Supplemental Figures:

Figure S1. Induction of the interferon response in B16-F10 cells protects from Maraba MG1 infection. 24000 B16-F10 cells were plated in wells of a 96-well plate and incubated overnight with 25 units of murine IFN-β. The following day, cells were washed in PBS and incubated with Maraba MG1-GFP at a multiplicity of infection of 5. Bright field pictures were taken at 48 hours. B16-F10 cells pretreated with type I IFN were protected from the cytopathic effect of Maraba MG1. IFN: interferon.

Figure S2. Maraba vector construction and expression of DCT in MG1-hDCT transduced Vero cells. (a) Maraba expressing hDCT or GFP was made by inserting the full-length sequence of human DCT or GFP between the *G* and *L* genes of the attenuated Maraba MG1 genome (diverging from the wild-type genome by two substitutions: L123W and Q242R in the M and G genes respectively). **(b)** Murine B16-F10 melanoma cells constitutively express endogenous DCT and were used as a positive control. Vero cells, that do not express DCT, were transduced with MG1-hDCT or its negative control GFP at multiplicity of infection of 25 and lysed 10 hours later. Detection of DCT protein in cell lysates was performed by western blot using PEP8h antibody. Beta-actin was detected using a monoclonal antibody (clone AC-15, ref. A5441, Sigma-Aldrich). Upper bands on DCT western blot corresponded to N-glycosylated forms of the protein. DCT: dopachrome tautomerase.

Figure S3. Tumor-specific immune memory resulting from the Ad-hDCT+MG1-hDCT treatment protects cured mice from tumor recurrence. Naive mice or mice that survived (>90 days) B16-F10 lung metastatic tumor after receiving Ad-hDCT+MG1-hDCT treatment were rechallenged by subcutaneous injection of 2e4 B16-F10 cells (n=6 per group). Tumor

development was closely monitored and displayed as a Kaplan-Meyer curve. Results demonstrated that the DCT-specific responses induced by the oncolytic prime-boost treatment generated tumor-specific immune memory that protects mice from cancer recurrence.

Figure S4. Ad-hDCT+MG1-hDCT heterologous prime-boost induces stronger antitumor immunity and shows better therapeutic efficacy than homologous prime-boost strategies or than the heterologous prime-boost involving VSV-hDCT. The therapeutic efficacy of the two heterologous prime-boost strategies involving Ad-hDCT with MG1-hDCT or VSV-hDCT and the two homologous prime-boost with either Ad-hDCT or MG1-hDCT was evaluated in the B16-F10 lung metastatic tumor model. Tumor-specific immunity was also measured. **(a)** Percentage of $CD8^+$ T-cells secreting IFN-γ in response to DCT-specific SVY peptide exposure measured 5 days post-boost in the blood. Box plots representing 25 to 75 percentile including median and whiskers illustrating the range between minimal and maximal values. n=5 per group except for the Ad-hDCT+MG1-hDCT and Ad-hDCT+VSV-hDCT groups where n=8. **(b)** Fold increase (boost) of the DCT(SVY)-specific T-cell response in the blood following systemic administration of MG1-hDCT ("MG1") or VSV-hDCT ("VSV") 9 days post-Ad-hDCT prime both in tumor-free (TF) and lung umor-bearing (TB) mice. **(c)** Survival data illustrated as Kaplan-Meyer curves. p values: considered non significant (NS) when >0.05, *p < 0.05, **p < 0.01, and ***p < 0.001. Ad: adenoviral vector. DCT: dopachrome tautomerase. IFN-γ: interferon-gamma.

