SUPPLEMENTARY INFORMATION

TITLE

Immune checkpoint proteins are conserved across 160 million years of evolution and are expressed on transmissible cancers

RUNNING TITLE

Transmissible cancers express evolutionarily conserved immune checkpoint molecules

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SUPPLEMENTARY MATERIALS AND METHODS

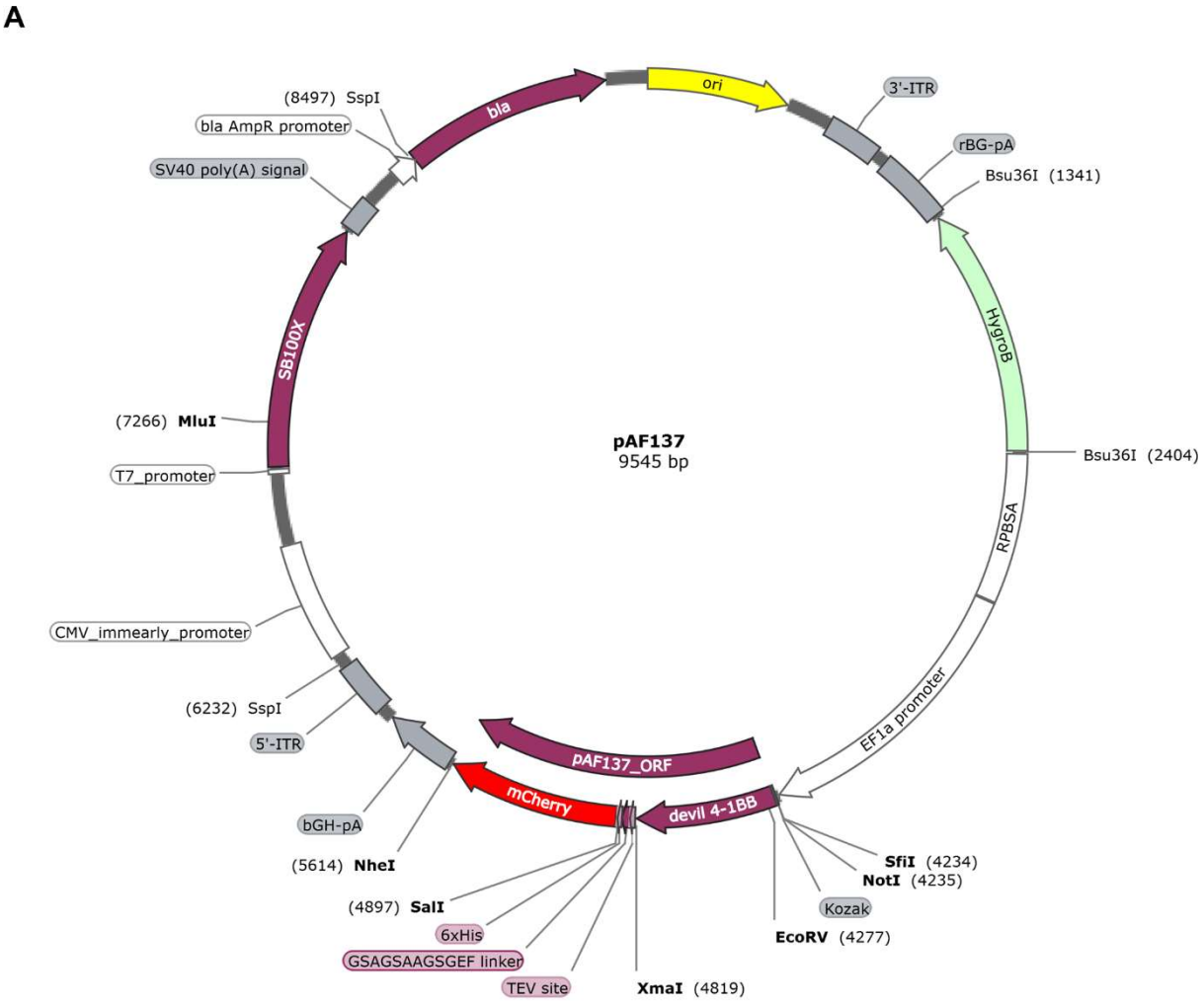
A
pAF137
9545 bp
ori
3'-ITR
rBG-pA
Bsu36I (1341)
Bsu36I (2404)
HygroB
RPBSA
EF1a promoter
devil 4-1BB
mCherry
pAF137_ORF
SB100X
bla
(8497) SspI
bla AmpR promoter
SV40 poly(A) signal
(7266) MluI
T7_promoter
CMV_immearly_promoter
(6232) SspI
5'-ITR
bGH-pA
(5614) NheI
(4897) SalI
6xHis
GSAGSAAGSGEF linker
TEV site
XmaI (4819)
EcoRV (4277)
Kozak
NotI (4235)
SfiI (4234)
B
NotI
SfiI
EF1a promoter
Devil 41BB SigP
Devil 41BB ECD
EcoRI
GSAGSAAGSGEF linker
TEV site
SmaI
XmaI
6xHis
SalI
mCherry
NheI
bGH-pA
pAF137_ORF
C
NotI
SfiI
EF1a promoter
Devil CD80 SigP
Devil CD80 ECD
EcoRV
Bsu36I
Devil IgG Fc region
GSAGSAAGSGEF linker
BamHI
TEV site
SmaI
XmaI
6xHis
SalI
mCherry
NheI
bGH-pA
pAF143_ORF
D
SalI
hamster IL2 signal peptide
NotI
EF1a promoter
mCherry
SmaI/XmaI
TEV site
GSAGSAAGSGEF linker
6xHis
NheI
sha_TNFSF9_ XM_003762879.1
bGH-pA
3'-SfiI
PmeI
pAF92_ORF

