

Supplementary information

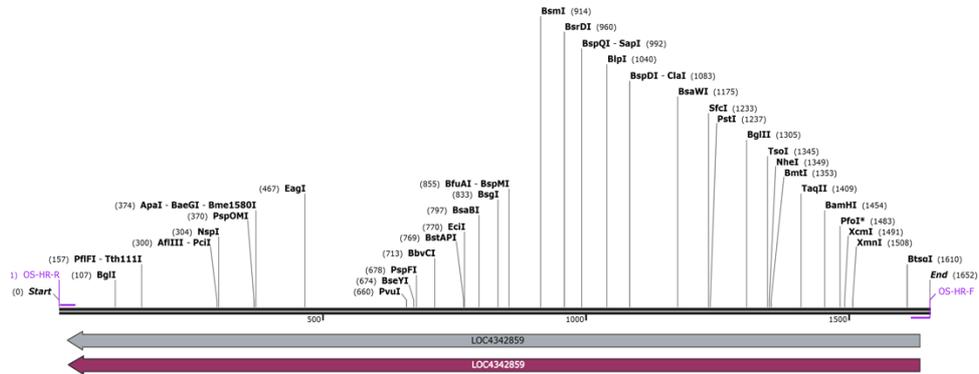


Fig. S1 The map of *OsNramp5*. The original sequence comes from *Oryza sativa Japonica Group* (Japanese rice, Gene ID:4342859; Gene symbol:LOC4342859). *OsNramp5* sequence was got from Chen lab, and constructed in shuttle plasmid pFL61.

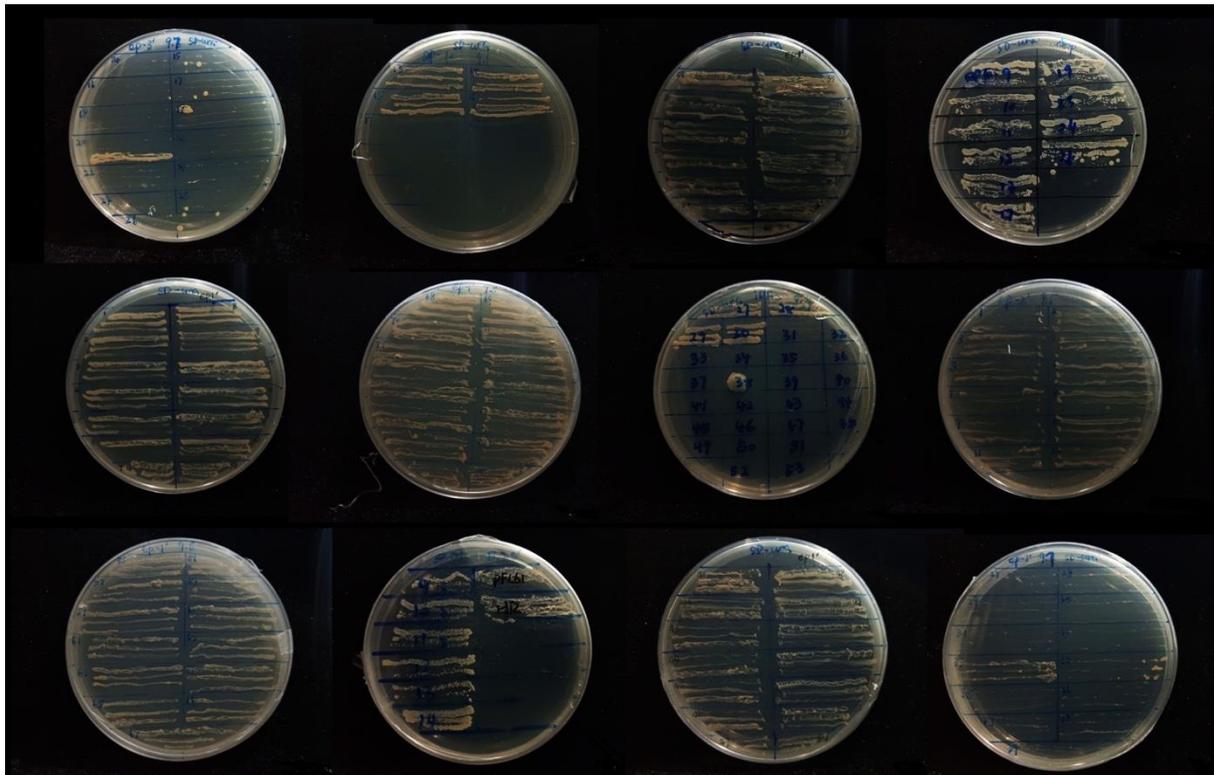


Fig.S2 Part of our library plates. Approximately 12 positive transformants were preserved on each plate, for a total of around 260. We redlined them for subsequent screening.