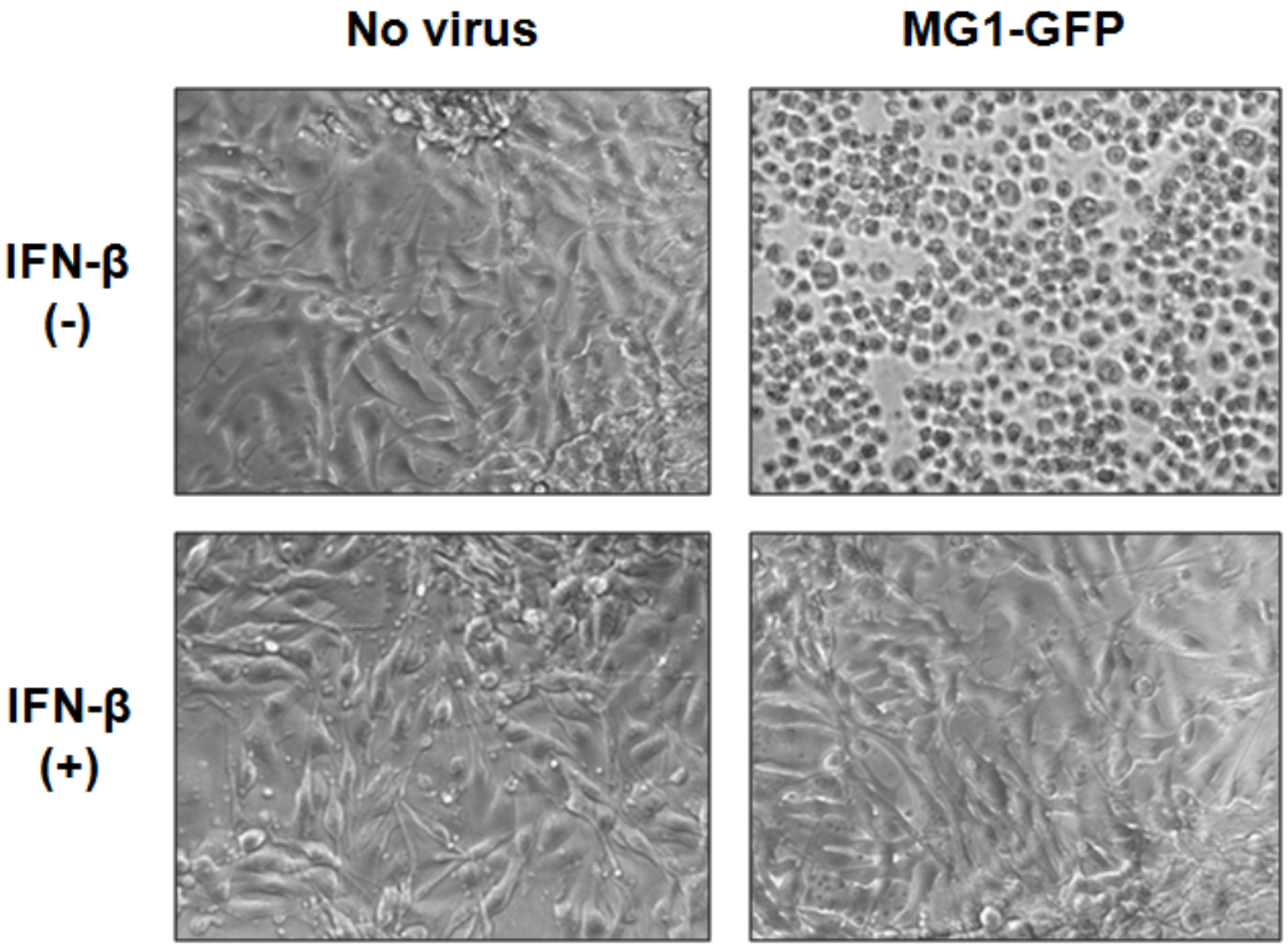
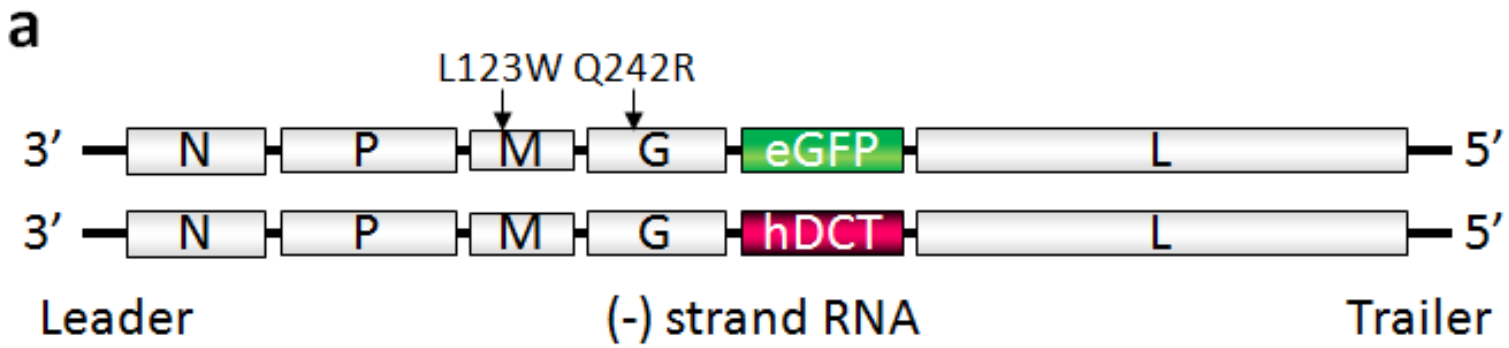


