ADVANCED HEALTHCARE MATERIALS

# Supporting Information

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Microfluidic T Cell Selection by Cellular Avidity

*Julian F. Ashby, Julien Schmidt, Neelima KC, Armand Kurum, Caroline Koch, Alexandre Harari, Li Tang and Sam H. Au**

# Supplementary Information

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Julian F. Ashby[1], Julien Schmidt[2], Neelima KC[1], Armand Kurum[3], Caroline Koch[1], Alexandre Harari[2], Li Tang[3,4] & Sam H. Au[1,5]

[1] Department of Bioengineering, Imperial College London, London, SW7 2AZ, UK.

[2] Center of Experimental Therapeutics, Department of Oncology, Lausanne University Hospital (CHUV), Lausanne, Switzerland.

[3] Institute of Materials Science and Engineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

[4] Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

[5] Cancer Research UK Convergence Science Centre, London, SW7 2AZ, UK

*Correspondence to: s.au@imperial.ac.uk

# Movies

Time-lapse movies taken using a Nikon Eclipse Ti2 Inverted Microscope (Nikon, Japan) attached with a monochrome Nikon DS-Qi2 camera and LED illuminator. Scale bar set at 250 μm.

**Movie S1:** 10 minute time-lapse of Fluo-8 stained SUPT1 DMβ T cells (high avidity, green) calcium flux activation on Me290 melanoma monolayers (NY-ESO-1$^+$)

**Movie S2:** 30 second time-lapse of SUPT1 T cell detachment off Me290 melanoma monolayers (NY-ESO-1$^+$) under 1 Pa (100 μL/min) of fluid flow. Double mutant beta T cells (high avidity, green), wild-type T cells (normal physiological avidity, red) and non-transduced T cells (non-specific avidity, blue).

**Movie S3:** 30 second time-lapse of SUPT1 T cell detachment off Me290 melanoma monolayers (NY-ESO-1$^+$) under 1.9 Pa (200 μL/min) of fluid flow. Double mutant beta T cells (high avidity, green), wild-type T cells (normal physiological avidity, red) and non-transduced T cells (non-specific avidity, blue).

**Movie S4:** 30 second time-lapse of SUPT1 T cell detachment off Me290 melanoma monolayers (NY-ESO-1$^+$) under 3.8 Pa (400 μL/min) of fluid flow. Double mutant beta T cells (high avidity, green), wild-type T cells (normal physiological avidity, red) and non-transduced T cells (non-specific avidity, blue).

**Movie S5:** 30 second time-lapse of SUPT1 T cell detachment off Me290 melanoma monolayers (NY-ESO-1$^+$) under 5.7 Pa (600 μL/min) of fluid flow. Double mutant beta T cells (high avidity, green), wild-type T cells (normal physiological avidity, red) and non-transduced T cells (non-specific avidity, blue).

**Movie S6:** 1 minute time-lapse of SupT1 T cell detachment from non-specific NY-ESO-1 antigen expressing NA8 monolayers (negative control) under 1.9 Pa (200 μL/min) of fluid flow. Double mutant beta T cells (green), wild-type T cells (red) and non-transduced T cells (blue).

# Tables

**Table S1.** List of volumetric flow rates inputted to calculate shear stresses computed at 4 microns above device surface within the T cell-tumour cell interaction region of device under laminar flow conditions.

| Volumetric flow rate [µL/min] | Volumetric flow rate [$mm^3/s$] | Shear stress [Pa] |
|---|---|---|
| 0 | 0 | 0.0 |
| 50 | 0.8335 | 0.5 |
| 100 | 1.667 | 1.0 |
| 200 | 3.334 | 1.9 |
| 400 | 6.668 | 3.8 |
| 600 | 10.002 | 5.7 |
| 800 | 13.336 | 7.7 |
| 1200 | 20.004 | 11.5 |
| 1500 | 25.005 | 14.4 |
| 2000 | 33.34 | 19.2 |

**Table S2.** List of entrance length values calculated by inputting the hydraulic diameter and Reynolds numbers for different input velocities

| Volumetric flow rate [µL/min] | Input velocity [m/s] | Reynolds [dimensionless] | Entrance length [mm] |
|---|---|---|---|
| 0 | 0 | 0 | 0.000 |
| 50 | 0.008335 | 1.515 | 0.014 |
| 100 | 0.01667 | 3.031 | 0.028 |
| 200 | 0.03334 | 6.062 | 0.055 |
| 400 | 0.06668 | 12.124 | 0.110 |
| 600 | 0.10002 | 18.185 | 0.165 |
| 800 | 0.13336 | 24.247 | 0.220 |
| 1200 | 0.20004 | 36.371 | 0.331 |
| 1500 | 0.25005 | 45.464 | 0.413 |
| 2000 | 0.3334 | 60.618 | 0.551 |

