

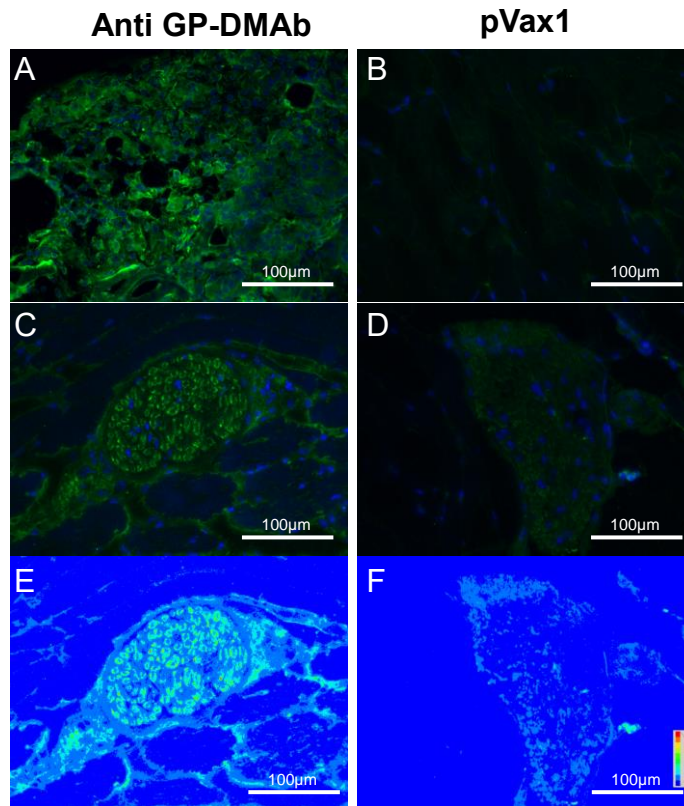
**Supplemental Information**

***In Vivo* Delivery of Synthetic Human DNA-Encoded  
Monoclonal Antibodies Protect  
against Ebolavirus Infection in a Mouse Model**

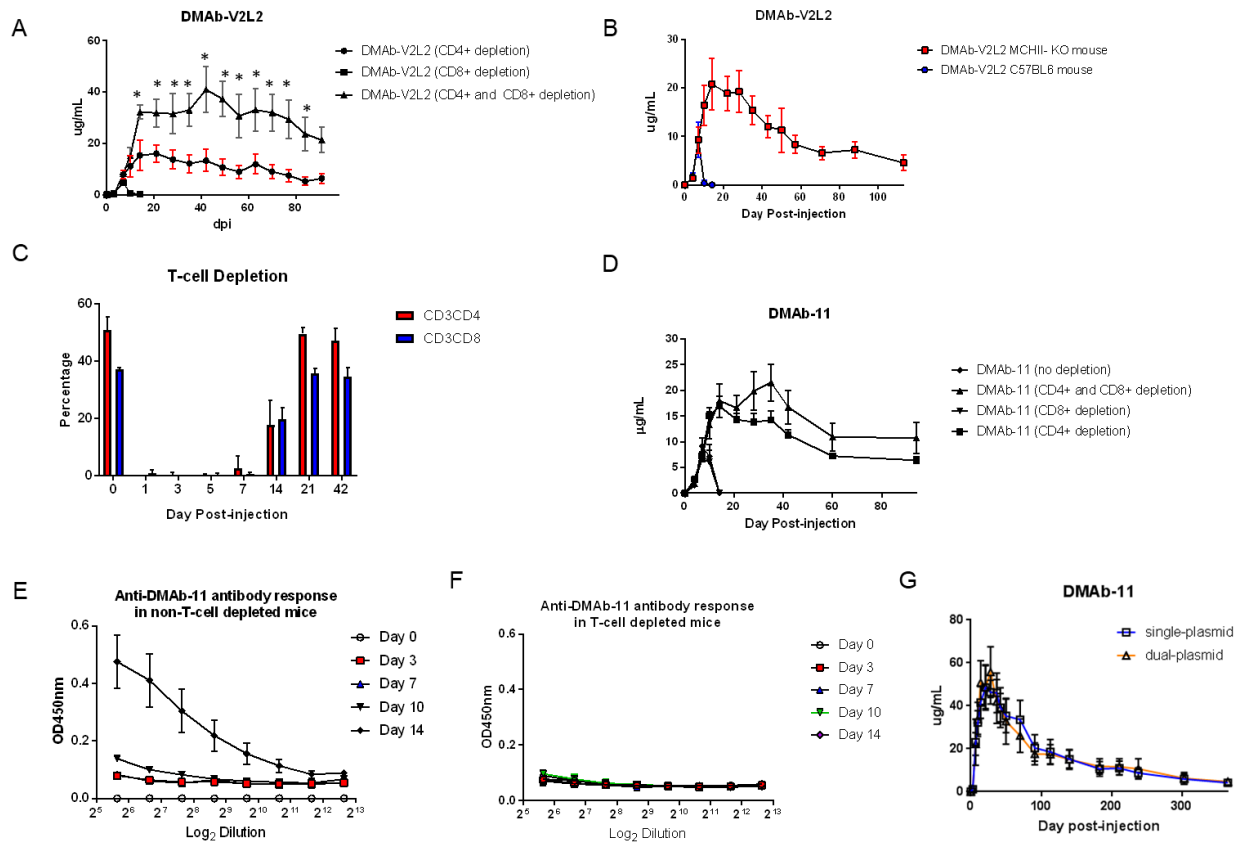
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## Supplemental Results