Figure S1


a
L123W Q242R
3′
N
P
M
G
eGFP
L
5′
3′
N
P
M
G
hDCT
L
5′
Leader
(-) strand RNA
Trailer

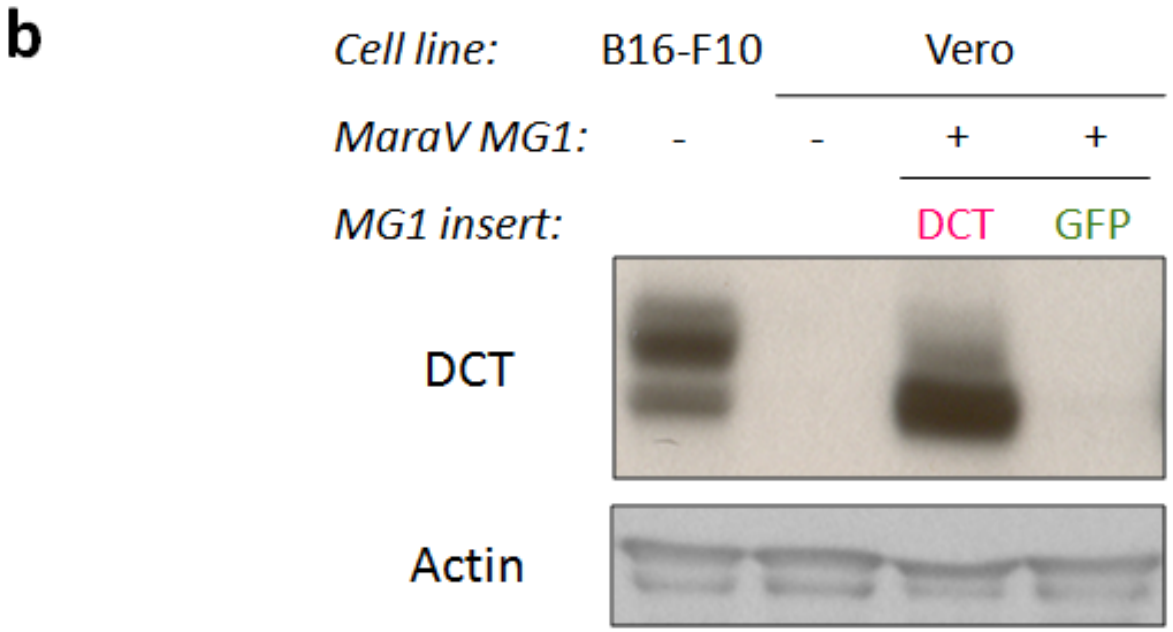

b
Cell line:
B16-F10
Vero
MaraV MG1:
