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Supplementary Materials for

Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival

Charles E. Gast, Alain D. Silk, Luai Zarour, Lara Riegler, Joshua G. Burkhart, Kyle T. Gustafson, Michael S. Parappilly, Minna Roh-Johnson, James R. Goodman, Brennan Olson, Mark Schmidt, John R. Swain, Paige S. Davies, Vidya Shasthri, Shinji Iizuka, Patrick Flynn, Spencer Watson, James Korkola, Sara A. Courtneidge, Jared M. Fischer, Jerry Jaboin, Kevin G. Billingsley, Charles D. Lopez, Julja Burchard, Joe Gray, Lisa M. Coussens, Brett C. Sheppard, Melissa H. Wong*

*Corresponding author. Email: wongme@ohsu.edu

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/9/eaat7828/DC1)

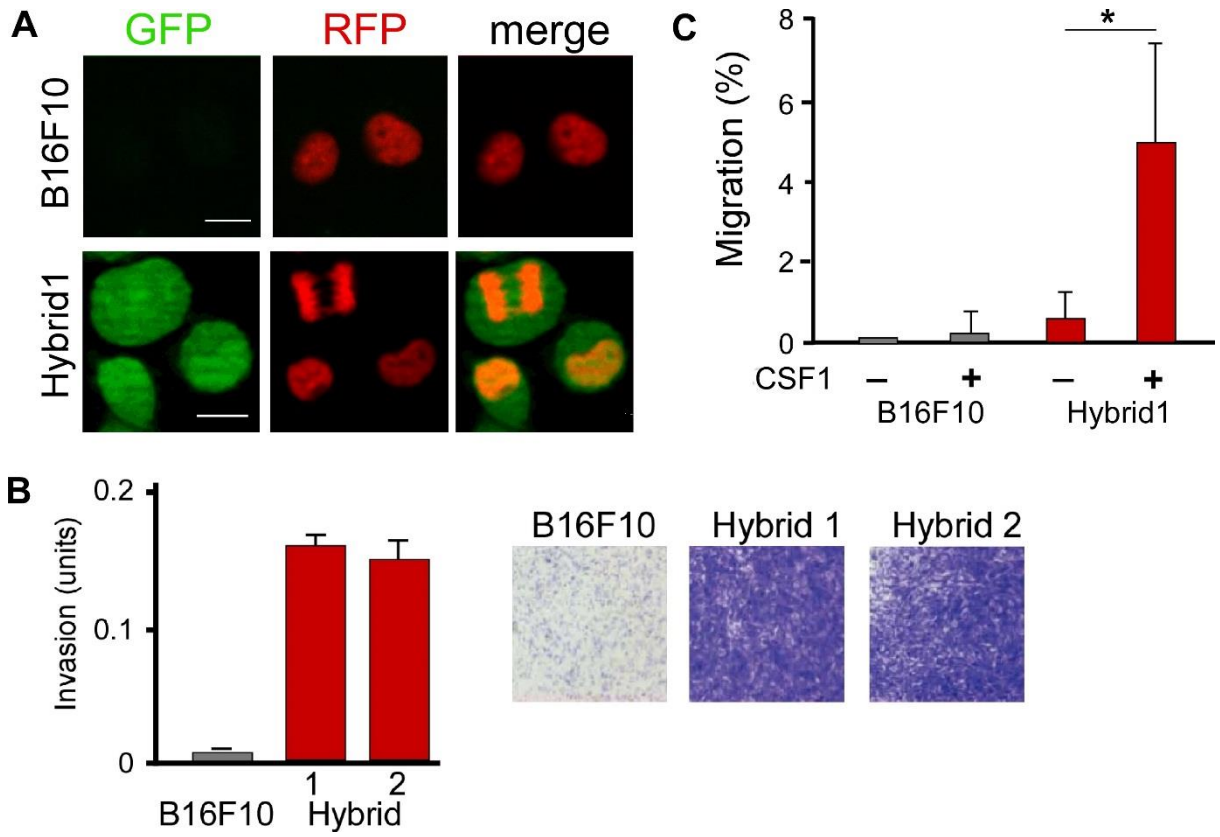

Fig. S1. Characterization of B16F10-derived fusion hybrids. (**A**) Unfused (B16F10) melanoma and hybrid cells analyzed for fusion marks (GFP and H2B-RFP). (**B**) B16F10-derived hybrids and unfused tumor cells subjected to an *in vitro* invasion assay in matrigel invasion chambers, stained with crystal violent after 15h. Data reflects the average of triplicate samples in biologic replicates. (**C**) Chemotaxis towards CSF1 ligand. B16F10-derived hybrid cells chemotax towards CSF1 after 24 hr ($p < 0.05$). Data represents the average of triplicate samples in 5 biological replicates (Hybrid=red bar, MC38=gray bar).

