Supplemental Figure 1: NIP-conjugated fluorochromes effectively identify memory B cells and memory B cell subsets expanded in response to immunization with NP-CGG.

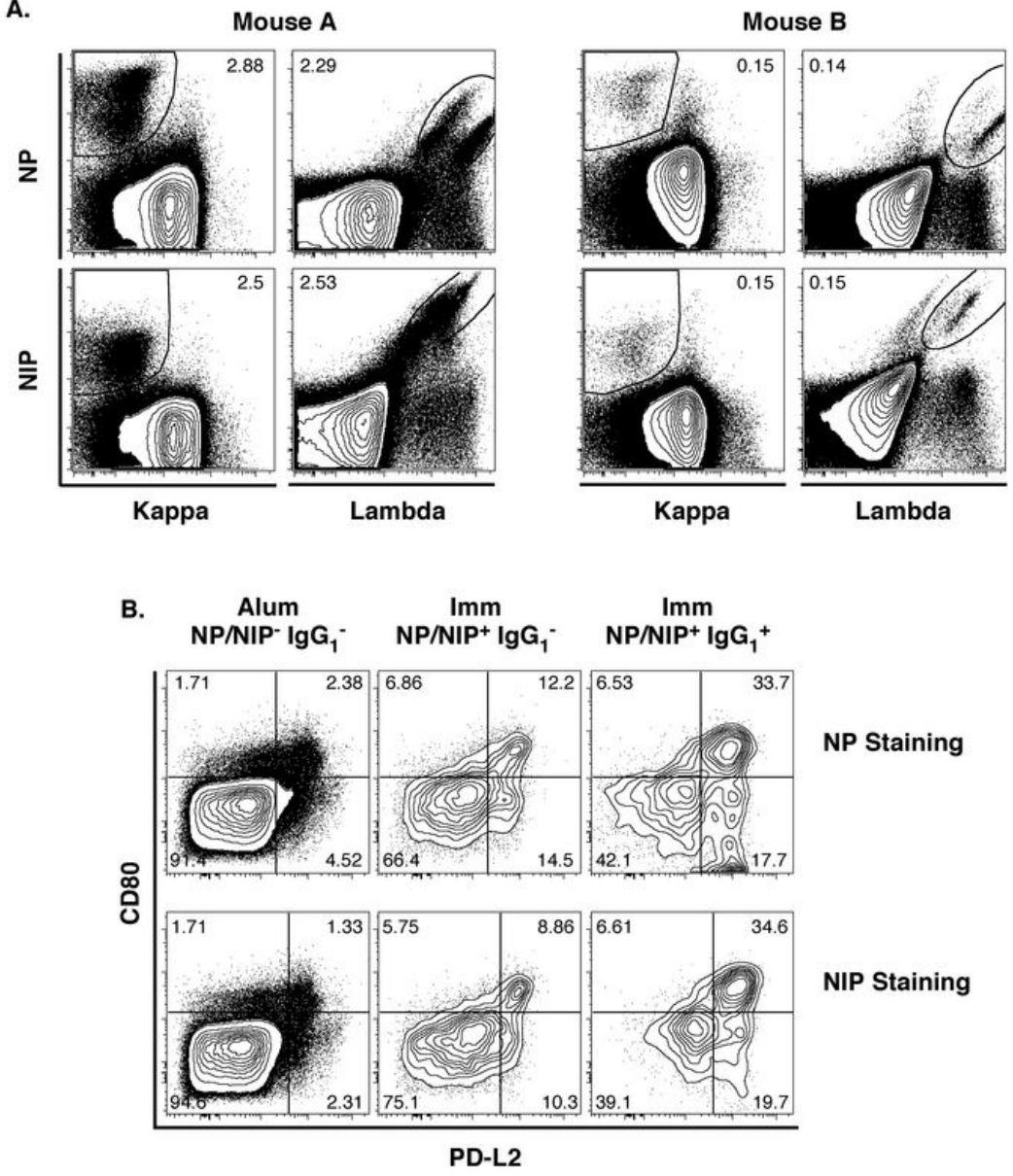
Supplemental Figure 2: Surface expression of PD-L2 (CD273), CD80 and CD73 define phenotypic subsets of both IgM and switched memory B cells in multiple systems, including immunized wild-type mice.

