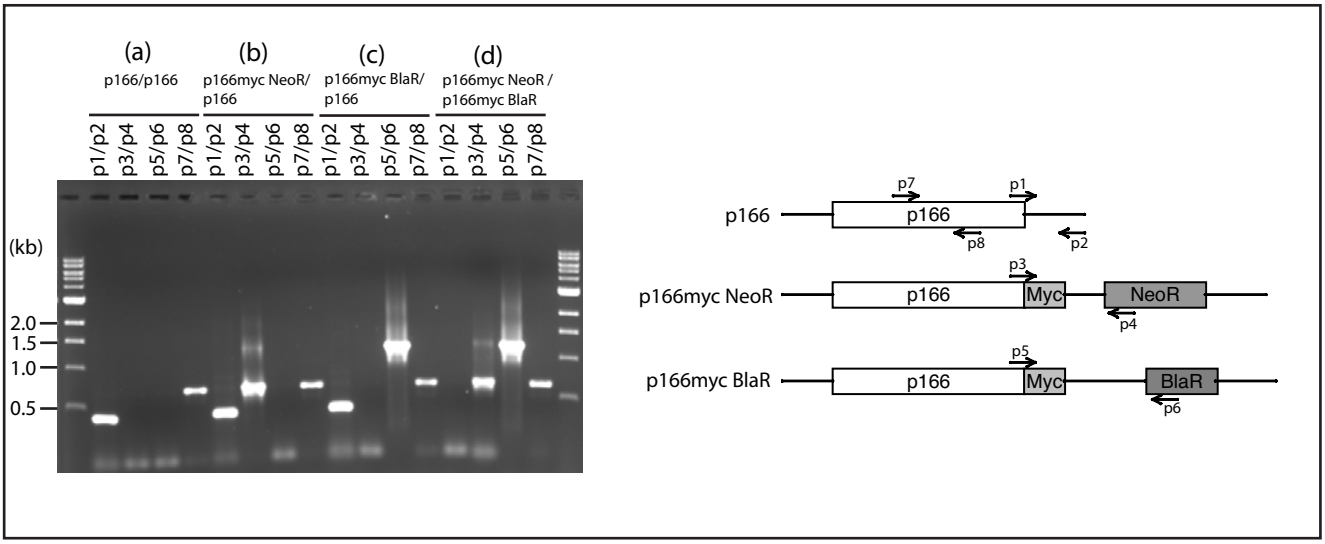
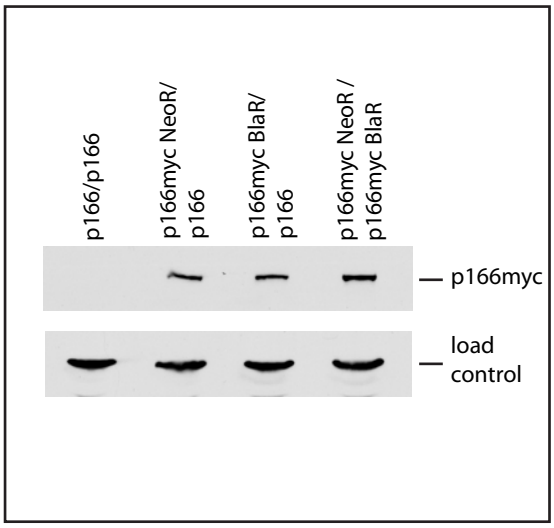
Figure S1.