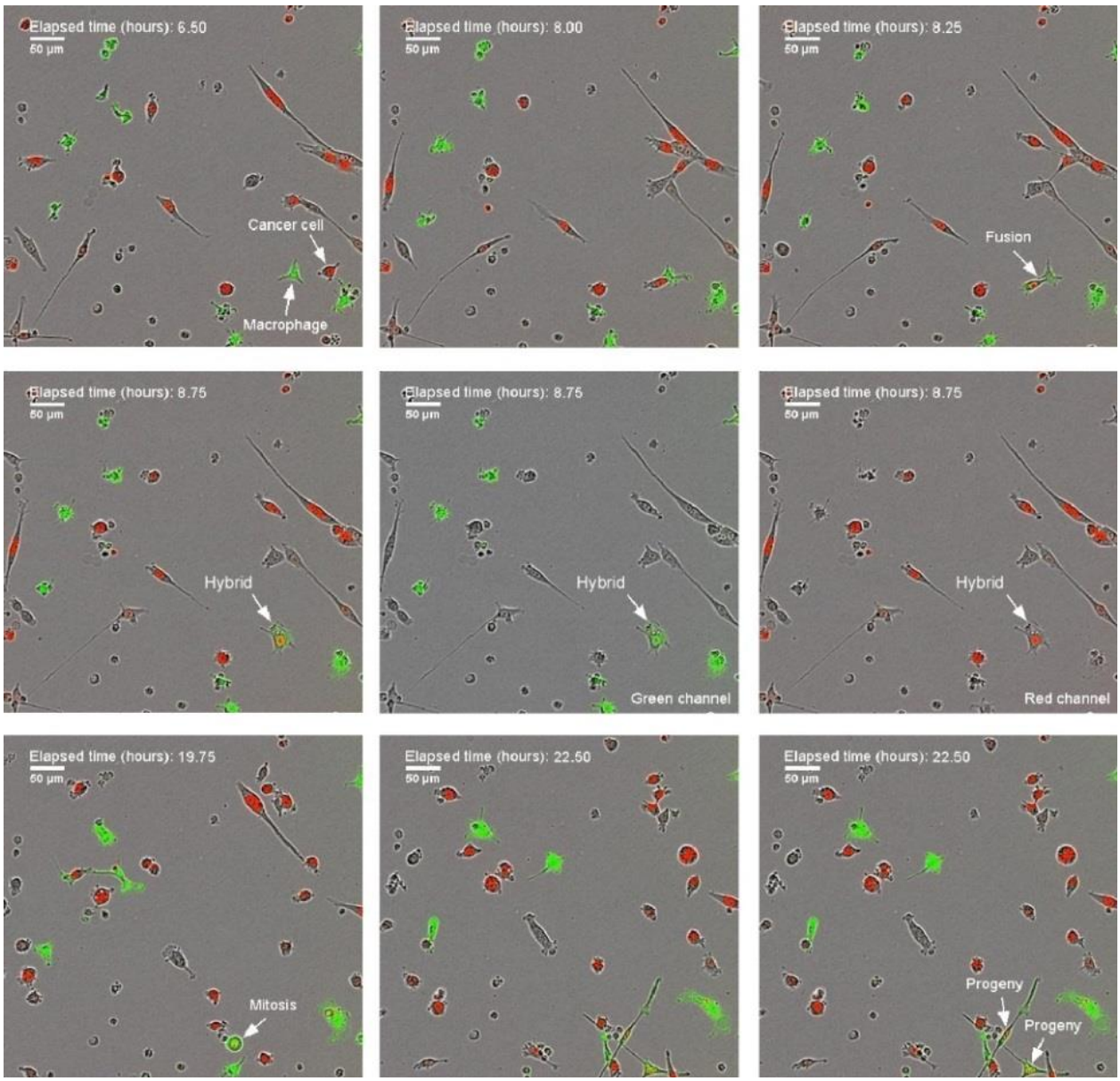

Fig. S2. Still images from cell fusion movie. Nine panels taken from Supplementary video file displays an MC38 cell (red nucleus) fusing with a GFP-expressing MΦ, then undergoing mitotic division.

