Supplementary

Table S1 Summary of previously reported key molecular findings in p40/TTF-1 double-positive non-small cell lung carcinoma (NSCLC) patients

No.	Study	Number of patients diagnosed with p40/TTF-1 NSCLC	Molecular findings
1	Pelosi *et al.* (12), Expert Rev Respir Med, 2016	1	*KRAS* (AAA>AAT, K117N, exon 4), *TP53* (GTG>GGG, V272G, exon 8), *FGFR1* amp
2	Hayashi *et al.* (21), *Human Pathology*, 2018	1	*TP53* (pVal272Leu), *PTEN* (pHis123Asp), phospho-AKT (Ser473)
3	Pelosi *et al.* (22), *JTO Clinical and Research Reports*, 2021	2	*EGFR* (pGlu746_ALA750del, c2235_2249del), *TP53* (pGlu224Asp), *RAD51B* (pPro365Arg), *CCND3* (pSer259Ala), NF1 (pArg1769Ter) CNVs for *IGF1R*, *MYC*, *CCND1*, *CDK2*
4	Spinelli *et al.* (26), *Pathologica*, 2019	1	polymorphism TP53 (pPro72Arg)
5	Savari *et al.* (20), *Histopathology*, 2023	7	*TP53* mut (7/7), *CDKN2A* mut (3/7), *KRAS* (G12C) mut (2/7), *KMT2D* mut (2/7), *KMT2A* mut (1/7), *ARID2* mut (1/7), *DNMT3A* mut (1/7), *FGFR1* amp (5/7), *MYC* amp (3/7), *AKT1* amp (2/7), *NKX2.1* amp (2/7), *EGFR* Ex20 insertion (N771-H773dup) (1/7)
6	Cai *et al.* (23), *Oncology Letters*, 2023	1	exon 6 of *EML4* and exon 20 of *ALK* fusion, *PIK3CA* mut
7	Guo *et al.* (24), *International Journal of Surgical Pathology*, 2023	1	*TP53* mut, *NOTCH2* mut, *STK11* mut

mut, mutation; amp, amplification; del, deletion; Ex, exon.

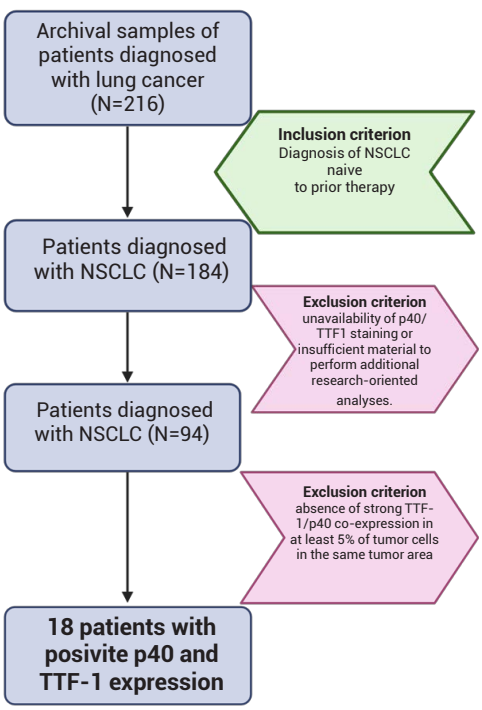

Figure S1 Flow diagram summarizing patient selection and inclusion in the study (May 2021–November 2022). Figure was created with BioRender.com (license to J.S.).

https://dx.doi.org/10.21037/tlcr-2025-690

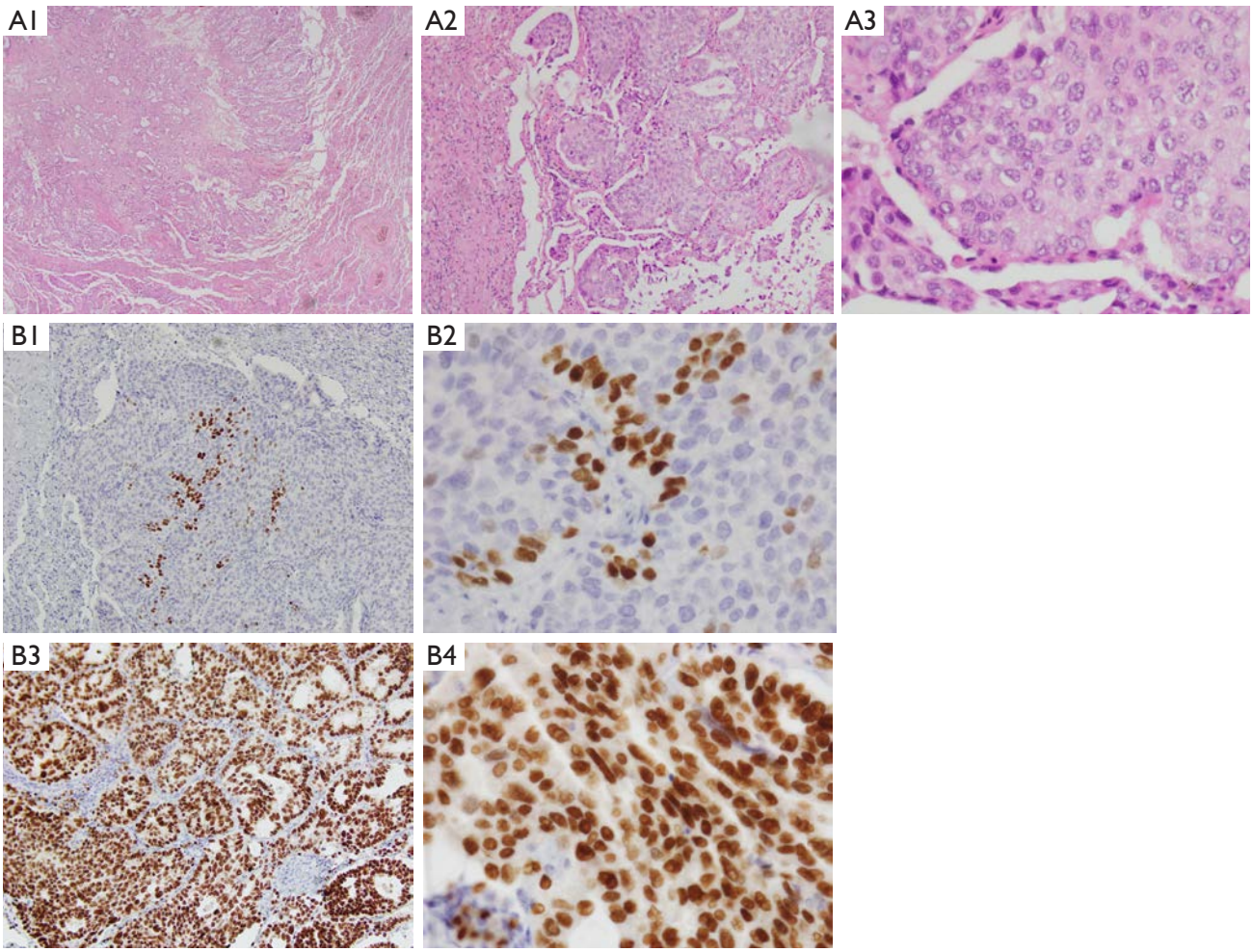

Figure S2 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 1. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4).

https://dx.doi.org/10.21037/tlcr-2025-690

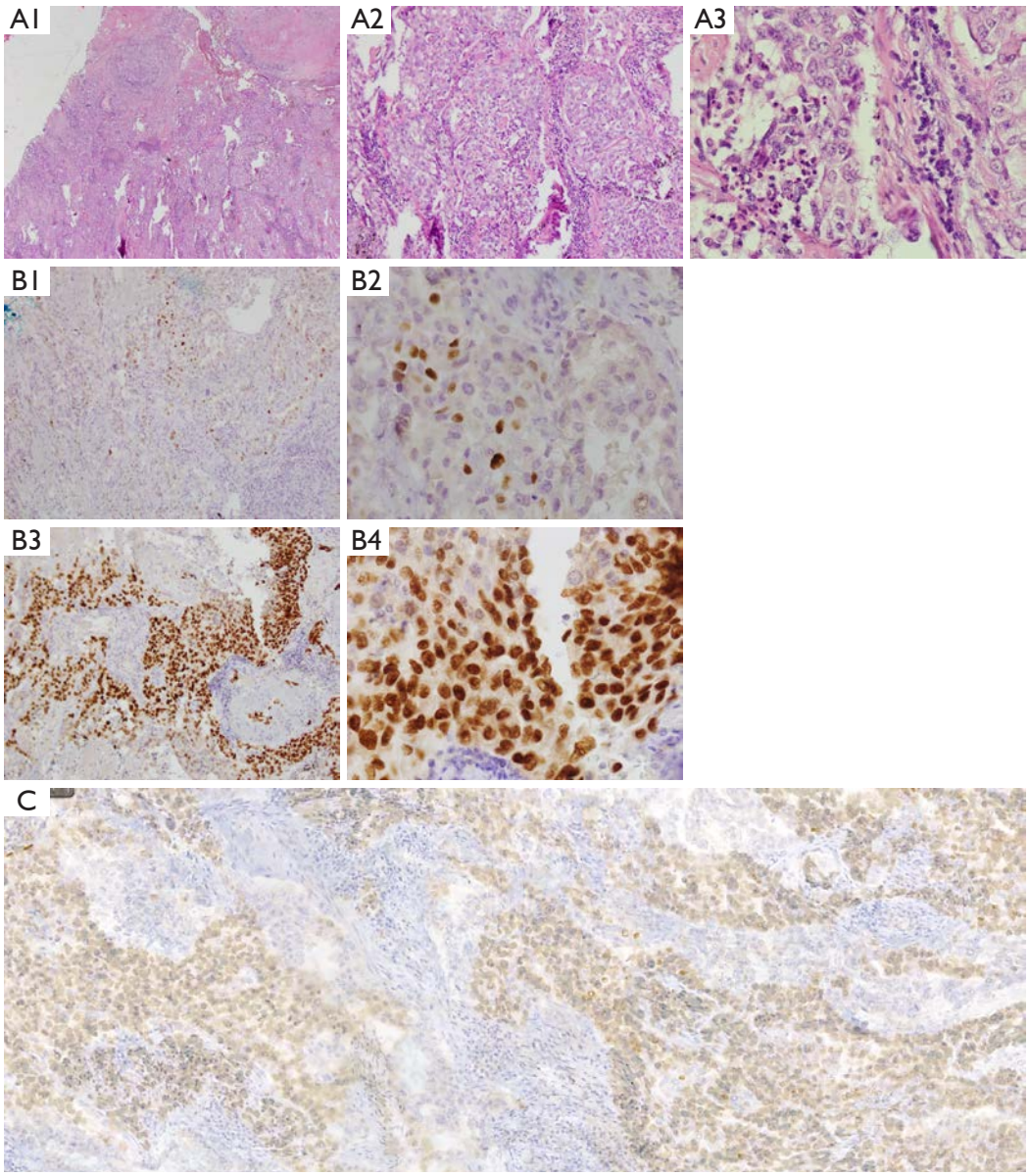

Figure S3 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 2. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 2. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

