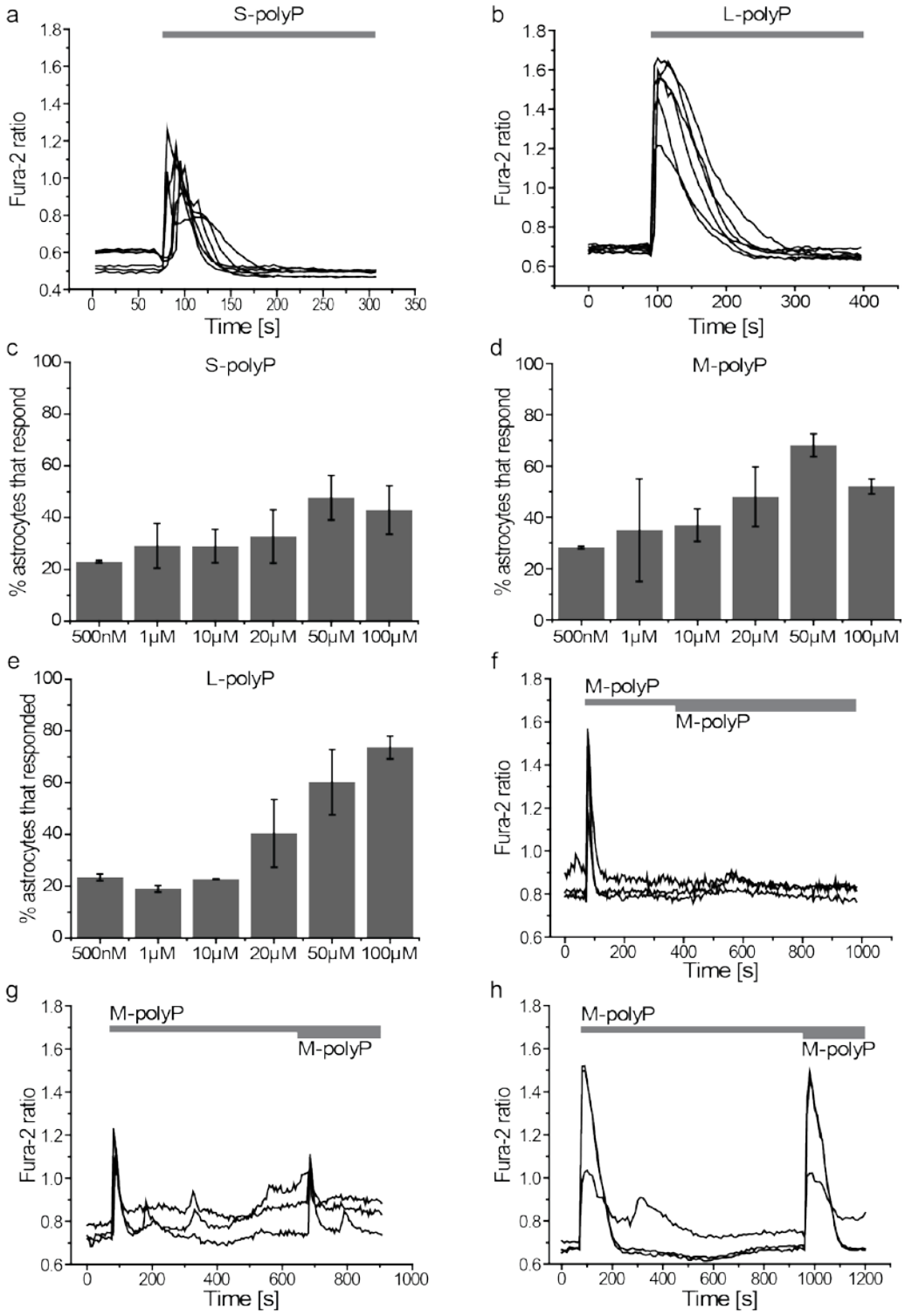
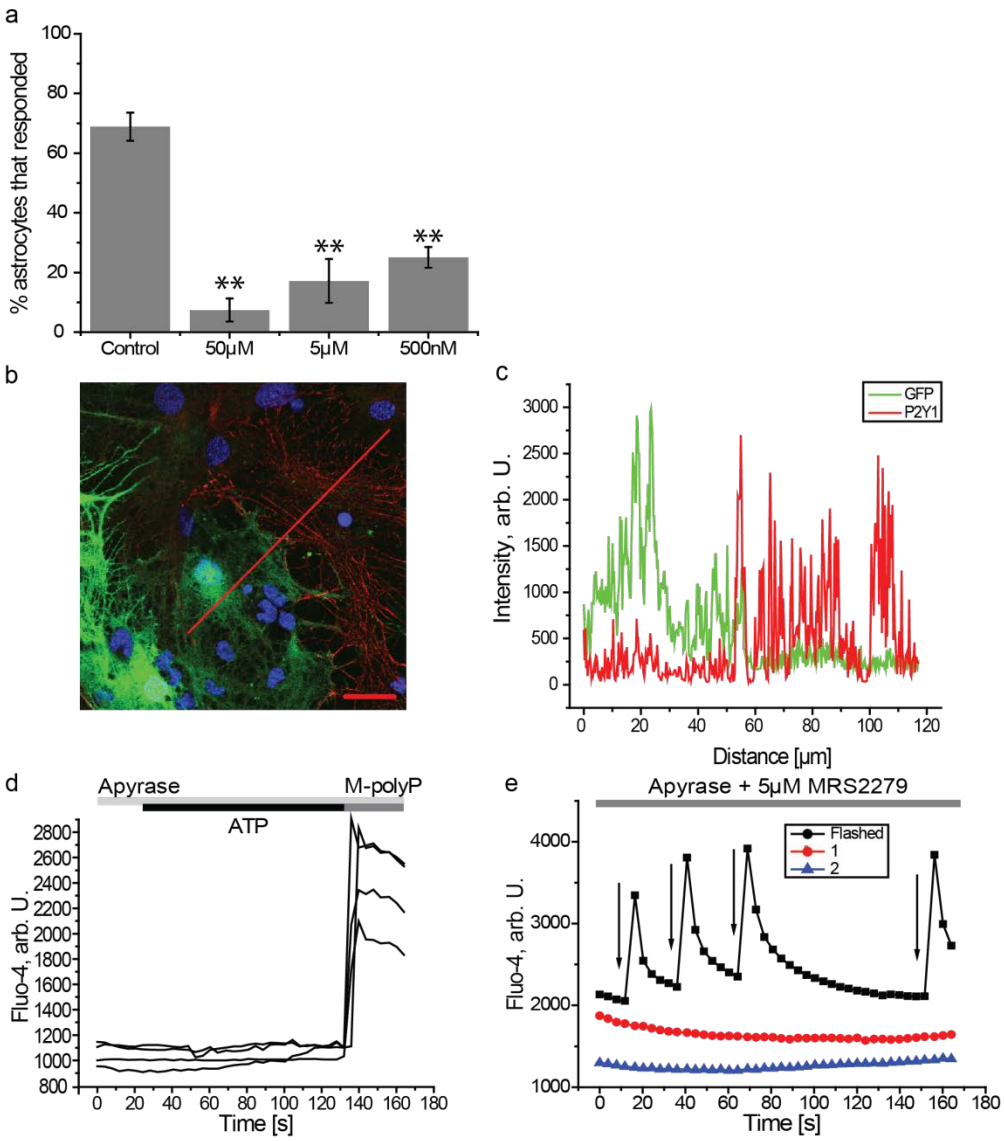
