Supplemental Material

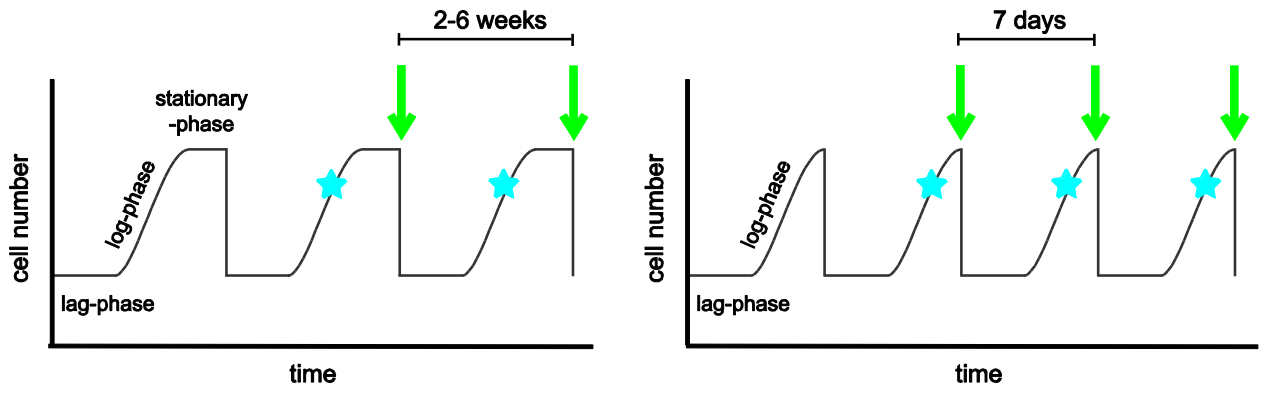

Figure S1. Culture Strategies. Arrows indicate the time point for sub-culturing, asterisks indicate the time point (5 days after the last sub-culturing step), when experiments started. In this study cells were kept under 'log-phase culturing'.

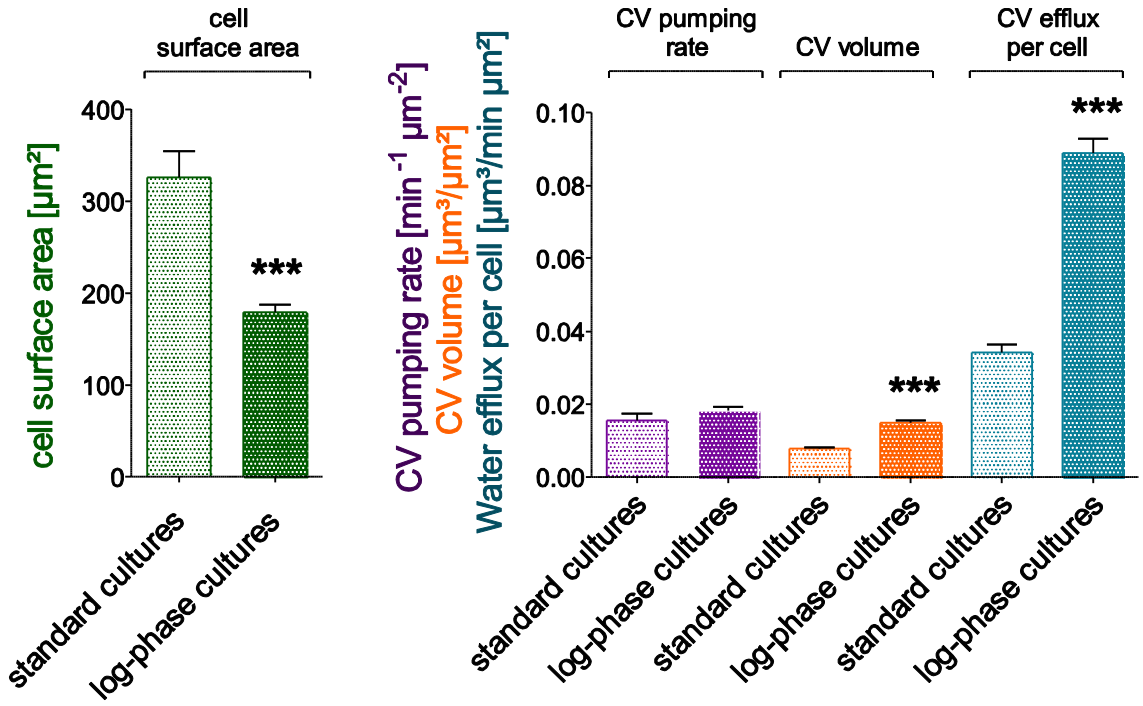

Figure S2. Effect of culture conditions on the physiology of the CV. A, Cells of 'standard cultures' were significant larger. B, CV properties of cells of 'standard' and 'log-phase' cultures

normalized to their cell surface area. CV pumping rate did not differ of cells of the different cultures. Strikingly, CV volume und thus CV efflux is tremendously increased in cells of 'log-phase' cultures.