Figure S1. FAST protein base vectors. (A) CMV promotor and SB100X transposase were inserted into a Sleeping Beauty vector (shown here with 41BB-mCherry FAST protein cassette pAF137). (B) Type I FAST protein cassette. The native signal peptide (SigP) and predicted extracellular domain (ECD) were used for all type I FAST proteins. The devil 41BB SigP-ECD was fused to mCherry via a TEV cleavage site (ENLYFQG), linker peptide (GSAGSAAGSGEF), and a 6x-His tag (HHHHHH). Restriction digest sites were included at the 5' and 3' ends of the gene-of-interest and the fluorescent protein to facilitate swapping of genes and fluorescent proteins. The human EF1α promotor is upstream and bovine growth hormone (bGH) poly(A) tail is downstream of the open reading frame. (C) Type I FAST protein cassette with Fc tag. This vector was the same as the type I FAST vectors, except that the Fc fragment of devil IgG was inserted between the gene-of-interest (e.g. CD80) and the TEV cleavage site. (D) Type II FAST protein cassette. To increase the probability of efficient secretion of type II FAST proteins from Chinese hamster ovary (CHO) cells, we used the hamster IL-2 signal peptide at the N-terminus of the protein, including a SalI restriction site, followed by mCherry, an NheI restriction site, 6x-His-tag, linker peptide, TEV cleavage site, XmaI/SmaI restriction site, the gene-of-interest, and a PmeI restriction site following the stop codon.

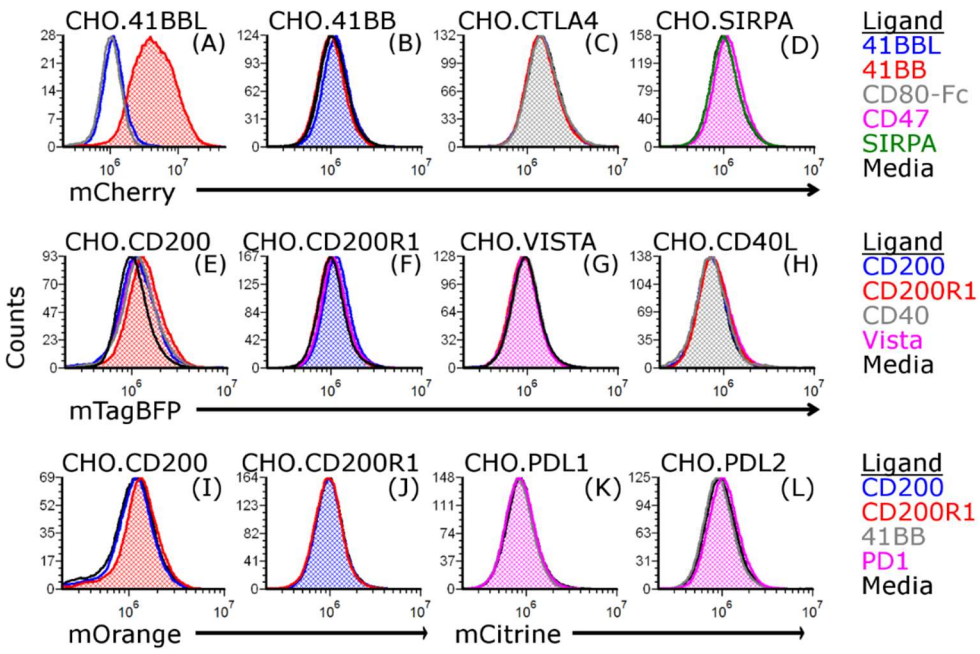

Figure S2. Screening FAST protein supernatants. Staining was performed using supernatants from FAST protein CHO cell lines; supernatants were collected 11 days post-transfection. Supernatants from FAST cell lines were incubated with CHO cells expressing full-length devil proteins at room temperature for 30 minutes before washing and fixing for flow cytometric analysis. Chloroquine was not used during these incubation steps.

