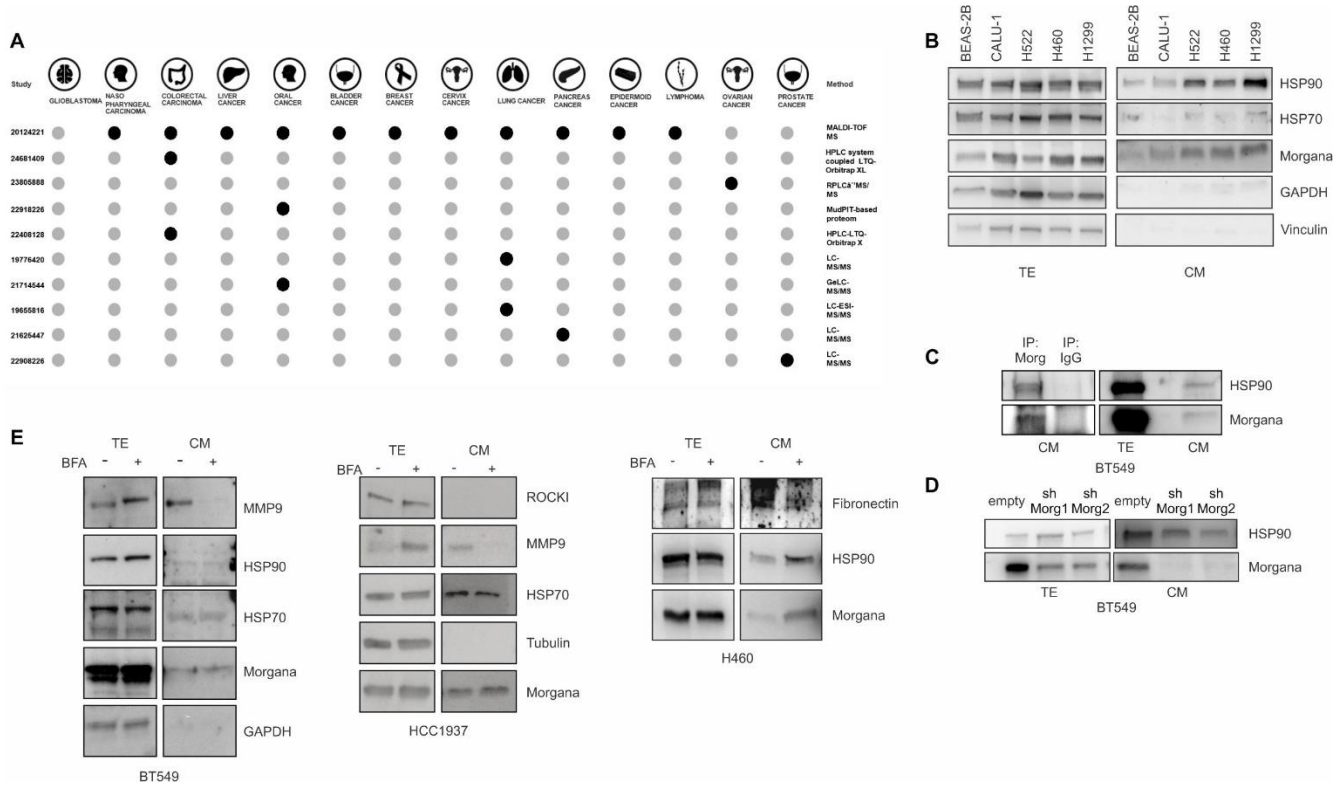
Supplementary Figures

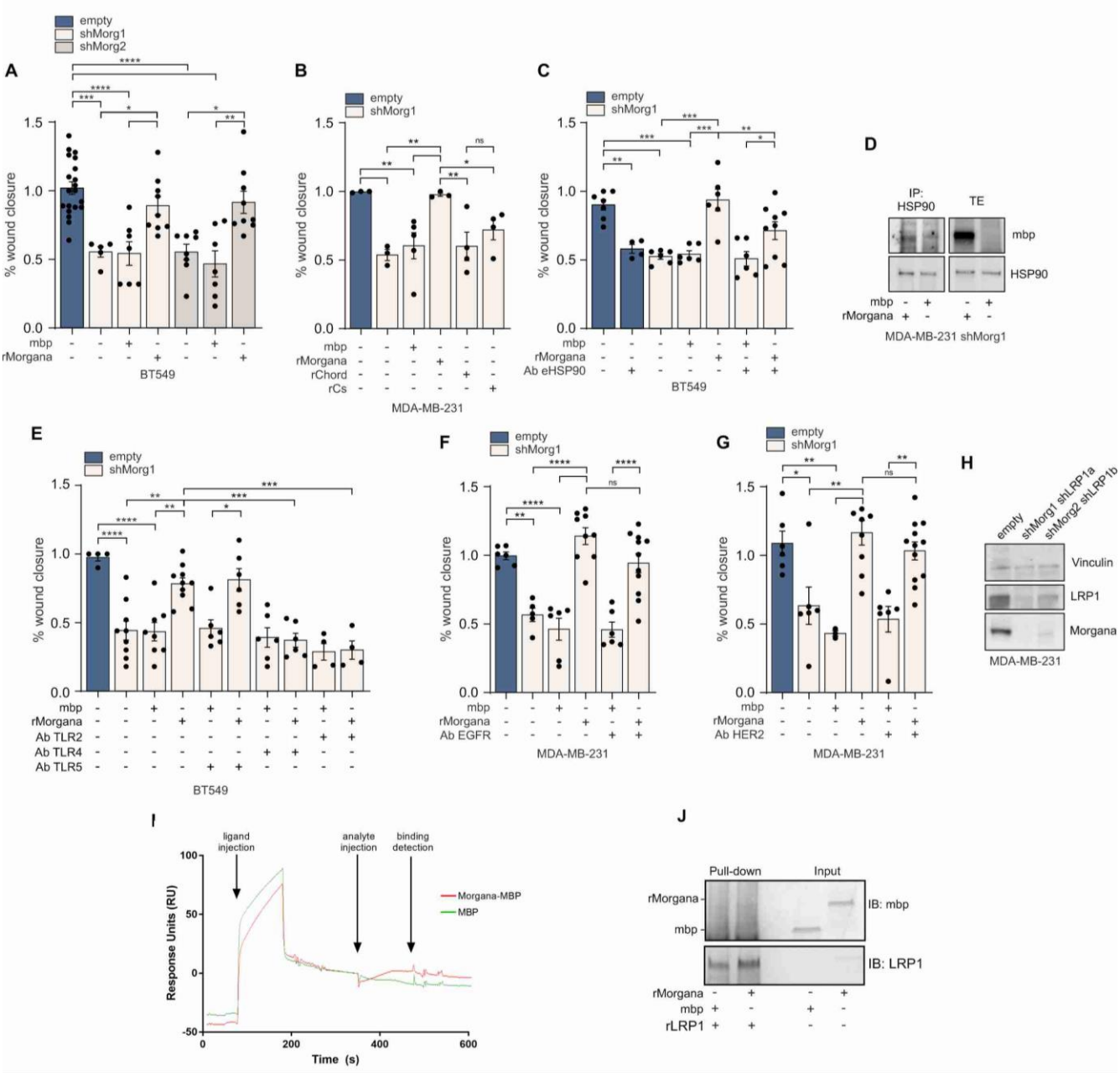
Supplementary Fig. S1



Supplementary Fig. S1. Morgana is secreted by cancer cells. (**A**) Data from Human Cancer Secretome Database indicated that Morgana is secreted by different types of cancer cells. Dark dots represent experiments in which Morgana has been identified in cancer cell secretomes. (**B**) Western blot analysis to detect Morgana, HSP70 and HSP90 in the total extracts (TE) and in the conditioned media (CM) of human lung normal and cancer cell lines. GAPDH and Vinculin were used as loading and negative control for TE and CM, respectively. (**C**) Co-immunoprecipitation experiments of Morgana and HSP90 in BT549 cell CM. An unrelated IgG was used as control. (**D**) BT549 empty and downregulated for Morgana (shMorg1/2) were analyzed by Western blotting to detect HSP90. (**E**) Western blot analysis of TE and CM of BT549, HCC1937 and H460 cells, treated with Brefeldin A (10 $\mu g\ ml^{-1}$) for 5 h. Proteins secreted through the conventional ER-Golgi pathway (MMP9 and Fibronectin) were used as control for the treatment. Proteins known to be secreted through

unconventional pathways (HSP70 and HSP90) were also analyzed. GAPDH and Tubulin were used as loading and negative control for TE and CM, respectively.