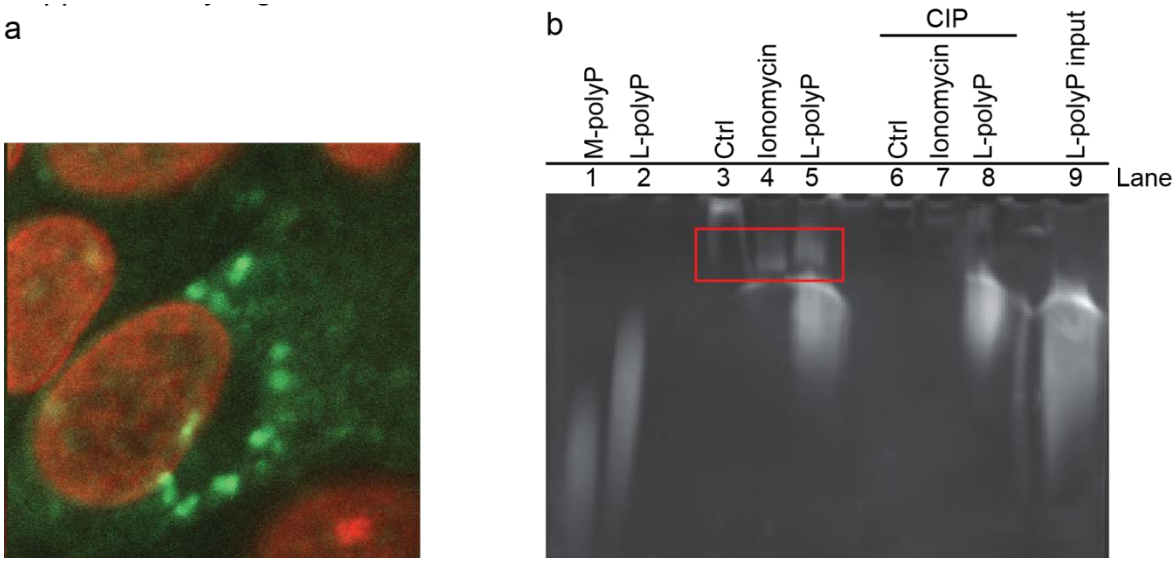


