Supplementary Information

Microscale Screening of Antibody Libraries as Maytansinoid Antibody-Drug Conjugates

Kalli C. Catcott[1], Molly A. McShea[1], Carl Uli Bialucha[2]*, Kathy L. Miller[3], Stuart W. Hicks[1], Parmita Saxena[2], Thomas G. Gesner[2], Mikias Woldegiorgis[3], Megan E. Lewis[1], Chen Bai[1], Michael S. Fleming[1], Seth A. Ettenberg[2, 4], Hans K. Erickson[1, 5], Nicholas C. Yoder[1,*]

[1]ImmunoGen, Inc.; Waltham, MA; [2]Novartis Institutes for Biomedical Research; Cambridge, MA;[3]Novartis Institutes for Biomedical Research; Emeryville, CA;[4]Current address: Unum Therapeutics; Cambridge, MA; [5]Current address: Genentech; South San Francisco, CA.

*Correspondence to: Nicholas C. Yoder; Email: nicholas.yoder@immunogen.com; Carl Uli Bialucha: carl_uli.bialucha@novartis.com.

Fig. S1A. Comparison of SE-HPLC and SE-UPLC (ADC #1)

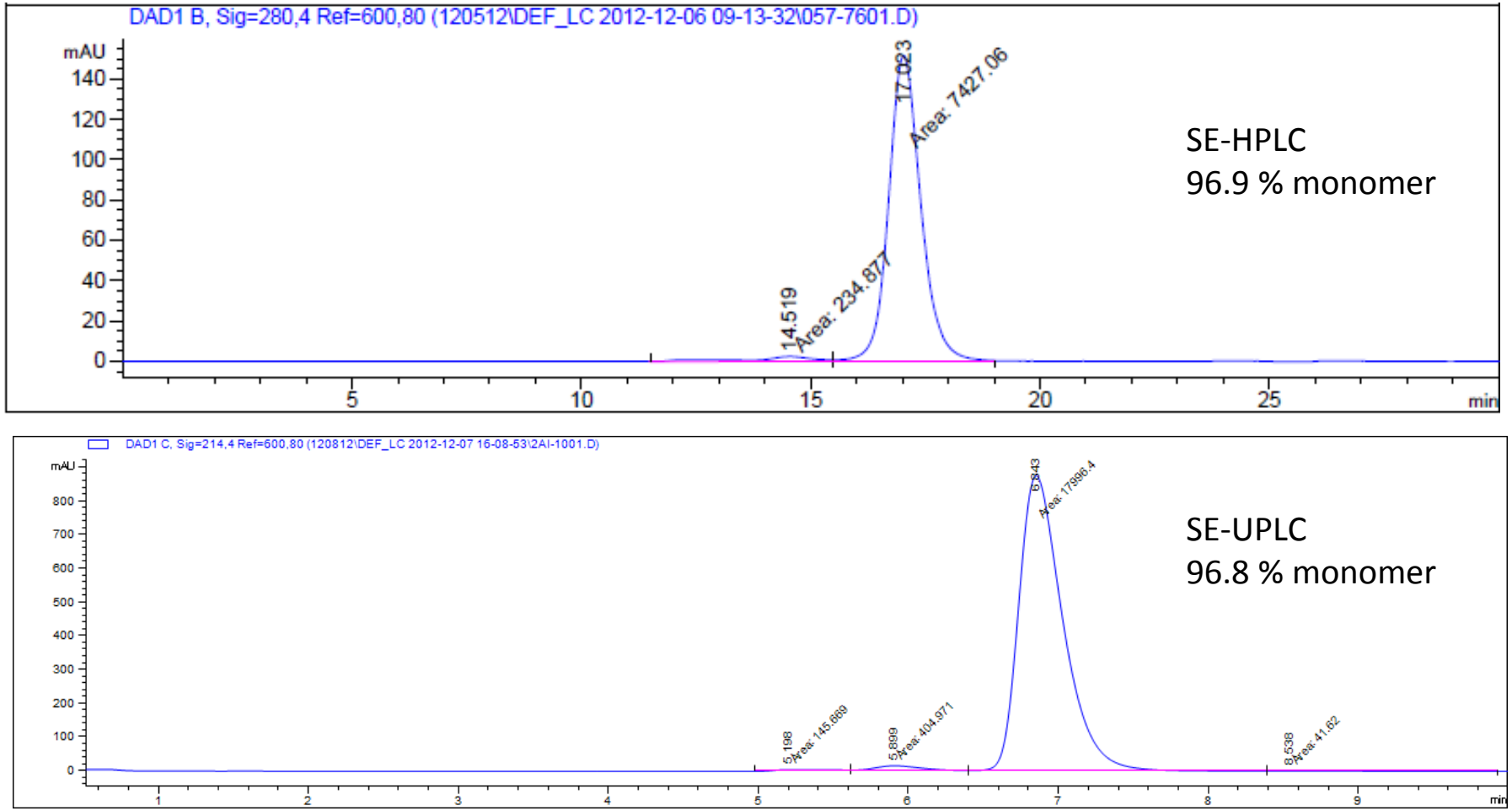

Figure S1A,B. Matched chromatograms showing the same maytansinoid ADC analyzed using SE-HPLC and SE-UPLC methods.

