

**Additional file 3: Table S3A: Intra-assay variability of antigen-specific IFN $\gamma$  responses ranging between 11-50 SFC/well<sup>a</sup>**

| Donor       | Antigen | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Mean | SD          | %CV          |
|-------------|---------|-------------|-------------|-------------|-------------|-------------|------|-------------|--------------|
| S10         | BZLF1   | 16          | 19          | 13          | ND          | ND          | 16   | 3.00        | 18.75        |
| S17         | BZLF1   | 22          | 29          | 28          | ND          | ND          | 26   | 3.79        | 14.38        |
| S24         | BZLF1   | 11          | 12          | 11          | ND          | ND          | 11   | 0.58        | 5.09         |
| S01         | EBNA3A  | 30          | 26          | 31          | ND          | ND          | 29   | 2.65        | 9.12         |
| S10         | EBNA3A  | 28          | 31          | 31          | ND          | ND          | 30   | 1.73        | 5.77         |
| S12         | EBNA3A  | 38          | 43          | 46          | ND          | ND          | 42   | 4.04        | 9.55         |
| S19         | PHA-L   | 35          | 35          | 45          | 44          | 38          | 39   | 4.83        | 12.25        |
| <b>Mean</b> |         |             |             |             |             |             |      | <b>2.94</b> | <b>10.70</b> |

**Additional file 3: Table S3B: Intra-assay variability of antigen-specific IL2 responses ranging between 11-50 SFC/well<sup>a</sup>**

| Donor       | Antigen | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Mean | SD          | %CV         |
|-------------|---------|-------------|-------------|-------------|-------------|-------------|------|-------------|-------------|
| S09         | BZLF1   | 39          | 39          | 43          | ND          | ND          | 40   | 2.31        | 5.73        |
| S11         | BZLF1   | 28          | 27          | 30          | ND          | ND          | 28   | 1.53        | 5.39        |
| S14         | BZLF1   | 37          | 31          | 34          | ND          | ND          | 34   | 3.00        | 8.82        |
| S16         | BZLF1   | 39          | 39          | 43          | ND          | ND          | 40   | 2.31        | 5.73        |
| S18         | BZLF1   | 32          | 37          | 37          | ND          | ND          | 35   | 2.89        | 8.17        |
| S01         | EBNA3A  | 33          | 29          | 21          | ND          | ND          | 28   | 6.11        | 22.08       |
| S10         | EBNA3A  | 26          | 26          | 20          | ND          | ND          | 24   | 3.46        | 14.43       |
| S12         | EBNA3A  | 47          | 44          | 51          | ND          | ND          | 47   | 3.51        | 7.42        |
| S21         | EBNA3A  | 42          | 38          | 38          | ND          | ND          | 39   | 2.31        | 5.87        |
| <b>Mean</b> |         |             |             |             |             |             |      | <b>3.05</b> | <b>9.29</b> |

**Additional file 3: Table S3C: Intra-assay variability of antigen-specific IFN $\gamma$ +IL2 responses ranging between 11-50 SFC/well<sup>a</sup>**

| Donor       | Antigen | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 | Replicate 5 | Mean | SD          | %CV          |
|-------------|---------|-------------|-------------|-------------|-------------|-------------|------|-------------|--------------|
| S11         | BZLF1   | 19          | 18          | 21          | ND          | ND          | 19   | 1.53        | 7.90         |
| S14         | BZLF1   | 24          | 18          | 18          | ND          | ND          | 20   | 3.46        | 17.32        |
| S16         | BZLF1   | 26          | 23          | 26          | ND          | ND          | 25   | 1.73        | 6.93         |
| S18         | BZLF1   | 23          | 29          | 29          | ND          | ND          | 27   | 3.46        | 12.83        |
| S01         | EBNA3A  | 18          | 11          | 11          | ND          | ND          | 13   | 4.04        | 30.31        |
| S10         | EBNA3A  | 13          | 18          | 11          | ND          | ND          | 14   | 3.61        | 25.75        |
| S12         | EBNA3A  | 23          | 25          | 27          | ND          | ND          | 25   | 2.00        | 8.00         |
| S20         | EBNA3A  | 10          | 14          | 16          | ND          | ND          | 13   | 3.06        | 22.91        |
| S21         | EBNA3A  | 22          | 17          | 21          | ND          | ND          | 20   | 2.65        | 13.23        |
| <b>Mean</b> |         |             |             |             |             |             |      | <b>1.70</b> | <b>16.13</b> |

<sup>a</sup> Values represent the number of detected antigen-specific IFN $\gamma$  (table 1A), IL2 (table 1B) and IFN $\gamma$ +IL2 (table 1C) SFC/2x10<sup>5</sup> PBMC (stimulated with 1 $\mu$ g/ml BZLF1 or EBNA3A peptide pools) or mitogen-specific IFN $\gamma$  (table 1A), IL2 (table 1B), and IFN $\gamma$ +IL2 (table 1C) SFC /5x10<sup>4</sup> PBMC (stimulated with 2 $\mu$ g/ml PHA-L) (data is not background subtracted) in the IFN $\gamma$ /IL2 FluoroSpot assay; ND = not done. SD = standard deviation. CV = coefficient of variation.