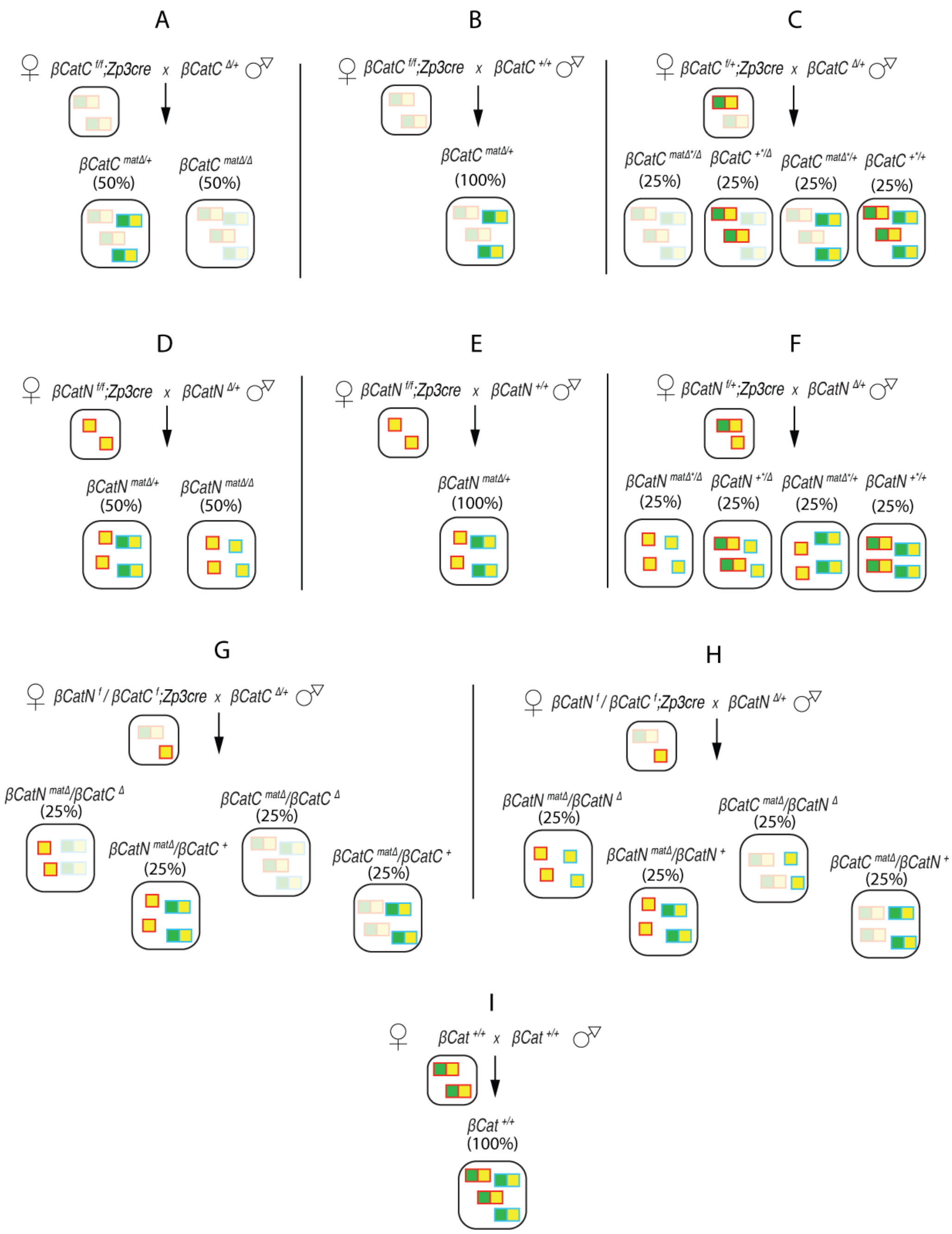

Figure S1. Schematic representation of mating schemes and CTNNB1 protein pools in oocytes and progeny blastocysts used in this work.

