

Article

Cell Populations Expressing Stemness-Associated Markers in Lung Adenocarcinoma

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Supplementary Materials

Table S1. Patient characteristics and smoking status.

| Patient | Age (years) | Gender | Ethnicity | Smoking Status |
|---------|-------------|--------|----------------|----------------|
| 1 | 49 | Male | NZ European | Current smoker |
| 2 | 67 | Female | NZ European | Ex-smoker |
| 3 | 75 | Male | NZ European | Non-smoker |
| 4 | 77 | Female | Pacific Island | Ex-smoker |
| 5 | 62 | Female | NZ European | Non-smoker |
| 6 | 77 | Female | NZ European | Ex-smoker |
| 7 | 58 | Male | Māori | Ex-smoker |
| 8 | 72 | Male | Pacific Island | Non-smoker |
| 9 | 66 | Female | Māori | Current smoker |
| 10 | 67 | Male | NZ European | Non-smoker |
| 11 | 50 | Female | NZ European | Current smoker |
| 12 | 69 | Male | NZ European | Non-smoker |

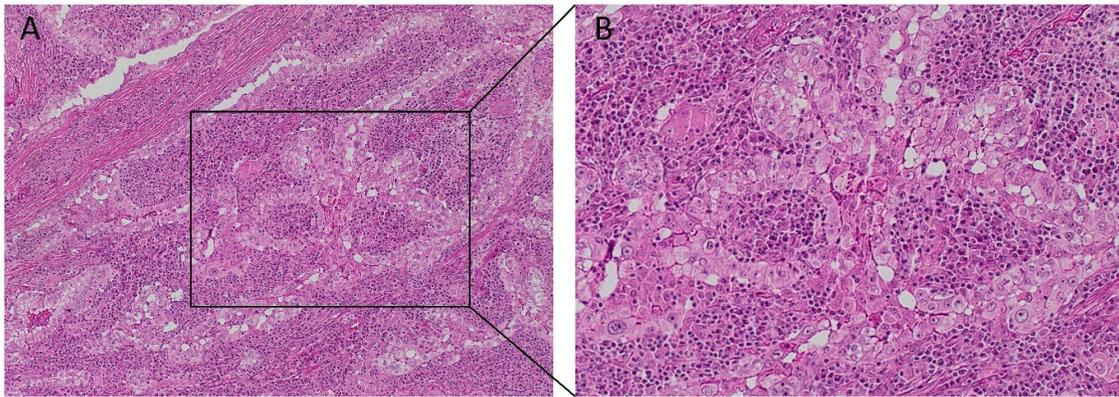


Figure S1. Representative hematoxylin and eosin images of lung adenocarcinoma demonstrating the tumor glands surrounded by stroma. Nuclei were counterstained with hematoxylin (A, B, blue). Original magnifications: 100× (A) and 400× (B).

