

Supplementary Material

Anti-Vascular Endothelial Growth Factor C Antibodies Efficiently Inhibit the Growth of Experimental Clear Cell Renal Cell Carcinomas

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MHLLGFFSVACSLAAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQLRSVSSV
DELMTVLYPEYWKMYKCQLRKGWQHNRQANLNSRTEETIKFAAAHYNTEILKSIDNEWKTKO
CMPREVCIDVGKEFVATNTEFKPPCVSVYRCGGCCNSEGLOCMNTSTSYLSKTLFEITVPLSOQPKP
VTISFANHTSCRCMSKLDVYRQVHSIIRSLPATLPQCQAANKTCPTNYMWNNHICRCLAQEDFM
FSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLRPASCGPHKELDRNSCQCVCCKNKLFPSC
GANREFDENTCQCVCCKRTCPRNQPLNPGKCACTESPQKCLLKGGKFFHHQTCSCYRRPCTNRQ
KACEPGFSYSEEVCRCPVSYWKRQMSL

XXX Signal peptide

XXX propeptide

XXX Mature peptide

Figure S1. The mature peptide (underlined) was chosen for the immunization protocol.

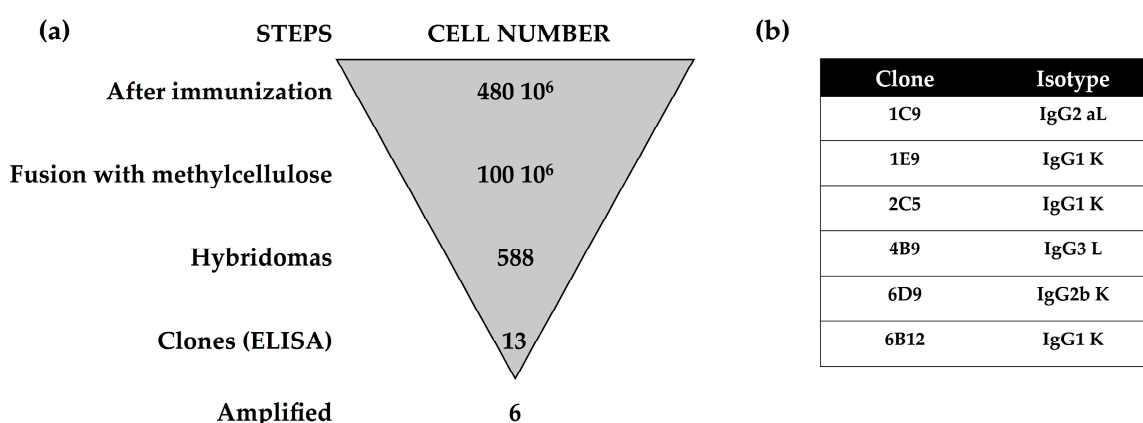


Figure S2. Description of the flow chart for the selection of the relevant hybridomas. (a) The bottlenecked, step-by-step procedure to select the best hybridomas (three rounds of subcloning). (b) Isotyping of the antibodies produced by the selected hybridomas.

(a) Light chain nucleotide sequence
 GATGTTGTGATGACCCAACTCCACTCTCCCTGACTGTCTGAGATCAAGCCTCCATCTCTTGAGATCTAGTCAGAGCCTTGT
 ACACAGTAATGGAAACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCGAAAGCTCCTGATCTACAAAGTTTTCAACCGA
 TTGTCTGGGGTCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTACACTCAAGATCAGTAGAGTGGAGGCTGAGGATCTG
 GGAGTTTATTTCTGCTCTCAAATAACGCATGTTCCGTGGACGTTCCGTGGAGGCACCAAGTTGGAATCAAA

Heavy chain nucleotide sequence
 CCAGGCCCAACTGCGGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTACACCTT
 CATCAGCTACTGGATGCACTGGGTGAAAACAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAGAGATTAATCCTAGCAACGGTCGTAC
 TAACTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAGATCCTCCAGCACAGCCTACATGCAACTCAGCAGCCTGA
 CATCTGAGGACTCTGCGGTCTATTACTGTGCAAGCACCTCTCTTTTACTATGCTATGAATTACTGGGGTCAAGGAACCTCAGTCACC
 GTCTCCTCA

(b) Light chain protein sequence

CDR1	CDR2	CDR3
DVVMQTPLSLTVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVFNRISGVDPDRFSGSGSDFTLKISRVEAEDLGVYFCSQNTHPVPTFGGGTKLEIK		

Heavy chain protein sequence

CDR1	CDR2	CDR3
QAQLRQPGAELVKPGASVKLSCKASGYTFISYWMHWVKQRPGQGLEWIGIEINPSNGRTNYNEKFKSKATLTVDSSSTAYMQLSSLTSEDSAVYYCASTSLFYAMNYWGQGSTVTVSS		

Figure S3. DNA and protein sequences of the variable light and heavy chains of the 1E9 antibodies: (a) DNA sequences; (b) protein sequences.