Supplemental Figure 3: Frequency of NP-reactive B cells among splenocytes of unimmunized wild-type Bl/6 mice.

Supplemental Figure 4: Unswitched memory B cell subset formation is influenced by naïve precursor frequency.

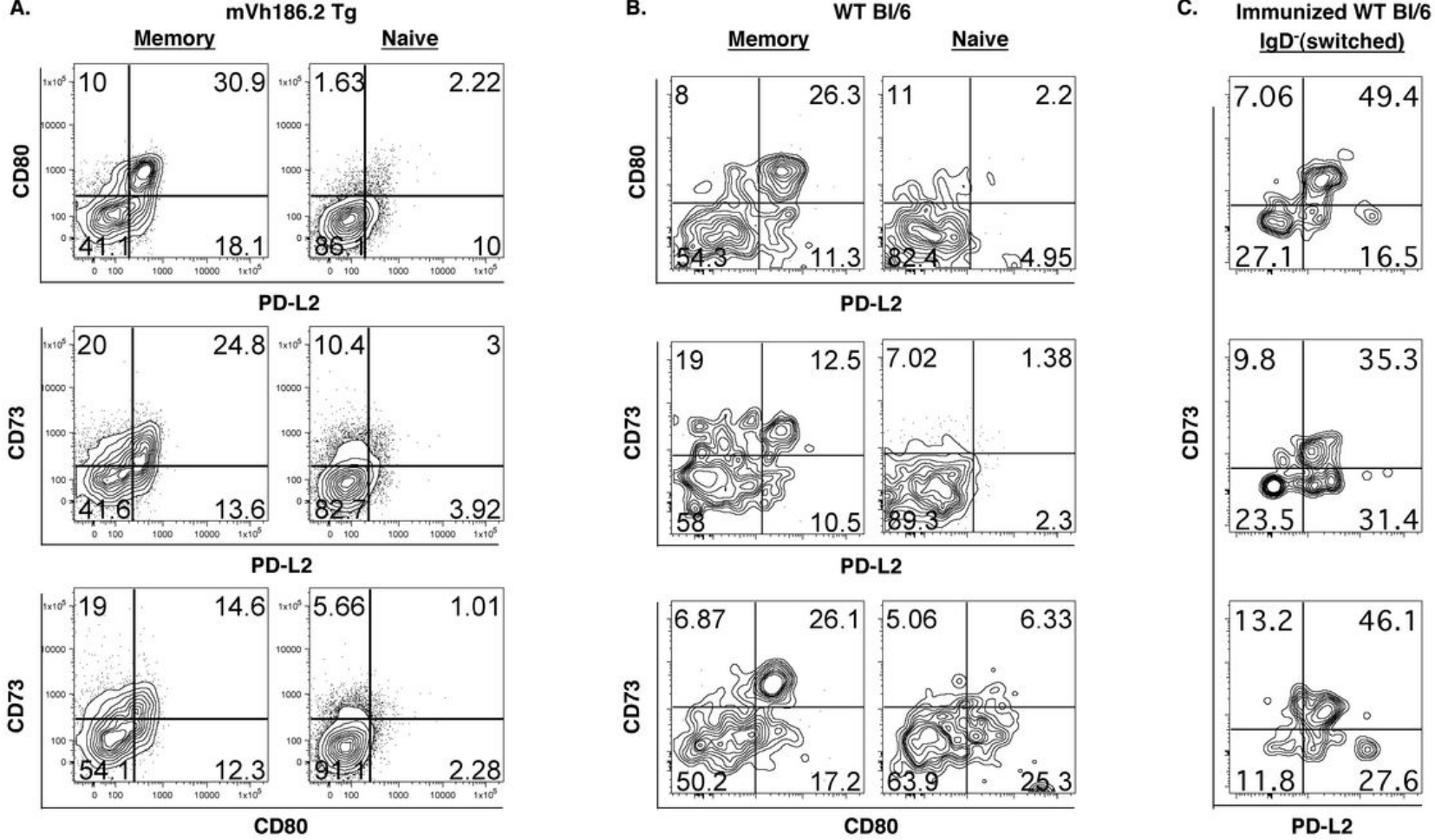
Supplemental Table 1: Precursor and progeny relationships in dose response experiments.