# Figures

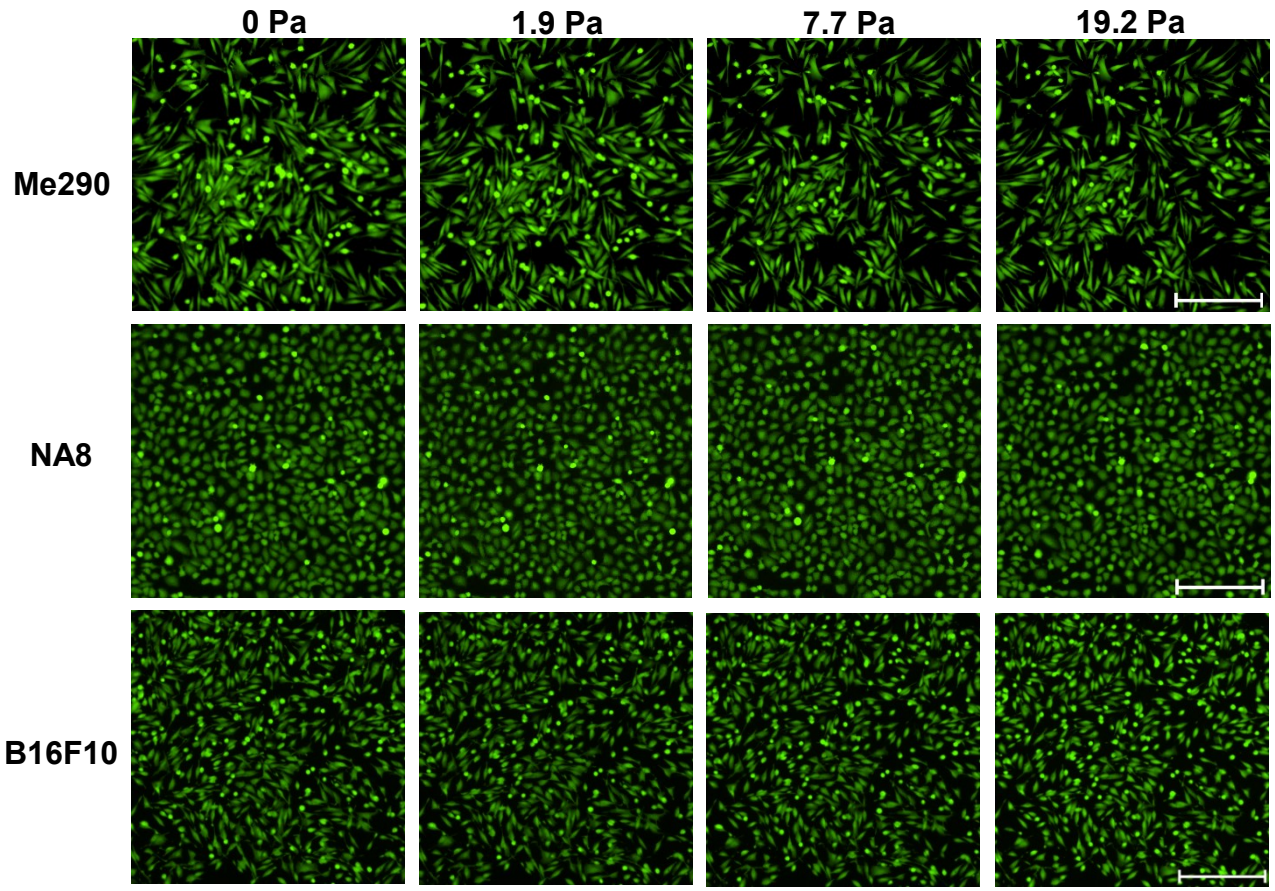
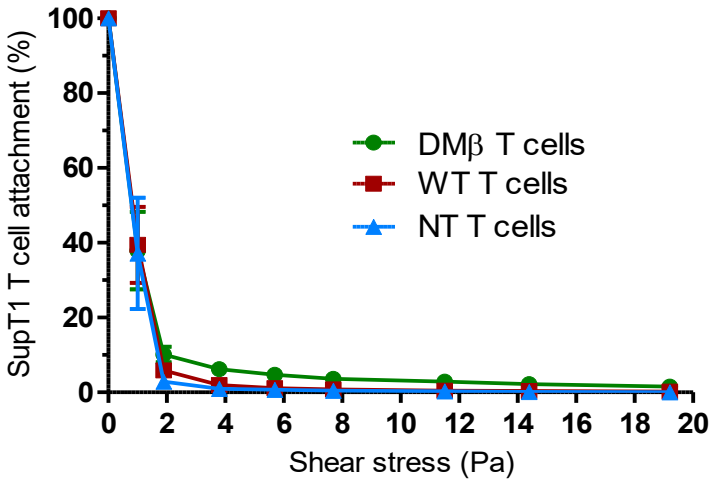
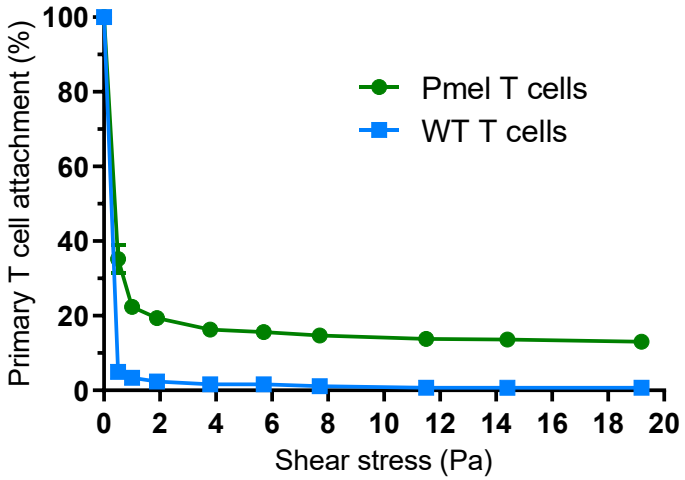