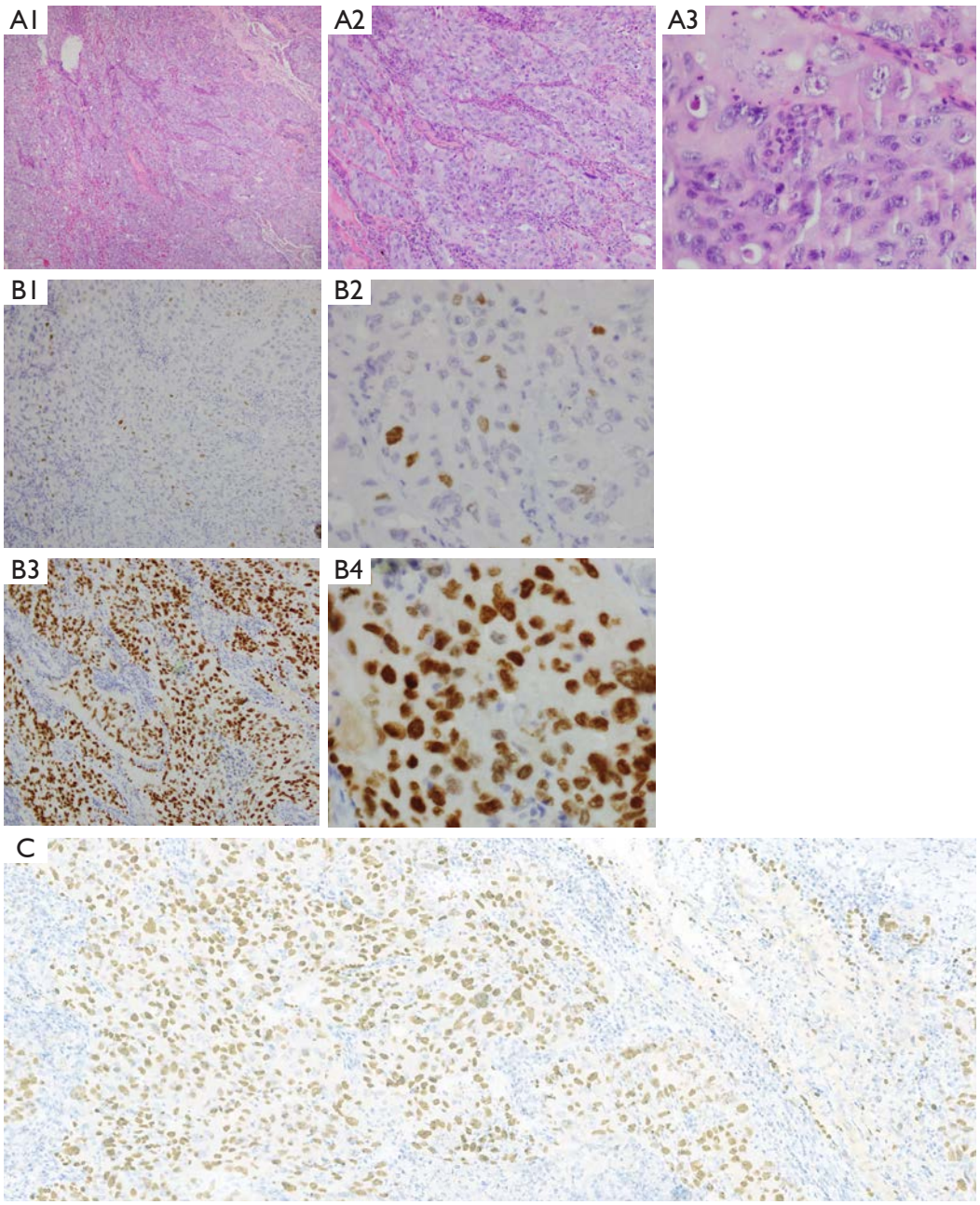

Figure S4 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 3. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 3. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

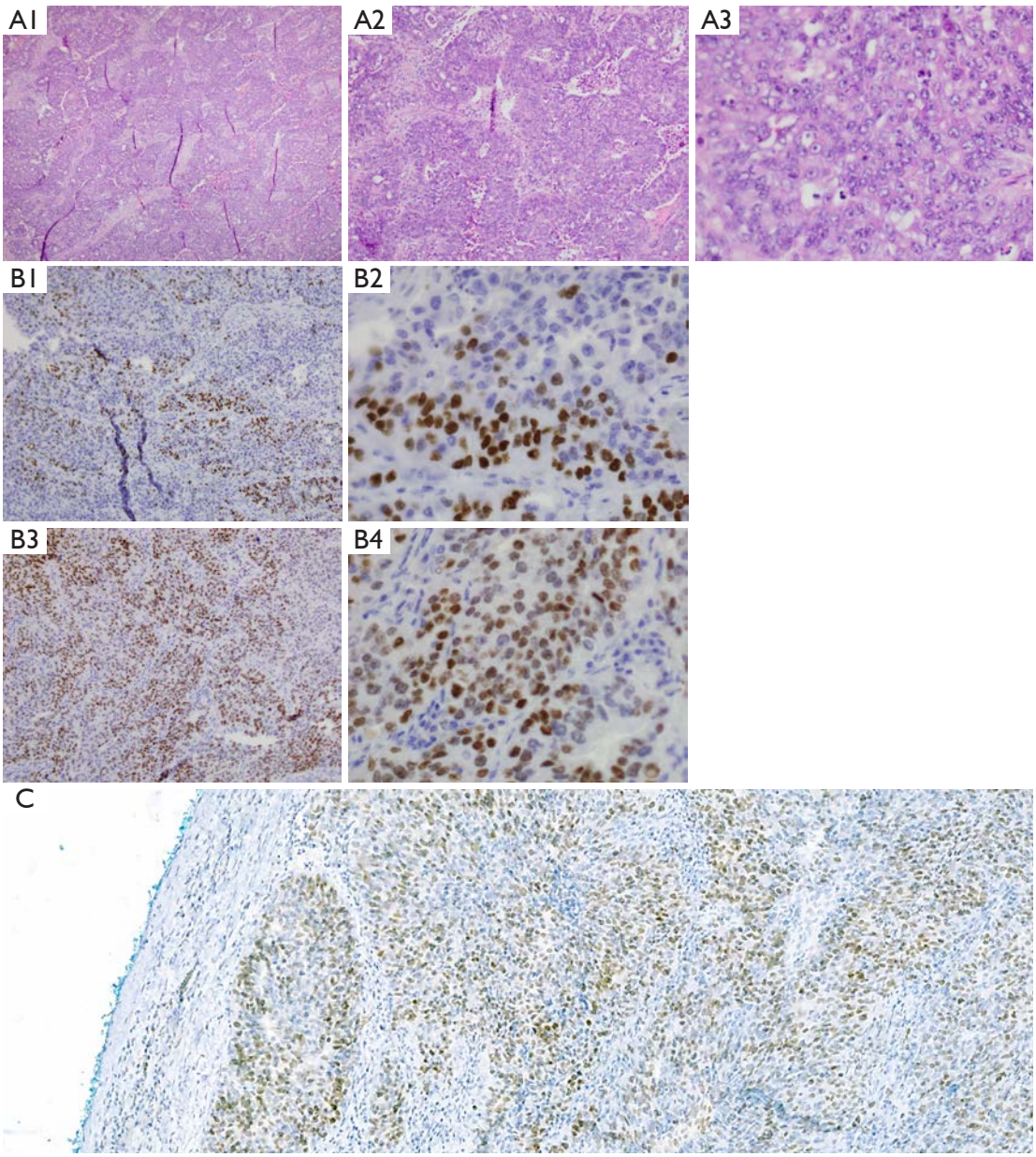

Figure S5 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 4. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 4. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

