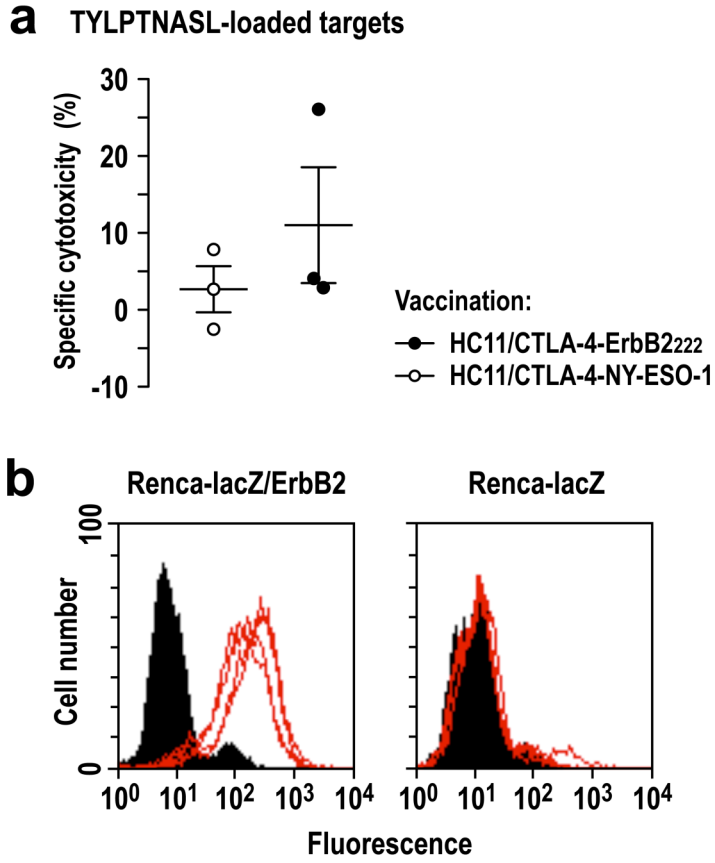
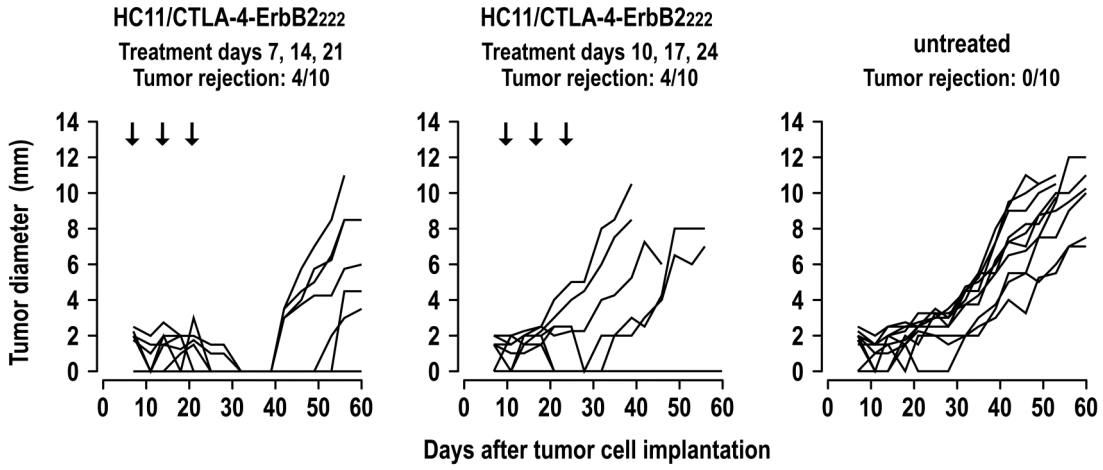
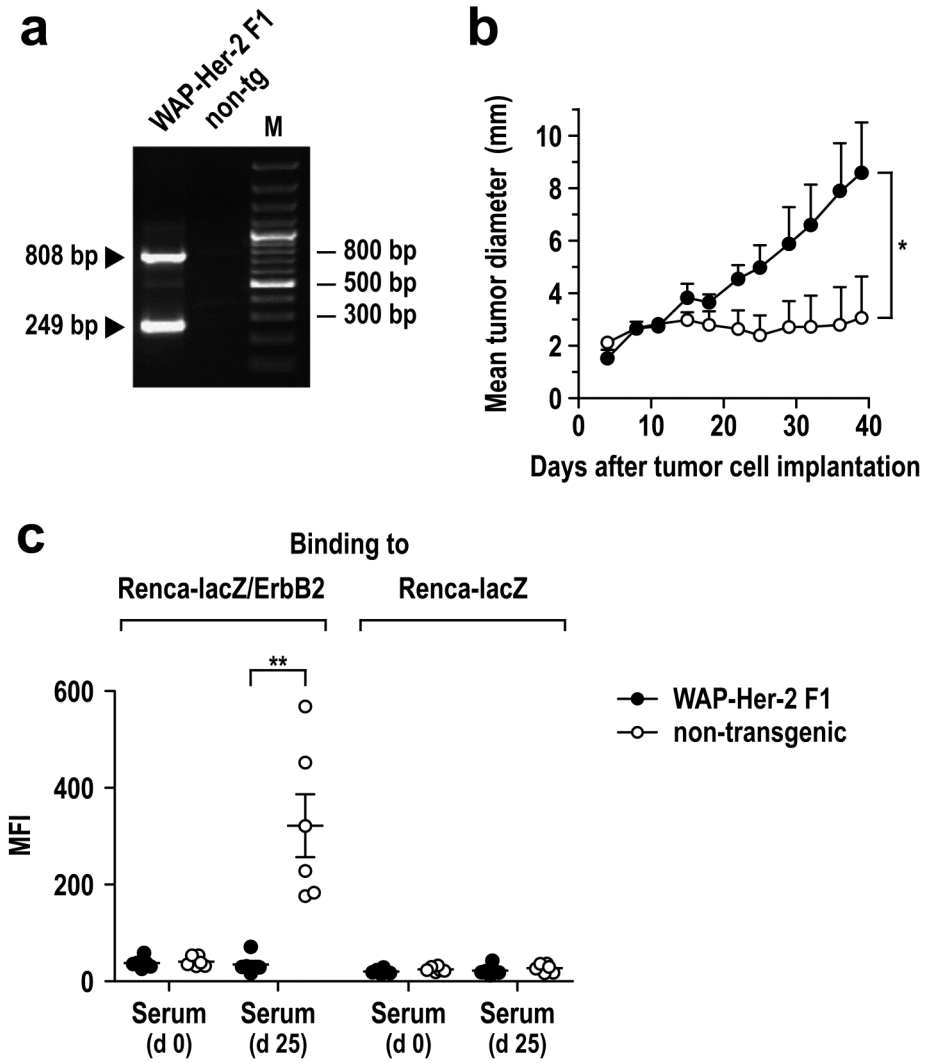


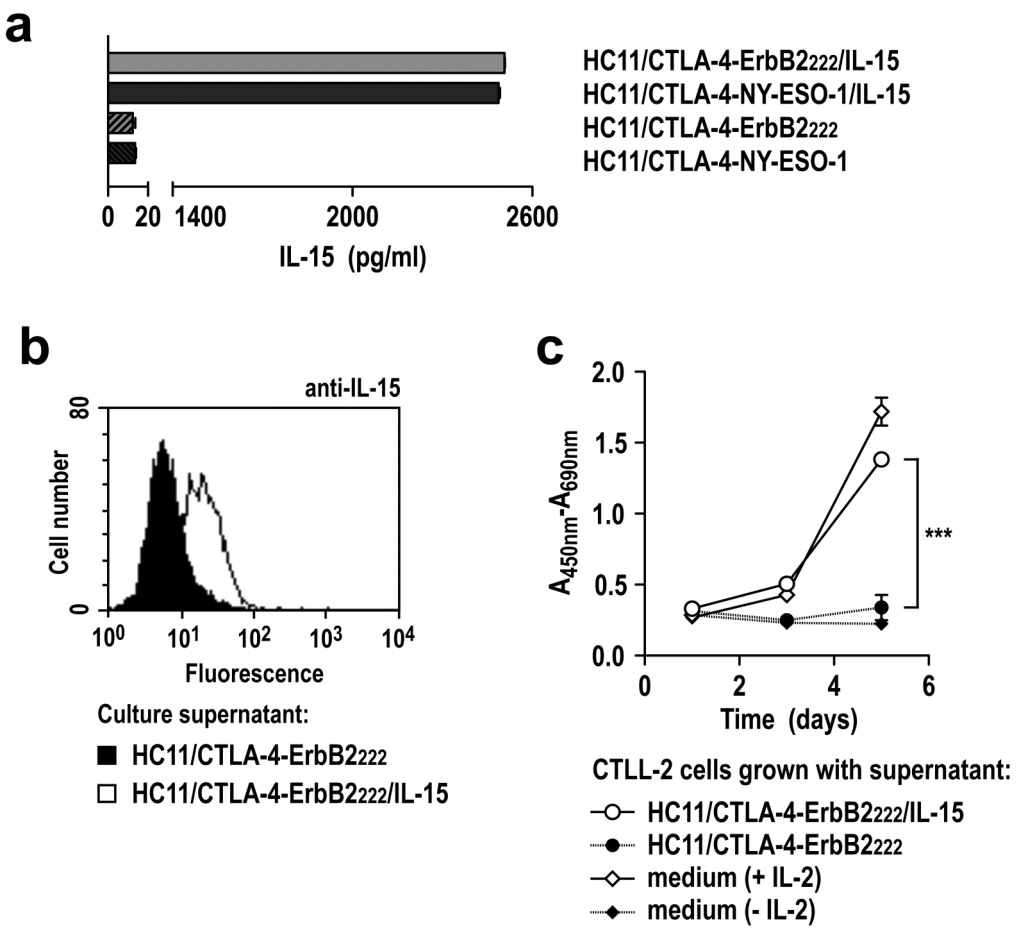
Supplementary Fig. 1 ErbB2-specific immune responses after treatment of BALB/c mice with vaccine-producing cells. **a** *In vivo* cytotoxicity assay. Naïve animals that had not received prior tumor cell inoculations were injected s.c. with HC11/CTLA-4-ErbB2$_{222}$ cells secreting the CTLA-4-ErbB2$_{222}$ vaccine (n=3), or HC11/CTLA-4-NY-ESO-1 control cells (n=3) at days 0, 7 and 14. One week after the last injection of vaccine-producing cells, *in vivo* cytotoxicity assays were performed