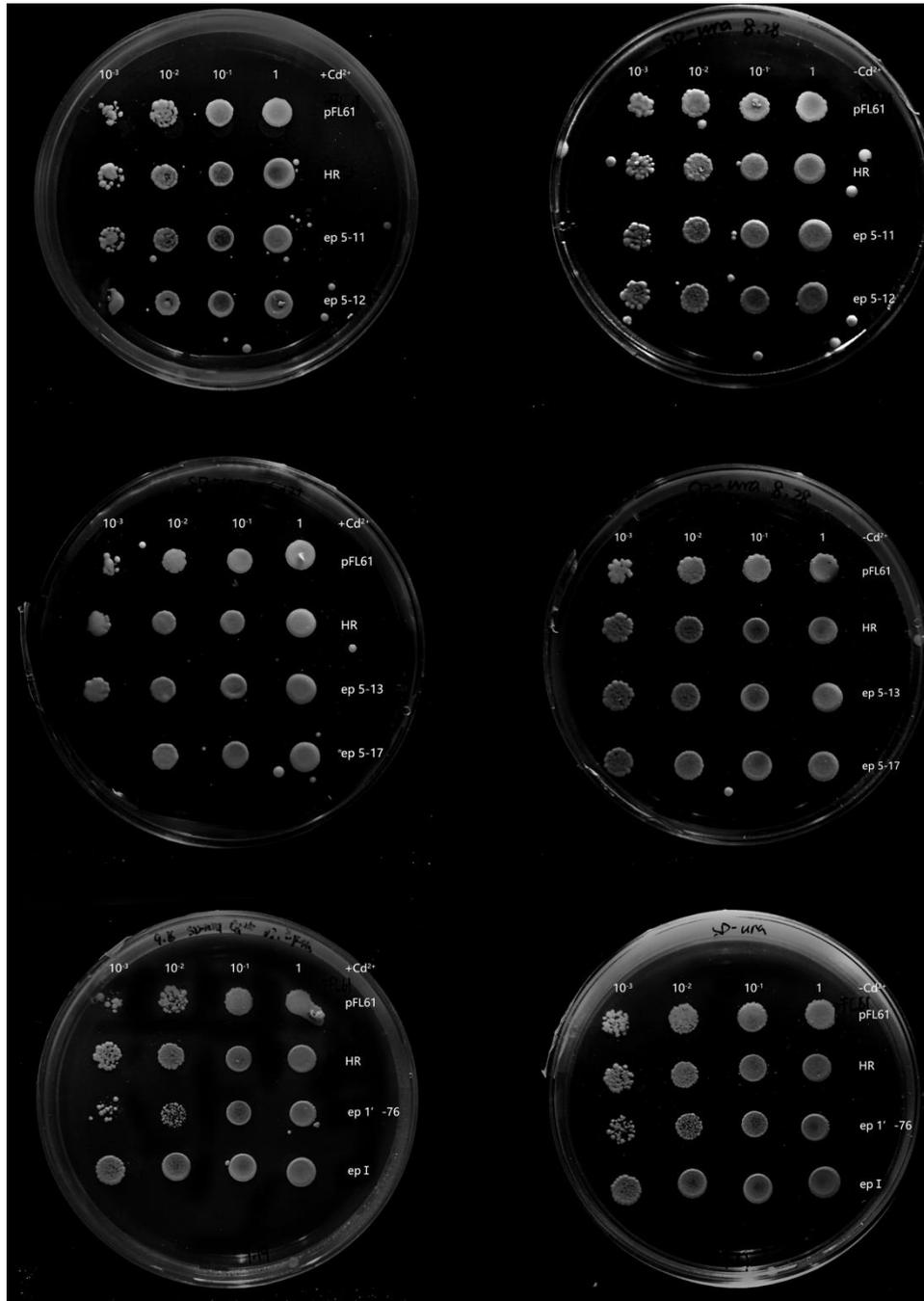


Fig. S3 Some typical potential benign mutant strains under Cd²⁺ stress. The growth of some mutant strains on SD-ura plates containing Cd²⁺ concentration of 30 μM was slightly better than that of *pFL61* and *HR* while they grew similarly to *pFL61* and *HR* on plates without Cd²⁺, indicating that they may be the potential *OsNramp5-mut* strains whose ability of transporting Cd²⁺ has successfully decreased or their *OsNramp5* has been damaged.

