

OMTN, Volume 8

Supplemental Information

**CRISPR/Cas9-Mediated Knockin Application
in Cell Therapy: A Non-viral Procedure
for Bystander Treatment of Glioma in Mice**

Oscar Meca-Cortés, Marta Guerra-Rebollo, Cristina Garrido, Salvador Borrós, Nuria Rubio, and Jeronimo Blanco

SUPPLEMENTAL FIGURES

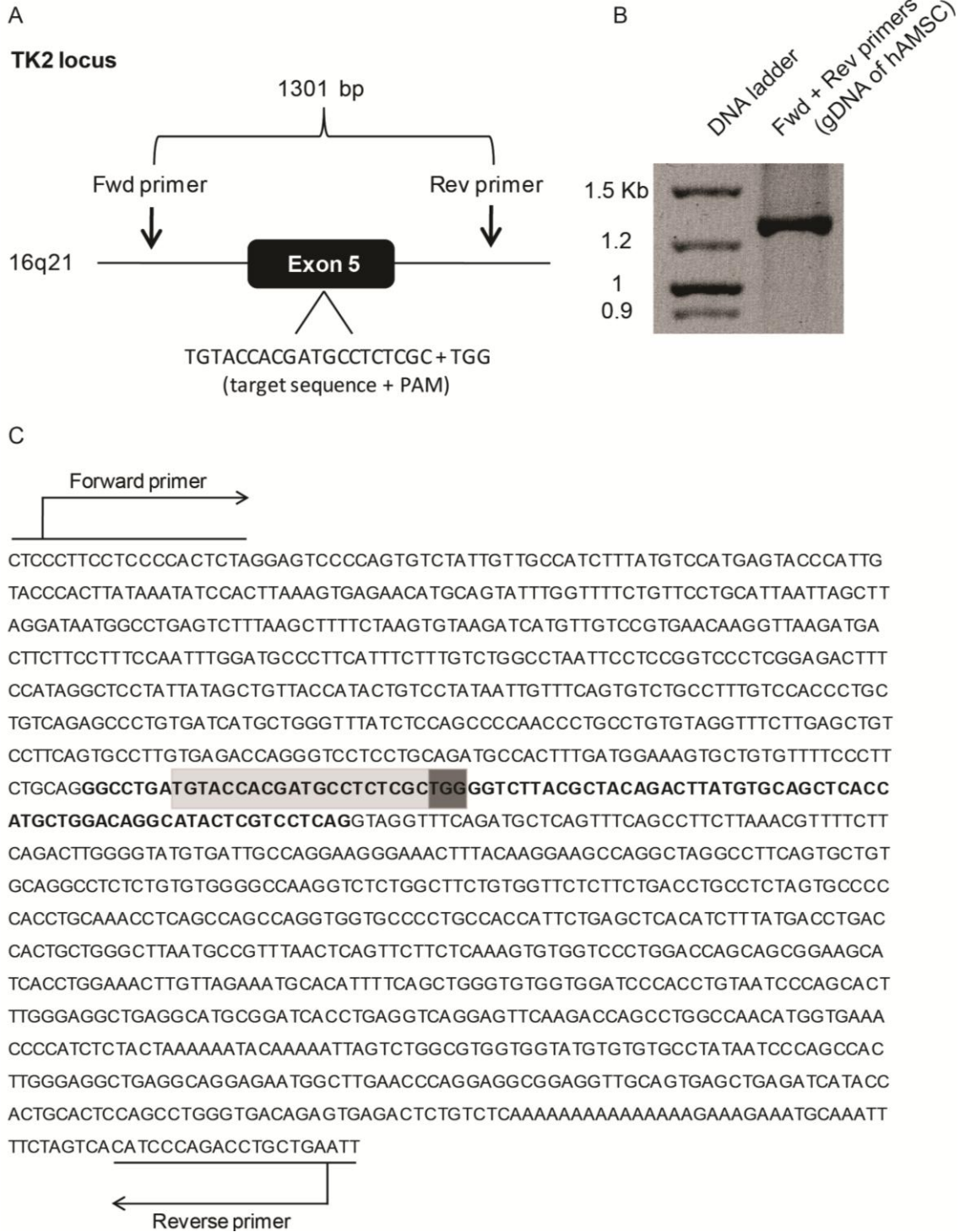


Figure S1. Assessment of the integrity of the selected target sequence in exon 5 of the *TK2* gene in hAMSCs. (A) Diagram showing the chosen Cas9 nuclease target sequence within exon 5 of *TK2* locus and the expected PCR amplicon size. (B) Agarose gel showing PCR generated amplicon. (C) Sequence corresponding to PCR amplicon. Exon 5 is highlighted (bold), including Cas9 nuclease target sequence (grey-shaded) and PAM (dark grey-shaded).