Supplemental Table 2: Comparison of $V_{\lambda 1}$ mutations across memory B cell subsets defined by PD-L2 and CD80 expression and Ig isotype



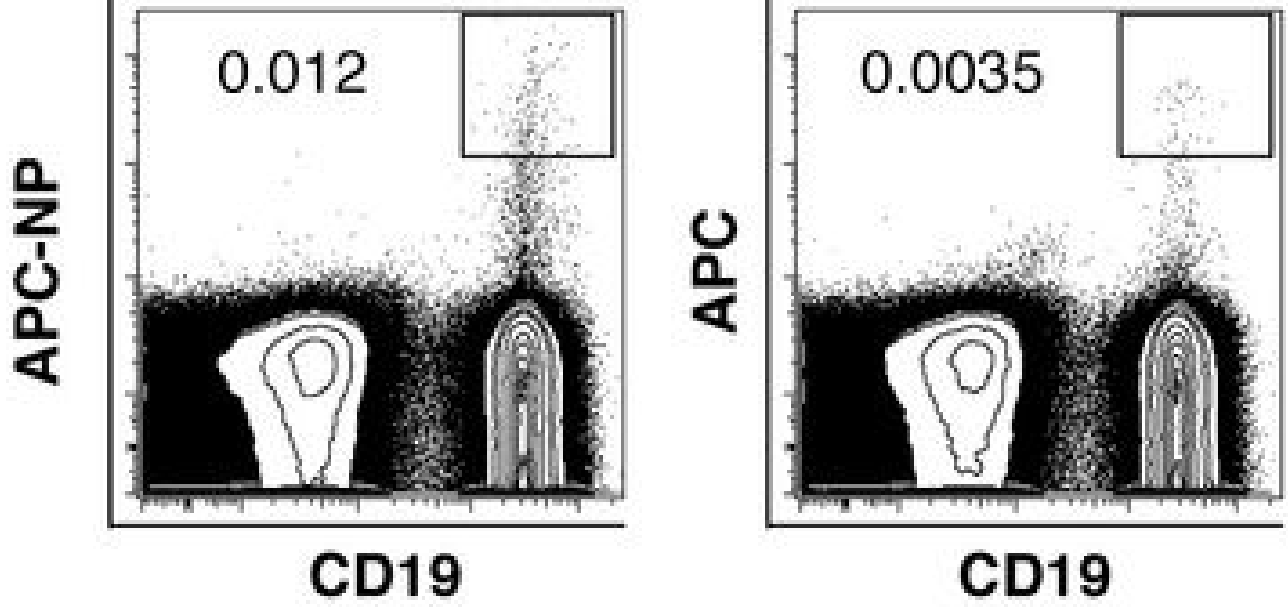
Suppl. Fig. 1. NIP- and NP-conjugated fluorochromes effectively identify memory B cells and memory B cell subsets expanded in response to immunization with NP-CGG. Memory B cells were generated in AM14 Tg x Vκ8R Sd.-Tg CB.17 recipients after adoptive transfer of B1.8 Sd.-Tg Jκ KO splenic B cells and immunization with NP-CGG in alum. Splenic B cells were stained with NP^- or NIP-APC in combination with other markers for memory B cells and analyzed by flow cytometry. A. Shown are FACS plots of live splenic B cells. NP- and NIP-APC identify similar fractions of B cells. These Ag-specific cells are predominately κ^{low} λ^{hi}. Mice A and B have different frequencies of Ag-specific cells, demonstrating that these properties are consistent over more than a 10-fold range. B. Shown are FACS plots of live splenic B cells gated by NP or NIP binding and by IgG_1 expression. For comparison, on the left are are plots of naïve NP^- or NIP^- IgG_1- B cells isolated from recipient mice immunized with alum alone. The protocol for simultaneous evaluation of PD-L2 and IgG_1 expression was developed subsequent to submission of the manuscript.