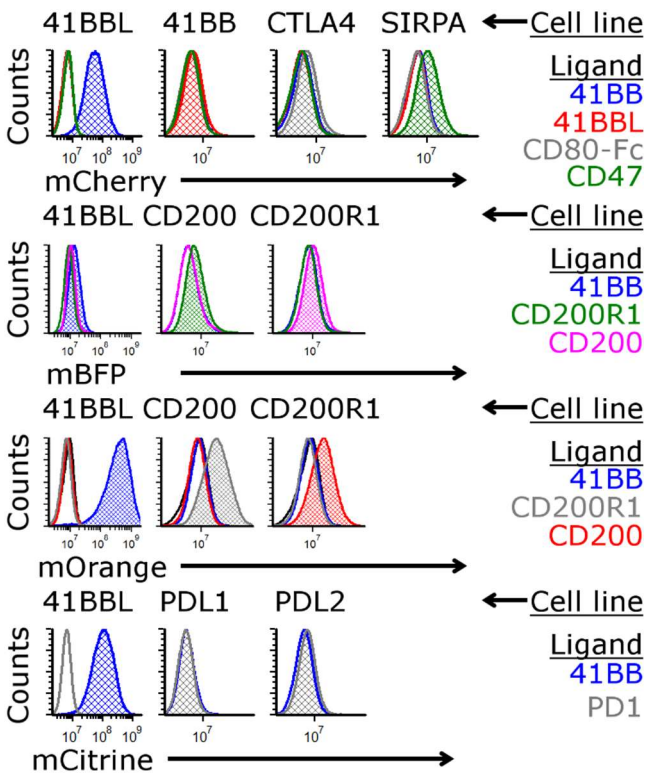

Figure S3. Screening FAST protein supernatants. Staining was performed using supernatants from FAST protein CHO cell lines; supernatants were collected around 2-3 weeks post-transfection and stored for 2 months at 4 °C. Supernatants from FAST cell lines were diluted 1:1 with 100 µM chloroquine (50 µM final concentration) in cRF5 without phenol red incubated with CHO cells expressing full-length devil proteins at room temperature for 60 minutes before washing and fixing for flow cytometric analysis.

