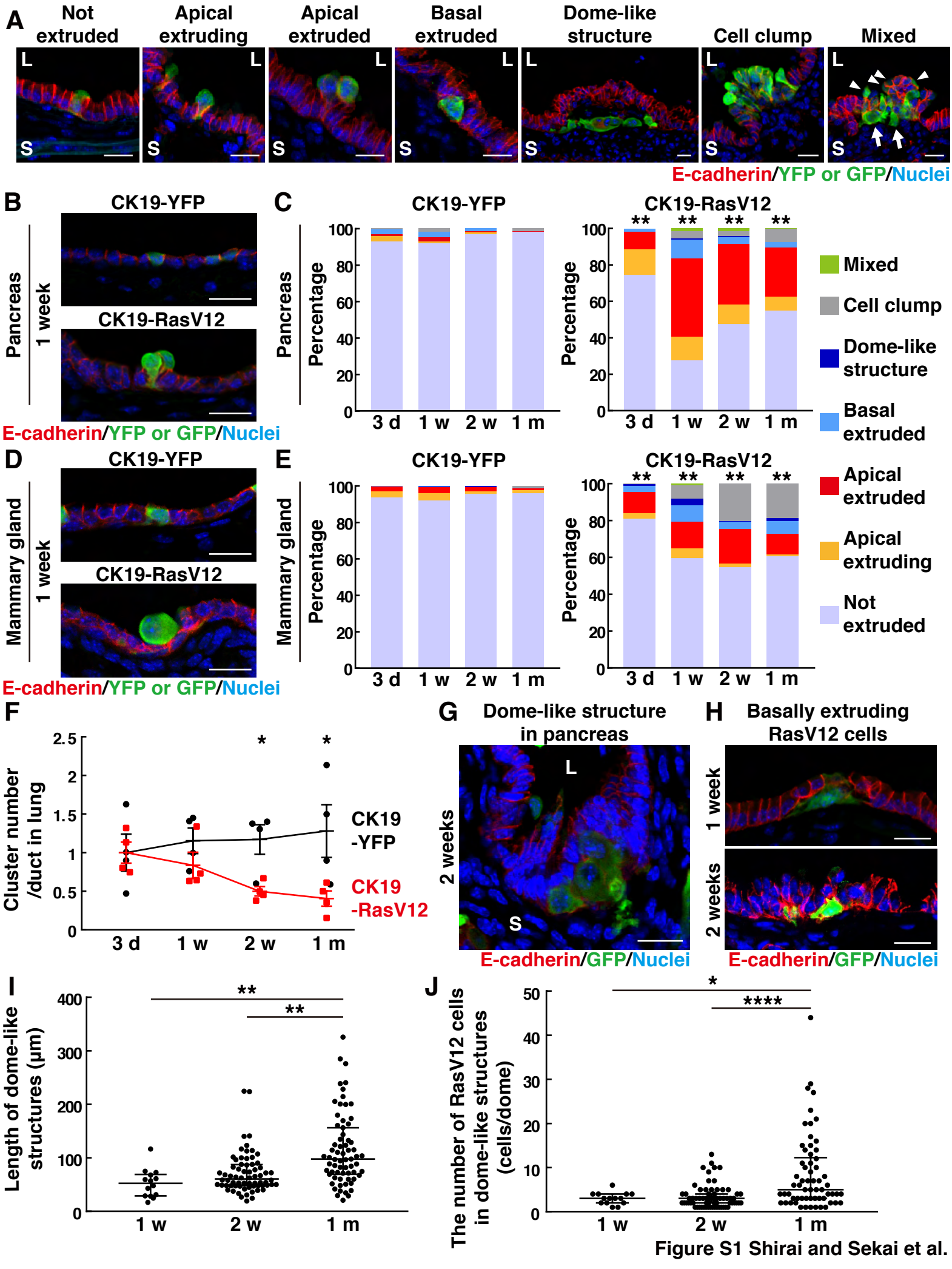

Figure S1 Shirai and Sekai et al.