Figure S1B. Comparison of SE-HPLC and SE-UPLC (ADC #2)

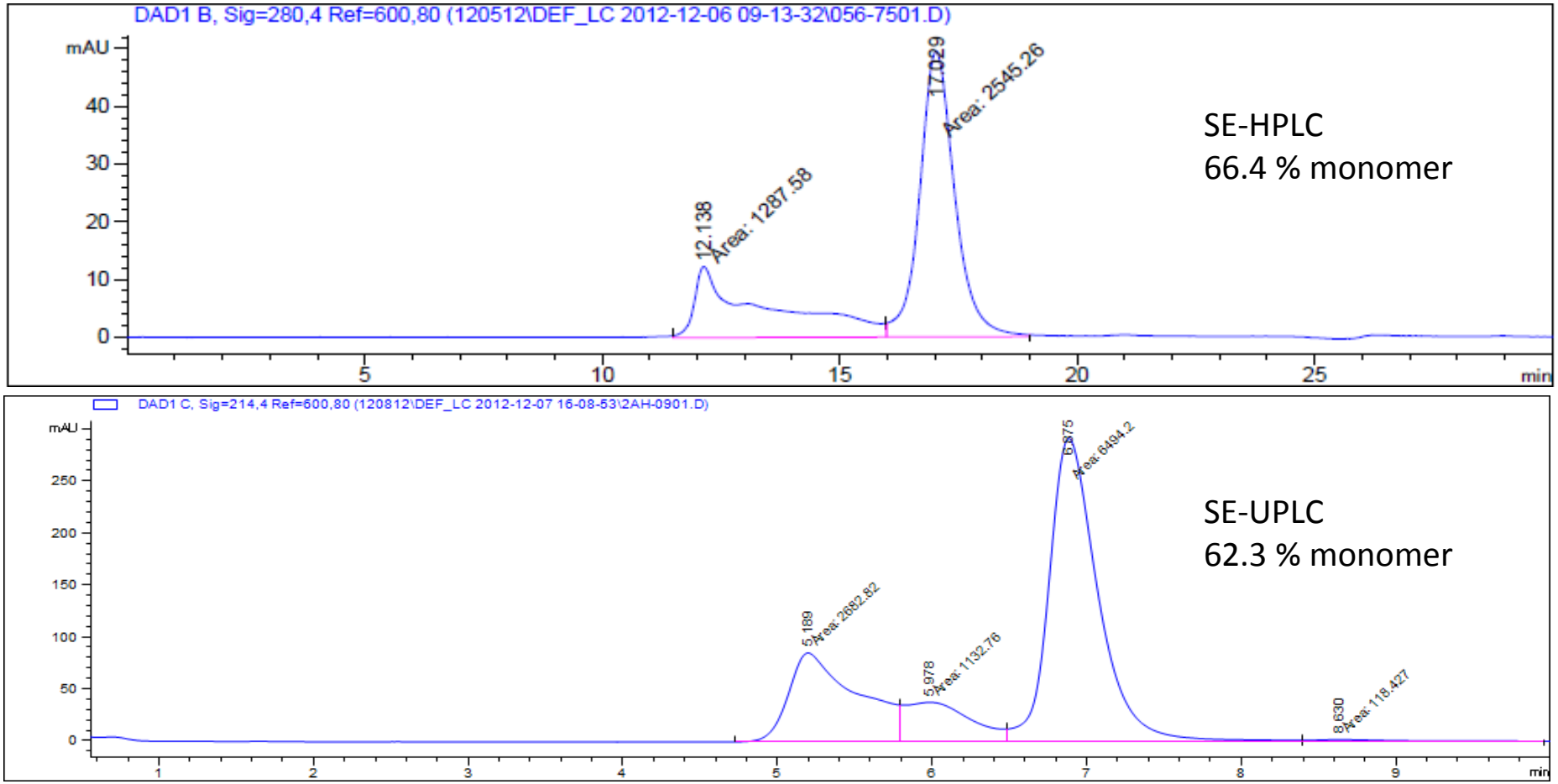

Figure S1C. Comparability of DAR, % monomer, concentration measurements using different analytical methods