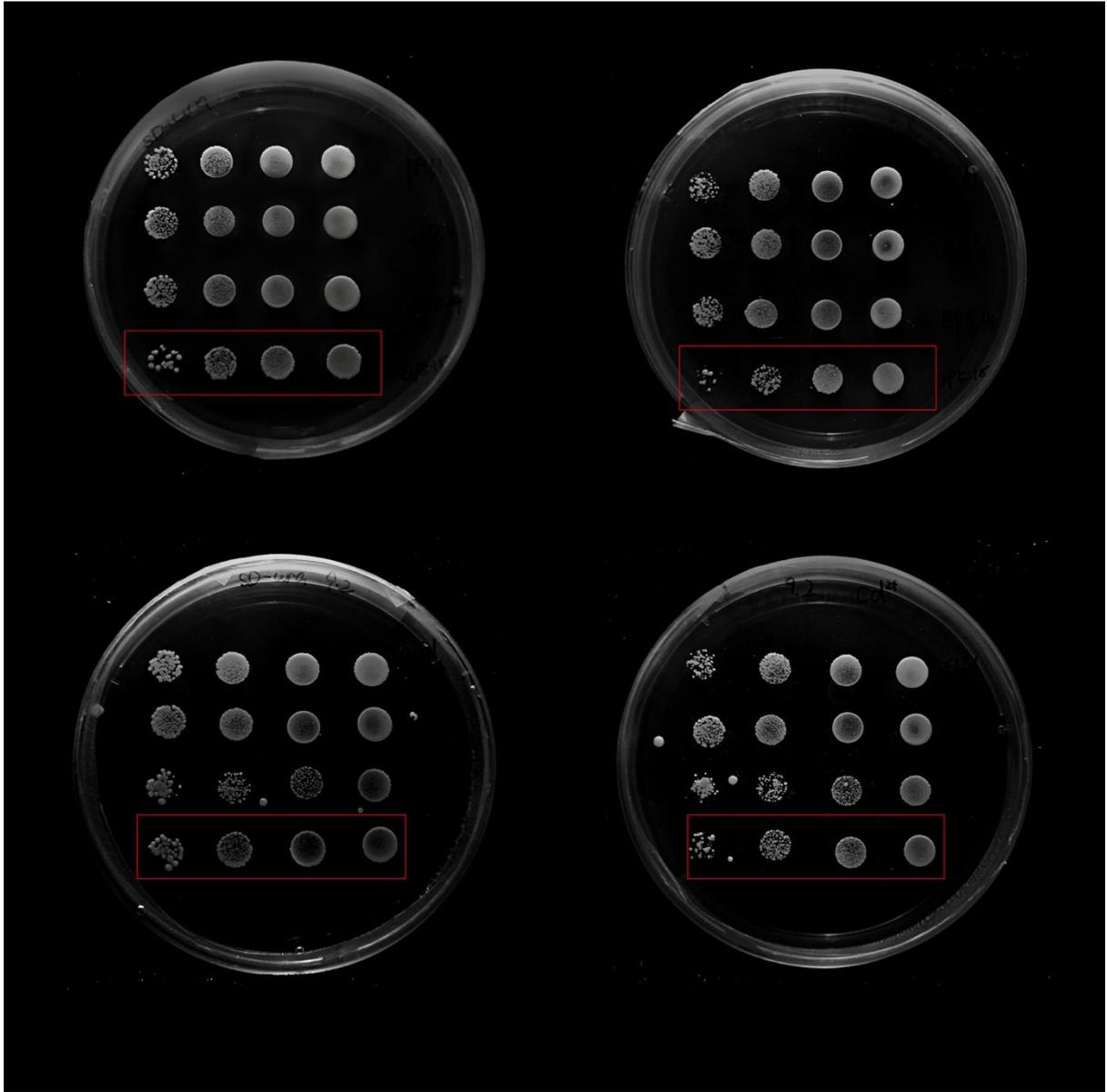


Fig. S4 *OsNramp5-mut* with enhanced ability of transporting Cd²⁺.