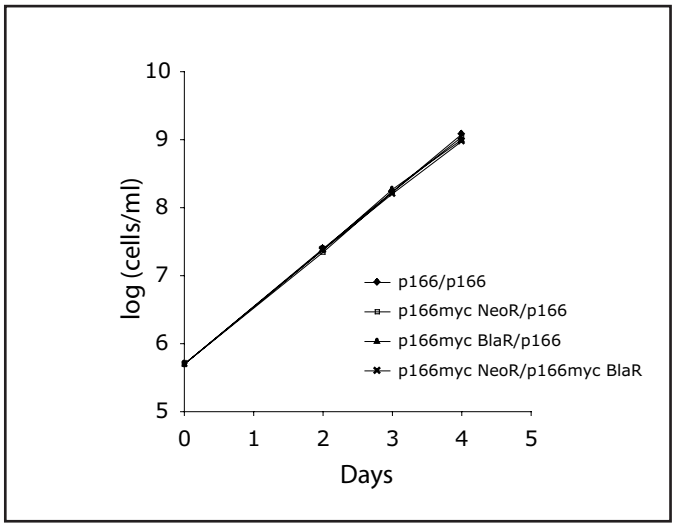
A



B



C



D

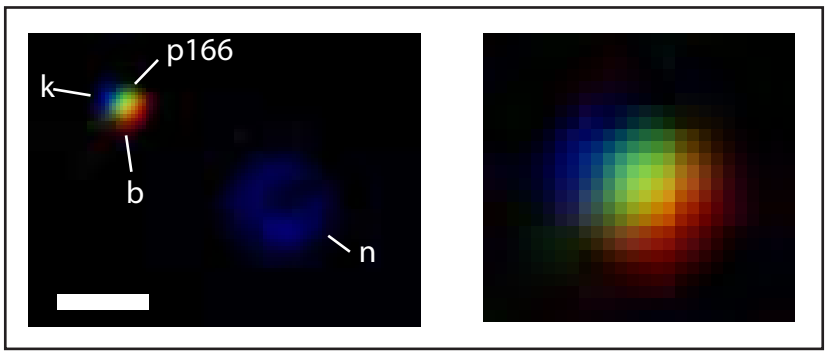


Figure S1

Myc-tagging both alleles of p166. (**A**) Right panel shows maps (not drawn to scale) of wild type p166 allele and alleles modified to encode p166myc (see Methods). Arrows indicate PCR primers used to demonstrate that one or both wild type alleles had been replaced by sequences encoding p166myc (see below for primer sequences; genomic DNA was template). Left panel shows PCR products using template from wild type (group a), from cells with a single p166 gene replaced by p166myc (groups b and c), and from cells with both p166 alleles replaced by p166myc (group d). Primer sequences were: p1: 5'-GGGTAATTCTATAACCTCC. p2: 5'-GGATATATGAGGGATAAG. p3: 5'-GGGTAATTCTAGGTACCG. p4: 5'-CACCCAAGCGGCCGGAGAAC. p5: 5'-GGGTAATTCTAGCTAGCG. p6: 5'-CAGAGATGGGGATGCTGTTG. p7: 5'-GGATAAATTGGACGCATTGG. p8: 5'-TCACCATTGCCTGTTCTAAG. (**B**) Western blot showing the expression of p166myc in the single- and double-tagged p166 cell lines. Cells (1×10^7) were centrifuged and the pellet was treated at 4 °C with 100 µl of SDS-PAGE loading buffer (containing 1% SDS) containing protease inhibitor (Pierce, catalog no. 78410, used at 2.5 times the recommended concentration). 20 µl of lysate containing trypanosome protein (2×10^6 cell equivalents) was fractionated by SDS-PAGE (10% gel), transferred to Immobilon-P (Millipore) and probed with a 1:1000 dilution of a rabbit anti-myc polyclonal antibody (Santa Cruz Biotech) to detect p166myc. Anti-myc antibody was detected with 1:10000 donkey anti-rabbit IgG labeled with horseradish peroxidase (Santa Cruz Biotech) and visualized using ECL western blotting detection reagents (Amersham Pharmacia Biotech). The same membrane was stripped by incubation in the stripping solution (100 mM 2-Mercaptoethanol, 2% SDS, 62.5 mM

Tris-HCl pH 6.7) at 50 °C for 30 min with occasional agitation and reprobed with an antibody recognizing mitochondrial HSP70 as the load control. (**C**) Growth of wild type, single- and double-myc-tagged p166 cell lines. Values of cells/ml on Y-axis are the measured value multiplied by the dilution factor. (**D**) Localization of p166myc in double myc-tagged p166 cell. Left panel, fluorescence micrographs used anti-myc antibody to recognize p166myc (green), anti-tyrosinated tubulin antibody for basal body (red), and DAPI to stain kDNA and nucleus (blue). Right panel, enlargement of the kDNA-p166myc-basal body complex in the left panel. k, kinetoplast; n, nucleus; b, basal body. Scale bar, 2μm.