**ONLINE SUPPLEMENTAL MATERIAL**

**Spheroid-type amyloid deposition: comprehensive analysis using immunohistochemistry, proteomic analysis, and a literature review**

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**List of Supplemental Materials**

Supplemental Table: 1

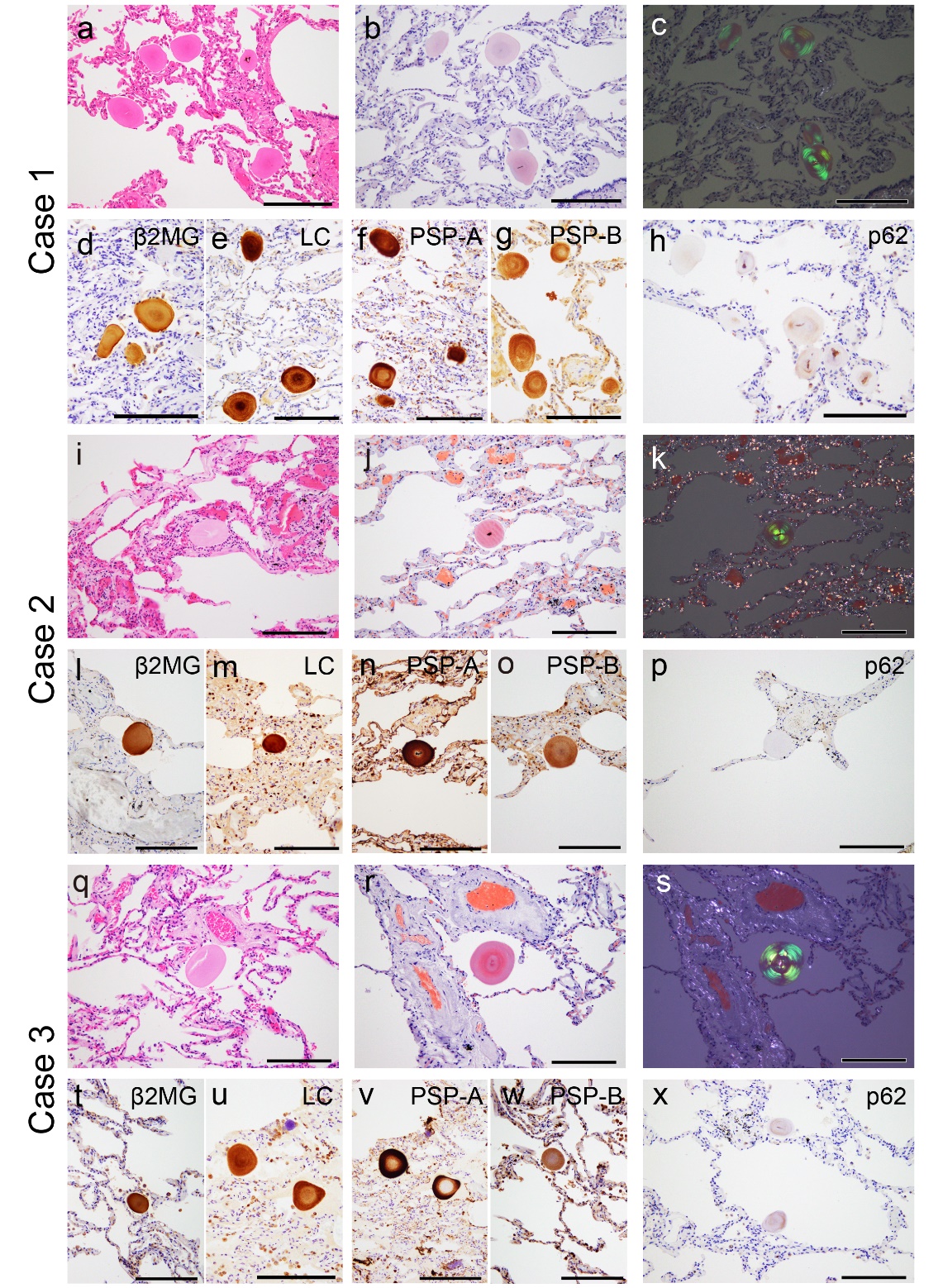
Supplementary Figure: 4

**Supplementary Table S1.** Details of the antibodies used in this study

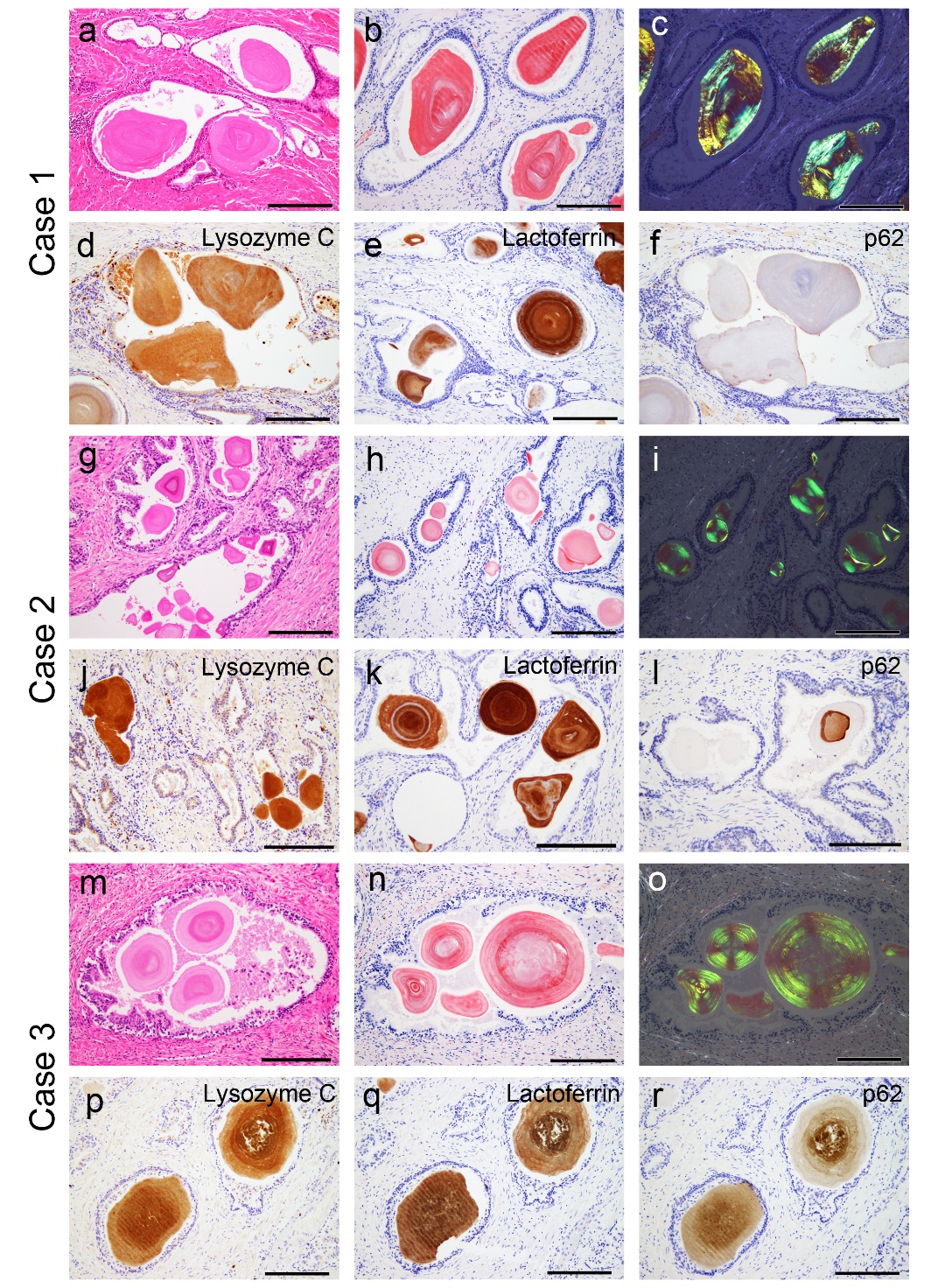
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| --- | --- | --- | --- | --- |
| **Antibody** | **Source** | **Clone** | **Dilution** | **Antigen retrieval** |
| Igλ | Abcam | polyclonal | 1:1600 | 98% FA (1 min) |
| β2-microglobulin | GeneTex | polyclonal | 1:3000 | 98% FA (1 min) |
| Lysozyme C | Santa Cruz Biotechnology | E-5 | 1:150 | 98% FA (1 min) |
| PSP-A | Abcam | 6F10 | 1:4000 | 98% FA (1 min) |
| PSP-B | Santa Cruz Biotechnology | F-2 | 1:4000 | 98% FA (1 min) |
| Lactoferrin | Santa Cruz Biotechnology | B97 | 1:150 | 98% FA (1 min) |
| Olfactomedin 4 | Abcam | polyclonal | 1:50 | Heat (20 min, pH6) |
| p62 | Biomol | polyclonal | 1:3000 | Heat (20 min, pH6) |