CTNNB1 protein is depicted as rectangle with an N- (green) and C-terminal (yellow) portion. Paternally encoded protein is boxed blue, maternal encoded protein is boxed in red. The βCatC allele deletion results in complete removal of the CTNNB1 protein (illustrated as semi-transparent squares), while βCatN allele deletion results in a C-terminal CTNNB1 fragment (C-terminal (yellow) square). Zp3cre recombines floxed alleles in the maternal germline. The content of rounded rectangles beneath the mothers in each cross depict the maternal protein contribution to the embryo through the oocyte. The content of the rounded rectangles beneath the F1 generation depict the CTNNB1 protein pool at the 3.5 dpc blastocyst stage. For simplicity sake display of ZP3cre inheritance is omitted as irrelevant to the resultant embryos. Progeny labeled with (*) received only half of the wild type maternal protein contribution at fertilization.

```
  WT  MATQADLMELDMAMEPDRKAAVSHWQQQSYLDSGIHSGATTTAPSLSGKGNPEEEDVDTSQVLYEWE
 Kem  -------------------------------------------------------------------
 Bir  -------------------------------------------------------------------
Bir2  -------------------------------------------------------------------

  WT  QGFSQSFTQEQVADIDGQYAMTRAQRVRAAMFPETLDEGMQIPSTQFDAAHPTNVQRLAEPSQMLKH
 Kem  -------------------------------------------------------------------
 Bir  -------------------------------------------------------------------
Bir2  -------------------------------------------------------------------

  WT  AVVNLINYQDDAELATRAIPELTKLLNDEDQVVVNKAAVMVHQLSKKEASRHAIMRSPQMVSAIVRT
 Kem  -------------------------------------------------------------------
 Bir  -------------------------------------------------------------------
Bir2  -------------------------------------------------------------------

  WT  MQNTNDVETARCTAGTLHNLSHHREGLLAIFKSGGIPALVKMLGSPVDSVLFYAITTLHNLLLHQEG
 Kem  -------------------------------------------------------------------
 Bir  -------------------------------------------------------------------
Bir2  -------------------------------------------------------------------

  WT  AKMAVRLAGGLQKMVALLNKTNVKFLAITTDCLQILAYGNQESKLIILASGGPQALVNIMRTYTYEK
 Kem  -----------------------------------------------------------MRTYTYEK
 Bir  -----------------------------------------------------------MRTYTYEK
Bir2  -------------------------------------------------------------------

  WT  LLWTTSRVLKVLSVCSSNKPAIVEAGGMQALGLHLTDPSQRLVQNCLWTLRNLSDAATKQEGMEGLL
 Kem  LLWTTSRVLKVLSVCSSNKPAIVEAGGMQALGLHLTDPSQRLVQNCLWTLRNLSDAATKQEGMEGLL
 Bir  LLWTTSRVLKVLSVCSSNKPAIVEAGGMQALGLHLTDPSQRLVQNCLWTLRNLSDAATKQEGMEGLL
Bir2  ---------------------MATQGGMQALGLHLTDPSQRLVQNCLWTLRNLSDAATKQEGMEGLL

  WT  GTLVQLLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICALR
 Kem  GTLVQLLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICALR
 Bir  GTLVQLLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICALR
Bir2  GTLVQLLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICALR

  WT  HLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLALCPANHAPLREQGAIPRL
 Kem  HLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLALCPANHAPLREQGAIPRL
 Bir  HLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLALCPANHAPLREQGAIPRL
Bir2  HLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLALCPANHAPLREQGAIPRL

  WT  VQLLVRAHQDTQRRTSMGGTQQQFVEGVRMEEIVEGCTGALHILARDVHNRIVIRGLNTIPLFVQLL
 Kem  VQLLVRAHQDTQRRTSMGGTQQQFVEGVRMEEIVEGCTGALHILARDVHNRIVIRGLNTIPLFVQLL
 Bir  VQLLVRAHQDTQRRTSMGGTQQQFVEGVRMEEIVEGCTGALHILARDVHNRIVIRGLNTIPLFVQLL
Bir2  VQLLVRAHQDTQRRTSMGGTQQQFVEGVRMEEIVEGCTGALHILARDVHNRIVIRGLNTIPLFVQLL

  WT  YSPIENIQRVAAGVLCELAQDKEAAEAIEAEGATAPLTELLHSRNEGVATYAAAVLFRMSEDKPQDY
 Kem  YSPIENIQRVAAGVLCELAQDKEAAEAIEAEGATAPLTELLHSRNEGVATYAAAVLFRMSEDKPQDY
 Bir  YSPIENIQRVAAGVLCELAQDKEAAEAIEAEGATAPLTELLHSRNEGVATYAAAVLFRMSEDKPQDY
Bir2  YSPIENIQRVAAGVLCELAQDKEAAEAIEAEGATAPLTELLHSRNEGVATYAAAVLFRMSEDKPQDY

  WT  KKRLSVELTSSLFRTEPMAWNETADLGLDIGAQGEALGYRQDDPSYRSFHSGGYGQDALGMDPMMEH
 Kem  KKRLSVELTSSLFRTEPMAWNETADLGLDIGAQGEALGYRQDDPSYRSFHSGGYGQDALGMDPMMEH
 Bir  KKRLSVELTSSLFRTEPMAWNETADLGLDIGAQGEALGYRQDDPSYRSFHSGGYGQDALGMDPMMEH
Bir2  KKRLSVELTSSLFRTEPMAWNETADLGLDIGAQGEALGYRQDDPSYRSFHSGGYGQDALGMDPMMEH

  WT  EMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDTDL
 Kem  EMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDTDL
 Bir  EMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDTDL
Bir2  EMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDTDL
```