**Figure S1. Representative images of melanoma monolayer adhesion under shear in the device**. Me290 (top), NA8 (middle) and B16F10 (bottom) cell attachment under 0-19 Pa shear stresses. Tumour cells stained with calcein-AM, scale bar: 250 μm.

a




b

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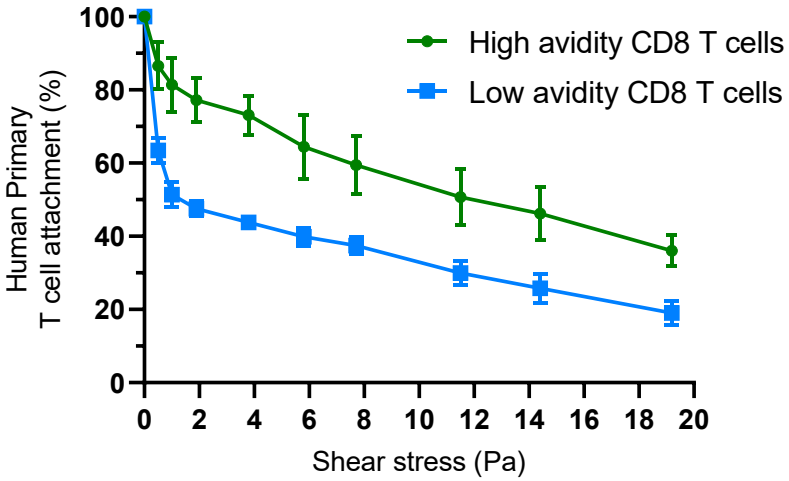



**Figure S2. Full data set for SUPT1, patient-derived T cells and primary mouse T cell attachment including non-specific binding. a,** SUPT1 T cell percent attachment quantification (0 – 1.9 Pa). **b,** Primary mouse T cell percent attachment quantification. **c,** Primary human T cell percent attachment quantification. Data are represented as mean ± SEM error bars, N=3.

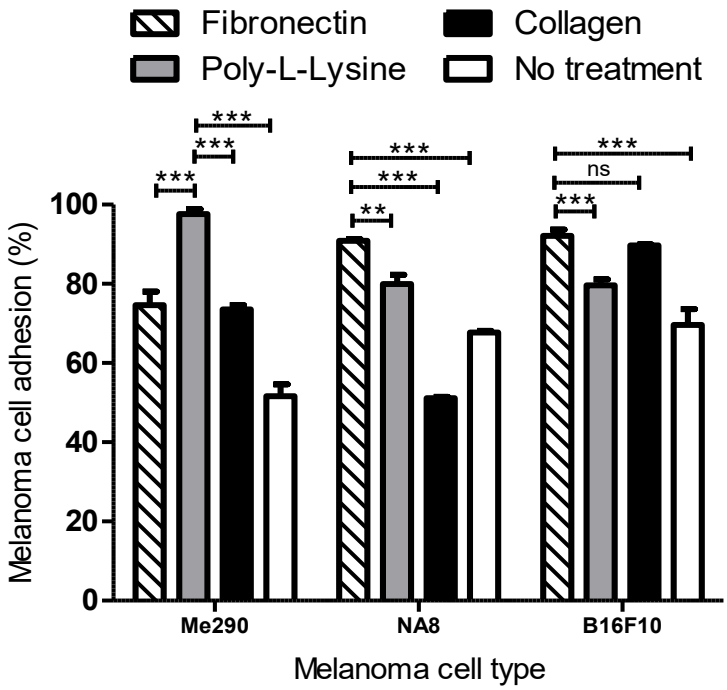



**Figure S3. Melanoma adhesion on varying surface treated devices**. Quantification of the initial percentage of melanoma cells adhered to device substrates coated with fibronectin, poly-L-lysine, collagen or untreated surfaces. Data are represented as mean ± SEM error bars, N=3, P-values are calculated using two-way ANOVA with Bonferroni's multiple comparisons test, *P < 0.05,**P < 0.01, ***P < 0.001.

