Table S2 Statistical analysis of hits derived from screening the bivalent/biparatopic Nanobody library (Fig. 4) and of the library quality control sample.

	Library design †	Screening hits §			Library QC sample ‡		
Criterion	**Matching criterion (counts out of 361)**	**Matching criterion (counts out of 65)**	**Fraction matching criterion (hits/design)**	**Chi Square *P*-value**	**Matching criterion (counts out of 65)**	**Fraction matching criterion (QC/design)**	**Chi Square *P*-value**
Combinations of different epitope bin representatives							
all	258	59	higher	0.001	46	lower	0.9086
1 x 1part	36	9	higher	0.3496	5	lower	0.5661
1 x 1&2	54	7	lower	0.3747	11	higher	0.6851
1 x 2	36	5	lower	0.5661	6	lower	0.8535
1 x 3	36	10	higher	0.1956	8	higher	0.569
1 x 4	18	0	lower	0.0658	2	lower	0.5029
2 x 1&2	12	10	higher	<0.0001	2	lower	0.918
2 x 1part	8	7	higher	0.0006	0	lower	0.2257
2 x 3	8	7	higher	0.0006	1	lower	0.7266
2 x 4	4	0	lower	0.3938	2	higher	0.215
3 x 1part	8	0	lower	0.2257	0	lower	0.2257
3 x 1&2	12	2	lower	0.918	2	lower	0.918
3 x 4	4	0	lower	0.3938	1	higher	0.7668
4 x 1part	4	0	lower	0.3938	1	higher	0.7668
4 x 1&2	6	0	lower	0.2952	0	lower	0.2952
1part x 1&2	12	2	lower	0.918	5	higher	0.0977
Combinations of same epitope bin representatives							
all	103	6	lower	0.001	19	higher	0.9086
1 x 1	81	5	lower	0.0064	13	lower	0.6626
1part x 1part	4	1	higher	0.7668	0	lower	0.3938
1&2 x 1&2	9	0	lower	0.1982	2	higher	0.7847
2 x 2	4	0	lower	0.3938	2	higher	0.215
3 x 3	4	0	lower	0.3938	1	higher	0.7668
4 x 4	1	0	lower	0.671	1	higher	0.1708
Combinations with epitope bin in Nter or Cter position							
1	261	36	lower	0.0063	45	lower	0.313
1part	72	19	higher	0.0927	11	lower	0.6127
1&2	105	21	higher	0.6003	22	higher	0.4399
2	72	29	higher	<0.0001	13	higher	0.9918
3	72	19	higher	0.0927	13	higher	0.9918
4	37	0	lower	0.0069	7	higher	0.8991
Combinations with epitope bin in Nter position							
1	171	14	lower	0.0001	30	lower	0.8567
1part	38	10	higher	0.2541	4	lower	0.2763
1&2	57	13	higher	0.3991	11	higher	0.8183
2	38	14	higher	0.0125	8	higher	0.6701
3	38	14	higher	0.0125	7	higher	0.9532

4	19	0	lower	0.0585	5	higher	0.4343
Combinations with epitope bin in Cter position							
1	171	29	lower	0.6822	28	lower	0.5232
1part	38	10	higher	0.2541	7	higher	0.9532
1&2	57	8	lower	0.4724	13	higher	0.3991
2	38	15	higher	0.0048	7	higher	0.9532
3	38	5	lower	0.485	7	higher	0.9532
4	19	0	lower	0.0585	3	lower	0.828
Combinations with building block in Nter or Cter position							
002B02	37	2	lower	0.0649	5	lower	0.5244
002B10	37	6	lower	0.8019	8	higher	0.6192
004C03	37	0	lower	0.0069	8	higher	0.6192
004G10	37	8	higher	0.6192	5	lower	0.5244
006B05	37	4	lower	0.3027	11	higher	0.1172
010C05	37	1	lower	0.0233	5	lower	0.5244
011B09	37	10	higher	0.2238	3	lower	0.1517
014E10	37	7	higher	0.8991	6	lower	0.8019
014B10	37	2	lower	0.0649	7	higher	0.8991
001E11	37	16	higher	0.0012	5	lower	0.5244
002G09	37	4	lower	0.3027	6	lower	0.8019
003B11	37	0	lower	0.0069	10	higher	0.2238
003E10	37	6	lower	0.8019	11	higher	0.1172
007C10	37	15	higher	0.0036	3	lower	0.1517
005E02	37	14	higher	0.0099	9	higher	0.3897
005H01	37	15	higher	0.0036	5	lower	0.5244
007E09	37	1	lower	0.0233	6	lower	0.8019
013F07	37	18	higher	0.0001	8	higher	0.6192
012B02	37	0	lower	0.0069	7	higher	0.8991

Shown are the numbers of bivalent/biparatopic Nanobodies that match different criteria. For instance, the criterion "Combinations of different epitope bin representatives | 2 x 4" refers to bivalent/biparatopic Nanobodies containing a monovalent building block belonging to bin 2 and a monovalent building block belonging to bin 4; the criterion "Combinations with epitope bin representatives in Nter or Cter position | 1part" refers to bivalent/biparatopic Nanobodies that contain a partially blocking bin 1 monovalent building block in either the N-terminal or C-terminal position; the criterion "Combinations with a given building block in Nter or Cter position | 002B02" refers to bivalent/biparatopic Nanobodies that contain building block 2B02 in the N-terminal or C-terminal position.

65 screening hits (§, see also Fig. 4) or 65 randomly picked quality control samples (‡) were compared to the 361 possible combinations of the library design (†) by means of chi square analysis. In case the fraction of screening hits or quality control samples matching a certain criterion differed significantly ($P < 0.05$) from the library design, the P-values are shown in blue; if not, the P-values are shown in red. The absence of significant differences between the randomly picked quality control sample and the library design, for any of the specified criteria, highlights the quality of the library as it was generated, matching the library design specifications. Consequently, significant differences between the screening hits and the library design suggest that these criteria (enriched or decreased) contribute to the improved inhibition of *Pseudomonas aeruginosa*-mediated cell death by the 65 hits compared to the remainder of the 960 clones screened. For a more detailed discussion of the significant changes, see Results section.