Figure S1 The fate of RasV12-transformed cells in the lung, pancreas and mammary gland. (A) Classification of the phenotypes of YFP- or RasV12-expressing cells. Single or clustered cells are classified into seven phenotypes. 'Not extruded': less than 6 cells (in a cluster) remaining within the epithelium. 'Apical extruding': more than 20% of cells (in a cluster) with their nuclei apically shifted, but still attached to the basement membrane. 'Apical extruded': more than 20% of cells completely detached from the basement membrane and translocated into the apical lumen. 'Basal extruded': more than 20% of cells basally delaminated beneath the basement membrane. 'Dome-like structure': more than 50% of cells basally delaminated with a bumped, overlying epithelial layer. 'Cell clump': a group of more than 5 cells remaining within epithelia (not including apically or basally extruded cells). 'Mixed': a cluster containing both apically extruded (arrowheads) and basally extruded (arrows) cells. L or S indicates a luminal or stromal region, respectively. Scale bars, 20 μm. (B, D) Immunofluorescence images of the pancreas (B) and mammary gland (D) from CK19-YFP or CK19-RasV12 mice with tamoxifen treatment. Scale bars, 20 μm. (C, E) Quantification of the phenotypes of YFP or RasV12 cells in the pancreas (C) and mammary gland (E). (C) n = 301 (3 d), 307 (1 w), 298 (2 w), and 298 (1 m) clusters in CK19-YFP. n = 307 (3 d), 315 (1 w), 241 (2 w), and 294 (1 m) clusters in CK19-RasV12. Data are from three mice. (E) n = 401 (3 d), 318 (1 w), 368 (2 w), and 403 (1 m) clusters in CK19-YFP. n = 408 (3 d), 261 (1 w), 421 (2 w), and 260 (1 m) clusters in CK19-RasV12. Data are from three (1 w in CK19-YFP; 1 w and 1 m in CK19-RasV12) or four (3 d, 2 w, and 1 m in CK19-YFP; 3 d and 2 w in CK19-RasV12) mice. Note that the distribution of the phenotypes is compatible between mice. $^{**}P < 0.01$, chi-square test. (F) Quantification of the number of YFP (black line) or RasV12 (red line) clusters in a bronchial epithelial duct from CK19-YFP or

CK19-RasV12 mice with tamoxifen treatment. Data are mean ± s.e.m. n = 335 (3 d), 334 (1 w), 315 (2 w), and 322 (1 m) ducts for CK19-YFP. n = 343 (3 d), 339 (1 w), 367 (2 w), and 352 (1 m) ducts for CK19-RasV12. The number of YFP or RasV12 cell clusters in three sequential 50-μm-thick sections was quantified and shown as 'Cluster number/duct in lung'. Data are from four mice for each group. *P < 0.05, unpaired two-tailed Student's t test. (G) Immunofluorescence image of a dome-like structure in the pancreatic duct from CK19-RasV12 mice with tamoxifen treatment. L or S indicates a luminal or stromal region, respectively. Scale bar, 20 μm. (H) Immunofluorescence images of basally extruding RasV12 cells from the bronchial epithelium. Scale bars, 20 μm. (I, J) Quantification of the length of dome-like structures (I) or the number of RasV12 cells within dome-like structures (J). For quantification of the length of dome-like structures, we measured the length of a straight line linking both bottom ends of the bump of epithelia. Data are median ± quartiles. (I) n = 14 (1 w), 68 (2 w), and 67 (1 m) dome-like structures from three mice. (J) n = 14 (1 w), 67 (2 w), and 62 (1 m) dome-like structures from three mice. *P < 0.05, **P < 0.01, ****P < 0.001, Kruskal-Wallis test followed by Dunn's multiple comparison test.