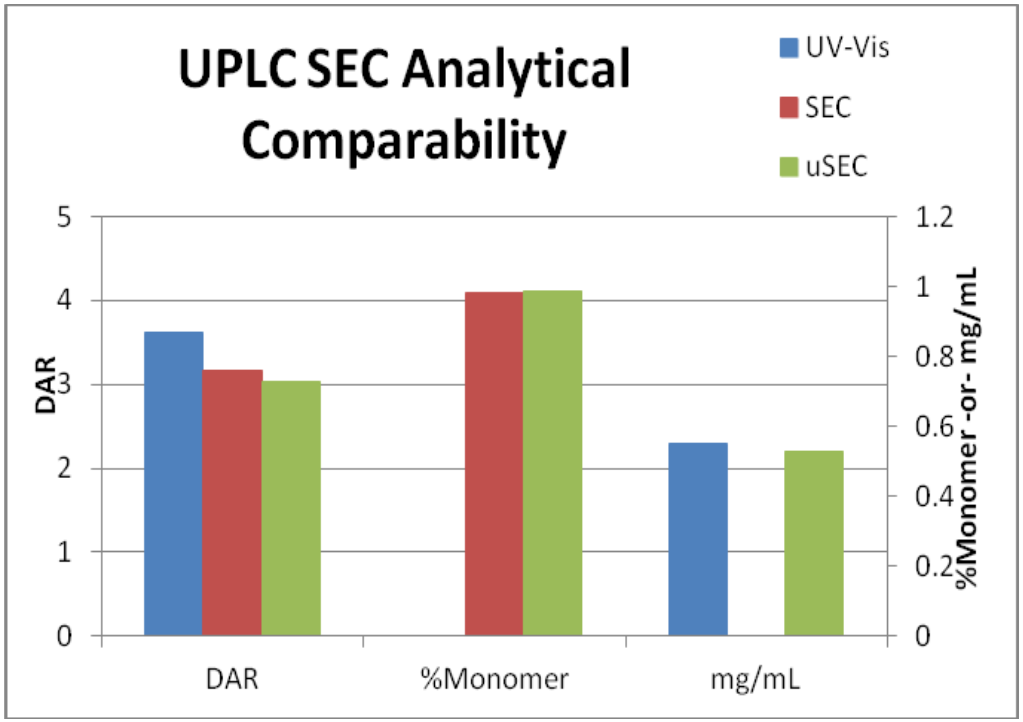

Figure S1C. Example comparison of DAR, % monomer, and concentration measurements made by the conventional combination of UV-Vis/SE-HPLC, and by SE-UPLC.

