Figure S1. Schematic diagram of the schedule of ICI administration and timing of PBMC analysis. Data from 44 patients with cancer who received anti-PD-1 monotherapy were retrospectively analyzed. PBMC fractions were evaluated before anti-PD-1 antibody therapy. Overall survival was defined as the time from the start of ICI treatment to either patient mortality from any cause or last follow-up. PBMC, peripheral blood mononuclear cell; ICI, immune checkpoint inhibitor; PD-1, programmed death-1; q2w, once every 2 weeks; q3w, once every 3 weeks.

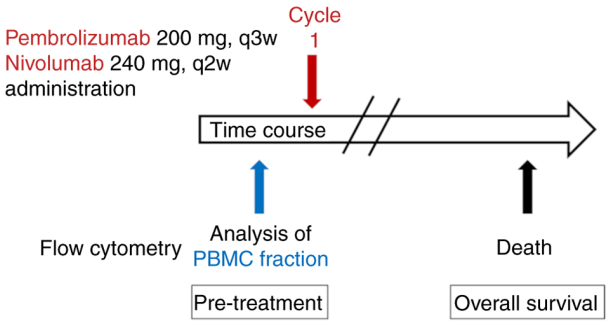

Figure S2. Association of each monocyte subset percentage with EGFR mutations in patients with NSCLC. Percentages of (A) non-classical, (B) intermediate and (C) classical monocytes were compared between patients with or without EGFR mutations. NSCLC, non-small cell lung cancer.

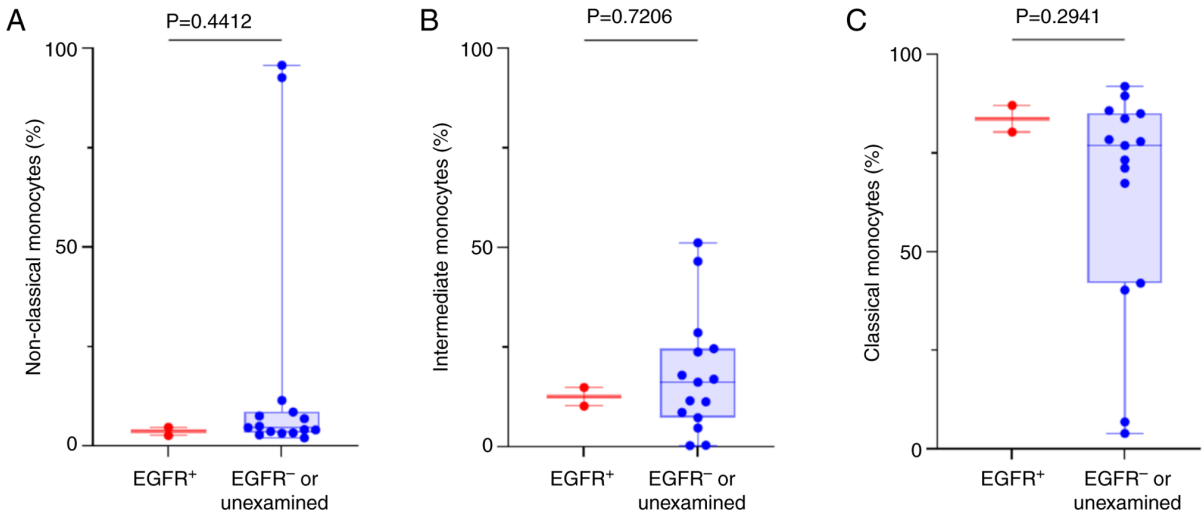

Figure S3. Linear correlation between each monocyte subset expressing PD-1 and OS. Graphs show the association between OS and the percentage of PD-1$^+$ (A) non-classical, (B) intermediate and (C) classical in the whole patient cohort (n=44); OS and the percentage of PD-1$^+$ (D) non-classical, (E) intermediate, (F) classical in the gastric cancer cohort (n=20); OS and the percentage of PD-1$^+$ (G) non-classical, (H) intermediate and (I) classical monocytes in the NSCLC cohort (n=17); and OS and the percentage of PD-1$^+$ (J) non-classical, (K) intermediate and (L) classical monocytes in the esophageal cancer cohort (n=7). Each dot represents a sample from each patient cohort. *P<0.05. PD-1, programmed death-1; OS, overall survival. NSCLC, non-small cell lung cancer.

