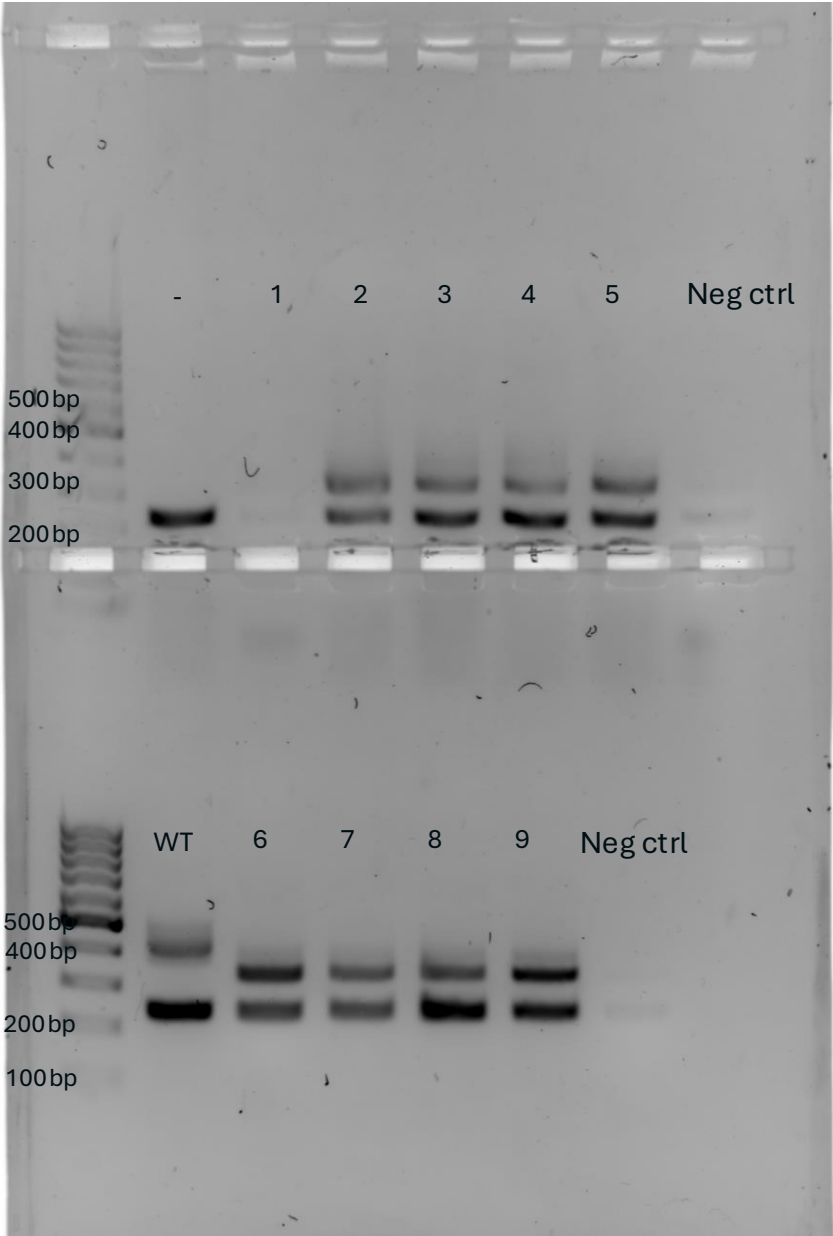
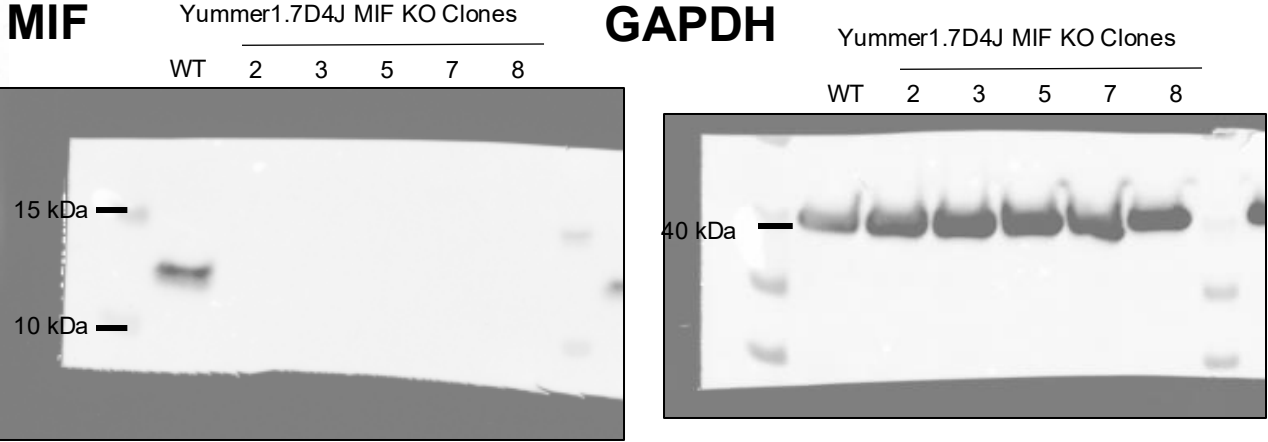


Supplemental Figure 3. CRISPR knockout of *Mif* in YUMMER1.7 diploid melanoma cells does not impact tumor growth in WT mice

(a) PCR analysis of genomic DNA from YUMMER1.7 diploid cells confirmed the presence of the expected band sizes corresponding to wild-type (WT, 450bp), *Mif* knockout (350bp), and *Mif* pseudogenes (~250bp).