Supplementary Fig. S2



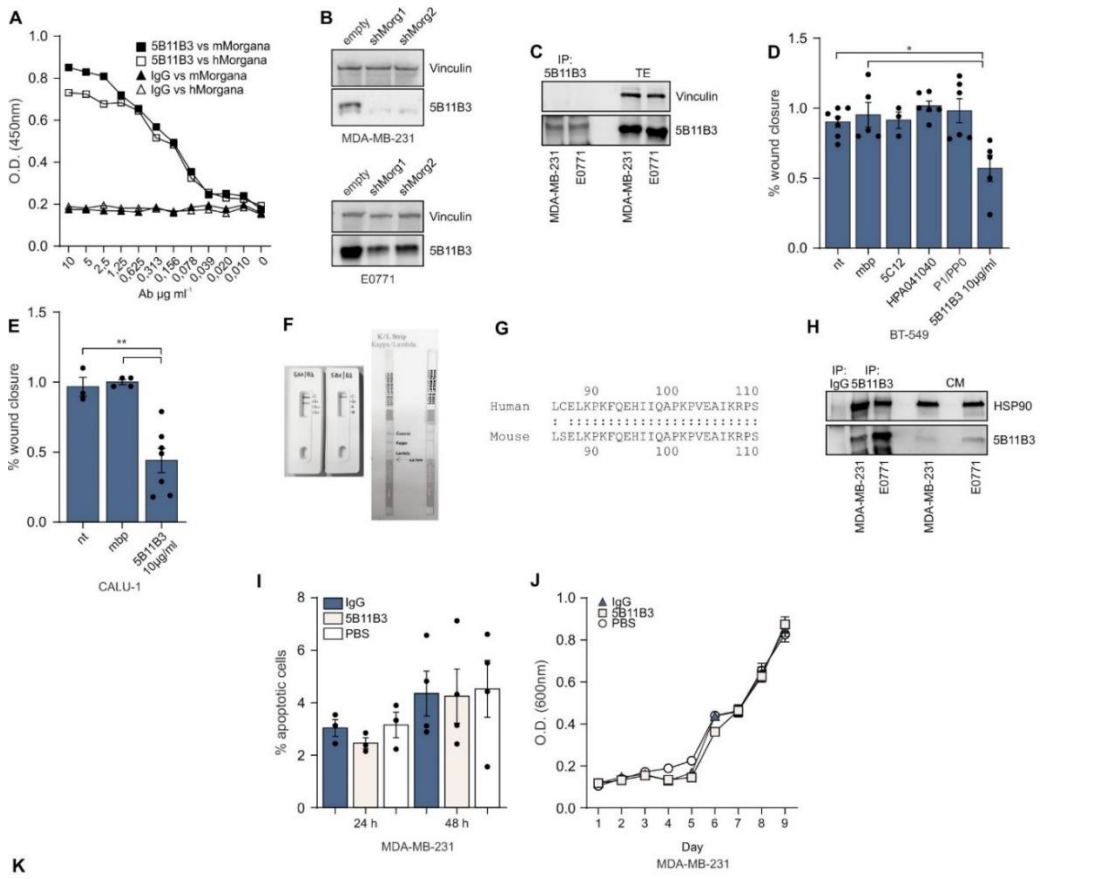
Supplementary Fig. S2. Extracellular Morgana induces migration in cancer cells. (**A**) BT549 empty and shMorg1/2 cells were treated or not with 0.1 μM of mbp or mbp-Morgana (rMorgana). Images were captured immediately after wounding and 24 h later. Bars represent the percentage of wound closure. Data are presented as median values ± SEM; n ≥ 5 experiments. One-way ANOVA tests

with Bonferroni post hoc test were performed to obtain P values. (**B**) MDA-MB-231 shMorg1 cells (treated with 0.1 μM of mbp, rMorgana, rChord or rCs) were wounded and images captured immediately and 24 h after wounding. Data are presented as median values ± SEM; n ≥ 3 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**C**) BT549 empty and shMorg1/2 cells were treated or not with 0.1 μM of mbp or rMorgana in combination or not with an HSP90-neutralizing antibody (100 ng ml^{-1}). Images were captured immediately after wounding and 24 h later. Data are presented as median values ± SEM; n ≥ 4 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**D**) MDA-MB-231 shMorg cells treated with 0.1 μM of mbp or rMorgana (2 h, 4°C) were washed and exposed to DTSSP cross-linking and lysed. Protein extracts were subjected to immunoprecipitation using anti-HSP90 antibodies. Total extracts (TE) were analyzed for the presence of rMorgana by Western blot. (**E**) BT549 shMorg1 cells, treated with 0.1 μM of mbp or rMorgana alone or in combination with a monoclonal antibody against TLR2 (30 ng ml^{-1}), TLR4 (100 ng ml^{-1}) or TLR5 (100 ng ml^{-1}) were wounded and images captured immediately and 24 h after wounding. Data are presented as median values ± SEM; n ≥ 4 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**F-G**) MDA-MB-231 shMorg1 cells, treated with 0.1 μM of mbp or rMorgana alone or in combination with a monoclonal antibody against EGFR (1 μM) or HER2 (1 μg ml^{-1}) were wounded and images captured immediately and 24 h after wounding. Data are presented as median values ± SEM; n ≥ 3 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**H**) Western blot analysis of MDA-MB-231 infected with an empty vector or with two different shRNAs against LRP1 and Morgana (shMorg1shLRP1a and shMorg2shLRP1b). Vinculin was used as loading control. (I) Upon TLR2 (ligand) immobilization onto a CM5 sensor chip through anti-His antibody, 1 μM of mbp-Morgana or mbp alone (analytes) were injected. A specific binding was detected only when mbp-Morgana fusion protein was used (120 s from analyte injection). (J) Pull down experiments testing the ability of a recombinant protein

containing the LRP1 cluster II domain to interact directly with rMorgana. The interaction has been tested in unconditioned medium (*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$; ****$p < 0.0001$).

