西京医院
XIJING HOSPITAL

A Novel SS18-SSX Fusion-specific Antibody for the Diagnosis of Synovial Sarcoma

汇报人：崔文静　　指导教师：徐玉乔

滑膜肉瘤（Synovial Sarcoma）

- 定义：具有不同程度上皮分化的间叶性肿瘤。
- t(X;18)(p11;q11)染色体易位，产生SS18-SSX1/SS18-SSX2/SS18-SSX4融合基因
- 可发生在任何年龄，15-20岁青壮年占50%以上
- 无性别差异
- 下肢膝关节 > 躯干 > 头颈部 > 生殖器官 > 肾 > 肾上腺

镜下形态

双相型

上皮样细胞&梭形细胞

单相梭形细胞型

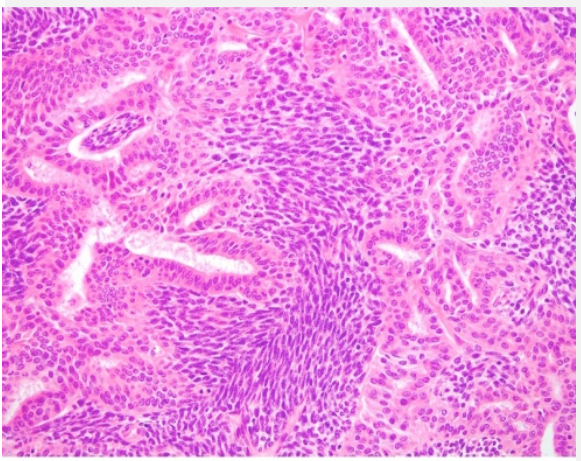
- 最常见，最易误诊
- 短条束状/长条束状的梭形细胞，呈旋涡状/鱼骨样排列

单相上皮型

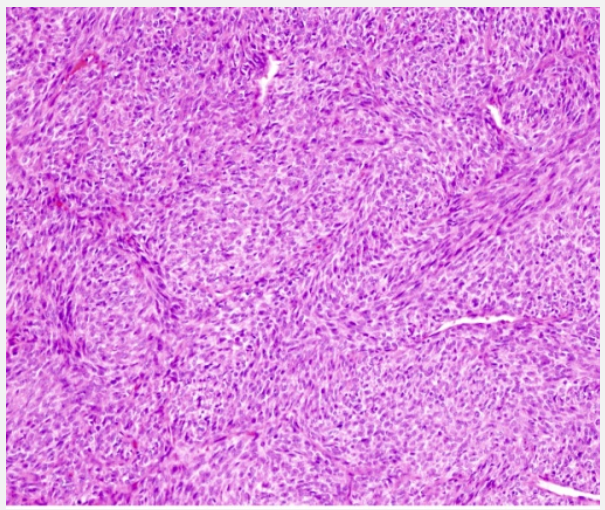
- 少见，好发于四肢
- 实性片块状、腺样、小管样
- 瘤细胞为肥胖的圆形或卵圆形上皮样细胞，梭形成分少

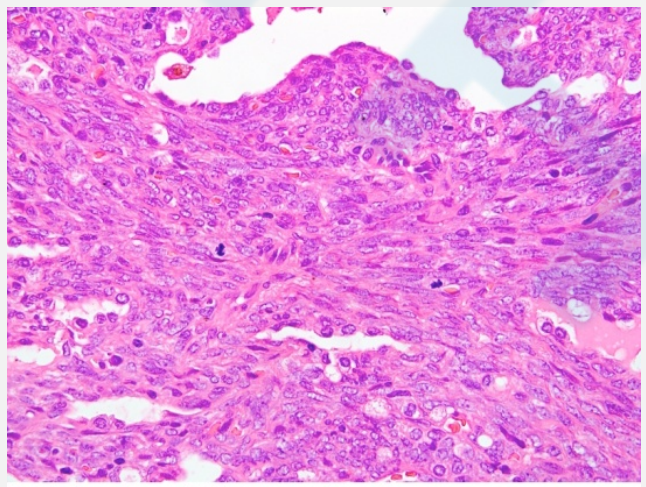
差分化型

- 更易侵袭和转移
- 分化差的小圆形细胞，大圆形细胞，高度恶性的胖梭形细胞

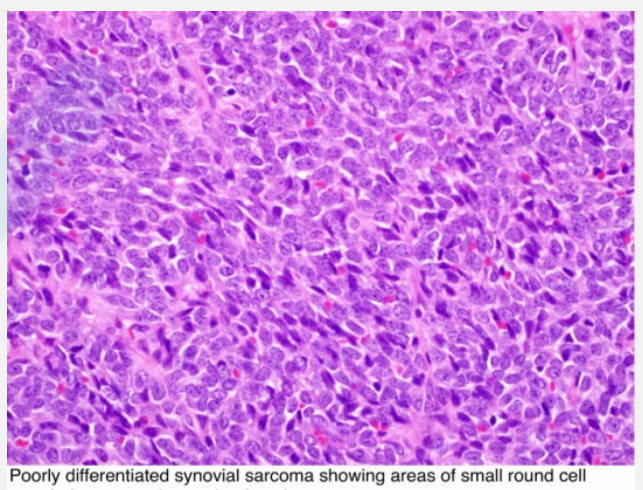


Biphasic synovial sarcoma, 20x: spindle cell areas show typical synovial sarcoma nuclear features; glands are composed of closely packed round to ovoid cells