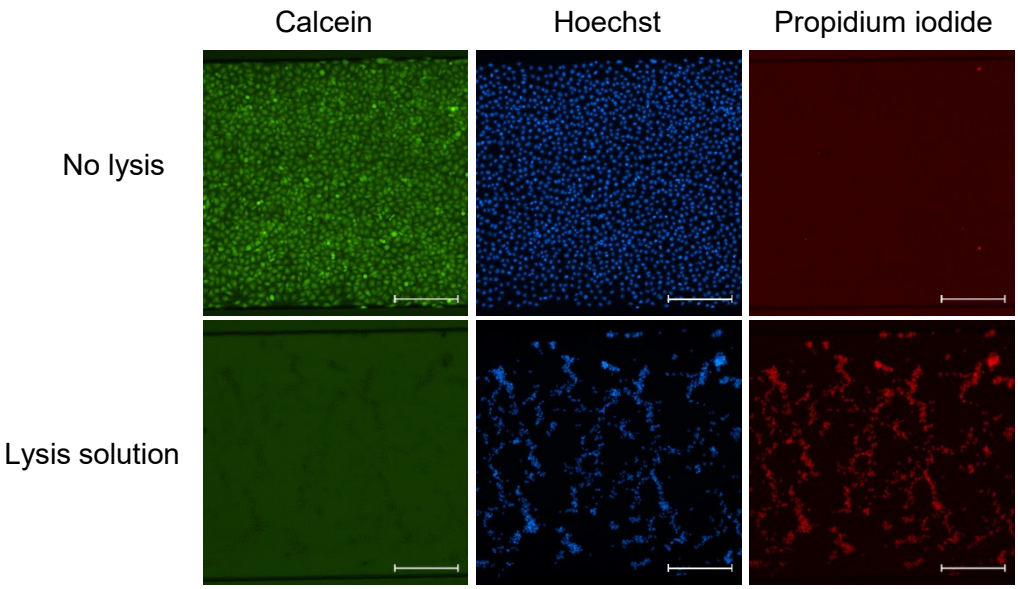



**Figure S4. Representative control images of melanoma monolayer viability.** Control to test the working mechanism of propidium iodide (red), Hoechst (blue) and calcein (green) using a cell lysis buffer to demonstrate live (top) and dead (bottom) B16F10 melanoma cells inside the device. . Scale bar: 250 μm

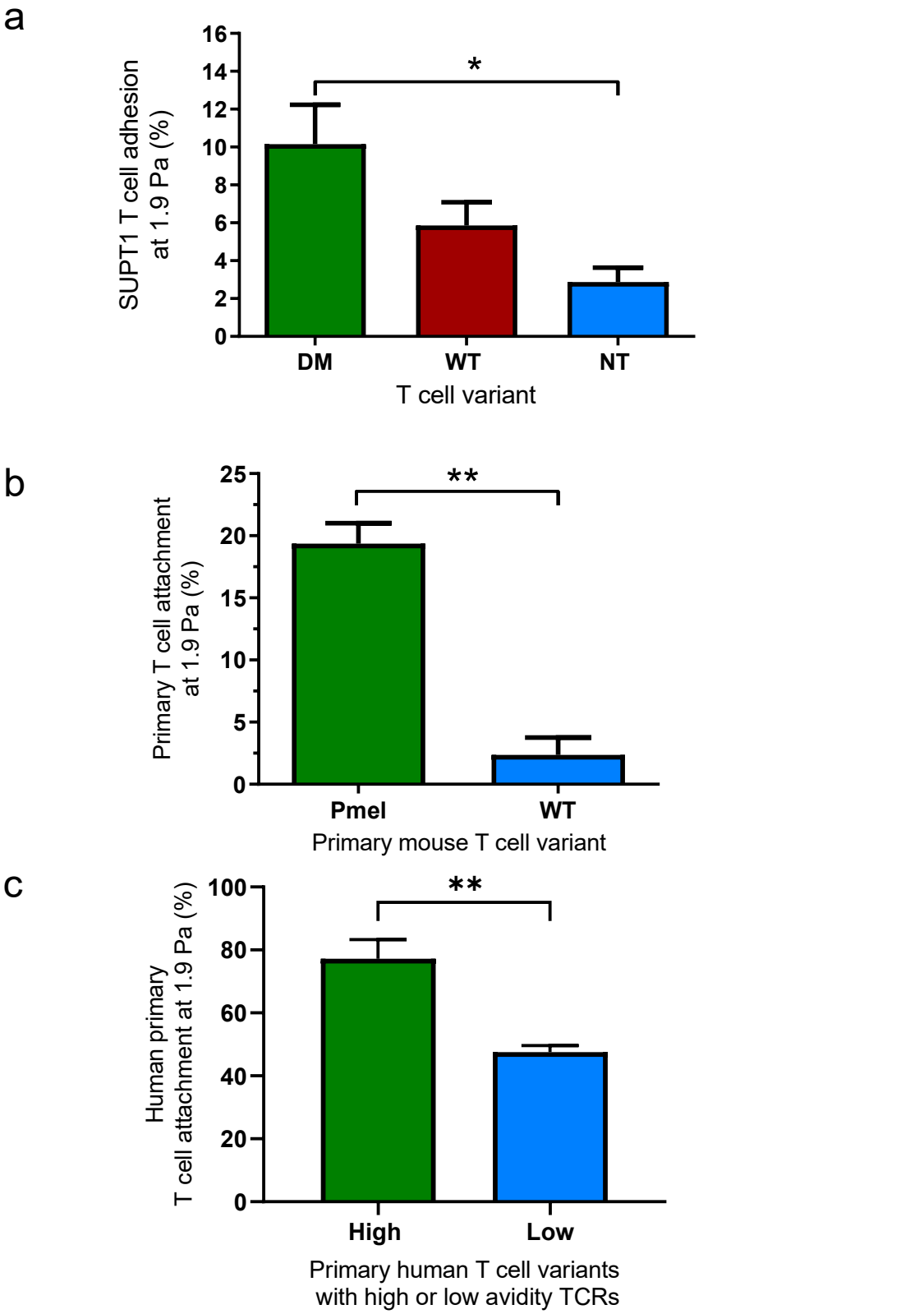
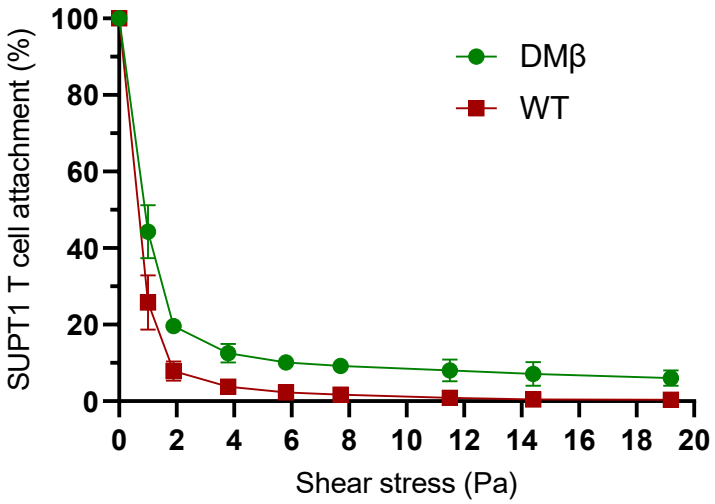



