mIF staining

For each marker, the staining conditions were optimized using monoplex-stained slides of positive control tissues and then re-examined on a multiplex-stained ES-SCLC slide. Prior to staining, all 4 µm thick FFPE tissue sections were deparaffinized by overnight baking at 56°C, immersion in BOND Dewax Solution at 72°C and rehydration in ethanol. Heat-induced epitope retrieval pretreatments were performed using BOND Epitope Retrieval (ER) Solutions citrate-based pH 6.0 ER1 or EDTA-based pH 9.0 ER2 (both Leica Biosystems), depending on the primary antibody. Tissue sections were blocked with serum-free block/antibody diluent (Akoya Biosciences) for 10 minutes prior to the application of each primary antibody. The anti-mouse+rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (Akoya Biosciences) was added for 10 minutes at room temperature, followed by incubation with different TSA-conjugated fluorophores for 10 minutes. Finally, spectral DAPI (Akoya Biosciences) was used as a nuclear counterstain and slides were mounted in ProLong Diamond Anti-fade Mountant (Life Technologies).