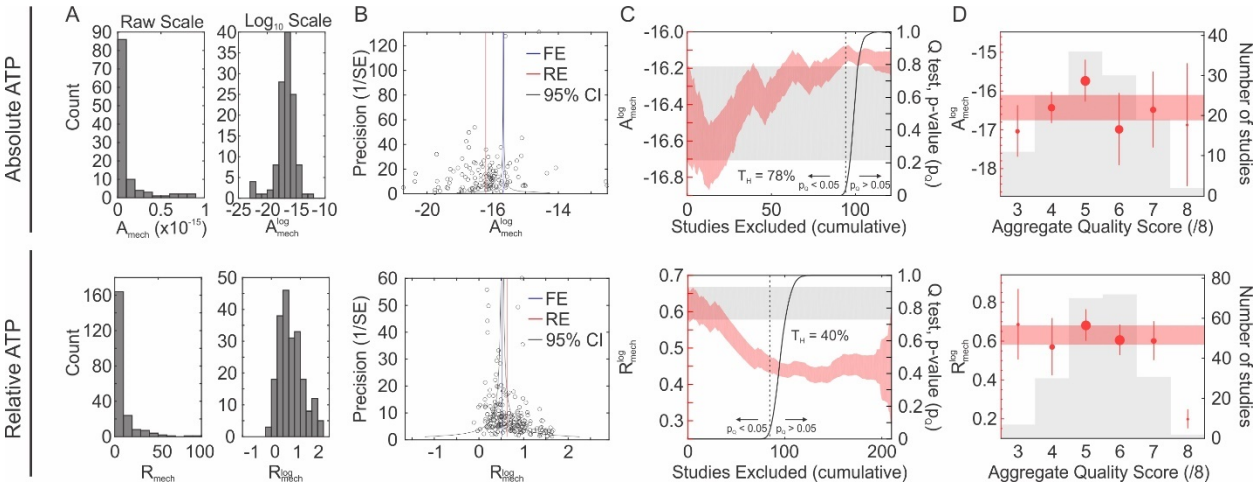

Figure S1. The distribution, bias, extent of heterogeneity and effect of study quality was evaluated for mechanically-stimulated ATP release (A_{mech}, *top row*) and relative ATP release above baseline (R_{mech}, *bottom row*) (A) Study-level outcome distributions on raw scale (*left*) and logarithmic (base 10) scale (*right*). **(B)** Funnel plots for log-transformed study-level effect sizes. *Black markers*: study-level data. *Blue lines*: fixed effect (FE) estimate. *Red lines*: random effects (RE) estimate. *Black lines*: theoretical 95% CI for FE estimate in absence of bias. **(C)** Effect of cumulative study exclusion on RE estimates and heterogeneity of log-transformed effect sizes. *Red band*: 95% CI for studies remaining after exclusion of the most heterogenous. *Grey band*: Overall 95% CI. *Black curve*: p-value p_Q for Q-test. *Dashed black line*: homogeneity threshold T_H. **(D)** Influence of aggregate study quality score on ATP release estimates. *Red band*: 95% CI for overall estimate, *red markers*: score-specific estimate ± 95% CI, *grey bars*: number of studies that received indicated aggregate quality score (also reflected in marker sizes).