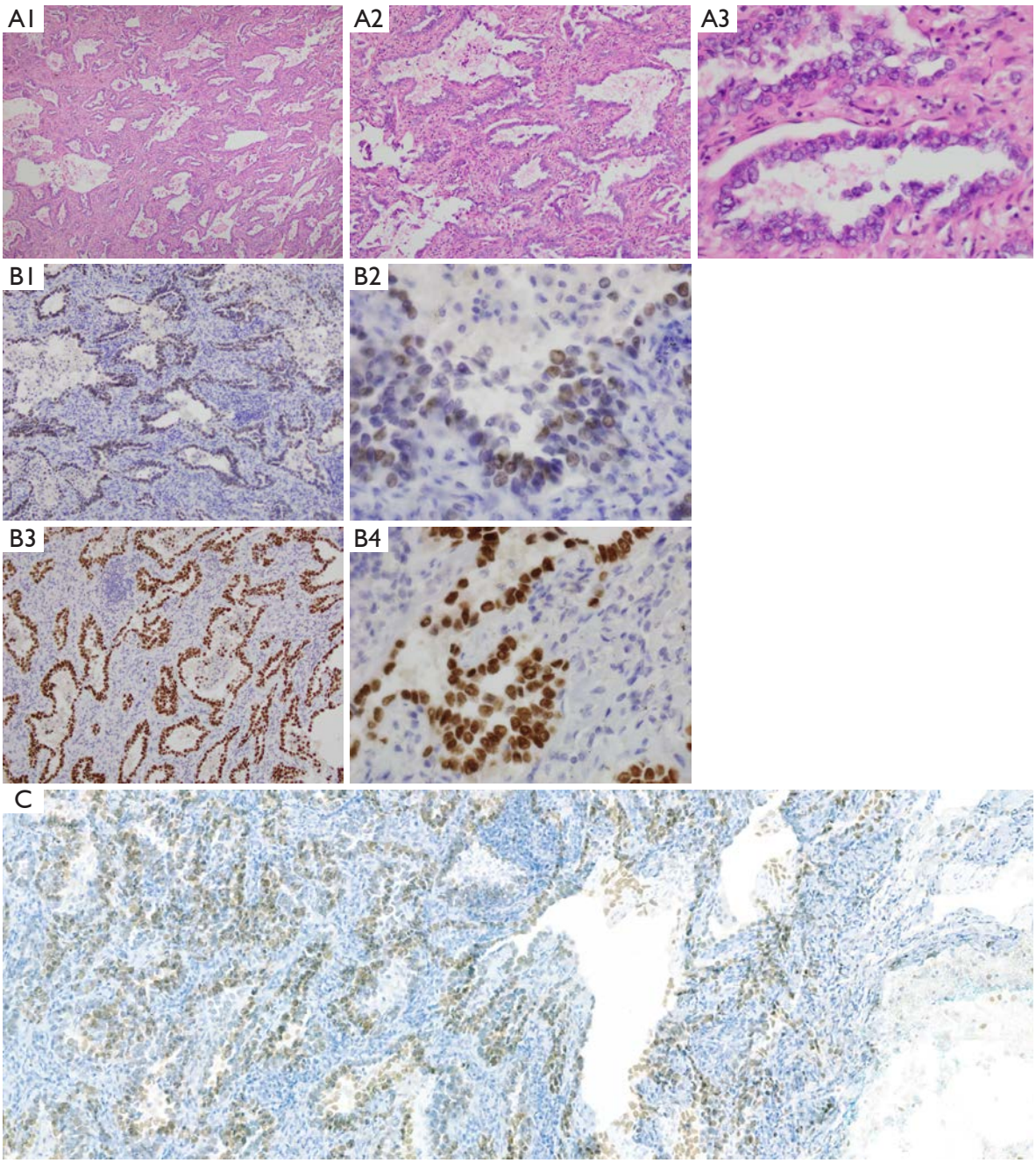

Figure S6 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 5. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 5. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

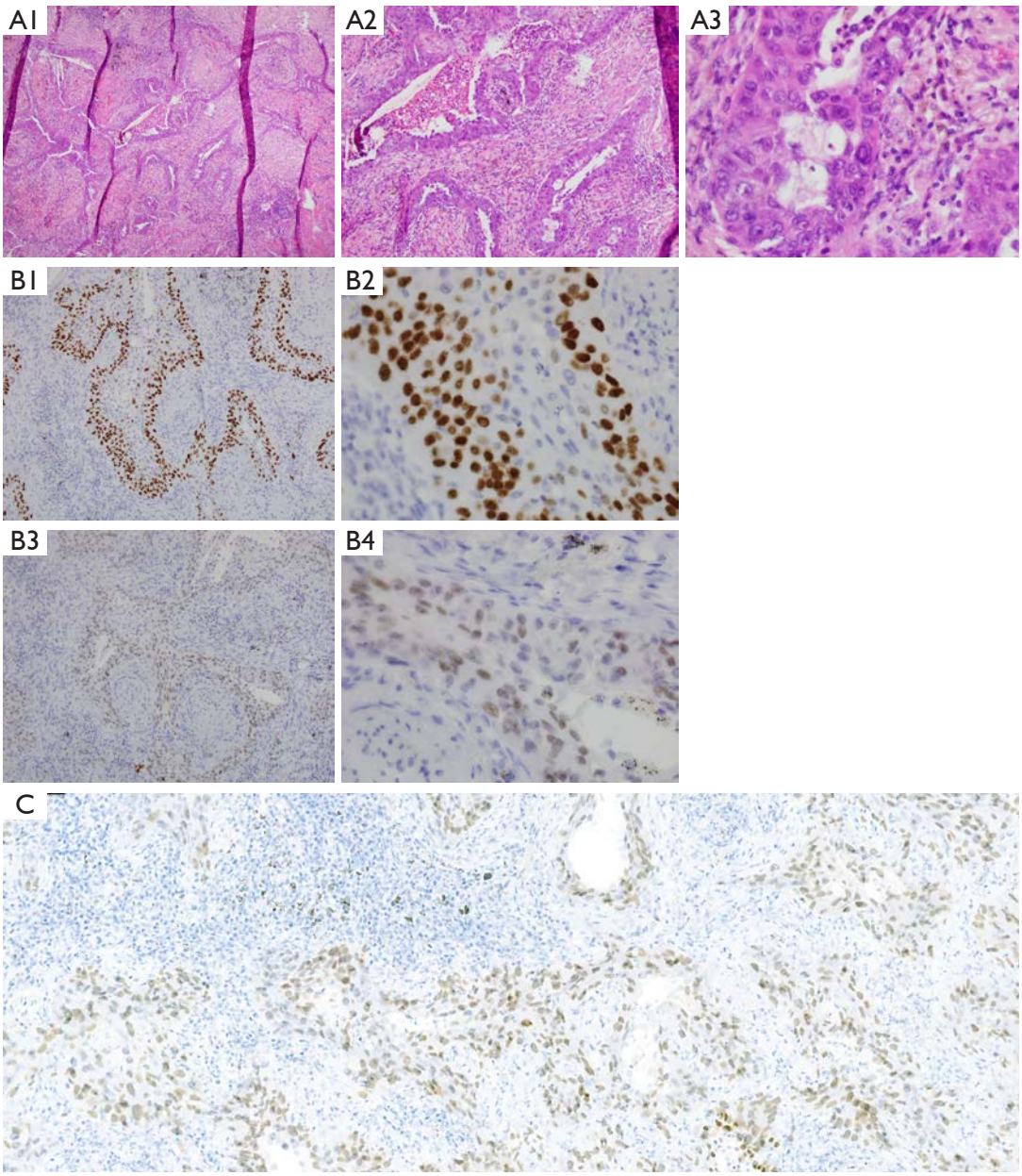

Figure S7 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 6. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 6. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

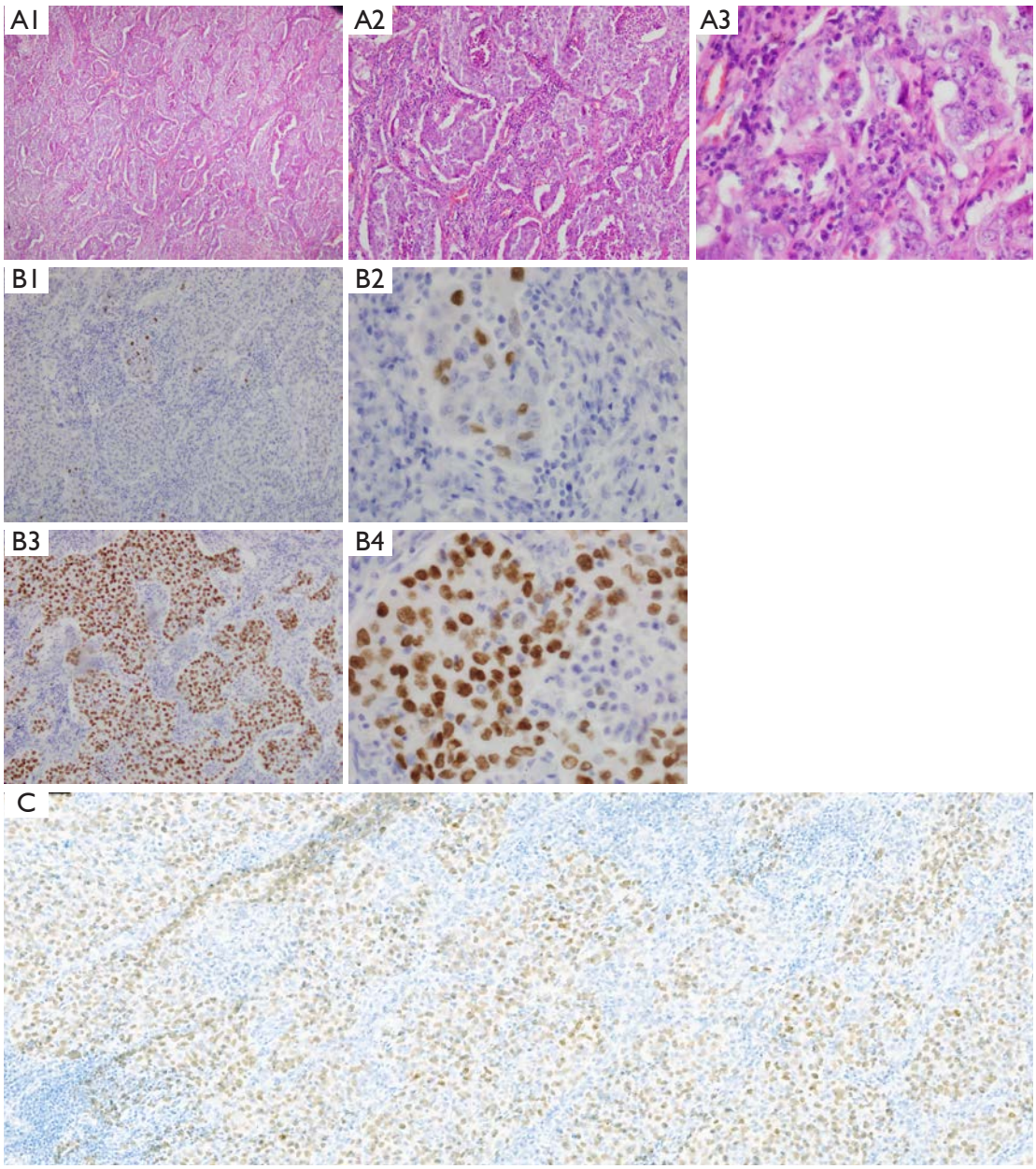

Figure S8 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 7. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 7. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