Monophasic synovial sarcoma, 20x: focally prominent mitotic activity



Poorly differentiated synovial sarcoma showing areas of small round cell morphology; note the retained nuclear monotony

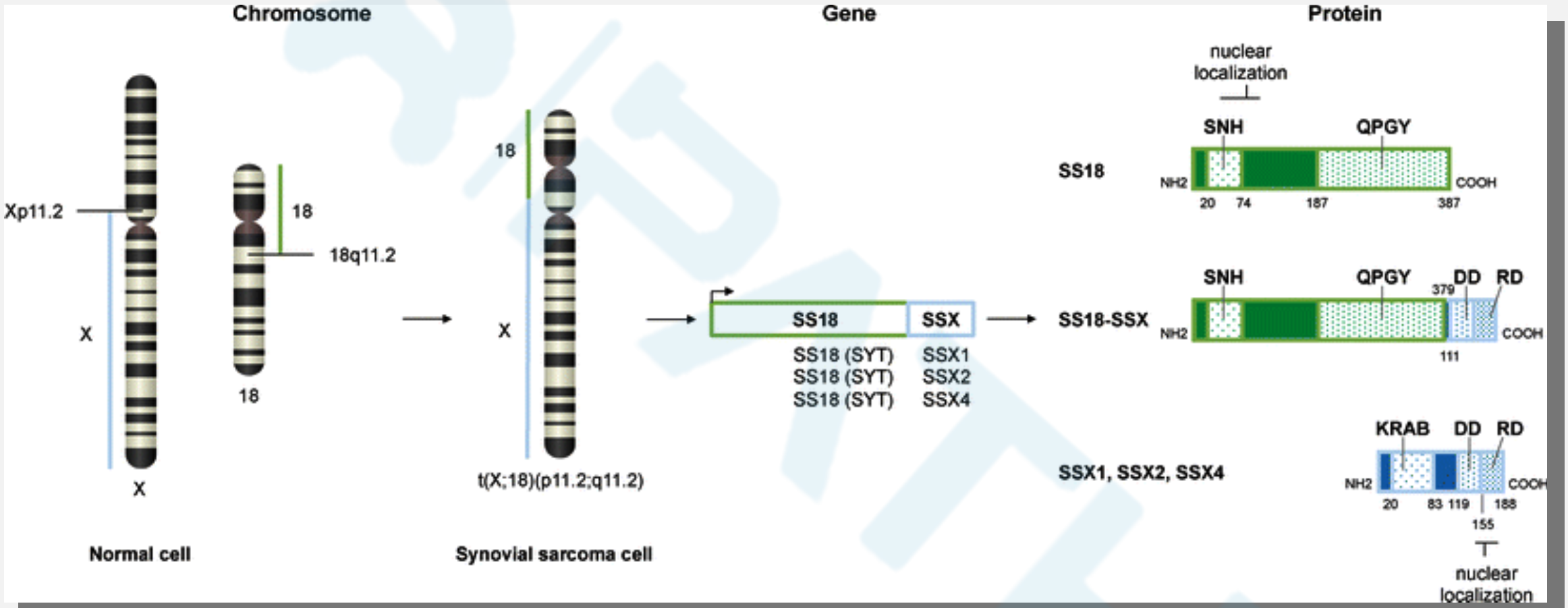
免疫组化

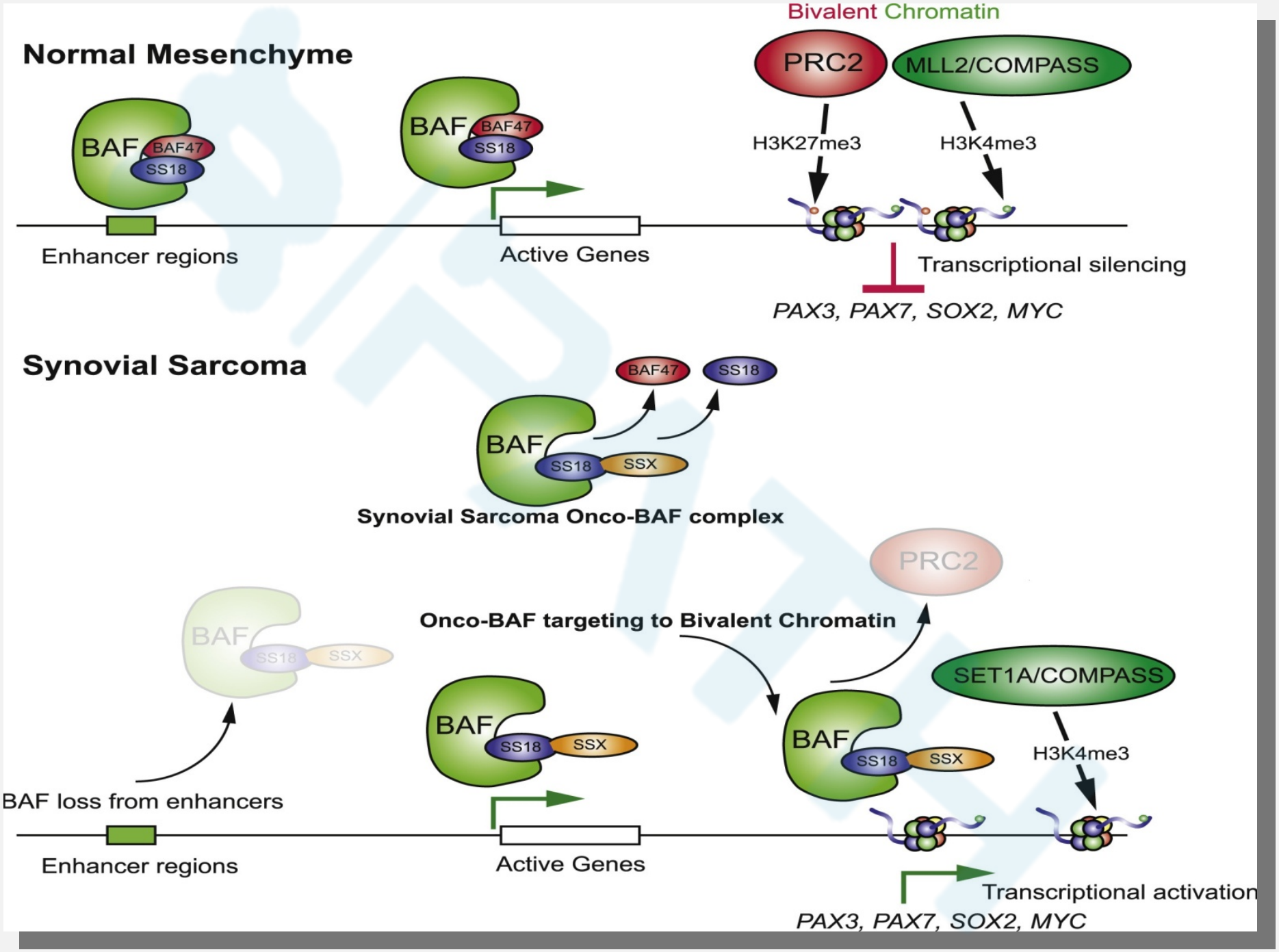
- CK，EMA， Vimentin 阳性
- TLE-1 阳性
- S-100，CD99 ，bcl-2，SMA，MSA可有不同程度的表达
- CD34、desmin阴性

t(X；18)（p11；q11）,涉及SS18-SSX基因融合

分子遗传学

SS18-SSX


Chromosome
Gene
Protein
Xp11.2
X
18
18q11.2
X
18
X
t(X;18)(p11.2;q11.2)
Normal cell
Synovial sarcoma cell
SS18
SSX
SS18 (SYT) SSX1
SS18 (SYT) SSX2
SS18 (SYT) SSX4
nuclear localization
SS18
SNH
QPGY
NH2
COOH
20 74 187 387
SS18-SSX
SNH
QPGY
DD
RD
379
NH2
COOH
111
SSX1, SSX2, SSX4
KRAB
DD
RD
NH2
COOH
20 83 119 188
155
nuclear localization



Epigenetic ConFUSION: SS18-SSX Fusion Rewires BAF Complex to Activate Bivalent Genes in Synovial Sarcoma. *Cancer Cell* .33, June 11, 2018

- 单纯形态对于诊断SS是具有挑战性的，也缺乏敏感性和特异性高的抗体。 FISH检测SS18 断裂点， RT-PCR或者NGS检测SS18-SSX融合基因成为诊断SS的金标准