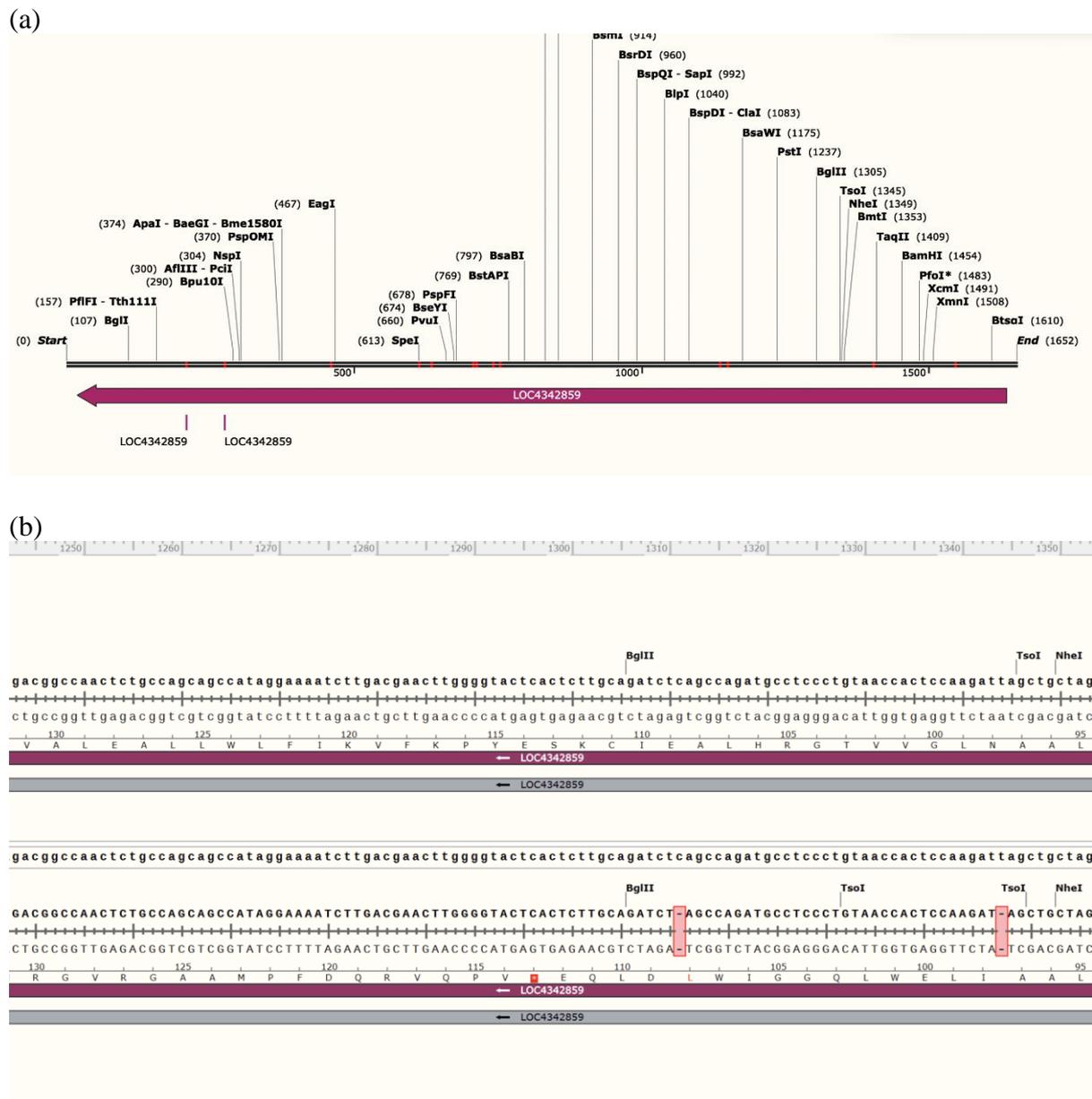


Fig. S5 (a) Sequencing analysis of *OsNramp5* ep-11. (b) Sequencing analysis of *OsNramp5* ep-17. The mutation sites of this gene were labeled red in the map. The mutants with poorer phenotypes than HR are probably caused by changes in crucial loci to Cd²⁺ transportation, so that the intracellular Cd²⁺ concentration increased and their growth was inhibited.