with donor splenocytes pulsed with ErbB2 peptide TYLPTNASL and the percentage of specific target cell killing was calculated. **b** ErbB2-specific antibody responses. In a separate experiment, peripheral blood was collected from mice three weeks after the last injection of HC11/CTLA-4-ErbB2$_{222}$ cells (n=4), and 1:100 dilutions of sera were analyzed by flow cytometry for binding to Renca-lacZ/ErbB2 (left panel) or ErbB2-negative Renca-lacZ cells (right panel) (red lines). Sera collected before vaccination served as controls (filled areas).



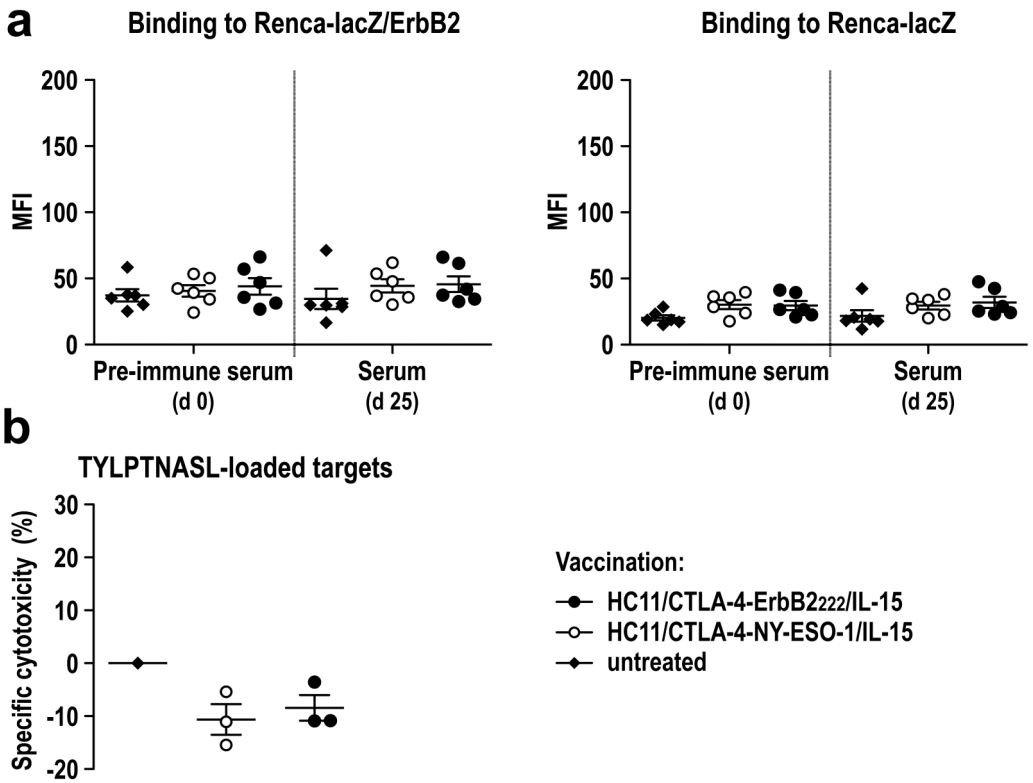
Supplementary Fig. 2 Therapeutic vaccination of tumor-bearing BALB/c mice with vaccine-producing HC11/CTLA-4-ErbB2$_{222}$ cells at later time points. Renca-lacZ/ErbB2 renal carcinoma cells were injected s.c. into both flanks of BALB/c mice. Established tumors were treated by s.c. injection of vaccine-producing HC11/CTLA-4-ErbB2$_{222}$ cells in the tumor vicinity at days 7, 14 and 21 (left), or at days 10, 17 and 24 (middle) after tumor cell inoculation. Control animals were left untreated (right). Tumor growth was followed by caliper measurements. Each line represents growth kinetics of an individual tumor. The fraction of rejected tumors at day 60 is indicated. Arrows specify the time points of vaccination.