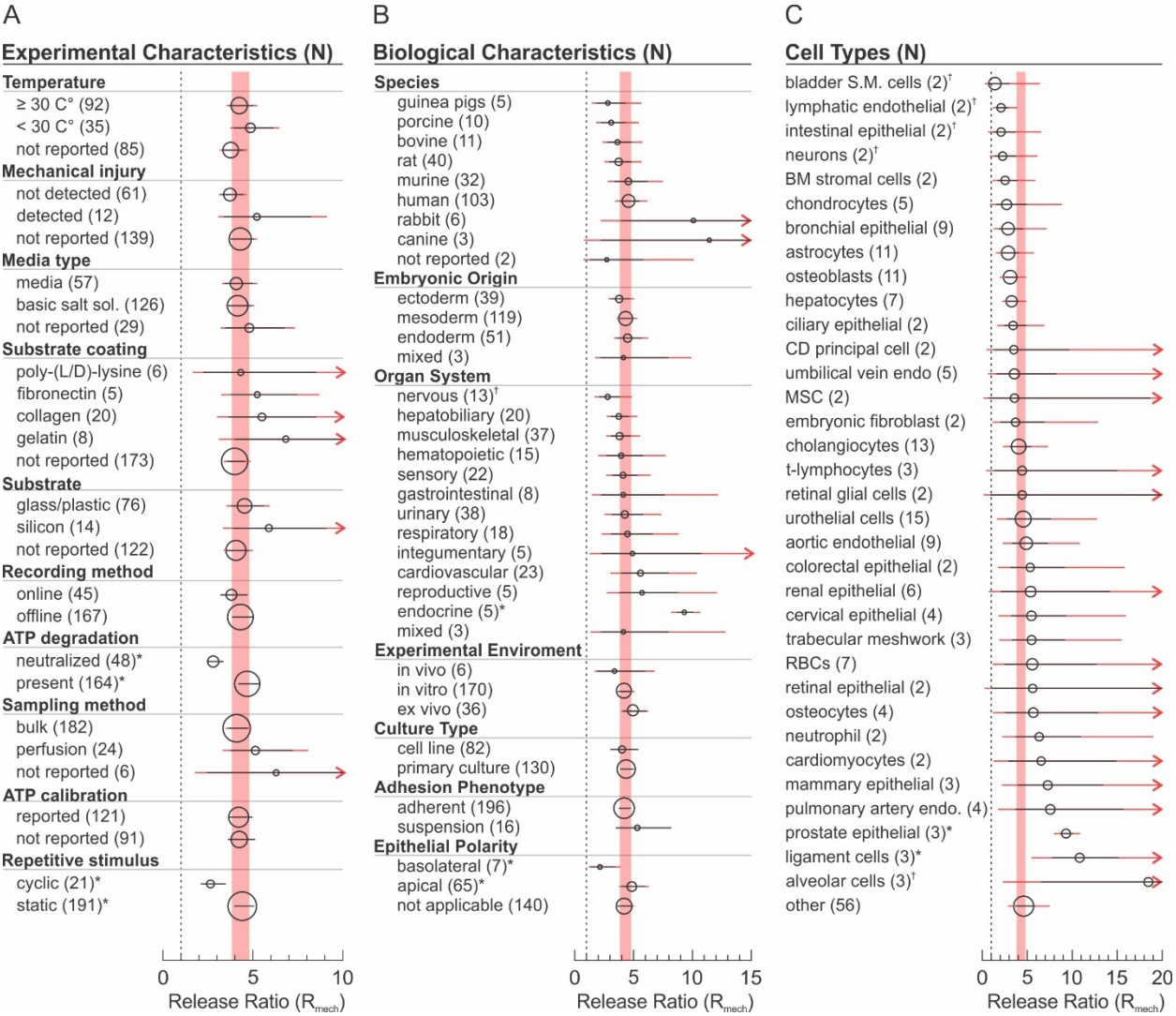

Figure S2. Influence of experimental and biological factors on the amount of ATP released following mechanical stimulation. Shown are estimates of relative ATP release above baseline following mechanical stimulation for different subgroups based on **(A)** experimental characteristics, **(B)** biological characteristics and **(C)** cell-types. *Round markers*: Subgroup-level estimates, markers sizes are proportional to number of datasets N in each subgroup (shown in parentheses), *Horizontal black lines*: ± 95% CI, *Horizontal red lines*: ± Bonferroni-adjusted 95% CI, *Red bands*: overall estimate ± 95% CI. † and * indicate significant differences ($p<0.05$) compared to overall estimate or to other subgroup (in case of two subgroups) before and after Bonferroni adjustment, respectively. Detailed statistics are in **Table S2**.