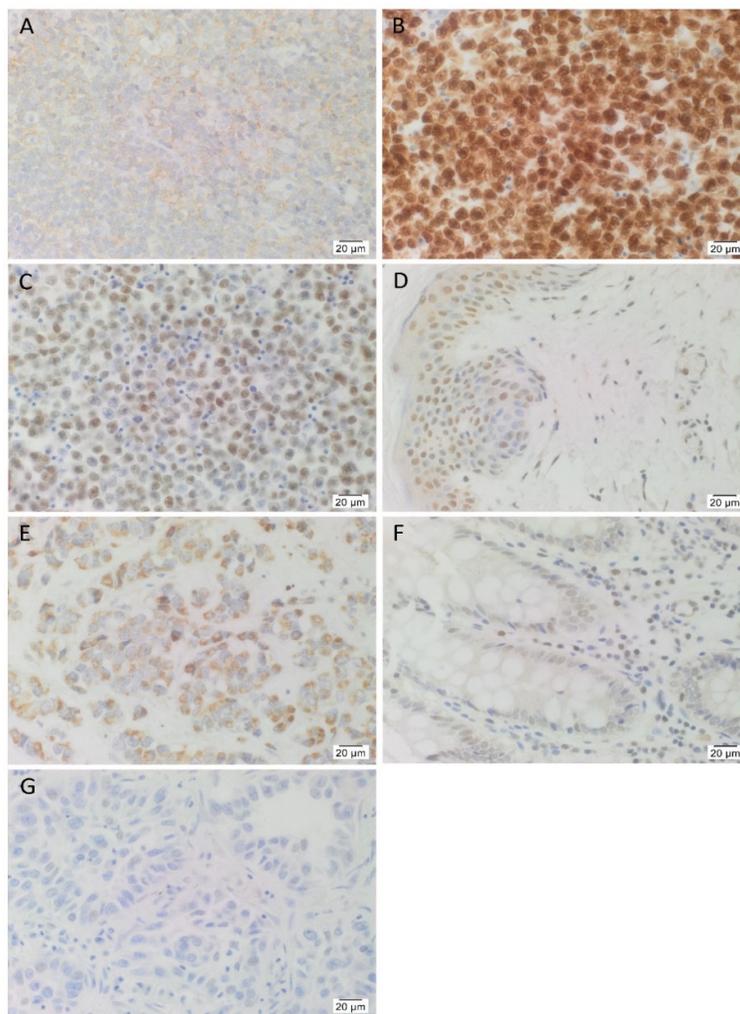
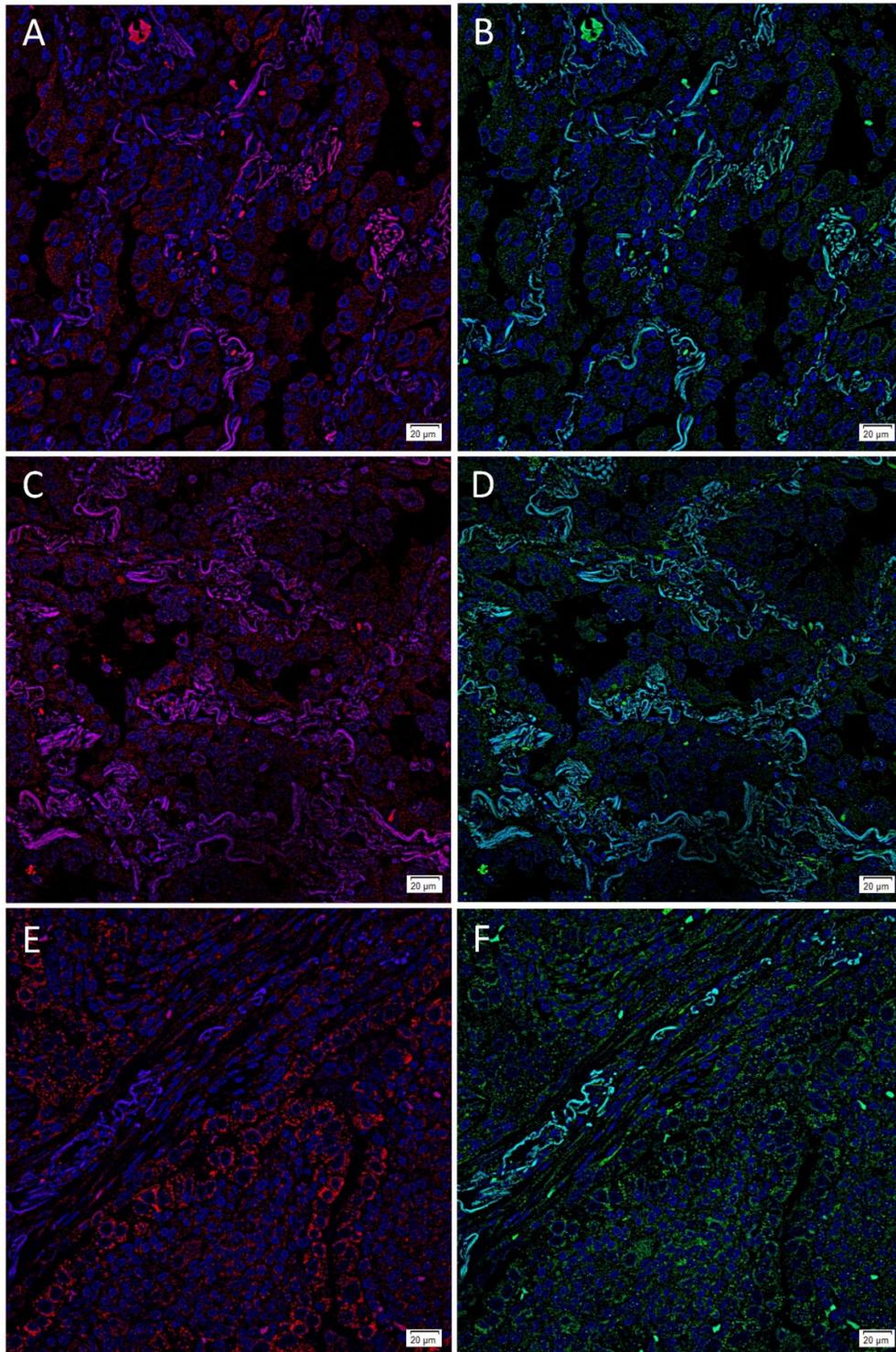