(b) Western blot analysis verified the complete absence of MIF protein in YUMMER1.7 diploid-*Mif*^{-/-} cells, indicating successful knockout of *Mif*.

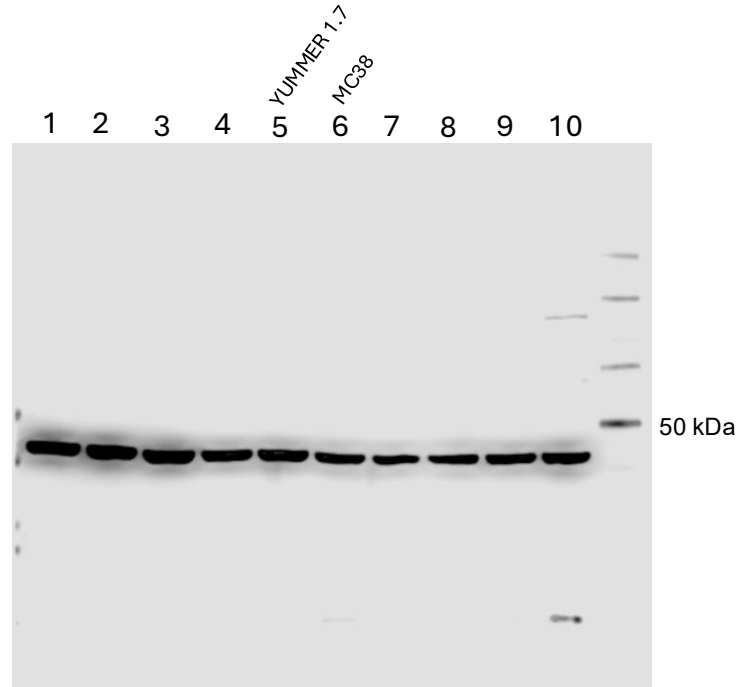


| Antibody | Catalog | Dilution | Antibody | Catalog | Dilution |
|-----------------------------|----------------------------|-------------------|-------------------------|----------------------------|-------------------|
| Rabbit anti-MIF (E7T1W) mAb | Cell Signaling, Cat#87501 | 1:10,000 dilution | Mouse GAPDH (D4C6R) mAb | Cell Signaling, Cat #97166 | 1:10,000 dilution |
| Anti-rabbit HRP | Cell Signaling, Cat #7074S | 1:1,000 dilution | Anti-mouse HRP | Cell Signaling, Cat #7076S | 1:1,000 dilution |

Figure. 1. Anti-PD-1/anti-MIF improves survival and tumor responses.

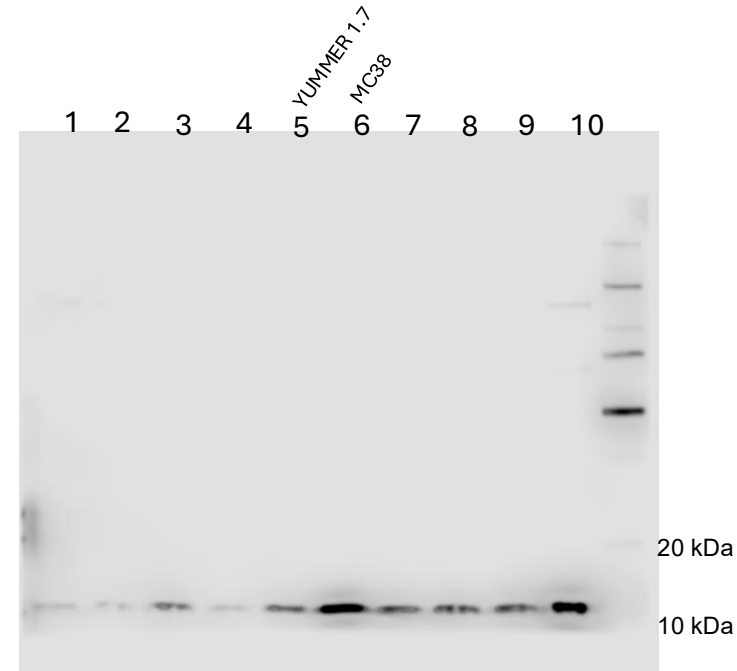
(i) MIF levels were detected in cell lysate of YUMMER1.7 and MC38 cells

Actin



| Antibody | Catalog | Dilution |
|----------------------|----------------------------|-------------------|
| Mouse anti-actin mAb | Cell Signaling, Cat #3700S | 1:10,000 dilution |
| Anti-mouse HRP | Cell Signaling, Cat #7076S | 1:1,000 dilution |

MIF



| Antibody | Catalog | Dilution |
|-----------------------------|----------------------------|-------------------|
| Rabbit anti-MIF (E7T1W) mAb | Cell Signaling, Cat#87501 | 1:10,000 dilution |
| Anti-rabbit HRP | Cell Signaling, Cat #7074S | 1:1,000 dilution |