Comparative Proteomics of Paired Vocal Fold and Oral Mucosa Fibroblasts

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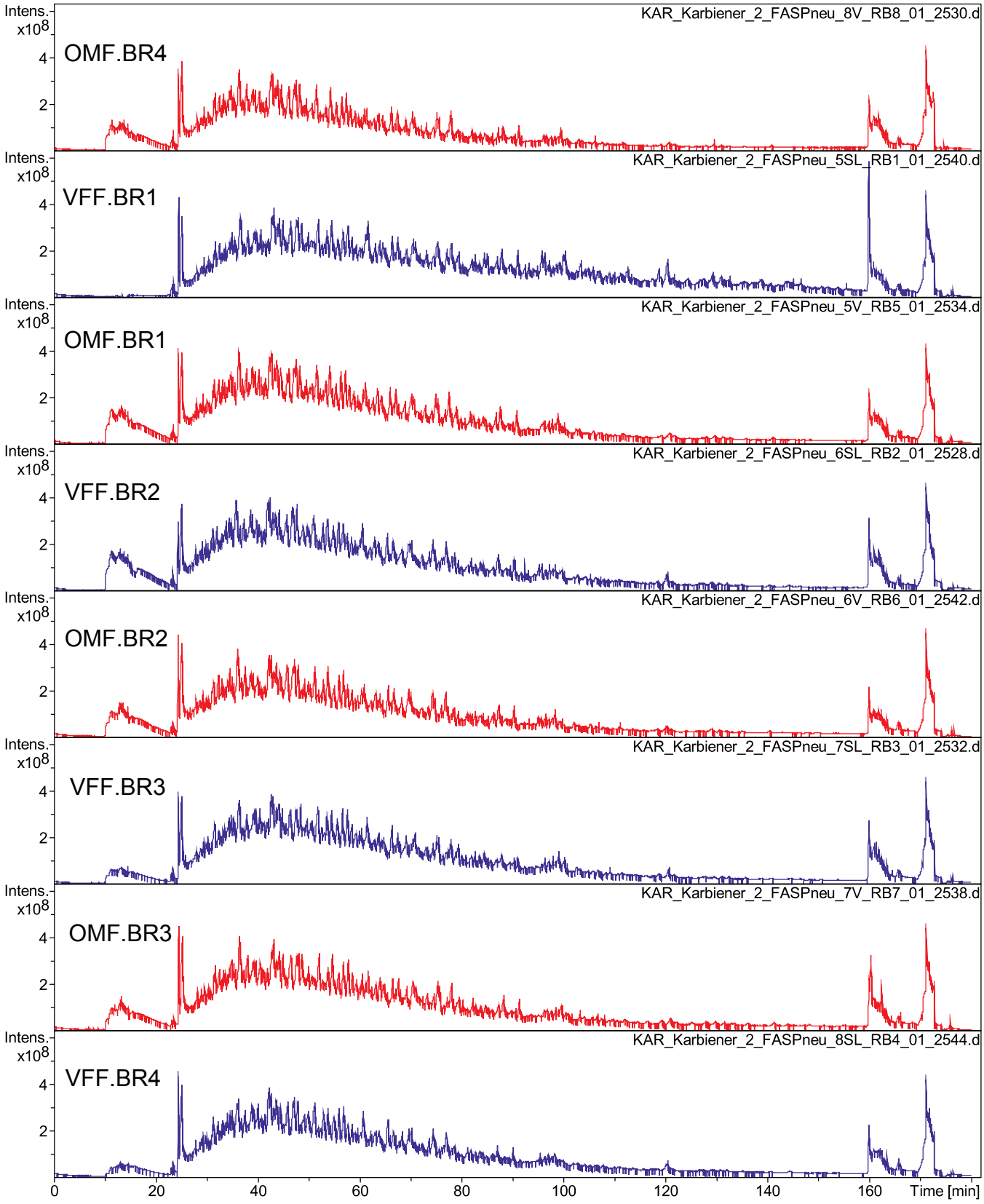
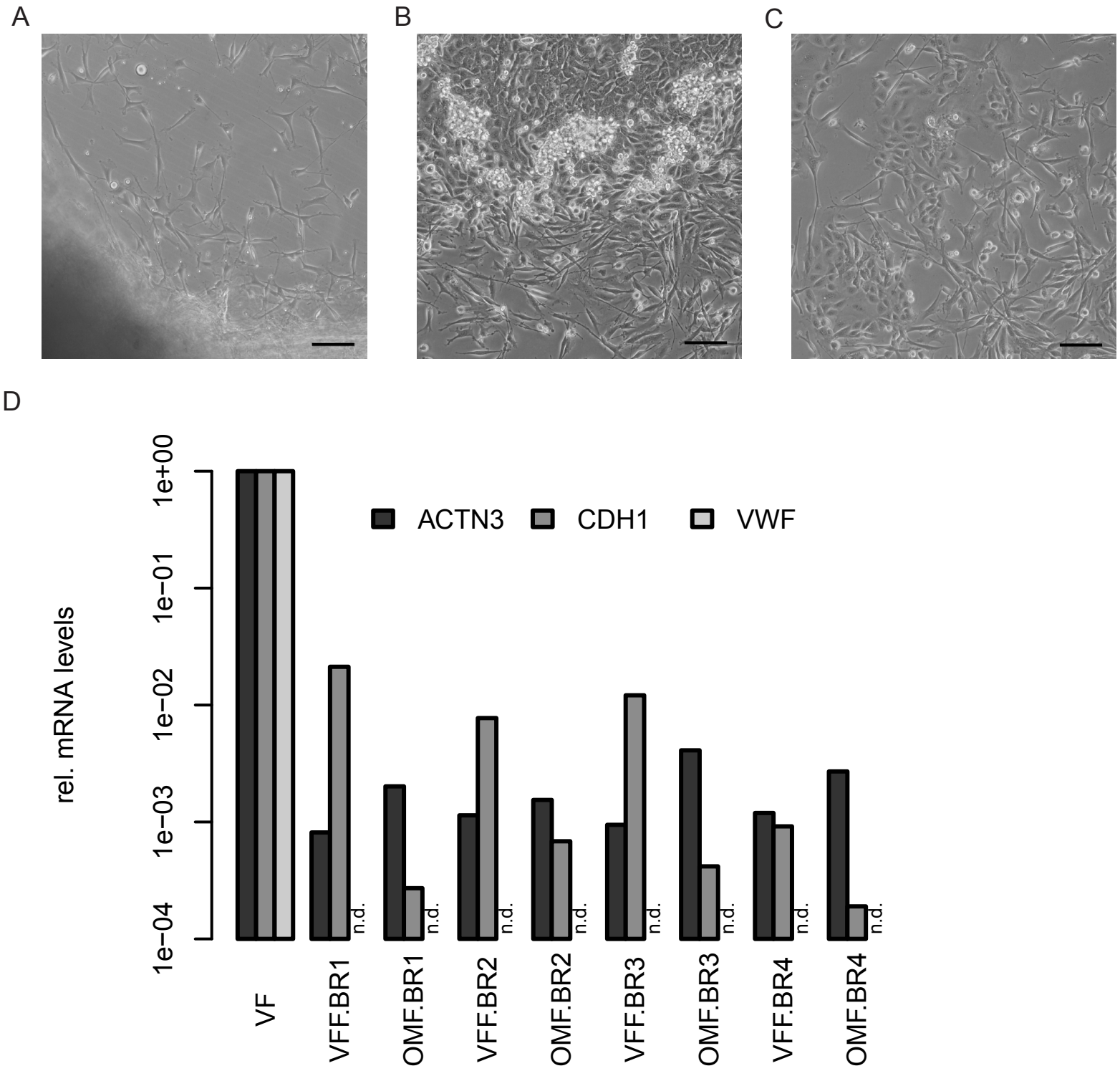
Supplemental Figures

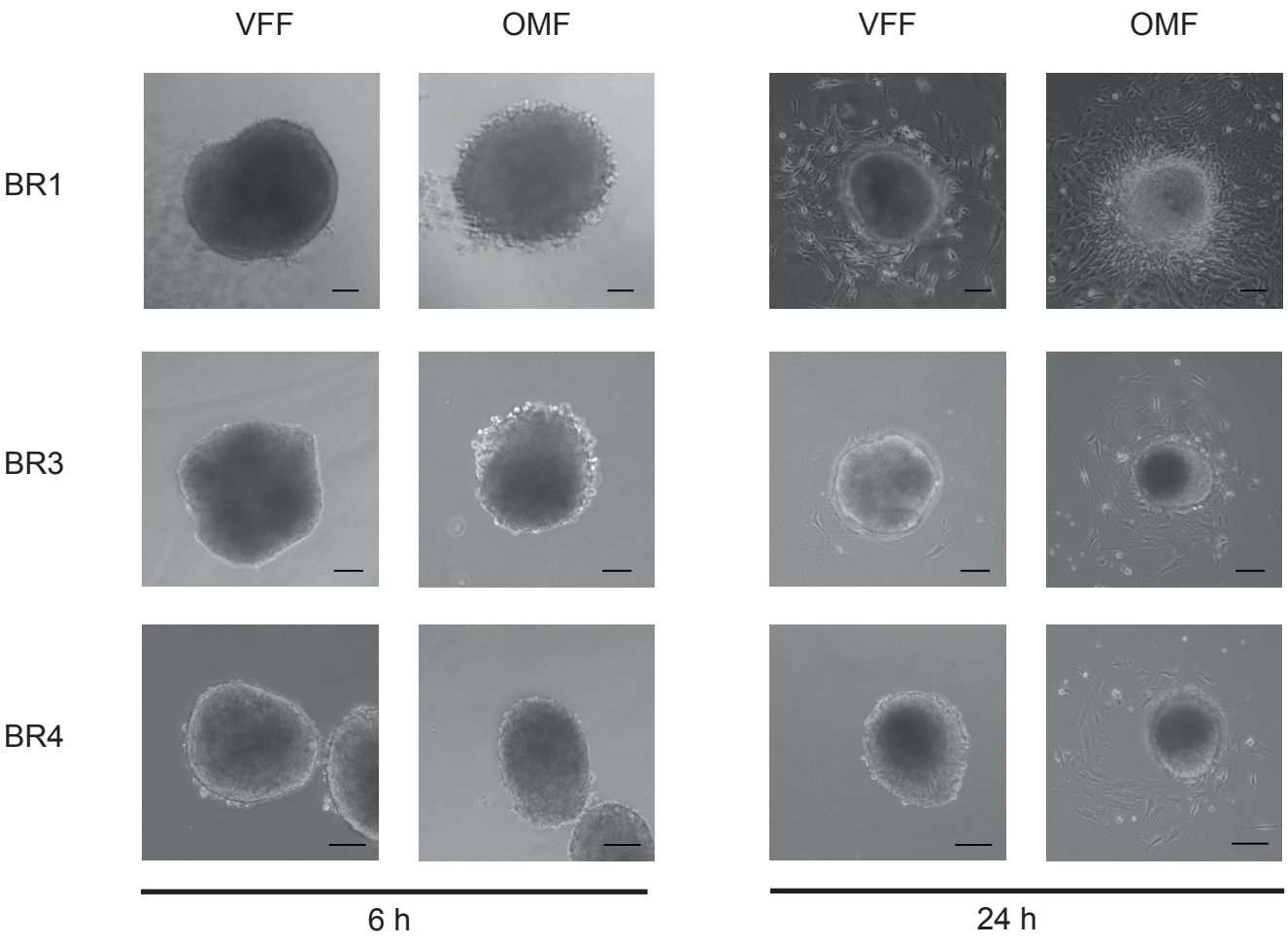
Window Display Report



