Supplementary Methods

Strain construction

The pilB\textsuperscript{D355N} and pilT\textsuperscript{H222A} chromosomal point mutations and the ΔchpB knockout mutant were generated using two-step allelic exchange [1]. Briefly, the cloning vectors were created by digesting the pEXG2 backbone with the HindIII HF restriction enzyme (NEB). The inserts were created the following: for pilB\textsuperscript{D355N}, the 500 bp flaking regions of the mutation site were PCR amplified using primers pilB_D355N_P1/2 and P3/4. Primers pilB_D355N_P2/3 are reverse complements to each other and contain the point mutation. Both fragments were then inserted into the backbone by Gibson assembly according to the manufacturers protocol using the NEB Gibson Assembly Master Mix (NEB). For pilT\textsuperscript{H222A}, an insert was created to complement back into a ΔpilTU mutant. This strategy makes screening for the correct point mutation on the chromosome faster compared to the direct mutation strategy used for pilB\textsuperscript{D355N}. The regions starting 1000 bp upstream of pilT to the point mutation site and from the point mutation site to 1000 bp downstream of pilU were PCR amplified using primers pilT_H222A_P1/2 and
Primers pilT_H222A2/3 are reverse complements to each other and contain the point mutation. Both fragments were joined using sewing PCR using the flanking primers. For ΔchpB, the 500 bp regions upstream and downstream of chpB were amplified with a 15 bp overlap using primers chpB_P1/P2 and chpB_P3/P4 and joined using sewing PCR using the flanking primers. For all constructs, the resulting fragments were either digested with HindIII HF and ligated into the pEXG2 backbone or directly inserted into the backbone using Gibson assembly. The cloning vectors were electroporated into E. coli and the correct mutation was PCR screened and sanger sequenced using primers pEXG2_Ver1/2.

For mating, 1.5 ml E. coli containing the vector were grown to OD 0.5. The P. aeruginosa parental strain was grown overnight, and 0.5 ml culture was diluted 1:2 into fresh LB and incubated for 3 hours at 42 °C. Both cultures were concentrated into 100 µl and spotted onto an LB agar plate and incubated overnight at 30 °C. The puddle was scraped off, resuspended into 150 µl PBS, spread onto a VBMM plate containing 30 µg/ml gentamycin and incubated 24 hours at 37 °C. Six single colonies from the VBMM plate were struck onto NSLB and incubated for 24 hours at 30 °C. Several single colonies from the NSLB plate were screened for the correct mutation using PCR amplification with the flanking primers and confirmed using sanger sequencing.

**Preparation of hydrogels**

Agarose gels were prepared according by microwaving and subsequent cool down to 60 C in a water bath. Agarose was prepared freshly every day. An approximately 3 mm thick gel was made with the help of adhesive silicone isolators (Grace Bio-Labs) between a microscope slide and a coverslip, cured for 20 min and dried for 3 min after removal of the cover slip to remove excess liquid on the surface and avoid planktonic cells, i.e., to increase interaction with the surface.

Polyacrylamide (PAA) gels were either prepared from commercially available acrylamide (AA) and bisacrylamide (BIS) solutions (Biorad) (for gels with G’ < 130 kPa) or solid AA and BIS by dissolving in ddH2O (for gels with G’ > 130 kPa) according to the ratios for total AA + BIS content \( T \left( \frac{w}{v} \right) = \frac{AA(g) + BIS(g)}{(total \ volume \ (ml))} \times 100\% \) and crosslinker content \( C \left( \frac{w}{w} \right) = \frac{BIS(g)}{(AA(g) + BIS(g))} \times 100\% \) given by Supplementary Table 1 and 2 [2]. For each gel, 50ml of total solution was prepared and polymerized adding 250 µl 10% APS (Biorad) and 75 µl Temed (Biorad). After mixing AA and BIS, the solution was vacuum degassed for 10 min to remove oxygen which hampers polymerization and polymerized for 60 min at room temperature in a 150 mm petri dish with another 145 mm petri dish on top to get a planar surface of an approximately 5 mm thick gel. Crosslinking of PAA is an exothermal process which becomes apparent
for gels with T > 20%. Such gels were polymerized in a water bath with the addition of small amounts of ice to keep the entire reaction at room temperature. Gels were then washed twice with LB medium and soaked overnight in LB. One centimeter of the edge of each gel was cut off before washing because gels swell slightly during soaking with growth medium. The next day, liquid was removed and the gel surface was dried for 20 min in a sterile flow hood. This removes excess liquid on the surface and avoids planktonic cells, i.e., increases interaction with the surface. Gels were prepared freshly every day.