Figure S2. Biochemical analysis of free drug removal

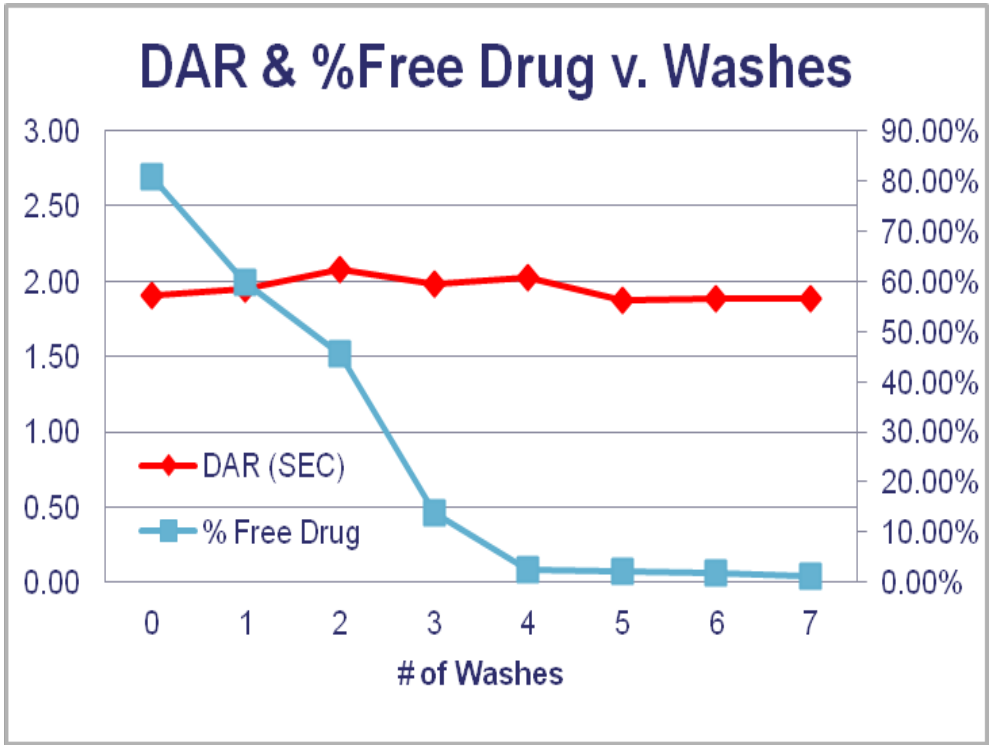

Figure S2. Removal of free maytansinoid species as a function of washes with 96 well ultrafiltration plates. Identical conjugates were prepared in parallel as described and then, after the indicated number of washes, assayed for free maytansinoid using a previously described mixed-mode HPLC assay{Fleming, 2005 #166}. ADC from four wells was combined to provide enough material for the free drug assay.

Figure S3: Effect of DAR of ML66 ADCs on cytotoxicity against MDA-MB-483 cells

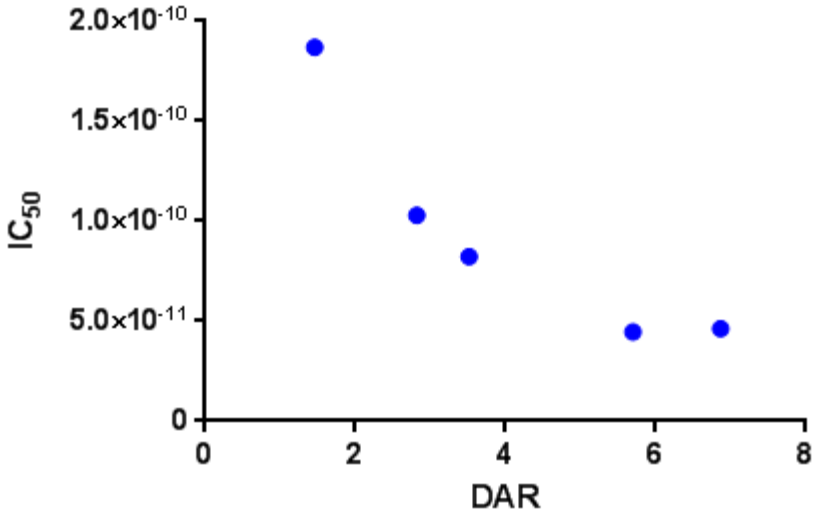

Figure S3. IC_{50} values for the variable DAR ML66 ADCs plotted against the DAR values.

Figure S4. Scatter plot comparing potency of research and microscale anti-[Antigen B] ADCs

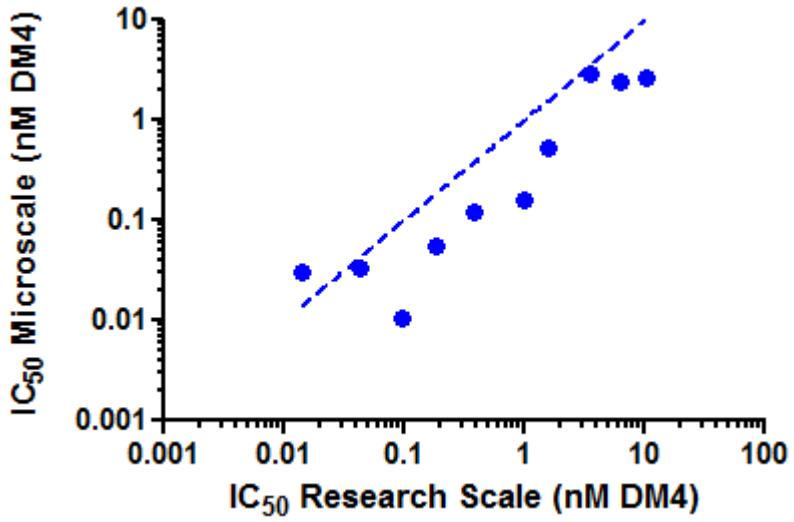

Figure S4. Same data as in Figure 6A, graphed as a linear correlation. The dashed line in this figure shows y = x.