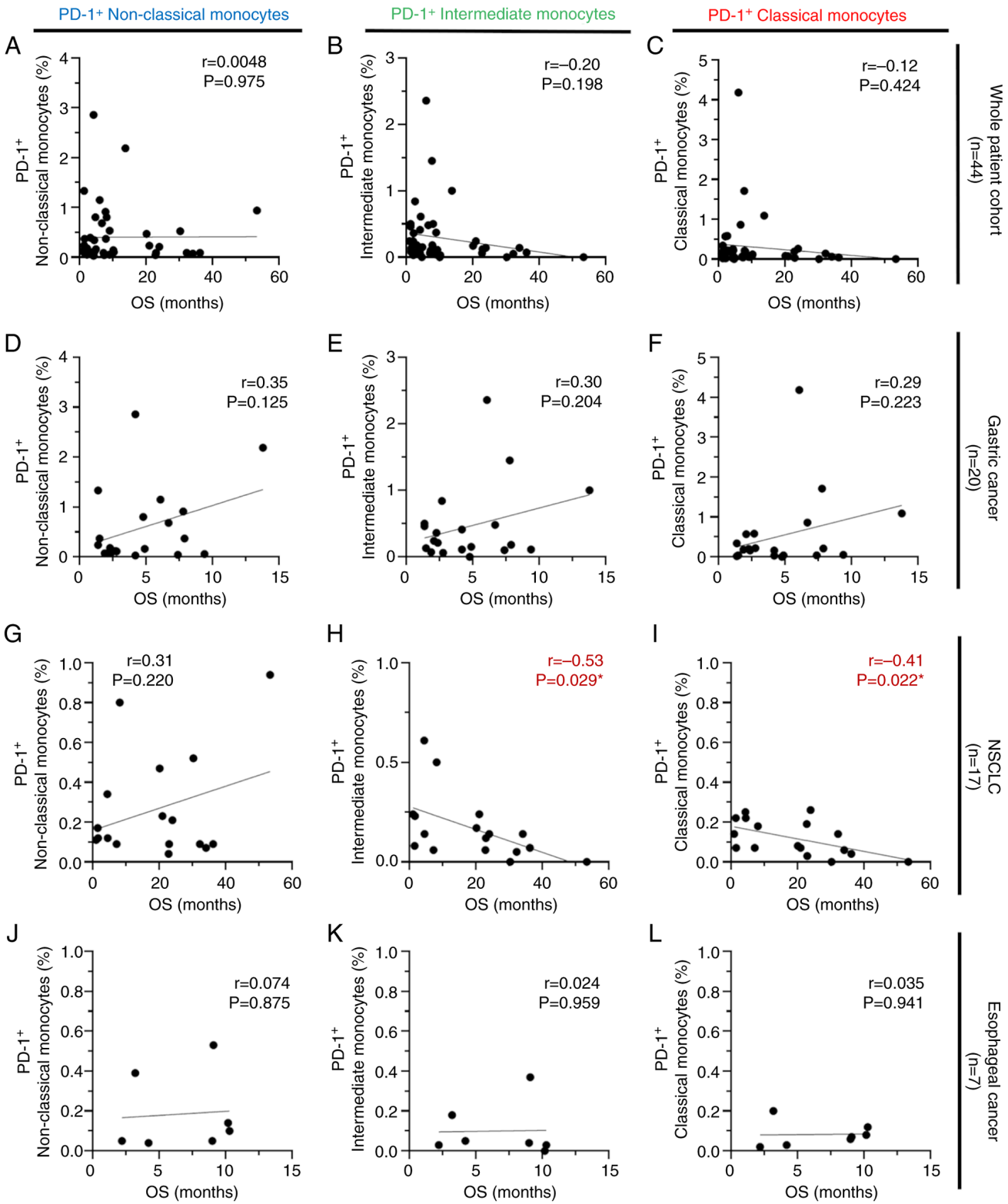

Figure S4. Correlation between each monocyte subset expressing PD-1 and PFS. Graphs showing the association between PFS and the percentage of (A) PD-1$^+$ non-classical, (B) PD-1$^+$ intermediate and (C) PD-1$^+$ classical monocytes in the whole patient cohort (n=44); PFS and the percentage of (D) PD-1$^+$ non-classical, (E) PD-1$^+$ intermediate and (F) PD-1$^+$ classical monocytes in the gastric cancer cohort (n=20); PFS and the percentage of (G) PD-1$^+$ non-classical, (H) PD-1$^+$ intermediate and (I) PD-1$^+$ classical monocytes in the NSCLC cohort (n=17); and PFS and the percentage of (J) PD-1$^+$ non-classical, (K) intermediate and (L) classical monocytes in the esophageal cancer cohort (n=7). Each dot represents a specimen from each patient cohort. PFS, progression-free survival; PD-1, programmed death-1.