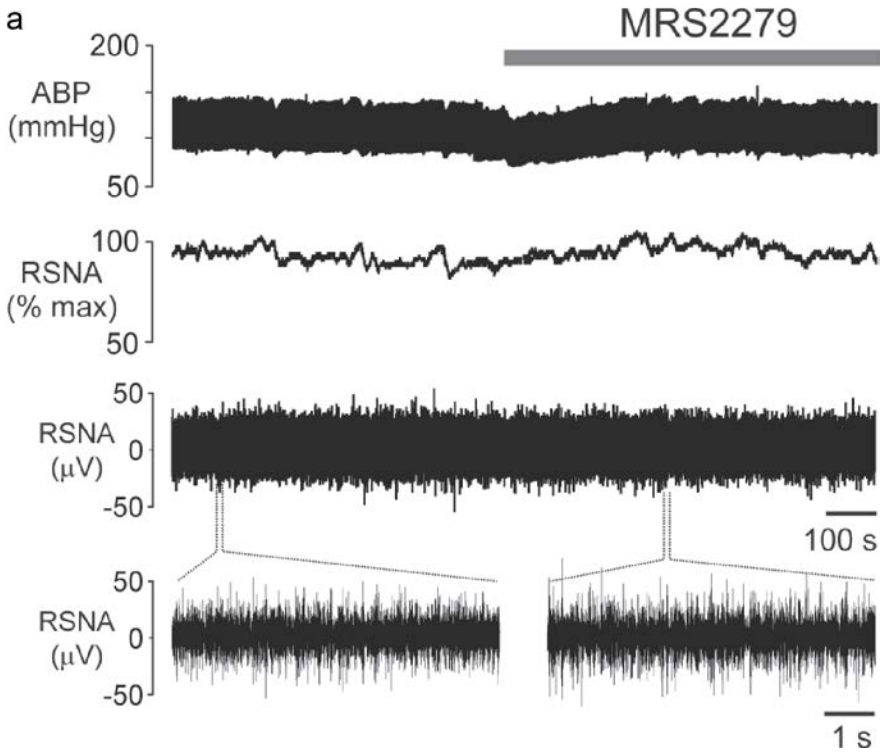
Supplementary Figure S1. PolyP induces Ca^{2+} responses in neurons and astrocytes. Short polyP (100 µM) induces $[Ca^{2+}]_c$ changes in cortical astrocytes (a) as does long polyP (100 µM) (b) as seen by an increase in the ratio of the Ca^{2+} dye Fura-2. The number of astrocytes that responded to varying concentrations of S-polyP (c), M-polyP (d) and L-polyP (e). n > 100 cells for each condition, error bars represent s.e.m.. Repeated exposure of astrocytes to M-polyP (50 µM); 5 min (f), 10 min (g) and 15 min (h), leads to a second Ca^{2+} signal only after 10 min in some cells and 15min in most cells.