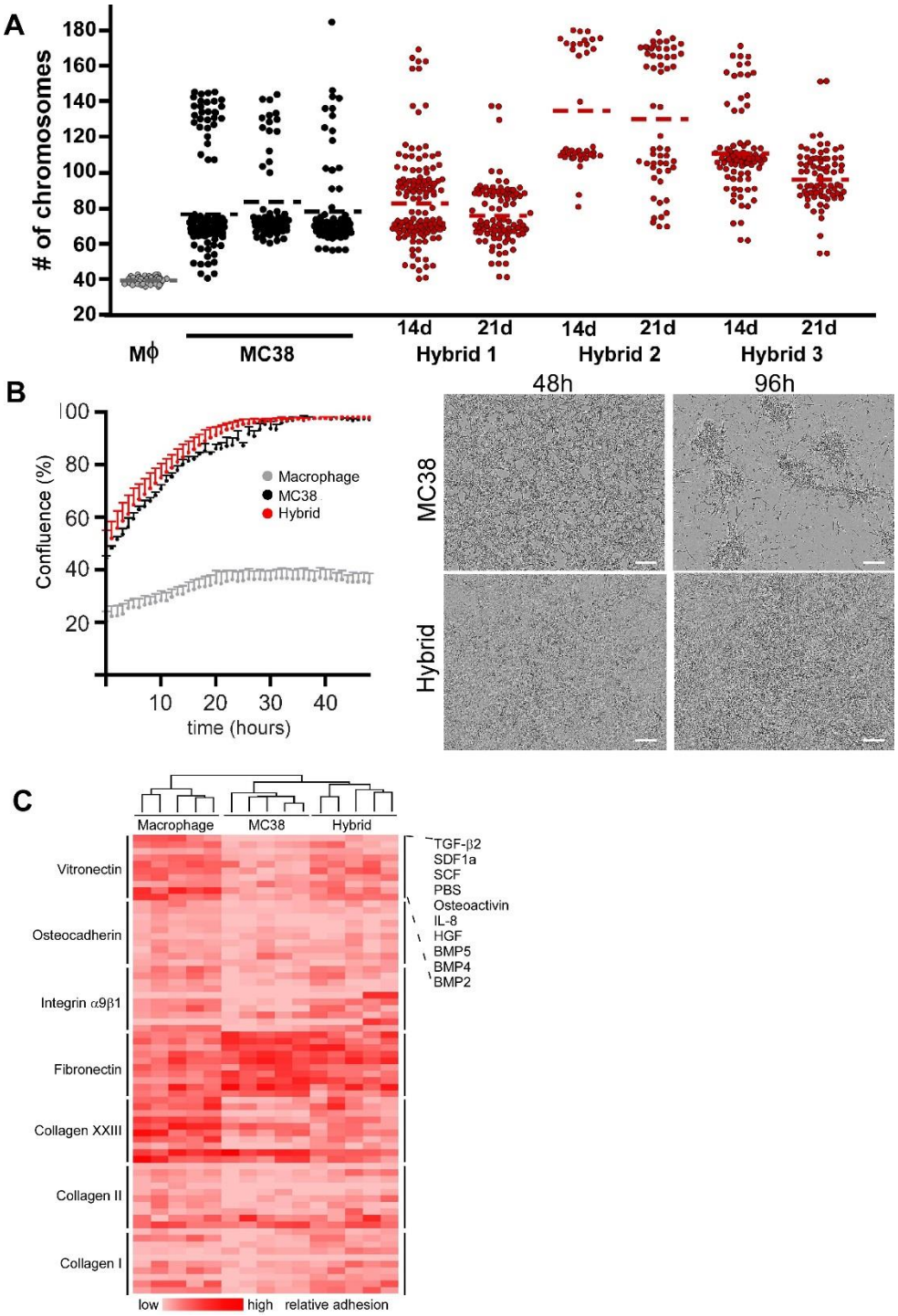

Fig. S3. Differential growth, adhesion, and cytokine response in hybrids. (**A**) Karyotype analyses in MΦ, MC38 cells and 14d and 21d hybrids. Three hybrid isolates are shown. (**B**) Cell confluence relative to time for MC38, MΦ, and hybrid cells. Still images from 48 and 96 h timepoints. (**C**) Heatmap of relative adhesive preference for replicate MC38, MΦ and independent hybrid isolates determined by microenvironment microarray assay; hierarchical clustering according to relative preference for adhesion under 70 different microenvironmental conditions.

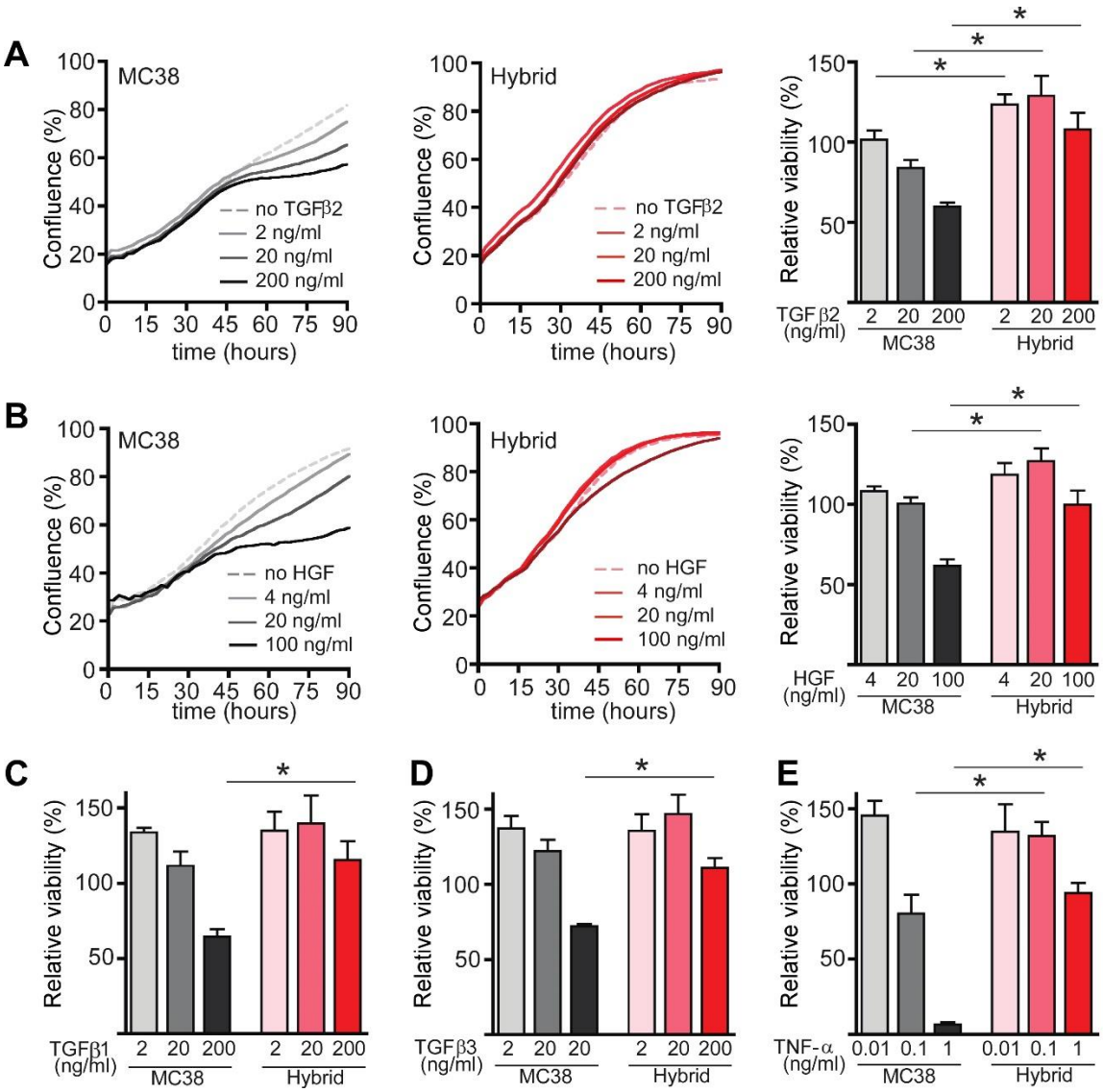

Fig. S4. Differential growth and cytokine response in hybrids. (**A**-**E**) Validation of microenvironment microarray results and cytokine evaluation of hybrid properties relative to unfused MC38 cells. Mean cell confluence over time, and mean viability relative to untreated cells, for replicate MC38 and independent hybrid populations in the presence of increasing concentrations of (A) TGFβ2, (B) HGF, (C) TGFβ1, (D) TGFβ3, and (E) TNF-α. *p<0.05, Student's t-test.