- RT-PCR 不能广泛检测 SS18-SSX1 、SS18-SSX2 、SS18-SSX4融合基因, 有可能丢失一些罕见的融合基因类型。FISH 也缺乏一定的敏感性

- 本文评估一个新的SS18-SSX 抗体和一个SSX C-terminal 抗体在SS诊断中的作用

Antibody Development

➢**an SS18-SSX fusion-specific antibody**

clone E9X9V; cat#72364; *Cell Signaling Technology*, Danvers,MA

designed to the breakpoint：Gln379 to Ile111 of human SSX

➢**an SSX-specific antibody**

clone E5A2C; cat# 23855; *Cell Signaling Technology*

SSX基因羧基端，即Gln173附近

Cell Lines and Cell Culture

The SS cell lines：HSSY2 、Aska and SYO1

The human fibroblast cell line：CRL7250

Immunoprecipitation

Western Blot Analysis

Chromatin Immunoprecipitation With Sequencing

Fluorescence In Situ Hybridization for SS18 Rearrangement

Immunohistochemistry

选取400个肿瘤对抗体进行特异性评估：

病例包括100 例SS (41 monophasic, 18 biphasic, and 41 PD)和300例形态需与SS鉴别的肿瘤。

免疫反应程度根据肿瘤细胞核染色百分率评分：

0 (0%), 1+ (<5%), 2+ (5% to 25%),3+ (26% to 50%), 4+ (51% to 75%), and 5+ (76% to 100%)