Supplementary Figure S2. Effect of polyP in astrocytes – the role of the $P2Y_1$ receptor. (a) The number of astrocytes that reacted to M-polyP (50 µM) with an increase in cytosolic Ca^{2+} in the presence of varying concentrations of MRS2279. Error bars represent s.e.m., n > 100 cells for each condition, ** = $p < 0.005$. (b) Primary neuroglia culture subjected to $P2Y_1$ specific shRNA (green) stained for $P2Y_1$ (red) and DAPI for nucleus (blue). Scale bar = 20 µm (c) Knock down efficiency after $P2Y_1$ shRNA. GFP (green) transfected cells show lower levels of $P2Y_1$ staining (red), compared to neighboring non-transfected cells. (d) Pre-incubation of cells with apyrase (25 U ml^{-1}) blocked ATP (100 µM), but not M-polyP (50 µM) induced Ca^{2+} signaling, as seen by an increase in the Ca^{2+} indicator Fluo-4. (e) Flash photolysis of a single cell (black trace, each arrow indicates a flash) uncages Ca^{2+} but pretreatement with 5 µM MRS2279 blocks transmission of the signal and a Ca^{2+} signal in neighboring cells (red and blue traces).



Supplementary Figure S3. Detection of polyP with DAPI. (a) Merged fluorescent image of DAPI-polyP (green) and DAPI-DNA (red). Cells were loaded with DAPI and excitated at 405 nm while emission was recorded at 460 nm for DAPI-DNA and at >550 nm for DAPI-polyP. (b) Release of polyP from cells treated with HBSS (Ctrl – lane 3), ionomycin (5 µM – lane 4) and L-polyP (100 µM – lane 5) can further be detected using TBE-Urea gel electrophoresis and DAPI staining. The red box indicates released endogenous polyP as does not occur in non-stimulated cells (Ctrl) and is of a different length than the exogenous polyP used (L-polyP). The media from the treated cells was collected, concentrated and subjected to gel electrophoresis. One set of samples were treated with calf intestinal phosphatase (CIP) to verify signal specificity (lanes 6-8), while the same amount of L-polyP as was added to the cells was included as a control for input (lane 9). Medium (60 – lane 1) and long (130 – lane 2) polyP were used as length standards.



Supplementary Figure S4. Effect of MRS2279 *in vivo*. (a) Application of ATP receptor antagonsit MRS2279 (50 μM) to the ventral surface of the brainstem had no significant effect on the arterial blood pressure (ABP) and renal sympathetic nerve activity (RSNA).

Supplementary Methods

PolyP electrophoresis

To detect polyP released from neuroglia, cultures cells were treated with 5 μM ionomycin or 100 μM long polyP (L-polyP) for 5 min in HBSS, or as a control only HBSS was added to the cells. The samples were concentrated in an Eppendorf concentrator until completely dry, after which they were re-constituted in 10 μl of sample buffer (89mM Tris, 89mM borate, 2mM EDTA [TBE], 2.5% ficoll and 0.004% xylene cyanol FF, pH 8.3). Alternatively, one set of samples were re-constituted in water and treated with 2 μg calf intestinal phosphatase (New England Biolabs, MA, USA) for 1hr at 37°C before six times sample buffer was added. This was to ensure the specificity of the polyP signal. To control for loading, each sample was obtained from the same amount of cells and the entire samples was loaded on the gel. As the L-polyP added to stimulate the cells (100 μM) tended to smear on the gel and distort the signal, the input amount of L-polyP, not added to cells, was included as a control (Supplementary Figure 3b, lane 9). Further, 10 μg medium and long polyP were added as size markers. The samples were run on a 10% TBE-Urea polyacrylamide gel (Invitrogen, Paisley, UK) with a TBE running buffer. The gel was fixed and stained for one hour at room temperature in (1mg/ml p-phenylenediamine, 10mM EDTA, pH 8.0 and 2μg/ml DAPI). They were further destained with DAPI-fixative (without DAPI) for two times one hour before they were visualised with a GelDoc-It Imaging system (UVP, Cambridge, UK).

Immunofluorescence

Cells were fixed with 4% PFA/PBS solution for 10 min at room temperature, then permeabilised using 0.5% Triton X-100/PBS solution for 5 min. Samples were blocked for 30 min in 10% FCS/PBS solution before addition of the primary antibody for 2 hours in blocking solution. Antibodies used were: rabbit anti-P2Y1 receptor (Almone labs, 1:500). The secondary antibody (AlexaFluor 568 secondary antibody from Invitrogen, 1:2000) was added in 10% FCS/PBS for 30 min, then cell nuclei were stained with 1 μM DAPI for 5 min. Samples were imaged using a using a Zeiss 710 inverted confocal microscope equipped with a META detection system and a 63x oil immersion objective.