### Supplementary Table 1.
Formulations of polyacrylamide gels of various stiffness. Stiffnesses estimated from [2].

| Stiffness G' (kPa) | 1    | 10   | 160  |
|-------------------|------|------|------|
| % T               | 4.4  | 10.0 | 25   |
| % C               | 9.0  | 0.2  | 7.9  |

### Supplementary Table 2.
Formulations for polyacrylamide gels of different pore size but similar stiffness. Based on [2, 3].

| Stiffness G' (kPa) | 0.21±0.04 | 0.73±0.18 | 1.7±1.1 | 4.7±0.6 | 16±1 | 45±5 | 90±30 | 132±23 | 222±50 | 311±50 | 410±50 |
|-------------------|-----------|-----------|---------|---------|------|------|-------|--------|--------|--------|--------|
| % T               | 3.1       | 5         | 7.6     | 7.7     | 12.1 | 12.6 | 16.4  | 20     | 25     | 29     | 33     |
| % C               | 2         | 1         | 0.7     | 2.6     | 1.2  | 4.8  | 4.9   | 5      | 6      | 7.5    | 9      |

### Microscopy, sample preparation, and data analysis

**TFP imaging.** A commercial Nikon Ti-E TIRF microscope was used in HILO mode where the TIRF angle is set to slightly below the critical angle. This configuration reduces background and allows to image TFP dynamics longer and with higher contrast compared to regular EPI fluorescence imaging. The microscope was used with perfect focus, a 100x NA 1.49 Apo TIRF lens (Nikon), an EMCCD camera (iXon Ultra DU-897U, Andor), a stage top incubator (INU, Tokai Hit) set to 37° C and controlled by Nikon Elements software. TFP were fluorescently labeled as described previously [4]. TFP dynamics were analyzed manually in ImageJ.

**P PaO₂ time-lapse imaging.** Movies were taken on a Nikon Ti-E inverted microscopes controlled by Nikon NIS elements software using a 40x NA 0.95 Plan Apo Phase lens, an Andor Clara CCD or Hamamatsu Orca Flash camera, equipped with an environmental chamber (In Vivo Scientific) for temperature control set to 37° C. For all experiments, a stationary phase overnight culture (> 18 hrs) in LB was diluted 1:2000 in fresh LB and grown for 120 min at 37 C in a shaking incubator. 1.5 µl of cell suspension was then
spread on an approx. 10 x 10 mm large gel pad. The cell suspension was given 3-5 min to allow drying. Excess liquid on the gel creates a thin liquid film between gel and cover slip and a significant number of cells swims rather than adhering to the surface. The pad was then transferred to a glass bottom dish (Matek) and 200µl of water was distributed to 8-10 small droplets at the edge of the dish to keep the sample moisturized and to minimize sample degradation over time. Each gel was imaged every 20 min for 4 hours. Each experiment is a multi-point acquisition of typically 8 different fields of view (FOV) on the same gel, each FOV containing 10 – 40 cells at the beginning. At least three independent replicates per substrate / stiffness were acquired. Individual cells were segmented using a custom written, threshold-based segmentation algorithm based on the constitutive reporter (P_roD::mkate2) image to create a binary mask. This allowed estimating the mean background and fluorescence signal of both channels, which were analyzed as the ratio mVenus2 / mKate2. The ratio was analyzed for each time point and the median and inter quantile ranges (IQR) for the distribution at each time point was calculated.