**Abbreviations:** FA, formic acid; PSP-A, pulmonary surfactant protein A; PSP-B, pulmonary surfactant protein B

Immunostaining was performed using the Leica Bond-IV automation and Leica Refine detection kits (Leica Biosystems, Bannockburn, IL, USA) according to the manufacturer’s instructions. All sections were counterstained with hematoxylin.

**Supplementary Figure S1**. Representative microphotographs of the pulmonary corpora amylacea (CA) in all three cases.

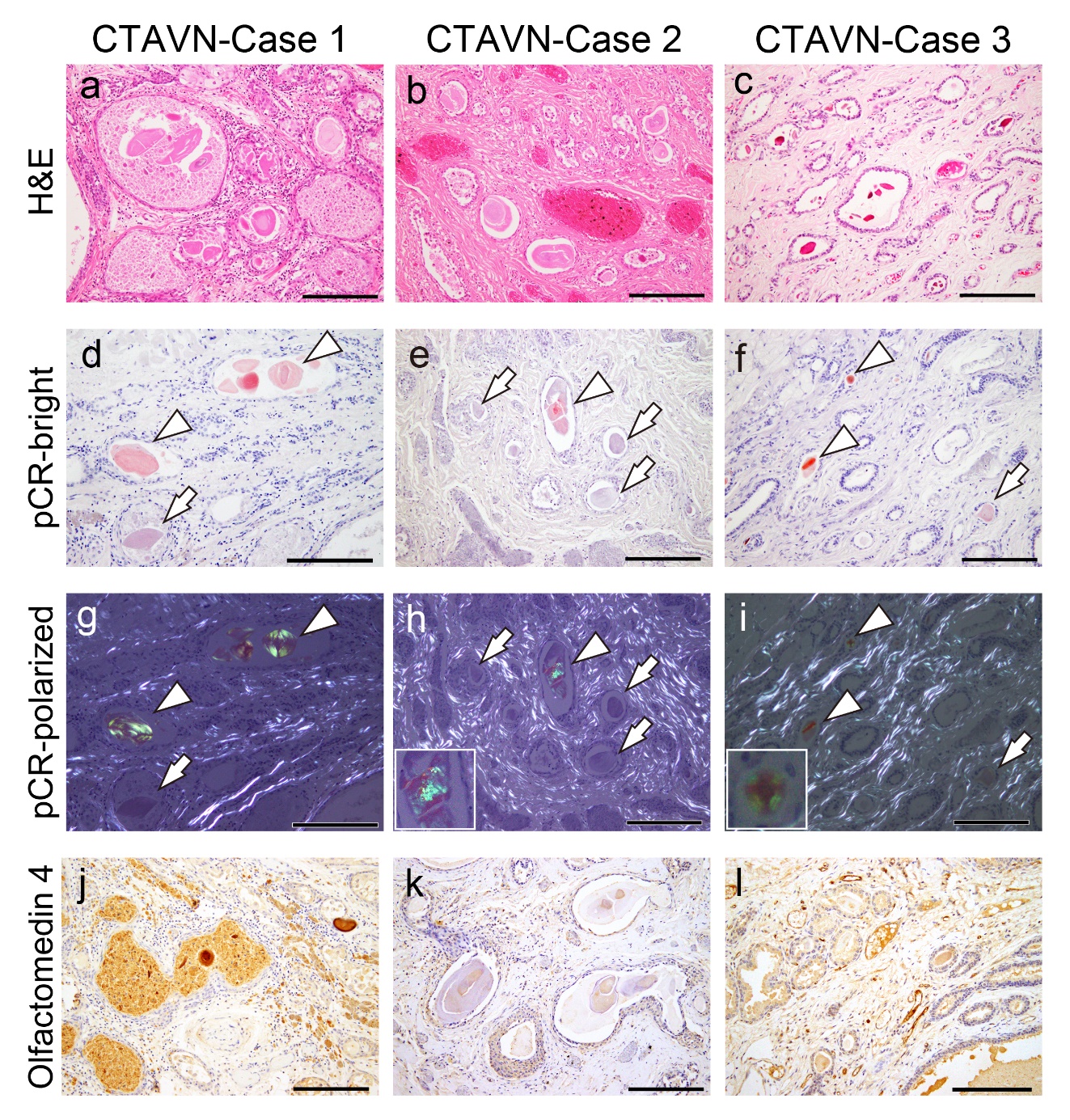
(a–h) Pulmonary CA Case 1; (i–p) Case 2; (q–x) Case 3. (a, i, q) Hematoxylin and eosin (H&E) staining; phenol Congo red (pCR) staining under bright field (b, j, r) and polarized light (c, k, s); Immunohistochemistry for β2-microglobulin (β2MG) (d, l, t); lysozyme C (LC) (e, m, u); pulmonary surfactant protein A (PSP-A) (f, n, v); PSP-B (g, o, w); and p62 (h, p, x). In all cases, CAs were round and eosinophilic deposits showed concentric laminations by H&E staining. In addition, all CAs exhibited congophilic and clear apple-green birefringence under polarized light. Furthermore, all CAs showed immunoreactivity for β2MG, LC, PAP-A, and PSP-B. In contrast, immunoreactivity for p62 was weak and focal in Cases 1 and 3, whereas it was almost negative in Case 2. Scale bar = 200 μm (a–x).