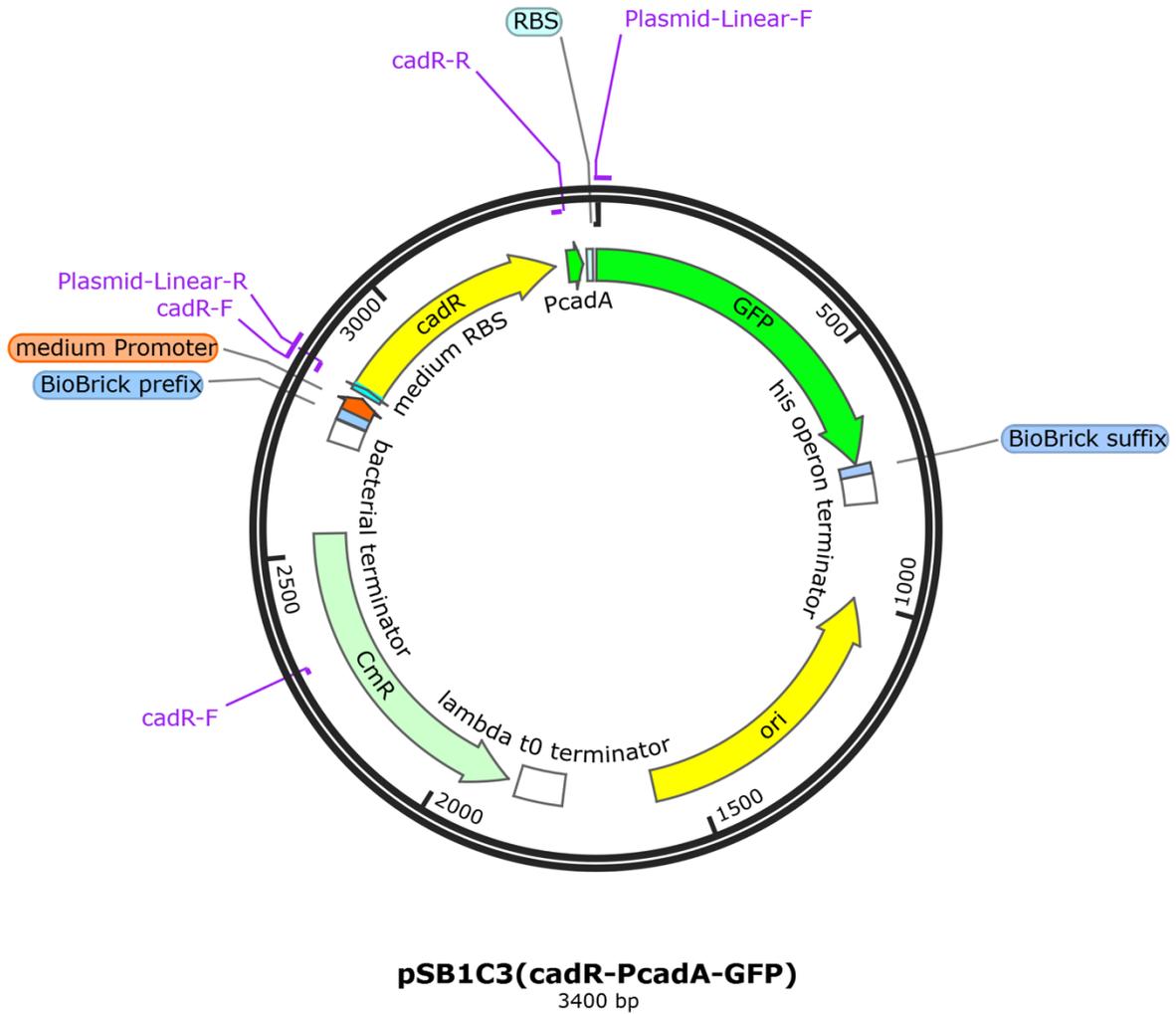


Fig.S6 The recombinant plasmid of Cd²⁺ responsive biosensor. The recombinant plasmid concludes CadR, PcadA and GFP(BBa_K608011) coding sequences. These are present in plasmid pSB1C3, which harbours a chloramphenicol resistant gene for the selection of our transformants.

Table.S1 The primers which were used in this study. “HRs” were used to constructed the plasmid pFL61-*OsNramp5*^{mut}. The “Linears” were used to constructed linearized plasmid pFL61 and sequencing. The “CadRs” and “Pcadas” were used to construct Cd²⁺ responsive biosensor.

