

SUPPLEMENTARY INFORMATION

A conserved phosphorylation switch controls the interaction between cadherin and β -catenin in vitro and in vivo

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HO/R1 (56) TQQQLKQSVMDLLTYEGSNNDMSGL..SLFDLVKLMCDH.....DESVVAVRHRAVYMLSRE
R1 (152) AIPPELTKLLN.....DE...DQVVVNKAAMVHVHQLSK

R2 (109) .D...PNFFN.....APGF.DHRSFVEALMAASK.....SS...NVNVRRNAIGALSHMSE
R2 (181) .K...EASRHA...IMR.....SPQMVSIVRTMQN.....TN...DVETARCTAGTLHNLSH

R3 (152) .Q...RGGPLL...IFRS.....GGLAEIIRML.Y.....DS...LESVVHYAVTTLRNLLMH
R3 (224) .H...REGLLA...IFKS.....GGIPALVKML.G.....SP...VDSVLFYAITTLHNLLL

R4 (194) V...SDSRAQ...ARAL.....NAVEALTPHL.HK.....T...NPKLLAQVADGLYFLLI
R4 (265) HQ...EGAKMA...VRLA.....GGLQKMAVALL.NK.....T...NVKFLAITTDCLQILAY

R5 (235) .D...DAPSKIT...FLSL.....LGGPQILVLSILREY.....SD...HRKLIYTVVRCIRSLSV
R5 (307) .G...NQESKLI...ILAS.....GGPQALVNIMRTY.....T...YEKLLWTTSRVLKVLVSV

R6 (279) .C...PSNKPA...LISL.....GCLPALYVELCT.....AK...DERSQTAILVAMRNLS.
R6 (350) .C...SSNKPA...IVEA.....GGMQALGLHLLT.....DP...SQRLVQNCLWTLRNLS.D

R7 (320) ....DSATN.EEN.....LTQLIIKLLEIIRV.....A...NDGMTACACGTLNLT.C
R7 (391) AA.....TK.QEG.....MEGLLGLTLVQLL.G.....SD...DINVVTCAAGILNLT.C

R8 (360) .N...NTRNKQT...VCSH.....GGIDALVTAIRRL.....PE...VEEVTEPALCALRHCTA
R8 (430) .N...NYKNKMM...VCQV.....GGIEALVRTVLRRA.....GD...REDITEPAICALRHLLTS

R9 (404) RHS LAEEAQSE...LRFC.....QAFPVILDQLE.....TL...RTPVIKAALGVIRNSAL
R9 (474) RHQEAEMAQNA...VRLH.....YGLPVVVKLLH.....PPS...HWPLIKATVGLIRNLAL

R10 (449) .L...QTNLIE...LTQEQ TANGH TAVSLTMDILRRAITAIEEN①GVPMWGVIEGAVSALHQLAN
R10 (520) .C...PANHAP...LREQ.....GAIPRLVQLLVRRAHQDTQR②.GVRMEEIVEGCTGALHILAR

R11 (512) .H...PAVAAA...CCDD③.....PFLDLLHRLLAHPRLG...S...MDDEVLEREILGLLYQLSK
R11 (583) .D...VHNRIV...IRGL.....NTIPLFVQLLY.....SP...IENIQRVAAGVLCELAQ

R12 (569) .R...PDGARA...VES T.....GVSALLMESRG.....SQ...YKSVVTYANGVLSNLKRGDS
R12 (624) .D...KEAAEA...IEAE.....GATAPLTELLH.....SR...NEGVATYAAAVLFRMSE

① PDIAVDGVP          ② RTSMGGTQQQFVE          ③ IGQVGNPECP

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Figure S1, related to Figure 1. Alignment of the armadillo domains of *C. elegans* HMP-2 and *M. musculus* β -catenin. The boxed regions correspond to α helices H1, H2 and H3 left to right, as observed in the respective crystal structures. Italicized residues are present in the crystallized construct but not visible. The circled numbers correspond to loop sequences inserted at those positions and shown at the bottom.