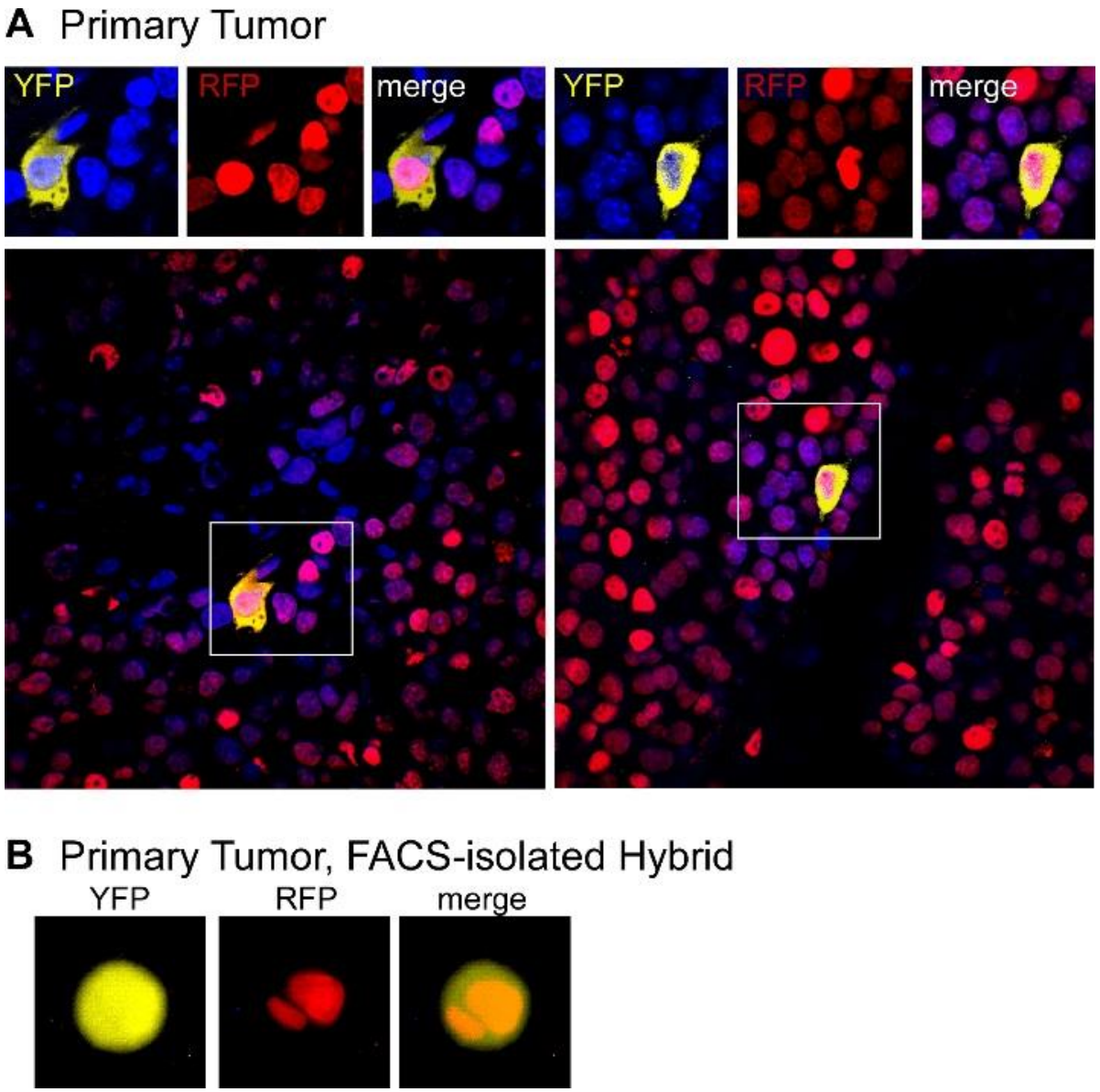

Fig. S5. Characterization of in vivo–derived B16F10 fusion hybrids. (**A**) Representative confocal micrographs of B16F10-derived fusion hybrid in a primary tumor visualized for YFP (yellow), and RFP (red). (**B**) FACS-isolated hybrid cell from the primary tumor visualized for YFP and RFP expression.

