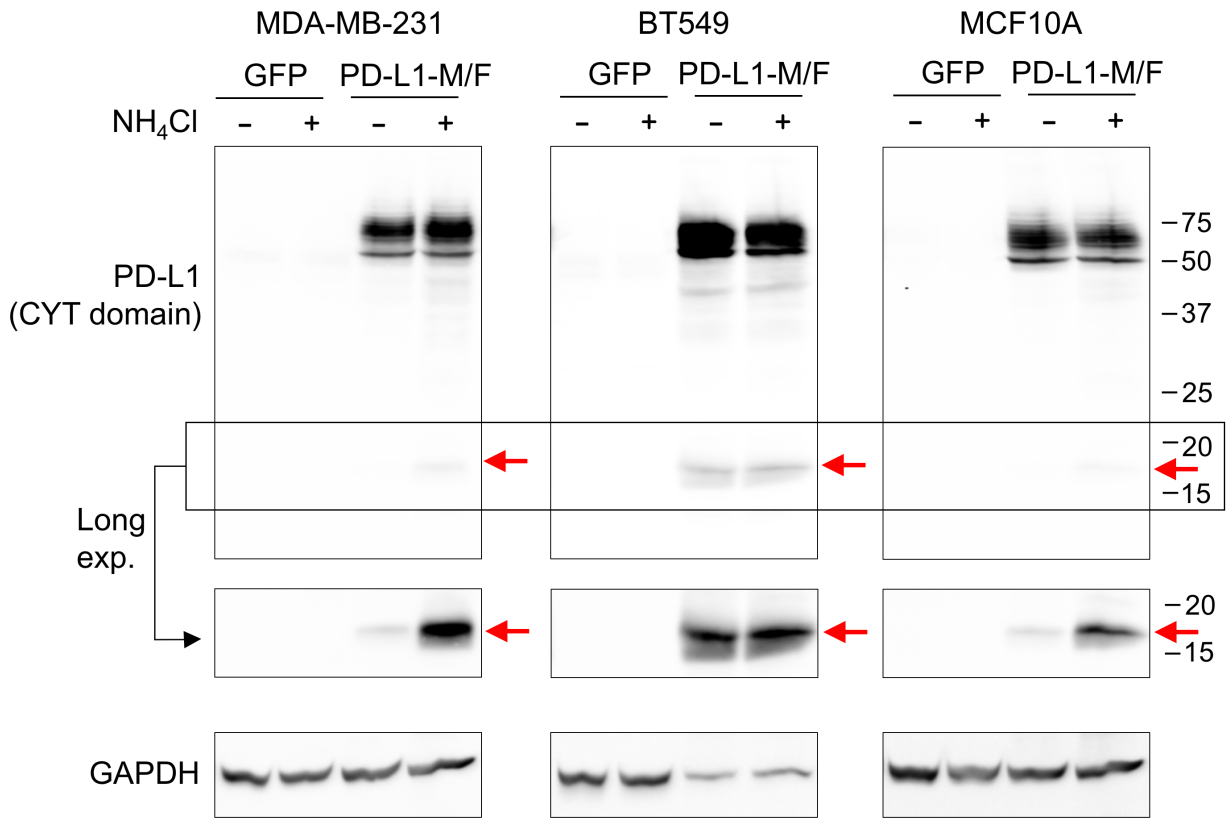
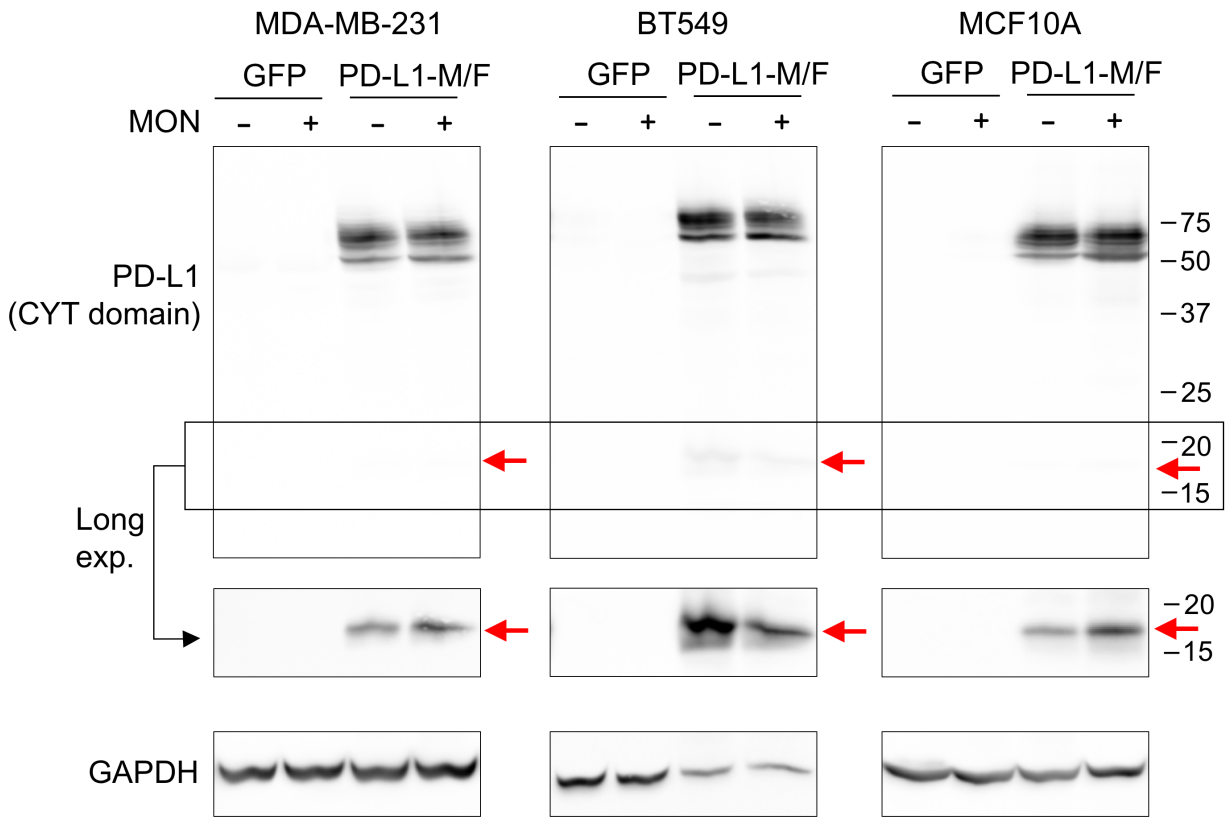
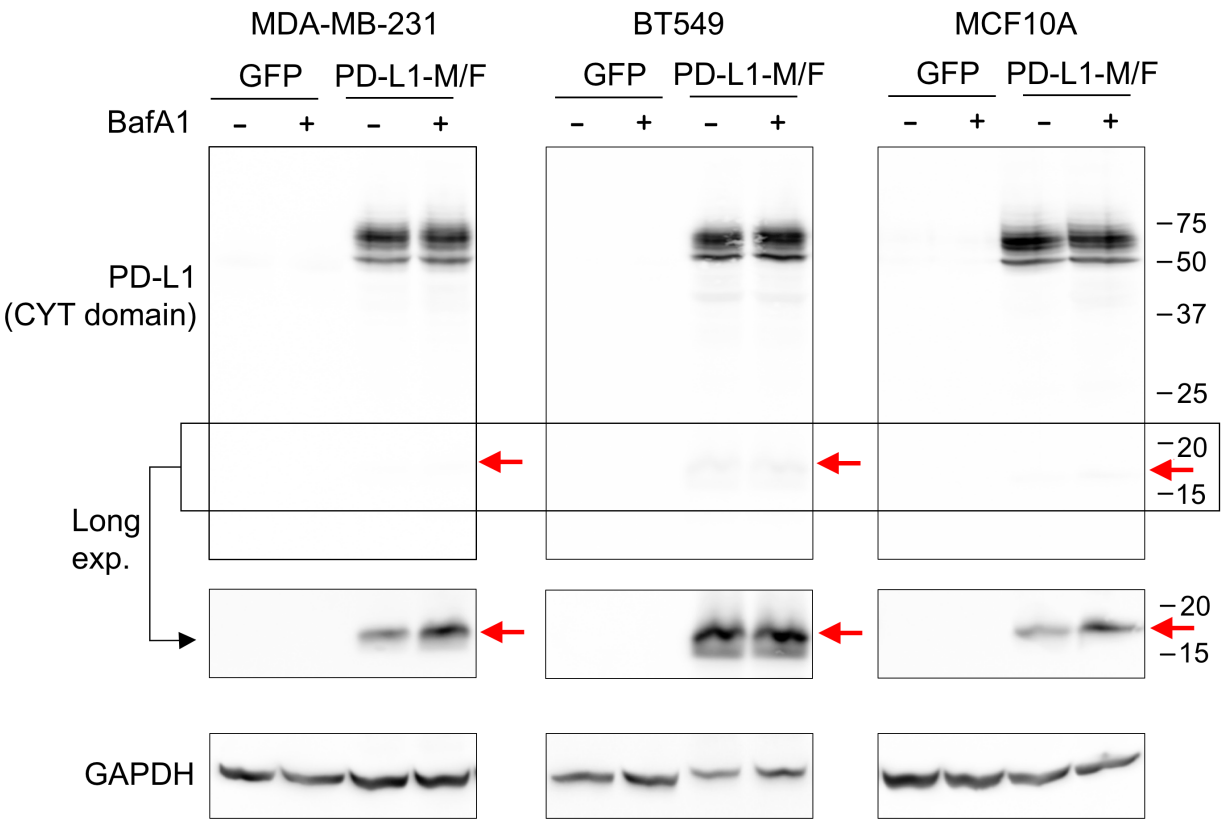


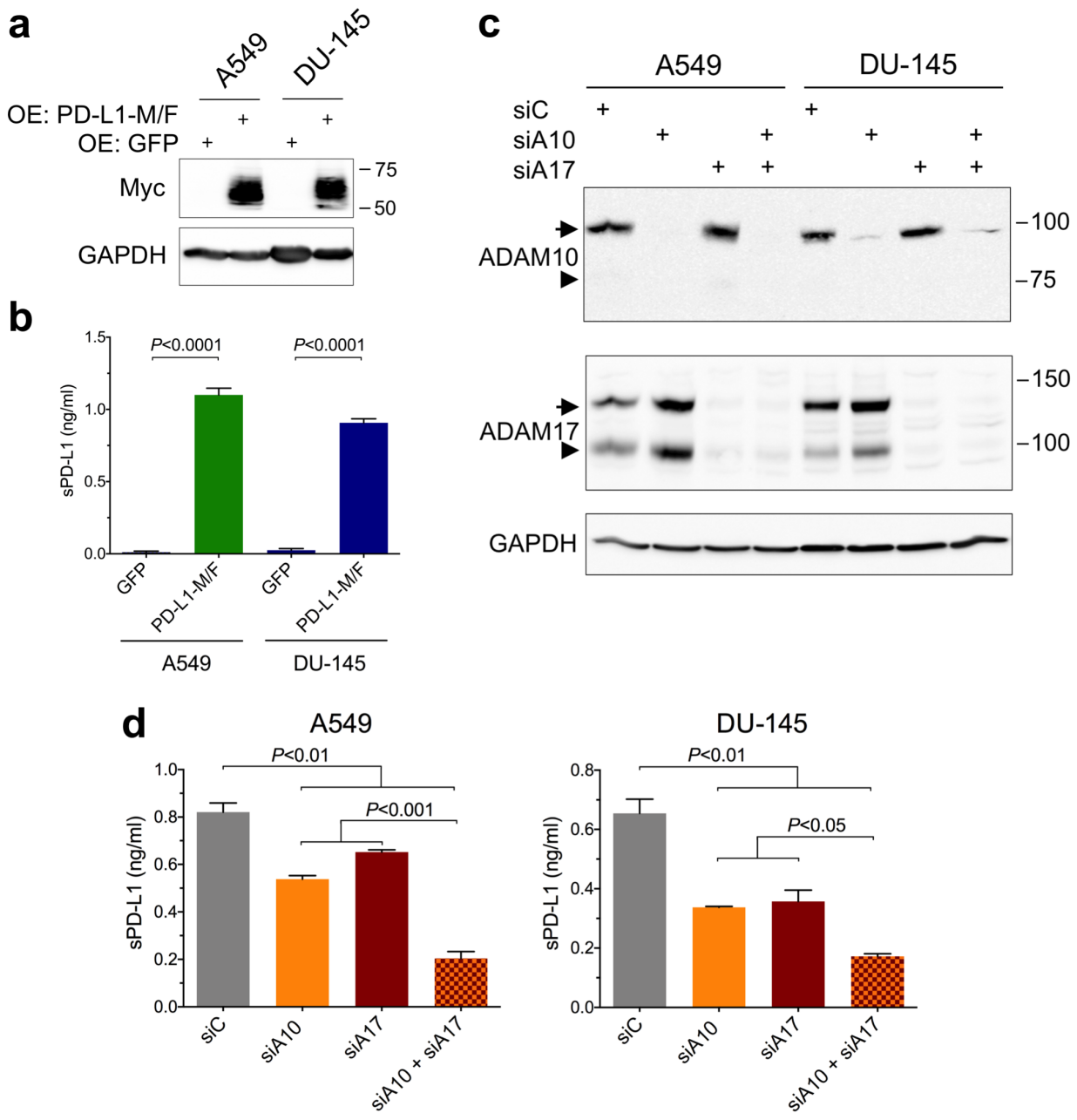
Supplementary Fig. 1 The effect of ammonium chloride treatment on the C-terminal proteolytic fragment of PD-L1-M/F. MDA-MB-231, BT549, or MCF10A cells overexpressing PD-L1-M/F or GFP (control) were incubated for 18 h in the absence or presence of 10 mM ammonium chloride (NH_4Cl). Cell lysates were analyzed by Western blotting using the E1L3N antibody specific for the cytoplasmic (CYT) domain of PD-L1. The low-molecular weight region of the blot (indicated by the box) is also shown after a longer exposure time. GAPDH is a gel loading control