**Figure S5. Bar plots of the percent of T cell attachment to melanoma monolayers at 1.9 Pa after initial wash phase. a,** SUPT1 T cells **b,** Primary mouse T cells and **c,** human primary T cell variants. Data are represented as mean ± SEM error bars, N=3, P-values are calculated using two-way ANOVA with Bonferroni's multiple comparisons test or a two-tailed Student's t-test, *P < 0.05,**P < 0.01, ***P < 0.001.

a

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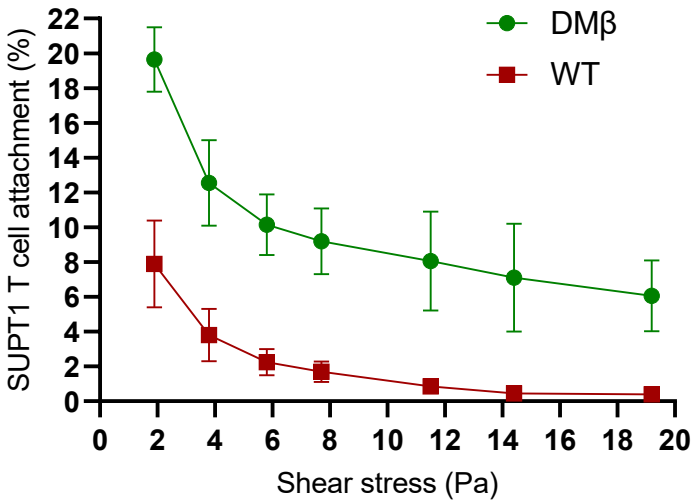
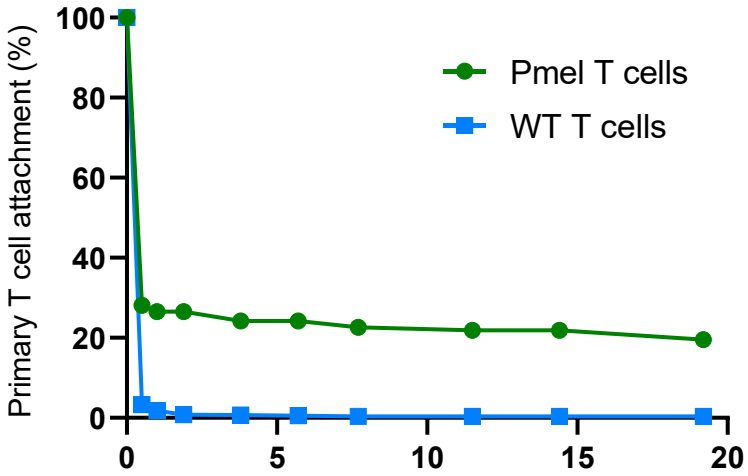



**Figure S6. SUPT1 T cell attachment under shear flow after 15 minutes of cell-cell interaction time before shear flow. a,** Full dataset including wash phase (0-19.2 Pa) and **b,** dataset after washing (1.9 – 19.2 Pa). Data are represented as mean ± SEM error bars, N=3.