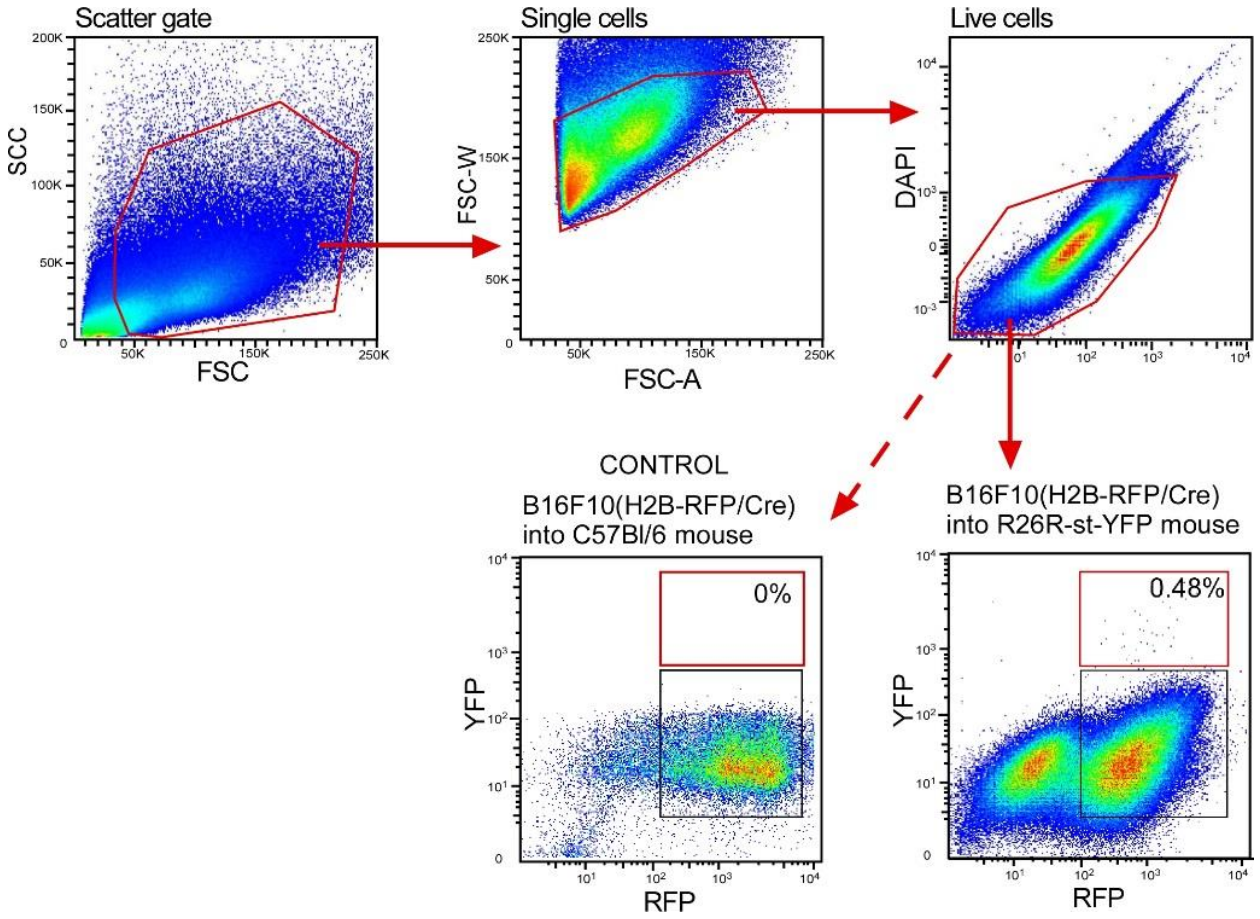

Fig. S6. Flow cytometry gating scheme for B16F10-derived cell fusion analyses. Dissociated tumor cells were stained and subjected to FACS. Gating scheme established based upon single color controls and/or FMO controls. RFP-expressing tumor cells injected into C57Bl/6 mice demonstrate no spurious YFP-expression. Representative data from Fig. 3D.