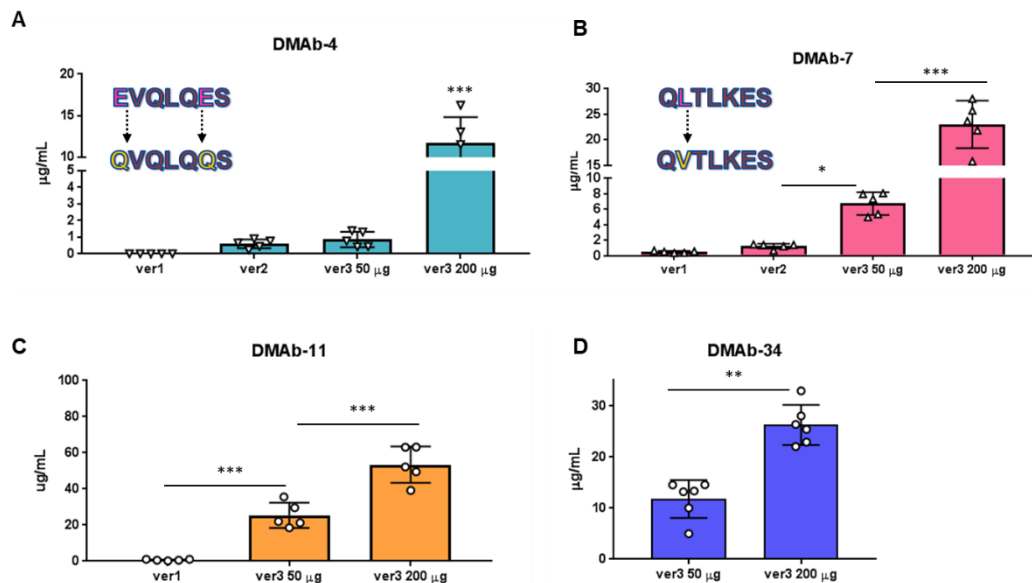
### Supplemental Figures



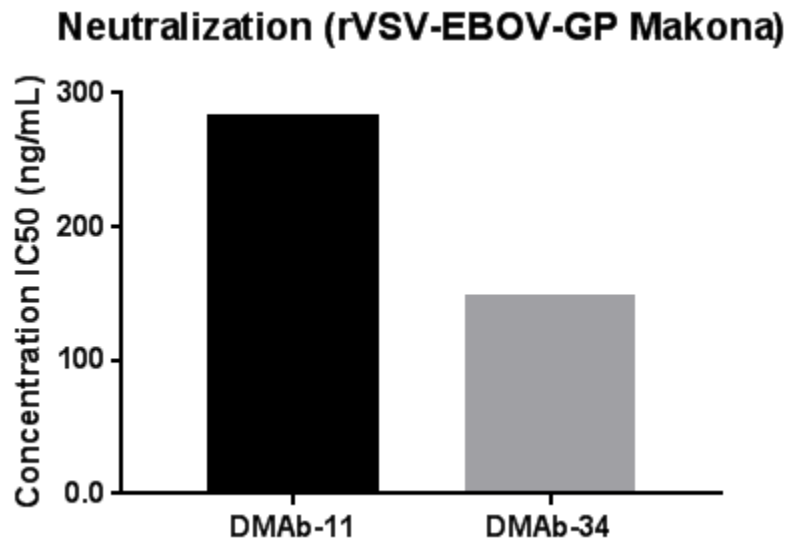
**Figure S1. Overview of DMAb *in vivo* expression.** Related to Figures 1-6. Anti-GP DMAb expression in mouse muscle. BALB/c mice were injected in the quadriceps muscle with an anti-GP DMAb-34 (50µg). The muscle was excised 48 hours later and frozen in O.T.C. compound before sectioning. Sections were stained with an unconjugated goat anti-human IgG-Fc antibody, followed by detection with a donkey anti-goat antibody conjugated to AF488 (green), and DAPI (blue) (Nikon 80i, magnification 40X). Sections show muscle expression (A, B) and expression within a muscle fiber (C, D). DMAb-34 expression is also shown as a pseudocolour image (red = highest expression intensity, dark blue = lowest expression intensity) to demonstrate the contrast in expression between DMAb expressing muscle cells and the negative control group (pVax1 vector alone) (E, F). A scale bar representing 100µm is shown.



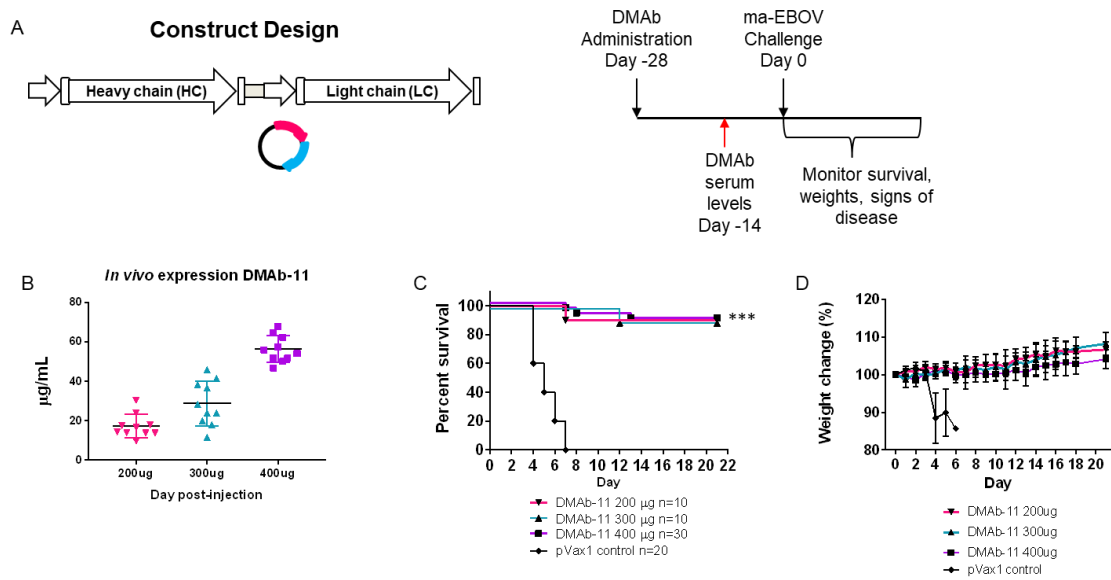