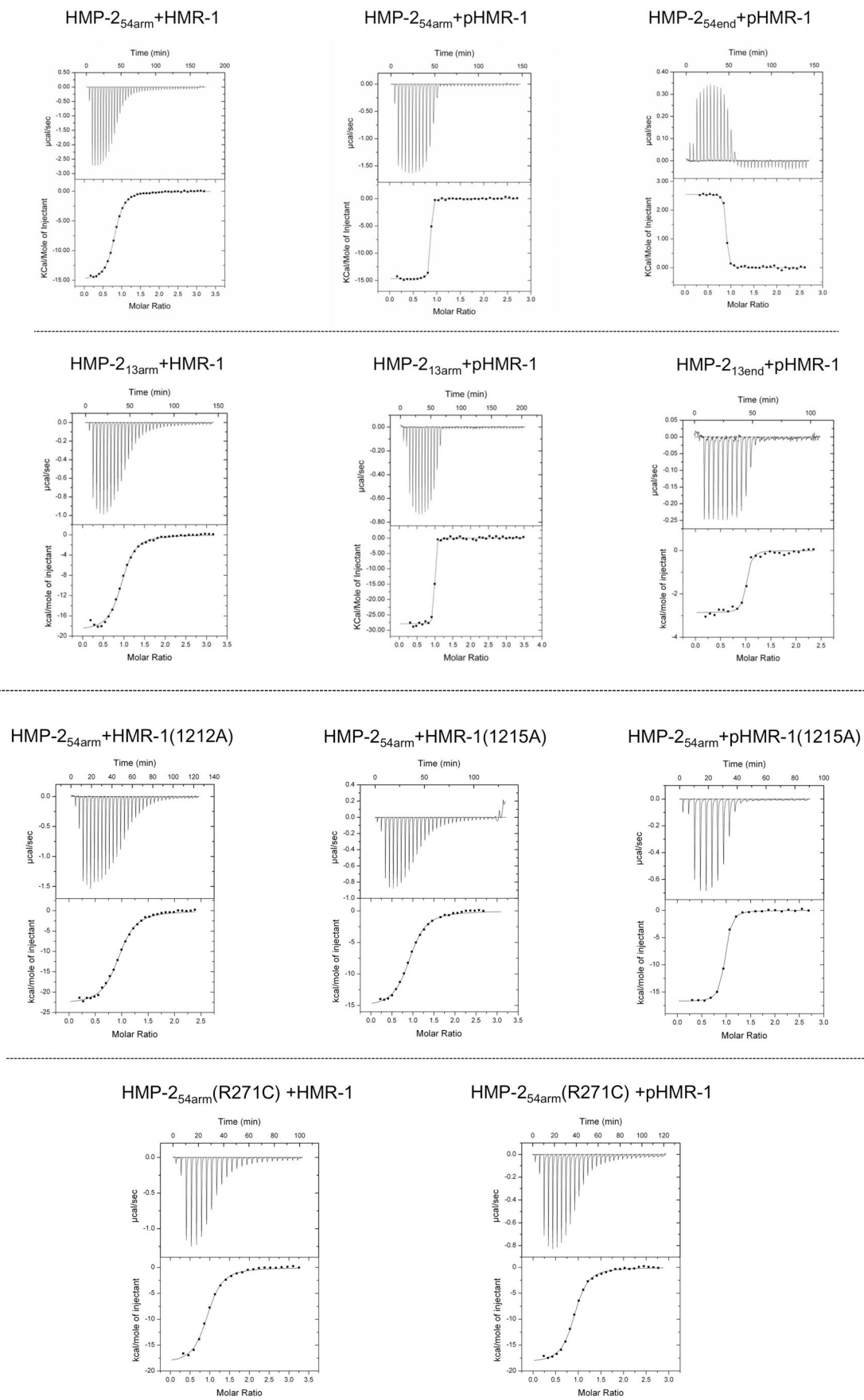


Figure S2, related to Figure 1 and Table S2. Isothermal titration calorimetry traces for the indicated binding reactions.