Name	Sequence 5'-3'
OS-HR-F	CAGGAAACAGCTATGACatggagattgagagagagag
OS-HR-R	GCTTGGCGTAATCATGTTctacctgggagc
pFL61-HR-F	GTCATAGCTGTTTCCTGTG
pFL61-HR-R	CATGATTACGCCAAGC
CadR-F	ctagagtcacacaggaagatgaagatcggtgagctgg
CadR-R	acacctccagtcactgag
Plasmid-Linear-F	gatgcgtaaaggagaagaac
Plasmid-Linear-R	ctttcctgtgtgactctag
Pcada-sense	ctcagtgactggaggtgttgactctgtagttgctacaggggtgcaattactagagaaagaggaga aatactaggatgcgtaaaggagaagaac
Pcada-antisense	gttcttctccttacgcatcctagatatttctcctcttctctagtaattgcacacctgtagcaactacaga gtcaaacacctccagtcactgag

Table S2. The TMHMM source data.

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# OsNramp5 Length: 538
# OsNramp5 Number of predicted TMHs: 12
# OsNramp5 Exp number of AAs in TMHs: 259.77649
# OsNramp5 Exp number, first 60 AAs: 12.79481
# OsNramp5 Total prob of N-in: 0.76362
# OsNramp5 POSSIBLE N-term signal sequence
OsNramp5      TMHMM2.0      inside      1      43
OsNramp5      TMHMM2.0      TMhelix    44      59
OsNramp5      TMHMM2.0      outside    60      78
OsNramp5      TMHMM2.0      TMhelix    79     101
OsNramp5      TMHMM2.0      inside    102     121
OsNramp5      TMHMM2.0      TMhelix   122     144
OsNramp5      TMHMM2.0      outside   145     147
OsNramp5      TMHMM2.0      TMhelix   148     170
OsNramp5      TMHMM2.0      inside   171     178
OsNramp5      TMHMM2.0      TMhelix   179     201
OsNramp5      TMHMM2.0      outside   202     220
OsNramp5      TMHMM2.0      TMhelix   221     243
OsNramp5      TMHMM2.0      inside   244     263
OsNramp5      TMHMM2.0      TMhelix   264     286
OsNramp5      TMHMM2.0      outside   287     323
OsNramp5      TMHMM2.0      TMhelix   324     346
OsNramp5      TMHMM2.0      inside   347     365
OsNramp5      TMHMM2.0      TMhelix   366     388
OsNramp5      TMHMM2.0      outside   389     391
OsNramp5      TMHMM2.0      TMhelix   392     414
OsNramp5      TMHMM2.0      inside   415     426
OsNramp5      TMHMM2.0      TMhelix   427     449
OsNramp5      TMHMM2.0      outside   450     463
OsNramp5      TMHMM2.0      TMhelix   464     486
OsNramp5      TMHMM2.0      inside   487     538
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Table S3. The “DPNG” conserved domain of *Nramp* family. The highly conserved "DPNG" region, located at the C-terminus of the first transmembrane region, is consistent with the calibration of 12 cadmium transmembrane residues in *Nramp* family homologue by Gros et al.

DdNR2	LWAFTGPGFLMSIAYLDPGNIESDIQAGAM
Pb1NR2	LMLYSGPGWLMAIAYLDPGNLES DLQSGAV
MbreNR	LWAFSGPSLLVSLAYLDPGNIESDLQAGVA
RenNR	LWAFSGPGFLMSIAYLDPGNIESDLQSGVT
NvecNR	LWAFTGPGFLMSIAYLDPGNIESDLRQGAV
DmeNR	LWAFTGPGFLMSIAYLDPGNIESDMQSGAA
SmNRa	LWTFTGPGFLMSIAYLDPGNIESDLQSGAI
MyesNR	LWAFTGPGFLMSIAYLDPGNVESDLRAGAS
SuperNRa	LWAFTGPGFLMSIAYLDPGNIESDLQSGFI
CinNR	LWAFTGPGFLMSIAYLDPGNIESDLQSGFI
DeeNR	LWAFTGPGFLMSIAYLDPGNIESDIQSGAI
PmaNR	LWAFTGPGFLMSIAYLDPGNIESDLQSGAK
XtrNR2	LWAFTGPGFLMSIAYLDPGNIESDIQSGAK
GgNR2	LWAFTGPGFLMSIAYLDPGNIESDIQSGAV
MmNR2	LWAFTGPGFLMSIAYLDPGNIESDLQSGAV
XlaeNR1	LWAFTGPGFLMSIAYLDPGNIESDLQCGAI
GgNR1	LWAFTGPGFLMSIAYLDPGNVESDLQCGAV
MmNR1	LWAFTGPGFLMSIAFLDPGNIESDLQAGAV

Table S4. The Mutant Library of *OsNramp5*.