-
-
+
+
MG1 insert:
DCT
GFP
DCT
Actin

Figure S2

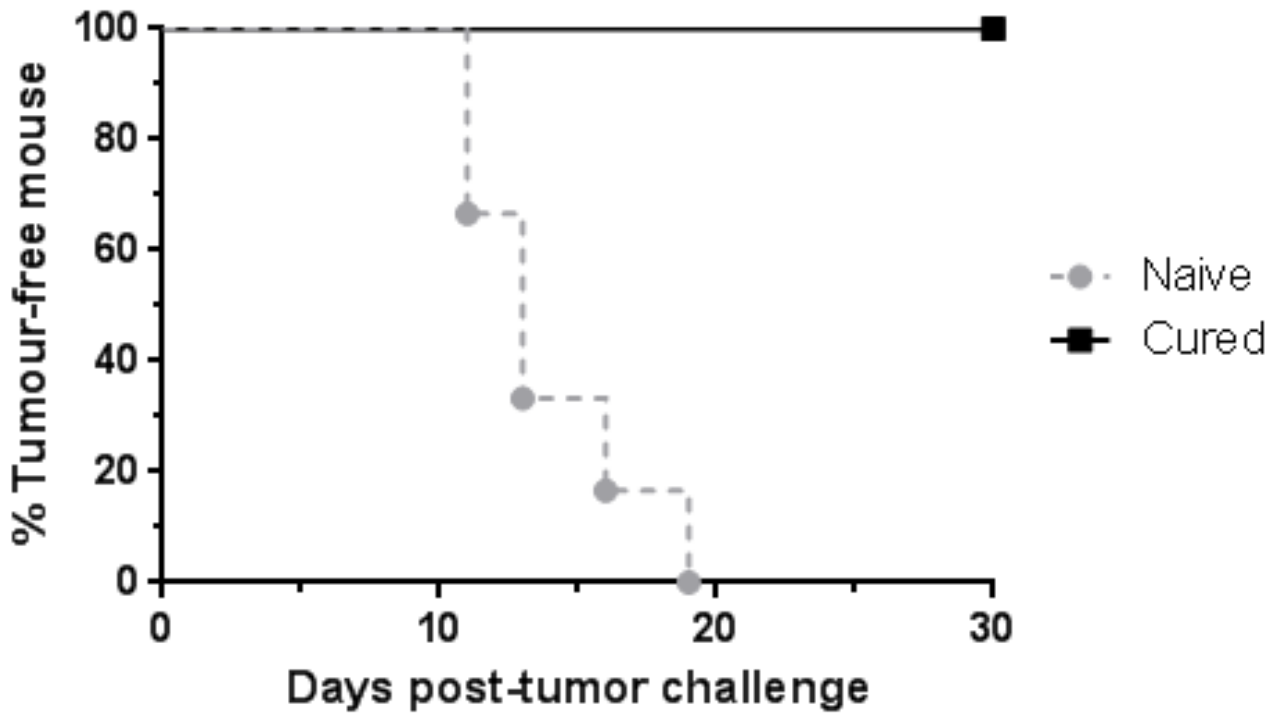
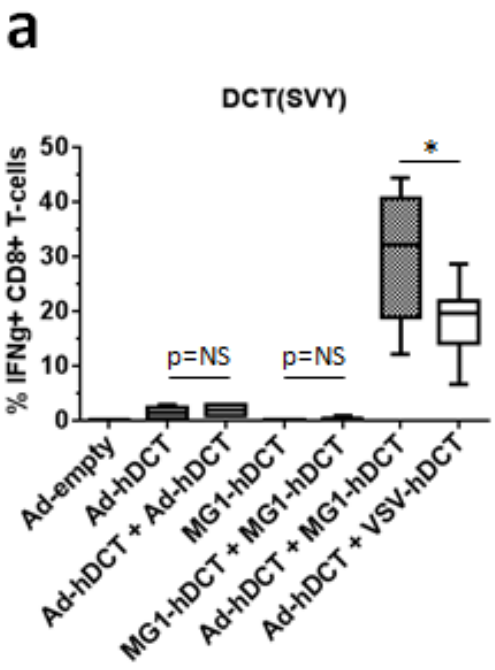
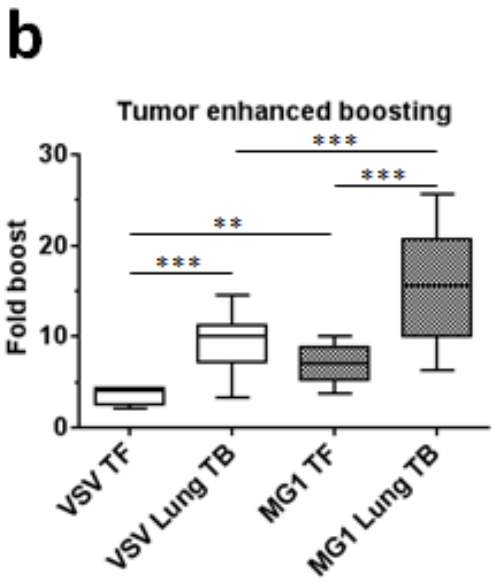