Optical trapping. The custom built optical trapping setup consists of a 5 W 1064 nm laser (Spectra Physics) focused in the focal plane of a 100x NA 1.49 Apo TIRF lens (Nikon) to create an optical trapping potential [5]. For detection, we used back focal plane interferometry using a 850nm laser (QPhotonics), a position sensitive photodetector (New Focus), and an analog to digital conversion card for data acquisition (National Instruments). The trap and detection focus were positioned onto each other using a tip-tilt piezo mirror (Mad City Labs). Samples were positioned using a three-axis piezo stage (Mad City Labs). Brightfield images were acquired using a water cooled EMCCD camera (iXon Ultra DU-897, Andor). The entire microscope was controlled using custom written software in National Instruments LabView. Experiments were performed using a custom built, laser-cut incubation chamber and a PID temperature controller (In Vivo Scientific) set to 37° C. For experiments, an overnight culture was diluted 1:50 in fresh LB and grown for 3 hrs at room temperature. 20 ul of cell suspension was mixed 1:10000 with 532 nm polystyrene beads (Bangs Labs) and flushed by capillary action into a tunnel slide made by taping a coverslip to a microscope slide using double sided sticky tape. A trapped bead was then brought close to the pole of individual cells and we waited until the bead was displaced by TFP retraction towards the cell. Individual traces of bead displacements were analyzed in Igor Pro (Wavemetrics). Traces were calibrated using the thermal fluctuations method [5]. As shown in Supplementary Fig. 6B, an exponential fit to the bead position and force was used to estimate the stalling force (Supplementary Fig. 6D), the force free velocity (Supplementary Fig. 6E), and the force-velocity relationship (Supplementary Fig. 6F). To obtain the force-velocity relationship of TFP retraction, the high frequency bead displacements and forces were down-sampled/smoothed and differentiated.
**Traction force measurements (TFM).** A commercial Nikon Ti-E TIRF microscope was used with a 100x NA 1.49 Apo TIRF lens (Nikon), an EMCCD camera (iXon Ultra DU-897U, Andor), a stage top incubator (INU, Tokai Hit) set to 37° C and controlled by Nikon Elements software. PAA gels were made as described above with the addition of 2.5 μl of red-orange (565/580) and dark red (660/680) 0.04 μm carboxylated beads (Thermo Fisher FluoSpheres) per 500 μl gel solution. Hydrogels were polymerized between two chemically modified coverslips [6]. A 22 x 40 mm coverslip (Fisherbrand) was methacrylate silanized by submerging plasma cleaned coverslips in 2% 3-(trimethoxysilyl) propylmethacrylate in 95% ethanol for 10 minutes. Hydrophobic round 12 mm coverslip (Fisherbrand) were prepared by submerging coverslips in Sigmacote (Sigma) for 10 minutes. All coverslips were rinsed three times in 100% ethanol and dried upright on kim wipes. About 10 ul of unpolymerized gel was added between both coverslips resulting in an approximately 50 μm tall gel. Gels were polymerized for 30 minutes at room temperature, followed by removal of the hydrophobic coverslip. Gels were washed three times and then incubated for 30 minutes in EZ rich medium. For experiments, overnight cultures were diluted 1:200 and grown to mid log phase at 37° C shaking in LB. Excess liquid was removed carefully from the gel using kim wipes and 1.5 μl of cell suspension was added. Cells were incubated on the gel for 5 minutes before excess liquid was removed carefully using kim wipes. A top 22 x 22 mm coverslip was added and sealed with Valap. Bead displacement caused by TFP retraction were analyzed manually in ImageJ. We specifically analyzed beads that were initially displaced towards the cell pole followed by at least a partial relaxation backwards to the initial position, thus indicative of a directed motion caused by pilus retraction.

**Fluorescence recovery after photobleaching (FRAP).** We used a custom built FRAP microscope. To allow quasi simultaneous widefield EPI fluorescence imaging and localized photo bleaching, we split a 488 nm laser (Coherent) into two separate beam paths using a motorized flip mirror (Thorlabs). One beam path was focused in the back focal plane of a 100x NA 1.49 Apo TIRF lens (Nikon) to allow even illumination of the focal plane. A second beam path was focused in the focal plane of the objective lens to enable diffraction limited localized photo bleaching. The flip mirror allows the sequential use of both path and flipping within less than 1 s. Samples were positioned using a three axis piezo stage (Mad City Labs) and images were acquired using a water cooled EMCCD camera (iXon Ultra DU-897, Andor). The entire microscope was controlled using custom written software in National Instruments LabView. Experiments were performed using a custom built, laser-cut incubation chamber and a PID temperature controller (In Vivo Scientific) set to 37° C. For experiments, the *pilA*<sup>A86C</sup> strain was grown and fluorescently labeled as described above. Cells were imaged for a few seconds to establish a pre-bleach fluorescence baseline, then bleached for 100 ms, and immediately imaged again at 1 s frame rate. To obtain the fraction of immobile fluorescence molecules, the experimental data have to be corrected for both bleaching due to
FRAP and bleaching during fluorescence imaging. The loss of fluorescence intensity due to FRAP bleaching was corrected by analyzing the total fluorescence of the entire cell immediately before and after the FRAP bleaching event, and images taken after FRAP bleaching were corrected respectively. Photobleaching due to imaging was corrected by analyzing the total fluorescence intensity of cells outside of the FRAP bleaching spot, fitted by an exponential which was applied to all images. The rise of fluorescence at the FRAP bleaching spot was then analyzed over time and fitted with an exponential. This yields the recovery plateau (immobile fraction) for \( t \to \infty \) and the time constant \( \tau \) of the recovery. The diffusion coefficient \( D \) can then be calculated by

\[
D = \frac{L_c^2}{4\pi \tau}
\]

using the length of the cell \( L_c \) [7].

Atomic force measurements (AFM). Polyacrylamide gels were prepared as described above. For each gel, we prepared 10 mL of total solution, made according to the proportions described above. Individual gels were made by placing an approximately 0.5 mm thick adhesive silicone isolater on top of a coverslip, adding 150 µL of pre-cure PAA solution, and covering with another coverslip before allowing the gel to cure. Gels were otherwise prepared as described above by subsequent washing and soaking in LB.

