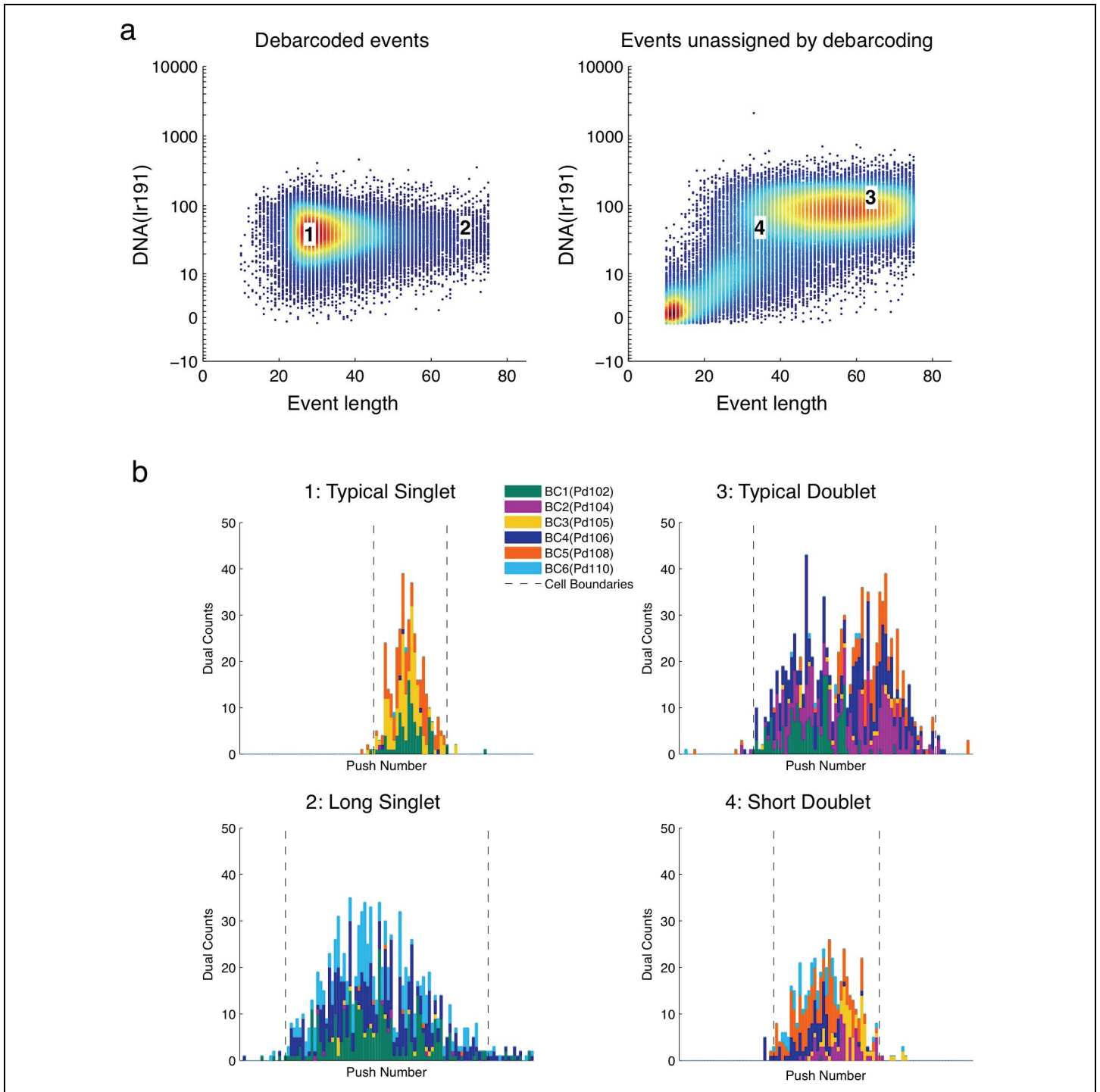


**Supplementary Figure 1**

Palladium barcode staining intensity across cell lines of different cell sizes.