Suppl. Fig. 1.



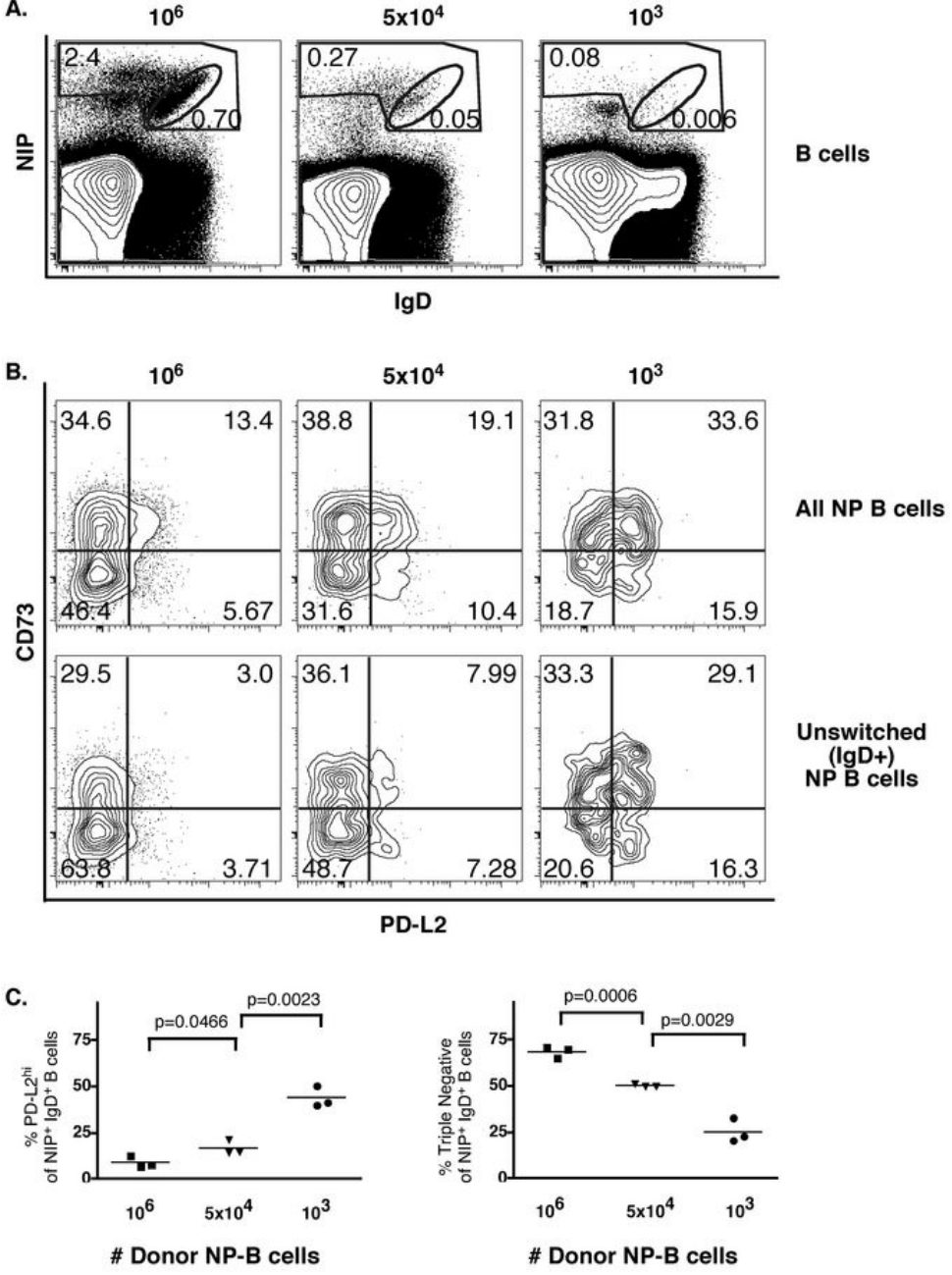
Suppl. Fig. 2. Surface expression of PD-L2 (CD273), CD80 and CD73 define phenotypic subsets of both IgM and switched memory B cells in multiple systems, including immunized wild-type mice. Shown are FACS plots of live NIP-binding κ^{neg} splenic B cells stained with antibodies to the indicated markers. The % of the parent population for the individual mice shown is indicated in the individual quadrants. *A.* Memory B cells were generated in mVh186.2 Tg mice after 2 i.p. immunizations with NP-CGG in alum, spaced 6 weeks apart, and subsequent rest for 12-20 weeks. Note that in this system, all memory cells are IgM-bearing and produce only membrane-bound Ab due to the use of the mVh186.2 IgH Tg. Naïve cells were isolated from unimmunized Vh186.2 Tg mice. Representative plots of at least 5 individual mice are shown. *B.* Memory B cells were generated in wild-type Bl/6 mice after i.p. immunization with NP-CGG in alum (memory) or alum alone (naïve) and 12.5 weeks rest. Representative plots of NIP-binding splenic B cells from 2-6 individual mice are shown. *C.* Memory B cells were generated in wild-type Bl/6 mice as described in *B.* Switched (IgD^-) NIP-binding splenic memory B cells are shown. PD-$L2^{low}$ $CD73^{low}$ Triple Low cells comprised an average of 19.6 % (+/- 7.0%) of the total switched memory B cell population. Plots are from individual mice and are representative of 4 mice total.

