

Supplementary information

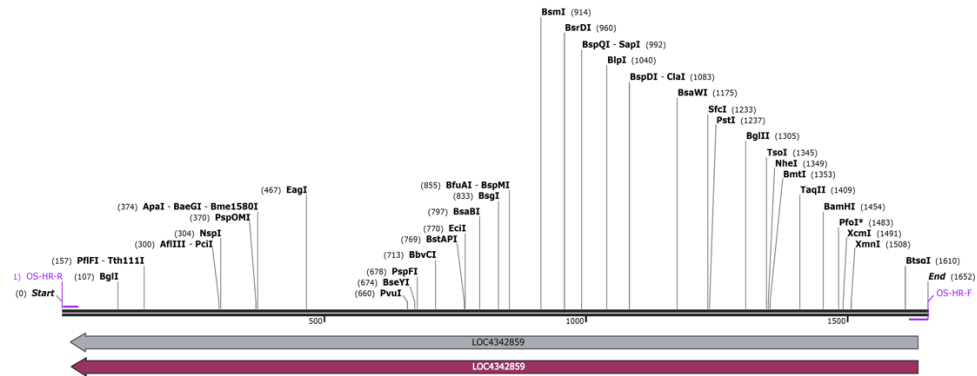


Fig. S1 The map of *OsNramp5*. The original sequence comes from *Oryza sativa Japonica Group* (Japanese rice, Gene ID:4342859; Gene symbol:LOC4342859). *OsNramp5* sequence was got from Chen lab, and constructed in shuttle plasmid pFL61.