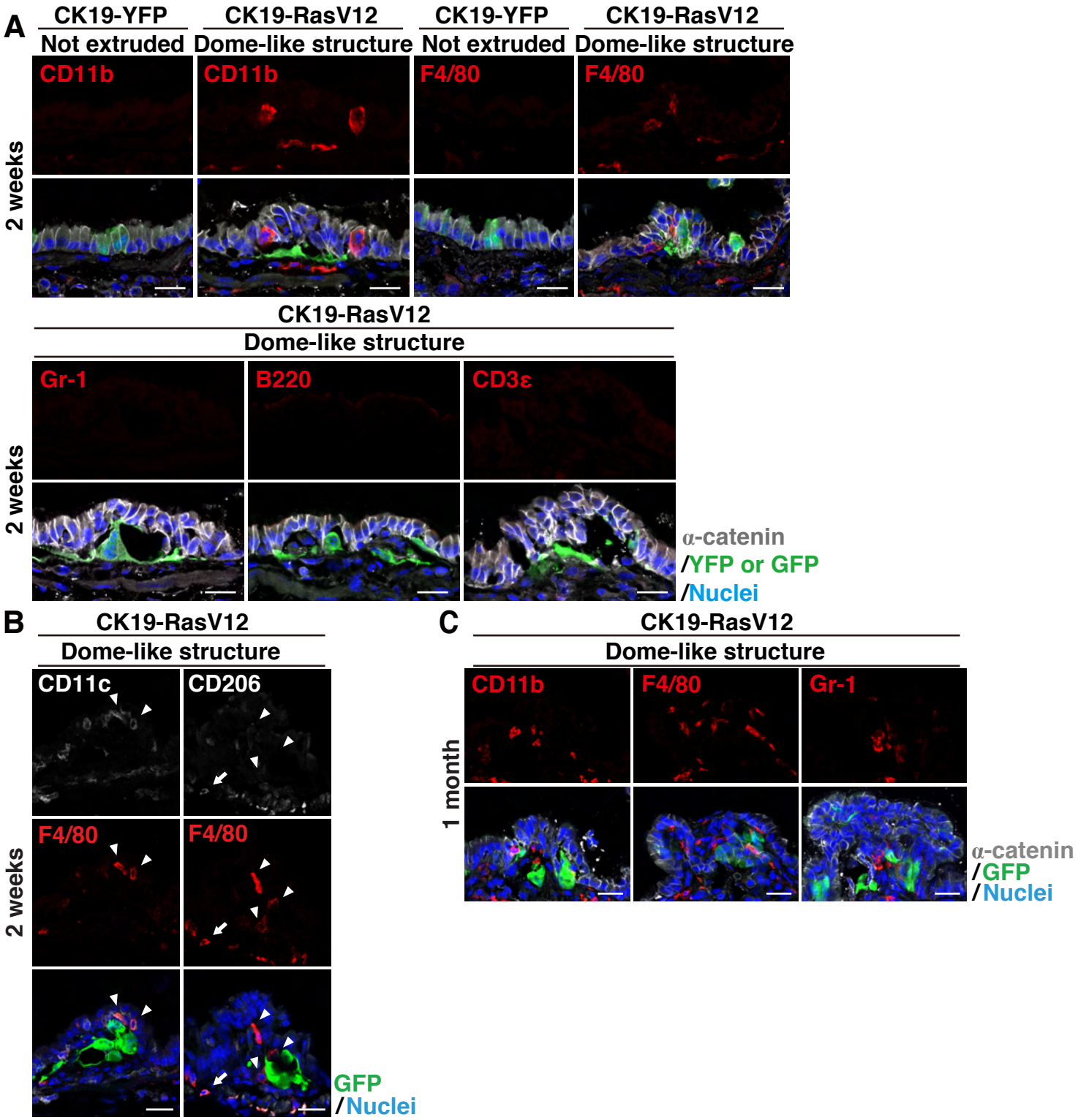

Figure S2 Shirai and Sekai et al.

Figure S2 Accumulation of immune cells in dome-like structures. (A-C) Immunofluorescence images of bronchial epithelia from CK19-YFP or CK19-RasV12 mice after tamoxifen injection. The following antibodies recognize a marker protein on specific immune cells: CD11b (macrophages and neutrophils), F4/80 (macrophages), Gr-1 (neutrophils), B220 (B lymphocytes), CD3ε (T lymphocytes), CD11c (M1 pro-inflammatory macrophages), and CD206 (M2 anti-inflammatory macrophages). (B) Arrowheads indicate F4/80-positive, CD11c-positive, CD206-negative macrophages in the dome-like structures that are supposed to be recruited from bloodstream or tissue-resident interstitial macrophages. Arrows indicate an F4/80-positive, CD206-positive tissue-resident alveolar macrophage outside the dome-like structure. Scale bars, 20 μm. (D, E) Correlations between the length of dome-like structures and the number of α-SMA$^+$ fibroblasts (D) or CD45$^+$ immune cells (E) within dome-like structures. n = 107 (D) and 82 (E) dome-like structures from four (D) or three (E) mice. Pearson correlation analysis. $P < 0.05$ was considered significant.