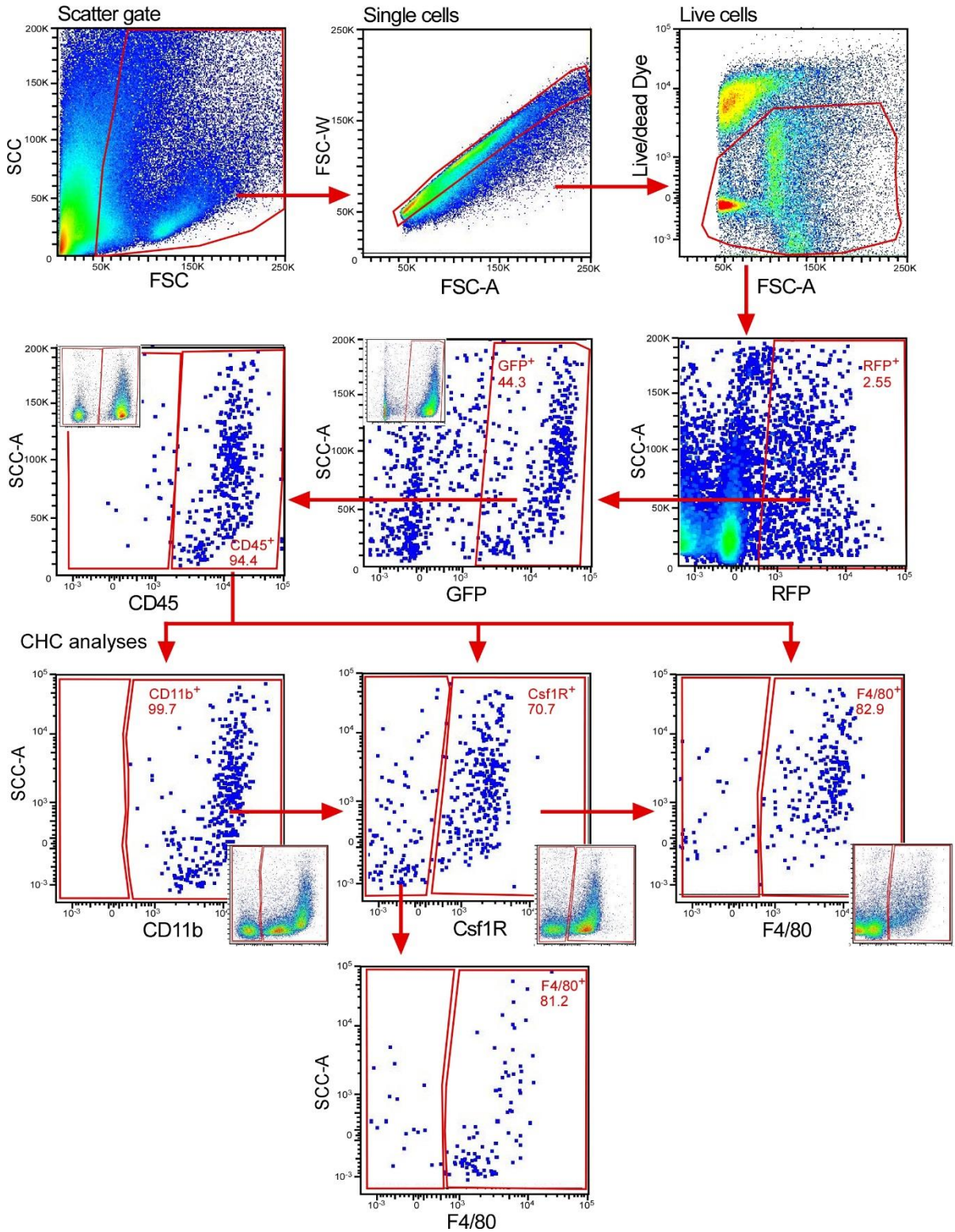

Fig. S7. Gating scheme for flow cytometry of in vivo–derived hybrids from primary tumor. Representative gating scheme for analysis in Figs. 4A and 3H.

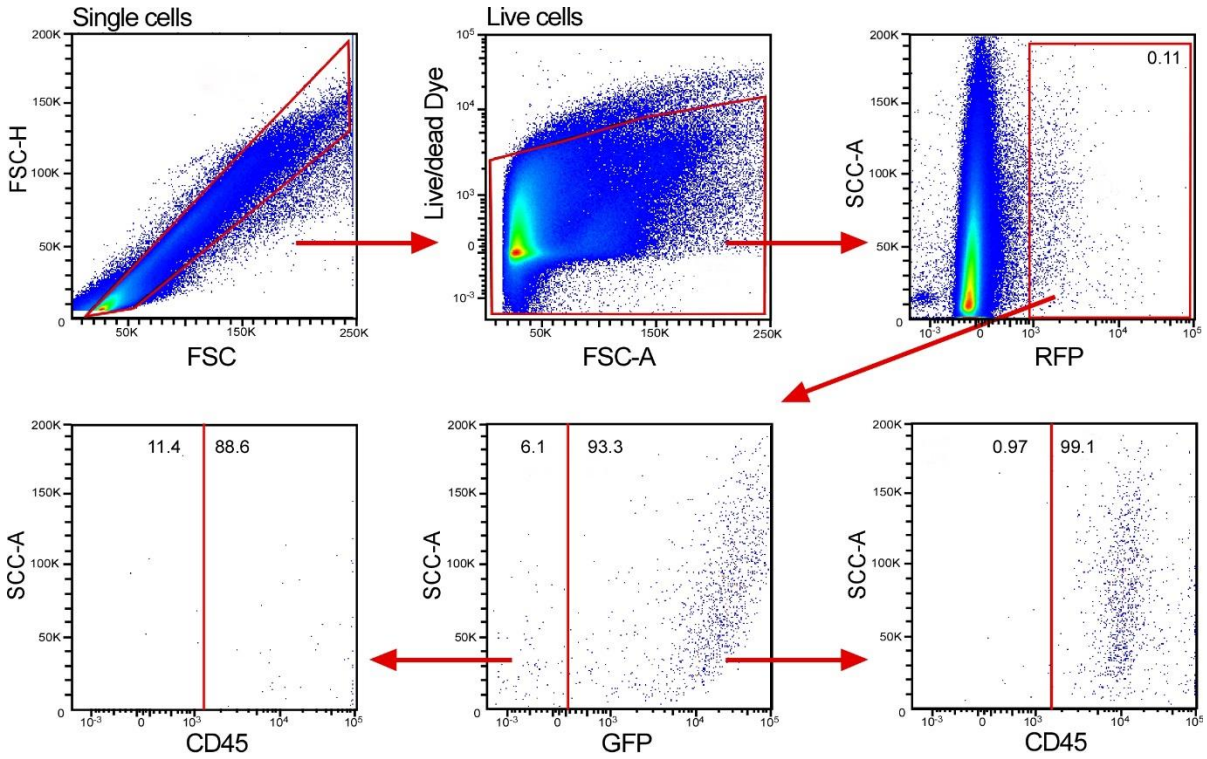

Fig. S8. Flow cytometry gating scheme for analyses of murine CHCs. Isolated murine peripheral blood cells were stained and subjected to FACS. Gating scheme established based upon single color controls and/or FMO controls. Representative data from Fig. 4B.