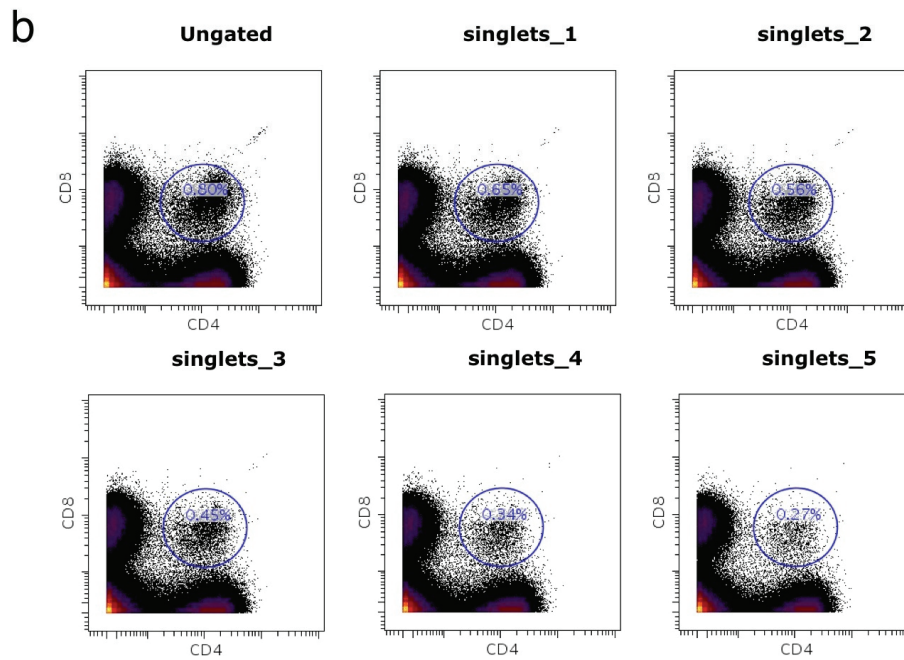
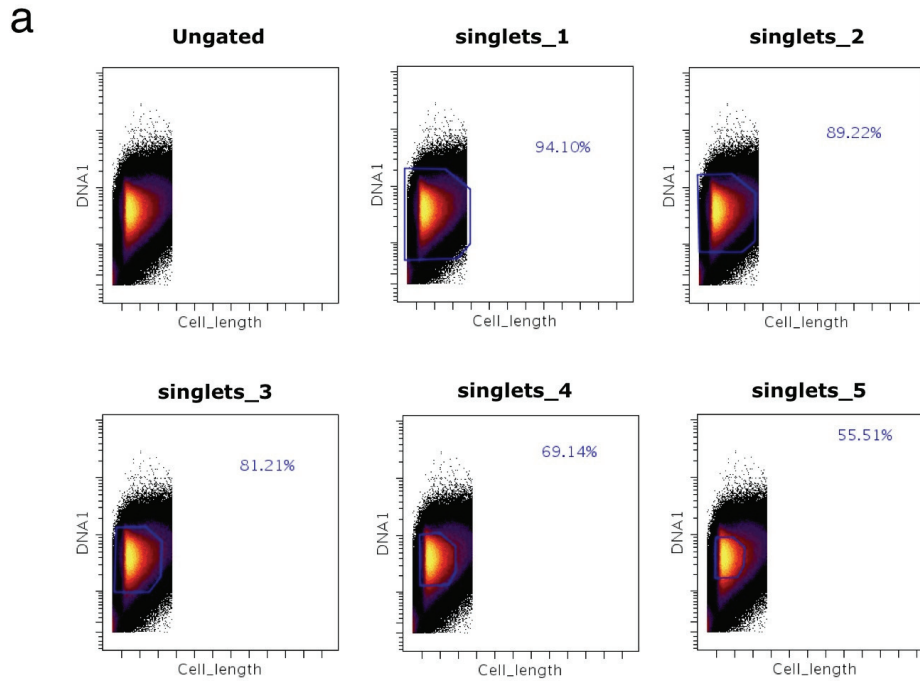
BC2 (Pd104) staining intensity in surface marker gated OVCAR and U937 cells from five barcoded samples, all of which are positive for BC2. Debarcoding was done independently of cell type gating.



**Supplementary Figure 2**

Time-of-flight traces of palladium barcodes of singlets and doublets with overlapping event lengths and Ir-intercalator intensities.