% Tumour-free mouse
100
80
60
40
20
0
0
10
20
30
Days post-tumor challenge
Naive
Cured

Figure S3


a
DCT(SVY)
% IFNg+ CD8+ T-cells
50
40
30
20
10
0
*
p=NS
p=NS
Ad-empty
Ad-hDCT
Ad-hDCT + Ad-hDCT
MG1-hDCT
MG1-hDCT + MG1-hDCT
Ad-hDCT + MG1-hDCT
Ad-hDCT + VSV-hDCT


b
Tumor enhanced boosting
Fold boost
30
20
10
0
***
***
**
***
VSV TF
VSV Lung TB
MG1 TF
MG1 Lung TB

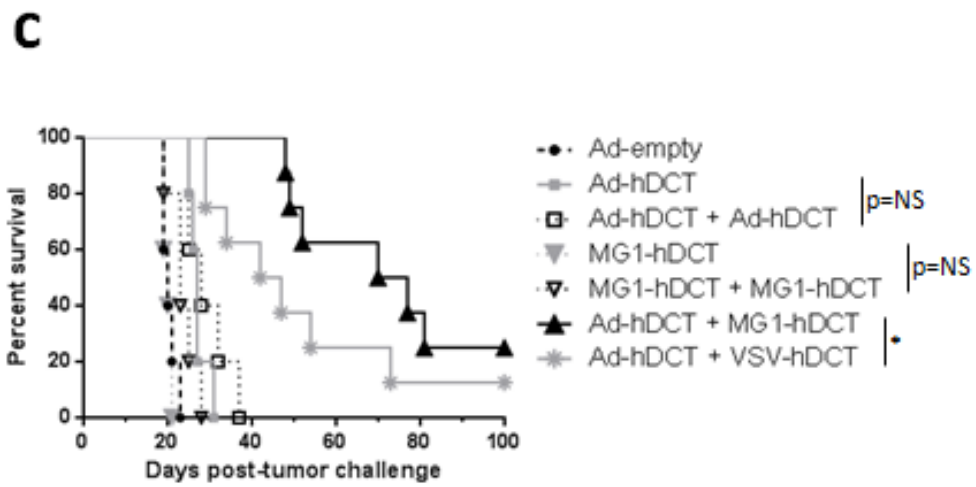

c
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days post-tumor challenge
Ad-empty
Ad-hDCT
Ad-hDCT + Ad-hDCT
MG1-hDCT
MG1-hDCT + MG1-hDCT
Ad-hDCT + MG1-hDCT
Ad-hDCT + VSV-hDCT
p=NS
p=NS
*

Figure S4