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Supplemental Information

Efficacy and Safety of Doubly-Regulated Vaccinia Virus in a Mouse Xenograft Model of Multiple Myeloma

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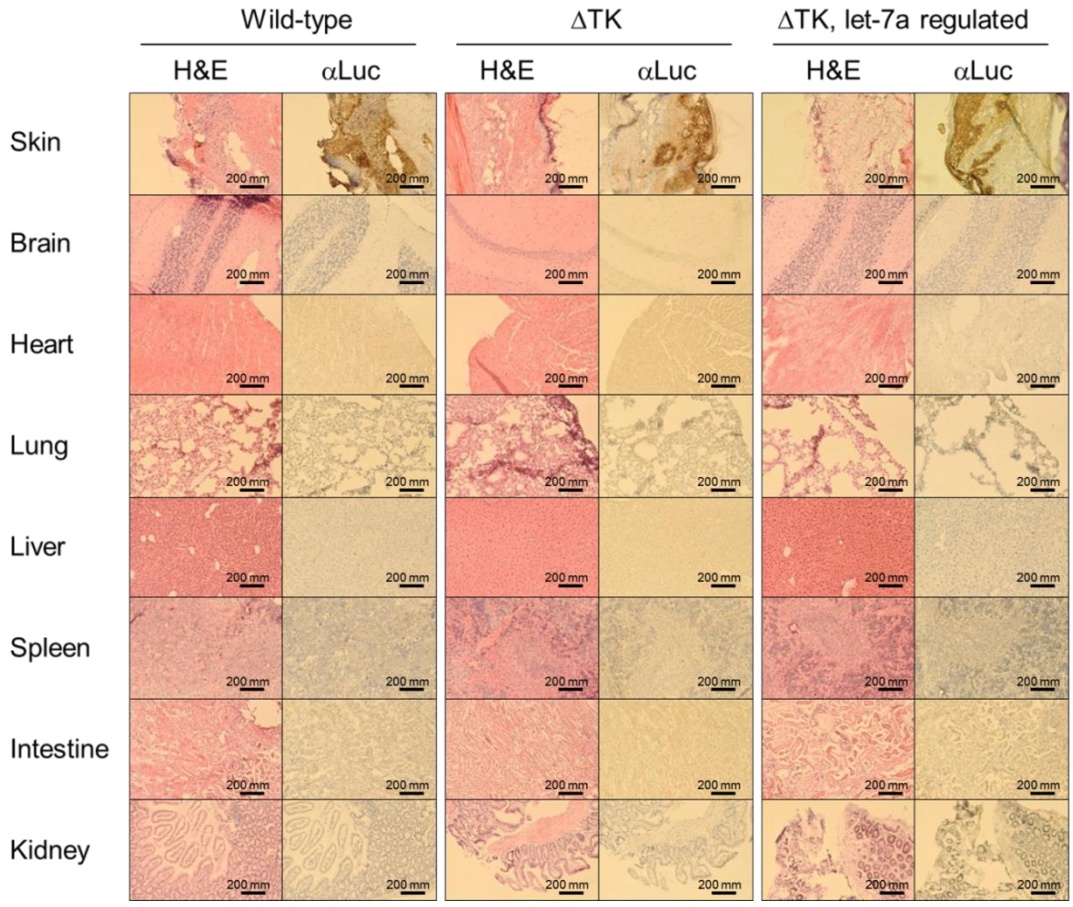

Figure S1

RPMI8226-bearing SCID mice were infected VVs (wild-type, ΔTK, and ΔTK, let-7a), and tissue samples were removed 28 days after infection. Immunostaining for firefly luciferase was performed on frozen sections following the manufacturer's instructions using an anti-firefly luciferase antibody (ab21176, Abcam, Cambridge, MA, USA).

Supplemental Materials and Methods

Plasmid Construction

Oligonucleotide pairs containing two copies of completed or mutated complementary target sequences for either miR-10a or miR-15a, plus the *Eco*RI site were annealed and subcloned into the pmirGLO vector (Promega) at the corresponding restriction sites *Nhe*I and *Xho*I, resulting in pmirGLO-miR-10a×2 or pmirGLO-miR-10a-mut×2, pmirGLO-miR-15a×2 or pmirGLO-miR-15a-mut×2, respectively.

