Supplements

Figure S1. Schematic representation of the conducted central composite design for the Passage viable cell density, the MTX concentration and the passage duration. The eight corner points represent the -1/1 levels. The three stars in the middle of the cube represent the replicate centre-point runs (0 level). The outer stars represent the -2/2 levels for two of the three parameters.

Table S1. Risk assessment and FMEA results.

| Process Parameter         | Probability | Severity | Detection | RPN |
|---------------------------|-------------|----------|-----------|-----|
| MTX concentration         | 1           | 5        | 4         | 20  |
| Passage duration          | 3           | 3        | 2         | 18  |
| Passage VCD               | 2           | 2        | 4         | 16  |
| pH                        | 2           | 3        | 2         | 12  |
| Thaw temperature          | 2           | 3        | 2         | 12  |
| Thaw duration             | 2           | 2        | 3         | 12  |
| Osmolality                | 1           | 3        | 3         | 9   |
| Media storage temperature | 1           | 4        | 2         | 8   |
| Media storage time        | 1           | 4        | 2         | 8   |
| Initial VCD               | 1           | 2        | 4         | 8   |
| Type of cryo conservation | 1           | 3        | 2         | 6   |
| Media temperature         | 1           | 3        | 2         | 6   |
| Cell line                 | 1           | 5        | 1         | 5   |
| Temperature               | 1           | 5        | 1         | 5   |
| Incubator CO₂             | 1           | 4        | 1         | 4   |
| Agitation speed           | 1           | 4        | 1         | 4   |
| Incubator humidity        | 1           | 3        | 1         | 3   |
| Non-/baffled flasks       | 1           | 3        | 1         | 3   |
| Working volume            | 1           | 2        | 1         | 2   |
### Table S2. Numerical coding of varied process parameters.

|                | -2 | -1 | 0  | 1  | 2  |
|----------------|----|----|----|----|----|
| MTX concentration [nM] | 0  | 7.5| 15 | 22.5| 30 |
| Passage duration [d]    | -  | 2  | 3.2| 5  | -  |
| Passage VCD [x10^6 cells/ml] | 0.1| 0.15| 0.2| 0.25| 0.3|

### Table S3. Experimental set up and responses for the DoE.

| Experiment number | MTX Concentration | Passage Duration | Passage VCD | Inoculum Viability [%] | Inoculum Growth Rate [1/d] | Production Viability [%] | Production Growth Rate [1/d] | Total mAb Titer [g/l] | Specific mAb Titer [g/l*(10^6 cells*d/ml)-1] |
|-------------------|-------------------|------------------|-------------|------------------------|---------------------------|--------------------------|--------------------------|---------------------|---------------------------------------------|
| 1                 | -1                | -1               | -1          | 98.5                   | 0.96                      | 98.6                     | 0.57                     | 1.10                | 14.78                                       |
| 2                 | 1                 | -1               | -1          | 98.6                   | 0.92                      | 98.1                     | 0.56                     | 1.12                | 16.41                                       |
| 3                 | -1                | 1                | -1          | 97.9                   | 0.75                      | 97.1                     | 0.55                     | 1.06                | 16.57                                       |
| 4                 | 1                 | 1                | -1          | 98.0                   | 0.79                      | 97.3                     | 0.51                     | 1.04                | 17.32                                       |
| 5                 | -1                | -1               | 1           | 98.7                   | 0.86                      | 98.7                     | 0.56                     | 1.11                | 17.26                                       |
| 6                 | 1                 | -1               | 1           | 98.7                   | 0.87                      | 98.0                     | 0.55                     | 1.09                | 16.47                                       |
| 7                 | -1                | 1                | 1           | 97.4                   | 0.69                      | 97.3                     | 0.49                     | 0.98                | 20.87                                       |
| 8                 | 1                 | 1                | 1           | 96.6                   | 0.68                      | 96.6                     | 0.44                     | 0.82                | 23.30                                       |
| 9                 | -2                | 0                | 0           | 98.8                   | 0.90                      | 98.7                     | 0.57                     | 1.19                | 16.72                                       |
| 10                | 2                 | 0                | 0           | 98.4                   | 0.82                      | 98.2                     | 0.52                     | 1.01                | 19.03                                       |
| 11                | 0                 | 0                | -2          | 98.2                   | 0.79                      | 97.4                     | 0.48                     | 0.99                | 20.21                                       |
| 12                | 0                 | 0                | 2           | 97.9                   | 0.72                      | 97.4                     | 0.48                     | 0.90                | 19.58                                       |
| 13                | 0                 | 0                | 0           | 98.9                   | 0.89                      | 98.3                     | 0.55                     | 1.16                | 17.12                                       |
| 14                | 0                 | 0                | 0           | 98.5                   | 0.88                      | 98.2                     | 0.57                     | 1.06                | 16.11                                       |
| 15                | 0                 | 0                | 0           | 98.8                   | 0.87                      | 98.6                     | 0.55                     | 1.08                | 17.08                                       |

### Table S4. Model statistics for the inoculum expansion and production process responses

|                   | R²   | R² adj | Q²   | p-value | Reproducibility |
|-------------------|------|--------|------|---------|-----------------|
| Inoculum viability | 0.95 | 0.91   | 0.79 | 0.0001  | 0.90            |
| Inoculum growth rate | 0.99 | 0.98   | 0.96 | < 0.0001 | 0.99            |
| Production viability | 0.89 | 0.81   | 0.62 | 0.0019  | 0.87            |
| Production growth rate | 0.99 | 0.90   | 0.69 | < 0.0001 | 0.92            |
| Total mAb titer | 0.93 | 0.88   | 0.70 | 0.0003  | 0.71            |
| Specific mAb titer | 0.94 | 0.914  | 0.827| < 0.0001| 0.90            |