Amplicon around 5' junction, 1079 bp

Fwd primer (gDNA)



Amplicon around 3' junction, 1361 bp

Fwd primer (tTK gene)

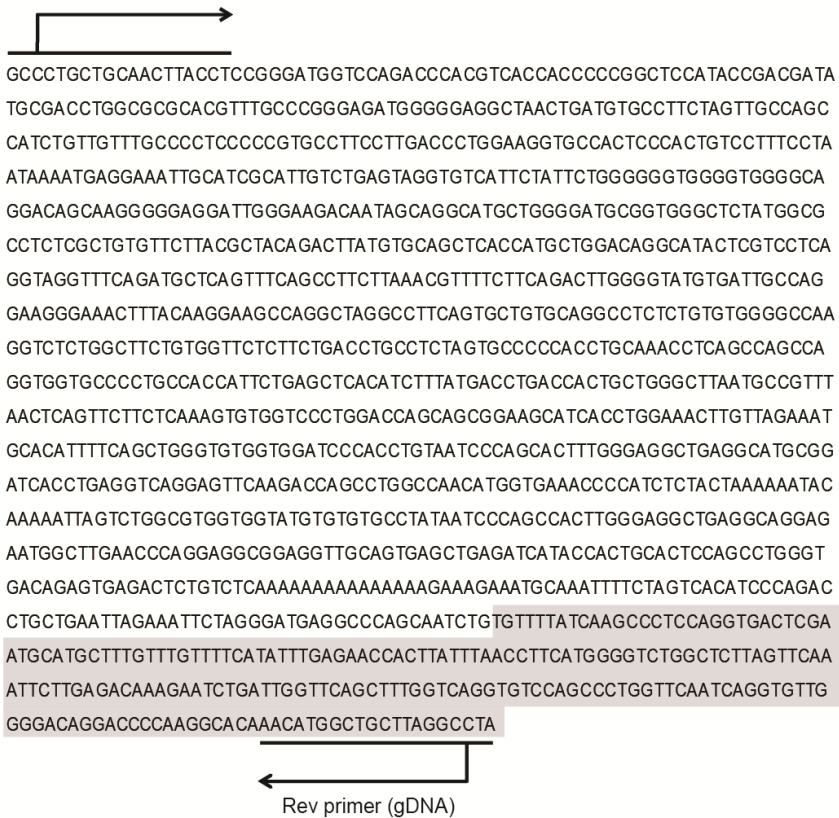
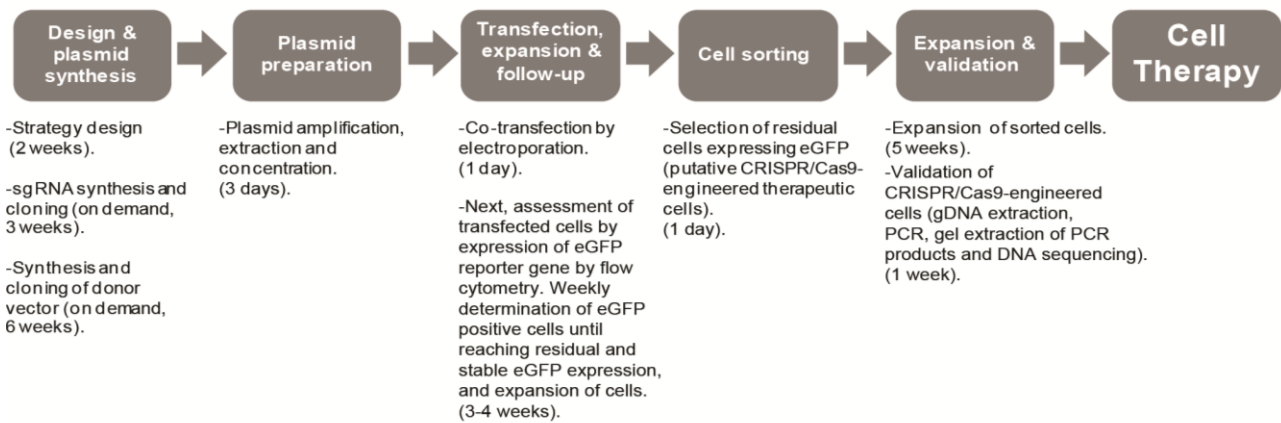


Figure S2. DNA sequence of predicted amplicons indicating integration of donor plasmid elements in exon 5 of *TK2* locus in hAMSCs. Amplicon sequences were obtained by PCR amplification of genomic DNA from putative CRISPR/Cas9-engineered hAMSCs using primers (underlined) flanking 5' and 3' junctions of integrated and genomic sequences (indicated as transition from non-shaded and grey-shaded nucleotides).