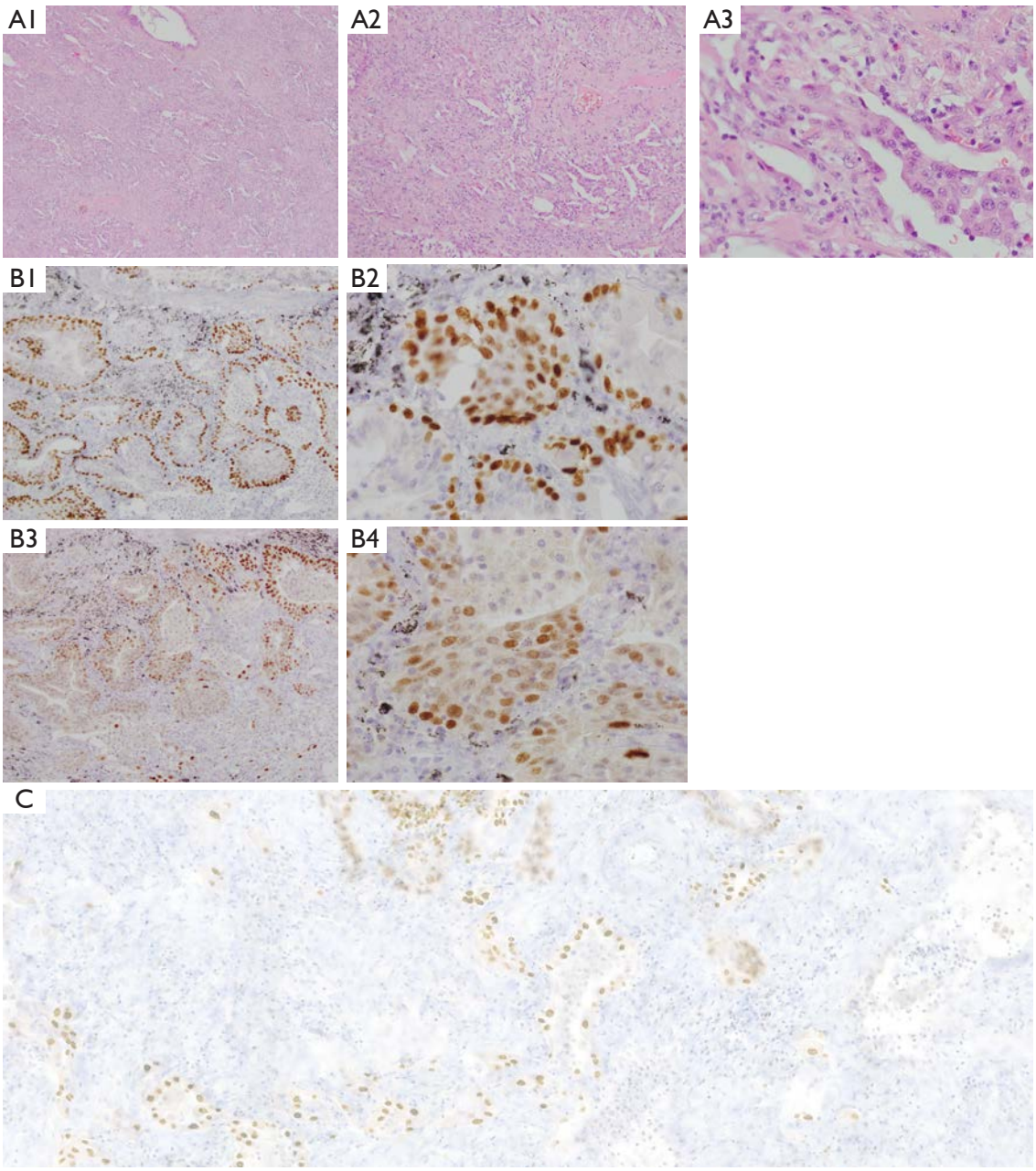

Figure S9 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 8. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 8. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

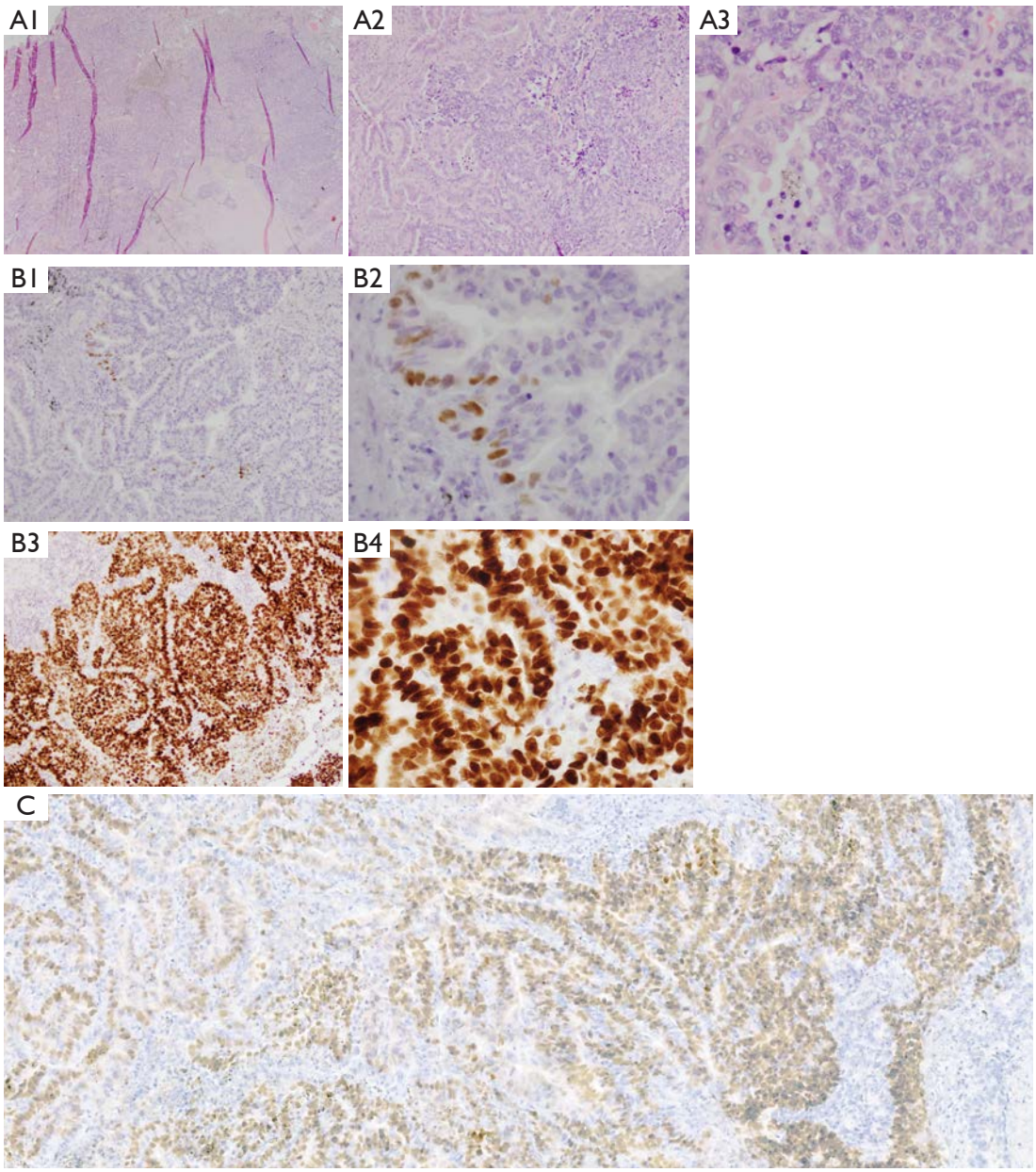

Figure S10 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 9. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 9. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