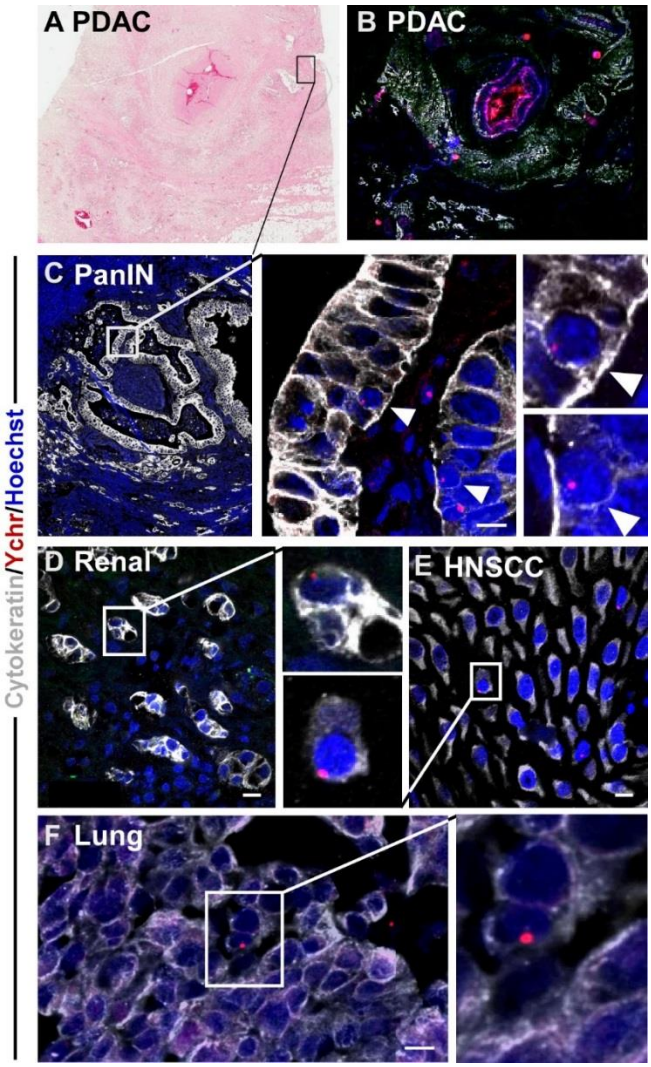

Fig. S9. Cell fusion in PanIN and tumors from other organ sites. Solid tumors from women with previous sex-mismatched bone marrow transplantation permits analysis of cell fusion. (**A**) Hematoxylin and Eosin stain of pancreatic ductal adenocarcinoma (PDAC) section, (**B**) with cytokeratin (gray), the Y-chromosome (Ychr, red) and Hoechst (blue) detection. Boxed region enlarged in (**C**) contains pancreatic intraepithelial neoplasia (PanIN). (**D**-**F**) Renal cell carcinoma, head and neck squamous carcinoma (HNSCC), and lung tumors analyzed for cytokeratin-positive cells with Y-chromosome-positive nuclei, white arrowhead. Representative areas boxed in white are enlarged. Bar = 10 µm.

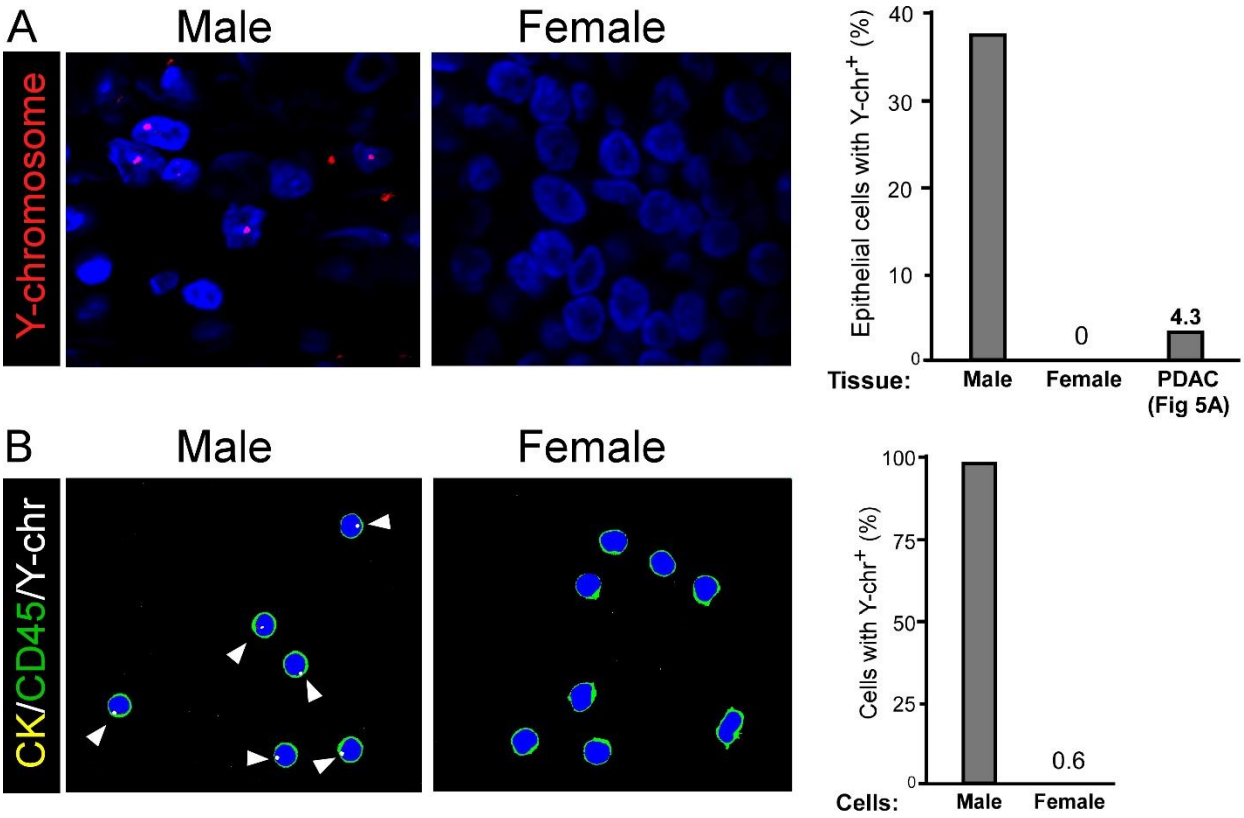

Fig. S10. Control blood samples for immunohistochemical and FISH analyses. (**A**) Male and female tissue stained with Y-chromosome FISH probe (red). Quantification of Y-chromosome-positive cells in male, female and the PDAC tumor from Fig. 5A using confocal microscopy to survey through nucleus. A total of 1532 nuclei in female tissue and 1057 nuclei in male tissue was analyzed. (**B**) Male and female peripheral blood analyzed for expression of cytokeratin (CK, yellow), CD45 (green) and the FISH probe to Y-chromosome (white). Only male cells are positive for the Y-chromosome. A total of 253 nuclei were analyzed in female cells and 638 nuclei in male cells.

