Table S1. Development of geometrical confinements *in vitro*.

Confinement	Method	Achieved sizes and shapes	References
2D free standing SLBs (open confinement)	Flat supported lipid bilayers (SLBs) were formed on a silica slide and glass cover slip to mimic the cell membrane.	Large patches of no specific size and orders of magnitude larger than the specific length scale of the Min pattern.	Loose et al., 2008
2D rectangular flowcell setup	Flat supported lipid bilayers were formed in a fused silica surface of a 25 µm deep flow cell. Flow cells consist of two silica slides that are fused together and contain a (usually) rectangular flow channel in between, which has an inlet or outlet hole on each side to apply liquid samples.	Rectangular flow cell: 4 mm x 30 mm	Ivanov and Mizuuchi, 2010; Vecchiarelli et al., 2014, 2016
2D confinements of various shapes and sizes	Supported lipid bilayers of various shapes and sizes, or with obstacles placed inside of them, were produced by photolithography. Hereby 2D gold microstructures were used as confinement borders and obstacles.	Rectangular shapes: 6-100 µm × 100-800 µm L-, ring, and wave-shaped confinements: approx. 30-50 µm wide and several hundred µm long	Schweizer et al., 2012
Semi-3D confinement	Supported lipid bilayers were produced on PDMS microcavities of 10 µm depth. The Min proteins were introduced via a buffer reservoir that was placed on top of the grooves. The buffer level was subsequently lowered below the upper rim of the microcavities.	Rectangular confinements: 10 µm x 12-245 µm	Zieske and Schwille, 2013, 2014
3D droplets	Microdroplets, made out of lipid monolayers, were used to mimic 3D cellular compartments. The droplets were pipetted on a glas cover slip.	Droplets with a diameter of approx. 10-70 µm.	Zieske et al., 2016
3D fully confined microfluidic chambers	Fully confined 3D microfluidic chambers were produced by lithography. They were lipid-bilayer coated and connected through valves with a protein reservoir. After protein injection, the valves are closed.	2.4 µm x 10-60 µm x 10-90 µm (height x width x length)	Caspi and Dekker, 2016

Table S2. Quantitative information on the parameter-influence on the Min protein dynamics gained through reconstituted systems.

Only one dataset is given per parameter; for further information see references in Fig. 2. For further details on the confinement methods, see Table S1. **E. coli* lipid extract includes the lipids PG, PC, CL. ** Higher diffusivity on the membrane was achieved through GUVs, who form a more fluid membrane (than SLBs). ***Lower cytosolic diffusivity was achieved through the use of a crowding agent: Ficoll70 (140 g/l). Abbreviations: CL: cardiolipin, GUV: free-standing giant uni-lamellar vesicle, MTS: Membrane-targeting sequence, ΔMTS: MinE lacking the entire MTS, PC: 1,2-dioleoyl-sn-glycero-3-phosphocholine, PG: 1,2-dielaidoyl-sn-glycero-3-phospho-(1'-rac-glycerol), RT: room temperature, SLB: supported lipid bilayer, WT: Wildtype.

Parameter	**Confinement**	**Pattern Wavelength [μm]**	**Wave velocity [μm/s]**	**Diffusion coefficient of MinD [μm²/s]**	**Min D conc. [μM]**	**MinE conc. [μM]**	**Temp.**	**Change of protein diffusivity**	**Membrane composition**	**Reference**
Confinement and Temperature	2D free standing SLBs	78±12	0.6±0.2	-	1.1	1.0	RT	-	PC:PG (67:33)	Caspi and Dekker, 2016
		48±6	1.4	-	1.1	1.0	37 °C	-	PC:PG (67:33)	Caspi and Dekker, 2016
	3D full confinement	43±6	0.4±0.1	-	1.1	1.0	RT	-	PC:PG (67:33)	Caspi and Dekker, 2016
		37±9	0.5±0.1	-	1.1	1.0	37 °C	-	*E. coli* lipid extract	Caspi and Dekker, 2016
MinD to MinE ratio	2D free standing SLBs	100	0.28	-	1.0	0.5	RT	-	*E. coli* lipid extract*	Loose et al., 2008
	2D free standing SLBs	55	0.80	-	1.0	5.0	RT	-	*E. coli* lipid extract*	Loose et al., 2008

MinE MTS	2D free standing SLBs	30±5	0.65±0.05	-	1.0	5.0 (WT)	RT	-	*E. coli* lipid extract*	Kretschmer et al., 2017
	2D free standing SLBs	10±2	0.65±0.10	-	1.0	5.0 (ΔMTS)	RT	-	*E. coli* lipid extract*	Kretschmer et al., 2017
Membrane Diffusivity	2D free standing SLBs	65 to 110	0.4 to 1.0	0.25±0.04	0.75	0.75	RT	-	*E. coli* lipid extract*	Martos et al., 2013
	GUV (2D surface)	120 to 420	1.1 to 3.7	1.0±0.2	0.75	0.75	RT	Higher membrane diffusivity **	*E. coli* lipid extract*	Martos et al., 2013
Diffusivity in the cytosol	2D free standing SLBs	70	0.75	-	0.75	0.75	RT	-	*E. coli* lipid extract*	Martos et al., 2015
	2D free standing SLBs	20	0.2	-	0.75	0.75	RT	Lower cytosolic diffusivity ***	*E. coli* lipid extract*	Martos et al., 2015
Membrane composition: Effect of CL	Flowcell with SLB (2D)	24	15±2	-	1.0	5.0	RT	-	*E. coli* lipid extract*	Vecchiarelli et al., 2014
	Flowcell with SLB (2D)	20	22±3	-	1.0	5.0	RT	-	PC:PG (67:33)	Vecchiarelli et al., 2014

Table S3. Examples on how quantitative information obtained by *in vitro* experiments on MTs led to deeper understanding of the underlying biological processes.