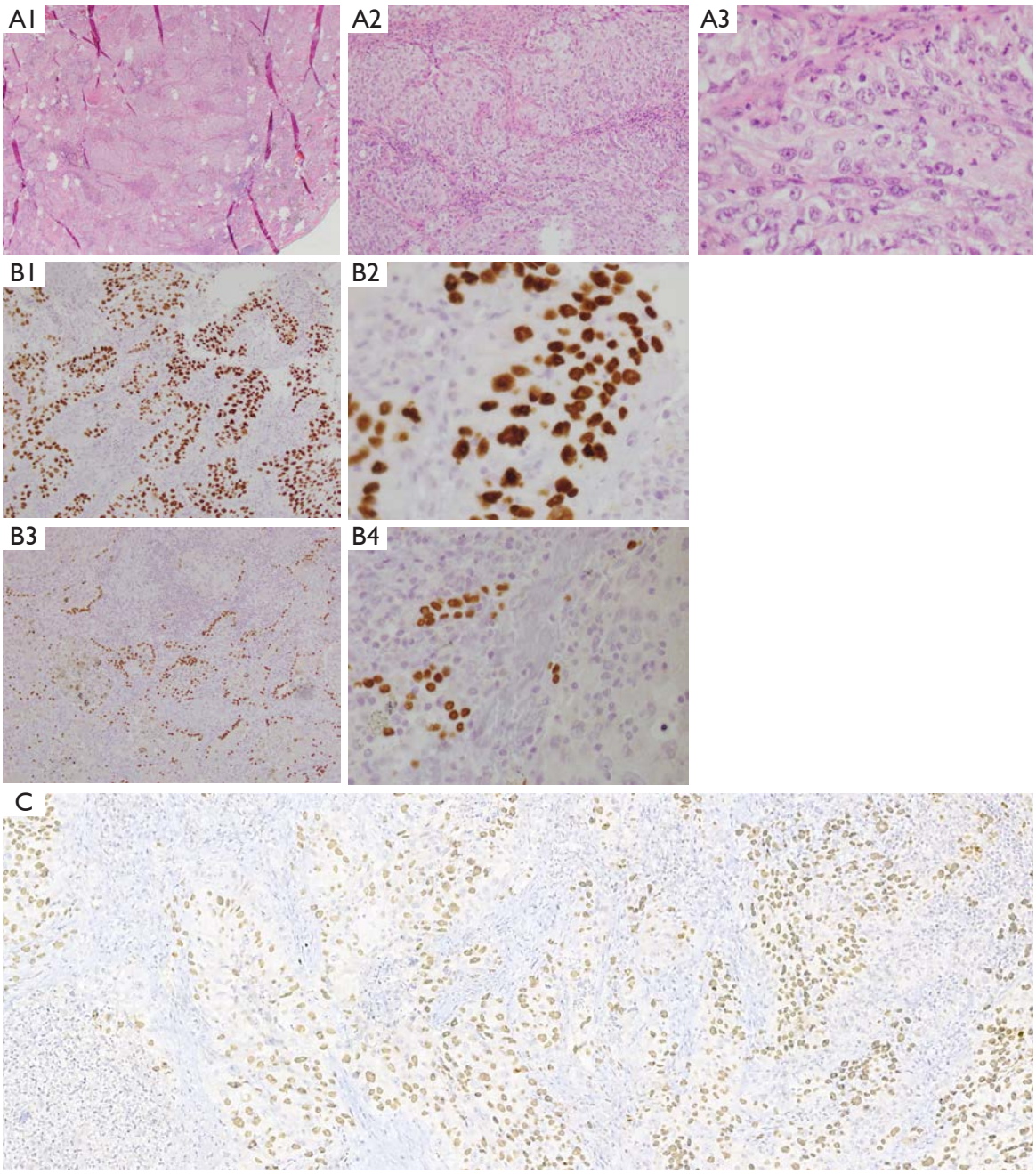

Figure S11 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 10. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 10. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

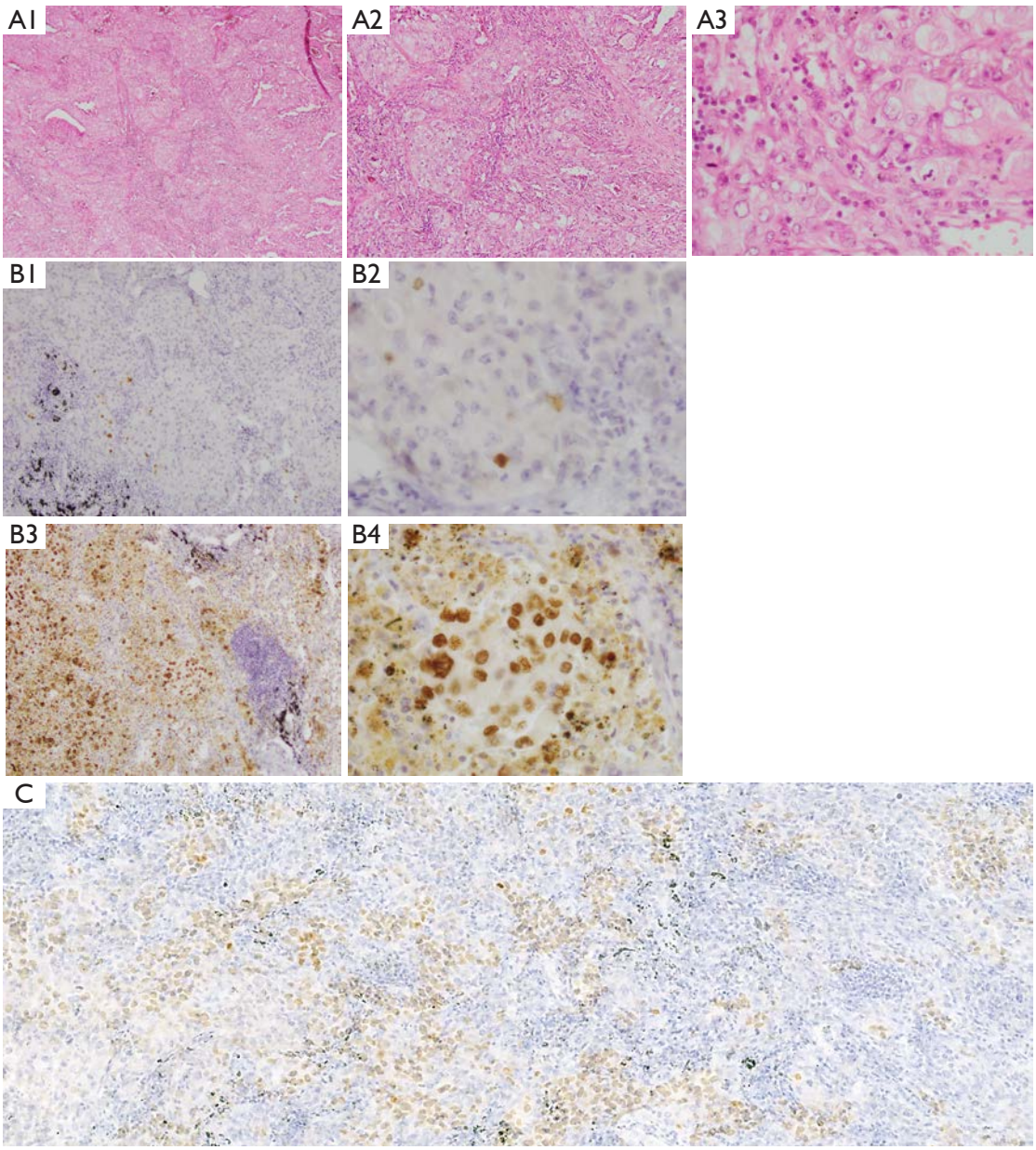

Figure S12 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 11. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 11. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

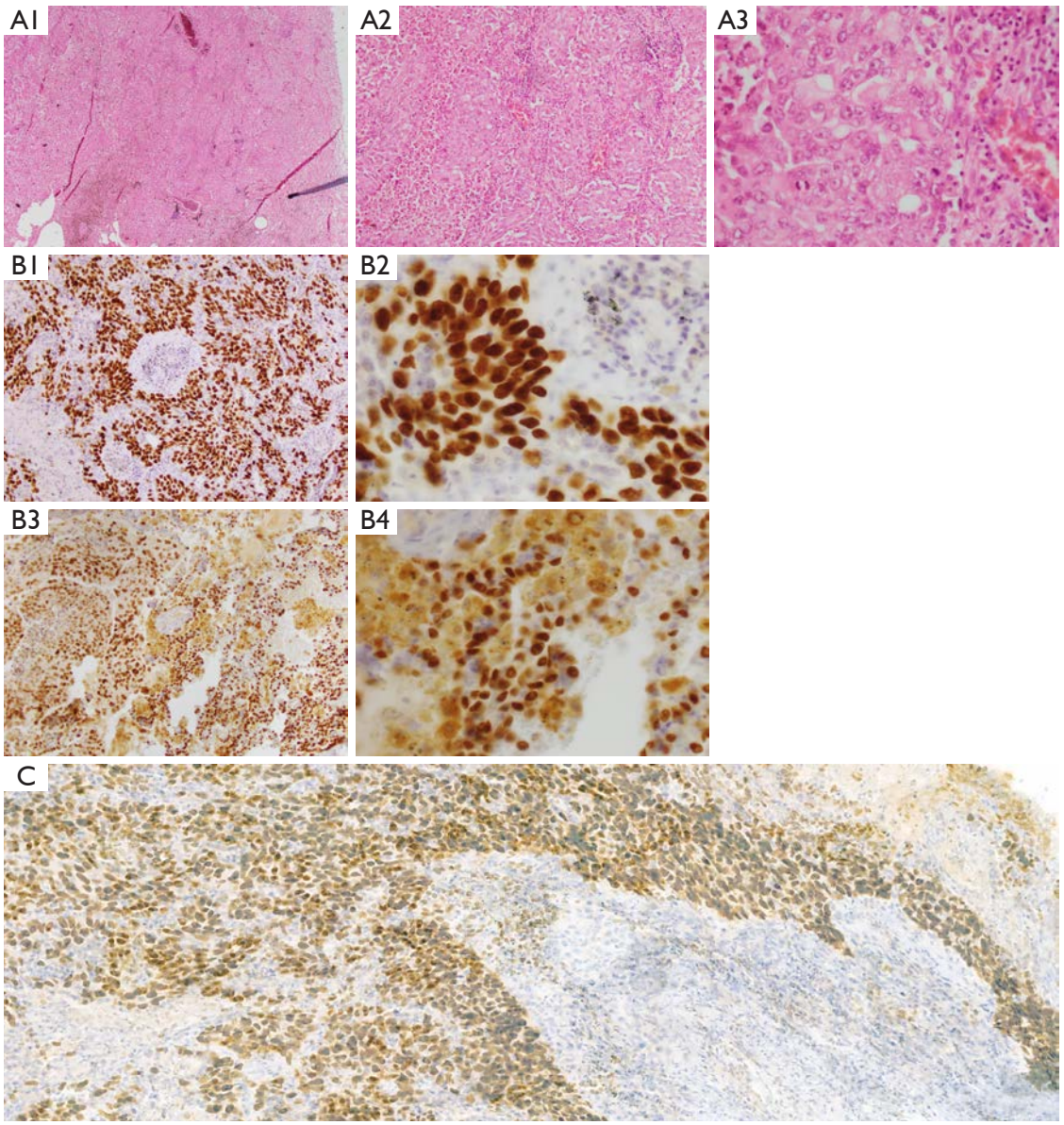

Figure S13 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 12. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 12. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