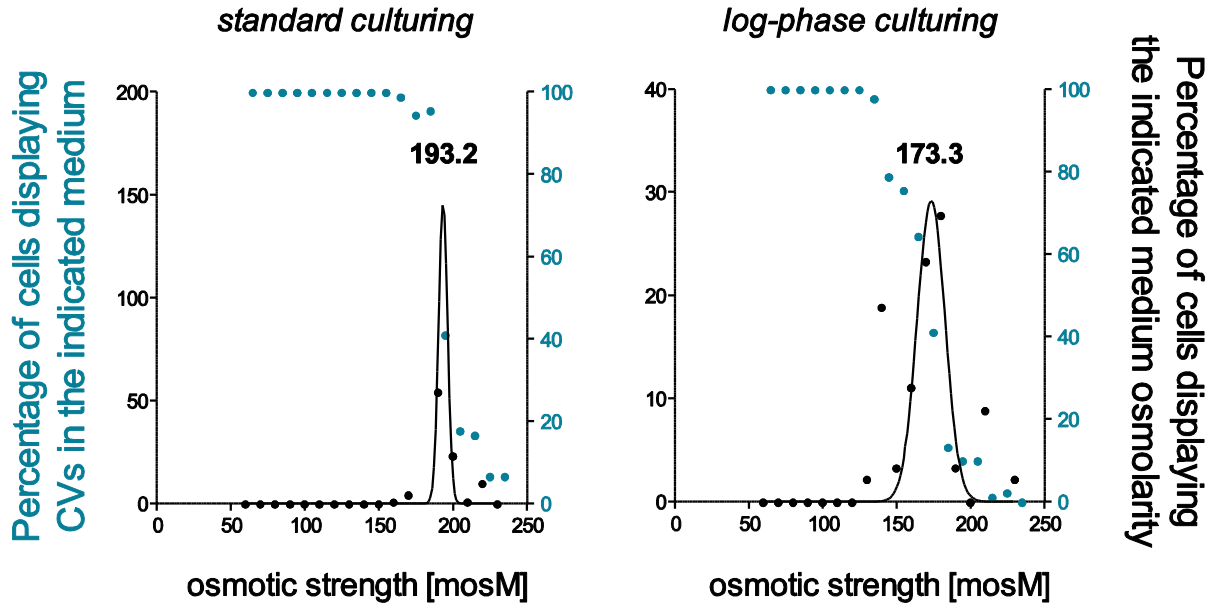

Figure S3. Determination of the cytosolic osmolarity of *Chlamydomonas reinhardtii* using the CV as sensor. The CVs also allow a simple determination of the cytosolic water potential. Under isotonic and hypotonic condition the CVs cease to function. *Chlamydomonas* cells were incubated with media of different osmotic strength and three times 30 cells were analyzed for the presence of light microscopically visible CVs in the cell. The mean number of CV/cell was plotted against the medium osmolarity (blue dots) and the obtained curve converted into a normal distribution of the cytosolic osmolarity (block dots). The obtained regression curve shows a maximum at 173.3 mosM. Interestingly, the cytosolic osmolarity is higher (193.2 mosM) when cells are cultured under standard conditions.

Table S1. Primers used in this study. Gene IDs are according to Phytozome 10. Primer were designed using primer3 or manually. For each target we made sure that melting curves had single peaks and that only one PCR product was visible on 2-4% agarose gels. Non-template controls were generally included.

Name	Gene ID	Sequence [5‘ to 3‘]	T_m [°C]
qMIP1-3’UTR f	Cre12.g549300.t1.2	GCGGAGATTGACATGACTGA	57.3
qMIP1-3’UTR r		CCCTCCACTTCCGAACACTA	59.4
qMIP1-CDS f		CATCTTCGCGGAGTTCTTTG	57.3
qMIP1-CDS r		GCCGTACAGGAAGATGGACA	59.4
qMIP3 f	Cre01.g038800.t1.2	ACAATTCTTGCGCACGGTGA	57.3
qMIP3 r		GCCGCTAAACCCCTTTGGTC	61.4
qSEC6 f	Cre17.g744847.t1.1	TGTTCAACCGCTGCTTCCAGAC	62.1
qSEC6 r		ACTTCTGCACCCAGTCCATCAC	62.1
qDynamin-like f	Cre13.g569000.t1.1	ATGTCGCGGGCTCACAGTTT	59.4
qDynamin-like r		CCCACACCACCAGTCACCAG	63.5
qRPL34 f	Cre16.g661050.t1.2	ATCATTCGGGCGTTCCTCATTGAG	62.7
qRPL34 r		TGACTTCCACCGCGTTTACTTGG	62.4

Movie S1. Fusion protein CreMIP1-GFP localized specifically to the CVs in *Chlamydomonas reinhardtii* UVM4-MIP1GFP-1. A confocal microscope (Leica TCS SP8 and a 60-fold water immersion objective) was used for generation of this time-lapse movie. The GFP-fluorescence is visible at the rim of the CVs. During the diastole phase the CV enlarges until systole takes place – then the CV collapses towards the plasma membrane. However, no intermingling of the CV

membrane with the plasma membrane is visible. Moreover, no GFP-fluorescence signal is visible at the plasma membrane.