Figure S5A: ADCs retain binding affinity of input antibody (scatter plot)

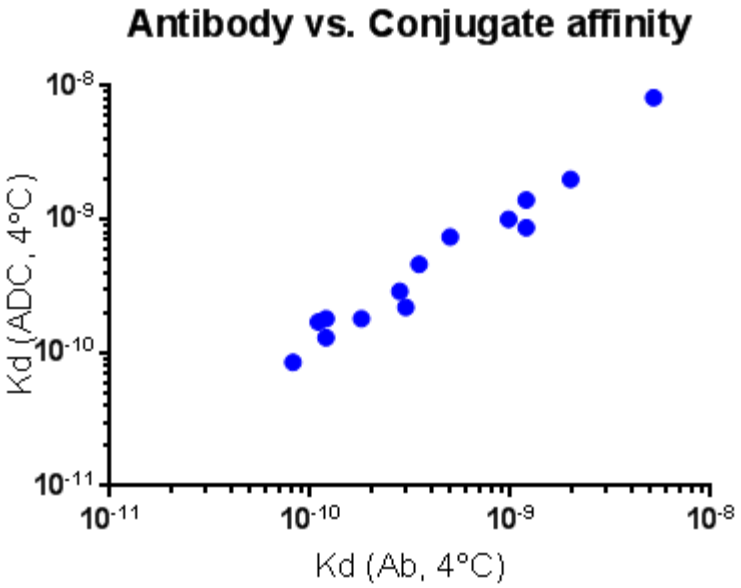

Figure S5. Correlation between binding affinity of 15 murine Abs and the corresponding SMCC-DM1 ADCs, depicted as (**A**) a scatter plot; or (**B**) a Bland-Altman plot.

Figure S5B: ADCs retain binding affinity of input antibody (Bland-Altman plot)

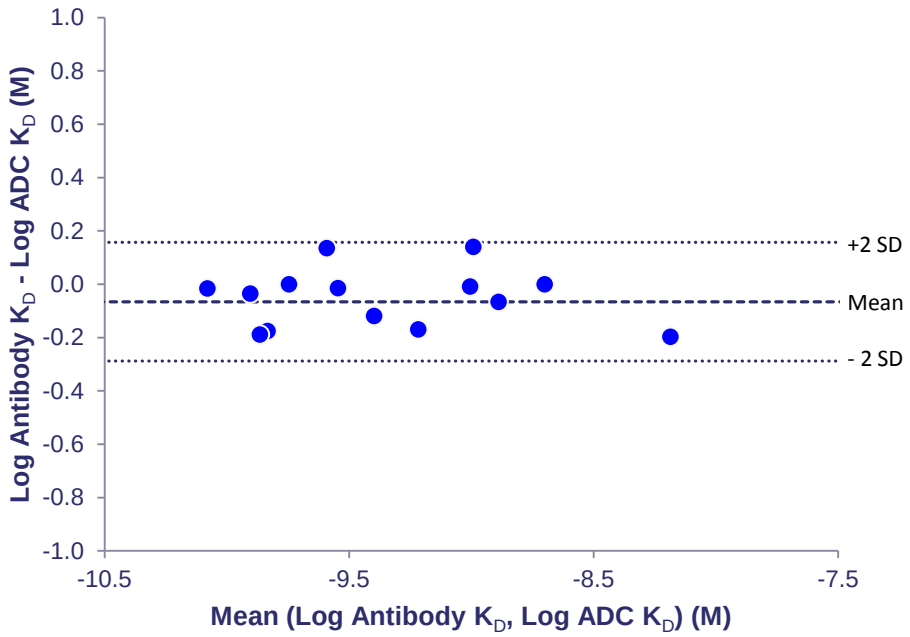

Table S1. Comparison of DAR values measured by different methods (SMCC-DM1 ADCs). The average of the absolute value of the percentage differences is 4%.

SE-UPLC	UV-vis	% Difference
3.1	3.2	-4%
3.1	3.3	-5%
2.9	3.1	-5%
2.2	2.1	5%
2.0	1.9	4%
1.9	2.0	-1%

Table S2. Comparison of concentration measurements of SMCC-DM1 ADCs made by different methods. The average of the absolute value of the percentage differences is 9%.

Concentration (mg Ab/mL)		
SE-UPLC	UV-vis	%Diff
0.51	0.61	-21%
0.37	0.31	16%
0.50	0.50	0%
0.24	0.23	3%
0.55	0.60	-10%
0.53	0.55	-5%

Table S3. Summary of the Antigen B conjugation campaign with SPDB-DM4. For this campaign, replicate conjugates were combined prior to analysis. The column "Comparison Assay" notes whether this particular ADC was included in the experiment summarized in Figure 6.