**Figure S2. DMAB mouse model development.** Related to Figures 1-6. **A)** BALB/c mice (n=5 mice/group) were *in vivo* depleted of CD4+, CD8+, or both CD4+CD8+ T cells using depletion antibodies (anti-CD4 = GK1.5, anti-CD8 = YTS169.4). Mice were administered anti-*Pseudomonas aeruginosa* DMAB-V2L2 (400  $\mu$ g) and monitored for expression of human IgG1 in sera. **B)** C57BL6 mice or MHC II- mice (n=5 mice/group) were administered DMAB-V2L2 (400  $\mu$ g) and monitored for expression of human IgG1 in sera. **C)** Percentage of CD3+CD4+ and CD3+CD8+ T cells during T cell depletion (BALB/c mice n=5 mice/group). **D)** BALB/c mice (n=5 mice/group) were administered anti-GP DMAB-11 (400  $\mu$ g) without T cell depletion or with CD4+, CD8+, and both CD4+CD8+ T cell depletion. Animals were monitored for expression of human IgG1 in sera. **E)** Mouse anti-DMAB-11 antibody responses in undepleted BALB/c mice. **F)** Mouse anti-DMAB-11 antibody responses in CD4+CD8+ T-cell depleted mice. **G)** Long-term expression of DMAB-11 single-plasmid (400  $\mu$ g) or dual-plasmid (200  $\mu$ g) in BALB/c mice (n=5 mice/group). Animals were monitored for human IgG1 in sera. (\* = p<0.01)