miR10a-SENSE1
5'-CTAGCCACAAATTCGGATCTACAGGGTAAACGATCGCACAAATTCGGATCTACAGGGTAAAGAATTCC-3',

miR10a-ANTISENSE1
5'-TCGAGGAATTCTTTACCCTGTAGATCCGAATTTGTGCGATCGTTTACCCTGTAGATCCGAATTTGTGG-3',

miR10a-mut-SENSE1
5'-CTAGCCATAAGTTTGGTTCAACTGGCTGAACGATCGCATAAGTTTGGTTCAACTGGCTGAAGAATTCC-3',

miR10a-mut-ANTISENSE1
5'-TCGAGGAATTCTTCAGCCAGTTGAACCAAACTTATGCGATCGTTCAGCCAGTTGAACCAAACTTATGG-3',

miR15a-SENSE1
5'-CTAGCCACAAACCATTATGTGCTGCTAAACGATCGCACAAACCATTATGTGCTGCTAAAGAATTCC-3',

miR15a-ANTISENSE1
5'-TCGAGGAATTCTTTAGCAGCACATAATGGTTTGTGCGATCGTTTAGCAGCACATAATGGTTTGTGG-3',

miR15a-mut-SENSE1
5'-CTAGCCATAAGCCTTTGTGCGCAGCAAAACGATCGCATAAGCCTTTGTGCGCAGCAAAAGAATTCC-3',

miR15a-mut-ANTISENSE1
5'-TCGAGGAATTCTTTTGCTGCGCACAAAGGCTTATGCGATCGTTTTGCTGCGCACAAAGGCTTATGG-3'.

Another pairs containing two copies of completed or mutated complementary target sequences for either miR-10a or miR-15a plus the *Age*I site were annealed and cloned into either pmirGLO-miR-10a×2 or pmirGLO-miR-10a-mut×2, pmirGLO-miR-15a×2 or pmirGLO-miR-15a-mut×2 at the corresponding restriction sites *Eco*RI and *Xba*I, resulting in pmrGLO-miR-10a×4 or pmirGLO-miR-10a-mut×4, pmrGLO-miR-15a×4 or pmirGLO-miR-15a-mut×4, respectively.

miR10a-SENSE2
5'-AATTCCACAAATTCGGATCTACAGGGTAAACATATGCACAAATTCGGATCTACAGGGTAAAACCGGTT-3',

miR10a-ANTISENSE2

5’-CTAGAACCGGTTTTACCCTGTAGATCCGAATTTGTGCATATGTTTACCCTGTAGATCCGAATTTGTGG-3’,

miR10a-mut-SENSE2
5’-AATTCCATAAGTTTGGTTCAACTGGCTGAACATATGCATAAGTTTGGTTCAACTGGCTGAAACCGGTT-3’,

miR10a-mut-ANTISENSE2
5’-CTAGAACCGGTTTCAGCCAGTTGAACCAAACTTATGCATATGTTCAGCCAGTTGAACCAAACTTATGG-3’,

miR15a-SENSE2
5’-AATTCCACAAACCATTATGTGCTGCTAAACATATGCACAAACCATTATGTGCTGCTAAAACCGGTT-3’,

miR15a-ANTISENSE2
5’-CTAGAACCGGTTTTAGCAGCACATAATGGTTTGTGCATATGTTTAGCAGCACATAATGGTTTGTGG-3’,

miR15a-mut-SENSE2
5’-AATTCCATAAGCCTTTGTGCGCAGCAAAACATATGCATAAGCCTTTGTGCGCAGCAAAAACCGGTT-3’,

miR15a-mut-ANTISENSE2
5’-CTAGAACCGGTTTTTGCTGCGCACAAAGGCTTATGCATATGTTTTGCTGCGCACAAAGGCTTATGG-3’.

To generate plasmid vectors in which B5R as well as green fluorescent protein (GFP) are regulated by either miR-10a or miR-15a, plasmid vectors pmrGLO-miR-10a×4 or pmirGLO-miR-10a-mut×4, pmrGLO-miR-15a×4 or pmirGLO-miR-15a-mut×4, were digested with *Nhe*I and *Age*I, and the fragment containing four copies of completed or mutated complementary target sequences for either miR-10a or miR-15a were cloned into the pTN-B5Rgfp at the corresponding restriction sites *Nhe*I and *Age*I, resulting in pTN-B5Rgfp-miR-10a×4 or pTN-B5Rgfp-miR-10a-mut×4, pTN-B5Rgfp-miR-15a×4 or pTN-B5Rgfp-miR-15a-mut×4, respectively. Plasmid vectors in which B5Rgfp is regulated by let-7a (pTN-B5Rgfp-let-7a×4 or pTN-B5Rgfp-let-7a-mut×4) were generated using the same method as described previously [1].

Supplemental References

1. Hikichi, M, Kidokoro, M, Haraguchi, T, Iba, H, Shida, H, Tahara, H, *et al.* (2011). MicroRNA regulation of glycoprotein B5R in oncolytic vaccinia virus reduces viral pathogenicity without impairing its antitumor efficacy. *Molecular therapy : the journal of the American Society of Gene Therapy* **19**: 1107-1115.