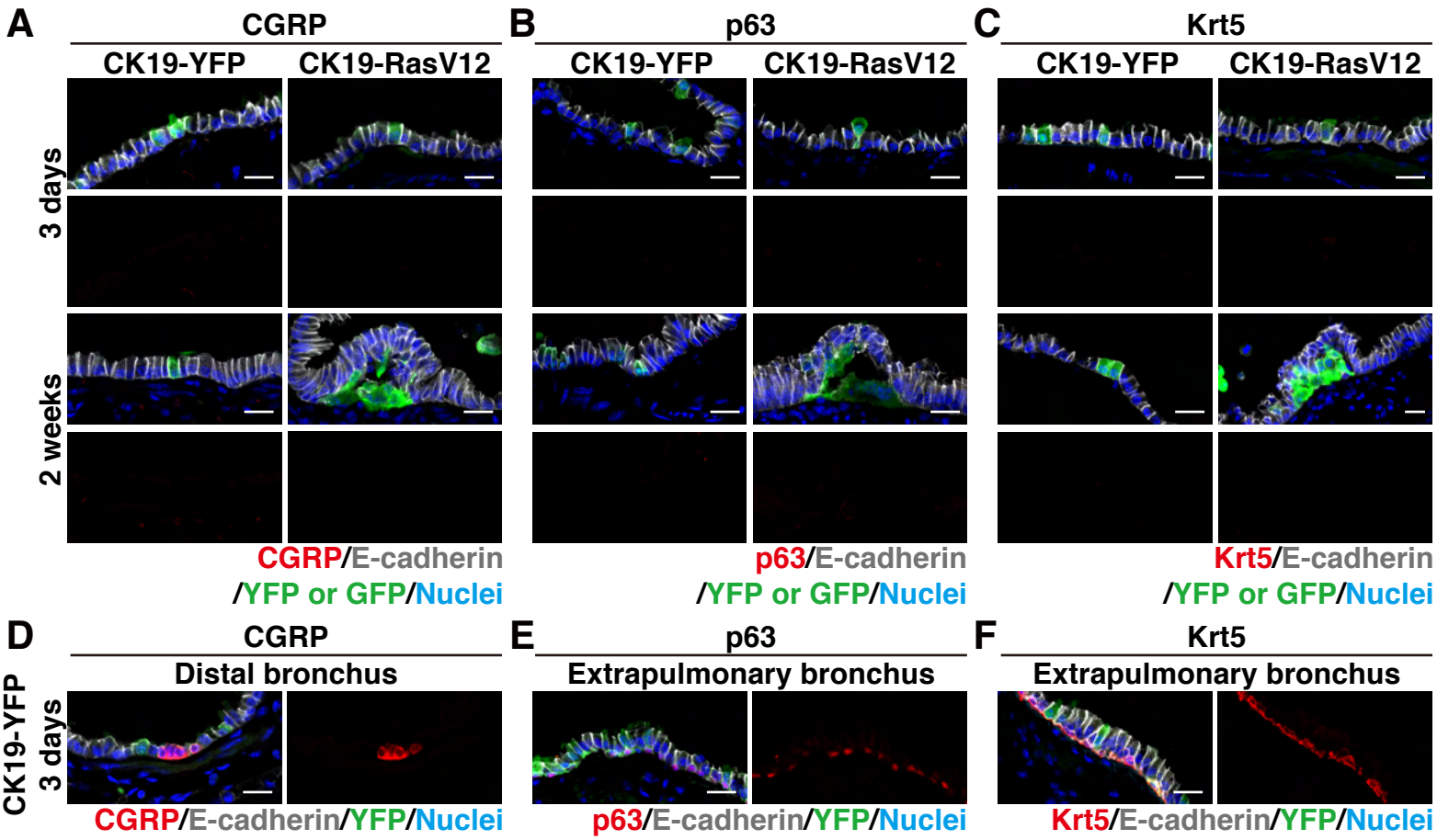

Figure S3 Shirai and Sekai et al.