**Figure S3. *In vivo* optimizations of anti-GP DMABs.** Related to Figure 1. *In vivo* expression of different optimizations of **A)** DMAB-4, **B)** DMAB-7, **C)** DMAB-11, and **D)** DMAB-34. BALB/c mice (n=5 mice/group) received injections with different optimized variants and formulations of each anti-GP DMAB. ver1= nucleotide optimization, ver2 = stabilizing amino acid modifications, ver3 = HYA formulation. The bar graphs display the Cmax expression levels at Day 7 post-DMAB administration and error bars represent the standard deviation. (\* = p<0.01, \*\* = p<0.01, \*\*\* = p<0.001)



**Figure S4. Neutralization IC50 using a recombinant VSV-EBOV-GP (rVSV-EBOVGP) pseudotype assay.** *Related to Figure 2.* Ebola virus neutralization IC50 from pooled sera obtained from mice injected with DMAb-11 or DMAb-34. The neutralization assays were performed with an rVSV-EBOV-GP (strain Makona) pseudotype expressing GFP.



**Figure S5. DMAb-11 single-plasmid protection.** *Related to Figures 4 and 6.* **A)** Overview of the single-plasmid injection design and regimen. DMAbs were administered on day -28 and serum was collected from animals on day -14 before lethal challenge with 1000 LD<sub>50</sub> of mouse-adapted EBOV (Mayinga). Animals were monitored for 21 days post-challenge for signs of disease and weight loss, **B)** Expression of increasing doses of DMAb-11 in mouse serum at day -14 before challenge. **C)** Survival, and **D)** Percent weight change. (\*\*\*) =  $p < 0.001$

**Table S1** *In vitro* expression levels of anti-GP DMAbs. Related to Figure 1.

GP-DMAb	Technical Replicate #			Average	SD
	1	2	3		
DMAb-1	1.86	2.01	2.22	2.03	0.18
DMAb-2	0.49	0.53	0.75	0.59	0.14
DMAb-3	2.16	1.83	2.05	2.01	0.16
DMAb-4	0.00	0.13	0.00	0.04	0.07
DMAb-5	2.44	2.46	2.34	2.41	0.06
DMAb-6	0.16	0.13	0.16	0.15	0.02
DMAb-7	0.51	0.45	0.47	0.48	0.03
DMAb-8	0.38	0.32	0.32	0.34	0.03
DMAb-9	1.21	1.47	1.19	1.29	0.16
DMAb-10	0.40	0.28	0.00	0.23	0.20
DMAb-11	6.32	5.12	7.59	6.34	1.24
DMAb-12	1.62	1.86	1.21	1.56	0.33
DMAb-13	0.85	0.64	0.96	0.82	0.17
DMAb-21	0.59	0.70	0.79	0.69	0.10
DMAb-22	0.63	0.67	0.79	0.70	0.08
DMAb-24	0.64	0.54	0.65	0.61	0.06
DMAb-25	14.63	12.27	10.50	12.47	2.07
DMAb-26	2.12	2.61	1.69	2.14	0.46
DMAb-27	1.94	1.90	1.90	1.91	0.02
DMAb-30	4.27	6.09	3.82	4.73	1.20
DMAb-31	2.59	2.32	2.36	2.42	0.15
DMAb-34	3.57	3.34	2.77	3.23	0.41
DMAb-35	10.58	11.75	6.06	9.46	3.00
DMAb-38	0.80	1.00	1.16	0.99	0.18
DMAb-39	3.73	3.30	3.46	3.49	0.22
DMAb-40	0.00	0.11	0.01	0.04	0.06
DMAb-41	2.10	1.81	1.59	1.83	0.25
pVax1	0.00	0.00	0.00	0.00	0.00