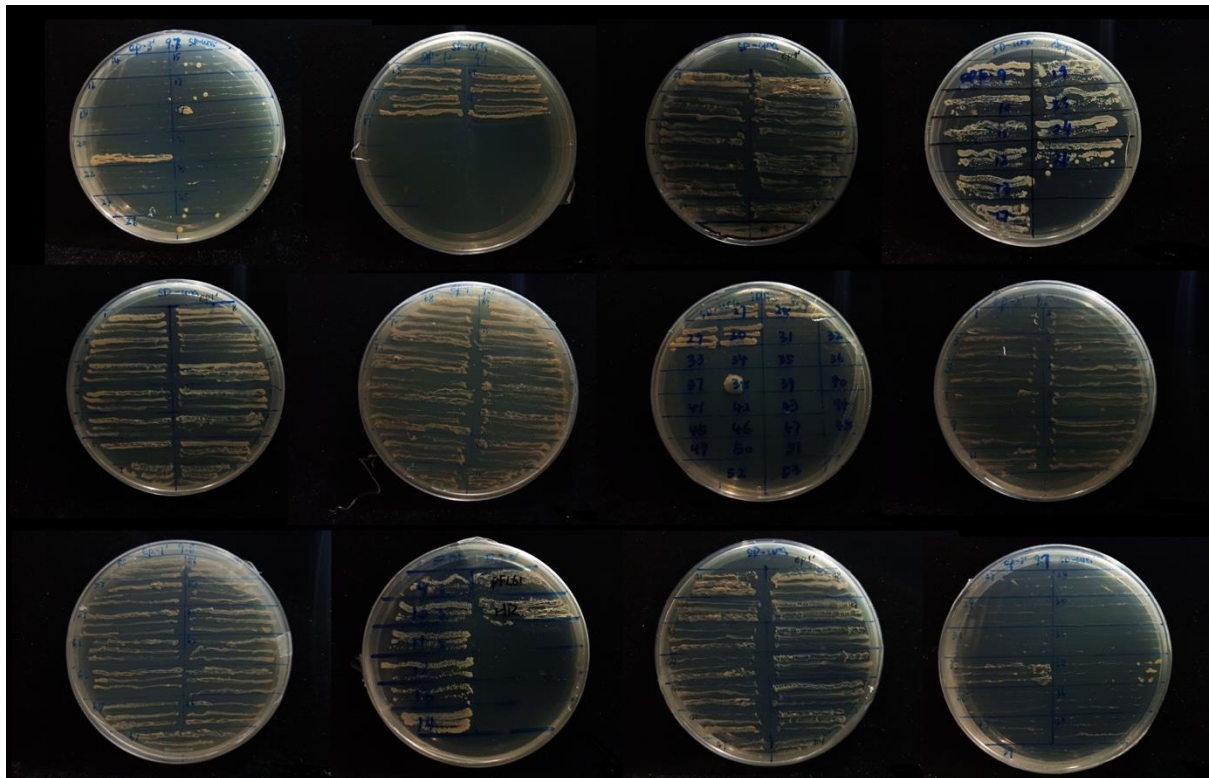


Fig.S2 Part of our library plates. Approximately 12 positive transformants were preserved on each plate, for a total of around 260. We redlined them for subsequent screening.