Supplementary Fig. S3

Figure S3



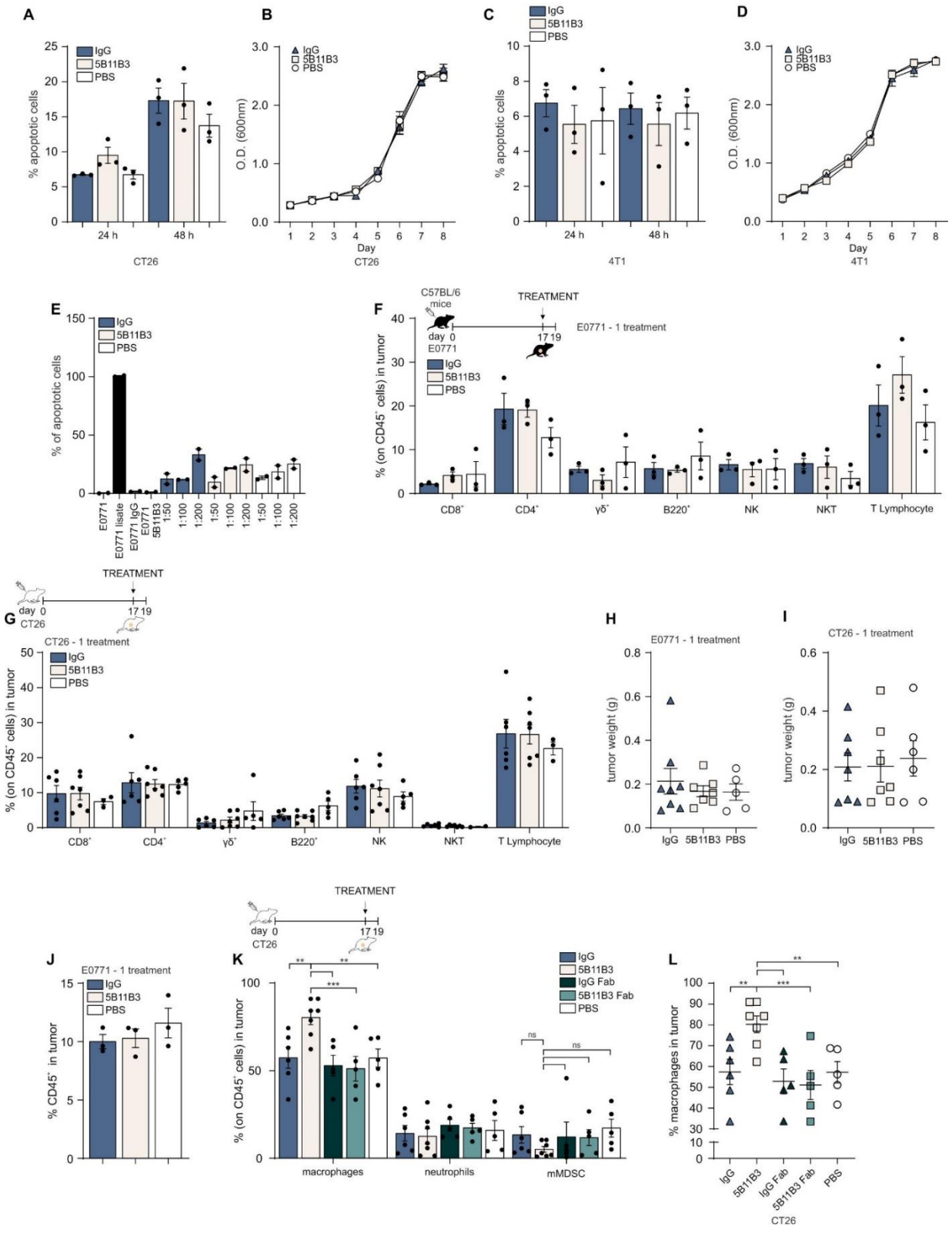
C57BL/6	Amylase (UI)	Aspartate Aminotransferase (UI)	Alanine Aminotransferase (UI)	Creatinine (mg dl^{-1})	Urea (mg dl^{-1})
IgG	417 ± 23	175 ± 11	38 ± 3	0.28 ± 0.1	68 ± 9
5B11B3	361 ± 18	160 ± 27	46 ± 7	0.36 ± 0.06	46 ± 3

BALB/c	Amylase (UI)	Aspartate Aminotransferase (UI)	Alanine Aminotransferase (UI)	Creatinine (mg dl^{-1})	Urea (mg dl^{-1})
IgG	620 ± 60	144 ± 32	42 ± 11	0.33 ± 0.09	38 ± 8
5B11B3	584 ± 24	138 ± 58	36 ± 10	0.38 ± 0.05	46 ± 4