(a) Example cell events are indicated on the event length x Ir-Intercalator biaxial plot. Event 1 is identified as a barcode singlet, but of similar length and Ir-intercalator intensity as event 4, which has been identified as a barcode doublet. Similarly, event 2 is of similar length and Ir-Intercalator intensity as event 3. (b) The per-spectrum traces of the barcoding channels from the IMD files confirm the barcode and single/doublet assignments.

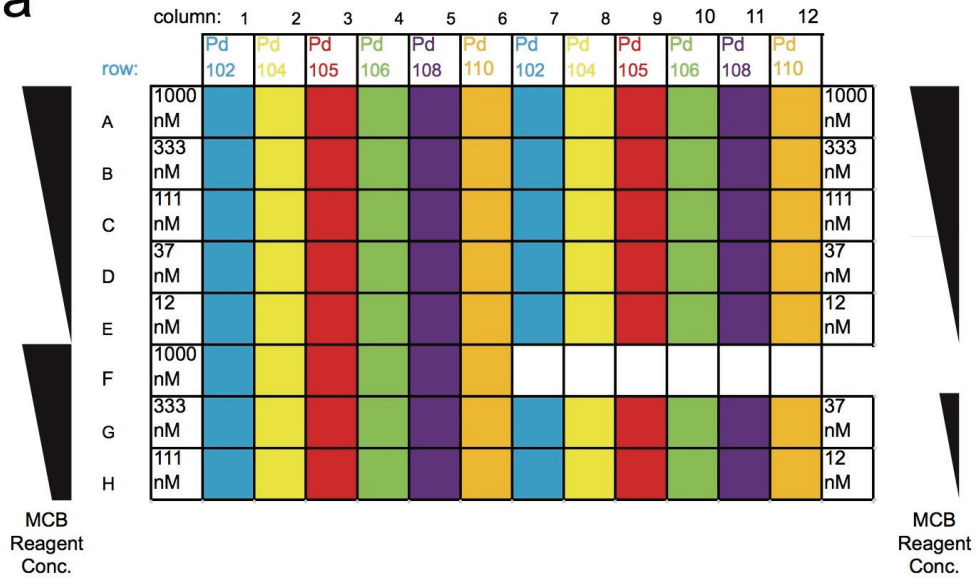


**Supplementary Figure 3**

Gates used for Figure 6b.

(a) Singlet gates of increasing stringency and their percent yields. (b) The percent of CD4+CD8+ cells within each of the singlet gates shown in (a).

**a**



**b**

row:	column:	1	2	3	4	5	6	7	8	9	10	11	12
A	1000 nM	Pd 102	Pd 104	Pd 105	Pd 106	Pd 108	Pd 110	Pd 102	Pd 104	Pd 105	Pd 106	Pd 108	Pd 110
B	333 nM	1000 nM Pooled Replicate 1						1000 nM Pooled Replicate 2					
C	111 nM	333 nM Pooled Replicate 1						333 nM Pooled Replicate 2					
D	37 nM	111 nM Pooled Replicate 1						111 nM Pooled Replicate 2					
E	12 nM	37 nM Pooled Replicate 1						37 nM Pooled Replicate 2					
F	1000 nM	12 nM Pooled Replicate 1						12 nM Pooled Replicate 2					
G	333 nM	1000 nM Pooled Replicate 3											
H	111 nM	333 nM Pooled Replicate 3						37 nM Pooled Replicate 3					
		111 nM Pooled Replicate 3						12 nM Pooled Replicate 3					

**Supplementary Figure 4**

96-well plate layout for MCB reagent titration in triplicate.

(a) Serial dilution layout for the 6 Palladium MCB reagents. (b) Wells to pool before for mass cytometry measurement.