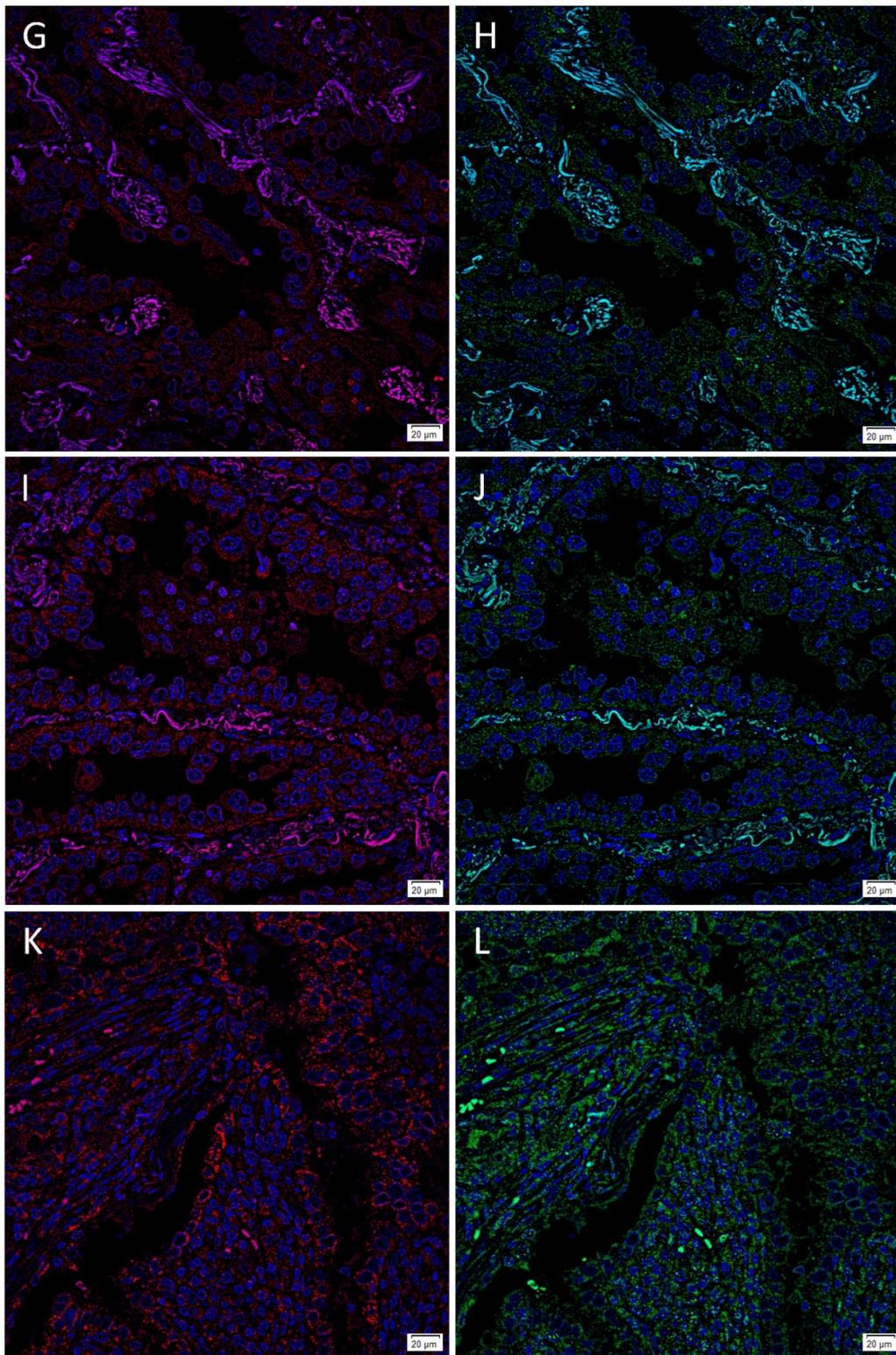