Supplementary Fig. 2 The effect of monensin treatment on the C-terminal proteolytic fragment of PD-L1-M/F. MDA-MB-231, BT549, or MCF10A cells overexpressing PD-L1-M/F or GFP (control) were incubated for 4 h in the absence or presence of 20 μM monensin (MON). Cell lysates were analyzed by Western blotting using the E1L3N antibody specific for the cytoplasmic (CYT) domain of PD-L1. The low-molecular weight region of the blot (indicated by the box) is also shown after a longer exposure time. GAPDH is a gel loading control



Supplementary Fig. 3 The effect of bafilomycin A1 treatment on the C-terminal proteolytic fragment of PD-L1-M/F. MDA-MB-231, BT549, or MCF10A cells overexpressing PD-L1-M/F or GFP (control) were incubated for 4 h in the absence or presence of 0.1 μM bafilomycin A1 (BafA1). Cell lysates were analyzed by Western blotting using the E1L3N antibody specific for the cytoplasmic (CYT) domain of PD-L1. The low-molecular weight region of the blot (indicated by the box) is also shown after a longer exposure time. GAPDH is a gel loading control



Supplementary Fig. 4 The effect of ADAM10 or ADAM17 knockdown on the cleavage of PD-L1-M/F in non-breast cancer cell lines. **a** A549 lung cancer cells and DU-145 prostate cancer cells were stably transduced to express PD-L1-M/F or GFP. PD-L1 overexpression was confirmed by Western blotting using total cell lysates and anti-Myc antibody. GAPDH is a gel loading control. **b** Concentrations of sPD-L1 in the conditioned media were measured by ELISA. Values are means ± S.D. from three replicate

measurements, normalized to equal protein concentration in all cell lysates. *P* values were determined by unpaired two-tailed Student's *t* test. **c**, **d** A549-PD-L1-M/F and DU-145-PD-L1-M/F cells were transfected with a mixture of siRNA#1 and #2 targeting ADAM10, a mixture of siRNA#1 and #2 targeting ADAM17, a combination of all four siRNAs targeting both ADAM10 and ADAM17, or with control siRNA. Three days after transfection, ADAM10 and ADAM17 expression levels were analyzed by Western blotting (**c**) and concentrations of sPD-L1 in the media were measured by ELISA (**d**). Arrows, the nascent full-length ADAMs; arrowheads, the processed forms. In **d**, values are means ± S.D. from duplicate measurements, normalized to equal protein concentration in all cellular lysates. *P* values were determined by one-way ANOVA, followed by Dunnett's multiple comparisons test. Results are representative of two independent experiments.