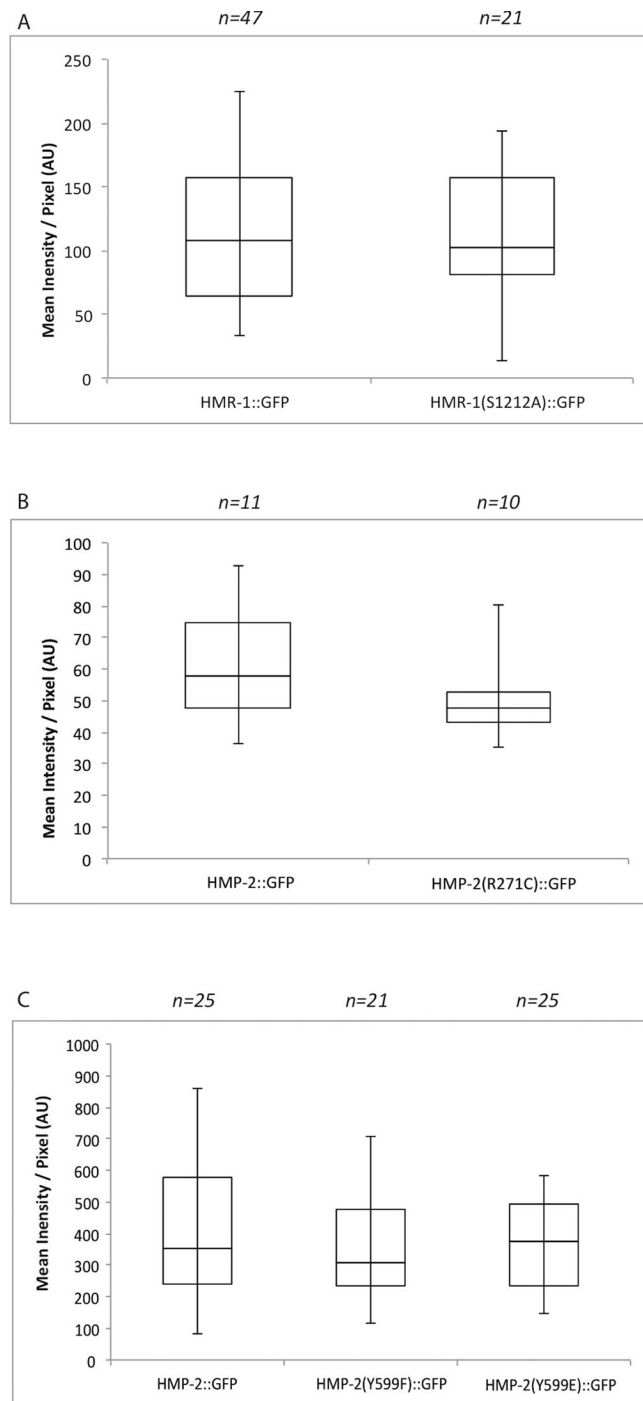


Figure S3, related to Figure 5 and 6. (A) Quantitation of HMR-1::GFP vs. HMR-1(S1212A)::GFP found no significant difference in expression levels ($p = 0.7100$, two-tailed t-test, $n =$ number of individual junctions measured). Z-projections of 2 focal planes were measured for each junction. (B) Quantitation of HMP-2::GFP vs. HMP-2(R271C)::GFP found no significant difference in expression levels ($p = 0.6549$, two-tailed t-test, $n =$ number of embryos scored). 20 focal planes were sum projected for each embryo. Mean fluorescence of each embryo was corrected for mean fluorescence of a background ROI in each image. (C) Quantitation of HMP-2::GFP vs. HMP-2(Y599F)::GFP vs. HMP-2(Y599E)::GFP found no significant difference in expression levels ($p = 0.4588$, one way ANOVA, $n =$ number of individual junctions measured). Z-projections of 2 focal planes were measured for each junction.