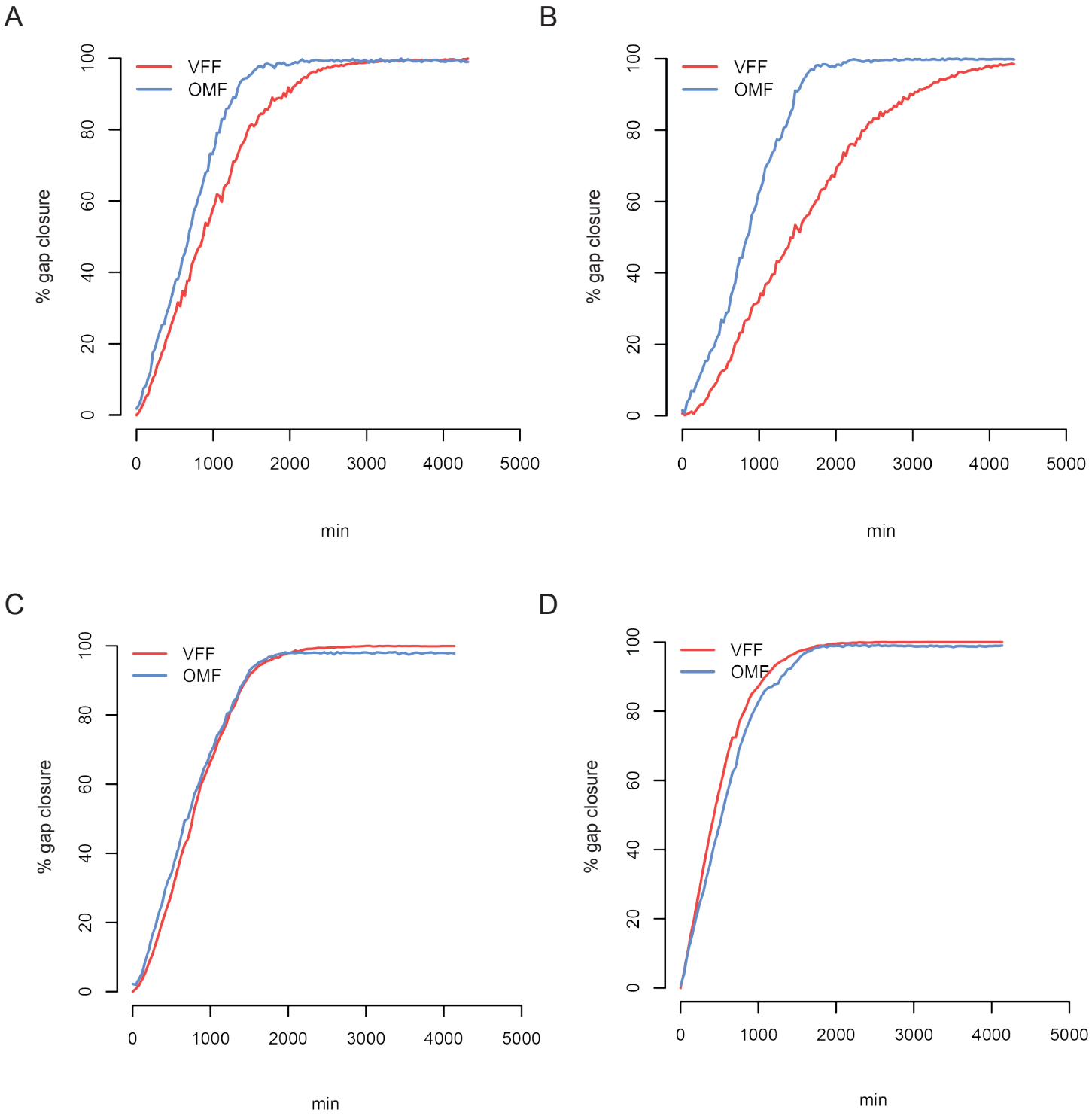
Supplemental Figure S1. Total ion chromatograms of individual samples are shown. OMF samples are depicted in red and VFF in blue.



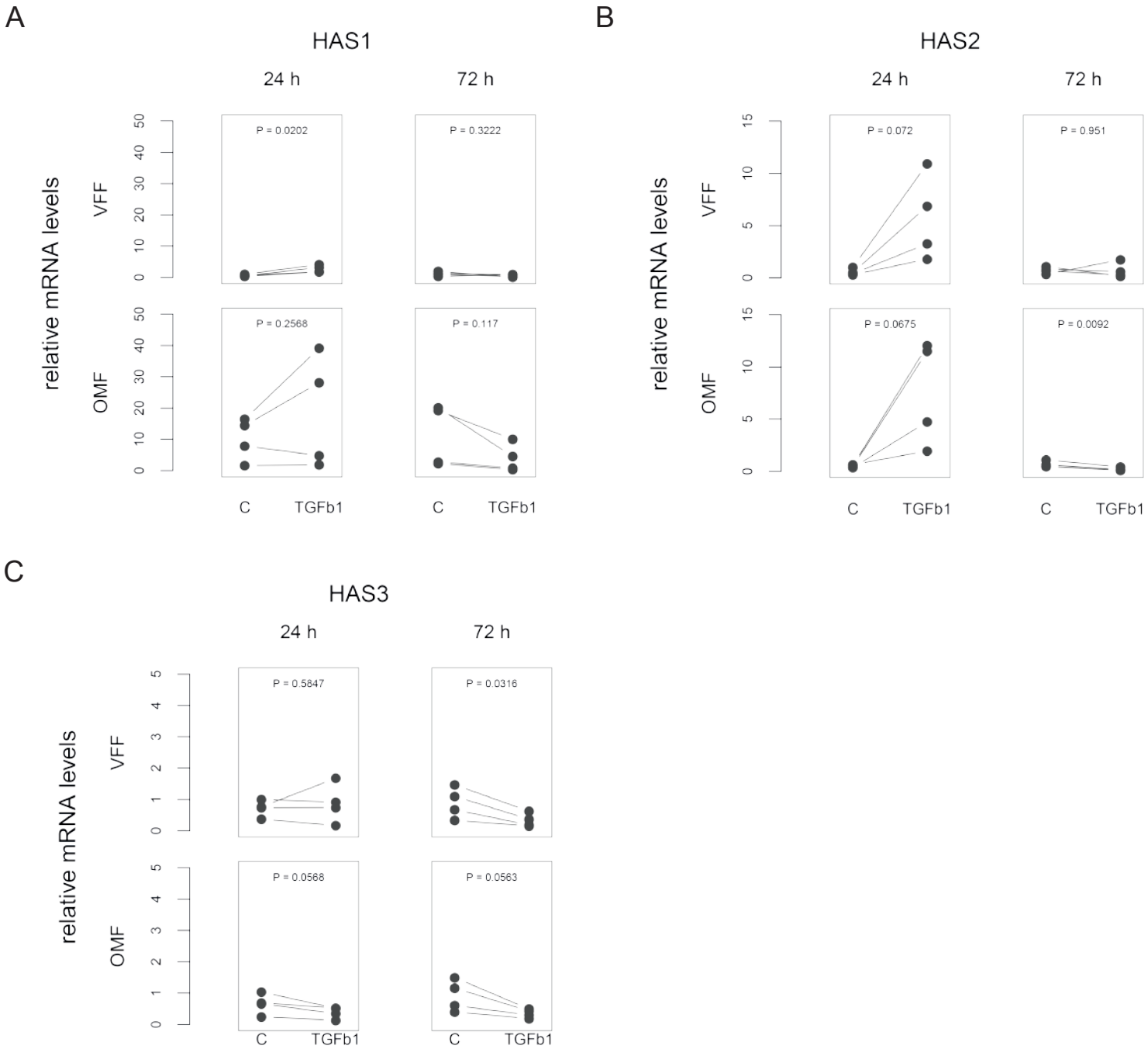
Supplemental Figure S2. Establishment of fibroblast populations from vocal fold and oral mucosa. Tissues were dissected to prepare pieces of approximately 2 mm x 2 mm x 2 mm, which were placed into cell culture plates, overlaid with growth medium and incubated at 37°C and 5%CO2. (A) Fibro¬blastoid cells started to emerge after 2-7 days (representative phase contrast microscopy image). Occasionally, the primary culture also contained mixtures of fibroblasts with cells that had epithelial (B) or endothelial (C) morphology; however, these cell types disappeared after 2-4 passages. (A-C) scale bar: 100 µm. (D) At passages 4-6, fibroblast populations from all 4 biological replicates (BR1–BR4) were analyzed by RT-qPCR for the presence of skeletal muscle (ACTN3), epithelial (CDH1), and endothelial (VWF) markers. Data is presented on a logarithmic scale relative to lysate from a total vocal fold (VF). n.d., not detectable.