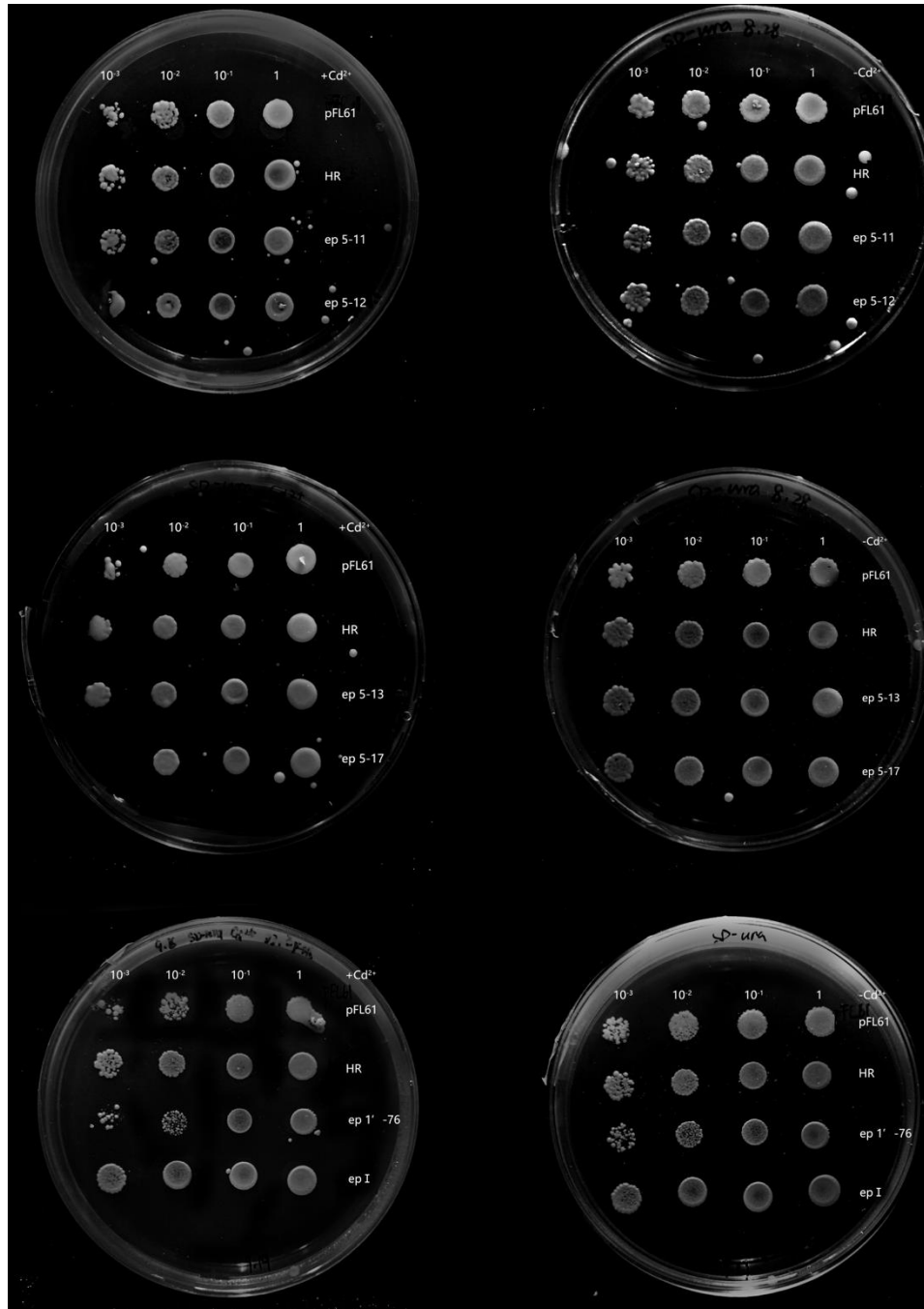


Fig. S3 Some typical potential benign mutant strains under Cd^{2+} stress. The growth of some mutant strains on SD-ura plates containing Cd^{2+} concentration of $30 \mu\text{M}$ was slightly better than that of *pFL61* and *HR* while they grew similarly to *pFL61* and *HR* on plates without Cd^{2+} , indicating that they may be the potential *OsNramp5-mut* strains whose ability of transporting Cd^{2+} has successfully decreased or their *OsNramp5* has been damaged.