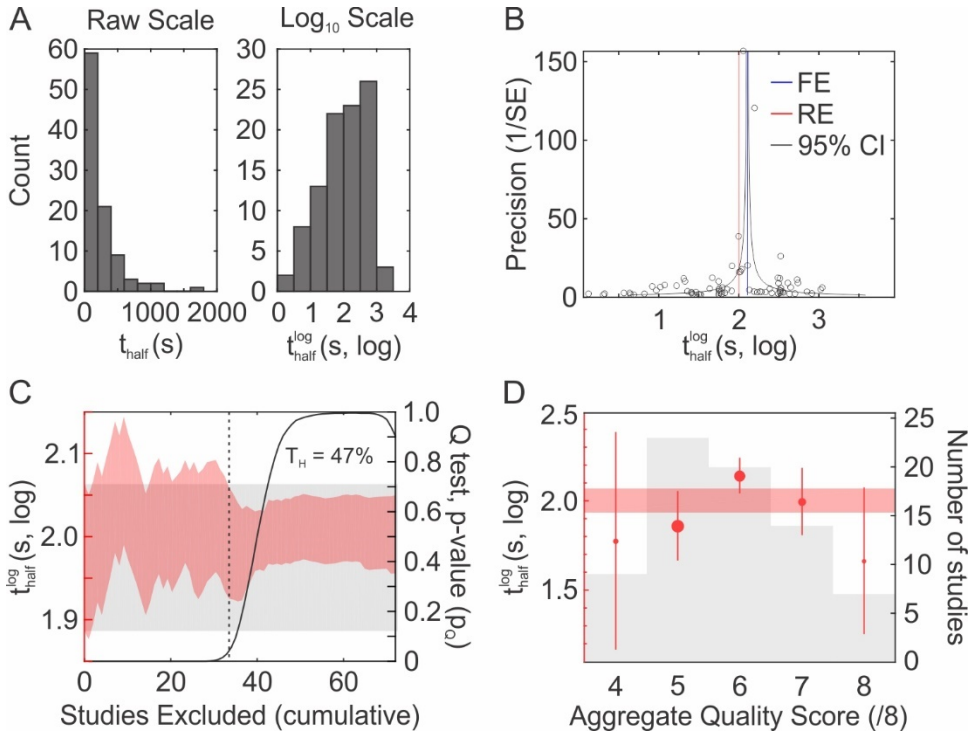

Figure S3. The distribution, bias, extent of heterogeneity and effect of study quality was evaluated for kinetic estimates of ATP release (t_{half}) (A) Study-level effect size distributions on raw scale (*left*) and logarithmic (base 10) scale (*right*). **(B)** Funnel plots for log-transformed study-level estimates. *Black markers*: study-level data. *Blue lines*: fixed effect (FE) estimate. *Red lines*: random effects (RE) estimate. *Black lines*: theoretical 95% CI for FE estimate in absence of bias. **(C)** Effect of cumulative study exclusion on RE estimates and heterogeneity of log-transformed effect sizes. *Red band*: 95% CI for studies remaining after exclusion of most heterogenous. *Grey band*: Overall 95% CI. *Black curve*: p-value p_Q for Q-test. *Dashed black line*: homogeneity threshold T_H. **(D)** Influence of aggregate study quality score on ATP release kinetic estimates. *Red band*: 95% CI for overall estimate, *red markers*: score-specific estimate ± 95% CI, *grey bars*: number of studies that received indicated aggregate quality score (also reflected in marker sizes).