Supplementary Fig. 3 Immunological tolerance of WAP-Her-2 F1 mice to human ErbB2. **a** Transgenic WAP-Her-2 F1 mice were obtained by crossing female BALB/c with male WAP-Her-2 mice. Transgene-positive animals were identified by PCR with oligonucleotide primers 5'-CCCCCACCCCACCCCCAAAGTC-3', 5'-ACAACCAAGTGAG-GCAGGTC-3', and 5'-CCTTCCACAAAATCGTGTCC-3' resulting in the amplification of 808 bp and 249 bp products. **b** WAP-Her-2 F1 mice (n=6) and non-transgenic littermates (n=8) were inoculated s.c. with Renca-lacZ/ErbB2 tumor cells and tumor growth was followed. Mean tumor diameters ± SEM are shown; *, $p < 0.05$. Five of 8 non-transgenic and 1 of 6 WAP-Her-2 F1 animals rejected their tumors. **c** ErbB2-specific antibody responses. Twenty-five days after tumor cell inoculation peripheral blood was collected from the mice, and 1:20 dilutions of sera were analyzed by flow cytometry for binding to Renca-lacZ/ErbB2 or ErbB2-negative Renca-lacZ cells. Sera collected before tumor cell injection served as controls. Mean fluorescence intensities (MFI) for sera from individual animals, and mean values ± SEM are shown; **, $p < 0.01$.



Supplementary Fig. 4 Generation of vaccine-producing HC11 cells that secrete IL-15. HC11/CTLA-4-ErbB2$_{222}$ and HC11/CTLA-4-NY-ESO-1 cells were transduced with a lentiviral vector encoding IL-15, and presence and functionality of IL-15 in culture supernatants was analyzed. **a** The concentration of IL-15 secreted into HC11 cell culture supernatants was determined by ELISA. Parental HC11 transfectants served as controls. Mean values ± SEM are shown (n=3). **b** Binding of IL-15 present in culture supernatant of HC11/CTLA-4-ErbB2$_{222}$/IL-15 cells to IL-15Rα-expressing Raji cells was confirmed by flow cytometry. Culture supernatant of HC11/CTLA-4-ErbB2$_{222}$ cells served as control. **c** Biological activity of IL-15 secreted by HC11/CTLA-4-ErbB2$_{222}$/IL-15 cells was analyzed in CTLL-2 proliferation assays. Culture supernatant of HC11/CTLA-4-ErbB2$_{222}$ cells, and medium containing or lacking IL-2 served as controls. Proliferation and survival of cells was followed by WST-1 assay, and absorption at 450 nm was determined. Mean values ± SEM are shown (n=3); ***, $p < 0.001$.



Supplementary Fig. 5 Evaluation of ErbB2-specific immune responses after treatment of tumor-bearing WAP-Her-2 F1 mice with vaccine- and IL-15-producing HC11/CTLA-4-ErbB2$_{222}$/IL-15 cells. **a** ErbB2-specific antibody responses. At day 25 of the treatment schedule peripheral blood was collected from 6 mice of each group treated with HC11/CTLA-4-ErbB2$_{222}$/IL-15 or HC11/CTLA-4-NY-ESO-1/IL-15 cells, or left untreated from the experiment shown in Fig. 5a. 1:20 dilutions of sera were analyzed by flow cytometry for binding to Renca-lacZ/ErbB2 (left) or ErbB2-negative Renca-lacZ cells (right). Pre-immune sera served as controls. Mean fluorescence intensities (MFI) for sera from individual animals, and mean values ± SEM are shown. **b** *In vivo* cytotoxicity assay. In a separate experiment, Renca-lacZ/ErbB2 renal carcinoma cells were injected s.c. into the interscapular region of ErbB2-transgenic WAP-Her-2 F1 mice and animals were treated with HC11/CTLA-4-ErbB2$_{222}$/IL-15 (n=3) or HC11/CTLA-4-NY-ESO-1/IL-15 cells (n=3) as described in the legend of Fig. 5a. An untreated animal was included as control. One week after the last treatment, *in vivo* cytotoxicity assays were performed with donor splenocytes pulsed with ErbB2 peptide TYLPTNASL and the percentage of specific target cell killing was calculated.