



**S18 Fig. Heterophilic binding assays of *hs* and *mm* PCDH24 and CDHR5.** (A-B) Images from binding assays of Fc-tagged *hs* PCDH24 (full-length extracellular domain and EC1-4Fc) mixed with the Fc-tagged full-length *hs* CDHR5 cadherin extracellular domain (A), and Fc-tagged *mm* PCDH24 (full-length extracellular domain and EC1-4Fc) mixed with Fc-tagged full-length *mm* CDHR5 cadherin extracellular domain (B). Green fluorescent protein A beads are coated with Fc-tagged PCDH24 fragments and red fluorescent protein A beads are coated with Fc-tagged CDHR5 in all panels. The white boxes indicate the regions shown in Fig 6. Images show bead aggregation observed after 60 min followed by rocking for 2 min in the presence of 2 mM  $\text{CaCl}_2$ . (C-F) Images from binding assays of truncations of Fc-tagged *hs* CDHR5 mixed with *hs* PCDH24 EC1-4Fc (C), truncations of Fc-tagged *hs* PCDH24 mixed with *hs* CDHR5 EC1-4Fc (D), truncations of Fc-tagged *mm* CDHR5 mixed with *mm* PCDH24 EC1-4Fc (E), and truncations of Fc-tagged *mm* PCDH24 mixed with *mm* CDHR5 EC1-4Fc (F). Images show bead aggregation observed after 60 min followed by rocking for 2 min in the presence of 2 mM  $\text{CaCl}_2$ . (G) Images from binding assays of minimum EC repeats required for heterophilic adhesion of *hs* and *mm* PCDH24 and CDHR5. The minimum units for heterophilic adhesion for the human proteins are CDHR5 EC1Fc and PCDH24 EC1-2Fc. The minimum units for heterophilic adhesion for the mouse proteins are CDHR5 EC1-2Fc and PCDH24 EC1-2Fc. Images show bead aggregation observed after 60 minutes followed by rocking for 2 minutes in the presence of 2 mM  $\text{CaCl}_2$ . (H) Protein A beads coated with Fc-tagged full-length *hs* and *mm* PCDH24 and CDHR5 cadherin extracellular domains (including MAD10 for PCDH24 when indicated and without the mucin-like domain for CDHR5) in the presence of 2 mM EDTA shown as in (A-B). Bar – 500  $\mu\text{m}$ . (I) Aggregate size (S11 Data) for minimum units of heterophilic adhesion of Fc-tagged *hs* and *mm* PCDH24 and CDHR5 at the start of the experiment (T0), after 60 min (T60) followed by rocking for 1 min (R1) and 2 min (R2). (J) Aggregate size (S12 Data) for full-length *hs* and *mm* PCDH24 and CDHR5 at the start of the experiment (T0), after 60 min (T60) followed by rocking for 1 min (R1) and 2 min (R2). Error bars in I and J are standard error of the mean (*n* indicated in S4 Table).