**Table S2.** Developability index comparison. *Related to Figure 1 and Supplemental Figure S4.*

DMAb	Expression		
	†Predicted <i>in vitro</i> DI (Ranked Highest = 1, Lowest = 8)	* <i>In vitro</i> Biochemical liabilities	<i>In vivo</i> (Cmax Dose #1) µg/mL
DMAb-4	1	Low	3.01
DMAb-9	2	Low	8.10
DMAb-7	3	Moderate	6.74
DMAb-11	4	High	9.44
DMAb-34	5	Moderate	6.59
DMAb-13	6	High	7.10
DMAb-12	7	Moderate	7.00
DMAb-30	8	High	1.02

†Biovia Discovery Studio (Accelrys) and the \*SAbPred algorithm



**Table S3.** Variable heavy and light chain families expressed in DMAb format. *Related to Figure 1 and Supplemental Tables S1 and S3.*

<b>GP-DMAb</b>	<b>Species</b>	<b>VH*</b>	<b>VL*</b>
DMAb-1	mouse	VH3-7	Vκ1-5
DMAb-2	human	VH4-34	Vκ3-20
DMAb-3	human	VH1-69	Vκ3-15
DMAb-4	mouse	VH1-42	Vκ12-44
DMAb-5	mouse	VH3-2	Vκ1-135
DMAb-6	mouse	VH14-3	Vκ4-55
DMAb-7	mouse	VH 8-8	Vκ 6-13
DMAb-8	human	VH4-59	Vλ3-19
DMAb-9	human	VH3-13	Vκ1-27
DMAb-10	human	VH3-13	Vκ1-39
DMAb-11	human	VH3-53	Vκ2-28
DMAb-12	human	VH1-69	Vλ3-19
DMAb-13	human	VH3-30	Vκ4-1
DMAb-21	human	VH4-4	Vκ1-39
DMAb-22	human	VH1-46	Vκ3-11
DMAb-24	human	VH1-46	Vκ3-11
DMAb-25	human	VH4-59	Vκ3-11
DMAb-26	human	VH1-46	Vκ3-11
DMAb-27	human	VH1-46	Vκ3-11
DMAb-28	human	VH1-46	Vκ3-11
DMAb-29	human	VH3-23	Vκ3-20
DMAb-30	human	VH1-46	Vκ3-11
DMAb-31	human	VH3-48	Vκ1-5
DMAb-34	human	VH1-18	Vκ2-28
DMAb-35	human	VH3-23	Vκ1-5
DMAb-38	human	VH3-23	Vκ3-20
DMAb-39	human	VH1-46	Vλ2-23
DMAb-40	human	VH1-46	Vλ3-25
DMAb-41	human	VH3-20	Vκ1-16

**\*families identified by IMGT DomainGapAlign (45, 46)**

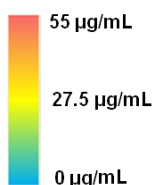
**Table S4.** *In vivo* Cmax expression data for individual mice receiving anti-GP DMABs. *Related to Figure 1.*

HR 50 ug	DMAb-21	DMAb-22	DMAb-24	DMAb-25	DMAb-26	DMAb-27	DMAb-28	DMAb-29	DMAb-30
	2.64	4.68	0.58	4.60	1.92	3.11	0.92	1.35	1.24
	1.42	3.36	0.39	10.60	1.14	1.71	1.49	1.81	1.21
	2.35	3.27	0.31	6.03	1.34	3.22	1.41	1.24	1.06
	3.78	2.98	0.83	7.29	0.62	2.43	1.72	0.99	1.24
	1.72	6.06	0.59	6.60	1.88	1.95	1.96	1.59	0.37
<b>Average</b>	<b>2.38</b>	<b>4.07</b>	<b>0.54</b>	<b>7.03</b>	<b>1.38</b>	<b>2.49</b>	<b>1.50</b>	<b>1.39</b>	<b>1.02</b>
<b>STDEV</b>	<b>0.92</b>	<b>1.29</b>	<b>0.20</b>	<b>2.23</b>	<b>0.54</b>	<b>0.67</b>	<b>0.39</b>	<b>0.32</b>	<b>0.37</b>

HR 200 ug	DMAb-21	DMAb-22	DMAb-28	DMAb-30
	18.83	18.64	4.70	5.92
	11.38	18.86	5.74	5.18
	11.78	17.44	6.50	5.22
	8.62	12.68	4.67	10.20
	18.03	11.56	5.54	6.47
<b>Average</b>	<b>13.73</b>	<b>15.84</b>	<b>5.43</b>	<b>6.60</b>
<b>STDEV</b>	<b>4.47</b>	<b>3.46</b>	<b>0.77</b>	<b>2.08</b>

