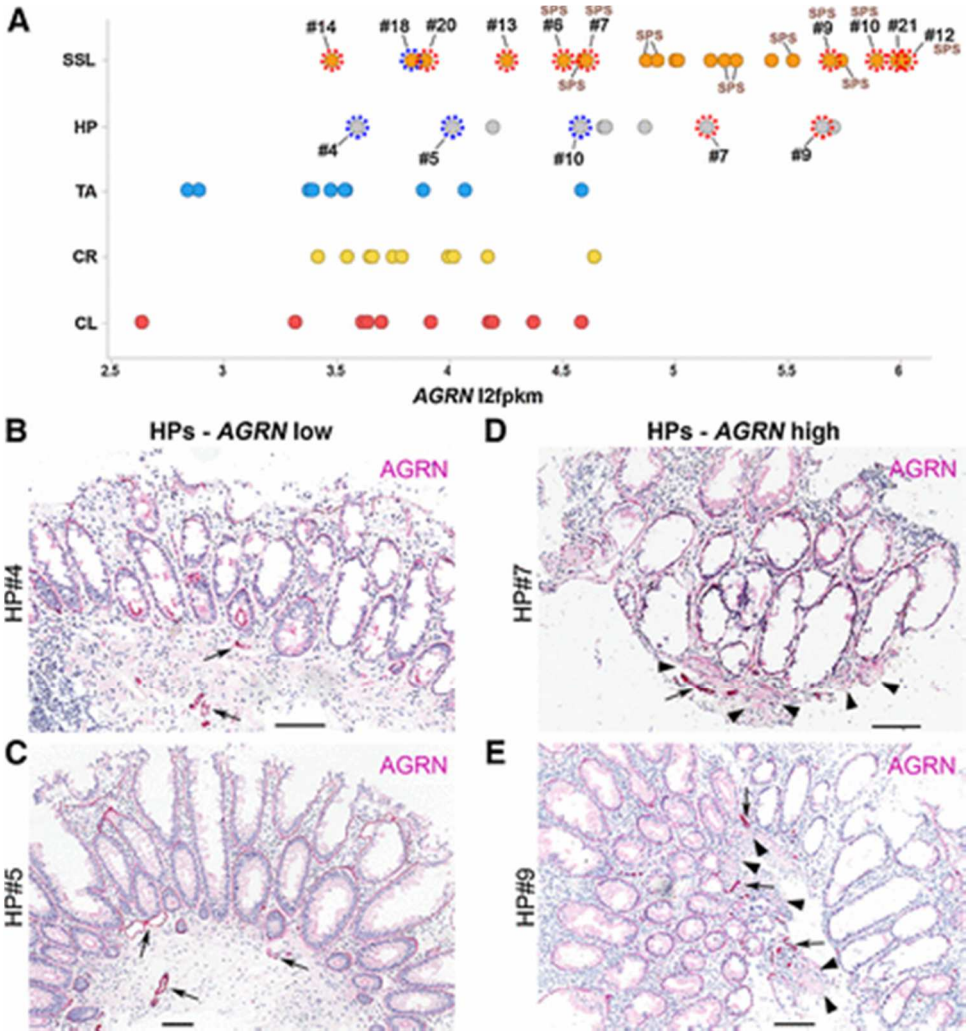
AGRN reactivity in the MM of SSLs. A, Representative H&E and IHC images of parallel sections of SSL, comparing AGRN reactivity to smooth muscle actin (SMA), desmin (DES), and vimentin (VIM). Scale bar: 300 μm. B, IHC images of immunostains for AGRN (brown), SMA, DES, and VIM (red) performed on consecutive sections. AGRN colocalizes with SMA and DES but not with VIM. Scale bar: 300 μm. C and D, Confocal immunofluorescence microscopy of SSL tissue sections, comparing the localization of AGRN (red) with that of SMA, DES, and VIM (all in green); yellow signal shows colocalization. Cell nuclei are shown in blue. Scale bars: 100 μm (C) and 50 μm (D). AGRN is localized to MM, which is also positive for SMA and DES (A–D) but negative for VIM (*; B and C). Arrows in B and C mark the colocalization of AGRN with SMA and DES (color overlay in B, and yellow merged color in C and D). Also note, AGRN is exclusively present in the MM adjacent to the abnormal crypts of SSL and ends, often abruptly, in the adjacent normal (N) crypts (black arrowheads in A). Magnified images reveal that AGRN mainly localizes to the upper half of the MM (white arrowheads in D).

