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| Primers | | |
| 1 | TcP21\_sgRNA\_BamHI Fw | GATCGGATCC***TAAATGGCATGATTGCCTGC***GTTTTAGAGCTAGAAATAGC | |
| 2 | Scrambled\_BamHI Fw | GATCGGATCC***GCACTACCAGAGCTAACTCA***GTTTTAGAGCTAGAAATAGC | |
| 3 | sgRNA\_BamHI\_ Rv all genes | CAGTGGATCCAAAAAAGCACCGACTCGGTG | |
| 4 | Tcp21 ultramer Fw | CAGCAGGAGGAGCCTGCGGTTTGTTTTTGTTCTTCTCGTCCTTTTTCTCG CCTGCAGCGTGTCGGCCGTGGAGGTGGTGAATCGGGGATACAACCACAAG GAGATGGCCAAGCCTTTGTC TCAAG | |
| 5 | Tcp21\_Ultramer Rv | TTACTGGCGTCTGTGGAATCCGACCACGGCATCTTTGTAGAATCTGGAGT TCCTGCATTCGTTGGGGATCGACTCTTTTTCTGAATGAAGCAGGTGCTTC ATTAGCCCTCCCACACATAACC | |
| 6 | P21 Fw | GATACAACCACAAGGAGCC | |
| 7 | P21 Rv | TTACTGGCGTCTGTGGAATC | |
| 8 | UTR P21 Fw | GCCTCCATCCACATTTCATG | |
| 9 | UTR P21 Rv | AACGTCCAATTAGGTCTTGTA | |
| 10 | BSD Fw | ATGGCCAAGCCTTTGTCTCA | |
| 11 | BSD Rv | AGGGCAGCAATTCACGAATC | |
| 12 | TcHGPRT Fw | CTACAAGGGAAAGGGTCTGC | |
| 13 | TcHGPRT Rv | ACCGTAGCCAATCACAAAGG | |
| 14 | TcMVK Fw | CGGCCGCGACATTTGGT | |
| 15 | TcMVK Rv | GGCACTTCTAGGGCACGCAG | |

**Table S1**. Primers used to generate CRISPR/Cas9 constructs for *T. cruzi*, knockout confirmation and RT-PCR.