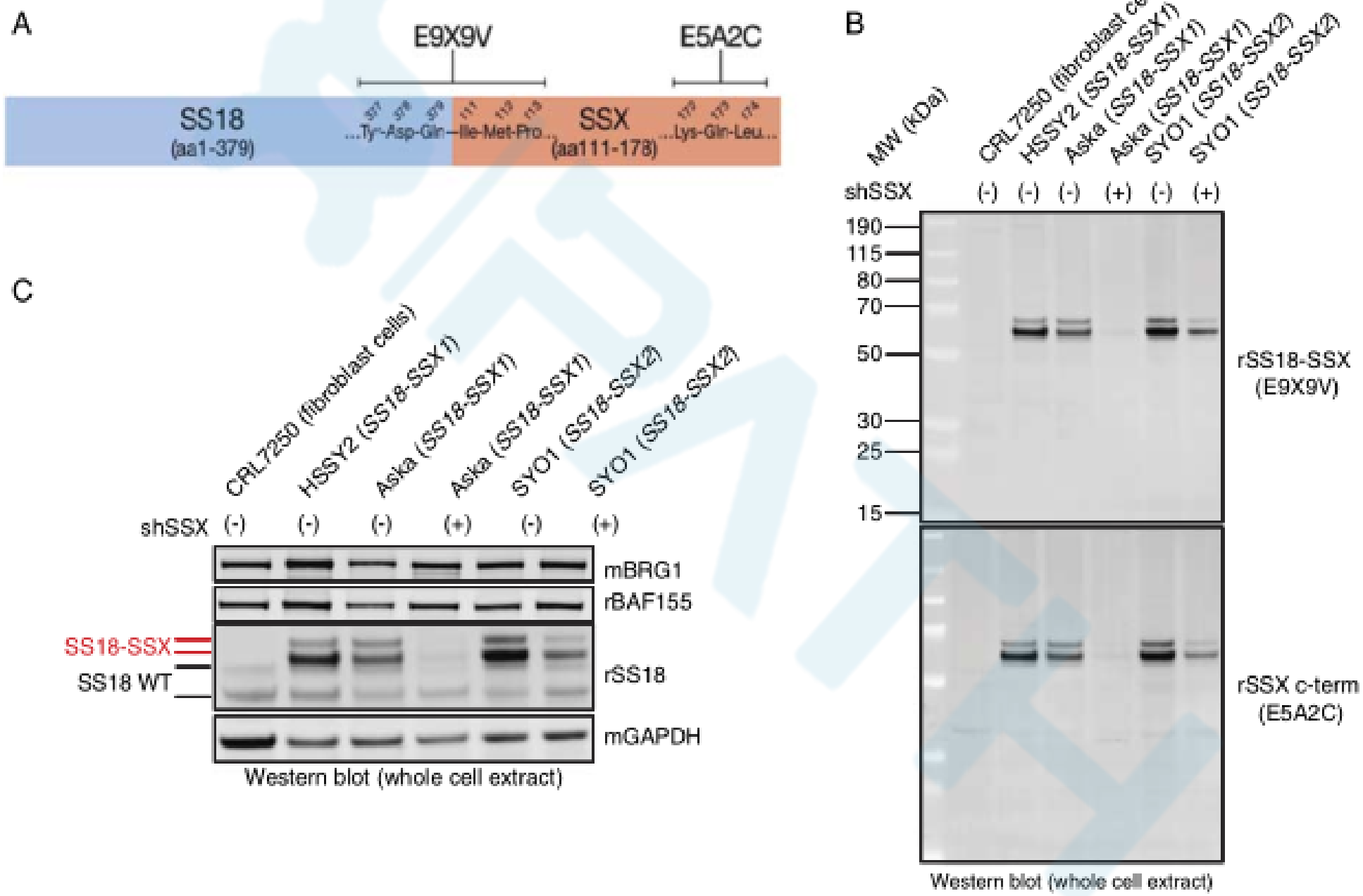


Fig 1. Development and Assessment of an Antibody Specific for the SS18-SSX Oncogenic Fusion