Figure 5.



Majority-based polyp validation among expert GI pathologists. Nine experts in GI pathology classified 50 diagnostically challenging polyps by H&E into (1) TSA, (2) HP, (3) SSL ± dysplasia, and (4) other, according to previous WHO criteria (see text for details). A, Presented are the results of 46 of these cases (for the four samples not shown, see Supplementary Fig. S7). Indicated are the original diagnoses, the site of polyp origin, and the individual GI expert opinions. For each case, the majority pathologist's opinion is compared with the corresponding MM-positivity for AGRN as scored by 4 nonpathologists (Supplementary Fig. S7A). Note the diagnostic variability among the individual GI experts as compared with the high concurrence of AGRN-positive MM staining with SSL (majority opinion), whereas most HPs and TSAs are negative. B–G, H&E (B, D, F) and corresponding AGRN IHC (C, E, G) images of three morphologically challenging cases (#25–27), as indicated by the three gray arrows in (A), in which the AGRN-positive stain of MM differs from the majority GI expert opinion, although those opinions were widely divergent. C, Note the absence of AGRN from the MM of sample #25 (~1.8 × 4.8 mm, left colon; 4/9 GI experts classified this as HP and 5/9 as SSL). E and G, Note AGRN-positive MM (arrowheads) in samples #26 (~2.1 × 1.6 mm, left colon; 4/9 GI experts classified this as HP, 3 as SSL, and 2 as TSA) and #27 (~0.55 × 1.8 mm, right colon; 5/9 GI experts classified this as HP and 4 as SSL). Notably, these polyps show two contiguous crypts with basal dilatation, meeting the WHO definition of SSLs. See also Supplementary Table S2 for individual samples. Scale bars: 300 μm (B, D, F), 100 μm (C, E, G).

Figure 6.



AGRN IHC validation on colonic polyp samples previously analyzed by RNA-seq. A, Scatter plot of AGRN mRNA expression values for SSL (n = 21), HP (n = 10), TA (n = 10), and colon controls (CL, control left, n = 10; CR, control right, n = 10) using publicly available RNA-seq data (see Supplementary Fig. S1; ref. 29). Expression values of AGRN (l2fpkm, log2 fragments per kilobase million) for the individual polyps and controls are indicated and the diagnosis is highlighted by a colored dot: orange (SSL), gray (HP), blue (TA), yellow (CR), red (CL). Note that many SSLs and a few HPs show high AGRN values (above 5) whereas all other samples show lower AGRN expression. Examples of SSL (n = 10) and HP (n = 5) for which sections were stained for AGRN are indicated by dotted circles; positive (red dotted circles) or negative (blue dotted circles) for MM-based AGRN reactivity. Note that the two designated HP samples showing high AGRN expression levels also scored positive for MM-based AGRN, whereas those with lower overall AGRN expression were also negative for AGRN MM staining. Most SSL samples scored positive for MM-based AGRN staining. SSL samples from patients with SPS are indicated. B–E, AGRN IHC images of sections from four HP samples from the RNA-seq dataset (29) presented in A. Shown are sections of HP samples in which the AGRN expression values are low [HP#4 (B), HP#5 (C)] or high [HP#7 (D), HP#9 (E)]. Note also in two of five HP samples the additional positivity for AGRN in the MM (HP#7, HP#9, arrowheads in D and E). Notably, these two cases cluster with the majority of SSLs, whereas HP#4 and HP#5 (negative for MM-based AGRN) cluster separately (Supplementary Fig. S1). Scale bars = 100 μm.