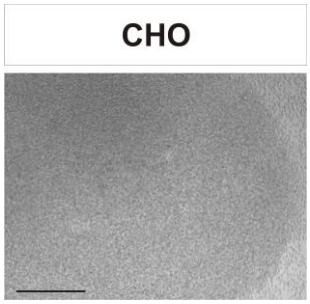
Electronic Supplementary Information:
Lanthanides compete with calcium for binding to cadherins and inhibit cadherin-mediated cell adhesion

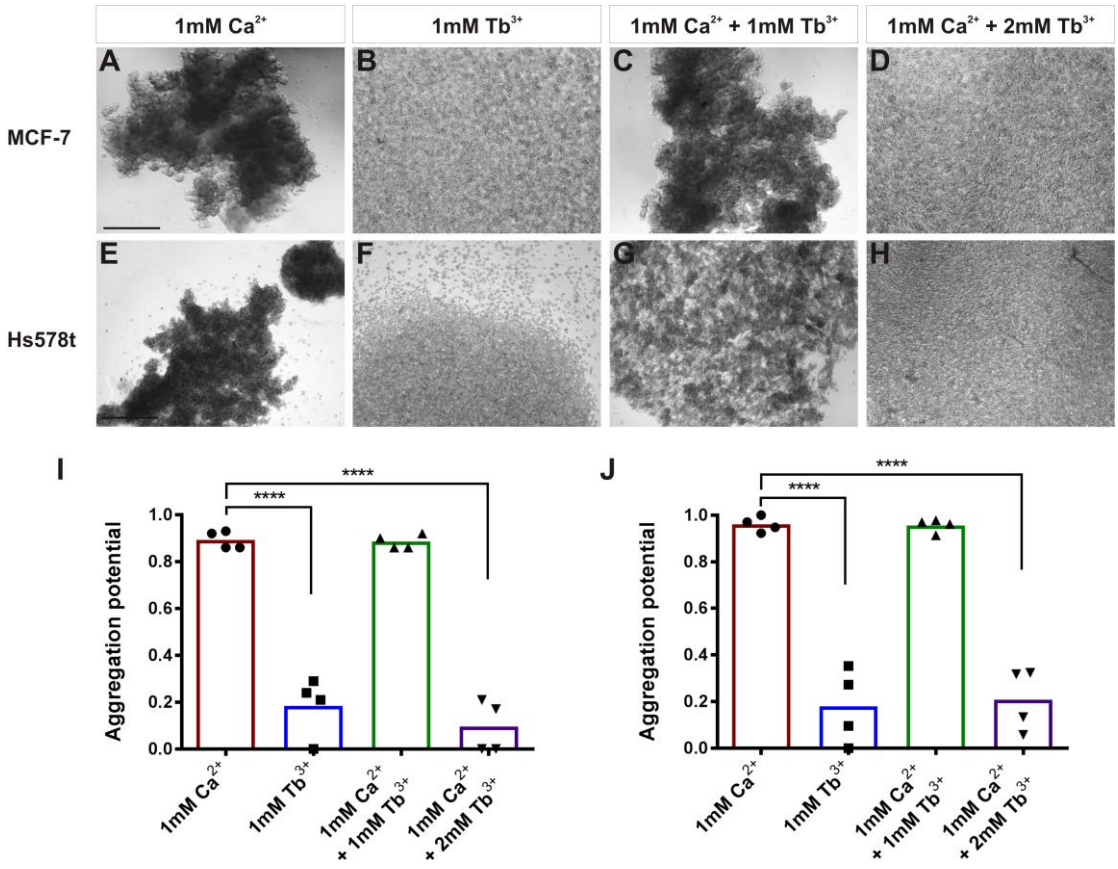
Lewis L. Brayshaw[a], Rosanna C. G. Smith[a, b], Magd Badaoui[c], James A. Irving[c], and Stephen R. Price*[a]

a. Research Department of Cell and Developmental Biology, UCL, Gower Street, London, WC1E 6BT
b. Centre for Human Development, Stem Cells, and Regeneration, University of Southampton, Southampton, SO17 1BJ
c. Research Department of Respiratory Medicine, UCL, Gower Street, London, WC1E 6BT