a

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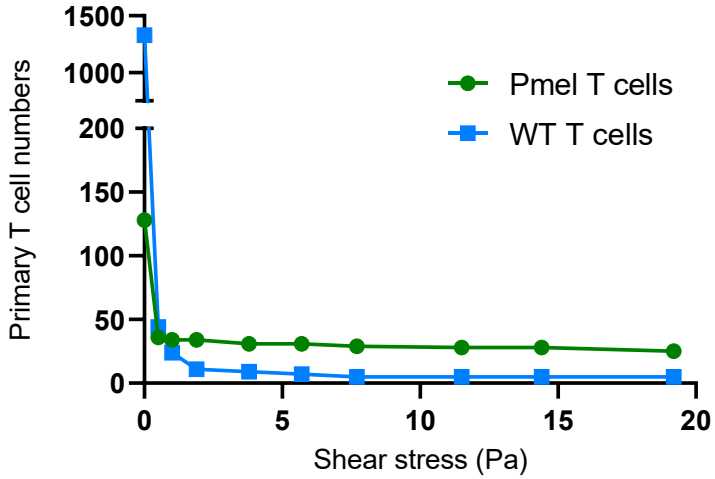
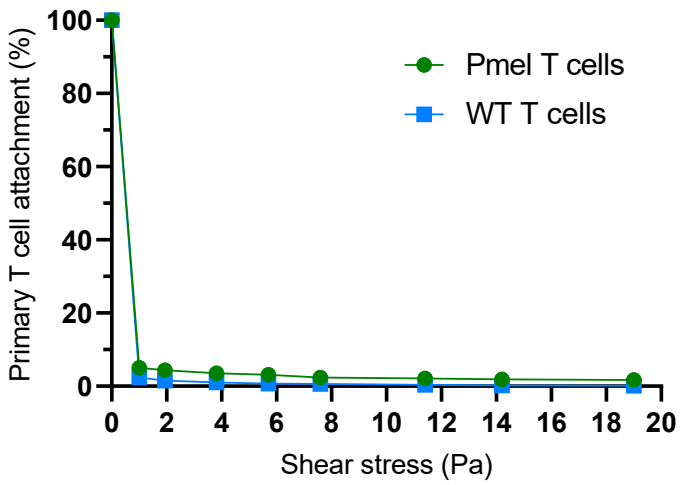



**Figure S7. Quantification of primary mouse T cell attachment under shear stress with a seeding ratio of 1:10 (Pmel:B6 T cells). a,** Primary T cell attachment percentage and **b,** primary T cell attachment absolute live T cell numbers. Data are represented as mean, N=1.

a




b

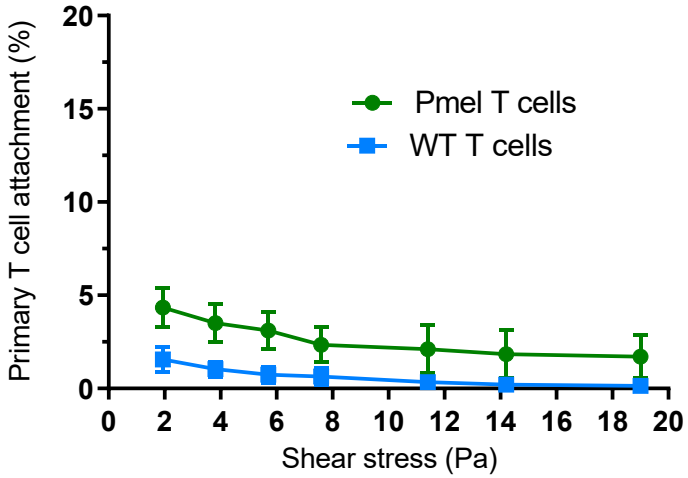



**Figure S8. Quantification of primary mouse T cell attachment on tumor monolayers with no hgp100 pre-treatment under shear. a,** Primary T cell attachment percentage including wash phase (0-19.2 Pa) and **b,** condensed primary T cell attachment percentage after wash phase (1.9 – 19.2 Pa). ). Data are represented as mean ± SEM error bars, N=3.