Suppl. Fig. 2.



Suppl. Fig. 3. Frequency of NP-reactive B cells among splenocytes of unimmunized wild-type Bl/6 mice. Shown are FACS plots of live-gated splenocytes stained with APC-NP or APC alone and PE-anti-CD19. A representative plot of 4 mice total is shown. Splenocytes ranged from 80-100 million per mouse.

Suppl. Fig. 3



Suppl. Fig. 4. Unswitched memory B cell subset formation is influenced by naïve precursor frequency. Memory B cells were generated after adoptive transfer of the indicated numbers (above each plot) of Vh186.2 Sd-Tg (Balb/c background) splenic B cells into AM14 x Vκ8R Tg Bl/6 x Balb/c F1 recipients, immunization with NP-CGG in alum, and 10 weeks rest. Shown are data from at least 3 individual mice per cell dose. *A*. FACS plots of NIP versus IgD staining of live splenic B cells. Unswitched memory cells are found within the oval-shaped IgD^{hi} NIP^{+} gate. The total NP-specific B cell gate was drawn to exclude the population of B cells that bind NIP via NP-specific cytophilic antibody bound to the F_cR. Averaged frequencies of the gated populations across replicates are shown. *B*. FACS plots of PD-L2 and CD73 staining of total (top row) versus IgD^{+} (unswitched, bottom row) NP-specific splenic B cells, gated as shown in *A*. The % of the parent population for the individual mice shown is indicated for each quadrant. *C*. Frequency of $PD\text{-}L2^{hi}$ (left) and $PD\text{-}L2^{low}$ $CD73^{low}$ (Triple Low, right) cells among the unswitched (IgD^{+}) NIP-binding splenic B cell population. P-values from 2-tailed Student's t-tests are indicated above the brackets.

Suppl. Fig. 4.