Supplementary Fig. S3. Characterization of mAb 5B11B3. (**A**) ELISA assays with serial dilution of mAb 5B11B3 or control IgG on human and mouse rMorgana proteins (10 μg ml^{-1}). (**B**) Western blot analysis with mAb 5B11B3 of MDA-MB-231 and E0771 empty or shMorg1 and shMorg2. Vinculin was used as loading control. (**C**) mAb 5B11B3 was used in an immunoprecipitation experiment from MDA-MB-231 and E0771 total extracts. Western blot analysis was performed using mAb 5B11B3 and Vinculin. (**D**) BT549 cells were treated with different antibodies against Morgana (10 μg ml^{-1}), wounded and images were captured immediately and 24 h later. An antibody against mbp was used as negative control. Data are presented as median values ± SEM; n ≥ 3 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**E**) CALU-1 cells were treated with mAb 5B11B3 (10 μg ml^{-1}), wounded and images were captured immediately and 24 h after wounding. An anti-mbp antibody was used as negative control. Data are presented as median values ± SEM; n ≥ 3 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**F**) Characterization of mAb 5B11B3 isotype and light chains. (**G**) Comparison of the amino acidic sequence of human and mouse Morgana from amino acid 85 to 110. (**H**) mAb 5B11B3 was used in an immunoprecipitation experiment from MDA-MB-231 and E0771 CM. Western blot analysis was performed using HSP90 and mAb 5B11B3. (**I**) Percentage of apoptotic MDA-MB-231 cells assessed by annexin V and propidium iodide staining at 24 and 48 h after treatment with control IgG or mAb 5B11B3 (10 μg ml^{-1}). Data are presented as median values ± SEM; n ≥ 3 experiments. (**J**) Proliferation assay on MDA-MB-231 cells treated with control IgG or mAb 5B11B3 (10 μg ml^{-1}). Data are presented as median values ± SEM; n ≥ 3 experiments. (**K-L**) Analysis of blood of C57BL/6 and BALB/c mice treated with mAb 5B11B3 or control IgG (100 μg, IV injection, 3 times per week). (**M**) CT26 cells were treated with mAb 5B11B3 or control IgG (10 μg ml^{-1}), wounded and images captured immediately and 24 h after wounding. Data are presented as median values ± SEM; n ≥ 9 experiments. One-way ANOVA tests with Bonferroni post hoc test were performed to obtain P values. (**N**) Lung metastatic burden of BALB/c mice injected with 1 x 10^5 4T1 and treated with intratumoral injection of rMorgana or mbp as control (100 μg, 4 injections, 2 times per week). Black arrow indicate

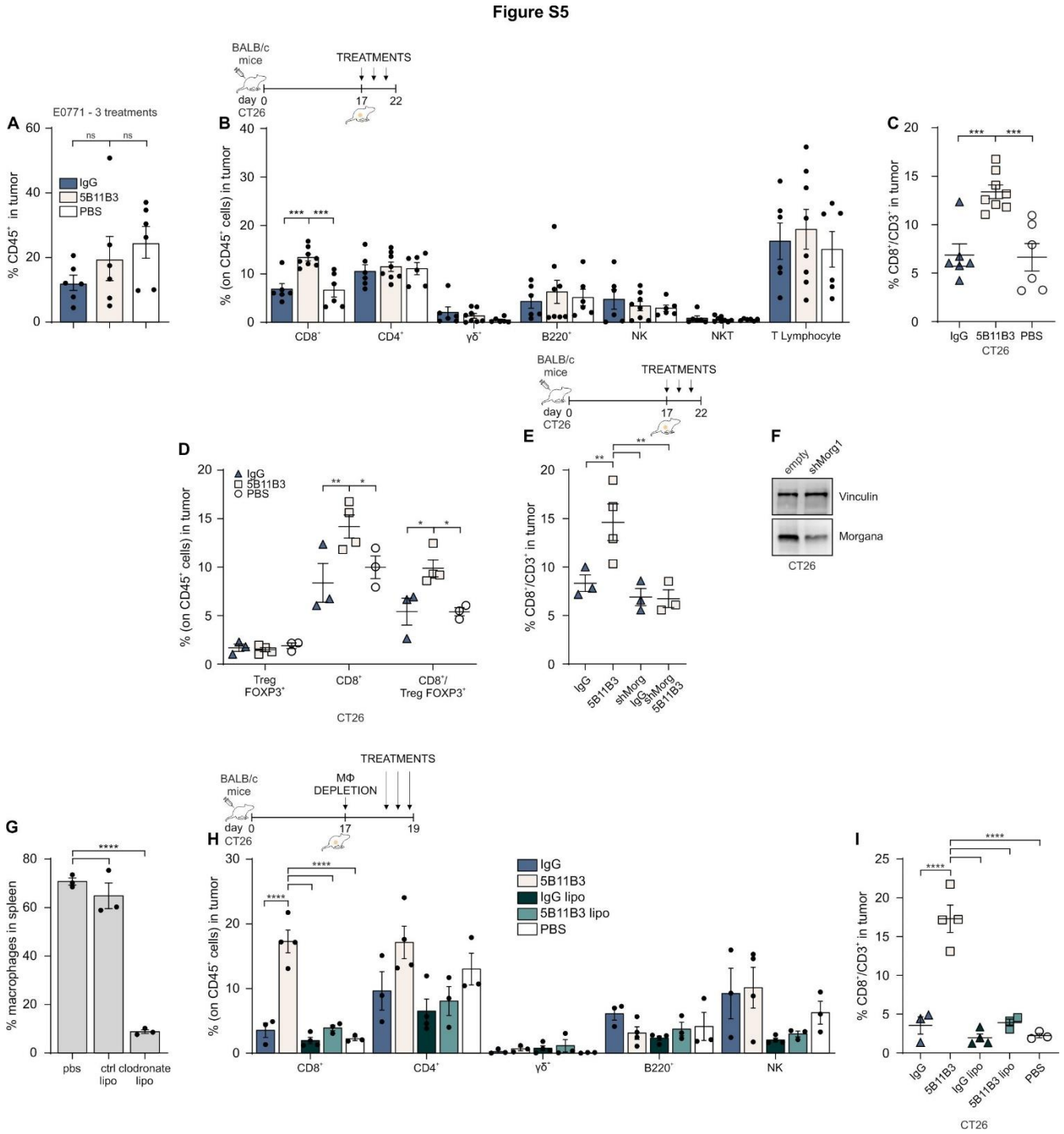
micrometastasis. Data are presented as median values ± SEM; n ≥ 7 lung lobes. One-way ANOVA tests with Fisher's LSD test post hoc were performed to obtain P values. (**O**) Proliferation assay on CT26 empty or shMorg1 and shMorg2 cells. Data are presented as median values ± SEM; n ≥ 3 experiments. (**P**) Tumor weight of BALB/c mice subcutaneously inoculated with 1 x 10^5 4T1 cells and treated with mAb 5B11B3 or control IgG (100 μg, IV injection, 3 times per week). Animals were sacrificed after 15 days. Data are presented as median values ± SEM; n ≥ 3 mice per group (*$p < 0.05$; **$p < 0.005$).