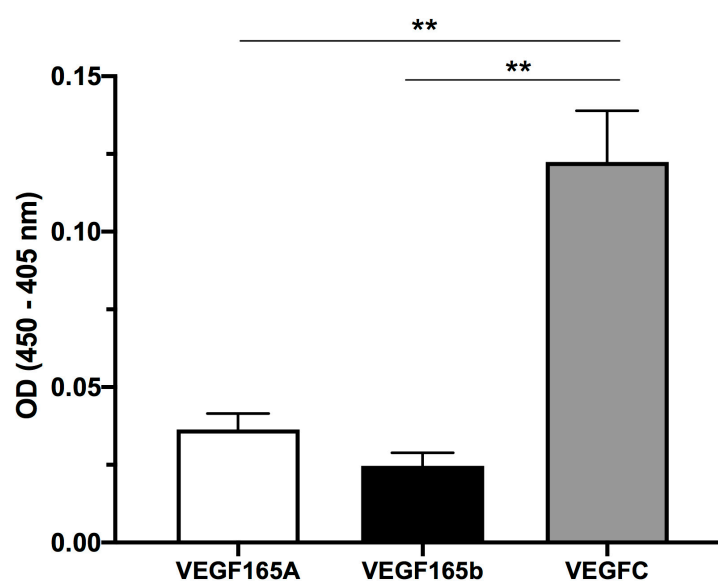


Figure S4. Specificity of 1E9 antibodies: an amount of 100 ng of recombinant VEGF165A, VEGF165b or VEGFC was immobilized overnight in a 96-well plate. 1E9 antibodies (10 µg/mL) were incubated for 1h, and revelation was performed with TMB substrate after incubation with a goat anti-human HRP antibody. OD was assessed at 450 nm and 405 nm after the addition of HCl solution to stop the reaction. $n = 4$; **, $p < 0.01$.

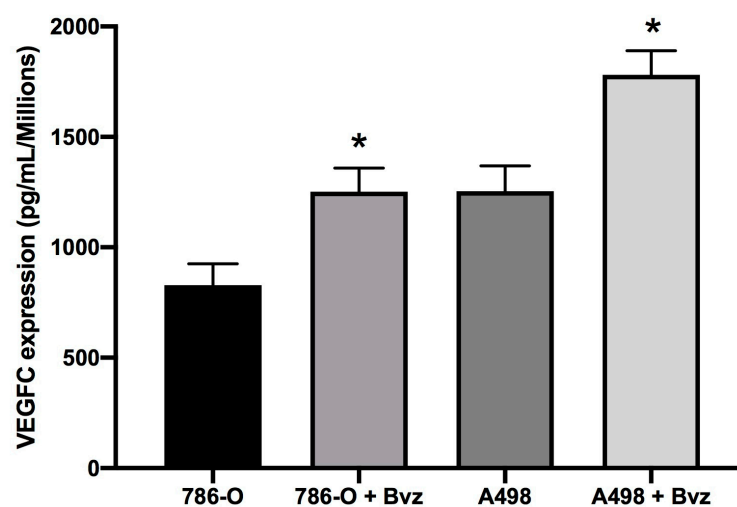


Figure S5. Bevacizumab increased VEGFC expression in 786-O and A498 cells. RCC cells were seeded in 6-well plates and treated for 48 hours with bevacizumab (BVZ, 10 μ g/mL). VEGFC levels were evaluated by ELISA with the R&D systems ELISA kit according to the manufacturer's recommendations. $n = 4$; *, $p < 0.05$ vs. untreated cells.