Measurements of PAA gel modulus were made on a custom-made atomic force microscope (AFM) mounted atop a Nikon TE-100 inverted microscope. Coverslips with attached gels were mounted in a liquid cell and resubmerged in LB before being placed into the AFM. All measurements were made using tipless cantilevers from BudgetSensors All-in-One tipless probes. These probes have four cantilevers of varying stiffness and thus allowed us to adjust the cantilever stiffness to best match that of the (theoretical) gel modulus under test. We assumed the manufacturer-specified spring constant, \( k \) for each cantilever. For softer gels (\( G' < 45 \text{ kPa} \)), we used probe A \( (k = 0.2) \), for gels of intermediate stiffness \( (45 \text{ kPa} \leq G' \leq 90 \text{ kPa}) \) we used either probe A or B \( (k = 2.7) \), and for the stiffest gels \( (G' > 100 \text{ kPa}) \) we used either probe B or C \( (k = 7.4) \). Cantilevers were modified by attaching approximately 80 µm borosilicate glass beads (Cospheric, Item No. BSGMS-2.2 75-90µm-10g) to the end of the cantilever. To prevent adhesion of the beads with the surface of the PAA gels, beads were first treated with a siliconizing agent (SigmaCote, Sigma). Beads were then attached to the cantilevers first dipping the end of the cantilever into uncured Norland Optical Adhesive 76 (NOA76, Norland Products Inc.), a UV-curable adhesive. The adhesive-coated cantilever end was then brought into contact with the bead and exposed to UV light for 10 minutes, causing the adhesive to cure and the bead to be directly attached to the cantilever.

Prior to each measurement, we calibrated the optical lever sensitivity of the cantilever by bending the probe with a glass surface and measuring the resulting change in detector voltage vs. deflection indented. We also measured the diameter of the cantilever-attached bead by capturing a preliminary image of the
mounted cantilever/bead in the AFM. Individual measurements of gel modulus were made by obtaining force-distance curves of 2 - 5 indentations, each made at a different area in the gel. These curves were obtained by an initial “coarse” surface detection where we approached the surface at fixed, 100nm step sizes until a target force (approximately 2σ the noise level) was reached. Upon initial surface detection, the cantilever was held at 0 force (this point being later treated as our “true” surface location) for 10 seconds before indentation and subsequent retraction into the sample for 3 seconds at a rate of 1 µm/s. Control was accomplished by tuning a PI-controller, operating at 20 Hz, to feedback on the initial coarse detection and the measured force at each time instant. All data was sampled at 500 Hz.

Data was then analyzed by treating the PAA as a flat, linear elastic substrate in contact with an infinitely-stiff sphere according to the Hertz model. Data from both the indentation and retraction portions were then fit to the Hertz relation

\[
F = \frac{4E_{r}Rc\delta^{3/2}}{3(1-v^{2})},
\]

where \(F\) is the applied force, \(E\) is the Young’s modulus of the gel under test, \(Rc\) is the radius of the cantilever-attached bead, \(\delta\) is the sample indentation, and \(v\) is the Poisson’s ratio of PAA (assumed incompressible, \(v = 0.5\)).

**Estimation of hydrogel stiffness and pore size distribution**

Data on agarose and PAA stiffness \(G'\) and pore size \(D\) of the gel network are available in the literature. For PAA stiffness as a function of \(T\) and \(C\), we used the data collected by the meta study by Denisin et al. [2] that summarizes stiffness data available in different publications. For a given formulation of \(T\) to \(C\), we averaged stiffness values from [2] that are +/- 10 % of the same composition (typically 2 – 4 reported values). Since the reported values of PAA gel stiffnesses sometimes vary for similar formulations, we further confirmed the stiffness of these gels using indentation with atomic force microscopy (see Supplementary Fig. 2A).

Holmes et al. [8], Weiss et al. [9], and Wen et al. [3] consistently report a pore size of up to 150 nm for soft gels and < 20 nm for stiff gels using electrophoresis. However, as explained in these studies, the pore size of PAA gels does not only depend on the gel stiffness but moreover on the relative concentration of \(T\) and \(C\). Holmes et al. [8] empirically determined that the pore size \(D\) of PAA gels follows a power law

\[
D(T, C) = A(C)e^{-pow(C)T}.
\]

Here, the prefactor \(A(C)\) and the exponent \(pow(C)\) depend on the concentration of crosslinker that were determined for various values of \(C\) [8]. In order to map stiffness of PAA gels to pore size, we thus identified all gel formulations of known stiffness reported by Denisin et al. that are within 5% of the same \(C\) for which Holmes and Stellwagen determined values for \(A(C)\) and \(pow(C)\). We then determined the corresponding pore sizes for these formulations according to \(D(T, C)\).
Together with data by Weiss et al., this allowed us to empirically describe the relation between stiffness and pore size by a single exponential function (see Supplementary Fig. 2B).