Supplemental Table I. Precursor and progeny relationships in dose response experiments

		CB.17 System			Bl/6 x Balb/c F1 System		
1	# Precursors transferred (tx'd)	1,000,000	50,000	1,000	1,000,000	50,000	1,000
2	Precursor dilution[a]	1	20	1000	1	20	1000
	Total NP Memory						
3	Average (Av.) # total memory B cells[b]	1,590,833	223,333	168,667	454,066	30,822	2,515
4	Relative total memory cell yield[c]	1.0	0.14	0.11	1.0	0.07	0.006
5	1/ Relative total memory cell yield	1.0	7.1	9.4	1.0	14.7	180.5
6	Average expansion per tx'd precursor[e]	1.6	4.5	168.7	0.5	0.6	2.5
7	Normalized Av. expansion per tx'd precursor[f]	1.0	2.8	106.0	1.0	1.4	5.5
	IgG_1 Memory						
8	Average # IgG_1 memory B cells[g]	145,456	170,865	110,887	88,585	12,452	1,457
9	Relative IgG_1 memory cell yield[c]	1.0	1.2	0.8	1.0	0.14	0.02
10	% IgG_1^+ of total memory pool[d]	9.1%	76.5%	65.7%	19.5%	40.4%	58.0%
11	Average expansion per tx'd precursor[e]	0.15	3.4	110.9	0.09	0.25	1.5
12	Normalized Av. expansion per tx'd precursor[f]	1.0	23.5	762.3	1.0	2.8	16.5
	Distribution of Memory Subsets (%)[h]						
13	PD-L2 High[i]	24.6	49.6	59.9	15.3	25.2	44.0
14	Triple Low[j]	56.7	37.0	31.9	48.8	34.1	21.4
	Distribution of Unswitched Memory Subsets (%)[k]						
15	PD-L2 High[i]	n.d.	n.d.	n.d.	8.4	16.4	43.6
16	Triple Low[j]	n.d.	n.d.	n.d.	68.1	49.9	24.9

[a]Compared with highest (1 million) dose.

[b]Calculated as (%NIP-binding κ^{low} $CD19^{+}$ B cells) * (# splenocytes). Alum corrected.

[c](# memory cells generated at given dose) / (# memory cells generated from 1 million precursors).

[d](# IgG_1^{+} memory cells generated) / (# total memory cells generated).

[e](# memory cells generated) / (# naïve precursors).

[f](Av. expansion per tx'd precursor at given dose) / (Av. expansion per tx'd precursor from 1 million dose).

[g]Calculated as (%IgG_1^{+} NIP-binding κ^{low} $CD19^{+}$ B cells) * (# splenocytes). Alum corrected.

[h]Calculated from anti-PD-L2 and anti-CD73 stain.

[i]PD-$L2^{hi}$ $CD73^{low}$ + PD-$L2^{hi}$ $CD73^{hi}$

[j]PD-$L2^{low}$ $CD73^{low}$, which are also $CD80^{low}$

[k]Gated as IgD^{+} from the anti-PD-L2 and anti-CD73 stain.

Data were calculated from averaged data from 3-6 individual mice per group.

Supplemental Table II. Comparison of $V\lambda_1$ mutations across memory B cell subsets defined by PD-L2 and CD80 expression and Ig isotype[a]

Subset	Total # Seq.	Total # Mut.	Av. Mut./Seq.	% Seq. with Mut.
IgM^+ $L2^{low}/80^{low}$	30	0	0.00	0.0%
IgM^+ $L2^{hi}/80^{low}$	36	49	1.36	50.0%
IgM^+ $L2^{hi}/80^{hi}$	40	106	2.65	82.5%
IgM^- $L2^{low}/80^{low}$	23	19	0.83	43.5%
IgM^- $L2^{hi}/80^{low}$	28	24	0.86	32.1%
IgM^- $L2^{hi}/80^{hi}$	31	147	4.74	90.3%

[a]1 million Vh186.2 Sd.-Tg naïve precursors were transferred into AM14 x Vκ8R F_1 CB.17 background recipients. 17-weeks post i.p. immunization with NP-CGG in alum, spleens from 3 mice were harvested and pooled for sorting. DNA was prepared from the indicated fractions by nested PCR as described in reference 3. PCR products were cloned into bacteria and the inserts in individual colonies were sequenced. The total number of individual colonies sequenced is listed in the second column.