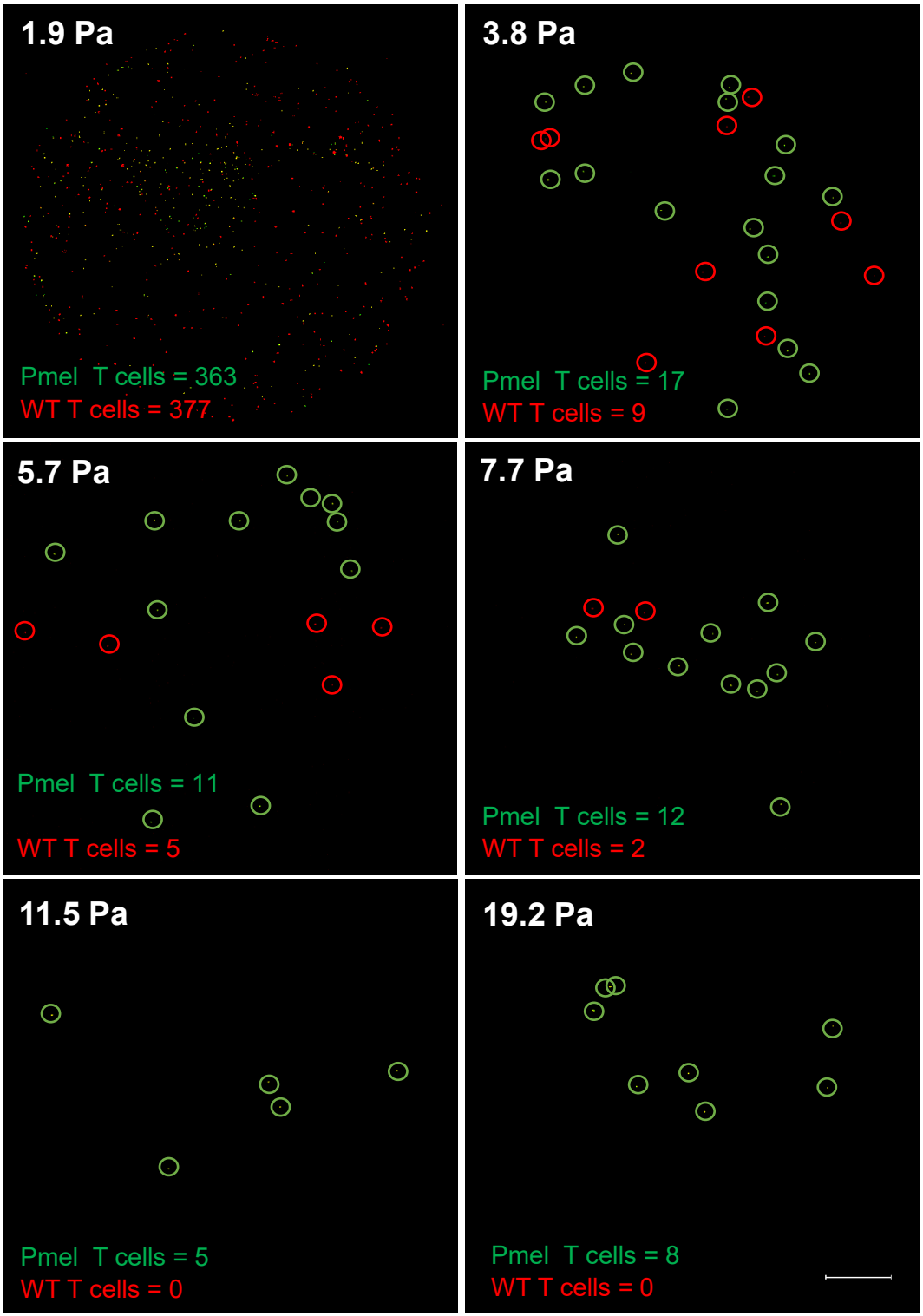



**Figure S9. Representative images of primary mouse T cell collection.** Pmel T cells (green with green circles) and WT T cells (red with red circles) collected from the product outlet reservoir and separated into 96 well plate fractions via outlet tubing after each shear stress condition (Pa). Automated cell detection was conducted using Nikon Elements Software. Scale bar:1 mm.