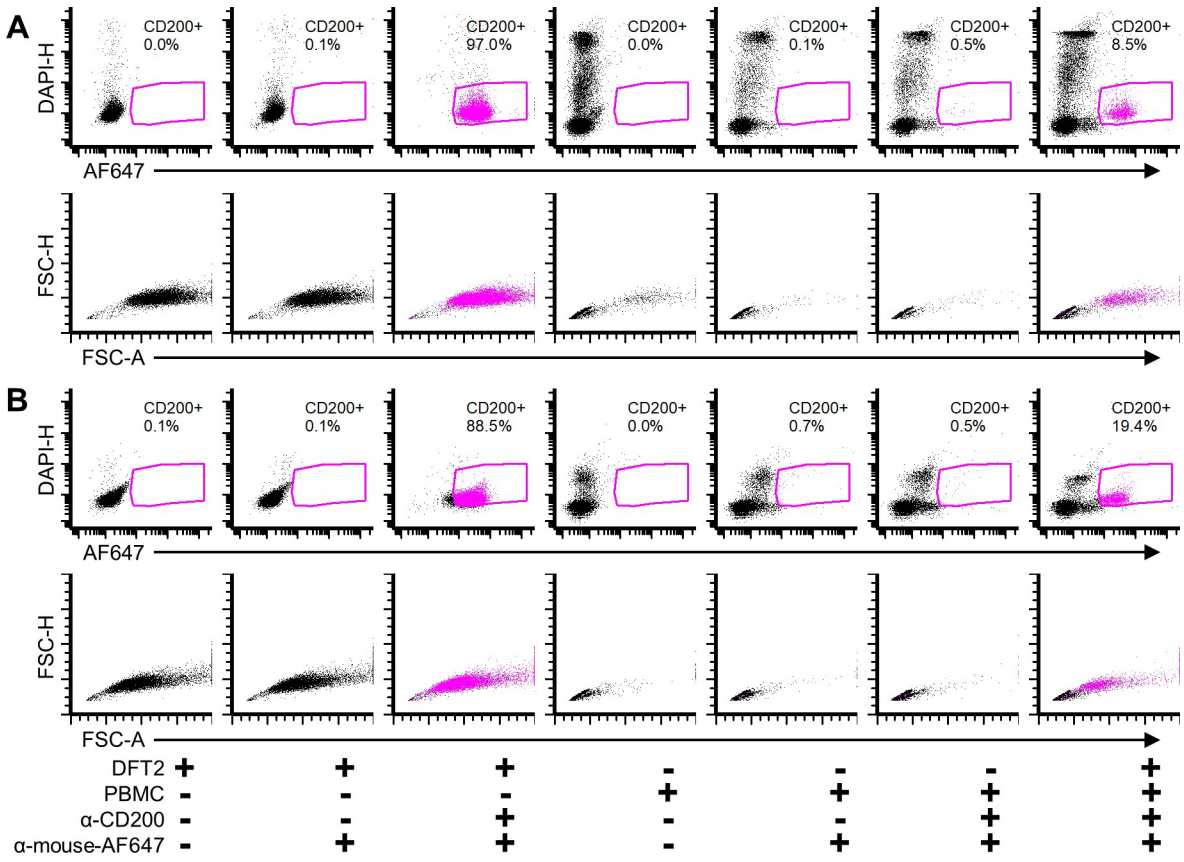

Figure S4. CD200 identifies DFT2 cells in PBMCs. (A) PBMCs isolated on histopaque, frozen, thawed, and cultured for 2 hours at 37 °C prior to use. DFT2 cells and PBMCs were stained separately by blocking with normal goat serum and then staining with anti-CD200 serum and anti-mouse IgG Alexa-647. Cells were then washed, stained with DAPI, and analyzed on a BD FACSCanto II. The stained DFT2 cells and PBMCs were then mixed at 1:10 to see if gating and CD200 signal could be used to distinguish DFT2 cells from PBMCs (n=1/treatment). (B) The procedure used for A was repeated except that DFT2 cells and PBMCs were mixed at 1:5.

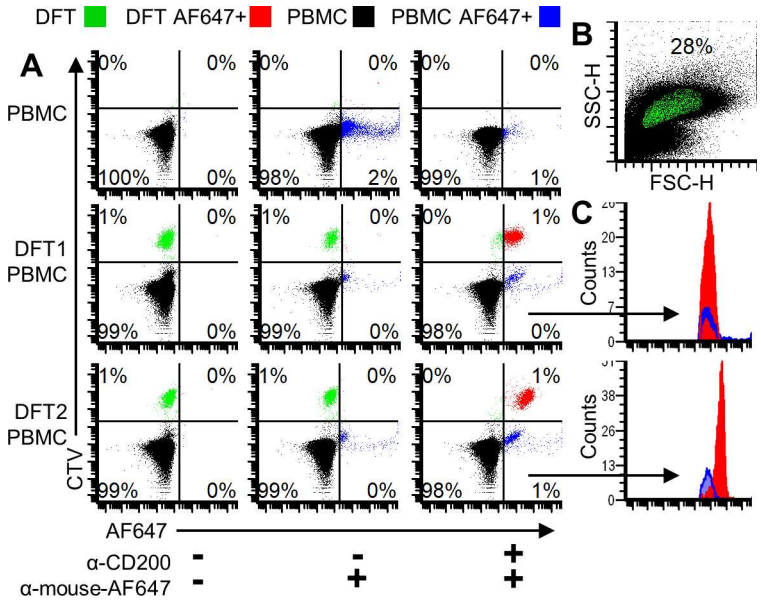

Figure S5. CD200 identifies DFT cells in whole blood. Color dot plots showing DFT cells in green (CellTrace Violet®), PBMCs in black, DFT Alexa Fluor 647+ (AF647) cells in red, and PBMC AF647+ in blue. (A) The top row shows unmixed PBMCs. The middle row and bottom row show DFT1.C5065 and DFT2.JV cells, respectively, mixed with PBMCs. Alexa Fluor 647+ DFT (red) and PBMC (blue) are in the right quadrants. (B) Forward- and side-scatter plot of DFT.JV cells mixed with PBMCs. Backgating of CFSE+ cells was used to create a forward-scatter by side-scatter gate thaat was used as the parent gate for all data shown here. (C) Histogram overlays to highlight AF647+ (right quadrants) from DFT1-PBMC and DFT2-PBMC mixtures. Cells were analyzed on the Beckman-Coulter MoFlo Astrios.

Table S1. Search terms for mammalian immune research studies.

Table S2. Summary and genes and plasmids.

Table S3. Vector backbones and insert cassette components.

Table S4. Primer sequences and gBlocks for plasmid assembly.

Table S5. gBlocks used for assembling plasmids.