Figure S2. Representative positive control immunohistochemical-stained images demonstrating the expression of CD44 (A, brown) in tonsil, OCT4, (B, brown) and NANOG (C, brown) in seminoma, SOX2 (D, brown) in skin epidermis, KLF4 (E, brown) in breast carcinoma, and c-MYC (F, brown) in normal colon mucosa. A negative control (G). Nuclei were counterstained with hematoxylin (A-G, blue). Original magnification: 400×.





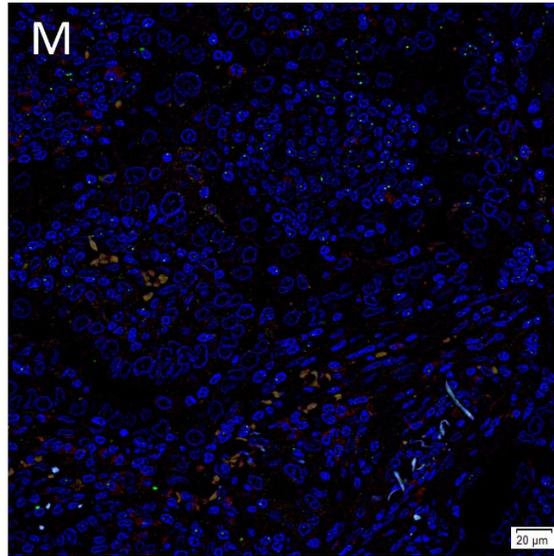


Figure S3. Split immunofluorescence-stained images shown in Figure 3 demonstrating the expression of NANOG (A, red) and OCT4 (B, green); SOX2 (C, red) and OCT4 (D, green); KLF4 (E, red) and OCT4 (F, green); NANOG (G, red) and c-MYC (H, green); SOX2 (I, red) and c-MYC (J, green); KLF4 (K, red) and c-MYC (L, green). A negative control (M). Cell nuclei were counterstained with 4',6 diamidino-2-phenylindole (A-M, blue). Original magnification: 400×.