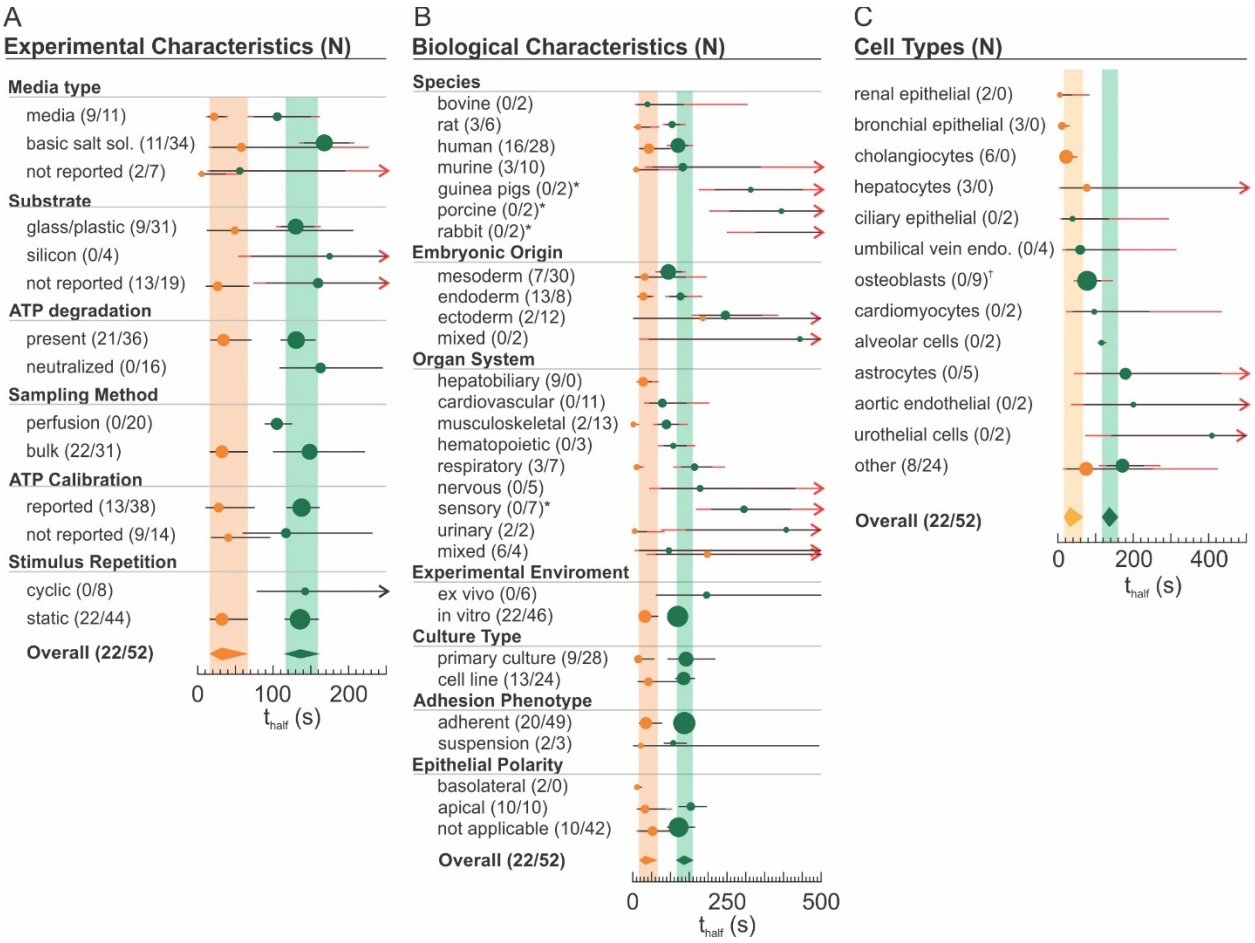

Figure S4. Influence of experimental and biological factors on kinetics of mechanically-stimulated ATP release. (A-E) ATP release kinetic estimates were stratified by online (*orange*) and offline (*green*) recording methods and subgroup analysis was conducted to evaluated influence of experimental **(A)** and biological **(B)** characteristics as well as differences between cell-types **(C)**. *Round markers*: Subgroup-level estimates, *Horizontal black lines*: ± 95% CI, *Horizontal red lines*: ± Bonferroni-adjusted 95% CI, *Bands/diamonds*: overall estimate ± 95% CI. Markers are proportional to number of studies N in each subgroup (shown in parentheses). † and * indicate significant differences (at least 5% level) compared to overall estimate before and after Bonferroni adjustment, respectively. Detailed statistics are in **Table S4**.

Table S1. Absolute estimates of ATP released from mechanically-stimulated mammalian cells (A_{mech}), intracellular ATP (A_{cell}), and basal extracellular ATP (A_{base}). Shown are meta-analytic outcomes and corresponding heterogeneity statistics I^2, H^2 and Q. CI: Confidence intervals, N: Number of datasets, P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect, Nucleated: Nucleated mammalian cells, RBC: Red blood cells.

	Meta-Analysis Summary Statistics					
	ATP released (± 95% CI), units	N	I^2 (%)	H^2	Q	P_Q
A_{mech}	38.5 (18.2, 81.8) amol cell^{-1}	123	99.9	1695.4	206843.1	<0.001
A_{cell}						
Nucleated	5.0 (2.6, 9.5) fmol cell^{-1}	4	89.2	9.2	27.7	<0.001
RBC	0.14 (0.12, 0.18) fmol cell^{-1}	4	0	0.5	1.6	0.66
A_{base}	8.1 (3.9, 16.6) amol cell^{-1}	84	99.8	657.9	54601.8	<0.001

Table S2. Subgroup analysis of the effects of experimental and biological factors on amount of ATP released following mechanical stimulation. Relative ATP release data were stratified by experimental or biological characteristics, or cell type, and amount of ATP released and heterogeneity was compared between subgroups. R_{mech}: relative ATP release compared to baseline, CI: confidence intervals, P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect, N: number of datasets per group.