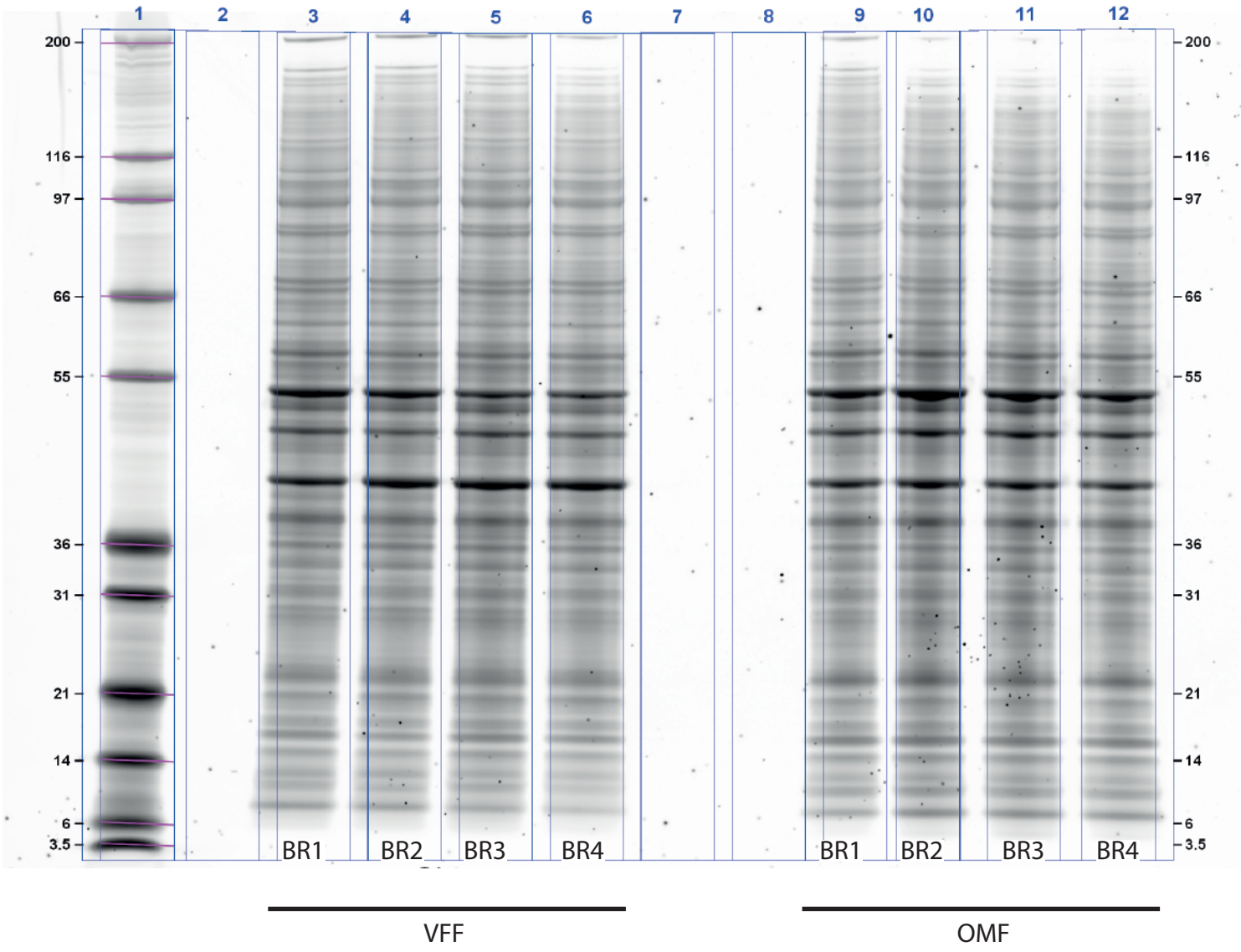
Supplemental Figure S3. 3D spheroid culture of paired vocal fold fibroblasts and oral mucosa fibroblasts. Phase contrast microscopy images that were acquired 6 h and 24 h post re-plating are shown for the biological replicates (BR) 1, 3, and 4. Scale bar: 100 µm.