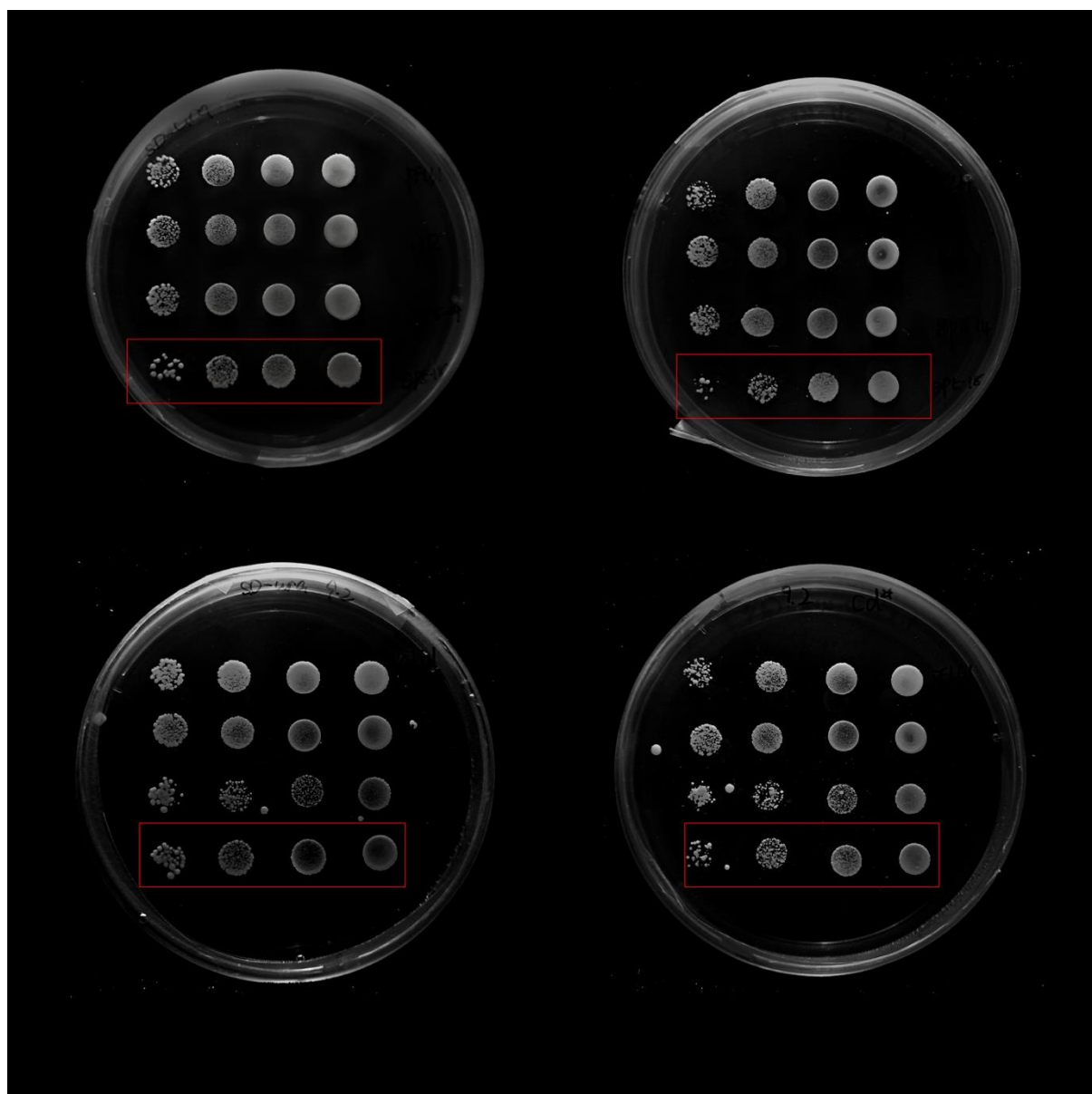


Fig. S4 *OsNramp5-mut* with enhanced ability of transporting Cd^{2+} .

