Figure S1. “Universal” beta-catenin reporters show disparate staining in the epithelial and stromal cells of the kidney. E14.5 wholemount and P1 sections of kidneys from BAT-Gal (A,B), NKD1-LacZ (C,D) and Axin2-LacZ (E,F) kidneys. In A-E, whole kidneys were stained with X-gal and photographed intact (A, C, E) or after sectioning (B,D). In F, an Axin2-LacZ kidney was sectioned and stained with antibodies to Beta-galactosidase (bgal, green), Six2 (red) and Topro (blue). Scale bar indicates 50 µm.
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<th>Lef/Tcf binding site</th>
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Lef/Tcf binding sites Myc binding site
Figure S2. Sequence of the Fam19a5-GRE. A. Sequence of the portion of intron 2 that was made to engineer the Fam19a5-GRE line. Consensus Lef/Tcf binding sites are in red, canonical Myc binding sites are in blue, STAT1 binding sites are in fuschia and the STAT3 binding sites are in green. The portion used to generate the Fam19a5-GRE-1-2160 is not underlined while the sequence used to generate the Fam19a5-GRE-2161-2934 is underlined. B. Sequence surrounding the Lef/Tcf (red) and Myc binding sites (blue). The mutated sequence used for the luciferase assays is shown below each site.
Figure S3. Chir stimulates Fam19a5-GRE reporter activity in vitro. (A) HEK 293 cells were transfected with a Fam19a5-GRE luciferase reporter and treated with Chir at different concentrations. (B) Graph of luciferase activity from HEK 293 cells transfected with or without a full length c-Myc expression plasmid as indicated. 24 hours after transfection, the media was replaced with fresh media (black bars) or media supplemented with 3uM Chir (gray bars) and luciferase activity was measured 18hrs later. Data are represented as mean ± SEM.
Figure S4. Expression and regulation of c- and N-Myc. Sections through E11.5 (A,B,E,F), E15.5 (C,G) and P1 (D,H) wild type (A,C,D,E,G,H) or Wnt9b mutant (B,F) kidneys stained with antisense probes to c-Myc (A-D) or N-Myc (E-H). All images are 40x.
Figure S5. Six2Cre efficiently ablates c- and N-Myc by e12.5. Sections through E12.5 wild type (c-Myc^{flox/flox};N-Myc^{flox/flox} in A,C and E) and mutant (Six2Cre;c-Myc^{flox/flox};N-Myc^{flox/flox}, B,D,F) kidneys hybridized with antisense mRNA probes to c-Myc (A,B), N-Myc (C,D) and Six2 (E,F). A-F, 20x. A’-F’, 40x. Note that c- and N-Myc expressions are greatly reduced in mutant kidneys while Six2 is unaffected.
Figure S6. NPCs of c/N-Myc double mutants have increased rates of apoptosis. Sections from E15.5 wild type (c-Myc<sup>flox/flox</sup>;N-Myc<sup>flox/flox</sup>) and mutant (Six2Cre;c-Myc<sup>flox/flox</sup>;N-Myc<sup>flox/flox</sup>) kidneys were stained with active caspase3, pH3 and Six2. The ratios of apoptotic (Active caspase3/Six2) and renewing (pHH3/Six2) cells are presented.