

**Table S1. Analysis of MT-dependent tracking by protein-coated beads, using the segmented MT assay.**

| Protein coating        | Bead diameter           | Coating confirm | Binding efficiency | Number of examined beads | Processive beads (%) | Rate of motion | Traveled distance              | Interaction time |
|------------------------|-------------------------|-----------------|--------------------|--------------------------|----------------------|----------------|--------------------------------|------------------|
| Control (HisAB)        | 1 $\mu$ m PS            | yes             | 0-5                | 20                       | 0                    | not applicable | not applicable                 | not applicable   |
| Control (streptavidin) | 1 $\mu$ m glass         | nd              | 2-5/ax.            | 80                       | 6                    | 42 $\pm$ 9     | 2.3 $\pm$ 0.8                  | 3 $\pm$ 1        |
| MAPs                   | 1 $\mu$ m glass         | nd              | 5-10/ax.           | 107                      | 7                    | 37 $\pm$ 14    | 2.3 $\pm$ 0.3                  | 3.7 $\pm$ 0.5    |
| Mis12 complex          | 1 $\mu$ m PS            | yes             | 2-5                | 48                       | 3                    | 15 $\pm$ 2     | 0.8 $\pm$ 0.1                  | 3.1 $\pm$ 0.4    |
| KNL1                   | 1 $\mu$ m PS            | nd              | 5-10               | 56                       | 16                   | 24 $\pm$ 6     | 1.7 $\pm$ 0.4                  | 4 $\pm$ 1        |
| Ndc80 head             | 1 $\mu$ m PS            | yes             | 20-30              | 17                       | 0                    | not applicable | not applicable                 | not applicable   |
| Ndc80 full length      | 1 $\mu$ m PS            | yes             | 20-30              | 211                      | 10                   | 18 $\pm$ 3     | 2.1 $\pm$ 0.4                  | 7 $\pm$ 1        |
| Dam1                   | 1 $\mu$ m PS            | yes             | 20-30              | 161                      | 34                   | 20 $\pm$ 2     | all available MT length (5-15) | 29 $\pm$ 3       |
| Dam1                   | 0.5 $\mu$ m PS          |                 | 20-30              | 420                      | 46                   | 30 $\pm$ 1     |                                | 20 $\pm$ 2       |
| Dam1 + sol.            | 0.5 $\mu$ m PS          |                 | 20-30              | 200                      | 48                   | 8 $\pm$ 1      |                                | 74 $\pm$ 7       |
| Ska1 minimal + sol.    | 0.5 $\mu$ m PS          | yes             | 1-3/ax.            | 68                       | 37                   | 23 $\pm$ 4     | 2.0 $\pm$ 0.3                  | 5.1 $\pm$ 0.8    |
| Ska1 full + sol.       | 0.5 $\mu$ m PS          | yes             | 1-3/ax.            | 112                      | 27                   | 15 $\pm$ 2     | 3.5 $\pm$ 0.5                  | 13 $\pm$ 2       |
| Klp5/6 kinesin         | 0.5 $\mu$ m PS          | yes             | 0-2/ax.            | 57                       | 7                    | 10 $\pm$ 1     | 7.2 $\pm$ 2.7                  | 41 $\pm$ 15      |
| NK350 kinesin chimera  | 0.5 and 1 $\mu$ m glass | nd              | 0-3                | 33                       | 6                    | 55 $\pm$ 14    | 1.7 $\pm$ 0.6                  | 1.8 $\pm$ 0.6    |

Abbreviations: ax. - axoneme; nd - not determined; PS - polystyrene; sol. - soluble