

# Supplement Figure 1

Human	MLSRKGIPEEYVLTRLAEDPAKPRYRARQRRARFVSKKGN CNVAHKNIREQGRFLQDVF	60
Mouse	MLSRKGIPEEYVLTRLAEDPAEPRYRTRERRARFVSKKGN CNVAHKNIREQGRFLQDVF	60
Dog	MLSRKGIPEEYVLTRLAEDPAEPRYRARERRARFVSKN GN CNVAHKNIREQGRFLQDVF	60
Cat	MLSRKGIPEEYVLTRLAEDPAEPRYRARERRARFVSKN GN CNVAHKNIREQGRFLQDVF	60
Horse	MLSRKGIPEEYVLTRLAEDPAEPRYRARERRARFVSKN GN CNVAHKNIREQGRFLQDVF	60
Pig	MLSRKGIPEEYVLTRLAEDPTEPRYRARERRARFVSKN GN CNVAHKNIREQGRFLQDVF	60
Cattle	MLSRKGIPEEYVLTRLAEDPAEPRYRARERKARFVSKN GN CNVAHKNIREQGRFLQDVF	60
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Human	TTLVDLKWPHTLLIFTMSFLCSWLLFAMAWWLI AFAHGD LAPSEGTAEPCVTSIHSFSSA	120
Mouse	TTLVDLKWPHTLLIFTMSFLCSWLLFAMVWVLI AFAHGD LAPGEGTNVPCVTSIHSFSSA	120
Dog	TTLVDLKWPHTLLIFTMSFLCSWLLFAMVWVLI AFAHGD LAPGEGTAVPCVTSIHSFSSA	120
Cat	TTLVDLKWPHTLLIFTMSFLCSWLLFAMVWVLI AFAHGD LAPGEGAAPCVTSIHSFSSA	120
Horse	TTLVDLKWPHTLLIFTMSFLCSWLLFAMAWWLI AFAHGD LAPDEGSAVPCVTSIHSFSSA	120
Pig	TTLVDLKWPYTLTLLIFTMSFLCSWLLFAMVWVLI AFAHGD LAPGEGASVPCVTSIHSFSSA	120
Cattle	TTLVDLKWPYTLTLLIFTMSFLCSWLLFAMVWVLI AFAHGD LAPGEGAAPCVTSIHSFSSA	120
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Human	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Mouse	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Dog	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Cat	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Horse	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Pig	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
Cattle	FLFSIEVQVTIGFGRMVTEECPLAILLILIVQNI VGLMINAIMLGCIFMKTAQHRAET	180
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Human	LIFSKHAVIALRHGR LCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
Mouse	LIFSKHAVITLRHGRLCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
Dog	LIFSKHAVIAVRHGRLCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
Cat	LIFSKHAVIAVRHGRLCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
Horse	LIFSKHAVIALRHSRLCFMLRVGDLRKSMIISATI RMQVVRK TTSPEGEVVPLHQVEIPM	240
Pig	LIFSKHAVIAVRHGRLCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
Cattle	LIFSKHAVIALRHGR LCFMLRVGDLRKSMIISATI HMQVVRK TTSPEGEVVPLHQVDIPM	240
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Human	ENGVGGNSIFLVAPLIIYHVIDANSPLYDLAPSDL HHHQDLEIIVILEGVVETTGITTTQA	300
Mouse	ENGVGGNGIFLVAPLIIYHVIDSNSPLYDLAPSDL HHHQDLEIIVILEGVVETTGITTTQA	300
Dog	ENGVGGNSIFLVAPLIIYHVIDANSPLYDLAPSDL HHHQDLEIIVILEGVVETTGITTTQA	300
Cat	ENGVGGNSIFLVAPLIIYHVIDANSPLYDLAPSDL HHHQDLEIIVILEGVVETTGITTTQA	300
Horse	ENGVGGNSIFLVAPLIIYHAIDANSPLYDLAPSDL HHHQDLEIIVILEGVVETTGITTTQA	300
Pig	ENGVGGNIFLVAPLIIYHVIDAKSPLYDLAPCDL HHHQDLEIIVILEGVVETTGITTTQA	300
Cattle	ENGVGGNSIFLVAPLIIYHVIDANSPLYDLAPCDL HHHQDLEIIVILEGVVETTGITTTQA	300
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Human	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLDEDHSLLEALT	360
Mouse	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLDEDRLLDALT	360
Dog	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLDEDRLLDALT	360
Cat	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLDEDRTLLDAVT	360
Horse	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLDEDHSLLDALT	360
Pig	RTSYLADEILWGQRFVPIVAEEDGRYTVDYSKFGNT IKVPTPLCSARQLDEDPSLLDVL T	360
Cattle	RTSYLADEILWGQRFVPIVAEEDGRYSVDYSKFGNT IKVPTPLCTARQLEEDPSLLDVL T	360
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Human	LASARGPLRKRSVMAKAKPKFSISPDLS	390
Mouse	LASSRGPLRKRSVAVAKAKPKFSISPDLS	390
Dog	LASARGPLRKRSVAVAKAKPKFSISPDLS	390
Cat	LASARGPLRKRSVAVAKAKPKFSISPDLS	390
Horse	LASARGPLRKRSVAVAKAKPKFSISPDLS	390
Pig	L--TRGPLRKRSMAVTKAKPKFSISPESLS	388
Cattle	L--VRGPLRKRTVAVAKAKPKFSISPDLS	388
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**Multi-species protein sequence alignment of the Kir6.2 protein, encoded by the *KCNJ11* gene.** Full multi-species sequence alignment for Kir6.2, for the same species shown in figure 1. Prepared using “The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024”.