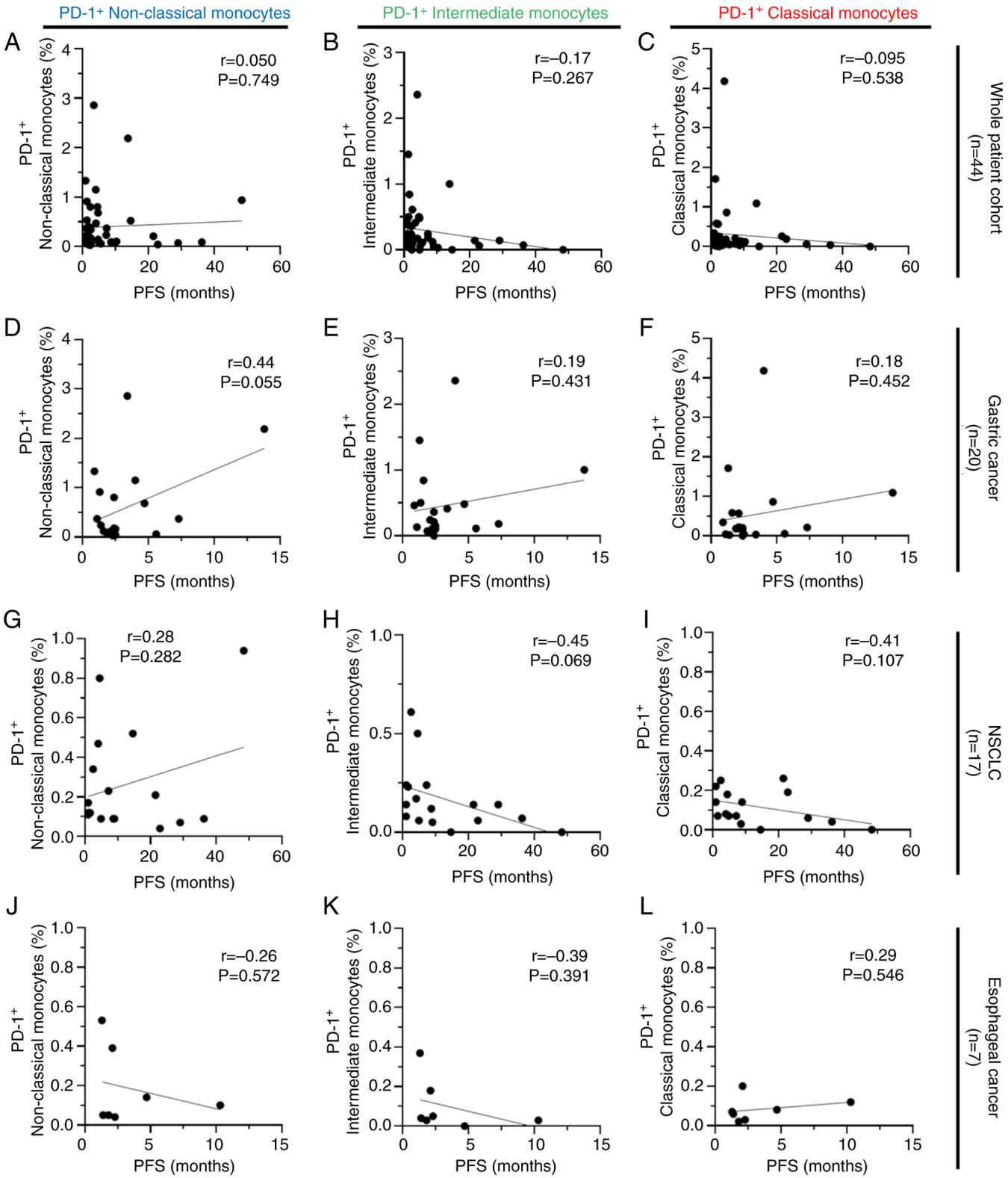

Figure S5. ROC curve analyses to determine cut-off values for PD-L1- and PD-1-expressing monocytes across the three monocyte subsets in the whole patient cohort (n=44). ROC curve for prediction of overall survival according to (A) non-classical, (B) intermediate, (C) classical, (D) PD-L1-expressing non-classical, (E) PD-L1-expressing intermediate, (F) PD-L1-expressing classical, (G) PD-1-expressing non-classical, (H) PD-1-expressing intermediate and (I) PD-1-expressing classical monocytes. ROC, receiver operating characteristic; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; AUC, area under the curve.