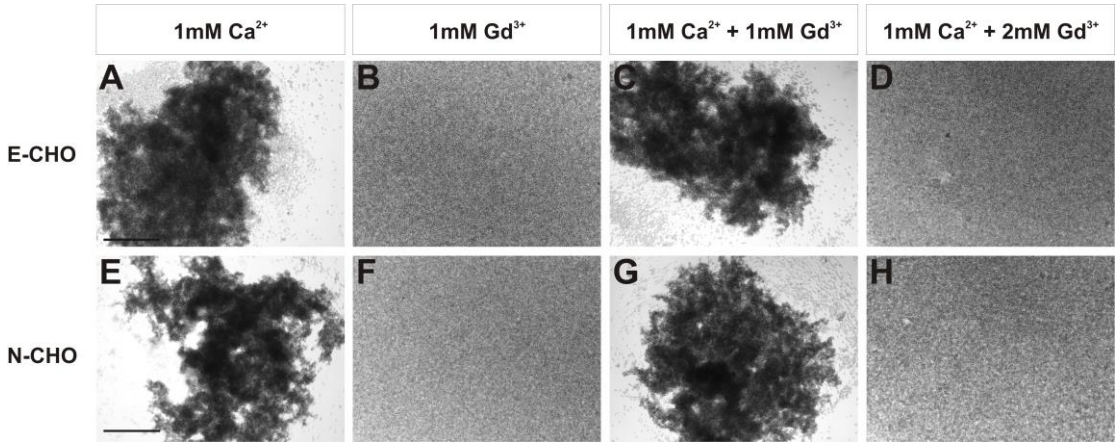
* Stephen.price@ucl.ac.uk



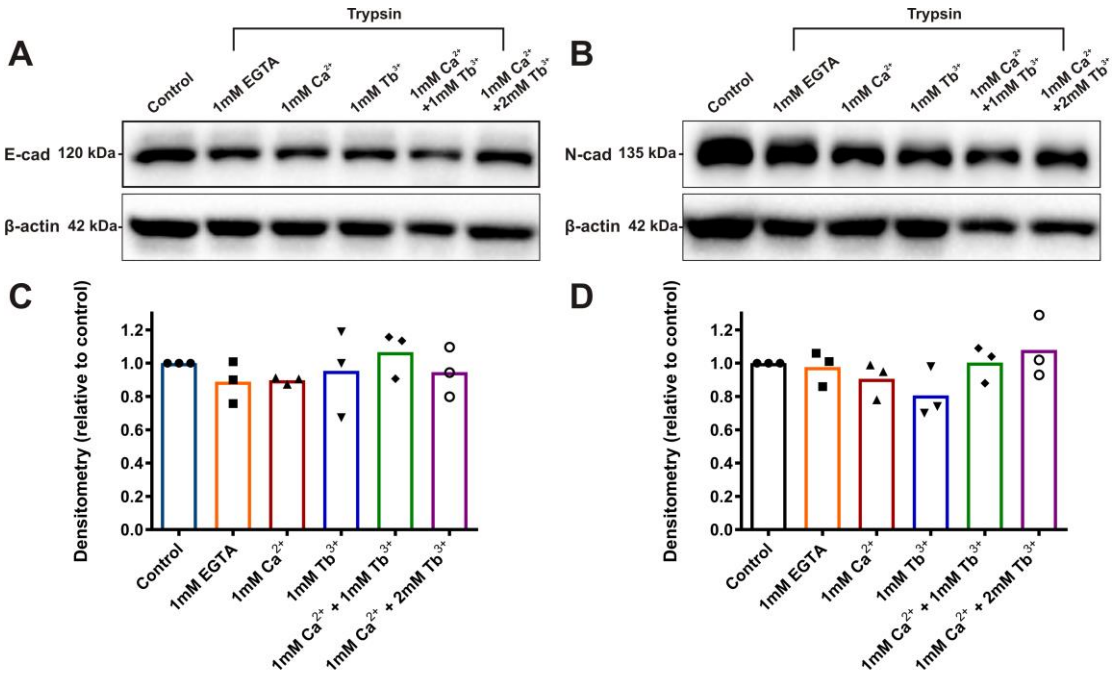
Supplementary Fig. 1 Cell aggregation assay using parental CHO cells with no cadherin expression. Prior to assay, cells were treated with 0.01% trypsin + 1 mM Ca^{2+}. Assay was performed in 1 mM Ca^{2+}. Scale bar = 200 μm.