## Supplementary tables

**Table 1.** Mean  $\pm$  SEM from Hill equation fits to individual ATP dose-responses for data in figures 1,3.

ATP.K <sub>2</sub>	hK <sub>ATP</sub> (n=13)	cK <sub>ATP</sub> (n=8)	hKir6.2 +cSUR1 (n=6)	cKir6.2 +hSUR1 (n=10)	hK <sub>ATP</sub> /K39N (n=7)	cK <sub>ATP</sub> /N39K (n=5)
IC <sub>50</sub> ( $\mu$ mol/L)	9.7 $\pm$ 0.6	31 $\pm$ 3	14 $\pm$ 1	23 $\pm$ 3	22 $\pm$ 2	19 $\pm$ 1
h	1.44 $\pm$ 0.05	1.19 $\pm$ 0.04	1.51 $\pm$ 0.12	1.29 $\pm$ 0.05	1.30 $\pm$ 0.03	1.43 $\pm$ 0.05

**Table 2.** Mean  $\pm$  SEM from Hill equation fits to individual MgATP dose-responses and residual currents at 1mmol/L and 3mmol/L MgATP for data in figures 1,3. Data for 3 mmol/L are extrapolated.

MgATP	hK <sub>ATP</sub> (n=9)	cK <sub>ATP</sub> (n=8)
IC <sub>50</sub> ( $\mu$ mol/L)	13 $\pm$ 1	43 $\pm$ 2
h	1.12 $\pm$ 0.05	0.81 $\pm$ 0.05
I/I <sub>c</sub> (%) (1mmol/L)	3.2 $\pm$ 0.4	12.0 $\pm$ 0.7
I/I <sub>c</sub> (%) (3mmol/L)	0.36 $\pm$ 0.07	5.8 $\pm$ 0.7

**Table 3.** Mean  $\pm$  SEM of the current fold change for MgADP activation shown in figure 2.

I/I <sub>c</sub>	hK <sub>ATP</sub> (n=5)	cK <sub>ATP</sub> (n=6)
MgADP (100 $\mu$ mol/L)	13 $\pm$ 2	11 $\pm$ 1
MgADP/ATP (100 $\mu$ mol/L each)	3.3 $\pm$ 0.3	2.8 $\pm$ 0.3