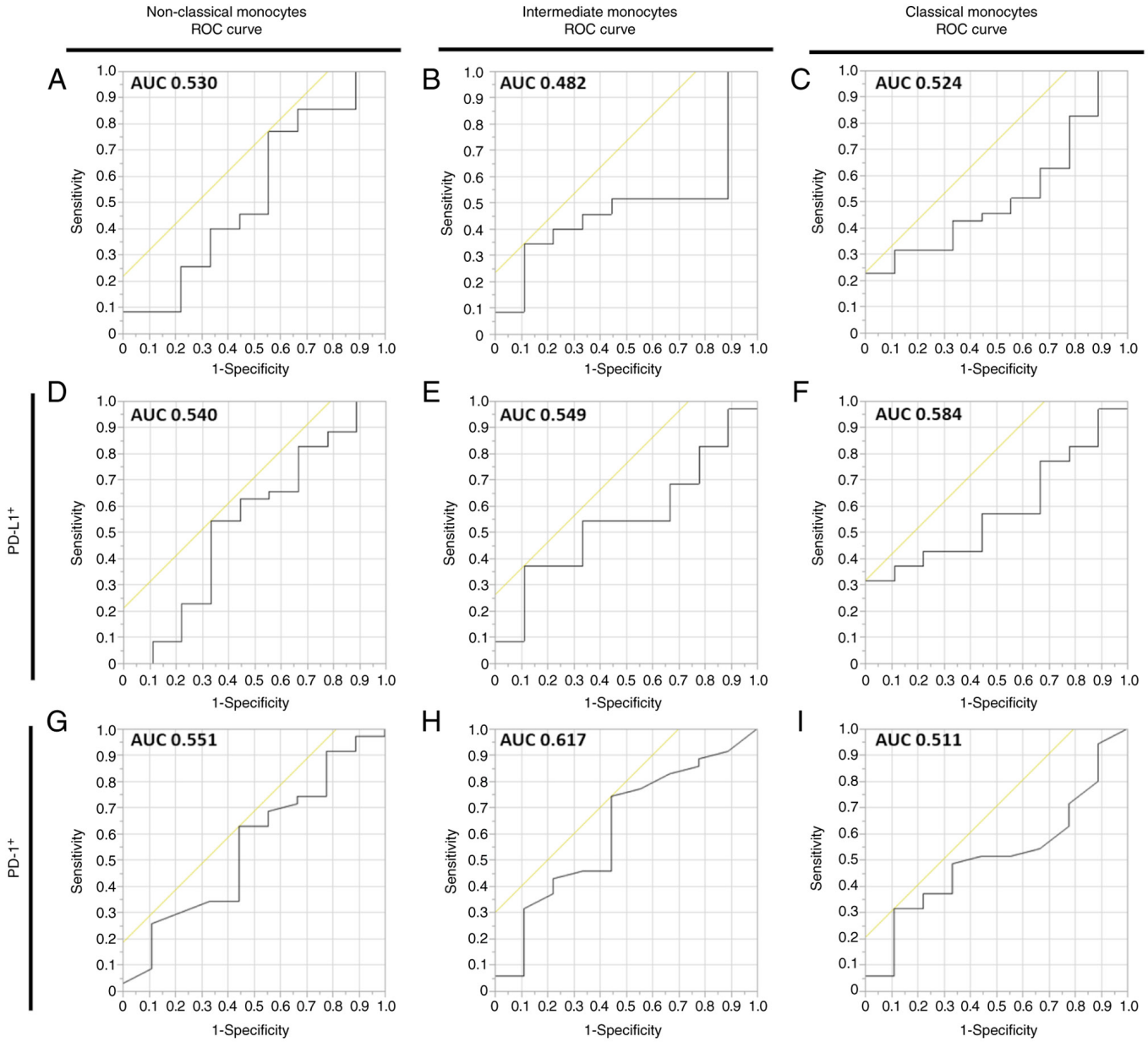

Figure S6. Kaplan-Meier curve analysis of the association between OS and monocyte subsets. The Kaplan-Meier analyses were conducted by dividing into two groups, i.e., high or low levels of each monocyte subset, to analyze the association between (A, D, G and J) non-classical monocytes, (B, E, H and K) intermediate monocytes and (C, F, I and L) classical monocytes, and OS in (A-C) the whole patient cohort (n=44), (D-F) patients with gastric cancer (n=20), (G-I) patients with NSCLC (n=17) and (J-L) patients with esophageal cancer (n=7). Red line, high level; blue line, low level. *P<0.05. OS, overall survival; NSCLC, non-small cell lung cancer.