Supplementary Fig. S4



Supplementary Fig. S4. mAb 5B11B3 recruits macrophages in the primary tumor. (**A**) Percentage of apoptotic CT26 cells assessed by annexin V and propidium iodide staining at 24 and 48 h after treatment with control IgG or mAb 5B11B3 (10 μg ml^{-1}). Data are presented as median values ± SEM; n = 3 experiments. **(B)** Proliferation assay on CT26 cells treated with control IgG or mAb 5B11B3 (10 μg ml^{-1}) Data are presented as median values ± SEM; n = 3 experiments. (**C**) Percentage of apoptotic 4T1 cells assessed by annexin V and propidium iodide staining at 24 and 48 h after treatment with control IgG or mAb 5B11B3 (10 μg ml^{-1}). Data are presented as median values ± SEM; n = 3 experiments. **(D)** Proliferation assay on 4T1 cells treated with control IgG or mAb 5B11B3 (10 μg ml^{-1}). Data are presented as median values ± SEM; n = 3 experiments. (**E**) ADCC: percentage of E0771 apoptotic cells after co-culture of E0771 cells with splenocytes from C57BL/6 mice using 3 different E:T ratios (50:1, 100:1 and 200:1) in the presence of the mAb 5B11B3, control IgG (10 μg) or vehicle (PBS). (**F-J**) C57BL/6 mice or BALB/c mice subcutaneously inoculated respectively with 2 x 10^5 E0771 or 2 x 10^5 CT26 and after tumor growth, treated with mAb 5B11B3, IgG or vehicle (PBS) (100 μg, IV injection) and sacrificed after 24 h. (**F-G**) Lymphoid immune composition of the primary tumor, (**H-I**) tumor weight, (**J**) identification of $CD45^+$ cells in E0771-derived tumors. (**K-L**) BALB/c mice inoculated subcutaneously with 2 x 10^5 CT26. After primary tumor development (size around 15 mm^3), animals were treated with mAb 5B11B3, control IgG, 5B11B3 Fab, control IgG Fab or vehicle (PBS) one time (100 μg, IV injection) and sacrificed after 24 h. (**K**) cytofluorimetric analysis of tumor-infiltrating myeloid cells; (**L**) percentage of tumor-infiltrating macrophages. Data are presented as median values ± SEM; n ≥ 3 mice per group. One-way ANOVA tests with Fisher's LSD test post hoc test were performed to obtain P values. (**p < 0.005; ***p < 0.001).