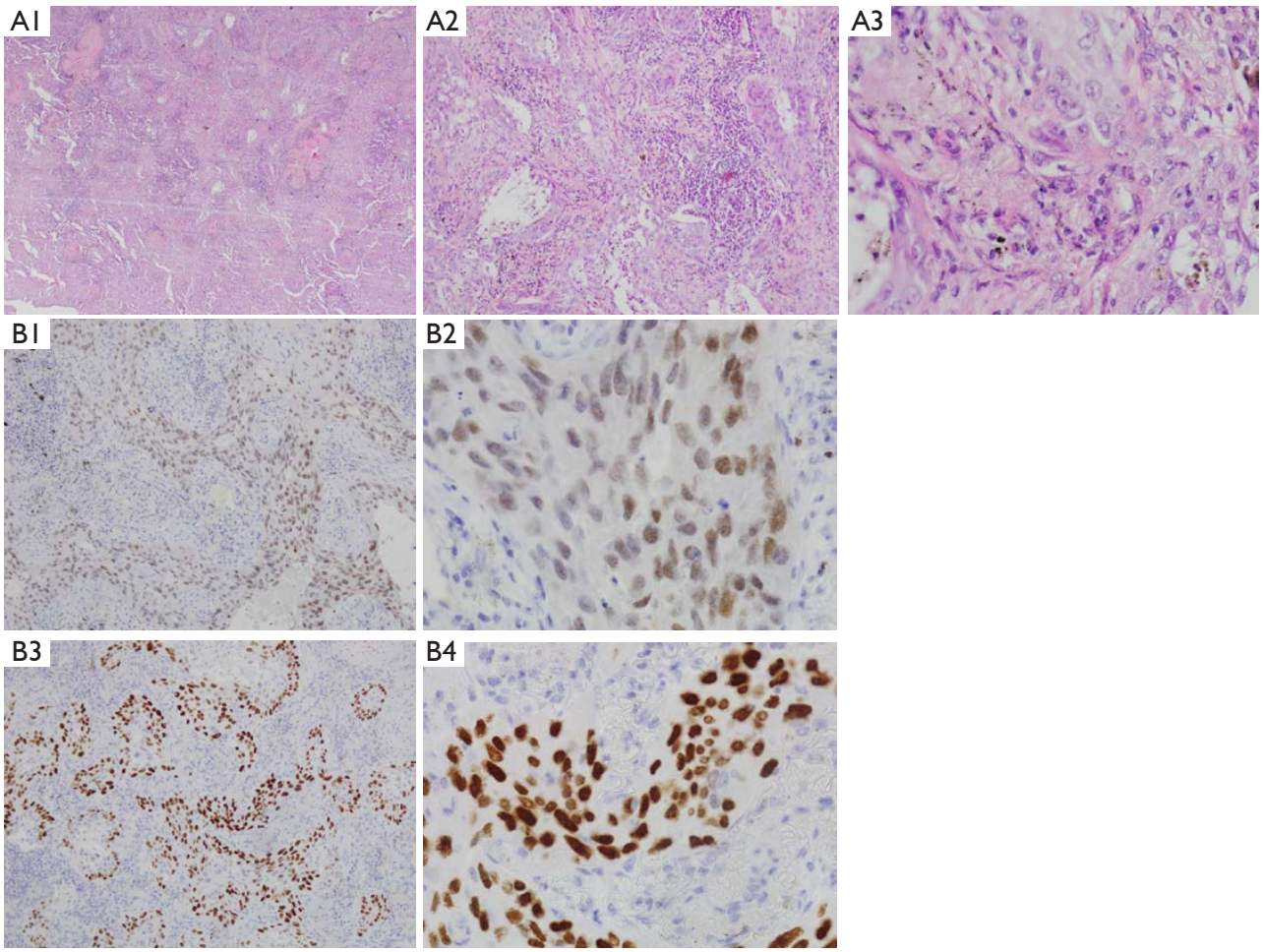

Figure S14 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 13. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4).

https://dx.doi.org/10.21037/tlcr-2025-690

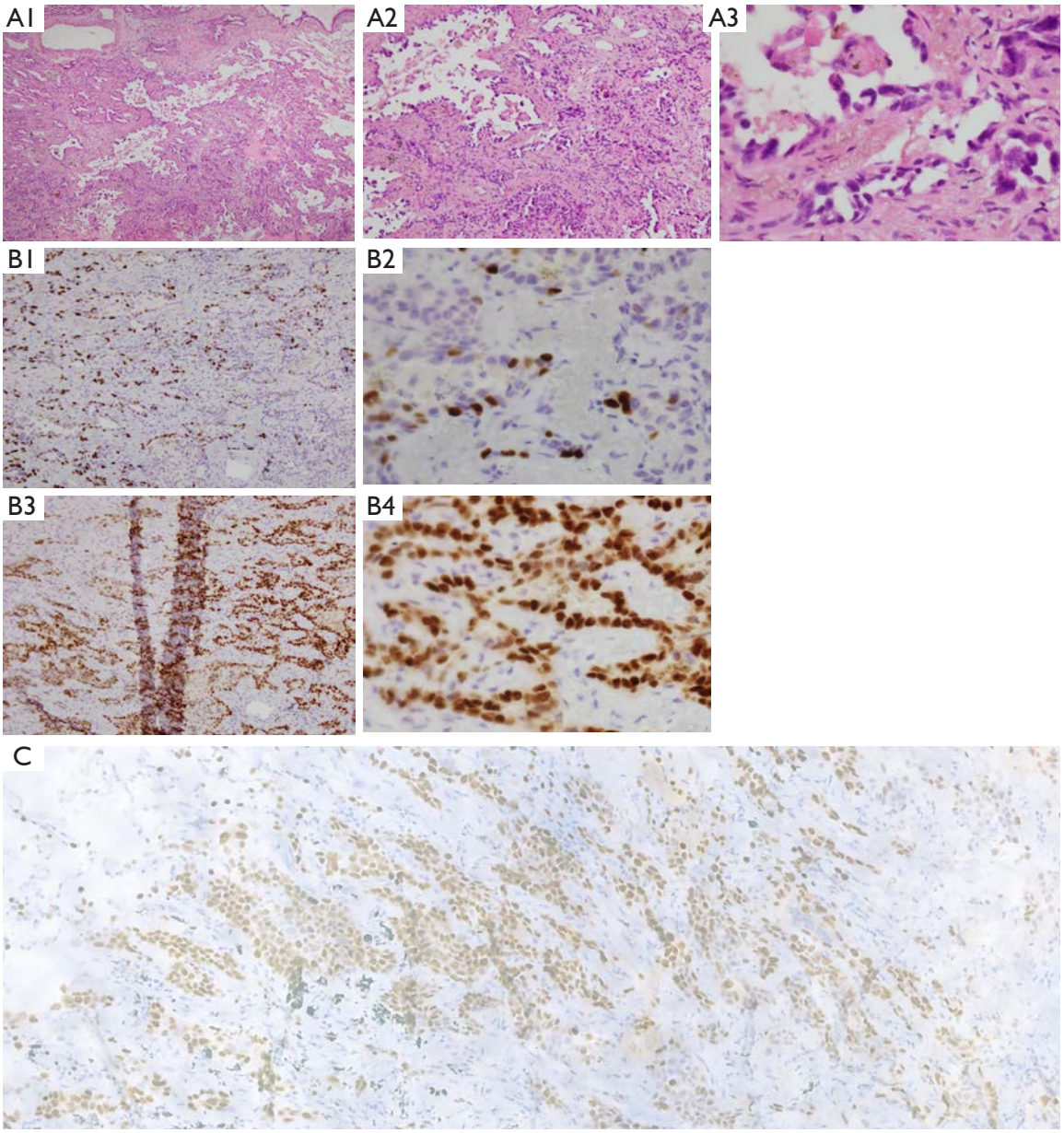

Figure S15 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 14. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 14. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