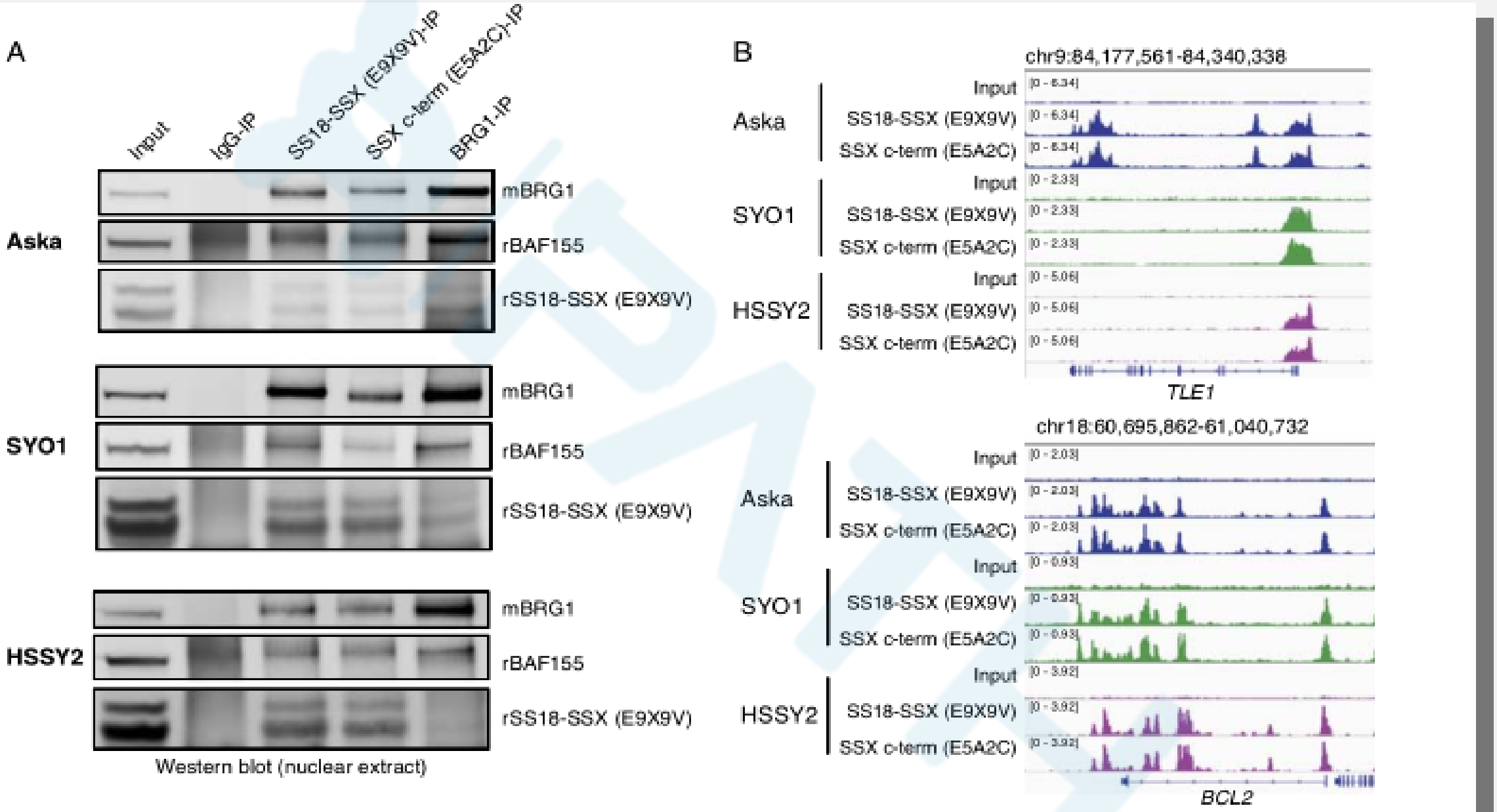


Fig 2. The SS18-SSX-specific Antibody Purifies mSWI/SNF (BAF) Complexes and Captures Chromatinbound Fusion Protein

TABLE 1. Summary of IHC Staining With SS18-SSX Fusion-specific and SSX C-terminus Antibodies

Tumor Type	Total Cases	n (%) SS18-SSX Positive	n (%) SSX C-terminus Positive
SS	100	95 (95)	100 (100)
Monophasic SS	41	39 (95)	41 (100)
Biphasic SS	18	18 (100)	18 (100)
PD SS	41	38 (93)	41 (100)
Non-SS tumors	300	0 (0)	13 (4)
MPNST	20	0 (0)	2 (10)
SFT	20	0 (0)	0 (0)
Dedifferentiated liposarcoma	20	0 (0)	2 (10)
Leiomyosarcoma	20	0 (0)	0 (0)
Fibrosarcomatous variant of DFSP	20	0 (0)	0 (0)
Ewing sarcoma	20	0 (0)	0 (0)
CIC sarcoma	20	0 (0)	0 (0)
Spindle cell rhabdomyosarcoma	20	0 (0)	0 (0)
Alveolar rhabdomyosarcoma	20	0 (0)	1 (5)
Embryonal rhabdomyosarcoma	20	0 (0)	1 (5)
Mesenchymal chondrosarcoma	20	0 (0)	2 (10)
Desmoplastic small round cell tumor	20	0 (0)	2 (10)
Clear cell sarcoma	20	0 (0)	0 (0)
Biphenotypic sinonasal sarcoma	10	0 (0)	1 (10)
BCOR-rearranged sarcoma	10	0 (0)	0 (0)
Sarcomatoid mesothelioma	10	0 (0)	2 (20)
Biphasic mesothelioma	10	0 (0)	0 (0)

DFSP indicates dermatofibrosarcoma protuberans.

Table 1. IHC Analysis With the SS18-SSX Fusion-specific and SSX C-terminus Antibodies