Antibody	Yield	DAR	% Monomer	Concentration (mg/mL ADC)	Comparison Assay
B1	25%	2.6	96%	0.25	N
B2	65%	3.7	98%	0.65	Y
B3	22%	2.7	95%	0.22	N
B4	12%	3.1	99%	0.12	Y
B5	73%	2.9	96%	0.73	N
B6	31%	3.0	74%	0.31	N
B7	53%	2.9	98%	0.53	Y
B8	59%	2.9	96%	0.59	Y
B9	65%	2.9	96%	0.65	Y
B10	22%	2.7	97%	0.22	Y
B11	35%	2.6	62%	0.35	N
B12	61%	2.9	96%	0.61	Y
B13	46%	2.8	87%	0.46	N
B14	61%	2.9	97%	0.61	N
B15	80%	3.1	98%	0.80	Y
B16	42%	3.1	97%	0.42	N

B17	73%	3.2	96%	0.73	Y
B18	75%	3.6	97%	0.75	Y
B19	2%	4.7	100%	0.01	N

Table S4. Summaries of the Antigen C conjugation campaign. For this campaign, replicate conjugates were combined before analysis.
(**A**) Characterization data for the entire set of ADCs generated at pH 6.

Antibody	Yield	DAR	% Monomer	Concentration (mg/mL ADC)	Success
C1	66%	5.7	91%	0.80	Y
C2	59%	4.0	96%	0.48	Y
C3	48%	3.7	94%	0.38	Y
C4	29%	3.5	95%	0.29	Y
C5	38%	4.0	91%	0.27	Y
C6	34%	4.3	96%	0.25	Y
C7	38%	4.5	96%	0.24	Y
C8	30%	4.9	95%	0.24	Y
C9	37%	5.2	93%	0.23	Y
C10	23%	3.2	93%	0.20	Y
C11	23%	5.1	95%	0.16	Y
C12	18%	3.9	94%	0.16	Y
C13	16%	6.0	94%	0.15	Y
C14	17%	4.0	94%	0.15	Y
C15	17%	3.3	90%	0.15	Y
C16	15%	4.1	95%	0.14	Y
C17	14%	4.8	94%	0.14	Y
C18	16%	3.2	91%	0.14	Y
C19	17%	3.4	93%	0.13	Y
C20	17%	4.7	91%	0.12	Y
C21	22%	5.0	91%	0.12	Y
C22	19%	3.8	92%	0.12	Y
C23	25%	5.7	93%	0.11	Y
C24	19%	5.6	92%	0.10	Y
C25	7%	4.9	84%	0.03	N
C26	30%	0.9	97%	0.38	N
C27	9%	4.8	89%	0.04	N
C28	34%	3.0	95%	0.28	N
C29	57%	2.7	88%	0.41	N
C30	9%	5.2	84%	0.04	N
C31	11%	5.6	92%	0.05	N
C32	48%	1.9	95%	0.39	N
C33	9%	5.4	84%	0.04	N

C34	5%	5.5	91%	0.04	N
C35	6%	4.9	81%	0.02	N
C36	12%	3.9	92%	0.08	N
C36	12%	5.3	90%	0.07	N
C37	16%	5.6	91%	0.09	N
C38	5%	5.3	83%	0.04	N
C39	5%	6.0	79%	0.03	N
C40	12%	6.5	87%	0.06	N
C41	8%	4.9	92%	0.06	N
C42	7%	6.8	89%	0.04	N
C43	5%	4.1	84%	0.03	N
C44	15%	5.8	93%	0.09	N
C44	1%	5.4	41%	0.01	N
C45	6%	4.4	89%	0.05	N
C46	8%	4.8	88%	0.04	N
C47	0%	ND	74%	0.00	N
C48	4%	6.1	80%	0.03	N
C49	8%	5.0	91%	0.05	N
C50	11%	3.4	93%	0.09	N
C51	14%	4.9	91%	0.08	N
C52	12%	6.4	92%	0.06	N
C53	12%	4.0	89%	0.08	N
C55	9%	3.5	90%	0.06	N
C56	9%	4.3	88%	0.06	N
C57	14%	1.8	92%	0.13	N
C58	10%	3.6	86%	0.05	N
C59	7%	5.1	84%	0.03	N
C60	11%	5.4	89%	0.06	N
C61	6%	7.8	85%	0.03	N
C63	6%	4.2	90%	0.05	N
C64	9%	4.6	88%	0.05	N
C65	3%	4.9	70%	0.02	N
C66	1%	9.9	23%	0.00	N
C67	7%	5.3	85%	0.03	N
C68	11%	4.5	92%	0.07	N
C69	1%	5.6	52%	0.01	N
C70	6%	5.4	86%	0.03	N
C71	9%	3.0	84%	0.04	N
C72	5%	4.7	82%	0.03	N
C73	7%	3.7	89%	0.03	N
C74	1%	5.9	52%	0.01	N

C75	4%	5.0	83%	0.03	N
C76	6%	4.2	86%	0.05	N
C77	0%	ND	11%	0.00	N
C78	3%	5.7	81%	0.02	N
C79	5%	2.9	84%	0.03	N
C80	4%	4.5	97%	0.02	N
C81	1%	15.4	19%	0.00	N
C82	1%	7.8	53%	0.00	N
C83	5%	4.1	85%	0.03	N
C84	6%	4.0	84%	0.02	N
C85	12%	5.3	92%	0.07	N

(**B**) Characterization data for the entire set of ADCs generated at pH 8.