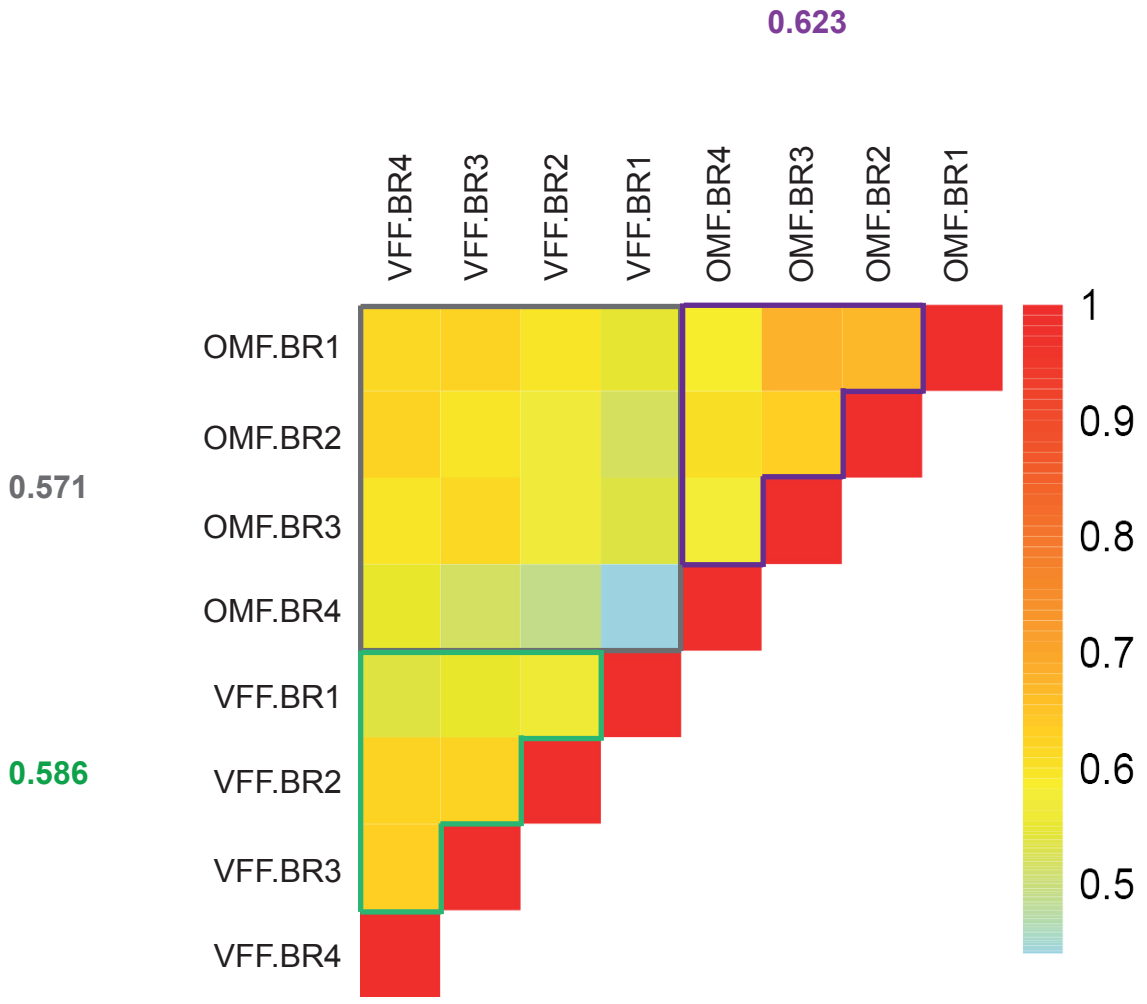
Supplemental Figure S4. 2D migration assay. Vocal fold fibroblasts (VFF) and oral mucosa fibroblasts (OMF) were seeded into a 2-well silicone insert (placed in wells of a 24 well plate). Inserts were removed after ~16 h, and cell migration (leading to closure of the 500 µm-gap between the two areas of confluent cells) was tracked over 72 h. Wound closure (expressed in % of the initial gap area) was subsequently calculated and is presented for pairs of VFF and OMF. (A) BR1, (B), BR2, (C) BR3, (D) BR4.



Supplemental Figure S5. Cellular response to pro-fibrotic stimulus. Paired VFF and OMF (n=4) were exposed to TGF-β1 (5 ng/mL) or vehicle ("C") and expression of hyaluronan synthase 1 (A), hyaluronan synthase 2 (B), and hyaluronan synthase 3 (C) was analyzed 24 h and 72 h later.



Supplemental Figure S6. Qualitative analysis of protein lysates used for LC-MS/MS analysis. Lysates from four biological replicates (BR) of VFF and OMF were subjected to denaturing gel electrophoresis (SDS-PAGE) followed by Krypton Fluorescent Protein Stain.



Supplemental Figure S7. Pairwise overlaps of proteins detected in VFF and OMF samples. For each possible pair of samples, the overlap index (OI = [no. of proteins detected in both samples] / [no. of proteins detected in both samples + no. of proteins detected in sample 1 only + no. of proteins detected in sample 2 only]) was calculated. Results are summarized as heat map. Average overlap indices within all VFF samples, within all OMF samples, or all possible VFF-OMF combinations are indicated as green, purple, and grey values, respectively.