Figure S2. Comparison of the wild type and truncated protein products produced by the *Ctnnb1* (wt), *Ctnnb1*^{tm2Kem} (Kem) and *Ctnnb1*^{tm4Wbm} (Bir and Bir2) alleles.

Analysis of the sequences of the different alleles revealed that the *Ctnnb1*^{tm2Kem} allele has one possible fragmented product produced from a alternative translation start site, whereas the *Ctnnb1*^{tm4Wbm} allele has two possible products. Western blot analysis (Fig 1 C) shows that the fragment, a splice product of exons 1 to exon 8, Bir2, gives rise to the truncated CTNNB1^{tm4Wbm}.

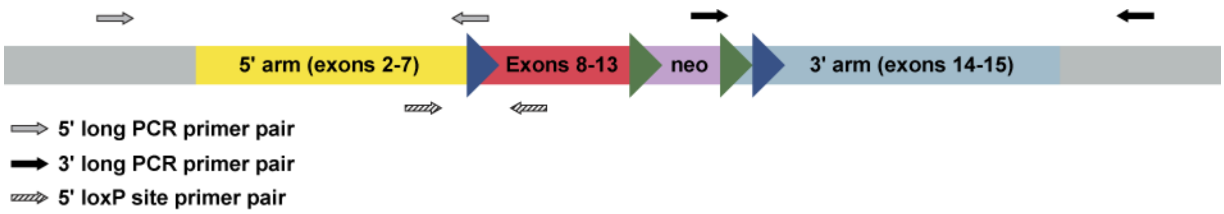

Figure S3. Schematic representation of the β-catenin floxed allele designed to remove the C-terminal portion of the protein (βCatC).

Floxed sites are indicated by blue arrowheads and frt sites by green arrowheads. Grey arrows indicate the position of the primer pairs used to determine correct recombination of the 5' arm of the construct. Black arrows indicate the position of the primer pairs used to determine correct recombination of the 3' arm of the construct. Patterned arrows indicate the primer pair used to determine whether or not the 5' loxP site was intact.

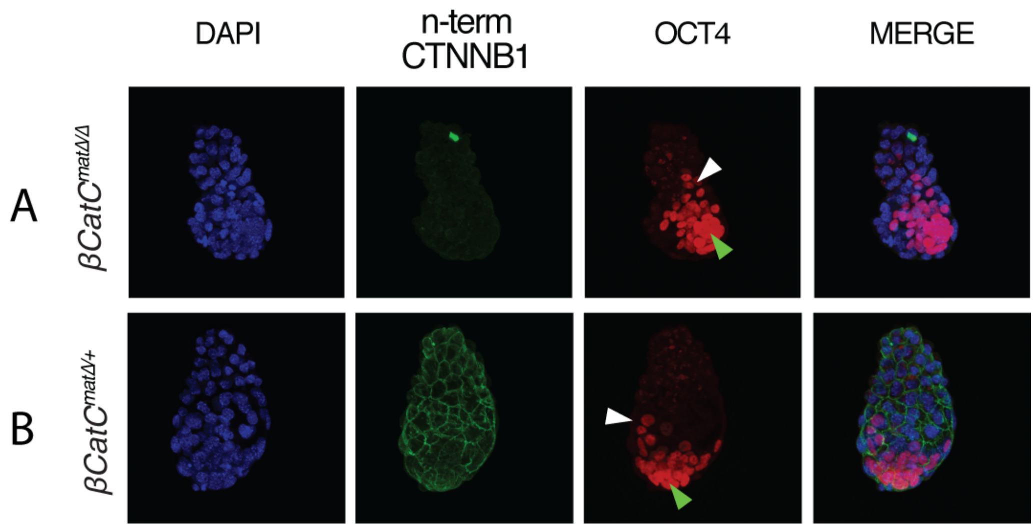

Figure S4. 4.5 dpc blastocysts derived from maternal-zygotic β-catenin null females.