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Table S3. Relationship between magnitude of mechanical stimulus and amount of ATP release evaluated by subgroup and meta-regression analyses. *For subgroup analysis*, Relative ATP release data were stratified by type of mechanical stimulus and amount of ATP released and heterogeneity was compared between subgroups. R_{mech}: relative ATP release compared to baseline. P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect. *For meta-regression*, strength of relationship (regression slope, β) was investigated between the magnitude of mechanical stimulus and the amount of relative ATP released on logarithmic scale. Magnitude of stimuli were % stretch for strain, cmH_2O for compression, absolute change in mOsm/L for hypotonic and hypertonic pressures, $dyne/cm^2$ for fluid shear stress (FSS) and μm^{-1} for RBC deformation. Regression slopes were compared between relationships observed within-studies (β_{intra}) and between-studies (β_{inter}). SE(β): standard error of β, P_β: Z-test derived p-value for comparison of β_{inter} and β_{intra}. N: Number of datasets per group.

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Table S4. Subgroup analysis of the effects of experimental and biological factors on ATP release kinetics. ATP release kinetics data were stratified by experimental or biological characteristics, cell type, or mechanical stimulus, and kinetics of ATP release and heterogeneity were compared between subgroups. t_{half}: Time to half max release, CI: confidence intervals, P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect, N: number of datasets per group.

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Table S5. Pharmacological Interventions used to study mechanically-stimulated ATP release. *Bolded interventions* are pharmacological agents that do not overlap with other known mechanisms of MSAR. Uncommon or unverified pharmacological interventions have been omitted. Drug pharmacology was obtained from literature identified in the current study.

Target	Pharmacological interventions
Release mechanisms	
Vesicular	**NEM**, **bafilomycin**, **monensin, brefeldin A**
Pannexins	carbenoxolone, 18α/β-glycyrrhetinic acid, probenecid, **10panx1**, NPPB, SITS, **DTT**, mefloquine
Connexins	carbenoxolone, 18 α/β -glycyrrhetinic acid, **octanol**, **heptanol, flufenamic acid** (little activity at Panx1)**,** arachidonic acid, mefloquine, **GAP26**, **GAP27**
VRAC	**tamoxifen**, **fluoxetine**, glybenclamide, **phloretin,** NPPB, SITS, **verapamil**
Maxi-anion	Gd^{3+}, NPPB, SITS, arachidonic acid
Auxiliary mechanisms	
ANK	probenecid
ATP synthase	**angiostatin, piceatannol**
CFTR	glybenclamide, **CFTR-172, Rp-cAMPS, niflumic acid**
ENaC	**amiloride**
L-type VSCC	**nifedipine**
P2X7	**brilliant blue G**, Gd^{3+}, **KN62, A10606120, A438079, A74003**
Piezo1	ruthenium red, Gd^{3+}, **GsMTx4**
TRPV4	**HC067047**, ruthenium red, Gd^{3+}
Regulatory mechanisms	
Intracellular Calcium	**BAPTA-AM, EGTA-AM, thapsigargin**
Extracellular Calcium	Calcium-free (Calcium omitted in solution, optionally chelated)
COX	**etodolac, indomethacin, NS398, ETYA**
PKC	**calphostin C, chelerythrine, myristoylated PKC ζ pseudosubstrate, GF 109203X, Gö6976, Gö6983**
P38 mitogen-activated protein	**SB203590**
Rho kinase	**Y27632, GSK269962, H1152**
MLC kinase	**ML-7**
Tyrosine kinase	**herbimycin A, tyrphostin 46**
PI3K	**wortmannin, LY294002**
f-actin	**cytochalasin B, cytochalasin D**
microtubules	**nocodazole**
cholesterol	**MβCD**
cilia	**chloral hydrate**

Table S6. Mechanisms of mechanically-stimulated ATP release. The effects of pharmacological and genetic interventions on MSAR for studied cell types were calculated as an inhibitory effect (%) ± 95% confidence intervals (CI) compared to vehicle control, according to random effects meta-analysis model. Positive effects (>0%) indicate that MSAR was inhibited and negative effects (<0%) indicate that MSAR was potentiated. Interventions for which 0% was not included in the 95% CI had a significant effect on MSAR. Involvement of studied mechanism in MSAR is indicated by green box (involved) or red box (not involved), and quality of evidence is indicated by dark green/red (finding replicated by separate study/method) or light green/red (not-replicated). Orange boxes: Interventions with inconsistent effects, reasoning for each case is provided in table. P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect, N: number of datasets. *indicates the interventions that were activators or agonists of MSAR, and therefore were not pooled with inhibitory interventions for calculation of overall inhibition.