**a**

Palladium MCB Reagent Isotope						
Well	102	104	105	106	108	110
1	1	1	1	0	0	0
2	1	1	0	1	0	0
3	1	1	0	0	1	0
4	1	1	0	0	0	1
5	1	0	1	1	0	0
6	1	0	1	0	1	0
7	1	0	1	0	0	1
8	1	0	0	1	1	0
9	1	0	0	1	0	1
10	1	0	0	0	1	1
11	0	1	1	1	0	0
12	0	1	1	0	1	0
13	0	1	1	0	0	1
14	0	1	0	1	1	0
15	0	1	0	1	0	1
16	0	1	0	0	1	1
17	0	0	1	1	1	0
18	0	0	1	1	0	1
19	0	0	1	0	1	1
20	0	0	0	1	1	1

**b**

Well:	1	2	3	4	5
1	1	2	3	4	5
2	6	7	8	9	10
3	11	12	13	14	15
4	16	17	18	19	20

**c**

Pd102:	1	2	3	4	5
1	1	1	1	1	1
2	1	1	1	1	1
3	0	0	0	0	0
4	0	0	0	0	0

Pd104:	1	2	3	4	5
1	1	1	1	1	0
2	0	0	0	0	0
3	1	1	1	1	1
4	1	0	0	0	0

Pd105:	1	2	3	4	5
1	1	0	0	0	1
2	1	1	0	0	0
3	1	1	1	0	0
4	0	1	1	1	0

Pd106:	1	2	3	4	5
1	0	1	0	0	1
2	0	0	1	1	0
3	1	0	0	1	1
4	0	1	1	0	1

Pd108:	1	2	3	4	5
1	0	0	1	0	0
2	1	0	1	0	1
3	0	1	0	1	0
4	1	1	0	1	1

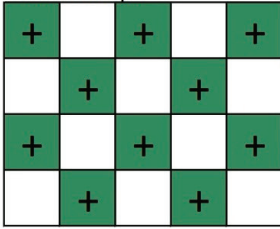
Pd110:	1	2	3	4	5
1	0	0	0	1	0
2	0	1	0	1	1
3	0	0	1	0	1
4	1	0	1	1	1

**Supplementary Figure 5**

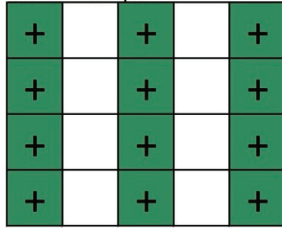
Plate layout for 6-choose-3 MCB combinatorial doublet-filtering scheme.

(a) MCB reagent combinations to use for a 20 sample 6-choose-3 combinatorial doublet-filtering scheme. (b) Mapping the 20 samples to a 5 x 4 grid. (c) Pipetting guide for each of the 6 Palladium MCB reagents into the 5 x 4 grid.

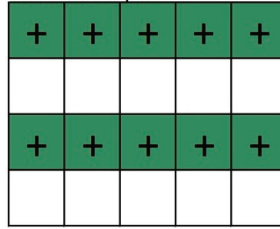
Pooled Group 1



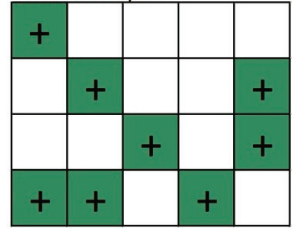
Pooled Group 2



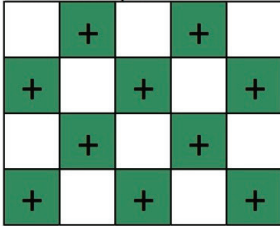
Pooled Group 3



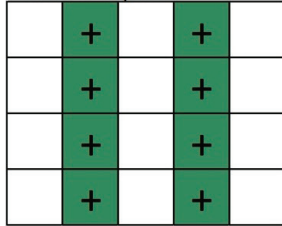
Pooled Group 4



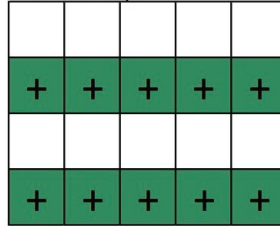
Pooled Group 5



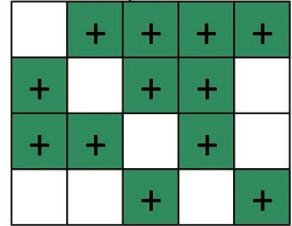
Pooled Group 6



Pooled Group 7



Pooled Group 8



**Supplementary Figure 6**

Pooled sample groups for 20-sample MCB combinatorial plate testing and validation.

Wells to pool for 8 pooled sample groups that will be used to validate the sample assignment and correct orientation of the tested 100X MCB combinatorial plate