Antibody	Yield	DAR	% Monomer	Concentration (mg/mL ADC)	Success
C25	52%	2.3	99%	0.25	Y
C26	18%	2.4	99%	0.23	Y
C27	45%	2.0	100%	0.22	Y
C28	25%	2.5	99%	0.21	Y
C29	29%	2.5	98%	0.21	Y
C30	54%	2.5	100%	0.21	Y
C31	37%	2.2	99%	0.19	Y
C32	22%	2.4	100%	0.18	Y
C33	40%	2.2	99%	0.18	Y
C34	18%	2.3	99%	0.17	Y
C35	43%	1.9	100%	0.17	Y
C36	28%	2.1	99%	0.17	Y
C37	26%	3.1	99%	0.16	Y
C38	20%	2.2	100%	0.14	Y
C39	25%	2.4	98%	0.12	Y
C40	25%	2.7	99%	0.12	Y
C41	16%	3.4	98%	0.12	Y
C42	20%	3.5	100%	0.12	Y
C43	22%	2.7	100%	0.12	Y
C44	20%	3.3	98%	0.11	Y
C45	12%	2.0	98%	0.11	Y
C46	22%	2.6	98%	0.11	Y
C47	18%	2.9	98%	0.11	Y
C48	16%	3.3	95%	0.10	Y
C49	15%	2.4	99%	0.10	Y
C50	17%	1.9	99%	0.14	N
C51	12%	2.7	99%	0.06	N

C52	19%	3.2	98%	0.10	N
C53	11%	3.3	97%	0.07	N
C54	12%	3.1	100%	0.09	N
C55	11%	2.3	100%	0.07	N
C56	11%	2.4	99%	0.07	N
C57	1%	3.5	95%	0.01	N
C58	11%	3.1	99%	0.05	N
C59	18%	2.9	99%	0.08	N
C60	13%	2.2	97%	0.06	N
C61	14%	2.9	99%	0.08	N
C62	4%	2.7	100%	0.03	N
C63	14%	2.4	100%	0.10	N
C64	9%	1.8	99%	0.05	N
C65	14%	1.9	100%	0.07	N
C66	12%	2.6	100%	0.07	N
C67	13%	2.3	100%	0.06	N
C68	9%	2.6	99%	0.06	N
C69	11%	1.8	99%	0.06	N
C70	14%	1.2	96%	0.06	N
C71	14%	2.4	100%	0.06	N
C72	9%	2.6	98%	0.05	N
C73	9%	2.7	94%	0.05	N
C74	8%	2.6	93%	0.04	N
C75	5%	3.3	96%	0.04	N
C76	5%	2.4	95%	0.03	N
C77	8%	3.0	87%	0.03	N
C78	5%	3.0	93%	0.03	N
C79	5%	1.0	98%	0.03	N
C80	4%	5.1	100%	0.02	N
C81	6%	1.9	94%	0.02	N
C82	5%	3.4	97%	0.02	N
C83	1%	3.2	100%	0.00	N
C84	1%	3.9	100%	0.00	N
C85	0%	ND	ND	0.00	N

(**C**) Characterization data for the ADCs generated by pooling material from the two rounds (all were assayed).

Antibody	Yield	DAR	% Monomer	Concentration (mg/mL ADC)
C50	14%	2.3	99%	0.31
C51	13%	3.4	98%	0.21
C52	16%	5.1	98%	0.16

C53	12%	3.4	97%	0.15
C54	12%	2.9	100%	0.11
C55	10%	2.3	98%	0.10
C56	10%	3.7	99%	0.10
C57	8%	2.0	99%	0.10
C58	10%	4.3	97%	0.09
C59	12%	3.1	98%	0.08
C60	12%	2.7	96%	0.08
C61	10%	4.0	97%	0.08
C62	10%	4.7	99%	0.08
C63	10%	2.8	99%	0.07
C64	9%	2.7	99%	0.06

Table S5. Summary of the Antigen D conjugation campaign. For this campaign, each reaction product was analyzed separately.