Figure S3. Initial homogenization of experimental groups for *in vivo* assessment of bystander glioblastoma therapy of CRISPR/Cas9-modified hAMSCs and tumor growth development in all mice until day 48. (A) Non-invasive BLI images were acquired as described above 5 days post-implantation of tumor cells and animals were separated in 3 groups with similar light emission capacity. (B) 48 day non-invasive BLI monitoring of tumor growth. Images show all mice from (A). BLI images (false color palette ranging from blue = low intensity, to red = high intensity light emission) are overlaid on bright field images.

Time-scale of CRISPR/Cas9-mediated knock-in in hAMSCs for glioma cell therapy



Working time approx. 8 weeks

Figure S4. Flow diagram showing the strategy for generating therapeutic hAMSCs through CRISPR/Cas9-mediated knock-in in this study.

SUPPLEMENTAL DATA FILES

5' homology arm, including 5'-end exon 5 of *TK2* gene (bold) and 11 of 20 nucleotides of target sequence (underlined)

AGGTTGTTCACTGTTGGTGTATATACATGCTACTGATTTTTATATGTTGATTTGTATCCTGTGG
TATTACTGAGTTCGTTTCATCAGTTCTAACAGTTGTTGGTGGAGTCTTTAAGGTTTTTTTTTTT
TTTTTTGGTCATTTCAACTTTTATTTTACTTTTAGCGGGTACATGTGCAGGTTTGTACCTG
GGTATATTTTCATGATGCTGAGGTTTAGGGTATGATGGATCCCATCACCTAGGTACTGAGCAT
AGTACCCAATAGGTACTTTTTCAACCTTTGCCCTCCCTTCTCCCACTCTAGGAGTCCCC
AGTGTCTATTGTTGCCATCTTTATGTCCATGAGTACCCATTGTACCCACTTATAAATATCCAC
TTAAAGTGAGAACATGCAGTATTTGGTTTTCTGTTCCCTGCATTAATTAGCTTAGGATAATGGC
CTGAGTCTTTAAGCTTTTCTAAGTGTAAGATCATGTTGTCCGTGAACAAGGTTAAGATGACTT
CTTCCTTTCCAATTTGGATGCCCTTCATTTCTTTGTCTGGCCTAATTCCTCCGGTCCCTCGGAG
ACTTTCCATAGGCTCCTATTATAGCTGTTACCATACTGTCCTATAATTGTTTCAGTGTCTGCCT
TTGTCCACCCTGCTGTGAGAGCCCTGTGATCATGCTGGGTTTATCTCCAGCCCCAACCTGCC
TGTGTAGGTTTCTTGAGCTGTCCTTCAGTGCCTTGTGAGACCAGGGTCTCCTGCAGATGCCA
CTTTGATGGAAAGTGCTGTGTTTTCCCTTCTGCAGGGCCTGATGTACCACGAT

EF1 α promoter

CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTT
GGGGGAGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGA
AAGTGATGTCGTGACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTG
CAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCC
GTGTGTGGTTCCCGCGGGCCTGGCCTCTTACGGGTTATGGCCCTTGCCTGCTTGAATTACT
TCCACCTGGCTGCAGTACGTGATTTCTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAG
TTCGAGGCCTTGCCTTAAGGAGCCCCTTCGCCTCGTGTGAGTTGAGGCCTGGCCTGGGC
GCTGGGGCCCGCGGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTTCGATAAG
TCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTCTGGCAAGATAGTCTT
GTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCCGGGCGGACCGG
GGCCCGTGCCTCCAGCGCACATGTTCCGGCAGGCGGGGCTGCGAGCGCGGCCACCCGAGA
ATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCTCGCGCCCGCTG
TATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGTTCGGCACAGTTGCGTGAGCGGAAAGA
TGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGGAGAGC
GGGCGGGTGAATCACCCACACAAAGGAAAAGGGCCTTCCGTCTCAGCCGTCGCTTCATGT

GACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTAC
GTCGTCTTTAGGTTGGGGGAGGGGTTTTATGCGATGGAGTTTCCCACACTGAGTGGGTGG
AGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAATTTGCCCTTTTTGAGT
TTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTTCTTCCATTTAGGT
GTCGTGAGGAATTAGCC