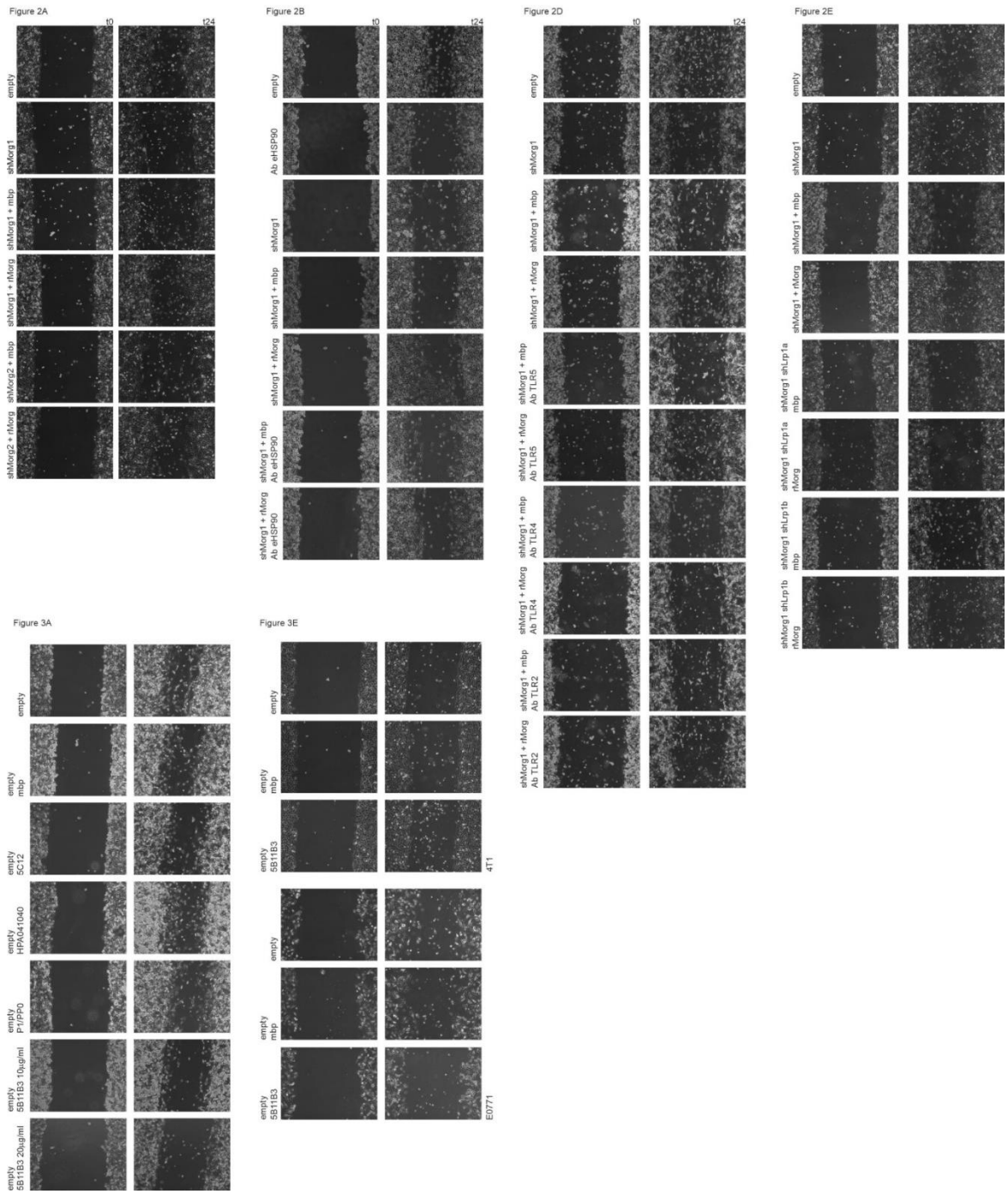
Supplementary Fig. S5



Supplementary Fig. S5. mAb 5B11B3 requires macrophages to induce $CD8^+$ T lymphocytes recruitment in the primary tumor. (**A**) Percentage of $CD45^+$ in the primary tumor of E0771 cancer