Figure S3 CK19 promoter-driven expressions in neuroendocrine or basal cells of bronchial epithelia. (A-F) Immunofluorescence images of distal bronchial epithelia (A-D) or extrapulmonary bronchial epithelia (E, F) from CK19-YFP or CK19-RasV12 mice with tamoxifen treatment. The following antibodies recognize a differentiation marker of specialized lung cells: CGRP (neuroendocrine cells), p63, or Krt5 (basal cells). (D-F) Validation of the antibodies for immunofluorescence. Note that basal cells are present in extrapulmonary bronchial epithelia, but not in distal bronchial epithelia where dome-like structures were analyzed in this study. Scale bars, 20 μm.

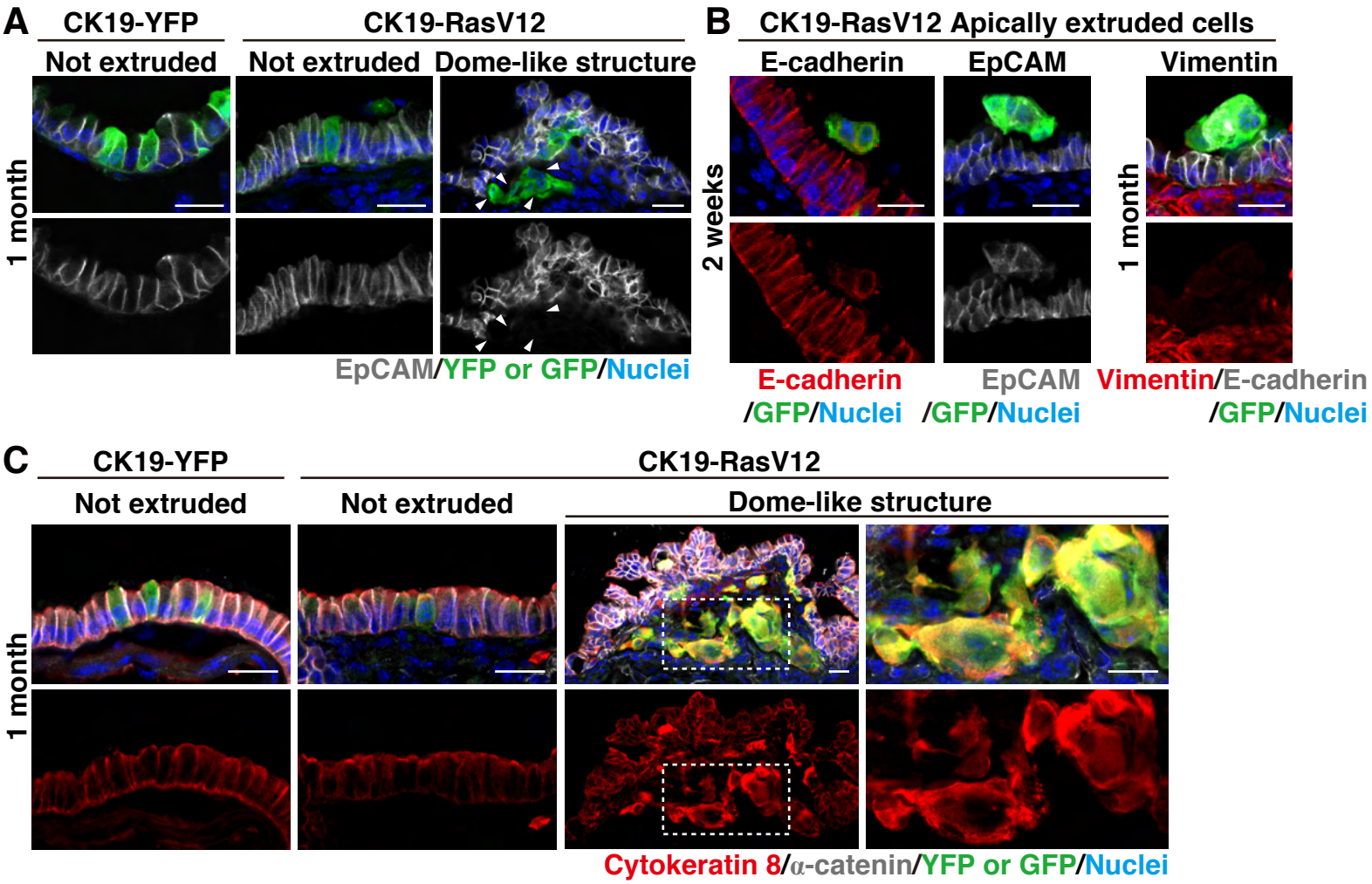

Figure S4 Shirai and Sekai et al.