Fusion loop 50 ug	DMAb-11	DMAb-39
	10.90	1.90
	7.08	1.60
	12.82	1.33
	9.10	0.64
	7.33	2.90
<b>Average</b>	<b>9.44</b>	<b>1.68</b>
<b>STDEV</b>	<b>2.43</b>	<b>0.83</b>

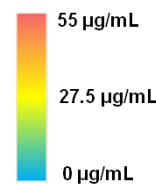
Fusion loop 200 ug	DMAb-11
	39.24
	63.12
	52.23
	49.57
	63.19
<b>Average</b>	<b>53.47</b>
<b>STDEV</b>	<b>10.08</b>



Glycan Cap 50 ug	DMAb-3	DMAb-7	DMAb-12	DMAb-10	DMAb-41
	0.99	8.06	5.35	0.54	6.60
	1.43	5.35	10.42	0.40	5.97
	0.89	7.34	11.64	0.07	6.48
	1.53	7.96	7.81	0.20	6.63
	1.57	4.98	5.73	0.97	6.59
<b>Average</b>	<b>1.28</b>	<b>6.74</b>	<b>8.19</b>	<b>0.43</b>	<b>6.46</b>
<b>STDEV</b>	<b>0.32</b>	<b>1.47</b>	<b>2.79</b>	<b>0.35</b>	<b>0.28</b>

Glycan Cap 200 ug	DMAb-7	DMAb-12	DMAb-10
	15.86	35.39	22.65
	27.97	27.44	16.48
	21.85	25.15	16.78
	25.69	20.66	14.95
	23.64	31.65	14.87
<b>Average</b>	<b>23.00</b>	<b>28.06</b>	<b>17.15</b>
<b>STDEV</b>	<b>4.60</b>	<b>5.71</b>	<b>3.20</b>

Base Dose 50 ug	DMAb-1	DMAb-4	DMAb-9	DMAb-31	DMAb-33	DMAb-34	DMAb-35
	1.37	3.43	8.33	2.19	3.41	7.80	0.00
	1.43	2.39	5.25	2.39	1.67	4.85	0.00
	1.39	3.77	9.55	1.45	3.07	7.13	0.99
	2.36	2.14	8.41	2.02	1.84	6.85	0.11
	2.35	3.31	8.98	2.11	2.41	6.33	0.41
<b>Average</b>	<b>1.78</b>	<b>3.01</b>	<b>8.10</b>	<b>2.03</b>	<b>2.48</b>	<b>6.59</b>	<b>0.30</b>
<b>STDEV</b>	<b>0.52</b>	<b>0.70</b>	<b>1.67</b>	<b>0.36</b>	<b>0.75</b>	<b>1.11</b>	<b>0.42</b>



Base Dose 200 ug	DMAb-1	DMAb-4	DMAb-9	DMAb-34
	8.06	16.25	32.69	28.00
	5.35	13.04	58.71	25.38
	7.34	8.93	44.60	32.90
	7.96	11.52	54.89	22.04
	4.98	8.83	23.10	22.91
<b>Average</b>	<b>6.74</b>	<b>11.71</b>	<b>42.80</b>	<b>26.25</b>
<b>STDEV</b>	<b>1.47</b>	<b>3.10</b>	<b>14.94</b>	<b>4.39</b>

Mucin Dose 200 ug	DMAb-40
	23.07
	48.76
	40.27
	25.12
	29.84
<b>Average</b>	<b>33.41</b>
<b>STDEV</b>	<b>10.85</b>

MPER 50 ug	DMAb-2	DMAb-13
	0.51	3.57
	0.69	4.65
	0.89	3.16
	0.76	8.85
	0.93	6.26
<b>Average</b>	<b>0.76</b>	<b>5.30</b>
<b>STDEV</b>	<b>0.17</b>	<b>2.32</b>

MPER 200 ug	DMAb-2	DMAb-13
	2.01	21.40
	2.73	23.24
	2.49	13.74
	1.85	15.37
	0.96	19.86
<b>Average</b>	<b>2.01</b>	<b>18.72</b>
<b>STDEV</b>	<b>0.68</b>	<b>4.03</b>