For agarose, the stiffness of a gel as a function of the agarose concentration is well established and given by \( G' \sim c^{1.8} \) that links the shear modulus \( G' \) with the w/w concentration \( c \) of agarose [10]. This estimate matches two other publications [11, 12] (see Supplementary Fig. 2C). Similar to the stiffness of agarose gels, the pore size of agarose gels has been reported by multiple studies [13-15]. We used and empirically determined a double exponential fit function to all of the available data to describe the pore size distribution as a function of agarose concentration \( c \) (see Supplementary Fig. 2D).

**Estimation of the total number of PilA proteins in a single cell**

From the fluorescent PilA concentration dynamics experiment described in the main text, we measured a total decrease of PilA fluorescence in the entire cell of 10% for the pilus that extend for 5 \( \mu \)m. The packing of PilA in the TFP fiber of the closely related *Pseudomonas K* strain (PAK) is approximately 1 PilA monomers per 1 nm of TFP fiber [16, 17]. This equals to 5000 PilA monomers for a 5 \( \mu \)m long TFP or 50000 PilA monomers total per cell. Based on this analysis we can model the local loss of PilA at the TFP base during extension and the local source of PilA at the base during retraction.

**Surface deformation, TFP retraction, and PilA diffusion models**

First, we analytically describe the relation between pilus retraction and surface deformation, which we will later use for our simulations. The surface displacement \( \Delta x \) generated by a force \( F \) applied uniformly in a circular area with radius \( R \) is given by [18]

\[
\Delta x = \int \int G(-x', -y')f(x', y')dx'dy' \\
= \int_0^R \int_0^{2\pi} \frac{1 + \nu}{\pi Er^3} \left( (1 - \nu)r^2 + \nu r^2 \cos^2 \theta \right) F \frac{\pi R^2}{r} dr d\theta \\
= (1 + \nu)(2 - \nu) \frac{F}{\pi Er}
\]

where \( \nu = 0.5 \) is the Poisson’s ratio of PAA and \( E = 3G \) is the Young’s modulus (see Supplementary Fig. 5A). This yields the force-deformation relation \( F = \frac{4}{3} \pi GR\Delta x \). Based on our optical trapping experiments, the force-velocity relation of TFP retraction can be described by \( \nu(F) = \nu_0 - \nu_F \cdot F \). The substrate
deformation is coupled to TFP retraction by $\frac{d}{dt} \Delta x = v(F)$. For a polymer with $N = N_0(t) + N_s(t)$ monomers, the transition rates of folded $N_0(t)$ to unfolded $N_s(t)$ protein can be described by $\frac{d}{dt} N_0(t) = r_{0s} \exp\left(\frac{F(t)}{w_0}\right) N_0(t) - r_{s0} \exp\left(-\frac{F(t)}{w_0}\right) N_s(t)$ and $\frac{d}{dt} N_s(t) = -\frac{d}{dt} N_0(t)$, where $r_{0s}$ and $r_{s0}$ are the force free equilibrium transition rates from unstretched to stretched, and stretched to unstretched, respectively, and $w_0 = k_B T / \delta_a$ reflects the shape of the potential along the reaction coordinate $\delta_a$ [19]. The current rest length of the TFP fiber is given by $l_{p0} = l_{p0} - \Delta x(t)$, where $l_{p0}$ is the maximum TFP length immediately prior to the start of retraction. At the same time, stretching of PilA monomers yields the length of the TFP fiber $l_p(t) = a_0 N_0(t) + a_s N_s(t)$, where $a_0 = 1$ nm and $a_s = 3 a_0$ are the length of an unstretched and stretched PilA monomer in the fiber, respectively [16, 17, 20]. Since $l_p(t) \geq l_{p0} - \Delta x(t)$, $\left(1 - \frac{l_{p0}(t) - \Delta x(t)}{l_p(t)}\right) N(t)$ monomers can be removed from the TFP fiber and retracted into the cell, given the maximum catalytic ratio (600nm/s ≡ 600 PilA/s) determined by ATP hydrolysis is not violated.

In step one of the simulations, the loss of PilA in the inner membrane due to TFP extension is described by the constant extension velocity $v_{ext} \cdot 1$ PilA/nm = 350 PilA / nm for WT with the TFP extension velocity $v_{ext} = 350$ nm/s. Next, we appended a simulation for the gain of PilA to the membrane during TFP retraction as described above. In each step, the substrate deformation force is calculated by the deformation of the gel and its stiffness. This is then used to calculate the current pilus retraction velocity, given the measured force-velocity relationship (Supplementary Fig. 6), and to update the number of currently stretched and unstretched monomers in the pilus fiber. Together, this yields the number of monomers that can be recycled back into the membrane.