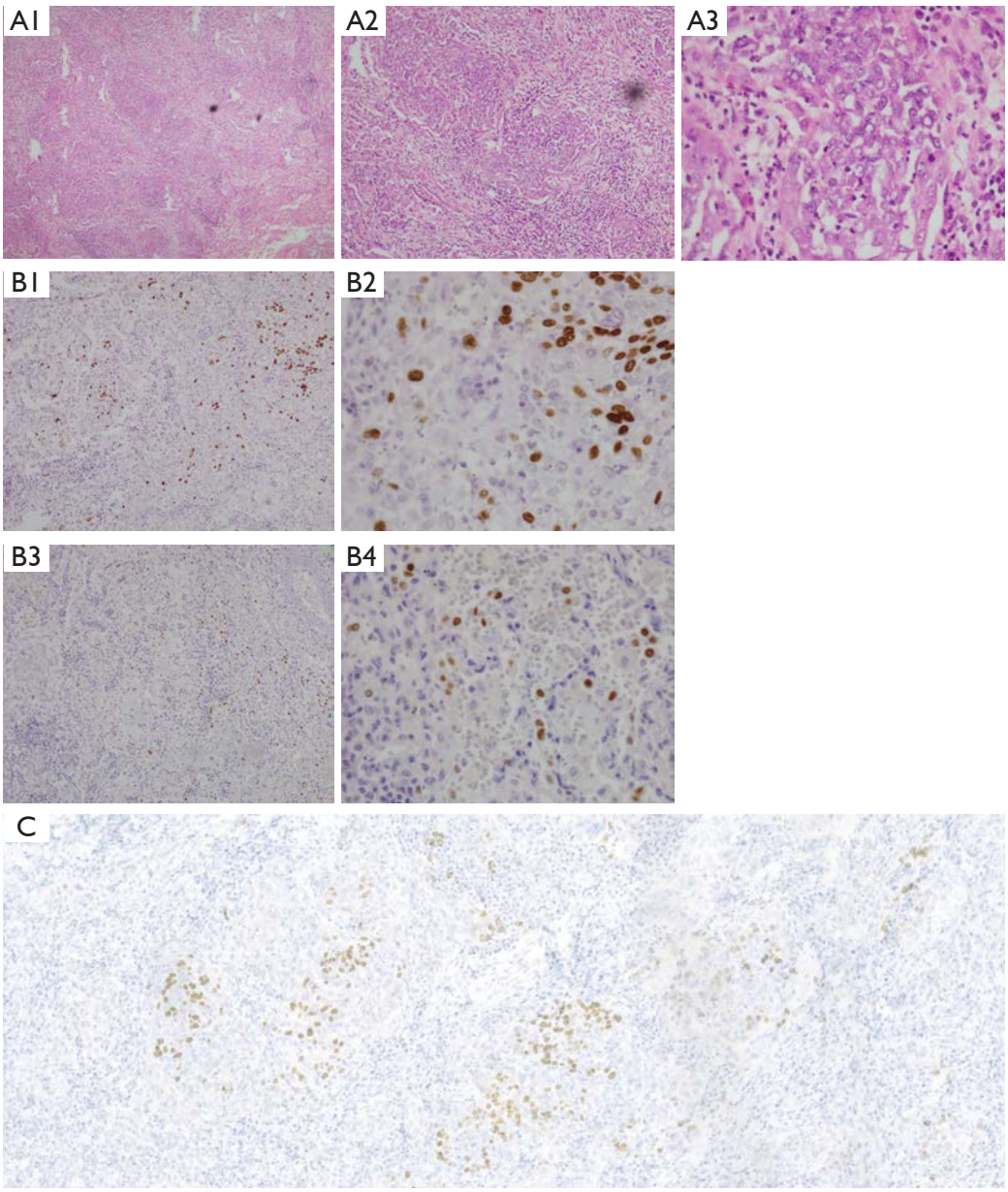

Figure S16 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 15. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 15. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

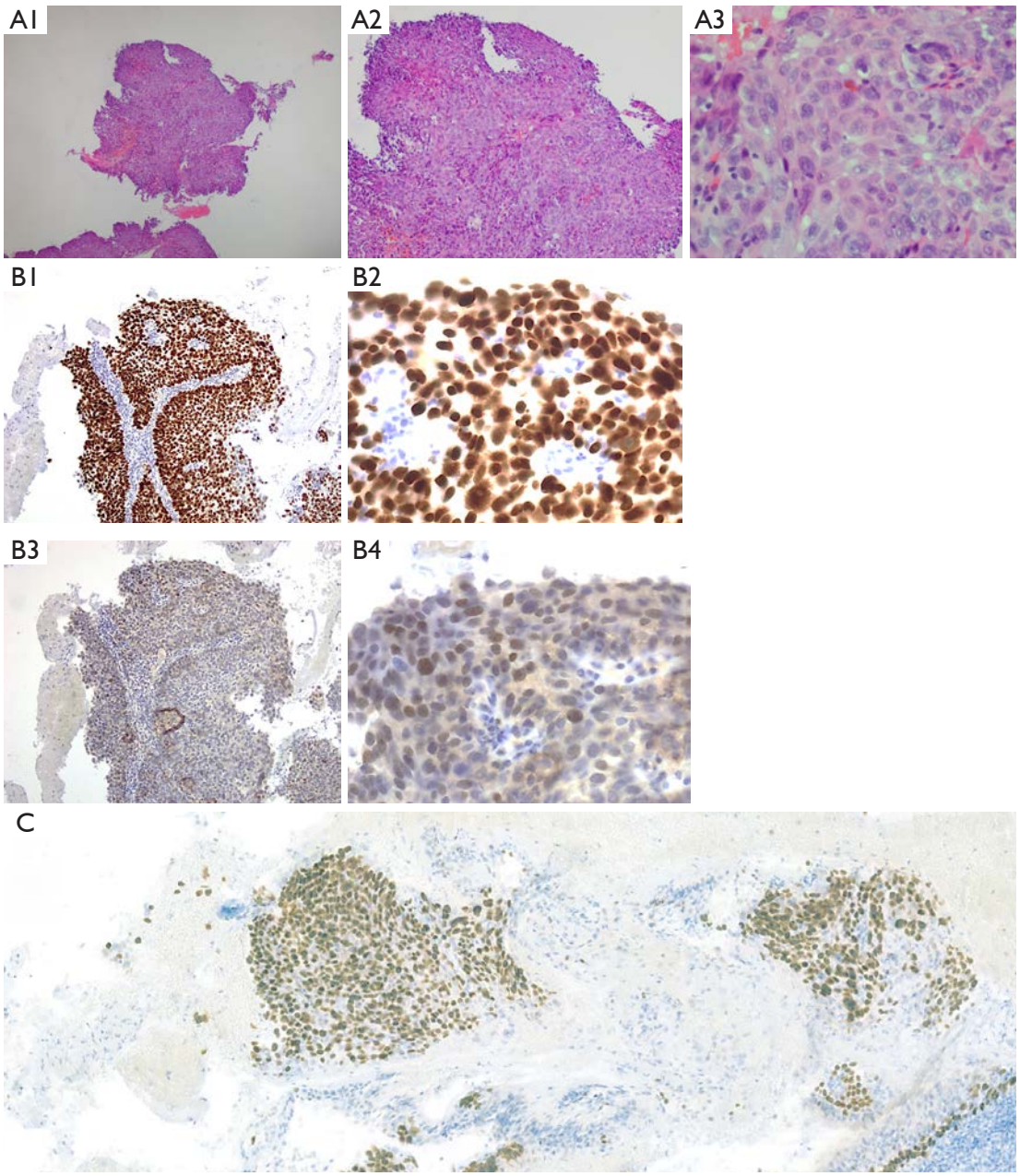

Figure S17 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 16. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 16. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

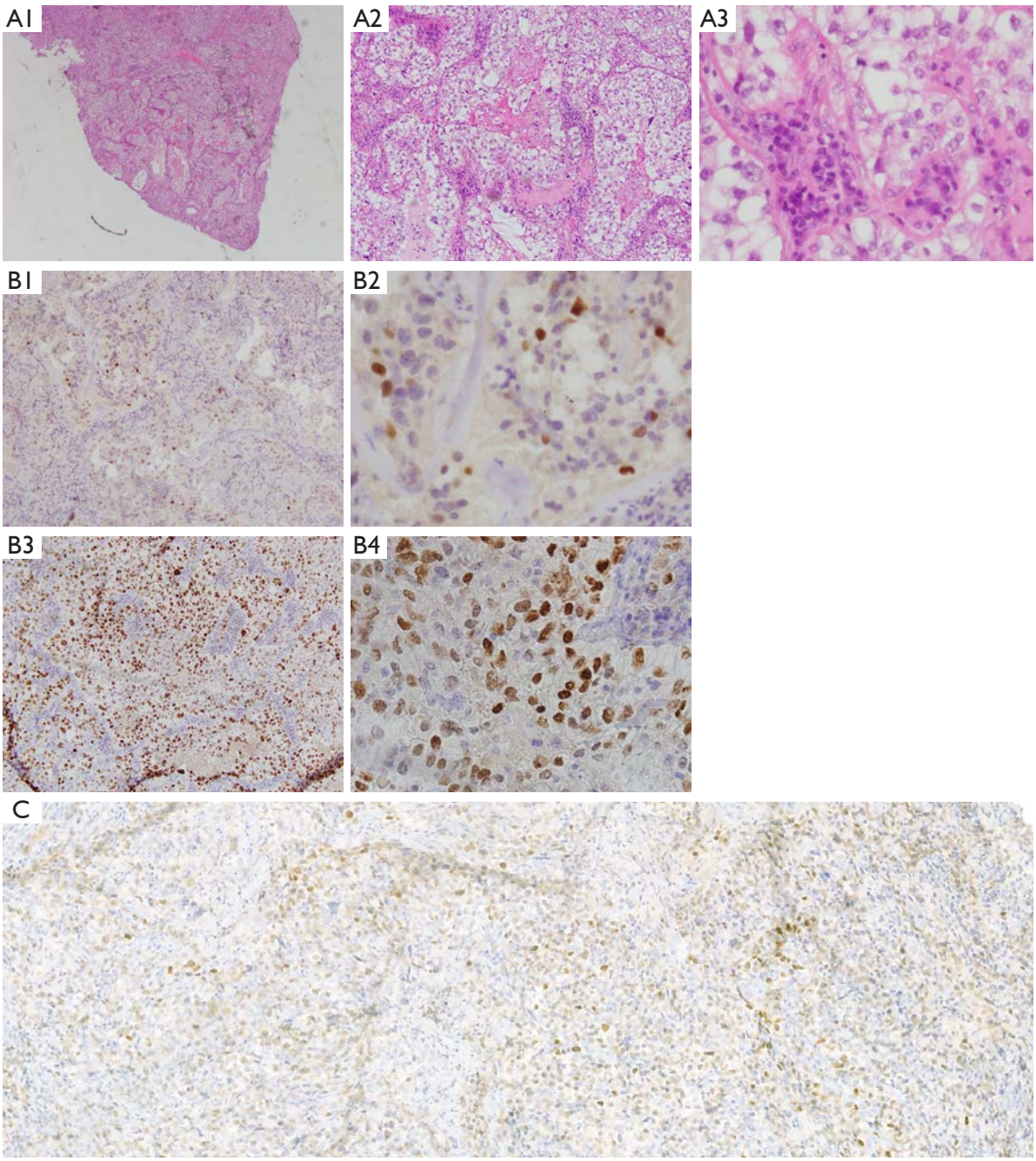

Figure S18 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 17. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 17. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