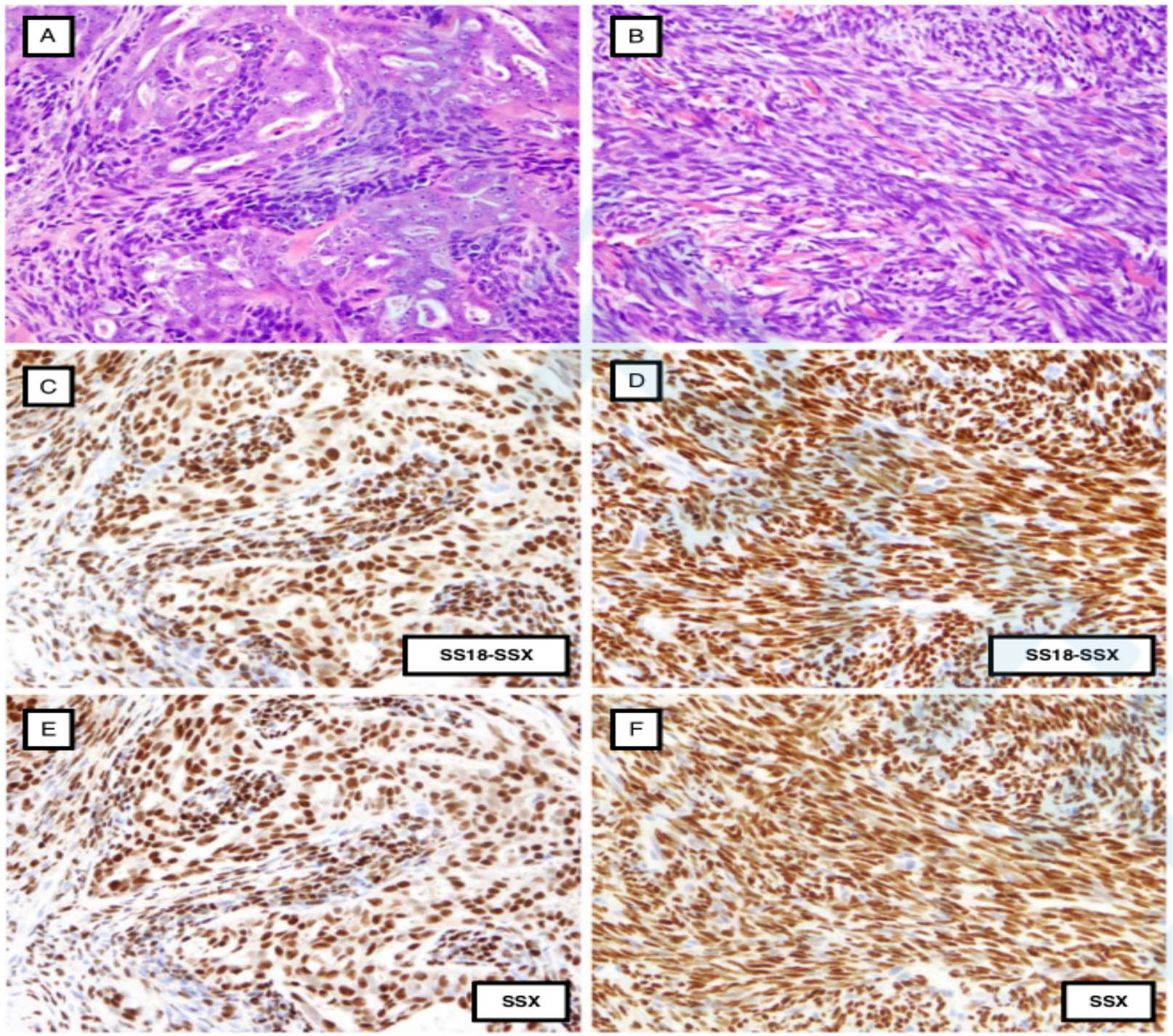


FIGURE 3
A：双相型的SS
B：单相梭形细胞型的SS

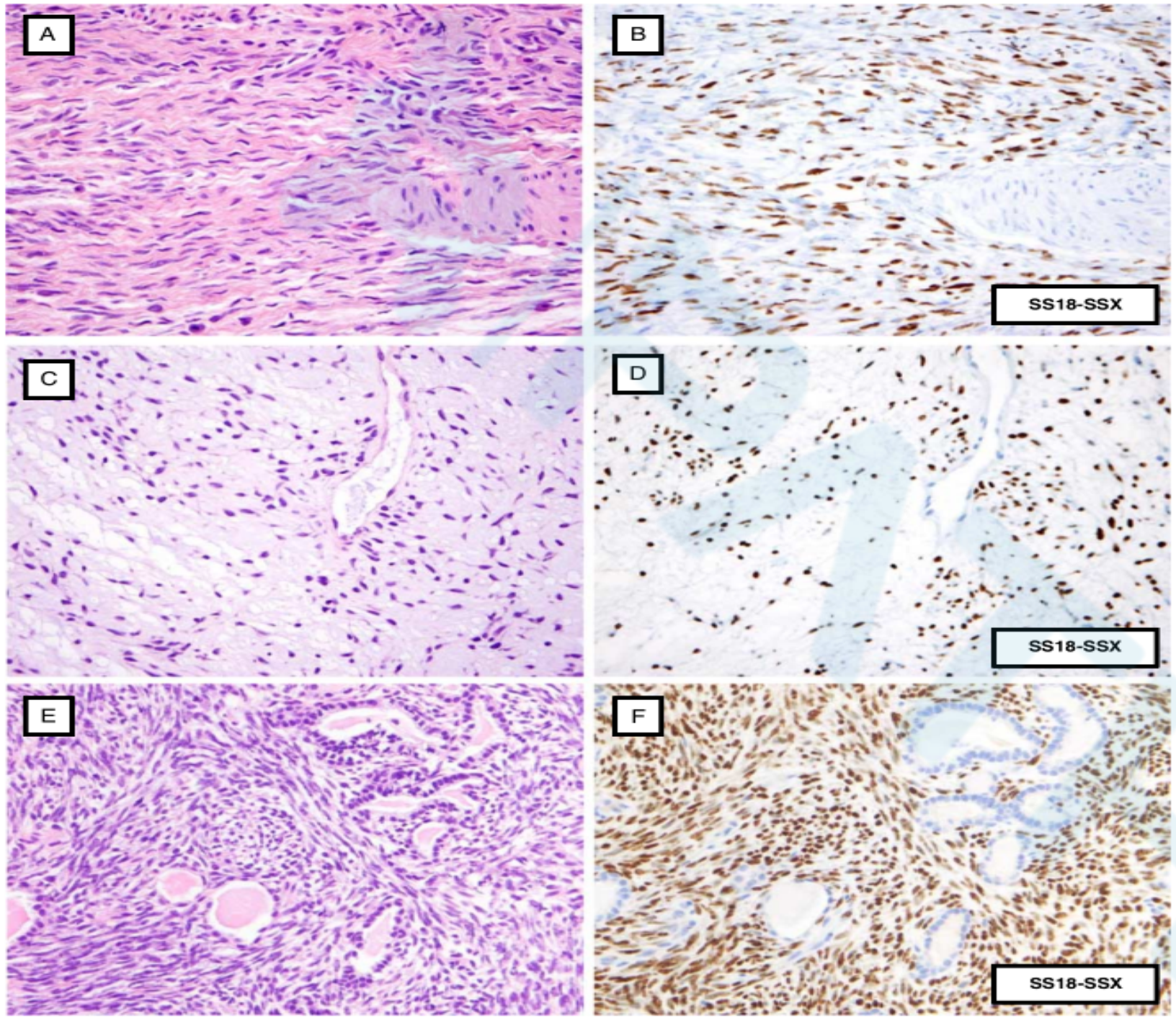


FIGURE 4
A：单相型SS
C：黏液样型SS
E：发生于肺的单相型SS

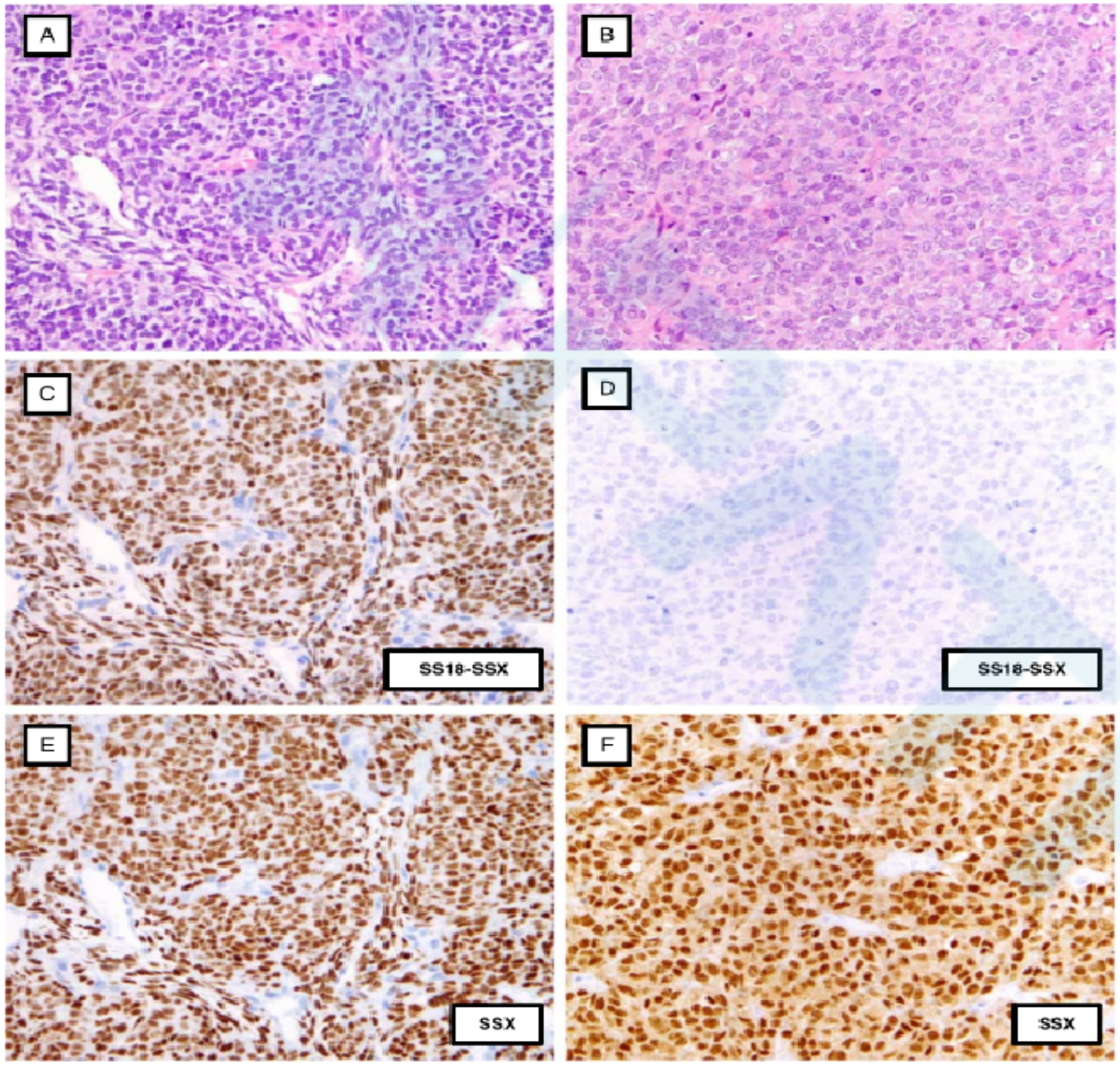


FIGURE 5
A、B：差分化SS