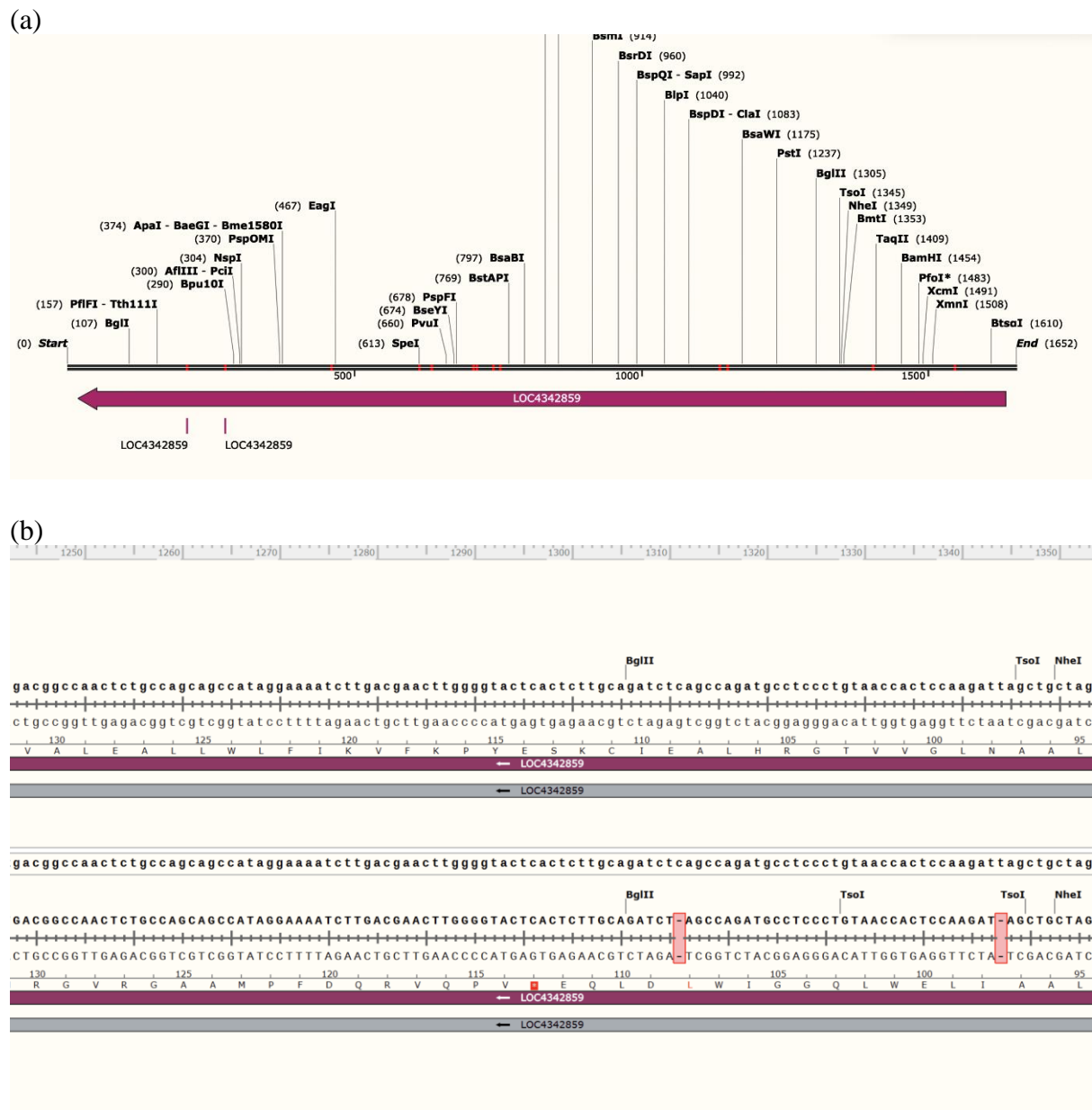


Fig. S5 (a) Sequencing analysis of *OsNramp5* ep-11. (b) Sequencing analysis of *OsNramp5* ep-17. The mutation sites of this gene were labeled red in the map. The mutants with poorer phenotypes than HR are probably caused by changes in crucial loci to Cd^{2+} transportation, so that the intracellular Cd^{2+} concentration increased and their growth was inhibited.