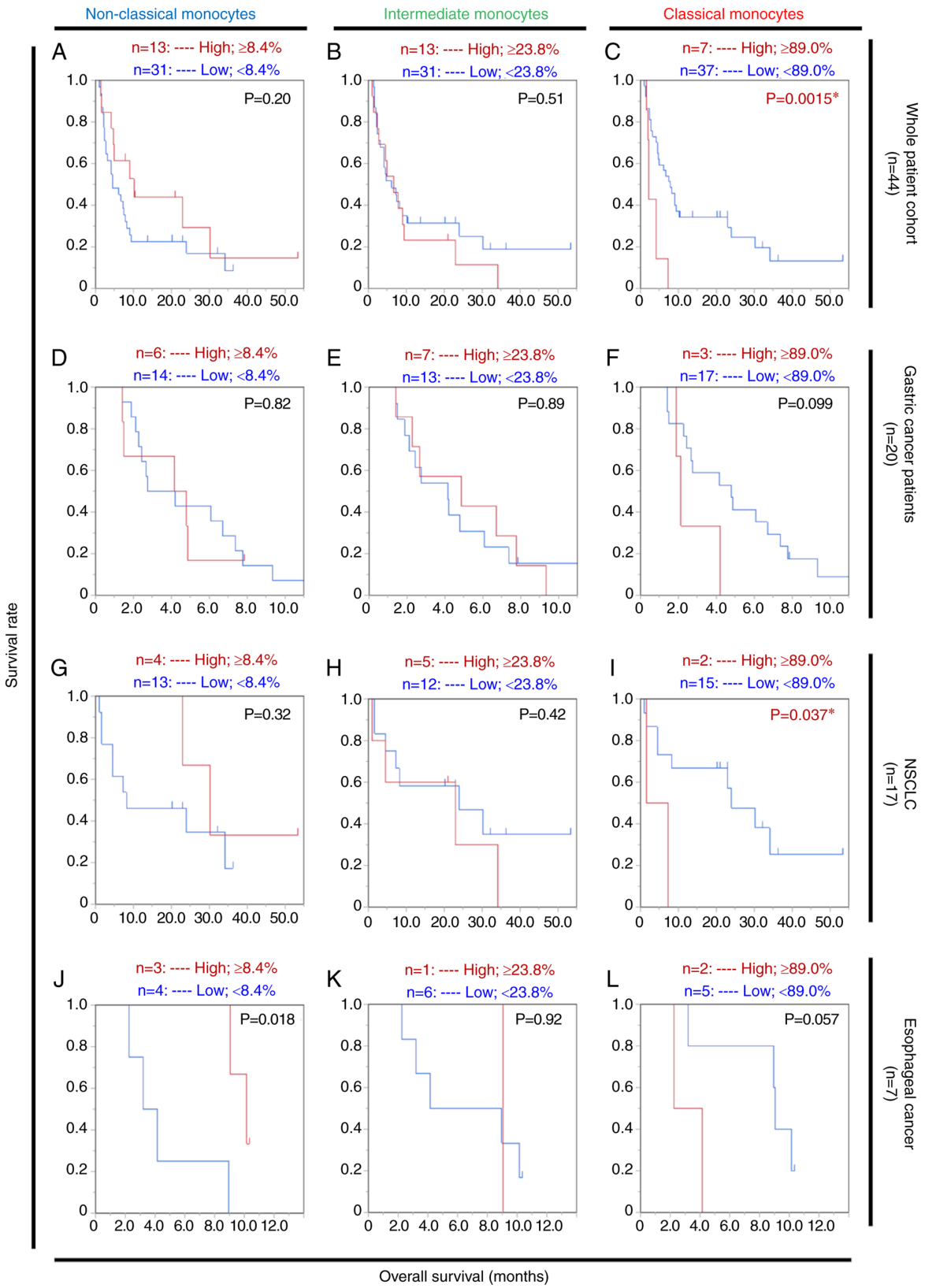

Figure S7. Kaplan-Meier curve analysis of the association between OS and the percentage of each monocyte subset expressing PD-L1. The Kaplan-Meier analyses were conducted by dividing into two groups, i.e., high or low levels of each monocyte subset expressing PD-L1, in order to analyze the association between (A, D, G and J) non-classical monocytes, (B, E, H and K) intermediate monocytes and (C, F, I and L) classical monocytes, and OS in (A-C) the whole patient cohort (n=44), (D-F) patients with gastric cancer (n=20), (G-I) patients with NSCLC (n=17) and (J-L) patients with esophageal cancer (n=7). Red line, high level; blue line, low level. *P<0.05. PD-L1, programmed death-ligand 1; OS, overall survival.