AU = arbitrary units.

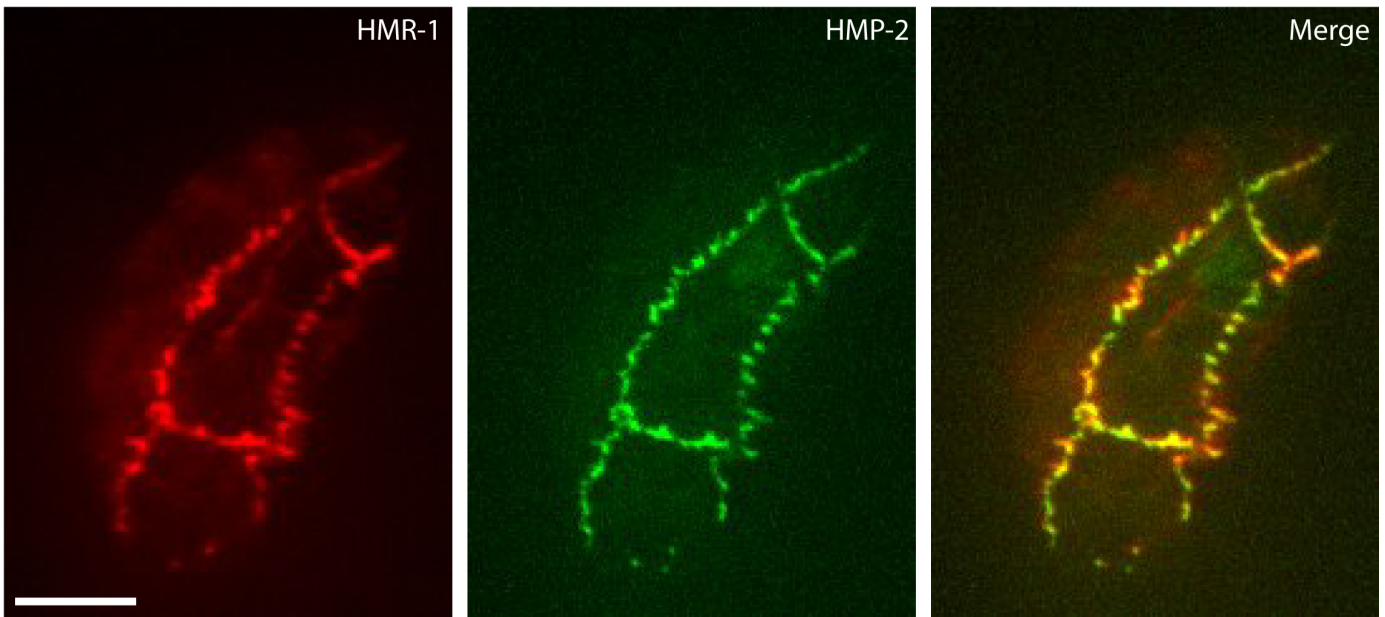


Figure S4, related to Figure 6. Co-immunostaining of a 4-fold *hmp-2(zu364); hmp-2(Y599E)::gfp* embryo with anti-HMR-1 antibody (red) and anti-GFP antibody (green). HMR-1 signal is discontinuous along cell-cell junctions and colocalizes with HMP-2(Y599E) excursions. Scale bar is 10 μ m.

Table S1, related to Figures 1, 3, 4 and 7. Crystallographic statistics.