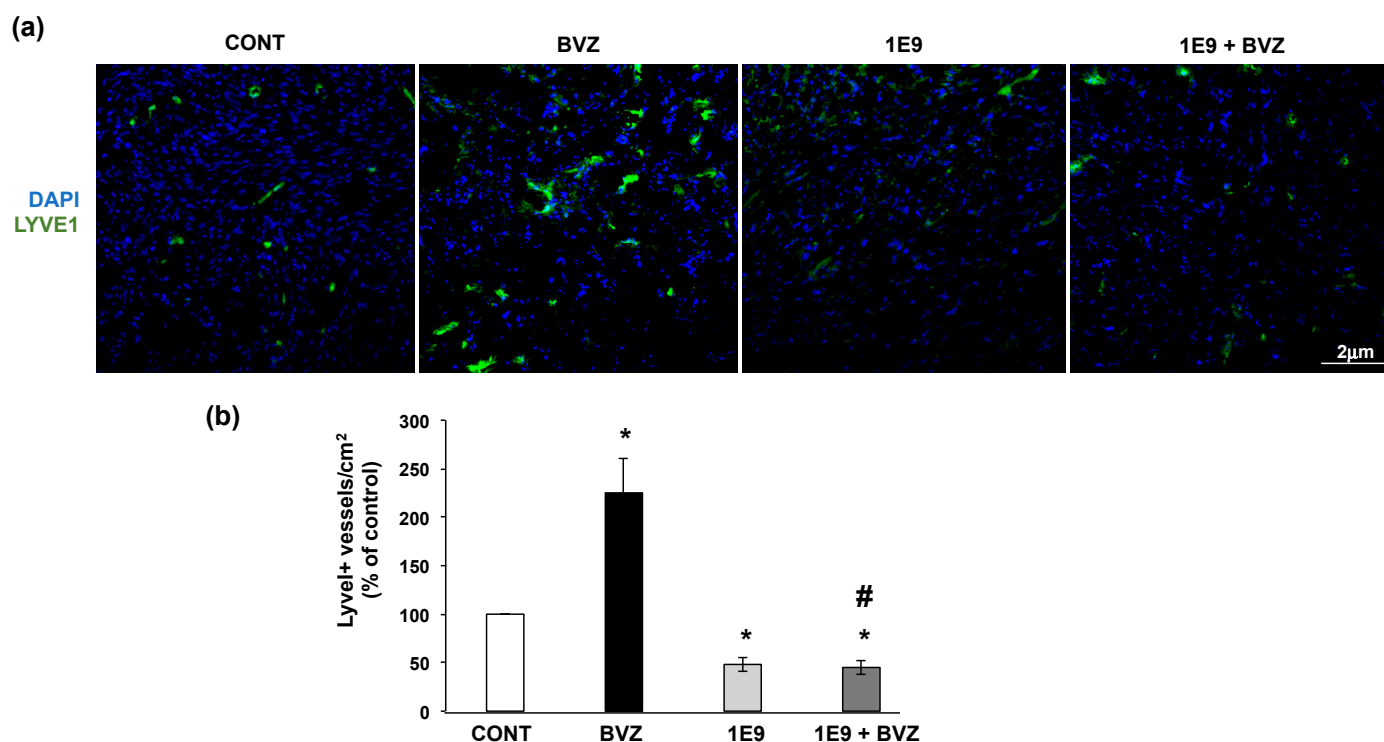


Figure S6. 1E9 antibodies prevent the bevacizumab-dependent development of lymphatic vessels (a). Lymphatic vessels were revealed by immunostaining using anti-LYVE1 antibodies. Tumor sections were counterstained with 40,6-diamidino-2-phenylindole (DAPI) (nucleus, blue) (b). Quantification of the number of lymphatic vessels is shown in (a). Statistics are indicated; *, $p < 0.05$ as compared to control; #, $p < 0.05$ as compared to bevacizumab condition.

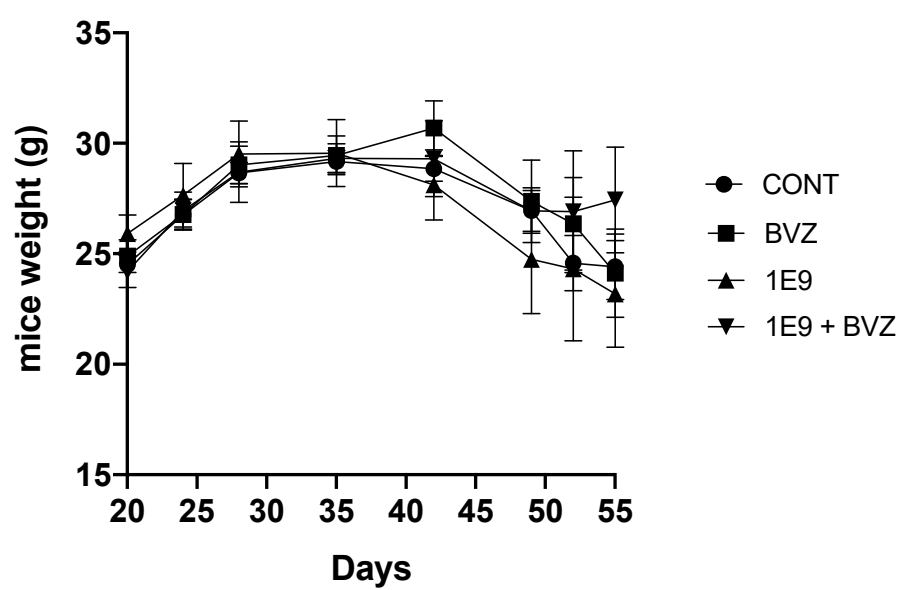


Figure S7. Body weight of mice throughout the experiment.