Number	Time of Culturing	Time of Spotting
ep4-1	8.27 a.m.	8.28 p.m.
ep4-2	8.27 a.m.	8.28 p.m.
ep5-1	8.24 p.m.	8.26 p.m.
ep5-2	8.24 p.m.	8.26 p.m.
ep5-3	8.24 p.m.	8.26 p.m.
ep5-4	8.24 p.m.	8.26 p.m.
ep5-5	8.24 p.m.	8.26 p.m.
ep5-6	8.24 p.m.	8.26 p.m.
ep5-7	8.24 p.m.	8.26 p.m.
ep5-8	8.24 p.m.	8.26 p.m.
ep5-9	8.27 p.m.	8.29 p.m.
ep5-10	8.27 p.m.	8.29 p.m.
ep5-11	8.27 p.m.	8.29 p.m.
ep5-12	8.27 p.m.	8.29 p.m.
ep5-13	8.27 p.m.	8.29 p.m.
ep5-14	8.27 p.m.	9.2 p.m.
ep5-15	8.26 a.m.	8.28 p.m.
ep5-16	8.26 a.m.	8.28 p.m.
ep5-17	8.26 a.m.	8.29 p.m.
ep5-18	8.26 a.m.	9.2 p.m.
ep5-19	8.26 a.m.	8.29 p.m.
ep5-20	8.26 a.m.	8.28 p.m.
ep5-21	8.26 a.m.	8.28 p.m.
ep5-22	8.26 a.m.	8.28 p.m.
ep5-23	8.26 a.m.	8.29 p.m.
ep5-24	8.26 a.m.	8.29 p.m.
ep5-25	8.26 a.m.	8.29 p.m.
ep5-26	8.26 a.m.	8.29 p.m.
ep5-27	8.30 p.m.	9.2 p.m.
ep5-28	8.30 p.m.	9.2 p.m.
ep5-29	8.30 p.m.	9.2 p.m.
ep5-30	8.30 p.m.	9.2 p.m.
ep5-31	8.28 p.m.	8.29 p.m.
ep5-32	8.28 p.m.	8.29 p.m.
ep5-33	8.28 p.m.	8.29 p.m.
ep5-34	8.28 p.m.	9.4 p.m.
ep5-35	8.28 p.m.	9.4 p.m.
ep5-36	8.28 p.m.	9.4 p.m.
ep5-37	8.28 p.m.	9.2 p.m.
ep5-38	8.28 p.m.	9.2 p.m.

ep5-39	9.1 p.m.	9.2 p.m.
ep5-40	9.1 p.m.	9.4 p.m.
ep5-41	9.1 p.m.	9.4 p.m.
ep5-42	9.1 p.m.	9.4 p.m.
ep5-43	9.1 p.m.	9.4 p.m.
ep5-44	9.1 p.m.	9.2 p.m.
ep5-45	9.2 p.m.	9.4 p.m.
ep5-46	9.2 p.m.	9.4 p.m.
ep5-47	9.2 p.m.	9.4 p.m.
ep5-48	9.2 p.m.	9.4 p.m.
ep5-49	9.2 p.m.	9.4 p.m.
ep5-50	9.2 p.m.	9.4 p.m.
ep5-51	9.2 p.m.	9.4 p.m.
ep5-52	9.1 p.m.	9.4 p.m.
ep5-53	9.1 p.m.	9.4 p.m.
ep5-54	9.2 p.m.	9.4 p.m.
ep5-56	9.5 p.m.	
ep5-57	9.5 a.m.	
ep5-58	9.5 a.m.	
ep5-59	9.6 p.m.	
ep5-60	9.6 p.m.	
ep5-61	9.6 p.m.	
ep5-62	9.5 a.m.	
ep5-63	9.5 a.m.	
ep5-64	9.6 p.m.	
ep5-65	9.5 a.m.	
ep5-66	9.5 a.m.	
ep5-67	9.6 p.m.	
ep5-68	9.6 p.m.	
ep5-69	9.6 p.m.	
ep5-70	9.6 p.m.	
ep5-71	9.6 p.m.	
ep5-72	9.6 p.m.	
ep5-73	9.6 p.m.	
ep5-74	9.6 p.m.	
ep5-75	9.6 p.m.	
ep10-1		
ep10-2		
ep10-3		9.11 p.m.
ep10-4		9.11 p.m.
ep10-5		
ep10-6		
ep10-7		9.11 p.m.
ep10-8		
ep10-9		