In the next step, we simulated the diffusion of each individual PilA monomer in the membrane by a Brownian dynamics simulation. Here, we modeled the inner membrane as a one-dimensional space with circular boundary conditions equivalent to the cross-sectional dimension of an average cylindrical cell with spherical endcaps with a length of 3 μm and a diameter of 0.5 μm. The diffusion of each individual monomer can then be described numerically by $\frac{d}{dt} x(t) = \frac{1}{\gamma} n_0 \sqrt{2 \pi k_B T} \Delta t^{-1}$, where $n_0$ is a random number drawn from a uniform distribution in the interval $-0.5 \leq n_0 \leq 0.5$. $\gamma = k_B T / D_{PilA}$ is the friction of PilA in the inner membrane given by the Einstein relation and the experimentally obtained diffusion coefficient $D = 0.22$ μm²/s, and $\Delta t = 1$ ms is the time step of the numerical simulation [21]. The loss or gain of monomer as simulated in step one was incorporated by either removing the monomer closest to the pole during TFP extension, or by adding a monomer at the pole during retraction. To analyze and plot the result of these simulations, we analyzed the PilA concentration in a spot of 500 nm diameter at the...
pole. Throughout the manuscript, relative concentrations are the concentration of this spot relative to the initial concentration in the whole cell.

We determined most of the parameters of this model experimentally and inferred $a_0$ and $a_s$ from published data (see above). Only the variables $r_0$, $r_s$, $w_0$, and $R$ remain as free fit parameters. These parameters were varied iteratively until we obtained a best match with the experimental data for WT cells on agarose. The best fit values are: $r_0 = 1 \cdot 10^{-6} \text{ s}^{-1}$, $r_s = 1000 \text{ s}^{-1}$, $w_0 = k_B T / \delta_0 = 3.4 \text{ pN}$, equivalent to $\delta_0 = 1.22 \text{ nm}$, and $R = 1 \text{ nm}$. 
Strains and plasmids used in this study

| Strain    | Description                                                      | Reference |
|-----------|------------------------------------------------------------------|-----------|
| E. Coli   | S17 Wild-type, used for cloning and conjugation                  |           |
| P. aeruginosa |                                                |           |
| PAO1      | Wild-type from Manoil transposon mutant library                  |           |
| ZG 1332   | Wild-type PAO1 with PPaQa-mVenus plasmid                         | Ref 26.   |
| ZG 1586   | PAO1 pilA-A86C chromosomal point mutation                        | Ref 39.   |
| ZG 1820   | PAO1 pilB-D355N chromosomal point mutation                       | This study|
| ZG 1821   | PAO1 pilB-D355N pilA-A86C chromosomal point mutations            | This study|
| ZG 1822   | PAO1 pilB-D355N chromosomal point mutation with PPaQa-mVenus plasmid | This study|
| ZG 1823   | PAO1 pilT-H222A chromosomal point mutation                       | This study|
| ZG 1824   | PAO1 pilT-H222A pilA-A86C chromosomal point mutations            | This study|
| ZG 1825   | PAO1 pilT-H222A chromosomal point mutation with PPaQa-mVenus plasmid | This study|
| ZG 1183   | PAO1 ΔpilTU, in frame deletion of pilTU                          | SI Ref 22.
| ZG 1337   | PAO1 ΔpilTU with PPaQa-mVenus plasmid                           | Ref 26.   |
| ZG 1186   | PAO1 Δvfr, in frame deletion of vfr                              | SI Ref 23.
| ZG 1826   | PAO1 Δvfr with PPaQa-mVenus plasmid                             | This study|
| ZG 1827   | PAO1 ΔchpB, in frame deletion of chpB                            | This study|
| ZG 1827   | PAO1 ΔchpB with PPaQa-mVenus plasmid                            | This study|

Supplementary Table 3. Strains used in this study.[22, 23]

| Plasmid    | Description                                                      | Reference |
|------------|------------------------------------------------------------------|-----------|
| pEXG2      | Vector for generating deletion mutants and point mutants         | SI Ref 1.
| ZG 1658 - pEXG2-pilA-A86C | Introduces A86C point mutation in PAO1 pilA                  | Ref 39.   |
| ZG XXXX - pEXG2-pilB-D355N | Introduces D355N point mutation in PAO1 pilB                  | This study|
| ZG XXXX - pEXG2-pilT-H222A | Introduces H222A point mutation in PAO1 pilT                  | This study|
| ZG XXXX - pEXG2-ΔchpB | ΔchpB kockout construct                                         | This study|
| ZG 1190 - pPaQa-mVenus | pUCP18 backbone with PPaQa::mVenus2, PrpoD::mKate2            | Ref 26.   |