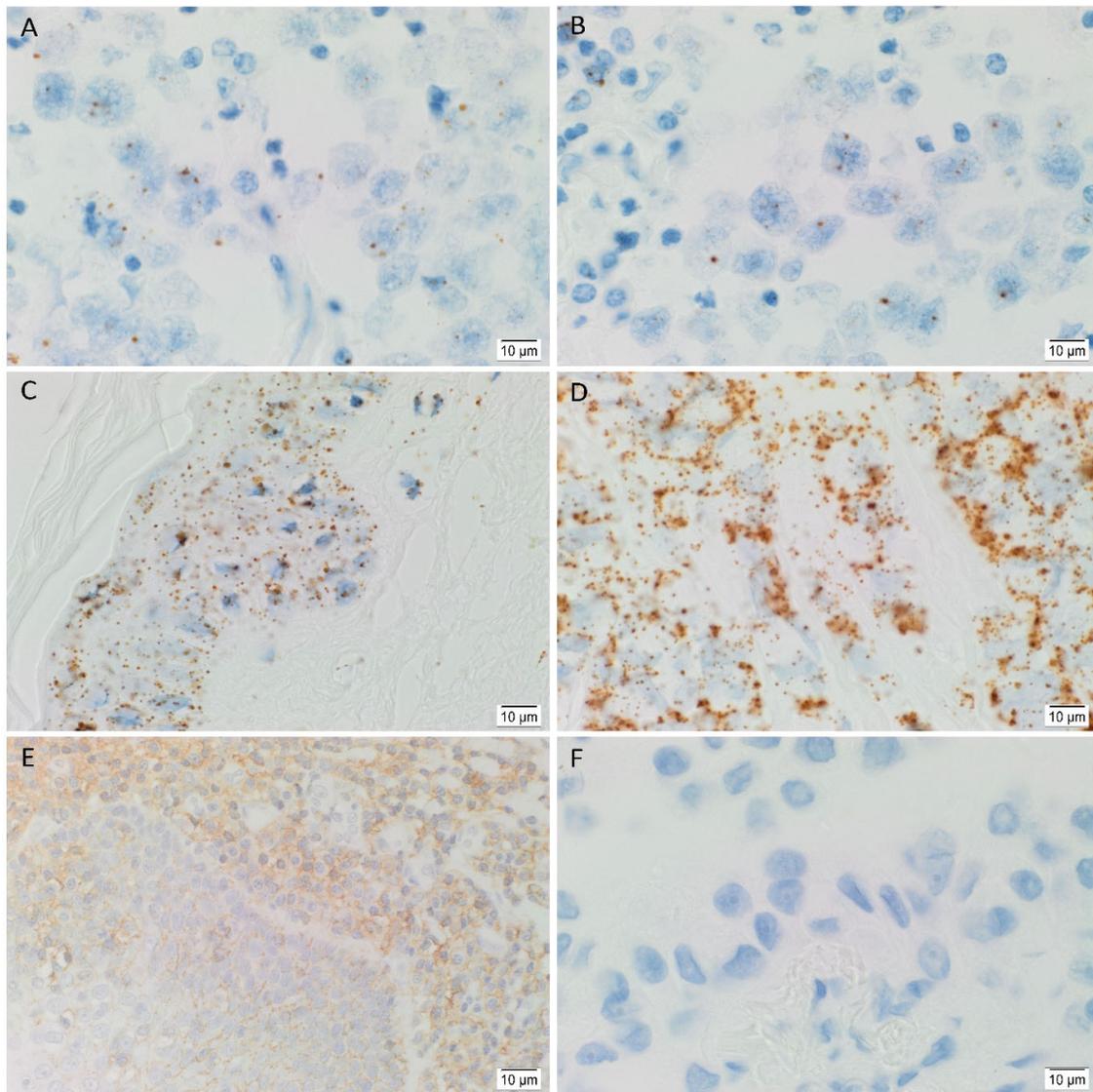


Figure S4. Representative *in situ* hybridization positive controls for OCT4 (A, brown) and NANOG (B, brown) in seminoma, SOX2 (C, brown) in normal skin, KLF4 (D, brown) breast carcinoma and c-MYC (E, brown) in normal prostatic tissue. Negative controls (F) performed on sections of lung adenocarcinoma tissue samples confirmed specificity of secondary antibody. Nuclei were counterstained with hematoxylin (A-F, blue). Original magnification: 1000×.