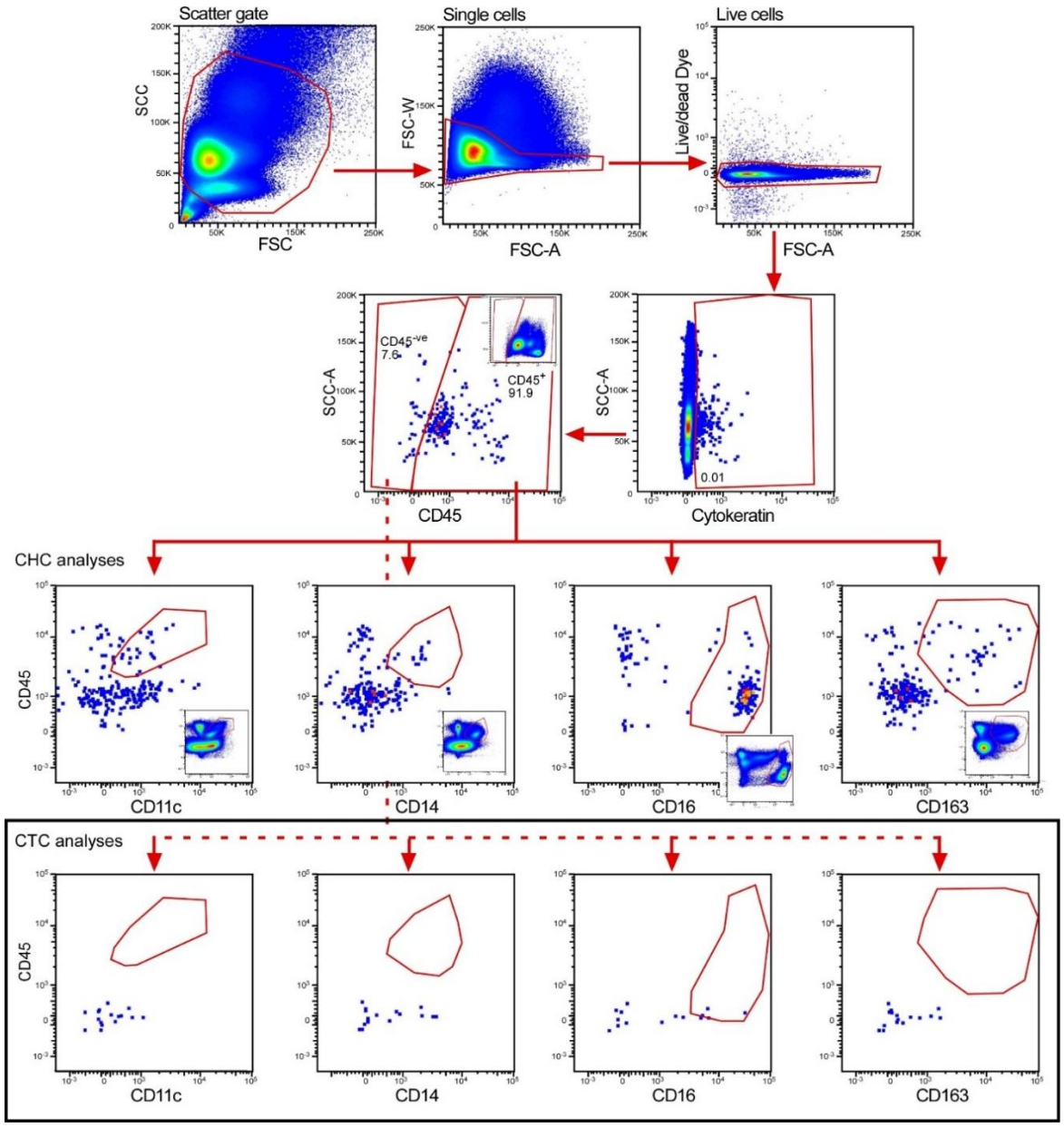

Fig. S11. Flow cytometry gating scheme for analyses of human CHCs. Isolated human peripheral blood cells were stained and subjected to FACS. Gating scheme established based upon single color controls and/or FMO controls.

Supplementary Table Captions

Table S1. GO terms derived from differentially expressed genes between MC38 and hybrid cells. Significantly enriched GO categories in genes significantly up-regulated in both Hybrid vs MC-38 and Macrophage vs MC-38 comparisons. GO category enrichment significance is shown as a -log10(p-value).

Table S2. GO category gene table. GO term log10(p-value), Affymetrix probe identifier, MGI Gene Symbol, Log2 fold-change in hybrid vs MC38 comparison, and Log2 fold-change in Macrophage vs MC38 comparison for genes significantly up-regulated in the hybrid vs MC38 comparison.

Table S3. MΦ-unique or MΦ-enriched genes. Significantly up-regulated genes in both Hybrid vs MC-38 and Macrophage vs MC-38 comparisons.

Supplementary Video Captions

Movie S1. Live imaging of MΦ–cancer cell fusion. Representative video of co-cultured MΦs isolated from Actin-GFP (YO1-GFP) mice and cancer cells expressing nuclear RFP (H2B-RFP). Green channel, red channel, and phase contrast images were captured every 15 minutes. MΦ-cancer cell fusion and subsequent mitosis are indicated by arrows. Hybrid cell expresses both cytoplasmic GFP and nuclear RFP. Bar = 50 μm.

Movie S2. Live imaging of cultured hybrid cells past confluence. Live imaging of proliferation of unfused MC38 cells (left) and MC38-derived hybrids (right) cell cultures. Phase contrast. Imaged every hour. Bar = 100 μm.