Supplementary Table 4. Plasmids used in this study.
| Primer         | Sequence                                                                 | Reference     |
|---------------|---------------------------------------------------------------------------|---------------|
| pEXG2.Ver1    | GTTGCATGGGCAATAAGGTTGCC                                                  | Ref 39.       |
| pEXG2.Ver2    | CCGGTCTCATAACGACAAGG                                                    |               |
| pilB_D355N_P1 | GGAAGCATATAATGTAAAAGGACAAGGCAATGATGTGACCC                              | This study.   |
| pilB_D355N_P2 | CAGGGTGATTCTTACCAGGGGTTTTTCCGGCGTTGAAAATGTGATG                           | This study.   |
| pilB_D355N_P3 | CATCAACATTTTTCCACCACCCGCAAACACCCGATGAGATCAACCTG                          | This study.   |
| pilB_D355N_P4 | GAGTCGAACCTGCAATAATCCGCTGCAAGGCGCG                                       | This study.   |
| pilB_D355N_Seq1| GCGAAAAGCTGCTGCAGC                                                    | This study.   |
| pilB_D355N_Seq2| CCTGAAGTGCGACAGTCG                                                       | This study.   |
| pilT_H222A_P1 | GATACAAAGCTTCAACGTGCAAGGGAACTCG                                         | This study.   |
| pilT_H222A_P2 | CAGGGTGACCAGATCAAGGACCCGCGCGTCTCCGCGCGC                                  | This study.   |
| pilT_H222A_P3 | GCGGCCGAGACCGCGCTGGCTCACTGACCTGCC                                       | This study.   |
| pilT_H222A_P4 | GATACAAAGCTTCAACGTGCAAGGGAACTCG                                         | This study.   |
| pilT_H222A_Seq1| GATACAAAGCTTCAACGTGCAAGGGAACTCG                                         | This study.   |
| pilT_H222A_Seq2| GATACAAAGCTTCAACGTGCAAGGGAACTCG                                         | This study.   |
| chpB_P1       | GGAAGCATATAATGTAAAAGGACAAGGCAATGATGTGACCC                              | This study.   |
| chpB_P2       | GCCCTCGGCGACGCGTCACTGACATGCT                                            | This study.   |
| chpB_P3       | GCCCTCGGCGACGCGTCACTGACATGCT                                            | This study.   |
| chpB_P4       | GAGTCGACCTGCAAGGACATGCAAGGCGCG                                          | This study.   |

**Supplementary Table 5.** Primers used in this study.
Supplementary Figures

Supplementary Fig. 1. Experimental response of *P*~pPQa~ to the stiffness of different agarose hydrogels.

(A) The responses of 27 technical replicates from 3 biological replicates on 0.2 % agarose (≈ 1.5 kPa).
(B) The responses of 19 technical replicates from 3 biological replicates on 1.0 % agarose (≈ 30 kPa).
(C) The responses of 31 technical replicates from 3 biological replicates on 1.5 % agarose (≈ 60 kPa).
(D) The responses of 30 technical replicates from 3 biological replicates on 2.5 % agarose (≈ 150 kPa). 
(E) The responses of 44 technical replicates from 4 biological replicates on 4.0 % agarose (≈ 350 kPa).
(F) The responses of 29 technical replicates from 3 biological replicates on 5.0 % agarose (≈ 520 kPa).

(A) – (F) The median and IQR are shown as solid black line and gray shaded area.

(G) The distributions of the raw *P*~pPQa~::yfp / *P*~pPQa~::mKate2 fluorescence intensity on various agarose hydrogels. “Liquid” is the zero time point and “Hydrogel” is the maximum response at 180 min after the experiment started. Boxes represent the median and 25/75% quantiles.
Supplementary Fig. 2. Estimation of pore size and gel stiffness for PAA and agarose gels (also see Supplementary Methods). (A) Comparison of the estimated stiffness of PAA gels based on [2] and the experimentally determined stiffness using AFM. The black line indicates the one-to-one correspondence. (B) Pore sizes of different PAA gels estimated by [8, 9]. An empirically determined double exponential fit was used to estimate the average pore size as a function of gel stiffness. (C) Stiffness of agarose gels as a function of agarose concentration. Experimental data points and the power law fit $G \sim c^{1.8}$ (black line) are taken from [10]. (D) Pore size of different agarose gels as estimated by [13-15]. An empirically determined double exponential fit was used to estimate the pore size as a function of gel stiffness.
Supplementary Fig. 3. Experimentally obtained doubling time of cells grown on different agarose and PAA hydrogels. (A) The doubling time as a function of substrate stiffness does not collapse onto each other for both gel types, indicating that the doubling time is not a function of substrate stiffness. (B) The doubling time as a function of substrate pore size does collapse onto each other for both gel types, indicating that the doubling time is a function of substrate stiffness.