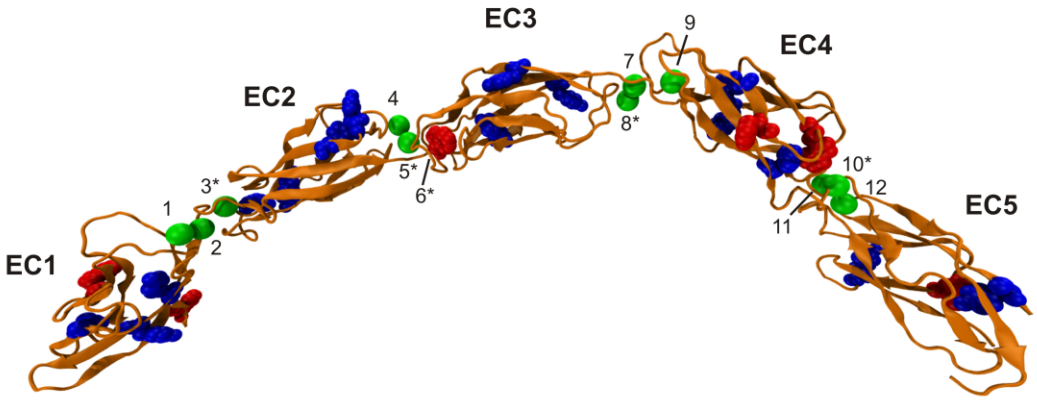
Supplementary Fig. 2 Influence of Gd^{3+} on E- and N-cadherin-mediated cell aggregation. Cell aggregation assays of (A-D) E-cadherin expressing CHO (E-CHO) and (E-H) N-cadherin expressing CHO (N-CHO) cells in different combinations of Ca^{2+} and Tb^{3+}. Prior to aggregation, cells were treated with 0.01% trypsin + 1 mM Ca^{2+}. Scale bar = 200 μm.



Supplementary Fig. 3 Influence of Tb^{3+} on cadherin-mediated cell aggregation of cancer cell lines. Cell aggregation assays of (A-D) MCF-7 and (E-H) Hs578t cells in different combinations of Ca^{2+} and Tb^{3+}. Prior to aggregation, cells were treated with 0.01% trypsin + 1 mM Ca^{2+}. Aggregation potential of (I) MCF-7 and (J) Hs578t cells. The aggregation potential was calculated by 1 minus the number of single cells at end of aggregation (Ne) divided by the number of single cells at the beginning of aggregation (Ns). Aggregation potential = 1 - Ne/Ns. Scatterplots show individual values and bars represent the mean from four biological replicates (one-way ANOVA with Tukey's multiple comparisons test, significant differences are to 1 mM $CaCl_2$, **** indicates $p \leq 0.0001$, *** indicates $p \leq 0.001$). Scale bar = 200 µm.



Supplementary Fig. 4 Cadherin expression levels after E-CHO and N-CHO cells are incubated with Ca^{2+} and Tb^{3+} in the absence of trypsin. Western blot of (A) E-cadherin after E-CHO cells and of (B) N-cadherin after N-CHO cells were incubated with no ions (control), 1 mM EGTA, 1 mM Ca^{2+}, 1 mM Tb^{3+}, 1 mM Ca^{2+} + 1 mM Tb^{3+}, and 1 mM Ca^{2+} + 2 mM Tb^{3+} for 80 minutes. β-actin was used as a loading control. (C) Quantification of E-cad expression normalised to β-actin expression and (D) N-cadherin expression normalised to β-actin expression. Densitometry is expressed as fold change relative to the no ion control. Scatterplots show individual values and bars represent the mean from three biological replicates (one-way ANOVA with Tukey's multiple comparisons test).



Tryptophan residues			Tyrosine residues		
Residue number		Distance to Ca^{2+}-binding site (Ca^{2+}-binding site number)	Residue number		Distance to Ca^{2+}-binding site (Ca^{2+}-binding site number)
Human	Mouse		Human	Mouse	
2	2		36	36	
59	59		74	74	
255	255	4.4Å (6) 8.3Å (5)		77	
372	372		142	142	8.3Å (3)
378	378	9.0Å (10)	148	148	
484	484			184	9.6Å (4)
			187	187	
			226	226	
			259	259	
			296	296	9.9Å (8)
			354	354	
			369	369	
			407	407	
			488	488	
			509	509	

Supplementary Fig. 5 Relative positions of tryptophan residues, tyrosine residues and Ca^{2+}-binding sites in E-cadherin EC1-5. (A) Mouse E-cadherin EC1-5 crystal structure (PDB 3Q2V) with tryptophan residues in red and tyrosine residues in blue (both represented in Van der Waals surface). Ca^{2+} ions are represented as green spheres. Ca^{2+}-binding sites are numbered 1-12 and are labelled with asterisks if they are within 10 Å of a tryptophan or tyrosine residue. (B) List of tryptophan and tyrosine residues in human and mouse E-cadherin EC1-5. Distances between tryptophan and tyrosine residues and proximal Ca^{2+}-binding sites were measured in the crystal structure of mouse E-cadherin EC1-5 (PDB 3Q2V). Distances shown only for residues within 10 Å of a Ca^{2+}-binding site.