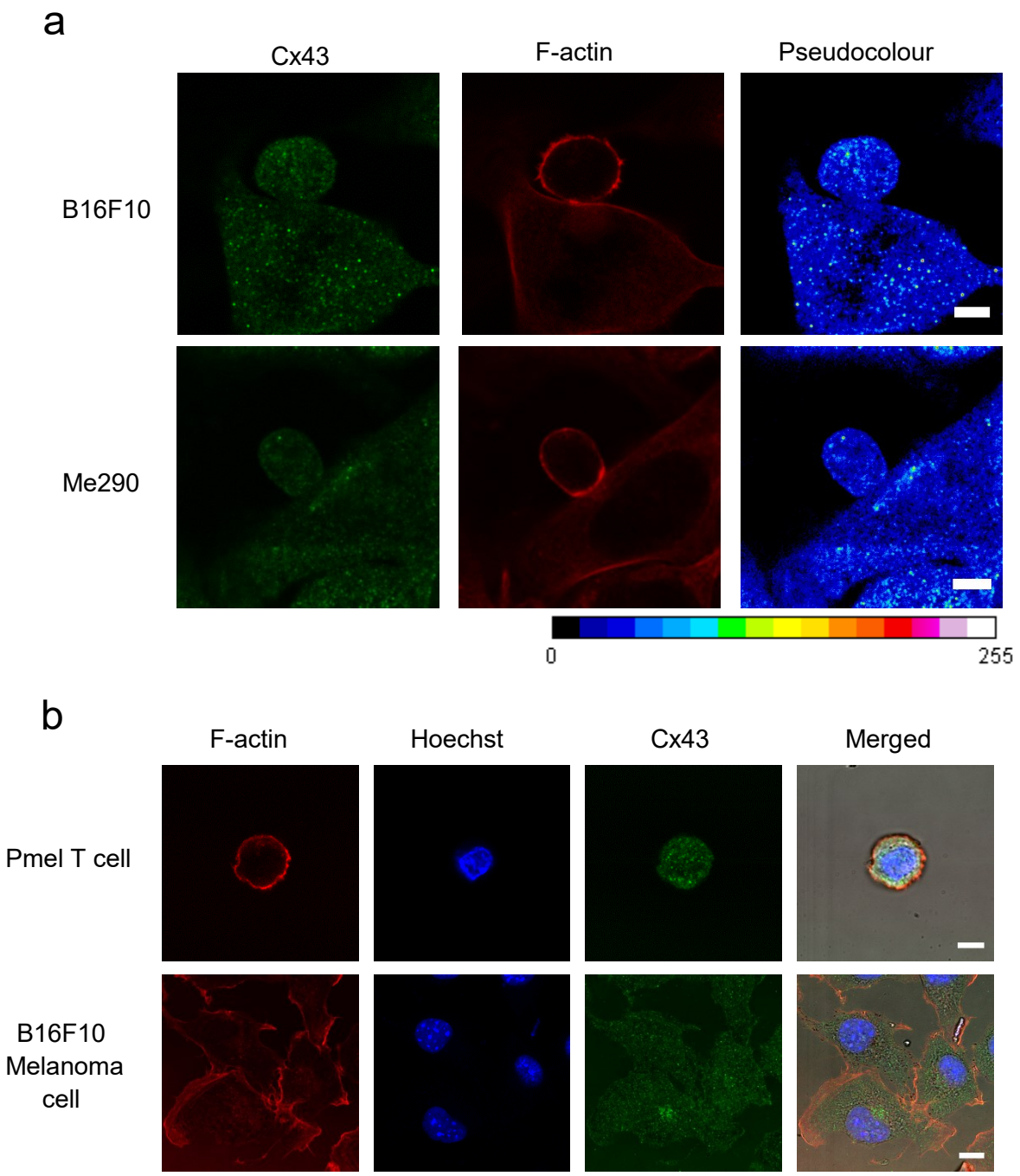
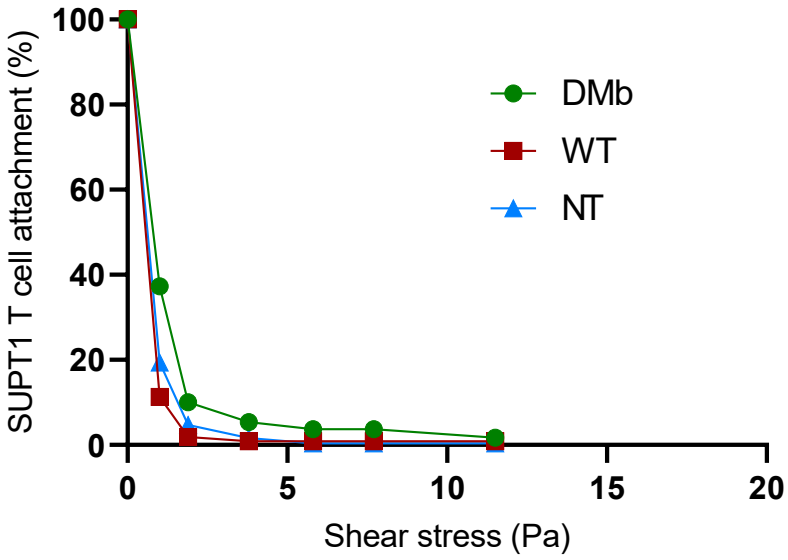



**Figure S10. Immunological synapse controls. a,** DMβ SUPT1 T cell bound to a B16F10 (top) and Me290 (bottom) melanoma cell inside the device. Cells stained for Cx43 (green) and F-actin (red) accumulation with pseudocolours (right panel) used to represent Cx43 intensity at the synapse, scale bar set at 5 μm. **b,** Pmel T cell (top) unbound and B16F10 cell bound to another B16F10 cell (bottom) stained for F-actin (red), Hoechst (blue) and Cx43 (green), scale bar set at 10 μm.

a

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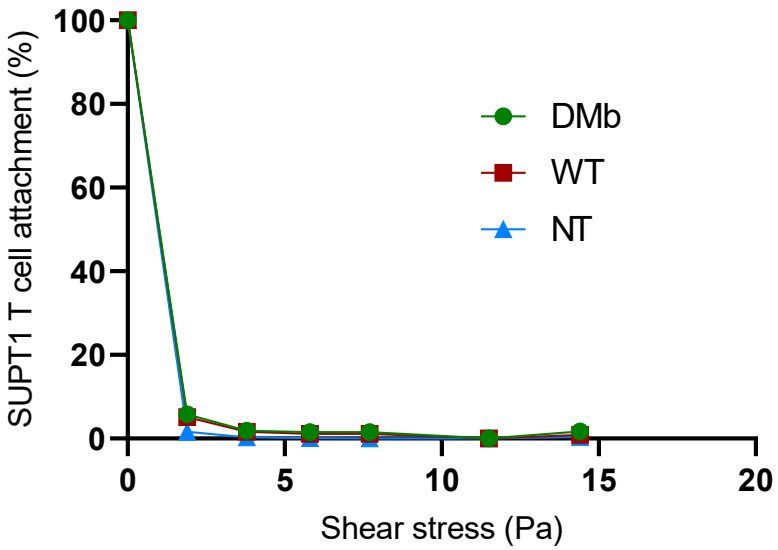


**Figure S11. SUPT1 T cell attachment under shear flow using different parameters to improve adhesion and separation between variants. a,** T cell media containing 1% bovine serum albumin and **b**, media containing E-cadherin blocking antibody (DECMA-1, Sigma). Data are represented as mean, N=1.