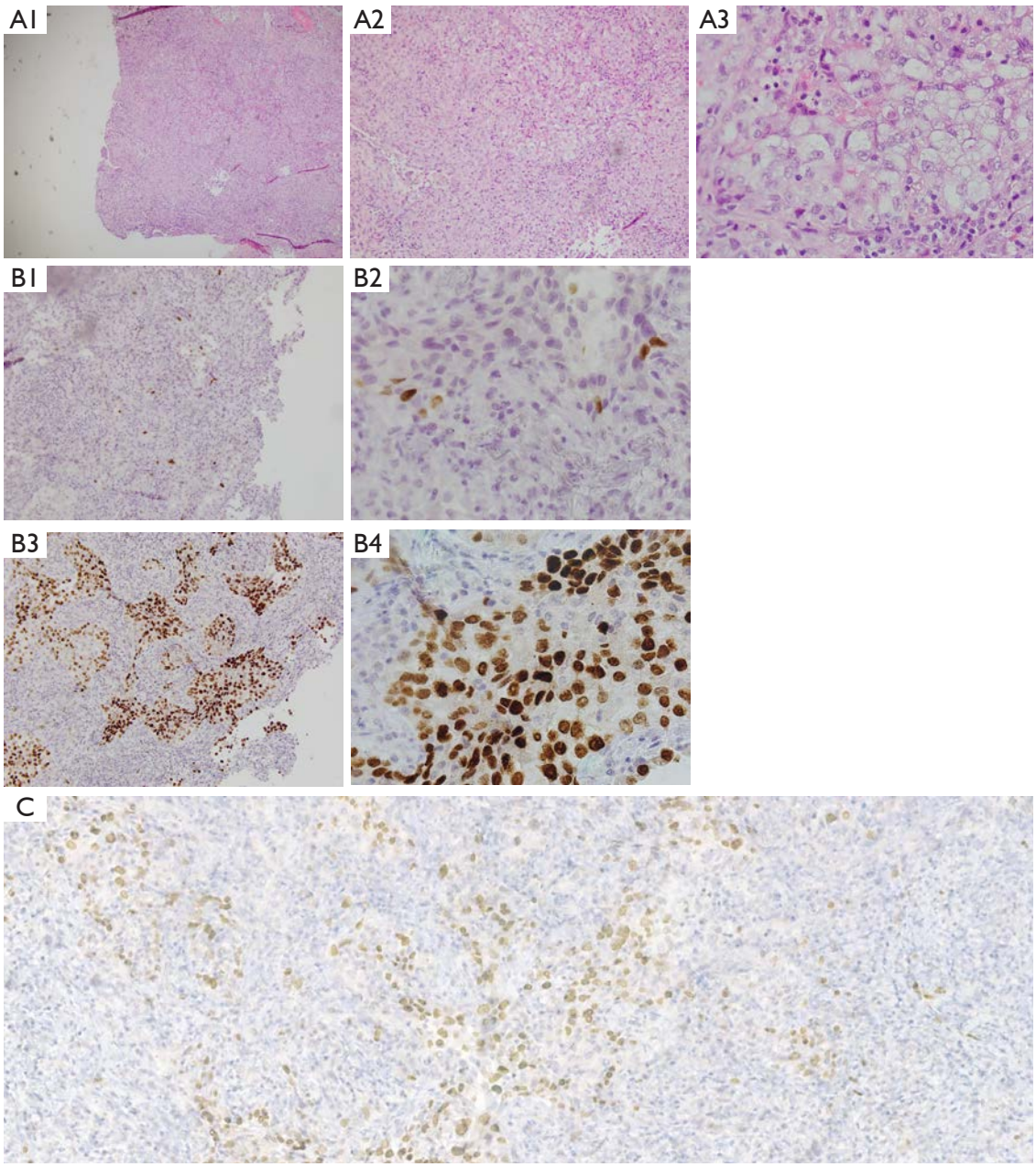

Figure S19 Representative histological and immunohistochemical images of p40/TTF-1 double-expressing NSCLC case No. 18. (A) Hematoxylin and eosin (H&E) histological images at 25×, 100×, and 400× magnification (panels A1–A3). (B) Immunohistochemical images of p40 at 100× and 400× magnification (panels B1,B2), and TTF-1 at 100× and 400× magnification (panels B3,B4). (C) Merged immunohistochemical images (SlideViewer, 3DHistech) showing overlay of p40 and TTF-1 stains in case No. 18. The software enables precise localization of individual cells within the same tissue region, highlighting tumor cells with clear co-expression of both markers.

https://dx.doi.org/10.21037/tlcr-2025-690

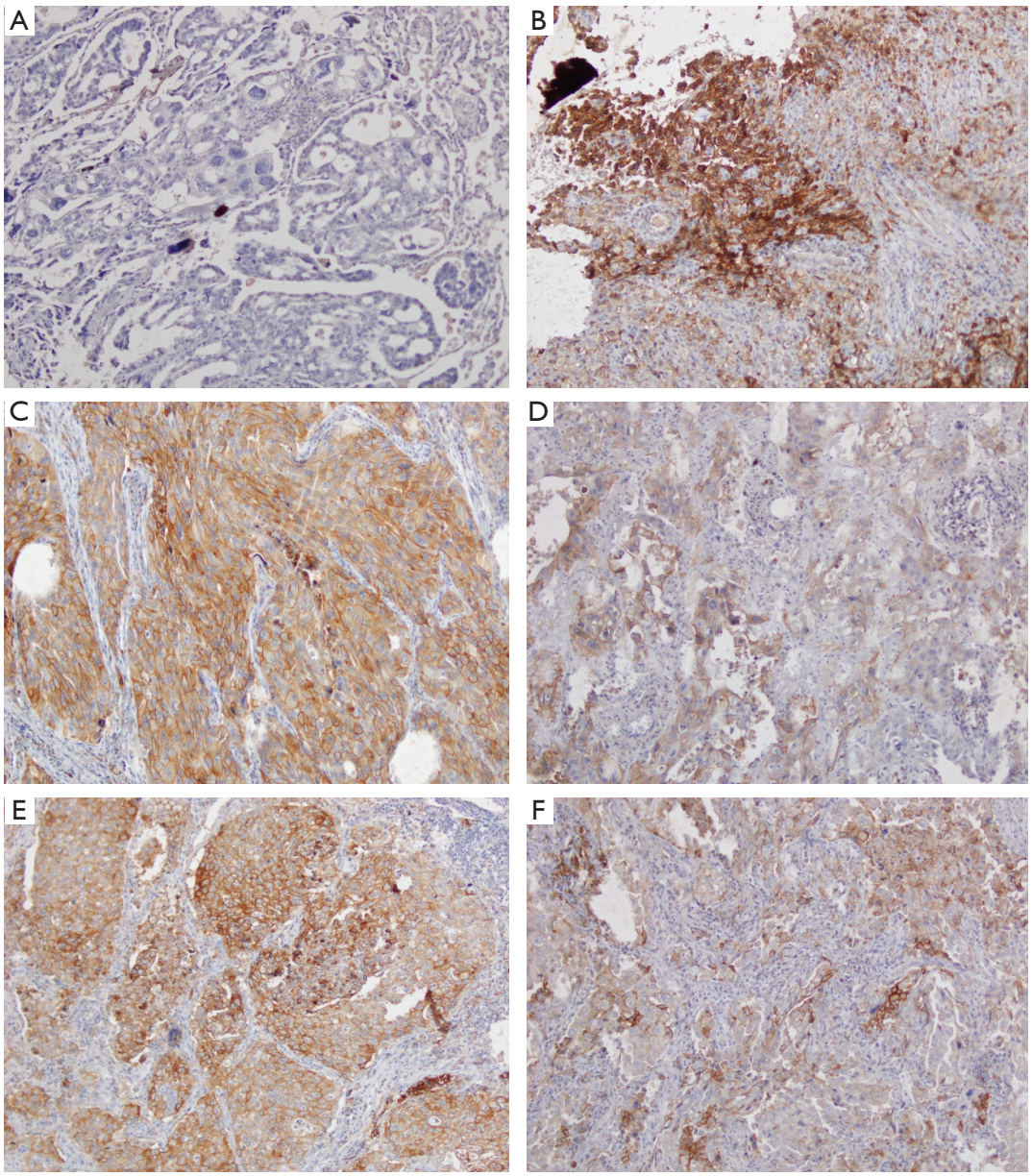

Figure S20 Examples of PD-L1 staining in selected patients at 100× magnification: (A) Patient No. 1 (TPS =0%, CPS =0). (B) Patient No. 18 (TPS =10%, CPS =10). (C) Patient No. 3 (TPS =20%, CPS =30). (D) Patient No. 6 (TPS =90%, CPS =100). (E) Patient No. 7 (TPS =30%, CPS =60). (F) Patient No. 17 (TPS =40%, CPS =50).

https://dx.doi.org/10.21037/tlcr-2025-690