ep10-10		
ep10-11		9.11 p.m.
ep10-12		
ep10-13		
ep10-14		
ep10-15		
ep10-16		
ep1'-1		9.11 p.m.
ep1'-2		9.12 p.m.
ep1'-3		9.12 p.m.
ep1'-4		9.12 p.m.
ep1'-5		9.12 p.m.
ep1'-6		9.11 p.m.
ep1'-7		9.11 p.m.
ep1'-8		9.11 p.m.
ep1'-9		9.11 p.m.
ep1'-10		9.11 p.m.
ep1'-11		9.13 p.m.
ep1'-12		9.12 p.m.
ep1'-13	9.11 p.m.	9.13 p.m.
ep1'-14	9.11 p.m.	9.13 p.m.
ep1'-15	9.11 p.m.	9.12 p.m.
ep1'-16	9.11 p.m.	9.12 p.m.
ep1'-17	9.11 p.m.	9.13 p.m.
ep1'-18	9.11 p.m.	9.13 p.m.
ep1'-19	9.11 p.m.	9.13 p.m.
ep1'-20	9.11 p.m.	9.13 p.m.
ep1'-21	9.11 p.m.	9.13 p.m.
ep1'-22	9.11 p.m.	9.12 p.m.
ep1'-23		
ep1'-24	9.11 p.m.	9.13 p.m.
ep1'-25	9.11 p.m.	9.13 p.m.
ep1'-26	9.11 p.m.	
ep1'-27	9.11 p.m.	
ep1'-28		
ep1'-29	9.11 p.m.	9.12 p.m.
ep1'-30	9.11 p.m.	9.13 p.m.
ep1'-31	9.11 p.m.	9.13 p.m.
ep1'-32	9.11 p.m.	
ep1'-33	9.11 p.m.	
ep1'-34	9.11 p.m.	
ep1'-35	9.11 p.m.	9.13 p.m.
ep1'-36	9.11 p.m.	
ep1'-37	9.11 p.m.	
ep1'-38	9.11 p.m.	

ep1'-39	9.12 p.m.	
ep1'-40	9.12 p.m.	
ep1'-41	9.12 p.m.	
ep1'-42	9.12 p.m.	
ep1'-43	9.12 p.m.	
ep1'-44	9.12 p.m.	
ep1'-45	9.12 p.m.	
ep1'-46	9.12 p.m.	
ep1'-47	9.12 p.m.	
ep1'-48	9.12 p.m.	
ep1'-49	9.12 p.m.	
ep1'-50	9.12 p.m.	
ep1'-51	9.12 p.m.	
ep1'-52	9.12 p.m.	
ep1'-53	9.12 p.m.	
ep1'-54	9.12 p.m.	
ep1'-55	9.12 p.m.	
ep1'-56	9.12 p.m.	
ep1'-57	9.12 p.m.	
ep1'-58	9.12 p.m.	
ep1'-59	9.12 p.m.	
ep1'-60	9.12 p.m.	
ep1'-61	9.12 p.m.	9.17 p.m.
ep1'-62	9.12 p.m.	
ep1'-63	9.12 p.m.	
ep1'-64	9.12 p.m.	9.17 p.m.
ep1'-65	9.12 p.m.	9.17 p.m.
ep1'-66	9.12 p.m.	9.17 p.m.
ep1'-67	9.12 p.m.	9.17 p.m.
ep1'-68	9.12 p.m.	9.19 a.m.
ep1'-69	9.12 p.m.	9.19 a.m.
ep1'-70	9.12 p.m.	9.20 a.m.
ep1'-71	9.12 p.m.	9.20 a.m.
ep1'-72	9.12 p.m.	9.20 a.m.
ep1'-73	9.12 p.m.	9.20 a.m.
ep1'-74	9.12 p.m.	9.20 a.m.
ep1'-75	9.12 p.m.	
ep1'-76	9.12 p.m.	9.20 a.m.
ep1'-77	9.12 p.m.	
ep1'-78	9.12 p.m.	
ep1'-79	9.12 p.m.	
ep1'-80	9.12 p.m.	
ep1'-81	9.12 p.m.	