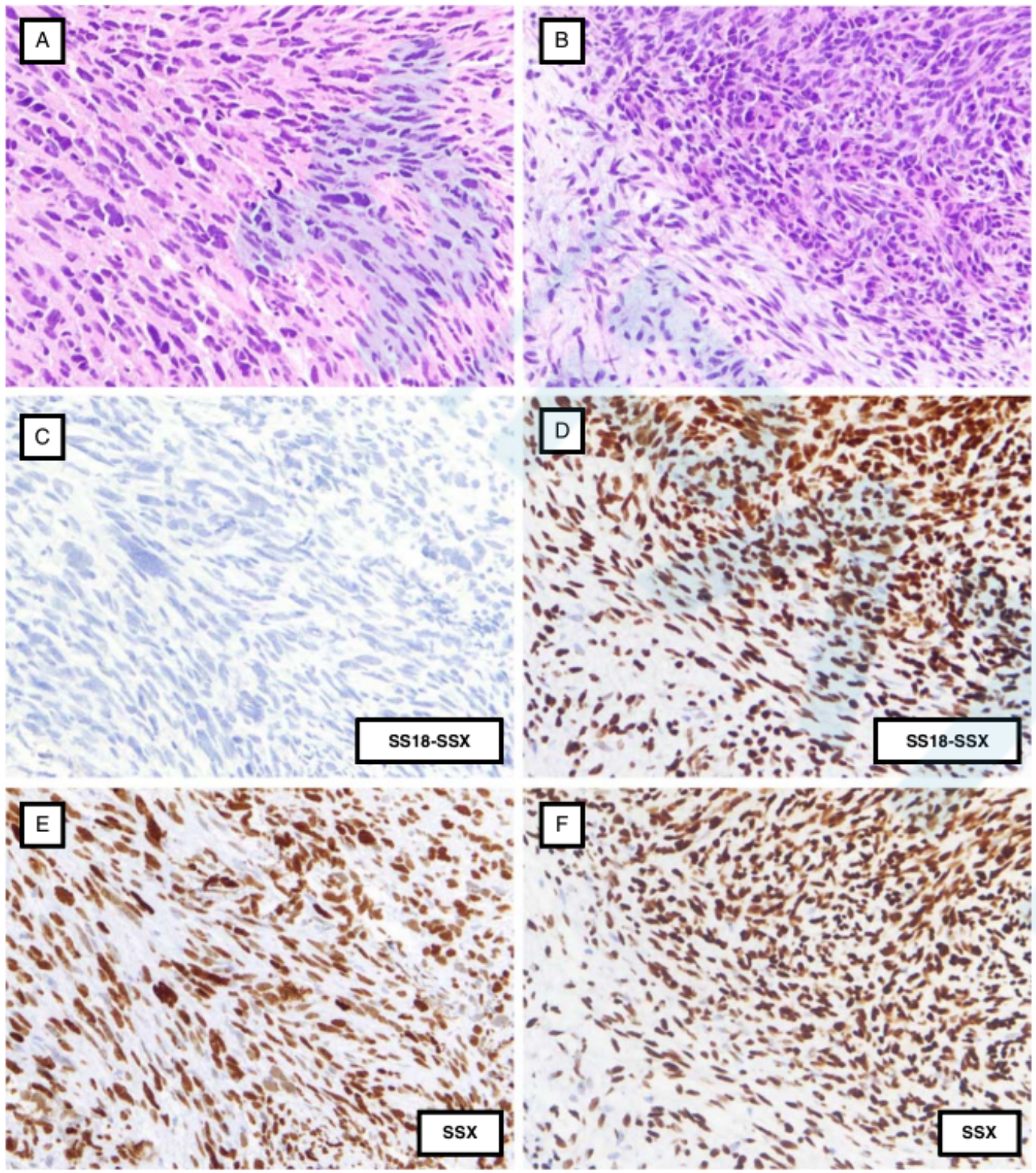


FIGURE 6
A：恶性外周神经鞘瘤
B：单相型SS

- SS18-SSX fusion-specific antibody (E9X9V)

特异性：100%　　敏感性：95%

- SSX C-terminus antibody (E5A2C)

特异性：96%　　敏感性：100%

- 本研究中5%的病例免疫组化结果E9X9V阴性有可能是不常见的融合变异。SSX尾部和其他断点变异的延长会改变融合基因的排列顺序，从而阻止与融合相关的特异性抗体所识别。这些病例可以被SSX C端抗体(E5A2C)识别

- 这些抗体在诊断中具有很高的价值，尤其是在RT-PCR、FISH漏掉的SS18-SSX融合基因相关病例中

- 这两种抗体可以取代分子遗传学或细胞遗传学检测的“金标准”，在大多数SS，可以使病理学家作出明确诊断，不需要花费更多的时间及使用较少的抗体

- 这两个试剂为进一步研究SS18-SSX融合蛋白的生物化学及基因组学提供了有价值的工具

谢谢

THANK YOU