Supplementary Fig. 4. Fluorescence expression of various promoters as a function of substrate stiffness. (A) – (F) Expression of PaQa (yellow) and the housekeeping gene rpoD (red) over time as a function of agarose concentration. The fluorescence is either stable or increasing over time as cells grow and divide thus demonstrating that fluorescent proteins are being made by cells on all substrates. Thick lines are the median and shaded areas the standard deviation of three independent biological replicates and at least six technical replicates each. (G) Fluorescence expression of the arabinose inducible pBad promoter (green: agarose, purple: PAA) and the tetracycline inducible pTet promoter (grey: arabinose) as a function of substrate stiffness. For pBad expression, 0.1% arabinose was incorporated in each gel. For pTet, 1000 ng/ml of anhydrous tetracycline was incorporated in each gel. Datapoints are the median and error bars the inter quantile range of three independent biological replicates and at least six technical replicates each.
Supplementary Fig. 5. Surface deformation and optical trapping. (A) Sketch of the surface deformation of a substrate. The force $F$ is uniformly applied in the circular area with diameter $2R$, yielding the deformation $\Delta x$ along $F$. (B) Exemplary displacement of an optical trapped bead by the retraction of a TFP. (C) Relation of the retraction velocity as a function of the retraction force from B).

Supplementary Fig. 6. Pilus retraction force obtained by optical tweezers experiment. (A) Sketch of optical trapping experiment. An optically trapped 500 nm polystyrene bead is brought close to the pole of a coverslip attached cell. TFP readily bind to polystyrene and retraction of bead bound TFP can be observed by a displacement of the bead towards the cell’s pole [24]. (B) Exemplary bead displacement/pilus retraction trajectory. The TFP retracted with initial velocity $v_0 \approx 600$ nm/s, then slowed down due to increasing resistance of the trapping force from the trap (equal to the retraction force of the pilus) until it stalls with the maximum stall force $F_0 \approx 60$ pN. (C) Relation of the retraction velocity as a function of the retraction force from B). (D) Distribution of retraction stall forces of individual pili (N = 56). This value agrees with the ranges of TFP retraction forces measured in other strains and species including P. aeruginosa PA14, Myxococcus xanthus, and Neisseria spp. [24-26]. (E) Distribution of load free velocities of individual TFP retractions (N = 56) with a median velocity $v_0 = 600$ nm/s, in good agreement with observations by fluorescence microscopy [4, 27]. (F) Distribution of the velocity-force relationship $v(F) = v_0 - f_0 \cdot F$, with $f_0 = -13$ nm/(s·pN) of individual pilus retractions (N = 56). See Supplementary Fig. 5 and Supplementary Methods for more details.
Supplementary Fig. 7. Mechanical surface deformation TFP retraction model. (A) - (F) Model result for a typical 0.8 µm long TFP on three different substrates (Low = 1 kPa, Peak = 50 kPa, High = 150 kPa). (A) Pilus length. (B) Substrate deformation. (C) Retraction force needed to deform the substrate. (D) Loss of PilA in the inner membrane for the non-stretching model. This value starts high because the TFP is fully extended at $t=0$. (E) The ratio of stretched PilA to all PilA in the TFP fiber in the stretching model. (F) Loss of PilA in the inner membrane for the stretching model. This value starts high because the TFP is fully extended at $t=0$. (H) Loss of PilA in the inner membrane at $t=0.75$ s after the start of retraction as a function of substrate stiffness for the non-stretching model (red dashed line), the stretching model (solid black line), and the experimental $P_{\text{PaQa}}$ data for WT on agarose.
Supplementary Fig. 8. Pilus extension velocity for the ATPase point mutant PilBD355N compared to WT. Boxes represent the median and 25/75% quantiles.

Supplementary Fig. 9. Simulation of the PilA loss function as a function of stiffness for a variation of the pilus-substrate adhesion spot size R.

Supplementary Movie 1: Traction force experiment of cells on 4.5 kPa PAA. Red arrows indicated areas of surface deformation mediated by pilus retraction. White rectangles indicate deformation free areas.

Supplementary Movie 2: Traction force experiment of cells on 16 kPa PAA. Red arrows indicated areas of surface deformation mediated by pilus retraction. White rectangles indicate deformation free areas.

Supplementary Movie 3: Traction force experiment of cells on 45 kPa PAA. Red arrows indicated areas of surface deformation mediated by pilus retraction. White rectangles indicate deformation free areas.

Supplementary Movie 4: Traction force experiment of cells on 132 kPa PAA. Red arrows indicated areas of surface deformation mediated by pilus retraction. White rectangles indicate deformation free areas.

Supplementary Movie 5: Crop of traction force experiment of one cell pole on 132 kPa PAA illustrating the retraction of a single pilus attached to the PAA surface.

Supplementary Movie 6: Retraction of a pilus attached to a 4.0% agarose hydrogel.
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