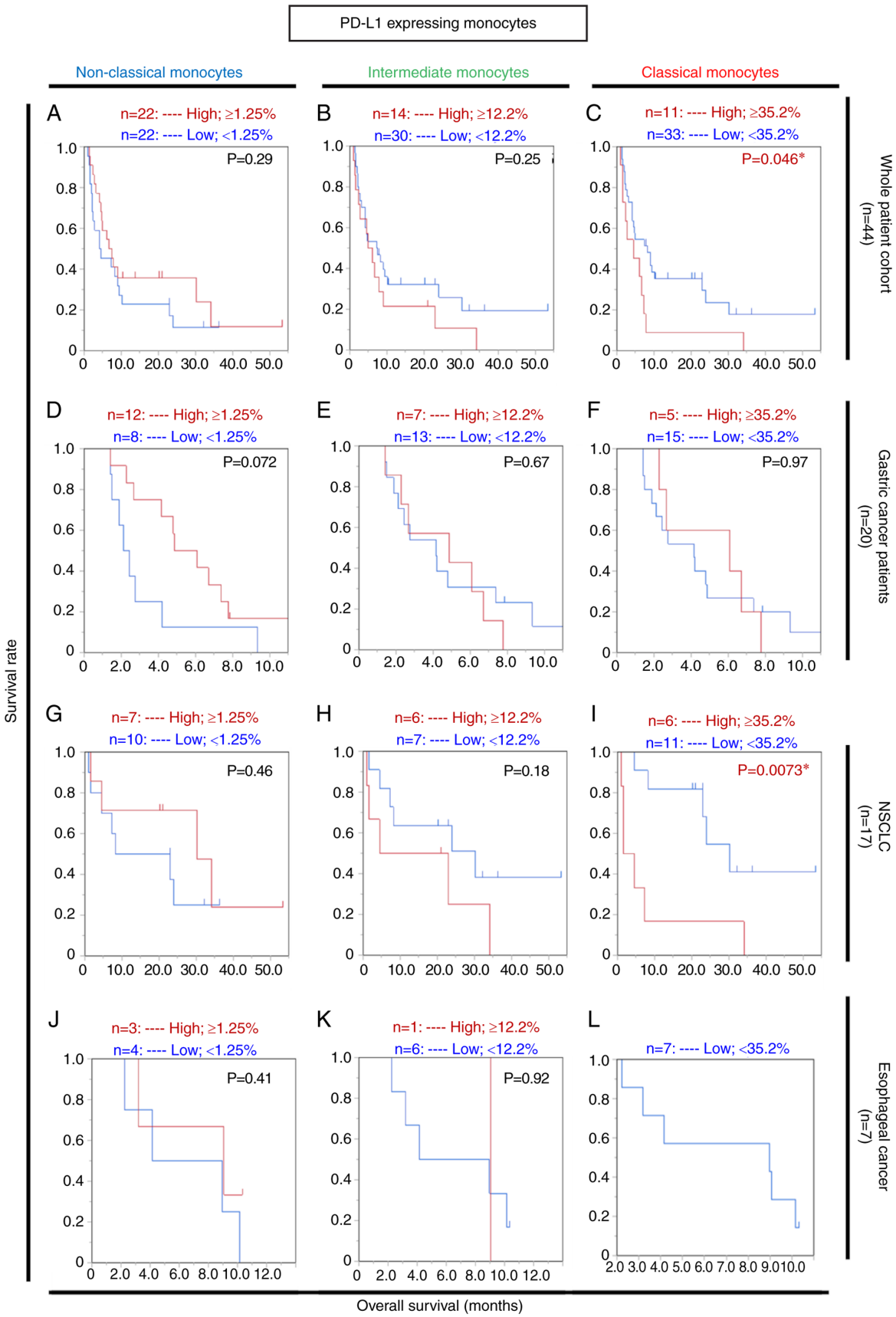

Figure S8. Kaplan-Meier curve analysis of the association between OS and the percentage of PD-1-expressing monocyte subset. The Kaplan-Meier analyses were performed by dividing into two groups, high or low levels of each monocyte subset expressing PD-1, in order to analyze the association between (A, D, G and J) non-classical monocytes, (B, E, H and K) intermediate monocytes and (C, F, I and L) classical monocytes, and OS in (A-C) the whole patient cohort (n=44), (D-F) patients with gastric cancer (n=20), (G-I) patients with NSCLC (n=17) and (J-L) patients with esophageal cancer (n=7). Red line, high level; blue line, low level. *P<0.05. OS, overall survival; PD-1, programmed death-1; NSCLC, non-small cell lung cancer.