Antibody	Yield	DAR	% Monomer	Concentration (mg/mL ADC)	Success
D1	29%	3.3	98	0.13	Y
D1	43%	3.1	98	0.24	Y
D1	0%	ND	ND	0.00	N
D2	57%	3.4	97	0.23	Y
D2	79%	2.6	98	0.45	N
D2	15%	2.7	97	0.08	N
D2	0%	ND	ND	0.00	N
D3	34%	2.6	97	0.16	N
D3	24%	2.5	97	0.13	N
D3	0%	ND	ND	0.00	N
D3	11%	3.5	97	0.05	N
D3	4%	3.8	96	0.02	N
D3	0%	3.6	88	0.00	N
D4	14%	3.4	96	0.06	N
D4	7%	3.8	95	0.02	N
D4	3%	4.1	91	0.01	N
D4	0%	4.6	90	0.00	N
D4	0%	ND	ND	0.00	N
D4	8%	4.5	93	0.03	N
D5	64%	3.4	94	0.24	Y
D5	81%	2.7	94	0.33	N
D5	41%	2.7	94	0.21	N
D5	5%	3.4	85	0.03	N
D5	11%	3.5	95	0.06	N
D5	16%	4.0	92	0.06	N

D6	59%	3.2	98	0.30	Y
D6	0%	ND	ND	0.00	N
D6	53%	2.4	98	0.37	N
D6	0%	2.6	95	0.00	N
D6	9%	3.3	98	0.05	N
D6	0%	ND	ND	0.00	N
D7	72%	3.7	97	0.32	Y
D7	7%	4.3	94	0.06	N
D7	4%	4.6	93	0.02	N
D7	1%	5.8	93	0.01	N
D7	13%	4.5	97	0.07	N
D7	64%	4.4	95	0.24	N
D8	41%	3.4	97	0.22	Y
D8	29%	3.3	97	0.15	Y
D8	74%	2.0	97	0.40	N
D8	87%	2.4	97	0.50	N
D8	58%	2.3	97	0.27	N
D8	17%	2.9	97	0.10	N
D9	66%	3.7	98	0.57	Y
D9	77%	3.5	98	0.47	Y
D9	90%	2.8	98	0.40	N
D9	41%	2.7	98	0.22	N
D9	42%	2.6	98	0.20	N
D9	12%	3.9	98	0.06	N
D10	59%	3.7	95	0.20	Y
D10	59%	3.8	94	0.24	Y
D10	0%	ND	ND	0.00	N
D10	64%	2.9	94	0.27	N
D10	19%	3.3	90	0.09	N
D10	16%	3.7	93	0.08	N
D11	47%	3.1	97	0.31	Y
D11	63%	3.5	97	0.26	Y
D11	30%	2.8	97	0.15	N
D11	3%	3.7	95	0.02	N
D11	0%	ND	ND	0.00	N
D11	0%	ND	ND	0.00	N
D12	59%	3.1	96	0.32	Y
D12	50%	3.8	95	0.20	Y
D12	0%	ND	ND	0.00	N
D12	52%	2.9	97	0.21	N
D12	12%	3.7	94	0.06	N

D12	18%	3.4	96	0.09	N
D13	76%	3.6	96	0.28	Y
D13	54%	3.9	96	0.20	Y
D13	34%	2.9	95	0.17	N
D13	5%	3.4	94	0.03	N
D13	0%	ND	ND	0.00	N
D13	0%	ND	ND	0.00	N
D14	64%	3.3	94	0.32	Y
D14	89%	3.3	93	0.34	Y
D14	10%	3.7	91	0.05	N
D14	0%	ND	ND	0.00	N
D14	89%	4.3	95	0.38	N
D14	16%	4.4	93	0.07	N
D15	71%	3.2	96	0.33	Y
D15	68%	3.8	95	0.32	Y
D15	0%	ND	ND	0.00	N
D15	3%	3.5	93	0.02	N
D15	18%	4.4	96	0.09	N
D15	0%	ND	ND	0.00	N
D16	75%	3.1	97	0.37	Y
D16	92%	3.2	97	0.36	Y
D16	73%	3.1	96	0.36	Y
D16	62%	2.4	97	0.36	N
D16	88%	2.3	97	0.38	N
D16	88%	2.4	97	0.43	N
D17	82%	3.4	95	0.34	Y
D17	48%	3.7	95	0.20	Y
D17	74%	3.4	95	0.28	Y
D17	78%	4.0	95	0.29	Y
D17	0%	ND	ND	0.00	N
D17	47%	4.1	94	0.17	N