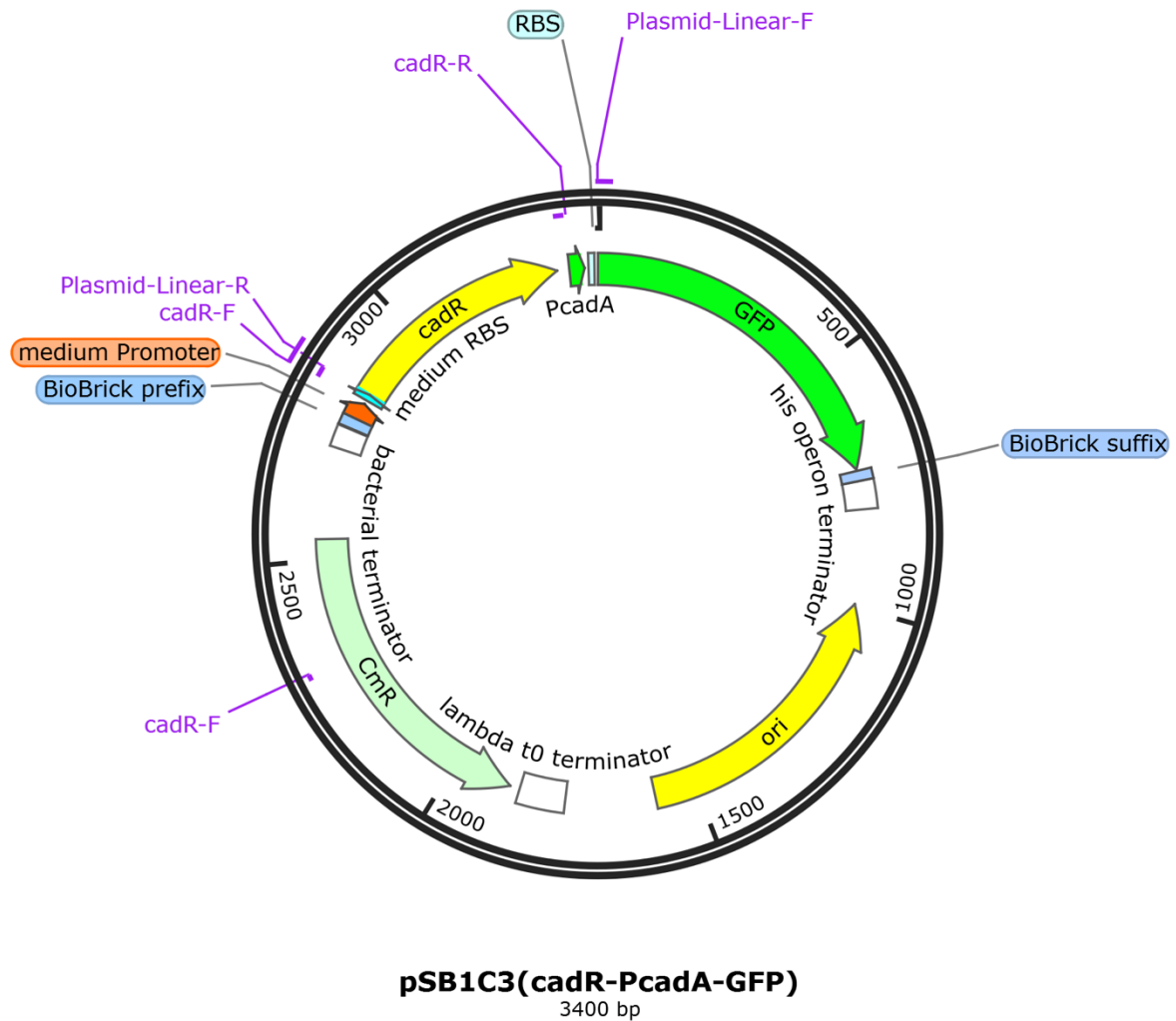


Fig.S6 The recombinant plasmid of Cd^{2+} responsive biosensor. The recombinant plasmid concludes CadR, PcadA and GFP([BBa_K608011](#)) coding sequences. These are present in plasmid pSB1C3, which harbours a chloramphenicol resistant gene for the selection of our transformants.

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	ctagagtcacacaggaaagatgaagatcggtgagctgg
CadR-R	acacctccagtcactgag
Plasmid-Linear-F	gatgcgtaaaggagaagaac
Plasmid-Linear-R	ctttcctgtgtgactctag
Pcada-sense	ctcagtgactggaggtgttgactctgtagttgctacaggggtgcaattactagagaaaggagaga aatactaggatgcgtaaaggagaagaac
Pcada-antisense	gttcttctcctttacgcacacctagtagtattctcctctttctctagtaattgcacacacctgtagcaactacaga gtcaaacacctccagtcactgag

Table S2. The TMHMM source data.

```
# 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	LWFTGPGFLMSIAYLDPGNIESDIQAGAM
Pb1NR2	LMLYSGPGWLMAIAYLDPGNLES DLQSGAV
MbreNR	LWAFSGPSLLVSLAYLDPGNIESDLQAGVA
Ren1NR	LWAFSGPGFLMSIAYLDPGNIESDLQSGVT
NvecNR	LWFTGPGFLMSIAYLDPGNIESDLRQGAV
DmeNR	LWFTGPGFLMSIAYLDPGNIESDMQSGAA
SmNRa	LWTFTGPGFLMSIAYLDPGNIESDLQSGAI
MyesNR	LWFTGPGFLMSIAYLDPGNVESDLRAGAS
SuperNRa	LWFTGPGFLMSIAYLDPGNIESDLQSGFI
CinNR	LWFTGPGFLMSIAYLDPGNIESDLQSGFI
DeeNR	LWFTGPGFLMSIAYLDPGNIESDIQSGAI
PmaNR	LWFTGPGFLMSIAYLDPGNIESDLQSGAK
XtrNR2	LWFTGPGFLMSIAYLDPGNIESDIQSGAK
GgNR2	LWFTGPGFLMSIAYLDPGNIESDIQSGAV
MmNR2	LWFTGPGFLMSIAYLDPGNIESDLQSGAV
XlaeNR1	LWFTGPGFLMSIAYLDPGNIESDLQCGAI
GgNR1	LWFTGPGFLMSIAYLDPGNVESDLQCGAV
MmNR1	LWFTGPGFLMSIAFLDPGNIESDLQAGAV

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.	