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Table S7. ATP release in pathologies. Relative effect (%) of pathology on ATP release compared to unaffected controls. $D_{\downarrow\uparrow}$: specifies direction of effect, CI: 95% confidence intervals, N: number of datasets per condition, I^2 and H^2: heterogeneity statistics, P_Q: p-value corresponding to Q heterogeneity statistic used to evaluate null hypothesis that all studies reported same effect, ADPKD: autosomal dominant polycystic kidney disease, ARPKD: autosomal recessive polycystic kidney disease, CF: cystic fibrosis, Epi.: epithelia, Glaucoma: primary acute angle closure glaucoma, FSS: fluid shear stress, RBC: red blood cells.

Covariates	Meta-Analysis Summary Statistics					
	$D_{\downarrow\uparrow}$	Rel. Effect, % (±95% CI)	N	I^2 (%)	H^2	P_Q
Pathology						
Cystic fibrosis	↓	-66.6 (-78.6, -54.5)	14	87.9		<0.001
RBC, pancreatic epi.	↓	-87.7 (-91.5, -83.8)	10	7.9	1.1	0.37
Airway epi., astrocyte	-	18.1 (-8.5, 44.7)	4	29.1	1.4	0.24
Colitis*	↑	248.1 (172.4, 323.8)	9	19.4	1.2	0.27
Diabetes, type II	↓	-49.6 (-75.0, -24.1)	1	-	-	-
Glaucoma	↑	1107.8 (539.0, 1676.6)	2	62.5	2.7	0.1
Hypoxia	-	20.3 (-55.9, 96.5)	8	97.4	37.8	<0.001
Acute hypoxia*	↑	60.9 (46.4, 75.4)	7	0	0.8	0.53
Chronic hypoxia	↓	-91.8 (-103.4, -80.2)	1	-	-	-
Ectopic ossification	-	4.5 (-38.4, 47.5)	1	-	-	-
Interstitial cystitis	↑	107.7 (53.3, 162.0)	7	0		0.99
Polycystic kidney disease	-	7.8 (-43.1, 58.8)	7	92.5	13.3	<0.001
FSS – AD/ARPKD	↓	-72.9 (-98.9, -46.9)	2	0	<0.001	1
Hypotonic – ADPKD	↑	92.9 (15.0, 170.7)	3	75.5		0.13
Hypotonic – ARPKD	↓	-25.2 (-147.8, 97.3)	2	88.2		<0.01
Pulmonary hypertension *	↓	-56.6 (-73.0, -40.2)	3	0	0.8	0.43
Spinal cord injury	↑	399.2 (-40.3, 838.6)	1	-	-	-
Xerocytosis, hereditary	↓	-61.9 (-71.9, -51.9)	1	-	-	-

*Pooled datasets are from same study

Table S8. Search Strategy

Click here to Download Table S8

Table S9. Systematically identified studies and their contributions to meta-analysis.

Click here to Download Table S9

Table S10. List of study-level characteristics extracted for each study and used in subgroup analyses

Click here to Download Table S10

Table S11: Experimental parameter assumptions for ATP unit conversion. *Top table* describes how unavailable (output) parameters were calculated based on available (input) parameters and assumed parameter values (assumption). Assumed cell-related parameters are shown in *middle table*, and culture dish-dependent parameters shown in the *bottom table*.

Input parameter(s)	Assumed parameter	Calculation	Output parameter
Cytocrit/hematocrit (CC, %), total volume (TV)	Cell volume (CV)	N = (CC x TV) / CV	Cell number (N)
Protein mass (PM)	Protein / cell (PC)	N = PM / PC	Cell number (N)
DNA mass (DM)	DNA / cell (DC)	N = DM / DC	Cell number (N)
Confluence (C, %), surface area (SA)	Confluent Cell Density (D)	N = SA x C x D	Cell number (N)
Culture Plate	Volume (V)	V	Volume (V)

Assumed parameters	Assumed values
Protein / cell (PC)	320 (pg/cell)
Mammalian cell volume (CV)	2.41 (pL)
RBC volume (CV)	0.10 (pL)
Platelet volume (CV)	0.10 (pL)
Cellular density at confluence (D)	10^5 cells / cm^2

Culture Dish	Surface area (SA, cm^2)	Assumed parameters Volume (V, mL)
10 cm	55	10
6 well (35mm)	9.6	2
48 well	1	1
96 well	0.3	0.2