<i>Data collection</i>	<i>HMP-2_{54end}</i>	<i>HMP-2_{54arm}+phosphoHMR-1_{cyto80}</i>	
wavelength (Å)	1.0332	0.97945	0.97945
Space group	C2	P2 ₁	P4 ₃
Unit cell parameters			
a(Å)	165.23	85.06	84.72
b(Å)	38.97	157.72	84.72
c(Å)	101.13	84.82	137.0
β(°)	116.7	94.2	90.0
Resolution (Å) (last shell)	50-2.0 (2.07-2.00)	50-2.8 (2.9-2.8)	50-2.3 (2.38-2.3)
Unique reflections	36385 (3154)	54788 (5272)	42695 (4189)
Completeness (%)	93.0 (82.7)	99.3 (95.9)	99.6 (99.3)
Multiplicity	4.9 (4.4)	3.6 (3.4)	3.9 (3.9)
I/σ(I)	19.7 (5.6)	11.8 (2.1)	17.9 (4.5)
R _{merge} ^a	0.038 (0.22)	0.048 (0.54)	0.03 (0.36)
CC _{1/2}	0.999 (0.978)	0.997 (0.810)	0.998 (0.873)
<i>Refinement</i>			
No. of reflections working set (test set)	36351 (2783)	54463 (4242)	42679 (3288)
R _{cryst} /R _{free} ^b	0.19 / 0.23	0.20/0.25	0.17 / 0.20
bond length rmsd from ideal (Å)	0.003	0.003	0.003
bond angle rmsd from ideal (°)	0.75	0.74	0.72
<i>Ramachandran analysis</i> ^c			
% favored regions	95.8	94.3	95.8
% allowed regions	4.2	5.7	4.2
% outliers	0.0	0.0	0.0

rmsd, root-mean square deviation.

^a $R_{\text{merge}} = \frac{\sum_h \sum_i |I_i(h) - \langle I(h) \rangle|}{\sum_h \sum_i I_i(h)}$, where $I_i(h)$ is the i^{th} measurement of reflection h , and $\langle I(h) \rangle$ is the weighted mean of all measurements of h .

^b $R = \frac{\sum_h |F_{\text{obs}}(h) - |F_{\text{calc}}(h)||}{\sum_h |F_{\text{obs}}(h)|}$. R_{cryst} and R_{free} were calculated using the working and test reflection sets, respectively.

^cAs defined in MolProbity

Table S2, related to Figures 1 and S2. ITC measurements of HMP-2 binding to phosphorylated and non-phosphorylated HMR-1. Representative traces are shown in Figure S2.

Proteins		K_D (nM)	ΔH (kcal mol ⁻¹)	$T\Delta S$ (kcal mol ⁻¹)	ΔG (kcal mol ⁻¹)
HMP-2 _{13end}	HMR-1 _{cyto80}	ND	-	-	-
HMP-2 _{13end}	pHMR-1 _{cyto80}	51.3	-2.9	7.1	-9.9
HMP-2 _{54end}	HMR-1 _{cyto80}	ND	-	-	-
HMP-2 _{54end}	pHMR-1 _{cyto80}	23.0	2.5	13.0	-10.5
HMP-2 _{13arm}	HMR-1 _{cyto80}	382	-19.0	-10.3	-8.7
HMP-2 _{13arm}	pHMR-1 _{cyto80}	7.9	-18.6	-7.5	-11.1
HMP-2 _{54arm}	HMR-1 _{cyto80}	380	-19.2	-10.5	-8.7
HMP-2 _{54arm}	pHMR-1 _{cyto80}	3.1	-27.9	-16.3	-11.6
HMP-2 _{54arm}	HMR-1 _{cyto80} (S1212A)	518	-23.0	-14.4	-8.6
HMP-2 _{54arm}	HMR-1 _{cyto80} (T1215A)	610	-15.4	-6.9	-8.5
HMP-2 _{54arm}	pHMR-1 _{cyto80} (T1215A)	25.6	-16.7	-6.4	-10.3
HMP-2 _{54arm} (R271C)	HMR-1 _{cyto80}	435	-18.7	-10.0	-8.7
HMP-2 _{54arm} (R271C)	pHMR-1 _{cyto80}	249	-18.5	-9.4	-9.1