**Supplementary Figure S2.** Representative microphotographs of prostatic CA in all three patients.

(a–f) Prostatic CA Case 1; (g–l) Case 2; and (m–r) Case 3. (a, g, m) H&E staining; pCR staining under bright field (b, h, n) and polarized light (c, i, o); Immunohistochemistry for lysozyme C (d, j, p); lactoferrin (e, k, q); and p62 (f, l, r). For all cases, the CAs were round and eosinophilic deposits showed concentric laminations by H&E staining. In addition, all CAs exhibited congophilic and clear apple-green birefringence under polarized light. Furthermore, all CAs showed immunoreactivity for lysozyme C and lactoferrin. Immunoreactivity for p62 was moderate in Case 3, but weak and focal in Cases 1 and 2. Scale bar = 200 μm (a–r).

**Supplementary Figure S3.** Proteomic analysis results for PSP-A and PSP-B in pulmonary CA lesions in Case 1.

(a) PSP-A and (b) PSP-B. Detected peptides with peptide scores of 30 or higher in the MASCOT analysis are shown in red.

**Supplementary Figure S4.** Representative microphotographs of cystic tumors of the atrioventricular node (CTAVN)-associated CA in all three cases.

(a, d, g, j) Prostatic CA Case 1; (b, e, h, k) Case 2; (c, f, i, l) Case 3. (a–c) H&E staining; pCR staining under bright field (d–f) and polarized light (g–i); (j–l) Immunohistochemistry for olfactomedin 4. For all cases, the CAs were round and eosinophilic deposits showed concentric laminations by H&E staining. For pCR staining, the CAs showed very weak (arrow) to moderate congophilia (arrowhead) (d–f), and those exhibiting moderate congophilia showed apple-green birefringence under polarized light (arrowhead) (g–i). Immunoreactivity of the CAs to olfactomedin 4 was strong in Case 1 (j), negative to weakly positive in Case 2 (k), and weakly positive in Case 3 (l). Scale bar = 200 μm (c–e)