Figure S4 Partial EMT phenotype in basally extruded RasV12-transformed cells in bronchial epithelia. (A-C) Immunofluorescence images of bronchial epithelia from CK19-YFP or CK19-RasV12 mice with tamoxifen treatment. Scale bars, 20 μm. The arrowheads indicate $EpCAM^{-}$ GFP^{+} RasV12 cells in the dome-like structure (A). The dotted areas are shown at higher magnification in the right panels (C).

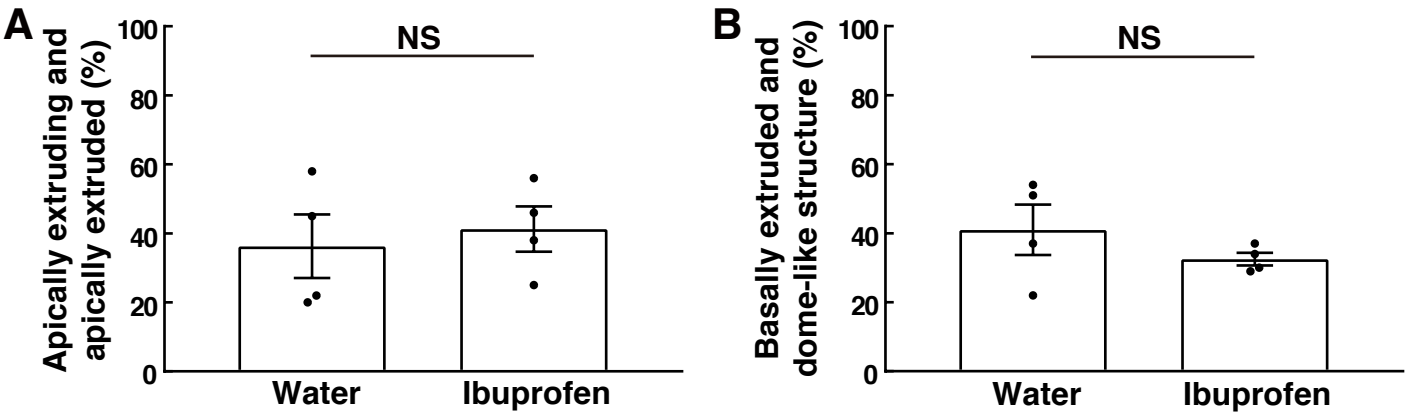

Figure S5 Shirai and Sekai et al.

Figure S5 The effect of ibuprofen treatment on the phenotypes of RasV12 cells. (A, B) Quantification of the effect of ibuprofen on the phenotypes of RasV12 cells: apically extruding/extruded (A) or basally extruded/dome-like structures (B). Ibuprofen was administered as shown in Figure 5C. Data are mean ± s.e.m. n = 433 (water) and 403 (ibuprofen) cells from four mice. NS, not significant, unpaired two-tailed Student's t test.

Primers used for genotyping

Genotyping primer	5' to 3' sequence
DNMT1-CAG-loxP-STOP-loxP-HRasV12-IRES-eGFP, forward	CACTGTGGAATCTCGGCAGG
DNMT1-CAG-loxP-STOP-loxP-HRasV12-IRES-eGFP, reverse	GCAATATGGTGGAAAATAAC
Rosa26-loxP-STOP-loxP-EYFP, forward	AAAGTCGCTCTGAGTTGTTAT
Rosa26-loxP-STOP-loxP-EYFP, WT reverse	GGAGCGGGAGAAATGGATATG
Rosa26-loxP-STOP-loxP-EYFP, mutant reverse	GCGAAGAGTTTGTCCTCAACC
Scgb1a1-CreERT, forward	ACTCACTATTGGGGGTGTGG
Scgb1a1-CreERT, WT reverse	AGGCTCCTGGCTGGAATAGT
Scgb1a1-CreERT, mutant reverse	CCAAAAGACGGCAATATGGT