CTNNB1 and OCT4 expression analysis in $\beta CatC^{mat\Delta/\Delta}$ (A) and $\beta CatC^{mat\Delta/+}$ (B) implanting embryos. At 4.5 dpc mutant ICMs remain OCT4 positive (green arrowhead). PE cells are also OCT4 positive and well visible in mutant and control (white arrowhead).

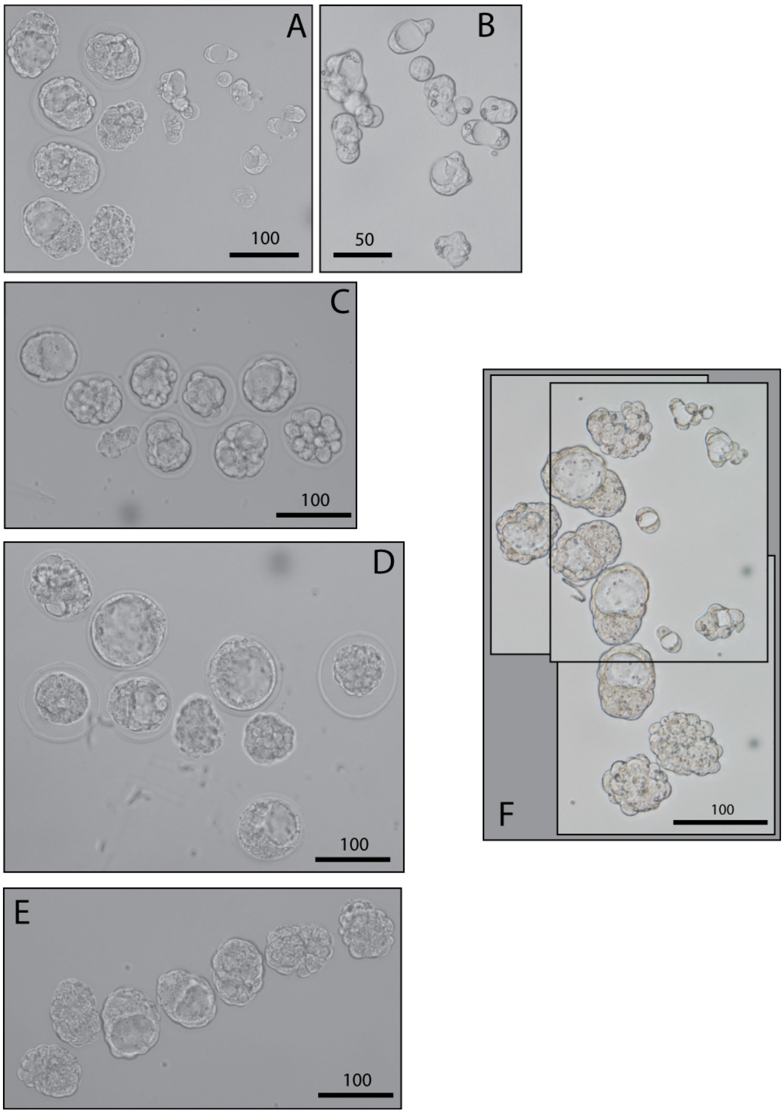

Figure S5. Representative images of litters isolated from maternal-zygotic β-catenin null females at 3.5 dpc (A, C-E) Representative 3.5 dpc litters of $\beta CatC^{f/f}$:*Zp3*-cre female mated with a $\beta CatC^{mat\Delta/+}$ male. (A) Litter containing presumably normal blastocysts, uncompacted morulae without a zona pellucida, and numerous trophoblastic vesicles. (B) Higher magnification of trophoblastic vesicles of litter shown in (A) (C-E) Litters containing normal blastocysts, compacted and uncompacted morulae with and without zona pellueidae. (F) Representative litter at 3.5 dpc of a $\beta CatN^{f}/\beta CatC^{f}$:*Zp3*-cre female mated with a $\beta CatC^{mat\Delta/+}$male. Litter contains normal and abnormal blastocysts, uncompacted morulae without zonas and several trophoblastic vesicles.