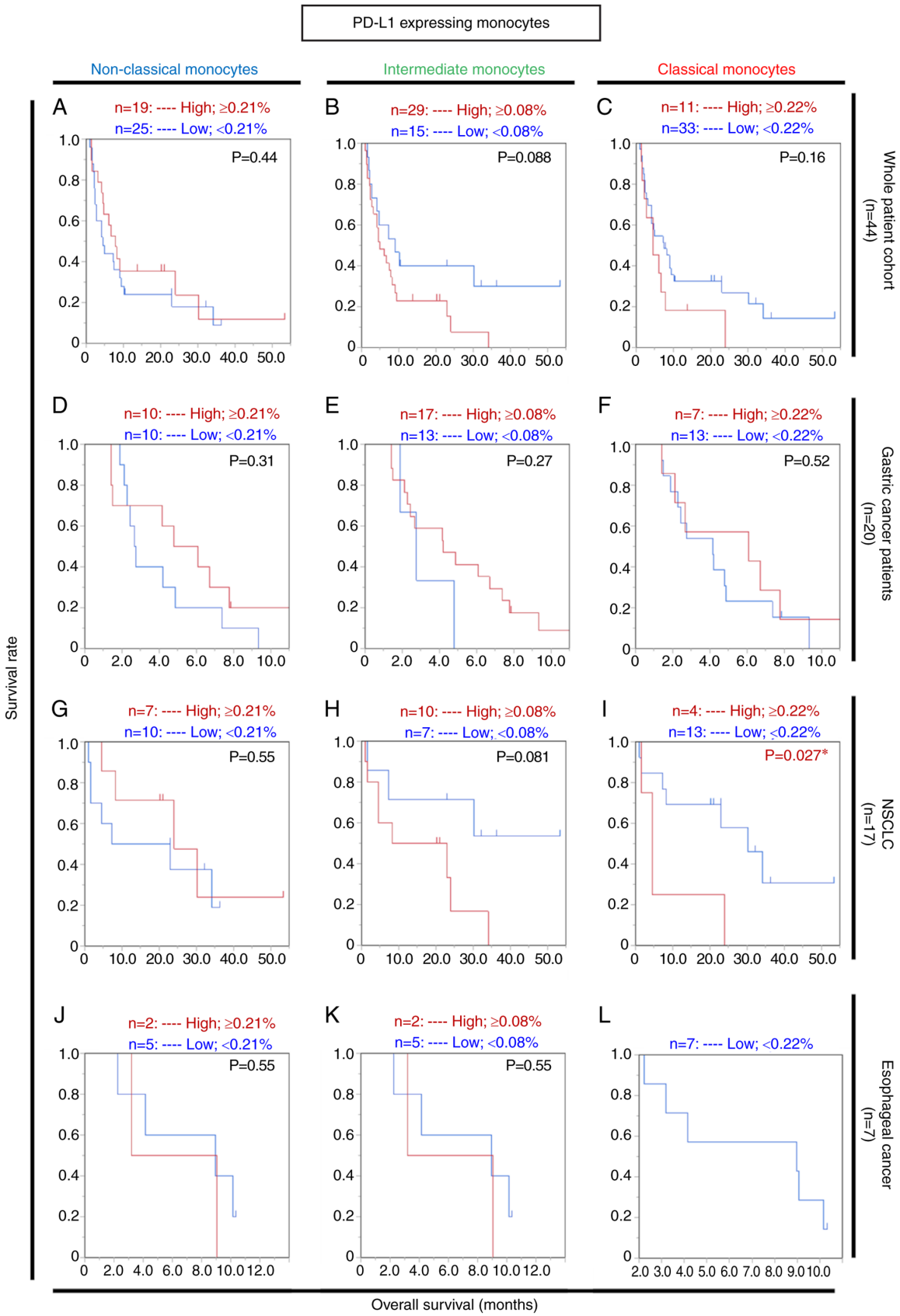

Figure S9. Representative image of PD-L1 expression analyzed by immunohistochemistry in a patient with NSCLC. Formalin-fixed paraffin-embedded tissue samples were obtained from the biopsy specimens and PD-L1 expression was assessed as the tumor proportion score in patients with NSCLC. PD-L1 expression level was determined by two independent pathologists to be 70-80% (case no. 3). NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1.

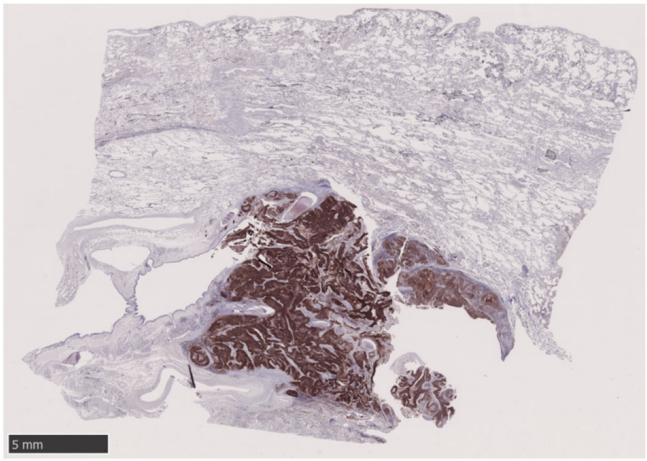

Figure S10. Correlation between CD68-stained area and monocyte percentage of each subset in all patients with cancer. The Pearson correlation coefficient was calculated to analyze the correlation between the CD68-stained area and monocyte percentages. (A-C) The correlation analyses between the CD68-positive area, the macrophage infiltrating area and the monocyte percentage of each monocyte subset, such as (A) non-classical, (B) intermediate and (C) classical monocytes, were conducted. The same analyses were conducted for each monocyte subset expressing both (D-F) PD-L1 and (G-H) PD-1. Correlation analyses between the CD68-positive area and the monocyte percentage of each monocyte subset, such as (D) PD-L1$^+$ non-classical, (E) PD-L1$^+$ intermediate and (F) PD-L1$^+$ classical monocytes, were conducted. Correlation analyses between the CD68-positive area and the monocyte percentage of each monocyte subset, such as (G) PD-1$^+$ non-classical, (H) PD-1$^+$ intermediate and (I) PD-1$^+$ classical monocytes, were conducted. PD-1, programmed death-1; PD-L1, programmed death-ligand 1.