***In vitro* technique**	**Measured parameter**	**Implication**	**Reference**
Optical trapping of beads coated with single kinesin molecules that can walk along MTs	Step size of 8 nm when kinesin moves along the MT	Answer to the question if motors make characteristic steps	Svoboda et al., 1993
Single molecule fluorescence using total internal reflection (TIRF) microscopy and photobleaching experiments on single dynein motors combined with axonemes	Mean velocity (85±30 nm/s), mean run length (1.9±0.2 µm), and 8 nm steps with a 0.8 probability of forward stepping	Molecular model for how processive motion is achieved by cytoplasmic dynein	Reck-Peterson et al., 2006
TIRF microscopy imaging of single MTs with purified fluorescent fission yeast MAPs	Mean velocities and dwell times of the different MAPs	Mechanistic understanding of MT plus-end tracking by Mal3 and the Tea2-Tip1-Mal3 complex in fission yeast	Bieling et al., 2007
TIRF imaging of single MTs with purified XMAP215 and EB1, titration of protein concentrations	MT growth rates and catastrophe frequencies	MT growth rates are increased to physiological (*in vivo*) levels by the synergistic effect of XMAP215 and EB1	Zanic et al., 2013
Optical trapping of beads attached to single MTs growing into a barrier coated with dynein	Pulling forces up to 5 pN, pulling by cortical dynein leads to centering of a MT aster in a 2D chamber	Demonstrate the intrinsic ability of cortical MT-dynein interactions to regulate MT dynamics and drive positioning processes in living cells	Laan et al., 2012

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Table S4. Examples of quantitative measurements necessary for modelling and experimentally building a minimal system for pattern formation by regulation of Cdc42, as is proposed in the discussion session of this paper

This list serves as an example, rather than being a complete list.

Protein	Parameter	Value	Technique	Reference
Cdc42	Protein abundance/cell	8690/cell	Proteomics	Kulak et al., 2014
Cdc42	nucleotide exchange rate	0.0002/s	*in vitro* Biochemistry	Zheng et al., 1994
Cdc42	GEF induced cellular nucleotide exchange rate	63.1/s	Model fitting	Freisinger et al., 2013
Cdc42	GAP induced cellular hydrolysis rate	2.74/s	Model fitting	Freisinger et al., 2013
Cdc42	Residence time of protein on the polarity site.	13.6 ± 1.5 (s.e.m.) s	Fluorescent recovery after photobleaching (FRAP) experiments in live cells	Gao et al., 2011
Rdi1	Protein abundance/cell	1670; 12395 proteins/cell	Proteomics	Ghaemmaghami et al., 2003; Kulak et al., 2014
Rdi1	Dissociation constant for the interaction between Cdc42-GDP and Rdi1 on the membrane	180 nM	*In vitro* biochemistry	Johnson et al., 2009
Rdi1	Dissociation constant for the interaction between Cdc42-GTP and Rdi1 on the membrane	1400 nM	*In vitro* biochemistry	Johnson et al., 2009
Rdi1	Association rate of Cdc42-GDP-Rdi1 to the membrane	0.03/s	*In vitro* biochemistry	Johnson et al., 2009

Rdi1	Dissociation rate of Cdc42-GDP-Rdi1 to the membrane	0.14/s	*In vitro* biochemistry	Johnson et al., 2009
Rdi1	Association rate of Cdc42-GTP-Rdi1 to the membrane	0.02/s	*In vitro* biochemistry	Johnson et al., 2009
Rdi1	Dissociation rate of Cdc42-GTP-Rdi1 to the membrane	0.13/s	*In vitro* biochemistry	Johnson et al., 2009
Bem1	Protein abundance per cell	1037; 6490 proteins/cell	Proteomics	Ghaemmaghami et al., 2003; Kulak et al., 2014
Bem1	Protein residence time on the polarity site.	10.2±1.6 (s.e.m.) s	FRAP experiments in live cells	Gao et al., 2011
Bem1–Cdc24	Cdc24 induced GDP exchange rate of Cdc42	[Bem1] dependent: 0.0024/s for 5 µM Bem1	*In vitro* biochemistry	Rapali et al., 2017
Cdc24	Protein abundance per cell	1010; 934 proteins/cell	Proteomics	Ghaemmaghami et al., 2003; Kulak et al., 2014
Cdc24	Protein residence time on the polarity site.	15.0±1.9 (s.e.m.) s	FRAP experiments in live cells	Gao et al., 2011
Cdc24	Cdc24 induced GDP exchange rate of Cdc42	0.0011/s	*In vitro* biochemistry	Rapali et al., 2017
Bem3	Protein abundance per cell	852; 599 proteins/cell	Proteomics	Ghaemmaghami et al., 2003; Kulak et al., 2014
Bem3	Protein residence time on the polarity site.	19.0±1.7 (s.e.m.) s	FRAP experiments in live cells	Gao et al., 2011
Bem3	GTPase activity for human Cdc42	100 nM	*In vitro* biochemistry	Zheng et al., 1993