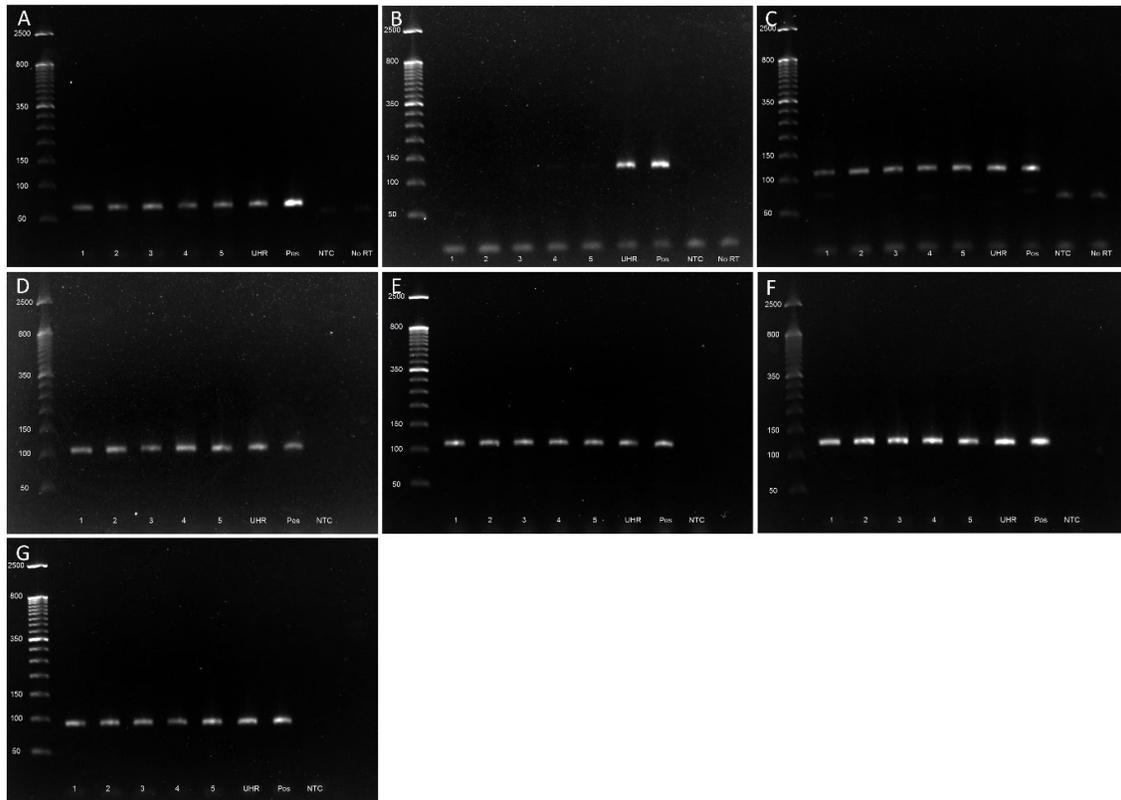


Figure S5. RT-qPCR amplification products from five lung adenocarcinoma-derived primary cell lines. The specificity of the primer probes was confirmed by running qPCR amplification products on agarose gels; OCT4 (A, 64bp), SOX2 (B, 121bp), NANOG (C, 91bp), KLF4 (D, 110bp) and c-MYC (E, 107bp). The reference genes GAPDH (F, 122bp) and PUM1 (G, 89bp) from cell lines were also checked. Ladder refers to the DNA marker in base pairs (bp); Lanes 1-5 refer to the cell line samples; Pos, positive control (NTERA-2 cell lines); NTC, no template control (RNase-free water) to confirm no contamination; No RT, reverse transcriptase negative control for primers that may detect genomic DNA.

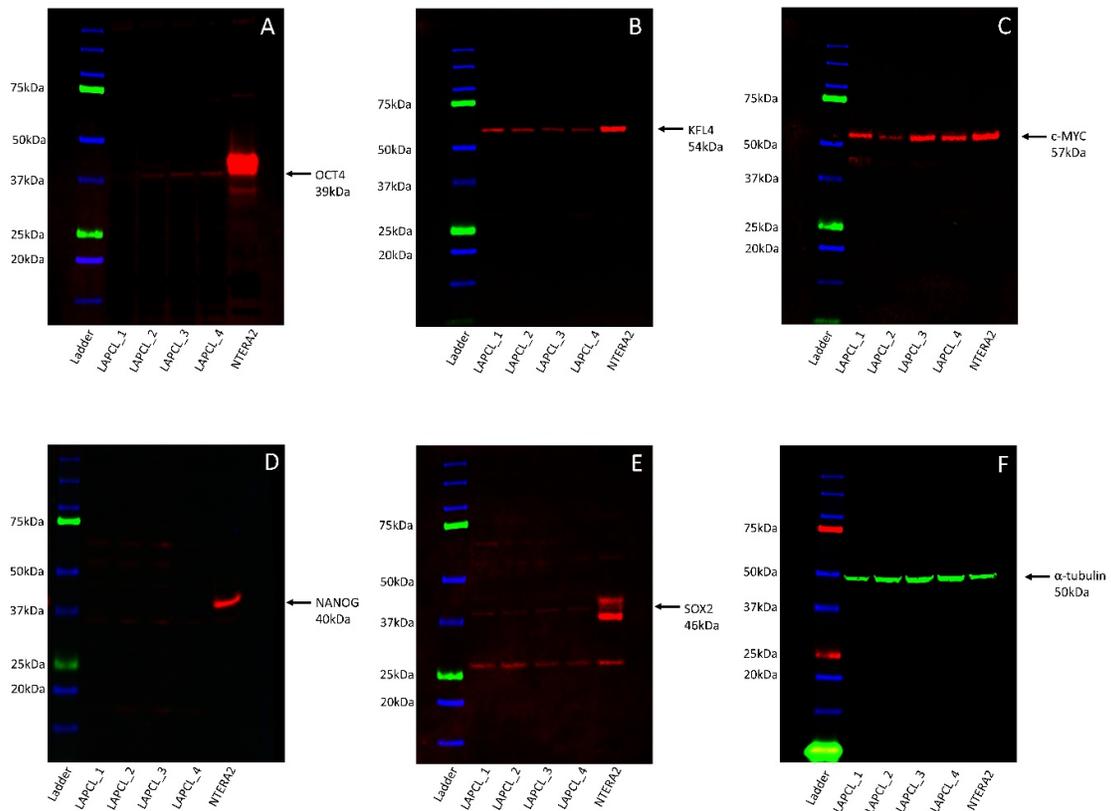


Figure S6. Full-length western blot images shown in Figure 7, of total protein extracted from four lung adenocarcinoma-derived primary cell lines demonstrating protein expression of stemness-associated markers. Arrows indicate the presence of the proteins with expected band sizes for OCT4 (A), KLF4 (B) and c-MYC (C). NANOG (D) and SOX2 (E) were below detectable levels. Bands for α-tubulin (F) confirmed approximately equal protein loading.