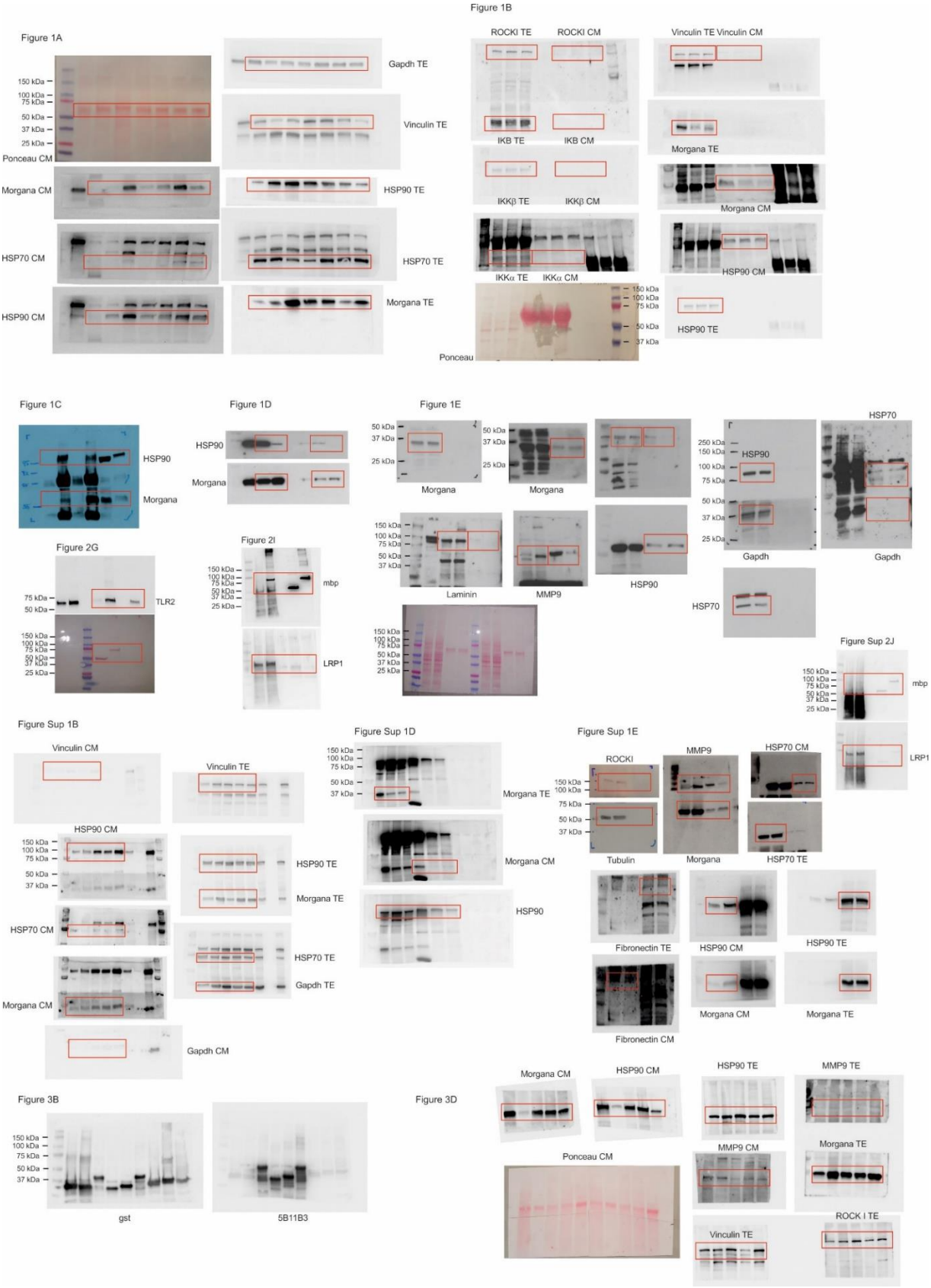
bearing mice, treated 3 times with mAb 5B11B3, IgG or PBS (100 μg, IV injection). Data are presented as median values ± SEM; n = 6 mice per group. (**B, C, D**) Identification of immune composition in the primary tumor after treatments with mAbs. (**B**) Percentage of immune cells, (**C**) of $CD8^+$ T lymphocytes and (**D**) of Treg $FOXP3^+$, $CD8^+$ T lymphocytes and the ratio of $CD8^+$ T lymphocytes/Treg $FOXP3^+$ in tumors of BALB/c mice subcutaneously inoculated with 2 x 10^5 CT26. After primary tumor development (size around 15 mm^3), animals were treated with mAb 5B11B3, IgG or PBS 3 times (100 μg, IV injection) and sacrificed. Data are presented as median values ± SEM; n ≥ 3 mice per group. One-way ANOVA tests with Fisher's LSD test post hoc test were performed to obtain P values. (**E-F**) Percentage of $CD8^+$ T lymphocytes in tumors of BALB/c mice subcutaneously inoculated with 2 x 10^5 CT26 empty or shMorg. (**E**) After primary tumor development (size around 15 mm^3), animals were treated with mAb 5B11B3 or control IgG 3 times (100 μg, IV injection) and sacrificed. Data are presented as median values ± SEM; n ≥ 3 mice per group. One-way ANOVA tests with Fisher's LSD test post hoc test were performed to obtain P values. (**F**) Western blot on CT26 infected with an empty lentiviral vector (empty) or a vector containing a shRNA for Morgana (shMorg1). Vinculin was used as loading control (**G**) Analysis of macrophage depletion in spleen of mice treated with clodronate liposomes, control liposomes (100 μg per 10 gr/mouse) or PBS. Data are presented as median values ± SEM; n = 3 mice per group. (**H**) Percentage of immune cells and (**I**) percentage of $CD8^+$ T lymphocytes in tumors of BALB/c mice subcutaneously inoculated with 2 x 10^5 CT26. After primary tumor development (size around 15 mm^3), animals were treated with clodronate liposomes in PBS (100 μg per 10 gr/mouse) and after 4 days mice were treated with mAb 5B11B3 or IgG (100 μg, IV injection) 3 times and sacrificed. Data are presented as median values ± SEM; n ≥ 3 mice per group. One-way ANOVA tests with Fisher's LSD test post hoc test were performed to obtain P values. (*$p < 0.05$; **$p < 0.005$; ***$p < 0.001$; ****$p < 0.0001$).

Supplementary Fig. S6



Supplementary Fig. S6. Wound healing representative images.

Supplementary Fig. S7



Supplementary Fig. S7. Uncropped images of Western blot analysis. Note that in some cases, nitrocellulose membranes had been probed with different antibodies in succession and residual bands of previous staining are visible.