***eGFP* gene**

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACG
GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGG
CAAGCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGCCCGTACCCTGGCCACCCTCG
TGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCGCTACCCCGACCACATGAAGCAGCAC
GACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGA
CGACGGCAACTACAAGACCCGCGGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGC
ATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGT
ACAACACTACAAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGT
GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
CAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCA
GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTG
ACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG

Spacer sequence

ACCGCGGGCCCGGGATCCGCCAC

Herpes simplex virus 1 truncated thymidine kinase gene (*tTK*)

ATGCCACGCTACTGCGGGTTATATAGACGGTCCCCACGGGATGGGGAAAACCACCACCA
CGAACTGCTGGTGGCCCTGGGTTTCGCGGACGATATCGTCTACGTACCCGAGCCGATGACT
TACTGGCGGGTGTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCGCCT
CGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACA
ATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAGGCTGG
GAGCTCATATGCCCCGCCCGGCCCTCACCATCTTCTCGACCGCCATCCCATCGCCTTCAT
GCTGTGCTACCCGGCCGCGCGGTACCTTATGGGCAGCATGACCCCCAGGCCGTGCTGGCGT
TCGTGGCCCTCATCCCCGCGACCTTGCCCGCACCAACATCGTGTGTTGGGGCCCTTCCGGAG
GACAGACACATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAGCGGCTGGACCTGGCTA
TGCTGGGTGCGATTGCGCCGCTTACGGGCTACTTGCCAATACGGTGCGGTATCTGCAGTGC
GGCGCTCGTGGCGGAGGACTGGGGACAGCTTTCGGGGACGGCCGTGCCGCCCGGGGTG
CCGAGCCCCAGAGCAACGCGGGCCACGACCCCATATCGGGGACACGTTATTTACCCTGTTT
CGGGCCCCGAGTTGCTGGCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCCTTGG
CGTCTTGCCAAACGCCTCCGTTCCATGCACGTCTTATCCTGGATTACGACCAATCGCCCCG
CGGCTGCCGGGACGCCCTGCTGCAACTTACCTCCGGGATGGTCCAGACCCACGTACCAACC
CCGGCTCCATACCGACGATATGCGACCTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGC
TAACTGA

BGH pA signal

TGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCCTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTA
GGTGTCAATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGA
CAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC

3' homology arm, including 3'-end exon 5 of *TK2* gene (bold), 9 of 20 nucleotides of target sequence (underlined) and PAM mutations (lower case)

GCCTCTCGCTGtGtTCTTACGCTACAGACTTATGTGCAGCTCACCATGCTGGACAGGCAT
ACTCGTCCTCAGGTAGGTTTCAGATGCTCAGTTTCAGCCTTCTTAAACGTTTTCTTCAGACTT
GGGGTATGTGATTGCCAGGAAGGGAACTTTACAAGGAAGCCAGGCTAGGCCTTCAGTGCT
GTGCAGGCCCTCTGTGTGGGGCCAAGGTCTCTGGCTTCTGTGGTTCTTCTGACCTGCCTC
TAGTGCCCCACCTGCAAACCTCAGCCAGCCAGGTGGTGCCCTGCCACCATCTGAGCTCA
CATCTTTATGACCTGACCACTGCTGGGCTTAATGCCGTTTAACTCAGTTCTTCTCAAAGTGTG
GTCCCTGGACCAGCAGCGGAAGCATCACCTGGAAACTTGTAGAAATGCACATTTTCAGCTG
GGTGTGGTGATCCACCTGTAATCCACACTTTGGGAGGCTGAGGCATCGGGATCGGGATCACCTG
AGGTCAGGAGTTCAAGACCAGCCTGGCCAACATGGTGAACCCCATCTCTACTAAAAATA

CAAAAATTAGTCTGGCGTGGTGGTATGTGTGTGCCTATAATCCCAGCCACTTGGGAGGCTGA
GGCAGGAGAATGGCTTGAACCCAGGAGGCGGAGGTTGCAGTGAGCTGAGATCATACCACTG
CACTCCAGCCTGGGTGACAGAGTGAGACTCTGTCTCAAAAAAAAAAAAAAAAAAGAAAGAAATG
CAAATTTTCTAGTCACATCCCAGACCTGCTGAATTAGAAATTCTAGGGATGAGGCCAGCAA
TCTG

Data file S1. Sequence of donor fragment designed for integration via HDR pathway after DNA cleavage exerted by CRISPR/Cas9 nuclease in hAMSCs.